Positive Cooperativity in Cation Binding by Novel Polyether Bis(β-diketone) Hosts

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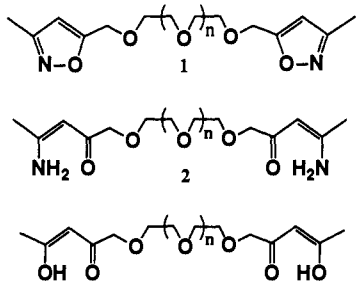
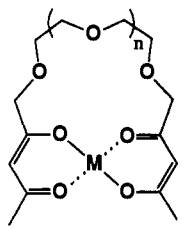
Cooperativity is an important biological device for controlling enzymatic functions insofar as the first step assists or suppresses the succeeding manifestation of the original function.[1] The first pioneering biomimetic models of the cooperative binding of two metal ions have been reported by Rebek, Jr., et al.[2] The host prepared had two metal binding sites which were either identical or different. Complexation to one of the sites was designed to transform the conformation of the second site into a geometry favorable for the metal binding. On using positive metal cations, however, Coulombic repulsion between the two metal centers overshadowed the other favorable factors and no assistance of the second metal binding was observed. Only non-ionic metals, which avoid the repulsion, showed enhancement of the second equilibrium constant by a factor on the order of 10.[2f] No positive cooperativity has been reported for the binding of *positive* metal ions so far.[2,3] Herein we report *positive* cooperativity in the binding of two *positive* metal cations and introduce a new concept of host organization.

β-Diketone is an excellent chelating agent toward various metal ions and has been incorporated into several macrocyclic hosts.[4] Here podands having two β-diketones at both terminals of polyethylene glycol were prepared. In the synthesis, di- or triethylene glycol was etherified (ca. 60% yield) using 5-(bromomethyl)-3-methylisoxazole, which was obtained by a regioselective bromination of 3,5-dimethylisoxazole.[5] the resulting bisisoxazolyl ether **1** was hydrogenated cleanly in the presence of platinum oxide to give rise to the formation of the β-enamino ketone moiety **2**, which was then converted quantitatively to β-diketones **3** and **4** by treatment with aqueous acid.[6]



3 (n=1), 4 (n=2)

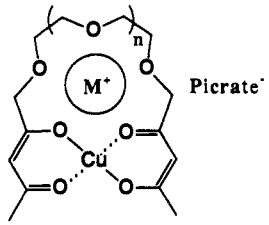


5 (M=Zn, n=2)
6 (M=Cu, n=1)
7 (M=Cu, n=2)

When Zn(II) sulfate was added to a 5 mM ethanolic solution of **4** followed by the addition of 2 equiv of KOH, a neutral Zn complex was isolated. The FAB mass spectrum gave a parent peak at m/z 408, suggesting the formation of a 1:1 complex (**4**-2H)·Zn (**5**). The NMR spectrum of **5** in $CDCl_3$ gave the largest upfield shift of 0.675 ppm at the olefinic keto enolate methine protons. Methyl, methylene α to keto enolate, and ether methylene protons showed a decreasing order of upfield shifts of 0.162, 0.108, and 0.081 ppm, respectively. These chemical shift data are in complete agreement with structure **5**, where Zn is coordinated by two β-keto enolate groupings in an intramolecular fashion.

A similar Cu(II) complex **7** was isolated from cupric sulfate and **4** in a 90% yield as deep-green crystals which were soluble in chloroform or ether and gave a UV absorption at 310 nm, characteristic of the β-keto enolate. The 1:1 complexation was ascertained also by the molecular peak at m/z 407 in the FAB mass spectrum. By using chloroform solutions of this and a shorter homologue (**3**-2H)·Cu^{II} (**6**), extraction of metal picrate from the aqueous phase was undertaken.[7] Partition coefficients between the chloroform and aqueous phases were determined as shown schematically in Figure 1.

The result clearly indicates that **6** was selective in the extraction of sodium ion, as was **7** for potassium, exhibiting a typical selectivity of matching between ionic diameters and the cavity size similar to those observed in macrocyclic antibiotics or crown ethers.[8] In a closed conformation of these complexes, where two β-keto enolate units are complexed in an intramolecular fashion with cupric ion probably similarly to planar cupric bis(acetylacetonate) complexes, contributions from two anionic oxygen atoms each from two β-keto enolate units and three or four neutral oxygens in the polyether linkage **3** or **4**, respectively, afford the most appropriate and selective binding site to alkali-metal ions as shown in **8**. The partition coefficients of Na^+ and K^+ picrates were negligibly small when **3** and **4** were used in their free or K^+ salt forms. Therefore, the cooperativity is absolute in the sense that the extraction of metal picrates takes place only with the assistance of another metal binding.



8 (M=Alkali Metal, n=1, 2)

The use of dissociated anionic ligands is crucial for the successful observation of positive cooperativity, since they coordinate strongly to the first transition-metal ion and compensate its positive charge to allow the acceptance of the second positive metal ion into the organized molecular arrangement constructed through the

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(5) 3,5-Dimethylisoxazole was treated with *N*-bromosuccinimide in the presence of benzoyl peroxide in carbon tetrachloride. Bromination occurred regioselectively at the 5-methyl group, as ascertained from ^{13}C-^1H 2D NMR measurements. ^1H NMR (δ, ppm, $CDCl_3$): 2.15 (s, 3 H, CH_3), 4.25 (s, 2 H, CH_2Br), 6.00 (s, 1 H, CH).

(6) Compounds **3** and **4** gave correct NMR, MS, and elemental analysis data. The transformation of isoxazole to β-diketone moieties according to this route has been developed by the following: (a) Yates, P.; Hand, E. S. *J. Am. Chem. Soc.* **1969**, *91*, 4749-4760. (b) Auricchio, S.; Ricca, A.; Vajna de Pava, O. *Gazz. Chim. Ital.* **1980**, *110*, 567-569.

(7) Chloroform solution (8 mL) containing 4×10^{-5} M host was shaken with an aqueous solution (0.8 mL) containing 2.5×10^{-4} M alkali metal picrate and the corresponding MCl at 1.0 M. Partition coefficients, *K*, were obtained from $K = ([Pic]_i - [Pic]_f)/[Pic]_f$, $[Pic]_i$ and $[Pic]_f$ being picrate concentrations in the aqueous phase before and after the partition, respectively.

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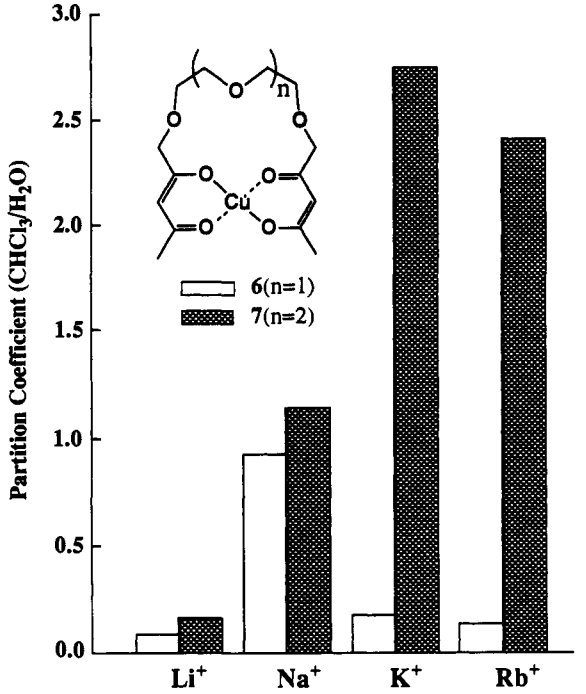

Figure 1. Partition coefficients[7] of alkali-metal picrates between $CHCl_3$ and H_2O with the use of copper-assisted coronands **6** and **7**.

structure-forming capacity of the first metal ion. This provides an interesting way of molecular organization in place of the "preorganization"[8e] which has long been a dominant principle in crown ether chemistry. The metal-assisted organization *after the chemical synthesis* may be an interesting alternative, because the ligand synthesized could be subjected further to a reversible organization to construct or destroy the structure as required. Therefore, the method is expected to lead to a new strategy for the design of supramolecular chemistry. Further studies are now actively under way.

The Structural Basis of Pancreatic Amyloid Formation: Isotope-Edited Spectroscopy in the Solid State

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The deposition of proteinaceous amyloid is characteristic of many diseases, including Alzheimer's disease (AD),[1] scrapie,[2] and type II diabetes.[3] Because amyloid is insoluble and noncrystalline, structural models of the constituent cross-β fibril are based solely on the low-resolution technique of X-ray fiber diffraction.[4] Consequently, there is little information regarding the molecular details of amyloidogenesis. We have developed a Fourier transform infrared spectroscopic (FTIR) method, based on isotropic substitution, which can discern details in amyloid structure which were previously unobservable.[5] We report herein the application of this method, which we call isotope-edited dipole coupling analysis, to a peptide amyloid related to the pancreatic amyloid of type II diabetes. An extension of the method allows the determination of the critical intermolecular interactions present in the antiparallel β-sheet structure which is the subunit of the cross-β fibril. The FTIR analysis and solid-state ^{13}C NMR studies carried out in parallel suggest that a sequence of at least four amino acids is critical in precipitating amyloidogenesis.

Table I[a]

analogue	A ^{12}C (cm^{-1})	B ^{13}C (cm^{-1})	C δ (ppm)	D line width (Hz)
S20	1628 (0)		172 (+1)	285
F23	1632 (+4)	1614 (+2)	172 (+4)	317
G24	1643 (+15)	1610 (+6)	170 (+3)	190
A25	1637 (+9)	1606 (+9)	174 (+2)	171
I26	1639 (+11)	1605 (+9)	171 (+4)	181
L27	1638 (+10)	1611 (+4)	173 (+4)	213
S28	1629 (+1)	1618 (ND)	173 (0)	322
S29	1629 (+1)		174.5 (-1.5)	386

[a] Column A lists the position of the ^{12}C amide band (± 2 cm^{-1}) and, in parentheses, the shift from the position in the unlabeled spectrum (1628 cm^{-1}). This shift reflects the total amount of dipole coupling (inter- and intramolecular) experienced by that amide. Column **B** lists the position of each ^{13}C amide I band and, in parentheses, the shift observed on isotopic dilution (5:1). The magnitude of the shift depends on the amount of intermolecular dipole coupling. For S28, the ^{13}C band was at 1618 cm^{-1} but was not observable on isotopic dilution. For S20 and S29, the ^{13}C band was not observable. Column C lists the chemical shift of each carbonyl carbon and, in parentheses, the deviation of that shift from the "unstructured" value.[13,14] For example, the chemical shift of the F carbonyl carbon in the multiconformational pentapeptide GGFGG is 176 ppm. Each value is the average of two separate experiments (deviation was <1 ppm) except for S20, which is the result of a single experiment. Column D lists the average line width of each carbonyl carbon line (two experiments; deviation $\leq$10%; S20 was a single experiment). Line width is related to several factors, including conformational disorder and structural rigidity.[14]

The presence of pancreatic amyloid may interfere with β cell function and cause insulin insensitivity or may be an epiphenomenon associated with type II diabetes.[3] Pancreatic amyloid comprises a 37-residue peptide known as the islet amyloid polypeptide (IAPP).[3] We synthesized[6] a 10 amino acid peptide (AcHN-SNNFGAILSS-$CONH_2$, IAPP 20-29) corresponding to a sequence from human IAPP which has been shown to form amyloid fibrils in vitro.[7,8] A film of the peptide IAPP 20-29 was analyzed by FTIR and shown to contain antiparallel β-sheet structure in the solid state (strong absorption at 1628 cm^{-1}).[9]

The antiparallel β-sheet, which is characteristic of amyloid, is unique among common peptide secondary structures in that extensive dipole-dipole coupling interactions occur.[9] This effect results in a splitting of the amide I band into a low-intensity band at high frequency (ca. 1695 cm^{-1}) and a diagnostic intense band at low frequency (ca. 1630 cm^{-1}).[9] The method of isotope-edited dipole coupling analysis depends on the substitution of a single amide carbonyl carbon with ^{13}C which essentially decouples that

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