[4] a) J. Chlistunoff, D. Cliffel, A. J. Bard, *Thin Solid Films* **1995**, *257*, 166 – 184; b) see reference [3a], pp. 390 – 394, 406 – 408; c) A. Deronzier, J.-C. Moutet, P. Seta, *J. Am. Chem. Soc.* **1994**, *116*, 5019 – 5020.
[5] For reversible electron transfer reactions of a C_{60}/γ-cyclodextrin complex dissolved in an aqueous solution, see P. Boulas, W. Kutner, M. T. Jones, K. M. Kadish, *J. Phys. Chem.* **1994**, *98*, 1282 – 1287.
[6] A.-E. F. Nassar, Y. Narikiyo, T. Sagara, N. Nakashima, J. F. Rusling, *J. Chem. Soc. Faraday Trans.* **1995**, *91*, 1775 – 1782, and references therein.
[7] N. Nakashima, Y. Yamaguchi, H. Eda, M. Kunitake, O. Manabe, *J. Phys. Chem. B* **1997**, *101*, 215 – 220.
[8] J. J. Davis, H. A. O. Hill, A. Kurz, A. D. Leighton, A. Y. Safronov, *J. Electroanal. Chem.* **1997**, *429*, 7 – 11.
[9] A. Szucs, A. Loix, J. B. Nagy, L. Lamberts, *J. Electroanal. Chem.* **1995**, *397*, 191 – 203.
[10] The peak separations between the cathodic and the anodic waves were 62 ± 3 mV. The electrochemistry of our system is governed by the diffusion of C_{60} and/or the electrolytes, and/or by electron hopping (self-exchange electron transfer between C_{60} molecules in the film).
[11] The total amount of electroactive C_{60} in the film should be greater than this because the redox reaction at a scan rate of 2 mV s^{-1} still involves diffusing species.
[12] C. Jehoulet, Y. S. Obeng, Y.-T. Kim, F. Zhou, A. J. Bard, *J. Am. Chem. Soc.* **1992**, *114*, 4237 – 4247.
[13] See reference [3a], pp. 386 – 390.
[14] A. Szucs, A. Loix, J. B. Nagy, L. Lamberts, *J. Electroanal. Chem.* **1996**, *402*, 137 – 148.
[15] The charge balance is maintained by incorporation of cations from the bulk solution.

Direct NMR Spectroscopic Observation of a Lanthanide-Coordinated Water Molecule whose Exchange Rate Is Dependent on the Conformation of the Complexes

Silvio Aime,* Alessandro Barge, Mauro Botta, Alvaro S. De Sousa, and David Parker

The development of magnetic resonance imaging (MRI) techniques for medical diagnosis has been accompanied by an explosive growth of interest in the study of water-soluble, paramagnetic metal complexes as contrast agents.[1] The contrast agents markedly improve the image contrast by enhancing the nuclear magnetic relaxation rates of the water protons in the tissues where they are distributed. The agents currently used in clinical practice are chelates of the Gd^{III} ion, which is particularly suitable because of its high magnetic moment and long electronic relaxation time (T_{1e}). The mechanism of the relaxation enhancement involves the modulation of the dipolar interaction between the magnetic moment of the electrons in Gd^{III} ions and the nuclear spins of the water protons in the inner and outer coordination sphere of the complex.[1, 2] The efficiency of the process depends primarily on the number q of water molecules bound to the metal ion, their exchange rate (k_{ex}) with the bulk water, and the rate of molecular reorientation. The residence lifetime τ_M ($\tau_M = 1/k_{ex}$) plays a particularly important role since it contributes directly to the modulation of the electron – nucleus dipolar interaction, and it controls the efficiency of the transfer of the paramagnetic effect to the bulk water. Thus, the issue of the water exchange rate in lanthanide(III) complexes is of paramount importance in the development of novel contrast agents for MRI. In fact, in a number of Gd^{III} chelates with $q = 1$ τ_M is much longer than that found for Ln^{III} aquo ions, which is of the order of nanoseconds,[3] and it may limit the relaxation efficiency of the contrast agents.[4]

Among the different complexes used as contrast agents for MRI, $[Gd(dota)]^-$ (dota = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) should be the candidate of choice because of its high thermodynamic and kinetic stability. The long electronic relaxation time of the Gd^{III} ion prevents the observation of the NMR spectra of its complexes, and its solution structure had to be inferred from the ^1H and ^{13}C NMR spectra of the related complex $[Eu(dota)]^-$. As previously reported, $[Eu(dota)]^-$ consists of a pair of isomers, namely, **M** and **m**, endowed with the same square [3333] conformation of the macrocyclic ring, but with a different layout of the acetate arms. This difference results in a square antiprismatic and a twisted antiprismatic geometry for **M** and **m**, respectively (see Scheme 1).[5] The two isomers are in slow exchange near room temperature and yield two distinct sets of six resonances in the ^1H NMR spectrum for the C_4-symmetric ring and the diastereotopic CH_2CO protons. The resonance of the coordinated water molecule has not been observed (nor in other $[Ln(dota)]^-$ chelates) because its exchange rate is too fast on the NMR time scale ($k_{ex} = 4.1 \times 10^6$ s^{-1} at 298 K, as inferred from ^{17}O NMR data on $[Gd(dota)]^-$).[6] Recently, we reported that a Gd^{III} complex with a dota-like ligand containing four *N*-methylcarboxamide groups in place of the four carboxylate groups displays a large increase in the residence lifetime of the coordinated water molecule.[7] The slow exchange rate is a consequence of the stronger $Gd-OH_2$ interaction and a stabilizing hydrogen-bonding interaction to the proximate anions. The slow exchange of the coordinated water in this type of complex may give further insight into the relationship between the solution structure of the Ln^{III} chelate complex and the water exchange rate.

Here we have considered the Eu^{III} complex with the macrocyclic ligand dotam (dotam = 1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane). Figure 1 shows the ^1H NMR spectrum of $[Eu(dotam)]^{3+}$ in CD_3CN at 232 K. The compound is present as a mixture of **M** and **m** isomers whose ^1H chemical shifts are similar to those reported for the parent complex $[Eu(dota)]^-$ (Scheme 1). A 2D-EXSY NMR experiment carried out at 274 K in D_2O confirmed this analogy, and enabled the resonances to be assigned.[5b] In addition to the expected set of ligand resonances, an extra resonance for each isomer (of relative intensity two) is found at $\delta = 84.11$ (**M** isomer) and $\delta = 19.03$ (**m** isomer), respectively, which are assigned to the water molecules coordinated to

[*] Prof. S. Aime, Dr. A. Barge, Dr. M. Botta
Dipartimento di Chimica I.F.M., Università di Torino
Via P. Giuria 7, I-10125 Torino (Italy)
Fax: (+390) 11-670-7524
E-mail: aime@ch.unito.it
Dr. A. S. De Sousa, Prof. D. Parker
Department of Chemistry, Durham University
South Road, DH1 3LE Durham (UK)

  1433-7851/98/3719-2673 $ 17.50+.50/0

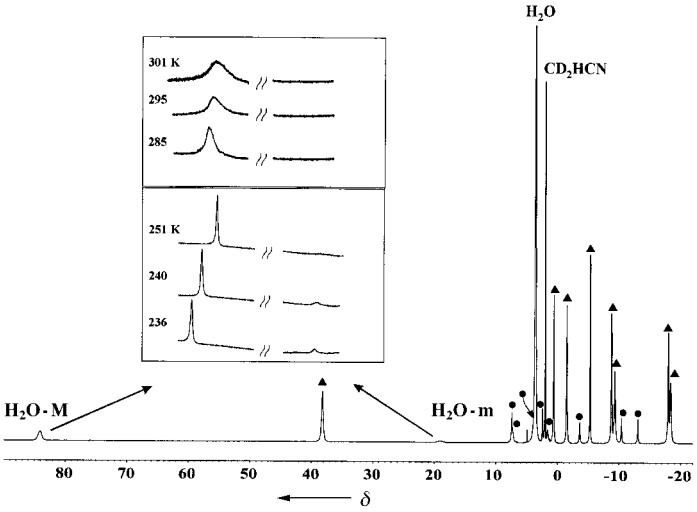
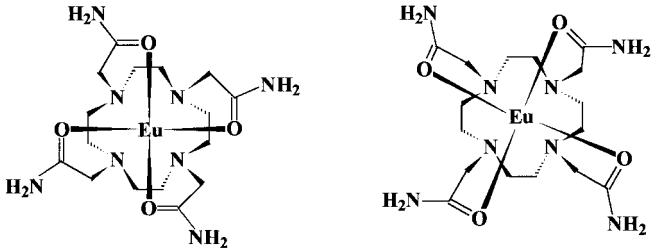


Figure 1. ^{1}H NMR spectrum (400 MHz) of [Eu(dotam)]$(SO_3CF_3)_3$ in CD_3CN at 232 K. The inset shows the expanded regions containing the bound water peaks for the two isomeric forms of the complex recorded at various temperatures. The symbols ▲ and ● indicate the ligand resonances of **M** and **m** isomers, respectively.



Scheme 1. Schematic representation of the two diastereoisomers of $[Eu(dotam)]^{3+}$. The water molecule, coordinated in the apical position above the plane of the four oxygen atoms, has been omitted for clarity.

the Eu^{III} ion. This assignment has been checked by measuring the ^{2}H NMR spectrum, in CH_3CN at 232 K, of a sample of $[Eu(dotam)]^{3+}$ that had undergone H/D exchange with D_2O. The ^{2}H resonances of free and **M**- and **m**-coordinated water molecules are observed at the same positions as in the ^{1}H NMR spectrum. In addition to the water signals, the ^{2}H NMR spectrum shows the resonances of the ^{2}H-exchanged $C(O)NH_2$ groups at $\delta = -5.6$ and 1.4, respectively.

A progressive broadening of the water resonance in the proton spectrum of the **m** isomer is observed upon increasing the temperature, which disappears at 243 K (see inset to Figure 1). The corresponding signal of the **M** isomer starts to broaden only at a temperature above 273 K. In the temperature range from 223 to 303 K the resonance of free water ($\delta_H = 3.57$) first broadens (maximum broadening at 248 K) then sharpens up and broadens again from 283 K. Selective irradiation of the free water resonance at 232 K results in the saturation of the **m** water signal only, whereas the same experiment carried out at 283 K caused the saturation of the corresponding **M** water peak. This behavior is consistent with the occurrence of a chemical exchange between free and bound water, which takes place at a rather different rate in the two isomers. Bandshape analysis has allowed the exchange rates at the various temperatures to be assessed and the following kinetic activation parameters to be determined: **M** isomer: $\Delta H^{\#} = 79.0(\pm 1.9)$ kJ mol^{-1}, $\Delta S^{\#} = 65.7(\pm 6.5)$ J mol^{-1} K^{-1}); **m** isomer: $\Delta H^{\#} = 79.0(\pm 2.5)$ kJ mol^{-1}, $\Delta S^{\#} = 110.8(\pm 10.4)$ J mol^{-} K^{-1}.

Under the assumption that the water exchange occurs through a dissociative mechanism, as is commonplace for nine-coordinate Ln^{III} complexes,[6] the observed behavior highlights the view that the release of water is much easier in the **m** isomer than in the **M** isomer (at 298 K τ_M is 1.88×10^{-5} s in **m** and 4.16×10^{-3} s in **M**). These results allows us to detect for the first time a dependence of the rate of water exchange on minor structural changes and are of relevance to the design of more efficent contrast agents for MRI. Indeed, until now the modulation of the exchange rate of the coordinated water, a crucial point for attaining an optimum relaxation efficiency, appeared possible only through variation of the overall residual charge of the complexes. It follows that the search for contrast agents of improved efficacy based on a dota-like coordination polyhedron may be addressed to systems endowed with a **m**-type structure. We have recently found that a shift towards this structural form occurs by introducing bulky substituents at the methylenic carbon atom of the acetate arms of $[Ln(dota)]^-$ complexes.[8]

Experimental Section

The ligand dotam was prepared by an established procedure.[9] Eu$(SO_3CF_3)_3$ was obtained by refluxing a stoichiometric amount of trifluoromethanesulfonic acid and Eu_2O_3 in water for 0.5 h.[10] Eu$(SO_3CF_3)_3$ (0.37 mmol) in dry acetonitrile (50 mL) was refluxed for 30 min under N_2 and then treated with a solution of dotam (0.37 mmol) in dry methanol (25 mL). The mixture was refluxed for 1.5 h, cooled to room temperature, and concentrated in vacuo. Addition of dichloromethane resulted in precipitation of the complex. Yield: 61 %. ^{1}H NMR (400 MHz, CD_3CN (500 μL) and H_2O (5 μL), 232 K, TMS): **M** isomer: $\delta = -18.37$ (CH_2CONH_2), -18.00 (CH_2CONH_2), -9.31 (ring CH_2, equatorial), -8.78 (ring CH_2, axial), -5.31 (CH_2CONH_2), -1.52 (CH_2CONH_2), 0.61 (ring CH_2, equatorial), 38.16 (ring CH_2, axial); ***m*** isomer: $\delta = -13.10$ (ring CH_2, equatorial), -10.41 (CH_2CONH_2), -3.64 (CH_2CONH_2), -1.52 (ring CH_2, axial), 2.43 (CH_2CONH_2), 3.57 (CH_2CONH_2), 7.40 (ring CH_2, axial), 7.41 (ring CH_2, equatorial).

The k_{ex} values were obtained by fitting the experimental spectra with the lineshape routine DNMR-SIM simulation program for dynamic NMR spectra (version 1.00, 1994) developed by G. Haegele and R. Fuhler, Institut für Anorganische and Strukturchemie I, Universität Düsseldorf. This program is available on the Internet.

Received: March 2,1998
Revised version: May 14, 1998 [Z11544IE]
German version: *Angew. Chem.* **1998**, *110*, 2819 – 2820

Keywords: lanthanides · macrocyclic ligands · NMR spectroscopy · water exchange

[1] a) R. B. Lauffer, *Chem Rev.* **1987**, *87*, 901 – 927; b) J. A. Peters, J. Huskens, D. J. Raber, *Prog. Nucl. Magn. Reson. Spectrosc.* **1996**, *28*, 283 – 350; c) S. Aime, M. Botta, M. Fasano, E. Terreno, *Chem. Soc. Rev.* **1998**, *27*, 19 – 29.

[2] a) I. Bertini, C. Luchinat, *NMR of Paramagnetic Molecules in Biological Systems*, Benjamin/Cummings, Menlo Park, **1996**; b) S. H. Koenig, R. D. Brown III, *Prog. Nucl. Magn. Reson. Spectrosc.* **1990**, *22*, 487 – 567.

  1433-7851/98/3719-2674 $ 17.50+.50/0

[3] a) R. V. Southwood-Jones, W. L. Earl, K. E. Newman, A. E. Merbach, *J. Chem. Phys.* **1980**, *73*, 5909 – 5917; b) C. Cossy, L. Helm, A. E. Merbach, *Inorg. Chem.* **1988**, *27*, 1973 – 1979.
[4] a) S. Aime, M. Botta, M. Fasano, S. Paoletti, P. L. Anelli, F. Uggeri, M. Virtuani, *Inorg. Chem.* **1994**, *33*, 4707 – 4711; b) G. Gonzalez, D. H. Powell, V. Tissières, A. E. Merbach, *J. Phys. Chem.* **1994**, *98*, 53 – 59; c) S. Aime, M. Botta, M. Fasano, S. Geninatti Crich, E. Terreno, *J. Biol. Inorg. Chem.* **1997**, *2*, 470 – 479.
[5] (a) S. Aime, M. Botta, G. Ermondi, *Inorg. Chem.* **1992**, *31*, 4291 – 4299; (b) S. Hoeft, K. Roth, *Chem. Ber.* **1993**, *126*, 869 – 873.
[6] D. H. Powell, O. M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer, A. E. Merbach, *J. Am. Chem. Soc.* **1996**, *118*, 9333 – 9346.
[7] S. Aime, A. Barge, M. Botta, A. De Sousa, D. Parker, *J. Am. Chem. Soc.* **1997**, *119*, 4767 – 4768.
[8] S. Aime, M. Botta, E. Terreno, unpublished results.
[9] S. Amin, J. R. Morrow, C. H. Lake, M. R. Churchill, *Angew. Chem.* **1994**, *106*, 824 – 826; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 773 – 775.
[10] P. H. Smith, K. N. Raymond, *Inorg. Chem.* **1985**, *24*, 3469 – 3477.

A Novel Aldol Condensation with 2-Methyl-4-pentenal and Its Application to an Improved Total Synthesis of Epothilone B**

Aaron Balog, Christina Harris, Kenneth Savin, Xiu-Guo Zhang, Ting Chao Chou, and Samuel J. Danishefsky*

Dedicated to Professor Ryoji Noyori

The recognition that the fermentation metabolites epothilones A and B (**1** and **2**, respectively) have potent in vitro antitumor properties and function through a paclitaxel-like (taxol-like) mechanism as inhibitors of microtubule disassembly has spurred a great deal of multidisciplinary research.[1, 2] Included in the pursuits which have followed in the wake of the exciting biology of the epothilones is the goal of total synthesis. Indeed, for those research groups (such as ours) for whom fermentation-derived epothilones are not available, chemical synthesis provides the only recourse to gain access to this series.

The total syntheses of epothilones A and B were accomplished by our group[3] and, shortly thereafter, by groups directed by Nicolaou[4a–e] and Schinzer.[5] A collection of fully synthetic epothilones from our laboratory,[6] as well as an even more extensive compendium from Nicolaou and co-workers,[7] were used to identify the zones of the epothilones that could undergo molecular modification with maintenance of biological function, at least at the in vitro level. The mapping exercises on structure – activity relationships performed by both groups provided very similar conclusions. An interesting finding first reported by our group,[6] and shortly thereafter by Nicolaou et al.,[7] was that the 12,13-deoxy versions of epothilones A and B (**3** and **4**, respectively) were quite active in in vitro assays. This discovery suggested the possibility that the epoxide linkages of the epothilones, which might be detrimental from the standpoint of peripheral toxicity, may not be crucial for eventual clinical function.

Our original synthesis of the epothilones, though quite long, had the feature of high stereoselectivity in each of the coupling steps.[3] While a disadvantage in enhancing access to multicomponent libraries,[4d,e, 7a] stereoselectivity allowed for accumulation of substantial quantities of fully synthetic key epothilones. Comparable harvesting of required amounts of material through the stereorandom olefin metathesis route, practiced by others[4, 5] as well as ourselves,[6a] would be virtually prohibitive. Its overall length notwithstanding, our first-generation total synthesis, which features the highly stereoselective LACDAC (*L*ewis *a*cid *c*atalyzed *d*iene – *a*ldehyde *c*yclocondensation) and *B*-alkyl Suzuki coupling steps, produced substantial quantities of epothilones. In fact, the only published in vivo data on epothilones available when this manuscript was submitted were obtained with our fully synthetic materials.[6c] These early findings in xenograft mice identified some significant toxicity problems with the highly potent epothilone B (**2**). Remarkably, in vivo studies in the interperitoneal mode of injection demonstrate that the less potent 12,13-deoxyepothilone B (**4**) is well tolerated and is virtually curative against human mammary tumor xenografts.[8] The lead compound **4** has many significant and clear advantages over paclitaxel in terms of efficacy against multiple drug resistent (MDR) tumors when administered intraperitoneally. We shall return to issues of bioactivity shortly.

These exciting early results[8] underscored the need for a greatly improved total synthesis which can sustain a serious and substantial discovery research program for assaying candidate structures in rodents as well as in higher animals. We now report major progress in this regard. Our new route, which retains the advantages of high stereoselectivity throughout, is totally reworked in the treatment of the once difficult C1 – C11 domain. Scheme 1 provides an overview of the problem.

The new route is based on four findings, each of which has implications well beyond epothilone. The first is the ease of formation and the synthetic utility of the (*Z*)-lithium enolate **10**, which is readily prepared from **8** (Scheme 2). The ethyl ketone unit, from which the critical enolate is formed, is part

[*] Prof. S. J. Danishefsky,[+] Dr. A. Balog, Dr. C. Harris, Dr. K. Savin
Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, Box 106, New York, NY 10021 (USA)
X.-G. Zhang, Dr. T.-C. Chou
Laboratory for Molecular Pharmacology and Therapeutics Program
Sloan-Kettering Institute for Cancer Research
[+] Further address:
Havemeyer Hall, Department of Chemistry, Columbia University
New York, NY 10027 (USA)

[**] This research was supported by the National Institutes of Health (NIH, grants CA-28824 and CA-08748). Postdoctoral Fellowship support is gratefully acknowledged by A.B. (NIH, CA-GM 72231), C.H. (American Cancer Society, PF-98 – 173 – 001), and K.S. (NIH, GM-18248). We gratefully acknowledge Dr. George Sukenick (NMR Core Facility, Sloan-Kettering Institute) for NMR and mass spectral analyses. Professor Dr. G. Höfle of the Gesellschaft für Biotechnologische Forschung is gratefully acknowledged for providing natural epothilones A and B for comparative analysis. We also thank Professor Gunda Georg of the University of Kansas for bringing the epothilone problem to our attention. We dedicate this paper to Professor Ryoji Noyori, whose accomplishments were critical to this work.

  1433-7851/98/3719-2675 $ 17.50+.50/0