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Rapid Transacylations of Acivated Ester Substrates Bound to the Primary Side β-Cyclodextrin-Cyclen Conjugate and its M^{2+} Complexes

by

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Background

Metal ions have long been recognized to afford accelerations in ester, amide, and phosphate hydrolyses, due in part to their ability to form metal-bound hydroxide under conditions that are not strongly basic. The first such artificial metalloenzyme was described by Breslow in 1970, in which a βCD·Ni(II) complex demonstrated accelerated transacylation of bound

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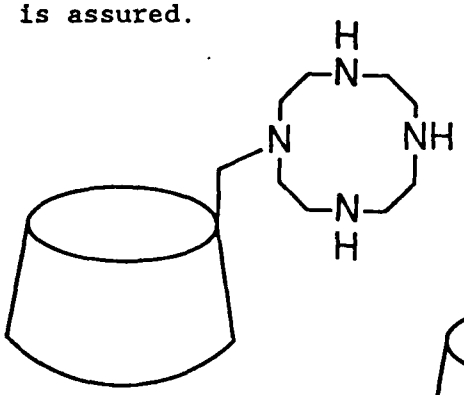
p-nitrophenyl acetate at pH 5.2. Subsequent descriptions of cyclodextrin-based artificial metalloenzymes have been reported by Matsui, Tabushi, and Breslow. In 1988, our group reported the synthesis of cyclodextrins with attached cyclen-Co(III) complexes; significant acceleration in the reaction with *p*-nitrophenyl acetate was observed only with the primary side derivative (Technical Report No. 1). Of course, metalloenzymes utilize M^{2+} and not M^{3+} catalytic centers; in addition, large rate accelerations in the transacylations of both activated and unactivated substrates have been observed previously in systems utilizing M^{2+} ions (e.g., Zn, Cu, Ni) as well as M^{3+} ions (e.g., Co, Ir, Cr). Therefore, we have investigated the ability of βCD-cyclen·M^{2+} conjugates to transacylate activated esters, amides, and phosphates. In addition, the ability of the apoenzyme mimic to effect transacylation was examined.

Results

The transacylation activities of cyclodextrin derivatives 1 and 2 towards *p*-nitrophenyl acetate, bis-*p*-nitrophenylcarbonate, and *p*-nitrotrifluoroacetanilide were evaluated. Values of k_{obs}/k_{buffer} at pH 7.0 were: 6.0-7.9 (bis-*p*-nitrophenylcarbonate), 2.8-4.3 (*p*-nitrotrifluoroacetanilide), and 8.6-16 (*p*-nitrophenyl acetate). In each case, a different divalent metal [Ni(II), Zn(II), Cu(II)] yielded the optimal acceleration.

Of particular interest is the activity of the metal-free conjugate (1). At pH 7, the reaction between 1 and *p*-nitrophenyl acetate was independent of buffer concentration, which we propose can be accounted for by the scheme depicted in host-guest complex 3. Comparing the reaction rates at zero buffer concentration, $k_{complex}$ is 150-times faster than $k_{uncomplex}$. This result is notable because simple monoamine derivatives of cyclodextrin do not accelerate this reaction at pH 7, as no free amine exists. However, cyclen (the azamacrocyclic portion of 1) exists almost completely in the +2 ionic form at pH 7. Therefore, the presence of a significant amount of an unprotonated amine is assured.



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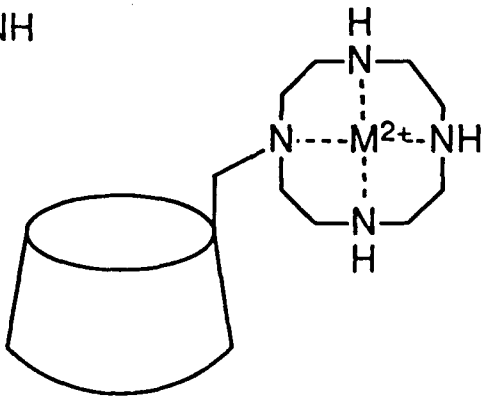

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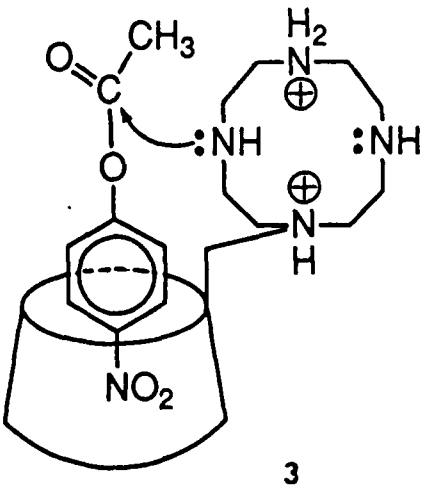

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RAPID TRANSACYLATIONS OF ACTIVATED ESTER SUBSTRATES BOUND TO THE PRIMARY SIDE β-CYCLODEXTRIN-CYCLEN CONJUGATE AND ITS M^{2+} COMPLEXES

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Introduction

The ability of cyclodextrins to affect rapid transacylations of bound substrates has been well studied.[1,2] One important difference between cyclodextrin and enzyme-mediated transacylation is the pH required. The hydrolytic cleavage of phenyl esters promoted by cyclodextrin has been extensively investigated[3] and the mechanism of the catalyzed reaction clearly established to involve nucleophilic attack by a deprotonated secondary-side hydroxyl group (~~pK_a 12~~) on the carbonyl carbon of the bound substrate.[4] As a result, transacylations are accelerated in the presence of cyclodextrin under basic conditions (pH > 10.5). Several derivatives of cyclodextrin have been prepared in efforts to provide a nucleophilic group near neutral pH. Pendant groups that have been used successfully in this regard include imidazole,[5] N-methylhydroxamic acid,[6] and histamine.[7]

Of particular pertinence to this manuscript are those cyclodextrins that have been substituted with metal ion complexes. Metal ions have long been recognized to afford accelerations in ester, amide, and phosphate hydrolyses, due in part to their ability to form metal-bound hydroxide under conditions that are not strongly basic. The first such artificial ~~metalloenzyme (1)~~ was described by Breslow in 1970, in which a Beta Cyclodextrin (βCD) Ni(II) complex demonstrated accelerated transacylation of bound *p*-nitrophenyl acetate at pH 5.2. Subsequent descriptions of cyclodextrin-based artificial metalloenzymes have been reported by Matsui,[8] Tabushi,[9] and Breslow.[10]

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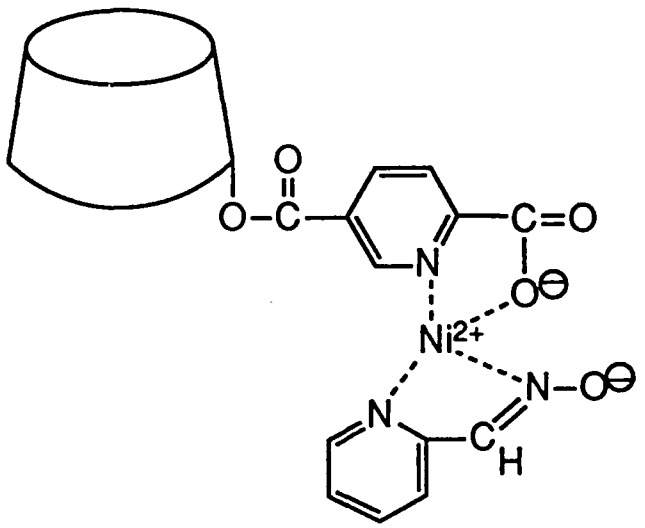

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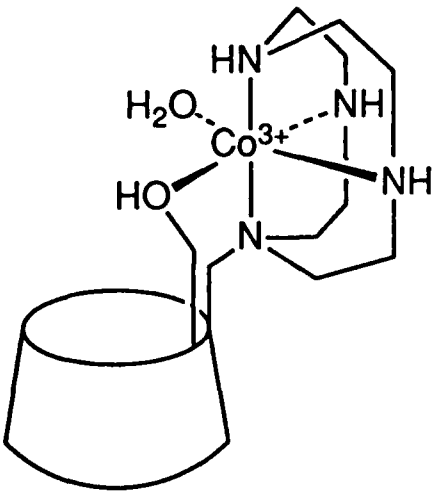

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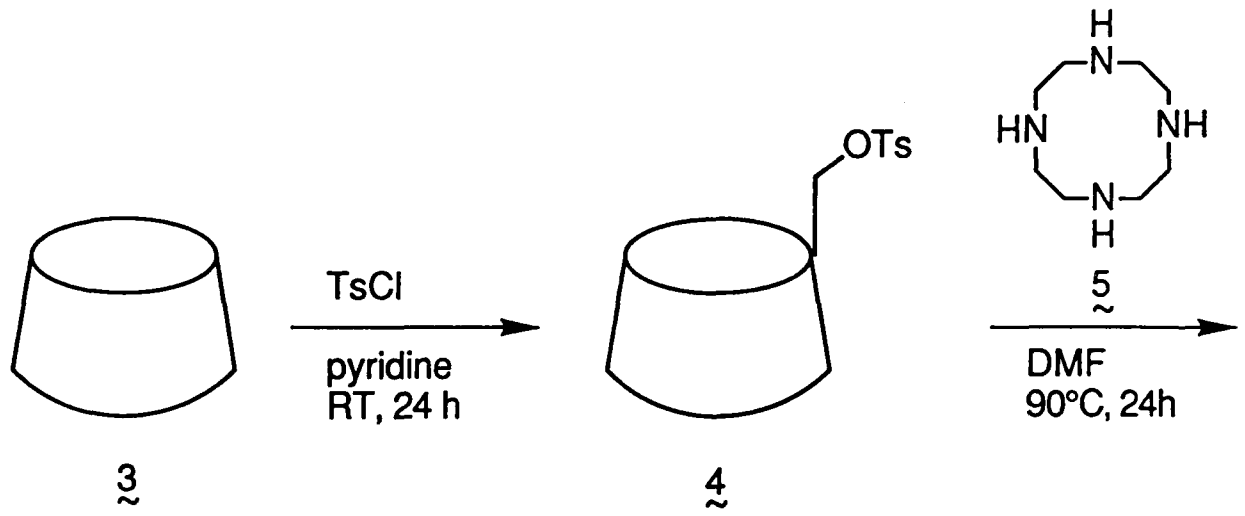
In 1988, our group reported the synthesis of cyclodextrins with attached cyclen-Co(III) complexes; significant acceleration in the reaction with *p*-nitrophenyl acetate was observed only with the primary side derivative (2).[11] Of course, metalloenzymes utilize M^{2+} and not M^{3+} catalytic centers; in addition, large rate accelerations in the transacylations of both activated and unactivated substrates have been observed previously in systems utilizing M^{2+} ions (e.g., Zn, Cu, Ni) as well as M^{3+} ions (e.g., Co, Ir, Cr).[12] Therefore, we have investigated the ability of βCD-cyclen·M^{2+} conjugates to transacylate activated esters, amides, and phosphates. In addition, the ability of the apoenzyme mimic to effect transacylation was examined.

Results

Syntheses were accomplished as shown in Scheme I. The βCD-cyclen conjugate (6) was prepared by displacement of the tosyl group of 4 by cyclen. Purification using cation-exchange chromatography[13] afforded 6 as a colorless solid after lyophilization. Complexation with divalent metals to give 7 (no geometry implied) was accomplished *in situ* by addition of an equimolar amount of the appropriate metal perchlorate.[14] Given the large complexation constants of cyclen for the metals used in this study,[15] complete complexation was assumed. We monitored the release of *p*-nitrophenolate from the following substrates: *p*-nitrophenyl acetate (8; "PNPA"), bis-*p*-nitrophenylcarbonate (9; "BIS-PNPC"), and *p*-nitrophenylphosphate (10; "PNPP"). We also monitored the release of *p*-nitroaniline from *p*-nitrotrifluoroacetanilide (11; "PNTFAA"). Rate data determined under various conditions are presented in Tables 1 and 2 (substrate: PNPA), Table 3 (substrate: BIS-PNPC), and Table 4 (substrate: PNTFAA).



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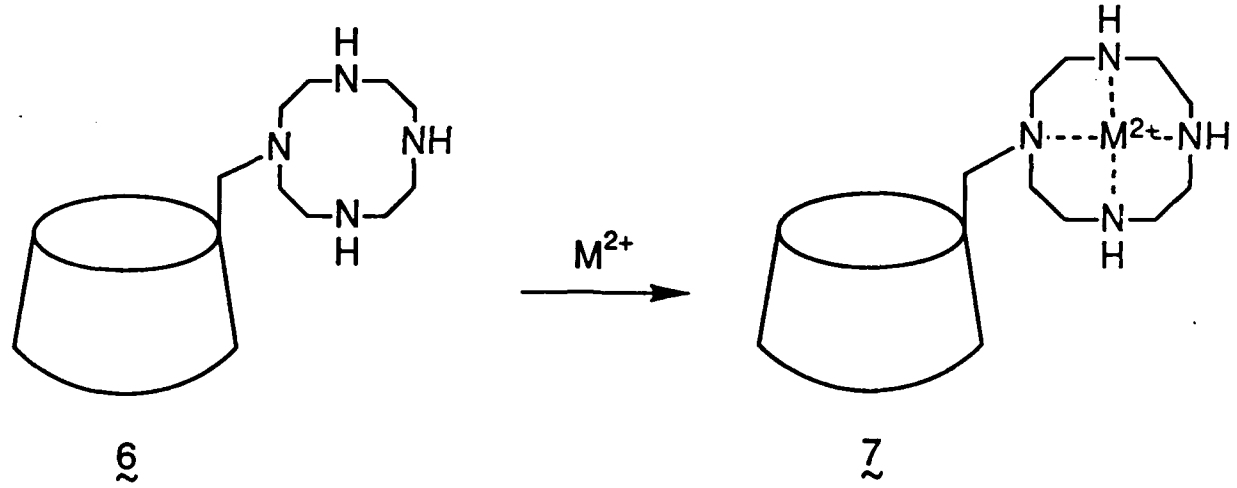

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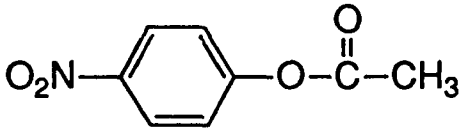

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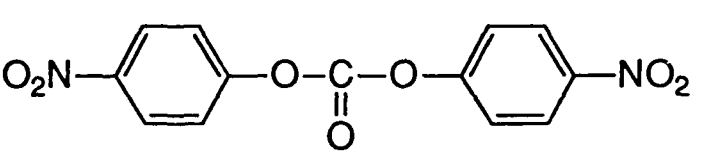

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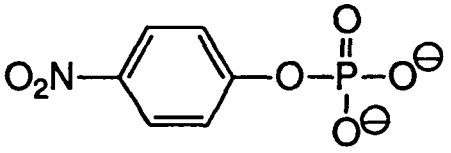

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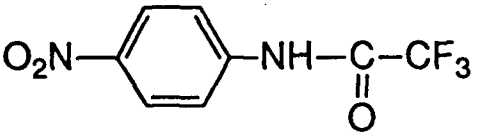

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TABLE 1

Transacylation reactions of *p*-nitrophenyl acetate at pH's 7.0-8.5

Rxn	acyl acceptor	M(II)	pH	$10^4 k_{obs}$, s^{-1}	k_{obs}/k_{buffer}	$t_{1/2}$, min
1	--	--	7.0	.22	1.0	530
2	CD	--	7.0	.24	1.1	480
3	N4	--	7.0	.31	1.4	370
4	CD + N4	--	7.0	.38	1.7	300
5	--	Ni(II)	7.0	.43	2.0	270
6	--	Zn(II)	7.0	.70	3.2	170
7	--	Cu(II)	7.0	.22	1.0	530
8	N4	Ni(II)	7.0	.64	2.9	180
9	N4	Zn(II)	7.0	.41	1.9	280
10	N4	Cu(II)	7.0	.30	1.4	390
11	CDN4	--	7.0	1.9	8.6	61
12	CDN4	Ni(II)	7.0	3.4	16	34
13	CDN4	Zn(II)	7.0	3.0	14	39
14	CDN4	Cu(II)	7.0	2.6	12	44
15	--	--	7.5	.58	1.0	200
16	CD	--	7.5	.63	1.1	180
17	--	Ni(II)	7.5	1.5	2.6	77
18	--	Zn(II)	7.5	2.3	3.9	50
19	--	Cu(II)	7.5	.52	0.9	220
20	N4	Ni(II)	7.5	2.2	3.8	53
21	N4	Zn(II)	7.5	1.4	2.4	83
22	N4	Cu(II)	7.5	.68	1.2	170
23	CDN4	--	7.5	3.6	6.1	33
24	CDN4	Ni(II)	7.5	4.5	7.8	26
25	CDN4	Zn(II)	7.5	3.7	6.4	31
26	CDN4	Cu(II)	7.5	1.9	3.3	61
27	--	--	8.0	1.4	1.0	83
28	CD	--	8.0	1.8	1.2	64
29	--	Ni(II)	8.0	2.5	1.8	46
30	--	Zn(II)	8.0	3.6	2.6	32
31	--	Cu(II)	8.0	.95	.63	120
32	N4	Ni(II)	8.0	6.1	4.4	19
33	N4	Zn(II)	8.0	3.3	2.4	35
34	N4	Cu(II)	8.0	1.5	1.1	77
35	CDN4	--	8.0	10	7.1	17
36	CDN4	Ni(II)	8.0	12	8.6	10
37	CDN4	Zn(II)	8.0	9.6	6.9	12
38	CDN4	Cu(II)	8.0	4.7	3.4	25
39	--	--	8.5	3.5	1.0	33
40	CD	--	8.5	4.9	1.4	24
41	--	Ni(II)	8.5	9.3	2.7	13
42	--	Zn(II)	8.5	8.4	2.4	14
43	--	Cu(II)	8.5	2.2	.63	53
44	N4	Ni(II)	8.5	12	3.4	10
45	N4	Zn(II)	8.5	6.2	1.8	19
46	N4	Cu(II)	8.5	3.5	1.0	33
47	CDN4	--	8.5	22	6.3	5.3
48	CDN4	Ni(II)	8.5	24	7.0	4.8
49	CDN4	Zn(II)	8.5	19	5.4	6.1
50	CDN4	Cu(II)	8.5	9.3	2.7	12

Legend: All solutions were 5.0×10^{-5} M in *p*-nitrophenyl acetate, 5.0×10^{-3} M in added acyl acceptor, and 5.0×10^{-3} M in metal ion concentration (perchlorate salts used). At each pH, the term "k_{buffer}" refers to the buffer-catalyzed reaction without added CD derivative, cyclen, or metal. N4 refers to cyclen (5) and CDN4 refers to compound 6. Reactions were carried out at 23°C in 0.1 M bis-tris-propane buffer solution.

TABLE 2

Relative rates of acyl transfer reactions of *p*-nitrophenyl acetate uncomplexed and complexed to CDN4 extrapolated to zero-buffer concentration

Exp	acyl acceptor	M(II)	pH	$10^4 k_{obs}, s^{-1}$	k_{obs}/k_{uncat}	$t_{1/2}$, min
1	--	--	7.0	.013	1.0	8900
2	CDN4	--	7.0	1.9	150	61

Legend: All solutions were 5.0×10^{-5} M in *p*-nitrophenyl acetate and 5.0×10^{-3} M in added acyl acceptor. CDN4 refers to compound 6. The term "k_{uncat}" refers to the rate of the reaction extrapolated to zero buffer concentration. Reactions were carried out at 23°C in 0.1 M bis-tris-propane buffer solution.

TABLE 3

Transacylation reactions of bis-(*p*-nitrophenyl)carbonate

Exp	acyl acceptor	M(II)	pH	$10^3 k_{obs}, s^{-1}$	k_{obs}/k_{buffer}	$t_{1/2}$, min
1	--	--	7.0	0.95	1.0	12
2	CDN4	--	7.0	6.6	6.9	1.8
3	CDN4	Cu(II)	7.0	5.7	6.0	2.0
4	CDN4	Zn(II)	7.0	7.5	7.9	1.5
5	CDN4	Ni(II)	7.0	6.9	7.3	1.7

Legend: All solutions were 5.0×10^{-5} M in bis-(*p*-nitrophenyl)carbonate, 5.0×10^{-3} M in added acyl acceptor, and 5.0×10^{-3} M in metal ion concentration (perchlorate salts used). At each pH, the term "k_{buffer}" refers to the buffer-catalyzed reaction without added CD derivative, cyclen, or metal. N4 refers to cyclen (5) and CDN4 refers to compound 6. Reactions were carried out at 23°C in 0.1 M bis-tris-propane buffer solution.

TABLE 4

Transacylation reactions of *p*-nitrotrifluoroacetanilide

Exp	acyl acceptor	M(II)	pH	$10^5 k_{obs}, s^{-1}$	k_{obs}/k_{buffer}	$t_{1/2}$, min
1	--	--	7.0	1.7	1.0	680
2	CDN4	--	7.0	4.8	2.8	240
3	CDN4	Ni(II)	7.0	5.0	2.9	230
4	CDN4	Zn(II)	7.0	5.5	3.2	210
5	CDN4	Cu(II)	7.0	7.3	4.3	160

Legend: All solutions were 5.0×10^{-5} M in *p*-nitrotrifluoroacetanilide, 5.0×10^{-3} M in added acyl acceptor, and 5.0×10^{-3} M in metal ion concentration (perchlorate salts used). At each pH, the term "k_{buffer}" refers to the buffer-catalyzed reaction without added CD derivative, cyclen, or metal. N4 refers to cyclen (5) and CDN4 refers to compound 6. Reactions were carried out at 23°C in 0.1 M bis-tris-propane buffer solution.

Discussion

It was of interest to observe the rapid acyl transfer of bound PNPA to βCD-cyclen itself even in the absense of added metal ions. Such behavior would not be expected for the simple primary side amine derivative of βCD because at pH 7 it would be completely protonated, and therefore unavailable as a nucleophile. It has been observed previously that the reactivity of the corresponding secondary side amine derivative of βCD at pH 7 is not greater than that of βCD itself.[16] However, cyclen exists almost completely in the +2 ionic form at pH 7. Therefore, the presence of a significant amount of an unprotonated amine (albeit deactivated by neighboring ammonium ions) is assured. The βCD-cyclen mediated reaction of PNPA was found to be buffer-independent; comparision of the non-buffer catalyzed rates of acyl transfer for complexed vs. uncomplexed forms of PNPA gave $k_{complex}/k_{un}$ =150. One mode for the attack of a βCD-cyclen nitrogen onto the acyl group of PNPA is shown in structure 12.

The fastest transacylation rates were observed when both the βCD-cyclen conjugate and divalent metal ion were present. Under the buffered conditions used for the reactions of PNPA, the addition of metal ions yielded transacylation rates greater than those observed using βCD-cyclen alone, but only marginally. At pH 7.0, the βCD-cyclen·Ni^{+2} conjugate ($5x10^{-3}$ M) yields a pseudo-first order rate constant for reaction with PNPA ($5x10^{-5}$ M) of 3.4×10^{-4} s^{-1}, which corresponds to a 16-fold increase as compared to the uncomplexed reaction carried out in 0.10 M BIS-TRIS-propane buffer. The complexes formed by Zn^{+2} (14-fold) and Cu^{+2} (12-fold) showed slightly smaller acclerations than the Ni^{+2} complex.

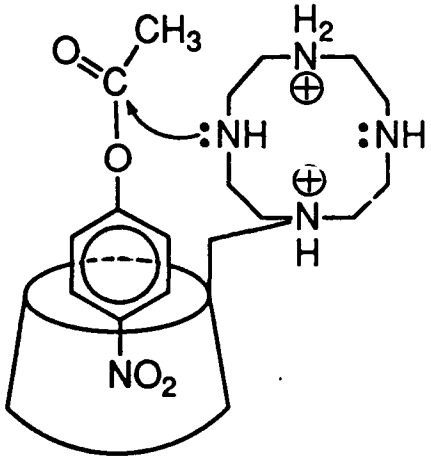
Over the pH range we examined, the largest acceleration by

βCD-cyclen·M^{+2} complexes occurred at pH 7.0. At pH's above 7.0, a decrease in the activity of the metal ions copper and zinc were observed; at higher pH's the formation of metal oxo dimers led to a decrease in the activity of the metal ions. Meriwether and Westheimer have reported that at pH >7.1 there is significant precipitation of zinc from buffered solutions containing Zn^{+2} ions.[17] Such oxo dimers do not promote ester hydrolysis. The metal hydroxide mechanism for accelerated transacylation, which has received support by the work of Bruice, Breslow, and Groves, could be envisioned as occurring through either attack by metal-bound hydroxide (13) or alkoxide (14) ions (the metal ion geometries shown are speculative only).

One unexpected observation was the apparent deceleration of PNPA hydrolysis upon addition of $Cu(ClO_4)_2$ without ligand. At pH's 7.5, 8.0, and 8.5 (reactions 19, 31, and 43 of Table 1) the observed hydrolysis rate was actually slower than the buffer-catalyzed rate. A possible explanation- precipitation of buffer by Cu(II) leading to less buffer catalysis- can account for part of this effect, but not all of it. The slower reaction with Cu(II) is reproducible, and as yet unexplained.

The effects of various metal ions with the βCD-cyclen conjugate were also studied for the hydrolysis of bis-*p*-nitrophenylcarbonate (9). The effects were shown to be small (under 10-fold) compared to the uncomplexed reaction carried out in 0.10 M buffer (Table 3).

p-Nitrotrifluoroacetanilide (11), an activated amide, has been used previously[18] in cyclodextrin promoted hydrolysis experiments. Effects of various metal ions with βCD-cyclen were also studied for the hydrolysis of this amide. The effects were even smaller than those observed in the carbonate hydrolysis experiments. Using the βCD-cyclen·Cu^{2+} complex, the rate of amide hydrolysis was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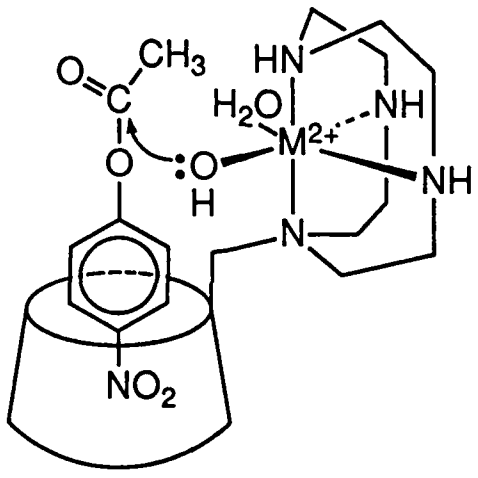
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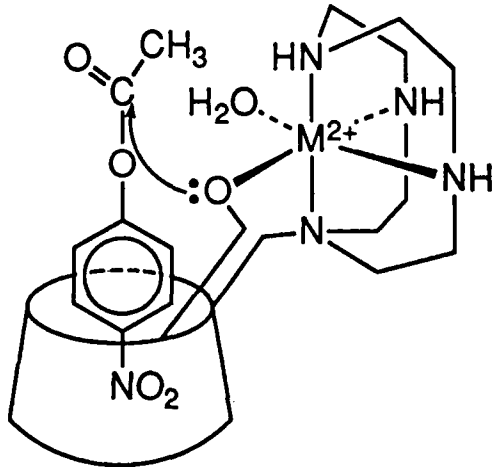
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increased by a factor of 4.3 as compared to the uncomplexed reaction carried out in 0.10 M buffer.

No detectable acceleration was observed for the hydrolysis of *p*-nitrophenylphosphate when comparing the uncomplexed reaction to the βCD-cyclen·M^{+2} complexed reactions at neutral pH.

Experimental Section

6-Deoxy-6-(1,4,7,10-tetraazacyclododecyl)-β-cyclodextrin (6). A solution of the 6-O-monotosyl derivative of βCD (4; 0.94 g, 0.73 mmol) and cyclen (5; 0.50 g, 2.9 mmol) in dry DMF (4 mL) was heated in a sealed tube at 90°C for 24 h. The reaction mixture was cooled, DMF was removed under reduced pressure, the residue was dissolved in water (3 mL) and added dropwise to ethanol (75 mL). After storage in the refrigerator overnight, the mixture of cyclodextrin containing compounds was collected by filtration. Unreacted azamacrocycle could be recovered from the ethanol filtrate (300 mg, 60%). The crude product was dissolved in H_2O (40 mL) and applied to a CM-Sephadex cation exchange column. Elution was started with water (800 mL), followed by a linear gradient of NH_4HCO_3 (from .05 M to .60 M).[13] The fractions (20 mL each) were analyzed by TLC (silica; AcOH/$CHCl_3$/H_2O, 8:1:1), and cyclodextrin containing spots were made visible using a MeOH/AcOH/H_2SO_4/p-anisaldehyde (200:20:10:1) spray. Fractions 81-150 were combined and lyophilized to obtain a white solid (6; 455 mg, 49%): ^1H NMR (D_2O) δ 2.5-3.0 (m, 16 H, azamacrocycle), 3.2-4.0 (m, 42 H, H_2, H_3, H_4, H_5, H_6), 4.9-5.2 (m, 7 H, H_1); FAB mass spectrum, m/e 1289 (M+1).

Anal. Calcd for $C_{50}H_{88}N_4O_{34}\cdot 7.5H_2O$: C, 42.16; H, 7.28; N, 3.93. Found: C, 41.90; H, 6.88; N, 4.32.

Kinetic Method

The hydrolysis reactions were followed by measuring the absorbance change at 398 nm (isosbestic point of *p*-nitrophenolate inclusion to cyclodextrin) for the reactions that produce *p*-nitrophenolate. In the *p*-nitroacetanilide derivative hydrolysis reactions, the formation of *p*-nitroaniline was followed at 400 nm. Reactions were monitored to >95% completion. Pseudo-first order kinetics were observed for most reactions, with an important exception being the phosphate hydrolysis reaction. Rate constants were determined using the program "ENZFITTER" (Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, UK). The UV spectrophotometer used was a Hewlett Packard 8451A Diode Array Spectrophotometer.

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