

## Rapid Transacylations of Activated Ester Substrates Bound to the Primary Side $\beta$ -Cyclodextrin–Cyclen Conjugate and Its $M^{2+}$ Complexes

MICHAEL I. ROSENTHAL and ANTHONY W. CZARNIK\*

*Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, U.S.A.*

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**Abstract.** The ability of cyclodextrins to effect rapid transacylations of bound substrates has been well studied. One important difference between cyclodextrin and enzyme-mediated transacylation is the pH required. Because the  $pK_a$  of a cyclodextrin secondary-side hydroxyl group is about 12, transacylations are accelerated in the presence of cyclodextrin under basic conditions ( $pH > 10.5$ ). In 1988, our group reported the synthesis of cyclodextrin with attached cyclen-Co(III) complexes; significant acceleration in the reaction with *p*-nitrophenyl acetate was observed only with the primary side derivative. 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). In this paper, we describe the ability of  $\beta$ CD-cyclen- $M^{2+}$  conjugates to transacylate activated esters, amides, and phosphates. In addition, the ability of the apoenzyme mimic to effect transacylations was examined.

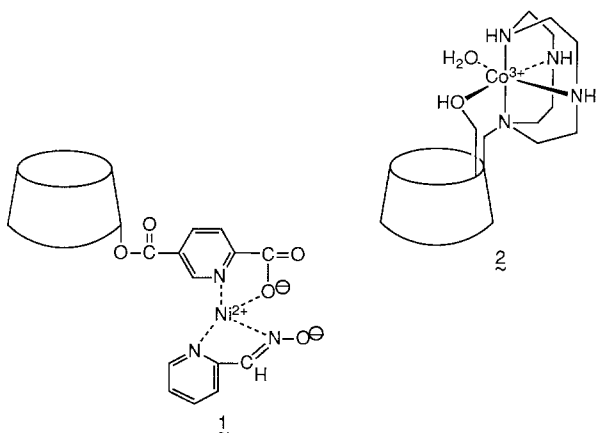
**Key words.** Cyclodextrin, transacylations, metal, catalysis, ester, amide, phosphate.

### 1. Introduction

The ability of cyclodextrins to effect 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 and the mechanism of the catalyzed reaction has been clearly established as involving nucleophilic attack by a deprotonated secondary-side hydroxyl group ( $pK_a$  12) on the carbonyl carbon of the bound substrate [3]. 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 [4], *N*-methylhydroxamic acid [5], and histamine [6].

Of particular relevance to this report 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

\* Author for correspondence.

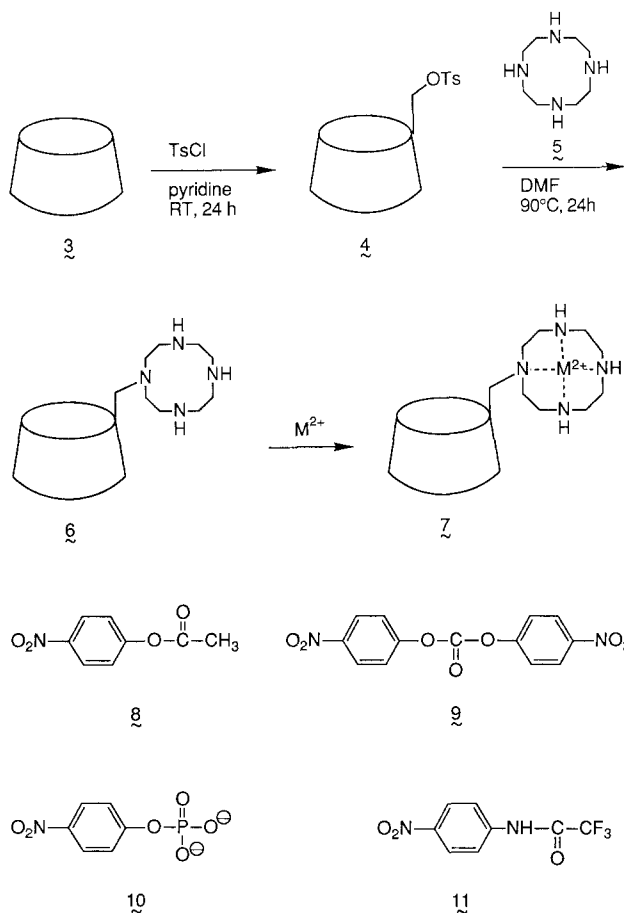


basic. The first such artificial metalloenzyme (**1**) was described by Breslow in 1970, in which a  $\beta$ CD-Ni(II) complex demonstrated accelerated transacylation of bound *p*-nitrophenyl acetate at pH 5.2 [7]. Subsequent descriptions of cyclodextrin-based artificial metalloenzymes have been reported by Matsui [8], Tabushi [9], and Breslow [10].

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  $\beta$ CD-cyclen- $M^{2+}$  conjugates to transacylate activated esters, amides, and phosphates. In addition, the ability of the apoenzyme mimic to effect transacylation was examined.

## 2. Results

Syntheses were accomplished as shown in Scheme I. The  $\beta$ CD-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 I and II (substrate: PNPA), Table III (substrate: BIS-PNPC), and Table IV (substrate: PNTFAA).



Scheme 1

### 3. Discussion

It was of interest to observe the rapid acyl transfer of bound PNPA to  $\beta$ CD-cyclen itself even in the absence of added metal ions. Such behavior would not be expected for the simple primary side amine derivative of  $\beta$ CD 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  $\beta$ CD at pH 7 is not greater than that of  $\beta$ CD itself [16]. However, cyclen exists almost completely in the 2+ ionic form at pH 7 [17], and it is likely that the cyclodextrin-cyclen conjugate will behave similarly. Therefore, the presence of a significant amount of an unprotonated amine (albeit deactivated by neighboring ammonium ions) is assured. The  $\beta$ CD-cyclen mediated reaction of PNPA was found to be buffer-independent; comparison of the non-buffer catalyzed rates of acyl transfer for complexed vs. uncomplexed forms of PNPA gave  $k_{\text{complex}}/k_{\text{un}} = 150$ . One mode for the attack of a  $\beta$ CD-cyclen nitrogen onto the acyl group of PNPA is shown in structure **12**.

Table I. Transacylation reactions of *p*-nitrophenyl acetate at pH 7.0–8.5<sup>a</sup>

Rxn	Acyl acceptor	M(II)	pH	$10^4 k_{\text{obs}}, \text{s}^{-1}$	$k_{\text{obs}}/k_{\text{buffer}}$	$t_{1/2}, \text{min}$
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

<sup>a</sup>All solutions were  $5.0 \times 10^{-5}$  M in *p*-nitrophenyl acetate,  $5.0 \times 10^{-3}$  M in added acyl acceptor, and  $5.0 \times 10^{-3}$  M in metal ion concentration (perchlorate salts used). At each pH, the term ' $k_{\text{buffer}}$ ' 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 II. Relative rates of acyl transfer reactions of *p*-nitrophenyl acetate uncomplexed and complexed to CDN4 extrapolated to zero-buffer concentration.<sup>a</sup>

Exp	acyl acceptor	M(II)	pH	$10^4 k_{\text{obs}}, \text{s}^{-1}$	$k_{\text{obs}}/k_{\text{uncat}}$	$t_{1/2}, \text{min}$
1	---	---	7.0	0.13	1.0	8900
2	CDN4	---	7.0	1.9	150	61

<sup>a</sup>All solutions were  $5.0 \times 10^{-5} \text{ M}$  in *p*-nitrophenyl acetate and  $5.0 \times 10^{-3} \text{ M}$  in added acyl acceptor. CDN4 refers to compound **6**. The term ' $k_{\text{uncat}}$ ' refers to the rate of the reaction extrapolated to zero buffer concentration. Reactions were carried out at 23°C.

Table III. Transacylation reaction of bis-(*p*-nitrophenyl)carbonate.<sup>a</sup>

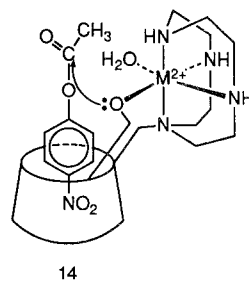
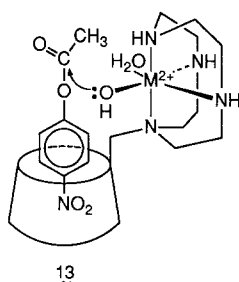
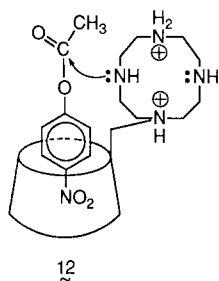
Exp	acyl acceptor	M(II)	pH	$10^3 k_{\text{obs}}, \text{s}^{-1}$	$k_{\text{obs}}/k_{\text{buffer}}$	$t_{1/2}, \text{min}$
1	---	---	7.0	0.95	1.0	12
2	CDN4	---	7.0	6.6	6.9	1.8
3	CDN4	Cu(II)	7.0	5.7	6.0	2.0
4	CDN4	Zn(II)	7.0	7.5	7.9	1.5
5	CDN4	Ni(II)	7.0	6.9	7.3	1.7

<sup>a</sup>All solutions were  $5.0 \times 10^{-5} \text{ M}$  in bis-(*p*-nitrophenyl)carbonate,  $5.0 \times 10^{-3} \text{ M}$  in added acyl acceptor, and  $5.0 \times 10^{-3} \text{ M}$  in metal ion concentration (perchlorate salts used). At each pH, the term ' $k_{\text{buffer}}$ ' 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 IV. Transacylation reaction of *p*-nitrotrifluoroacetanilide.<sup>a</sup>

Exp	acyl acceptor	M(II)	pH	$10^3 k_{\text{obs}}, \text{s}^{-1}$	$k_{\text{obs}}/k_{\text{buffer}}$	$t_{1/2}, \text{min}$
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

<sup>a</sup>All solutions were  $5.0 \times 10^{-5} \text{ M}$  in *p*-nitrotrifluoroacetanilide,  $5.0 \times 10^{-3} \text{ M}$  in added acyl acceptor, and  $5.0 \times 10^{-3} \text{ M}$  in metal ion concentration (perchlorate salts used). At each pH, the term ' $k_{\text{buffer}}$ ' 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.



The fastest transacylation rates were observed when both the  $\beta$ CD–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  $\beta$ CD–cyclen alone, but only marginally. At pH 7.0, the  $\beta$ CD–cyclen·Ni<sup>2+</sup> conjugate ( $5 \times 10^{-3}$  M) yields a pseudo first-order rate constant for reaction with PNPA ( $5 \times 10^{-5}$  M) of  $3.4 \times 10^{-4}$  s<sup>-1</sup>, 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<sup>2+</sup> (14-fold) and Cu<sup>2+</sup> (12-fold) showed slightly smaller accelerations than the Ni<sup>2+</sup> complex.

Over the pH range we examined, the largest acceleration by  $\beta$ CD–cyclen·M<sup>2+</sup> complexes occurred at pH 7.0. At pH > 7.0, a decrease in the activity of the metal ions copper and zinc were observed. At higher pH the formation of metal oxo dimers commonly leads to a decrease in the activity of these metal ions; for example, Meriwether and Westheimer have reported that at pH > 7.1 there is significant precipitation of zinc from buffered solutions containing Zn<sup>2+</sup> ions [18]. 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<sub>4</sub>)<sub>2</sub> without ligand. At pH 7.5, 8.0, and 8.5 (reactions 19, 31, and 43 of Table I) the observed hydrolysis rate was actually slower than the buffer-catalyzed rate. A possible explanation – precipitation of buffer by Cu<sup>2+</sup> leading to less buffer catalysis – could account only for a small part of this effect given the large buffer to Cu<sup>2+</sup> molar ratio. The slower reaction with Cu<sup>2+</sup> is reproducible, and as yet unexplained.

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

*p*-Nitrotrifluoroacetanilide (11), an activated amide, has been used previously [19], in cyclodextrin promoted hydrolysis experiments. Effects of various metal ions with  $\beta$ CD–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  $\beta$ CD–cyclen·Cu<sup>2+</sup> complex, the rate of amide hydrolysis was 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  $\beta$ CD–cyclen·M<sup>2+</sup> complexed reactions at neutral pH.

## Experimental Section

### 4.1. 6-DEOXY-6(1,4,7,10-TETRAAZACYCLODODECYL)- $\beta$ -CYCLODEXTRIN (6).

A solution of the 6-*O*-monotosyl derivative of  $\beta$ CD (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 azamacrocyclic could be recovered from the ethanol filtrate (300 mg, 60%). The crude product was dissolved in H<sub>2</sub>O (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<sub>4</sub>HCO<sub>3</sub> (from 0.05 M to 0.60 M) [13]. The fractions (20 mL each) were analyzed by TLC (silica; AcOH/CHCl<sub>3</sub>/H<sub>2</sub>O, 8:1:1), and cyclodextrin-containing spots were made visible using a MeOH/AcOH/H<sub>2</sub>SO<sub>4</sub>/*p*-anisaldehyde (200:20:10:1) spray. Fractions 81–150 were combined and lyophilized to obtain a white solid (6; 455 mg, 49%): <sup>1</sup>H NMR (D<sub>2</sub>O) δ 2.5–3.0 (*m*, 16 H, azamacrocyclic), 3.2–4.0 (*m*, 42 H, H<sub>2</sub>, H<sub>3</sub>, H<sub>4</sub>, H<sub>5</sub>, H<sub>6</sub>), 4.9–5.2 (*m*, 7 H, H<sub>1</sub>); FAB mass spectrum, *m/z* 1289 (*M* + 1).

*Anal.* Calculated for C<sub>50</sub>H<sub>88</sub>N<sub>4</sub>O<sub>34</sub>·7.5 H<sub>2</sub>O: C, 42.16; H, 7.28; N, 3.93. Found: C, 41.90; H, 6.88; N, 4.32.

#### 4.2. 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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