

Advances in
Inorganic Chemistry

Volume 61



Advances in
INORGANIC CHEMISTRY

Volume 61

ADVISORY BOARD

I. Bertini

*Università degli Studi di Firenze
Florence, Italy*

D. J. Darensbourg

*Texas A&M University
College Station
Texas, USA*

J. H. Espenson

*Iowa State University
Ames, Iowa, USA*

L. H. Gade

*Universität Heidelberg
Germany*

H. B. Gray

*California Institute of Technology
Pasadena, California, USA*

M. L. H. Green

*University of Oxford
Oxford, United Kingdom*

P. A. Lay

*University of Sydney
Sydney, Australia*

A. E. Merbach

*Laboratoire de Chimie et
Bioinorganique, EPFL
Lausanne, Switzerland*

J. Reedijk

*Leiden University
Leiden, The Netherlands*

P. J. Sadler

*University of Warwick
Warwick, England*

Y. Sasaki

*Hokkaido University
Sapporo, Japan*

K. Wieghardt

*Max-Planck Institut
Mülheim, Germany*

Advances in **INORGANIC CHEMISTRY**

EDITED BY

Rudi van Eldik and Colin D. Hubbard

Department of Chemistry and Pharmacy
University of Erlangen-Nürnberg
91058 Erlangen
Germany

VOLUME 61: Metal Ion Controlled Reactivity



AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD
PARIS • SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
ACADEMIC PRESS IS AN IMPRINT OF ELSEVIER



Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

First edition 2009

Copyright © 2009 Elsevier Inc. All rights reserved

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the publisher

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone (+44) (0) 1865 843830; fax (+44) (0) 1865 853333; email: permissions@elsevier.com. Alternatively you can submit your request online by visiting the Elsevier web site at <http://www.elsevier.com/locate/permissions>, and selecting *Obtaining permission to use Elsevier material*

Notice

No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made

Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN: 978-0-12-375033-4

ISSN: 0898-8838

For information on all Academic Press publications visit our website at elsevierdirect.com

Printed and bound in USA

09 10 11 12 13 10 9 8 7 6 5 4 3 2 1

Working together to grow
libraries in developing countries

www.elsevier.com | www.bookaid.org | www.sabre.org

ELSEVIER

BOOK AID
International

Sabre Foundation

CONTENTS

LIST OF CONTRIBUTORS	ix
PREFACE	xi

Controlling Platinum, Ruthenium, and Osmium Reactivity for Anticancer Drug Design

PIETER C.A. BRUIJNINCX and PETER J. SADLER

I. Introduction	1
II. Platinum Anticancer Prodrugs: A Photoactivation Strategy . . .	4
III. Ruthenium–Arene Anticancer Drugs	21
IV. Osmium(II)–Arenes: A Cytotoxic Family of the Heavier Congener	51
V. Concluding Remarks	57
Acknowledgments	58
References	58

Design and Function of Metal Complexes as Contrast Agents in MRI

VOJTĚCH KUBÍČEK and ÉVA TÓTH

I. Introduction	63
II. Relaxivity of Gd ^{III} Complexes: Optimization of the Relaxivity . .	67
III. Stability Aspects	92
IV. Paramagnetic CEST Agents	98
V. Responsive Imaging Probes	101
VI. Mn ^{II} Complexes in the Context of MRI	109
VII. Non-Classical Gd ^{III} -Based MRI Contrast Agents	115
VIII. Conclusions and Outlook	119
References	119

Design Considerations Towards Simultaneously Radiolabeled and Fluorescent Imaging Probes Incorporating Metallic Species

SOFIA I. PASCU, PHILIP A. WAGHORN, TIMOTHY CONRY,
BONITA LIN, CATRIN JAMES and JAMEEL M. ZAYED

I. Introduction	131
II. Small-Molecule Metal-Based Probes and Their Biomedical Imaging Capabilities	137

III. Conclusions	171
Acknowledgments	172
References	172

Iron Sequestration by Small Molecules: Thermodynamic and Kinetic Studies of Natural Siderophores and Synthetic Model Compounds

ALVIN L. CRUMBLISS and JAMES M. HARRINGTON

I. Chemistry of Iron and Siderophores	179
II. Siderophore Structure	182
III. Thermodynamics of Iron–Siderophore Interactions	186
IV. Chelation Kinetics of Iron–Siderophore Systems	220
V. Siderophore Recognition and Cellular Uptake	231
VI. Applications and Future Directions in Siderophore Research	235
List of Structure Numbers, Figures, and Abbreviations	240
Acknowledgments	242
References	242

Calcium in Biological Systems

JOHN BURGESS and EMMA RAVEN

I. Introduction	252
II. The Ca^{2+} Aqua-Cation	268
III. Calcium-Binding Metalloproteins	289
IV. Ca^{2+} in Ionophores, Channels, and Pumps	308
V. Carbohydrates and Carboxylates	316
VI. Phosphates, Phosphonates, Nucleosides, and Nucleotides	321
VII. Stability Constants: An Overview	326
VIII. Calcium-Containing Rigid Materials	326
Ligand Abbreviations	338
Acknowledgments	339
References	339
Appendix: The Term Ligand	365

New Developments in Synthetic Nitrogen Fixation with Molybdenum and Tungsten Phosphine Complexes

AMELI DREHER, GERALD STEPHAN and FELIX TUCZEK

I. Introduction	367
II. Mechanism of the Chatt Cycle: Experimental Investigations	371
III. Mechanism of the Chatt Cycle: Theoretical Investigations	382
IV. Molybdenum Dinitrogen Complexes with Polydentate Phosphine and Mixed P/N Ligands	386

V. Conclusions	401
Acknowledgments	402
References	402

Chemistry of Metalated Container Molecules

BERTHOLD KERSTING and ULRIKE LEHMANN

I. Introduction	407
II. Metalated Container Molecules: Host Systems and Complex Types	409
III. Metalated Container Molecules of Binucleating Polyaza-Dithiophenolate Macrocycles	430
IV. Conclusion	457
Acknowledgments	458
References	458

Mechanistic Considerations on the Reactivity of Green Fe^{III}-TAML Activators of Peroxides

ALEXANDER D. RYABOV and TERRENCE J. COLLINS

I. Introduction	472
II. Speciation of Tetraamide Iron(III) Macrocycles in Solution and the Solid State	473
III. Kinetics and Mechanisms of Demetalation of Fe ^{III} -TAML Activators	478
IV. Catalysis-Relevant Oxidized Forms Derived from Fe ^{III} -TAMLs . .	487
V. Mechanism of Catalysis by Tetraamide Macrocyclic Fe ^{III} -TAML Activators of Hydrogen Peroxide, Functional Catalase-Peroxidase Replicas	494
VI. The Activity-Stability Parameterization of Homogeneous Green Oxidation Catalysts	510
VII. Concluding Remarks	516
Acknowledgment	517
References	517

Ligand Exchange Processes on the Smallest Solvated Alkali and Alkaline Earth Metal Cations: An Experimental and Theoretical Approach

RALPH PUCHTA, EWA PASGRETA and RUDI VAN ELDIK

I. Introduction	523
II. Solvation of Metal Ions	526
III. Solvent Exchange Mechanism	533

IV. Conclusion	564
Acknowledgments	564
References	564

Spin-State Changes and Reactivity in Transition Metal Chemistry:
Reactivity of Iron Tetracarbonyl

MARIA BESORA, JOSÉ-LUIS CARREÓN-MACEDO,
ÁLVARO CIMAS and JEREMY N. HARVEY

I. Introduction	573
II. Chemistry of $\text{Fe}(\text{CO})_5$ Photofragments	577
III. Other Spin-Forbidden Reactions of Transition Metal Compounds .	606
IV. Spin-Forbidden Reactions: General Trends	609
V. Conclusions	616
Acknowledgments	618
References	618
INDEX	625
CONTENTS OF PREVIOUS VOLUMES.	649

LIST OF CONTRIBUTORS

Maria Besora

School of Chemistry and Centre for Computational Chemistry, University of Bristol, Bristol, United Kingdom

Pieter C.A. Bruijninx

Department of Chemistry, University of Warwick, Coventry, United Kingdom

John Burgess

Department of Chemistry, University of Leicester, Leicester, United Kingdom

José-Luis**Carreón-Macedo**

School of Chemistry and Centre for Computational Chemistry, University of Bristol, Bristol, United Kingdom; Department of Chemistry, University of Alberta, Edmonton, AB, Canada

Álvaro Cimas

School of Chemistry and Centre for Computational Chemistry, University of Bristol, Bristol, United Kingdom

Terrence J. Collins

Department of Chemistry, Institute for Green Science, Carnegie Mellon University, Pittsburgh, PA, USA

Timothy Conry

Oxford Siemens Molecular Imaging Laboratory, Chemistry Research Laboratory, University of Oxford, Oxford, United Kingdom

Alvin L. Crumbliss

Department of Chemistry, Duke University, Durham, NC, USA

Ameli Dreher

Institut für anorganische Chemie der Christian Albrechts Universität zu Kiel, Max-Eyth-Strasse 2, Kiel, Germany

James M. Harrington

Department of Chemistry, Duke University, Durham, NC, USA

Jeremy N. Harvey

School of Chemistry and Centre for Computational Chemistry, University of Bristol, Bristol, United Kingdom

Catrin James

Chemistry Department, University of Bath, Claverton Down, United Kingdom

Berthold Kersting

Institut für Anorganische Chemie, Universität Leipzig, Leipzig, Germany

Vojtěch Kubíček

Centre de Biophysique Moléculaire, CNRS, Orléans Cedex 2, France

Ulrike Lehmann

Institut für Anorganische Chemie, Universität Leipzig, Leipzig, Germany

Bonita Lin

Oxford Siemens Molecular Imaging Laboratory, Chemistry Research Laboratory, University of Oxford, Oxford, United Kingdom

Sofia I. Pascu

Chemistry Department, University of Bath, Claverton Down; Oxford Siemens Molecular Imaging Laboratory, Chemistry Research Laboratory, University of Oxford, Oxford, United Kingdom

Ewa Pasgreta

Inorganic Chemistry, Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Erlangen, Germany

Ralph Puchta

Inorganic Chemistry, Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Erlangen; Computer-Chemistry-Center, Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Erlangen, Germany

Emma Raven

Department of Chemistry, University of Leicester, Leicester, United Kingdom

Alexander D. Ryabov

Department of Chemistry, Institute for Green Science, Carnegie Mellon University, Pittsburgh, PA, USA

Peter J. Sadler

Department of Chemistry, University of Warwick, Coventry, United Kingdom

Gerald Stephan

Institut für anorganische Chemie der Christian Albrechts Universität zu Kiel, Max-Eyth-Strasse 2, Kiel, Germany

Éva Tóth

Centre de Biophysique Moléculaire, CNRS, Orléans Cedex 2, France

Felix Tuczek

Institut für anorganische Chemie der Christian Albrechts Universität zu Kiel, Max-Eyth-Strasse 2, Kiel, Germany

Rudi van Eldik

Inorganic Chemistry, Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Erlangen, Germany

Philip A. Waghorn

Oxford Siemens Molecular Imaging Laboratory, Chemistry Research Laboratory, University of Oxford, Oxford, United Kingdom

Jameel Zayed

Oxford Siemens Molecular Imaging Laboratory, Chemistry Research Laboratory, University of Oxford, Oxford; University Chemical Laboratories, University of Cambridge, Cambridge, United Kingdom

PREFACE

It was noted in the preface of Volume 60 that the next volume (61) would be a thematic issue, entitled "Metal Ion Controlled Reactivity." For this volume, as for Volume 54, I have invited Colin D. Hubbard, Professor Emeritus of Chemistry, University of New Hampshire, Durham, USA and University of Erlangen-Nürnberg, Erlangen, Germany, to serve as co-editor. The theme, in a wide variety of manifestations has been addressed by an outstanding group of contributors, and ranges from what may be termed real-life chemistry to prediction of metal complex reactivity from sophisticated calculations. As could be anticipated, kinetics and mechanism, or reactivity relationships of metal ion based systems, although not exclusively, feature in the authors' deliberations.

The initial trio of chapters is of undoubted significance in medicinal chemistry or in actual medical application. Chapter 1, by Pieter C.A. Bruijninx and Peter J. Sadler, is "Controlling platinum, ruthenium and osmium reactivity for anticancer drug design." This is followed by "Design and function of metal complexes as contrast agents in MRI", by Vojtěch Kubiček and Éva Tóth, and "Design considerations towards simultaneously radiolabeled and fluorescent imaging probes incorporating metallic species," by Sofia I. Pascu and her colleagues. Chapters 4 and 5 are "Iron sequestration by small molecules: Thermodynamic and kinetic studies of natural siderophores and synthetic model compounds" by Alvin L. Crumbliss and James M. Harrington and "Calcium in biological systems" by John Burgess and Emma Raven, respectively. Both chapters present biological and bioinorganic reaction chemistry of the respective metal ions and with considerable medicinal relevance. In Chapter 6 Ameli Dreher, Gerald Stephan, and Felix Tuczek describe "New developments in synthetic nitrogen fixation with molybdenum and tungsten phosphine complexes." This is followed by the "Chemistry of metalated container molecules," by Berthold Kersting and Ulrike Lehmann, and Chapter 8 is an account "Mechanistic considerations on the reactivity of green Fe^{III} -TAML activators of peroxide" by Alexander D. Ryabov and Terrence J. Collins. Ralph Puchta, Ewa Pasgreta, and Rudi van Eldik have authored a chapter, "Ligand exchange processes on the smallest solvated alkali and alkaline earth metal cations: An

experimental and theoretical approach.” A final contribution, Chapter 10, by Jeremy N. Harvey and colleagues is entitled “Spin-state changes and reactivity in transition metal chemistry: Reactivity of iron tetracarbonyl.”

The authors’ endeavors in generating these topical reviews are acknowledged. We trust the readers in the inorganic and bioinorganic chemistry communities will find this volume informative and useful.

Rudi van Eldik
Department of Chemistry and Pharmacy
University of Erlangen-Nürnberg
Erlangen
Germany
June 2009

CONTROLLING PLATINUM, RUTHENIUM, AND OSMIUM REACTIVITY FOR ANTICANCER DRUG DESIGN

PIETER C.A. BRUIJNINCX and PETER J. SADLER

Department of Chemistry, University of Warwick, Coventry CV4 7AL, United Kingdom

I. Introduction	1
II. Platinum Anticancer Prodrugs: A Photoactivation Strategy	4
A. Prodrug Strategies	5
B. Photoactivated Platinum Anticancer Drugs	9
III. Ruthenium–Arene Anticancer Drugs	21
A. General Features of Ruthenium–Arene Anticancer Drugs	24
B. Cytotoxicity Studies: Towards Establishing Structure–Activity Relationships	29
C. Reactivity of Ruthenium–Arene Anticancer Drugs	32
IV. Osmium(II)–Arenes: A Cytotoxic Family of the Heavier Congener	51
V. Concluding Remarks	57
Acknowledgments	58
References	58

I. Introduction

The main task of the medicinal chemist is to design molecules that interact specifically with derailed or degenerating processes in a diseased organism, translating the available knowledge of pathobiochemical and physiological data into chemically useful information and structures (*1*). Current knowledge of the biological and chemical processes underlying diseases is vast and rapidly expanding. In particular the unraveling of the genome in combination with, for instance, the rapid development of structural biology has led to an explosion in available information and identification of new targets for chemotherapy. The task of translating this wealth of data into active and selective new drugs is an enormous, but realistic, challenge. It requires knowledge from many different fields, including

molecular biology, chemistry, pharmacology, physiology, and medicine and as such requires a truly interdisciplinary approach.

Ultimately, the goal is to design molecules that satisfy all the requirements for a candidate drug to function therapeutically. Therapeutic activity can then be achieved by an understanding of and control over structure and reactivity of the candidate drug through molecular manipulation.

Metals in Medicine. Our involvement in drug design comes from our interest in the development of metal-based chemotherapeutics. The use of metals in medicine is as old as human civilization and has, in more recent years, led to the establishment of the field of medicinal inorganic chemistry. Initially fuelled by the discovery of the cytotoxic activity of cisplatin, the world's most widely used chemotherapeutic anticancer drug today, the field has developed into a thriving area of research, now with several other notable successes such as the gadolinium-based MRI agents. We and others have recently discussed the use of metals in medicine in more comprehensive overviews (2–6). The use of metals is attractive as they offer a unique spectrum of reactivity and indeed a structural diversity that is not readily available in the more common organic-based drugs. The wide range of coordination numbers and geometries, accessible redox states, thermodynamic and kinetic characteristics, and the intrinsic properties of both the cationic metal ion and ligand itself offer the medicinal chemist a wide spectrum of reactivities that can be exploited. The (transition) metals provide a rich palette from which to choose and offer the real possibility of the discovery of truly novel drugs with new mechanisms of action (2).

In our group, a major part of our research is devoted to the design of new anticancer drugs. Our recent efforts towards the discovery of new platinum-, ruthenium- and osmium-based anticancer agents provide the topic for this account and a section is devoted to each metal. We focus on recent results from our lab in the context of other developments and related research in this field (hence our coverage of the field is focused on these areas and is not comprehensive).

Platinum and Ruthenium Anticancer Drugs. In the field of anticancer drug design and in particular with metallochemotherapeutics, DNA has most commonly served as the major target, either by direct interaction with the drug or indirectly by inhibition of DNA synthesis and replication. The anticancer agents thus work by inhibiting cell growth and/or by hastening cell death. The cytotoxic effects of complexes are experienced by the rapidly dividing tumor cells, but this mode of action invariably means that normal cells (especially rapidly dividing

healthy cells of bone marrow, gut, and the skin epithelium) are affected as well. This lack of selectivity of cancer drugs is one of the main problems of cancer chemotherapy. Addressing this problem of selectivity, together with that of (multi)drug resistance, is one of the major goals of research in this field. The genomic revolution has identified many new targets, for instance elevated levels of particular enzymes in tumor cells or their involvement in pathways significant for proliferation, that hold some promise for the development of more selective drugs. Other strategies to alleviate systemic toxicity and resistance make use of prodrugs that can either be activated locally by an *internal* stimulus, e.g. a physiological difference in the environment (pH, salt concentration, redox potential) or an enzyme-catalyzed chemical transformation, or, alternatively, by an *external* stimulus, such as light. Examples of both approaches will be discussed.

Considerations for Metal-Based Drug Design. Regardless of the target or approach chosen, many considerations for drug design apply to all metal-based therapeutics. As with organic drugs, the ADME properties (absorption, distribution, metabolism, and excretion) are of prime importance as the pharmacological and pharmacokinetic parameters of the drug must be such for the drug to be able to reach the site of action in a timely manner, in a sufficient concentration, and be effectively cleared from the system (1). Additionally, for a potent and selective drug, the possibility for strong association with the intended target preferably via multiple specific interactions should be incorporated into the design of the drug. Metal-based drugs have additional possibilities for such molecular recognition, as reactive sites might be available that can result in direct coordination to the target. In this respect, stereochemical arguments play an important role as well.

Some aspects of drug design need to be addressed specifically for metal-based therapeutics. The reactivity that offers so many options for new mechanisms of action, also poses some inherent difficulties in delivering the drug to its target. It is therefore important to constrain the reactivity of the complex sufficiently for it to reach its target, without being deactivated by reactions with the large number of biomolecules encountered along the way. If they are designed to bind directly to the target then the compounds should also not be too stable, as too high kinetic inertness in general will render them nontoxic. This is a feature that, however, can be harnessed for the design of activatable prodrugs. The prodrug approaches briefly mentioned above attempt to address these challenges. Other characteristics

specific to metal-containing compounds that can have a major influence on their cytotoxic properties include geometrical isomerism and redox properties.

To understand fully processes such as molecular recognition, reactivity and bioactivity, it is therefore imperative to obtain a detailed insight into the thermodynamic and kinetic properties of the metal-based anticancer agent at hand.

An important part of our work is concerned with the study of the coordination chemistry of the anticancer drugs under physiological conditions and in the presence of biomolecules that can either be the intended target or a possible influence on deactivation or activation of the drug. Identification of the key processes for activity, such as hydrolysis or photoreduction, and subsequent manipulation by systematic ligand variation proved to be valuable for obtaining potent anticancer agents. Indeed, the following sections will show that it is possible to control metal-ion reactivity for drug design.

II. Platinum Anticancer Prodrugs: A Photoactivation Strategy

Introduction to Platinum Anticancer Drugs. Platinum anticancer drugs such as the archetypical cisplatin and second-generation drugs carboplatin and oxaliplatin (Fig. 1) are widely employed in cancer chemotherapy and are amongst the most effective chemotherapeutic agents in clinical use. Cisplatin is particularly active against testicular cancer and, if tumors are discovered early, an impressive cure rate approaching 100% is achieved. The clinical use of cisplatin against this and other malignancies is, however, limited. The low-efficacy stems from a combination of uni- and multicellular resistance, poor whole-body or cellular pharmacokinetic profiles, and systemic toxicity (7). Dose-limiting side effects include neuro-, hepato- and nephrotoxicity. Hence,

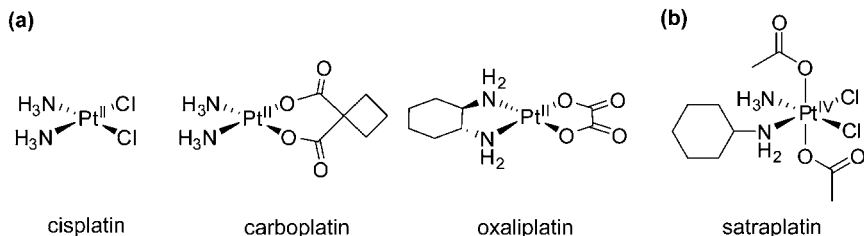


FIG. 1. Molecular structures of (a) some platinum(II)-based anticancer drugs currently used in the clinic; (b) satraplatin, a platinum(IV) prodrug currently in clinical trials.

much effort has been devoted to the development of new platinum drugs and to the elucidation of cellular responses to them to alleviate these limitations (8,9). An excellent review on the current status of platinum-based cancer chemotherapy is available (10). Of the various strategies that have been developed (11), those using platinum(IV) complexes as *prodrugs* appear to have particular potential (7). Some general properties of metal-based prodrugs are briefly discussed below.

A. PRODRUG STRATEGIES

Prodrugs are derivatives of drug molecules that undergo an enzymatic or other chemical transformation *in vivo* to release the active parent drug (12), or indeed to generate an active species that could not be delivered by itself (because it is too reactive). Prodrug strategies are aimed at improving physicochemical, biopharmaceutical and pharmacokinetic properties of pharmacologically active agents (12). Major applications include the ability to improve oral bioavailability, for instance by increasing aqueous solubility or lipophilicity, and to achieve site-selective drug delivery. The latter is a central aim in cancer therapy: targeting an inactive prodrug selectively to tumor cells, followed by its release, so avoiding toxicity to normal, healthy tissue (12).

Prodrug strategies are common for metal-based anticancer drugs as well; in fact cisplatin itself can be regarded as a prodrug since hydrolysis of the Pt–Cl bonds activates the drug for DNA binding (Fig. 2). Prodrug activation mechanisms of metal-based prodrugs are usually different from organic prodrug activation mechanisms, which typically involve bioconversion by enzymes (e.g. esterases, phosphodiesterases, reductive enzymes). Metal-based prodrugs are commonly activated by ligand substitution, a change in oxidation state, a photochemical process, or combinations of these (Figs. 2 and 3). Ligand substitution can result in the creation of a (more) reactive site on the metal, as occurs during hydrolysis of cisplatin. Alternatively, substitution can result in the controlled release of a biologically active ligand, a typical example being nitric oxide delivery by photolabile metal nitrosyls (13), or CO delivery by metal carbonyls (14). Drug activation by substitution requires detailed knowledge of exchange kinetics. The ligand-exchange rates depend not only on the metal employed and its oxidation state, but also on the other ligands in the complex (often stereospecifically) and can vary over many orders of magnitude. For example, the exchange rates for aqua ligands on metal ions vary over some 19 orders of magnitude, even though the M–OH₂ bonds can have similar

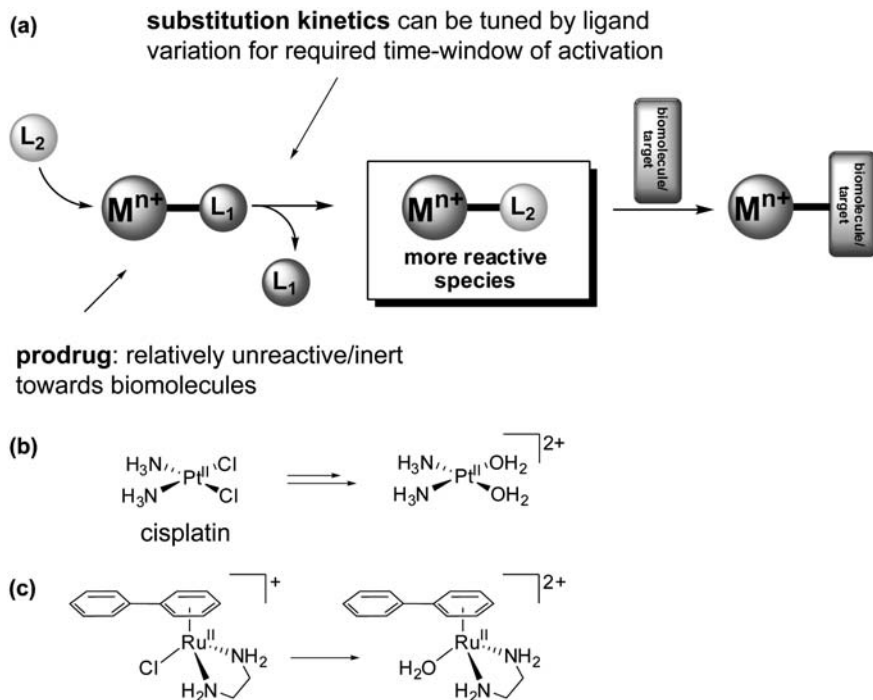


FIG. 2. Ligand substitution as a prodrug strategy for metallochemotherapeutics; (a) general scheme of prodrug activation by ligand substitution; hydrolysis of a metal–halide bond is a typical activation pathway of metal-based anticancer drugs, as exemplified by the activation of cisplatin (b) and a ruthenium–arene complex (c).

thermodynamic strengths (15,16). Consideration of exchange kinetics is therefore of prime importance in drug design. The timescale of substitution needs to be long enough to allow the drug to reach its target site before it is activated, but activation needs to take place before excretion, leaving a relatively narrow window. Furthermore, the ligand-exchange rates can depend on pH, the stereochemistry of the complex (cf. hydrolysis rates of cisplatin vs. its geometric isomer transplatin), and the other ligands coordinated to the metal, therefore providing many options to exert control over this process. Such considerations have played a major role in the development of ruthenium- and osmium–arene anticancer complexes in our group, a topic that will be discussed further in [Sections III and IV](#).

A redox activation mechanism usually involves a change in oxidation state of the metal (but could also be ligand-centered). Typically, the oxidation state changes from a state in which

ligand-exchange is very slow, i.e. the complex is kinetically inert, to a state in which ligand-exchange can occur. Again, reduction can either result in a reactive metal complex, such as reduction of platinum(IV) complexes to yield reactive platinum(II) species (7), or in controlled release of bioactive ligands. An example of the latter is Co(III) acting as a chaperone for an analog of marimastat, a cytotoxic inhibitor of matrix metalloproteinases, which is then released upon reduction of the metal to Co(II) (17). The redox change can be triggered by the properties of the local environment. The cobalt chaperone complexes, for instance, exploit high-level hypoxia, a property both common in and unique to solid tumors, for activation, as low levels of oxygen prevent reoxidation to the inert Co(III) complex. Other physiological characteristics that can be used to advantage are the reducing environment in the cell, tissue permeability, and pH (18). These properties differ between healthy and tumor cells, which provides a strategy for tumor-selective activation of the prodrug. Cancer cell selectivity can furthermore be achieved by targeting of biomarkers and receptors that are specifically expressed on tumor cell surfaces. Additionally, levels of certain enzymes are often elevated in these cells and this can also be exploited in targeted prodrug-tumor delivery. Conjugation of a bioactive tumor-targeting group to the metal-based prodrug thus provides another interesting strategy for tumor cell-selective activation to combat systemic toxicity.

Photoactivation as a Prodrug Strategy. Alternatively, prodrug activation by ligand substitution or redox change can also be brought about by photochemical means (19). In general, the combination of light and metal complexes has been widely employed in medicine (20). Phototherapy of cancer, i.e. the treatment of a tumor with a drug and light, is particularly attractive as it allows for local treatment of the tumor (i.e. site-specific activation of the photodrug), thereby minimizing the side effects of chemotherapy. Recent advances in laser and fiber optic technologies have made it possible now to reach almost any tissue in the body with light of highly defined intensity and wavelength, expanding the applicability of this approach beyond only those cancers that are easily accessible. A key parameter in these studies is the wavelength of the light. In general, for efficient phototherapy, light with wavelengths between 300 and 900 nm should be employed, as shorter wavelengths are typically damaging to tissue, DNA, and proteins, and longer wavelengths are of insufficient energy for chemical transformation (19). Within this window, longer wavelengths are preferred if deep penetration into tissue is required. Photodynamic therapy (PDT) is a common technique used in the clinic that selectively damages

tissue of easily accessible cancers (e.g. skin, neck and head, mouth, oesophagus, and bladder) by using a photosensitizing drug and light. In PDT, a nontoxic photosensitizer catalytically generates cytotoxic singlet oxygen after electronic excitation by light. This oxygen-dependence for cytotoxicity is a major drawback of PDT as many malignant and most aggressive cancer cells are hypoxic (21). There is therefore an incentive to develop photochemotherapy that is not dependent on oxygen. We have recently reviewed the different approaches to photochemotherapy with metal-based anticancer complexes (19).

Pt(IV) Prodrugs. Platinum(IV) complexes have been widely studied as potential prodrugs that avoid the limitations of the cisplatin class of anticancer drugs. Indeed, the Pt(IV) compound satraplatin $[\text{Pt}(\text{cha})\text{Cl}_2(\text{OAc})_2(\text{NH}_3)]$ (cha, cyclohexylamine) is currently in clinical trials for treatment of hormone-refractory prostate cancer (Fig. 1) (22). Satraplatin is the first orally bioavailable platinum derivative under active clinical investigation and is particularly attractive because of the convenience of administration, milder toxicity profile, and lack of cross-resistance with cisplatin. These results are promising and support the idea that platinum(IV) complexes offer the opportunity to overcome some of the problems associated with cisplatin and its analogs.

In general, the high kinetic inertness of Pt(IV) complexes lowers reactivity and the prospect of side reactions (7). In fact, substitution reactions of the ligands take place very slowly or not at all under physiological conditions. Hence, intracellular reduction to Pt(II), for instance by cellular reducing agents, is thought to be essential for cytotoxic activity. In addition, the octahedral geometry of Pt(IV) introduces two extra ligand sites, which offer many possibilities for drug design. They can be used to modify the pharmacokinetic parameters of the prodrugs, including the rate of reduction and lipophilicity, and to allow the inclusion of biologically active ligands that either target the complexes to tumor cells or, alternatively, are themselves cytotoxic upon release (7). Recent advances in the design and development of Pt(IV) anticancer complexes have been reviewed (7).

An interesting alternative mechanism of activation is the photochemical reduction of Pt(IV) to Pt(II) (Fig. 3). In addition to photoreduction, photosubstitution and photoisomerization can also occur, making the photochemistry of Pt complexes difficult to predict and a careful analysis of the photoproducts imperative (21). We have been involved particularly in the development of photochemotherapeutic agents based on Pt(IV) and the study of their photodecomposition and (subsequent) interactions with

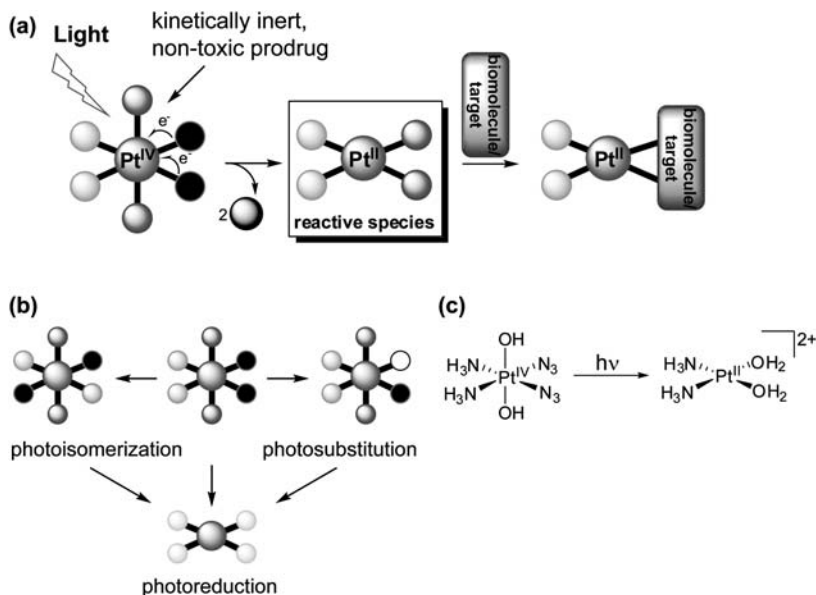


FIG. 3. Photoactivation of Pt(IV) complexes as a prodrug strategy for metallochemotherapeutics; (a) general scheme of prodrug activation by photoreduction; (b) photosubstitution and photoisomerization are competing photoreaction pathways, which can result in different reactive species upon reduction; (c) an example of a photoactive platinum(IV) diazido complex developed in our lab.

biomolecules (21). Our aim is the development of Pt(IV) prodrugs that are stable towards reduction, nontoxic in the dark, and activated only by light and not by cellular processes or responses to other stimuli, such as pH or biological reducing agents. In the following sections, a detailed overview is given on how rational ligand variation led to control over metal ion reactivity and to the development of highly cytotoxic, selectively activatable platinum anticancer drugs.

Ultimately, for Pt(IV) anticancer drugs, a combination of incorporation of bioactive ligands that specifically target cancer cells, control over ligand-exchange kinetics, and selective activation by light would allow for temporal and spatial control of drug delivery and activation.

B. PHOTOACTIVATED PLATINUM ANTICANCER DRUGS

Pt(IV)–Iodido Complexes. The first photoactive Pt(IV) prodrugs were reported by Bednarski *et al.* in the 1990s (23). The complex

trans,cis-[PtCl₂(en)I₂] (**1**; en = ethylenediamine) (Fig. 4) can be photolyzed with visible light to give species that bind to DNA and inhibit the growth of human cancer cells *in vitro*. The chelating ligand en was chosen as a nonlabile ligand (as opposed to NH₃) to avoid photoisomerization to the thermodynamically more stable but originally considered inactive *trans*-isomer (*vide infra*). This compound, however, suffered from a very limited stability in serum even in the absence of light. Consequently, no difference in dark and light toxicity was seen towards human cancer cells. This was most likely due to the relatively high reduction potential of this complex, which leads to facile intracellular reduction (21). Electron transfer to the metal can readily occur through donation from the coordinated iodo ligands (24). The nature of the axial ligand exerts a major influence on the reduction potential and indeed, altering these ligands led to complexes with better dark stability (23). In the dark, the complex *cis,trans*-[Pt(en)I₂(OAc)₂] (**2**) (Fig. 4) showed no binding of Pt to CT DNA after 6 h, but, on irradiation ($\lambda_{\text{irr}} > 375$ nm)

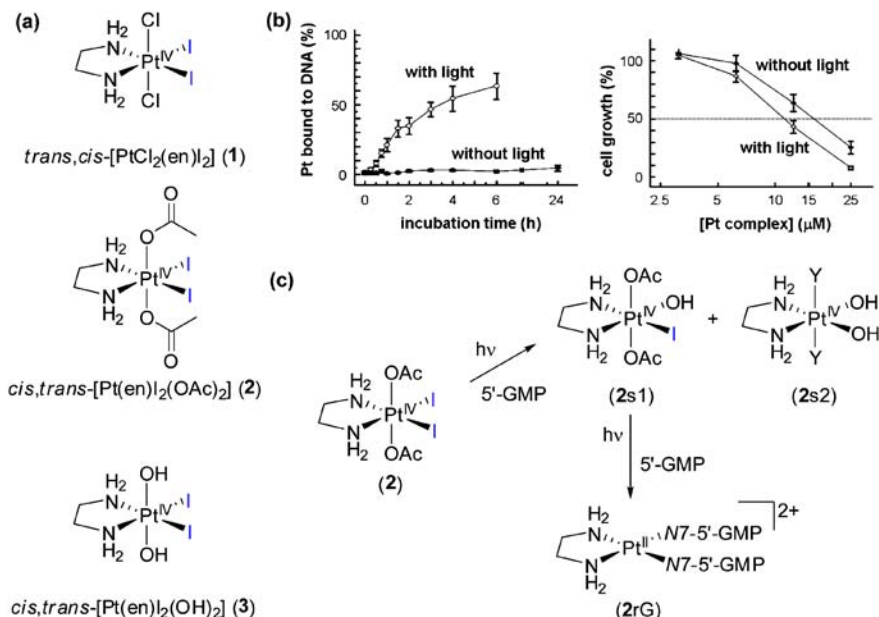


FIG. 4. Photoactive Pt(IV)-iodido complexes. (a) Molecular structures of complexes **1**–**3**; (b) influence of visible light on CT DNA binding of **2** and cytotoxicity of **2** against a TCCSUP human bladder cell line (data from Ref. (23)); (c) NMR studies showed that photosubstitution precedes photoreduction in the reaction of **2** with 5'-GMP upon irradiation Ref. (25).

resulted in 63% of the platinum being bound to CT DNA after 6 h, suggesting photoreduction to Pt(II) and loss of the iodido ligands. The electronic spectra of diiodido-Pt(IV) complexes are dominated by relatively low-energy LMCT transitions, which allow irradiation of these complexes with visible light. Cancer cell growth-inhibition studies showed a small, but significant difference in light and dark cytotoxicity, with a 35% greater growth inhibition activity with irradiation. The complex *cis,trans*-[Pt(en)I₂(OH)₂] (**3**) (Fig. 4), on the other hand, gave rise to very limited DNA platination on irradiation and a smaller enhancement of antiproliferative activity (22%). The nonphotoalyzed complex could, however, be chemically reduced quite readily by addition of the major cellular reducing-agent glutathione (γ -L-Glu-L-Cys-Gly; GSH). This led to the conclusion that photolysis resulted predominately in photosubstitution rather than photoreduction in this case, illustrating the influence of the axial ligands on photodecomposition pathways (23).

As intracellular reduction mechanisms are important for Pt(II) and Pt(IV) drugs alike, the chemical reduction of *cis,trans*-[Pt(en)I₂(OH)₂] (**3**) by GSH was further explored under biologically relevant conditions (24), which led to the unexpected detection of a long-lived chelate-ring-opened Pt(II) complex capable of forming DNA-Pt adducts.

These initial results showed for the first time that it is feasible to design photoactivated platinum anticancer drugs by proper choice of the coordinating ligands (23). Cisplatin exerts its cytotoxic effect through binding to DNA, which is thought to be a major target of the Pt(IV) cisplatin-type prodrugs after activation as well. It is therefore important to gain insight into the reactivity of photoactive prodrugs towards DNA and model compounds, such as nucleobases and nucleotides. In further collaboration with the Bednarski group, we therefore studied the interaction of the photoactive platinum complexes **2** and **3** with nucleotides. Stereospecific reactions between guanosine 5'-monophosphate (5'-GMP) and *cis,trans*-[Pt(en)I₂(OH)₂] (**3**) or *cis,trans*-[Pt(en)I₂(OAc)₂] (**2**) could be induced by visible light (λ_{irr} 457.9 nm) and photoactivation could be controlled by the axial ligands, allowing fine-tuning of the photoreactivity of this class of complexes (25). NMR studies on nucleotide binding of ¹⁵N-labelled complexes confirmed the previously observed differences in DNA-binding properties of the two complexes and the suggested differences in the photolysis mechanism. 1D ¹H, 2D [¹H, ¹⁵N] HSQC and 2D [¹H, ¹⁵N] HSQC-TOCSY NMR studies of photoreactions of *cis,trans*-[Pt(en)I₂(OH)₂] (**3**) showed that neither the dihydroxido complex nor its photosubstitution

product *mer*-[Pt(en)I(OH)₃] react with 5'-GMP. On the contrary, similar experiments on *cis,trans*-[Pt(en)I₂(OAc)₂] (**2**) revealed two steps in the photoactivation. First, photosubstitution of the labile iodo ligands resulted in formation of two complexes, *trans*-[Pt(en)I(OAc)₂(OH)] (**2s1**) and *cis,trans*-[Pt(en)(OH)₂Y₂] (**2s2**) (Y might be I⁻ or OH⁻). The latter complex does not react any further, but kinetic analysis revealed that in a second photoactivation step *trans*-[Pt(en)I(OAc)₂(OH)] (**2s1**) is photoreduced and the bis-nucleotide adduct [Pt(en)(5-GMP-N7)₂] (**2rG**) is observed.

The iodo-Pt(IV) complexes thus provided a proof-of-principle being photoactive, but the complexes still suffered from slow photoreactions and, importantly, limited stability in the dark especially against biological reducing agents such as glutathione, which results in undesired toxicity of the anticancer agents in the dark.

Pt(IV)–Azido Complexes. We sought to address this issue and reasoned that replacement of the iodo ligands for other photolabile ligands could improve stability against reducing agents. Pt(IV)–azido coordination complexes are well known to be photoactive (26,27) and previous studies on Pt(IV)–azido photochemistry had shown a two-electron reduction from Pt(IV) to Pt(II) with concomitant liberation of two unstable azide radicals, which rapidly decompose in water to form N₂ (Fig. 5) (27). Halide radicals, on the contrary, do not readily decompose in water, and therefore recombination to generate the starting material is a competing process. Azides therefore promised to be an attractive alternative to iodides for our drug design.

We synthesized and reported the first crystal structures of Pt(IV)–diazidodiam(m)ine compounds and showed that they also could be activated with visible light to give rapid and stereo-specific binding of the nucleotide 5'-GMP (28) (Fig. 6). The X-ray crystal structures of *cis,cis,trans*-[Pt(N₃)₂(NH₃)₂(OH)₂] (**4**) and *cis,trans*-[Pt(en)(N₃)₂(OH)₂] (**5**) show that the metal ion has a close to octahedral coordination geometry with almost linear

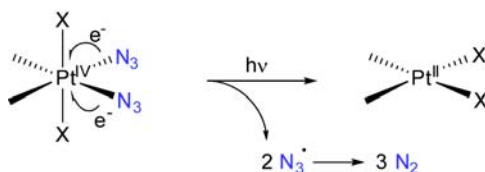


FIG. 5. Originally perceived, possible mechanism for photoactivation of platinum(IV) diazido complexes.

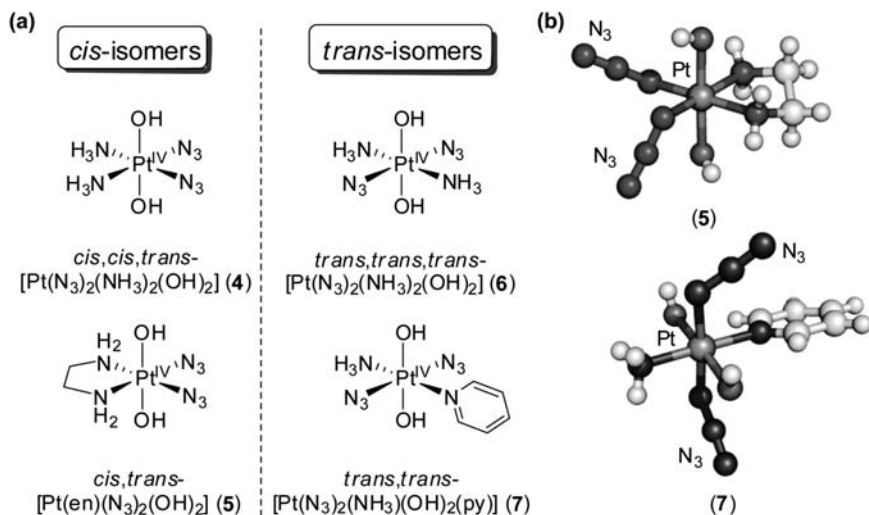


FIG. 6. (a) Molecular structures of Pt(IV)–diazido complexes 4–7; (b) X-ray crystal structures of a *cis*- (5) and *trans*-diazido complex (7).

azides in *cis*-position in the equatorial plane. Importantly, the complexes are stable in human blood plasma and the presence of even 5 mM GSH has little effect on their stability over a period of several weeks. No reactions with either 5'-GMP or the dinucleotide d(GpG) occurred over a week at 298 K in the dark, further illustrating that these complexes possess the low chemical reactivity that is desired in the dark. The electronic absorption spectra of complexes 4 and 5 contain a strong LMCT transition centered on 255 nm, but tailing into the visible. Irradiation of *cis,trans*-[Pt(en)(N₃)₂(OH)₂] (5) (λ_{irr} 457.9 nm, 15 mW) in the presence of 5'-GMP resulted in metal coordination of 60% of the nucleotide present after 20 h and the bis-nucleotide Pt(II) adduct [Pt^{II}(en)(5'-GMP-N7)₂]²⁺ was identified as the major product. Photoreaction with d(GpG) resulted in the rather clean, rapid, and stereospecific formation of [Pt^{II}(en){d(G¹pG²-N7¹,N7²)₂}]²⁺ (charges on the nucleotide are not considered). Similar photo-reactivity was observed for *cis,cis,trans*-[Pt(N₃)₂(NH₃)₂(OH)₂] (4). Photolysis of the complexes in water alone revealed that the photoreaction pathway involved both photoisomerization and photoreduction to highly reactive Pt(II) species of the kind implicated as capable of killing cancer cells. High power red light (λ_{irr} 647.1 nm, 75 mW), which penetrates tissue more effectively, was also able to activate the complexes.

DNA platination experiments with *cis,trans*-[Pt(N₃)₂(OH)₂(en)] (5) were performed both in the dark and with irradiation in

collaboration with the group of Brabec (29). DNA platination of a 212 base pair (bp) fragment of plasmid DNA and transcription mapping by RNA polymerase *in vitro* revealed that whereas no DNA binding occurred in the dark, DNA binding in the irradiated sample occurred to the same extent and produced similar stop sites as cisplatin, appearing at guanine residues mainly contained in GG sequences (29). These results indicate that the prodrugs indeed form cisplatin-like reactive intermediates upon irradiation, although other reactive species might be formed as well.

These promising results prompted us, in collaboration with the groups of Woods and Bednarski, to study the photocytotoxicity of platinum diazido complexes (30). The effect of photoactivation of *cis,trans*-[Pt(en)(N₃)₂(OH)₂] (**5**) and *cis,cis,trans*-[Pt(N₃)₂(NH₃)₂(OH)₂] (**4**) on the growth of 5637 human bladder cancer cells was quite dramatic. The complexes were nontoxic in the dark (IC₅₀ > 300 μM), yet irradiation resulted in selective inhibition of cell growth, with IC₅₀ values of 63 (**5**) and 49 μM (**4**) (Fig. 7). Growth inhibition by cisplatin, on the other hand, was unaffected by light. Importantly, the platinum diazido complexes were equally cytotoxic to 5637 and cisplatin-resistant 5637 (5637-CDDP) cell lines, showing no cross-resistance with

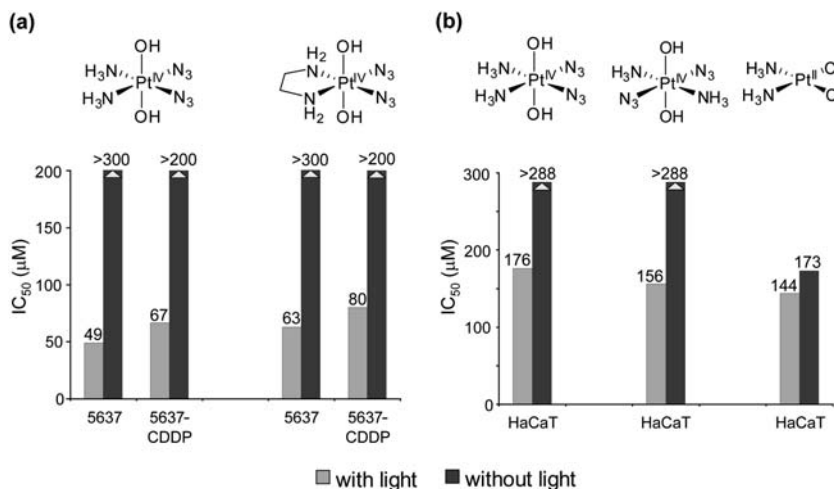


FIG. 7. Effect of light on the IC₅₀ values for the inhibition of cell growth of various cancer cell lines by platinum(IV) diazido complexes. (a) toxicity of the *cis*-complexes **4** and **5** on human bladder cancer cell lines (5637-CDDP, cisplatin-resistant cell line); (b) comparison of cytotoxicities of the *cis*- and *trans*-isomers **4** and **6** in the dark and upon irradiation; cisplatin is included for comparison. Data from Ref. (30).

cisplatin. This latter result suggests that the mechanism of action of the photoactive drug is actually different from cisplatin. This is an important observation, as overcoming tumor resistance is a major goal in anticancer drug design. Photoactivation of the prodrug resulted in dramatic changes in cell morphology, i.e. shrinking of the cancer cells, loss of adhesion, packing of nuclear material and disintegration of the nuclei. Such changes in morphology are not observed with cisplatin, providing further evidence that the light-activated complexes cause a very different kind of cytotoxicity than cisplatin (30). This suggests that additional cytotoxic species not available to cisplatin might be generated. CT DNA platination studies showed that photolysis rates matched closely the rates of irreversible DNA platination, indicating that the photolysis products react directly with DNA and notably faster than cisplatin, for which the platination rate is determined by slow hydrolysis steps.

It is important to note here that the activity of the platinum diazido complexes does not depend on the presence of oxygen, in contrast to conventional PDT, which is a potential advantage, as some tumors are oxygen-deficient.

The photoactive platinum compounds discussed so far have their labile, photoinduced electron-donating ligands (I^- , N_3^-) and their nitrogen donor ligands (NH_3 , en) positioned *cis* with respect to each other. They can therefore, at least in principle, be regarded as prodrugs of cisplatin-like complexes. It is well established that whereas cisplatin is highly cytotoxic to cancer cells, its *trans*-isomer, transplatin, is relatively nontoxic and clinically ineffective (31). Our observation that the *trans*-isomer of a platinum(IV) diazido complex, *trans,trans,trans*-[Pt(N_3)₂(NH_3)₂(OH)₂] (**6**), is as cytotoxic when photoactivated as cisplatin was therefore somewhat unexpected and quite remarkable (Fig. 7) (32). The stereochemistry of the octahedral complex was again confirmed by X-ray crystallography, and bond lengths and angles comparable to the *cis*-isomer were found. The *trans* complex has a much higher aqueous solubility than its *cis*-isomer. This is noteworthy as not only the reactivity of the drug needs to be tuned, but also other parameters, such as (oral) bioavailability.

Complex **6** exhibits an intense LMCT absorption band at 285 nm, which is shifted to longer wavelength and is more intense compared to the *cis*-isomer. Irradiation led to the disappearance of this band, indicating loss of azide. Photoactivation of the *trans*-isomer initially resulted in the appearance of new Pt(IV) complexes (probably substitution of N_3 for OH), as judged by 2D [^1H , ^{15}N] HSQC NMR, and after 60 min peaks for Pt(II) species appeared, including *trans*-[Pt(NH_3)₂(OH)₂]²⁺ (**6r**).

However, after 120 min of irradiation the majority of Pt was still in the +4 oxidation state. One of the new Pt(IV) complexes was identified as the monoazido species *trans,mer*-[Pt(N₃)(NH₃)₂(OH)₃] (**6s**), which indicates that the azides do not necessarily leave the platinum metal center together (33). Remarkably, photoreduction proceeded much faster in the presence of 5'-GMP, and after 1 h over 75% had converted to the major product, the bis-GMP adduct *trans*-[Pt^{II}(5'-GMP-N7)₂(NH₃)₂]²⁺. The absence of the range of side-products observed on photoactivation of *trans,trans,trans*-[Pt(N₃)₂(NH₃)₂(OH)₂] (**6**) alone, suggests that 5'-GMP can readily trap reactive intermediates produced during the photoexcitation process. No reaction with 5'-GMP is observed without irradiation. These results illustrate the importance of studying interactions with biomolecules as part of the attempt to elucidate the mechanism of action of these new anticancer complexes. The photoreactivity products are remarkable in that they not only form very fast compared to reactions of transplatin, but also the formation of bis-guanine adducts is rarely observed for transplatin. The lack of progression from mono- to bis-guanine adducts is thought to be responsible for its lack of anticancer activity (32).

Cytotoxicity studies (32) on HaCaT cells showed that both the *cis*- and *trans*-isomers of [Pt(N₃)₂(NH₃)₂(OH)₂] were equally active upon irradiation and as effective as cisplatin (Fig. 7). Again, the complexes are nontoxic in the absence of light. A "comet"-assay showed that the complexes produced DNA cross-linking in living cells on irradiation. An appreciation of the intensity of exposure to light in these experiments, can be obtained from the comparison that the intensity of the UVA irradiation used (5 J cm⁻² for 50 min) is equivalent to about 15–60 min of exposure to sunlight at midday in the United Kingdom. These results suggest that further studies also on the *trans*-isomers of the platinum diazido complexes are merited.

As systematic ligand variation provides an attractive method to explore and indeed tune the properties of metal-based anticancer drugs (see also the section on ruthenium–arenes, Section III), we tried to improve further our platinum diazido prodrug design by varying one of the amines in *trans,trans,trans*-[Pt(N₃)₂(OH)₂(NH₃)₂] (**6**) for different *N*-donor ligands, such as aliphatic amines (methylamine, ethylamine, cyclohexylamine) and aromatic *N* donors (pyridine, picolines, quinoline, and thiazole) (34). Replacement of the ammine by the π -acceptor pyridine ligand had a dramatic effect. The complex *trans,trans*-[Pt(N₃)₂(NH₃)(OH)₂(py)] (**7**) was found to be highly phototoxic (35). In the dark, no cytotoxicity towards HaCaT keratinocytes,

SH-SY5Y neuroblastoma, cisplatin-sensitive A2780 and cisplatin-resistant A2780cis human ovarian cancer cells was observed, but upon irradiation **7** strongly reduced the viability of the cancer cells (Fig. 8). In the A2780 cell line, the complex was 80× more toxic than cisplatin under identical conditions, and ca. 15× more effective against the cisplatin-resistant A2780cis cell line (33). The *trans* diazido-Pt(IV) complex therefore has remarkable cytotoxic properties.

Complex **7** is stable in water, does not react with 5'-GMP in the dark, and only 5% of Pt(IV) was reduced by GSH after 21 days. Irradiation of the complex alone in water yielded little reduction to Pt(II) and photosubstitution of one or both of the azides was observed. In the presence of 5'-GMP, however, rapid photoreduction resulted in the formation of the mono-adduct (SP-4-4)-[Pt^{II}(5'-GMP-N7)(N₃)(NH₃)(py)]⁺ (**7rG**) and bis-adduct *trans*-[Pt^{II}(5'-GMP-N7)₂(NH₃)(py)]²⁺ (**7rG2**) (Fig. 9). These results again argue for stepwise (photo)dissociation of the two azides, rather than reductive elimination of both azides in a concerted step.

Several biological assays pointed to an unusual mechanism of action for **7**, which is clearly different from cisplatin, as expected from its remarkable cytotoxic activity. For example, different levels of p53 and caspase activity were observed. Unusual

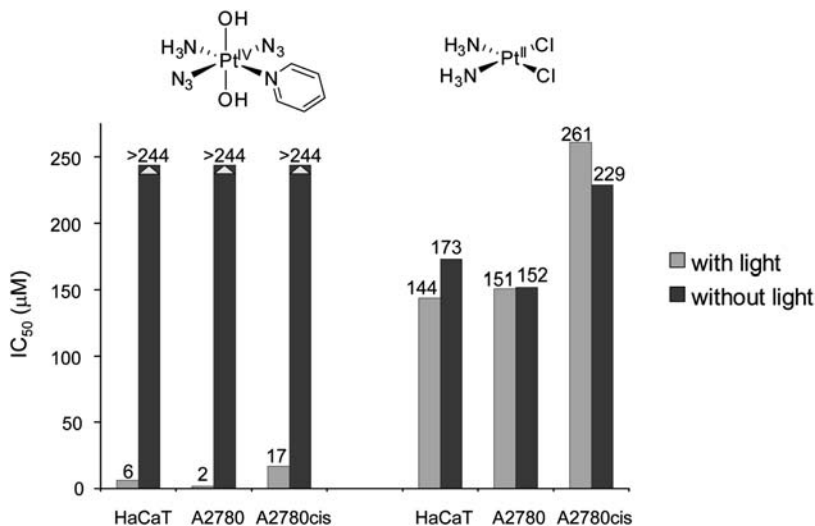


FIG. 8. Complex **7** is nontoxic in the dark, yet shows remarkable cytotoxicity with irradiation against several cancer cell lines; cisplatin is included for comparison. Data from Ref. (35).

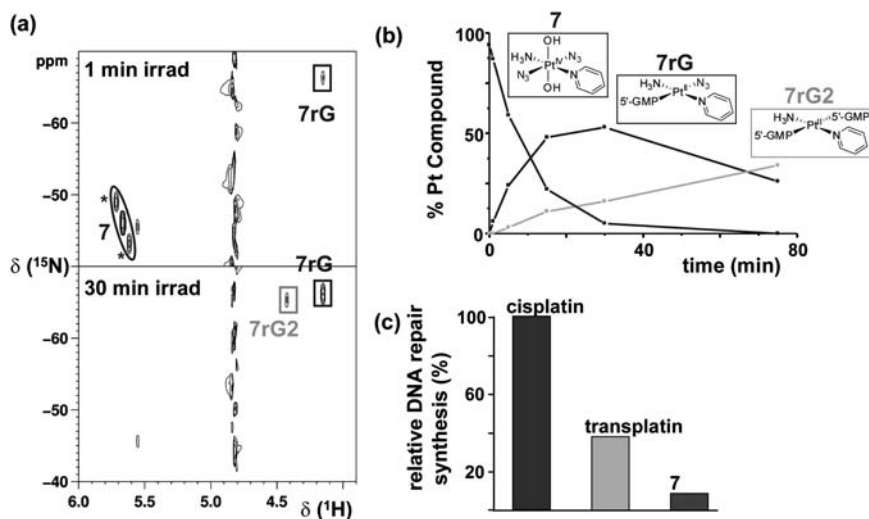


FIG. 9. (a) [^1H , ^{15}N] HSQC NMR spectra of ^{15}N -labeled **7** and 2 mol equiv of 5'-GMP after 1 min and 30 min of irradiation (*, ^{195}Pt satellites); (b) time dependent decrease in concentration of **7** and formation of **7rG** and **7rG2** (lines drawn merely to connect points); (c) quantification of *in vitro* DNA repair synthesis using an extract prepared from the repair-proficient HeLa cell line. Cisplatin was taken as 100%. Adapted from Ref. (35).

crosslinks were detected upon DNA platination, mainly intras-trand *trans*-guanine Pt(II) adducts. Importantly, DNA repair synthesis of the platinated lesion resulting from photo-platination was markedly lower than for cisplatin or transplatin (Fig. 9) (33). All these results show that *trans,trans*-[Pt(N_3)₂(NH_3)(OH)₂(py)] (**7**) exerts a rather unique cytotoxic effect and renders the complex a candidate for use in photoactivated cancer chemotherapy.

A quick survey of the photochemistry of the different complexes described above shows that the mechanism of photoactivation and the subsequent nature of the observed photoproducts varies from complex to complex and from one geometric isomer to another. Photochemical pathways often involve a combination of photosubstitution, photoisomerization, and photoreduction steps. In general, photolysis is rather slow in water and many different products are obtained if the complex is irradiated alone. The presence of nucleophilic biomolecules, on the other hand, can have a major influence, as photoreduction is usually rapid and accompanied by simpler reaction pathways. NMR methods

including 2D [^1H , ^{15}N] NMR spectroscopy in particular, have been instrumental in studying the photochemical decomposition of the platinum(IV) prodrugs. However, full understanding of the mechanistic pathways of photoactivation has not yet been achieved and remains an important goal of this research. The influence of biomolecules other than the nucleobase derivatives is still relatively unexplored and deserves more attention. Photochemically induced interactions with abundant extra- and intracellular constituents, e.g. proteins such as serum albumin and cytochrome *c*, are currently underway in our laboratory. Elucidation of photoactivation pathways and, as a result, predictable photochemistry would greatly aid further improvement of drug design.

All things considered, the originally proposed mechanism of a concerted, photoinduced reductive elimination of the two azide ligands to give highly reactive Pt(II) species and dinitrogen seems somewhat oversimplified. Although this mechanism appears to operate under some conditions, other pathways of activation should be considered as well. Indeed, recent photodecomposition studies paint a more complex picture (36). NMR studies of the UVA-induced photodecomposition of *cis,cis,trans*-[Pt(N₃)₂(NH₃)₂(OH)₂] (**4**) under anaerobic conditions do indeed confirm N₂ formation and photoreduction to Pt(II), but a tentative assignment of the major product (ca. 56%) as *trans*-[Pt(NH₃)₂(OH)₂]²⁺, suggests that photoisomerization accompanies photoreduction (36). Surprisingly, O₂ was also detected as a product in the sealed NMR tube, suggesting that alternative pathways can operate (Fig. 10). Evidence for the involvement of nitrene intermediates was also obtained. Trapping experiments with dimethylsulfide gave rise to an unusual *N,N'*-bis(ethyl)-sulfurousdiamide ligand involving an apparently unprecedented carbon–carbon bond formation. This is an intriguing observation since dialkylsulfides (e.g. methionine derivatives) are readily available in biological components. The production of dioxygen and detection of oxidized sulfur species points to the photochemical generation of reactive oxygen species (ROS). Hydroxyl radicals might, for instance, result from homolytic cleavage of Pt(IV)–OH bonds upon reduction. The formation of nitrenes and ROS might contribute to the mechanism of action of these photoactivated platinum anticancer drugs.

In addition to key factors that have guided improvements in our design of drugs, such as stability in the dark and controlled photoactivation, efforts are aimed particularly at shifting the absorption band relevant for photoactivation towards higher wavelengths for better tissue penetration, while maintaining the

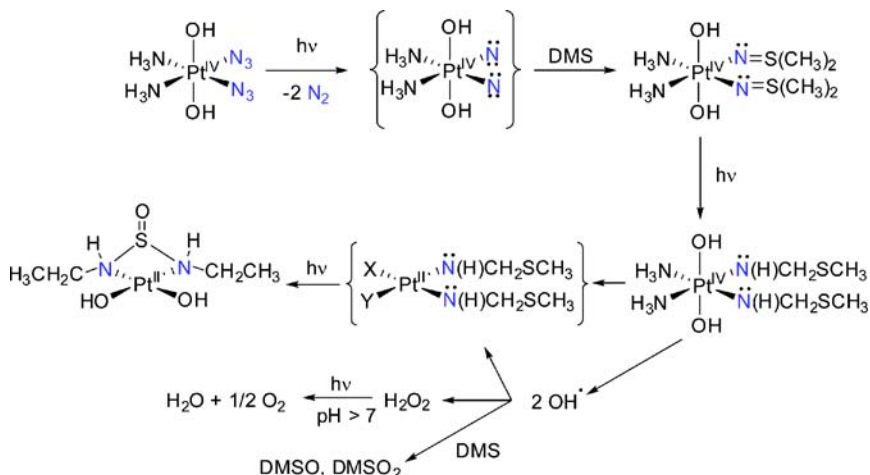


FIG. 10. Possible photoactivation pathway of **4**: mechanism for nitrene formation and subsequent trapping with dimethylsulfide (DMS) results in C–C bond formation, DMS oxidation, and oxygen evolution.

stability of the complexes towards reducing agents and the like. The latter is key for nontoxicity in the absence of light and hence for site-selective activation. As ligand variation can give only a limited bathochromic shift, other strategies need be explored as well. An interesting alternative pathway for photoaction would be multiphoton excitation (19). This way, for molecules with a large multiphoton cross-section, a higher energy transition may be obtained by the absorption of more than one photon of lower energy (19,37). Preliminary studies on some of the platinum(IV) diazido complexes showed no photoactivation by two- or three-photon absorption (33). Current efforts are therefore directed at increasing the multiphoton cross-section to achieve photoactivation by this method.

In summary, the overview given here of our work on photoactive platinum anticancer drugs shows how stepwise improvements transformed an interesting lead compound into a highly active new anticancer drug. Rational drug design allowed us to tackle initial problems of the photoactive prodrugs, such as the limited stability against reducing agents and nonselective activation of the drug leading to cytotoxicity also in the absence of light. Systematic ligand variation resulted in a high degree of control over the reactivity of the prodrug complex and the discovery of a stable, nontoxic and selectively photoactivated potent prodrug that is a promising candidate for photoactivated cancer chemotherapy.

III. Ruthenium–Arene Anticancer Drugs

The field of bioorganometallic chemistry (38) has now established itself as an exiting, vibrant area of research at the interface of biology and organometallic chemistry (39). The compatibility of organometallic compounds with aqueous conditions, water being the essential biological component, has long been considered improbable, many organometallic compounds being sensitive to water and oxygen. Yet, the gradual discovery of different biomolecules with metal–carbon bonds such as methylcobalamin and the enzyme acetyl-CoA synthase has shown that organometallic chemistry and biology are not mutually exclusive. Indeed, the development of aqueous organometallic chemistry shows that these limitations can be overcome and has expanded the toolbox of the medicinal chemist by introducing many structures (M–C bonds with σ -, π -, and/or δ -bonding) and reactivities unique to organometallics (40). This expansion of the field is reminiscent of the introduction of inorganic drugs to medicinal chemistry by way of cisplatin in the early 70s, which has had an enormous impact on medicine and resulted in an active and successful research community. Notable examples of the application of organometallic compounds to medicinal chemistry include the work of Jaouen on tamoxifen-derivatives as chemotherapeutics against breast cancer (41) and the development of organometallic $^{99\text{m}}\text{Tc}$ complexes as radioimaging agents by Alberto (42). Our contribution to this field has been the study of ruthenium–arene and recently osmium–arene complexes as potential anticancer drugs (43,44).

Introduction to Ruthenium Anticancer Drugs. The discovery of the antitumoral properties of cisplatin (Fig. 1), as discussed above, marked the advent of modern medicinal inorganic chemistry (45). The subsequent clinical success of cisplatin and related platinum-based anticancer drugs showed the viability of this approach, but the limitations associated with the platinum drugs (mainly required or inherent drug resistance and severe dose-limiting side effects) provided the impetus for the search for alternative chemotherapeutic strategies based on different complexes with other metals. The transition metals offer a rich palette to choose from and indeed many different transition-metal compounds have been explored for potential anticancer activity. Ruthenium, however, stands out as a particularly attractive alternative to platinum. The rich and well-established synthetic and coordination chemistry of ruthenium compounds combined with the fact that the metal has several oxidation states available under physiological conditions, e.g. Ru(II),

Ru(III), and Ru(IV) (46), make ruthenium compounds in general well-suited for medicinal applications. Indeed, ruthenium compounds have been investigated as immunosuppressants, nitric oxide scavengers, antimicrobial agents, and antimalarials (44,47). The potential ability of ruthenium to mimic iron in binding to various biomolecules, such as human serum albumin and the iron-transport protein transferrin, may aid a more effective delivery of ruthenium complexes to cancer cells as such rapidly dividing cells have a greater demand for iron and over-express transferrin receptors (46). This property may be responsible for the markedly lower toxicity of ruthenium-based anticancer agents compared to platinum drugs (48).

Finally, a key advantage of ruthenium-based metallodrugs is the ability to tune the metal–ligand exchange kinetics over many orders of magnitude via ligand variation. Such influence over kinetic stability is critical for drug development and our work on ruthenium–arene anticancer drugs that is described in this section shows that such control can indeed be exerted.

The first report on the anticancer properties of ruthenium was published in 1976 when the Ru(III) compound *fac*-[RuCl₃(NH₃)₃] (Fig. 11) was found to induce filamentous growth of *Escherichia coli* at concentrations comparable to those at which cisplatin generates similar effects (49). This Ru(III) complex and related compounds such as *cis*-[RuCl₂(NH₃)₄]Cl illustrated the potential anticancer activity of ruthenium complexes, but insolubility prevented further pharmacological use. Since these initial studies, other Ru(III) complexes have been studied for potential anticancer activity, and two compounds, NAMI-A (50) and KP1019 (51), are currently undergoing clinical trials. Remarkably,

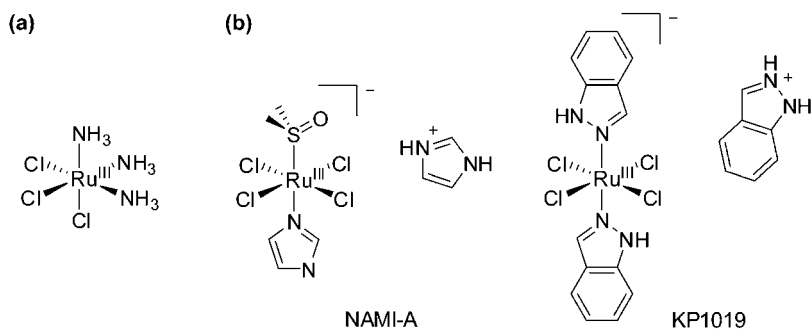


FIG. 11. Molecular structures of (a) *fac*-[RuCl₃(NH₃)₃], the first reported ruthenium complex with anticancer activity, and (b) NAMI-A and KP1019, two ruthenium compounds currently in clinical trials.

although the structures of NAMI-A ($[\text{Him}][\text{trans-RuCl}_4(\text{dmsO})(\text{im})]$, im = imidazole) and KP1019 ($[\text{Hind}][\text{trans-RuCl}_4(\text{ind})_2]$, ind = indazole) are quite similar (Fig. 11), they exhibit contrasting therapeutic effects. KP1019 is significantly cytotoxic to cancer cells, whereas NAMI-A is virtually devoid of cytotoxicity *in vitro*, but has antimetastatic activity *in vivo*. Understandably, this remarkable difference has spurred a widespread interest in the chemistry of these and related Ru(III) complexes, and elucidation of their mechanisms of action. Several reviews on this topic are available (46,48,52,53).

These relatively inert Ru(III) compounds are essentially prodrugs (see section on prodrugs above, Section II.A): they are “activated by reduction” *in vivo* to their more labile and reactive Ru(II) counterparts, which in turn are responsible for the observed cytotoxicity (cf. the Pt(IV)/Pt(II) redox couple) (54,55). With this in mind, we concentrated on the design of ruthenium(II) anticancer complexes. Initial studies showed that aminophosphine ruthenium(II) complexes were cytotoxic to cancer cells (56), but these complexes suffered from low aqueous solubility and were not further pursued (57,58). We synthesized the organometallic ruthenium(II) half-sandwich complex $[\text{Ru}(\eta^6\text{-benzene})\text{Cl}(\text{Me}_2\text{NCH}_2\text{CH}_2\text{PPh}_2)](\text{PF}_6)$ in which Ru(II) is stabilized not only by the phosphine but also by an η^6 -bonded arene ligand. This complex has the characteristic “piano-stool” geometry typical of half-sandwich ruthenium(II)–arene complexes (57). This structural element turned out to be key to our later discovery of potent organometallic ruthenium anticancer drugs. The inclusion of an η^6 -arene in our ruthenium anticancer drug design held some promise, since arene ligands are known to stabilize ruthenium in its +2 oxidation state and they provide the complex with a hydrophobic face, which might enhance biomolecular recognition processes and transport of ruthenium through cell membranes. In an attempt to increase the aqueous solubility of the ruthenium–arene complexes (an advantage for clinical use) the aminophosphine ligand was replaced with *N,N*-bidentate ethylenediamine (en), which indeed resulted in complexes that are reasonably soluble in water (59). Most importantly, the new complexes showed significant inhibition of human ovarian cancer cell growth and thus led to our discovery of a new family of organometallic ruthenium(II)–arene anticancer drugs (59). The study of this family of ruthenium anticancer drugs is the topic of this section.

Other active areas of research into the anticancer properties of ruthenium(II) complexes, include, amongst several other examples, the related work on RAPTA ruthenium–arene

1,3,5-triaza-7-phosphaadamantane complexes by Dyson and coworkers (46,60). The recent work by Meggers *et al.* (61) shows an interesting departure from the central paradigm of utilizing the transition metal in metallotherapeutics for its inherent reactivity. Their approach focuses on the use of the metal as a building block, relying on the kinetic inertness of certain coordination/organometallic bonds, for well-defined three-dimensional bioactive constructs, which are not accessible through purely organic, carbon-based compounds (61). The potential of this strategy is underlined by the reported organometallic indolocarbazole ruthenium(II) complexes that were designed as staurosporine mimetics and are highly cytotoxic towards human melanoma cancer cells (62).

A. GENERAL FEATURES OF RUTHENIUM–ARENE ANTICANCER DRUGS

General Structural Features. The general structure of half-sandwich ruthenium(II)–arene complexes is shown in Fig. 12. The structural, stereochemical and electronic features of metal–arene complexes have been discussed (63). A typical “piano-stool” geometry consists of an η^6 -arene occupying three coordination sites of the pseudo-octahedral complex, leaving the three “legs” **X**, **Y**, and **Z** available for coordination. The sites **X** and **Y** can be taken up by two monodentate ligands, but are more commonly

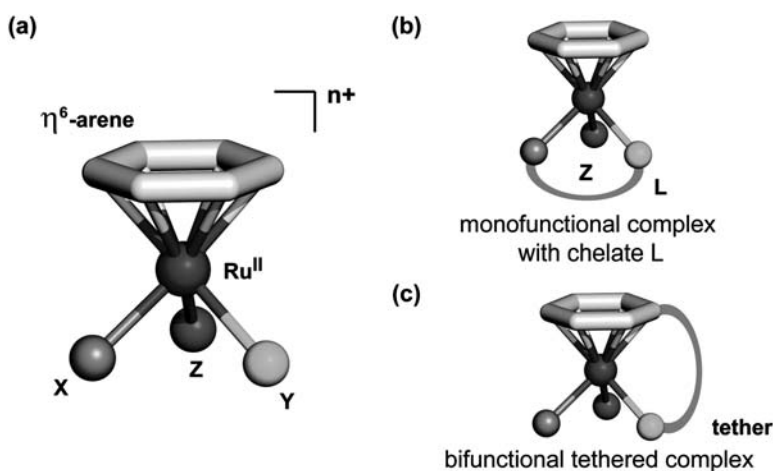


FIG. 12. (a) General structure of the half-sandwich, piano-stool ruthenium–arene complexes; (b) **X** and **Y** are commonly occupied by a bidentate ligand **L** giving a monofunctional complex; (c) tethering of a monodentate ligand to the arene results in a bifunctional complex.

occupied by a bidentate ligand **L** with e.g. nitrogen and/or oxygen donor atoms (e.g. *NN*, *NO*, or *OO* coordination), typical examples being ethylenediamine (*en*) or acetylacetonate (*acac*) (Fig. 12). The chelating nature of the bidentate ligand seems to be advantageous for anticancer activity. Linking two chelating ligands together allows for the synthesis of dinuclear ruthenium–arene complexes.

The sixth and final coordination site **Z** is usually a halide and endows the molecule with a reactive site. The arene ligand, on the other hand, is relatively inert towards displacement under physiological conditions (*vide infra* for some exceptions). As a result of this single-site reactivity, the complexes are considered monofunctional, in this case meaning that the metal is capable of forming one direct coordinative bond with a biological target. Other additional noncovalent interactions, such as hydrogen bonding and π – π stacking, are available to some of the complexes and can be advantageous for high cytotoxicity. Depending on the nature of both the chelating ligand **L** and ligand **Z**, the complexes are either neutral, or mono- or dications and as such isolated as salts. The charge on the complex and the nature of the anion can have a pronounced influence on important pharmacological properties, such as aqueous solubility and partition coefficients.

The general structure allows the nature of the arene, the type of chelate, and the monodentate ligand to be varied. This gives access to a vast library of compounds that can be synthesized and screened for anticancer activity (see Fig. 13 for the cross-section of ruthenium–arene anticancer agents discussed in this section). Indeed, variation of each of the building blocks allows us to modify key thermodynamic and kinetic parameters, and in this way tailor the pharmacological properties of the complexes. The general reactivity will be discussed in a following section.

Whereas the general structure described above can yield monofunctional adducts with biomolecules (*vide infra*), it is interesting to explore bifunctional ruthenium–arene complexes as well, as a different profile of reactivity can be expected. The use of a monodentate ligand to achieve this is, however, inadvisable as such complexes readily undergo substitution reactions and are, as such, unstable in solution. Tethering the monodentate ligand to the arene provides an attractive strategy (Fig. 12), such chelation stabilizes the molecule towards substitution. In this way, we have synthesized some nitrogen-containing, bifunctional tethered ruthenium(II)–arene complexes (64). Alternatively, if such an *N*-donor tethered ruthenium–arene is complexed with a bidentate chelating ligand, e.g. *en*, a molecular switch can be designed to work by selective ring-opening of the tether under certain conditions (65).

We have also recently explored some ruthenium–arene complexes that depart markedly from the general structure described above. For instance, full-sandwich ruthenium complexes have been synthesized, in which the positions **X**, **Y**, and **Z** are taken by an η^6 -arene ring of a biologically active ligand, such as aspartame, to assess the influence of a metal complex as a modulating substituent on the properties of the bioactive ligand (66).

Synthesis. The various synthetic routes to these complexes have been recently reviewed (67). Usually, the synthesis involves the reaction of dimeric Ru(II)–arene complexes of the type $[\text{Ru}(\eta^6\text{-arene})\text{Cl}_2]$ with an appropriate (chelating) ligand. The dimers are generally prepared by the redox reaction of RuCl_3 and a cyclic 1,4-diene as arene precursor, but other routes are available as well. The redox reaction between RuCl_3 and a cyclic 1,4-diene usually gives the ruthenium–arene dimers in high yield and purity, but this synthetic route is limited by the commercial availability or ease of synthesis of diene precursors. Thermal displacement of a coordinated arene provides another valuable synthetic route. The latter method has been employed for the synthesis of ruthenium–arenes with sterically demanding arenes such as hexamethylbenzene (hmb) and for the synthesis of amine-tethered complexes (64,68). We have also synthesized the ^{106}Ru radiolabeled complex $[\text{}^{106}\text{RuCl}(\text{en})(\eta^6\text{-fluorene})](\text{PF}_6)$ (**8**) to facilitate pharmacological (ADME) studies (69).

Stereochemistry. It is important to note here that the ruthenium–arene complexes are inherently chiral if the three legs of the piano-stool are nonequivalent. For instance, the use of an *N,O*-bidentate mixed chelate such as glycine or hydroxyquinoline gives rise to a chiral ruthenium centre. Alternatively, the coordination of a secondary amine, such as in *N*-ethyldiaminoethane (Et-en), to a Ru(II)–arene leads to the formation of four diastereoisomers with stereogenic centers at nitrogen and ruthenium. This realization is important as the enantiomers (diastereomers), in principle, have different biological properties. Knowledge of epimerization rates and, if possible, resolution of the enantiomers may then be important for formulation and mechanism of action. In this light, the stereochemistry of $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{Et-en})](\text{PF}_6)$ (**9**) (bip, biphenyl) was studied in detail (70). The synthesis of $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{Et-en})](\text{PF}_6)$ (**9**) gave rise to two diastereomers (with relative configurations $R_{\text{Ru}}^*R_{\text{N}}^*$ (A) and $S_{\text{Ru}}^*R_{\text{N}}^*$ (B), see Fig. 14), which were separable by crystal picking. Dissolution of the diastereomerically-pure compounds resulted in the dynamic interconversion of the A or B diastereomers, resulting in an equilibrium mixture of 72:28% of $R_{\text{Ru}}^*R_{\text{N}}^*$ (A) and $S_{\text{Ru}}^*R_{\text{N}}^*$ (B). The preferred isomer has the ethyl group pointing away from the arene ring, thus minimizing steric repulsion. Our studies thus suggested that isomer A ($R_{\text{Ru}}^*R_{\text{N}}^*$) is thermodynamically preferred, and that each ruthenium complex is in dynamic equilibrium between the A ($R_{\text{Ru}}^*R_{\text{N}}^*$) and B ($S_{\text{Ru}}^*R_{\text{N}}^*$) configurations. Interestingly, the reaction of the diastereomeric mixture with 9-ethylguanine (9-EtG) resulted in the formation of a single diastereomeric pair B ($S_{\text{Ru}}^*R_{\text{N}}^*$), apparently made possible by the

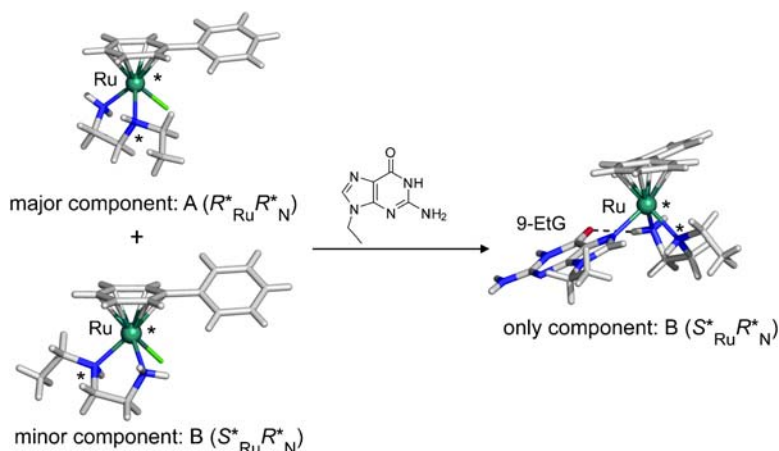


FIG. 14. Dynamic chiral recognition of 9-ethylguanine by chiral ruthenium–arene complex **9**.

facile epimerization at the nitrogen center (Fig. 14). This dynamic chiral recognition of guanine provides the possibility of highly diastereoselective DNA recognition by ruthenium anticancer agents by an induced fit mechanism (70).

Hydrophobicity. An important pharmacological characteristic of the complexes is their hydrophobicity (partition coefficients), as it is strongly related to both influx and efflux of anticancer drugs. Transport and sequestration of ruthenium complexes, i.e. drug uptake, into tumor cells may involve both active and passive diffusion, and may be mediated by protein receptors. Active drug efflux on the other hand is sometimes mediated by P-glycoprotein, a membrane protein that is overexpressed in cancer cells. Hydrophobicity is thought to be a major determinant in substrate specificity and is therefore another structural parameter that can be explored and, in principle, optimized (71). Additionally, hydrophobic interactions are important in binding to biomolecules such as DNA and proteins.

A convenient and easily accessible way to quantify hydrophobicity is the determination of the octanol/water partition coefficient ($\log P$) and we have determined the hydrophobicity of 13 selected ruthenium–arene complexes (71). As expected, hydrophobicity increases with an increase of the size of the coordinated arene ring, but decreases significantly when the chloride is replaced by neutral ligands such as pyridine and 4-cyanopyridine. The latter observation is somewhat counter intuitive at first inspection, but correlates with replacement of anionic chloride to yield a dicationic complex. The hydrophobicity

correlates significantly with the biological activity of these complexes (71).

B. CYTOTOXICITY STUDIES: TOWARDS ESTABLISHING STRUCTURE–ACTIVITY RELATIONSHIPS

Our *in vitro* and *in vivo* activity studies have been carried out in collaboration with Jodrell's group at the Western General Hospital in Edinburgh and with the help of Oncosense Ltd., and more recently also with the group of Brabec.

Initial cancer cell growth inhibition tests done on the human ovarian cancer cell line A2780 revealed the cytotoxic activity of some of the tested ruthenium–arene complexes (59). IC₅₀ values as low as 6 μM were obtained for the complexes [Ru(η⁶-bip)Cl(en)](PF₆) (**10**) and [Ru(η⁶-bip)Cl(Et-en)](PF₆) (**9**). This was an order of magnitude higher than cisplatin (0.5 μM in the same test), but comparable to the second-generation anticancer drug carboplatin, illustrating the potential of this new family of metallo-anticancer drugs. The complexes [Ru(η⁶-bip)Cl(en)](PF₆) (**10**) and [Ru(η⁶-bip)Cl(Et-en)](PF₆) (**9**) did not inhibit the catalytic activity of topoisomerase I or II over the concentration range of observed cytotoxicity (1–50 μM), making it unlikely that the anticancer activity is related to inhibition of these enzymes (59).

Complexes with other arenes showed slight differences in activity, which already indicated that the structure of the arene is important for cytotoxicity. Indeed, increasing the size and overall hydrophobicity of the arene increased cytotoxicity and IC₅₀ values of 2 and 0.4 μM were found for [RuCl(η⁶-dha)(en)](PF₆) (**11**) (dha, dihydroanthracene) and [RuCl(en)(η⁶-tha)](PF₆) (**12**) (tha, tetrahydroanthracene), respectively, the latter thus being equipotent to cisplatin (Fig. 15) (67,72). Complexes with three monodentate ligands, i.e. devoid of the chelate, were essentially inactive. These complexes are probably too reactive towards components of the cell culture medium and/or the cells and do not reach the target site. Indeed, the bifunctional tethered complexes [Ru(η⁶:η¹-C₆H₅CH₂(CH₂)_nNH₂)Cl₂] (*n* = 1,2) (**13a,b**) possessing two reactive Ru–Cl bonds, are also not cytotoxic against the A2780 cell line (64). A more hydrophobic arene and a single ligand-exchange site therefore seem associated with high cytotoxicity.

Cross-resistance profiles of selected Ru(II)-arene complexes in cisplatin-resistant A2780^{cis} cells and multi-drug-resistant A2780^{AD} cells showed a relatively high degree of cross-resistance in A2780^{AD}. By contrast, the organometallic complexes tested were completely non-cross-resistant in A2780^{cis} cells (72) (Fig. 15).

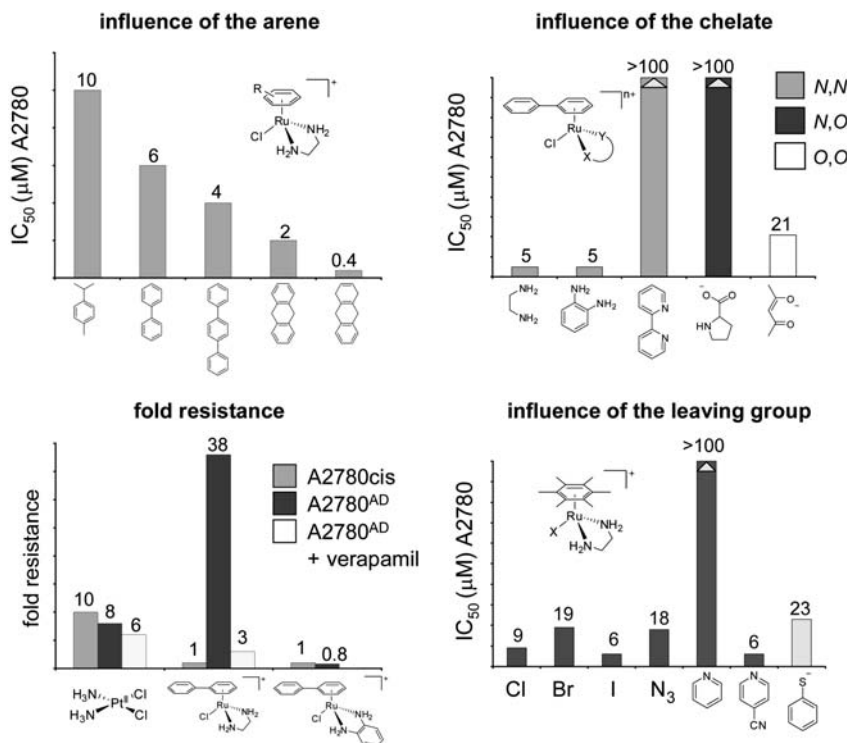


FIG. 15. Trends illustrating the influence of the arene, the chelate, and the leaving group on the cytotoxicity and cross-resistance of ruthenium-arene complexes developed in the Sadler lab. The complexes are not cross-resistant with cisplatin.

Cross-resistance to [Ru(η⁶-bip)Cl(en)]PF₆ in A2780^{AD} fell from a factor of 38 to only threefold upon co-administration of verapamil, indicating that P-glycoprotein mediated active efflux of the anticancer drug was predominantly responsible for the observed cross-resistance and could be abrogated by addition of the competitive inhibitor. Such behavior is common for lipophilic positively charged drugs.

The patterns of activity established *in vitro* for **10** were mirrored to a large degree *in vivo*, with high activity in an A2780 xenograft together with non-cross-resistance in an A2780cis xenograft and a lack of activity in the A2780^{AD} xenograft (72). [Ru(η⁶-bip)Cl(en)](PF₆) (**10**) was further evaluated against a 13-cell line panel (Freiburg screen). The results demonstrated a broad spectrum of activity with a “compare-negative” score versus other common anticancer compounds suggesting a unique mode of action (73). Particular sensitivity

was noted in a breast cancer cell line (401NL) and a non-small cell lung cancer (NSCLC) cell line (LXFL 529L). The activity of $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})](\text{PF}_6)$ (**10**) in two other NSCLC cell lines (H520 (3.5 μM) and A549 (3 μM)) was similar to cisplatin (9.5 and 2.6 μM , respectively). $[\text{RuCl}(\text{en})(\eta^6\text{-tha})](\text{PF}_6)$ (**12**) was even more potent with IC_{50} values of 0.53 and 0.5 μM , respectively. *In vivo* experiments showed antitumor activity of both complexes against an A549 xenograft. A preliminary assessment of the potential therapeutic index for these agents, showed that liver toxicity is a concern (73).

We recently tested a more extended range of these organometallic Ru(II) complexes for cytotoxicity to elaborate on the structure–activity relationships (67). Some general trends could be discerned, but the structure–activity relationships turned out to be quite complex. Variation of the *arene ring* revealed that inclusion of polar substituents, such as amides, esters, and alcohols lowered cytotoxicity. Nonpolar, sterically demanding substituents, however, resulted in more potent complexes with IC_{50} values as low as 3 μM (compared to 0.5 μM for cisplatin). Fused ring systems showed good activity, with the polycyclic aromatic hydrocarbons being the most active, emphasizing the importance of lipophilicity, and possible hydrophobic interactions with the nucleobases of DNA (*vide infra*).

Data for a series of $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})](\text{PF}_6)$ complexes with the isomeric *p*-, *o*-, and *m*-terphenyls mirrored these observations (74). The complex with the most extended arene (*p*-terphenyl, **14**) was the most potent, with potency similar to cisplatin, but is not cross-resistant, and has a much higher activity than its isomeric complexes. Again, no cross-resistance with cisplatin was observed for these complexes (74).

Variation of the *chelated ligand* resulted in complexes with vastly differing cytotoxicities. Aliphatic diamines generally showed good cytotoxicity (e.g. **9–12**), provided they contain a primary amine for stereospecific hydrogen bonding with guanine (*vide infra*). 1,2-Diaminobenzene (dab) complexes such as $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{dab})]^+$ (**15**) also showed good activity and, interestingly, dab complexes overcome the cross-resistance to A2780^{AD}, previously observed for en-containing complexes. Bipyridine and phenanthroline derivatives are inactive, again perhaps indicating the importance of NH groups.

Surprisingly, replacement of the *N,N*-chelating ligand en by *N,O*-chelating ligands of some amino acids (amino acidates) proved detrimental for activity. Reactivity studies indicated that fast hydrolysis rates and a relatively large proportion of a reactive aquated species at physiological chloride concentration might be

responsible for the lack of activity. Finally, complexes with *O*, *O*-chelating ligands such as acac and its derivatives (**16a,b**) showed good to moderate activity, despite the lack of hydrogen-bond donors. Some of the trends described above, but not all, can be explained by the general reactivity profile of ruthenium–arene complexes, which will be discussed in more detail below.

In the series of diamine complexes, changing the *monodentate ligand Z* from chlorido to iodido or even the pseudohalide N_3^- has little effect on activity; despite significant differences in rate and extent of hydrolysis they are all potential leaving groups (59,75). This contrasts dramatically with the chlorido and iodido ruthenium(II) arene complexes that contain σ -donor/ π -acceptor 2-phenylazopyridines (azpy) as *N,N*-chelating ligands (**17a,b**). These chlorido complexes are inactive against the A2780 and A549 cell lines (76). Some of the corresponding phenylazopyridine/iodido complexes, on the other hand, proved highly cytotoxic to the same cell lines (see section on catalytic anticancer drugs and Fig. 23) (77). These complexes exhibit a different mechanism of cancer cell cytotoxicity, involving catalytic redox reactions (*vide infra*), in which the reactivity is ligand-based rather than metal-based. The complexes provide a nice example of how metal ions can also tune the reactivity of coordinated ligands, rather than the other way round. The use of a transition metal as a modulating substituent of a ligand is an interesting strategy and should be explored further. It takes advantage of properties of the metal center other than its direct reactivity and is in this way related to the metal-as-scaffold approach in which the metal serves the structural function of spatially orienting the coordinated ligands (61).

The cytotoxicity data cited above and illustrated in Fig. 15 show that ligand variation can have dramatic effects on the biological activity of these ruthenium–arenes. Certain trends can be discerned and such structure–activity relationships then carry some predictive value for further drug design. An important next step is then to rationalize these relationships by correlating biological activity to chemical reactivity. Hence, much attention has been given to the study of the reactivity of this family of ruthenium–arene anticancer drugs and their interaction with biomolecules that are considered a possible target or that can be encountered by the complex before reaching its (final) target.

C. REACTIVITY OF RUTHENIUM–ARENE ANTICANCER DRUGS

Aquation. The principal reactivity of our family of ruthenium–arene complexes is the exchange of the leaving group **Z**, usually a

halide, with water to form the more reactive aqua complexes (Fig. 16). This latter species undergoes substitution reactions, for instance DNA nucleobase binding, much faster than the corresponding chlorido precursors and is therefore considered as the active species that exerts the pharmacological effect. The ruthenium–arene anticancer complexes $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{L})]$ can therefore be regarded as *prodrugs* (Fig. 2). Understanding the thermodynamic and kinetic parameters that govern prodrug activation by aquation has been an important part of our research, as detailed knowledge of the factors that control such ligand substitutions under physiological conditions is very valuable in drug design. The aqueous chemical reactivity of the complexes can be chosen so as to balance the inertness required for the drug to reach its target site and minimize attack on other sites, yet allow activation necessary for binding to the target (75).

The rate of hydrolysis depends strongly on the nature of all three building blocks, i.e. the leaving group, the coordinated arene, and the chelate, and can be varied over several orders of magnitude, opening a time-window of activation. A detailed study of the aquation and the reverse, anation reactions of three $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})](\text{PF}_6)$ complexes (arene = bip (10), dha (11), and tha (12)) showed that the rates of aquation

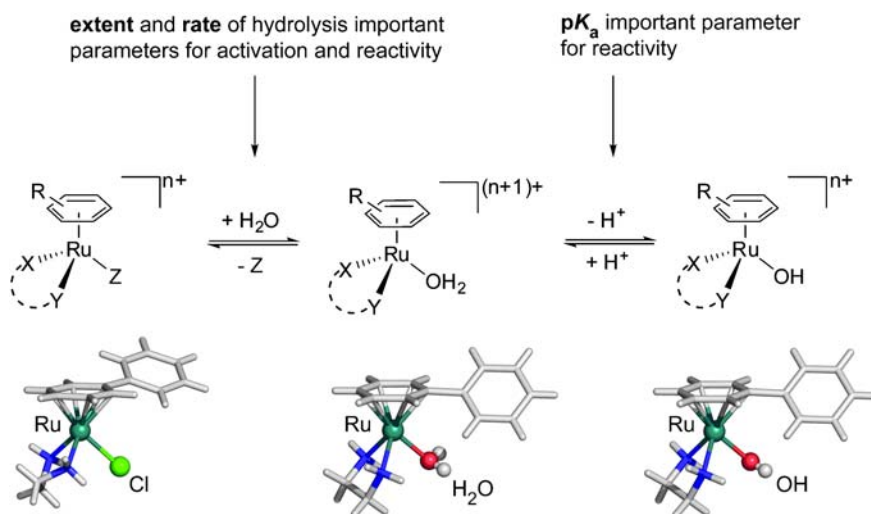


FIG. 16. General reactivity of the ruthenium(II)–arenes. Hydrolysis of the Ru–Z bond gives the more reactive aqua species. The pK_a of the coordinated water molecule is important, as the hydroxido complex is less reactive. The different structures are exemplified by the reactivity of $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (10) for which Z = Cl.

($k_{\text{H}_2\text{O}}$ $1.23\text{--}2.59 \times 10^{-3} \text{ s}^{-1}$, at 298 K and $I = 0.1 \text{ M}$) with half-lives of $<10 \text{ min}$ are an order of magnitude (>20 times) faster than that of cisplatin (78). The anation reactions in the presence of 100 mM NaCl were very rapid (k_{Cl} $0.127\text{--}0.306 \text{ M}^{-1} \text{ s}^{-1}$, at 298 K and $I = 0.1 \text{ M}$), resulting in small equilibrium constants for aquation ($7.3\text{--}9.7 \times 10^{-3} \text{ M}$). This is significant for the speciation of the ruthenium complexes under physiological conditions (Fig. 17). The results indicate that in blood plasma, where the $[\text{Cl}^-]$ is high (about 104 mM), the complexes would exist primarily in their chlorido forms ($>89\%$). In contrast, the chloride concentrations are much lower in the cell cytoplasm (about 23 mM) and cell nucleus (4 mM) which would increase the amount of more reactive aqua species to ca. 30% and 70%, respectively. As a result, the extracellular suppression of aquation followed by activation upon entering the cell provides a selective mechanism of activation.

The mean values of the activation parameters for both aquation and anation show that $\Delta S^\ddagger$ is negative, indicative of an associative pathway (78). Density functional theory calculations also suggest that aquation occurs via a more associative pathway in an $\text{I}_a \leftrightarrow \text{I}_d$ mechanistic continuum for which bond-making is of greater importance than bond-breaking (75). The electron-accepting effect of the strong π -acid arene ligands is thought to be responsible for the shift towards a more associative I_a pathway, as it increases the charge on the metal

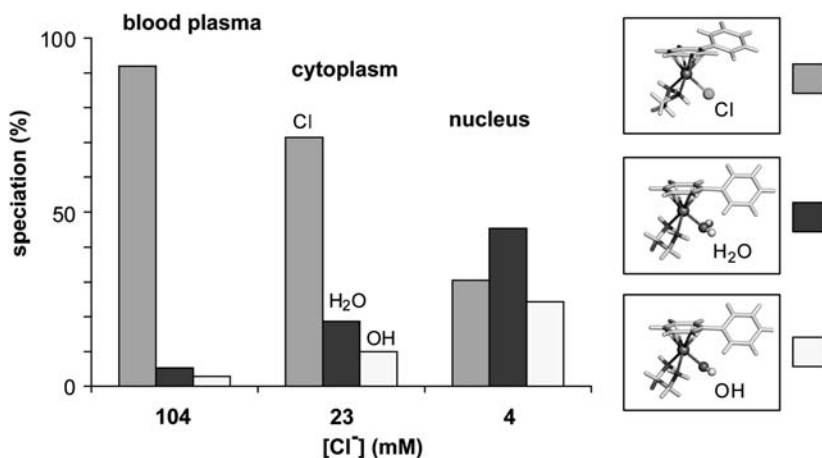


FIG. 17. Speciation of $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (10) $5 \mu\text{M}$ in blood plasma, cytoplasm, and nucleus at equilibrium, based on the equilibrium constants of aquation, $\text{p}K_{\text{a}}$, $[\text{Cl}^-]$, and pH of the environments. Data from Ref. (78).

(75). Ru(II) in $\{\text{Ru}(\eta^6\text{-arene})\}$ complexes may therefore behave more like a Ru(III) center, which usually reacts via associative pathways (79).

Both aquation and anation reactions are about twice as fast for the dha (**11**) and tha (**12**) complexes compared to the biphenyl complex **10**. Consequently, the hydrolysis rates decrease with increase in the electron-accepting ability of the arene, an observation that was later confirmed for a more extensive series of compounds (75). We also studied the influence of the type of leaving group on the hydrolysis rate and found that it has a large effect. For the halides, the aquation rate was found to decrease in the order $\text{Cl} \approx \text{Br} > \text{I}$. Replacement of the chloride ligand with the pseudohalide N_3^- even slowed down the hydrolysis rate 40-fold, while changing it for other monodentate ligands such as (substituted) pyridines or thiophenol made the hydrolysis too slow to observe. Generally, but not always, complexes that readily hydrolyze are cytotoxic. An interesting exception to this rule is $[\text{Ru}(\text{en})(\eta^6\text{-hmb})(\text{SPh})]^+$ (**18**), which hydrolyzes extremely slowly yet shows significant activity. Closer inspection of the reactivity of this and related compounds suggests an “activation-by-ligand-oxidation” mechanism and led to our studies on ruthenium–sulfenate and –sulfinate complexes that will be further discussed below.

The chelating ligand also influences the hydrolysis rate. In general, the effect on substitution reactions depends on the nature of the chelate and the position of the ligand relative to the leaving group. The *cis*-positioning of the en chelate to the leaving group in $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})]^+$ slows down the aquation and anation reactions due to electronic and steric effects. Replacement of the neutral en ligand by monoanionic acac increases the rate and extent of hydrolysis (80). The presence of a π -acceptor azopyrazole ligand on the other hand decreases the rate of hydrolysis by more than an order of magnitude (76).

The *bifunctional* amine-tethered ruthenium(II) arene complexes $[\text{Ru}(\eta^6\text{-}\eta^1\text{-C}_6\text{H}_5\text{CH}_2(\text{CH}_2)_n\text{NH}_2)\text{Cl}_2]$ ($n = 1, 2$) (**13a,b**) show two consecutive hydrolysis steps to yield the mono- and bis-aqua complexes (64). At extracellular chloride concentrations, the majority of the complexes could be expected to be present as the mono-aqua adduct. Equilibrium constants were determined for both steps (for **13b**, $K_1 = 145 \text{ mM}$ $K_2 = 5.4 \text{ mM}$) and found to be considerably higher than those of cisplatin, which also has two reactive sites available.

An important characteristic for the activity of the hydrolysis product, the aqua species, is the $\text{p}K_a$ of the coordinated water molecule (Fig. 16). Acid dissociation gives the hydroxido complex $[\text{Ru}(\eta^6\text{-arene})(\text{L})(\text{OH})]^+$ which is less susceptible to substitution

reactions than the corresponding aqua complex (81). The pK_a values of the aqua complexes can be determined by UV-Vis or NMR spectroscopy. The pK_a value of the coordinated water molecule in $[\text{Ru}(\eta^6\text{-arene})(\text{en})(\text{OH}_2)]^+$ was found to be 7.71, 7.89, and 8.01 for the bip, dha, and tha complexes, respectively (78). This relatively low acidity of the water ligand is important, since under physiological conditions only small amounts ($<10\%$ of the total Ru(II)-arene complexes) are predicted to exist as the less reactive hydroxido complexes. Changing the bidentate ligand to monoanionic oxygen chelates such as acac or tropolonate increases the pK_a further to 9.12 ($[\text{Ru}(\eta^6\text{-}p\text{-cym})(\text{OH}_2)(\text{trop})]^+$) (**19H₂O**) (82) and 9.41 ($[\text{Ru}(\text{acac})(\eta^6\text{-}p\text{-cym})(\text{OH}_2)]^+$) (**16aH₂O**) (80), most likely as a consequence of electronic effects exerted by the ligand on the metal center (82). In contrast, the pK_a of the aqua adduct in $[\text{Ru}(\text{azpyz-NMe}_2)(\eta^6\text{-}p\text{-cym})(\text{OH}_2)]^+$ (**20H₂O**) (azpyz-NMe₂, 4-(1H-pyrazol-3-ylazo)-*N,N* dimethylaniline) is 4.60, indicative of the low electron density at ruthenium and consistent with the π -acidic nature of the ligand. Indeed, the complex has a low affinity for DNA bases (see below) as it would predominately exist in the more inert hydroxido form at physiological pH (76).

These results clearly show that the hydrolysis rate, and with that drug activation, and the pK_a of the aqua adduct can be tuned, a strategy that has previously been successful for Pt anticancer drugs. This opens the possibility of drugs with tailor-made properties. As an aside, it is interesting to note that careful control over the kinetics of exchange or, alternatively, the redox potential of a drug might ultimately lead to personalized anticancer drugs. The characteristics of the drugs could be adapted to fit physical parameters particular to an individual and would allow activation in specific cells. If individual variations in response to a certain drug have a genetic basis, dividing patients into subgroups with a similar genetic profile would allow more efficient treatment, if a series of anticancer drugs with slightly different properties is available.

Loss of Coordinated Arene. We previously stated that the arene ligand in ruthenium(II)-arene complexes is relatively inert towards displacement under physiological conditions. While this is generally true, there are a few exceptions to this rule and this type of reactivity can be used to advantage. Weakly bound arenes, for instance, can be thermally displaced, a property convenient for the synthesis of ruthenium-arene complexes that are not readily available through more common synthetic routes. This way, the reaction of a precursor dimer, $[\text{RuCl}_2(\text{etb})]_2$ (etb, ethylbenzoate) (68), with either 3-phenyl-1-propylamine or

2-phenylethylamine results in arene replacement and the isolation of the complexes $[\text{Ru}(\eta^6:\eta^1\text{-C}_6\text{H}_5\text{CH}_2(\text{CH}_2)_n\text{NH}_2)\text{Cl}_2]$ ($n = 1, 2$) (**13a,b**) in which the amine tether is coordinated to ruthenium as well (64).

The nature of the chelating ligand exerts a strong influence on the lability of the coordinated arene. The presence of strong π -acceptor chelate ligands in $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{L})]^+$ where L is an azopyridine (**17**) or azopyrazole (**20**), can lead to the thermal displacement of the arene under mild conditions (low temperatures, aqueous solution) The arene loss is significant, up to 67% after 24 h at 310 K, depending on the arene. The competition for π -electron density with the π -acceptor chelate ligands is responsible for the weakening of the Ru(II)–arene bonds and subsequent loss of the arene (76).

Alternatively, arene displacement can also be photo- rather than thermally-induced. In this respect, we studied the photoactivation of the dinuclear ruthenium–arene complex $[\{\text{RuCl}(\eta^6\text{-indane})_2(\mu\text{-}2,3\text{-dpp})\}^{2+}]$ (2,3-dpp, 2,3-bis(2-pyridyl)pyrazine) (**21**). The thermal reactivity of this compound is limited to the stepwise double aquation (which shows biexponential kinetics), but irradiation of the sample results in photoinduced loss of the arene. This photoactivation pathway produces ruthenium species that are more active than their ruthenium–arene precursors (Fig. 18). At the same time, free indane fluoresces 40 times more strongly than bound indane, opening up possibilities to use the arene as a fluorescent marker for imaging purposes. The photoactivation pathway is different from those previously discussed for photoactivated Pt(IV) diazido complexes, as it involves photosubstitution rather than photoreduction. Importantly, the photoactivation mechanism is independent of oxygen (see Section II on photoactivatable platinum drugs) (83).

Interactions with Nucleobases. Although it has not yet been unequivocally established, the primary cellular target for these organometallic Ru(II) complexes is thought to be DNA, as for many metal-based anticancer drugs including the archetypical drug cisplatin. The study of this interaction and its possible significant effects on DNA structure has therefore been of prime importance in our studies of the ruthenium–arene complexes. Initial studies showed a strong selective binding to N7 of the guanine bases (the most electron-dense site on DNA) on a DNA 14-mer oligonucleotide (59). To gain additional insight into the potential modes of interaction, we have studied in depth the binding of the ruthenium–arene complexes to nucleic acid derivatives as models of DNA. A detailed study of the complexes $[(\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en}))^+]$ (arene = bip, dha, tha (**10–12**)) with

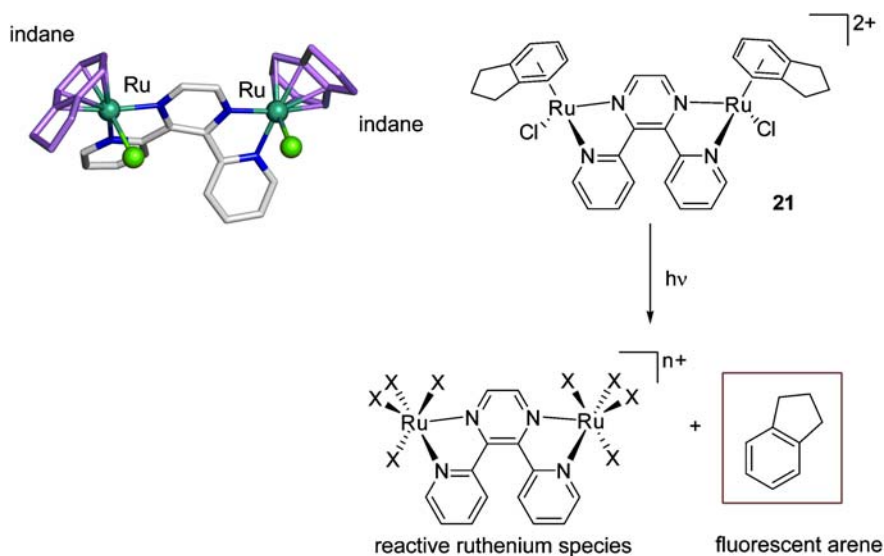


FIG. 18. The dinuclear complex $[\{\text{RuCl}(\eta^6\text{-indane})\}_2(\mu\text{-}2,3\text{-dpp})]^{2+}$ (**21**) can be photoactivated to yield highly reactive and potentially cytotoxic ruthenium species and the arene indane, which could be used as a fluorescent probe.

the guanine derivatives 9-EtG, guanosine (Guo), and 5'-guanosine monophosphate (5'-GMP) both in the solid state (by X-ray crystallography) and in solution (by NMR studies) elucidated the interactions that give rise to such high specificity (84). Non-covalent interactions turned out to play key roles in the biological molecular recognition process. In addition to direct monofunctional coordination of ruthenium to N7, the crystal structures of $[\text{Ru}(\eta^6\text{-dha})(\text{en})(9\text{-EtG})]^+$ and $[\text{Ru}(\text{en})(9\text{-EtG})(\eta^6\text{-tha})]^+$ also show strong arene–nucleobase stacking and strong stereospecific intramolecular hydrogen bonding. The hydrogen-bonding interaction and the arene–base stacking are illustrated in Fig. 19.

The π – π stacking interaction between the outer ring of dha or tha and the guanine base is close to ideal, with interplanar distances and dihedral angles of 3.45 Å/3.3° (tha) and 3.31 Å/3.1° (dha). The extensive arene–nucleobase stacking in the absence of arene–arene or base–base stacking in $[\text{Ru}(\eta^6\text{-arene})(\text{en})(\text{G-N}7)]^+$ represented a new structural feature in adducts of metal-based anticancer drugs with nucleobases. The observed stacking indicates the potential of ruthenium–arenes for intercalation or hydrophobic interactions of the arene ring with duplex DNA (84). Such hydrophobic interactions could provide a major contribution

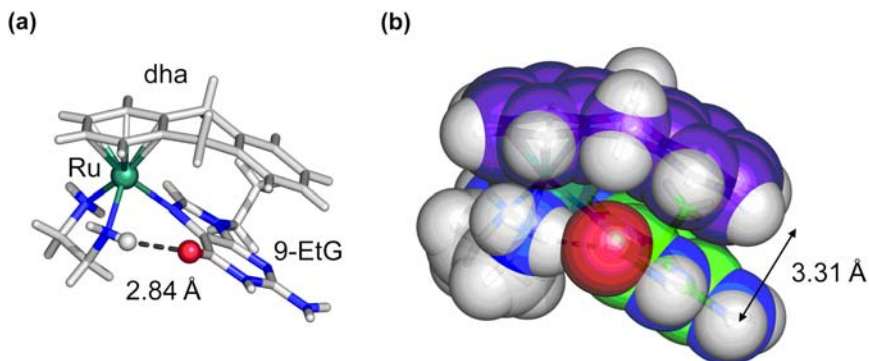


FIG. 19. The combination of (a) a strong stereospecific hydrogen-bonding interaction of the C6O carbonyl of 9-EtG with an en NH in $[\text{Ru}(\eta^6\text{-dha})(\text{en})(9\text{-EtG})]^+$ and (b) a strong π - π arene-nucleobase stacking interaction is responsible for the high preference of G over A observed for such ruthenium-arene complexes.

to the driving force for DNA binding. Comparison of the conformations of the G-adducts with the parent chlorido complexes revealed a significant reorientation and conformational change of the arene, as a result of the arene-nucleobase interaction. The changes involve rotation around the arene-Ru π -bonds, twisting around the Ph-Ph bond (bip), and ring bending (dha, tha). The arene ligands thus possess the flexibility to optimize the geometry for simultaneous covalent binding and arene-base stacking. This may reduce the steric demands of the Ru drug and enhance the DNA affinity.

The second strong noncovalent interaction observed was the stereospecific hydrogen bonding of an amine NH to the C6O carbonyl of G (average $\text{N} \cdots \text{O}$ distance 2.8 Å, $\text{N-H} \cdots \text{O}$ angle 163°). This strong hydrogen bonding interaction partly accounts for the high preference for binding of $\{\text{Ru}(\eta^6\text{-arene})(\text{en})\}^+$ to G versus A. Indeed, subsequent studies on the thermodynamics and kinetics of binding of $([\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})])^+$ (arene = bip, dha, tha) to mononucleotides and mononucleosides (G, A, C, and T) confirmed the importance of these specific interactions. For the mononucleosides, selective binding to N7 of guanosine (100%), moderate binding to N3 of thymidine (35%), weak binding to N3 of cytidine (14%), and hardly any binding to adenosine ($<5\%$ to N1/N7) was observed (81). Similar affinities for Ru were found for the corresponding mononucleotides, except for an additional, significant amount (40–60%) of 5'-phosphate binding with 5'-AMP, 5'-CMP, and 5'-TMP, but not with 5'-GMP (although initially phosphate bound intermediates were detected for

5'-GMP, bound phosphate was displaced by N7). No binding to the phosphodiester groups of 3',5'-cyclic guanosine monophosphate (3',5'-cGMP) or 3',5'-cAMP was observed, suggesting that the complexes do not bind to the phosphodiester backbone of DNA. Kinetic experiments also indicated that the binding of Ru(II)-arene complexes with arenes that can take part in π - π stacking (dha, tha, bip) are up to an order of magnitude faster than those containing arenes which cannot (*p*-cym (**22**), benzene). This demonstrates the significant role arene-purine base-stacking plays in stabilizing the transition state in the associative substitution reactions.

The differences in the extent of binding to Ru(II) can be explained by analyzing the possible hydrogen bonding and nonbonding repulsive interactions, in addition to the electronic properties of the nucleobase binding sites themselves. We previously saw the contribution of noncovalent interactions of amino ruthenium-arenes with the exocyclic carbonyl group of G. In contrast, adenosine has a C6 NH₂ group and as a consequence binding at either N7 or N1 is weakened by repulsive interactions. The reactivity of the various binding sites of the nucleobases decreases in the order G(N7) > T(N3) > C(N3) > A(N1, N7). This strong preference for G was confirmed by competition studies with 5'-GMP versus either 5'-AMP or 5'-CMP or 5'-TMP; in each case, essentially only the 5'-GMP adduct forms. The ability of the NH proton of en to act as an H-bond donor toward an exocyclic oxo group but not toward an amino group therefore plays an important role in the selective site recognition of these ruthenium-arene anticancer complexes (81). Extensive NMR studies mirrored the observations made for the solid state structures of the ruthenium-arene nucleobase adducts. ¹H 2D NOESY NMR experiments, for instance, confirm that the pendant phenyl ring in [Ru(η^6 -bip)(en)(9EtG-N7)]⁺ adopts a syn conformation with respect to the G base.

It is therefore not surprising that replacement of en by acac results in changes in the nucleobase selectivity. The complex [Ru(acac)Cl(η^6 -*p*-cym)] (**16a**) binds equally well to guanosine and adenosine and no binding to either thymidine or cytidine was observed (80). The N7/N1 coordination ratio for adenosine binding was about 4:1 at pH 5.8. Molecular models demonstrate that adenine coordination can be stabilized by hydrogen bonding between N6H₂ as a donor and an acac oxygen as hydrogen-bond acceptor. This hydrogen-bonding interaction was later observed in the crystal structure of [Ru(η^6 -*p*-cym)(9-EtA)(Ph₂acac)]⁺ (**16b**) (Ph₂acac, diphenylacetylacetonate) (85). In contrast, the crystal structure of [Ru(η^6 -*p*-cym)(9-EtG)(Ph₂acac)]⁺ reveals a repulsive

interaction between the exocyclic C6O carbonyl and the acac oxygen, which further explains the weaker affinity for G compared with the en complexes (85). Indeed, competitive binding of Guo and Ado showed a slightly higher affinity for Ado, but the adducts were found to be kinetically labile.

Interactions with Oligonucleotides. Binding studies of ruthenium–arene complexes with oligonucleotides have provided insight into their modes of interaction with duplex DNA. The reaction of $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**10**) with the single-strand DNA 14-mer d(ATACATGGTACATA) or its complementary strand d(TATGTACCATGTAT) gave rise to monoruthenated and/or diruthenated species depending on the Ru:oligonucleotide ratio, consistent with selective binding at G (86). Surprisingly, when a ruthenated single strand was annealed with its complementary strand, the product was not simply a double strand ruthenated at a single site. 2D NOESY NMR data showed that all four guanine residues were ruthenated at N7. Hence, we concluded that ruthenation is indeed highly specific, but also that $\{\text{Ru}(\eta^6\text{-bip})(\text{en})\}^{2+}$ is mobile at elevated temperatures (353 K) and migration between guanine residues can be facile. This contrasts with observations for DNA with bound Pt(II) am(m)ines for which migration is rare.

Intramolecular NOEs and other NMR data were consistent with arene ring intercalation between DNA bases. Molecular models suggested two different interactions (Fig. 20). One in which the ruthenium complex was bound to G and its pendant arene ring is intercalated between a G and T residue, and a second in which the ruthenium–arene is stacked on a flipped-out T on the surface of the major groove, i.e. is nonintercalated, forcing a T base to stack underneath it by tilting. Furthermore the data suggest that arene intercalation is dynamic: equilibria can exist between the intercalated and nonintercalated conformers. We also reported on the ruthenation of single- and double-stranded self-complementary hexameric DNA (d(CGGCCG)) (87). The complexes $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})]^+$, arene = *p*-cym (**22**) and bip (**10**), in this study were chosen to compare potential intercalators (bip) with nonintercalators (*p*-cym). For ss-DNA, all three G's were readily ruthenated, but for duplex DNA a preferential ruthenation of G3 and G6, and no binding to G2 was detected. This was attributable to unfavorable steric interactions between the duplex and arene for binding at G2. The differences between the two arenes manifested themselves in different strengths of hydrogen bonding between the amine NH and the C6O carbonyl of G. The intercalation of the pendant phenyl ring in the bip adduct resulted in weakening of

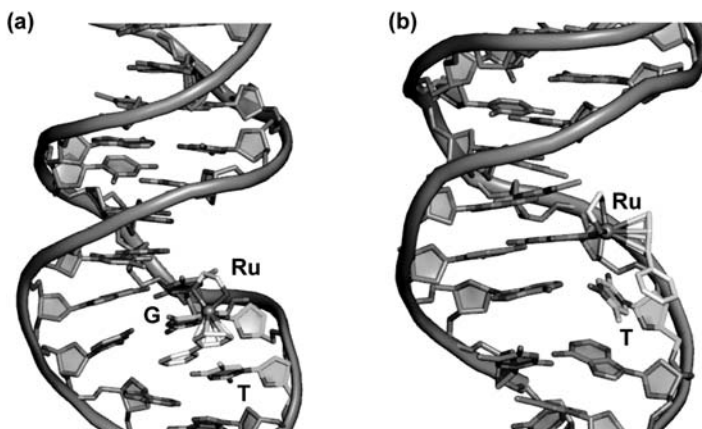


FIG. 20. Molecular models of two conformers of 14-mer duplex d(ATACATGGTACATA) ruthenated at N7 of one of the guanine residues with $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**10**). Conformer (a) shows the intercalation of the arene (b) shows the nonintercalated phenyl ring of the arene stacked on a thymine residue.

the stereospecific hydrogen bond compared to the *p*-cymene adduct. The arene ligand plays a major role in distorting the duplex either through steric interaction (*p*-cymene) or through intercalation (biphenyl) (87).

This work provides important evidence for elucidating the cytotoxic effect of the ruthenium–arene complexes and the influence of the arene thereon, for instance with respect to excision repair of DNA lesions and DNA destabilization. It also established two different classes of Ru(II) arene anticancer drugs, i.e. those bearing an arene that has the possibility to intercalate and those that do not. This distinction is important as we will see further differences in DNA binding interactions for these two classes (*vide infra*).

Interactions with DNA. In collaboration with the Brabec group, we studied the DNA interactions of several organometallic $[\text{RuCl}(\eta^6\text{-arene})(\text{en})]^+$ complexes in cell-free media. Binding studies using calf thymus (CT) double-helical DNA ($r_i = 0.1$) showed that the bip, dha, and tha complexes **10–12** bind about an order of magnitude faster than cisplatin, with $t_{1/2}$ values of 10, 15, and 10 min, respectively (88). The influence of the arene is remarkable as for *p*-cymene (**22**) a $t_{1/2}$ of 3.5 h was obtained. These results correlate well with the ability of the arene for intercalation/ π – π stacking. DNA binding was almost quantitative and irreversible and transcription mapping experiments

identified G as the preferential binding site, consistent with our previous studies on the DNA models. DNA binding of the achiral Ru(II) complexes with bip, dha, or tha as the arenes induced a sizeable circular dichroism (CD) spectrum for Ru(II)–arene absorption bands, whereas *p*-cym binding did not result in an induced CD band. Linear dichroism (LD) data showed that the *p*-cym complex stiffened DNA, while the other complexes bend it. Differential pulse polarography measurements demonstrated nondenaturational alterations in DNA with bip, dha, and tha and denaturational alterations with *p*-cym (88). All these observations point to differences in binding interactions between ruthenium–arene complexes that can intercalate and those that cannot. Interestingly, the adducts of the *p*-cym complex distort the conformation and thermally destabilize DNA distinctly more than the other three adducts (88). This is remarkable since an intercalating arene enhances cytotoxicity in a number of tumor cell lines (59,89). It is known that biological activity is modulated by the “downstream” effects of damaged DNA, such as recognition of damaged DNA by specific proteins and/or repair. The different distortions might lead to different “downstream” effects, which could eventually explain the differences in observed cytotoxicity.

Further experiments focused therefore on $[\text{RuCl}(\text{en})(\eta^6\text{-tha})]^+$ (**12**) and $[\text{RuCl}(\eta^6\text{-p-cym})(\text{en})]^+$ (**22**), which represent the two different classes, and their conformational distortion of short oligonucleotide duplexes. Chemical probes demonstrated that the induced distortion extended over at least seven base pairs for $[\text{RuCl}(\eta^6\text{-p-cym})(\text{en})]^+$ (**22**), whereas the distortion was less extensive for $[\text{RuCl}(\text{en})(\eta^6\text{-tha})]^+$ (**12**). Isothermal titration calorimetry also showed that the thermodynamic destabilization of the duplex was more pronounced for $[\text{RuCl}(\eta^6\text{-p-cym})(\text{en})]^+$ (**22**) (89). DNA polymerization was markedly more strongly inhibited by the monofunctional Ru(II) adducts than by monofunctional Pt(II) compounds. The lack of recognition of the DNA monofunctional adducts by HMGB1, an interaction that shields cisplatin–DNA adducts from repair, points to a different mechanism of antitumor activity for the ruthenium–arenes. DNA repair activity by a repair-proficient HeLa cell-free extract (CFE) showed a considerably lower level of damage-induced DNA repair synthesis (about six times) for $[\text{RuCl}(\text{en})(\eta^6\text{-tha})]^+$ compared to cisplatin. This enhanced persistence of the adduct is consistent with the higher cytotoxicity of this compound (89).

DNA-binding studies on the series of isomeric $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})]^+$ compounds with *o*-, *m*-, or *p*-terphenyl as the arene further illustrate the importance of the hydrophobicity and

intercalation potential of the arene. The more extended *p*-terphenyl isomer **14** showed faster binding, a larger unwinding angle, and a more significant distortion of the duplex compared to the *m*-terphenyl isomer (**74**).

The tethered bifunctional complexes $[\text{Ru}(\eta^6\text{:}\eta^1\text{-C}_6\text{H}_5\text{CH}_2(\text{CH}_2)_n\text{NH}_2)\text{Cl}_2]$ ($n = 1, 2$) (**13a,b**) readily bound to CT DNA but failed to produce stop sites on the pSP73KB plasmid DNA for RNA synthesis and very low amounts of cross-linking were observed, indicating the formation mainly of monofunctional adducts on DNA. This, together with a small observed unwinding angle, may explain why these complexes exhibit low cytotoxicities (**64**).

Dinuclear ruthenium–arene complexes can also bind rapidly to CT DNA, again preferentially to G bases. DNA-directed RNA synthesis was inhibited more effectively than by the corresponding mononuclear complex (**70**). A large unwinding angle of 31° induced by binding of the dinuclear complex $[\{\text{RuCl}(\eta^6\text{-bip})\}_2(\text{en})_2\text{-(CH}_2)_6\text{]}^{2+}$ (**23**) to pSP3KB DNA, twice that of the mononuclear complex (14°), is attributable to DNA cross-linking and structure perturbation by the two pendant phenyl rings (**70**). These results illustrate the synergistic effects of tethering the complexes together.

Interactions with Biomolecules. Candidate metallodrugs encounter a plethora of biomolecules upon administration, be it in, for instance, the blood plasma, the cell membrane, or the cytosol, before they reach the proposed target site (e.g. DNA in the nucleus or mitochondrion). The study of the interactions between the metal-based anticancer agent and proteins, antioxidants and other cellular components is therefore of key importance in understanding the biological activity both *in vitro* and *in vivo*. Such interactions can be responsible for drug inactivation (related to resistance) or activation (e.g. in the case of prodrugs) and drug delivery. Indeed, the biomolecule can even be the actual target of the metallodrug. Whereas the interaction of ruthenium–arene complexes with (models of) DNA has been extensively investigated, interactions with proteins are much less well characterized. Advances in particular in mass spectrometric methods, in combination with NMR and single crystal X-ray diffraction studies, now provide an opportunity to map the interactions of ruthenium–arene anticancer drugs with the proteome and identify key protein targets. This area is currently attracting much interest (**53**).

We reported the first crystal structure of a half-sandwich arene ruthenium(II)–enzyme complex (**Fig. 21**) (**90**). The crystal structure showed a $\{\text{Ru}(\eta^6\text{-}p\text{-cym})\text{X}_2\}$ half-sandwich fragment

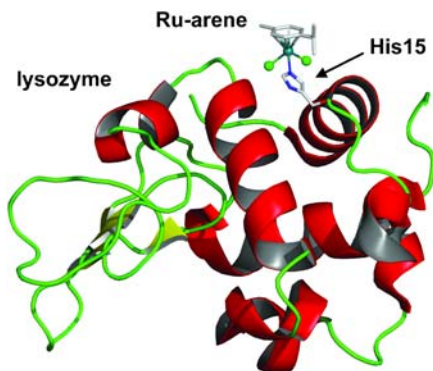


FIG. 21. X-ray crystal structure of a half-sandwich arene ruthenium(II)-enzyme complex: $[\text{RuCl}_2(\eta^6\text{-}p\text{-cym})(\text{lysozyme})]$.

bound selectively to N_ϵ of the imidazole ring of the only histidine residue (His15) in egg-white lysozyme, a small 14 kDa single-chain protein. The electron density for the additional two ligands on ruthenium was modeled as chloride, although partial occupancy by water cannot be ruled out. The selective binding of the Ru fragment places the complex in an environment that is asymmetric by nature. Since there is much current interest in ruthenium-catalyzed enantioselective synthesis, these results suggest that such sites can provide a basis for the design of novel catalytic centers (90).

Horse heart cytochrome *c* also has two solvent-accessible histidine residues, His26 (interior residue), and His33 (surface exposed), but our NMR and ESI-MS binding studies of $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**10**) show no binding to the histidine residues even with a 10-fold excess of the ruthenium complex. Mono-ruthenated enzymes were detected both in water (pH 8.7) and buffer (pH 7.6) and ICP-AES revealed that 50% of cytochrome *c* was ruthenated. Surprisingly, 2D ^1H , ^{15}N] HSQC NMR data suggest that ruthenium is bound to the N-terminal amino group or to a carboxylate group (Glu, Asp, or C-terminus), rather than to the histidines (91).

Histidine residues are, however, generally regarded as major possible binding sites for ruthenium-arene complexes in proteins. To model this interaction, we also studied the reaction of $[\text{RuCl}(\text{en})(\eta^6\text{-bip})]^+$ (**10**) with L-histidine at 310 K in aqueous solution (91). The reaction was quite sluggish and did not reach equilibrium until 24 h at 310 K, by which time only about 22% of the complex had reacted. Two isomeric imidazole-bound histidine adducts could be discerned, with more or less equal binding of N_ϵ

and N_8 to ruthenium. Competitive binding experiments of $[Ru(\eta^6\text{-bip})Cl(en)]^+$ with an oligonucleotide and either L-histidine or cytochrome *c* showed almost selective binding to the oligonucleotide and neither the amino acid nor the protein can compete effectively with the guanine site in the oligonucleotide (91).

Reaction with the sulfur-containing amino acids L-cysteine and L-methionine appear to play a major role in the biological chemistry of both Pt(II), Pt(IV), and Ru(III) anticancer agents, making it important to study these interactions with Ru(II)–arenes as well. $[Ru(\eta^6\text{-bip})Cl(en)]^+$ (**10**) reacts slowly with L-cysteine with only 50% of the complex reacting after 48 h (92). Initially, *S*- or *O*-bound 1:1 $[Ru(\eta^6\text{-bip})(\text{L-Cys})(en)]^+$ adducts are formed after hydrolysis of the chlorido complex. The mononuclear adducts then convert to the final products, which could be identified as unusual dinuclear ruthenium complexes, in which one or two en chelates have been displaced to form singly or doubly *S*-bridged adducts. Methionine binding was found to be equally slow, with only 23% of the ruthenium complex reacted after 48 h to give *S*-bound $[Ru(\eta^6\text{-bip})(en)(\text{S-L-Met})]^{2+}$.

These amino acid and protein binding results might account for the low toxic side effects of this class of anticancer agents (72). On the other hand, the relatively weak binding to amino acids and proteins could perhaps aid in transport and delivery of active species to cancer cells prior to binding to DNA or RNA (75).

Of special interest is the interaction of the family of ruthenium–arenes with the tripeptide glutathione (γ -L-Glu-L-Cys-Gly; GSH). This major intracellular antioxidant, present at millimolar concentrations in the cell, is known for its ability to detoxify heavier transition-metal ions, including some platinum and ruthenium anticancer complexes (93). Given the abundance of GSH in the cell, competition experiments with GSH and cGMP were undertaken to obtain more insight into the binding selectivity of ruthenium–arene anticancer complexes and to determine whether a large molar excess of GSH could prevent nucleobase binding. Our initial studies on the reaction of $[Ru(\eta^6\text{-bip})Cl(en)]^+$ and GSH gave some surprising results (Fig. 22) (94). Under physiological and anaerobic conditions (20 μ M [Ru], 5 mM [GSH], pH = 7 buffered solution, 310 K), the reaction yielded the thiolato adduct $[Ru(\eta^6\text{-bip})(en)(\text{S-GS})]^+$ as the major product. Unexpectedly, under an O_2 atmosphere, the thiolato adduct fully converted to a sulfenato complex in 48 h, strongly suggesting that the sulfenato complex originates from oxidation of the thiolato complex. The sulfenato is likely to be coordinated through *S* rather than *O*, as indicated by the observed SO stretching

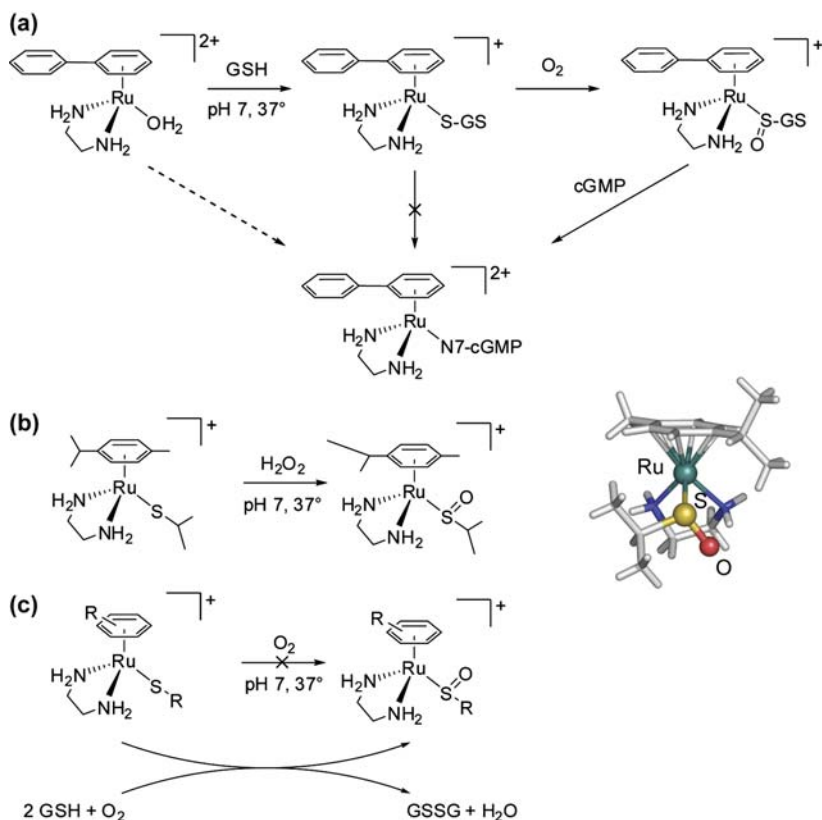


FIG. 22. Remarkable “activation-by-ligand-oxidation” pathways for the reaction of ruthenium–arenes with thiolates. (a) Reaction of $[\text{Ru}(\eta^6\text{-bip})(\text{en})(\text{OH}_2)]^+$ with GSH; (b) direct synthesis of ruthenium–arene sulfenato complexes; (c) the air-stable thiolato complexes are oxidized in the presence of the antioxidant GSH.

frequency. Sulfenates are generally too reactive to be isolated, but can be stabilized by coordination to a transition metal.

Competition experiments with 250 mol equiv GSH and 25 mol equiv cGMP subsequently showed that although initially both glutathione (thiolato and sulfenato) and cGMP adducts were formed, after 72 h the cGMP adduct was identified as the dominant product of the reaction. Our results indicated that the sulfenato ligand is readily displaced by cGMP, whereas the thiolato ligand is not (Fig. 22). The oxidation to sulfenato (perhaps followed by protonation, *vide infra*) could possibly weaken the ruthenium–sulfur bond and in this way introduce a good leaving group. X-ray absorption studies that provide insight into the

covalency of the metal–sulfur bonds of the thiolato, sulfenato, and sulfinato complexes are currently under way (95). This “activation-by-ligand-oxidation” is remarkable and clearly distinguishes these complexes from, for instance, cisplatin. It provides a facile route for DNA and RNA ruthenation even in the presence of a large excess of GSH (94). These observations also shed more light on the activity of $[\text{Ru}(\text{en})(\eta^6\text{-hmb})(\text{SPh})]^+$ (**18**), a complex of significant, but also initially somewhat unexpected cytotoxicity, given that the complex does not hydrolyze (75).

We further explored the oxidation of a ruthenium-coordinated thiolate and the chemistry of the resulting sulfenato complexes (Fig. 22) (96). Sulfenato complexes could be obtained directly from the oxidation of $[\text{Ru}(\eta^6\text{-arene})(\text{en})(\text{SR})]^+$ with hydrogen peroxide. The resulting sulfenato complexes are stable towards hydrolysis at pH 7 at ambient temperature. The ruthenium–sulfenato complex is rather basic ($\text{p}K_{\text{a}}$ of 3.37 for $[\text{Ru}(\text{en})(\eta^6\text{-hmb})(\text{S(O)iPr})]^+$) (**24b**) and protonation at lower pH leads to hydrolysis (96). The thiolato complexes are not sensitive to air, in contrast to the analogous GSH adducts. Surprisingly, however, we found that $[\text{Ru}(\text{en})(\eta^6\text{-hmb})(\text{SiPr})]^+$ (**24a**) could be fully oxidized to $[\text{Ru}(\text{en})(\eta^6\text{-hmb})(\text{S(O)iPr})]^+$ (**24b**) by dioxygen in the presence of GSH, with concomitant conversion of the latter to GSSG. The oxygen atom transfer from an intermediate of GSH autooxidation to the organometallic ruthenium arene thiolato complex was remarkably efficient (97).

Our work on thiolato oxidation is of broader relevance, as there is much current interest in the function of protein cysteinyl sulfenates in signal transduction, oxygen metabolism, oxidative stress, and their role in the activity of nitrile hydratase (98–100).

Catalytic Activity of Ruthenium–Arene Complexes. Although a different mechanism of action than cisplatin is assumed for the family of cytotoxic ruthenium–arene compounds, the general reactivity assumed for most of the cytotoxic ruthenium–arene complexes is consistent with the classic cisplatin paradigm of aquation followed by DNA binding, which in turn leads to “downstream” effects that ultimately trigger apoptosis. The reactivity of the cytotoxic agent is therefore limited to a one-off event. It would be desirable to develop metallo-anticancer agents that exert their cytotoxic effect in a catalytic manner, rather than the common single reactivity. The possibility of multiple turn-overs would potentially increase the potency of a drug and thus allow lower doses to be administered to the patient.

The exceptional ability of bioorganometallics to act as catalysts has not yet been widely explored (101). Half-sandwich ruthenium

complexes, for instance, are highly active catalysts in a wide variety of chemical transformations, most notably transfer hydrogenations, amongst many others. With this in mind, we have studied the regioselective reduction of NAD^+ to NADH by $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})]^+$ complexes using formate as the hydrogen donor under physiological conditions (102). $\text{NAD(P)}^+/\text{NAD(P)H}$ is one of the major redox couples found in the cell involved in many electron-transfer reactions. Off-setting the $\text{NAD(P)}^+/\text{NAD(P)H}$ equilibrium by catalytic conversion might be a way of exerting a cytotoxic effect through disturbing the cell's redox state. The reactivity was again found to be highly dependent on the arene, with $[\text{RuCl}(\text{en})(\eta^6\text{-hmb})]^+$ (**25**) giving the best results. Turnover frequencies were modest (1.46 h^{-1} at saturation, pH 7.4 and 37°C), however, and formation of the ruthenium-hydride species proved rate-limiting. Lung cancer cells were found to be remarkably tolerant to formate even at millimolar concentrations, opening up the possibility of *in vivo* biocatalysis if more active organometallic complexes would become available (102).

One point of concern for *in vivo* activity is that metal-based catalysts might readily be inactivated by poisoning. The myriad of cellular components such as thiols that are present in millimolar concentrations, e.g. GSH, provide a challenge for organometallic complexes. It is therefore intriguing that $[\text{Ru}(\eta^6\text{-arene})(\text{azpy})\text{I}]^+$ complexes can act as catalysts for the oxidation of the major intracellular reducing-agent glutathione to glutathione disulfide, in a cycle which appears to involve ligand-centered redox reactions (Fig. 23) (77). The influence of the halide is remarkable in this case as the chloride complex is rather unstable and not cytotoxic/catalytically active. The $[\text{Ru}(\eta^6\text{-arene})(\text{azpy})\text{I}]^+$ complexes do not hydrolyse, but do deplete millimolar amounts of GSH over 24 h by catalytic oxidation to GSSG under physiological conditions. Concomitant hydrogenation of dissolved dioxygen leads to the formation of hydrogen peroxide, a ROS, which are thought to be ultimately responsible for cell death. The catalytic ruthenium anticancer agents thus lead to oxidative stress by simultaneously producing ROS and consuming its antioxidant defenses in the cell (77). It is important to note that the azpy ligands themselves do not show this type of reactivity and the ligands are catalytically active only upon coordination to the metal center, i.e. controlling ligand reactivity by metal-ion coordination (77).

We started this section by stating that the advent of bioorganometallics provides the medicinal chemist with access to new types of reactivity and therefore with new opportunities for anticancer drug design. Our studies on the ruthenium-arene

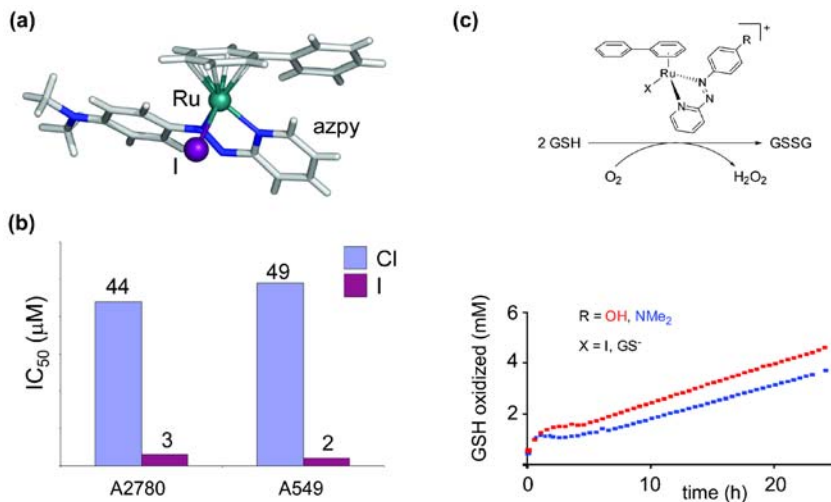


FIG. 23. (a) The $[\text{Ru}(\eta^6\text{-arene})(\text{azpy})\text{I}]^+$ complex (**17a**) is a catalytically active ruthenium–arene anticancer agent; (b) remarkable influence of the leaving group on cytotoxic activity; (c) $[\text{Ru}(\eta^6\text{-arene})(\text{azpy})\text{I}]^+$ complexes can act as catalysts for the oxidation of the major intracellular reducing agent glutathione to glutathione disulfide with concomitant production of reactive oxygen species. Data from Ref. (77).

anticancer drugs and results by others show that ruthenium-based compounds in particular do live up to this expectation. The general structure of the ruthenium–arenes provides a versatile platform for structural modification and activity optimization. Correlating structure and reactivity with cytotoxicity is complicated, but some general trends can be discerned. This way, control can be exerted over some kinetic (e.g. hydrolysis rate) and thermodynamic ($\text{p}K_{\text{a}}$, specific binding interactions) parameters that govern both activation of the drug, and the strength of the interaction with cellular targets. It is interesting to note here that not only the complexes on the (relatively) fast side of the scale of ligand-exchange exhibited good cytotoxicity. Some kinetically inert complexes proved equally cytotoxic and presumably operate through completely different, nonclassical mechanisms of action. A good example of the latter is the simultaneous depletion of GSH with concomitant generation of ROS by a catalytically active Ru(II)–arene anticancer agent (77). These results show the scope for design of ruthenium–arene anticancer drugs, and in particular their ability to operate in ways beyond the classical cisplatin paradigm of selective DNA-binding.

A major point that needs to be addressed in future work is the targeted delivery of organometallic anticancer drugs to cancer cells only. Also, additional features that generate cytotoxic activity other than disruption of DNA replication, such as the inclusion of molecular fragments that can interfere in cancer cell-specific cellular pathways, could be explored (11,103).

IV. Osmium(II)–Arenes: A Cytotoxic Family of the Heavier Congener

The application of organometallic complexes of the other group 8 elements, iron and osmium, in anticancer drug design has until recently been almost exclusively focused on iron, with the ferrocenyl derivative of tamoxifen (ferrocifen) being the most prominent example (104). Organometallic osmium compounds have been little explored in this respect.

We have recently extended our interest to the analogous half-sandwich osmium–arene complexes and are exploring the chemical and biological properties of $[\text{Os}(\eta^6\text{-arene})(\text{XY})\text{Z}]^{n+}$ complexes (Fig. 25) (105). Both the aqueous chemistry and the biological activity of osmium complexes have been little studied. Third-row transition metals are usually considered to be more inert than those of the first and second rows. Similar to the five orders of magnitude decrease in substitution rates of Pt(II) complexes compared to Pd(II), the $[\text{Os}(\eta^6\text{-arene})(\text{L})\text{X}]^{n+}$ complexes were expected to display rather different kinetics than their Ru(II)–arene analogs. A few other reports on the anticancer activity of osmium–arene complexes have also appeared recently (106–108).

Structure and Reactivity. In our initial studies (105), we looked at the structure and reactivity of $[\text{OsCl}(\text{L})(\eta^6\text{-arene})]^{n+}$ with *N*, *N*-chelating ligand en (**26**) and *O*, *O*-chelating ligand acac (**27a,b**) (Fig. 25). X-ray crystal structure determinations showed the complexes to be isostructural with their ruthenium analogs, with the same M–Cl bond lengths, even though the ligand-exchange rates are different (*vide infra*) (Fig. 24). The hydrolysis of $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**) is ca. 40 times slower ($t_{1/2}$ 6.4 h) than that of its Ru(II) analog **10**, consistent with the often-observed slower exchange rates on osmium compared to ruthenium.

It is interesting to note, however, that similar water exchange rates have been reported for the Os(II) and Ru(II) complexes $[\text{M}(\eta^6\text{-benzene})(\text{OH}_2)_3]^{2+}$ (109), indicative of a major influence of the chelating ligand. Indeed, changing the chelating ligand to acac resulted in a significant increase in the rate and extent of aquation. DFT calculations show that aquation of $[\text{Os}(\eta^6\text{-arene})\text{Cl}(\text{L})]$ is

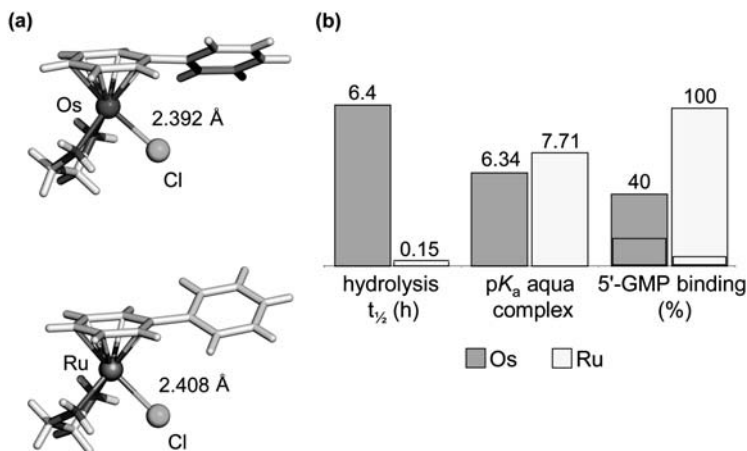


FIG. 24. Comparison between the osmium- and ruthenium-arenes, exemplified by the respective $[M(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ complexes. Although the crystal structures show the complexes to be isostructural with similar $M\text{-Cl}$ bond lengths (a), the properties of the complexes are quite different, illustrated by the differences in hydrolysis rate ($t_{1/2}$), pK_a , and 5'-GMP binding (the black box denotes the amount of OPO_3 -bound 5'-GMP) (b).

indeed more facile for $L = \text{acac}$ compared to $L = \text{en}$, and the mechanism is more dissociative in nature (110). However, hydroxido-bridged dimers $[\text{Os}_2(\eta^6\text{-arene})_2(\mu\text{-OH})_3]^+$ play a dominant role in the aqueous chemistry of $[\text{Os}(\text{acac})(\eta^6\text{-arene})\text{Cl}]$ (27a,b), whereas they are only a minor component, and then only at high pH, in the ruthenium systems (Fig. 25). The hydroxido-bridged dimer is the only species present on dissolution of $[\text{Os}(\text{acac})\text{Cl}(\eta^6\text{-p-cym})]$ (27a) at micromolar concentrations, conditions relevant for biological testing. The pK_a values of the aqua complexes are all significantly lower (ca. 1.5 units) than those for the corresponding ruthenium-arene complexes, e.g. 6.34 for $[\text{Os}(\eta^6\text{-bip})(\text{en})(\text{OH}_2)]^{2+}$ and 7.12 for $[\text{Os}(\text{acac})(\eta^6\text{-bip})(\text{OH}_2)]^+$. Therefore, at physiological pH almost all of the hydrolyzed en complex would be present in the hydroxide form $[\text{Os}(\eta^6\text{-bip})(\text{en})(\text{OH})]^+$. The higher acidity of osmium arene aqua complexes proved to be general and can be attributed to the increased mixing of the $\delta\sigma^*(\text{Os}) \rightarrow \sigma(\text{OH}^-)$ orbitals (105).

The aqueous chemistry of N,O -chelated complexes appeared to be intermediate between that of the neutral N,N -chelates and anionic O,O -chelates, with significant effects of the chosen N - and O -group (111). The aminoacidate complexes hydrolyzed

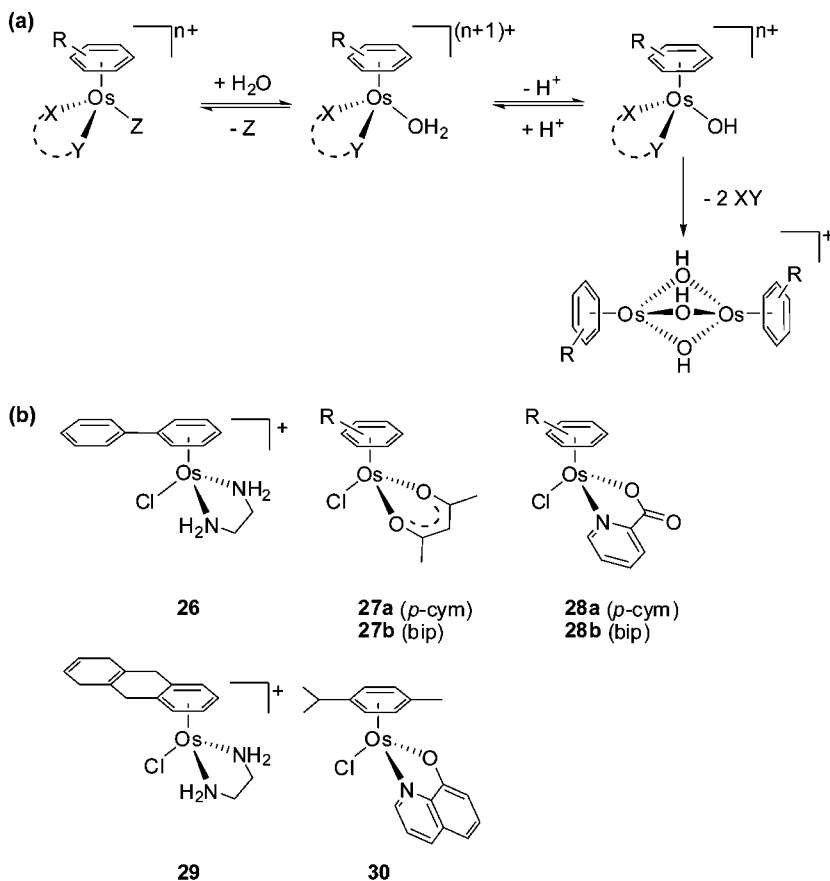


FIG. 25. (a) General aqueous reactivity of osmium(II)–arenes: the formation of an hydroxido-bridged dimer can play a major role in the aqueous chemistry of osmium(II)–arenes, especially if the chelate XY is an *O,O*-bidentate ligand; (b) molecular structures of the osmium–arene anticancer agents **26–30**.

rapidly, but replacement of the primary amine by the π -acceptor pyridine slowed down the rate of hydrolysis. Important was the observation that the inactive aminoacidate complexes again proved unstable with respect to hydroxido-bridged dimer formation, whereas no such dimer formation was observed with the active pico (**28**) and oxine (**30**) complexes (*vide infra* for cytotoxicity). The strength of the Os–N(pyridine) bond seems crucial for the stability of the complex. At high chloride concentrations typical of blood plasma (ca. 100 mM) the complex [OsCl(η^6 -*p*-cym)(pico)] (**28a**) is likely to be mainly present as the less reactive intact

chlorido species. At lower $[\text{Cl}^-]$ concentration (4 mM, typical of cell nucleus), the complex is activated by hydrolysis. As previously stated for the ruthenium–arenes this presents an interesting prodrug activation strategy for DNA binding.

Nucleobase Binding. Nucleobase reactions showed slow binding of $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**) to 9-EtG and then only to a limited extent (45% after 22 h). No binding to Ado, Cyt, or Thy was observed. $[\text{Os}(\text{acac})(\eta^6\text{-bip})\text{Cl}]$ (**27b**) on the other hand binds rapidly to both 9-EtG and Ado (<10 min), but not to Cyt or Thy. Notably, the hydroxo-bridged dimer was inert towards nucleobases Fig. 25 (105). Two major adducts were observed in the reaction between $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**) and 5'-GMP attributable to N7 and phosphate adducts, which formed in a 2:1 N7/phosphate ratio (112) (Fig. 24). Only about 40% of 5'-GMP is bound after 30 h. This contrasts with 5'-GMP binding to $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**10**), for which quantitative and almost selective binding to N7 is observed after 1 d (81).

Nucleobase competition experiments showed binding of $[\text{OsCl}(\eta^6\text{-p-cym})(\text{pico})]$ (**28a**) to both G and A, but with a strong preference for G (111). Intriguingly, a modest binding constant for G binding was found ($\log K$ 3.95), but the slow dissociation of 9-EtG at micromolar concentrations, makes it likely for G adducts on DNA or RNA to persist once formed. Little and no binding to the pyrimidine bases Cyt and Thy was observed.

Cytotoxicity. $[\text{OsCl}(\text{acac})(\eta^6\text{-arene})]$ (**27a,b**) showed no cytotoxicity towards A2780 and A549 cell lines, probably attributable to the formation of the inert hydroxido-bridged dimer. Replacement of acac for the potentially more stable five-membered chelate ring of maltolato did provide some stabilization towards dimer formation compared to the acac complex, but under biologically relevant conditions the hydroxido-bridged dimer remained the dominant species (110). Although the complexes were not active, the results did show that kinetics of ligand substitution on osmium can be controlled by variation of the ligand and thus proved very valuable for our further studies.

Initial experiments showed that $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**) was not cytotoxic towards cancer cells (105), but a later reassessment of the cytotoxic activity of this compound showed that it indeed was active at micromolar concentrations (IC_{50} values of 7.6 (A2780) and 10 μM (A549)) (112). A possible explanation for the initial lack of activity may be the partial decomposition of the complex in stock test solutions prepared in DMSO, as was evidenced in subsequent studies (112). The cytotoxicity data are now more in line with the chemical properties of the complex, i.e. observed hydrolysis rate and guanine binding.

The use of an extended arene (tetrahydroanthracene) in $[\text{OsCl}(\text{en})(\eta^6\text{-tha})]^+$ (**29**) gave rise to a similar potency (112). This is in contrast with the data for ruthenium–arenes, where the same substitution gave rise to a 10-fold increase in activity. Further work therefore needs to determine if the extended Os–arenes can intercalate into DNA in a manner similar to Ru–arenes. Replacement of the *N,N*-chelating ligand en for other *N,N*-bidentates with pyridine, aliphatic amine, or azopyridine donor atoms leads to loss of activity, probably because of slower hydrolysis and higher acidity of the coordinated water (112).

Further ligand variation led to the isolation of other osmium–arenes with moderate to high cytotoxicity towards cancer cells (111). We found that the aqueous chemistry of the organometallic osmium complexes could be fine-tuned so as to achieve cancer cell cytotoxicity by using mixed *N,O*-chelate ligands, Fig. 26. Several aminoacidates, picoline (pico), and 8-hydroxyquinoline (oxine) were tested, but inclusion of pyridine as the *N* donor rather than a primary amine proved key for cytotoxic activity. The complexes $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{pico})]$ (**28b**), $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{pico})]$ (**28a**), and $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{oxine})]$ (**30**) exhibit cytotoxic activity against human ovarian (A2780) and lung (A549) cancer cells, with IC_{50} values of 5–60 μM . Notable is the high activity of $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{pico})]$ (**28b**) with IC_{50} values of 4.2 (A2780) and 8 μM (A549) (111). The four active complexes $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**), $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{pico})]$ (**28b**), $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{pico})]$ (**28a**), and $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{oxine})]$ (**30**) all show similar potency towards cisplatin-sensitive and resistant A2780 cell lines, indicating a different detoxification mechanism than cisplatin. Intriguingly, $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**) shows an even greater activity in the resistant cell line (resistance factor 0.55) (113).

DNA binding. An in-depth study of osmium–arene anticancer drug binding to DNA was carried out in collaboration with the group of Brabec (113). It is notable that the tested complexes all bind polymeric DNA. The complexes $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{pico})]$ (**28b**) and $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{oxine})]$ (**30**) bind rapidly to CT DNA ($t_{1/2}$ ca. 2 h at $r_i = 0.1$), whereas $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**) and $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{pico})]$ (**28a**) bind up to two and four times more slowly with $t_{1/2}$ values of 4.6 and 8.3 h, respectively (113). For comparison, the $t_{1/2}$ of DNA binding of the ruthenium–arene $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ is ca. 10 min, approximately 28 times faster than its osmium analog, which might allow more of the latter to reach its target site (88). The extent of DNA binding ranges from ca. 72% for $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{pico})]$ (**28a**) to 95% for $[\text{Os}(\eta^6\text{-}p\text{-bip})\text{Cl}(\text{pico})]$ (**28b**), illustrating the importance of an extended arene ring for DNA binding. RNA synthesis transcription mapping showed the

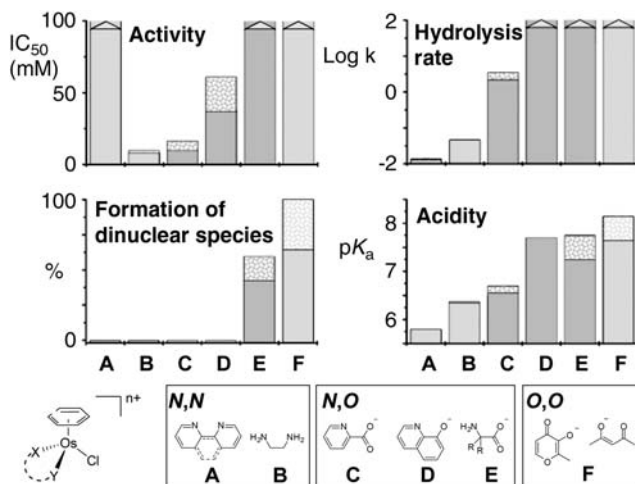


FIG. 26. Bar charts relate the influence of different chelates in $[\text{Os}(\eta^6\text{-arene})\text{Cl}(\text{XY})]^{n+}$ ($\text{XY} = \text{N,N-}$, N,O- or O,O-) on cytotoxicity, stability with respect to hydroxido-dimer formation, hydrolysis rates, and pK_a of the aqua adduct for osmium–arene complexes. Shading indicates the range in observed values. Adapted from Ref. (111).

major stop sites to be guanine residues (with some minor ones at adenine), similar to cisplatin and the ruthenium analogs. Electrophoretic mobility experiments on osmium-adducts of DNA duplex oligonucleotides (up to 21 bp) showed that no DNA bending is induced upon osmium binding. The binding of the osmium–arene complex leads to a large degree of unwinding ($21\text{--}27^\circ$) (except for the oxine complex) (113), much larger than that observed for ruthenium(II) complexes (88). This large unwinding angle might be explained by an additional interaction of the arene ligand with the duplex upon strong binding of osmium. The complex $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{oxine})]$ (30) on the other hand shows hardly any significant unwinding of DNA ($<2.5^\circ$). This suggests that this complex interacts with DNA in a different manner, which could correlate with its markedly lower cytotoxic activity (113).

In line with expectations of kinetic inertness for third-row transition metals, little interest has been vested in the development of osmium anticancer drugs, as ligand-exchange rates did not seem favorable on the timescale of cellular processes. Our work, however, shows that the kinetic lability of such complexes can be tuned to such extent that anticancer activity comes within range. We have demonstrated how rational chemical design can thus be applied to osmium–arene complexes resulting in specific

windows of reactivity, stability, and cancer cell cytotoxicity (111). This has allowed us to design complexes with cytotoxicities comparable to their ruthenium analogs, but with reactivities that are 100 times less. Such a range of kinetic effects can be useful for balancing cytotoxicity and unwanted side effects of anticancer drugs, as illustrated previously by the clinical profiles of cisplatin and the less-labile second-generation drug carboplatin (112). The osmium complexes are therefore interesting candidates for further investigation.

V. Concluding Remarks

In general transition-metal complexes provide enormously versatile platforms for drug design. Many variations in the metal itself, the types, and numbers of coordinated ligands and hence in the strengths of coordination bonds and in the kinetics of ligands substitution processes are available. In this review, we have highlighted our efforts towards the development of photoactive platinum anticancer agents and the ruthenium- and osmium-arene families of anticancer agents. The key to successful design of clinically useful drugs is effective activity whilst minimizing side effects. Targeting is important to ensure that sufficient amounts of the active drug reach the target site and that unwanted reactions do not occur along the way. Hence both the thermodynamics and kinetics of ligand-exchange and redox processes must be carefully controlled. This presents a major challenge for the transition-metal medicinal chemist – an exciting one too. We have shown that such control is indeed possible by systematic ligand variation. This way we were able to design photoactive platinum(IV) complexes that are stable and nontoxic in the dark. The strategy of activation by light to yield highly cytotoxic species at irradiated spots only allows for local, targeted treatment and holds the promise of less-invasive chemotherapy. Similarly, our work on the ruthenium- and osmium-arenes showed that thermodynamic and kinetic parameters, such as those associated with aquation, can vary over several orders of magnitude, thus allowing the chemist to fine-tune the properties of the agent for increased cytotoxicity. Not only is direct coordination to the metal important in biological recognition processes but so are second coordination sphere interactions, e.g. hydrogen bonding, hydrophobic interactions, as exemplified by the stereospecific hydrogen-bonding interactions of the ruthenium-arenes and arene intercalation upon coordination of the complex to DNA.

While for the photoactive Pt(IV) complexes both metal- and ligand-centered redox reactions can be involved in biological mechanisms of action, it is interesting to note that ligand-centered redox processes can also dominate reactions of arene complexes. The catalytic depletion of GSH by azopyridine complexes with concomitant generation of ROS, for instance, is a ligand-based process, which is nonetheless modulated and made possible by coordination of the ligand to ruthenium. Such reactions are often not possible for the ligands alone in purely organic drugs. Additionally, metal complexes themselves are renowned as catalysts and the possibility of designing catalytically active metallodrugs which are not prematurely poisoned in the biological system represents a significant challenge.

ACKNOWLEDGMENTS

Pieter C.A. Bruijninx thanks the Netherlands Organization for Scientific Research (NWO) for financial support through a Rubicon Scholarship. We thank the EPSRC, BBSRC, Royal Society, Wellcome Trust, EC (Marie Curie and COST), Scottish Enterprise, and Oncosense Ltd. for support of our recent research on therapeutic metal complexes.

The University of Edinburgh (former employer of Peter J. Sadler) has filed patent applications relating to the ruthenium–arene, and platinum diazido complexes and University of Warwick for osmium–arene complexes under study in the Peter J. Sadler laboratory.

REFERENCES

1. Krogsgaard-Larsen, P.; Liljefors, T.; Madsen, U.; (Eds.) “*Textbook of Drug Design and Discovery*”; Taylor & Francis: London, **2002**.
2. Guo, Z.; Sadler, P. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 1513–1531.
3. Hambley, T. W. *Dalton Trans.* **2007**, 4929–4937.
4. Mukherjee, A.; Sadler, P. J. In: “*Wiley Encyclopedia of Chemical Biology*”; Ed. Begley, T. P.; **2009**, in press.
5. Ronconi, L.; Sadler, P. J. *Coord. Chem. Rev.* **2007**, *251*, 1633–1648.
6. Storr, T.; Thompson, K. H.; Orvig, C. *Chem. Soc. Rev.* **2006**, *35*, 534–544.
7. Hall, M. D.; Mellor, H. R.; Callaghan, R.; Hambley, T. W. *J. Med. Chem.* **2007**, *50*, 3403–3411.
8. Jung, Y.; Lippard, S. J. *Chem. Rev.* **2007**, *107*, 1387–1407.
9. Van Zutphen, S.; Reedijk, J. *Coord. Chem. Rev.* **2005**, *249*, 2845–2853.
10. Kelland, L. *Nat. Rev. Cancer* **2007**, *7*, 573–584.
11. Bruijninx, P. C. A.; Sadler, P. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197–206.

12. Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Jarvinen, T.; Savolainen, J. *Nat. Rev. Drug Discov.* **2008**, *7*, 255–270.
13. Rose, M. J.; Mascharak, P. K. *Curr. Opin. Chem. Biol.* **2008**, *12*, 238–244.
14. Johnson, T. R.; Mann, B. E.; Clark, J. E.; Foresti, R.; Green, C. J.; Motterlini, R. *Angew. Chem. Int. Ed.* **2003**, *42*, 3722–3729.
15. Reedijk, J. *Platinum Metals Rev.* **2008**, *52*, 2–11.
16. Helm, L.; Nicolle, G. M.; Merbach, A. E. *Adv. Inorg. Chem.* **2005**, *57*, 327–379.
17. Hall, M. D.; Failes, T. W.; Yamamoto, N.; Hambley, T. W. *Dalton Trans.* **2007**, 3983–3990.
18. Kratz, F.; Muller, I. A.; Ryppa, C.; Warnecke, A. *Chem. Med. Chem.* **2008**, *3*, 20–53.
19. Farrer, N. J.; Sadler, P. J. *Austr. J. Chem.* **2008**, *61*, 669–674.
20. Szacilowski, K.; Macyk, W.; Drzewiecka-Matuszek, A.; Brindell, M.; Stochel, G. *Chem. Rev.* **2005**, *105*, 2647–2694.
21. Bednarski, P. J.; Mackay, F. S.; Sadler, P. J. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 75–93.
22. Choy, H.; Park, C.; Yao, M. *Clin. Cancer Res.* **2008**, *14*, 1633–1638.
23. Kratochwil, N. A.; Zabel, M.; Range, K.-J.; Bednarski, P. J. *J. Med. Chem.* **1996**, *39*, 2499–2507.
24. Kratochwil, N. A.; Guo, Z.; Del Socorro Murdoch, P.; Parkinson, J. A.; Bednarski, P. J.; Sadler, P. J. *J. Am. Chem. Soc.* **1998**, *120*, 8253–8254.
25. Kratochwil, N. A.; Parkinson, J. A.; Bednarski, P. J.; Sadler, P. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 1460–1463.
26. Vogler, A.; Hlavatsch, J. *Angew. Chem. Int. Ed.* **1983**, *22*, 154–155.
27. Vogler, A.; Kern, A.; Huttermann, J. *Angew. Chem. Int. Ed.* **1978**, *17*, 524–525.
28. Muller, P.; Schroder, B.; Parkinson, J. A.; Kratochwil, N. A.; Coxall, R. A.; Parkin, A.; Parsons, S.; Sadler, P. J. *Angew. Chem. Int. Ed.* **2003**, *42*, 335–339.
29. Kasparkova, J.; Mackay, F. S.; Brabec, V.; Sadler, P. J. *J. Biol. Inorg. Chem.* **2003**, *8*, 741–745.
30. Bednarski, P. J.; Grunert, R.; Zielzki, M.; Wellner, A.; Mackay, F. S.; Sadler, P. J. *Chem. Biol.* **2006**, *13*, 61–67.
31. Blisard, K. S.; Harrington, D. A.; Long, D. A.; Jackson, J. E. *J. Comp. Pathol.* **1991**, *105*, 367–375.
32. Mackay, F. S.; Woods, J. A.; Moseley, H.; Ferguson, J.; Dawson, A.; Parsons, S.; Sadler, P. J. *Chem. Eur. J.* **2006**, *12*, 3155–3161.
33. Mackay, F. S. Photoactive platinum azide anticancer complexes, PhD Thesis, University of Edinburgh, Edinburgh, **2006**.
34. Mackay, F. S.; Moggach, S. A.; Collins, A.; Parsons, S.; Sadler, P. J. *Inorg. Chim. Acta*, doi:10.1016/j.ica.2008.02.039.
35. Mackay, F. S.; Woods, J. A.; Heringova, P.; Kasparkova, J.; Pizarro, A. M.; Moggach, S. A.; Parsons, S.; Brabec, V.; Sadler, P. J. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20743–20748.
36. Ronconi, L.; Sadler, P. J. *Chem. Commun.* **2008**, 235–237.
37. Albot, M.; Beljonne, D.; Bredas, J.-L.; Ehrlich, J. E.; Fu, J.-Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M. D.; Marder, S. R.; McCord-Maughon, D.; Perry, J. W.; Rockel, H.; Rumi, M.; Subramaniam, G.; Webb, W. W.; Wu, X.-L.; Xu, C. *Science* **1998**, *281*, 1653–1656.
38. Fish, R. H.; Jaouen, G. *Organometallics* **2003**, *22*, 2166–2177.
39. Jaouen, G. (Ed.) *"Bioorganometallics"*; Wiley-VSH Verlag GmbH & Co: Weinheim, **2006**.

40. Halpern, J. *Pure Appl. Chem.* **2001**, 73, 209–220.
41. Vessieres, A.; Top, S.; Beck, W.; Hillard, E.; Jaouen, G. *Dalton Trans.* **2006**, 529–541.
42. Alberto, R.; Schibli, R.; Waibel, R.; Abram, U.; Schubiger, A. P. *Coord. Chem. Rev.* **1999**, 190–192, 901–919.
43. Dougan, S. J.; Sadler, P. J. *Chimia* **2007**, 61, 704–715.
44. Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. *Chem. Commun.* **2005**, 4764–4776.
45. Rosenberg, B.; VanCamp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, 222, 385–386.
46. Ang, W. H.; Dyson, P. J. *Eur. J. Inorg. Chem.* **2006**, 4003–4018.
47. Clarke, M. J. *Coord. Chem. Rev.* **2002**, 232, 69–93.
48. Jakupec, M. A.; Galanski, M.; Arion, V. B.; Hartinger, C. G.; Keppler, B. K. *Dalton Trans.* **2008**, 183–194.
49. Durig, J. R.; Danneman, J.; Behnke, W. D.; Mercer, E. E. *Chem.-Biol. Interact.* **1976**, 13, 287–294.
50. Rademaker-Lakhai, J. M.; Van Den Bongard, D.; Pluim, D.; Beijnen, J. H.; Schellens, J. H. M. *Clin. Cancer Res.* **2004**, 10, 3717–3727.
51. Hartinger, C. G.; Zorbas-Seifried, S.; Jakupec, M. A.; Kynast, B.; Zorbas, H.; Keppler, B. K. *J. Inorg. Biochem.* **2006**, 100, 891–904.
52. Bergamo, A.; Sava, G. *Dalton Trans.* **2007**, 1267–1272.
53. Timerbaev, A. R.; Hartinger, C. G.; Aleksenko, S. S.; Keppler, B. K. *Chem. Rev.* **2006**, 106, 2224–2248.
54. Reisner, E.; Arion, V. B.; Keppler, B. K.; Pombeiro, A. J. L. *Inorg. Chim. Acta* **2008**, 361, 1569–1583.
55. Brindell, M.; Stawoska, I.; Supel, J.; Skoczowski, A.; Stochel, G.; van Eldik, R. *J. Biol. Inorg. Chem.* **2008**, 13, 909–918.
56. Habtemariam, A.; Kelland, L.; Sadler, P. J. unpublished results.
57. Morris, R.; Habtemariam, A.; Guo, Z.; Parsons, S.; Sadler, P. J. *Inorg. Chim. Acta* **2002**, 339, 551–559.
58. Guo, Z.; Habtemariam, A.; Sadler, P. J.; James, B. R. *Inorg. Chim. Acta* **1998**, 273, 1–7.
59. Morris, R. E.; Aird, R. E.; Del Socorro Murdoch, P.; Chen, H.; Cummings, J.; Hughes, N. D.; Parsons, S.; Parkin, A.; Boyd, G.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem.* **2001**, 44, 3616–3621.
60. Allardyce, C. S.; Dyson, P. J.; Ellis, D. J.; Heath, S. L. *Chem. Commun.* **2001**, 1396–1397.
61. Meggers, E.; Atilla-Gokcumen, G. E.; Bregman, H.; Maksimoska, J.; Mulcahy, S. P.; Pagano, N.; Williams, D. S. *Synlett* **2007**, 1177–1189.
62. Smalley, K. S. M.; Contractor, R.; Haass, N. K.; Kulp, A. N.; Atilla-Gokcumen, G. E.; Williams, D. S.; Bregman, H.; Flaherty, K. T.; Soengas, M. S.; Meggers, E.; Herlyn, M. *Cancer Res.* **2007**, 67, 209–217.
63. Muetterties, E. L.; Bleeke, J. R.; Wucherer, E. J.; Albright, T. *Chem. Rev.* **1982**, 82, 499–525.
64. Melchart, M.; Habtemariam, A.; Novakova, O.; Moggach, S. A.; Fabbiani, F. P. A.; Parsons, S.; Brabec, V.; Sadler, P. J. *Inorg. Chem.* **2007**, 46, 8950–8962.
65. Pizarro, A. M.; Melchart, M.; Sadler, P. J. unpublished results.
66. Gray, J. C.; Habtemariam, A.; Winnig, M.; Meyerhof, W.; Sadler, P. J. *J. Biol. Inorg. Chem.* **2008**, 13, 1111–1120.
67. Habtemariam, A.; Melchart, M.; Fernandez, R.; Parsons, S.; Oswald, I. D. H.; Parkin, A.; Fabbiani, F. P. A.; Davidson, J. E.; Dawson, A.; Aird, R. E.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem.* **2006**, 49, 6858–6868.

68. Therrien, B.; Ward, T. R.; Pilkington, M.; Hoffmann, C.; Gilardoni, F.; Weber, J. *Organometallics* **1998**, *17*, 330–337.
69. Hoeschele, J. D.; Habtemariam, A.; Muir, J.; Sadler, P. J. *Dalton Trans.* **2007**, 4974–4979.
70. Chen, H.; Parkinson, J. A.; Novakova, O.; Bella, J.; Wang, F.; Dawson, A.; Gould, R.; Parsons, S.; Brabec, V.; Sadler, P. J. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14623–14628.
71. Wang, F.; Aird, R. E.; Habtemariam, A.; Jodrell, D. I.; Sadler, P. J.; Guichard, S. M. unpublished results.
72. Aird, R. E.; Cummings, J.; Ritchie, A. A.; Muir, M.; Morris, R. E.; Chen, H.; Sadler, P. J.; Jodrell, D. I. *Br. J. Cancer* **2002**, *86*, 1652–1657.
73. Guichard, S. M.; Else, R.; Reid, E.; Zeitlin, B.; Aird, R.; Muir, M.; Dodds, M.; Fiebig, H.; Sadler, P. J.; Jodrell, D. I. *Biochem. Pharmacol.* **2006**, *71*, 408–415.
74. Bugarcic, T.; Novakova, O.; Halamikova, A.; Zerzankova, L.; Vrana, O.; Kasparkova, J.; Habtemariam, A.; Parsons, S.; Sadler, P. J.; Brabec, V. *J. Med. Chem.* **2008**, *51*, 5310–5319.
75. Wang, F.; Habtemariam, A.; Van Der Geer, E. P. L.; Fernandez, R.; Melchart, M.; Deeth, R. J.; Aird, R.; Guichard, S.; Fabbiani, F. P. A.; Lozano-Casal, P.; Oswald, I. D. H.; Jodrell, D. I.; Parsons, S.; Sadler, P. J. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 18269–18274.
76. Dougan, S. J.; Melchart, M.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *Inorg. Chem.* **2006**, *45*, 10882–10894.
77. Dougan, S. J.; Habtemariam, A.; McHale, S. E.; Parsons, S.; Sadler, P. J. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11628–11633.
78. Wang, F.; Chen, H.; Parsons, S.; Oswald, I. D. H.; Davidson, J. E.; Sadler, P. J. *Chem. Eur. J.* **2003**, *9*, 5810–5820.
79. Fairhurst, M. T.; Swaddle, T. W. *Inorg. Chem.* **1979**, *18*, 3241–3244.
80. Fernandez, R.; Melchart, M.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *Chem. Eur. J.* **2004**, *10*, 5173–5179.
81. Chen, H.; Parkinson, J. A.; Morris, R. E.; Sadler, P. J. *J. Am. Chem. Soc.* **2003**, *125*, 173–186.
82. Melchart, M.; Habtemariam, A.; Parsons, S.; Moggach, S. A.; Sadler, P. J. *Inorg. Chim. Acta* **2006**, *359*, 3020–3028.
83. Magennis, S. W.; Habtemariam, A.; Novakova, O.; Henry, J. B.; Meier, S.; Parsons, S.; Oswald, I. D. H.; Brabec, V.; Sadler, P. J. *Inorg. Chem.* **2007**, *46*, 5059–5068.
84. Chen, H.; Parkinson, J. A.; Parsons, S.; Coxall, R. A.; Gould, R. O.; Sadler, P. J. *J. Am. Chem. Soc.* **2002**, *124*, 3064–3082.
85. Melchart, M.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *J. Inorg. Biochem.* **2007**, *101*, 1903–1912.
86. Liu, H. K.; Berners-Price, S. J.; Wang, F.; Parkinson, J. A.; Xu, J.; Bella, J.; Sadler, P. J. *Angew. Chem. Int. Ed.* **2006**, *45*, 8153–8156.
87. Liu, H. K.; Wang, F.; Parkinson, J. A.; Bella, J.; Sadler, P. J. *Chem. Eur. J.* **2006**, *12*, 6151–6165.
88. Novakova, O.; Chen, H.; Vrana, O.; Rodger, A.; Sadler, P. J.; Brabec, V. *Biochemistry* **2003**, *42*, 11544–11554.
89. Novakova, O.; Kasparkova, J.; Bursova, V.; Hofr, C.; Vojtiskova, M.; Chen, H.; Sadler, P. J.; Brabec, V. *Chem. Biol.* **2005**, *12*, 121–129.
90. McNae, I. W.; Fishburne, K.; Habtemariam, A.; Hunter, T. M.; Melchart, M.; Wang, F.; Walkinshaw, M. D.; Sadler, P. J. *Chem. Commun.* **2004**, *10*, 1786–1787.

91. Wang, F.; Bella, J.; Parkinson, J. A.; Sadler, P. J. *J. Biol. Inorg. Chem.* **2005**, *10*, 147–155.
92. Wang, F.; Chen, H.; Parkinson, J. A.; Murdoch, P. D. S.; Sadler, P. J. *Inorg. Chem.* **2002**, *41*, 4509–4523.
93. Reedijk, J. *Chem. Rev.* **1999**, *99*, 2499–2510.
94. Wang, F.; Xu, J.; Habtemariam, A.; Bella, J.; Sadler, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 17734–17743.
95. Sriskandakumar, T.; Petzold, H.; Bruijninx, P. C. A.; Habtemariam, A.; Sadler, P. J.; Kennepohl, P. unpublished results.
96. Petzold, H.; Xu, J.; Sadler, P. J. *Angew. Chem. Int. Ed.* **2008**, *47*, 3008–3011.
97. Petzold, H.; Sadler, P. J. *Chem. Commun* **2008**, 4413–4415.
98. Harrop, T. C.; Mascharak, P. K. *Acc. Chem. Res.* **2004**, *37*, 253–260.
99. Claiborne, A.; Conn Mallett, T.; Yeh, J. I.; Luba, J.; Parsonage, D.; Klinman, J. P.; Dove, J. E. *Adv. Protein Chem.* **2001**, *58*, 215–236.
100. Claiborne, A.; Yeh, J. I.; Mallett, T. C.; Luba, J.; Crane, E. J.; Charrier, V.; Parsonage, D. *Biochemistry* **1999**, *38*, 15407–15416.
101. Streu, C.; Meggers, E. *Angew. Chem. Int. Ed.* **2006**, *45*, 5645–5648.
102. Yan, Y. K.; Melchart, M.; Habtemariam, A.; Peacock, A. F. A.; Sadler, P. J. *J. Biol. Inorg. Chem.* **2006**, *11*, 483–488.
103. Wang, D.; Lippard, S. J. *Nat. Rev. Drug Discov.* **2005**, *4*, 307–320.
104. Hillard, E.; Vessieres, A.; Thouin, L.; Jaouen, G.; Amatore, C. *Angew. Chem. Int. Ed.* **2006**, *45*, 285–290.
105. Peacock, A. F. A.; Habtemariam, A.; Fernandez, R.; Walland, V.; Fabbiani, F. P. A.; Parsons, S.; Aird, R. E.; Jodrell, D. I.; Sadler, P. J. *J. Am. Chem. Soc.* **2006**, *128*, 1739–1748.
106. Dorcier, A.; Ang, W. H.; Bolano, S.; Gonsalvi, L.; Juillerat-Jeannerat, L.; Laurenczy, G.; Peruzzini, M.; Phillips, A. D.; Zanolini, F.; Dyson, P. J. *Organometallics* **2006**, *25*, 4090–4096.
107. Dorcier, A.; Dyson, P. J.; Gossens, C.; Rothlisberger, U.; Scopelliti, R.; Tavernelli, I. *Organometallics* **2005**, *24*, 2114–2123.
108. Schmid, W. F.; John, R. O.; Arion, V. B.; Jakupec, M. A.; Keppler, B. K. *Organometallics* **2007**, *26*, 6643–6652.
109. Stebler-Rothlisberger, M.; Hummel, W.; Pittet, P. A.; Buerger, H. B.; Ludi, A.; Merbach, A. E. *Inorg. Chem.* **1988**, *27*, 1358–1363.
110. Peacock, A. F. A.; Melchart, M.; Deeth, R. J.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *Chem. Eur. J.* **2007**, *13*, 2601–2613.
111. Peacock, A. F. A.; Parsons, S.; Sadler, P. J. *J. Am. Chem. Soc.* **2007**, *129*, 3348–3357.
112. Peacock, A. F. A.; Habtemariam, A.; Moggach, S. A.; Prescimone, A.; Parsons, S.; Sadler, P. J. *Inorg. Chem.* **2007**, *46*, 4049–4059.
113. Kostrhunova, H.; Florian, J.; Novakova, O.; Peacock, A. F. A.; Sadler, P. J.; Brabec, V. *J. Med. Chem.* **2008**, *51*, 3635–3643.

DESIGN AND FUNCTION OF METAL COMPLEXES AS CONTRAST AGENTS IN MRI

VOJTĚCH KUBÍČEK and ÉVA TÓTH

Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron,
45071 Orléans Cedex 2, France

I.	Introduction	63
II.	Relaxivity of Gd^{III} Complexes: Optimization of the Relaxivity	67
	A. Inner Sphere Proton Relaxivity	68
	B. Second and Outer Sphere Relaxivity	89
	C. Contrast Agents Optimized for Application at High Magnetic Field	90
III.	Stability Aspects	92
	A. Thermodynamic Stability Constants of the Complexes	93
	B. Kinetic Inertness of Gd^{III} Complexes	95
IV.	Paramagnetic CEST Agents	98
	A. Principle of CEST	98
	B. Application of Paramagnetic Ions: PARACEST	99
	C. LIPOCEST	100
V.	Responsive Imaging Probes	101
	A. Enzyme-Activated Contrast Agents	102
	B. Contrast Agents Responsive to Metal Ions	105
VI.	Mn^{II} Complexes in the Context of MRI	109
	A. MRI Properties of Mn^{II} Complexes	109
	B. Stability of Mn^{II} Complexes	113
VII.	Non-Classical Gd^{III} -Based MRI Contrast Agents	115
	A. Gadofullerenes	115
	B. Carbon Nanotubes Loaded with Gd^{III}	117
VIII.	Conclusions and Outlook	119
	References	119

I. Introduction

From its commercial appearance in the early 1980s, magnetic resonance imaging (MRI) has blossomed into a standard component of modern diagnostic clinical services. MRI has many advantages: it is non-invasive, it has an exceptional temporal and

spatial resolution (in contrast to PET), unlimited tissue penetration (in contrast to optical methods), and it does not involve any ionizing radiation (in contrast to X-ray, SPECT, or PET). Magnetic resonance (MR) images are based on proton density and proton relaxation dynamics, parameters that vary according to the tissue under examination and reflect its physical and chemical properties. MRI affords anatomic images in multiple planes. The images are obtained by placing the patient or area of interest within a powerful, highly uniform, static magnetic field. Magnetized protons within the patient align like small magnets in this field. Radiofrequency pulses are then utilized to create an oscillating magnetic field perpendicular to the main field, from which the nuclei absorb energy and move out of alignment with the static field, in a state of excitation. As the nuclei return from excitation to the equilibrium state, a signal induced in the receiver coil of the instrument is transformed into diagnostic images. Images based on different tissue characteristics can be obtained by varying the number and sequence of pulsed radiofrequency fields in order to take advantage of magnetic relaxation properties of the tissues.

The main disadvantage in MRI is its relatively low sensitivity, which can be compensated by the use of contrast agents. These paramagnetic, superparamagnetic, or ferromagnetic substances are catalysts of water proton relaxation. They shorten the relaxation times of water protons and therefore shorten the examination time and most importantly, result in an improved contrast of the images. The use of contrast agents has largely contributed to the spectacular development of MRI (1–7). Today, around 30–40% of all medical MR images are generated with the use of a contrast medium.

They are commonly described as T_1 - or T_2 -agents depending on whether the relative reduction in relaxation times caused by the agent is greater for the longitudinal (T_1) or transverse (T_2) relaxation. Signal intensity increases with increasing $1/T_1$ (a bright spot on T_1 -weighted images) and decreases with increasing $1/T_2$ (decreased brightness on T_2 -weighted images). Given that proton $T_1 \gg T_2$ for most tissues, usually the relative effect of the contrast agent on T_1 is larger than on T_2 . In MRI practice, T_1 agents are favored since a positive contrast is more easily detected than a negative one.

In 1948, Bloch, Hansen, and Packard reported first the use of a paramagnetic salt, ferric nitrate to enhance the relaxation rates of water protons (8). In 1987, Lauterbur *et al.* (9) applied a manganese(II) salt to distinguish between different tissues based on the differential relaxation times. The first commercial MRI

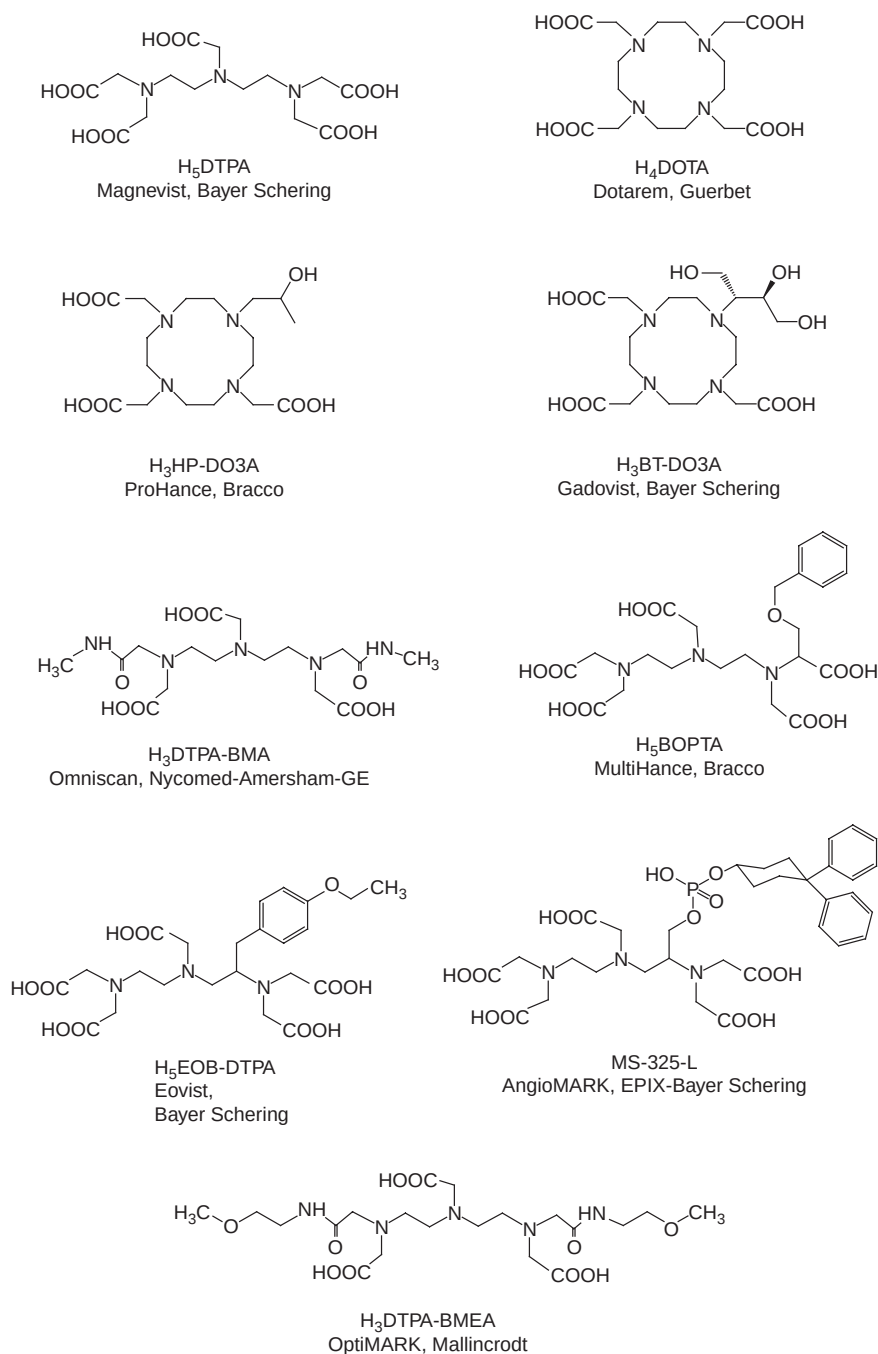
contrast material, $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$, was approved in 1988. It was soon followed by the macrocyclic $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ and various DTPA- or DOTA-derivatives (Scheme 1). Gadolinium(III) complexes, which belong to the family of T_1 agents, are the most widely used contrast media in clinical practice (around 95% of all contrast enhanced MRI examinations), with over 200 million injections so far. The free gadolinium ion, Gd^{III} , induces toxicity in many ways and cannot be injected into the blood. Gd^{III} has a tendency to form hydroxo complexes at physiological pH, it can also bind to donor groups of proteins, replace other metal ions in enzymes, or, due to its similar size to Ca^{II} (107.8 pm for Gd^{3+} vs. 114 pm for Ca^{2+}), it may interfere in Ca^{II} -regulated signal transmission processes. In order to circumvent toxicity, Gd^{III} is complexed with appropriate multidentate ligands, preferentially poly(aminocarboxylates), which are able to ensure a high thermodynamic stability and kinetic inertness of the complex.

The choice of Gd^{III} is explained by the seven unpaired electrons, which renders it the most paramagnetic stable metal ion and its relatively slow electronic relaxation, originating from a symmetric S-state. Nevertheless, other paramagnetic metal ions, in particular manganese(II) (five unpaired electrons), have also been considered as contrast agents, and one Mn^{II} chelate is commercialized for clinical use (10). Clinical agents today are all of low molecular weight, extracellular and non-specific. They are administered intravenously and distribute through the extracellular and intravascular spaces. Typical doses of the currently used, low molecular weight complexes are 0.1–0.3 mmol kg⁻¹ body weight, with a Gd^{III} concentration of the injected solution about 0.5 M.

The efficiency of a paramagnetic chelate to act as a contrast agent is expressed by its proton relaxivity, r_1 or r_2 , referring to the paramagnetic enhancement of the longitudinal or transverse water proton relaxation rate, $1/T_1$ and $1/T_2$, respectively, by a unity concentration of the agent (1 mM):

$$\frac{1}{T_{i,\text{obs}}} = \frac{1}{T_{i,d}} + \frac{1}{T_{i,p}} = \frac{1}{T_{i,d}} + r_i[\text{Gd}] \quad i = 1, 2 \quad (1)$$

The observed proton relaxation rate, $1/T_{i,\text{obs}}$ is the sum of a diamagnetic contribution, $1/T_{i,d}$, and the paramagnetic relaxation rate enhancement, $1/T_{i,p}$, which is linearly proportional to the concentration of the paramagnetic species, $[\text{Gd}]$. This relation is valid in dilute solutions and in the absence of intermolecular interactions between the contrast agent molecules. Given the



SCHEME 1. Ligands of the Gd^{III} complexes approved for clinical use as MRI contrast agents.

preponderance of T_1 -agents in practice, “proton relaxivity” most often refers to longitudinal relaxation (r_1).

In the last two decades considerable effort has been devoted to gain insight into the relationship between the structure and dynamics and the relaxivity of the Gd^{III} complexes. This understanding led to the rational development of novel, more efficient potential agents. In the first part of this chapter, we will survey the mechanisms that relate the relaxivity to the microscopic parameters of the chelate, and the mainstreams of efforts to increase longitudinal relaxivity for Gd^{III} complexes, involving probes optimized for imaging at high magnetic fields. In addition to high relaxivity, a potential contrast agent has to fulfill several additional requirements related to safety, non-toxicity, osmolality, biodistribution, elimination, etc. The physiological toxicity of a Ln^{III} poly(aminocarboxylate) complex depends mainly on the release of free Ln^{III} , controlled by its thermodynamic stability and kinetic inertness. These aspects will be also addressed. The second part of the chapter will be devoted to “new generation” MRI probes, discussing representative examples of responsive agents. We will also discuss paramagnetic chemical exchange saturation (PARACEST) agents, and review the recent results on Mn^{II} complexes in the context of MRI. Finally, we include a short overview of “non-classical” potential contrast agents such as gadofullerenes and carbon nanotubes.

II. Relaxivity of Gd^{III} Complexes: Optimization of the Relaxivity

The general theory of solvent nuclear relaxation in the presence of paramagnetic substances was described by Bloembergen, Solomon, Morgan, and others (11–16). During the last 10 years, it has been substantially reviewed in the context of MRI contrast agents (1–7,17,18).

The paramagnetic relaxation of the water protons originates from the dipole–dipole interactions between the proton nuclear spins and the fluctuating local magnetic field caused by the Gd^{III} unpaired electron spins. For Gd^{III} complexes, the proton relaxivity has two main contributions. (i) Inner sphere relaxivity arises from protons on the inner sphere water molecule(s), directly bound to the metal ion (Fig. 1). The paramagnetic effect is then transmitted to the bulk water via the exchange of these protons or of the entire water molecules. (ii) Bulk solvent molecules experience a paramagnetic effect also when they diffuse near the paramagnetic complex. The effect of this random translational diffusion is defined as outer sphere relaxation.

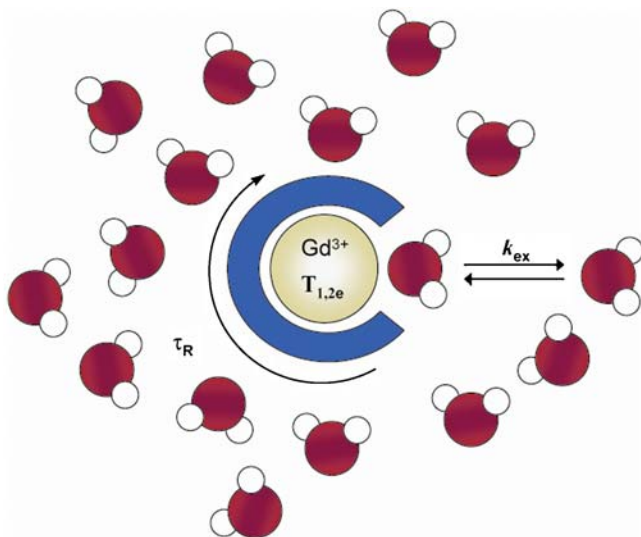


FIG. 1. Schematic representation of a Gd^{III} complex with one inner sphere water molecule, which is the origin of the inner sphere contribution to proton relaxivity. The complex is surrounded by bulk water, giving rise to the outer sphere relaxation mechanism.

The separation of inner and outer sphere terms is based on the intra- and intermolecular nature of the interaction, respectively. Water molecules may be also bound via hydrogen bridges to the ligand (e.g., to carboxylates or phosphonates) or to the inner sphere water. They give rise to a “second sphere” relaxivity term, described by the same theory as the inner sphere term, and which, in certain cases, represents a non-negligible contribution to the overall relaxivity. The contribution of the inner and outer sphere relaxation mechanisms to the overall relaxivity is around 50–50% for low molecular weight Gd^{III} -based contrast agents. The inner sphere term can be substantially improved by optimization of the influencing parameters via an appropriate ligand design, and then becomes preponderant.

A. INNER SPHERE PROTON RELAXIVITY

The longitudinal inner sphere relaxation rate, $1/T_1$, of bulk water protons is given by Eq. (2) (19):

$$\left(\frac{1}{T_1}\right)^{\text{IS}} = \frac{cq}{55.5} \frac{1}{T_{1\text{m}} + \tau_{\text{m}}} = P_{\text{m}} \frac{1}{T_{1\text{m}} + \tau_{\text{m}}} \quad (2)$$

where c is the molal concentration, q the number of bound water nuclei per Gd^{III} (hydration number), P_m the mole fraction of the bound water nuclei, τ_m the lifetime of a water molecule in the inner sphere (equal to the reciprocal water/proton exchange rate, $1/k_{\text{ex}}$), and $1/T_{1m}$ the relaxation rate in the bound water. The longitudinal relaxation of bound water protons originates from the magnetic field-dependent dipole–dipole mechanism, modulated by the reorientation of the molecule, the electron spin relaxation and the water/proton exchange. The relaxation rate is expressed by the modified Solomon–Bloembergen equation:

$$\frac{1}{T_{1m}} = \frac{2}{15} \frac{\gamma_I^2 g^2 \mu_B^2}{r_{\text{GdH}}^6} S(S+1) \left(\frac{\mu_0}{4\pi} \right)^2 \left[7 \frac{\tau_{c2}}{1 + \omega_s^2 \tau_{c2}^2} + 3 \frac{\tau_{c1}}{1 + \omega_I^2 \tau_{c1}^2} \right] \quad (3)$$

Here, γ_I is the nuclear gyromagnetic ratio, g the electron g factor, μ_B the Bohr magneton, r_{GdH} the electron spin–proton distance, and ω_I and ω_s are the nuclear and electron Larmor frequencies, respectively ($\omega = \gamma B$, where B is the magnetic field). The correlation times, τ_{ci} , are defined as $1/\tau_{ci} = 1/\tau_R + 1/T_{ie} + 1/\tau_m$ ($i = 1, 2$), where τ_R is the rotational correlation time, and T_{1e} and T_{2e} are the longitudinal and transverse electron spin relaxation times of Gd^{III} . The electronic relaxation rates, interpreted in terms of zero-field splitting (ZFS) interactions, also depend on the magnetic field (Bloembergen–Morgan theory of paramagnetic electron spin relaxation, valid if $\tau_v^2 \Delta^2 \ll 1$) (20):

$$\left(\frac{1}{T_{1e}} \right) = \frac{1}{25} \Delta^2 \tau_v \{ 4S(S+1) - 3 \} \left(\frac{1}{1 + \omega_S^2 \tau_v^2} + \frac{4}{1 + 4\omega_S^2 \tau_v^2} \right) \quad (4)$$

$$\left(\frac{1}{T_{2e}} \right) = \frac{1}{50} \Delta^2 \tau_v \{ 4S(S+1) - 3 \} \left(\frac{5}{1 + \omega_S^2 \tau_v^2} + \frac{2}{1 + 4\omega_S^2 \tau_v^2} + 3 \right) \quad (5)$$

In Eqs. (4) and (5), Δ^2 is the mean square ZFS energy and τ_v the correlation time for the modulation of the ZFS interaction, resulting from the transient distortions of the complex. The combination of Eqs. (3)–(5) constitutes a complete theory to relate the paramagnetic relaxation rate enhancement to microscopic properties (Solomon–Bloembergen–Morgan (SBM) theory) (21,22).

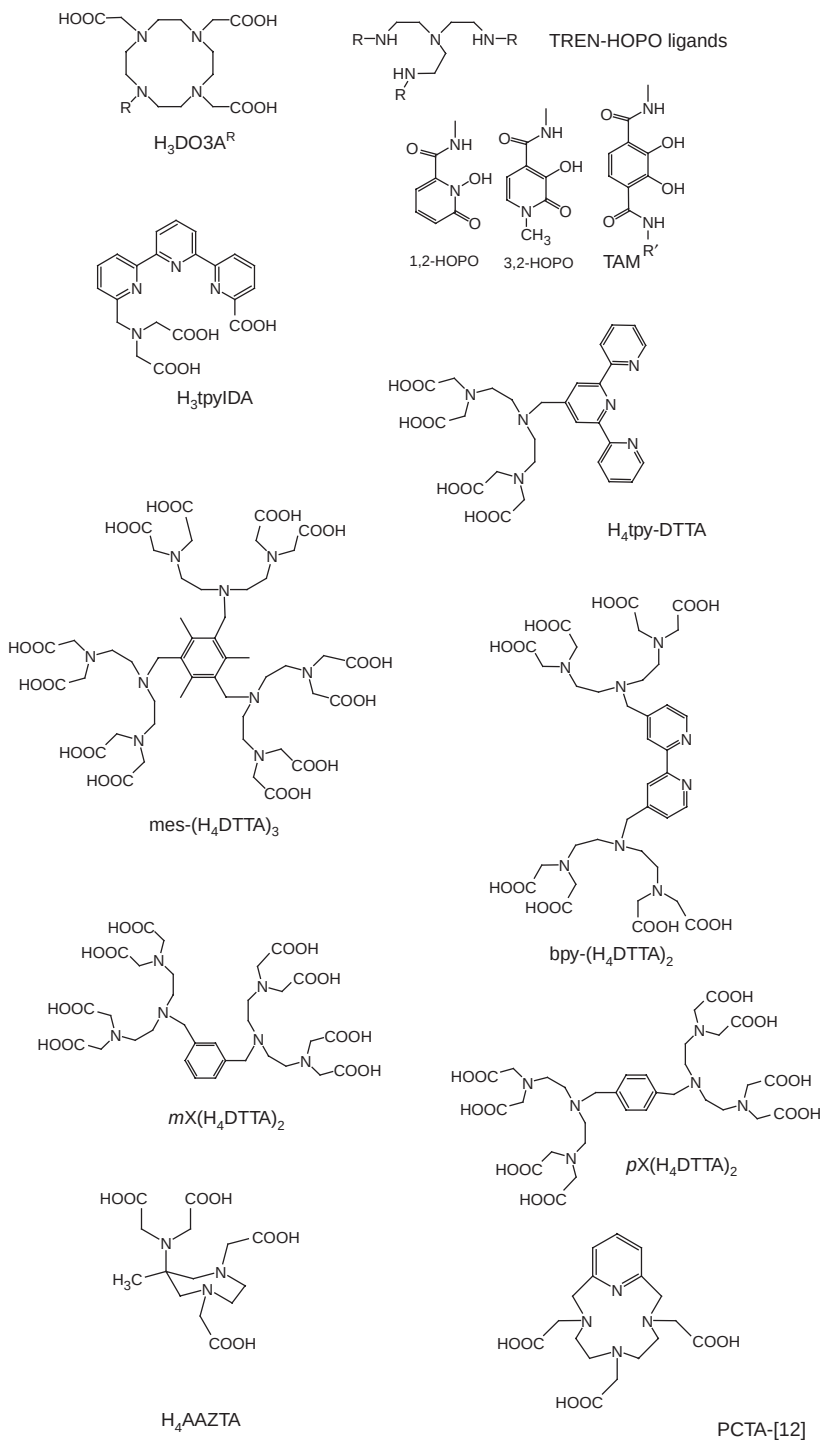
A.1. Hydration number: bishydrated Gd^{III} chelates as potential MRI contrast agents

The hydration state of lanthanide(III) chelates can be assessed by different techniques which have been reviewed in details (1,2).

Among these, luminescence lifetime measurements made in H_2O and D_2O are most widely used to determine the hydration number q for Eu^{III} and Tb^{III} chelates, via an empirical equation (23–26). ^{17}O NMR chemical shift measurements in solution of lanthanide(III) (most often Dy or Gd) complexes also give information on q (27). More recently, pulsed ^{17}O electron-nuclear double resonance (ENDOR) spectroscopy has been used for the direct determination of the water coordination number of gadolinium-based MRI contrast agents. Spectra of metal complexes in frozen aqueous solutions at approximately physiological concentrations were obtained either in the presence or absence of protein targets (28). This method is an improvement over the ^1H ENDOR method (29), which involved the difference ENDOR spectrum of exchangeable protons from spectra taken in H_2O and D_2O . In addition to exchangeable water protons, the ^1H ENDOR method is also sensitive to other exchangeable protons, thus it can overestimate hydration numbers for complexes with exchangeable protons at Gd–H distances similar to that of the coordinated water. The ^{17}O method does not suffer from this limitation. ^{17}O ENDOR spectroscopy was applied to Gd^{III} complexes containing zero, one, or two inner sphere water molecules.

All Gd^{III} chelates approved for clinical application are nine-coordinated with one inner sphere water molecule. As the inner sphere relaxivity is linearly proportional to the hydration number, bishydrated chelates would represent a clear advantage. Although the thermodynamic stability and kinetic inertness of bishydrated Gd^{III} complexes have often been considered to be insufficient to allow for human applications, there is an intensive research to identify stable chelates with two inner sphere water molecules. Bishydrated Gd^{III} chelates based on TREN-HOPO (hydroxypyridinonate, [Scheme 2](#)) derivatives have been reported to possess high thermodynamic stability, with stability constants, $\log K_{\text{ML}} = 19\text{--}21$ (30–32). However, their kinetic inertness can be expected to be considerably low. Aime *et al.* reported a novel heptadentate ligand, the 6-amino-6-methylperhydro-1,4-diazepine-1,4, N^6 , N^6 -tetraacetate (AAZTA) which also forms relatively stable bishydrated lanthanide complexes (33). Among bishydrated Gd^{III} complexes, those formed with the pyridine-containing 12-membered macrocycle, such as PCTA-[12], are likely kinetically to be the most inert (34).

Bishydrated complexes formed with diethylenetriamine tetraacetate (DTTA^{4-} , [Scheme 2](#)) ligands attached to various scaffolds have been also investigated (35–37). The stability constants determined for the Gd^{III} complexes formed with DTTA-chelators attached to a benzene or a bipyridine core were all in the range of

SCHEME 2. Ligands forming bishydrated Gd^{III} complexes.

$\log K_{\text{GdL}} = 17\text{--}19$, in comparison to $\log K_{\text{GdL}} = 22.5$ for $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$. This decrease of the stability would clearly prevent any human application. Some of these chelates have been injected to mice to perform *in vivo* imaging experiments and showed no apparent toxicity. The contrast agents were well tolerated by the animals; no side effects were observed during injection, immediately or days after the experiment (38,39).

Another important aspect to consider in relation with bishydrated chelates is the possible formation of ternary complexes in biological medium. The two water molecules can be replaced by small endogenous ligands such as carbonate, phosphate, citrate, or coordinating functions of proteins, which can seriously reduce the *in vivo* relaxivity of the compound. For instance, it has been directly proven by ENDOR measurements on a protein binding DO3A-type chelate that the number of inner sphere water molecules decreased from 2 to 0 on passing from phosphate-buffered saline to an HSA solution (29). In general, DO3A-type Ln^{III} chelates that have two water molecules in geminal positions tend to form ternary complexes with a variety of endogenous carboxylate donors (40). The position of the two inner sphere water molecules in the chelate is indeed primordial for the formation of ternary complexes. Ideally, ligands which offer planar coordination such as texaphyrins (41) can present vacant coordination sites at the opposite hemispheres of the plane defined by the ligand, avoiding therefore bidentate anion binding. Several of the non-macrocyclic bishydrated chelates show also resistance to anion binding. Among these, complexes in the HOPO family were proven not to be affected by various anions (42). DTTA-type Ln^{III} chelates show also little tendency to form ternary complexes. The high relaxation efficiency of $[\text{Gd}_3(\text{mes-DTTA}_3)(\text{H}_2\text{O})_6]^{3-}$ and the metallostar $\{\text{Fe}[\text{Gd}_2(\text{bpy-DTTA})(\text{H}_2\text{O})_2]_3\}^{4-}$ (Scheme 2) under *in vivo* conditions confirmed that the two inner sphere water molecules are not (or not substantially) replaced by endogenous anions or other potential donors from proteins, etc. (38,39). The disymmetric terpyridine based, bishydrated Eu-tpyIDA presented in Scheme 2 (43), and complexes of the AAZTA ligand (33) have been also found to be resistant to anion binding.

A.2. Gd–H distance

The Gd–H distance, r_{GdH} , which enters on the inverse sixth power into the expression of inner sphere relaxivity, remains a difficult parameter to obtain experimentally. Most often it has been estimated on the basis of Gd^{III} – coordinated water–oxygen

distances, determined by solid state X-ray analysis. Recently, ENDOR spectroscopy was used to assess directly r_{GdH} which was found to be around 3.1 Å for a series of eight- and nine-coordinate complexes, and independent of the ligand structure or complex charge (44). We should note that, due to the influence of the very large number of parameters on relaxivity, the determination of the Gd–H distance from the fit of NMRD profiles is not reliable.

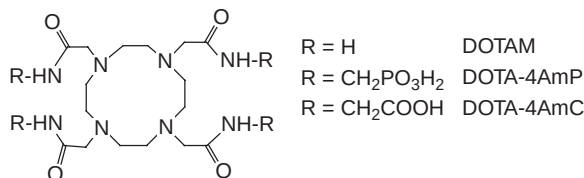
A.3. *Water/proton exchange: routes to accelerate water exchange on Gd^{III} complexes*

The proton exchange rate, k_{ex} , has a dual role in determining relaxivity. It governs the chemical exchange between the inner sphere and the bulk (Eq. (2)), and it also enters the overall correlation time, τ_c , that modulates the relaxation of inner sphere protons (Eq. (3)). Proton exchange can occur either via the exchange of the water molecules or independently (or both). For current Gd^{III}-based contrast agents, inner sphere protons exchange only via water exchange, at least at physiological pH. Nevertheless, on certain Gd^{III} complexes proton exchange can proceed considerably faster than water exchange (45–47).

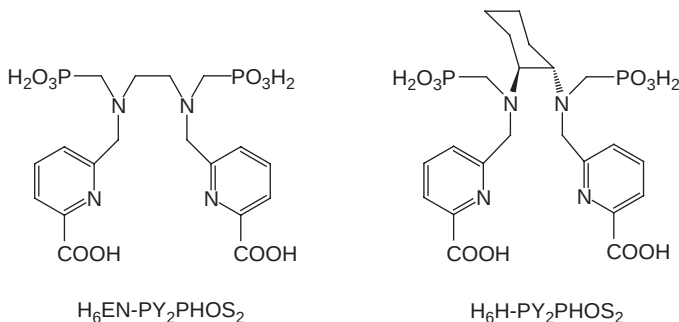
By now, water exchange has been studied on more than one hundred Gd^{III} complexes with the help of ¹⁷O NMR, and the large body of data available has been reviewed recently (48). Variable temperature ¹⁷O transverse relaxation rate measurements provide the rate of the water exchange, whereas the mechanism can be assessed by determining the activation volume, $\Delta V^\ddagger$, from variable pressure ¹⁷O T_2 measurements (49,50). The technique of ¹⁷O NMR has been described in detail (51).

Although water exchange rate constants reported for different Gd^{III} complexes cover a range of more than 5 orders of magnitude, from the lowest for tetraamide derivatives of DOTA such as [Gd(DOTA-4AmP)(H₂O)] ($k_{\text{ex}}^{298} = 4 \times 10^3 \text{ s}^{-1}$, Scheme 3) (52) to the highest for the aqua-ion ($k_{\text{ex}}^{298} = 8 \times 10^8 \text{ s}^{-1}$) (53) and for [Gd(EN-PY₂PHOS₂)(H₂O)_{0.6}][−] ($k_{\text{ex}}^{298} = 7.0 \times 10^8 \text{ s}^{-1}$; Scheme 4) (54), the medically relevant Gd^{III} chelates have similar water exchange rates. Table I shows water exchange rates for selected macrocyclic and linear Gd^{III} complexes.

In the inner sphere relaxation mechanism, first the paramagnetic ion has to relax the water protons which are in the first coordination sphere, and then the exchange of the relaxed water has to be fast enough to efficiently transmit the paramagnetic effect toward the bulk water. While the water exchange is usually not limiting relaxivity for the small molecular weight complexes, such as the contrast agents currently used in the clinics, it



SCHEME 3. Tetraamide derivatives of DOTA.



SCHEME 4.

becomes critical when the rotational motion is slow. Therefore, in recent years, much interest has been devoted to the modulation of water exchange rate on Gd^{III} complexes, and in particular to the search for chelates with optimal water exchange for MRI contrast agent applications. The optimal value of the water exchange rate to attain maximum proton relaxivities has been reported typically for the traditionally used MRI field strengths. At 20–60 MHz, this value is around $5\text{--}10 \times 10^7 \text{ s}^{-1}$. Nowadays higher and higher magnetic fields are entering the clinical practice and for experimental animal research in particular (400 MHz–9.4 T, or even higher) (55,56). The maximum relaxivities attainable at those high fields for Gd^{III} -based contrast agents are much lower than at 20 MHz (*vide supra*). This makes more important the optimization of the proton relaxation properties of Gd^{III} complexes, involving the optimization of the water exchange rate. It is noteworthy to examine how the optimal value of the water exchange rate to attain the maximum relaxivity is varying with the magnetic field strength. Fig. 2 shows calculated relaxivities as a function of the water exchange rate, for three different frequencies and two rotational correlation times. The optimal water exchange rate varies significantly with magnetic field, namely it shifts to considerably higher values with increasing magnetic field. In addition, it also depends

TABLE I

WATER EXCHANGE RATES OF SELECTED MONOHYDRATED Gd^{III} COMPLEXES

Ligands	$k_{\text{ex}}^{298}/10^6 \text{ s}^{-1}$	Refs.
[Gd(H ₂ O) ₈] ³⁺	804	(53)
DOTA	4.1	(61)
DO3A-bz-NO ₂	1.6	(63)
DOTAM	0.053 ^a	(265) ^a
DOTA-4AmP	0.015 ^a	(266) ^a
DO3A-Nprop	61	(65)
TRITA	270	(64)
DO3AP	71	(66)
DO3AP ^{OE} t	20	(67)
DTPA	3.3	(61)
BOPTA	3.45	(47)
EOB-DTPA	3.6	(60)
MP-2269	4.2	(69)
COPTA	3.4	(70)
DTPA-BMA	0.45	(61)
EPTPA	330	(64)
DTTA-Nprop	80	(65)
DTTA-N'prop	31	(64)

^aDetermined for the Eu^{III} complex.

on the rotational correlation time. At high frequencies, extremely fast water exchange is needed to reach maximum relaxivities.

The majority of the Gd^{III} poly(aminocarboxylate) complexes studied has been found to undergo a dissociatively activated water exchange, implying that the rate determining step is the leaving of the coordinated water molecule (48). In these processes, the negative charge of the complex and an increased steric crowding around the water-binding site have been identified as the two main factors to contribute to the acceleration of the water exchange. Inversely, the replacement of one negatively charged carboxylate in the complex with a neutral amide decreases the exchange rate to about one third. This empirical rule has been found valid for essentially all DTPA- and DOTA-amide complexes studied (57–63). While increasing the negative charge on the complex is not a viable route to optimize water exchange, the design of ligands ensuring steric compression has proved to be a successful strategy. Steric compression has been induced in the acyclic DTPA- or the macrocyclic DOTA-type complexes by the intercalation of an additional CH₂ group either in the amine backbone of the ligand (EPTPA, TRITA), or in the carboxylate arm (DTTA-N'prop, DTTA-Nprop; Scheme 5). While the elongation of the amine backbone results in an almost 2 orders of magnitude

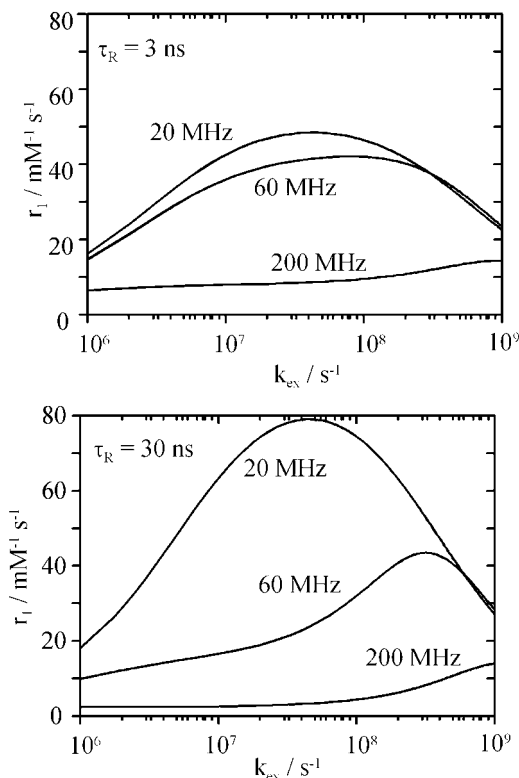
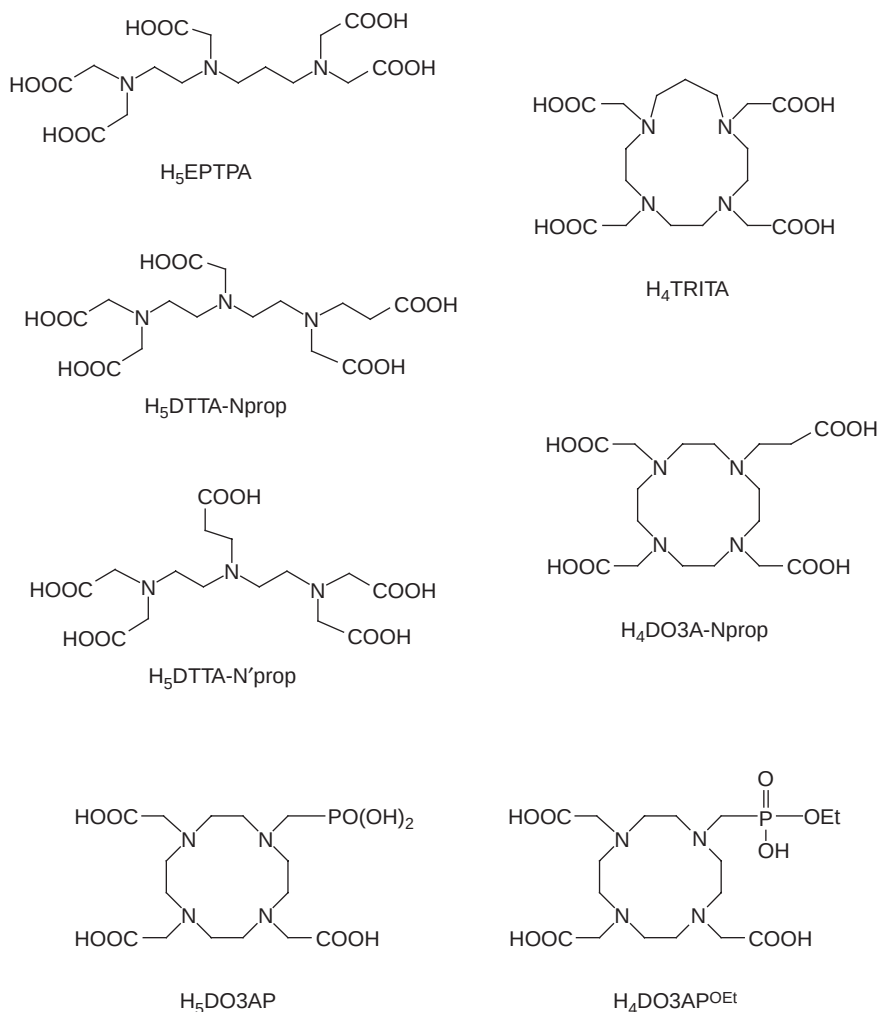


FIG. 2. Calculated relaxivities as a function of the water exchange rate for various proton Larmor frequencies and rotational correlation times, τ_R . The simulations have been performed by using the common Solomon–Bloembergen–Morgan theory of paramagnetic relaxation.

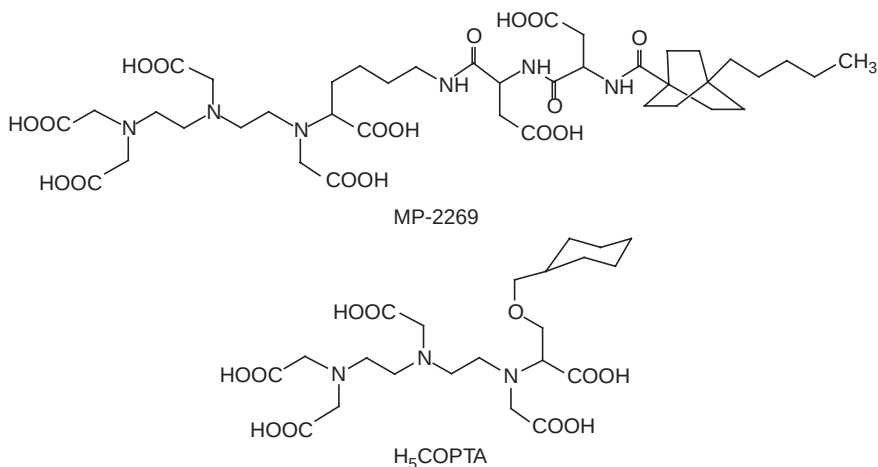
increase in the water exchange rate of the Gd^{III} complex in comparison to the parent $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ or $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$, with the propionate derivative ligands DTTA-Nprop, DO3A-Nprop we observe a moderate increase of the water exchange rate (Table I) (64,65). This demonstrates that by minor, appropriate changes in the ligand structure one can fine-tune the steric compression around the water-binding site in nine-coordinate, monohydrated Gd^{III} poly(amino carboxylate) complexes, which translates to the fine-tuning of the rate of water exchange. The introduction of one six-membered chelate ring in the complex always gives rise to an increased steric crowding. The steric crowding and the consequent acceleration of the water exchange depend more on the elongation of the amine backbone (EPTPA, TRITA) than on the elongation of the carboxylate pendant arm (DTTA-Nprop, DO3A-Nprop). The water exchange is also



SCHEME 5.

accelerated when an acetate function is replaced by a sterically more demanding phosphorus containing pendant arm, as it was illustrated by several examples for both macrocyclic and linear ligands (66,67).

We should note that the water exchange rate remains relatively constant on introducing different substituents on the ligand, which do not directly interfere in the inner coordination shell. Similar rates have been reported for the different bisamide DTPA derivatives (57,59,60,68). Even bulky substituents on the carbon backbone of the DTPA had limited influence on the water



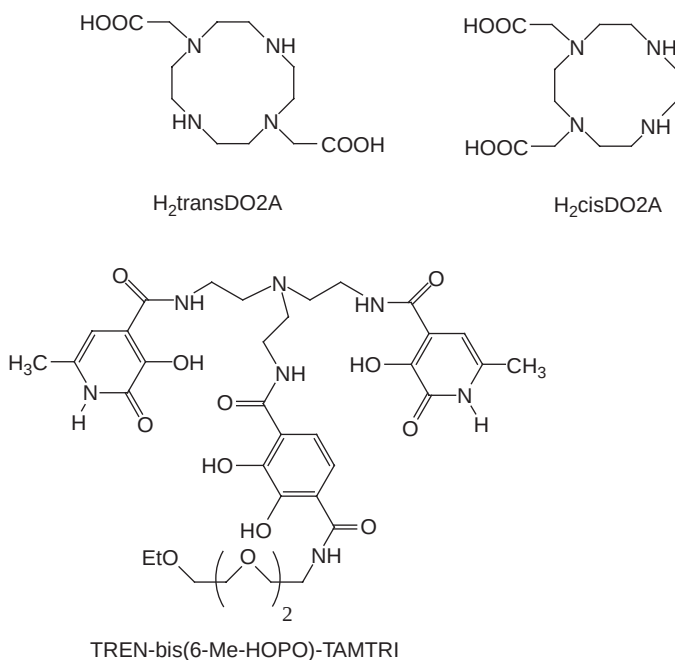
SCHEME 6.

exchange kinetics: k_{ex} is similar for the Gd^{III} complexes of DTPA, EOB-DTPA (Scheme 1), COPTA, and MP-2269 (Scheme 6) (60,61,69,70). We should mention that on DOTA-type complexes, the water exchange rate is strongly dependent on the relative abundance of the TSA/SA isomers, the TSA isomers having a much faster exchange (SA = square-antiprismatic; TSA = twisted square-antiprismatic). As an example, k_{ex}^{298} is 40 times higher on the TSA isomer of the Eu^{III} complex of DOTAM (Scheme 3) than on the SA analog (71). According to the same tendency, the water exchange rate monotonously decreases with decreasing abundance of the TSA isomer in a series of Gd^{III} DO3A-monophosphinate complexes (66,67,72).

The water exchange is not much affected when the Gd^{III} chelate is attached to a macromolecular scaffold. The rate and mechanism have been found to be similar for different generations (gen. 3, 4, and 5) of PAMAM dendrimers functionalized with the same Gd^{III} DO3A-monoamide chelate and for the monomer Gd^{III} DO3A-monoamide complex itself (63). Similar results have been obtained later on other dendrimeric systems (73,74), linear polymers (75–77), or micellar complexes (78–80). Non-covalent binding to a protein, however, can have an influence. Due to the interaction between the Gd^{III} chelate and the protein, the access to the water-binding site can be limited, and consequently, the rate of water exchange is significantly reduced (81,82). On the other hand, if the distance is relatively large between the metal- and the protein-binding site of the ligand, the water exchange is not affected by protein binding (69).

In general, bishydrated complexes have faster water exchange than monohydrated complexes, mainly related to the flexible nature of the inner sphere. For instance, DTTA-derived Gd^{III} complexes all have water exchange rates in the order of $k_{\text{ex}}^{298} = 8 - 9 \times 10^6 \text{ s}^{-1}$ (35–37) with respect to $k_{\text{ex}}^{298} = 3.3 \times 10^6 \text{ s}^{-1}$ on the monohydrated $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$. Likewise, $[\text{Gd}(\text{AAZTA})(\text{H}_2\text{O})_2]^-$ (Scheme 2) has a water exchange rate of $1 \times 10^7 \text{ s}^{-1}$ (33). The Gd^{III} complex formed with the hexadentate heterotripodal HOPO-type ligand, $[\text{Gd}(\text{TREN-bis}(6\text{-Me-HOPO})\text{-TAMTRI})(\text{H}_2\text{O})_2]$ (Scheme 7), has an even faster water exchange ($k_{\text{ex}}^{298} = 5.3 \times 10^7 \text{ s}^{-1}$), which is related to a complex with a coordination number of eight resulting in an associative exchange mechanism (83).

In contrast to these examples, one order of magnitude slower water exchange has been reported for the bishydrated MS325 derivative Gd^{III} complex, in which one acetate function of MS325 has been replaced by a methyl group, with respect to the parent MS325 (84). The exact reasons of this decrease of k_{ex} have not been identified; in addition to the reduced charge, the decreased stability of an eight-coordinate transition state in a dissociative mechanism has been hypothesized.



SCHEME 7.

An extremely fast exchange has been measured for Gd^{III} complexes formed with ligands based on ethane-1,2-diamine ($\text{EN-PY}_2\text{PHOS}_2$) or cyclohexane-1,2-diamine backbones ($\text{CH-PY}_2\text{PHOS}_2$) and containing pyridine units and phosphonate pendant arms (Scheme 4) (54). The water exchange rate on $[\text{Gd}(\text{EN-PY}_2\text{PHOS}_2)(\text{H}_2\text{O})_q]^{3-}$, $k_{\text{ex}}^{298} = 7.0 \times 10^8 \text{ s}^{-1}$, is comparable to that on the Gd^{III} aqua-ion, while it is slightly reduced for $[\text{Gd}(\text{CH-PY}_2\text{PHOS}_2)(\text{H}_2\text{O})_q]^{3-}$, $k_{\text{ex}}^{298} = 1.5 \times 10^8 \text{ s}^{-1}$. This very fast exchange was explained in terms of a hydration equilibrium (between non-hydrated and monohydrated species; $q = 0.6$ at 298 K for both complexes), and more importantly, of the very flexible inner coordination sphere around the metal ion. The hydration equilibrium itself does not necessarily result in a very fast water exchange on the complex, and other Gd^{III} complexes which also possess hydration equilibrium do not have extreme water exchange rates. For instance, $[\text{Gd}(\text{DO3A})(\text{H}_2\text{O})_q]$ and $[\text{Gd}(\text{transDO2A})(\text{H}_2\text{O})_q]^+$ both have differently hydrated species in aqueous solution ($q_{\text{ave}} = 1.8$ and 2.8, respectively), but they show only a limited increase of the water exchange rate as compared to the monohydrated $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ ($k_{\text{ex}}^{298} = 11 \times 10^6 \text{ s}^{-1}$ for $[\text{Gd}(\text{DO3A})(\text{H}_2\text{O})_q]$ and $10 \times 10^6 \text{ s}^{-1}$ for $[\text{Gd}(\text{transDO2A})(\text{H}_2\text{O})_q]^+$) (Scheme 7; 85). In addition to the decreased negative charge, which has a “slowing” effect in dissociatively activated mechanisms, the rigidity of the inner sphere of these macrocyclic complexes is the main factor responsible for the limited increase in k_{ex}^{298} . The inner sphere structure is always less rigid for complexes of non-macrocyclic ligands. The rigidity of the inner sphere is a very important factor in determining the water exchange rate. A direct illustration of this phenomenon is the diminution of k_{ex} by a factor of 5 from $[\text{Gd}(\text{EN-PY}_2\text{PHOS}_2)(\text{H}_2\text{O})_q]^{3-}$ to $[\text{Gd}(\text{CH-PY}_2\text{PHOS}_2)(\text{H}_2\text{O})_q]^{3-}$ related to the introduction of the cyclohexyl ring in the amine backbone in $\text{CH-PY}_2\text{PHOS}_2$ which rigidifies the structure.

A.4. Rotational dynamics: application of the Lipari–Szabo approach to describe internal flexibility of macromolecular chelates

The optimization of the rotational motion of Gd^{III} complexes in the objective of increasing their proton relaxivity has been in the center of very intensive research in the last two decades. At intermediate magnetic fields typically used in the clinics (20–60 MHz), the SBM theory predicts a significant relaxivity increase with respect to small molecular weight chelates when the rotation is slowed down. In this perspective, a large number of macromolecular chelates have been investigated. The three

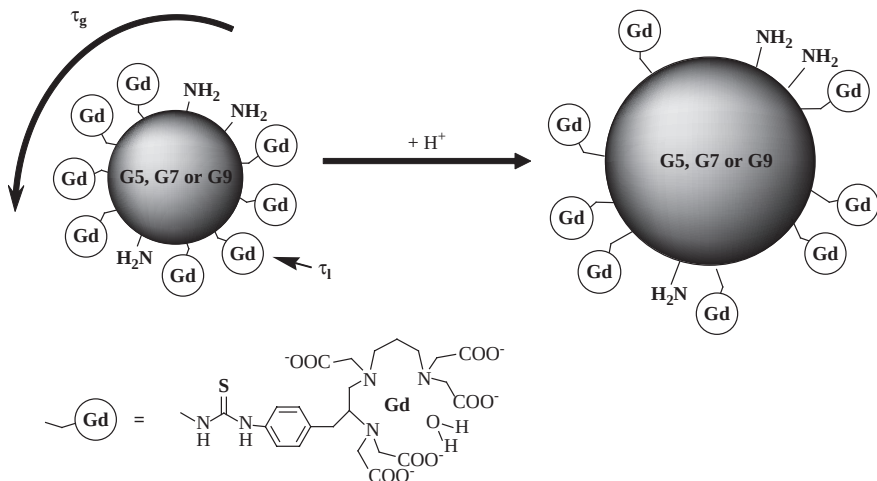
main strategies involve (i) covalent binding of the Gd^{III} complex to a macromolecular scaffold, such as dendrimers, linear polymers, etc., (ii) exploiting non-covalent interactions between an appropriate ligand and a macromolecule, mainly the endogenously abundant serum albumin, and (iii) approaches based on self-assembly, including metal-templated self-assembly and micellar structures. These macromolecular systems and their relaxivity properties have been reviewed in the last years (1,2,4,7). Here, instead of a systematic overview, we will rather discuss the description of the rotational dynamics of high molecular weight Gd^{III} complexes by using the “model-free” Lipari–Szabo approach. We will illustrate via a few examples how the Lipari–Szabo analysis of the longitudinal ^{17}O and ^1H relaxation rates can be particularly useful to gain insight into the relation between structure and relaxation behavior of macromolecular systems.

While the assumption of an isotropic rotational motion is reasonable for low molecular weight chelates, macromolecules have anisotropic rotation involving internal motions. In the Lipari–Szabo approach, two kinds of motion are assumed to affect relaxation: a rapid, local motion, which lies in the extreme narrowing limit and a slower, global motion (86,87). Provided they are statistically independent and the global motion is isotropic, the reduced spectral density function can be written as:

$$J(\omega) = \left(\frac{S^2 \tau_g}{1 + \omega^2 \tau_g^2} + \frac{(1 - S^2) \tau}{1 + \omega^2 \tau^2} \right) \quad \tau^{-1} = \tau_g^{-1} + \tau_l^{-1} \quad (6)$$

where τ_g is a correlation time for the global motion, common to the whole molecule, τ_l the correlation time for the fast local motion, specific for the individual relaxation axis which is related to the motion of the Gd^{III} chelate unit. The generalized order parameter, S^2 , is a model-independent measure of the degree of spatial restriction of the local motion, $S^2 = 0$ if the internal motion is isotropic; $S^2 = 1$ if the motion is completely restricted. This analysis has then been applied to a series of macromolecular Gd^{III} complexes, including linear polymers (74–77), dendrimers (73,88), micellar systems (79,80), protein bound chelates (89), or self-assemblies (35).

The Lipari–Szabo approach was used to understand the cause of the pH-dependent relaxivities of PAMAM-type dendrimeric Gd^{III} complexes (74). Three different generations (5,7,9) of PAMAM dendrimers loaded with the $[\text{Gd}(\text{EPTPA})(\text{H}_2\text{O})]^{2-}$ chelate via a benzyl-thiourea linkage have been investigated (Scheme 8). The relaxivities show a strong and reversible pH dependency for all three dendrimer complexes,



SCHEME 8. Generation 5, 7, and 9 PAMAM dendrimers bearing $[\text{Gd}(\text{EPTPA})(\text{H}_2\text{O})]^-$ complexes. The increase of relaxivity with decreasing pH is related to the protonation of the internal amines which, via electrostatic repulsion, leads to an increasing size of the dendrimer.

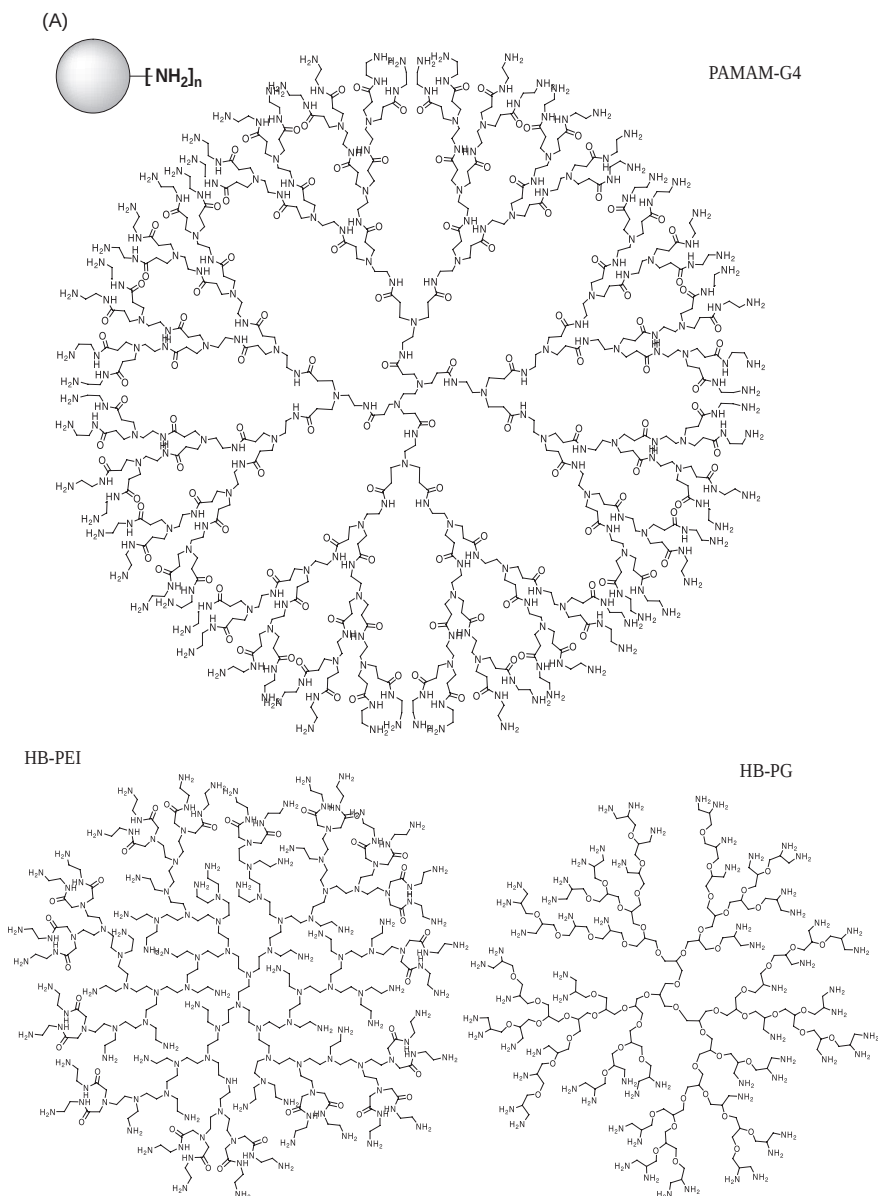
$\text{G5-}[\text{Gd}(\text{EPTPA})(\text{H}_2\text{O})]_{111}$, $\text{G7-}[\text{Gd}(\text{EPTPA})(\text{H}_2\text{O})]_{253}$, and $\text{G9-}[\text{Gd}(\text{EPTPA})(\text{H}_2\text{O})]_{1157}$ (r_1 increases by 50–60% from pH 10.8 to 6.2). It has been evidenced by ^{17}O NMR studies that neither the hydration state of the dendrimer complex nor the exchange rate of the coordinated water changes with pH. On the other hand, the pH-invariant behavior of the monomeric complex clearly indicated that the pH effect observed on the longitudinal proton and ^{17}O relaxation rate of the dendrimer complexes is linked to the PAMAM skeleton. A combined variable temperature and multiple field ^{17}O NMR and ^1H relaxivity study has been performed at pH 6.0 and 9.9 on $\text{G5-}[\text{Gd}(\text{EPTPA})(\text{H}_2\text{O})]_{111}$ which proved that the pH dependency of the relaxivity originates from the pH-dependent rotational dynamics of the dendrimer scaffold itself. The longitudinal ^{17}O and ^1H relaxation rates have been analyzed by the Lipari–Szabo spectral density functions and correlation times have been calculated for the global motion of the entire macromolecule (τ_g) and the local motion of the Gd^{III} chelates on the surface (τ_l), correlated via the order parameter S^2 . The dendrimer complex $\text{G5-}[\text{Gd}(\text{EPTPA})(\text{H}_2\text{O})]_{111}$ has a considerably higher τ_g under acidic than under basic conditions ($\tau_g^{298} = 4040$ and 2950 ps, respectively), while local motions are less influenced by pH ($\tau_l^{298} = 150$ and 125 ps). The order parameter, characterizing the rigidity of the macromolecule, is also higher at pH 6.0 than pH 9.9 ($S^2 = 0.43$ vs. 0.36). The pH dependence of the global

correlation time was related to the protonation of the tertiary amine groups in the PAMAM skeleton, which leads to an expanded and more rigid dendrimeric structure at lower pH. The increase of τ_{gO} with decreasing pH is responsible for the pH-dependent proton relaxivities.

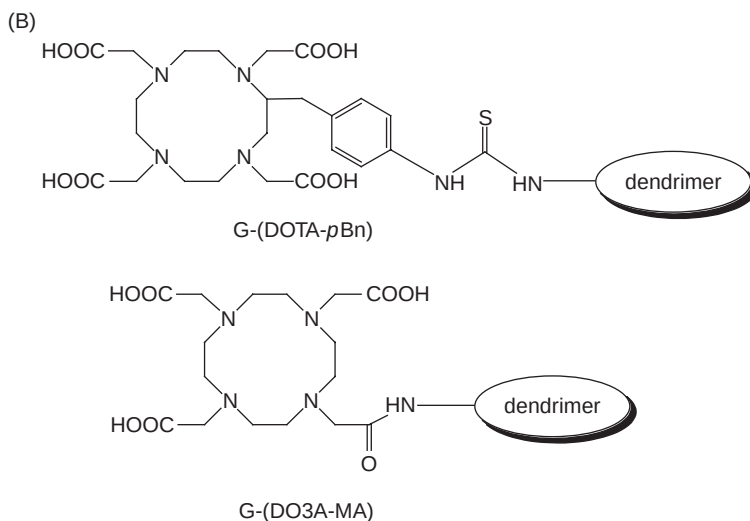
In another example, a series of generation 4 PAMAM dendrimers and hyperbranched (HB) dendrimeric structures loaded with macrocyclic Gd^{III} chelates on their surface have been described with respect to their rotational dynamics by using the Lipari–Szabo approach (90). The Gd^{III} was complexed either by the tetraaza-tetracarboxylate DOTA unit (DOTA-*p*Bn) or by the tetraaza-tricarboxylate-monoamide DO3A-MA chelator (Scheme 9). More than twice as high proton relaxivities have been found at frequencies below 200 MHz for the dendrimers loaded with the negatively charged $[\text{Gd}(\text{DOTA-}p\text{Bn})(\text{H}_2\text{O})]^-$ in comparison to the dendrimeric complex bearing the neutral $[\text{Gd}(\text{DO3A-MA})(\text{H}_2\text{O})]$ moieties (Table II). The analysis of the field-dependent proton relaxivities in terms of local and global rotational motion allowed the conclusions that it is almost exclusively the different rotational dynamics, which are responsible for the different proton relaxivities (Table II). The slower rotation of the $[\text{Gd}(\text{DOTA-}p\text{Bn})(\text{H}_2\text{O})]^-$ -loaded dendrimers, and the consequently higher relaxivity was related to a negative charge of the complex which creates more rigidity and increases the overall size of the macromolecule as compared to the dendrimer loaded with the neutral $[\text{Gd}(\text{DO3A-MA})(\text{H}_2\text{O})]$ complex. On the other hand, PEGylation of the dendrimers did not influence the rotational dynamics, and consequently the proton relaxivity. HB dendrimers have been proven to be as good macromolecular scaffolds for Gd^{III} complexes with respect to proton relaxivity as the regular PAMAM dendrimers.

We should note that the use of the Lipari–Szabo analysis implies that relaxation data are available at multiple magnetic fields. It provides a phenomenological description of the rotational motion that can be very useful for comparing systems with similar structure. Nevertheless, one should be aware of the limits of this approach and avoid direct comparison of local or global rotational correlation times for structurally very different compounds.

The above examples of the dendrimeric Gd^{III} complexes clearly illustrate how flexibility of the macromolecule is important in limiting proton relaxivity. This flexibility can originate from the intrinsic flexibility of the macromolecule itself, or/and from the non-rigid coupling of the chelate to the dendrimer surface. In both cases, the surface chelate benefits only partially from the slow motion of the dendrimer (or other macromolecule). In order



SCHEME 9. (A) Different dendrimers as macromolecular scaffolds for MRI contrast agents: ethylenediamine cored polyamido amine, generation 4 (PAMAM G4), top; hyperbranched, ethylenediamine cored polyethylene imine (HB-PEI), bottom left; hyperbranched, amino functionalized polyglycerol (HB-PG), bottom right. (B) Different moieties attached to the respective dendrimers via amide or thiourea bonds.



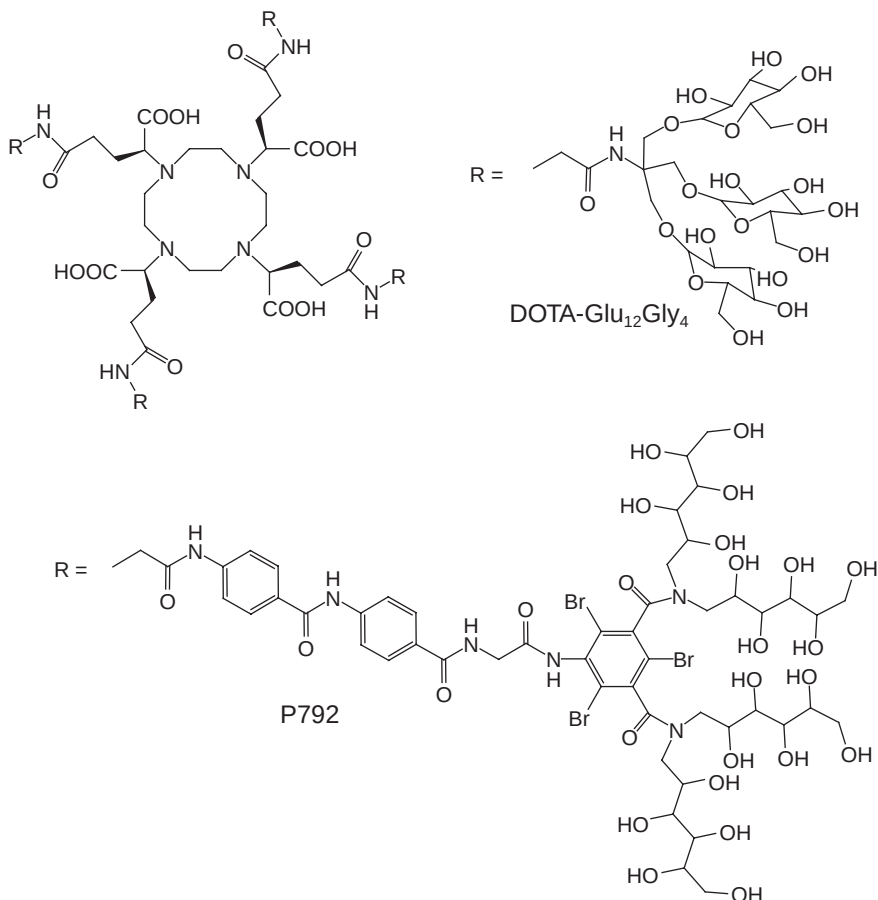
SCHEME 9. (Continued).

TABLE II

PARAMETERS OBTAINED FOR THE MACROMOLECULAR Gd^{III} COMPLEXES BY FITTING THE ^1H NMRD DATA WITH THE USE OF THE LIPARI-SZABO APPROACH (90)

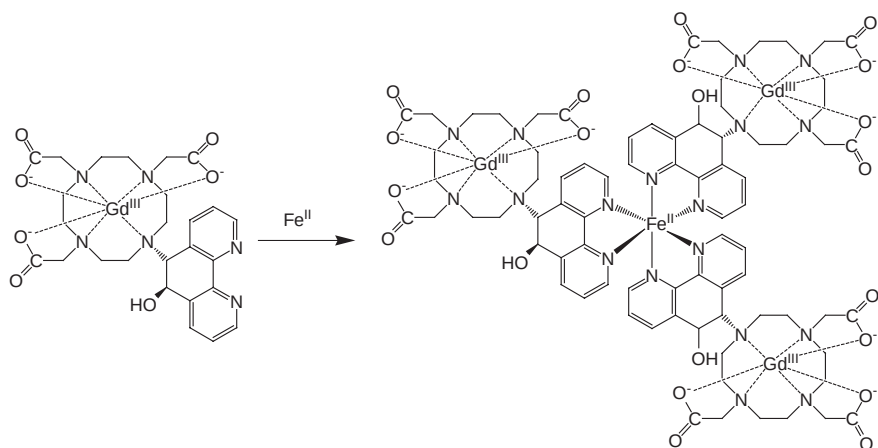
Dendrimers	$k_{\text{ex}}^{298}/10^6 \text{ s}^{-1}$	τ_g^{298}/ps	τ_1^{298}/ps	S^2	$r_1 (20 \text{ MHz})/\text{mM}^{-1} \text{ s}^{-1}$
PAMAM-G4-[Gd(DOTA- <i>p</i> Bn)(H ₂ O)] ₃₃ [−]	6.6	3100	550	0.39	31.2
PAMAM-G4-[Gd(DO3A-MA)(H ₂ O)] ₃₁ [−]	3.4	970	140	0.33	13.2
PAMAM-G4-PEG-[Gd(DOTA- <i>p</i> Bn)(H ₂ O)] ₃₈ [−]	6.6	2400	620	0.43	30.2
HB-PEI-[Gd(DOTA- <i>p</i> Bn)(H ₂ O)] ₅₂ [−]	6.6	3800	390	0.39	34.2
HB-PG-[Gd(DOTA- <i>p</i> Bn)(H ₂ O)] ₆₈ [−]	6.6	4000	530	0.36	34.2

to take full advantage of the slow tumbling of the macromolecule, the Gd^{III} ion can be placed into the barycenter of the molecule. The success of this approach is nicely demonstrated with $[\text{Gd}(\text{DOTA-Glu}_{12}\text{Gly}_4)(\text{H}_2\text{O})]^-$ (91,92) or with P792 (93–96), the latter being currently under clinical trials (Scheme 10). In both cases, a GdDOTA^- unit is in the center of the molecule, to which bulky and strongly hydrophilic groups are attached. The $\text{Gd-H}_{\text{water}}$ vector cannot rotate independently of the entire macromolecule which leads to a very important increase in relaxivity ($r_1 = 39.0 \text{ mM}^{-1} \text{ s}^{-1}$ for P792 at 37°C, 20 MHz, in comparison to $4.74 \text{ mM}^{-1} \text{ s}^{-1}$ for $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$).



SCHEME 10.

Another promising way of reducing flexibility is based on self-assembly between metal ions and appropriate ligands. The first system was proposed by Desreux *et al.* (97), where the [Gd(PhenHDO3A)(H₂O)_q] (Scheme 11) features two complexing units of totally different nature. The macrocyclic-binding site forms highly stable lanthanide chelates, while the phenanthroline-like unit is a good complexing agent of Fe^{II}. The [Gd(PhenHDO3A)(H₂O)_q] chelate spontaneously associates with Fe^{II} to form a tris-complex of higher molecular weight giving rise to a more than three-fold relaxivity increase (from 3.7 mM⁻¹s⁻¹ to 12.2 mM⁻¹s⁻¹; 20 MHz, 37°C). The group in Lausanne reported a poly(aminocarboxylate)-functionalized terpyridine ligand (tpy-DTTA) with distinct binding sites for Fe^{II} and Gd^{III} (Scheme 2)



SCHEME 11.

(98,99). In aqueous solution and in the presence of these metals, the ligand is capable of self-assembling to a rigid supramolecular structure, $\{\text{Fe}^{\text{II}}[\text{Gd}(\text{tpy-DTTA})(\text{H}_2\text{O})_2]_2\}$. In the heteroditopic ligand tpy-DTTA^{4-} , the central amine nitrogen of the Gd^{III} binding poly(aminocarboxylate) unit is directly linked to the Fe^{II} binding terpyridine part. This design efficiently eliminates internal flexibility, and largely contributes to the remarkable rigidity of the self-assembled molecule. Unfortunately, the direct linkage of the aromatic ring to the amine nitrogen reduces the basicity of the amine, resulting in a large decrease of the thermodynamic stability of the Gd^{III} complex. The heteroditopic ligand, bpy-DTTA_2 has a 2,2'-bipyridine moiety for specific Fe^{II} , and two poly(aminocarboxylates) for Gd^{III} binding which are bound to the bipyridine core via a CH_2 linkage to improve the stability of the lanthanide complex (Scheme 2). The ligand self-assembles with Fe^{II} and Gd^{III} to a metallostear $\{\text{Fe}[\text{Gd}_2(\text{bpy-DTTA})(\text{H}_2\text{O})_2]_3\}^{4-}$ structure (Fig. 3) with exceptionally high relaxivity both per molecule and per Gd ($r_1 = 27.0 \text{ mM}^{-1} \text{ s}^{-1}$; 298 K; 20 MHz). In addition to the rigid supramolecular scaffold, the two inner sphere water molecules with an exchange rate close to optimal are also important for the remarkable relaxivity. The presence of six efficiently relaxing Gd^{III} centers within one metallostear leads to an exceptionally high relaxivity confined to a small molecular space (high density of relaxivity). Nevertheless, flexibility is not entirely eliminated in the metallostear structure; the local motion of the Gd-coordinated water proton vector could be separated from the overall motion of the molecule, and is found to be ~ 5 times faster than the tumbling of the entire metallostear

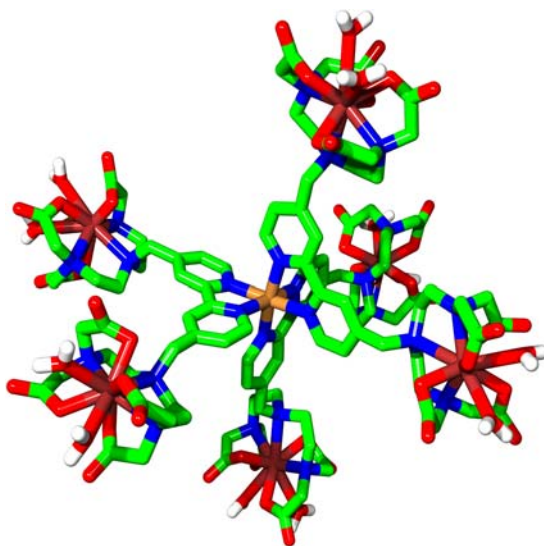


FIG. 3. Structure of the metallosstar $\{\text{Fe}[\text{Gd}_2(\text{bpy}-\text{DTTA}_2)(\text{H}_2\text{O})_4]_3\}^{4-}$ formed by self-assembly.

($\tau_g^{298} = 930$ ps vs. $\tau_l^{298} = 190$ ps). In the metallosstar, the $\text{Fe}(\text{bpy})_3$ core can be considered as fully rigid. This will also remain valid for extended, metallodendrimeric edifices, which is one clear advantage of such self-assembled systems. The only point of flexibility in the metallosstar is the methylene group connecting the bipyridine moiety to the poly(aminocarboxylate) part, which was introduced in order to maintain a sufficient thermodynamic stability.

A.5. Electronic relaxation

Electronic relaxation is a crucial and difficult issue in the analysis of proton relaxivity data. The difficulty resides, on the one hand, in the lack of a theory valid in all real conditions, and, on the other hand, by the technical problems of independent and direct determination of electronic relaxation parameters. At low fields (below 0.1 T), electronic relaxation is fast and dominates the correlation time τ_c in Eq. (3), however, at high fields its contribution vanishes. The basic theory of electron spin relaxation of Gd^{III} complexes, proposed by Hudson and Lewis, uses a transient ZFS as the main relaxation mechanism (100). For complexes of cubic symmetry Bloembergen and Morgan developed an approximate theory, which led to the equations generally

used in the context of electronic relaxation of Gd^{III} complexes (15). A similar approximate treatment was used by Powell *et al.* to describe temperature and magnetic field dependence of T_{2e} (101). Later, Rast *et al.* developed a refined model of electronic relaxation of the S states of metal complexes in solution (102,103). Beside the usual transient crystal ZFS caused by vibration, intramolecular rearrangement and collision, this model includes a contribution of the static crystal field surrounding the Gd^{III} which is modulated by random rotation of the complex. The approach is able to provide a complete and satisfactory description of the full EPR line shapes over a wide range of magnetic fields and temperatures. It also allows a calculation of longitudinal electronic relaxation rates relevant to nuclear magnetic relaxation. A limitation of this model is the Redfield's approximation used in the line shape calculation, which is inadequate for slowly rotating complexes and for low magnetic fields. Recently this model has been extended beyond the electronic Redfield limit using Monte Carlo simulations (104,105). The application of this theory in a combined analysis of ^{17}O NMR, ^1H NMRD, and EPR data is not straightforward and needs EPR spectra over a wide frequency and temperature range. Therefore, the common Bloembergen–Morgan theory continues to be applied in current research to generate electron spin relaxation rates, though one should be aware of its limitations and renounce the theory in discussing the resulting electronic parameters τ_v and Δ^2 .

B. SECOND AND OUTER SPHERE RELAXIVITY

Outer sphere relaxation arises from the dipolar intermolecular interaction between the water proton nuclear spins and the gadolinium electron spin whose fluctuations are governed by random translational motion of the molecules (106). The outer sphere relaxation rate depends on several parameters, such as the closest approach of the solvent water protons and the Gd^{III} complex, their relative diffusion coefficient, and the electron spin relaxation rate (107–109). Freed and others (110–112) developed an analytical expression for the outer sphere longitudinal relaxation rate, $(1/T_1)^{\text{OS}}$, for the simplest case of a force-free model. The force-free model is only a rough approximation for the interaction of outer sphere water molecules with Gd^{III} complexes.

Phosphonate or carboxylate groups of the ligand are capable of tightly binding water molecules that cannot be treated any more using only translational diffusion. For such cases, a second sphere contribution (r_1^{2nd}) has to be added to the overall proton

relaxivity (113,114):

$$r_1 = r_1^{\text{IS}} + r_1^{2\text{nd}} + r_1^{\text{OS}} \quad (7)$$

where r_1^{IS} is the inner sphere relaxivity and r_1^{OS} represents the contribution from water molecules diffusing in the proximity of the paramagnetic complex. $r_1^{2\text{nd}}$ can be calculated with equations similar to those applied for first sphere relaxation.

C. CONTRAST AGENTS OPTIMIZED FOR APPLICATION AT HIGH MAGNETIC FIELD

In MRI, the amount of available signal is inextricably associated with the static magnetic field strength. Higher field strength offers considerable improvement in the signal-to-noise ratio (proportional to $B_0^{7/4}$ at mid-fields, with more linear dependence at high fields), which translates to an increased spatial and temporal resolution. In addition, T_1 relaxation times of gray and white matter also increase with field strength (~ 30 – 40% between 1.5 T and 3 T), thus at high field the uptake of a contrast agent will result in a more significant shortening of T_1 . This can allow for decreasing the contrast agent dose to generate the same contrast. Indeed, the image contrast was similar at 1.5 T and at 3 T using only a half-dose of GdDTPA (115). Until recently, common clinical scanners operated at ≤ 1.5 T. Due to recent improvements in magnet design, 3 T scanners became widely available in the clinics, and for experimental animal studies, much higher fields (≥ 9.4 T; 400 MHz) enter everyday practice (55,56).

We have seen that in the last two decades much effort has been devoted to the optimization of all factors that determine relaxivity on Gd^{III} complexes with the objective of increasing the efficiency of contrast agents. In particular, slowing down the rotation by using macromolecules resulted in a remarkable relaxivity improvement at intermediate frequencies (20–60 MHz) as compared to commercial agents (1–7). For macromolecular agents, however, above 60 MHz r_1 drops sharply with increasing magnetic field and, at high frequencies (above 100 MHz), they are hardly superior to small chelates. The optimization of the relaxivity at high magnetic field requires the fine-tuning of the microscopic parameters of the Gd^{III} complex to optimal values, which are different from those at intermediate fields. Namely, as the SBM theory predicts, at proton Larmor frequencies above 200 MHz r_1 increases with the inverse rotational correlation time $1/\tau_R$, in contrast to lower frequencies, where it is proportional to

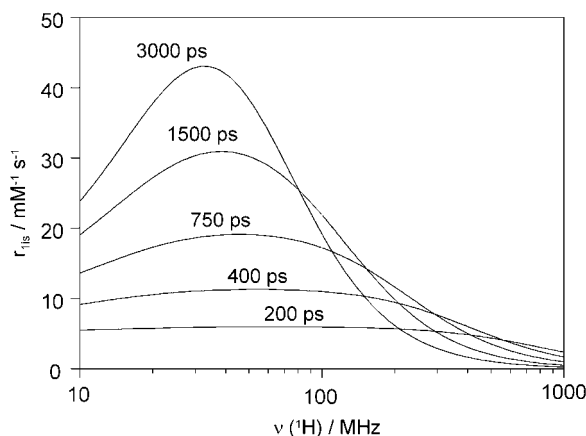


FIG. 4. Inner sphere contribution to the proton relaxivity as a function of the proton Larmor frequency. The curves were calculated on the basis of the Solomon–Bloembergen–Morgan theory for different values of the rotational correlation time, τ_R , and $q = 1$, $k_{\text{ex}} = 10 \times 10^6 \text{ s}^{-1}$, $\tau_v = 20 \text{ ps}$, $\Delta^2 = 0.1 \times 10^{20} \text{ s}^{-2}$.

τ_R (Fig. 4). Thus at high frequencies, intermediate-size, rigid molecules are favored over large ones, with an optimal τ_R of $\sim 400 \text{ ps}$ at 400 MHz (the exact value of the optimal rotational correlation time will also be dependent on the other influencing parameters). One peculiarity of high-field optimization is that the optimal rotational correlation time is very sensitive to the magnetic field, but remains, nevertheless, in the range of 400 – 1000 ps . The optimal value of the water exchange rate will also be different (and considerably higher) from that for current clinical fields (Fig. 2; 54). The development of contrast agents specifically designed for high-field applications is an emerging domain, and so far only a few dedicated, potential agents have been reported (35–37,99,116,117). High-field relaxivity values of some of them, in comparison to those of commercial agents, are reported in Table III. Their common feature is that they have an “intermediate size” and a relatively rigid structure.

In particular, the self-assembled metallostare system, $\{\text{Fe}[\text{Gd}_2(\text{bpy-DTTA}_2)(\text{H}_2\text{O})_4]_3\}^{4-}$, has remarkable relaxivity at high magnetic fields. MRI studies conducted at 4.7 T in mice have confirmed that the metallostare displays approximately four times higher *in vivo* relaxivity than the commercially available $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ (38). $[\text{Gd}_3(\text{mes-DTTA}_3)(\text{H}_2\text{O})_6]^{3-}$ contains three DTTA units covalently linked to a trimethyl-benzene core and has a smaller size than the metallostare. It displays considerably high

TABLE III

HIGH FIELD RELAXIVITIES OF SELECTED Gd^{III} COMPLEXES

$r_1/\text{mM}^{-1}\text{s}^{-1}$	Refs.	200 MHz		400 MHz	
		25°C	37°C	25°C	37°C
$[\text{Gd}_3(\text{mes-DTTA}_3)(\text{H}_2\text{O})_6]^{3-}$	(37)	17.0	14.1	10.7	10.2
$\{\text{Fe}[\text{Gd}_2(\text{bpy-DTTA}_2)(\text{H}_2\text{O})_4]_3\}^{4-}$	(35)	16.4	15.9	9.32	8.53
$[\text{Gd}_2(m\text{X}(\text{DTTA})_2)(\text{H}_2\text{O})_4]^{2-}$	(36)	13.4	10.2	9.18	8.12
$[\text{Gd}_2(p\text{X}(\text{DTTA})_2)(\text{H}_2\text{O})_4]^{2-}$	(36)	14.4	11.9	10.7	9.59
$[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$	(37)	4.02	3.04	3.86	2.95
$[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$	(37)	4.22	3.20	4.06	3.13

relaxivities, although at medium field they are still limited by fast rotation. An important characteristic of the trinuclear complex is its exceptionally broad relaxivity hump centered around 30–60 MHz. In fact, the relaxivity is almost constant between 20 MHz ($15.7\text{ mM}^{-1}\text{s}^{-1}$, 37°C) and 200 MHz ($14.1\text{ mM}^{-1}\text{s}^{-1}$, 37°C).

III. Stability Aspects

When a Gd^{III} complex is injected in the body, it must stay intact and not dissociate to free metal ion and ligand, both toxic alone (LD₅₀ values for free Gd^{III} and for uncomplexed ligands are in the range $0.1\text{--}0.2\text{ mmol kg}^{-1}$) (118–120). Upon complexation, the LD₅₀ increases dramatically to e.g. 6 mmol kg^{-1} for $[\text{Gd}(\text{DPTA})(\text{H}_2\text{O})]^{2-}$; 0.35 mmol kg^{-1} for $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$, or 12 mmol kg^{-1} for $[\text{Gd}(\text{HP-DO3A})(\text{H}_2\text{O})]$. The most important criteria for safe *in vivo* application thus include high thermodynamic stability and kinetic inertness (121). Sufficiently stable lanthanide chelates form only with multidentate ligands.

In general, the safety profile of Gd^{III}-based MRI contrast agents is considered to be superior to that of iodinated X-ray agents. Today, however, particularly much attention is focused on the stability and toxicity issues of Gd^{III}-based agents (122), as the recently described nephrogenic systemic fibrosis (NSF), identified in patients with severe renal failure, might be associated with the use of certain Gd^{III} complexes (123,124). NSF is a rare and potentially mortal syndrome that involves fibrosis of skin, joints, eyes, and internal organs. Out of the few hundred cases reported so far, most of them are associated with the administration of $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$. The clinical contrast agents are excreted by passive glomerular filtration (125). The plasma elimination half-life of the commercialized, non-specific chelates is around

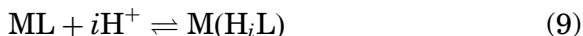
1.5 h (119,126); however, it can be increased by a factor of 20 or more in patients with renal insufficiency (127). Gadolinium has been indeed detected in skin and blood vessels of patients with NSF (128).

A. THERMODYNAMIC STABILITY CONSTANTS OF THE COMPLEXES

The thermodynamic stability of an ML complex is expressed by the equilibrium constant of the formation reaction, K_{ML} , or known as the stability constant:

$$K_{ML} = \frac{[ML]}{[M][L]} \quad (8)$$

where $[M]$, $[L]$, and $[ML]$ are the equilibrium concentrations of metal ion, deprotonated ligand, and complex, respectively (charges are omitted for simplicity). Complexes formed with multidentate ligands can be protonated at low pH:



This equilibrium is characterized by the protonation constant of the complex:

$$K_{MH_iL} = \frac{[M(H_iL)]}{[M(H_{i-1}L)][H^+]} \quad (10)$$

The protonation constants of the ligand, K_i , are also necessary for the calculation of the complex stability constant, K_{ML} :

$$K_i = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad (11)$$

Complex stability constants are often determined by pH-potentiometric titration of the ligand in the presence and absence of the metal ion (129). This method works well when equilibrium is reached rapidly (within few minutes), which is usually the case for linear ligands. For macrocyclic compounds, such as DOTA and its derivatives, complex formation is slow, especially at pH-s where the formation is not yet complete, therefore a batch method is used instead of direct titration (130,131). A few representative examples of stability constant data mainly collected from Ref. (132), on MRI relevant Gd^{III} complexes are presented in Table IV.

To predict the complexation behavior of ligands at physiological pH, the protonation constants of the ligand have to be considered by using conditional stability constants. Conditional stability

TABLE IV

STABILITY CONSTANTS, $\log \beta_{\text{ML}}$, OF SELECTED Gd^{III} COMPLEXES AT 298 K

Ligands	Gd^{III}	Cu^{II}	Zn^{II}	Ca^{II}
DOTA	24.7	22.7	18.7	16.4
HP-DO3A	23.8	22.84	19.37	14.83
DOTAM	10.05	16.3	10.47	7.54
TRITA	19.2	21.5	18.7	12.0
DO3A	22	22.9	19.3	11.7
DTPA	22.39	21.2	18.2	10.75
DTPA-BMA	16.86	13.05	12.04	7.17
EPTPA ^a	19.20	18.07	16.01	9.38
DTTA-Nprop ^b	19.74	—	17.85	—

For comparison, complex stabilities with endogenous divalent metal ions are also given. Data are collected from Ref. (132), except those indicated.

^aRef. (64).

^bRef. (152).

constants reflect competition between the metal ion and protons for the ligand. K_{ML}^c is defined by the total free ligand concentration ($[\text{L}]_t = [\text{L}] + [\text{HL}] + [\text{H}_2\text{L}] + \dots + [\text{H}_n\text{L}]$) instead of the free ligand concentration, $[\text{L}]$,

$$K_{\text{ML}}^c = \frac{[\text{ML}]}{[\text{M}][\text{L}]_t} = \frac{[\text{ML}]}{[\text{M}][\text{L}]^{\alpha_{\text{H}}}} \quad (12)$$

where, $\alpha_{\text{H}} = 1 + K_1[\text{H}^+] + K_1K_2[\text{H}^+]^2 + \dots + K_1K_2 \dots K_n[\text{H}^+]^n$.

Body fluids are very complex systems from the coordination chemistry point of view. In addition to protonation, the free ligand, the free metal ion, or the complex itself can participate in many side reactions. Among these, the most important are interaction with endogenous metal ions (Mg^{II} , Ca^{II} , Zn^{II} , Cu^{II} , etc.), the complexation of free Gd^{III} by biological ligands (citrate, phosphate, bicarbonate, transferrin, oxalic acid, etc.), and the interaction of the Gd^{III} complex with protons or with small ligands like carbonate, phosphate, dicarboxylic acids to form ternary complexes. Gd^{III} complexes can also be protonated or form ternary complexes with other small ligands like carbonate, phosphate, dicarboxylic acids. By including the most probable side reactions that can occur with the participation of all three species, another conditional stability constant, K^{c*} , can be defined as:

$$K^{c*} = \frac{[\text{ML}^*]}{[\text{M}^*][\text{L}^*]} \quad (13)$$

where the concentrations of $[M^*]$, $[L^*]$, and $[ML^*]$ are expressed as

$$[M^*] = [M] + [MA] + [MB] + \dots \quad (14)$$

$$[L^*] = [L] + [HL] + [H_2L] + \dots + [H_nL] + [M'L] + [M''L] + \dots \quad (15)$$

$$[ML^*] = [ML] + [MHL] + [MLX] + [MLY] + \dots \quad (16)$$

This conditional stability constant, K^* , is related to the thermodynamic stability constant (K_{ML}) by (133):

$$K^* = K_{ML} \frac{\alpha_{ML}}{\alpha_M \alpha_L} \quad (17)$$

where

$$\alpha_M = 1 + K_{MA}[A] + K_{MB}[B] + \dots$$

$$\alpha_L = 1 + K_1[H^+] + K_1K_2[H^+]^2 + \dots + K_1K_2 \dots K_n[H^+]^n \\ + K_{M'L}[M'] + K_{M''L}[M''] + \dots$$

$$\alpha_{ML} = 1 + K_{MHL}[H^+] + K_{MLX}[X] + \dots + K_{MLY}[Y] + \dots$$

Correlation between complex stability and *in vivo* toxicity has always been an important question in contrast agent development. Conditional stability constants were often correlated to the selectivity of a given ligand for Gd^{III} over endogenous metals, such as Zn^{II} or Cu^{II} (134), and therefore they are widely used to compare the behavior of different complexes at physiological pH.

In practice, some contrast agent formulations also include a small amount of the corresponding Ca^{II} chelate ($\sim 5\%$) which acts as a sacrificial complex to pre-empt displacement of Gd^{III} from GdL by adventurous Zn^{II} . As a result, the LD_{50} value for $[Gd(DTPA-BMA)(H_2O)]$ increased from $14.8 \text{ mmol kg}^{-1}$ to $38.2 \text{ mmol kg}^{-1}$ upon addition of the adjuvant Ca^{II} complex (121).

B. KINETIC INERTNESS OF Gd^{III} COMPLEXES

It is evident that competitive equilibria alone cannot explain the *in vivo* behavior of Gd^{III} complexes and kinetic factors also have to be considered. The excretion of low molecular weight chelates from the body is very rapid (119,126), whereas the dissociation and transmetallation of the Gd^{III} complexes is relatively slow. Therefore, the system is not in equilibrium even in the case of linear chelates and kinetic factors are important. We should mention that for renal impaired patients the elimination rate becomes much slower which might lead to an

equilibrium situation for linear Gd^{III} complexes (macrocyclic chelates dissociate more slowly, see below). In such a case, the thermodynamic stability (selectivity and conditional stability constants) becomes the physiologically relevant factor.

The kinetic inertness of the Gd^{III} complex is related to exchange reactions that take place in plasma. The most important one is probably the displacement of Gd^{III} by the endogenous metals Cu^{II} and Zn^{II} , though the possible transmetallation with Ca^{II} and Fe^{III} has been also evoked (135). This can occur either via the direct attack of the endogenous metal on GdL , or via the proton-assisted dissociation of the complex, followed by the fast recombination of the ligand with the endogenous metal ion (or both). Ligand exchange reactions between GdL and ligands present in the blood plasma are usually considered to be less probable.

When characterizing the kinetic inertness of Gd^{III} -based contrast agents, the main difficulty resides in the very long half-lives at physiological pH, relevant to medical applications. Therefore, the *in vitro* experiments to assess the kinetic inertness of the complexes are usually carried out either in strongly acidic (typically pH 1) solutions (136–140), or in the presence of an exchanging metal ion, typically Zn^{II} , present at much higher than the physiological concentration (141–143). Muller *et al.* proposed a relaxometric method to evaluate the transmetallation of Gd^{III} chelates with Zn^{II} . It is based on the precipitation of the free Gd^{III} ion released in the transmetallation process via the highly insoluble GdPO_4 (144).

It has become clear for some time that the two main classes of Gd^{III} -based MRI agents, namely the linear and the macrocyclic chelates, have considerably different behavior with respect to dissociation kinetics. Complexes with macrocyclic ligands dissociate much more slowly than those formed with linear chelators. The rate constants which characterize the dissociation of the Gd^{III} chelates and which can be used to compare the kinetic stabilities are about 10^3 times lower for macrocyclic DOTA derivative complexes than for the linear DTPA family (121,145). The high inertness of DOTA-type chelates is usually related to the rigidity of the 12-membered macrocyclic ring. Indeed, $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$ is stable even at the low pH of the stomach and therefore, it was suggested for oral use as a gastrointestinal contrast agent (146).

In the case of DTPA derivative complexes, both proton- and metal-assisted (Zn^{2+} or Cu^{2+}) pathways are important for the dissociation (143). For the DTPA-amide derivatives, the proton-assisted dissociation is much faster, while the metal-assisted pathway is slower (147,148), with a dependency of the

dissociation rates on the nature of the amide substituents. The overall result is a one order of magnitude faster dissociation for $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$ than for $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$. Concerning the tetraamide derivatives of DOTA, the kinetic inertness is usually higher than that of the DOTA complexes, mainly due to the lower basicity of macrocyclic nitrogens and to the overall positive charge of the complexes (149,150). DTPA- or DOTA-derivatives bearing substituents on carbon atoms are usually more kinetically inert. The replacement of acetate pendants on either DOTA or DTPA by alcoholic OH (151), propionate (152), or methylphosphonic or methylphosphinic (153) arms reduces the kinetic inertness of the complexes.

Experimental biodistribution studies in humans or animals generally indicate little Gd^{III} displacement from the contrast agent. Nevertheless, in animal experiments using radioactive labeled Gd^{III} complexes considerably higher Gd^{III} retention was found for $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$ than for other linear compounds, while it was very low for all macrocyclic chelates (154,155). In humans, the Gd^{III} retention has been evaluated by ICP in bone tissue after administration of clinical doses of $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$ and $[\text{Gd}(\text{HP-DO3A})(\text{H}_2\text{O})]$ (156). In the case of the linear chelate, four times more Gd^{III} was retained in the femoral head bone than in the case of $[\text{Gd}(\text{HP-DO3A})(\text{H}_2\text{O})]$. The limited kinetic inertness of $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$ was further demonstrated in a cell labeling study by Cabella *et al.*, which showed one order of magnitude more important cell internalization with $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$ than with $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$, $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$, or $[\text{Gd}(\text{HP-DO3A})(\text{H}_2\text{O})]$. This result was explained in terms of dissociation of $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$ and an easier internalization of the free Gd^{III} ion released.

In a normal situation, the blood pH is 7.4, as regulated by the kidneys via H^+ excretion to urine and re-absorption of hydrogen carbonate, which makes urine slightly acidic (pH 5–7). In the case of acidosis, the pH of the blood is below 7.35, and consequently the urine pH drops below 5.5. This pH decrease can substantially accelerate the dissociation of the Gd^{III} complexes. Indeed, acidosis has been suggested to play a role in the development of NSF (157).

In conclusion, Gd^{III} complexes currently used as MRI contrast agents can be considered as safe drugs, due to their high thermodynamic stability, kinetic inertness as well as to an efficient excretion from the body. Nevertheless, in case of renal impairment, the stability issues become much more important and macrocyclic chelates, which have considerably higher kinetic inertness, should be used instead of linear complexes. We should

also mention that for the macromolecular contrast agents, which have prolonged clearance time, kinetic inertness will become a more significant issue.

IV. Paramagnetic CEST Agents

Beside “classical” MRI, based on the measurement of water abundance in tissues and variation of its relaxation rates, other techniques such as chemical exchange saturation transfer (CEST) and its improved form using paramagnetic compounds (PARACEST) are currently investigated to improve the potential of MRI diagnosis.

A. PRINCIPLE OF CEST

The principle of the CEST was first described by Wolf and Balaban in 1989 (158). A CEST effect can be detected for compounds containing protons in chemical exchange with the bulk water protons. These protons are typically situated either on functional groups of the molecule (such as amides, amines, hydroxyls) or on a very slowly exchanging water molecule of a lanthanide chelate. When the NMR signal of these exchangeable protons is selectively saturated (the populations of both energy levels are equilibrated and the NMR signal is reduced or disappears), the chemical exchange will also transfer the saturation to the second proton pool (bulk water proton) and, consequently, the intensity of the NMR signal of the second pool is decreased. Compounds showing a CEST effect are commonly characterized by the so-called Z-spectrum that displays the intensity of the bulk water signal as a function of the saturation pulse frequency. The spectra always show an intensive peak corresponding to the direct saturation of the bulk water signal, while the other peaks in the spectrum represent the CEST effect of the molecule studied. The CEST effect for a simple system with proton pools A (bulk water) and B (CEST agent) in chemical exchange can be described by the following equation:

$$Z = \frac{M_Z^a}{M_0^a} = \frac{\tau_a}{(T_{1a} + \tau_a)} = \frac{\tau_b}{(T_{1a}(M_0^b/M_0^a) + \tau_b)} = \frac{\tau_b}{(T_{1a}(nc/110) + \tau_b)} \quad (18)$$

Here, M_0 is magnetization of the pool given by the Boltzmann distribution, M_Z the fraction of the magnetization resulting from CEST, c the concentration, and n the number of exchangeable

protons of the CEST agent, τ the residence time and T_1 the longitudinal relaxation time of the proton in the pool. It is necessary to note, that Z is expressed as a decrease of the water intensity signal and, thus, a lower Z value represents a larger CEST effect. From Eq. (18), it is evident that to observe a high CEST effect, one needs slow relaxation, high concentration of the CEST agent, and fast exchange. On the other hand, for selective saturation of one pool, the chemical exchange must be slow on the NMR time scale, which brings a higher limit for the exchange rate (k_{ex}). The exchange rate must be no greater than the frequency difference ($\Delta\omega$) between the two proton pools:

$$\Delta\omega \geq \frac{1}{\tau_b} = k_{\text{ex}} \quad (19)$$

Since $\Delta\omega$ is proportional to the magnetic field, higher fields allow for exploiting faster exchanges which consequently leads to a more important CEST effect. More detailed explanation of the CEST theory (52,159) and numerical solution of the Bloch equations describing the CEST effect (160) can be found in the literature.

B. APPLICATION OF PARAMAGNETIC IONS: PARACEST

The chemical shifts of protons in diamagnetic compounds cover a range of 1–10 ppm. As the signal of the bulk water can be found in the middle of this range, $\Delta\omega$ values are always limited. In order to increase the $\Delta\omega$ value, Aime *et al.* (161) and Sherry *et al.* (162,163) applied paramagnetic complexes as CEST agents (PARACEST). The presence of a paramagnetic ion results in dramatic changes in the proton chemical shifts of the ligand, which cover then a range of up to hundreds of parts per million. The disadvantage of paramagnetic complexes inducing large $\Delta\omega$ is in shortening of the T_1 relaxation time of bulk water.

The first PARACEST effect was observed for lanthanide complexes of DOTA tetraamides. In these complexes, the water exchange is extremely slow and a highly shifted signal of the coordinated water molecule can be observed in the ^1H NMR spectra. Selective saturation of the coordinated water signal can be used to induce the CEST effect. The CEST effect was reported for complexes of various lanthanide ions, including Nd^{III} , Eu^{III} , Tb^{III} , Dy^{III} , Tm^{III} , and Yb^{III} . Variation of the metal ions brings differences in all three parameters (chemical shift, relaxation rate, and proton exchange) that determine the CEST effect. For one ligand, the water exchange rate is dependent on the size,

whereas the values of T_1 and $\Delta\omega$ are dependent on the electronic parameters of the metal ion.

The CEST effect was observed also for the signal of amide protons. Numerous DOTA amides bearing different substituents have been studied as potential PARACEST agents. Due to a very slow water exchange, mainly amides derived from DOTA-4AmC (Scheme 3) (162–164) and dipeptide derivatives (165,166) were studied. Modulation of the water exchange and CEST effect was investigated on *para*-substituted phenylamides (167).

The hydroxyethyl moiety, used as a pendant arm in DOTA-like ligands, also contains hydrogen that is in chemical exchange with bulk water. The CEST effect was observed when the complex was dissolved in acetonitrile. This functional group has so far not proved to be suitable for practical application as in aqueous solution the exchange is too fast to produce a CEST effect (159).

The detection limits for off-resonance saturation are in the low millimolar concentration range of PARACEST agents and are comparable with those for “classical” MRI. Recently, the application of the exchange-sensitive, low-power RF pulses positioned on the bulk water resonance for imaging of the effects of PARACEST agents has been proposed as an alternative to the standard CW off-resonance irradiation (168). The technique decreases the detection limit of the PARACEST agents to low micromolar concentrations.

PARACEST agents are particularly well adapted for the development of responsive probes, since the rate of exchange and the chemical shift of the protons leading to a PARACEST effect depend on various physico-chemical parameters. We will discuss several examples from this field in the following section on “Responsive Imaging Probes.”

We should mention that similarly to other imaging agents, PARACEST agents can also be targeted to specific biological structures. Bifunctional PARACEST agents allowing for attachment of targeting functionalities were studied (169) and, later, used for attachment of fibrin-targeting nanoparticles (170). Another bifunctional DOTA amide was used for labeling of adenovirus particles (171). Several DOTA amides containing albumin-binding groups were studied in order to extend the blood retention of the PARACEST agent (172).

C. LIPOCEST

An innovative approach to CEST agents was introduced by Aime *et al.* (173). The CEST effect is proportional to the number of exchangeable protons or water molecules. When a lanthanide

ion is entrapped in a liposome, the signal of the water protons inside the liposome is shifted and two separate water signals can be found in the ^1H NMR spectra. As the inner water molecules are in slow exchange with the bulk, such a structure could be used as a PARACEST (LIPOCEST) agent. The high number of water molecules entrapped in the liposome increases efficiency of the LIPOCEST agent and the detection limit is several orders of magnitude lower than for other PARACEST agents. Later, the properties of LIPOCEST agents were improved by application of non-spherical liposomes showing higher chemical shift of the inner water signal (174) and by application of two different complexes with different CEST properties allowing their colocalization (175). Recently, the efficiency of the LIPOCEST agent as a function of the liposome size was studied and optimized (176).

V. Responsive Imaging Probes

Classical MRI provides anatomical images of the living organism. A very important step further is represented by molecular imaging, which seeks for the *in vivo* visualization of molecular events at cellular levels. Approaches that visualize molecules which can be considered as the signature of a given disease represent an outstanding breakthrough with respect to the current diagnostic modalities. Any molecular imaging procedure requires an imaging probe that is specific for a given molecular event, which places the development of appropriate probes in the center of molecular imaging applications. Molecular imaging probes in general involve responsive (smart) agents and targeted agents. Here, we only focus on responsive probes that most often imply a rational design of the chelate from the coordination chemistry point of view. The search for responsive contrast agents capable of reporting on various physical-chemical parameters in tissues such as temperature, pH, concentration of metal ions and biomolecules is a permanent challenge for molecular imaging. By now, the field of responsive probes has become extremely wide and an exhaustive review exceeds the scope of this work. An overview of responsive agents was published in this series by Aime *et al.* in 2005 (4); more recent and extensive reviews include Refs. (177) and (178).

For Gd^{III} -based agents, the relaxivity response is most often related to a change in water accessibility or to the variation of the size and consequently of the rotational correlation time of the complex. In addition to Gd^{III} complexes, PARACEST agents are

extensively investigated as responsive probes. Indeed, one of the difficulties with Gd^{III} -based agents is that they always have a T_1 effect due to the outer sphere relaxation contribution. In contrast, in PARACEST imaging, it is possible to administer and independently visualize two PARACEST agents in the same experiment, which makes it possible to develop methods which eliminate the concentration dependence in the determination of a given physico-chemical parameter. On the other hand, the PARACEST properties can be influenced by various factors (pH, temperature, metabolite concentration, etc.) via the modulation of the proton exchange rate or the alteration of the proton resonance frequency. In addition to the enzyme-activated and metal ion responsive probes that we will discuss more in detail, several examples of smart PARACEST agents have been reported in the field of temperature sensing (179–181) or pH-mapping (182). The detection of metabolites is also a very active field. Binding of such molecules to a PARACEST agent can switch on/off the CEST effect. For instance, the CEST effect of the DOTA amides containing boronic functionalities in the side chains was reported to be sensitive to the concentration of glucose (183). The efficiency of the sensing is dependent on the number and positions of the boronic acid groups in the molecule (184) as specific geometry of glucose binding is reflected in modulation of the water exchange rate. The triamide derivative of DO3A was reported as another metabolite responsive PARACEST agent. The probe is sensitive to lactate concentration (185). As in the complex with the heptadentate ligand the lactate molecule can replace the two coordinated water molecules, and the binding of lactate and formation of the ternary structure results in a change of the complex CEST properties. An irreversible change can also be used for PARACEST sensing. A DOTA amide containing an *ortho*-amino-phenyl group was studied as a PARACEST agent for sensing of nitric oxide. Efficient binding of the nitric oxide to the aromatic amino group decreases the CEST effect (186).

A. ENZYME-ACTIVATED CONTRAST AGENTS

In the field of responsive agents, enzyme targeting has specific advantages. A small concentration of the enzyme can convert a relatively high amount of the probe in multiple catalytic cycles which considerably decreases the detection limit for the enzyme as compared to other biomolecules. Moreover, enzymatic reactions are usually highly specific; therefore, the observed change

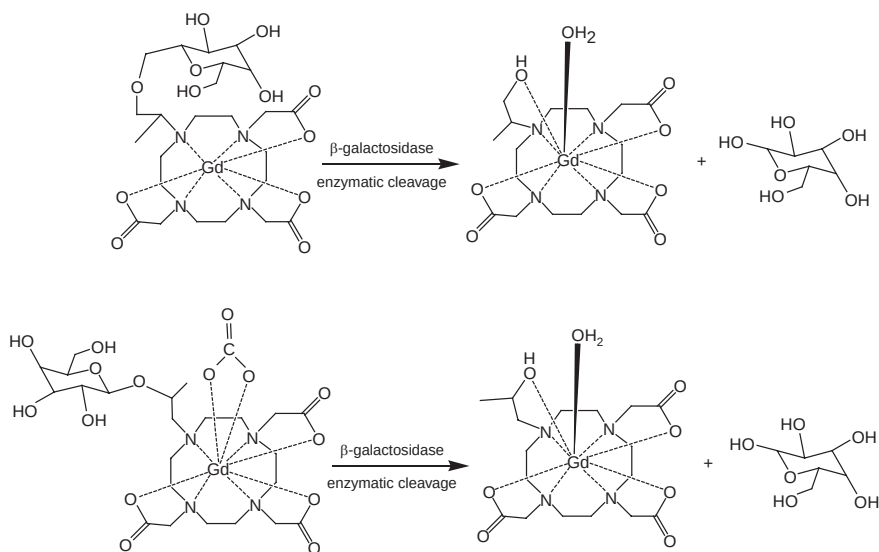
in the MRI properties can be attributed with high certitude to the targeted enzyme.

The polymerization process was used to follow the activity of peroxidases. Gd^{III} complexes bearing phenolic side chains are polymerized in the presence of peroxides by action of the enzyme (187–191). Another example of an enzyme-assisted increase of the molecular weight is the incorporation of the Gd^{III} -based contrast agent into a more complex biological structure. For this purpose, a complex containing a short peptide chain acting as a transglutaminase substrate was used (192). Transglutaminases play a role in remodeling the tissue by cross-linking between proteins of the extracellular matrix. The rate of the increase of the relaxivity due to the attachment to the macromolecules in biological media was found proportional to the enzyme activity.

The degradation of a macromolecular Gd^{III} conjugate could also be used for enzyme detection. A conjugate of GdDTPA derivative with hyaluronan, a high molecular weight polysaccharide present in the extracellular matrix, was studied to follow hyaluronidase activity (193,194). In the conjugate, the complex is shielded from bulk water. The degradation of the polymer by the enzyme exposes the complex to bulk water and, consequently, increases relaxivity. Another example of a complex activated by enzymatic cleavage was reported for DTPA amides containing long aliphatic side chains (195). After internalization into dendritic cells, the insoluble material undergoes cleavage by lipases to produce a soluble complex. The increase of the relaxivity is a direct response to the solubilization of the complex due to the enzyme action.

Another approach relies on the enzymatic cleavage of a group that blocks the protein binding functionality of the complex. This method was used mainly for DOTA and DTPA derivatives capable of HSA binding. After the enzymatic cleavage of the blocking group, the binding of the complex to HSA resulted in a relaxivity increase. HSA binding groups blocked by phosphate (196), β -galactose (197,198), or trilylsine chain (199) were reported as sensors for activity of the corresponding hydrolytic enzymes. Masking groups can also be used for blocking different processes. Cathecolic derivatives of DTPA were studied as reporters of esterase activity (200). Upon cleavage of the ester groups protecting the cathecholic moiety, the complex polymerizes to yield melanine-like macromolecules.

Beside changes in the rotational correlation time, modifications in the hydration state of the chelate were also used to follow enzymatic activity. $\text{Gd}(\text{HP-DO3A})$ -derivative complexes bearing a galactopyranose moiety were studied as reporters for

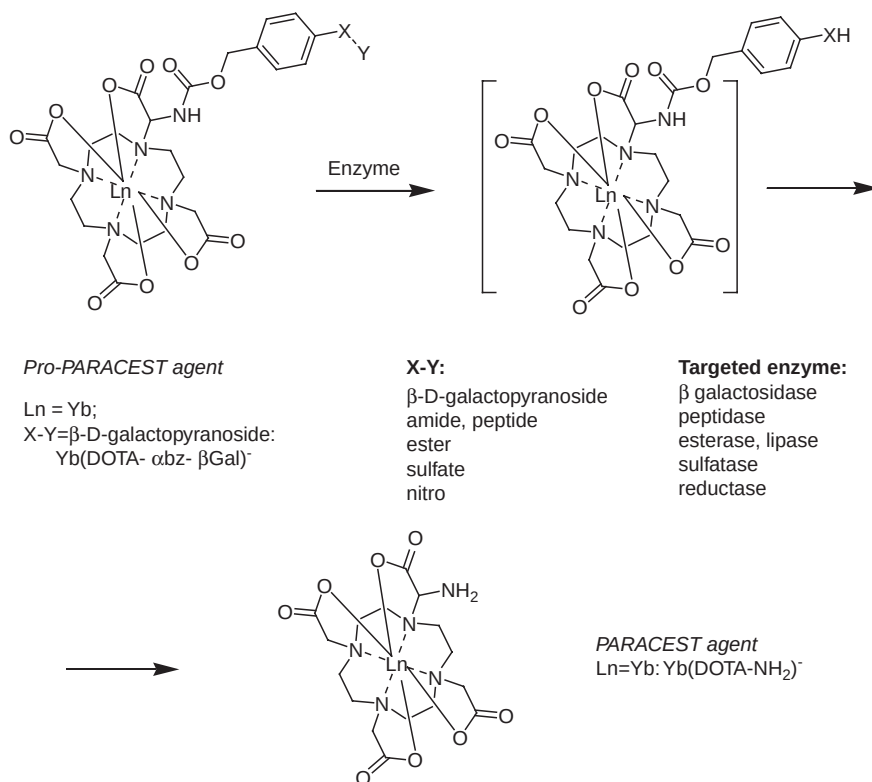


SCHEME 12.

β -galactosidase activity (Scheme 12) (201–203). Before the enzymatic cleavage, depending on the linker, the galactopyranose or a carbonate in bidentate coordination block the water access to the Gd^{III} ion. After enzymatic cleavage, one water molecule enters the inner sphere and results in an increased relaxivity.

PARACEST agents with a response to enzymatic activity have also been reported. Pagel *et al.* used a Tm^{III} DOTA monoamide complex containing a peptide chain which is hydrolyzed by the Caspase-3 enzyme (204,205). Following enzymatic cleavage, the PARACEST effect originating from the amide proton disappears due to the hydrolysis of the amide bond.

In another example, the lanthanide complex has been linked to an enzyme-specific substrate via a self-immolative spacer (Scheme 13) (206). With benzyloxycarbamates acting as self-immolative units, the substrate can be any enzyme-recognized moiety capable of transitionally reducing the electron donor capabilities of the phenyl substituent. The enzymatic cleavage of the substrate initiates an electron cascade and leads to the spontaneous elimination of the spacer. This results in the appearance of a PARACEST effect attributed to the exchange of the amine protons. This platform represents several positive features: the substrate is at the extremity of a spacer that facilitates the enzymatic cleavage, and the PARACEST properties will not be affected by the variation of the substrate. Since



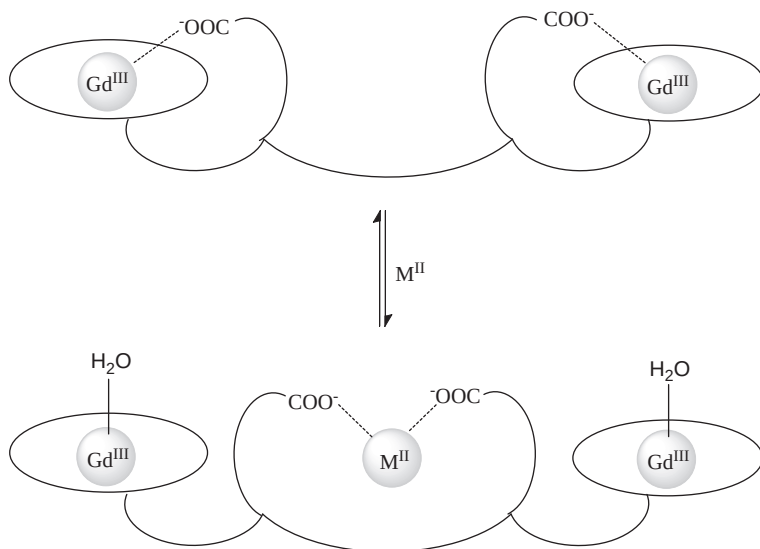
SCHEME 13. Self-immolative degradation of the enzyme-specific agents.

the PARACEST effect is observed after enzymatic activation, it can be optimized once for the whole family. The same Ln^{III} chelate and spacer will be applied for the detection of diverse enzymes, by varying the substrate. The system works as a switch off/on probe that can be a further advantage in practical *in vivo* or *in vitro* applications.

B. CONTRAST AGENTS RESPONSIVE TO METAL IONS

For sensing of metal ions, the Gd^{III} complex has to contain another coordination site, specific to the metal to be detected. This coordination center must show a high selectivity for the ion in question to avoid an undesired response due to interaction with other metal ions.

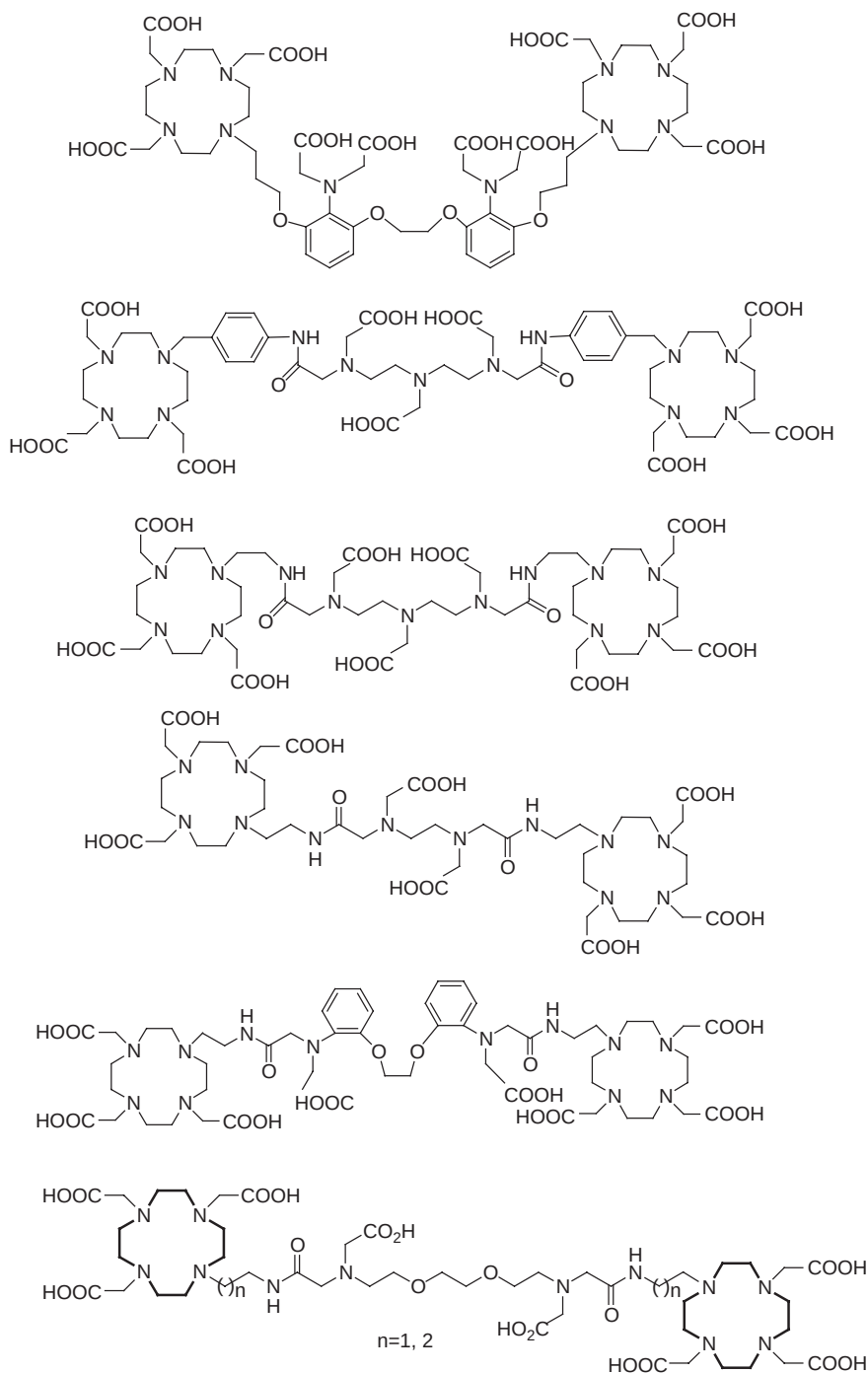
The most common design of metal ion sensitive probes involves changes in the coordination sphere of the Gd^{III} ion following



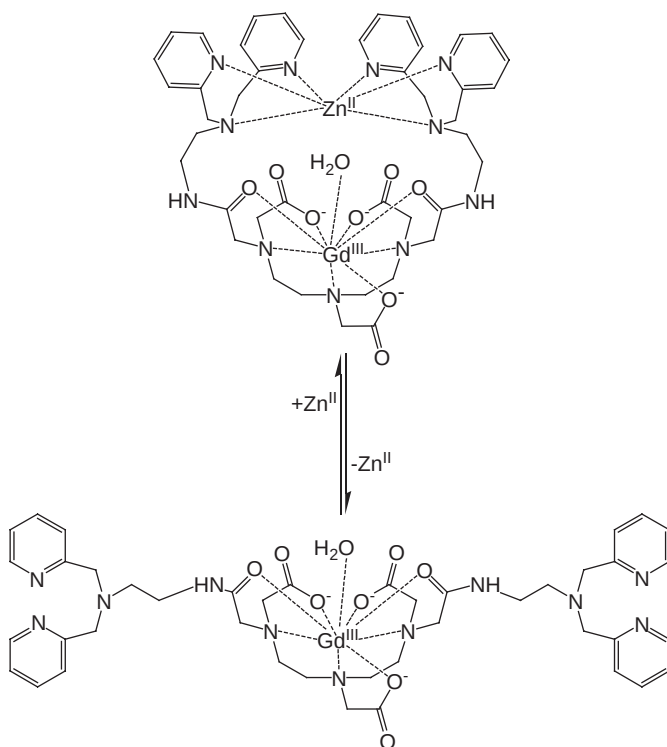
SCHEME 14. Metal ion sensing based on the change of the hydration state of the Gd^{III} complex.

coordination of the other metal. Most of these probes were DO3A derivatives containing a second coordination site linked to one of the macrocycle nitrogens. In the absence of the sensed ion, one (or more) of the donor groups of the second chelating center is weakly coordinated to the Gd^{III} ion. Upon interaction with the sensed metal ion, this donor group will participate in its coordination, and the free coordination position on the Gd^{III} ion will be occupied by a water molecule (Scheme 14). Consequently, the increase of the hydration number results in a relaxivity increase. This approach was used in the design of compounds with two DO3A chelators connected by a spacer containing a calcium selective chelating unit (Scheme 15; 207–211). Recently, DO3A derivatives attached to aniline-acetate chelators were reported as efficient zinc(II) (212), copper(II) (213), and calcium sensors (214).

In another approach, the water access to the Gd^{III} is modified by coordination of Zn^{II} in a DTPA bisamide complex bearing bis(methylpyridine) chelating groups (215,216). Upon interaction with Zn^{II} the relaxivity decreases. It was explained by the formation of a Zn^{II} complex involving the pyridine groups which blocks the water access to the Gd^{III} (Scheme 16). A similar approach was reported for a DTPA-amide derivative containing two crown-ethers in the side chains, which exhibits a significant efficiency for sensing potassium, whereas the β -diketone derivative was responsive to the concentration of Ca^{II} and Mg^{II} (217).



SCHEME 15. Bismacrocyclic ligands whose Gd^{III} complexes have been studied for Ca^{II} sensing.



SCHEME 16.

Another example of this mechanism is represented by a Zn^{II} -sensitive PARACEST agent. Trokowski *et al.* (218) reported a DOTA amide containing a Zn^{II} chelating unit attached to the amide nitrogen. The coordination of Zn^{II} modulates the water exchange rate and switches off the CEST effect.

The relaxivity of a metal ion sensitive probe can be also modified via changes of the molecular size (rotational correlation time). Fe^{II} ions form stable complexes with bipyridine, terpyridine, and phenantroline ligands in 1:2 or 1:3 metal:ligand ratios. Such ligands can be attached to a Gd^{III} chelate and act as an assembling unit when interacting with Fe^{II} (Scheme 11). Formation of these assemblies results in an increased molecular size and, consequently, improves relaxivity. The systems based on this approach were mainly studied as contrast agents for high magnetic fields (see Section II.C) (97,99,116); they could be also used for sensing iron(II) ions. In another family of bifunctional ligands using less specific complexation, bisphosphonate derivatives of DOTA were studied as vectors for bone tissue (219,220).

Their Gd^{III} complexes were found to show significantly increased relaxivity upon interaction with Zn^{II} , Ca^{II} , and Mg^{II} ions. The relaxivity increase was related to an increase of the rotational correlation time, and the mechanism was ascribed to the formation of coordination oligomers or polymers that are typical for bisphosphonate complexes.

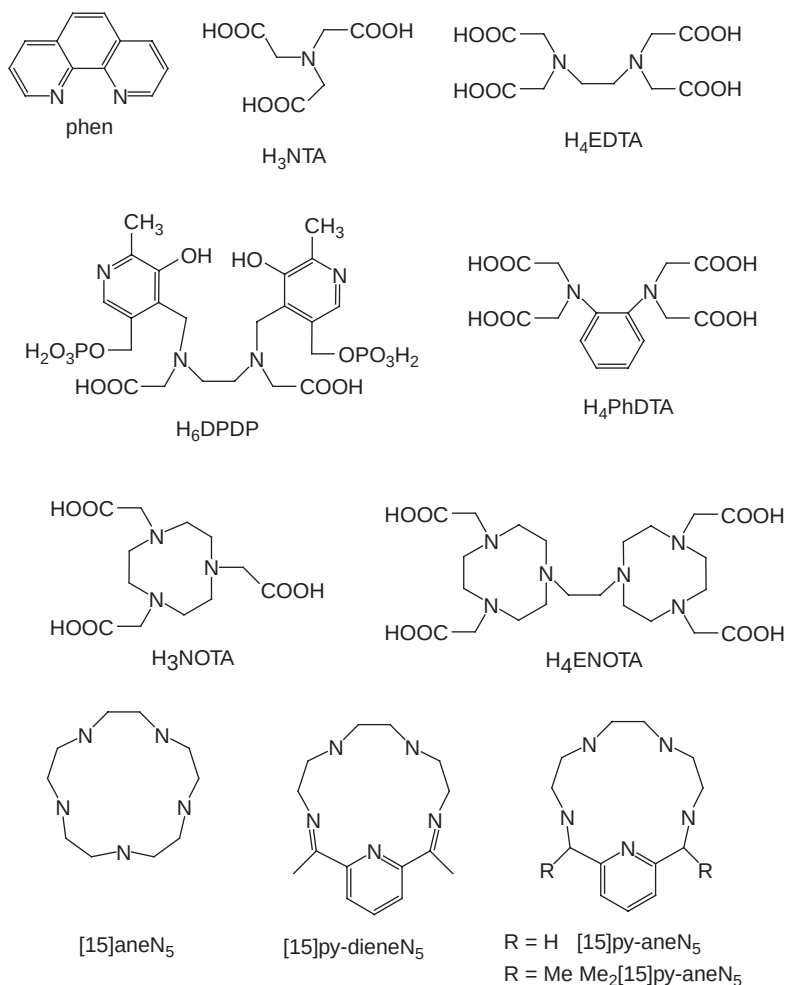
VI. Mn^{II} Complexes in the Context of MRI

In the MRI research, transition metal ions and their complexes have attracted much less attention as compared to gadolinium-based contrast agents. Among them, Mn^{II} is the most studied one due to its relatively high spin ($S = 5/2$), long electronic relaxation time and labile water exchange, which make it an attractive candidate for contrast agent applications. The Mn^{II} aqua-ion was applied already in the early days of MRI (9,221,222). Manganese is a biogenic element with a serum concentration of $0.5\text{--}1.2\ \mu\text{g L}^{-1}$ (223). It plays an important role as a cofactor in a number of enzymes, such as manganese superoxide dismutase and glutamine synthetase. At higher concentrations, manganese shows significant cell toxicity. Chronic exposure to and brain accumulation of manganese, result in disorders resembling Parkinson's disease (224), which strongly limits its application. Nevertheless, Mn^{II} salts are widely used in animals for functional mapping of neural pathways and brain activity and for imaging of the heart. The advances in the MRI applications of the Mn^{II} aqua-ion – manganese-enhanced magnetic resonance imaging (MEMRI) – were recently reviewed (225).

Among commercial contrast agents, Mn^{II} -dipyridoxyldiphosphate $[\text{Mn}(\text{DPDP})]^{2-}$ is the only Mn^{II} complex approved for liver imaging (Scheme 17; 10,226–229). The functioning of this non-hydrated complex relies on a slow transmetallation of Mn^{II} by biogenic metal ions and the released free Mn^{II} ion is then taken up by the hepatocytes.

A. MRI PROPERTIES OF Mn^{II} COMPLEXES

In general, Mn^{II} has two different coordination numbers. A coordination number of six is found for the aqua-ion and for complexes of ligands having low denticities. For ligands having more than six donor groups, coordination number seven is usually expected. Hexadentate ligands can form complexes with both coordination numbers. Whereas in $[\text{Mn}(\text{NOTA})]^-$ (Scheme 17) (230), the metal ion is hexacoordinated, in $[\text{Mn}(\text{EDTA})(\text{H}_2\text{O})]^{2-}$



SCHEME 17.

(231) a seventh coordination site is occupied by a water molecule. The situation is different for [15]aneN₅ and its analogs (Scheme 17) (232). Despite the presence of only five donor groups, the Mn^{II} ion forms heptadentate complexes with two coordinated water molecules. This could be ascribed to geometrical factors: the five nitrogen atoms are arranged in the equatorial plane and the pentagonal bipyramid is accomplished with two water molecules coordinated in axial positions. In general, the relation between ligand geometry and the coordination number of the Mn^{II} ion is not easily predictable.

In the case of Mn^{II} complexes as potential MRI contrast agents, the presence of a water molecule in the first coordination sphere is crucial to obtain reasonable relaxivity. Given the lower spin of Mn^{II} , the outer sphere contribution to the overall relaxivity is lower than for Gd^{III} complexes. Thus, for complexes that lack inner sphere water the relaxivity is less than 50% as compared to complexes with coordinated water (233–235).

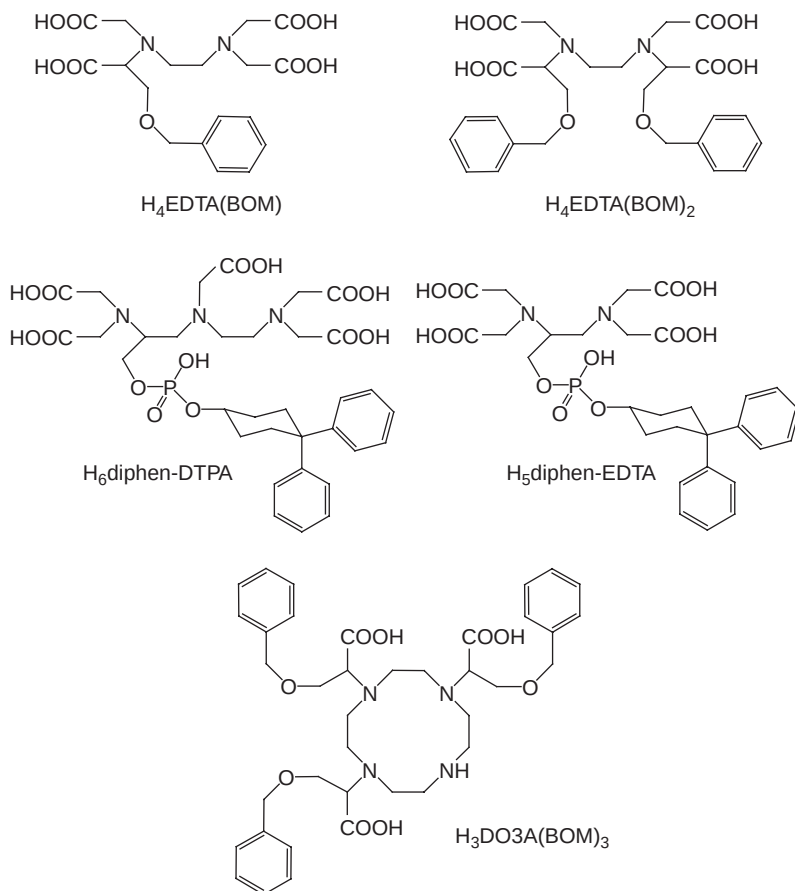
For Mn^{II} complexes, the disadvantage of the lower spin, as compared to Gd^{III} , can be partially compensated with a shorter distance between the metal ion and the coordinated water molecule, and with a more labile bond of the coordinated water molecule, both favorable for relaxivity. The water exchange rate of Mn^{II} complexes is close to the optimum values to attain maximum relaxivities at intermediate fields. Contrary to Gd^{III} , the complexation of Mn^{II} does not result in a significant decrease of the water exchange rate (231–238). For Mn^{II} complexes both higher and lower water exchange rates, as compared to the aqua-ion, have been observed (Table V). The mechanism of the water exchange depends on the coordination number. For coordination number six, an associative mechanism and for coordination number seven, a dissociative mechanism is expected. For the Mn^{II} aqua-ion, an associative interchange mechanism is operative (239), for $[\text{Mn}_2(\text{E-NOTA})(\text{H}_2\text{O})_2]$ (Scheme 17) an associative mechanism prevails (240), and for $[\text{15}] \text{aneN}_5$ analogs a dissociative (232) mechanism for water exchange was confirmed by variable pressure ^{17}O NMR.

TABLE V
WATER EXCHANGE RATES OF Mn^{II} COMPLEXES

Complexes	q^b	$k_{\text{ex}}^{298}/10^7 \text{ s}^{-1}$	Refs.
$[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$	6	2.1	(239)
$[\text{Mn}(\text{phen})]^{2+}$	4	0.13 ^a	(236)
$[\text{Mn}(\text{phen})_2]^{2+}$	2	0.31 ^a	(236)
$[\text{Mn}(\text{ATP})]$	3	5	(231)
$[\text{Mn}(\text{NTA})]^-$	2	150	(237)
$[\text{Mn}(\text{EDTA})]^{2-}$	1	44	(231)
$[\text{Mn}(\text{PhDTA})]^{2-}$	1	35	(238)
$[\text{Mn}(\text{EDTA})(\text{BOM})]^{2-}$	1	9.3	(233,234)
$[\text{Mn}(\text{EDTA})(\text{BOM})_2]^{2-}$	1	13	(233,234)
$[\text{Mn}(\text{diphen-EDTA})]^{2-}$	1	23	(235)
$[\text{Mn}_2(\text{ENOTA})]$	1	5.5	(240)
$[\text{Mn}([\text{15}] \text{py-dieneN}_5)]^{2+}$	2	1.7	(232)
$[\text{Mn}([\text{15}] \text{py-aneN}_5)]^{2+}$	2	5.3	(232)
$[\text{Mn}(\text{Me}_2[\text{15}] \text{py-aneN}_5)]^{2+}$	2	4.7	(232)

^a0°C.

^bNumber of coordinated water molecules.



SCHEME 18.

In the context of MRI, Aime *et al.* studied one DO3A- and two EDTA-derivative Mn^{II} complexes bearing benzyloxymethyl (BOM) substituents, which allow for efficient protein binding (Scheme 18, 233). In an ^{17}O NMR and NMRD study, they found that the macrocyclic $[\text{Mn}(\text{DO3A})(\text{BOM})_3]^-$ complex had no inner sphere water, while both the EDTA-derivatives contained one water molecule. In the presence of serum albumin, a significant enhancement of the proton relaxivity was observed for the EDTA-derivatives (up to $\sim 58 \text{ mM}^{-1} \text{ s}^{-1}$ at 20 MHz). The field-dependent NMRD profiles of these systems have been interpreted by Kruk and Kowalewski using theoretical models that take into consideration a multiexponential electron spin relaxation (234). Caravan *et al.* reported another EDTA-derivative Mn^{II} complex

(diphen-EDTA), which contains the same protein binding diphenylcyclohexyl moiety as the Gd^{III} -based contrast agent MS325 (235). The high relaxivity under biological conditions ($46 \text{ mM}^{-1} \text{ s}^{-1}$ at 20 MHz, 37°C in human plasma) could be rationalized in terms of an efficient exchange of the coordinated water and a slow tumbling of the system due to protein binding.

It is interesting to note that for low-molecular Mn^{II} complexes two different shapes of the ^1H NMRD profile were reported. For the Mn^{II} aqua-ion (241) and for $[\text{Mn}_2(\text{ENOTA})(\text{H}_2\text{O})_2]$ (240), two dispersions are observed. The low magnetic field dispersion at $\sim 0.1 \text{ MHz}$ is known to arise from contact interaction, while the high-field dispersion at $\sim 10 \text{ MHz}$ originates from the dipolar contribution. The profiles of other Mn^{II} complexes studied from the EDTA, DTPA, NOTA, and DOTA families show only the high-field dispersion. The lack of the low-field dispersion has been mainly attributed to a shortening of the electronic relaxation times (233).

Recently, Lanza *et al.* suggested the use of nanoparticles labeled with Mn^{III} -protoporphyrin. The relaxivities measured for these nanobialys were $r_1 = 3.7 \text{ mM}^{-1} \text{ s}^{-1}$ and $r_2 = 5.2 \text{ mM}^{-1} \text{ s}^{-1}$ per Mn^{III} (1.5 T; 298 K) (242).

Aime *et al.* took advantage of the different redox states of manganese and of the difference in the related relaxation properties to design a pO_2 responsive contrast agent. The adducts formed between $\text{Mn}^{\text{III}}/\text{Mn}^{\text{II}}$ tpps complexes and poly- β -cyclodextrin have considerably different relaxivities depending on the redox state of the metal, itself determined by the partial oxygen pressure of the solution (tpps = 5,10,15,20-tetrakis-(*p*-sulfonatophenyl porphinate) (243).

B. STABILITY OF Mn^{II} COMPLEXES

Among transition metal ions, Mn^{II} shows relatively low stability of the complexes (132). The ion lacks the ligand field stabilization energy due to the totally symmetrical d^5 configuration. As the Mn^{II} ion is usually hexa or heptacoordinated, the ligand should contain the corresponding number of donor groups to reach a reasonable stability of the complex. The stability of the complexes with bidentate, tridentate, or tetradentate ligands (e.g., phen, NTA, or ATP, Scheme 17) is quite low (a comparison of the stability constants for a selection of complexes is shown in Table VI). Higher stabilities are obtained with ligands of the EDTA and DTPA family. In the EDTA family, Mn^{II} ion is heptacoordinated with six positions occupied by the ligand and one by a water molecule. In the DTPA family a higher stability is

TABLE VI

THERMODYNAMIC STABILITY (LOGK) OF SELECTED Mn^{II} COMPLEXES AND THEIR COMPARISON WITH OTHER BIOGENIC METAL IONS AT 298 K

Ligands	Mn^{II}	Cu^{II}	Zn^{II}	Ca^{II}	Refs.
Phen	4.09	9.13	6.38	1.0	(132)
	7.52 ^a	15.84 ^a	12.08 ^a		
ATP	4.76	6.34	5.16	3.76	(132)
NTA	7.27	12.7	10.45	6.44	(132)
	10.44 ^a	17.4 ^a	14.24 ^a	8.81 ^a	
EDTA	13.89	18.78	16.5	10.65	(132)
PhDTA	11.37	15.11	12.89	8.60	(132)
DTPA	15.2	21.2	18.2	10.75	(132)
NOTA	14.9	21.63	18.6	8.92	(132)
ENOTA	24.06 ^b	36.93 ^b	35.88 ^b	12.45 ^b	(240)
DOTA	19.89	22.7	18.7	16.8	(132)
DO3A	19.40	—	—	11.7	(132,244)
<i>cis</i> DO2A	16.13	—	—	—	(244)
<i>trans</i> DO2A	14.54	—	—	—	(244)
TRITA	16.7	21.5	18.7	12.0	(132)
[15]aneN ₅	10.75	27.0	17.9	—	(132)
[15]py-aneN ₅	11.46	—	—	—	(232)

^aFor ML_2 .^bFor M_2L .

observed, but all the coordination positions are occupied by the ligand and no coordinated water molecule is present. More stable complexes are formed with NOTA or DOTA-type macrocyclic ligands. In $[\text{Mn}(\text{NOTA})]^-$, the Mn^{II} is hexacoordinated, whereas in $[\text{Mn}(\text{DOTA})]^{2-}$, heptacoordination is expected. Unfortunately, the geometry of these complexes does not allow for coordination of a water molecule. A good compromise between high stability of the complex and requirement for an inner sphere water molecule could be found in macrocyclic ligands with a reduced number of the pendant arms. Recently, the NOTA-like ligand ENOTA containing two pentadentate chelators was reported. In the $[\text{Mn}_2(\text{ENOTA})(\text{H}_2\text{O})_2]$ complex, each metal ion is hexacoordinated with the last coordination site occupied by a water molecule. Despite the lower denticity of the ligand, a good complex stability has been found.

In the cyclen family, similar stability was found for the Mn^{II} complex of DOTA and DO3A indicating heptacoordination of the metal ion (244). For both, no coordinated water molecule is present. By further decreasing the ligand denticity ($[\text{Mn}(\text{cisDO2A})]$), the stability is decreased (Table VI). The heptacoordination of Mn^{II} ion found for the complex in the solid state can be promising for future interest.

Another promising structural motif for Mn^{II} complexation could be the $[\text{15}] \text{aneN}_5$ core. With ligands based on a $[\text{15}] \text{aneN}_5$ structure, quite high stabilities have been found for the Mn^{II} complexes despite the absence of negatively charged pendant arms. Such high stability of the complexes containing two inner sphere molecules can be an interesting starting point for future research on Mn^{II} based MRI contrast agents.

Despite a high thermodynamic stability of the complex, the release of Mn^{II} ion might occur and can be a problem for practical application. Due to the lack of the ligand field stabilization energy for Mn^{II} , and given the similar charge and ionic radius of Cu^{II} or Zn^{II} , no ligands showing significant selectivity for Mn^{II} could be expected. For instance, even using strong chelators like DTPA did not prevent the brain accumulation of the Mn^{II} ion, and a similar regional distribution was found upon application of both – MnCl_2 as well as $[\text{Mn}(\text{DTPA})]^{3-}$ complex (245). Therefore, the attention should be focused on the kinetic inertness of the Mn^{II} complexes. Unfortunately, data describing this aspect of manganese coordination chemistry are not available.

VII. Non-Classical Gd^{III} -Based MRI Contrast Agents

Beside the stable Gd^{III} complexes that we discussed throughout this chapter, other Gd^{III} -based systems have been also investigated as potential contrast agents for MRI. Here, we will shortly review data on gadofullerenes and carbon nanotubes loaded with Gd^{III} .

A. GADOFULLERENES

Endohedral metallofullerenes, $\text{Gd}@\text{C}_{82}$ and $\text{Gd}@\text{C}_{60}$ that encapsulate a paramagnetic Gd^{III} within the fullerene interior space were proposed as contrast agents from the end of the 1990s (246–250). Early phantom and *in vivo* MRI studies have both shown promising results. Water-soluble derivatives of endohedral metallofullerenes could possess unique potential for medicinal applications since the fullerene cage protects the encapsulated metal ion from external chemical attack and metal ion release in the body. The electronic structure of these gadofullerenes involves the transfer of three electrons from the Gd atom to the cage resulting in seven unpaired electrons on the Gd^{III} center and one unpaired electron on the cage. Similarly to empty fullerenes, metallofullerenes can be derivatized via chemical reactions to provide water solubility and biological targeting.

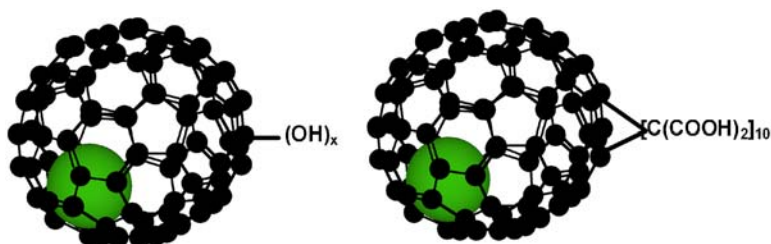


FIG. 5. Depiction of $\text{Gd@C}_{60}(\text{OH})_x$ (left) and $\text{Gd@C}_{60}[\text{C}(\text{COOH})_2]_{10}$ (right).

The gadofullerenes studied in the context of MRI were mainly malonate- or OH-derivatives (Fig. 5).

The early interest in gadofullerenes has been largely promoted by the remarkably high values of the longitudinal proton relaxivities, though the published data showed important variations. Zhang *et al.* (246) reported a relaxivity of $r_1 = 47 \text{ mM}^{-1} \text{ s}^{-1}$ (9.4 T), Wilson (247) found $r_1 = 20 \text{ mM}^{-1} \text{ s}^{-1}$ (0.47 T, 40°C), and Shinohara *et al.* (248) measured a particularly high longitudinal proton relaxivity for $\text{Gd@C}_{82}(\text{OH})_{40}$ ($81 \text{ mM}^{-1} \text{ s}^{-1}$ at 25°C , $B = 1.0 \text{ T}$).

The large relaxivity of the gadofullerenes has been attributed to their slow tumbling in solution and to the large number of surrounding water molecules (251,252). This slow tumbling/rotation was related to aggregation phenomena in aqueous solution. A variable-pH proton relaxation and dynamic light scattering (DLS) study confirmed the pH-dependent aggregation of the gadofullerenes. In fact, self-aggregation is likely a common feature of all water-soluble fullerene chemistry (253–255). A ^1H NMRD study combined with DLS has shown that aggregates formed in aqueous solution of water-soluble gadofullerenes can be disrupted by addition of salts (256). For instance, increasing the NaCl concentration in a $\text{Gd@C}_{60}(\text{OH})_x$ solution from 0 mM to 150 mM results in a decrease of the hydrodynamic diameter from 810 nm to 121 nm and a concomitant drop in the relaxivity from $83.2 \text{ mM}^{-1} \text{ s}^{-1}$ to $31.6 \text{ mM}^{-1} \text{ s}^{-1}$ (37°C , 60 MHz). Phosphate was found much more efficient than sodium halides. This study also gave an explanation for the diversity of r_1 values reported in the literature. The different synthetic methods of preparing water-soluble gadofullerenes may involve varying amounts of salts and some may remain in the sample after purification, which could influence the observed relaxivities.

In the aim of a better understanding of paramagnetic relaxation mechanisms in water-soluble gadofullerenes, variable

temperature and multiple field ^{17}O and ^1H relaxation rates have been recorded for $\text{Gd@C}_{60}(\text{OH})_x$ and $\text{Gd@C}_{60}[\text{C}(\text{COOH})_y\text{Na}_{1-y}]_{10}$ in both aggregated and disaggregated state (monomers) (257). In the aggregated solutions, very different ^{17}O T_1 and T_2 values were measured. This proved the confinement of water molecules in the interstices of the aggregates that is more important for the OH than for the malonate derivative. The rapid exchange of these water molecules with bulk contributes to the relaxivity of the aggregated gadofullerenes. After disruption of the aggregates into distinct gadofullerene molecules, the temperature-dependent proton relaxivities could be described as the sum of an outer-sphere and an inner-sphere-like mechanism. The inner-sphere-like term originates from proton exchange between the bulk and protonated OH or COOH sites. The high-field relaxivity peak in the NMRD profile also evidenced the partial protonation of the malonate groups at pH 7.4. The rotational correlation times were long (~ 1.2 ns) and identical for the two gadofullerenes. The larger high-field relaxivity of $\text{Gd@C}_{60}(\text{OH})_x$ as compared to $\text{Gd@C}_{60}[\text{C}(\text{COOH})_x\text{Na}_{1-x}]_{10}$ was related to the larger number of protonated sites.

B. CARBON NANOTUBES LOADED WITH Gd^{III}

Carbon nanotubes have been also explored as “nanocapsules” for Gd^{III} ions. Single-walled carbon nanotubes (SWNTs) possess unique characteristics that might be desirable for biomedical applications (258). The ideal length for medical applications is uncertain, but ultra-short (US) nanotubes are probably best suited for cellular uptake, biocompatibility, and eventual elimination from the body (259,260). Additionally, the US-tube exterior surface provides a versatile scaffold for attachment of chemical groups for solubilizing or targeting purposes, while its interior space allows for encapsulation of atoms and/or ions whose cytotoxicity may be sequestered within the short carbon nanotube. An US carbon nanotube-based system (gadonanotubes) has been reported to outperform significantly clinical MRI contrast agents (261,262). At standard clinical field strength of 1.5 T, the gadonanotubes demonstrate a 40-fold increase in efficacy (relaxivity) compared to contrast agents in current clinical use. These gadonanotubes are 20–80 nm segments of single-walled, full-length carbon nanotubes that have been cut chemically via fluorination and pyrolysis, followed by aqueous internal loading with Gd^{III} ions. A depiction of the gadonanotube structure is shown in Fig. 6. Due to the sidewall defects created in the US-tubes as a consequence of the chemical cutting

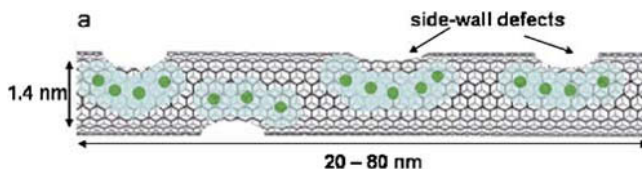


FIG. 6. Schematic representation of the gadonanotubes. Small clusters of Gd^{3+} are situated within the sidewall defects of the nanotube.

procedure, the Gd^{III} ions load and exist as small (*ca.* $1 \times 5 \text{ nm}$) superparamagnetic clusters, which produce an extremely high T_1 -weighted relaxivity. The extremely high values of relaxivity (ranging from *ca.* $180 \text{ mM}^{-1} \text{ s}^{-1}$ at 1.5 T to $>600 \text{ mM}^{-1} \text{ s}^{-1}$ at 0.2 mT) and the variable-field NMRD profiles cannot be interpreted using current SBM theory, indicating that gadonanotubes might possess special (magnetic/relaxivity) properties arising from the nanoscalar confinement of Gd^{III} -ion clusters within their carbon capsule sheaths. The gadonanotubes show a dramatic response to pH and thermal change under physiologically relevant conditions (263). Indeed, the relaxivity of the gadonanotubes undergoes more than a three-fold increase from pH = 8.3 ($40 \text{ mM}^{-1} \text{ s}^{-1}$) to pH = 6.7 ($133 \text{ mM}^{-1} \text{ s}^{-1}$) at 37°C , with the slope of the change between pH 7.4 and 7.0 being $98 \text{ mM}^{-1} \text{ s}^{-1}/\text{pH unit}$. No other pH-responsive MRI contrast agent has demonstrated such a dramatically large change in relaxivity over such a narrow pH range. DLS data provided evidence that aggregation of the gadonanotubes does not play a role in their relaxivity characteristics. The integrity of these clusters has also been proved as a function of pH, temperature, as well as against various physiological challenges, such as phosphate-buffered saline or bovine serum albumin. With their high relaxivity alone, the gadonanotubes make for an attractive contrast agent candidate. Due to their sensitivity to pH, they are also potential probes for cancer detection, as the extracellular pH of tumors can drop to pH 7 or below.

Carbon nanotubes have been also used as a macromolecular scaffold for Gd^{III} complexes. An amphiphilic gadolinium(III) chelate bearing a C16 chain was adsorbed on multiwalled carbon nanotubes (264). The resulting suspensions were stable for several days. Longitudinal water proton relaxivities, r_1 , showed a strong dependence on the GdL concentration, particularly at low field. The relaxivities decreased with increasing field as predicted by the SBM theory. Transverse water proton relaxation times, T_2 , were practically independent of both the frequency and the GdL concentration. An *in vivo* feasibility MRI study has been

performed at 300 MHz in mice. A negative contrast could be well observed after injection of a suspension of functionalized nanotubes into the muscle of the legs of the mice.

VIII. Conclusions and Outlook

Thanks to the biomedical applications of lanthanide complexes, and of Gd^{III} chelates in particular as MRI contrast agents, the last two decades witnessed the renaissance of lanthanide coordination chemistry. Our understanding of the relationships between the structure of a Gd^{III} complex and its relaxation efficacy has largely increased and it is continuously leading to the conception of improved contrast agents. The most important parameters to consider in the design are rotation, water exchange, and hydration number. The optimization of the rotational dynamics by controlling the internal rigidity and the size of the agents will certainly occupy a central position in future contrast agent development, especially for applications at high magnetic fields. By now, we have good tools to modulate water exchange on Gd^{III} complexes. The design of stable, bishydrated complexes, which are inert to anion binding is a quickly growing field. Novel Mn^{II} chelates, possibly with improved kinetic inertness, will also appear. These efforts in optimizing relaxivity will also contribute to the successful development of efficient responsive imaging probes which open new perspectives in the biomedical and clinical use of MRI contrast agents. We believe that coordination chemistry offers practically unlimited possibilities to translate the interaction of a metal-based imaging probe with another metal ion, an enzyme, a metabolite, etc. into a magnetic response which ensures a bright future of chemistry in the development of new molecular imaging approaches.

REFERENCES

1. Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.
2. Tóth, É.; Merbach, A. E. (Eds.) *"The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging"*; Wiley: Chichester, **2001**.
3. Krause, W. (Ed.), *"Topics in Current Chemistry: Contrast Agents I. Magnetic Resonance Imaging"*; vol. 221; Springer-Verlag: Berlin, **2002**.
4. Aime, S.; Botta, M.; Terreno, E. *Adv. Inorg. Chem.* **2005**, *57*, 173.
5. Aime, S.; Geninatti Crich, S.; Gianolio, E.; Giovenzana, G.; Tei, L.; Terreno, E. *Coord. Chem. Rev.* **2006**, *250*, 1562.
6. Caravan, P. *Chem. Soc. Rev.* **2006**, *35*, 512.

7. Hermann, P.; Kotek, J.; Kubíček, V.; Lukeš, I. *Dalton Trans.* **2008**, 3027.
8. Bloch, F.; Hansen, W. W.; Packard, M. *Phys. Rev.* **1948**, 70, 474.
9. Lauterbur, P. C.; Mendoca-Dias, M. H.; Rudin, A. M. In: *"Frontier of Biological Energetics"*; Eds. Dutton, P. L.; Leigh, L. S.; Scarpa, A.; Academic: New York, **1978**, p. 752.
10. Rocklage, S. M.; Cacheris, W. P.; Quay, S. C.; Hahn, F. E.; Raymond, K. N. *Inorg. Chem.* **1989**, 28, 477.
11. Bloembergen, N.; Purcell, E. M.; Pound, R. V. *Phys. Rev.* **1984**, 73, 678.
12. Solomon, I. *Phys. Rev.* **1955**, 99, 559.
13. Solomon, I.; Bloembergen, N. *J. Chem. Phys.* **1956**, 25, 261.
14. Bloembergen, N. *J. Chem. Phys.* **1957**, 27, 572.
15. Bloembergen, N.; Morgan, L. O. *J. Chem. Phys.* **1961**, 34, 842.
16. Connick, R. E.; Fiat, D. *J. Chem. Phys.* **1966**, 44, 4103.
17. Helm, L.; Tóth, É.; Merbach, A. E. In: *"Lanthanides and Their Interrelations with Biosystems"*; vol. 40; Eds. Sigel, H.; Sigel, A.; Marcel Dekker Inc: New York, **2003**, p. 589.
18. Helm, L. *Progr. Nucl. Magn. Res. Spectr.* **2006**, 49, 45.
19. Luz, Z.; Meiboom, S. *J. Chem. Phys.* **1964**, 40, 2686.
20. McLachlan, A. D. *Proc. R. Soc. Lond.* **1964**, A280, 271.
21. Kowalewski, J.; Nordenskiöld, L.; Benetis, N.; Westlund, P.-O. *Progr. NMR Spectrosc.* **1985**, 17, 141.
22. Kowalewski, J. In: *"Encyclopedia of NMR"*; vol. 8; Eds. Grant, D. M.; Harris, R. K.; Wiley: Chichester, **1996**, p. 3456.
23. Horrocks, W. D., Jr.; Sudnick, D. R. *J. Am. Chem. Soc.* **1979**, 101, 334.
24. Parker, D.; Williams, J. A. G. *J. Chem. Soc. Dalton Trans.* **1996**, 3613.
25. Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc. Perkin Trans.* **1999**, 2, 493.
26. Supkowski, R. M.; Horrocks, W., Jr.; De, W. *Inorg. Chim. Acta* **2002**, 340, 44.
27. Alpoim, M. C.; Urbano, A. M.; Geraldès, C. F. G. C.; Peters, J. A. *J. Chem. Soc. Dalton Trans.* **1992**, 463.
28. Raitsimring, A. M.; Astashkin, A. V.; Baute, D.; Goldfarb, D.; Poluektov, O. G.; Lowe, M. P.; Zech, S. G.; Caravan, P. *Chem. Phys. Chem.* **2006**, 7, 1590.
29. Zech, S. G.; Sun, W. C.; Jacques, V.; Caravan, P.; Astashkin, A. V.; Raitsimring, A. M. *Chem. Phys. Chem.* **2005**, 6, 2570.
30. Hajela, S.; Botta, M.; Giraudo, S.; Xu, J.; Raymond, K. N.; Aime, S. *J. Am. Chem. Soc.* **2000**, 122, 11228.
31. Pierre, V. C.; Botta, M.; Aime, S.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, 128, 5344.
32. Jocher, J. C.; Botta, M.; Avedano, S.; Moore, E. G.; Xu, J.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2007**, 46, 4796.
33. Aime, S.; Calabi, L.; Cavallotti, C.; Gianolio, E.; Giovenzana, G. B.; Losi, P.; Maiocchi, A.; Palmisano, G.; Sisti, M. *Inorg. Chem.* **2004**, 43, 7588.
34. Tircso, G.; Kovacs, Z.; Sherry, A. D. *Inorg. Chem.* **2006**, 45, 9269.
35. Livramento, J. B.; Sour, A.; Borel, A.; Merbach, A. E.; Tóth, É. *Chem. Eur. J.* **2006**, 12, 989.
36. Costa, J.; Tóth, É.; Helm, L.; Merbach, A. E. *Inorg. Chem.* **2005**, 44, 4747.
37. Livramento, J. B.; Helm, L.; Sour, A.; O'Neil, C.; Merbach, A. E.; Tóth, É. *Dalton Trans.* **2008**, 1195.
38. Livramento, J. B.; Weidensteiner, C.; Prata, M. I. M.; Allegrini, P. R.; Geraldès, C. F. G. C.; Helm, L.; Kneuer, R.; Merbach, A. E.; Santos, A. C.; Schmidt, P.; Tóth, É. *Contrast Med. Mol. Imag.* **2006**, 1, 30.

39. Loureiro de Sousa, P.; Livramento, J. B.; Helm, L.; Merbach, A. E.; Mème, W.; Doan, B.-T.; Beloeil, J.-C.; Prata, M. I. M.; Santos, A. C.; Geraldès, C. F. G. C.; Tóth, É. *Contrast Med. Mol. Imag.* **2008**, *3*, 78.
40. Aime, S.; Botta, M.; Bruce, J. I.; Mainiero, V.; Parker, D.; Terreno, E. *Chem. Commun.* **2001**, 115.
41. Lisowski, J.; Sessler, J. L.; Lynch, V.; Mody, T. D. *J. Am. Chem. Soc.* **1995**, *117*, 2273.
42. Raymond, K. N.; Pierre, V. C. *Bioconj. Chem.* **2005**, *16*, 3.
43. Charbonnière, L. J.; Mameri, S.; Flot, D.; Waltz, F.; Zandanel, Ch.; Ziessel, R. F. *Dalton Trans.* **2007**, 2245.
44. Astashkin, A. V.; Raitsimring, A. M.; Caravan, P. J. *Phys. Chem.* **2004**, *A 108*, 1900.
45. Aime, S.; Barge, A.; Botta, M.; Parker, D.; De Sousa, A. S. *J. Am. Chem. Soc.* **1997**, *119*, 4767.
46. Aime, S.; Botta, M.; Fasano, M.; Paoletti, S.; Terreno, E. *Chem. Eur. J.* **1997**, *3*, 1499.
47. Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Accounts Chem. Res.* **1999**, *32*, 941.
48. Helm, L.; Merbach, A. E. *Chem. Rev.* **2005**, *105*, 1923.
49. Lincoln, S. F.; Merbach, A. E. *Adv. Inorg. Chem.* **1995**, *42*, 1.
50. Helm, L.; Nicolle, G.; Merbach, A. E. *Adv. Inorg. Chem.* **2005**, *57*, 327.
51. Tóth, E.; Helm, L.; Merbach, A. E. In: "The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging"; Eds. Tóth, E.; Merbach, A. E.; Wiley: Chichester, **2001**, p. 45.
52. Zhang, S.; Matthew, M.; Woessner, D. E.; Lenkinski, R. E.; Sherry, A. D. *Acc. Chem. Res.* **2003**, *36*, 783.
53. Micskei, K.; Powell, D. H.; Helm, L.; Brücher, E.; Merbach, A. E. *Magn. Reson. Chem.* **1993**, *31*, 1011.
54. Balogh, E.; Mato-Iglesias, M.; Platas-Iglesias, C.; Tóth, É.; Djanashvili, K.; Peters, J. A.; de Blas, A.; Rodríguez-Blas, T. *Inorg. Chem.* **2006**, *45*, 8719.
55. Pautler, R. G.; Fraser, S. E. *Curr. Opin. Immunol.* **2003**, *15*, 363, and references therein.
56. Pautler, R. G. *Physiology* **2004**, *19*, 168.
57. Gonzalez, G.; Powell, D. H.; Tissièrès, V.; Merbach, A. E. *J. Phys. Chem.* **1994**, *48*, 53.
58. Lammers, H.; Maton, F.; Pubanz, D.; Van Laren, M. W.; Van Bekkum, H.; Merbach, A. E.; Muller, R. N.; Peters, J. A. *Inorg. Chem.* **1997**, *36*, 2527.
59. Tóth, É.; Connac, F.; Helm, L.; Adzamlı, K.; Merbach, A. E. *Eur. J. Inorg. Chem.* **1998**, 2017.
60. Tóth, É.; Burai, L.; Brücher, E.; Merbach, A. E. *J. Chem. Soc. Dalton Trans.* **1997**, 1587.
61. Powell, H. D.; Ni Dhubhghaill, O. M.; Pubanz, D.; Helm, L.; Lebedev, Y.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333.
62. Tóth, E.; Vauthey, S.; Pubanz, D.; Merbach, A. E. *Inorg. Chem.* **1996**, *35*, 3375.
63. Tóth, É.; Pubanz, D.; Vauthey, S.; Helm, L.; Merbach, A. E. *Chem. Eur. J.* **1996**, *2*, 1607.
64. Laus, S.; Ruloff, R.; Tóth, É.; Merbach, A. E. *Chem. Eur. J.* **2003**, *9*, 3555.
65. Jászberényi, Z.; Sour, A.; Tóth, É.; Benmelouka, M.; Merbach, A. E. *Dalton Trans.* **2005**, 2713.
66. Rudovský, J.; Cígler, P.; Kotek, J.; Hermann, P.; Vojtíšek, P.; Lukeš, I.; Peters, J. A.; Vander Elst, L.; Muller, R. N. *Chem. Eur. J.* **2005**, *11*, 2373.

67. Lebdušková, P.; Hermann, P.; Helm, L.; Tóth, É.; Kotek, J.; Binnemans, K.; Rudovský, J.; Lukeš, I.; Merbach, A. E. *Dalton Trans.* **2007**, 493.
68. Botteman, F.; Nicolle, G. M.; Vander Elst, L.; Laurent, S.; Merbach, A. E.; Muller, R. N. *Eur. J. Inorg. Chem.* **2002**, 2686.
69. Tóth, E.; Connac, F.; Helm, L.; Adzamlı, K.; Merbach, A. E. *J. Biol. Inorg. Chem.* **1998**, 3, 606.
70. Aime, S.; Crich, S. G.; Gianolo, E.; Terreno, E.; Beltrami, A.; Uggeri, F. *Eur. J. Inorg. Chem.* **1998**, 1283.
71. Dunand, F. A.; Aime, S.; Merbach, A. E. *J. Am. Chem. Soc.* **2000**, 122, 1506.
72. Rudovský, J.; Kotek, J.; Hermann, P.; Lukeš, I.; Mainero, V.; Aime, S. *Org. Biomol. Chem.* **2005**, 3, 112.
73. Nicolle, G. M.; Tóth, É.; Schmitt-Willich, H.; Radüchel, B.; Merbach, A. E. *Chem. Eur. J.* **2002**, 8, 1040.
74. Laus, S.; Sour, A.; Ruloff, R.; Tóth, É.; Merbach, A. E. *Chem. Eur. J.* **2005**, 11, 3064.
75. Tóth, E.; van Uffelen, I.; Helm, L.; Merbach, A. E.; Ladd, D.; Briley-Saebo, K.; Kellar, K. E. *Magn. Res. Chem.* **1998**, 36, S125.
76. Tóth, É.; Helm, L.; Kellar, K. E.; Merbach, A. E. *Chem. Eur. J.* **1999**, 5, 1202.
77. Dunand, F. A.; Tóth, É.; Hollister, R.; Merbach, A. E. *J. Biol. Inorg. Chem.* **2001**, 6, 247.
78. André, J. P.; Tóth, É.; Maecke, H. R.; Merbach, A. E. *Chem. Eur. J.* **1999**, 5, 2977.
79. Nicolle, G. M.; Tóth, É.; Eisenwiener, K.-P.; Maecke, H.; Merbach, A. E. *J. Biol. Inorg. Chem.* **2002**, 7, 757.
80. Torres, S.; André, J. P.; Martins, J. A.; Geraldès, C. F. G. C.; Merbach, A. E.; Tóth, É. *Chem. Eur. J.* **2006**, 12, 940.
81. Muller, R. N.; Radüchel, B.; Laurent, S.; Platzek, J.; Pierart, C.; Mareski, P.; Vander Elst, L. *Eur. J. Inorg. Chem.* **1999**, 1949.
82. Aime, S.; Chiaussa, M.; Digilio, G.; Gianolio, E.; Terreno, E. *J. Biol. Inorg. Chem.* **1999**, 4, 766.
83. Thompson, M. K.; Botta, M.; Nicolle, G.; Helm, L.; Aime, S.; Merbach, A. E.; Raymond, K. N. *J. Am. Chem. Soc.* **2003**, 125, 14274.
84. Caravan, P.; Amedio, J. C., Jr.; Dunham, S. U.; Greenfield, M. T.; Cloutier, N. J.; McDermid, S. A.; Spiller, M.; Zech, S. G.; Looby, R. J.; Raitsimring, A.; McMurry, T.; Lauffer, R. B. *Chem. Eur. J.* **2005**, 11, 5866.
85. Tóth, É.; Ni Dhubhghaill, O. M.; Besson, G.; Helm, L.; Merbach, A. E. *Magn. Res. Chem.* **1999**, 37, 701.
86. Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, 104, 4546.
87. Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, 104, 4559.
88. Rudovský, J.; Botta, M.; Hermann, P.; Hardcastle, K. I.; Lukeš, I.; Aime, S. *Bioconj. Chem.* **2006**, 17, 975.
89. Avedano, S.; Tei, L.; Lombardi, A.; Giovenzana, G. B.; Aime, S.; Longo, D.; Botta, M. *Chem. Commun.* **2007**, 4726.
90. Jászberényi, Z.; Moriggi, L.; Schmidt, P.; Weidensteiner, C.; Kneuer, R.; Merbach, A. E.; Helm, L.; Tóth, É. *J. Biol. Inorg. Chem.* **2007**, 12, 406.
91. Fulton, D. A.; O'Halloran, M.; Parker, D.; Senanayake, K.; Botta, M.; Aime, S. *Chem. Commun.* **2005**, 474.
92. Fulton, D. A.; Elemento, E. M.; Aime, S.; Chaabane, L.; Botta, M.; Parker, D. *Chem. Commun.* **2006**, 1064.

93. Vander Elst, L.; Port, M.; Raynal, I.; Simonot, C.; Muller, R. N. *Eur. J. Inorg. Chem.* **2003**, 2495.
94. Laurent, S.; Botteman, F.; Vander Elst, L.; Muller, R. N. *Helv. Chim. Acta* **2004**, 87, 1077.
95. Laurent, S.; Botteman, F.; Vander Elst, L.; Muller, R. N. *Magn. Reson. Mater. Phys. Biol. Med. (MAGMA)* **2004**, 16, 235.
96. Vander Elst, L.; Raynal, I.; Port, M.; Tisnès, P.; Muller, R. N. *Eur. J. Inorg. Chem.* **2005**, 1142.
97. Comblin, V.; Gilsoul, D.; Hermann, M.; Humblet, V.; Jacques, V.; Mesbahi, M.; Sauvage, C.; Desreux, J. F. *Coord. Chem. Rev.* **1999**, 185–186, 451.
98. Ruloff, R.; van Koten, G.; Merbach, A. E. *Chem. Commun.* **2004**, 842.
99. Costa, J.; Ruloff, R.; Burai, L.; Helm, L.; Merbach, A. E. *J. Am. Chem. Soc.* **2005**, 127, 5147.
100. Hudson, A.; Lewis, L. W. E. *Trans. Faraday Soc.* **1970**, 66, 1297.
101. Powell, D. H.; Merbach, A. E.; González, G.; Brücher, E.; Micskei, K.; Ottaviani, M. F.; Köhler, K.; von Zelewsky, A.; Grinberg, O. Y.; Lebedev, Y. S. *Helv. Chim. Acta* **1993**, 76, 2129.
102. Rast, S.; Fries, H. P.; Belorizky, E. *J. Chem. Phys.* **2000**, 113, 8724.
103. Rast, S.; Borel, A.; Helm, L.; Belorizky, E.; Fries, H. P.; Merbach, A. E. *J. Am. Chem. Soc.* **2001**, 123, 2637.
104. Rast, S.; Fries, H. P.; Belorizky, E.; Borel, A.; Helm, L.; Merbach, A. E. *J. Chem. Phys.* **2001**, 123, 2637.
105. Borel, A.; Yerly, F.; Helm, L.; Merbach, A. E. *J. Am. Chem. Soc.* **2002**, 124, 2042.
106. McConnell, J. R. *“The Theory of Nuclear Magnetic Relaxation in Liquids”*; University Press: Cambridge, **1987**.
107. Abragam, A. *“The Principles of Nuclear Magnetism”*; Oxford University: Oxford, **1961**.
108. Fries, P. H.; Belorizky, E. *J. Phys. (Paris)* **1978**, 39, 1263.
109. Albrand, J. P.; Taieb, M. C.; Fries, P. H.; Belorizky, E. *J. Chem. Phys.* **1983**, 78, 5809.
110. Hwang, L.-P.; Freed, J. H. *J. Chem. Phys.* **1975**, 63, 1975.
111. Freed, J. H. *J. Chem. Phys.* **1978**, 68, 4034.
112. Polnaszek, C. F.; Bryant, R. G. *J. Chem. Phys.* **1984**, 81, 4038.
113. Aime, S.; Botta, M.; Geninatti Crich, S.; Giovenzana, G.; Pagliarin, R.; Piccinini, M.; Sisti, M.; Terreno, E. *J. Biol. Inorg. Chem.* **1997**, 2, 470.
114. Botta, M. *Eur. J. Inorg. Chem.* **2000**, 399.
115. Trättnig, S.; Pinker, K.; Ba-Ssalamh, A.; Nöbauer-Huhmann, I.-M. *Eur. Radiol.* **2006**, 16, 1280.
116. Livramento, J. B.; Tóth, É.; Sour, A.; Borel, A.; Merbach, A. E.; Ruloff, R. *Angew. Chem. Int. Ed.* **2005**, 44, 1480.
117. Pierre, V. C.; Botta, M.; Raymond, K. N. *J. Am. Chem. Soc.* **2005**, 127, 504.
118. Tweedle, M. F. In: *“Lanthanide Probes in Life, Chemical and Earth Sciences: Theory and Practice”*; Eds. Bünzli, J.-C. G.; Choppin, G. R.; Elsevier: Amsterdam, **1989**, p. 127.
119. Weinmann, R. J.; Laniado, M.; Muetzel, W. *Physiol. Chem. Phys. Med. NMR* **1984**, 16, 167.
120. Chang, C. A. *Eur. J. Solid State Inorg. Chem.* **1991**, 28, 237.
121. Brücher, E.; Sherry, A. D. In: *“The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging”*; Eds. Tóth, É.; Merbach, A. E.; Wiley: Chichester, **2001**, p. 243.

122. (a) Morcos, S. K. *Br. J. Radiol.* **2007**, *80*, 73. (b) Schmitt-Willich, H. *Br. J. Radiol.* **2007**, *80*, 581. (c) Tweedle, M. F. *Br. J. Radiol.* **2007**, *80*, 583. (d) Morcos, S. K. *Br. J. Radiol.* **2007**, *80*, 584.
123. Markmann, P.; Skov, L.; Rossen, K.; Dupont, A.; Damholt, M. B.; Heaf, J. G.; Thomsen, H. S. *J. Am. Soc. Nephrol.* **2006**, *17*, 2359.
124. Cheng, S.; Abramova, L.; Saab, G.; Turabelidze, G.; Patel, P.; Arduino, M.; Hess, T.; Kallen, A.; Jhung, M. *J. Am. Chem. Soc.* **2007**, *297*, 1542.
125. Bellin, M. F.; Vasile, M.; Morel-Precetti, S. *Eur. Radiol.* **2003**, *13*, 2688.
126. Staks, T.; Schuhmann-Giampieri, G.; Frenzel, T.; Weinmann, H. J.; Lange, L.; Platzek, J. *Invest. Radiol.* **1994**, *29*, 709.
127. Joffe, P.; Thomsen, H. S.; Meusel, M. *Acad. Radiol.* **1998**, *5*, 491.
128. High, W. A.; Ayers, R. A.; Chandler, J.; Zito, G.; Cowper, S. E. *Acad. Dermatol.* **2007**, *56*, 21.
129. Beck, M. T.; Nagypál, I. "Chemistry of Complex Equilibria"; Ellis Horwood Ltd: Chichester, **1990**.
130. Burai, L.; Fabián, I.; Kiraly, R.; Szilagyi, E.; Brücher, E. *J. Chem. Soc. Dalton Trans.* **1998**, 243.
131. Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *190*, 37.
132. Martell, A. E.; Smith, R. M. "NIST Standard Reference Database 46 (Critically Selected Stability Constants of Metal Complexes), Version 7.0"; **2003**.
133. Ringbom, A. (Ed.), "Complexation in Analytical Chemistry"; Interscience Publishers: New York, **1963**.
134. Cacheris, W. P.; Quay, S. C.; Rocklage, S. M. *Magn. Reson. Imag.* **1990**, *8*, 467.
135. Idee, J.-M.; Port, M.; Raynal, I.; Schaefer, M.; Le Greneur, S.; Corot, C. *Fundam. Clin. Pharmacol.* **2006**, *20*, 563.
136. Wedeking, P.; Kumar, K.; Tweedle, M. F. *Magn. Reson. Imag.* **1992**, *10*, 641.
137. Broan, C. J.; Cox, J. P. L.; Craig, A. S.; Katakya, R.; Parker, D.; Ferguson, G.; Harrison, A.; Randall, A. M. *J. Chem. Soc. Perkin Trans.* **1991**, *2*, 87.
138. Lazar, I.; Sherry, A. D.; Ramasamy, R.; Brücher, E.; Kiraly, R. *Inorg. Chem.* **1991**, *30*, 5016.
139. Harrison, A.; Royle, L.; Walker, C.; Pereira, C.; Parker, D.; Pulukkody, K.; Norman, T. J. *Magn. Res. Imag.* **1993**, *11*, 761.
140. Pulukkody, K. P.; Norman, T. J.; Parker, D.; Royle, L.; Broan, C. J. *J. Chem. Soc. Perkin Trans.* **1993**, 605.
141. Tweedle, M. F.; Hagan, J. J.; Kumar, K.; Mantha, S.; Chang, C. A. *Magn. Res. Imag.* **1991**, *9*, 409.
142. Magerstadt, M.; Gansow, O. A.; Brechbiel, M. W.; Colcher, D.; Baltzer, L.; Knop, R. H.; Girton, M. E.; Naegele, M. *Magn. Reson. Med.* **1986**, *3*, 808.
143. Sarka, L.; Burai, L.; Brücher, E. *Chem. Eur. J.* **2000**, *6*, 719.
144. (a) Laurent, S.; Vander Elst, L.; Copoix, F.; Muller, R. N. *Invest. Radiol.* **2001**, *36*, 115. (b) Laurent, S.; Vander Elst, L.; Muller, R. N. *Contrast Med. Mol. Imag.* **2006**, *1*, 128.
145. Brücher, E. In: "Topics in Current Chemistry: Contrast Agents I. Magnetic Resonance Imaging"; vol. 221; Ed. Krause, W.; Springer-Verlag: Berlin, **2002**, p. 104.
146. Schwizer, W.; Fraser, R.; Maecke, H.; Siebold, K.; Funk, R.; Fried, M. *Magn. Reson. Med.* **1994**, *31*, 388.
147. Jászberényi, Z.; Tóth, É.; Kálai, T.; Király, R.; Burai, L.; Brücher, E.; Merbach, A. E.; Hideg, K. *Dalton Trans.* **2005**, 694.

148. Sarka, L.; Burai, L.; Király, R.; Zékány, L.; Brücher, E. *J. Inorg. Biochem.* **2002**, *91*, 320.
149. Baranyai, Z.; Brücher, E.; Ivanyi, T.; Király, R.; Lázár, I.; Zékány, L. *Helv. Chim. Acta* **2005**, *88*, 604.
150. Pasha, A.; Tircsó, G.; Benyó, E. T.; Brücher, E.; Sherry, A. D. *Eur. J. Inorg. Chem.* **2007**, 4340.
151. Tóth, É.; Király, R.; Platzek, J.; Radüchel, B.; Brücher, E. *Inorg. Chim. Acta* **1996**, *249*, 191.
152. Balogh, E.; Tripier, R.; Fousková, P.; Reviriego, F.; Handel, H.; Tóth, É. *Dalton Trans.* **2007**, 3572.
153. Kotek, J.; Kálmán, F. K.; Hermann, P.; Brücher, E.; Binnemans, K.; Lukeš, I. *Eur. J. Inorg. Chem.* **2006**, 1976.
154. Tweedle, M. F. *Magn. Res. Imag.* **1992**, *10*, 641.
155. Tweedle, M. F.; Wedeking, P.; Kumar, K. *Invest. Radiol.* **1995**, *30*, 372.
156. (a) Gibby, W. A.; Gibby, K. A. *Invest. Radiol.* **2004**, *39*, 138. (b) Gibby, W. A.; Gibby, K. A.; Tweedle, M. F. *Invest. Radiol.* **2006**, *41*, 272.
157. Grobner, T. *Nephrol. Dial. Transplant.* **2006**, *21*, 1104.
158. Wolf, S. D.; Balaban, R. S. *Magn. Reson. Med.* **1989**, *10*, 135.
159. Woods, M.; Woessner, D. E.; Sherry, A. D. *Chem. Soc. Rev.* **2006**, *35*, 500.
160. Woessner, D. E.; Zhang, S.; Merritt, M. E.; Sherry, A. D. *Magn. Reson. Med.* **2005**, *53*, 790.
161. Aime, S.; Delli Castelli, D.; Terreno, E. *Angew. Chem. Int. Ed.* **2002**, *41*, 4334.
162. Zhang, S.; Winter, P.; Wu, K.; Sherry, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 1517.
163. Zhang, S.; Michaudet, L.; Burgess, S.; Sherry, A. D. *Angew. Chem. Int. Ed.* **2002**, *41*, 1919.
164. Aime, S.; Carrera, C.; Delli Castelli, D.; Geninatti Cich, S.; Terreno, E. *Angew. Chem. Int. Ed.* **2005**, *44*, 1813.
165. Yoo, B.; Pagel, M. D. *Bioconj. Chem.* **2007**, *18*, 903.
166. Wojciechowski, F.; Suchy, M.; Li, A. X.; Azab, H. A.; Bartha, R.; Hudson, R. H. E. *Bioconj. Chem.* **2007**, *18*, 1625.
167. Ratnakar, S. J.; Woods, M.; Lubag, A. J. M.; Kovács, Z.; Sherry, A. D. *J. Am. Chem. Soc.* **2008**, *130*, 6.
168. Vinogradov, E.; Zhang, S.; Lubag, A.; Balschi, J. A.; Sherry, A. D.; Lenkinski, R. E. *J. Magn. Res.* **2005**, *176*, 54.
169. Adair, C.; Woods, M.; Zhao, P.; Pasha, A.; Winter, P. M.; Lanza, G. M.; Athey, P.; Sherry, A. D.; Kiefer, G. E. *Contrast Med. Mol. Imag.* **2007**, *2*, 55.
170. Winter, P. M.; Cai, K.; Chen, J.; Adair, C. R.; Kiefer, G. E.; Athey, P. S.; Gaffney, P. J.; Buff, C. E.; Robertson, J. D.; Caruthers, S. D.; Wickline, S. A.; Lanza, G. M. *Magn. Reson. Med.* **2006**, *56*, 1384.
171. Vasalatiy, O.; Gerard, R. D.; Zhao, P.; Sun, X.; Sherry, A. D. *Bioconj. Chem.* **2008**, *19*, 598.
172. Ali, M. M.; Woods, M.; Suh, E. H.; Kovacs, Z.; Tircsó, G.; Zhao, P.; Kodibagkar, V. D.; Sherry, A. D. *J. Biol. Inorg. Chem.* **2007**, *12*, 855.
173. Aime, S.; Delli Castelli, D.; Terreno, E. *Angew. Chem. Int. Ed.* **2005**, *44*, 5513.
174. Terreno, E.; Cabella, C.; Carrera, C.; Delli Castelli, D.; Mazzon, R.; Rollet, S.; Stancanella, J.; Visigalli, M.; Aime, S. *Angew. Chem. Int. Ed.* **2007**, *46*, 966.
175. Terreno, E.; Delli Castelli, D.; Milone, L.; Rollet, S.; Stancanella, J.; Violante, E.; Aime, S. *Contrast Med. Mol. Imag.* **2008**, *3*, 38.

176. Zhao, J. M.; Harel, Y.; McMahon, M. T.; Zhou, J.; Sherry, A. D.; Sgouros, G.; Bulte, J. W. M.; van Zijl, P. C. M. *J. Am. Chem. Soc.* **2008**, *130*, 5178.
177. Querol, M.; Bogdanov, A. *J. Magn. Reson. Imag.* **2006**, *24*, 971.
178. Yoo, B.; Pagel, M. D. *Front. Biosci.* **2008**, *13*, 1733.
179. Terreno, E.; Delli Castelli, D.; Cravotto, G.; Milone, L.; Aime, S. *Invest. Radiol.* **2004**, *39*, 235.
180. Zhang, S.; Malloy, C. R.; Sherry, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 17572.
181. Li, A. X.; Wojciechowski, F.; Suchy, M.; Jones, C. K.; Hudson, R. H. E.; Menon, R. S.; Bartha, R. *Magn. Reson. Med.* **2008**, *59*, 374.
182. Aime, S.; Barge, A.; Delli Castelli, D.; Fedeli, F.; Mortillaro, A.; Nielsen, F. U.; Terreno, E. *Magn. Res. Med.* **2002**, *47*, 639.
183. Zhang, S.; Trokowski, R.; Sherry, A. D. *J. Am. Chem. Soc.* **2003**, *125*, 15288.
184. Trokowski, R.; Zhang, S.; Sherry, A. D. *Bioconj. Chem.* **2004**, *15*, 1431.
185. Aime, S.; Delli Castelli, D.; Fedeli, F.; Terreno, E. *J. Am. Chem. Soc.* **2002**, *124*, 9364.
186. Liu, G.; Li, Y.; Pagel, M. D. *Magn. Reson. Med.* **2007**, *58*, 1249.
187. Chen, J. W.; Pham, W.; Weissleder, R.; Bogdanov, A. *Magn. Reson. Med.* **2004**, *52*, 1021.
188. Querol, M.; Chen, J. W.; Weissleder, R.; Bogdanov, A. *Org. Lett.* **2005**, *7*, 1719.
189. Chen, J. W.; Querol, M.; Bogdanov, A.; Weissleder, R. *Radiology* **2006**, *240*, 473.
190. Querol, M.; Chen, J. W.; Bogdanov, A. *Org. Biomol. Chem.* **2006**, *4*, 1887.
191. Querol, M.; Bennett, D. G.; Sotak, C.; Won Kang, H.; Bogdanov, A. *ChemBioChem.* **2007**, *8*, 1637.
192. Mazooz, G.; Mehlman, T.; Lai, T.-S.; Greenberg, C. S.; Dewhirst, M. W.; Neeman, M. *Cancer Res.* **2005**, *65*, 1369.
193. Shiftan, L.; Israely, T.; Cohen, M.; Frydman, V.; Dafni, H.; Stern, R.; Neeman, M. *Cancer Res.* **2005**, *65*, 10316.
194. Shiftan, L.; Neeman, M. *Contrast Med. Mol. Imag.* **2006**, *1*, 106.
195. Himmelreich, U.; Aime, S.; Hieronymus, T.; Justicia, C.; Uggeri, F.; Zenke, M.; Hoehna, M. *NeuroImage* **2006**, *32*, 1142.
196. Lauffer, R.; McMurry, T. J.; Dunham, S. O.; Scott, D. M.; Parmelee, D. J.; Dumas, S.; "WIPO Patent Application 97/36619"; **2001**.
197. Hanaoka, K.; Kikuchi, K.; Terai, T.; Komatsu, T.; Nagano, T. *Chem. Eur. J.* **2008**, *14*, 987.
198. Chang, Y.-T.; Cheng, Ch.-M.; Su, Y.-Z.; Lee, W.-T.; Hsu, J.-S.; Liu, G.-Ch.; Cheng, T.-L.; Wang, Y.-M. *Bioconj. Chem.* **2007**, *18*, 1716.
199. Nivorozhkin, A. L.; Kolodziej, A. F.; Caravan, P.; Greenfield, M. T.; Lauffer, R. B.; McMurry, T. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 2903.
200. Aime, S.; Cabella, C.; Colombatto, S.; Geninatti Crich, S.; Gianolio, E.; Maggioni, F. *J. Magn. Reson. Imag.* **2002**, *16*, 394.
201. Moats, R. A.; Fraser, S. E.; Meade, T. J. *Angew. Chem. Int. Ed.* **1997**, *36*, 726.
202. Louie, A. Y.; Hüber, M. M.; Ahrens, E. T.; Rothbächer, U.; Moats, R.; Jacobs, R. E.; Fraser, E.; Meade, T. J. *Nat. Biotechnol.* **2000**, *18*, 321.
203. Urbanczyk-Pearson, L. M.; Femia, F. J.; Smith, J.; Parigi, G.; Duimstra, J. A.; Eckermann, A. L.; Luchinat, C.; Meade, T. J. *Inorg. Chem.* **2008**, *47*, 56.
204. Yoo, B.; Pagel, M. D. *J. Am. Chem. Soc.* **2006**, *128*, 14032.

205. Yoo, B.; Raam, M. S.; Rosenblum, R. M.; Pagel, M. D. *Contrast Med. Mol. Imag.* **2007**, 2, 189.
206. Chauvin, T.; Durand, P.; Bernier, M.; Meudal, H.; Doan, B. T.; Noury, F.; Badet, B.; Beloeil, J. C.; Tóth, É. *Angew. Chem. Int. Ed.* **2008**, 47, 4370.
207. Li, W.; Fraser, S. E.; Meade, T. J. *J. Am. Chem. Soc.* **1999**, 121, 1413.
208. Li, W.; Parigi, G.; Fragai, M.; Luchinat, C.; Meade, T. J. *Inorg. Chem.* **2002**, 41, 4018.
209. Dhingra, K.; Fousková, P.; Angelovski, G.; Maier, M. E.; Logothetis, N. K.; Tóth, É. *J. Biol. Inorg. Chem.* **2008**, 13, 35.
210. Mishra, A.; Fousková, P.; Angelovski, G.; Balogh, E.; Mishra, A. K.; Logothetis, N. K.; Tóth, É. *Inorg. Chem.* **2008**, 47, 1370.
211. Angelovski, G.; Fousková, P.; Mamedov, I.; Canals, S.; Tóth, É.; Logothetis, N. K. *ChemBioChem.* **2008**, 9, 1729.
212. Major, J. L.; Parigi, G.; Luchinat, C.; Meade, T. J. *Proc. Natl. Acad. Sci. USA* **2007**, 104, 13881.
213. Que, E. L.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, 128, 15942.
214. Dhingra, K.; Maier, M. E.; Beyerlein, M.; Angelovski, G.; Logothetis, N. K. *Chem. Commun.* **2008**, 3444.
215. Hanaoka, K.; Kikuchi, K.; Urano, Y.; Nagano, T. *J. Chem. Soc. Perkin Trans.* **2001**, 2, 1840.
216. Hanaoka, K.; Kikuchi, K.; Urano, Y.; Narazaki, M.; Yokawa, T.; Sakamoto, S.; Yamaguchi, K.; Nagano, T. *Chem. Biol.* **2002**, 9, 1027.
217. Hifumi, H.; Tanimoto, A.; Citterio, D.; Komatsua, H.; Suzuki, K. *Analyst* **2007**, 132, 1153.
218. Trokowski, R.; Ren, J.; Kálmán, F. K.; Sherry, A. D. *Angew. Chem. Int. Ed.* **2005**, 44, 6920.
219. Kubíček, V.; Rudovský, J.; Kotek, J.; Hermann, P.; Vander Elst, L.; Muller, R. N.; Kolar, Z. I.; Wolterbeek, H. T.; Peters, J. A.; Lukeš, I. *J. Am. Chem. Soc.* **2005**, 127, 16477.
220. Vitha, T.; Kubíček, V.; Hermann, P.; Vander Elst, L.; Muller, R. N.; Kolar, Z. I.; Wolterbeek, H. T.; Breeman, W. A. P.; Lukeš, I.; Peters, J. A. *J. Med. Chem.* **2008**, 51, 677.
221. Mamourian, A. C.; Burnett, K. R.; Goldstein, E. J.; Wolf, G. L.; Kressel, H. Y.; Baum, S. *Physiol. Chem. Phys. Med. NMR* **1984**, 16, 123.
222. Kang, Y. S.; Gore, J. C.; Armitage, I. M. *Magn. Reson. Med.* **1984**, 1, 396.
223. Crossgrove, J.; Zheng, W. *NMR Biomed.* **2004**, 17, 544.
224. Wolf, G. L.; Baum, L. *Am. J. Roentgenol.* **1983**, 141, 193.
225. *NMR Biomed.* **2004**, 17(8): volume dedicated to manganese-enhanced magnetic resonance imaging.
226. Elizondo, G.; Fretz, C. J.; Stark, D. D.; Rocklage, S. M.; Quay, S. C.; Worah, D.; Tsang, Y. M.; Chen, M. C.; Ferrucci, J. T. *Radiology* **1989**, 178, 73.
227. Pomeroy, O. H.; Wendland, M.; Wagner, S.; Derugin, N.; Holt, W. W.; Rocklage, S. M.; Quay, S.; Higgins, C. B. *Invest. Radiol.* **1989**, 24, 531.
228. Tirkkonen, B.; Aukrust, A.; Couture, E.; Grace, D.; Haile, Y.; Holm, K. M.; Hope, H.; Larsen, A.; Lunde, H. S.; Sjogren, C. E. *Acta Radiol.* **1997**, 38, 780.
229. Brurok, H.; Skoglund, T.; Berg, K.; Skarra, S.; Karlsson, J. O. G.; Jynge, P. *NMR Biomed.* **1999**, 12, 364.
230. Gerald, C. F. G. C.; Sherry, A. D.; Brown, R. D.; Koenig, S. H. *Magn. Reson. Med.* **1986**, 3, 242.
231. Zetter, M. S.; Dodgen, H. W.; Hunt, J. P. *Biochemistry* **1973**, 12, 778.

232. Dees, A.; Zahl, A.; Puchta, R.; van Eikema Hommes, N. J. R.; Heinemann, F. W.; Ivanovic-Burmazovic, I. *Inorg. Chem.* **2007**, *46*, 2459.
233. Aime, S.; Anelli, P. L.; Botta, M.; Brocchetta, M.; Canton, S.; Fedeli, F.; Gianolio, E.; Terreno, E. *J. Biol. Inorg. Chem.* **2002**, *7*, 58.
234. Kruk, D.; Kowalewski, J. *J. Biol. Inorg. Chem.* **2003**, *8*, 512.
235. Troughton, J. S.; Greenfield, M. T.; Greenwood, J. M.; Dumas, S.; Wiethoff, A. J.; Wang, J.; Spiller, M.; McMurry, T. J.; Caravan, P. *Inorg. Chem.* **2004**, *43*, 6313.
236. Grant, M.; Dodgen, H. W.; Hunt, J. P. *Inorg. Chem.* **1971**, *10*, 71.
237. Zetter, M. S.; Grant, M.; Wood, E. J.; Dodgen, H. W.; Hunt, J. P. *Inorg. Chem.* **1972**, *11*, 2701.
238. Liu, G.; Dodgen, H. W.; Hunt, J. P. *Inorg. Chem.* **1977**, *16*, 2652.
239. Ducommun, Y.; Newmann, K. E.; Merbach, A. E. *Inorg. Chem.* **1980**, *19*, 3696.
240. Balogh, E.; He, Z.; Hsien, W.; Liu, S.; Tóth, É. *Inorg. Chem.* **2007**, *46*, 238.
241. Bertini, I.; Briganti, F.; Xiu, Z.; Luchinat, C. *J. Magn. Reson. Imag.* **1993**, *101*, 198.
242. Pan, D.; Caruthers, S. D.; Hu, G.; Senpan, A.; Scott, M. J.; Gaffney, P. J.; Wickline, S. A.; Lanza, G. M. *J. Am. Chem. Soc.* **2008**, *130*, 9186.
243. Aime, S.; Botta, M.; Gianolio, E.; Terreno, E. *Angew. Chem. Int. Ed.* **2000**, *39*, 747.
244. Bianchi, A.; Calabi, L.; Giorgi, C.; Losi, P.; Mariani, P.; Palano, D.; Paoli, P.; Rossi, P.; Valtancoli, B. *J. Chem. Soc. Dalton Trans.* **2001**, 917.
245. Gallez, B.; Baudelet, C.; Geurts, M. *Magn. Reson. Imag.* **1998**, *16*, 1211.
246. Zhang, S.; Sun, D.; Li, X.; Pei, F.; Liu, S. *Fullerene Sci. Technol.* **1997**, *5*, 1635.
247. Wilson, L. J. *The Electrochemical Society Interface*, **1999**, winter, p. 24.
248. Mikawa, M.; Kato, H.; Okumura, M.; Narazaki, M.; Kanazawa, Y.; Miwa, N.; Shinohara, H. *Bioconj. Chem.* **2001**, *12*, 510.
249. Kato, H.; Kanazawa, Y.; Okumura, M.; Taninaka, A.; Yokawa, T.; Shinohara, H. *J. Am. Chem. Soc.* **2003**, *125*, 4391.
250. Bolskar, R. D.; Benedetto, A. F.; Husebo, L. O.; Price, R. E.; Jackson, E. F.; Wallace, S.; Wilson, L. J.; Alford, J. M. *J. Am. Chem. Soc.* **2003**, *125*, 5471.
251. Tóth, É.; Bolskar, R. D.; Borel, A.; Gonzalez, G.; Helm, L.; Merbach, A. E.; Sitharaman, B.; Wilson, L. J. *J. Am. Chem. Soc.* **2005**, *127*, 799.
252. Sitharaman, B.; Bolskar, R. D.; Rusakova, I.; Wilson, L. J. *Nano. Lett.* **2004**, *4*, 2373.
253. Jeng, U.-S.; Lin, T.-L.; Chang, T.-S.; Lee, H.-Y.; Hsu, C.-H.; Hsieh, Y.-W.; Canteenwala, T.; Chiang, L. Y. *Prog. Colloid Polym. Sci.* **2001**, *118*, 232.
254. Rudalevige, T.; Francis, A. H.; Zand, R. *J. Phys. Chem. A* **1998**, *102*, 9797.
255. Guldi, D. M. *J. Phys. Chem. A* **1997**, *101*, 3895.
256. Laus, S.; Sitharaman, B.; Tóth, É.; Bolskar, R. D.; Helm, L.; Asokan, S.; Wong, M. S.; Wilson, L. J.; Merbach, A. E. *J. Am. Chem. Soc.* **2005**, *127*, 9368.
257. Laus, S.; Sitharaman, B.; Tóth, É.; Bolskar, R. D.; Helm, L.; Wilson, L. J.; Merbach, A. E. *J. Phys. Chem. C* **2007**, *111*, 5633.
258. Martin, C. R.; Kohli, P. *Nat. Rev. Drug Discov.* **2003**, *2*, 29.
259. Gu, Z.; Peng, H.; Hauge, R. H.; Smalley, R. E.; Margrave, J. L. *Nano Lett.* **2002**, *2*, 1009.
260. Mackeyev, Y. A.; Marks, J. W.; Rosenblum, M. G.; Wilson, L. J. *J. Phys. Chem. B* **2005**, *109*, 5482.
261. Sitharaman, B.; Kissell, K. R.; Hartman, K. B.; Tran, L. A.; Baikalov, A.; Rusakova, I.; Sun, Y.; Khant, H. A.; Ludtke, S. J.; Chiu, W.; Laus, S.; Tóth, É.; Helm, L.; Merbach, A. E.; Wilson, L. J. *Chem. Commun.* **2005**, 3915.

- 262. Sitharaman, B.; Wilson, L. J. *Int. J. Nanomed.* **2006**, *1*, 291.
- 263. Hartman, K. B.; Laus, S.; Bolskar, R. D.; Muthupillai, R.; Helm, L.; Tóth, É.; Merbach, A. E.; Wilson, L. J. *Nano Lett.* **2008**, *8*, 415.
- 264. Richard, C.; Doan, B.-T.; Beloeil, J.-C.; Bessodes, M.; Tóth, É.; Scherman, D. *Nano Lett.* **2008**, *8*, 232.
- 265. Aime, S.; Barge, A.; Bruce, J. I.; Botta, M.; Howard, J. A. K.; Moloney, J. M.; Parker, D.; De Sousa, A. S.; Woods, M. *J. Am. Chem. Soc.* **1999**, *121*, 5762.
- 266. Zhang, S.; Sherry, A. D. *J. Solid State Chem.* **2003**, *171*, 38.

This page intentionally left blank

DESIGN CONSIDERATIONS TOWARDS SIMULTANEOUSLY RADIOLABELED AND FLUORESCENT IMAGING PROBES INCORPORATING METALLIC SPECIES

SOFIA I. PASCU^{a,b}, PHILIP A. WAGHORN^b, TIMOTHY CONRY^b, BONITA LIN^b, CATRIN JAMES^a and JAMEEL M. ZAYED^{b,c}

^aChemistry Department, University of Bath, Claverton Down BA2 7AY, United Kingdom

^bOxford Siemens Molecular Imaging Laboratory, Chemistry Research Laboratory,
University of Oxford, Mansfield Road OX1 3TA, Oxford, United Kingdom

^cUniversity Chemical Laboratories, University of Cambridge, Lensfield Road CB2 1EW,
Cambridge, United Kingdom

I. Introduction	131
A. The Problem	131
B. Probe Design Towards a Solution	135
II. Small-Molecule Metal-Based Probes and Their Biomedical Imaging Capabilities	137
A. SPECT Imaging Reagents Suitable for the Fluorescence Imaging of Living Cells	137
B. Nonconventional Metallic PET Isotopes and Their Fluorescence Imaging	143
C. Towards Hypoxic Selective Imaging Probes	148
D. Applications of Metalloporphyrins as Multimodal, Optical, and/or Radioactive Imaging Probes	156
E. Radiolabeled and Optically Tagged Targeted Nanoparticles	163
III. Conclusions	171
Acknowledgments	172
References	172

I. Introduction

A. THE PROBLEM

Molecular imaging is emerging as an expanding and exciting area of research with wide interest in designing novel imaging probes for biological targets. Molecular imaging of biological

systems has the potential to provide information on soft-tissue structure and function without resorting to surgery, and as such is a powerful noninvasive technique in oncology (1,2). Targets can be imaged *in vivo* by a range of molecular imaging methods to attain research and clinical objectives. Although magnetic resonance imaging (MRI) is the dominant soft-tissue imaging technique (3) *on the basis of its* high resolution, there is a great interest in designing ligands labeled with radioisotopes for delineating diseased tissues due to the increased sensitivity of radioimaging methods (4,5). The use of coordination compounds as contrast agents in diagnostics for medical imaging (positron emission tomography (PET), single-photon emission computed tomography (SPECT)) and MRI is receiving increasing attention. Metallic elements possessing radionuclides suitable for medical imaging include ^{99m}Tc , $^{186/188}\text{Re}$, ^{111}In , $^{66/67/68}\text{Ga}$ and $^{62/64/67}\text{Cu}$, and for neutron-capture therapy, B and Gd. The past decade in particular has witnessed a significant increase in activity in the area of radiopharmaceuticals suitable for both imaging and therapy, especially those containing Re, Cu, and Y isotopes.

Developments in both *radiometal-tagged* and *radiometal-essential vehicles* have been reviewed extensively (6–8).

Radiolabeled metallic complexes offer promising resolution, for high quality delineated imaging, using radioisotopes that are readily isolated from generators or cyclotrons (Fig. 1). Importantly, the concentration of isotope necessary for imaging is extremely low and as such relatively non-toxic doses of compound can be injected without compromising the sensitivity of the imaging method. This is crucial in terms of immediate applications since the health risks for patients are minimized. The main two techniques currently used in clinical practice for medical imaging utilizing radioisotopes to obtain the image are

- (a) SPECT, and
- (b) PET.

Currently, SPECT with ^{99m}Tc is the most widely used radioisotope in nuclear medical diagnostics due to its favorable nuclear properties (140 keV, γ emitter, half life ca. 6h), low cost and availability. The γ rays emitted can be detected by a γ camera, and images are then obtained using conventional mapping techniques. Commercial developments in the production of radiopharmaceuticals for diagnostic imaging using SPECT include Myoview[®] (which localizes in myocardial muscle) and Ceretec[®] (for SPECT scans of the brain showing cerebral

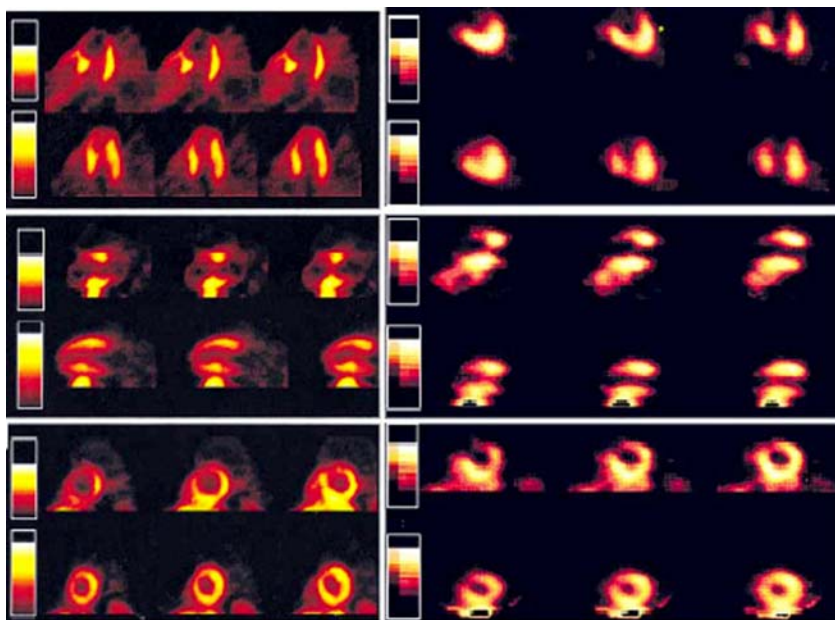


FIG. 1. Comparison of heart perfusion measurements made with the SPECT agent Cardiolite™, $[^{99\text{m}}\text{Tc}(\text{CNR})_6]^+$, where $\text{R} = -\text{CH}_2\text{C}(\text{Me})_2\text{OMe}$ (right) and the PET agent $[^{62}\text{Cu}(\text{PTSM})]$ (PTSM = Cu(II)-pyruvaldehyde bis(N^4 -methylthiosemicarbazonato), left). The higher resolution possible with PET is evident in LHS images (9). *Cited references for this, and following images, contain images of higher clarity.*

blood flow which can be used to image and locate areas of impaired flow due to strokes) (6–8).

PET uses positron (β^+)-emitting isotopes as the signaling unit. Positrons are antimatter and annihilate when they encounter an electron, giving rise to two photons 180° to one another. The emission of the photon pairs must be detected simultaneously to give rise to images showing the localization of the isotope (Fig. 2).

PET has been gaining in popularity as a result of its enhanced sensitivity and resolution with respect to SPECT at the millimetre scale, allowing clinicians to study drug distribution *in vivo* (10). Indeed, it is estimated that by 2010 there will be approximately two million PET scans per year, of which the majority will image the metabolism of the ^{18}F -labeled fluoro-deoxyglucose (FDG) (11). The increased sensitivity in turn reduces the concentration of the administered radiopharmaceutical required to 10^{-8} – 10^{-10} M. The much lower concentrations of administered radioactive probe employed reduce the overall

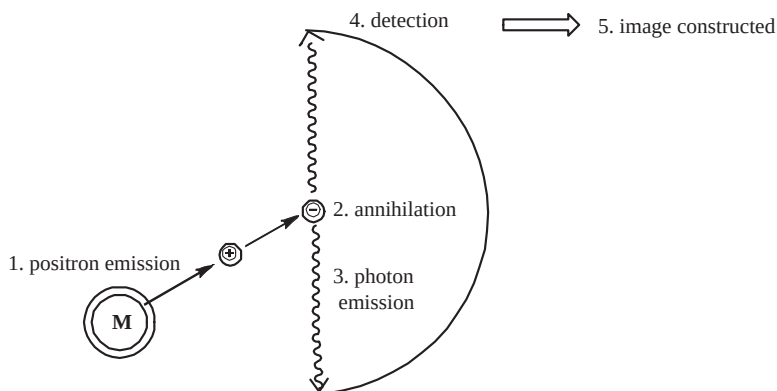


FIG. 2. From positron emission to image reconstruction in a PET scan.

radiation dose to the patient and hospital staff and lowers the likelihood of radiotoxic or cytotoxic effects after imaging (6, 8).

Common radioisotopes used for PET are ^{11}C (half-life 20 min), ^{18}F (half-life 110 min) and ^{15}O (half-life 2.05 min). Unconventional metallic radioisotopes in clinical settings are growing in popularity due to longer half-lives, hence bringing transportation, handling and derivatization advantages. Use of long-lived PET radioisotopes such as, for example, ^{55}Co (half-life 18 h), ^{64}Cu (half-life 12.7 h), ^{62}Cu (half-life 9 h), ^{66}Ga (half-life 9.5 h), ^{83}Sr (half-life 33 h), ^{94g}Tc (half-life 5 h), in diagnosis of degenerative diseases may be advantageous due to their matching of the slow pharmacokinetics. In particular, copper has several positron-emitting isotopes (^{60}Cu , ^{61}Cu , ^{62}Cu , and ^{64}Cu) with various half-lives, ranging from 9.7 min for ^{62}Cu to 12.7 h for ^{64}Cu . A variety of half-lives and radiation energy levels can be tailored for specific diagnosis needs. Importantly *positron emitting copper radioisotopes* are beginning to be of interest in the context of their high sensitivity and spatial resolution. Gallium is also of interest in radiodiagnostic medicines and is the basis of SPECT and PET agents with relatively long half-lives and availability from generators. Other emerging metallic isotopes for medical imaging are ^{90}Y (a high-energy beta emitter) used in conjunction *with* ^{111}In – a SPECT and Auger emitter – or more recently with ^{86}Y , an impure positron emitter. ^{89}Zr is a positron emitter with a long half-life (3.27 days) allowing for high tumor/background ratios, immuno-PET imaging, and antibody targeting (6, 8).

Other advantages of PET imaging with metallic radionuclides are facile radiolabeling (i.e. certain ^{64}Cu complexes radiolabel in less than 1 min at room temperature (12)) and generator availability (for ^{68}Ga).

PET and SPECT imaging methods may be used to follow the tissue/organ uptake and distribution of metal complexes of interest but, in both cases, the resolution of the imaging method is only appropriate for millimetre-level imaging: radiochemical imaging is highly effective for whole body imaging but it offers limited spatial resolution (6, 8). Thus subcellular processes cannot be investigated with accuracy using such methods. An understanding of the mechanisms of the delivery and localization of the imaging reagent at the cellular level will be beneficial for optimizing drug design. Whilst optical microscopy suffers from poor contrast, it offers the possibility of much greater sensitivity suitable for imaging work with nano- and micro-meter resolutions (13) than the radio-imaging methods.

There is currently significant interest in developing new imaging modalities capable of addressing imaging resolutions ranging from the millimetre to the nanometer scale, and in developing complementary imaging techniques towards multi-modality imaging/therapy applications.

B. PROBE DESIGN TOWARDS A SOLUTION

General considerations on common dual-modality imaging methods have been discussed in a recent review (14). The most commonly described dual-mode imaging methods have the ability to correlate anatomical information (for example from X-ray computed tomography (CT)) with functional measurements from radioimaging methods, such as SPECT or PET. This would play a role in improving disease diagnosis, for instance in the staging of cancer.

Dual-modality imaging is emerging as a method capable of improving the visual quality as well as increasing the quantitative accuracy of radioimaging for diagnosis of a variety of diseases including neurodegenerative disorders, cancer and heart disease. For mechanistic studies of the uptake and biodistribution in cells, the coupling of radioimaging with optical imaging in the same imaging probe is desirable. This new direction in dual mode imaging is still in its infancy. Although biological chromophores result in cell autofluorescence, this is predominantly in the blue region of the spectrum and can therefore be bypassed by using compounds that fluoresce at longer wavelengths as contrast agents. A number of fluorescent sensors have thus been designed for cell-imaging purposes with applications as selective metal ion detectors (15–17). Transition metals anchored on fluorescent ligands, as well as lanthanide complexes, have found potential as

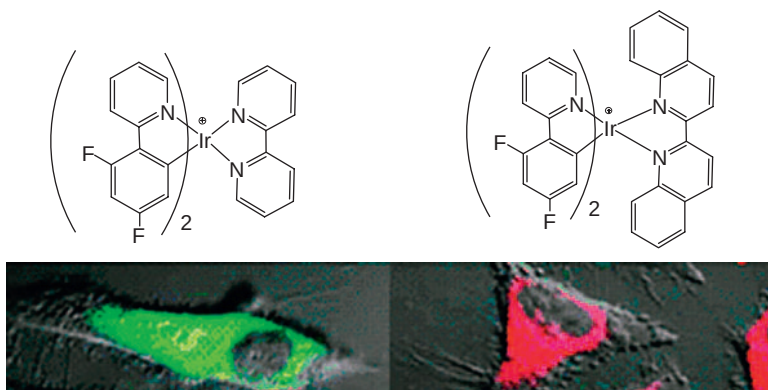


FIG. 3. Phosphorescence staining of the cytoplasm in living cells by iridium(III) complexes. Image adapted from Ref. (19).

markers for biological systems (Fig. 3) by virtue of their long fluorescence/luminescence lifetimes (18,19).

Our limited understanding with regard to subcellular-level events makes the use of fluorescence microscopy a desirable complementary technique to PET and SPECT. Currently there is only limited direct *in vitro* and *in vivo* comparison, and there has been little development in the underlying chemistry of suitable imaging agents. A system that could deliver simultaneous imaging at the cellular and tissue level with the same chemical species and thus identical pharmacokinetics is desirable. Several key design features of small molecule metal complexes suitable for simultaneous optical/PET imaging probes are:

- (a) Solubility in biologically compatible media.
- (b) High kinetic stability with respect to metal loss to nonspecific sites.
- (c) Rapid and clean conversion to radiolabeled analogs.
- (d) Incorporation of functional groups suitable for acting as an “address” for targeted delivery (if not self-targeted).
- (e) Strong intrinsic fluorescence intensity and long lifetime, coupled with solubility and stability in biologically compatible medium, facilitating the monitoring of cell delivery and bio-distribution of imaging probes *in vitro* as well as *in vivo*.

In the schematic representation shown in Fig. 4, the radio-nuclide core is supported by a bifunctional chelating ligand or encapsulating unit, which may be intrinsically fluorescent and decorated with one (or more) biologically active molecules that can bind to specific receptor sites *in vivo*. Such examples

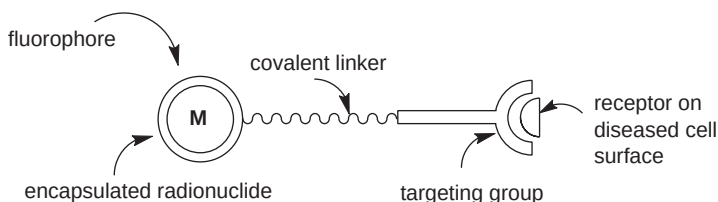


FIG. 4. General representation of the design concept for multimodal, targeted, radioimaging probes.

include favorable binding to antibody fragments for targeted delivery.

Some of the recent developments in molecular imaging are outlined here with a view to describe the overall design considerations for probes which may be simultaneously radiolabeled (for PET or SPECT) and investigated in cells by means of optical imaging. These systems are opening the way for dual-mode optical imaging/PET or optical imaging/SPECT methods. Systems ranging from small-molecule-based imaging probes, to supramolecular assemblies for imaging and/or therapy and advances in nanotechnology for multimodal imaging are under current exploration and intense interdisciplinary research in this field is currently underway in the UK and Worldwide.

In this review, the fluorescent agent suitable for radiolabeling is often a metal complex-based small molecule having the ability to enter living cells (intrinsically or when anchored on a targeting vector such as biomolecules, antibodies, or suitably-modified biocompatible nanoparticles). We explore the optical imaging in living cells (such as fluorescence and luminescence) as a complementary tool to PET and SPECT imaging methods. Therefore, its main current role is with respect to enhancing our understanding of the delivery mechanisms and activity of radiolabeled imaging agents within cells.

II. Small-Molecule Metal-Based Probes and Their Biomedical Imaging Capabilities

A. SPECT IMAGING REAGENTS SUITABLE FOR THE FLUORESCENCE IMAGING OF LIVING CELLS

The widest investigated species are those where ^{99m}Tc is conjugated to targeting biomolecules such as cyclic glycoprotein receptor antagonists, monoclonal antibodies, oligonucleotides, dopamine transporter ligands, targeting peptides, etc., which have

been reviewed extensively (20,21). Their main application (and, indeed, biomedical imaging potential) is in imaging tumors, neurodegenerative diseases, and hypoxia but many other applications have been considered (20,21). To date, the underlining synthetic chemistry developed has mainly been concerned with developing simple and efficient methods of linking technetium radioisotopes to the carrier molecule. Work on derivatizing peptides with a coordination framework for the metal center is dominated by the use of DOTA (DOTA is 1,4,7,10-tetraazacyclododecane-tetracetate) and TETA (TETA is triethylenetetramine also abbreviated as Trien) linkers, particularly in the context of ^{99m}Tc radiolabeling (e.g. Demotate (22)).

Peptide-derivatization of radionuclides has been used extensively, the advantages of these systems include their high affinity for the target, low toxicity, small size, hydrophilicity, and favorable pharmacokinetics, in addition to easy synthesis (and radiolabeling) routes.

Disadvantages include rapid degradation and high kidney uptake. Several peptide receptors are over-expressed in tumors, e.g. receptors for somatostatin (SST, with 14 or 28 amino acid cycle), vasoactive intestinal peptide (VIP), bombesin, CCK-B, neurotensin, opiod etc., and these peptides have been used to deliver SPECT imaging drugs to these targets (20,21). Imaging simultaneously with somatostatin and VIP has offered the possibility of concomitant multireceptor tumour targeting. Rapid degradation of SST *in vivo* has led to the development of octreotide. So far, interest in conjugating radioisotopes has been limited to SST and neuroendocrine tumors (Fig. 5) (23–26).

Recent work by the Valliant (27–29), Alberto (30–32), and Pelecanou (33) groups has aimed to combine fluorescence microscopy with SPECT radioimaging, mainly through the design of bifunctional chelate ligands specific for Tc. The synthetic chemistry work on Tc(I) has been carried out in parallel with that on the intrinsically fluorescent “cold” Re(I) analogues. New “cold” Re(I) fluorescent probes possessing long lifetimes and large stokes shifts were synthesized recently, with an aim to radiolabel these with radioisotopes in a further design step. Research by the Valliant group involves incorporating fluorophores into the coordination sphere of the metal as well as linking the resulting complex to peptides for achieving both targeting and biocompatibility. The Re(I) compound shown in Fig. 6a is a promising imaging probe with an excitation wavelength of 360 nm and emission between 400 and 550 nm range, since this window is favorable for bypassing the cell autofluorescence (Fig. 6b).

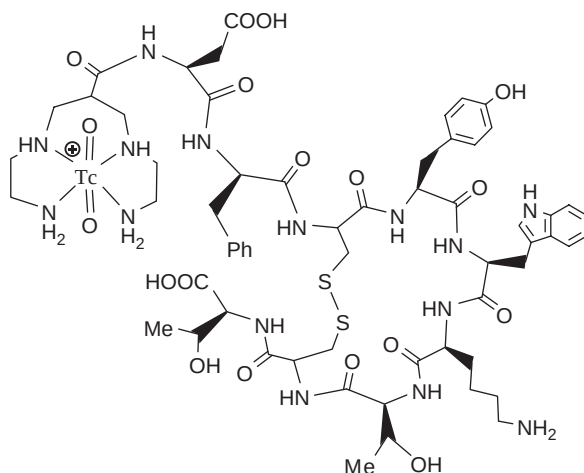


FIG. 5. Schematic representation of a ^{99m}Tc bioconjugate incorporating ocreotide.

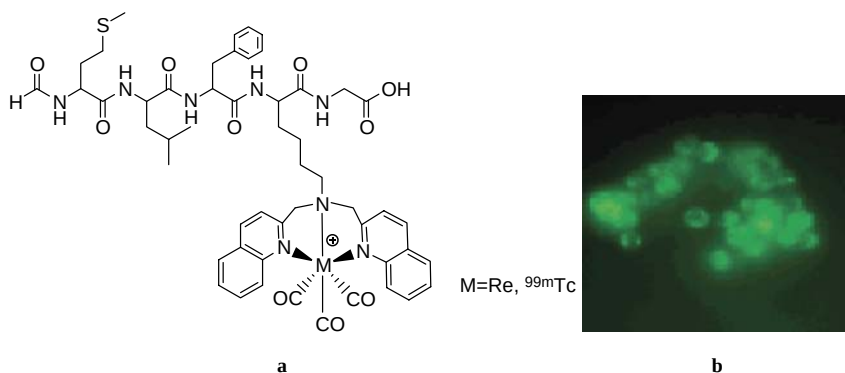


FIG. 6. Metallic imaging probes (a) and the fluorescence imaging (b) at the uptake of the Re(I) analog in tumor cells. Images adapted from Ref. (29).

Directly analogous (nonfluorescent), radiolabeled, ^{99m}Tc systems have also been described (27–29).

Trifunctional metal-based conjugates have been developed by the Alberto group, with probes consisting of a metallic radioisotope, a targeting group (i.e. bombesin, a gastrin releasing peptide (GRP) receptor) and a DNA intercalator (acridine orange). ^{99m}Tc compounds were shown to be radiotoxic when

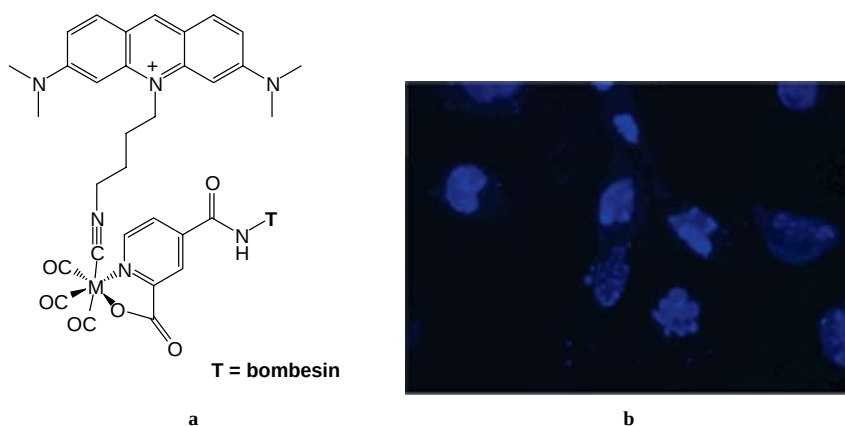


FIG. 7. A fluorescent Tc compound incorporating targeting unit (a) and its uptake in tumor cells by fluorescence imaging (b). Images adapted from Ref. (31).

located in close proximity to cellular DNA (Fig. 7a). Cell studies with and without the inclusion of the targeting unit (bombesin) were performed, in addition to studies on cells incorporating GRP receptors. Proof-of-principle studies suggested that the cell uptake of the conjugate is possible but its biodistribution into the cell nucleus was not evident (Fig. 7b).

Simultaneous imaging and therapy was not fully demonstrated, although a versatile design for the multifunctional probe was reported (30–32).

The charged derivatives of pyrene conjugated to a peptide capable of crossing the cell nucleus membrane have been synthesised (30,32) (Fig. 8). Studies with the $^{99\text{m}}\text{Tc}$ labelled species showed positive nuclear uptake and radiotoxicity. This probe, designed by the Alberto group, comprises a nuclear localizing peptide, a triamine tridentate ligand, and a DNA intercalating pyrene moiety and may be relevant to dual imaging and therapy methods. This is a small, monopositively charged complex capable of DNA interaction where the d^6 Tc(I) core is chemically inert. Furthermore, $^{99\text{m}}\text{Tc}$ provides Auger electrons that are known to induce double strand breaks in circular dsDNA, when the probe is directed to the cell nucleus. Cell uptake was followed by pyrene fluorescence showing that no nuclear uptake takes place in the absence of the targeting peptide. No decomposition was observed in biological medium. Studies with $^{99\text{m}}\text{Tc}$ uptake by cell lysing after 5 h incubation showed that 20% of the complex reaches the nucleus, unlike the pertechnetate control which showed a negligible nuclear uptake. Radiotoxicity studies

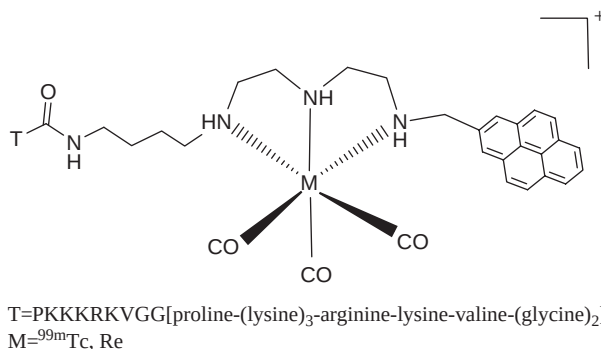


FIG. 8. Schematic representation of a pyrene-conjugated multi-modality imaging probe prototype.

were carried out on the “hot” ^{99m}Tc complex (nanomolar range with 36 h incubation) followed by five and nine days recovery time. The fraction of surviving cells was determined by a colorimetric assay to show that the use of 35 MBq/100 μl leads to 100% cell death after nine days. Staining suggests nucleus damage and micronuclei formation, which infers a radiation-induced mitosis or senescence resulting from DNA damage. LD₅₀ of the complex was estimated as ca. 10 times that of the pertechnetate standard. These were amongst the first systematic observations of ^{99m}Tc showing simultaneous radionuclide therapy and imaging although the ^{99m}Tc levels used at the proof-of-principle stage were not sufficient for significant therapeutic effect (30–32).

The Pelecanou group synthesized a Re(I) complex that enters MCF-7 cancer cells but not normal HFFF-2 and MRC-5 cells. The analogous ^{99m}Tc complex produced fast soft tissue and blood clearance. This system incorporates thiazole which, as well as a known, potent antitumor agent, has also been used as binding agent of amyloid plaques for Alzheimer’s disease diagnosis (Fig. 9a). These complexes are believed to be useful platforms as radiopharmaceuticals for imaging (^{99m}Tc) and targeted radiotherapy (¹⁸⁶Re and ¹⁸⁸Re) of breast cancer, and the coupling of the *in vivo* imaging with *in vitro* studies shows their promise as multimodal imaging reagents. Fluorescence imaging *in vitro* (after 24 h incubation) was obtained with excitation at 332 nm and emission at 407 nm (Fig. 9b), with no apparent probe decomposition (33).

Noninvasive imaging strategies play a critical role in assessing the efficacy of angiogenesis therapies and a role in early diagnosis towards personalised medicine. Angiogenic gene expression is regulated by proteins such as hypoxia-inducible factor-1

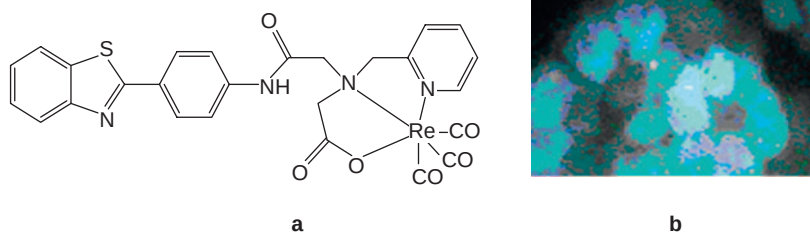


FIG. 9. A fluorescent Re(I) complex (a) and its uptake in tumor cells imaged by fluorescence microscopy methods. (b). Images adapted from Ref. (33).

(HIF-1), which are produced in response to low tissue oxygen concentrations, i.e. hypoxia (*vide infra*). The integrin (i.e. integral protein attached to the cellular membrane) is activated in angiogenic vessels and represents a potential target for angiogenesis imaging. A ^{99m}Tc -labeled peptide (NC100692) targeting integrin (after murine hindlimb ischemia) has been investigated (34). A fluorescent analog of NC100692 was used to confirm specificity and localization of the targeted tracer in cultured endothelial cells. Specificity was also confirmed on tissue sections with the use of immunofluorescent staining of the endothelium in combination with targeting the fluorescent analog at the integrin. The ^{99m}Tc -labeled peptide selectively localized to endothelial cells in regions of increased angiogenesis and could be used for noninvasive serial “hot spot” imaging. This targeted radiotracer imaging approach is a major advance in tracking therapeutic myocardial angiogenesis and has important clinical potential (34).

^{111}In is routinely used in labeling proteins and other macromolecules for SPECT imaging (e.g. ^{111}In -labeled octreotide analogs) and recent developments have been reviewed (20) (N.B. octreotide is an octapeptide that mimics natural somatostatin pharmacologically, and is a potent inhibitor of the growth hormone, glucagon, and insulin). In addition to emitting γ photons, ^{111}In also emits Auger electrons that are highly cytotoxic when emitted close to DNA. ^{111}In -labeled octreotide is under clinical evaluation for treatment as well as imaging of tumors. ^{111}In conjugated to DOTA or related ligands, and derivatized with octreotide, PNA (peanut agglutinin, receptors found in malignant tissues) and CCK (cholecystokinin receptors, a group of G-protein coupled receptors that bind the gastrin receptors) have been developed and used for intestinal imaging. Other examples of targeting include disulfide-cross-linked anti-myosin F antibody fragment aggregates,

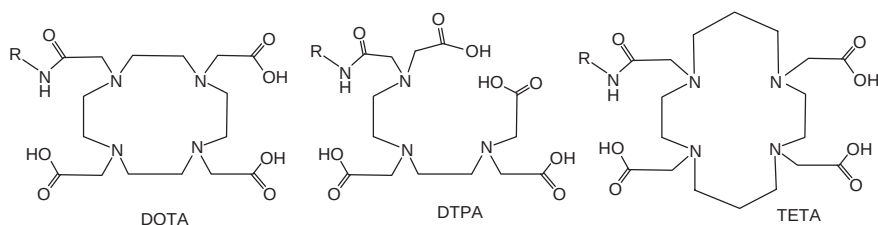


FIG. 10. Typical chelating agents for radionuclides having the ability for further derivatization with biomolecules for targeted delivery.

and folate, etc. (35–39). It appears that indium supported by a biomolecule-targeted DTPA (Fig. 10) chelate remains intact and still linked to the amino acid (usually lysine) to which it was originally bound while the remainder of the protein vector is destroyed after exposure to cells (20). It has also been demonstrated that when ^{111}In is bound to bleomycin (a glycopeptide antibiotic used as a chemotherapeutic having the ability to induce DNA strand breaks) with the purpose of DNA targeting and irradiation, the cytotoxicity of the resulting probe is greater than for the bleomycin alone or “free,” unchelated ^{111}In (40). Although this work clearly achieved the potential of targeted imaging with ^{111}In for dual-mode imaging/therapy, it has not yet been investigated by complementary optical imaging techniques, using “all-in-one” probes.

Optical imaging is likely to emerge as a useful tool to identify the metabolic fate of the ^{111}In radiolabel during the intracellular uptake of the carrier peptide or protein. This would be an important mechanistic aspect to address and solve by multi-modality imaging approaches as it could enhance the current understanding of the nature of binding and of the residence time of the radiolabeled probe within the tissue. Several examples of ^{111}In -radiolabeled porphyrins, with applications in SPECT and having intrinsic fluorescence have been reported (41,42) and these will be discussed below (Section 2.D).

B. NONCONVENTIONAL METALLIC PET ISOTOPES AND THEIR FLUORESCENCE IMAGING

The development of PET radiopharmaceuticals labeled with generator-produced radionuclides has facilitated greater use of PET in clinical nuclear medicine. The $^{68}\text{Ge}/^{68}\text{Ga}$ parent/daughter pair is ideal as a source of PET radiopharmaceuticals as a result of the favorable half-lives of both the parent and daughter radionuclides (43–45). The 271 days half-life of the ^{68}Ge parent

gives the generator a long shelf life while the 68 min half-life of the ^{68}Ga daughter is long enough to achieve the synthesis of a wide variety of radiopharmaceuticals and allow for long image acquisition thus enhancing image quality. The main gallium radionuclides are ^{67}Ga (a γ -emitter used for imaging lymphoma and inflammation by SPECT) and ^{68}Ga (a generator-produced positron emitter for PET imaging of, for example, neuroendocrine tumours). The similarity of gallium and iron has led in the past to imaging of iron transport processes such as transferrin receptor activity in tumors (46). This analogy led to the use of typical iron chelators (such as deferoxamine (47)) both as a bifunctional chelators to label targeting agents with gallium radionuclides, and as an aid in the clearance of nontumor bound gallium to improve tumor imaging (48). The citrate complex of gallium has found widespread clinical application in tumor and inflammation imaging (49,50). The Gallium citrate complex can be rapidly bound by transferrin and slowly delivered to tumor cells over-expressing transferrin receptors. Clinical use is affected by the slow tracer clearance from non-target organs, leading to delays in imaging and poor contrast (49,50).

Research interest has focused on investigating the tumor localizing ability of small monomeric gallium complexes and bifunctional species where the gallium center is coupled to a range of targeting antibodies and peptides for directed uptake (51–56). Complexation and structural studies with the chelators tris(2-mercaptobenzyl)amine and tris(2-hydroxybenzyl)amine (57,58) have been reported. A chelate of ^{68}Ga was used in a pre-targeting approach, using a bi-specific antibody (with one arm specific for a human colon carcinoma antigen and the other specific for one of the enantiomers of the gallium-bound bifunctional chelator), leading to improved PET detection of tumors (59). The preparation of ^{66}Ga - and ^{68}Ga -labeled Ga(III)-deferoxamine-folate as potential folate-receptor-targeted PET radiopharmaceuticals was reported (39,60). The linear trihydroxamate ligand; deferoxamine (Fig. 11) forms stable octahedral coordination complexes with trivalent Ga that resist ligand exchange with transferrin. This work explored the use for noninvasive diagnostic imaging of folate receptor bearing tumors using Ga-radiolabeled folate conjugates. Folic acid is an essential dietary vitamin used in DNA synthesis and one-carbon metabolism (Fig. 11). The folate receptor is a tumor marker over-expressed in many epithelial cancer cell surfaces but restricted in normal tissues. When folate is covalently linked to a macrocycle (via its γ carboxylate moiety), its affinity for cell surface receptors is maintained. This property had been exploited to deliver folate-conjugated protein toxins and

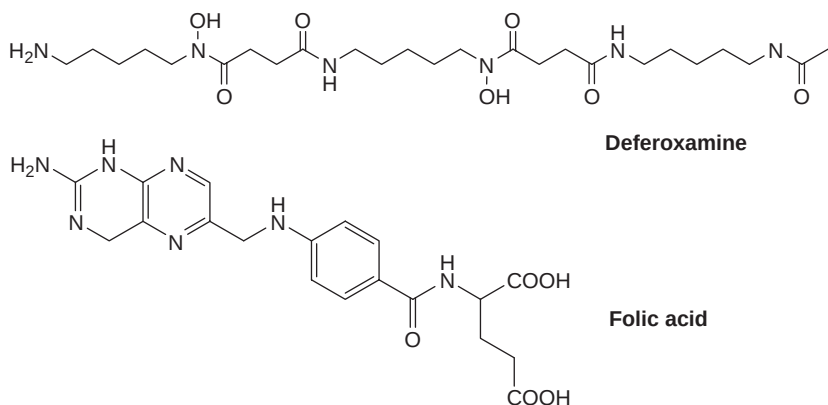


FIG. 11. Schematic representations of imaging probe components for ^{68}Ga delivery.

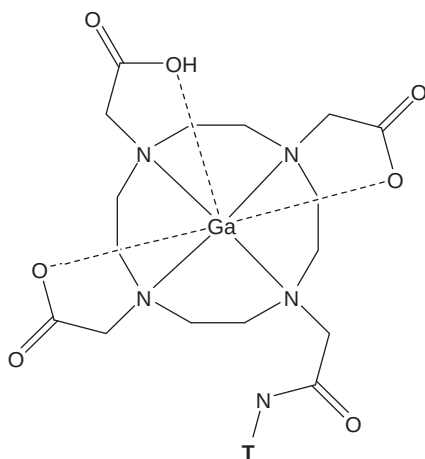


FIG. 12. Schematic representation of a $[\text{Ga}(\text{DOTA})]$ derivative incorporating a targeting unit.

drug/antisense oligonucleotide and also for carrying liposomes into cultured tumor cells over-expressing the folate receptor. This study suggested that a range of tumor-directed folate/chelate conjugates may be developed in which the chelating agent and metal ion radiolabel can be manipulated to optimize radio-pharmaceutical biodistribution, pharmacokinetics, and nuclear properties of the resulting probe (39,60).

The high metal-binding stability of the DOTA framework has led to a large number of complex systems formed by a radionuclide and target unit (Figs. 10 and 12). However, no simultaneously

radiolabeled and fluorescent systems have been investigated so far. A variety of [Ga(DOTA)]-linked peptides, antibodies, and proteins have been reported, and both the “cold” and “hot” chemistry of these highly kinetically stable systems have been explored and reviewed extensively (20). Specific radioactivity determination is key for binding-site affinity studies to improve imaging resolution and ascertain dosimetry. Issues with radiolabeling can be bypassed by the use of microwave-based synthesis techniques. Alternative ligand frameworks offering high complex stabilities, due to the availability of the 5th coordination site at gallium were explored. Despite demonstrating the targeting and stability *in vivo*, no *in vitro* imaging for mechanistic studies of uptake and biodistribution have been carried out.

The use of the β emitting radionuclides ^{57}Co and ^{55}Co for PET applications has been reported (61–63). It is believed that cobalt species (administered as CoCl_2) mimic the distribution of calcium in the body, and may be deposited at sites of tissue damage resulting from ischemia, thus offering the opportunity to image these sites *in vivo*. Imaging of ischemic brain damage in stroke patients has thus been reported, as was the use of $^{57}\text{CoCl}_2$ to radiolabel lymphocytes for imaging inflammatory processes *in vivo* (61–63). To the best of our knowledge, mechanistic studies in cells via optical imaging methods have not yet been coupled with PET imaging for this radionuclide.

The potential use of the β emitting radionuclides ^{186}Re and ^{188}Re for targeted radionuclide therapy has provided the main driving force towards the development of rhenium-based pharmaceuticals (Fig. 13). Much of the chemistry (especially involving fluorescence tracing in cells in addition to radiolabeling) was done in parallel with that of technetium analogs (as discussed above). Developments in rhenium radiopharmaceutical chemistry (for both imaging and therapeutic applications) have been discussed in a comprehensive review (8). For coupling to biomolecules, the search and development of new bifunctional chelating systems for Re(V)

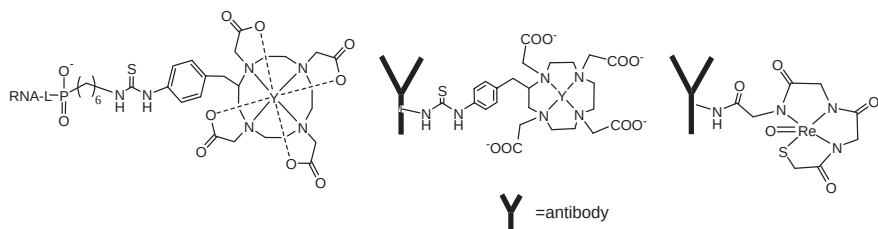


FIG. 13. Typical immunoconjugates for some PET-relevant elements (81).

continues, and representative ligands include tridentate and tetradentate frameworks such as thiolate/amide/thioether donors (64,65). The rhenium-tagged class of imaging probes also includes antibodies and/or antibody fragments with Re(V) bound via reduced disulfide bonds (66–69). A bifunctional chelator for ReO_3^- has been developed comprising of 1,3,5-triamino-1,3,5-trideoxyinositol labeled with antibody fragments (32). In addition, targets for dopamine transporters and steroid hormone receptors based on a wide range of neutral and cationic rhenium–thiol or water-soluble rhenium-phosphine complexes, have been developed (70–73). Rhenium analogs of technetium-essential tracers, including $[\text{}^{188}\text{ReO}(\text{DMSA})]_2$ (where DMSA = dimercaptosuccinic acid) (74,75) are being developed and evaluated towards bone metastases and soft-tissue tumors. However, no study of the mechanism of uptake in cells of this species using optical imaging has been reported thus far.

Yttrium has a number of β emitting radionuclides and is viewed as one of the leading metallic probes with applications (in preliminary clinical and animal evaluations) towards targeted radionuclide therapy of cancer. The tracer has been coordinated via bifunctional chelators (Fig. 13) to targeting agents (e.g. octreotide, monoclonal antibodies, or peptides having the ability to bind to somatostatin receptors and to be internalized (76–79)). Compounds in which ^{89}Y is coordinated with DOTA ligands are shown to have high kinetic stability and have been functionalized with amide linkers suitable for the attachment of modified RNA sequences. This has generated molecular probes for gene expression at the transcription level; *in vivo* PET imaging has showed high uptake in the kidneys and adrenal glands. A multimodal delivery system was also reported whereby a DOTA–adenine conjugate labeled with ^{90}Y (or ^{111}In) was injected after pre-administration of an antibody having the ability to bind simultaneously to a tumor antigen and the metal complex (80).

The search for the ideal design of new bifunctional chelators specific for yttrium continues as does the development of new strategies for the attachment of fluorophors for uptake and distribution studies in cells. A complete study of the cell uptake and biodistribution by fluorescence, coupled with radiolabeling studies has not yet been reported for this radionuclide. Recently, the *in vivo* imaging of (mice) tumors with ^{64}Cu - and ^{86}Y -DOTA targeted using the gastrin-releasing peptide (GRP) receptor (Pro(1),Tyr(4))-bombesin(1–14) was reported (82).

Some of the copper-labeled bifunctional chelators reported to date have shown an enhanced stability in a biological environment and decreased transchelation (Fig. 14) with respect

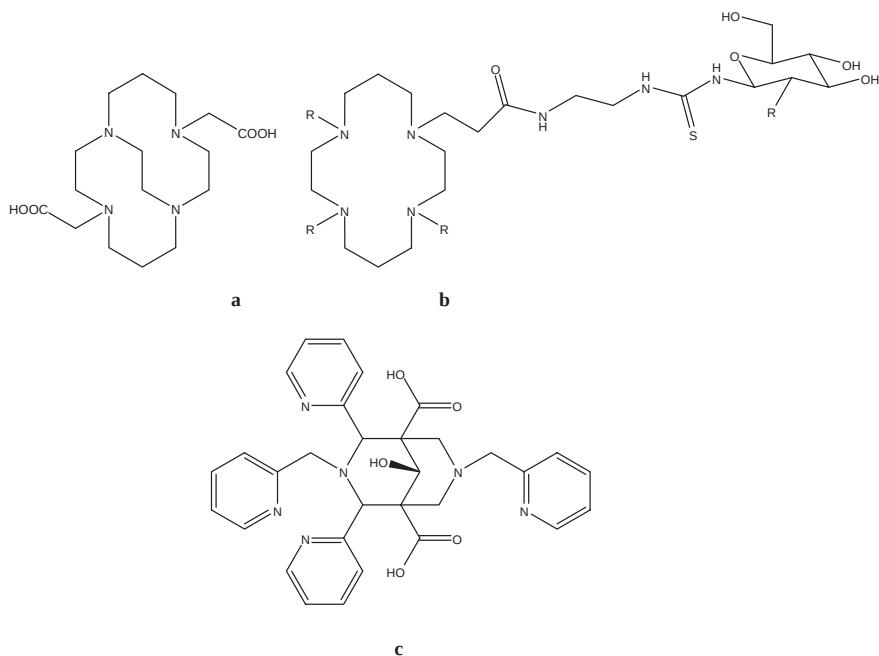


FIG. 14. Some examples of chelating ligands for enhanced kinetic stability suitable for encapsulating both Cu(II) and Cu(I) centres and complexes and derivatization with targeting groups and/or solubilising units R, such as -CH₂COOH (65).

to the simpler DOTA-based frameworks. Complexes supported by a “bridged” ligand (Fig. 14a) showed enhanced blood and organ clearance *in vivo*. Electrochemical reduction is possible (at very negative $E_{1/2} < -0.5$ V) and ligand folding results in a favorable coordination for both Cu(I) and Cu(II). This may be of relevance for selective retention in hypoxic tissues and therefore PET imaging of hypoxia (*vide infra*), but their potential in this context has not been explored. A cyclam derivative tagged with a sugar unit has also been synthesised, as well as a hexadentate bispidine (i.e. a bis(2-pyridylmethyl) derivative of 1,4,7-triazacyclononane). These showed strong biostability and bioavailability coupled with enhanced blood and organ clearance of the corresponding ⁶⁴Cu PET probe (65).

C. TOWARDS HYPOXIC SELECTIVE IMAGING PROBES

Currently, a range of imaging methods (including PET tracer-based ones) has been used clinically to image hypoxia, a condition

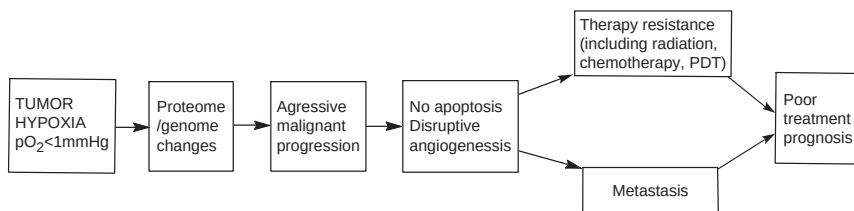


FIG. 15. Influence of hypoxia on cancer cell progression and treatment (83–88).

occurring in solid tumors as a result of rapid growth and insufficient blood supply. Normal cells have pO_2 values in the range 20–80 mmHg, whereas in hypoxic cells this value may reach <1 mmHg. The presence of hypoxia is a major cause of resistance to cancer treatment. Poor oxygenation of cells and tissues (hypoxia) is thought to be one of the main reasons for the ineffectiveness of radiotherapy and chemotherapy (Fig. 15). It is believed that hypoxic cells are three times less sensitive to radiation than oxygenated (normoxic) cells. It is also thought that if the amount of hypoxic cells in a tumor could be monitored, radiotherapy could be optimized for each patient on the grounds of the oxygen status of their tumors (83–88). Tumor hypoxia is known to be heterogeneous but the tracers currently available on the market (e.g. ^{18}F -FDG) do not image this condition per se. The fraction of hypoxic tissue present in a tumor is closely linked to the long-term prognosis for a cancer patient. Therefore, the development of noninvasive methods for assessing this is highly desirable, as is the development of a range of tracers having the ability to detect different levels of hypoxia. Most tumors are characterized by impaired perfusion and regions. Selective imaging agents for levels of hypoxia and distinguishing poorly perfused from necrotic tissue (e.g. in heart diseases). Methods for imaging hypoxia using MRI are currently employed, and include approaches such as direct measurements of oxygen concentration by BOLD MRI (blood oxygen level dependent MRI) and ^{19}F MRI using reporter molecules such as perfluorohydrocarbons.

Optical imaging methods for hypoxia are not yet applicable *in vivo* but immunohistochemical antibody detection of nitroimidazoles (e.g. pimonidazole) has been used extensively for *in vitro* work and biopsy tissue staining (89).

The hypoxia-targeting potential of $^{99\text{m}}\text{Tc}(\text{CO})_3$ core labelled with 2-nitro and 4-nitroimidazoles has been studied. Phosphate coupled metronidazole gave fast clearout from major organs but high accumulation in bone. There is no tumor uptake study to date or

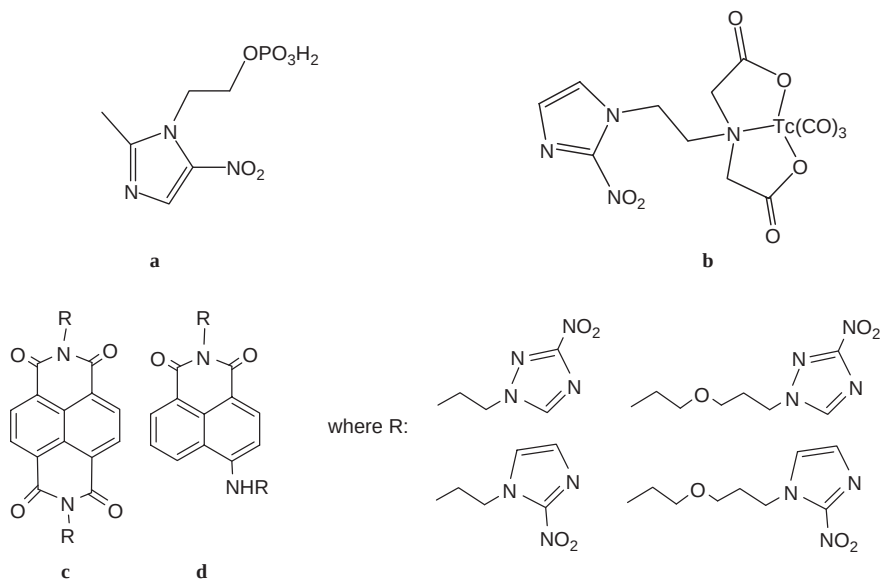


FIG. 16. Example of fluorescent markers for hypoxic cells (90–92).

mechanistic imaging of hypoxia in cells (Fig. 16) (90). The aminodiacetic acid derivative of 2-nitroimidazole (Fig. 16a) showed a good tumor/muscle ratio, but poor tumor/blood ratio.

A noninvasive method for the identification of hypoxic cells is the use of fluorescent nitroaromatic compounds. The nitro group quenches the fluorescence of the ring system, but when bioreduction occurs in the hypoxic cells, the group fluoresces. Liu *et al.* reported the use of naphthalimides with single side chains of 2-nitroimidazole as hypoxic cell markers (91,92) (Fig. 16c and d). It was found that the variation of the side chain had little effect on cell uptake but a large influence on the fluorescence properties of the marker (91). No coupling with radiochemistry has been reported to these fluorescent hypoxic selective systems so far. Lucifer dyes are alternative examples of an intensely fluorescent group of markers that were already being used to image living cells (93). Several research groups have researched the use of nitroaromatic compounds to help identify the hypoxic cells (94–96). An example is the binding of fluorescent nitrofurans, such as NFVO. This is advantageous as the fluorescence can be visualized in sections and the amount of hypoxic cells can also be easily monitored as the systems are susceptible to quantisation (94–96). Fluorescent sulfonamides were designed as probes of tumor-associated carbonic anhydrase IX (CAIX), a target for the detection and therapy of hypoxic tumors. CAIX is a

transmembrane isoform with predominant association with hypoxia and limited distribution in normal tissue. Hypoxia increases the catalytic performance of CAIX contributing to microenvironmental acidosis. Sulfonamides inhibit the catalytic activity of carbonic anhydrases disrupting the regulation of acid-base balance and ion transport in hypoxic tissue. Fluorescent sulfonamides have been developed and shown to inhibit the activity of CAIX preferentially. These also show reduced penetration through the plasma membranes and binding to hypoxic cells. These inhibitors were believed to represent promising candidates for developing anticancer therapies and offer interesting tools for imaging hypoxic tumors. However, these studies have not yet been coupled with PET imaging of tumors (97).

PET imaging using ^{18}F -fluoromisonidazole (FMISO) and ^{18}F -fluoroazomycinarabinofuranoside (FAZA) is well established for human application, allowing the measurement of hypoxia status. Their limitations include poor availability of the radiolabeled product and short lifetime of the ^{18}F PET isotope (half life ca. 110 min).

There has been great interest in Cu(II) as a result of its role in biology, and the versatility in its available radioactive isotopes. The chemistry of *bis*(thiosemicarbazonato) metal complexes has received much interest over the last decade with particular interest in the copper complexes that are known blood perfusion tracers and also display hypoxic selectivity. Biomedical applications revolve around its redox chemistry (12,83–88,98–104).

$^{64}\text{Cu}(\text{ATSM})$, $^{64}\text{Cu}(\text{II})$ -diacetyl-*bis*(N^4 -methylthiosemicarbazonato), is a recently developed PET imaging agent for tumor hypoxia which has been shown to be predictive of radiotherapy treatment outcome in several small clinical series. ^{62}Cu or ^{64}Cu labeled *bis*(thiosemicarbazones) for hypoxia imaging are showing promise although there are some limitations such as high liver uptake and low response for some cell types, i.e. prostate cancer cells. Despite demonstrations of biological activity, the mechanism of action of such complexes at the cellular level remains unknown but it is believed to be due to a subtle interplay between one-electron reduction, protonation, and ligand dissociation processes. (12,83–88,98–104). It is believed that the hypoxic tissue targeting may be achieved on the basis of the intrinsic properties of the complex, and not using receptor targeting as in the case of bifunctional chelators. Studies on $[\text{Cu}(\text{ATSM})]$ uptake and retention have been conducted, and experimented on tumor cells both *in vitro* and *in vivo* (12,83–88,98–104). However, to date, there is no direct evidence to assist the understanding of the mechanism of cellular uptake. Zinc *bis*(thiosemicarbazones)

have been used as a model for the [Cu(ATSM)] and related probes (101–105). The advantage of [Zn(ATSM)], which is isoelectronic with the reduced Cu(I) species is that it is diamagnetic, and therefore can be characterized in solution by NMR and fluorescence spectroscopies. Work on the analogous Zn(II) species, which are intrinsically fluorescent and allow for *in vitro* uptake investigations, has helped further develop the ligand design: this has involved tuning the solubility, lipophilicity, fluorescence, and potentially the hypoxia range available (101–105). Cu(II) is d^9 paramagnetic and as a result tends to quench the fluorescence of adjacent fluorophores. This generally hampers the direct observation of the copper complexes of interest by fluorescence imaging techniques. By direct substitution with zinc, however, the ability to image via fluorescence is a practical possibility. Despite this, fluorescent, molecular zinc complexes are not well known. The complex [Zn(ATSM)] is weakly fluorescent and there is no evidence of significant cytotoxicity for this, or related aliphatic compounds (Fig. 17) at low concentrations (smaller than ca. 10 μM). To date, the limited solubility of this class of compounds in biocompatible media has hampered detailed biodistribution studies. Another limitation in the use of [Zn(ATSM)] as a fluorescent model for the hypoxic selective [Cu(ATSM)] (Figs. 17 and 18, where $Q^1 = Q^2 = \text{Me}$, $M = \text{Cu(II)}$, $R^1 = R^3 = \text{H}$ and $R^2 = R^4 = \text{Me}$), is the availability of a 5th coordination site in the zinc complex versus the striking planarity of the copper complex making it susceptible for binding ligands and/or donors from neighbouring molecules. Structural

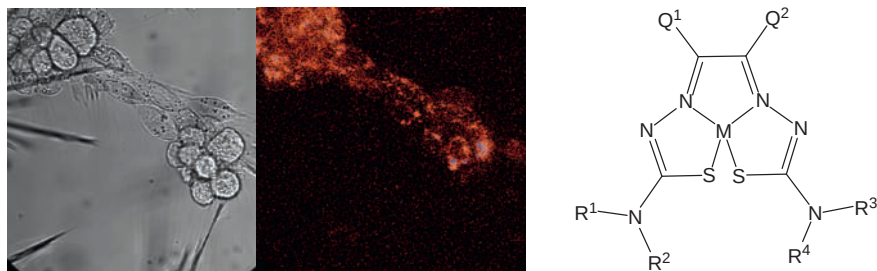


FIG. 17. Confocal fluorescence imaging of [Zn(ATSM)] in IGROV cells (100 μM , where $Q^1 = Q^2 = \text{Me}$, $M = \text{Zn(II)}$, $R^1 = R^3 = \text{H}$ and $R^2 = R^4 = \text{Me}$, $\lambda_{\text{ex}} = 488 \text{ nm}$, DMEM with 1% DMSO). Brightfield image shows formation of needle-like crystalline material on the cell plate (*N.B.* Small crystallites may be endocytosed by the cells rather than passively diffuse through the cell membrane).

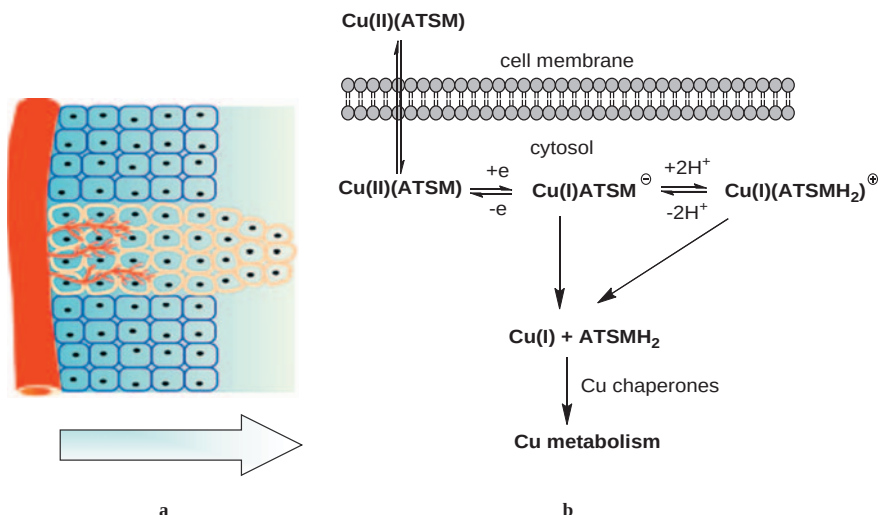


FIG. 18. (a) Representation of the tumor hypoxic state (diagram adapted from Ref. (83a). Arrow direction indicates decrease in $p\text{O}_2$ (<1 mmHg), achieved for tumor depths larger than $100\text{ }\mu\text{m}$; (b) proposed mechanism for redox-mediated retention of $[\text{Cu(ATSM)}]$ in hypoxic cells (101–105). *Note:* Contrary to common belief cell membrane crossing solely by direct diffusion is unlikely for compounds of this family is unlikely, as indicated by fluorescence imaging work on aromatic Zn(II) analogs (*vide infra*). Endocytosis is the more likely uptake mechanism (112–113).

aspects of this class of compounds are well understood, mainly via X-ray diffraction studies.

A wide range of Zn(II) and Cu(II) bis(thiosemicarbazonato) complexes featuring a variety of organic groups/biomolecules at the exocyclic nitrogens have been synthesised and characterized in solution by spectroscopic methods. Their solid-state structures have been determined by single crystal X-ray diffraction. These $[\text{Zn(ATSM)}]$ analogs are intrinsically weakly fluorescent but show enhanced solubility in cell media. Their uptake and biodistribution in HeLa, MCF-7 and IGROV cancer cells have been monitored by fluorescence microscopies (epi- and confocal-fluorescence imaging) (Fig. 19). Radiolabeling to the $^{64}\text{Cu(II)}$ bis(thiosemicarbazonato) complex has been performed cleanly by transmetallation from the corresponding Zn(II) species using $^{64}\text{Cu(OAc)}_2$ (Fig. 20) (101–105).

Over the past few decades, many transition-metal complexes have been screened for cytotoxicity, but surprisingly zinc complexes have seldom been investigated in this context. Detailed investigations of their distribution in cancer cells are extremely

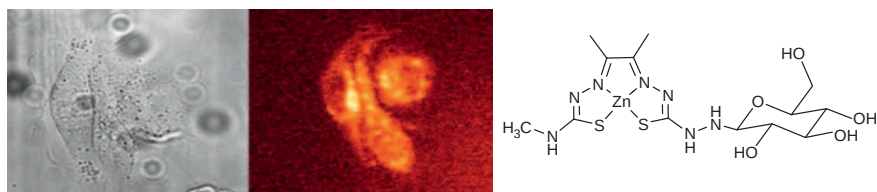


FIG. 19. Typical bright-field and confocal fluorescence images of the glucose-ATSM Zn(II) derivative in cancer cells ($\lambda_{\text{ex}} = 360$ nm, $\lambda_{\text{em}} = 530$ nm). Diagrams adapted from Ref. (102).

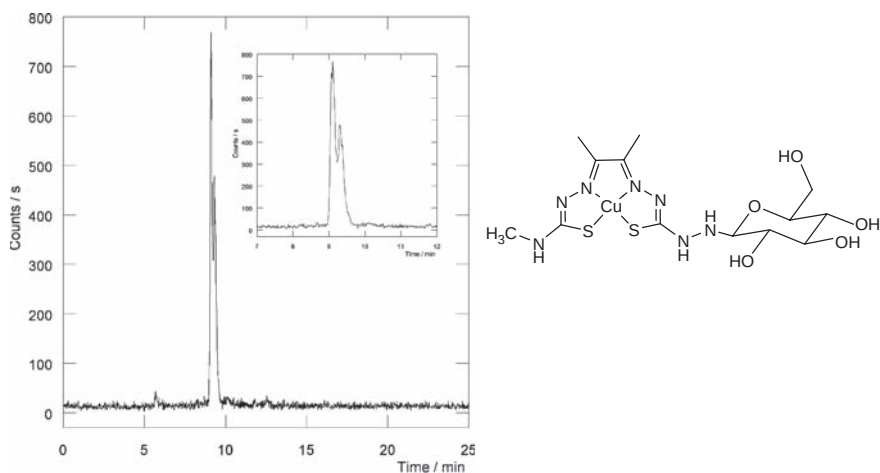


FIG. 20. ^{64}Cu radiolabeling experiment with a glucose-conjugate [Cu(ATSM)] analog. Diagrams adapted from Ref. (102).

limited. Although fluorescent ligands have been used to monitor the presence of Zn(II) in cells (106,107), there are only a few reports of intrinsically fluorescent zinc complexes used to investigate the mechanism of cytotoxicity with relevance to chemotherapy (108). Zinc complexes of tridentate thiosemicarbazones (derived from pyridine-2-aldehyde) showed cytotoxic activities against a range of cancer cells and the cell uptake mechanism was investigated qualitatively (109). New aromatic bis(thiosemicarbazone)-based zinc and copper complexes were designed with the goal to enhance both fluorescence and cytotoxicity by the incorporation of a planar aromatic group which is structurally related to naphthyl-based DNA intercalators (Fig. 21) (110,111). This class of compounds is soluble in biocompatible media and displays cytotoxicity comparable to that of *cis*-platin within a

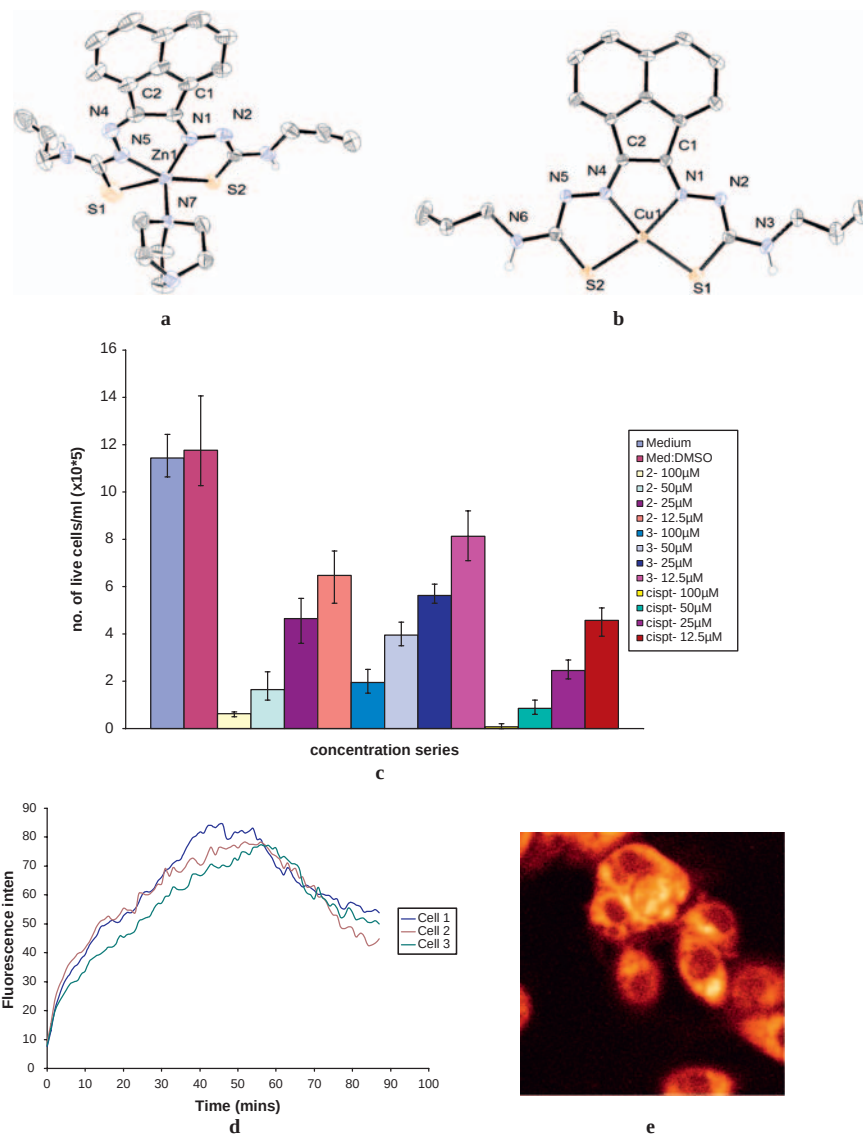


FIG. 21. Molecular structures of new aromatic [M(ATSM)] analogs: (a) M = Zn(II) and (b) M = Cu(II), (c) cytotoxicity tests in MCF-7 cells for the Zn(II) complex (group 2) and Cu(II) complex (group 3) and comparison with control and with cis-platin over a range of concentrations, (d) cell uptake profile monitored over 90 min, (e) confocal fluorescence imaging of Zn(II) complex in MCF-7 cells, at 100 μ M conc. in DMEM, 1% DMSO (112,113).

range of human cancer cell lines. Their intrinsic fluorescence has enabled the measurement of the cellular distribution which has suggested that their activity is associated with both disruption of the mitochondria and slower uptake into the nucleus (over more than 12 h). These complexes are closely related to the “aliphatic” [$^{64}\text{Cu}(\text{ATSM})$] hypoxia PET tracer. We are currently investigating whether the (radiolabeled) aromatic Cu(II) analogs of the complexes reported by us show hypoxic selectivity. It would be of interest to observe the uptake of the radiolabeled copper complex in tumor cells by comparing the radiation count present in the cell and that in the washout under normoxic and hypoxic conditions and ultimately to obtain *in vivo* images (110,111).

The main design features for intrinsically fluorescent and hypoxia selective next generation thiosemicarbazone-based tracers are:

- (1) Retain redox characteristics – no major changes to ligand backbone – mainly use exocyclic nitrogens.
- (2) Retain as much of ATSM structure as possible – single site modification preferred.
- (3) Simple, rapid, reproducible, and flexible chemistry.
- (4) Stability in biological medium at least equivalent to that of [$\text{Cu}(\text{ATSM})$].
- (5) Facility to attach functional targeting or probe molecules – sugars, proteins, 2-nitroimidazoles, fluorophores, ^{18}F radiolabel (for multinuclide imaging), cytotoxins (for dual mode imaging/therapy – is desirable).

The questions which remain under investigation are: (a) What does the chemistry of these complexes tell us about possible mechanisms for hypoxic selectivity? (b) How far can these complexes be modified without compromising their hypoxic selectivity? (c) Can they be optimized for different degrees of hypoxia?

D. APPLICATIONS OF METALLOPORPHYRINS AS MULTIMODAL, OPTICAL, AND/OR RADIOACTIVE IMAGING PROBES

Over the past 20 years, the principal biomedical application for (free or metal-substituted) porphyrins has been Photodynamic Therapy (PDT), with extensive literature in the area including a number of comprehensive reviews (114–116). Porphyrins offer scope for optical imaging as they are potent fluorophores in the red region of the electromagnetic spectrum. Although, to date, the emphasis has been on their therapeutic effects, due to their

specific localization and sensitizing ability, they have the potential for use in tumor imaging and biodistribution studies after radiolabeling with the radionuclide of choice. Early work with ^3H and ^{14}C radiolabeling allowed the observation of the specific uptake in tumors of haematoporphyrin (117). These isotopes are not suitable for *in vivo* scintigraphic imaging, but have the advantage of not altering the intrinsic biodistribution of the probe. There is little imaging work using radiolabeled porphyrins, and less still taking advantage of the suitably intense fluorescence as an alternative, complementary, source for drug localisation analysis (116,118). This is surprising since the ligands shown in Figs. 22–26 are all biologically compatible, their metal complexes are both thermodynamically and kinetically stable, and they show apparent high specificity for tumors (Figs. 22–26). In addition to the emphasis on their role as therapeutics, only limited information on the use of porphyrin as fluorophors for imaging purposes exists to date (1). The uptake of

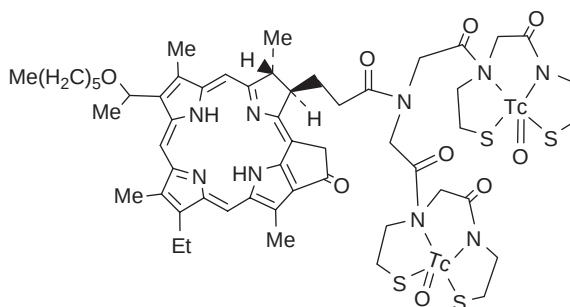


FIG. 22. $^{99\text{m}}\text{Tc}$ -labeled PDT system for “see and treat” approach.

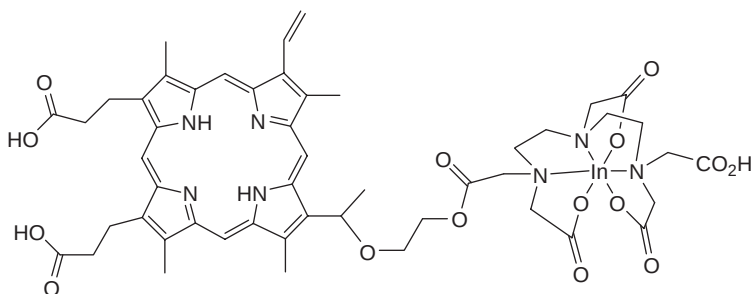


FIG. 23. Structure of the $[\text{In}(\text{DTPA})]$ core coupled to haematoporphyrin.

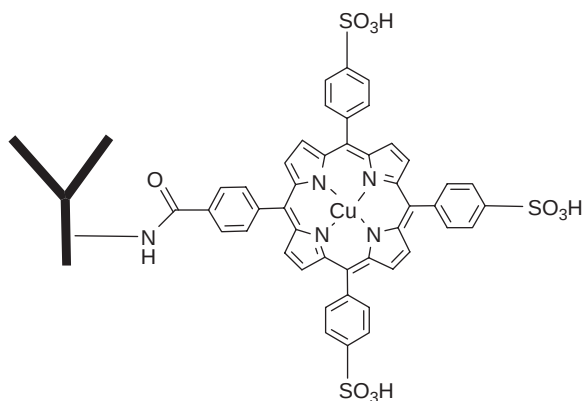


FIG. 24. ^{67}Cu radiolabeled porphyrin coupled to an antibody.

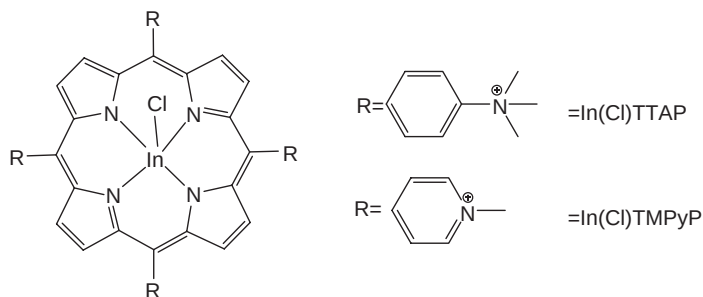


FIG. 25. Structure of water-soluble indium porphyrins with relevance to ^{111}In SPECT.

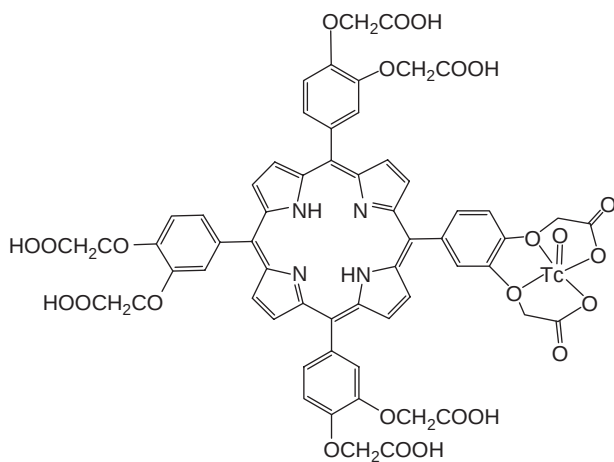


FIG. 26. Structure of tetrakis[3,4-bis(carboxymethyleneoxy)phenyl]porphyrin indirectly labeled with $^{99\text{m}}\text{Tc}$.

the porphyrins in tumors can be amplified by conjugation to a wide range of biomolecules (2) resulting in significantly enhanced therapeutic effects upon irradiation with light.

An early report (119) describes the ^{64}Cu labeling of naturally occurring porphyrins, but PET images of tumors in humans showed poor specific uptake. Wrenn and co-workers have developed a method for radiolabeling a tetrasulfonated porphyrin with ^{67}Cu for intrinsic porphyrin localization. However, the human *in vivo* studies with this compound did not yield promising neoplasma imaging results (119). Copper porphyrins have also been investigated as bifunctional chelating agents for antibody labeling as the copper porphyrins are nontoxic and exhibit good *in vivo* stability with limited copper exchange with other serum-bound metal ions. A carboxyl porphyrin coupled to an antirenal carcinoma antibody was labeled giving favourable *in vivo* tumour to blood ratios; four fold higher than with the free copper porphyrin alone. (120). ^{64}Cu tetracarboxytetraphenylporphyrin was studied as a potential radiopharmaceutical for inflamed lymph node detection (121,122). A *N*-4-nitrobenzyl-5-(carboxyphenyl) porphyrin has been conjugated to the antirenal carcinoma antibody A6H and labeled with ^{67}Cu , and a preliminary *in vivo* study indicated efficient targeting (123). Haematoporphyrin derivatives can readily be metallated with $^{99\text{m}}\text{Tc}$ (30 min at room temperature) using a direct labeling route from $[\text{}^{99\text{m}}\text{TcO}_4]^-$ and SnCl_2 (124). Haematoporphyrins were shown to localize in tumors and neoplasms by *in vivo* imaging in tumor-bearing rats. The complex formed was not chemically characterised but by analogy with known Re porphyrins (125,126) and a $^{99\text{m}}\text{Tc}$ octaethylporphyrin complex (127) it is believed that it contains a Tc(V)oxo core. Indirect labeling of porphyrins with $^{99\text{m}}\text{Tc}$ has been reported using 5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxy)phenyl-porphyrin] where the Tc is bound to the peripheral $-\text{OCH}_2\text{CO}_2$ groups (128). Sufficient uptake in gliomas and $\text{C}_3\text{H}/5$ breast tumors indicated that these complexes may be suitable as imaging agents. The analogous ^{186}Re complex was also described (129). An analogous approach was used to bind $^{99\text{m}}\text{Tc}$ to cyclam groups attached to the tetraphenyl porphyrin (TPP) (130). For rhenium, nitride complexes of the general type $[\text{ReN}(\text{porphyrin})]$ have been reported (125,126) and the nitride ligand is sufficiently nucleophilic to be reacted with acyl chlorides giving stable acylimido-complexes (131). Complexes of the type $[\text{M}(\text{CO})_3]_n(\text{porphyrin})$ ($\text{M} = ^{99}\text{Tc}$, Re, $n = 1$ or 2) have been structurally characterized to show that three of the porphyrin nitrogens bind to the tricarbonyl fragment (132,133).

Gallium and indium porphyrins have been studied in some detail in the context of PDT (2). There is comparatively little literature on their radiolabeled analogs, but the major focus has been on ^{111}In complexes. A range of symmetrical tetra-arylporphyrins have been prepared and their biodistributions established by SPECT (41,42,134). A tetra(4-trimethylaminophenyl) porphyrin complex was used to visualise lymph nodes in rabbits, and a tetra(methylpyridinium) analog provided clear images of malignant melanoma in hamster models (42,124,137). As with $^{99\text{m}}\text{Tc}$, ^{111}In was coordinated to specific chelators such as DTPA which in turn was conjugated to a porphyrin with 95% labeling efficiency. The porphyrin substituents may be varied to optimize uptake (135). The water-soluble pyridinium porphyrins labeled with ^{111}In have been conjugated to a monoclonal antibody and shown to retain immuno-reactivity (136).

The principle focus has been on ^{111}In as this is also an Auger electron emitter, and thus of interest for therapy: Photofrin (a mixture of naturally occurring haematoporphyrins which has been approved as a photosensitizing agent in the clinical treatment of selected solid tumors) has also been labeled with indium and evaluated for tumor uptake in animal models (137). As with technetium, ^{111}In has been coordinated to specific chelators such as DTPA conjugated to haematoporphyrin with 95% labeling efficiency and showed strong tumor accumulation (136). The water-soluble pyridinium porphyrins labeled with ^{111}In have been conjugated to monoclonal antibodies and shown to retain immunoreactivity (136).

Haematoporphyrin and its derivatives can readily be radiolabeled at room temperature with $^{99\text{m}}\text{Tc}$, using a standard method from pertechnetate and SnCl_2 (124,138). These were shown to localize in tumors and neoplasma (by *in vivo* imaging in tumor-bearing rats). Indirect labeling of porphyrins with $^{99\text{m}}\text{Tc}$ has been reported using tetrakis[3,4-bis(carboxymethylene-oxy)-phenyl-porphyrin] where the $^{99\text{m}}\text{Tc}$ is bound to the peripheral carboxy groups (128,130). There was sufficient uptake in gliomas and breast tumors to suggest that these complexes might be useful as imaging agents. An analogous approach has been used to bind $^{99\text{m}}\text{Tc}$ to cyclam groups attached to the periphery of TPPH_2 (130). There are several reports of Re tricarbonyl complexes, shown to have three porphyrin nitrogens bound to the tricarbonyl core (132,133). Despite this, there are no reports of radiolabeling studies *in vitro* or *in vivo* with this derivative.

To date, the use of radiolabeled metalloporphyrins as imaging agents has focused on the intrinsic tumor localizing properties of porphyrins with variation in the porphyrin peripheral substructure

to modulate the lipophilicity and hence biodistribution *in vivo*. Interest in bifunctional radiolabeled porphyrins for specific pharmacological targets is in its infancy and holds potential for much investigation.

Porphyrins have been investigated in the context of multifunctional modality for simultaneous dual imaging and photosensitization for PDT. As with most photosensitizing agents, porphyrins are endowed with high quantum yields, but limitations apply since this can be influenced by the aggregation of the porphyrin in cells or tissues. To overcome this problem, Zn(II)phthalocyanines labelled with ^{131}I (a β and γ emitting diagnostic and therapeutic radionuclide with half life 8.02 days) have been used as photosensitizing agents (139) since radio-diagnostic measurements are much less sensitive to the aggregation of the dye and the nature of the microenvironment. Thus, PET has allowed the study of the drug distribution *in vivo*, complementary to fluorescence imaging at the cellular level to achieve earlier diagnosis. ^{124}I (half life 4.2 days, 25% positron abundance) was also used as a radiolabel since it is suitable both for targeting with antibodies work and as a photosensitizer attachment.

Developing dye conjugates of small bioactive molecules is also of interest in the context of the development of Near Infra-Red (NIR) molecular probes with high specificity, selectivity, stability, and sensitivity. A trifunctional agent for dual PET/fluorescence and PDT using pyropheophorbide porphyrins was also reported in the context of targeted delivery (13,118). Elevated peripheral benzodiazepine expression is associated with certain tumors (breast, colon, prostate etc.) and is a known binding site for pyropheophorbide. Competitive studies have been performed and fluorescence imaging shows initial localization in mitochondria with a shift towards Golgi complexes and no uptake in lysosomes. Also, it was shown to give PDT *in vitro* and *in vivo*, with excitation at 662 nm and emission at 667 nm (strong) and 710 nm (weak). Although a promising bio-localization was found *in vivo*, due to limited penetration of visible light the fluorescence uptake of organs could not be measured directly. However, this was evaluated by radioimaging. To make up for time delay differences in PET and PDT, ^{124}I -labeled probe (50 μCi) was administered, followed by a therapeutic dose (1.5 $\mu\text{mol/kg}$) 24 h later. This allowed for accumulation on a long timescale and time for wash-out from organs and demonstrated the potential for dual imaging and therapy. Although better receptor binding is required and the probe showed only a limited potential as a photosensitizer for tumors of the liver and kidneys, it nevertheless appeared

promising for lung, breast, skin and neck tumors. The quest for multimodal probes suitable for longer wavelength absorbers and with larger Stokes shifts continues as does that for multimodality agents suitable for tumor imaging (PET, fluorescence) and simultaneous PDT, i.e. a method identified as the “see and treat” approach (13,118).

Although some of the complexes investigated *in vivo* showed better tumor-to-tissue ratios for selected tissues with respect to the ratios observed using a well-known SPECT marker for tumors and inflammation (^{67}Ga citrate, where ^{67}Ga is a γ emitter with half life 7.8 h) none of the radiolabeled porphyrin derivatives investigated *in vivo* showed true tumor selectivity. Usually the porphyrin-based reagents showed high uptake in organs such as the liver, kidneys, and spleen, which particularly limits their usefulness for the imaging of the abdomen. An exception may be tetraphenyl porphyrin sulfonate which showed only limited retention in these organs (due to enhanced hydrophilicity). It was also noted that the role of the metal ion in altering porphyrin biodistribution *in vivo* may be linked to its effect on porphyrin aggregation. The use of antibody-conjugated radiopharmaceuticals in PDT enables pharmaceutical specificity, but the limiting factor is that it takes ca. 1–3 days for the antibodies to localise at tumor tissue (13,118).

Texaphyrins (i.e. metal-coordinating expanded porphyrins, Fig. 27) have recently been investigated as new drugs for applications in radioimaging and PDT (140).

A water-solubilized texaphyrin based on gadolinium(III) (XCYTRINTM) is under current testing in a new Phase III clinical trial with the role to enhance the effects of radiation therapy for

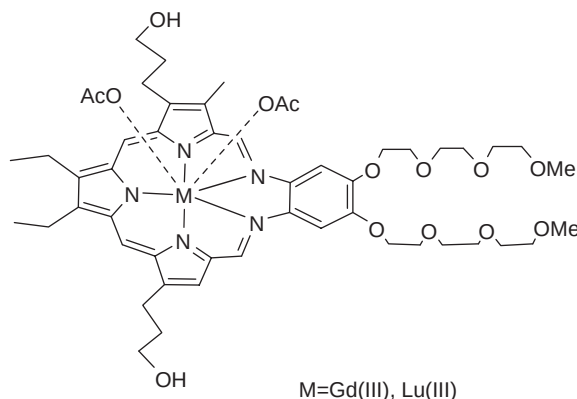


FIG. 27. Schematic representation of texaphyrin-based drugs (140).

brain tumors which metastasised (140). Its lutetium analog is being tested (in Phase I and/or Phase II clinical trials) as a photosensitizer for use in the photodynamic treatment of recurrent breast cancer, for photoangioplastic reduction of the peripheral arteries, for atherosclerosis and for light-based treatment of age-related macular degeneration (a vision-threatening disease of the retina). These systems are highly kinetically stable and biocompatible, show favorable tissue biolocalization characteristics, and their mechanisms of action in cells have been determined with a view to clarify radiation therapy mechanisms and the pathophysiology of atherosclerosis (140). They show promise as platforms for multimodality imaging/therapy applications.

E. RADIOLABELED AND OPTICALLY TAGGED TARGETED NANOPARTICLES

Increased attention has been drawn recently to new methods of drug delivery emerging from the new, interdisciplinary field of nanotechnology. Administering drugs continually for prolonged time periods and in a controlled manner is a holy grail, as the rapid release of drugs may lead to incomplete absorption, gastrointestinal disorders, and other side effects. Several drug delivery and targeting strategies have been developed that are based on coupling drugs to receptor-specific ligands and/or protection of the drug by wrapping it in a polymer or lipid coat (141–143).

Designing nanoprobe whereby imaging agents (for PET, SPECT, and/or optical imaging methods, as well as for simultaneous imaging and therapy) are anchored to (or encapsulated by) nanosize carriers is a topical issue (144).

The aim is to deliver multiple molecules of an imaging reagent (within a carrier) to a target while keeping the administered dose of the overall probe extremely small. Nanodimensional carriers such as vesicles, liquid crystal dispersions, and shell–core nanoparticle dispersions are promising drug delivery carriers (Fig. 28). The ultimate goal is to achieve the optimum radioimaging and/or therapy drug loading and release properties, lowest toxicity, and longest shelf-life (141,145–147).

Desirably, a probe designed for delivery of a radiopharmaceutical to cells will have the following components (Fig. 29):

- (a) Address = an antibody or a related biological molecule with recognition motifs, linked covalently or noncovalently to the main “carrier.”



FIG. 28. A variety of nanomaterial scaffolds with potential for dual mode imaging methods.

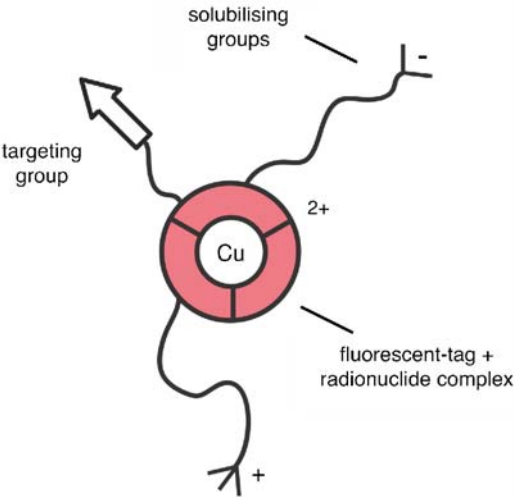


FIG. 29. A schematic representation of a copper-based radiopharmaceutical encapsulated in a tailored multifunctional nanocarrier.

- (b) Carrier = single walled carbon nanotube (SWNT), vesicle, nanocapsule etc. for stable containment to prevent radionuclide loss en route to target
- (c) Solubilizing groups = carboxylate units, also suitable for further synthetic modifications.
- (d) Fluorescent tag = dansyl chloride, nitrobenzoxadiazoles, fluorescein, rhodamine etc.

A series of liposome constructs (MLV – multilamellar vesicles, SUV – small unilamellar vesicles and monosialoganglioside [GM1]-coated, sterically stabilized liposomes) have been used to encapsulate diagnostic and /or therapeutic radionuclides e.g. ^{67}C (β^- and γ emitter, $t_{1/2} = 61.5$ h), ^{188}Re (γ emitter, $t_{1/2} 16.9$ h), ^{90}Y (β^- emitter, 64.1 h), ^{131}I for example, and used to obtain tumour and background biodistribution dose estimates in a range of carcinomas (148). The biodistribution, pharmacokinetics, and imaging of ^{188}Re (BMEDA)-labeled pegylated liposomes (BMEDA: *N,N*-bis(2-mercaptoethyl)-*N',N'*-diethylethylenediamine) have been reported by Chen *et al.* However, to date no study involving both (a) *in vitro* (fluorescence) and (b) *in vivo* (radioimaging) has been reported (149). $^{99\text{m}}\text{Tc}/^{188}\text{Re}$ -labeled lipid nanocapsules have been designed as promising radiotracers for imaging and therapy, where radiolabeling proceeded with high purity. Lipophilic complexes [$^{99\text{m}}\text{Tc}/^{188}\text{Re}(\text{S}_3\text{CPh})_2(\text{S}_2\text{CPh})$] (Fig. 30) have been encapsulated within these nanoparticles and their biodistributions were evaluated both *in vitro* and *in vivo*. The radiolabeling purity and

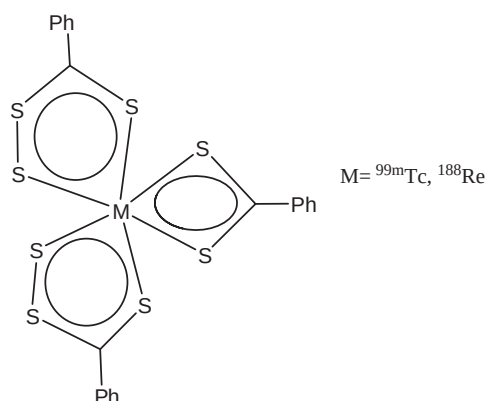


FIG. 30. Schematic representation of lipophilic, radiolabeled complexes which have been encapsulated in lipid-based nanoparticles (150).

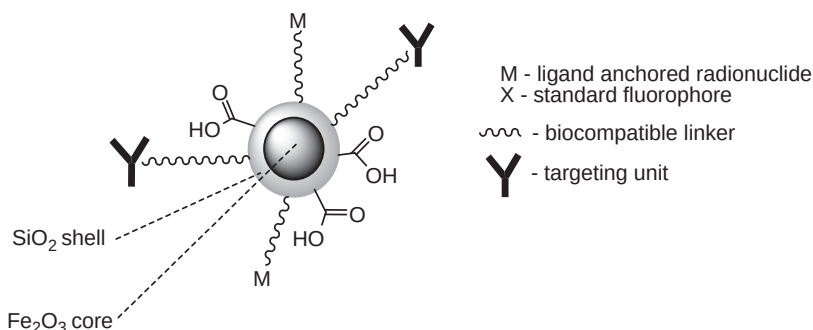


FIG. 31. Proposed design for core-shell multimodal imaging probes incorporating iron nanoparticles.

biostability compared favorably to that of known PEG-coated $^{188}\text{Re}/^{99\text{m}}\text{Tc}$ -containing particles (150).

Systems where the radionuclide (therapeutic or imaging agent) is targeted via a magnetic carrier have been investigated. A functionalized magnetic nanoparticle contains a ca. 7–9 nm ferrite core encased in silica. Functional groups may be attached to this shell (or encapsulated within it) including radionuclides and/or fluorophores for *in vitro* and *in vivo* surveillance (Fig. 31) (143,151).

If the radionuclide is targeted to a region near the tumor site, the radiation is likely to affect all surrounding tissue. *In vivo* and *in vitro* tests on mouse models (using a magnetic carrier coupled with a beta-emitter, e.g. ^{90}Y or ^{188}Re) were reported. In addition, ^{188}Re has been attached directly to microspheres for intratumoral injection (152). Shell cross-linked nanoparticles labeled with ^{64}Cu and conjugated with folate were synthesized, radiolabeled, and their biodistribution evaluated both *in vivo* and *in vitro*. Biological evaluation for tumor imaging and radiotherapy have also been performed (153). This multifunctional probe, comprising a $^{64}\text{Cu}(\text{TETA})$ -radiolabeled folate-conjugated shell cross-linked nanoparticle, was designed and found to be a promising platform to shuttle radionuclides and drugs into tumors over-expressing the folate receptor. The nanoparticle carriers were obtained by cross-linking the shell of micelles obtained from amphiphilic diblock copolymers. SCKs were subsequently functionalized with folate, fluorescein thiosemicarbazide (FTSC), and TETA (1,4,8,11-tetraazacyclotetradecane-*N'*,*N'*,*N'*,*N'*-tetraacetic acid). The targeted delivery of the multifunctional probe was demonstrated by epifluorescence microscopy (Fig. 32). ^{64}Cu labeling of TETA proceeded with high radiochemical purity

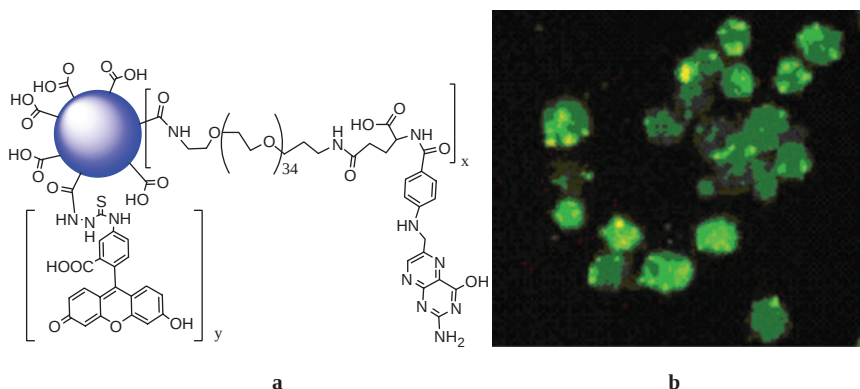


FIG. 32. (a) Folate-conjugated nanoparticles; (b) Fluorescence micrograph of KB cells incubated with the fluorescent probe for 4 h at 37°C. Figure adapted from Ref. (153).

(95%). These probes showed long circulation in blood and were able to accumulate passively in tumors, but this was highest in small-size tumors. The targeted uptake was competitively blocked by excess folate in small-size solid tumors, suggesting positive interaction with the corresponding folate receptor. In addition, these nanoparticles are versatile for further derivatization and their surface can easily be functionalized with other chelating systems, or prodrugs and genes, and this targeting approach may be generalized. Targeted multimodal PET imaging (with ^{18}F and ^{64}Cu) was demonstrated using the probes shown in Figs. 32 and 33 (153).

The preparation and high-yield radiolabeling of a water-soluble fullerene $^{67}\text{Ga}@C_{60}(\text{OH})_x$ has been reported. The biodistribution in mice showed a high localization of $^{67}\text{Ga}@C_{60}(\text{OH})_x$ in the bone marrow, bone, liver, and spleen with slow clearance and a negligible accumulation in the blood. Data suggested that this water-soluble probe has similar properties to microcolloids and that it may be used as a drug carrier for lymphatic targeting (154).

Functionalized carbon nanotubes are currently investigated for biological applications (155). Although such nanoarchitectures for targeting *in vivo* were designed, and the labeling of carbon-based nanoparticles with ^{64}Cu anchored on DOTA was reported, limitations include poor control over the number of DOTA groups available for radionuclide binding (92). In an experiment by Kam *et al.* the carrier used was a SWNT and the biological targeting moiety used was folic acid (covalently attached). A fluorescent tag

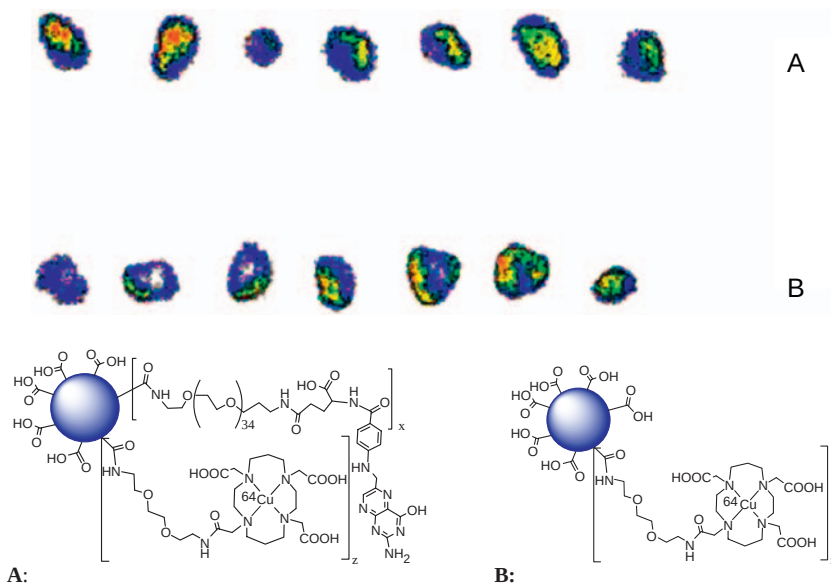


FIG. 33. PET imaging of mice tumor after administration of compounds **A** and **B** respectively. Top images show regional concentration uptake of the folate-targeted (A) or nontargeted (B) ^{64}Cu radiotracers. Figure adapted from Ref. (153).

was used to monitor cell delivery to HeLa cells, which are folic acid receptor rich. These cells endocytosed the functionalized SWNTs, subsequently identified inside the cells by confocal fluorescence microscopy (156). Investigations into biodistribution, and its dependency on size, coatings, and flexibility of the nanocarrier were carried out. Imaging *in vivo* confirmed that uptake is dominated by strong liver localization. Long-circulating nanoparticles functionalized with ligands for receptors over-expressed by tumor cells have promising applications for active and passive tumor targeting (156,157). Further functionalities, such as amino acid modified peptide nucleic acid conjugates (to show specific tumor targeting *in vivo* for non-Hodgkin lymphoma) and/or therapeutic radioisotopes may be attached to nanodimensional scaffolds.

SWNTs have attracted growing interest due to their potential applications in the fields of cancer diagnosis and therapy. To trace and diagnose the effects of the treatment in single cells or tissues, SWNTs need to be derivatized covalently or noncovalently with fluorescent tags. The main derivatization method has

involved adsorbing different molecules and antigens to their surfaces so that they may target tumor cells specifically. Intrinsically, carbon nanotubes exhibit weak near-infrared emissions, but these are not intense enough to be useful in diagnosis. Covalent functionalization with fluorophores may be problematic due to the strong quenching qualities of the electron-rich SWNTs, which is likely to change the properties of both the imaging probe and the nanotube scaffold itself. It is believed that the supramolecular wrapping of the carbon nanotubes with fluorescent dyes, or the use of a very long linker to separate the fluorophore and the SWNT may be advantageous in avoiding the fluorescence quenching. Recently, Dai *et al.* demonstrated the noncovalent functionalization of SWNTs by fluorescein-polyethylene glycol (Fluor-PEG) (158). Water-soluble nanotube conjugates (Fluor-PEG/SWNT), simultaneously attached to fluorescent labels have been developed. The same study showed that fluorescein, a widely used fluorophore, can strongly adsorb onto the sidewall of the SWNTs, likely via π -stacking, and the hydrophilic PEG chain imparts high aqueous solubility. Interestingly, the interaction between SWNTs and fluorescein is pH-dependent: it weakens when pH increases, therefore the Fluor-PEG/SWNT composite is less stable at high pH. Fluorescence emission intensity was observed to be quenched by $\sim 67\%$ due to interactions between fluorescein and SWNT. Despite the quenching of fluorescence, the remaining fluorescence is still sufficient to render the probe useful as a fluorescent marker. Fluor-PEG/SWNT was subsequently investigated for its uptake in BT474 breast cancer cells, and fluorescent signals were observed by confocal fluorescence microscopy and flow cytometry (158).

The same group reported the simultaneous radiolabeling (with DOTA-anchored ^{64}Cu) and fluorescence studies, coupled with biodistribution *in vivo* and *in vitro* (92). It is believed that appropriately functionalized SWNTs can efficiently reach tumor tissues in mice with no apparent toxicity (159). Furthermore, water-solubilised carbon nanotubes are nontoxic when taken up by cells even in high concentration (92). These studies have been complemented by the recent PET imaging of water-soluble ^{86}Y labelled carbon nanotubes *in vivo* (mice) (160,161), to explore the potential usefulness of carbon nanocarriers as scaffolds for drug delivery. The tissue biodistribution and pharmacokinetics of model DOTA functionalized nanotubes have been explored *in vivo* (mouse model). MicroPET images indicated accumulation of activity mainly in the kidney, liver, spleen, and to a much less

extent the bone. Since blood clearance was rapid, it is believed that it could be more beneficial to use shorter-lived radionuclides in diagnostic applications of SWNTs. Several studies that incorporated antibody-functionalized and radiolabeled carbon nanotubes for tumor targeting were also reported and aspects of carbon nanotubes translocation in living cells and organisms have been reviewed recently (161,162).

Promising results have thus been obtained relating to the delivery of radionuclides, chemotherapeutics, and optical imaging tags by anchoring these onto the surfaces of SWNTs. However, the search continues for identifying the most effective method for the loading of imaging and therapeutic drugs onto or inside the nanotubes (Fig. 34) (150, 163). Further cellular uptake studies are in progress as are studies relating to the most effective encapsulation, targeting, and triggering drug release at the therapeutic site for carbon nanotubes and related materials. There are only very few radiolabeled nanotubes to date, although

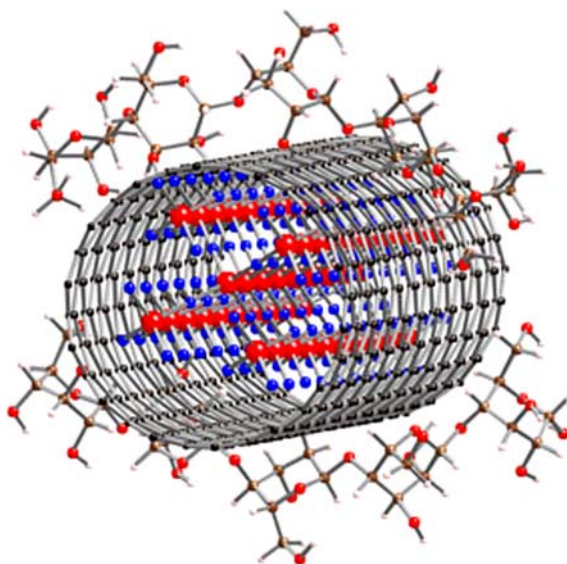


FIG. 34. Model of a functionalised (internally or externally) SWNT, which may be closed, open and/or corked reversibly or permanently after filling. Potentially the inner cavity may be filled with a (radio)-imaging agent (for PET/SPECT and/or MRI) or a (radio)therapeutic agent. Such functionalized SWNTs may conceivably act as probe prototype for multimodal imaging/therapy and targeted drug delivery (155).

the functionalisation methods for carbon nanomaterials for biomedical purposes in general has received huge attention over the past 5 years (162,164,165). Furthermore, the long-term fate of the nanoparticles in the body remains unknown and their biocompatibility and cytotoxicity (particularly for carbon nanotubes) is still much debated (166).

III. Conclusions

The development of new candidates for use as imaging agents, either for the radioactive imaging techniques such as PET and SPECT or for the nonradioactive optical imaging techniques, such as fluorescence or luminescence imaging, is highly desirable. When combined with optical imaging, within the same probe, radioactive imaging techniques such as PET and SPECT could provide an extremely powerful tool in the clinical diagnosis and treatment of disease in addition to cell-level imaging for mechanistic understanding of metabolic processes.

Furthermore, the challenge is to design and deliver to targets new imaging drug candidates for use as dual- or multi-modality imaging and/or therapy and imaging agents, for example in the treatment and imaging of cancer tumors and other conditions characterised by undesirable cellular proliferation.

There is therefore an ongoing interest to synthesize biologically active metal complexes for use as imaging probes and/or as therapeutic agents, and to study their mechanism of action at the cellular level. The field of nanomedicine is advancing rapidly and a variety of nanomaterials are beginning to find their applications with relevance to multimodal imaging and therapy. Although the delivery of imaging reagents and drugs using certain type of nanoparticles (especially the coated, core-shell superparamagnetic materials for MRI applications (167)) has reached preclinical stages of research, such delivery systems are unlikely to be widely available in a commercial sense in the immediate future: for example, PET imaging alone remains a very expensive technique and, at present, ^{18}F -FDG remains the only PET radiopharmaceutical licensed for routine clinical use.

The use of complementary, multimodal imaging techniques to analyze probes both *in vivo* and *in vitro* may be helpful in speeding up this process; this should ensure that laboratory-based experiments mimic and are able to predict more closely the expected behavior of the probe *in vivo*.

ACKNOWLEDGMENTS

The authors thank the Royal Society (SIP, JMZ, PAW), St Anne's College (SIP), Merton College (PAW), The University of Bath and EPSRC for funding. The authors thank Professor Jonathan R. Dilworth for support and many constructive and stimulating discussions in preparing the material for this manuscript.

REFERENCES

1. Massoud, T. F.; Gambhir, S. S. *Genes Dev.* **2003**, *17*, 545.
2. Rudin, M.; Weissleder, R. *Nat. Rev. Drug Disc.* **2003**, *2*, 123.
3. Watanabe, T. *Nucl. Magn. Res.* **1998**, *27*, 431.
4. Parker, D. *Chemistry in Britain* **1994**, *30*, 818.
5. Storr, T.; Thompson, K. H.; Orvig, C. *Chem. Soc. Rev.* **2006**, *35*, 534.
6. Anderson, C. J.; Welch, M. J. *Chem. Rev.* **1999**, *99*, 2219.
7. Liu, S. *Chem. Soc. Rev.* **2004**, *33*, 445.
8. Dilworth, J. R.; Parrott, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43.
9. <http://www.proportionaltech.com/cuptsm.htm>
10. Blower, P. J.; Lewis, J. S.; Zweit, J. *Nucl. Med. Biol.* **1996**, *23*, 957.
11. Delgado-Bolton, R. C.; Carreras, J. L.; Perez-Castejon, M. J. *Curr. Med. Imag. Rev.* **2006**, *2*, 215.
12. Holland, J. P.; Green, J. C.; Dilworth, J. R. *Dalton Trans.* **2006**, 783.
13. Ntziachristos, V.; Ripoll, J.; Wang, L. V.; Weissleder, R. *Nat. Biotech.* **2005**, *23*, 313.
14. Hasegawa, B.; Tang, H. R.; Da Silva, A. J.; Wong, K. H.; Iwata, K.; Wu, M. C. *Nucl. Instrum. Meth. Phys. Res. A* **2001**, *471*, 140.
15. Wolfbeis, O. S. *J. Mater. Chem.* **2005**, *15*, 2657.
16. Godwin, H. A.; Berg, J. M. *J. Am. Chem. Soc.* **1996**, *118*, 6514.
17. Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3.
18. Pandya, S.; Yu, J.; Parker, D. *Dalton Trans.* **2006**, 2757.
19. Yu, M.; Zhao, Q.; Shi, L.; Li, F.; Zhou, Z.; Yang, H.; Yi, T.; Huang, C. *Chem. Commun.* **2008**, 2115.
20. Blower, P. *Annu. Rep. Prog. Chem. Sect. A* **1999**, *95*, 631.
21. Blower, P. J. *Dalton Trans.* **2006**, 1705.
22. Maina, T.; Nock, B. A.; Cordopatis, P.; Bernard, B. F.; Breeman, W. A.; Gameren, A.; Berg, R.; Reubi, J. C.; Krenning, E. P.; Jong, M. *Eur. J. Nucl. Med. Molec. Imag.* **2006**, *33*(7), 831.
23. Wang, M.; Caruano, A. L.; Lewis, M. R.; Meyer, L. A.; VanderWaal, R. P.; Anderson, C. J. *Cancer Res.* **2003**, *63*(20), 6864.
24. Smith-Jones, P. M.; Bischof, C.; Leimer, M.; Gludovacz, D.; Angelberger, P.; Pangerl, T.; Peck-Radosavljevic, M.; Hamilton, G.; Kaserer, K.; Kofler, A. *Endocrinology* **1999**, *140*(11), 5136.
25. Wangberg, B.; Nilsson, O.; Johanson, V.; Kolby, L.; Forssell-Aronsson, E.; Andersson, P.; Fjalling, M.; Tisell, L.-E.; Ahlman, H. *Oncologist* **1997**, *2*, 50.
26. Storch, D.; Behe, M.; Walter, M. A.; Chen, J.; Powell, P.; Mikolajczak, R.; Macke, H. R. *J. Nucl. Med.* **2005**, *46*, 1561.
27. a) James, S.; Maresca, K. P.; Babich, J. W.; Valliant, J. F.; Doering, L. J. *Z. Bioconj. Chem.* **2006**, *17*, 590. b) James, S.; Maresca, K. P.; Allis, D.;

- Valliant, J. F.; Eckelman, W.; Babich, J. W.; Zubieta, J. *Bioconjug. Chem.* **2006**, *17*, 579.
28. Wei, L.; Babich, J. W.; Ouellette, W.; Zubieta, J. *Inorg. Chem.* **2006**, *45*, 3057.
29. Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. *J. Am. Chem. Soc.*, **2004**, *126* (28), 8598.
30. a) Haeffliger, P.; Agorastos, N.; Renard, A.; Giambonini-Brugnoli, G.; Marty, C.; Alberto, R. *Bioconjug. Chem.* **2005**, *16*, 582. b) Haeffliger, P.; Agorastos, N.; Spingler, B.; Georgiev, O.; Viola, G.; Alberto, R. *Chem. Bio. Chem.* **2005**, *6*, 414.
31. Agorastos, N.; Borsig, L.; Renard, A.; Antoni, P.; Viola, G.; Spingler, B.; Kurz, P.; Alberto, R. *Chem. Eur. J.* **2007**, *13*, 3842.
32. Kramer, A.; Alberto, R.; Egli, A.; NovakHofer, I.; Hegetschweiler, K.; Abram, U.; Bernhardt, P. V.; Schubiger, P. A. *Bioconjug. Chem.* **1998**, *9*, 691.
33. Tzanopoulou, S.; Pirmettis, I. C.; Patsis, G.; Paravatou-Petsotas, M.; Livaniou, E.; Papadopoulos, M.; Pelecanou, M. *J. Med. Chem.* **2006**, *49*, 5408.
34. Hua, J.; Dobrucki, L. W.; Sadeghi, M. M.; Zhang, J.; Bourke, B. N.; Cavaliere, P.; Song, J.; Chow, C.; Jahanshad, N.; van Royen, N.; Buschmann, I.; Madri, J. A.; Mendizabal, M.; Sinusas, A. J. *Circulation* **2005**, 3256.
35. Yao, Z.; Zhang, M.; Sakahara, H.; Saga, T.; Arano, Y.; Konishi, J. *J. Natl. Cancer Inst.* **1998**, *90*, 25.
36. Nakamoto, Y.; Saga, T.; Sakahara, H.; Yao, Z. S.; Zhang, M. L.; Sato, N.; Zhao, S. J.; Nakada, H.; Yamashina, I.; Konishi, J. *Nucl. Med. Biol.* **1998**, *25*, 95.
37. Dalkara, S.; Petrov, A.; Trubetskoy, V. S.; Khaw, B. A.; Torchilin, V. P. *J. Drug Targeting* **1998**, *6*, 45.
38. Mathias, C. J.; Green, M. A. *Nucl. Med. Biol.* **1998**, *25*, 585.
39. Mathias, C. J.; Wang, S.; Waters, D. J.; Turek, J. J.; Low, P. S.; Green, M. A. *J. Nucl. Med.* **1998**, *39*, 1579.
40. JaarskelaSaari, H. A.; Kairemo, K. J. A.; Ramsay, H. A.; Grenman, R. *Int. J. Radiat. Biol.* **1998**, *73*, 565.
41. Foster, N.; Woo, D. V.; Kaltovich, F.; Emrich, J.; Ljungquist, C. *J. Nucl. Med.* **1985**, *26*, 756.
42. Robinson, G. D., Jr.; Alavi, A.; Vaum, R.; Staum, M. *J. Nucl. Med.* **1986**, *27*, 239.
43. Yang, D. J.; Azhdarinia, A.; Kim, E. E. *Curr. Med. Imag. Rev.* **2005**, *1*, 25.
44. Moerlein, S. M.; Welch, M. J. *Int. J. Nucl. Med. Biol.* **1981**, *8*, 277.
45. Green, M. A.; Welch, M. J. *Nucl. Med. Biol.* **1989**, *16*, 435.
46. Luttrupp, C. A.; Jackson, J. A.; Jones, B. J.; Sohn, M.-H.; Lynch, R. E.; Morton, K. A. *J. Nucl. Med.* **1998**, *39*, 1405.
47. Caraco, C.; Aloj, L.; Eckelman, W. C. *Appl. Radiat. Isot.* **1998**, *49*, 1477.
48. Ryser, J. E.; Rose, K.; Jones, R.; Pelegrin, A.; Donath, A.; Egeli, R.; Smith, A.; Oord, R. E. *Nucl. Med. Biol.* **1998**, *25*, 261.
49. Watanabe, R.; Iizuka, H.; Kaira, K.; Mori, T.; Takise, A.; Ito, J.; Motegi, A.; Onozato, Y.; Ishihara, H. *Radiat. Med.* **2006**, *24*, 456.
50. Nieweg, O. E.; Beekhuis, H.; Piers, D. A.; Sluiter, H. J.; van der Wal, A. M.; Woldring, M. G. *Thorax* **1983**, *38*, 16.
51. Zhang, Z.; Lyster, D. M.; Webb, G. A.; Orvig, C. *Int. J. Rad. Appl. Instr. B: Nucl. Med. Biol.* **1992**, *19*, 327.

52. Tsang, B. W.; Mathias, C. J.; Fanwick, P. E.; Green, M. A. *J. Med. Chem.* **1994**, *37*, 4400.
53. Kung, H. F.; Liu, B. L.; Mankoff, D.; Kung, M. P.; Billings, J. J.; Francesconi, L.; Alavi, A. *J. Nucl. Med.* **1990**, *31*, 1635.
54. Mohammadi, K.; Thompson, K. H.; Patrick, B. O.; Storr, T.; Martins, C.; Polishchuk, E.; Yuen, V. G.; McNeill, J. H.; Orvig, C. *J. Inorg. Biochem.* **2005**, *99*, 2217.
55. Griffiths, G. L.; Chang, C.-H.; McBride, W. J.; Rossi, E. A.; Sheerin, A.; Tejada, G. R.; Karacay, H.; Sharkey, R. M.; Horak, I. D.; Hansen, H. J.; Goldenberg, D. M. *J. Nucl. Med.* **2004**, *45*, 30.
56. Forster, G. J.; Santos, E. B.; Smith-Jones, P. M.; Zanzonico, P.; Larson, S. M. *J. Nucl. Med.* **2006**, *47*, 140.
57. Motekaitis, R. J.; Martell, A. E.; Koch, S. A.; Hwang, J. W.; Quarless, D. A.; Welch, M. J. *Inorg. Chem.* **1998**, *37*, 5902.
58. Karanikas, G.; Rodrigues, M.; Grannegger, S.; Havlik, E.; Sinzinger, H. *Nucl. Med. Biol.* **1998**, *25*, 165.
59. Klivenyi, G.; Schuhmacher, J.; Patzelt, E.; Hauser, H.; Matys, R.; Moock, M.; Regiert, T.; Maier-Borst, W. *J. Nucl. Med.* **1998**, *39*, 1769.
60. Mathias, C. J.; Lewis, M. R.; Reichert, D. E.; Laforest, R.; Sharpe, T. L.; Lewis, J. S.; Yang, Z.-F.; Waters, D. J.; Snyder, P. W.; Low, P. S.; Welch, M. J.; Green, M. A. *Nucl. Med. Biol.* **2003**, *30*, 725.
61. Stevens, H.; Knollema, S.; Piers, D. A.; Van der Wiele, C.; Jansen, H. M. L.; De Jager, A. E. J.; De Reuck, J.; Dierckx, R. A.; Korf, J. *Nucl. Med. Commun.* **1998**, *19*, 573.
62. Stevens, H.; Van der Wiele, C.; Santens, P.; Jansen, H. M. L.; De Reuck, J.; Dierckx, R.; Korf, J. *J. Nucl. Med.* **1998**, *39*, 495.
63. Korf, J.; Veenma-van der Duin, L.; Brinkman-Medema, R.; Niemarkt, A.; de Leij, L. F. F. H. *J. Nucl. Med.* **1998**, *39*, 836.
64. AlJeboori, M. J.; Dilworth, J. R.; Zheng, Y. F. *J. Chem. Soc. Dalton Trans.* **1998**, 3215.
65. Gasser, G.; Tijoe, L.; Belousoff, M. J.; Spiccia, L.; Graham, B.; Juran, S.; Walther, M.; Künstler, J.-U.; Bergmann, R.; Stephan, H. *J. Label. Compd. Radiopharm.* **2007**, *50*, S230.
66. Juweid, M.; Sharkey, R. M.; Swayne, L. C.; Griths, G. L.; Dunn, R.; Goldenberg, D. M. *J. Nucl. Med.* **1998**, *39*, 34.
67. Schmidt, P. F.; Smith, S. V.; Bundesen, P. G. *Nucl. Med. Biol.* **1998**, *25*, 639.
68. Iznaga Escobar, N. *Nucl. Med. Biol.* **1998**, *25*, 441.
69. Cattabriga, M.; Scatturin, A.; Bertolasi, V.; Ferretti, V. *J. Chem. Soc. Dalton Trans.* **1998**, 1453.
70. Hoepfing, A.; Brust, P.; Berger, R.; Leibnitz, P.; Spies, H.; Machill, S.; Scheller, D.; Johannsen, B. *Bioorg. Med. Chem.* **1998**, *6*, 1663.
71. Wust, F.; Scheller, D.; Spies, H.; Johannsen, B. *Eur. J. Inorg. Chem.* **1998**, 789.
72. Maresca, K. P.; Femia, F. J.; Babich, J. W.; Zubieta, J. *Inorg. Chem. Commun.* **1998**, *1*, 209.
73. Papadopoulos, M.; Pirmettis, I.; Raptopoulou, C.; Chiotellis, E.; Friebe, M.; Berger, R.; Spies, H.; Johannsen, B. *Appl. Radiat. Isot.* **1998**, *49*, 961.
74. Hashimoto, K.; Baghiawati, S.; Islam, S. *Radiochemistry* **1998**, *39*, 389.
75. Dadachova, E.; Chapman, J. *Nucl. Med. Commun.* **1998**, *19*, 173.
76. De Jong, M.; Bernard, B. F.; De Bruin, E.; Van Gameren, A.; Bakker, W. H.; Visser, T. J.; R., M. H.; Krenning, E. P. *Nucl. Med. Commun.* **1998**, *19*, 283.

77. Leimer, M.; Kurtaran, A.; Smith-Jones, P.; Raderer, M.; Havlik, E.; Angelberger, P.; Vorbeck, F.; Niederle, B.; Herold, C.; Virgolini, I. *J. Nucl. Med.* **1998**, *39*, 2090.
78. Kobayashi, H.; Wu, C.; Yoo, T. M.; Sun, B.-F.; Drumm, D.; Pastan, I.; Paik, C. H.; Gansow, O. A.; Carrasquillo, J. A.; Brechbiel, M. W. *J. Nucl. Med.* **1998**, *39*, 829.
79. Smith-Jones, P. M.; Stolz, B.; Albert, R.; Ruser, G.; Briner, U.; Macke, H. R.; Bruns, C. *Nucl. Med. Biol.* **1998**, *25*, 181.
80. Kruger, K.; Jochum, C.; Glusenkamp, K.-H.; Krusemann, C.; Lorenz, P.; Eberle-Adamkiewicz, G.; Drosdziok, W.; Beelen, D. W.; Coenen, H. H.; Rajewsky, M. E. *Int. J. Cancer* **1998**, *77*, 610.
81. Verel, I.; Visser, G. W. M.; Boerman, O. C.; van Eerd, J. E. M.; Finn, R.; Boellaard, R.; Vosjan, M. J. W. D.; Stigter-van Walsum, M.; Snow, G. B.; van Dongen, G. A. M. S. *Cancer Biother. Radiopharm.* **2003**, *18*, 655.
82. Biddlecombe, G.; Rogers, B. E.; Visser, M.; Parry, J.; Jong, M.; Erion, J.; Lewis, J. S. *Bioconjug. Chem.* **2007**, *18*, 724.
83. (a) Carroll, V. A.; Ashcroft, M. *Expert Rev. Mol. Med.* **2005**, *7*, 1. (b) Maurer, R. I.; Blower, P. J.; Dilworth, J. R.; Reynolds, C. A.; Zheng, Y.; Mullen, G. E. D. *J. Med. Chem.* **2002**, *45*, 1420.
84. Blower, P. J.; Castle, T. C.; Cowley, A. R.; Dilworth, J. R.; Donnelly, P. S.; Labisbal, E.; Sowrey, F. E.; Teat, S. J.; Went, M. J. *Dalton Trans.* **2003**, 4416.
85. Fujibayashi, Y.; Taniuchi, H.; Yonekura, Y.; Ohtani, H.; Konishi, J.; Yokoyama, A. *J. Nucl. Med.* **1997**, *38*, 1155.
86. Dearling, J. L. J.; Lewis, J. S.; Mullen, G. E. D.; Welch, M. J.; Blower, P. J. *J. Biol. Inorg. Chem.* **2002**, *7*, 249.
87. Dearling, J. L. J.; Lewis, J. S.; Mullen, G. E. D.; Rae, M. T.; Zweit, J.; Blower, P. J. *Eur. J. Nucl. Med.* **1998**, *25*, 788.
88. Lewis, J. S.; McCarthy, D. W.; McCarthy, T. J.; Fujibayashi, Y.; Welch, M. J. *J. Nucl. Med.* **1999**, *40*, 177.
89. Padhani, A. R.; Krohn, K. A.; Lewis, J. S.; Alber, M. *Eur. Radiol.* **2007**, *17*, 861.
90. Mallia, M. B.; Subramanian, S.; Mathur, A.; Sarma, H. D.; Venkatesh, M.; Banerjee, S. *J. Label. Compd. Radiopharm.* **2007**, *51*, 308.
91. Liu, Y.; Xu, Y.; Qian, X.; Liu, J.; Shen, L.; Li, J.; Zhang, Y. *Bioorg. Med. Chem.* **2006**, *14*, 2935.
92. Liu, Z.; Cai, W.; He, L.; Nakayama, N.; Chen, K.; Sun, X.; Chen, X.; Dai, H. *Nat. Nanotechnol.* **2007**, *2*, 47.
93. Stewart, W. W. *Nat. Biotech.* **1981**, *292*, 17.
94. Hodgkiss, R. J.; Jones, G. W.; Long, A.; Middleton, R. W.; Parrick, J.; Stratford, M. R.; Wardman, P. G. D. W. *Biochem. Pharmacol.* **1991**, *41*, 533.
95. Hodgkiss, R. J.; Jones, G. W.; Long, A.; Middleton, R. W.; Parrick, J.; Stratford, M. R.; Wardman, P. G. D. W. *J. Med. Chem.* **1991**, *34*, 2268.
96. Olive, P. L.; Durand, R. E. *Cancer Res.* **1983**, *43*, 3276.
97. Cecchi, A.; Hulikova, A.; Pastorek, J.; Pastoreková, S.; Scozzafava, A.; Winum, J.-Y.; Montero, J.-L.; Supuran, C. T. *J. Med. Chem.* **2005**, *48*, 4834.
98. Chao, K. S.; Bosch, W. R.; Mutic, S.; Lewis, J. S.; Dehdashti, F.; Mintun, M. A.; Dempsey, J. F.; Perez, C. A.; Purdy, J. A.; Welch, M. J. *Int. J. Radiat. Oncol. Biol. Phys.* **2001**, *49*, 1171.
99. Fujibayashi, Y.; Cutler, C. S.; Anderson, C. J.; McCarthy, D. W.; Jones, L. A.; Sharp, T.; Yonekura, Y.; Welch, M. J. *Nucl. Med. Biol.* **1998**, *26*, 117.

100. Cowley, A. R.; Dilworth, J. R.; Donnelly, P. S.; Labisbal, E.; Sousa, A. *J. Am. Chem. Soc.* **2002**, *124*, 5270.
101. Barnard, P.; Bayly, S.; Betts, H.; Bonnitcha, P.; Christlieb, M.; Dilworth, J.; Holland, J.; Pascu, S. Q. *J. Nucl. Med. Mol. Imag.* **2008**, *52*, 174.
102. Holland, J.; Aigbirhio, F.; Betts, H.; Bonnitcha, P.; Burke, P.; Christlieb, M.; Churchill, G.; Cowley, A.; Dilworth, J.; Donnelly, P.; Green, J.; Peach, J.; Vasudevan, S.; Warren, J. *Inorg. Chem.* **2007**, *46*, 465.
103. Bonnitcha, P. D.; Vavere, A. L.; Lewis, J. S.; Dilworth, J. R. *J. Med. Chem.* **2008**, *51*, 2985–2991.
104. Holland, J. P.; Barnard, P. J.; Bayly, S. R.; Betts, H. M.; Churchill, G. C.; Dilworth, J. R.; Edge, R.; Green, J. C.; Hueting, R. *Eur. J. Inorg. Chem.* **2008**, 1985–1993.
105. Cowley, A. R.; Davis, J. J.; Dilworth, J. R.; Donnelly, P. S.; Dobson, R.; Nightingale, A.; Peach, J. M.; Shore, B.; Kerr, D.; Seymour, L. *Chem. Commun.* **2005**, 845.
106. Hirano, T.; Kikichi, K.; Urano, Y.; Higuchi, T.; Nagano, T. *Angew. Chem. Int. Ed.* **2000**, *39*, 1052.
107. Liu, Y.; Zhang, N.; Chen, Y.; Wang, L.-H. *Org. Lett.* **2007**, *9*, 315.
108. Rodriguez-Arguelles, M. C.; Ferrari, M. B.; Fava, G. G.; Pelizzi, C.; Pelosi, G.; Albertini, R.; Bonati, A.; Dall'Aglia, P. P.; Lunghi, P.; Pinelli, S. *J. Inorg. Biochem.* **1997**, *66*, 7.
109. Kovala-Demertzi, D.; Yadav, P. N.; Wiecek, J.; Skoulia, S.; Varadinova, T.; Demertzis, M. A. *J. Inorg. Biochem.* **2006**, *100*, 1558.
110. Li, F.; Cui, J.; Guo, L.; Qian, X.; Ren, W.; Wang, K.; Liu, F. *Bioorg. Med. Chem.* **2007**, *15*, 5114.
111. Lu, H.-L.; Liang, J.-J.; Zeng, Z.-Z.; Xi, P. X.; Liu, X.-H.; Chen, F.-J.; Xu, Z.-H. *Trans. Met. Chem.* **2007**, *32*, 564.
112. Pascu, S. I.; Waghorn, P. A.; Conry, T. D.; Lin, B.; Betts, H. M.; Dilworth, J. R.; Sim, R. B.; Churchill, G. C.; Aigbirhio, F. I.; Warren, J. E. *Dalton Trans.* **2008**, 2107.
113. Pascu, S. I.; Waghorn, P. A.; Conry, T. D.; Betts, H. M.; Dilworth, J. R.; Churchill, G. C.; Pokrovskaya, T.; Christlieb, M.; Aigbirhio, F. I.; Warren, J. E. *Dalton Trans.* **2007**, 4988.
114. Bonnett, R. *Chem. Soc. Rev.* **1995**, *24*, 19.
115. Ali, H.; Van Lier, J. E. *Chem. Rev.* **1999**, *99*, 2379.
116. Berg, K.; Selbo, P. K.; Weyergang, A.; Dietze, A.; Prasmickaite, L.; Bonsted, A.; Engesaeter, B. O.; Angell-Petersen, E.; Warloe, T.; Frandsen, N.; Hogset, A. *J. Microsc.* **2005**, *218*, 133.
117. Gomer, C. J.; Dougherty, T. J. *Cancer Res.* **1979**, *39*, 146.
118. a) Pandey, S. K.; Gryshuk, A. L.; Sajjad, M.; Zheng, X.; Chen, Y.; Abouzeid, M. M.; Morgan, J.; Charamisinau, I.; Nabi, H. A.; Oseroff, A.; Pandey, R. K. *J. Med. Chem.* **2005**, *48*, 6286. b) Ma, B.; Li, G.; Kanter, P.; Lamonica, D.; Grossman, Z.; Pandey, R. K. *J. Porphyrins Phthalocyanines* **2003**, *7*, 500.
119. Wrenn, J. F. R.; Good, M. L.; Handler, P. *Science* **1951**, *113*, 525.
120. Roberts, J. C.; Figard, S. D.; Mercer-Smith, J. A.; Svitra, Z. V.; Anderson, W. L.; Lavalley, D. K. *J. Immunol. Meth.* **1987**, *105*, 153.
121. Bases, R.; Brodie, S. T.; Rubinfeld, S. *Cancer* **1958**, *11*, 259.
122. Cole, D. A.; Mercer-Smith, J. A.; Schreyer, S. A.; Norman, J. K.; Lavalee, D. K. *Nucl. Med. Biol.* **1990**, *17*, 457.
123. Mercer-Smith, J. A.; Cole, D. A.; Roberts, J. A.; Lewis, D.; Behr, M. J.; Lavallee, D. K. *Adv. Exp. Med. Biol.* **1989**, *258*, 103.

124. Wong, D. W.; Mandal, A.; Reese, I. C.; Brown, J.; Siegler, R. *Int. J. Nucl. Med. Biol.* **1983**, *10*, 211.
125. Buchler, J. W.; De Cian, A.; Fischer, J.; Kruppe, S. B.; Weiss, R. *Chem. Ber.* **1990**, *123*, 2247.
126. Buchler, J. W.; Kruppe, S. M. S.; Prescher, G. *Patent Ger Offen*; Germany, **1989**.
127. Lawrence, A. J.; Thornback, J. R.; Zanelli, G. D.; Lawson, A. *Inorg. Chim. Acta* **1988**, *141*, 165.
128. Subbarayan, M.; Shetty, S. J.; Srivastava, T. S.; Noronha, O. P. D.; Samuel, A. M. *J. Porph. Phthalocyan.* **2001**, *5*, 824.
129. Banerjee, D. T.; Das, T.; Samuel, G.; Sarma, H.; Pillai, M. R. A. *Nucl. Med. Commun.* **2001**, *22*, 1101.
130. Murugesan, S.; Shetty, S. J.; Srivastava, T. S.; Noronha, O. P. D.; Samuel, A. M. *Appl. Rad. Isot.* **2001**, *55*, 641.
131. Tong, C.; Bottomley, L. A. *J. Porph. Phthalocyan.* **1998**, *B2B*, 261.
132. Collman, J. P.; Garner, J. M.; Kim, F.; Ibers, J. *Inorg. Chem.* **1989**, *28*, 1610.
133. Tsutsui, M.; Hsung, C. P.; Ostfeld, D.; Srivastava, T. S.; Cullen, D. L.; Meyer, E. F., Jr. *J. Am. Chem. Soc.* **1975**, *97*, 3952.
134. Vaum, R.; Heindel, N. D.; Burns, H. D.; Emrich, J.; Foster, N. *J. Pharm. Sci.* **1982**, *71*, 1223.
135. Nakajima, S.; Hayashi, H.; Ohshima, K.; Yamazaki, K.; Kubo, Y.; Samejima, N.; Kakiuchi, Y.; Shindoh, Y.; Koshimizu, H. *Photochem. Photobiol.* **1987**, *46*, 783.
136. Bedel-Cloutour, C. H.; Mauclaire, L.; Saux, A.; Pereyre, M. *Bioconjug. Chem.* **1996**, *7*, 617.
137. Wong, D. W. *Int. J. Appl. Rad. Isot.* **1984**, *35*, 691.
138. Babbar, A. K.; Singh, A. K.; Goel, H. C.; Chauhan, U. P. S.; Sharma, R. K. *Nucl. Med. Biol.* **2000**, *27*, 587.
139. Polo, L.; Segalla, A.; Jori, G.; Bocchiotti, G.; Verna, G.; Franceschini, R.; Mosca, R.; De Filippi, P. G. *Cancer Lett.* **1996**, *109*, 57.
140. Sessler, J. L.; Miller, R. A. *Biochem. Pharma.* **2000**, *59*, 733.
141. van Vlerken, L. E.; Amiji, M. M. *Expert Opin. Drug. Deliv.* **2006**, *3*, 205.
142. Molema, G. "Organ-Specific Strategies"; Wiley-VCH: Weinheim, **2001**.
143. Adschiri, T.; Naka, T.; Takami, S.; Umetsu, M.; Ohara, S. *Baioimejingu* **2005**, *14*, 85.
144. Maysinger, D. *Org. Biomol. Chem.* **2007**, *5*, 2335.
145. Cuenca, A. G.; Jiang, H.; Hochwald, S. N.; Delano, M.; Cance, W. G.; Grobmyer, S. R. *Cancer* **2006**, *107*, 459.
146. Jain, K. K. *Clin. Chim. Acta* **2005**, *358*, 37.
147. a) Oh, J. M.; Choi, S.-J.; Lee, G.-E.; Han, S.-H.; Choy, J.-H.; *Adv. Funct. Mater.* **2009**, *19*, DOI:10.1002/adfm.200801127. b) Park, K.; Lee, S.; Kang, W.; Kim, K.; Choi, K.; Kwon, I. C. *Adv. Funct. Mater.* **2009**, *19*, DOI:10.1002/adfm.200801655.
148. Emfietzoglou, D.; Kostarelos, K.; Sgouros, G. *J. Nucl. Med.* **2001**, *42*, 499.
149. Chen, L. C.; Chang, C. H.; Yu, C. Y.; Chang, Y. J.; Hsu, W. C.; Ho, C. L.; Yeh, C. H.; Luo, T. Y.; Lee, T. W.; Ting, G. *Nucl. Med. Biol.* **2007**, *34*, 415.
150. Ballot, S.; Noiret, N.; Hindré, F.; Denizot, B.; Garin, E.; Rajerison, H.; Benoit, J.-P. *Eur. J. Nucl. Med. Mol. Imag.* **2006**, *33*, 602.
151. Pankhurst, O. A.; Connolly, J.; Jones, S. K.; Dobson, J. *J. Phys. D: Appl. Phys.* **2003**, *36*, R167.

152. Wang, S. J.; Lin, W. Y.; Chen, M. N.; Chi, C. S.; Chen, J. T.; Ho, W. L.; Hsieh, B. T.; Shen, L. H.; Tsai, Z. T.; Ting, G.; Mirzadeh, S.; Knapp, F. F. *J. Nucl. Med.* **1998**, *39*, 1752.
153. Rossin, R.; Pan, D.; Qi, K.; Turner, J. L.; Sun, X.; Wooley, K. L.; Welch, M. J. *J. Nucl. Med.* **2005**, *46*, 1210.
154. Li, Y.-G.; Huang, X.; Liu, R.-L.; Li, Q.-n.; Zhang, X.-D.; Li, W.-X. *J. Radioanal. Nucl. Chem.* **2005**, *265*, 127.
155. a) www.cnanotech.com b) S.I. Pascu; The 1st European Chemistry Congress, Budapest **2006**.
156. Kam, N. W. S.; O'Connell, M.; Wisdom, J. A.; Dai, H. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11600.
157. Yehia, H. N.; Draper, R. K.; Mikoryak, C.; Walker, E. K.; Bajaj, P.; Musselman, I. H.; Daigrepont, M. C.; Dieckmann, G. R.; Pantano, P. J. *Nanobiotech.* **2007**, *5*, 8.
158. Nakayama-Ratchford, N.; Bangsaruntip, S.; Sun, X.; Welsher, K.; Dai, H. *J. Am. Chem. Soc.* **2007**, *129*, 2448.
159. Liu, Y.; Wang, H. *Nat. Nanotechnol.* **2007**, *2*, 20.
160. McDevitt, M. R.; Chattopadhyay, D.; Jaggi, J. S.; Finn, R. D.; Zanzonico, P. B.; Villa, C.; Rey, D.; Mendenhall, J.; Batt, C. A.; Njardarson, J. T.; Scheinberg, D. A. *Int. J. Radiat. Oncol. Biol. Phys.* **2006**, *64*, 1503.
161. a) McDevitt, M. R.; Chattopadhyay, D.; Kappel, B. J.; Jaggi, J. S.; Schiffman, S. R.; Antczak, C.; Njardarson, J. T.; Brentjens, R.; Scheinberg, D. A. *J. Nucl. Med.* **2007**, *48*, 1180. b) Escorcia, F. E.; McDevitt, M. R.; Villa, C. H.; Scheinberg, D. A. *Nanomedicine* **2007**, *2*, 805.
162. Lacerda, L.; Soundararajan, A.; Singh, R.; Pastorin, G.; Al-Jamal, K. T.; Turton, J.; Frederik, P.; Herrero, M. A.; Li, S.; Bao, A.; Emfietzoglou, D.; Mather, S.; Phillips, W. T.; Prato, M.; Bianco, A.; Goins, B.; Kostarelos, K. *Adv. Mater.* **2008**, *20*(2), 225.
163. a) Mackeyev, Y. A.; Marks, J. W.; Rosenblum, M. G.; Wilson, L. J. *J. Phys. Chem. B* **2005**, *109*, 5482. b) Prato, M.; Kostarelos, K.; Bianco, A. *Acc. Chem. Res.* **2008**, *41*(1), 60.
164. Chan, H. B. S.; Ellis, B. L.; Sharma, H. L.; Frost, W.; Caps, V.; Shields, R. A.; Tsang, S. C. *Adv. Mater.* **2004**, *16*, 144.
165. Davis, J. J.; Green, M. L. H.; Hill, H. A. O.; Leung, Y. C.; Sadler, P. J.; Sloan, J.; Xavier, A. V.; Tsang, S. C. *Inorg. Chim. Acta* **1998**, *272*, 261.
166. a) Lacerda, L.; Pastorin, G.; Wu, W.; Wieckowski, S.; Luangsivilay, J.; Godefroy, S.; Pantarotto, D.; Briand, J. P.; Muller, S.; Prato, M.; Bianco, A. *Nature Nanotech.* **2007**, *2*, 108. b) Schulze, C.; Kröll, A.; Lehr, C.-M.; Schaefer, U. F.; Becker, K.; Schnekenburger, J.; Schulze Isfort, C.; Landsiedel, R.; Wohlleben, W. *Nanotoxicology* **2008**, *2*, 51.
167. van Kasteren, S. I.; Campbell, S. J.; Serres, S.; Anthony, D. C.; Sibson, N. R.; Davis, B. G. *Proc. Natl. Acad. Sci.*, **2009**, *106*(1), 18.

IRON SEQUESTRATION BY SMALL MOLECULES: THERMODYNAMIC AND KINETIC STUDIES OF NATURAL SIDEROPHORES AND SYNTHETIC MODEL COMPOUNDS

ALVIN L. CRUMBLISS and JAMES M. HARRINGTON

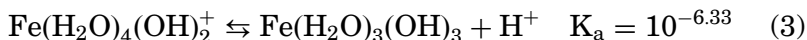
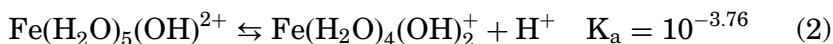
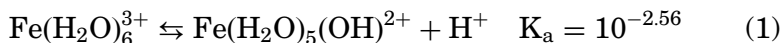
Department of Chemistry, Duke University, Durham, NC, 27708-0346, USA

I. Chemistry of Iron and Siderophores	179
II. Siderophore Structure	182
A. Common Binding Groups	182
B. Common Architectures	183
III. Thermodynamics of Iron–Siderophore Interactions	186
A. Siderophore Complex Stability Constants	186
B. Methods of Stability Constant Determination	190
C. Survey of Thermodynamic Stability Constants	194
D. Redox Chemistry of Iron–Siderophore Complexes	210
IV. Chelation Kinetics of Iron–Siderophore Systems	220
A. Siderophore Complex Formation Kinetics and Mechanism	220
B. Siderophore Complex Hydrolysis Kinetics and Mechanism	223
C. Siderophore Complex Exchange Kinetics and Mechanism	228
V. Siderophore Recognition and Cellular Uptake	231
VI. Applications and Future Directions in Siderophore Research	235
A. Iron Chelation Therapy	236
B. Siderocalin and Evasion by Siderophores	237
C. Future Research in Siderophore Coordination Chemistry	238
List of Structure Numbers, Figures, and Abbreviations	240
Acknowledgments	242
References	242

I. Chemistry of Iron and Siderophores

Iron is the second most abundant metal on the planet. Probably due to its abundance, virtually all forms of life have evolved to depend on iron to support essential biological processes, which include a range of catalytic processes, electron transfer, and small molecule storage and transport (1). However, ferric

iron (Fe^{3+}) is a moderately strong Brønsted acid, with pK_a 's of 2.56, 3.76, and 6.33 (Eqs. (1)–(3)), on an order with many organic



acids (2). This results in easy deprotonation of the metal ion and formation of a variety of insoluble hydroxy containing species including $\text{Fe}(\text{OH})_3$ ($K_{\text{sp}} = 10^{-39}$). In an aqueous aerobic environment iron exists as the ferric ion and as a result of these deprotonation reactions has a maximum solubility of all hydrolytic forms at a pH of 7 of approximately 10^{-10} M, much lower than the minimum concentration required to support microbial life (1,3–5). Another challenge for aerobic living systems is that many processes produce superoxide and hydrogen peroxide, which can participate in redox cycles involving iron(III) and iron(II). Ferrous iron can react with O_2 present in the system to produce reactive oxygen species (ROS) in coupled reactions known as the Fenton cycle (6). These ROS's are potentially harmful to the cell as they can cause oxidative damage to membranes, DNA, and other cellular components (7,8).

Due to these complications, microorganisms have developed methods to aid in obtaining environmental iron. Environmental microbes and some pathogenic bacteria use small molecules approximately 1 kDa in size, called siderophores (from the Greek term for “iron-carrying”), to make iron bioavailable. Siderophores are specific for binding environmental iron and carrying it back to the cell, where it is recognized and transported across the outer cell membrane (3,4,9,10). Siderophores manifest their high and specific affinity for iron through very large stability constants for the formation of the ferric complex, generally in the range of $\beta = 10^{25}$ to $\beta = 10^{50}$. This high stability is required so that siderophore sequestration can compete effectively with the precipitation of iron (hydr)oxides. Siderophores must also be able to compete with chelating agents produced by other sources. The sequestration of iron by siderophores serves three important purposes: (i) it will increase the aqueous solubility of iron; (ii) it will control the redox activity of the metal ion (moving it out of the range of potentials that will react with hydrogen peroxide

and superoxide); and (iii) it will provide characteristic surface features for recognition so that the target cell can selectively bring the iron across the outer membrane.

Some bacteria in a mammalian host use a receptor mediated iron acquisition process whereby a receptor is expressed on the cell surface that recognizes a host protein iron carrier (e.g., transferrin), strips the iron, and brings it into the cell. Other bacteria excrete siderophores to compete with host carriers in the extracellular fluids (11,12). Environmental microbes also produce siderophores that must compete with other environmental chelators to sequester the iron and bring it back to the cell. In some cases these organisms must liberate the iron that is trapped in mineral rocks (e.g., goethite and hematite) in the form of oxides and other insoluble salts, which dissolve very slowly and to a limited degree (13,14). One mechanism for extracting iron from the minerals is by increasing the solubility of the metal ion through reductase release by the organism (15). The iron is reduced from the less soluble 3+ oxidation state to the more soluble 2+ oxidation state, which both increases the solubility of the metal ion and increases the rate of dissolution of the metal. It has been reported that iron(II) in solution can interact with siderophores through a redox reaction to produce iron(III), providing a pathway for reoxidation of the iron in addition to air oxidation (16,17). Another route of extracting the metal is through complexation by siderophores that have adsorbed on the surface of the insoluble minerals. When siderophores adsorb onto the surface of minerals, they displace water and hydroxide coordinated to the exposed face of the insoluble metal. This partial coordination of the metal by a siderophore may be sufficient to increase the dissolution kinetics of the metal center (4,13,18).

This review will discuss research on natural siderophores and synthetic siderophore-like molecules, with an emphasis on results from our laboratory over the past decade in the context of the overall field. These findings will be discussed in terms of siderophore structure, the thermodynamics of iron sequestration, the kinetics of iron exchange reactions in ferri-siderophore systems, and molecular recognition and transport. We will also discuss the expanding field of ferri-siderophore chemistry and its application to the design of synthetic iron chelators for the study of siderophore binding and uptake in mimics of biological systems, and in therapeutic applications. The reader is also referred to other recent reviews, which cover various aspects of the chemistry and the biology of siderophore mediated iron transport (3-5,9,10,19-41).

II. Siderophore Structure

Siderophores have evolved in nature to bind selectively to iron, in the presence of other metal ions that may be present in higher concentration, and transport it as iron(III) to the cell. This selectivity is achieved through iron(III) binding group selectivity and ligand architectural features that enhance the stability of the complexes formed with iron. Ferric iron, which is the more stable oxidation state in aerobic aqueous medium, is a hard acid due to its high charge-to-radius ratio, low polarizability and high affinity for OH^- (42,43). As a hard Lewis acid, iron(III) will generally bind strongly to hard donor atoms, such as negatively charged oxygen and amine nitrogen atoms. Most microbes make use of this characteristic by producing siderophores with amine, catechol, hydroxamic acid, and α -hydroxycarboxylic acid binding groups. These groups form five-membered chelate rings upon binding, an effective chelate ring size for iron complexes due to the size of the metal and the bite angles formed in chelation (44).

The identity of the hard donor group and how it is incorporated in a molecular structure has a bearing on the affinity of a siderophore for iron(III). An analysis of siderophore structure and its relationship to iron(III) binding affinity as expressed by the thermodynamic stability constant is useful in understanding structure/function relationships and in the design of siderophore mimics for specific applications.

A. COMMON BINDING GROUPS

The most common siderophore binding groups that are found in nature are shown in Fig. 1: catechol, hydroxamic acid, and α -hydroxycarboxylic acid. Hexadentate siderophores may incorporate a single type of donor group, such as the tris-catecholate siderophore, enterobactin (**1** in Fig. 2), or the tris-hydroxamate siderophore, desferrioxamine B (**4** in Fig. 2), or may contain mixed donor group systems, such as the marine siderophore aerobactin, which contains two hydroxamate binding moieties and one α -hydroxycarboxylate moiety (**2** in Fig. 2). Other less-frequently observed binding moieties are the hydroxypyridinone donor group, the carboxylic acid donor group, and the amine moiety (Fig. 1) (45). While there are many examples of natural siderophores that feature catechol and hydroxamic acid donor groups, there is at present only one known example with the hydroxypyridinone donor group; cepabactin produced by the bacterium *Burkholderia cepacia* (**8** in Fig. 2) (46). Hydroxamate

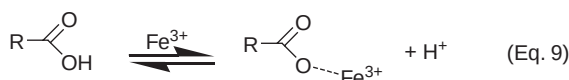
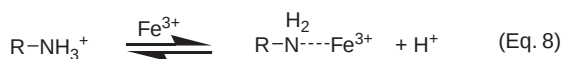
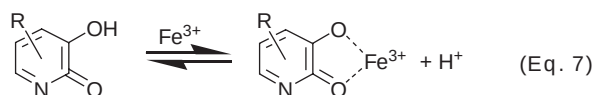
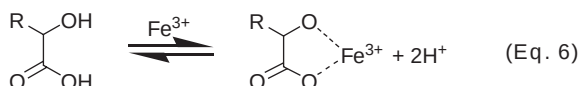
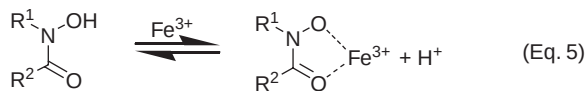
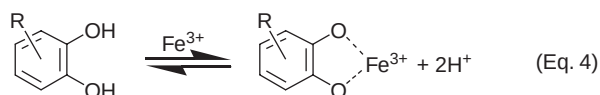


FIG. 1. Common siderophore iron-binding groups: catechol (Eq. (4)), hydroxamic acid (Eq. (5)), α -hydroxycarboxylic acid (Eq. (6)), and hydroxypyridinone (Eq. (7)). Additional siderophore binding groups include amine (Eq. (8)) and carboxylic acid (Eq. (9)).

donor groups are particularly prevalent in fungal siderophores, such as rhodotorulic acid, neurosporin, and ferrichrome (**3**, **5**, and **6** in Fig. 2) (36). While most of these binding groups form five-membered chelate rings, there are exceptions, such as mycobactin J, which forms a six-membered chelate ring with iron in addition to two five-membered rings (**7** in Fig. 2) (47).

B. COMMON ARCHITECTURES

There are a variety of factors concerning siderophore architecture that can contribute to the stability of iron-siderophore complexes. The overall architecture for natural multidentate siderophores can be any one of a number of different types: linear,

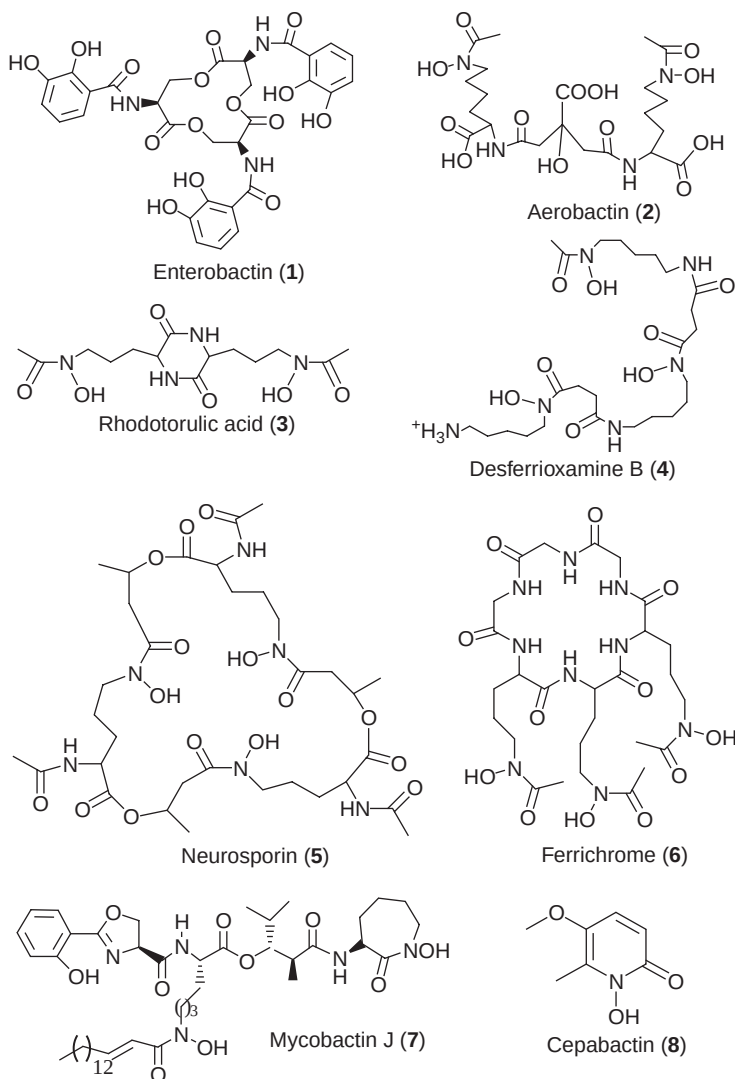


FIG. 2. Chemical structures of representative natural siderophores.

exocyclic, tripodal, and endocyclic (Fig. 3). Most desferrioxamines are examples of linear acyclic siderophores where the donor groups are part of a linear molecule (e.g., desferrioxamine B; **4** in Fig. 2), while enterobactin (**1**) and ferrichrome (**6**) are examples of exocyclic siderophores (Fig. 2), where the donor groups are attached to pendant arms radiating from a central ring system. Endocyclic siderophores feature donor groups that are part of macrocyclic molecules, such as alcaligin, desferrioxamine E, and neurosporin

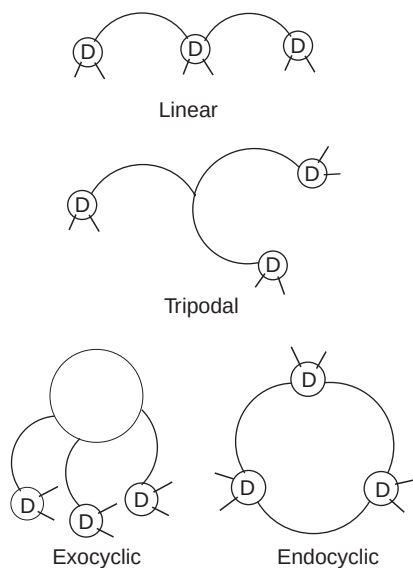


FIG. 3. Different general architectures for hexadentate siderophores. The circles marked by "D" represent general locations of bidentate donor groups on the molecule.

(5 in Fig. 2). These structures will behave differently in solution due to binding site preorganization and other steric effects (10). The thermodynamics and kinetics of exchange for different architectures will also vary depending on the type of donor group incorporated in the molecule. Endocyclic siderophores have been found to exhibit a degree of preorganization that promotes stronger complexation with hydroxamate donor groups than with their linear analogs (48,49). There are no known examples of natural catechol-based endocyclic siderophores to date, likely due to the steric strain inherent in an endocyclic catechol structure.

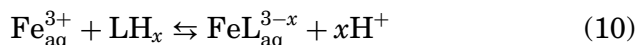
Another factor that relates complex stability and siderophore architecture is the chelate effect. The chelate effect is represented by an increase in complex stability for a multidentate ligand when compared to complexes with homologous donor atoms of lower denticity. The effect can be observed when comparing the stability of complexes of mono-hydroxamate ligands to their tris-hydroxamate analogs, such as ferrichrome (6) or desferrioxamine B (4). However, the increase in stability alone is not sufficient to explain the preponderance of hexadentate siderophores over tetradentate or bidentate siderophores in nature, and the chelate effect is not observed to a great extent in some siderophore structures (10,22,50,51).

III. Thermodynamics of Iron–Siderophore Interactions

As mentioned previously, siderophores must selectively bind iron tightly in order to solubilize the metal ion and prevent hydrolysis, as well as effectively compete with other chelators in the system. The following discussion will address in more detail the effect of siderophore structure on the thermodynamics of iron binding, as well as different methods for measuring and comparing iron–siderophore complex stability. The redox potentials of the ferri-siderophore complexes will also be addressed, as ferri-siderophore reduction may be important in the iron uptake process in biological systems.

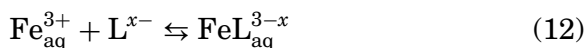
A. SIDEROPHORE COMPLEX STABILITY CONSTANTS

Siderophore binding sites for iron(III) are for the most part negatively charged and therefore, in aqueous solution there is a competition between H^+ and Fe^{3+} binding. Consequently, the equilibrium expression for the formation of the iron–siderophore complex must take into account proton participation in the reaction.



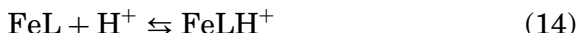
$$K = \frac{[FeL^{3-x}][H^+]^x}{[Fe^{3+}][LH_x]} \quad (11)$$

This means that the sequestration equilibrium reaction will be pH-dependent. The constant K is known as the “conditional” equilibrium constant. However, for stability comparisons between complexes of the same denticity, it may be more convenient to compare the equilibrium constant for the proton independent reaction between iron and siderophore. This can also be useful in a theoretical sense, as it allows comparison of complex stability where siderophores have different protonation constants. However, this approach does not account for competition between H^+ and Fe^{3+} for binding, which is always present in a real situation in aqueous solution.



$$\beta_{110} = \frac{[FeL^{3-x}]}{[Fe^{3+}][L^{x-}]} \quad (13)$$

In Eq. (13), β_{110} corresponds to the overall stability constant of the complex, following the β_{MLH} notation. β_{MLH} is the symbol reserved for an overall stability constant, and the subscripts denote the metal (M), ligand (L), and proton (H) stoichiometry of the equilibrium expression for the complex. So, the overall stability constant expression for an $\text{Fe}_2\text{L}_3^{x-}$ complex formed from the reaction stoichiometry 2Fe^{3+} and 3L^{n-} would be referred to as β_{230} . In many cases, a hexadentate siderophore (L) complex can be protonated at low pH, resulting in a tetracoordinate FeLH complex with a single binding group protonated, as shown in Eq. (14) (coordinated water molecules not shown for clarity).



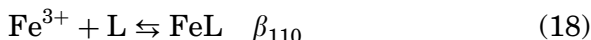
$$K = \frac{[\text{FeLH}^+]}{[\text{FeL}][\text{H}^+]} \quad (15)$$

However, Eqs. (14) and (15) can be combined with the proton independent overall stability constant of the FeL complex to give an overall stability constant for the FeLH complex, β_{111} .



$$\beta_{111} = \frac{[\text{FeLH}^{4-x}]}{[\text{Fe}^{3+}][\text{L}^{x-}][\text{H}^+]} \quad (17)$$

Complexes formed by tetradentate siderophores involve stepwise complex formation and therefore, have somewhat different equilibria from their hexadentate analogs. Initial chelation will occur with a tetracoordinate FeL complex forming. A subsequent equilibrium then occurs, where the FeL complexes will react in a 2:1 stoichiometry with free ligands in solution to form a single Fe_2L_3 complex (coordinated water and charges not shown for clarity).



In some cases where the siderophore architecture permits, the tetradentate siderophore will form a dimeric Fe_2L_2 complex with iron instead of the monomeric FeL form. These dimeric complexes are not discernible from their monomer counterparts

by their UV-visible spectra, although mass spectrometric methods can distinguish between the two forms (52).

An examination of equilibrium expressions for different siderophore denticities will illustrate nature's preference toward hexadentate siderophores. There is a concentration effect when comparing lower denticity siderophores to their higher denticity analogs. pH-independent equilibrium expressions for generic bidentate and hexadentate siderophores are shown below, where Hx represents a hexadentate siderophore and Bd a bidentate siderophore (charges are omitted for clarity).

$$\beta_{110} = \frac{[\text{FeHx}]}{[\text{Fe}][\text{Hx}]} \quad (21)$$

$$\beta_{130} = \frac{[\text{Fe}(\text{Bd})_3]}{[\text{Fe}][\text{Bd}]^3} \quad (22)$$

Rearranging these expressions to a form that displays the ratio of complexed to unbound iron gives the following equations.

$$\beta_{110}[\text{Hx}] = \frac{[\text{FeHx}]}{[\text{Fe}]} \quad (23)$$

$$\beta_{130}[\text{Bd}]^3 = \frac{[\text{Fe}(\text{Bd})_3]}{[\text{Fe}]} \quad (24)$$

The stability constant equation for the bidentate complex features ligand concentration raised to the third power (Eq. (24)), while the hexadentate complex stability constant expression only features the ligand concentration raised to the first power (Eq. (23)). Consequently, assuming equivalent values for β_{110} and β_{130} , there is a much larger concentration of free bidentate siderophore required to obtain the same degree of iron binding (e.g., 50% of total iron present where $[\text{Fe}]_{\text{bound}} = [\text{Fe}]_{\text{free}}$) than in the case of a hexadentate siderophore. Therefore, it is generally more efficient for organisms to exert the extra metabolic effort to produce hexadentate siderophores rather than their lower denticity analogs.

Stability comparisons between siderophore complexes with different binding stoichiometries are complicated by the fact that the units for the concentration equilibrium constants are different. Also, since the Fe^{3+} binding moieties have different pK_a values; competition for binding with H^+ differs, which will not be reflected in the pH-independent β_{MLH} values. Therefore, it is important to have a scale for iron-siderophore complex

stability that is independent of metal/ligand stoichiometry and that accounts for H^+ competition. Raymond *et al.* developed the pFe scale to compare stability for a variety of complexes with different denticities and ligand pK_a values in a meaningful way at physiological conditions (53). The pFe value is the negative logarithm of the free aqueous iron(III) concentration at arbitrary fixed $[Fe^{3+}]$, $[ligand]$, and $[H^+]$ concentrations (Eq. (25)). The concentrations for pFe calculations normally found in the literature

$$pFe = -\log[Fe_{aq}^{3+}] \quad (25)$$

are $[Fe^{3+}]_{tot} = 1 \mu M$, $[L]_{tot} = 10 \mu M$, and $pH = 7.4$. The calculation of pFe values takes into account the protonation constants of the siderophore and the equilibrium constants of all forms of the iron–siderophore complexes. It is important to note that these values are not a complete measure of the soluble iron concentration, as pFe values only take into account the non-hydrolyzed metal ion. As the pH increases, the proportion of non-chelated iron present in the non-hydrolyzed form will decrease as the relative complex stability decreases and bis-hydroxy- and tetra-hydroxy-species will increase. At physiological pH, this difference will be significant, with the bis-hydroxy-species present at a concentration generally a few orders of magnitude higher than the fully aquated metal ion.

It is also important to state the conditions at which the pFe is determined, as the value will change with the conditions (54). Fig. 4 shows an example of how the pFe value can vary over a range of pH values. This variation is due to the inclusion of a value known as the Ringbom coefficient in the determination of pFe values. The Ringbom coefficient, α , is determined from the mass balance equation as shown below for the ligand Ringbom coefficient.

$$L_{tot} = [L] + [LH] + [LH_2] + \dots \quad (26)$$

$$L_{tot} = [L] + K_{a1}[L][H^+] + \beta_{a2}[L][H^+]^2 + \dots \quad (27)$$

$$L_{tot} = [L](1 + K_{a1}[H^+] + \beta_{a2}[H^+]^2 + \dots) \quad (28)$$

$$\alpha_L = 1 + K_{a1}[H^+] + \beta_{a2}[H^+]^2 + \dots \quad (29)$$

This variation in complex stability with change in pH is particularly important in the context of biological systems, as it can potentially play a role in the iron uptake mechanism of some organisms. In some cases, the iron–siderophore complex is taken

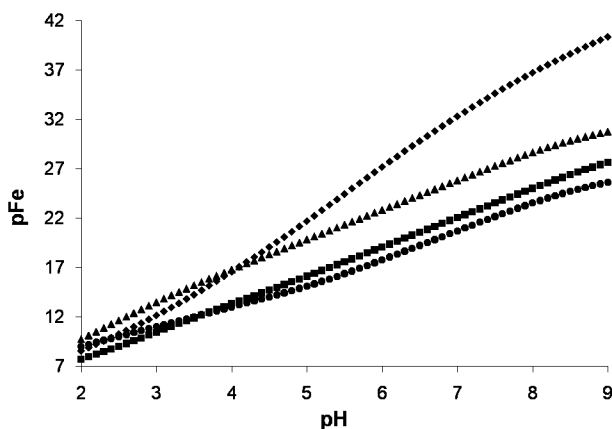


FIG. 4. Variation of pFe with pH for (◆) enterobactin (1), (■) aerobactin (2), (●) rhodotorulic acid (3), and (▲) ferrioxamine B (4). Conditions: $[\text{Fe}]_{\text{tot}} = 10^{-6} \text{ M}$ and $[\text{Siderophore}]_{\text{tot}} = 10^{-5} \text{ M}$.

up into compartments where the pH is lower than the surrounding environment of the organism. This change in pH can lead to lower relative stability of the iron–siderophore complex compared to other competing ligands, and can potentially facilitate the exchange of iron from one siderophore to another.

B. METHODS OF STABILITY CONSTANT DETERMINATION

There are a variety of experimental methods available for determination of iron–siderophore complex stability constants. As stated above, experiments to determine the pH-independent constants β_{MLH} must be based on indirect methods due to the proton-dependence of the actual equilibria observed in solution. There are several issues involved. In order to deprotonate the siderophore to the extent that it can efficiently complex iron requires the solution to be at a pH that will cause the metal to hydrolyze and precipitate. Also, at higher pH values, the equilibrium constant for siderophore complex formation reaction is generally too high to be determined by direct titration. In order to determine equilibrium constants correctly, measurable amounts of reactants and products must be present in solution. These factors necessitate the use of competition reactions to observe the exchange of iron from one well-characterized sequestering agent to the desired siderophore system with measurable amounts of both present (10,55,56). The competing chelator that is generally used for competition experiments is ethylenediaminetetraacetate,

EDTA, due to its well-characterized pK_a values, hexadentate binding mode, high stability with Fe(III), and well-behaved equilibria (57).



The hexadentate EDTA complex has a low absorbance in the visible range, while iron–siderophore complexes generally have relatively high-intensity ligand-to-metal charge transfer bands, resulting in colored solutions (10). This makes spectrophotometric methods ideal for monitoring the competition reaction; one can determine the relative amounts of each species present over a range of concentrations of reactants. Eqs. (31)–(32) illustrate the exchange of iron between the siderophore (L) and EDTA.



$$K_{\text{comp}} = \frac{[\text{FeEDTA}][\text{L}]}{[\text{FeL}][\text{EDTA}]} \quad (32)$$

This equation can then be used in conjunction with the β_{110} expression for the reaction of ferric iron with EDTA to determine the β_{110}^L for the desired siderophore.

$$\beta_{110}^L = \frac{\beta_{110}^{\text{EDTA}}}{K_{\text{comp}}} \quad (33)$$

Note that Eq. (33) is simplified, as the equilibria observed in solution must take into account the pH at which the experiment is performed. Corrections must be made using the Ringbom coefficients for the ligand systems.

Monitoring the stepwise complexation of iron(III) by siderophores where multiple protonation states and/or multiple complex stoichiometries are possible can often be performed by pH-dependent spectrophotometric methods (55,56). The spectrum of the siderophore complex generally involves a ligand-to-metal charge transfer band, which is sensitive to first coordination shell composition and is readily monitored in the visible region of the spectrum. Upon a change in the inner coordination environment of the iron center, the wavelength of maximum absorbance and molar absorptivity of the complex will shift. This allows distinction of the coordination environment of the iron in solution based on comparison with appropriate models, which can in turn lead to determination of the stability constants when solution spectra are measured over a range of pH values. An example of this can be observed in the pH-dependent spectrophotometric titration of

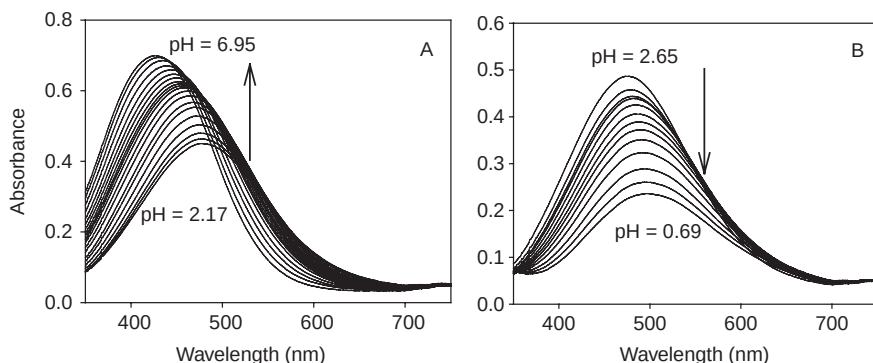
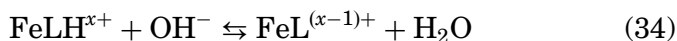


FIG. 5. Spectrophotometric titration of the $\text{Fe}^{3+}/\text{H}_3\text{L}_\text{R}^{234}$ (**11**) equilibrium system over the pH range 0.69–6.95. Arrows indicate the direction of movement of the spectra (A) with increasing pH and (B) with decreasing pH. Conditions: $[\text{Fe}^{3+}] = [\text{H}_3\text{L}_\text{R}^{234}] = 2.0 \times 10^{-4} \text{ mol dm}^{-3}$, $T = 25^\circ\text{C}$, $\mu = 0.10$. Two equilibria appear over two distinct pH ranges, indicating two separate protonation/deprotonation events occurring. Reprinted with permission from Ref. (58). Copyright 2007 American Chemical Society.

the $\text{Fe}^{3+}/\text{H}_3\text{L}_\text{R}^{234}$ equilibrium system, shown in Fig. 5 (58). $\text{H}_3\text{L}_\text{R}^{234}$ is an exocyclic tris-hydroxamate siderophore model built around a saccharide platform (**11** in Fig. 6), which forms a hexacoordinate complex with iron. Over the pH range from 3 to 9, the coordination environment of the metal center changes due to deprotonation of a donor group, resulting in a change in the visible spectrum of the complex solution, corresponding to the reaction equilibrium shown in Eq. (34). By using the pH range of the spectral change and the



previously determined β_{110} of the complex, it is possible to determine the protonation constant for the complex and the overall β_{111} . A second equilibrium is also observed in the titration above pH 9 for the $\text{Fe}^{3+}/\text{H}_3\text{L}_\text{R}^{234}$ system, corresponding to a second deprotonation reaction.

Potentiometric titrations can also be used to determine stability constants for complexes in some cases, which can be useful as a substitute or complementary approach to spectrophotometric titrations (56). By starting at a low pH, where the relative amount of complex formation is low, and then measuring the pH upon addition of standardized base, one can determine the relative amounts of the species in solution or how many equivalents of H^+

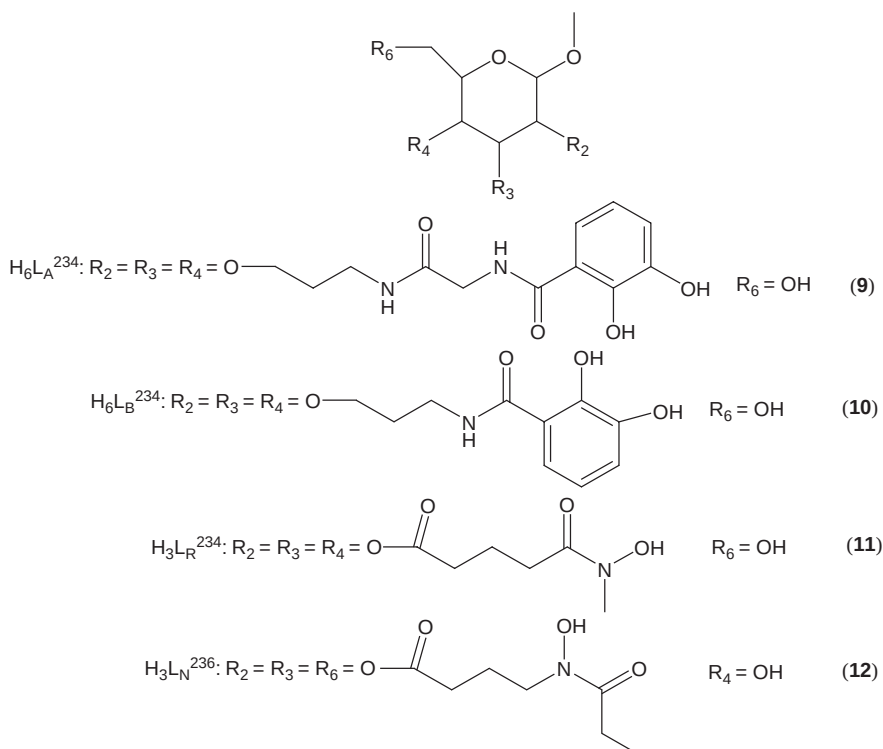
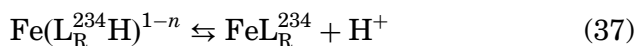
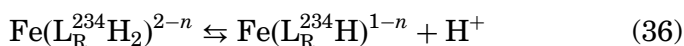
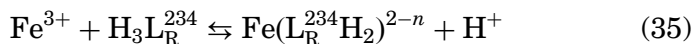


FIG. 6. Saccharide-platform siderophore mimics with catechol and hydroxamic acid donor groups, $\text{H}_6\text{L}_\text{A}^{234}$ (9), $\text{H}_6\text{L}_\text{B}^{234}$ (10), $\text{H}_3\text{L}_\text{R}^{234}$ (11), and $\text{H}_3\text{L}_\text{N}^{236}$ (12).

are displaced by chelation. This will allow determination of the stability constants, as well as the stepwise formation constants for the H^+ dependent complexation equilibria. An example of this is shown below for the $\text{Fe}^{3+}/\text{H}_3\text{L}_\text{R}^{234}$ equilibrium system (coordinated water omitted for clarity) (Fig. 7) (58).



Two separate titrations are shown in Fig. 7, one of the ligand alone and one of the ligand with an equivalent concentration of iron(III) present. More equivalents of base are required to reach

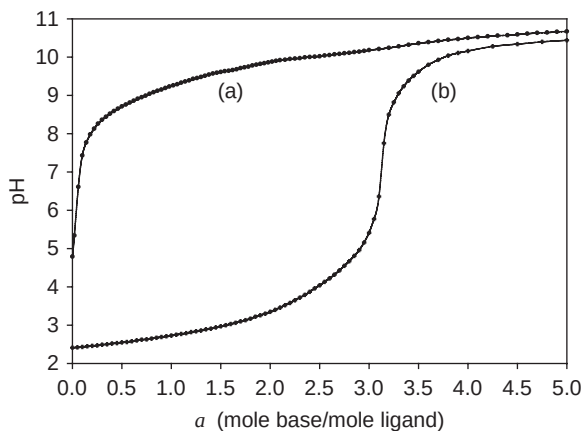


FIG. 7. Potentiometric titration curves for the $\text{Fe}^{3+}/\text{H}_3\text{L}_\text{R}^{234}$ (11) equilibrium system. (a) Titration of the ligand alone. Conditions: $[\text{H}_3\text{L}_\text{R}^{234}] = 5.9 \times 10^{-4} \text{ mol dm}^{-3}$, $T = 25^\circ\text{C}$, $\mu = 0.10$ (NaClO_4). (b) Titration of the ligand in the presence of one equivalent of metal ion. Conditions: $[\text{H}_3\text{L}_\text{R}^{234}] = [\text{Fe}^{3+}] = 5.9 \times 10^{-4} \text{ mol dm}^{-3}$, $T = 25^\circ\text{C}$, $\mu = 0.10$ (NaClO_4). Reprinted with permission from Ref. (58). Copyright 2007 American Chemical Society.

the titration's endpoint when the metal ion is present because coordination of the ligand to iron(III) will release protons from the binding moieties into solution more readily than for the ligand alone.

Measurement of pH-dependent equilibria can also be used to identify coordination isomerization reactions in addition to stepwise dissociation, such as in the case of the iron(III) complex of exochelin MN (59). Here, a combination of spectrophotometric and potentiometric titration characterized multiple equilibria involving second-sphere protonation, coordination isomerization, and stepwise dechelation, and is illustrated in Fig. 8.

Electrochemical experiments allow the determination of complex stability constants for Fe^{2+} by measuring complex redox potentials over a range of pH values. The $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox potential of the siderophore complex, as with the spectral characteristics of the complex, is dependent on the inner coordination environment of the iron. These considerations will be addressed later (Section III.D).

C. SURVEY OF THERMODYNAMIC STABILITY CONSTANTS

Much work has been performed in determining the stability constants of a variety of siderophores and synthetic siderophore

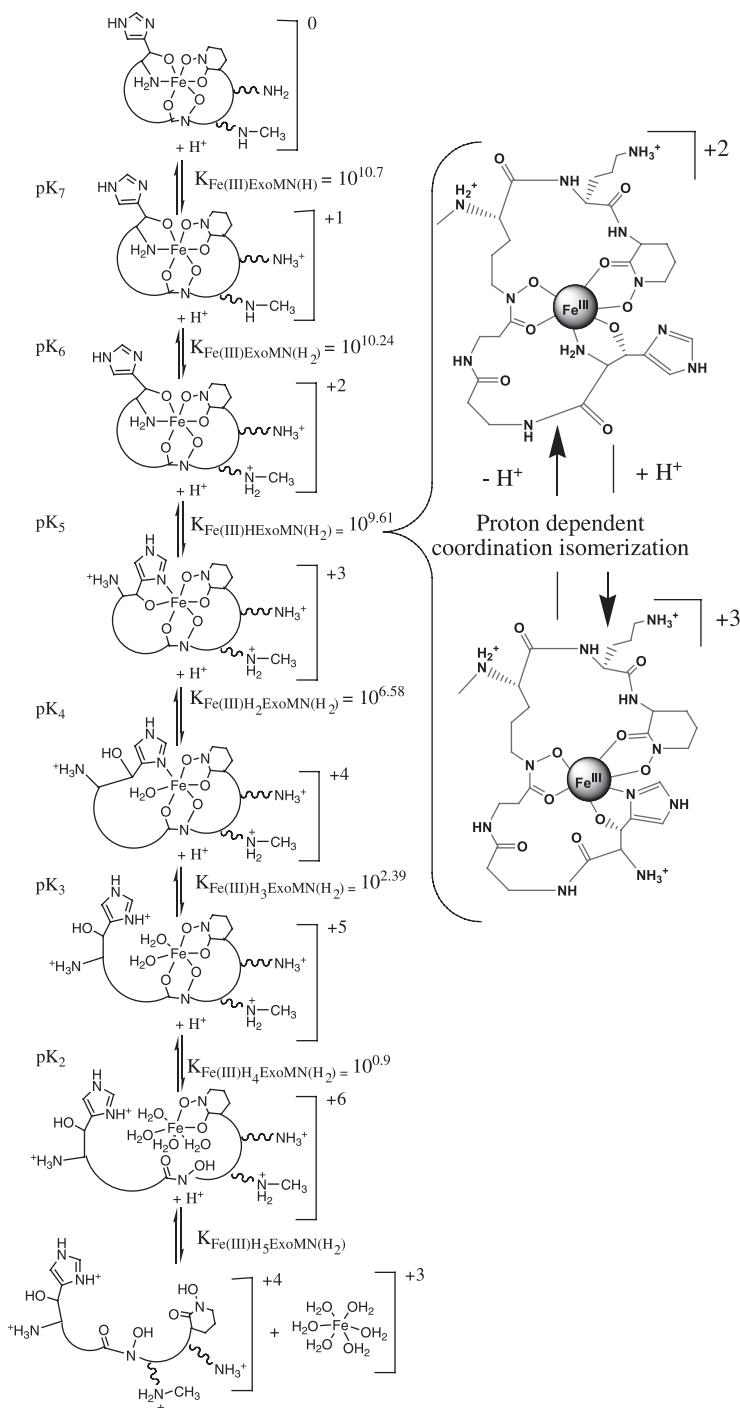


FIG. 8. Reaction scheme for the pH-dependent titration of the Fe^{3+} /exochelin MN equilibrium system, showing the pH ranges of coordination isomerization reactions. Reprinted with permission from Ref. (59). Copyright 2003 American Chemical Society.

mimics to gain information about the effects of structural features on iron(III) sequestering ability. Natural siderophores have been isolated with a variety of different binding groups, which affect the stability of the iron–siderophore complex. Iron–siderophore complex stability is related to a number of factors including siderophore architecture and binding moiety. Tables I–III are a representative compilation of stability constants from the literature.

Catechol siderophores have the highest iron(III) affinity of any binding group class, as they incorporate two hard oxygen donor atoms to form a five-membered chelate ring, with electron density delocalized around the phenyl ring (Fig. 1). This is illustrated by high β_{110} and pFe values for the natural siderophores enterobactin and bacillibactin (**1** and **14** in Fig. 9, respectively; Table IA), as well as the synthetic enterobactin analog, TRENCAM (**17** in Fig. 10; Table IIA).

Enterobactin (**1**) is the most stable known natural siderophore, a triscatechol exocyclic structure based around a trilactone central ring system. Structural analogs have been studied to attempt to identify the features that lead to its high stability, although comparison to the recently discovered structural analog natural product, bacillibactin (**14**), may aid in clarifying the high stability of the Fe–enterobactin complex (Fig. 9) (60). The structural difference between enterobactin and bacillibactin is the addition of a glycine residue between the trilactone central ring system and the catechol donor groups, as well as methylation of the trilactone central ring. This variation in the structure is proposed to move the catechol donor group arms out of position, moving iron away from its ideal position in the binding cavity of enterobactin (60). The result is a lower stability and pFe value for the bacillibactin complex than the enterobactin complex by more than an order of magnitude (Table IA).

The effect of the amino acid spacer on iron(III) affinity was investigated using a series of enterobactin-mimic TRENCAM-based siderophores (82). While TRENCAM (**17**) has structural similarities to enterobactin, in that it is a tripodal tris-catechol iron-binding molecule, the addition of amino acid spacers to the TRENCAM frame (Fig. 10) increases the stability of the iron(III) complexes of the analogs in the order of bAla (**19**) < Gly (**18**) < Glu (**21**) < Ser (**20**) < Lys (**22**) (Table IIA). This increase in complex stability is attributed to the intramolecular interactions of the additional amino acid side chains that stabilize the iron–siderophore complex slightly.

Siderophores undergo a conformational change on sequestering iron(III) in solution, which is reflected in strain energy

TABLE I

THERMODYNAMIC STABILITY CONSTANTS FOR IRON(III)-SIDEROPHORE COMPLEXES,
MEASURED AT 25°C

Siderophore	No. ^a	Arch. ^b	log β_{110}	pFe ^{3+ c}	Refs.
A. Tris-catecholate					
Enterobactin	1	X	~49	34.3	(61)
Bacillibactin	14	X	47.6	33.1	(60)
B. Tris-hydroxamate					
Coprogen		L	29.35	25.6	(62,63)
Ferricrocin		X	30.4	26.5	(62)
Ferrichrysin		X	29.96	25.8	(64)
Ferrichrome	6	X	29.07	25.2	(62,64)
Ferrichrome A		X	32.0		(65)
Ferrioxamine B	4	L	30.6	26.6	(50)
Ferrioxamine D ₁		L	30.76		(64)
Ferrioxamine D ₂		L	31.40	26.69	(66)
Ferrioxamine E		N	32.21	27.27	(66)
Exochelin MS		L	28.86	25.0	(67)
C. α -Hydroxycarboxylate					
Mugineic acid			32.5 ^d		(68)
Rhizoferrin		L	25.3	19.7	(69)
D. Bis-hydroxamate					
Rhodotorulic acid	3	L	62.2 ^e 21.99 ^f	21.9	(48,51)
Alcaligin		N	64.7 ^e 23.5 ^f	23.0	(48,70)
Bisucaberin		N	64.3 ^e 23.5 ^f	22.5	(49)
E. Mixed					
Aerobactin ^{g,h}	2	L	27.6	23.3	(71)
Alterobactin A ⁱ		X	42.2		(72)
Azotobactin ^j		L	28.1	27.8	(73)
Ferrithiocin ^k		L	31.04 ^l	22.14	(74)
Norferrithiocin ^k		L	29.09 ^l	20.51	(74)
Parabactin ^m		T	~48		(75)
Pyoverdin PaA ⁿ		X	30.8	27.0	(76)
Pyoverdin Pp ^j		L	31.06	25.13	(77)
Exochelin MN ^o		L	39.12	31.1	(59)
Mycobactin S ^p		L	26.6		(78)
Quinolobactin ^q		L	28.20 ^l	18.2	(79)
Yersiniabactin ^r		L	36.6		(80)

^aStructure number which may be found in a figure.^bSiderophore architecture. X = exocyclic, N = endocyclic, L = linear, T = tripodal.^cCalculated for [Fe³⁺]_{tot} = 10⁻⁶ M, [L]_{tot} = 10⁻⁵ M, and pH = 7.4.^dEstimated to be in the range of 32.5–33.3.^elog β_{230} for 2Fe³⁺_{aq} + 3 L²⁻ $\rightleftharpoons$ Fe₂L₃.^flog β_{110} for the ferric complex of a tetradentate siderophore.^gDonor groups: 2 hydroxamate and 1 α -hydroxycarboxylate.^hThe photoproduct of aerobactin has a reported log β_{110} value of 28.6 (71).ⁱDonor groups: 1 catecholate and 2 α -hydroxycarboxylate.^jDonor groups: 1 hydroxamate, 1 catecholate, and 1 α -hydroxycarboxylate.^kDonor atoms: 1 carboxylate O, 1 phenolate O, and 1 thiazoline N.^llog β_{120} for Fe³⁺ + 2L²⁻ $\rightleftharpoons$ FeL₂⁻. Ferrithiocin, norferrithiocin, and quinolobactin are tridentate siderophores.^mDonor groups: 2 catecholate and 1 phenolate.ⁿDonor groups: 2 hydroxamate and 1 catecholate.^oDonor groups: 2 hydroxamate and 1 β -hydroxyhistidine.^pDonor groups: 2 hydroxamate and 1 oxazoline.^qDonor groups: 1 8-hydroxyquinoline and 1 carboxylate.^rDonors: 1 phenolate O, 2 thiazole N, 1 oxazoline, and 1 carboxylate.

TABLE II

THERMODYNAMIC STABILITY CONSTANTS FOR IRON(III)-HEXADENTATE SIDEROPHORE
MIMIC COMPLEXES, MEASURED AT 25°C

Ligand	No. ^a	Arch. ^a	log β_{110}	pFe ^{3+^b}	Refs.
A. Tris-catecholate					
TRENCAM	17	T	43.6	27.8	(81)
TRENBAlaCAM	19	T	42.6	28.0	(82)
TRENGlyCAM	18	T	45.2	30.9	(82)
TRENGluCAM	21	T	45.1	30.9	(82)
TRENSerCAM	20	T	45.2	31.6	(82)
TRENLysCAM	22	T	46.4	32.8	(82)
Capped TRENCAM		E	43.1	30.7	(81)
TRENCAMS ^c		T	43.6	29.6	(83)
MECAM		X	43.6	29.1	(81)
Me ₃ MECAM		X	43		(84)
Et ₃ MECAM		X	47		(85)
Linear enterobactin		L	43		(85)
CYCAM		X	40	23.0	(86)
H ₆ L _A ²³⁴	9	X	41.38	28.6	(87)
H ₆ L _B ²³⁴	10	X	46.38	28.3	(87)
B. Tris-hydroxamate					
DFOCAM		L	35.4	28.2	(88)
DFOCAMC		L	34.9	26.7	(88)
DFOMTA		L	34.8	28.5	(88)
DFOHOPO		L	30.7	26.7	(88)
TRENDROX		T	32.9	27.8	(89)
TAGE		T	28.6	25	(90)
H ₃ L _R ²³⁴	11	X	31.86	27.1	(91)
H ₃ L _N ²³⁶	12	X	31.14	26.1	(58)
C. Tris-hydroxypyridinonate					
HOPObactin	16	X	26.4	27.4	(92)
N ³ (LH) ₃	59	X	27.34	23.5	(93)
L-Ser ₃ -TREN-Me-3,2-HOPO		T	26.3	26.8	(94)
TREN-Me-3,2-HOPO	27	T	26.8	26.8	(95)
TR322-Me-3,2-HOPO		T	26.2	26.6	(95)
TR332-Me-3,2-HOPO		T	26.42	25.8	(95)
TRPN-Me-3,2-HOPO		T	24.48	23.8	(95)
3,4-LI-(Me-3,2-HOPO)		L	25.67	25.5	(96)
D. Various ^d					
TBPAS		T	28.7	22.8	(97)
TBPANS		T	31.8	22.1	(97)
N-TRENsoX	25	T	25.3	22.2 ^e	(98)
O-TRENsoX	26	T	30.9	29.5 ^e	(99)
SERSAM	13	X	39		(100)
SER(3M)SAM	15	X	38		(100)
3,4-LI-(TAMmeg)		L	43.78	32.5	(96)
TRENTAM		T	45.2	34.2	(101)

TABLE II (Continued)

Ligand	No. ^a	Arch. ^a	log β_{110}	pFe ^{3+ b}	Refs.
E. Mixed ^f					
TRENSEX2CAMS		T	36.8	31.6	(83)
TRENSEXCAMS2		T	41.3	32.3	(83)
L(cat ₂ hydroxamate) ^g		L	31.4	18.3	(102)
5LIOHOPO ₂ TAM		L	32.1	30.4	(103)
5-LIO-(TAMmeg) ₂ -TAM		L	44.99	34.4	(96)
3,4-LI(Me-3,2-HOPO) ₂ -(TAMmeg)		L	30.65	27.3	(96)
3,4-LI(Me-3,2-HOPO)-(TAMmeg) ₂		L	36.25	28.7	(96)
TREN-Me-3,2-HOPOIAM		T	26.51	26.7	(104)
TREN-Me-3,2-HOPOTAM		T	33.14	30.2	(104)
TRENHOPO ₂ TAM		T	34.6	30.9	(101)
TRENHOPOTAM ₂		T	41.0	33.6	(101)

^aColumns are as defined in Table I.

^bCalculated for $[\text{Fe}^{3+}]_{\text{tot}} = 10^{-6} \text{ M}$, $[\text{L}]_{\text{tot}} = 10^{-5} \text{ M}$, and pH = 7.4.

^c5-Sulfonated version of TRENAM (17).

^dHomopodal hexadentate siderophore mimics with various binding groups.

^eThese pFe values were calculated for $[\text{Fe}^{3+}]_{\text{tot}} = 10^{-3} \text{ M}$, $[\text{L}]_{\text{t}} = 10^{-2} \text{ M}$, and pH = 7.4.

^fHeteropodal hexadentate siderophore mimics with various binding groups.

^gDonor groups: 2 catechol and 1 hydroxamate.

calculations. It has been found that the stability constant of a complex can be related to the change in strain energy of the molecule due to this conformational change. These calculations can reflect the degree of preorganization inherent in the design of a molecule that will predispose it to bind a metal ion, resulting in a higher stability constant (123,124). One such study demonstrated that enterobactin exhibited significant preorganization for binding iron(III) due to the conformation of the protonated donor groups, but suggested that additional stability was possible through the use of 2,3-dihydroxy-*N,N*-dimethylbenzamide, DMBA, donor groups attached to a rigid backbone in a synthetic mimic (124). The endocyclic tris-hydroxamate siderophore, desferrioxamine E, which has a higher β_{110} value than its linear analog, desferrioxamine B (4), by ~ 1.5 orders of magnitude (Table IB), is a good example of this, where the preorganization inherent in the endocyclic ligand architecture results in more stable binding.

As mentioned previously, in many siderophore complexes, a decrease in pH will result in protonation and dissociation of a donor group. This decrease in effective denticity of the chelator will lead to a corresponding decrease in complex stability and the opening of available coordination sites for the formation of ternary complexes, and/or exchange with other chelators. However, in the case of catecholamide donor group siderophores, such

TABLE III

THERMODYNAMIC STABILITY CONSTANTS FOR IRON(III)-TETRADENTATE SIDEROPHORE MIMIC COMPLEXES, MEASURED AT 25°C

Ligand	No. ^a	log β_{110}	log β_{230}	pFe ^{3+ b}	Refs.
A. Bis-hydroxamates					
MNDHA-1 ^c		19.85	59.22	21.5	(105)
MRDHA-1 ^c		20.03	59.45	21.8	(105)
MRDHA-2 ^c		19.69	58.91	21.4	(105)
BRDHA-1 ^c		19.85	58.46	20.9	(105)
3-IPHA	33	22.84	62.1		(106)
4-IPHA	34	22.83	62.2		(106)
5-IPHA	35	22.76	62.4		(106)
6-IPHA	36	22.63	62.3		(106)
2,2-DIHA	41	20.32	57.80		(107)
2,3-DIHA	42	20.26	59.03		(107)
2,4-DIHA	43	20.84	58.09		(107)
2,5-DIHA	44	21.05	61.01		(107)
3,4-DIHA	45	19.87	57.53		(107)
3,3-DIHA	46	19.84	58.99		(107)
DOCYDMAHA		21.33	60.50	21.1	(108)
PIPDMAHA			61.7	21.5	(108)
B. Bis-hydroxypyridinonate					
N ² (prLH) ₂	58	20.39	60.46	22.07	(93)

^aStructure number which may be found in a figure.^bCalculated for [Fe³⁺]_{tot} = 10⁻⁶ M, [L]_{tot} = 10⁻⁵ M, and pH = 7.4.^cLinear dihydroxamic acids with a tetraamide connecting chain. The first letter designates analogs with methyl (M) or butyl (B) backbone substituents, the second letter represents normal (N) or retro (R) hydroxamate donor groups; DHA represents dihydroxamate donor groups, and the number represents the length of the carbon spacer chain between the donor group and the main chain.

as enterobactin (**1**), a decrease in pH will lead to a shift in binding mode of the donor groups. One of the oxygen donors from the catechol-binding group will become protonated and the binding mode to iron will shift to the salicylate-binding mode (Fig. 11). This has been observed in the case of the natural siderophore enterobactin (**1**), as well as the synthetic analog, TRENCAM (**17**) (100). Protonation of the enterobactin complex results in a change in binding mode and a change in absorption spectrum of the molecule due to a change in the primary coordination environment. Studies of salicylate-binding mode in the synthetic mimics, SERSAM and TRENSAM (**13** in Fig. 9 and **23** in Fig. 12, respectively), shows their spectral properties to be consistent with the triply-protonated enterobactin complex (100). This change in binding mode from catecholate to salicylate may be useful *in vivo*, as catecholamide donor group siderophores can

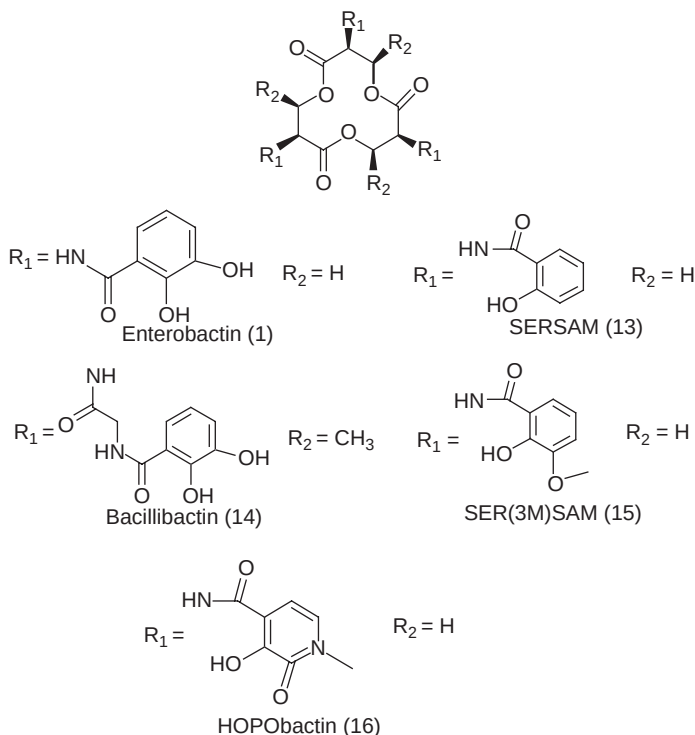


FIG. 9. Chemical structures of enterobactin and analogs.

experience lower environmental pH values without leaving binding sites on the iron center open.

Another study of a synthetic siderophore analog of mixed catechol and hydroxamate donor groups exhibited similar spectral shifts with pH as observed for tris-catecholate siderophores, consistent with a salicylate-binding mode shift (102). While this synthetic siderophore mimic supports the growth of many species of bacteria, it also exhibits a low iron-binding affinity relative to other hexadentate siderophores, with a pFe value of 18.3 (see L(cat₂hydroxamate); Table IIE).

Serratrice *et al.* published reports of the properties of the siderophore mimic, O-TRENSEX (26), which incorporates 5-sulfonato-8-hydroxyquinoline donor groups into a TREN structure, and compared its binding efficiency to its analog, N-TRENSEX (25 in Fig. 12) (99). O-TRENSEX exhibited stable complex formation with iron(III) and was found to exhibit a shift from the 8-hydroxyquinoline binding mode to the salicylate-binding mode at lower pH. The structure of O-TRENSEX differs from N-TRENSEX in that its donor groups are arranged in the

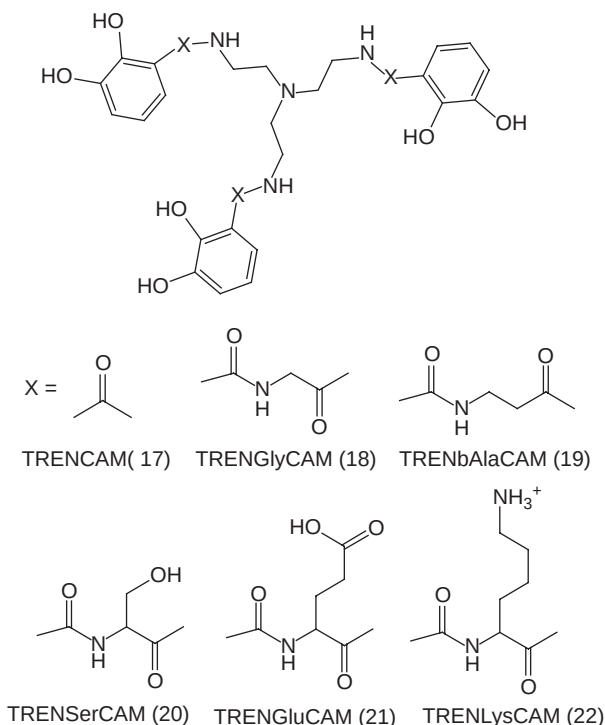


FIG. 10. TREN family of catechol siderophore mimics.

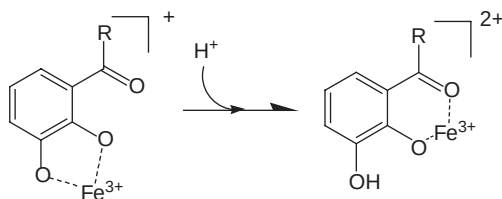


FIG. 11. Catecholate-to-salicylate-binding mode shift in catechola-mide donor group siderophores, such as enterobactin (1) and enterobactin analogs.

opposite direction from that in N-TRENsOX. This configuration allows the formation of the salicylate-binding mode in O-TRENsOX, which stabilizes the ferric complex over a pH range relative to that of N-TRENsOX (Table IID) (99).

In designing siderophore mimics, solubility and molecular recognition are key issues. A saccharide can provide a chiral

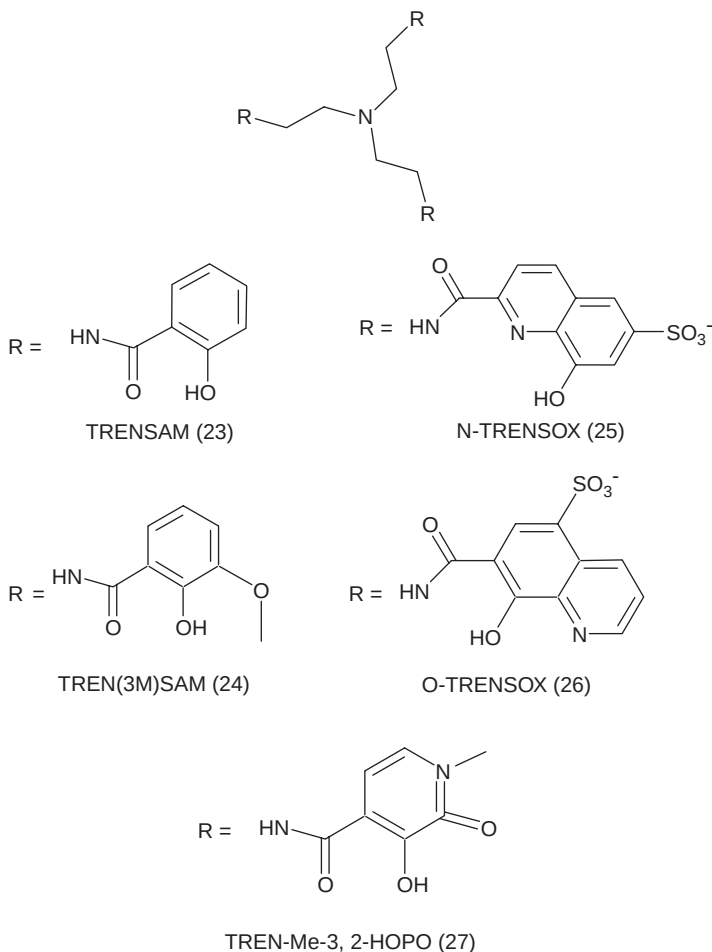


FIG. 12. Non-catecholate donor group TREN family of synthetic siderophore mimics.

synthetic platform for an exocyclic siderophore mimic with excellent aqueous solubility (125). In the pyranose form, saccharide scaffolds featuring pendant arms terminating with donor groups that can bind iron are good mimics for the exocyclic siderophores ferrichrome (**6**) and enterobactin (**1**). Two catechola-mide-substituted saccharide-backbone analogs of enterobactin (**9** and **10**; Fig. 6) were found to exhibit a catechol to salicylate shift at relatively high pH, and a binding affinity for iron(III) equal or superior to that of other enterobactin mimics (Table IIA) (87). These two versions of the saccharide-backbone catechola-mide siderophores were compared featuring spacer chain lengths

that differed by a glycine residue, similar to the comparison between enterobactin (**1**) and bacillibactin (**14**). The pH-independent stability constant (β_{110}) for the shorter chain mimic, $H_6L_B^{234}$ (**10**) is 5 log units greater than the longer chain analog, $H_6L_A^{234}$ (**9**, Table IIA). However, due to the lower acidity of $H_6L_B^{234}$ (**10**), the pFe values for both mimics are similar (28.6 and 28.3). Both pFe values are higher than those for similar catecholamide analogs, such as TRENCAM (**17**) and CYCAM (Table IIA) (87).

Siderophores with exocyclic architecture are likely to be sensitive to pendant arm placement and iron-binding moiety attachment site. In enterobactin (**1**), the pendant arms are placed at equivalent intervals around the central ring system, whereas in ferrichrome (**6**), they are clustered on adjacent residues of the central ring (Fig. 2). A model study using an exocyclic saccharide platform investigated the effects of arm placement and normal vs. retro orientation of the hydroxamate moiety (**11** and **12**; Fig. 6) (58). The results (Table IIB), which include experimental determination of iron(III) affinity constants as well as strain energy calculations, suggest that distributing the arms in the 236 rather than 234 positions provides higher affinity for Fe(III). However, the effect of pendant arm position on strain energy is less than the retro/normal hydroxamate configuration change, which favors the retrohydroxamate isomer. The latter effect is consistent with literature comparisons of retro- and normal hydroxamate versions with the same siderophore architecture (105,126).

The terephthalamide moiety (Fig. 13) is similar in structure to catechol, but has a higher affinity for iron(III) at physiological conditions and consequently has been used in the synthesis of siderophore mimics. The higher pFe values are due to the

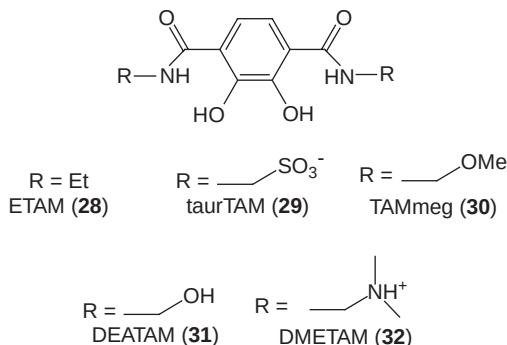


FIG. 13. A series of bidentate terephthalamide-based siderophores with differently charged substituents.

increased electron-withdrawing characteristic of the additional amide substituent located on the 4-carbon of the phenyl ring. Affinity constants and pFe values for terephthalamide siderophore mimics are generally at least 3 log units higher than catechol analogs for bidentate siderophores, and on the order of 7 log units higher in tris-terephthalamide siderophore mimics (Table IID) (96,101,122). A variety of mono-terephthalamide siderophore mimics has been studied, with different substituents connected to the amide arms (Fig. 13) (121,122). A substituent charge effect was observed whereby negatively charged substituents had the lowest pFe values, while positively charged substituents yielded the highest pFe values (Table IVD). This substituent charge effect was explained by comparing the stability constants of the stepwise reactions (122). While the β_{110} values were the highest for the negatively charged compound, taurTAM (29), the stability of higher order bis- and tris-complexes was lower than with the positively charged compounds because of electrostatic repulsion after binding by the ligand. In taurTAM (29), the overall charge of the FeL complex was -1 , as opposed to $+1$ for DEATAM (31), TAMmeg (30), and ETAM (28), and $+3$ for DMETAM (32). This results in lower repulsion of the uncomplexed ligand in the case of DMETAM and the neutral-substituent terephthalamide chelators than negatively charged taurTAM (122). Comparison of the β_{110} values of the mono-catecholate siderophores with those of the mono-terephthalamide siderophores (Table IVA and IVD, respectively) shows higher values for the catecholate siderophores than for the terephthalamide siderophores; however, this effect quickly decreases for the bis- and tris-complexes due to a similar charge effect as was observed for the terephthalamide siderophores.

As mentioned previously, tetradentate siderophores will form binuclear M_2L_3 complexes to saturate the preferred hexacoordination of iron by forming a singly- or triply bridged species (Fig. 14). Preference for either form depends on siderophore architecture and donor group steric considerations. For some labile systems of appropriate architecture the solution structures may vary with pH (52,127,128). Rhodotorulic acid (3) has been proposed to form a triply bridged Fe_2L_3 complex in solution based on crystallographic and ESI mass spectrometric studies, while the natural cyclic dihydroxamate siderophore alcaligin has been shown to form a singly bridged Fe_2L_3 complex (49,51,70). The thermodynamic stability constants for tetradentate siderophores (Table III) reflect the effect of changing siderophore architecture on the affinity for iron(III).

TABLE IV

THERMODYNAMIC STABILITY CONSTANTS FOR IRON(III)-BIDENTATE SIDEROPHORE AND SIDEROPHORE MIMIC COMPLEXES, MEASURED AT 25 °C

	No. ^a	log β_{110}	log β_{120}	log β_{130}	pFe ^{3+ b}	Refs.
A. Mono-catecholate						
Chrysobactin				35.4		(109)
Catechol		20.4	35.5	44.9	15.1	(57)
Dopamine		21.63	35.02	45.8	14.1	(110)
Aminochelin		19.1	30.8	41.3	17.6	(111)
B. Mono-hydroxamate						
AHA ^c		11.42	21.10	28.33	12.5	(57)
NMAHA ^d		11.70	21.50	29.44	16.2	(112)
Ly-AHA ^e		6.80	12.4	16.1	7.1	(113)
C. Mono-hydroxypyridinonate						
1H2P	47	10.3	19.3	26.9	17.41	(45)
3H4P	48	14.26	25.73	34.91	20.72	(114)
MHP	49	15.4	27.43	37.2		(115)
DMHP	50	15.1	26.61	35.88	19.4	(116)
DEHP	51	15.2	26.96	36.8		(117)
EMHP	52	15.33	27.83	37.7		(115)
PMHP	53	15.34	27.91	37.7		(115)
3H2P	54	11.48	21.25	29.26	16.26	(114)
MH2P	55	11.8	21.63	29.99		(114)
EH2P	56			32.3		(118)
DH2P	57	10.22	17.70	21.66		(114)
APM-3,4-HOPY ^f				51.25 ^g	21.34	(119,120)
D. Mono-terephthalamide						
ETAM	28	17.3	31.6	42.7	22.8	(121)
taurTAM	29	18.2	31.7	40.3	20.4	(121)
TAMmeg	30	17.9	32.1	43.0	24.7	(122)
DEATAM	31	17.0	31.0	42.1	22.9	(121)
DMETAM	32	17.4	32.7	45.3	25.4	(121)

^aStructure number which may be found in a figure.^bCalculated for [Fe³⁺]_{tot} = 10⁻⁶ M, [L]_{tot} = 10⁻⁵ M, and pH = 7.4.^cAHA = acetohydroxamic acid.^dNMAHA = *N*-methylacetohydroxamic acid.^eLy-AHA = lysineacetohydroxamic acid.^fAPM-3,4-HOPY is a bidentate 3-hydroxy,4-pyridinone featuring a propylamine moiety attached to the pyridine N and a methyl group on carbon 2 of the pyridine ring.^gThis value is reported as a β_{131} .

Synthetic linear dihydroxamic acids may be considered as models of rhodotorulic acid (**3**) to investigate steric effects on the formation of mono- or di-iron(III) species (127,128). pH-dependent electrospray ionization-mass spectrometry (ESI-MS) methods show that ferric complexes with dihydroxamates with short linker chains (**37** and **38** in Fig. 15) will prefer the dimeric

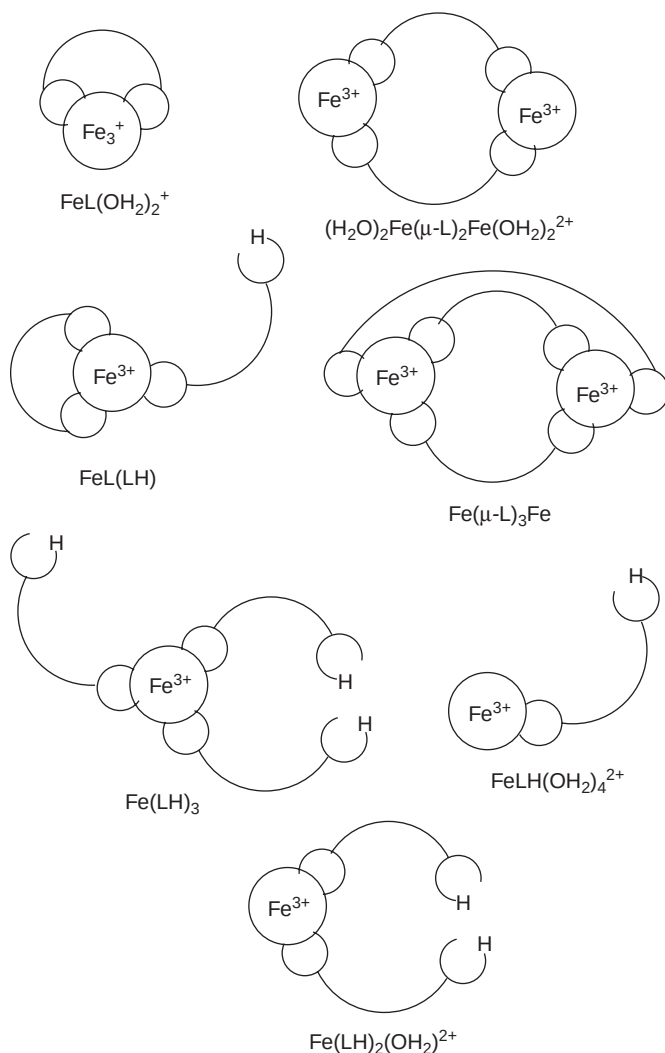
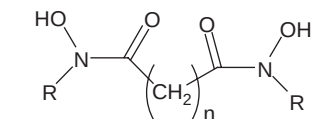


FIG. 14. Schematic representations of linear tetradentate siderophore (H₂L) complexes with different siderophore/iron stoichiometries. Coordinated water not shown for clarity.

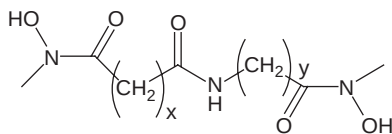
(Fe₂L₂) and longer chain the monomeric (FeL) form. When the intermediate connecting chain is $-(\text{CH}_2)_6-$ (**39**), a crossover point between Fe₂L₂ and FeL is reached where both are present (52).

In addition to structure, the dihydroxamate connecting chain length will affect the affinity of linear siderophores and siderophore mimics for iron(III) (Table IIIA). If the chain connecting the hydroxamates is long, there will be a significant entropic



R	n	Name	No
CH(CH ₃) ₂	3	3-IPHA	(33)
CH(CH ₃) ₂	4	4-IPHA	(34)
CH(CH ₃) ₂	5	5-IPHA	(35)
CH(CH ₃) ₂	6	6-IPHA	(36)
CH ₃	2	2-MHA	(37)
CH ₃	4	4-MHA	(38)
CH ₃	6	6-MHA	(39)
CH ₃	8	8-MHA	(40)

FIG. 15. Linear dihydroxamic acid siderophore mimics with alkyl connecting chains.



x	y	Name	No
2	2	2,2-DIHA	(41)
2	3	2,3-DIHA	(42)
2	4	2,4-DIHA	(43)
2	5	2,5-DIHA	(44)
3	4	3,4-DIHA	(45)
3	3	3,3-DIHA	(46)

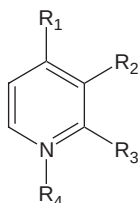
FIG. 16. Linear dihydroxamic acid siderophore mimics with amide connecting chains.

price to pay for iron sequestration. However, in many natural hydroxamate siderophores, the chain length between hydroxamate donor groups is very long. This is presumably why there is little to no observed chelate effect in hydroxamate siderophores, because the length of the spacer chain allows the hydroxamate donor groups to act independently of each other (10). The placement of amide links in the connecting chain of dihydroxamate siderophores can affect iron(III) affinity. Farkas *et al.* investigated this phenomenon using dihydroxamate models where spacer chains of varying lengths connected two hydroxamate binding groups around an amide group connector (Fig. 16). The Fe(III) complex stability of a dihydroxamate model for ferrioxamine B, 2,5-DIHA (44), was found to be higher than that

of 3,4-DIHA (**45**) (Table IIIA), a result that was corroborated by theoretical strain energy calculations (107). However, comparison of the stability of the ferric complex of 2,5-DIHA (**44**) with those of dihydroxamates of shorter chain length, 2,2-DIHA (**41**), 2,3-DIHA (**42**), and 2,4-DIHA (**43**) showed less variation in iron(III) affinity (Table IIIA) (129).

Another important factor in the stability of hydroxamate complexes is the identity of the substituents on the nitrogen and carbon of the hydroxamate. Electron-withdrawing substituents and inductive electron-donating substituents connected to the N-terminus, as well as electron-donating substituents on the C-terminus will stabilize the most favorable resonance form of the hydroxamate donor group, where a positive formal charge is located on the nitrogen and a negative formal charge is located on the carbonyl oxygen atom. This topic has been covered extensively in previous reviews of iron-siderophore chemistry (10,22).

One donor group that is only rarely observed in nature is hydroxypyridinone (Fig. 17). As mentioned previously, the sole example found in nature is cepabactin, a bidentate 1-hydroxy-2-pyridinone siderophore (**8** in Fig. 2) (46). The bidentate 1,



R ₁	R ₂	R ₃	R ₄	Name	No
H	H	=O	OH	1H2P	(47)
=O	OH	H	H	3H4P	(48)
=O	OH	CH ₃	H	MHP	(49)
=O	OH	CH ₃	CH ₃	DMHP	(50)
=O	OH	C ₂ H ₅	C ₂ H ₅	DEHP	(51)
=O	OH	CH ₃	C ₂ H ₅	EMHP	(52)
=O	OH	CH ₃	C ₃ H ₇	PMHP	(53)
H	OH	=O	H	3H2P	(54)
H	OH	=O	CH ₃	MH2P	(55)
H	OH	=O	C ₂ H ₅	EH2P	(56)
OH	H	=O	OH	DH2P	(57)

FIG. 17. A series of bidentate hydroxypyridinone ligands.

2-dimethyl-3-hydroxy-4-pyridinone (DMHP, L1, Ferriprox[®], **50**; Fig. 17) is currently in use in Europe as a treatment for iron overload (130). Studies of the hydroxypyridinone donor groups have shown them to have high affinity for iron(III) at physiological conditions. The ferric complex of DMHP (**50**) has been found to have a higher pFe value than catechol or similar bidentate siderophores (Table IVC). However, this trend does not continue to higher denticity systems. The hydroxypyridinone analog of enterobactin, HOPObactin (**16** in Fig. 9), was found to have a pFe value ten orders of magnitude lower than that of enterobactin (**1**). TREN-Me-3,2-HOPO (**27** in Fig. 12) has a pFe value one order of magnitude lower than its catecholamide analog, TRENCAM (**17**) (Table IIC and IIA) (92,95). However, the iron(III) binding properties of the hydroxypyridinone donor group is sensitive to the identity and location of the substituent. For example, the pFe values for nonsubstituted hydroxypyridinones increase in the order of 3H2P (**54**) < 1H2P (**47**) < 3H4P (**48**) (Table IVC) (45). The 1,2-HOPO moiety forms a stronger complex with iron(III) than the 3,2-HOPO moiety due to the higher acidity of the hydroxyl donor. Meanwhile, the 3,4-HOPO moiety forms more stable complexes with iron(III) due to the location of the ketone oxygen trans to the pyridyl N-atom and localization of a formal negative charge on the carbonyl oxygen. The 1-hydroxy,2-pyridinone donor group can be viewed as a cyclic hydroxamic acid, and comparison of pFe values for 1H2P (**47**) with acetohydroxamic acid shows a five log unit improvement of the binding affinity for iron(III) due to the greater electron delocalization present in the hydroxypyridinone structure (Tables IVB and IVC).

One interesting aspect of the hydroxypyridinone donor group siderophores is the dependence of the chelate effect on siderophore structure. Comparison of the pFe values of the complex of the bidentate EH2P (**56** in Fig. 17) with pFe values of the complex of the hexadentate TREN-Me-3,2-HOPO (**27** in Fig. 12) shows a decrease in apparent stability (Tables IIC and IVC), implying no chelate effect present in the system (118). This is likely due to a lack of preorganization in the ligand. However, in the tris-hydroxypyridinone siderophore mimic, N³(LH)₃ (**59** in Fig. 18), the pFe value is greatly increased over that for the bidentate analog (**58**), apparently due to the chelate effect (Table IIC) (93).

D. REDOX CHEMISTRY OF IRON-SIDEROPHORE COMPLEXES

One aspect of microbial iron metabolism that remains unclear in many cases is the mechanism for iron release from tight sequestration once the siderophore complex arrives at its

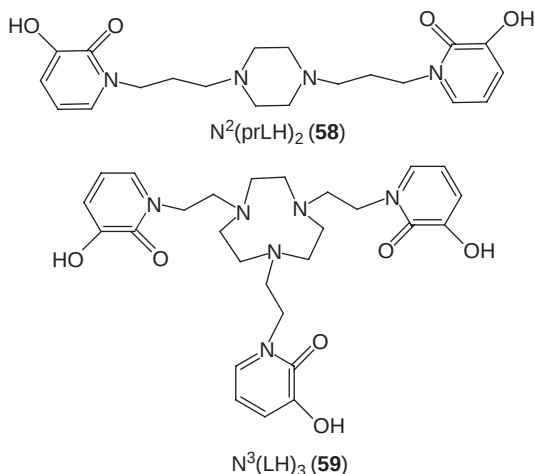


FIG. 18. 3-Hydroxy,2-pyridinone siderophore mimics based on an azamacrocycle central ring structure.

destination, the cell surface or cell interior. Three broad hypotheses may be envisioned to describe the mechanism of iron release to the cell: (i) simple release of iron driven by binding site protonation and/or conformational change; (ii) siderophore ligand cleavage or hydrolysis leaving a binding site with lower iron affinity and leading to subsequent iron release; or (iii) reduction of Fe^{3+} to Fe^{2+} , followed by dissociation of the siderophore (4,10,131). The second mechanism has been observed in the case of enterobactin (**1**), where an enterobactin-specific esterase cleaves the backbone (132). This results in a tris-bidentate ligand complex, which has a lower affinity for iron than the hexadentate analog, resulting in facile release of the iron. However, it has been proposed that the first and third mechanisms are more efficient, due to the metabolic cost of producing new siderophores after hydrolysis (3,4,8,133). Reduction of the bound iron to the 2+ state results in a lower charge-to-radius ratio, making the metal center a borderline soft acid. This results in lower affinity of the metal ion for the hard donor atoms contained in most siderophores (9). Reduction also will increase the lability of the iron as high-spin Fe^{2+} undergoes ligand exchange more rapidly than high-spin Fe^{3+} (134,135). The efficacy of the reductive iron release hypothesis depends heavily on the redox potential, $E_{1/2}$, of the ferri-siderophore complex at cellular conditions.

Table V shows redox potentials ($E_{1/2}$) for ferric complexes of a series of natural and synthetic siderophores. The first coordination shell of the complex formed between iron and siderophore

TABLE V

FORMAL $\text{Fe}^{3+}/\text{Fe}^{2+}$ REDOX POTENTIALS FOR FERRI-SIDEROPHORE AND SIDEROPHORE
MIMIC COMPLEXES AT POTENTIAL-LIMITING, HIGH-pH CONDITIONS

Siderophore	No. ^a	$E_{1/2}^b$	Refs.
A. Tris-catecholate			
Enterobactin	1	−0.990	(136–139)
TRENCAM	17	−1.040	(139)
3,4-LI(TAMmeg)		−1.032	(96)
B. Mono-catecholate			
2,3-Dihydroxybenzoylserine		−0.350	(140)
C. Tris-hydroxamate			
Ferrioxamine B	4	−0.468	(48,50,141)
Ferrioxamine E		−0.477	(48,64)
Ferricrocin		−0.412	(62)
Ferrichrome	6	−0.400	(142)
Ferrichrome A		−0.440	(142)
Coprogen		−0.447	(62)
Exochelin MS		−0.380	(67)
N,N',N''-triacetylfulvarinine C		−0.468	(143)
H ₃ L _R ²³⁴	11	−0.436	(91)
H ₃ L _N ²³⁶	12	−0.454	(58)
TAGE		−0.230	(90)
D. Bis-hydroxamate			
Rhodotorulic acid	3	−0.419	(48,51)
Alcaligin		−0.446	(48)
DACODMAHA		−0.261 ^c	(108)
DOCYDMAHA		−0.362 ^c	(108)
PIPDMAHA		−0.319 ^c	(108)
E. Mono-hydroxamate			
Acetohydroxamic acid		−0.293	(113)
N-methylacetohydroxamic acid		−0.348	(48)
L-lysinehydroxamic acid		−0.214	(113)
F. α -Hydroxycarboxylic acid			
Mugineic acid		−0.102	(144)
Rhizoferrin		−0.082	(69)
G. Hydroxypyridinone			
HOPObactin	16	−0.342	(92)
TRENHOPO		−0.435	(92)
3,4-LI(Me-3,2-HOPO)		−0.276	(96)
TREN-Me-3,2-HOPO	27	−0.177	(95)
TR322-Me-3,2-HOPO		−0.233	(95)
TR332-Me-3,2-HOPO		−0.170	(95)
TRPN-Me-3,2-HOPO		−0.185	(95)
H. Tris-salicylate			
TRENSAM	23	−0.551	(100)
SERSAM	13	−0.357	(100)
TREN(3M)SAM	24	−0.450	(100)
SER(3M)SAM	15	−0.089	(100)

TABLE V (Continued)

Siderophore	No. ^a	$E_{1/2}$ ^b	Refs.
I. Mixed			
Aerobactin ^d	2	−0.336	(53)
Pyoverdin PaA ^e		−0.510	(76)
Pyoverdine Pp		−0.480	(77)
Norferriethiocin		−0.097	(74)
Ferriethiocin ^f		−0.166	(74)
Azotobactin σ^g		−0.391	(73)
Alterobactin A ^h		−0.614	(72)
Alterobactin B ^h		−0.672	(145)
Exochelin MN ⁱ		−0.595	(59)
Parabactin ^j		−0.673 ^c	(146)
Parabactin A ^j		−0.400 ^c	(146)
Pseudobactin ^g		−0.482	(147)
3,4-LI(Me-3,2-HOPO) ₂ -(TAMmeg)		−0.327	(96)
3,4-LI(Me-3,2-HOPO)(TAMmeg) ₂		−0.553	(96)
5-LIO(TAMmeg) ₂ (TAM)		−0.773	(96)

^aStructure number which may be found in a figure.^bVolts relative to NHE.^cMeasured at pH 7.0, which may not necessarily be the potential-limiting pH.^dDonor groups: 2 hydroxamate and 1 α -hydroxycarboxylate.^eDonor groups: 2 hydroxamate and 1 catecholate.^fDonor atoms: 1 carboxylate O, 1 phenolate O, and 1 thiazoline N.^gDonor groups: 1 hydroxamate, 1 catecholate, and 1 α -hydroxycarboxylate.^hDonor groups: 1 catecholate and 2 α -hydroxycarboxylate.ⁱDonor groups: 2 hydroxamate and 1 β -hydroxyhistidine.^jDonor groups: 2 catecholate and 1 phenolate.

will be the major determinant of the redox potential of the complex. Data in Table V demonstrate that the most negative redox potentials are observed for the catecholate siderophores, such as enterobactin (**1**) and TRENCAM (**17**), while the least negative redox potentials are observed in α -hydroxycarboxylate siderophores, such as rhizoferrin. $E_{1/2}$ values for mixed siderophore systems and hydroxamate donor group siderophores are in the intermediate range. A lower redox potential denotes a greater thermodynamic difficulty to reduce, implying that the siderophore stabilizes iron in the 3+ oxidation state. This is demonstrated by the low redox potentials observed in the cases of tris-catechol siderophores like enterobactin (**1**), where the hard oxygen donor atoms and the delocalized electron density of the catechol donor groups stabilize the ferric state. Investigation of the aqueous redox potentials also allows estimation of the values of the stability constants of the complexes of the siderophores with iron(II). Assuming ideal behavior and applying the Nernst equation, the relationship between β^{III} and β^{II} , the stability

constants of the iron(III) and iron(II) complexes, respectively, is as follows, where E_0^{complex} represents the iron–siderophore complex redox potential and E_{aq} is the redox potential of the

$$E_0^{\text{complex}} - E_{\text{aq}} = -59.16 \log \left(\frac{\beta^{\text{III}}}{\beta^{\text{II}}} \right) \quad (38)$$

aquo-complex couple, $\text{Fe}(\text{H}_2\text{O})_6^{3+}/\text{Fe}(\text{H}_2\text{O})_6^{2+}$. Eq. (38) also illustrates that the more negative the formal redox potential, the greater the differences in siderophore affinity for iron(III) vs. iron(II).

Another factor that will affect the complex redox potential is the architecture of the siderophore. A plot of ferri-siderophore redox potentials as a function of pFe for a series of hydroxamate complexes of differing denticity (shown in Fig. 19) exhibits a trend. The trend demonstrates that hydroxamate siderophores of higher denticity will form complexes with more negative $E_{1/2}$ values than analogous siderophores of lower denticity.

A similar plot of $E_{1/2}$ vs. pFe is shown in Fig. 20, which is limited to hexadentate siderophores, but includes a wide variety of donor groups. Although there is significant scatter, a linear

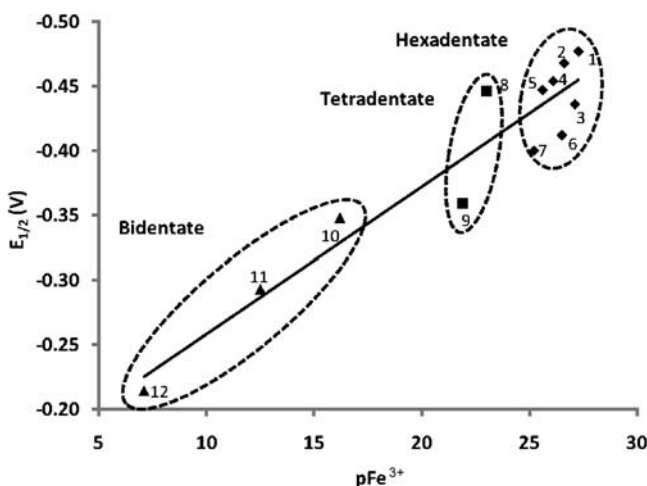


FIG. 19. Plot of redox potentials ($E_{1/2}$) as a function of pFe^{3+} values for a series of hexadentate, tetradentate, and bidentate hydroxamic acid siderophores and siderophore mimics. Data from Table V. Legend: 1 – ferrioxamine E; 2 – ferrioxamine B (4); 3 – $\text{H}_3\text{L}_\text{R}^{234}$ (11); 4 – $\text{H}_3\text{L}_\text{N}^{236}$ (12); 5 – coprogen; 6 – ferricrocin; 7 – ferrichrome (6); 8 – alcaligin; 9 – rhodotorulic acid (3); 10 – NMAHA; 11 – AHA; 12 – Ly-AHA.

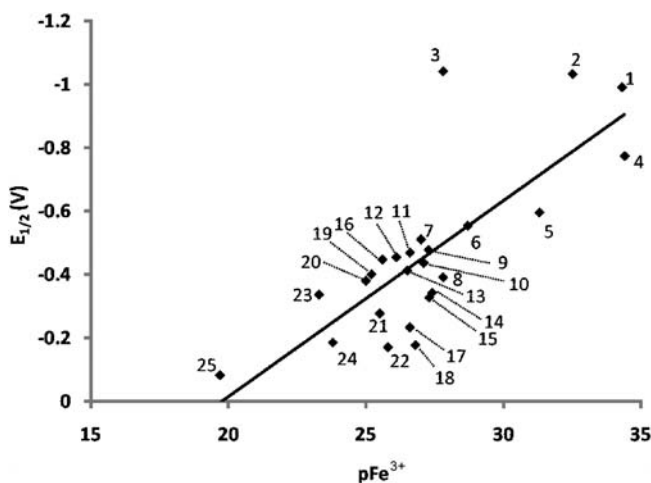


FIG. 20. Plot of redox potentials ($E_{1/2}$) as a function of pFe^{3+} values for hexadentate siderophore and siderophore model complexes. Solid line represents a linear least squares fit to the data with slope = 0.0615 V. Legend: 1 – enterobactin (1); 2 – 3,4-LI(TAMmeg); 3 – TRENCAM (17); 4 – 5-LIO(TAMmeg)₂TAM; 5 – exochelin MN; 6 – 3,4-LI(Me-3,2-HOPO)(TAMmeg)₂; 7 – pyoverdine; 8 – azotobactin; 9 – ferrioxamine E; 10 – $\text{H}_3\text{L}_\text{R}^{234}$ (11); 11 – ferrioxamine B (4); 12 – $\text{H}_3\text{L}_\text{N}^{236}$ (12); 13 – ferricrocin; 14 – HOPObactin (16); 15 – 3,4-LI(Me-3,2-HOPO)₂(TAMmeg); 16 – coprogen; 17 – TR322-Me-3,2-HOPO; 18 – TREN-Me-3,2-HOPO (27); 19 – ferrichrome (6); 20 – exochelin MS; 21 – 3,4-LI(Me-3,2-HOPO); 22 – TR332-Me-3,2-HOPO; 23 – aerobactin (2); 24 – TRPN-Me-3,2-HOPO; 25 – rhizoferrin. $E_{1/2}$ values taken from Table V and pFe values taken from Tables I and II.

trend is evident with higher iron affinity siderophores exhibiting more negative $E_{1/2}$ values. Using the Nernst equation and the standard redox potential for the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple,

$$E_{1/2} = E^0 - \frac{59.16}{n} \log \frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} \quad (39)$$

it can be shown that a linear relationship is possible between the pFe^{3+} value and the solution redox potential if pFe^{2+} is a constant for all of the complexes considered.

$$-E_{1/2} = -(0.77) - c \text{pFe}^{2+} + c \text{pFe}^{3+} \quad (40)$$

This ideal plot should have a slope of $c = 59 \text{ mV/log unit}$ and a y -intercept related to the standard redox potential of the $\text{Fe}_{(\text{aq})}^{3+}/\text{Fe}_{(\text{aq})}^{2+}$ couple and the pFe^{2+} for the complexes. Linear

regression of the plot in Fig. 20 shows a line fit to the points with a slope of approximately 60 mV/log unit, close to that expected for the ideal plot. This implies limited variation of the $p\text{Fe}^{2+}$ values from 7.4 for the siderophores included in the plot. Of interest is the fact that the hexadentate catechol siderophore complexes exhibit more negative $E_{1/2}$ values than predicted from Eq. (40), while hydroxypyridinone donor group siderophores exhibit less negative complex $E_{1/2}$ values than predicted.

The redox potential and reversibility of the redox of many iron–siderophore complexes will also vary with pH. This variation is due to a number of factors, including the speciation of the Fe(III)–siderophore and Fe(II)–siderophore complex in solution over the pH range studied (3,4,59). Reduction of the Fe^{3+} results in diminished competition with protons for binding to ionizable donor groups. Fig. 21 shows the variation of the redox potential of a series of hydroxamate siderophore complexes with pH. At higher pH values, the redox potential of the complex reaches a plateau, as the protonation states of the complexes do not change above this pH. This variation of $E_{1/2}$ with pH may be advantageous, as at physiological conditions the redox potential of many complexes are out of the range of biological reducing agents (3,4). Therefore, lower pH regions may contribute to the increased ability of an organism to obtain iron through a reductive mechanism. This is most notably observed in

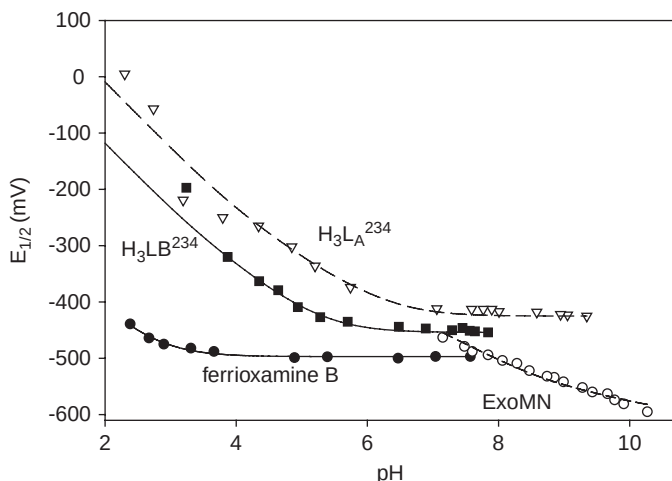


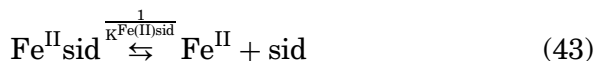
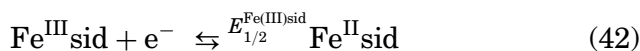
FIG. 21. Redox potentials ($E_{1/2}$) of Fe^{3+} siderophore and siderophore mimic complexes as a function of pH. Lines represents a fit of Eq. (41) to the data, which are taken from Refs. (3,58,59,91). Adapted with permission from Ref. (4).

hydroxamate siderophores, such as alcaligin, desferrioxamine B (4), and rhodotorulic acid (3), although studies have shown the phenomenon in other donor group systems (48,136,141,148). The variation of $E_{1/2}$ with pH can be derived from the protonation constant expressions of the oxidized and reduced forms of the complexes and the Nernst equation (Eq. (41)):

$$E_{\text{rxn}} = E_{\text{complex}} + 59.16 \log(1 + K_{\text{a1}}^{\text{II}}[\text{H}^+] + \beta_{\text{a2}}^{\text{II}}[\text{H}^+]^2 + \dots) - 59.16 \log(1 + K_{\text{a1}}^{\text{III}}[\text{H}^+] + \dots) \quad (41)$$

Using linear regression, it is possible to estimate the protonation constants of the Fe(II) complexes of siderophore complexes where the redox potentials have been measured over a range of pH values (59). This also explains the variation in reversibility of reduction as the pH changes, as the stability of the ferro-siderophore complex is much lower than the ferric complex, and the increased lability of ligand exchange and increased binding site competition from H^+ may result in dissociation of the complex before the iron center can be reoxidized.

Another factor that can possibly affect the redox potential in biological systems is the presence of secondary chelating agents that can participate in coupled equilibria (3). When other chelators are present, coupled equilibria involving iron-siderophore redox occur and a secondary ligand will cause the siderophore complex effective redox potential to shift. The decrease in stability of the iron-siderophore complex upon reduction results in a more facile release of the iron. Upon release, the iron(II) is available for complexation by the secondary ligand, which results in a corresponding shift in the redox equilibrium toward production of iron(II). In cases where iron(II) is stabilized by the secondary chelators, there is a shift in the redox potential to more positive values, as shown in Eqs. (42)–(45).



$$E_{\text{rxn}} = E_{1/2}^{\text{Fe(III)sid}} + \frac{0.0592}{n} \log \left(1 + \frac{K^{\text{Fe(II)L}}[\text{L}]}{K^{\text{Fe(II)sid}}[\text{sid}]} \right) \quad (45)$$

In Eq. (45), $K^{\text{Fe(II)L}}$ is the stability constant for iron(II) complexation by the competing ligand, $K^{\text{Fe(II)sid}}$ the stability constant for the complex formed between iron(II) and the siderophore, n the number of electrons transferred, E_{rxn} the observed redox potential for the iron(III)–siderophore system coupled with iron(II) chelation, and $E_{1/2}^{\text{Fe(III)sid}}$ the redox potential of the iron(III)–siderophore complex.

Ternary complex formation in ferrioxamine B has been shown to facilitate the reduction and release of iron by ascorbate, glutathione, and NADH. Biological reductants glutathione and ascorbate will not reduce the iron(III) in ferrioxamine B, but will do so in the presence of sulfonated bathophenanthroline (BPDS), enabling the iron to be readily released from the siderophore as Fe(II) (149). BPDS was shown to form a ternary complex with ferrioxamine B, and while BPDS is not a biologically relevant ternary ligand, it can be viewed as a model for *in vivo* small molecule soft chelators or protein receptor side chains. A second study with the mugineic acid family of phytosiderophores suggested a similar mechanism using nicotinamine as a model biological reductant (150). Evidence for the biological relevance of a hypothetical ternary complex route leading to reduction and release of siderophore bound iron comes from the siderophore pyochelin (60), where the tetradentate siderophore forms a complex with iron, leaving two available coordination sites. The hydroxypyridinone siderophore, cepabactin (8) can bind to the remaining two coordination sites and form a ternary complex (61 in Fig. 22). Ternary complex formation may be important in the recognition of the iron(III)–pyochelin complex by the pyochelin receptor protein, FptA (151,152). Ferrioxamine B ternary complex formation was shown to be stabilized in the presence of SDS micelles, suggesting the possible involvement of low pH regions and cell-receptor surfaces in the formation and transport of ternary complexes in biological systems (153).

Another possible route for reduction of the iron center is photoreduction. This has been studied in a variety of marine siderophore systems, such as aquachelin, marinobactin, and aerobactin (2), where it was demonstrated that photolytic reduction was due to a ligand-to-metal charge transfer band of the Fe(III)–siderophore complex, eventually resulting in reduction of iron(III) and cleavage of the siderophore (31,154,155). This suggests a possible role for iron reduction in iron release (71,155).

The redox strategy can become useful in catecholamide donor siderophores. As mentioned earlier, complexes formed with catecholamide donor group siderophores are generally very stable, and as shown in Table VA, have very low redox potentials.

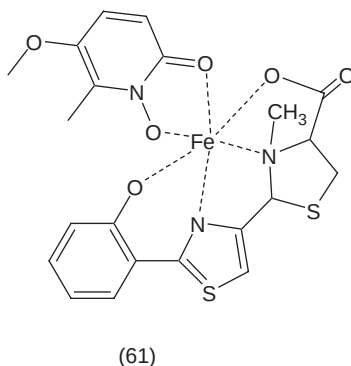
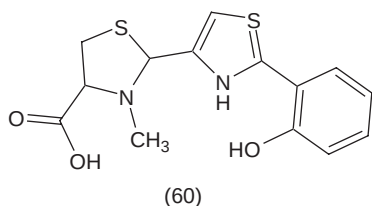


FIG. 22. Pyochelin (**60**) and the ternary complex formed with ferric iron, pyochelin, and cepabactin (**61**).

However, the redox potential is particularly sensitive to changes in pH. As mentioned previously, protonation of the catecholamide donor group can lead to a salicylate shift, which results in weaker binding of iron. Studies of salicylate analogs of enterobactin (**13** and **15**) and TRENCAM (**23** and **24**) show that their ferric complexes have much less negative redox potentials than the limiting potential of the catecholamide analogs (Table V.H), on the order of 400 mV less negative for TRENCAM (**17**) and 600 mV less negative for SERSAM (**13**), much closer to the range of redox potentials observed in biological reductants (100).

There are two general mechanisms for electron transfer reactions in metal complex chemistry. The first is the outer sphere electron transfer mechanism, where the ligand donors of the inner coordination sphere of the metal remain unchanged during the electron transfer process. The second mechanism is inner sphere electron transfer, where the redox agent enters the inner coordination shell of the metal complex through direct coordinate covalent bond formation, leading to an oxidation state change for the metal (135,156). Very little research has been done into the mechanism of iron(III)–siderophore complex redox mechanisms. Kazmi *et al.* report some investigations into the mechanism of electron transfer for the redox of some

iron(III)–hydroxamate complexes (157–159). A more thorough discussion of the topic of iron–siderophore complex redox mechanisms can be found elsewhere (41).

Reduction of the iron center may be described as a molecular “switch” to indicate its possible role in the mechanism of iron release from the siderophore complex. Reduction from Fe(III) to Fe(II) results in lower siderophore selectivity, as evidenced by a decrease in the overall complex stability with iron(III) compared to iron(II), as well as an increase in H^+ competition for the binding group resulting in iron dissociation. Reduction also acts as a kinetic “switch,” as the rate of ligand exchange at the iron center increases greatly upon reduction, as will be addressed further below (134). All of these factors lead to much easier release of the iron from the siderophore binding site, making the process of reduction of the metal center a molecular “switch.” Although many ferri-siderophores have very negative redox potentials, these thermodynamic potentials may be shifted to higher values through change in the dielectric constant of the medium such as experienced in a receptor interior, a drop in pH, ternary complex formation, coupled reactions that trap reduced iron, and photolysis.

IV. Chelation Kinetics of Iron–Siderophore Systems

An understanding of the kinetics and mechanism of iron chelation and release involving siderophores and siderophore mimics is important, as siderophores function as an extracellular transporter, which involves two key steps: the sequestration and release of iron. In order to understand the chemical basis of siderophore mediated iron bioavailability it is necessary to understand the thermodynamics and the kinetics of both processes. Controlling the rate of iron release from its tightly held siderophore binding site is a particularly interesting and challenging problem, since an issue is site and time specific iron release from a binding site with a very deep thermodynamic well ($\log \beta$ values >30).

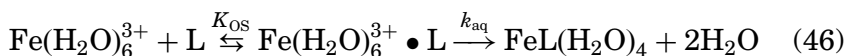
Additional relevant details of the kinetics and mechanisms of iron chelation reactions may be found in other recent reviews (10,22,134,160,161).

A. SIDEROPHORE COMPLEX FORMATION KINETICS AND MECHANISM

The kinetics and mechanism of iron–siderophore complex formation are influenced by the oxidation state and composition of the first coordination shell of iron. The iron sequestration

process involves replacing one ligand (an ambient ligand such as water) with a siderophore donor group. In general, ligand substitution at an iron(III) center is a concerted interchange process with a small degree of dissociative or associative character (I_d or I_a) depending on the exchanging ligands (160). This means that the kinetics of iron-siderophore complex formation are largely controlled by the kinetics of iron-leaving ligand bond cleavage. If we consider the simplest environmental iron ion as $\text{Fe}(\text{H}_2\text{O})_6^{n+}$, then the kinetics of sequestration by a siderophore are essentially controlled by water exchange kinetics. For $\text{Fe}(\text{H}_2\text{O})_6^{3+}$ this exchange rate constant (k_{aq}) is $1.60 \times 10^2 \text{ s}^{-1}$ and for $\text{Fe}(\text{H}_2\text{O})_6^{2+}$ it is $4.4 \times 10^6 \text{ s}^{-1}$ at 25°C (162–164). This suggests (generally correctly) that aqueous ferrous ion sequestration is expected to proceed more rapidly than ferric ion sequestration. This also means that we expect very similar values for initial iron–ligand bond formation rates for different siderophore donor groups and architectures.

These general comments regarding iron sequestration kinetics can be placed in the context of the Eigen–Wilkins mechanism for complexation (135). Initial complex formation proceeds by way of the formation of an encounter complex, $\text{Fe}(\text{H}_2\text{O})_6^{3+} \bullet \text{L}$,



which enables us to express a second order sequestration rate constant as follows:

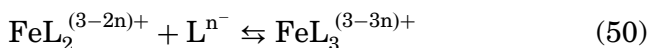
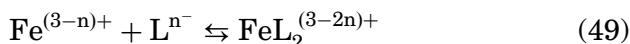
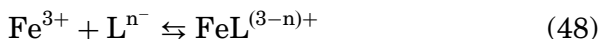
$$k_{\text{obs}} = K_{\text{OS}} S k_{\text{aq}} \quad (47)$$

This demonstrates that the second order sequestration rate constant (k_{obs}) is controlled by the water exchange rate constant (k_{aq}), modulated by a pre-equilibrium constant for the formation of the encounter complex (K_{OS}). The pre-equilibrium reaction brings the entering ligand and metal ion within reaction distance at a diffusion controlled rate and the value of K_{OS} is largely controlled by electrostatics (134,165). In the case of uncharged ligands binding to iron(III), the value of K_{OS} may be calculated or experimentally found to range from 10^{-1} M^{-1} to 10^2 M^{-1} for entering ligand charges ranging from 0 to 2– (161,166–168). Of course the K_{OS} value for charged ligands with metal ions will decrease as the ionic strength increases (161,169). In Eq. (47), a statistical factor (S) corrects for six dissociating water ligands and other steric considerations regarding the entering ligand.

Charge density is known to influence water exchange rates at a metal center (160). Since OH^- is a stronger donor than OH_2 , this

means that the hydroxy forms of the aquated Fe cation will undergo sequestration faster than $\text{Fe}(\text{H}_2\text{O})_6^{n+}$. For example, water exchange for $\text{Fe}(\text{H}_2\text{O})_5(\text{OH})^{2+}$ occurs $\sim 10^3$ times faster than for $\text{Fe}(\text{H}_2\text{O})_6^{3+}$ (160,162,163). The tendency of iron to hydrolyze (see Eqs. (1)–(3)) makes this contribution significant in natural systems, as the hexaaqua ion is not likely to be present at ambient conditions. The effect of strong charge donors in the first coordination shell on water exchange rates also means that for polydentate siderophores, initial Fe–siderophore bond formation will likely be the slow step, with rapid ring closure due to enhanced water exchange rate and increased proximity of subsequent donor groups to the iron center.

The effect of charge donors in the first coordination shell on water/ligand exchange rates is illustrated by the observation that in the sequential formation of mono-, bis-, and tris-*N*-methylacetohydroxamato iron(III) complexes from $\text{Fe}(\text{H}_2\text{O})_6^{3+}$ or $\text{Fe}(\text{H}_2\text{O})_5\text{OH}^{2+}$, the rate constant progressively increases (112,170). This is illustrated below for the generic case of a tris-bidentate ligand (L) complex formation where coordinated water



is omitted for clarity. This analysis is further illustrated by the fact that reaction of $\text{Fe}(\text{OH})_2^{3+}$ with hexadentate desferrioxamine B (4) to form ferrioxamine B proceeds at the same rate as with acetohydroxamic acid to form mono(acetohydroxamato)tetra(aqua) iron(III) (170–172).

The implications of these mechanistic studies for our understanding of environmental iron sequestration by siderophores is as follows. The hydroxyl containing aqua ferric ions will tend to form ferri-siderophore complexes more rapidly than the hexaaqua ion and ferrous ion will be sequestered more rapidly than the ferric ion. However, once in a siderophore binding site the ferrous ion will be air oxidized to the ferric ion, due to the negative redox potentials (see Section III.D). This also means that Fe dissolution from rocks will be influenced by mineral composition (other donors in the first coordination shell) as well as surface reductases in contact with the rock, and of course surface area (4,13).

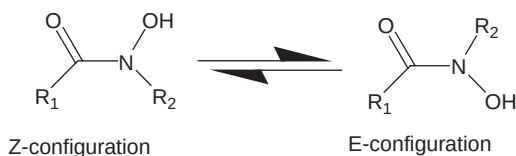
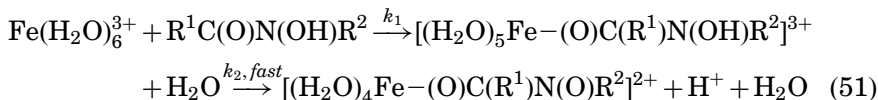


FIG. 23. Rotation around the hydroxamic acid C–N bond allows two possible configurations of the donor group, Z and E.

In some complexes of higher denticity, another factor in complex formation can be the ring closure of the donor group. In most cases, this step is rapid, as the donor groups are in close proximity to the metal center and therefore, ring closure is favorable. However, ring closure can play a major role in the complex formation mechanism where steric features may hinder the process. One such example is in hydroxamate systems, where the hydroxamate donor group can have two configurations. In solution, the C–N bond is not rigid, which allows rotation between the Z and E configurations (Fig. 23). This can make the ring closure step a kinetically important step in the formation of hydroxamate complexes, where the initial binding step occurs with the carbonyl oxygen, followed by rotation of the hydroxamate moiety from the E to the Z configuration, followed by rapid ring closure. The ring closure reaction has been found to occur in a single observable step in the case of mono-hydroxamates, but in two observable steps for trihydroxamates, such as desferrioxamine B (4), Eq. (51) (112,170–174).



B. SIDEROPHORE COMPLEX HYDROLYSIS KINETICS AND MECHANISM

As noted at the beginning of this section, an important and challenging process is investigating and characterizing the release of tightly bound iron from its siderophore carrier. This can be brought about by the redox initiated dissociation of Fe(II) as presented in Section III.D. It may also be brought about by metabolic decomposition of the complex, thereby weakening the siderophore's affinity for iron (140). Alternatively, iron dissociation may be driven by a pH drop, such as at the cell membrane surface or in a vesicular compartment, and/or a conformational change in the ferri-siderophore complex. Here, we will review some relevant model kinetics work for iron(III) dissociation,

which is largely studied through pH-jump experiments. The removal of a donor group from iron is facilitated by low pH, as the proton will bind to the free donor group in competition with iron, preventing it from re-associating with the iron center.

A kinetic study of tris-(L-lysinehydroxamato)iron(III) showed the effect of electrostatic repulsion on the rates of complex dissociation (113). The hydroxamate ligand dissociation kinetics for L-lysinehydroxamate (LyHA) were faster than that for aceto-hydroxamate (AHA) or *N*-methylacetohydroxamate (NMAHA) ligands, presumably due to the electrostatic repulsion between the dissociating 2+ charged ligand and the positive iron(III) center in the former case, relative to the dissociating neutral ligand in the latter two cases (112,113,170,171). These results also demonstrate the difference in complex dissociation kinetics due to different substituents on the N-terminus of the hydroxamate group. The kinetics of stepwise ligand dissociation for NMAHA is much slower than either AHA or LyHA, due to the increased inductive effect of the *N*-alkyl substituent of NMAHA compared to AHA or Ly-AHA (112,113,170,171).

In linear hexadentate siderophore systems, such as ferrioxamine B (4), dissociation proceeds in a sequential manner, with one of the terminal hydroxamates dissociating first upon protonation, followed by the second donor group at a slower rate, and finally the third group dissociating at a much slower rate (Fig. 24) (172). In this case, however, the rate of each successive dissociation is slower than the analogous dissociation in the model mono-hydroxamate system, NMAHA (173). This is likely due to a number of factors, including solvation and denticity effects. Dissociating bidentate units are less likely to recombine with the metal when not tethered, as is the case for NMAHA. The proton driven hydrolysis of the endocyclic tris-hydroxamato-iron(III) complex with desferrioxamine E was studied by pH-jump methods. These data show a single stepwise dissociation pathway involving the proton driven sequential removal of each hydroxamate group, resulting in aqueous iron(III) (175). The reaction proceeds at a slower rate than the complex with the linear analogue desferrioxamine B and the tris-bidentate complex $\text{Fe}(\text{NMAHA})_3$, probably due to the tight preformed cavity of desferrioxamine E which leads to a proximity effect enhancing complex formation.

Serratrice *et al.*, studied the hydrolysis kinetics of the iron(III) complexes of the tripodal hexadentate siderophore mimics, O-TRENSEX (26) and TRENCAMS, and their heteropodal analogs, TRENSOX2CAMS and TRENSOXCAMS2 (176). Protonation of the complexes proceeds in four stages: (i) the initial

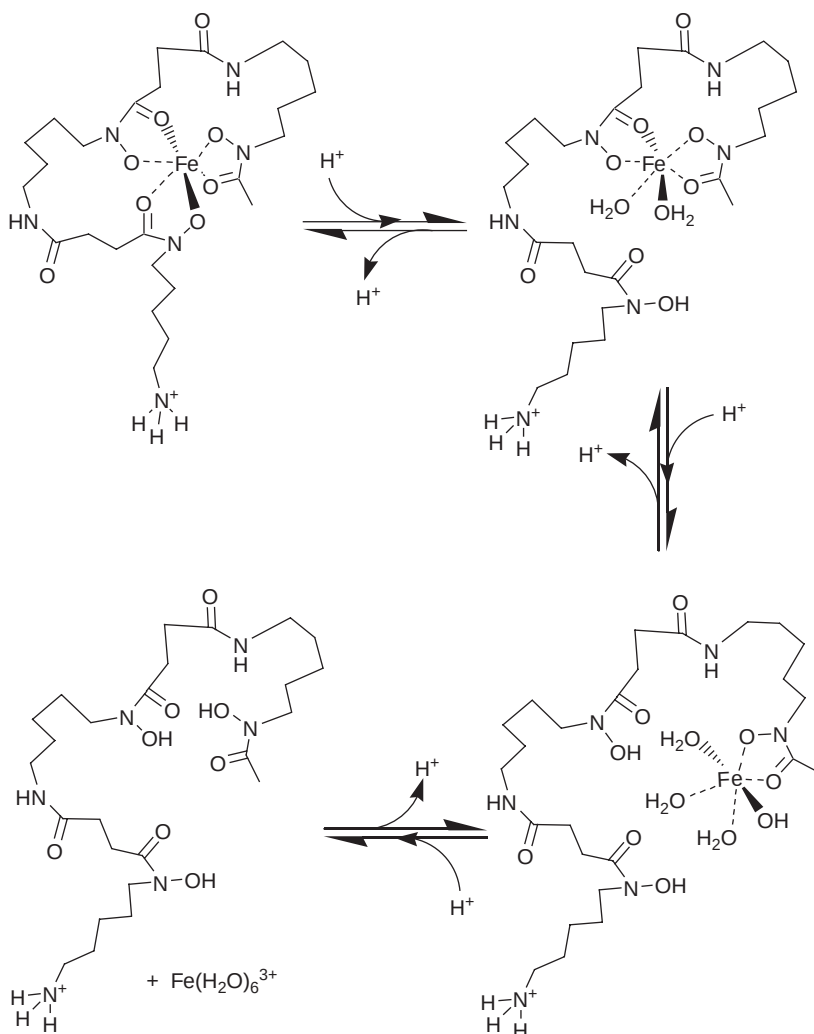


FIG. 24. Stepwise process for H^+ -assisted dechelation of iron from ferrioxamine B.

release of a single arm; (ii) and (iii) protonation events at the two remaining coordinated arms resulting in salicylate shifts; and (iv) complete dissociation of the siderophore from the iron (Fig. 25). In the initial stage, dissociation of the catecholate arm of TRENCAMS was more rapid than dissociation of the 8-hydroxyquinolyl (8-HQ) arm of O-TRENSEX. This was proposed to be due to the lower effective charge on iron when bound by the catecholate donor group (-1 when in the tetracoordinate form)

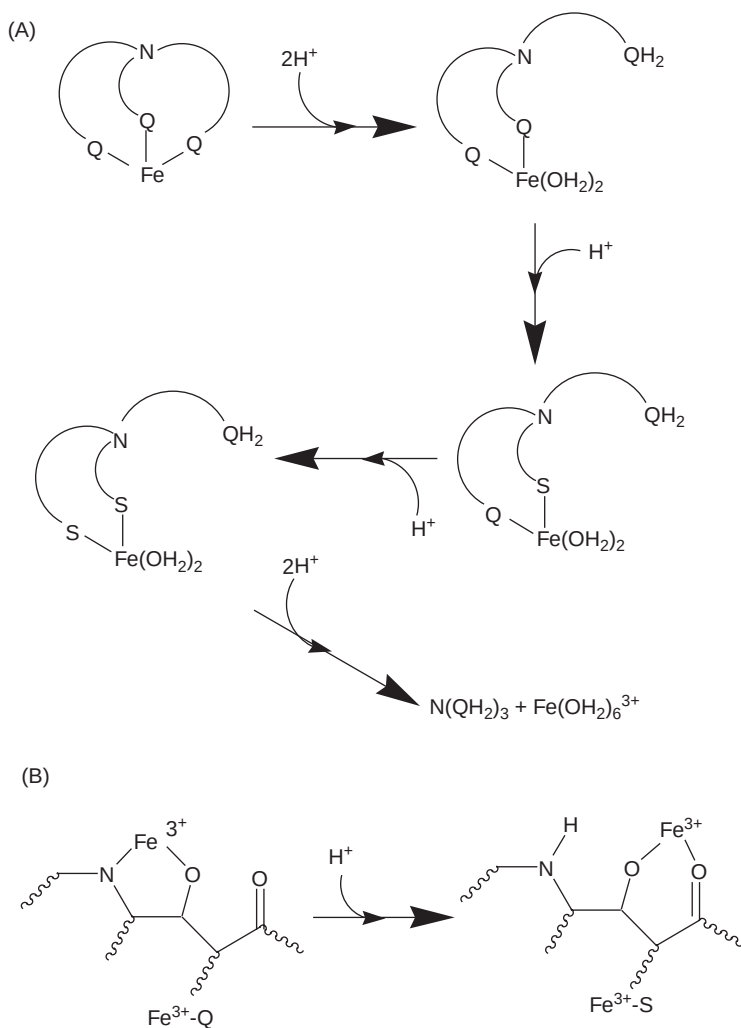


FIG. 25. Protonation and ligand dissociation mechanism for O-TRENSEX (**26**) (176). (A) Four-step hydrolysis mechanism of O-TRENSEX where Q represents 8-hydroxyquinolyl and S the corresponding salicylate coordinating moiety. Charge on the complex has been omitted for clarity. (B) Schematic representation of the salicylate shift of the 8-hydroxyquinolyl donor groups of O-TRENSEX.

compared to that when bound by the 8-HQ donor group (+1 when in the tetracoordinate form) (99). The salicylate shift was more rapid for TRENCAMS than O-TRENSEX, likely due to the sterics of ligand rearrangement in the larger 8-HQ donor group

and the electron-withdrawing properties of the 8-HQ donor group. In the TRENSEX2CAMS system, the single catecholate group was the first to dissociate, and conversion to the mono-salicylate, mono-8-HQ complex was not observed. However, conversion to the mono-salicylate complex was observed in the TRENSEXCAMS2 complex, which features two catecholamide donor groups and a single 8-HQ donor group. Dissociation of one salicylate-binding group was also observable in the TRENSEX-CAMS2 system, resulting in two separate observable dissociation events.

The dissociation of iron from a tetradentate siderophore complex is more rapid than from the analogous hexadentate system (3). This may be a reason for some organisms to produce tetradentate siderophores instead of hexadentate siderophores despite the concentration effect noted in Section III.A. As was illustrated in Fig. 19, it is also thermodynamically easier to reduce iron(III) in tetradentate complexes than hexadentate complexes, making it easier to induce release of iron from the complex by a redox mechanism.

The kinetics of iron(III) dissociation from a series of dihydroxamate siderophores and siderophore mimics, including rhodotorulic acid (3) and alcaligin, have been investigated (52,127,128, 177,178). ESI-MS studies show that these systems form multiple species as a function of pH and siderophore/iron ratio (128). The lability of these systems and the resultant multiple species leads to several parallel paths to iron(III) dissociation (177). Both the distribution of structures and kinetics of dissociation were shown to be dependent on the length of the spacer chain between the dihydroxamate donor groups (52,127).

A detailed kinetic study was performed on the natural siderophore systems, rhodotorulic acid (3; $\text{H}_2\text{L}^{\text{RA}}$) and alcaligin ($\text{H}_2\text{L}^{\text{ALC}}$) (178). It was shown that in solution with excess $\text{H}_2\text{L}^{\text{RA}}$, iron(III) is present as the hexacoordinated $\text{FeL}^{\text{RA}}(\text{L}^{\text{RA}}\text{H})$ complex, which initially reacts in response to a pH drop in two parallel pathways to give the tetra-coordinated intermediate products, $\text{FeL}^{\text{RA}}(\text{OH}_2)_2^+$ and $\text{Fe}(\text{L}^{\text{RA}}\text{H})_2(\text{OH}_2)_2^+$. $\text{Fe}(\text{L}^{\text{RA}}\text{H})_2(\text{OH}_2)_2^+$ then rapidly dissociates to form $\text{Fe}(\text{L}^{\text{RA}}\text{H})(\text{OH}_2)_4^{2+}$. A separate study of the 1:1 complex at lower pH showed dissociation of the complex through a multistep reaction, where $\text{Fe}(\text{L}^{\text{RA}})(\text{OH}_2)_2^+$ is rapidly protonated to produce $\text{Fe}(\text{L}^{\text{RA}}\text{H})(\text{OH}_2)_4^{2+}$, which then dissociates at different rates through parallel pathways to produce $\text{Fe}(\text{OH}_2)_6^{3+}$ and $\text{Fe}(\text{OH}_2)_5(\text{OH})^{2+}$. These kinetic studies are consistent with the 3:2 ligand:metal complex having a mono-bridged structure, $(\text{L}^{\text{RA}})\text{Fe}(\mu\text{-L}^{\text{RA}})\text{Fe}(\text{L}^{\text{RA}})$ (178). This is in

contrast to the proposed solution structure of the complex based on ESI experiments and crystal structural data for a model triply bridged $\text{Fe}(\mu\text{-L})_3\text{Fe}$ complex (128,179).

The alcaligin complex exhibits two parallel pathways for dissociation of its iron(III) complex (177). One path represents dissociation from the $\text{Fe}(\text{L}^{\text{ALC}})(\text{L}^{\text{ALCH}})$ complex to produce $\text{Fe}(\text{L}^{\text{ALC}})(\text{OH}_2)_2^+$ and the other represents the reaction of $(\text{L}^{\text{ALC}})\text{Fe}(\mu\text{-L}^{\text{ALC}})\text{Fe}(\text{L}^{\text{ALC}})$ to give one equivalent each of $\text{Fe}(\text{L}^{\text{ALC}})(\text{OH}_2)_2^+$ and $\text{Fe}(\text{L}^{\text{ALC}})(\text{L}^{\text{ALCH}})$. Both of these pathways, however, occur at the same rate, due to the lack of interaction between the two metal centers in the di-iron complex. Incomplete dissociation of the $\text{Fe}(\text{L}^{\text{ALC}})(\text{OH}_2)_2^+$ complex is observed, likely due to its high stability and the significant amount of preorganization of the cyclic alcaligin structure.

The process of ligand dissociation may be affected by anions in solution. This may be relevant in biological systems, where there are a variety of environmental anions present. Boukhalfa *et al.* investigated the effects of anions on the dissociation kinetics of iron–dihydroxamate complexes and found that the rate of acid-dependent complex hydrolysis is accelerated in the presence of three anions, in the order: $\text{Cl}^- \gg \text{Br}^- > \text{NO}_3^-$ (180). The rate enhancement was attributed to the formation of transient mixed anion/siderophore complexes, with the coordinating anion displacing water bound to the metal center. The process can be compared to the acceleration of the dissociation due to hydrolysis of the metal center, where the deprotonation of the bound water increases the electron density at the metal center, resulting in more rapid ligand exchange rates. This anion catalysis of ligand exchange is most viable for the dihydroxamate complexes where there are multiple parallel paths to dissociation. Thus as a secondary effect of lower denticity, tetradentate and bidentate siderophores are more labile systems than hexadentate counterparts due to multiple parallel paths to dissociation, which can also lead to anion catalysis.

It has also been observed that the ionic strength of the solution or the presence of SDS micelles will affect the rate of dissociation of iron–siderophore complexes (22,181).

C. SIDEROPHORE COMPLEX EXCHANGE KINETICS AND MECHANISM

While studying the formation kinetics of complexes gives useful mechanistic information about the reactivity of the iron center when bound to a particular siderophore, it is not necessarily a good model for how environmental iron will react in the siderophore system of interest. In biological systems,

environmental iron is not usually found as the bare hydrated metal ion, but is complexed by various competing natural chelating agents. For example, the process of iron uptake at the cell surface may involve the exchange of iron from one chelating agent to another, and subsequent transfer across the cellular membrane. This uptake mechanism has been observed in some bacteria, where studies have shown that the receptor protein binds an apo-siderophore, and subsequently, an iron-bound siderophore will enter the open β -barrel and exchange iron with the bound apo-siderophore, which is then taken into the cell. Similar uptake systems have been observed in both the amonabactin uptake system of *Aeromonas hydrophila*, and in the pyoverdinin receptor protein of *Pseudomonas aeruginosa* (182–184). Therefore, rather than studying the kinetics of complex formation from the hydrated metal ion, it is sometimes more relevant to study the kinetics and mechanism of iron exchange from one chelator coordination environment to another. Of relevance in a bacterial system is iron exchange from transferrin to a siderophore, which may be a virulence factor.

An important step in the reaction mechanism for iron exchange between polydentate ligands is the formation of a ternary complex (135,185). This may happen where the first step of iron release from the complex is dissociation of a single binding group from iron. This will leave open coordination sites on the metal center, which can then be filled by a second chelator. This new ligand binding will contribute to the exchange of chelators at the iron center, as the lower denticity expressed by the original chelator will exhibit a lower iron affinity and accelerated kinetics of release due to the iron being bound by another donor group. The importance of ternary complex formation in multidentate complex iron exchange has been observed, for example, in the cases of tripodal trihydroxamic acid siderophore models and cyclic polypeptides (186–188).

Matsumoto *et al.* demonstrated that the removal of iron from diferric transferrin by the tris-hydroxamate siderophore mimic TAGE occurs in two discreet steps (90). The slower step corresponds to iron removal from the more stable C-lobe site on transferrin and the faster step to removal from the N-lobe. The rates of removal are similar to the rates of removal of iron from diferric transferrin by desferrioxamine B (4), signifying similar mechanisms of removal between the two systems (90).

The exchange of iron from transferrin to desferrioxamine B (4), some catecholate siderophores, and some hydroxypyridinone-based siderophore mimics has been investigated (139,189,190). Turcot *et al.* found that at concentrations similar to those that would be observed in biological settings or clinical treatments,

desferrioxamine B removes iron from transferrin very slowly or not at all (189). Enterobactin (**1**) and its synthetic analog, TRENAM (**17**), have also been found to remove iron slowly (139,190). The hydroxypyridinone siderophore mimics, TREN-Me-3,2-HOPO (**27**), deferiprone, 5LIO-3,2-HOPO, and TREN-1,2,3-HOPO were all found to remove iron more quickly than the catecholate siderophores with higher stability constants (189). Clearly, both kinetic and thermodynamic factors are relevant in removing iron from transferrin.

Serratrice *et al.* investigated the mechanism of iron exchange between EDTA and O-TRENSEX (**26**), TRENSEX2CAMS, TRENSEXCAMS2, and TRENSEX, all of which proceed in a single observable step (191). It was also observed that the reactions between iron-TRENSEX or TRENSEX and heteropodal siderophores proceeded in a single observable step. The iron(III) exchange reaction between TRENSEX and TRENSEX was found to proceed in two detectable steps, indicating that the exchange mechanism proceeds via a ternary complex, where one of the arms of the bound siderophore dissociates and is replaced with an arm of the incoming siderophore (191). The formation of the ternary complex is then followed by sequential exchange of the two remaining arms, resulting in exchange of iron from the initial to the final siderophore, as shown in Fig. 26.

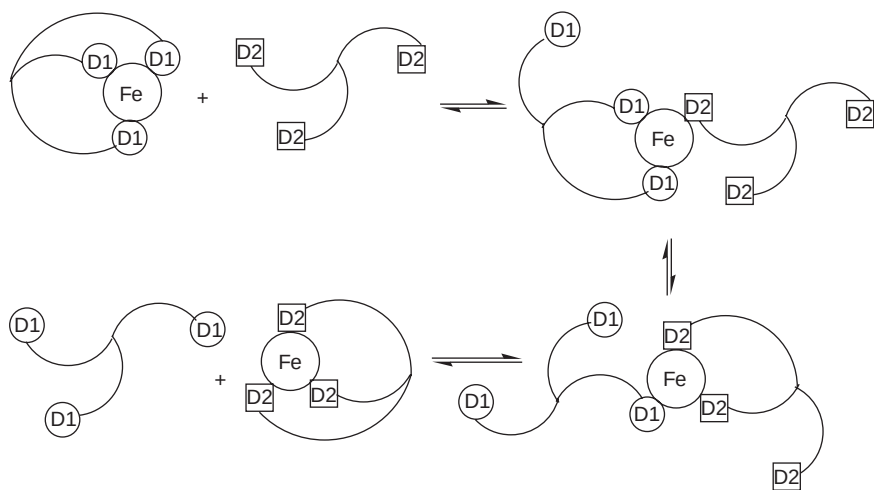


FIG. 26. Cartoon illustrating the exchange of Fe(III) between two hexadentate chelators through a ternary complex intermediate.

V. Siderophore Recognition and Cellular Uptake

In order for siderophores to be effective transporters of iron in nature, they must be recognized by the target cell. This process generally involves the expression of specific receptor proteins that interact with the iron–siderophore complex and either release the iron or transport the ferri-siderophore complex across the membrane into the interior of the cell. Developing an understanding of the principles behind the recognition/uptake process of siderophore complexes in natural systems will enhance our understanding of iron metabolism and allow more effective development of synthetic siderophore mimics and antibiotic agents.

Structures of membrane receptor proteins have been difficult to determine and consequently there are few structures available. Because of this, it is difficult to characterize the structural features that are important for iron–siderophore complex recognition. However, a few structures are available including the ferric enterobactin receptor protein, FepA, the ferric pyochelin receptor, FptA, and the ferrichrome receptor, FhuA (152,192,193). The general structural features common among these three receptors are a β -barrel tertiary structure and the presence of a “plug,” or a globular domain, which presumably prevents passage into the cell until a conformational change is brought about through binding to the iron–siderophore complex.

Insights into siderophore structural features relevant to recognition can be obtained from comparative growth studies of siderophores with structural differences. An example is the case of the desferrioxamines B, D, and E, where desferrioxamine B (**4**) has been demonstrated to support microbial growth more efficiently than desferrioxamines D and E (194). Since a structural difference between these siderophores is the protonated pendant amine tail in ferrioxamine B, the amide tail moiety in ferrioxamine D, and the endocyclic structure in ferrioxamine E, it has been proposed that the protonated pendant amine tail plays a role in the recognition process, which results in the higher growth efficiency for ferrioxamine B (195). The available structures of the tris-hydroxamate desferrioxamines show a *N-cis,cis* enantiomeric conformation where the coordinating CO bonds are all facing in the same direction, producing a trigonal carbonyl face in the octahedral coordination sphere of iron(III) (Fig. 27) (195–197). It has been proposed that this face also plays a role in the cellular recognition of ferric tris-hydroxamate complexes (195).

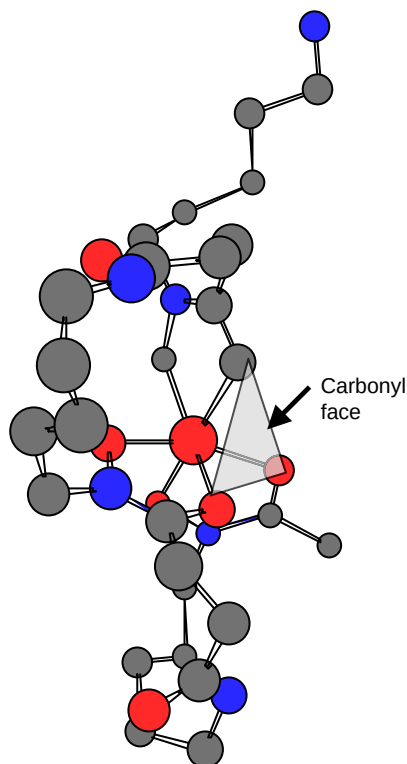


FIG. 27. Stereochemistry observed for ferrioxamine B resulting in a “carbonyl face” for the inner coordination shell of Fe(III) that may play a role in recognition. Legend: dark grey = O atom; light grey = N atom; black = Fe atom; white = C atom. Figure based on X-ray crystal structure and adapted from Fig. 4b in Ref. (195) and used with kind permission of Springer Science & Business Media.

Gaspar *et al.* report the growth promotion of a series of bis- and tris-hydroxamate siderophore mimics in *Escherichia coli* mutated to be deficient in some siderophore receptor proteins (120). They found that the bis-hydroxamate siderophores promoted the most growth of all of the siderophore models. The siderophore model with the highest growth promotion was DOCYDMAHA, and it was proposed that this was due to the amide portion of the central ring system, which might aid in interacting with the involved receptor proteins. PIPDMAHA and APM-3,4-HOPY, a 3,4-hydroxypyridinone, were also found to effectively promote growth of a variety of mutants, suggesting that the two siderophores are taken up via multiple pathways. Based on their results, it was suggested that the hydroxypyridinone APM-3,

4-HOPY and the bis-hydroxamate PIPDMAHA used FoxA and FhuA receptors, while the bis-hydroxamate DOCYDMAHA could potentially enter through a number of receptor proteins.

A series of model siderophore molecular recognition studies coupled with host–guest carrier facilitated model membrane transport studies was reported (198–202). Three approaches were taken which incorporate: (i) second coordination shell host–guest complexation, (ii) ternary complex formation, and (iii) a combination of ternary complex – second coordination shell host–guest complex formation. Examples of these approaches are described below.

Siderophore–ionophore supramolecular assembly formation via host–guest complexation of the pendant protonated amine arm of ferrioxamine B has been confirmed by X-ray crystallography (Fig. 28) (203). The stability and selectivity of this interaction as a function of ionophore structure, metal ion identity, and counter anion identity were determined by liquid–liquid extraction, isothermal calorimetry, and MS (204–211). Second-sphere host–guest complexation constants fall in the range 10^3 – 10^6 M^{−1} in CHCl₃ and methanol depending on ionophore structure.

Second coordination shell complexation of hydrophilic ferrioxamine B produces a hydrophobic supramolecular siderophore–ionophore assembly. The hydrophobic characteristic in concert

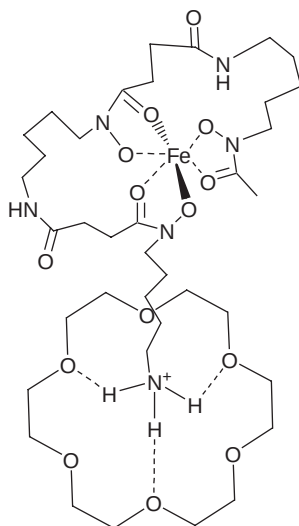


FIG. 28. Host–guest complex formed between ferrioxamine B (4) and 18-crown-6 crown ether; an ionophore–siderophore supramolecular assembly.

with labile host-guest complexation/dissociation and a moderate association constant lead to selective bulk liquid membrane (BLM) transport of the hydrophilic ferrioxamine B (212,213). The flux for ionophore carrier facilitated BLM transport of ferrioxamine B based on second coordination shell host-guest complexation was found to vary with host carrier structure. Use of an ionizable ionophore, lasalocid A, permits the operation of a ferrioxamine B active transport system driven by a pH gradient or the presence of desferrioxamine B on the interior side of the membrane (214).

BLM transport systems for ferrioxamine B were also devised based on first coordination shell recognition via ternary complex formation utilizing vacant coordination sites on the Fe(III) center (Fig. 29) (199). The tetra-coordinated substrate complex selectively transported was partially dechelated diaqua-ferrioxamine B and coordinately unsaturated di-hydroxamato iron(III) complexes, which utilized a hydrophobic membrane bound bidentate chelator as a carrier for selective transport. Active transport for these systems was accomplished using a pH gradient (199).

A combination first coordination shell-second coordination shell based recognition BLM transport system was devised, including active transport (200). This is based on a labile dihydroxamic acid system, including alcaligin, and a free lysine hydroxamic acid ligand capable of ternary complex formation to

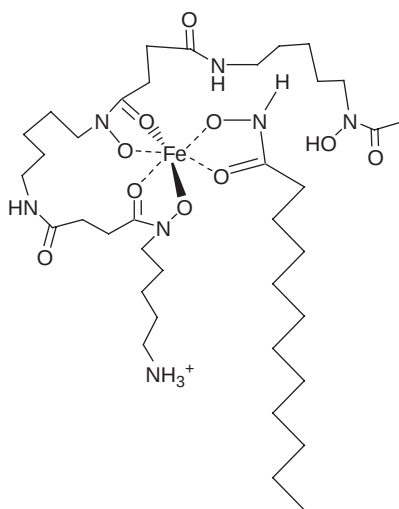


FIG. 29. Ternary complex formed between ferrioxamine B (4) and lauroyl hydroxamic acid.

afford the iron(III)–dihydroxamate complex a second coordination shell protonated amine recognition “handle.”

One paradigm for membrane transport of iron is the binding of the receptor protein to an iron-free siderophore molecule, followed by exchange of iron from an external ferri-siderophore to the receptor bound iron-free siderophore, and subsequent transfer across the cellular membrane. This shuttle mechanism has been explored in the transport system of ferric pyoverdine in *P. aeruginosa* (215,216). It is unclear why the bacterial system behaves in this manner, but mutagenesis studies of the protein suggest that residues involved in the closure of the β -barrel will not interact in the same way with the iron-free siderophore as they do with the ferri-siderophore. A similar mechanism has been suggested for *A. hydrophila* and *E. coli* (182).

The recognition of siderophores in many biological systems is also stereospecific, as binding to the receptor is promoted frequently by a certain configuration of the binding groups or the siderophore (217). This is demonstrated by the receptors from a variety of bacteria, including the rhodotorulic acid transporter in *Rhodotorula pilmanae* and the enterobactin receptor in a number of bacteria (218–220). Enantiomeric preference lends to the specificity of the receptor proteins, as the architecture of many siderophores will contribute to the formation of the least sterically constrained enantiomer in solution. This has been observed in the case of the desferrioxamines, as desferrioxamine B (4) as noted above maintains a *N-cis,cis* conformation in the solid state, despite the possibility of 16 conformations available to the tris-hydroxamate siderophore complex (195).

VI. Applications and Future Directions in Siderophore Research

The use of iron chelators with high stability and specificity can be linked to the study of iron–siderophore chemistry, as an understanding of the structural and architectural features microbes use to obtain iron from their environment can be used to design synthetic siderophore-like molecules for chelating iron in biological and engineered systems. Design of molecules with high binding affinity for iron and with high bioavailability, and that are not biologically active in the case of iron overload therapeutic agents, or are biologically active in the case of antimicrobial siderophores, requires an intimate knowledge of the binding and iron uptake mechanisms of a variety of biological systems. The design of synthetic iron chelators and their applications in medical treatments of diseases is covered in previous reviews (221–223).

A. IRON CHELATION THERAPY

Genetic disorders resulting in the mutation of hemoglobin, such as β -thalassemia and sickle cell, can result in anemia, for which a treatment is frequent blood transfusions. An indirect result of these blood transfusions is a high concentration of non-transferrin bound iron (NTBI) in the body, which over time can accumulate in organs, such as the liver (224,225). Accumulation of NTBI will cause oxidative damage to the organs and, after an average of 20 years, results in death of the patient (226). Treatment with small molecule chelating agents can aid the body in removal of this excess iron (227,228). An understanding of iron-siderophore and siderophore mimic interactions has been a guiding principle in the development of chelators for the treatment of iron overload.

Current chelation therapy treatments for iron overload that have been approved for clinical use by the US FDA are DesferalTM, or desferrioxamine B mesylate salt (**4**), and ExjadeTM, or deferasirox (**62**) (229,230). A third treatment that has been approved for use in Europe is DeferiproneTM, or 1,2-dimethyl-3-hydroxy-4-pyridinone (**50**, Fig. 30) (231). However, there are problems associated with all three of these treatments. Desferrioxamine B, a tris-hydroxamate siderophore (**4**), has low bioavailability and must be administered through frequent subcutaneous transfusions. Treatments are long, painful, and expensive, resulting in low user compliance rates. While Exjade has a higher bioavailability than desferrioxamine B and can be administered orally, its structure features a variety of binding groups, which are not specific for binding iron. This can

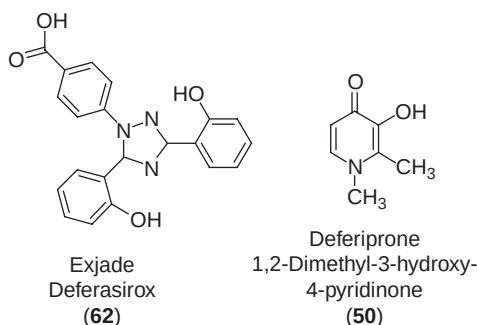


FIG. 30. Chemical structures of two siderophore mimic molecules presently in clinical use for treatment of iron overload, Exjade and Deferiprone.

potentially lead to binding of other metal ions that may result in undesirable side effects (232). Deferiprone (**50**) is a bidentate iron chelator, a hydroxypyridinone, which has high and specific affinity for iron and is administered orally. However, concerns have been raised regarding its toxicity and possible links between its use and diseases such as hepatic fibrosis, casting doubts on its efficacy (233,234). Future development of chelators for use as therapeutic agents in the treatment of iron overload diseases will likely rely on our understanding of iron–siderophore chemistry and biology.

Another potential use of iron chelators in biological systems is as antimicrobial agents. Siderophores and siderophore-like molecules can act as antimicrobial agents by multiple routes. The ability of bacteria to uptake iron has been linked to the virulence of some species of bacteria. If a chelating agent can compete effectively with bacterial siderophores for iron and not be taken up by the bacterial cell receptors, the bacterium will be deprived of essential iron, resulting in death (37,235). Another route of depriving the bacterium of iron is to use a siderophore mimic that will bind to the siderophore uptake protein, but not be taken up itself, preventing the bacterial siderophores from being transported across the cell membrane (19,37). A third route of antimicrobial activity is the attachment of an antimicrobial agent to a siderophore backbone that will bind iron and then be taken up by the bacterium. Once inside the cell, the molecule will release iron and undergo a reaction to activate its antimicrobial activity, killing the cell. This is known as the “Trojan Horse” approach (37,236).

Iron chelators can also be used to selectively bind iron in areas where oxidative stress is observed, thereby preventing the iron from taking part in Fenton reactions without interfering with normal iron homeostasis. Charkoudian *et al.* have developed boronic acid and boronic ester masked prochelators, which do not bind metals unless exposed to hydrogen peroxide (237,238). The binding of these chelators to iron(III) prevents redox cycling. Similar studies of these systems have been performed by a separate group (239,240).

B. SIDEROCALIN AND EVASION BY SIDEROPHORES

Pathogenic bacteria often make use of siderophores to obtain iron from their host organisms, and consequently the iron-binding capacity of the siderophore is closely related to the bacterial virulence. Enterobactin (**1**) is the natural siderophore

of a variety of pathogenic bacteria, and the high stability of its ferric complex (Table IA) makes it a particularly effective virulence factor. However, the biological activity of enterobactin may be neutralized in the presence of serum albumin, which decreases its effectiveness in human serum (241). As a defense mechanism, many mammals have developed a protein, siderocalin, a lipocalin-type protein that specifically binds siderophores, such as enterobactin and carboxymycobactins, and prevents their use as an iron delivery agent by the bacterium (242–244).

To counter the neutralization of siderophores by the host, some bacteria have evolved the ability to produce siderophores with structural variations that allow evasion of host defense mechanisms. One such example is the C-glucosylated analog of enterobactin, the salmochelin family (**63** in Fig. 31) (245). The glucosylated donor groups prevent recognition of the siderophore by siderocalin due to increased steric interactions, allowing its utilization by the bacteria. It is presumed that while the glucosylation will affect the sterics of the iron complex, it will have a minimal effect on the iron sequestering ability of the siderophore, due to the placement of the glucosyl substituents on the position 5-carbons of the catechol groups.

Another bacterial strategy of siderocalin evasion is the use of 3,4-catecholate donor groups in the structure of the siderophore. This is evidenced by the inability of siderocalin to recognize petrobactin (**64** in Fig. 31) or its ferric complex (246,247). Petrobactin is one of two major siderophores produced by *Bacillus anthracis*, the other being bacillibactin (**14**). Bacillibactin is recognized by siderocalin, due to its structural similarity to enterobactin, and the ability of petrobactin to evade binding by siderocalin makes it a major virulence factor of *B. anthracis*. The major forces that enable the recognition of siderophores are: (i) electrostatic, where the negatively charged complex is attracted to the positively charged binding pocket of siderocalin and (ii) cation- π interactions between the binding pocket and the siderophore. Since the petrobactin complex is negatively charged, both of these factors likely play a role in recognition, although another structural factor may be involved, such as the presence of 3,4-catechol groups (248).

C. FUTURE RESEARCH IN SIDEROPHORE COORDINATION CHEMISTRY

As research continues into the field of siderophore production and iron uptake, the knowledge gained will be invaluable to the development of medical treatments for bacterial infection,

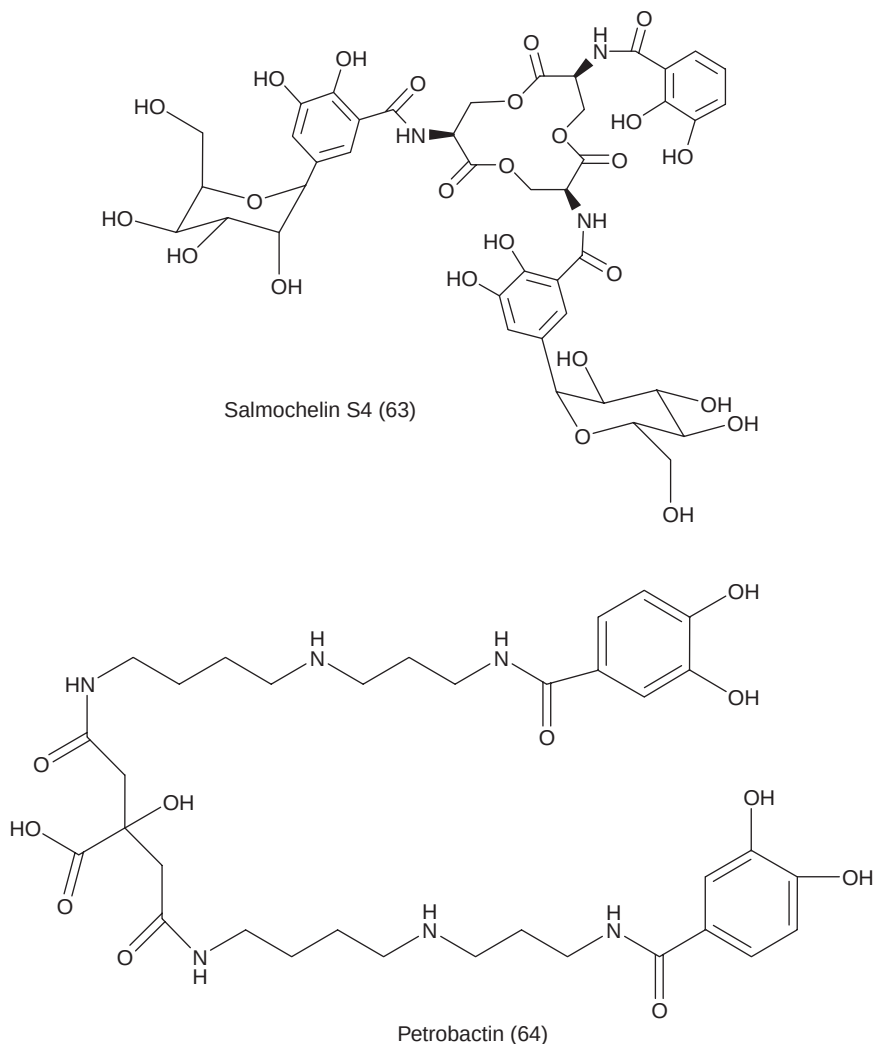


FIG. 31. Bacterial siderophores that evade recognition by siderocalin, salmochelin S4, and petrobactin.

iron overload, iron deficiency, and cancer (e.g., desferrioxamine B is currently used as an anticancer agent, as well as a chelation therapy agent) (228,249,250). An understanding of the chelator structural factors that result in selectivity for iron, as well as the formation of more stable iron complexes, while still making use of bioavailable chelating agents, may allow treatments to become less expensive, less painful, and more convenient, which may enhance compliance. An understanding

of the uptake systems used in bacterial and fungal organisms can be applied to the development of therapeutic agents as the uptake mechanism in bacteria is related to the virulence of the organism.

There is currently expanding interest in the interaction of siderophores with metals other than iron. Due to their chemical structures, siderophores have a strong affinity for high charge-to-radius ratio hard metal cations. The biological significance of siderophore interactions with manganese and molybdenum has been explored (251–254). Siderophores can play a role in the environmental distribution of radioactive actinides, such as Pu(IV) (255–257). Such studies aim to develop an understanding of the speciation of radioactive nuclides in the environment. They also give useful information regarding the toxicity of these nuclides in the presence of natural siderophores. Siderophores have recently also been reported to sequester boron in the ocean (258). Siderophore mimics featuring hydroxypyridinone and terephthalamide donor groups loaded with gadolinium ion are being explored for application as MRI agents (259–261).

While much work has been done to gain an understanding of the iron coordination chemistry involved with siderophore binding, much remains to be done with respect to characterizing the uptake mechanisms in a variety of organisms, for which the receptor proteins and their mode of action have not yet been clarified. Microbial growth studies, receptor protein crystal structures, and mutagenesis experiments will aid in elucidating microbial iron transport systems, while studies of the coordination chemistry of iron in siderophores and siderophore mimics will aid in expanding our knowledge of the chemistry and biology of siderophore complexes.

List of Structure Numbers, Figures, and Abbreviations

Structure number	Figures	Names
1	2, 9	Enterobactin
2	2	Aerobactin
3	2	Rhodotorulic acid
4	2	Desferrioxamine B
5	2	Neurosporin
6	2	Ferrichrome
7	2	Mycobactin J
8	2	Cepabactin

Structure number	Figures	Names
9	6	H ₆ L _A ²³⁴
10	6	H ₆ L _B ²³⁴
11	6	H ₃ L _R ²³⁴
12	6	H ₃ L _N ²³⁶
13	9	SERSAM
14	9	Bacillibactin
15	9	SER(3M)SAM
16	9	HOPObactin
17	10	TRENCAM
18	10	TRENGlyCAM
19	10	TRENbAlaCAM
20	10	TRENSerCAM
21	10	TRENGluCAM
22	10	TRENLysCAM
23	12	TRENSAM
24	12	TREN(3M)SAM
25	12	N-TRENSEX
26	12	O-TRENSEX
27	12	TREN-Me-3,2-HOPO
28	13	ETAM
29	13	taurTAM
30	13	TAMmeg
31	13	DEATAM
32	13	DMETAM
33	15	3-IPHA
34	15	4-IPHA
35	15	5-IPHA
36	15	6-IPHA
37	15	2-MHA
38	15	4-MHA
39	15	6-MHA
40	15	8-MHA
41	16	2,2-DIHA
42	16	2,3-DIHA
43	16	2,4-DIHA
44	16	2,5-DIHA
45	16	3,4-DIHA
46	17	3,3-DIHA
47	17	1H2P
48	17	3H4P
49	17	MHP

Structure number	Figures	Names
50	17, 30	DMHP, deferiprone
51	17	DEHP
52	17	EMHP
53	17	PMHP
54	17	3H2P
55	17	MH2P
56	17	EH2P
57	17	DH2P
58	18	N ² (prLH) ₂
59	18	N ³ (LH) ₃
60	22	Pyochelin
61	22	Fe(pyochelin) (cepabactin)
62	30	Exjade, Deferasirox
63	31	Salmoachelin S4
64	31	Petrobactin

ACKNOWLEDGMENTS

We thank our coworkers and collaborators, whose names appear in the references, for their intellectual and experimental contributions to the work described here. We also thank the National Science Foundation (CHE 0418006 and CHE 0809466) for their support of our research.

REFERENCES

1. Crichton, R. *"Inorganic Biochemistry of Iron Metabolism: From Molecular Mechanisms to Clinical Consequences"*; 2nd edn. Wiley: Chichester, **2001**.
2. Baes, C. J.; Mesmer, R. C. *"The Hydrolysis of Cations"*; Wiley: New York, **1976**.
3. Boukhalfa, H.; Crumbliss, A. L. *Biomaterials* **2002**, *15*, 325.
4. Dhungana, S.; Crumbliss, A. L. *Geomicrobiol. J.* **2005**, *22*, 87.
5. Chipperfield, J. R.; Ratledge, C. *Biomaterials* **2000**, *13*, 165.
6. Fenton, H. J. H. *J. Chem. Soc. Trans.* **1894**, *65*, 899.
7. Pierre, J. L.; Fontecave, M. *Biomaterials* **1999**, *12*, 195.
8. Pierre, J. L.; Fontecave, M.; Crichton, R. R. *Biomaterials* **2002**, *15*, 341.
9. Raymond, K. N.; Dertz, E. A. In: *"Iron Transport in Bacteria"*; Eds. Crosa, J. H.; Rey, A. R.; Payne, S. M.; ASM Press: Washington, D.C., **2004**, p. 3.

10. Albrecht-Gary, A. M.; Crumbliss, A. L. In: "Metal Ions in Biological Systems"; Eds. Sigel, A.; Sigel, H.; Marcel Dekker Inc.: New York, **1998**, p. 234.
11. Mietzner, T. A.; Tencza, S. B.; Adhikari, P.; Vaughan, K. G.; Nowalk, A. J. *Curr. Top. Microbiol. Immunol.* **1998**, *225*, 113.
12. Clarke, E. T.; Tari, L. W.; Vogel, H. J. *Curr. Top. Med. Chem.* **2001**, *1*, 7.
13. Kraemer, S. M. *Aquat. Sci.* **2004**, *66*, 3.
14. Hersman, L. E. In: "Environmental Microbe-Metal Interactions"; Ed. Lovely, D. R.; ASM Press: Washington, D.C., **2000**, p. 145.
15. Dhungana, S.; Anthony, C. R., III; Hersman, L. E. *Appl. Environ. Microbiol.* **2007**, *73*, 3428.
16. Farkas, E.; Enyedy, E. A.; Zekany, L.; Deak, G. J. *Inorg. Biochem.* **2001**, *83*, 107.
17. Farkas, E.; Enyedy, E. A.; Fábíán, I. *Inorg. Chem. Commun.* **2003**, *6*, 131.
18. Dhungana, S.; Anthony, C. R., III; Hersman, L. E. *Geochim. Cosmochim. Acta* **2007**, *71*, 5651.
19. Miethke, M.; Marahel, M. A. *Microbiol. Mol. Biol. Rev.* **2007**, *71*, 413.
20. Stintzi, A.; Raymond, K. N. In: "Molecular and Cellular Iron Transport"; Ed. Templeton, D. M.; Marcel Dekker Inc.: New York, **2002**, p. 273.
21. Meyer, M.; Trowitzsch-Kienast, W. *Rec. Res. Devel. Phys. Chem.* **2004**, *7*, 127.
22. Crumbliss, A. L. In: "Handbook of Microbial Chelates"; Ed. Winkelmann, G.; CRC Press: Boca Raton, FL, **1991**, p. 177.
23. Matzanke, B. F.; Müller-Matzanke, C.; Raymond, K. N. In: "Physical Bioinorganic Chemistry Series: Iron Carriers and Iron Proteins"; Ed. Loehr, T. M.; VCH Publishers: New York, **1989**, p. 3.
24. Neilands, J. B. *J. Biol. Chem.* **1995**, *270*, 26723.
25. Raymond, K. N.; Telford, J. R. In: "NATO ASI Series C, Mathematical and Physical Sciences"; vol. 459; Ed. Kessissoglous, D. P.; Kluwer Academic Publishers: Dordrecht, The Netherlands, **1995**, p. 25.
26. Winkelmann, G. "Handbook of Microbial Iron Chelates"; CRC Press: Boca Raton, FL, **1991**.
27. Ratledge, C.; Dover, L. G. *Annu. Rev. Microbiol.* **2000**, *54*, 881.
28. Philpott, C. C. *Biochim. Biophys. Acta* **2006**, *1763*, 636.
29. Grass, G. *Biomaterials* **2006**, *19*, 159.
30. Butler, A.; Martin, J. D. In: "Metal Ions in Biological Systems"; vol. 44; Eds. Sigel, A.; Sigel, H.; Marcel Dekker, Inc.: New York, **2005**, p. 21.
31. Kraemer, S. M.; Butler, A.; Borer, P.; Cervini-Silva, J. *Rev. Mineral. Geochem.* **2005**, *59*, 53.
32. Wandersman, C.; Delepelaire, P. *Ann. Rev. Microbiol.* **2004**, *58*, 611.
33. Dertz, E. A.; Raymond, K. N. In: "Comprehensive Coordination Chemistry II"; vol. 8; Eds. McCleverty, J. A.; Meyer, T. J.; Elsevier, Ltd: Oxford, UK, **2004**, p. 141.
34. Winkelmann, G. In: "Iron Transport in Bacteria"; Eds. Crosa, J. H.; Rey, A. R.; Payne, S. M.; ASM Press: Washington, D.C., **2004**, p. 437.
35. Haas, H. *Appl. Microbiol. Biotech.* **2003**, *62*, 316.
36. Renshaw, J. C.; Robson, G. D.; Trinci, A. P. J.; Wiebe, M. G.; Livens, F. R.; Collison, D.; Taylor, R. J. *Mycol. Res.* **2002**, *106*, 1123.
37. Roosenberg, J. M., II; Lin, Y. M.; Lu, Y.; Miller, M. J. *Curr. Med. Chem.* **2000**, *7*, 159.
38. Granger, J.; Price, N. M. *Limnol. Oceanogr.* **1999**, *44*, 541.
39. Dreschel, H.; Winkelmann, G. In: "Transition Metals in Microbial Metabolism"; Eds. Winkelmann, G.; Carrano, C. J.; Harwood Academic Publishing: Amsterdam, **1997**, p. 1.

40. Telford, J. R.; Raymond, K. N. In: "Comprehensive Supramolecular Chemistry"; vol. 1; Ed. Gokel, G. W.; Elsevier: Oxford, UK, **1996**, p. 245.
41. Harrington, J. M.; Crumbliss, A. L. *Biometals* **2009**, doi: 10.1007/s10847-009-9536-7.
42. Crumbliss, A. L.; Garrison, J. M. *Comments Inorg. Chem.* **1988**, 8, 1.
43. Pearson, R. G. "Chemical Hardness"; Wiley-VCH: Weinheim, Germany, **1997**.
44. McDougall, G. J.; Hancock, R. D.; Boeyens, J. C. A. *J. Chem. Soc. Dalton Trans.* **1977**, 1438.
45. Scarrow, R. C.; Riley, P. E.; Abu-Dari, K.; White, D. L.; Raymond, K. N. *Inorg. Chem.* **1985**, 24, 954.
46. Thomas, M. S. *Biometals* **2007**, 20, 431.
47. Luo, M.; Fadeev, E. A.; Groves, J. I. *Nat. Chem. Biol.* **2005**, 1, 149.
48. Spasojević, I.; Armstrong, S. K.; Brickman, T. J.; Crumbliss, A. L. *Inorg. Chem.* **1999**, 38, 449.
49. Hou, Z.; Raymond, K. N.; O'Sullivan, B.; Esker, T. W.; Nishio, T. *Inorg. Chem.* **1998**, 37, 6630.
50. Schwarzenbach, G.; *Helv. Chim. Acta* **1963**, 46, 1390.
51. Carrano, C. J.; Cooper, S. R.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, 101, 599.
52. Caudle, M. T.; Stevens, R. D.; Crumbliss, A. L. *Inorg. Chem.* **1994**, 33, 6111.
53. Harris, W. R.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, 101, 2722.
54. Valdebenito, M.; Crumbliss, A. L.; Winkelmann, G.; Hantke, K. *Int. J. Med. Microbiol.* **2006**, 296, 513.
55. Meloun, M.; Havel, J.; Hogfeldt, E. "Computation of Solution Equilibria"; Halstead Press: New York, **1988**.
56. Martell, A. E.; Motekaitis, R. J. "Determination and Use of Stability Constants"; 2nd edn. VCH: New York, **1992**.
57. Martell, A. E.; Smith, R. M. "Critical Stability Constants. vol. 6; Plenum: New York, **1989**.
58. Dhungana, S.; Harrington, J. M.; Gebhardt, P.; Möllmann, U.; Crumbliss, A. L. *Inorg. Chem.* **2007**, 46, 8362.
59. Dhungana, S.; Miller, M. J.; Dong, L.; Ratledge, C.; Crumbliss, A. L. *J. Am. Chem. Soc.* **2003**, 125, 7654.
60. Dertz, E. A.; Xu, J.; Stintzi, A.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, 128, 22.
61. Loomis, L. D.; Raymond, K. N. *Inorg. Chem.* **1991**, 30, 906.
62. Wong, G. B.; Kappel, M. J.; Raymond, K. N.; Matzanke, B.; Winkelmann, G. *J. Am. Chem. Soc.* **1983**, 105, 810.
63. Enyedy, E. A.; Posci, I.; Farkas, E. *J. Inorg. Biochem.* **2004**, 98, 1957.
64. Anderegg, G.; L'Eplattenier, F.; Schwarzenbach, G. *Helv. Chim. Acta* **1963**, 46, 1409.
65. Raymond, K. N.; Müller, G.; Matzanke, B. F. In: "Topics in Current Chemistry"; vol. 123; Ed. Boschke, F. L.; Springer Berlin: Heidelberg, **1984**.
66. Konetschny-Rapp, S.; Jung, G.; Raymond, K. N.; Miewes, K. N.; Zahner, H. *J. Am. Chem. Soc.* **1992**, 114, 2224.
67. Dhungana, S.; Ratledge, C.; Crumbliss, A. L. *Inorg. Chem.* **2004**, 43, 6274.
68. Murakimi, T.; Ise, K.; Hayakawa, M.; Kamei, S.; Takagi, S. *Chem. Lett.* **1989**, 18, 2137.

69. Carrano, C. J.; Dreschel, H.; Kaiser, D.; Jung, G.; Matzanke, B.; Winkelmann, G.; Rochel, N.; Albrecht-Gary, A. M. *Inorg. Chem.* **1996**, *35*, 6429.
70. Hou, Z.; Sunderland, C. J.; Nishio, T.; Raymond, K. N. *J. Am. Chem. Soc.* **1996**, *118*, 5148.
71. Küpper, F. C.; Carrano, C. J.; Kuhn, J. U.; Butler, A. *Inorg. Chem.* **2006**, *45*, 6028.
72. Holt, P. D.; Reid, R. R.; Lewis, B. L.; Luther, G. W., III; Butler, A. *Inorg. Chem.* **2005**, *44*, 7671.
73. Palanché, T.; Blanc, S.; Hennard, C.; Abdallah, M. A.; Albrecht-Gary, A. M. *Inorg. Chem.* **2004**, *43*, 1137.
74. Langemann, K.; Heineke, D.; Rupprecht, S.; Raymond, K. N. *Inorg. Chem.* **1996**, *35*, 5663.
75. Neilands, J. B.; Peterson, T.; Leong, S. A. *ACS Symp. Ser.* **1980**, *140*, 263.
76. Albrecht-Gary, A. M.; Blanc, S.; Ockatan, A. Z.; Abdallah, M. A. *Inorg. Chem.* **1994**, *32*, 6391.
77. Boukhalfa, H.; Reilly, S. D.; Michalczyk, R.; Iver, S.; Neu, M. P. *Inorg. Chem.* **2006**, *45*, 5607.
78. MacCordick, H. J.; Schleiffer, J. J.; Duplatre, G. *Radiochim. Acta* **1985**, *38*, 43.
79. du Moulinet d'Hardemare, A.; Serratrice, G.; Pierre, J.-L. *Biometals* **2004**, *17*, 691.
80. Perry, R. D.; Balbo, P. B.; Jones, H. A. *Microbiology* **1999**, *145*, 1181.
81. Garrett, T. M.; McMurray, T. J.; Hosseini, M. W.; Reyes, Z. E.; Hahn, F. E.; Raymond, K. N. *J. Am. Chem. Soc.* **1991**, *113*, 2965.
82. Dertz, E. A.; Xu, J.; Raymond, K. N. *Inorg. Chem.* **2006**, *45*, 5465.
83. Albrecht-Gary, A. M.; Blanc, S.; Biaso, F.; Thomas, F.; Baret, P.; Gellon, G.; Pierre, J.-L.; Serratrice, G. *Eur. J. Inorg. Chem.* **2003**, 2596.
84. Raymond, K. N. *Pure Appl. Chem.* **1994**, *66*, 773.
85. Stack, T. D. P.; Hou, Z.; Raymond, K. N. *J. Am. Chem. Soc.* **1993**, *115*, 6466.
86. Harris, W. R.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6534.
87. Dhungana, S.; Heggemann, S.; Heinisch, L.; Möllmann, U.; Boukhalfa, H.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 7079.
88. Hou, Z.; Whisenhunt, J. D. W.; Xu, J.; Raymond, K. N. *J. Am. Chem. Soc.* **1994**, *116*, 840.
89. Ng, C. Y.; Rodgers, S. J.; Raymond, K. N. *Inorg. Chem.* **1989**, *28*, 2062.
90. Matsumoto, K.; Ozawa, T.; Jitsukawa, K.; Masuda, H. *Inorg. Chem.* **2004**, *43*, 8538.
91. Dhungana, S.; Heggemann, S.; Gebhardt, P.; Möllmann, U.; Crumbliss, A. L. *Inorg. Chem.* **2003**, *42*, 42.
92. Meyer, M.; Telford, J. R.; Cohen, S. M.; White, D. J.; Xu, J.; Raymond, K. N. *J. Am. Chem. Soc.* **1997**, *119*, 10093.
93. Harrington, J. M.; Dhungana, S.; Chittamuru, S.; Jacobs, H. K.; Gopalan, A. S.; Crumbliss, A. L. *manuscript in preparation*.
94. Hajela, S. P.; Johnson, A. R.; Xu, J.; Sunderland, C. J.; Cohen, S. M.; Caulder, D. L.; Raymond, K. N. *Inorg. Chem.* **2001**, *40*, 3208.
95. Xu, J.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2002**, *41*, 6731.
96. Abergel, R. J.; Raymond, K. N. *Inorg. Chem.* **2006**, *45*, 3622.
97. Serratrice, G.; Mourral, C.; Zeghli, A.; Beguin, C. G.; Baret, P.; Pierre, J.-L. *New J. Chem.* **1994**, *18*, 749.

98. Baret, P.; Beguin, C. G.; Boukhalfa, H.; Caris, C.; Laulhere, J. P.; Pierre, J.-L.; Serratrice, G. *J. Am. Chem. Soc.* **1995**, *117*, 9760.
99. Serratrice, G.; Boukhalfa, H.; Beguin, C.; Baret, P.; Caris, C.; Pierre, J.-L. *Inorg. Chem.* **1997**, *36*, 3898.
100. Abergel, R. J.; Warner, J. A.; Shuh, D. K.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, *128*, 8920.
101. Jurchen, K. M. C.; Raymond, K. N. *Inorg. Chem.* **2006**, *45*, 1078.
102. Mies, K. A.; Gebhardt, P.; Möllmann, U.; Crumbliss, A. L. *J. Inorg. Biochem.* **2008**, *102*, 850.
103. Jurchen, K. M. C.; Raymond, K. N. *J. Coord. Chem.* **2005**, *58*, 55.
104. Cohen, S. M.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2000**, *39*, 4339.
105. Nguyen-van-Duong, M. K.; Guillot, V.; Nicolas, L.; Gaudemer, A.; Lowry, L.; Spasojević, I.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 5948.
106. Barclay, S. J.; Huynh, B. H.; Raymond, K. N. *Inorg. Chem.* **1984**, *23*, 2011.
107. Farkas, E.; Buglyo, P.; Enyedy, E. A.; Gerlei, V. A.; Santos, A. M. *Inorg. Chim. Acta* **2002**, *339*, 215.
108. Gaspar, M.; Telo, J. P.; Santos, M. A. *Eur. J. Inorg. Chem.* **2003**, 4025.
109. Persmark, M.; Nielands, J. B. *Biometals* **1992**, *5*, 29.
110. Charkoudian, L. K.; Franz, K. J. *Inorg. Chem.* **2006**, *45*, 3657.
111. Khodr, H. H.; Hider, R. C.; Duhme-Klair, A.-K. *J. Biol. Inorg. Chem.* **2002**, *7*, 891.
112. Caudle, M. T.; Crumbliss, A. L. *Inorg. Chem.* **1994**, *33*, 4077.
113. Wirgau, J. I.; Spasojević, I.; Boukhalfa, H.; Batinić-Haberle, I.; Crumbliss, A. L. *Inorg. Chem.* **2002**, *41*, 1464.
114. Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1992**, *196*, 185.
115. Dobbin, P. S.; Hider, R. C.; Hass, A. D.; Taylor, P. D.; Sargon, P.; Porter, J. B.; Xiao, G.; van der Helm, D. *J. Med. Chem.* **1993**, *34*, 1749.
116. Motekaitis, R. J.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *183*, 71.
117. Ma, R.; Reibenspies, J. J.; Martell, A. E. *Inorg. Chim. Acta* **1994**, *223*, 21.
118. Streator, M.; Taylor, P. D.; Hider, R. C. *J. Med. Chem.* **1990**, *33*, 1749.
119. Grazina, R.; Pinto, M.; Rodrigues, E.; Santos, M. A. "In XII Encontro Galego Portugues de Quimica"; Porto: Portugal, **1998**.
120. Gaspar, M.; Santos, M. A.; Krauter, K.; Winkelmann, G. *Biometals* **1999**, *12*, 209.
121. Van Horn, J. D.; Gramer, C. J.; O'Sullivan, B.; Jurchen, K. M. C.; Doble, D. M. J.; Raymond, K. N. *C. R. Chimie* **2002**, *5*, 395.
122. Jurchen, K. M. C.; Raymond, K. N. *Inorg. Chem.* **2006**, *45*, 2438.
123. Hay, B. P.; Hancock, R. D. *Coord. Chem. Rev.* **2001**, *212*, 61.
124. Hay, B. P.; Dixon, D. A.; Vargas, R.; Garza, J.; Raymond, K. N. *Inorg. Chem.* **2001**, *40*, 3922.
125. Gebhardt, P.; Crumbliss, A. L.; Miller, M. J.; Möllmann, U. *Biometals* **2008**, *21*, 41.
126. Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1991**, *30*, 1554.
127. Caudle, M. T.; Caldwell, C. D.; Crumbliss, A. L. *Inorg. Chim. Acta* **1995**, *240*, 519.
128. Spasojević, I.; Boukhalfa, H.; Stevens, R. D.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 49.
129. Farkas, E.; Buglyo, P.; Enyedy, E. A.; Santos, A. M. *Inorg. Chim. Acta* **2004**, *357*, 2451.
130. Cohen, A. R. *Hematology* **2006**, 42–47.
131. Barchini, E.; Cowart, R. E. *Arch. Microbiol.* **1996**, *166*, 51.
132. Greenwood, K. T.; Luke, R. K. *J. Biochem. Biophys. Acta* **1978**, *525*, 209.

133. Matzanke, B. F.; Anemuller, S.; Schunemann, V.; Trautwein, A. X.; Hantke, K. *Biochemistry* **2004**, *43*, 1386.
134. Richens, D. T. *Chem. Rev.* **2005**, *106*, 1961.
135. Wilkins, R. G. "Kinetics and Mechanism of Reactions of Transition Metal Complexes"; 2nd edn. VCH: Weinheim, Germany, **1991**.
136. Cooper, S. R.; McArdle, J. V.; Raymond, K. N. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 3551.
137. Lee, C. W.; Ecker, D. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1985**, *107*, 6920.
138. Pecoraro, V. L.; Harris, W. P.; Wong, G. B.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1983**, *105*, 4623.
139. Rodgers, S. J.; Lee, C. W.; Ng, C. Y.; Raymond, K. N. *Inorg. Chem.* **1987**, *26*, 1622.
140. O'Brien, I. G.; Cox, G. B.; Gibson, F. *Biochem. Biophys. Acta* **1971**, *237*, 537.
141. Helman, R.; Lawrence, G. D. *Biochem. Bioenerg.* **1989**, *22*, 187.
142. Wawrousek, E. F.; McArdle, J. V. *J. Inorg. Biochem.* **1982**, *15*, 169.
143. Adjimani, J. P.; Emery, T. *J. Bacteriol.* **1987**, *169*, 3664.
144. Sugiura, Y.; Nomoto, K. *Struct. Bond.* **1984**, *58*, 107.
145. Lewis, B. L.; Holt, P. D.; Taylor, S. W.; Wilhelm, S. W.; Trick, C. G.; Butler, A.; Luther, G. W., III *Marine Chem.* **1995**, *50*, 179.
146. Robinson, J. P.; McArdle, J. V. *J. Inorg. Nucl. Chem.* **1981**, *43*, 1951.
147. Bar-Ness, E.; Chen, Y.; Hadar, Y.; Marschner, H.; Romheld, V. In: "Iron Nutritional Interest in Plants"; Eds. Chen, Y.; Hadar, Y.; Kluwer: Dordrecht, the Netherlands, **1991**.
148. El-Jammal, A.; Templeton, D. M. *Inorg. Chim. Acta* **1996**, *245*, 199.
149. Mies, K. A.; Wirgau, J. I.; Crumbliss, A. L. *Biometals* **2006**, *19*, 115.
150. Weber, G.; von Wiren, N.; Hayen, H. *Biometals* **2008**, *21*, 503–513.
151. Klumpp, C.; Burger, A.; Mislin, M. L.; Abdallah, M. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1721.
152. Cobessi, D.; Celia, H.; Pattus, F. *J. Mol. Biol.* **2005**, *352*, 893.
153. Olmstead, E. G.; Harman, S. W.; Choo, P. L.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 5420.
154. Barbeau, K.; Rue, E. L.; Bruland, K. W.; Butler, A. *Nature* **2001**, *413*, 409.
155. Barbeau, K.; Rue, E. L.; Trick, C. G.; Bruland, K. W.; Butler, A. *Limnol. Oceanogr.* **2003**, *48*, 1069.
156. Henderson, R. A. "The Mechanisms of Reactions at Transition Metal Sites"; Oxford University Press: Oxford, **1994**.
157. Kazmi, S. A.; Shorter, A. L.; McArdle, J. V. *J. Inorg. Biochem.* **1982**, *17*, 269–281.
158. Kazmi, S. A.; Shorter, A. L.; McArdle, J. V. *Inorg. Chem.* **1984**, *23*, 4331–4332.
159. Kazmi, S. A.; Shorter, A. L.; McArdle, J. V. In: "New Trends in Natural Products Chemistry"; vol. 26; Eds. Atta-ur-Rahman, ; Le Quesne, P. W.; Elsevier Science Publishers: Amsterdam, **1986**.
160. Helm, L.; Merbach, A. E. *Chem. Rev.* **2005**, *105*, 1923.
161. Biruš, M.; Kujundžić, N.; Pribanić, M. *Prog. React. Kin.* **1993**, *18*, 171.
162. Swaddle, T. W.; Merbach, A. E. *Inorg. Chem.* **1981**, *20*, 4212.
163. Grant, M.; Jordan, R. B. *Inorg. Chem.* **1981**, *20*, 55.
164. Dodgen, H. W.; Liu, G.; Hunt, J. P. *Inorg. Chem.* **1981**, *20*, 1002.
165. Prue, J. E. *J. Chem. Ed.* **1969**, *46*, 12.
166. Mentasti, E.; Pelizzetti, E.; Saini, G. *J. Inorg. Nucl. Chem.* **1976**, *38*, 785.
167. Mentasti, E.; Pelizzetti, E. *J. Chem. Soc. Dalton Trans.* **1973**, 2605.

168. Fuoss, R. M. *J. Am. Chem. Soc.* **1958**, *80*, 5059.
169. Mentasti, E.; Secco, F.; Venturini, M. *Inorg. Chem.* **1982**, *21*, 2314.
170. Monzyk, B.; Crumbliss, A. L. *J. Am. Chem. Soc.* **1979**, *101*, 6203.
171. Biruš, M.; Bradić, Z.; Kujundžić, N.; Pribanić, M.; Wilkins, P. C.; Wilkins, R. G. *Inorg. Chem.* **1985**, *24*, 3980.
172. Monzyk, B.; Crumbliss, A. L. *J. Am. Chem. Soc.* **1982**, *104*, 4921.
173. Biruš, M.; Bradić, Z.; Krznarić, G.; Kujundžić, N.; Pribanić, M.; Wilkins, P. C.; Wilkins, R. G. *Inorg. Chem.* **1987**, *26*, 1000.
174. Biruš, M.; Bradić, Z.; Kujundžić, N.; Pribanić, M. *Inorg. Chem.* **1984**, *23*, 2170.
175. Spasojević, I.; Crumbliss, A. L. *manuscript in preparation*.
176. Serratrice, G.; Biaso, F.; Thomas, F.; Beguin, C. *Eur. J. Inorg. Chem.* **2004**, 1552.
177. Boukhalfa, H.; Crumbliss, A. L. *Inorg. Chem.* **2000**, *39*, 4318.
178. Boukhalfa, H.; Brickman, T. J.; Armstrong, S. K.; Crumbliss, A. L. *Inorg. Chem.* **2000**, *39*, 5591.
179. Scarrow, R. C.; White, D. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1985**, *107*, 6540.
180. Boukhalfa, H.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 4183.
181. Batinić-Haberle, I.; Spasojević, I.; Crumbliss, A. L. *Inorg. Chem.* **1994**, *33*, 3151.
182. Stintzi, A.; Barnes, C.; Xu, J.; Raymond, K. N. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 10691.
183. Dhungana, S.; Crumbliss, A. L. *CHEMTRACTS* **2001**, *14*, 258.
184. Schalk, I. J.; Kyslik, P.; Prome, D.; van Dorsselaer, A.; Poole, K.; Abdallah, M. A.; Patus, F. *Biochemistry* **1999**, *38*, 9357.
185. Margerum, D. W.; Cayley, G. R.; Weatherburn, D. C.; Pagenkopf, G. K. In: "Coordination Chemistry"; vol. 2; Ed. Martell, A. E.; American Chemical Society: Washington, D.C., **1978**, p. 1.
186. Katoh, A.; Akiyama, M. *J. Chem. Soc. Perkin Trans.* **1991**, 1839.
187. Hara, Y.; Akiyama, M. *Inorg. Chem.* **1996**, *35*, 5173.
188. Akiyama, M.; Katoh, A.; Iijima, M.; Takagi, T.; Natori, K.; Kojima, T. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1356.
189. Turcot, I.; Stintzi, A.; Xu, J.; Raymond, K. N. *J. Biol. Inorg. Chem.* **2000**, *5*, 634.
190. Harris, W. R.; Carrano, C. J.; Cooper, S. R.; Sofen, S. R.; Avdeef, A.; McArdle, J. V.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 6097.
191. Serratrice, G.; Biaso, F.; Pierre, J.-L.; Blanc, S.; Albrecht-Gary, A.-M. *Eur. J. Inorg. Chem.* **2007**, 3681.
192. Ferguson, A. D.; Hofmann, E.; Coulton, J. W.; Diedrichs, K.; Welte, W. *Science* **1998**, *282*, 2215.
193. Locher, K. P.; Rees, B.; Koebnik, R.; Mitschler, A.; Moulinier, L.; Rosenbush, J. P.; Moras, D. *Cell* **1998**, *95*, 771.
194. Müller, G.; Raymond, K. N. *J. Bacteriol.* **1984**, *160*, 304.
195. Dhungana, S.; White, P. S.; Crumbliss, A. L. *J. Biol. Inorg. Chem.* **2001**, *6*, 810.
196. van der Helm, D.; Poling, M. *J. Am. Chem. Soc.* **1976**, *98*, 82.
197. Hossain, M. B.; Jalal, M. A. F.; van der Helm, D. *Acta Cryst. C* **1986**, *42*, 1305.
198. Spasojević, I.; Batinić-Haberle, I.; Choo, P. L.; Crumbliss, A. L. *J. Am. Chem. Soc.* **1994**, *116*, 5714.
199. Wirgau, J. I.; Crumbliss, A. L. *Inorg. Chem.* **2003**, *42*, 5762.
200. Wirgau, J. I.; Crumbliss, A. L. *J. Chem. Soc. Dalton Trans.* **2003**, 3680.

201. Trzaska, S. M.; Kim, M.; Bartsch, R. A.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 5823.
202. Crumbliss, A. L.; Batinić-Haberle, I.; Caldwell, C.; Dunkel, R.; Hammels, D. E.; Spasojević, I. *J. Inorg. Biochem.* **1995**, *59*, 114.
203. Dhungana, S.; White, P. S.; Crumbliss, A. L. *J. Am. Chem. Soc.* **2003**, *125*, 14760.
204. Batinić-Haberle, I.; Crumbliss, A. L. *Inorg. Chem.* **1995**, *34*, 928.
205. Trzaska, S. M.; Toone, E. J.; Crumbliss, A. L. *Inorg. Chem.* **2000**, *39*, 1071.
206. Batinić-Haberle, I.; Spasojević, I.; Bartsch, R. A.; Crumbliss, A. L. *J. Chem. Soc. Dalton Trans.* **1995**, 2503.
207. Batinić-Haberle, I.; Spasojević, I.; Crumbliss, A. L. *Inorg. Chem.* **1996**, *35*, 2352.
208. Crumbliss, A. L.; Batinić-Haberle, I.; Spasojević, I. *Pure Appl. Chem.* **1996**, *68*, 1225.
209. Batinić-Haberle, I.; Spasojević, I.; Jang, Y.; Bartsch, R. A.; Crumbliss, A. L. *Inorg. Chem.* **1998**, *37*, 1438.
210. Batinić-Haberle, I.; Spasojević, I.; Crumbliss, A. L. *Inorg. Chim. Acta* **1997**, *260*, 35.
211. Tristani, E. M.; Dubay, G. R.; Crumbliss, A. L. *J. Incl. Phenom.* **2009**, doi: 10.1007/s10534-009-9233-4.
212. Spasojević, I.; Crumbliss, A. L. *J. Chem. Soc. Dalton Trans.* **1998**, 4021.
213. Caldwell, C. D.; Crumbliss, A. L. *Inorg. Chem.* **1998**, *37*, 1906.
214. Spasojević, I.; Crumbliss, A. L. *Inorg. Chem.* **1999**, *38*, 3248.
215. Nader, M.; Dobbelaere, W.; Vincent, M.; Journet, L.; Adams, H.; Cobessi, D.; Gallay, J.; Schalk, I. J. *Biochemistry* **2007**, *46*, 11707.
216. Greenwald, J.; Hoegy, F.; Nader, M.; Journet, L.; Mislin, G. L. A.; Graumann, P. L.; Schalk, I. J. *J. Biol. Chem.* **2006**, *282*, 2987.
217. Winkelman, G.; Hushka, H. In: *"Iron Transport in Microbes, Plants and Animals"*; Eds. Winkelman, G.; van der Helm, D.; Nielands, J. B.; VCH: Weinheim, Germany, **1987**, p. 317.
218. Müller, G.; Isowa, Y.; K.N., R. *J. Biol. Chem.* **1985**, *260*, 13921.
219. Winkelman, G. *FEBS Lett.* **1979**, *97*, 43.
220. Winkelman, G.; Braun, V. *FEMS Microbiol. Lett.* **1981**, *11*, 237.
221. Liu, Z. D.; Hider, R. C. In: *"Molecular and Cellular Iron Transport"*; Ed. Templeton, D. M.; Marcel Dekker, Inc.: New York, **2002**, p. 321.
222. Richardson, D. R. *Crit. Rev. Oncol./Hematol.* **2002**, *42*, 267.
223. Liu, Z. D.; Hider, R. C. *Coord. Chem. Rev.* **2002**, *232*, 151.
224. Deugnier, Y.; Turlin, B. *World J. Gastroenterol.* **2007**, *13*, 4755.
225. Porter, J. B. *Am. J. Haematol.* **2007**, *82*, 1136.
226. Borgna-Pignatti, C.; Rugolotto, S.; De Stefano, P.; Zhao, H.; Cappellini, M. D.; Del Vecchio, G. C.; Romeo, M. A.; Forni, G. L.; Gamberini, M. R.; Ghilardi, R.; Piga, A.; Cnaan, A. *Haematologica* **2004**, *89*, 1187.
227. Bernhardt, P. V. *Dalton Trans.* **2007**, 3214.
228. Faa, G.; Crisponi, G. *Coord. Chem. Rev.* **1999**, *184*, 291.
229. Jacobs, A. J. In: *"Development of Iron Chelators for Clinical Use"*; Eds. Martell, A. E.; Anderson, W. F.; Badman, D. B.; Elsevier: North Holland, **1981**, p. 29.
230. Vichinsky, E. *Am. J. Haematol.* **2008**, *83*, 398.
231. Galanello, R. *Ther. Clin. Risk Manag.* **2007**, *3*, 795.
232. Kontoghiorghes, G. J. *Expert Opin. Drug Saf.* **2007**, *6*, 235.
233. Olivieri, N. F.; Brittenham, G. M.; McLaren, C. E.; Templeton, D. M.; Cameron, R. G.; McClelland, R. A.; Burt, A. D.; Fleming, K. A. *N. Eng. J. Med.* **1998**, *339*, 417.

234. Wu, S. F.; Peng, C. T.; Wu, K. H.; Tsai, C. H. *Hemoglobin* **2006**, 30, 215.
235. Jones, R. L.; Gray, R. W. *Eur. J. Clin. Microbiol.* **1983**, 2, 411.
236. Kline, T.; Fromhold, M.; McKennon, T. E.; Cai, S.; Treiberg, J.; Ihle, N.; Sherman, D.; Schwan, W.; Hickey, M. J.; Warrenner, P.; Witte, P. R.; Brody, L. L.; Goltry, L.; Barker, L. M.; Anderson, S. U.; Tanaka, S. K.; Shawar, R. M.; Nguyen, L. Y.; Langhorne, M.; Bigelow, A.; Embuscado, L.; Naeemi, E. *Bioorg. Med. Chem.* **2000**, 8, 73.
237. Charkoudian, L. K.; Pham, D. M.; Kwon, A. M.; Vangeloff, A. D.; Franz, K. J. *Dalton Trans.* **2007**, 5031.
238. Charkoudian, L. K.; Pham, D. M.; Franz, K. J. *J. Am. Chem. Soc.* **2006**, 128, 12424.
239. Wei, Y.; Guo, M. *Angew. Chem. Int. Ed.* **2007**, 46, 4722.
240. Wei, Y.; Guo, M. *Angew. Chem. Int. Ed.* **2007**, 46, 6948.
241. Konopka, K.; Niellands, J. B. *Biochemistry* **1984**, 23, 2122.
242. Holmes, M. A.; Paulsene, W.; Jide, X.; Ratledge, C.; Strong, R. K. *Structure* **2005**, 13, 29.
243. Goetz, D. H.; Holmes, M. A.; Borregaard, N.; Bluhm, M. E.; Raymond, K. N.; Strong, R. K. *Mol. Cell* **2002**, 10, 1033.
244. Borregaard, N.; Cowland, J. B. *Biometals* **2006**, 19, 211.
245. Fischbach, M. A.; Lin, H.; Zhou, L.; Yu, Y.; Abergel, R. J.; Liu, D. R.; Raymond, K. N.; Wanner, B. L.; Strong, R. K.; Walsh, C. T.; Aderem, A.; Smith, K. D. *Proc. Natl. Acad. Sci. USA* **2006**, 103, 16502.
246. Abergel, R. J.; Wilson, M. K.; Arceneaux, J. E. L.; Hoette, T. M.; Strong, R. K.; Byers, B. R.; Raymond, K. N. *Proc. Natl. Acad. Sci. USA* **2006**, 103, 18499.
247. Abergel, R. J.; Moore, E. G.; Strong, R. K.; Raymond, K. N. *J. Am. Chem. Soc.* **2006**, 128, 10998.
248. Wilson, M. K.; Abergel, R. J.; Raymond, K. N.; Arceneaux, J. E. L.; Byers, B. R. *Biochem. Biophys. Res. Comm.* **2006**, 348, 320.
249. Buss, J. L.; Greene, B. T.; Turner, J.; Torti, F. M.; Torti, S. V. *Curr. Top. Med. Chem.* **2004**, 4, 1623.
250. Gupta, A.; Crumbliss, A. L. *J. Lab. Clin. Med.* **2000**, 136, 371.
251. Parker, D. L.; Sposito, G.; Tebo, B. M. *Geochim. Cosmochim. Acta* **2004**, 68, 4809.
252. Parker, D. L.; Morita, T.; Mozafarzadeh, M. L.; Verity, R.; McCarthy, J. K.; Tebo, B. M. *Geochim. Cosmochim. Acta* **2007**, 71, 5672.
253. Bellenger, J. P.; Wichard, T.; Kustka, A. B.; Kraepiel, A. M. L. *Nat. Geosci.* **2008**, 1, 243.
254. Bellenger, J. P.; Arnaud-Neu, F.; Asfari, Z.; Myeni, S. C. B.; Stiefel, E. I.; Kraepiel, A. M. L. *J. Biol. Inorg. Chem.* **2007**, 12, 367.
255. Boukhalfa, H.; Reilly, S. D.; Neu, M. P. *Inorg. Chem.* **2007**, 46, 1018.
256. Boukhalfa, H.; Reilly, S. D.; Smith, W. H.; Neu, M. P. *Inorg. Chem.* **2004**, 43, 5816.
257. Neu, M. P.; Icopini, G. A.; Boukhalfa, H. *Radiochim. Acta* **2005**, 93, 705.
258. Harris, W. R.; Amin, S. A.; Kuepper, F. C.; Green, D. H.; Carrano, C. J. *J. Am. Chem. Soc.* **2007**, 129, 12263.
259. Jocher, C. J.; Botta, M.; Avedano, S.; Moore, E. G.; Xu, J.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2007**, 46, 4796.
260. Pierre, V. C.; Botta, M.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2006**, 45, 8355.
261. Pierre, J. L.; Melchior, M.; Doble, D. M. J.; Raymond, K. N. *Inorg. Chem.* **2004**, 43, 8520.

CALCIUM IN BIOLOGICAL SYSTEMS

JOHN BURGESS and EMMA RAVEN

Department of Chemistry, University of Leicester, Leicester LE1 7RH, United Kingdom

I.	Introduction	252
A.	General	252
B.	Calcium in Biology	253
C.	Monitoring Calcium	257
D.	Structures, Coordination Numbers, and Stereochemistry	262
II.	The Ca^{2+} Aqua-Cation	268
A.	Introduction	268
B.	The Nature of Ca^{2+}aq	269
C.	Stability Constants	273
D.	Kinetics and Mechanisms	283
III.	Calcium-Binding Metalloproteins	289
A.	General	289
B.	Structures and Calcium-Binding	292
C.	Stability Constants	299
D.	Kinetics	302
E.	Ca^{2+} in Blood Clotting	307
IV.	Ca^{2+} in Ionophores, Channels, and Pumps	308
A.	Trans-Membrane Transport	308
B.	Ionophores	309
C.	Calcium Channels	313
D.	Calcium Pumps	315
V.	Carbohydrates and Carboxylates	316
A.	Structures	316
B.	Stability Constants	319
VI.	Phosphates, Phosphonates, Nucleosides, and Nucleotides	321
A.	Introduction	321
B.	Structures	322
C.	Stability Constants	322
D.	Kinetic Aspects	326
VII.	Stability Constants: An Overview	326
VIII.	Calcium-Containing Rigid Materials	326
A.	Introduction	326
B.	Salts Soluble and Insoluble	328
C.	Bones and Teeth	332
D.	Calcium-Linked Disorders	334
	Ligand Abbreviations	338

Acknowledgments	339
References	339
Appendix: The Term Ligand	365

I. Introduction

A. GENERAL

This review¹ deals with calcium in biological systems, in particular its interaction with its immediate environment. It also deals with various aspects of the inorganic chemistry of calcium which are of relevance to the numerous and highly important roles of this element in biological systems – what might more accurately but unacceptably inelegantly called “Biorelevant Calcium Chemistry”. In keeping with the title of this volume we have devoted a considerable proportion of the contents to kinetic aspects and to stability constants. The latter are particularly relevant to the former for calcium, since the very rapid formation reactions characteristic of Ca^{2+} mean that stabilities are often determined by dissociation rates. However, we have also devoted major sections to the structures of the biochemical entities whose kinetic behavior and stabilities we discuss, and indeed to their place in the overall scheme of calcium in biological systems. The great advances in recent years in the knowledge of the part played by calcium in biology indicate that the importance of calcium is probably second only to that of iron amongst the metallic elements. We have tried to give some idea of the range of roles of calcium in biological systems, to highlight areas of current interest and activity, and to place these in their context. We have assumed a readership of inorganic chemists and of biochemists who are aware of the importance of calcium in biology but who do not have detailed knowledge of this area on the borders of chemistry and biochemistry. We have cited key references to seminal research and to significant earlier papers and reviews as well as references from recent literature, so that interested readers can access context and fundamental aspects as well as current research.

¹Values given for stability constants, rate constants, and other temperature- and medium-dependent parameters refer to 298 K and ionic strength of 0.1 M (mol dm^{-3}) or slightly higher unless stated otherwise; logarithms of stability constants, $\log K$, are to base 10, with concentrations on the molar scale. Abbreviations that appear only once are generally defined at the appropriate point in the text; abbreviations that appear more than once are listed and defined at the end of this Chapter.

B. CALCIUM IN BIOLOGY

B.1. Introduction

Calcium plays an important role in a great variety of biological compounds, processes, and systems (1,2), occurring in mammals, plants, and a number of living species in the oceans. It regulates many key biochemical and physiological processes, such as enzymic reactions, muscle action, blood coagulation, and secretion. Its compounds are central to conferring rigidity and to maintaining membranes. In vertebrates, the majority of the calcium is in the skeleton. Adult humans have 1 to 1.5 kg in their skeleton and only a few grams elsewhere in the body. However, the relatively small amounts in cells and body fluids are of enormous importance in a multitude of ways. It is this latter small amount which is responsible for such functions as controlling muscle and nerve action, regulating cell division, buffering blood pH, and triggering hormone release. The resting intracellular concentration of Ca^{2+} in mammals is in the region of 0.01–0.05 μM , rising towards 1 mM on stimulation. In blood plasma the concentration is about 3 mM, some as Ca^{2+}aq , some complexed by organic anions such as citrate, and some protein-bound, for example by albumin. In human milk, the concentration of Ca^{2+} is about 8 mM.

B.2. Rigid materials

Many extended rigid materials of biological importance, especially in endo- and exo-skeletons, have a major inorganic content (3–5). These include materials for frameworks, for protection, for feeding, and for detection and regulation – bones, shells, defensive spikes, teeth, and sensors (Table I). The most important and widespread are based on calcium carbonate or phosphates. Generally the inorganic material is incorporated into an organic matrix, as for instance in the combination of calcium phosphates with collagen in forming bone, or of calcium carbonate with the polysaccharide chitin in crab and lobster shells.

B.3. Structural effects

The previous paragraph stated the most obvious structural role of calcium. However Ca^{2+} , like several other ions, for example Zn^{2+} and K^{+} , is also important in stabilizing a range of less rigid biological structures, such as membranes, and determining and maintaining the secondary, tertiary, and quaternary structures

TABLE I

EXAMPLES OF INORGANIC MATERIALS WHICH CONFER FIRMNESS, STRUCTURE, AND RIGIDITY IN BIOLOGICAL SYSTEMS^a

Compound (mineral forms)	Examples of biological function or occurrence
Calcium carbonate (calcite, aragonite, vaterite)	Exoskeletons (mollusc and egg shells; coral)
Calcium hydroxyl phosphate (hydroxyapatite)	Bones and teeth
Calcium fluorophosphate (fluorapatite)	Brachiopod shells
Calcium oxalate (whewellite, weddellite)	Gallstones; plant defenses
Calcium sulphate (gypsum)	Gravity sensors
Strontium sulphate (celestite)	Exoskeletons of a few marine organisms
Barium sulphate (barytes)	Gravity sensors
Silica	Diatom valves; defence spikes
Iron (hydr)oxides	Teeth of chitons ^b

^aFrom Ref. (5); the solubilities of many of these compounds are given in Table XXI in Section VIII.B below.

^bChitons are marine molluscs with shells consisting of movable plates.

of large biomolecules such as polypeptides, proteins, and polynucleotides (RNA, DNA). The stereochemical requirements of the cation and cation–bioligand interactions play a key role in determining overall structure. Such structural stabilization may involve electrostatic interactions (complex formation) and, often, osmotic effects (many structured biomolecules are denatured, i.e. lose their active conformation, in deionized water). Nucleic acids are polyanions – associated cations are necessary to enable them to adopt compact structures through charge compensation, the cations shielding neighboring negatively charged phosphate groups from each other. Coordination of Ca^{2+} , or of Mg^{2+} , to carboxylate and phosphate substituents contributes to structural stabilization of membranes through the resulting cross-linking between sugar molecules. Structural stabilization in plants may also be promoted by Ca^{2+} and Mg^{2+} .

B.4. Calcium-containing metalloproteins

A large number of these (6,7) are involved in stabilization, activation, catalysis, triggering, regulation, and storage, as detailed in Section III.A. At one extreme Ca^{2+} acts simply as a structure stabilizer, as in its complexes with trypsin, with sugar adducts of D-galactose-binding protein (DGBP), and phospholipid adducts of annexins. Several metalloproteins contain more than one metal ion – sometimes more than one Ca^{2+} , sometimes Ca^{2+} and a second metal such as iron, copper, or zinc (see Section III.A).

Often the activating role of Ca^{2+} is purely structural – Ca^{2+} can activate a reaction site some distance from its binding site, transmitting its effect through the conformational changes in the protein caused by its binding. However there are several enzymes, generally catalyzing reactions involving phosphate in some form, in which Ca^{2+} plays a direct activating role.

The importance of calcium in signaling (8–10) was first adumbrated towards the end of the 19th century, when the importance of calcium in rat heart contraction was established (11). Early work on physiological responses to calcium was documented in 1956 (12). Often an extracellular primary messenger binds to a receptor on the outer surface of a cell and transmits its message across the cell wall to a secondary messenger, which in turn initiates a process within the cell – Ca^{2+} is an important and widespread secondary messenger. Its weak cation–solvent and cation–ligand interactions, with the consequent ease and rapidity of complex formation and dissociation make it eminently suitable for information transfer. Ca^{2+} as secondary messenger is responsible for triggering, *inter alios*, release of thyroxine, amylose, adrenalin, and pituitary hormone, secretion of acid in the stomach, and muscle contraction.

Calcium channel proteins control changes in concentration gradients across membranes; the opening and closing of these channels transmit signals for the regulation of cellular function, as for example in muscle and nerve action (13) or the secretion of insulin by the pancreas. Ca^{2+} is by far the most important metal ion regulator (14); it is itself regulated by a range of agents such as inositol-1,4,5-triphosphate, IP_3 (15,16), nicotinic acid adenine dinucleotide phosphate, NAADP (17), or sphingosine-1-phosphate, $\text{Me}(\text{CH}_2)_{12}\text{CH}=\text{CHCH}(\text{OH})\text{CH}(\text{NH}_3)\text{CH}_2\text{OPO}_3\text{H}_2$. The last-named is a calcium regulator in plants as well as in animal cells (18,19). Indeed an array of calcium-binding proteins regulates concentrations of calcium – parathyroid hormone enhances absorption of calcium from the gastrointestinal tract (GIT) and controls calcium excretion by the kidneys; calcitonin controls the release of calcium from bone. Calcium- and integrin-binding protein (CIB1) may be redox-regulated; it binds to reduced glutathione (20). Regulation is essential not only in relation to all the calcium-dependent processes within the cell, but is also essential to prevent the precipitation of calcium carbonate, oxalate, or phosphates in inappropriate places. The toxicity of some venoms, such as that of the black widow spider, is due to their effect on calcium regulation. Channel defects or malfunctions are the cause of many disorders, especially muscle dysfunction. Thus, for a recent example, defects in Ca^{2+}

homeostasis associated with voltage-gated Ca^{2+} channels cause the increase in neurotransmission and neurodegeneration which characterizes the progression of Huntington's disease (21). A dramatic malfunction of calcium regulation is the cause of malignant hyperthermia, which can be triggered by halothane anesthetics in patients with abnormal release channels and which has been introduced into pigs in the course of breeding for lean meat. Permanent channel opening causes a large release of calcium from muscle stores, prompting spasms and a marked rise in body temperature (22,23).

B.5. Calcium-carbohydrate species

The role played by Ca^{2+} in membrane stabilization was noted in [Section I.B.3](#) above. Calcium-carbohydrate interactions are important in the glycocalyx that covers the outer membrane of most types of cells; calcium-sugar complexes have long been known to be involved in cell adhesion (24). Chondroitin-4- and -6-sulfates, which are mucopolysaccharides (glycosaminoglycans), are abundant in soft and in skeletal connective tissue, endowing cartilage with flexibility; they are involved in cartilage to bone transformation. Chondroitin sulfates form water-soluble complexes with several M^{n+} at high pH, presumably by binding through their carboxylate groups (25). The ability of these sulfated polysaccharides to complex divalent cations, especially calcium, is an important biological function (26). Sialic acids (27,28) are constituents as terminal sugar moieties of numerous oligosaccharides, glycoproteins, and glycolipids, and thus important constituents of membranes and cell surfaces. They are *N*-acylneuraminic acids, which are mannose derivatives with carboxylate, $-\text{NHCOR}$, and $-\text{CHOHCHOHCH}_2\text{OH}$ groups attached. The glycerol moiety attached to the sugar ring is important for binding Ca^{2+} . Calcium-sugar interactions are also important in relation to lectins and their role in biological recognition and in the rejection of invading microorganisms.

Galactose is the major carbohydrate component of collagen, i.e. of bone. Lactose enhances absorption of Ca^{2+} from the GIT, presumably by forming a complex which can more easily cross the GIT wall. Many sugar acids and their derivatives are of biological importance, with their calcium salts often involved in biochemical systems. Thus D-glucuronic acid is present in some bacterial polysaccharides and D-glucuronolactone is found in plant gums and animal connective tissues. Sugar acids such as galactaric and glucaric acids have been used to model the

binding of ions to carboxylate-containing polysaccharides (see [Section V.A](#)).

Calcium-carbohydrate species and calcium salts of sugar-derived hydroxycarboxylic acids are also important in several areas of applied biochemistry. Thus, for example, the calcium-induced gelling of polysaccharides is important in the preparation of foodstuffs such as ice cream and preserves (29), while calcium alginate is used both as a food-thickener and as an arresting agent ("Sorbsan") for superficial hemorrhaging. Alginic acid is a polymannuronic acid, a hydrophilic, colloidal polysaccharide found, in association with calcium and magnesium, in the cell walls of seaweed.

C. MONITORING CALCIUM

The biological importance and roles of cations such as Ca^{2+} , Mg^{2+} , Na^+ , and K^+ were appreciated considerably more slowly than those of the transition metals, despite the demonstration as early as 1882 that Ca^{2+} was essential for heart function (*v.s.*). Only in recent decades has it become apparent that calcium may rival iron in the number and range of roles it plays in living systems. One of the main reasons for this tardiness in appreciating the roles of Group 1 and Group 2 cations in biology was the relative difficulty of detecting their participation and monitoring their concentrations, dynamics, and functions. They do not register in normal electronic (visible/ultraviolet) spectroscopy as their λ_{max} values are well below 200 nm, and are EPR-silent. ^{43}Ca has a natural abundance of only 0.13% and low sensitivity, making NMR monitoring difficult. So in the formative years of bioinorganic chemistry, and still in some areas today, the structure and function of biological species containing Ca^{2+} have been probed by replacing it by another cation, preferably of similar chemical properties, and monitoring the resultant analog by appropriate techniques. Replacement of Ca^{2+} reduces but often does not eliminate the biological activity of a calcium-binding protein. Structure determinations have shown that Ca^{2+} can be replaced isomorphously by Ln^{3+} in thermolysin and in parvalbumin, while it has been demonstrated that Ln^{3+} cations can activate, for example, aequorin, concanavalin A, or prothrombin, albeit to a limited extent. In structure determination by X-ray diffraction, the heavier cations Sr^{2+} , Ba^{2+} (30–32), or Ln^{3+} (31,33) – or even Hg^{2+} or UO_2^{2+} – have proved useful replacements. Sr^{2+} -for- Ca^{2+} substitution has also been assessed for the monitoring by secondary ion mass spectrometry (SIMS) of active mineralization in bones and teeth (see [Section VIII.C.2](#))

(34), but such substitution was found to be fraught with complications for probing calcium-induced calcium release (35). The use of Ba^{2+} as a probe for Ca^{2+} in assessing the permeability of calcium channels looks more promising (36). The use of luminescence probes such as Eu^{3+} or Tb^{3+} (37) and of paramagnetic transition-metal or lanthanide cations in NMR and EPR spectroscopy has proved considerably more valuable. Pr^{3+} can be used as a relaxation probe, Mn^{2+} or Gd^{3+} as a shift probe, in such NMR studies (38), while Mn^{2+} -for- Ca^{2+} substitution is useful for EPR. These approaches proved particularly useful in estimating interatomic distances (37,39). However with all these substitution techniques there must always be concerns that the favored coordination environment of the substituted cation may differ significantly from that of the Ca^{2+} , in ionic radius, coordination number, or stereochemistry. There can also be complications with proteins with more than one calcium-binding site, in that the substituting M^{n+} may occupy a different site or sites from Ca^{2+} . Thus Gd^{3+} is believed to occupy different sites from Ca^{2+} in bovine serum albumin (40), while even when a lanthanide Ln^{3+} ion binds at the same site as Ca^{2+} (as shown for, e.g., Tb^{3+} at troponin C (41)) its different geometrical requirements result in conformational change and a significant difference in coordinates (see also the case of thermolysin in [Section III.B](#) below). Higher lanthanides, which are smaller and thus cause less conformational change, are to be preferred to the earlier members of the $4f$ series (33).

Calcium-selective electrodes have long been in use for the estimation of calcium concentrations – early applications included their use in complexometric titrations, especially of calcium in the presence of magnesium (42). Subsequently they have found use in a variety of systems, particularly for determining stability constants. Examples include determinations for ligands such as chloride, nitrate, acetate, and malonate (mal) (43), several diazacrown ethers (44,45), and methyl aldofuranosides (46). Other applications have included the estimation of Ca^{2+} levels in blood plasma (47) and in human hair (where the results compared satisfactorily with those from neutron activation analysis) (48). Ion-selective electrodes based on carboxylic polyether ionophores are mentioned in [Section IV.B](#) below. Though calcium-selective electrodes are convenient they are not particularly sensitive, and have slow response times.

Spectrophotometric analysis and complexometric titrations have long been used to determine calcium, with much effort devoted to developing reagents and indicators that permit the

determination of calcium in the presence of other cations, especially magnesium. Many of these reagents and indicators are of the form $(\text{HO}_2\text{CCH}_2)_2\text{N}\cdots\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$, the parent compound with the spacer $\cdots = \text{CH}_2\text{CH}_2$ being the familiar ethylenediamine-*N,N,N',N'*-tetraacetic acid, edtaH_4 (49). The spacer $\cdots = (\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2$ gives ethylene glycol bis-(2-aminoethyl ether)tetraacetic acid, egtaH_4 , a particularly selective reagent for Ca^{2+} (Section II.C.6). These reagents, developed for classical analysis methods, are generally inappropriate for biological systems. For biochemical investigations, a range of sensitive and specific fluorescent indicators has been developed for calcium analysis and intracellular assay (50). These reagents generally have a fluorescent moiety fused to a calcium-chelating unit, as in long-established quin-2 = 2-[[2-[bis(carboxymethyl)amino]-5-methylphenoxy]methyl]-6-methoxy-8-[bis(carboxymethyl)amino]quinoline), which exhibits a UV shift and an enhancement of fluorescence on binding to Ca^{2+} (51). Many reagents use a Ca^{2+} -chelating unit based on ethylene glycol bis-(2-aminoethyl ether)-tetraacetate (egta), for example the series of appropriately substituted derivatives of 1,2-bis-(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (baptaH_4), which is $(\text{HO}_2\text{CCH}_2)_2\text{N}\cdots\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$ with the spacer $\cdots$ being $\text{C}_6\text{H}_4\text{OCH}_2\text{CH}_2\text{OC}_6\text{H}_4$. Thus fura-1, fura-2 (52), and fura-3 (53) combine the eight-coordinating chelating site of bapta with stilbene chromophores. They have considerably improved fluorescence properties; fura-3 is particularly suited to long-term monitoring of Ca^{2+} in biological systems. Although they have a slightly lower Ca^{2+} affinity than quin-2 they give a marked wavelength shift, not just a modest change in intensity on binding Ca^{2+} . They are also more selective for Ca^{2+} vs. Zn^{2+} than quin-2 (54); there is currently some disagreement over suitable fluorescent probes for the analysis of this pair of ions (55). Fluo-1, fluo-2, fluo-3, rhod-1, rhod-2 all have a xanthene chromophore on bapta , to give fluorescein-like or rhodamine-like fluorophores (56); their somewhat lower affinity than the fura chelators for Ca^{2+} permits easier monitoring of transient concentrations. Fura-3 is an *N,N,O*-triacetate analog, again with a conveniently low stability constant. Rhod-5N is a nonfluorescent indicator which gives a fluorescent Ca^{2+} complex (57). Several of these fluorescence-monitoring chelators, such as fluo-3, fura-2, and fura-3, are commercially available, both as their potassium salts and as their acetoxymethyl ester derivatives (e. g. fura-2 AM *vice* fura-2) with $-\text{N}(\text{CH}_2\text{COCH}_2\text{OCOCH}_3)_2$ in place of $-\text{N}(\text{CH}_2\text{CO}_2^-)_2$. The relatively lipophilic acetoxymethyl ester derivatives show cell permeability, whereas the ionic forms are cell-impermeable; the

former undergo enzymatic hydrolysis within the cell to give the latter. The fluorescent probe KCM-1 has been developed for imaging both Ca^{2+} and Mg^{2+} in biological systems. It combines a coumarin fluorophore, a bapta moiety to provide a Ca^{2+} -selective binding site, and a β -diketone unit to bind Mg^{2+} (58). The calcium-binding protein aequorin, 2-(4'-hydroxyphenylacetyl)-amino-3-benzyl-5-(4'-hydroxyphenyl)pyrazine (59,60), has also been used as a fluorescent chelator to monitor Ca^{2+} concentrations. Stability constants for a few of these fluorescent ligands are given in Section II.C.3 below.

The development of genetically targeted fluorescent Ca^{2+} indicator proteins (FCIP) for probing structure and dynamics started in the early 1990s. Aequorin was the first Ca^{2+} indicator to be so targeted, but it exhibits rather feeble luminescence and is slowly attacked by Ca^{2+} . Next came the so-called "cameleons," such as Green Fluorescent Protein (GFP) attached to calmodulin (61). These indicators proved too bulky and too slow, so were superseded by analogs based on troponin C. Troponin fragments sandwiched between cyan fluorescent protein (CFP) as donor and citrine, a novel yellow fluorescent protein (62), as acceptor offered improved subcellular targetting and permitted dynamic imaging within live cells (63). However, these species still responded too slowly ($\tau_{1/2} = \text{e. g. } 142, 867 \text{ ms}$ for the double-exponential dissociation, whereas simple indicators can have $\tau < 40 \mu\text{s}$) and have been superseded by a species with $\tau < 1 \text{ ms}$ (64). The tailoring of the latest ligand, "Calcium Green FAsH" (Fluorescein Arsenical Hairpin binder) maximizes its properties by the use of the relatively low-affinity pentadentate chelator *o*-amino-phenyl-*N,N,O*-triacetic acid rather than octadentate bapta, with one of the carboxylates amidated (morpholine) to improve Ca^{2+} vs. Mg^{2+} selectivity. The chelator plus indicator is attached through its two arsenic atoms to a tetracysteine-containing motif in the peptide. Its response time is sufficiently short for monitoring rapid localized changes in calcium concentration in such situations as the mouth of a calcium channel or in fluxes within neurons (65). Another important requirement is to establish calcium concentrations in various regions of interest within a system, which may require the use of more than one indicator (66). The measurement of intracellular calcium concentrations has been extensively reviewed (440 references) (67), difficulties and artifacts arising in intracellular Ca^{2+} estimation by fluorescent chelators considered (68), and the importance of allowing for Ca^{2+} -indicator interactions in interpreting kinetic data obtained using such indicators emphasized (69). It is

interesting to note that classical CaF_2 gravimetric analysis is still in use in verifying novel analytical procedures, as for the recent ICP-MS determination for Ca^{2+} in fish otoliths (70).

Despite the drawbacks mentioned above, ^{43}Ca NMR spectroscopy, generally with considerable isotope enrichment, has occasionally been used to study systems of biological interest. Indeed the potential of this approach was demonstrated, for investigating ligand exchange through line broadening in the $\text{Ca}^{2+}/\text{ATP}$ system, as long ago as 1969 (71). Subsequently, the first ^{43}Ca NMR study of calcium-containing proteins suggested the calcium-binding sites in calmodulin, parvalbumin, and troponin C to have a comparatively rigid structure (72). Soon after this, kinetic parameters for exchange at calmodulin, troponin C, and phospholipase A2 were estimated from ^{43}Ca NMR spectra (73). ^{43}Ca NMR showed two types of sites in S100 proteins – nerve-specific members of the calmodulin and troponin C protein family containing numerous calcium-binding sites – with very different Ca^{2+} exchange rates (74). A few years ago it was shown that natural abundance ^{43}Ca magic-angle spinning (MAS) NMR could be carried out on solids of biogeochemical interest such as apatite, calcite, and perovskite (75), as well as on binary compounds (oxide, halides) and salts (sulfate, nitrate) (76), though data collection took some hours. Natural abundance ^{43}Ca NMR spectra have recently been published for biorelevant Ca salts such as citrate, gluconate, and oxalate (77).

Calcium has several radioactive isotopes. ^{37}Ca , ^{38}Ca , and ^{39}Ca all have half-lives of less than a second; ^{41}Ca has a half-life of 8×10^4 years. The radioisotopes which have proved useful in tracer studies of biological systems are ^{45}Ca (β^- ; $\tau_{1/2}$ 165 days) and, to a much smaller extent, ^{47}Ca (β^- , γ ; $\tau_{1/2}$ 4.5 days). Early applications, mainly concerned with studying Ca^{2+} -protein interactions using ^{45}Ca , have been conveniently documented (78). Both radioisotopes have been used to monitor calcium metabolism, for example in nutrition studies involving milk (79). ^{45}Ca has been used in a variety of other applications, for instance in the initial study of calsequestrin (80), to estimate the number of Ca^{2+} -binding sites in phospholipase A2 (81) and in prothrombin (82), to monitor calcium channels (83) and pumps (84), in investigations of Ca^{2+} -binding to mitochondrial glycoproteins (85), to prothrombin (86), to the phosphoglycoprotein phosphovitin (87), and to hemolysin (88), to probe accumulation of calcium in tumor cells (89), to monitor bisphosphonate treatment of malignancy-induced hypercalcemia (90), and to monitor crystal growth and calcium uptake in biomineralization (91).

D. STRUCTURES, COORDINATION NUMBERS, AND STEREOCHEMISTRY

D.1. General

Coordination numbers of Ca^{2+} range from six to twelve in inorganic compounds in the solid state, as documented in Table II. Most of the entries in this table are for compounds of long-established structures (92), but the structure of $[\text{Ca}(\text{XeF}_2)_5](\text{PF}_6)_2$ is recent (93). Table III (94–98) illustrates, using the example of the hydrates of calcium chloride, how small differences in composition can have a significant effect on coordination number and stereochemistry. Ca^{2+} exhibits coordination numbers of both seven and eight in anhydrous CaHPO_4 (99), in $\text{Ca}[\text{Ca}(\text{edta})] \cdot 7\text{H}_2\text{O}$ (detailed in Section I.D.4.c below), and in $\text{Ca}(\alpha\text{-memal})_2 \cdot 4\text{H}_2\text{O}$ (Section I.D.4.a); it exhibits coordination numbers of seven and nine in hydroxyapatite (100). Its highest coordination number in salts and salt hydrates appears to be ten, in $\text{Ca}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ (101), though it is 12-coordinated in perovskite. In complexes, as in

TABLE II

COORDINATION NUMBERS (CN) OF Ca^{2+} IN A SELECTION OF BINARY COMPOUNDS AND SALTS

Compound	CN of Ca^{2+}	Compound	CN of Ca^{2+}
CaO ; CaS	6	$\text{Ca}(\text{tartrate}) \cdot 4\text{H}_2\text{O}$	8
CaCO_3 (calcite)	6 ^a	CaH_2	9
$\text{CaNa}(\text{H}_2\text{PO}_2)_3$	6	$\text{Ca}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$	9
$\text{CaTiCl}_5 \cdot 7\text{H}_2\text{O}$	7	$[\text{Ca}(\text{XeF}_2)_5](\text{PF}_6)_2$	9
CaF_2	8 ^a	CaCO_3 (aragonite)	9 ^a
$\text{CaO}_2 \cdot 8\text{H}_2\text{O}$	8	$\text{Ca}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$	10
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	8	CaTiO_3 (perovskite)	12

^aCoordination numbers increase under high pressure, with calcite converting into aragonite and the coordination number of Ca^{2+} in fluorite increasing from 8 to 9 (PbCl_2 structure) (see pp. 326, 415 of Ref. (92)).

TABLE III

COORDINATION NUMBERS (CN) OF Ca^{2+} IN HYDRATES OF CALCIUM CHLORIDE

Compound	Structure	CN of Ca^{2+}	Donor set	Ref.
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	Layer	6	<i>trans</i> - Cl_4O_2	(94)
$\alpha\text{-CaCl}_2 \cdot 4\text{H}_2\text{O}$	Dimeric	7	Cl_3O_4	(95)
$\beta\text{-CaCl}_2 \cdot 4\text{H}_2\text{O}$	Chain	6; 8	Cl_6 ; ClO_7	(96)
$\gamma\text{-CaCl}_2 \cdot 4\text{H}_2\text{O}$	Molecular	6	<i>trans</i> - Cl_4O_2	(97)
$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	Chain	9	O_9	(98)

simple compounds, Ca^{2+} favors coordination numbers higher than six (though it is only six-coordinated in $\text{Cu}(\text{acac})_2 \cdot 3\text{H}_2\text{O}$ (102)), especially eight. However in complexes it does not attain the particularly high coordination numbers of 11 (103–105) or 12 (106) found in some Ba^{2+} complexes, and certainly not the formal coordination numbers of 12 (107) or 14 (108) for uranium(IV) or 15 for zirconium(IV) (109) – though admittedly these high numbers are only permitted by the particularly small H atoms of η^3 -coordinated BH_4^- ligands. At the other coordination extreme, Ca^{2+} is three-coordinate in $\text{Ca}(\text{N}[(\text{SiPh}_2^t\text{Bu})(\text{SiMe}_3)])_2 \cdot \text{THF}$ (110), such a low coordination number imposed by the bulkiness of the silicon-containing ligands, and in Ca_2N , which has the anti- CdCl_2 structure (111). It has been claimed that the average coordination number of Ca^{2+} in complexes which do not have bulky ligands is 7.2; the corresponding values for Mg^{2+} , Sr^{2+} , and Ba^{2+} are 6.0, 8.3, and 9.1 (105). Coordination numbers greater than six provide a mechanism, additional to the size differential, for the selection of Ca^{2+} in preference to Mg^{2+} , as the latter has a very strong preference for octahedral coordination. The coordination preferences of Ca^{2+} also distinguish this cation from the first-row transition-metal 2+ cations and from Fe^{3+} – though not from lanthanide cations such as Gd^{3+} . Ca–O bond distances for ligands such as carboxylate, alcohols, and water are collected in a review of complexes of Ca^{2+} – alongside Mg^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} analogs – which provide models for Ca^{2+} –protein complexes (112).

D.2. Oxides

Calcium in biological systems is normally bonded to oxygen-donor atoms. For this reason, and in the light of the range of coordination numbers and stereochemistries involved, it seemed appropriate to devote a section of this chapter to compounds of the simplest oxygen ligand, viz. oxide. Furthermore there is an interesting parallel between the calcium channels of biology and the calcium tunnels and channels of mixed oxides, particularly as in CaTa_2O_6 and CaMo_5O_8 (*v.i.*).

Calcium oxide, CaO , adopts the sodium chloride structure; the Ca^{2+} is thus octahedrally coordinated with six oxygen atoms. Calcium forms a wide range of mixed or ternary oxides, in which Ca^{2+} exhibits a wide variety of coordination numbers and geometries (92). This aspect of the solid-state chemistry of calcium reveals marked parallels with the variety of coordination numbers and geometries shown by Ca^{2+} in biological systems. CaTa_2O_6 contains Ca^{2+} ions in tunnels running through an assembly of TaO_6 octahedra sharing edges and vertices.

Each calcium has six oxygens at the corners of a slightly distorted trigonal prism, at Ca–O distances of 2.45–2.56 Å, and two oxygens above faces of the trigonal prism, at 2.43 and 2.57 Å – but this straightforward picture of eight-coordination is complicated by the presence of a ninth oxygen above the third face at 3.28 Å (113). Calcium aluminate, $\text{CaAl}_2\text{O}_4 \cdot 10\text{H}_2\text{O}$, one of the main components of high-alumina cement, has been much studied, by X-ray and neutron diffraction techniques (114). The calcium ions are eight-coordinated, with Ca–O distances between 2.4 and 2.6 Å. Several mixed oxides CaMO_4 (M = e.g. V, Fe, Ti) have similar structures and Ca^{2+} environments, but mixed oxides Ca_2MO_4 show a greater variety. Ca^{2+} is six-coordinated in Ca_2GeO_4 , whereas in Ca_2IrO_4 the IrO_6 octahedra share edges, with Ca^{2+} ions in three different types of site. These have coordination numbers 6, 7, and 9 – in this last case there are six oxygens at 2.37 Å, three at 2.75 Å; at the six- and seven-coordinate sites the Ca–O distances are all within the range 2.39–2.42 Å (115). Ca_3UO_6 has a perovskite-related structure ($\equiv \text{Ca}_2[\text{CaU}]\text{O}_6$), with a wide range of Ca–O and U–O distances (116) – the shortest U–O distances, 1.91 and 2.02 Å, are considerably longer than U–O ~ 1.80 Å in UO_2^{2+} . Structures of some mixed oxides change with pressure. Thus the coordination number of Ca^{2+} in Ca_2GeO_4 increases from 6 to 9 or 10 on increasing the pressure, while CaGeO_3 changes its form from wollastonite (6-coordination for the Ca^{2+}) through garnet (6- and 8-coordination) to perovskite (12-coordination) as the pressure is increased.

CaMo_5O_8 provides a link to calcium channels in biological systems. This mixed oxide contains Mo/O columns, of which groups of four define channels in which the Ca^{2+} cations reside. There is no clearly defined coordination number for these cations; Ca–O distances range from 2.39 Å upwards, there being ten oxygens at fairly regularly spaced distances up to 3.04 Å from the Ca^{2+} (117).

D.3. Crystal hydrates

The occurrence of the species $[\text{Ca}(\text{H}_2\text{O})_n]^{2+}$, $n = 6, 7, 8$, and 9, in crystal hydrates (118) is of particular relevance to the nature of Ca^{2+}aq in aqueous solution. Of these homoleptic species $[\text{Ca}(\text{H}_2\text{O})_8]^{2+}$ is the most common, occurring in square-antiprismatic form in, for example, $\text{CaO}_2 \cdot 8\text{H}_2\text{O}$, $\text{CaK}[\text{AsO}_4] \cdot 8\text{H}_2\text{O}$ (119), and $\text{CaI}_2 \cdot 6.5\text{H}_2\text{O}$ (120). The pairs of square-antiprismatic $[\text{Ca}(\text{H}_2\text{O})_8]^{2+}$ units in $\text{CaI}_2 \cdot 6.5\text{H}_2\text{O}$ are joined by triangular faces (Ca–O = 2.39 to 2.42 Å to terminal waters, 2.52–2.60 Å to

$\mu\text{-H}_2\text{O}$). The rarely found dodecahedral form of $[\text{Ca}(\text{H}_2\text{O})_8]^{2+}$ occurs in $\text{CaHgI}_4 \cdot 8\text{H}_2\text{O}$ (all $\text{Ca-O} = 2.49 \text{ \AA}$) (121) and in $2\text{CaCl}_2 \cdot 11\text{HgCl}_2 \cdot 16\text{H}_2\text{O}$ (122). The $[\text{Ca}(\text{H}_2\text{O})_7]^{2+}$ units in $\text{CaTiCl}_5 \cdot 7\text{H}_2\text{O}$ are square-capped trigonal prisms, with $\text{Ca-O} = 2.39, 2.40 \text{ \AA}$ to prism-corner waters, $2.45 \text{ \AA}$ to the capping water (123). The trihydrate of the calcium salt of the reluctant ligand methanedisulfonate contains seven-coordinate Ca^{2+} , with bidentate methanedisulfonate and five waters (two axial) in a pentagonal bipyramidal arrangement (124). A convenient summary of the extensive data available in 1993 on Ca-O distances in salt hydrates reports that for $[\text{Ca}(\text{H}_2\text{O})_n]^{2+}$, $n = 6, 7, 8$, and 9 , the mean Ca-O increases from 2.33 to $2.52 \text{ \AA}$ as n increases from 6 to 9 . For ternary systems $[\text{CaL}_m(\text{H}_2\text{O})_n]^{n+}$, the mean $\text{Ca-O}(\text{water})$ increases from 2.44 to $2.49 \text{ \AA}$ as the coordination number of the Ca^{2+} (CN) increases from 6 to 9 ; for $\text{CN} = 10$ the two available values are $2.40, 2.42 \text{ \AA}$. Also for $[\text{CaL}_m(\text{H}_2\text{O})_n]^{n+}$, the minimum $\text{Ca-O}(\text{water})$ distance is $2.30 \text{ \AA}$ ($\text{CN} = 6$), the maximum $2.64 \text{ \AA}$ ($\text{CN} = 8$) (118). A later review based on data assembled from the Cambridge Crystallography Database concluded that mean $\text{Ca-O}(\text{water})$ bond distances in compounds of relevance to protein chemistry increased from 2.38 for $\text{CN} = 6$ to $2.43 \text{ \AA}$ for $\text{CN} = 8$; a somewhat tidier picture for alcohol ligands showed mean $\text{Ca-O}(\text{alcohol})$ bond distances of $2.35, 2.42$, and $2.45 \text{ \AA}$ for $\text{CN} = 6, 7$, and 8 (112).

D.4. Carboxylate salts and complexes

a Dicarboxylates. A number of the α,ω -dicarboxylic acids, $[\text{O}_2\text{C}(\text{CH}_2)_n\text{CO}_2]^{2-}$, and of their calcium salts are of biological and pharmacological relevance (125,126). Oxalate ($n = 0$) occurs, often as its calcium salt (Section VIII.B.3 below), in a range of plant species, for example rhubarb, spinach, sorrel, and eucalypts. Oxalic acid is a minor product of animal metabolism; its calcium salt may occur in the urinary tract. Malonic ($n = 1$), glutaric ($n = 3$), and adipic ($n = 4$) are rarely found in nature, but all occur in sugar beet juice. Azelaic acid ($n = 7$) occurs in rancid fats. Thapsic acid ($n = 14$) occurs in the form of esters in plants such as *Thapsia garganica*, *Thuja occidentalis*, and *Juniperus sabina*. It has some therapeutic value, being a constituent of Oil of Savin, used variously for combating intestinal worms, for hastening menstrual flow, to induce sweating, and as an anti-rheumatic agent. It is also a constituent of herbal remedies derived from mullein (*Verbascum thapsus*), recommended for a variety of ailments. The cyclic analog cyclobutane-1,1-dicarboxylate is the ligand in the platinum anti-tumor agents

carboplatin, zeniplatin, and enloplatin. Calcium 7-oxabicycloheptane-2,3-dicarboxylate is a herbicide (Endothall) (127).

Both calcium oxalate monohydrate and dihydrate – whewellite and weddellite – contain eight-coordinated Ca^{2+} in distorted square-antiprismatic geometry, but there is one coordinated water in the former, two in the latter. Whewellite has a layer structure through the sharing of three edges of each coordination square-antiprism with three adjacent polyhedra; weddellite has a chain structure (128). The trihydrate also contains eight-coordinated Ca^{2+} (129).

In calcium malonate dihydrate the Ca^{2+} is eight-coordinate (bicapped trigonal prismatic (130)), but if this compound is crystallized from a gel then the crystals contain seven-coordinated Ca^{2+} in a pentagonal bipyramidal environment (131). Calcium bis(hydrogen malonate), $\text{Ca}(\text{O}_2\text{CCH}_2\text{CO}_2\text{H})_2$, crystallizes as a three-dimensional framework built up from face-sharing Ca_2O_{12} units. The Ca^{2+} ions are eight-coordinated, with the eight oxygens coming from six mals. The Ca–O distances to oxygens of bridging mals are 2.85 Å, the Ca–O distances to the other oxygens are normal, at 2.32–2.42 Å (132). Coordination numbers of Ca^{2+} in its α -methylmalonate and α -ethylmalonate salts, $\text{Ca}(\alpha\text{-memal})_2 \cdot 4\text{H}_2\text{O}$ (133) and $\text{Ca}(\alpha\text{-etmal})_2$ (134) are both seven and eight in the former, eight in the latter. In $\text{Ca}(\alpha\text{-etmal})_2$ each Ca^{2+} is coordinated by four monodentate and two bidentate ethylmalonate ligands, one of which binds through two oxygens of a single carboxylate, the other through one oxygen from each carboxylate. In calcium succinate trihydrate, $[\text{Ca}(\text{succ})(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O}$, the Ca^{2+} is eight-coordinate, with similar stereochemistry to the malonate dihydrate (*v.s.*) (135); Ca^{2+} in calcium fumarate trihydrate is also eight-coordinate (136). The dihydrate of the calcium salt of 7-oxabicycloheptane-2,3-dicarboxylic acid (Endothall) contains seven-coordinated Ca^{2+} (127).

b Hydroxycarboxylates. The Ca^{2+} in calcium tartrate tetrahydrate is at the center of a distorted dodecahedron of oxygens (137), while in the malate dihydrate it is in an irregular eight-vertices polyhedron (138). Four forms of hydroxycitrate – $\text{HO}_2\text{CCH}(\text{OH})\text{C}(\text{OH})(\text{CO}_2\text{H})\text{CH}_2\text{CO}_2\text{H}$ – are natural products. The lactones of two of these form calcium salts of established structure. The tetrahydrate of the calcium salt of the lactone of garcinia acid has the Ca^{2+} in a square-antiprismatic environment (139), whereas the tetrahydrate of the calcium salt of the lactone of hibiscus acid contains nine-coordinate Ca^{2+} , in a square-capped square-antiprism (140). Glyceric acid, $\text{HO}_2\text{CCH}(\text{OH})\text{CO}_2\text{H}$, furnishes a link to the sugar acids mentioned in Section V.B.2 below – $\text{Ca}(\text{DL-glycerate}) \cdot 2\text{H}_2\text{O}$ has

seven-coordinated Ca^{2+} in pentagonal bipyramidal geometry with axial water ligands (141). Calcium ascorbate dihydrate exhibits the familiar square-antiprismatic surroundings for the Ca^{2+} (142).

Several rather elderly reviews summarize a lot of data relating to the structures of hydrated carboxylates of Ca^{2+} , documenting the geometry of Ca^{2+} -carboxylate bonding (143,144) and the geometry of Ca^{2+} -water bonding (145). The mean Ca-O(water) distance in these aqua-carboxylate complexes is very close to the mean value, 2.42 Å, calculated from distances determined in 150 crystalline hydrates. A more recent collation states that Ca-O(monodentate $-\text{CO}_2^-$) distances range from a minimum of 2.25 Å (average 2.31 Å for six-coordination) to 2.43 Å for nine-coordination. Ca-O(bidentate $-\text{CO}_2^-$) distances range from 2.35 to 2.94 Å, with a mean value of 2.51 Å for seven-coordination, 2.52 Å for nine-coordination (118).

c Aminocarboxylates. The anionic calcium complex of nitrilotriacetate, whose structure was determined in the form of its sodium salt $\text{Na}[\text{Ca}(\text{nta})]$, contains seven-coordinated Ca^{2+} in capped trigonal prismatic geometry. The structure of the calcium salt of edta, $\text{Ca}[\text{Ca}(\text{edta})] \cdot 7\text{H}_2\text{O}$, is more complicated. One Ca^{2+} is eight-coordinated, in approximately square-antiprismatic geometry, with a hexadentate edta ligand, while the other is seven-coordinated (approximately pentagonal bipyramidal) with mono- and bidentate edta ligands and two water ligands (146). In the 1,3-propylenediaminetetraacetate (pdta) complex $\text{Ca}[\text{Ca}(\text{pdta})] \cdot 6\text{H}_2\text{O}$ both Ca^{2+} are eight-coordinated, but one is in square-prismatic and the other in dodecahedral, geometry (147). The calcium salt of anilic acid (2,5-dianilinoterephthalic acid) crystallizes as dimers, $[\text{Ca}_2(\text{anilate})_2(\text{H}_2\text{O})_8] \cdot 4\text{H}_2\text{O}$, in which the two Ca are joined by two anilate ligands and two bridging waters. Ca-O distances to the latter oxygens are 2.46, 2.48 Å, to the terminal waters 2.37–2.47 Å; the Ca^{2+} ions are seven-coordinate (148). Amide derivatives of aminocarboxylic acids have been suggested to be models for calcium-binding proteins; the tetraamide from edta (edtam) forms a complex $[\text{Ca}(\text{edtam})(\text{NO}_3)](\text{NO}_3)$ in which the Ca^{2+} is eight-coordinated, with the edtam acting as an N_2O_4 donor ligand and the nitrate ligand bidentate (149).

d Amino acids. A series of amino acid salts $[\text{CaL}_2] \cdot x\text{H}_2\text{O}$ provides examples of a variety of hydrate stoichiometries, structures, and coordination numbers (150). The amino acid anion ligands are *N,O*-bonded, and are often bridging; the donor sets are N_2O_5 for seven-coordination (*L* = ala, val), N_2O_6 for eight-coordination (*L* = gly, leu). $\text{Ca}(\text{ala})_2 \cdot 3\text{H}_2\text{O}$ is a mononuclear

complex; others are one-dimensional coordination polymers – thereby lending support to suggestions of the existence of polynuclear species $[\text{Ca}_x(\text{aa})_{2x}(\text{H}_2\text{O})_n]$ in solution. The diiodide salt $[\text{Ca}(\text{glyH})_2(\text{H}_2\text{O})_3]\text{I}_2$ also crystallizes as a one-dimensional coordination polymer; the Ca^{2+} is seven-coordinate, each pair of ions being bridged by carboxylate groups from protonated glycine, $\text{Ca}-\{\text{OC}(\text{CH}_2\text{NH}_3)\text{O}\}_2-\text{Ca}$ (151). Ca^{2+} is seven-coordinate (ligating oxygens from two carbonyl groups, three carboxyls, and two waters; $2.30 < \text{Ca}-\text{O} < 2.5$, average $2.39 \text{ \AA}$) in the glycyglycylglycinate $\text{CaCl}_2(\text{glyglygly}) \cdot 3\text{H}_2\text{O}$ (152).

Aspartate, $[\text{O}_2\text{CCH}_2\text{CH}(\text{NH}_3)\text{CO}_2]^-$, and glutamate, $[\text{O}_2\text{CCH}_2\text{CH}_2\text{CH}(\text{NH}_3)\text{CO}_2]^-$, are effective bridging ligands as they have “hard” O-donors at both ends. Ca^{2+} is six-coordinated, with all six donor atoms being oxygen, in the glutamate salts $[\text{Ca}(\text{glu})\text{Cl}]$ (153) and in $\text{Ca}(\text{glu})_2 \cdot 4\text{H}_2\text{O}$ (154), but in $\text{Ca}(\text{glu})_2 \cdot 3\text{H}_2\text{O}$ the Ca^{2+} is eight-coordinate, with three bidentate glutamate ligands, two *O,O*-bonded through $-\text{CO}_2^-$ and one *N,O*-bonded, plus two water ligands (155). Interestingly, imidazole carboxylate is also *N,O*-bonded in its calcium salt, in which the Ca^{2+} is eight-coordinated (156). Imidazole is one of the few nitrogen-donor ligands that bond to Ca^{2+} in biological systems; $\text{Ca}-\text{N}$ bond distances in several Ca^{2+} -imidazole and Ca^{2+} -imidazole-chloride complexes range from 2.44 to $2.51 \text{ \AA}$. The Ca^{2+} in these complexes is six-coordinate (157).

e Mixed-metal complexes. It is interesting that one Ca^{2+} can be substituted into the triangular hexacarboxylato- Fe_3O unit so familiar from iron chemistry – thus linking the two most biorelevant metals. There is a $\mu^3\text{-O}$ -centered CaFe_2 unit in the tetrahydrofuran (thf) solvate of the trichloroacetate $[\text{CaFe}_2(\text{CCl}_3\text{CO}_2)_6(\text{thf})_4] \cdot \text{thf}$, in which the Ca is seven-coordinated, with $\text{Ca}-\text{O} = 2.53 \text{ \AA}$ for the center-oxygen, 2.34 – 2.42 (i.e. normal length) to the other coordinated oxygens (158).

II. The Ca^{2+} Aqua-Cation

A. INTRODUCTION

The hydrated cation $\text{Ca}^{2+}_{\text{aq}}$ is of prime importance to the aqueous solution chemistry of calcium, and to most of its various roles in biological systems. The relation between lattice energy and hydration energies of the constituent ions determine solubilities, the size of the hydrated cation controls selectivity and the passage of ions through channels, and the work required to remove some or all of the water of hydration is relevant both to

forming complexes and to entering channels. Some of the relevant properties and parameters are well established, but such fundamental features as the hydration number of Ca^{2+} and kinetic parameters for water exchange at Ca^{2+}aq are still ill-defined. Ion-pairing is of little significance, except in concentrated solutions of very soluble salts such as the chloride, but complex formation can be important, especially in systems containing anionic oxygen-donor ligands (see next section). Several books and reviews (159–162) offering detailed and extensively referenced treatments of ions in solution include many mentions of Ca^{2+}aq .

B. THE NATURE OF Ca^{2+}aq

B.1. Hydration numbers

Whereas the primary hydration numbers of Be^{2+} and Mg^{2+} have long been established as four and six respectively, the NMR techniques used to determine primary solvation numbers are not applicable to the fast-exchange cations lower down Group 2 of the Periodic Table. Various indirect methods have led to estimates of between 5.5 and 12 for the hydration number of Ca^{2+} (161–164). X-ray crystallography has demonstrated the existence of species $[\text{Ca}(\text{H}_2\text{O})_n]^{2+}$, $n = 6, 7, 8$, and 9, in crystal hydrates (118). $[\text{Ca}(\text{H}_2\text{O})_8]^{2+}$ is the most common and thus perhaps the most likely formulation for Ca^{2+}aq , but $[\text{Ca}(\text{H}_2\text{O})_7]^{2+}$ should not be ruled out in the light of evidence in favor of $[\text{Sc}(\text{H}_2\text{O})_7]^{2+}$ (165) and of $[\text{Hg}(\text{H}_2\text{O})_7]^{2+}$ (166). $[\text{Ca}(\text{H}_2\text{O})_9]^{2+}$ is implausible in view of its extreme rarity in salt hydrates. X-ray and neutron diffraction and scattering methods can give estimates of hydration numbers (167–169), though such estimates are generally ± 1 , sometimes ± 2 . Indeed hydration numbers of 6 (170–172), 7 (170,173), 8 (171,172,174), and 10 (169) have been proposed or assumed in such investigations, with one group claiming that the hydration number increases from 5.5 to 10 as Ca^{2+} concentration decreases (175). A problem with these techniques, and with NMR until recently, is that rather or very concentrated solutions are necessary – though as a by-product it has been shown that even in concentrated solutions of calcium chloride there is no evidence for contact ion pairs between Ca^{2+} and Cl^- . However in molten $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, alias saturated CaCl_2 solution, there are solvent-separated ion pairs whose structure is closely related to fragments of solid $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (170,172,176). In contrast to this considerable reluctance of Ca^{2+} to ion-pair with chloride, it associates with

the $\text{Zr}(\text{OH})_6^{2-}$ or $\text{Th}(\text{OH})_6^{2-}$ anions generated by dissolving hydrated ZrO_2 or ThO_2 in alkaline CaCl_2 solution. EXAFS showed the $\text{Zr}(\text{OH})_6^{2-}$ and $\text{Th}(\text{OH})_6^{2-}$ to have 3 Ca^{2+} at 3.4 Å or 4 Ca^{2+} at 4.0 Å respectively (177), putting the hydroxoanions within the secondary coordination shell of Ca^{2+}aq . These cation–cation distances are comparable with $\text{Ca}\cdots\text{Mn}$ distances of 4–5 Å in concanavalin A and pea lectin, and indeed of shortest $\text{Ca}\cdots\text{Ca}$ distances in thermolysin and in C_2 domains (see Section III.B below).

In dilute solutions of calcium salts, the Ca^{2+} will be surrounded by a secondary hydration shell even less well-defined than its primary hydration shell. However it would be useful to have some idea of the effective size of Ca^{2+} with all its associated waters, for example for discussing its passage through ion channels. This question has been addressed by a variety of approaches that attempt to estimate the number of water molecules significantly associated with the cation (entropies, volumes) or which move with the cation under spontaneous (diffusion) or forced (e.g. ionic conductivity, ionic mobility, transference numbers, viscosity) conditions (178–181). A wealth of early information and experimental data may be found in the extensive, though noncritical presentation, of Hinton and Amis (180). Large-angle X-ray scattering (LAXS) studies indicated a mean $\text{Ca}\cdots\text{O}$ distance of 4.6 Å to the secondary hydration shell (174), and an estimate of 16–18 for the secondary hydration number (cf. estimates between 17 and 21 from computer modeling reported in Section II.B.3 below). Recent attempts to quantify total or secondary hydration have been based on freezing point depression (182) and on electrostriction reflected in the partial molar ionic volume of Ca^{2+}aq (183). These approaches gave estimates of 12 ± 2 and 10 respectively for primary plus secondary hydration. Several estimates for the total hydration number (180,181) seem to be little more than those for the number of waters in the primary solvation shell, indicating rather weak attractions between the Ca^{2+} cation and its secondary hydration shell. Indeed estimation of dynamic hydration numbers has been claimed to show that only two of the water molecules in the primary hydration shell of Ca^{2+} are tightly bound and that it is the radius of this minimally hydrated ion that is important in relation to biological behavior (184). The results of neutron and X-ray diffraction investigations of Ca^{2+} -containing solutions have been discussed in terms of the nature of the bonding and of the orientation of the water molecules in both the first and second coordination shells (185).

B.2. *Ca–O distances*

Although X-ray and neutron diffraction and scattering methods give only approximate estimates of hydration numbers they can provide precise measures of ion–water distances in solution. In calcium chloride and bromide solutions of various concentrations, Ca–O distances of between 2.40 and 2.44 Å have been reported (167,168,171,172); Ca–O = 2.26 Å was claimed in an early X-ray investigation of molar calcium nitrate solution (167,186). EXAFS and LAXS studies showed a broad and asymmetric distribution of Ca–O distances centered on a mean value of 2.46 Å (174).

B.3. *Computation and modeling*

In view of the difficulties of characterizing Ca^{2+}aq by the usual experimental methods there have been several attempts to model and compute the hydration shell of this cation. An early molecular dynamics (MD) study estimated a total hydration number of 64 (187), which indicated a much larger secondary solvation shell than that suggested by any of the experimental approaches alluded to above. Later simulations and calculations have tended to focus on the primary hydration shell. A Monte Carlo approach suggested a primary hydration number of 7 (188), while Born–Oppenheimer *ab initio* molecular quantum mechanics calculations indicated a primary hydration number of 8.3; various earlier calculations and computations had resulted in estimates between 8 and 10 (189). Probing the structure and dynamics of Ca^{2+}aq by Car–Parrinello MD methods suggested that the number of waters in the primary hydration shell fluctuates between 7 and 8 (190). Density Functional Theory (DFT) has been used to probe the relation between the primary and secondary hydration shells of several cations, including Ca^{2+}aq ; up to eight waters were indicated in the primary hydration shell (191). MD simulations, based on ionic mobilities, indicated primary and secondary hydration numbers of 7.9 and 17.6; the effective radius of the secondary hydration shell was just under 4.5 Å (192). Quantum mechanics/molecular mechanics-molecular dynamics (QM/MM-MD) simulations, carried out according to the Hartree–Fock (HF) or DFT approaches, have been compared with classical MD simulations to clarify the primary and secondary hydration numbers of Ca^{2+}aq . Estimates for the primary hydration number were 7.1 (MD), 7.6 (HF), or 8.1 (DFT); the second shell contains 17.9, 19.1, or 20.5 water molecules. The authors favored the QM/MM-MD/HF results (193). A discrete solvation model and two continuum models – spherical cavity and molecular shape cavity – for M^{n+}aq

TABLE IV

ENTHALPIES OF HYDRATION (kJ mol^{-1}) OF SELECTED CATIONS^a

Na ⁺	−405
K ⁺	−321
Mg ²⁺	−1922
Ca ²⁺	−1592
Sr ²⁺	−1445
Ba ²⁺	−1304
TM1 ^{2+b}	−1845 to −2100
Zn ²⁺	−2044

^aRelative to a value of $-1091 \text{ kJ mol}^{-1}$ for H^+ .^bTM1²⁺ = Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} .

have been discussed; the authors favor² the shape cavity model. This article provides good referencing of earlier published work but only the briefest mention of Ca^{2+}aq (194). *Ab initio* calculations combined with results from infrared spectroscopy have been employed to probe second shell hydration of Ca^{2+} , albeit in the gas phase (193).

B.4. Thermodynamic parameters

Unlike hydration numbers, thermodynamic parameters for Ca^{2+}aq can be established with some confidence, subject to the small uncertainties in setting single ion values (160,194). The enthalpy of hydration of Ca^{2+} , $\Delta H^\circ_{\text{hydr}}(\text{Ca}^{2+}) = -1592 \text{ kJ mol}^{-1}$, is given, with values for a number of other cations for comparison, in Table IV. These values are based on the long-established convention that the single ion enthalpy of hydration of the proton is $-1091 \text{ kJ mol}^{-1}$. A recent alternative route to $\Delta H^\circ_{\text{hydr}}(\text{Ca}^{2+})$, based on the assumption $S^\circ(\text{H}^+\text{aq}) = -5.5 \text{ J K}^{-1} \text{ mol}^{-1}$ and thence using Krestov's approach to ion hydration enthalpies (195), leads to $\Delta H_{\text{hydr}}(\text{Ca}^{2+}) = -1551 \text{ kJ mol}^{-1}$ (196), significantly different from, more recent than, but not necessarily preferable to, the value given in Table IV. Marcus's 1987 value (197), $\Delta H^\circ_{\text{hydr}}(\text{Ca}^{2+}) = -1602 \text{ kJ mol}^{-1}$, based on the widely accepted TATB/TPTB assumption $\{\Delta H^\circ_{\text{hydr}}(\text{Ph}_4\text{As}^+) = \Delta H^\circ_{\text{hydr}}(\text{Ph}_4\text{P}^+) = \Delta H^\circ_{\text{hydr}}(\text{Ph}_4\text{B}^-)\}$, is in better agreement with the Table IV value.

²The underlying arguments are detailed in Schwenk, C. F.; Loeffler, H. H.; Rhode, B. M. *Chem. Phys. Lett.* **2001**, 349, 99–103 and Schwenk, C. F.; Loeffler, H. H.; Rhode, B. M. *J. Chem. Phys.* **2001**, 115, 10808–10813, for the QM/MM-MD/HF results, and Martinez, J. M.; Pappalardo, R. R.; Marcos, E. S.; Mennucci, B.; Tomasi, J. *J. Phys. Chem. B* **2002**, 106, 1118–1123, for the shape cavity models.

The partial molar volume of Ca^{2+}aq , based on the long-established assumption of a value of $-5\text{ cm}^3\text{ mol}^{-1}$ for H^+aq , is $-29\text{ cm}^3\text{ mol}^{-1}$ (198,199), the negative value reflecting electrostriction of bulk water on transfer to the hydration shell of the cation. Comparative values ($\text{cm}^3\text{ mol}^{-1}$) are +16 for Cs^+aq , -23 for Mg^{2+}aq , -23 for Ba^{2+}aq , -61 for Al^{3+}aq . In recent years, it has been argued that it would be preferable to use the TATB $\{V^\circ(\text{Ph}_4\text{As}^+) = V^\circ(\text{Ph}_4\text{B}^-)\}$ (or essentially identical TPTB) assumption value (200), but this makes a difference of only $1\text{ cm}^3\text{ mol}^{-1}$ for H^+aq (201), $2\text{ cm}^3\text{ mol}^{-1}$ for Ca^{2+}aq . Partial molar volumes are of relevance to such matters as the properties of sea water, the behavior of membranes, for example skin, and the buoyancy of marine organisms such as elasmobranch fishes (e.g. shark, skate). Ca^{2+} , like Mg^{2+} , contributes a small negative buoyancy, in contrast to the large positive buoyancy contributed by for example chloride $\{V^\circ(\text{Cl}^-) = +23\text{ cm}^3\text{ mol}^{-1}\}$ (202). Partial molar volumes reflect the effective radii of hydrated ions in solution, hence the use of these quantities to assess hydration numbers (178). The effective volume of Ca^{2+}aq controls such properties as diffusion; as long ago as 1905 it was estimated that Ca^{2+}aq diffuses through saline at a rate of $40\text{ }\mu\text{m s}^{-1}$ (203), whereas diffusion of Ca^{2+} in the cytosol is at least a hundred times slower (10).

The $\text{p}K$ of Ca^{2+}aq (204), 12.6 at zero ionic strength, rising to over 13 as ionic strength increases, means that concentrations of CaOH^+aq will be negligible in body fluids ($1 < \text{pH} < 8$), in all natural and polluted waters, and under all conditions of biological relevance, from the very low pHs of 0.5 (*Thiobacillus thiooxidans*) to 1.5 at which bacteria used for oxidative metal extraction operate (205), through acid soils and acid rain (pH 3 to 6), streams, rivers, and oceans (pH 6 to 8), soda lakes (pH ~ 10), up to the pHs of 11 or more in Jamaican Red Mud slurry ponds (206) (cf. Section II.C.1 below).

Knowledge of complex permittivities of appropriate electrolyte solutions is useful in assessing interactions of microwave radiation with biological tissues. A full study and analysis of complex permittivities of sodium chloride solutions as a function of concentration, temperature, and microwave frequency (207) has laid the foundations for a similar investigation of calcium salt solutions.

C. STABILITY CONSTANTS

C.1. General

Extensive information on stability (formation) constants has been conveniently collected together in several reference texts

and databases, both comprehensive (208–210) and selective (211–213). Values without specific attribution in the tables in this section have been taken from one or the other of these publications, which detail conditions and primary sources. The valuable compilations published by IUPAC of stability constants for specific ligands or groups of ligands are cited where appropriate later in this section. A recent copiously referenced review (225 references (214)) of weak complexes ($\log K < \sim 3$) of alkali metal and alkaline earth metal cations contains many data for calcium. Stability constants for calcium complexes are available not only for normal electrolyte solutions of various ionic strengths, but also in such biogeo-relevant media as sea water (215,216), Italian red wine (217), and urine (218).

The stoichiometry and concentration of species present in a metal ion–ligand–complex system depends on the initial concentrations of the metal and ligand and on pH. The parameters defining the system are the pK values for ligand and aqua-metal ion and the stability constants for the complexes involved; the distribution of species depends on the interrelation of these parameters. The pK of Ca^{2+}aq is sufficiently high (Section II.B.4 above) for hydrated Ca^{2+} to be present as $\text{Ca}(\text{H}_2\text{O})_n^{2+}$, with negligible amounts of CaOH^+aq , at all pHs of biological interest, but many ligands exhibit acid–base properties within the pH range of interest. For such ligands tabulated stability constant data, which usually refer to fully deprotonated ligands, may not be directly applicable. Normally stability constants decrease as pH decreases, since the degree of protonation increases and ligand charge decreases. Thus, for example, $\log K_1$ values for the Ca^{2+} /citrate system decrease from 3.6 for ligand cit^{3-} at high pH to 2.0 for citH^{2-} and 1.0 for citH_2^- (at 298 K, $I = 0.1$ M (219)), while $\log K_1$ values for Ca^{2+} /edta and Ca^{2+} /egta decrease from 10.7 for edta^{4-} to 3.5 for edtaH^{3-} , from 11.0 for egta^{4-} to 5.3 for egtaH^{3-} (at 293 K, $I = 0.1$ M (220)). These considerations also apply to indicators – for example for *o*-cresolphthalein complexone (cpcH_6) $\log K_1$ values for $\text{Ca}^{2+}/\text{cpc}^{6-}$, cpcH^{5-} , cpcH_2^{4-} are 7.8, 6.9, and 3.1 (221) – and to biological substrates, where protonation levels may well be ill-defined or unknown. Conditional (effective) stability constants were therefore introduced to provide a quantitative measure of stability in specified conditions, for example neutral solution. Such conditional stability constants are often available for ligands of biological relevance. Fig. 1 presents some information on pH values and ranges for a selection of biochemical and geochemical environments, and relates this to successive pK values for the aminocarboxylate ligand ethylene glycol bis-(2-aminoethyl ether)tetraacetate, egta, and to the pK for Ca^{2+}aq .

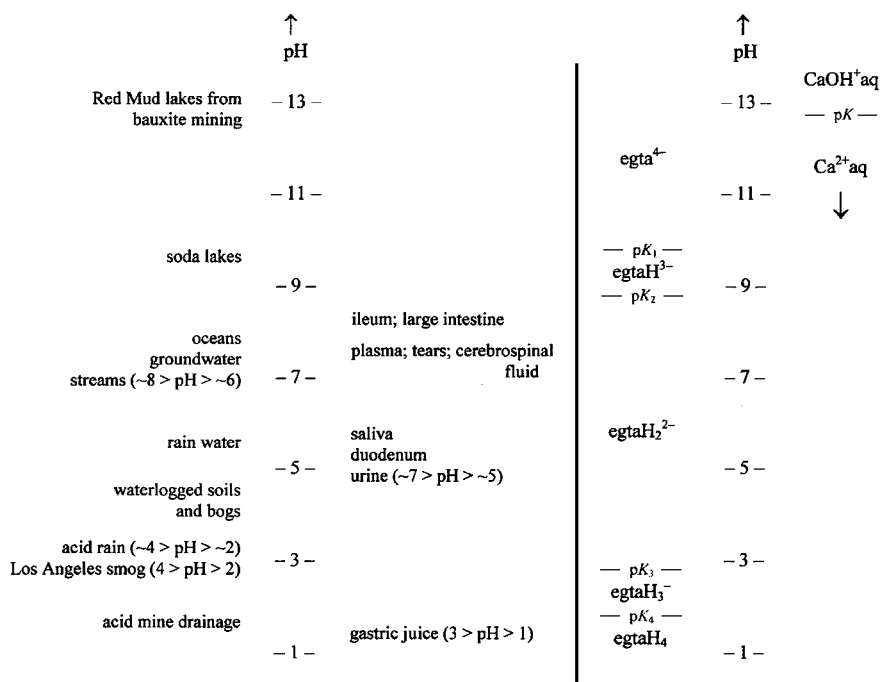


FIG. 1. Approximate pH values and ranges for a selection of biochemical and geochemical environments, with their relation to successive pK values for the aminocarboxylate ligand ethylene glycol bis-(2-aminoethyl ether)-tetraacetate, egta.

Ca^{2+} is a "hard" (222,223) cation with a moderate propensity to form complexes in aqueous media. It has a marked preference for oxygen donors over nitrogen donors, though stability constants for oxoanion complexes are generally quite small, as is apparent from entries in Table V (43,208,209,224–230). Reported stability constants for formation of complexes with such common nitrogen-donor ligands as ammonia (223), imidazole (231), pyridine (208, 232), 2,2'-bipyridyl (232,233), and 1,10-phenanthroline (234) are as low as 0.6, 0.1, ≤ 1 , ~ 0 , and ~ 0.6 respectively. Even the potentially tetradentate pyridine ligand with a $-\text{CH}_2\text{CH}_2\text{NHCOCH}_2\text{NHCOCH}_2\text{NH}_2$ substituent exhibits no detectable interaction with Ca^{2+} (235).

The complexing behavior of Ca^{2+} is put into context in Table VI (49,208,211,236–246), which provides a comparison of stability constants ($\log_{10} K_1$, on the molar scale, in aqueous media at 298 K (a few at 293 K), generally at $I \sim 0.1 \text{ M}$) for a selection of complexes of Ca^{2+} with those for a range of other metal cations. Ionic strength effects are often significant, especially for ionic

TABLE V

STABILITY CONSTANTS, $\text{LOG}_{10}K_1$, FOR Ca^{2+} COMPLEXES WITH INORGANIC LIGANDS^a

	$\log_{10}K_1$
Cl^-	$\sim -1^b$
NO_3^-	-0.4^c
$\text{S}_2\text{O}_3^{2-}$	0.4^d
F^-	0.7^e
OH^-	1.2
SO_4^{2-}	2.3^f
SiO_3^{2-}	3.1
$\text{Fe}(\text{CN})_6^{4-}$	3.6
HPO_4^{2-}	1.5^g
$\text{HP}_2\text{O}_7^{3-}$	3.4^g
$\text{HP}_3\text{O}_{10}^{4-}$	4.1^g
$\text{HP}_4\text{O}_{13}^{5-}$	3.3^h
$\text{P}_3\text{O}_9^{3-}$	2.1^i
$\text{P}_4\text{O}_{12}^{4-}$	3.3^i
$\text{P}_6\text{O}_{18}^{6-}$	3.0^i
$\text{P}_8\text{O}_{24}^{8-}$	2.9^i

^aFrom Ref. (209) except where otherwise indicated; values refer to aqueous solution at 298 K, $I \sim 0.1$ M unless otherwise indicated.

^bFrom Ref. (43); an earlier estimate from diffusion studies was $\log K_1 = -0.2$ at $I = 1.0$ M (PAT).

^cFrom Ref. (43); earlier estimates arising from vibrational spectroscopy studies of the anion in $\text{Ca}(\text{NO}_3)_2$ solutions were $\log K_1 = -0.8$ to -0.9 , depending on ionic strength (228). A $\log K_1$ value of -0.9 has been reported for the iodate complex (224,225).

^dFrom Ref. (208).

^eThis value is the mean of several remarkably consistent estimates. Ref. (223) recommends 0.6. Ref. (226) gives $\log K_1$ values of 0.7 and 0.6 at $I = 0.4$ and 1.0 M (298 K); $\log K_1$ values for Mg^{2+} are 0.7 higher.

^fThis value is the mean of several consistent estimates.

^gFrom Ref. (229).

^hAt $I = 1.0$ M.

ⁱFrom Ref. (230).

ligands. Thus for several carboxylate ligands it has been shown that $\log_{10}K_1$ values are between 0.5 and 1.0 larger at zero ionic strength than at $I = 0.25$ M (246). The more highly charged cyclic phosphates on the right-hand side of Table V show larger ionic strength dependences. $\log K$ values increased from 1.8 at $I = 0.2$ M to an estimated 2.3 at $I = 0$ for $\text{P}_3\text{O}_9^{3-}$, and from 4.6 at $I = 0.2$ M to about 8 at $I = 0$ for $\text{P}_8\text{O}_{24}^{8-}$ (230).

C.2. Amino acids and peptides

Stability constants for Tyr, Ser, Asp, Glu (247–250) are very similar to the values given for Gly and Ala in Table VI. The value

TABLE VI

STABILITY CONSTANTS, $\text{Log}_{10} K_1$, FOR A SELECTION OF COMPLEXES OF Ca^{2+} AND OF OTHER M^{2+} AND M^{3+} IONS^a

	Mg^{2+}	Ca^{2+}	Ba^{2+}	$\text{Mn}^{2+} \rightarrow \text{Cu}^{2+}$	Zn^{2+}	Fe^{3+}	Ln^{3+}	Ref.
Acetate	0.8	0.8^b	0.4	$\sim 1 \rightarrow 2$			$1.8 \rightarrow 1.9$	(211,226)
Oxalate	2.6	3.0	2.3	$3.9 \rightarrow 6.3$	4.7	7.6	$\text{La}^{3+} 4.5$	(208)
Glycinate	2.2	1.4^c	0.8	$3.2 \rightarrow 8.6$	5.4	(8)	$\text{Gd}^{3+} (4)$	(237)
α -alaninate ^d	2.0	1.2	0.8	$3.0 \rightarrow 8.4$	5.0	10.7	$4.5 \rightarrow 4.8$	(238)
Lactate ^e	0.7	0.9	0.4	$1.0 \rightarrow 2.5$	1.6	6.8	$\text{La}^{3+} 2.4$; $\text{Gd}^{3+} 2.7$	(239)
ida ^f	2.9	2.6	1.7	$(5)^g \rightarrow 10.6$	7.3	10.7^h	$5.9 \rightarrow 7.6$	(208,240)
nta	5.4	6.6ⁱ	4.7	$8.6 \rightarrow 13.1$	10.5	16.3^j	$10.4 \rightarrow 12.5$	(241)
edta ^k	8.7	10.7^l	7.7	$14.0 \rightarrow 18.9$	16.7	25.1	$15.5 \rightarrow 20.0$	(49)
dtpa	9.3	10.7^m	8.8	$15.5 \rightarrow 21.5$	18.6 ^j	27.8	$\text{Gd}^{3+} 22.5$	(240)
dota ⁿ	11.9	17.2	12.9	$20.0 \rightarrow 22.3$	20.8	29.4	$\text{Gd}^{3+} 25.0^o$	(240,242,243)
Kojate	2.9	2.5		$3.7 \rightarrow 6.6$	5.0	26	$\text{Gd}^{3+} 5.5^p$	(244)
trenhopo ^q		7.4			11.9	27.9	$\text{Gd}^{3+} 20.3$	(245)

^aIn aqueous solution at 298 K; $I \sim 0.1$ M, unless otherwise stated.^bRef. (272) give $K = 7$ (i.e. $\log K = 0.8$); Ref. (246) gives $\log K = 0.93$, 0.57 ($I = 0$, 0.25 M respectively); (43) give $\log K = 1.1$, 0.8 ($I = 0$, 0.216 M respectively); Ref. (211) recommends $\log K = 0.5$, at $I \sim 0.1$ M, 298 K; Ref. (214) recommends $\log K = 1.0$ at $I = 0$, 298 K.^cRef. (246) gives $\log K = 1.51$, 1.05 for glycinate at $I = 0$, 0.25 M respectively, while Harju, L. *Talanta* **1987**, 34, 817–820 gives $\log K = 1.34$, 0.55 at $I = 0.5$, 1.0 M respectively.^d $I \rightarrow 0$ ($I = 1.0$ M for Fe(III) ; $I = 0.1$ M for Ln(III)).^eIn 1 M NaClO_4 ; uncertainties are relatively large as K_1 values are so small; $\log K_1$ values for glycolate are almost identical (see also Ref. (239)).^f $\log K$ values for Mg^{2+} , Ca^{2+} , Sr^{2+} , and Ba^{2+} complexes of a variety of iminodiacetate derivatives are listed in Irving, H.; da Silva, J. J. R. F. *J. Chem. Soc.* **1963**, 3308–3320.^gValue for Fe^{2+} .^hRef. (210).ⁱFor $[\text{Co}(\text{NH}_3)_5(\text{nta})]$ acting as a ligand, $\log K(\text{Ca}) = 4.4$ (Cannon, R. D.; Gardiner, J. *Inorg. Chem.* **1974**, 13, 390–399) – the (uncharged) cobalt complex binds a hundred times less strongly than the $(3-)$ free anion.^jAt 293 K.^kAll values at 293 K; $I = 0.1$ M.^lFor edta, pdta, bdta, cydta values are 10.3, 11.4, 13.1, 13.7 at 298 K, $I = 0.5$ (Ref. (267)); for cydta $\log K = 13.2$ at 298 K, $I = 0.1$ (Delgado, R.; da Silva, J. J. R. F. *Talanta* **1982**, 29, 815–822); pdta = 1,2-diaminopropane tetraacetate, bdta = 2,3-diaminobutane tetraacetate, cydta = 1,2-diaminocyclohexane tetraacetate.^m $\log K = 9.8$ at 310 K, $I = 0.15$ M (Duffield, J. R.; May, P. M.; Williams, D. R. *J. Inorg. Biochem.* **1984**, 20, 199–214).ⁿValues for nota are also documented in Ref. (237); values for nota and dota complexes of several M^{2+} ions are given in Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, 190, 27–36, for M^{3+} ions in Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, 190, 37–46.^oFor Ho^{3+} , Sm^{3+} plus dota (and trita, teta, see Marques, F.; Gano, L.; Campello, M. P.; Lacerda, S.; Santos, I.; Lima, L. M. P.; Costa, J.; Antunes, P.; Delgado, R. *J. Inorg. Biochem.* **2006**, 100, 270–280.^pYoneda, H.; Choppin, G. R.; Bear, J. L.; Quagliano, J. V. *Inorg. Chem.* **1964**, 3, 1642–1644.^qTrenhopo is a hexadentate tripodal ligand $\text{N}(\text{CH}_2\text{CH}_2\text{NHCOR})_3$ with X being 6-methyl-3-hydroxy-2-pyridinonate.

for hydroxyproline is slightly lower (231), that for L-dopa (3-hydroxytyrosine) slightly higher (251). Log K values for complex formation with Gly, Ala, and Asp under physiological conditions, viz. 310 K and $I = 0.15$ M NaCl, are available (252). Calcium complexes of γ -carboxyglutamate, its N -acetyl derivative, the α -methylamide derivative of the latter (a model for carboxyglutamate incorporated in a polypeptide chain), and γ -carboxyglutamate-containing di- and tri-peptides have surprisingly small stability constants. Log K values for three such di- and tri-peptides are about 3 (253), considerably smaller than the Ca^{2+} -binding constants for the blood coagulation factors that contain moieties of this type (254).

There is a little quantitative information on stabilities of Ca^{2+} complexes of di-, oligo-, and polypeptides. Log K values, at 298 K and $I = 1.0$ M KNO_3 , for Ca^{2+} complexes of gly, trigly, and tetragly are similar – 1.25, 1.20, and 1.49 respectively – while for AlaGlu log K is slightly higher, at 2.03 (255). Log K for the dipeptide GlyGlu, often found at Ca^{2+} -binding sites, 2.67 at 298 K, $I = 0.1$ M KNO_3 (256), is also significantly higher than log K values for most amino acid anions. An early review of the biological activity of cyclic peptides (257) makes very little mention of their interactions with Ca^{2+} . Subsequently stability constants were determined for the Ca^{2+} complexes of the dodecapeptide cyclo-(–LVal–Gly–Gly–LPro–)₃ (log $K = 8.5$ (258)) and of several more cyclic polypeptides (12-, 18-, 24-, and 36-membered rings; $1.5 < \log K < 7$ in various solvents (259)). Log K values, again in acetonitrile, for two naturally occurring thiazole- and oxazoline-containing cyclic octapeptides, ascidiacyclamide and patellamide, are 2.8 and 2.9. For a synthetic analog cyclo-(–Thr–Val–Abu–Ile–)₂ containing α -aminobutyrate, Abu, log K is appreciably higher (5.5) (260). Several synthetic cyclic octa- and deca-peptides, especially cyclo-(–LPro–LPro–LLeu–LLeu–LLeu–)₂ show significant selectivity for Mg^{2+} over Ca^{2+} (261), while a 24-membered cyclic peptide has been shown to provide selective transport of Ca^{2+} through a liquid membrane (259). Stability constants for complexes of ionophores containing both peptide and ester linkages are documented in [Section IV.B](#).

C.3. Aminocarboxylates

Stability constants for edta and its α,ω -homologs show the expected decrease as chelate ring sizes increase (cf. dicarboxylates, below) (262). Log K values are 10.57, 7.3, 5.7, 5.2, 4.6, and 4.6 (293 K; $I = 0.1$ M KNO_3 (220)) for $n = 2, 3, 4, 5, 6, 8$ in the ligands $[(\text{O}_2\text{CCH}_2)_2\text{N}(\text{CH}_2)_n\text{N}(\text{CH}_2\text{CO}_2)]^{4-}$. Log K is

very nearly the same for the complex of ttha, $[(O_2CCH_2)_2NCH_2\{CH_2N(CH_2CO_2)CH_2\}_2CH_2N(CH_2CO_2)_2]^{6-}$, as for that of edta (220,263). Multidentate aminocarboxylate ligands such as edta or ttha can act as bridging ligands, giving homo- or hetero-bi- or trinuclear complexes, for example $[Ca(edta)Hg(OH)_2]^{2-}$ (264), $[Ca_2(ttha)]^{2-}$, and $[Ca_3(ttha)]$ (265), for all of which stability data are available.

Many of the fluorescent chelators used to monitor Ca^{2+} concentrations are edta derivatives, based on 1,2-bis-(2-amino-phenoxy)ethane-*N,N,N',N'*-tetraacetic acid, $(HO_2CCH_2)_2NC_6H_4OCH_2CH_2OC_6H_4N(CH_2CO_2H)_2$ (baptaH₄). $\log K$ for the Ca^{2+} complex of baptaH³⁻, the form of the ligand in the physiological pH region, is 7.0 (266), equal to the value for $[Ca(edtaH)]^-$ (267). Quin-2 is also an *N,N,N',N'*-tetraacetic acid, but with a quinoline-containing spacer. The stability constant for its Ca^{2+} complex is markedly dependent on ionic strength; $\log K = 6.9$ in 0.15 M KCl, 8.3 at $I \sim 0$ (268). $\log K$ values for the calcium complexes of most of the bapta-based fluorescent chelators lie between 6 and 7 (65,66,269). Appropriate tailoring can reduce $\log K$ considerably, which is an advantage for many biochemical investigations. Thus replacement of the ethane-*N,N,N',N'*-tetraacetate moiety of bapta by an *N,N,O*-triacetate analog results in a marked reduction in $\log K$, which for the widely used fura-2 complex is 4.4. The reagent 7-amino-3-(2-benzothiazolyl)-6-[2-(2,3-difluoro-6-aminophenoxy)ethoxy]-coumarin-*N,N,N',N'*-tetraacetate has a particularly low $\log K$ of 3.3 and has been proposed as a suitable reagent for the assay of calcium in living cells of the endoplasmic reticulum (270). $\log K(Ca^{2+})$ values for the large number of indicators of this type which have been developed over several decades can be traced through reports of recent applications (58,65,66,271). Values of $\log K$ for Mg^{2+} analogs of these complexes are very much lower.

C.4. Other carboxylates

Stability constants for calcium complexes of a selection of hydroxycarboxylate ligands are listed in Table VII (239,246,272–274). For tartrate, malate, and citrate stabilities decrease in the expected order $Ca^{2+} > Ba^{2+} > Ra^{2+}$ (231,275). The stability constant for the complex of pyruvate ($\log_{10} K_1 \sim 0.8$ (273)) is similar to that for acetate; calcium complexes of α -ketoglutarate and of oxaloacetate are somewhat more stable ($\log_{10} K_1 = 1.3, 1.6$ respectively (273)). The sequence $\log_{10} K_1 = 3.0, 1.4, \sim 1.1, \sim 0.6$ for the dicarboxylate ligands oxalate, malonate, succinate,

TABLE VII

STABILITY CONSTANTS, $\text{Log}_{10} K_1$, FOR HYDROXYCARBOXYLATES (298 K; $I = 0.15$ – 0.16 M)

Parent acid		$\text{Log}_{10} K_1$	Ref.
Lactic	$\text{CH}_3\text{CH}(\text{OH})\text{CO}_2\text{H}$	0.9	(239,272)
Gluconic	$\text{HO}_2\text{C}(\text{CH}(\text{OH}))_4\text{CO}_2\text{H}$	1.2	(272,273)
Tartaric	$\text{HO}_2\text{CCH}(\text{OH})\text{CH}(\text{OH})\text{CO}_2\text{H}$	2.1 ^a	(246)
Malic	$\text{HO}_2\text{CCH}(\text{OH})\text{CH}_2\text{CO}_2\text{H}$	2.0 ^a	(246)
Citric	$\text{HO}_2\text{CCH}_2\text{C}(\text{OH})(\text{CO}_2\text{H})\text{CH}_2\text{CO}_2\text{H}$	3.2 ^b	(273)
Isocitric	$\text{HO}_2\text{CCH}(\text{OH})\text{CH}(\text{CO}_2\text{H})\text{CH}_2\text{CO}_2\text{H}$	2.5	(273)
Salicylic	$o\text{-C}_6\text{H}_4(\text{OH})(\text{CO}_2\text{H})$	0.6	(239,246,274)
Mandelic	$\text{C}_6\text{H}_5\text{CH}(\text{OH})\text{CO}_2\text{H}$	1.5	(239)

^aRef. (214) gives $\log K_1$ values for tartrate and malate at $I = 0$, 298 K.^bRef. (249) report $\log K_1 = 3.4$, $\log K_2 = 1.6$, at 310 K; (214) give $\log K_1 = 2.8$, 1.1 for Hcitrate^{2-} , $\text{H}_2\text{citrate}^-$ ($I = 0$, 298 K), while (219) quotes $\log K_1 = 3.6$, 2.0, 1.0 for citrate^{3-} , Hcitrate^{2-} , $\text{H}_2\text{citrate}^-$ ($I = 0.1$ M, 298 K), and estimates that $\log K_1 \sim 4.8$ for citrate^{3-} at $I = 0$.

glutarate (at pH 7.2–7.3, $I = 0.16$ M, 298 K (208,273))³ conforms well with the normal sequence of decreasing stability with increasing ring size for chelate complexes. $\text{Log}_{10} K_1$ values for a range of dicarboxylates, including fumarate, maleate, citraconate and itaconate) and tricarboxylates (tricarballylate, α -carboxypimelate, aconitate)⁴ are, again at pH 7.2–7.3, $I = 0.16$ M, 298 K, between 1 and 2 (273). $\text{Log}_{10} K_1$ values are between 0.8 and 1.0 larger at zero ionic strength; the temperature variation of $\log_{10} K_1$ has also been established for several di- and tricarboxylate ligands (246). $\log K$ values for Ca^{2+} complexes of several dicarboxylates have been compared with $\log K$ values for their respective Mg^{2+} complexes (276). The naturally occurring anti-neoplastic bis-indole alkaloid vincristine may be mentioned here in view of its carboxylate ester component. Its calcium complex has $\log K = 3.27$ at 298 K, $3.4 \leq \text{pH} \leq 5.5$; Ca^{2+} binds through acetoxy and formyl as well as methyl ester oxygen atoms (277).

C.5. Crown ethers and azamacrocycles

Ca^{2+} interacts only weakly in aqueous solution with crown ethers (278,279), as indeed with polyethers such as

³Ref. (43) reports $\log K = 2.5$, 1.8, 1.8 at $I = 0$, 0.1, 0.16 M respectively for oxalate; while Ref. (214) recommends averaged $\log K$ values of 3.2, 2.4, 2.3 for oxalate, malonate, succinate at $I = 0$, 298 K.

⁴Citraconic acid: $\text{CH}_3\text{C}(=\text{CHCO}_2\text{H})\text{CO}_2\text{H}$; itaconic acid: $\text{HO}_2\text{CCH}_2\text{C}(=\text{CH}_2)\text{CO}_2\text{H}$; tricarballic acid: $\text{HO}_2\text{CCH}_2\text{CH}(\text{CO}_2\text{H})\text{CH}_2\text{CO}_2\text{H}$; α -carboxypimelic acid: $\text{HO}_2\text{CCH}_2\text{CH}_2\text{CH}(\text{CO}_2\text{H})\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$; aconitic acid: *trans*- $\text{HO}_2\text{CC}(=\text{CHCO}_2\text{H})\text{CH}_2\text{CO}_2\text{H}$.

pentaethyleneglycol (280). $\log K$ values are reported to be between 0.5 and 1.3 for the 18-crown-6 complex in water (279,281,282). Complexes are rather more stable in nonaqueous media (279,282–284). $\log K$ values are 2.2 and 4.0 for the 15-crown-5 and for the 18-crown-6 complex in methanol at 298 K (279,285), 3.4 and >5 for the 18-crown-6 complex in acetonitrile and in nitromethane respectively (283).

The stabilities of 1,10-diaza-18-crown-6 complexes (45,283, 286) are similar to those of their 18-crown-6 analogs; $\log K = 3.4$ in methanol for the Ca^{2+} complex of the parent ligand with NH in the 1,10 positions. Stabilities may be significantly increased by attachment of appropriate groups, such as $-\text{CH}_2\text{CH}_2\text{OH}$ or $-\text{CH}_2-2\text{-py}$ on the ring nitrogen atoms, giving so-called lariat crown ether ligands. These complexes are nearly as stable as that with the ligand having the two nitrogens joined by $-\text{CH}_2\text{CH}_2\text{OCH}_2\text{-CH}_2\text{OCH}_2\text{CH}_2-$, i.e. the cryptand [2,2,2] (see Section II.C.6 below). Considerably increased stability is shown by the attachment of methyl groups (286a) or of dipeptide arms to the two ring nitrogens. Stability constants for the calcium complexes of the ligands with pendant arms $-(\text{aa})_2\text{OEt}$, where aa = for example GlyGly, GlyAla, or GlyVal, are given by $\log K(\text{Ca}^{2+}) = 6.6, 7.8$, and 7.7 in aqueous solution at 298 K (44). Yet more stable is the diaza-18-crown-6 complex with $-\text{CH}_2\text{CO}_2-$ on each nitrogen, for which $\log K(\text{Ca}^{2+}) = 8.4$ in aqueous solution (287); the mal analog, with $-\text{CH}(\text{CO}_2^-)_2$ on each nitrogen, has $\log K(\text{Ca}^{2+}) = 7.5$ (288).

C.6. Selectivity

Table VI showed that several ligands exhibited selectivity for Ca^{2+} over Mg^{2+} and Ba^{2+} – this selectivity is small for oxalate and lactate, dramatic for the tetraazamacrocyclic tetracarboxylate dota. The familiar aminocarboxylate ligand edta is moderately selective for Ca^{2+} over Mg^{2+} ; replacement of the central $-(\text{CH}_2)_2-$ of hexadentate edta with $-(\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2-$, to give the potentially octadentate egta, leads to a very large increase in selectivity, with $\log K$ values for Ca^{2+} and Mg^{2+} of 11.0 and 5.2 respectively (220). Table VIII presents further stability constant data (240,281,282,289–292) relating to ligand selectivity for the alkaline earth metal cations; the maximum value for each ligand is highlighted. The central section of this table illustrates the prime importance of matching ligand cavity size to cation radius for cryptands. In the case of the oxine carboxylate complexes, it is the steric constraint imposed by the two linked five-membered chelate rings that causes the low

TABLE VIII

SELECTIVITY: STABILITY CONSTANTS, $\text{Log}_{10} K_1$, FOR A SELECTION OF COMPLEXES OF THE ALKALINE EARTH CATIONS^a

	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Ref.
dota	11.9	17.2	15.0	12.0	(240)
nota	9.7	8.9	6.8	5.1	(240)
Cryptands [2,1,1]	2.5	2.7	<2	<2	^b
[2,2,1]	<2	6.9	7.4	6.3	^b
[2,2,2]	<2	4.5	8.1	9.6	^b
[3,2,2]	<2	~2	3.4	6.0	^b
Oxine-2-carboxylate	4.1	6.0	4.5	3.9	(292)

^a $\text{Log } K_1$, in aqueous solution at 298 K, $I = 0.1$ M for dota, nota, and [2.2.2] complexes, $I = 0.05$ or 0.1 M for the other cryptates; the maximum value for each ligand is in **bold italics**.

^bRefs. (281,282,289–291).

stability of complex of the too-small Mg^{2+} ion. Comparison of the sequences of values for dota and for its triaza-tricarboxylate analog nota suggests that the strong favoring of Ca^{2+} over Mg^{2+} for the former is due both to size effects and to the matching of the potentially octadentate and hexadentate dota and nota to Ca^{2+} and to Mg^{2+} respectively.

Knowledge of stability constants is also important when considering speciation in multicomponent systems of pharmacological relevance. Thus the use of hydroxypyranones and hydroxypyridinones in chelation therapy for controlling iron or aluminum levels in the body (293) may lead to undesirable removal of calcium. Relative stabilities have also been established for Ca^{2+} , Zn^{2+} , and Gd^{3+} complexes in respect to the use of hexadentate hydroxypyridinonate ligands for administering gadolinium for enhancing MRI scans; selectivities can be tuned by small changes in the ligands (245,294,295). Relevant stability constants may be found in Table VI above. Similar concerns led to the establishment of stability constants for the Ca, Zn, and Cu complexes of a pentadentate ligand containing three pyridine rings linked through two $-\text{CONH}-$ bridges. These complexes exhibit both low stabilities and low selectivity. In this case the relevance was to the use of copper in the treatment of rheumatoid arthritis (296).

C.7. Enthalpies and entropies of complexation

The temperature dependence of stability constants has been documented for a few calcium complexes, giving estimates for

TABLE IX

ENTHALPIES AND ENTROPIES OF COMPLEX FORMATION WITH Ca^{2+}aq

	$\Delta H^\circ{}^a$	$\Delta S^\circ{}^a$
Acetate	+3.5	+30
Malonate	+7	+69
Succinate	+10	+76
Salicylate	+5	+31
Malate	-7	+30
Tartrate	-9	+27
Glycinate	-2	+22
L-Histidinate	+5	+44

^aUnits: ΔH° kJ mol⁻¹; ΔS° JK⁻¹ mol⁻¹.

enthalpies and entropies of complex formation – and also enabling access to values for the physiological temperature of 310 K as opposed to the standard temperature of 298 K. An example of the provision of stability constants at 310 K is provided by the modeling of the formation of calcium complexes (e.g. with sulfate, phosphate, oxalate) in urine (297). Enthalpies and entropies for the formation of a selection of complexes are given in Table IX (246). Formation of many of these rather weak complexes is entropy-controlled.

D. KINETICS AND MECHANISMS

D.1. Water exchange

Just as water exchange is too fast for the determination of a primary hydration number for Ca^{2+} so also it is too fast to be monitored by standard experimental techniques (298). Estimates from measured rate constants for complex formation (*v.i.*) range from 10⁸ s⁻¹ upward (236,299–301), a value of $\sim 5 \times 10^9$ s⁻¹ often being quoted (302–305). These estimates, along with those for Sr^{2+} and Ba^{2+} and in marked contrast to Mg^{2+} ($k_{298} = 6 \times 10^5$ s⁻¹), are amongst the largest for water exchange at aquacations (303,304). They are comparable with the rate constant derived from ¹⁷O NMR spectroscopy for Eu^{2+} , which is 5×10^9 s⁻¹ (304–306), and with the rate constant estimated for water exchange between the primary and secondary hydration shells of Cr^{3+}aq (307). Limited information on dynamics of ionic solutions can be obtained from IQENS (incoherent quasi-elastic neutron scattering); the neutron scattering spectrum reflects the exchange of water protons in and out of the first coordination sphere of metal ions. Unfortunately this technique only gives orders of

magnitude of timescales in the region of $\sim 10^{-10}$ s (308); Ca^{2+}aq , like the alkali metal cations, has $\tau \leq 10^{-10}$ s (consistent with other estimates of this upper limit (190,309)), whereas Zn^{2+}aq has $\tau > 10^{-10}$ s and Mg^{2+}aq (like many M^{3+}aq) has $\tau > 5 \times 10^{-9}$ s (310). There is also the complication that the rate of proton exchange may be significantly faster than the rate of exchange of whole water molecules. As for hydration numbers, the paucity of experimental information on exchange kinetics has encouraged computational approaches (311). Of particular interest is the estimation of a timescale of the order of 10^{-11} s for exchange of water molecules between the primary and secondary hydration shells of Ca^{2+} (190,192,312). Ca^{2+}aq makes the occasional appearance in a useful and comprehensible review of molecular orbital (MO) and DFT studies of water-exchange mechanisms of aqua-metal ions (313).

D.2. Complex formation and dissociation

a Formation. Reactions of Ca^{2+}aq with simple ligands are very fast. Very early T-jump and ultrasonics experiments indicated values of k approaching or exceeding 10^8 s^{-1} for e.g., chromate or glycinate (236), presumably for the ligand-for-water interchange step in the Eigen–Tamm–Wilkins mechanism (ACS).⁵ Subsequently values between 10^8 and 10^9 s^{-1} were reported for acetate (314), glycinate, oxinate, and iminodiacetate (236), while formation rate constants of $2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ have been quoted both for sulfate and for chromate (221,301), $7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ for the complexometric titration indicator xylenol orange (301,315), and again $7 \times 10^8 \text{ s}^{-1}$ for *o*-cresolphthalein complexone (221,236,315) and for the edta-phthalein analytical reagent for Ca^{2+} , Sr^{2+} , and Ba^{2+} (316). Rate constants between 2 and $8 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ have been reported for reaction of Ca^{2+}aq with eight fluorescent chelators, and their parent ligand bapta (T-jump, at pH 7.2, $I \sim 0.1 \text{ M}$, 293 or 295 K) (269,317).

At pH 7.2, 295 K, rate constants ($\text{M}^{-1} \text{ s}^{-1}$) for reaction of Ca^{2+}aq with the appropriately protonated forms of egta, hedta, and dtpa are 2.7×10^6 , 4.5×10^6 , and $\geq 4 \times 10^7$ (269). Rate

⁵It is sometimes unclear precisely what process these rate constants refer to, since units are given variously as s^{-1} or $\text{M}^{-1} \text{ s}^{-1}$. The former suggests that the quantity documented is the interchange rate constant (k_i) of the Eigen–Tamm–Wilkins mechanism (804); the latter suggests an experimentally based second-order rate constant (k_p). Chapters 4 and 5 of Caldin, E. F. “*Fast Reactions in Solution*”; Blackwell Scientific: Oxford, 1964 discuss the derivation of kinetic data from relaxation experiments. Most values were determined at 298 K but several at 293 K.

constants between 1 and $24 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ have been reported for reaction of Ca^{2+}aq with edta^{4-} and its analogs pdta^{4-} , bdta^{4-} , and cydta^{4-} (267,318,319); activation parameters are $\Delta H^\ddagger = 10 \text{ kJ mol}^{-1}$ and $\Delta S^\ddagger = -25 \text{ J K}^{-1} \text{ mol}^{-1}$ for reaction with edta^{4-} (318). The rate constant for formation of the sixth $\text{Ca}-\text{edta}^{4-}$ bond was estimated from ultrasonic experiments to be $3.6 \times 10^7 \text{ s}^{-1}$ (320). The similarity of this value to those for the analogous Sr^{2+} and Ba^{2+} complexes is consistent with conformational constraints by the edta ligand to the formation of the sixth link to the metal ion determining closure of this last chelate ring. As expected, reaction of Ca^{2+}aq with edtaH^{3-} is slower than with edta^{4-} . A T-jump determination at 295 K gave $k_f = 4.5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (269), NMR at 298 K gave $k_f = 3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ ($\Delta H^\ddagger = 13 \text{ kJ mol}^{-1}$ and $\Delta S^\ddagger = -56 \text{ J K}^{-1} \text{ mol}^{-1}$) (318), and estimation from stopped-flow determination of k_d and the known stability constant gave $k_f = 1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at 298 K (267,321). Reaction with egtaH_2^{2-} is somewhat slower again ($k_f \cong 2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$) (322,323). Reaction of Ca^{2+}aq with cydtaH^{3-} is about 500 times slower than replacement of coordinated water, suggesting an SCS (i.e. rate-limiting chelate ring closure) mechanism in this case (324). Reaction of Ca^{2+}aq with the macrocyclic aminocarboxylate dota is slower than with linear aminocarboxylates – $k_f = 7.9 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for reaction with dotaH^{3-} , $1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ for reaction with edtaH^{3-} under the same conditions (321). Added Ca^{2+} has a retarding effect on the Cu^{2+} plus edta reaction (319) and a marked effect on the kinetics of reactions of Pb^{2+} , Zn^{2+} , and Cd^{2+} with edta. Rate constants for these reactions have been measured in various real and model sea-water media (325).

Rate constants for reaction of Ca^{2+}aq with macrocycles and with cryptands (281,282,291) reflect the need for conformational changes, considerably more difficult for cryptands than for crown ethers, which may be considerably slower than formation of the first Ca^{2+} -ligand bond. Ca^{2+}aq reacts with crown ethers such as 18-crown-6 with rate constants of the order of $5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, with diaza crown ethers more slowly (286,326). The more demanding cryptands complex Ca^{2+} more slowly than crown ethers ($k_f \leq 10^5 \text{ M}^{-1} \text{ s}^{-1}$, Table X (290,327–329)), with particularly slow reaction for cryptands with benzene rings fused to the macrocycle. The dominance of k_d over k_f in determining stability constants is well illustrated by the cryptates included in Table X. Whereas for formation of the [2,1,1], [2,2,1], and [2,2,2] cryptates k_f values increase in order smoothly and gently, the k_d sequence $\text{Ca}[2,1,1]^{2+} \gg \text{Ca}[2,2,1]^{2+} \ll \text{Ca}[2,2,2]^{2+}$ determines the very marked preference of Ca^{2+} for the cryptand [2,2,1] (290).

TABLE X

KINETIC PARAMETERS FOR FORMATION AND DISSOCIATION OF Ca^{2+} COMPLEXES OF CRYPTATES^{a,b}

	$k_f/\text{M}^{-1} \text{s}^{-1}$	$\Delta H^\ddagger$	$\Delta S^\ddagger$	Ref.	k_d/s^{-1}	$\Delta H^\ddagger$	$\Delta S^\ddagger$	Ref.
[2,1,1]	260			(290)	0.82	51	-22	(290)
	410	1		(327)				
[2,2,1]	5900			(290)	0.00066			(290)
[2,2,2]	7300	33	-59	(328)	0.19 ^c	45 ^c	-110 ^c	(328)
					0.26	35	-140	(329)
[2 _B ,2,2]	2400	50	-12	(329)	0.38	38	-125	(329)
[2 _B ,2 _B ,2]	110	45	-59	(329)	0.24	41	-110	(329)

^aIn aqueous solution at 298 K; $\Delta H^\ddagger$ in kJ mol^{-1} ; $\Delta S^\ddagger$ in $\text{J K}^{-1} \text{mol}^{-1}$.^bValues also available for Sr^{2+} , Ba^{2+} plus [2,2,2], [2_B,2,2], and [2_B,2_B,2] (328,329).^cDietrich, B.; Lehn, J. M.; Sauvage, J. P. *Tetrahedron Lett.* **1969**, 2889-2892 gives similar values (and is the reference in which the name "cryptates" was first proposed).

Similarly, the considerably greater stability of $\text{Ca}[2,2,2]^{2+}$ than of $\text{Ca}[\text{diaza-18-crown-6}]^{2+}$ is almost entirely due to its much ($\sim 10^5$ -fold in methanol) slower dissociation (284,326).

The chapter on kinetics and mechanisms of complex formation and ligand substitution at alkali metal and alkaline earth cations elsewhere in this volume provides context and complementary discussion of these processes in relation to calcium.

b *Dissociation.* A relaxation study of Ca^{2+} - and CrO_4^{2-} -containing solutions indicated a rate constant of $2 \times 10^8 \text{s}^{-1}$ for dissociation of the complex $[\text{CaCrO}_4]$ (221). Dissociation rate constants (k_d/s^{-1} at pH ~ 7 , 295 to 298 K) for aminocarboxylate complexes decrease dramatically on going from dtpa ($\geq 3 \times 10^3$ (269)) through bapta (79 (269)), hedta (20 (269)), edta (1.28 (267) or 0.5 (318)), egta (0.4 (322) or 0.5 (269)), pdta (6.6×10^{-2} (267)), bdta (10^{-4} (267)), and cydta (3.0×10^{-5} (267)) respectively. The decrease in k_d is ascribed to increasing barriers to the internal rotation required to enable Ca-N bond breaking as one goes from edta through to cydta (267,324); a rate constant of $1.5 \times 10^7 \text{s}^{-1}$ has been estimated for breaking the first Ca^{2+} -ligand bond in dissociation of $[\text{Ca}(\text{edta})]^{2-}$ (320).

Rate constants for dissociation of calcium complexes of fluorescent chelating indicators cover a wide range, from 120s^{-1} to $27,600 \text{s}^{-1}$ (at pH 7.2, $\sim 295 \text{K}$) (269,317,330); $k > 2000 \text{s}^{-1}$ for Calcium Green FLAsH detailed in the calcium-monitoring section above (Section I.C) (65). Dissociation of the furaptra complex is fast enough, $k_d = 26,760 \text{s}^{-1}$ (269), to follow changes in $[\text{Ca}^{2+}]$ with a very advantageous delay time of less than $150 \mu\text{s}$.

D.3. Metal exchange and ligand exchange

Dissociation of a ligand from a complex may be spontaneous, acid-catalyzed, or consequent on the addition of an appropriate metal ion or ligand. Metal exchange, *viz.* $ML + M' \rightarrow M'L + M$, involves either dissociation of a complex ($ML \rightleftharpoons M + L$) followed by formation of a new complex ($M' + L \rightleftharpoons M'L$), or reaction through a binuclear ligand-bridged transition state or intermediate $M \cdots L \cdots M'$ (I_a or A). Ligand exchange is the complementary reaction $ML + L' \rightarrow ML' + L$. Again this may involve separation of L from M before bonding to L' commences, or may involve a binuclear metal-bridged transition state or intermediate $L \cdots M \cdots L'$. The dissociative pathway is also known as a *disjunctive* mechanism, the metal- or ligand-bridged pathways as *adjunctive*. These types of reaction are relevant to a number of biological systems involving metalloproteins, and to the biogeochemistry of natural waters (331).

An early example of metal exchange was provided by the replacement of Ca^{2+} in $[Ca(cydtA)]^{2-}$ by Pb^{2+} , which takes place by a dissociative mechanism. Rate constants ($k_2/M^{-1} s^{-1}$) for the acid-catalyzed path increase rapidly on descending the alkaline earth series, from 6.3×10^4 for Mg^{2+} through 4.1×10^5 for Ca^{2+} and 6.1×10^6 for Sr^{2+} to 1.1×10^8 for Ba^{2+} . These marked differences in reactivity formed the basis for a stopped-flow kinetic method for analysis of members of this series of cations (332). Cu^{2+} also replaces Ca^{2+} in $[Ca(cydtA)]^{2-}$, and in $[Ca(edta)]^{2-}$; the former reaction is sluggish and again dissociative in mechanism. Metal-exchange reactions of $[Ca(cydtA)]^{2-}$ are perforce slow and dissociative (disjunctive) due to the impossibility of binding two metal ions simultaneously to one cydtA ligand – though coordinated cydtA can be protonated and there is marked acid catalysis of $[Ca(cydtA)]^{2-}$ dissociation (324). The reaction of $Ca(edta)^{2-}$ with Cu^{2+} (at pH = 8.2) involves parallel disjunctive and adjunctive paths, demonstrated by the concentration dependence of k_{obs} in the presence of a varying excess of Ca^{2+} (329). The second-order rate constant, k_2 , is $970 M^{-1} s^{-1}$ for the associative pathway. Reaction of $[Ca(edta)]^{2-}$ with Cd^{2+} also involves parallel disjunctive and adjunctive paths, the former involving acid-catalyzed dissociation of $[Ca(edta)]^{2-}$ followed by rate-limiting reaction of Cd^{2+} with $edtaH^{3-}$, the latter having $k_2 = 230 M^{-1} s^{-1}$ (at 298 K) and proceeding through an intermediate with both Ca^{2+} and Cd^{2+} bonded to the edta (333). The kinetics of reaction of $[Ca(edta)]^{2-}$ with Tb^{3+} are consistent with a parallel two-step associative mechanism with a $[Ca(edta)Tb]^+$ intermediate and two-step dissociation of $[Ca(edtaH)]^-$ to Ca^{2+}

plus edtaH^{3-} followed by Tb^{3+} scavenging the released ligand. The $[\text{edtaTb}]^-$ entity dissociates from $[\text{Ca}(\text{edta})\text{Tb}]^+$ rather slowly; k_d is only $\sim 18 \text{ s}^{-1}$ at 298.2 K, much smaller than k_d for dissociation of Ca^{2+} from $[\text{Ca}(\text{edta})]^{2-}$ (334).

Ligand exchange at $[\text{CaL}]^{2-}$ is a second-order, i.e. associative, process for $\text{L} = \text{edta}$, with $k_2 = 74$, $\sim 4 \text{ M}^{-1} \text{ s}^{-1}$ at 298 K for $[\text{Ca}(\text{edta})]^{2-} + \text{edta}^{4-}$, edtaH^{3-} respectively) or 1,2-pdta, but first-order (i.e. k independent of $[\text{L}]$; dissociative mechanism) for cydta (267,318). The effects of Ca^{2+} on the kinetics of the reaction of $[\text{Cu}(\text{egta})]^{2-}$ with par (pyridine-2-azo-4'-dimethylaniline) show that a par-Cu-egta-Ca intermediate is important, indeed dissociation of such a complex is the rate-limiting step of one major pathway (335). Several metal- and ligand-exchange reactions are involved in the markedly retarding effect of Ca^{2+} , in sea-water concentrations, on a number of fast complex formation reactions of transition-metal ions (336).

D.4. Template reactions

The Ca^{2+} cation plays a relatively minor, but not insignificant, role in the large field of template synthesis of macrocyclic compounds – Ba^{2+} , Pb^{2+} , and a number of first-row transition metal cations dominate this field (337). The main area of application is in the synthesis of macrocycles from di- and polyamines and dicarbonyl compounds such as 2,6-dicarbonylpyridines. Thus, for example, Ca^{2+} is an effective template for the condensation of 1,3-diaminopropane (104) or of phenylenediamine (106) with 2,5-diformylfuran to give N_4O_2 -donor macrocycles. The Ba^{2+} complexes of these macrocyclic products are 11- and 12-coordinate respectively, but the Ca^{2+} complexes appear to be eight-coordinate. Here, as often, Ca^{2+} is a less effective template than Ba^{2+} , but elsewhere Ca^{2+} sometimes offers the advantage of leading to different products. Thus reaction of 2,6-diacetylpyridine with 1,3-diaminopropan-2-ol on a Ba^{2+} template gives a 20-membered macrocycle, whereas reaction on a Ca^{2+} template yields an 18-membered macrocycle (338), a difference consistent with the difference in ionic radii. Interestingly, reaction of these same two reagents on an La^{3+} template gives either the same 20-membered macrocycle as Ba^{2+} -templating or the tris- La^{3+} complex of a 30-membered macrocycle, depending on conditions (339). In other circumstances a Ba^{2+} template may give a macrocyclic product, a Ca^{2+} template a mixture of macrocycle and garland analog (340).

It is surprising that rather little use has been made of template reactions in relation to calcium biochemistry, possibly since the

majority of template procedures have been designed to give nitrogen-rich macrocycles. However, there have recently been several developments in this area. Large binucleating macrocycles with up to 52 ring atoms have been prepared on a Ca^{2+} template from *o*-phenylenediamine and α,ω -bis(3'-hydroxy-4'-phenoxy)alkenes (341), cryptate ligands have been templated on Ca^{2+} (342), and channel models have been developed on the basis of template synthesis of their constituents (see [Section IV.C](#)). Calcium may well play a templating role in the formation of structural features of melanin granules (343), while the organic dicarboxylates malonate, succinate, glutarate, and adipate act as structure-directing templating species on the hydrothermal formation of calcium carbonate; calcite, aragonite, or vaterite are formed depending on conditions (344). Squaraine dyes self-assemble in acetonitrile solution in the presence of Ca^{2+} to give spherical micellar species. A 1:1 complex has been characterized which, over a matter of hours, undergoes reorganization to give fiber-like cylinders – yet another example of the classical sequence of kinetic control giving an intermediate, but thermodynamics controlling the ultimate product (345).

The kinetics and mechanisms of template reactions are rarely studied in depth – they are often merely assumed to be genuine template reactions rather than a metal-activated reaction at a coordinated ligand. The best evidence for a true template mechanism is the isolation and characterization of intermediates with both reactants and with the macrocyclic product complexed with the template cation.

III. Calcium-Binding Metalloproteins

A. GENERAL

[Table XI \(346–390\)](#) lists a number of calcium-binding proteins and indicates very succinctly their role in biological systems. This table both illustrates the range of functions of calcium-binding proteins and serves to introduce those which appear subsequently in this chapter. The structures and functions of particularly important calcium-binding proteins such as calmodulin, parvalbumin, and troponin C are described in standard texts on biochemistry. The minimal [Table XI](#) entry for the particularly important calmodulins is amplified in the next paragraph. [Table XI](#) provides a sprinkling of references to enable readers to gain entry into the literature, for these and for most of the less-familiar species.

TABLE XI

INTERACTIONS AND FUNCTIONS OF SOME CALCIUM-BINDING PROTEINS

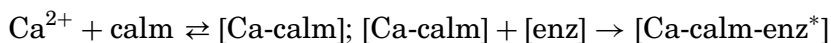
Protein ^a	Function or interaction
Aequorin	Bioluminescent protein which emits light on binding Ca^{2+} (346); has been used as a fluorescent chelator to monitor Ca^{2+} concentrations.
α -Amylase	Polysaccharide hydrolysis
Androcam	Signal transducer, similar to calmodulin in structure and function (347)
Annexins	Phospholipid- and membrane-binding proteins involved in the regulation of cell growth, coagulation, mediation of secretion, signal transduction, and ion channel activity; link signaling to membrane dynamics (348,349)
Ca^{2+} -ATPase	Provides the means of pumping Ca^{2+} selectively across membranes (350,351)
Cadherins	Transmembrane glycoproteins (352) promoting cell-cell adhesion (353,354); linked to integrins (355) and via catenins to muscle action (356)
Calbindin	Provides storage and buffering capacity for the regulation of Ca^{2+} concentrations (357)
Calcineurin	Calmodulin-dependent protein phosphatase; an important signal transducer (13,358,359)
CIB1	Regulates platelet aggregation in hemostasis (20,360,361)
Calmodulins	Activate a variety of enzymes and pumps – see text (362–364)
Calpactins; calactrin	Membrane-binding proteins (365)
Calpain	Protease controlling cell adhesion (366)
Calsequestrin	Sequesters calcium within the sarcoplasmic reticulum when muscles relax (80,367)
Calumin	Membrane-spanning calcium-binding protein involved in Ca^{2+} -handling and signaling in the endoplasmic reticulum (368)
DGBP	Galactose activation (369)
Lactalbumins	Lactose biosynthesis
Lysozymes	Catalyze degradation of peptidoglycan, e.g. of bacterial cell walls (370,371)
Melanins	Heavily pigmented chromoproteins in e.g. hair and eyes (366,372); may act as Ca^{2+} reservoir (373) and regulator (374)
Oncomodulin	β -Parvalbumin (375)
Osteocalcin	Extracellular protein abundant in bone and in dentin (376,377)
Parvalbumins	High-affinity calcium buffers
Phospholipase A ₂	Hydrolysis of phospholipids with the release of free fatty acids (378,379)
Pmr1	Yeast Golgi $\text{Ca}^{2+}/\text{Mn}^{2+}$ -ATPase; ion pump (380,381)
Proteinase K	Peptide fragmentation enzyme (382)
Prothrombin	Thrombin precursor; extracellular trigger; involved in blood clotting

TABLE XI (Continued)

Protein ^a	Function or interaction
Relaxins	Inhibit smooth muscle contraction; soften connective tissue (383,384)
S100 proteins	Triggering in nerve cells (74)
Staphylococcal nuclease	Catalysis of nucleic acid (RNA, DNA) hydrolysis to 3'-phosphonucleotides (385,386)
Surfactin	Bacterial lipopeptide biosurfactant; inhibits clot formation (387)
Synaptotagmins	Neurotransmitter release at synaptic membrane (388)
Synexin	Membrane-binding protein (365)
Troponin C	Regulates muscle contraction; complex formation triggers actin to realign and interact with myosin (389,390) – inhibited by relaxins, which are themselves Ca ²⁺ -binding proteins (<i>v.s.</i>)

^aCIB1 = Calcium- and integrin-binding protein; DGBP = D-Galactose binding protein; Pmr1 = plasma membrane ATP-ase related protein.

The calmodulins are perhaps the most important and widely-occurring family of calcium-binding proteins (364,391). They constitute some 0.1% of the total protein in cells; their location and level can be monitored by tagging with GFP (50). Calmodulins contain about 150 amino acid residues (bovine calmodulin contains 148 residues), having molecular weights of about 17 kDa. The structures of calcium-bound calmodulin, Ca₄calm, from mammalian calmodulin (392) and from *Drosophila melanogaster* (393) have been established, as has the structure of Ca-free calmodulin in solution (394). Calmodulin is dumbbell-shaped, with a seven-turn α -helix at its center. Ca²⁺-calmodulin complexes ([Ca-calm]) are trigger proteins, being the principal calcium-dependent regulators (391). They activate many enzymes ([enz] $\rightarrow$ [enz*]):



coupling changes in intracellular calcium concentrations to the state of activation of these enzymes. Examples of Ca²⁺-calmodulin-activated enzymes include nitric oxide synthase (395), many kinases (396,397), such as protein kinases (398,399), myosin light chain kinase (MLCK) (400), NAD kinase (for NADP synthesis), and phosphoryl kinase, phosphodiesterases, and adenylate cyclase (for cyclic-AMP formation). They also inhibit cyclic nucleotide-gated channels, which themselves mediate sensory transduction in the visual and olfactory systems (401). Binding Ca²⁺ to the calmodulin affects the

three-dimensional structure of the protein, increasing the degree of folding (402). The effect of these structural changes is for a larger hydrophobic area to be exposed for binding to substrates (403). The substrate is enclosed and clamped, and the system thus activated. This was demonstrated for a 20-residue peptide analog of MLCK, which occupies a tunnel lying diagonally within the Ca^{2+} -calmodulin ellipsoid (404). The X-ray structural determination of the C-terminal association domain of CaMKII (Ca^{2+} /calmodulin-dependent kinase II) revealed a pocket that could be a substrate docking site (405).

B. STRUCTURES AND CALCIUM-BINDING

Early structural determinations of calcium complexes of peptides and proteins have been collected in several reviews (6,78). Most calcium-binding proteins contain polypeptide chains of 100 or more amino acid residues, often consisting of several helical sections joined by loops containing potential Ca^{2+} -binding regions. Many have several such Ca^{2+} -binding sites (Table XII (82,348,406–412)). Some simple extracellular calcium-binding proteins bind only one Ca^{2+} , whereas structural and, especially, storage proteins tend to bind several or many Ca^{2+} ions. Often

TABLE XII

NUMBERS OF CALCIUM-BINDING SITES IN SELECTED METALLOPROTEINS

	Number of Ca^{2+} -binding sites
Trypsin; staphylococcal nuclease; concanavalin A	1
Phospholipase A_2 ; calbindin (ICBP ^a); parvalbumin	2
Calmodulin; troponin C; thermolysin; annexins	4 ^b
Synaptotagmins	5
Cadherins	8 or 9
Blood clotting proteins	5–20 ^c
S100 proteins	7–20
Calsequestrin	40–50 ^d
Phosphodentine	Many

^aIntestinal calcium-binding protein.

^bTroponin C from skeletal sources has four binding sites (406), but cardiac troponin C only three (407); rat annexin V has four binding sites (348), chicken annexin V has three (408).

^cProthrombin has been reported to have six strong and four or five weak Ca^{2+} -binding sites (82), though a later report suggests, on the basis of its Eu^{3+} -binding behavior, that it may only bind four Ca^{2+} strongly (409). Other blood coagulation glycoproteins have two high-affinity and 6 or 7 low-affinity Ca^{2+} -binding sites (Factor VIIa (411)) and 16 Ca^{2+} -binding sites (Human Factor IX (411)).

^dSpecifically 43 according to Ref. (412).

not all the potential Ca^{2+} -binding sites are filled. Parvalbumins and calmodulins have a dozen or so such sites, but generally bind only two, three, or four Ca^{2+} ions. In contrast, the storage protein calsequestrin can bind 40 or more Ca^{2+} ions (367). The number of occupied Ca^{2+} -binding sites may be source dependent (cf. Table XII footnotes). Bound Ca^{2+} has a significant effect on the structure of the protein, for this must adopt a conformation compatible with the coordination requirements of the metal ion. Often the coordination of successive calcium ions brings about a series of conformational changes in the protein, a sequence of events that may play a key role in the functioning of a particular calcium-protein control or messenger species. This has been demonstrated for, for example, annexin, troponin C, and calmodulin. In the case of calmodulin the conformational effects of binding Ca^{2+} have been monitored by a range of techniques, including NMR, circular dichroism, Raman spectroscopy, and fluorescence (413). Binding Ca^{2+} to calmodulin produces a large increase in the fluorescence of the two tyrosines, which are bonded to two of the bound Ca^{2+} ions (414). Ca^{2+} is implicated in some cases of protein folding and unfolding (415).⁶

Several metalloproteins contain more than one metal, Ca^{2+} to confer structural stability and a second metal ion for its catalytic properties. Examples include horseradish peroxidase and hemocyanin (Ca with Fe, Cu respectively to facilitate electron transfer), thermolysin (Zn for peptide hydrolysis) (416), and concanavalin A (417) (Mn for sugar activation (418)). Ca^{2+} is involved with binuclear Cu, Zn superoxide dismutase in the latter's secondary function as a promoter of nucleolytic hydrolysis, for example in hydrolysis of DNA. Here Ca^{2+} is presumed to act as a charge neutralization agent by interacting with the phosphate moiety of the substrate – however Ca^{2+} is inferior to Mg^{2+} in such a role (419). The plant kingdom provides an example of a three-metal system involving Ca^{2+} as a structural stabilizer. The blue color of cornflowers is due to protocyanin, an anthocyanin pigment incorporated into a supramolecular entity containing two flavone-bonded Ca^{2+} ions, one Mg^{2+} , and one Fe^{3+} . The Ca^{2+} ions stabilize the overall structure, while the Fe^{3+} and Mg^{2+} determine and tune the blue color – anthocyanins normally color flowers red (420).

Many calcium-binding proteins can be described in terms of loops containing potential metal-binding sites linked by helices of various shapes and lengths. There are several families of closely related proteins, in particular the groups of EF-hand species and

⁶The dynamics of Ca^{2+} -bound calbindin have been investigated by ^{13}C NMR relaxation measurements (Mulder, F. A. A.; Akke, M. *Magn. Reson. Chem.* 2003, 41, 853–865).

of C_2 domains. EF hands, which incorporate a well-conserved Ca^{2+} -binding site group, generally occur in pairs, or in pairs of pairs, in proteins (6). Included in the EF-hand group (421–423), which includes about three-quarters of calcium proteins, are calbindin (the simplest member), calmodulin, troponin C (70% of whose amino acid sequence closely corresponds with part of calmodulin), parvalbumin, protein kinase C, S100 proteins, CIB1, aequorin, and the Ca^{2+} -binding sites of the major Ca^{2+} trans-membrane crossing channels. They are characterized by four potential Ca^{2+} -binding sites in loops connecting six α -helical segments, termed AB, CD, and EF. The EF-hand portion consists of 29 amino acid residues – an α -helix, then the Ca^{2+} -binding loop, then a second α -helix (424). It gets its name (425) from a somewhat fanciful visualization of this section of the structure as a human hand holding a Ca^{2+} -binding loop in its palm with the E and F helices representing its thumb and forefinger outstretched. Despite the structural similarity between calmodulins and troponin C, the two high-affinity Ca^{2+} -binding sites in the former are different from those in the latter (426). The Ca^{2+} -binding sites of annexins and of phospholipase A_2 are similar, but differ from the EF sites in calmodulins. Overall there is a marked contrast between EF-hand proteins such as calbindin and parvalbumin which undergo only small conformational changes on binding Ca^{2+} and the group of Ca^{2+} -sensors, including calmodulin, troponin C, and S100 proteins, which change their conformation greatly on binding Ca^{2+} (391).

The C_2 domain group of calcium-binding proteins includes several membrane proteins, kinases, and phospholipases (427). The first C_2 domain was recognized, in a protein kinase, in 1988 – ten years later, more than 100 C_2 -domains had been registered in the collective database. Synaptotagmins (Syts), a family of more than a dozen proteins localized on the membranes of synaptic vesicles and involved in the release of neurotransmitters, are much-studied members of this group (388). They consist of an N-terminal trans-membrane region, a linker, and two C-terminal Ca^{2+} -binding domains where the Ca^{2+} is bound by aspartate and serine residues. The Ca^{2+} -binding sites are clustered together in the top loop of the protein. They are formed primarily by aspartate side chains that serve as bidentate and bridging ligands for two or three Ca^{2+} ions. The C_2A domain (428) consists of a compact $Ca_3(asp)_5$ unit linking two protein chains, each of about 130 amino acids; the C_2B domain (429,430) has three Ca^{2+} -binding sites but usually contains only two Ca^{2+} ions. The Ca^{2+} ions are in strongly distorted octahedral coordination (431). Binding Ca^{2+} to these proteins does not cause significant conformational

changes. These C₂-domain arrangements contrast markedly with the helix-loop-helix with one Ca²⁺ per loop of the EF-hand metalloproteins. The binding of Ca²⁺ in the C₂ domain of protein kinase C increases its affinity for anionic membranes; the Ca²⁺ probably acts as a bridge linking the C₂ domain with phospholipid head groups (399). Phospholipase C has both C₂ and EF-hand Ca²⁺-binding sites.

In all the calcium-protein complexes mentioned in this review the Ca²⁺ is bound solely through oxygen-donor atoms. This is in contrast to Mg²⁺, where both nitrogen donors (e.g. chlorophyll) and oxygen donors (e.g. phosphate groups) are biologically important. Ca²⁺ is manifesting its somewhat harder, in the hard and soft acids and bases (HSAB(222)) sense, character than Mg²⁺. Similarly, Ca²⁺, like Zn²⁺ and (this time) Mg²⁺, bind only to carboxylates in melanin, whereas Cu²⁺ can bind both to carboxylate and amino (432). In general, the oxygen donors to Ca²⁺ are provided mainly by carboxylate groups in protein side chains, carbonyl groups in peptide backbones, and water molecules. Usually the carboxylate groups come from aspartate or glutamate residues, but blood-clotting proteins such as prothrombin contain γ -carboxyglutamate residues which offer an additional CO_2^- -binding site and thus stronger interaction with Ca²⁺.

In biological species and systems, as in its inorganic compounds and complexes, Ca²⁺ may be six-, seven-, eight-, or even nine-, perhaps ten-, coordinate. Geometries for coordination numbers 6 and 8 are an octahedron and a square-antiprism respectively, for coordination number 7 usually a (somewhat distorted) pentagonal bipyramidal (occasionally a trigonal prism with a rectangular face capped, as in the EF site of parvalbumin, or an octahedron with one face capped). In practically all cases there are small, sometimes marked, distortions from the respective idealized geometries. In a few cases it is difficult to decide on the exact coordination number. Such a situation can arise when a carboxylate group has its two oxygen atoms at somewhat different distances from the Ca²⁺ with the more distant oxygen in a position suggesting significant Ca²⁺-O interaction but at a distance slightly more than normal. This is the case for a number of EF-hand proteins earlier described as octahedral, but whose inclusion of a bidentate carboxylate group as a single ligand disguises the essentially seven-coordinate state of the Ca²⁺. Similarly proteinase K is variously described as having seven- or eight-coordinated Ca²⁺, depending on whether the bidentate aspartate is counted as a single or chelating group. There can also be problems with difficult-to-locate or disordered water ligands, as in the case of annexins, where an apparent coordination number of 5

was found for the majority of calcium sites. In the rare cases where Ca^{2+} acts as a coordination catalyst, as in the case of staphylococcal nuclease, then at least one donor from the substrate or from water (or hydroxide) must be coordinated in the activated complex. The environment of Ca^{2+} in staphylococcal nuclease was probed by a model in which the enzyme was bound by the inhibitor thymidine-3',5'-diphosphate. The Ca^{2+} here was six-coordinated, by two aspartate carboxylates, one threonine (peptide-link-carbonyl), two waters, and phosphate (385).

C-type, i.e. Ca^{2+} -dependent, animal lectins are proteins whose interactions with carbohydrates on cell surfaces are central to cell adhesion (433). They contain carbohydrate-recognition domains that bind Ca^{2+} , which in turn binds carbohydrate moieties. Ca^{2+} binds at two sites in the carbohydrate-recognition domains of galactose- and of mannose-binding proteins, but sugars bind at only one of these Ca^{2+} ions (434). A crystal structure determination of the calcium complex with a rat mannose-binding protein (435) showed the Ca^{2+} ions to be seven-coordinated. On reaction with an asparaginyl-oligomannose substrate the water in an apical position on the potentially sugar-binding Ca^{2+} is replaced by the oxygens from the 3- and 4-hydroxyl groups of the mannose effectively occupying one of the apices of the pentagonal bipyramid, making this Ca^{2+} eight-coordinated.

Coordination numbers and coordination environments for a small number of calcium-binding proteins are given in Table XIII (369,378,382,425,436,437). The four Ca^{2+} -binding sites in EF-hand proteins consist of various combinations of glutamate (in some cases bidentate), aspartate, asparagine, threonine, tyrosine, serine, glycine, and water. Thus, for example, in calmodulin all four Ca^{2+} -binding sites are seven-coordinating, with one water ligand, two glutamates, and either two or three aspartates. The two aspartate sites also have an asparagine ligand; the seventh ligand is variously Thr, Phe, or Gln (392). The environments of all four calcium-binding sites in thermolysin have been established in detail; there are several bridging amino acid residues (416,438,439).

There is a considerable range in the number of waters coordinated to Ca^{2+} in its protein complexes. In most cases there are 0, 1, or 2 water ligands – generally 1 at EF-hand Ca^{2+} sites. Staphylococcal nuclease has $3\text{H}_2\text{O}$ per Ca^{2+} (385); hemocyanin has, probably, $5\text{H}_2\text{O}$ (440). The Ca^{2+} in the calcium complex of the aspartic proteinase rhizopuspepsin has $6\text{H}_2\text{O}$ (441), so that the metal site here is almost Ca^{2+}aq – the only other ligand is a main-chain glycyl carbonyl group in one of the axial sites of the pentagonal bipyramidal coordination shell of the Ca^{2+} .

TABLE XIII

Ca²⁺ COORDINATION ENVIRONMENTS IN SOME CALCIUM-PROTEIN COMPLEXES

Protein	CN ^a of Ca ²⁺	Coordination environment of the Ca ²⁺ ^b	Ref.
Concanavalin A	6	2 aspartates (bridging) 1 asparagine 1 tyrosine 2 waters	(436)
D-Galactose binding protein	7	2 aspartates 1 glutamate (bidentate) 1 asparagine 2 glutamine	(369)
Phospholipase A ₂	7	2 glycinate 1 aspartate (bidentate) 1 tyrosine 2 waters	(378)
Parvalbumin (CD site)	7 ^c	2 aspartates 2 glutamates (1 bidentate) 1 serine 1 phenylalanine	(425,437)
Parvalbumin (EF site)	7 ^d	3 aspartates 1 glutamate (bidentate) 1 lysine 1 water	(425,437)
Proteinase K	8 ^e	1 aspartate (bidentate) 1 proline 1 valine 4 waters	(382)

^aCN = Coordination number.^bCarboxylates are monodentate unless otherwise stated.^cPentagonal bipyramid.^dMonocapped trigonal prism.^eBut see text.

Ca–O distances in calcium–protein complexes cover a significant range as a result of variation in coordination number, irregular geometries around the cation, and varying strengths of metal–ligand bonding. Thus, for example, Ca–O distances in the CD and EF sites of parvalbumin range from 2.23 to 2.60 and 2.25 to 2.51 Å respectively, while Ca²⁺ in its rhizopuspepsin complex has five water-oxygens (and the carbonyl-oxygen) within the 2.29–2.51 Å range, but the sixth water-oxygen is at 2.70 Å (441). There is a very rough correlation between Ca–O distances and coordination number, though the ranges of values for each

coordination number are much wider than the differences between the means. Thus Ca–O distances for complexes of small biological molecules range from 2.29 to 2.51 Å for seven-coordinate Ca^{2+} and from 2.35 to 2.61 Å for eight-coordinate Ca^{2+} . Mean values in inorganic salts and complexes are 2.33, 2.39, and 2.45 Å for six-, seven-, and eight-coordination respectively; Ca^{2+} –O(water) distances range from 2.30 to 2.49 Å in various crystal hydrates (2.41 Å in aqueous solution).

Intercation distances between Ca^{2+} ions, and between Ca^{2+} and other metal ions, in metalloproteins containing more than one metal ion cover a wide range, as do distances between Ca^{2+} and active sites. In, for instance, Syts (442) and Ca^{2+} -ATPase (443), where pairs of ions are bridged by aspartate ligands, $\text{Ca}\cdots\text{Ca}$ distances are less than 6 Å. $\text{Ca}\cdots\text{Mn}$ distances are even less, between 4 and 5 Å, in concanavalin A (444,445) and in pea lectin (446). $\text{Ca}\cdots\text{Ca}$ distances are considerably greater in EF-hand proteins – the pair of Ca^{2+} ions near the C-terminal of troponin C are 11.3 Å apart (447). $\text{Ca}\cdots\text{Ca}$ distances, from Ca(1) to Ca(2), Ca(3), and Ca(4) in thermolysin (33,416) are 3.8, 11.7, and 29.6 Å; the shortest $\text{Ca}\cdots\text{Zn}$ is 13.7 Å (37). The pair of adjacent Ca^{2+} ions in thermolysin are bridged by three carboxylate groups, in concanavalin A the adjacent Ca^{2+} and Mn^{2+} ions are bridged by two carboxylates (416). Some of the interionic distances in calcium-containing proteins have been derived from X-ray diffraction structure determinations, but others have been estimated from fluorescence studies on samples in which some or all of the Ca^{2+} has been replaced by, for example, Pr^{3+} , Eu^{3+} , or Tb^{3+} . A degree of caution is required in relation to distances derived from these lanthanide-for-calcium substitution studies, as it has been shown that in the case of thermolysin Eu^{3+} binds 0.3–0.7 Å away from the Ca^{2+} it replaces, a change arising from the different coordination requirements of the two cations, Ca^{2+} favoring coordination numbers of 7 or 8, Eu^{3+} 8–9; higher (i.e. smaller) lanthanides cause smaller displacements (439). However, these luminescence methods, especially if results can be averaged over two or three different lanthanides binding at a given calcium site, can give useful estimates for $\text{Ca}\cdots\text{Ca}$ or $\text{Ca}\cdots\text{M}$ distances (448).

Calcium-binding sites are often some distance from the active site of a metalloprotein. The binding of Ca^{2+} has effects on protein structure many Å from its point of attachment, and can in this way affect substrate binding at a distance through conformational changes at the substrate interaction site. Thus, for instance, Ca^{2+} binding at the strong binding site of proteinase K affects the secondary substrate binding site 25 Å away, as well

as the catalytic Asp-His-Ser triad which constitutes the active site at about 17 Å (382). The polysaccharide-binding sites of concanavalin A and of DGBP are ~ 8 Å (445) and ~ 30 Å (369) from the Ca^{2+} , while the pair of Ca^{2+} ions in Ca^{2+} -ATPase are about 50 Å and 80 Å from the nucleotide binding and phosphorylation sites in the cytoplasmic domain (443). Long-range effects on conformation and binding sites are also evident in the Ca^{2+} -calmodulin-MLCK system (391).

C. STABILITY CONSTANTS

Stability constants for a selection of calcium-binding proteins are listed, roughly in order of ascending $\log K$ for the strongest Ca^{2+} -binding site, in Table XIV (449–466); a selection of enthalpies and entropies for such complexation is shown in Table XV (467–475). The values given in Table XIV have been obtained in various conditions, especially of ionic strength, and sometimes represent mean or recommended values – the references cited should be consulted for details both of experimental conditions and sources of the protein samples used. Stability constants for Ca^{2+} -protein interaction cover a wide range, from a value of $K \sim 1$ (i.e. $\log K \sim 0$) for interaction with gramicidin channels (476), through the rather low K values of 10^2 – 10^3 for albumin and storage proteins such as calsequestrin and melanin, up to values of K of 10^7 to perhaps 10^9 for lactalbumins, calbindin, and parvalbumins. $\log K$ values for EF-hand proteins cover a range from about 5 to 9 (460,477). $\log K$ for Ca^{2+} binding at the C-domain sites of EF hands varies more widely than $\log K$ for Ca^{2+} binding at N-domains. For calmodulin the difference between the C- and N-domain values of $\log K$ is 0.8, but for S100P the difference is just over 3.4 (478). Estimates have been made for the intrinsic affinities for Ca^{2+} binding at the various binding positions in Syt 1 (430). For the three sites in the C_2A domain $\log K(\text{Ca}1) = 4.3$, $\log K(\text{Ca}2) = 3.3$, and $\log K(\text{Ca}3) < 1.7$. For the two sites in the C_2B domain $\log K$ values are between 3.2 and 3.5; precise values could not be derived as they are too close together. Studies of Ca^{2+} interactions with muscle sarcoplasmic reticulum and with placenta plasma membranes have identified several Ca^{2+} -binding sites with various binding constants, though a site characterized by $\log K$ between 4.5 and 4.9 and associated with Ca^{2+} -ATPase seems to be common to all three systems (479–481).

$\log K$ values for Ca^{2+} -protein complexes do not seem to vary greatly according to source. Thus the Ca^{2+} complex of pigeon lysozyme is ten times more stable than that of equine lysozyme,

TABLE XIV

APPROXIMATE STABILITY CONSTANTS, $\text{Log}_{10} K$, FOR SOME CALCIUM-BINDING PROTEINS

	$\text{Log}_{10} K^a$	Ref.		$\text{Log}_{10} K^a$	Ref.
Albumin	ca. 2	(449)	Annexins	~ 6	(366)
Phosphodentine	3	(305)	Thermolysin	4.7, > 6	(450, 456)
Thrombin	3	(305)	Calmodulins	5.4 ^g , 6.2 ^h	(391, 449)
Calumin	3.1	(368)	CIB1 ⁱ	5.7, 6.3	(360)
Trypsin	3.4	(450)	IP ₃ receptor	6.1 ^j , 6.5 ^k	(16)
Osteocalcin	2.5–3.5	(376)	S100P	~ 4 , ~ 7	(458)
Phospholipase A ₂	3.4	(81, 450)	Glycoproteins ^l	~ 5 , ~ 7	(85)
Prothrombin	2.7, 3.5 ^b	(82)	Lysozyme	6–7	(459)
Melanin	~ 3 –3.5 ^c	(366, 451)	Calbindin	6.6, 7 ^m	(460)
α -Haemolysin	~ 3.8	(452)	Proteinase K	7.1 ⁿ	(382)
Calsequestrin	3–4 ^d	(367)	Prm1 ^o	7.2	(381, 461)
Concanavalin A	3.5 or 4.0	(453, 454)	Troponin C	5.5 ^g , 7.3 ^h	(406)
Calpactin	4 ^e	(365)	Oncomodulin ^p	6.1, 7.3	(462, 463)
Synaptotagmins	3.2–4.3	(388, 430, 431)	α -Lactalbumin	7.3 ^q	(459)
DGBP ^f	4.9	(455)	Myosin	7.5	(407)
Calpain	~ 5	(366)	Androcam	7.5 ^r	(347)
Surfactin	5.2	(387)	Parvalbumins	7–9 ^s	(464–466)

^aIn aqueous medium at (or close to) 298 K, $I = 0.1$ – 0.16 M, on the molar scale; quoted to a precision which reflects uncertainties, differences in conditions, and disagreements between different authors.

^bOther blood coagulation proteins have $\log K = 3.2$ (Human Factor IX; (411)) and $\log K = 3.0, 3.8$ (Factor VIIa; (410)).

^cSee Potts, A. M.; Au, P. C. *Exptl. Eye Res.* **1976**, 22, 487–491 and Larsson, B.; Tjalve, H. *Acta Physiologica Scandinavica* **1978**, 104, 479–484 for earlier reports on interactions of melanin with metal ions, including Ca^{2+} and other alkaline earth cations.

^dThe first description of calsequestrin (Ref. (80)) gave a significantly higher estimate of 4.4 for $\log K$. Interestingly, some use was made of hydroxyapatite in protein fractionation in this work.

^eSimilar values have been reported for other membrane-binding proteins, e.g. $\log K = 3.7$ for synexin I; $\log K$ for the electric fish protein calcitrin is between 5 and 6 (365).

^fDGBP = D-Galactose binding protein; for $\text{DGBP} + \text{Mg}^{2+}$, Ca^{2+} , Sr^{2+} , Ba^{2+} $\log K = 0.2, 4.9, 2.2, < 0.1$ respectively, making DGBP markedly selective for Ca^{2+} .

^gAt the N-domain.

^hAt the C-domain.

ⁱCIB1 = Calcium- and integrin-binding protein.

^jAt the cytosolic binding site.

^kAt the luminol binding site.

^lMitochondrial.

^m $\log K$ values are considerably higher at $I \sim 0$ – see text.

ⁿAt the strong binding site.

^oPrm1 = Plasma Membrane ATP-ase Related Protein.

^pOncomodulin = β -Parvalbumin

^qPrevious authors have reported $\log K$ values ranging from 6 to 9 (see pp. 216–218 of (371) – values seem to be strongly dependent on source and conditions).

^rAt 293 K; two calcium ions bind cooperatively at the C-domain, with $\log K = 7.3, 7.6$.

^sFor the two high-affinity sites – $\log K$ values between 5 and 6 have been estimated for the lower affinity sites of hake muscle parvalbumin (Ref. (464)).

TABLE XV
ENTHALPIES AND ENTROPIES OF COMPLEX FORMATION

	$\Delta H^{\circ a}$	$\Delta S^{\circ a}$	Ref.
Parvalbumins ^b	-16 to -56	-8 to +80	(467-469)
Equine lysozyme	-76	-131	(470)
α -Lactalbumin	-110 to -145	-273 to -325	(471-474)
Oncomodulin	-19, -19 ^c	+77, +56 ^c	(462)
Calbindin	-8	+134	(460)
Calmodulin	+5	+112	(475)
Calmodulin/mellitin adduct	+7	+158	(475)

^aUnits: ΔH° kJ mol⁻¹ or kJ site⁻¹; ΔS° J K⁻¹ mol⁻¹ or J K⁻¹ site⁻¹.

^bFrom carp muscle, bullfrog skeletal muscle, or toad; site 1 or site 2.

^cThe two values refer to addition of the first and second Ca²⁺ ions respectively.

but Ca²⁺ complexes of bovine and human lactalbumin are of equal stability (459). Stability constants for Ca²⁺ binding at bovine, goat, and human α -lactalbumin are very similar -2.6×10^8 , 2.8×10^8 , and 3.0×10^8 respectively (470). Table XV suggests that such similarities may arise from enthalpy-entropy compensation. For the parvalbumins, and indeed for the range of proteins in this table, complex formation with Ca²⁺ ranges from favorable enthalpy-dominated behavior to favorable entropy-dominated behavior. Mutant forms of calcium-binding proteins have dramatically lower stability constants (482).

Ionic strength may have a significant effect on stability constants. This is particularly the case for calbindin, where $\log K = 8.2, 8.6$ at $I \sim 0$, but decreasing to $\log K = 6.3, 6.5$ as ionic strength increases to 0.15 M KCl. This large ionic strength effect is attributable to the high charge product, about -14 , resulting from the negatively charged groups, whose resultant effective charge is about $7-$, in the vicinity of the Ca²⁺-binding site. These $|\Delta(\log K)|$ values of 1.9, 2.1 may be compared with $|\Delta(\log K)| = 1.4$ for analogous ionic strength dependences for complexes of 5,5'-Br₂bapta and of a related fluorescent chelator, for which the ionic charge product is $8-$ (268).

Several proteins with more than one Ca²⁺-binding site exhibit cooperativity, with $\log K$ for binding a second Ca²⁺ slightly higher than $\log K$ for binding the first Ca²⁺ ion. Such behavior can be ascribed to the conformational change in the protein induced by binding the first Ca²⁺ producing a favorable change in molecular geometry in the vicinity of the binding site for the second Ca²⁺. Cooperativity has been described for calbindin (483,484), where $\log K$ values are 8.2 and 8.6 for binding the first and second Ca²⁺ ions (268,460), while for androcam the values are 7.3 and 7.6

(at 293 K) (347). For sequential binding of four Ca^{2+} to calmodulin successive $\log K$ values of 5.5, 5.9, 5.1, and 4.7 (at 298 K, pH 7.5, $I = 0.1 \text{ M KCl}$) (485) and of 5.2, 6.4, 4.3, 5.3 (at 293 K, pH 8, $I = 0.1 \text{ M KCl}$) (347) also indicate marked cooperativity. The binding of Ca^{2+} to thermolysin is cooperative for the first two Ca^{2+} , which go into the Ca-1 and Ca-2 sites which are only $3.8 \text{ \AA}$ apart (456). Cooperativity precludes the generation of a 1:1 Ca^{2+} to calbindin complex, since adding limited amounts of Ca^{2+} to calbindin merely gives the 2:1 species. However the binding of Cd^{2+} to calbindin is not cooperative, so it is possible to make the 1:1 Cd^{2+} to calbindin complex and thus have access to a one-site-filled model for calmodulin (357,486).

A different sort of cooperativity is shown by the increased affinities for Ca^{2+} of some protein-substrate combinations over those of the respective proteins themselves. Thus K for association of Ca^{2+} with the 1:1 adduct of calmodulin with melittin, a strongly basic polypeptide from honey bee venom, is $\geq 10^7$, at least a hundred times higher than K for calmodulin alone under similar conditions (475). Analogous, though smaller, stability enhancements have been reported for the Ca^{2+} -calmodulin-cyclic nucleotide phosphodiesterase (485), Ca^{2+} -calmodulin-MLCK (487), Ca^{2+} -calmodulin-cytoskeleton protein model (488), Ca^{2+} -DGBP-galactose (455), and Ca^{2+} -Syt-phospholipid (388,431,489). The high stability of the adduct species in the last-named case can be ascribed to the fact that the Ca^{2+} can bind firmly to both components, the C_2 domain and the phospholipid docked over it (431). In other cases increased stability is the result of conformational changes in the protein consequent on binding the addendum – as in the cooperativity outlined in the preceding paragraph. In contrast some protein adducts have very low affinities for Ca^{2+} . Such a situation may be due to the addendum, for instance the antipsychotic drug trifluoperazine, binding strongly at the hydrophobic binding site on the calmodulin surface. This prevents the normal occupancy of this site by the activated polypeptide and suppresses Ca^{2+} binding to the calmodulin moiety (403).

D. KINETICS

D.1. General

Most calcium-binding proteins react rapidly with Ca^{2+}aq , though often the conformational changes in the protein required to effect binding to the metal ion have a significant, occasionally a dramatic, effect on formation rates. Some formation rate

constants, k_f , have been measured directly, others, particularly for fast formation reactions, have been estimated from binding constants and dissociation rate constants. Stopped-flow techniques may be used for moderately fast Ca^{2+}aq -protein reactions (481,490). Reactions of α -lactalbumin with Ca^{2+} can be monitored by stopped-flow fluorescence spectroscopy as this protein has four tryptophan residues whose exposure to solvent and thus level of fluorescence are greatly affected by the binding of Ca^{2+} to the protein (491) – similarly for parvalbumins (492). Faster processes can be studied by relaxation or NMR methods. Rate constants for loss or removal of Ca^{2+} from protein-bound species are often established by monitoring reactions with fluorescent chelators or ligands of the edta type, which bind Ca^{2+} considerably more strongly than do Ca^{2+} -binding proteins (cf. Fig. 2 in Section VII below).

Kinetic patterns may not be straightforward for those proteins which bind more than one Ca^{2+} ; biphasic kinetics are often observed, for formation and for dissociation. Metal exchange and replacement reactions at peptides and proteins may be complicated by possibilities of preferential binding of different metals at various sites on the substrate. For reactions involving the association or separation of charged species – i.e. practically all reactions in this section as $\text{Ca}^{2+}/\text{CO}_2^-$ interactions are usually a central feature – ionic strength will affect rates. A general approach to modeling reactions of Ca^{2+} with proteins has been developed with particular reference to calmodulin, parvalbumin, troponin C, and myosin (407). This paper, as also a similar paper dealing just with troponin C (493), contains useful tables summarizing numbers of Ca^{2+} -binding sites, stability constants, and rate constants for formation and dissociation.

D.2. Formation

Ca^{2+} reacts with bovine calbindin $\text{D}_{9\text{k}}$ with a rate constant (k_f) of $9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ at zero ionic strength; in 0.1M KCl k_f is considerably lower at $2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. The large ionic strength effect is attributable to the high effective charge product arising from the cluster of negative charges in the vicinity of the binding site (357). Reduction of the charge in the vicinity of the calcium-binding site in mutants with fewer carboxylate groups in this region leads to a marked reduction in k_f , which is reduced to $2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ when three of the negative charges have been neutralized. Lower stability constants for the binding of Ca^{2+} to such mutants (cf. Section III.C above) are determined by this k_f variation (484). Higher and lower affinity sites of calbindin-28k

bind Ca^{2+} with $k_f = 1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and $8 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, in 0.12 M KCl at pH 7.3 – rates very similar to those for Ca^{2+} reacting with egta under similar conditions (494).

The kinetics of the reaction between calmodulin and Ca^{2+} have been examined by stopped-flow and relaxation techniques, which revealed a two-stage process even when an excess of Ca^{2+} ensured the binding of four Ca^{2+} per calmodulin molecule (495). Rate constants were $\sim 1.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. These values are similar to Ca^{2+} -binding rate constants at the various sites of skeletal and cardiac troponin, which range from 4×10^7 to $3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (407). It is probably regulation by Ca^{2+} of troponin rather than myosin-ATP hydrolysis which is rate-determining in the onset of muscle contraction (496). Ca^{2+} binding at myosin is slightly slower ($k_f = 1.4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$) (407); for aequorin the kinetics of binding calcium are complicated by the series of processes which follow an initial fast step (346).

Reaction of Ca^{2+} with parvalbumin is believed to involve binding at the EF site first, then at the CD site, the order first established for reaction with Tb^{3+} (497). Early preliminary estimates for k_f were $1.4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ for binding the first Ca^{2+} , $10^7 \text{ M}^{-1} \text{ s}^{-1}$ for the second (498). Subsequent determinations of k_f gave values slightly greater than $10^8 \text{ M}^{-1} \text{ s}^{-1}$ at temperatures between 288 and 298 K (407, 493). Later, a complete set of eight rate constants was derived based on a stopped-flow fluorescence study carried out at 293 K, pH 9, $I = 0.1 \text{ M KCl}$, for the forward and reverse reactions for the four equilibria comprising the reaction of Ca^{2+} with whiting parvalbumin via initial binding to one or the other of the two Ca^{2+} -binding sites (499).

The kinetics of reaction of Ca^{2+} with apobovine α -lactalbumin, monitored by stopped-flow fluorescence, suggested a rate law intermediate between first- and second-order in $[\text{Ca}^{2+}]$. In 0.5 mM Ca^{2+} k_{obs} is 14 s^{-1} at 293 K, the activation energy for the reaction being about 70 kJ mol^{-1} (491). Subsequently, this reaction was reported to be biphasic with rate constants 7 and 2 s^{-1} in 0.5 and 0.1 mM Ca^{2+} at 293 K. The determined activation energy of 58 kJ mol^{-1} may be compared with $E_a = 48, 51, 93 \text{ kJ mol}^{-1}$ for reaction with $\text{Zn}^{2+}, \text{Mg}^{2+}, \text{Mn}^{2+}$ respectively – though not necessarily for binding at the Ca^{2+} -binding site (500). These rates are extraordinarily slow for complexation at Ca^{2+} , and may be presumed to be due to major conformational changes in the protein prior to binding. Indeed the kinetics of Ca^{2+} -induced refolding of α -lactalbumin have been established, and indicate a two-stage process with $k_1 = 6.0 \text{ s}^{-1}$; $k_2 = 1.3 \text{ s}^{-1}$ (501).

Apoconcanavalin reacts with Ca^{2+} (in the absence of Mn^{2+}) with a rate constant of about $10^5 \text{ M}^{-1} \text{ s}^{-1}$ over the pH range

5.0–7.2; the reverse reaction has $k_d = 3 \text{ s}^{-1}$. The rate constant for the initial reaction of apoconcanavalin with Mn^{2+} is $1.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$; subsequent reaction with Ca^{2+} ($k = 7 \text{ M}^{-1} \text{ s}^{-1}$, at 294 K) gives $[\text{MnCa}(\text{conA})]$ in its “unlocked” form. This slowly converts into the “locked” (native) form with $k \cong 0.02 \text{ s}^{-1}$. Loss of Ca^{2+} from the unlocked form is rapid ($k_{\text{off}} = 2.1 \times 10^4 \text{ s}^{-1}$, at 294 K), from the locked form is on the timescale of several hours. The magnitude of the free-energy change for the locked $\rightleftharpoons$ unlocked equilibrium is 92 kJ mol^{-1} (454,502–504). Reactions of $[\text{MnCa}(\text{conA})]$ with α - and β -D-monosaccharides are characterized by $k_f = 7\text{--}13 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, $k_d = 4\text{--}220 \text{ s}^{-1}$ (at pH 5.0, 298.2 K); as so often the stability constants of the adducts are controlled by dissociation rates (505).

D.3. Dissociation, displacement, and exchange

The kinetics of Ca^{2+} displacement or dissociation from calcium metalloproteins containing more than one Ca^{2+} are often bi- or polyphasic, reflecting stepwise loss of Ca^{2+} . Thus removal of Ca^{2+} from the EF-hand binding sites in its calmodulin complex by the fluorescent chelator quin-2 is characterized by two time constants. Loss of Ca^{2+} from the high-affinity sites (sites I and II) was reported to have $k = 24 \text{ s}^{-1}$, from the low-affinity sites (III and IV) $k = 240 \text{ s}^{-1}$, values later refined to 11.0 and 389 s^{-1} (506). ^{43}Ca NMR spectroscopy had earlier given estimates for rate constants for Ca^{2+} exchange at calmodulin of $k_{296} = 30, 1000 \text{ s}^{-1}$, with $\Delta H^\ddagger = 51$ and 43 kJ mol^{-1} (73). Loss of Ca^{2+} from the high-affinity sites of mutants in which one of the four binding glutamates is replaced by glutamine or by lysine occurs more rapidly, with k values up to 126 s^{-1} . Conformational changes consequent on these mutations are responsible for the modest decreases in calcium bonding which permit faster dissociation of the metal ion (506). The kinetics of dissociation of Ca^{2+} from calbindin (357) and from troponins (73,407,493) are less clear-cut, but are probably biphasic, and reaction is certainly rather slow, with k_d values reported as between 0.3 and 350 s^{-1} . As with calmodulin, dissociation of Ca^{2+} from troponin C is much faster, up to nearly 2000 times, for derivatives with amino acid substitutions or deletions (460).

Dissociation or displacement of the two Ca^{2+} ions from parvalbumin occur at rates amenable to study by stopped-flow techniques. The kinetics of these reactions have generally been found to be biphasic, as for example in the displacement of Ca^{2+} by Yb^{3+} (507), or dissociation engendered by the addition of edta or egta (498,508) or of a fluorescent indicator (508). Fluorescent

chelating indicators have also been used to establish decay time constants for Ca^{2+} transients and extrusion rates for parvalbumin-expressing cells (509). Rate constants are somewhat species dependent, though comparisons are tricky due to differences in temperature between various studies. However values are in the region of 5 and 1 s^{-1} at 298 K for the faster and slower steps (407,466,493,497–499,508,510), corresponding to dissociation from the CD and EF sites. The kinetics of displacement of Ca^{2+} from parvalbumin by a second metal ion M^{3+} ($\text{M} = \text{Eu}, \text{Tb}, \text{Yb}$) obey a two-term rate law, viz.:

$$\text{rate} = \{k_1 + k_2[\text{M}^{3+}]\}[\text{Ca}(\text{parv})]$$

The rate constant for the k_1 term equals that for reaction of $[\text{Ca}(\text{parv})]$ with cydta, consistent with rate-determining dissociation of $[\text{Ca}(\text{parv})]$ in both cases; the k_2 term may be assigned to an associative (adjunctive) process (497). This mechanism parallels that of parallel associative and dissociative pathways established for displacement of edta from $\text{Ca}(\text{edta})^{2-}$ by Tb^{3+} (cf. Section II.D.3 (334)).

Displacement of Ca^{2+} from bovine α -lactalbumin by bapta, monitored by NMR spectroscopy and stopped-flow techniques, is characterized by $k \sim 2\text{--}5\text{ s}^{-1}$ at 298 K (511). CD and DSC experiments confirm that partial unfolding of bovine α -lactalbumin is necessary before Ca^{2+} can be released (512). ^{43}Ca NMR has been used to obtain rate constants for Ca^{2+} exchange, displacement, or dissociation from several proteins (cf. calmodulin above). Ca^{2+} dissociation from myosin is rather slow, with $k = 0.5\text{ s}^{-1}$ (407), from chymotrypsin rather faster ($k = 70\text{ s}^{-1}$; for trypsin $k = 3\text{ s}^{-1}$, from stopped-flow experiments on displacement by an edta derivative (513)). ^{43}Ca NMR showed two types of calcium-binding site in S100 proteins (see above) with very different Ca^{2+} exchange rates – $k_{\text{off}} \sim 3 \times 10^{-3}$ and $\sim 10^2\text{ s}^{-1}$ (74). Variable-temperature ^{43}Ca NMR spectroscopy indicated the activation enthalpy for Ca^{2+} exchange at the moderately strong Ca^{2+} -binding site of phospholipase A2 to be 52 kJ mol^{-1} (73).

Materials such as humates, fulvates, and melanins are related to, or contain, peptide or protein molecules or moieties. Kinetic patterns for their interactions with metal ions may be complicated by the probability that more than one complex species will be involved. Thus it has been demonstrated that Ni^{2+} -fulvate solutions contain at least four kinetically distinct species, i.e. Ni^{2+}aq and three complexes, as indicated in reactions of such solutions with for example par (514). Moreover in a metal-exchange reaction the metal ions M and M' may complex at some

or all different sites (515,516). Therefore displacement of Ca^{2+} by Cu^{2+} from its humate complex may well have a different mechanism from the $\text{Ca}(\text{edta})^{2-}$ plus Cu^{2+} reaction mentioned above. There is some qualitative kinetic information on the $\text{Ca}^{2+}/\text{Cu}^{2+}/\text{edta}/\text{humate}$ system, in the form of a figure showing the time course of the Cu -humate plus Ca -edta system (331); the $\text{Ca}^{2+}/\text{Cu}^{2+}/\text{edta}/\text{par}$ system (335) may be an appropriate model. Calcium uptake, release, and binding in melanosomes has been investigated; calcium uptake is increased by ionophores (Section IV.B below) such as nigericin or A23187 but much reduced by the addition of Ruthenium Red (517).

Ruthenium Red (518,519), first described in 1892 (520) and soon thereafter shown to be a selective and permanent stain for vegetable tissues (521), is an intensely colored complex. Attempts to characterize this elusive species (522) were eventually successful – it is the trinuclear mixed valence complex $[(\text{H}_3\text{N})_5\text{RuORu}(\text{NH}_3)_4\text{ORu}(\text{NH}_3)_5]^{6+}$, which readily undergoes one-electron oxidation to the less strongly colored Ruthenium Brown $[(\text{H}_3\text{N})_5\text{RuORu}(\text{NH}_3)_4\text{ORu}(\text{NH}_3)_5]^{5+}$ (518). In recent decades it has been widely used as a sensitive oxidation indicator and a cytological (523,524) and polysaccharide stain (85). It has long been known to affect biological systems and models, for example mitochondrial calcium transport (85,525–528). It inhibits Ca^{2+} binding at calmodulins (528) and at calsequestrin – $\log K$ for the calsequestrin complex with Ruthenium Red is 7.2 (524) – and inhibits the effects of capsaicin on voltage-gated calcium channels (529) (cf. Section IV.C below). The $[(\text{H}_3\text{N})_5\text{RuORu}(\text{NH}_3)_4\text{ORu}(\text{NH}_3)_5]^{6+}$ cation is twisted but with a nearly linear NRuORuORuN axis in its thiosulfate salt, and almost certainly retains this near-linearity of the NRuORuORuN axis in solution (530) – and presumably on approaching a biosubstrate. It was suspected in some quarters (523,531), then demonstrated, that other ruthenium complexes, such as the binuclear $[\text{X}(\text{H}_3\text{N})_4\text{RuORu}(\text{NH}_3)_4\text{X}]^{3+}$ ($\text{X} = \text{OH}$ or Cl), may be rather better inhibitors than Ruthenium Red itself (528,532). Complexes of this type are present in technical Ruthenium Red. Ruthenium Red is still widely used in biochemical studies (cf. Sections III.D.2, IV.C, and V.B.2); its ethane-1,2-diamine analog is also available and has been characterized (533).

E. Ca^{2+} IN BLOOD CLOTTING

In blood clotting, the binding of Ca^{2+} by prothrombin anchors it to phospholipid membranes derived from blood platelets, thus bringing the prothrombin close to the proteins that mediate its

conversion into thrombin. Blood proteins involved in the clotting process contain γ -carboxyglutamate (gla) residues, whose additional carboxylate moiety enables them to bind Ca^{2+} significantly more strongly than glutamate residues. The blood-clotting mechanism is extremely complicated. An 80-reaction model has been proposed to cover the various activation, regulation, and feedback processes, with their associated loops, cascades, and other components (534), though progress is being made with much simpler models (535). Polyphosphate- Ca^{2+} interactions may well be important in activating blood clotting; polymers with $n > ca. 45$ stimulate blood coagulation. Polyphosphate seems both to accelerate formation of blood clots and to prolong their life (536).

IV. Ca^{2+} in Ionophores, Channels, and Pumps

A. TRANS-MEMBRANE TRANSPORT

There are three mechanisms for the transfer of metal ions across membranes. The first involves complexation of the ion by a suitable ligand (ionophore) that can carry the encapsulated ion in the form of a predominantly hydrophobic complex through the membrane. The second involves membrane-spanning proteins (537) that provide a hydrophilic channel with ligating atoms on its walls through which ions may pass. Such channels (350,538–540) often provide a high level of selectivity, and are subject to regulation by their opening and shutting. This regulation, or gating, may be controlled by membrane polarization changes (*voltage-gating*) or by the action of a messenger molecule or ion (*ligand-gating*).⁷ Transfer of ions across membranes by ionophores or through channels takes place down concentration gradients, i.e. is thermodynamically favorable. The third mechanism involves enzymes bound to the membrane which can pump ions through the membrane against the prevailing concentration gradient. Such ion transfer is driven by the energy provided by hydrolysis of ATP. Ion pumps transfer ions across membranes much more slowly than ionophores or channels – rates of membrane crossing are $\sim 10^2$ – 10^3 s^{-1} for pumps, $\sim 10^6$ – 10^7 s^{-1} for channels (though channel gating may slow transfer considerably). The rate at which Ca^{2+} exits channels has been estimated to be of the order of $1 \mu\text{s}^{-1}$ (10).

⁷See the [Appendix](#) for a discussion of the different meanings of the term “ligand” in relation to ion channels and in coordination chemistry.

For trans-membrane crossing of metal ions ionophores or ion-binding channel proteins or ligands of high coordinating power and high selectivity are needed. High stability is often achieved through electrostatic interactions between cations and negatively charged centers, often carboxylate groups, through utilization of the HSAB principle (222), the chelate and macrocyclic effects and, in the limit, encapsulation. Selectivity involves the matching of ionophore and channel geometry and configuration, and cavity size where appropriate, to cation size and coordination geometry preferences. There is also the need for peripheries with an appropriate hydrophiliclipophilic balance (HLB (541)) to match the surroundings, either for binding into, or for diffusion through, the membrane. Current work on cell-penetrating peptides (542) may lead to novel means for assisting the passage of metal complexes into cells.

Channels and ionophores are closely linked, as ionophores generally have fairly lipophilic peripheries, so they can be incorporated into membranes to form ion channels. Thus the channel protein gramicidin is a close relation of the ionophore valinomycin – the former consists of 15 amino acid residues, the latter 12. In fact membrane-spanning proteins need to contain rather more than 20 amino acid residues to span a normal double-layer membrane. The pores of these cation channels, defined by four, five, or six linked trans-membrane units, have walls bearing a number of hydrophilic charged groups, especially carboxylate, to facilitate the passage of ions such as Ca^{2+} , with any accompanying water molecules.

B. IONOPHORES

The ionophores of biological chemistry include peptides, catechols, hydroxamates, and monocarboxylic acids containing five- and six-membered cyclic ether rings – sometimes in a spiro system, sometimes with a potentially coordinating azaheterocyclic ring. Many ionophores are macrocyclic or encapsulating. The polypeptide and polyether ionophores are sophisticated versions of crown ethers and cryptands, having oxygen donors as far as possible for maximum hardness, and often a carboxylate group to augment their complexing ability. Several have been isolated from various *Streptomyces* species, including ionomycin (from *Streptomyces ribosidicus* (543)), septamycin (from *Streptomyces hygroscopicus* (544)), lasalocid A (X-537A (545,546); from *Streptomyces lasaliensis* (547)), lysocellin (from *Streptomyces cacaoi* (548)), ionomycin (from *Streptomyces globatus* (549)), and calcimycin (550). The last-named (A23187; from *S. chartreusensis*

(551)) was the first M^{2+} -selective ionophore (552). Many ionophores are effective antibiotics (553–555), affecting ion transport across bacterial membranes much more than across the more sophisticated membranes of human cells. Ionophores have proved useful in the investigation of such cell physiology processes as membrane permeability, secretion, and contraction (556).

The group of ionophores containing five- and six-membered cyclic ether rings and a carboxylic acid moiety includes monensin, nigericin, calcimycin, and lysocellin. Of these the last two are M^{2+} -selective ionophores; for the latter $K_1(Ca^{2+})$ is ~ 100 times larger than $K_1(K^+)$ (548). In the hydrated Ca^{2+} complex of calcimycin $[Ca(A23187)_2] \cdot 2H_2O$, each Ca^{2+} is bonded to two calcimycin anions which are hydrogen-bonded to each other; the cation is seven-coordinated. Each ligand provides two donor oxygens, from carbonyl and carboxyl groups, and a nitrogen from the benzoxazole moiety, while a coordinated water molecule completes the coordination shell (557). In $[Ca(A23187)_2] \cdot 2EtOH$ the Ca^{2+} has a coordination number of six – ethanol, in contrast to water in the dihydrate, does not act as a ligand (558). Lasalocid A, X-537A, forms $[Ca(X-537A)]$ and $[Ca(X-537A)_2]$ (559); NMR spectra of CD_3CN solutions indicate conformational and binding characteristics for the ligand and suggest a sandwich structure for $[Ca(X-537A)_2]$ (560). Ionomycin is, unusually, a diacidic ionophore as there is a second acidic proton, from a β -diketone group, as well as the customary $-CO_2H$ group; its Ca^{2+} complex has an octahedrally coordinated cation, though a seventh oxygen is nearby, at $Ca-O \sim 2.8 \text{ \AA}$ (549). Ba^{2+} -for- Ca^{2+} substitution has been used as an aid to structure-solving by X-ray diffraction techniques (cf. e.g. for lasalocid A, X-537A), but it would be dangerous to assume a coordination number of nine for Ca^{2+} in $Ca(X-537A)_2$ on the basis of nine-coordinate Ba^{2+} in both the monoclinic (30) and orthorhombic (32) forms of $Ba(X-537A)_2$. However, comparison between these structures and that of X-537A itself (545,561) does give some idea of conformational changes required for metal-ion binding for this ionophore. A series of ion-selective electrodes has been developed making use of a number of ionophores of this type, including lasalocid, monensin, and nigericin (562).

Tetracycline and several related antibiotics (aureomycin = chlorotetracycline, terramycin = oxytetracycline dihydrate) are also obtained from various species of *Streptomyces*, but are of very different structure, being based on the fused polycyclic arene naphthacene. The presence of five hydroxy substituents (six for terramycin) and an NMe_2 group, and possibilities for various conformations and tautomers, complicate the study of their

solution chemistry. However the speciation of tetracycline as a function of pH has recently been established (at pH 7 LH_3 and LH_2^- are present in almost equal concentrations) and stability constants as a function of pH determined, along with ΔH° and ΔS° by isothermal titration calorimetry (*v.s.*) (563). Plicamycin (mithramycin; aureolic acid) is a tetracycline-related oligosaccharide antibiotic, also from *Streptomyces* species, which lowers serum calcium levels. It, and several other antibiotics, are antineoplastic agents, possibly acting through interaction of a calcium–antibiotic complex with DNA (564).

There is also a group of cyclic oligopeptides that can act as neutral ionophores. This group includes the enniatins, beauvericin, and the cyclosporins, and hybrid species such as valinomycin, which has both peptide and ester linkages. Most are predominantly alkali metal cation carriers, but beauvericin (565,566) is a preferential Ca^{2+} carrier (567), showing moderate antimicrobial activity. It is an 18-membered-ring macrocycle comprising three L-*N*-methylphenylalanyl and three D- α -hydroxyisovaleryl residues alternating around the ring (568). When complexed it has a very hydrophobic exterior, thanks to peripheral methyl, isopropyl, and phenyl groups. Beauvericin acts by causing an influx of Ca^{2+} to increase intracellular Ca^{2+} concentrations two- to threefold, thereby causing cell death (569). The behavior of Ca^{2+} –valinomycin–acetonitrile and Ca^{2+} –lasalocid A–acetonitrile systems has been investigated, by NMR spectroscopy and by circular dichroism. Mono- and bis-ligand complexes are formed in both cases; the latter are of sandwich form (559,560,570).

Stability constants, measured in methanol solution, for alkaline earth complexes of a number of ionophores are given in Table XVI (280,289,571–577).⁸ The values for the complexes of valinomycin and enniatin B lie between the values for the crown ethers 15C5 and 18C6 (cf. Section II.C.5 above), for the middle four entries the values are slightly higher. Stabilities of enniatin B complexes show a modest maximum for Ca^{2+} , and of valinomycin complexes show stabilities increasing up to Ba^{2+} (281). Log K_1 values for the Ca^{2+} complexes of acetate, benzoate, and salicylate are between 4.5 and 4.7 in methanol (578) – the

⁸Stability constants for a series of M^{2+} cations, including Ca^{2+} , with A23187 and with ionomycin have been determined in methanol–water mixtures (Taylor, R. W.; Pfeiffer, D. R.; Chapman, C. J.; Craig, M. E.; Thomas, T. P. *Pure Appl. Chem.* **1993**, 65, 579–584); the estimation of stability constants for complexes of the anti-fungal antibiotic pradimicin (BMV-28864) with alkaline earth cations is complicated by the tendency of this antibiotic to aggregate (Hu, M.; Ishizuka, Y.; Igarashi, Y.; Oki, T.; Nakanishi, H. *Spectrochim. Acta A* **1999**, 56, 181–191).

TABLE XVI

STABILITY CONSTANTS ($\text{LOG}_{10} K_1$; IN METHANOL AT 298 K) FOR COMPLEXES OF IONOPHORES

	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Ref.
Valinomycin	<0.7	2.7	2.2	3.3	(281,289)
Enniatin B	1.2	3.0	2.7	2.9	(281,289)
Monensin ^a	5.2	4.5	6.0	7.1	(571)
Lasalocid (X-537A) ^a	4.0	4.8 ^b	5.6	6.6	(571)
X-14547A	4.8	4.8	4.1	3.6	(572)
Lysocellin	4.6	5.1			(573)
X-14885A	7.2	7.9	6.8	5.8	(574)
Calciomycin (A23187)	7.6	8.2	6.7	6.2	(572,575)

^aStability constants have also been reported for complexes of monensin and lasalocid in several other nonaqueous solvents (571).

^b $\Delta H^\circ = -20 \text{ kJ mol}^{-1}$; $\Delta S^\circ = -163 \text{ J K}^{-1} \text{ mol}^{-1}$ (576); $\Delta V^\circ = 40 \text{ cm}^3 \text{ mol}^{-1}$ (577); Ref. (562) gives values for K_1 and K_2 (in acetonitrile).

stability of the lasalocid (X-537A) complex (Table XVI) is thus very similar to that of its salicylate component.⁹ Pseudostability constants have been estimated for alkaline earth complexes of lasalocid in hexane, which solvates the predominantly hydrophobic exterior of the respective complexes; circular dichroism studies suggest that these complexes have different shapes in methanol and in hexane (579). For membrane-bound calciomycin (A23187), $\log K_1 = 5.2, 4.8, 3.9, 3.7$ for $\text{Mg}^{2+}, \text{Ca}^{2+}, \text{Sr}^{2+}, \text{Ba}^{2+}$ respectively; for vesicle-bound lasalocid $\log K(\text{Ca}^{2+}) = 3.5$ (580). The stability constant for the Ca^{2+} -lysocellin complex in water is very much smaller than in methanol (548); the tetracycline antibiotics adriamycin and daunomycin form considerably more stable complexes than lysocellin in aqueous media ($\log K_1 = 3.0$ (581), 3.4 (582)). The stability of Ca^{2+} -tetracycline increases markedly with pH; complex formation here is enthalpy-driven ($\Delta H^\circ = -58 \text{ kJ mol}^{-1}$, $T\Delta S^\circ = -40 \text{ kJ mol}^{-1}$ at pH 7.5; $\Delta H^\circ = -35 \text{ kJ mol}^{-1}$, $T\Delta S^\circ = -7 \text{ kJ mol}^{-1}$ at pH 11.7) (563). Several of the references cited give $\log K_2$ or β_2 values as well as $\log K_1$. For the Mg^{2+} and Sr^{2+} systems $\log K_2$ values are significantly higher than $\log K_1$, indicating cooperativity; for Ca^{2+} $\log K_2$ is just slightly smaller than $\log K_1$, but the smallness of the difference suggests a little cooperativity here too (572,575).

⁹However, the stability of the Ca^{2+} complex of the antibiotic squalastatin-1 is significantly greater than that of its citrate component (Bal, W.; Drake, A. F.; Jezowska-Bojczuk, M.; Kozłowski, H.; Pettit, L. D.; Sadler, P. J. *J. Chem. Soc. Chem. Commun.* **1994**, 555–556).

Ionophores such as A-23187 and X-14885A are flexible, so despite the need for conformational change, established for A-23187 by a ^1H and ^{13}C NMR solution study (556), their complex formation reactions can take place quite quickly as they can change their conformations rapidly as required for sequential bonding to the cation, and thus proceed in a series of energetically not-too-demanding steps. Formation rate constants for the Ca^{2+} complexes of A-23187 and X-14885A are 6×10^5 and $1.2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ respectively, for the Mg^{2+} complexes about ten times faster. However these complexes dissociate very slowly – $k_d = 2$ and 7 s^{-1} for A-23187 and X-14885A respectively; the Mg^{2+} complexes have $k_d = 0.3$ and 3 s^{-1} (574).

C. CALCIUM CHANNELS

Ca^{2+} may enter a cell by voltage-gated, ligand-gated, or store-operated channels (583,584) through the cell wall membrane; L-type voltage-gated channels may also be fine-tuned through secondary ligand modulation by protein kinases, which themselves may be stimulated by Ca^{2+} (398). There are also channels for the release of Ca^{2+} from the calcium stores within the cell (585). This release is either IP_3 -activated or Ca^{2+} -activated, according to which second messenger they respond. The IP_3 -release path is blocked by Ca^{2+} at high $[\text{Ca}^{2+}]$, weakly inhibited by decavanadate (586), and unaffected by Al^{3+} (587). All types of calcium channels are modulated by arachidonic acid, $\{\text{Me}(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4\text{CH}_2\text{CH}_2\text{CO}_2\text{H}\}$ (588), which is a constituent of membrane phospholipids, released by phospholipases (589).

Voltage-gated Ca^{2+} channels consist of four linked domains each consisting of six trans-membrane α -helices. The six helices plus all the various linking sections form a single polypeptide chain consisting of about 1600 amino acids; both C- and N- termini are inside the cell. The voltage sensor is in a helical section that contains an unusually large number of positively charged residues (arginine; lysine); most of the channel-cation interactions take place with a nonhelical portion of the polypeptide. Pore sizes for voltage-gated Ca^{2+} channels range from 5.1 to 6.2 Å (590). Ruthenium Red (Section III.D.3) affects the performance of calcium channels (529,591) by interaction with carboxylates at protein-binding sites. The Ruthenium Red is said to block the channel by forming a 1:1 complex at a site in the extracellular entrance to the channel pore, at least for certain neuronal voltage-gated calcium channels (592).

Store-operated Ca^{2+} channels (593), of which CRACs (Ca^{2+} -release activated channels) are the best characterized (594),

provide endoplasmic reticulum-to-plasma signaling. These channels are very specific for Ca^{2+} transport, showing a thousandfold selectivity for Ca^{2+} over alkali metal cations. Their minimum pore diameter is 3.9 Å, though they may be somewhat wider, nearer the voltage-gated channel diameter of 5–6 Å. The effective $\log K$ value for intrapore Ca^{2+} binding is nearly 5 (595). They deliver calcium at a slow but sustained rate ($\sim 10^4$ Ca^{2+} ions per second) over periods of minutes or hours, in contrast to voltage-gated L-type channels, which provide short bursts of Ca^{2+} ions ($\sim 5 \times 10^5$ Ca^{2+} ions per second). This IP_3 -connected system involves two Ca^{2+} -binding units, a trans-membrane protein Ca^{2+} -sensor and CRAC channel activator, STIM1 (593,596), and a novel Ca^{2+} channel subunit, Orai1 (597). The stromal interaction molecule STIM1 contains an EF-hand Ca^{2+} -binding site in the lumen of the endoplasmic reticulum, while Orai1 has four trans-membrane segments with Glu residues to provide Ca^{2+} selectivity (598). These CRACs are blocked by sphingosine, $\text{Me}(\text{CH}_2)_{12}\text{CH}=\text{CHCH}(\text{OH})\text{CH}(\text{NH}_2)\text{CH}_2\text{OH}$, whereas sphingosine-1-phosphate, a sphingolipid metabolite, is a calcium-mobilizer in cells (19,599).

Ca^{2+} is not only important in respect of its transport through channels, but also in that it can control ion channels. It has a self-monitoring role in the Ca^{2+} -controlled Ca^{2+} -release channels already mentioned, and also acts as “ligand” for one of the ligand-gated K^+ channels (the MthK channel) (600). Here it is bound by three carboxylate groups, from serine and glutamate or aspartate; the binding and release of the Ca^{2+} at this site cause the conformational changes that open and close the channel. Ca^{2+} can also control inter-cell channels – so-called gap junctions (601) – through which a variety of species can pass from the cytoplasm of one cell directly into the cytoplasm of another. These channels consist of six trans-membrane proteins (connexins) forming a cylinder with a channel, about 20 Å across, in the middle. In the presence of high cytosolic concentrations of Ca^{2+} the Ca^{2+} binds at one end of the gap junction, causing the array of connexins to tilt and thus greatly reduce the channel diameter and effectively close it.

An inorganic model for ion channels is provided by CaMo_5O_8 (Section I.D.2 (117)), a more biochemical model by the G-quadruplexes built up from the Ca^{2+} -templated G-quartets generated by self-assembly of guanosine molecules. Biology, supramolecular chemistry, and nanoengineering meet in G-quartets and quadruplexes (602,603). The first G-quartet was characterized in 1962, being a macrocyclic ionophore prepared by guanosine assembly on a cationic template (604). Since then, similar processes involving cation-templated self-assembly of

various nucleosides and oligonucleotides have been reported, and the biological significance of the products in relation to DNA and RNA noted (605). Guanosine-rich nucleic acid sequences can, through hydrogen-bonding, form planar G-quartets, which can stack to form G-quadruplexes. These have a central channel in which cations can reside, indeed the presence of metal cations is probably necessary to stabilize such arrays (602). Most of the work to date on these quartets and quadruplexes has been concerned with the alkali metal cations, especially K^+ , and Ba^{2+} , but recent studies have shown participation of Ca^{2+} ions. There is ^{43}Ca NMR evidence for the binding of Ca^{2+} in G-quartets derived from 2',3',5'-*O*-triacetylguanosine (TAG). A composition $Ca(TAG)_8$ was used to model the assembly, with the Ca^{2+} sandwiched between two G-quartets in eight-coordination, the eight oxygens coming from guanine-carbonyls at the corners of a square-antiprism (606). Ca^{2+} promotes the formation of the antiparallel form of the $d(T_4G_4T_4)$ quadruplex, induces an antiparallel $\rightarrow$ parallel structural change, and finally gives a parallel-stranded G-wire. This sequence involves a multistage mechanism involving the Ca^{2+} in binding, isomerization, and oligomerization of $d(T_4G_4T_4)$. The antiparallel $\rightarrow$ parallel step is induced by a pair of Ca^{2+} ions coordinating to guanine-O6 atoms. The stability constant for the Ca^{2+} - $d(T_4G_4T_4)$ complex is low (as are the stability constants for alkali-metal cation complexes of $d(G_3T_4G_3)$), at 60 M^{-2} (607).

Recently, the presence of two different ions in a quadruplex channel has been reported. Four Ca^{2+} ions and three Na^+ ions were detected, 3–4 Å apart, in a four-stranded DNA G-quadruplex ion channel assembled from $d(TG_4T)$. Both the Ca^{2+} and the Na^+ were found to be coordinated by six guanine-oxygens (608). It is interesting to compare these quadruplexes with species such as the stacked multimacrocyclic polyether complexes with spacers arranged to keep the mean planes roughly parallel and sufficiently far apart for each to bind a cation (609).

D. CALCIUM PUMPS

Ca^{2+} pumps are needed for the removal of calcium from cells, as in the maintenance of low Ca^{2+} levels in resting muscle, in bone deposition, and in biomineralization. The Na^+/Ca^{2+} exchanger and Ca^{2+} -ATPase (a Ca^{2+}/H^+ -exchanger) act in parallel to pump Ca^{2+} across membranes, the latter being considerably more effective than the former. X-ray structure determinations have been carried out both for Ca^{2+} -ATPase and for its Ca^{2+} -bound form (443). The latter contains two Ca^{2+} ions

in close proximity (aspartate-bridged; $\text{Ca}^{2+} \dots \text{Ca}^{2+} = 5.7 \text{ \AA}$), surrounded by four of the ten α -helices of the trans-membrane domain. This pair of Ca^{2+} ions is at least $50 \text{ \AA}$ from both the nucleotide binding and phosphorylation sites (cf. [Section III.B](#) above). Comparison of the structures of the free and Ca^{2+} -bound forms reveals the major structural changes involved in binding and releasing Ca^{2+} .

V. Carbohydrates and Carboxylates

A. STRUCTURES

There has long been interest in metal complexes of carbohydrates, particularly in view of the importance of the latter in food (610,611), and in calcium complexing with polysaccharides such as heparin, alginate, and glucans (612). Early structural determinations of calcium complexes of carbohydrates were reviewed in 1976 (78). By 1979 it had become clear, as detailed in an exhaustive (773 references) review of the coordination chemistry of alkaline earth and alkali metal cations, that coordination numbers of seven and eight were favored in carbohydrate complexes of Ca^{2+} (613). By 1983 more than 20 crystal structures of carbohydrate and sugar acid complexes of Ca^{2+} had been reported (612), and the general picture of Ca^{2+} -carbohydrate structures began to take shape – in particular the prevalence of coordination numbers greater than six, the frequently-encountered coordination of three-hydroxyl binding units, and the presence of waters coordinated to the Ca^{2+} . Almost all the complexes were of the form $\text{Ca}(\text{carbohydrate})\text{X}_2 \cdot n\text{H}_2\text{O}$ with $\text{X} = \text{Cl}$ or Br and n ranging from 0 (in just one case) to 7. The coordination number of the calcium ion is seven in four cases, eight in 14 cases, and nine in three cases. Except in the obvious exceptional case of the anhydrous compound, between one and four water molecules are in the primary coordination shell of the calcium; in only one case is there a halide coordinated to the calcium. This reluctance of Ca^{2+} to bind chloride or bromide rather than oxygen-donor ligands is reminiscent of crown ether complexes, as in the eight-coordinate $[\text{Ca}(15\text{C}5)(\text{H}_2\text{O})_3]\text{X}_2$ and nine-coordinate $[\text{Ca}(18\text{C}6)(\text{H}_2\text{O})_3]\text{X}_2$, where the halide ions $\text{X} = \text{Br}$ or I are outside the primary coordination shell of the Ca^{2+} (614). However perchlorate occasionally, and nitrate frequently, enter the coordination shell, as in eight-coordinate $[\text{Ca}(12\text{C}4)(\text{H}_2\text{O})_3\text{X}]\text{X}$, where $\text{X} = \text{ClO}_4$ or NO_3 (615), or nine-coordinate $[\text{Ca}(15\text{C}5)(\text{NO}_3)_2]$ (616).

The geometry around the calcium in the carbohydrate complexes may be described as distorted square-antiprismatic for the eight-coordinate complexes, and may be derived from the square-antiprism for the nine-coordinated and three of the seven-coordinated complexes. In its fructose complexes CaX_2 (β -D-fructose) $\cdot 2\text{H}_2\text{O}$, $\text{X} = \text{Cl}$ (617) or Br (618), Ca^{2+} is seven-coordinated, in geometry very close to pentagonal bipyramidal. Each calcium has two bidentate fructose ligands, one monodentate fructose, and two waters. The sugar conformation in these complexes differs significantly from that in the free state to accommodate this extensive interaction with the cation – every hydroxyl is coordinated to Ca^{2+} . In the great majority of monosaccharide complexes the sugar ligand is terdentate, but di- and polysaccharides offer possibilities for sugar ligands of higher denticity. Thus, for example, lactose is tetradentate in $\text{Ca}(\text{lactose})\text{Br}_2 \cdot 7\text{H}_2\text{O}$, binding the Ca^{2+} through O(3) and O(4) of the galactose moiety and O(2') and O(3') of the glucose moiety (619). In $\text{Ca}(\alpha$ -D-allopyranosyl α -D-allopyranoside) $\text{Cl}_2 \cdot 5\text{H}_2\text{O}$ the sugar is pentadentate, the Ca^{2+} being nine-coordinate (4 water ligands) (620). There are several other examples of crystal structure determinations for monosaccharide (Table XVII (621–624)) and disaccharide complexes. Disaccharide complexes include $\text{Ca}(\beta$ -D-mannofuranose) $\text{Cl}_2 \cdot 4\text{H}_2\text{O}$ (625), $\text{Ca}(\text{saccharose})\text{Cl}_2 \cdot 2\text{H}_2\text{O}$, and $\text{Ca}(\text{saccharose})_2\text{Br}_2 \cdot 2\text{H}_2\text{O}$ (626) – the last-named

TABLE XVII

COORDINATION NUMBERS OF Ca^{2+} IN A SELECTION OF ITS COMPLEXES WITH CARBOHYDRATES AND WITH SUGAR ACIDS

Coordination number	Ligand	Compound ^a	Ref.
Seven	D-Xylose	$\text{CaCl}_2(\text{xylose}) \cdot 3\text{H}_2\text{O}$	(621)
Eight	Arabinose	$\text{CaX}_2(\text{arabinose}) \cdot 4\text{H}_2\text{O}$	(622, 623)
	Galactose	$\text{CaBr}_2(\text{galactose}) \cdot 3\text{H}_2\text{O}$	(624)
	D-Galactarate ^b	$\text{Ca}(\text{galactarate}) \cdot 4\text{H}_2\text{O}$	(628, 629)
	D-Glucuronate ^c	$\text{CaBr}(\text{glucuronate}) \cdot 3\text{H}_2\text{O}$	(630)
	5-keto-D-Gluconate ^d	$\text{Ca}(\text{5-keto-D-gluconate})_2 \cdot 2\text{H}_2\text{O}$	(631)
Nine	D-Galacturonate ^c	$\text{NaCa}(\text{D-galacturonate})_3 \cdot 6\text{H}_2\text{O}$	(632)
	D-Glucuronate ^c	$\text{K}[\text{Ca}(\text{D-glucuronate})_3(\text{H}_2\text{O})_3] \cdot 2\text{H}_2\text{O}$	(633)

^aThe Ca^{2+} has between 1 and 4 water ligands in all of these compounds.

^bGalactarate and galacturonate are isomers of $^-\text{O}_2\text{C}(\text{CHOH})_4\text{CO}_2^-$; $\text{Ca}(\text{galactarate}) \cdot 4\text{H}_2\text{O}$ also contains eight-coordinate Ca^{2+} .

^cGlucuronate and galacturonate are isomeric forms of $\text{OHC}(\text{CHOH})_4\text{CO}_2^-$.

^d5-Keto-D-gluconate is $\text{HO}_2\text{C}(\text{CHOH})_3\text{COCO}_2^-$.

is unusual in having Ca^{2+} :sugar stoichiometry 1:2 rather than 1:1.

The salt $\text{Ca}(\text{lactobionate})\text{Br} \cdot 4\text{H}_2\text{O}$, where lactobionate = 4- β -D-galactosido-D-gluconate, provides a link between sugar and sugar acid complexes. Its Ca^{2+} ions are, as so often, in (slightly distorted) square-antiprismatic coordination (627). Table XVII gives the coordination numbers for Ca^{2+} in several sugar acid complexes (628–633). Whereas $\text{NaCa}(\text{D-galacturonate})_3 \cdot 6\text{H}_2\text{O}$ contains nine-coordinate (tricapped trigonal prism) Ca^{2+} (632), it is thought that the salts $\text{Na}[\text{Ca}(\text{D-galacturonate})_3] \cdot 4\text{H}_2\text{O}$ and $\text{K}[\text{Ca}(\text{D-galacturonate})_3] \cdot 3\text{H}_2\text{O}$ contain six-coordinate Ca^{2+} , with each D-galacturonate ligand bidentate and all the waters of hydration present as lattice water (633). Calcium glucuronate is important because it models Ca^{2+} interactions with oligo- and polysaccharides; while acids such as galactaric (galactosaccharic, mucic) and glucaric (glucosaccharic, saccharic), two of the isomers of $\text{HO}_2\text{C}(\text{CHOH})_4\text{CO}_2\text{H}$, have been used to model the bonding of ions to carboxylate-containing polysaccharides. The tetrahydrates of calcium galactarate and calcium glucarate both contain eight-coordinated Ca^{2+} in square-antiprismatic stereochemistry (628, 629, 634), as does calcium arabonate, $\text{Ca}(\text{O}_2\text{C}(\text{CHOH})_3\text{CO}_2)$ (635). There is FTIR evidence that galactarate bonds to Ca^{2+} through both $-\text{CO}_2^-$ groups (both monodentate) and through hydroxyl-oxygen (636). $\text{Ca}(\text{5-keto-D-gluconate})_2 \cdot 2\text{H}_2\text{O}$, identified in soils in the vicinity of roots of certain plants grown in chalky soil, has Ca^{2+} in the unusual geometry of a triakistetrahedron (631).

Carbohydrates and their derivatives can act as bridging ligands, as for example fructose in $\text{CaX}_2(\beta\text{-D-fructose}) \cdot 2\text{H}_2\text{O}$, $\text{X} = \text{Cl}$ or Br (617, 618) and galacticol, $\text{HOCH}_2(\text{CHOH})_4\text{CH}_2\text{OH}$, a metabolic derivative of galactose, in $\text{CaCl}_2(\text{galactitol}) \cdot 4\text{H}_2\text{O}$. This has a chain structure, with the Ca^{2+} eight-coordinate; all eight oxygen-donor atoms are from the galactitol (637). Both the mono- and the bis-dihydroxyacetone, $\text{HOCH}_2\text{COCH}_2\text{OH}$ (dha), complexes $\text{CaCl}_2(\text{dha}) \cdot 4\text{H}_2\text{O}$ and $\text{CaCl}_2(\text{dha})_2 \cdot \text{H}_2\text{O}$ are bis-dha-bridged dimers with both the Ca^{2+} ions eight-coordinated. Both the water and the chloride are in the first coordination sphere of the Ca^{2+} in $\text{CaCl}_2(\text{dha})_2 \cdot \text{H}_2\text{O}$; $\text{Ca} \cdots \text{Ca}$ distances are 4.31–4.33 Å in these two complexes (638).

NMR spectroscopy can give information about calcium-carbohydrate interactions in solution (639), demonstrating that cations such as Ca^{2+} , Sr^{2+} , and Ba^{2+} bind predominantly to 1,2,3-*ax,eq,ax* units of monosaccharides; this configuration giving three donor oxygens at the corners of an equilateral triangle in appropriate geometry for *fac* coordination to Ca^{2+} (640–642). However, even with this favorable geometry stabilities are low.

B. STABILITY CONSTANTS

B.1. Carbohydrate ligands

The coordination chemistry of carbohydrates has been reviewed from time to time, for example the early brief discussion of complex formation between metal ions and sugars (641) and a comprehensive (571 references) coverage of complexes of natural carbohydrates (643). Potential ligands with one or two hydroxyl groups – alcohols, diols, phenols – interact only weakly with Ca^{2+} ; even for glycerol and erythritol K_1 values of zero have been reported (272). Likewise sugars are reluctant to act as ligands (644), at least in solution (contrast, e.g., the above crystal structure section) unless they have at least three suitably disposed hydroxyl groups. K_1 values for formation of Ca^{2+} –sugar complexes are generally within the range 0 (e.g. xylose, mannose, galactose) to 10 – many are close to 1 (i.e. $\log_{10} K_1 \sim 0$) (Table XVIII (272,642,645–651)). The D-ribose system has been examined several times (647,652,653); the magnitude of the value quoted

TABLE XVIII

STABILITY CONSTANTS, K_1 , FOR Ca^{2+} –CARBOHYDRATE COMPLEXES^{a,b}

	K_1	Ref.
Lactose	0.2	(645)
L-Arabitol	0.4	(646)
D-Mannitol	0.3, 0.9	(272,646)
Maltitol	0.5	(646)
D-Lyxose	0.7	(647)
Sorbitol	0.8 ^c	(646)
D-Arabinitol	1.2	(272)
Xylitol	0.7, 1.2	(272,646)
D-Talose	1.4	(648)
D-Glucitol	1.5 ^d	(272)
Epi-inositol	2.2	(642)
D-Allose	3.2	(648)
Glucose	~5, 8.7	(648,649)
Fructose	~6, 8.7	(648,649)

^aIn aqueous solution at 298 K.

^bValues from Refs. (272,646) are derived from measurements of the solubility of calcium sulfate in solutions of the respective sugars (at 298 K, $0.2 < I < 0.8$ M). They are about ten times higher than earlier estimates in Ref. (650). The determination of stability constants from solubility measurements has been reviewed – see Ref. (225).

^c $K_1 = 1.2$ at 277 K, 0.6 at 309 K, determined by ^{13}C NMR in D_2O (651).

^dA considerably higher value of K_1 , approximately 10, was claimed for Ca^{2+} /glucitol interaction in the system 0.1 M $\text{Ca}(\text{OH})_2$ in 1 M D-glucitol.

depends on whether, and how, allowance has been made for the differing interactions between Ca^{2+} and the various anomers of this sugar. NMR spectroscopy suggested values of K to be 5.3, 3.4, 0.8, and ~ 0 for the α -furanose, α -pyranose, β -pyranose, and β -furanose forms (in D_2O at 300 K) (647). Of course the addition of Ca^{2+} to a solution containing the equilibrium mixture of sugar conformers will affect the equilibrium composition; equilibrium may be well be reestablished slowly. The low value of K for D-lyxose (Table XVII) reflects the unfavorable orientation for binding of the hydroxyl groups in its major conformer – the interaction-favorable *ax,eq,ax* conformer is a minor conformer. Interestingly, the sugars that form the Ca^{2+} complexes with the lowest stability constants are naturally occurring monosaccharides (654). The low stability constants for these carbohydrate complexes mean that the determination of their kinetic behavior is difficult. However it did prove possible to monitor reaction of Ca^{2+}aq with sorbitol – the estimated value for k_f , $1\text{--}2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ at 313 K (651), is similar to k_f values for other reactions of Ca^{2+}aq with simple uncharged ligands (Section II.D.2 above).

Methyl aldofuranosides are reluctant to complex Ca^{2+} . In aqueous solution, at 298.2 K, K_1 values range from 0.1 for methyl α -D-xylofuranoside to 1.4 for methyl α -D-ribofuranoside (46), though in methanol $K_1 = 120$ for the latter (655).

Enthalpies of interaction of Ca^{2+} with several aldopentoses and aldohexoses have been determined calorimetrically (652,653,656–658). Complex formation is generally characterized by the balance between a fairly large favorable enthalpy term and an almost equal but unfavorable entropy term – for ribose both ΔH° and $T\Delta S^\circ$ are -24 kJ mol^{-1} ; a value of $+5 \text{ cm}^3 \text{ mol}^{-1}$ for ΔV° can be understood in terms of electrostriction (658).

B.2. Hydroxycarboxylate ligands

Oxidation of D-glucose gives D-gluconate (terminal $-\text{CHO} \rightarrow -\text{CO}_2^-$), which forms a slightly stronger complex, with $K_1 = 17$ (273) or 23 (272). Despite the apparent weakness of Ca^{2+} -D-gluconate interaction, Ca^{2+} interacts with iron(III)-gluconate species to give modestly stable binuclear complexes, in which the Ca^{2+} has a significant effect ($|\Delta E| \sim 0.15 \text{ V}$) on the iron(III)/(II) redox potential (659). The determination of stability constants in this Fe^{3+} -D-gluconate- Ca^{2+} system adds a quantitative dimension to earlier reports of the isolation of solid calcium ferrigluconates (660). The Ca^{2+} -D-gluconate complex is slightly more stable than the 1:1 Ca^{2+} -D-lactate complex ($\log K_1 \sim 1$; Table VII).

Sialic acids (see [Section I.B.5](#)) are slightly better ligands for Ca^{2+} , with $K_1 = 121$ for *N*-acetylneuraminate, the only member of the group to have been found in human tissues (661). Binding of Ca^{2+} to the β -anomer is stronger than to the biologically relevant α -anomer (662). The stability constant for the Ca^{2+} complex of glucose-1-phosphate ($\log K_1 = 2.5$ (208)) is similar, of glycerol-1-phosphate ($\log K_1 = 1.4$ (663)) lower. Polysaccharide derivatives containing sulfate groups (sulfated galactans), carboxylate groups (hyaluronic acid), or both (chondroitin sulfate) give somewhat more stable Ca^{2+} complexes with $\log K$ values between 3 and 4 (28,664). Certain ruthenium and cobalt complexes have been reported to inhibit Ca^{2+} transport in mitochondria by selectively blocking membrane mucopolysaccharides (526). $[\text{Co}(\text{NH}_3)_6]^{3+}$ was found to be 100 times less active, $[\text{Co}(\text{en})_3]^{3+}$ 10 times less active, than Ruthenium Red ([Section III.D.3](#)).

Humic acid and fulvic acid both contain carboxylic acid groups – about 4 and 8 mmol g^{-1} respectively (665) – as well as phenolic hydroxy groups. Humate and fulvate bind Ca^{2+} , though much less strongly than transition-metal cations. Notional values for $\log K$ depend on how ligand binding and concentration (and molecular weight distribution (666)) are defined for these complex materials (667); $\log K$ values in the range 3.2–4.7 have been suggested (668). However it is clear that stability constants for humates and fulvates binding Ca^{2+} are similar to those for Mg^{2+} , but are around 10^5 times smaller than similarly-defined values for Cu^{2+} (516,668). It has been suggested that Ca^{2+} and Cu^{2+} bind to different sites (516,669); Ca^{2+} has a small but significant effect on Cu^{2+} -fulvate binding (669). Species containing both ions bound to a transiently bonded bridging humate or fulvate are likely intermediates in the adjunctive metal-exchange pathway mentioned in [Section II.D.3](#) above.

VI. Phosphates, Phosphonates, Nucleosides, and Nucleotides

A. INTRODUCTION

Most of this section will be devoted to summarizing information relating to the stability constants reported for complexes of this group of Ca^{2+} -binding ligands. However, we shall precede this main part with a short mention of a few relevant structures. Other properties of calcium phosphates and phosphonates will be mentioned in [Sections VIII.B.4 and VIII.D](#) below. An overall view of complexes of nucleosides, nucleotides, and nucleic acids is available (670).

B. STRUCTURES

B.1. Phosphates

Coordination numbers for the Ca^{2+} in various phosphates range from six, for example $\text{CaNa}(\text{H}_2\text{PO}_2)_3$, to eight, for example $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (671), with eight-coordination favored here as in many other areas of calcium chemistry. Anhydrous CaHPO_4 is of interest in that the Ca^{2+} occurs both in seven- and eight-coordination (99). Many calcium phosphates have sheet-like structures, including CaHPO_4 , $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, $\text{Ca}_2\text{PO}_4\text{Cl}$, and $\text{CaCl}(\text{H}_2\text{PO}_4) \cdot \text{H}_2\text{O}$ – this last having a corrugated sheet structure (119). There have been many reports of structures of various apatite species since the early structure determinations of fluorapatite (672) and hydroxyapatite (673); more recent structural work on a range of modified apatites is referenced in [Section VIII.C.2](#) below. Overall reviews of calcium phosphate structures range from the extremely succinct (674) to the comprehensive (675).

B.2. Phosphonates

Calcium methylphosphonate and hexylphosphonate both adopt layer structures. The Ca^{2+} in $\text{Ca}(\text{O}_3\text{PCH}_3) \cdot \text{H}_2\text{O}$ is seven-coordinated, approximately pentagonal bipyramidally, with one water-oxygen and five phosphonate-oxygens at 2.40–2.45 Å from the Ca^{2+} , and one phosphonate-oxygen at 2.71 Å. The hexyl compound, $\text{Ca}(\text{HO}_3\text{PC}_6\text{H}_{13})_2$, has its Ca^{2+} in a distorted octahedral environment (676). A detailed study of Ca^{2+} , Mg^{2+} , and Zn^{2+} complexes of a series of piperid-1-yl-methane-1, 1-bisphosphonates with methyl and ethyl groups variously substituted in the piperidine ring showed that these ligands bind only through oxygen – the ligand is in the *N*-protonated, $\equiv\text{NH}^+$, form in the complexes (677). Ca^{2+} is seven-coordinate in the dichloromethylenebisphosphonate (clodronate) complex/salt $\text{Ca}[\text{Cl}_2\text{C}(\text{PO}_3\text{H})_2] \cdot 5\text{H}_2\text{O}$ (678), but eight-coordinate in the phenylphosphonate $\text{Ca}(\text{HO}_2\text{CC}_6\text{H}_4\text{PO}_3\text{H})_2$ – where hydrogen-bonding to the carboxylates holds the 1-D ribbons together (679) – as in $\text{Ca}(\text{C}_6\text{H}_5\text{PO}_3\text{H})_2$ (680).

C. STABILITY CONSTANTS

Stability constants for Ca^{2+} complexes of several inorganic phosphate ligands have been given in [Table V](#) ([Section II.C.1](#) above), for Ca^{2+} complexes of glucose-1-phosphate and glycerol-1-phosphate ($\log_{10} K_1 = 2.5, 1.4$ respectively) in [Sections V.B.2](#). For

Ca^{2+} complexes of phenyl phosphate, 4-nitrophenyl phosphate, and *n*-butyl phosphate $\log K_1 = 1.5, 1.3,$ and 1.6 respectively, at $298\text{ K}, I = 0.1\text{ M NaNO}_3$ (681). The stability constants for several phosphate derivatives of amino acids and peptides, such as *O*-phosphoryl-serine, $^{-}\text{O}_2\text{CCH}(\text{NH}_2)\text{CH}_2\text{OPO}_3^{2-}$, and glycyl-*O*-phosphorylseryl-glycine, are in the range $1.5 < \log K < 2.2$ (682) – cf. $\log K = 1.5$ for the parent HPO_4^{2-} ligand (Table V). *O*-Phosphorylserine is the prosthetic group in the phosphoprotein phosvitin, which occurs in egg yolk. The caseins, phosphoproteins containing around 300 amino acid residues, occur in milk to the extent of about 3%. Casein-containing micelles are stabilized by colloidal calcium phosphate, CCP (683), presumably by interaction between $-\text{NH}_3^+$ groups of lysine or arginine in the casein and Ca^{2+} -coordinated phosphate. Two-thirds of the calcium in milk is in the form of CCP, the rest (“diffusible calcium”) is in solution – 8–10% as Ca^{2+}aq , the remainder complexed by ligands such as citrate. The effects of heat, pressure, low temperature, pH, and ultrafiltration on the balance between colloidal and diffusible calcium has been reviewed (684). Pressure effects on milk have been examined from time to time for over a century (685); a recent investigation focused on the role of pressure in gelation (686). Here the equilibrium between the casein–CCP micelles and their casein, Ca^{2+} , and inorganic phosphate components was probed by studying the effects of applied pressure on milk, milk–sugar, and milk– CaCl_2 solutions.

Table XIX contains stability constants for complexes of Ca^{2+} and of several other M^{2+} ions with a selection of phosphonate and nucleotide ligands (681,687–695). There is considerably more published information, especially on ATP (and, to a lesser extent, ADP and AMP) complexes at various pHs, ionic strengths, and temperatures (229,696,697), and on phosphonates (688) and bisphosphonates (688,698). The metal–ion binding properties of cytidine have been considered in detail in relation to stability constant determinations for its Ca^{2+} complex and complexes of seven other M^{2+} cations (232), and for ternary M^{2+} –cytidine–amino acid and –oxalate complexes (699). Stability constant data for Ca^{2+} complexes of the nucleosides cytidine and uridine, the nucleoside bases adenine, cytosine, uracil, and thymine, and the 5′-monophosphates of adenosine, cytidine, thymidine, and uridine, have been listed along with values for analogous complexes of a wide range of other metal ions (700). Unfortunately comparisons are sometimes precluded by significant differences in experimental conditions.

There has been much discussion of complexes between M^{2+} cations and nucleoside phosphates, which ligands can in principle

TABLE XIX

STABILITY CONSTANTS ($\text{LOG}_{10}K$, AT 298 K AND $I = 0.1$ M) FOR PHOSPHATE, PHOSPHONATE, NUCLEOSIDE, AND NUCLEOTIDE COMPLEXES OF SELECTED M^{2+} CATIONS

	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	$\text{Mn}^{2+} \rightarrow \text{Cu}^{2+}$	Zn^{2+}	Ref.
MeOPO_3^{2-a}	1.6	1.5			2.2 ^b	2.6	(687)
MePO_3^{2-c}	1.9	1.6	1.4	1.3	2.5 $\rightarrow$ 3.5	2.6	(688)
$\text{EtOCH}_2\text{PO}_3^{2-} (\text{PME}^{2-})$	2.0	1.7 ^d	1.4	1.3	2.6 $\rightarrow$ 3.7	2.7	(689)
PMEA^{2-e}	1.9	1.7	1.4	1.3	2.5 $\rightarrow$ 4.0	(2.7)	(689,690)
$\text{MeP}(\text{O}_2)\text{OPO}_3^{3-} (\text{MePP}^{3-})$	3.5	3.0	2.3	2.2	4.4 $\rightarrow$ 5.7	4.5	(691)
$\text{HOCH}_2\text{CH}_2\text{CH}(\text{PO}_3)_2^{4-}$	4.5	6.5	4.8	3.4	6.4 ^f	7.4	(692)
ribmp^{2-g}	1.6	1.5	1.3	1.2	2.2 $\rightarrow$ 3.0	3.2	(681)
$\text{AMP}^{2-h,i}$	2.0	1.9	1.8	1.8	2.5 $\rightarrow$ 3.2	2.8	(693)
ADP^{3-}	3.4	3.1	2.8	2.6	4.3 $\rightarrow$ 6.0	4.4	(693)
$\text{ATP}^{4-j,k}$	4.6	4.2	3.8	3.6	5.1 $\rightarrow$ 6.4	5.2	(693)

^aAt 293 K.

^bFor Mn^{2+} .

^c $\text{Log } K$ values for the analogous EtPO_3^{2-} complexes are almost identical (Ref. (689)); stability constants for methylene diphosphonate complexes are considerably higher – for Ca^{2+} $\text{log } K = 4.7$.

^dThe aminoalkylphosphonate, $\text{H}_2\text{N}(\text{CH}_2)_n\text{PO}_3^{2-}$ complexes have $\text{log } K = 1.7$ for $n = 1, 2$, or 3 (Woźniak, M.; Nowogrocki, G. *Talanta* **1979**, 26, 1135–1141).

^e PMEA^{2-} = adenine- $\text{CH}_2\text{CH}_2\text{OCH}_2\text{PO}_3^{2-}$.

^fFor Cu^{2+} .

^g ribmp = D-ribose 5'-monophosphate.

^h $\text{Log } K$ values for analogous complexes derived from cytidine, thymidine, or uridine are slightly lower – see Ref. (681).

ⁱ $\text{Log } K$ values for the isomeric 2', 3', and 5'-monophosphate complexes are almost identical.

^j $\text{Log } K$ values for analogous complexes derived from cytidine, thymidine, uridine, inosine, or guanosine are slightly lower; the extent of metal-to-ligand back-bonding to the pyrimidine bases in cytidine, thymidine, or uridine differs somewhat from that to the purine base in adenine (Refs. (229,694)). GTP^{4-} and ITP^{4-} occupy an intermediate position (Ref. (695)).

^k $\text{Log } K = 1.2$ for K^+ , 6.3 for La^{3+} (Ref. (693)).

bind through either of the imidazole-nitrogens, through phosphate-oxygen, or as bidentate O,N -donors (695,697,701,702). Bidentate behavior has been termed “macrochelation” (703), and may be exemplified by the model ligand ethoxymethylphosphonate, $\text{EtOCH}_2\text{PO}_3^{2-}$. This can be monodentate: $\text{EtOMCH}_2\text{PO}_2\text{---O}\cdots\text{M}$ or bidentate, bonding also through the ethoxy-oxygen. The percentage of the chelate or closed form for $\text{M} = \text{Ca}^{2+}$ is 28, for Mg^{2+} 40, and for Cu^{2+} 67 (689). The situation is similar, with similar percentages of the closed form, for e.g., $\text{M}(\text{ATP})^{2-}$ (695). Stability constants for complexes of Ca^{2+} and of several other M^{2+} with (1*H*-benzimidazole-2-yl-methyl)phosphonate (704) are similar to those for

MePO_3^{2-} or PME^{2-} (Table XIX), but the open $\rightleftharpoons$ closed equilibrium lies very much on the side of the chelated form of the complex (87% for the Ca^{2+} complex – compare 15% for $[\text{Ca}(\text{atp})]^{2-}$ and just 7% for $[\text{Ca}(\text{amp})]$ (695)). The availability of stability constants both for methylphosphonate and for benzimidazole (a purine model) complexes means that the chelate effect for complexes of (1*H*-benzimidazol-2-yl-methyl)phosphonate can be discussed without the usual complications, such as the differences between ethane-1,2-diamine and two ammonia or two methylamine ligands and disparities between units (704).

PMEApp, the diphosphorylated derivative of PMEA^{2-} {adenine- $\text{CH}_2\text{CH}_2\text{OCH}_2\text{PO}_3^{2-}$ or 9-[2-(phosphonomethoxy)ethyl]adenine}, is an antiviral agent, known as Adefovir. Its bis(pivaloyloxymethyl ester, Adefovir dipivoxil, has been approved by the US FDA for the treatment of hepatitis B (702,705). Microchelation in PMEA and in PMEApp complexes has been documented in a review of antiviral nucleotide analogs (706). $\log K$ values for methylphosphonylphosphonate, $[\text{MePO}_2\text{OPO}_3]^{3-}$, MePP^{3-} , complexes (691) are important because combining these $\log K$ values with $\log K$ for PMEA^{2-} complexes (690) is the nearest one can get experimentally to $\log K$ for PMEApp^{4-} complexes.

Enthalpies for complex formation of Ca^{2+} (229) and of several other cations of biological relevance (K^+ (707), Mg^{2+} (229), Mn^{2+} (708), Zn^{2+} (709)) with a selection of nucleotides are listed in Table XX (693); enthalpies of complexation of Ca^{2+} by several other nucleotides are within the range 4–14 kJ mol^{-1} (693). There are rather large disagreements between the calorimetrically determined values given in this table and values calculated from the temperature variation of stability constants, though admittedly there are often differences in ionic strength or added salt. Slightly disturbingly, the former, which may be traced through references given in Ref. (693), are in some cases intuitively less plausible than the latter.

TABLE XX

ENTHALPIES ($\Delta H^\circ/\text{kJ mol}^{-1}$) AND ENTROPIES ($\Delta S^\circ/\text{J K}^{-1}\text{mol}^{-1}$) OF COMPLEX FORMATION BETWEEN Ca^{2+} AND NUCLEOTIDE 5'-PHOSPHATES

	Mg^{2+}		Ca^{2+}		Mn^{2+}	Zn^{2+}	K^+
	ΔH°	ΔS°	ΔH°	ΔS°	ΔH°	ΔH°	ΔH°
ATP	18.1	138	13.5	116	19.2	16.3	1.3
ADP	17.5	121	13.8	101	14.7		
AMP-5	5.7	59	4.2	53	9.6		

D. KINETIC ASPECTS

Ca^{2+}aq reacts very rapidly with adp^{3-} and with atp^{4-} (236,710). Rate constants for onward reaction of $[\text{M}(\text{adp})]^{-}$ and $[\text{M}(\text{atp})]^{2-}$, $\text{M} = \text{Ca}^{2+}$, Mg^{2+} , or Mn^{2+} , with creatine phosphotransferase are similar – 1.7×10^6 , 5.3×10^6 , $7.4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ respectively (at 284 K) for the adp complexes, and indeed are similar to the value of $23 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ for the reaction of adp itself with creatine phosphotransferase (711). Kinetic parameters and binding constants have been established for interaction of the antiparallel form of the G-quadruplex $\text{d}(\text{T}_4\text{G}_4\text{T}_4)$ with Ca^{2+}aq and for the subsequent Ca^{2+} -promoted antiparallel $\rightleftharpoons$ parallel equilibrium (cf. Section IV.C above) (607).

VII. Stability Constants: An Overview

Values for stability (formation) constants for complexes of simple inorganic and organic ligands, of proteins, of carbohydrates and sugar acids, and of analytical reagents, have appeared at various points in the previous sections. In Fig. 2 we compare ranges of values and individual stability constants so that qualitative comparisons can easily be made. The values for biological ligands and analytical reagents on the left-hand side are conditional values for $\text{pH} \sim 7$ (cf. Section II.C.1); the values for the ligands in the ladder on the right are standard $\log K$ values (note that charges on the ligands have been omitted in the interests of clarity).

VIII. Calcium-Containing Rigid Materials

A. INTRODUCTION

CaCO_3 , CaF_2 , $\text{Ca}_3(\text{PO}_4)_2$, and its derivatives hydroxyapatite and fluorapatite, and calcium oxalate are insoluble in water. Several of these salts and their derivatives are of considerable importance in biological systems, conferring rigidity or firmness (cf. Section I.B.2, Table I above). Calcium carbonate and the ternary phosphates hydroxyapatite and fluorapatite are widespread in the animal kingdom, whereas calcium oxalate is found in various plants. Calcium carbonate and phosphates are important in the biogeochemistry of natural waters; under certain conditions of high organic content there is precipitation of such complex apatite materials as francolite, a fluorapatite in

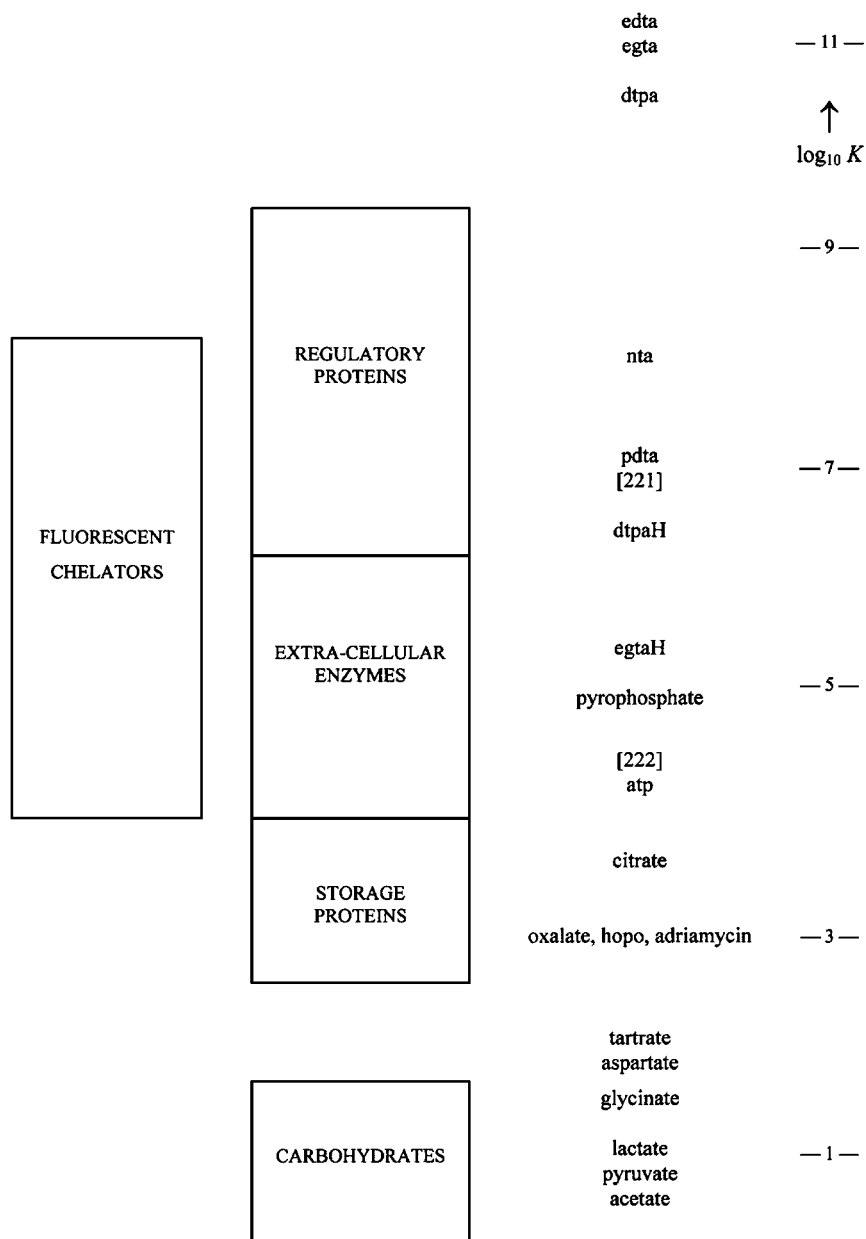


FIG. 2. Overview of stability constants ($\log_{10} K_1$, on the molar scale) for formation of calcium complexes in aqueous solution, at (or close to) 298 K and in ionic strengths in the region of 0.1–0.15 M.

which carbonate replaces some of the phosphate (712), from lakes or the ocean (713). These compounds are covered in individual sections below. Recently there has been considerable activity in relation to the treatment of disorders in bones and teeth, as outlined separately in [Section VIII.C](#) below. Conversely, several soluble salts are of importance in pharmaceutical preparations, as detailed briefly in [Section VIII.B.1](#) below. A wide-ranging, if somewhat uneven and dated, set of reviews deals with several aspects of calcification, covering bones, teeth, geochemical features, and metabolic disorders (714).

B. SALTS SOLUBLE AND INSOLUBLE

There are wide variations in the solubility of various classes of calcium salts in aqueous media. Thus, for example, the fluoride is insoluble, but the other halides are freely soluble. Among inorganic oxoanion salts the phosphate is insoluble, the sulfate and iodate sparingly soluble, but the chlorate and perchlorate dissolve in less than their own weight of water. The pH of the system is relevant, as hydrogen phosphate and hydrogen carbonate salts are water soluble. This is reflected in, for example, the widespread occurrence of hard water and in the technology for the manufacture of phosphate fertilizers from phosphate rocks. The solubilities of a number of inorganic biomaterials are given in [Table XXI](#) (715). The solubilities of several calcium salts, soluble and insoluble, have been measured,

TABLE XXI

SOLUBILITIES OF SOME INORGANIC BIOMINERALS

Compound	Formula	Solubility ^a	pK _{sp} (pH 7)
Calcium carbonate – calcite	CaCO ₃	2.5	8.42
Calcium carbonate – aragonite	CaCO ₃	3.2	8.22
Calcium carbonate – vaterite	CaCO ₃	2	7.6
Hydroxyapatite	Ca ₁₀ (OH) ₂ (PO ₄) ₆	~6 ^b	–
Fluoroapatite	Ca ₁₀ F ₂ (PO ₄) ₆	~0.1 ^b	–
Calcium oxalate	Ca(O ₂ CCO ₂)	^c	^c
Calcium sulphate	CaSO ₄ · 2H ₂ O	320	4.2
Strontium sulphate	SrSO ₄	49	6.5
Barium sulphate	BaSO ₄	1.4	10.0

^aAt 298 K; expressed as milligrams of M²⁺ per litre to permit direct comparison between the apatites and the other calcium salts.

^bPerforce approximate due to measurement difficulties.

^cpK_{sp} values are 8.6, 8.1, and 8.3 at 310 K for the mono-, di-, and tri-hydrate (Ref. (730)).

at 310 K, in relation to the elaboration of a computer model for complexation in (218), and precipitation from (297), urine.

Carboxylates in various forms are widespread in biological systems. The majority of their calcium salts are water soluble, though the presence of large hydrocarbon components, such as alkyl chains or phenyl rings, reduces solubility markedly – calcium cinnamate has water-solubility very similar to that of sparingly soluble calcium hydroxide (both $\sim 2 \text{ g dm}^{-3}$ at around ambient temperature). Calcium oxalate forms three hydrates, all of which are very sparingly soluble in water (91,716). The solubility of calcium oxalate in water is 0.167 mM, increasing to 0.27 mM in 5 mM MgCl_2 , and to 0.44 mM in 5 mM sodium citrate solution (at 310 K (91)). Solubilities of the calcium dicarboxylates $\text{Ca}[\text{O}_2\text{C}(\text{CH}_2)_n\text{CO}_2]$ increase greatly as n increases from 0 (oxalate) through 1 (mal) and 2 (succinate) to 4 (adipate), then decrease as n increases further. Presumably the initial increase is due to decreasing lattice energy as the anion increases in size, but at higher n the increasing hydrophobicity of the anions begins to dominate (126).

B.1. Soluble salts

Several of these are important in the treatment of hypocalcemia (cf. Section VIII.D below), or simply as tonics to help maintain healthy calcium levels in the body. Salts such as the gluconate, lactate, citrate, or lactobionate are probably effective through ease of absorption of organically complexed Ca^{2+} through the walls of the GIT, but such hydrophilic salts as the hypophosphite – and even insoluble calcium carbonate – are also used to treat calcium deficiency. Calcium glycerophosphate (water-solubility $\sim 20 \text{ g dm}^{-3}$) offers the double advantage of supplying both calcium and phosphorus; freely soluble calcium ascorbate similarly provides vitamin C as a bonus.

A number of water-soluble calcium salts provide convenient vehicles for the administration of therapeutic anions. Probably the most widely encountered is the acetylsalicylate of soluble aspirin (patented 1935); the urea complex of calcium acetylsalicylate (water-solubility 231 g dm^{-3} at 310 K, pH 4.8 (717)) is also widely used. Other examples include calcium bromide and bromolactobionate (sedatives), calcium 2-hydroxy-3-mercapto-1-propanesulfonateaurate(III) (chrysanol, antiarthritic), and calcium *N*-carbamoylaspartate (tranquillizer). Calcium iron(II) citrate has been used to remedy iron deficiency – it has the advantage of being stable to air oxidation of the iron(II). The cyclamate anion is certainly not therapeutic, but is relevant here.

Its freely water-soluble calcium salt (patented 1957) was a much-used and very effective sweetener, as was the sodium salt, whose sweetness was first noted in 1937; cyclamic acid is ineffective as it is essentially insoluble in aqueous media.

B.2. Calcium carbonate

Calcium carbonate is a minor component of several gravity and inertia sensors, for example in the human inner ear. It is the principle inorganic component of the shells of invertebrates. In the case of molluscs (e.g. limpets, mussels, cockles) and marine arthropods (e.g. crabs, lobsters, shrimps) the calcium carbonate forms a hard casing, whereas in echinoderms (e.g. starfish) the calcium carbonate is incorporated into numerous plates that are embedded in soft organic material. Small amounts of Mg^{2+} and/or Sr^{2+} may be incorporated into the CaCO_3 (which is somewhat reminiscent of the admixture of MgCO_3 and CaCO_3 in dolomite). All three forms of calcium carbonate – calcite, aragonite, and vaterite – are found in biological systems. Sophisticated control of precipitation must operate, for the energy and solubility differences between the three forms are extremely small – the Gibbs free energy of formation, ΔG°_f , of calcite is just 1.1 kJ mol^{-1} more negative than that of aragonite. Solubilities (718), stabilities (719), and rates of dissolution (720) of various mineral forms of calcium carbonate have been documented. Variation of the carbon dioxide concentration in the ocean has a significant effect on calcification – increasing partial pressure of carbon dioxide in the atmosphere may increase the efficiency of some marine CaCO_3 -producing organisms through increase in the concentration of the bicarbonate which is their carbonate source (721). Precipitation of calcium carbonate may be inhibited by fulvic acid and fulvates (722).

B.3. Calcium oxalate

Calcium oxalate (723) occurs as the monohydrate (whewellite, the thermodynamically stable form under ambient conditions (724)), the dihydrate (weddelite) in plant calcium stores and in sap, or the trihydrate (725). Calcium oxalate also plays a structural role in plants. Oxalate, for example from excessive amounts of rhubarb or spinach, inhibits absorption of Ca^{2+} from the GIT; precipitation of calcium oxalate is the reason for the toxicity of oxalates. Calcium oxalate may also occur in man, where it can appear as minute star-shaped crystals in the urine. It is the main constituent of the majority of urinary calculi in man (726,727). The relationships between dietary calcium

intake, urinary oxalate excretion, the deposition of calcium oxalate in urinary calculi, and inhibition of such deposition can be traced through Ref. (728) and the references cited in this paper. The taking of large amounts of vitamin C ($> \sim 0.5$ g per day) may lead to significant increase in oxalate levels in the urine, as oxalate is a metabolic oxidation product of ascorbic acid (729). Urine is supersaturated with respect to all three hydrates; studies of the nucleation and crystal growth of the trihydrate suggest that this may be the initial crystallizing phase when calcium oxalate precipitates from urine (730). Crystallization of calcium oxalate is inhibited by such species as polyaspartate and polyglutamate (731). Additives such as heparin have no significant effect on solubility (contrast citrate, Mg^{2+} above) but do affect crystal growth (91). In general which hydrate of calcium oxalate is the final product of crystallization depends on the absolute and relative concentrations of calcium and of oxalate, and indeed of any added solute(s), and on pH (727). Studies of crystallization of the various hydrates (725) have been complemented by studies of their kinetics of dissolution (727,732).

B.4. Calcium phosphates

Apatite (hydroxyapatite), $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$, along with some tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, is the main constituent of the skeletons, bones and teeth, of vertebrates (675,733,734). It is deposited in a matrix of the flexible fibrous protein collagen (three intertwined helices, with potential donor oxygens at the same spacing as in solid hydroxyapatite) to give rigidity; bone is $\sim 70\%$ by weight hydroxyapatite. As almost all the calcium in the human body is in the skeleton, this means that the calcium content of a child or adult is in the region of 1–1.5 kg. There is significant exchange between calcium in the skeleton and the small but vitally important amount of calcium in body fluids (less than 1 g). This exchange is regulated by three hormones, the steroid 1,25-dihydroxycholecalciferol (calcitriol; a metabolite of vitamin D) and two proteins, parathyroid hormone and calcitonin, secreted by the thyroid gland. A daily turnover of about 0.4 g of calcium per day corresponds to a mean residence time of the order of 8 years in the skeleton.

Solubilities and stabilities of calcium phosphates in natural waters have been described (735), as have the nucleation and growth of calcium phosphate from solution (736). Several species have long been known to inhibit the precipitation of calcium phosphates, for example carbohydrates (646) and statherin, the tyrosine-rich peptide which occurs in saliva (737). The role of

CCP in milk is mentioned in connection with casein above (Section VI.C). Fluorapatite is a major constituent of phosphate rocks, and a constituent, probably important, of human tooth enamel for those whose drinking water contains significant amounts of naturally occurring or added fluoride. Fluorapatite is significantly less soluble than hydroxyapatite – the relationship between the solubilities of fluorapatite and hydroxyapatite parallels (but is much less extreme than) that between calcium fluoride ($K_{\text{sp}} = 3.9 \times 10^{-11} \text{ mol}^3 \text{ dm}^{-9}$) and calcium hydroxide ($K_{\text{sp}} = 7.9 \times 10^{-6} \text{ mol}^3 \text{ dm}^{-9}$). Calcium diphosphate, $\text{Ca}_2\text{P}_2\text{O}_7$, is believed to be the least soluble of the calcium phosphates.

C. BONES AND TEETH

C.1. *Properties*

Collagen–mineral (hydroxyapatite, $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$; tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$) interactions are of primary importance to the strength of calcified tissues such as bones and teeth (738). The compositions of the dentin, enamel, and cementum of human teeth – in terms of apatite, organic material, water, and hydroxyl content – have recently been detailed in connection with the determination of their hydroxyl contents by magic-angle spinning NMR (739). Tooth enamel may contain up to 90% hydroxyapatite. The structure, nucleation, and growth of dentin have been studied by SAXS (small-angle X-ray scattering) (740); even now very little is known about mechanisms of regulation in dentin formation (741). The effects of various dietary constituents on dental caries have been outlined (742), in particular the cariostatic effects of cations – especially Ca^{2+} – and of a range of inorganic and organic phosphate species (743).

Organic matrices may induce elaborate morphologies, as for example the nanocluster structure of calcitic sponge spicules, which despite their complexity show some characteristics of calcite single crystals (744). The organic dicarboxylates malonate, succinate, glutarate, and adipate act as templates in the hydrothermal formation of calcium carbonate, controlling the formation of calcite, aragonite, or vaterite. Hydroxyaminocarboxylates such as hdtpa, 1,3-diamino-2-hydroxypropane-*N,N,N',N'*-tetraacetate have an even more marked effect than the dicarboxylates on the morphology of the calcite produced (344).

C.2. *Substitution of constituent ions*

The properties of apatites can be modified by partial or complete replacement of constituent ions – structural variations

in natural hydroxy-, fluoro-, and chloro-apatites have been described (745,746). The importance of fluoride replacing hydroxide in the hydroxyapatite of teeth has long been recognized. Fluorapatite, $\text{Ca}_{10}\text{F}_2(\text{PO}_4)_6$, is both less soluble and harder than hydroxyapatite, hence the use of fluoride (actually often fluorophosphate, hexafluorosilicate, or tin(II) fluoride) in toothpastes. Crystalline fluorapatite with a structure very similar to human dental enamel has recently been prepared hydrothermally (747). It has emerged that chloride substitution into hydroxyapatite has the effect of improving bone resorption (748) – the optimum chloride content is only a little more than the level in natural bone (where the natural chloride content is slightly more than 0.1%), very much less than in chloroapatite (6.8% chloride). The effect of chloride may arise from its effect on local pH. Cosubstitution of chloride and fluoride into hydroxyapatite can be achieved by controlled precipitation techniques. This may offer the best of both worlds – dental protection and bone resorption – especially as this approach gives very small crystallites, comparable in size with natural bone mineral crystallites (749). It is quite difficult to determine such small dimensions, $< 50 \text{ nm}$, because of interference from lattice imperfections which, along with the small crystallite dimensions, produce line broadening in samples from biological sources such as bone (750).

Strontium can be incorporated up to 100% into hydroxyapatite with merely a small increase in lattice dimensions, but it is not possible to incorporate more than about 10% of magnesium without significant structural change (751). The structures of strontium- and manganese-containing apatites have been investigated (752). The incorporation of small amounts of strontium into hydroxyapatite increases its solubility, and can have a beneficial effect on bone formation (100). The incorporation of Sr^{2+} into hydroxyapatite permits monitoring of active biomineralization in bones or teeth by SIMS. This approach obviates the problems associated with conventional monitoring with calcium-binding fluorescent dyes (see Section I.C) – Ca^{2+} -dye complexes are generally designed for rapid response to changes in Ca^{2+} concentration, so are inconveniently labile for monitoring the longer term effects of active biomineralization (34). Energy-dispersive X-ray fluorescence spectrometry (EDXRF) can also be used for the determination of strontium levels in teeth (753).

C.3. *Repair and replacement*

Brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, is more soluble and more readily resorbed under physiological conditions than hydroxyapatite.

Conventional bone grafting cements produce brushite from β -tricalcium phosphate and orthophosphoric acid, but set too rapidly. The replacement of orthophosphoric acid by pyrophosphoric acid gives a slower-setting cement, the resulting microstructure of whose brushite crystals gives a more readily resorbable material (754). The role of connexins and gap junctions in the resorption of bone and of bone substitutes has been studied (755); certain gap junctions may be under Ca^{2+} control through the Ca^{2+} causing tilting to close the gap junction (601) (cf. Section IV.C).

There are at least 14 synthetic or biological bone substitution materials, of which half are 100% inorganic – various routes to hydroxyapatite lead to materials with significantly different physical characteristics (756,757). Thus, for instance, whereas most synthetic hydroxyapatites have proved disappointing for tooth repair, nanoparticulate hydroxyapatite shows considerable promise for repairing dental enamel (758). Porous composite microspheres of hydroxyapatite in gelatin have potential value in bone tissue engineering; they may also provide a vehicle for controlled drug release (759). Structures of hydroxyapatite–fluorapatite composite materials have been considered, in relation to the use of fluorapatite in biocompatible bone replacement materials (760). Both fluorapatite and hydroxyapatite have been assessed as bone coating materials, with their effects on bone growth and resorption compared (761). Hydroxyapatite coated with porous silica and a heat-sensitive *N*-isopropylacrylamide polymer may prove a suitable bone mimic for mending bone. This ensemble can be used as a scaffold or template, and also can have drugs incorporated for controlled slow release for therapy or palliative use (cf. Section VIII.D below); the system proved to be biocompatible (762). Alternatively, bone repair may be facilitated by providing a polylactate scaffold on which bone grows, with the biodegradable polylactate slowly being metabolized (763). The successful trapping of live bacteria within alumina matrices (764) might form the first step on a path to bone repair and regeneration involving encapsulation in a nanocrystalline hydroxyapatite matrix (765).

D. CALCIUM-LINKED DISORDERS

Calcium is essential for bones, teeth, metabolic signaling and regulation, and other bodily functions. Adults require an intake of around 1 g per day; only about 10% of the calcium ingested is absorbed into the body – less from a low-protein diet, more from a high-protein diet. Vitamin D facilitates absorption, as does

lactose. Oxalate (from e.g. rhubarb), phytate (inositol hexaphosphate, from cereals), and other phosphates inhibit absorption through formation of sparingly soluble salts. Calcium deficiency, especially in childhood, leads to skeletal problems and to disorders in other calcium-dependent processes. However an excess of calcium may lead to increases in concentrations of sparingly soluble derivatives and consequent problems from their deposition. Whereas extracellular calcification is normal, intracellular calcification leads to disorders resulting from abnormal calcification of soft tissues (766). Hypercalcemia may thus lead to a variety of malfunctions, for example of the heart, kidneys, and central nervous system (767).

Calcium plays a central role in cardiac function, ultimately controlling the heartbeat (768). Several drugs for cardiovascular disorders act through their effects on calcium channels. For some years there has been a debate over whether low calcium intake may result in high blood pressure and therefore whether calcium supplementation should be used in the control of hypertension (769). It has been suggested that magnesium rather than calcium has a hypertensive effect (770), and that calcium supplementation may result in magnesium deficiency (771). There is also some evidence that calcium supplements may significantly increase the chances of the elderly developing cardiovascular problems (772).

Sustained abnormally high levels of calcium in the cytosol lead to cell death (773). Indeed free $\text{Ca}^{2+}_{\text{aq}}$ ions probably play a critical role in cell death, particularly in the central nervous and immune systems (774). The extent of their contribution in cellular response to irradiation, as in cases of excessive X-irradiation (775), has been assessed (68).

Clearly it is necessary to maintain appropriate levels of calcium in the body, and therefore to treat hypo- or hyper-calcemia if these conditions develop. It is relatively easy to treat hypocalcemia by administering a calcium salt (see [Section VIII.B.1](#) above), though the problem may be one of calcium absorption rather than of lack of dietary calcium – under normal conditions only around 10% of ingested calcium is absorbed (767). Calcium supplements, though generally felt to be beneficial in the management of osteoporosis and reducing the incidence of fractures (776), and possibly of value in preventing hypertensive disorders in pregnancy (777), may thus sometimes prove of little value. An example of disappointing results relates to maintaining bone density by reducing bone turnover in postmenopausal women (778). However, if vitamin D is also administered then such supplements may achieve the desired objective (779). Rickets may be due to vitamin D deficiency or to calcium

deficiency (780). Vitamin D deficiency is common, and is a major problem in relation to bone loss and fractures (781). Hypercalcemia is more difficult to treat – chelating agents are contraindicated by the lower stability constants for almost all calcium complexes than for the respective complexes of most other cations (cf. e.g. Table VI in Section II.C.1). However treatment with bisphosphonates (*v.i.*), calcitonin (*v.i.*), or plicamycin (564,767) is effective. It is difficult to dissolve calculi once formed (782), though solubilization of urinary calculi by bis-pentaaza-crown ether derivatives has been claimed (783). It is preferable to try to prevent their formation – several patents claim that pyruvate discourages their development (782). A recent illustration of the need to control calcium levels reports the need to feed ammonium chloride to goats being given calcium supplements to prevent the formation of urinary calculi (784).

Calcium-linked disorders may arise from secondary causes such as the presence of an excess of magnesium or aluminum reducing calcium levels well below normal. An example is provided by recent investigations of manganese toxicity (785), which may be caused by manganese reducing calcium availability (786) – the ATP-dependent calcium transporter Pmr1 has high affinities both for calcium and for manganese (381,461,787).

Methylenebisphosphonate derivatives, which are pyrophosphate analogs – $\text{O}_3\text{PCX}_2\text{PO}_3^{4-}$; cf. $\text{O}_3\text{PPO}_3^{4-}$ – have proved of value in the treatment of various diseases of bones and teeth, particularly osteoporosis and related disorders, and in the treatment of hypercalcemia (788). They bind strongly to hydroxyapatite crystals, are potent inhibitors of bone resorption, and inhibit calcification of soft tissues. Many bisphosphonic acids have therefore been synthesized and studied extensively over the past few decades (697,789) – various pharmacopoeias list about a dozen bisphosphonates. They have been developed mainly as possible therapeutic agents for bone resorption disorders such as osteoporosis, tumor-associated bone loss, and Paget's disease. Some also have palliative value, while a couple have been investigated as vehicles for delivering ^{99m}Tc to bone for imaging purposes. They bind strongly to hydroxyapatite surfaces by complexing Ca^{2+} , discouraging bone resorption and encouraging bone growth and regeneration. 1-Hydroxyethylidene-1,1-bisphosphonic acid (hedp), $\text{MeC}(\text{OH})(\text{PO}_3\text{H}_2)_2$, forms $\text{Ca}(\text{hedp})_2 \cdot 2\text{H}_2\text{O}$ at interfaces with bone or hydroxyapatite (790). Sodium alendronate ($\text{O}_3\text{PCX}_2\text{PO}_3^{4-}$ with $\text{X}_2 = \text{OH}; (\text{CH}_2)_3\text{NH}_2$), etidronate ($\text{X}_2 = \text{OH}; \text{CH}_3$), and clodronate ($\text{X}_2 = \text{Cl}_2$ (678)) have been in clinical use since the 1970s, but have gradually been superseded by more effective analogs, many containing an $\equiv\text{NH}^+$ center. Some

bisphosphonates may have undesirable side effects – alendronate can cause gastric ulcers (788) and may also induce heart problems (791). The binding and resorption properties of various pyridyl-methylene derivatives, including risedronate, have been investigated, and structure–activity relationships established (792). There have recently been efforts to find bisphosphonates that can be administered at long intervals. The aminobisphosphonate ibandronate (789) is a possibility for once-monthly (oral) or thrice-monthly (intravenous) administration, while zoledronic acid, an imidazole-containing bisphosphonate, has been shown to have considerable potential as a once-a-year injection against postmenopausal osteoporosis (793). Zoledronic acid is currently marketed for the treatment of Paget's disease and of tumor-induced bone loss, and for pain-reduction in chemotherapy of bone metastases. An alternative approach involves administration of a monoclonal antibody preparation (794), which in clinical trials has been shown to increase bone density to an extent comparable with bisphosphonates (795).

A recent extension of the therapeutic use of bisphosphonates has been to cancer treatment – administration of zoledronic acid after treatment with doxorubicin has been shown to have a significant ameliorative effect of subcutaneous breast tumors (796). Another recent extension has been to the characterization of arthritic conditions involving crystalline calcium phosphate in joints (797). The difficulties associated with isolating samples of these crystals for diagnostic tests have been mitigated by the use of superparamagnetic beads with attached bisphosphonate groups. These bind to the calcium phosphate, and for inspection can be removed using a magnet.

An alternative therapeutic approach to osteoporosis is through the use of calcitonin (798), a peptide hormone containing 32 amino acids (the sequence is species dependent) secreted by the thyroid gland, which stimulates the production of new bone. Synthetic calcitonins corresponding to human, eel, and salmon variants, and natural calcitonin extracted from pig thyroid, are all used in medicine – they have slightly different efficacies, side effects, and tolerance levels (799).

From time to time it is suggested that Ca^{2+} may be involved in Alzheimer's disease (800), perhaps in the guise of a Ca^{2+} -conducting amyloid protein channel (801). Now at the time of writing this review it has emerged that calcium channels and their regulation may indeed be involved in both early-onset (802) and late-onset (803) forms of Alzheimer's disease, thus providing yet another example of the key role played by calcium in biological systems.

Ligand Abbreviations

acac	acetylacetonate (pentane-2,4-dionate)
amp, adp, atp	adenosine mono-, di-, triphosphate
amp-5	adenosine-5'-monophosphate
bapta	1,2-bis-(2-aminophenoxy)ethane- <i>N,N,N',N'</i> -tetraacetate
bdt	butane-2,3-diaminetetraacetate
CIB1	calcium- and integrin-binding protein
cydta	cyclohexane-1,2-diaminetetraacetate
dota	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate
dtpa	diethylenetriamine- <i>N,N,N',N'',N''</i> -pentaacetate
edta	ethylenediamine- <i>N,N,N',N'</i> -tetraacetate
egta	ethylene glycol bis-(2-aminoethyl ether)-tetraacetate
fluo-3	1-[2-amino-5-(2,7-dichloro-6-hydroxy-3-oxy-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane- <i>N,N,N',N'</i> -tetraacetate
furaptra	2-[2-(5-carboxyoxazole)]-5-hydroxy-6-amino-benzofuran- <i>N,N,O</i> -triacetate
GFP	green fluorescent protein
hopo	hydroxypyridinon(at)e
ICBP	intestinal calcium-binding protein
IP ₃	inositol 1,4,5-triphosphate
Ln ³⁺	a lanthanide(III) cation
mal	malonate
memal	methylmalonate
MLCK	myosin light chain kinase
nota	1,4,7-triazacyclononane-1,4,7-triacetate
oxine	8-hydroxyquinoline
par	pyridine-2-azo-4'-dimethylaniline
pdta	propylene-1,2-diaminetetraacetate
pmea	9-[2-(phosphonomethoxy)ethyl]adenine
py	pyridine; pyridyl
quin-2	8-amino-2-[(2-amino-5-methylphenoxy)methyl]-6-methoxyquinoline- <i>N,N,N',N'</i> -tetraacetate {2-[[2-[bis(carboxymethyl)amino]-5-methyl-phenoxy]methyl]-6-methoxy-8-[bis(carboxymethyl)amino]quinoline}
Syt	synaptotagmin
thf	tetrahydrofuran (as solvent, ligand)
ttha	triethylenetetramine- <i>N,N,N',N',N'',N''</i> -hexaacetate

18C6 etc 18-crown-6 etc
 [2,2,1], [2,2,2] cryptands ([2_B,2,2], [2_B,2_B,2] have one, two fused benzo groups)

ACKNOWLEDGMENTS

The authors are most grateful to Colin Hubbard for his interest and input throughout the preparation of this chapter, and his invaluable assistance in polishing and submitting the manuscript. We also record our appreciation of the enthusiasm and encouragement of Jonathan Crowe, of Oxford University Press, in relation to a parallel project without which the present review would not have been possible.

REFERENCES

1. "Metal Ions in Biological Systems. Volume 17. Calcium and its Role in Biology"; Ed. Sigel, H.; Marcel Dekker: New York, **1984**.
2. Wilkins, R. G.; Wilkins, P. C. "The Role of Calcium and Comparable Cations in Animal Behaviour"; The Royal Society of Chemistry: Cambridge, **2003**.
3. Mann, S. "Biom mineralization: Principles and Concepts in Bioinorganic Materials Chemistry"; OUP: Oxford, **2001**.
4. Krampitz, G.; Graser, G. *Angew. Chem. Int. Ed.* **1988**, 27, 1145–1156.
5. Kaim, W.; Schwederski, B. "Bioinorganic Chemistry: Inorganic Elements in the Chemistry of Life"; Wiley: Chichester, **1994**, Chap. 15.
6. Glusker, J. P. *Adv. Prot. Chem.* **1991**, 42, 1–76.
7. "Guidebook to the Calcium-Binding Proteins"; Eds. Celio, M.R.; Pauls, T.; Schwaller, B.; OUP: Oxford, **1996**.
8. Carafoli, E. *Proc. Natl. Acad. Sci. USA* **2002**, 99, 1115–1122.
9. Whitfield, J. F.; Chakravarthy, B. "Calcium: The Grand-Master Signaler"; NRC Research Press: Ottawa, **2002**.
10. Clapham, D. *Cell* **2007**, 131, 1047–1058.
11. Ringer, S. *J. Physiol.* **1883**, 4, 29–43.
12. Heilbrunn, L. V. "The Dynamics of Living Protoplasm"; Academic Press: New York, **1956**.
13. Burdett, S. C.; Lippard, S. J. *Proc. Natl. Acad. Sci. USA* **2003**, 100, 3605–3610.
14. "Calcium as a Cellular Regulator"; Eds. Carafoli, E.; Klee, C.; OUP: New York, **1999**.
15. Berridge, M. J.; Irvine, R. F. *Nature* **1989**, 341, 197–205.
16. Sienart, I.; De Smedt, H.; Parys, J. B.; Missiaen, L.; Vanlingen, S.; Sipma, H.; Casteels, R. *J. Biol. Chem.* **1996**, 271, 27005–27012.
17. Lee, H. C. *J. Biol. Chem.* **2005**, 280, 33693–33696.
18. Ng, C. K.-Y.; Carr, K.; McAinsh, M. R.; Powell, B.; Hetherington, A. M. *Nature* **2001**, 410, 596–599.
19. Spiegel, S.; Milstien, S. *J. Biol. Chem.* **2002**, 277, 25851–25854.

20. Blamey, C. J.; Ceccarelli, C.; Naik, U. P.; Bahnson, B. *J. Prot. Sci.* **2005**, *14*, 1214–1221.
21. Romero, E.; Cha, G.-H.; Verstreken, P.; Ly, C. V.; Hughes, R. E.; Bellen, H. J.; Botas, J. *Neuron* **2008**, *57*, 27–40.
22. MacLennan, D. H. *Eur. J. Biochem.* **2000**, *267*, 5291–5297.
23. East, M. *Chem. Brit.* **2002**, (3), 42.
24. Weiss, L. *J. Natl. Cancer Inst.* **1973**, *50*, 3–19.
25. Balt, S.; de Bolster, M. W. G.; Visser-Luirink, G. *Inorg. Chim. Acta* **1983**, *78*, 121–127.
26. Ruggiero, J.; Fosey, M. A.; Santos, J. A.; Mourão, P. A. S. *Carbohydrate Res.* **1998**, *306*, 545–550.
27. Benzing-Nguyen, L.; Perry, M. B. *J. Org. Chem.* **1978**, *43*, 551–554.
28. Csuk, R.; Hugener, M.; Vasella, A. *Helv. Chim. Acta* **1988**, *71*, 609–618.
29. Whitfield, D. M.; Stojkovski, S.; Sarkar, B. *Coord. Chem. Rev.* **1993**, *122*, 171–225.
30. Johnson, S. M.; Herrin, J.; Liu, S. J.; Paul, I. C. *Chem. Commun.* **1970**, 72–73.
31. Colman, P. M.; Weaver, L. H.; Matthews, B. W. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 1999–2005.
32. Suh, I.-H.; Aoki, K.; Yamazaki, H. *Acta Cryst.* **1989**, *C45*, 415–418.
33. Matthews, B. W.; Weaver, L. H. *Biochemistry* **1974**, *13*, 1719–1725.
34. Balter, V.; Reynard, B. *Bone* **2008**, *42*, 229–234.
35. Zoghbi, M. E.; Copello, J. A.; Villalba-Galea, C. A.; Vélez, P.; Diaz Sylvester, P. L.; Bolaños, P.; Marciano, A.; Fill, M.; Escobar, A. L. *Cell Calcium* **2004**, *36*, 119–134.
36. Bakowski, D.; Parekh, A. B. *Cell Calcium* **2007**, *42*, 333–339.
37. Horrocks, W. DeW.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384–392.
38. Williams, R. J. P. *Chem. Brit.* **1978**, *14*, 25–29.
39. (a) Mildvan, A. S. *Acc. Chem. Res.* **1977**, *10*, 246–252. (b) Mildvan, A. S.; Gupta, R. K. *Meth. Enzymol.* **1978**, *49*, 322–359.
40. Reuben, J. *Biochemistry* **1971**, *10*, 2834–2838.
41. Leavis, P. C.; Nagy, B.; Lehrer, S. S.; Bialkowska, H.; Gergely, J. *Arch. Biochem. Biophys.* **1980**, *200*, 17–21.
42. (a) Rechnitz, G. A.; Lin, Z. F. *Anal. Chem.* **1968**, *40*, 696–699. (b) Tackett, S. L. *Anal. Chem.* **1969**, *41*, 1703–1705. (c) Maj-Zurawska, M.; Rouilly, M.; Morf, W. E.; Simon, W. *Anal. Chim. Acta* **1989**, *218*, 47–59.
43. De Robertis, A.; Di Giacomo, P.; Foti, C. *Anal. Chim. Acta* **1995**, *300*, 45–51.
44. Trafton, J. E.; Li, C.; Mallen, J.; Miller, S. R.; Nakano, A.; Schall, O. F.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1990**, 1266–1268.
45. Tsukube, H.; Yamashita, K.; Iwachido, T.; Zenki, M. *J. Org. Chem.* **1991**, *56*, 268–272.
46. Lönnberg, H.; Vesala, A. *Carbohydrate Res.* **1980**, *78*, 53–59.
47. Buckley, B. M.; Russell, L. J. *Ann. Clin. Biochem.* **1988**, *25*, 447–465.
48. Pilecká, N.; Raković, M.; Obrušník, I.; Broulik, P. *J. Radioanal. Nucl. Chem.* **1989**, *135*, 429–434.
49. Anderegg, G. In: “*Comprehensive Coordination Chemistry*”; vol. 2; Eds. Wilkinson, G.; Gillard, R. D.; McCleverty, J. A.; Oxford: Pergamon, **1987**, Chap. 20.3.
50. Bevan, N.; Rees, S. In: “*Green Fluorescent Protein: Properties, Applications, and Protocols*”; 2nd edn.; Eds. Chalfie, M.; Kain, S. R.; Wiley-Interscience: Hoboken, NJ, **2006**, Chap. 16.
51. Tsien, R. Y.; Pozzan, T.; Rink, T. J. *J. Cell Biol.* **1982**, *94*, 325–334.
52. (a) Poenie, M.; Alderton, J.; Tsien, R. Y.; Steinhardt, R. A. *Nature* **1985**, *315*, 147–149. (b) Shelanski, M.; Ratan, R. *Meth. Enzymol.* **1987**, *139*, 824–834.

53. Vorndran, C.; Minta, A.; Poenie, M. *Biophys. J.* **1995**, *69*, 2112–2124.
54. Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440–3450.
55. (a) Dinely, K. E. *Cell Calcium* **2007**, *42*, 341–342. (b) Stork, C. J.; Li, Y. V. *Cell Calcium* **2007**, *42*, 343–344, and references therein.
56. Minta, A.; Kao, J. P. Y.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 8171–8178.
57. Ribou, A.-C.; Salmon, J.-M.; Vigo, J.; Goyet, C. *Talanta* **2007**, *71*, 437–442.
58. Komatsu, H.; Miki, T.; Citterio, D.; Kubota, T.; Shindo, Y.; Kitamura, Y.; Oka, K.; Suzuki, K. *J. Am. Chem. Soc.* **2005**, *127*, 10798–10799.
59. Shimomura, O. In: “*Green Fluorescent Protein: Properties, Applications, and Protocols*”; 2nd edn.; Eds. Chalfie, M.; Kain, S. R.; Wiley-Interscience: Hoboken, NJ, **2006**, Chap. 1.
60. Hastings, J. W.; Morin, J. G. In: “*Green Fluorescent Protein: Properties, Applications, and Protocols*”; 2nd edn.; Eds. Chalfie, M.; Kain, S. R.; Wiley-Interscience: Hoboken, NJ, **2006**, Chap. 2.
61. Miyawaki, A.; Llopis, J.; Heim, R.; McCaffery, J. M.; Adams, J. A.; Ikura, M.; Tsien, R. Y. *Nature* **1997**, *388*, 882–887.
62. Griesbeck, O.; Baird, G. S.; Campbell, R. E.; Zacharias, D. A.; Tsien, R. Y. *J. Biol. Chem.* **2001**, *276*, 29188–29194.
63. Heim, N.; Griesbeck, O. *J. Biol. Chem.* **2004**, *279*, 14280–14286.
64. Garaschuk, O.; Griesbeck, O.; Konnerth, A. *Cell Calcium* **2007**, *42*, 351–361.
65. Tour, O.; Adams, S. R.; Kerr, R. A.; Meijer, R. M.; Sejnowski, T. J.; Tsien, R. W.; Tsien, R. Y. *Nat. Chem. Biol.* **2007**, *3*, 423–431.
66. Hyrc, K. L.; Rzeszutnik, Z.; Kennedy, B. R.; Goldberg, M. P. *Cell Calcium* **2007**, *42*, 576–589.
67. Takahashi, A.; Camacho, P.; Lechleiter, J. D.; Herman, B. *Physiol. Rev.* **1999**, *79*, 1089–1125.
68. See, e.g.; Szumiel, I.; Buraczewska, I.; Sochanowicz, B.; Wojewódzka, M. *Nukleonika* **1993**, *38*, 21–30, and references therein.
69. Neher, E. *J. Physiol.* **2008**, *586*, 2031.
70. Arslan, Z. *Talanta* **2005**, *65*, 1326–1334.
71. Bryant, R. G. *J. Am. Chem. Soc.* **1969**, *91*, 1870–1871.
72. Andersson, T.; Drakenberg, T.; Forsén, S.; Thulin, E.; Swärd, M. *J. Am. Chem. Soc.* **1982**, *104*, 576–580.
73. Drakenberg, T.; Forsén, S.; Lilja, H. *J. Magn. Reson.* **1983**, *53*, 412–422.
74. Ogoma, Y.; Shimizu, T.; Hatano, M.; Fujii, T.; Hachimori, A.; Kondo, Y. *Inorg. Chem.* **1988**, *27*, 1853–1855.
75. Dupree, R.; Howes, A. P.; Kohn, S. C. *Chem. Phys. Lett.* **1997**, *276*, 399–404.
76. Lin, Z.; Smith, M. E.; Sowrey, F. E.; Newport, R. J. *Phys. Rev. B* **2004**, *69*, 224107-1–224107-7.
77. Wong, A.; Howes, A. P.; Dupree, R.; Smith, M. E. *Chem. Phys. Lett.* **2006**, *427*, 201–205.
78. Kretsinger, R. H.; Nelson, D. J. *Coord. Chem. Rev.* **1976**, *18*, 29–124, see Sections III.B.7 and III.C.1–3.
79. Recker, R. R. *New Engl. J. Med.* **1985**, *313*, 70–73.
80. MacLennan, D. H.; Wong, P. T. S. *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 1231–1235.
81. Pieterse, W. A.; Volwerk, J. J.; de Haas, G. H. *Biochemistry* **1974**, *13*, 1439–1445.
82. Bajaj, S. P.; Butkowski, R. J.; Mann, K. G. *J. Biol. Chem.* **1975**, *250*, 2150–2156.

83. Lennon, V. A.; Kryzer, T. J.; Griesmann, G. E.; O'Suilleabhain, P. E.; Windebank, A. J.; Woppmann, A.; Miljanich, G. P.; Lambert, E. H. *New Engl. J. Med.* **1995**, *332*, 1467–1475.
84. Monteith, G. R.; Kable, E. P. W.; Chen, S.; Roufogalis, B. D. *J. Hypert.* **1996**, *14*, 435–442.
85. Sottocasa, G.; Sandri, G.; Panfili, E.; de Bernard, B.; Gazzotti, P.; Vasington, F. D.; Carafoli, E. *Biochem. Biophys. Res. Commun.* **1972**, *47*, 808–813.
86. Prendergast, F. G.; Mann, K. G. *J. Biol. Chem.* **1977**, *252*, 840–850.
87. Grizzuti, K.; Perlmann, G. E. *Biochemistry* **1973**, *12*, 4399–4403.
88. Frey, J.; Meier, R.; Gygi, D.; Nicolet, J. *Infect. Immun.* **1991**, *59*, 3026–3032.
89. Anghileri, L. J. *Int. J. Nucl. Med. Biol.* **1973**, *1*, 93–95.
90. Lumachi, F.; Brunello, A.; Roma, A.; Basso, U. *Curr. Med. Chem.* **2008**, *15*, 415–421.
91. Kok, D. J.; Papapoulos, S. E.; Blomen, L. J. M. J.; Bijvoet, O. L. M. *Kidney Int.* **1988**, *34*, 346–350.
92. Wells, A. F. “*Structural Inorganic Chemistry*”; 5th edn. OUP: Oxford, **1984**.
93. Bunič, T.; Tavčar, G.; Tramšek, M.; Žemva, B. *Inorg. Chem.* **2006**, *45*, 1038–1042.
94. Leclaire, A.; Borel, M. M. *Acta Cryst.* **1977**, *B33*, 1608–1610.
95. Leclaire, A.; Borel, M.-M. *Acta Cryst.* **1979**, *B35*, 585–588.
96. Leclaire, A.; Borel, M.-M. *Acta Cryst.* **1978**, *B34*, 900–902.
97. Leclaire, A.; Borel, M.-M.; Monier, J. C. *Acta Cryst.* **1980**, *B36*, 2757–2759.
98. Leclaire, A.; Borel, M.-M. *Acta Cryst.* **1977**, *B33*, 2938–2940.
99. MacLennan, G.; Beevers, C. A. *Acta Cryst.* **1955**, *8*, 579–583.
100. Bigi, A.; Boanini, E.; Capuccini, C.; Gazzano, M. *Inorg. Chim. Acta* **2007**, *360*, 1009–1016.
101. Leclaire, A. *Acta Cryst.* **1974**, *B30*, 605–607.
102. Sahbari, J. J.; Olmstead, M. M. *Acta Cryst.* **1983**, *C39*, 208–211.
103. Metz, B.; Moras, O.; Weiss, R. *Acta Cryst.* **1973**, *B29*, 1388–1393.
104. Drew, M. G. B.; Esho, F. S.; Nelson, S. M. *J. Chem. Soc., Dalton Trans.* **1983**, 1653–1659.
105. Hancock, R. D.; Siddons, C. J.; Oscarson, K. A.; Reibenspies, J. M. *Inorg. Chim. Acta* **2004**, *357*, 723–727.
106. Nelson, S. M.; Esho, F. S.; Drew, M. G. B. *J. Chem. Soc., Dalton Trans.* **1983**, 1857–1864.
107. Shinomoto, R.; Gamp, E.; Edelstein, N. M.; Templeton, D. H.; Zalkin, A. *Inorg. Chem.* **1983**, *22*, 2351–2355.
108. Brennan, J.; Shinomoto, R.; Zalkin, A.; Edelstein, N. *Inorg. Chem.* **1984**, *23*, 4143–4146.
109. Császár, A. G.; Hedberg, L.; Hedberg, K.; Burns, R. C.; Wen, A. T.; McGlinchey, M. J. *Inorg. Chem.* **1991**, *30*, 1371–1376.
110. Tang, Y.; Zakharov, L. N.; Kassel, W. S.; Rheingold, A. L.; Kemp, R. A. *Inorg. Chim. Acta* **2005**, *358*, 2014–2022.
111. Baker, C. F.; Barker, M. G.; Blake, A. J. *Acta Cryst.* **2001**, *E57*, i6–i7.
112. Harding, M. M. *Acta Cryst.* **1999**, *D55*, 1432–1443.
113. Jahnberg, L. *Acta Chem. Scand.* **1963**, *17*, 2548–2559.
114. Christensen, A. N.; Lebech, B.; Sheptyakov, D.; Hanson, J. C. *Acta Cryst.* **2007**, *63B*, 850–861; and references therein.
115. Babel, D.; Rüdorff, W.; Tschöpp, R. *Z. Anorg. Allg. Chem.* **1966**, *347*, 282–288.

116. Rietveld, H. M. *Acta Cryst.* **1966**, 20, 508–513.
117. Gougeon, P.; Gall, P. *Acta Cryst.* **2002**, E58, i65–i67.
118. Carugo, O.; Djinić, K.; Rizzi, M. *J. Chem. Soc., Dalton Trans.* **1993**, 2127–2135.
119. Dickens, B.; Brown, W. E. *Acta Cryst.* **1972**, B28, 3056–3065.
120. Thiele, G.; Putzas, D. *Z. Anorg. Allg. Chem.* **1984**, 519, 217–218.
121. Thiele, G.; Brodersen, K.; Pezzei, G. *Z. Anorg. Allg. Chem.* **1982**, 491, 308–318.
122. Putkas, D.; Rotter, H. W.; Thiele, G.; Brodersen, K.; Pezzei, G. *Z. Anorg. Allg. Chem.* **1991**, 595, 193–202.
123. Thiele, G.; Rotter, H. W.; Faller, M. *Z. Anorg. Allg. Chem.* **1984**, 508, 129–135.
124. Karipides, A. *Acta Cryst.* **1981**, B37, 2232–2234.
125. Carafoli, E.; Inesi, G.; Rosen, B. P. In: “*Metal Ions in Biological Systems. Volume 17. Calcium and its Role in Biology*”; Ed. Sigel, H.; Marcel Dekker: New York, **1984**, pp. 129–186.
126. Burgess, J.; Drasdo, D. N. *Polyhedron* **1993**, 12, 2905–2911.
127. Smith, G.; Lynch, D. E.; Kennard, C. H. L.; Mak, T. C. W. *Polyhedron* **1991**, 10, 1569–1572.
128. (a) Deganello, S. *Z. Krist.* **1980**, 152, 247–252. (b) Deganello, S. *Acta Cryst.* **1981**, B37, 826–829. (c) Tazzoli, V.; Domeneghetti, C. *Am. Mineralogist* **1980**, 65, 327–334.
129. Deganello, S.; Kampf, A. R.; Moore, P. B. *Am. Mineralogist* **1981**, 66, 859–865.
130. (a) Karipides, A.; Reed, A. T. *Inorg. Chem.* **1977**, 16, 3299–3302. (b) Briggman, B.; Oskarsson, Å. *Acta Cryst.* **1977**, B33, 1900–1906. (c) Marsh, R. E.; Schomaker, V. *Inorg. Chem.* **1979**, 18, 2332–2336.
131. Albertsson, J.; Oskarsson, Å.; Svensson, C. *Acta Cryst.* **1978**, B34, 2343–2743.
132. Aliouane, K.; Rahahia, N.; Guehria-Laidoudi, A.; Dohaoui, S.; Lecomte, C. *Acta Cryst.* **2007**, E63, m1834–m1836.
133. Curry, M. E.; Eggleston, D. S.; Hodgson, D. J. *J. Am. Chem. Soc.* **1985**, 107, 8234–8238.
134. Zell, A.; Einspahr, H.; Bugg, C. E. *Biochemistry* **1985**, 24, 533–537.
135. Karipides, A.; Reed, A. T. *Acta Cryst.* **1980**, B36, 1377–1381.
136. Gupta, M. P.; Prasad, S. M.; Sahu, R. G.; Sahu, B. N. *Acta Cryst.* **1972**, B28, 135–139.
137. Ambady, G. K. *Acta Cryst.* **1968**, B24, 1548–1557.
138. Brändén, C.-I.; Söderberg, B.-O. *Acta Chem. Scand.* **1966**, 20, 730–738.
139. Glusker, J. P.; Minkin, J. A.; Casciato, C. A. *Acta Cryst.* **1971**, B27, 1284–1293.
140. Glusker, J. P.; Minkin, J. A.; Soule, F. *Acta Cryst.* **1972**, B28, 2499–2505.
141. Meehan, E. J.; Einspahr, H.; Bugg, C. E. *Acta Cryst.* **1979**, B35, 828–832.
142. (a) Hearn, R. A.; Bugg, C. E. *Acta Cryst.* **1968**, B24, 2705–2711. (b) Hvorslev, J.; Kjellef, K. E. *Acta Cryst.* **1968**, B24, 2711–2716.
143. Einspahr, H.; Bugg, C. E. *Acta Cryst.* **1981**, B37, 1044–1052.
144. (a) Einspahr, H.; Bugg, C. E. In: “*Metal-Ligand Interactions in Organic Chemistry and Biochemistry*”; vol. 2; Eds. Pullman, B.; Goldblum, W.; Reidel: Dordrecht, **1977**, pp. 231–266. (b) Einspahr, H.; Bugg, C. E. In: “*Calcium Binding Proteins and Calcium Function*”; Ed. Wasserman, R. H.; Elsevier: Amsterdam, **1977**, pp. 13–20. (c) Einspahr, H.; Bugg, C. E. In: “*Metal Ions in Biological Systems. Volume 17. Calcium and its Role in Biology*”; Ed. Sigel, H.; Marcel Dekker: New York, **1984**, pp. 52–97.
145. Einspahr, H.; Bugg, C. E. *Acta Cryst.* **1980**, B36, 264–271.

146. Barnett, B. L.; Uchtman, V. A. *Inorg. Chem.* **1979**, *18*, 2674–2678.
147. Radanović, D. D.; Rychlewska, U.; Djuran, M. I.; Warzajtis, B.; Drašković, N. S.; Gurešić, D. M. *Polyhedron* **2004**, *23*, 2183–2192.
148. Schmidt, M. U.; Schmiermund, T.; Bolte, M. *Acta Cryst.* **2007**, *E63*, m360–m362.
149. Clapp, L. A.; Siddons, C. J.; Whitehead, J. R.; VanDerveer, D. G.; Rogers, R. D.; Griffin, S. D.; Jones, S. B.; Hancock, R. D. *Inorg. Chem.* **2005**, *44*, 8495–8502.
150. Fox, S.; Busching, I.; Barklage, W.; Strasdeit, H. *Inorg. Chem.* **2007**, *46*, 818–824.
151. Natarajan, S.; Shanmugam, G.; Dhas, S. A. M. B.; Athimoolam, S. *Acta Cryst.* **2007**, *E63*, m2897–m2898.
152. Van Der Helm, D.; Willoughby, T. V. *Acta Cryst.* **1969**, *B25*, 2317–2326.
153. Einspahr, H.; Gartland, G. L.; Bugg, C. E. *Acta Cryst.* **1977**, *B33*, 3385–3390.
154. Einspahr, H.; Bugg, C. E. *Acta Cryst.* **1979**, *B35*, 316–321.
155. Einspahr, H.; Bugg, C. E. *Acta Cryst.* **1974**, *B30*, 1037–1043.
156. Gao, S.; Zhang, X. F.; Huo, L.-H.; Zhao, H. *Acta Cryst.* **2004**, *E60*, m1790–m1792.
157. Unger, Y.; Taige, M. A.; Ahrens, S.; Strassner, T. *Inorg. Chim. Acta* **2007**, *360*, 3699–3704.
158. Prodius, D.; Turta, C.; Mereacre, V.; Shova, S.; Gdaniec, M.; Simonov, Y.; Lipkowski, J.; Kuncser, V.; Filoti, G.; Caneschi, A. *Polyhedron* **2006**, *25*, 2175–2182.
159. Burgess, J. *“Metal Ions in Solution”*; Ellis Horwood: Chichester, **1978**.
160. Marcus, Y. *“Ion Solvation”*; Wiley: New York, NY, **1985**.
161. Richens, D. T. *“The Chemistry of Aqua Ions”*; Wiley: Chichester, **1997**; pp. 136–138.
162. Ohtaki, H.; Radnai, T. *Chem. Rev.* **1993**, *93*, 1157–1204.
163. See pp. 143–144 of Ref. (159).
164. *“Water and Aqueous Solutions”*. Proceedings of the 37th Symp. Colston Res. Soc., Bristol, 1985; Eds. Neilson, G. W.; Enderby, J. E.; Adam Hilger; Bristol, **1986**.
165. See pp. 173–178 of Ref. (161).
166. Chillensi, G.; Mancini, G.; Sanna, N.; Barone, V.; Della Longa, S.; Benfatto, M.; Pavel, N. V.; D'Angelo, P. *J. Am. Chem. Soc.* **2007**, *129*, 5430–5436.
167. Neilson, G. W.; Enderby, J. E. *Annu. Rep. Chem., Section C* **1979**, *76*, 185–220.
168. (a) Neilson, G. W.; Enderby, J. E. *Adv. Inorg. Chem.* **1989**, *34*, 195–218. (b) Neilson, G. W.; Tromp, R. H. *Annu. Rep. Chem., Section C* **1991**, *88*, 45–75. (c) Enderby, J. E. *Chem. Soc. Rev.* **1995**, *24*, 159–168.
169. Enderby, J. E.; Cummings, S.; Herdman, G. J.; Neilson, G. W.; Salmon, P. S.; Skipper, N. *J. Phys. Chem.* **1987**, *91*, 5851–5858.
170. Fulton, J. L.; Heald, S. M.; Badyal, Y. S.; Simonson, J. *J. Phys. Chem. A* **2003**, *107*, 4688–4696.
171. (a) Licheri, G.; Piccaluga, G.; Pinna, G. *J. Chem. Phys.* **1975**, *63*, 4412–4414. (b) Licheri, G.; Piccaluga, G.; Pinna, G. *J. Chem. Phys.* **1976**, *64*, 2437–2441.
172. Caminiti, R.; Licheri, G.; Piccaluga, G.; Pinna, G. *Rev. Inorg. Chem.* **1979**, *1*, 333–385.
173. Probst, M.; Radnai, T.; Heinzinger, K.; Bopp, P.; Rode, B. M. *J. Phys. Chem.* **1985**, *89*, 753–759.

174. Jalilehvand, F.; Spångberg, B.; Lindqvist-Reis, P.; Hermansson, K.; Persson, I.; Sandström, M. *J. Am. Chem. Soc.* **2001**, *123*, 431–444.
175. Cummings, S.; Enderby, J. E.; Howe, R. A. *J. Phys. C* **1980**, *13*, 1–8.
176. Caminiti, R.; Licheri, G.; Paschina, G.; Piccaluga, G.; Pinna, G. *Z. Naturforsch.* **1980**, *35A*, 1361–1367.
177. Brendenbach, B.; Altmaier, M.; Rothe, J.; Neck, V.; Denecke, M. A. *Inorg. Chem.* **2007**, *46*, 6804–6810.
178. Marcus, Y. *Chem. Rev.* **1988**, *88*, 1475–1498.
179. Hinton, J. F.; Amis, E. S. *Chem. Rev.* **1967**, *67*, 367–425.
180. Hinton, J. F.; Amis, E. S. *Chem. Rev.* **1971**, *71*, 627–674.
181. See Chaps. 4 and 5 (especially p. 144) of Ref. (159).
182. Zavitsas, A. A. *J. Phys. Chem. B* **2001**, *105*, 7805–7817.
183. Marcus, Y. *Biophys. Chem.* **2006**, *124*, 200–207.
184. Kiriukhin, M. Y.; Collins, K. D. *Biophys. Chem.* **2002**, *99*, 155–168.
185. Collins, K. D.; Nielson, G. W.; Enderby, J. E. *Biophys. Chem.* **2007**, *128*, 95–104.
186. Bol, W.; Gerrits, G. J. A.; van Panthaleon van Eck, C. L. *J. Appl. Cryst.* **1970**, *3*, 486–492.
187. Bounds, D. G. *Mol. Phys.* **1985**, *54*, 1335–1355.
188. Bernal-Uruchurtu, M. I.; Ortega-Blake, I. *J. Chem. Phys.* **1995**, *103*, 1588–1598.
189. Tongraar, A.; Liedl, K. R.; Rode, B. M. *J. Phys. Chem. A* **1997**, *101*, 6299–6309.
190. Naor, M. M.; Van Nostrand, K.; Dellago, C. *Chem. Phys. Lett.* **2003**, *369*, 159–164.
191. Pavlov, M.; Siegbahn, P. E. M.; Sandström, M. *J. Phys. Chem. A* **1998**, *102*, 219–228.
192. Koneshan, S.; Rasaiah, J. C.; Lynden-Bell, R. M.; Lee, S. H. *J. Phys. Chem. B* **1998**, *102*, 4193–4204.
193. Bush, M. F.; Saykally, R. J.; Williams, E. R. *Chem. Phys. Chem.* **2007**, *8*, 2245–2253.
194. Blandamer, M. J.; Burgess, J. *Transition Met. Chem.* **1988**, *13*, 1–18.
195. (a) Krestov, G. A. “*Thermodynamics of Ionic Processes in Solution*”; Khimiya: Moscow, **1985**. (b) Krestov, G. A. “*Thermodynamics of Solvation – Solution and Dissolution; Ions and Solvents; Structure and Energetics*”; Ellis Horwood: Chichester, **1991**.
196. Schmid, R.; Miah, A. M.; Sapunov, V. N. *Phys. Chem. Chem. Phys.* **2000**, *2*, 97–102.
197. Marcus, Y. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 339–349.
198. Swaddle, T. W.; Mak, M. K. S. *Can. J. Chem.* **1983**, *61*, 473–480.
199. Barta, L.; Hepler, L. G. *J. Phys. Chem.* **1989**, *93*, 5588–5595.
200. Hefter, G.; Marcus, Y. *J. Solution Chem.* **1997**, *26*, 249–266.
201. Hedwig, G. R.; Hakin, A. W. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4690–4700.
202. Withers, P. C.; Morrison, G.; Hefter, G. T.; Pang, T. S. *J. Exptl. Biol.* **1994**, *188*, 175–189.
203. Einstein, A. *Ann. Physik* **1905**, *17*, 549–560.
204. (a) See p. 264 of Ref. (159). (b) Baes, C. F.; Mesmer, R. E. “*The Hydrolysis of Cations*”; Wiley: New York, **1976**.
205. Barrett, J.; Hughes, M. N.; Karavaiko, G. I.; Spencer, P. A. “*Metal Extraction by Bacterial Oxidation of Minerals*”; Ellis Horwood: Chichester, **1993**; pp. 74, 123, 132.
206. Wagh, A. S.; Pinnock, W. R. *Econ. Geol.* **1987**, *82*, 757–761.

207. Peyman, A.; Gabriel, C.; Grant, E. H. *Bioelectromagnetics* **2007**, *28*, 264–274.
208. Sillen, L.G.; Martell, A.E. “*Stability Constants of Metal-Ion Complexes*”, Special Publications 17 and 25; The Chemical Society: London, **1964**, **1971**.
209. Högfeldt, E. “*Stability Constants of Metal-Ion Complexes. Part A: Inorganic Ligands*”; Pergamon Press: Oxford, **1982**.
210. Perrin, D. D. “*Stability Constants of Metal-Ion Complexes. Part B: Organic Ligands*”; Pergamon Press: Oxford, **1979**.
211. Smith, R. M.; Martell, A. E. “*Critical Stability Constants*”, vols. 1 – 6; Plenum Press: New York, 1974, 1975, 1976, 1977, 1982, 1989.
212. Pettit, L. D.; Powell, H. K. J. IUPAC Stability Constants Database, Academic Software, **1993**; Version 5, 2003; scdbase@acadsoft.co.uk.
213. Martell, A. E.; Smith, R. M.; Motekaitis, R. J. NIST Critical Stability Constants of Metal Complexes Database, US Department. of Commerce, Gaithersburg, **1993**; NIST Standard Reference Database 46, version 8.0, 2004.
214. Daniele, P. G.; Foti, C.; Gianguzza, A.; Prenesti, E.; Sammartano, S. *Coord. Chem. Rev.* **2008**, *252*, 1093–1107.
215. Stumm, W.; Morgan, J. J. “*Aquatic Chemistry*”; Wiley-Interscience: New York, **1970**, Chap. 6.
216. De Stefano, C.; Foti, C.; Gianguzza, A.; Sammartano, S. *Chem. Spec. Bioavail.* **1998**, *10*, 27–30.
217. Prenesti, E.; Daniele, P. G.; Toso, S.; Zelano, V.; Berto, S. *Chem. Spec. Bioavail.* **2004**, *16*, 17–24.
218. Daniele, P. G.; Marangella, M. *Ann. Chim. (Rome)* **1982**, *72*, 25–38.
219. Pearce, K. N. *Aust. J. Chem.* **1980**, *33*, 1511–1517.
220. Anderegg, G. *Helv. Chim. Acta* **1964**, *47*, 1801–1814.
221. Czerlinski, G.; Eigen, M. *Z. Elektrochem.* **1959**, *63*, 652–661.
222. “*Hard and Soft Acids and Bases*”; Ed. Pearson, R. G.; Dowden, Hutchinson, and Ross: Stroudsburg, Pennsylvania, **1973**.
223. Martin, R. B. *Inorg. Chim. Acta* **2002**, *339*, 27–33.
224. Bell, R. P.; George, J. H. B. *Trans. Faraday Soc.* **1953**, *49*, 619–627.
225. Johansson, L. *Coord. Chem. Rev.* **1968**, *3*, 293–318.
226. Elgquist, B. *J. Inorg. Nucl. Chem.* **1970**, *32*, 937–944.
227. Patil, S. K.; Sharma, H. D. *Can. J. Chem.* **1969**, *47*, 3851–3858.
228. (a) Hester, R. E.; Plane, R. A. *J. Chem. Phys.* **1964**, *40*, 411–414. (b) Irish, D. E.; Walrafen, G. E. *J. Chem. Phys.* **1967**, *46*, 378–384.
229. Shanbhag, S. M.; Choppin, G. R. *Inorg. Chim. Acta* **1987**, *138*, 187–192.
230. Kura, G.; Ohashi, S.; Kura, S. *J. Inorg. Nucl. Chem.* **1974**, *36*, 1605–1609.
231. Schubert, J. *J. Am. Chem. Soc.* **1954**, *76*, 3442–3444.
232. Kinjo, Y.; Ji, L.; Corfù, N. A.; Sigel, H. *Inorg. Chem.* **1992**, *31*, 5588–5596.
233. Capone, S.; De Robertis, A.; De Stefano, C.; Scarcella, R. *Talanta* **1985**, *32*, 675–677.
234. McBryde, W. A. E. “*A Critical Review of Equilibrium Data for Proton- and Metal Complexes of 1,10-Phenanthroline, 2,2-Bipyridyl and Related Compounds*”; Pergamon: Oxford, **1978**.
235. Zvimba, J. M.; Jackson, G. E. *Polyhedron* **2007**, *26*, 2395–2404.
236. Diebler, H.; Eigen, M.; Ilgenfritz, G.; Maass, G.; Winkler, R. *Pure Appl. Chem.* **1969**, *20*, 93–115.
237. Kiss, T.; Sóvágó, I.; Gergely, A. *Pure Appl. Chem.* **1991**, *63*, 597–638.
238. Sóvágó, I.; Kiss, T.; Gergely, A. *Pure Appl. Chem.* **1993**, *65*, 1029–1080.

239. Portanova, R.; Lajunen, L. H. J.; Tolazzi, M.; Piispanen, J. *Pure Appl. Chem.* **2003**, *75*, 495–540.
240. Anderegg, G.; Arnaud-Neu, F.; Delgado, R.; Felcman, J.; Popov, K. *Pure Appl. Chem.* **2005**, *77*, 1445–1495.
241. Anderegg, G. *Pure Appl. Chem.* **1982**, *54*, 2693–2758.
242. Delgado, R.; Quintino, S.; Teixeira, M.; Zhang, A. *J. Chem. Soc., Dalton Trans.* **1997**, 55–63.
243. Bianchi, A.; Calabi, L.; Giorgi, C.; Losi, P.; Mariani, P.; Paoli, P.; Rossi, P.; Valtancoli, B.; Virtuani, M. *J. Chem. Soc., Dalton Trans.* **2000**, 697–705.
244. Burgess, J.; Rangel, M. *Adv. Inorg. Chem.* **2008**, *60*, 167–243.
245. Pierre, V. C.; Melchior, M.; Doble, D. M. J.; Raymond, K. N. *Inorg. Chem.* **2004**, *43*, 8520–8525.
246. Daniele, P. G.; De Robertis, A.; De Stefano, C.; Sammartano, S.; Rigano, C. *J. Chem. Soc., Dalton Trans.* **1985**, 2353–2361.
247. Lumb, R. F.; Martell, A. E. *J. Phys. Chem.* **1953**, *57*, 690–693.
248. Laurie, S. H. In: “*Comprehensive Coordination Chemistry*”; vol. 2; Eds. Wilkinson, G.; Gillard, R. D.; McCleverty, J. A.; Pergamon: Oxford, **1987**, Chap. 20.2.
249. Blaquiere, C.; Berthon, G. *Inorg. Chim. Acta* **1987**, *135*, 179–189.
250. Kiss, T. In: “*Biocoordination Chemistry*”; Ed. Burger, K.; Ellis Horwood: Chichester, **1990**, Chap. III.
251. Rajan, K. S.; Mainer, S.; Davis, J. M. *Bioinorg. Chem.* **1978**, *9*, 187–203.
252. Maeda, M.; Okada, K.; Tsukamoto, Y.; Wakabayashi, K.; Ito, K. *J. Chem. Soc., Dalton Trans.* **1990**, 2337–2339.
253. Robertson, P.; Koehler, K. A.; Hiskey, R. G. *Biochem. Biophys. Res. Commun.* **1979**, *86*, 265–270.
254. Burger, K.; Sipos, P.; Véber, M.; Horváth, I.; Noszál, B.; Löw, M. *Inorg. Chim. Acta* **1988**, *152*, 233–239.
255. Rajan, K. S.; Jaw, R.; Grecz, N. *Bioinorg. Chem.* **1978**, *8*, 477–491.
256. Ferrari, R.; Bernés, S.; de Barbarín, C. R.; Mendoza-Díaz, G.; Gasque, L. *Inorg. Chim. Acta* **2002**, *339*, 193–201.
257. Ovchinnikov, Yu. A.; Ivanov, V. T. *Tetrahedron* **1975**, *31*, 2177–2209.
258. Baron, D.; Pease, L. G.; Blout, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8299–8306.
259. Miyake, H.; Kojima, Y. *Coord. Chem. Rev.* **1996**, *148*, 301–314.
260. Cusack, R. M.; Grøndahl, L.; Abbenante, G.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Hambley, T. W. *J. Chem. Soc., Perkin Trans.* **2000**, *2*, 323–331.
261. Behm, F.; Ammann, D.; Simon, W.; Brunfeldt, K.; Halstrøm, J. *Helv. Chim. Acta* **1985**, *68*, 110–118.
262. Beck, M. T. “*Chemistry of Complex Equilibria*”; Van Nostrand Reinhold: London, **1970**; pp. 229–231.
263. Anderegg, G. In: “*Comprehensive Coordination Chemistry*”; vol. 1; Eds. Wilkinson, G.; Gillard, R. D.; McCleverty, J. A.; Pergamon: Oxford, **1987**, p. 782.
264. Schmid, R. W.; Reilley, C. N. *J. Am. Chem. Soc.* **1956**, *78*, 5513–5518.
265. Bohigian, T. A.; Martell, A. E. *Inorg. Chem.* **1965**, *4*, 1264–1270.
266. Tsien, R. Y. *Biochemistry* **1980**, *19*, 2396–2404.
267. Carr, J. D.; Swartzfager, D. G. *J. Am. Chem. Soc.* **1975**, *97*, 315–321.
268. Linse, S.; Johansson, C.; Brodin, P.; Grundström, T.; Drakenberg, T.; Forsén, S. *Biochemistry* **1991**, *30*, 154–162.
269. Naraghi, M. *Cell Calcium* **1997**, *22*, 255–268.

270. Takesako, K.; Sasamoto, K.; Ohkura, Y.; Hirose, K.; Iino, M. *Anal. Commun.* **1997**, 34, 391–392.
271. Devinney, M. J.; Reynolds, I. J.; Dinely, K. E. *Cell Calcium* **2005**, 37, 225–232.
272. Kieboom, A. P. G.; Buurmans, H. M. A.; van Leeuwen, L. K.; Benschop, H. *J. Rec. Trav. Chim.* **1979**, 98, 393–394.
273. Schubert, J.; Lindenbaum, A. *J. Am. Chem. Soc.* **1952**, 74, 3529–3532.
274. Lajunen, L. H. J.; Portanova, R.; Piispanen, J.; Tolazzi, M. *Pure Appl. Chem.* **1997**, 69, 329–381.
275. Schubert, J.; Russell, E. R.; Myers, L. S. *J. Biol. Chem.* **1950**, 185, 387–398.
276. Smith, R. M.; Martell, A. E.; Motekaitis, R. J. *Inorg. Chim. Acta* **1985**, 99, 207–216.
277. Burger, K.; Véber, M.; Sipos, P.; Galbács, Z.; Horváth, I.; Szepesi, G.; Takácsi-Nagy, G.; Siemroth, J. *Inorg. Chim. Acta* **1986**, 124, 175–179.
278. Buschmann, H.-J. *Z. Anorg. Allg. Chem.* **1985**, 523, 107–111.
279. Arnaud-Neu, F.; Delgado, R.; Chaves, S. *Pure Appl. Chem.* **2003**, 75, 71–102.
280. Buschmann, H.-J. *Z. Phys. Chem. (Neue Folge)* **1984**, 139, 113–121.
281. Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J.; Sen, D. *Chem. Rev.* **1985**, 85, 271–339.
282. Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1991**, 91, 1721–2085.
283. Amini, M. K.; Shamsipur, M. *J. Phys. Chem.* **1991**, 95, 9601–9604.
284. Cox, B. G.; Schneider, H. “*Coordination and Transport Properties of Macrocyclic Compounds in Solution*”; Elsevier: Amsterdam, **1992**.
285. (a) Lamb, J. D.; Izatt, R. M.; Swain, C. S.; Christensen, J. J. *J. Am. Chem. Soc.* **1980**, 102, 475–479. (b) Gokel, G. W.; Goli, D. M.; Minganti, C.; Echegoyen, L. J. *Am. Chem. Soc.* **1983**, 105, 6786–6788.
286. (a) Cox, B. G.; Firman, P.; Schneider, I.; Schneider, H. *Inorg. Chem.* **1989**, 27, 4018–4021. (b) de Jong, F.; Reinhoudt, D. N. “*Stability and Reactivity of Crown-Ether Complexes*”; Academic Press: London, **1981**; p. 15.
287. Chang, C. A.; Rowland, M. E. *Inorg. Chem.* **1983**, 22, 3866–3869.
288. Brücher, E.; Györi, B.; Emri, J.; Solymosi, P.; Sztanyik, L. B.; Varga, L. J. *Chem. Soc., Chem. Commun.* **1993**, 574–575.
289. Lehn, J. M.; Sauvage, J. P. *J. Am. Chem. Soc.* **1975**, 97, 6700–6707.
290. Cox, B. G.; Schneider, H. *J. Am. Chem. Soc.* **1977**, 99, 2809–2811.
291. Izatt, R. M.; Pawlak, K.; Bradshaw, J. S.; Bruening, R. L. *Chem. Rev.* **1995**, 95, 2529–2586.
292. McDonald, F. C.; Applefield, R. C.; Halkides, C. J.; Reibenspies, J. H.; Hancock, R. D. *Inorg. Chim. Acta* **2008**, 361, 1937–1946.
293. See pp. 217–223 of Ref. (244).
294. Xu, J.; Franklin, S. J.; Whisenhunt, D. W.; Raymond, K. N. *J. Am. Chem. Soc.* **1995**, 117, 7245–7246.
295. Jocher, C. A.; Moore, E. G.; Xu, J.; Avedano, S.; Botta, M.; Aime, S.; Raymond, K. N. *Inorg. Chem.* **2007**, 46, 9182–9191.
296. Odisitse, S.; Jackson, G. E. *Polyhedron* **2008**, 27, 453–464.
297. Marangella, M.; Daniele, P. G.; Ronzani, M.; Sonogo, S.; Linari, F. *Urol. Res.* **1985**, 13, 189–193.
298. (a) Caldin, E. F. “*Fast Reactions in Solution*”; Blackwell Scientific: Oxford, **1964**. (b) Caldin, E. F. “*The Mechanisms of Fast Reactions in Solution*”; IOS Press: Oxford, **2001**.
299. Eigen, M. *Pure Appl. Chem.* **1963**, 6, 97–115.
300. Eigen, M.; Wilkins, R. G. *Adv. Chem. Ser.* **1965**, 49, 55–65.

301. (a) Budarin, L. I.; Yatsimirskii, K. B. *Uspekhi Khimii* **1968**, *37*, 209–225.
(b) Budarin, L. I.; Yatsimirskii, K. B. *Russ. Chem. Rev.* **1968**, *37*, 469–503, and references therein.
302. Helm, L.; Merbach, A. E. *Coord. Chem. Rev.* **1999**, *187*, 151–181.
303. Dunand, F. A.; Helm, L.; Merbach, A. E. *Adv. Inorg. Chem.* **2003**, *54*, 1–69.
304. Helm, L.; Merbach, A. E. *Chem. Rev.* **2005**, *105*, 1923–1960.
305. Cowan, J. A. *“Inorganic Biochemistry: An Introduction”*; 2nd edn. VCH: New York, **1997**; see, e.g., p. 374.
306. Caravan, P.; Merbach, A. E. *Chem. Commun.* **1997**, 2147–2148.
307. Lincoln, S. F. *Helv. Chim. Acta* **2005**, *88*, 523–545.
308. Salmon, P. S. *J. Phys. C* **1987**, *20*, 1573–1587.
309. Salmon, P. S.; Howells, W. S.; Mills, R. *J. Phys. C* **1987**, *20*, 5727–5747.
310. Herdman, G. J.; Salmon, P. S. *J. Am. Chem. Soc.* **1991**, *113*, 2930–2939.
311. See, e.g., Newman, K. E.; Adamson-Sharp, K. M. *Inorg. Chem.* **1984**, *23*, 3818–3822.
312. (a) Schwenk, C. F.; Loeffler, H. H.; Rhode, B. M. *Chem. Phys. Lett.* **2001**, *349*, 99–103. (b) Schwenk, C. F.; Loeffler, H. H.; Rhode, B. M. *J. Chem. Phys.* **2001**, *115*, 10808–10813.
313. Erras-Hanauer, H.; Clark, T.; van Eldik, R. *Coord. Chem. Rev.* **2003**, *239*, 233–253.
314. Atkinson, G.; Emara, M. M.; Fernandez-Prini, R. *J. Phys. Chem.* **1974**, *78*, 1913–1917.
315. Czerlinski, G.; Diebler, H.; Eigen, M. *Z. Phys. Chem. (Neue Folge)* **1959**, *16*, 246–249.
316. Pollard, F. H.; Martin, J. V. *Analyst* **1956**, *81*, 348–353.
317. Kao, J. P.; Tsien, R. Y. *Biophys. J.* **1988**, *53*, 635–639.
318. Bryson, A.; Fletcher, I. S. *Aust. J. Chem.* **1970**, *23*, 1095–1110.
319. Hering, J. G.; Morel, F. M. M. *Environ. Sci. Technol.* **1988**, *22*, 1469–1478.
320. Harada, S.; Funaki, Y.; Yasunaga, T. *J. Am. Chem. Soc.* **1980**, *102*, 136–139.
321. Kasprzyk, S. P.; Wilkins, R. G. *Inorg. Chem.* **1982**, *21*, 3349–3352.
322. Hellam, D. C.; Podolsky, R. J. *J. Physiol.* **1969**, *200*, 807–819.
323. Smith, P. D.; Liesegang, G. W.; Berger, R. L.; Czerlinski, G.; Podolsky, R. J. *Anal. Biochem.* **1984**, *143*, 188–195.
324. Margerum, D. W.; Menardi, P. J.; Janes, D. L. *Inorg. Chem.* **1967**, *6*, 283–289.
325. Raspor, B.; Nurnberg, H. W.; Valenta, P.; Branica, M. *J. Electroanal. Chem.* **1980**, *115*, 293–308.
326. Cox, B. G.; Firman, P.; Schneider, H. *Inorg. Chim. Acta* **1983**, *69*, 161–166.
327. Liesegang, G. W. *J. Am. Chem. Soc.* **1981**, *103*, 953–955.
328. Loyola, V. M.; Pizer, R.; Wilkins, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 7185–7188.
329. Bemtgen, J. M.; Springer, M. E.; Loyola, V. M.; Wilkins, R. G.; Taylor, R. W. *Inorg. Chem.* **1984**, *23*, 3348–3353.
330. Eberhard, M.; Erne, P. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 209–215.
331. Hering, J. G.; Morel, F. M. M. In: *“Aquatic Chemical Kinetics”*; Ed. Stumm, W.; Wiley-Interscience: New York, **1990**, Chap. 5.
332. Pausch, J. B.; Margerum, D. W. *Analyt. Chem.* **1969**, *41*, 226–232.
333. Kuempel, J. R.; Schaap, W. B. *Inorg. Chem.* **1968**, *7*, 2435–2442.

334. Breen, P. J.; Horrocks, W. DeW.; Johnson, K. A. *Inorg. Chem.* **1986**, *25*, 1968–1971.
335. Funahashi, S.; Yamada, S.; Tanaka, M. *Inorg. Chem.* **1971**, *10*, 257–263.
336. Hering, J. G.; Morel, F. M. M. *Geochim. Cosmochim. Acta* **1989**, *53*, 611–618.
337. See, e. g., Gerbeleu, N. V.; Arion, V. B.; Burgess, J. “*Template Synthesis of Macrocyclic Compounds*”; Wiley-VCH: Weinheim, **1999**.
338. Adams, H.; Bailey, N. A.; Fenton, D. E.; Good, R. J.; Moody, R.; Rodriguez de Barbarin, C. *J. Chem. Soc., Dalton Trans.* **1987**, 207–218.
339. Aspinall, H. C.; Black, J.; Dodd, I.; Harding, M. M.; Winkley, S. J. *J. Chem. Soc., Dalton Trans.* **1993**, 709–714.
340. Drew, M. G. B.; Yates, P. C.; Murphy, B. P.; Nelson, J.; Nelson, S. M. *Inorg. Chim. Acta* **1986**, *118*, 37–47.
341. Yuan, Z.-L.; Zhang, Q.-L.; Liang, X.; Zhu, B.-X.; Lindoy, L. F.; Wei, G. *Polyhedron* **2008**, *27*, 344–348.
342. Hu, X.-L.; Li, Y.-Z.; Luo, Q.-H.; Pan, Z.-Q. *J. Coord. Chem.* **2007**, *60*, 2519–2526.
343. Liu, Y.; Simon, J. D. *Pigment Cell Res.* **2005**, *18*, 42–48.
344. Mukkamala, S. B.; Anson, C. E.; Powell, A. K. *J. Inorg. Biochem.* **2006**, *100*, 1128–1138.
345. Ajayghosh, A.; Chithra, P.; Varghese, R.; Divya, K. P. *Chem. Commun.* **2008**, 969–971.
346. Hastings, J. W.; Mitchell, G.; Mattingly, P. H.; Blinks, J. R.; Van Leeuwen, M. *Nature* **1969**, *222*, 1047–1050.
347. Martin, S. R.; Lu, A. Q.; Xiao, J.; Kleijung, J.; Beckingham, K.; Bayley, P. M. *Prot. Sci.* **1999**, *8*, 2444–2454.
348. Concha, N. O.; Head, J. F.; Kaetzel, M. A.; Dedman, J. R.; Seaton, B. A. *Science* **1993**, *261*, 1321–1324.
349. Gerke, V.; Creutz, C. E.; Moss, S. E. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 449–461.
350. Gouaux, E.; MacKinnon, R. *Science* **2005**, *310*, 1461–1465.
351. Lacapère, J.-J.; Pebay-Peyroula, E.; Neumann, J.-M.; Etchebest, C. *Trends Biochem. Sci.* **2007**, *32*, 259–270.
352. Shapiro, L.; Colman, D. R. *Curr. Opin. Neurobiol.* **1998**, *8*, 593–599.
353. Hynes, R. O.; Lander, A. *Cell* **1992**, *68*, 303–322.
354. Sallee, J. L.; Wittchen, E. S.; Burridge, K. *J. Biol. Chem.* **2006**, *281*, 16189–16192.
355. Springer, T. A.; Wang, J.-H. *Adv. Prot. Chem.* **2004**, *68*, 29–63.
356. Weis, W. I.; Nelson, W. J. *J. Biol. Chem.* **2006**, *281*, 35593–35597.
357. Forsén, S.; Kördel, J.; Grundström, T.; Chazin, W. J. *Acc. Chem. Res.* **1993**, *26*, 7–14.
358. Aramburu, J.; Rao, A.; Klee, C. B. *Curr. Top. Cell Regul.* **2000**, *36*, 237–295.
359. Mumby, M. *ACS Chem. Biol.* **2007**, *2*, 99–103.
360. Yamniuk, A. P.; Nguyen, L. T.; Hoang, T. T.; Vogel, H. J. *Biochemistry* **2004**, *43*, 2558–2568.
361. Yamniuk, A. P.; Ishida, H.; Vogel, H. J. *J. Biol. Chem.* **2006**, *281*, 26455–26464.
362. Klee, C. B.; Vanaman, T. C. *Adv. Prot. Chem.* **1982**, *35*, 213–321.
363. Bachs, O.; Agell, N.; Carafoli, E. *Biochim. Biophys. Acta* **1992**, *1113*, 259–270.
364. “*Calmodulin*”, Eds. Cohen, P.; Klee, C. B.; Elsevier: Amsterdam, **1988**.
365. Klee, C. B. *Biochemistry* **1988**, *27*, 6645–6653.

366. Hong, L.; Simon, J. D. *J. Phys. Chem. B* **2007**, *111*, 7938–7947.
367. Ohnishi, M.; Reithmeier, R. A. F. *Biochemistry* **1987**, *26*, 7458–7465.
368. Zhang, M.; Yamazaki, T.; Yazawa, M.; Treves, S.; Nishi, M.; Murai, M.; Shibata, E.; Zorzato, F.; Takeshima, H. *Cell Calcium* **2007**, *42*, 83–90.
369. Vyas, N. K.; Vyas, M. N.; Quiocho, F. A. *Nature* **1987**, *327*, 635–638.
370. Kachalova, G.; Morozov, V. N.; Morozova, T. Ya.; Myachin, E. T.; Vagin, A. A.; Strokopytov, B. V.; Nekrasov, Yu. V. *FEBS Lett.* **1991**, *284*, 91–94.
371. McKenzie, H. A.; White, F. H. *Adv. Prot. Chem.* **1991**, *41*, 173–315.
372. Prota, G. *Med. Res. Rev.* **1988**, *8*, 525–556.
373. Panessa, B. J.; Zadunaisky, J. A. *Exp. Eye Res.* **1981**, *32*, 593–604.
374. Hoogduijn, M. J.; Smit, N. P.; Van Der Laarse, A.; Van Nieuwpoort, A. F.; Wood, J. M.; Thoday, A. J. *Pigment Cell Res.* **2003**, *16*, 127–132.
375. Ahmed, F. R.; Przybylska, M.; Rose, D. R.; Birnbaum, G. L.; Pippy, M. E.; MacManus, J. P. *J. Mol. Biol.* **1990**, *216*, 127–140.
376. Hauschke, P. V.; Lian, J. B.; Cole, D. E. C.; Gundberg, C. M. *Physiol. Rev.* **1989**, *69*, 990–1047.
377. Nielsen-Marsh, C. M.; Richards, M. P.; Hauschka, P. V.; Thomas-Oates, J. E.; Trinkaus, E.; Pettitt, P. B.; Karavanic, I.; Poinar, H.; Collins, M. J. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4409–4413.
378. Dijkstra, B. W.; Kalk, K. H.; Hol, W. G. J.; Drenth, J. *J. Mol. Biol.* **1981**, *147*, 97–123.
379. Dijkstra, B. W.; Renetseder, R.; Kalk, K. H.; Hol, W. G. J.; Drenth, J.; Huber, R. *J. Mol. Biol.* **1983**, *168*, 163–179.
380. Sorin, A.; Rosas, G.; Rao, R. *J. Biol. Chem.* **1997**, *272*, 9895–9901.
381. Wei, Y.; Marchi, V.; Wang, R.; Rao, R. *Biochemistry* **1999**, *38*, 14534–14541.
382. (a) Bajorath, J.; Raghunathan, S.; Hinrichs, W.; Saenger, W. *Nature* **1989**, *337*, 481–484. (b) Bajorath, J.; Hinrichs, W.; Saenger, W. *Eur. J. Biochem.* **2005**, *176*, 441–447.
383. Schwabe, C.; Büllesbach, E. E. *Comp. Biochem. Physiol., B: Comp. Biochem.* **1990**, *96*, 15–21.
384. Fields, P. A. *Ann. NY Acad. Sci.* **2005**, *1041*, 328–331.
385. (a) Cotton, F. A.; Bier, C. J.; Day, V. W.; Hazen, E. E.; Larson, S. *Cold Spring Harbor Symp. Quant. Biol.* **1971**, *36*, 243–249. (b) Cotton, F. A.; Hazen, E. E.; Legg, M. J. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2551–2555.
386. Shortle, D. *Adv. Prot. Chem.* **1995**, *46*, 217–247.
387. Thimon, L.; Peypoux, F.; Wallach, J.; Michel, G. *Coll. Surf. B: Biointerf.* **1993**, *1*, 57–62.
388. Rizo, J.; Südhof, T. C. *J. Biol. Chem.* **1998**, *273*, 15879–15882.
389. Satyshur, K. A.; Rao, S. T.; Pyzalska, D.; Drendel, W.; Greaser, M.; Sundaralingam, M. *J. Biol. Chem.* **1988**, *263*, 1628–1647.
390. Fujimori, K.; Sorenson, M.; Herzberg, O.; Moulton, J.; Reinach, F. C. *Nature* **1990**, *345*, 182–184.
391. Chin, D.; Means, A. R. *Trends Cell Biol.* **2000**, *10*, 322–328.
392. (a) Babu, Y. S.; Sack, J. S.; Greenhough, T. J.; Bugg, C. E.; Means, A. R.; Cook, W. J. *Nature* **1985**, *315*, 37–40. (b) Babu, Y. S.; Bugg, C. E.; Cook, W. J. *J. Mol. Biol.* **1988**, *204*, 191–204.
393. Taylor, D. A.; Sack, J. S.; Maune, J. F.; Beckingham, K.; Quiocho, F. A. *J. Biol. Chem.* **1991**, *266*, 21375–21380.
394. Kuboniwa, H.; Tjandra, N.; Grzesiek, S.; Ren, H.; Klee, C. B.; Bax, A. *Nat. Struct. Biol.* **1995**, *2*, 768–776.

395. Stuehr, D. J.; Santolini, J.; Wang, Z.-Q.; Wei, C.-C.; Adak, S. *J. Biol. Chem.* **2004**, *279*, 36167–36170.
396. Hook, S. S.; Mearns, A. R. *Ann. Rev. Pharmacol. Toxicol.* **2001**, *41*, 471–505.
397. Ishida, A.; Sueyoshi, N.; Shigeri, Y.; Kameshita, I. *Br. J. Pharmacol.* **2008**, *154*, 729–740.
398. Soderling, T. R.; Stull, J. T. *Chem. Rev.* **2001**, *101*, 2341–2352.
399. Newton, A. C. *Chem. Rev.* **2001**, *101*, 2353–2364.
400. Hartshorne, D. J.; Ito, M.; Erdödi, F. *J. Biol. Chem.* **2004**, *279*, 37211–37214.
401. Trudeau, M. C.; Zagotta, W. N. *J. Biol. Chem.* **2003**, *278*, 18705–18708.
402. Lodish, H. F.; Kong, N.; Wilkstrom, L. *J. Biol. Chem.* **1992**, *267*, 12753–12760.
403. Cheung, W. Y. *Rec. Trav. Chim. Pays-Bas* **1987**, *106*, 262.
404. Meador, W. E.; Mearns, A. R.; Quirocho, F. A. *Science* **1992**, *257*, 1251–1255.
405. Hoelz, A.; Naim, A. C.; Kuriyan, J. *Mol. Cell* **2003**, *11*, 1241–1251.
406. Tsuda, S.; Ogura, K.; Hasegawa, Y.; Yagi, K.; Hikichi, K. *Biochemistry* **1990**, *29*, 4951–4958.
407. Robertson, S. P.; Johnson, J. D.; Potter, J. D. *Biophys. J.* **1981**, *34*, 559–569.
408. Bewley, M. C.; Bousted, C. M.; Walker, J. H.; Waller, D. A.; Huber, R. *Biochemistry* **1993**, *32*, 3923–3929.
409. Rhee, M.-J.; Horrocks, W. DeW.; Kosow, D. P. *Biochemistry* **1982**, *21*, 4524–4528.
410. Sabharwal, A. K.; Birktoft, J. J.; Gorka, J.; Wildgoose, P.; Peterson, L. C.; Bajaj, S. P. *J. Biol. Chem.* **1995**, *270*, 15523–15530.
411. Bajaj, S. P. *J. Biol. Chem.* **1982**, *257*, 4127–4132.
412. Lodish, H.; Baltimore, D.; Berk, A.; Zipursky, S. L.; Matsudaira, P.; Darnell, J. “*Molecular Cell Biology*”; 3rd edn. W. H. Freeman: New York, **1995**; p. 646.
413. Hughes, M. N. In: “*Comprehensive Coordination Chemistry*”; vol. 1; Eds. Wilkinson, G.; Gillard, R. D.; McCleverty, J. A.; Pergamon: Oxford, **1987**, p. 574; and references therein.
414. Kilhoffer, M.-C.; Demaille, J. G.; Gerard, D. *Biochemistry* **1981**, *20*, 4407–4414.
415. Schmid, F. X. *Adv. Prot. Chem.* **2002**, *59*, 243–282.
416. Matthews, B. W.; Weaver, L. H.; Kester, W. R. *J. Biol. Chem.* **1974**, *249*, 8030–8044.
417. Lin, S. L.; Stern, E. A.; Kalb, A. J.; Zhang, Y. *Biochemistry* **1990**, *29*, 3599–3603.
418. Kalb, A. J.; Habash, J.; Hunter, N. S.; Price, H. J.; Raftery, J.; Helliwell, J. R. In: “*Metal Ions in Biological Systems. Volume 37. Manganese and its Role in Biological Processes*”; Eds. Sigel, A.; Sigel, H.; Marcel Dekker: New York, **2000**.
419. Jiang, W.; Han, Y.; Pan, Q.; Shen, T.; Liu, C. *J. Inorg. Biochem.* **2007**, *101*, 667–677.
420. Shiono, M.; Matsugaki, N.; Takeda, K. *Nature* **2005**, *436*, 791.
421. Strynadka, N. C. J.; James, N. M. G. *Annu. Rev. Biochem.* **1989**, *58*, 951–958.
422. Persechini, A.; Moncrief, N. D.; Kretsinger, R. H. *Trends Neurosci.* **1989**, *12*, 462–467.
423. Dudev, T.; Lim, C. *Chem. Rev.* **2003**, *103*, 773–787.

424. Babitch, J. *Nature* **1990**, 346, 321–322.
425. Kretsinger, R. H.; Nockolds, C. E. *J. Biol. Chem.* **1973**, 248, 3313–3326.
426. Kilhoffer, M.-C.; Demaille, J. G.; Gerard, D. *FEBS Lett.* **1980**, 116, 269–272.
427. Nalefski, E. A.; Falke, J. J. *Prot. Sci.* **1996**, 5, 2375–2390.
428. Sutton, R. B.; Davletov, B. A.; Berghuis, A. M.; Südhof, T. C.; Sprang, S. R. *Cell* **1995**, 80, 929–938.
429. Ubach, J.; Leo, Y.; Fernandez, I.; Arac, D.; Südhof, T. C.; Rizo, J. *Biochemistry* **2001**, 40, 5854–5860.
430. Fernandez, I.; Araç, D.; Ubach, J.; Gerber, S. H.; Shin, O.; Gao, Y.; Anderson, R. G. W.; Südhof, T. C.; Rizo, J. *Neuron* **2001**, 32, 1057–1069.
431. Südhof, T. C. *J. Biol. Chem.* **2002**, 277, 7629–7632.
432. Hong, L.; Simon, J. D. *Photochem. Photobiol.* **2006**, 82, 1265–1269.
433. Drickamer, K. *Curr. Opin. Struct. Biol.* **1999**, 9, 585–590.
434. Ng, K. K.-S.; Park-Snyder, S.; Weis, W. I. *Biochemistry* **1998**, 37, 17965–17976.
435. Weis, W. I.; Drickamer, K.; Hendrickson, W. A. *Nature* **1992**, 360, 127–134.
436. Szebenyi, D. M. E.; Obendorf, S. K.; Moffat, K. *Nature* **1981**, 294, 327–332.
437. (a) Swain, A. L.; Amma, E. L. *Inorg. Chim. Acta* **1989**, 163, 5–7.
(b) Kretsinger, R. H. *CRC Crit. Rev. Biochem.* **1980**, 8, 119–174.
438. Matthews, B. W.; Colman, P. M.; Jansonius, J. N.; Titani, K.; Walsh, K. A.; Neurath, H. *Nat. New Biol.* **1972**, 238, 41–43.
439. Colman, P. M.; Jansonius, J. N.; Matthews, B. W. *J. Mol. Biol.* **1972**, 70, 701–724.
440. Hwang, Y. T.; Andrews, L. J.; Solomon, E. I. *J. Am. Chem. Soc.* **1984**, 106, 3832–3838.
441. Sugana, K.; Bott, R. R.; Padlan, E. A.; Subramanian, E.; Sheriff, S.; Cohen, G. H.; Davies, D. R. *J. Mol. Biol.* **1987**, 196, 877–900.
442. Ubach, J.; Zhang, X.; Shao, X.; Südhof, T. C.; Rizo, J. *EMBO J.* **1998**, 17, 3921–3930.
443. (a) Toyoshima, C.; Nakasako, M.; Nomura, H.; Ogawa, H. *Nature* **2000**, 405, 647–655. (b) Toyoshima, C.; Nomura, H. *Nature* **2002**, 418, 605–611.
444. Hardman, K. D.; Agarwal, R. C.; Freiser, M. J. *J. Mol. Biol.* **1982**, 157, 69–86.
445. Deacon, A.; Gleichmann, T.; Kalb, A. J.; Price, H.; Raftery, J.; Bradbrook, G.; Yariv, J.; Helliwell, J. R. *J. Chem. Soc., Faraday Trans. 1* **1997**, 93, 4305–4312.
446. Einspahr, H.; Suguna, K.; Suddath, F. L.; Ellis, G.; Helliwell, J. R.; Papiz, M. Z. *Acta Cryst.* **1985**, B41, 336–341.
447. Herzberg, O.; James, M. N. G. *Nature* **1985**, 313, 653–659.
448. Horrocks, W. DeW.; Rhee, M.-J.; Snyder, A. P.; Sudnick, D. R. *J. Am. Chem. Soc.* **1980**, 102, 3650–3652.
449. Martin, R. B. *Clin. Chem.* **1986**, 32, 1797–1806.
450. Szebenyi, D. M.; Moffat, K. *J. Biol. Chem.* **1986**, 261, 8761–8777.
451. Bush, W. D.; Simon, J. D. *Pigment Cell Res.* **2007**, 20, 134–139.
452. Sánchez-Magraner, L.; Viguera, A. R.; Garcia-Pacios, M.; Garcillán, M. P.; Arrondo, J.-L. R.; de la Cruz, F.; Goñi, F. M.; Ostolaza, H. *J. Biol. Chem.* **2007**, 282, 11827–11835.
453. Kalb, A. J.; Levitzki, A. *Biochem. J.* **1968**, 109, 669–672.
454. Harrington, P. C.; Wilkins, R. G. *Biochemistry* **1978**, 17, 4245–4250.

455. Falke, J. J.; Snyder, E. E.; Thatcher, K. C.; Voertler, C. S. *Biochemistry* **1991**, *30*, 8690–8697.
456. Voordouw, G.; Roche, R. S. *Biochemistry* **1974**, *13*, 5017–5027.
457. Martin, S. R.; Masino, L.; Bayley, P. M. *Prot. Sci.* **2000**, *9*, 2477–2488.
458. Gribenko, A. V.; Makhatadze, G. I. *J. Mol. Biol.* **1998**, *283*, 679–694.
459. Aramini, J. M.; Drakenberg, T.; Hiraoki, T.; Ke, Y.; Nitta, K.; Vogel, H. J. *Biochemistry* **1992**, *31*, 6761–6768.
460. Linse, S.; Brodin, P.; Drakenberg, T.; Thulin, E.; Sellers, P.; Elmdén, K.; Grundström, T.; Forsén, S. *Biochemistry* **1987**, *26*, 6723–6735.
461. Mandal, D.; Woolf, T. B.; Rao, R. J. *Biol. Chem.* **2000**, *275*, 23933–23938.
462. Cox, J. A.; Milos, M.; MacManus, J. P. *J. Biol. Chem.* **1990**, *265*, 6633–6637.
463. Henzl, M. T.; Hapak, R. C.; Goodpasture, E. A. *Biochemistry* **1996**, *35*, 5856–5869.
464. Benzonana, G.; Caponey, J.-P.; Pechere, J.-F. *Biochim. Biophys. Acta* **1972**, *278*, 110–116.
465. Pauls, T. L.; Cox, J. A.; Berchtold, M. W. *Biochim. Biophys. Acta* **1996**, *1306*, 39–54.
466. Lee, S.-H.; Schwaller, B.; Neher, E. *J. Physiol.* **2000**, *525*, 419–432.
467. Moeschler, H. J.; Schaer, J. J.; Cox, J. A. *Eur. J. Biochem.* **1980**, *111*, 73–78.
468. Tanokura, M.; Yamada, K. *FEBS Lett.* **1985**, *185*, 165–169.
469. Tanokura, M.; Imaizumi, M.; Yamada, K. *FEBS Lett.* **1986**, *209*, 77–82.
470. Desmet, J.; Van Dael, H.; Van Cauwelaert, F.; Nitta, K.; Sugai, S. *J. Inorg. Biochem.* **1989**, *37*, 185–191.
471. Schaer, J. J.; Milos, M.; Cox, J. A. *FEBS Lett.* **1985**, *190*, 77–80.
472. Van Ceunebroeck, J. C.; Hanssens, I.; Joniau, M.; Van Cauwelaert, F. *J. Biol. Chem.* **1985**, *260*, 10944–10947.
473. Desmet, J.; Hanssens, I.; Van Cauwelaert, F. *Biochim. Biophys. Acta* **1987**, *912*, 211–219.
474. Desmet, J.; Haezebrouck, P.; Van Cauwelaert, F. *J. Inorg. Biochem.* **1991**, *42*, 139–145.
475. Milos, M.; Schaer, J. J.; Comte, M.; Cox, J. A. *J. Biol. Chem.* **1987**, *262*, 2746–2749.
476. Hinton, J. F.; Koeppe, R. E. In: “*Metal Ions in Biological Systems. Volume 19. Antibiotics and Their Complexes*”; Ed. Sigel, H.; Marcel Dekker: New York, **1985**, Chap. 7.
477. See, e.g., Seaman, K. B.; Kretsinger, R. H. In: “*Calcium in Biology*”; Ed. Spiro, T. G.; Wiley: New York, **1983**, pp. 3–51.
478. Table 7 of Ref. (423).
479. Butow, R. A.; Chevallier, J. *Biochemistry* **1971**, *10*, 2733–2737.
480. Shami, Y.; Messer, H. H.; Copp, D. H. *Biochim. Biophys. Acta* **1974**, *339*, 323–333.
481. Chiu, V. C. K.; Haynes, D. H. *Biophys. J.* **1977**, *18*, 3–22.
482. Maune, J. F.; Klee, C. B.; Beckingham, K. *J. Biol. Chem.* **1992**, *267*, 5286–5295.
483. Hofmann, T.; Eng, S.; Lilja, H.; Drakenberg, T.; Vogel, H. J.; Forsén, S. *Eur. J. Biochem.* **1988**, *172*, 307–313.
484. Martin, S. R.; Linse, S.; Johansson, C.; Bayley, P. M.; Forsén, S. *Biochemistry* **1990**, *29*, 4188–4193.
485. Crouch, T. H.; Klee, C. B. *Biochemistry* **1980**, *19*, 3692–3698.
486. Vogel, H. J.; Drakenberg, T.; Forsén, S.; O'Neill, J. D. J.; Hofmann, T. *Biochemistry* **1985**, *24*, 3870–3876.

487. Olwin, B. B.; Edelman, A. M.; Krebs, E. G.; Storm, D. R. *J. Biol. Chem.* **1984**, *259*, 10949–10955.
488. Yazawa, M.; Ikura, M.; Hikichi, K.; Ying, L.; Yagi, K. *J. Biol. Chem.* **1987**, *262*, 10951–10954.
489. Sugita, S.; Shin, O.-K.; Han, W.; Lao, Y.; Südhof, T. C. *EMBO J.* **2002**, *21*, 270–280.
490. See, e.g., Billo, E. J.; Brito, K. K.; Wilkins, R. G. *Bioinorg. Chem.* **1978**, *8*, 461–475.
491. Noyelle, K.; Van Dael, H. *J. Inorg. Biochem.* **2002**, *88*, 69–76.
492. Breen, P. J.; Hild, E. K.; Horrocks, W. DeW. *Biochemistry* **1985**, *24*, 4991–4997.
493. Baylor, S. M.; Chandler, W. K.; Marshall, M. W. *J. Physiol.* **1983**, *344*, 625–666.
494. Nägerl, U. V.; Novo, D.; Mody, I.; Vergera, J. L. *Biophys. J.* **2000**, *79*, 3009–3018.
495. Czerlinski, G. H. *Physiol. Chem. Phys. Med. NMR* **1992**, *24*, 213–226.
496. Ashley, C. C.; Barsotti, R. J.; Ferenczi, M. A.; Lea, T. J.; Mulligan, I. P.; Tsien, R. Y. *J. Physiol.* **1987**, *390*, 144P.
497. Breen, P. J.; Johnson, K. A.; Horrocks, W. DeW. *Biochemistry* **1985**, *24*, 4997–5004.
498. White, H.; Closset, J. *Biophys. J.* **1979**, *25*, 247a.
499. White, H. *Biochemistry* **1988**, *27*, 3357–3365.
500. Van Dael, H.; Chedad, A. *J. Fluoresc.* **2006**, *16*, 361–365.
501. Wirmer, J.; Kühn, T.; Schwalbe, H. *Angew. Chem. Int. Ed.* **2001**, *40*, 4248–4251.
502. Koenig, S. H.; Brewer, C. F.; Brown, R. D. *Biochemistry* **1978**, *17*, 4251–4260.
503. Harrington, P. C.; Moreno, R.; Wilkins, R. G. *Israel J. Chem.* **1981**, *21*, 48–51.
504. Stroupe, S. D.; Doyle, R. J. *J. Inorg. Biochem.* **1980**, *12*, 173–178.
505. Farina, R. D.; Wilkins, R. G. *Biochim. Biophys. Acta* **1980**, *631*, 428–438.
506. (a) Martin, S. R.; Andersson Teleman, A.; Bayley, P. M.; Drakenberg, T.; Forsén, S. *Eur. J. Biochem.* **1985**, *151*, 543–550. (b) Martin, S. R.; Maune, J. F.; Beckingham, K.; Bayley, P. M. *Eur. J. Biochem.* **1992**, *2005*, 1107–1114.
507. Corson, D. C.; Lee, L.; McQuaid, G. A.; Sykes, B. D. *Can. J. Biochem. Cell Biol.* **1983**, *61*, 860–867.
508. Müller, M.; Felmy, F.; Schwaller, B.; Schneggenburger, R. *J. Neurosci.* **2007**, *27*, 2261–2271.
509. Aponte, Y.; Bishofberger, J.; Jonas, P. *J. Physiol.* **2008**, *586*, 2061–2075.
510. Hou, T. T.; Johnson, J. D.; Rall, J. A. *J. Physiol.* **1992**, *449*, 399–410.
511. Aramini, J. M.; Hiraoki, T.; Grace, M. R.; Swaddle, T. W.; Chiancone, E.; Vogel, H. J. *Biochim. Biophys. Acta* **1996**, *1293*, 72–82.
512. Vanderheeren, G.; Hanssens, I.; Meijberg, W.; Van Aerschot, A. *Biochemistry* **1996**, *35*, 16753–16759.
513. Chiancone, E.; Drakenberg, T.; Teleman, O.; Forsén, S. *J. Mol. Biol.* **1985**, *185*, 201–207.
514. Lavigne, J. A.; Langford, C. H.; Mak, M. K. S. *Anal. Chem.* **1987**, *59*, 2520–2616.
515. Cabaniss, S. E.; Shuman, M. S. *Geochim. Cosmochim. Acta* **1988**, *52*, 185–200.
516. Hering, J. G.; Morel, F. M. M. *Environ. Sci. Technol.* **1988**, *22*, 1234–1237.
517. Salceda, R.; Sánchez-Chávez, G. *Cell Calcium* **2000**, *27*, 223–229.

518. Fletcher, J. M.; Greenfield, B. F.; Hardy, C. J.; Scargill, D.; Woodhead, J. L. *J. Chem. Soc.* **1961**, 2000–2006.
519. (a) Charonnat, R. In: “*Nouveau Traité de Chimie Minérale*”; vol. 19; Ed. Pascal, P.; Masson: Paris, **1958**, pp. 127–129. (b) Cotton, S. A. “*Chemistry of Precious Metals*”; Chapman & Hall: London, **1997**; p. 25.
520. (a) Joly, A. *Comptes Rend. Hebd. Seances Acad. Sci.* **1892**, 114, 291–293. (b) Joly, A. *Comptes Rend. Hebd. Seances Acad. Sci.* **1892**, 115, 1299–1301.
521. Mangin, L. *Comptes Rend. Hebd. Seances Acad. Sci.* **1893**, 116, 653–656.
522. Morgan, G. T.; Burstall, F. H. *J. Chem. Soc.* **1936**, 41–45.
523. See, e.g., Luft, J. H. *Anat. Rec.* **1971**, 171, 347–368, 369–416.
524. Charuk, J. H. M.; Pirraglia, C. A.; Reithmeier, R. A. F. *Anal. Biochem.* **1990**, 188, 123–131.
525. Gomez-Puyou, A.; de Gomez-Puyou, M. T.; Becker, G.; Lehninger, A. L. *Biochem. Biophys. Res. Commun.* **1972**, 47, 814–819.
526. Tashmukhamedov, B. A.; Gagelgans, A. I.; Mamatkulov, K.; Makhmudova, E. M. *FEBS Lett.* **1972**, 28, 239–242.
527. Mitani, M.; Ōtake, N. *J. Antibiot.* **1978**, 31, 888–893.
528. Emerson, J.; Clarke, M. J.; Ying, W.-L.; Sanadi, D. R. *J. Am. Chem. Soc.* **1993**, 115, 11799–11805.
529. Bleakman, D.; Brorson, R. R.; Miller, R. J. *Br. J. Pharmacol.* **1990**, 101, 423–431.
530. Carrondo, M. A. A. F. de C. T.; Griffith, W. P.; Hall, J. P.; Skapski, A. C. *Biochim. Biophys. Acta* **1980**, 627, 332–334.
531. Reed, K. C.; Bygrave, F. L. *FEBS Lett.* **1974**, 46, 109–114.
532. Ying, W.-L.; Emerson, J.; Clarke, M. J.; Sanadi, D. R. *Biochemistry* **1991**, 30, 4949–4952.
533. Smith, P. M.; Fealey, T.; Earley, J. E.; Silverton, J. V. *Inorg. Chem.* **1971**, 10, 1943–1947.
534. Basmadjian, D.; Sefton, M. V.; Baldwin, S. A. *Biomaterials* **1997**, 18, 1511–1522.
535. See, e.g., (a) Epstein, I. R. *Proc. Natl. Acad. Sci. USA* **2006**, 103, 15727–15728, (b) Kastrop, C. J.; Runyon, M. K.; Shen, F.; Ismagilov, R. F. *Proc. Natl. Acad. Sci. USA* **2006**, 103, 15747–15752; and references therein.
536. Smith, S. A.; Mutch, N. J.; Baskar, D.; Rohloff, P.; Docampo, R.; Morrissey, J. H. *Proc. Natl. Acad. Sci. USA* **2006**, 103, 903–908.
537. Bezanilla, F.; Perozo, E. *Adv. Prot. Chem.* **2003**, 63, 211–241.
538. *Ann. N. Y. Acad. Sci.* **1989**, 568 – several articles in this volume deal with calcium in cells and membranes.
539. “*Ion Channel Pharmacology*”; Eds. Soria, B.; Ceña, V.; OUP: Oxford, **1998**.
540. Alexander, S. P. H.; Mathie, A.; Peters, J. A. “*Guide to Receptors and Channels (GRAC)*”, 2nd edn., *Br. J. Pharmacol.* **2007**, 150 (Suppl. 1), S1–S168.
541. (a) Griffin, W. C. *J. Soc. Cosmet. Chem.* **1954**, 5, 249–256. (b) McGowan, J. C. *Tenside Surf. Det.* **1990**, 27, 229–230. (c) Van de Waterbeemd, H.; Testa, B. *Adv. Drug Res.* **1987**, 16, 85–225. (d) “*Calculation of Drug Lipophilicity*”; Rekker, R. F.; Mannhold, R.; Eds., VCH: Weinheim, **1992**.
542. Stewart, K. M.; Horton, K. L.; Kelley, S. O. *Org. Biomol. Chem.* **2008**, 2242–2255.
543. (a) Ōmura, S.; Shibata, M.; Machida, S.; Sawada, J.; Ōtake, N. *J. Antibiotics* **1976**, 29, 15–20. (b) Ōtake, N.; Koenuma, M.; Miyamae, H.; Sato, S.; Saito, Y. *J. Chem. Soc., Perkin Trans.* **1977**, 2, 494–496.

544. Petcher, T. J.; Weber, H.-P. *J. Chem. Soc., Chem. Commun.* **1974**, 697–698.
545. Westley, J. W.; Evans, R. H.; Williams, T.; Stempel, A. *Chem. Commun.* **1970**, 71–72.
546. Lindoy, L. F. *Coord. Chem. Rev.* **1996**, *148*, 349–368.
547. Berger, J.; Rachlin, A. I.; Scott, W. E.; Sterbach, L. H.; Goldberg, M. W. *J. Am. Chem. Soc.* **1951**, *73*, 5295–5298.
548. Mitani, M.; Umetsu, T.; Yamanishi, T.; Ōtake, N. *J. Antibiotics* **1977**, *30*, 239–243.
549. Toeplitz, B. K.; Cohen, A. I.; Funke, P. T.; Parker, W. L.; Gougoutas, J. Z. *J. Am. Chem. Soc.* **1979**, *101*, 3344–3353.
550. Negri, D. P.; Kishi, Y. *Tetrahedron Lett.* **1987**, *28*, 1063–1066.
551. Chaney, M. O.; Demarco, P. V.; Jones, N. D.; Occolowitz, J. L. *J. Am. Chem. Soc.* **1974**, *96*, 1932–1933.
552. Evans, D. A.; Sacks, C. E.; Kleschick, W. A.; Taber, T. R. *J. Am. Chem. Soc.* **1979**, *101*, 6789–6791.
553. “Metal Ions in Biological Systems. Volume 19. Antibiotics and Their Complexes”, Ed. Sigel, H.; Marcel Dekker: New York, **1985**.
554. Riddell, F. G. *Chem. Brit.* **1992**, 533–537.
555. “Handbook of Antibiotics”, 3rd edn., Ed. Reece, R.E.; Lippincott Williams & Wilkins: Philadelphia, **2000**.
556. Deber, C. M.; Pfeiffer, D. R. *Biochemistry* **1976**, *15*, 132–141.
557. Smith, G. D.; Duax, W. L. *J. Am. Chem. Soc.* **1976**, *98*, 1578–1580.
558. Chaney, M. O.; Jones, N. D.; Debono, M. *J. Antibiot.* **1976**, *29*, 424–427.
559. Vishwanath, C. K.; Easwaran, K. R. K. *FEBS Lett.* **1983**, *153*, 320–324.
560. Vishwanath, C. K.; Easwaran, K. R. K. *J. Chem. Soc., Perkin Trans.* **1985**, *2*, 65–68.
561. Duesler, E. N.; Paul, I. C. In: “Polyether Antibiotics: Naturally Occurring Acid Ionophores”, vol. 2; Ed. Westley, J. W.; Marcel Dekker: New York, **1983**, pp. 138–164.
562. (a) Suzuki, K.; Tohda, K.; Aruga, H.; Matsuzoe, M.; Inoue, H.; Shirai, T. *Anal. Chem.* **1988**, *60*, 1714–1721. (b) Suzuki, K.; Tohda, K. *Trends Anal. Chem.* **1993**, *12*, 287–296.
563. Jin, L.; Amaya-Mazo, X.; Apel, M. E.; Sankisa, S. S.; Johnson, E.; Zbyszynska, M. A.; Han, A. *Biophys. Chem.* **2007**, *128*, 185–196.
564. Kowal, C. D.; Larnier, J. M. In: “Human Pharmacology: Molecular-to-Clinical”; Eds. Wingard, L. B.; Brody, T. M.; Larnier, J.; Schwartz, A.; Mosby-Year Book: St. Louis, **1991**, Chap. 43.
565. Roeske, R. W.; Isaac, S.; King, T. E.; Steinrauf, L. K. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 554–561.
566. Steinrauf, L. K. In: “Metal Ions in Biological Systems. Volume 19. Antibiotics and Their Complexes”; Ed. Sigel, H.; Marcel Dekker: New York, **1985**, Chap. 6.
567. Prince, R. C.; Crofts, A. R.; Steinrauf, L. K. *Biochem. Biophys. Res. Comm.* **1974**, *59*, 697–703.
568. (a) Hammill, R. L.; Higgins, C. E.; Boaz, H. E.; Gorman, M. *Tetrahedron Lett.* **1969**, *49*, 4255–4258. (b) Geddes, A. J.; Akrigg, D. *Acta Cryst., B* **1976**, *32*, 3164–3171. (c) Hamilton, J. A.; Steinrauf, L. K.; Braden, B. *Biochem. Biophys. Res. Comm.* **1975**, *64*, 151–156. (d) Braden, B.; Hamilton, J. A.; Sabesan, M. N.; Steinrauf, L. K. *J. Am. Chem. Soc.* **1980**, *102*, 2704–2709.
569. Chen, B.-F.; Tsai, M.-C.; Jow, G.-M. *Biochem. Biophys. Res. Comm.* **2006**, *340*, 134–139.

570. Vishwanath, C. K.; Easwaran, K. R. K. *Biochemistry* **1982**, *21*, 2612–2620.
571. Cox, B. G.; Van Truong, N.; Rzeszotarska, J.; Schneider, H. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 3275–3284.
572. Bolte, J.; Demuynek, C.; Jeminet, G.; Juillard, J.; Tissier, C. *Can. J. Chem.* **1982**, *60*, 981–989.
573. Juillard, J.; Tissier, C.; Jeminet, G. *J. Chem. Res. (S)* **1985**, 324–325.
574. Albrecht-Gary, A. M.; Blanc-Parasote, S.; Boyd, D. W.; Dauphin, G.; Jeminet, G.; Juillard, J.; Prudhomme, M.; Tissier, C. *J. Am. Chem. Soc.* **1989**, *111*, 8598–8609.
575. Tissier, C.; Juillard, J.; Jeminet, G.; Dupin, M. *J. Chim. Phys.* **1979**, *76*, 611–617.
576. Albin, M.; Cader, B. M.; Horrocks, W. DeW. *Inorg. Chem.* **1984**, *23*, 3045–3050.
577. Woźnicka, J.; Lhermet, C.; Morel-Desrosiers, N.; Morel, J.-P.; Juillard, J. *J. Chem. Soc., Faraday Trans. 1* **1989**, *85*, 1709–1721.
578. Juillard, J.; Tissier, C.; Jeminet, G. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 951–958.
579. Degani, H.; Friedman, H. L. *Biochemistry* **1974**, *24*, 5022–5032.
580. (a) Kolber, M. A.; Haynes, D. H. *Biophys. J.* **1981**, *36*, 369–391. (b) Painter, G. R.; Pressman, B. C. In: “*Metal Ions in Biological Systems. Volume 19. Antibiotics and Their Complexes*”; Ed. Sigel, H.; Marcel Dekker: New York, **1985**, Chap. 9.
581. May, P. M.; Williams, G. K.; Williams, D. R. *Inorg. Chim. Acta* **1980**, *46*, 221–228.
582. Kiraly, R.; Martin, R. B. *Inorg. Chim. Acta* **1982**, *67*, 13–18.
583. (a) Glossmann, H.; Striessnig, J.; Knaus, H.-G.; Müller, J.; Grassegger, A.; Höltje, H.-D.; Marrer, S.; Hymel, L.; Schindler, H. G. *Ann. NY Acad. Sci.* **1989**, *560*, 198–214. (b) Several other articles in this volume also deal with or mention various aspects of calcium channels. Tsien, R. W.; Tsien, R. Y. *Ann. Rev. Cell Biol.* **1990**, *6*, 715–760.
584. See Chaps. 4, 5, and 6 of Ref. (539).
585. Kostyuk, P.; Verkhratsky, A. In: “*Ion Channel Pharmacology*”; Eds. Soria, B.; Ceña, V.; OUP: Oxford, **1998**, Chap. 14.
586. Strupish, J.; Wojcikiewicz, R. J. H.; Challiss, R. A. J.; Safrany, S. T.; Willcocks, A. L.; Potter, B. V.; Nahorski, S. R. *Biochem. J.* **1991**, *277*, 294.
587. Wood, P. C.; Wojcikiewicz, R. J. H.; Burgess, J.; Castleden, C. M.; Nahorski, S. R. *J. Neurochem.* **1994**, *62*, 2219–2223.
588. (a) Liu, L.; Rittenhaus, A. R. *J. Physiol.* **2000**, *525*, 391–404. (b) Liu, L.; Rittenhaus, A. R. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 295–300.
589. Rychkov, R. Y.; Litjens, T.; Roberts, M. L.; Barritt, G. J. *Biochem. J.* **2005**, *385*, 551–556.
590. Cataldi, M.; Perez-Reyes, E.; Tsien, R. W. *J. Biol. Chem.* **2002**, *271*, 45969–45976.
591. E.g. (a) Aurisano, N.; Bertani, A.; Reggiani, R. *Plant Cell Physiol.* **1995**, *36*, 1525–1529. (b) Trollinger, D. R.; Cascio, W. E.; Lemasters, J. J. *Biophys. J.* **2000**, *79*, 39–50.
592. Cibulsky, S. M.; Sather, W. A. *Pharm. Exp. Ther.* **1999**, *289*, 1447–1453.
593. Putney, J. W. *J. Cell Biol.* **2005**, *169*, 381–382.
594. (a) Parekh, A. B.; Penner, R. *Physiol. Rev.* **1977**, *77*, 901–930. (b) Parekh, A. B.; Putney, J. W. *Physiol. Rev.* **2005**, *85*, 757–810.
595. Prakriya, M.; Lewis, R. S. *J. Gen. Physiol.* **2006**, *128*, 373–386.

596. (a) Liou, J.; Kim, M. L.; Heo, W. D.; Jones, J. T.; Myers, J. W.; Ferrell, J. E.; Meyer, T. *Curr. Biol.* **2005**, *15*, 1235–1241. (b) Zhang, S. L.; Yu, Y.; Roos, J.; Kozak, J. A.; Deerinck, T. J.; Ellisman, M. H.; Stauderman, K. A.; Cahalan, M. D. *Nature* **2005**, *437*, 902–905.
597. Feske, S.; Gwack, Y.; Prakriya, M.; Srikanth, S.; Puppel, S.-H.; Tanasa, B.; Hogan, P. G.; Lewis, R. S.; Daly, M.; Rao, A. *Nature* **2006**, *441*, 179–185.
598. Hogan, P. G.; Rao, A. *Trends Biochem. Sci.* **2007**, *32*, 235–245.
599. Choi, O. H.; Kim, J.-H.; Kinet, J.-P. *Nature* **1996**, *380*, 634–636.
600. Hucho, F.; Weise, C. *Angew. Chem. Int. Ed.* **2001**, *40*, 3100–3116.
601. Bennett, M. V. L.; Barrio, L. C.; Bargiello, T. A.; Spray, D. C.; Hertzberg, E.; Sáez, J. C. *Neuron* **1991**, *6*, 305–320.
602. Davis, J. T. *Angew. Chem. Int. Ed.* **2004**, *43*, 668–698.
603. “*Quadruplex Nucleic Acids*”; Eds. Neidle, S.; Balasubramanian, S.; Royal Society of Chemistry: Cambridge, **2006**.
604. Gellert, M.; Lipsett, M. N.; Davies, D. R. *Proc. Natl. Acad. Sci. USA* **1962**, *48*, 2013–2018.
605. Burge, S.; Parkinson, G. N.; Hazel, P.; Todd, A. K.; Neidle, S. *Nucl. Acids Res.* **2006**, *34*, 402–415.
606. Kwan, I. C. M.; Wong, A.; She, Y.-M.; Smith, M. E.; Wu, G. *Chem. Commun.* **2008**, 682–684.
607. Miyoshi, D.; Nakao, A.; Sugimoto, N. *Nucl. Acid Res.* **2003**, *31*, 1156–1163.
608. Lee, M. P. H.; Parkinson, G. M.; Hazel, P.; Neidle, S. *J. Am. Chem. Soc.* **2007**, *129*, 10106–10107.
609. Lehn, J.-M.; Potvin, P. G. *Can. J. Chem.* **1988**, *66*, 195–200.
610. (a) Rendleman, J. A. *Adv. Carbohydrate Chem.* **1966**, *21*, 209–271. (b) Rendleman, J. A. *Food Chem.* **1978**, *3*, 47–79.
611. See pp. 185–191 of Ref. (29).
612. Dheu-Andries, M. L.; Péres, S. *Carbohydrate Res.* **1983**, *124*, 324–332.
613. Poonia, N. S.; Bajaj, A. V. *Chem. Rev.* **1979**, *79*, 389–445.
614. Junk, P. C.; Steed, J. W. *J. Coord. Chem.* **2007**, *60*, 1017–1028.
615. Junk, P. C.; Louis, L. M.; Smith, M. K. *Z. Anorg. Allg. Chem.* **2002**, *628*, 1196–1209.
616. (a) Junk, P. C.; Steed, J. W. *J. Chem. Soc., Dalton Trans.* **1999**, 407–414. (b) Brown, M. D.; Levason, W.; Webster, M. *Acta Cryst.* **2005**, *E61*, m1015–m1017.
617. Cook, W. J.; Bugg, C. E. *Acta Cryst.* **1976**, *B32*, 656–659.
618. Craig, D. C.; Stephenson, N. C.; Stevens, J. D. *Cryst. Struct. Commun.* **1974**, *3*, 277–281.
619. Bugg, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 908–913.
620. Ollis, J.; James, V. J.; Angyal, S. J.; Pojer, P. M. *Carbohydrate Res.* **1978**, *60*, 219–228.
621. Richards, G. F. *Carbohydrate Res.* **1973**, *26*, 448–449.
622. Terzis, A. *Cryst. Struct. Commun.* **1978**, *7*, 95–99.
623. Tajmir-Riahi, H. A. *Carbohydrate Res.* **1984**, *127*, 1–8.
624. Cook, W. J.; Bugg, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 6442–6446.
625. Craig, D. C.; Stephenson, N. C.; Stevens, J. D. *Carbohydrate Res.* **1972**, *22*, 494–495.
626. Tajmir-Riahi, H. A. *J. Inorg. Biochem.* **1987**, *31*, 255–265.
627. Cook, W. J.; Bugg, C. E. *Acta Cryst.* **1973**, *B29*, 215–222.
628. Sheldrick, B.; Mackie, W.; Akrigg, D. *Acta Cryst.* **1989**, *C45*, 191–194.
629. Burden, C.; Mackie, W.; Sheldrick, B. *Acta Cryst.* **1985**, *C41*, 693–695.

630. DeLucas, L.; Bugg, C. E.; Terzis, A.; Rivest, R. *Carbohydrate Res.* **1975**, *41*, 19–29.
631. Balchin, A. A.; Carlisle, C. H. *Acta Cryst.* **1965**, *B19*, 103–111.
632. (a) Hjortås, J.; Larsen, B.; Thanomkul, S. *Acta Chem. Scand.* **1974**, *B28*, 689. (b) Gould, S. E. B.; Gould, R. O.; Rees, D. A.; Scott, W. E. *J. Chem. Soc., Perkin Trans. 2* **1975**, 237–242.
633. Kmiecik, S.; Maciejewska, G.; Adach, A.; Grabowski, T.; Gulonowski, B.; Cieślak-Golonka, M. *J. Inorg. Biochem.* **2006**, *100*, 143–151.
634. Taga, T.; Osaki, K. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1517–1520.
635. Furberg, S.; Helland, S. *Acta Chem. Scand.* **1962**, *16*, 2373–2383.
636. Tian, W.; Yang, L.-M.; Xu, Y.-Z.; Weng, S.-F.; Wu, J.-G. *Carbohydrate Res.* **2000**, *324*, 45–52.
637. Yang, L.; Su, Y.; Liu, W.; Jin, X.; Wu, J. *Carbohydrate Res.* **2002**, *337*, 1485–1493.
638. Šlepokura, K.; Lis, T. *Carbohydrate Res.* **2004**, *339*, 1995–2007.
639. Symons, M. C. R.; Benbow, J. A.; Pelmore, H. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 1999–2016.
640. (a) Angyal, S. J. *Aust. J. Chem.* **1972**, *25*, 1957–1966. (b) Angyal, S. J. *Tetrahedron* **1974**, *30*, 1695–1702.
641. Angyal, S. J. *Pure Appl. Chem.* **1973**, *35*, 131–146.
642. Angyal, S. J.; Hickman, R. J. *Aust. J. Chem.* **1975**, *28*, 1279–1287.
643. Alekseev, Yu. E.; Gamovskii, A. D.; Zhdanov, Yu. A. *Russ. Chem. Rev.* **1998**, *67*, 649–669.
644. Gyurcsik, B.; Nagy, L. *Coord. Chem. Rev.* **2000**, *203*, 81–149.
645. Swartz, M. L.; Bernhard, R. A.; Nickerson, T. A. *J. Food Sci.* **1978**, *43*, 93–97.
646. Mäkinen, K. K.; Söderling, E. *Calcif. Tissue Int.* **1984**, *36*, 64–71.
647. Lenkinski, R. E.; Reuben, J. *J. Am. Chem. Soc.* **1976**, *98*, 3089–3094.
648. Morel, J.-P.; Lhermet, C.; Morel-Desrosiers, N. *J. Chem. Soc., Faraday Trans. 1* **1988**, *84*, 2567–2571.
649. Grebbernikov, V. N.; Vyskrebtssov, B. V.; Budarin, L. I. *Zh. Neorg. Khim.* **1984**, *29*, 51–55; *Russ. J. Inorg. Chem.* **1984**, *29*, 27–30.
650. Moulik, S. P.; Khan, D. P. *Carbohydrate Res.* **1974**, *36*, 147–157.
651. Beattie, J. K.; Kelso, M. T. *Aust. J. Chem.* **1981**, *34*, 2563–2568.
652. Morel, J.-P.; Lhermet, C. *Can. J. Chem.* **1985**, *63*, 2639–2643.
653. Alvarez, A. M.; Morel-Desrosiers, N.; Morel, J.-P. *Can. J. Chem.* **1986**, *65*, 2656–2660.
654. Angyal, S. J. *Chem. Soc. Rev.* **1981**, *9*, 415–428.
655. Lönnberg, H.; Vesala, A.; Käppi, R. *Carbohydrate Res.* **1980**, *86*, 137–142.
656. Evans, W. J.; Frampton, V. L. *Carbohydrate Res.* **1977**, *59*, 571–574.
657. Morel-Desrosiers, N.; Morel, J.-P. *J. Chem. Soc., Faraday Trans. 1* **1989**, *85*, 3461–3469.
658. Morel, J.-P.; Lhermet, C.; Morel-Desrosiers, N. *Can. J. Chem.* **1986**, *64*, 996–1001.
659. Bechtold, T.; Burtcher, E.; Turcanu, A. *J. Chem. Soc., Dalton Trans.* **2002**, 2683–2688.
660. Traube, W.; Kuhbier, F. *Chem. Ber.* **1936**, *69*, 2655–2663.
661. Jaques, L. W.; Brown, E. B.; Barrett, J. M.; Brey, W. S.; Weltner, W. *J. Biol. Chem.* **1977**, *252*, 4533–4538.
662. Czarniecki, M. F.; Thornton, E. R. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 553–558.
663. Liang, G.; Chen, D.; Bastian, M.; Sigel, H. *J. Am. Chem. Soc.* **1992**, *114*, 7780–7785.

664. Ruggiero, J.; Vieira, R. P.; Mourão, P. A. S. *Carbohydrate Res.* **1994**, 256, 275–287.
665. Schnitzer, M. *Agrochimica* **1978**, 22, 216–225.
666. Plechanov, N. *Org. Geochem.* **1983**, 5, 143–149.
667. See, e.g., Dzombak, D. A.; Fish, W.; Morel, F. M. M. *Environ. Sci. Technol.* **1986**, 20, 669–675.
668. Williams, P. A. “Oxide Zone Geochemistry”; Ellis Horwood: Chichester, **1990**; p. 115.
669. Cabaniss, S. E.; Shuman, M. S. *Geochim. Cosmochim. Acta* **1988**, 52, 185–193.
670. “Metal Ions in Biological Systems. Volume 32. Interactions of Metal Ions with Nucleotides, Nucleic Acids, and their Constituents”; Ed. Sigel, A.; Sigel, H.; Marcel Dekker: New York, **1996**.
671. MacLennan, G.; Beevers, C. A. *Acta Cryst.* **1956**, 9, 187–190.
672. Beevers, C. A.; MacIntyre, D. *Miner. Mag.* **1946**, 27, 254–257.
673. Kay, I.; Young, R. A.; Posner, A. S. *Nature* **1964**, 204, 1050–1052.
674. Beevers, C. A.; Raistrick, B. *Nature* **1954**, 173, 542–543.
675. Elliott, J. C. “Structure and Chemistry of the Apatites and Other Calcium Orthophosphates”; Elsevier: Amsterdam, **1994**.
676. Cao, G.; Lynch, V. M.; Swinnea, J. S.; Mallouk, T. E. *Inorg. Chem.* **1990**, 29, 2112–2117.
677. Matczak-Jon, E.; Kurzak, B.; Kawarski, P.; Woźna, A. *J. Inorg. Biochem.* **2006**, 100, 1155–1166.
678. Nardelli, M.; Pelizzi, G.; Staibano, G.; Zucchi, E. *Inorg. Chim. Acta* **1983**, 80, 259–271.
679. Svoboda, J.; Zima, V.; Beneš, L.; Melánová, K.; Vlček, M. *Inorg. Chem.* **2005**, 44, 9968–9976.
680. Mahmoudkhani, A. H.; Langer, V. *Solid State Sci.* **2001**, 3, 519–525.
681. Massoud, S. S.; Sigel, H. *Inorg. Chem.* **1988**, 27, 1447–1453.
682. See pp. 156–157 of Ref. (262).
683. Visser, J.; Schaier, R. W.; van Gorkom, M. *J. Dairy Res.* **1979**, 46, 333–335.
684. de la Fuente, M. A. *Trends Food Sci. Technol.* **1998**, 9, 281–288.
685. Hite, B. H. *Bull. W. Va. Univ. Agric. Exp. Stn.* **1899**, 58, 15–35.
686. Keenan, R. D.; Young, D. J.; Tier, C. M.; Jones, A. D.; Underdown, J. J. *Agric. Food Chem.* **2001**, 49, 3394–3402.
687. Brintzinger, H. *Helv. Chim. Acta* **1965**, 48, 47–54.
688. Popov, K.; Rönkkömäki, H.; Lajunen, L. H. *J. Pure Appl. Chem.* **2001**, 73, 1641–1677.
689. Sigel, H.; Chen, D.; Corfù, N. A.; Gregáň, F.; Holý, A.; Strašák, M. *Helv. Chim. Acta* **1992**, 75, 2634–2656.
690. Sigel, H. *Pure Appl. Chem.* **1999**, 71, 1727–1740.
691. Song, B.; Zhao, J.; Gregáň, F.; Pronáyová, N.; Sajadi, S. A. A.; Sigel, H. *Metal-Based Drugs* **1999**, 6, 321–328.
692. Rizkalla, E. N.; Zaki, M. T. M.; Ismail, M. I. *Talanta* **1980**, 27, 715–719.
693. Smith, R. M.; Martell, A. E.; Chen, Y. *Pure Appl. Chem.* **1991**, 63, 1015–1080.
694. Sigel, H. *Eur. J. Biochem.* **1987**, 165, 65–72.
695. Sigel, H.; Griesser, R. *Chem. Soc. Rev.* **2005**, 34, 875–900.
696. (a) Watanabe, S.; Evenson, L.; Gulz, I. *J. Biol. Chem.* **1963**, 238, 324–327.
(b) Granot, J.; Fiat, D. *J. Am. Chem. Soc.* **1977**, 99, 70–79.
697. Sigel, H.; Tribolet, R.; Malini-Balakrishnan, R.; Martin, R. B. *Inorg. Chem.* **1987**, 26, 2149–2157.

698. (a) Matkovskaya, T. A.; Nikolaeva, L. S.; Mezhonova, E. A.; Baleshova, T. M.; Evseev, A. M.; Dyatlova, N. M. *Zh. Neorg. Khim.* **1989**, *34*, 1935–1939; *Russ. J. Inorg. Chem.* **1989**, *34*, 1100–1102. (b) Matczak-Jon, E.; Kurzak, B.; Kamecka, A.; Kafarski, P. *Polyhedron* **2002**, *21*, 321–332. (c) Matczak-Jon, E.; Videnova-Adrabińska, V. *Coord. Chem. Rev.* **2005**, *249*, 2458–2488.
699. Reddy, P. R.; Rao, V. B. M. *Polyhedron* **1985**, *4*, 1603–1609.
700. Lönnberg, H. In: “*Biocoordination Chemistry*”; Ed. Burger, K.; Ellis Horwood: Chichester, **1990**, Chap. VII.
701. Sigel, H.; Scheller, K. H. *Eur. J. Biochem.* **1984**, *138*, 291–299.
702. Sigel, H. *Pure Appl. Chem.* **2004**, *76*, 375–388.
703. Sigel, H. *Chem. Soc. Rev.* **1993**, *22*, 255–267.
704. Sánchez-Moreno, M. J.; Fernández-Botello, A.; Gómez-Coca, R. B.; Griesser, R.; Ochocki, A.; Kotynski, A.; Nicolás-Gutiérrez, J.; Moreno, V.; Sigel, H. *Inorg. Chem.* **2004**, *43*, 1311–1322.
705. Holý, A.; Votruba, I.; Masojídková, M.; Andrei, G.; Snoek, R.; Naesens, L.; De Clerq, E.; Balzarini, J. *J. Med. Chem.* **2002**, *45*, 1918–1929.
706. Sigel, H. *Chem. Soc. Rev.* **2004**, *33*, 191–200.
707. Cali, R.; Musumeci, S.; Rigano, C.; Sammartano, S. *Inorg. Chim. Acta* **1981**, *56*, L11–L13.
708. Ragot, M.; Sari, J. C.; Belaich, J. P. *Biochim. Biophys. Acta* **1977**, *499*, 411–420, 421–431.
709. Arena, G.; Cali, R.; Cucinotta, V.; Musumeci, S.; Rizzarelli, E.; Sammartano, S. *J. Chem. Soc., Dalton Trans.* **1983**, 1271–1278.
710. (a) Eigen, M.; Hammes, G. G. *J. Am. Chem. Soc.* **1960**, *82*, 5951–5952. (b) Eigen, M.; Hammes, G. G. *J. Am. Chem. Soc.* **1961**, *83*, 2786.
711. Hammes, G. G.; Hurst, J. K. *Biochemistry* **1969**, *8*, 1083–1094.
712. (a) Matthews, A.; Nathan, Y. *Am. Miner.* **1977**, *62*, 565–573. (b) McArthur, J. M. *Geochim. Cosmochim. Acta* **1985**, *49*, 23–35.
713. See Chap. 10–6 of Ref. (215).
714. “*Calcification of Biological Systems*”; Ed. Bonnucci, E.; CRC Press: Baton Rouge, **1992**.
715. See p. 304 of Ref. (5).
716. Molzon, J. A.; Lausier, J. M.; Paruta, A. N. *J. Pharm. Sci.* **1978**, *67*, 733–735; and references therein.
717. Parrott, E. L. *J. Pharm. Sci.* **1962**, *51*, 897–900.
718. Pankow, J. F. “*Aquatic Chemistry Concepts*”; Lewis Publishers: Chelsea, MI, **1991**, Chap. 12.
719. See Chap. 5 of Ref. (215).
720. Wollast, R. In: “*Aquatic Chemical Kinetics*”; Ed. Stumm, W.; Wiley-Interscience: New York, **1990**, Chap. 15.
721. Iglesias-Rodriguez, M. D.; Halloran, P. R.; Rickaby, R. E. M.; Hall, I. R.; Colmenero-Hidalgo, E.; Gittins, J. R.; Green, D. R. H.; Tyrrell, T.; Gibbs, S. J.; von Dassow, P.; Rehm, E.; Armbrust, E. V.; Boessenkool, K. P. *Science* **2008**, *320*, 336–340.
722. Stewart, A. J.; Wetzel, R. G. *Arch. Hydrobiol.* **1981**, *92*, 265–286.
723. “*Calcium Oxalate in Biological Systems*”; Ed. Khan, S.R.; CRC Press: Boca Raton, FL, **1995**.
724. See pp. 76–77 of Ref. (668).
725. Walter-Lévy, L.; Laniepce, J. *Comptes Rend. Hebd. Seances Acad. Sci.* **1962**, *254*, 296–298.
726. Prien, E. L.; Frondel, C. *J. Urol.* **1947**, *57*, 949–994.
727. Tomazić, B.; Nancollas, G. H. *J. Cryst. Growth* **1979**, *46*, 355–361.

728. Lemann, J.; Pleuss, J. A.; Worcester, E. M.; Hornick, L.; Schrab, D.; Hoffmann, R. G. *Kidney Int.* **1996**, *49*, 200–208.
729. Urivetzky, M.; Kessaris, D.; Smith, A. D. *J. Urol.* **1992**, *147*, 1215–1218.
730. Gardner, G. L. *J. Cryst. Growth* **1975**, *30*, 158–168.
731. Tomažić, B.; Sheehan, M. E.; Nancollas, G. H. *World J. Urol.* **1992**, *10*, 216–225.
732. See, e.g., Tomažić, B. B.; Nancollas, G. H. *Invest. Urol.* **1980**, *18*, 97–101.
733. Currey, J. D. “*Bones – Structure and Mechanics*”; Princeton University Press: Princeton, **2002**.
734. Fratzl, P.; Weinkamer, R. *Progr. Mater. Sci.* **2007**, *52*, 1263–1334.
735. Stumm, W.; Morgan, J. J. “*Aquatic Chemistry*”; Wiley-Interscience: New York, **1970**, Chap. 10–2.
736. Nancollas, G. H.; Koutsoukos, P. G. *Prog. Cryst. Growth Charac.* **1980**, *3*, 77–102.
737. Schlesinger, D. H.; Hay, D. I. *J. Biol. Chem.* **1977**, *252*, 1689–1695.
738. Landis, W. J. *Bone* **1995**, *16*, 533–534.
739. Kolmas, J.; Kolodziejewski, W. *Chem. Commun.* **2007**, 4390–4392.
740. Kinney, J. H.; Pople, J. A.; Marshall, G. W.; Marshall, S. J. *Calcif. Tissue Int.* **2001**, *69*, 31–37.
741. Heyeraas, K. J.; Haug, S. R.; Bukowski, R. D.; Awumey, E. M. *Calcif. Tissue Int.* **2008**, *82*, 57–65.
742. Harris, R. S. *Adv. Chem. Ser.* **1970**, *94*, 1–6.
743. Harris, R. S. *Adv. Chem. Ser.* **1970**, *94*, 116–122.
744. Sethmann, I.; Hinrichs, R.; Wörheide, G.; Putnis, A. *J. Inorg. Biochem.* **2006**, *100*, 88–96.
745. Sudarsanan, K.; Young, R. A. *Acta Cryst.* **1978**, *B34*, 1401–1407.
746. Hughes, J. M.; Cameron, M.; Crowley, K. D. *Am. Miner.* **1989**, *74*, 870–876.
747. Chen, H.; Tang, Z.; Liu, J.; Sun, K.; Chang, S.-R.; Peters, M. C.; Mansfield, J. F.; Czajka-Jakubowska, A.; Clarkson, B. H. *Adv. Mater.* **2006**, *18*, 1846–1851.
748. Schlesinger, P. H.; Blair, H. C.; Teitelbaum, S. L.; Edwards, J. C. *J. Biol. Chem.* **1997**, *272*, 18636–18643.
749. Kannan, S.; Rebelo, A.; Ferreira, J. M. F. *J. Inorg. Biochem.* **2006**, *100*, 1692–1697.
750. Danilchenko, S. N.; Kukhareenko, O. G.; Moseke, C.; Protsenko, I. Y.; Sukhodub, L. F.; Sulkio-Cleff, B. *Cryst. Res. Technol.* **2002**, *37*, 1234–1240.
751. Bigi, A.; Falini, G.; Foresti, E.; Gazzano, M.; Ripmonti, A.; Roveri, N. *Acta Cryst.* **1996**, *B52*, 87–92.
752. Hughes, J. M.; Cameron, M.; Crowley, K. D. *Am. Miner.* **1991**, *76*, 1857–1862.
753. Da Silva, E.; Pejović-Milić, A.; Heyd, D. V. *J. Anal. At. Spectrom.* **2008**, *23*, 527–534.
754. Grover, L. M.; Gbureck, U.; Young, A. M.; Wright, A. J.; Barralet, J. E. *J. Mater. Chem.* **2005**, *15*, 4955–4962.
755. Herde, K.; Hartmann, S.; Brehm, R.; Kilian, O.; Heiss, C.; Hild, A.; Alt, V.; Bergmann, M.; Schnettler, R.; Wenisch, S. *Biomaterials* **2007**, *28*, 4912–4921.
756. Posner, A. S.; Betts, F.; Blumenthal, N. C. *Prog. Cryst. Growth Charac.* **1980**, *3*, 49–64.
757. Tadic, D.; Epple, M. *Biomaterials* **2004**, *25*, 987–994; and references therein.

758. Li, L.; Pan, H.; Tao, J.; Xu, X.; Mao, C.; Gu, X.; Tang, R. *J. Mater. Chem.* **2008**, *18*, 4079–4084.
759. Teng, S.; Chen, L.; Guo, Y.; Shi, J. *J. Inorg. Biochem.* **2007**, *101*, 686–691.
760. Leroy, N.; Bres, E. *Eur. Cells Mater.* **2001**, *2*, 36–48.
761. Overgaard, S.; Søballe, K.; Lind, M.; Bünger, C. *J. Bone Joint Surg.* **1997**, *79B*, 654–659.
762. Chang, J. H.; Park, M. E.; Shin, Y.; Exarhos, G. J.; Kim, K. J.; Lee, S. C.; Oh, K. S. *J. Mater. Chem.* **2007**, *17*, 238–242.
763. Kanczler, J. M.; Ginty, P. J.; Barry, J. J. A.; Clarke, N. M. P.; Howdle, S. M.; Shakesheff, K. M.; Oreffo, R. O. C. *Biomaterials* **2008**, *29*, 1892–1900.
764. Amoura, M.; Nassif, N.; Roux, C.; Livage, J.; Coradin, T. *Chem. Commun.* **2007**, 4015–4017.
765. Crow, J. M. *Chem. World* **2007**, *4*(11), 33.
766. Azari, F.; Vali, H.; Guerquin-Kern, J.-L.; Wu, T.-D.; Croisy, A.; Sears, S. K.; Tabrizian, M.; McKee, M. D. *J. Struct. Biol.* **2008**, *162*, 468–479.
767. Stern, P. H. In: “*Human Pharmacology: Molecular-to-Clinical*”; Eds. Wingard, L. B.; Brody, T. M.; Lerner, J.; Schwartz, A.; Mosby-Year Book: St. Louis, **1991**, Chap. 42.
768. Vega, R. B.; Bassel-Duby, R.; Olson, E. N. *J. Biol. Chem.* **2003**, *278*, 36981–36984.
769. (a) Kotchen, T. A.; McCasson, D. A. *Circulation* **1998**, *98*, 613–617. (b) Sacks, F. M.; Willett, W. C.; Smith, A.; Brown, L. E.; Rosner, B.; Moore, T. *J. Hypertension* **1998**, *31*, 131–138. (c) Rinner, M. D.; Spliet v. Laar, L.; Kromhout, D. *J. Hypert.* **1989**, *7*, 977–981. (d) Burgess, E.; Lewanczuk, R.; Bolli, P.; Chockalingam, A.; Cutler, H.; Taylor, G.; Hamet, P. *Can. Med. Assoc. J.* **1999**, *160*(9 Suppl.), 535–545. (e) Hajjar, I. M.; Grim, C. E.; George, V.; Kotchen, T. A. *Arch. Intern. Med.* **2001**, *161*, 589–593.
770. Yang, C.-Y.; Chiu, H.-F. *Am. J. Hypert.* **1999**, *12*, 894–899.
771. Evans, G. H.; Weaver, C. M.; Harrington, D. D.; Babbs, C. F. *J. Hypert.* **1990**, *8*, 327–337.
772. Bolland, M. J.; Barber, P. A.; Doughty, R. N.; Mason, B.; Horne, A.; Ames, R.; Gamble, G. D.; Grey, A.; Reid, I. R. *Br. Med. J.* **2008**, *336*, 262–266.
773. (a) Orrenius, S.; McConkey, D. J.; Bellomo, G.; Nicotera, P. *Trends Pharm. Sci.* **1989**, *10*, 281–285. (b) Orrenius, S.; Nicotera, P. *J. Neural. Transm. Suppl.* **1994**, *43*, 1–11.
774. Orrenius, S.; Nicotera, P.; McCabe, M. J. *Toxicol. Lett.* **1992**, *65*, 357–364.
775. Szumiel, I.; Buraczewska, I.; Sochanowicz, B. *Int. J. Radiat. Biol.* **1990**, *58*, 125–131.
776. Prince, R. L.; Devine, A.; Dhaliwal, S. S.; Dick, I. M. *Arch. Intern. Med.* **2006**, *166*, 869–875.
777. Hofmeyr, G. J.; Atallah, A. N.; Duley, L. *Int. J. Epidemiol.* **2007**, *36*, 290–291.
778. Zhu, K.; Devine, A.; Dick, I. M.; Wilson, S. G.; Prince, R. L. *J. Clin. Endocrin. Metab.* **2008**, *93*, 743–749.
779. Rizzoli, R.; Boonen, S.; Brandi, M. L.; Burlet, N.; Reginster, J. Y. *Bone* **2008**, *42*, 246–249.
780. Okonofua, F.; Gill, D. S.; Alabi, Z. O.; Thomas, M.; Bell, J. L.; Dandona, P. *Metabolism* **1991**, *40*, 209–213.
781. See, e.g., Thomas, M. K.; Lloyd-Jones, D. M.; Thadhani, R. I.; Shaw, A. C.; Deraska, D. A.; Kitch, B. T.; Vamvakas, E. C.; Dick, I. M.; Prince, R. L.; Finkelstein, J. S. *New Engl. J. Med.* **1998**, *338*, 777–783.
782. Kimura, E. *Topics Curr. Chem.* **1985**, *128*, 113–141.

783. Kuramoto, Y.; Kimura, E. *Nippon Kagaku Kaishi* **1987**, 288–292; *Chem. Abstr.* **1988**, 108, 130928t.
784. McGregor, B. A. *Anim. Feed Sci. Technol.* **2006**, 128, 53–66.
785. See p. 227 of Ref. (244).
786. Quintanar, L. *Inorg. Chim. Acta* **2008**, 361, 875–884.
787. Xiang, M.; Mohamalawari, D.; Rao, R. *J. Biol. Chem.* **2005**, 280, 11608–11614.
788. Papapoulos, S. E.; Schimmer, R. C. *Ann. Rheum. Dis.* **2007**, 66, 853–858.
789. Fleisch, H. In: “*Bisphosphonates and Tumor Osteolysis*”; Eds. Brunner, K. W.; Fleisch, H.; Senn, H.-J.; Springer: Berlin, **1989**, Chap. 1.
790. Cukrowski, I.; Popović, L.; Barnard, W.; Paul, S. O.; van Rooyen, P. H.; Liles, D. C. *Bone* **2007**, 41, 668–678.
791. Heckbert, S. R.; Li, G.; Cummings, S. R.; Smith, N. L.; Psaty, B. M. *Arch. Intern. Med.* **2008**, 168, 826–831.
792. van Beek, E. R.; Löwrick, C. W. G. M.; Ebentino, F. H.; Papapoulos, S. E. *Bone* **1998**, 23, 437–442.
793. Black, D. M.; Delmas, P. D.; Eastell, R.; Reid, I. R.; Boonen, S.; Cauley, J. A.; Cosman, F.; Lakatos, P.; Leung, P. C.; Man, Z.; Mautalen, C.; Mesenbrink, P.; Hu, H.; Caminis, J.; Tong, K.; Rosario-Jansen, T.; Krasnov, J.; Hue, T. F.; Sellmeyer, D.; Eriksen, E. F.; Cummings, S. R. *New Engl. J. Med.* **2007**, 356, 1809–1822.
794. Favus, M. J. *Nat. Clin. Prac. Endocrin. Med.* **2006**, 2, 600–601.
795. McClung, M. R.; Lewiecki, E. M.; Bekker, P. J. *New Engl. J. Med.* **2006**, 354, 821–831.
796. Ottewell, P. D.; Mönkkönen, H.; Jones, M.; Lefley, D. V.; Coleman, R. E.; Holen, I. *J. Natl. Cancer Inst.* **2008**, 100, 1167–1178.
797. Hernandez-Santana, A.; Yavorsky, A.; Olinyole, A.; McCarthy, G. M.; McMahon, G. P. *Chem. Commun.* **2008**, 2686–2688.
798. MacIntyre, I.; Alevizaki, M.; Bevis, P. J. R.; Zaidi, M. *Clin. Orthop. Relat. Res.* **1987**, 217, 45–55.
799. Fowler, S. B.; Poon, S.; Muff, R.; Chiti, F.; Dobson, C. M.; Zurdo, J. *Proc. Natl. Acad. Sci. USA* **2005**, 102, 10105–10110.
800. Peterson, C.; Ratan, R.; Shelanski, M.; Goldman, J. *Ann. NY Acad. Sci.* **1989**, 568, 262–270.
801. Pollard, H. B.; Arispe, N.; Rojas, E. In: “*Ion Channel Pharmacology*”; Eds. Soria, B.; Ceña, V.; OUP: Oxford, **1998**, Chap. 6.
802. Cheung, K.-H.; Shineman, D.; Müller, M.; Cárdenas, C.; Mei, L.; Yang, J.; Tomita, T.; Iwatsubo, T.; Lee, V. M.-Y.; Foskett, J. K. *Neuron* **2008**, 58, 871–883.
803. Dreses-Werringloes, U.; Lambert, J.-C.; Vingtdeux, V.; Zhao, H.; Vais, H.; Siebert, A.; Jain, A.; Koppel, J.; Rovelet-Lecrux, A.; Hannequin, D.; Pasquier, F.; Galimberti, D.; Scarpini, E.; Mann, D.; Lendon, C.; Campion, D.; Amouyel, P.; Davies, P.; Foskett, J. K.; Campagne, F.; Marambaud, P. *Cell* **2008**, 133, 1149–1161.
804. Eigen, M. In: “*Advances in the Chemistry of the Coordination Compounds*”; Ed. Kirschner, S.; Macmillan: New York, **1961**, pp. 371–378.

Appendix: The Term Ligand

In the context of ion channel biochemistry, and indeed in other areas of biochemistry, the term “ligand” has a different, and more

inclusive, meaning from that in coordination chemistry. In biology a "ligand" is an entity which binds to a particular site on a host molecule, as for example a substrate to its enabling enzyme. In relation to channel gating the term "ligand" applies to any gating species, including Ca^{2+} . Current biochemical usage is not dissimilar from that of Stock, who coined the term (from the Latin *ligare* = to bind) in 1916 (see Stock, A. *Ber. Dtsch. Chem. Ges.* **1917**, 50, 170–182), and others in the 1920s and 1930s who used the term, for example in organic chemistry, to describe a group or radical attached to a central atom of formal positive or negative charge. The first use of the term "ligand" in coordination chemistry was by Bjerrum in 1941 (Bjerrum, N. "*Metal Ammine Formation in Aqueous Solution: Theory of Reversible Step Reactions*"; P. Haase and Son: Copenhagen, 1941); there is a three-page abstract of what is essentially Bjerrum's doctoral thesis in *Chem. Abs.* **1941**, 35, 6527–6534, its use in this context quickly becoming widespread in the 1950s. The origin, and changes in usage and meaning, of the term "ligand" over the years are outlined in Brock, W. H.; Jensen, K. A.; Jørgensen, C. K.; Kauffman, G. B. *J. Coord. Chem.* **1982**, 11, 261–262; Brock, W. H.; Jensen, K. A.; Jørgensen, C. K.; Kauffman, G. B. *J. Chem. Inf. Comp. Sci.* **1982**, 22, 125–129, fully detailed and documented in Brock, W. H.; Jensen, K. A.; Jørgensen, C. K.; Kauffman, G. B. *Ambix* **1981**, 28, 171–183.

NEW DEVELOPMENTS IN SYNTHETIC NITROGEN FIXATION WITH MOLYBDENUM AND TUNGSTEN PHOSPHINE COMPLEXES[☆]

AMELI DREHER, GERALD STEPHAN and FELIX TUCZEK

Institut für anorganische Chemie der Christian Albrechts Universität zu Kiel,
Max-Eyth-Strasse 2, D-24098 Kiel, Germany

I. Introduction	367
II. Mechanism of the Chatt Cycle: Experimental Investigations	371
A. Protonation of Coordinated N ₂	372
B. N–N Cleavage	374
C. Electronic Structure and Reactivity of Molybdenum Nitrido and Imido Complexes	378
III. Mechanism of the Chatt Cycle: Theoretical Investigations	382
IV. Molybdenum Dinitrogen Complexes with Polydentate Phosphine and Mixed P/N Ligands	386
A. Tetraphos Complexes	386
B. Complexes with Pentaphosphine Coordination	391
C. Mo Complexes with Mixed P/N Ligands	397
V. Conclusions	401
Acknowledgments	402
References	402

I. Introduction

One of the great challenges of biological, inorganic, and organometallic chemistry is a biomimetic process of ammonia synthesis under ambient conditions (1). A historic breakthrough in this respect has been the synthesis and characterization of the Mo(III) triamidoamine complex [Mo(HIPTN₃N)] by Schrock *et al.* (2). This complex for the first time allowed the catalytic synthesis of NH₃ from N₂. Many years before, Pickett and Talarmin had already achieved a cyclic conversion of N₂ to NH₃ on the basis of a tungsten phosphine complex. However, fewer cycles and a much smaller yield of NH₃ were achieved (3). Nevertheless, the underlying type of dinitrogen complexes provided the first

[☆]Dedicated to Prof. Dr. B. Krebs on the occasion of his 70th birthday.

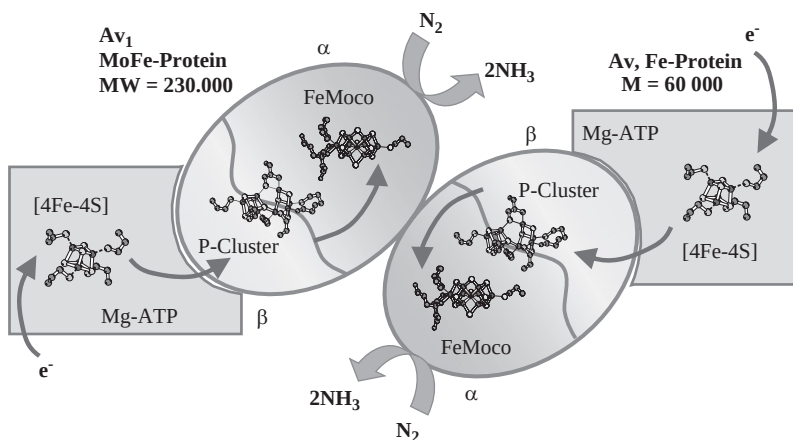


FIG. 1. Schematic illustration of the enzyme nitrogenase being composed of the molybdenum-iron (MoFe) protein, an $\alpha_2\beta_2$ tetramer with two unique iron-sulfur clusters (P-cluster) and two iron-molybdenum cofactors (FeMoco), and the iron protein with one [4Fe-4S]-cluster and two ATP binding sites.

rational and complete reactive scheme for synthetic nitrogen fixation, the Chatt cycle (4), and the realization of a truly catalytic¹ ammonia synthesis on the basis of Mo/W phosphine complexes still represents a significant goal (1,5).

In Nature, nitrogen fixation is mediated by the enzyme nitrogenase according to Eq. (1) (6)



The energy consumption of this process is very high (180 kcal/(mol N_2)) (7), which is mostly due to the hydrolysis of 16 ATP molecules. Moreover, dinitrogen reduction is always accompanied by H_2 evolution, which cannot be suppressed. Nitrogenase consists of two proteins (Fig. 1). The larger one, called molybdenum-iron (MoFe) protein, is an $\alpha_2\beta_2$ tetramer which contains two unique iron-sulfur clusters, the P-clusters and two iron-molybdenum cofactors (FeMoco) (8). The electrons necessary for the reduction of N_2 are provided by the iron protein which contains one iron-sulfur cluster located between its two subunits. During turnover the iron protein forms a complex with the MoFe protein and reduces it by one electron, which is transferred to the FeMoco via the P-cluster. After this process the complex

¹“Truly catalytic” means here that the amount of converted substrate (in moles) exceeds that of the added catalyst (in moles).

dissociates and the Fe protein is recharged, being able to reduce the MoFe protein with one electron again. This process occurs eight times for a full catalytic cycle (9).

In order to determine the reaction mechanism of nitrogenase on a molecular level, a number of spectroscopic studies have been performed (10). However, no full mechanistic picture of the enzymatic reaction has emerged yet from these studies. Moreover, a large number of theoretical studies on the binding and reduction of N_2 at the FeMoco has been published (11). These investigations established some plausible mechanistic scenarios for the biological nitrogen fixation reaction. Model chemistry approaches, finally, have focused on binding and activating dinitrogen at mono-, di-, or polynuclear metal centers (1–5). Importantly, some of these systems also allowed information to be obtained on further elementary reactions necessary for synthetic nitrogen fixation. In this context, two reaction schemes exist which comprise the full set of elementary steps for the conversion of N_2 to ammonia: the Schrock and the Chatt cycles.

Yandulov and Schrock were the first to realize a truly catalytic ammonia synthesis proceeding under ambient conditions and through a series of well-defined intermediates (2). The corresponding reactive system is based on the molybdenum(III) complex $[Mo(HIPTN_3N)]$ containing the triamidoamine ligand $HIPTN_3N$ (hexaisopropylterphenyl-triamidoamine), which provides a sterically shielded site for dinitrogen bonding and transformation to ammonia (Fig. 2). Using lutidinium tetrakis(3,5-bis-trifluoromethyl-phenyl)-borate ($LutHBAr'_4$) as a proton source and decamethylchromocene (Cp_2^*Cr) as a reductant, ammonia was formed from N_2 at room temperature and under normal pressure, achieving six cycles with an overall yield of 65%. DFT calculations were performed on all possible intermediates occurring in the catalytic transformation of N_2 to ammonia (12), including those characterized and invoked, respectively, by Schrock *et al.* (2). Based on these calculations a detailed mechanism and a free enthalpy profile of the entire cycle were derived. The calculations indicate that the catalytic cycle consists of a sequence of strictly alternating protonation and reduction steps. This agrees with the mechanism postulated initially by Schrock *et al.* (2), except for the transformation of the dinitrogen complex to the neutral NNH complex (13). All relevant intermediates thus are either neutral or singly positively charged, while formation of negatively charged species appears to be excluded. Furthermore, most of the reactions are exergonic, the cleavage of the N–N bond being by far the most exergonic step which, in addition, proceeds spontaneously. Only three steps

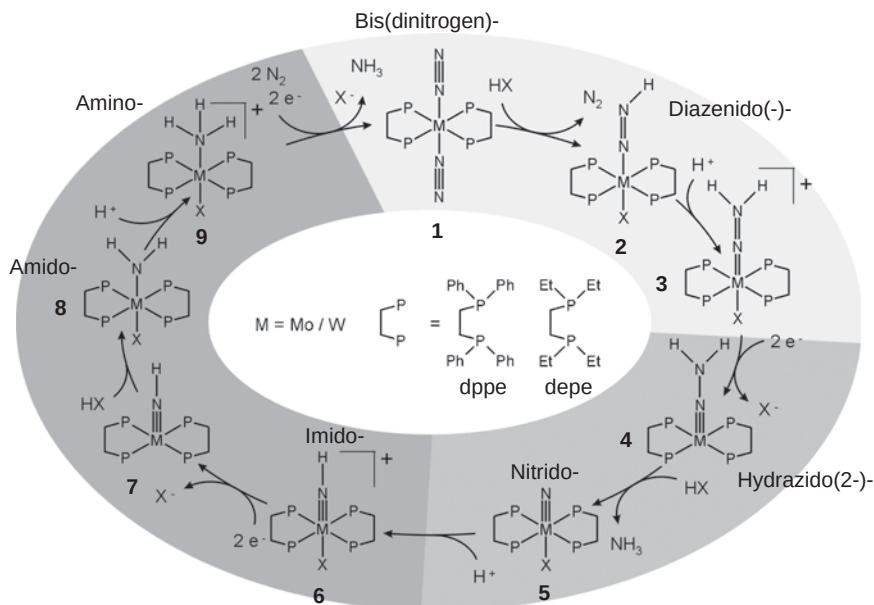


FIG. 3. The Chatt cycle with its intermediates. The cycle is divided in three stages: (a) protonation of bound N_2 , (b) cleavage of the N–N bond, releasing one molecule of NH_3 , and (c) reduction and protonation of nitrido complexes generating the second molecule of NH_3 .

spectroscopy coupled to DFT calculations. The results of these studies are presented in the following section. Calculations on the Chatt cycle in analogy to those performed for the Schrock cycle are presented in [Section III](#). As our goal is to achieve a catalytic cycle on the basis of Mo and W phosphine complexes we have designed new ligands, which are presented in [Section IV](#). General conclusions from these studies are, finally, presented in [Section V](#).

II. Mechanism of the Chatt Cycle: Experimental Investigations

For a consideration of its mechanistic details it is meaningful to divide the Chatt cycle into three stages: (a) the protonation of bound N_2 , (b) the cleavage of the N–N bond, generating the first molecule of NH_3 , and (c) the reduction and protonation of nitrido complexes, leading to the formation of the second equivalent of ammonia. In the meantime we have performed spectroscopic, synthetic, and mechanistic investigations on all of these stages of the Chatt cycle that are presented in the following.

A. PROTONATION OF COORDINATED N₂

In order to gain insight into the factors determining the protonation of dinitrogen we investigated the tungsten N₂, N₂H, and N₂H₂ complexes [W(N₂)₂(dppe)₂] (**1**^W_{dppe}), [WF(NNH)(dppe)₂] (**2**^W_{dppe,F}), and [WF(NNH₂)(dppe)₂]⁺ (**3**^W_{dppe,F}) (dppe = 1,2-bis(diphenylphosphino)ethane)) with infrared and Raman spectroscopy coupled to DFT calculations (15). More recently, these studies were complemented by the investigation of the Mo and W hydrazidium complexes [MF(NNH₃)(depe)₂](BF₄)₂, M = Mo (**4**^{Mo}_{depe,F}) and W (**4**^W_{depe,F}); depe = 1,2-bis(diethylphosphino)ethane (16). The analogous NNH₃ compounds with dppe do not exist, as protonation of the corresponding N₂ precursors stops at the NNH₂ stage; we, therefore, had to switch to depe in the latter investigation. The diazenido(-) and hydrazido(2-) complexes **2**^W_{dppe,F} and **3**^W_{dppe,F}, respectively, were prepared from complex **1**^W_{dppe} by treatment with HBF₄. The hydrazidium complexes **4**^{Mo}_{depe,F}, **4**^W_{depe,F} were obtained from the N₂ depe complexes [M(N₂)₂(depe)₂], M = Mo and W (**1**^{Mo}_{depe}, **1**^W_{depe}), by protonation with HBF₄ as well. On the other hand, protonation of **1**^{Mo}_{depe} and **1**^W_{depe}, respectively, with HCl leads to the NNH₂ complexes [MCl(NNH₂)(depe)₂]Cl, M = Mo and W (**3**^{Mo}_{depe,Cl}, **3**^W_{depe,Cl}) (17).

DFT calculations were performed on Mo dinitrogen, hydrazido(2-) and hydrazidium complexes. The calculations are based on available X-ray crystal structures, simplifying the phosphine ligands by PH₃ groups. Vibrational spectroscopic data were then evaluated with a quantum chemistry-assisted normal coordinate analysis (QCA-NCA) which involves calculation of the *f* matrix by DFT and subsequent fitting of important force constants to match selected experimentally observed frequencies, in particular ν(NN), ν(MN), and δ(MNN) (M = Mo, W). Furthermore time-dependent (TD-) DFT was employed to calculate electronic transitions, which were then compared to experimental UV/Vis absorption spectra (16). As a result, a close check of the quality of the quantum chemical calculations was obtained. This allowed us to employ these calculations as well as to understand the chemical reactivity of the intermediates of N₂ fixation (*cf.* Section III).

The N–N and metal–N force constants resulting from normal coordinate analysis of the N₂-, NNH-, NNH₂-, and NNH₃-complexes **1**^W_{dppe}, **2**^W_{dppe,F}, **3**^W_{dppe,F}, and **4**^W_{depe,F} as well as the nitrido and imido complexes **5**^{Mo}_{dppe,N₃} and **6**^{Mo}_{dppe,Cl} are graphically represented in Fig. 4 (15–17). Upon protonation of **1**^W_{dppe} to the NNH complex **2**^W_{dppe,F}, the N–N force constant decreases from

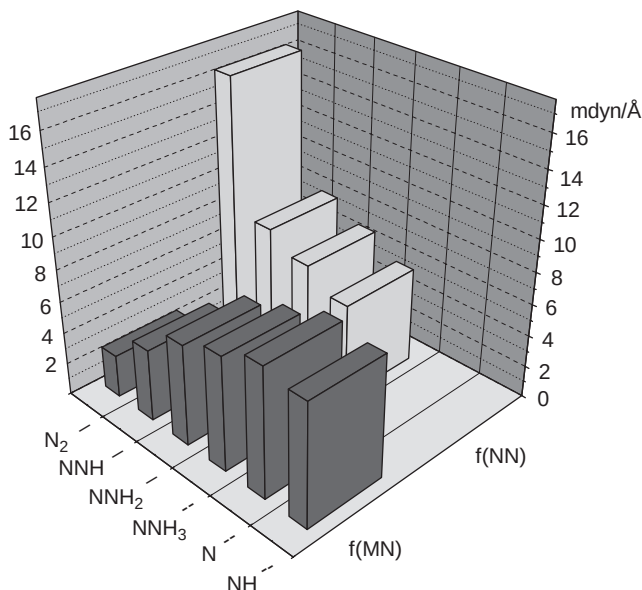


FIG. 4. Force constants $f/\text{mdyn}\text{\AA}^{-1}$ of the MN and the NN bonds in the intermediates of the Chatt cycle $1_{\text{dppe}}^{\text{W}}$, $2_{\text{dppe,F}}^{\text{W}}$, $3_{\text{dppe,F}}^{\text{W}}$, $4_{\text{depe,F}}^{\text{W}}$, $5_{\text{dppe,N}_3}^{\text{Mo}}$, and $6_{\text{dppe,Cl}}^{\text{Mo}}$ (15,17).

about $16 \text{ mdyn}\text{\AA}^{-1}$ to a value of $8.3 \text{ mdyn}\text{\AA}^{-1}$ and the M–N force constant increases from a value of about 2.5 to $4.5 \text{ mdyn}\text{\AA}^{-1}$. This trend continues in the next protonation steps; i.e., for $3_{\text{dppe,F}}^{\text{W}}$ the N–N force constant is further reduced to a value of $7.2 \text{ mdyn}\text{\AA}^{-1}$ and the metal–N force constant further increases to $6.3 \text{ mdyn}\text{\AA}^{-1}$. In the NNH_3 complexes $4_{\text{depe,F}}^{\text{Mo}}$, $4_{\text{depe,F}}^{\text{W}}$, the N–N force constant is found to be $6.0 \text{ mdyn}\text{\AA}^{-1}$, close to the value of an N–N single bond, while the metal–N force constants (8.0 and $7.3 \text{ mdyn}\text{\AA}^{-1}$, respectively) reach values which are typical for a metal–N triple bond (see below) (16). In contrast to the N–N and metal–N force constants, the MNN bending force constants remain approximately constant upon successive protonation (around $0.7 \text{ mdyn}\text{\AA}^{-1}$), with exception of the in-plane bending force constants in the NNH_2 systems which exhibit values of about $0.4 \text{ mdyn}\text{\AA}^{-1}$.

The evolution of N–N force constants upon stepwise protonation indicates a successive decrease in N–N bond order, thus initiating bond cleavage, whereas the corresponding rise of metal–N force constants reflects a successive strengthening of the metal–N bond, indicative of an increase of metal–ligand covalency. Besides providing an energetic driving force for the

reduction of the N–N triple bond, this also acts to prevent loss of partly reduced NNH_x substrate, $x = 1\text{--}3$, in the course of the conversion of N_2 to ammonia.

A characteristic feature of the classic Chatt cycle is the fact that it starts with bis(dinitrogen) complexes and after the first protonation of the N_2 complex one dinitrogen ligand is replaced by the conjugate base of the employed acid. In our investigated model systems and in the corresponding calculations the conjugate base has mostly been a halide, in particular fluoride. In order to obtain an impression of the activation of N_2 in mono-dinitrogen complexes with alternative *trans*-ligands, a series of tungsten nitrilo-dinitrogen complexes has been prepared (18). These complexes are of significant interest to synthetic nitrogen fixation as they can be protonated to the corresponding N_2H_2 complexes under retention of the *trans* ligand. Importantly, the N_2 ligand was found to be activated to a higher degree in the *trans* nitrilo than in corresponding bis(dinitrogen) systems. On the other hand, the N_2H_2 ligand is less activated toward further protonation in the *trans* nitrilo than in the analogous *trans*-fluoro complexes. Moreover, bonding of the nitrile group becomes labile at the N_2H_2 stage of N_2 reduction. The implications of these results with respect to synthetic nitrogen fixation were discussed.

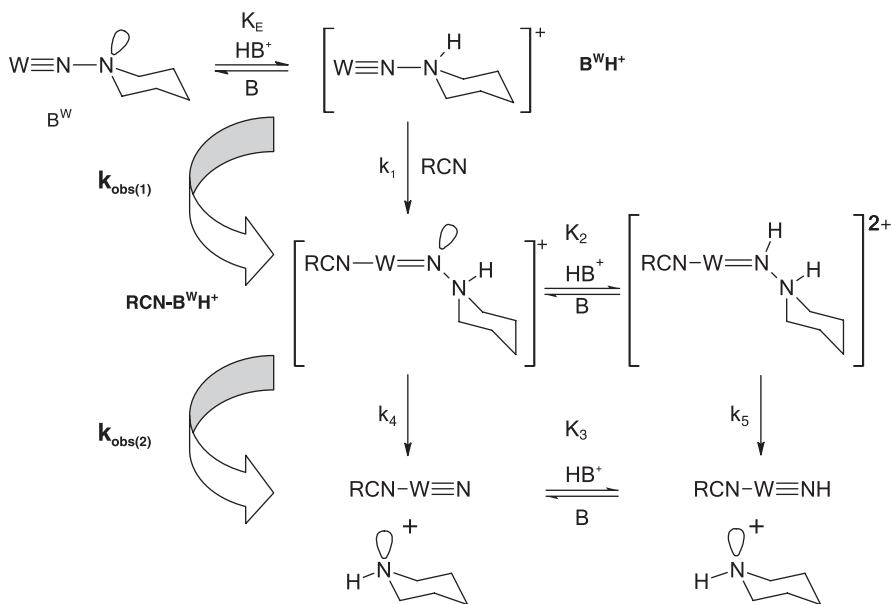
B. N–N CLEAVAGE

The ultimate stage of N_2 reduction and protonation at d^6 Mo/W centers in the absence of external reductants is represented by Mo(IV)/W(IV) hydrazidium complexes. These systems exhibit certain instability toward cleavage of the metal–P bonds (16). However, if the phosphine coligands remain bound to the metal, the NNH_3 complexes are stable toward N–N splitting. This was supported by a DFT simulation of the heterolytic N–N cleavage of $[\text{MoF}(\text{NNH}_3)(\text{PH}_3)_4]^+$ leading to NH_3 and the Mo(VI) nitrido complex $[\text{MoF}(\text{N})(\text{PH}_3)_4]^{2+}$. The calculation indicates that this process is endothermic ($\Delta H^\circ > +40 \text{ kcal mol}^{-1}$, $\Delta G^\circ > +30 \text{ kcal mol}^{-1}$) (16). Breaking the N–N single bond therefore requires the addition of electrons from an external source. As NNH_3 compounds are strongly acidic, this reduction is better performed at the NNH_2 stage. Moreover, it is useful to employ alkylated (NNR_2) derivatives in order to obtain mechanistic insight into the N–N cleavage process (19). Detailed investigations along this line have been performed by Henderson *et al.* (20). Thus, protonation of the five-coordinate complex $[\text{Mo}(\text{NNC}_5\text{H}_{10})(\text{dppe})_2]$ (compound **B^{Mo}**) with HBr in THF was found to lead to N–N splitting with formation of $\text{HNC}_5\text{H}_{10}$ (piperidine) and the imido complex

[MoBr(NH)(dppe)₂]Br. Compound **B^{Mo}** was prepared by treatment of the Mo(IV) dialkylhydrazido(2-) complex [MoBr{NN(C₅H₁₀)}(dppe)₂]Br (compound **A^{Mo}**) with *tert*-butyllithium or electrochemically at -1.62 V vs. a saturated calomel electrode (21). Compound **A^{Mo}**, in turn, is accessible through alkylation of [Mo(N₂)₂(dppe)₂] with Br-(CH₂)₅-Br.

In order to obtain information with respect to the metal dependence of the N-N cleavage reaction we investigated the protolytic N-N splitting of the tungsten analog of **B^{Mo}**, **B^W**. First the spectroscopic properties and electronic structures of **B^W** and its W(IV) analog **A^W** were evaluated and compared to those of their Mo counterparts **B^{Mo}** and **A^{Mo}**, respectively. A single-crystal X-ray structure determination of the five-coordinate W(II) complex **B^W** was reported and the vibrational properties of compound **A^W** and **B^W** were evaluated. Infrared and Raman data were analyzed using the QCA-NCA procedure (*vide supra*) (22). Finally, the electronic structures of compounds **A^M** and **B^M** (M = Mo, W) were determined using DFT, with special emphasis on the nature of the frontier orbitals.

The N-N cleavage reaction of the dialkylhydrazido complex [W(NNC₅H₁₀)(dppe)₂] (**B^W**) upon treatment with acid (HNEt₃BPh₄), leading to the nitrido complex and piperidine, was investigated experimentally and theoretically (23). Most importantly, **B^W** reacts orders of magnitude faster with HNEt₃BPh₄ in acetonitrile and propionitrile at room temperature than its Mo analogue, [Mo(NNC₅H₁₀)(dppe)₂] (**B^{Mo}**). Therefore, a stopped-flow experiment was performed at -70°C for the reaction of **B^W** with HNEt₃BPh₄ in propionitrile. Evaluation of the kinetic data indicated biphasic characteristics, which were interpreted in the following way: protonation of **B^W** is completed within the dead-time of the stopped-flow apparatus, leading to the primary protonated intermediate **B^WH⁺** (Scheme 1). Propionitrile coordination to this species proceeds at a rate $k_{\text{obs}(1)}$ of $1.5 \pm 0.4 \text{ s}^{-1}$, generating intermediate **RCN-B^WH⁺** (R = Et) that subsequently mediates N-N bond splitting in a slower reaction ($k_{\text{obs}(2)} = 0.35 \pm 0.08 \text{ s}^{-1}$, 6 equivalents of acid). Obviously these findings differ from the results obtained on the Mo analogue **B^{Mo}** where only *one* phase corresponding to the N-N cleavage step had been observed (20). Moreover, $k_{\text{obs}(1)}$ and $k_{\text{obs}(2)}$ were found to be independent of the acid concentration, again in contrast to the Mo system where an acid-dependent rate constant had been observed. This suggests that the protonation equilibrium K_2 in Scheme 1 is saturated in the W system already at low acid concentrations, leading to a higher activation toward N-N cleavage in **B^W** than in **B^{Mo}**.



SCHEME 1.

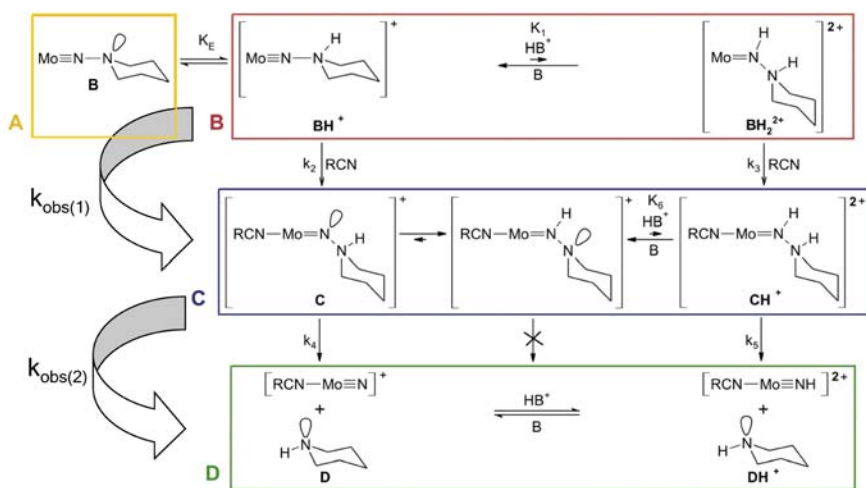
In the meantime temperature-dependent stopped-flow measurements were conducted on the latter complex in order to determine the activation parameters of the N–N cleavage reaction (24). Plots of the absorption intensity at 418 nm vs. time at $T = -35$ to $+15^\circ\text{C}$ indicate biphasic kinetics with two rate constants $k_{obs(1)}$ and $k_{obs(2)}$, in analogy to our measurements of the tungsten complex. This time, however, both rates depended upon the acid concentration. Interestingly much smaller rate constants $k_{obs(1)}$ and $k_{obs(2)}$, were found for all acid concentrations than given by Henderson *et al.* for his (single) rate constant k_{obs} (up to 1 order of magnitude). Furthermore plots of $k_{obs(1)}$ and $k_{obs(2)}$ vs. the acid concentration showed no saturation behavior but linear dependencies with slopes k_1^s and k_2^s and intercepts k_1^i and k_2^i , respectively (s = acid dependent and i = acid independent), Eq. (2):

$$\begin{aligned} k_{obs(1)} &= k_1^i + k_1^s \cdot [H^+] \\ k_{obs(2)} &= k_2^i + k_2^s \cdot [H^+] \end{aligned} \quad (2)$$

On the basis of the reaction scheme given in Scheme 1 we again assume that the first protonation occurs very rapidly (within the dead-time of the apparatus), and that subsequently the complex is attacked by the solvent; this would correspond to the rate

constant $k_{\text{obs}(1)}$. The second rate, $k_{\text{obs}(2)}$, is associated with the actual N–N cleavage step. In qualitative agreement with Henderson *et al.* the latter process exhibits an acid-dependent and an acid-independent component; these are denoted in Scheme 1 by k_4 and k_5 .

In order to determine the activation parameters of the reactions corresponding to $k_{\text{obs}(1)}$ and $k_{\text{obs}(2)}$ temperature-dependent stopped-flow measurements on the N–N cleavage of $[\text{Mo}(\text{NNC}_5\text{H}_{10})(\text{dppe})_2]$ were performed. An evaluation of the kinetic data indicated that all four rate constants in Eq. (2) – k_1^i , k_1^s , k_2^i , and k_2^s – correspond to thermally activated processes. On the basis of the modified reaction scheme shown in Scheme 2 these results can be explained consistently. In any case cleavage of the N–N bond requires one proton. We assume that for steric reasons this proton is bound to N_β . In case no further proton is added (limiting case of small $[\text{H}^+]$), the subsequent reaction path is determined by the rates k_1^i and k_2^i . The process corresponding to k_1^i is thermally activated because the protonated intermediate initially exhibits a linear Mo–N–N unit with a lone-pair at the metal center and therefore, does not allow coordination of a Lewis base at the metal center. This can only occur after bending at N_α which proceeds via an activation barrier; this way the metal center becomes Lewis acidic and a solvent molecule can coordinate, generating intermediate C. After bending of the N_β -protonated NNR₂-ligand at N_α this intermediate, in principle, should undergo a *spontaneous* N–N cleavage, as suggested by



SCHEME 2.

DFT calculations. The fact that this is *not* observed and this process (corresponding to $k_{\text{obs}(2)}$) exhibits thermally activated behavior initially surprised us. DFT, however, also indicated that an NNR_2 ligand protonated at N_α and bent at N_α should be stable toward N–N cleavage. Therefore, we assume that after bending of the N_β -protonated intermediate at N_α a rapid proton shift from N_β to N_α occurs, leading to an intermediate where the splitting of the N–N bond is blocked. This hypothesis is corroborated by a DFT–population analysis indicating a higher negative charge at N_α than at N_β . N–N cleavage can only occur if the proton shifts back to N_β (which represents the barrier of the cleavage process at small $[\text{H}^+]$) or if another proton is added to N_β , generating a doubly protonated intermediate.

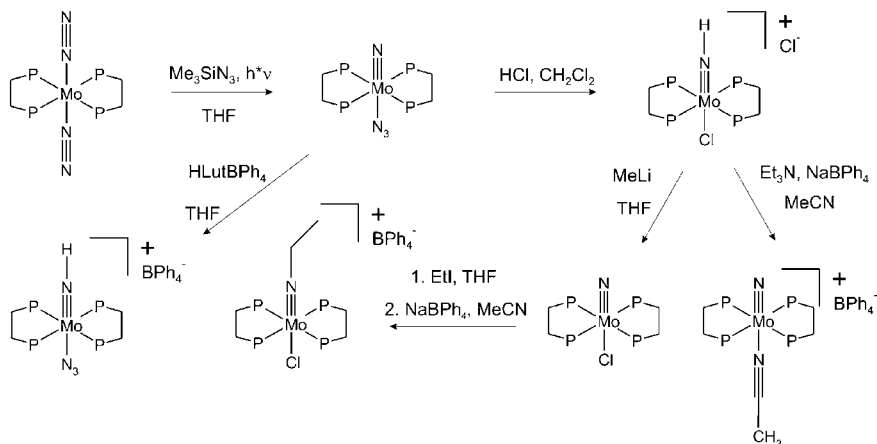
The latter reaction course proceeds in the regime of higher acid concentrations. In this case secondary protonation can also occur already at the level of the solvent-free, single-protonated intermediate BH^+ : if *this* intermediate adds a second proton, bending at N_α occurs and a solvent molecule can coordinate. Protonation in this case lowers the activation barrier for solvent attack, leading to an increase of $k_{\text{obs}(1)}$ via k_1^s . If, on the other hand, the solvent-coordinated, bent intermediate **C** adds a second proton the actual N–N cleavage process is accelerated, leading to an increase of $k_{\text{obs}(2)}$ via k_2^s . In both cases the experimentally determined activation parameters reflect the respective protonation pre-equilibria, which are endergonic.

Thus, in summary our investigations have led to a complete mechanistic understanding of the N–N cleavage in Mo- and W-dialkylhydrazido complexes. Both for the tungsten and for the molybdenum complex biphasic kinetics have been observed which can be explained by a very rapid initial protonation, subsequent solvent coordination and slow N–N cleavage reaction, in agreement with the overall picture already given by Henderson *et al.* For the Mo complex however, the rates are much smaller than reported by these authors. In any case, the W complex reacts order of magnitudes faster than the Mo complex, which can be explained by an increased basicity of the latter system. This is also reflected by the fact that for the W complex no dependence of the decay rate upon the acid concentration has been found: the protonation equilibria are already saturated at a low excess of acid.

C. ELECTRONIC STRUCTURE AND REACTIVITY OF MOLYBDENUM NITRIDO AND IMIDO COMPLEXES

After cleavage of the N–N bond the parent dinitrogen complex has been converted to a Mo(IV) or W(IV) nitrido or imido complex

which after four-electron reduction and further protonation can in principle be converted back to the dinitrogen complex, giving a second molecule of ammonia. A major problem at this stage of the Chatt cycle is the strongly negative reduction potential, which is needed to regenerate the low-valent molybdenum or tungsten species capable of binding N_2 (25). In a first step, we thus wanted to obtain information on the reactivity of Mo and W nitrido and imido complexes toward acids and reductants. In this context, we synthesized a number of such systems with various coligands and investigated their electronic structures and spectroscopic as well as electrochemical properties (26). The starting compounds for these studies were the Mo azido-nitrido complexes $[Mo(N)(N_3)(diphos)_2]$ (diphos = depe ($5^{Mo}_{depe,N_3}$) or dppe ($5^{Mo}_{dppe,N_3}$)) which are accessible from the corresponding bis(dinitrogen) complexes by reaction with trimethylsilylazide (Scheme 3). These nitrido complexes can be converted to the corresponding imido-azido systems $6^{Mo}_{depe,X}$ and $6^{Mo}_{dppe,X}$ by treatment with acids. For strong acids, HX the *trans*-azido ligand is exchanged for the conjugated base X of that acid; otherwise protonation occurs under retention of the *trans* ligand (N_3^-). The former applies to the reaction with HCl whereas the latter is found for the protonation with HLutBPh₄. The imido-chloro complexes $6^{Mo}_{depe,Cl}$ and $6^{Mo}_{dppe,Cl}$ can in turn be deprotonated with, e.g., MeLi, to their nitrido-chloro counterparts. Moreover, if the chloro-imido Mo-dppe complex $6^{Mo}_{dppe,Cl}$ is deprotonated in acetonitrile, the corresponding cationic nitrido-acetonitrile complex $5^{Mo}_{dppe,MeCN}$ is obtained.



SCHEME 3.

The above-mentioned nitrido and imido complexes **5** and **6** provided the basis for an extensive spectroscopic investigation, correlated with DFT. In the nitrido complexes with *trans*-azido and -chloro coligands the metal-N stretch is found at about 980 cm^{-1} , shifting to about 920 cm^{-1} upon protonation. As for the N_2 and NNH_x compounds, QCA-NCA normal coordinate analyses were performed. The resulting metal-N force constants are $f(\text{Mo}\equiv\text{N}) = 7.1\text{ mdyn}\text{\AA}^{-1}$ and $f(\text{Mo}\equiv\text{NH}) = 6.7\text{ mdyn}\text{\AA}^{-1}$, indicative of metal-ligand triple bonds. Force constants of similar magnitude had already been observed for the Mo hydrazidium complexes (*vide supra*). The metal-N vibration was also observed in the optical spectra. A typical feature of the Mo(IV) and W(IV) nitrido complexes is the ${}^1\text{A}_1 \rightarrow {}^1\text{E}(n \rightarrow \pi^*)$ electronic transition which corresponds to the excitation of an electron from the nonbonding d_{xy} orbital to the metal-ligand π -antibonding d_{xz}/d_{yz} orbitals. This transition is observed for complex **5**^{Mo}_{depe.N₃} at 398 nm, showing a progression in the metal-N stretch of 810 cm^{-1} . This frequency corresponds to $\nu(\text{MoN})$ in the excited state and reflects the lowering of the metal-N force constant due to excitation of an electron from a nonbonding to an antibonding orbital. The corresponding elongation of the metal-N bond was determined from a bandshape analysis based on the relationship in Eq. (3).

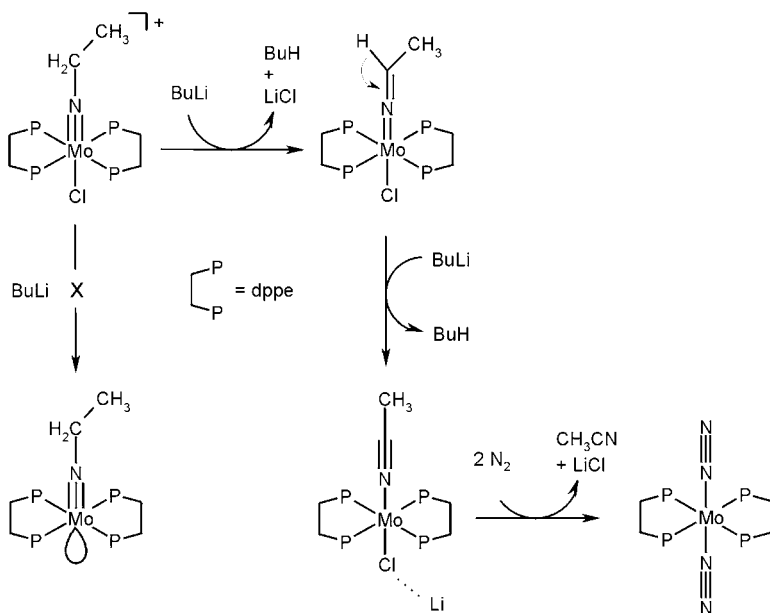
$$\text{Shc}\tilde{\nu} = \frac{1}{2}k\Delta R^2 \quad (3)$$

With a Huang-Rhys factor S of 2.5, a frequency $\tilde{\nu} = 810\text{ cm}^{-1}$ and a force constant k in the excited state of $4.9\text{ mdyn}\text{\AA}^{-1}$ an excited-state bond elongation ΔR of $0.12\text{ \AA}$ was derived. Fortunately, the analogous electronic transition could also be observed in the luminescence spectrum. In this case the ${}^3\text{E} \rightarrow {}^1\text{A} (\pi^* \rightarrow n)$ emission band was observed at 542 nm, exhibiting a progression in the metal-N stretch of 980 cm^{-1} . This value corresponds to the metal-N stretching frequency in the electronic ground state, in agreement with the results from infrared and Raman spectroscopy. A bandshape analysis of the emission band gave a Huang-Rhys factor of 2.25 and an ΔR value of $0.11\text{ \AA}$, in good agreement with the information from the absorption spectrum.

In the imido systems the $n \rightarrow \pi^*$ transition is shifted to lower energy (518 nm) and markedly decreases in intensity. On the other hand, upon substitution of the anionic *trans*-ligands by acetonitrile the $n \rightarrow \pi^*$ transition is found at 450 nm, shifting to 525 nm upon protonation. Moreover, the metal-N(nitrido) stretching frequency increases to 1016 cm^{-1} . From a chemical viewpoint it is important that the nitrido-nitrile complex can be

reduced at -1.5 V vs. Fc^+/Fc , indicating that reduction of this system is drastically facilitated compared to its counterparts with anionic *trans*-ligands ($E_{\text{red}} \leq -2.4\text{ V}$ vs. Fc^+/Fc) (26). However, the basicity of the nitrido group also decreased with respect to the nitrido complexes with *trans*-halide ligands, as evident from an increased acidity of $[\text{Mo}(\text{NH})(\text{NCCH}_3)(\text{dppe})_2](\text{BPh}_4)_2$ (**6^{Mo}_{dppe,MeCN}**) ($\text{pK}_a = 5$). Nevertheless, the basicity of the nitrido-nitrile complex should be sufficient to warrant further reaction of the Mo(IV) nitride to the Mo(II) amido complex.

In order to study the following steps of the Chatt cycle, it again appeared useful to employ alkylated Mo nitrido complexes, in analogy with the investigation of the two-electron reduction of the Mo(IV)- NNH_2 compounds **4^{Mo}** (*vide supra*). We, therefore, prepared the Mo(IV) chloro-ethylimido complex $[\text{MoCl}(\text{NEt})(\text{dppe})_2]\text{Cl}$ and reacted it with *n*-butyllithium in order to prepare the corresponding five-coordinate Mo(II) ethylimido complex. Instead of the desired reaction product, however, the bis(dinitrogen) complex **1^{Mo}_{dppe}** was obtained (Scheme 4). We ascribed this to a double deprotonation of the ethylimido group at the β -carbon atom, leading via the neutral Mo(II) azavinylidene complex $[\text{MoCl}(\text{N}=\text{C}(\text{CH}_3)_2)(\text{dppe})_2]$ to the anionic Mo(0) chloro-acetonitrile complex $[\text{MoCl}(\text{CH}_3\text{CN})(\text{dppe})_2]^-$ which under an N_2



SCHEME 4.

atmosphere replaces its two ligands by dinitrogen (27). In order to avoid deprotonation we decided to substitute the ethyl for a *tert*-butyl group. The *tert*-butylimido complex $[\text{MoX}(\text{N}t\text{Bu})(\text{depe})_2]$ ($\text{X} = \text{N}_3$) was successfully prepared and characterized, but it turned out to be unstable toward elimination of isobutene (28). We therefore currently try to synthesize directly Mo amido complexes with *trans*-halide ligands and investigate their reactivity toward protonation and reduction, leading to amines.

III. Mechanism of the Chatt Cycle: Theoretical Investigations

Having studied the elementary reactions of the Chatt cycle as described in the previous sections, it appeared of interest to obtain also a global picture of the energetics of this cycle in analogy to the Schrock cycle. To this end, the employed acid, the reductant and the intermediates of the "classic" Chatt cycle, starting from bis(dinitrogen) bis(diphosphine) complexes and proceeding via Mo fluoro- NNH_x and $-\text{NH}_x$ complexes ($x = 1-3$), were treated with DFT, applying the same methodology as in the Schrock cycle (12a). For the simulation of the reaction course, simplified models of molybdenum diphosphine complexes were applied. In particular, the dppe ligands were simplified to $\text{H}_2\text{PCH}_2\text{CH}_2\text{PH}_2$ (diphosphinoethane, dpe) groups. As for the Schrock cycle, decamethylchromocene was employed as reductant. For the protonation reactions two acids were considered, $\text{HBF}_4/\text{diethylether}$ and lutidinium (HLut^+). The free reaction enthalpy changes of all protonation and reduction steps were calculated (29).

For $\text{HBF}_4/\text{diethylether}$ as a proton source the derived energy profile and corresponding reaction mechanism bear strong similarities to the Schrock cycle (*vide supra*) (12). In particular, the most endergonic reaction is the first protonation of the N_2 complex and the most exergonic reaction is the cleavage of the N–N bond. If lutidinium is employed as acid and Cp_2^*Cr as reductant, however, the classic Chatt cycle involves steps that are not thermally allowed. Only if $\text{HBF}_4/\text{diethylether}$ is employed as acid and Cp_2^*Cr as reductant, a cycle consisting of thermally allowed reactions is predicted. This cycle, however, involves a Mo(I) fluoro complex as a dinitrogen intermediate. Reduction of Mo(I) fluoro to the Mo(0) bis(dinitrogen) complex is *not* possible in this system. Although the Mo(I)- N_2 complex is able to activate dinitrogen toward protonation just as the Mo(0) bis(dinitrogen) complex is, it is at significantly higher energy ($\sim 20 \text{ kcal mol}^{-1}$) than the latter. Bonding of N_2 to a Mo(I) fluoro complex thus is thermodynamically not particularly favorable, and the resulting

intermediate is unstable toward disproportionation (30). We determined theoretically a free energy change of 30 kcal mol^{-1} for the reaction of two $[\text{Mo}^{\text{I}}\text{F}(\text{N}_2)(\text{dpe})_2]$ to $[\text{Mo}^{\text{0}}(\text{N}_2)_2(\text{dpe})_2]$ and $[\text{Mo}^{\text{II}}\text{F}_2(\text{dpe})_2]$. The resulting Mo(0) bis(dinitrogen) complex gives another two molecules of NH_3 along the Chatt cycle, but the Mo(II) difluoro complex cannot be converted back to a Mo(I) or Mo(0) dinitrogen complex by the applied reductant. Disproportionation at the Mo(I) level thus leads to 50% loss of catalyst per cycle.

If a truly catalytic action of the Chatt system is intended, strategies have to be developed to avoid disproportionation of the Mo(I) dinitrogen complex to a Mo(II) complex carrying two anionic ligands. Important points in this respect are (i) avoiding the presence of strongly Lewis-basic species in solution (such as F^-) and (ii) employing multidentate ligands which also occupy the *trans*-position of coordinated N_2 , in contrast to the conventional Mo and W diphosphine systems. Strategies for the synthesis of such systems are described in Section IV. In order to obtain an impression of the corresponding energetics the analogous calculations as described above were performed for a MoN_2 complex with pentaphosphine coordination. The reaction course with reduction steps being mediated by decamethylchromocene and the protonation reactions being mediated by HLut^+ is shown in Fig. 5 and Scheme 5 (31).

The reactive cycle starts with a protonation of the dinitrogen complex **1a** to the Mo(II) diazenido complex **2a**. Further reaction to the Mo(I) NNH_2 complex **3b** either proceeds via the Mo(I) diazenido complex **2b** or the Mo(II) NNH_2 complex **3a**; in both cases an activation energy of $\sim 25 \text{ kcal}$ is involved. In analogy to the fluoro system the reduced NNH_2 complex **3b** exhibits a bent Mo- NNH_2 unit and can be protonated at the terminal (β) or the coordinating (α) nitrogen. As a consequence there are in principle two possible reaction pathways for N-N splitting. The first alternative involves formation of the hydrazidium complex **4a** by protonation of **3b** at N_β . Due to the high energy of this intermediate ($\sim 40 \text{ kcal mol}^{-1}$), however, this pathway is practically excluded. Protonation at N_α , on the other hand, generates intermediate **10a** which has a bent $\text{HN}=\text{NH}_2$ ligand. Reduction of this species generates **10b** which cleaves the N-N bond, leading to the Mo(IV) imido complex **6a** and NH_3 .

Further reduction and protonation of the imido group leads to the formation of a Mo(I) ammine complex (**8b**) in a mechanism similar to the fluoro system in the classic Chatt cycle. The Mo(I) ammine complex **8b** is first reduced to the corresponding Mo(0) complex **8c** at which stage the ammine ligand is exchanged with

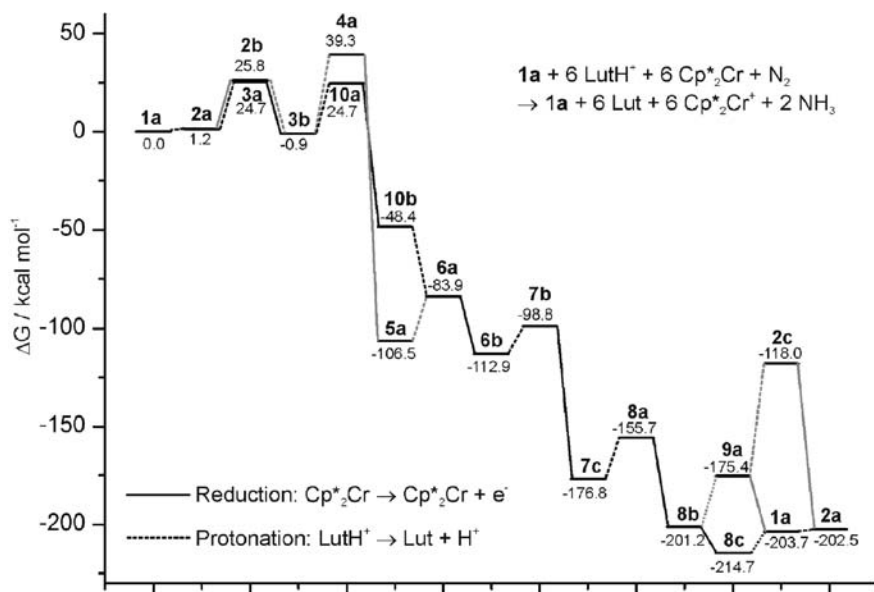
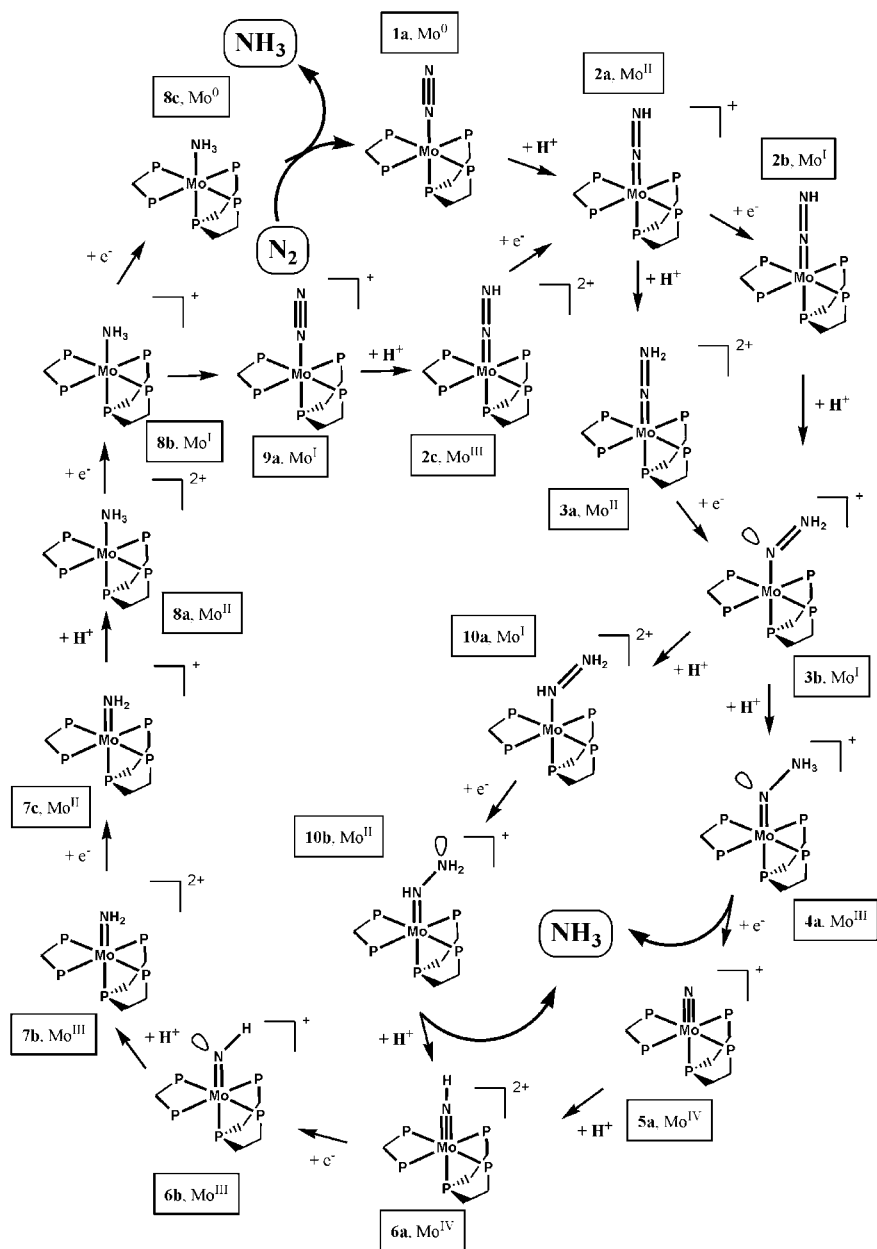


FIG. 5. Energy profile ($\Delta G/\text{kcal mol}^{-1}$) of a catalytic cycle of N₂ in a Mo-pentaphosphine complex with reduction by decamethylchromocene and protonation by HLut⁺ obtained by DFT calculations (31).

N₂, leading to the dinitrogen complex **1a**. The alternative scenario, exchange of NH₃ by N₂ at the Mo(I) level leading to **9a** is energetically less favorable; moreover, the Mo(I) dinitrogen complex does not allow protonation under thermal conditions. This is in contrast to the Mo-fluoro system where the Mo(I) fluoro-ammine complex cannot be reduced to the corresponding Mo(0) complex and exchange of NH₃ with N₂ occurs at the level of the Mo(I) complex (the resulting Mo(I) fluoro-dinitrogen complex can be protonated with a strong acid, but is unstable with respect to disproportionation, *vide supra*). These differences in reactivity of course reflect the influence of *trans* ligand (anionic fluoro vs. neutral phosphine).

In sum, the calculations thus indicate that a catalytic cycle of ammonia synthesis on the basis of a Mo-pentaphosphine complex should be feasible in the presence of decamethylchromocene as reductant and lutidinium as acid; i.e., under the same conditions as employed for the Mo(triamidoamine) system by Schrock *et al.* (*vide supra*). Interestingly the most endergonic step of the cycle this time is not the first protonation of N₂ but the protonation or one-electron reduction of the Mo(II)-NNH₂ complex (both of these reactions are endergonic by $\sim 25 \text{ kcal mol}^{-1}$, a limiting value for



SCHEME 5.

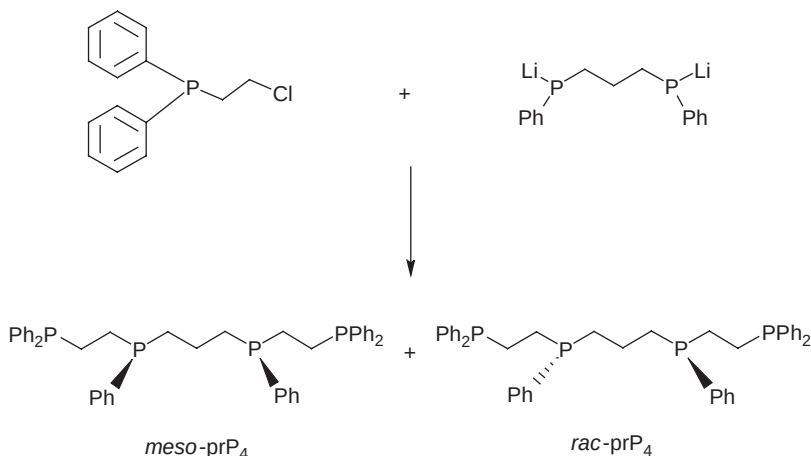
thermally allowed reactions). In contrast, the “classic” Chatt cycle starting from bis(dinitrogen) complexes should not be catalytic under these conditions. These theoretical predictions are presently checked experimentally. Presumably the number of catalytic cycles of the pentaphosphine system will be limited by the fact that the *trans*-phosphine ligand of N_2 (being contained in the linear triphos ligand) becomes labile at higher oxidation states of the metal and sooner or later will be replaced by another Lewis-basic ligand or a solvent molecule. Therefore, the necessity persists to bind the *trans* ligand to the metal center in a way that it cannot dissociate at any stage of the catalytic cycle. Concepts to achieve such an architecture are presented in the following sections.

IV. Molybdenum Dinitrogen Complexes with Polydentate Phosphine and Mixed P/N Ligands

Based on the mechanistic insight into the Chatt cycle obtained in the experimental and theoretical investigations presented in the previous sections alternative phosphine ligands or corresponding ligand systems can be designed which allow an improvement of the performance of Mo and W dinitrogen complexes toward a catalytic reaction mode. With this goal in mind three types of ligands were considered: (a) tetraphos ligands; (b) pentaphosphine ligand systems; and (c) mixed phosphorus/nitrogen ligands. The results of our efforts toward synthesizing and coordinating these ligands to transition-metal centers (in particular, Mo and W) are presented in the following.

A. TETRAPHOS COMPLEXES

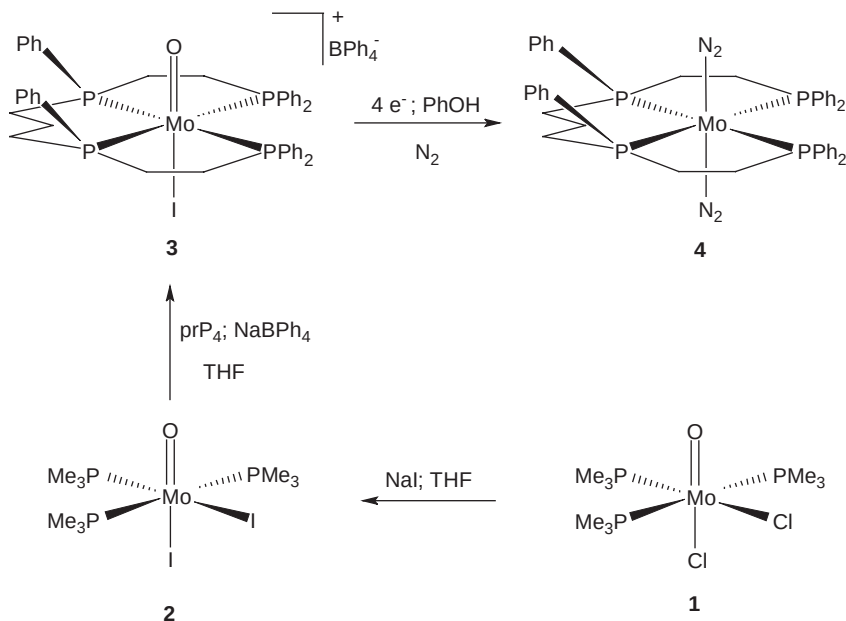
An obvious strategy to increase the thermal stability of Mo and W dinitrogen complexes and their protonated derivatives is to employ tetraphosphine instead of diphosphine ligands which conventionally have been used in this type of systems. Against this background the tetradentate phosphine ligand prP_4 (1,1,4,8,11,11-hexaphenyl-1,4,8,11-tetraphosphaundecane) was prepared and its coordination properties in mononuclear complexes were explored (32). The synthesis of the ligand is achieved in the following manner (Scheme 6): In a first step chloroethyl (diphenyl)phosphine is prepared by reaction of lithium diphenylphosphide with dichloroethane. This precursor is reacted with 1,3-dilithium diphenylphosphide (Lipp) obtained from



SCHEME 6.

1,3-bis(diphenylphosphino)propane (dppp) by treatment with lithium in THF to give a mixture of *meso* and *rac* prP₄. Note that the *meso* isomer has the two phenyl rings of the central phosphorus atoms oriented to the same side whereas in the *rac* isomer the two phenyl rings are located on the opposite sides of the ligand. Correspondingly the ³¹P-NMR spectrum of the reaction product exhibits two (AX)₂ schemes with equal chemical shifts for the terminal and slightly different chemical shifts for the central phosphorus atoms. The J_{AX} coupling constants for both isomers are 28.5 Hz. No coupling is observed between the central P-atoms, indicating that spin-spin interaction via the propylene bridge is negligible.

In the course of our investigations of the prP₄ ligand the stereospecific synthesis of the molybdenum dinitrogen complex *trans*-[Mo(N₂)₂(*meso*-prP₄)] (**4**) was achieved (Scheme 7) (33). The intermediates and the product of the synthesis were characterized by ³¹P-NMR, vibrational and UV/Vis spectroscopy as well as X-ray structure analysis. As a matter of fact **4** is the first molybdenum bis(dinitrogen) complex with a tetraphosphine ligand. The synthetic problem of coordinating a tetraphos ligand to a mononuclear Mo dinitrogen complex was solved by applying the ligand exchange chemistry of molybdenum(IV) oxo-halide complexes with PMe₃ coligands established by Carmona *et al.* (34). As demonstrated by these authors the neutral Mo(IV) complex [Mo(O)Cl₂(PMe₃)₃] (**1**) is able to exchange two PMe₃ groups and one chloro ligand for two 1,2-bis(dimethylphosphino)ethane ligands (dmpe), giving *trans*-[Mo(O)Cl(dmpe)₂]Cl (**35**).



SCHEME 7.

We successfully reproduced the synthesis of the Mo(IV) oxo-chloro complex **1** from $[\text{MoCl}_4(\text{thf})_2]$ and PMe_3 in aqueous THF as well as the ligand exchange reaction with dmpe. Reaction of **1** with the tetradentate ligand prP_4 , however, did not lead to an isolable product.

In order to make the Mo(IV) oxo precursor more reactive toward the coordination of prP_4 , the chloro ligands of **1** were replaced by iodides, leading to the complex $[\text{Mo}(\text{O})\text{I}_2(\text{PMe}_3)_3]$ (**2**) which had been prepared and characterized by Poli *et al.* recently (Scheme 7) (36). Complex **2** reacts cleanly with the tetradentate prP_4 ligand in thf, affording crystalline $[\text{Mo}(\text{O})\text{I}(\text{prP}_4)]\text{BPh}_4^-$ (**3**) after addition of NaBPh_4 to the reaction mixture. Importantly, ^{31}P -NMR spectroscopy indicates the presence of only one isomer, showing that the reaction product either contains the *meso* or the *rac* ligand but not a mixture of both. In contrast to the free prP_4 ligand which shows no coupling between the central phosphorus atoms, the spectrum now exhibits an AA'XX' pattern. The *trans* (J_{AX}) coupling constant is 110.4 Hz. The central and the terminal phosphorus atoms are coupled with constants J_{AA} and J_{XX} of -18.4 Hz and -28.2 Hz, respectively. We assume the *cis*-interaction with the larger absolute value (28.2 Hz) corresponds to the interaction between the central P-atoms where the bonding

angle is about 90° (see below). For the terminal P-atoms where the bonding angle is increased to 108° the absolute value of the coupling constant is decreased and thus corresponds to the smaller coupling constant, -18.4 Hz. The terminal and bridging P-atoms, finally, are coupled via the metal and the ethylene bridge whence the coupling constant is small (J_{AX} ; -4.9 Hz) (37). Fortunately, single crystals could be grown, allowing determination of an X-ray structure of **3** (33). The structure of the complex cation shown in Fig. 6 reveals that the reaction product contains the *meso* and not the *rac* ligand. This finding can be attributed to the steric influence of the iodo ligand, which prevents formation of the *rac* complex in which one of the phenyl substituents on the central phosphorus atoms would point toward this ligand. Evidently the Mo center fits nicely within the cavity provided by the prP_4 ligand, a small steric strain only being visible by an increase of the P-Mo-P angle to 108° and a bending of the terminal phosphine groups out of the equatorial plane.

The $[\text{MoOI}(\text{prP}_4)]$ precursor **3** was finally converted to the corresponding dinitrogen complex by electrochemical reduction with a Hg pool electrode in the presence of dinitrogen and phenol. The latter reagent was added as a weak acid to induce protonation of the oxo group and subsequent elimination as water. The blue solution of the Mo oxo complex thereby turned

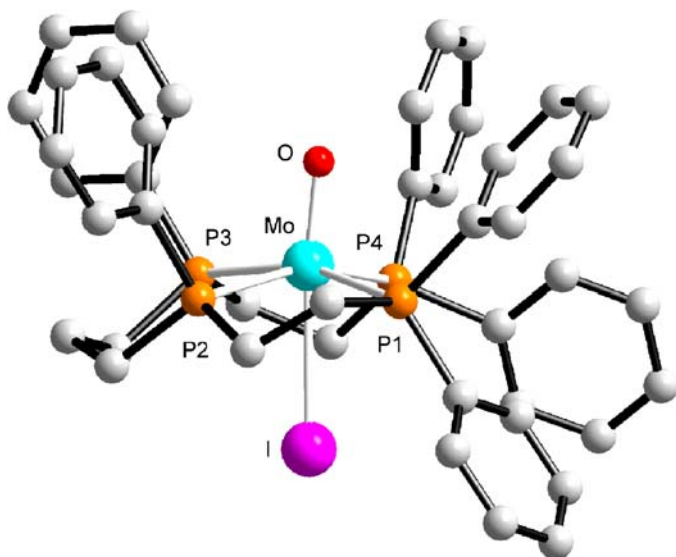


FIG. 6. X-ray structure of $[\text{Mo}(\text{O})\text{I}(\text{prP}_4)]\text{BPh}_4$ (**3**) containing the *meso* PrP_4 ligand (33).

yellow, indicating the formation of the dinitrogen complex. The ^{31}P -NMR spectrum of the reaction product, **4** again exhibits a single AA'XX' pattern, indicating a *trans* geometry of the complex and the presence of only one isomer. On the basis of the structural information obtained on the precursor **3**, the dinitrogen complex **4** should contain the *meso* ligand. The ^{31}P -NMR-spectrum of **4** can be reproduced perfectly by simulation, employing parameters that are quite similar to those of the precursor **3**.

A second, independent spectroscopic proof of the identity of **4** as *trans*-[Mo(N₂)₂(*meso*-prP₄)] was provided by vibrational spectroscopy. The comparison of the infrared and Raman spectrum (Fig. 7) shows the existence of two N–N vibrations, a symmetric combination at 2044 cm⁻¹ and an antisymmetric combination at 1964 cm⁻¹, indicating the coordination of *two* dinitrogen ligands. In the presence of a center of inversion the symmetric combination is Raman-allowed and the antisymmetric combination IR allowed. The intensities of ν_s and ν_{as} as shown in Fig. 2 clearly reflect these selection rules. Moreover, these findings fully agree with results obtained in studies of other Mo(0) bis(dinitrogen)

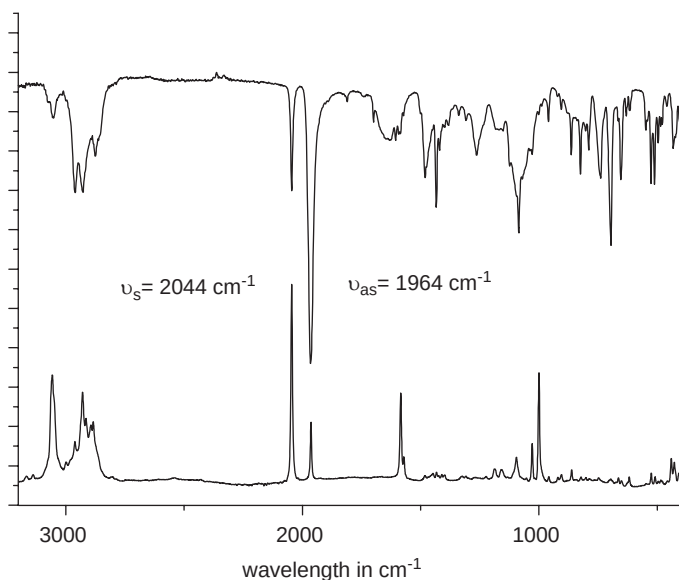


FIG. 7. Comparison of the infrared (top) and Raman (bottom) spectrum of *trans*-[Mo(N₂)₂(*meso*-prP₄)] showing the symmetric N–N vibration at 2044 cm⁻¹ and the antisymmetric combination at 1964 cm⁻¹ (33).

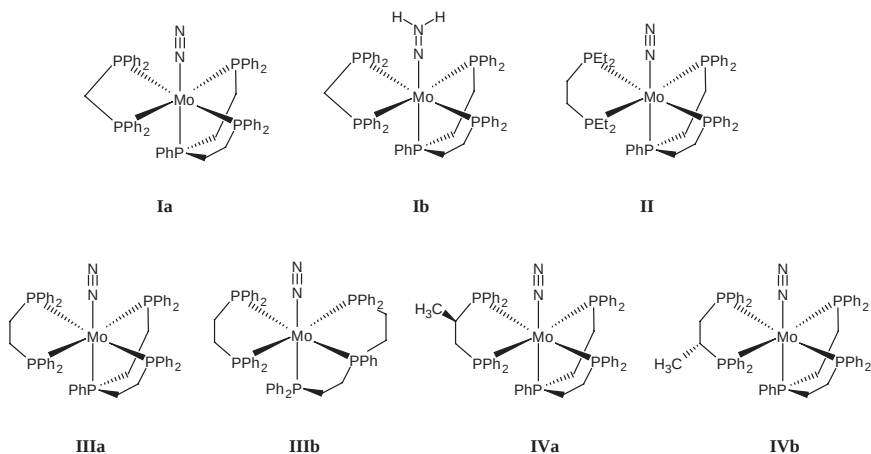
complexes with a *trans* configuration (15,17). For bis(dinitrogen) complexes with a *cis*-configuration, in contrast, no vibrational exclusion principle applies and both vibrations are observed with comparable intensities in the infrared and the Raman spectrum. Complex **4** therefore, is unambiguously associated with a bis-(dinitrogen) complex exhibiting a *trans* configuration, as already inferred from NMR evidence.

The synthesis of the dinitrogen complex **4** has been a major step forward as for the first time the synthesis of a low-valent molybdenum complex with a tetraphosphine ligand was achieved, using a generally applicable synthetic strategy. However, this complex does not solve the basic problem of the classic Chatt cycle, the coordination of Lewis bases in *trans*-position to the dinitrogen ligand. Nevertheless, this complex should be of some interest in the context of synthetic nitrogen fixation, as it should exhibit a higher thermodynamic stability in the reactions of the Chatt cycle. Further experimental and theoretical studies are underway to check this assumption and characterize the intermediates resulting from protonation and reduction of **4**.

B. COMPLEXES WITH PENTAPHOSPHINE COORDINATION

In order to both enhance the stability of Mo diphos systems with respect to the cleavage of metal-phosphine bonds at higher metal oxidation states and to suppress the *trans*-ligand exchange reactions occurring in the classic Chatt cycle (*vide supra*), a number of Mo dinitrogen complexes containing a combination of a diphosphine and a triphosphine ligand had been prepared by George *et al.* (38). As a triphosphine component the ligand dpepp (bis(diphenylphosphinoethyl)phenylphosphine) was employed, and as diphosphine ligands dpmm (1,2-bis(diphenylphosphino)-methane) and dppe were chosen (Scheme 8). We augmented this series by the dialkyl-diphosphine ligand depe and the optically active diphos ligand 1,2-dppp ($\text{Ph}_2\text{PCH}(\text{CH}_3)\text{CH}_2\text{PPh}_2$) containing a stereocenter at the C2-atom (37). In analogy to the Mo triamidoamine complexes, these systems exhibit only one binding site for N_2 and can be protonated to give NNH_2 complexes. Moreover, these systems allow the assessment of the influence of strongly σ -donating *trans*-phosphorus ligands on the bonding and reduction of N_2 . Importantly, DFT indicated the possibility of a catalytic synthesis of NH_3 from N_2 on the basis of these systems (*cf.* Section III).

First, the complex $[\text{Mo}(\text{N}_2)(\text{dpepp})(\text{dpmm})]$ (**1a**) and its hydrazido(2-) derivative **1b** were investigated with ^{15}N - and ^{31}P -NMR as well as vibrational spectroscopy (39), coupled to DFT calculations



SCHEME 8.

and QCA-NCA (*vide supra*) (40). In the dinitrogen complex **Ia** the N–N and Mo–N stretching frequencies were found at ~ 1980 , and 454 cm^{-1} , which correspond to N–N and metal–N force constants of 16.3 and $2.8\text{ mdyn \AA}^{-1}$. These values indicate that the activation of the N_2 ligand of the parent N_2 complex is *lower* than in the *trans*-acetonitrile complex $[\text{Mo}(\text{N}_2)(\text{NCCH}_3)(\text{dppe})_2]$, but *higher* than in the bis(dinitrogen) complex $[\text{Mo}(\text{N}_2)_2(\text{dppe})_2]$, which was interpreted in terms of the relative σ/π donor and π -acceptor strengths of the respective *trans*-ligands. In general, mono-dinitrogen complexes exhibit a stronger activated ligand than corresponding bis(dinitrogen) complexes owing to the lack of a second strong π -acceptor. Comparison of the two mono- N_2 complexes, on the other hand, showed that the *trans*-nitrile system exhibits a stronger activation than the pentaphosphine system. This effect is due to the different σ -donor capabilities of nitrile and phosphine: in the presence of a weaker σ -donor in a *trans*-position (acetonitrile) N_2 acts as a stronger σ -donor, which lowers its π^* orbitals in energy. This enhances metal–ligand backbonding and overcompensates the effect of charge loss due to σ -donation, resulting in a stronger activation. In the presence of a strong σ -donor like phosphine in a *trans*-position, this synergistic effect is absent; i.e., N_2 becomes a weaker σ -donor, its π^* orbitals are less lowered in energy and as a consequence a weaker activation of N_2 is found.

Another interesting result obtained from the NMR investigations was the strong low-field shift of the ^{15}N signals in the complex **Ia**. The terminal N-atom N_β shows with -16.7 ppm the

strongest low-field shift for end-on coordinated N_2 complexes. With the help of DFT GIAO calculations, it could be shown that the origin of this unusual shift is the paramagnetic term. The magnitude of the paramagnetic shift is determined by the nitrogen contribution in the backbonding metal t_{2g} orbitals. This effect is stronger for N_β than for N_α as a larger negative charge resulting from backbonding is located on the former atom. In the case of the pentaphosphine complex, besides the comparatively strong backbonding interaction another effect contributes to the strong low-field shift of the ^{15}N signals, in particular of N_β : the d_{xy} orbital, which in the bis(dinitrogen) and in the *trans*-nitrile dinitrogen complex is nonbonding, is included in the backbonding interaction with the N_2 ligand in the P_5 system. For this reason, all $t_{2g} \rightarrow e_g$ excitations contribute to the paramagnetic shift. This effect is basically a consequence of the symmetry reduction from (approximately) tetragonal in the bis(diphos) systems to mirror symmetry in the P_5 complex.

The results of the NMR and vibrational spectroscopic investigation of the hydrazido complex $[Mo(NNH_2)(dpepp)(dppm)](OTf)_2$ (OTf = triflate) **1b** confirm the structure of a pentaphosphine complex with a linearly coordinated NNH_2 unit. On the basis of vibrational spectra and DFT calculations a normal coordinate analysis has been performed. The resulting force constants ($f(NN) = 7.65 \text{ mdyn } \text{\AA}^{-1}$, $f(MoN) = 5.94 \text{ mdyn } \text{\AA}^{-1}$) demonstrate the weakening of the N–N bond and the enhancement of the Mo–N bond strength as a consequence of the protonation (*cf.* Section II.A). The force constants of the hydrazido ligand are comparable with those of other hydrazido complexes such as, e.g., $\mathbf{3}_{depe,Cl}^{Mo}$ ($f(NN) = 7.16 \text{ mdyn } \text{\AA}^{-1}$, $f(MoN) = 5.52 \text{ mdyn } \text{\AA}^{-1}$) (17) (no force constants exist for analogous Mo-dppe complexes with anionic or nitrile coligands which precludes a direct comparison of the spectroscopic data of the NNH_2 complexes).

With the help of DFT calculations on the model complexes $[Mo(NNH_2)(PH_3)_5]^{2+}$, $[MoF(NNH_2)(PH_3)_4]^+$, and $[Mo(NNH_2)(NCMe)(PH_3)_4]^+$ it could be shown that the NPA charge of the NNH_2 ligand in the *trans*-fluoro complex (-0.03) is lower than in the P_5 complex ($+0.20$). This leads to a lower activation of the NNH_2 ligand in the latter system toward further protonation. The reason for this finding is a lower charge transfer from the metal due to the exchange of a negative (F^-) for a neutral (i.e., phosphine) ligand.

In a second study, we performed similar investigations on molybdenum dinitrogen complexes containing a combination of dpepp with diphos ligands exhibiting C_2 bridges; i.e., depe, dppe, and $R-(+)-1,2\text{-dppp}$ ($Ph_2PCH(CH_3)CH_2PPh_2$) (37). Specifically,

the three compounds $[\text{Mo}(\text{N}_2)(\text{dpepp})(\text{depe})]$ (**II**), $[\text{Mo}(\text{N}_2)(\text{dpepp})(\text{dppe})]$ (**III**), and $[\text{Mo}(\text{N}_2)(\text{dpepp})(1,2\text{-dppp})]$ (**IV**) were prepared and investigated by vibrational and ^{31}P -NMR spectroscopy. Complex **II** was found to exist in only one form. Compound **III** had already been synthesized by George *et al.* (38). In agreement with these authors, two isomers were identified, $[\text{Mo}(\text{N}_2)(\text{dpepp})(\text{dppe})]$ (**IIIa**) and *iso*- $[\text{Mo}(\text{N}_2)(\text{dpepp})(\text{dppe})]$ (**IIIb**). For compound **IV** evidence for the existence of two diastereomers (**IVa** and **IVb**) was obtained as well. In this case, however, the isomerism is not induced by the coordination geometry of the complex but by an optically active ligand: the methyl group of the 1,2-dppp ligand either points into the direction of the N_2 ligand (**IVa**) or the opposite direction (**IVb**). These results were found to be compatible with the corresponding vibrational (infrared and Raman) data, showing an unsplit N–N stretch for **II**, strongly split N–N stretching vibrations for compound **III** and weakly split N–N vibrations for compound **IV**.

The frequencies of the N–N stretching vibrations (1952 cm^{-1} for **II**, 1953 cm^{-1} for **IIIa**, 2007 cm^{-1} for **IIIb**, and 1957 cm^{-1} for **IVa/b**) reflect the different degrees of activation imparted to the dinitrogen ligand by the phosphine coligands. Compounds **II**, **IVa**, and **IVb** have dinitrogen ligands in a *trans*-position to the *central* phosphorus atom of the dpepp ligand. All of these complexes exhibit the N–N stretch at $1950\text{--}1960\text{ cm}^{-1}$. The same applies to compound **IIIa**. Compound **IIIb**, in contrast, has a *terminal* phosphine group of the dpepp ligand coordinated in a *trans*-position to the N_2 ligand. Correspondingly the N_2 stretch is observed at higher frequency than in **II**, **IVa**, **IVb**, and **IIIa**; i.e., at $2010\text{--}2020\text{ cm}^{-1}$. This demonstrates the *lower* activation of N_2 in a complex with a *trans*-diphenylalkyl compared to a complex with a *trans*-dialkylphenylphosphine group. As expected, a much smaller difference in $\nu(\text{NN})$ is observed between the two isomers **IVa** and **IVb** than in the case in which the methyl group of the equatorial 1,2-dppp ligand points into different directions (Scheme 8).

The ^{31}P -NMR spectra of all compounds could be analyzed and perfectly reproduced by simulation, giving a complete set of chemical shifts and coupling constants. A consistent set of coupling constants and corresponding signs was derived for compounds **II–IV** and the related complex **I** which has been studied previously. The ^{31}P -NMR *trans*-coupling constants were found to be in the range of $\sim 100\text{ Hz}$. For *cis*-coupling, two cases have to be distinguished. If the interaction exclusively occurs via the metal, the coupling constant is $\sim 20\text{ Hz}$ and negative. If the interaction occurs simultaneously over the metal center and an

ethylene bridge, the coupling constant is small (sometimes in the order of the line-width), indicating that the corresponding interactions relate to coupling constants with almost identical absolute values, but opposite signs. This is in agreement with literature data indicating that the ^2J coupling constant via a metal has a negative sign (41) and the ^3J coupling constant via an ethylene bridge a positive sign (42). It is also known that for second- and third-row metals $^2J(P,P)$ *trans*-coupling constants are large and positive (43).² This in turn has been used to determine the absolute signs of coupling constants for compounds **III** and **IV**.

The ^{31}P spectra also sensitively reflect the symmetry of the complexes. Quasi-tetragonal symmetry is exhibited by the dpepp-dppe complex **IIIa** which has one phosphine group in an axial position and four diphenylphosphine endgroups in equatorial positions. Here, the signals deriving from the equatorial phosphines (a,b,c,d) appear almost unsplit. A similar geometry is present in the dpepp-depe complex **II**. In this case, however, the equatorial diethylphosphine and diphenylphosphine groups P_a/P_b and P_c/P_d , respectively, appear at different chemical shifts (48.80 and 62.12 ppm, respectively). Diethylphosphine is a stronger σ -donor than diphenylphosphine and the latter is a stronger π -acceptor than diethylphosphine; P_a and P_b are therefore more shielded than P_c and P_d . In complex **IV**, finally, the symmetry of complexes **II** and **IIIa** is broken by an additional methyl group attached to the ethylene bridge of the equatorial diphos ligand. This leads to different environments for the phosphorus donors of the 1,2-dppp ligand, P_a and P_b . Moreover, the methyl group can point "upwards" (i.e., in the direction of the N_2 ligand; isomer **IVa**) or "downwards" (i.e., in the opposite direction; isomer **IVb**). In the downward position the methyl group comes close to the phenyl ring of the axial phosphine, whereas in the upward position no such interaction exists (there is only the N_2 ligand). We thus assume that the equatorial coordination of isomer **IVb** is more distorted than in the case of **IVa** and, correspondingly, a larger chemical shift difference between P_a and P_b results in the former as compared to the latter isomer.

²This does not apply to first-row metals where $^2J(P,P)$ for *trans*-coupling may be negative. Likewise, $^2J(P,P)_{cis}$ and $^2J(P,P)_{trans}$ values for metal complexes in the first transition-metal series can be of similar magnitude whereas in the second and third series the *trans*-coupling is usually much larger.

For complex **III** an *iso*-form (**IIIb**) has been identified in which the central phosphorus atom of the dpepp ligand is in an equatorial position; i.e., *cis* to the dinitrogen group. From the relative intensities in the ^{31}P -NMR spectrum, **IIIa** and **IIIb** occur in about equal amounts. Surprisingly, however, no corresponding *iso*-forms were in evidence for compounds **II** and **IV**. For the depe complex **II** this may have *electronic* reasons; i.e., a configuration where the diethylphosphine groups are in a *trans*-position to diphenylphosphine groups may be particularly favorable as strongly electron-donating groups are arranged in a *trans*-position to strongly electron-accepting groups. For the 1,2-dppp complex **IV**, on the other hand, *steric* reasons may account for the lack of the *iso*-form. Specifically, for **IVb** (with the methyl group pointing downwards) an *iso*-form may be unfavorable as in a *trans*-position of the dinitrogen ligand there would be two phenyl groups instead of one coming in close contact with the methyl group. For **IVa**, on the other hand, this argument would not apply, and no principal reasons are discernible which would prevent the formation of an *iso*-form in this geometry.

To conclude, the vibrational and ^{31}P -NMR spectroscopic properties of three complexes with five coordinating phosphine groups have been determined and correlations between their geometries and their spectroscopic properties have been derived. In the first study, it had been shown that the activation of N_2 in these complexes is higher than in corresponding bis(diphosphine) complexes with two dinitrogen ligands, but lower than in *trans*-acetonitrile dinitrogen complexes with two diphosphine coligands (39). The latter systems, on the other hand, have the disadvantage that the nitrile coligands are labile. As dinitrogen complexes with a pentaphosphine ligation have also been shown to be protonatable at the N_2 ligand, these systems appear to represent a good compromise between sufficient activation of the dinitrogen ligand on the one hand and thermodynamic/kinetic stability of the ligand environment on the other. This agrees with our theoretical finding that a catalytic cycle of ammonia synthesis on the basis of a Mo pentaphosphine complex should be feasible (*vide supra* and Ref. (44)). Practically this concept is limited by the fact that the phosphine ligand in the *trans*-position to N_2 becomes labile at higher oxidation states of the metal (e.g., in the nitrido complex) and sooner or later will be replaced by another Lewis-basic ligand or a solvent molecule. Therefore, the necessity persists to attach covalently the *trans* ligand to the complex in a way that it cannot dissociate at any stage of the catalytic cycle.

C. Mo COMPLEXES WITH MIXED P/N LIGANDS

In an effort to prepare Mo/W dinitrogen complexes with tetraphosphine ligands additionally occupying the *trans*-position to N_2 we also synthesized a series of mixed phosphine/amine ligands, which we wanted to coordinate to low-valent Mo centers in order to bind, activate, and reduce dinitrogen. In the present case, we additionally wanted to combine the advantage of phosphine ligands – stability vs. acids – with the presence of Brønsted basic groups that would have the potential of assisting the protonation of the N_2 ligand. The resulting target structure is shown in Fig. 8. An important side-effect of the presence of basic groups in the ligand framework would also be the fact that a “secondary” (= ancillary ligand) protonation should shift the reduction potentials of these systems toward less negative values and thus make the NNH_x and NH_x ($x = 1-3$) intermediates of the Chatt cycle easier to reduce (45).

In the framework of this concept a series of new mixed P/N ligands was prepared, employing the phosphorus-analogous Mannich reaction (Scheme 9). This transformation permits the substitution of primary or secondary amines by methylenephosphine residues $-CH_2PRR'$ ($R, R' = \text{alkyl, aryl}$) through reaction with a secondary phosphine and formaldehyde (46). Based on the

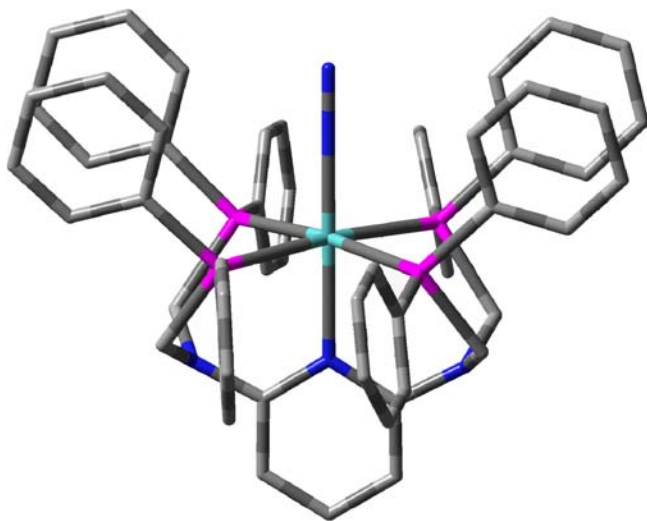
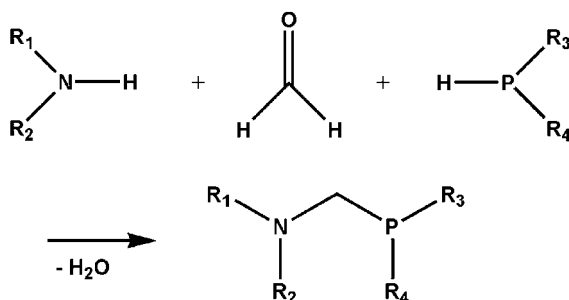
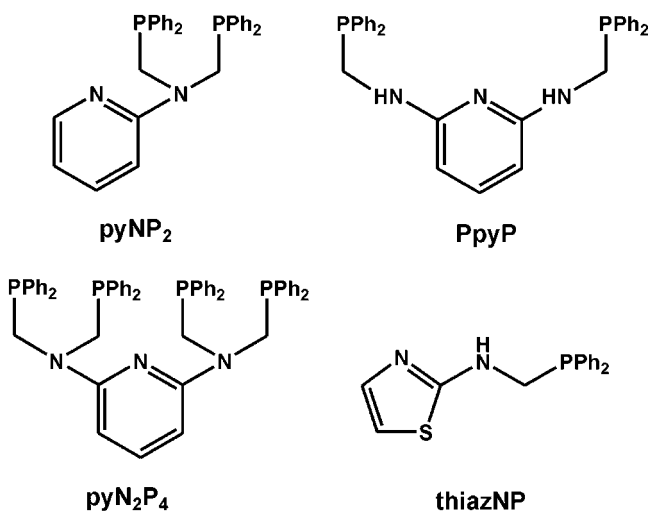


FIG. 8. Mo/W dinitrogen complex with the P/N ligand N,N,N',N' -tetrakis-(diphenylphosphinomethyl)-2,6-diaminopyridine (pyN_2P_4) as a target structure for a catalyst in the Chatt cycle.



SCHEME 9.

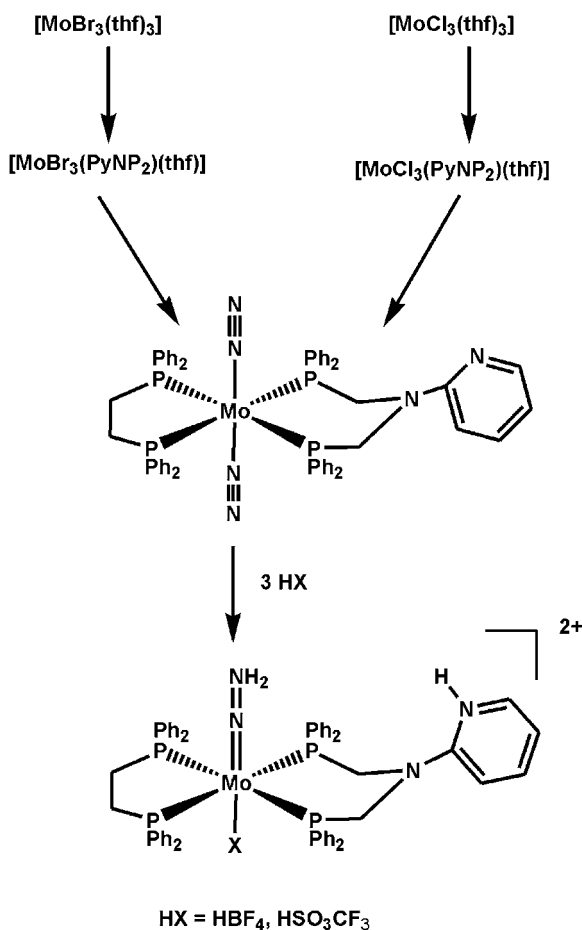


SCHEME 10.

heterocyclic amines, 2-aminopyridine, 2,6-diaminopyridine, and 2-aminothiazole, the new mixed P/N ligands N,N,N',N'-tetrakis-(diphenylphosphinomethyl)-2,6-diaminopyridine (pyN₂P₄); N, N-bis(diphenylphosphinomethyl)-2-aminopyridine (pyNP₂); N,N'-bis(diphenylphosphinomethyl)-2,6-diamino-pyridine (PpyP); and N-diphenylphosphinomethyl-2-aminothiazole (thiazNP) were obtained (Scheme 10) (45). In contrast to aliphatic and aromatic amines, however, aminopyridines react sluggishly in the P-analogous Mannich reaction. Moreover, while aniline and its

derivatives give bidentate PNP-ligands in high yields (47), aminopyridines prefer a monosubstitution of the amine, leading to $\text{pyNHCH}_2\text{PR}_2$ derivatives (Scheme 10) (48). This may be due to the deactivating effect of the pyridine ring, considerably reducing the nucleophilicity of the amino group. For this reason the reaction under aprotic conditions was found to lead to the monosubstituted amines while the disubstituted amines could be isolated under protic/acidic conditions.

The pyNP_2 ligand was used for the synthesis of a molybdenum(0) dinitrogen complex (Scheme 11) (45). First the ligand was coordinated to a Mo(III) halide precursor to form $[\text{MoX}_3(\text{pyNP}_2)(\text{thf})]$ ($\text{X} = \text{Cl}^-$, Br^-). Reduction of the Mo(III) complex



SCHEME 11.

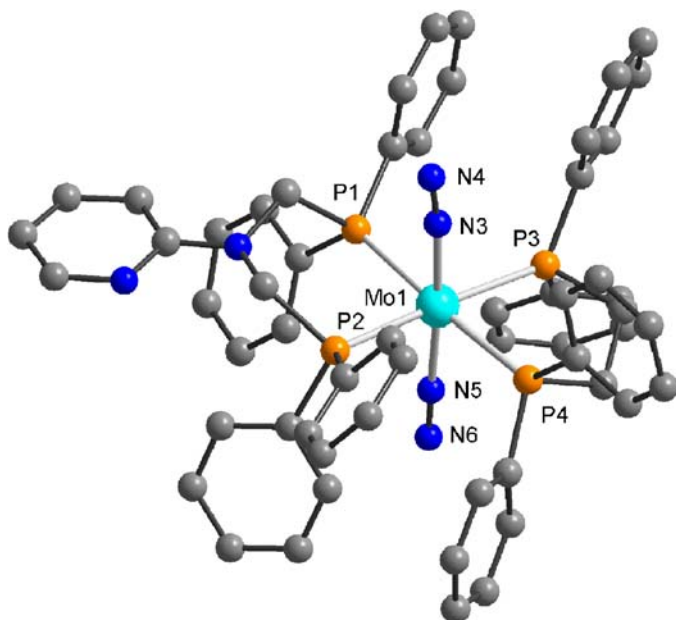


FIG. 9. X-ray structure of $[\text{Mo}(\text{N}_2)_2(\text{dppe})(\text{pyNP}_2)]$ in which the *fac*-coordinating pyNP_2 ligand is bonded only in a bidentate fashion to the molybdenum center (45).

under N_2 with an additional diphosphine such as 1,2-bis(diphenylphosphino)ethane (dppe) leads to the $\text{Mo}(0)$ bis(dinitrogen) complex $[\text{Mo}(\text{N}_2)_2(\text{dppe})(\text{pyNP}_2)]$ (Fig. 9). The formation of a mono- N_2 complex with pyNP_2 acting as a tridentate ligand was not observed. Obviously, the *fac*-coordinating pyNP_2 cannot bind completely to the $\text{Mo}(\text{III})$ precursor which has *mer*-geometry (49). After the subsequent reduction step, the free pyridine group then competes with a stronger backbonding ligand like N_2 for metal coordination. Formation of a mono- N_2 complex with pyNP_2 acting as a tridentate ligand thus probably requires a complete coordination of this ligand already at the level of the $\text{Mo}(\text{III})$ -precursor. Protonation of the $\text{Mo}(0)$ pyNP_2 dinitrogen complex with HBF_4 and triflic acid leads to secondary protonated hydrazido complexes: $[\text{MoF}(\text{NNH}_2)(\text{dppe})(\text{pyHNP}_2)](\text{BF}_4)_2$ and $[\text{Mo}(\text{OTf})(\text{NNH}_2)(\text{dppe})(\text{pyHNP}_2)](\text{OTf})_2$, respectively (Scheme 11). The crystal structure of the former complex was solved (Fig. 10) (50). Each Mo atom is surrounded by four P-atoms of one dppe and one pyNP_2 ligand. The remaining coordination sites of the distorted octahedron are occupied by a hydrazido(2-) and a fluoride ligand. Importantly, the complex is also protonated at

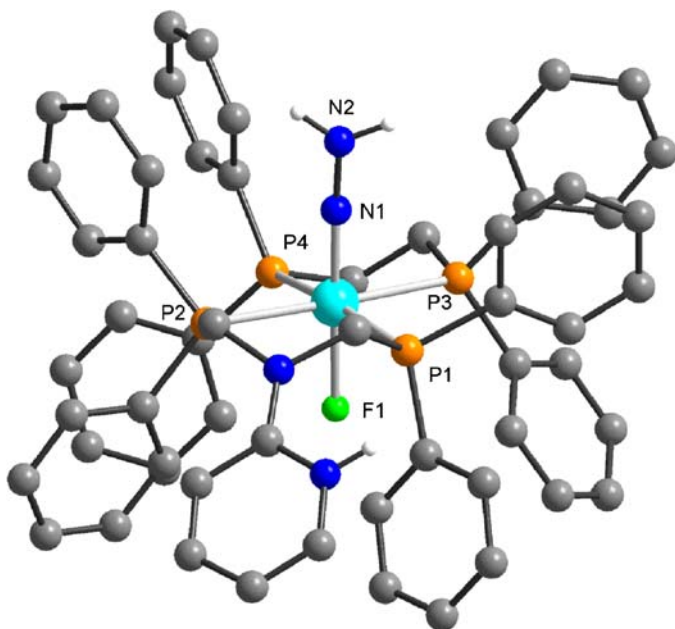


FIG. 10. X-ray structure of $[\text{MoF}(\text{NNH}_2)(\text{dppe})(\text{pyHNP}_2)](\text{BF}_4)_2$ containing the protonated pyNP_2 ligand (50).

the pyridine nitrogen, besides the double protonation of the N_2 ligand.

Although a dinitrogen complex with a protonatable ligand has thus been obtained, the final goal of these studies remains the synthesis of a dinitrogen complex with the full pyN_2P_4 ligand (Fig. 8). So far all known methods for the synthesis of $\text{Mo}(0)/\text{W}(0)$ dinitrogen complexes have failed in this regard. Therefore, we have investigated the reactivities of low-valent metal compounds with respect to exchange reactions with phosphines. Moreover, we want to evaluate further the concept of protonatable ligands by careful stepwise protonation and deprotonation of the pyNP_2 dinitrogen and corresponding hydrazido complexes, respectively.

V. Conclusions

In the preceding sections, experimental and theoretical studies on the mechanism and various modifications of the Chatt cycle have been presented. Our investigations have started with the detailed spectroscopic and theoretical characterization of the intermediates of the classic Chatt cycle. These studies are not yet

finished; i.e., we are currently investigating the reduction and protonation of Mo(II) and W(II) amido complexes to give NH_3 and the corresponding dinitrogen complexes. The mechanistic insight obtained through these investigations allows the design of new ligands, which should improve the performance of the Chatt cycle with respect to a catalytic reaction mode. In this respect, we have considered tetraphosphine ligands, pentaphosphine ligand systems as well as polydentate mixed P/N ligands. With P_5 ligand systems a catalytic reaction mode employing Cp_2^*Cr and HLut^+ (in analogy to the Schrock cycle) is predicted by DFT. The coordination of more elaborate polydentate phosphorus or mixed P/N ligands to Mo and W centers in order to prepare the corresponding dinitrogen complexes is far from trivial and always requires the development of new synthetic routes. In the case of a tetraphos ligand this has been achieved, and the first Mo(0) dinitrogen complex (as a matter of fact, the first low-valent mononuclear Mo complex) with a tetraphos ligand was prepared and characterized spectroscopically. This strategy is now extended to the synthesis of Mo/W complexes with other polydentate P or mixed P/N ligands. Another possibility for an improved reaction mode of the Chatt cycle is to fix the Mo and W complexes to surfaces, an approach which has not been covered by this review. Studies toward achieving this goal are also underway (51).

ACKNOWLEDGMENTS

Felix Tuczek thanks all students and postdocs who have been involved in this research for their valuable contributions and Deutsche Forschungsgemeinschaft (DFG) for continuous financial support of this research.

REFERENCES

1. Fryzuk, M. D.; MacKay, B. A. *Chem. Rev.* **2004**, *104*, 385.
2. (a) Yandulov, D. V.; Schrock, R. R. *Science* **2003**, *76*, 301. (b) Yandulov, D. V.; Schrock, R. R. *J. Am. Chem. Soc.* **2002**, *124*, 6252. (c) Yandulov, D. V.; Schrock, R. R.; Rheingold, A. L.; Ceccarelli, C.; Davis, W. A. *Inorg. Chem.* **2003**, *42*, 796. (d) Yandulov, D. V.; Schrock, R. R. *Inorg. Chem.* **2005**, *44*, 1103. (e) Weare, W. W.; Dai, C.; Byrnes, M. J.; Chin, J.; Schrock, R. R. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17099. (f) Weare, W. W.; Schrock, R. R.; Hock, A. S.; Müller, P. *Inorg. Chem.* **2006**, *45*, 9185. (g) Schrock, R. R. *Acc. Chem. Res.* **2005**, *38*, 955. (h) Ritleng, V.; Yandulov, D. V.; Weare, W. W.; Schrock, R. R.; Hock, A. S.; Davis, W. M. *J. Am. Chem. Soc.* **2004**, *126*, 6150.

3. Pickett, C. J.; Talarmin, J. *Nature* **1985**, 317, 652.
4. (a) Pickett, C. J. *J. Biol. Chem.* **1996**, 1, 601. (b) Henderson, R. A.; Leigh, G. J.; Pickett, C. J. *Adv. Inorg. Chem. Radiochem.* **1983**, 27, 177.
5. (a) Hidai, M. *Coord. Chem. Rev.* **1999**, 185–186, 99. (b) Kozak, C. M.; Mountford, P. *Angew. Chem.* **2004**, 116, 1206. (c) Kozak, C. M.; Mountford, P. *Angew. Chem. Int. Ed.* **2004**, 43, 1186. (d) Chatt, J.; Dilworth, J. R.; Richards, R. L. *Chem. Rev.* **1978**, 78, 589. (e) Hidai, M.; Mizobe, Y. *Chem. Rev.* **1995**, 95, 1115. (f) Leigh, G. J. *Acc. Chem. Res.* **1992**, 25, 177. (g) Henderson, R. A.; Leigh, G. J.; Pickett, C. J. *Adv. Inorg. Chem. Radiochem.* **1983**, 27, 197.
6. Burgess, B. K.; Lowe, D. J. *Chem. Rev.* **1996**, 96, 2983.
7. Alberty, R. A.; Goldberg, R. N. *Biochemistry* **1992**, 31, 10610.
8. (a) Burgess, B. K. *Chem. Rev.* **1990**, 90, 1377. (b) Kim, J.; Rees, D. C. *Nature* **1992**, 360, 553.
9. Thorneley, R. N. F.; Lowe, D. J. In: “*Molybdenum Enzymes*”; Ed. Spiro, T. G.; Wiley: New York, **1985**.
10. (a) Barney, B. M.; Lee, H. I.; Dos Santos, P. C.; Hoffman, B. M.; Dean, D. R.; Seefeldt, L. C. *Dalton Trans.* **2006**, 2277. (b) Lee, H. I.; Sørle, M.; Christiansen, J.; Yang, T. C.; Shao, J.; Dean, D. R.; Hales, B. J.; Hoffman, B. M. *J. Am. Chem. Soc.* **2005**, 127, 15880. (c) Barney, B. M.; Yang, T. C.; Igarashi, R. Y.; Dos Santos, P. C.; Laryukhin, M.; Lee, H. I.; Hoffman, B. M.; Dean, D. R.; Seefeldt, L. C. *J. Am. Chem. Soc.* **2005**, 127, 14960. (d) Lee, H. I.; Igarashi, R. Y.; Laryukhin, M.; Doan, P. E.; Dos Santos, P. C.; Dean, D. R.; Seefeldt, L. C.; Hoffman, B. M. *J. Am. Chem. Soc.* **2004**, 126, 9563. (e) Igarashi, R. Y.; Laryukhin, M.; Dos Santos, P. C.; Lee, H. I.; Dean, D. R.; Seefeldt, L. C.; Hoffman, B. M. *J. Am. Chem. Soc.* **2004**, 127, 6231.
11. (a) Studt, F.; Tucek, F. *J. Comp. Chem.* **2006**, 27, 1278. (b) Kästner, J.; Blöchl, P. E. *J. Am. Chem. Soc.* **2007**, 129, 2998. (c) Dance, I. *J. Am. Chem. Soc.* **2007**, 129, 1076.
12. (a) Studt, F.; Tucek, F. *Angew. Chem.* **2005**, 117, 5783. (b) Studt, F.; Tucek, F. *Angew. Chem. Int. Ed.* **2005**, 44, 5639. (c) Neese, F. *Angew. Chem.* **2005**, 118, 202. (d) Neese, F. *Angew. Chem. Int. Ed.* **2005**, 45, 196.
13. Schrock, R. R. *Angew. Chem.* **2008**, 120, 2.
14. (a) Reiher, M.; Le Guennic, B.; Kirchner, B. *Inorg. Chem.* **2005**, 44, 9640. (b) Schenk, S.; Le Guennic, B.; Kirchner, B.; Reiher, M. *Inorg. Chem.* **2008**, 47, 3634.
15. (a) Lehnert, N.; Tucek, F. *Inorg. Chem.* **1999**, 38, 1659. (b) Lehnert, N.; Tucek, F. *Inorg. Chem.* **1999**, 38, 1671.
16. Horn, K. H.; Lehnert, N.; Tucek, F. *Inorg. Chem.* **2003**, 42, 1076.
17. Tucek, F.; Horn, K. H.; Lehnert, N. *Coord. Chem. Rev.* **2003**, 245, 107.
18. Habeck, C. M.; Lehnert, N.; Näther, C.; Tucek, F. *Inorg. Chim. Acta* **2002**, 337, 11.
19. Ishino, H.; Tokunaga, S.; Seino, H.; Ishii, Y.; Hidai, M. *Inorg. Chem.* **1999**, 38, 2489.
20. Henderson, R. A.; Leigh, G. J.; Pickett, C. J. *J. Chem. Soc. Dalton Trans.* **1989**, 425.
21. Pickett, C. J.; Leigh, G. J. *J. Chem. Soc. Dalton Trans.* **1981**, 1033.
22. Horn, K. H.; Böres, N.; Lehnert, N.; Mersmann, K.; Näther, C.; Peters, G.; Tucek, F. *Inorg. Chem.* **2005**, 44, 3016.
23. Mersmann, K.; Horn, K. H.; Böres, N.; Lehnert, N.; Studt, F.; Paulat, F.; Peters, G.; Ivanovic-Burmazovic, I.; van Eldik, R.; Tucek, F. *Inorg. Chem.* **2005**, 44, 3031.

24. Dreher, A.; Mersmann, K.; Näther, C.; Ivanovic-Burmazovic, I.; van Eldik, R.; Tucek, F. *Inorg. Chem.* **2009**, *48*, 2078–2093.
25. Alias, Y.; Ibrahim, S. K.; Queiros, M. A.; Fonseca, A.; Talarmin, J.; Volant, F.; Pickett, C. J. *J. Chem. Soc. Dalton Trans.* **1997**, 4807.
26. Mersmann, K.; Hauser, A.; Lehnert, N.; Tucek, F. *Inorg. Chem.* **2006**, *45*, 5044.
27. Sivasankar, C.; Tucek, F. *Dalton Trans.* **2006**, *28*, 3396.
28. Dreher, A.; Barboza da Silva, C.; Mersmann, K.; Peters, G.; Tucek, F. *manuscript in preparation*.
29. Stephan, G. C.; Sivasankar, C.; Studt, F.; Tucek, F. *Chem. Eur. J.* **2008**, *14*, 644.
30. Elson, C. M. *Inorg. Chim. Acta* **1976**, *18*, 209.
31. Stephan, G. C. *PhD thesis*, University of Kiel **2007**.
32. Habeck, C. M.; Hoberg, C.; Peters, G.; Näther, C.; Tucek, F. *Organometallics* **2004**, *23*, 3252.
33. Römer, R.; Stephan, G.; Peters, G.; Tucek, F. *Eur. J. Inorg. Chem.* **2008**, *21*, 3258.
34. Carmona, E.; Galindo, A.; Sanchez, L. *Polyhedron* **1984**, *3*, 347.
35. Carmona, E.; Galindo, A.; Guille-Photin, C.; Sanchez, L. *Polyhedron* **1988**, *7*, 1767.
36. Mata, J. A.; Maria, S.; Daran, J.-C.; Poli, R. *Eur. J. Inorg. Chem.* **2006**, *2006*, p. 2624.
37. Klatt, K.; Stephan, G.; Peters, G.; Tucek, F. *Inorg. Chem.* **2008**, *47*, 6541.
38. (a) George, T. A.; Tisdale, R. C. *Inorg. Chem.* **1988**, *27*, 2909. (b) George, T. A.; Tisdale, R. C. *J. Am. Chem. Soc.* **1985**, *107*, 5157. (c) George, T. A.; Ma, L.; Shailh, S. N.; Tisdale, R. C.; Zubietta, J. *Inorg. Chem.* **1990**, *29*.
39. Stephan, G. C.; Peters, G.; Lehnert, N.; Habeck, C. M.; Näther, C.; Tucek, F. *Can. J. Chem.* **2005**, *83*, 385.
40. Studt, F.; MacKay, B. A.; Fryzuk, M. D.; Tucek, F. *J. Am. Chem. Soc.* **2004**, *126*, 280.
41. Pregosin, P. S.; Kunz, R. W. “*NMR Basic Principles and Progress. 31P and 13C NMR of Transition Metal Phosphine Complexes*”; Springer-Verlag: Berlin, **1979**.
42. Friebolin, H. “*Ein- und zweidimensionale NMR-Spektroskopie*”; Wiley VCH: Weinheim, **2006**.
43. (a) Bertrand, R. D.; Ogilie, F. B.; Verkade, J. G. *J. Am. Chem. Soc.* **1970**, *92*, 1916. (b) Ogilie, F. B.; Jenkins, J. M.; Verkade, J. G. *J. Am. Chem. Soc.* **1970**, *92*, 1908. (c) Crumbliss, A. L.; Topping, R. J. “*Phosphorus-31 NMR: Spectral Properties in Compound Characterization and Structural Analysis*”; vol. 15; Wiley VCH: Weinheim, **1987**. (d) Hughes, A. N. “*Phosphorus-31 NMR: Spectral Properties in Compound Characterization and Structural Analysis*”; vol. 19; Wiley VCH: Weinheim, **1987**. (e) Clark, H. C.; Kapoor, P. N.; McMahon, I. J. *J. Organomet. Chem.* **1984**, *190*, C101. (f) Airey, A. A.; Swiegers, G. F.; Willis, A. C.; Wild, S. B. *Inorg. Chem.* **1997**, *36*, 1588.
44. Stephan, G.; Tucek, F. In: “*Activating Unreactive Substrates*”; Eds. Bolm, C.; Hahn, E.; Wiley VCH: Weinheim, **2009**.
45. Stephan, G.; Näther, C.; Sivasankar, C.; Tucek, F. *Inorg. Chim. Acta* **2008**, *361*, 1008.
46. Keller, K.; Tzschach, A. *Z. Chem.* **1984**, *10*, 365.
47. Märkl, G.; Jin, G. Y.; Schoerner, C. *Tetrahedron Lett.* **1980**, *21*, 1845.

48. (a) Durran, S. E.; Smith, M. B.; Slawin, A. M. Z.; Steed, J. W. *J. Chem. Soc. Dalton Trans.* **2000**, 2771. (b) Coles, S. J.; Durran, S. E.; Hursthouse, M. B.; Slawin, A. M. Z.; Smith, M. B. *New J. Chem.* **2001**, 25, 416.
49. (a) Owens, B. E.; Poli, R.; Rheingold, A. L. *Inorg. Chem.* **1989**, 28, 1456. (b) Poli, R.; Gordon, J. C. *Inorg. Chem.* **1991**, 30, 4550.
50. Stephan, G. C.; Näther, C.; Sivasankar, C.; Tucek, F. *Acta Cryst. E.* **2008**, 64, M629-U241.
51. Hallmann, L.; Bashir, A.; Strunskus, R.; Adelung, R.; Staemmler, V.; Wöll, H.; Tucek, F. *Langmuir* **2008**, 24, 5726.

This page intentionally left blank

CHEMISTRY OF METALATED CONTAINER MOLECULES

BERTHOLD KERSTING and ULRIKE LEHMANN

Institut für Anorganische Chemie, Universität Leipzig, Johannisallee 29,
D-04103 Leipzig, Germany

I.	Introduction	407
II.	Metalated Container Molecules: Host Systems and Complex Types	409
	A. Calixarenes	409
	B. Cyclodextrins	412
	C. Sterically Demanding Tripodal Ligands	414
	D. Molecular Squares	418
	E. Molecules of the Type "Complex in a Complex"	421
	F. Other Supporting Ligands	427
III.	Metalated Container Molecules of Binucleating Polyaza-Dithiophenolate Macrocycles	430
	A. Macrocyclic Thiophenolate Schiff-Base Ligands	430
	B. Polyamine-Dithiophenolate Macrocycles	431
	C. Coordination Chemistry of Polyamine-Dithiophenolate Ligands	435
	D. Effects of N-alkylation on the Molecular and Electronic Structures of the Complexes	442
	E. The Ligand Matrix as a Medium	445
	F. Variation, Coordination Modes, and Activation of Coligands	447
	G. Reactivity of the Complexes	452
IV.	Conclusion	457
	Acknowledgments	458
	References	458

I. Introduction

Since the seminal work of Cram on cyclophanes and resorcinarenes (*1*) the host–guest chemistry of container molecules has been extensively investigated and more sophisticated examples with other forms and larger cavities have been reported. Thus a large number of host–guest complexes containing neutral,

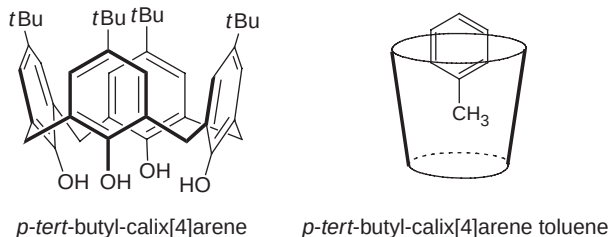


FIG. 1. *p*-*Tert*-butyl-calix[4]arene and schematic representation of the structure of its 1:1 complex with toluene.

cationic, and anionic species have been prepared and characterized (2–5). The 1:1 complex between *p*-*tert*-butyl-calix[4]arene and toluene is an early example (Fig. 1) (6,7).

Container molecules are of great interest because their encapsulated guests often exhibit novel and unusual properties, which are not observed in the free or solvated state (8,9). They are used today as probes of isolated molecules and of the intrinsic characteristics of the liquid state, and are capable of enantioselective recognition (10), reversible polymerization (11), isolation of reactive species (12–14), and promoting reactions within their interiors (15–18). For a valuable introduction to this area the reader is directed to some excellent review articles (15,19–21).

Several types of closed-shell host molecules with fascinating inner-phase binding and reactivity properties have been described in the literature. These include covalently constructed molecular containers such as the carcerands (22), which encapsulate their guests irreversibly, and the calixarenes or resorcinarenes (23,24), which have shell holes large enough to permit the exchange of guest molecules (25). During the last two decades, many examples of noncovalent molecular capsule complexes formed by spontaneous aggregation via H-bonds (16,26,27), hydrophobic interactions (28), or metal–ligand coordinative bonds have also been described (29–34).

In recent years, the development of metalated container molecules has also become an attractive research goal (35–38). This is mainly due to the fact that such compounds allow for an interplay of molecular recognition and transition-metal catalysis (39–41). Consequently, several research groups are involved in the development of new receptor molecules that create confined environments about active metal coordination sites. The aim of this review is to highlight recent advances in this area.

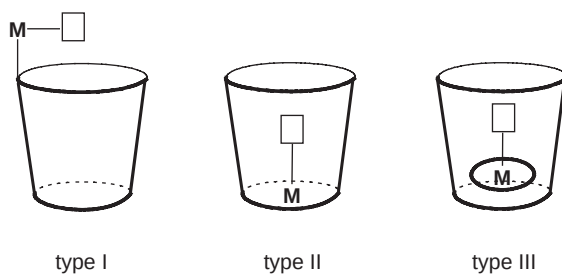


FIG. 2. Schematic representation of the most common architectures of metalated container molecules. The open boxes represent active coordination sites at the metal ions. The bowl-shaped representation of the host molecules should not be confused with the one used for the cyclodextrins.

II. Metalated Container Molecules: Host Systems and Complex Types

Metalated container molecules can be viewed as a class of compounds that have one or more active metal coordination sites anchored within or next to a molecular cavity (Fig. 2). A range of host systems is capable of forming such structures. The majority of these compounds represent macrocyclic molecules and sterically demanding tripod ligands, as for instance calixarenes (42), cyclodextrins (43,44), and trispyrazolylborates (45–48), respectively. In the following, selected types of metalated container molecules and their properties are briefly discussed and where appropriate the foundation papers from relevant earlier work are included. Porphyrin-based hosts and coordination cages with encapsulated metal complexes have been reviewed previously (49–53) and, therefore, only the most recent examples will be described. Thereafter, our work in this field is reported.

A. CALIXARENES

Calixarenes, when in their cone-conformation (54), represent versatile host systems for metalated container molecules and many examples have been reported in the literature (55–61). Reinaud and coworkers have carried out extensive work concerned with calix[6]arenes that are functionalized at the small rim by nitrogen arms (62), aiming to reproduce the hydrophobic binding site of mononuclear zinc and copper metalloenzymes. A recent example is the calix[6]arene ligand L^1 (Fig. 3), in which a tris(2-methylpyridyl)amine unit covalently caps the calixarene small rim (63). The ligand forms copper complexes of

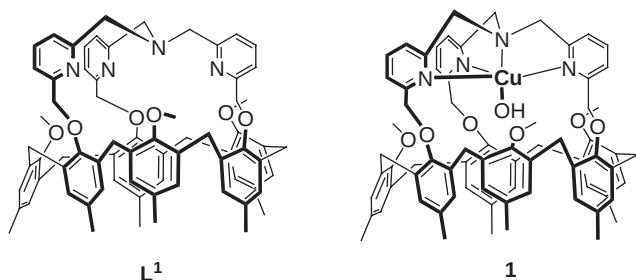


FIG. 3. The structures of L^1 (63) and $[Cu(L^1)(OH)]^+$ (1) (64).

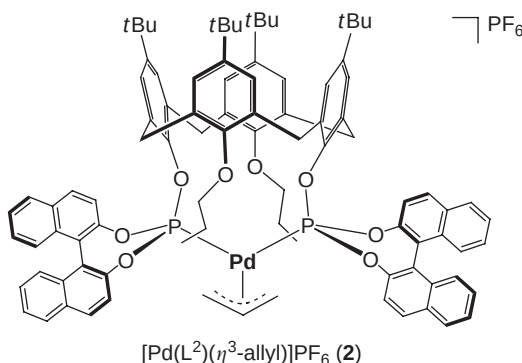


FIG. 4. The structure of $[Pd(L^2)(\eta^3\text{-allyl})]PF_6$ (2) (67).

the type $[Cu(L^1)(L')^{2+}]$ with an open binding site (hereafter referred to as L') that is directed towards the center of the macrocyclic cavity. The complex binds a range of neutral and anionic guest ligands, several of which are of biological importance. Thus a copper(II) complex, $[Cu(L^1)(OH)]^+$ (1), with a terminally bound hydroxo-ligand ($L' = OH$) has been isolated (64). The formation of this unique complex (hydroxide acts normally as a bridge) can be traced to the protection offered by the calix[6]arene cavity.

The smaller *p*-*tert*-butyl-calix[4]arenes have a rich coordination chemistry as well (65). Of these, however, only the upper-rim modified calixarenes seem to support metal complexes with confined binding sites (66), except in those cases where the lower-rim substituents form an appended cavity. Thus, Matt and coworkers have reported (67) a pocket-shaped calix[4]arene ligand L^2 bearing two lower-rim [(*S*)-1,1'-binaphthalene-2,2'-diyl]phosphite moieties (Fig. 4). This ligand reacts readily with $[Pd(\eta^3\text{-C}_3\text{H}_5)\text{Cl}]_2/\text{NH}_4\text{PF}_6$ to produce the complex $[Pd(L^2)(\eta^3\text{-allyl})]PF_6$ (2). In contrast to the calix[6]arenes described above,

the Pd-bound coligand is not situated in the calixarene cavity, but is embraced within a cleft formed by the naphthyl units. The calix[4]arene cavity remains unoccupied in this case.

So far, only few calixarene hosts have been reported which contain catalytically active transition-metal fragments inside their cavities (68). Reek and coworkers have reported a new approach to such systems. Their strategy makes use of template-ligands that have a bifunctional character in that they coordinate to the active metal center and function as a template for the capsule formation (69–74). A representative compound is the 1:1 complex **3** between a tetracationic Pd complex of a concave xantphos-type diphosphine ligand L^3 and a tetraanionic sulfonatocalix[4]arene (Fig. 5). A Job plot analysis of a titration experiment in CD_3OD proved unambiguously the 1:1 stoichiometry of **3** ($K_a(CD_3OD) = 6 \times 10^3 M^{-1}$), and the 1D-NOESY spectrum clearly establishes that the aryl-substituents of the diphosphine ligand L^3 and the upper rim of the sulfonatocalix[4]arene are facing one another to form a capsular structure (75). Most importantly, the metal center is not involved in the assembly process and is therefore available for catalytic processes.

Recently, Atwood and coworkers reported a series of metal-organic analogs of multimeric nanocapsules based on resorcin[4]arenes (76) and pyrogallol[4]arene units (77). These containers contain several metal atoms in the hydrogen-bonded seam connecting the capsule units and it was demonstrated that metal

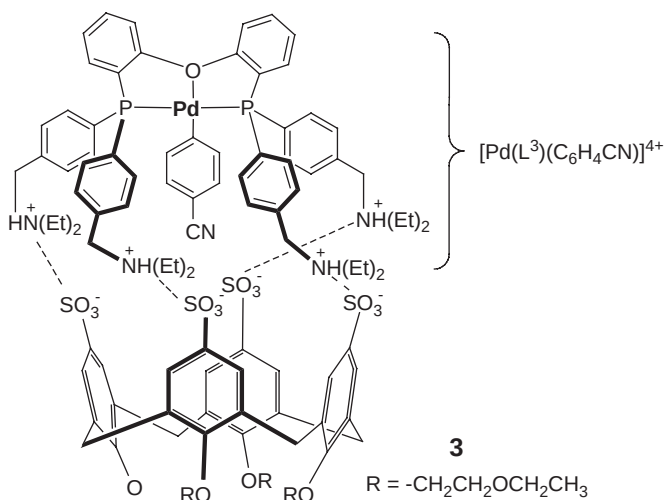


FIG. 5. The structure of the 1:1 complex between **3** and tetra-sulfonatocalix[4]arene (75).

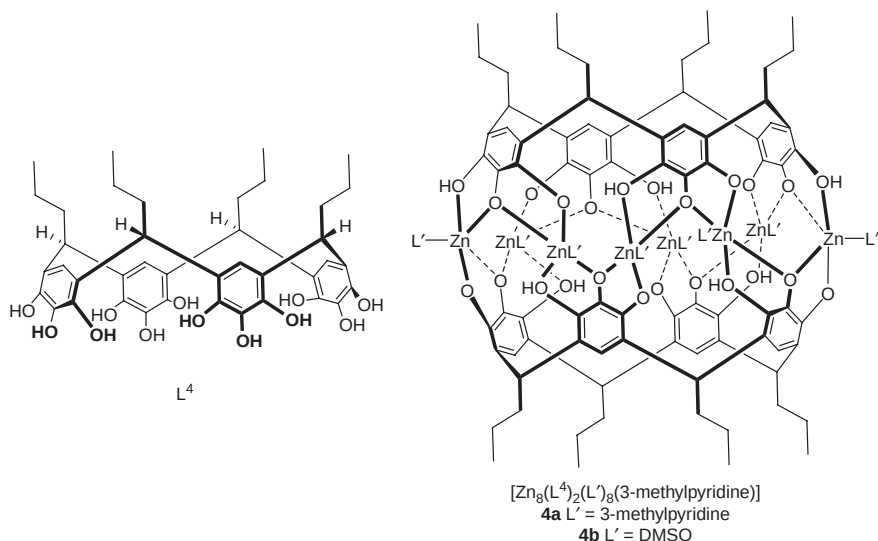


FIG. 6. The structures of pyrogallol[4]arene L^4 and capsule complexes **4a,b** (77). The encapsulated 3-methylpyridine in **4a,b** is omitted for clarity.

ligand exchange reactions proceed without disintegration of the nanocapsules. Thus, replacement of the 3-methylpyridines (L') by DMSO in the metalated capsule $[Zn_8(L^4)(L')_8(3\text{-methylpyridine})]$ (**4a**, $L' = 3\text{-methylpyridine}$) could be accomplished with ease either by dialysis in a solution of DMSO in methanol or by repeated azeotropic vacuum distillation with DMSO to form the complex $[Zn_8(L^4)(L')_8(3\text{-methylpyridine})]$ (**4b**, $L' = \text{DMSO}$) (Fig. 6). The two bowl-shaped pyrogallol entities in **4b** are arranged in a staggered capsule structure and are equatorially seamed by eight zinc(II) centers, each of which are coordinated by two phenoxy groups from the pyrogallol monomers and one O-bound DMSO ligand. For more detailed summaries on such nanoreactors, especially for the period before 2005, the reader is referred to an excellent review by Nolte and coworkers (9).

B. CYCLODEXTRINS

Cyclodextrins (CDs) are a class of chiral cyclic oligosaccharides that have molecule-sized cavities. They commonly comprise between six and eight D-glucopyranoside units that are linked via α -1,4-glycosidic links. Their bowl-shaped form is generally represented as a cylindrical funnel by analogy to the calixarenes family. There is a large number of cyclodextrin derivatives in the

literature. Derivatization improves the hosts' solubility, increases their affinity for a particular guest target, and allows the attachment of specific catalytic groups which is of importance for many applications (78–80). For the use of cyclodextrins in biomimetic chemistry, for example, the reader is directed to an excellent review article by Breslow (81).

A relatively new field in cyclodextrin chemistry focuses on the design and synthesis of receptors in which a catalytically active 4d metal ion is rigidly fixed at the CD entrance. Reetz *et al.*, for example, described a rhodium catalyst based on a β -CD-derivative which displays shape selectivity in hydrogenation and hydroformylation reactions thus opening the way to supramolecular catalysis of industrial relevance (39). Metallocavitands based on phosphanyl-substituted cyclodextrins also constitute valuable objects for studying the influence of a second coordination sphere on the properties of a metal center and for achieving catalytic reactions in a confined environment. A fascinating set of such receptors was reported by Matt and coworkers (44,82). The complexation of the α -CD-based ligand L^5 with $AgBF_4$ yields $[Ag(L^5)(NCMe)]BF_4$ (**5**), the crystal structure of which confirms the coordinated MeCN to be situated inside the cavity (Fig. 7). The complex binds another MeCN molecule to give a bis-acetonitrile complex $[Ag(L^5)(NCMe)_2]BF_4$. With benzonitrile, on the other hand, only 1:1 adducts are supported. Clearly, the CD cavity does not allow the coordination of more than one benzonitrile molecule, but can easily accommodate two smaller nitrile ligands such as MeCN.

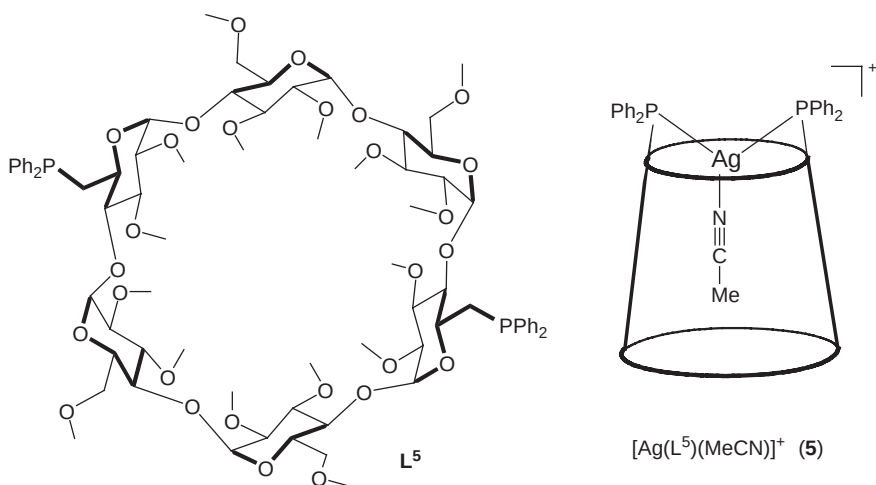


FIG. 7. The structures of L^5 and $[Ag(L^5)(CH_3CN)]^+$ (**5**).

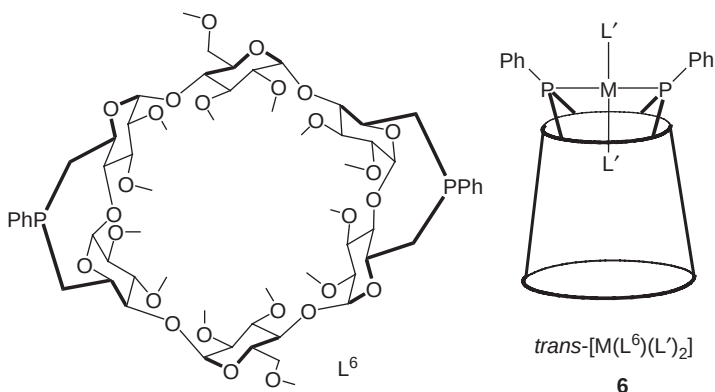


FIG. 8. The structures of L^6 and $[M(L^6)(L')_2]^+$ (**6**, $M = Ni, Pd, Pt$; $L' = Cl, Br$) (83).

Matt and coworkers have also reported the synthesis of the α -CD-based ligand L^6 . This ligand is more rigid than L^5 , since the two "PPh" units are now anchored to two sugar units of the α -CD (Fig. 8). L^6 forms square-planar chelate complexes $trans-[M(L^6)(L')_2]$ (**6**) with $M = Ni, Pd$, and Pt and $L' = Cl$ or Br , respectively, such that one $M-L'$ bond points towards the center of the cavitand. The favorable $P \cdots P$ separation and the directional control of the lone pairs on the phosphorus atoms prevent the system from forming binuclear complexes or higher oligomers. L^6 can thus be regarded as an authentic *trans*-spanning diphosphane ligand. Interestingly, in the complex $[Ni(L^6)(Br)_2]$, the cavity provides effective protection of the encapsulated $Ni-Br$ bond towards nucleophilic attack by $MeLi$, while the same complex, upon activation with methylaluminoxane, efficiently dimerizes ethene and propene (83) and is also capable of encapsulating fully medium-sized inorganic anions (84). The latter study also nicely illustrates the fact that a number of secondary $CH \cdots X$ ($X = F, Cl, Br$) interactions can contribute to the stabilization of encapsulated anions inside a cyclodextrin cavity.

Recently, a ternary complex of cytochrome *c* with a polyanionic β -cyclodextrin has been reported (85). A self-assembled supramolecular bidentate N,P ligand for aqueous organometallic catalysis was also described (86). Although related, such systems are out of the scope of this review and will not be discussed further here.

C. STERICALLY DEMANDING TRIPODAL LIGANDS

The activation and transformation of small molecules by metal complexes with sterically demanding tripod ligands, whose

substituents serve to form a concave surface around the active coordination site, currently receive widespread attention (46–48, 87–93). Yandulov and Schrock's recent work has provided impetus for such studies by establishing the synthetic viability of a $\text{Mo}^{\text{III/VI}}$ N_2 fixation cycle (94). This section will highlight some recent achievements in the field in which small molecules are bound and activated by such metallocavitands.

Since both iron and molybdenum are present in the FeMo cofactor of MoFe nitrogenases (95,96), an understanding of the reactivity of the enzymes, isolation, and stabilization of the hypothetical intermediates in the N_2 -fixation mechanism are highly desirable. Recently, Meyer and co-workers reported a discrete iron(IV) nitride complex, $[\text{Fe}(\text{L}^7)(\text{N})][\text{BPh}_4]$ (**8**), stabilized by the sterically encumbering N-anchored tris(carbine) ligand, tris[2-(3-aryl-imidazolyl-2-ylidene)ethyl]amine (L^7 , $\text{R} = \text{mesityl}$) (Fig. 9). The complex was prepared by photolysis of the Fe(II) complex $[\text{Fe}^{\text{II}}(\text{L}^7)(\text{N}_3)][\text{BPh}_4]$ (**7**) in THF (97). The Fe^{IV} ion in **8**, a hypothetical intermediate of an Fe^{IV} N_2 fixation mechanism (98), is coordinated in a trigonal-pyramidal fashion, with a $\text{Fe} \equiv \text{N}$ bond distance of 1.57(2) Å consistent with a triply bonded nitride ligand.

Holland and Peters earlier reported examples of three- and four-coordinate N_2 complexes with iron in the formal +1 oxidation state, but in neither case were terminally bonded N_2 species identified or isolated; dinuclear end-on $\text{Fe}-\text{NN}-\text{Fe}$ species were inevitably obtained (99,100). Therefore, Peters and co-workers recently began to turn their attention to new derivatives of the classic Sacconi-type tripodal ligands to achieve access to a terminally bound $\text{Fe}^{\text{I}}-\text{N}_2$ complex. Indeed, by employing the mono-anionic ligand $\text{L}^8 = [(2-\text{Ph}_2\text{PC}_6\text{H}_4)_3\text{Si}]^-$ (Fig. 10) they were able to generate the desired complex $[\text{Fe}(\text{L}^8)(\text{N}_2)]$ (**10**) (101).

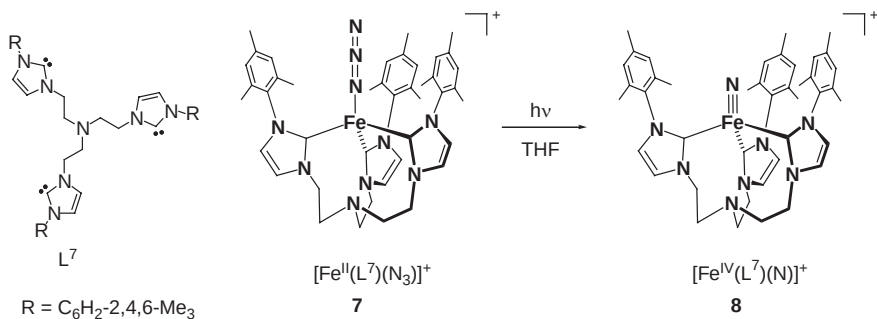


FIG. 9. The structures of L^7 , $[\text{Fe}^{\text{II}}(\text{L}^7)(\text{N}_3)]^+$ (**7**) and $[\text{Fe}^{\text{IV}}(\text{L}^7)(\text{N})]^+$ (**8**) (97).

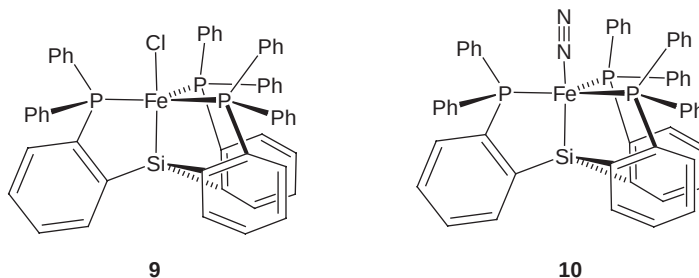


FIG. 10. The structures of $[\text{Fe}^{\text{II}}(\text{L}^8)(\text{Cl})]$ (**9**) and $[\text{Fe}^{\text{I}}(\text{L}^8)(\text{N}_2)]$ (**10**) (101).

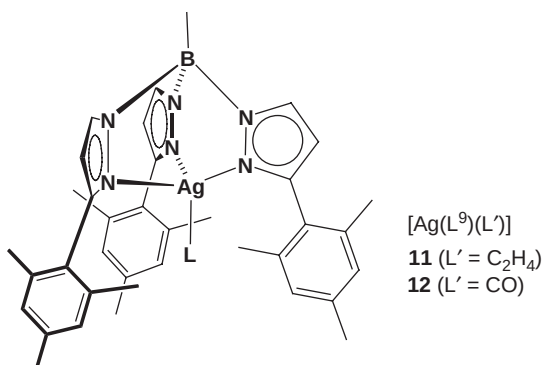


FIG. 11. The structures of $[\text{Ag}^{\text{I}}(\text{L}^9)(\text{C}_2\text{H}_4)]$ (**11**) and $[\text{Ag}^{\text{I}}(\text{L}^9)(\text{CO})]$ (**12**) (102).

Initially, the precursor $[\text{Fe}(\text{L}^8)(\text{Cl})]$ (**9**) was prepared. The $\text{Fe}^{\text{I}}\text{--N}_2$ complex was then generated upon reduction of **9** with Na/Hg in THF. The N_2 -complex **10** has an $\text{Fe}\text{--N}$ bond length of 1.819(2) Å and a short $\text{N}\text{--N}$ bond of 1.106(3) Å, consistent with the weak N_2 activation implied by the relatively high-energy $\text{N}\text{--N}$ stretching vibrations observed by IR spectroscopy. The N_2 ligand is almost completely buried in the pocket provided by L^8 , thus precluding formation of a dinuclear N_2 -bridged species. This clearly demonstrates that otherwise unstable molecules can be efficiently trapped and isolated within molecular cavities.

Using a similar strategy, Dias and coworkers were able to prepare classical $\text{Ag}^{\text{I}}\text{--CO}$ and $\text{Ag}^{\text{I}}\text{--C}_2\text{H}_4$ complexes supported by the mesityl-substituted tris(pyrazolyl)borate ligand L^9 (Fig. 11) (102). The isolation of such complexes is a challenging task because silver(I) is a poor σ -acceptor, a poor π -donor, and in

general forms extremely labile adducts with π -acidic ligands. The light sensitivity of silver adducts, the oxidizing nature of silver, and the easy displacement of the coordinated CO and C₂H₄ ligands by many other weakly donating species (which includes common solvents) present further synthetic challenges. It is thus surprising that the complex [Ag(L⁹)(C₂H₄)] (**11**), which is obtained through a metathesis reaction between the thallium salt [Tl(L⁹)] and CF₃SO₃Ag in CH₂Cl₂ under an ethylene atmosphere, exhibits remarkable stability. The formation of the ethylene complex can be monitored by NMR spectroscopy. The bound guest experiences a high-field shift owing to the anisotropy of the benzene ring current. For example, the coordinated ethylene gives a ¹H NMR signal at 3.42 ppm which is significantly upfield when compared with free ethylene (5.40 ppm).

The carbonyl complex [Ag(L⁹)(CO)] (**12**), also of quite remarkable stability, is obtained by reaction of [Ag(L⁹)(C₂H₄)] (**11**) with CO in hexane. Nevertheless, the CO can be easily removed by increasing the temperature of the solution or by purging with an inert gas. Hence, such a reversible guest encapsulation within a molecular container might find applications for gas separation and storage. Again, one likely reason for the stability of the complexes is the protection offered by the bulky mesityl substituents that surround the ethylene or CO unit.

Synthetic bioinorganic copper dioxygen complexes have attracted great interest in the past decades because of their prominent role as oxidants in biochemistry and catalysis (103–105). Thus, end-on copper dioxygen adduct complexes have been proposed as reactive intermediates in the catalytic cycle of mononuclear copper enzymes, such as peptidylglycine α -hydroxylating monooxygenase (PHM) or dopamine β -monooxygenase (D β H) (104,105); however, the isolation of 1:1 Cu/O₂ complexes is complicated by the intrinsic tendency of these species to dimerize in solution or to decompose after irreversible ligand oxidation. Very recently, Schindler, Holthausen, and Sundermeyer reported a stable Cu^I complex of the sterically congested superbasic tren derivative L¹⁰ (*tren* = tris(2-amino-ethyl)amine, Fig. 12), which, in striking contrast to former experiments with sterically less demanding *tren* derivatives, reversibly forms a stable Cu–O₂ adduct [Cu(L¹⁰)(O₂)]SbF₆ (**13**) (106). The crystal structure of this complex reveals an end-on O₂ coordination mode, the O–O bond distance being 1.280(3) Å.

The geometric requirements imposed by the ligand substituents can not only be utilized to force a molecule to adopt a specific binding mode. Moreover, within the capsule, the guest is isolated from the surrounding, which significantly affects its chemical

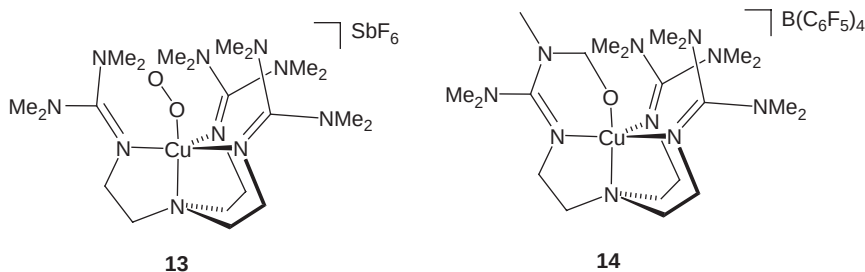


FIG. 12. The structures of $[\text{Cu}^{\text{II}}(\text{L}^{10})(\text{O}_2)]\text{SbF}_6$ (**13**) (106) and $[\text{Cu}^{\text{II}}(\text{L}^{10})]\text{B}(\text{C}_6\text{F}_5)_4$ (**14**) (107).

reactivity. Thus, complex **13** further reacts with 4-MeO-2,6-*t*Bu₂-phenol to form the alkoxide complex $[\text{Cu}(\text{L}^{10})]^+$ **14** (Fig. 12), where the alkoxide O atom is derived from dioxygen (107). The oxidative reactivity of this complex is thus similar to the Cu_M-centered action at the PHM active site, in which oxygenation of a prohormone peptide substrate C–H group adjacent to an amide nitrogen occurs. The results clearly demonstrate that end-on coordination of O₂ to copper ions can lead to quite stable intermediates, which lends support to their assumed role as important intermediates in catalytic oxidation reactions.

D. MOLECULAR SQUARES

In the field of porous supramolecular metal complexes, both molecular and extended-solid materials have been extensively studied in recent years. A particularly well-studied class of compounds is the metal-containing “molecular squares,” that is, square-shaped porous tetrameric structures (30,108). These have been prepared by several approaches, the most common being the reaction of an organic bridging ligand with a metal complex that has available *cis*-coordination sites (109–113) (Fig. 13). However, the resulting metal centers are usually coordinatively saturated, which makes it difficult for guest molecules to interact directly with the metal atoms.

Recently, several groups reported molecular squares prepared by an alternative approach, in which bifunctional metalloligands serve as the linkers thereby placing the metal ions into the walls of the container molecules (114–116). Hupp and coworkers, for example, have designed square-shaped macrocycles based on salen-type components (117). Thus, the molecular square **15** was prepared by the directed assembly of *cis*-[(PEt₃)₂Pt(OTf)₂] and

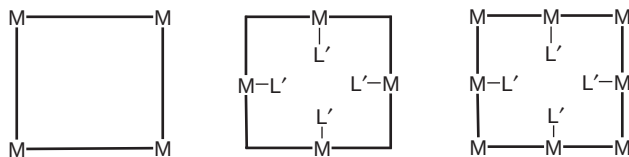


FIG. 13. Schematic illustration of metal-organic molecular squares, assembled from linear organic linkers and 90° metal units (left), linear metal units and organic “corners” (middle), or linear metal units and 90° metal units (113). The latter two classes have several inner-cavity binding sites and thus fit the definition of metalated container molecules.

bis(4-pyridyl)-functionalized Zn(II)-salen-type ligands in THF (117) (Fig. 14). The THF presumably coordinates axially to Zn(II), thereby preventing aggregation of the bridging salen moieties (coordination of distal pyridines of one ligand by the electrophilic Zn(II) centers of others). Attempts to grow crystals of **15** were not successful. Nevertheless, the incorporation of multiple reactive metal sites into a single, cavity-containing supramolecular structure points toward catalytic applications for these new assemblies. Indeed, the same group reported some squares incorporating Zn and Mn porphyrin ligands that are powerful epoxidation catalysts (113).

The unsubstituted 9-methylpurine ligand (L^{11}) has been utilized by Lippert and coworkers as a “ 90° ”-organic building block of a Pt_4 -square (115). Thus, *trans*- $[Pt(NH_3)_2(L^{11})(H_2O)](ClO_4)_2$ (**16**) spontaneously self-assembles to give $[Pt_4(NH_3)_8(L^{11})_4](ClO_4)_8$ (**17**), the single crystal X-ray structure of which confirms the presence of a Pt_4 square (Fig. 15). The purine ligands and the Pt^{II} atoms in **17** are essentially coplanar, and the ammine ligands are perpendicular to this plane. The distances between the Pt^{II} atoms on opposing sides of the square are $9.03(1)\text{ \AA}$ and $9.09(1)\text{ \AA}$. Interestingly, **17** shows a pronounced affinity for sulfate anions. For the binding of sulfate in D_2O , an association constant of $(9.9 \pm 0.6) \times 10^3 \text{ L mol}^{-1}$ was determined. This value indicates very strong sulfate binding in water and is in the range known for very strong anion receptors such as aza crown ethers and cryptands (118,119).

Maverick and coworkers have reported a number of square structures derived from β -diketonate ligands (116). The dark green complex $[Cu_4(L^{12})_4]$ is a representative example. This complex was obtained by reaction of the β -diketone HL^{12} with $[Cu(NH_3)_4]^{2+}$ in almost quantitative yield and has a diameter of about $14\text{ \AA}$. The $[Cu_4(L^{12})_4]$ square functions effectively as a host

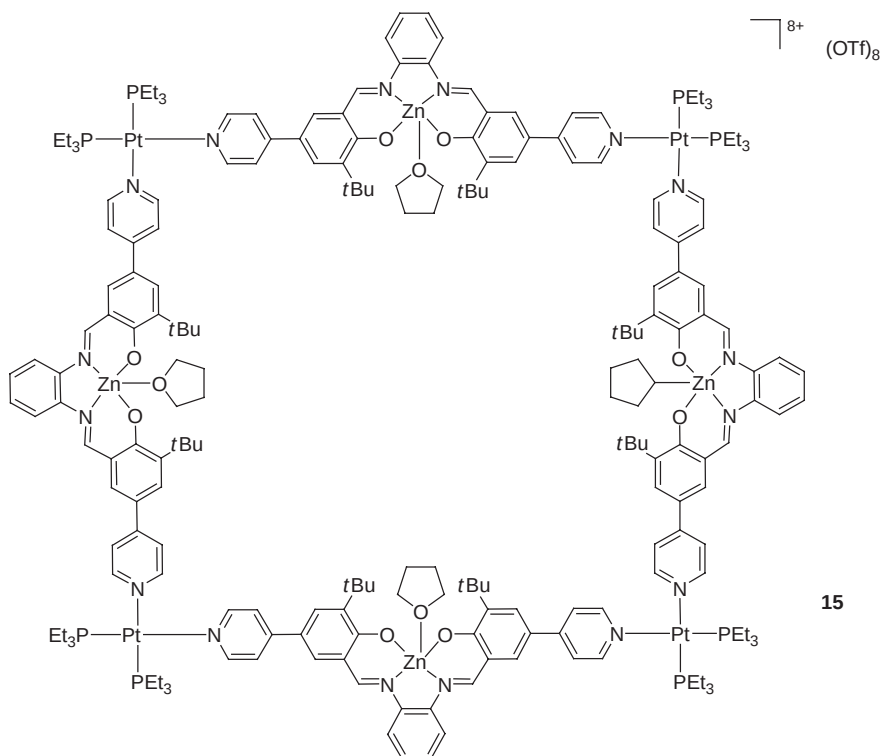


FIG. 14. The structure of the molecular square **15** (117).

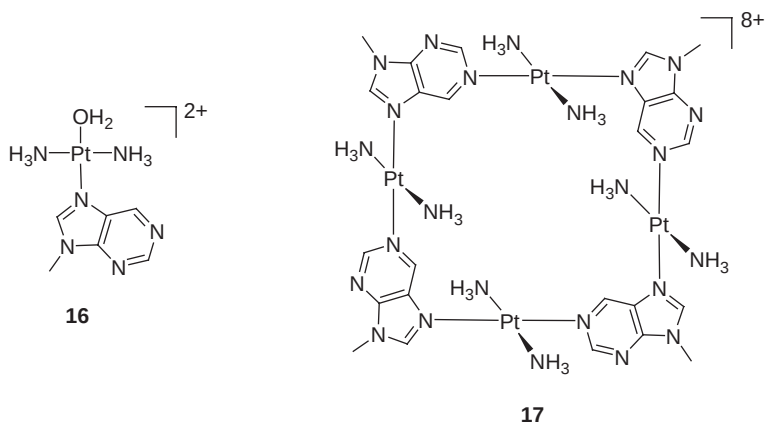


FIG. 15. The structures of the Pt complexes **16** and **17** (115).

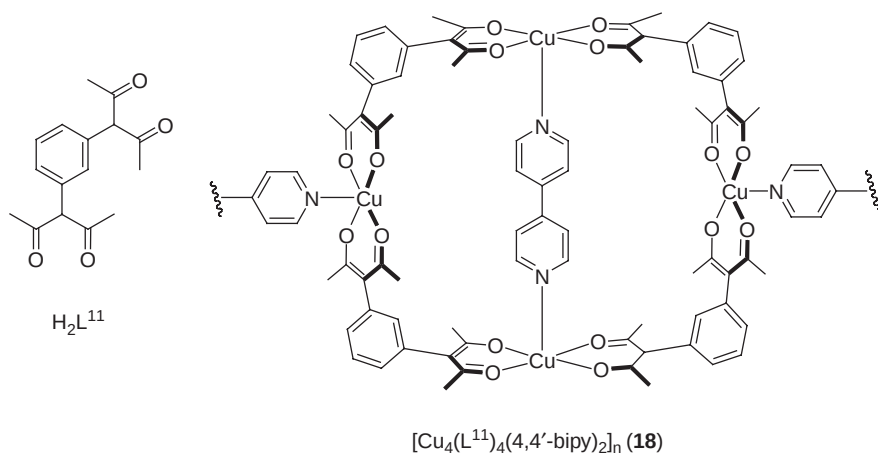


FIG. 16. The structures of H_2L^{11} and the Cu_4 complex **18** (116).

for various guest molecules. For example, $[Cu_4(L^{12})_4]$ reacts with 4,4'-*bipy* (*bipy* = bipyridine) to produce the polymeric complex $[Cu_4(L^{12})(4,4'-bipy)_2]_n$ (**18**). In this compound, the 4,4'-*bipy* molecules are intra- and intermolecularly bonded to the molecular square as shown in Fig. 16.

E. MOLECULES OF THE TYPE "COMPLEX IN A COMPLEX"

The encapsulation of classical and organometallic transition-metal complexes to yield molecules of the type "complex in a complex" is a very attractive research area. A variety of inclusion complexes of this type has been reported. Of relevance for this review are reports on the encapsulation of coordinatively unsaturated transition-metal complexes inside self-assembled coordination cages (120), cyclodextrins (121,122), and cucurbiturils (123).

The cucurbit[*n*]uril family (CB[*n*]) of molecular containers possess remarkable binding affinities and selectivities (K_a values up to $10^{12} M^{-1}$, K_{rel} values up to 10^6) which renders them useful as a component of molecular machines, sensors, and biomimetic systems (123–125). Recently, Wagner and coworkers have reported (126) that CB[10] – with its spacious $870\text{\AA}^3$ cavity – is capable of acting as a host for free base and metalated tetra (*N*-methylpyridinium)porphyrins **19a–d** (Fig. 17). Despite the large ellipsoidal deformation of CB[10] upon complexation, the complexed porphyrins retain their fundamental UV/VIS, fluorescence, and electrochemical properties. The CB[10] · porphyrin

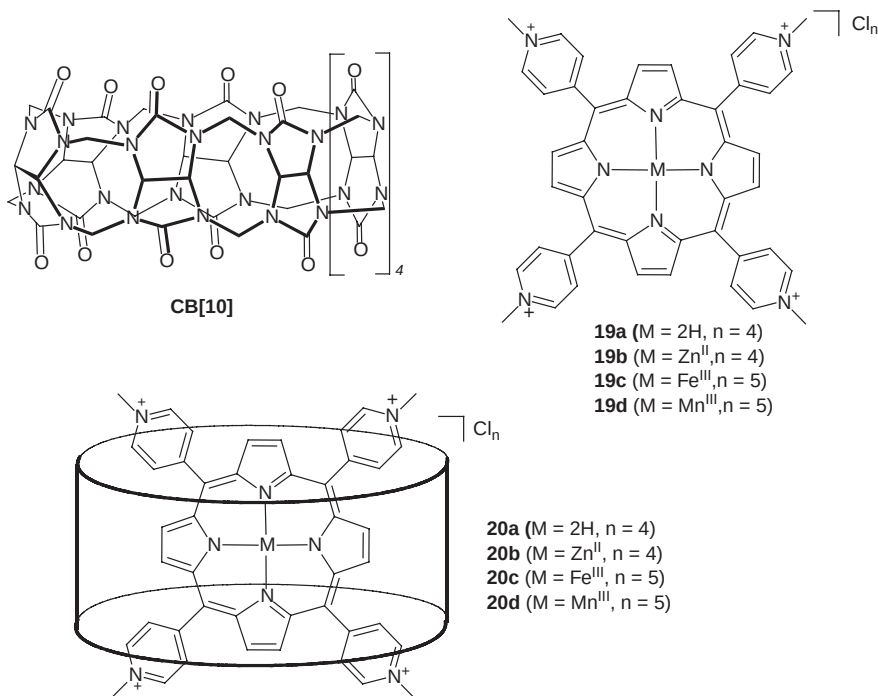


FIG. 17. The structures of CB[10] and its inclusion complexes **20a–d** with the corresponding free base and metalated tetra(N-methylpyridinium)porphyrins **19a–d** (126).

complexes **20a–d** thereby combine the useful recognition properties of the CB[n] family with the function of the porphyrins. The ability of CB[10] to protect and preserve the properties of encapsulated porphyrins paves the way for many applications in a wide variety of areas including enzyme-mimetic catalysts for oxidation chemistry, targeted phototherapeutic agents, as an insulating unit for conjugated molecular wires and in the construction of light-harvesting materials and photovoltaic devices.

Porphyrin units represent versatile building blocks for coordination cages and a number of such complexes have been reported (127–130). Katagiri and coworkers were able to isolate the cylindrical complex **23** via a multicomponent self-assembly process of the amidinate **21** and zinc 5,10,15,20-tetrakis (4-carboxyphenyl)porphyrin **22** (Fig. 18). In **23**, the individual components are held together by eight complementary amidinium-carboxylate salt bridges. Molecular-mechanics calculations suggest that **23** adopts a cylindrical structure in which each column is nearly perpendicular to the two porphyrin units, which

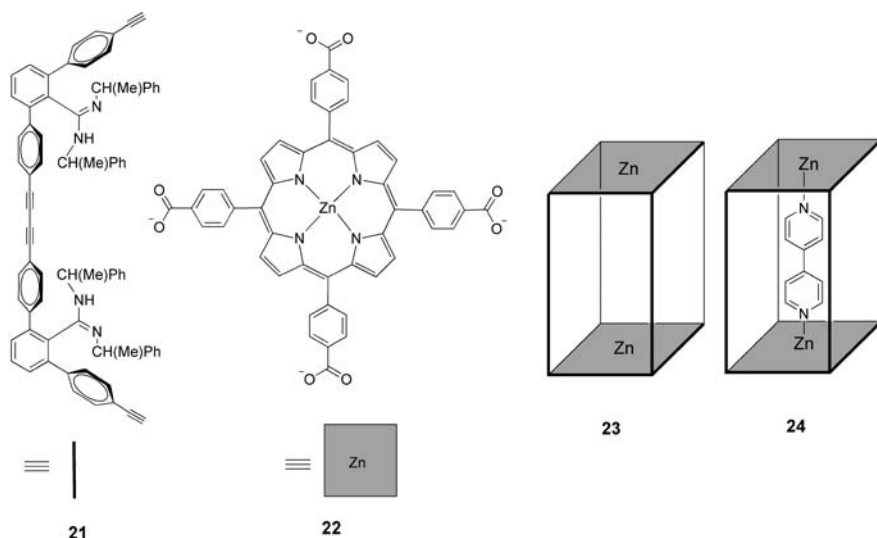


FIG. 18. The structures of the building blocks **21** and **22** and schematic representation of the structures of cage complexes **23** and **24** (131).

are separated by approximately 1.3 nm from each other. The complex provides two open coordination sites and binds nitrogen bases of appropriate size and shape. Upon addition of *bipy* into a solution of **23**, for example, a gradual red shift in the Soret band was observed indicative of the formation of the sandwich complex **24** (131). The encapsulation of transition-metal complexes within the cavity of **24** has not yet been reported.

Recently, Bergman and Raymond reported on the stabilization of highly reactive organometallic species inside self-assembled $[\text{M}_4\text{L}_6]$ coordination cages (120). The very reactive ion $[\text{CpRu}(\text{cis-1,3,7-octatriene})]\text{BF}_4$, for example, decomposes rapidly in aqueous solution (within minutes), but is stable for weeks in the same solvent when encapsulated in the tetrahedral $[\text{Ga}_4(\text{L}^{13})_6]^{12-}$ cage **25** (where L^{13} represents a bis(bidentate) catecholamide ligand, Fig. 19). Despite its stabilization, the $[\text{CpRu}(\text{cis-1,3,7-octatriene})]^+$ cation in **26** is still able to react stoichiometrically with CO.

The encapsulation of reactive organometallic complexes is not restricted to the anionic $[\text{Ga}_4(\text{L}^{13})_6]^{12-}$ cages. Thus, Fujita and coworkers were able to generate the coordinatively unsaturated complex $[\text{Cp}'\text{Mn}(\text{CO})_2]$ ($\text{Cp}' = \text{C}_5\text{H}_4\text{Me}$) within a self-assembled $[\text{M}_6\text{L}_6]$ coordination cage **28** (Fig. 20) (132). Photoirradiation of solid **27** gave complex **28**, the crystal structure of which confirms the presence of the unsaturated pyramidal $[\text{Cp}'\text{Mn}(\text{CO})_2]$ fragment. The direct observation of such intermediates is

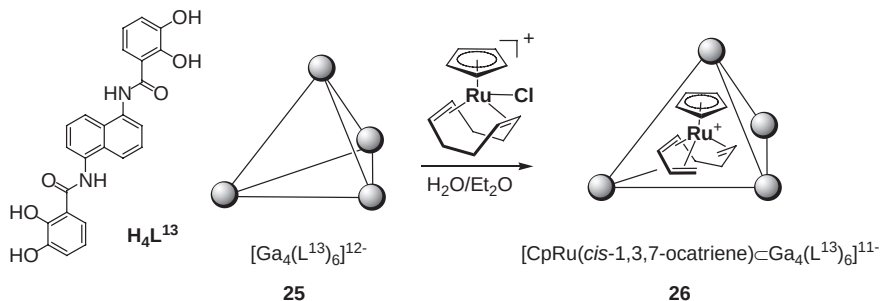


FIG. 19. Stabilization of $[\text{CpRu}(\text{cis-1,3,7-octatriene})]^+$ in a tetrahedral $[\text{Ga}_4(\text{L}^{13})_6]^{12-}$ coordination cage (120).

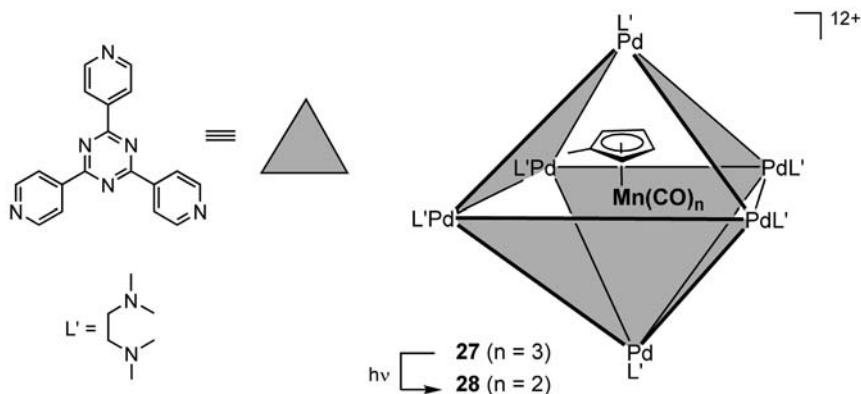


FIG. 20. Stabilization of $[\text{Cp}'\text{Mn}(\text{CO})_2]^+$ in an octahedral $[\text{Pd}_6(\text{L}^{14})_4]^{12+}$ coordination cage (132).

particularly important for understanding the mechanism of various catalytic transformations. These results clearly illustrate that supramolecular coordination cages may soon find use in organometallic chemistry.

Fujita and coworkers have also reported the encapsulation of multimetric porphyrin assemblies in the box-shaped cavities of ternary Pd_6 coordination cages. Two types of cofacial porphine dimers A and B could be stabilized (133). In the smaller $[\text{Pd}_6(\text{L}^{14})_2(\text{L}^{15})_6]^{12+}$ cage **29**, whose diameter is 10.4 Å (Fig. 21), two porphyrin molecules can be stacked directly on top of each other with an interplane distance of 3.4 Å. In the larger cage **30**, an additional molecule of L^{14} is intercalated between the two porphyrin bases. All complexes were found to be water-soluble in contrast to other π -stacked porphyrin dimers. The encapsulation

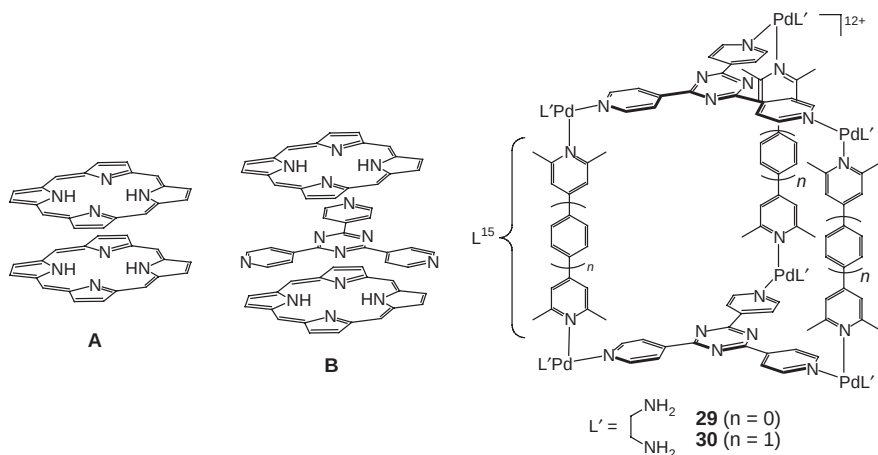


FIG. 21. The two types of cofacial porphyrin dimers **A** and **B** and the chemical structures of the coordination cages **29** and **30** (133).

of metalloporphyrins within these cages has not yet been reported.

Polynuclear clusters can also be stabilized inside coordination cages. By combining the molecular clip strategy developed by Stang (134,135) with the “molecular paneling” approach pioneered by Fujita (136), Therrien and coworkers earlier synthesized trigonal-prismatic coordination cages in which six (η^6 -arene) ruthenium or (η^5 -pentamethyl-cyclopentadienyl)-rhodium units are held together by two trigonal 2,4,6-tris(pyridine-4-yl)-1,3,5-triazine (L^{14}) panels and three dichloro or oxalate bridges (137,138). They have now extended this principle to construct the larger hexanuclear coordination cage $[\text{Ru}_6(p\text{-iPrC}_6\text{H}_4\text{Me})_6(\text{L}^{14})_2(\text{L}^{16})_3]^{6+}$ (**31**), in which the *p*-cymene ruthenium units are connected by three 2,5-dihydroxy-1,4-benzoquinonato (L^{16}) and two L^{14} subunits (Fig. 22). The synthesis of this complex can be carried out in the presence of $[\text{Pd}(\text{acac})_2]$ (or $[\text{Pt}(\text{acac})_2]$) to give cations of composition $[(\text{acac})_2\text{Pd}]\{\text{Ru}_6(p\text{-iPrC}_6\text{H}_4\text{Me})_6(\text{L}^{14})_2(\text{L}^{16})_3\}^{6+}$ (**32**) (139).

Koshevoy *et al.* have recently reported the stabilization of an unprecedented luminescent Au_6Cu_6 cluster in the cavity of a trinuclear gold diphosphane complex (140). Thus, reaction of $[\text{Au}_2(\text{C}_2\text{Ph})_2(\mu\text{-}4,4'\text{-Ph}_2\text{P}(\text{C}_6\text{H}_4)_2\text{PPh}_2)_2]$ with $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ and PhC_2H yields, via reshuffling of the ligands, $[\text{Au}_3(\text{PPh}_2(\text{C}_6\text{H}_4)_2\text{PPh}_2)_3\{\text{Au}_6\text{Cu}_6(\text{C}_2\text{Ph})_{12}\}](\text{PF}_6)_3$ (**33**) (Fig. 23). The molecule contains a central $[\text{Au}_6\text{Cu}_6(\text{C}_2\text{Ph})_{12}]$ fragment which is wrapped around by a $[\text{Au}(\text{Ph}_2\text{P}(\text{C}_6\text{H}_4)_2\text{PPh}_2)]_3$ belt. Six Cu^{I} ions are disposed above and below the plane through the six Au^{I} ions,

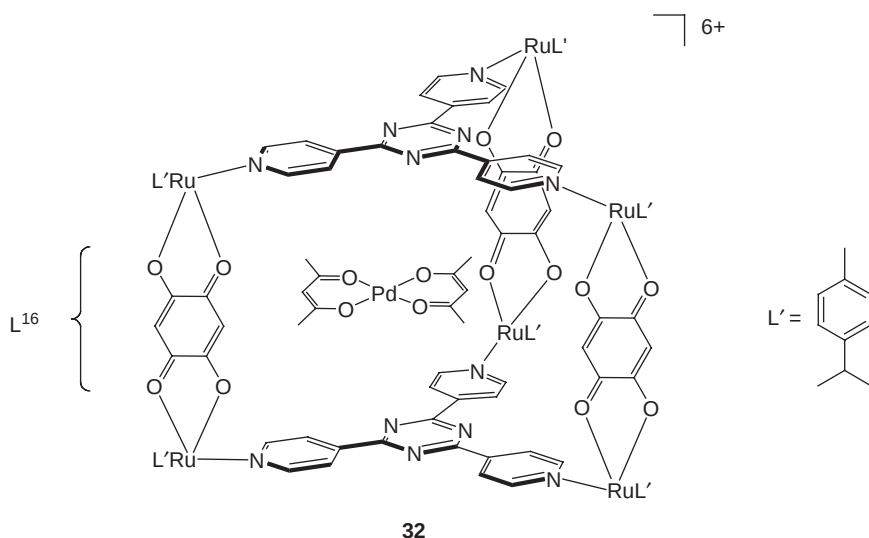


FIG. 22. The structure of $[(\text{acac})_2\text{Pd}]\mathbf{31}]^{6+}$ (**32**) (139).

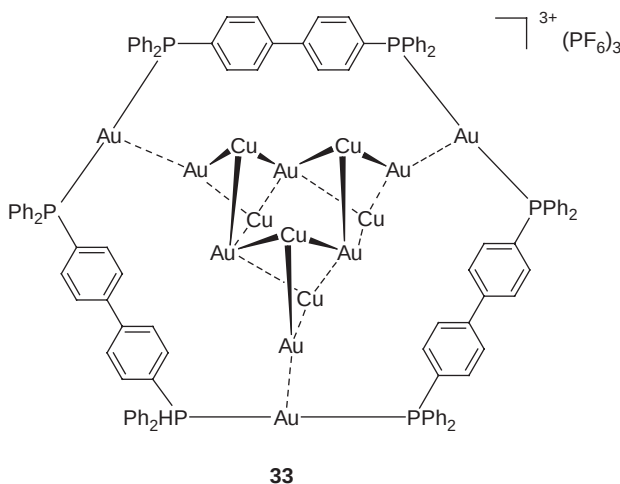


FIG. 23. Schematic structure of $[\text{Au}_3(\text{PPh}_2(\text{C}_6\text{H}_4)_2\text{PPh}_2)_3\{\text{Au}_6\text{Cu}_6(\text{C}_2\text{Ph})_{12}\}]^{3+}$ (**33**, C_2Ph units omitted for clarity) (140).

so that each is also coordinated to three alkynyl units (not shown in Fig. 23). The encapsulation of the neutral $\text{Au}_6\text{Cu}_6(\text{C}_2\text{Ph})_{12}$ cluster (which can be prepared as an individual complex) increases the stability of the Au_6Cu_6 core significantly, particularly under irradiation. This allows for a better understanding of the unique photophysical properties of such cluster complexes

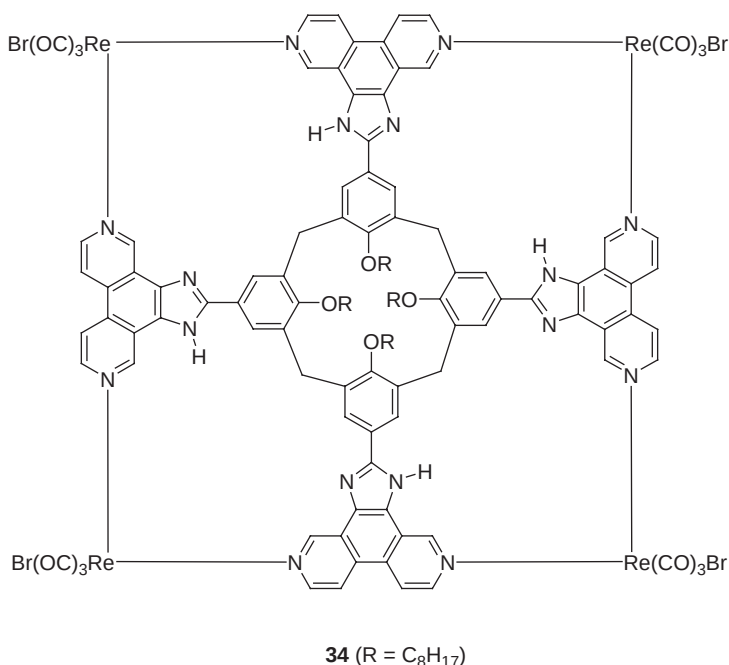


FIG. 24. Structure of the metallobridged cavitand **34** (141).

and may provide the basis for the future design and synthesis of luminescent materials.

Finally, a metallobridged cavitand $[(Re(CO)_3Br)_4(L^{17})]$ (**34**), where L^{17} represents 3,8-phenanthroline-imidazole-calix[4]arene, has been reported by Mendoza and coworkers (141). This complex reveals a basket-like structure with the four rhenium atoms situated at the corners of an almost perfect square, the diagonal distances being 16.0 and 16.7 Å (Fig. 24). Molecular modeling studies suggest that the complex could act as a receptor for the complexation of guests of complementary shape and size. Indeed, **34** reacts with a variety of calix[4]arenes forming 1:1 complexes, with association constants K_a up to $1.5 \times 10^5 M^{-1}$. Computer simulations and NMR data suggest that the complexes are held together by face-to-face stacking interactions between the electron-rich guest and the electron-deficient metallohost. The use of the deep cavity of the Re_4 complex **34** is under active investigation.

F. OTHER SUPPORTING LIGANDS

Recently, new ligand systems which support discrete dinuclear cage structures of type II have appeared in the literature

(142–145). Most of these ligands have a macrobinucleating architecture and selected examples are reviewed in this section.

Severin and coworkers reported (146) the reaction of tris(2-aminoethyl)amine and 4-formylphenylboronic acid with pentaerythritol to give, via multicomponent assembly, the boronic acid based macrobicyclic cage **35** (Fig. 25). The cage has the form of an ellipsoid with a diameter of 20.5 Å and binds two Cu(I) ions in a fashion similar to the smaller tren-based cryptands. The reversible formation of boronic esters has also been employed to build other hollow structures such as nanotubes (147) and porous covalent organic frameworks (148,149).

Love and coworkers have reported a series of dinuclear cobalt complexes derived from a rigid binucleating macrocycle H_4L^{18} as shown in Fig. 26 (150). The synthesis of the dicobalt complex $[Co_2(L^{18})]$ (**36**) was achieved by an anaerobic transamination reaction between H_4L^{18} and $[Co(thf)\{N(SiMe_3)_2\}_2]$ in THF. The unsaturated species **36** forms a bis(pyridine) adduct, $36 \cdot py_2$ (py = pyridine), which has a cleft-like structure reminiscent of pacman diporphyrin complexes (151,152). Both cobalt ions are square pyramidal, with Co1 and Co2 displaced out of the N_4 -basal planes by 0.17 and 0.18 Å, respectively. The apical sites are occupied by pyridine nitrogen atoms that are *exo* and *endo* to the cleft. Interestingly the *endo* pyridine is canted and reflects the

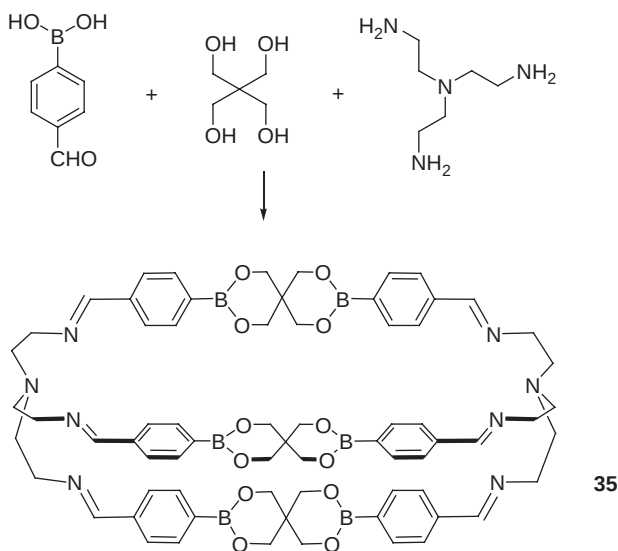


FIG. 25. Structure of macrobicyclic cage **35** (146).

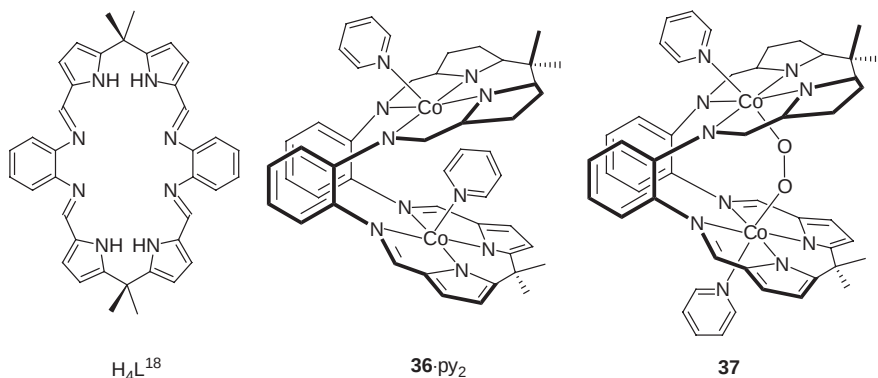


FIG. 26. Structures of H_4L^{18} , $36 \cdot py_2$ and 37 (150).

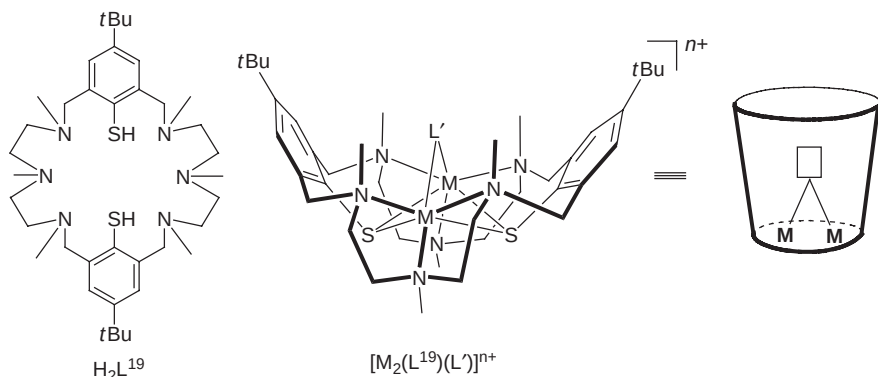


FIG. 27. Structure of H_2L^{19} and schematic representation of the structure of its corresponding $[M_2(L^{19})(L')]^{n+}$ complexes (154).

inability of the binuclear cleft to expand. This is in contrast to the pacman diporphyrin complexes which have the capacity to undergo considerable vertical expansion (153). The ability of complex **36** to react with small molecules such as O_2 has also been demonstrated. Thus **36** reacts spontaneously with O_2 forming a $Co^{III}Co^{III}$ peroxo complex which was isolated as $[Co_2(O_2)(py)_2(L^{18})]$ (**37**). The structure shows a bridging peroxide ($d(O-O) = 1.361(3) \text{ \AA}$) situated in the cleft of the cavitand.

The 24-membered Robson-type hexaaza-dithiophenolate ligand H_2L^{19} and its various derivatives have been utilized by Kersting and coworkers (154) as supporting ligands for metalated container molecules with a biocuboidal $N_3M(\mu-S_2)_2(L')MN_3$ core structure (Fig. 27). The remainder of this article will now focus on

the coordination chemistry of these ligand systems and their corresponding complexes.

III. Metalated Container Molecules of Binucleating Polyaza-Dithiophenolate Macrocycles

Owing to their biological relevance as simple model compounds for the active sites of metalloenzymes (155–161) and their intriguing magnetic and electronic properties (162–164), there has been much interest over the years in the synthesis and characterization of mono- and polynuclear transition-metal thiolate complexes (165–170). Macrocyclic ligands have turned out to be particularly useful for the synthesis of such complexes as they form more stable complexes than their acyclic counterparts and since they provide control over structures, coordination geometries, and nuclearity. Moreover, in the case of binucleating macrocycles the two metal ions can be fixed in close proximity which has important implications for the metal–metal interactions (171) and the binuclear metal reactivity (172–174). In the past, most of the pertinent studies had focused on binucleating phenolate-based ligands and it is only recently that the coordination chemistry of the corresponding thiophenolate-based macrocycles has been investigated (175). Some of these ligands, in particular the N_6S_2 ligand H_2L^{19} (Fig. 27), adopt capsular cleft-like structures which are reminiscent of the “partial cone” and “cone” conformations of the calixarenes. The presence of these binding pockets affects the coordination chemistry of the corresponding complexes considerably and these aspects are now discussed in detail.

A. MACROCYCLIC THIOPHENOLATE SCHIFF-BASE LIGANDS

One of the most efficient syntheses of macrobinucleating phenolate-based ligands such as the tetraimine-diphenol H_2L^{20} (Fig. 28) involves a Schiff-base condensation of 2,6-diformyl-4-methyl-phenol with an aliphatic α,ω -diamine in the presence of a labile first-row transition-metal ion. Since its discovery by Robson and co-workers in 1970 (176), this reaction has been successfully applied to a large number of other phenolate-based Schiff-bases (177–182). This method was also employed to prepare the ligand H_2L^{21} , the first thiophenolate-based Schiff-base macrocycle of which was reported by Schröder and co-workers (183) in 1993 (Fig. 28). The ligand was isolated as its

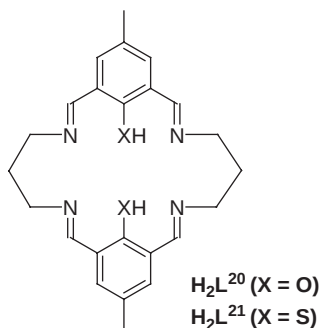


FIG. 28. Structure of macrobinucleating ligands H_2L^{20} and H_2L^{21} .

dinickel complex $[\text{Ni}_2(\text{L}^{21})]^{2+}$ in low yields by reaction of $[\text{Ni}(\text{OAc})_2] \cdot 4\text{H}_2\text{O}$ with 2,6-diformyl-4-methyl-thiophenol and 1,3-diaminopropane in acetonitrile. An improved synthesis of H_2L^{21} has subsequently been developed by Brooker and co-workers (184). Their strategy makes use of the thiophenolate precursor *S*-(2,6-diformyl-4-methylphenyl)dimethylthiocarbamate which is readily deprotected *in situ* during complex synthesis. The same protocol has been used for the synthesis of various other macrobinucleating thiophenolate Schiff-base ligands (185–195), but in contrast to the phenolate ligands, the free thiophenols have not yet been reported in the literature. The synthesis and coordination chemistry of such thiophenolate Schiff-base macrocycles has been described in recent reviews by Brooker (175), Schröder (155), and Tamburini (196) and will not be discussed further here. This review will focus specifically on the synthesis and coordination chemistry of binucleating polyamine-dithiophenolate ligands, the reduced versions of thiophenolate-based Schiff-bases.

B. POLYAMINE-DITHIOPHENOLATE MACROCYCLES

The conversion of the N^{imine} to N^{amine} donors is expected to affect the ligating properties of Schiff-base thiophenolate ligands significantly due to a significantly decreased N^{amine} ligand field strength and the sterically more demanding (sp^3 -hybridized) NR_3 functions of the resulting polyamine-dithiophenolates (197). In addition, the amine macrocycles are not susceptible towards hydrolysis and remain intact under various reaction conditions (198–201). Brooker and coworkers reported the first such ligand, H_2L^{22} , isolated as its dinickel complex $[\text{Ni}_2(\text{L}^{22})](\text{ClO}_4)_2$ (**38**), in

1998 (Fig. 29). The complex was prepared in good yields by the reduction of $[\text{Ni}_2(\text{L}^{21})](\text{ClO}_4)_2$ with NaBH_4 in MeOH followed by acidic workup.

Recently, Kersting and coworkers have developed an alternative procedure for preparing polyamine-dithiophenolate macrocycles. In this case, the ligands are obtained without the need of metal templates. Scheme 1 illustrates the general procedure for the hexaaza-dithiophenols H_2L^{19} and H_2L^{23} . The key step is the reductive amination of the tetraaldehyde **39** with diethylene triamine under medium-dilution conditions to produce a macrobicyclic hexaaza-dithioether intermediate **40** in almost quantitative yield (202,203). The high yield of this double macrocyclization reaction can be traced to the presence of the aliphatic thioether linkage that functions as a template in predisposing the four aldehyde groups in close proximity during

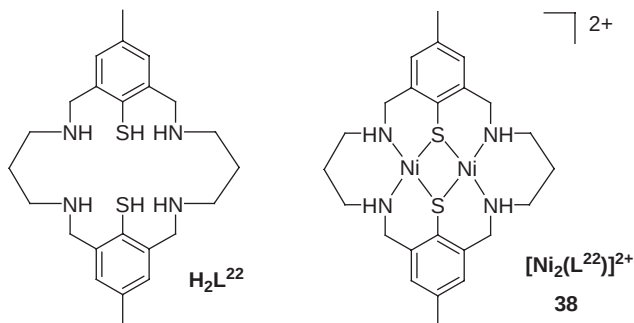
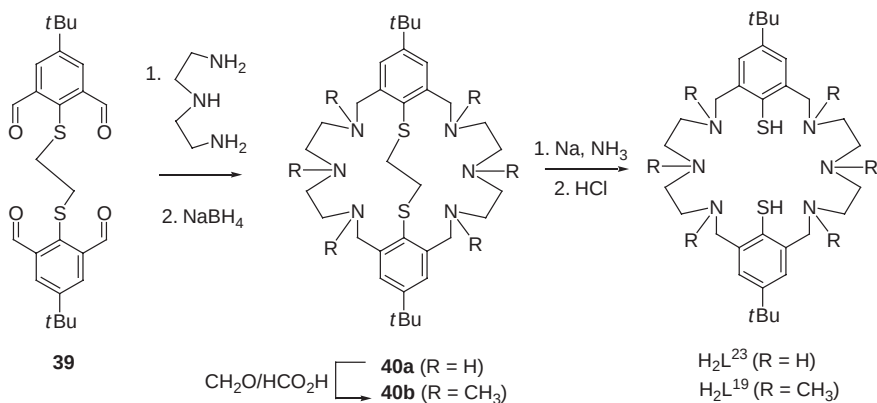


FIG. 29. Structure of H_2L^{22} and its corresponding dinickel(II) complex **38**.



SCHEME 1. Synthesis of **40a,b** and H_2L^{23} and H_2L^{19} (202,154).

the Schiff-base formation step (204–206). In addition, the ethylene-linkage serves also as a protecting group for the air-sensitive thiophenolate groups. Similar reactions of **39** with ethylenediamine or propylenediamine yielded tricyclic macrocycles because these smaller diamines cannot easily span the two aldehyde functions (207,208).

An attractive feature of this route is that the macrobicyclic intermediate **40a** can be readily N-alkylated without affecting the masked thiolate functions (209). Thus, reductive methylation of **40a** with formaldehyde and formic acid under Eschweiler–Clarke conditions, followed by deprotection of **40b** with Na/NH₃ provides the otherwise inaccessible N₆S₂ ligand H₂L¹⁹ in nearly quantitative yield (Scheme 1). Several other aza-thioethers **40c–p** and their corresponding thiophenolate ligands were prepared by this route (see Fig. 30 and Table I) and some of them will be discussed further in later sections (210–215).

Three macrobicycles **40b** (216), **40h** (212), and **40k** (211) were characterized by X-ray crystallography. Fig. 31 displays the structure of **40h**. The compounds adopt a highly folded conformation, reminiscent of calixarenes (23) and related Schiff-base macrocycles (181).

The deprotection of the polyamine-thioethers **40a,b** can also be accomplished in similar good yields with Li/naphthalene in THF (214). Cleavage of the thioether functions also commenced with [PdCl₂(NCMe)₂] in the case of **40b** (216), and it is likely that the other macrobicycles will also undergo this metal-ion mediated cleavage reaction as well (Scheme 2). Note that this reaction cleaves only one of the two thioether bonds forming a macrocyclic ligand with one thiophenolate and one vinyl-thioether moiety, as

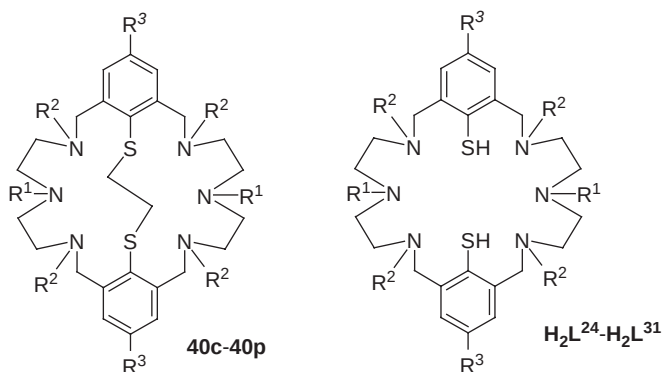


FIG. 30. Binucleating aza-thioethers **40c–40p** and amine-thiophenolate macrocycles H₂L²⁴–H₂L³¹ (see Table I for ligand designations).

TABLE I

BINUCLEATING AZA-THIOETHERS **40c–40p** AND AMINE-THIOPHENOLATES H_2L^{24} – H_2L^{31}

	R ¹	R ²	R ³	Ref.	R ¹	R ²	R ³	Ref
40c	H	H	H	(215)				
40d	H	H	Ph	(212)				
40e	H	H	4- <i>t</i> Bu-Ph	(212)				
40f	CH ₃	CH ₃	H	(215)	H₂L²⁴	CH ₃	CH ₃	H (215)
40g	CH ₃	CH ₃	Ph	(212)				
40h	CH ₃	CH ₃	4- <i>t</i> Bu-Ph	(212)				
40i	C ₂ H ₅	C ₂ H ₅	<i>t</i> Bu	(210)	H₂L²⁵	C ₂ H ₅	C ₂ H ₅	<i>t</i> Bu (210)
40j	<i>n</i> C ₃ H ₇	<i>n</i> C ₃ H ₇	<i>t</i> Bu	(210)	H₂L²⁶	<i>n</i> C ₃ H ₇	<i>n</i> C ₃ H ₇	<i>t</i> Bu (210)
40k	CH ₃	C ₂ H ₅	<i>t</i> Bu	(211)	H₂L²⁷	CH ₃	C ₂ H ₅	<i>t</i> Bu (211)
40l	CH ₃	H	<i>t</i> Bu	(211)	H₂L²⁸	CH ₃	H	<i>t</i> Bu (211)
40m	(CH ₂) ₂ OMe	(CH ₂) ₂ OMe	<i>t</i> Bu	(214)	H₂L²⁹	(CH ₂) ₂ OMe	(CH ₂) ₂ OMe	<i>t</i> Bu (214)
40n	(CH ₂) ₂ OH	(CH ₂) ₂ OH	<i>t</i> Bu	(214)	H₂L³⁰	(CH ₂) ₂ OH	(CH ₂) ₂ OH	<i>t</i> Bu (214)
40o	(CH ₂) ₃ NH ₂	(CH ₂) ₃ NH ₂	<i>t</i> Bu	(214)	H₂L³¹	(CH ₂) ₃ NH ₂	(CH ₂) ₃ NH ₂	<i>t</i> Bu (214)
40p	(CH ₂) ₂ CN	(CH ₂) ₂ CN	<i>t</i> Bu	(214)				

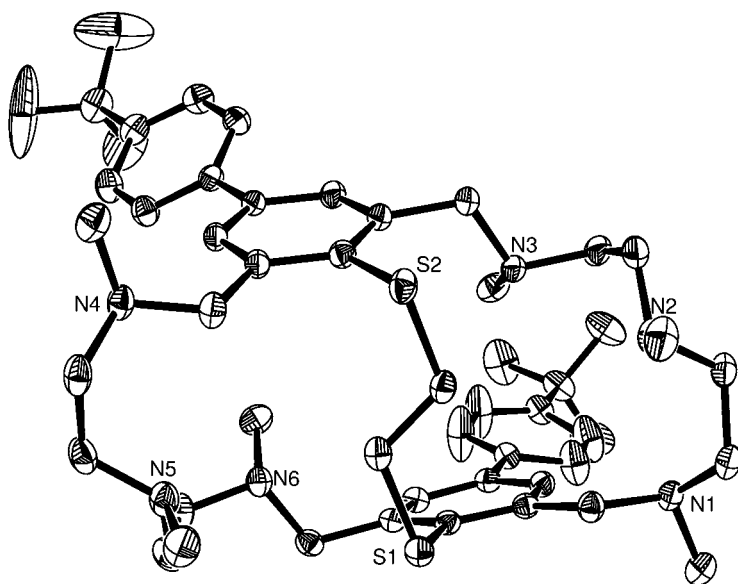
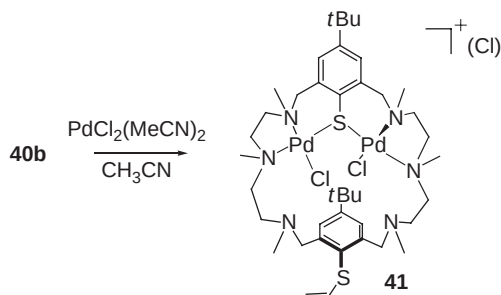


FIG. 31. Molecular structure of **40h** in the crystal. This figure was generated using data downloaded from The Cambridge Crystallographic Data Centre (CCDC) and corresponds to a structure originally reported in Ref. (212).

confirmed by the crystal structure of $[\text{Pd}_2(\text{L}^{32})]\text{Cl}$ (**41**). The structure of this dipalladium complex reveals two distorted square planar PdN_2ClS units that are bridged by the deprotected thiophenolate donor of $(\text{L}^{32})^-$. The vinyl-thioether group and the



SCHEME 2. Partial deprotection of **40b** with $[\text{PdCl}_2(\text{MeCN})_2]$ (216).

two remaining amine donors of $(\text{L}^{32})^-$ do not interact with the two palladium ions.

C. COORDINATION CHEMISTRY OF POLYAMINE-DITHIOPHENOLATE LIGANDS

A large number of mono- and dinuclear metal complexes of the ligands H_2L^{19} and H_2L^{23} has been synthesized and characterized within the last five years. Selected examples originating from this work will now be presented and discussed.

C.1. Mononuclear complexes

Given the fact that phenolate-based Schiff-bases such as H_2L^{21} are capable of forming mononuclear complexes (217–221), one might expect that analogous complexes are supported by the amine-thiophenolates. Mononuclear complexes of binucleating ligands are of interest because they can be used as starting materials for the preparation of heterodinuclear complexes (173, 222, 223). Recently, Kersting and coworkers have described the first examples of mononuclear complexes of the hexaamine-dithiophenolate ligands H_2L^{19} and H_2L^{26} , respectively (224). The perchlorate salts of the complexes $[\text{NiH}_2(\text{L}^{19})]^{2+}$ (**42**), $[\text{ZnH}_2(\text{L}^{19})]^{2+}$ (**43**), and $[\text{NiH}_2(\text{L}^{26})]^{2+}$ (**44**) (Fig. 32) were obtained in high yields by treating the free ligands with the corresponding free ligands with the corresponding metal dichlorides and NEt_3 in a 1:1 ratio, followed by the addition of LiClO_4 . On the basis of the τ -values (225), which range from 0.01 to 0.41 in **42–44**, the metal ions are coordinated in a distorted square-pyramidal fashion. The two hydrogen atoms are attached to the noncoordinating benzylic amine functions and are intramolecularly hydrogen bonded to the metal-bound thiophenolate groups. A comparison with the structures of analogous complexes **45** and **46** of the acyclic ligands H_2L^{33} and

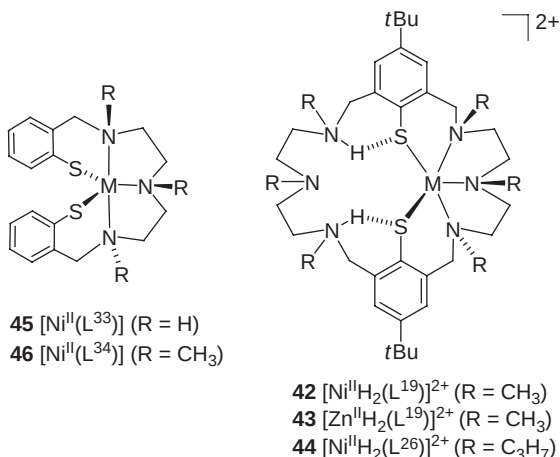


FIG. 32. Mononuclear Ni(II) and Zn(II) complexes of acyclic and macrocyclic polyamine-thiophenolate ligands (224).

H₂L³⁴, respectively, shows that the macrocycles do not confer unusual coordination geometries on the metal ions.

Another example of a mononuclear complex is the dicationic Cr(III) complex [Cr^{III}H₂L¹⁹(OAc)]²⁺ (**47**). This complex is obtained when (L¹⁹)²⁻ is treated with CrCl₂ and NaOAc in methanol followed by oxidation in air (226). The Cr^{III} ion in **47** is coordinated to three N and two S atoms of (L¹⁹)²⁻ and an O atom of a monodentate acetate coligand in an octahedral manner as shown in Fig. 33. To our knowledge, mononuclear complexes of macrocyclic thiophenolate Schiff-bases have not yet been documented in the literature (227).

The ability of the monometallic complexes **42** and **43** to serve as starting materials for homo- and heterodinuclear complexes has also only recently been demonstrated (224). Thus, the reaction of **42** with Ni(OAc)₂ · 4H₂O proceeds smoothly and yields the homodinuclear complex [Ni₂(L¹⁹)(OAc)]⁺ (**48**) in almost quantitative yield (Scheme 3). The Zn(II) complex **43** reacts similarly yielding [Zn₂L¹⁹(OAc)]⁺ (**49**) upon treatment with Zn(OAc)₂ · 2H₂O. When **43** is reacted with Cd(OAc)₂ · 2H₂O in methanol, a 8:1:1 mixture of the heterodinuclear [ZnCd(L¹⁹)(OAc)]⁺ (**50**) and homodinuclear Zn₂ (**49**) and Cd₂ complexes (**51**) is produced as established by ¹H NMR and ESI MS spectroscopy (224,228). Heterodinuclear transition-metal complexes analogous to **51** would be of considerable interest, in particular with regard to their relevance to bioinorganic chemistry (229–232) and their magnetic properties (175,233–237).

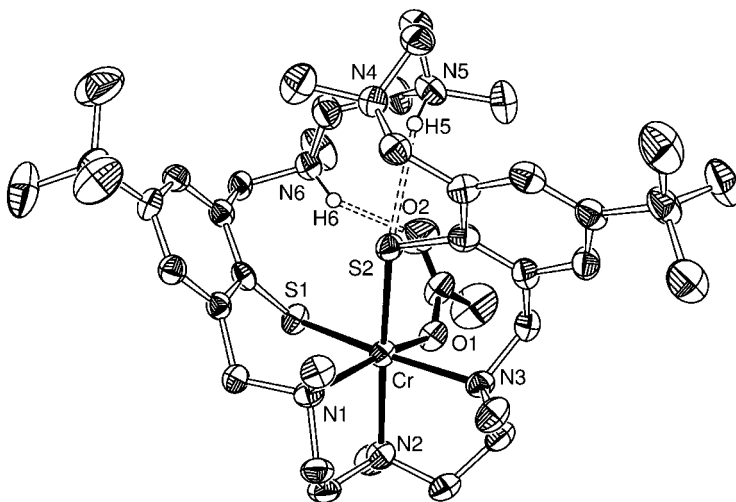
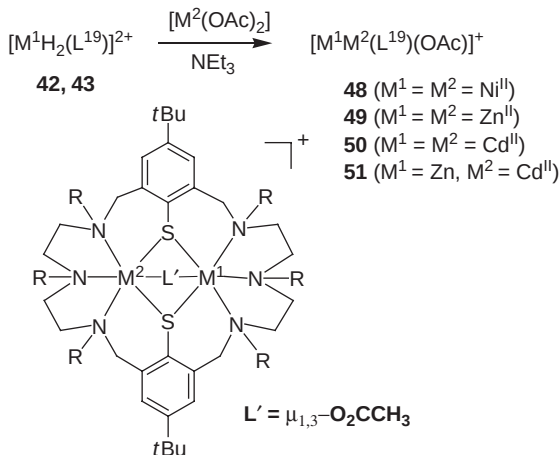


FIG. 33. Molecular structure of **47**. This figure was generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and corresponds to a structure originally reported in Ref. (226).



SCHEME 3. Synthesis of homo- and dinuclear complexes **48-51** (224).

C.2. Binuclear complexes: complex types and ligand conformations

The ligands H_2L^{19} , H_2L^{23} – H_2L^{31} are effective dinucleating ligands towards various divalent metal ions. Complexes of composition $[M^{II}_2(L^R)(\mu\text{-}L')]^+$ ($M = Mn, Fe, Co, Ni, Zn, Cd$)

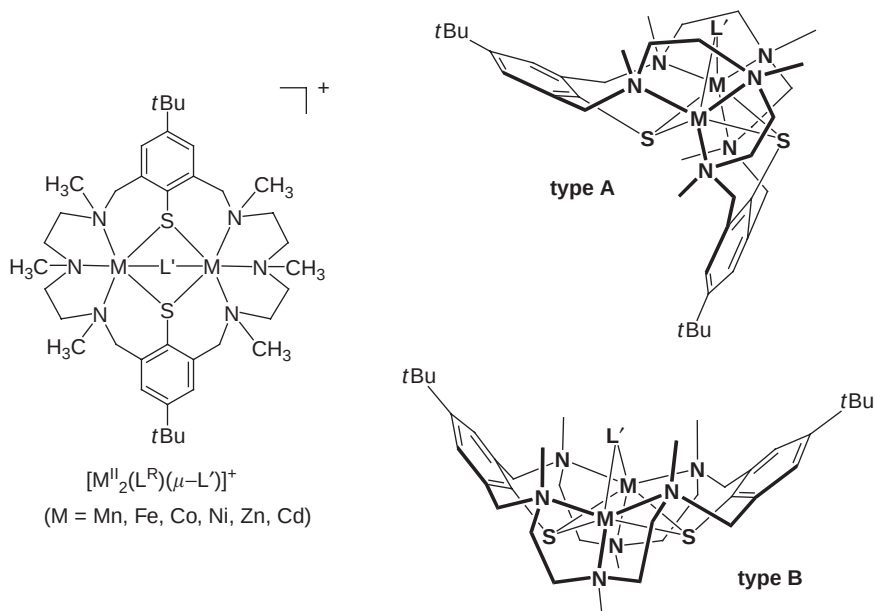


FIG. 34. Cationic $[M^{II}_2L^R(\mu-L')]^+$ complexes supported by the ligands $(L^R)^{2-}$ and schematic representation of their structures ($L' = \text{coligand}$).

bearing Cl^- and OAc^- coligands (L') (Fig. 34) can be readily obtained by the treatment of the free ligands H_2L^R with two equivalents of the corresponding metal(II) dihalogenides (MCl_2) or acetates ($\text{M}(\text{OAc})_2$) in methanolic solution in the presence of a base. Table II lists a selection of the synthesized complexes and their labels.

All cations **48–67** feature a bioctahedral $\text{N}_3\text{M}(\mu\text{-S})_2(\mu\text{-L}')\text{MN}_3$ core structure, irrespective of the type of the coligand or supporting ligand. Fig. 35 illustrates the molecular structures of the bioctahedral nickel complexes **52** and **48**, which are representative for all complexes. The divalent metal ions are coordinated in a square-pyramidal fashion by the two *fac*- N_3 ($\mu\text{-S}$)₂ donor sets of the macrocycles. Upon coordination of the exogenous coligands (L') distorted octahedral environments result for the metal atoms. The $\text{M}\cdots\text{M}$ distances vary from 3.037 to 3.512 Å. Complexes of type B are amongst the first prototypes for binuclear complexes with confined binding cavities (238). The two aromatic rings of the amine-thiophenolates H_2L^R form the walls of the binding pockets.

Interestingly, the macrocycles can adopt two different conformations A and B (Fig. 34), which are reminiscent of the “partial cone” and “cone” conformations of the calixarenes. A detailed

TABLE II

SYNTHESIZED COMPLEXES, THEIR LABELS, AND STRUCTURE TYPES^a

Complex		Structure	[d(M...M)/Å]	Ref.
48	$[\text{Ni}_2(\text{L}^{19})(\mu\text{-OAc})]^+$	B	3.483(1)	(239)
49^d	$[\text{Zn}_2(\text{L}^{19})(\mu\text{-OAc})]^+$	B	3.427(1)	(239)
50	$[\text{Cd}_2(\text{L}^{19})(\mu\text{-OAc})]^+$	B	3.402(2)	(240)
52	$[\text{Ni}_2(\text{L}^{23})(\mu\text{-Cl})]^+$	A	3.098(2)	(209)
53	$[\text{Co}_2(\text{L}^{23})(\mu\text{-Cl})]^+$	n.d. ^b		(202)
54	$[\text{Ni}_2(\text{L}^{19})(\mu\text{-Cl})]^+$	A	3.184(1) [3.217(2)] ^c	(209)
55	$[\text{Co}_2(\text{L}^{19})(\mu\text{-Cl})]^+$	A	3.165(1) [3.194(1)] ^c	(239)
56	$[\text{Ni}_2(\text{L}^{28})(\mu\text{-Cl})]^+$	A	3.074(1)	(211)
57	$[\text{Cd}_2(\text{L}^{23})(\mu\text{-Cl})]^+$	A	3.267(2)	(211)
58	$[\text{Cd}_2(\text{L}^{19})(\mu\text{-Cl})]^+$	B	3.3584(9)	(211)
59	$[\text{Cd}_2(\text{L}^{28})(\mu\text{-Cl})]^+$	A	3.267(1)	(211)
60	$[\text{Cd}_2(\text{L}^{27})(\mu\text{-Cl})]^+$	B	3.3550(8) [3.3387(8)] ^c	(211)
61	$[\text{Mn}_2(\text{L}^{19})(\mu\text{-OAc})]^+$	B	3.456(1)	(226)
62	$[\text{Fe}_2(\text{L}^{19})(\mu\text{-OAc})]^+$	B	3.421(1)	(226)
63	$[\text{Co}_2(\text{L}^{19})(\mu\text{-OAc})]^+$	B	3.448(1)	(241)
64	$[\text{Co}_2(\text{L}^{25})(\mu\text{-OAc})]^+$	B	3.482(1)	(210)
65	$[\text{Zn}_2(\text{L}^{25})(\mu\text{-OAc})]^+$	B	3.460(1)	(210)
66	$[\text{Ni}_2(\text{L}^{26})(\mu\text{-OAc})]^+$	B	3.512(1)	(210)
67	$[\text{Ni}_2(\text{L}^{19})(\mu\text{-OH})]^+$	A	3.037(3)	(209)

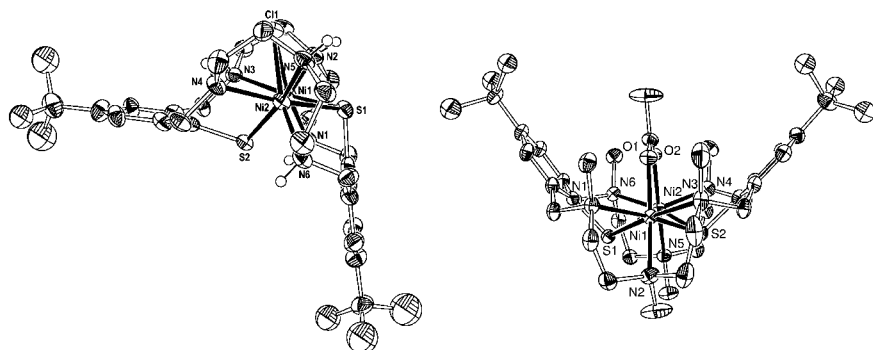
^aThe complexes were isolated as ClO_4^- or BPh_4^- salts.^bNot determined.^cThere are two independent molecules in the unit cell. The values in square brackets correspond to the second molecule.^dSee Table III.

FIG. 35. Molecular structures of **52** (left) and **48** (right) in the crystal. The figure was generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and corresponds to the structures originally reported in Refs. (209) and (239).

comparison of the individual structures has shown that the conformation of the amino-thiophenolates (L^R)²⁻ is coupled to the size of the coligand L' and the metal-ion radii (211,242). For the complexes of the 3d elements the type A conformation is only seen for small monoatomic bridging ligands such as $L' = OH^-$ and Cl^- . For larger monoatomic coligands (such as SH^-) or multiatom bridging ligands (such as OAc^-) the bowl-shaped conformation B is assumed, the driving force being the more regular octahedral coordination environments about the M^{II} ions (243).

Martell and coworkers have investigated the coordination chemistry of the analogous phenolate ligand H_2L^{35} (244) (Fig. 36). The complexes $[Ni_2(L^{35})]Br_2$ (245), $[Ni_2(L^{35})(H_2O)_2]Br_2$ (244), $[Zn_2(L^{35})(H_2O)_2]Br_2$ (246), and $[Cu_2(L^{35})]ClBr$ (247) were prepared and structurally characterized. Despite the identical ligand backbones, only doubly bridged $N_3M(\mu-OR)_2MN_3$ core structures are supported (248). In addition, these compounds have flat structures in striking contrast to the bowl- or cleft-like structures of the amine-thiophenolate complexes.

Another highly significant difference is that the nickel atoms in the phenolate complexes do not tend to bind exogeneous coligands, such that they remain five-coordinated. Similar differences have been noted by Brooker and coworkers, who compared the coordination chemistries of analogous phenolate and thiophenolate Schiff-bases (175). The structural differences can be traced to the different hybridizations of the phenolate-oxygen (sp^2 , trigonal-planar) and the thiophenolate-sulfur atoms (sp^3 , tetrahedral). The former macrocycles tend to enforce a planar $M(\mu-OR)_2M$ core structure, while the latter feature a bent $M_2(\mu-SR)_2$ structure, which can be spanned more easily by the coligands.

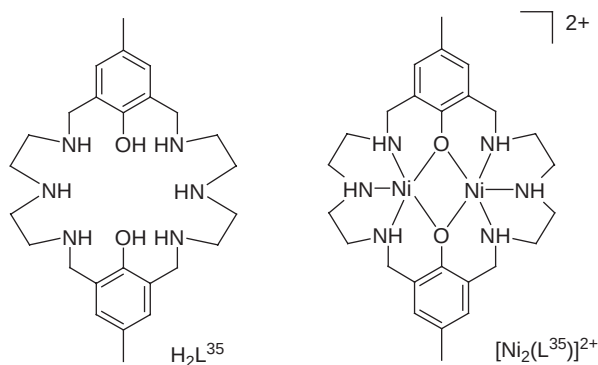


FIG. 36. Structures of the macrocycle H_2L^{35} and its dinuclear nickel complex, $[Ni_2(L^{35})]^{2+}$ (244).

A different coordination behavior of the hexaamine-dithiophenolate ligand H_2L^{19} was observed in the case of Hg^{II} and Pb^{II} . The complexation reaction of H_2L^{19} with $\text{Hg}(\text{OAc})_2$ and $\text{Pb}(\text{OAc})_2$ provided the dicationic complexes $[\text{Hg}_2(\text{L}^{19})]^{2+}$ (**68**) and $[\text{Pb}_2(\text{L}^{19})]^{2+}$ (**69**), respectively, which were isolated as their ClO_4^- or BPh_4^- salts (240). These compounds also show no tendency to bind further coligands. The $\text{Hg}(\text{II})$ ions in **68** are thus surrounded by three nitrogen and two sulfur donor atoms of $(\text{L}^{19})^{2-}$ (coordination number of Hg^{II} : 4+1) (Fig. 37), while the coordination environments of the lead(II) ions in **69** consist of three amine donors and one thiophenolate-sulfur atom (coordination number of Pb^{II} : 3+1). The coordination geometry of Pb^{II} is best considered as distorted square pyramidal, with the lead ion sitting ca. 1.3 Å above the plane through the four donor atoms. The empty space above the lead(II) ion on the apex of the pyramid is indicative of a stereochemically active lone pair, as in PbO (yellow modification) (249), and this might be responsible for the different coordination behavior. Nevertheless, these results have also established the ability of the macrobinucleating polyaza-dithiophenolate ligand to form stable binuclear complexes of toxic heavy metals.

The S-oxygenation of the hexaamine-dithiophenolate macrocycles should provide a potential entry into the novel class of binucleating polyamine-disulfonate and -disulfinate macrocycles. Indeed, such ligands can be prepared by the oxidation of dinuclear nickel complexes of the parent hexaaza-dithiophenolate macrocycles followed by the decomposition of the oxidation products in acidic solution. The dinuclear nickel complexes $[\text{Ni}^{\text{II}}_2(\text{L}^{36})(\text{L}')^+]$ ($\text{L}' = \text{Cl}^-$ (**70**) and OAc^- (**71**)) of the hexaaza-diphenylsulfonate ligand $(\text{L}^{36})^{2-}$ (Fig. 38) are obtained by

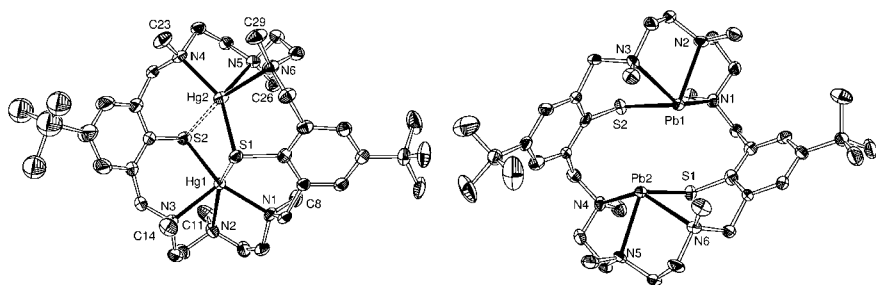


FIG. 37. Molecular structures of **68** (left) and **69** (right) in the crystal. This figure was generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and corresponds to the structures originally reported in Ref. (240).

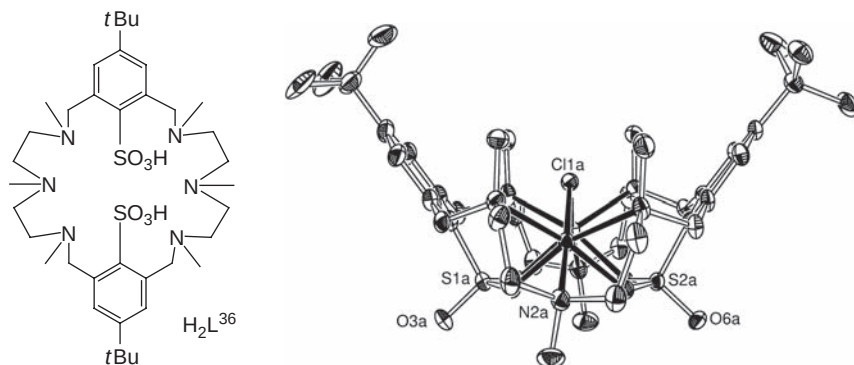


FIG. 38. (Left) Polyamine-sulfonate macrocycle H_2L^{36} . (Right) Molecular structure of the dinickel(II) complex **70**. This figure was generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and corresponds to the structure originally reported in Ref. (250).

oxidation of their respective $[\text{Ni}^{\text{II}}_2(\text{L}^{19})(\mu\text{-L})]^+$ parents ($\text{L}' = \text{Cl}^-$ (**54**), OAc^- (**48**)) with *meta*-chloro-peroxybenzoic acid or hydrogen peroxide (250). Decomposition of the sulfonate complexes gives the free macrocycle H_2L^{36} , which upon treatment with $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ produces diamagnetic $[\text{Zn}^{\text{II}}_2(\text{L}^{36})(\text{OAc})]^+$ (**72**). The crystal structure determination of **70** (Fig. 38) reveals a binuclear complex with bridging sulfonate and chloride groups and an overall bowl-shaped geometry.

A dinuclear Cu(II) complex of the hexaaza-disulfinate derivative $(\text{L}^{37})^{2-}$, i.e. $[\text{Cu}^{\text{II}}_2(\text{L}^{37})]^{2+}$ (**73**), is produced by air-oxidation of $(\text{L}^{19})^{2-}$ in the presence of Cu(I) (Fig. 39). The crystal structure determination of **73** reveals two Cu(II) ions with pseudo-square pyramidal N_3O_2 coordination spheres. The $\text{Cu} \cdots \text{Cu}$ distance is 4.993(1) Å.

D. EFFECTS OF N-ALKYLATION ON THE MOLECULAR AND ELECTRONIC STRUCTURES OF THE COMPLEXES

It is well-established that the molecular and electronic structures of metal complexes of azamacrocycles are greatly affected upon N-alkylation (197). This is mainly due to two factors: (a) the decrease of the ligand field strength and (b) the increase in the steric requirements upon going from a secondary to a tertiary amine donor function (251). To examine whether the properties of the dinuclear amine-thiophenolate complexes are affected by the N-alkyl substituents, analogous complexes of the

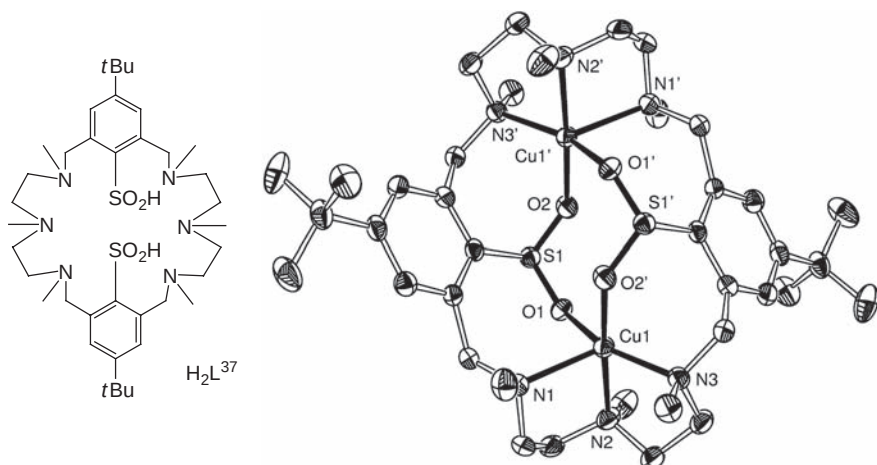


FIG. 39. (Left) Polyamine-disulfinate macrocycle H_2L^{37} . (Right) Molecular structure of **73**. This figure was generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and corresponds to the structure originally reported in Ref. (250).

two macrocycles H_2L^{19} and H_2L^{23} were prepared and characterized by various spectroscopic and other analytical methods (IR, UV/Vis, EPR, ^{57}Fe -Mössbauer spectroscopy), cyclic voltammetry, temperature-dependent magnetic susceptibility measurements, and X-ray crystallography. The most important findings of these investigations are as follows.

- The conversion of the six secondary into tertiary amine donor functions does not change the overall structure of the $[M_2(L^R)(\mu-L')^+]$ complexes. That is the complexes retain their bioctahedral $N_3M(\mu-SR)_2(\mu-L')MN_3$ core structures and the macrocycle conformation remains constant for a given coligand, see for instance the isostructural compounds **52–55** (211). This offers the opportunity to extend the rims of the binding pocket of the $[M_2(L^R)(L')]$ complexes.
- The complexes of the permethylated macrocycle H_2L^{19} exhibit longer MN and shorter ML' bond distances than their homologous complexes of the unfunctionalized parent H_2L^{23} . For instance, upon going from **52** to **54** the average Ni–N bond length increases from 2.107(6) Å to 2.239(2) Å ($\Delta = +0.132$ Å) and the average Ni–Cl bond length decreases from 2.621 to 2.442 Å ($\Delta = -0.179$ Å). In other words, N-alkylation of the amine-thiophenolate ligand H_2L^{23} results in stronger metal–coligand bonding interactions. This in turn results in

a stronger polarization of the bridging coligands and thus in a higher reactivity (Lewis-acidity) of the $[M_2(L^{19})(\mu-L')]^+$ complexes (239).

- The alkylation of the secondary amine functions generates a more hydrophobic microenvironment about the active coordination site L' . For example, in the cations **52** and **53** the coligands and the NH functions act as hydrogen bond acceptors and donors, respectively, and are involved in inter- and intramolecular hydrogen bonding interactions (209). No such interactions are observed for analogous complexes **54** and **55** of $(L^{19})^{2-}$. This also drastically alters the ease of the substitution of the bridging coligands. Thus, while the μ -Cl complex **54** reacts readily with NBu_4OH in acetonitrile to produce the hydroxo-bridged complex **67**, complex **52** was found to be unreactive (209). Even the addition of a halide scavenger such as lead perchlorate did not lead to substitution of the Cl^- ion in **52**.
- The donor strength of H_2L^{19} is significantly weaker (by ca. 650 cm^{-1}) than its unalkylated derivative H_2L^{23} as determined by analysis of the UV/Vis spectra of the octahedral nickel complexes **52** and **54**. The ν_1 band (${}^3A_{2g} \rightarrow {}^3T_{2g}$) in an octahedral $Ni^{II}L_6$ complex is directly related to the ligand field strength of L (252,253). For **52**, this band is observed at 941 nm, whereas for **54** it occurs at much lower energies at 1002 nm (209), showing that the donor strength of H_2L^{19} is significantly weaker than H_2L^{23} . From the ν_1 transition one can obtain rough estimates of the octahedral splitting parameters Δ_{oct} for **52** ($\approx 10,627\text{ cm}^{-1}$) and for **54** ($\approx 9980\text{ cm}^{-1}$), respectively (253). Such low values for Δ_{oct} (i.e. $\Delta_{oct}[Ni(H_2O)_6]^{2+} = 8500\text{ cm}^{-1}$) are quite typical for $N_3Ni^{II}(\mu-SR)_3$ chromophores. In general, thiolates induce only weak ligand field strengths due to their poor σ -donor bonding abilities (254).
- The different ligand field strengths are also reflected in the redox potentials. Cyclic voltammetry experiments have shown that the dinickel(II) complex **52** undergoes two one-electron oxidations at +0.27 and at +1.05 V vs. SCE which correspond to the formation of the mixed-valent $Ni^{II}Ni^{III}$ and the fully oxidized $Ni^{III}Ni^{III}$ forms (209). The cyclic voltammogram of **54** also reveals two redox waves, but this complex is oxidized at more positive potentials at +0.43 and +1.37 V vs. SCE which is in good agreement with the weaker ligand field strength of $(L^{19})^{2-}$. Thus, in the $[M_2(L^{19})(\mu-L')]^{n+}$ complexes the M^{III} oxidation state is enormously destabilized over the M^{II} oxidation state, even in the case of Co^{III} (241, 255).
- As a consequence of the weak ligand field of $(L^{19})^{2-}$, the Mn^{II} , Fe^{II} , Co^{II} , and Ni^{II} ions in the acetato-bridged complexes **61–63**

and **48**, respectively, adopt high-spin configurations (226). Weak intramolecular antiferromagnetic exchange interactions are present in the Mn^{II}_2 , Fe^{II}_2 , and Co^{II}_2 complexes where $J = -5.1$, -10.6 , and -2.0 cm^{-1} , respectively ($H = -2JS_1S_2$). In contrast, in the dinickel complex **48** a ferromagnetic exchange interaction is present with $J = +6.4 \text{ cm}^{-1}$. There appears to be a magneto-structural correlation between the sign of the exchange parameter J and the M–S–M bond angle (226). For **61–63** the average M–S–M angles are significantly smaller than 90° and J is negative. For **47**, however, the mean Ni–S–Ni bridging angle is very close to 90° and J has a positive value.

Finally, the presence of the substituents on the nitrogen donors also influences the reactivity of the corresponding complexes significantly. Thus, utilization of the permethylated derivative in place of H_2L^{23} drastically alters the Lewis-acidity of the metal ions, that is the coligands in the $[\text{Ni}_2(\text{L}^{19})(\text{L}')^+]^+$ compounds become more polarized than in the $[\text{Ni}_2(\text{L}^{23})(\text{L}')^+]^+$ complexes. In the latter complexes, the Lewis-acidity of the divalent nickel(II) ions is in large part consumed for the polarization of the six secondary NH functions. This explains why only the complexes of the permethylated macrocycle are able to activate and transform small molecules such as H_2O or CO_2 (210,239,241).

Overall, the use of the permethylated derivative H_2L^{19} in place of H_2L^{23} influences many properties of the binuclear $[\text{M}^{\text{II}}_2(\text{L}^{\text{R}})]^{2+}$ complex fragments, including color, molecular and electronic structure, hydrogen bonding interactions, redox potential, complex stability, and ground spin-state. As shown below the reactivity is also greatly affected (256).

E. THE LIGAND MATRIX AS A MEDIUM

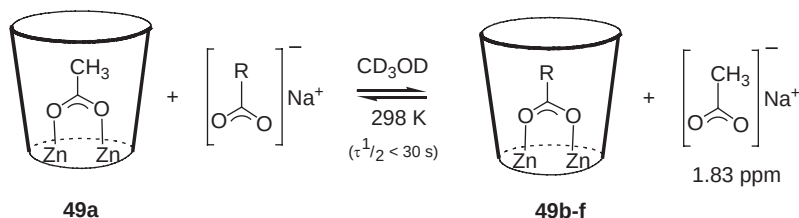
To probe whether the binding pocket exerts an effect on the stability of the complexes, a series of diamagnetic zinc complexes $[\text{Zn}_2(\text{L}^{19})(\text{O}_2\text{CR})^+]^+$ **49a–f** bearing different carboxylate coligands was prepared and analyzed by NMR spectroscopy in the solution state (Table III). The most important findings of these investigations are as follows:

- All complexes exist as discrete species in solution.
- The NMR signals of the coligands are shifted considerably (by $\sim 1 \text{ ppm}$) to higher field from their values in the uncomplexed form (224,239). The shift to higher field can be explained by the ring current effect. The coligands are located above the center of the two aryl rings of $(\text{L}^{19})^{2-}$ in the shielding region (250). This results in the observed chemical shift change to

TABLE III

CARBOXYLATO-BRIDGED ZINC COMPLEXES **49a–49f**

Complex		Coligand	Structure	pK_s	K_{rel}
49a	$[Zn^{II}_2(L^{19})(O_2CH)]^+$	Formate	B	3.75	0.1
49b	$[Zn^{II}_2(L^{19})(O_2CCH_3)]^+$	Acetate	B	4.75	1.0
49c	$[Zn^{II}_2(L^{19})(O_2CCH_2Cl)]^+$	Chloroacetate	B	2.86	1.1
49d	$[Zn^{II}_2(L^{19})(O_2CCH_2CH_3)]^+$	Propionate	B	4.87	2.7
49e	$[Zn^{II}_2(L^{19})(O_2CC_6H_5)]^+$	Benzoate	B	4.20	5.4
49f	$[Zn^{II}_2(L^{19})(O_2CC_6H_4-p-CH_3)]^+$	4-Methyl-benzoate	B	4.38	9.0



SCHEME 4. Exchange of carboxylate ligands in dizinc complexes **49a–f**. The bowl-shaped representation of the host molecules should not be confused with the one used for the cyclodextrins.

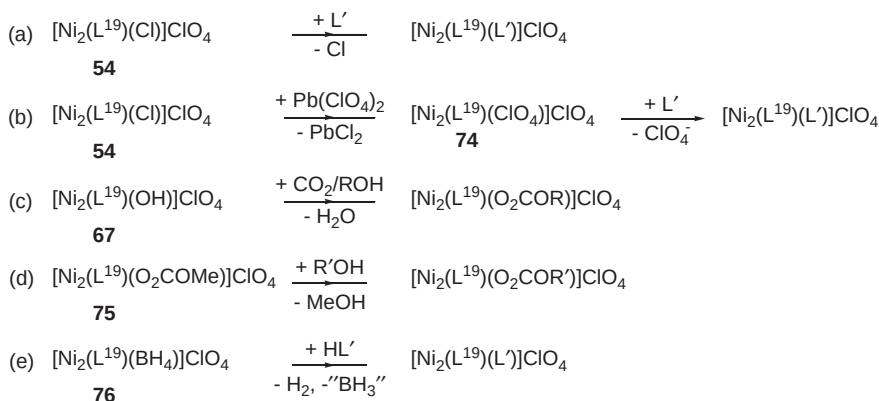
higher field. It clearly indicates that the ligand matrix functions as a medium.

- Ligand exchange reactions of the zinc complexes are quite rapid (Scheme 4). The equilibrium concentrations of the individual species are attained within the time scale of sample preparation (i.e. <2 min).
- To probe the hydrophobicity of the binding cavity of the $[Zn_2(L^{19})]^{2+}$ fragment (257), the relative stability constants (K_{rel}) of the carboxylato-bridged zinc complexes were determined (239,258). The concentrations of the species were measured by integration of the respective NMR signals and the relative stability constants were determined with the mass action law. Table III lists the calculated values. As can be seen, the stability constants differ by ca. two orders of magnitude and are not correlated with the pK_a values of the acids. Rather, they increase in the order **49a** < **49b** < **49c** < **49d** < **49e** < **49f**. Thus the larger the organic residue R of the carboxylate anion (RCO_2^-) the larger the binding constant. The observed trend is indicative of hydrophobic effects (van der Waals interactions) between the substituents of the carboxylate ion and the macrocyclic ligand. These interactions also contribute to the stability of the complexes.

F. VARIATION, COORDINATION MODES, AND ACTIVATION OF COLIGANDS

As outlined in the previous sections, the coordination chemistry of metalated container molecules is of interest in many respects. In particular, by adjusting the size and form of the binding cavity it is often possible to stabilize reactive intermediates or to coordinate coligands in unusual coordination modes. Such assemblies also offer the opportunity to study secondary host–guest interactions between the coordinated coligands and the walls of the host. For systematic investigations it is of importance that various coligands can be accommodated in the binding pocket of the container molecules. Single crystals suitable for X-ray structure determinations are also required such that one can study the effects of the size and form of the binding pocket on the coordination mode of the coligands and *vice versa*.

In this context it is to be noted that a large number of coligands can be readily accommodated in the binding pocket of the $[\text{Ni}_2^{\text{II}}(\text{L}^{19})]^{2+}$ fragment. These include Cl^- , OH^- (209), NO_2^- , NO_3^- , N_3^- (242), BH_4^- (259), various carboxylates and alkylcarbonates (239, 256), N_2H_4 , pyrazolate, pyridazine (242), H_2PO_4^- (239), SH^- (243), and some biologically relevant species such as HCO_3^- (239), $(p\text{-NO}_2\text{C}_6\text{H}_4\text{O})_2\text{PO}_2^-$, and proline (260), to name but a few. The methods used to synthesize these complexes include (exemplified here only for the dinickel complexes, Scheme 5): (a) ligand exchange using the chloro-bridged complex **54** or (b) the more substitutionally labile perchlorato complex **74** (itself prepared by Cl^- abstraction from **54** with $\text{Pb}(\text{ClO}_4)_2$ (261)), (c) CO_2 -fixation by the hydroxo-bridged complex **67**, (d) transesterification reactions



SCHEME 5. Different methods used to synthesize $[\text{Ni}_2(\text{L}^{19})(\text{L}')]^+$ complexes.

using the methylcarbonato complex **75**, and (e) reduction of the boranato-bridged complex **76**. In general, these reactions were complete within a few hours at room temperature and provided the desired complexes (as their ClO_4^- or BPh_4^- salts) in good to excellent yields. They are stable in a range of organic solvents and their charged nature confers on them a good solubility.

A selection of the complexes prepared by these methods is listed in Table IV. Single crystals of X-ray quality were obtained in each case, except for **88**. In all cases, except complex **67**, the macrocycle adopts the conical-calixarene like conformation B, as illustrated by two representative examples, $[\text{Ni}_2(\text{L}^{19})(\text{BH}_4)]^+$ (**76**) and $[\text{Ni}_2(\text{L}^{19})(\text{SH})]^+$ (**85**) (Fig. 40). The complexes are thus invariably bioctahedral with either a face-sharing or edge-sharing $\text{N}_3\text{M}(\mu\text{-SR})_2(\mu\text{-L}')\text{MN}_3$ core structure depending on the coordination mode of the coligands. The metal-metal separations ($d(\text{M}\cdots\text{M})$) correlate with the bridging mode of the coligands, i.e. ($d(\mu\text{L}') < d(\mu_{1,2}\text{L}') < d(\mu_{1,3}\text{L}')$).

TABLE IV

SYNTHESIZED $[\text{Ni}_2(\text{L}^{19})(\text{L}')^{N+}]$ COMPLEXES AND THEIR LABELS

L'	Preparation method ^b	Structure type ^b	Coordination mode	$d(\text{M}\cdots\text{M})/\text{\AA}$	Ref.
48 CH_3CO_2^-	<i>a</i>	B	$\mu_{1,3}$	3.483(1)	(239)
67 OH^-	<i>a</i>	A	μ	3.037(3)	(209)
74 ClO_4^-	<i>b</i>	B	$\mu_{1,3}$	3.547(1)	(261)
75 $\text{CH}_3\text{OCO}_2^-$	<i>c</i>	B	$\mu_{1,3}$	3.491(1)	(239)
76 BH_4^-	<i>b</i>	B	$\mu_{1,3}$	3.458(1)	(259)
77 EtOCO_2^-	<i>d</i>	B	$\mu_{1,3}$	3.485(1)	(239)
78 O_2CPh^-	<i>a</i>	B	$\mu_{1,3}$	3.470(1)	(242)
79 NO_3^-	<i>a</i>	B	$\mu_{1,3}$	3.492(1)	(242)
80 NO_2^-	<i>a</i>	B	$\mu_{1,2}$	3.398(1)	(242)
81 N_3^-	<i>a</i>	B	$\mu_{1,3}$	3.683(1)	(242)
82 pydz^a	<i>b</i>	B	$\mu_{1,2}$	3.392(1)	(242)
83 N_2H_4	<i>a</i>	B	$\mu_{1,2}$	3.441(1)	(242)
84 HCO_2^-	<i>b</i>	B	$\mu_{1,3}$	3.480(1)	(259)
85 SH^-	<i>e</i>	B	μ	3.295(1)	(243)
86 $\text{CpFeC}_5\text{H}_4\text{CO}_2^-$	<i>a</i>	B	$\mu_{1,3}$	3.485(1)	(262)
87 SO_4^{2-}	<i>a</i>	B	$\mu_{1,3}$	3.506(1)	(261)
88 MoO_4^{2-}	<i>a</i>	B	$\mu_{1,3}$	n.d. ^c	(261)
89 WO_4^{2-}	<i>a</i>	B	$\mu_{1,3}$	3.655(1)	(261)
90 $\text{PhCH}_2\text{NCO}_2^-$	<i>d</i>	B	$\mu_{1,3}$	3.472(1)	(263)
91 HCN_4^-	<i>a</i>	B	$\mu_{1,2}$	3.455(1)	(264)

^apydz = pyridazine.

^bFor the meaning of the label, see Scheme 5 and Fig. 34.

^cNot determined.

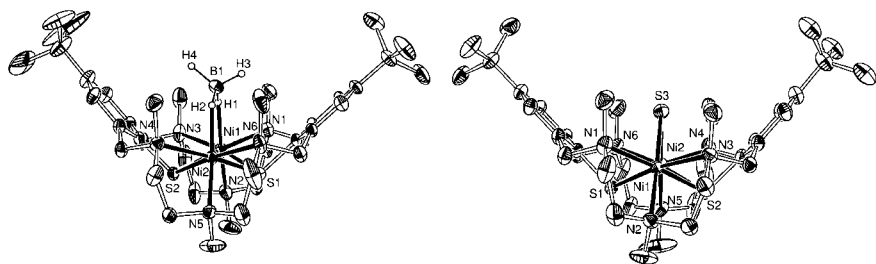


FIG. 40. Molecular structures of **76** (left) and **85** (right) in the crystal. The figures were generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and correspond to the structures originally reported in Refs. (259) and (243), respectively.

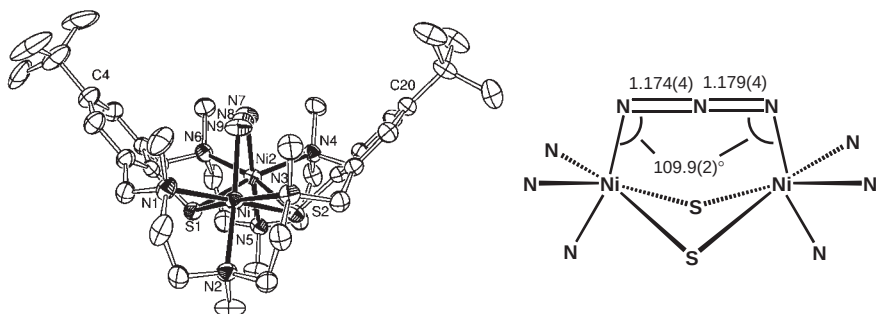


FIG. 41. (Left) Molecular structure of the azide complex **81** in the crystal. (Right) Central $\text{N}_3\text{Ni}(\mu\text{-S})_2(\mu_{1,3}\text{-N}_3)\text{NiN}_3$ core in **81**. The figures were generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and correspond to the structure originally reported in Ref. (242).

Some of the complexes feature unusual ligand coordination modes. The azide complex **81** is a representative example (Fig. 41). The planarity of the $\text{Ni-N}_3\text{-Ni}$ assembly (torsional angle $\tau = 0^\circ$) together with the remarkably obtuse Ni-N-N angles at $109.9(2)^\circ$ has never been observed before for $\text{M}-\mu_{1,3}\text{-N}_3\text{-M}$ linkages (265–267). The presence of this distinct binding mode can be traced back to the complementary size and form of the binding pocket of the $[\text{Ni}_2(\text{L}^{19})]^{2+}$ complex. Similar effects have been observed by McKee and Nelson, who stabilized a nearly linear $\text{Ni}-(\mu_{1,3}\text{-N}_3)\text{-Ni}$ linkage in the cavity of a dinuclear nickel cryptate complex (268,269). The magnetic properties of dinuclear nickel complexes with such extreme azide coordination modes are of interest, because the theory predicts unusual large

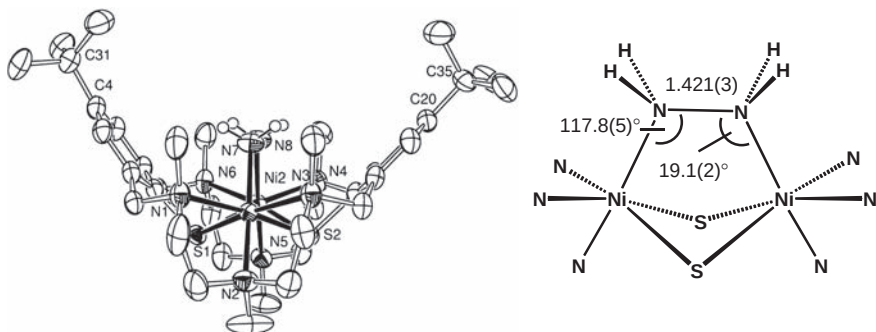


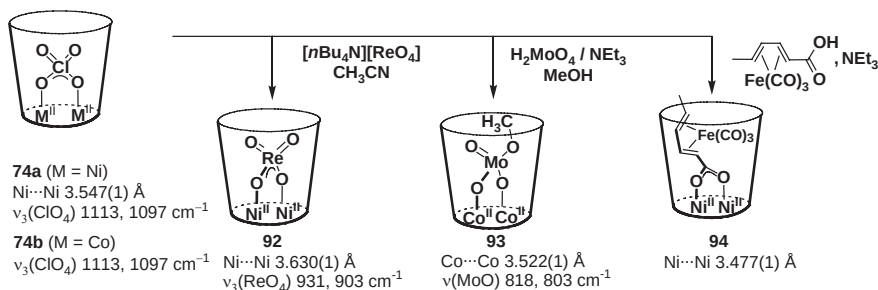
FIG. 42. (Left) Molecular structure of the hydrazine complex **83** in the crystal. (Right) Central $\text{N}_3\text{Ni}(\mu\text{-S})_2(\mu_{1,2}\text{-N}_2\text{H}_4)\text{NiN}_3$ core in **83**. The figure was generated using data downloaded from The Cambridge Crystallographic Data Center (CCDC) and corresponds to the structure originally reported in Ref. (242).

intramolecular magnetic exchange interactions for such species (270,271).

The hydrazine complex **83** is an example of a complex that features an unusual conformation of a small neutral inorganic molecule. Free hydrazine exists predominantly in the *gauche* conformation at room temperature (dihedral angle $\tau \sim 100^\circ$) (272), also most commonly seen in dinuclear hydrazine complexes (273,274). In **83**, the N_2H_4 ligand can only adopt the *cis* (ecliptic) conformation ($\tau = 3.7^\circ$) (Fig. 42). To the best of our knowledge, such a coordination mode is without precedence in dinuclear transition-metal hydrazine complexes (275–279), albeit it is documented for mononuclear species (280,281). The ecliptic N_2H_4 conformation is presumably a consequence of repulsive $\text{NH}\cdots\text{C}_{\text{aryl}}$ van der Waals interactions between the N_2H_4 molecule and the phenyl rings of $(\text{L}^{19})^{2-}$.

More recent examples of metalated container molecules based on the polyamine-dithiophenolate macrocycle $(\text{L}^{19})^{2-}$ are **92–94** bearing classical (255,261) or organometallic metallo coligands (Scheme 6) in their binding pockets (260). Such compounds are readily accessible from the labile ClO_4^- complexes **74a,b** (Scheme 6). They are stable in a range of organic solvents and their charged nature confers on them a good solubility.

In complex **95**, an unusual conformation of an organic molecule is present as shown in Fig. 43 (215). The substituents in cyclohexene rings generally assume equatorial positions. In **95**, they are axial. The container molecules can also stabilize resonance extremes. Complex **96**, bearing a *p*-nitrophenolate in the quinoid *aci*-form is an example (228). Again, these unusual coordination



SCHEME 6. Preparation of the complexes **92-94**. The bowl-shaped representation of the ligand (L^{19}) $^{2-}$ should not be confused with the one used for the cyclodextrins.

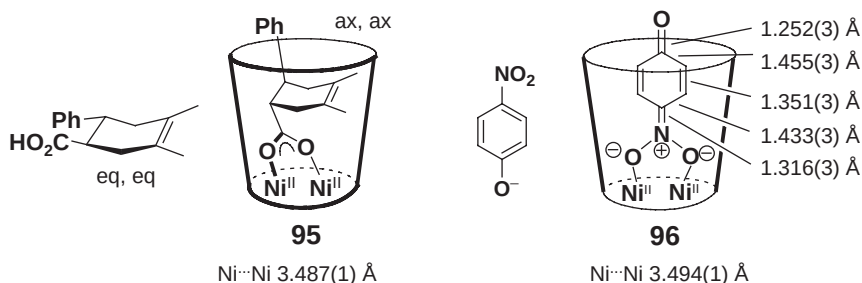


FIG. 43. Binding mode of the coligands in **95** and **96**. The bowl-shaped representation of the ligand (L^{19}) $^{2-}$ should not be confused with the one used for the cyclodextrins.

modes are directly related to the restricted space in the binding pocket of the $[\text{Ni}_2(\text{L}^{19})]^{2+}$ complex.

Finally, reactive molecules can also be incorporated in the binding cavities. The BH_4^- complex **76**, for example, is stable for weeks both in the solid state and in solution in the absence of air and protic reagents. This stability is quite remarkable given that nickel(II) complexes of sterically less demanding ligands are readily reduced to nickel boride. Nevertheless, the BH_4^- becomes activated upon coordination, as indicated by IR by the shifts of the B–H stretching frequencies (Fig. 44). The complex thus reacts rapidly with protic reagents (HCl , H_2O), with electrophiles (CO_2 , S_8) and halogenated solvents (CCl_4) as shown in Scheme 7.

In summary, a large number of coligands can be accommodated in the binding pockets of the $[\text{M}_2(\text{L}^{19})]^{2+}$ complexes. In each case only one of several possible coordination modes of the coligand is realized. In some cases, the binding pocket confers very unusual

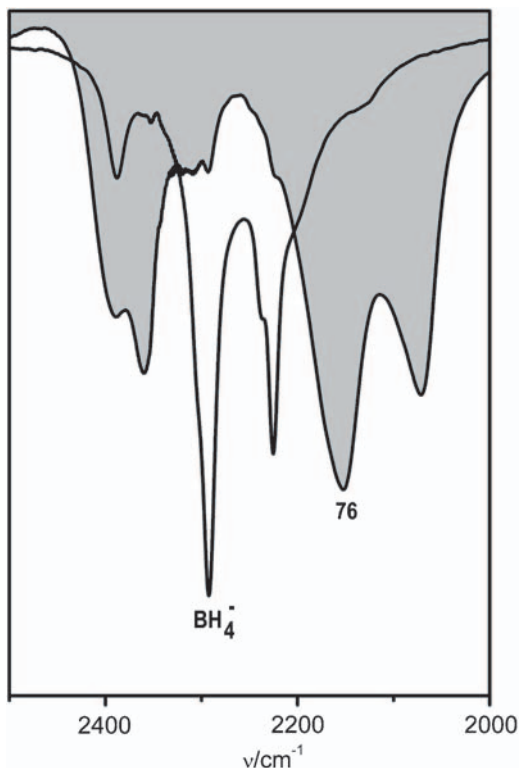
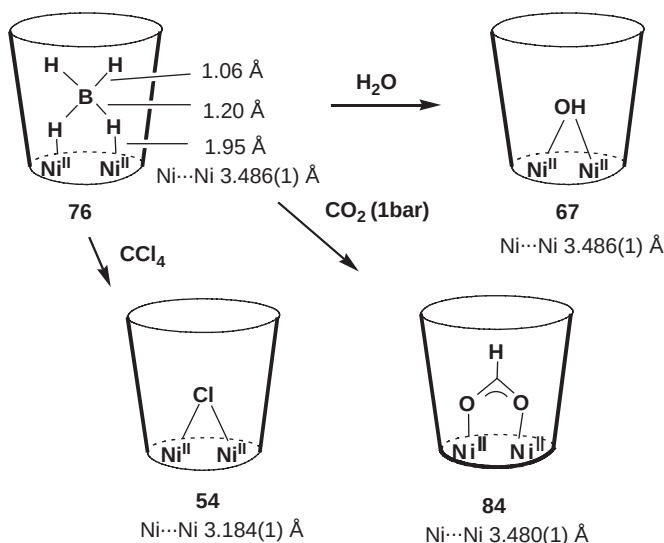


FIG. 44. Section of the IR spectrum of **76** showing the B-H stretching region (2500-2000 cm^{-1}).

coordination modes or conformations on the guest molecules. Stabilization and activation of reactive molecules are observed in other instances. Since the complex integrity is retained in the solution state, the reactivity of these compounds can be examined (see below).

G. REACTIVITY OF THE COMPLEXES

It has already been mentioned that metal complexes with confined binding pockets often display unusual chemical reactivities (see [Section II](#)). Thus, complexes of substituted hydrotris (pyrazolyl)borates, in which the substituents serve to form a hydrophobic binding pocket, have already been shown to exhibit enhanced chemical reactivity when compared with their unmodified analogs (282,283). Likewise, cyclodextrin and calixarene-based metallocavitands have been used as catalysts for selective organic transformations, and even as catalysts for reactions that

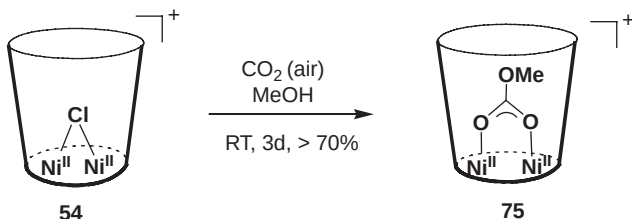
SCHEME 7. Reactions of **76**.

depend on the reaction medium. Some of these complexes were designed to mimic the hydrophobic binding site of metalloproteins (58). In contrast, the study of the reactivity of related binuclear complexes is very limited. This led us to study the reactivity of the $[\text{M}_2(\text{L}^{19})(\mu\text{-L}')]^+$ complexes, hoping that the bowl-shaped ligand matrix exerts an effect on the rate and course of the substrate transformations.

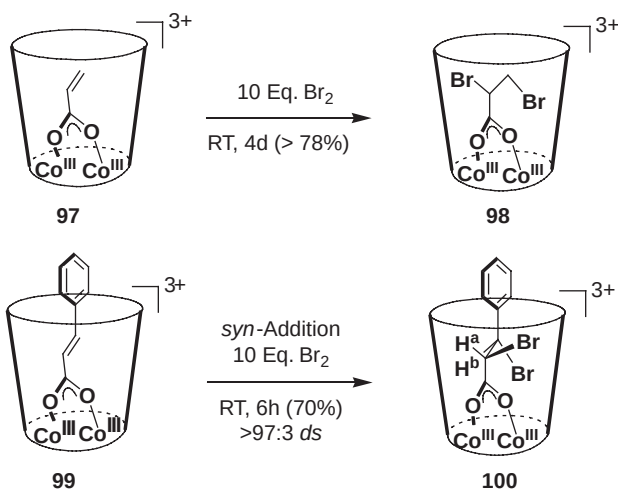
It has already been mentioned that utilization of the permethylated ligand $(\text{L}^{19})^{2-}$ in place of $(\text{L}^{23})^{2-}$ drastically alters the ease of substitution reactions of the $[\text{M}_2(\text{L}^{19})(\text{Cl})]^+$ complexes (Section III.D). Further studies revealed a remarkable influence of the hydrophobic pocket on the rate and course of several substrate transformations, as for instance the fixation of carbon dioxide (239) (Scheme 8), the *cis*-bromination of α,β -unsaturated carboxylate ligands (256), and some Diels–Alder reactions (215). Of these the latter two reactions will now be discussed.

The use of a metalated container in stereoselective transformations has only recently begun to be explored (284). This prompted us to study the bromination of the α,β -unsaturated carboxylate ligands in dicobalt(III) complexes of the type $[\text{Co}_2(\text{L}^{19})(\mu\text{-O}_2\text{CCH}=\text{CHR})]^{3+}$ (256).

The acrylate-bridged dicobalt(III) complex **97** (241) was selected in an orienting experiment (Scheme 9). Indeed, bromination of the kinetically inert complex proceeded smoothly and produced complex **98** as the sole product, as established by IR



SCHEME 8. Fixation of CO_2 by **54**. The bowl-shaped representation of the ligand $(\text{L}^{19})^{2-}$ should not be confused with the one used for the cyclodextrins.



SCHEME 9. Bromination of α,β -unsaturated carboxylate ligands in **97** and **99** (256). The bowl-shaped representation of the ligand $(\text{L}^{19})^{2-}$ should not be confused with the one used for the cyclodextrins.

and NMR spectroscopy. The bromination of the cinnamato-bridged dicobalt(III) complex **99** was examined next. In this case, the reaction was complete after 6 h at ambient temperature and yielded a single addition product **100** in nearly quantitative yield (Scheme 9).

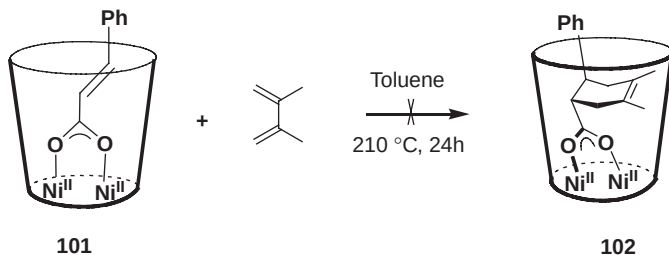
NMR-spectroscopic studies and a single crystal X-ray structure determination of the reduced Co_2^{II} form of **100** revealed the presence of a bridging 2,3-dibromo-3-phenyl-propionato ligand (*threo dl* pair). The complex bearing the *erythro* form of 2,3-dibromo-3-phenyl-propionate is only produced in minor yields <3%. Therefore, the bromination of the encapsulated alkene is a highly diastereoselective *syn*-addition. This is rather

unusual and is in striking contrast to the bromination of the free acid, which is an *anti*-addition (285). Further, the bromination of the encapsulated alkene is 2–3 orders of magnitude slower. The decrease in the rate presumably relates to steric effects. This is true in particular, when one recalls that the bromination of free alkenes begins with the formation of olefine–Br₂ π -complexes with T-shaped structures (286,287). In our case, such a π -complex cannot form due to steric interactions with the ligand matrix. We assume that the hydrophobic ligand matrix also contributes to the decrease in the rate in that it destabilizes the hypothetical bromonium ion intermediate. The fact that the binding pocket of our complexes favors uncharged species over charged ones is in good agreement with this assumption (242). We also note that it is possible to liberate the brominated product from the binding pocket. This shows that these container molecules are also of synthetic value.

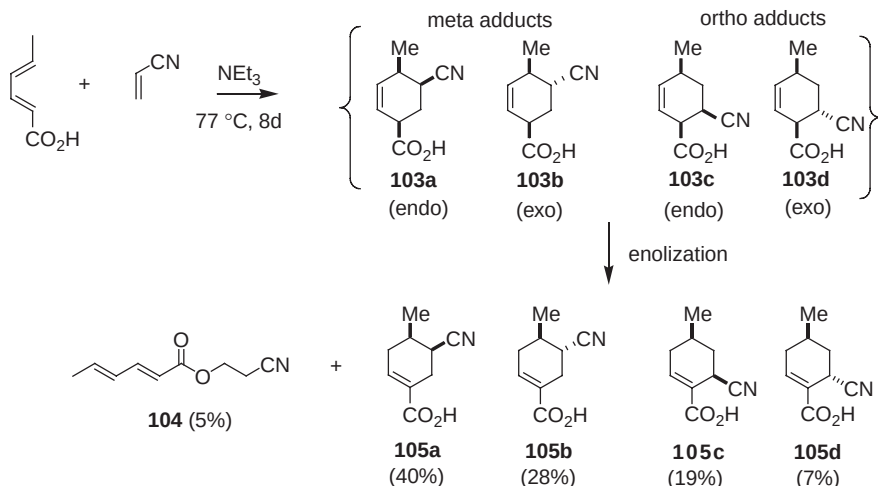
Recently, it has been found that the outcome of some cycloadditions can be altered remarkably when performed inside the cavity of cyclodextrins (288), self-assembled molecular capsules (289), or coordination cages (290). This fact intrigued us greatly and stimulated our interest in the Diels–Alder reactivity of the “calixarene-like” $[M_2(L^{19})(L')]^+$ complexes bearing unsaturated carboxylate coligands L' (215).

The observation that α,β -unsaturated carboxylate ligands can be readily accommodated in the binding pocket led us to study an orienting reaction between the dinickel complex **101** and 2,3-dimethylbutadiene (Scheme 10). However, the reaction did not proceed, even when the reaction mixture was heated at 210 °C for 24 h. The inhibition of the Diels–Alder reaction can be traced to the limited space in the binding pocket of **101**.

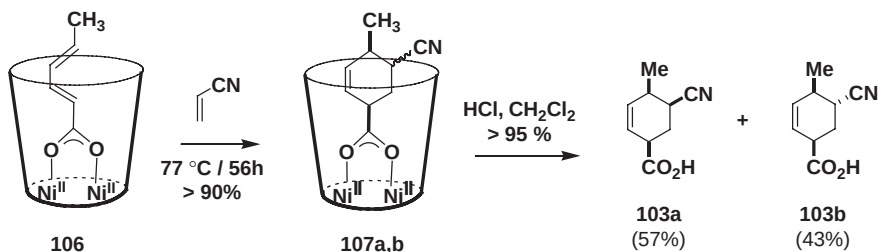
The above findings prompted us to study the reaction between a coordinated dienolate ligand and an external alkene. The



SCHEME 10. Attempted Diels–Alder reaction between **101** and 2,3-dimethylbutadiene (215).



SCHEME 11. The Diels–Alder reaction between sorbic acid and acrylonitrile (215).

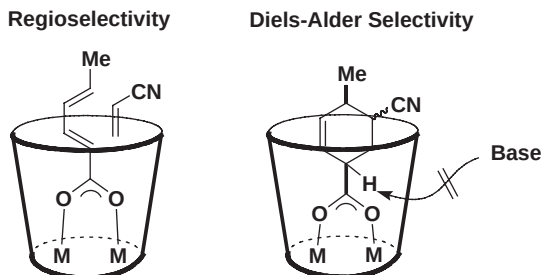


SCHEME 12. The Diels–Alder reaction between **106** and acrylonitrile (215).

reaction between sorbic acid and acrylonitrile was selected (Scheme 11).

The free reaction is rather slow (pseudo-first-order rate constant $k' = 4.8 \times 10^{-6} \text{ s}^{-1}$, $\tau_{1/2}$ ca. 2 days) and produces a mixture of the four possible Diels–Alder adducts **103a–d** along with the byproduct **104**. Note that all acids isomerize under the basic reaction conditions to give the α,β -unsaturated derivatives **105a–d**.

Thereafter, the reaction between the coordinated dienolate ligand in **106** and acrylonitrile was examined (Scheme 12). Surprisingly, this reaction is complete within 56 h (pseudo-first-order rate constant $k' = 1.4 \times 10^{-5} \text{ s}^{-1}$, $\tau_{1/2}$ ca. 0.5 day) and affords only two products **107a** and **107b** in a ratio of 57:43 (or the corresponding acids **103a,b** via acid hydrolysis). Thus, in



SCHEME 13. Directing and protecting effect of the binding cavity.

striking contrast to the low regioselectivity observed in the background reaction (Scheme 11), the Diels–Alder reaction between the encapsulated dienoate and acrylonitrile proceeds with strict “*meta*” regioselectivity. In addition, no byproducts are detected.

This and the fact that the Diels–Alder adducts **103a,b** do not isomerize in the binding pocket of the complexes can be attributed to the directing and protecting effect of the binding cavity as schematically represented in Scheme 13.

Similar results were obtained for analogous complexes of the de-(*tert*-butyl)ated macrocycle ($L^{24})^{2-}$. The *tert*-butyl substituents do not affect the regiochemistry of this particular Diels–Alder reaction, but they clearly increase its rate. The observed trend is indicative of a small stabilization of the transition-state by hydrophobic effects ($\Delta\Delta G^\ddagger \approx 3$ kJ/mol; $k'_{\text{complex}}/k'_{\text{background}} = \exp(\Delta\Delta G^\ddagger/RT)$). This would be consistent with our earlier observation that complexes bearing less polar carboxylate anions have the higher stability constants (see Section III.E).

Overall, the reaction between the coordinated sorbate coligand and acrylonitrile is controlled by the binding cavity of the complexes and is highly regioselective. The new method is currently only applicable to dienes with anchoring carboxylate groups, but expansion of this approach to a general concept for the control of the regioselectivity of Diels–Alder reactions between unsymmetrical dienes and dienophiles appears to be in reach.

IV. Conclusion

The development, synthesis, and the coordination chemistry of novel metal complexes with deep binding cavities have been described. It was demonstrated that the doubly deprotonated forms of the macrocycles ($L^R)^{2-}$ support the formation of

complexes of the type $[M_2(L^R)(L')^+]^+$ with bowl-shaped, "calixarene-like" structures. The compounds are amongst the first prototypes for metalated container molecules with open binding cavities. Despite the fact that the active coordination site L' is deeply buried in the center of the molecules, it is readily accessible for a wide range of exogenous guest molecules. A large number of crystal structure determinations have provided insights into the binding modes of the coligands. These sense the size and form of the binding pocket of the $[M_2(L^R)]^{2+}$ fragments as indicated by distinctive binding modes and unusual conformations. The use of the N-alkylated macrocycles in place of the unmodified analogs influences many properties of the binuclear complexes, including color, molecular and electronic structure, hydrogen bonding interactions, redox potential, complex stability, and ground spin-state. The reactivity is also greatly affected as shown by the activation and transformation of small molecules such as CO_2 , the highly diastereoselective *cis*-bromination of α,β -unsaturated carboxylate coligands, and some regioselective Diels–Alder reactions. Future work will be directed to the coordination chemistry of functionalized derivatives of H_2L .¹⁹

ACKNOWLEDGMENTS

The authors thank several coworkers named in their joint publications who made major contributions to the chemistry described here. This chemistry would not have reached its current level of broad synthetic utility without their dedication. This work was also supported by financial assistance by the Deutsche Forschungsgemeinschaft (SPP 1118, "Sekundäre Wechselwirkungen"), the Universität Freiburg, and the Universität Leipzig.

REFERENCES

1. Cram, D. J. *"Container Molecules and their Guests"*; RSC: Cambridge, **1999**.
2. Vögtle, F. *"Supramolekulare Chemie"*; Teubner: Stuttgart, **1992**.
3. Lehn, J. M. *"Supramolecular Chemistry"*; Wiley: Chichester, **1995**.
4. Steed, J. W.; Atwood, J. L. *"Supramolecular Chemistry"*; Wiley: Chichester, **2000**.
5. Steed, J. W.; Turner, D. R.; Wallace, K. J. *"Core Concepts in Supramolecular Chemistry and Nanochemistry"*; Wiley-VCH: Weinheim, **2007**.

6. Andreetti, G. D.; Ungaro, R.; Pochini, A. *J. Chem. Soc., Chem. Commun.* **1979**, 1005–1007.
7. In this article, all species with a binding cavity (open or closed) are referred to as container molecules.
8. (a) Hecht, S.; Fréchet, J. M. J. *Angew. Chem.* **2001**, *113*, 76–94. (b) Hecht, S.; Fréchet, J. M. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 74–91.
9. Vriezema, D. M.; Aragonès, M. C.; Elemans, J. A. A. W.; Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M. *Chem. Rev.* **2005**, *105*, 1445–1489.
10. Rivera, J. M.; Martín, T.; Rebek, J., Jr. *Science* **1998**, *279*, 1021–1023.
11. (a) Ritter, H.; Sadowski, O.; Tepper, E. *Angew. Chem.* **2003**, *115*, 3279–3281. (b) Ritter, H.; Sadowski, O.; Tepper, E. *Angew. Chem. Int. Ed.* **2003**, *42*, 3171–3173.
12. (a) Warmuth, R.; Kerdelhué, J.-L.; Carrera, S. S.; Langenwalter, K. J.; Brown, N. *Angew. Chem.* **2002**, *114*, 102–105. (b) Warmuth, R.; Kerdelhué, J.-L.; Carrera, S. S.; Langenwalter, K. J.; Brown, N. *Angew. Chem. Int. Ed.* **2002**, *41*, 96–99.
13. (a) Roach, P.; Warmuth, R. *Angew. Chem.* **2003**, *115*, 3147–3150. (b) Roach, P.; Warmuth, R. *Angew. Chem. Int. Ed.* **2003**, *42*, 3039–3042.
14. (a) Nakabayashi, K.; Ozaki, Y.; Kawano, M.; Fujita, M. *Angew. Chem.* **2008**, *120*, 2076–2078. (b) Nakabayashi, K.; Ozaki, Y.; Kawano, M.; Fujita, M. *Angew. Chem. Int. Ed.* **2008**, *47*, 2046–2048.
15. (a) Hof, F.; Craig, S. L.; Nuckolls, C.; Rebek, J., Jr. *Angew. Chem.* **2002**, *114*, 1556–1578. (b) Hof, F.; Craig, S. L.; Nuckolls, C.; Rebek, J., Jr. *Angew. Chem. Int. Ed.* **2002**, *41*, 1488–1508.
16. (a) Rebek, J., Jr. *Angew. Chem.* **2005**, *117*, 2104–2115. (b) Rebek, J., Jr. *Angew. Chem. Int. Ed.* **2005**, *44*, 2068–2078.
17. Rousseau, C.; Christensen, B.; Bols, M. *Eur. J. Org. Chem.* **2005**, 2734–2739.
18. (a) Yamaguchi, T.; Fujita, M. *Angew. Chem.* **2008**, *120*, 2097–2099. (b) Yamaguchi, T.; Fujita, M. *Angew. Chem. Int. Ed.* **2008**, *47*, 2067–2069.
19. (a) Rissanen, K. *Angew. Chem.* **2005**, *117*, 3718–3720. (b) Rissanen, K. *Angew. Chem. Int. Ed.* **2005**, *44*, 3652–3654.
20. (a) Oshovsky, G. V.; Reinhoudt, D. N.; Verboom, W. *Angew. Chem.* **2007**, *119*, 2418–2445. (b) Oshovsky, G. V.; Reinhoudt, D. N.; Verboom, W. *Angew. Chem. Int. Ed.* **2007**, *46*, 2366–2393.
21. (a) Schmuck, C. *Angew. Chem.* **2007**, *119*, 5932–5935. (b) Schmuck, C. *Angew. Chem. Int. Ed.* **2007**, *46*, 5830–5833.
22. (a) Cram, D. J.; Tanner, M. E.; Thomas, R. *Angew. Chem.* **1991**, *103*, 1048–1051. (b) Cram, D. J.; Tanner, M. E.; Thomas, R. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1024–1027.
23. Gutsche, C. D. Calixarenes, revisited. In: “*Monographs in Supramolecular Chemistry*”; Ed. Stoddart, J. F.; Royal Society of Chemistry: Cambridge, **1998**.
24. (a) McKinlay, R. M.; Atwood, J. L. *Angew. Chem.* **2007**, *119*, 2446–2449. (b) McKinlay, R. M.; Atwood, J. L. *Angew. Chem. Int. Ed.* **2007**, *46*, 2394–2397.
25. (a) Lützen, A. *Angew. Chem.* **2005**, *117*, 1022–1025. (b) Lützen, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 1000–1002.
26. Warmuth, R.; Yoon, J. *Acc. Chem. Res.* **2001**, *34*, 95–105.
27. (a) Custelcean, R.; Remy, P.; Bonnesen, P. V.; Jiang, D.; Moyer, B. A. *Angew. Chem.* **2008**, *120*, 1892–1896. (b) Custelcean, R.; Remy, P.; Bonnesen, P. V.; Jiang, D.; Moyer, B. A. *Angew. Chem. Int. Ed.* **2008**, *47*, 1866–1870.

28. Gibb, C. L. D.; Gibb, B. C. *J. Am. Chem. Soc.* **2004**, *126*, 11408–11409.
29. Caulder, D. L.; Raymond, K. N. *Acc. Chem. Res.* **1999**, *32*, 975–982.
30. Seidel, S. R.; Stang, P. J. *Acc. Chem. Res.* **2002**, *35*, 972–983.
31. Fujita, M.; Tominaga, M.; Hori, A.; Therrien, B. *Acc. Chem. Res.* **2005**, *38*, 369–378.
32. (a) Cronin, L. *Angew. Chem.* **2006**, *118*, 3656–3658. (b) Cronin, L. *Angew. Chem. Int. Ed.* **2006**, *45*, 3576–3578.
33. (a) Hiraoka, S.; Harano, K.; Shiro, M.; Ozawa, Y.; Yasuda, N.; Toriumi, K.; Shionoya, M. *Angew. Chem.* **2006**, *118*, 6638–6641. (b) Hiraoka, S.; Harano, K.; Shiro, M.; Ozawa, Y.; Yasuda, N.; Toriumi, K.; Shionoya, M. *Angew. Chem. Int. Ed.* **2006**, *45*, 6488–6491.
34. Yoshizawa, M.; Tamura, M.; Fujita, M. *Science* **2006**, *312*, 251–254.
35. Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146–153.
36. Canary, J. W.; Gibb, B. C. *Prog. Inorg. Chem.* **1997**, *45*, 1–81.
37. Armspach, D.; Matt, D. *Chem. Commun.* **1999**, 1073–1074.
38. Cameron, B. R.; Loeb, S. J.; Yap, G. P. A. *Inorg. Chem.* **1997**, *36*, 5498–5504.
39. (a) Reetz, M. T.; Waldvogel, S. R. *Angew. Chem.* **1997**, *109*, 870–873. (b) Reetz, M. T.; Waldvogel, S. R. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 865–867.
40. Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev.* **2000**, *29*, 75–86.
41. Breslow, R. (Ed.), “*Artificial Enzymes*”; Wiley-VCH: Weinheim, Germany, **2005**.
42. S  n  que, O.; Campion, M.; Giorgi, M.; Le Mest, Y.; Reinaud, O. *Eur. J. Inorg. Chem.* **2004**, 1817–1826.
43. Lejeune, M.; S  meril, D.; Jeunesse, C.; Matt, D.; Peruch, F.; Lutz, P. J.; Ricard, L. *Chem. Eur. J.* **2004**, *10*, 5354–5360.
44. (a) Engeldinger, E.; Armspach, D.; Matt, D.; Jones, P. G.; Welter, R. *Angew. Chem.* **2002**, *114*, 2705–2708. (b) Engeldinger, E.; Armspach, D.; Matt, D.; Jones, P. G.; Welter, R. *Angew. Chem. Int. Ed.* **2002**, *41*, 2593–2596.
45. Hammes, B. S.; Ramos-Maldonado, D.; Yap, G. P. A.; Liab  -Sands, L.; Rheingold, A. L.; Young, V. G., Jr.; Borovik, A. S. *Inorg. Chem.* **1997**, *36*, 3210–3211.
46. Vahrenkamp, H. *Acc. Chem. Res.* **1999**, *32*, 589–596.
47. Kitajima, N.; Tolman, W. B. *Prog. Inorg. Chem.* **1995**, *43*, 419–531.
48. Trofimenko, S. “*Scorpionates: The Coordination Chemistry of Polypyrazolylborate Ligands*”; Imperial College Press: London, U.K., **1999**.
49. Collman, J. P.; Boulatov, R.; Sunderland, C. J.; Fu, L. *Chem. Rev.* **2004**, *104*, 561–588.
50. Sanders, J. K. M. *Chem. Eur. J.* **1998**, *4*, 1378–1383.
51. Brady, P. A.; Sanders, J. K. M. *Chem. Soc. Rev.* **1997**, *26*, 327–336.
52. Sanders, J. K. M. In: “*Comprehensive Supramolecular Chemistry*”; vol. 9; Eds. Atwood, J. L.; Davies, J. E. D.; Macnicol, D. D.; V  gtle, F.; Elsevier, **1996**.
53. Marty, M.; Clyde-Watson, Z.; Twyman, L. J.; Nakash, M.; Sanders, J. K. M. *Chem. Commun.* **1998**, 2265–2266.
54. (a) B  hmer, V. *Angew. Chem.* **1995**, *107*, 785–818. (b) B  hmer, V. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 145–713.
55. (a) Blanchard, S.; Clainche, L. L.; Rager, M.-N.; Chansou, B.; Tuchagues, J.-P.; Duprat, A. F.; Le Mest, Y.; Reinaud, O. *Angew. Chem.* **1998**, *110*, 2861–2864. (b) Blanchard, S.; Clainche, L. L.; Rager, M.-N.; Chansou, B.;

- Tuchagues, O.; Duprat, A. F.; Le Mest, Y.; Reinaud, O. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2732–2735.
56. (a) Rondelez, Y.; Bertho, G.; Reinaud, O. *Angew. Chem.* **2002**, *114*, 1086–1088. (b) Rondelez, Y.; Bertho, G.; Reinaud, O. *Angew. Chem. Int. Ed.* **2002**, *41*, 1044–1046.
 57. Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2000**, *122*, 6183–6189.
 58. Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2001**, *123*, 8442–8443.
 59. Coquière, D.; Cadeau, H.; Rondelez, Y.; Giorgi, M.; Reinaud, O. *J. Org. Chem.* **2006**, *71*, 4059–4065.
 60. Coquière, D.; Marrot, J.; Reinaud, O. *Chem. Commun.* **2006**, 3924–3926.
 61. (a) LeGac, S.; Marrot, J.; Reinaud, O.; Jabin, I. *Angew. Chem.* **2006**, *118*, 3195–3198. (b) LeGac, S.; Marrot, J.; Reinaud, O.; Jabin, I. *Angew. Chem. Int. Ed.* **2006**, *45*, 3123–3126.
 62. Reinaud, O.; Le Mest, Y.; Jabin, I. In: “*Calixarenes Enter The Nano-world*”; Eds. Harrowfield, J.; Vicens, J.; Springer: Dordrecht, **2007**.
 63. Zeng, X.; Coquière, D.; Alenda, A.; Garrier, E.; Prangé, T.; Li, Y.; Reinaud, O.; Jabin, I. *Chem. Eur. J.* **2006**, *12*, 6393–6402.
 64. Izzet, G.; Zeng, X.; Akdas, H.; Marrot, J.; Reinaud, O. *Chem Commun.* **2007**, 810–812.
 65. Wieser, C.; Dieleman, C. B.; Matt, D. *Coord. Chem. Rev.* **1997**, *165*, 93–161.
 66. (a) Wieser-Jeunesse, C.; Matt, D.; De Cian, A. *Angew. Chem.* **1998**, *110*, 3027–3030. (b) Wieser-Jeunesse, C.; Matt, D.; De Cian, A. *Angew. Chem. Int. Ed.* **1998**, *37*, 2861–2864.
 67. (a) Sémeril, D.; Jeunesse, C.; Matt, D.; Toupet, L. *Angew. Chem.* **2006**, *118*, 5942–5946. (b) Sémeril, D.; Jeunesse, C.; Matt, D.; Toupet, L. *Angew. Chem. Int. Ed.* **2006**, *45*, 5810–5814.
 68. Fiedler, D.; Leung, D. H.; Bergman, R. G.; Raymond, K. N. *Acc. Chem. Res.* **2005**, *38*, 349–358.
 69. (a) Slagt, V. F.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Angew. Chem.* **2001**, *113*, 4401–4404. (b) Slagt, V. F.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Angew. Chem. Int. Ed.* **2001**, *40*, 4271–4274.
 70. (a) Slagt, V. F.; van Leeuwen, P. W. N. M.; Reek, J. N. H. *Angew. Chem.* **2003**, *113*, 5777–5781. (b) Slagt, V. F.; van Leeuwen, P. W. N. M.; Reek, J. N. H. *Angew. Chem. Int. Ed.* **2003**, *42*, 5619–5623.
 71. Slagt, V. F.; Kamer, P. C. J.; van Leeuwen, P. W. N. M.; Reek, J. N. H. *J. Am. Chem. Soc.* **2004**, *126*, 1526–1536.
 72. Kleij, A. W.; Kuil, M.; Tooke, D. M.; Spek, A. L.; Reek, J. N. H. *Inorg. Chem.* **2005**, *44*, 7696–7698.
 73. Kleij, A. W.; Reek, J. N. H. *Chem. Eur. J.* **2006**, *12*, 4218–4227.
 74. (a) Flapper, J.; Reek, J. N. H. *Angew. Chem.* **2007**, *119*, 8744–8746. (b) Flapper, J.; Reek, J. N. H. *Angew. Chem. Int. Ed.* **2007**, *46*, 8590–8592.
 75. Koblenz, T. S.; Dekker, H. L.; de Koster, C. G.; van Leeuwen, P. W. N. M.; Reek, J. N. H. *Chem. Commun.* **2006**, 1700–1702.
 76. (a) Cave, G. W. V.; Antesberger, J.; Barbour, L. J.; McKinlay, R. M.; Atwood, J. L. *Angew. Chem.* **2004**, *116*, 5375–5378. (b) Cave, G. W. V.; Antesberger, J.; Barbour, L. J.; McKinlay, R. M.; Atwood, J. L. *Angew. Chem. Int. Ed.* **2004**, *43*, 5263–5266.
 77. (a) Power, N. P.; Dalgarno, S. J.; Atwood, J. L. *Angew. Chem.* **2007**, *119*, 8755–8758. (b) Power, N. P.; Dalgarno, S. J.; Atwood, J. L. *Angew. Chem.* **2007**, *46*, 8601–8604.

78. Del Valle, E. M. M. *Proc. Biochem.* **2004**, 39, 1033–1046.
79. The entire July/August volume of *Chem. Rev.* **1998**, 98 is devoted to cyclodextrins.
80. (a) Yuan, D.-Q.; Kitagawa, Y.; Aoyama, K.; Douke, T.; Fukudome, M.; Fujita, K. *Angew. Chem.* **2007**, 119, 5112–5115. (b) Yuan, D.-Q.; Kitagawa, Y.; Aoyama, K.; Douke, T.; Fukudome, M.; Fujita, K. *Angew. Chem. Int. Ed.* **2007**, 46, 5024–5027.
81. Breslow, R. In: “Artificial Enzymes”; Ed. Breslow, R.; Wiley-VCH: Weinheim, **2005**, pp. 1–35.
82. (a) Engeldinger, E.; Armspach, D.; Matt, D. *Angew. Chem.* **2001**, 113, 2594–2597. (b) Engeldinger, E.; Armspach, D.; Matt, D. *Angew. Chem.* **2001**, 40, 2526–2529.
83. Poorters, L.; Armspach, D.; Matt, D.; Toupet, L.; Choua, S.; Turek, P. *Chem. Eur. J.* **2007**, 13, 9448–9461.
84. (a) Poorters, L.; Armspach, D.; Matt, D.; Toupet, L.; Jones, P. G. *Angew. Chem.* **2007**, 119, 2717–2719. (b) Poorters, L.; Armspach, D.; Matt, D.; Toupet, L.; Jones, P. G. *Angew. Chem. Int. Ed.* **2007**, 46, 2663–2665.
85. (a) Kano, K.; Ishida, Y. *Angew. Chem.* **2007**, 119, 741–744. (b) Kano, K.; Ishida, Y. *Angew. Chem.* **2007**, 46, 727–730.
86. (a) Machut, C.; Patriceon, J.; Tilloy, S.; Bricout, H.; Hapiot, F.; Monflier, E. *Angew. Chem.* **2007**, 119, 3100–3102. (b) Machut, C.; Patriceon, J.; Tilloy, S.; Bricout, H.; Hapiot, F.; Monflier, E. *Angew. Chem. Int. Ed.* **2007**, 46, 3040–3042.
87. Chaudhuri, P.; Wiegardt, K. *Prog. Inorg. Chem.* **1987**, 35, 329–436.
88. (a) Chaudhuri, P.; Wiegardt, K.; Nuber, B.; Weiss, J. *Angew. Chem.* **1985**, 97, 774–775. (b) Chaudhuri, P.; Wiegardt, K.; Nuber, B.; Weiss, J. *Angew. Chem., Int. Ed. Engl.* **1985**, 24, 778–779.
89. Diebold, A.; Elboudili, A.; Hagen, K. S. *Inorg. Chem.* **2000**, 39, 3915–3923.
90. Tolman, W. B. *Acc. Chem. Res.* **1997**, 30, 227–237.
91. Trofimenko, S. *Chem. Rev.* **1993**, 93, 943–980.
92. MacBeth, C. E.; Golombek, A. P.; Young, V. G., Jr.; Yang, C.; Kuczera, K.; Hendrich, M. P.; Borovik, A. S. *Science* **2000**, 289, 938–941.
93. Castro-Rodriguez, I.; Nakai, H.; Zakharov, L. N.; Rheingold, A. L.; Meyer, K. *Science* **2004**, 305, 1757–1759.
94. Yandulov, D. V.; Schrock, R. R. *Science* **2003**, 301, 76–78.
95. Seefeldt, L. C.; Dance, I. G.; Dean, D. R. *Biochemistry* **2004**, 43, 1401–1409.
96. Einsle, O.; Tezcan, F. A.; Andrade, S. L. A.; Schmid, B.; Yoshida, M.; Howard, J. B.; Rees, D. C. *Science* **2002**, 297, 1696–1700.
97. (a) Vogel, C.; Heinemann, F. W.; Sutter, J.; Anthon, C.; Meyer, K. *Angew. Chem.* **2008**, 120, 2721–2724. (b) Vogel, C.; Heinemann, F. W.; Sutter, J.; Anthon, C.; Meyer, K. *Angew. Chem. Int. Ed.* **2008**, 47, 2681–2684.
98. Berry, J. F.; Bill, E.; Bothe, E.; George, S. D.; Mienert, B.; Neese, F.; Wiegardt, K. *Science* **2006**, 312, 1937–1941.
99. Smith, J. M.; Sadique, A. R.; Cundari, T. R.; Rodgers, K. R.; Lukat-Rodgers, G.; Lachicotte, R. J.; Flaschenriem, C. J.; Vela, J.; Holland, P. L. *J. Am. Chem. Soc.* **2006**, 128, 756–769.
100. Betley, T. A.; Peters, J. C. *J. Am. Chem. Soc.* **2003**, 125, 10782–10783.
101. (a) Mankad, N. P.; Whited, M. T.; Peters, J. C. *Angew. Chem.* **2007**, 119, 5870–5873. (b) Mankad, N. P.; Whited, M. T.; Peters, J. C. *Angew. Chem. Int. Ed.* **2007**, 46, 5768–5771.

102. (a) Dias, H. V. R.; Fianchini, M. *Angew. Chem.* **2007**, *119*, 2238–2241.
(b) Dias, H. V. R.; Fianchini, M. *Angew. Chem. Int. Ed.* **2007**, *46*, 2188–2191.
103. Hatcher, L. Q.; Karlin, K. D. *J. Biol. Inorg. Chem.* **2004**, *9*, 669–683.
104. Lewis, E. A.; Tolman, W. B. *Chem. Rev.* **2004**, *104*, 1047–1076.
105. Mirica, L. M.; Ottenwaelder, X.; Stack, T. D. P. *Chem. Rev.* **2004**, *104*, 1013–1046.
106. (a) Würtele, C.; Gaoutchenova, E.; Harms, K.; Holthausen, M. C.; Sundermeyer, J.; Schindler, S. *Angew. Chem.* **2006**, *118*, 3951–3954.
(b) Würtele, C.; Gaoutchenova, E.; Harms, K.; Holthausen, M. C.; Sundermeyer, J.; Schindler, S. *Angew. Chem. Int. Ed.* **2006**, *45*, 3867–3869.
107. (a) Maiti, D.; Lee, D.-H.; Gaoutchenova, K.; Würtele, C.; Holthausen, M. C.; Sarjeant, A. A. N.; Sundermeyer, J.; Schindler, S.; Karlin, K. D. *Angew. Chem.* **2008**, *120*, 88–91. (b) Maiti, D.; Lee, D.-H.; Gaoutchenova, K.; Würtele, C.; Holthausen, M. C.; Sarjeant, A. A. N.; Sundermeyer, J.; Schindler, S.; Karlin, K. D. *Angew. Chem. Int. Ed.* **2008**, *47*, 82–85.
108. Stang, P. J.; Olenyuk, B. *Acc. Chem. Res.* **1997**, *30*, 502–518.
109. (a) Holliday, B. J.; Mirkin, C. A. *Angew. Chem.* **2001**, *113*, 2076–2097.
(b) Holliday, B. J.; Mirkin, C. A. *Angew. Chem. Int. Ed.* **2001**, *40*, 2022–2043.
110. Schweiger, M.; Seidel, S. R.; Arif, A. M.; Stang, P. J. *Inorg. Chem.* **2002**, *41*, 2556–2559.
111. Forniés, J.; Gómez, J.; Lalinde, E.; Moreno, M. T. *Chem. Eur. J.* **2004**, *10*, 888–898.
112. Addicott, C.; Oesterling, I.; Yamamoto, T.; Müllen, K.; Stang, P. J. *J. Org. Chem.* **2005**, *70*, 797–801.
113. (a) Merlau, M. L.; del Pilar Mejia, M.; Nguyen, S. T.; Hupp, J. T. *Angew. Chem.* **2001**, *113*, 4369–4372. (b) Merlau, M. L.; del Pilar Mejia, M.; Nguyen, S. T.; Hupp, J. T. *Angew. Chem. Int. Ed.* **2001**, *40*, 4239–4242.
114. Lee, S. J.; Kim, J. S.; Lin, W. *Inorg. Chem.* **2004**, *43*, 6579–6588.
115. (a) Roitzsch, M.; Lippert, B. *Angew. Chem.* **2006**, *118*, 153–156.
(b) Roitzsch, M.; Lippert, B. *Angew. Chem. Int. Ed.* **2006**, *45*, 147–150.
116. (a) Pariya, C.; Sparrow, C. R.; Back, C.-K.; Sandí, G.; Fronczek, F. R.; Maverick, A. W. *Angew. Chem.* **2007**, *119*, 6421–6424. (b) Pariya, C.; Sparrow, C. R.; Back, C.-K.; Sandí, G.; Fronczek, F. R.; Maverick, A. W. *Angew. Chem. Int. Ed.* **2007**, *46*, 6305–6308.
117. Sun, S.-S.; Stern, C. L.; Nguyen, S. T.; Hupp, J. T. *J. Am. Chem. Soc.* **2004**, *126*, 6314–6326.
118. Sessler, J. L.; Katayev, E.; Pantos, G. D.; Ustynyuk, Y. A. *Chem. Commun.* **2004**, 1276–1277.
119. Grell, D.; Grell, E.; Bugnon, P.; Dietrich, B.; Lehn, J.-M. *J. Therm. Anal. Calorim.* **2004**, *77*, 483–495.
120. (a) Fiedler, D.; Bergman, R. G.; Raymond, K. N. *Angew. Chem.* **2006**, *118*, 759–762. (b) Fiedler, D.; Bergman, R. G.; Raymond, K. N. *Angew. Chem. Int. Ed.* **2006**, *45*, 745–748.
121. Hapiot, F.; Tilloy, S.; Monflier, E. *Chem. Rev.* **2006**, *106*, 767–781.
122. Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743–1753.
123. Lee, J. W.; Samal, S.; Selvapalam, N.; Kim, H.-J.; Kim, K. *Acc. Chem. Res.* **2003**, *36*, 621–630.
124. (a) Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem.* **2005**, *117*, 4922–4949. (b) Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem. Int. Ed.* **2005**, *44*, 4844–4870.

125. Kim, K.; Selvapalam, N.; Ko, Y. H.; Park, K. M.; Kim, D.; Kim, J. *Chem. Soc. Rev.* **2007**, *36*, 267–279.
126. (a) Liu, S.; Shukla, A. D.; Gadde, S.; Wagner, B. D.; Kaifer, A. E.; Isaacs, L. *Angew. Chem.* **2008**, *120*, 2697–2700. (b) Liu, S.; Shukla, A. D.; Gadde, S.; Wagner, B. D.; Kaifer, A. E.; Isaacs, L. *Angew. Chem. Int. Ed.* **2008**, *47*, 2657–2660.
127. Bampos, N.; Marvaud, V.; Sanders, J. K. M. *Chem. Eur. J.* **1998**, *4*, 335–343.
128. Ballester, P.; Costa, A.; Castilla, A. M.; Deyà, P. M.; Frontera, A.; Gomila, R. M.; Hunter, C. A. *Chem. Eur. J.* **2005**, *11*, 2196–2206.
129. Ballester, P.; Oliva, A. I.; Costa, A.; Deyà, P. M.; Frontera, A.; Gomila, R. M.; Hunter, C. A. *J. Am. Chem. Soc.* **2006**, *128*, 5560–5569.
130. (a) Halime, Z.; Lachkar, M.; Roisnel, T.; Furet, E.; Halet, J.-F.; Boitrel, B. *Angew. Chem.* **2007**, *119*, 5212–5216. (b) Halime, Z.; Lachkar, M.; Roisnel, T.; Furet, E.; Halet, J.-F.; Boitrel, B. *Angew. Chem. Int. Ed.* **2007**, *46*, 5120–5124.
131. (a) Katagiri, H.; Tanaka, Y.; Furusho, Y.; Yashima, E. *Angew. Chem.* **2007**, *119*, 2487–2491. (b) Katagiri, H.; Tanaka, Y.; Furusho, Y.; Yashima, E. *Angew. Chem. Int. Ed.* **2007**, *46*, 2435–2439.
132. Kawano, M.; Kobayashi, Y.; Ozeki, T.; Fujita, M. *J. Am. Chem. Soc.* **2006**, *128*, 6558–6559.
133. (a) Ono, K.; Yoshizawa, M.; Kato, T.; Watanabe, K.; Fujita, M. *Angew. Chem.* **2007**, *119*, 1835–1838. (b) Ono, K.; Yoshizawa, M.; Kato, T.; Watanabe, K.; Fujita, M. *Angew. Chem. Int. Ed.* **2007**, *46*, 1803–1806.
134. Kuehl, C. J.; Kryshchenko, Y. K.; Radhakrishnan, U.; Seidel, S. R.; Huang, S. D.; Stang, P. J. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4932–4936.
135. Yang, H.-B.; Ghosh, K.; Northrop, B. H.; Stang, P. J. *Org. Lett.* **2007**, *9*, 1561–1564.
136. Fujita, M.; Umemoto, K.; Yoshizawa, M.; Fujita, N.; Kusakawa, T.; Biradha, K. *Chem. Commun.* **2001**, 509–518.
137. Govindaswamy, P.; Süß-Fink, G.; Therrien, B. *Organometallics* **2007**, *26*, 915–924.
138. Govindaswamy, P.; Linder, D.; Lacour, J.; Süß-Fink, G.; Therrien, B. *Chem. Commun.* **2006**, 4691–4693.
139. (a) Therrien, B.; Süß-Fink, G.; Govindaswamy, P.; Renfrew, A. K.; Dyson, P. J. *Angew. Chem.* **2008**, *120*, 3833–3836. (b) Therrien, B.; Süß-Fink, G.; Govindaswamy, P.; Renfrew, A. K.; Dyson, P. J. *Angew. Chem. Int. Ed.* **2008**, *47*, 3773–3776.
140. (a) Koshevoy, I. O.; Koskinen, L.; Haukka, M.; Tunik, S. P.; Serdobintsev, P. Y.; Melnikov, A. S.; Pakkanen, T. A. *Angew. Chem.* **2008**, *120*, 4006–4009. (b) Koshevoy, I. O.; Koskinen, L.; Haukka, M.; Tunik, S. P.; Serdobintsev, P. Y.; Melnikov, A. S.; Pakkanen, T. A. *Angew. Chem.* **2008**, *47*, 3942–3945.
141. (a) Botana, E.; Da Silva, E.; Benet-Buchholz, J.; Ballester, P.; de Mendoza, J. *Angew. Chem.* **2007**, *119*, 202–205. (b) Botana, E.; Da Silva, E.; Benet-Buchholz, J.; Ballester, P.; de Mendoza, J. *Angew. Chem. Int. Ed.* **2007**, *46*, 198–201.
142. (a) Fontecha, J. B.; Goetz, S.; McKee, V. *Angew. Chem.* **2002**, *114*, 4735–4738. (b) Fontecha, J. B.; Goetz, S.; McKee, V. *Angew. Chem. Int. Ed.* **2002**, *41*, 4553–4556.
143. Du Bois, J.; Mizoguchi, T. J.; Lippard, S. J. *Coord. Chem. Rev.* **2000**, *200–202*, 443–485.

144. Dieleman, C. B.; Matt, D.; Harriman, A. *Eur. J. Inorg. Chem.* **2000**, 831–834.
145. Copp, S. B.; Holman, K. T.; Sangster, J. O. S.; Subramanian, S.; Zaworotko, M. J. *J. Chem. Soc. Dalton Trans.* **1995**, 2233–2243.
146. (a) Christinat, N.; Scopelliti, R.; Severin, K. *Angew. Chem.* **2008**, *120*, 1874–1878. (b) Christinat, N.; Scopelliti, R.; Severin, K. *Angew. Chem. Int. Ed.* **2008**, *47*, 1848–1852.
147. (a) Abrahams, B. F.; Price, D. J.; Robson, R. *Angew. Chem.* **2006**, *118*, 820–824. (b) Abrahams, B. F.; Price, D. J.; Robson, R. *Angew. Chem. Int. Ed.* **2006**, *45*, 806–810.
148. Côté, A. P.; Benin, A. I.; Ockwig, N. W.; O’Keeffe, M.; Matzger, A. J.; Yaghi, O. M. *Science* **2005**, *310*, 1166–1170.
149. El-Kaderi, H. M.; Hunt, J. R.; Mendoza-Cortés, J. L.; Côté, A. P.; Taylor, R. E.; O’Keeffe, M.; Yaghi, O. M. *Science* **2007**, *316*, 268–272.
150. (a) Givaja, G.; Volpe, M.; Edwards, M. A.; Blake, A. J.; Wilson, C.; Schröder, M.; Love, J. B. *Angew. Chem.* **2007**, *119*, 590–592. (b) Givaja, G.; Volpe, M.; Edwards, M. A.; Blake, A. J.; Wilson, C.; Schröder, M.; Love, J. B. *Angew. Chem. Int. Ed.* **2007**, *46*, 584–586.
151. (a) Collman, J. P.; Wagenknecht, P. S.; Hutchison, J. E. *Angew. Chem.* **1994**, *106*, 1620–1639. (b) Collman, J. P.; Wagenknecht, P. S.; Hutchison, J. E. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1537–1554.
152. Deng, Y.; Chang, C. J.; Nocera, D. G. *J. Am. Chem. Soc.* **2000**, *122*, 410–411.
153. Guillard, R.; Jérôme, F.; Barbe, J.-M.; Gros, C. P.; Ou, Z.; Shao, J.; Fischer, J.; Weiss, R.; Kadish, K. M. *Inorg. Chem.* **2001**, *30*, 4856–4865.
154. Kersting, B. Z. *Anorg. Allg. Chem.* **2004**, *630*, 765–780.
155. Marr, A. C.; Spencer, D. J. E.; Schröder, M. *Coord. Chem. Rev.* **2001**, *219–221*, 1055–1074.
156. Harrop, T. C.; Mascharak, P. K. In: “*Concepts and Models in Bioinorganic Chemistry*”; Eds. Kraatz, H.-B.; Metzler-Nolte, N.; Wiley-VCh, **2006**, pp. 460–469.
157. Sellmann, D.; Sutter, J. *Acc. Chem. Res.* **1997**, *30*, 460–469.
158. Grapperhaus, C. A.; Darensbourg, M. Y. *Acc. Chem. Res.* **1998**, *31*, 451–459.
159. For biological, dithiolate-bridged Cu/Cu systems, see: (a) Tsukihara, T.; Aoyama, H.; Yamashita, E.; Tomizaki, T.; Yamaguchi, H.; Shinzawa-Itoh, K.; Nakashima, R.; Yaono, R.; Yoshikawa, S. *Science* **1995**, *269*, 1069–1074. (b) Iwata, S.; Ostermeier, C.; Ludwig, B.; Michel, H. *Nature* **1995**, *376*, 660–669. (c) Wilmanns, M.; Lappalainen, P.; Kelly, M.; Saver-Eriksson, E.; Saraste, M. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11955–11959.
160. For biological, dithiolate-bridged Fe/Ni systems, see: (a) Volbeda, A.; Charon, M.-H.; Piras, C.; Hatchikian, E. C.; Frey, M.; Fontecilla-Camps, J. C. *Nature* **1995**, *373*, 580–587. (b) Volbeda, A.; Garcin, E.; Piras, C.; de Lacey, A. L.; Fernandez, V. M.; Hatchikian, E. C.; Frey, M.; Fontecilla-Camps, J. C. *J. Am. Chem. Soc.* **1996**, *118*, 12989–12996. (c) Halcrow, M. A. *Angew. Chem.* **1995**, *107*, 1307–1310. (d) Halcrow, M. A. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1193–1197.
161. For biological, dithiolate-bridged Fe/Fe systems, see: (a) Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. *Science* **1998**, *282*, 1853–1858. (b) Cammack, R. *Adv. Inorg. Chem.* **1992**, *38*, 281–322.
162. Beissel, T.; Bürger, K. S.; Voigt, G.; Wieghardt, K.; Butzlaff, C.; Trautwein, A. X. *Inorg. Chem.* **1993**, *32*, 124–126.

163. Glaser, T.; Beissel, T.; Bill, E.; Weyhermüller, T.; Schünemann, V.; Meyer-Klaucke, W.; Trautwein, A. X.; Wieghardt, K. *J. Am. Chem. Soc.* **1999**, *121*, 2193–2208.
164. Krüger, H.-J.; Holm, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 2955–2963.
165. Dance, I. G. *Polyhedron* **1986**, *5*, 1037–1104.
166. (a) Krebs, B.; Henkel, G. *Angew. Chem.* **1991**, *105*, 785–804. (b) Krebs, B.; Henkel, G. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 769–788.
167. Arnold, J. *Prog. Inorg. Chem.* **1995**, *43*, 353–417.
168. Blower, P. J.; Dilworth, J. R. *Coord. Chem. Rev.* **1987**, *76*, 121–185.
169. Halcrow, M. A.; Christou, G. *Chem. Rev.* **1994**, *94*, 2421–2481.
170. Henkel, G.; Krebs, B. *Chem. Rev.* **2004**, *104*, 801–824.
171. Zanello, P.; Tamburini, S.; Vigato, P. A.; Mazzocchin, G. A. *Coord. Chem. Rev.* **1987**, *77*, 165–273.
172. (a) Sträter, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B. *Angew. Chem.* **1996**, *108*, 2158–2191. (b) Sträter, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B. *Angew. Chem. Int. Ed.* **1996**, *35*, 2024–2055.
173. McCollum, D. G.; Yap, G. P. A.; Rheingold, A. L.; Bosnich, B. *J. Am. Chem. Soc.* **1996**, *118*, 1365–1379.
174. Gavrilova, A. L.; Qin, C. J.; Sommer, R. D.; Rheingold, A. L.; Bosnich, B. *J. Am. Chem. Soc.* **2002**, *124*, 1714–1722.
175. Brooker, S. *Coord. Chem. Rev.* **2001**, *222*, 33–56.
176. Pilkington, N. H.; Robson, R. *Aust. J. Chem.* **1970**, *23*, 2225–2236.
177. Fenton, D. E.; Vigato, P. A. *Chem. Soc. Rev.* **1988**, *17*, 69–90.
178. Vigato, P. A.; Tamburini, S.; Fenton, D. E. *Coord. Chem. Rev.* **1990**, *106*, 25–170.
179. Fenton, D. E. *Chem. Soc. Rev.* **1999**, *28*, 159–168.
180. Okawa, H.; Furutachi, H.; Fenton, D. E. *Coord. Chem. Rev.* **1998**, *174*, 51–75.
181. Atkins, A. J.; Black, D.; Blake, A. J.; Marin-Becerra, A.; Parsons, S.; Ruiz-Ramirez, L.; Schröder, M. *Chem. Commun.* **1996**, 457–464.
182. Borisova, N. E.; Reshetova, M. D.; Ustynyuk, M. D. *Chem. Rev.* **2007**, *107*, 46–79.
183. Atkins, A. J.; Blake, A. J.; Schröder, M. *J. Chem. Soc. Chem. Commun.* **1993**, 1662–1665.
184. Brooker, S.; Croucher, P. D.; Roxburgh, F. M. *J. Chem. Soc. Dalton Trans.* **1996**, 3031–3037.
185. Brooker, S.; Croucher, P. D. *J. Chem. Soc. Chem. Commun.* **1995**, 1493–1494.
186. Brooker, S.; Croucher, P. D. *J. Chem. Soc. Chem. Commun.* **1995**, 2075–2076.
187. Brooker, S.; Croucher, P. D. *Chem. Commun.* **1997**, 459–460.
188. Brooker, S.; Davidson, T. C. *Chem. Commun.* **1997**, 2007–2008.
189. Brooker, S.; Croucher, P. D.; Davidson, T. C.; Dunbar, G. S.; McQuillan, A. J.; Jameson, G. B. *Chem. Commun.* **1998**, 2131–2132.
190. Brooker, S.; Caygill, G. B.; Croucher, P. D.; Davidson, T. C.; Clive, D. L. J.; Magnuson, S. R.; Cramer, S. P.; Raston, C. Y. *J. Chem. Soc., Dalton Trans.* **2000**, 3113–3121.
191. Brooker, S.; Croucher, P. D.; Davidson, T. C.; Dunbar, G. S.; Beck, C. U.; Subramanian, S. *Eur. J. Inorg. Chem.* **2000**, 169–179.
192. Christensen, A.; Jensen, H. S.; McKee, V.; McKenzie, C. J.; Munch, M. *Inorg. Chem.* **1997**, *36*, 6080–6085.
193. Kruger, P. E.; McKee, V. *Chem. Commun.* **1997**, 1341–1342.

194. Branscombe, N. D. J.; Blake, A. J.; Marin-Becerra, A.; Li, W. S.; Parsons, S.; Ruiz-Ramirez, L.; Schröder, M. *Chem. Commun.* **1996**, 2573–2574.
195. Branscombe, N. D. J.; Atkins, A. J.; Marin-Becerra, A.; McInnes, E. J. L.; Mabbs, F. E.; McMaster, J.; Schröder, M. *Chem. Commun.* **2003**, 1098–1099.
196. Vigato, P. A.; Tamburini, S.; Bertolo, L. *Coord. Chem. Rev.* **2007**, 251, 1311–1492.
197. Suh, M. P. In: “*Adv. Inorg. Chem.*”; vol. 44; Ed. Sykes, A. G.; Academic Press: New York, **1997**, pp. 93–146.
198. Constable, E. C. “*Metals and Ligand Reactivity*”; VCH: Weinheim, **1996**.
199. Adams, H.; Bailey, N. A.; Fenton, D. E.; Good, R. J.; Moody, R.; de Barbarin, C. O. R. *J. Chem. Soc. Dalton Trans.* **1987**, 207–218.
200. Harding, C. J.; Lu, Q.; Malone, J. F.; Marrs, D. J.; Martin, N.; McKee, V.; Nelson, J. *J. Chem. Soc. Dalton Trans.* **1995**, 1739–1747.
201. Brooker, S.; McKee, V. *Inorg. Chim. Acta* **1990**, 173, 69–83.
202. Klingele, M. H.; Steinfeld, G.; Kersting, B. *Z. Naturforsch.* **2001**, 56b, 901–907.
203. Klingele, M. H.; Kersting, B. *Z. Naturforsch.* **2001**, 56b, 437–439.
204. Illuminati, G.; Mandolini, L. *Acc. Chem. Res.* **1981**, 14, 95–102.
205. Diederich, F.; Stang, P. J. “*Templated Organic Synthesis*”; Wiley-VCH: Weinheim, Germany, **2000**.
206. Busch, D. H.; Stephenson, N. A. *Coord. Chem. Rev.* **1990**, 100, 119–154.
207. Kersting, B.; Steinfeld, G.; Fritz, T.; Hausmann, J. *Eur. J. Inorg. Chem.* **1999**, 2167–2172.
208. Kersting, B. *Z. Naturforsch.* **2000**, 55b, 961–965.
209. Kersting, B.; Steinfeld, G. *Chem. Commun.* **2001**, 1376–1377.
210. Gressenbuch, M.; Lozan, V.; Steinfeld, G.; Kersting, B. *Eur. J. Inorg. Chem.* **2005**, 2223–2234.
211. Gressenbuch, M.; Kersting, B. *Eur. J. Inorg. Chem.* **2007**, 90–102.
212. Gregor, T.; Weise, C. F.; Lozan, V.; Kersting, B. *Synthesis* **2007**, 3706–3712.
213. Lozan, V.; Kersting, B. *Z. Anorg. Allg. Chem.* **2008**, 634, 941–945.
214. Gressenbuch, M., PhD thesis, “*Darstellung und Koordinationsverhalten N-funktionalisierter Hexaamin-Dithiophenolatmakrozyklen*”; University of Leipzig, Germany, **2008**.
215. (a) Käss, S.; Gregor, T.; Kersting, B. *Angew. Chem.* **2006**, 118, 107–110.
(b) Käss, S.; Gregor, T.; Kersting, B. *Angew. Chem. Int. Ed.* **2006**, 45, 101–104.
216. Siedle, G.; Kersting, B. *Z. Anorg. Allg. Chem.* **2003**, 629, 2083–2090.
217. Okawa, H.; Nishio, J.; Ohba, M.; Tadokoro, M.; Matsumoto, N.; Koikawa, M.; Kida, S.; Fenton, D. E. *Inorg. Chem.* **1993**, 32, 2949–2957.
218. Yamami, M.; Furutachi, H.; Yokoyama, T.; Okawa, H. *Inorg. Chem.* **1998**, 37, 6832–6838.
219. Yonemura, M.; Matsumura, Y.; Furutachi, H.; Ohba, M.; Okawa, H.; Fenton, D. E. *Inorg. Chem.* **1997**, 36, 2711–2717.
220. He, H.; Martell, A. E.; Motekaitis, R. J.; Reibenspies, J. H. *Inorg. Chem.* **2000**, 39, 1586–1592.
221. Nishio, J.; Okawa, H.; Ohtsuka, S.; Tomono, M. *Inorg. Chim. Acta* **1994**, 218, 27–32.
222. Gagne, R. R.; Spiro, C. L.; Smith, T. J.; Hamann, C. A.; Thies, W. R.; Shiemke, A. D. *J. Am. Chem. Soc.* **1981**, 103, 4073–4081.

223. Lambert, S. L.; Spiro, C. L.; Gagne, R. R.; Hendrickson, D. N. *Inorg. Chem.* **1982**, *21*, 68–72.
224. Fritz, T.; Steinfeld, G.; Käss, S.; Kersting, B. *Dalton Trans.* **2006**, 3812–3821.
225. Addison, A. W.; Rao, T. N.; Reedijk, J.; Van Rijn, J.; Verschoor, G. C. *J. Chem. Soc. Dalton Trans.* **1984**, 1349–1356.
226. Journaux, Y.; Glaser, T.; Steinfeld, G.; Lozan, V.; Kersting, B. *Dalton Trans.* **2006**, 1738–1748.
227. Christensen, A.; Mayer, C.; Jensen, F.; Bond, A. D.; McKenzie, C. J. *Dalton Trans.* **2006**, 108–120.
228. Steinfeld, G., PhD thesis, “*Makrozyklische Zweikernkomplexe als Synthetische Rezeptoren zur Aktivierung kleiner Moleküle*”; University of Freiburg, Germany, **2004**.
229. Fontecilla-Camps, J. C.; Volbeda, A.; Cavazza, C.; Nicolet, Y. *Chem. Rev.* **2007**, *107*, 4273–4303.
230. Doukov, T. I.; Iverson, T. M.; Seravalli, J.; Ragsdale, S. W.; Drennan, C. L. *Science* **2002**, *298*, 567–572.
231. Liu, X.; Ibrahim, S. K.; Tard, C.; Pickett, C. J. *Coord. Chem. Rev.* **2005**, *249*, 1641–1652.
232. Bouwman, E.; Reedijk, J. *Coord. Chem. Rev.* **2005**, *249*, 1555–1581.
233. (a) Mealli, C.; Rauchfuss, T. B. *Angew. Chem.* **2007**, *119*, 9100–9102. (b) Mealli, C.; Rauchfuss, T. B. *Angew. Chem. Int. Ed.* **2007**, *46*, 8942–8944.
234. Kersting, B.; Siebert, D.; Volkmer, D.; Kolm, M. J.; Janiak, C. *Inorg. Chem.* **1999**, *38*, 3871–3882.
235. Kersting, B.; Siebert, D. *Inorg. Chem.* **1998**, *37*, 3820–3828.
236. Kersting, B.; Siebert, D. *Eur. J. Inorg. Chem.* **1999**, 189–193.
237. Steinfeld, G.; Kersting, B. *Chem. Commun.* **2000**, 205–206.
238. Harding, C. J.; McKee, V.; Nelson, J.; Lu, Q. *J. Chem. Soc. Chem. Commun.* **1993**, 1768–1770.
239. (a) Kersting, B. *Angew. Chem.* **2001**, *113*, 4110–4112. (b) Kersting, B. *Angew. Chem. Int. Ed.* **2001**, *40*, 3988–3990.
240. Lozan, V.; Kersting, B. *Eur. J. Inorg. Chem.* **2005**, 504–512.
241. Kersting, B.; Steinfeld, G. *Inorg. Chem.* **2002**, *41*, 1140–1150.
242. Hausmann, J.; Klingele, M.-H.; Lozan, V.; Steinfeld, G.; Siebert, D.; Journaux, Y.; Girerd, J.-J.; Kersting, B. *Chem. Eur. J.* **2004**, *10*, 1716–1728.
243. Lozan, V.; Kersting, B. *Inorg. Chem.* **2008**, *47*, 5386–5393.
244. Kong, D.; Martell, A. E.; Motekaitis, R. J.; Reibenspies, J. H. *Inorg. Chim. Acta* **2001**, *317*, 243–251.
245. Wang, J.; Martell, A. E.; Reibenspies, J. H. *Inorg. Chim. Acta* **2002**, *328*, 53–60.
246. Kong, D.; Martell, A. E.; Reibenspies, J. H. *Inorg. Chim. Acta* **2002**, *333*, 7–14.
247. Gao, J.; Martell, A. E.; Reibenspies, J. H. *Inorg. Chim. Acta* **2003**, *346*, 32–42.
248. Gao, J.; Martell, A. E.; Reibenspies, J. H. *Inorg. Chim. Acta* **2002**, *329*, 122–128.
249. Greenwood, N. N.; Earnshaw, A. “*Chemistry of the Elements*”; VCH: Weinheim, **1988**.
250. Hausmann, J.; Käss, S.; Kersting, B.; Klod, S.; Kleinpeter, E. *Eur. J. Inorg. Chem.* **2004**, 4402–4411.
251. Barefield, E. K.; Freeman, G. M.; Van Derveer, D. G. *Inorg. Chem.* **1986**, *25*, 552–558.

252. Cotton, F. A.; Wilkinson, G.; Murillo, C. A.; Bochman, M. *Advanced Inorganic Chemistry*; 6th edn. Wiley-Interscience: New York, **1999**; pp. 838–840.
253. Lever, A. B. P. *Inorganic Electronic Spectroscopy*; 2nd edn. Elsevier Science: Amsterdam, **1984**.
254. Beissel, T.; Glaser, T.; Kersting, F.; Wieghardt, K.; Nuber, B. *Inorg. Chem.* **1996**, *35*, 3936–3947.
255. Lozan, V.; Kersting, B. *Inorg. Chem.* **2006**, *45*, 5630–5634.
256. (a) Steinfeld, G.; Lozan, V.; Kersting, B. *Angew. Chem.* **2003**, *115*, 2363–2365. (b) Steinfeld, G.; Lozan, V.; Kersting, B. *Angew. Chem. Int. Ed.* **2003**, *42*, 2261–2263.
257. Smithrud, D. B.; Sanford, E. M.; Chao, I.; Ferguson, S. B.; Carcanague, D. R.; Evanseck, J. D.; Houk, K. N.; Diederich, F. *Pure. Appl. Chem.* **1990**, *62*, 2227–2236.
258. Hausmann, J. *“Diplomarbeit”*; Universität Freiburg, **2001**.
259. Journaux, Y.; Hausmann, J.; Lozan, V.; Kersting, B. *Chem. Commun.* **2006**, 83–84.
260. Lozan, V.; Kersting, B., *unpublished results*.
261. Lozan, V.; Kersting, B. *Eur. J. Inorg. Chem.* **2007**, 1436–1443.
262. Lozan, V.; Buchholz, A.; Plass, W.; Kersting, B. *Chem. Eur. J.* **2007**, *13*, 7305–7316.
263. Lozan, V.; Holldorf, J.; Kersting, B., *Inorg. Chim. Acta.* **2009**, *362*, 793–798.
264. Lozan, V.; Votekovich, S. V.; Gaponik, P. N.; Ivashkevich, O. A.; Kersting, B. *Z. Naturforsch.* **2008**, *63b*, 496–502.
265. Pierpont, C. G.; Hendrickson, D. N.; Duggan, D. M.; Wagner, F.; Barefield, E. K. *Inorg. Chem.* **1975**, *14*, 604–610.
266. Chaudhuri, P.; Guttmann, M.; Ventur, D.; Wieghardt, K.; Nuber, B.; Weiss, J. *J. Chem. Soc. Chem. Commun.* **1985**, 1618–1620.
267. Ribas, J.; Escuer, A.; Monfort, M.; Vicente, R.; Cortés, R.; Lezama, L.; Rojo, T. *Coord. Chem. Rev.* **1999**, *193–195*, 1027–1068.
268. Harding, C. J.; Mabbs, F. E.; MacInnes, E. J. L.; McKee, V.; Nelson, J. *J. Chem. Soc. Dalton Trans.* **1996**, 3227–3230.
269. Escuer, A.; Harding, C. J.; Dussart, Y.; Nelson, J.; McKee, V.; Vicente, R. *J. Chem. Soc. Dalton Trans.* **1999**, 223–227.
270. Escuer, A.; Vicente, R.; Ribas, J.; El Fallah, M. S.; Solans, X.; Font-Bardia, M. *Inorg. Chem.* **1993**, *32*, 3727–3732.
271. Escuer, A.; Vicente, R.; Ribas, J.; El Fallah, M. S.; Solans, X.; Font-Bardia, M. *Inorg. Chem.* **1994**, *33*, 1842–1847.
272. Hollemann, A. F.; Wiberg, E. *“Lehrbuch der Anorganischen Chemie”*; 91–100 edn. Walter de Gruyter: Berlin, **1985**; pp. 557–559.
273. Sellmann, D.; Kunstmann, H.; Knoch, F.; Moll, M. *Inorg. Chem.* **1988**, *27*, 4183–4190.
274. (a) Sellmann, D.; Soglowek, W.; Knoch, F.; Moll, M. *Angew. Chem.* **1989**, *101*, 1244–1245. (b) Sellmann, D.; Soglowek, W.; Knoch, F.; Moll, M. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1271–1272.
275. Henderson, R. A.; Leigh, G. J.; Pickett, C. J. *Adv. Inorg. Chem. Radiochem.* **1983**, *27*, 197–292.
276. Sutton, D. *Chem. Rev.* **1993**, *93*, 995–1022.
277. Sellmann, D.; Engl, K.; Heinemann, F. W.; Sieler, J. *Eur. J. Inorg. Chem.* **2000**, 1079–1089.
278. Matsumoto, K.; Koyama, T.; Koide, Y. *J. Am. Chem. Soc.* **1999**, *121*, 10913–10923.

279. Matsukawa, S.; Kuwata, S.; Ishii, Y.; Hidai, M. *J. Chem. Soc. Dalton Trans.* **2002**, 2737–2746.
280. Blum, L.; Williams, I. D.; Schrock, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 8316–8317.
281. Murray, R. C.; Schrock, R. R. *J. Am. Chem. Soc.* **1985**, *107*, 4557–4558.
282. Parkin, G. *Adv. Inorg. Chem.* **1995**, *42*, 291–393.
283. Ruf, M.; Vahrenkamp, H. *Inorg. Chem.* **1996**, *35*, 6571–6578.
284. Yoshizawa, M.; Kusukawa, T.; Fujita, M.; Sakamoto, S.; Yamaguchi, K. *J. Am. Chem. Soc.* **2001**, *123*, 10454–10459.
285. Carman, R. M.; Derbyshire, R. P. C.; Hansford, K. A.; Kadirvelraj, R.; Robinson, W. T. *Aust. J. Chem.* **2001**, *54*, 117–126.
286. Lenoir, D.; Chiappe, C. *Chem. Eur. J.* **2003**, *9*, 1044–1936.
287. (a) Herges, R. *Angew. Chem.* **1995**, *107*, 57–59. (b) Herges, R. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 51–53. (c) Bianchini, R.; Chiappe, C.; Lenoir, D.; Lemmen, P.; Herges, R.; Grunenberg, J. *Angew. Chem.* **1997**, *109*, 1340–1343. (d) Bianchini, R.; Chiappe, C.; Lenoir, D.; Lemmen, P.; Herges, R.; Grunenberg, J. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1284–1287.
288. Rideout, D. C.; Breslow, R. *J. Am. Chem. Soc.* **1980**, *102*, 7816–7817.
289. Kang, J.; Rebek, J., Jr. *Nature* **1997**, *385*, 50–52.
290. (a) Yoshizawa, M.; Takeyama, Y.; Kusukawa, T.; Fujita, M. *Angew. Chem.* **2002**, *114*, 1403–1405. (b) Yoshizawa, M.; Takeyama, Y.; Kusukawa, T.; Fujita, M. *Angew. Chem. Int. Ed.* **2002**, *41*, 1347–1349.

MECHANISTIC CONSIDERATIONS ON THE REACTIVITY OF GREEN Fe^{III} -TAML ACTIVATORS OF PEROXIDES

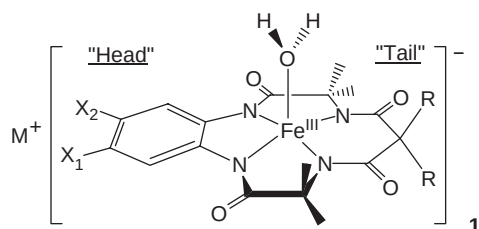
ALEXANDER D. RYABOV and TERRENCE J. COLLINS

Department of Chemistry, Institute for Green Science, Carnegie Mellon University,
4400 Fifth Avenue, Pittsburgh, PA 15213, USA

I.	Introduction	472
II.	Speciation of Tetraamide Iron(III) Macrocycles in Solution and the Solid State	473
	A. Solid State Structure and Speciation in Water	473
	B. Binding of Axial Ligands in Water	475
III.	Kinetics and Mechanisms of Demetalation of Fe^{III} -TAML Activators	478
	A. Specific Acid Catalysis	478
	B. General Acid "Coordinative" Catalysis	482
IV.	Catalysis-Relevant Oxidized Forms Derived from Fe^{III} -TAMLs	487
	A. $\text{Fe}(\text{IV})$: Oxidation by Dioxygen in Aprotic Organic Solvents	487
	B. $\text{Fe}(\text{IV})$: Oxidation by Peroxides in Water	489
	C. $\text{Fe}(\text{V})$: Oxidation by Peroxides in Organic Solvents	493
V.	Mechanism of Catalysis by Tetraamide Macrocyclic Fe^{III} -TAML Activators of Hydrogen Peroxide, Functional Catalase-Peroxidase Replicas	494
	A. General Scheme	494
	B. Peroxidase-Like Activity	495
	C. Catalase-Like Activity	504
	D. Other Primary Oxidants, Mechanisms of Benzoyl Peroxide Activation, and Degradation of Orange II	507
VI.	The Activity-Stability Parameterization of Homogeneous Green Oxidation Catalysts	510
	A. Kinetic Model for Parameterization	510
	B. Model Verifications	514
VII.	Concluding Remarks	516
	Acknowledgment	517
	References	517

I. Introduction

Enzymes hold a universal reputation for being the best green catalysts. Their existence challenges chemists to design and develop small molecule mimics that compete catalytically while possessing comparable environmental acceptability. We have introduced Fe^{III}-TAML activators, a family of low-molecular weight green catalysts for the activation of H₂O₂ and other peroxides to oxidize a wide spectrum of targeted substrates (1–3) including toxic polychlorophenols (4), thiophosphate pesticides and nitrophenols (5), azo dyes (6), dibenzothiophenes (7), as well as an anthrax surrogate (8) and natural or synthetic estrogens (9). These iron catalysts contain a central ferric iron coordinated to the cavity of a tetraanionic *tetra-amido-macrocylic-ligand* (TAML[®]), Chart 1. Fe^{III}-TAML activators of H₂O₂ display



1	X ₁	X ₂	R
a	H	H	Me
b	Me	Me	Me
c	Me	H	Me
d	MeO	MeO	Me
e	NO ₂	H	Me
f	COOMe	H	Me
g	COOH	H	Me
h	CONH(CH ₂) ₂ NMe ₃ ⁺	H	Me
i	Cl	Cl	Me
j	Cl	Cl	Et
k	Cl	Cl	F
l	H	H	F
m	H	H	
n	NO ₂	H	F

CHART 1. Fe^{III}-TAML activators mentioned in this account are shown as pentacoordinated species with an axial aqua ligand as obtained in the solid state. The aqua complexes are synthesized as such or as the corresponding chloro species with Cl[−] instead of H₂O (with two M⁺ = Li⁺, Na⁺, NR₄⁺ counter ions). The subscript “Cl” as in 1_{Cl} is used throughout to indicate the corresponding chloro species.

peroxidase-like activities and longevities (10) that are remarkable for low-molecular weight synthetic catalysts. They are functional analogues of catalase-peroxidase enzymes (11). This review summarizes the results of mechanistic studies on various aspects of Fe^{III} -TAML peroxide activation obtained in our laboratory over the last decade. We also describe kinetic properties of **1** that are essential for understanding intrinsic reactivity features to build a solid mechanistic picture of Fe^{III} -TAML catalysis and to show that they are a family of successful artificial enzymes; “tamlzymes” if you like. To the reader, we make the point that significant tracts of this article have been extracted directly from our publications. In these cases, the publications were developed carefully, usually over many years, and we believe the presentations to be optimal. This review combines such tracts with linking ideas and with certain sections further developed to explain particular connections and to collect the entire body of work in one place. This story has a strong physico-chemical flavor. We believe that the present book is an ideal forum for describing our mechanistic views. Concluding remarks highlight how this knowledge has led us to a smart design of new Fe^{III} -TAML activators with enhanced performance. If although the reader is more interested in green and synthetic applications of tamlzymes rather than in their mechanistic jungles, the very recent account published in *Handbook of Green Chemistry* is recommended (12).

II. Speciation of Tetraamide Iron(III) Macrocycles in Solution and the Solid State

A. SOLID STATE STRUCTURE AND SPECIATION IN WATER

Solid iron(III)-TAML activators are five-coordinated tetragonal pyramidal species (Fig. 1) (13). The base amide Fe-N bonds are short (mean 1.897, 1.894, and 1.885 Å in **1i**_{Cl}, **1l**_{Cl}, and **1a**, respectively) and the macrocyclic ligands have a high degree of planarity. Iron(III) is out of the plane by 0.448 and 0.403 Å toward chloride in **1i**_{Cl} and **1l**_{Cl}, respectively, and 0.364 Å toward oxygen in **1a**. The Fe-Cl bond distance is significantly longer (2.359 and 2.361 Å in **1i**_{Cl} and **1l**_{Cl}, respectively) than in $[\text{FeCl}_4]^-$ (2.182–2.187 Å) (14,15), iron(III) porphyrins (2.218 (16), 2.192 (17), and 2.223 Å (18)), and in the five-coordinate Fe^{III} complexes (mean 2.235 Å) (19). A smaller metal cation in the Fe^{IV} analogue of **1i**_{Cl} is immersed deeper into the macrocyclic cavity (0.04 Å

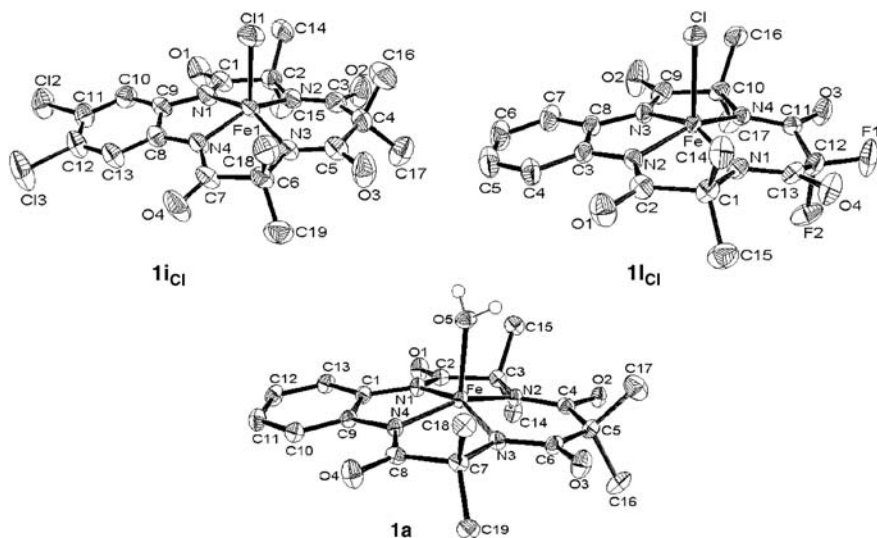
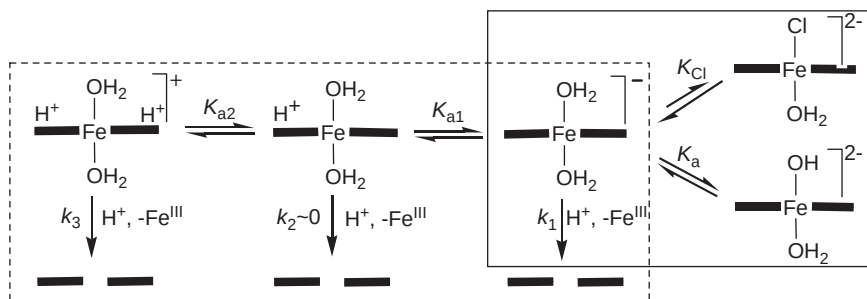


FIG. 1. Crystal structures of complexes **1iCl**, **1lCl**, and **1a**. H atoms omitted for clarity. From Ref. (13).

displacement) and the Fe–Cl bond length reduces to 2.203 Å (20). The Fe^{III}–Cl bond is elongated in **1Cl** due to the ground state destabilization of the axial positions by the four deprotonated strongly σ -donating and significantly π -donating (21) equatorial amide nitrogens which quench the axial Lewis acidity (22).

Structures in Fig. 1 differ in the vicinity of the “tail” R groups. The six-membered rings in **1iCl** and **1a** have a boat conformation and the ligand plane is roughly a plane of symmetry for the methyl groups. The **1lCl** complex adopts a chair conformation. The F1 atom is virtually in the ligand plane; F2 is perpendicular to it. The separation between F1 and two adjacent amide oxygens O3 and O4 (2.550 and 2.546 Å, respectively) is lower than the sum of the van der Waals radii of O and F (2.75 Å) (23).

Elongated M–Cl bonds in transition metal complexes are readily cleaved by water to afford the corresponding aqua/hydroxo species (24–26). This is also true for the **1Cl** complexes. The speciation of Fe^{III}-TAMLs in water was studied by UV/Vis spectrophotometry and EPR spectroscopy to indicate the following. The chloro ligands of **1Cl** undergo fast hydrolysis (Scheme 1); the equilibrium constants K_{Cl} (Table I) indicate insignificant coordination at $[Cl^-] \leq 0.5$ M. The complexes in water are six-coordinated species with two axial aqua ligands. UV/Vis and EPR spectra are pH independent at 5–8, but vary strongly and reversibly at pH 8–11 due to deprotonation of an aqua ligand to



SCHEME 1. Speciation of Fe^{III}-TAML activators in aqueous solution (solid rectangle) and suggested mechanism of the H⁺-induced demetalation (dashed rectangle); — — = free base ligand. From Ref. (13).

TABLE I

EQUILIBRIUM AND KINETIC PARAMETERS FOR Fe-TAML ACTIVATORS AT 25°C (13)

Complex	pK _a	K _{Cl} (M ⁻¹) ^a	k ₁ [*] (M ⁻¹ s ⁻¹) ^b	k ₃ [*] (M ⁻³ s ⁻¹) ^b
1a	10.1 ± 0.6	0.18 ± 0.04	2.2 ± 0.7 6.1 ± 0.5 ^c	(6.7 ± 0.2) × 10 ⁵ (6.4 ± 0.3) × 10 ^{5a}
1i	10.0 ± 0.2	0.21 ± 0.09	5.19 ± 0.06	(1.13 ± 0.01) × 10 ⁶
1d	10.5 ± 0.5	> 0.05	7.1 ± 0.5	(6.7 ± 0.5) × 10 ⁵
1j	10.4 ± 0.3		0.31 ± 0.02	(1.03 ± 0.05) × 10 ³
1l	9.5 ± 0.4	1.9 ± 0.2	(1.6 ± 0.1) × 10 ⁻⁴	(1.6 ± 0.2) × 10 ⁻⁴
1k	9.4 ± 0.6	2.4 ± 0.4	(3.7 ± 0.4) × 10 ⁻⁵	(4.8 ± 0.6) × 10 ⁻⁵

^apH 7.

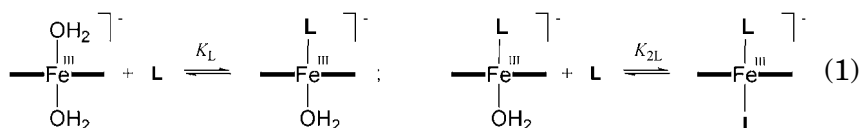
^b0.1 M KPF₆.

^cIn DCl/D₂O.

produce an hydroxo ligand. The corresponding pK_a's are in the range 9.4–10.5 (Table I) (13).

B. BINDING OF AXIAL LIGANDS IN WATER

Spectral changes (Fig. 2) obtained on addition of pyridine to an aqueous solution of **1a** indicate stepwise substitution of both aqueous ligands (Eq. (1)).



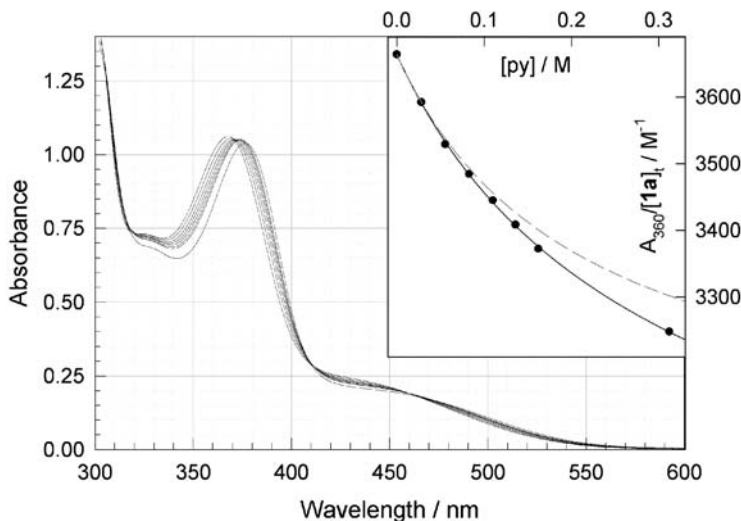


FIG. 2. Spectral changes of **1a** (2.75×10^{-4} M) in the presence of pyridine: 0.1 M KPF₆, pH 6.3 and 25°C. *Inset*: Absorbance at 360 nm as a function of [py]; solid line is calculated curve using best-fit parameters of Eq. (2); broken line is a calculated curve with $K_{2L} = 0$ validating binding of the second axial ligand. From Ref. (27).

The pyridine-induced spectral changes were quantified in terms of Eq. (2).

$$A = \frac{\varepsilon_M + \varepsilon_{ML}K_L[L] + \varepsilon_{ML_2}K_LK_{2L}[L]^2}{1 + K_L[L] + K_LK_{2L}[L]^2} [M]_t \quad (2)$$

Here, ε_M , ε_{ML} , and ε_{ML_2} are the respective extinction coefficients of all participants of Eq. (1) and $[M]_t$ is the total concentration of **1**. The values of K_L and K_{2L} are summarized in Table II. The solid line in the inset was calculated using the best-fit values of ε_{ML} , ε_{ML_2} , K_L , and K_{2L} , while the broken line is the calculated prediction if $K_{2L} \sim 0$. The broken line deviates systematically from the experimental points confirming the binding of the second pyridine-type ligand. Imidazole behaves similarly (27).

The relation $K_L > K_{2L}$ holds for both pyridine and imidazole, but the magnitude of the difference between K_L and K_{2L} is larger for pyridine. An opposite trend ($K_L < K_{2L}$) is observed for Fe^{II} and Fe^{III} porphyrins, for which only β_2 ($K_L \times K_{2L}$) is usually determined in water (28) and nonaqueous solutions (29,30). In water, only bis-ligated species are observed. These are, however, formed from iron-porphyrin dimers, which are the dominating iron-porphyrin species in aqueous solutions (31,32).

TABLE II

 EQUILIBRIUM CONSTANTS FOR Fe^{III}-TAML ACTIVATORS **1** REACTING WITH LIGANDS, L, IN AQUEOUS SOLUTION AT 25°C, 0.1 M KPF₆ (27)

Complex	L	pH	K_1/M^{-1}	K_2/M^{-1}
1a	Pyridine	5.0	4.7 ± 0.1	0.42 ± 0.03
1d			4.3 ± 0.3	^b
1e			9.6 ± 1.0	^b
1a	Imidazole	6.3	69 ± 10	4.7 ± 4.5
1d			28.4 ± 0.8	16 ± 2
1e			24.1 ± 0.6	2.1 ± 0.1
1a	Azide (N ₃ ⁻)	9.17	67 ± 6^a	~ 0

^aWith 1.0 M NaClO₄ as a background electrolyte.

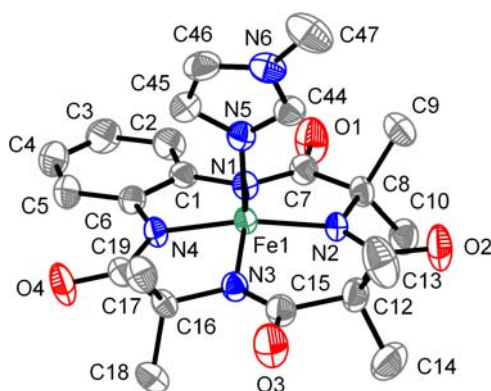
^bCould not be determined.


FIG. 3. The molecular structure of the anionic part of **1a-MeIm**. H atoms omitted for clarity. Fe–N(1) 187.4(2) pm, Fe–N(2) 187.5(2) pm, Fe–N(3) 188.5(3) pm, Fe–N(4) 187.2(2) pm, Fe–N(5) 212.6(2) pm. From Ref. (27).

The structure of the mono-ligated 1-methylimidazole (MeIm) adduct (**1a-MeIm**) was determined by X-ray crystallography (Fig. 3) (27). Five-coordinate iron(III) in a square pyramidal environment is typical of Fe^{III}-TAMLs in the solid state (13,20,33) including the imidazole derivative as demonstrated by this structure (27). There is an interesting orientation of the imidazole ligand relative to the four equatorial amide nitrogens, N1–N4. The imidazole plane is nearly parallel to the plane passing through atoms Fe1, N2, and N4 (dihedral angle 11.2(2)°).

III. Kinetics and Mechanisms of Demetalation of Fe^{III}-TAML Activators

A. SPECIFIC ACID CATALYSIS

Aqueous solutions of **1** (R = Me) at neutral pH are stable for months, but at pH 3–4 the distinctive UV/Vis band around 360 nm fades irreversibly. The free macrocyclic ligand was isolated in >95% yield by treating **1a** at pH 1. For this reaction, plots of the pseudo-first-order rate constants (k_{obs}) vs. [HClO₄] or [HCl] are curved (Fig. 4) and the expression for the pseudo-first-order rate constant is given by Eq. (3) for all compounds investigated (13).

$$k_{\text{obs}} = k_1^*[\text{H}^+] + k_3^*[\text{H}^+]^3 \quad (3)$$

The k_1^* pathway is three times faster in D⁺/D₂O than in H⁺/H₂O for **1a**. The reverse kinetic isotope effect suggests that the rate-limiting event for the k_1^* pathway could involve protonation of an amido-nitrogen or an N–Fe bond, forming the stronger N–H bond as the weaker N–Fe bond is cleaved. The k_3^* pathway is rationalized as involving pre-equilibrium peripheral protonations of the TAML macrocycle (Scheme 1). The dependence of k_{obs} on [H⁺] is then given by Eq. (4), which corresponds

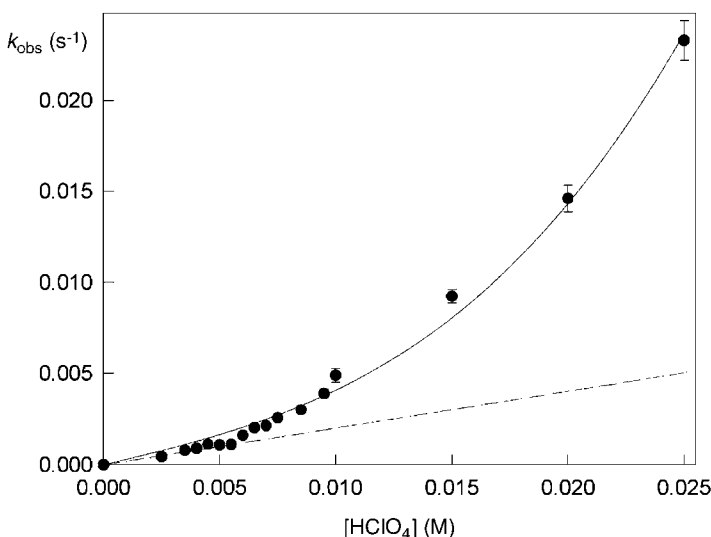


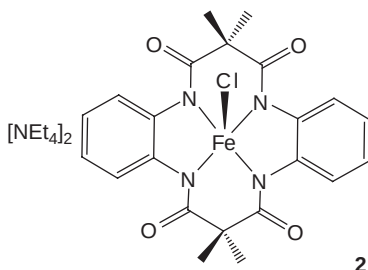
FIG. 4. Dependence of k_{obs} vs. [H⁺] for **1j** demetalation; 25°C, 0.1 M KPF₆. The solid line is calculated using the best-fit k_1^* and k_3^* values. The broken line shows the first-order pathway dominating at lower [HClO₄]. From Ref. (13).

to the experimental rate law when K_{a1} and K_{a2} are high (i.e., **1** complexes are weak bases) ensuring $K_{a1}K_{a2} \gg (K_{a2}[H^+] + [H^+]^2)$ and k_2 is negligible compared to k_1 and k_3 , i.e., $(k_1K_{a1}K_{a2}[H^+] + k_3[H^+]^3) \gg k_2K_{a2}[H^+]^2$, with $k_1^* = k_1$ and $k_3^* = k_3(K_{a1}K_{a2})^{-1}$ (13).

$$k_{\text{obs}} = \frac{k_1K_{a1}K_{a2}[H^+] + k_2K_{a2}[H^+]^2 + k_3[H^+]^3}{K_{a1}K_{a2} + K_{a2}[H^+] + [H^+]^2} \quad (4)$$

The rate of acid-induced demetalation depends only slightly on the nature of the head substituents X (Table I). In contrast, the tail-R groups dramatically affect k_1^* and, for the most part, k_3^* , suggesting that tail amide O-atoms are sites of peripheral protonation. Thus, the acid tolerant Fe^{III}-TAML catalysts with tail electron-withdrawing groups should be more acid resistant and replacement of R = Me with R = F results in a remarkable stabilization. The rate constants (Table I) show that under weakly acidic conditions (pH ~ 2–3), when the k_1^* pathway dominates over k_3^* , fluorinated **1k** is 10⁵-fold more H⁺-tolerant than **1a**.

Under more acidic conditions when the k_3^* pathway dominates ($[H^+] = 1\text{M}$), the reactivity difference is 11 orders of magnitude. The pre-equilibrium protonations associated with this third-order in $[H^+]$ pathway most likely involve tail amide oxygens. The O...F bond separation in **1l**_{Cl} is lower than the sum of the van der Waals radii. A *sterically assisted electronic effect*, which arises from electronic flows both through bond and through space (due to steric constraints and hence the close proximity of the tail amide oxygen and fluorine atoms), appears to play a role in producing the very large stabilizing effect (13).



A mechanism involving a pre-equilibrium M–N cleavage for the third-order pathway for **1** is unlikely because the cleavage of a single M–N bond would possibly cause a significant distortion of the planar complex and induce rapid cleavage of the remaining M–N bonds such that higher order $[H^+]$ dependencies would not

be attained – distorted TAML ligands tend to have non-planar amides and higher hydrolytic instability than their planar analogues. For example, in complex **2** the move from a 5,5,5,6-chelate ring system for the smaller macrocycles that comprise the complexes of [Chart 1](#) to a 5,6,5,6-chelate ring system appears to make the macrocyclic cavity too large for iron(III) such that one amido-*N* ligand becomes significantly non-planar with the result that **2** is unstable in water at pH 7.13. This finding signals a relatively rigid design constraint over the type of ring systems that can be incorporated into TAMLs to produce ferric complexes of acceptable hydrolytic stability. The rate constant k_1^* for **2** is > 1000 times larger than for the most reactive complex in [Table I](#).

DFT studies (in vacuum) for the six-coordinate diaqua species derived from **1a** ([Fig. 5](#)) are consistent with the mechanistic conclusions (27). Although the calculated negative charge density at the amide nitrogens is the highest, one can imagine that the amide oxygens would be more prone to protonation because they are more exposed to the solvent and because H-bonding to water might proceed to protonation with greater facility. For **1a**, the

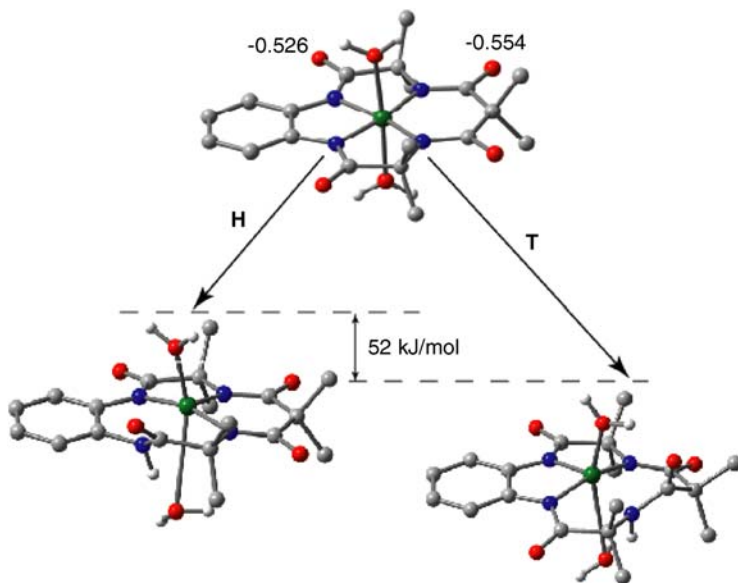


FIG. 5. DFT calculated Mulliken charges (vacuum) of tail and head amide oxygens (top structure) of the diaqua form of **1a** and the relative energies of postulated intermediates of demetalation with cleaved Fe–N bonds in the head (**H**) and tail part (**T**) of the molecule (lower structures). Hydrogens attached to carbon atoms are not shown for clarity. From Ref. (27).

Mulliken charges on the amide oxygens are -0.554 (tail) and -0.526 (head) (Fig. 5). This difference can be inferred as amounting to a substantial determiner of the reactivity, an assessment that is supported by comparing the Mulliken charges at the tail amido oxygens of **1a** (-0.554) and with those of **1k** (-0.491). Complexes **1a** and **1k** exhibit significantly different hydrolytic sensitivities, the latter being vastly more stable (13). DFT calculations of the energetics of protonation indicate that the proton-induced cleavage of the Fe–N_{TAIL} bond is more exergonic than that of the Fe–N_{HEAD} bond (Fig. 5). While there are obvious limitations to a study performed in vacuum, the energy difference between these selectivity options for the diaqua form of **1a** equals 52 kJ mol^{-1} . This significant energy difference indicates a likely favoring of the Fe–N_{TAIL} cleavage in solution that might reduce the free energy of activation in favor of the T-pathway.

Mulliken charges are helpful for mechanistic choices, but frontier molecular orbitals of reacting molecules are more relevant (34,35). The HOMO and the second highest occupied (sHOMO) molecular orbitals of the diaqua form of **1a** are shown in Fig. 6 (27). The HOMO is primarily a “redox active” orbital (36). It is noticeably delocalized over the aromatic ring and two adjacent amide moieties (36). While proton addition to this orbital could occur with significant restructuring at either the head amide nitrogen or oxygen atoms, the electronic density of the sHOMO is much less diffuse and *solely* localized at the nitrogen and oxygen atoms of the tail amide fragment (Fig. 6). The sHOMO is thus arguably a good target for the N-protonation followed by Fe–N bond cleavage and demetalation and for an O-protonation associated with the third-order process in $[\text{H}^+]$. Although the overall energy of the sHOMO is slightly lower than that of HOMO, this orbital could well be more susceptible to the electrophilic

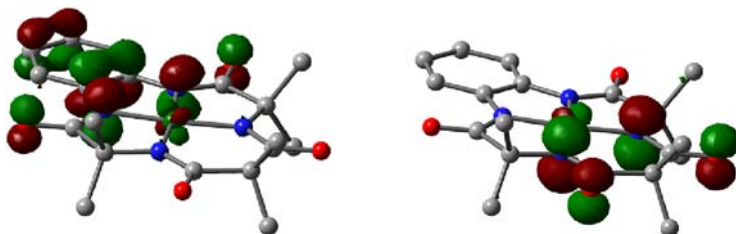


FIG. 6. HOMO (left) and second highest occupied molecular orbital, sHOMO (right) of **1a** predicted by DFT calculations. Hydrogen atoms and axial oxygens are not shown for clarity. From Ref. (27).

proton addition. Note again that solvation could influence the relative energies of these molecular orbitals.

B. GENERAL ACID “COORDINATIVE” CATALYSIS

The Fe^{III} -TAML activators display catalytic activity in neutral and basic aqueous solutions, but it turns out that they are also subject to an unusual catalyzed demetalation by certain, but not all, Brønsted acid buffer components (H_2PO_4^- , HSO_3^- , $\text{CH}_3\text{CO}_2\text{H}$, $\text{HO}_2\text{CCH}_2\text{CO}_2^-$) (27). Buffers based on pyridine (py) and tris(hydroxymethyl)aminomethane (TRIS) are inactive. Where reactions proceed, the products are demetalated TAMLs and iron species of variable composition. Pseudo-first-order rate constants for the demetalation (k_{obs}) depend linearly on total phosphate (Fig. 7A). The effective second-order rate constants $k_{1,\text{eff}}$ depend on pH as shown in Fig. 7B. The right part of the sigmoid profile of $k_{1,\text{eff}}$ approaches zero with an inflection point at ~ 6.5 , coincident with the pK_a of dihydrogen phosphate. This implies that H_2PO_4^- is a major participant in the demetalation process (27).

$$k_{1,\text{eff}} = \frac{a_1[\text{H}^+]}{b_1 + [\text{H}^+]} \quad (5)$$

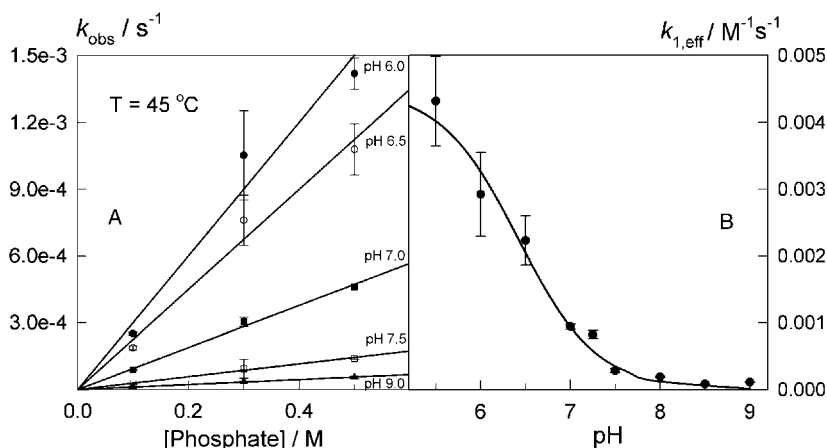
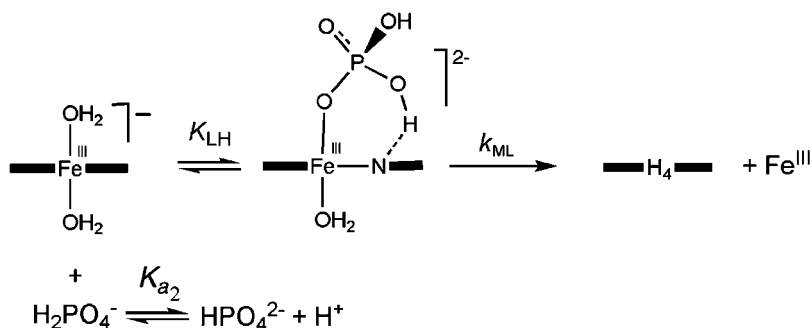


FIG. 7. (A) Pseudo-first-order rate constants for demetalation of **1a** vs. phosphate concentration at different pHs and 45°C. (B) Second-order rate constants $k_{1,\text{eff}}$ for demetalation of **1a** as a function of pH at 45°C. The solid line is a theoretical curve calculated using the best-fit values of parameters of Eq. (5). From Ref. (27).



SCHEME 2. Proposed mechanism for the phosphate-induced demetalation of Fe^{III}-TAMLs.

The data in Fig. 7B quantified in terms of Eq. (5) agree with the mechanism shown in Scheme 2. The rate of demetalation of **1a** in H₂PO₄[−]/HPO₄^{2−} buffer is appreciable, but the *k*_{obs} values for **1k** and **1m** are immeasurably low showing that the rates are strongly affected by the tail CR₂ fragments. The reactivities of **1** depend insignificantly on the aromatic ring or “head” group of **1** (27).

The demetalating abilities of buffer species depend on both their structures and their acidities. Thus, while pyridine-2-carboxylic (picolinic) acid catalyzes the demetalation even of the rather inert **1m**, its 3- and 4-isomers (nicotinic and isonicotinic acids) are inactive. The difference is rationalized to result from the ability that only *coordinated* picolinic acid has to deliver a proton to an amidato nitrogen in an intramolecular manner. The reaction order in picolinic acid equals one for **1a** and two for **1m**. For **1m**, “inactive” pyridine and nicotinic acid speed up the demetalation in the presence of picolinic acid (Fig. 8).

The observed hyperbolic dependences suggest a mechanism that involves a pre-equilibrium binding of two pyridine carboxylates to the Fe^{III} of **1m**, followed by the intramolecular proton transfer from the coordinated acid (Scheme 3). This option has been supported by measurements of the binding constants for py and related ligands. The data in Fig. 8 agree with the mechanism in Scheme 3. The reactive intermediate for robust **1m** is the diaxially coordinated species, one of the two ligands being picolinic acid (L).

Scheme 3 leads to Eq. (6) for the pseudo-first-order rate constant for demetalation of **1m** at a given pH when the concentration of total iron (*M*_t) is much less than that of L and P.

$$k_{\text{obs}} = \frac{k_{2\text{L}}K_{\text{L}}K_{2\text{L}}[\text{L}]^2 + k_{\text{LP}}K_{\text{L}}K_{\text{LP}}[\text{L}][\text{P}]}{1 + K_{\text{L}}[\text{L}] + K_{\text{P}}[\text{P}] + K_{\text{L}}K_{2\text{L}}[\text{L}]^2 + K_{\text{P}}K_{2\text{P}}[\text{P}]^2 + K_{\text{L}}K_{\text{LP}}[\text{L}][\text{P}]}$$

(6)

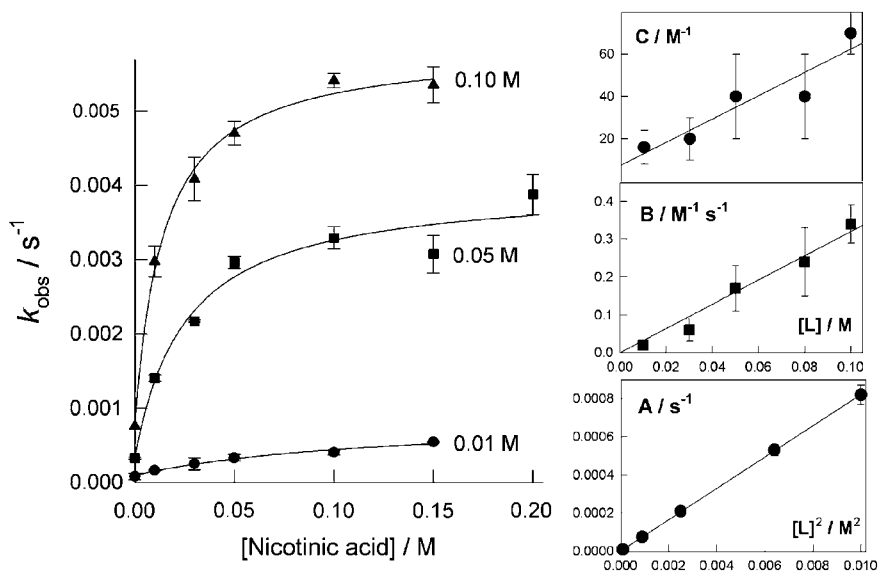
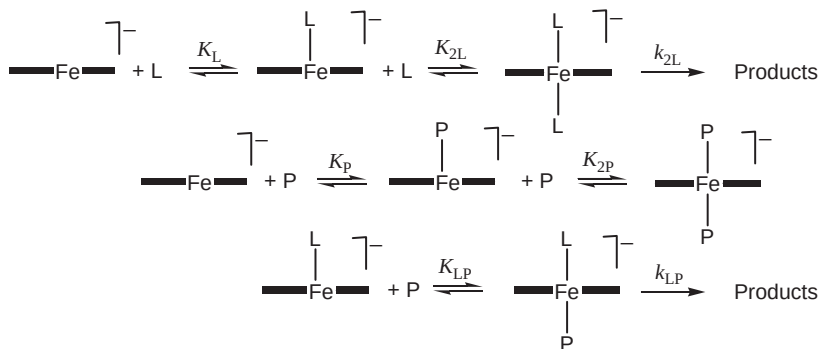


FIG. 8. Effect of nicotinic acid on demetalation of **1m** in the presence of various concentrations of picolinic acid (0.01, 0.05, and 0.1 M) at pH 4.5 and 65°C. Boxes on the right show dependencies of parameters *A*, *B*, and *C* of Eq. (10) on [picolinic acid]^{*m*} (*m* = 2 for *A* and 1 for *B* and *C*). See text for details. From Ref. (27).



SCHEME 3. Stoichiometric mechanism of demetalation of robust Fe^{III}-TAMLs such as **1m** by picolinic acid (L) in the presence of other pyridine bases (P). Axial aqua ligands are omitted for clarity.

In the absence of added P, Eq. (6) becomes Eq. (7).

$$k_{\text{obs}} = \frac{k_{2\text{L}}K_{\text{L}}K_{2\text{L}}[\text{L}]^2}{1 + K_{\text{L}}[\text{L}] + K_{\text{L}}K_{2\text{L}}[\text{L}]^2} \quad (7)$$

The experimentally observed second-order dependence on L for **1m** implies that $1 \gg K_{\text{L}}[\text{L}] + K_{\text{L}}K_{2\text{L}}[\text{L}]^2$ and $k_{\text{obs}} = k_{2\text{L}}K_{\text{L}}K_{2\text{L}}[\text{L}]^2$. Therefore, in the presence of P, Eq. (6) appears in the form

$$k_{\text{obs}} = \frac{k_{2\text{L}}K_{\text{L}}K_{2\text{L}}[\text{L}]^2 + k_{\text{LP}}K_{\text{L}}K_{\text{LP}}[\text{L}][\text{P}]}{1 + K_{\text{P}}[\text{P}] + K_{\text{P}}K_{2\text{P}}[\text{P}]^2 + K_{\text{L}}K_{\text{LP}}[\text{L}][\text{P}]} \quad (8)$$

The dependence of k_{obs} on [P] is hyperbolic (Fig. 8) and hence the term $K_{\text{P}}K_{2\text{P}}[\text{P}]^2$ in the denominator is negligible. Consequently,

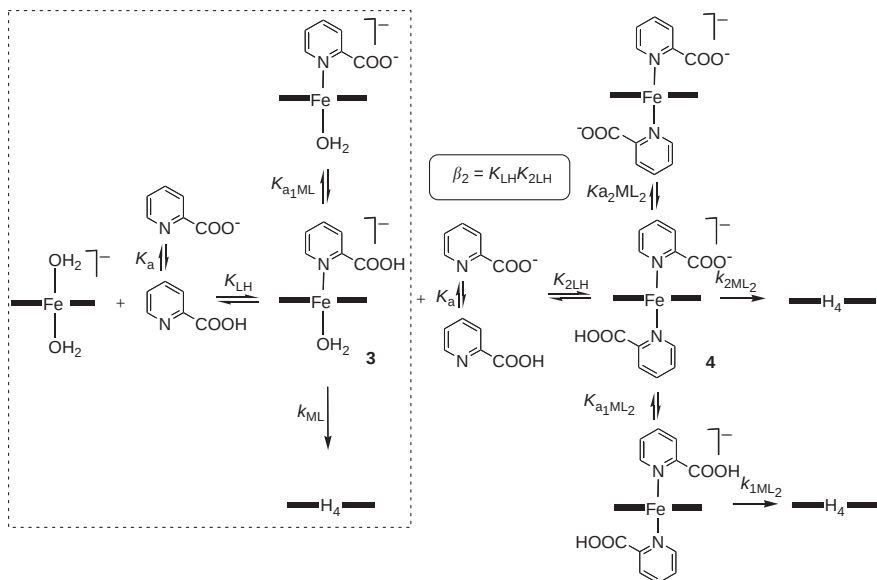
$$k_{\text{obs}} = \frac{k_{2\text{L}}K_{\text{L}}K_{2\text{L}}[\text{L}]^2 + k_{\text{LP}}K_{\text{L}}K_{\text{LP}}[\text{L}][\text{P}]}{1 + (K_{\text{P}} + K_{\text{L}}K_{\text{LP}}[\text{L}])[\text{P}]} \quad (9)$$

At constant L, the dependence of k_{obs} on [P] appears as

$$k_{\text{obs}} = \frac{A + B[\text{P}]}{1 + C[\text{P}]} \quad (10)$$

Here, $A = k_{2\text{L}}K_{\text{L}}K_{2\text{L}}[\text{L}]^2$, $B = k_{\text{LP}}K_{\text{L}}K_{\text{LP}}[\text{L}]$, and $C = K_{\text{P}} + K_{\text{L}}K_{2\text{L}}[\text{L}]$. Both B and C should be linear functions of [L], whereas A should be proportional to $[\text{L}]^2$. The graphs in the small boxes of Fig. 8 show such dependencies. The fits of the plots of A and B vs. [L] and $[\text{L}]^2$, respectively, intersect with the origin. The intercept of the plot of C against [L], which should equal K_{P} , is close to 8 M^{-1} and falls in the range of values found for K_{L} (Table II).

Scheme 4 presents the most general mechanistic picture of demetalation by picolinic acid of all investigated complexes **1**, which actually holds for any Brønsted acid capable of intramolecularly delivering a proton. The boxed component corresponds to those **1** complexes that obey first-order kinetics in picolinic acid (**1a**, for example). The boxed part leads to kinetic Eq. (11) that is valid at pH 4–6.5 because the pK_{a} of the pyridine nitrogen of picolinic acid equals 1.60 (37). It is interesting to note that Eq. (11) indicates that the reaction rate in such cases is determined by the pK_{a} s of both the free and coordinated ligand in the case of weak (formally $[\text{L}] \rightarrow 0$) or “saturative” ($[\text{L}] \rightarrow \infty$)



SCHEME 4. Proposed general mechanism of demetallation of **1** by picolinic acid accounting for first (**1a**, in the box) and second (**1m**) orders in the buffer acid concentration. The charge of the Fe^{III} -TAML complex is shown outside the bracket and localized charges are shown for the deprotonated pyridine carboxylates. From Ref. (27).

binding, respectively.

$$k_{\text{obs}} = \frac{k_{\text{ML}}K_{\text{LH}}[\text{L}][\text{H}^+]}{(K_{\text{a}} + [\text{H}^+]) + K_{\text{LH}}[\text{L}][\text{H}^+] + K_{\text{a1ML}}K_{\text{LH}}[\text{L}]} \quad (11)$$

The entire Scheme 4 accounts for the second-order kinetics in picolinic acid for **1m** in terms of Eq. (12) under the assumption that $k_{\text{ML}} \approx 0$ and setting $\beta_2 = K_{\text{LH}}K_{2\text{LH}}$. Eq. (12) rationalizes both the second-order in picolinic acid concentration and the observed pH profile for k_{obs} in the case of **1m**.

$$k_{\text{obs}} = \frac{(k_{1\text{ML}_2}[\text{H}^+]^2 + k_{2\text{ML}_2}K_{\text{a1ML}_2}[\text{H}^+])\beta_2[\text{L}]^2}{(K_{\text{a}} + [\text{H}^+])^2 + ([\text{H}^+]^2 + K_{\text{a1ML}_2}[\text{H}^+] + K_{\text{a1ML}_2}K_{\text{a2ML}_2})\beta_2[\text{L}]^2} \quad (12)$$

Species **3** and **4** in Scheme 4 are key intermediates in the demetallation of **1a** and **1m**, respectively. Picolinic acid binds to the axial site of Fe^{III} and then delivers the carboxylic proton to a Fe–N bond. This is more difficult in the case of **1m** where the amidato-N donor atoms are less basic. Therefore, the second py

ligand may increase the electron density at the Fe–N bonds and it may also affect the location of the iron atom with respect to the plane of the four amidato ligands in a manner that favors the demetalation. It is significant that the picolinic acid α -carboxylic group is clearly not essential for this second pyridine ligand because both py and nicotinic acid also accelerate the demetalation.

The mechanism of demetalation shows how buffer components can be chosen based on their structures and acidities so that they will be inactive in the demetalation in the vicinity of neutral pH. This demetalation is a case of *coordinative* general acid catalysis, because the free proton is not implicated, and while we do not object to the general acid catalysis label, it is important to note that only one of all the proton sources in any given solution contributes to driving the hydrolyses and, most unusually, the catalysis is also predicated upon precoordination of the buffer ion. Preferably, buffers chosen to avoid demetalation should not have pK_a's in the range 4–8, but if there is a particular advantage for any such buffer, the proton-bearing buffer ion should not have a structure that enables it to bind to the iron(III) of **1** and deliver a proton in an intramolecular fashion to a coordinated amidato-N atom, i.e., avoid buffer ions capable of forming 6- and probably also 5-membered rings for proton delivery as in [Scheme 2](#). The knowledge developed in this study (27) is especially valuable for avoiding unfavorable catalyst degradation processes in functioning Fe^{III}-TAML activator systems as well as for storing Fe^{III}-TAML catalyst solutions.

IV. Catalysis-Relevant Oxidized Forms Derived from Fe^{III}-TAMLS

In 2006, a review was published that summarized the structural and spectroscopic properties of oxidized forms of Fe-TAML activators produced under non-catalytic conditions (36). In the conclusion section, we emphasized that the earlier results “provide the basis for the unambiguous identification of the high-valent intermediates, which are generated from H₂O₂, organic hydroperoxides, peroxides, dioxygen, or other oxidizing agents”. In recent years as articulated in this section, we have been able to characterize additionally some of the high-valent iron compounds (we believe there are others) that are essential for understanding the mechanisms of catalysis by compounds **1**.

A. Fe(IV): OXIDATION BY DIOXYGEN IN APROTIC ORGANIC SOLVENTS

Compounds **1** with alkali metal counter ions M⁺ are insoluble in aprotic organic solvents. The phosphonium salts [PPh₄]**1** are

readily soluble in solvents such as dichloromethane to form orange or reddish orange solutions which remain orange under a nitrogen atmosphere. However, for **1a** or **1d**, the solutions turn black in minutes (≤ 10) on standing under air or more rapidly upon bubbling of air or O_2 through the solution (38). UV/Vis data indicate that the conversion in solution is quantitative and isolated yields of these black compounds are greater than 80%. Compounds **1** with methyl tails groups are oxidized by O_2 ; the fluorine-tailed molecules such as **11** are significantly less reactive. X-ray crystallographic studies demonstrate that the products formed from **1a,d** are bis(Fe^{IV})- μ -oxo complexes **5** (Fig. 9). Each Fe^{IV} center is coordinated by four deprotonated amide nitrogens and the axial bridging oxo ligand. The average Fe–N bond distances in **5a** (1.887(13) Å) and **5d** (1.891(12) Å) are comparable to the reported distances for Fe^{III} and Fe^{IV} mononuclear TAML complexes (20,36,39,40). The mean deviation of each iron atom from the 4N plane in **5a** and **5d** equals 0.50 Å. The Fe–O bond distances in **5a** (1.7284(8) Å, symmetric) and **5d** (1.744(3) and 1.730(2) Å, asymmetric) are shorter than those in reported bis(μ -oxo)-bridged complexes, $Fe^{IV}(O)_2Fe^{IV}$ (1.78 Å) (41) and $Fe^{III}(O)_2Fe^{IV}$ (1.80, 1.860 Å) (42). The Fe–O distance in **5a** is slightly shorter than the average (1.77 Å) reported for μ -oxo-bridged Fe^{III} compounds (43) and significantly longer than the $Fe=O$ distances in various protein and synthetic $Fe^{IV}=O$

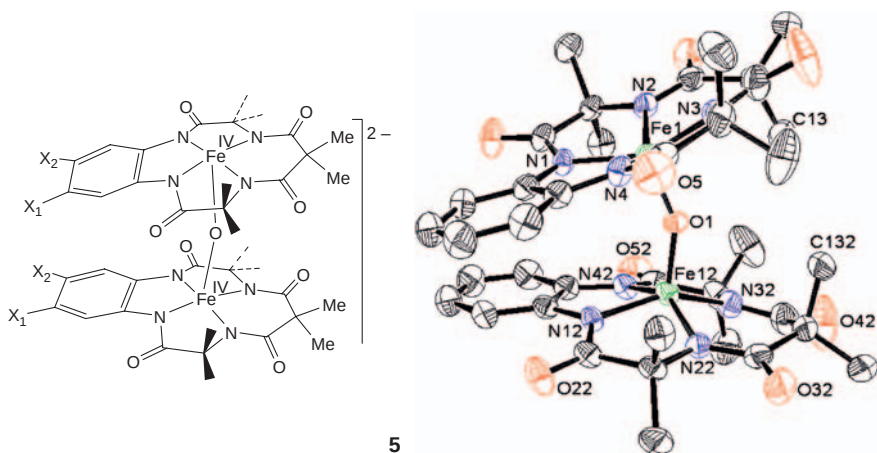


FIG. 9. Composition (**5a**, $X_{1,2} = H$; **5d**, $X_{1,2} = OCH_3$) and ORTEP (**5a**) structures of the black μ -oxo-bridged diiron(IV) complex rapidly formed in a high yield from **1a,d** and O_2 in CH_2Cl_2 or other weakly coordinating solvents. The ellipsoids are drawn at the 50% probability level. From Ref. (38).

heme derivatives (1.60–1.69 Å, EXAFS) (44–46) or non-heme mononuclear Fe^{IV}=O complexes of tetradentate-N4 macrocyclic and tripodal ligands (1.65–1.67 Å, X-ray and EXAFS) (47). The Fe^{IV}–O–Fe^{IV} bond angles in **5** are in the range of 151.4(2)–153.8(2)° and are comparable to reported Fe^{III}–O–Fe^{III} bond angles (43). The planes defined by the four N atoms surrounding the iron(IV) sites are tilted toward the benzene rings, perhaps to avoid steric crowding of the methyl groups at the chelate rings. There is a short C13...O1 contact in complex **5a** suggesting an intramolecular hydrogen bond between the bridging oxygen and the corresponding hydrogen at C13; the C–H...O1 separation is 2.319 Å. The Mössbauer spectra of solid **5a** obtained at 4.2 K reveal a high-valent character for the iron centers. The spectral parameters ($\Delta E_Q = 3.3 \text{ mm s}^{-1}$, $\delta = -0.07 \text{ mm s}^{-1}$) are characteristic of an $S = 1$ Fe^{IV} site. The Mössbauer data do not exclude the possibility that the local sites of **5** are high-spin ($S = 2$) Fe^{IV}.

The reaction between **5a** and PPh₃ that affords **1a** and OPPh₃ was used to probe whether or not the bridging μ -oxo ligand originates from O₂, and the experimental evidence convincingly demonstrates that it does (38). The oxidation of PPh₃ into OPPh₃ is a catalytic process. In addition, **5** compounds catalyze the oxidation of benzylic alcohols (benzyl, 4-chlorobenzyl, 4-nitrobenzyl, 4-methoxybenzyl, cinnamyl) by O₂ into the corresponding aldehydes in 1,2-dichlorobenzene or nitrobenzene at 100°C. The highest conversion thus far involves transformation of 60 equivalents of benzyl alcohol to benzaldehyde per equivalent of **5a** within 1 h without production of benzoic acid. As the alcohols are oxidized, **5a** returns to **1a**. The oxidation also occurs at 40–50°C where benzyl alcohol is oxidized to benzaldehyde. The oxidation of alcohols was also achieved by *in situ* generation of **5a** from [PPh₄]**1a** in 1,2-dichlorobenzene with subsequent addition of alcohols. When complex **5a**, dissolved in acetone or acetonitrile, is added to an aqueous solution of Orange II, rapid bleaching of this commercial dye occurs, but it is not catalytic under these conditions.

B. Fe(IV): OXIDATION BY PEROXIDES IN WATER

Addition of H₂O₂ or *t*-BuOOH to aqueous solutions of iron(III)-TAML complexes **1** produces brownish-green colors. The spectral changes can be measured by UV/Vis spectroscopy (Fig. 10). Less than a stoichiometric amount of peroxide causes a major increase in the absorbance in the range 350–550 nm. It requires several minutes to obtain invariable spectra at pH below 8.5, but the reaction becomes much faster at pH > 9. Two isosbestic points

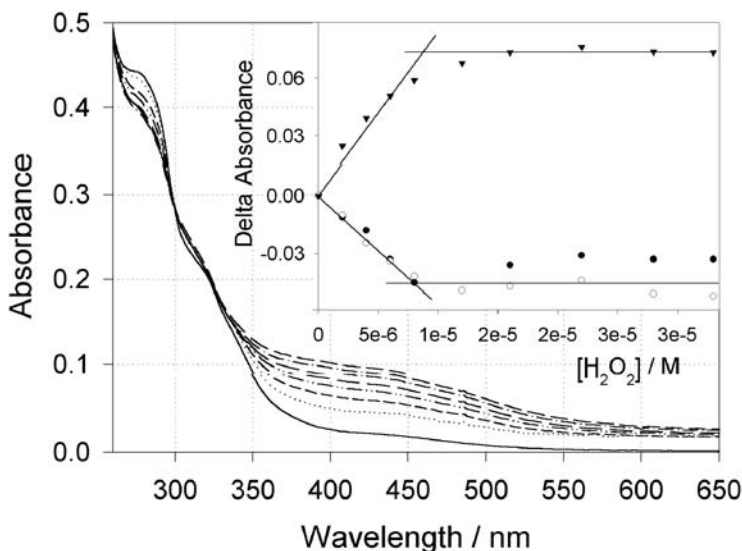


FIG. 10. Spectral changes of **1i** (2.5×10^{-5} M) in the presence of 0, 2×10^{-6} , 4×10^{-6} , 6×10^{-6} , 8×10^{-6} , 12×10^{-6} , and 16×10^{-6} M H_2O_2 at pH 11.4, 25°C . Inset: Titration curves at 242 (●), 280 (○), and 420 (▼) nm showing a 2:1 ($\text{Fe}^{\text{III}}:\text{H}_2\text{O}_2$) stoichiometry.

hold at 298 and 324 nm for **1i**. At pH 11, the changes are fast and the final spectrum remains practically unchanged within 30 min. The inset in Fig. 10 shows that the absorbing species are by 1 oxidation equivalent above the resting state.

When an aqueous solution of *t*-BuOOH is added to a solution of **1a** at pH 14 (0.01 M phosphate, 1 M KOH), a red color appears promptly that is stable at room temperature for hours. The same species can be obtained at any pH higher than 12. A titration of **1a** by *t*-BuOOH followed by UV/Vis spectroscopy revealed that *ca.* 0.5 equivalent of the peroxide per Fe^{III} is needed to obtain the maximum change. Similar results have been obtained with H_2O_2 under identical conditions all suggesting the formation of an Fe^{IV} species (48).

The zero-field Mössbauer spectrum of a frozen aqueous solution of the red compound (generated from 1×10^{-3} M ^{57}Fe -enriched **1a** and 0.5 equivalent *t*-BuOOH) at 4.2 K consists of one doublet with a quadrupole splitting, $\Delta\text{EQ} = 4.2 \text{ mm s}^{-1}$ and isomer shift (vs. Fe metal at 298 K) $\delta = -0.19 \text{ mm s}^{-1}$ (Fig. 11). This negative isomer shift is indicative of an Fe^{IV} formulation in the TAML systems. Variable high field (1.5, 6.5, and 8 T) and high temperature (150 K) studies indicated an $S = 1$ species. The Mössbauer data quantitatively account for $\sim 100\%$ of the total iron at pH 14

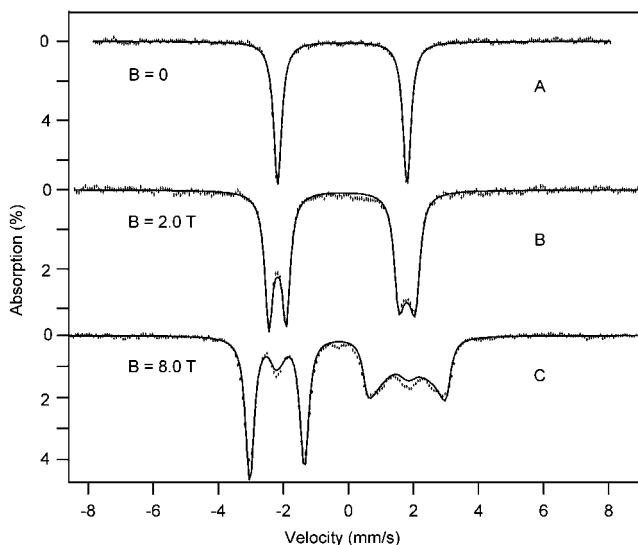
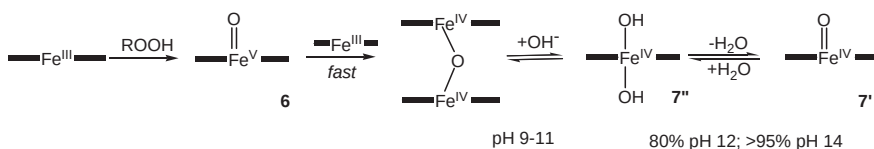


FIG. 11. 4.2 K Mössbauer spectra of ⁵⁷Fe-enriched **7'** in aqueous solution, pH 14, recorded in parallel applied fields as indicated. The solid lines are spectral simulations. At least 95% of the iron in the sample belongs to ⁵⁷Fe-**1a**. From Ref. (48).



SCHEME 5. Speciation of oxidized TAML species derived from **1** in aqueous solution. Axial aqua ligands are not shown.

as this Fe(IV) species (*ca.* 80% at pH 12). Minor instability of the oxidized species has been detected at pH < 12 (48).

The UV/Vis, Mössbauer, EXAFS, and EPR spectroscopic data suggest a rather complicated picture regarding the speciation of oxidized TAML species derived from **1** and various oxidants *in aqueous solution* (Scheme 5). Peroxides ROOH have the capacity to function as two-electron oxidants and usually do. In cases where prior coordination occurs, they can oxidize metal ions via one-electron processes where the O–O bond is cleaved homolytically or two-electron processes where it is cleaved heterolytically. The two-electron oxidation of **1** presumably would give the iron-oxo intermediate **6**, two electrons oxidized above the iron(III) state (see below). Before **6** was actually isolated, there

was considerable reason to believe it would be formed and that it actually would be an iron(V) compound, as opposed to an iron(IV)-oxo-ligand radical cation ensemble (20). This was based on intuitive thinking about the donor capacities of TAMLs to iron vs. those of porphyrins where two-electron oxidation leads to the metal-ligand oxidized ensemble and other ligand systems. In the actual chemistry of **1**, as has been found for sterically hindered anionic porphyrin chemistry (49), the highly oxidized intermediate **6**, if it indeed forms in water as it does in organic solvents which is reasonable to assume (see below), is rapidly quenched by **1** with iron(V) and iron(III) undergoing comproportionation (the reverse of disproportionation, also sometimes called “synproportionation” or “conproportionation”) to the room temperature observable, less oxidized iron(IV) species, the speciation of which is significantly pH dependent. At pH 14, the iron (*ca.* 95%) is to be found in the form of the monomeric oxoiron(IV) species **7'**, although theoretical DFT simulations (see below) do not exclude its aquated formulation as **7''**.

The nature of **7'** has been analyzed by DFT using Gaussian 03 (50). The B3LYP functional and the 6-31G level of basis function is known to predict reliably the electronic and structural properties of complexes **1** (36). Similar calculations show for **7'** that the $S = 1$ spin state is energetically more favorable than both high or low-spin states (Fig. 12). Interestingly, the theory predicts that 5- and 6-coordinated iron(IV) can be formulated as **7'** and **7''**, respectively. The central metal in **7'** does not hold axial water. This is obviously due to a strong trans influence of the axial oxo ligand with its intense donor capacity and its of

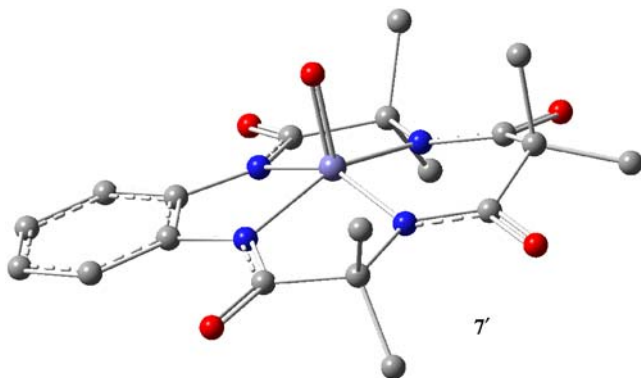


FIG. 12. DFT-predicted lowest energy structure of oxidized species **7'**. From Ref. (48).

charge -2 . It should, however, be added that the six-coordinated iron-oxo species with weakly bound water cannot be excluded.

C. Fe(V): OXIDATION BY PEROXIDES IN ORGANIC SOLVENTS

An oxoiron(V) species such as **6** derived from **1** and peroxides is accessible in nonaqueous media (51). The reaction of the tetraphenylphosphonium salt of **1a** with 2 to 5 equivalents of *m*-chloroperbenzoic acid (*m*CPBA) at -60°C in *n*-butyronitrile produces within about 10 s a bis-iron(IV)- μ -oxo dimer followed by an as – yet uncharacterized EPR – silent iron(IV) intermediate. After 15 min, the deep green oxoiron(V) species **6** forms with distinct absorption maxima at 445 nm ($\epsilon = 5400 \text{ M}^{-1} \text{ cm}^{-1}$) and 630 nm ($\epsilon = 4200 \text{ M}^{-1} \text{ cm}^{-1}$). At -60°C , **6** decays by 10% in 90 min, but it is stable for at least one month at 77 K. Selected spectral data for the oxoiron(V) species are shown in Fig. 13. DFT calculations favor the low-spin ($S = 1/2$) configuration of the ground state. The calculated Fe–O bond length of 1.60 Å is in excellent agreement with the EXAFS results. The Fe atom is displaced out of the 4-N plane by 0.5 Å.

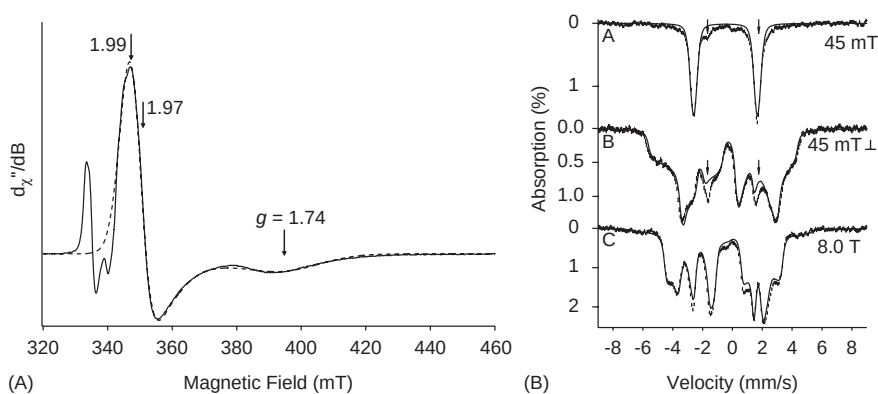
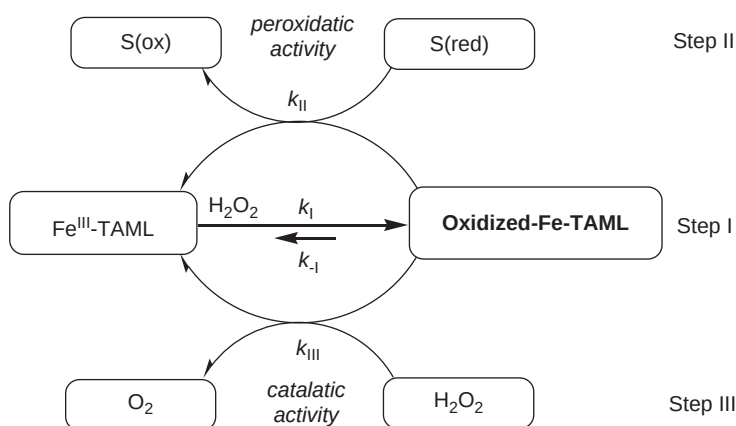


FIG. 13. X band EPR (A) and Mössbauer (B) spectra of *ca.* 2 mM ⁵⁷Fe-enriched oxoiron(V) compound **6** in *n*-butyronitrile. EPR: 28 K; frequency, 9.66 GHz; microwave power, 0.02 mW; modulation, 1 mT. The dashed line is a spectral simulation. Mössbauer: 140 K (A) and 4.2 K (B, C) in magnetic fields indicated; incident γ -beam perpendicular (B) and parallel (C) to the applied field. The solid lines are spectral simulations. They represent 95% of the total absorption. Arrows (in A and B) indicate a decay product (3% of Fe) with $\Delta\text{EQ} = 3.4 \text{ mm s}^{-1}$ and $\delta = 0.08 \text{ mm s}^{-1}$. From Ref. (51).

V. Mechanism of Catalysis by Tetraamide Macrocyclic Fe^{III}-TAML Activators of Hydrogen Peroxide, Functional Catalase-Peroxidase Replicas

A. GENERAL SCHEME

As noted in the opening paragraph, Fe^{III}-TAML activators catalyze the oxidation by H₂O₂ and other peroxides of a wide spectrum of targetable substrates (1–3). Extensive mechanistic information on catalysis by **1** has been published recently (6,52,53) and is reviewed here in this section. The overall reactivity can be captured by the statement that Fe^{III}-TAML activators are functioning mimics of the peroxidase enzymes that also possess significant catalase-like activity. The general mechanistic summary of the major directions of catalysis in its most simple form is in Scheme 6. This scheme represents what may be in the final analysis a number of overlapping cycles. Several catalyst degradation processes (not shown) are well understood and have been parameterized in time against directed peroxidase-like activity (see Section V.B). We will show that the catalase-like activity of the catalysts is practically negligible in the presence of a suitable target substrate S(red), and the **1** catalysts transfer both oxidation equivalents from H₂O₂ onto S(red) with high efficiency.



SCHEME 6. General mechanistic picture of catalysis by Fe^{III}-TAML activators of peroxides that underscores the peroxidase-like (peroxidatic) and catalase-like (catalatic) activities of the catalysts. Here, S(red) is an oxidizable target substrate. All rate constants are conditional (pH dependent).

Oxidized Fe-TAML could be the iron(V)oxo complex **6**, which as noted above can be produced from **1a** and *m*-chloroperoxybenzoic acid at low temperatures (−60°C) in a nonaqueous solvent (51). Presumably such an iron(V)oxo complex can behave in a substrate-dependent way as both a two-electron or one-electron oxidant. In the former case, it is returned in one step to the iron(III) state. In the latter, it must first pass through an iron(IV) intermediate. At pH > 12, the likely iron(IV) species would be the same compound as is formed from **1a** and H₂O₂, (48) namely the iron(IV)-oxo complex **7'**, which has similar features with [(H₂O)₅Fe^{IV}=O]²⁺, (54) or its water adduct **7''**. At other pHs, other iron(IV) compounds are known to be formed (48). Both the iron(V)-oxo and iron(IV)-oxo complexes as well as the other iron(IV) species could be involved in catalysis by **1** (see Section V.B). The possible involvement of complexes that are in a higher oxidation state than **6** cannot be ruled out.

B. PEROXIDASE-LIKE ACTIVITY

If step III in Scheme 6 is ignored, a simplification is reached that has been demonstrated to be reasonable with certain substrates (see below). As a consequence, then the **1** catalysts should oxidize targeted reductants in accordance with Eq. (13) (here [Fe-TAML] is the total concentration of all TAML iron in solution). Eq. (13) implies that the catalysis by **1** mimics the steady-state oxidation by peroxidase enzymes, where Compound I is much more reactive than Compound II (55).

$$-\frac{d[\text{S(red)}]}{dt} = \frac{k_{\text{I}}k_{\text{II}}[\text{Fe-TAML}][\text{H}_2\text{O}_2][\text{S(red)}]}{k_{-\text{I}} + k_{\text{I}}[\text{H}_2\text{O}_2] + k_{\text{II}}[\text{S(red)}]} \quad (13)$$

Eq. (13) has been tested in extensive investigation of the **1a**-catalyzed oxidation of the Orange II dye (Chart 2) at different concentrations of the dye and H₂O₂ (Fig. 14) (6). The data suggest that *k*_{−I} is negligible and the reaction rate levels off with increasing concentrations of both reagents. When the concentration of H₂O₂ is low, the formation of oxidized Fe-TAML is rate-limiting and the initial rates are independent of [Orange II]. Speeding up the formation of oxidized Fe-TAML by increasing [H₂O₂] moves the oxidation into a different kinetic regime where the step driven by *k*_{II} is the slowest and hence the reaction rate is almost proportional to the Orange II concentration. These properties give rise to characteristic 3D plots such as Fig. 14 for two-substrate catalytic reactions that mark in this case the

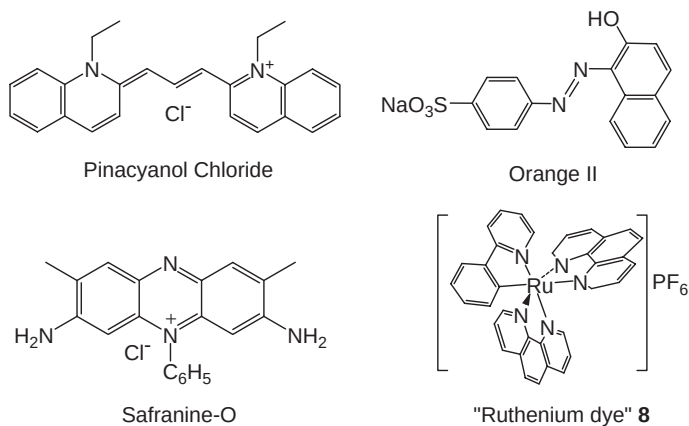


CHART 2. Water-soluble organic dyes and a ruthena(II)cyclic dye used in these studies.

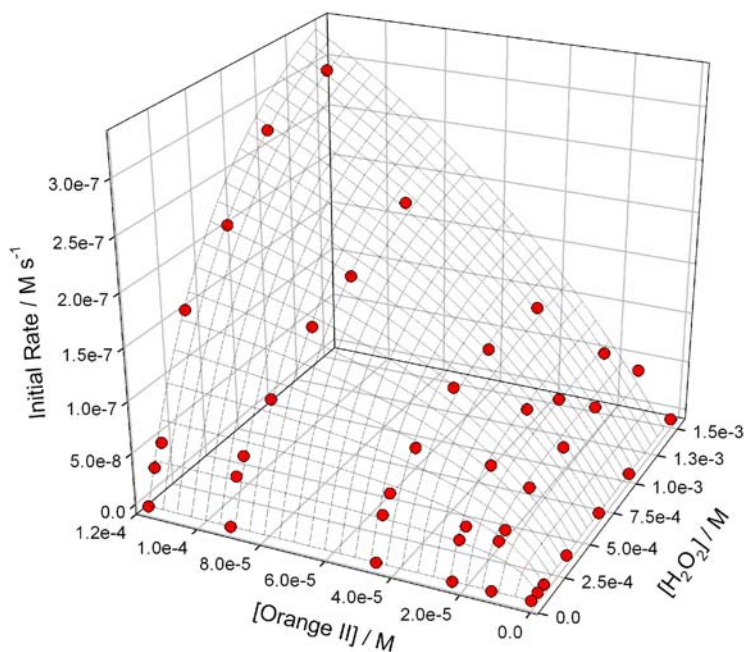
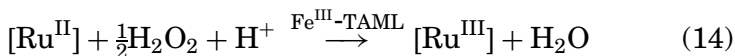


FIG. 14. 3D-plot showing the dependence of initial rates of **1a**-catalyzed bleaching of Orange II by H_2O_2 as a function of $[\text{H}_2\text{O}_2]$ and $[\text{Orange II}]$. Conditions: **[1a]** 2×10^{-7} M, pH 11, 25°C . From Ref. (6).

numerical values of the products $k_I[\text{H}_2\text{O}_2]$ and $k_{II}[\text{S}(\text{red})]$ as comparable (56).

The rate constants k_I and k_{II} for **1a** calculated from the data in Fig. 14 of *ca.* 3.5×10^3 and $1.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (pH 11, 25°C), respectively, illustrate a very high activity of Fe^{III}-TAML activators particularly in terms of k_{II} . For example, Oakes and Gratton reported the value of $0.08 \text{ M}^{-1} \text{ s}^{-1}$ for the oxidation of Orange II by *p*-sulfonated perbenzoic acid under the same conditions (57).

A key challenge for parameterizing the peroxidase-like behavior was to find reaction conditions where the kinetics of the interactions of Fe^{III}-TAML catalysts with H₂O₂ would be rate-determining. It was essential to find a fast reacting substrate where the oxidation process could also be followed in a straightforward manner over all pHs and temperatures of interest. The cyclometalated Ru^{II} complexes [Ru(C~N)(LL)₂]⁺PF₆⁻ (**8**; C~N = cyclometalated 2-phenylpyridine or 4-tolylpyridine, LL = bpy or phen) are very reactive electron donors toward peroxidases from different sources – the Ru^{III}/Ru^{II} reduction potentials reside around 200 mV (vs. SCE) (56,58,59). The rate constants for oxidation to their Ru^{III} forms measured under the steady-state conditions are higher than $10^7 \text{ M}^{-1} \text{ s}^{-1}$. Complexes **8** are inert to ligand substitution and their redox potentials are pH invariant in the range 6–11.5 and therefore, they were selected for kinetic studies of the peroxidase-like activity of **1**. Exactly 1 mole of H₂O₂ oxidizes 2 moles of Ru^{II} in accordance with the stoichiometry of Eq. (14). Both oxidation equivalents of H₂O₂ are thus delivered by Fe^{III}-TAMLs to the ruthenium complex and competition from catalase activity has been eliminated by the rapidity of the peroxidase-like process (53). Moreover, since complexes **8** are one-electron reductants, they were expected to direct the reactivity around a catalytic cycle from a Compound I-like intermediate, assuming it is indeed also formed in water, via Compound II-like intermediate on route to the Fe^{III}-TAML.



The kinetics of reaction (14) using different TAML activators **1** have been investigated over a wide pH range. The rates are directly proportional to the concentrations of Fe^{III}-TAML activators. First-order dependence has also been observed in the H₂O₂ concentration. The steady-state rate is independent of the **8** concentration. The zero-order dependency in the Ru^{II} complex and the first-order dependence in H₂O₂ both hold over the *entire pH range* investigated (6–11.5). The experimental rate law

(Eq. (15)) suggests that the rate-limiting step involves the interaction between **1** and H_2O_2 (53).

$$\text{Rate} = k_{\text{obs}}^{\text{per}}[\mathbf{1}][\text{H}_2\text{O}_2] \quad (15)$$

The rate law of Eq. (15) holds at all pHs, despite the fact that $k_{\text{obs}}^{\text{per}}$ is strongly pH dependent (see below). Free radical oxidation chemistry (60) appears not to be involved in these Fe^{III} -TAML catalyzed oxidations to any detectable degree. The efficient hydroxyl radical scavenger, mannitol (61,62), when added over the concentration range $(0.5\text{--}2.0) \times 10^{-3} \text{ M}$ has no effect on the rate. This peroxide oxidation catalyzed by **1** does not proceed extensively via the hydroxyl free radical serving as the reactive intermediate.

The dependencies of $k_{\text{obs}}^{\text{per}}$ on pH for **11** at 25–45°C are shown in Fig. 15. The rate constant increases dramatically at $\text{pH} > 9$ reaching a maximum around pH 10 and declines at higher pHs. These dependencies are analyzed in what follows using a conventional approach as shown in Scheme 7 using the known pK_a 's of the catalysts (13) and H_2O_2 (63). The diaqua and aqua/hydroxo Fe^{III} complexes are likely reactive species that interact pairwise with either H_2O_2 or its conjugate base ($\text{pK}_{a_2} \sim 11.2\text{--}11.6$ (63)) with the rate constants $k_1\text{--}k_4$ to give an oxidized Fe-TAML, which then converts rapidly Ru^{II} into Ru^{III} . Scheme 7 leads to

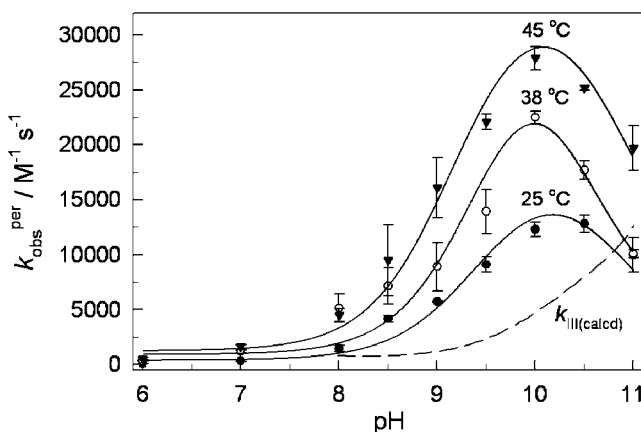
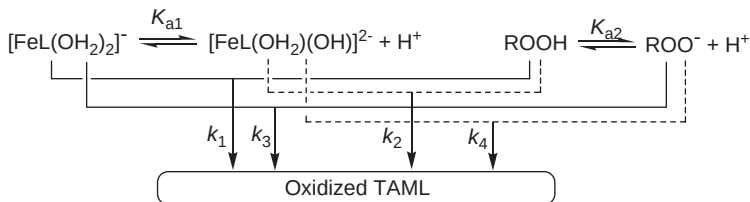


FIG. 15. pH profiles of the rate constants $k_{\text{obs}}^{\text{per}}$ for the oxidation of **8** by H_2O_2 catalyzed by **1c** at different temperatures. Conditions: [**11**] $1.28 \times 10^{-7} \text{ M}$, [**8**] $5.81 \times 10^{-5} \text{ M}$, $[\text{H}_2\text{O}_2]$ $3.3 \times 10^{-4} \text{ M}$, 0.01 M phosphate buffer. Dashed line shows the calculated dependence of k_{III} vs. pH, see text for details. From Ref. (53).



SCHEME 7. The mechanism of reactions of **1** with peroxides in water that accounts for the bell-shaped pH profiles with maxima around pH 10. Oxidized TAML reacts rapidly with **8**.

Eq. (16) with kinetically indistinguishable k_2 and k_3 pathways.

$$k_{\text{obs}}^{\text{per}} = \frac{k_1[\text{H}^+]^2 + (k_2K_{a_1} + k_3K_{a_2})[\text{H}^+] + k_4K_{a_1}K_{a_2}}{[\text{H}^+]^2 + (K_{a_1} + K_{a_2})[\text{H}^+] + K_{a_1}K_{a_2}} \quad (16)$$

The numerical values of the rate constants are shown in Table III. If one assumes that $k_2 = 0$, then $k_3 \sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$. This is perhaps too high for non-enzymatic activation of H_2O_2 (see below). A more realistic rate constant $k_2 = 1.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at 25°C (if $k_3 = 0$) is still very high for a low-molecular weight synthetic activator of H_2O_2 . The rate constants k_1 and k_4 are by a factor of *ca.* 100 and 10 lower compared to k_2 , respectively, and this accounts for the sharp maxima in Fig. 15. Since the different **1** catalysts contain TAMLs of significantly dissimilar donor capacities, a trend in variation of the intrinsic rate constants k_1 – k_4 is anticipated because the interaction between **1** and H_2O_2 based on all current experimental evidence is a redox reaction combining both binding of H_2O_2 to the Fe^{III}-TAMLs and decomposition of the peroxide to an oxo ligand and water. The deprotonated species $[\text{Fe}(\text{TAML})(\text{OH})(\text{H}_2\text{O})]^{2-}$ is more electron rich than $[\text{Fe}(\text{TAML})(\text{H}_2\text{O})_2]^-$ and therefore, it is reasonable to conclude that H_2O_2 would oxidize the former much faster than the latter ($k_2 \gg k_1$). Deprotonated hydrogen peroxide HO_2^- is more electron rich than H_2O_2 and therefore, it may react slowly with $[\text{FeL}(\text{OH})(\text{H}_2\text{O})]^{2-}$ ($k_2 > k_4$) because a coulombic factor may also affect this step. Of course, these arguments are posteriori rationalizations of the observed pH dependencies.

The values of $\log k_{\text{obs}}^{\text{per}}$ do not correlate as might be expected with the reduction potentials of **1**, which have been measured in acetonitrile by cyclic voltammetry. The Hammett plot reveals that **1** activators that are less electron rich react slightly faster with H_2O_2 at pH 9 – the parameter ρ equals +0.3. This appears to be perplexing at first glance because while the substituent

TABLE III

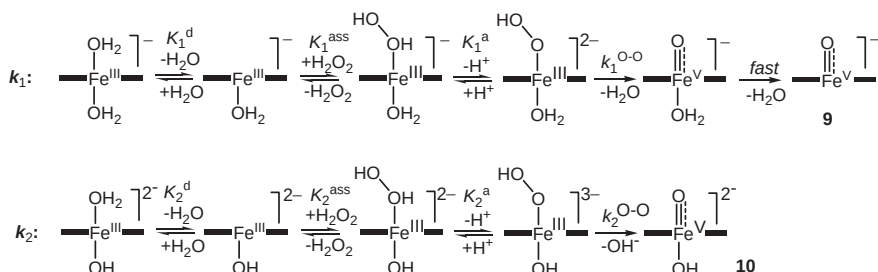
EQUILIBRIUM CONSTANTS, RATE CONSTANTS (IN $\text{M}^{-1}\text{s}^{-1}$) AND ACTIVATION PARAMETERS FOR THE Fe^{III} -TAML-CATALYZED OXIDATION OF **8** (Eq. (15)) BY HYDROGEN PEROXIDE (0.01 M PHOSPHATE) (53)

Fe^{III} -TAML	Temperature (in $^{\circ}\text{C}$)/ activation parameter	$10^{-2} \times k_1$	$10^{-4} \times k_2$	$10^{-6} \times k_3$	$10^{-3} \times k_4$	$\text{pK}_{\text{a}1}$	$\text{pK}_{\text{a}2}$
1a	25	1.3 ± 1.0	1.2 ± 0.5	0.4 ± 0.2	^a	9.7 ± 1.0	10.8 ± 2.0
1l	25	4.0 ± 1.8	1.8 ± 0.7	0.8 ± 0.1	1.5 ± 1.0	9.5 ± 0.4	10.9 ± 0.4
	32	8.5 ± 11.6	3.3 ± 0.2	1.3 ± 0.1	4.0 ± 2.4	9.3 ± 0.1	10.4 ± 0.1
	38	9.0 ± 8.8	3.5 ± 0.2	1.2 ± 0.1	3.8 ± 1.7	9.6 ± 0.7	10.4 ± 0.1
	45	12.5 ± 0.7	3.6 ± 0.1	1.8 ± 0.1	5.1 ± 2.3	9.2 ± 0.1	10.9 ± 0.1
	$\Delta H^{\ddagger}$ (kJ mol^{-1})	42 ± 17	4 ± 4	24 ± 4	38 ± 30		
	$\Delta S^{\ddagger}$ ($\text{J mol}^{-1} \text{K}^{-1}$)	-54 ± 17	-145 ± 12	-49 ± 14	-55 ± 95		

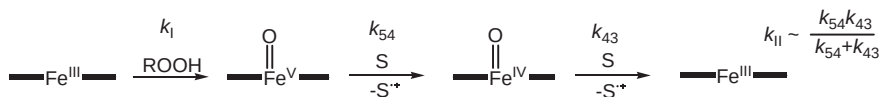
^aCould not be reliably estimated.

effect is minor, it suggests that the “head” ring-substituents are controlling the relative rates of a process that is favored by a more Lewis acidic metal center. The rates of both the ligand exchange and oxidation reactions ought to be favored by a greater electron density at the metal. However, the oxidation process that results in splitting of the O–O bond of H₂O₂ after its coordination needs also to be considered, as this presumably also requires proton transfers from the coordinated hydrogen peroxide. Scheme 8 presents a more detailed analysis than the minimalist version used for the kinetic treatment (Scheme 7). The details in Scheme 8 depict suggestions for the underlying sequence of additional events between the starting Fe^{III}-TAML and the final iron(V)oxo complex, suggested here in water and observed in nonaqueous solvents (51). The peroxide ligand deprotonation associated with the equilibria K_1^a and K_2^a should be enhanced by an increase in the Lewis acidity of the iron. Such a deprotonation would result in a rehybridization of the coordinated O-atom and an increase in bonding with iron favorable for liberation of water or hydroxide by heterolytic cleavage of the O–O bond. An alternative process whereby the proton on the coordinated O-atom tautomerizes by moving to the uncoordinated O-atom to facilitate release of water is also possible, but this process seems less likely at pH 9 given that the [Fe–O–OH₂] species formed could be expected to be quite acidic.

It is not known if the proposed **9** complex observed in nonaqueous solvents, is formed in water. An alternative formulation would have the hydroxo ligand still coordinated to iron and this hypothetical species **10** could also be deprotonated to give a *trans*-dioxo species. In fact, our mechanistic theory for the pH dependent rate of interaction Fe^{III}-TAML complexes with H₂O₂ is



SCHEME 8. A more extensive picture of the mechanistic events proposed to play a role in the k_1 and k_2 pathways of formation of an iron(V)oxo intermediate.



SCHEME 9. Postulated reaction mechanism of oxidation involving **1** with Fe^{V} and Fe^{IV} oxo intermediates.

consistent with their being more than one iron(V) reactive intermediate. When Fe^{III} -TAML complexes reacted with excess peroxide in water and in the absence of substrates, only Fe^{IV} complexes can be observed, produced possibly by the rapid comproportionation of the Fe^{III} and Fe^{V} species, or perhaps even from **9** or **10** via catalase type reduction. Scheme 9 visualizes the reaction mechanism involving both Fe^{V} and Fe^{IV} oxo intermediates in the presence of S(red). If the reactivity closely maps that of peroxidases, the reactivity of the former exceeds noticeably that of the latter, the reaction rate will follow Eq. (13), in which the rate constant k_{II} should be associated with k_{43} .

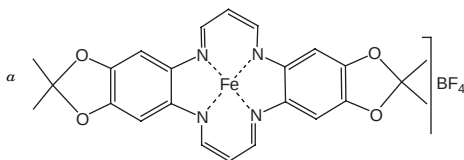
The rate constants for the enzymatic activation of H_2O_2 are in the range 5.4×10^5 – $8.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ under the optimal pH at 25°C (Table IV). The rate constant k_2 for **11** equals $1.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (Table III). Thus, **11** is only one order of magnitude less reactive than some of the slower reported enzymes such as lignin peroxidase *H8* (Table IV, entry 11). Note that the peroxidases consist of 290–350 amino acid residues and range in weight from 27,000 to 50,000 Da (64). The Fe^{III} -TAML catalysts are 50–100 times lighter and considerably more synthetically tractable from the perspective of developing commercially viable peroxide activators. If the rate constants are expressed in units of $\text{L g}^{-1} \text{ s}^{-1}$, i.e., when they refer to the activity of 1 g of catalyst, the rate constant for activator **11** is $17 \text{ L g}^{-1} \text{ s}^{-1}$ which is higher than the corresponding rate constant for lignin peroxidase ($13 \text{ L g}^{-1} \text{ s}^{-1}$, Table IV, entry 11: mw 41,000 g mol^{-1} (65)) and is only 23 times slower than horseradish peroxidase *C* ($390 \text{ L g}^{-1} \text{ s}^{-1}$, entry 4: mw 44,000 g mol^{-1} (55,70)). Although the most reactive ascorbate peroxidase ($2900 \text{ L g}^{-1} \text{ s}^{-1}$, entry 1: mw 27,194 g mol^{-1} (66)) still has a 170-fold advantage, the values determined, clearly indicate high comparative reactivity for Fe^{III} -TAML activators.

Peroxidase enzymes exhibit low enthalpies of activation $\Delta H_{12}^\ddagger$ for the formation of Compound I (k_{12} step), 12, 9.6, and 22 kJ mol^{-1} for horseradish (86), turnip (75), and lignin (77) peroxidases, respectively. Similarly low activation barriers have been found for Fe^{III} -TAML activators (Table III). The k_3 pathway for **11** is characterized by $\Delta H_3^\ddagger$ of 24 kJ mol^{-1} , which is only

TABLE IV

RATE CONSTANTS k_{12} FOR THE FORMATION OF COMPOUND I FROM H₂O₂ AND SELECTED PEROXIDASES OR CATALASES AND FOR *NON-FENTON* PEROXIDASE- OR CATALASE-RELATED ACTIVATION OF H₂O₂ SUCH AS k_1 BY THE MOST REACTIVE LOW-MOLECULAR WEIGHT IRON(III) COMPLEXES IN WATER

Entry	Enzyme or metal complex with catalase- or peroxidase-like activity	pH (T/°C)	Rate constant/ M ⁻¹ s ⁻¹	Refs.
1	Pea cytosolic ascorbate peroxidase	7.8 (20)	8.0×10^7	(67)
2	Yeast cytochrome <i>c</i> peroxidase	6.1 (25)	3.4×10^7	(68)
3	Yeast cytochrome <i>c</i> peroxidase	6.1 (25)	1.4×10^7	(69)
4	Horseradish peroxidase	4.7 (25)	1.7×10^7	(55,70)
5	Myeloperoxidase (<i>Homo sapiens</i>)	7.0 (25)	2.3×10^7	(71)
6	Manganese peroxidase (<i>Phanerochaete chrysosporium</i>)	4.5 (28)	2.0×10^6	(72)
7	Soybean cytosolic ascorbate peroxidase	7.0 (20)	3.3×10^7	(73)
8	Chloroperoxidase (<i>Caldariomyces fumago</i>)	4.7 (25)	2.4×10^6	(74)
9	Hog thyroid peroxidase	7.5 (20)	7.8×10^6	(75)
10	Turnip peroxidase P7	3.5 (25)	1.6×10^6	(76)
11	Lignin peroxidase H8 (<i>P. chrysosporium</i>)	3.5 (25)	5.4×10^5	(77)
12	Catalase (horse erythrocyte)	6.7 (25)	3×10^7	(78)
13	Catalase (beef liver)	7.0 (25)	5.6×10^6	(79)
14	Microperoxidase-8	5.0 (25)	680	(80,81)
15	Hemin	10 (25)	~1	(82)
16	Fe ^{III} -tetraaza[14] annulene ^a	7.2 (32)	1.5×10^3	(83)
17	Fe ^{III} -5,10,15,20-tetrakis(2,6-Me ₂ -3-sulfonatophenyl)porphyrinato	10.0 (30)	1.4×10^{4b}	(84)
18	[Fe ^{III} (octaphenylsulfonato)porphyrazine] ⁵⁻	10.0 (25)	15	(85)
19	Fe ^{III} -TAML activator 11	10.0 (25)	1.8×10^4	(53)

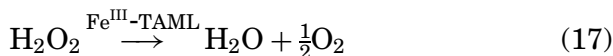


^b 1.05×10^5 at pH 12.

slightly higher than that observed for lignin peroxidase. These mechanistic similarities emphasize that Fe^{III}-TAML activators are the best functioning models of the catalase-peroxidase enzymes known to date (87).

C. CATALASE-LIKE ACTIVITY

In the absence of electron donors, complexes **1** display a catalase-like activity (Eq. (17)). Dioxygen evolution can be visually observed at $[\text{H}_2\text{O}_2] > 0.01 \text{ M}$. This catalytic feature has been studied kinetically by monitoring the initial rates of O_2 formation with a Clark electrode (53).



The reaction stoichiometry is given in Eq. (17). The reaction in Eq. (17) follows first-order kinetics in **1** in the absence of an electron donor. Thus, the rate expression (Eq. (18)) holds for the catalase-like activity, which is identical to Eq. (15).

$$\text{Rate} = k_{\text{obs}}^{\text{cat}}[\mathbf{1}][\text{H}_2\text{O}_2] \quad (18)$$

The rate constants $k_{\text{obs}}^{\text{cat}}$ are again pH dependent and the pH profiles for reactions in Eqs. (14) (Fig. 15) and (17), both catalyzed by **1l**, are similar. Eq. (16) has therefore also been applied for fitting the data for reaction in Eq. (17). The best-fit rate and equilibrium constants are summarized in Table V. For the reasons discussed above, the rate constants k_3 (obtained by setting $k_2 = 0$) are not included in Table V. The rate constants in Tables III and V indicate that the catalase-like rate constants are somewhat lower than the peroxidase-like rate constants.

The similar rate laws (Eqs. (15) and (18)), pH profiles, and the values of the observed second-order rate constants ($k_{\text{obs}}^{\text{per}}$ and $k_{\text{obs}}^{\text{cat}}$) suggest a common reactive intermediate in reactions of Eqs. (14) and (17) (oxidized Fe-TAML in Scheme 6). Taking all three steps

TABLE V

RATE CONSTANTS (IN $\text{M}^{-1}\text{s}^{-1}$) AND EQUILIBRIUM CONSTANTS AND ACTIVATION PARAMETERS FOR IRON(III)-TAML CATALYZED DISPROPORTIONATION OF HYDROGEN PEROXIDE (Eq. (17)) AT 25°C AND 0.1 M PHOSPHATE

$\text{Fe}^{\text{III}}\text{-TAML}$	$10^{-2} \times k_1$	$10^{-4} \times k_2$	$10^{-3} \times k_4$	$\text{pK}_{\text{a}1}$	$\text{pK}_{\text{a}2}$
1a	1.4 ± 1.2	0.51 ± 0.03^a	^b	11.0 ± 0.2	12.1 ± 0.4
1e	4.3 ± 1.8	0.90 ± 0.06	0.87 ± 0.6	11.0 ± 0.2	12.15 ± 0.35
1l	1.3 ± 0.7	0.64 ± 0.04^c	1.6 ± 0.2	9.75 ± 0.11	11.3 ± 0.3
1k	6 ± 3	1.68 ± 0.08^d	3.2 ± 0.2	10.5 ± 0.2	11.6 ± 0.2

^a $\Delta H^\ddagger = 23 \pm 3 \text{ kJ mol}^{-1}$; $\Delta S^\ddagger = -95 \pm 20 \text{ J mol}^{-1} \text{ K}^{-1}$.

^b Could not be reliably estimated.

^c $\Delta H^\ddagger = 14.6 \pm 0.7 \text{ kJ mol}^{-1}$; $\Delta S^\ddagger = -123 \pm 13 \text{ J mol}^{-1} \text{ K}^{-1}$.

^d $\Delta H^\ddagger = 8.1 \pm 3.3 \text{ kJ mol}^{-1}$; $\Delta S^\ddagger = -137 \pm 63 \text{ J mol}^{-1} \text{ K}^{-1}$.

into consideration, the rate of the peroxidase-like activity of **1** is given by Eq. (19),

$$\text{Rate} = \frac{k_I k_{II} [\text{Fe-TAML}] [\text{H}_2\text{O}_2] [\text{ED}]}{k_{-I} + [\text{H}_2\text{O}_2] (k_I + k_{III}) + k_{II} [\text{ED}]} \quad (19)$$

where ED is any appropriate electron donor, an organic dye or ruthenium dye **8**. The catalase-like activity is described in the rate expression of Eq. (20).

$$\text{Rate} = \frac{k_I k_{III} [\text{Fe-TAML}] [\text{H}_2\text{O}_2]^2}{k_{-I} + [\text{H}_2\text{O}_2] (k_I + k_{III}) + k_{II} [\text{ED}]} \quad (20)$$

Eqs. (19) and (20) were derived applying the steady-state approximation to the oxidized Fe-TAML species and using the mass balance equation $[\text{Fe-TAML}] = \mathbf{1} + [\text{oxidized Fe-TAML}]$ ($[\text{Fe-TAML}]$ is the *total* concentration of all iron species, which is significantly lower than the concentrations of H_2O_2 and ED). The oxidation of ruthenium dye **8** is a zeroth-order reaction in **8**. This implies that $k_{II} [\text{ED}] \gg \{k_{-I} + [\text{H}_2\text{O}_2] (k_I + k_{III})\}$. Eq. (19) becomes very simple, i.e.,

$$\frac{\text{Rate}}{[\text{Fe-TAML}] [\text{H}_2\text{O}_2]} = k_{\text{obs}}^{\text{per}} = k_I \quad (21)$$

First-order dependence in hydrogen peroxide for the catalase-like activity holds when $[\text{H}_2\text{O}_2] (k_I + k_{III}) \gg k_{-I} + k_{II} [\text{ED}]$. Then, in the absence of added ED, Eq. (20) becomes

$$\frac{\text{Rate}}{[\text{Fe-TAML}] [\text{H}_2\text{O}_2]} = k_{\text{obs}}^{\text{cat}} = \frac{k_I k_{III}}{k_I + k_{III}} \quad (22)$$

Thus, both the peroxidase-like and catalase-like reactions have similar rate laws and the effective second-order rate constant $k_{\text{obs}}^{\text{cat}}$ should practically equal $k_{\text{obs}}^{\text{per}}$ (when $k_{III} > k_I$) or it should be lower than $k_{\text{obs}}^{\text{per}}$ when otherwise, provided $k_{\text{obs}}^{\text{per}}$ is zeroth-order in ED. The conditional rate constants k_I and k_{III} are pH dependent. These data allow us to estimate the dependence of the rate constant k_{III} on pH using Eqs. (21) and (22). Thus, the calculated plot of k_{III} vs. pH in the range 8–11, where the accuracies in measuring $k_{\text{obs}}^{\text{per}}$ and $k_{\text{obs}}^{\text{cat}}$ are the highest, is shown as a dashed line in Fig. 15. Under these conditions, k_{III} is somewhat lower than k_I . This is consistent with the fact that characterization of the product/s of interaction between complexes **1** and H_2O_2 by spectral techniques is feasible even in the presence of an excess of the oxidizing agent (48). The shape of the pH profile for k_{III} in Fig. 15 can be qualitatively understood as follows. Hydrogen

peroxide becomes a better reducing agent upon deprotonation and therefore, the rate constant k_{III} starts to rise at $\text{pH} > 10$ (88).

The postulated identities of the oxidized Fe-TAMLs in the peroxidase- and catalase-like reactions sets up the question of whether 100% oxidative efficacy (quantitative peroxidase-like activity) can be achieved for **1**. If peroxidase- and catalase-like reactions involve the same intermediate, ED and H_2O_2 should compete for it and the peroxide could be wasted. At a given pH, 100% efficacy is observed if $k_{\text{II}}[\text{ED}] \gg k_{\text{III}}[\text{H}_2\text{O}_2]$, i.e., when the oxidized Fe-TAML reacts faster with ED than with H_2O_2 . The ratio $k_{\text{II}}/k_{\text{III}}$ can be estimated by measuring the rate of O_2 evolution in the presence of variable ED concentrations using Eq. (20). The rate of O_2 formation should be retarded hyperbolically with increasing [ED] and this has been confirmed by using the dyes Safranin O and Orange II as electron donors.

The catalase-like activity of Fe^{III} -TAML activators does decrease in the presence of Safranin O (Fig. 16). The inset to Fig. 16 shows that the plot of the inverse rate vs. [Safranin O] is a straight line consistent with Eq. (23).

$$\frac{[\text{Fe-TAML}][\text{H}_2\text{O}_2]}{\text{Rate}} = \frac{k_{-\text{I}} + [\text{H}_2\text{O}_2](k_{\text{I}} + k_{\text{III}})}{k_{\text{I}}k_{\text{III}}[\text{H}_2\text{O}_2]} + \frac{k_{\text{II}}[\text{ED}]}{k_{\text{I}}k_{\text{III}}[\text{H}_2\text{O}_2]} \quad (23)$$

$$\approx \frac{k_{\text{I}} + k_{\text{III}}}{k_{\text{I}}k_{\text{III}}} + \frac{k_{\text{II}}[\text{ED}]}{k_{\text{I}}k_{\text{III}}[\text{H}_2\text{O}_2]}$$

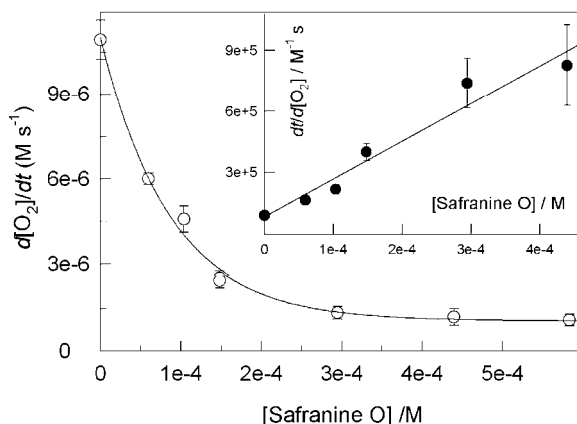


FIG. 16. Retardation of the catalase activity of Fe-TAML activators by the dye Safranin O as an electron donor. Conditions: $[\text{H}_2\text{O}_2]$ 2.65×10^{-3} M; $[\mathbf{1k}]$ 1.18×10^{-6} M; pH 10, 25°C. Inset shows that the rate of O_2 evolution is inversely proportional to [Safranin O]. From Ref. (53).

The intercept and slope equal $(k_I + k_{III})/k_I k_{III}$ and $k_{II}/k_I k_{III}[\text{H}_2\text{O}_2]$, respectively. An estimate for the ratio k_{II}/k_{III} is derived from the slope using the known value of k_I (e.g., $4.4 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ for **1k**). The k_{II}/k_{III} ratio equals 52 for **1k** and indicates that the oxidized Fe-TAML is significantly more reactive to the ED, Safranin O, than to H_2O_2 . This extremely important feature of the Fe^{III}-TAML activators eliminates unproductive decomposition of H_2O_2 in the oxidation of Safranin O and, similarly, provides a rationale for the 100% efficacy with respect to H_2O_2 in reactions with complex **8** and with ferrocene.

The molecular masses of heme catalases are usually significantly higher as compared with peroxidases. If expressed in $\text{L g}^{-1} \text{ s}^{-1}$, rate constants for the Fe^{III}-TAML activators when compared with catalase from beef liver, which has a molecular weight $250,000 \text{ g mol}^{-1}$ (Table IV, entry 13) (89), look very impressive, *viz.* $17 \text{ L g}^{-1} \text{ s}^{-1}$ for **1l** vs. $22 \text{ L g}^{-1} \text{ s}^{-1}$ for the enzyme. Nevertheless, the catalase-like activity of the Fe^{III}-TAML activators can be suppressed by the addition of electron donors – it is negligible in the presence of the substrates tested in this work. In Nature, catalases display only minor peroxidase-like activity (79) because electron donors bulkier than H_2O_2 cannot access the deeply buried active sites of these massive enzymes (90). The comparatively unprotected Fe^{III}-TAML active sites are directly exposed to electron donors such that the overall behavior is determined by the inherent relative reactivity of the substrates.

D. OTHER PRIMARY OXIDANTS, MECHANISMS OF BENZOYL PEROXIDE ACTIVATION, AND DEGRADATION OF ORANGE II

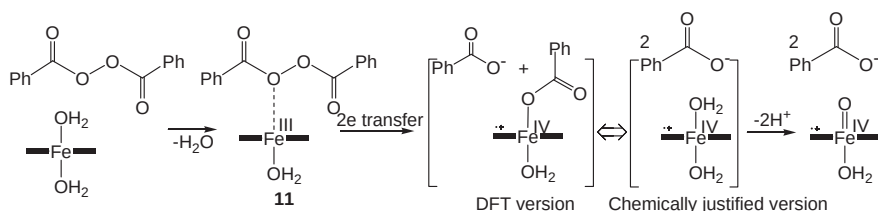
The Fe^{III}-TAML activators catalyze oxidations by organic peroxides, i.e., *tert*-butyl hydroperoxide, cumyl hydroperoxide, and benzoyl peroxide. The results of kinetic studies of the catalyzed oxidation of Orange II by organic peroxides agree with Eq. (13) ($k_{-I} \sim 0$) at pH 9 and 25°C (6). The rate constants k_I and k_{II} in Table VI demonstrate a closeness of the rate constants k_{II} for all oxidants and an exceptionally large value of k_I for benzoyl peroxide, which is by a factor of 53 higher than that for H_2O_2 . The reactivity of peroxides in terms of k_I decreases in the series benzoyl peroxide (6.8×10^3) > H_2O_2 (130) > *t*-BuOOH (2) > cumyl hydroperoxide (1).

Benzoyl peroxide is much larger than H_2O_2 , but the steric effect is apparently not a factor for the highest rate constant k_I . Assuming that the activation of hydroperoxides by Fe^{III}-TAMLS **1** occurs as it has been suggested for peroxidases, i.e., involving

TABLE VI

THE RATE CONSTANTS k_I AND k_{II} (IN $M^{-1}S^{-1}$) FOR THE **1A**-CATALYZED OXIDATION OF ORANGE II AT PH 9 AND 25°C (6)

Peroxide	$10^{-3} \times k_I$	$10^{-4} \times k_{II}$
H ₂ O ₂	1.40 ± 0.01	3.8 ± 0.3
Benzoyl peroxide	74 ± 3	3.7 ± 0.9
<i>t</i> -BuOOH	0.024 ± 0.002	3.2 ± 1.5
Cumyl hydroperoxide	0.011 ± 0.001	1.8 ± 0.8



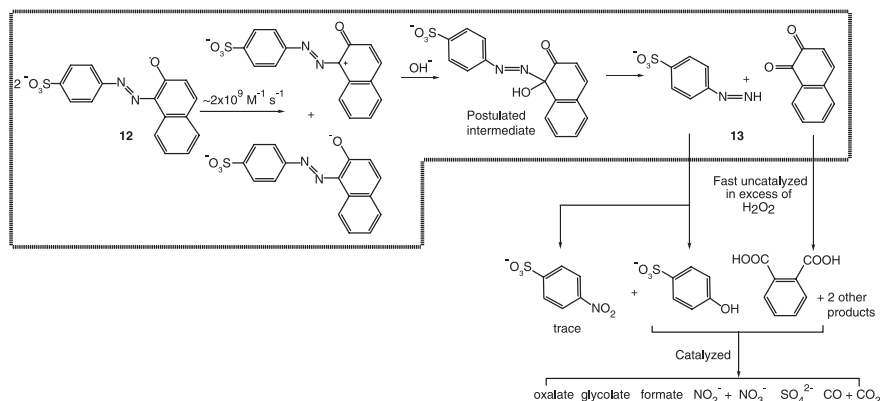
SCHEME 10. Plausible mechanism for early steps for activation of benzoyl peroxide by Fe^{III}-TAMLs. For details, see text. From Ref. (6).

the formation of the Fe^{III}-O-OR intermediate followed by the heterolytic cleavage of the O-O bond (55,91), the reaction of benzoyl peroxide differs (Scheme 10). Benzoyl peroxide does not have an H-O fragment and therefore, its coordination to iron(III) should involve either carbonyl or peroxo oxygen. The DFT calculations suggest that both oxygen atoms may coordinate to iron(III) because their effective negative charges are similar, i.e., -0.38 and -0.30 for carbonyl and peroxo oxygen, respectively. The peroxo oxygen seems to be a better candidate because the σ^* orbital of the O-O fragment is thus much closer to the reducing Fe^{III} center. The DFT calculations (in vacuum) support this mechanistic hypothesis (6). If the carbonyl oxygen is a donor center, the complexation between **1** and benzoyl peroxide is a dead-end pathway, which does not result in the O-O bond cleavage. Intermediate **11** is on the reaction coordinate and the following energy minimum is found for the system consisting of free benzoate and benzoate coordinated to Fe^{IV} or Fe^V. Benzoate coordination to iron of TAML does not occur in water (13) and the mechanism involves two free benzoates and oxidized iron (Scheme 10). The formation of two benzoate anions needs two electrons that could be moved concertedly or stepwise.

The mechanistic aspects of peroxidase-like activity of Fe^{III}-TAMLs discussed so far concerned mainly the activation by **1** of primary oxidizing agents, such as in Table VI, characterized by

the rate constant k_I (Scheme 6). This does reflect the current state of art. The mechanistic vision of the chemistry associated with oxidation of target substrates driven by k_{II} is narrower due to the fact that the first step is slower. As a point is raised by a reviewer, intriguing aspects of the catalysis regarding a role of H-atom abstraction and/or oxo-transfer mechanism of oxidation are still under investigation. There was just one detailed attempt to sketch a mechanism of decolorization of an azo dye (Orange II) by H₂O₂ catalyzed by **1** (6). The mechanism was proposed on the basis of kinetic data, investigation of organic fragments derived from Orange II (6), and the results of dye bleaching by one-electron oxidizing agents studied by pulse radiolysis (92). The latter results are consistent with diffusion-controlled disproportionation of two primary dye-radicals formed after the initial, rate-limiting electron transfer. The disproportionation affords a starting dye and the product of its 2-electron oxidation, which is further involved in a series of transformations. Early steps of this mechanism are shown in Scheme 11 (92). This mechanism is useful for describing the results of catalysis by **1** assuming that the oxidized catalysts **1** generate the primary radical **12** shown in Scheme 11 in a pathway described by the rate constant k_{II} .

Compounds 4-diazenylbenzenesulfonate (**13**) and 1,2-naphthoquinone were not detected by HPLC under the catalytic conditions. The quinone was not detected because under these conditions 1,2-naphthoquinone is rapidly oxidized into phthalic acid plus two additional products just by H₂O₂ without participation of **1**. 4-Diazenylbenzenesulfonate **13** should be



SCHEME 11. Plausible scheme of **1**-catalyzed oxidative degradation of Orange II. Steps in the dotted box were suggested previously (92). From Ref. (6).

unstable as well. Formation of 4-nitrobenzo- and 4-hydroxybenzosulfonate detected by HPLC could presumably result from the oxidation of 4-diazenylbenzosulfonate. Phthalic acid and 4-hydroxybenzosulfonate undergo catalyzed oxidation by H_2O_2 giving a variety of small biodegradable, non-toxic organic products with substantial mineralization.

VI. The Activity-Stability Parameterization of Homogeneous Green Oxidation Catalysts

A. KINETIC MODEL FOR PARAMETERIZATION

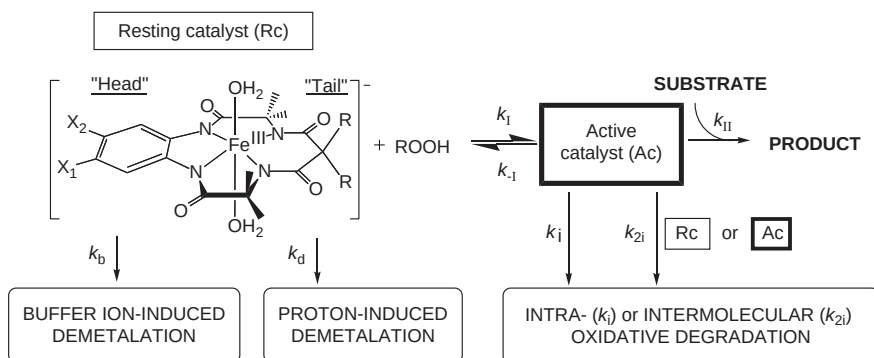
The performance of catalysts is characterized by *turnover numbers* (TON) and *turnover frequencies* (TOF) (93). TON, the molar ratio of a product formed to the amount of a catalyst used (the number of catalytic turnovers), may not report directly on the catalyst efficacy from a kinetic point of view. Expressed in per second, TOF is TON per second. In our opinion, the estimation of TON is to a certain extent arbitrary. Effective TON may be artificially increased by lowering the catalyst concentration and/or by increasing reaction time. As effective macro characteristics of a catalyst, just TON and TOF may provide insufficiently deep insight into a catalytic process; they are silent with respect to the *operational stability* of the catalyst, which is influenced by both the catalytic activity and the stability under the operating conditions. Activity loss or inactivation as the catalyst functions as a result of catalystocidal (a combination of the human equivalents of suicide and homicide) oxidation or reduction, solvolysis, ligation by medium components, and/or reaction product/s, etc., have not been as prioritized by chemists as by biochemists, enzymologists, and microbiologists. Understanding of thermal denaturing, autolysis, aggregation, chemical transformation of key functional groups, etc., became a powerful tool for increasing the functional performance of biocatalysts (94–97).

The progress of *Green Chemistry* impacts the established scenarios for the chemical industry (98,99). Future catalysts will likely become more enzyme-like in the sense that they will more efficiently balance the properties of effectiveness and fragility. An overarching environmental consideration holds: a synthetic catalyst should simply “go away” by some mechanism when its work is done. Nature has developed enzymes with limited lifetimes. After their task is completed, their constituent molecules and atoms become recyclable to serve as useful resources for the

ongoing processes of the ecosphere. Industrial catalysts, and particularly those intended for use in *environmental* applications such as Fe^{III}-TAML activators, should also degrade to harmless and preferably beneficial substances for nature.

Enzymes are protected from overly rapid inactivation by their polypeptide chains. Low-molecular weight synthetic catalysts are not. The tendency of synthetic homogenous oxidation catalysts to degrade too quickly has been a particular challenge that Fe^{III}-TAML activators begin to address. While synthetic catalysts should have a lifetime that is sufficient to afford economically useful technical performance, environmentally persistent catalysts could become a serious problem if they become widely distributed in the environment. Catalyzed oxidation is a two-substrate reaction. The first substrate, the oxidizing agent, delivers oxidizing equivalent/s (k_I) to the catalyst followed by oxidation of a target substrate (k_{II}) by the active form(s) of the catalyst (Scheme 12). The catalyst lifetime as exemplified by the free energies of activation involved with k_i and/or k_{2i} should not be much shorter than the time of catalytic turnovers associated with k_{II} . The approach developed for Fe^{III}-TAMLs allows for the simultaneous determination of k_{II} and k_i (52). It holds when the formation of the active catalyst associated with k_I is fast and the catalyst concentration is very low such that the k_{2i} pathways can be neglected.

If the oxidation process is monitored spectrophotometrically, Step I (as in Scheme 6) is fast, and the oxidation is set up to be



SCHEME 12. Adopted mechanism of catalysis. The rate constant k_d describes the proton-induced demetalation of **1** (13,27); k_b characterizes the buffer ion-induced demetalation of **1** as described above (27); k_i and k_{2i} refer to catalyticidal intra- or intermolecular inactivation (52). The catalysis is commonly run using very low concentrations of **1** and under these circumstances the k_{2i} -driven pathway can be negligible (52).

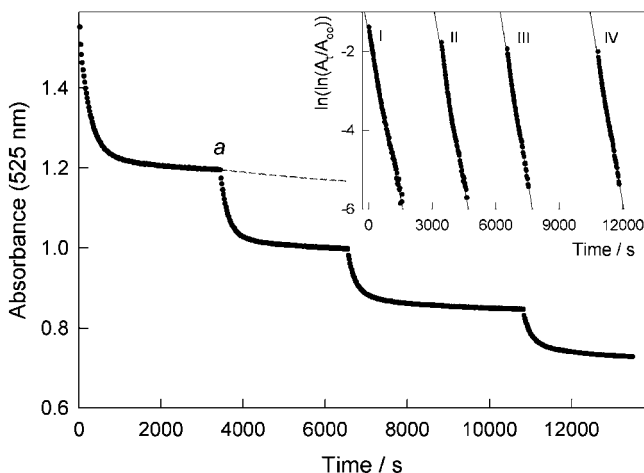


FIG. 17. Kinetics of **1a**-catalyzed bleaching of Safranin O (4.3×10^{-5} M) by 0.012 M H_2O_2 . Initial concentration of **1a** (7.5×10^{-8} M); aliquots of the same amount of **1a** were added after complete inactivation of the catalyst giving rise to the stepped dependence. The dashed line shows that addition of 0.012 M H_2O_2 does not resume the catalytic bleaching. *Inset* shows linearization of the data obtained after each addition of **1a** in terms of Eq. (6) to give consistent values of k_i ($\sim 3.3 \times 10^{-3} \text{ s}^{-1}$). Conditions: pH 11, 25°C . From Ref. (52).

incomplete (Fig. 17), the kinetic trace can be analyzed using Eq. (24)¹ thereby allowing for determination of k_{II} and k_i . Here, A_t and A_∞ are absorbances at time t and at the end of reaction ($t = \infty$), respectively, and $[\text{Fe}^{\text{III}}]_t$ is a total catalyst concentration (52).

$$\ln\left(\ln \frac{A_t}{A_\infty}\right) = \ln\left(\frac{k_{\text{II}}}{k_i} [\text{Fe}^{\text{III}}]_t\right) - k_i t \quad (24)$$

The rate constants of k_{II} and k_i obtained using Eq. (24) reveal that: (i) the activity of Fe^{III} -TAMLs in bleaching Safranin O (k_{II}) increases more than 10-fold when the tail ethyl groups of **1a** are replaced by fluorine atoms in **1k**. The rate constant k_{II} for **1k** equals $10^5 \text{ M}^{-1} \text{ s}^{-1}$ at 25°C , a value that corresponds to those found for the reactivity of horseradish peroxidase Compound II

¹Eq. (24) has been obtained by integration $-(d(D_t - x))/dt = k_{\text{II}}(D_t - x)([\text{Fe}^{\text{III}}]_t e^{-k_i t})$. Here, D_t and $[\text{Fe}^{\text{III}}]_t$ are total concentrations of the dye and a Fe^{III} -TAML catalyst, respectively, and x the concentration of a bleached dye at time t . The boundary conditions $x = x_\infty$ (x_∞ is the concentration of bleached dye obtained with single catalyst aliquot), when $t = \infty$.

toward anilines and phenols (100). (ii) The rate constants k_i also increase on going from **1a** to **1k** and a similar 10-fold gap holds. Under the operating conditions, i.e., $[\text{H}_2\text{O}_2] = 0.012 \text{ M}$, $[\text{Safranin O}] = 4.3 \times 10^{-5} \text{ M}$, pH 11, and 25°C , the half-lives, $\tau_{1/2}$, for catalysts **1a** and **1k** equal 7.7 and 0.88 min, respectively.

Eight structurally similar, but electronically different Fe^{III}-TAML catalysts **1**, all with $\text{R} = \text{Me}$, have been investigated by applying Eq. (24). Surprising results demonstrate a LFER plot in Fig. 18. Electron-withdrawing substituents (NO_2 , NMe_3^+ , COOEt , Cl) increase the oxidizing power of the catalysts with respect to Safranin O (k_{II}), but retard the intramolecular inactivation (k_i). The most resistant-to-inactivation catalyst is the nitro-substituted Fe^{III}-TAML, **1e**. This suggests the aromatic component of Fe^{III}-TAML catalysts as a vulnerable fragment. Presumably the active catalyst destroys itself via the oxidative damage at this group and electron-withdrawing substituents protect the ligand system from the damage. The k_i value for **1a** is higher than the other data in the LFER would predict – if this datum is neglected, the slope of the resulting straight line equals -1.0 ± 0.3 . This is unusual because a positive slope might be anticipated with more reactive catalysts decomposing more rapidly but useful as a tool

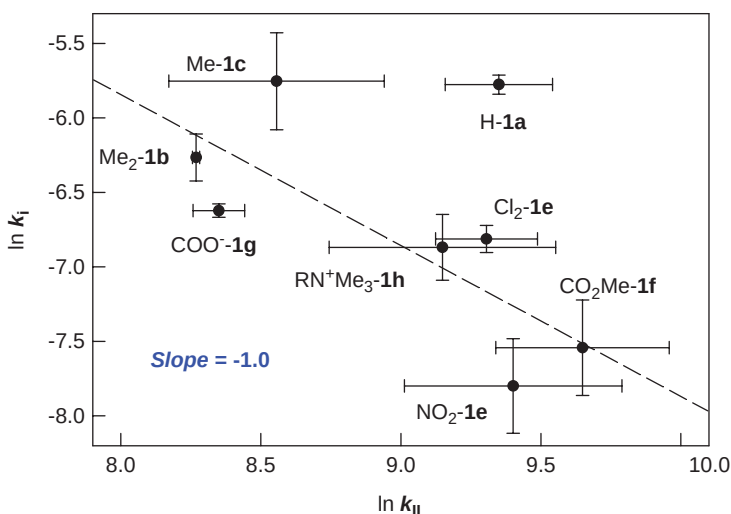


FIG. 18. LFER between the rate constants k_{II} for bleaching Safranin O and k_i for intramolecular inactivation in the series of “head”-substituted Fe^{III}-TAML catalysts with methyl tail groups. Conditions: $[\text{H}_2\text{O}_2]$ 0.012 M, 25°C , pH 11.0. The data point for **1a** was ignored in the linear regression. From Ref. (52).

for tuning the catalytic activity and stability of Fe^{III} -TAML catalysts.

Bleaching of Safranin O in excess of H_2O_2 relative to the dye is affected by $[\text{H}_2\text{O}_2]$. Deeper **1a**-catalyzed bleaching of Safranin O is observed at *lower* concentrations of H_2O_2 ; 19% bleaching is achieved at $[\text{H}_2\text{O}_2] = 0.12\text{ M}$ and 31% at 0.012 M concentration of H_2O_2 . Similar behavior for heme chloroperoxidase from *C. fumago* results from the H_2O_2 -induced irreversible inactivation of the enzyme (101). The rate constants k_{II} are virtually insensitive to $[\text{H}_2\text{O}_2]$ – the reactivity of the oxidized TAML should not depend on the H_2O_2 concentration. The rate constants k_{i} for **1a** and **1e** increase linearly with increase in the H_2O_2 concentration with a positive intercept: $k_{\text{i}} = k_{\text{i}\alpha} + k_{\text{i}\beta}[\text{H}_2\text{O}_2]$. The equation guides one to keep $[\text{H}_2\text{O}_2]$ at the lowest possible level because in addition to the catalase-like activity displayed by Fe^{III} -TAML catalysts at high $[\text{H}_2\text{O}_2]$ (resulting in waste of H_2O_2) (53), hydrogen peroxide is an inactivator of the Fe^{III} -TAML catalysts. The value of $k_{\text{i}\alpha}$ is almost six times lower for the NO_2 -ring substituted catalyst **1e** compared to unsubstituted **1a**. Thus, changing the electronic properties of the head component of the catalysts is a simple tool for the protection from the intramolecular inactivation (52).

B. MODEL VERIFICATIONS

Eq. (24) can be rearranged to a form (Eq. (25)) that describes a relative conversion of substrate (A_t/A_0) as a function of time (52). Eq. (25) is convenient for simulating the catalyst performance using the current model. Theoretical predictions can be compared with the experimental data. Eq. (25) eliminates the illusion that Fe^{III} -TAML-catalyzed bleaching is always incomplete as in Fig. 17.

$$\frac{A_t}{A_0} = \exp \left\{ \frac{k_{\text{II}}}{k_{\text{i}}} [\text{Fe}^{\text{III}}]_t (1 - e^{-k_{\text{i}}t}) \right\}^{-1} \quad (25)$$

The “regime of incompleteness” is a tool for evaluating k_{i} . Fig. 19A demonstrates different regimes of bleaching calculated using Eq. (25). The bleaching is complete when the catalyst concentration reaches 10^{-6} M and $k_{\text{i}} \leq 10^{-3}\text{ s}^{-1}$ (Fig. 19Ae). At lower concentrations of Fe^{III} -TAML, the bleaching is slower and incomplete. It is also incomplete at higher catalyst concentrations ($[\text{Fe}^{\text{III}}] \geq 10^{-6}\text{ M}$) and large k_{i} (Fig. 19Ac,d).

Support for the mechanistic concept is a match between the experimental and calculated data using known k_{i} and k_{II} .

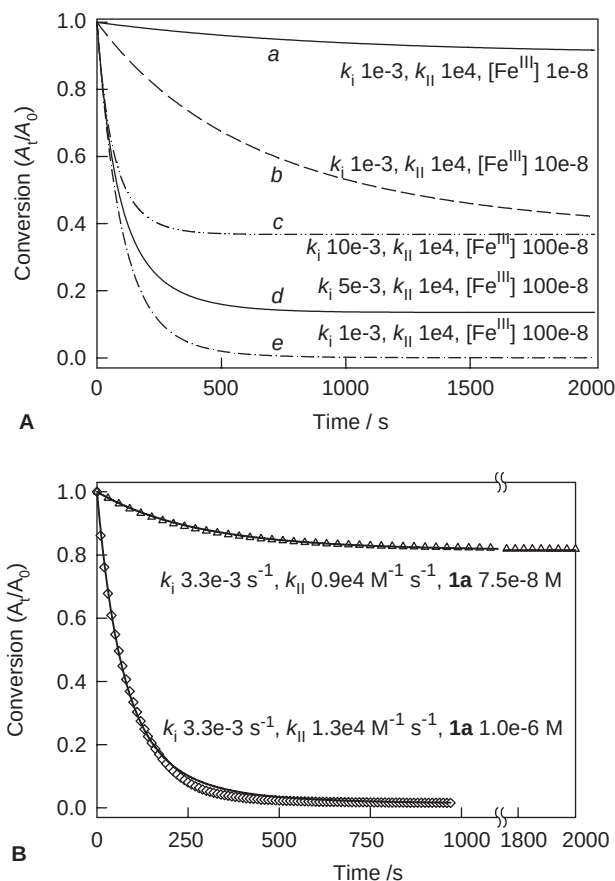


FIG. 19. (A) Simulated bleaching of a hypothetical dye using Eq. (24) at different concentrations of Fe^{III}-TAML catalyst (in M) with the rate constants k_i (in s^{-1}) and k_{II} (in $M^{-1}s^{-1}$). The numerical values are indicated on the graph. (B) Normalized experimental and simulated bleaching of Safranin O (4.3×10^{-5} M) by H₂O₂ (0.012 M) catalyzed by **1a** at pH 11 and 25°C. Experimental data are shown as $\triangle$ and $\diamond$. The simulations, shown as solid lines, were made as in (A). From Ref. (52).

Fig. 19B shows the **1a**-catalyzed bleaching of Safranin O at different **[1a]** for comparison. The experimental and calculated curves agree and prove that complete bleaching of the dye is achievable by just increasing the **1a** concentration. The required amount is still very low, specifically, 10^{-6} M for the difficult-to-oxidize dye Safranin O.

Work is ongoing to quantify the relative importance of intermolecular pathways of Fe^{III}-TAML catalyst inactivation

and this should also be significant in eventual process optimization procedures. A further development involves the most general case without putting limitations on the rate constants k_I and k_{II} . This is vital when significantly higher catalyst concentrations are used and where the bimolecular suicidal oxidative inactivation of Fe^{III} -TAML catalysts is driven by the value of k_{2i} to the point that it cannot be neglected in the modeling – an example is the killing of spores (8).

VII. Concluding Remarks

The general features of hydrogen peroxide catalytic activation by iron(III)-TAML activators which display both peroxidase- and catalase-like activity are being revealed. The hydrolytic sensitivity of Fe^{III} -TAML activators is now well understood. Hydrolysis is promoted both by the proton and by certain buffer ions that are able to deliver protons intramolecularly following coordination to the iron. The lifetime of Fe^{III} -TAML activators under operating conditions is an intimate function of the multiple oxidation processes that are unleashed when peroxide is added to a solution of one of the catalysts. Catalyst suicidal degradation involves at least two processes that are unimolecular in the Fe^{III} -TAML activator (suicide equivalent) and at least one that is bimolecular (homicide equivalent). For certain substrates such as **8**, the rate-determining step of the peroxidase-like activity is the interaction between **1** and H_2O_2 . The rate is strongly pH dependent and the maximum activities for the different catalysts are observed over a fairly narrow pH range near 10. The rate constants for the activation of H_2O_2 under the optimal conditions ($>10^4 \text{ M}^{-1} \text{ s}^{-1}$) approach the lower limit of the corresponding enzymatic peroxidase-catalyzed reactions for the same step, namely the reaction of the enzyme with peroxide. The catalyst/enzyme rate comparisons made give worst-case estimates for the comparable reactivity of Fe^{III} -TAML activators against peroxidase enzymes (the enzymes may have other rate-determining steps) and define the former as exhibiting comparable performance to the enzymes they were designed to mimic. The activation of H_2O_2 to oxidize the one-electron transfer reducing agent such as **8** is believed to proceed via both iron(V)oxo and iron(IV)oxo (or iron(IV)dihydroxo) and perhaps other species are also involved. In the absence of peroxidase-like substrates, Fe^{III} -TAML activators display catalase-like activity with the production of dioxygen. In addition to underscoring the basic scientific features behind the significant technological

importance of Fe^{III}-TAML activators (2,4), these results signify that the activators are prominent functional models of the catalase-peroxidase enzymes as they display both high catalase- and peroxidase-like activities, but the latter dominates in the presence of the electron donor substrates studied other than H₂O₂ itself, thus eliminating unproductive consumption of hydrogen peroxide. This property results in high efficiency in peroxide use for the peroxidase-like reaction channel and this is an important finding for the technological significance of Fe^{III}-TAML activators. The evolving mechanistic understanding of the Fe^{III}-TAML degradation processes and reactivity enabled the design of novel Fe^{III}-TAML catalysts with improved reactivity properties. The *quantitative* understanding of (i) hydrolytic and (ii) operational stabilities, (iii) comparative speed of peroxide activation, and (iv) the pHs of highest activities has led us to design, synthesize, and evaluate the superior properties regarding the characteristics i–iv Fe^{III}-TAML activator **1n** (102). It is the most medium- and operationally stable, the most reactive with a pH of maximum activity closest to 7 among current Fe^{III}-TAMLs. At this point in time, the technical performance of the activators is so effective that the pursuit of precautionary logic is defining the cutting edge in their further design and development.

ACKNOWLEDGMENT

Terrence J. Collins thanks the Heinz Endowments, the Eden-Hall Foundation, NSF (9612990), DOE (NETL), and the Institute for Green Science for support.

REFERENCES

1. Collins, T. J. *Acc. Chem. Res.* **1994**, 27, 279–285.
2. Collins, T. J. *Acc. Chem. Res.* **2002**, 35, 782–790.
3. Collins, T. J.; Walter, C. *Sci. Am.* **2006**, 294, 83–88, 90.
4. Sen Gupta, S.; Stadler, M.; Noser, C. A.; Ghosh, A.; Steinhoff, B.; Lenoir, D.; Horwitz, C. P.; Schramm, K.-W.; Collins, T. J. *Science* **2002**, 296, 326–328.
5. Chanda, A.; Khetan, S. K.; Banerjee, D.; Ghosh, A.; Collins, T. J. *J. Am. Chem. Soc.* **2006**, 128, 12058–12059.
6. Chahbane, N.; Popescu, D.-L.; Mitchell, D. A.; Chanda, A.; Lenoir, D.; Ryabov, A. D.; Schramm, K.-W.; Collins, T. J. *Green Chem.* **2007**, 9, 49–57.
7. Mondal, S.; Hangun-Balkir, Y.; Alexandrova, L.; Link, D.; Howard, B.; Zandhuis, P.; Cugini, A.; Horwitz, C. P.; Collins, T. J. *Catal. Today* **2006**, 116, 554–561.

8. Banerjee, D.; Markley, A. L.; Yano, T.; Ghosh, A.; Berget, P. B.; Minkley, E. G., Jr.; Khetan, S. K.; Collins, T. J. *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 3974–3977.
9. Shappell, N. W.; Vrabell, M. A.; Madsen, P. J.; Harrington, G.; Billey, L. O.; Hakk, H.; Larsen, G. L.; Beach, E. S.; Horwitz, C. P.; Ro, K.; Hunt, P. G.; Collins, T. J. *Environ. Sci. Technol.* **2008**, *42*, 1296–1300.
10. Horwitz, C. P.; Fooksman, D. R.; Vuocolo, L. D.; Gordon-Wylie, S. W.; Cox, N. J.; Collins, T. J. *J. Am. Chem. Soc.* **1998**, *120*, 4867–4868.
11. Smulevich, G.; Jakopitsch, C.; Droghetti, E.; Obinger, C. *J. Inorg. Biochem.* **2006**, *100*, 568–585.
12. Collins, T. J.; Khetan, S. K.; Ryabov, A. D. In: “*Handbook of Green Chemistry*”; Eds. Anastas, P. T.; Crabtree, R. H.; Wiley-VCH Verlag GmbH & KgaA: Weinheim, **2009**, pp. 39–77.
13. Ghosh, A.; Ryabov, A. D.; Mayer, S. M.; Horner, D. C.; Prasuhn, D. E., Jr.; Sen Gupta, S.; Vuocolo, L.; Culver, C.; Hendrich, M. P.; Rickard, C. E. F.; Norman, R. E.; Horwitz, C. P.; Collins, T. J. *J. Am. Chem. Soc.* **2003**, *125*, 12378–12379.
14. Kistenmacher, T. J.; Stucky, G. D. *Inorg. Chem.* **1968**, *7*, 2150–2155.
15. Nelson, S. M. In: “*Comprehensive Coordination Chemistry. The Synthesis, Reactions, Properties and Application of Coordination Compounds*”; Vol. 4; Eds. Wilkinson, G.; Gillard, R. D.; McCleverty, J. A.; Pergamon Press: Oxford, **1987**, pp. 217–276.
16. Koenig, D. F. *Acta Cryst.* **1965**, *18*, 663–673.
17. Hoard, J. L.; Cohen, G. H.; Glick, M. D. *J. Am. Chem. Soc.* **1967**, *89*, 1992–1996.
18. Ayougou, K.; Mandon, D.; Fischer, J.; Weiss, R.; Muther, M.; Schunemann, V.; Trautwein, A. X.; Bill, E.; Terner, J.; Jayaraj, K.; Gold, A.; Austin, R. N. *Chem. Eur. J.* **1996**, *2*, 1159–1163.
19. Orpen, A. G.; Brammer, L.; Allen, F. H.; Kennard, O.; Watson, D. G.; Taylor, R. J. *Chem. Soc. Dalton Trans.* **1989**, S1–S89.
20. Bartos, M. J.; Gordon-Wylie, S. W.; Fox, B. G.; Wright, L. J.; Weintraub, S. T.; Kauffmann, K. E.; Münck, E.; Kostka, K. L.; Uffelman, E. S.; Rickard, C. E. F.; Noon, K. R.; Collins, T. J. *Coord. Chem. Rev.* **1998**, *174*, 361–390.
21. Conradie, M. M.; Conradie, J.; Ghosh, A. *J. Inorg. Biochem.* **2006**, *100*, 620–626.
22. Popescu, D.-L.; Chanda, A.; Stadler, M.; Tiago de Oliveira, F.; Ryabov, A. D.; Bominaar, E. L.; Münck, E.; Collins, T. J. *Coord. Chem. Rev.* **2008**, *252*, 2050–2071.
23. Pauling, L. “*The Nature of the Chemical Bond*”; 3rd edn. Cornell University Press: Ithaca, NY, **1960**.
24. Ryabov, A. D.; Kazankov, G. M.; Yatsimirsky, A. K.; Kuz'mina, L. G.; Burtseva, O. Y.; Dvortsova, N. V.; Polyakov, V. A. *Inorg. Chem.* **1992**, *31*, 3083–3090.
25. Schmülling, M.; Ryabov, A. D.; van Eldik, R. *J. Chem. Soc. Dalton Trans.* **1994**, 1257–1263.
26. Schmülling, M.; Grove, D. M.; van Koten, G.; van Eldik, R.; Veldman, N.; Spek, A. L. *Organometallics* **1996**, *15*, 1384–1391.
27. Polshin, V.; Popescu, D.-L.; Fischer, A.; Chanda, A.; Horner, D. C.; Beach, E. S.; Henry, J.; Qian, Y.-L.; Horwitz, C. P.; Lente, G.; Fabian, I.; Münck, E.; Bominaar, E. L.; Ryabov, A. D.; Collins, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 4497–4506.
28. Fleischer, E. B.; Fine, D. A. *Inorg. Chim. Acta* **1978**, *29*, 267–271.

29. Coyle, C. L.; Rafson, P. A.; Abbott, E. H. *Inorg. Chem.* **1973**, *12*, 2007–2010.
30. Nasset, M. J. M.; Shokhirev, N. V.; Enemark, P. D.; Jacobson, S. E.; Walker, F. A. *Inorg. Chem.* **1996**, *35*, 5188–5200.
31. Shack, J.; Clark, W. M. *J. Biol. Chem.* **1947**, *171*, 143–187.
32. Cowgill, R. W.; Clark, W. M. *J. Biol. Chem.* **1952**, *198*, 33–61.
33. Bartos, M. J.; Kidwell, C.; Kauffmann, K. E.; Gordon-Wylie, S. W.; Collins, T. J.; Clark, G. C.; Münck, E.; Weintraub, S. T. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1216–1219.
34. Fukui, K. *Angew. Chem.* **1982**, *94*, 852–861.
35. Woodward, R. B.; Hoffmann, R. “*The Conservation of Orbital Symmetry*”; Academic Press: New York, **1970**.
36. Chanda, A.; Popescu, D.-L.; Tiago de Oliveira, F.; Bominaar, E. L.; Ryabov, A. D.; Münck, E.; Collins, T. J. *J. Inorg. Biochem.* **2006**, *100*, 606–619.
37. Jellinek, H. H. G.; Urwin, J. R. *J. Phys. Chem.* **1954**, *58*, 548–550.
38. Ghosh, A.; Tiago de Oliveria, F.; Toshihiro Yano, T.; Nishioka, T.; Beach, E. S.; Kinoshita, I.; Münck, E.; Ryabov, A. D.; Horwits, C. P.; Collins, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 2505–2513.
39. Collins, T. J.; Kostka, K. L.; Münck, E.; Uffelman, E. S. *J. Am. Chem. Soc.* **1990**, *112*, 5637–5639.
40. Kostka, K. L.; Fox, B. G.; Hendrich, M. P.; Collins, T. J.; Rickard, C. E. F.; Wright, L. J.; Münck, E. *J. Am. Chem. Soc.* **1993**, *115*, 6746–6757.
41. Xue, G.; Wang, D.; De Hont, R.; Fiedler, A. T.; Shan, X.; Münck, E.; Que, L., Jr. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20713–20718.
42. Hsu, H.-F.; Dong, Y.; Shu, L.; Young, V. G., Jr.; Que, L., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 5230–5237.
43. Kurtz, D. M., Jr. *Chem. Rev.* **1990**, *90*, 585–606.
44. Chance, M.; Powers, L.; Poulos, T.; Chance, B. *Biochemistry* **1986**, *25*, 1266–1270.
45. Penner-Hahn, J. E.; Smith Eble, K.; McMurry, T. J.; Renner, M.; Balch, A. L.; Groves, J. T.; Dawson, J. H.; Hodgson, K. O. *J. Am. Chem. Soc.* **1986**, *108*, 7819–7825.
46. Wolter, T.; Meyer-Klaucke, W.; Muther, M.; Mandon, D.; Winkler, H.; Trautwein, A. X.; Weiss, R. *J. Inorg. Biochem.* **2000**, *78*, 117–122.
47. Rohde, J.-U.; In, J.-H.; Lim, M. H.; Brennessel, W. W.; Bukowski, M. R.; Stubna, A.; Münck, E.; Nam, W.; Que, L., Jr. *Science* **2003**, *299*, 1037–1039.
48. Chanda, A.; Shan, X.; Chakrabarti, M.; Ellis, W.; Popescu, D.; Tiago de Oliveria, F.; Wang, D.; Que, L., Jr.; Collins, T. J.; Münck, E.; Bominaar, E. L. *Inorg. Chem.* **2008**, *47*, 3669–3678.
49. Hodges, G. R.; Lindsay Smith, J. R.; Oakes, J. *J. Chem. Soc. Perkin Trans.* **1998**, *2*, 617–628.
50. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J.; Vreven, J. A. T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels,

- A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. "Gaussian 03 (release B.05)"; Gaussian, Inc.: Wallingford, CT, **2004**.
51. Tiago de Oliveira, F.; Chanda, A.; Banerjee, D.; Shan, X.; Mondal, S.; Que, L., Jr.; Bominaar, E. L.; Münck, E.; Collins, T. J. *Science* **2007**, *315*, 835–838.
52. Chanda, A.; Ryabov, A. D.; Mondal, S.; Alexandrova, L.; Ghosh, A.; Hangun-Balkir, Y.; Horwitz, C. P.; Collins, T. J. *Chem. Eur. J.* **2006**, *12*, 9336–9345.
53. Ghosh, A.; Mitchell, D. A.; Chanda, A.; Ryabov, A. D.; Popescu, D. L.; Upham, E.; Collins, G. J.; Collins, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 15116–15126.
54. Pestovsky, O.; Stoian, S.; Bominaar, E. L.; Shan, X.; Münck, E.; Que, L., Jr.; Bakac, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 6871–6874.
55. Dunford, H. B. "Heme Peroxidases"; Wiley-VCH: New York, **1999**.
56. Ryabov, A. D.; Sukharev, V. S.; Alexandrova, L.; Le Lagadec, R.; Pfeffer, M. *Inorg. Chem.* **2001**, *40*, 6529–6532.
57. Oakes, J.; Gratton, P. *J. Chem. Soc. Perkin Trans.* **1998**, *2*, 1857–1864.
58. Alpeeva, I. S.; Soukharev, V. S.; Alexandrova, L.; Shilova, N. V.; Bovin, N. V.; Csoregi, E.; Ryabov, A. D.; Sakharov, I. Y. *J. Biol. Inorg. Chem.* **2003**, *8*, 683–688.
59. Ryabov, A. D. *Adv. Inorg. Chem.* **2004**, *55*, 201–270.
60. Walling, C. *Acc. Chem. Res.* **1975**, *8*, 125–131.
61. Halliwell, B.; Gutteridge, J. M. *Arch. Biochem. Biophys.* **1986**, *246*, 501–514.
62. Halliwell, B.; Gutteridge, J. M. *Methods Enzymol.* **1990**, *186*, 1–85.
63. Jones, C. W. "Applications of Hydrogen Peroxide and Derivatives"; The Royal Society of Chemistry: Cambridge, **1999**.
64. Sae, A. S. W.; Cunningham, B. A. *Phytochemistry* **1979**, *18*, 1785–1787.
65. Piontek, K.; Glumoff, T.; Winterhalter, K. *FEBS Lett.* **1993**, *315*, 119–124.
66. Hill, A. P.; Modi, S.; Sutcliffe, M. J.; Turner, D. D.; Gilfoyle, D. J.; Smith, A. T.; Tam, B. M.; Lloyd, E. *Eur. J. Biochem.* **1997**, *248*, 347–354.
67. Marquez, L. A.; Quitoriano, M.; Zilinskas, B. A.; Dunford, B. H. *FEBS Lett.* **1996**, *389*, 153–156.
68. Ohlsson, P. I.; Yonetani, T.; Wold, S. *Biochem. Biophys. Acta* **1986**, *874*, 160–166.
69. Balny, C.; Anni, H. S.; Yonetani, T. *FEBS Lett.* **1987**, *221*, 349–354.
70. Dolman, D.; Newell, G. A.; Thurlow, M. D.; Dunford, H. B. *Can. J. Biochem.* **1975**, *53*, 495–501.
71. Furtmüller, P. G.; Obinger, C.; Hsuanyu, Y.; Dunford, H. B. *Eur. J. Biochem.* **2000**, *267*, 5858–5864.
72. Kuan, I. C.; Johnson, K. A.; Tien, M. *J. Biol. Chem.* **1993**, *268*, 20064–20070.
73. Lad, L.; Mewies, M.; Raven, E. L. *Biochemistry* **2002**, *41*, 13774–13781.
74. Araiso, T.; Rutter, R.; Palcic, M. M.; Hager, L. P.; Dunford, H. B. *Can. J. Biochem.* **1981**, *59*, 233–236.
75. Ohtaki, S.; Nakagawa, H.; Nakamura, M.; Yamazaki, I. *J. Biol. Chem.* **1982**, *257*, 761–766.
76. Job, D.; Ricard, J.; Dunford, H. B. *Can. J. Biochem.* **1978**, *56*, 702–707.

77. Andrawis, A.; Johnson, K. A.; Tien, M. *J. Biol. Chem.* **1988**, 263, 1195–1198.
78. Chance, B. *Acta Chem. Scand.* **1947**, 1, 236–267.
79. Kremer, M. L. *Biochim. Biophys. Acta* **1970**, 198, 199–209.
80. Casella, L.; De Gioia, L.; Silvestri, G. F.; Monzani, E.; Redaelli, C.; Roncone, R.; Santagostini, L. *J. Inorg. Biochem.* **2000**, 79, 31–39.
81. Dallacosta, C.; Monzani, E.; Casella, L. *J. Biol. Inorg. Chem.* **2003**, 8, 770–776.
82. Uno, T.; Takeda, A.; Shimabayashi, S. *Inorg. Chem.* **1995**, 34, 1599–1607.
83. Paschke, J.; Kirsch, M.; Korth, H.-G.; de Groot, H.; Sustmann, R. *J. Am. Chem. Soc.* **2001**, 123, 11099–11100.
84. Zipplies, M. F.; Lee, W. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1986**, 108, 4433–4445.
85. Theodoridis, A.; Maignut, J.; Puchta, R.; Kudrik, E. V.; van Eldik, R. *Inorg. Chem.* **2008**, 47, 2994–3013.
86. Hewson, W. D.; Dunford, H. B. *Can. J. Chem.* **1975**, 53, 1928–1932.
87. Meunier, B. “*Models of Heme Peroxidases and Catalases*”; Imperial College Press: London, **2000**; pp. 171–214.
88. Lide, D. R. “*CRC Handbook of Chemistry and Physics*”; 87th edn. CRC Taylor & Francis: New York, **2006–2007**.
89. Weber, K.; Sund, H. *Angew. Chem.* **1965**, 77, 621.
90. Nicholls, P.; Fita, I.; Loewen, P. C. *Adv. Inorg. Chem.* **2001**, 51, 51–106.
91. Dunford, H. B. *Adv. Inorg. Biochem.* **1982**, 4, 41–80.
92. Coen, J. J. F.; Smith, A. T.; Candeias, L. P.; Oakes, J. *J. Chem. Soc. Perkin Trans.* **2001**, 2, 2125–2129.
93. Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, 100, 3009–3066.
94. Cornish-Bowden, A. “*Fundamentals of Enzyme Kinetics*”; Portland Press: London, **1995**.
95. Dixon, M.; Webb, E. C. “*Enzymes*”; Academic Press: New York, NY, **1958**; 782 pp.
96. Dixon, M.; Webb, E. C.; Thorne, C. J. R.; Tipton, K. F. “*Enzymes*”; 3rd edn. Academic Press: New York, NY, **1979**.
97. Fersht, A. “*Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*”; Freeman: New York, **1999**.
98. Anastas, P. T.; Warner, J. C. “*Green Chemistry: Theory and Practice*”; Oxford University Press: Oxford, **1998**.
99. Horvath, I. T. *Acc. Chem. Res.* **2002**, 35, 685.
100. Job, D.; Dunford, H. B. *Eur. J. Biochem.* **1976**, 66, 607–614.
101. Shevelkova, A. N.; Ryabov, A. D. *Biochem. Mol. Biol. Int.* **1996**, 39, 665–670.
102. Popescu, D.-L.; Chanda, A.; Stadler, M. J.; Mondal, S.; Tehranchi, J.; Ryabov, A. D.; Collins, T. J. *J. Am. Chem. Soc.* **2008**, 130, 12260–12261.

This page intentionally left blank

LIGAND EXCHANGE PROCESSES ON THE SMALLEST SOLVATED ALKALI AND ALKALINE EARTH METAL CATIONS: AN EXPERIMENTAL AND THEORETICAL APPROACH[☆]

RALPH PUCHTA^{a,b}, EWA PASGRETA^a and RUDI VAN ELDIK^a

^aInorganic Chemistry, Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany

^bComputer-Chemistry-Center, Department of Chemistry and Pharmacy, University of Erlangen-Nürnberg, Nögelsbachstr. 25, 91052 Erlangen, Germany

I. Introduction	523
II. Solvation of Metal Ions	526
A. Solvation of Li ⁺ and Be ²⁺	526
B. Selective Solvation of Li ⁺	531
III. Solvent Exchange Mechanism	533
A. Water Exchange	533
B. Ammonia Exchange	539
C. Dimethylsulfoxide Exchange	543
D. Hydrogen Cyanide and Acetonitrile Exchange	545
E. Exchange of Other Ligands: The Influence of Hybridization	551
F. Ligand Exchange on Chelated Beryllium Dications	555
G. Ligand Exchange on Five Coordinated Beryllium Dications	560
IV. Conclusion	564
Acknowledgments	564
References	564

I. Introduction

In the present contribution we focus mainly on our own recent work, as well as on literature published during the past few years. Earlier contributions dealing mainly with experimental studies are already summarized in different reviews and will therefore not be discussed in detail (1).

Lithium and beryllium are the first elements in the periodic table that are solid metals under ambient conditions. As the first

[☆]Dedicated to Prof. Dr. Kurt Dehnicke in recognition of his outstanding contributions to inorganic chemistry.

members of the alkali and alkaline earth metals, they can serve as models for other group members and for metals in general. In terms of mechanistic and computational studies, these metals and their ions are excellently suited as the simplest examples with clear defined spin states, and as closed shell species that allow, e.g., NMR studies and application of restricted quantum chemical methods, such as RHF, RB3LYP, RMP2, etc.

Whereas the application of lithium salts is in general well known, e.g., in industrial applications, synthesis, and medicine, e.g., in psychiatry (2–6), the application of beryllium and beryllium compounds is severely hampered by their high toxicity (7,8). Nevertheless, as already taught in high school (9), beryllium is not only important for gems, but also plays a key role in a number of modern technologies (10,11), such as neutron moderator, reflector in nuclear reactors, in alloys, e.g., for springs, electrical contacts, gyroscopes, satellites, metal foil in X-ray windows (12) or ceramics.

The chemistry of alkali and alkaline earth metals can be separated into two main areas: (i) organometallic chemistry, commonly used in synthesis and extensively investigated. By way of example, we only refer to important organolithium (13) and Grignard reagents (14). (ii) Coordination chemistry, strongly influenced by the Nobel prize-winning discovery by Pedersen, Lehn, and Cram of macrocyclic ligands such as “crown” ethers, cryptands, and spherands (15–17), together with the metalla topomers discovered later (18–25). Such ligands wrap around the metal ion, encapsulate ions to a larger or lesser degree, and are highly selective regarding the metal cation that can be accommodated. These features have led to important applications in cation separation (26–30), ion-specific measurement devices, phase-transfer catalysis (31–33), and as mimics of naturally occurring macrocyclic antibiotics (34).

Although not at the focus of recent research trends, the most important coordination compounds with alkali and alkaline earth ions are their aqua complexes. Here, we focus on $[\text{Li}(\text{OH}_2)_4]^+$ and $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$. To learn more about the role of the solvent and solvent exchange processes, we followed the approach of Jander (35–39) and also investigated solvent complexes with water-like solvents, such as NH_3 , HCN , etc., all possessing an O- or N-donor. Motivated by the ongoing discussion on sustainability in chemistry (40), which partly focuses on the role of solvents and the negative properties of molecular organic solvents (e.g., toxicity, flammability, etc.), we investigated common organic solvents and their alternatives such as (supercritical) carbon dioxide (41–43).

Why are we interested in different solvents and solvent exchange reactions? Detailed knowledge of elementary reactions, such as solvent exchange processes in different solvents or solvent mixtures, is an important prerequisite for selecting, optimizing, and tailoring the ideal solvent and/or solvent mixture. Solvent exchange is the simplest ligand exchange process, because there is no *net* change in the composition of the first coordination sphere of the metal ion. However, this process helps to understand ligand exchange and substitution on Li^+ or Be^{2+} in different environments, i.e., between solvent and other ligands. To study this topic in more detail, we combined computational and experimental methods, as they can complement each other extremely well. Whereas experimental methods investigate reactions under realistic conditions in a whole ensemble of molecules, quantum chemical calculations can focus on a single molecule in the presence of a small number of solvent molecules, e.g., only those in the first coordination sphere. This was already demonstrated earlier for different systems (44,45).

To investigate the different solvent exchange mechanisms of $[\text{Li}(\text{S})_4]^+$ and $[\text{Be}(\text{S})_4]^{2+}$ theoretically, we applied the common hybrid density functional B3LYP (46–48), as implemented in Gaussian (49), almost throughout in combination with the Pople style all electron basis set 6-311+G^{**} to calculate the structures and correct for zero point vibrational energies. Where possible, we compare calculated structures with experimental data, e.g., X-ray structures. This approach is sometimes hampered by neighborhood effects that can influence the experimental structure. Being well aware of the limitations of DFT calculations (50–54), we evaluated the energies by MP2(full)/6-311+G^{**} calculations (MP2(full)/6-311+G^{**}//B3LYP/6-311+G^{**}+ZPE(B3LYP/6-311+G^{**})) (55). When employing this approach (54), one has to keep in mind that activation barriers are generally somewhat underestimated by MP2 (56). In general, we did not find any significant deviations between DFT and MP2 energies. Solvent effects were probed via single point calculations employing implicit solvation models, where the solvent is represented as a continuous medium. We applied two models, *viz.*, IPCM (57,58) and CPCM (59,60), both as implemented in Gaussian.

During the past few decades, it has been shown that multinuclear NMR is a very powerful experimental technique to study alkali complexes particularly in non-aqueous solutions. The experimental method used throughout this work was ^7Li NMR. This method possesses features that make it convenient for this kind of investigation. Particular attention was given to high-pressure ^7Li NMR, which includes the first example reported for

this kind of research. High-pressure NMR is a sensitive tool employed to elucidate details of the solvent exchange mechanism. The determination of activation volumes from the pressure dependence of exchange rate constants for solvated metal ions is a useful method for the diagnosis of the intimate reaction mechanism. This has been demonstrated for numerous solvent exchange processes in the literature (1).

II. Solvation of Metal Ions

A. SOLVATION OF Li^+ AND Be^{2+}

As most of the reactions carried out by chemists, as well as most reactions observed in nature, are reactions in solution, solutions have been studied since the days of the alchemists (61). In nature and in industry the most important solvent is water. Therefore, our first and main interest concerns metal ions in aqueous medium. In addition, water serves as an excellent reference for water-like solvents according to Jander's approach and for solvents in general.

The first and very fundamental question we had to address was how many solvent molecules coordinate to a metal ion. In the case of the Be^{2+} cation, the coordination of four water molecules to form $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$ (at $\text{pH} < 3$) is corroborated based on NMR (62–68), X-ray (69–74), or even neutron diffraction data (75). In parallel, these observations are also made by different types of computer-based simulations (76–79). In the case of Li^+ one can find different values in the literature. While most X-ray structures demonstrate the existence of $[\text{Li}(\text{H}_2\text{O})_4]^+$ (80–82), $[\text{Li}(\text{H}_2\text{O})_5]^+$ (83), and $[\text{Li}(\text{H}_2\text{O})_6]^+$ (84) are also found. Even if one is doubtful and sceptical from a modern crystallographic point of view, e.g., $[\text{Li}(\text{H}_2\text{O})_5]^+$ and $[\text{Li}(\text{H}_2\text{O})_6]^+$ were studied at room temperature, we need to clarify the coordination number before the water exchange mechanism can be investigated.

To determine the coordination number of Li^+ experimentally, the cation should be dissolved in a solvent mixture that consists of a weak and strong coordinating component. The method is based on monitoring the resonance frequency of Li^+ as a function of the concentration of the stronger coordinating solvent while keeping the metal ion concentration constant. This kind of study might be hampered by limited solubility of lithium salts in solvent mixtures or limited miscibility of the components. There are a few conditions that must be satisfied. A weak coordinating solvent must be available that will dissolve all components, a

measurable parameter must exist that is a function of solvent composition, and the formation constant of the solvated metal ion must be sufficiently large so that a limiting value of the parameter can be obtained. In this case, the parameter was the Li^+ NMR signal. Its position in the NMR spectrum depends on the composition of the solvation shell of the solvated ion. Typical alkali metal salts are not appreciably soluble in truly non-coordinating solvents. Therefore, it was necessary to select a solvent with a weak interaction toward strongly coordinating solvents and the alkali metal ions (85).

Parallel to the NMR study we tried to determine the number of water molecules that coordinate to Li^+ by DFT calculations by adding stepwise additional water molecules. In the gas phase the addition of a further water molecule is in all cases exothermic up to $[\text{Li}(\text{H}_2\text{O})_6]^+$ and the resulting structures are also local minima (see Fig. 1).

The overestimation of complexation energies is clearly an artifact of the quantum chemical calculation performed in the "gas phase" without environmental effects, since the positive charge of Li^+ can be better stabilized if the system is enlarged by polarizable molecules such as water. To overcome this problem and to test the influence of the bulk solvent, we performed energy calculations applying the IPCM solvent model ($\text{B3LYP}(\text{IPCM}:\text{H}_2\text{O})/6\text{-}311+\text{G}^{**}/\text{B3LYP}/6\text{-}311+\text{G}^{**} + \text{ZPE}(\text{B3LYP}/6\text{-}311+\text{G}^{**})$). The bulk solvent clearly stabilizes the partially hydrated lithium cations. The tetrahedrally coordinated $[\text{Li}(\text{H}_2\text{O})_4]^+$ is the only system where the addition of the fourth water molecule is exothermic. In all other systems the addition of a further water molecule is endothermic. This coordination mode is corroborated by different MD simulations performed by Rode and Loeffler (86–89).

The combination of NMR investigations with published X-ray studies and in combination with quantum chemical calculations, convincingly show that in solution $[\text{Li}(\text{H}_2\text{O})_4]^+$ is expected to be the reactant species for the water exchange process.

In addition to pure water, we extended our investigation on solvated Li^+ to NH_3 (90), $\text{NH}_3\text{-H}_2\text{O}$ mixtures (91), dimethylsulfoxide (DMSO) (92), $\text{DMSO-H}_2\text{O}$ mixtures (92), HCN and CH_3CN , and their mixtures with water (93). For NH_3 as a water-like solvent, all published X-ray structures are tetrahedrally coordinated, $[\text{Li}(\text{NH}_3)_4]^+$ (94–97). Therefore, we expect to have $[\text{Li}(\text{NH}_3)_4]^+$ in solution. The validity of this assumption is also supported by the exchange mechanism. In the case of DMSO, a variety of theoretical and experimental studies on liquid DMSO (98–104), DMSO-water mixtures (105–110), DMSO-non-aqueous

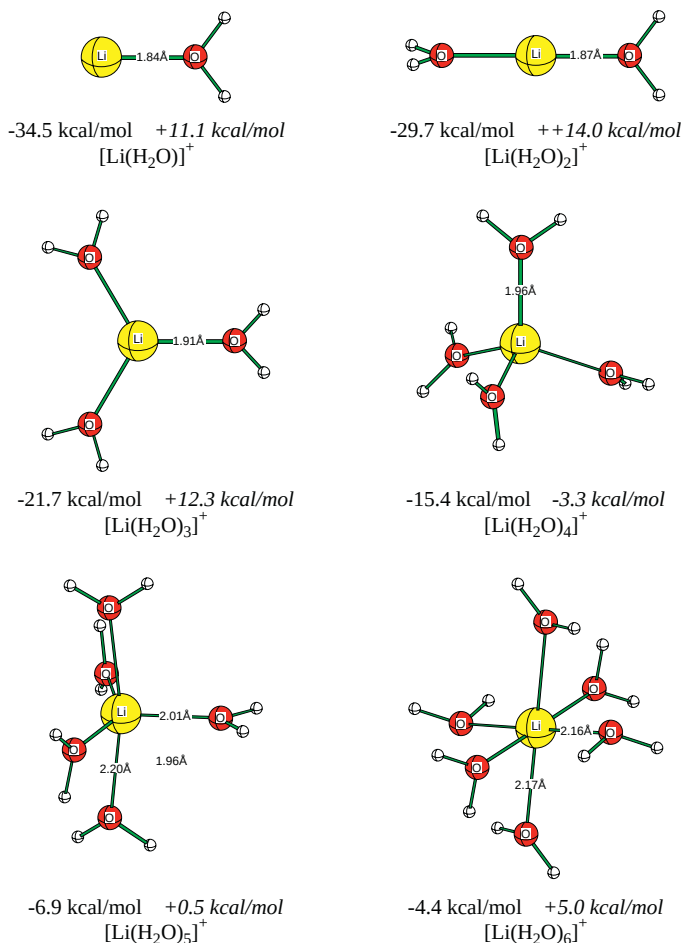


FIG. 1. Structures of $[\text{Li}(\text{H}_2\text{O})_n]^+$ ($n = 1-6$), calculated at the B3LYP/6-311+G^{**}+ZPE (B3LYP/6-311+G^{**}) and B3LYP(IPCM:H₂O)/6-311+G^{**}//B3LYP/6-311+G^{**}+ZPE (B3LYP/6-311+G^{**}) (values in italics) levels.

electrolyte solutions (111,112), and solutions of biological molecules in DMSO (113) are known.

It could be shown (Fig. 2) that the Li^+ ion in DMSO is coordinated by four solvent molecules. γ -Butyrolactone was found to be a convenient diluent in this kind of study. Contrary to DMSO ($DN = 29.8$), it is a rather weak donor ($DN = 18.0$) which appreciably dissolves alkali metal salts. Its interaction with DMSO is much weaker than that between DMSO and a metal cation. By plotting the chemical shift of the ^7Li NMR signal vs.

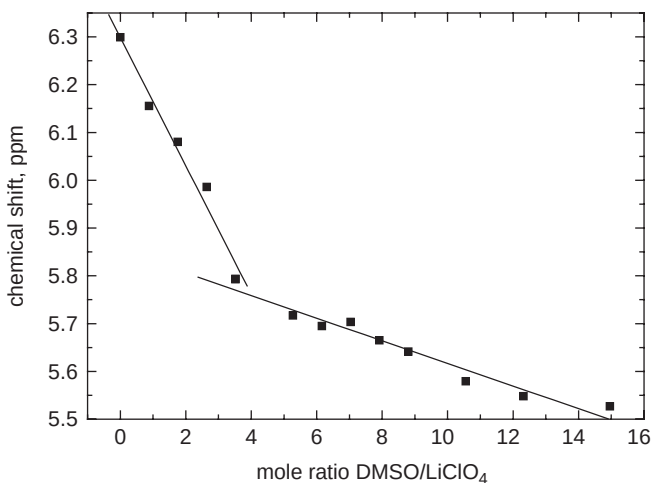


FIG. 2. ^7Li NMR chemical-shift variation as a function of the DMSO/ LiClO_4 molar ratio in γ -butyrolactone solution at 25°C (92).

the DMSO/ LiClO_4 mole ratio, a clear break point could be observed at a DMSO: Li^+ ratio of 4:1, indicating that the solvation number of Li^+ in DMSO is 4 (92).

The behavior of pure acetonitrile and mixtures of acetonitrile with other solvents have been well studied experimentally (114,115) and computationally (114,116). Since an ion in solution strongly disturbs the local solvent structure (117), detailed knowledge and special investigations are an essential prerequisite to understand reactions in the neighborhood of the ion. Several studies investigated solvated Li^+ ions in acetonitrile, both experimentally with various spectroscopic techniques (118–120) and computationally with different classical molecular dynamics methods (121–124) and Monte Carlo simulations on solvated alkali and halide ions in CH_3CN (125). Over the past decade DFT and *ab initio* calculations on $[\text{Li}(\text{CH}_3\text{CN})_n]^+$ and related clusters were also performed by different groups (120, 126–129).

Compared to the structures of Li^+ -water solvates, the structures of Li^+ -acetonitrile solvates are in general less studied. The Li^+ ion was found to be four coordinate with the use of different techniques, e.g., by NMR where acetonitrile was gradually replaced by water in a 1.6 M solution of LiClO_4 (130), or based on IR intensities measured for the acetonitrile C–N stretching vibration (131,132). Even mixed coordination of a counter ion and acetonitrile were reported to be four coordinate, *viz.*, in $[\text{Li}(\text{CH}_3\text{CN})_3\text{Br}]$ for 0.58 M LiBr in CH_3CN (133). Extensive

concentration dependent ion pairing was also found for weak coordinating counter ions as in LiBF_4 (120) and LiClO_4 in CH_3CN (131), e.g., by studying the C–N vibration (134). The reported coordination numbers are in good agreement with published X-ray structures (94,135–140).

On the other hand, however, Sajeevkumar and Singh found the average coordination number to be 4.7 (141), whereas Megyes *et al.* found up to six acetonitrile molecules bound to the Li^+ ion in the gas phase by means of mass spectrometry (142). Therefore, we reinvestigated the maximum coordination number of $[\text{Li}(\text{CH}_3\text{CN})_n]^+$ and also of $[\text{Li}(\text{NCH})_n]^+$ purely by quantum chemical calculations, which resulted in $[\text{Li}(\text{NCH})_4]^+$ and $[\text{Li}(\text{CH}_3\text{CN})_4]^+$ (93).

On application of the NMR method mentioned above for the solvent mixture consisting of acetonitrile ($DN = 14.1$) and nitromethane ($DN = 2.7$), it was found that also in this case the Li^+ ion is coordinated by four acetonitrile molecules (Fig. 3).

In the case of $[\text{Be}(\text{S})_4]^{2+}$, the situation is in general more clear as the less available data do not contradict one other. Beside the X-ray structures of $[\text{Be}(\text{OH}_2)_4]^{2+}$ (67–74) and $[\text{Be}(\text{DMSO})_4]^{2+}$, (143,144) no structure determinations by X-ray are known and NMR experiments with solvents or similar ligands, e.g., *N,N*-dimethylformamide, 1,1,3,3-tetramethylurea, etc. (67,145) all hint at $[\text{Be}(\text{S})_4]^{2+}$.

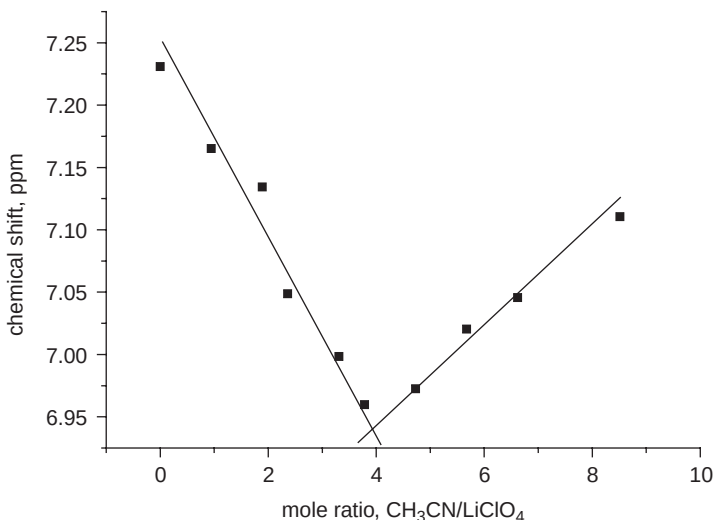


FIG. 3. ^7Li NMR shift measured for 0.05 M LiClO_4 in acetonitrile–nitromethane solvent mixtures at 25°C (93).

B. SELECTIVE SOLVATION OF Li^+

Because of the significant importance of lithium ions and the availability of data from earlier published studies, we investigated the selectivity of Li^+ between water and DMSO, and water and acetonitrile. Selective coordination occurs in general when the solvate prefers one of the solvent components over the other. The exchange of ions between different environments is usually rapid with respect to the NMR time scale and therefore, difficult to investigate. Only one resonance signal is observed at an average frequency determined by the magnetic shielding and lifetime of the nucleus in each of the sites. Variation of parameters such as concentration, counter ions, and solvent, produces changes in the relative composition and type of the environment, which may be reflected by the NMR spectra in terms of chemical shift and/or line shape, and/or line width of the observed resonance (146).

Selective solvation of alkali metal ions in mixed solvents can be monitored by the extent to which the chemical shift of the alkali metal nucleus varies with solvent composition. In the binary solvent mixture of water and DMSO, no selective solvation was observed, indicating that on increasing the water content of the solvent mixture, DMSO is gradually displaced by water in the coordination sphere of Li^+ . Any rapid change in the position of the NMR signal, shown in Fig. 4, when the amount of water was increased was not observed. DMSO is a solvent of high solvation ability, which can strongly coordinate to the Li^+ ion and can compete with water in the first coordination sphere of the Li^+ ion (92). It could be concluded that the composition of the first coordination sphere in mixtures of $\text{H}_2\text{O}/\text{DMSO}$ gradually changes from $[\text{Li}(\text{DMSO})_4]^+$ to $[\text{Li}(\text{H}_2\text{O})_4]^+$ on increasing the water content of the solvent. This is a consequence of the rather similar donor strength of these solvents as expressed by their donor numbers.

In the binary water/acetonitrile mixture, however, water coordinates much stronger to Li^+ than acetonitrile, such that addition of water immediately leads to the formation of $[\text{Li}(\text{H}_2\text{O})_4]^+$ (93). It was observed that the position of the NMR signal moved significantly (from 4.5 to 6.4 ppm) even if the amount of added water was very small (only 10% H_2O). Then the chemical shift increased slightly and stayed almost constant while the amount of water was increased from 10 to 100%. This observation confirms that water coordinates much stronger to the Li^+ ion than acetonitrile (Fig. 5).

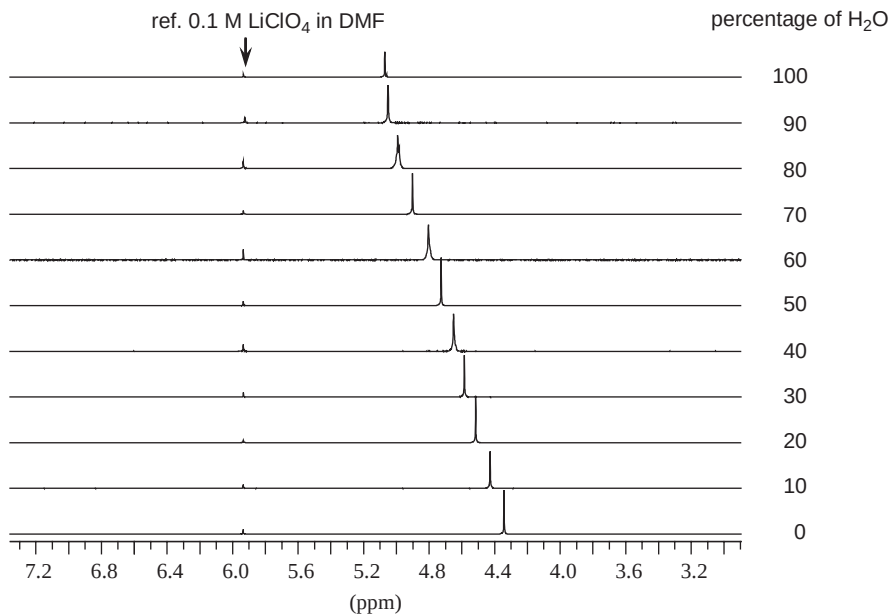


FIG. 4. ^7Li NMR spectra of LiClO_4 in H_2O –DMSO mixtures of different compositions at 25°C (92).

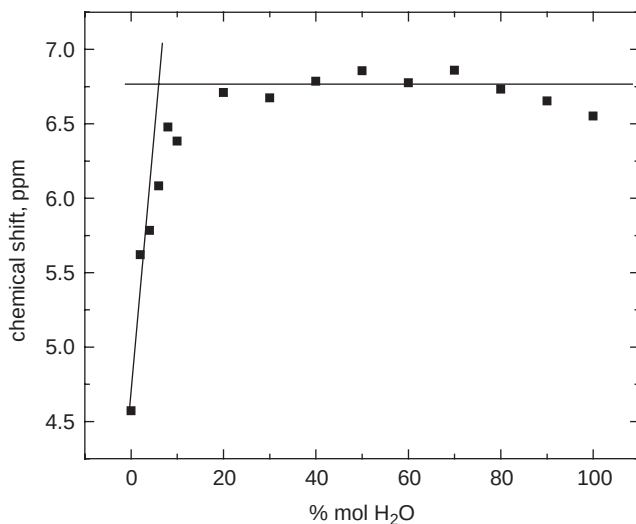


FIG. 5. ^7Li NMR shifts for 0.1 M LiClO_4 as a function of the $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ composition at 25°C (93).

III. Solvent Exchange Mechanism

A. WATER EXCHANGE

Exchange of a solvent molecule between the first and second coordination sphere is the simplest reaction that can occur on a solvated metal ion in aqueous and non-aqueous solution. This reaction is fundamental to understand not only the reactivity of metal ions in chemical and biological systems, but also the interaction between the metal ion and the solvent molecules. The displacement of a solvent molecule from the first coordination sphere represents an important step in complex-formation reactions of metal cations and in many redox processes (147). The measured rates of solvent exchange vary extensively with the nature of the metal ion and, to a lesser extent, with that of the solvent. Metal ions of main group elements have filled electron shells, and they differ mainly in electric charge and ionic radius. The number of solvent molecules in the first coordination sphere around the metal ion ranges from 4 to 10. Alkali group ions are very labile, due to the low surface charge density and the absence of ligand field stabilization effects (1). Solvent exchange at Li^+ in water is extremely fast, which made it difficult to study directly. An exchange rate constant of approximately 10^9 s^{-1} at 25°C was predicted on the basis of complex-formation rate constants (148,149).

Previous theoretical studies focused on the structures of various aqua complexes of Li^+ (150,151). Hermansson *et al.* reported evidence for an associative water exchange processes for 512 water molecules and one lithium cation in a cubic simulation box by employing the reactive flux method in combination with very long molecular dynamics simulations based on effective three-body ion-water potentials (152). They reported that both the simulation and reactive flux exchange events show that in about 80% of the cases the exchange mechanisms have an associative (A) or an associative interchange (I_a) character to almost the same degree. However, only schematic pictures of the exchange mechanisms could be presented, and detailed structures of the transition states involved were not available.

Our B3LYP/6-311+ G^{**} calculations supported a limiting associative exchange mechanism on the aquated lithium cation as shown in Fig. 6 (90).

Water exchange proceeds through a trigonal bipyramidal reactive intermediate $[\text{Li}(\text{H}_2\text{O})_5]^+$, reached via a late transition state. In accordance with the above-mentioned experimental observation of a very fast exchange process, the activation barrier

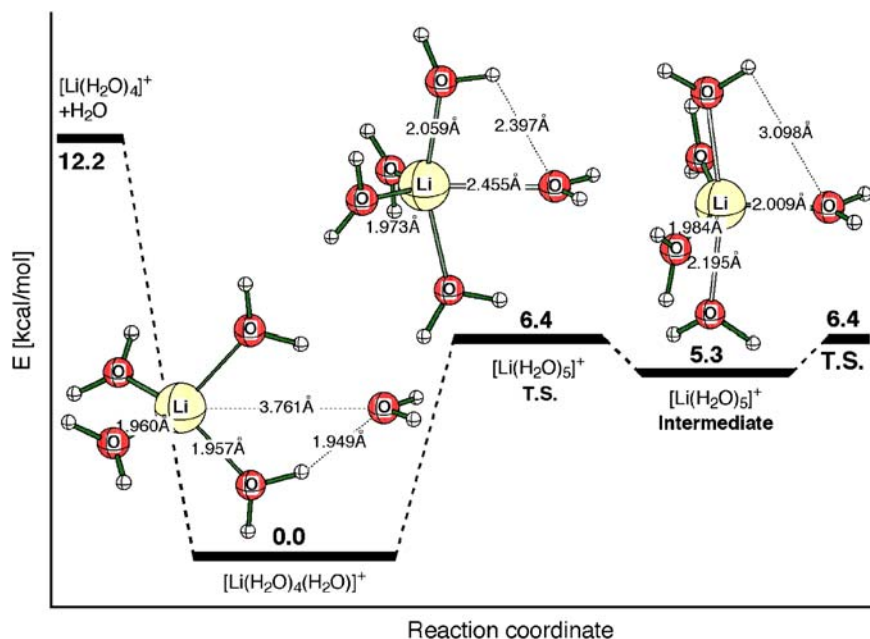


FIG. 6. Energy profile for water exchange around the lithium cation (relative energies: B3LYP/6-311+G^{**}) (90).

is only $6.4 \text{ kcal mol}^{-1}$, while the five-coordinate intermediate is $5.3 \text{ kcal mol}^{-1}$ less stable than the precursor complex.

It is well known for more than 25 years that the reactivity of beryllium salts in solution is in general controlled by the lability of coordinated solvent molecules attached to the Be^{2+} ions (153). Earlier mechanistic assignments were based on correlations between both the stereochemistry and the electron donor ability of the solvent and its tendency to exchange with free solvent molecules through a first-order and/or a second-order pathway in non-coordinating polar diluents (154). Available temperature dependent data from which mechanistic conclusions were drawn on the basis of activation enthalpy and entropy data were at that point still subjected to criticism and not clear cut to interpret. Therefore, Merbach *et al.* determined an activation volume of $-13.6 \text{ cm}^3 \text{ mol}^{-1}$ for water exchange around Be^{2+} by high-pressure NMR and interpreted this as a clear indication of an associative (A or I_a) exchange mode (67). Based on semi-empirical calculations developed by Swaddle (155), they favored an associative A mechanism, but a definite conclusion was not possible.

Our computations revealed unequivocally that an A mechanism does not operate (156). The trigonal bipyramidal pentaqua

complex $[\text{Be}(\text{H}_2\text{O})_5]^{2+}$ is not an intermediate, but represents the transition state structure for water exchange, energetically ($\text{B3LYP}/6\text{-}311+\text{G}^{**}$) $15.6 \text{ kcal mol}^{-1}$ above the hydrogen-bonded pentaqua complex $[\text{Be}(\text{H}_2\text{O})_4(\text{H}_2\text{O})]^{2+}$. The energy profile for the water exchange process and the structures of the complexes are shown in Fig. 7.

The hydrogen-bonded water molecule from the second solvation sphere approaches the beryllium dication, during which the previously hydrogen-bonded ligands assume equatorial positions. The ligand exchange process proceeds in a *trans* mode, similar to the well-known $\text{S}_{\text{N}}2$ mechanism. The ligand being expelled remains in the second solvation sphere, hydrogen bonded to two metal-coordinated water molecules.

The three water ligands that are not directly involved in the exchange process remain strongly bound to Be^{2+} during the reaction. The Be–O distances are essentially the same as in the precursor complex and the distances to the entering and leaving water molecules are only $0.3 \text{ \AA}$ longer, indicating that the bonds to these ligands are strong, in line with an associative interchange (I_{a}) mechanism (156).

To test the reliability of the B3LYP hybrid density functional, we computed the $\text{MP2}(\text{full})/6\text{-}311+\text{G}^{**}$ energies based on the structures obtained at the $\text{B3LYP}/6\text{-}311+\text{G}^{**}$ level. The activation barrier at this level is only $12.6 \text{ kcal mol}^{-1}$. This is not

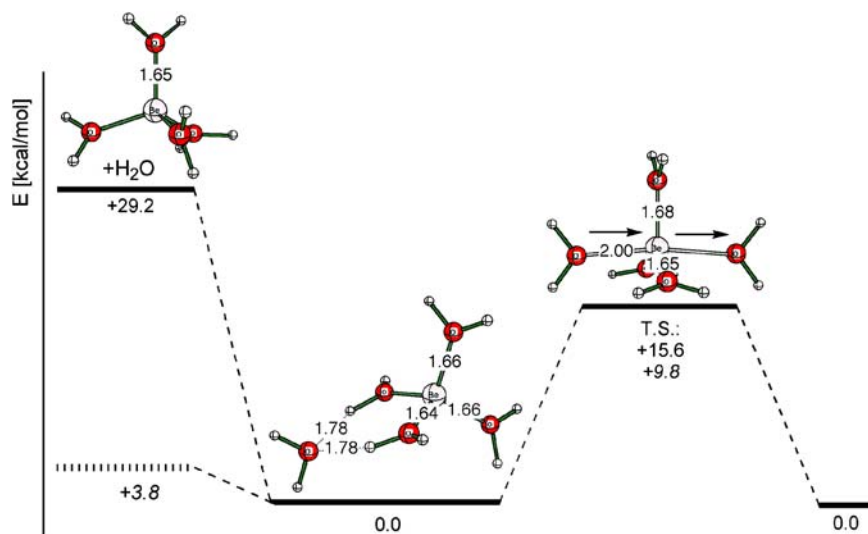


FIG. 7. Energy profile for water exchange around the beryllium dication ($\text{B3LYP}/6\text{-}311+\text{G}^{**}$, italic: $\text{B3LYP}(\text{IPCM})/6\text{-}311+\text{G}^{**}$) (156).

unexpected, as activation barriers are generally somewhat underestimated by MP2 (56). Inclusion of the influence of the bulk solvent leads to a moderate reduction of the activation energy ($9.8 \text{ kcal mol}^{-1}$).

In the IPCM calculations, the molecule is contained inside a cavity within the polarizable continuum, the size of which is determined by a suitable computed isodensity surface. The size of this cavity corresponds to the molecular volume allowing a simple, yet effective evaluation of the molecular activation volume, which is not based on semi-empirical models, but also does not allow a direct comparison with experimental data as the second solvation sphere is almost completely absent. The volume difference between the precursor complex $[\text{Be}(\text{H}_2\text{O})_4(\text{H}_2\text{O})]^{2+}$ and the transition structure $[\text{Be}(\text{H}_2\text{O})_5]^{2+}$, *viz.*, $-4.5 \text{ \AA}^3$, represents the activation volume of the reaction. This value can be compared with the value of $-6.1 \text{ \AA}^3$ calculated for the corresponding water exchange reaction around Li^+ , for which we concluded the operation of a limiting associative mechanism. In the present case, both the nature of $[\text{Be}(\text{H}_2\text{O})_5]^{2+}$ and the activation volume clearly indicate the operation of an associative interchange mechanism (156).

The weakest point of our approach is the treatment of the bulk solvent. The energies derived from an implicit solvent model like IPCM are mainly based on energy calculations on gas-phase structures and effects of explicit solvent molecules are not included.

For the first time the influence of an explicit solvent sphere on a water exchange process was studied for the dissociative water exchange on $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$ (157). We considered up to four additional water molecules explicitly in the second coordination sphere. The additional four hydrogen-bonded water molecules acted as spectator ligands, not directly involved in the exchange mechanism. For $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$ we found that even an incomplete solvent sphere shows a stabilizing effect of the additional solvent molecules for the dissociative exchange process for all systems, e.g., the activation energy was reduced nearly to 50% by taking only two extra water molecules into account. On the other hand, the energy was not lowered systematically as this approach was spoiled by secondary steric effects (157).

For the small system involved in the water exchange on $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$, we evaluated the effect of an implicit and approximated explicit treatment of the bulk water while investigating water exchange on $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$. For the implicit treatment, the CPCM and PCM models were applied as implemented in Gaussian, and geometry optimizations and

vibrational frequencies were calculated within the B3LYP (PCM:H₂O)/6-311+G^{**} (57) and B3LYP(CPCM:H₂O)/6-311+G^{**} (59,60) solvent models. For the explicit treatment we adopted the approach of Bertrán, Lledós and Revetllat, who connected the entering water molecule via hydrogen-bonded water molecules to the leaving halogen ion of tetrahedral CH₃F (158). We furnished tetrahedral [Be(H₂O)₄]²⁺ not only with five extra water molecules that do not directly participate in the reaction, but also modeled the surrounding solvent shell by connecting the entering and leaving water molecules (159).

A structural comparison of the calculated (B3LYP/6-311+G^{**}) **ts** (transition state in the gas phase), **ts-wc** (transition state in the cluster of five extra water molecules), **ts-CPCM** (transition state within the CPCM-solvent model (B3LYP(CPCM)/6-311+G^{**})) and **ts-PCM** (transition state optimized within the PCM-solvent model (B3LYP(PCM)/6-311+G^{**})), shows no large differences (see Fig. 8), which is also valid for the precursor complexes (see Fig. 9). Modeling solvent effects shrinks in all cases the Be–O bonds of the entering/leaving water molecules (159).

While the transition states could all be confirmed as transition states, only the precursor in the gas phase **pc**, and for the water cluster approach **pc-wc**, was confirmed as a local minimum, and despite intensive search no minima could be found within the CPCM and PCM model approximation.

Since experimental mechanistic investigations measure activation volumes, Rotzinger proposed to use in calculations, the currently well-accepted quantum chemical descriptor “difference of sums of $r(\text{M}-\text{O})$ ”, $\Delta\Sigma r$, for the volume of activation, $\Delta V^\ddagger$ (160). As the structures are all similar (Table I) this descriptor is in all four cases identical.

The highest activation energy for water exchange on [Be(H₂O)₄(H₂O)]²⁺ was calculated in the gas phase, independent of the selected method. All approaches to include solvent effects clearly reduced the energy barrier. While the approach of connecting the entering and leaving water ligands reduces the activation barrier to *ca.* 5/6 (13.5 kcal mol⁻¹), single point calculations applying implicit solvent models reduce the barrier to less than 4/6 (CPCM: 9.4 kcal mol⁻¹; IPCM: 9.8 kcal mol⁻¹, see Table II). The lowest barrier (8.3 kcal mol⁻¹), a little more than 3/6 of the barrier, can be obtained by optimizing the precursor and transition states within the PCM- and CPCM-solvent models (see Fig. 10) (159).

All employed methods to include solvent effects corroborate the nature of the transition state for water exchange via an interchange mechanism and show very satisfactorily the mode

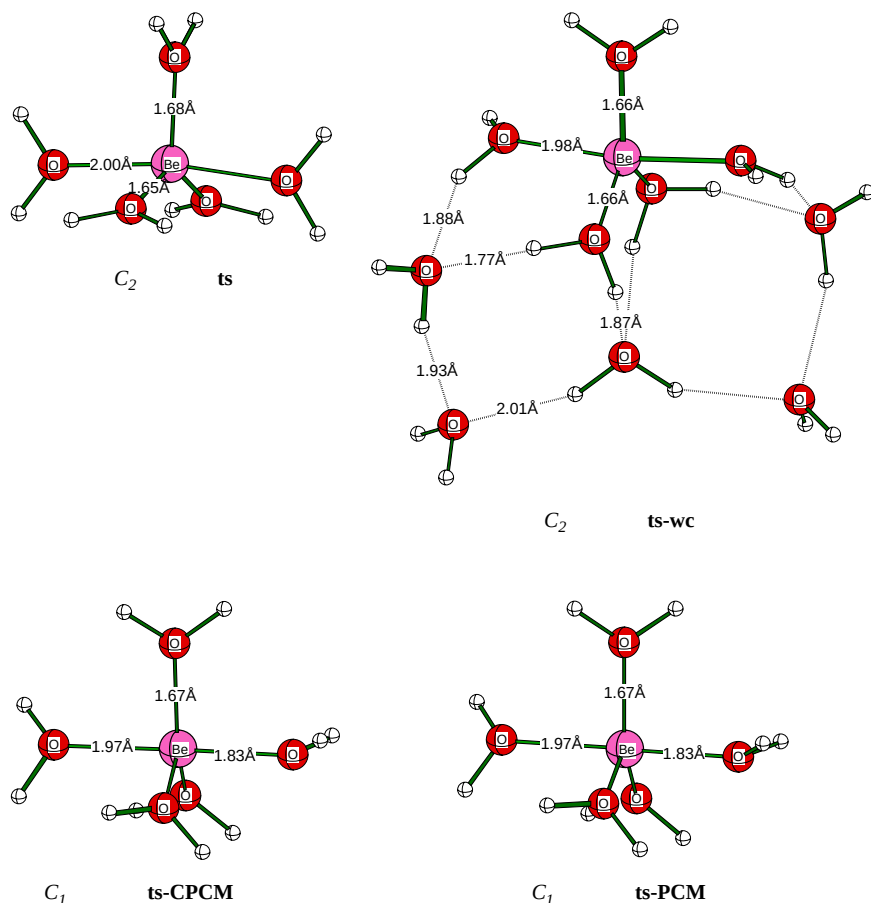


FIG. 8. Calculated structures of the transition states within different environmental models (159).

of the interchange process. Since it is an absolute prerequisite for studying an unknown reaction to obtain a local minimum as a precursor, we do not consider optimization within the CPCM or PCM model to be a favorable approach. In the case of the explicit treatment, we fear that a larger water cluster can lead to artifacts of conformation and unbalanced reaction equations. Hopefully, this can be solved in the future by *ab initio* molecular dynamics simulations. Motivated by these observations and earlier successful studies, we consider investigations of solvent exchange reactions with traditional small solvent clusters, where the second coordination sphere is denoted, e.g., by a single solvent molecule, presently as a good approach. Solvent effects can be included in single point calculations of implicit solvent models.

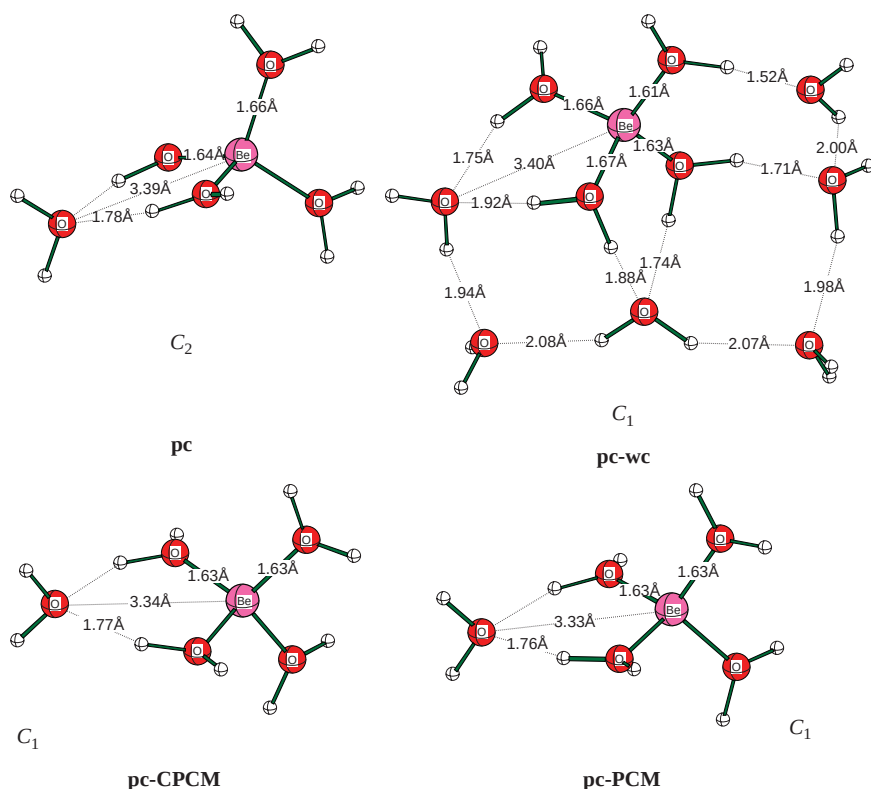


FIG. 9. Calculated structures of the precursor complex within different environment models (159).

TABLE I

CALCULATED CHANGES OF THE VOLUME DESCRIPTOR $\Delta\Sigma R$ FOR THE WATER EXCHANGE PROCESS

pc	pc-wc	pc-CPCM	pc-PCM
1.01 Å	1.03 Å	1.05 Å	1.04 Å

B. AMMONIA EXCHANGE

Whereas ligand exchange reactions in aquated complexes of Li^+ have been studied extensively, the corresponding ammine complexes have so far received very little attention. The interaction of an ammonia molecule (belonging to the second coordination sphere) with $[\text{Li}(\text{NH}_3)_4]^+$ is weaker than in the

TABLE II

CALCULATED ACTIVATION ENERGIES IN kcal mol^{-1} FOR THE WATER EXCHANGE PROCESS ON $\text{Be}^{2+}_{(\text{aq})}$

Method	ts	ts-wc	ts-CPCM	ts-PCM
B3LYP	+15.6	+13.5	—	—
MP2(full)	+12.7	+9.7	—	—
CPCM-SP	+9.4	—	—	—
IPCM-SP (156)	+9.8	—	—	—
CPCM	—	—	+8.3	—
PCM	—	—	—	+8.3

B3LYP: B3LYP/6-311+G**//B3LYP/6-311+G**+ZPE (B3LYP/6-311+G**); MP2(full): MP2(full)/6-311+G**//B3LYP/6-311+G**+ZPE (B3LYP/6-311+G**); CPCM-SP: B3LYP (CPCM: H_2O)/6-311+G**//B3LYP/6-311+G**+ZPE (B3LYP/6-311+G**); IPCM-SP: B3LYP(IPCM: H_2O)/6-311+G**//B3LYP/6-311+G**+ZPE (B3LYP/6-311+G**); CPCM: B3LYP(CPCM: H_2O)/6-311+G**//B3LYP(CPCM: H_2O)/6-311+G**+ZPE (B3LYP (CPCM: H_2O)/6-311+G**); PCM: B3LYP(PCM: H_2O)/6-311+G**//B3LYP(PCM: H_2O)/6-311+G**+ZPE (B3LYP(PCM: H_2O)/6-311+G**).

corresponding coordination by water, and in contrast to water the fifth ammonia molecule with only one lone-pair forms two long, weak bonds to two different NH_3 ligands in the first coordination sphere (see Fig. 11) (90). The activation barrier is only $3.8 \text{ kcal mol}^{-1}$. As in the case of water, the influence of the bulk solvent is negligible and ammonia exchange at Li^+ thus might be even faster than in the case of water. The molecular volume changes very little, i.e., the transition state structure is only slightly larger than the precursor complex. Thus, the exchange is best described as proceeding via an associative interchange (I_a) mechanism.

To learn more about potential reasons for the different reaction mechanisms for water and ammonia exchange at $[\text{Li}(\text{L})_4]^+$, we investigated the stepwise substitution of NH_3 by H_2O at $[\text{Li}(\text{NH}_3)_4]^+$ (91). In all cases, we computed an interchange mechanism. The activation energies are all of the same order of magnitude as the pure water exchange reaction (Table III). An interesting observation only possible in such mixed exchange processes is that the reactants and products can form different isomers by different hydrogen-bonding modes of the solvent in the second coordination sphere. For our test, we only consider the lowest lying local minima.

If a NH_3 molecule was included in the exchange process, we obtained an interchange type of mechanism. Therefore, we conclude that the larger molar volume of ammonia compared to water is responsible for the mechanistic changeover from an associative (A) to an associative interchange (I_a) type of mechanism.

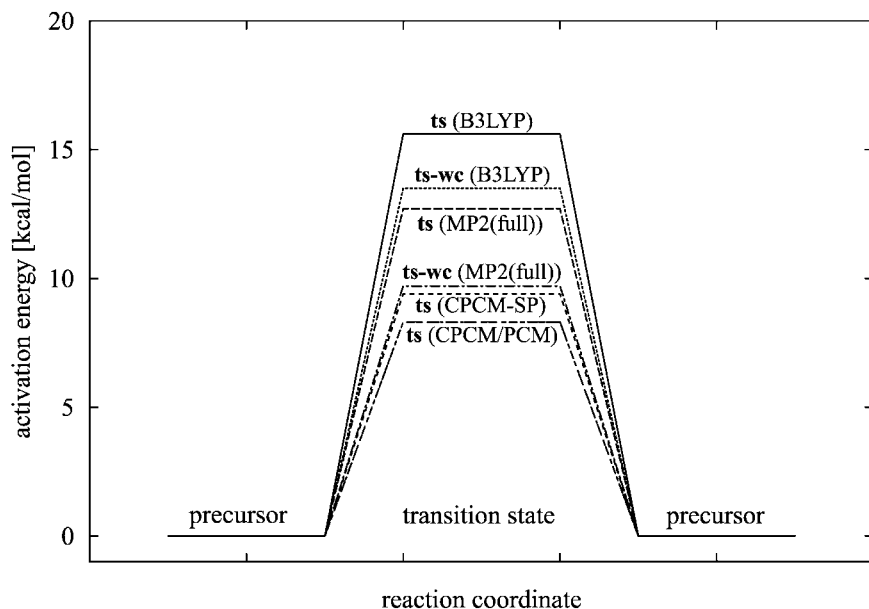


FIG. 10. Energy profiles for the water exchange processes on Be^{2+} species (159). **ts** (B3LYP): **ts** B3LYP/6-311+ G^{**} //B3LYP/6-311+ G^{**} + ZPE (B3LYP/6-311+ G^{**}); **ts-wc** (B3LYP): **ts-wc** B3LYP/6-311+ G^{**} //B3LYP/6-311+ G^{**} + ZPE (B3LYP/6-311+ G^{**}); **ts** (MP2(full)): **ts** MP2(full)/6-311+ G^{**} //B3LYP/6-311+ G^{**} + ZPE (B3LYP/6-311+ G^{**}); **ts-wc** (MP2(full)): **ts-wc** MP2(full)/6-311+ G^{**} //B3LYP/6-311+ G^{**} + ZPE (B3LYP/6-311+ G^{**}); **ts** (CPCM-SP): **ts** B3LYP(CPCM: H_2O)/6-311+ G^{**} //B3LYP/6-311+ G^{**} + ZPE (B3LYP/6-311+ G^{**}); **ts** (CPCM): **ts-CPCM** B3LYP(CPCM: H_2O)/6-311+ G^{**} //B3LYP(CPCM: H_2O)/6-311+ G^{**} + ZPE (B3LYP(CPCM: H_2O)/6-311+ G^{**}); **ts** (PCM): **ts-PCM** B3LYP(PCM: H_2O)/6-311+ G^{**} //B3LYP(PCM: H_2O)/6-311+ G^{**} + ZPE (B3LYP(PCM: H_2O)/6-311+ G^{**}). The “product” is denoted as “precursor” since the overall reaction does not include a net chemical conversion.

This is further corroborated by the smaller Be^{2+} ion. $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$ already shows an interchange mechanism, and one can predict that ammonia exchange at $[\text{Be}(\text{NH}_3)_4]^{2+}$ will also proceed according to an interchange mechanism. As in the case of the aqua complex, $[\text{Be}(\text{NH}_3)_4]^{2+}$ has a tetrahedral symmetry, the additional NH_3 molecules form a second solvation sphere and the large electrostatic contribution leads to an exaggerated binding energy, as confirmed by the IPCM calculations ($1.9 \text{ kcal mol}^{-1}$) (156).

The ammonia exchange proceeds as in the case of water exchange through an associative interchange mechanism, showing a trigonal bipyramidal transition state structure $[\text{Be}(\text{NH}_3)_5]^{2+}$,

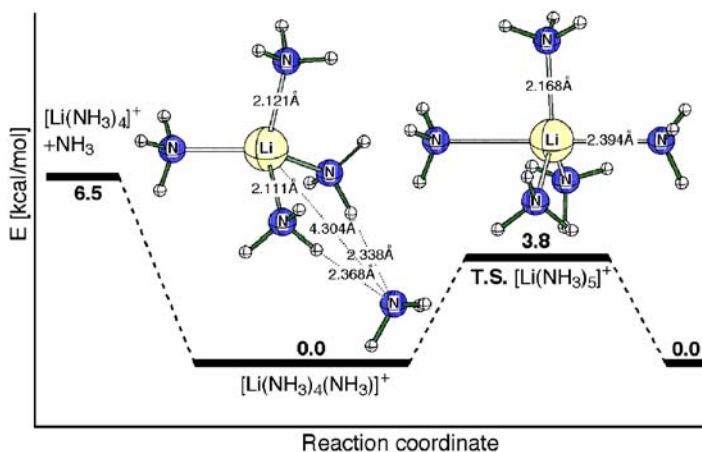


FIG. 11. Energy profile for ammonia exchange around the lithium cation (relative energies: B3LYP/6-311+G^{**}) (90).

TABLE III

CALCULATED ACTIVATION ENERGIES (B3LYP/6-311+G^{**}) FOR THE STEPWISE SUBSTITUTION OF AMMONIA BY WATER (91)

$[\text{Li}(\text{NH}_3)_4]^+$	$[\text{Li}(\text{NH}_3)_3(\text{H}_2\text{O})]^+$	$[\text{Li}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^+$	$[\text{Li}(\text{NH}_3)(\text{H}_2\text{O})_3]^+$
6.1 kcal mol ⁻¹	8.0 kcal mol ⁻¹	8.9 kcal mol ⁻¹	8.4 kcal mol ⁻¹

with an activation barrier of 18.8 kcal mol⁻¹ (Fig. 12). Thus, the rate of the exchange reaction will be similar to or somewhat slower than in the case of water. The Be–N distances for the entering and leaving ammonia molecule, 2.29 Å, are more than 0.5 Å longer than the other Be–N bonds, which vary very little throughout the reaction. This elongation, however, reflects the higher steric demand of ammonia, compared to water, for which an elongation of only 0.28 Å was computed, rather than a changeover in mechanism.

The MP2(full)/6-311+G^{**}//B3LYP/6-311+G^{**} energy calculation resulted in a somewhat lower activation barrier of 15.7 kcal mol⁻¹, as expected for MP2. The influence of the bulk solvent on the activation barrier appears to be small, *viz.*, 17.3 kcal mol⁻¹ (B3LYP(IPCM)/6-311+G^{**}//B3LYP/6-311+G^{**}). The molecular volume of the transition state structure is 3.1 Å³ larger than that of the precursor $[\text{Be}(\text{NH}_3)_4(\text{NH}_3)]^{2+}$. Since this increase was also found for ammonia exchange around Li⁺, we concluded that the exchange process follows an associative interchange (I_a) mechanism.

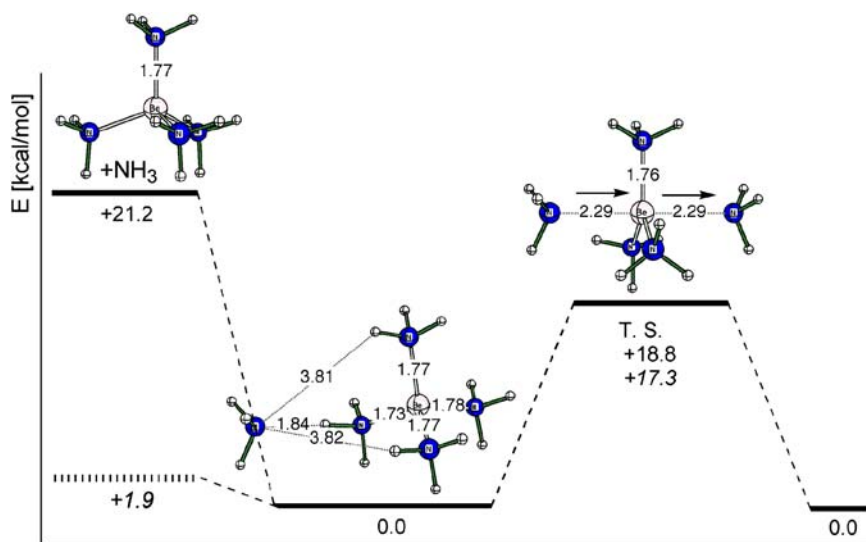


FIG. 12. Energy profile for ammonia exchange around the beryllium dication. Distances in Å, energies in kcal mol^{-1} (B3LYP/6-311+G^{**}, italic: B3LYP(IPCM)/6-311+G^{**}) (156).

C. DIMETHYLSULFOXIDE EXCHANGE

A common organic solvent that is easier to handle in the laboratory than NH_3 is DMSO. Ligand exchange on $[\text{Li}(\text{DMSO})_4]^+$ (101) was found to follow a limiting associative mechanism (Fig. 13), whereas the displacement of coordinated H_2O by DMSO in $[\text{Li}(\text{H}_2\text{O})_4]^+$ follows an associative interchange mechanism (Fig. 14). An additional DMSO molecule is only weakly bound mainly via electrostatic interactions, with a distance of over 5.5 Å. It does not coordinate to the lithium cation. The computed gas-phase binding energy for the fifth DMSO molecule in $[\text{Li}(\text{DMSO})_4(\text{DMSO})]^+$ is $7.6 \text{ kcal mol}^{-1}$. As observed in similar cases before, this binding energy vanishes when the influence of the bulk solvent is taken into account (B3LYP (CPCM)/6-311+G^{**}//B3LYP/6-311+G^{**}).

Mechanistic details are very similar for DMSO and water exchange. The reaction proceeds through a distorted trigonal bipyramidal reactive intermediate $[\text{Li}(\text{DMSO})_5]^+$ that is reached via a late transition state. The enthalpy profile (see Fig. 13) is in line with the experimentally observed very fast exchange process. The five-coordinate intermediate is computed to be $7.9 \text{ kcal mol}^{-1}$ less stable than $[\text{Li}(\text{DMSO})_4]^+$ and free DMSO, while an overall activation barrier of only $8.4 \text{ kcal mol}^{-1}$ is computed. Obviously,

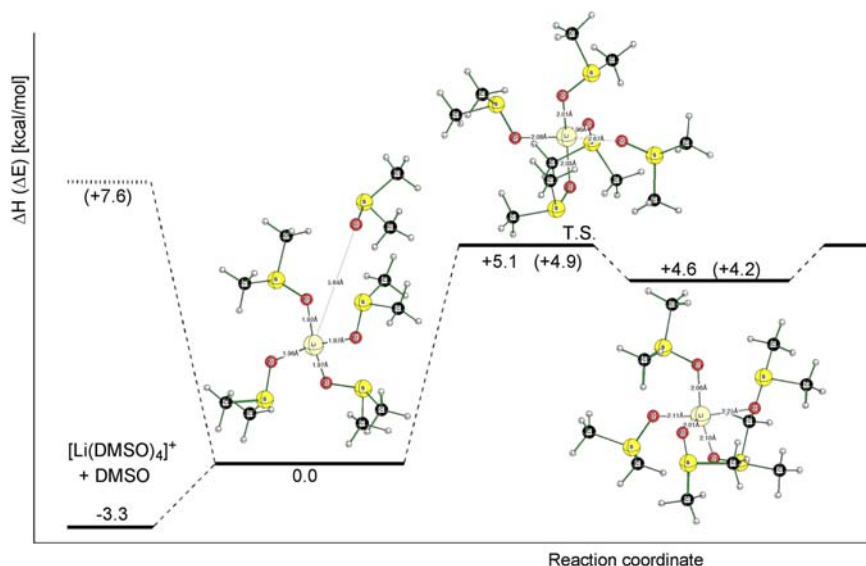


FIG. 13. Reaction profile for DMSO exchange on solvated Li^+ ; enthalpy: B3LYP(CPCM)/6-311+ G^{**} including thermodynamic contributions at B3LYP/LANL2DZp; gas-phase energy values: B3LYP/6-311+ G^{**} including ΔZPE at B3LYP/LANL2DZp, in parentheses; T.S. = transition state (92).

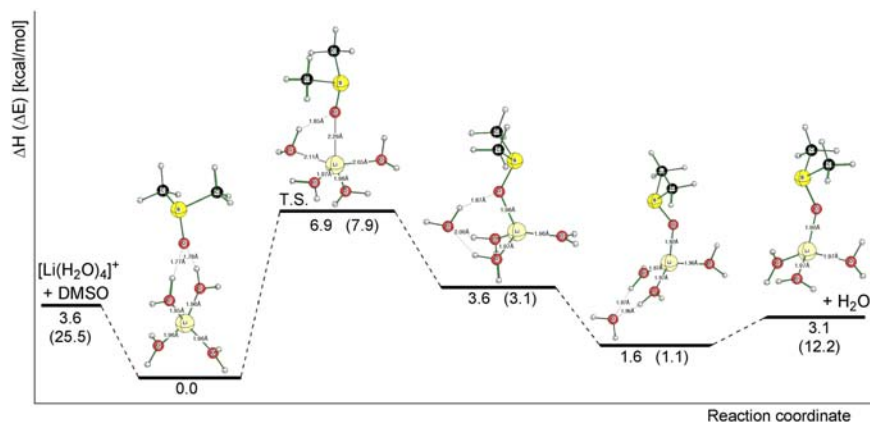


FIG. 14. Reaction profile (enthalpy: B3LYP(CPCM)/6-311+ G^{**} , including thermodynamic contributions at B3LYP/6-311+ G^{**} ; gas phase energy values: B3LYP/6-311+ G^{**} including ΔZPE in parentheses) for DMSO/water exchange around $[\text{Li}(\text{H}_2\text{O})_4]^+$ (92).

the intermediate will be extremely short-lived, its barrier toward loss of DMSO being only $0.5 \text{ kcal mol}^{-1}$.

At a first glance the energy difference is smaller than the accuracy of the computational method and would thus be meaningless. While the value of the (small) barrier may vary, the shape of the potential energy surface usually does not. In the case of the DMSO exchange, zero point energy and thermodynamic corrections do not change the energetic order of the structures. Inclusion of entropy contributions (we judge them to be sufficiently accurate in view of the similarity of the computed structures for intermediate and transition states) even stabilizes the intermediate: a free energy barrier of $1.8 \text{ kcal mol}^{-1}$ was computed.

We also investigated the mechanism of the exchange of a water ligand by DMSO in detail (Fig. 14). In the precursor the entering DMSO molecule is weakly bound by hydrogen bonds to two water ligands. The two water ligands, hydrogen bonded to the DMSO oxygen atom, move to axial positions while DMSO approaches the lithium cation. In the distorted trigonal bipyramidal transition structure, one hydrogen bond to the leaving water molecule remains, enabling *cis* stereochemistry in this reaction. The gas phase activation barrier lies $7.9 \text{ kcal mol}^{-1}$ above that of the precursor complex. From this energetic data we can conclude that the reaction will be very fast, and from the absence of a stable five-coordinate species we can conclude that the reaction proceeds through an associative interchange (I_a) mechanism. The reaction proceeds stepwise to a complex in which the leaving water molecule is completely part of an outer solvent sphere.

Experimentally Merbach *et al.* (67) found for the DMSO exchange at $[\text{Be}(\text{DMSO})_4]^{2+}$ (144) an associative interchange or an associative mechanism by NMR studies. Up to now no adequate computational study investigated the mechanism in detail, but exploratory calculations suggest an associative interchange mechanism (161).

D. HYDROGEN CYANIDE AND ACETONITRILE EXCHANGE

In contrast to the strong and bulky DMSO nucleophile, the small HCN molecule is a weak donor compared to water (*vide supra*) and can be easily replaced by H_2O . HCN on the one hand is a water-like solvent, without much application because of its toxicity. On the other hand, it is employed as a simplified model to perform quantum chemical studies on CH_3CN . Our calculations corroborated the experimental work that the first coordination sphere around Li^+ consists of four tightly bound CH_3CN and HCN

activation barrier is only $5.5 \text{ kcal mol}^{-1}$, while the five-coordinate intermediate is $4.6 \text{ kcal mol}^{-1}$ less stable than the precursor complex. The very low barrier toward dissociation of only $0.9 \text{ kcal mol}^{-1}$ means that the five-coordinate intermediate will be very short-lived. The evaluation of the energy at the MP2(full)/6-311+G**//B3LYP/6-311+G** level shows only minor changes in the computed energies, except for the intermediate $[\text{Li}(\text{L})_4(\text{L})]^+$ (see Table IV). This can be attributed to an incorrectly balanced description of the metal-ligand and hydrogen-bond strengths by DFT as observed earlier by Rotzinger and others in studies on water exchange reactions (157,162). Inclusion of solvent effects at the B3LYP(CPCM)/6-311+G**//B3LYP/6-311+G** and B3LYP(IPCM)/6-311+G**//B3LYP/6-311+G** level clearly favors a four-coordinate structure with NCH in the second coordination sphere or uncoordinated. Therefore, the transition state is also stabilized since the entering HCN molecule can best be addressed as uncoordinated (see Table IV and Fig. 15). Consequently, the transition state is modeled to be too stable in the B3LYP(IPCM)/6-311+G**//B3LYP/6-311+G** approximation, no matter if acetonitrile or water is modeled by the IPCM formalism.

In trigonal bipyramidal $[\text{Li}(\text{NCH})_5]^+$, the axial Li–N distances are $0.11 \text{ \AA}$ longer than the equatorial ones, which may be taken as a clear indication that one of the axial ligands will be expelled. The energy profile for the exchange of acetonitrile on $[\text{Li}(\text{NCCH}_3)_4]^+$ shows the same topology; only the energy differences are somewhat smaller. We attribute this difference to stabilization effects caused by the methyl group and to the absence of hydrogen bonding; a detailed investigation was

TABLE IV

CALCULATED RELATIVE ENERGIES (IN kcal mol^{-1}) FOR THE EXCHANGE OF L AT $[\text{Li}(\text{L})_4]^+$ (L = HCN AND CH_3CN) (93)

	$[\text{Li}(\text{L})_4]^+ + \text{L}$	$[\text{Li}(\text{L})_4 \cdots (\text{L})]^+$	ts	$[\text{Li}(\text{L})_4(\text{L})]^+$
L = HCN				
B3LYP	8.9	0.0	5.5	4.6
MP2(full)	9.5	0.0	3.2	0.5
IPCM	-2.8	0.0	-2.6	3.2
CPCM	-0.4	0.0	1.8	1.4
L = H_3CCN				
B3LYP	6.1	0.0	4.1	3.7

B3LYP: B3LYP/6-311+G**//B3LYP/6-311+G**; MP2(full): MP2(full)/6-311+G**//B3LYP/6-311+G**; IPCM: B3LYP(IPCM: Acetonitrile)/6-311+G**//B3LYP/6-311+G**; IPCM: B3LYP(CPCM: Water)/6-311+G**//B3LYP/6-311+G**.

prevented as no local minimum could be found for $[\text{Li}(\text{NCCH}_3)_4(\text{NCCH}_3)]^+$ owing to the rotation of the methyl groups, and IPCM calculations already show well-documented problems to reach a converged energy (163,164).

Since under actual experimental conditions water can never be excluded, and because of the importance of solvent mixtures in technical and laboratory applications, we modeled by way of example the HCN/ H_2O exchange process for $[\text{Li}(\text{NCH})_4]^+$ to $[\text{Li}(\text{NCH})_3(\text{H}_2\text{O})]^+$. HCN can again be considered as a solvent itself and as a model for acetonitrile.

From experiments as well as from the Gutmann donor number for acetonitrile (only 50% of that for water), it is well known that addition of water to solutions of lithium ions in acetonitrile leads to the formation of a water coordinated Li^+ ion. This can be reproduced for HCN as modeled by quantum chemical calculations (RB3LYP/6-311+ G^{**}).

Including solvent effects employing B3LYP(IPCM)/6-311+ G^{**} //B3LYP/6-311+ G^{**} energy calculations, significantly increases the reaction energies, independent of whether water or acetonitrile is selected as solvent (see Table V) (93).

For the first substitution process, we investigated the mechanism in detail (see Fig. 16). The additional solvent (water) molecule does not coordinate to the Li^+ ion; it forms a second coordination sphere and is bound by one linear hydrogen bond (1.88 Å) to $[\text{Li}(\text{NCH})_4]^+$, forming $[\text{Li}(\text{NCH})_4 \cdots (\text{OH}_2)]^+$, which is $9.5 \text{ kcal mol}^{-1}$ lower in energy. Here again, the entering solvent, now water, approaches the Li^+ ion directly through a face of the $[\text{Li}(\text{NCH})_4]^+$ tetrahedron and pushes three coordinated HCN molecules away into the trigonal plane. From the movement of the water molecule

TABLE V

CALCULATED ENERGIES IN kcal mol^{-1} FOR THE SUBSEQUENT REACTIONS OF $[\text{Li}(\text{NCH})_4]^+$ WITH WATER (93)

B3LYP(IPCM)/6-311+ G^{**} //B3LYP/ 6-311+ G^{**}	Gas phase	IPCM: acetonitrile	IPCM: water
$[\text{Li}(\text{NCH})_4]^+ + \text{H}_2\text{O} \rightarrow$ $[\text{Li}(\text{H}_2\text{O})(\text{NCH})_3]^+ + \text{NCH}$	-0.5	+1.7	+1.6
$[\text{Li}(\text{H}_2\text{O})(\text{NCH})_3]^+ + \text{H}_2\text{O} \rightarrow$ $[\text{Li}(\text{H}_2\text{O})_2(\text{NCH})_2]^+ + \text{NCH}$	-0.5	-1.4	-1.6
$[\text{Li}(\text{H}_2\text{O})_2(\text{NCH})_2]^+ + \text{H}_2\text{O} \rightarrow$ $[\text{Li}(\text{H}_2\text{O})_3(\text{NCH})]^+ + \text{NCH}$	-0.3	-3.6	-3.9
$[\text{Li}(\text{H}_2\text{O})_3(\text{NCH})]^+ + \text{H}_2\text{O} \rightarrow$ $[\text{Li}(\text{H}_2\text{O})_4]^+ + \text{NCH}$	+0.1	-0.9	-1.2
Σ	-1.3	-4.2	-5.1

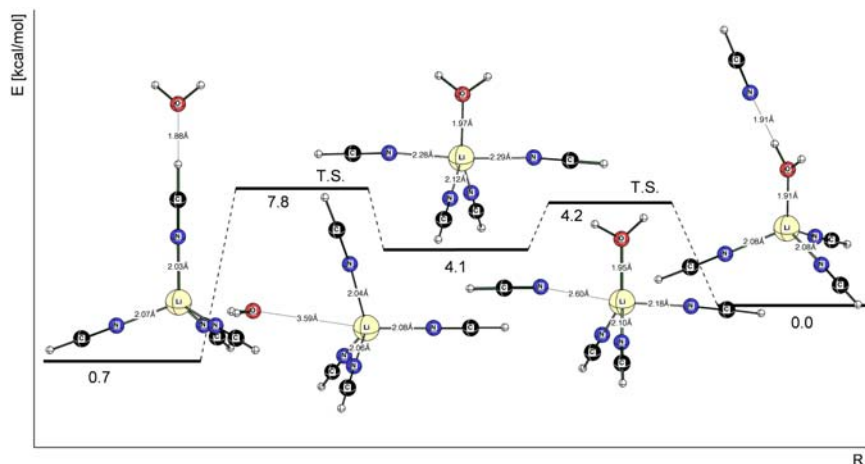


FIG. 16. Energy profile (RB3LYP/6-311+G^{**}) for the HCN/water exchange around [Li(NCH)₄]⁺ (93).

in the 7.1 kcal mol⁻¹ higher transition state than the reactant state, one would expect an intermediate with three HCN molecules in equatorial positions and the fourth one together with H₂O in the axial positions. During the optimization steps the structure rearranged and ended up in an intermediate state 3.7 kcal mol⁻¹ lower in energy, with two HCN molecules in the axial positions and the H₂O molecule together with the two other HCN molecules in the equatorial position. The bond distances for the axial molecules are 0.16 Å longer than for the equatorial HCN ligands, from which it was concluded that one axial molecule would be expelled to reach a four-coordinate product. The activation barrier for the release of an axial HCN molecule is rather low, *viz.*, 0.1 kcal mol⁻¹, which means that the five-coordinate intermediate will be very short-lived. On the assumption that the expelled HCN will form a hydrogen bond with the nearest hydrogen atom, one ends up with [Li(NCH)₃(H₂O)⋯(NCH)]⁺ being 4.2 kcal mol⁻¹ lower in energy than the transition state. A total of 9.7 kcal mol⁻¹ is required to break this hydrogen bond (Table VI) (93).

While the MP2(full)/6-311+G^{**}//B3LYP/6-311+G^{**} energies show typical discrepancies, the application of the IPCM- and CPCM-solvent models dramatically lowers the energy for the intermediate and the transition states. The barriers of 2.8 and 1.8 kcal mol⁻¹ corroborate the experimental findings for an efficient formation of a water coordinated Li⁺ complex.

In the case of the beryllium dication, we again found an associative interchange (I_a) mechanism for HCN exchange at

TABLE VI

CALCULATED RELATIVE ENERGIES IN kcal mol^{-1} FOR THE EXCHANGE OF $\text{H}_2\text{O}/\text{NCH}$ AT $[\text{Li}(\text{NCH})_4]^+$ (93)

L: HCN; W: H_2O	$[\text{Li}(\text{L})_4 \cdots \text{W}]^+$	ts	$[\text{Li}(\text{L})_2\{\text{W}(\text{L})_2\}]^+$	ts	$[\text{Li}(\text{L})_3(\text{W}) \cdots \text{L}]^+$
B3LYP	0.7	7.8	4.1	4.2	0.0
MP2(full)	0.9	6.1	0.6	1.2	0.0
IPCM	0.1	2.8	1.3	1.8	0.0
CPCM	0.1	5.6	1.0	1.5	0.0

B3LYP: B3LYP/6-311+G**//B3LYP/6-311+G**; MP2(full): MP2(full)/6-311+G**//B3LYP/6-311+G**; IPCM: B3LYP/IPCM: acetonitrile/6-311+G**//B3LYP/6-311+G**; CPCM: B3LYP/CPCM: water/6-311+G**//B3LYP/6-311+G**.

$[\text{Be}(\text{NCH})_4]^{2+}$ (156). The addition of a fifth HCN molecule to $[\text{Be}(\text{NCH})_4]^{2+}$ (T_d), forming a second coordination sphere, lowers the structure by $12.7 \text{ kcal mol}^{-1}$ in the gas phase (MP2(full)/6-311+G**//B3LYP/6-311+G**: $16.3 \text{ kcal mol}^{-1}$, B3LYP/(IPCM: H_2O)/6-311+G**//B3LYP/6-311+G**: $-4.1 \text{ kcal mol}^{-1}$). The activation barrier for HCN exchange is relatively low with $4.3 \text{ kcal mol}^{-1}$, as the fifth solvent molecule in the C_{3v} symmetric precursor was not bound by a hydrogen bond. As expected, the MP2 (full)/6-311+G**//B3LYP/6-311+G** value of $1.5 \text{ kcal mol}^{-1}$ is too low and the B3LYP/(IPCM: H_2O)/6-311+G**//B3LYP/6-311+G** values of $3.9 \text{ kcal mol}^{-1}$ are again similar. The transition state structure has D_{3h} symmetry. The equatorial HCN–Be bonds ($1.74 \text{ \AA}$) are very similar to the Be bonds in the precursor, whereas the axial entering and leaving ones have a bond length of $2.04 \text{ \AA}$.

Since the precursor and the transition state in the HCN exchange at Be^{2+} have structures of high symmetry, we tested the influence of different substituents on the exchange reaction by variation of H (see Fig. 17) (165). We substituted H by CH_3 , F, Cl, and Br. The incoming fifth ligand in the precursor complex is bound solely by electrostatic interactions and the complexation energy is close to 12 kcal mol^{-1} for all substituents X. The Be–N distances for the incoming ligand are $3.15 \text{ \AA}$ for HCN and $3.6 \text{ \AA}$ in all other cases. Hydrogen bonding, which might be possible for HCN, plays no role.

For $\text{X} = \text{H}$, an activation barrier for the ligand exchange of $4.3 \text{ kcal mol}^{-1}$ was computed, while barriers of 6.5 – $7.5 \text{ kcal mol}^{-1}$ were computed for $\text{X} = \text{CH}_3$, F, Cl, and Br. This shows that the nature of the substituent, electron withdrawing in the case of halogens, or electron donating such as the methyl group, has no influence in this case.

To test this result, we extended our initial water exchange study on $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$ to the exchange of CH_3OH at $[\text{Be}(\text{CH}_3\text{OH})_4]^{2+}$

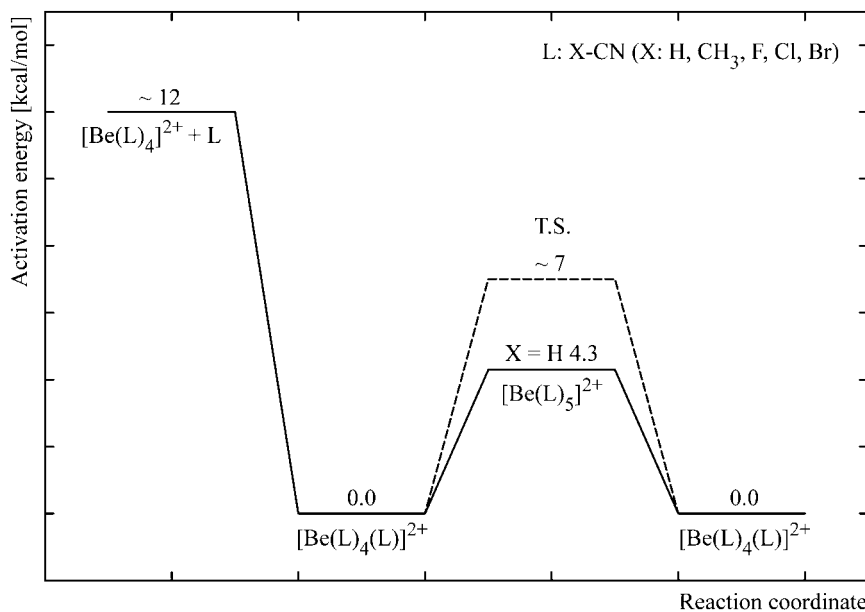


FIG. 17. Energy profile (RB3LYP/6-311+G**) for the ligand exchange around $[\text{Be}(\text{X-CN})_4]^{2+}$ (X: H, CH_3 , F, Cl, Br) (165).

and of CH_3OCH_3 at $[\text{Be}(\text{CH}_3\text{OCH}_3)_4]^{2+}$ (165). As in the case of water, the beryllium dication is coordinated by four methanol molecules or four dimethyl ether ligands, and the fifth solvent molecule is bound by electrostatic forces. Of course in the case of H_2O and CH_3OH , additional hydrogen bonding stabilizes the precursor, as illustrated by the more than double the binding energy of the fifth ligand. As clearly depicted in Fig. 18, the electron donating effect of the methyl group strengthens the Be–O bonds in the methanol and in the dimethyl ether complexes and raises the activation barrier for solvent exchange. However, the higher steric demand of dimethyl ether counteracts this to some degree. As a result, the transition state structure of $[\text{Be}(\text{CH}_3\text{OCH}_3)_5]^{2+}$ has less associative character: the bonds for the entering and leaving CH_3OCH_3 molecules (3.1 Å) are more than 1 Å longer and thus weaker than in the case of water or methanol.

E. EXCHANGE OF OTHER LIGANDS: THE INFLUENCE OF HYBRIDIZATION

Besides water and the water-like solvents NH_3 , HCN , and DMSO , we tested some other molecules that are potential or are already applied as solvents, or may serve as models. We tested

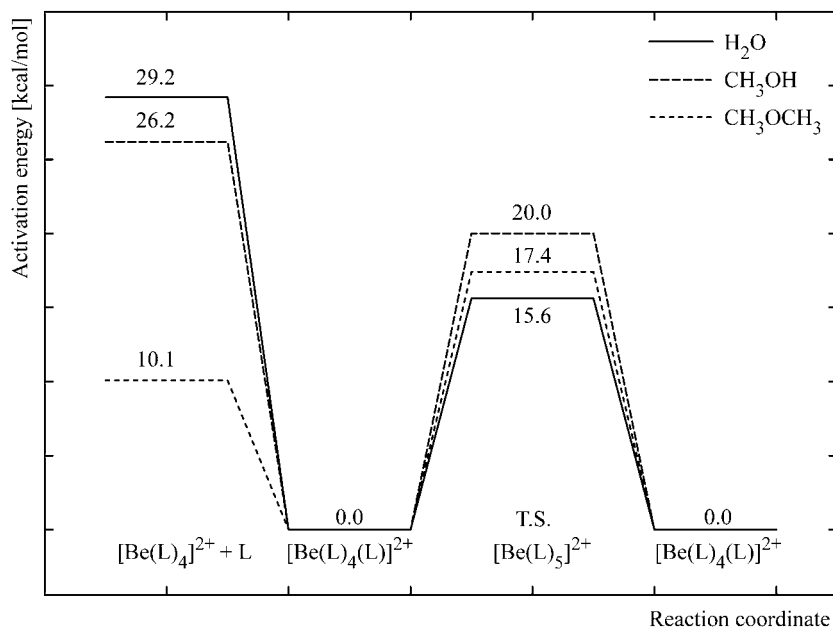


FIG. 18. Energy profile (RB3LYP/6-311+G**) for the ligand exchange around $[\text{Be}(\text{L})_4]^{2+}$ ($\text{L} = \text{H}_2\text{O}, \text{CH}_3\text{OH}, \text{CH}_3\text{OCH}_3$) (165).

the smallest carbonyl compound, formaldehyde, carbon dioxide (166), used as solvent in the supercritical phase, molecular nitrogen, and carbon monoxide. For CO, both coordination modes to carbon and to oxygen were investigated. This series of ligands, together with the solvents already discussed (*vide supra*), allow us to compare the coordinating properties of oxygen, nitrogen, and carbon donor atoms, as well as to study the influence of the chemical environment of the coordinating atom in terms of the differences between sp^3 , sp^2 , and sp hybridization around the Be^{2+} .

Starting from the precursor complexes $[\text{Be}(\text{L})_4(\text{L})]^{2+}$, in all cases the calculation showed that $[\text{Be}(\text{L})_5]^{2+}$ is the transition state structure for a ligand exchange proceeding via an interchange type of mechanism. Since no hydrogen bonds are possible between the incoming ligand L and the already beryllium-bound ligands in the case of N_2 , CO_2 , CO, or H_2CO , L is bound to $[\text{Be}(\text{L})_4]^{2+}$ by electrostatic forces only. Bonding is caused by the attractive interaction between the beryllium ion and the dipole of the ligand (i.e., sum of the induced dipole and the dipole moment of the ligand itself). Compared to the hydrogen-bonded cases discussed above, the interaction energy

is much weaker. Inclusion of the influence of the bulk solvent strongly reduces the interaction energies. The structures of the precursor complexes $[\text{Be}(\text{L})_4\text{L}]^{2+}$, where $\text{L} = \text{N}_2$, CO (oxygen bound to Be^{2+}), and OC (carbon bound to Be^{2+}), already show the characteristics of the corresponding transition state structures, *viz.*, the Be-L distance for the ligand that is *trans* to the incoming ligand is elongated by 0.02 to 0.03 Å. No lengthening is apparent for $\text{L} = \text{CO}_2$, but the picture is blurred here by the lack of symmetry in the complex. Except for $[\text{Be}(\text{CO}_2)_5]^{2+}$, the transition state structures are highly symmetrical. The Be-L bonds to the incoming and the leaving ligand are between 0.23 Å and 0.40 Å longer than the other beryllium ligand bonds. Such elongations are typical for an associative interchange mechanism. The molecular volumes support this, since except for $\text{L} = \text{N}_2$, the transition state structures $[\text{Be}(\text{L})_5]^{2+}$ are more compact than the precursor complexes $[\text{Be}(\text{L})_4\text{L}]^{2+}$. The activation barriers are relatively low, indicating fast exchange reactions.

Formaldehyde, a working model for acetone, is exceptional. The precursor complex is almost 20 kcal mol^{-1} more stable than $[\text{Be}(\text{formaldehyde})_4]^{2+}$ and an unbound formaldehyde molecule, despite the very long beryllium oxygen distance of 3.74 Å (156). However, the fifth formaldehyde molecule is bound indirectly. Instead of a direct oxygen- Be^{2+} interaction, the fifth molecule binds to the carbonyl carbon of one of the coordinated formaldehyde molecules. As the high positive charge of the beryllium cation polarizes the $\text{C}=\text{O}$ bond of the coordinated formaldehyde molecule, the positive charge on the carbonyl carbon is increased, which in turn leads to an attraction of the oxygen of the incoming formaldehyde molecule. As a consequence, the computed O-C distance is only 2.20 Å, while the O-Be bond is shortened by 0.06 Å. Unfortunately, a flawless estimate of the energetic preference was not possible without leading to a biased activation barrier. The unusual structure of the precursor complex, however, has no influence on the course of the exchange reaction, which proceeds via a similar transition structure as found for the other ligands discussed here.

Comparison of the calculated activation energies enables to answer the question – which factors determine the height of the activation barrier for exchange on Be^{2+} ? Little, if any, influence comes from the coordinating donor element. In the case of oxygen donor ligands, the computed barriers vary between $2.6 \text{ kcal mol}^{-1}$ and $15.6 \text{ kcal mol}^{-1}$, while a range from $1.7 \text{ kcal mol}^{-1}$ to $18.8 \text{ kcal mol}^{-1}$ is obtained for nitrogen donor ligands (see Table VII).

A detailed comparison of the computed activation barriers (Table VII) for all solvents considered, and establishment of a

TABLE VII

SUMMARY OF THE RELATIVE ENERGIES AND MOLECULAR VOLUME CHANGES ADDRESSED IN
Fig. 19 (156)

	P.G.	B3LYP (kcal mol ⁻¹)	MP2(full) (kcal mol ⁻¹)	IPCM (kcal mol ⁻¹)	Δ Volume (Å ³)
[Be(H ₂ O) ₄] ²⁺ +H ₂ O	C ₁	+29.2	+29.3	+3.8	+2.7
[Be(H ₂ O) ₄ H ₂ O] ²⁺	C ₂	0.0	0.0	0.0	0.0
T.S. [Be(H ₂ O) ₅] ²⁺	C ₂	+15.6	+12.6	+9.8	-4.5
[Be(NH ₃) ₄] ²⁺ +NH ₃	T _d	+21.2	+21.8	+1.9	+7.3
[Be(NH ₃) ₄ NH ₃] ²⁺	C ₁	0.0	0.0	0.0	0.0
T.S. [Be(NH ₃) ₅] ²⁺	C _{3h}	+18.8	+15.8	+17.3	+3.2
[Be(OCH ₂) ₄] ²⁺ +OCH ₂	C ₁	+19.8	+19.3	+3.9	+10.2
[Be(OCH ₂) ₄ OCH ₂] ²⁺	C ₁	0.0	0.0	0.0	0.0
T.S. [Be(OCH ₂) ₅] ²⁺	C ₁	+10.1	+3.3	+9.8	+4.6
[Be(CO ₂) ₄] ²⁺ +CO ₂	C ₁	+8.2	+11.2	+1.2	-2.5
[Be(CO ₂) ₄ CO ₂] ²⁺	C ₁	0.0	0.0	0.0	0.0
T.S. [Be(CO ₂) ₅] ²⁺	C ₁	+7.6	+5.9	-0.7	-7.7
[Be(NCH) ₄] ²⁺ +HCN	T _d	+12.7	+16.3	-4.1	-2.6
[Be(NCH) ₄ NCH] ²⁺	C _{3v}	0.0	0.0	0.0	0.0
T.S. [Be(NCH) ₅] ²⁺	D _{3h}	+4.3	+1.5	+3.9	-3.7
[Be(N ₂) ₄] ²⁺ +N ₂	T _d	+5.5	+8.8	-3.1	+4.5
[Be(N ₂) ₄ N ₂] ²⁺	C _{3v}	0.0	0.0	0.0	0.0
T.S. [Be(N ₂) ₅] ²⁺	D _{3h}	+1.7	-0.3	+9.9	0.0
[Be(OC) ₄] ²⁺ +CO	T _d	+4.7	+6.2	-15.7	-3.2
[Be(OC) ₄ OC] ²⁺	C _{3v}	0.0	0.0	0.0	0.0
T.S. [Be(OC) ₅] ²⁺	D _{3h}	+2.6	+0.3	-0.7	-5.3
[Be(CO) ₄] ²⁺ +CO	T _d	+7.5	+10.5	-11.5	+0.5
[Be(CO) ₄ CO] ²⁺	C _{3v}	0.0	0.0	0.0	0.0
T.S. [Be(CO) ₅] ²⁺	D _{3h}	+4.7	+4.3	+3.7	-2.9

P.G., Point group; B3LYP, B3LYP/6-311+G^{**}//B3LYP/6-311+G^{**}; MP2, MP2(full)/6-311+G^{**}//B3LYP/6-311+G^{**}; IPCM, B3LYP(IPCM: H₂O)/6-311+G^{**}//B3LYP/6-311+G^{**}; all energies are corrected for Δ ZPE/B3LYP/6-311+G^{**}; Δ Volume, volume based on B3LYP(IPCM: H₂O)/6-311+G^{**}//B3LYP/6-311+G^{**} calculations.

relationship with the hybridization of the donor atom, leads to an interesting observation. Relatively high activation barriers are calculated for the exchange of water and ammonia on Be²⁺, whereas very low barriers are computed for exchange of hydrogen cyanide, carbon monoxide, and molecular nitrogen. Carbon dioxide and formaldehyde lie in between. This suggests that the activation barrier may be determined by the nature of the lone pair, *viz.*, the hybridization of the coordinating atom (Fig. 19). This hypothesis was verified by NLMO analysis (167).

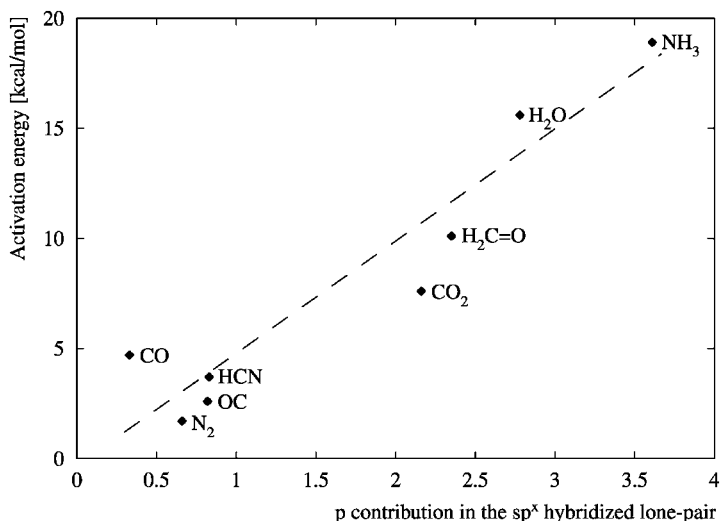


FIG. 19. Relationship between computed activation energies (B3LYP/6-311+G^{**}) and p-orbital contribution in the NLMO hybridization of the lone pairs on the coordinating atoms (156).

Formally, the lone pairs on molecular nitrogen, hydrogen cyanide, and carbon monoxide are sp hybrid orbitals, whereas NLMO hybridizations calculated even lower p contributions. Hence, these lone pairs have low directionality, the electron density remains close to the coordinating atom and interaction between the lone pair and the Be^{2+} is comparatively weak. The Be–L bonds are easily disrupted and ligand exchange consequently can proceed with a low activation barrier. A high degree of p character, on the other hand, means that the lone pair is directed toward beryllium, with electron density close to the metal center, and thus well suited for coordination.

F. LIGAND EXCHANGE ON CHELATED BERYLLIUM DICATIONS

In Nature chelating ligands are employed to bind, dissolve, and transport metal cations, e.g., histidine, malate, and phytochelatins are typical chelators used by plants to avoid having toxic free metal ions (168–170). Plieger *et al.* have been interested in selectively binding Be^{2+} to chelate ligands (171). This approach seems encouraging since most of the traditional cryptands like [2.2.2] and related derivatives including topomers do not complex beryllium cations selectively in a facile manner (172–175).

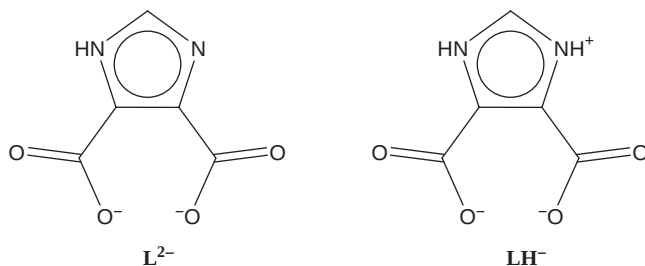


FIG. 20. Chelating ligands 1H-imidazole-4,5-dicarboxylate ($\mathbf{L}^{2-}$) and the N-protonated form 1H-imidazol-3-ium-4,5-dicarboxylate ($\mathbf{LH}^-$) (177).

As a chelating ligand system to bind Be^{2+} , Plieger *et al.* applied 1-methyl-1H-imidazol-3-ium-4,5-dicarboxylate and investigated soluble polymer structures. Motivated by their work, we applied 1H-imidazol-3-ium-4,5-dicarboxylate ($\mathbf{LH}^-$), as an excellent model to test the influence of a different charged ligand system on the water exchange mechanism. In addition the beryllium complex of the unprotonated form 1H-imidazole-4,5-dicarboxylate ($\mathbf{L}^{2-}$) (see Fig. 20) can also be investigated by quantum chemical methods, and the coordination sphere of Be^{2+} is not directly affected. Further, by calculating the NICS (176) values, whether the electron delocalization on the aromatic imidazolium ring is affected by the ligand exchange reaction can be tested. Both resulting model complexes, $[\text{Be}(\text{H}_2\text{O})_2(\mathbf{L})]$ and $[\text{Be}(\text{H}_2\text{O})_2(\mathbf{LH})]^+$, are tetrahedrally coordinated, as the other two coordination sites are occupied by water molecules.

Since Plieger *et al.* (171) were able to obtain a crystal structure for $[\text{Be}(\text{H}_2\text{O})_2(1\text{-methyl-1H-imidazol-3-ium-4,5-dicarboxylate})]^+$, we compared the calculated and measured bond lengths and found satisfactory agreement between experimental and calculated structures. However, there is a clear influence of the strong hydrogen-bonding network in the X-ray structure.

As expected, the structural differences between the monocationic $[\text{Be}(\text{H}_2\text{O})_2(\mathbf{LH})]^+$ species and the neutral species are not very large. Whereas the $\text{Be}-\text{O}_{\text{ligand}}$ bonds in $[\text{Be}(\text{H}_2\text{O})_2(\mathbf{LH})]^+$ are slightly elongated (+0.1 Å) as compared to $[\text{Be}(\text{H}_2\text{O})_2(\mathbf{L})]$, the $\text{Be}-\text{O}_{\text{water}}$ bonds are somewhat shorter (Fig. 21).

In aqueous solution, a metal complex is enclosed by several solvation spheres involving many solvent molecules. As recently shown, for mechanistic studies a reduced coordination sphere is sufficient (157,159), and therefore here inclusion of a third solvent molecule is sufficient. Addition of a third water molecule results in the exothermic formation of $[\text{Be}(\text{H}_2\text{O})_2(\mathbf{L})\cdots\text{H}_2\text{O}]$ and $[\text{Be}(\text{H}_2\text{O})_2(\mathbf{LH})\cdots\text{H}_2\text{O}]^+$, where the third hydrogen-bonded

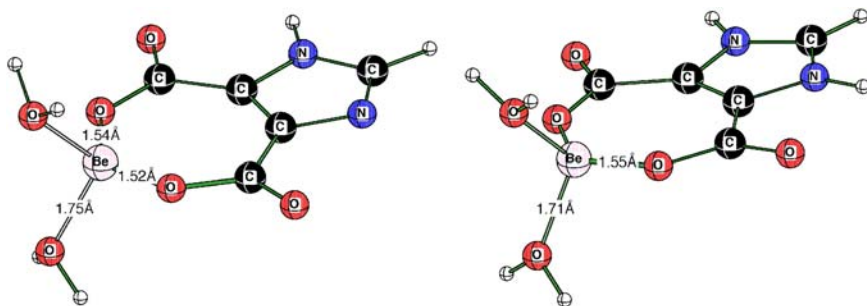


FIG. 21. Calculated (B3LYP/6-311+G^{**}) structures for $[\text{Be}(\text{H}_2\text{O})_2(\text{L})]$ and $[\text{Be}(\text{H}_2\text{O})_2(\text{LH})]^+$ (177).

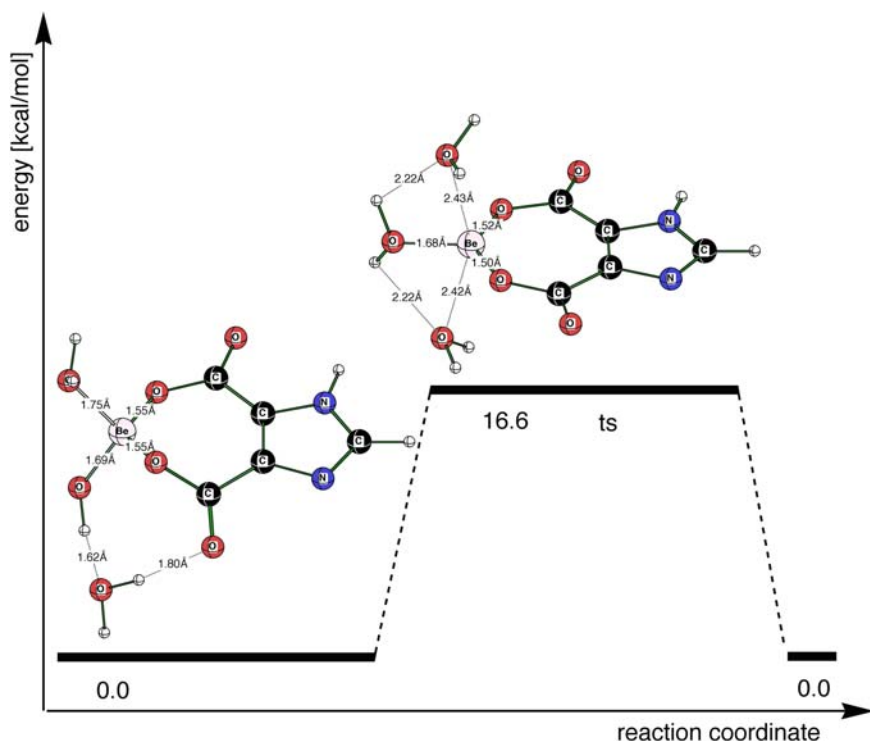


FIG. 22. Calculated (B3LYP/6-311+G^{**}) reaction profile for water exchange on $[\text{Be}(\text{H}_2\text{O})_2(\text{L})]$ (177).

solvent molecule forms part of a second coordination sphere. Whereas two strong hydrogen bonds are formed in $[\text{Be}(\text{H}_2\text{O})_2(\text{L})\cdots\text{H}_2\text{O}]$ (1.62 and 1.80 Å), $[\text{Be}(\text{H}_2\text{O})_2(\text{LH})\cdots\text{H}_2\text{O}]^+$ has only one strong hydrogen bond (1.64 Å) (see Figs. 22 and 23).

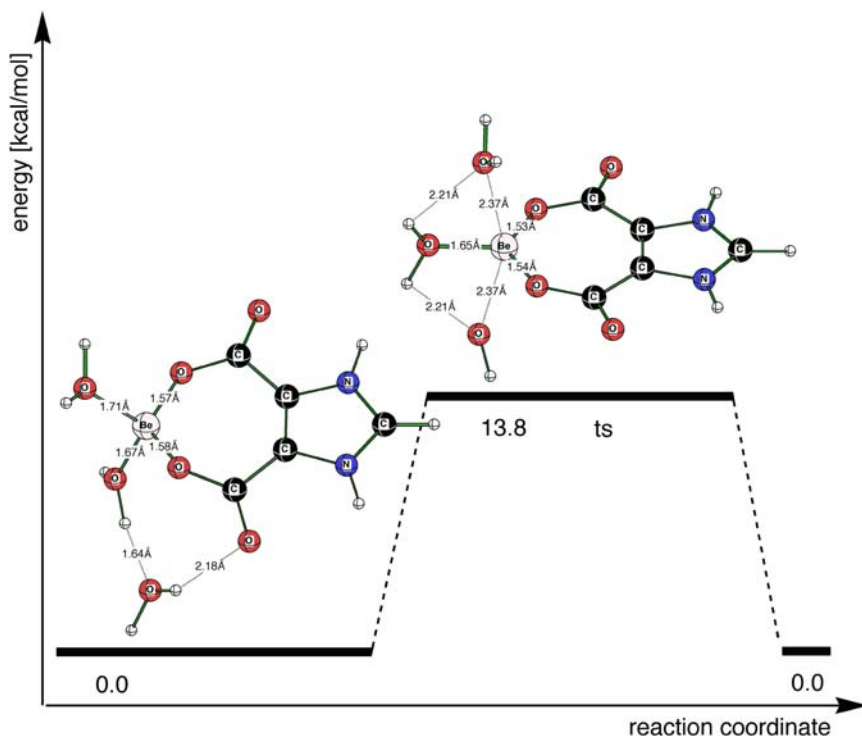


FIG. 23. Calculated (B3LYP/6-311+G^{**}) reaction profile for water exchange on $[\text{Be}(\text{H}_2\text{O})_2(\text{LH})]^+$ (177).

In contrast to the hydrogen bonds formed, the stabilization of the entering water molecule in the neutral species $[\text{Be}(\text{H}_2\text{O})_2(\text{L})\cdots\text{H}_2\text{O}]$ is -17 kcal mol^{-1} , almost 3 kcal mol^{-1} larger than for $[\text{Be}(\text{H}_2\text{O})_2(\text{LH})\cdots\text{H}_2\text{O}]^+$. The effect of the negatively charged ligand systems can be clearly seen: (a) as indicated by the longer $\text{Be}-\text{O}_{\text{water}}$ bonds, the water molecules are more loosely bound compared to $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$ ($1.65\text{ \AA}$); (b) the energy liberated by an additional water molecule to form the precursor species for the water exchange reaction is reduced to around 50% ($-29.2\text{ kcal mol}^{-1}$ for $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$ (156) (Table VIII) and is similar to $-12.2\text{ kcal mol}^{-1}$ for $[\text{Li}(\text{H}_2\text{O})_4]^+$ (90). Inclusion of bulk solvent effects by the CPCM model presents a more realistic picture and the binding energy decreases to nearly zero (see Table VIII). Interestingly, the published X-ray structure has a third water molecule bound to a coordinated water molecule in $[\text{Be}(\text{H}_2\text{O})_2(1\text{-methyl-1H-imidazol-3-ium-4,5-dicarboxylate})]^+$ and is included in the whole network of hydrogen bonds as in solution. The hydrogen bond between this water molecule and

TABLE VIII

RELATIVE ENERGIES (KCAL MOL⁻¹) OF THE STATIONARY POINTS WITHIN THE SOLVENT EXCHANGE REACTION (177)

[kcal mol ⁻¹]	P.G.	B3LYP	MP2	CPCM
[Be(H ₂ O) ₃ (LH)] ⁺ +H ₂ O	C ₁ /C _{2v}	14.4	6.7	0.7
[Be(H ₂ O) ₂ (LH)...H ₂ O] ⁺	C ₁	0.0	0.0	0.0
T.S. [Be(H ₂ O) ₃ (LH)] ⁺	C _s	13.8	13.5	10.5
[Be(H ₂ O) ₂ (L)+H ₂ O	C ₁ /C _{2v}	17.1	19.3	0.4
[Be(H ₂ O) ₂ (L)...H ₂ O]	C ₁	0.0	0.0	0.0
T.S. [Be(H ₂ O) ₃ (L)]	C _s	16.6	16.7	10.3
[Be(H ₂ O) ₄] ²⁺ +H ₂ O	C ₂ /C _{2v}	29.2 (156)	29.3 (156)	3.7 (159)
[Be(H ₂ O) ₄ ...H ₂ O] ²⁺	C ₂	0.0	0.0	0.0
T.S. [Be(H ₂ O) ₅] ²⁺	C ₂	15.6 (156)	12.6 (156)	9.4 (159)

P.G., Point group; B3LYP, B3LYP/6-311+G^{**}//B3LYP/6-311+G^{**}; MP2, MP2(full)/6-311+G^{**}//B3LYP/6-311+G^{**}; CPCM, B3LYP((CPCM: H₂O))/6-311+G^{**}//B3LYP/6-311+G^{**}; all energies are corrected for ΔZPE//B3LYP/6-311+G^{**}.

the coordinated one is 1.68 Å, almost similar to the calculated one.

Our earlier investigated reactions for the four- and five-coordinate Be²⁺ cation (156,159,178) show for both solvent exchange reactions an interchange (I) mechanism. To reach the transition state the hydrogen bonds between the third water molecule and the coordinated water of the precursor complexes [Be(H₂O)₂(**L**)...H₂O] and [Be(H₂O)₂(**LH**)...H₂O]⁺ are broken and the third water molecule enters the first coordination sphere. The Be–O_{water} bond *trans* to the coordinated water ligand is clearly weakened and elongated by *ca.* 0.7 Å. This moderate elongation and the resulting Be–O distances suggest that the exchange process follows an associative interchange mechanism. The calculated barriers for the investigated systems are in the same range as the barrier for water exchange at [Be(H₂O)₄]²⁺ (15.6 kcal mol⁻¹) (*vide supra*), whereas the Be–O_{water} bonds for entering/leaving water in the transition state [Be(H₂O)₅]²⁺ are clearly shorter (2.00 Å) (156,159).

These results illustrate that the activation energies of the investigated four-coordinate systems [Be(H₂O)₂(**L**)...H₂O] and [Be(H₂O)₂(**LH**)...H₂O]⁺ are close in value to the activation barrier for the four-coordinate [Be(H₂O)₄]²⁺ independent of the overall charge. Since [Be(H₂O)₂(**L**)...H₂O], [Be(H₂O)₂(**LH**)...H₂O]⁺, and [Be(H₂O)₄]²⁺, stabilize the third and fifth water molecules in the precursor, respectively, by strong hydrogen bonding and have similar activation energies, the impression is gained that the activation barrier depends more on the

coordination number and stabilization effects of the precursor, than on the charge on the complex.

Testing the aromaticity of the imidazole-based ligands by NICS calculations showed nearly no change throughout the reaction, indicating that the aromaticity of the ligand is not affected. Together with the very similar activation barriers for $[\text{Be}(\text{H}_2\text{O})_2(\text{L})\cdots\text{H}_2\text{O}]$, $[\text{Be}(\text{H}_2\text{O})_2(\text{LH})\cdots\text{H}_2\text{O}]^+$, and $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$, the impression is gained that the aromatic system and the Be^{2+} ions do not influence each other.

G. LIGAND EXCHANGE ON FIVE COORDINATED BERYLLIUM DICATIONS

Beside four-coordinate Be^{2+} structures, a couple of five-coordinate structures are known, e.g., Dehnicke's $[\text{BeCl}(\text{12-crown-4})]^+$ (179). The combination of beryllium cations and different crown ethers is of particular interest, not only because the Nobel Prize was awarded in part for the development of crown ethers (15), but also because crown ethers are common building blocks in all kinds of chemistry (180,181), with a wide range of applications. Since Be^{2+} is the smallest metal ion, the binding modes of Be^{2+} and crown ethers can lead to unexpected structural motifs.

While 12-crown-4 acts as a regular crown ether that tetra-coordinates to beryllium as shown by Neumüller and Dehnicke to form $[\text{BeCl}(\text{12-crown-4})]^+$ (179), 15-crown-5 can only coordinate as a bidentate ligand $[\text{BeCl}_2(\text{15-crown-5})]$. The complex was reinvestigated with the purpose of establishing a new X-ray structure by Dehnicke *et al.* (Fig. 24) (182,183).

From the method of preparation of $[\text{BeCl}(\text{12-crown-4})]^+$ (179), it is known that the Cl^- ligand can be substituted by a solvent molecule. We applied our most common test solvents water and NH_3 to a Be^{2+} cation, where most coordination sites are occupied by a chelating ligand, in this case the crown ether 12-crown-4. In contrast to the tetrahedral $[\text{Be}(\text{solvent})_4]^{2+}$ solvated complexes, the precursor complexes $[\text{Be}(\text{solvent})(\text{12-crown-4})]^{2+}$ are quadratic pyramidal, where four oxygen donor atoms of the crown ether form the quadratic basis, while Cl^- or a coordinating solvent molecule occupies the apical position. Addition of one water or ammonia molecule to $[\text{Be}(\text{12-crown-4})]^{2+}$ is exothermic (see Table IX).

Addition of a second water or ammonia molecule results in the formation of $[\text{Be}(\text{solvent})(\text{12-crown-4})\cdots(\text{solvent})]^{2+}$, where the second solvent molecule, is regarded as present in the second solvation sphere and is very loosely attached ($[\text{Be}(\text{H}_2\text{O})(\text{12-crown-4})\cdots(\text{H}_2\text{O})]^{2+}$: 3.71 Å; $[\text{Be}(\text{NH}_3)(\text{12-crown-4})\cdots(\text{NH}_3)]^{2+}$: 5.06 Å)

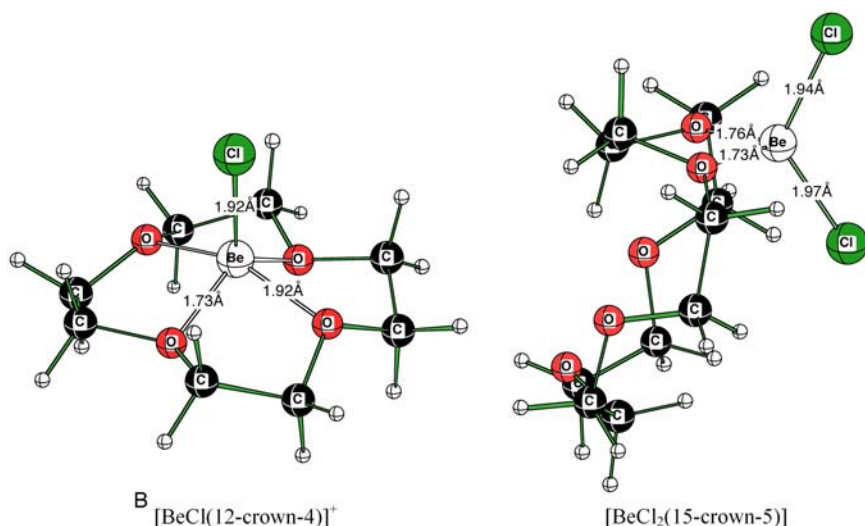


FIG. 24. Calculated (B3LYP/6-311+G^{**}) structure of crown ether Be²⁺ complexes (178,182).

TABLE IX

CALCULATED ENERGIES FOR THE ADDITION OF A SOLVENT MOLECULE TO $[\text{BeCl}(\text{12-crown-4})]^+$ (178)

kcal mol ⁻¹	P.G.	B3LYP	MP2	CPCM
$[\text{Be}(\text{12-crown-4})]^{2+} + \text{H}_2\text{O}$	D_2/C_{2v}	-25.3	-33.0	-14.9
$[\text{Be}(\text{12-crown-4})]^{2+} + \text{NH}_3$	D_2/C_{3v}	-31.5	-40.0	-21.0

P.G., Point group; B3LYP, B3LYP/6-311+G^{**}//B3LYP/6-311+G^{**}; MP2, MP2(full)/6-311+G^{**}//B3LYP/6-311+G^{**}; CPCM, B3LYP(CPCM: H₂O)/6-311+G^{**}//B3LYP/6-311+G^{**}; all energies are corrected for $\Delta\text{ZPE}/\text{B3LYP}/6\text{-}311\text{+G}^{**}$.

(Figs. 25 and 26) (178). The negligible elongation of the bond between Be²⁺ and the coordinated solvent molecule compared to the precursor $[\text{Be}(\text{solvent})(\text{12-crown-4})]^{2+}$, and the reduced stabilization of the second solvent molecule in the second coordination sphere, originating from the missing hydrogen bonds, clearly show the weakness of this interaction (Table X). Inclusion of the influence of bulk solvent by means of the CPCM model led to slightly endothermic values (Table X).

Both solvent exchange reactions show an interchange (I) mechanism (Figs. 25 and 26). Therefore, the quadratic pyramidal ground state with a second solvent molecule in the second

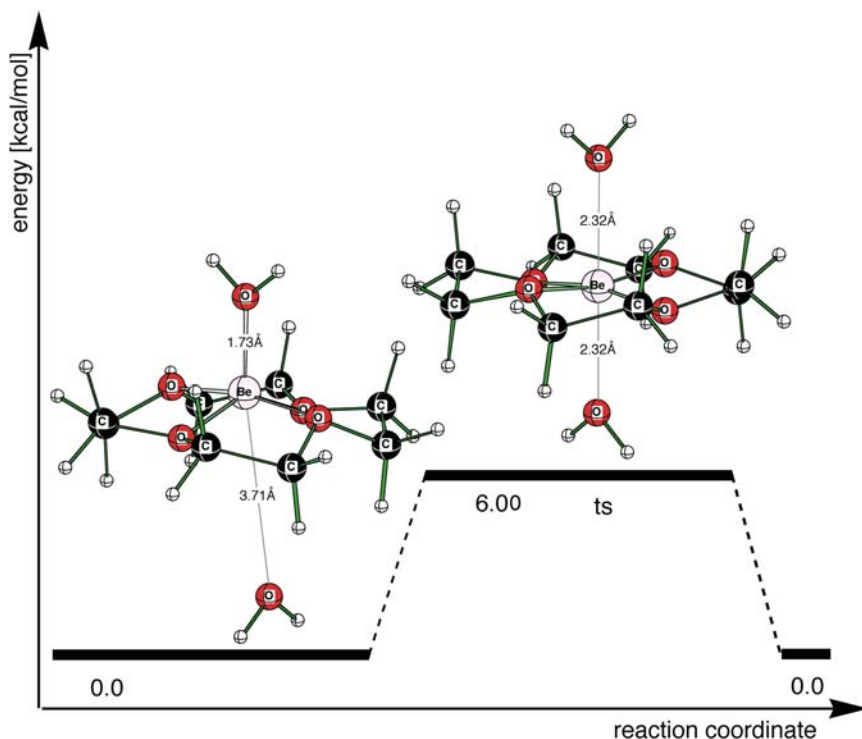


FIG. 25. Calculated (B3LYP/6-311+G^{**}) reaction profile for water exchange on $[\text{Be}(\text{H}_2\text{O})(12\text{-crown-4})]^{2+}$ (178).

coordination sphere is reorganized to form the C_2 symmetric, chiral transition state. The Be^{2+} cation becomes the center of the 12-crown-4 ligand whereas the second solvent molecule enters the first coordination sphere and the bond of the Be-coordinated solvent is weakened and elongated by *ca.* 0.7 Å (Figs. 25 and 26). As in the case of the four-coordinate Be^{2+} ion, the five-coordinate species shows the same type of mechanism. Of course the activation energies for $[\text{Be}(\text{H}_2\text{O})(12\text{-crown-4})]^{2+}$ and $[\text{Be}(\text{NH}_3)(12\text{-crown-4})]^{2+}$ are clearly smaller due to the absence of extra stabilization by hydrogen bonding in the precursor complex. Whereas for the water exchange the activation energy was reduced to 6 kcal mol⁻¹ (15.6 kcal mol⁻¹ for $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$), the barrier was only lowered by 3.5 kcal mol⁻¹ to 15.3 kcal mol⁻¹ for the exchange of ammonia. This energetic difference can be attributed (*vide supra*) to the larger p-orbital contribution from the lone

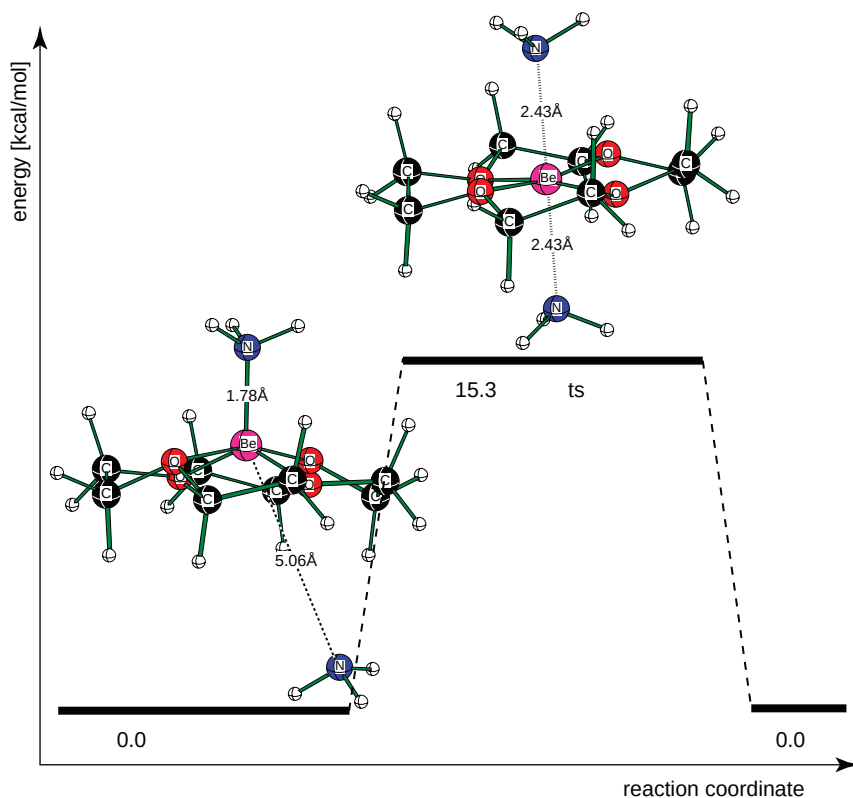


FIG. 26. Calculated (B3LYP/6-311+G^{**}) reaction profile for ammonia exchange on $[\text{Be}(\text{NH}_3)(12\text{-crown-4})]^{2+}$ (178).

TABLE X

RELATIVE ENERGIES (KCAL.MOL⁻¹) OF THE STATIONARY POINTS WITHIN THE SOLVENT EXCHANGE REACTION (178)

kcal mol ⁻¹	P.G.	B3LYP	MP2	CPCM
$[\text{Be}(\text{H}_2\text{O})(12\text{-crown-4})]^{2+} + \text{H}_2\text{O}$	C_1/C_{2v}	+9.3	+12.6	-1.3
$[\text{Be}(\text{H}_2\text{O})(12\text{-crown-4}) \cdots \text{H}_2\text{O}]^{2+}$	C_1	0.0	0.0	0.0
T.S. $[\text{Be}(\text{H}_2\text{O})_2(12\text{-crown-4})]^{2+}$	C_2	+6.0	+3.6	+7.3
$[\text{Be}(\text{NH}_3)(12\text{-crown-4})]^{2+} + \text{NH}_3$	C_1/C_{3v}	+10.0	+11.5	-0.9
$[\text{Be}(\text{NH}_3)(12\text{-crown-4}) \cdots \text{NH}_3]^{2+}$	C_1	0.0	0.0	0.0
T.S. $[\text{Be}(\text{NH}_3)_2(12\text{-crown-4})]^{2+}$	C_2	+15.3	+10.4	+13.5

P.G., Point group; B3LYP, B3LYP/6-311+G^{**}//B3LYP/6-311+G^{**}; MP2, MP2(full)/6-311+G^{**}//B3LYP/6-311+G^{**}; CPCM, B3LYP((CPCM: H₂O))/6-311+G^{**}//B3LYP/6-311+G^{**}; all energies are corrected for ΔZPE /B3LYP/6-311+G^{**}.

pair of the nitrogen donor than from the lone pair of the oxygen donor (156).

IV. Conclusion

Quantum chemical methods are well established, accepted and of high potential for investigation of inorganic reaction mechanisms, especially if they can be applied as a fruitful interplay between theory and experiment. In the case of solvent exchange reactions their major deficiency is the limited possibility of including solvent effects. We demonstrated that with recent DFT- and *ab initio* methods, reaction mechanisms can be successfully explored. To obtain an idea about solvent effects, implicit solvent models can be used in the calculations, when their limitations are kept in mind. In future, more powerful computers will be available and will allow more sophisticated calculations to be performed. This will enable scientists to treat solvent molecules explicitly by *ab initio* molecular dynamics (e.g., Car-Parrinello simulations). The application of such methods will in turn complement the quantum chemical toolbox for the exploration of solvent and ligand exchange reactions.

ACKNOWLEDGMENTS

This work was supported by the EC TMR network HPRN-CT-2000-19 ("Solvation Dynamics and Ionic Mobility in Conventional and Polymer Solvents") and the Deutsche Forschungsgemeinschaft. We would like to thank Dr. Lothar Helm (EPFL Lausanne) for introducing Ewa Pasgreta to *NMRICMA* 2.7, Dr. Nico van Eikema Hommes for helpful discussions, Dr. Achim Zahl for measuring innumerable NMR spectra, Sonja Gubo (BSc) and Michael Galle for their contributions in particular parts of the work, Prof. Tim Clark for hosting this work in the CCC and the Regionales Rechenzentrum Erlangen (RRZE) for a generous allotment of computer time.

REFERENCES

1. (a) Lincoln, S. F. *Coord. Chem. Res.* **1997**, *166*, 255–289. (b) Helm, L.; Merbach, A. E. *Chem. Rev.* **2005**, *105*, 1923–1960.
2. Bauer, R. *Chem. uns. Zeit* **1985**, *19*, 167–173.
3. Cade, J. *Med. J. Aus.* **1949**, *36*, 349–352.
4. Birch, N. J. *Chem. Rev.* **1999**, *99*, 2659–2682.

5. Müller-Oerlinghausen, B.; Greil, W.; Berghöfer, A. (Eds.) *“Die Lithiumtherapie”*; Springer: Berlin, **1997**.
6. Deberitz, J.; Boche, G. *Chem. uns. Zeit* **2003**, *37*, 258–266.
7. Kumberger, O.; Schmidbaur, H. *Chem. uns. Zeit* **1993**, *27*, 310–316.
8. Newmann, L. S. *Chem. Eng. News* **2003**, *81*, 83.
9. Schmidkunz, H. *Unterricht Chem.* **2007**, *18*, 42–43.
10. Illner, P.; Puchta, R.; Schiessl, W. *Unterricht Chem.* **2007**, *18*, 4–8.
11. Dehnicke, K.; Neumüller, B. *Anorg. Allg. Chem.* **2008**, *634*, 2703–2728.
12. Suzuki, Y.; Momose, A.; Sugiyama, H. *J. Synchrotron Radiat.* **1998**, *5*, 596–599.
13. Wu, G.; Huang, M. *Chem. Rev.* **2006**, *106*, 2596–2616.
14. Garst, J. F.; Soriagab, M. P. *Coord. Chem. Rev.* **2004**, *248*, 623–652.
15. (a) Pedersen, C. J. *Angew. Chem.* **1988**, *100*, 1053–1059. (b) Pedersen, C. J. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1021–1027.
16. (a) Lehn, J.-M. *Angew. Chem.* **1988**, *100*, 91–116. (b) Lehn, J.-M. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89–112.
17. (a) Cram, D. J. *Angew. Chem.* **1988**, *100*, 1041–1052. (b) Cram, D. J. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1009–1020.
18. Puchta, R.; Seitz, V.; van Eikema Hommes, N. J. R.; Saalfrank, R. W. *J. Mol. Model.* **2000**, *6*, 126–132.
19. Saalfrank, R. W.; Dresel, A.; Seitz, V.; Trummer, S.; Hampel, F.; Teichert, M.; Stalke, D.; Stadler, C.; Daub, J.; Schünemann, V.; Trautwein, A. X. *Chem. Eur. J.* **1997**, *3*, 2058–2062.
20. Saalfrank, R. W.; Seitz, V.; Caulder, D. L.; Raymond, K. N.; Teichert, M.; Stalke, D. *Eur. J. Inorg. Chem.* **1998**, *9*, 1313–1317.
21. Saalfrank, R. W.; Seitz, V.; Heinemann, F. W.; Göbel, C.; Herbst-Irmer, R. *J. Chem. Soc. Dalton Trans.* **2001**, *5*, 599–603.
22. Saalfrank, R. W.; Scheurer, A.; Puchta, R.; Hampel, F.; Maid, H.; Heinemann, F. W. *Angew. Chem.* **2007**, *119*, 269–272.
23. Saalfrank, R. W.; Scheurer, A.; Puchta, R.; Hampel, F.; Maid, H.; Heinemann, F. W. *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 265–268.
24. (a) Saalfrank, R. W.; Scheurer, A.; Maid, H. *Angew. Chem.* **2008**, *120*, 8924–8956. (b) Saalfrank, R. W.; Scheurer, A.; Maid, H. *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 8794–8824.
25. (a) Saalfrank, R. W.; Scheurer, A.; Maid, H.; Puchta, R.; Bauer, W. *Angew. Chem.* **2008**, *120*, 9073–9077. (b) Saalfrank, R. W.; Scheurer, A.; Maid, H.; Puchta, R.; Bauer, W. *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 8941–8945.
26. See, e.g., Müller, W. H. *Naturwissenschaften* **1970**, *57*, 248.
27. Lehn, J.-M.; Montavon, F. *Helv. Chim. Acta* **1978**, *61*, 67–76.
28. (a) Lehn, J.-M.; Kirch, M. *Angew. Chem.* **1975**, *87*, 542–543. (b) Lehn, J.-M.; Kirch, M. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 555–556.
29. See, e.g., Ajgaonkar, H. S.; Khopkar, S. M. *Chemia Analityczna (Chem. Anal.)* **1999**, *44*, 61–66.
30. Tait, D.; Haase, G.; Wiechen, A. *Kieler Milchwirtschaftliche Forschungsberichte* **1998**, *50*, 207–211.
31. See, e.g., Dehmlow, E. V. *“Phasen Transfer Catalysts”*. MERCK-Schuchardt: Darmstadt, Germany.
32. (a) See, e.g. Dehmlow, E. V. *Angew. Chem.* **1977**, *89*, 521–533, (b) See, e.g. Dehmlow, E. V. *Angew. Chem. Int. Ed. Engl.* **1977**, *17*, 493–505,
33. See, e.g., Li, J.-H.; Shih, J. S. *J. Chin. Chem. Soc.* **1999**, *46*, 885–888,
34. Farahbakhsh, M.; Schmidt, H.; Rehder, D. *Chem. Ber.* **1997**, *130*, 1123–1127.

35. Jander, G.; Klemm, W. "Die Chemie in Wasserähnlichen Lösungsmitteln"; Springer-Verlag: Berlin, **1949**.
36. Jander, J.; Lafrenz, C. "Wasserähnliche Lösungsmittel. vol. 3; Springer-Verlag: Berlin, **1968**.
37. Jander, J.; Tuerk, G. *Chem. Ber.* **1962**, *95*, 881–888.
38. Jander, J.; Tuerk, G. *Chem. Ber.* **1962**, *95*, 2314–2320.
39. Jander, J.; Tuerk, G. *Chem. Ber.* **1965**, *98*, 894–903.
40. Anastas, P. T.; Williamson, T. C. (Eds.) "Green Chemistry: Designing Chemistry for the Environment (Symposium at the 208th National Meeting of the American Chemical Society" Washington, DC, August 21–25, 1994.) [In: ACS Symp. Ser. 1996, p. 626], 1996, p. 251.
41. Clifford, A.; Clifford, T. "Fundamentals of Supercritical Fluids"; Oxford University Press: Oxford, **1999**; p. 228.
42. Erkey, C. *J. Supercrit. Fluids* **2000**, *17*, 259–287.
43. Venter, M. J.; Willems, P.; Kareth, S.; Weidner, E.; Kuipers, N. J. M.; de Haan, A. B. *J. Supercrit. Fluids* **2007**, *41*, 195–203.
44. Erras-Hanauer, H.; Clark, T.; van Eldik, R. *Coord. Chem. Rev.* **2003**, *238–239*, 233–253.
45. Rotzinger, F. *Chem. Rev.* **2005**, *105*, 2003–2038.
46. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
47. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B Condens. Matter Mater. Phys.* **1988**, *37*, 785–789.
48. Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623–11627.
49. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. "Gaussian 03, Revision B.02"; Gaussian, Inc: Wallingford, CT, **2004**.
50. Wodrich, M. D.; Corminboeuf, C.; von Ragué Schleyer, P. *Org. Lett.* **2006**, *8*, 3631–3634.
51. Schreiner, P. R.; Fokin, A. A.; Pascal, R. A.; de Meijere, A. *Org. Lett.* **2006**, *8*, 3635–3638.
52. Grimme, S.; Steinmetz, M.; Korth, M. *J. Org. Chem.* **2007**, *72*, 2118–2126.
53. Wodrich, M. D.; Corminboeuf, C.; Schreiner, P.; Fokin, A. A.; von Ragué Schleyer, P. *Org. Lett.* **2007**, *9*, 1851–1854.
54. Schreiner, P. R. *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 4217–4219.
55. Hofmann, H.; Hänsele, E.; Clark, T. *J. Comp. Chem.* **1990**, *111*, 1147–1150.

56. Hehre, W. J.; Radom, L.; von Ragué Schleyer, P. *“Ab Initio Molecular Orbital Theorie”*; Wiley: New York, **1986**.
57. (a) Miertus, S.; Scrocco, E.; Tomasi, J. *J. Chem. Phys.* **1981**, *55*, 117–129. (b) Miertus, S.; Tomasi, J. *J. Chem. Phys.* **1982**, *65*, 239–245. (c) Cossi, M.; Barone, V.; Cammi, R.; Tomasi, J. *J. Chem. Phys. Lett.* **1996**, *255*, 327–335. (d) Cancès, M. T.; Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3032–3041. (e) Barone, V.; Cossi, M.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3210–3221. (f) Cossi, M.; Barone, V.; Mennucci, B.; Tomasi, J. *J. Chem. Phys. Lett.* **1998**, *286*, 253–260. (g) Barone, V.; Cossi, M.; Tomasi, J. *J. Comp. Chem.* **1998**, *19*, 404–417. (h) Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *106*, 5151. (i) Mennucci, B.; Cancès, E.; Tomasi, J. *J. Phys. Chem. B* **1997**, *101*, 10506–10517. (j) Tomasi, J.; Mennucci, B.; Cancès, E. *J. Mol. Struct. (THEOCHEM)* **1999**, *464*, 211–226. (k) Cammi, R.; Mennucci, B.; Tomasi, J. *J. Phys. Chem. A* **1999**, *103*, 9100–9108. (l) Cossi, M.; Barone, V.; Robb, M. A. *J. Chem. Phys.* **1999**, *111*, 5295–5302. (m) Cammi, R.; Mennucci, B.; Tomasi, J. *J. Phys. Chem. A* **2000**, *104*, 5631–5637. (n) Cossi, M.; Barone, V. *J. Chem. Phys.* **2000**, *112*, 2427–2435. (o) Cossi, M.; Barone, V. *J. Chem. Phys.* **2001**, *115*, 4708–4717. (p) Cossi, M.; Rega, N.; Scalmani, G.; Barone, V. *J. Chem. Phys.* **2001**, *114*, 5691–5701. (q) Cossi, M.; Scalmani, G.; Rega, N.; Barone, V. *J. Chem. Phys.* **2002**, *117*, 43–54. (r) Silla, E.; Tunon, I.; Pascual-Ahuir, J. L. *J. Comp. Chem.* **1994**, *15*, 1127–1138.
58. Foresman, J. B.; Keith, T. A.; Wiberg, K. B.; Snoonian, J.; Frisch, M. J. *J. Phys. Chem.* **1996**, *100*, 16098–16104.
59. Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995–2001.
60. Cossi, M.; Scalmani, G.; Rega, N.; Barone, V. *J. Comp. Chem.* **2003**, *24*, 669–796.
61. Jander, G. *Die Naturwissenschaften* **1938**, *26*, 779–783.
62. Connick, R. E.; Fiat, D. N. *J. Chem. Phys.* **1963**, *39*, 1349–1351.
63. Fratiello, A.; Lee, R. E.; Nishida, V. M.; Schuster, R. E. *J. Chem. Phys.* **1968**, *48*, 3705–3711.
64. Frahm, J.; Földner, H.-H. *Ber. Bunsen-Ges. Phys. Chem.* **1980**, *84*, 173–176.
65. Akitt, J. W.; Duncan, R. H. *J. Chem. Soc. Faraday I* **1980**, *76*, 2212–2220.
66. Akitt, J. W.; Elders, J. M. *Bull. Soc. Chim. Fr.* **1988**, 466–469.
67. Pittet, P.-A.; Elbaze, G.; Helm, L.; Merbach, A. E. *Inorg. Chem.* **1990**, *29*, 1936–1942.
68. Inada, Y.; Nakayama, Y.; Funahashi, S. *J. Solut. Chem.* **2004**, *33*, 847–862.
69. Dance, I. G.; Freeman, H. C. *Acta Crystallogr.* **1969**, *B25*, 304–310.
70. Yamaguchi, T.; Ohtaki, H.; Spohr, E.; Palinkas, G.; Heinzinger, K.; Probst, M. M. *Z. Naturforsch.* **1986**, *41a*, 1175–1185.
71. Robl, C.; Hentschel, S. *Z. Naturforsch. B Chem. Sci.* **1990**, *45*, 1499–1502.
72. Robl, C.; Hentschel, S.; McIntyre, G. J. *J. Solid State Chem.* **1992**, *96*, 318–323.
73. Moers, O.; Friedrichs, S.; Blaschette, A.; Jones, P. G. *Z. Anorg. Allg. Chem.* **2002**, *628*, 589–595.
74. Yasodha, V.; Govindarajan, S.; Low, J. N.; Glidewell, C. *Acta Crystallogr. Sect. C Cryst. Struct. Commun.* **2007**, *63*, m207–m215.
75. Sikka, S. K.; Chidambaram, R. *Acta Crystallogr.* **1969**, *B25*, 310–315.
76. Gnanakaran, S.; Scott, B.; McCleskey, M.; Garcia, A. E. *J. Chem. Phys. B* **2008**, *112*, 2958–2963.
77. D’Incal, A.; Hofer, T. S.; Randolph, B. R.; Rode, B. M. *Phys. Chem. Chem. Phys.* **2006**, *8*, 2841–2847.

78. Rozmanov, D. A.; Sizova, O. V.; Burkov, K. A. *J. Mol. Struct. (THEOCHEM)* **2005**, 756, 127–132.
79. Rozmanov, D. A.; Sizova, O. V.; Burkov, K. A. *J. Mol. Struct. (THEOCHEM)* **2004**, 712, 123–130.
80. Meske, W.; Babel, D. Z. *Anorg. Allg. Chem.* **1998**, 624, 1751–1755.
81. (a) Benner, K.; Ihringer, J.; Klüfers, P.; Marinov, D. *Angew. Chem.* **2006**, 118, 5950–5954. (b) Benner, K.; Ihringer, J.; Klüfers, P.; Marinov, D. *Angew. Chem. Int. Ed. Engl.* **2006**, 45, 5818–5822.
82. Steyl, G. *Acta Crystallogr. Sect. E Struct. Rep. Online* **2007**, 63, m2343.
83. Bruggmann, R. C.; Thewalt, U. Z. *Naturforsch. B Chem. Sci.* **1994**, 49, 1531–1538.
84. Tang, K.; Li, A.; Jin, X.; Tang, Y. *Chem. J. Chin. Univ.* **1993**, 14, 310–313.
85. Maxey, B. W.; Popov, A. I. *J. Am. Chem. Soc.* **1968**, 90, 4470–4472.
86. Loeffler, H. H.; Inada, Y.; Funahashi, S. *J. Phys. Chem. B* **2006**, 110, 5690–5696.
87. Loeffler, H. H.; Mohammed, A. M.; Inada, Y.; Funahashi, S. *Chem. Phys. Lett.* **2003**, 379, 452–457.
88. Loeffler, H. H.; Rode, B. M. *J. Chem. Phys.* **1998**, 117, 110–117.
89. Tongraar, A.; Liedl, K. R.; Rode, B. M. *Chem. Phys. Lett.* **1998**, 286, 56–64.
90. Puchta, R.; Galle, M.; van Eikema Hommes, N.; Pasgreta, E.; van Eldik, R. *Inorg. Chem.* **2004**, 43, 8227–8229.
91. Gubo, S.; Puchta, R.; van Eikema Hommes, N.; van Eldik, R. *in preparation*.
92. Pasgreta, E.; Puchta, R.; Galle, M.; van Eikema Hommes, N.; van Eldik, R. *Chem. Phys. Chem.* **2007**, 8, 1315–1320.
93. Pasgreta, E.; Puchta, R.; Zahl, A.; van Eldik, R. *Eur. J. Inorg. Chem.* **2007**, 1815–1822.
94. Bach, A.; Hoyer, M.; Hartl, H. Z. *Naturforsch. B Chem. Sci.* **1997**, 52, 1497–1500.
95. Flacke, F.; Jacobs, H. *Eur. J. Solid. State Inorg. Chem.* **1997**, 34, 495–501.
96. Scotti, N.; Zachwieja, U.; Jacobs, H. Z. *Anorg. Allg. Chem.* **1997**, 623, 1503–1505.
97. Wiesler, K.; Korber, N. Z. *Kristallogr.* **2005**, 220, 188–191.
98. Luzar, A.; Soper, A. K.; Chandler, D. *J. Chem. Phys.* **1993**, 99, 6836–6847.
99. Skaf, M. S. *Mol. Phys.* **1997**, 90, 25–43.
100. Liu, H.; Müller-Plathe, F.; van Gunsteren, W. F. *J. Am. Chem. Soc.* **1995**, 117, 4363–4366.
101. Onthong, U.; Bako, I.; Radnai, T.; Hermansson, K.; Probst, M. *Int. J. Mass Spectr.* **2003**, 263, 223–224.
102. Onthong, U.; Megyes, T.; Bako, I.; Radnai, T.; Grosz, T.; Hermansson, K.; Probst, M. *Phys. Chem. Chem. Phys.* **2004**, 6, 2136–2144.
103. Gabrielian, L. S.; Markarian, S. A. *J. Mol. Liq.* **2004**, 112, 137–140.
104. Clark, T.; Murray, J. S.; Lane, P.; Politzer, P. *J. Mol. Mod.* **2008**, 14, 689–697.
105. Vaisman, I. I.; Berkowitz, M. L. *J. Am. Chem. Soc.* **1992**, 114, 7889–7896.
106. Kirchner, B.; Reiher, M. *J. Am. Chem. Soc.* **2002**, 124, 6206–6215.
107. Luzar, A.; Chandler, D. *J. Chem. Phys.* **1993**, 98, 8160–8173.
108. Laria, D.; Skaf, M. S. *J. Chem. Phys.* **1999**, 111, 300–309.
109. Skaf, M. S. *J. Phys. Chem. A* **1999**, 103, 10719–10729.
110. Borin, I. A.; Skaf, M. S. *J. Chem. Phys.* **1999**, 110, 6412–6420.
111. Rao, B. G.; Singh, U. C. *J. Am. Chem. Soc.* **1990**, 112, 3803–3811.
112. Vechi, S. M.; Skaf, M. S. *J. Chem. Phys.* **2005**, 123, 1–8.

113. Mierke, D. F.; Kessler, H. *J. Am. Chem. Soc.* **1991**, *113*, 9466–9470.
114. Bako, I.; Megyes, T.; Palinkas, G. *Chem. Phys.* **2005**, *316*, 235–244.
115. Lu, H.; Wang, J.; Zhao, Y.; Xuan, X.; Zhuo, K. *J. Chem. Eng. Data* **2001**, *46*, 631–634.
116. Rivelino, R.; Cabral, B. J. C.; Coutinho, K.; Canuto, S. *Chem. Phys. Lett.* **2005**, *407*, 13–17.
117. Hojo, M. *Bunseki Kagaku* **2004**, *53*, 1279–1293.
118. Mollner, A. K.; Brooksby, P. A.; Loring, J. S.; Bako, I.; Palinkas, G.; Fawcett, W. R. *J. Phys. Chem. A* **2004**, *108*, 3344–3349.
119. Barthel, J.; Buchner, R.; Wismeth, E. *J. Solution Chem.* **2000**, *29*, 937–954.
120. Xuan, X.; Zhang, H.; Wang, J.; Wang, H. *J. Phys. Chem. A* **2004**, *108*, 7513–7521.
121. Maroncelli, M. *J. Chem. Phys.* **1991**, *94*, 2084–2103.
122. Richardi, J.; Fries, P. H.; Krienke, H. *J. Chem. Phys.* **1998**, *108*, 4079–4089.
123. Guardia, E.; Pinzon, R. *J. Mol. Liq.* **2000**, *85*, 33–44.
124. Spångberg, D.; Hermansson, K. *Chem. Phys.* **2004**, *300*, 165–176.
125. Fischer, R.; Richardi, J.; Fries, P. H.; Krienke, H. *J. Chem. Phys.* **2002**, *117*, 8467–8478.
126. Dimitrova, Y. *J. Mol. Struct. (THEOCHEM)* **1995**, *334*, 215–222.
127. Cabaleiro-Lago, E. M.; Rios, M. A. *Chem. Phys.* **2000**, *254*, 11–23.
128. Pejov, L. *Int. J. Quantum Chem.* **2002**, *86*, 356–367.
129. Bako, I.; Megyes, T.; Radnai, T.; Palinkas, G.; Probst, M.; Fawcett, W. R. *Z. Phys. Chem.* **2004**, *218*, 643–658.
130. Keil, R. G.; Johnson, D. W.; Fryling, M. A.; O'Brien, J. F. *Inorg. Chem.* **1989**, *28*, 2764–2766.
131. Fawcett, W. R.; Liu, G.; Faguy, P. W.; Foss, C. A., Jr.; Motheo, A. J. *J. Chem. Soc. Faraday Trans.* **1993**, *89*, 811–816.
132. Barthel, J.; Deser, R. *J. Solut. Chem.* **1994**, *23*, 1133–1146.
133. Cartailier, T.; Kunz, W.; Turq, P.; Bellisent-Funel, M. C. *J. Phys. Condens. Matter* **1991**, *3*, 9511–9520.
134. Solovieva, L. A.; Akopyan, S. K.; Vilaseca, E. *Spectrochim. Acta Part A* **1994**, *50*, 683–688.
135. Brooks, N. R.; Henderson, W. A.; Smyrl, W. H. *Acta Crystallogr. Sect. E Struct. Rep. Online* **2002**, *58*, m176–m177.
136. Chitsaz, S.; Pauls, J.; Neumüller, B. *Z. Naturforsch. B Chem. Sci.* **2001**, *56*, 245–248.
137. Yokota, Y.; Young, V. G., Jr.; Verkade, J. G. *Acta Crystallogr. Sect. C Cryst. Struct. Commun.* **1999**, *55*, 196–197.
138. Raston, C. L.; Whitaker, C. R.; White, A. H. *Aust. J. Chem.* **1989**, *42*, 201–207.
139. Jacoby, D.; Floriani, C.; Chiesi-Villa, A.; Rizzoli, C. *J. Chem. Soc., Chem. Commun.* **1991**, 220–222.
140. Hoyer, M.; Hartl, H. *Z. Anorg. Allg. Chem.* **1992**, *612*, 45–50.
141. Sajeevkumar, V. A.; Singh, S. *J. Mol. Struct.* **1996**, *382*, 101–110.
142. Megyes, T.; Radnai, T.; Wakisaka, A. *J. Mol. Liq.* **2003**, *103–104*, 319–329.
143. Ullstroem, A.-S.; Warminska, D.; Persson, I. *J. Coord. Chem.* **2005**, *58*, 611–622.
144. Neumüller, B.; Petz, W.; Dehnicke, K. *Z. Anorg. Allg. Chem.* **2008**, *634*, 662–668.
145. Aizawa, S.; Kato, T.; Funahashi, S. *Anal. Sci.* **1997**, *13*, 541–544.

146. Cahen, Y. M.; Handy, P. R.; Roach, E. T.; Popov, A. I. *J. Phys. Chem.* **1975**, 79, 80–85.
147. Richens, D. T. *"The Chemistry of Aqua Ions"*; Wiley: Chichester, **1997**.
148. Helm, L.; Merbach, A. E. *Coord. Chem. Rev.* **1999**, 187, 151–181.
149. Dunand, F. A.; Helm, L.; Merbach, A. E. *Adv. Inorg. Chem.* **2003**, 54, 1–69.
150. Lee, H. M.; Tarakeshwar, P.; Park, J.; Kolaski, M. R.; Yoon, Y. J.; Yi, H.-B.; Kim, W. Y.; Kim, K. S. *J. Phys. Chem. A* **2004**, 108, 2949–2958.
151. Müller, I. B.; Cederbaum, L. S.; Tarantelli, F. *J. Phys. Chem. A* **2004**, 108, 5831–5844.
152. Spångberg, D.; Rey, R.; Hynes, J. T.; Hermansson, K. *J. Phys. Chem. B* **2003**, 107, 4470–4477.
153. Lincoln, S. F.; Tkaczuk, M. N. *Ber. Bunsen. Ges. Phys. Chem.* **1981**, 85, 433–437.
154. Lincoln, S. F.; Tkaczuk, M. N. *Ber. Bunsen. Ges. Phys. Chem.* **1982**, 86, 221–225.
155. Swaddle, T. W. *Inorg. Chem.* **1983**, 22, 2663–2665.
156. Puchta, R.; van Eikema Hommes, N.; van Eldik, R. *Helv. Chim. Acta* **2005**, 88, 911–922.
157. Hanauer, H.; Puchta, R.; Clark, T.; van Eldik, R. *Inorg. Chem.* **2007**, 46, 1112.
158. Bertrán, J.; Lledós, A.; Revetllat, J. A. *Int. J. Quant. Chem.* **1983**, 23, 587–593.
159. Puchta, R.; van Eldik, R. *Helv. Chim. Acta* **2008**, 91, 1063–1071.
160. Rotzinger, F. P. *J. Am. Chem. Soc.* **1996**, 118, 6760–6766.
161. Puchta, R.; van Eldik, R., unpublished results.
162. Rotzinger, F. P. *J. Phys. Chem.* **2005**, 109, 1510.
163. Illner, P.; Zahl, A.; Puchta, R.; van Eikema Hommes, N.; Wasserscheid, P.; van Eldik, R. *J. Organomet. Chem.* **2005**, 690, 3567–3576.
164. Puchta, R.; Bauer, W.; Clark, T. *J. Mol. Mod.* **2006**, 12, 739–747.
165. Galle, M.; Puchta, R.; van Eikema Hommes, N.; van Eldik, R., *in preparation*.
166. (a) Wells, S. L.; DeSimone, J. M. *Angew. Chem.* **2001**, 113, 518–527. (b) Wells, S. L.; DeSimone, J. M. *Angew. Chem. Int. Ed. Engl.* **2001**, 29, 535–544.
167. Reed, A. E.; Curtis, L. A.; Weinhold, F. *Chem. Rev.* **1988**, 88, 899–926, and refs. *loc. cit.*
168. Krämer, U.; Cotter-Howells, J. D.; Charnock, J. M.; Baker, A. H. J. M.; Smith, J. A. C. *Nature* **1996**, 379, 635–638.
169. Magalhaes, J. V. *Proc. Natl. Acad. Sci. USA* **2006**, 103, 9749–9750.
170. Ha, S.-B.; Smith, A. P.; Howden, R.; Dietrich, W. M.; Bugg, S.; O'Connell, M. J.; Goldsbrough, P. B.; Cobbett, C. S. *Plant Cell* **1999**, 11, 1153–1164.
171. Plieger, P. G.; Ehler, D. S.; Duran, B. L.; Taylor, T. P.; John, K. D.; Keizer, T. S.; McCleskey, T. M.; Burrell, A. K.; Kampf, J. W.; Haase, T.; Rasmussen, P. G.; Karr, J. *Inorg. Chem.* **2005**, 44, 5761–5769.
172. Galle, M.; Puchta, R.; van Eikema Hommes, N.; van Eldik, R. *Z. Phys. Chem.* **2006**, 220, 511–523.
173. Puchta, R.; van Eldik, R. *Eur. J. Inorg. Chem.* **2007**, 1120–1127.
174. Puchta, R.; Meier, R.; van Eldik, R. *Aust. J. Chem.* **2007**, 60, 889–897.
175. Puchta, R.; van Eldik, R. *J. Incl. Phenom. Macrocycl. Chem.* **2008**, 60, 383–392.
176. (a) von Ragué Schleyer, P.; Maerker, C.; Dransfeld, A.; Jiao, H.; van Eikema Hommes, N. J. R. *J. Am. Chem. Soc.* **1996**, 118, 6317–6318.

- (b) von Ragué Schleyer, P.; Jiao, H.; van Eikema Hommes, N. J. R.; Malkin, V. G.; Malkina, O. L. *J. Am. Chem. Soc.* **1997**, *119*, 12669–12670.
- (c) von Ragué Schleyer, P.; Manoharan, M.; Wang, Z.-X.; Kiran, B.; Jiao, H.; Puchta, R.; van Eikema Hommes, N. J. R. *Org. Lett.* **2001**, *3*, 2465–2468.
- (d) Chen, Z.; Wannere, C. S.; Corminboeuf, C.; Puchta, R.; von Ragué Schleyer, P. *Chem. Rev.* **2005**, *105*, 3842–3888.
- (e) Fallah-Bagher-Shaidaei, H.; Wannere, Ch. S.; Corminboeuf, C.; Puchta, R.; von Ragué Schleyer, P. *Org. Lett.* **2006**, *8*, 863–866.
177. Puchta, R.; van Eldik, R. *Z. Anorg. Allg. Chem.* **2008**, *634*, 1915–1920.
178. Puchta, R.; van Eldik, R. *Z. Anorg. Allg. Chem.* **2008**, *634*, 735–739.
179. Neumüller, B.; Dehnicke, K. *Z. Anorg. Allg. Chem.* **2006**, *632*, 1681.
180. (a) Clark, D. L.; Keogh, D. W.; Palmer, P. D.; Scott, B. L.; Tait, C. D. *Angew. Chem.* **1998**, *110*, 173–175. (b) Clark, D. L.; Keogh, D. W.; Palmer, P. D.; Scott, B. L.; Tait, C. D. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 164–166.
181. (a) Holdt, H.-J.; Müller, H.; Pötter, M.; Kelling, A.; Schilde, U.; Starke, I.; Heydenreich, M.; Kleinpeter, E. *Eur. J. Inorg. Chem.* **2006**, 2377–2384. (b) Holdt, H.-J.; Müller, H.; Pötter, M.; Kelling, A.; Drexler, H.-J.; Müller, T.; Schwarze, T.; Schilde, U.; Starke, I. *Z. Anorg. Allg. Chem.* **2006**, *631*, 114–122. (c) Holzberger, A.; Holdt, H.-J.; Kleinpeter, E. *J. Phys. Org. Chem.* **2004**, *17*, 257–265. (d) Holzberger, A.; Holdt, H.-J.; Kleinpeter, E. *Org. Biomol. Chem.* **2004**, *2*, 1691–1697.
182. Neumüller, B.; Dehnicke, K.; Puchta, R. *Z. Anorg. Allg. Chem.* **2008**, *634*, 1473–1476.
183. Bel'skii, V. K.; Bulychev, B. M.; Strel'tsova, N. R.; Ivakina, L. V. *Zh. Obshch. Khim. (Russ. J. Gen. Chem.)* **1989**, *59*, 1806–1812.

This page intentionally left blank

SPIN-STATE CHANGES AND REACTIVITY IN TRANSITION METAL CHEMISTRY: REACTIVITY OF IRON TETRACARBONYL

MARIA BESORA^a, JOSÉ-LUIS CARREÓN-MACEDO^{a,b},
ÁLVARO CIMAS^a and JEREMY N. HARVEY^a

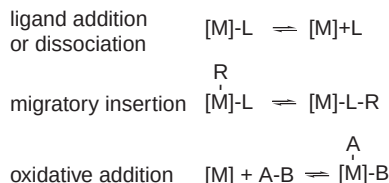
^aSchool of Chemistry and Centre for Computational Chemistry, University of Bristol,
Cantock's Close, Bristol BS8 1TS, United Kingdom

^bCurrent address: Department of Chemistry, University of Alberta, Edmonton, AB,
T6G 2G2, Canada

I. Introduction	573
II. Chemistry of Fe(CO) ₅ Photofragments	577
A. Experimental Background	577
B. Computational Studies: Addition to Fe(CO) ₄	581
C. Computational Studies: Addition to Fe(CO) ₃	594
D. Computational Studies: Addition to Fe(CO) ₃ L Species	599
E. Fe(CO) ₅ Photofragment Chemistry: Conclusions	605
III. Other Spin-Forbidden Reactions of Transition Metal Compounds	606
IV. Spin-Forbidden Reactions: General Trends	609
V. Conclusions	616
Acknowledgments	618
References	618

I. Introduction

Metal complexes, especially involving transition metals, are known for their role as catalysts in a broad variety of chemical processes including isomerization, oxidization, hydrogenation, and polymerization. Such catalytic reactions play an important role not only in many industrial processes, such as petroleum and polymer industries, but also in many biological systems, e.g., a variety of selective oxidation catalysts with heme (1) and nonheme (2) iron centers. The transition metals in these systems usually constitute a fundamental part of the catalyst, due to their



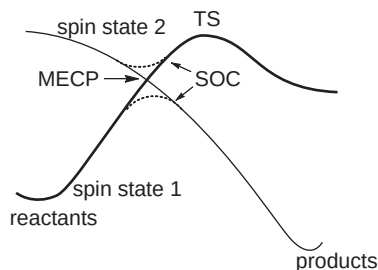
SCHEME 1.

flexible chemistry, with many different coordination geometries and oxidation states available.

Many reactions involving transition metal compounds, especially those in which there is a change in oxidation state or in the number of ligands at the metal center (e.g., the typical processes shown in Scheme 1), are accompanied by a change in spin-state. This is related to the well-known fact that transition metal complexes, especially those that are coordinatively unsaturated, quite often have several different spin-states quite close in energy. Reactions in which there is a spin-state change are usually referred to as spin-forbidden, as they cannot occur in the absence of spin-orbit coupling or other effects that lead to mixing of the electronic wave functions. In practice, some of these reactions are indeed slower than similar spin-allowed reactions (in which there is no change on the spin-state), while others have higher or similar reaction rates (3).

The elementary reactions in Scheme 1 all involve a change in the formal electron count at the metal center; they typically comprise interconversion of a saturated (18 electron) and an unsaturated (16 electron) species (or maybe interconversion of 15- and 17-electron complexes). Such reactions are particularly prone to being spin-forbidden, as the unsaturated 16- or 15-electron species often have close-lying spin-states and high-spin ground states. For example, in the ligand dissociation process in Scheme 1, if $[M]-L$ is a singlet 18-electron complex then the dissociation product $[M]$ will be a 16-electron complex, and may have a triplet ground state, so that dissociation is spin-forbidden.

We (as well as many others (4–8), for reviews see (9,10)) have been interested for some time (11,12) in the effect of spin-state changes on reactivity. In particular, we have used computational methods to explore these effects in transition metal chemistry ((13–15), for reviews see (10,16)). The key factor affecting reactivity is the relative energy at which the zeroth-order potential energy surfaces corresponding to the individual spin-states cross (Scheme 2). This factor will determine, among other things, whether spin-forbidden reactivity is competitive with



SCHEME 2.

adiabatic behavior, staying on the same potential energy surface and passing over a transition state (TS). Note that the full adiabatic potential energy surfaces *do not* cross, as spin-orbit coupling and other effects cause the different electronic wave functions to mix, leading to avoided crossings, as shown in dotted lines in Scheme 2. However, in typical spin-forbidden reactions, even with transition metal compounds, the extent of the mixing is small (less than 1 kcal mol^{-1} usually), so the zeroth-order picture is not badly wrong in terms of the energy of the crossing region.

As potential energy surfaces are multidimensional, they cross in many different points, among which the most important for reactivity is the minimum energy crossing point (MECP). An MECP is not a stationary point on either of the potential energy surfaces, and cannot be located using straightforward energy minimization with the standard methods available in the common electronic structure codes. However, by combining the energies and the energy gradients on the two potential energy surfaces, which can be calculated straightforwardly by these programs, it is possible to locate the MECPs, and several groups have reported techniques to do so (see e.g., (11,17)).

The energy of the MECP does not, on its own, determine reactivity; one also needs to take into account the nonadiabatic behavior in the vicinity of the surface crossing. In terms of the zeroth-order potential energy surfaces with defined spin, in the limit of small spin-orbit coupling, the most likely outcome when the system reaches the crossing seam is that it remains on the reactant surface and then returns to the reactant side. Note that in terms of the adiabatic surfaces, which have a narrowly avoided crossing around the MECP, this same behavior is described differently, in terms of a high probability of hopping *twice*, once from the lower surface to the upper one and then again back to the lower one and back to reactants. These two descriptions sound very different but are in fact identical. In either case, there

is only a small probability p_{sh} of moving toward products on the lower-energy surface. This probability of crossing between surfaces depends on the spin-orbit coupling matrix element between the two electronic wave functions, the relative slope at the crossing point between surfaces, the reduced mass of the system along the hopping coordinate, and the kinetic energy of the system as it passes through the crossing seam. It can be calculated according to a number of different approximations (4,18). The key point is that this probability increases with increasing strength of spin-orbit coupling (or other coupling terms), but remains significantly smaller than unity, even for transition metal compounds.

The combined effect of the two factors can be computed, thereby yielding an expression for the rate constants of spin-forbidden reactions in terms of a nonadiabatic-transition state theory (NA-TST) (5,6,19,20). It is only meaningful to apply this theory where accurate calculations of the energy surfaces can be carried out, as small errors on the relative energy of the MECP will affect computed rates quite significantly. In most cases, expected reactivity behavior can be estimated qualitatively, simply by comparing the energy of the MECP with the energy of competing adiabatic transition states. We have applied this theory to a number of cases (21), including organometallic spin-forbidden reactions (22–24).

Here we would like to review our recent work concerning qualitative and semiquantitative characterization of reactivity, with a particular focus on a specific family of spin-forbidden reactions: ligand addition to unsaturated iron carbonyl fragments (23–27). Photolysis of iron pentacarbonyl and related species leads to loss of one or more molecules of carbon monoxide, and to unsaturated 16- and 14-electron complexes that usually have high-spin ground states. These can then react with CO or other ligands to regenerate stable 18-electron singlet species. These simple reactions represent excellent models for understanding spin-forbidden reactivity, as there is extensive experimental data available and the molecules involved are small enough that it is possible to carry out accurate computational studies. As well as this fundamental interest, the reactions have some practical importance, e.g., in olefin hydrogenation and isomerization processes. The review will also cover other examples of spin-forbidden reactions in inorganic chemistry that have been published since our earlier reviews (10,16). Finally, it will include a general discussion about the rates of different types of spin-forbidden reactions, based on the work carried out on iron pentacarbonyl-derived species and other systems.

II. Chemistry of $\text{Fe}(\text{CO})_5$ Photofragments

In this section, we start by describing the main features of the photolytic chemistry of iron pentacarbonyl and related species, focusing on the experimental data. We then describe our and other people's computational work aimed at understanding the reactivity of the fragments formed upon photolysis, with a focus on the spin-forbidden steps. We consider, in turn, the addition of ligands to $\text{Fe}(\text{CO})_4$, $\text{Fe}(\text{CO})_3$, and $\text{Fe}(\text{CO})_3\text{L}$ (where L is a ligand such as H_2 or ethene). For some of the reactions, we include some new computational results as well as a review of existing work.

A. EXPERIMENTAL BACKGROUND

Iron pentacarbonyl is a very well-known organometallic species that has been extensively studied since its discovery in 1891 (28,29). In particular, the photochemical behavior of $\text{Fe}(\text{CO})_5$ has been of great interest, and has been reviewed many times (30–32). In one of the first studies of $\text{Fe}(\text{CO})_5$, Mond showed that upon exposure to light, CO is lost and a crystalline species containing two iron atoms is formed (28), and suggested the formula $\text{Fe}_2(\text{CO})_7$; this was reexamined by Dewar and Jones a few years later and the species reassigned as $\text{Fe}_2(\text{CO})_9$ (33). These authors also proposed a mechanism involving initial photodissociation of CO to form $\text{Fe}(\text{CO})_4$, followed by reaction of this fragment with a second molecule of $\text{Fe}(\text{CO})_5$ to form the diiron nonacarbonyl (33).

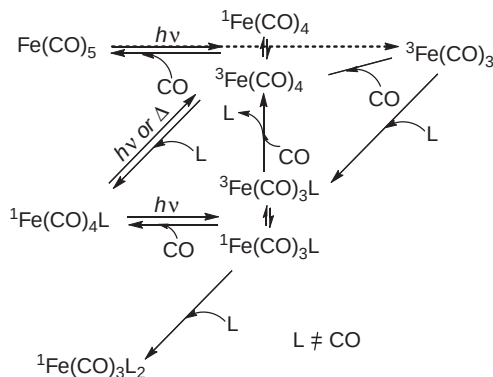
The presence of $\text{Fe}(\text{CO})_4$ on photolysis of iron pentacarbonyl was confirmed in later works (34,35), and this species was proposed to have a triplet ground state based on computational work (36). This assignment was later supported by experimental findings using magnetic circular dichroism (37). More recently, the course of the photodissociation process has been studied theoretically and experimentally. Using ultrafast electron diffraction in the gas phase, the primary photolysis product observed was assigned as the $^1\text{A}_1$ state of $\text{Fe}(\text{CO})_4$ (38). Gas-phase femtosecond experiments (39) confirm that the singlet state of iron tetracarbonyl is formed rapidly, and then relaxes more slowly on a picosecond timescale to the triplet, in competition with further dissociation to give lower carbonyls. Solution experiments tend to lead to loss of fewer carbonyl fragments, with the main product being $^3\text{Fe}(\text{CO})_4$, which is formed with a timescale of 26 ps (40), and the singlet species that is initially observed in the gas phase is not detected (40). However, other photolysis studies lead to the detection of solvated $\text{Fe}(\text{CO})_3$ species (41–43) and the

dependence of the yield of these species on laser power suggests that they are primary photoproducts (42). As pointed out by Poliakoff (32), the fact that $^1\text{Fe}(\text{CO})_4$ cannot be detected at short times in solution could mean either that the photolysis has a different mechanism in the gas phase and in solution, or that relaxation of $^1\text{Fe}(\text{CO})_4$ to $^3\text{Fe}(\text{CO})_4$ is faster in solution.

The photolysis experiments have also received theoretical attention, with electronic structure methods used to calculate the nature of the excited states (44), as well as the potential energy curves for loss of CO (45). Theoretical models for the excited-state dynamics leading to dissociation have also been proposed (46).

Rather than focusing on the short-time photochemical reactivity, our interest in the spin-forbidden reactions of iron carbonyl fragments has been mainly in the longer-time thermal chemistry of the fragments produced. This is summarized in Scheme 3. As already stated, iron tricarbonyl and tetracarbonyl are known to have triplet ground states, and for many ligands, it is assumed that $\text{Fe}(\text{CO})_3\text{L}$ would also have a triplet ground state. Hence many of the indicated processes are spin-forbidden.

A variety of experimental methods has been used to study the thermal chemistry of the unsaturated iron fragments produced by photolysis. For example, regeneration of $^1\text{Fe}(\text{CO})_5$ was observed upon heating low-temperature matrices in which $\text{Fe}(\text{CO})_5$ had been photolyzed (35). These condensed-phase reactions are rather complex, as in some cases, components of the "inert" matrix may form adducts $\text{Fe}(\text{CO})_4\text{L}$ or $\text{Fe}(\text{CO})_3\text{L}$ ($\text{L} = \text{N}_2, \text{Xe}, \text{CH}_4$), so that the reaction observed is not simply CO addition to an unsaturated iron tetracarbonyl fragment. The same reactions were studied in the gas phase, using flash



SCHEME 3.

photolysis/time-resolved infrared studies, at room temperature. In these conditions, genuine, adduct-free, ${}^3\text{Fe}(\text{CO})_4$ and lower carbonyls are formed, and the rate of addition of CO or other ligands can be measured without interference from matrix or solvent effects (47–59). For example, the rate constant for the addition of CO to ${}^3\text{Fe}(\text{CO})_4$ was measured as $k_{\text{CO}} = 5.8(\pm 1.5) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (52,53), while for ${}^3\text{Fe}(\text{CO})_3$ and ${}^3\text{Fe}(\text{CO})_2$, the corresponding rate constants were reported as $2.2(\pm 0.3) \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ and $3.0(\pm 0.5) \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, respectively (52,53). The difference of almost three orders of magnitude between the rate constants for the first reaction and the other two was suggested to be linked to the fact that for ${}^3\text{Fe}(\text{CO})_4$ there is a spin-state change to form the product ${}^1\text{Fe}(\text{CO})_5$, while for ${}^3\text{Fe}(\text{CO})_3$ and ${}^3\text{Fe}(\text{CO})_2$ carbon monoxide addition preserves spin (47). The rate constant for addition of CO to ${}^3\text{Fe}(\text{CO})_4$ in the condensed phase, also at room temperature, using a genuinely “inert” solvent, supercritical (sc) Ar, has recently been reevaluated and a rather similar value of $k_{\text{CO}} = 2.0 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ was obtained (42,43).

As well as carbon monoxide, other ligands such as N_2 (35,51,54), O_2 (58), H_2 (35), heptane (42,43), Xe (35,42,59), CH_4 (35,42,57,59), and C_6H_6 (56) have been observed to undergo addition to ${}^3\text{Fe}(\text{CO})_4$ and form the species ${}^1\text{Fe}(\text{CO})_4\text{L}$. Many of these reactions have been observed in solution, low-temperature matrices, and/or the gas phase. It appears that at least some species are unable to form such adducts. For example, in low-temperature Ar (35,42,59,60) or Ne (35,59) matrices, neither Ar nor Ne adducts are observed, and where present, other ligands add to the metal center instead. For many of the gas-phase studies and some of the solution studies, rate constants for the ligand addition to ${}^3\text{Fe}(\text{CO})_4$ were reported. In many cases, but not all, these are notably slower than the gas-kinetic limit, e.g., k_{obs} for N_2 addition is $2.8(\pm 0.2) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (51), for H_2 it is $1.97(\pm 0.15) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (55), and for ethene it is $1.7(\pm 0.2) \times 10^{-13} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (50). For some ligands, such as O_2 , the measured rate constant is significantly larger and is close to the expected collisional limit ($k_{\text{obs}} = 3.1 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$) (58). For the experiments carried out in solution, where the ligand is the solvent, it is not possible to vary the concentration; hence, it is not straightforward to measure bimolecular rate constants, and the first-order decay process of ${}^3\text{Fe}(\text{CO})_4$ is all that can be measured, yielding (apparent) first-order rate constants of $7.8(\pm 0.3) \times 10^7 \text{ s}^{-1}$ for heptane (42), of $2.1(\pm 0.1) \times 10^7 \text{ s}^{-1}$ for Xe (42), and of $5(\pm 1) \times 10^6 \text{ s}^{-1}$ for CH_4 (42). In reactions carried out in inert solvents such as sc Ar,

with an additional ligand, it is possible to measure apparent first-order rate constants for reaction with the added ligand, e.g., $3.3 \times 10^6 \text{ s}^{-1}$ for H_2 (24).

One of the most frequently studied species that reacts with $^3\text{Fe}(\text{CO})_4$ is dihydrogen. This does not strictly speaking yield a product corresponding to the general $^1\text{Fe}(\text{CO})_4\text{L}$ formula, as the product is an Fe(II) dihydride species $\text{Fe}(\text{CO})_4(\text{H})_2$ (55). However, the initial step does correspond to simple ligand addition, leading to formation of a dihydrogen complex $^1\text{Fe}(\text{CO})_4(\text{H}_2)$ that converts over a low barrier into the final product (61,62). The rate constants for the addition of H_2 to $^3\text{Fe}(\text{CO})_4$ and $^3\text{Fe}(\text{CO})_3$ were reported as $1.97(\pm 0.15) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (55,58) and $2.7 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (49), respectively. The difference of three orders of magnitude between the rate constants for these processes, the first of which is spin-forbidden whereas the second is presumably not, again suggests that spin-forbiddenness can affect the rate of the first process quite significantly. Over the fairly narrow temperature range accessible to the experiment, no significant change in the rate constant for the first reaction was observed, leading to an experimental activation energy close to zero (55). For the reverse reaction, loss of H_2 from the dihydride, an activation energy of $20.5 \pm 2.1 \text{ kcal mol}^{-1}$ (55) was measured. This is an example of a common reaction occurring for weakly or moderately bound $\text{Fe}(\text{CO})_4\text{L}$ species, namely loss of L, and addition of a more strongly bound species such as CO. Indeed, in the condensed phase, under an atmosphere of CO, the dihydride evolves to $\text{Fe}(\text{CO})_5$ (63).

Photolysis of $\text{Fe}(\text{CO})_4\text{L}$ (including the dihydride) also leads to loss of a ligand, either CO or L, leading to an unsaturated 16-electron species, and both the photodissociation and the thermal kinetics of the dissociation products have been studied experimentally also. In the case of $\text{Fe}(\text{CO})_4(\text{H})_2$, dihydrogen is lost, leading to the same $\text{Fe}(\text{CO})_4$ fragment as formed upon photolysis of iron pentacarbonyl (63). Theoretical electronic structure and dynamical studies of this photochemical process have been published (62,64), but are not discussed here as our focus is on the thermal chemistry of the fragments. However, for other complexes, such as $\text{Fe}(\text{CO})_4(\text{C}_2\text{H}_4)$, CO loss is preferred, leading to species such as $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$ (50,65). Such reactive $\text{Fe}(\text{CO})_3\text{L}$ species can also be formed by addition of L to $\text{Fe}(\text{CO})_3$ formed upon dissociation of two carbonyl ligands from $\text{Fe}(\text{CO})_5$.

Fragments derived from photolysis of $\text{Fe}(\text{CO})_5$ have been observed to catalyze olefin isomerization and hydrogenation (65–67). The key active species for the isomerization is iron tricarbonyl which can easily add alkene to give $\text{Fe}(\text{CO})_3(\text{alkene})$.

Rate constants for the addition of small ligands including alkene to $^3\text{Fe}(\text{CO})_3$ have been reported, with values of $2.2(\pm 0.3) \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ for the addition of CO, $> 2.7 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ for addition of H_2 , and $2.2(\pm 0.2) \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ for the addition of C_2H_4 (48,49,68). With larger alkenes, the species $\text{Fe}(\text{CO})_3(\text{alkene})$ can undergo oxidative addition of an allylic C–H bond to the iron center, to give an $\text{Fe}(\text{II})(\text{allyl})(\text{H})$ complex, which can then reductively eliminate to give an isomeric iron alkene complex (69). Addition of H_2 to $\text{Fe}(\text{CO})_3(\text{alkene})$ can instead lead to dihydride species, which can undergo migratory insertion and reductive elimination processes to yield alkanes (67). The mechanisms involved in these alkene isomerization and hydrogenation processes are complex and are not discussed here in detail.

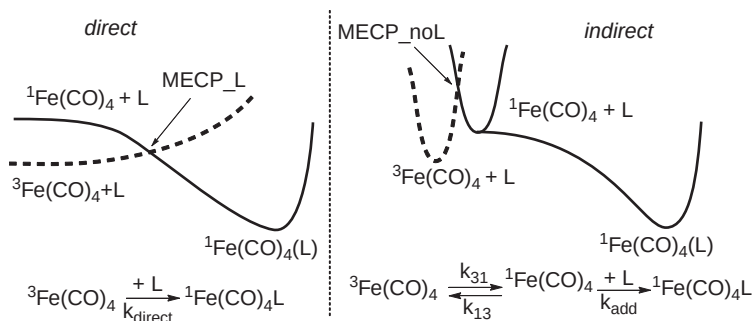
We will however discuss some examples of the general process whereby $\text{Fe}(\text{CO})_3(\text{L})$ reacts with a second ligand L' to form $^1\text{Fe}(\text{CO})_3\text{LL}'$, or, in the case where L' is CO, $^1\text{Fe}(\text{CO})_4(\text{L})$ (see Scheme 3). Formation of $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)_2$ and $\text{Fe}(\text{CO})_4(\text{C}_2\text{H}_4)$ by addition of the relevant ligand to $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$ in the gas phase was studied using time-resolved infrared spectroscopy by Weitz *et al.* (49,50). Large rate constants were obtained in both cases: $4.3(\pm 0.7) \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ and $1.08(\pm 0.06) \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ for addition of CO and ethene, respectively (49,50). These rate constants are however smaller than that for the spin-allowed addition of CO to $^3\text{Fe}(\text{CO})_3$ ($2.2(\pm 0.3) \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$). This observation was rationalized by the authors as being due to the supposed spin-forbidden character of ligand addition to $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$, which was assumed to have a triplet ground state by analogy to $\text{Fe}(\text{CO})_4$.

B. COMPUTATIONAL STUDIES: ADDITION TO $\text{Fe}(\text{CO})_4$

We discuss here our and other groups' computational studies of the addition of ligands to $\text{Fe}(\text{CO})_4$ to give $\text{Fe}(\text{CO})_4\text{L}$ (Scheme 3). This reaction has been studied experimentally for a wide range of ligands under a variety of conditions. Only the addition of carbon monoxide and dihydrogen will be covered in detail, as these represent typical cases. Both reactions are known to be spin-forbidden, as experiment and computation unequivocally show $\text{Fe}(\text{CO})_4$ to have a triplet ground state, and both $\text{Fe}(\text{CO})_5$ and $\text{Fe}(\text{CO})_4\text{H}_2$ clearly have singlet ground states. As discussed in the Introduction, the kinetics of ligand addition can be understood in terms of the properties of the MECPs between singlet and triplet potential energy surfaces.

A key property in computational studies of these reactions is the energy splitting between the triplet and the singlet states of $\text{Fe}(\text{CO})_4$. This needs to be reproduced accurately to obtain accurate rate constant predictions, as the reactions involve crossing from the triplet ground state of $\text{Fe}(\text{CO})_4$ to the singlet surface, so a larger energy splitting is expected to lead to MECPs at higher energy and hence to a lower reactivity. Unfortunately, the magnitude of the splitting is not known from experiment, and different calculations give very different values (23,25,70–74), ranging from $0.5 \text{ kcal mol}^{-1}$ (71) to 19 kcal mol^{-1} (74). Density functional theory (DFT) methods give very different values for the splitting, mostly depending on the amount of exact exchange included in the functional (23). Complete active space self-consistent field (CASSCF) and perturbation theory calculations suggest a splitting of between 5 kcal mol^{-1} and 10 kcal mol^{-1} but results are very dependent on the active space used and the details of the CASPT2 treatment (23). The most accurate estimate of this splitting comes from benchmark CCSD(T) coupled cluster calculations with extrapolation to the infinite basis set limit and treatment of effects such as core correlation and relativity (25). This approach leads to a predicted excitation energy to the singlet of just $2.3 \text{ kcal mol}^{-1}$ (75), and it was also shown that only quite small energy splittings close to this value are consistent with the observation of addition of Xe or CH_4 to $^3\text{Fe}(\text{CO})_4$ to form stable singlet adducts (25). Such correlated *ab initio* methods are too expensive to be used to explore potential energy surfaces for ligand addition, but with an accurate value for the splitting available, it becomes possible to select a density functional that reproduces this property reasonably well. We have used a modified form of the B3PW91 functional, with a reduced 10% contribution of exact exchange (B3PW91**), in recent studies of the reactivity (24). This functional gives an energy splitting of $4.2 \text{ kcal mol}^{-1}$, relatively close to the accurate CCSD(T) value mentioned above (23,24).

One complication that arises for these ligand addition reactions is the existence of two different mechanisms, *direct* and *indirect*. The former of these is a single-step mechanism, in which the change from the triplet to the singlet surface occurs simultaneously with ligand addition (Scheme 4, left). As the ligand approaches the metal, the triplet surface rises in energy while the singlet surface goes down in energy, and the two can cross at an MECP, which we will refer to here as MECP_L (in some cases, we will more specifically refer to MECP_CO or MECP_H₂). The *indirect* mechanism occurs in two steps, with initial spin-state change from the triplet to the singlet in the absence of the



SCHEME 4.

TABLE I

B3PW91**/TZVP CALCULATED ENERGIES (kcal mol⁻¹ WITH ZPE CORRECTION, REF. (24)) FOR SPECIES ON THE SINGLET AND TRIPLET POTENTIAL ENERGY SURFACES FOR THE Fe(CO)₄+L LIGAND ADDITION REACTIONS (L = CO, H₂)

Species	L = CO	L = H ₂
³ Fe(CO) ₄ +L	0.0	0.0
¹ Fe(CO) ₄ +L	4.2	4.2
MECP_noL	6.3	6.3
¹ Fe(CO) ₄ L	-41.2	-16.3
MECP_L	-1.3	6.3

ligand, followed by spin-allowed ligand addition on the singlet surface (see Scheme 4, right). The first step requires distortion of triplet Fe(CO)₄ away from its equilibrium geometry toward an MECP with the singlet surface, which we refer to here as MECP_noL. As discussed below, addition of the ligand on the singlet surface is found to be barrierless.

The calculated data for the potential energy surfaces for addition of CO and H₂ are summarized in Table I (23,24,26). The B3PW91** functional gives reasonable agreement with CCSD(T) for the energy splitting in Fe(CO)₄, and with experiment for the bond energies of CO and H₂. Concerning the addition mechanism, it can be seen that the energy of the MECP for addition by the direct mechanism, MECP_L, is very different for the two ligands. With carbon monoxide, the triplet surface is weakly attractive, so the MECP lies slightly *below* the energy of ³Fe(CO)₄+CO, while for H₂, the triplet surface is more strongly repulsive, and MECP_H₂ lies higher in energy than the reactants. Note that the values in Table I are from Ref. (24), and are very slightly different from those presented in earlier

work (23,26), due to the use of a different functional (B3PW91**, instead of B3PW91* in which 15% of exact exchange is used). The initial product for the addition of H₂ is the singlet dihydrogen complex ¹Fe(CO)₄(H₂), which can convert over a low TS to the more stable isomeric dihydride, ¹Fe(CO)₄(H)₂ (25,26,71).

For the *indirect* mechanism, we located MECP_noL in more recent work (24). As can be seen, it lies somewhat higher in energy than ¹Fe(CO)₄, and at a very similar energy to MECP_H₂. This implies that the energy of the latter species is determined mainly by the requirement that the Fe(CO)₄ fragment needs to adopt a geometry at which the singlet and triplet states are very close in energy – the H₂ fragment interacts rather weakly with iron in both electronic states, as shown by the fairly large Fe–H distances of 2.384 and 2.313 Å at MECP_H₂. We checked that there is no barrier on the singlet potential energy surfaces for addition of H₂ or CO to ¹Fe(CO)₄.

Turning to the calculation of rate constants, our results are summarized in Table II. We consider first the direct addition mechanism. This is expected to lead to second-order kinetics, and

TABLE II

CALCULATED RATE CONSTANTS, ACTIVATION ENERGIES, EQUILIBRIUM CONSTANTS, AND RELATED EXPERIMENTAL DATA, FOR ADDITION OF CO AND H₂ TO ³Fe(CO)₄ AT 300 K

Experimental and computed data	CO	H ₂
Direct mechanism		
$k_{\text{direct}}/\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$	7.04×10^{-14}	1.71×10^{-17}
$E_{\text{A}}/\text{kcal mol}^{-1}$	−0.6	6.3
Indirect mechanism, low-concentration limiting conditions		
k_{31}/s^{-1}	5.12×10^6	5.12×10^6
k_{13}/s^{-1}	8.68×10^8	8.68×10^8
K_{31}	5.90×10^{-3}	5.90×10^{-3}
$k_{\text{add}}/\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$	2.20×10^{-11a}	1.03×10^{-11b}
$k_{\text{app}} (=K_{31}k_{\text{add}})/\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$	1.30×10^{-13}	6.07×10^{-14}
$E_{\text{A}}/\text{kcal mol}^{-1}$	2.9	2.9
$k_{\text{exp}}/\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$	5.2×10^{-14c}	1.97×10^{-14d}
Indirect mechanism, high-concentration limiting conditions		
$k_{\text{app}} (=k_{31})$	5.12×10^6	5.12×10^6
$E_{\text{A}}/\text{kcal mol}^{-1}$	6.3	6.3
$k_{\text{exp}}/\text{s}^{-1}$	$1.2\text{--}4 \times 10^{6e}$	3.3×10^{6e}

^aRef. (53,58).

^bRef. (76).

^cRef. (52,53) 5–55 Torr CO (10–55 Torr total), 295 K.

^dRef. (55) 50–500 Torr CO (550 Torr total), 296.5 K.

^eRef. (24).

this is indeed what had been observed for addition of CO and H₂ to Fe(CO)₄ in the gas phase (52,53,55,58). For addition of CO in *sc* Ar, it appears that second-order kinetics are also observed (42). Using a nonadiabatic form of TST (6,23), it is possible to calculate the second-order rate constant k_{direct} based on various properties of the MECP and the reactants. Specifically, one needs the relative energy of the MECP, the strength of coupling between the potential energy surfaces at the MECP, vibrational and rotational properties of the reactants and MECP, and various properties of the potential energy surfaces at the MECP such as the relative slope of the two surfaces (23). All these data are available, and hence it is possible to calculate k_{direct} for both addition of CO (23,24) and H₂ (24).

For addition of CO, it can be seen in Table II that the calculated (24) rate constant for the direct mechanism is in quite good agreement with the experimental value at room temperature. An earlier study, using a slightly different DFT method to characterize the MECP, reached a similar conclusion (23). As well as being close to the value measured in the gas-phase experiments, $5.8(\pm 1.5) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (52,53), it is also similar to the value measured in supercritical fluids, $2.0 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (42), suggesting that the gas-phase treatment used in the TST is broadly applicable to reactions in solution also, at least where the solvent does not play a major role as here. A more stringent test of the experimental data would be to try to reproduce the temperature dependence of the rate constant. Unfortunately, this is not available experimentally. Calculation of k_{direct} at a range of temperatures around 300 K and fitting to the Arrhenius equation yields very small temperature dependence, with an activation energy of $-0.6 \text{ kcal mol}^{-1}$. As there is only a negligible temperature dependence, the fact that the calculated rate constant is quite far from the gas-phase collisional rate must be due to an anomalously unfavorable activation entropy. It has been proposed that such unfavorable activation entropies are typical of spin-forbidden reactions (19).

We note at this point that the nonadiabatic-transition state method used here (6,19,77) is not expected to be able to give *quantitative* agreement with experimental rate constants. There are too many factors that are treated approximately (or not at all) in this theory for such performance to be possible. One of the key difficulties is that calculated rate constants are very sensitive to the accuracy of the potential energy surface: at room temperature, an error of 1 kcal mol^{-1} on the relative energy of the MECP relative to reactants will equate, roughly speaking, to an error by a factor of five on the calculated rate constant. Even though we

have taken great care to calibrate the B3PW91** DFT functional chosen here to explore potential energy surfaces, it is clear that in some cases at least, our calculated key energies will be wrong by at least this amount. There are also many errors similar to those found in regular TST (78,79), as well as specific errors associated with the treatment of the nonadiabatic behavior at the MECP (23,77). Hence, we treat agreement with experiment to within one order of magnitude for rate constants as being about as good as is really possible for what is ultimately a relatively simple theory. This accuracy is nevertheless enough to explore trends, predict mechanisms, and gain detailed insight into the effects determining reactivity in spin-forbidden processes.

The ability to interpret the calculated rate constant is one of the benefits of carrying out the calculations. It was noted (53) that the $^3\text{Fe}(\text{CO})_4 + \text{CO}$ reaction has a rate coefficient that is *ca.* 500 times smaller than that for $^3\text{Fe}(\text{CO})_3 + \text{CO}$. The NA-TST studies (23,24) suggest that this is due to two factors. First, it is possible to calculate the average probability for hopping from the reactant surface to the product surface, and this is found to be only of 1/20 (23) or 1/13 (24). The other factor is associated with differences in the potential energy profile along the reaction coordinate (monotonously attractive for $\text{CO} + \text{Fe}(\text{CO})_3$, weakly attractive then rising slightly to an MECP for $\text{CO} + \text{Fe}(\text{CO})_4$), and to differences in how the other degrees of freedom behave along the reaction coordinate (e.g., the vibrational frequencies are quite stiff at the MECP for the spin-forbidden reaction).

Calculation of the rate constant k_{direct} for addition of H_2 assuming the direct mechanism yields very poor agreement with experiment, as shown in Table II – the calculated value is more than three orders of magnitude smaller than the experimental one. This is mainly due to the high energy of MECP_ H_2 relative to reactants, which leads to a large predicted activation energy, and a small predicted rate constant. When MECP_ H_2 was first reported ((26), without a calculation of k_{direct}), it was already anticipated that its relatively high energy was not consistent with the observed significant reactivity of $\text{Fe}(\text{CO})_4$ with H_2 .

These observations, combined with experimental data concerning the activation energy in solution experiments (see below), led to the consideration of the indirect mechanism. As will be seen below, this mechanism does account for the observed reactivity with H_2 , and indeed with other species also, either on its own or in combination with the direct mechanism (24). In the context of this review, it is interesting to expend a few words regarding why this mechanism was not considered in the earlier work on this topic (23,26). There are in fact several reasons for this oversight

of what is in many respects a fairly obvious mechanism. First, the gas-phase experiments on the reaction with CO and other ligands yield second-order kinetics (50,52,53,58), and this is at first sight not what one would expect for a mechanism involving initial – presumably somewhat slow – spin-state change of the metal fragment followed by rapid barrierless ligand addition.

Second, many of the initial DFT calculations, especially those using the *a priori* quite reliable B3LYP functional (see e.g., (23)), and all of the older *ab initio* calculations (45,72), yield a large spin-state splitting for $\text{Fe}(\text{CO})_4$, such that the singlet state is kilocalories per mole than the triplet. Given the kinetic observation that ligand addition involves only a small activation energy (55), this seems to rule out any mechanism in which formation of the $^1\text{Fe}(\text{CO})_4$ intermediate is required. Third, for the first spin-forbidden reaction of $\text{Fe}(\text{CO})_4$ that was studied using NA-TST, addition of CO, the direct mechanism was assumed and the calculations gave good agreement with experiment (23). Fourth, it was found that the quantitative description of the singlet and triplet potential energy surfaces for $\text{Fe}(\text{CO})_4$ and $\text{Fe}(\text{CO})_5$ was very sensitive to the level of theory used (23), with little clear-cut experimental data available with which to discriminate between different methods. Given the known difficulties of DFT in particular with respect to providing an accurate description of potential energy surfaces for transition metal compounds (see (80,81) and Refs. therein), it was easy to assume that the difficulties found with respect to accounting for reactivity (26) were to be attributed to the calculated potential energy surfaces rather than to the assumed mechanism.

We now consider the results obtained for the *indirect* mechanism (for a reminder of this mechanism, see [Scheme 4](#)). The overall rate law for this mechanism is given by Eq. (1):

$$v = \frac{-d[^3\text{Fe}(\text{CO})_4]}{dt} = \frac{k_{31}k_{\text{add}}}{k_{13} + k_{\text{add}}[\text{L}]}[^3\text{Fe}(\text{CO})_4][\text{L}] \quad (1)$$

The first step of the reaction is not dependent on which ligand L is being considered, and to calculate the quantities k_{31} , k_{13} , and $K_{31}(=k_{31}/k_{13})$ that describe this initial step, only the properties of $^3\text{Fe}(\text{CO})_4$, $^1\text{Fe}(\text{CO})_4$, and MECP_noL are needed. In contrast, the second step involves L, so k_{add} is different for different ligands. As discussed below, however, this elementary rate constant adopts similar values for different ligands.

As mentioned above, MECP_noL lies $6.3 \text{ kcal mol}^{-1}$ above $^3\text{Fe}(\text{CO})_4$ at the B3PW91** level of theory, and its energy is

rationalized by the need for $^3\text{Fe}(\text{CO})_4$ to distort toward a geometry where it has the same energy as the singlet state. Using the NA-TST, it is possible to calculate the forward rate constant k_{31} , and we obtained a value of $5.12 \times 10^6 \text{ s}^{-1}$ at room temperature. Some care is needed to calculate the reverse rate constant, k_{13} , due to deficiencies in the calculated potential energy surfaces. High-level *ab initio* calculations (25) suggest a smaller singlet-triplet energy difference ($3.0 \text{ kcal mol}^{-1}$) than obtained at the B3PW91** level ($4.2 \text{ kcal mol}^{-1}$). Hence in the NA-TST calculations, the energy difference between $^1\text{Fe}(\text{CO})_4$ and the MECP was adjusted to $3.3 \text{ kcal mol}^{-1}$ so that, together with the energy difference between $^3\text{Fe}(\text{CO})_4$ and the MECP ($6.3 \text{ kcal mol}^{-1}$), it is consistent with the high-level CCSD(T) calculations (24). In principle, a different approach could have been used in which it was the energy difference between the $^3\text{Fe}(\text{CO})_4$ minimum and the MECP that was adjusted – or indeed both energy differences were adjusted. High-level CCSD(T) optimization of MECP_noL could in principle determine the energies to use unambiguously. Such calculations are not possible, although some test calculations at the geometry of the B3PW91**/TZV MECP_noL suggested that the option chosen here was of reasonable accuracy. Taking this approach, the value of k_{13} obtained at room temperature is $8.68 \times 10^8 \text{ s}^{-1}$, yielding an equilibrium constant K_{31} of 5.9×10^{-3} (24). Both forward and reverse rate constants are rather large, as expected given the relatively low energy barrier constituted by MECP_noL. K_{31} is as expected smaller than one, reflecting the higher stability of $^3\text{Fe}(\text{CO})_4$.

The next step required to predict the kinetic behavior is to compute k_{add} . For both CO and H_2 , it was found that there was no barrier or saddle point on the singlet potential energy surface to addition of the ligand to $^1\text{Fe}(\text{CO})_4$. This was demonstrated by carrying out a series of partial geometry optimizations of the system while keeping the Fe–C or the Fe–H distance frozen. The absence of a saddle point does not mean that there is no “transition state,” in the sense of a generalized geometry that represents a bottleneck to reaction. However, standard TST cannot be used to compute addition rate constants. Instead, these values can be obtained in two different ways. First, one can use some or other version of variational TST (78,79). We performed this using a simple rigid rotor–harmonic oscillator approach for both cases (24). At several values of the metal–ligand distance, projected vibrational frequencies orthogonal to the reaction coordinate were computed using the reaction path Hamiltonian (82) and combined with rotational constants to give a trial value of the calculated rate constant according to TST. The calculated

trial values at different values of the reaction coordinate are not all the same, and indeed a minimum value is obtained, which is taken to be an approximation to the correct rate constant.

For addition of CO, this procedure yields a room temperature value for k_{add} of $1.05 \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, whereas for H_2 , a value of $1.31 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ is obtained. Some caution is however required when using the results of these calculations, due to the use of the rigid rotor-harmonic oscillator approximation. The variational TSs located here both occur at fairly large Fe-ligand distances, 3.7 Å for the Fe-C distance in addition of CO, and 3.0 Å for the Fe-H distances in addition of H_2 . At these large distances, some of the harmonic frequencies in the directions orthogonal to the reaction coordinate, especially those corresponding to translation and rotation of the incoming ligand with respect to the metal fragment, are extremely small. For CO, the smallest harmonic frequencies at the variational TS are of 10, 17, and 46 cm^{-1} , whereas for H_2 , values of 47, 74, and 87 cm^{-1} are obtained. Such small frequencies, especially those found for the CO TS, are not well described in the harmonic oscillator limit, as they are better described as corresponding, e.g., to hindered rotations. Klippenstein *et al.* (79,83) have shown that rate constants calculated using the rigid rotor-harmonic oscillator approximation for barrierless processes can deviate from experiment quite considerably, even when very accurate potential energy surfaces are used. In most cases, the error leads to an overestimate of the rate constant. More advanced variational TST calculations are possible, but we instead chose the pragmatic route of using experimental values of the rate constants of similar barrierless processes for k_{add} .

For CO, this choice gives a rather different value of $2.20 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (the rate constant for the addition of CO to $^3\text{Fe}(\text{CO})_3$ (53,58)), which is approximately five times smaller than the value calculated using TST. For H_2 , the value chosen for k_{add} is $1.03 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, which corresponds to the rate constant for addition of H_2 to the $\text{Ru}(\text{dmpe})_2$ complex (76). In this case, the variational TST value was not very different. In both cases, the error involved in determining k_{add} can reliably be estimated to be smaller than that involved in calculating other properties.

The next difficulty in comparing the predictions of Eq. (1) with experiment is that experimental values are reported in terms of either second-order rate constants for the gas-phase experiments or pseudo-first-order rate constants for the solution experiments. According to Eq. (1), neither pure reaction order is correct nor should the apparent rate constant depend on the concentration or

pressure of ligand L. Analyzing the different experimental conditions, we realized that they correspond quite closely to the two limiting conditions for Eq. (1), namely the low and high concentration limits (24).

In the gas-phase experiments carried out by Weitz (52,53, 55,58), the range of ligand pressures used was of 5–50 Torr (CO) and 50–500 Torr (H₂). With these pressures, the concentration of ligand is such that the term $k_{\text{add}}[\text{L}]$ appearing in the denominator of Eq. (1) is significantly smaller than the term k_{13} . For example, for the highest pressure used in the experiments with CO, the density of CO was 2×10^{18} molecules cm⁻³, which makes the maximum value for $k_{\text{add}}[\text{CO}]$, *ca.* 4×10^7 s⁻¹, still significantly smaller than the calculated value of k_{13} of 8.68×10^8 s⁻¹. This means that Eq. (1) can be simplified:

$$\begin{aligned} v &= \frac{k_{31}k_{\text{add}}}{k_{13} + k_{\text{add}}[\text{L}]} [\text{}^3\text{Fe}(\text{CO})_4][\text{L}] \approx \frac{k_{31}k_{\text{add}}}{k_{13}} [\text{}^3\text{Fe}(\text{CO})_4][\text{L}] \\ &= K_{31} \cdot k_{\text{add}} [\text{}^3\text{Fe}(\text{CO})_4][\text{L}] \end{aligned} \quad (2)$$

At low concentrations, the kinetics should therefore be second-order, first-order with respect to both iron tetracarbonyl and ligand, as is indeed experimentally observed (52,53,55,58). Another way of viewing the simplification in Eq. (2) is that it assumes that the rate-limiting step in the mechanism of [Scheme 4](#) is the addition of L to singlet Fe(CO)₄. This might appear to be a strange assumption, given the barrierless nature of this addition, but it must be borne in mind that this is a bimolecular step, whereas the competing spin-state change back to ³Fe(CO)₄ is unimolecular, and has a large rate constant. In the relatively dilute gas phase, it can therefore compete effectively with the addition, thereby setting up a preequilibrium between singlet and triplet states of Fe(CO)₄, out of which the singlet can be trapped by L.

For CO, calculation according to Eq. (2) yields an apparent rate constant at 300 K, k_{app} of 1.30×10^{-13} cm³ molecule⁻¹ s⁻¹ ([Table II](#)), which is in good agreement with the experimental value reported under these conditions. For H₂, the calculated k_{app} is 6.07×10^{-14} cm³ molecule⁻¹ s⁻¹, which is in quite good agreement with the experimental rate constant of $1.97(\pm 0.15) \times 10^{-14}$ cm³ molecule⁻¹ s⁻¹ (see [Table II](#)). In both cases, the activation energies around 300 K were computed (making the reasonable assumption that the temperature dependence of k_{add} is negligible, and using Arrhenius analysis). For addition of both CO and H₂, a small value of 2.9 kcal mol⁻¹ is obtained ([Table II](#)). Experimentally, an increase in the rate constant for addition of

H_2 to $\text{Fe}(\text{CO})_4$ from $(1.97 \pm 0.15) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ to $(2.47 \pm 0.49) \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ was measured upon increasing the temperature from 296.5 K to 315 K (55). Although the experimental error bars preclude reaching quantitative conclusions about the activation energy based on these two results, it is remarkable that the ratio of these values, 1.25, is very close to the ratio of the calculated k_{app} values at these temperatures, 1.32. Additional measurements of the temperature dependence could provide a more stringent test of these theoretical predictions.

One prediction of the theoretical model described here is that almost *all* ligands should add to $^3\text{Fe}(\text{CO})_4$ in the gas phase under low-concentration conditions with second-order kinetics and a rate constant close to $5 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$. The only exceptions would be ligands that are able to add by a direct mechanism with a larger rate constant (see a discussion of the case of CO below). Some variation in the rate constant is expected, as k_{add} is not identical for all ligands, but this is a small effect. Considering the rate constants reported for several ligands that we have not studied computationally, it can be seen that this prediction is indeed confirmed experimentally. C_2H_4 , C_2F_4 , and N_2 all react with $^3\text{Fe}(\text{CO})_4$ with rate constants very close to the predicted value: 1.7×10^{-13} , 1.3×10^{-14} , and $2.8 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, respectively (51). Some larger ligands react slightly faster, e.g., 1,3-pentadiene and triethylamine (1.16×10^{-12} and $8 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, respectively) (51). We note that these ligands also react much faster with $\text{Fe}(\text{CO})_3$ than does CO, so k_{add} for these species probably is also quite large.

In other experiments, the kinetics were studied in supercritical fluids (24,42) in the presence of very high partial pressures or concentrations of ligand. Under these conditions, the term $k_{\text{add}}[\text{L}]$ is somewhat larger than k_{13} , and Eq. (1) can be simplified to:

$$v = \frac{k_{31}k_{\text{add}}}{k_{13} + k_{\text{add}}[\text{L}]} [^3\text{Fe}(\text{CO})_4][\text{L}] \approx k_{31}[^3\text{Fe}(\text{CO})_4] \quad (3)$$

This equation applies provided that it is the initial spin-state change from $^3\text{Fe}(\text{CO})_4$ to $^1\text{Fe}(\text{CO})_4$ that is rate-limiting, with rapid and quantitative trapping by the ligand present in high concentration. In this case, the observed kinetics will be independent of the ligand L and of its concentration, and one will simply obtain first-order decay with a rate constant equal to k_{31} , of $5 \times 10^6 \text{ s}^{-1}$ at 300 K. This predicted behavior is quite well supported by experiment. Trapping of $^3\text{Fe}(\text{CO})_4$ by Xe occurs with a k_{obs} of $2.0 \pm 0.2 \times 10^7 \text{ s}^{-1}$ in both *sc* Xe, and Xe doped into *sc*

Ar (42), in both cases within a factor of four of the theoretical prediction. Also, a similar decay rate was obtained in *sc* CH₄ ($6.2 \pm 0.6 \times 10^6 \text{ s}^{-1}$), and in heptane ($8.1 \pm 0.7 \times 10^7 \text{ s}^{-1}$) (42). The observed decay rate constant in *sc* Ar doped with H₂, $3.3 (\pm 0.1) \times 10^6 \text{ s}^{-1}$, mentioned above, is also close to these values. Finally, in *sc* Ar doped with carbon monoxide, the rate of decay is $5.3 \times 10^6 \text{ s}^{-1}$ (42). All of these values are in reasonable agreement with the computed rate constant k_{31} considering the many uncertainties involved in predicting the latter, associated with errors in the computed energy of MECP_noL and in the NA-TST itself.

We note that $k_{\text{add}}[\text{L}]$ is certainly larger than k_{13} in pure supercritical solvent; however, in other experiments, an inert solvent such as *sc* Ar is used, and the ligand is present in lower (if still substantial and much higher than stoichiometric) concentration. In these cases, $k_{\text{add}}[\text{L}]$ is not much larger than k_{13} , and a small proportion of the $^1\text{Fe}(\text{CO})_4$ formed may return to $^3\text{Fe}(\text{CO})_4$ rather than undergoing trapping by L. In this intermediate concentration regime, the pseudo-first-order rate constant for disappearance of $^3\text{Fe}(\text{CO})_4$ and appearance of $^1\text{Fe}(\text{CO})_4\text{L}$ may vary slightly with ligand concentration. For example, the experiments with H₂ use dihydrogen doped into *sc* Ar, with a hydrogen density of *ca.* $1.5 \times 10^{20} \text{ molecules cm}^{-3}$ (24), leading to a $k_{\text{add}}[\text{H}_2]$ value of *ca.* $1.5 \times 10^9 \text{ s}^{-1}$, just 1.7 times larger than the calculated k_{13} . Using these values, and applying the full Eq. (1), leads to a slightly smaller predicted pseudo-first-order rate constant for disappearance of $^3\text{Fe}(\text{CO})_4$, of $3.1 \times 10^6 \text{ s}^{-1}$, in remarkably good agreement with the experiment value of $3.3 (\pm 0.1) \times 10^6 \text{ s}^{-1}$.

For CO, the decay rate was measured in *sc* Ar for five different CO concentrations between *ca.* $7 \times 10^{19} \text{ molecule cm}^{-3}$ and $2 \times 10^{20} \text{ molecule cm}^{-3}$ (42). These concentrations correspond to $k_{\text{add}}[\text{CO}]$ values of 1.6×10^9 to $4.4 \times 10^9 \text{ s}^{-1}$, respectively – again larger than k_{13} , but not much so. Applying the full Eq. (1) to the five concentration values gives predicted pseudo-first-order decay rate constants varying almost linearly from $3.3 \times 10^6 \text{ s}^{-1}$ to $4.3 \times 10^6 \text{ s}^{-1}$. The experimental results also show a near-linear variation in the rate constant, from *ca.* $3.8 \times 10^6 \text{ s}^{-1}$ to *ca.* $6.3 \times 10^6 \text{ s}^{-1}$, fairly close to the values predicted by the calculations, especially when taking into account the errors involved in the TST method. We note that in the experimental paper (42), the measured pseudo-first-order decay rates were interpreted as corresponding to second-order behavior, with a rate constant of $2.0 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, but within the experimental error. The errors on the computed rate constants, Eq. (1) provides an equally good fit.

It should be noted that some experiments lead to significantly faster observed decay of $^3\text{Fe}(\text{CO})_4$ than is predicted here. Reactions in neat methanol (84) and triethylsilane (40) occur with a first-order decay rate of $2.4 \times 10^{10} \text{ s}^{-1}$ and $8.3 \times 10^8 \text{ s}^{-1}$, respectively. Reaction of $\text{Fe}(\text{CO})_4$ with triethylphosphine (84) is apparently too fast to measure. The latter observation may be due to trapping of $^1\text{Fe}(\text{CO})_4$ before it can relax to the triplet (38). It is also possible that for some of these species, there are significant solvent effects that lead to the faster decay. Indeed, the potential energy surfaces and rate constants are calculated for the gas phase, and are used to try to account for experiments carried out in solution. We must distinguish between two types of solvent effect here. First, "solvents" such as heptane or Xe are clearly not at all inert and change the potential energy surfaces in a significant way by binding to $^1\text{Fe}(\text{CO})_4$. In some experimental papers, such solvents are described as having a significant effect on the observed rate of conversion of $\text{Fe}(\text{CO})_4$ (singlet and triplet) to $\text{Fe}(\text{CO})_5$ in the presence of CO. We prefer to describe this as the outcome of a sequence of elementary steps including " $\text{Fe}(\text{CO})_4$ " species that are in fact better described as $\text{Fe}(\text{CO})_4\text{L}$. This type of effect *is* included in the calculations. But a second type of effect is *not* included: although singlet and triplet $\text{Fe}(\text{CO})_4$ are rather nonpolar species, they will interact with solvent (e.g., by hydrogen bonding, etc.) and this interaction may be different for the singlet than for the triplet. This effect can shift the potential energy surfaces with respect to each other by fractions of a kilocalorie per mole, or even more in the case of methanol solvent. In turn, this can make significant changes to k_{31} .

We close this section by discussing the special case of addition of CO. As for the other ligands, the calculated k_{app} for the indirect mechanism, under low-concentration gas-phase conditions ($1.30 \times 10^{-13} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$), gives good agreement with experiment ($5.2 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, Table II). However, for this ligand only, as MECP_L is quite low in energy (Table I), the calculated k_{direct} ($7.04 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$) is also in good agreement with experiment, although it is slightly smaller. Both calculated rate constants are within much better than one order of magnitude of experimental values, and as mentioned earlier, such accuracy is somewhat better than can really be expected from these methods. Hence it is difficult to conclude which of the two mechanisms is actually favored, and this would be true even if new, accurate measurements of the gas-phase rate constant at several different temperatures could be carried out. The conservative conclusion we reach is that either mechanism could apply, or indeed that both could contribute to the observed reactivity.

C. COMPUTATIONAL STUDIES: ADDITION TO $\text{Fe}(\text{CO})_3$

As discussed above, iron tricarbonyl is a very reactive species which is formed upon single-photon photodissociation of iron pentacarbonyl both in the gas phase and in solution. It has a triplet ground state, and is known to undergo many facile ligand addition reactions. In this section, we discuss three typical reactions of $\text{Fe}(\text{CO})_3$, namely addition of the three species, CO, C_2H_4 , and H_2 . Experimentally, all three reactions are known to proceed very rapidly, with rate constants of the order of 10^{-10} – $10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (49,53). At first sight, this suggests that these reactions are spin-allowed and barrierless.

We have explored the energy surface for the addition of CO to $\text{Fe}(\text{CO})_3$ at the same B3PW91**/TZV level we used in the other work on iron carbonyls discussed here (85). We find a similar C_{3v} structure for $\text{Fe}(\text{CO})_3$ as found in previous work on this species (70,72,74), with a $^3\text{A}_2$ ground state. The bond energy for the fourth carbonyl ligand is $35.3 \text{ kcal mol}^{-1}$, again similar, as expected, to that found by others in previous work (70,72). We have shown that the energy surface for binding the fourth ligand does not involve a barrier, by carrying out a series of geometry optimization calculations while freezing the Fe–C distance for the incoming ligand. The minimum energy path involves approach of the CO ligand along the threefold axis of symmetry of $\text{Fe}(\text{CO})_3$ until the Fe–C distance falls below $2.4 \text{ \AA}$, at which point the C_{3v} symmetry is broken, as the CO moves off the axis to lead toward the C_{2v} geometry of the $^3\text{Fe}(\text{CO})_4$ minimum.

We also carried out variational TST calculations of the high-pressure limiting rate constant for the addition by computing vibrational frequencies orthogonal to the reaction path using the reaction path Hamiltonian, as discussed above (82). We located a variational TS for an Fe–C distance of $3.69 \text{ \AA}$, rather similar to that for the analogous $\text{CO} + ^1\text{Fe}(\text{CO})_4$ reaction mentioned above. The calculated rate constant corresponding to this TS is $3 \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, similar to but somewhat larger than the experimentally determined value of $2.2 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ obtained by Weitz *et al.* (53). The overestimate of the rate constant is probably due to the approximate nature of the harmonic oscillator approximation for the very small harmonic frequencies found for the motion of the CO away from the minimum energy path; the smallest of these at the variational TS are of only 13 cm^{-1} . As already mentioned above, the fact that using a reaction-path-based harmonic oscillator model for calculating variational rate constants for barrierless processes tends to lead to incorrect results, usually larger than experiment,

has been noted before (79,83). Nevertheless, the present calculations confirm the picture whereby addition of CO to $\text{Fe}(\text{CO})_3$ is a barrierless, spin-allowed process that occurs with a near gas-kinetic rate constant, close to the high-pressure limit, under the experimental conditions used to study it (53).

We now turn to the addition of hydrogen to $^3\text{Fe}(\text{CO})_3$. This is hypothesized to be the process that leads to the short-lived species formed in *sc* Ar upon photolysis of iron pentacarbonyl in the presence of H_2 (24). It is however possible that $\text{Fe}(\text{CO})_3$ is present instead in the form of the weakly bound $^3\text{Fe}(\text{CO})_3(\text{Ar})$ species under these conditions. However, the reaction has also been studied in the gas phase, where a room-temperature rate constant of $2.7 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ was reported (49,55), again corresponding to a very large, near collision-limit value.

Here too, we have explored this reaction using B3PW91**/TZV calculations (85), and this shows that this process is more complex than the CO addition. The initial addition can lead to a triplet dihydrogen complex, $^3\text{Fe}(\text{CO})_3(\text{H}_2)$, which lies 9.8 kcal mol^{-1} lower in energy than the reactants. The geometry for this species is similar to that described previously by others (86), roughly corresponding to a truncated trigonal bipyramid, with an equatorial H_2 ligand and a missing axial CO. As for the case of CO, we carried out a series of geometry optimizations along the coordinate for H_2 addition to $^3\text{Fe}(\text{CO})_3$, by holding one of the Fe–H distances frozen at a set of increasing smaller values. This confirms that there is no energy barrier to addition, and rationalizes the observed large rate constant for addition. In this case, we have not carried out variational TST calculations as we expect a pattern similar to that observed for the other similar barrierless processes we have studied.

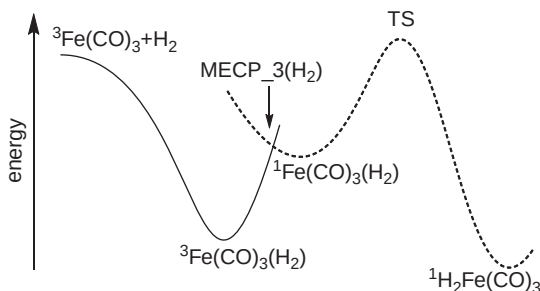
The $^3\text{Fe}(\text{CO})_3(\text{H}_2)$ species is the one that can be formed most readily from the separated reactants, but there are several other minima on the $[\text{Fe}, \text{C}_3, \text{O}_3, \text{H}_2]$ singlet and triplet potential energy surfaces that could conceivably also be formed in the reaction. Most notably, we located isomers $^1\text{Fe}(\text{CO})_3(\text{H}_2)$, $^3\text{Fe}(\text{CO})_3(\text{H})_2$, and $^1\text{Fe}(\text{CO})_3(\text{H})_2$, located, respectively, at 4.8, 14.9, and -1.4 kcal mol^{-1} with respect to $^3\text{Fe}(\text{CO})_3(\text{H}_2)$ (see Table III and Scheme 5, and Ref. (24)). The triplet dihydride species lies higher in energy than separated H_2 and $\text{Fe}(\text{CO})_3$, and hence is unlikely to be formed in this system. However, the two singlet species are lower in energy and might be formed; hence, we have also explored the pathways for interconversion.

The first important step involves conversion of triplet $\text{Fe}(\text{CO})_3(\text{H}_2)$ to the corresponding singlet; this occurs through an MECP (MECP_3(H_2)) which is located 6.6 kcal mol^{-1} higher in

TABLE III

B3PW91** CALCULATED RELATIVE POTENTIAL ENERGIES (kcal mol⁻¹ WITH ZPE CORRECTION) FOR Fe(CO)₃(H)₂ AND Fe(CO)₃(H₂) SPECIES (24)

³ Fe(CO) ₃ (H ₂)	0.0	MECP_3(H ₂)	6.6
¹ Fe(CO) ₃ (H ₂)	4.8		
³ Fe(CO) ₃ (H) ₂	14.9	¹ TS[Fe(CO) ₃ (H ₂) → Fe(CO) ₃ (H) ₂]	10.6
¹ Fe(CO) ₃ (H) ₂	-1.4		



SCHEME 5.

energy than the triplet minimum. Next, there is a TS for oxidative addition of H₂ to iron, leading to the dihydride species ¹Fe(CO)₃(H)₂. This TS lies 10.6 kcal mol⁻¹ higher in energy than the triplet dihydrogen complex, and is hence *higher* in energy than the separated reactants ³Fe(CO)₃+H₂. It seems likely, therefore, that the ultimate product formed in the addition of H₂ to iron tricarbonyl is the triplet dihydrogen complex. This is supported by experimental observations both in the gas phase (49) and in supercritical Ar (24).

Finally, we discuss the addition of ethylene to triplet iron tricarbonyl, and again report here new results at the B3PW91**/TZV level of theory (87), together with some correlated *ab initio* calculations. The initial step here is addition on the triplet surface to form ³Fe(CO)₃(C₂H₄) in an exothermic process associated with a ligand binding energy of 25.7 kcal mol⁻¹. The geometry of this species is shown below in Fig. 1; it is very similar to that previously reported by Kismartoni *et al.* (86). It corresponds loosely to a truncated trigonal bipyramid structure, with a missing axial CO ligand, and a roughly equatorial ethylene ligand. The C–C bond is oriented along the notional C₃ axis of the trigonal bipyramid. The Fe–C bond lengths to the ethene ligand are quite short (2.134 and 2.197 Å), and there is

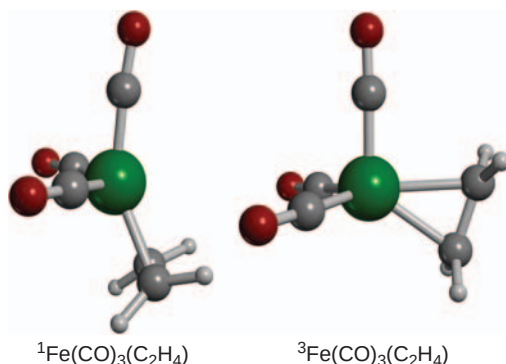
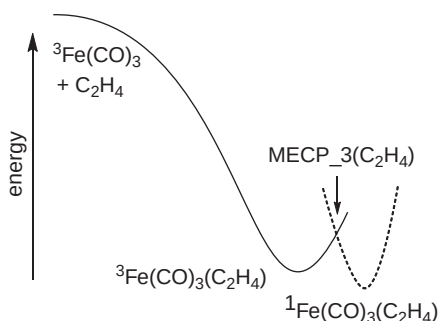


FIG. 1. Optimized geometries for the singlet (left) and triplet (right) states of $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$.



SCHEME 6.

extensive pyramidalization of the alkene carbons, corresponding to strong donation of π electrons from the ligand to the metal, and strong back-bonding from a metal d orbital into the alkene π^* orbital. The electronic state is $^3A''$, corresponding to singly occupied orbitals of a' and a'' symmetry, respectively. Based on the previous examples, we expect addition to be barrierless, and this also fits in well with the observed experimental rate constant of $2.2 \times 10^{-10} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (49).

In previous work, it has been assumed that $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$ has a triplet ground state, by analogy to $\text{Fe}(\text{CO})_4$. A previous computational study indeed predicted a triplet ground state (86). This would also explain the rapid nature of the ligand addition, since it would then be spin-allowed (49,50). At the B3PW91**/TZV level of theory, however, we find that there is also a singlet minimum for this species, lying $0.6 \text{ kcal mol}^{-1}$ lower in energy than the triplet ((87), Table IV and Scheme 6). This species can

TABLE IV

CALCULATED B3PW91** RELATIVE POTENTIAL ENERGIES (kcal mol⁻¹ WITH ZPE CORRECTION) OF Fe(CO)₃(C₂H₄) SPECIES

³ Fe(CO) ₃ (C ₂ H ₄)	0.0	MECP Fe(CO) ₃ (C ₂ H ₄)	4.2
¹ Fe(CO) ₃ (C ₂ H ₄)	-0.6		

also be loosely described as a truncated trigonal bipyramid, but it is now an equatorial CO that is missing, and the ethene ligand is better described as being axial. The Fe–C bond lengths to the ethene ligand are shorter than for the triplet (2.068 Å). The calculated spin-state splitting with a singlet ground state is surprising at first sight, given the widespread assumption that this complex has a triplet ground state, and we therefore sought to attempt to confirm the B3PW91** result.

First, we recalculated the spin-state splitting using three different DFT functionals: B3LYP, B3PW91, and B3PW91* (like B3PW91**, this is a modified form of the B3PW91 functional, in this case with 15% of exact exchange, as compared to 20% for B3PW91 and 10% for B3PW91**). As found for many metal complexes (80), larger amounts of exact exchange, as here for B3LYP, B3PW91, and B3PW91*, lead to a lower energy for the triplet, which is therefore calculated to be the ground state with these other three functionals, with an energy splitting of, respectively, 8.3, 4.6, and 1.9 kcal mol⁻¹. This casts some doubt on our assignment, based on B3PW91** calculations, of a singlet ground state.

However, for Fe(CO)₄, we found previously (23,25) that these functionals with 20% or 15% of exact exchange overestimate the relative stability of the triplet state. B3PW91** is more accurate, but if anything, it too underestimates the energy of the triplet. Given the very similar electronic structures of the two complexes, one might also have more confidence in the B3PW91** prediction for Fe(CO)₃(C₂H₄). Preliminary CCSD(T) calculations similar to those carried out for Fe(CO)₄ (25) also suggest a singlet ground state for the alkene complex. As discussed below, we have indirect evidence also for the singlet ground state from reactivity of Fe(CO)₃(C₂H₄) (Table VI).

Based on this tentative assignment of Fe(CO)₃(C₂H₄) as having a singlet ground state, addition of ethylene to Fe(CO)₃ now becomes a spin-forbidden process, which might seem inconsistent with the observed rapid addition. However, as mentioned above, addition on the triplet surface should be barrierless, and hence rapid. Conversion of the initially formed triplet adduct to the

singlet can also occur readily, as there is an MECP between the singlet and triplet potential energy surfaces lying $22.1 \text{ kcal mol}^{-1}$ lower in energy than separated $^3\text{Fe}(\text{CO})_3$ and C_2H_4 , and thereby just $4.2 \text{ kcal mol}^{-1}$ above the triplet $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$ minimum ((87) and Scheme 6). This reaction is similar to some other spin-forbidden reactions that we have discussed in previous work, such as addition of CO to $^3\text{Tp}'\text{Co}(\text{CO})$ (15), in which there is no barrier to the initial addition on the high-spin surface, and a very low energy crossing enabling the system to reach the final low-spin adduct.

D. COMPUTATIONAL STUDIES: ADDITION TO $\text{Fe}(\text{CO})_3\text{L}$ SPECIES

Photolysis of $\text{Fe}(\text{CO})_4\text{L}$ species can lead to loss of CO, with formation of $\text{Fe}(\text{CO})_3(\text{L})$, and the latter can also be formed, as just discussed, by addition of L to iron tricarbonyl. In this section, we discuss the kinetics of addition of a second ligand L' to form singlet species of general formula $^1\text{Fe}(\text{CO})_3(\text{L})(\text{L}')$. We consider three cases: addition of CO to $^3\text{Fe}(\text{CO})_3(\text{H}_2)$, and addition of CO or ethylene to $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$.

With reference to Scheme 3, it can be seen that the net process of ligand addition can occur in several different ways. Where the species $\text{Fe}(\text{CO})_3\text{L}$ has a triplet ground state, one can have either: (i) direct spin-forbidden addition of L' (Scheme 3); (ii) spin-crossover from $^3\text{Fe}(\text{CO})_3(\text{L})$ to $^1\text{Fe}(\text{CO})_3(\text{L})$, followed by rapid addition of L' (Scheme 3); or (iii) spin-allowed substitution reaction between L' and $^3\text{Fe}(\text{CO})_3(\text{L})$ to give $^3\text{Fe}(\text{CO})_3(\text{L}')$ and L, crossover to the singlet surface to form $^1\text{Fe}(\text{CO})_3(\text{L}')$, followed by L addition (Scheme 3). Some precedent for the key substitution step in the latter mechanism is available from experiments in which photolysis of $\text{Fe}(\text{CO})_5$ in the presence of phosphines L led to $\text{Fe}(\text{CO})_3(\text{L})_2$, apparently due to substitution of CO by L in the intermediate $\text{Fe}(\text{CO})_4$ (31,84,88). If instead $\text{Fe}(\text{CO})_3\text{L}$ has a singlet ground state, then one expects that $\text{Fe}(\text{CO})_3(\text{L})(\text{L}')$ formation to occur simply by spin-allowed ligand addition.

Net addition of CO to $^3\text{Fe}(\text{CO})_3(\text{H}_2)$ to form $\text{Fe}(\text{CO})_4(\text{H})_2$ was observed upon photolysis of $\text{Fe}(\text{CO})_5$ in *sc* Ar in the presence of H_2 (24). The intermediate species $^3\text{Fe}(\text{CO})_3(\text{H}_2)$ is involved as a minor product of the photolysis, and was proposed to arise from addition of H_2 to $\text{Fe}(\text{CO})_3$ or $\text{Fe}(\text{CO})_3\text{Ar}$ (24). Experimentally, this species was shown to decay in the presence of excess CO with a pseudo-first-order rate constant $k_{\text{obs}} = 4.1(\pm 0.3) \times 10^7 \text{ s}^{-1}$. The mechanism for this spin-forbidden process was however unclear from experiment alone, and computation was used to explore the various possibilities (24).

TABLE V

B3PW91**/TZV CALCULATED RELATIVE POTENTIAL ENERGIES (kcal mol⁻¹ WITH ZPE CORRECTION) FOR Fe(CO)₃+H₂+CO SPECIES (24,85)

³ Fe(CO) ₃ (H ₂)+CO	0.0	¹ Fe(CO) ₄ H ₂	-48.2
³ Fe(CO) ₄ +H ₂	-25.4	¹ Fe(CO) ₄ (H ₂)	-41.7
¹ Fe(CO) ₄ +H ₂	-21.3	MECP	6.1
		(Fe(CO) ₃ (H ₂)-CO)	
TS[³ Fe(CO) ₃ (H ₂)+CO → ³ Fe(CO) ₄ +H ₂]	-0.2	³ Fe(CO) ₃ +H ₂ +CO	9.8
		¹ Fe(CO) ₃ +H ₂ +CO	24.0

Three different mechanisms were considered: (i) direct spin-forbidden addition of CO; (ii) spin-state change from ³Fe(CO)₃(H₂) to ¹Fe(CO)₃(H₂) or ¹Fe(CO)₃(H)₂, followed by rapid addition of CO; and (iii) spin-allowed substitution reaction between CO and ³Fe(CO)₃(H₂) to give ³Fe(CO)₄ and H₂, followed by spin-forbidden addition of H₂ to ³Fe(CO)₄. The results are summarized in Table V.

For the first proposed mechanism (i) the MECP between singlet and triplet Fe(CO)₃(H₂)+CO surfaces was found. As the triplet surface is repulsive the MECP lies higher in energy at 6.9 kcal mol⁻¹. As discussed above for direct spin-forbidden addition of H₂ to Fe(CO)₄, such a high MECP inevitably leads to very low bimolecular rate constants, of the order of 10⁻¹⁷ cm³ molecule⁻¹ s⁻¹, which under the conditions of the experiment (CO concentration of the order of 10²⁰ molecule cm⁻³) would lead to a decay much slower than that observed experimentally. For mechanism (ii) which is analogous to the indirect mechanism discussed above, with spin change and then fast addition of CO, an MECP between the singlet and triplet energy surfaces of the dihydrogen complex Fe(CO)₃(H₂) was found lying 7.4 kcal mol⁻¹ above the triplet minimum. This is somewhat higher than the relative energy of the MECP for Fe(CO)₄, so a rate constant for spin-crossover *k*₃₁ somewhat smaller than that calculated in that case (5 × 10⁶ s⁻¹), can be expected. Assuming that trapping by CO on the singlet surface is fast, the spin-crossover would be irreversible, and *k*₃₁ should then match the observed decay rate. The observed rate constant of 4 × 10⁷ s⁻¹ is eight times larger than this, so while mechanism (ii) is clearly more plausible than mechanism (i), a significant error in the calculated MECP energy would be required for this to account for the experimental observations.

This leaves mechanism (iii), triplet state substitution of H₂ to form ³Fe(CO)₄ and then reaction of the latter with H₂, as discussed in a previous section. We found a TS for a concerted

substitution process lying $0.2 \text{ kcal mol}^{-1}$ lower in energy than separated ${}^3\text{Fe}(\text{CO})_3(\text{H}_2)$ and CO. This TS has a very early geometry, similar to that of the starting $\text{Fe}(\text{CO})_3(\text{H}_2)$, with the incoming CO approaching roughly along the axis of the "missing" axial CO ligand, but slightly displaced so as to interact with the H_2 ligand, and with an Fe–C distance that is still very large, at $4.081 \text{ \AA}$. It is possible to calculate a bimolecular rate constant for the substitution process using standard TST, and the properties of the reactants as well as this TS. The value obtained at 300 K is $3.07 \times 10^{-11} \text{ cm}^3 \text{ molecules}^{-1} \text{ s}^{-1}$, which, with the number density of CO in the experiments (24) of $1.06 \times 10^{20} \text{ molecules cm}^{-3}$, would lead to a predicted pseudo-first-order rate of decay of $3.25 \times 10^9 \text{ s}^{-1}$, almost 80 times faster than observed experimentally.

Although the located TS is a saddle point on the energy surface, it occurs in a very flat region of the potential energy surface, as shown by the small magnitude of the imaginary frequency at the TS ($-9i \text{ cm}^{-1}$) and by the presence of two very low vibrational frequencies (4 and 22 cm^{-1}). This suggested that variational effects might be important, and accordingly, we carried out variational TST calculations at a number of points along the reaction coordinate, defined here as the Fe–C distance. For this purpose, geometry optimization was carried out at several values of this coordinate, and vibrational frequencies orthogonal to the reaction path (82) were computed (85). The variational minimum for the calculated rate constant at room temperature occurs for a much "tighter" TS, with an Fe–C distance of $2.88 \text{ \AA}$ (vs. $4.081 \text{ \AA}$ for the saddle point). At this point, the motion of the H_2 ligand away from the iron is more pronounced than at the TS, with Fe–H distances of 1.739 and $1.751 \text{ \AA}$ in ${}^3\text{Fe}(\text{CO})_3(\text{H}_2)$, of 1.747 and $1.759 \text{ \AA}$ at the saddle point, and of 1.769 and $1.782 \text{ \AA}$ in this variational transition state. The potential energy at this point ($1.1 \text{ kcal mol}^{-1}$ below reactants) is somewhat lower than that at the saddle point ($0.2 \text{ kcal mol}^{-1}$ below reactants). Due to much tighter vibrational motions at this point, the calculated rate constant is however significantly smaller than that at the saddle point, $1.7 \times 10^{-12} \text{ cm}^3 \text{ molecules}^{-1} \text{ s}^{-1}$. Combined with the experimental CO number density, this leads to a predicted pseudo-first-order rate of decay of $1.8 \times 10^8 \text{ s}^{-1}$, in much better agreement with the experimentally observed rate of decay of $4.1 \times 10^7 \text{ s}^{-1}$.

Clearly, mechanism (iii) can indeed account for the loss of ${}^3\text{Fe}(\text{CO})_3(\text{H}_2)$ in the presence of CO. Other support for this mechanism comes from the experimental observation (24) of an increase in concentration of $\text{Fe}(\text{CO})_4$ with a timescale similar to that of decay of $\text{Fe}(\text{CO})_3(\text{H}_2)$.

TABLE VI

B3PW91**/TZV CALCULATED RELATIVE ENERGIES (kcal mol⁻¹ WITH ZPE CORRECTION)
FOR ¹Fe(CO)₃(C₂H₄)+CO AND ¹Fe(CO)₃(C₂H₄)+C₂H₄ REACTIONS

Addition of CO	Relative energies	Addition of C ₂ H ₄	Relative energies
¹ Fe(CO) ₃ (C ₂ H ₄)+CO	0.0	¹ Fe(CO) ₃ (C ₂ H ₄)+C ₂ H ₄	0.0
TS _L ¹ Fe(CO) ₃ (C ₂ H ₄) · CO	1.7	TS _L ¹ Fe(CO) ₃ (C ₂ H ₄) · C ₂ H ₄	1.5
¹ Fe(CO) ₄ (C ₂ H ₄)	-37.9	¹ Fe(CO) ₃ (C ₂ H ₄) ₂	-22.2
¹ Fe(CO) ₄ (C ₂ H ₄) (ax)	-32.1	¹ Fe(CO) ₃ (C ₂ H ₄) ₂ (ax, eq)	-17.4

We now consider addition of CO and C₂H₄ to Fe(CO)₃(C₂H₄), for which the computational results are presented in Table VI. In the experimental report of the kinetics of these reactions in the gas phase (49,50), it was suggested that these processes were spin-forbidden, as the observed rate constants ($4.3(\pm 0.8) \times 10^{-12}$ cm³ molecules⁻¹ s⁻¹ (50) and $1.1(\pm 0.3) \times 10^{-11}$ cm³ molecules⁻¹ s⁻¹ (49)) are somewhat smaller than those of similar spin-allowed reactions (e.g., addition of CO to ³Fe(CO)₃, $k = 2.2(\pm 0.3) \times 10^{-11}$ cm³ molecules⁻¹ s⁻¹). The difference between these three rate constants is however rather small, and all are significantly larger than the values measured for addition of ligands to ³Fe(CO)₄, which are well known to involve spin-state change and hence be spin-forbidden. As discussed above, although there has been a general assumption that Fe(CO)₃(C₂H₄) would have a triplet ground state, like Fe(CO)₄ (see e.g., (86)), our B3PW91** calculations (87) in fact predict a singlet ground state. This raises the question of why the rate constants for spin-allowed addition of CO or C₂H₄ would be smaller than those for some related spin-allowed processes. Accordingly, we have explored the potential energy surface for addition.

Addition of CO leads to singlet ¹Fe(CO)₄(C₂H₄). This can be described as having a trigonal bipyramidal structure, with the ethene ligand occupying one of the coordination sites. Accordingly, there are two isomers, one in which the alkene lies in an axial position and one in which it is in the equatorial positions (see Fig. 2). The latter of these two isomers lies lower in energy, at 37.9 kcal mol⁻¹ below the reactants at the B3PW91**/TZV level of theory, with the other isomer lying 5.8 kcal mol⁻¹ higher, at 32.1 kcal mol⁻¹ below Fe(CO)₃(C₂H₄)+CO. The geometrical parameters obtained for the equatorial isomer are similar to those previously reported computationally (89) and experimentally by gas-phase electron diffraction (60). The two carbons of the ethene are found in the equatorial plane with Fe–C distances

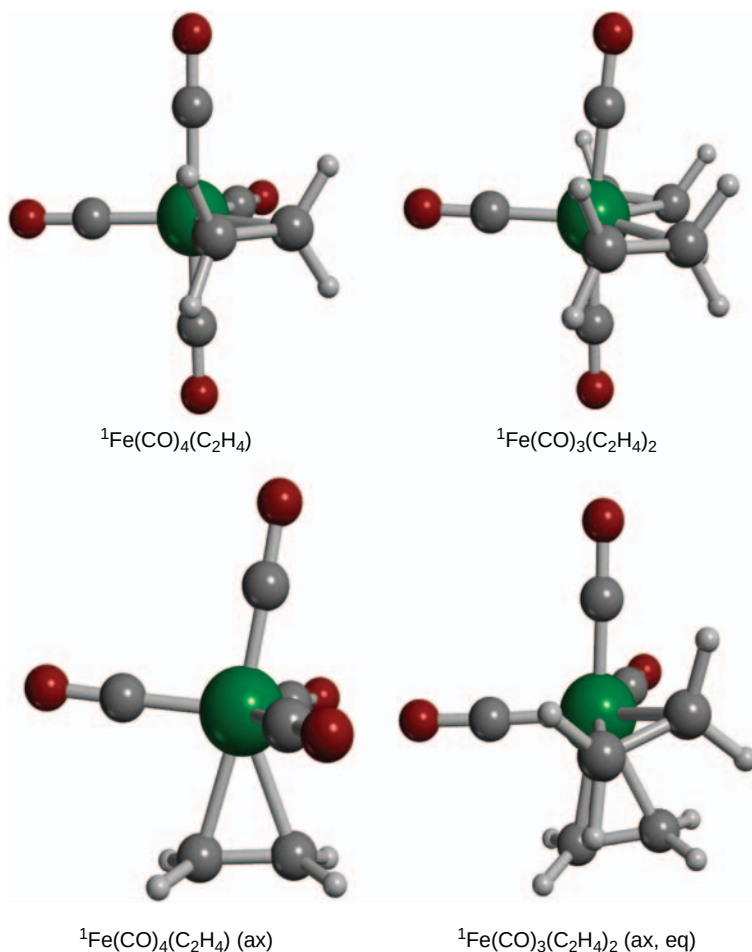


FIG. 2. Optimized geometries of ${}^1\text{Fe}(\text{CO})_4(\text{C}_2\text{H}_4)$ (left) and ${}^1\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)_2$ (right).

of 2.115 Å (exp. value 2.117 Å). The less stable isomer has not been reported previously and has longer Fe–C distances. A pseudorotation TS must exist that connects these two minima, and by analogy to the case of $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)_2$ described below, this TS should lie very low in energy, so that whichever isomer is formed initially, conversion to the more stable product will be rapid.

As mentioned earlier, ${}^1\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$ can be described as having a distorted trigonal bipyramidal structure, with C_2H_4 in the axial position, and a missing equatorial CO ligand (see Fig. 2). Accordingly, the most facile process for addition of CO

involves approach in the equatorial plane, leading to the less stable axial isomer of $\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$. In agreement with the spin-allowed character of the addition, we found that CO addition along this direction occurs without a barrier on the singlet potential energy surface. This was demonstrated by carrying out a series of geometry optimizations while holding the Fe–C distance for the incoming carbonyl ligand fixed. Vibrational frequencies orthogonal to the reaction path were computed and used together with the energies and rotational constants to carry out variational TST calculations of the room-temperature rate constant (in the high-pressure limit) for CO addition. This yields a value of $3.99 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, for a variational TS structure corresponding to an Fe–C distance of 3.15 Å, and a potential energy relative to reactants of $-0.94 \text{ kcal mol}^{-1}$. Bearing in mind the inaccuracies associated with carrying out rigid rotor–harmonic oscillator calculations of the partition functions required for the TST calculations (79,83), this value is in remarkable agreement with the experimental rate constant of $4.3(\pm 0.7) \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ (50).

As already mentioned, the rate constant for this reaction is somewhat smaller than that for CO addition to $\text{Fe}(\text{CO})_3$, which was used as a benchmark for how fast spin-allowed ligand additions to unsaturated iron complexes should occur. The present calculations suggest that this benchmark should be used with some caution, as small variations in rate constant can occur even for rather similar barrierless spin-allowed ligand addition processes. This is in fact well known for barrierless reactions (79), and can be caused by features such as the degree of long-range attraction in the potential energy curve, the extent to which the rotational modes of the incoming ligand are impeded by approach to the metal center, or the changes in the stiffness of the “spectator” vibrational degrees of freedom in the ligand and the metal fragment along the reaction coordinate.

Addition of a second ethylene molecule to the $^1\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)$ complex occurs in a manner very similar to the CO addition process just discussed, as shown by our calculations (87). Again, there are several isomers of the product $^1\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)_2$ complex to consider, with the global minimum corresponding to a trigonal bipyramid structure with two equatorial alkene ligands and C_{2v} symmetry (Fig. 2). This has two strongly bound alkene ligands, with Fe–C bond lengths of 2.104 and 2.108 Å, and lies $22.2 \text{ kcal mol}^{-1}$ below the reactants (see Table VI). This is in good agreement with the experimental dissociation energy reported for loss of C_2H_4 from $^1\text{Fe}(\text{CO})_3(\text{C}_2\text{H}_4)_2$ (50). The geometry of the most stable isomer was reported in a previous computational

study (86), and very similar geometrical parameters were obtained here. The lowest energy pathway for addition of C_2H_4 to $^1Fe(CO)_3(C_2H_4)$ leads, however, to a slightly less stable isomer, which lies only $17.4 \text{ kcal mol}^{-1}$ below the reactants, with one alkene in an axial position and one in an equatorial position. Again, there is a pseudorotation TS leading from this isomer to the global minimum. As this TS lies only $1.5 \text{ kcal mol}^{-1}$ above the axial-equatorial isomer, the latter, if formed, can rearrange very easily to the global minimum. A series of geometry optimizations with the Fe-C distance for the incoming alkene frozen at successively smaller values demonstrates that there is no barrier to addition. Variational TST calculations for the rate constant at 300 K for addition show the existence of a variational TS at a point corresponding to Fe-C distances for the incoming alkene of $3.7 \text{ \AA}$, and with an energy relative to the separated reactants of $-2.3 \text{ kcal mol}^{-1}$. The corresponding calculated rate constant is $8.8 \times 10^{-13} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, which in this case is significantly (30 times) smaller than the experimental value ($1.1 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$). This discrepancy may be due to deficiencies in the variational TST method, or to a somewhat insufficiently attractive B3PW91**/TZV potential energy surface in the region corresponding to the variational TS. Nevertheless, the existence of a barrierless pathway to addition confirms that this reaction should be fairly rapid. As for the previous case, the fact that the rate constant is smaller than for the benchmark $CO+Fe(CO)_3$ reaction cannot be taken as proof for a spin-forbidden character to this process.

E. $Fe(CO)_5$ PHOTOFRAGMENT CHEMISTRY: CONCLUSIONS

Iron tetracarbonyl and various related unsaturated iron complexes show a remarkably rich diversity of spin-forbidden chemistry. Various reactions of these species have been the object of a large number of experimental studies, including many careful studies of reaction kinetics. These experiments provide an excellent set of data with which to evaluate the power of computation to rationalize observations in a qualitative way, as well as to account in a more quantitative way for experimental observations. The computational work described above is largely drawn from our own published work (23-27), including some unpublished data for some of the reactions (85,87). Nevertheless, many other groups have carried out insightful *ab initio* and DFT studies of many of the species and processes described.

As well as being of great interest in their own right, the computations reported here are important for a second reason.

The ability of theory to account for the wide range of spin-forbidden reactivity observed in a near-quantitative way means that the same theoretical models can be trusted to give insight into more complex transition metal systems. For these other systems, detailed experimental data are not always present for comparison, and it is not always possible to carry out high-level *ab initio* computations in order to calibrate DFT methods. Nevertheless, the dual approach of locating MECPs and using NA-TST will clearly be able to provide lots of qualitative and semiquantitative insight into reactivity.

III. Other Spin-Forbidden Reactions of Transition Metal Compounds

In 2003, we presented two reviews concerning spin-forbidden reactions in transition metal chemistry (10,16). A number of other reviews in 2004 addressed closely related topics (90,91). However, since then, we are not aware of any review on this topic. We, therefore, present a short discussion of a number of recent studies of reactivity in transition metal chemistry for processes involving changes in spin-state. The aim here is not to be exhaustive, as there are now quite a lot of studies of transition metal chemistry that address the topic of spin-state changes. Instead, this is an attempt to give a flavor of the types of question that are being addressed now and the techniques used to investigate them.

Gas-phase chemistry of metal-containing ions continues to be a fruitful area for identifying reactions involving changes in spin-state (8,90,92). Zhang and Bowers carried out an elegant study of the reaction of high-spin metal hydride cations MH^+ ($\text{M} = \text{Fe}, \text{Co},$ and Ni) with CH_4 to produce MCH_3^+ and dihydrogen (93). The reaction with NiH^+ was found to proceed very rapidly and without an activation barrier, whereas the FeH^+ and CoH^+ reactions involved barriers of 11.7 and 1.9 kcal mol⁻¹, respectively. The preexponential factor in each case was large, of the order of 10⁻¹² cm³ molecule⁻¹ s⁻¹. DFT calculations at the B3LYP level of theory were used to understand these results, and it was shown that all of the reactions must involve a change in spin-state, with qualitative potential energy surfaces as shown in Fig. 3. All three metal hydride cations have high-spin ground states ^{HS}MH⁺ (respectively ⁵Δ, ⁴Φ, and ³Φ for FeH^+ , CoH^+ , and NiH^+) with relatively low-lying low-spin-states ^{LS}MH⁺. In the gas phase, the ions can form fairly strongly bound ion-molecule complexes $\text{MH}^+ \cdot \text{CH}_4$, and then undergo a σ-metathesis process yielding a product ion complex that dissociates to give

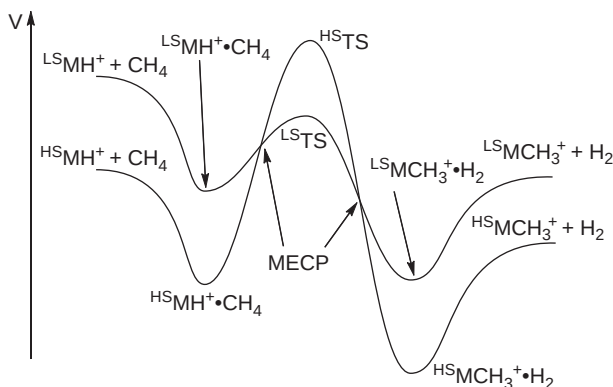


FIG. 3. Schematic potential energy surfaces for reaction of FeH^+ , CoH^+ , and NiH^+ with CH_4 (93).

the metal–methyl ion. In each case, the TS is much lower on the low-spin surface than on the high-spin surface, and the calculated energy of the $^{\text{LS}}\text{TS}$ agrees very well with the measured activation parameters. Although no MECPs were located, it is reasonable to suppose that these will be much lower in energy than the $^{\text{LS}}\text{TS}$, so that the spin-forbidden crossover process should be more facile than the reaction itself. Certainly, it is clear that the sequence of spin-crossover, low-spin metathesis, and spin-crossover will be much more favorable than spin-allowed reactivity on the high-spin surface. Similar mechanisms have been invoked for many other C–H bond activation processes with small metal ion reactants, with the key novelty in the study mentioned here (93) being the measurement of activation parameters, which, together with computation, demonstrate the involvement of the low-spin TS for each metal.

Another notable study we highlight here concerns the catalysis of the trimerization of alkynes to form arenes by cobalt(I) cyclopentadiene complexes (94). The general features of the mechanism of this reaction were understood for some time, and there had been a number of computational studies in the literature. However, many aspects of the experimental studies remained poorly understood before the elegant computational work described in this and previous papers (95). The generic mechanism is shown in Fig. 4. The key contribution of this study was to treat the spin-state change processes that can occur for the 16-electron complexes in this catalytic cycle in a systematic way, and assess e.g., how rapidly spin-state change from singlet to triplet occurs upon formation of the metallacyclopentadiene species, how rapidly ligand binding to the species to form an

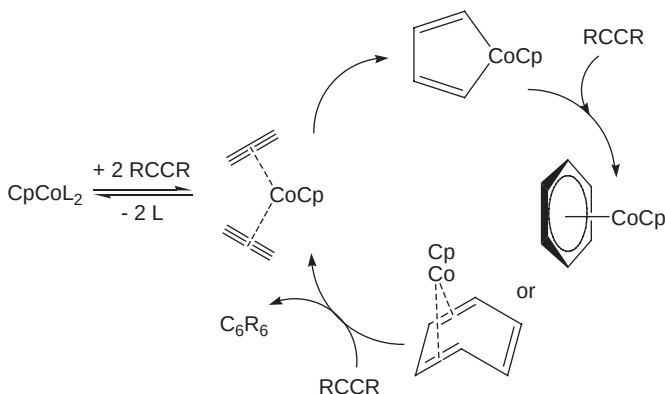


FIG. 4. Generic mechanism for trimerization of alkynes to provide arenes by CpCoL_2 catalysts (94).

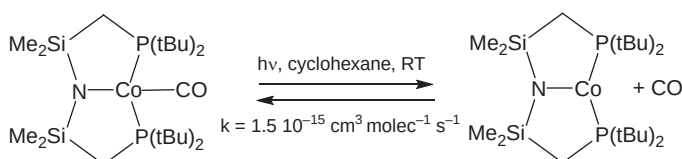


FIG. 5. Flash photolysis of $(\text{PNP})\text{CoCO}$ leading to a triplet three-coordinate cobalt species, with slow rebinding of carbon monoxide (96).

18-electron singlet species occurs, and so on. These different steps were explored using appropriate computations to provide the first general picture of the overall mechanism that accounts for nearly all the experimental observations.

A final example to be discussed here is a recent study of the flash photolysis of a cobalt carbonyl complex (96) $(\text{PNP})\text{CoCO}$, as shown in Fig. 5. This leads to a transient three-coordinate cobalt(I) species $(\text{PNP})\text{Co}$ that was already known to have a triplet ground state (97). Time-resolved infrared study of the transient in the presence of variable concentrations of carbon monoxide enables the study of the recombination kinetics, leading to a rate constant of *ca.* $10^{-15} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$, much smaller than the expected diffusional limit. This is, therefore, another example of a spin-forbidden ligand addition process that appears to be significantly slower than the limiting diffusion-controlled value found for spin-allowed ligand addition to unsaturated metal fragments.

In conclusion to this short section, it can be seen that the literature now contains quite a lot of examples of reactions

involving spin-state changes. The increasing level of consciousness of spin-state changes and their effect on kinetics in recent years is manifested by the fact that in each of the cases mentioned, there is a careful analysis of the reaction mechanism and the effect of the spin-state change. It is notable that the impact of the spin-state changes is different in the different cases.

The example of the metal hydride cations mentioned first (93) is a case in which spin-state changes occur prior to and after the rate-limiting step, and have no major effect on reactivity. In fact, it can be argued in such cases that the possibility to change spin makes the reaction *faster* than it would otherwise have been. The second example, of alkyne trimerization (94), is one in which many of the relevant MECPs are quite accessible, and hence the elementary steps involving spin-state change can occur relatively easily. In this case, the computational exploration of MECPs provides a means to map out complex reaction pathways on multiple potential energy surfaces of different spin, and thereby provide an interpretation of experimental results. Finally, we discussed the case of CO addition to triplet (PNP)Co (96), in which spin-state change appears to make a reaction slower than analogous spin-allowed reactions.

IV. Spin-Forbidden Reactions: General Trends

In the preceding text, we have discussed in some detail the features of the potential energy surfaces and their crossings for a number of spin-forbidden reactions, with a particular focus on the chemistry of iron carbonyl unsaturated fragments. The energy of the MECPs between potential energy surfaces provides a qualitative interpretation of reactivity. We have also discussed the application of NA-TST to the calculation of rate constants for spin-forbidden processes and shown that semiquantitative agreement with experiment is possible, provided accurate potential energy surfaces can be computed. In this section, we want to bring together some of these results and make some general predictions concerning the rate constants of spin-forbidden reactions of transition metal compounds.

This discussion will be restricted to two common classes of elementary reactions at transition metal centers that often involve spin-state change: spin-crossover (98,99) and ligand addition. We define spin-crossover as being a change in spin-state at a metal center without any associated change in bonding (although of course some structural changes may occur). Several

examples of such processes have been given above, e.g., the spin-state change ${}^3\text{Fe}(\text{CO})_4 \rightarrow {}^1\text{Fe}(\text{CO})_4$. This class of reactions naturally includes the very well known spin-crossover events for Fe(II) bipyridine complexes and related species. By "ligand addition," we understand exclusively addition of a ligand to an unsaturated metal center, without prior loss of another ligand. However, we include in this class reactions in which the added ligand then undergoes bond cleavage and oxidative addition to the metal. Addition of H_2 to ${}^3\text{Fe}(\text{CO})_4$ to give iron tetracarbonyl dihydride is an example of such a reaction. Again, many examples of these ligand addition reactions have been given.

Spin-crossover is by definition a reversible process in principle, so there are always *two* rate constants to consider, for the high-spin $\rightarrow$ low-spin conversion and its reverse. The ratio of these two rate constants is defined by the thermodynamics of the conversion, which means in practice that the conversion of the more stable form to the less stable form can be characterized by a rate constant spanning a huge range of values. We consider here only the reverse process, relaxation of the less stable form at a given temperature to the more stable form. Based on our experience, the rate constant for this process is usually rather large, of the order of 10^6 – 10^{10} s^{-1} . To give some examples, high-spin to low-spin spin relaxation in typical iron(II) complexes occurs with a rate constant of 10^6 – 10^8 s^{-1} at room temperature (98). At lower temperatures, trapping of the metastable excited state can occur for longer times, the light-induced excited spin-state trapping (LIESST) phenomenon. We note that spin-crossover in these compounds has also been extensively studied in the solid phase, and the effect of the environment can cause deviation from the simple kinetic behavior obtained in solution. The ${}^1\text{Fe}(\text{CO})_4 \rightarrow {}^3\text{Fe}(\text{CO})_4$ relaxation discussed above is another example, with a predicted rate constant of $9 \times 10^8 \text{ s}^{-1}$ at room temperature (24). The principle that spin-state relaxation is fast extends beyond transition metal chemistry to the, in some ways, analogous process of singlet to triplet conversion in carbenes, for which rate constants of the order of 10^9 s^{-1} have been measured (100).

Why is the relaxation process in spin-crossover compounds so fast? This can be readily understood based on the features of the potential energy surfaces, and indeed in terms of Marcus theory (101). Given that both spin-states have the same connectivity, the only changes in geometry are slight adjustments in bond lengths and angles associated with ligand field effects. This means that the reorganization energy required to distort the higher-energy species from its equilibrium geometry to that of the lower-energy species is typically small. Hence, the potential energy surfaces

cross at MECPs that are quite low in energy relative to the higher-energy minimum. For example, the MECP lies only *ca.* 3–4 kcal mol⁻¹ above ¹Fe(CO)₄ (24), and the measured activation energies for relaxation in spin-crossover complexes are of the order of 3–5 kcal mol⁻¹ (98). The energy of the MECP is the key factor in determining reactivity in spin-forbidden processes, and a low MECP inevitably leads to a large rate constant. The example of carbene spin-state change referred to above (100) shows that even when the coupling between the surfaces is expected to be fairly small (spin-orbit coupling is smaller for carbon than for iron), large rate constants can be obtained.

We now turn to ligand addition. Here one needs to distinguish between a number of different situations, depending on the strength of the interactions between the ligand and the metal center in both the high-spin and low-spin-states, and on the magnitude of the spin-state splitting in the metal fragment. The first case to consider (a) is when a ligand addition process to a high-spin metal fragment is not favorable for the simple reason that it is not exothermic (or exothermic enough). Where the spin-state splitting is large, and/or the bond energy to the low-spin fragment is small, the bound state of the ligand on the low-spin energy surface is higher in energy than the separated ligand and high-spin metal fragment, as shown in Fig. 6. Note that the loss of translational and rotational degrees of freedom upon ligand binding means that ligand addition is entropically unfavorable in most cases, so binding needs to be significantly exothermic before the free energy of binding becomes negative.

In such cases, the binding energy on the low-spin surface may of course be significant. There may also be analogous systems in which the spin-state splitting is much smaller or indeed the ground state of the metal fragment is low-spin, and for those

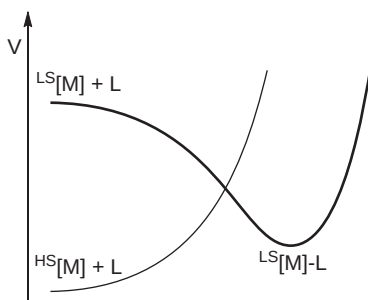


FIG. 6. Potential energy surfaces for ligand addition. Case (a): bonding is not energetically favorable.

analogous systems, the analogous ligand binding process may be rapid and favorable. This may suggest that the ligand binding is “spin-forbidden,” and in one sense this is correct as it is the high-spin nature of the metal fragment that prevents binding. However, in the more precise meaning where “spin-forbidden” refers to a kinetic barrier to binding, this does not apply here as the main reason for which ligand addition is not observed to occur is thermodynamics.

We have studied two examples of such cases computationally. $\text{Cp}^*\text{Rh}(\text{CO})$ and $\text{Cp}^*\text{Ir}(\text{CO})$ are known to form alkane complexes and subsequently undergo C–H bond insertion to give $\text{M}(\text{III})$ alkyl hydrides (102). Both of these 16-electron fragments have singlet ground states or close-lying singlet and triplet states (103). The related $\text{CpCo}(\text{CO})$ species, which has a triplet ground state, with the singlet lying significantly higher in energy, does not form either the adduct or the C–H activation product (103,104). We showed in our computational study of this reaction (15) that both potential products at best have the same energy as the reactants. Given the entropic cost of addition, addition will be unfavorable in free energy terms, and this explains why it cannot occur. In this case, the reaction energy on the singlet surface is not too dissimilar for the Rh, Ir, and Co cases, but the large spin-state splitting in $\text{CpCo}(\text{CO})$ makes the overall process unfavorable. Another case is the addition of Ar, Kr, and Ne to $^3\text{Fe}(\text{CO})_4$. These species typically do not add to the metal center in low-temperature matrices or in supercritical fluids, whereas ligands such as Xe, N_2 , and CH_4 do (see (42) and Refs. therein). In our computational study (25), we found that the bond energy of Xe and CH_4 to $^1\text{Fe}(\text{CO})_4$ is just large enough to compensate for the singlet-triplet splitting in the metal fragment. In contrast, we found that Kr and Ar barely bind at all to $^1\text{Fe}(\text{CO})_4$, let alone having a large enough net bond energy to $^3\text{Fe}(\text{CO})_4$ to undergo addition (25,105). Another example is given by an $\text{Fe}(\text{II})$ diimine complex, which was synthesized with the view of preparing a biomimetic hydrogenase system (106). For this, binding of CO was required, but this did not occur due to the very large spin-state splitting.

The next case to consider is when the incoming ligand L is a typically strongly bound ligand such as CO, a phosphine, or similar, the spectator ligands in the high-spin 16-electron metal fragment [M] are somewhat weaker, the high-spin/low-spin splitting is not too large, and there is not too much steric hindrance. In such cases, there often exists a metastable high-spin 18-electron adduct. Formally, this type of species violates the 18-electron rule but its existence can be accounted for by

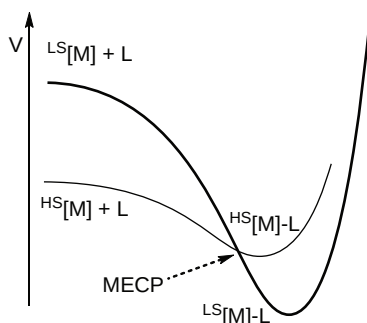


FIG. 7. Potential energy surfaces for ligand addition. Case (b): formation of a metastable adduct on the high-spin surface.

considering that the incoming ligand can bind to the metal by partially breaking the bonds to the other ligands. This leads to a local minimum on the high-spin energy surface, and there is most usually no energy barrier to forming this minimum from the separated fragments. Spin-state change to form the final product is then a special case of spin-crossover that can occur rapidly through a low-lying MECP (Fig. 7).

Examples of this behavior are addition of CO to $\text{Tp}'\text{Co}(\text{CO})$, where Tp' is a substituted trispyrazolylborate ligand (15,107) as well as addition of CO, ethene or trimethylphosphine to $\text{CpCo}(\text{CO})$ (15). In all these cases, very rapid ligand addition, at the gas-phase collision rate or the diffusion-limited rate in solution, should occur despite the overall spin-forbiddenness of the process, as the rate-limiting step is the initial barrierless approach of the ligand to the high-spin metal fragment. Note also that a related case occurs when the spin-state splitting is large, but the ligand bond energy on the high-spin surface is large also. In this case, the high-spin adduct is simply stable enough on its own, and no spin-state change occurs. This case is very common in coordination chemistry, with maybe the simplest example being addition of water to a partially hydrated, high-spin metal ion such as aqueous Fe^{2+} with one vacant coordination site.

A slight modification to the previous scenario is expected when the incoming ligand is no longer significantly more strongly bound than the existing, spectator ligands. In this case, the high-spin potential energy surface may not be attractive, and approach of the ligand to the metal will lead to a steady increase of energy (with the possible exception of a region at long distance, where dispersion or other nonbonded interactions may lead to the formation of a weakly bound species). If the incoming ligand is a strong ligand, the key point here is that the high-spin

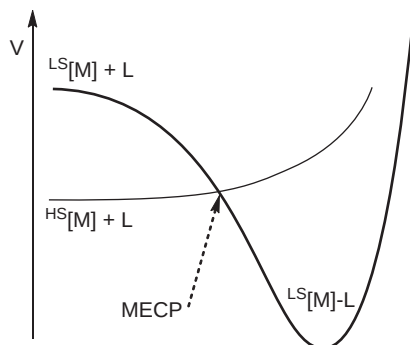


FIG. 8. Potential energy surfaces for ligand addition. Case (c): weakly repulsive potential energy surface for approach of the incoming ligand to the high-spin metal fragment.

potential energy surface may be only *weakly* repulsive across a quite broad range of geometries (Fig. 8).

In this case, the MECP between the two potential energy surfaces will lie higher in energy than the separated fragments, but only by a small amount. Hence, the reaction will be a few orders of magnitude slower than the diffusion rate in solution, but will still be relatively fast. Importantly, as the rate-limiting step in these reactions is expected to be the concerted spin-state change and ligand addition step, slightly unusual kinetic behavior is expected. The probability of hopping from high-spin to low-spin surface at the MECP is much smaller than one, unless the spin-orbit coupling is very large. Typically, for the transition metal systems we have considered, this probability is in the range 0.001–0.1. This translates into a significant negative contribution to the activation entropy (19), on top of any contribution from translational, rotational, and vibrational degrees of freedom. Hence, such reactions are expected to have more negative entropies of activation, and less positive enthalpies of activation, than related spin-allowed ligand addition processes that have similar rate constants. Examples of such behavior are the addition of CO to $\text{Fe}(\text{CO})_4$ and the addition of CO to triplet (PNP)Co (96) discussed previously, or the addition of CO to the heme group in proteins such as myoglobin (14,22,108,109). At least in the case of CO addition to myoglobin (109), it is noteworthy that experiment shows a very negative entropy of addition, although it is clear that for such a complex system, the origin of this term may not be the one suggested above.

The fourth and final common situation for spin-forbidden ligand addition occurs for addition of weak-field ligands. In many

cases, of course, such ligands are weakly bound and one will simply be in case (a) discussed above where addition is unfavorable. However, in some cases, ligands that are intrinsically rather weakly bound may end up bonding quite strongly to a given metal in a given coordination environment. An example of a ligand that conforms to this pattern fairly frequently is dihydrogen, which is a relatively weak ligand, but one that can lead to stable products due to oxidative addition (as noted above, we include such oxidative reactions under the general heading of "ligand addition" although this clearly stretches the definition somewhat). In these cases, the high-spin potential energy surface may be quite strongly repulsive, and the MECP with the attractive low-spin surface may therefore occur significantly higher in energy than the ground state of the reactant fragments (Fig. 9).

In fact, although this is not shown in Fig. 9, it is even possible that the MECP lies higher in energy than the low-spin metal fragment. Based on the two-dimensional "cartoon" representations of the potential energy surfaces, such as Fig. 9, this does not appear to be possible if the low-spin potential energy surface is attractive toward the incoming ligand, as it nearly always is. However, one must remember that the potential energy surfaces are highly multidimensional. In particular, the equilibrium geometry for the low-spin and high-spin metal fragments are not the same, so that to reach the MECP from the high-spin fragments, one must not only approach the ligand to the metal, but one must also distort the metal fragment so as to bring the high-spin and low-spin species to the same geometry. We have found several cases where this leads to MECPs higher in energy than the high-energy, low-spin, fragments (26,27).

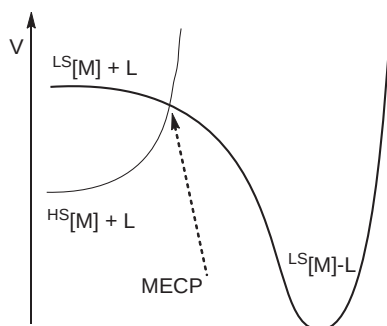


FIG. 9. Potential energy surfaces for ligand addition. Case (d): strongly repulsive potential energy surface for approach of the incoming ligand to the high-spin metal fragment.

Reactivity in such cases can clearly be quite low, such that addition is very noticeably slower than the diffusion limit. As discussed above for the case of H_2 or Xe addition to $^3\text{Fe}(\text{CO})_4$, it appears that in such cases, there may be a competition between the “direct” mechanism, in which ligand addition and spin-state change occur in a single step, and an indirect one in which spin-state change (or spin-crossover) to give the high-energy, low-spin form of the metal fragment occurs first. This can then be followed by barrierless addition of the ligand on the low-spin surface. Which of those two mechanisms dominates may depend on the details of the system, such as exactly how high in energy the MECF for the direct addition is, how high the MECF for spin-crossover in the absence of ligand is, and other factors.

What is noticeable is that many of the examples of spin-forbidden ligand addition processes that are observed to be very slow despite being exothermic involve hydrogen. For several such cases, there is evidence that spin does indeed play a role, as e.g., in the addition to tungsten triamidoamine complexes (15,110) or the addition to the iron carbonyl species (24,26,27). Other cases of slow hydrogen addition to high-spin metal species, such as $\text{Rh}_6(\text{PR}_3)_6\text{H}_{14}$, leading to a low-spin adduct, have been noted to be slow (111), and it may well be that spin-state change plays a role here too. Given the importance of hydrogen binding in catalysis as well as biocatalysis (e.g., in hydrogenases), this apparently relatively common kinetic feature of dihydrogen binding to metals is of significant interest (112).

The preceding discussion shows that with increasing experience of the shapes of potential energy surfaces in spin-forbidden processes, it becomes possible to make qualitative predictions about the role of spin-state change in transition metal chemistry for a range of different reaction types, without the need for much computation. As a general rule, elementary reaction steps involving spin-state change occur fast or very fast provided they are thermochemically favorable. For example, spin-crossover from the less stable spin-state of a complex to the more stable form tends to occur very quickly. Many ligand addition steps involving spin-state change also occur at or close to the diffusion limit. Only a few reactions seem to be best categorized as ligand additions of case (d), and hence to be notably slow due to the need for spin-state change.

V. Conclusions

In this review, we have described some of the contributions that computational chemistry has made to understanding

transition metal reactivity involving multiple spin-states. Although we have included, where appropriate, work from other groups, we have focused mainly on work from our group. A large part of the review discussed many of the spin-forbidden steps involved in the reactions of small molecules with unsaturated fragments formed upon photolysis of iron pentacarbonyl, especially triplet iron tetracarbonyl. This system is of great interest because extensive experimental data concerning its reactivity are available, making it an excellent test system for theoretical models. The relative energy of MECPs between potential energy surfaces corresponding to different spin-states has been shown to provide a good qualitative basis to understand reactivity for this system. A form of NA-TST that accounts for the energy required to reach MECPs as well as for the net probability of surface crossing at the MECP has also been used for many of the reactions, and been shown to give very good agreement with experiment for many of the reactions.

A particular point of success of the theory has been its ability to predict two different behavior regimes for reactions such as $\text{H}_2 + \text{Fe}(\text{CO})_4$ (24). In the gas phase, with low H_2 concentration, the reaction involves rapid preequilibration of ground state $^3\text{Fe}(\text{CO})_4$ with its low-lying singlet excited state, followed by slower bimolecular trapping by H_2 . In supercritical Ar doped with high concentrations of hydrogen, in contrast, the second step is predicted to be much faster, so that spin-state change from triplet to singlet becomes effectively rate determining. This accounts for many of the differences between the gas phase and supercritical fluid kinetics.

The review also discusses some recent examples of other spin-forbidden chemistry of transition metal compounds. Many different factors have combined to increase the awareness of spin-state changes in inorganic chemistry, and the examples are partly chosen to emphasize this. One important aspect is the availability of more and more high-quality kinetic data for reactions involving spin-state changes. This allows the long-standing question of whether spin-state changes affect reactivity to be addressed in a more informed way. Another important aspect is that more and more experimental studies of spin-forbidden reactions are accompanied by computational studies, in some cases involving the location of MECPs. This too contributes to the better understanding of the effect of spin-state changes, as it means that the topology of the potential energy surfaces and the regions where they cross becomes better appreciated.

A final aspect of the review is an attempt to make some general statements and predictions concerning the rate of spin-forbidden reactions of transition metal compounds. Doing this for *all* such

reactions would be a very considerable task, so we have limited ourselves to considering two very important examples of spin-forbidden reaction steps: spin-crossover and ligand addition. The former is a special case of an isomerization process in which no bonds are broken or formed, with only the electronic configuration on the central metal atom changing. It is argued that in most cases, spin-crossover from the less stable spin-state to the more stable one (spin relaxation) should occur rapidly, on a timescale of picoseconds to microseconds, as the MECP usually lies quite close in energy to the higher-energy minimum configuration.

For ligand addition, reactivity is predicted to span a wide range, from very fast reactions that occur at the collisional or diffusional rate, to much slower processes, which in some cases may even not occur due to favorable competition from other processes. The position of a particular reaction across this broad spectrum can be predicted based on the bonding strength of the incoming ligand, the magnitude of the energy gap between the spin-states of the metal fragment to which the ligand binds, and the nature of the "spectator" ligands already present on the metal.

We expect that these qualitative insights will be strengthened (and perhaps modified) as more and more experience concerning spin-forbidden reactivity accumulates in the inorganic chemistry literature. We also expect that computational studies of potential energy surfaces, and of MECPs, will play an important role in developing and extending these more qualitative insights.

ACKNOWLEDGMENTS

Jeremy Harvey thanks the EPSRC for an Advanced Research Fellowship, and acknowledges many helpful conversations on spin-state changes with Helmut Schwarz, Detlef Schröder, Sason Shaik, Massimiliano Aschi, Rinaldo Poli, James Mayer, and many others. A special thanks is due to Prof. Michael George for the collaborative work discussed in the text. Maria Besora thanks the Department of Education and Universities (DEiU) of the Autonomous Government of Catalonia for financial support. JLCM thanks Mexico's National Council of Science and Technology (CONACYT) for a scholarship.

REFERENCES

1. Shaik, S.; Kumar, D.; de Visser, S. P.; Altun, A.; Thiel, W. *Chem. Rev.* **2005**, *105*, 2279–2328.

2. Solomon, E. I.; Brunold, T. C.; Davis, M. I.; Kemsley, J. N.; Lee, S.-K.; Lehnert, N.; Neese, F.; Skulan, A. J.; Yang, Y.-S.; Zhou, J. *Chem. Rev.* **2000**, *100*, 235–349.
3. Poli, R. *Acc. Chem. Res.* **1997**, *30*, 494–501.
4. Delos, J. B. *J. Chem. Phys.* **1973**, *59*, 2365–2369.
5. Zahr, G. E.; Preston, R. K.; Miller, W. H. *J. Chem. Phys.* **1975**, *62*, 1127–1135.
6. Lorquet, J. C.; Leyh-Nihant, B. *J. Phys. Chem.* **1988**, *92*, 4778–4783.
7. (a) Yarkony, D. R. *J. Chem. Phys.* **1990**, *92*, 320–323. (b) Cui, Q.; Morokuma, K. *J. Chem. Phys.* **1997**, *107*, 4951–4959.
8. Shaik, S.; Danovich, D.; Fiedler, A.; Schroder, D.; Schwarz, H. *Helv. Chim. Acta* **1995**, *78*, 1393–1407.
9. (a) Carpenter, B. K. *Chem. Soc. Rev.* **2006**, *35*, 736–747. (b) Forster, L. S. *Coord. Chem. Rev.* **2006**, *250*, 2023–2033. (c) Yarkony, D. R. *J. Phys. Chem. A* **2001**, *105*, 6277–6293.
10. Poli, R.; Harvey, J. N. *Chem. Soc. Rev.* **2003**, *32*, 1–8.
11. Harvey, J. N.; Aschi, M.; Schwarz, H.; Koch, W. *Theor. Chem. Acc.* **1998**, *99*, 95–99.
12. (a) Harvey, J. N.; Schroder, D.; Schwarz, H. *Bull. Soc. Chim. Belg.* **1997**, *106*, 447–453. (b) Schalley, C. A.; Harvey, J. N.; Schroder, D.; Schwarz, H. *J. Phys. Chem. A* **1998**, *102*, 1021–1035.
13. (a) Smith, K. M.; Poli, R.; Harvey, J. N. *New J. Chem.* **2000**, *24*, 77–80. (b) Smith, K. M.; Poli, R.; Harvey, J. N. *Chem. Eur. J.* **2001**, *7*, 1679–1690.
14. Harvey, J. N. *J. Am. Chem. Soc.* **2000**, *122*, 12401–12402.
15. Carreon-Macedo, J. L.; Harvey, J. N. *J. Am. Chem. Soc.* **2004**, *126*, 5789–5797.
16. Harvey, J. N.; Poli, R.; Smith, K. M. *Coord. Chem. Rev.* **2003**, *238*, 347–361.
17. (a) Koga, N.; Morokuma, K. *Chem. Phys. Lett.* **1985**, *119*, 371–374. (b) Bearpark, M. J.; Robb, M. A.; Schlegel, H. B. *Chem. Phys. Lett.* **1994**, *223*, 269–274.
18. (a) Zener, C. *Proc. R. Soc. Lond. Ser. A* **1932**, *137*, 696–702. (b) Wittig, C. *J. Phys. Chem. B* **2005**, *109*, 8428–8430.
19. Harvey, J. N. *Phys. Chem. Chem. Phys.* **2007**, *9*, 331–343.
20. Cui, Q.; Morokuma, K.; Bowman, J. M.; Klippenstein, S. J. *J. Chem. Phys.* **1999**, *110*, 9469–9482.
21. Besora, M.; Harvey, J. N. *J. Chem. Phys.* **2008**, *129*, art. 044303.
22. Harvey, J. N. *Faraday Discuss.* **2004**, *127*, 165–177.
23. Harvey, J. N.; Aschi, M. *Faraday Discuss.* **2003**, *124*, 129–143.
24. Besora, M.; Carreón-Macedo, J.-L.; Cowan, A. J.; George, M. W.; Harvey, J. N.; Portius, P.; Ronayne, K. L.; Sun, X.-Z.; Towrie, M. *J. Am. Chem. Soc.* **2009**, *131*, 3583–3592.
25. Carreon-Macedo, J. L.; Harvey, J. N. *Phys. Chem. Chem. Phys.* **2006**, *8*, 93–100.
26. Harvey, J. N.; Poli, R. *Dalton Trans.* **2003**, 4100–4106.
27. Schott, D.; Callaghan, P.; Dunne, J.; Duckett, S.; Godard, C.; Goicoechea, J. M.; Harvey, J. N.; Lowe, J. P.; Mawby, R. J.; Muller, G.; Perutz, R. N.; Poli, R.; Whittlesey, M. K. *Dalton Trans.* **2004**, 3218–3224.
28. Mond, L.; Langer, C. *J. Chem. Soc. Trans.* **1891**, 1090–1093.
29. Mond, L.; Quincke, F. *J. Chem. Soc. Trans.* **1891**, 604–607.
30. (a) Wrighton, M. *Chem. Rev.* **1974**, *74*, 401–430. (b) Poliakoff, M. *Chem. Soc. Rev.* **1978**, *7*, 527–540. (c) Poliakoff, M.; Weitz, E. *Acc. Chem. Res.* **1987**, *20*, 408–414. (d) Weitz, E. *J. Phys. Chem.* **1987**, *91*, 3945–3953.
31. Leadbeater, N. *Coord. Chem. Rev.* **1999**, *188*, 35–70.

32. Poliakoff, M.; Turner, J. J. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 2809–2812.
33. Dewar, J.; Jones, H. O. *Proc. R. Soc. Lond. Ser. A* **1905**, *76*, 558–577.
34. (a) Stolz, I. W.; Dobson, G. R.; Sheline, R. K. *J. Am. Chem. Soc.* **1963**, *85*, 1013–1014. (b) Poliakoff, M.; Turner, J. J. *J. Chem. Soc. Dalton Trans.* **1973**, 1351–1357.
35. Poliakoff, M.; Turner, J. J. *J. Chem. Soc. Dalton Trans.* **1974**, 2276–2285.
36. (a) Burdett, J. K. *J. Chem. Soc. Faraday Trans. 2* **1974**, *70*, 1599–1613. (b) Elian, M.; Hoffmann, R. *Inorg. Chem.* **1975**, *14*, 1058–1076.
37. Barton, T. J.; Grinter, R.; Thomson, J.; Davies, B.; Poliakoff, M. *J. Chem. Soc. Chem. Commun.* **1977**, 841–842.
38. (a) Ihee, H.; Cao, J.; Zewail, A. H. *Chem. Phys. Lett.* **1997**, *281*, 10–19. (b) Ihee, H.; Cao, J. M.; Zewail, A. H. *Angew. Chem. Int. Ed.* **2001**, *40*, 1532–1536.
39. (a) Banares, L.; Baumert, T.; Bergt, M.; Kiefer, B.; Gerber, G. *Chem. Phys. Lett.* **1997**, *267*, 141–148. (b) Banares, L.; Baumert, T.; Bergt, M.; Kiefer, B.; Gerber, G. *J. Chem. Phys.* **1998**, *108*, 5799–5811. (c) Trushin, S. A.; Fuss, W.; Kompa, K. L.; Schmid, W. E. *J. Phys. Chem. A* **2000**, *104*, 1997–2006.
40. Snee, P. T.; Payne, C. K.; Kotz, K. T.; Yang, H.; Harris, C. B. *J. Am. Chem. Soc.* **2001**, *123*, 2255–2264.
41. Bachler, V.; Grevels, F. W.; Kerpen, K.; Olbrich, G.; Schaffner, K. *Organometallics* **2003**, *22*, 1696–1711.
42. Portius, P.; Yang, J. X.; Sun, X. Z.; Grills, D. C.; Matousek, P.; Parker, A. W.; Towrie, M.; George, M. W. *J. Am. Chem. Soc.* **2004**, *126*, 10713–10720.
43. Sun, X. Z.; Portius, P.; Grills, D. C.; Cowan, A. J.; George, M. W. *Appl. Spectrosc.* **2008**, *62*, 24–29.
44. Rubner, O.; Engel, V.; Hachey, M. R.; Daniel, C. *Chem. Phys. Lett.* **1999**, *302*, 489–494.
45. Daniel, C.; Benard, M.; Dedieu, A.; Wiest, R.; Veillard, A. *J. Phys. Chem.* **1984**, *88*, 4805–4811.
46. (a) Erdmann, M.; Rubner, O.; Shen, Z.; Engel, V. *Chem. Phys. Lett.* **2001**, *341*, 338–344. (b) Rubner, O.; Baumert, T.; Bergt, M.; Kiefer, B.; Gerber, G.; Engel, V. *Chem. Phys. Lett.* **2000**, *316*, 585–592.
47. Ouderkirk, A. J.; Wermer, P.; Schultz, N. L.; Weitz, E. *J. Am. Chem. Soc.* **1983**, *105*, 3354–3355.
48. (a) Cedeno, D. L.; Weitz, E. *J. Phys. Chem. A* **2000**, *104*, 8011–8026. (b) Wang, J. Q.; Weitz, E. *J. Phys. Chem. A* **2001**, *105*, 5410–5419.
49. Hayes, D. M.; Weitz, E. *J. Phys. Chem.* **1991**, *95*, 2723–2727.
50. House, P. G.; Weitz, E. *J. Phys. Chem. A* **1997**, *101*, 2988–2995.
51. House, P. G.; Weitz, E. *Chem. Phys. Lett.* **1997**, *266*, 239–245.
52. Ouderkirk, A. J.; Weitz, E. *J. Chem. Phys.* **1983**, *79*, 1089–1091.
53. Seder, T. A.; Ouderkirk, A. J.; Weitz, E. *J. Chem. Phys.* **1986**, *85*, 1977–1986.
54. (a) Wang, J. Q.; Long, G. T.; Weitz, E. *J. Phys. Chem. A* **2001**, *105*, 3765–3772. (b) Cooper, A. I.; Poliakoff, M. *Chem. Phys. Lett.* **1993**, *212*, 611–616.
55. Wang, W. H.; Narducci, A. A.; House, P. G.; Weitz, E. *J. Am. Chem. Soc.* **1996**, *118*, 8654–8657.
56. Church, S. P.; Grevels, F. W.; Hermann, H.; Kelly, J. M.; Klotzbucher, W. E.; Schaffner, K. *J. Chem. Soc. Chem. Commun.* **1985**, 594–596.
57. McNeish, A.; Poliakoff, M.; Smith, K. P.; Turner, J. J. *J. Chem. Soc. Chem. Commun.* **1976**, 859–860.
58. Ryther, R. J.; Weitz, E. *J. Phys. Chem.* **1991**, *95*, 9841–9852.

59. Turner, J. J.; Burdett, J. K.; Perutz, R. N.; Poliakoff, M. *Pure Appl. Chem.* **1977**, *49*, 271–285.
60. Davies, B.; McNeish, A.; Poliakoff, M.; Turner, J. J. *J. Am. Chem. Soc.* **1977**, *99*, 7573–7579.
61. (a) Ewens, R. V. G.; Lister, M. W. *Trans. Faraday Soc.* **1939**, *35*, 681–690. (b) McNeill, E. A.; Scholer, F. R. *J. Am. Chem. Soc.* **1977**, *99*, 6243–6249.
62. Heitz, M. C.; Daniel, C. *J. Am. Chem. Soc.* **1997**, *119*, 8269–8275.
63. Sweany, R. L. *J. Am. Chem. Soc.* **1981**, *103*, 2410–2412.
64. Vallet, V.; Strich, A.; Daniel, C. *Chem. Phys.* **2005**, *311*, 13–18.
65. Miller, M. E. *J. Am. Chem. Soc.* **1987**, *109*, 7951–7960.
66. (a) Schroeder, M. A.; Wrighton, M. S. *J. Am. Chem. Soc.* **1976**, *98*, 551–558. (b) Mitchener, J. C.; Wrighton, M. S. *J. Am. Chem. Soc.* **1981**, *103*, 975–977. (c) Chase, M. W. *J. Phys. Chem. Ref. Data* **1998**, *27*. (d) Fleckner, H.; Grevels, F. W.; Hess, D. *J. Am. Chem. Soc.* **1984**, *106*, 2027–2032.
67. (a) Whetten, R. L.; Fu, K. J.; Grant, E. R. *J. Am. Chem. Soc.* **1982**, *104*, 4270–4272. (b) Miller, M. E.; Grant, E. R. *J. Am. Chem. Soc.* **1984**, *106*, 4635–4636. (c) Miller, M. E.; Grant, E. R. *J. Am. Chem. Soc.* **1985**, *107*, 3386–3387.
68. Chase, D. B.; Weigert, F. J. *J. Am. Chem. Soc.* **1981**, *103*, 977–978.
69. (a) Cedenio, D. L.; Weitz, E. *Organometallics* **2003**, *22*, 2652–2659. (b) Glascoe, E. A.; Sawyer, K. R.; Shanoski, J. E.; Harris, C. B. *J. Phys. Chem. C* **2007**, *111*, 8789–8795. (c) Long, G. T.; Wang, W. H.; Weitz, E. *J. Am. Chem. Soc.* **1995**, *117*, 12810–12818. (d) Long, G. T.; Weitz, E. *J. Am. Chem. Soc.* **2000**, *122*, 1431–1442. (e) Pearson, A. J.; Alimardanov, A.; Pinkerton, A. A.; Parrish, D. A. *J. Org. Chem.* **1998**, *63*, 6610–6618.
70. Ricca, A. *Chem. Phys. Lett.* **2001**, *350*, 313–317.
71. Wang, W. H.; Weitz, E. *J. Phys. Chem. A* **1997**, *101*, 2358–2363.
72. Barnes, L. A.; Rosi, M.; Bauschlicher, C. W. *J. Chem. Phys.* **1991**, *94*, 2031–2039.
73. (a) Delley, B.; Wrinn, M.; Luthi, H. P. *J. Chem. Phys.* **1994**, *100*, 5785–5791. (b) Ehlers, A. W.; Frenking, G. *Organometallics* **1995**, *14*, 423–426. (c) Decker, S. A.; Klobukowski, M. *J. Am. Chem. Soc.* **1998**, *120*, 9342–9355. (d) Li, J.; Schreckenbach, G.; Ziegler, T. *J. Am. Chem. Soc.* **1995**, *117*, 486–494.
74. Gonzalez-Blanco, O.; Branchadell, V. *J. Chem. Phys.* **1999**, *110*, 778–783.
75. Here and elsewhere, all relative energies in this review are potential energies including a correction for zero-point vibration.
76. Hall, C.; Jones, W. D.; Mawby, R. J.; Osman, R.; Perutz, R. N.; Whittlesey, M. K. *J. Am. Chem. Soc.* **1992**, *114*, 7425–7435.
77. Harvey, J. N.; Aschi, M. *Phys. Chem. Chem. Phys.* **1999**, *1*, 5555–5563.
78. Truhlar, D. G.; Garrett, B. C.; Klippenstein, S. J. *J. Phys. Chem.* **1996**, *100*, 12771–12800.
79. Fernandez-Ramos, A.; Miller, J. A.; Klippenstein, S. J.; Truhlar, D. G. *Chem. Rev.* **2006**, *106*, 4518–4584.
80. (a) Reiher, M.; Salomon, O.; Hess, B. A. *Theor. Chem. Acc.* **2001**, *107*, 48–55. (b) Harvey, J. N. *Struct. Bond.* **2004**, *112*, 151–183.
81. Harvey, J. N. *Ann. Rep. Prog. Chem. Sect. C Phys. Chem.* **2006**, *102*, 203–226.
82. Miller, W. H.; Handy, N. C.; Adams, J. E. *J. Chem. Phys.* **1980**, *72*, 99–112.
83. Klippenstein, S. J.; Georgievskii, Y.; Harding, L. B. *Phys. Chem. Chem. Phys.* **2006**, *8*, 1133–1147.

84. Snee, P. T.; Payne, C. K.; Mebane, S. D.; Kotz, K. T.; Harris, C. B. *J. Am. Chem. Soc.* **2001**, *123*, 6909–6915.
85. Besora, M.; Harvey, J. N. *Unpublished work*.
86. Kismartoni, L. C.; Weitz, E.; Cedeno, D. L. *Organometallics* **2005**, *24*, 4714–4720.
87. Besora, M.; Cimas, A.; Harvey, J. N. *Unpublished work*.
88. Nayak, S. K.; Farrell, G. J.; Burkey, T. J. *Inorg. Chem.* **1994**, *33*, 2236–2242.
89. Cedeno, D. L.; Weitz, E.; Berces, A. *J. Phys. Chem. A* **2001**, *105*, 8077–8085.
90. Schwarz, H. *Int. J. Mass Spec.* **2004**, *237*, 75–105.
91. (a) Matsunaga, N.; Koseki, S. *Rev. Comp. Chem.* **2004**, *20*, 101–152. (b) Poli, R. *J. Organomet. Chem.* **2004**, *689*, 4291–4304.
92. Schroder, D.; Shaik, S.; Schwarz, H. *Acc. Chem. Res.* **2000**, *33*, 139–145.
93. Zhang, Q.; Bowers, M. T. *J. Phys. Chem. A* **2004**, *108*, 9755–9761.
94. Agenet, N.; Gandon, V.; Vollhardt, K. P. C.; Malacria, M.; Aubert, C. *J. Am. Chem. Soc.* **2007**, *129*, 8860–8871.
95. Gandon, V.; Agenet, N.; Vollhardt, K. P. C.; Malacria, M.; Aubert, C. *J. Am. Chem. Soc.* **2006**, *128*, 8509–8520.
96. Rimmer, R. D.; Grills, D. C.; Fan, H.; Ford, P. C.; Caulton, K. G. *J. Am. Chem. Soc.* **2007**, *129*, 15430–15431.
97. Ingleson, M.; Fan, H. J.; Pink, M.; Tomaszewski, J.; Caulton, K. G. *J. Am. Chem. Soc.* **2006**, *128*, 1804–1805.
98. Gutlich, P.; Hauser, A.; Spiering, H. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2024–2054.
99. Halcrow, M. A. *Chem. Soc. Rev.* **2008**, *37*, 278–289.
100. (a) Griller, D.; Nazran, A. S.; Scaiano, J. C. *Acc. Chem. Res.* **1984**, *17*, 283–289. (b) Eisenthal, K. B.; Turro, N. J.; Sitzmann, E. V.; Gould, I. R.; Hefferon, G.; Langan, J.; Cha, Y. *Tetrahedron* **1985**, *41*, 1543–1554. (c) Peon, J.; Polshakov, D.; Kohler, B. *J. Am. Chem. Soc.* **2002**, *124*, 6428–6438. (d) Wang, J.; Kubicki, J.; Peng, H.; Platz, M. S. *J. Am. Chem. Soc.* **2008**, *130*, 6604–6609.
101. Marcus, R. A. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155–196.
102. (a) Bengali, A. A.; Schultz, R. H.; Moore, C. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1994**, *116*, 9585–9589. (b) Schultz, R. H.; Bengali, A. A.; Tauber, M. J.; Weiller, B. H.; Wasserman, E. P.; Kyle, K. R.; Moore, C. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1994**, *116*, 7369–7377.
103. Siegbahn, P. E. M. *J. Am. Chem. Soc.* **1996**, *118*, 1487–1496.
104. Bengali, A. A.; Bergman, R. G.; Moore, C. B. *J. Am. Chem. Soc.* **1995**, *117*, 3879–3880.
105. Carreón-Macedo, J.-L.; Harvey, J. N. *Unpublished work*, **2006**.
106. Hardman, N. J.; Fang, X. G.; Scott, B. L.; Wright, R. J.; Martin, R. L.; Kubas, G. J. *Inorg. Chem.* **2005**, *44*, 8306–8316.
107. Detrich, J. L.; Reinaud, O. M.; Rheingold, A. L.; Theopold, K. H. *J. Am. Chem. Soc.* **1995**, *117*, 11745–11748.
108. (a) Austin, R. H.; Beeson, K. W.; Eisenstein, L.; Frauenfelder, H.; Gunsalus, I. C. *Biochemistry* **1975**, *14*, 5355–5373. (b) Taube, D. J.; Projahn, H. D.; van Eldik, R.; Magde, D.; Traylor, T. G. *J. Am. Chem. Soc.* **1990**, *112*, 6880–6886.
109. Projahn, H. D.; van Eldik, R. *Inorg. Chem.* **1991**, *30*, 3288–3293.

- 110. Schrock, R. R.; Shih, K. Y.; Dobbs, D. A.; Davis, W. M. *J. Am. Chem. Soc.* **1995**, *117*, 6609–6610.
- 111. Brayshaw, S. K.; Harrison, A.; McIndoe, J. S.; Marken, F.; Raithby, P. R.; Warren, J. E.; Weller, A. S. *J. Am. Chem. Soc.* **2007**, *129*, 1793–1804.
- 112. (a) Kubas, G. J. *Chem. Rev.* **2007**, *107*, 4152–4205. (b) Weller, A. S.; McIndoe, J. S. *Eur. J. Inorg. Chem.* **2007**, 4411–4423.

This page intentionally left blank

INDEX

A

- A549 cell line, 32
- A2780 cell line, 17, 29, 32, 54–55
- Acetonitrile, Li^+ ions, 529–530
 - $[\text{Li}(\text{NCCH}_3)_4]^+$, energy profile, 547–548
 - $[\text{Li}(\text{NCCH}_3)_4(\text{NCCH}_3)]^+$, 546
 - and water, 548
- Acid-induced demetalation, 479
- A2780cis cells, 29
- A2780cis xenograft, 30
- Acrylato-bridged dicobalt(III) complex, 453–454
- Acrylonitrile
 - Diels–Alder reactions of, 456
- Activation-by-ligand-oxidation mechanism, 35
- N*-acylneuraminic acids, 256
- Adenylate cyclase, 291
- Aequorin (2-(4'-hydroxyphenylacetyl)-amino-3-benzyl-5-(4'-hydroxyphenyl)pyrazine), 260, 290
 - kinetics of binding calcium, 304
- Aerobactin, 182, 184, 218
- Aeromonas hydrophila*, 229, 235
- $\text{Ag}^{\text{I}}\text{-C}_2\text{H}_4$ complexes, 416–417
- $\text{Ag}^{\text{I}}\text{-CO}$ complexes, 416–417
- $[\text{Ag}(\text{L}^9)(\text{C}_2\text{H}_4)]$, 417
- $[\text{Ag}(\text{L}^5)(\text{CH}_3\text{CN})]^+$
 - structure of, 413
- $[\text{Ag}(\text{L}^9)(\text{CO})]$, 417
- $[\text{Ag}(\text{L}^5)(\text{NCMe})]\text{BF}_4$, 413
- AlaGlu, $\log K$ value for, 278
- Alcaligin, 217, 227–228
- Alginic acid, 257
- $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$, dissociative water exchange on, 536
- “aliphatic” $[\text{}^{64}\text{Cu}(\text{ATSM})]$ hypoxia PET tracer, 156
- Alkaline earth metal cations,
 - stability constant relating to ligand selectivity for, 281–282
- Alzheimers diagnosis, 141
- Alzheimer's disease, 337
- Amine-thiophenolates, 433–434
- 7-amino-3-(2-benzothiazolyl)-6-[2-(2,3-difluoro-6-aminophenoxy)ethoxy]-coumarin-*N,N,N',N'*-tetraacetate, 279
- Aminobisphosphonate ibandronate, 337
- Aminocarboxylate complexes,
 - dissociation rate constants for, 286
- 6-amino-6-methylperhydro-1,4-diazepine-1,4,*N*⁶,*N*⁶-tetraacetate (AAZTA), 70
- o*-aminophenyl-*N,N,O*-triacetic acid, 260
- Ammonia exchange, ligand exchange process, 539–543
- beryllium (Be^{2+})
 - activation barrier, 542
 - Be–N distances, 542
 - $[\text{Be}(\text{NH}_3)_4]^{2+}$, 541
 - $[\text{Be}(\text{NH}_3)_5]^{2+}$, 541–542
 - $[\text{Li}(\text{NH}_3)_4]^+$, 539–540
 - substitution of NH_3 by H_2O , 540
- Androcam, 301
- Annexins, 254
- Anticancer drugs
 - ADME properties of metal-based drugs, 3
 - cytotoxic effects of complexes, 2
 - and ligand substitution, 5–6
 - metal-based therapeutics, 3
 - osmium-based, 2
 - platinum, 2–3
 - prodrug activation mechanisms, 5
 - prodrug approaches, 3–4
 - role of DNA synthesis and replication, 2
 - ruthenium, 2–3
- Apatite (hydroxyapatite), $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$, 331

- APM-3,4-HOPY, 232–233
 Apobovine α -lactalbumin, kinetics of reaction of Ca^{2+} with, 304
 Apoconcanavalin, reaction of Ca^{2+} with, 304–305
 Arachidonic acid, 313
 Aspartate, $[\text{O}_2\text{CCH}_2\text{CH}(\text{NH}_3)\text{CO}_2]^-$, 268
 Asp–His–Ser triad, calcium binding site of, 299
 $[\text{Au}_2(\text{C}_2\text{Ph})_2(\mu\text{--}4,4'\text{--Ph}_2\text{P}(\text{C}_6\text{H}_4)_2\text{PPh}_2)_2]$, 425
 $[\text{Au}_3(\text{PPh}_2(\text{C}_6\text{H}_4)_2\text{PPh}_2)_3]$, schematic structure of, 426
 Azelaic acid, 265
 Azide complex, molecular structure, 449
 (azpyz–NMe₂, 4-(1H-pyrazol-3-ylazo)-N,N dimethylaniline), 36
- B**
 Beauvericin, 311
 $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$, water exchange on, 536–537
 $\text{Be}(\text{H}_2\text{O})_4(\text{H}_2\text{O})]^{2+}$, water exchange on, 537
 Benzoyl peroxide
 plausible mechanism for early steps for activation of, 508
 steric effect, 507
 Beryllium (Be^{2+})
 ammonia exchange
 activation barrier, 542
 Be–N distances, 542
 $[\text{Be}(\text{NH}_3)_4]^{2+}$, 541
 $[\text{Be}(\text{NH}_3)_5]^{2+}$, 541–542
 application of, 524
 chelating ligands. *See* Chelating ligands, and beryllium (Be^{2+})
 12-crown-4, 560–564
 DMSO, 530, 545
 hydrogen cyanide (HCN), 545–551
 activation barrier, 550
 bonds, 550
 gas phase, 550
 substituents, 550
 water exchange study, 550–551
 overview, 523–524
 solvation, 526
 water exchange
 $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$, 536–537
 $[\text{Be}(\text{H}_2\text{O})_5]^{2+}$, 534–535
 $[\text{Be}(\text{H}_2\text{O})_4(\text{H}_2\text{O})]^{2+}$, 537
 B3LYP hybrid density functional, testing, 535–536
 hydrogen-bonded ligands, 535
 IPCM calculation, 536
 Binary compounds and salts, selection of
 coordination numbers (CN) of Ca^{2+} in, 262–263
 Binding mode, of coligands, 451
 Binuclear complexes of binucleating ligands, 437–442
 hexamine-dithiophenolate ligand H_2L_{19} , coordination of, 441
 $[\text{M}_2^{\text{II}}\text{L}^{\text{R}}(\mu\text{--L}')^+]$, 438
 molecular structure of dinickel(II) complex, 442
 $\text{N}_3\text{M}(\mu\text{--S})_2(\mu\text{--L}')\text{MN}_3$, 438
 synthesized complexes and their labels, 439
 Binucleating aza-thioethers, 433–434
 Binucleating Polyaza-dithiophenolate macrocycles, metalated container molecules, 430
 effects of N-alkylation on molecular and electronic structures of complexes, 442–445
 ligand matrix as medium, 445–446
 macrocyclic thiophenolate Schiff-base ligands, 430–431
 polyamine-dithiophenolate macrocycles, 431–442
 coordination chemistry of, 435–442
 reactivity of complexes, 452–457
 variation, coordination modes, and activation of coligands, 447–452
 Bipyridine, 31
 bisamide DTPA derivatives, 77
 1,2-bis-(2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid (baptaH₄), 259, 279
 Bis(Fe^{IV})-m-oxo complexes, 488
 α,ω -bis(3'-hydroxy-4'phenoxy) alkenes, 289

Bis(4-pyridyl)-functionalized Zn(II)-salen-type ligands, in THF, 419
 Bloembergen–Morgan theory, 69, 89
 Blood clotting, Ca^{2+} in, 307–308
 Bombesin, 138
 Bones and teeth
 properties, 332
 repair and replacement, 333–334
 substitution of constituent ions, 332–333
 Born–Oppenheimer approach, for hydration number, 271
 Bovine, Ca^{2+} complexes of, 301
 Bovine α -lactalbumin, displacement of Ca^{2+} from, 306
 5,5'-Br₂bapta, 301
 Bromination, of α, β -unsaturated carboxylate ligands, 454
 Brønsted acid buffer components, 482
 Brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 333
Burkholderia cepacia, 182

C

Ca^{2+}
 in blood clotting, 307–308
 carbohydrate complexes of
 stability constants, 319–321
 structures, 316–318
 in ionophores, 309–313
 phosphates, complexes of
 kinetics, 326, 328
 stability constants for, 323–324
 structures, 322–323
 phosphonates, complexes of
 kinetics, 326, 328
 stability constants for, 324–325
 structures, 322
 trans-membrane transport and, 309–310
⁴³Ca, 257
 NMR spectroscopy, 261
 Ca(α -D-allopyranosyl
 α -D-allopyranoside) $\text{Cl}_2 \cdot 5\text{H}_2\text{O}$, 317
 Ca(α -etmal)₂, 266
 Ca(α -Memal)₂, 266
 [Ca₂ (anilate)₂(H₂O)₈] $\cdot$ 4H₂O, 267

Ca^{2+} aqua-cation (Ca^{2+} aq), 268
 calcium-binding proteins reactivity with, 302–303
 Ca–O distances, 271
 computation and modeling, 271–272
 hydration numbers, 269–270
 kinetics and mechanisms
 complex formation and dissociation, 284–286
 metal exchange and ligand exchange, 287–288
 template reactions, 288–289
 water exchange, 283–284
 rate constants, 284–285
 stability constants, 273–275
 amino acids and peptides, 276–278
 aminocarboxylates, 278–279
 complex formation, enthalpies and entropies of, 282–283
 crown ethers and
 azamacrocycles, 280–281
 other carboxylates, 279–280
 selectivity, 281–282
 thermodynamic parameters, 272–273
⁴⁵Ca (β^- ; $\tau_{1/2}$ 165 days), 261
⁴⁷Ca (β^- , γ ; $\tau_{1/2}$ 4.5 days), 261
 Ca(β -D-mannofuranose) $\text{Cl}_2 \cdot 4\text{H}_2\text{O}$, 317
 Ca[Ca(edta)] $\cdot$ 7H₂O, 267
 Ca²⁺–calmodulin–cyclic nucleotide phosphodiesterase, 302
 Ca²⁺/calmodulin-dependent kinase II (CaMKII), 292
 Ca²⁺/Cu²⁺/edta/humate system, kinetics of, 307
 [Ca(cydtA)]²⁻, metal-exchange reactions of, 287
 Ca²⁺–DGBP–galactose, 302
 Ca²⁺-D(T₄G₄T₄) complex, stability constant for, 315
 [Ca(edta)]²⁻, metal-exchange reactions of, 287
 [CaFe₂(CCl₃CO₂)₆(thf)₄] $\cdot$ thf, 268
 CaF₂ gravimetric analysis, 261
 Ca₂GeO₄, 264
 Ca₂IrO₄, 264

- CAIX, 150–151
- Ca(5-keto-D-gluconate)₂ · 2H₂O, 318
- Ca(lactobionate)Br · 4H₂O, 318
- Calbindin, 294
- kinetics of Ca²⁺ in
 - dissociation, 303–306
 - formation, 303–305
 - log*K* values for, 299
 - ionic strength and, 301
- Calcimycin (A23187), 309–310
- formation rate constants for, 313
 - log*K* value for, 312–313
- Calcium, in biology
- calcium–carbohydrate species, 256–257
 - carboxylate salts and complexes, 265–268
 - coordination numbers
 - in hydrates of calcium chloride, 262–263
 - in selection of binary compounds and salts, 262–263
 - crystal hydrates, 264–265
 - metalloproteins, 254–256
 - monitoring, 257–261
 - oxides, 263–264
 - radioactive isotopes of, 261
 - rigid materials, 253
 - role in signaling, 255
 - structural effects, 253–254
- Calcium alginate, use of, 257
- Calcium aluminate
- (CaAl₂O₄ · 10H₂O), 264
- Calcium-and integrinbinding protein (CIB1), 255
- Calcium arabonate, 318
- Calcium ascorbate dihydrate, 267
- Calcium-binding metalloproteins, 254–256, 289
- and Ca²⁺ aq. reactivity with, 302–303
 - Ca²⁺ in blood clotting, 307–308
 - Ca–O distances of, 297–298
 - coordination numbers (CN) of, 297–298
 - interactions and functions of, 290–291
 - kinetics, 302
 - complex formation, 303–305
 - dissociation, displacement, and exchange, 305–307
 - stability constants, 299–302
 - structures and, 292–299
- Calcium bis(hydrogen malonate) (Ca(O₂CCH₂CO₂H)₂), 266
- Calcium–carbohydrate species, 256–257
- Calcium carbonate, 330
- Calcium channels, 313–315
- Calcium chloride, hydrates of
- coordination numbers (CN) of Ca²⁺ in, 262–263
- Calcium-containing rigid materials, 326
- bones and teeth
 - properties, 332
 - repair and replacement, 333–334
 - substitution of constituent ions, 332–333
 - calcium-linked disorders, 334–337
 - salts, 328
 - calcium carbonate, 330
 - calcium oxalate, 330–331
 - calcium phosphates, 331–332
 - soluble, 329–330
- Calcium glucuronate, 318
- Calcium glycerophosphate, 329
- “Calcium Green FIAsh”(Fluorescein Arsenical Hairpin binder), 260
- Calcium 2-hydroxy-3-mercapto-1-propanesulfonateaurate(III), 329
- Calcium iron(II) citrate, 329
- Calcium malonate dihydrate, 266
- Calcium *N*-carbamoylaspartate, 329
- Calcium 7-oxabicycloheptane- 2, 3-dicarboxylate, 266
- calcium oxalate, 330–331
- Calcium oxide (CaO), 263–264
- Calcium phosphates, 331–332
- Calcium pumps, 315–316
- calcium selective chelating unit, 106
- Calcium succinate trihydrate
- [Ca(succ)(H₂O)₂] · H₂O, 266
- Calcium tartra tetrahydrate, 266
- Calixarenes, 409–412
- copper complexes, 409–410

- diphosphine ligand (L^3), 411
 hydroxo-ligand ($L' = OH$) in, 410
 pocket-shaped ligand (L^2), 410–411
p-tert-butyl-calix[4]arenes, 410
 tris(2-methylpyridyl)amine ligand (L^1), 409–410
- Calmodulins (Ca_4calm), 291
 and Ca^{2+} , kinetics of, 304
 Ca^{2+} -binding sites in, 293, 294, 296
 dissociation of Ca^{2+} in, 305
 log*K* values for, 299, 301
- Calsequestrin
 log*K* values for, 299
- Cambridge Crystallographic Data Centre (CCDC), 434
- “Cameleons”, 260
- $CaMoO_4$, 264
- $CaMo_5O_8$, 263, 264
- Cancer cell selectivity, 7
- Ca NMR spectroscopy, 305, 306
- Ca–O distances
 of Ca^{2+} aq, 271
 of calcium–protein complexes, 297–298
- Carbohydrate complexes of Ca^{2+}
 stability constants, 319–321
 structures, 316–319
- carbon nanotubes loaded with Gd^{III} ions, 117–119
- Carbonyl compound, ligand exchange, 552–553
- carboplatin, 4
- γ -carboxyglutamate (gla), 308
- Carboxylate coligands, NMR spectroscopy of, 445–446
- Carboxylate ligands
 bromination of α, β -unsaturated, 454
 in dizinc complexes, exchange of, 446
- Carboxylato-bridged zinc complexes, 446
- Cardiac function, role of calcium in, 335
- Car–Parrinello MD methods, 271
- $Ca(saccharose)_2Br_2 \cdot 2H_2O$, 318
- $Ca(saccharose)Cl_2 \cdot 2H_2O$, 318
- Ca^{2+} –Syt–phospholipid, 302
- $CaTa_2O_6$, structural dimensions, 263–264
- Ca_3UO_6 , 264
- CCDC. *See* Cambridge Crystallographic Data Centre (CCDC)
- CCK-B, 138
- Cell death, role of calcium in, 335
- Cepabactin, 182, 184
- Chatt cycle, and N_2 fixation
 Mo nitrido/imido complexes, 379–382
 N–N bond cleavage, 374–378
 overview, 370–371
 protonation of bound N_2 , 372–374
 theoretical investigations, 382–386
- Chelate effect, 185
- Chelating ligands, and beryllium (Be^+), 555–560
 1H-imidazole-4,5-dicarboxylate (L^{2-}), 556
 1H-imidazol-3-ium-4, 5-dicarboxylate (LH^-), 556
 water molecules, 556–560
 activation energies, 559–560
 aromaticity, 560
 hydrogen bonds, 556–557, 558–559
 stabilization, 558
- Chelation therapy
 hydroxypyranones and hydroxypyridinones, use in, 282
- chemical exchange saturation transfer (CEST), 98–99
- chemotherapeutic anticancer drug. *See* Anticancer drugs
- chemotherapy, 1
- Chondroitin-4- and -6-sulfates, 256
- $CH-PY_2PHOS_2$, 80
- cis*,*trans*-[Pt(en)I₂(OH)₂] complex, 11
- cis*,*cis*,*trans*-[Pt(N₃)₂(NH₃)₂(OH)₂] (4), 12, 13–14, 19
- Cis-[(PET₃)₂Pt(OTf)₂], 418
- cisplatin, 2, 4
- cisplatin-resistant A2780cis human ovarian cancer cells, 17
- cisplatin-resistant 5637 cell lines, 14
- cisplatin-sensitive A2780, 17
- cis*-[RuCl₂(NH₃)₄]Cl, 22

- cis,trans*-[Pt(en)I₂(OAc)₂], 10, 11–12
cis,trans-[Pt(en)I₂(OH)₂], 11
cis,trans-[Pt(en)(N₃)₂(OH)₂], 13–14
cis,trans-[Pt(N₃)₂(OH)₂(en)], 13
 cobalt chaperone complexes, 7
 Cofacial porphyrin dimers
 chemical structures, 425
 “cold” Re(I) fluorescent probes,
 138
 Coligands
 variation, coordination modes, and
 activation of, 447–452
 Colloidal calcium phosphate (CCP),
 323
 1:1 complex
 between $K_a(\text{CD}_3\text{OD})$ and
 tetrasulfonatocalix[4]arene,
 structure, 411
 Complex dissociation
 with Ca^{2+}aq , 286
 of Ca^{2+} from calcium
 metalloproteins’ kinetics of,
 305–307
 Complex formation
 with Ca^{2+}aq , 284–286
 cryptands, 285–286
 enthalpies and entropies of,
 282–283
 of calcium-binding proteins,
 303–305
 “complex in a complex”, metalated
 container molecules, 421–427
 encapsulation of reactive
 organometallic complexes,
 423–425
 metallobridged cavitand, 427
 polynuclear clusters stabilized in
 coordination cages, 425
 porphyrin units in, 422–423
 stabilization luminescent Au_6Cu_6
 cluster, 425–427
 structures of the building blocks of,
 423
 Concanavalin A, 293
 Ca...Ca distances, 298
 polysaccharide-binding sites of,
 299
 “conditional” equilibrium constant,
 186
 Conditional stability
 constant (K^*), 94–95
 Connexins, 314
 Coordination chemistry, of
 polyamine-dithiophenolate
 macrocycles, 435–442
 binuclear complexes, 437–441
 dinickel complexes in, 431–432
 mononuclear complexes, 435–437
 Coordination numbers (CN) of Ca^{2+}
 of calcium–protein complexes, 297
 of carbohydrate complexes of Ca^{2+} ,
 316–317
 in hydrates of calcium chloride,
 262–263
 in phosphates, 322
 in phosphonates, 322
 in selection of binary compounds
 and salts, 262–263
 Copper isotopes, 134
 [Co(thf)₂], 428
 [Cp⁺Mn(CO)₂]⁺
 stabilization in octahedral
 [Pd₆(L¹⁴)₄]₁₂⁺ coordination
 cage, 424
 [CpRu(cis-1,3,7-octatriene)]⁺
 stabilization in tetrahedral
 [Ga₄(L¹³)₆]₁₂[−] coordination
 cage, 424
 CRACs (Ca^{2+} -release activated
 channels), 313–314
 o-cresolphthalein complexone
 (cpcH₆), 274
 o-cresolphththalein complexone,
 284
 [Cr^{III}H₂L¹⁹(OAc)]²⁺, 436
 12-crown-4, and beryllium (Be^{2+}),
 560–564
 Crown ether, and beryllium. *See*
 12-crown-4, and beryllium (Be^{2+})
 Cryptands, formation and
 dissociation of Ca^{2+} complexes of,
 285–286
 Crystal hydrates, of calcium,
 264–265
 CT DNA platination, 15
 C-type (Ca^{2+} -dependent) animal
 lectins, 296
 [Cu(ATSM)], 151–154

[⁶⁴Cu(ATSM)], ⁶⁴Cu(II)-diacetyl-bis(*N*⁴-methylthiosemicarbazonato), 151
 Cucurbit[n]uril family (CB[n]), 421–422
 structures of, 422
⁶⁴Cu(II) bis(thiosemicarbazonato) complex, 153
 [Cu^{II}(L¹⁰)]B(C₆F₅)₄, structure, 418
 [Cu^{II}(L¹⁰)(O₂)]SbF₆, structure of, 418
 [Cu(L¹)(OH)]⁺, 410
 [Cu(L¹⁰)(O₂)]SbF₆, 417
 [Cu(NH₃)₄]²⁺
 β-diketonate ligands reaction with, 419–421
⁶⁷Cu radiolabeled porphyrin, 158
⁶⁴Cu(TETA)-radiolabeled folate-conjugated shell cross-linked nanoparticle, 166
 Cyan fluorescent protein (CFP), 260
 Cyclam derivative, 148
 3',5'-cyclic guanosine monophosphate (3',5'-cGMP), 40
 Cyclodextrins (CD), 412–414
 complexation of ligands with AgBF₄, 413
 derivatization of, 413
 metallocavitands based on phosphanyl-substituted of, 413
 synthesis of ligands, 414
 cyclohexane-1,2-diamine backbones (CHPY₂PHOS₂), 80
 Cyclo(–LPro–LPro–LLeu–LLeu–LLeu–)₂, 278
 Cyclosporins, 311
 Cyclo(–Thr–Val–Abu–Ile–)₂, logK value for, 278
 CydtaH^{3–}, reaction of Ca²⁺aq with, 285
 Cytotoxicity and oxygen-dependence, 8

D

Decamethylchromocene, 382
 Deferiprone (**50**), 237
 DeferiproneTM, 236
 Deferoxamine, 144, 145

Demetalation of Fe^{III}-TAML activators
 coordinative general acid catalysis, 487
 dependence of *k*_{obs} *vs.* [H⁺] for, 478
 DFT studies, Mulliken charges, 480–481
 effect of nicotinic acid on, in presence of picolinic acid, 484
 H⁺-induced, 475
 hydrolytic instability, 480
 N-protonation target, 481
 phosphate-induced, 483
 phosphate role in, 482
 by picolinic acid, 485–487
 pre-equilibrium M–N cleavage, 479
 pseudo-first-order rate constants for, 482–484
 rate of acid-induced, 479
 specific acid catalysis, 478–482
 sterically assisted electronic effect, 479
 stoichiometric mechanism of, 484–485
 Density Functional Theory (DFT), 271
 DesferaltTM, 236
 Desferrioxamine B, 184, 185, 217, 223, 229, 234
 D-galactose-binding protein (DGBP), 254
 D-glucuronic acid, 256
 D-glucuronolactone, 256
 1D ¹H, 2D [¹H, ¹⁵N] HSQC, 11
 2D [¹H, ¹⁵N] HSQC, 11
 1,2-Diaminobenzene (dab)
 complexes, 31
 1,3-diamino-2-hydroxypropane-*N,N,N,N'*-tetraacetate, 332
 1,3-diaminopropane, 288
 1,10-diaza-18-crown-6 complexes,
 stability constant for, 281
 4-Diazenylbenzosulfonate, oxidation of, 509–510
 2,6-dicarbonylpyridines, 288
 Diels–Alder reactions, 453, 455–457
 between complexes and acrylonitrile, 456

- between complexes and dimethylbutadiene, 455
 - between sorbinic acid and acrylonitrile, 456
 - Diethylenetriamine tetraacetate (DTTA⁴⁻) ligands, 70
 - 2,5-diformylfuran, 288
 - 2,3-dihydroxy-*N,N*-dimethylbenzamide, 199
 - Diiodide salt [Ca(glyH)₂(H₂O)₃]₂, 268
 - Diiodido-Pt(IV) complexes, 11
 - β -diketonate ligands reaction with [Cu(NH₃)₄]²⁺, 419–421
 - β -diketone, 260
 - Dimethylsulfoxide (DMSO), 412
 - Dimethylsulfoxide (DMSO), ligand
 - exchange process, 543–545
 - [Be(DMSO)₄]²⁺, 545
 - lithium (Li⁺), 527–529
 - [Li(DMSO)₄]⁺, 543
 - [Li(DMSO)₅]⁺, 543
 - Dinickel(II) complex
 - molecular structure of, 442
 - Diphosphine ligand (L³), in calixarenes, 411
 - Displacement and exchange of Ca²⁺ from calcium metalloproteins' kinetics of, 305–307
 - Dissociation
 - with Ca²⁺ aq, 286
 - of Ca²⁺ from calcium metalloproteins' kinetics of, 305–307
 - rate constants, of calcium complexes, 286
 - Dizinc complexes
 - exchange of carboxylate ligands in, 446
 - DMETAM, 205
 - DMHP (50), 210
 - DMSO. *See* Dimethylsulfoxide (DMSO)
 - 1D-NOESY spectrum, of aryl-substituents of diphosphine ligand, 411
 - DO3A-type chelate, 72
 - DOCYDMAHA, 232, 233
 - Dodecapeptide cyclo-(-LVal-Gly-Gly-LPro-)₃, log*K* value for, 278
 - Dota, selectivity for, 281–282
 - DOTA-amide complexes, 74–75
 - DOTA framework, metal stability, 145–148
 - Drosophila melanogaster*, 291
 - DTPA-amide derivatives, 96–97
 - DTPA- or DOTA-derivatives, 65–66
 - Dual-modality imaging methods, 135
- E**
- Edta
 - with Ca²⁺ aq, rate constant for, 285
 - selectivity for Ca²⁺, 281
 - stability constants for, 278–279
 - EDTA-derivative Mn^{II} complexes, 112–113
 - Eigen-Tamm-Wilkins mechanism (ACS), 284
 - Electron spin relaxation, of Gd^{III} complexes, 88–89
 - Energydispersive X-ray fluorescence spectrometry (EDXRF), 333
 - Enniatins, 311
 - Enterobactin, 184
 - Enthalpies of complex formation
 - between Ca²⁺ and nucleotide 5'-phosphates, 325
 - with Ca²⁺ aq, 282–283
 - calcium-protein complexes, 301
 - Entropies of complex formation
 - between Ca²⁺ and nucleotide 5'-phosphates, 325
 - with Ca²⁺ aq, 282–283
 - calcium-protein complexes, 301
 - Enzymes, 472
 - Enzyme-specific agents, self-immolative degradation of, 105
 - Equilibrium and kinetic parameters for Fe^{III}-TAML activators, 475
 - Equine lysozyme, Ca²⁺ complex of, 299
 - 9-EtG, 38
 - Ethane-1,2-diamine (EN-PY₂PHOS₂), 80

Ethoxymethylphosphonate, 324
 ethylenediaminetetraacetate, 190
 Ethylene glycol bis-(2-aminoethyl ether)tetraacetate (egta), 259, 274–275
 Exjade™, 236

F

- fac*-[RuCl₃(NH₃)₃], 22
 Fe–Cl bonds, in Fe^{III}-TAML
 activators, 473–474
 Fe³⁺–D-gluconate–Ca²⁺ system
 stability constants, 320
⁵⁷Fe-enriched oxoiron(V) compound, 493
 Fe[Gd₂(bpy-DTTA)(H₂O)₂]₃^{4–}, 87
 Fe[Gd₂(bpy-DTTA₂)(H₂O)₄]₃^{4–}, 90
 Fe³⁺/H₃L_R²³⁴ equilibrium system, 192
 Fe^{II}[Gd(tpy-DTTA)(H₂O)₂]₂, 87
 Fe^{III}-TAML activators
 addition of H₂O₂/t-BuOOH to
 aqueous solutions of, 489–490
 aromatic component of, 513
 catalysis-relevant oxidized forms
 derived from
 aprotic organic solvents, 487–488
 benzylic alcohols, 489
 brownish-green colors, 489–490
 ⁵⁷Fe-enriched oxoiron(V)
 compound, 493
 iron-oxo intermediate, 491–492
 monomeric oxoiron(IV) species,
 492
 μ-oxo-bridged diiron(IV)
 complex, 488–489
 peroxides ROOH, 490–491
 red compound, 490–491
 demetalation of
 coordinative general acid
 catalysis, 487
 dependence of *k*_{obs} vs. [H⁺] for,
 478
 DFT studies, Mulliken charges,
 480–481
 effect of nicotinic acid on, in
 presence of picolinic acid, 484
 H⁺-induced, 475
 hydrolytic instability, 480
 N-protonation target, 481
 phosphate-induced, 483
 by picolinic acid, 485–487
 pre-equilibrium M–N cleavage,
 479
 pseudo-first-order rate constants
 for, 482
 rate of acid-induced, 479
 specific acid catalysis, 478–482
 sterically assisted electronic
 effect, 479
 stoichiometric mechanism of,
 484–485
 equilibrium and kinetic
 parameters for, 475
 of H₂O₂, 472–473
 hydrogen peroxide catalytic
 activation by, 516
 mechanism of catalysis by
 benzoyl peroxide, 507–510
 catalase-like activity, 504–507
 general mechanistic picture of,
 494–495
 peroxidase-like activity, 495–503
 oxidized, 495
 solid state structure of
 Fe–Cl bonds, 474
 Fe–N bonds, 473
 speciation in aqueous solution
 binding of axial ligands, 475–477
 bis-ligated species, 476
 chloro ligands, 474
 Fe–Cl bonds, 473–474
 Fe–N bonds, 473
 Fe^{III}-TAML catalyzed bleaching, 514
 [Fe^{II}(L⁸)(Cl)], structure of, 416
 [Fe^{II}(L⁷)(N₃)]⁺
 structure of, 415
 [Fe^I(L⁸)(N₂)], structure of, 416
 [Fe^{IV}(L⁷)(N)]⁺
 structure of, 415
 [Fe(L⁷)(N)][BPh₄], 415
 Fe–N bonds, 473
 Ferrichrome, 183, 184
 Ferric iron, 182
 Ferrioxamine B, 218, 233–234
 Ferrous iron. *See* Iron, chemistry of
¹⁸F-fluoroazomycinarabinofuranoside
 (FAZA), 151
¹⁸F-fluoromisonidazole (FMISO), 151

Fluo-3, fura-2, 259
 Fluorapatite, 331, 332, 333
 Fluorescent agents, for radiolabeling
 $^{64}\text{Cu}(\text{II})$ bis(thiosemicarbazonato)
 complex, 153
 PET isotopes, nonconventional
 metallic, 143–148
 SPECT reagents, 137–143
 uptake of the Re(I) analog in tumor
 cells, 139
 Fluorescent Ca^+ indicator proteins
 (FCIP), 260
 Fluorescent chelating indicators,
 305–306
 Fluorescent nitroaromatic
 compounds, use of, 150
 Fluorescent sensors, 135–136
 Fluorescent sulfonamides, 150–151
 Folic acid, 144, 145, 167, 168
 Formaldehyde, ligand exchange
 processes, 553
 Fructose, in $\text{CaX}_2(\beta\text{-D-}$
 $\text{fructose}) \cdot 2\text{H}_2\text{O}$, 318
 Fulvic acid, 321
 Furaptra, 259, 279
 dissociation of, 286

G

^{66}Ga - and ^{68}Ga -labeled Ga(III)-
 deferoxamine-folate, 144
 ^{67}Ga citrate, 162
 $^{67}\text{Ga}@C_{60}(\text{OH})_x$, 167
 Gadofullerenes, 115–117
 Gadolinium(III) complexes, 65
 bishydrated MS325 derivative, 79
 DO3A-monoamide complex, 78
 DTTA-derived, 79
 kinetic inertness, 95–98
 ligands forming bishydrated, 71
 ligands of, 66
 Lipari–Szabo analysis of the
 longitudinal ^{17}O and ^1H
 relaxation rates, 81–83
 non-Classical Gd^{III} -based MRI
 contrast agents
 carbon nanotubes, 117–119
 gadofullerene, 115–117
 PAMAM-type dendrimeric,
 81–85

poly(amino carboxylate)
 complexes, 76
 relativity
 electronic relaxation, 88–89
 Gd–H distance, 72–73
 hydration numbers for
 complexes, 69–72
 MRI optimization using contrast
 agent, 90–92
 paramagnetic relaxation, 67–68
 rotational motion, 80–88
 second and outer sphere, 89–90
 water/proton exchange, 73–80
 schematic representation of, 68
 stability aspects, 92–93
 thermodynamic stability
 constants, 93–95
 T_1 relaxation times of gray and
 white matter, 90
 Gadolinium(III) (XCyTRINt), 162
 [Ga(DOTA)] derivative, 145
 Galacticol ($\text{HOCH}_2(\text{CHOH})_4$
 CH_2OH), 318
 Galactose, 256
 $[\text{Ga}_4(\text{L}^{13})_6]^{12-}$ coordination cage
 [CpRu(cis-1,3,7-octatriene)]⁺
 stabilization in tetrahedral, 424
 Gap junctions, defined, 314
 Gastrointestinal tract (GIT), 255
 $[\text{Gd}(\text{CH-PY}_2\text{PHOS}_2)(\text{H}_2\text{O})_q]^{3-}$, 80
 $[\text{Gd}(\text{DO}_3\text{A-MA})(\text{H}_2\text{O})]$ moieties, 83
 $[\text{Gd}(\text{DOTA-4AmP})(\text{H}_2\text{O})]$
 $(k_{\text{ex}}^{298} = 4 \times 10^3 \text{ s}^{-1})$, 73
 $[\text{Gd}(\text{DOTA-Glu}_{12}\text{Gly}_4)(\text{H}_2\text{O})]^-$, 85
 $[\text{Gd}(\text{DOTA})(\text{H}_2\text{O})]^-$, 80
 $[\text{Gd}(\text{DTPA-BMA})(\text{H}_2\text{O})]$, 92, 97
 $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$, 65
 $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$, 79
 $[\text{Gd}(\text{EN-PY}_2\text{PHOS}_2)(\text{H}_2\text{O})_{0.6}]^-$
 $(k_{\text{ex}}^{298} = 7.0 \times 10^8 \text{ s}^{-1})$, 73
 Gd(HP-DO3A)-derivative complexes,
 103–104
 $[\text{Gd}(\text{HP-DO}_3\text{A})(\text{H}_2\text{O})]$, 92, 97
 Gd– H_{water} vector, 85
 $[\text{Gd}_3(\text{mes-DTTA}_3)(\text{H}_2\text{O})_6]^{3-}$, 91
 $[\text{Gd}(\text{PhenHDO}_3\text{A})(\text{H}_2\text{O})_q]$ chelate, 86
 $[\text{Gd}(\text{transDO}_2\text{A})(\text{H}_2\text{O})_q]^+$, 80
 $[\text{Gd}(\text{TREN-bis}(6\text{-Me-HOPO})\text{-}$
 $\text{TAMTRI})(\text{H}_2\text{O})_2]$, 79

- $^{68}\text{Ge}/^{68}\text{Ga}$ parent/daughter pair, 143–144
 $\text{G5}[\text{Gd}(\text{EPTA})(\text{H}_2\text{O})]_{111}$, 82
 Glucose-ATSM derivative, 154
 Glutamate, $[\text{O}_2\text{CCH}_2\text{CH}_2\text{CH}(\text{NH}_3)\text{CO}_2]^-$, 268
 Glutathione (γ -L-Glu-L-Cys-Gly; GSH), 11
 Glyceric acid, 266
 Glycylglycyl glycinate
 $\text{CaCl}_2(\text{glyglygly}) \cdot 3\text{H}_2\text{O}$, 268
 GlyGlu, $\log K$ value for, 278
 G-quartets and quadruplexes, 314–315
 Green Fluorescent Protein (GFP), 260
 Guanosine (Guo), 38
 Guanosine 5'-monophosphate (5'-GMP), 11
 5'-guanosine monophosphate (5'-GMP), 38
- H**
 HaCaT cells, 16
 HaCaT keratinocytes, 16
 Haematoporphyrin, 157, 159, 160
 Half-sandwich ruthenium(II)-arene complexes, 23
Handbook of Green Chemistry, 473
 Hartree-Fock (HF) approaches, 271
 H^+ -assisted dechelation of iron, 225
 $\text{HBF}_4/\text{diethylether}$, 382
 H_5BOPTA , 66
 $\text{H}_3\text{BT-DO3A}$, 66
 $\text{H}_2\text{cisDO2A}$, 79
 HCN. *See* Hydrogen cyanide (HCN)
 H_5COPTA , 78
 $\text{H}_4\text{DO3A-Nprop}$, 77
 $\text{H}_5\text{DO3AP}$, 77
 H_4DOTA , 66
 H_5DTPA , 66
 $\text{H}_3\text{DTPA-BMA}$, 66
 $\text{H}_3\text{DTPA-BMEA}$, 66
 $\text{H}_5\text{DTTA-Nprop}$, 77
 $\text{H}_5\text{DTTA-N'prop}$, 77
 HeLa cancer cell, 153, 168
 Hemocyanin, 293
 $\text{H}_5\text{EOB-DTPA}$, 66
 H_5EPTA , 77
 Hexamine-dithiophenolate macrocycles, S-oxygenation, 441–442
 Hexaaza-disulfinate derivative, dinuclear Cu(II) complex, 442
 Hexadentate EDTA complex, 191
 Hexadentate TREN-Me-3,2-HOPO (27), 210
 Hexaisopropylterphenyl-triamidoamine (HIPTN₃N), 369
 $\text{H}_3\text{HP-DO3A}$, 66
 1H-imidazole-4,5-dicarboxylate (L^{2-}), 556
 1H-imidazol-3-ium-4,5-dicarboxylate (LH^-), 556
 Histidine residues, 45
 H_2L^{19} , structure of, 429
 H_4L^{18} , structure of, 429
 HOMO and sHOMO orbital, 481
 Homogeneous green oxidation catalysts, activity-stability parameterization of kinetic model for parameterization LFER plot, 513
 Safranine O bleaching, 512–514
 synthetic homogenous oxidation catalysts, 511
 TON and TOF, 510
 model verifications catalyst performance, 514
 simulated bleaching of hypothetical dye, 515
 HOPO family, 72
 3,4-HOPO moiety forms, 210
 Horse heart cytochrome c, 45
 Horseradish peroxidase, 293
 1H2P (47), 210
 $\text{H}_2\text{transDO2A}$, 79
 H_4TRITA , 77
 Human lactalbumin, Ca^{2+} complexes of, 301
 Humic acid, 321
 Huntington's disease, 256
 Hydration numbers, of Ca^{2+} aq, 269–270
 Hydrazine complex, molecular structure, 450

- Hydrogen cyanide (HCN), 545–551
 beryllium (Be^+), 549–551
 activation barrier, 550
 bonds, 550
 gas phase, 550
 substituents, 550
 water exchange study, 550–551
 lithium (Li^+), 545–546
 water, 548–549
- Hydrophobicity
 of binding cavity of $[\text{Zn}_2(\text{L}^{19})]^{2+}$
 fragment, 446
- Hydroxamate donor group, 182–183
- Hydroxo-ligand ($\text{L}'=\text{OH}$), in
 calixarenes, 410
- Hydroxycarboxylate, stability
 constants for, 279–280
- Hydroxycarboxylate ligands
 stability constants for, 320–321
- 1-Hydroxyethylidene-1,1-bisphosphonic acid (hedp), 336
- Hydroxypyranones, use in chelation
 therapy, 282
- Hydroxypyridinone donor group, 182
- Hydroxypyridinones, use in chelation
 therapy, 282
- Hypercalcemia, 335
- Hypoxia-inducible factor-1 (HIF-1),
 141–142
- Hypoxic selective imaging probes,
 148–156
- I**
- IGROV cancer cell, 153
- Imidazole
 carboxylate, 268
 orientation in 1-methylimidazole,
 477
- Imidazole ligand
 orientation in 1-methylimidazole,
 477
- Imido complexes, molybdenum (Mo)
 imido-azido complexes, 379
 imido-chloro complexes, 379
 $n-\pi^*$ transition shift, 380
- Incoherent quasi-elastic neutron
 scattering (IQENS), 283–284
- ^{111}In dium, 142–143
- Inositol-1,4,5-triphosphate (IP3), 255
- Integrin, 142
- Ionic strength
 stability constants of calcium-
 binding proteins and, 301
- Ionomycin, 309, 310
- Ionophores, Ca^{2+} in, 309–313
 complexes of, stability constants
 for, 311–312
- Iron
 chelators, 237
 chemistry of, 179–181
 iron-siderophore complex. *See*
 Siderophores
- Iron chelation therapy, 236–237
- Iron(III) dissociation, kinetics,
 227
- Iron(III)-TAML activators. *See* Fe^{III} -
 TAML activators
- Iron pentacarbonyl, photolytic
 chemistry of
 addition reactions of, 594–599
 kinetics of, 599–605
 computational studies of the
 addition of ligands to, 581–593
 experimental background, 577–581
- IR spectrum
 of BH_4^- complex, 452
- J**
- Job plot analysis
 of titration in CD_3OD , 411
- Juniperus sabina*, 265
- K**
- $K_a(\text{CD}_3\text{OD})$, 411
- $\text{K}[\text{Ca}(\text{D-galacturonate})_3] \cdot 3\text{H}_2\text{O}$, 318
- KCM-1, fluorescent probe for Ca^+
 and Mg^+ , 260
- KP1019, 22, 23
- Krestov's approach, 272
- L**
- Lactalbumins
 $\log K$ values for, 299
- Lactose, 256
- Lanthanide(III) chelates, hydration
 state of, 69–70
- Large-angle X-ray scattering (LAXS),
 270

- Lariat crown ether ligands, 281
Lasalocid A (X-537A), 310, 311, 312
L-dopa (3-hydroxytyrosine), $\log K$ value for, 278
Ligand exchange processes, 525
 ammonia exchange, 539–543
 carbonyl compound, 552
 chelated beryllium diactions. *See*
 Chelating ligands, and beryllium (Be^+)
 DMSO. *See* Dimethylsulfoxide (DMSO), ligand exchange process
 five coordinated beryllium diactions, 560–564
 formaldehyde, 553
 HCN, 545–551
 water exchange. *See* Water exchange, ligand exchange process
Ligand-exchange rates, in metal ions, 5–7
Ligand exchange reactions, of
 calcium complexes, 287–288
Ligand matrix, as medium, 445–446
Li/naphthalene, in THF, 433
LIPOCEST, 100–101
Liposome constructs, 165
Lithium (Li^+)
 acetonitrile exchange, 529–530
 $[\text{Li}(\text{NCCH}_3)_4]^+$, energy profile, 547–548
 $[\text{Li}(\text{NCCH}_3)_4(\text{NCCH}_3)]^+$, 546
 and water, 548
 ammonia exchange, 539–540
 substitution of NH_3 by H_2O , 540
 application, 524
 DMSO exchange, 527–529
 $[\text{Li}(\text{DMSO})_4]^+$, 543
 $[\text{Li}(\text{DMSO})_5]^+$, 543
 overview, 523–524
 solvation
 acetonitrile, 529–530, 531
 binary solvent mixture, 531
 chemical shift, 531
 coordination number of, 526–527
 DMSO, 527–529, 531
 NMR study, 527
 selective coordination, 531
 water molecules, 527
 water exchange
 aqua complexes, 533
 $[\text{Li}(\text{H}_2\text{O})_5]^+$, 533–534
L-lysinehydroxamate (LyHA), 224
 Ln^{III} poly(aminocarboxylate)
 complex, 67
Lonomycin, 309
low-energy LMCT transitions, 11
L-type voltage-gated channels, 313
Lucifer dyes, 150
Lutetium analog, 163
Lutidinium tetrakis(3,5-bis-trifluoromethyl-phenyl)-borate ($\text{LutHBAr}'_4$), 369
LXFL 529L, 31
Lysocellin, 309, 310, 312
- M**
Macrobicyclic cage, structure of, 428
Macrocyclic aminocarboxylate dota, reaction of Ca^{2+}aq with, 285
Macrocyclic polyamine-thiophenolate ligands, 436
Macrocyclic thiophenolate Schiff-base ligands, 430–431
 dinickel complex, 431
Magnetic resonance (MR) images
 advantages, 63–64
 disadvantages, 64
[M(ATSM)] analogs, 155
MCF-7 cancer cells, 141
M–Cl bonds
 in transition metal complexes, 474
Medicinal chemist, task of, 1
Melanin
 $\log K$ values for, 299
Melittin, 302
24-membered Robson-type hexaazadithiophenolate ligand (H_2L^{19}), 429
Metalated container molecules, 409
 of binucleating Polyazadithiophenolate macrocycles, 430
 effects of N-alkylation on
 molecular and electronic structures of complexes, 442–445

- ligand matrix as medium, 445–446
- macrocyclic thiophenolate Schiff-base ligands, 430–431
- polyamine-dithiophenolate macrocycles, 431–442
- reactivity of complexes, 452–457
- variation, coordination modes, and activation of coligands, 447–452
- calixarenes, 409–412
- “complex in a complex” type, 421–427
- cyclodextrins, 412–414
- molecular squares, 418–421
- other supporting ligands, 427–430
- schematic representation of architectures of, 409
- sterically demanding tripod ligands, 414–418
- Metal carbonyls, 5
- Metal complexes, chemical reactivity, 452–457
 - acrylato-bridged dicobalt(III) complex, 453–454
- Metal exchange reactions, of calcium complexes, 287–288
- Metal ion sensitive probes, 105–109
- Metallobridged cavitand, structure of, 427
- Metalloporphyrins, applications of, 156–163
 - carboxyl porphyrin, 159
 - copper porphyrins, 159
 - ^{64}Cu labeling porphyrins, 159
 - ^{64}Cu tetracarboxytetraphenylporphyrin, 159
 - gallium and indium porphyrins, 160
 - haematoporphyrin, 157, 159, 160
 - indirect labeling of porphyrins with $^{99\text{m}}\text{Tc}$, 159–160
 - N*-4-nitrobenzyl-5-(carboxyphenyl) porphyrin, 159
 - PDT using pyropheophorbide porphyrins, 161
 - photofrin, 160
 - Re porphyrins, 159
 - Tc octaethylporphyrin, 159
 - tetraphenyl porphyrins (TPPH₂), 159, 162
 - tetra(4-trimethylaminophenyl) porphyrin complex, 160
 - uptake of porphyrins in tumors, 159
 - use of radiolabeled, 160–161
- Metalloproteins, calcium-binding, 254–256, 289
 - Ca^{2+} aq, reactivity with, 302–303
 - Ca^{2+} in blood clotting, 307–308
 - Ca–O distances of, 297–298
 - coordination numbers (CN) of, 296–297
 - interactions and functions of, 290–291
 - kinetics, 302–308
 - complex formation, 303–305
 - dissociation, displacement, and exchange, 305–307
 - stability constants, 299–302
 - structures and, 292–299
- Metallostear $\{\text{Fe}[\text{Gd}_2(\text{bpy}-\text{DTTA})(\text{H}_2\text{O})_2]_3\}^{4-}$, 72
- Metal nitrosyls, 5
- Metals, in medicine, 2–4
- Methyl aldofuranosides, 320
- Methylenebisphosphonate derivatives, for treatment of osteoporosis and related disorders, 336
- 1-methyl-1H-imidazol-3-ium-4, 5-dicarboxylate, 556
- 1-Methylimidazole (MeIm) adduct structure of, 477
- Methylphosphonylphosphonate, $[\text{MePO}_2\text{OPO}_3]^{3-}$, 325
- $[\text{M}_2^{\text{II}}\text{L}^{\text{R}}(\mu\text{-L}')^+]$, structure of, 438
- Mixed phosphorus/nitrogen (P/N) ligands, 397–401
 - pyNP₂ ligand, 399–401
- β_{MLH} notation, 187
- $[\text{M}(\text{L}^6)(\text{L}')_2]^+$ structure of, 414
- $[\text{M}_2(\text{L}^{19})(\text{L}')^{\text{n}+}]$ complexes, structure of, 429
- $[\text{MnCa}(\text{conA})]$, 305

- Mn^{II} complexes
 MRI properties, 109–113
 stability, 113–115
 Mn^{II}-dipyridoxylidiphosphate
 [Mn(DPDP)]²⁻, 109
 [Mn(NOTA)]⁻, 110
 Mo-fluoro system, 384
 M–OH₂ bonds, 5
 Mo(I) ammine complex, 383–384
 Mo(I) fluoro-ammine complex,
 384
 Mo(I) fluoro complex, 382
 Mo(II) azavinylidene complex
 [MoCl(NQC(CH₃)₂)(dppe)₂], 381
 Mo(II) difluoro complex, 383
 Mo(III) triamidoamine complex
 [Mo(HIPTN₃N)], 367
 Mo(IV) chloro-ethylimido complex
 [MoCl(NEt)(dppe)₂]Cl, 381
 Mo(IV)-NNH₂ compounds, 381
 Molecular dynamics (MD) studies, of
 Ca²⁺ aq, 271
 Molecular imaging
 of biological systems, 135–136
 developments in, 137
 procedure, 101
 Molecular squares, 418–421
 β-diketonate ligands reaction with
 [Cu(NH₃)₄]²⁺, 419–421
 Pt complexes, 418–419
 structure of, 420
 schematic illustration of metal-
 organic, 419
 structure of, 420
 Molybdenum diphosphine complexes,
 382
 Molybdenum-iron (MoFe) protein,
 368
 Molybdenum (Mo), N₂ fixation with
 imido complexes
 imido-azido complexes, 379
 imido-chloro complexes, 379
 n-π* transition shift, 380
 mixed P/N ligands, 397–401
 nitrido complexes, 379–381
 azido-nitrido complexes
 [Mo(N)(N₃)(diphos)₂], 379
 metal-N stretch, 380
 metal-N vibration, 380
 nitrido-acetonitrile complex, 379
 nitrido-chloro complexes, 379
 N–N cleavage reaction, 374–375
 biphasic kinetics, 376, 378
 pentaphosphine ligands, 391–396
 protonation, 372
 five-coordinate complex, 374
 N–N force constant, 373
 tetraphos ligands. *See* Tetraphos
 ligands, and Mo dinitrogen
 complexes
 Monensin, 310
 Monodentate ligand Z, 32
 Mono-ligated 1-methylimidazole
 (MeIm), structure, 477
 Mononuclear complexes of
 binucleating ligands
 coordination chemistry, 435–437
 dicationic Cr(III) complex, 436
 Ni(II) and Zn(II) complexes, 436
 Zn(II) complex, 436
 Monte Carlo approach, for hydration
 number, 271
 Mössbauer spectra
 of ⁵⁷Fe-enriched oxoiron(V)
 compound, 493
 of frozen aqueous solution of red
 compound, 490
 of solid μ-oxo-bridged diiron(IV)
 complex, 488
 MP-2269, 78
 MS-325-L, 66
 MthK channel, 314
 Mullein (*Verbascum thapsus*),
 265
 Mulliken charges, 480–481
 mycobactin J, 183, 184
 Myosin, Ca²⁺ binding at, 304
 Myosin light chain kinase (MLCK),
 291, 292

N
 N, N-bis(diphenylphosphinomethyl)-
 2-aminopyridine (pyNP₂),
 399–401
 N, N-chelates, 52
 NaCa(D-galacturonate)₃ · 6H₂O,
 318
 NAD kinase, 291

- N-alkylation effects
on molecular and electronic
structures of binucleating
complexes, 442–445
- NAMI-A, 22, 23
- Nanodimensional carriers, 163
- Nanotechnology, drug delivery based
on, 163–170
- Neuroendocrine tumors over-express
SST receptors, 138
- Neurosporin, 183, 184
- Neurotensin, 138
- NFVO, 150
- Nicotinic acid adenine dinucleotide
phosphate (NAADP), 255
- Nigericin, 310
- $[\text{Ni}_2(\text{L}^{21})]^{2+}$, 431
- $[\text{Ni}_2(\text{L}^{19})(\text{L}')^+]$
methods used synthesize, 447
- Nitric oxide synthase, 291
- Nitrido complexes, molybdenum
(Mo), 379–382
azido-nitrido complexes
 $[\text{Mo}(\text{N})(\text{N}_3)(\text{diphos})_2]$, 379
metal-N stretch, 380
metal-N vibration, 380
nitrido-acetonitrile complex, 379
nitrido-chloro complexes, 379
- Nitrogenase, 368–369
energy consumption, 368
iron protein, 368
MoFe, 368
reaction mechanism, 369
- Nitrogen donor ligands, 553, 554
- Nitrogen fixation
Chatt cycle and. *See* Chatt cycle
enzyme nitrogenase, 368
with Mo complexes. *See*
Molybdenum (Mo), N₂
fixation with
Schrock cycle and, 370–371
- 2-nitroimidazole, aminodiacetic acid
derivative of, 150
- $\text{N}^3(\text{LH})_3$ (**59**), 210, 211
- NLMO hybridizations, 554–555
- N-methylacetohydroxamate
(NMAHA), 224
- $\text{N}_3\text{M}(\mu\text{-S})_2(\mu\text{-L})\text{MN}_3$, 438
- NMR spectroscopy
of carboxylate coligands, 445–446
reactivity of complexes and,
454–455
- N,N*-bidentate ethylenediamine, 23
- N,N'*-bis(ethyl)-sulfurousdiamide
ligand, 19
- N,N*-bis(2-mercaptoethyl)-*N',N'*-
diethylethylenediamine, 165
- N–N bond cleavage, Chatt cycle,
374–378
biphasic kinetics, 376, 378
dialkylhydrazido complex, 375
- N,N*-chelating ligands (**17a,b**), 32
- nontransferrin bound iron (NTBI), in
the body, 236
- Nucleotide 5'-phosphates and Ca^{2+} ,
complex formation between
entropies and enthalpies of, 325
- O**
- O*-chelating ligands, 32
- ocreotide, 139
- ^{17}O electron-nuclear double
resonance (ENDOR)
spectroscopy, 70, 73
- O,O*-chelates, 52
- Orail, 314
- Orange II dye
bleaching of, 496
oxidation of, 495, 497
oxidative degradation of, 509
rate constants for catalyzed
oxidation of, 508
structure of, 496
- Organometallic ruthenium(II)–arene
anticancer drugs, 23
- $[\text{Os}(\text{acac})(\eta^6\text{-arene})\text{Cl}]$ (**27a,b**),
52
- $[\text{Os}(\text{acac})\text{Cl}(\eta^6\text{-}p\text{-cym})]$ (**27a**), 52
- $[\text{Os}(\eta^6\text{-arene})(\text{L})\text{X}]^{n+}$ complexes, 51
- $[\text{Os}(\eta^6\text{-arene})(\text{XY})\text{Z}]^{n+}$
complexes, 51
- $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**26**), 55
- $[\text{Os}(\eta^6\text{-bip})\text{Cl}(\text{pico})]$ (**28b**), 55
- $[\text{OsCl}(\text{acac})(\eta^6\text{-arene})]$ (**27a,b**), 54
- $[\text{OsCl}(\text{en})(\eta^6\text{-tha})]^+$ (**29**), 55
- $[\text{OsCl}(\text{L})(\eta^6\text{-arene})]^{n+}$, 51
- $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{oxine})]$ (**30**), 55–56
- $[\text{OsCl}(\eta^6\text{-}p\text{-cym})(\text{pico})]$ (**28a**), 53, 55

- Osmium-based anticancer agents,
2, 6
cytotoxicity, 54–55
DNA binding, 55–57
nucleobase interactions, 54
structure and reactivity, 51–54
- Osteoporosis, 335
- 7-oxabicycloheptane-2,3-
dicarboxylic acid (Endothall),
266
- Oxalic acid, 265
- Oxaliplatin, 4
- Oxidized TAML, 499
- μ -oxo-bridged diiron(IV) complex,
488–489
- P**
- Pacman diporphyrin complexes,
428–429
- Paget's disease, 336, 337
- PAMAM dendrimers, 78, 81–83
- Paramagnetic chelate, 65
- Paramagnetic chemical exchange
saturation (PARACEST)
agents, 67
applications, 99–100
principle of chemical exchange
saturation transfer (CEST),
98–99
- Par-Cu-egta-Ca, 288
- Partial molar volume, of Ca^{2+} aq,
273
- Parvalbumins, 292, 293
Ca–O distances in, 297
dissociation/displacement of Ca^{2+}
ions from, 305–306
 $\log K$ values for, 299
reaction of Ca^{2+} with, 304
- p*-cym complex, 43
- $[\text{PdCl}_2(\text{NCMe})_2]$, 433
partial deprotection, 435
- $[\text{Pd}_6(\text{L}^{14})_4]^{12+}$ coordination cage
 $[\text{Cp}^*\text{Mn}(\text{CO})_2]^+$ stabilization in
octahedral, 424
- $[\text{Pd}(\text{L}^2)(\eta^3\text{-allyl})]\text{PF}_6$
structure of, 410
- $[\text{Zr}]/\text{NH}_4\text{PF}_6$, 410
- PEGcoated $^{188}\text{Re}/^{99\text{m}}\text{Tc}$ -containing
particles, 166
- Pentaethyleneglycol, stability
constants for, 281
- Pentaphosphine ligands, Mo
dinitrogen complexes, 391–396
with C2 bridges, 393–396
 ^{31}P -NMR spectra, 394–396
quasi-tetragonal symmetry, 395
 $[\text{Mo}(\text{N}_2)(\text{dpepp})(\text{dppm})]$ complex
DFT GIAO calculations, 393
NMR investigations, 392–393
N–N and Mo–N stretching, 392
 $[\text{Mo}(\text{NNH}_2)(\text{dpepp})(\text{dppm})(\text{OTf})_2]$,
393
- Peptidylglycine α -hydroxylating
monooxygenase (PHM),
417, 418
- P-glycoprotein, 28
- Phenanthroline derivatives, 31
- o*-phenylenediamine, 289
- 2-phenylethylamine, 37
- 3-phenyl-1-propylamine, 36
- PHM. *See* Peptidylglycine
 α -hydroxylating monooxygenase
(PHM)
- Phosphate-induced demetalation,
482–483
- Phosphates, Ca^{2+} complexes of
kinetics, 326–328
stability constants for, 322–325
structures, 322
- Phosphodiesterases, 291
- Phospholipase C, 295
- Phosphonates, Ca^{2+} complexes of
kinetics, 326–328
stability constants for, 322–325
structures, 322
- Phosphoryl kinase, 291
- O*-Phosphorylserine, 323
- O*-phosphorylserylglycine, 323
- Photoactivation, as prodrug strategy,
7–9
- Photodynamic therapy (PDT), 7–8,
156–157
- Picolinic acid, demetalation by,
485–487
- Pigeon lysozyme, Ca^{2+} complex of,
299
- Pinacyanol chloride, 496
- PIPDMAHA, 232, 233

- pK , of Ca^{2+} aq, 273
 Platinum anticancer drug, 2–3
 application, 4
 dose-limiting side effects, 4
 molecular structures, 4–5
 prodrug strategies, 5–9
 Pt(IV)–azido complexes, 12–21
 Pt(IV)–iodido complexes, 9–12
 Platinum (IV) complexes, 7, 8–9
 Pllicamycin, 311
 PMEApp (Adefovir dipivoxil), 325
 Pocket-shaped ligand (L^2), in
 calixarenes, 410–411
 Polyamine-dithiophenolate
 macrocycles, 431–442
 coordination chemistry of, 435–442
 binuclear complexes, 437–441
 mononuclear complexes,
 435–437
 deprotection of polyamine-
 thioethers, 433–435
 dinickel complexes in, 431–432
 procedure to prepare, 432–433
 Porphyrin complexes, 421–423
 Porphyrins. *See* Metalloporphyrins,
 applications of
 Positron emission tomography (PET),
 132–135, 161
 advantages of, 134
 imaging of mice tumor, 168
 molecules metal complexes
 suitable for, 136–137
 nonconventional metallic isotopes,
 143–148
 radioisotopes used for, 134
 time delay differences, 161–162
 Prodrugs, 3–5, 33
 and cell morphology, 15
 and ligand substitution, 6
 photoactivation and, 7–8, 15
 Pt(IV) compounds, 8–9
 strategies, 5–9
 1,3-propylenediaminetetraacetate
 (pdta) complex, 267
 Proteinase K, 295
 calcium binding site for, 298
 Protein kinases, 291, 293, 313
 Protocyanin, 293
 Protonation, of bound N_2 , 372–374
 DFT calculations, 372
 metal-N force constants, 372, 373
 N-N force constants, 372–373
 trans-ligands, 374
 Proton-induced demetalation, 511
 Proton relaxation rate, 65
Pseudomonas aeruginosa, 229
 [Pt(en)(5-GMP-N7) $_2$] (**2rG**), 12
 [Pt(en)I(OAc) $_2$ (OH)] (**2s1**), 12
p-tert-butyl-calix[4]arene, 410
 structure of, 408
p-tert-butyl-calix[4]arene toluene
 structure of, 408
 Pt(IV)–azido coordination complexes,
 12–20
 Pt(IV) cisplatin-type prodrugs, 11
 Pt(IV)–diazidodiam(m)ine
 compounds, 12
 Pt(IV)–iodido complexes, 9–12
 [Pt $_4$ (NH $_3$) $_8$ (L^{11}) $_4$](ClO $_4$) $_8$, 419
 Pyochelin (**60**), 218–219
 Pyrene fluorescence, 140
 Pyridine-2-aldehyde, 154
 Pyridine carboxylates, pre-
 equilibrium binding of, 483
 Pyridine-induced spectral changes,
 476
 Pyrogallol[4]arene L^4 , structure of,
 412
- ## Q
- Quantum mechanics/molecular
 mechanics-molecular dynamics
 (QM/MM-MD) simulations, 271
 Quin-2, stability constant for, 279
 Quin-2 = 2-[[2-[bis(carboxymethyl)
 amino]- 5-methylphenoxy]
 methyl]-6-methoxy-8-
 [bis(carboxymethyl) amino]
 quinoline, 259, 305
- ## R
- Radioactive isotopes, of calcium
 ^{47}Ca (β^- , γ ; $\tau_{1/2}$ 4.5 days), 261
 Radiochemical imaging, 135
 Radioisotopes of calcium
 ^{45}Ca (β^- ; $\tau_{1/2}$ 165 days), 261
 Radiolabeled metallic complexes, 132
 Radionuclides, 166

- Radiopharmaceutical complexes, 163–165
- Rat mannose-binding protein, Ca^{2+} binding and, 296
- reactive oxygen species (ROS), 180
- $^{188}\text{Re}(\text{BMEDA})$ -labeled pegylated liposomes, 165
- $[(\text{Re}(\text{CO}_3)\text{Br})_4(\text{L}^{17})]$, 427
- Redfield's approximation, 89
- Redox activation mechanism, 6–7
- $^{188}\text{ReO}(\text{DMSA})_2$, 147
- Responsive imaging probes
enzyme-activated, 102–105
metal ion sensitive probes, 105–109
- Rhod-5N, 259
- Rhodotorula pilmanae*, 235
- Rhodotorulic acid, 183, 184, 205, 217
- D-ribose system, 319
- Rickets, 335
- Rigid materials, 253
- Ringbom coefficient, 189
- $[\text{Ru}(\text{acac})\text{Cl}(\eta^6\text{-p-cym})]$ (**16a**), 40
 $([\text{Ru}(\text{acac})(\eta^6\text{-p-cym})(\text{OH}_2)]^+)$
 (**16aH₂O**), 36
- $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{en})](\text{PF}_6)$
 complexes, 31
- $[\text{Ru}(\eta^6\text{-arene})\text{Cl}(\text{L})]^+$, 37
- $[\text{Ru}(\eta^6\text{-arene})(\text{en})(\text{G-N7})]^+$, 38
- $[\text{Ru}(\eta^6\text{-arene})(\text{L})(\text{OH})]^+$, 35
- $[\text{Ru}(\text{azpyz-NMe}_2)(\eta^6\text{-p-cym})(\text{OH}_2)]^+$
 (**20H₂O**), 36
- $[\text{Ru}(\eta^6\text{-benzene})\text{Cl}(\text{Me}_2\text{NCH}_2\text{CH}_2\text{PPh}_2)](\text{PF}_6)$, 23
- $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{dab})]^+$ (**15**), 31
- $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]^+$ (**10**), 40, 45, 46
- $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})]\text{PF}_6$, 30
- $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})](\text{PF}_6)$ (**10**), 29
- $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{en})](\text{PF}_6)$ (**10**), 30–31
- $[\text{Ru}(\eta^6\text{-bip})\text{Cl}(\text{Et-en})](\text{PF}_6)$ (**9**), 29
- $[\text{Ru}(\eta^6\text{-bip})(\text{en})(9\text{EtG-N7})]^+$, 40
- $[\text{Ru}(\eta^6\text{-}\eta^1\text{-C}_6\text{H}_5\text{CH}_2(\text{CH}_2)_n\text{NH}_2)\text{Cl}_2]$
 ($n = 1, 2$) (**13a,b**), 29, 35, 37, 44
- $[\text{RuCl}(\eta^6\text{-arene})(\text{en})]^+$ complexes, 42
- $[\text{RuCl}(\eta^6\text{-dha})(\text{en})](\text{PF}_6)$ (**11**) (dha, dihydroanthracene), 29
- $[\text{RuCl}(\text{en})(\eta^6\text{-bip})]^+$ (**10**), 45
- $[\text{RuCl}(\text{en})(\eta^6\text{-tha})]^+$ (**12**), 43
- $[\text{RuCl}(\text{en})(\eta^6\text{-tha})](\text{PF}_6)$ (**12**) (tha, tetrahydroanthracene), 29, 31
- $[\text{RuCl}_2(\text{etb})_2]$ (etb, ethylbenzoate), 36
- $[\text{Ru}(\mu\text{-}2,3\text{-dpp})]^{2+}$ (2,3-dpp, 2,3-bis(2-pyridyl)pyrazine) (**21**), 37
- $[\text{RuCl}(\eta^6\text{-p-cym})(\text{en})]^+$ (**22**), 43
- $[\text{Ru}(\text{en})(\eta^6\text{-hmb})(\text{SiPr})]^+$ (**24a**), 48
- $[\text{Ru}(\text{en})(\eta^6\text{-hmb})(\text{S(O)iPr})]^+$ (**24b**), 48
- $[\text{Ru}(\eta^6\text{-p-cym})(9\text{-EtA})(\text{Ph}_2\text{acac})]^+$
 (**16b**) (Ph_2acac , diphenylacetylacetonate), 40
- $[\text{Ru}(\eta^6\text{-p-cym})(9\text{-EtG})(\text{Ph}_2\text{acac})]^+$, 40
- $[\text{Ru}(\eta^6\text{-p-cym})(\text{OH}_2)(\text{trop})]^+$ (**19H₂O**), 36
- $[\text{Ru}(\eta^6\text{-p-cym})\text{X}_2]$ half-sandwich complex, 44
- $[\text{Ru}_6(p\text{-iPrC}_6\text{H}_4\text{Me})_6(\text{L}^{14})_2(\text{L}^{16})_3]^{6+}$, 425
- Ruthena(II)cyclic dye, 496
- Ruthenium anticancer drug, 2–3, 6
 advantages, 22
 catalytic activity of, 48–51
 cytotoxic activities, 29–32
 dinuclear ruthenium–arene complexes, 44
 general structure features, 24–29
 half-sandwich arene ruthenium(II)–enzyme complex, 44, 48–49
 hydrophobicity, 28–29
 interactions with biomolecules, 44–48
 interactions with DNA, 42–44
 interactions with nucleobases, 36, 37–41
 interactions with oligonucleotides, 41–42
 interactions with tripeptide glutathione, 46–48
 loss of coordinated arene, 36
 methionine binding, 46
 potential ability of, 21–24
 reactivities, 29–32
 ruthenium–arene complexes, 29
 stereochemistry, 27–28
 and sulfenato complexes, 48
 synthesis, 27

- Ruthenium Brown, 307
Ruthenium Red, 307
affects on calcium channels, 313
- S**
S. chartreusensis, A23187 from, 309–310
Safranin O
activity of Fe^{III}-TAMLs in
bleaching, 512–513
Fe^{III}-TAML activators catalase-like
activity in, 506–507
kinetics of 1a-catalyzed bleaching
of, 512, 515
structure of, 496
Salts, 328
calcium carbonate, 330
calcium oxalate, 330–331
calcium phosphates, 331–332
soluble, 329
Satraplatin [Pt(cha)Cl₂(OAc)₂(NH₃)], 8
[(S)-1,1'-binaphthalene-2,2'-diyl], 410
Schiff-base condensation, 430
Schrock cycle, 369–371
Secondary ion mass spectrometry (SIMS), 257
Selective solvation, of alkali metal ions, 531–532
Septamycin, 309
SH-SY5Y neuroblastoma, 17
Sialic acids, 256
stability constants, 321
Siderophores
as antimicrobial agents, 237
applications and future directions, 235–242
chelation treatments, 236–237
siderocalin and evasion by, 237–238
bacillibactin (**14**), 196, 201
catechol, 196
cepabactin (**8**), 218, 219
chemistry of, 179–181
designing of mimics, 202–203
effect of dihydroxamate, 207–209
endocyclic, 185
enterobactin (**1**), 196, 200–201, 203, 213, 230, 237
and evasion of host defense mechanisms, 237–238
with exocyclic architecture, 204
Fe³⁺/Fe²⁺ reduction potential of, 194
hexadentate, 182
hexadentate (L) complex, 187
HOPObactin (**16**), 201, 210
kinetics and mechanisms of iron chelation reactions
complex formation, 220–223
exchange process, 228–230
hydrolysis, 223–228
list of structure numbers, figures, and abbreviations, 240–242
mono-terephthalamide, 205
natural, 196
O-TRENTOX (**26**), 201, 203, 226, 230
pyochelin (**60**), 218, 219
recognition and cellular uptake, 231–235
role in environmental distribution
of radioactive actinides, 240
saccharide-platform, 193
SER(3M)SAM (**15**), 201
SERSAM (**13**), 201, 219
structure
common architecture, 183–185
common binding groups, 182–183
tetradentate, 205
thermodynamics of iron binding
conformational changes, 196
determination of stability constants, 190–194
effect of the amino acid spacer, 196
measurement of pH-dependent equilibria, 194–195, 199–200
pH-independent equilibrium expressions, 188
redox activities, 210–220
siderophore complex stability constants, 186–190

- survey of stability constants, 194–210
 - use of potentiometric titrations, 192–194
 - TRENCAM-based, 196, 200, 202, 213, 219, 230
 - Single-photon emission computed tomography (SPECT), 132–133
 - reagents suitable for fluorescence imaging in living cells, 137–143
 - Single walled carbon nanotube (SWNT), 165, 167–170
 - Sodium alendronate, 336
 - Solomon–Bloembergen–Morgan (SBM) theory, 69, 76, 91
 - Soluble salts, 329–330
 - Somatostatin (SST), 138, 142, 147
 - S- or O-bound 1:1 [Ru(η^6 -bip)(L-Cys(en))]⁺, 46
 - S-oxygenation, of hexamine-dithiophenolate macrocycles, 441–442
 - Sphingosine-1-phosphate, 314
 - Spin-forbidden reactions
 - potential energy surfaces and their crossings for, 609–616
 - in transition metal chemistry, 606–609
 - (SP-4-4)-[Pt^{II}(5'-GMP-N7)(N₃)(NH₃(py))]⁺ (**7rG**), 17
 - S100 proteins, 294, 306
 - log*K* values for, 299
 - Squaraine dyes, 289
 - Stability constant (*K*_{ML}), 93
 - Stability constants
 - of Ca²⁺ aq, 273–275
 - amino acids and peptides, 276–278
 - aminocarboxylates, 278–279
 - complex formation, enthalpies and entropies of, 282–283
 - crown ethers and azamacrocycles, 280–281
 - other carboxylates, 279–280
 - selectivity, 281–282
 - calcium-binding proteins, 299–302
 - ionic strength and, 301
 - carbohydrate ligands, 319–320
 - for complexes of ionophores, 311–312
 - general overview, 326
 - hydroxycarboxylate ligands, 320–321
 - phosphates, Ca²⁺ complexes of, 322–325
 - phosphonates, Ca²⁺ complexes of, 322–325
 - π - π stacking interaction, 38
 - Staphylococcal nuclease, Ca²⁺
 - binding and, 296
 - Sterically assisted electronic effect, 479
 - Sterically demanding tripod ligands, 414–418
 - Ag^I-CO and Ag^I-C₂H₄ complexes, 416–417
 - carbonyl complex, 417
 - [Fe(L⁷(N))][BPh₄], 415
 - iron(IV) nitride complex in, 415–416
 - synthetic bioinorganic copper dioxygen complexes, 417–418
 - STIM1, 314
 - Stopped-flow fluorescence spectroscopy, 303, 304
 - Store-operated Ca²⁺ channels, 313
 - Streptomyces cacaoi*, lysocellin from, 309
 - Streptomyces conglobatus*, ionomycin from, 309
 - Streptomyces hygroscopicus*, septamycin from, 309
 - Streptomyces lasaliensis*, lasalocid A (X-537A) from, 309
 - Streptomyces ribosidicus*, lonomycin from, 309
 - Strontium, bone formation and, 333
 - structural biology, 1
 - Synaptotagmins (Syts), 294
 - Ca–O distances for, 298
 - Synthetic bioinorganic copper dioxygen complexes, 417–418
 - Synthetic linear dihydroxamic acids, 206
- T**
- Tamlzymes, 473
 - taurTAM (**29**), 204, 205

- ^{99m}Tc -labeled peptide (NC100692), 142
- $^{99m}\text{Tc}/^{188}\text{Re}$ -labeled lipid nanocapsules, 165
- $^{99m}\text{Tc}/^{188}\text{Re}(\text{S}_3\text{CPh})_2(\text{S}_2\text{CPh})$, 165
- Tc-labeled PDT system, 157
- Tc radioisotopes, 138
- Template reactions, calcium complexes and, 288–289
- tert*-butylimido complex $[\text{MoX}(\text{NtBu})(-\text{depe})_2]$, 382
- Tetraamide derivatives, of DOTA, 74
- Tetracycline, 310–311
stability constant for, 312
- Tetradentate phosphine ligand (prP_4), 386–387
meso isomer, 387
 $[\text{Mo}(\text{O})\text{I}_2(\text{PMe}_3)_3]$, 388
 ^{31}P -NMR spectroscopy, 388–389
rac isomer, 387
- 5,10,15,20-tetrakis[3,4-bis(carboxymethyleneoxy)phenylporphyrin], 158
- $\alpha_2\beta_2$ tetramer. *See* Molybdenum-iron (MoFe) protein
- tetraphos ligands, and Mo dinitrogen complexes, 386–391
- tetradentate phosphine ligand prP_4 , 386–387
meso isomer, 387
 $[\text{Mo}(\text{O})\text{I}_2(\text{PMe}_3)_3]$, 388
 ^{31}P -NMR spectroscopy, 388–389
rac isomer, 387
trans- $[\text{Mo}(\text{N}_2)_2(\text{meso-prP}_4)]$, synthesis, 387–391
vs. diphosphine ligands, 386
- Tetrasulfonatocalix[4]arene, 411
- Texaphyrins, 72, 162
- Thapsic acid, 265
- Therapeutic activity, 2
- Thermolysin, 293
Ca...Ca distances, 298
calcium-binding sites in, 296, 302
- THF
anaerobic transamination reaction between H_4L^{18} and $[\text{Co}(\text{thf})_2]$, 428
bis(4-pyridyl)-functionalized $\text{Zn}(\text{II})$ -salen-type ligands in, 419
Li/naphthalene in, 433
- Thiobacillus thiooxidans*, 273
- Thuja occidentalis*, 265
- Thymidine-3',5'-diphosphate, 296
- Tm^{III} DOTA monoamide complex, 104
- T_1 - or T_2 -agents, 64–65
- Transition metal chemistry, spin-forbidden reactions in, 606–609
- Transition state, for water exchange, 537–538
- Trans-membrane transport, Ca^{2+} and, 308–309
- trans,mer*- $[\text{Pt}(\text{N}_3)(\text{NH}_3)_2(\text{OH})_3]$ (**6s**), 16
- trans*- $[\text{Pt}(\text{en})\text{I}(\text{OAc})_2(\text{OH})]$ (2s1), 12
- trans*- $[\text{Pt}^{\text{II}}(5'\text{-GMP-}N7)_2(\text{NH}_3)_2]^{2+}$, 16
- trans*- $[\text{Pt}^{\text{II}}(5'\text{-GMP-}N7)_2(\text{NH}_3)(\text{py})]^{2+}$ (**7rG2**), 17
- Trans*- $[\text{Pt}(\text{NH}_3)_2(\text{L}^{11})(\text{H}_2\text{O})](\text{ClO}_4)_2$, 419
- trans*- $[\text{Pt}(\text{NH}_3)_2(\text{OH})_2]^{2+}$, 19
- trans*- $[\text{Pt}(\text{NH}_3)_2(\text{OH})_2]^{2+}$ (**6r**), 15
- trans,trans*- $[\text{Pt}(\text{N}_3)_2(\text{NH}_3)(\text{OH})_2(\text{py})]$ (**7**), 16
- trans,trans,trans*- $[\text{Pt}(\text{N}_3)_2(\text{NH}_3)_2(\text{OH})_2]$, 15
- trans,trans,trans*- $[\text{Pt}(\text{N}_3)_2(\text{NH}_3)_2(\text{OH})_2]$ (**6**), 16
- trans,trans,trans*- $[\text{Pt}(\text{N}_3)_2(\text{OH})_2(\text{NH}_3)_2]$ (**6**), 16
- TREN-bis(6-Me-HOPO)-TAMTRI, 79
- TREN-HOPO (hydroxypyridinonate) derivatives, 70–71
- TRENSEX2CAMS, 224
- TRENSEX2CAMS2, 224
- TRENSEX2CAMS system, 227
- 2',3',5'-*O*-triacetylguanosine (TAG), 315
- trifunctional metal-based conjugates, 139
- TRIS. *See* Tris(hydroxymethyl)aminomethane (TRIS)
- tris(2-hydroxybenzyl)amine, 144

- Tris(hydroxymethyl)aminomethane (TRIS), 482
tris(2-mercaptobenzyl)amine, 144
Tris(2-methylpyridyl)amine, 409
Tris(pyrazolyl)borate ligand, 416
Trispyrazolylborates, 409
“Trojan Horse” approach, 237
Troponin C, 294
 Ca...Ca distances, 298
 kinetics of dissociation of Ca^{2+} from, 305
TSA isomer, 78
ttha, stability constants for, 279
Tumor-associated bone loss, 336, 337
Tumor hypoxic state, 153
Tungsten (W), N_2 fixation with. *See also* Molybdenum (Mo), N_2 fixation with
 N–N cleavage reaction
 acid concentration, 375
 biphasic kinetics, 376–378
 of dialkylhydrazido complex, 375
 and protonation of dinitrogen hydrazidium complexes, 372
 N–N bond order, 373–374
 N–N force constant, 372–373
 quantum chemical calculations, 372
- U**
 α,β -unsaturated carboxylate ligands, bromination, 454
- V**
Valinomycin, 311
Vasoactive intestinal peptide (VIP), 138
Verapamil, 30
Vitamin D deficiency, 335, 336
Voltage-gated Ca^{2+} channels, 313
- W**
Water exchange, ligand exchange process, 533–539
 $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$, 536
beryllium (Be^{2+})
 $[\text{Be}(\text{H}_2\text{O})_4]^{2+}$, 536–537
 $[\text{Be}(\text{H}_2\text{O})_5]^{2+}$, 534–535
 $[\text{Be}(\text{H}_2\text{O})_4(\text{H}_2\text{O})]^{2+}$, 537
 B3LYP hybrid density functional, testing, 535–536
 hydrogen-bonded ligands, 535
 IPCM calculation, 536
lithium (Li^+)
 aqua complexes, 533
 $[\text{Li}(\text{H}_2\text{O})_5]^+$, 533–534
 transition states, 537–538
 in gas phase, 537
 PCM model, 538
Water exchange reactions, of calcium complexes, 283–284
Water/proton exchange rate, 69, 73–80
 of Mn^{II} complexes, 111
water proton relaxation rate, 65
Water-soluble organic dyes, 496
Weddelite, 266
Whewellite, 266
- X**
X-14885A
 formation rate constants for, 313
- Y**
Yttrium, 147
- Z**
Zero-field splitting (ZFS)
 interactions, 69
Zinc bis(thiosemicarbazones), 151–152
 $[\text{Zn}(\text{ATSM})]$ complex, 152–153
 $[\text{ZnCd}(\text{L}^{19})(\text{OAc})]^+$, 436
Zn(II) complexes, 152–153
Zn(II)phthalocyanines, 161
 $[\text{Zn}_8(\text{L}^4)(\text{L}')_8(3\text{-methylpyridine})]$, 412
 $[\text{Zn}_2\text{L}^{19}(\text{OAc})]^+$, 436
Zoledronic acid, 337
Z-spectrum, 98

This page intentionally left blank

CONTENTS OF PREVIOUS VOLUMES

VOLUME 42

Substitution Reactions of Solvated Metal Ions

*Stephens F. Lincoln and
André E. Merbach*

Lewis Acid–Base Behavior in Aqueous Solution: Some Implications for Metal Ions in Biology

Robert D. Hancock and Arthur E. Martell

The Synthesis and Structure of Organosilanols

Paul D. Lickiss

Studies of the Soluble Methane Monooxygenase Protein System: Structure, Component Interactions, and Hydroxylation Mechanism

*Katherine E. Liu and
Stephen J. Lippard*

Alkyl, Hydride, and Hydroxide Derivatives in the *s*- and *p*-Block Elements Supported by Poly(pyrazolyl)borato Ligation: Models for Carbonic Anhydrase, Receptors for Anions, and the Study of Controlled Crystallographic Disorder

Gerard Parkin

INDEX

VOLUME 43

Advances in Thallium Aqueous Solution Chemistry

Julius Glaser

Catalytic Structure–Function: Relationships in Heme Peroxidases

Ann M. English and George Tsaprailis

Electron-, Energy-, and Atom-Transfer Reactions between Metal Complexes and DNA

H. Holden Thorp

Magnetism of Heterobimetallics: Toward Molecular-Based Magnets

Olivier Kahn

The Magnetochemistry of Homo- and Hetero-Tetranuclear First-Row *d*-Block Complexes

Keith S. Murray

Diiron–Oxygen Proteins

*K. Kristoffer Andersson and
Astrid Graslund*

Carbon Dioxide Fixation Catalyzed by Metals Complexes

Koji Tanaka

INDEX

VOLUME 44

Organometallic Complexes of Fullerenes

*Adam H. H. Stephens and
Malcolm L. H. Green*

Group 6 Metal Chalcogenide Cluster Complexes and Their Relationships to Solid-State Cluster Compounds

Taro Saito

Macrocyclic Chemistry of Nickel

Myunghyun Paik Suh

Arsenic and Marine Organisms

*Kevin A. Francesconi and
John S. Edmonds*

The Biochemical Action of Arsonic Acids Especially as Phosphate Analogues

Henry B. F. Dixon

Intrinsic Properties of Zinc(II) Ion Pertinent of Zinc Enzymes

Eiichi Kimura and Tbhru Koike

Activation of Dioxygen by Cobalt Group Metal Complexes

Claudio Bianchini and Robert W. Zoellner

Recent Developments in Chromium
Chemistry

Donald A. House

INDEX

VOLUME 45

Syntheses, Structures, and Reactions of
Binary and Tertiary Thiomolybdate
Complexes Containing the (O)Mo(S_x)
and (S)Mo(S_x) Functional Groups
(*x* = 1,2,4)

Dimitri Coucouvanis

The Transition Metal Ion Chemistry of
Linked Macrocyclic Ligands

Leonard F. Lindoy

Structure and Properties of Copper–Zinc
Superoxide Dismutases

*Ivano Bertini, Stefano Mangani, and
Maria Silvia Viezzoli*

DNA and RNA Cleavage by Metal
Complexes

*Genevieve Pratviel, Jean Bernadou, and
Bernard Meunier*

Structure–Function Correlations in High
Potential Iron Problems

J. A. Cowan and Siu Man Lui

The Methylamine Dehydrogenase Electron
Transfer Chain

*C. Dennison, G. W. Canters, S. de Vries,
E. Vijgenboom, and R. J. van Spanning*

INDEX

VOLUME 46

The Octahedral M₆Y₆ and M₆Y₁₂ Clusters
of Group 4 and 5 Transition Metals

Nicholas Prokopuk and D. F. Shriver

Recent Advances in Noble–Gas Chemistry

John H. Holloway and Eric G. Hope

Coming to Grips with Reactive
Intermediates

*Anthony J. Downs and Timothy M.
Greene*

Toward the Construction of Functional
Solid-State Supramolecular Metal
Complexes Containing Copper(I)
and Silver(I)

*Megumu Munakata, Liang Ping Wu, and
Takayoshi Kuroda-Sowa*

Manganese Redox Enzymes and Model
Systems: Properties, Structures, and
Reactivity

*Neil A. Law, M. Tyler Caudle, and
Vincent L. Pecoraro*

Calcium-Binding Proteins

*Bryan E. Finn and
Torbjörn Drakenberg*

Leghemoglobin: Properties and
Reactions

*Michael J. Davies, Christel Mathieu,
and Alain Puppo*

INDEX

VOLUME 47

Biological and Synthetic [Fe₃S₄]
Clusters

*Michael K. Johnson, Randall E.
Duderstadt, and Evert C. Duin*

The Structures of Rieske and Rieske-Type
Proteins

Thomas A. Link

Structure, Function, and Biosynthesis
of the Metallosulfur Clusters in
Nitrogenases

Barry E. Smith

The Search for a “Prismane” Fe–S
Protein

*Alexander F. Arendsen and
Peter F. Lindley*

NMR Spectra of Iron–Sulfur Proteins

*Ivano Bertini, Claudio Luchinat, and
Antonio Rosato*

Nickel–Iron–Sulfur Active Sites:
Hydrogenase and CO Dehydrogenase

*Juan C. Fontecilla-Camps and
Stephen W. Ragsdale*

FeS Centers Involved in Photosynthetic
Light Reactions

*Barbara Schoepp, Myriam Brugna,
Evelyne Lebrun, and
Wolfgang Nitschke*

Simple and Complex Iron–Sulfur
Proteins in Sulfate Reducing
Bacteria

*Isabel Moura, Alice S. Pereira, Pedro
Tavares, and José J. G. Moura*

Application of EPR Spectroscopy to the
Structural and Functional Study of
Iron–Sulfur Proteins

*Bruno Guigliarelli and
Patrick Bertrand*

INDEX

VOLUME 48

Cumulative Index for Volumes 1–47

VOLUME 49

Inorganic and Bioinorganic Reaction
Mechanisms: Application of
High-Pressure Techniques

*Rudi van Eldik, Carlos Dücker-Benfer,
and Florian Thaler*

Substitution Studies of Second- and
Third-Row Transition Metal Oxo
Complexes

*Andreas Roodt, Amira Abou-Hamdan,
Hendrik P. Engelbrecht, and Andre E.
Merbach*

Protonation, Oligomerization, and
Condensation Reactions of
Vanadate(V), Molybdate(VI),
and Tungstate(VI)

J. J. Cruywagen

Medicinal Inorganic Chemistry

Zijian Guo and Peter J. Sadler

The Cobalt(III)-Promoted Synthesis of
Small Peptides

*Rebecca J. Browne, David A.
Buckingham, Charles R. Clark, and
Paul A. Sutton*

Structures and Reactivities of
Platinum-Blues and the Related
Amidate-Bridged Platinum^{III}
Compounds

Kazuko Matsumoto and Ken Sakai

INDEX

VOLUME 50

The Reactions of Stable Nucleophilic
Carbenes with Main Group
Compounds

Claire J. Carmalt and Alan H. Cowley

Group 1 Complexes of P- and As-Donor
Ligands

Keith Izod

Aqueous Solution Chemistry of Beryllium

*Lucia Alderighi, Peter Gans, Stefano
Midollini, and Alberto Vacca*

Group 2 Element Precursors for the
Chemical Vapor Deposition of
Electronic Materials

Jason S. Matthews and William S. Rees Jr.

Molecular, Complex Ionic, and Solid-State
PON Compounds

*Roger Marchand, Wolfgang Schnick, and
Norbert Stock*

Molecular Clusters of Dimetalated
Primary Phosphanes and Arsanes

Matthias Driess

Coordination Complexes of Bismuth(III)
Involving Organic Ligands with
Pnictogen or Chalcogen Donors

Glen G. Briand and Neil Burford

Phanes Bridged by Group 14 Heavy
Elements

Hideki Sakurai

INDEX

VOLUME 51

Clinical Reactivity of the Active Site of
Myoglobin

*Emma Lloyd Raven and
A. Grant Mauk*

Enzymology and Structure of Catalases
*Peter Nicholls, Ignacio Fita, and
 Peter C. Laeven*

Horseradish Peroxidase
Nigel C. Veitch and Andrew T. Smith

Structure and Enzymology of Diheme
 Enzymes: Cytochrome *cd₁* Nitrate and
 Cytochrome *c* Peroxidase
*Vilmos Fulöp, Nicholas J. Watmough,
 and Stuart J. Ferguson*

Binding and Transport of Iron-Porphyrins
 by Hemopexin
William T. Morgan and Ann Smith

Structures of Gas-Generating Heme
 Enzymes: Nitric Oxide Synthase and
 Heme Oxygenase
*Thomas L. Poulos, Huiying Li, C. S.
 Raman, and David J. Schuller*

The Nitric Oxide-Releasing Heme Proteins
 from the Saliva of the Blood-Sucking
 Insect *Rhodnius prolixus*
F. Ann Walker and William R. Montfort

Heme Oxygenase Structure and Mechanism
*Paul R. Ortiz de MonteBano and
 Angela Wilks*

De Novo Design and Synthesis of Heme
 Proteins
Brian R. Gibney and P. Leslie Dutton

INDEX

VOLUME 52

High-Nuclearity Paramagnetic *3d*- Metal
 Complexes with Oxygen- and
 Nitrogen-Donor Ligands
Richard E. P. Winpenny

Transition Metal–Noble Gas Complexes
D. C. Grills and M. W. George

The Materials Chemistry of
 Alkoxy stilbazoles and their Metal
 Complexes
Duncan W. Bruce

Tetra- and Trinuclear Platinum(II)
 Cluster Complexes
Tadashi Yamaguchi and Tasuku Ito

Complexes of Squaric Acid and Its
 Monosubstituted Derivatives
Lincoln A. Hall and David J. Williams

Applications for Polyaza Macrocycles
 with Nitrogen-Attached
 Pendant Arms
Kevin P. Wainwright

Perfluorinated Cyclic Phosphazenes
Anil J. Elias and Jean'ne M. Shreeve
 INDEX

VOLUME 53

Wheel-Shaped Polyoxo and
 Polyoxothiometalates: From the
 Molecular Level to Nanostructures
Anne Dolbecq and Francis Sécheresse

Redox Chemistry and Functionalities of
 Conjugated Ferrocene Systems
Hiroehi Nishihara

New Aspects of Metal–Nucleobase
 Chemistry
Andrew Houlton

Advances in the Chemistry of
 Chlorocyclophosphazenes
*Vadapalli Chandrasekhar and
 Venkatasubbaiah Krishnan*

Self-Assembly of Porphyrin Arrays
*Laura Baldini and
 Christopher A. Hunter*

INDEX

VOLUME 54

Solvent Exchange on Metal Ions
*Frank A. Dunand, Lathar Helm, and
 Andre E. Merbach*

Ligand Substitution Reactions
John Burgess and Colin D. Hubbard

Oxygen Transfer Reactions: Catalysis by
Rhenium Compounds

James H. Espenson

Reaction Mechanisms of Nitric Oxide with
Biologically Relevant Metal Centers

*Peter C. Ford, Leroy E. Laverman and
Ivan M. Lorkovic*

Homogeneous Hydrocarbon C–H Bond
Activation and Functionalization with
Platinum

Ulrich Fekl and Karen I. Goldberg

Density Functional Studies of Iridium
Catalyzed Alkane Dehydrogenation

Michael B. Hall and Hua-Jun Fan

Recent Advances in Electron-Transfer
Reactions

David M. Stanbury

Metal Ion Catalyzed Autoxidation
Reactions: Kinetics and Mechanisms

István Fabian and Viktor Csordds

INDEX

VOLUME 55

Dioxygen Activation by Transition
Metal Complexes. Atom Transfer
and Free Radical Chemistry in
Aqueous Media

Andreja Bakac

Redox Reactivity of Coordinated Ligands
in Pentacyano(L)Ferrate Complexes

José A. Olabe

Carbonato Complexes: Models for
Carbonic Anhydrase

*Achyuta N. Acharya, Arabinda Das and
Anadi C. Dash*

Transition Metal Chemistry of Glucose
Oxidase, Horseradish Peroxidase,
and Related Enzymes

Alexander D. Ryabov

Properties of Transition Metal Complexes
with Metal–Carbon Bonds in Aqueous

Solutions as Studied by Pulse
Radiolysis

*Alexandra Masarwa and
Dan Meyerstein*

Transition Metal Complexes with
Bis(Hydrazone) Ligands of 2,
6-Diacetylpyridine. Hepta-Coordination
of 3d Metals

*Ivana Ivanović-Burmazovic and
Katarina Andjelkovic*

Potential Applications for the Use of
Lanthanide Complexes as
Luminescent Biolabels

*Graham R. Motson, Jean S. Fleming and
Sally Brooker*

INDEX

VOLUME 56

Synergy Between Theory and Experiment
as Applied to H/D Exchange
Activity Assays in [Fe]H₂ase
Active Site Models

*Jesse W. Tye, Michael B. Hall,
Irene P. Georgakaki and Marcetta Y.
Darensbourg*

Electronic Structure and Spectroscopic
Properties of Molybdenum and
Tungsten N₂, NNH, NNH₂, and NNH₃
Complexes with Diphosphine
Co-Ligands: Insights into the End-on
Terminal Reduction Pathway of
Dinitrogen

Felix Tuczek

Quantum Chemical Investigations
into the Problem of Biological
Nitrogen Fixation: Sellmann-Type
Metal–Sulfur Model Complexes

Markus Reiher and Bernd A. Hess

Proton and Electron Transfers in [NiFe]
Hydrogenase

Per E. M. Siegbahn

Heterolytic Splitting of H–H, Si–H, and
Other sigma Bonds on Electrophilic
Metal Centers

Gregory J. Kubas

Tetrapodal Pentadentate Nitrogen
Ligands: Aspects of Complex
Structure and Reactivity
Andreas Grohmann

Efficient, Ecologically Benign, Aerobic
Oxidation of Alcohols
*István E. Mark, Paul R. Giles, Masao
Tsukazaki, Isabelle Chelle-Regnaut,
Arnaud Gautier, Raphael Dumeunier,
Freddi Philippart, Kanae Doda,
Jean-Luc Mutoinkole, Stephen
M. Brown and Christopher J. Urch*

Visible Light Photocatalysis by a Titania
Transition Metal Complex
*Horst Kisch, Gerald Burgeih and
Wojciech Macyk*

INDEX

VOLUME 57

Introduction: General Theory of Nuclear
Relaxation
Daniel Canet

NMR Relaxation in Solution of
Paramagnetic Complexes: Recent
Theoretical Progress for $S \geq 1$
*Jozef Kowalewski, Danuta Kruk and
Giacomo Parigi*

^1H NMRD Profiles of Paramagnetic
Complexes and Metalloproteins
*Ivano Bertini, Claudia Luchinat and
Giacomo Parigi*

Gd(III)-Based Contrast Agents for MRI
*Silvio Aime, Mauro Botta
and Enzo Terreno*

Relaxation by Metal-containing
Nanosystems
*R. N. Midler, L. Vander Elst, A. Roch,
J. A. Peters, E. Csajbok, P. Gillis
and Y. Gossuin*

Magnetic Relaxation Dispersion in Porous
and Dynamically Heterogeneous
Materials
Jean-Pierre Korb and Robert G. Bryant

Water and Proton Exchange Processes
on Metal Ions
*LotharHelm, Gaëlle M, Nicolle and
André E. Merbach*

Nuclear Magnetic Relaxation Studies on
Actinide Ions and Models of Actinide
Complexes
Jean F Desreux

Technical Aspects of fast
Field Cycling
Gianni Fermnte and Stanislav Sykora

INDEX

VOLUME 58

Diversity-Based Approaches to Selective
Biomimetic Oxidation Catalysis
Albrecht Berkessel

Selective Conversion of Hydrocarbons with
 H_2O_2 Using Biomimetic Non-heme
Iron and Manganese Oxidation
Catalysts
*Stefania Tanase and
Elisabeth Bouwman*

DNA Oxidation by Copper and Manganese
Complexes
*Marguerite Pitié, Christophe Boldron
and Geneviève Pratviel*

Ligand Influences in Copper-Dioxygen
Complex-Formation and Substrate
Oxidations
*Lanying Q. Hatcher and
Kenneth D. Karlin*

Biomimetic Oxidations by Dinuclear and
Trinuclear Copper Complexes
*Giuseppe Battaini, Alessandro Granata,
Enrico Monzani, Michele Gullotti and
Luigi Casella*

Green Oxidation of Alcohols using
Biomimetic Cu Complexes and Cu
Enzymes as Catalysts
*Isabel W.C.E Arends, Patrick Gamez and
Roger A. Sheldon*

INDEX

VOLUME 59

Self-Assembled Metallo-Supramolecular
Systems Incorporating β -Diketone
Motifs as Structural Elements

*David J. Bray, Jack K. Clegg, Leonard F.
Lindoy and David Schilter*

Coordination Polymer Open Frameworks
Constructed of Macrocyclic
Complexes

Myunghyun Paik Suh and Hoi Ri Moon

Molecular Devices Based on Metallocyclam
Subunits

*Luigi Fabbrizzi, Francesco Foti,
Maurizio Licchelli, Antonio Poggi, Angelo
Taglietti and Miguel Vázquez*

Molecular Recognition of Neutral
and Charged Guests using
Metallomacrocyclic Hosts

*Ivan V. Korendovych, Rebecca A. Roesner
and Elena V. Rybak-Akimova*

Supramolecular Chemistry of

Environmentally Relevant Anions

*Bruce A. Moyer, Lætitia H. Delmau,
Christopher J. Fowler, Alexandre Ruas,
Debra A. Bostick, Jonathan L. Sessler,
Evgeny Katayev, G. Dan Pantos, José
M. Llinares, MD. Alamgir Hossain,
Sung O. Kang and Kristin
Bowman-James*

Role of Cation Complexants in the Synthesis
of Alkalides and Electrides

*James L. Dye, Mikhail Y. Redko,
Rui H. Huang and James
E. Jackson*

Structure–Activity Studies and the
Design of Synthetic Superoxide
Dismutase (SOD) Mimetics as
Therapeutics

Dennis P. Riley and Otto F. Schall

Electronic Tuning of the Lability
of Inert Co(III) and Pt(II)
Complexes

Rudi Van Eldik

INDEX

VOLUME 60

Tripodal Carbene and Aryloxide
Ligands for Small-Molecule

Activation at Electron-Rich
Uranium and Transition

Metal Centers

*Karsten Meyer and
Suzanne C. Bart*

β -Cyclodextrin-Linked Ru Complexes for
Oxidations and Reductions

*W.-D. Woggon, Alain Schlatter and
Hao Wang*

Catalytic Dismutation vs. Reversible
Binding of Superoxide

Ivana Ivanović-Burmazović

Tripodal *N,N,O*-Ligands for

Metalloenzyme Models and
Organometallics

Nicolai Burzlaff

Hydroxypyranones, Hydroxypyridinones,
and their Complexes

John Burgess and Maria Rangel

Late Transition Metal-Oxo Compounds
and Open-Framework Materials that
Catalyze Aerobic Oxidations

*Rui Cao, Jong Woo Han, Travis M.
Anderson, Daniel A. Hillesheim, Kenneth
I. Hardcastle, Elena Slonkina, Britt
Hedman, Keith O. Hodgson, Martin L.
Kirk, Djamaladdin G. Musaev, Keiji
Morokuma, Yuri V. Geletii and Craig L.
Hill*

INDEX