formation of the interdigitating β-sheet between the metalloinhibitor and the monomeric protein.

In conclusion we have demonstrated that the activity of a dimerization inhibitor of HIV-1 protease derived from cross-linked interfacial peptides can be enhanced by controlling the conformation of their connecting unit through Zn^{II} complexation.[17] This provides a straightforward example of allosteric regulation of the activity of a synthetic inhibitor by a metal ion and shows that although the Zn^{II}-binding process occurs at a remote position, it in fact amplifies the chemical information present in the system (i.e. its ability to recognize a monomer of HIV-1 protease).

Experimental Section

Compound **2** was synthesized according to the route reported in Scheme 1. **4**: ESI-MS (*m*/*z*): 837 [M^+]. **2**: amino acid analysis: Phe(2) 2.01, Asn(1) 1.1, Leu(2) 2.06, Thr(2) 2.09, Ser(1) 1.03, Gln(1) 0.9, Pro(1) 0.98, Ile(1) 0.87; MALDI-MS (*m*/*z*): 1815 [M+1], 1321, 1115; HR-ESI-MS (*m*/*z*): 908.056 [MH_2^{2+}]. **2**–Zn^{II}: HR-ESI-MS (*m*/*z*) 939.013 [MZn^{2+}]. (HR = high-resolution, ESI = electrospray-ionization, MALDI = matrix-assisted laser-desorption ionization).

Enzyme assay: Inhibition experiments where performed by using 250 μL of a standard HIV-1 protease solution [50 nM in a buffer prepared with phosphate (20 mM), glycerol (20 %), dithiothreitol (DTT; 1 mM) and 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) (0.1 %)], which was incubated for 1 h with a solution of the inhibitor with the required concentration (50 μL). The solution was then added to the substrate (Abz-Thr-Ile-Nle-*p*-NO_2Phe-Glu-ArgNH_2; 200 μL) in the same buffer diluted with dimethyl sulfoxide (DMSO; 10 %) to give a substrate concentration of 60 μM. The amount of DMSO was kept constant at 14 %. The concentration of HIV-1 protease in the kinetic runs of Figure 1 was 25 nM while in the experiments of Figure 2 it was varied within the 10–80 nM interval. For the determination of K_m and k_{cat}, the substrate was varied within the 10–100 μM interval. Kinetic runs were performed by following the increase of fluorescence emission at 430 nm (λ_{ex} = 360 nm) for 400 s.

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Regioselective Tetrametalation of Ferrocene in a Single Reaction: Extension of s-Block Inverse Crown Chemistry to the d-Block**

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s-Block metal inverse crowns constitute an emerging family of special compounds in which polymetallic amide cationic rings complex anionic guests.[1] They are "inverse" crowns in the sense that their Lewis acidic/Lewis basic sites have been interchanged relative to those in conventional crown ether complexes,[2] that is, here the metal atoms belong to the ring and not to the core. However, the chemistry controlling their formation goes far beyond that of simple macrocyclic host–

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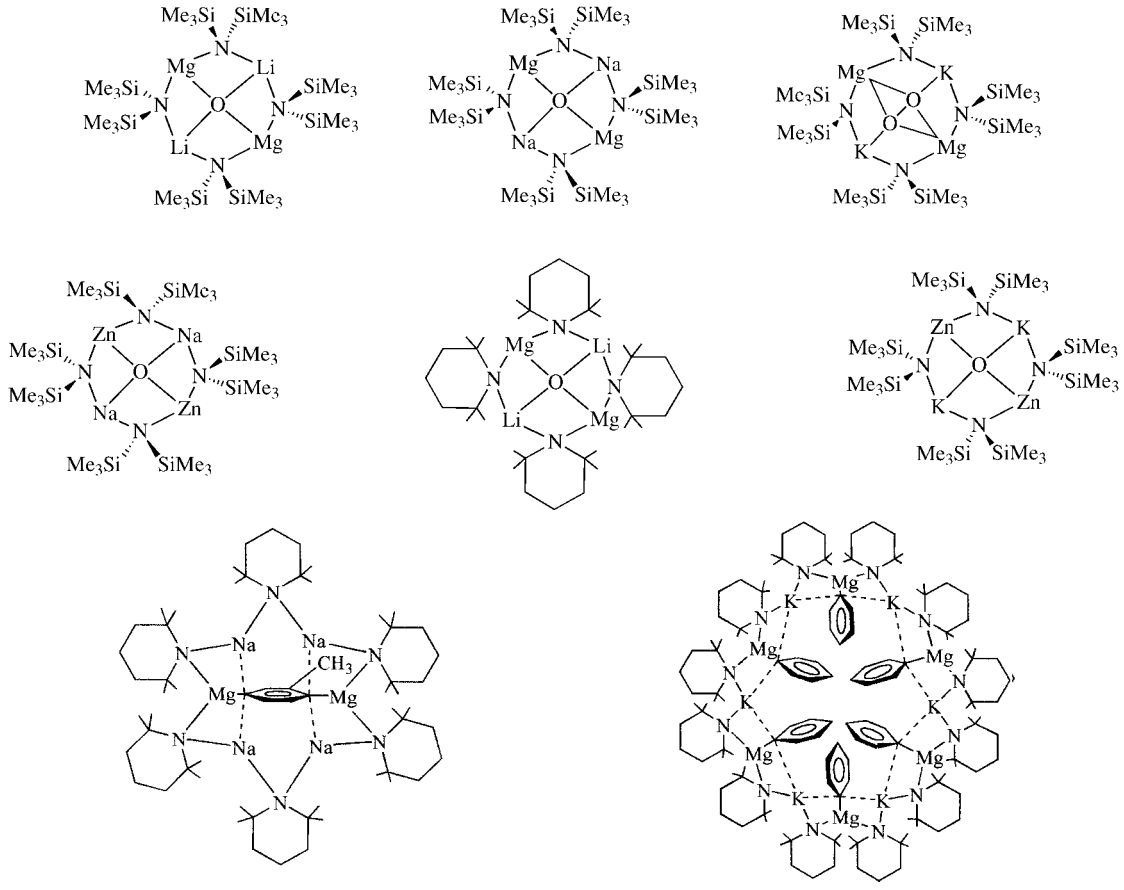
guest combinations. It appears that a powerful ring template effect brings about the regioselective deprotonation of certain substrates to create and to encapsulate unusual anions, which are inaccessible through the use of mainstream bases. The best example of this phenomenon to date is in the transformation of toluene to the arenediide $(C_6H_3CH_3)^{2-}$ with twofold deprotonation occurring at thermodynamically unfavorable ring sites (2,5-positions), while the more acidic methyl substituent is left untouched.[3] Scheme 1 shows the membership of the inverse crown family as it stood prior to the work reported here. These compounds of eight, twelve, or twenty-four ring atoms were the subject of a recent feature article.[4] A product of a synthetic and structural synergism which is triggered by pairing together two distinct metal types (Li, Na, or K with Mg or Zn[5]) within the same amide environment, these mixed-valent compounds exhibit a unique chemistry that cannot be replicated by complexes containing one or the other metal type on its own. Herein we describe the entry of inverse crown complexes into the domain of transition metal organometallic chemistry.[6] The entrance is eye catching with ferrocene transformed regioselectively to a 1,1′3,3′-tetrayl derivative by the encapsulating action of a new sixteen-membered tetrasodium – tetramagnesium amide cationic ring.

Previously the amide component of inverse crowns had been limited to either hexamethyldisilazide (HMDS) or 2,2,6,6-tetramethylpiperidide (TMP). Here a third sterically demanding amide is added to the list of inverse crowns in diisopropylamide (iPr$_2$N$^-$). This anion was generated by subjecting three equivalents of the parent amine iPr$_2$NH to an equimolar mixture of nBuNa and Bu$_2$Mg. The resulting synergic solution[7] of "[NaMg{NiPr$_2$}$_3$]" smoothly converts ferrocene to the spectacular new addition to the inverse crown family **1**. Remarkably this amounts to a "simultaneous" fourfold regioselective deprotonation. This contrasts greatly with the behavior of conventional alkali metal bases such as nBuLi/*N*,*N*,*N*′,*N*′-tetramethyl-1,2-ethylenediamine (TMEDA),[8] which generally strip only one or two hydrogen atoms from ferrocene.

$[\{Fe(C_5H_3)_2\}Na_4Mg_4\{i Pr_2N\}_8]$ **1**

Figure 1 shows a view of the molecular structure of **1** through the face of its sixteen-membered ring.[9] This centrosymmetric ring is made up of alternating nitrogen and metal atoms, with the latter atoms themselves alternately sodium and magnesium. A severe flexing of this ring is necessary to anchor the dideprotonated edge of each Cp ring (Figure 2). The missing hydrogen atoms of the Cp rings are lost from the 1,1′,3,3′ positions (corresponding to C27, C27′, C29, C29′). Formally therefore the ferrocenyl captive carries a 4− charge, which is balanced by the 4+ charge of its cyclic $Na_4Mg_4N_8$ captor. This captivity is enforced by a combination



Scheme 1. The inverse crown family.

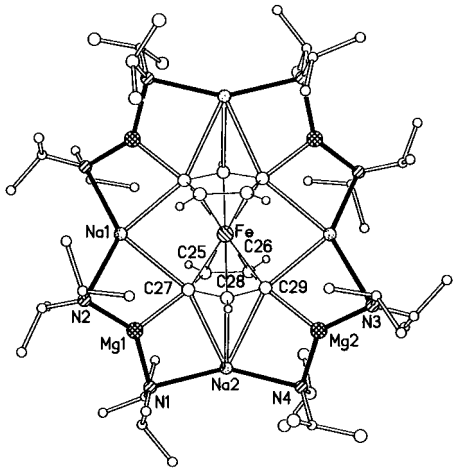


Figure 1. Molecular structure of **1**. Hydrogen atoms, except those belonging to the C_5H_3 rings, are omitted for clarity. Selected bond lengths [Å]: Mg1-N1 2.0397(13), Mg1-N2 2.0163(14), Mg2-N3 1.9965(13), Mg2-N4 2.0411(13), Mg1-C27 2.1469(15), Mg2-C29 2.1580(15), Na1-N2 2.5347(15), Na1-N3′ 2.7113(15), Na2-N1 2.5068(14), Na2-N4 2.4661(14), Na1-C27 2.7546(16), Na1-C29′ 2.6287(15), Na2-C27 2.8329(15), Na2-C28 2.6080(15), Na2-C29 2.9232(15).

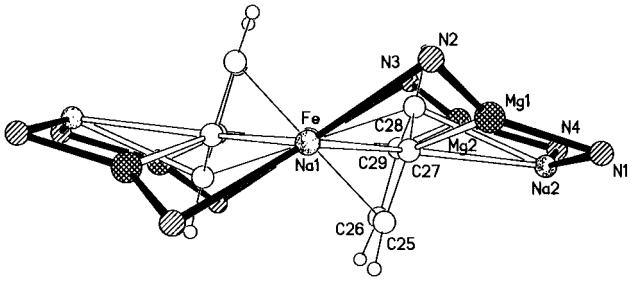


Figure 2. Side view of **1** looking along the Na1···Fe1···Na1′ line. Isopropyl groups are omitted for clarity.

of Na–C and Mg–C bonds. Part of a linear Na···Fe···Na arrangement, Na1 occupies a μ_2-bonding site between the Cp rings (mean Na–C bond length, 2.670 Å) and completes a distorted tetrahedral environment by binding to two N atoms (mean Na–N bond length, 2.623 Å). In contrast Na2 binds to the π face of one Cp ring. Involving the three C atoms of the long doubly deprotonated edge, this interaction is asymmetric (η^3) with the central C atom (C28) providing the shortest contact distance (2.608 Å). Two N atoms also complete the coordination sphere of Na2, but at significantly shorter distances (mean, 2.486 Å) than those to Na1. Following the pattern set in the other arene-based inverse crowns, the privileged positions within the Cp ring undergoing deprotonation are dictated by the positions of the Mg atoms within the host ring. This reflects the stronger, more covalent nature of the Mg–C bonding (mean, 2.153 Å), and the possibility (suggested in a previous paper[4]) that the amide anion performing the deprotonation step (and subsequently leaving as an amine molecule) originates from a terminal position on Mg within a reactive mixed-metal tris(amide) cyclic intermediate. Unlike in the benzene- and toluene-based inverse crowns, the Mg–C bonds in **1** do not lie coplanar with the plane of the aromatic ring, but tilt away from it by 63.1 and 58.4° for the two independent Mg–C bonds. Clearly it is inherently more difficult for a host ring to accommodate a molecule as large as ferrocene, and it appears that a "best fit" arrangement can only be reached at the expense of compromising the σ contribution to the Mg–C bonding. However, this has a negligible effect on the lengths of the Mg–C bonds as those in the toluene-based inverse crown are actually slightly longer at 2.200 Å. The structure of **1** also exhibits two distinct types of amide ligand in N2/N3 and N1/N4, which is a consequence of their attachment to the two distinct Na atoms (Na1 and Na2, respectively).

Crystalline **1** dissolves in toluene thus enabling a ^{1}H NMR spectrum to be recorded, whose appearance indicates a molecular structure exactly matching that found in the solid state. To elaborate, there are three Cp H resonances, two of which have nearly identical, but well-resolved chemical shifts (0.02 ppm apart), while the third one lies 0.66 ppm to lower frequency. Using the crystal structure labels, these can be assigned to the hydrogen atoms attached to C25/C26 and C28, respectively. Two sets of *i*Pr$_2$N resonances are also discernible. Each set contains four diastereotopic Me groups, which leads to a complicated pattern of overlapping doublets caused by coupling to the CH units. The prochiral nature of their attached N atoms is a consequence of the geminal heterobimetallic bonding. It can therefore be concluded that the structure of **1** is retained in arene solution.

Looking to the future, the propensity of s-block metals to undergo exchange in reactions with electrophiles makes **1**, in effect, an invaluable synthon[10] for a 1,1′,3,3′-tetraanion of unsubstituted ferrocene. Thus in theory it offers chemists scope for designing new regioselectively tetrasubstituted ferrocenes. The potential utility of **1** could also have important implications for the synthesis of new ferrocene polymers. This field is currently being studied in the search for polymers with unusual properties (e.g., conductive, magnetic, electronic, optical). Also, following this first venture into organometallic chemistry the development prospects for inverse crown chemistry as a whole look decidedly bright with a vast pool of metallocenes now available as potential candidates for exploitation in this exciting new area.

Experimental Section

1: Freshly prepared *n*BuNa (5 mmol) was suspended in hexane (10 mL) in an argon-filled Schlenk tube. The tube was then placed in an ultrasonic bath for 10 min to obtain a more reactive fine suspension. Commercial (from Aldrich) Bu$_2$Mg (5 mmol in heptane solution) was then added to give a brown mass. This was subsequently dissolved upon the addition of *i*Pr$_2$NH (15 mmol) with gentle warming. Next, ferrocene (2.5 mmol) was introduced to give an orange solution, and the mixture was heated to reflux for 15 min by which time it had turned from orange to red. Much of the hydrocarbon solvent was removed in vacuo and replaced by toluene (2–3 mL), and the mixture was heated until complete dissolution was achieved. The resulting deep red solution was placed in a Dewar flask of hot water and allowed to cool slowly to ambient temperature. This yielded red cubelike crystals of **1** (yield, 42%). M.p. 161–164 °C. Satisfactory (C,H,N) analysis. ^{1}H NMR (400 MHz,[D$_8$] toluene, 25 °C): $\delta = 4.20$ (s; C_5H_3), 4.18 (s; C_5H_3), 3.52 (s; C_5H_3), 3.48 (m, CH × 8; *i*Pr), 3.12 (m, CH′ × 8; *i*Pr), 1.42 (m, CH_3 × 16; *i*Pr), 1.06 (m, CH_3' × 8; *i*Pr), 0.97 (m, CH_3' × 8; *i*Pr). Small traces of free ferrocene and diisopropylamine were also present due to slight hydrolysis. Repeating the preparation using a stoichiometry of ferrocene commensurate with that in the formula of **1** (1.25 mmol) also produced **1** in similar yields. Note that **1** is air- and moisture-sensitive, and pyrophoric.

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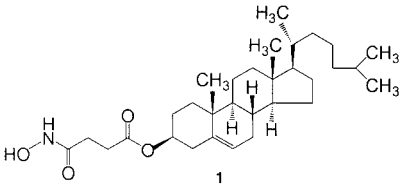
Chemical Reaction between Colliding Vesicles**

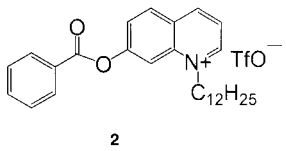
Fredric M. Menger,* Kevin L. Caran, and Victor A. Seredyuk

It is difficult to say exactly when the reaction kinetics of water-soluble agents with colloidal assemblies, now a venerable sector of chemistry, first came into prominence. Perhaps it occurred in the l960s when scientists worldwide began studying the reactions of hydroxide and hydronium ions with micelle-adsorbed substrates.[1] Modest rate increases or rate inhibitions (depending upon the electrostatics of the situation) were the general rule. For example, hydroxide-catalyzed ester hydrolysis is accelerated by cationic micelles and inhibited by anionic micelles.[2] A quantitative understanding of such rate effects is now in hand.[3] Later on, attention turned to bimolecular reactions between species in solution and vesicular reactants. For example, rates of reduction between external dithionite and vesicle-bound dye were established.[4] Owing in part to the hundreds of publications on the kinetics of reagent/colloid reactions, we now have an appreciation of colloids' interfacial polarity,[5] concentration of bound counterions,[6] molecular dynamics of assembly components,[7] and various size, shape, and phase properties.[8]

The next higher level of complexity, namely the reaction kinetics between two discrete molecular assemblies, has never received much attention. This is surprising considering the biological relevance of inter-assembly reactivity. One merely has to consider a sperm penetrating an ovum, or a virus penetrating a cell, to recognize the importance of membrane/membrane reactivity.[9] It is for this reason that we began examining vesicle/vesicle reactions. The idea was to place a nucleophilic lipid in one phospholipid vesicle, and an electrophilic lipid in another, and to monitor the ensuing reaction efficiency as the two vesicles collided. In preliminary experiments, we found that vesicle/vesicle reactivity is indeed an observable phenomenon, and that its rates depend upon the exposure of the membrane-bound functionalities to the external water.[10] Encouraged by these results, we continued our investigation and now report a reaction whose molecular features—including vesicle-to-vesicle transfer processes during collision—were revealed by the kinetics.

The reaction in question involves two amphiphilic compounds: a steroidal nucleophile **1** and a long-chain quinolium ester **2**. Nucleophile **1** is a known hydroxamic acid[10] (pK_a = ca. 9.4)[11] which, under the basic conditions of our experiments (pH 9.0), partially transforms into a powerful esterolytic species: hydroxymate.[12] The cholesterol moiety of **1**, with its known affinity for bilayer membranes,[13] serves to "anchor" the molecule in the vesicles. Substrate **2** is a labile ester whose cleavage leads to a marked increase in absorbance and fluorescence. Membrane affinity, in this case, derives from a hydrophobic dodecyl chain.





Reactant **2** possesses a useful trait that, to our knowledge, is rare or nonexistent among reactive fluorescent probes. Hydrolysis of **2** produces a fluorescent hydroxyquinolinium ion that retains its hydrophobic chain. Consequently, this ion tends to remain within the membrane where it is initially formed. This facilitates direct visualization of membrane reactions (as by fluorescence microscopy) compared to systems in which the reactive probes eject their incipient

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