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SYNTHESIS AND TRANSACYLATING ACTIVITY OF ISOMERIC Co(III)-CYCLODEXTRIN ARTIFICIAL METALLOENZYMES

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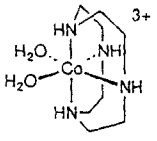
Although the cyclen–Co(III) complex has exhibited amongst the greatest rate accelerations in acyl and phosphoryl transfer reactions, this catalytic unit has not been used previously in the design of an artificial metalloenzyme. For this study, β-cyclodextrin derivatives of cyclen–Co(III) with attachments to the primary and secondary sides of the cyclodextrin torus were synthesized. The primary-side cyclodextrin–cyclen–Co(III) conjugate accelerates the hydrolysis of *p*-nitrophenylacetate by a factor of 1000 (pH 7·0, 25 °C) in comparison with the water-catalyzed reaction. Maximum reactivity occurs at pH 7, consistent with the known pK_a values and hypothesised mechanism of action of Co(III) complexes. The secondary-side cyclodextrin–cyclen–Co(III) conjugate is less reactive towards *p*-nitrophenylacetate hydrolysis under saturating conditions. Reactivities towards an azide, a phosphonate and a phosphate triester were in each case less than five times greater than the buffer-catalyzed rate.

INTRODUCTION

Metal ions can catalyze a variety of reaction types, either by super-acid catalysis (including metal ion-bound hydroxide mechanisms) that has a directional or template effect, or by acting as a carrier of electrons in the catalysis of redox reactions. These properties of metal ions are exploited by enzymes and other proteins. Metalloenzymes are often involved in biochemical acyl, and always in phosphoryl, transfer reactions. Most of the enzymes which act on nucleic acids or nucleotides require a divalent metal ion (typically Zn^{2+} or Mg^{2+}) for optimum activity. Whereas enzymes demonstrate such desirable properties as large rate enhancements, substrate selectivity, activity under neutral aqueous conditions and turnover behavior, virtually all enzyme mimics studied to date succeed in mimicking only one or two of these properties at a time. The compounds described in this paper are no exception, but we are encouraged by their unique activity under neutral conditions.

One way of improving the specificity of metal-catalyzed processes is to attach the reactive metal ion center to a molecule that can selectively bind substrate molecules. Cyclodextrins (CDs) have been used for this purpose, as they have a hydrophobic cavity and can form inclusion complexes. Also, these cyclic oligomers of glucose can be modified on both primary and secondary sides, making them versatile molecules for this purpose. CD-based metalloenzyme mimics have been reported to catalyze carbon dioxide hydration,[1] phophotriester hydrolysis,[2] activated ester hydrolysis,[3] furoin oxidation[4] and decarboxylation.[5] The cyclen–Co(III) complex **1** has exhibited amongst the greatest rate accelerations in acyl and phosphoryl transfer reactions; accordingly, we have synthesized two cyclen–Co(III) complexes positioned alternately on the primary and secondary sides of β-CD.[6]



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RESULTS AND DISCUSSION

Synthesis

6-Deoxy-6-monotosyl-β-CD is a useful intermediate for functionalizing β-CD on its primary side. The tosylation reaction has been carried out both in dry pyridine[7] and in a biphasic mixture of diethyl ether and 0·1 M aqueous NaOH solution.[8] In both procedures, the reaction yields a mixture of monotosylate (major product), ditosylates and unreacted β-CD. In our hands, the first method results in better yields. Repeated recrystallizations from warm water (60 °C) did not remove the ditosylate impurity completely. Therefore, this tosylate mixture was reacted with cyclen (1,4,7,10-tetraazacyclododecane; **3**), prepared by following the literature procedures[9] with some modifications. A

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similar reaction of β-CD monotosylate with cyclam (1,4,7,11-tetraazacyclotetradecane) has been reported previously,[10] but without experimental detail. Purification of the 6-cyclenyl derivative (**4**) was accomplished using CM-Sephadex ion-exchange chromatography, eluting with an NH_4HCO_3 linear gradient. The ditosylate impurity in the 'monotosylate' sample, when reacted with an excess of cyclen, will form a dicyclenyl–CD that can be separated easily from the monosubstituted product. The pK_a values of cyclen are >1, 1·73, 9·7 and 10·7.[10] Therefore we predict the monocyclen compound to be +2 charged and the dicyclen compound to be +4 charged at pH 7. A solid sample of 6-deoxy-6-cyclenyl-β-CD was obtained after lyophilization, whose NMR and mass spectra and microanalytical characterization were supportive of the structure assignment.

When β-CD is treated with *m*-nitrophenyl tosylate at pH 10, the isolated organic product is almost exclusively the 3-deoxy-3-tosyl derivative.[12a] On heating with NH_4HCO_3 in aqueous solution, the mannoepoxide **8** forms. This has been the most useful synthetic intermediate for the secondary side derivatization (alternative, higher yielding syntheses of the secondary side tosylate have been reported[13]). The literature procedure affords an epoxide that is >50% salt by weight, which has been used successfully for further reactions.[12b] We have effected a 'desalting' of the CD-epoxide by passing an aqueous solution through an Amberlite MB-3 column (a mixture of cation- and anion-exchange resins). Lyophilization afforded a fluffy white product that was show to be 'salt free' by elemental analysis. Epoxide **8** is not as reactive as the primary-side tosylate, probably for steric reasons; nucleophiles must approach from the inside of the CD cavity to open the epoxide ring. When **8** was reacted with excess cyclen at 100–110 °C, substitution took place together with some hydrolytic opening of the epoxide ring. As with the primary-side derivative, compound **9** was purified by ion-exchange chromatography. Again, the NMR and mass spectra and microanalysis data are supportive of the structure assignment.

The preparations of various Co(III) complexes are documented in the literature.[14,15] Our target Co(III) complexes were the diaqua forms. These complexes have been prepared mainly via two routes: by conversion of the dinitro complex to the dichloro complex followed by hydrolysis, or by preparation of the carbonate complex followed by direct hydrolysis. Carbonate complexes treated with concentrated acids bearing non-nucleophilic counterions can be converted in the diaqua complexes directly. However, reactions under strongly acidic conditions are sometimes not applicable to the preparation of CD derivatives because the glycosidic linkages are hydrolyzed with strong acids, especially when heated.[16]

We first reproduced the complex formation reaction using cyclen itself. As a stable source of Co(III) with labile ligands, sodium triscarbonatocobaltate(III) was prepared by oxidizing Co(II) with H_2O_2 in the presence of $NaHCO_3$.[17] The olive-green complex is stable for a few weeks if properly kept dry. This complex, when reacted with cyclen hydrochloride (as described for the analogous cyclam complex[18]), gave the carbonate complex as pink microcrystals. The same reaction was then repeated using **4**, affording carbonato complex **5** in 89% yield. The literature procedure for conversion of the cyclam–carbonato complex to the dichloro complex suggests heating for 1 h on a steam-bath.[18] We have found that 5 min at 65 °C is sufficient to complete the conversion of the carbonato complexes into the corresponding dichloro complexes. As with the cyclen–carbonato complex, a suspension of **5** in methanolic HCl, when heated at 60–65 °C for 5 mins, forms the dichloro complex (**6**). Lyophilization affords **6** as a pink fluffy solid. The UV spectra of the cyclenyl and primary-CD–cyclenyl dichloro complexes were identical at $\lambda > 300$ nm (see Table 1). In addition, the fast atom bombardment (FAB) mass spectrum and the elemental analysis were consistent with the structure assignment (**6**). The dichloro complex is unstable in aqueous solutions, hydrolyzing to the monoaquachloro complex. Dichloro complex **6** was therefore isolated as a hydroscopic purple powder.

The aqua complex of cyclen–Co(III) (i.e., **1**) was obtained by hydrolysis of the dichloro complex; this was achieved simply by passing an aqueous solution of the dichloro complex through a strong anion exchange column followed by acidification and then trituration using diethyl ether. We found that Dowex resin (OH^- form) works very well for the cyclen compound, but that β-CD interacts strongly with the resin (inclusion of the aromatic portion of the resin is a possibility); in fact, no CD derivative could be eluded from the ion-exchange column. We therefore switched to a Sephadex resin (QAE-Sephadex) and were able to obtain the hydrolysis product as a solid material; however, the UV spectrum was different from that of the non-CD complex. In order to show that decomposition had not occurred during the ion-exchange process, another method of conversion to the aqua complex was examined. The carbonato–Co(III) complexes of the

Table 1. UV data ($\lambda_{max} \pm 1$ nm of the Co(III) complexes of cyclen derivatives in water

Ligand	Non-CD	Primary-CD	Secondary-CD
Carbonato	530	530	531
Dichloro	560	562	— [a]
(Di)aqua	504	524	508
Aquahydroxo	522	534	522

[a] The secondary-side Co(III) dichloro complex hydrolyses rapidly to the diaqua complex.

tetraamine ligands hydrolyze almost immediately to the aqua complexes when treated with acids. The color change due to hydrolysis is apparent and can be also followed by UV spectrophotometry. As expected, on acidification the cyclen–carbonato complex changes color immediately. However, the same color change is not observed for the CD derivative. The UV spectra indicate a hydrolysis-induced spectral shift only to 524 nm. Since no other potential external ligand exists in the solution that is not also available to the parent complex, we assign structure **6** to the compound obtained in this way. We postulate that one of the primary hydroxyl groups of an adjacent glucose unit is involved in coordination to the Co(III) cation. In fact, coordination to CD by metal ions has been shown previously for Mn^{3+} and Cu^{2+},[19] and recently for Co(III) itself.[20]

With some modifications, the yield of **6** was improved. It was found that the final ion exchange can be avoided altogether if the carbonato complex has

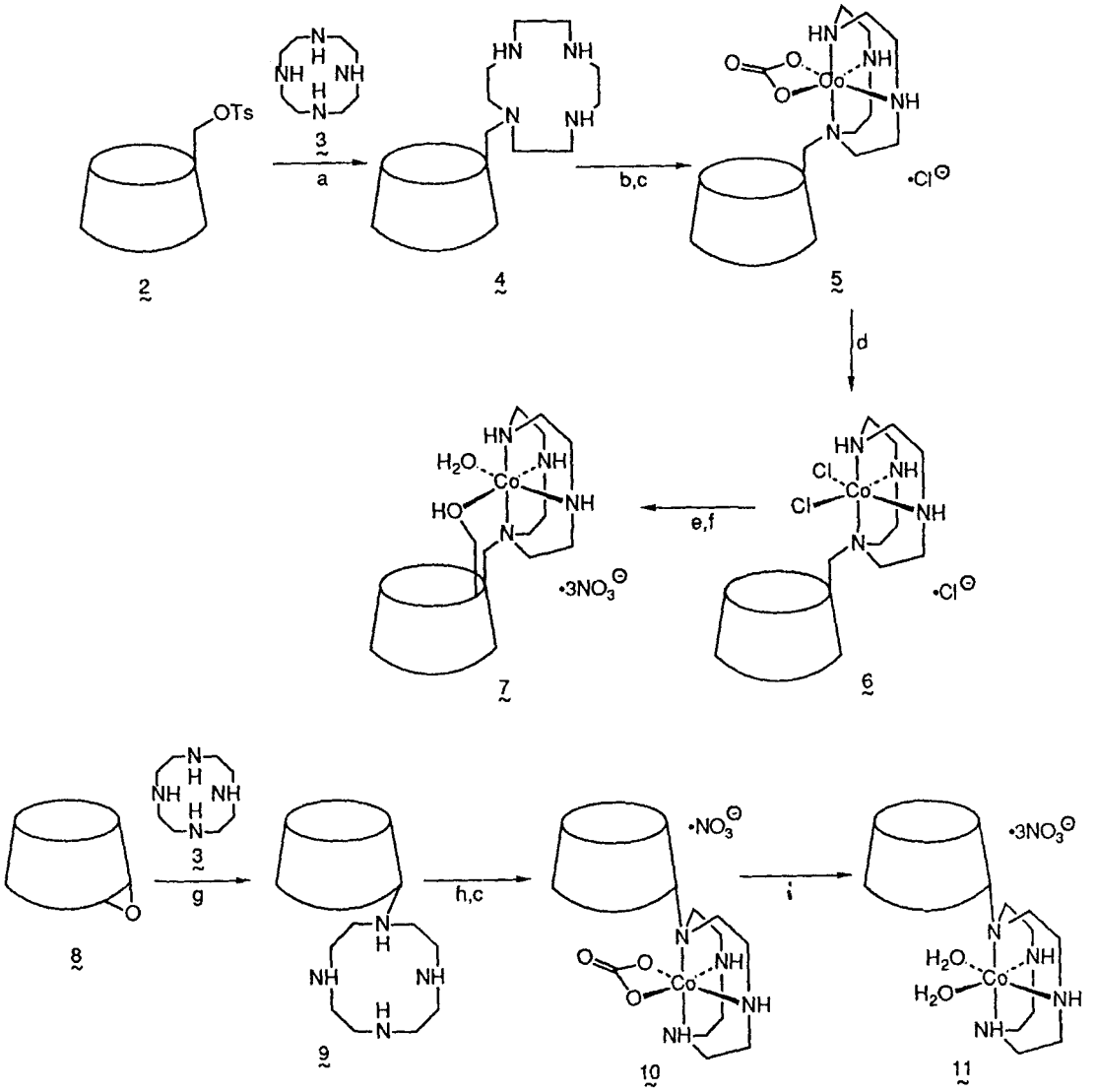


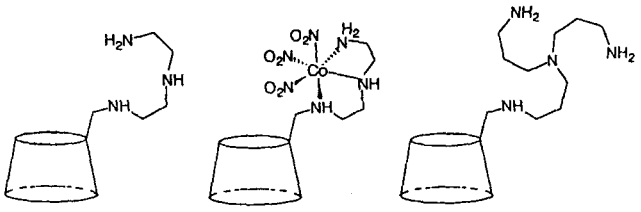
Figure 1. Synthesis of CD–cyclen–Co(III) complexes. (a) DMF, 80 °C, CM-Sephadex (HCO_3^- form); (b) aq. HCl; (c) $Na_3[Co(CO_3)_3]$; (d) HCl–MeOH; (e) QAE-Sephadex (OH^- form); (f) acidification (HNO_3); (g) DMF, 105 °C; (h) aq. HNO_3; (i) methanolic HNO_3

nitrate or perchlorate as the counterion simply by warming an acidic (acidified with HNO_3 or $HClO_4$) solution in MeOH for 5 min; conversion to the aqua complex is achieved efficiently. By this method we were able to prepare 0·8 g of the primary-side complex.

Preparation of the Co(III) complex with the corresponding secondary-side cyclen derivative was achieved similarly, although with an important modification (Figure 1). Carbonato complex **10** can be prepared from the corresponding triscarbonato complex exactly as described for the primary-side complex. MeOH–HCl treatment, however, produced some interesting results. The dichloro compound is purple, but reaction of the secondary-side derivative produced instead a bright red product. In methanol, the dichloro compound is stable, but when dissolved in water it immediately changes color. The λ_{max} of the resulting species was found to be 504 nm, the same as for the diaqua complex. In fact, it was observed that when an attempt was made to collect the solid purple dichlorides complex by filtration, the color changed from purple to red; the solid seemed to be hydroscopic and it hydrolyzed as it absorbed water from the air. Larger amounts of the secondary-side aqua complex were prepared by direct hydrolysis of the carbonato complex in MeOH–HNO_3. More importantly, the reactivities of secondary-side aqua samples differ, depending on the method of isolation.

Our first communication of this work[6] noted that the secondary-side complex obtained by hydrolysis of the dichloro complex, followed by hydrolysis on a QAE-Sephadex column, was unreactive towards activated ester and carbonate substrates. We now report that QAE-Sepahadex chromatography itself, by an as yet undetermined reaction, results in the reactivity loss. Alternatively, direct hydrolysis of the carbonato nitrate complex with methanolic $HN0_3$ affords a sample that is spectroscopically identical with, but more reactive than, the QAE-Sephadex-treated material. Thus, the kinetic results reported in this paper are those obtained using the new synthesis method. This effect is not observed for the primary-side complex.

In an attempt to prepare a primary-CD–Co(III) complex with at least two aqua ligands coordinated to the Co(III) center (as required for catalytic activity in phosphate hydrolysis[21]), primary-dien–CD (**12**) was synthesized by the reaction of dien (diethylenetriamine) with primary-CD–tosylate. It was thought that since the Co(III) complex of dien bears three water ligands, even if one of the H_2O molecules was displaced by a CD hydroxyl group there would be two more labile ligands as required to form the cyclic phosphacohalt intermediate. We have been successful in preparing the dien–Co(III) complex, although only in the trinitro form (i.e. **13**). We have found that the parent trinitro complex can be converted into the trischloro complex only under strongly acidic conditions. Therefore, although **13** was prepared successfully, conversion



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attempts with **13** failed because significant acidic hydrolysis of the CD took place.

As part of this work, we also prepared the primary-CD derivative of tris(3-aminopropyl)amine (TRPN). Co(III) complexes of this ligand have been studied recently and found to have exceptional reactivity towards phosphodiesters such as bis-*p*-nitrophenylphosphate.[22] The polyamine ligand TRPN was synthesized using literature procedures.[23] Primary-TRPN–CD (**14**) was then synthesized and characterized successfully. Two different attempts were made to prepare a Co(III) complex, but each failed. In related work, we have likewise found that anthrylmethyl substitution on TRPN results in an inability to form Co(III) complexes. It appears that when the amine is substituted, our complexes cannot form. Hence, although cyclen as a ligand does not afford Co(III) complexes of maximum activity, it seems to be (at present) the optimum compromise between activity and the ability to synthesize the Co(III) complex in the first place.

Circular dichroism studies

Additional support for our structural assignment came from spectropolarimetric work done with complexes **7** and **11**. The primary-side complex showed one positive peak at 19 500 cm^{-1} in the first d–d absorption band region, whereas the secondary-side complex showed three peaks in the same region (Figure 2). As expected, circular dichroism contributions due to the cyclodextrin unit on the primary side, although mainly lined with achiral methylene carbons, are larger as the Co(III) center is fixed at a shorter distance from the CD by hydroxymethyl coordination. The observed secondary-side induced circular dichroism is less, suggesting the absence of a direct coordination of Co(III) center to the CD. It is also interesting that the circular dichroism spectrum for **11** is very similar to the published[20] spectrum of Co(III)en_2 complexed to the CD secondary side, CD supplying two vicinal hydroxyls of a glucose residue as a bidentate ligand.

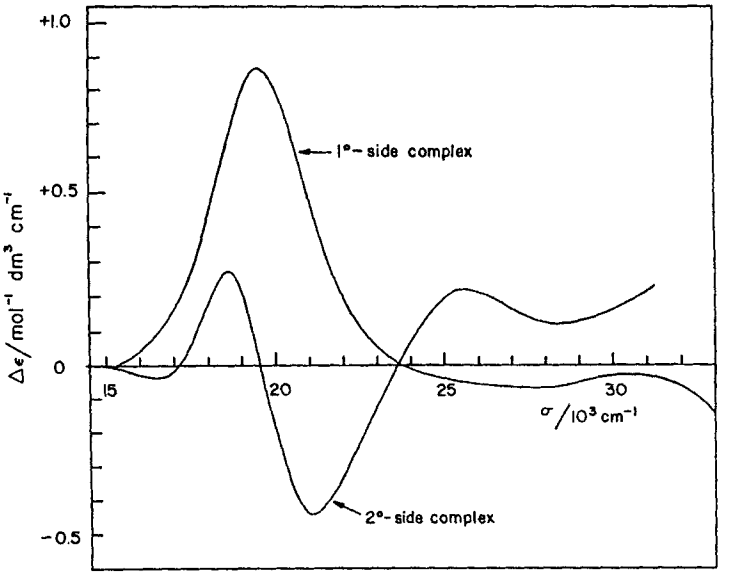

Figure 2. Circular dichroism spectra of primary and secondary side complexes

Reactions with ester, carbonate, amide and phosphate substrates

The reactivity of compounds **7** and **11** was evaluated with the activated substrates shown in Figure 3: *p*-nitrophenylacetate (PNPA), bis-*p*-nitrophenylcarbonate (BPNPC), *p*-nitrotrifluoroacetanilide (PNTFAA), bis-*p*-nitrophenylphenylphosphonate (BPNPPP) and bis-*p*-nitrophenylethylphosphate (BPNPEP). Although activated substrates may not be good surrogates for the more interesting unactivated varieties, it is unlikely that an enzyme mimic inactive towards these reactants will prove active towards, e.g., an ethyl ester. Because the reactions of *p*-nitrophenyl substrates are easy to monitor experimentally, and because they do provide a yardstick for comparison against previously published work, we employed them in the current study.

As shown in Table 2, the transacylation reaction of PNPA bound to **7** at neutral pH is faster than that derived from the buffer. As both buffer and **7** are present in excess, pseudo-first-order rate constants are obtained. Because the reaction of PNPA is strongly accelerated by buffer whereas that of PNPA–**7** is not, the intracomplex rate advantage increases with decreasing buffer concentration. Moreover, the reactivity of Co(III) complexes is well known to be decelerated by buffer owing to a reversible complexation that effectively decreases the concentration of the reactive aquahydroxo species. Thus, the intracomplex reaction is almost twice as fast with 0·1 M than with 0·2 M buffer. Whereas the zero buffer rate can be safely extrapolated for the uncomplexed reaction as

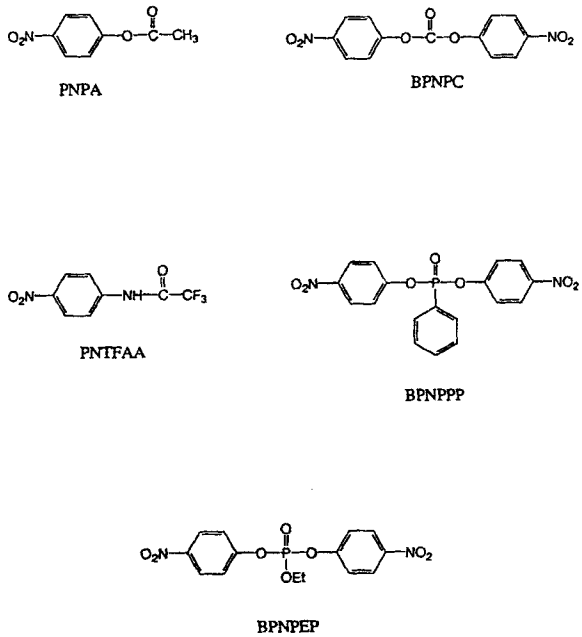

Figure 3. Structures of substrates used in this study

$1{\cdot}3 \times 10^{-6}\ s^{-1}$, extrapolation of the intracomplex reaction to zero buffer is more tenuous. The intracomplex rate advantage thus calculated ranges from 1000 to 1400-fold, with the former comparison the

Table 2. pH and buffer effects in PNPA reactions with 7[a]

[7] (mM)	pH	[Buffer] (M)	k_{buffer}	k_7	k_7/k_{buffer}
5	7	0·2	0·5	7·3	15
	7	0·1	0·26	13	49
	7	0·0[b]	0·013	(18)	1000 – 1400
	7	0·1	—	1·9 (0·1 M cyclohexanol)	
1	6	0·05	0·01	0·20	20
	7	0·05	0·028	2·0	75
	8	0·05	0·24	2·8	12
	9	0·05	0·95	5·5	5·8

[a] All rate constants are $k_{obs} \times 10^4$ s^{-1}. [PNPA] = 5 μM. The buffer materials used are listed under Experimental.
[b] Obtained by extrapolation to zero buffer concentration.

more secure one. This proves to be the largest acceleration seen to date at pH 7 for a cyclodextrin–metal conjugate, and is attributable to the high reactivity of the cyclen–Co(III) complex. The acceleration is not due simply to reaction with **4**, which demonstrates only an 8·6-fold acceleration over buffer under these conditions.[24]

Table 2 also indicates a bell-shaped pH–rate profile for the intracomplex reaction (while comparisons of zero buffer rates would be much preferable, the amounts of **7** required for extrapolation were prohibitive). The apogee occurs at pH 7, consistent with two facts: (1) the *cis* water molecules on the cyclen-Co(III) complex have pK_a values of *ca* 6 and 8; and (2) it is the singly deprotonated (i.e. the aquohydroxo) form that shows the greatest reactivity. It is precisely this set of properties that portended the utility of Co(III) complexes in artificial metalloenzyme design.

Table 3 summarizes the effect of CD conjugate concentration on the reaction rate. Two conclusions may be drawn. First, the secondary-side conjugate achieves saturation (by 3 mM) before the primary-side conjugate does; this is consistent with an observed preference of *p*-nitrophenyl compounds to bind at the secondary-side of cyclodextrin itself. Second, although the primary-side conjugate binds PNPA more weakly, it is nonetheless more reactive. Although we might deliberate on the origin of this observation, the rate ratio is really too small to warrant such speculation.

Table 3. Effect of changing Co(III) complex concentration in PNPA reactions at pH 8·0[a]

[CD] (mM)	k_7	k_{11}
1·0	4·8	2·4
2·0	6·8	3·0
3·0	8·4	3·5
4·0	9·7	3·5

[a] Buffer: 0·1 M bis-trispropane. All rate constants are $k_{obs} \times 10^4$ s^{-1}. [PNPA] = 5 μM.

Bis-*p*-nitrophenylcarbonate (BPNPC) is one of the substrates most commonly used with carbonic anhydrase models. Both of the Co(III) complexes that we have prepared had some promotional activity towards BPNPC. Carbonate is a very good ligand for Co(III), and catalysis of CO_2 hydration by Co(III) complexes has been reported.[25] Again, with this substrate the primary-side complex was more reactive. Table 4 summarizes the reactions of **7** with BPNPC at various pH values. It is important to note that, although several buffering materials were used to obtain k_{buffer} rates at different pH, no corrections have been made (such as extrapolation to zero buffer concentration). Nevertheless, because the intracomplex reactions are much faster than the buffer reactions, a bell-shaped pH–rate profile

Table 4. Reactions of BPNPC with 7[a,b]

pH	[*Buffer*] (M)	k_{buffer}	k_7	k_7/k_{buffer}
6	0·1	11	76	7·1
6·5	0·1	15	150	10
7	0·1	10	290	29
7·5	0·1	9·5	370	38
8	0·1	30	600	20
8·5	0·1	17	380	22
9	0·1	120	400	3·4

[7] = 2 mM.
[a] All rate constants are $k_{obs} \times 10^4$ s^{-1}. [BPNPC] = 5 μM.
[b] The buffer materials used are listed under Experimental. Buffer rates shown are those for that buffer at 0·1 M concentration.

is observed. Again, this is consistent with the reaction proceeding via the aquohydroxo Co(III) species.

The reactions of **7** and **11** with amide (PNTFAA), phosphonate (BPNPPP) and phosphate (BPNPEP) substrates were also examined. Although rate increases over the buffer-catalyzed rates were observed in every case, the intracomplex advantage was never more than fivefold (which occurred using 4 mM **7** with BPNPPP at pH 8). *p*-Nitrophenylphosphate, which is of interest because of its structural analogy to nucleoside monophosphates, reacts about 20 times more slowly with **7** than with **1**. It therefore appears that **7** and **11** show little or no intracomplex rate advantage towards these functional groups, even though non-CD Co(III) complexes demonstrate activity towards each substrate type. The most likely explanation is that the intracomplex reaction cannot achieve the conformation required for productive metal–functional group interaction. This is an issue that can be addressed; structure–reactivity relationships are, in principle, possible using synthetic catalysts; the structures of these compounds are known.

CONCLUSION

Although the cyclen–Co(III) complex has exhibited amongst the greatest rate accelerations in acyl and phosphoryl transfer reactions, this catalytic unit has not been used previously in the design of an artificial metalloenzyme. For this study, we have synthesized β-cyclodextrin derivatives of cyclen–Co(III) with attachments to the primary and secondary sides of the cyclodextrin torus. The primary-side cyclodextrin–cyclen–Co(III) conjugate accelerates the hydrolysis of *p*-nitrophenylacetate by a factor of 1000 (pH 7·0, 25 °C) as compared with the water-catalyzed reaction. Maximum reactivity occurs at pH 7, consistent with the known pK_a values and hypothesized mechanism of action of Co(III) complexes. The secondary-side cyclodextrin–cyclen–Co(III) conjugate is less reactive towards in *p*-nitrophenylacetate hydrolysis under saturating conditions, perhaps because of strain that requires the metal to point away from the CD cavity (as predicted by space-filling models). Reactivities towards an amide, a phosphonate and a phosphate triester were smaller. Artificial enzymes that can act on an unactivated substrates remain elusive but important targets. It appears likely that a hydrolytically active Co(III) complex attached to a strong and selective binding-cavity bearing molecule may well be a good candidate for this purpose.

EXPERIMENTAL

General. Melting points were taken on an electrothermal melting point apparatus and are uncorrected. Microanalyses were carried out at Canadian Microanalytical Services (New Westminster, BC). Mass spectra were obtained by use of a Kratos-30 mass spectrometer. FT-NMR spectra were obtained at 11·75 T (500 MHz) or 7·0 T (300 MHz). UV spectra were obtained on a Hewlett-Packard Model 8451A diode-array spectrophotometer; all wavelength data reported are ±1 nm. 6-Monotosyl-β-cyclodextrin (**2**) was prepared as described previously.[7] Most of the chemicals used in this study were obtained from Aldrich Chemical (Milwaukee, WI). Biological buffers (pH/buffer: 6/MES, 6·5/HEPES, 7–8/bis-trispropane, 8·5–9/CHES) and *p*-nitrophenylphosphate were obtained from Sigma Chemical (St. Louis, MO).

Reactions that produced p-nitrophenolate were followed by measuring the change in absorbance at 398 nm and 25 °C. For the reactions carried out at pH 6 and 6·5, the change in absorbance at 340 nm was followed (λ_{max} of *p*-nitrophenol). *p*-Nitroacetanilide reactions were followed at 400 nm. Reactions were monitored to >95% completion; pseudo-first-order behaviour was observed in most of the reactions, one important exception being that of *p*-nitrophenylphosphate hydrolysis.

6-Deoxy-6-(1',4',7',10'-tetrazacyclododecyl)-β-cyclodextrin (**4**). A solution of the 6-monotosyl derivative of β-cyclodextrin (1·3 g, 1·0 mmol) and cyclen (1,4,7,10-tetraazacyclododecane; 695 mg, 4·0 mmol) in dry DMF (4 ml; stirred with KOH and distilled from BaO) was heated in a sealed tube at 90 °C for 24 h. The reaction mixture was cooled, DMF was removed under reduced pressure and the residue was dissolved in water (2 ml) and added dropwise to ethanol (40 ml). The precipitated CD-containing compounds were collected by filtration, dissolved in 0·05 M NH_4HCO_3 buffer (25 ml) and applied to a CM-Sephadex cation-exchange column (30 × 7 cm i.d.). A linear gradient of NH_4HCO_3 (from 0·05 to 0·5 M) was used for elution. The fractions (25 ml each) were checked by TLC; CD-containing spots were made visible using a MeOH–AcOH–H_2SO_4–*p*-anisaldehyde (200 : 20 : 10 : 1) spray and developing with heat. Fractions 50–110 were combined and lyophilized to afford a fluffy white solid (**4**; 675 mg, 52%). ^{1}H NMR (D_2O): δ 2·35–3·10 (m, 12 H, azamacrocycle), 3·24–4·12 (m, 42 H, H_2, H_3, H_4, H_5, H_6 and 4 H azamacrocycle), 4·87–5·15 (m, 7 H, H_1). FAB mass spectrum: *m/z* 1290 ($M^+ + 1$). Analysis: calculated for $C_{50}H_{88}N_4O_{34} \cdot 6H_2O \cdot NH_4HCO_3$, C 41·49, H 7·17, N 4·74; found, C 41·71, H 7·12, N 4·7%.

6-Deoxy-6-(1',4',7',10'-tetraazacyclododecyl)-β-cyclodextrin($N^{1'}$,$N^{4'}$, $N^{7'}$,$N^{10'}$)carbonatocobalt(III) chloride (**5**). To a solution of **4** (500 mg, 0·39 mmol) in 1·2 M HCl (1·0 ml) was added sodium triscarbonato-Co(III) (159 mg, 0·44 mmol) in portions. After CO_2

evolution had ceased, the solution was warmed to 66 °C for 5 min. The solution was cooled to room temperature, filtered and acetone (50 ml) was added. The product separated as pink microcrystals (**5**; 498 mg, 89%). UV (λ_{max}, H_2O): 368, 530 nm. ^1H NMR (D_2O): δ 2·45–4·42 (m, 16 H, azamacrocycle; 42 H, H_2, H_3, H_4, H_5, H_6), 4·80–5·18 (m, 7 H, H_1). FAB mass spectrum: m/z 1347 ($M^+ - CO_3 - Cl$).

6-Deoxy-6-(1',4',7',10'-tetraazacyclododecyl)-β-cyclodextrin($N^{1'}$,$N^{4'}$,$N^{7'}$,$N^{10'}$)bischlorocobalt(III) chloride (6). Compound **5** (498 mg, 0·34 mmol) was suspended in methanol (8 ml) and conc. HCl (1·0 ml) was added. The mixture was warmed to 65 °C for 5 min. A purple solid precipitated on cooling (**6**; 454 mg, 90%). UV (λ_{max}, H_2O): 390, 562 (unstable in aqueous solutions). FAB mass spectrum, m/z 1347 ($M^+ - 3Cl$). Analysis: calculated for $C_{50}H_{88}Cl_3CoN_4O_{34} \cdot NH_4Cl \cdot 7H_2O$, C 36·75, H 6·54, N 4·28, Cl 8·68; found, C 37·05, H 6·51, N 3·89, Cl 8·71%

6-Deoxy-6-(1',4',7',10'-tetraazacyclododecyl)-β-cyclodextrin($N^{1'}$,$N^{4'}$,$N^{7'}$,$N^{10'}$,O^{6B})aquacobalt(III) nitrate (7). Compound **6** (400 mg, 0·28 mmol) was dissolved in water (5 ml) and the solution was applied to a QAE-Sephadex anion-exchange column (OH^- form, 15 × 3 cm i.d.) and eluted with water. The pink eluate was concentrated under reduced pressure to 5 ml The solution was acidified with 4 M HNO_3 (1 ml) and triturated with Et_2O until the product separated as a reddish powder, which was filtered and then dried *in vacuo* at room temperature (**7**; 251 mg, 59%). ^1H NMR (D_2O): δ 2·30–4·15 (m, 16 H, azamacrocycle; 42 H, H_2, H_3, H_4, H_5, H_6), 4·90–5·25 (m, 6 H, H_1), 5·28–5·45 (br d, 1 H, H_1). UV (λ_{max}): 366, 522 nm (0·1 M HNO_3), 368, 540 nm (pH 7 buffer), 362, 576 nm (0·1 M NaOH). FAB mass spectrum: m/z 1347 ($M^+ - H_2O - 3NO_3$). Analysis: calculated for $C_{50}H_{90}CoN_7O_{44} \cdot 6H_2O \cdot HNO_3$, C 34·53, H 5·97, N 6·44; found, C 34·80, H 6·12, N 6·29%.

Direct conversion of the primary-carbonatocobalt(III) complex into the aqua complexes. We have found out that carbonato–cyclenyl–CD–Co(III) complexes can be converted directly into the aqua complexes with high yields. Both primary- and the secondary-side complexes can be reacted in this way. This procedure will be exemplified here with the primary-side complex. First, the carbonato complex was prepared in the nitrate or perchlorate form. The carbonato complex (0·8 g) was then suspended in MeOH (10 ml) and concentrated HNO_3 (0·5 ml) was added. The bright pink color of the aqua complex was observed immediately. To remove any dissolved CO_2, the solution was heated on a steam-bath for 2 min. The mixture was then cooled to room temperature and the bright pink precipitate was collected by filtration. The precipitate was dissolved in water (30 ml) and lyophilized to afford a fluffy pink solid (0·585 g, 50%), identical in all respects with material prepared by initial conversion to the primary-side dichloro complex.

β-Cyclodextrin-manno-2,3-epoxide (8). The literature procedure[12a] was used to prepare the epoxide. However, the white solid obtained in this way is a mixture of product and various salts. The salts were removed by passing an aqueous solution of the epoxide sample through a cation–anion-exchange column (Amberlite MB-3). The salt-free epoxide was obtained after lyophilization. TLC indicated the presence of small amounts of CD and and of the diepoxide. Analysis: calculated for $C_{42}H_{68}O_{34} \cdot 5H_2O$, C 41·79, H 6·51, found, C 41·66, H 6·43%.

3-Deoxy-3-(1',4',7',10'-tetraazacyclododecyl)-β-cyclodextrin (9). To a solution of **8** (655 mg, 0·59 mmol) dissolved in dry DMF (4·0 ml) was added cyclem (1,4,7,10-tetraazacyclododecane; 400 mg, 2·3 mmol). The solution was heated at 100 °C for 48 h, then DMF was removed under reduced pressure. The residue was dissolved in water (2·0 ml) and added to EtOH (45 ml) dropwise. The resulting precipitate was collected by filtration, dissolved in water (50 ml) and then applied to a CM-Sephadex cation-exchange column (30 × 7 cm i.d.). Elution was performed using a linear gradient of NH_4HCO_3 buffer (0·05 to 0·6 M). Fractions were analyzed by TLC as described for compound **2**. Appropriate fractions (55–100) were pooled, concentrated and lyophilized to afford the secondary-side derivative (**9**; 187 mg, 25%). ^1H NMR (D_2O): δ 2·50–3·15 (m, 12 H, azamacrocycle), 3·19–4·08 (m, 42 H, H_2, H_3, H_4, H_5, H_6; 4 H azamacrocycle), 4·85–5·22 (m, 7 H, H_1). FAB mass spectrum: m/z 1289 (M^+). Analysis: calculated for $C_{50}H_{88}N_4O_{34} \cdot NH_4HCO_3 \cdot 6H_2O$, C 41·49, H 7·17, N 4·74; found, C 41·11, H 7·12, N 5·06%.

3-Deoxy-3-(1',4',7',10'-tetraazacyclododecyl)-β-cyclodextrin($N^{1'}$,$N^{4'}$,$N^{7'}$,$N^{10'}$)diaquacobalt(III) nitrate (11). To a solution of **9** (415 mg, 0·32 mmol) in 1·2 M HNO_3 (0.80 ml) was added $Na_3Co(CO_3)_3 \cdot 3H_2O$ (132 mg, 0·37 mmol). After the effervescence had stopped, the mixture was heated at 60 °C for 5 min, cooled and filtered. The solid was washed with cold water (5 ml), then to the clear red, combined filtrate were added acetone (5 ml) and EtOH (50 ml). The precipitate was collected by filtration, suspended in MeOH (6 ml), and conc. HNO_3 (six drops) was added. The mixture thus obtained was heated at 60 °C for 4 mins and cooled to room temperature. The reaction vessel was evacuated to remove dissolved CO_2, and the resulting precipitate was collected by filtration, dissolved in water (100 ml) and lyophilized to provide **11** as a red

fluffy solid (213 mg, 24%). UV (λ_{max}): 508 (0·1 M HNO_3), 522 nm (pH 7 buffer), 530 nm (0·1 M NaOH): ^1H NMR (D_2O): δ 2·20–4·40 (m, 16 H, azamacrocycle, 42 H, H_2, H_3, H_4, H_5, H_6), 4·50–5·32 (m, H_1, 7 H). FAB mass spectrum: *m/z* 1409 ($M^+ - 2NO_3 - 2H_2O$), 1347 ($M^+ - 2H_2O - 3NO_3$). Analysis: calculated for $C_{50}H_{92}CoN_7O_{45} \cdot HNO_3 \cdot 6H_2O$, C 34·49, H 6·08, N 6·43; found, C 34·28, H 5·93, N 6·80%.

6-Deoxy-6-(1',4',7'-triazaheptyl)-β-cyclodextrin (12). To a solution of **2** (655 mg, 0·59 mmol) dissolved in dry DMF (4·0 ml) was added dien (1,4,7-triazaheptane; 400 mg, 2·3 mmol). The solution was heated at 70 °C for 24 h, then DMF and most of the diethylenetriamine were removed under reduced pressure. The residue was dissolved in water (2·0 ml) and the solution was added to EtOH (45 ml) dropwise. The precipitate was collected by filtration, dissolved in water (50 ml) and applied to a CM-Sephadex cation-exchange column (30 × 7 cm i,d,). Elution was performed using a linear gradient of NH_4HCO_3 buffer (0·05 to 0·5 M). Fractions were analyzed by TLC as for compound **4**. Appropriate fractions (50–90) were pooled, concentrated and lyophilized to afford the primary-side derivative (**12**; 187 mg, 25%). ^1H NMR (D_2O): δ 2·55–3·13 (m, 6 H, polyamine), 3·19–4·08 (m, 42 H, H_2, H_3 H_4 H_5, H_6; 2 H polyamide), 4·85–5·22 (m, 7 H, H_1).

6-Deoxy-6-[5'-N-(3"-aminopropyl)-1',5',9'-triazanony]-β-cyclodextrin(14). To a solution of **2** (1·3 g, 0·587 mmol) dissolved in dry DMF (4·0 ml) was added TRPN [5-*N*-(3'-aminopropyl)-1,5,9-triazanonane; 1·0 g, 2·3 mmol]. The solution was heated at 70 °C for 12 h, then DMF and some of the polyamide were removed by vacuum distillation. The residue was dissolved in water (6·0 ml) and added to EtOH (75 ml) dropwise. The precipitate was collected by filtration, dissolved in water (40 ml) and applied to a CM-Sephadex cation exchange column (30 × 7 cm i.d.). Elution was performed using a linear gradient of NH_4HCO_3 buffer (0·05 to 0·8 M). Fractions were analysed by TLC as for compound **4**. Appropriate fractions (61–120) were pooled, concentrated and lyophilized to afford the primary-side derivative (**14**; 480 mg, 25%). ^1H NMR (D_2O): δ 2·52–3·11 (m, 16 H, polyamide), 3·20–4·11 (m, 42 H, H_2, H_3, H_4, H_5, H_6; 2 H polyamide), 4·83–5·19 (m, 7 H, H_1).

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