to anomeric configuration, with the α-anomers typically resonating some 16–20 ppm downfield from the β-anomers. Fortunately this enzyme also exhibits significant mannosidase activity[11] and is efficiently inactivated by 2-deoxy-2-fluoro-β-D-mannosyl fluoride (**2**).[8] Thus, the 2-deoxy-2-fluoromannosyl enzyme can be formed and its anomeric configuration determined by ^{19}F NMR.

Figure 2a shows the ^{19}F NMR spectrum of pABG5 β-glucosidase (0.74 mM) inactivated with 2-deoxy-2-fluoro-β-D-mannosyl fluoride (1.52 mM). Resonances at δ 121.0, 149.5, and 224.4 ppm arise from released fluoride and F-1 and F-2, respectively, of excess inhibitor. The broad peak at δ 201.0 ppm is the signal from the 2-fluoromannosyl enzyme, a chemical shift which is consistent with an α-anomeric configuration. In order to ensure that the shift observed is not in part due to its local environment,[12] the inactivated 2-deoxy-2-fluoromannosyl enzyme was denatured by overnight dialysis against 8 M urea and the ^{19}F NMR spectrum of this sample determined, Figure 2b. The presence of the peak due to bound inhibitor clearly indicates the covalent nature of the linkage and the fact that only a small shift back upfield ($\Delta\delta$ = 1.6 ppm) is observed indicates that environmental effects were indeed small. Peaks at δ 206.2 and 224.5 ppm arise from α- and β-2-deoxy-2-fluoro-D-mannose which is produced upon nonenzymic hydrolysis of the exposed sugar residue after denaturation.

These experiments have provided the first room temperature spectroscopic evidence, with intact enzyme, for the existence and the α-stereochemistry of this covalent glycopyranosyl intermediate. Such information has only been available previously from indirect trapping methods involving denaturation,[13] derivatization with highly reactive substrate analogues,[3] or to a certain extent by low-temperature trapping.[14]

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Synthesis and Reactivity of Cobalt(III) Complexes Bearing Primary- and Secondary-Side Cyclodextrin Binding Sites.† A Tale of Two CD's

Engin U. Akkaya and Anthony W. Czarnik*

Department of Chemistry, The Ohio State University Columbus, Ohio 43210

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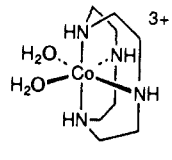
In recent years, efforts to focus the catalytic power of metals by connection to a binding moiety have received considerable attention.[1] Although the cyclen–Co(III) complex (**I**) has exhibited



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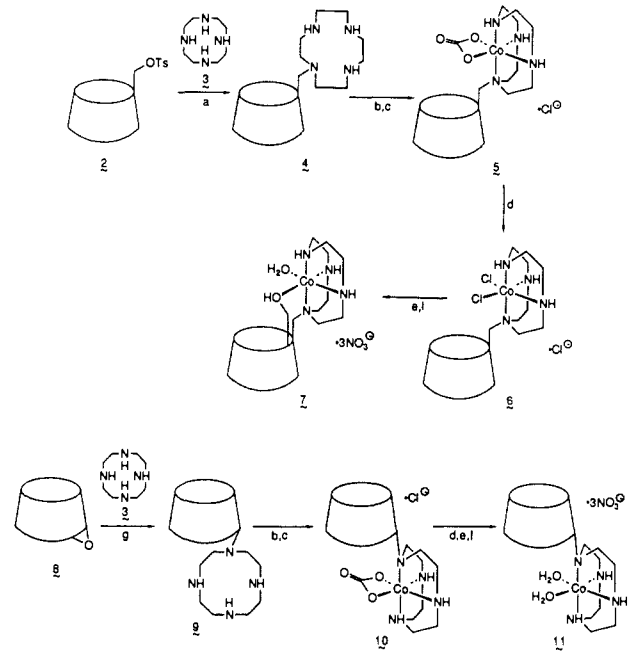
†Dedicated to the memory of Myron L. Bender.

Scheme I[a]



[a] (a) DMF, 90 °C, 24 h; (b) acidification with 1.2 M HCl; (c) $Na_3Co(CO_3)_3 \cdot 3H_2O$, 65 °C, 5 min; (d) MeOH, HCl, 65 °C, 5 min; (e) QAE-Sephadex chromatography (OH^- form); (f) acidification with HNO_3; (g) DMF, 100 °C, 48 h.

amongst the greatest rate accelerations in acyl-[2] and phosphoryl-[3,4]transfer reactions, this catalytic unit has not been used previously in the design of a preassociating artificial metalloenzyme. Cyclodextrin (CD) has been used previously for the synthesis of metalloprotein models,[1a,b,j,5] but incorporation of a Co(III) metal center onto CD has not been achieved to date. Accordingly, we have synthesized two cyclen–Co(III) complexes positioned alternately on the primary- and secondary-sides of β-CD. We now report that artificial metalloenzyme **7** demonstrates a 900-fold enhancement of metal-promoted ester hydrolysis attributable entirely to the presence of the CD binding unit. Furthermore, we find that the properties of primary- and secondary-side CD's are unexpectedly quite different, in part because the primary-side derivative is able to involve an adjacent hydroxymethyl group in chelation of the metal ion.

The syntheses were accomplished as shown in Scheme I. Reaction of cyclen (**3**) with β-CD monotosylate (**2**)[6] afforded, after

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(4) Recent work by Chin indicates that such Co(III) complexes can be made even more reactive by thoughtful choice of ligand. For example, see: Chin, J.; Zou, X. *J. Am. Chem. Soc.* **1988**, *110*, 223.
(5) (a) Matsui, Y.; Yokoi, T.; Mochida, K. *Chem. Lett.* **1976**, 1037. (b) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* **1977**, *99*, 7100. (c) Siegel, B. *J. Inorg. Nucl. Chem.* **1979**, *41*, 609. (d) Kuroda, Y.; Sasaki, Y.; Shiroiwa, Y.; Tabushi, I. *J. Am. Chem. Soc.* **1988**, *110*, 4049.

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Table I. UV Data (λ_{max} ± 1 nm) for Aquo Cobalt Complexes

complex	0.1 M HNO_3	pH 7 buffer	0.1 M NaOH
1	506	526	532
7	522	540	576
11	508	528	534

CM-Sephadex chromatography, the primary-side cyclen-CD conjugate (**4**); in the same way, β-CD monoepoxide (**8**)[7] was converted to the secondary-side cyclen-CD conjugate (**9**). Both compounds gave satisfactory microanalytical, mass spectral, and (albeit complex) NMR data. Cyclen-CD's **4** and **9** were converted to complexes **7** and **11**, respectively, by modification of a literature procedure.[8] Both were obtained as fluffy pink, totally water-soluble solids after lyophilization; each yielded NMR, microanalytical, and mass spectral data consistent with structures **7** and **11**.[9,10]

Kinetic studies were carried out at 25 °C in buffered solution. At pH 7.0, the cyclen–Co(III) complex itself (**1**) (10^{-3} M) does not promote the hydrolysis of *p*-nitrophenyl acetate[11] ("PNP-acetate"; 10^{-4} M), which occurs with a water-catalyzed rate constant of 2.0×10^{-6} s^{-1} at 25 °C. However, addition of primary-side complex **7** (5×10^{-3} M) results in accelerated hydrolysis with a pseudo-first-order rate constant of 1.8×10^{-3} s^{-1} (extrapolated to zero buffer concentration), yielding $k_{complexed}/k_{uncomplexed} = 900$. We note that this represents the largest reported acceleration attributable to CD-induced binding to a metal complex (a 4-fold acceleration has been reported previously[1b]); as expected, the CD-mediated reaction is inhibited competitively by 0.1 M cyclohexanol ($k_I = 1.9 \times 10^{-4}$ s^{-1}). Because previous studies have found rather small rate differences in reactions catalyzed by primary- and secondary-side derivatives of CD, we were quite surprised to observe that secondary-side complex **11** is an ineffectual catalyst for PNP-acetate hydrolysis. At pH 7.0, complex **11** (10^{-3} M) yields a pseudo-first-order rate constant for PNP-acetate (10^{-4} M) hydrolysis of 7.2×10^{-6} s^{-1}, only 3.6 times faster than the water-catalyzed rate. We postulate that hindered rotation of the cyclen–Co(III) group in complex **11** makes its interaction with bound PNP-acetate less favorable; indeed, it is physically impossible to construct a space-filling model of **11** without some distortion of the cavity.[12]

Complexes **7** and **11** (10^{-3} M) accelerate the hydrolysis of PNP-phosphate (10^{-4} M) at pH 7.0 (25 °C) by factors of 2900- and 3700-fold, respectively, as compared to the uncatalyzed[13] reaction (1.9×10^{-8} s^{-1}). Surprisingly, however, each CD complex is ca. 20 times less effective than is **1** itself in accelerating the PNP-phosphate hydrolysis reaction. In the case of complex **11**, this diminished reactivity can be rationalized as for the ester hydrolysis reaction by freezing the conformation of the Co(III) complex away from the binding site. The rationale for the lessened reactivity of **7** proves more interesting. While the UV spectra of **11** and of **1** are virtually superimposable, the spectrum of **7** indicates a change in its ligand substitution pattern (Table I).[14] It seems likely that an adjacent hydroxymethyl group also coordinates to the cobalt in **7**. Because the proposed mechanism for Co(III)-catalyzed phosphate hydrolysis involves formation of a bidentate phosphacycle intermediate, elimination of one exchangeable coordination site in **7** reduces the activity of the CD-complex even though the PNP acetate result foreshadowed an entropic advantage from precomplexation to the CD binding site. The single exchangeable ligand site on **7** does allow for "monodentate" association with the ester carbonyl group, which can then react either with solvent or with the activated adjacent hydroxyl group to cleave the phosphomonoester bond. Modifications leading to enhanced phosphate hydrolyses may be envisioned, and are the focus of ongoing work.

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(7) Breslow, R.; Czarnik, A. W. *J. Am. Chem. Soc.* **1983**, *105*, 1390. Passage of epoxide **8**, obtained as described in this paper, through an Amberlite MB-3 desalting column affords a salt-free sample as the pentahydrate (by microanalysis) after lyophilization.
(8) Poon, C. K.; Tobe, M. L. *J. Chem. Soc. A* **1968**, 1549. This procedure for the preparation of a cyclam–Co(III) complex was readily adapted for use with the cyclodextrin derivatives by incorporating the modifications shown in Scheme I.
(9) Characterization of **7**: ^1H NMR (D_2O) δ 2.30–4.15 (m, 16 H, azamacrocycle and 42 H, H_2-H_6), 4.90–5.25 (m, 6 H, H_1), 5.28–5.45 (br d, 1 H, H_1); FAB mass spectrum, *m/e* 1347 ($M^+ - H_2O - 3NO_3$). Anal. Calcd for $C_{50}H_{90}CoN_7O_{44}\cdot HNO_3\cdot 6H_2O$: C, 34.53; H, 5.97; N, 6.44. Found: C, 34.80; H, 6.12; N, 6.29.
(10) Characterization of **11**: ^1H NMR (D_2O) δ 2.20–4.40 (m, 16 H, azamacrocycle and 42 H, H_2-H_6), 4.50–5.32 (m, 7 H, H_1); FAB mass spectrum, *m/e* 1347 ($M^+ - 2H_2O - 3NO_3$). Anal. Calcd 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.
(11) Menger has pointed out that PNP-acetate is an unusually activated ester, reacting more like an anhydride or perhaps a metal-bound ester (Menger, F. M.; Ladika, M. *J. Am. Chem. Soc.* **1987**, *109*, 3145).
(12) While the position at which *manno*-epoxide **8** is attacked by **3** cannot be determined with the PMR spectrum of **9**, analogy to a similar reaction (ref 7) suggests opening at C-3. On the basis of coupling constant data from ref 7, distortion of the substituted sugar residue (and, therefore, of the CD cavity) from the usual $^4C_1$ conformation is indicated.
(13) Extrapolated from the data provided in the following: Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209.
(14) Geometrically induced shifts in the λ_{max}'s of similarly substituted Co(III) complexes have been observed previously: Collman, J. P.; Schneider, P. W. *Inorg. Chem.* **1966**, *5*, 1380.

Diastereotopically Distinct Secondary Deuterium Kinetic Isotope Effects on the Thermal Isomerization of *cis*-Hexatriene to 1,3-Cyclohexadiene

John E. Baldwin* and V. Prakash Reddy

Department of Chemistry, Syracuse University
Syracuse, New York 13244

B. A. Hess, Jr.,* and L. J. Schaad*

Department of Chemistry, Vanderbilt University
Nashville, Tennessee 37235

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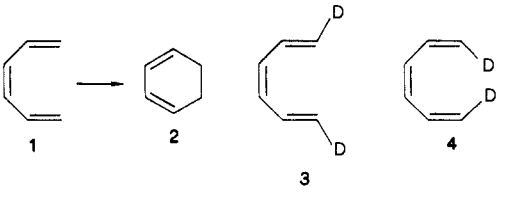
In many pericyclic reactions hydrogens that are equivalent by symmetry in either starting material or product may be diastereotopically related in the transition-state structure and might be associated with different secondary deuterium kinetic isotope effects. These possibilities have not been investigated thoroughly,[1] even though kinetic isotope effects are generally considered one of the most promising means for studying the details of potential surfaces in these reactions.[2,3] We report here a first demonstration



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