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Synthesis of a Dinucleating Ligand with Addressed Ion Binding Sites

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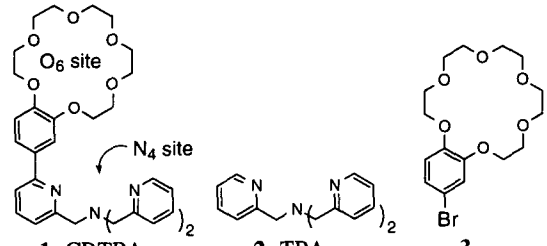
Abstract: A ligand was synthesized that contains two binding sites, differentiated on the basis of size and donor atom. The binding profile for a series of amino acid guests suggest that the two binding sites are 6.5 Å apart. Kinetic studies of hydrolysis of a model phosphodiester substrate by heterodinuclear complexes of the ligand suggest that one metal ion delivers a hydroxide nucleophile and the other increases substrate binding although not in the ideal geometry. © 1997 Elsevier Science Ltd.

Much interest has been drawn to the design of coordination complexes that bind organic substrates selectively through both metal-guest and ligand-guest interactions.[1] Similarly, the design of coordination complexes composed of two different metal ions is currently an active research area.[2,3] We recently synthesized compound **1** (CRTPA), which was designed to possess two different binding sites for cations such as metals or ammonium ions.



1, CRTPA **2**, TPA **3**

There are several interesting applications for ligands possessing multiple binding sites. One problem that has attracted much recent interest is catalysis of phosphodiester hydrolysis. Many nucleases utilize two different metal ions,[4] and recent studies have shown that homodinuclear[5-9] and heterodinuclear[10] model complexes are extremely effective catalysts. However, designed ligands that fix two different metal ions in addressed binding sites with concave spatial arrangements are scarce.[11,12]

Compound **1** (CRTPA, Figure 1) contains two differentiated metal ion binding sites, expected to discriminate between metal ions on the basis of hardness (N vs. O ligation) and cavity size.[13] One binding site (the N_4 site) is composed of the elements of the ligand tris(2-pyridylmethyl)amine (TPA, **2**), which forms stable complexes with ions of small atomic radii (~0.8 Å) such as Zn(II) and Cu(II).[13,14] In aqueous solution, $[Zn(TPA)OH_2]^{2+}$ displays an acidic water molecule with pK 8.0.[14] Homodinuclear Zn/TPA complexes with bridging phosphates[15] and carbonate[16] have been reported. The other metal ion binding site in **1** (the O_6 site) contains the elements of 18-crown-6, which forms reasonably stable complexes with a variety of ions, including alkyl ammonium ions and alkali metal and alkaline earth ions with radii similar to that of K^+.[17]

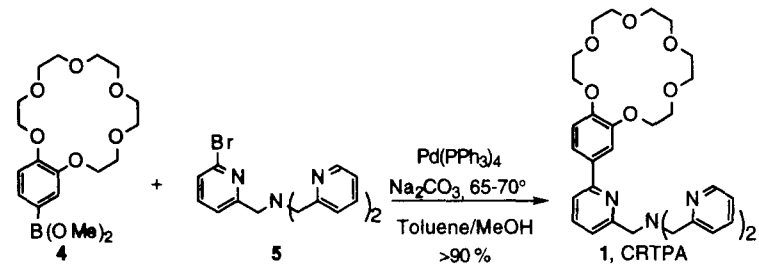


Figure 1. Synthesis of CRTPA.

The synthesis of CRTPA was completed as shown in Figure 1.[18] The strategy parallels syntheses of ligands related to TPA that have been reported from our laboratory in that a Suzuki coupling reaction is used to append a substituted phenyl group to the TPA ligand.[19-21] Commercially available 4-bromobenzo-18-crown-6 **3** was converted to the dimethyl boronic acid ester **4** by careful lithiation and low-temperature quench with trimethyl borate. Coupling[19] with bromo(TPA)[20] **5** catalyzed by Pd^0 proceeded efficiently to yield CRTPA in good yield.

In order to probe the geometry of the two binding sites with respect to one another, the association constants for $[Cu(CRTPA)]^{2+}$ with a series of amino acids were measured. UV/vis titrations were carried out in 50% aqueous acetonitrile (pH = 5.8, 1 M $NaClO_4$). The titrations were successful under a narrow pH range, as precipitation occurred at higher pH and binding was much weaker at lower pH. The results (Table 1) indicate optimal complexation with 5-aminohexanoic acid, consistent with a cleft-like arrangement of the binding sites approximately 6.5 Å apart, with coordination of the carboxylate to the Cu^{II} ion and concomitant binding of the ammonium ion to the oxygens of the crown ether ring. The 6.5 Å intersite distance is consistent with that predicted by CPK molecular models in combination with recent X-ray crystallographic results on related monophenyl-substituted TPA complexes.[22] The K_a for 6-aminohexanoic acid increased to 251 ± 26 M^{-1} in 1 M $LiClO_4$ and was > 500 M^{-1} in 0.1 M Me_4NClO_4, which is higher than can be measured by the present method. Thus the 1 M $NaClO_4$ conditions served to modulate the binding constant within a measurable range.

Amino Acid	K_a (M^{-1})
NaOAc	40 ± 5
^+H_3N-$(CH_2)_2$–CO_2^-	< 40
^+H_3N-$(CH_2)_3$–CO_2^-	100 ± 6
^+H_3N-$(CH_2)_4$–CO_2^-	91 ± 5
^+H_3N-$(CH_2)_5$–CO_2^-	131 ± 5
^+H_3N-$(CH_2)_6$–CO_2^-	57 ± 4
^+H_3N-$(CH_2)_7$–CO_2^-	22 ± 2

Table 1. Association constants for anionic and neutral guests.

The ligand was shown to bind two different metal ions in differentiated sites. ^1H NMR spectra (aqueous DMSO) of the ligand reveal metal ion binding by downfield shifts of CH_2 or Ar-H signals upon binding of Zn^{2+} or Ba^{2+}. Addition of one equivalent of Zn^{2+} to a solution of CRTPA showed downfield shifts in NCH_2 and Ar-H but not OCH_2 resonances. Addition of Ba^{2+} ion to $[Zn(CRTPA)]^{2+}$ resulted in downfield shifts of the OCH_2 resonances ($\Delta\delta$=0.2 ppm, identical to the maximum shift observed upon addition of $Ba(ClO_4)_2$ to **3**). In order to observe this behavior, the binding of the Ba^{2+} to the O_6 site must be at least 100 M^{-1}, consistent with reported stability constants of this ion with other crown ethers.[17] Potentiometric titrations of CRTPA in the

absence and presence of $Zn(ClO_4)_2$ confirmed strong binding of Zn^{II} to the N_4 site ($\log[K_{LZn}] = 8.1$), almost identical to that for TPA[14] and other phenyl-substituted derivatives of TPA.[22]

A rate study was conducted for the reaction of metal complexes of compound **1** with BNPP.[23] Pseudo-first order rate constants were determined at 55°C in 20% DMSO/water solution at pH 8.5 (50 mM CHES, 50 mM Me_4NClO_4). Ligand concentrations were 2.5 mM. Observed rates were determined by initial rates analysis and corrected for background. The complex $[ZnBa(CRTPA)]^{4+}$ gave rate enhancements of 1120-fold over the background rate at pH 8.5. The complex $[ZnSr(CRTPA)]^{4+}$ gave a very similar enhancement, while that for $[ZnCa(CRTPA)]^{4+}$ was 1060. Reaction with $[Zn(CRTPA)]^{2+}$ without any second metal gave a rate acceleration of 570, while that of $[Zn(TPA)]^{2+}$ was 340. Reaction with $[Ba(CRTPA)]^{2+}$ gave an acceleration of 150. An evaluation of rate as a function of pH indicated that the reaction mechanism involves a titratable species with pK_a~9.0, slightly higher than the pK_a determined for zinc-bound water in $[Zn(CRTPA)OH_2](ClO_4)_2$ by potentiometric titration (8.0, determined in 50 mM Me_4NClO_4 at 25°C). The maximum rate enhancement with $[ZnBa(CRTPA)]^{4+}$ was observed at pH 9.3 (2800-fold). These data are consistent with a mechanism of hydrolysis involving a zinc-bound hydroxide ion as nucleophile with the second metal ion playing a less important role.

A determination of rate as a function of substrate concentration for the complexes $[Zn(CRTPA)]^{2+}$ and $[ZnBa(CRTPA)]^{4+}$ provided useful information. The dinuclear complex clearly showed curvature with kinetic saturation consistent with $k_{cat} = 5.98 \times 10^{-6}\ s^{-1}$ and $K_M = 14.5$ mM. The $[Zn(CRTPA)]^{2+}$ complex did not saturate under the measurable reaction conditions (solubility limits would preclude observation of saturation with $K_M > 40$ mM). At low substrate concentrations, the dinuclear complex was slightly more efficient than the mononuclear $[Zn(CRTPA)]^{2+}$ complex, but at high substrate concentrations, the latter complex actually showed better rate enhancement. Thus, the Ba^{2+} ion appeared to facilitate the binding of the substrate, but the reaction within the dinuclear complex was not very efficient. This may be a result of the inability of the Ba^{2+} to activate the substrate or, most likely, an indication of a non-ideal geometry in the dinuclear complex, as many dinculear enzymes display shorter intermetal distances.[4]

In summary, we have demonstrated: 1) the successful synthesis of a heterodinucleating ligand by a heterobiaryl coupling strategy; 2) cooperative binding of amino acids with selectivity consistent with geometrical considerations; 3) binding of two different ions at addressed sites with discrimination on the basis of atomic radii; 4) hydrolysis of an activated phosphodiester by N_4-Zn–OH complexes; and 5) cooperation between the two metal ions, although the geometric relationship between the metals appears non-ideal. Further studies with other metal ions and different ligand geometries are underway.[24]

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