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Remote Substituents Influence Both the Thermodynamics and Kinetics of Zinc Binding to Tris-pyridyl Methanol Derivatives

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ABSTRACT

Three families of tris-pyridyl methanol ligands were synthesized. An analysis of the Zn^{2+} binding properties of the ligands revealed that both steric and electronic properties of the pyridine substituents, as well as the nature of the group on the tertiary alcohol oxygen, control the thermodynamics and kinetics of complex formation.

Our current knowledge of the carbonic anhydrase (CA) family of enzymes derives from studies of the enzymes themselves,[1] complementary computational investigations,[2−4] and the synthesis and analysis of ligands designed to mimic the essential $H_2O-Zn(His)_3$ active site.[5−7] On paper, tris-ligands possessing sp^2 nitrogen donors[6,8−14] represent the ideal type of mimic. However, as a result of a highly Lewis acidic zinc ion in the corresponding $[ZnL]^{2+}$ complex, they frequently suffer from an inherent problem not observed in sp^3-hybridized aza-ligands.[7] Thus, the initially formed $[ZnL]^{2+}$ complex undergoes further reaction to form the thermodynamically more stable $[ZnL_2]^{2+}$ complex or hydroxy-bridged dimers.[6,8−16] Without a zinc-bound hydroxide, these complexes cannot act as CA mimics. To circumnavigate this problem, steric barriers around the metal center have been introduced to many ligands. However, in addition to inhibiting these deleterious processes, such barriers can also inhibit

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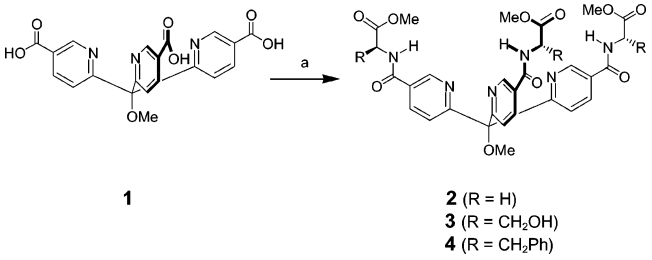
substrate approach. Consequently, with hydrolyase catalysts in mind, efforts have turned toward shielding the zinc ion but at the same time providing it with enough space for coordination changes[14] and substrate complexation. Thus, both calixarenes[10] and dendrimers[11] have been used to place the "active site" at the base of a large concave surface. Such complexes will provide valuable information about how the binding pocket influences the properties of CA. These considerable advances aside, there remains a dearth of information regarding what controls the interplay between the 1:1 and 2:1 complexes of tris-sp^2−N ligands. Toward a better understanding of tris-pyridyl methane ligands, we report here on the binding of three families of ligands. Our results demonstrate that the relationship between the different complexes is a rich and subtle one and suggest that it may ultimately be possible to use these types of ligands as both successful CA mimics and catalysts.

Tris-(2-nicotinic acid)methanol methyl ether **1**[17] was chosen as a starting point to investigate how structure and solvent affected zinc complexation. Use of standard peptide bond-forming technologies afforded the tris-glycine, tris-serine, and tris-phenylalanine derivatives **2**−**4** in good to excellent yield (Scheme 1). Ligands **2** and **4** offer minimal

Scheme 1. Synthesis of Ligands **2**−**4**[a]

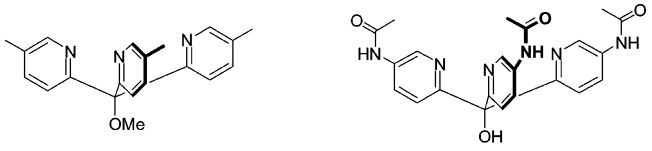


1

2 (R = H)
3 (R = CH_2OH)
4 (R = CH_2Ph)

[a] Conditions: (a) HBTU, Et_3N, DMF, respective amino acid methyl esters, 65−95%.

and maximal amounts of steric protection, respectively, while ligand **3** has two sides to its character: it offers some steric protection against the formation of $[ZnL_2]^{2+}$ complexes while also promoting the possibility of $[ZnL_2]^{2+}$ formation via hydrogen bonding. We used ^1H NMR to examine 1:1 mixtures of these ligands and zinc perchlorate, in a number of pure solvents. As a reference, we used the tris-(3-picoline) methanol methyl ether **5** (Figure 1).[17]



5 **22**

Figure 1. Structures of ligands **5** and **22**.

The equilibrium distribution of species, in three different solvents, is shown in Table 1. These results demonstrate that

Table 1. Equilibrium Distribution (25 °C) for 1:1 Mixtures of Zn^{2+} and the Ligands (Free L:ZnL:ZnL_2)[a]

ligand	DMSO	acetonitrile	D_2O
2	c	0:82:18	70:30:0
3	c	0:67:33	74:26:0
4	c	0:85:15	d
5	77:23:0	0:67:33	e
6	0:80:20	0:69:31	0:0:100
9	c	0:76:24	18:78:4
10	c	0:71:29	22:72:6
11	c	0:80:20	d
18	c	0:80:20[f]	0:53:47
20	c	0:86:24[f]	d
21	c	0:70:30[f]	0:59:41

[a] Typical procedure: 200 μL of a 5 mM stock solution of ligand was added to an NMR tube. To this was added 1 equiv of a 20 mM $ZnClO_4$ stock solution, and the sample was diluted to 1 mM complex. Each determination was repeated three times using fresh stock solutions. [b] Pyridine H-6 signal was used to determine the ratio of products (see Supporting Information and refs 11 and 15). [c] No binding was observed. [d] Ligand was insufficiently soluble in water. [e] Precipitate formed upon mixing **5** and Zn^{2+}. [f] D_2O (10%) was added as a cosolvent.

a combination of electron-withdrawing substituents on the pyridine rings and strongly donating solvents such as DMSO inhibit the binding of zinc to ligands **2**−**4**. In contrast, ligand **5**, with its mildly electron-donating methyl groups, binds zinc, albeit rather weakly. In less competitive solvents such as acetonitrile, binding is stronger and no free ligand is observed. Of the amino acid derivatives, the serine species forms the most $[ZnL_2]^{2+}$, suggesting that in acetonitrile, hydrogen bonding can stabilize the $[ZnL_2]^{2+}$ species. The similar complexation properties of **2** and **4** suggests that the phenylalanine side chain does not inhibit formation of the $[ZnL_2]^{2+}$ complex.

From an enzyme-mimicking point of view, water is the most relevant of solvents. In aqueous solution, only ligands **2** and **3** gave a homogeneous solution. The composition of these solutions was as expected considering the intermediate donating ability of water relative to DMSO and acetonitrile. Thus, binding was weak, with the predominant species in the mixture being free ligand.

These data suggest that the formation of ZnL_2 complexes can in part be controlled by limited shielding. However, zinc binding to ligands **2** and **3** in water is quite weak. Why is this the case? We hypothesized that two factors in the complex were important: (1) a steric interaction between the H-3 on each pyridine ring and the MeO methyl group and (2) the electron-withdrawing groups on the pyridine rings.

To test the idea that the remote methyl group diminishes binding, we examined the properties of **5** and demethylated derivative **6** (Scheme 2).[18] In DMSO, the latter complexed

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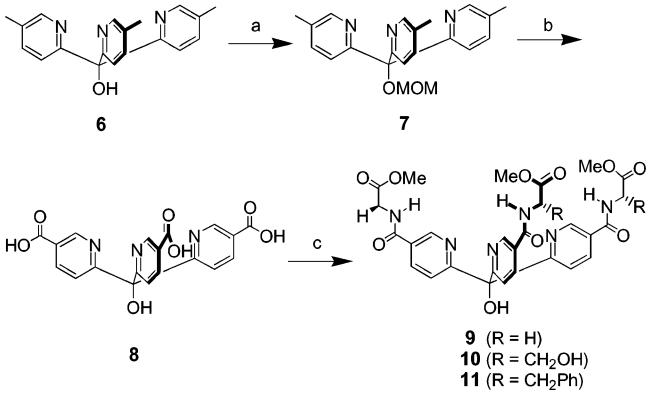
Scheme 2. Synthesis of Ligands **9**−**11**[a]



[a] Conditions: (a) NaH, MOMBr, THF, 98%; (b) $KMnO_4$, NaOH, H_2O, 60 °C, 16 h, then 20% aq HCl 85%; (c) HBTU, Et_3N, DMF, respective amino acid methyl esters, 60−88%.

Zn^{2+} more strongly, but the ligands were indistinguishable in acetonitrile. With these mixed results in hand, we synthesized **9**−**11** (Scheme 2). The required **8** could not be isolated in good yield by the oxidation of previously reported **6**.[17] Thus, **6** was protected as its MOM ether **7**, which was oxidized with $KMnO_4$, and deprotected to yield **8**. Coupling reactions gave "second-generation" ligands **9**−**11** in good to excellent yield.

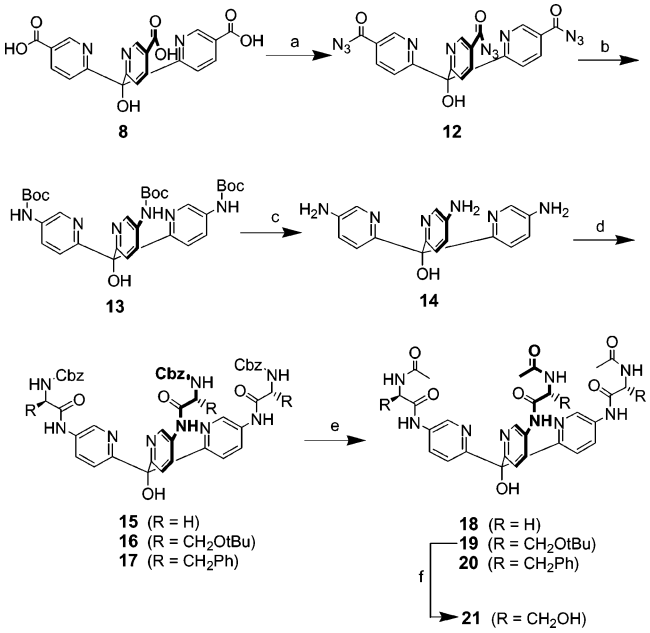
In DMSO and acetonitrile, the binding properties of **9**−**11** were similar to those of ligands **2**−**4**. Gratifyingly, however, Zn^{2+} binding was noted to be stronger in water. A comparison of the binding constants of **2** and **9** reveals a 40-fold increase by simply removing the methoxy methyl group (6.0×10^2 and 2.4×10^4 M^{-1} for ligands **2** and **9**, respectively).

To investigate the electronic effects of the substituents we designed a third family of ligands with electron-donating groups (Scheme 3). Thus, **8** was treated with DPPA in THF to afford tris-azide **12**. Curtius rearrangement of **12** by refluxing in dry *t*-BuOH gave tris-BOC **13** in good yield. Attempts to convert **8** to **13** directly by reflux in *t*-BuOH in the presence of DPPA were unsuccessful. Free amine **14** was then obtained in high yield by acid hydrolysis of **13**. No reactions occurred between **13** and *N*-acetyl-protected amino acids. However, coupling with N-Cbz-protected amino acids went smoothly to afford ligands **15**−**17** in high yield. The Cbz groups were then removed by hydrogenation, and the amine intermediates reacted with acetic anhydride to give tris-acetates **18**−**20**. Removal of the *t*-Bu group on **19** with TFA afforded ligand **21**.

As expected, in water the new generation of ligands complexed zinc ion more strongly (Table 1). Thus, no free Zn^{2+} was present upon mixing ligand and metal ion.

Interestingly, the kinetics of complex formation also differed for these ligands. Thus, whereas ligands **2**−**4** and

Scheme 3. Synthesis of Ligands **18**, **20**, and **21**[a]



[a] Conditions: (a) DPPA, Et_3N, THF, 75%; (b) *t*-BuOH, reflux, 80%; (c) 20% aq HCl, 90%; (d) HBTU, HOBT, Et_3N, DMF, respective N-Cbz amino acids, 45 °C, 70−75%; (e) H_2, Pd/C, acetic anhydride, MeOH, 80%; (f) TFA, 65%.

9−**11** gave equilibrated mixtures in a matter of minutes, approximately 9 h were required for ligands **18** and **22**. In these cases, the amount of the $[ZnL]^{2+}$ complex decreased slowly over this time period to hold constant at around 50−60% (Figure 2), a decrease that was mirrored by a corresponding increase in the proportion of $[ZnL_2]^{2+}$ sandwich complex (see Supporting Information). To examine this kinetic phenomenon further, the less sterically hindered

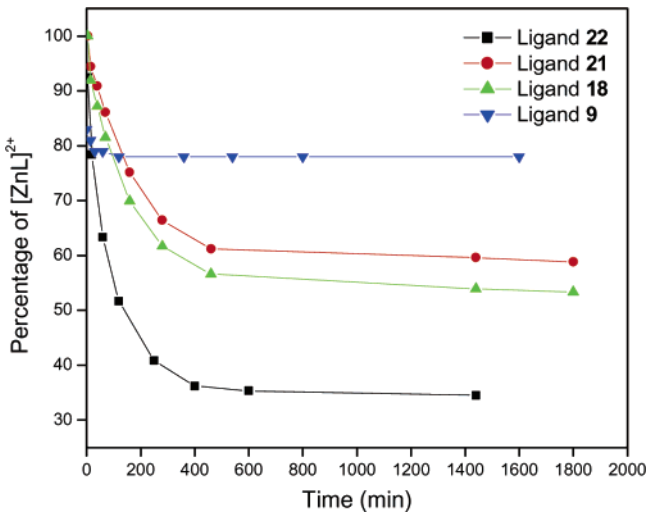


Figure 2. Change of the percentages of $[ZnL]^{2+}$ in aqueous solution as a function of time.

(18) We are unaware of previous work documenting that alkylation of the alcohol group in Pyr_3COH changes the binding constant of metal ion binding. However, it is well documented that methylation inhibits the N,N′,O coordination that is often displayed by Pyr_3COH. See for example: Szalda, D. J.; Keene, F. R. *Inorg. Chem.* **1996**, *25*, 2795−2799.

ligand **22** (Figure 1) was synthesized by N-acylation of tris-amine **14**. For this ligand, the kinetics of equilibration between $[ZnL]^{2+}$ and $[ZnL_2]^{2+}$ were similar to ligands **18** and **21**. However, devoid of any steric shielding, the corresponding equilibrated mixture contained a much larger proportion of the $[ZnL_2]^{2+}$ sandwich complex. Thus, for these ligands, relatively small steric barriers influence the position of the equilibrium between $[ZnL]^{2+}$ and $[ZnL_2]^{2+}$, whereas the electronic properties of the substitutents influence both this equilibrium and the kinetics of complexation.

In summary, our investigations of the zinc ion binding properties of a series of tris-pyridyl methanol ligands demonstrate a deep and complex relationship between the different electronic and structure features of these ligands and the species they form in solution. The richness of the physicochemical properties of these ligands suggests avenues for the development of novel catalysts modeled on CA. Current studies are focusing on the catalytic properties of these ligands, as well as the synthesis of other tris-pyridyl ligands. These results will be reported in due course.

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Supporting Information Available: Experimental details for the synthesis and characterization of the products, representative NMR and MS spectra of complex mixtures, and titration data. This material is available free of charge via the Internet at http://pubs.acs.org.

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