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Bifunctional Cyclodextrin Metalloenzyme Mimics

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Abstract: A variety of regioisomeric bifunctionalized β-cyclodextrins derivatized with an imidazole and a metal ligand tris(2-aminoethyl)amine (tren) group were prepared. As their Zn^{2+} complexes, these compounds show bifunctional catalysis of phosphate hydrolysis with a regioisomeric preference.

Keywords: tren, zinc, phosphate hydrolysis

Many enzymes employ both a metal ion and a basic group to carry out bifunctional catalysis. In model systems, we have previously shown that Zn^{2+} in imidazole buffer performs cooperative bifunctionally catalyzed hydrolysis of the RNA analog *p*-nitrophenyl hydroxypropyl phosphate and of 3',5'-uridyluridine.[1] Zn^{2+} acts as a Lewis acid, coordinating and stabilizing the phosphate oxyanion, while imidazole participates in catalysis as a general base. We also synthesized macrocyclic metal ligands carrying rigidly oriented auxiliary general bases such as thiophenoxide or imidazole groups that could not coordinate to the bound zinc.[2] These catalysts displayed pH vs. rate behavior consistent with bifunctional catalysis of hydrolysis and were 10-20 times more effective than the simple macrocycle without auxiliary groups. Related bifunctional compounds catalyze the hydrolysis of small RNA oligomers.[3] Cyclodextrin derivatives with metal ions coordinated to attached metal binding groups have also been shown to be effective hydrolytic catalysts.[4] We have now improved the catalytic activity of such metallocyclodextrins by installing an additional imidazolyl group to act as a general base.

To explore the geometric preferences, we prepared all possible regioisomers[5] of catalysts in which a β-cyclodextrin carries one tris(2-aminoethyl)amine (tren) group attached to C-6 of a glucose residue of the cyclodextrin by a primary amino group, and also an imidazole group N-linked to C-6 of another glucose residue (compounds **7-10**). As substrate we used 4-*tert*-butylcatechol cyclic phosphate **15** (Scheme 1), which binds into the cyclodextrin cavity and whose catalytic hydrolysis we had previously examined using cyclodextrins carrying only imidazole base and acid (imidazolium) catalytic groups.[6-9]

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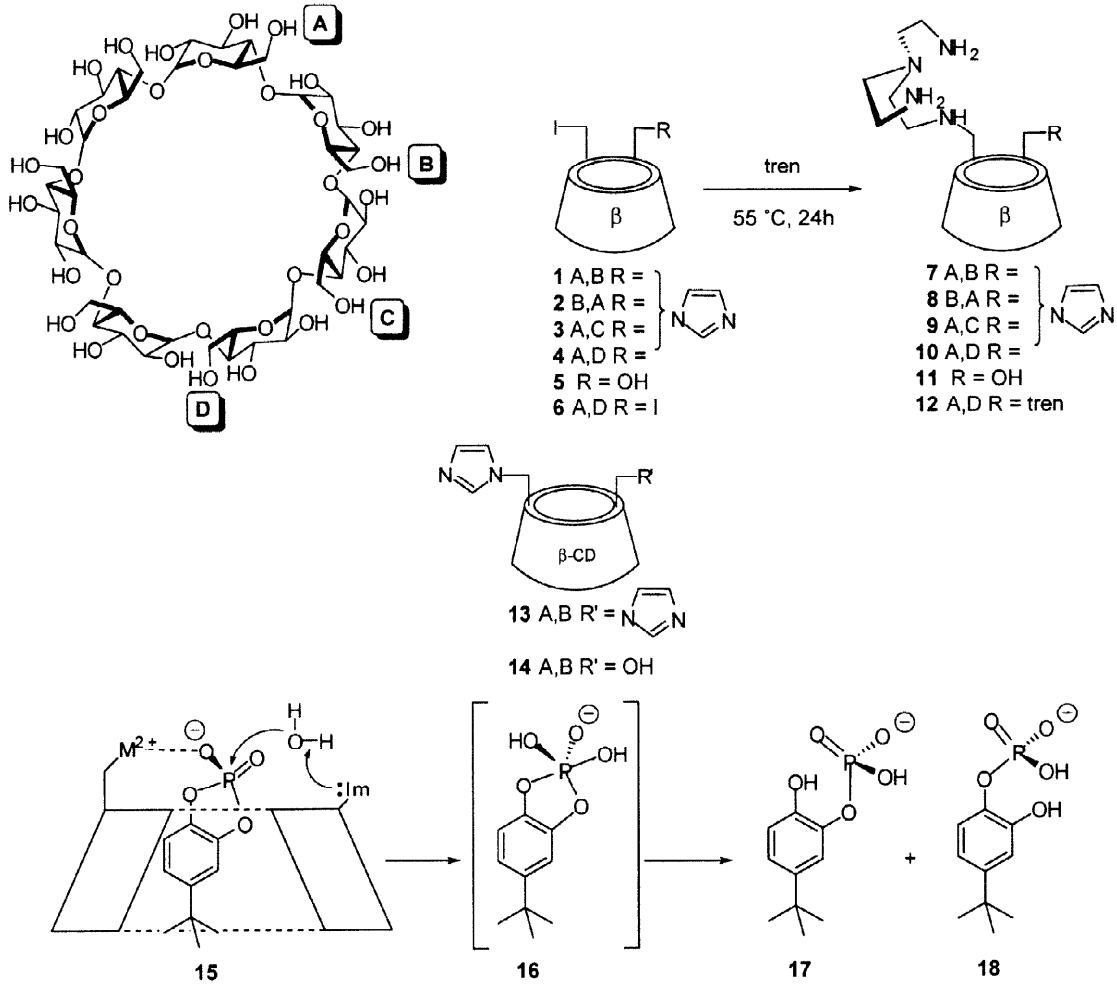
The bifunctionalized cyclodextrins were prepared by capping β-cyclodextrin with appropriately spaced disulfonate capping reagents,[10,11] followed by treatment with NaI and then imidazole to yield the mono-iodo,mono-imidazolyl-β-cyclodextrins **1-4**. As previously reported,[12] we separated the two A,B-iodo,imidazolyl isomers **1** and **2**, while **3** and **4** remained a mixture of the regioisomers (A,C and A,F for **3**; A,D and A,E for **4**). The A,B-, B,A-, A,C- and A,D-tren,imidazolyl-cyclodextrin catalysts **7-10** were prepared by treating the mono-iodo precursors with neat tren (Scheme 1).[13] Mono-tren **11** and A,D-bis-tren-β-cyclodextrin **12** were prepared from mono-iodide **5** and A,D-diiodide **6**.[13-15] Lincoln *et al.*[14] have shown that simple mono-tren derivatized β-cyclodextrin **11** has a high affinity for Zn^{2+} ($K_f = 10^{12}$ M^{-1}). We saw that ^1H NMR titration of A,D-tren,imidazolyl **10** with $ZnCl_2$ resulted in down field shifting of imidazole and tren group protons.



Scheme 1.

From the pH-rate profiles in Figure 1, we observe that the rate of hydrolysis of cyclic phosphate **15** catalyzed by A,D-tren,imidazolyl **10** is fastest when the tren group carries a bound Zn^{2+} (Figure 1A) and when the catalyst carries an imidazole group as well as a tren-Zn^{2+} (Figure 1B). As expected, there is a plateau in the region of pH 7-8 in the

plots of Figure 1 where the imidazole group is acting as the base catalyst, while above this pH region OH^- takes over as the base/nucleophile. At pH 8.5 the catalyst with Zn^{2+} is an order of magnitude faster than the catalyst without zinc (Figure 1A). B,A-tren,imidazolyl **8** is 67 times more effective in the presence of zinc than in its absence (Table 1). A,D-tren,imidazolyl **10** with zinc is approximately 10 fold better than mono-tren **11** with zinc in the pH range 7.0-8.5 (Figure 1B). B,A-tren,imidazolyl **8** is 4 fold better at pH 8.5 than the mono-tren compound **11**. Reactions performed with the mono-tren- or mono-imidazolyl-β-cyclodextrin **11** or **14** in the presence of an equivalent of free *N*-methylimidazole (NMI) or tren were 3 and 300 times less effective than **8**, respectively (Table 1). A,D-bis-tren-β-cyclodextrin **12** is twice as effective as mono-tren-β-cyclodextrin **11** but **12** is a poorer catalyst than is B,A-tren,imidazolyl-β-cyclodextrin **8**.

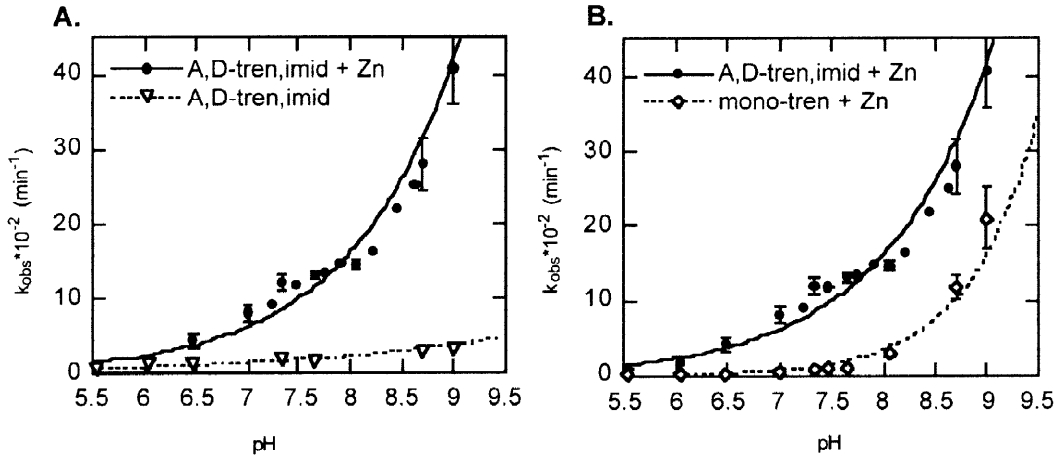


Figure 1. Observed pseudo-first order rate constants for the hydrolysis of catechol cyclic phosphate **15** by (A) A,D-tren,imidazolyl **10** with (●) and without (▽) Zn^{2+} and by (B) A,D-tren,imidazolyl **10** (●) and mono-tren **11** (◇) with Zn^{2+} as a function of pH. Arbitrary curves are fit to the data, which actually show plateaus in the pH 7-8 region. (10 mM MES, HEPES, CHES, CAPS buffer, 1.0 mM catalyst, 1.0 mM $Zn(ClO_4)_2$, 0.2 mM substrate).

We examined the different isomers of the tren,imidazole catalysts to see if there was any geometric preference (Table 1). B,A-tren,imidazolyl **8** is the most effective catalyst at pH 8.5 with a rate acceleration 937 fold better than β-cyclodextrin. B,A-tren,imidazolyl **8** is 2 fold better than the A,C- and A,D-isomers **9** and **10**. This is the same general geometric preference observed with the previously reported RNase A mimic A,B-bis-imidazolyl **13** and suggests that a mechanism involving pentacoordinated phosphorane intermediate **16** may be involved (Scheme 1). At pH 8.5, B,A-tren,imidazolyl **8** is 80 fold better than A,B-bis-imidazolyl **13**.[7] Intriguingly, A,B-tren,imidazolyl **7** is 7 times less effective than the B,A regioisomer **8**. This result speaks to a definite orientational preference for the general base group to reside at the A position and the general acid at the B position. All bifunctionalized catalysts favored formation of the C-2 phosphate hydrolysis product **17** over the isomer **18**, as with other catalysts.[6-9]

Table 1.

Entry	Catalyst	$[Zn^{2+}]$ (mM)	$k_{obs} * 10^3$ (min-1)[a]	k_{obs}/k_{back}[b]
1	**8**, BA-tren,imid	5	93.4 ± 10.5	937
2	**8**, BA-tren,imid	0	1.35	14
3	**7**, AB-tren,imid	5	14.0 ± 0.8	141
4	**9**, AC-tren,imid	5	55.7 ± 4.6	558
5	**10**, AD-tren,imid	5	59.2 ± 3.3	594
6	**12**, AD-bis-tren	10	46.8 ± 7.0	470
7	**11**, mono-tren	5	23.3 ± 1.2	233
8	**11**, monotren+NMI[c]	5	27.3	274
9	**13**, AB-bis-imid	5	1.17 ± 0.30	12
10	**14**, monoimid+tren[d]	5	0.277 ± 0.030	3
11	β-CD	0	0.0997 ± 0.0058	1

a. Pseudo-first order rate constants for the hydrolysis of **15**. The progress of the hydrolysis was monitored by UV by following product formation at 290 nm. The pseudo-first order rate constants (k_{obs}) for the reaction within the complex were determined by initial rate analysis. (50 mM pH 8.5 EPPS buffer, 5.0 mM catalyst, 5.0-25.0 mM $Zn(ClO4)2$ and 1.0 mM **15** in 1% DMSO, 25 °C). b. Relative to entry 11. c. 5.0 mM N-methylimidazole. d. 5.0 mM.

There are few examples of bifunctionalized cyclodextrins such as **7-10** containing two different catalytic groups. The new compounds reported here display cooperative bifunctional catalysis involving specifically located metal and imidazole groups. Such bifunctionalized compounds show promise in the catalysis of other hydrolytic reactions and may have potential in enantioselective substrate recognition.

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