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Synthesis and Transacylating Reactivity of β-Cyclodextrin Ethylenediamines

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Abstract—The synthesis of the ethylenediamine-connected cyclodextrin dimer is reported, together with the syntheses of several reference cyclodextrinylamines. Each compound displayed enhanced transacylation or transphosphorylation of activated substrates, with the primary amine-bearing monocyclodextrin compound showing the greatest activity. No special rate advantage was observed for this cyclodextrin dimer, although such effects do exist in other cycloextrin dimers reported previously.

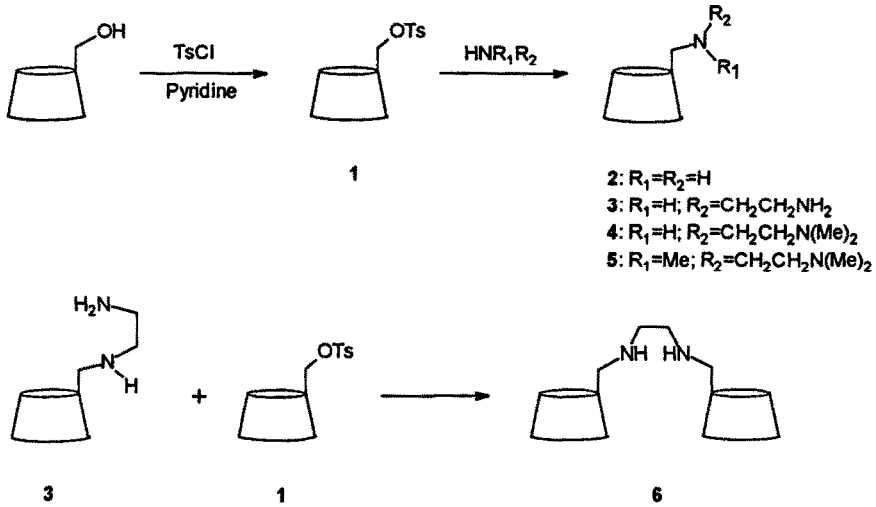
Introduction

The employment of cyclodextrin derivatives as enzyme models in biomimetic chemistry continues to provide insight for the design of artificial enzymes.[1] One goal of this field is to develop synthetic species that will hydrolyze unactivated esters, amides, and phosphoesters at ambient temperatures in neutral aqueous solutions. Additionally, the ability to perform selective cleavage while observing true catalytic behavior is highly desirable. Towards this goal, cyclodextrins have been synthesized containing imidazole which show reactivity at neutral pH,[2] and cyclodextrins containing metal coordination groups have similarly been utilized.[3] Systematic studies exploiting cyclodextrins derivatized with simple amines have not been reported heretofore. Our interest in this type of study stems from the potential use of a cyclodextrin dimer linked by an ethylenediamine unit as a metalloenzyme mimic. Cyclodextrin dimers that allow synergistic binding have been described previously,[4] and enzyme-like turnover behavior has been recently reported by Breslow. Multiple binding interactions in such hosts may locate a reactive functional group into close proximity to a potential catalytic group. In this regard, we now report the syntheses, characterizations, and reactivities of aminocyclodextrins **2**–**5** and of cyclodextrin dimer **6**.

Synthesis

Compounds **2**–**6** were synthesized as outlined in Scheme I. The synthesis of 6-amino-β-cyclodextrin via an azide derivative has been reported previously.[5] Mono-azide-β-cyclodextrin was formed from substitution of **1** with sodium azide and conversion to **2** was achieved with sodium borohydride using this method in overall 8 % yield. Our approach involved substitution of **1** directly to the desired product **2**. β-Cyclodextrin was stirred in pyridine and treated with tosyl chloride; crystallization from water afforded the monotosylate. This mixture was dissolved in concentrated ammonium hydroxide to effect substitution. Purification was achieved by cation exchange chromatography to afford pure monoamine **2** in 13 % yield based on β-cyclodextrin.

The ethylenediamine derivatives, **2**–**5**, were all synthesized in an adaptation of Matsui's procedure.[6] The tosyl group of **1** was displaced by reaction in neat solution of the appropriate amine precursor while heating at 75 °C in a



Scheme I. Synthetic scheme for the preparation of the cyclodextrin amines described in this study.

sealed tube. The reaction solution was concentrated, precipitated from alcohol and purified by cation exchange chromatography. *N*-Methylated derivatives **4** and **5** were crystallized from water to provide clear needles. The ^1H NMR of these derivatives exhibit peaks shifted upfield for the substituted C(6) hydrogens, which are resolved from the bulk of the cyclodextrin peaks. Additionally, the ethylene bridge protons are well resolved, allowing for their easy assignment. Shifts in the ^{13}C NMR spectra were as expected with the methylenes next to primary amines furthest upfield.

Forming target compound **6** was attempted by direct condensation of a single diaminoethane bridge with **1** under numerous conditions without evidence (TLC or NMR) of efficient product formation. Based on Tabushi's work with duplex cyclodextrin,[4a] a sequential approach was utilized to provide the desired product. Compound **3** was reacted with 2 mole equivalents of **1** in DMF. Multiple precipitations and cation exchange chromatography afforded **6**. Initial evidence for product formation was provided by TLC; further structural proof was observed in the ^1H NMR, which showed the appearance of a new singlet at ~2.2, assigned to the linking methylenes concurrent with a disappearance of ethylene peaks observed for **3**. This peak's shift was sensitive to the pH of the solution, indicating the proximity of these hydrogens to a protonation site. A single peak at 45.6 ppm was observed in the ^{13}C NMR consistent with a compound symmetric about a rotational axis through the ethylene bridge. Additional confidence in the structural assignment was achieved using the ^{13}C–^1H correlation spectrum; a signal was observed indicating correlation of the ^1H peak at 2.2 ppm to the carbon at 45.6 ppm.

The final compound synthesized during, the course of this work was the substrate *N*-(*p*-nitrophenyl)-*p*-*tert*-butylbenzamide (PNPTBA), seen in Figure 1. This compound was synthesized by conversion of *p*-*tert*-butylbenzoic acid to the related acid chloride with thionyl chloride. *p*-Nitroaniline was introduced in the presence of pyridine to effect the condensation. Excellent yields of PNPTBA as clear crystals were thus achieved.

Kinetic Evaluation

Our initial kinetic evaluations of transacylation were performed using *p*-nitrophenylacetate (PNPA). The *p*-nitrophenol substrates were chosen for this and subsequent studies for a number of reasons. First, this system provides a convenient chromophore for monitoring the reaction. The deprotonated phenoxide ion has a UV peak maximum at 398 nm and is strongly absorbing (ε = 9,000 M^{-1} cm^{-1} at pH 7.0). The system is also activated, which provides reaction within a reasonable time frame for preliminary studies.

The transacylation reactions were performed with a large excess of acyl acceptor so that pseudo-first order reaction behavior was followed. Using **2** and PNPA at 5.0 x 10^{-5} M, a saturation study was executed at room temperature and pH 7.0 in 0.1 M bis-tris-propane (BTP) buffer. The resulting data (Figure 2) indicates that at 20 mM **2**, nearly all substrate is associated with a host molecule.

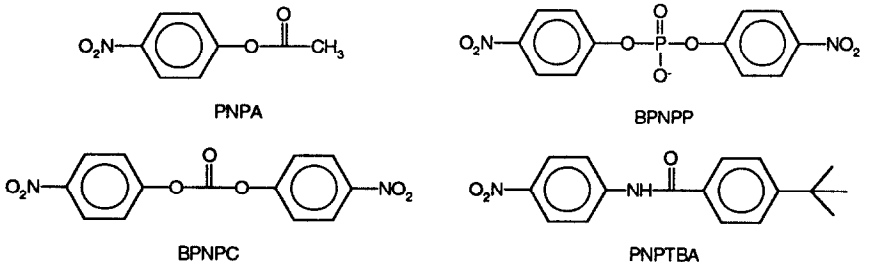


Figure 1. Substrates examined in this study.

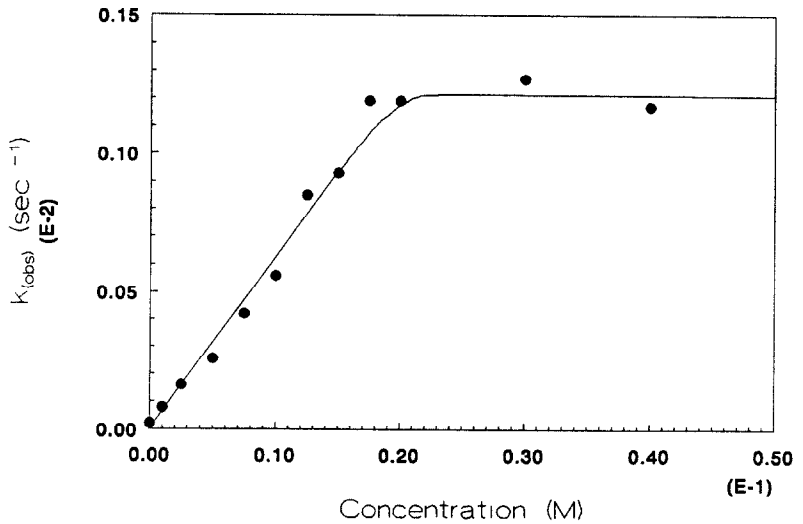


Figure 2. Saturation study of transacylation of PNPA with **2**.

The reactivities of compounds **2–5**, β-cyclodextrin, and methylated ethylenediamine derivatives were examined under these conditions. The results are summarized in Table 1. Under these saturating conditions, cyclodextrinamine **3** afforded the greatest rate enhancement (k_{obs}/k_{buffer} = 150). Interestingly, *N,N*-dimethyl analogs **4** and **5** showed very little rate enhancement, and their reactions were slower than the non-cyclodextrin control reaction using *N,N,N'*-trimethylethylenediamine. *N,N,N',N'*-Tetramethylethylenediamine (TMEDA) afforded almost no acceleration. TMEDA, **4** and **5** may be too sterically congested for efficient attack at the carbonyl site. The pK_a's for **3** are predicted to be 9.1 and 5.8[7] with the first protonation at the secondary amine, which may provide general acid catalysis to stabilize the tetrahedral intermediate formation. Evidence for formation of an acylated cyclodextrin product was seen by TLC and mass spectra.

In order to investigate the ability of our duplex cyclodextrin to enhance rates of reaction, suitable ditopid substrates were needed. The readily available *bis*-(*p*-nitrophenyl)carbonate (BPNPC) fulfilled the requirement for two hydrophobic moieties that bind in the cyclodextrin cavity situated at either end of a potential reactive center. Additionally, the reactivity of this substrate makes it ideal for initial investigations. The results obtained were averages of at least three kinetic runs. Only consistently reproducible experiments are described in Table 2. The copper containing reaction conditions are a result of some trial and error. Ideally, we would investigate this reaction at saturation levels, but this would require copper ion concentrations in the 20 mM range. Solutions of this concentration can be made in BTP, but the buffer binds the metal and decreases its availability to chelate with the ethylenediamine units of our catalyst. To lessen buffer competition for the metal, a non-chelating buffer, HEPES, was employed. Initially, mM level concentrations were used, but precipitation of the metal hydroxide forced the use of concentrations no greater than 0.5 mM. As long as the [Cu^{2+}] >> [substrate], first order behavior should be observed.

The last substrate examined was a phosphodiester because of its obvious relevance in biological transformations. The substrate examined was *bis*-(*p*-nitrophenol)-phosphate, which should bind in the cavity of **6** based on CPK models. The results of these inquiries are presented in Table 3. The rate enhancements observed for **3** and **6** are very large, 10^6-fold over the uncatalyzed reaction. The copper(II) containing reactions were slowed once again, but these systems still improved the rate of reaction.

The transacylation reaction with PNPTBA was also investigated. Typically, amide bonds are very stable ($t_{1/2}$ = 2 years). PNPTBA proved exceptionally stable, with no cleavage of the C–N bond observed, utilizing any of our cyclodextrin models with or without copper(II) after one month. Further investigation of this substrate was not pursued. A brief inspection of **3**'s ability to hydrolyze the pesticide parathion was executed. No catalysis was observed under the conditions typically used for these reactions.

Conclusion

The synthesis and complete characterization of a series of β-cyclodextrin ethylenediamines has been accomplished along with an investigation into their rate enhancing abilities for transacylation and transphosphorylation reactions. Compound **3**, which presents a primary amine group, distinguished itself as the most effective promoter of these reactions. While rate advantages in different cyclodextrin duplexes have been observed, the previously unreported duplex **6** did not display enhanced reactivity towards the substrates investigated even at subsaturation concentrations.

Table 1. Transacylation reaction of *p*-nitrophenyl acetate at pH 7.0.[a]

Acyl Acceptor	$10^4 k_{obs}$, s^{-1}	k_{obs}/k_{buffer}	$t_{1/2}$, min
---------	0.22[b]	1.0	530
CD[c]	0.24[b]	1.1	480
Me-en[d]	5.7	26	20
Me_3-en[e]	3.9	16	30
TMEDA[f]	.33	1.5	350
2	16.0	73	7.2
3	34	150	3.4
4	0.95	4.3	120
5	0.97	4.4	120

[a]All solutions were 50 μM in *p*-nitrophenyl acetate and 20 mM in acyl acceptor. Reactions were carried out at 23 °C in 0.2 M bis-tris-propane buffer solutions at pH 7.0. [b]*J. Incl. Phenom. Mol Rec. Chem.* **1991**, *10*, 119. [c]CD refers to β-cyclodextrin. [d]Me-en refers to *N*-methylethylenediamine. [e]Me-en refers to *N,N,N'*-trimethylethylenediamine. [f]TMEDA refers to *N,N,N',N'*-tetramethylethylenediamine.

Table 2. Transacylation reaction of *bis*-(*p*-nitrophenyl)carbonate at pH 7.0.

Acyl Acceptor	Acyl Acceptor Concentration (mM)	$10^3 k_{obs}$, s^{-1}	k_{obs}/k_{buffer}	$t_{1/2}$, s
---------[a]	20	0.95[b]	1.0	730
Me-en[a,c]	20	3.7	3.9	190
2[a]	20	110	120	6.1
3[a]	20	190	200	3.7
5[a]	20	3.4	3.6	200
---------[d]	0.5	0.36	1.0	1900
CD[d,e]	0.5	0.50	1.4	1400
3[d]	0.5	9.4	26	74
5[d]	0.5	0.77	2.1	900
6[d]	0.5	9.8	27	71
Cu^{++}[f]	0.5	0.51	1.4	1400
3, Cu^{++}[d,f]	0.1	6.0	17	120
6, Cu^{++}[d,f]	0.1	3.2	8.9	220
6, Cu^{++}[d,f]	0.01	0.85	2.4	820

[a]Solutions were 50 μM in *bis*-(*p*-nitrophenyl)carbonate. Reactions were carried out at 23 °C in 0.2 M bis-tris-propane buffer solutions at pH 7.0. [b]*J. Incl. Phenom. Mol Rec. Chem.* **1991**, *10*, 119. [c]Me-en refers to *N*-methylethylenediamine. [d] Solutions were 25 μM in *bis*-(*p*-nitrophenyl)carbonate. Reactions were carried out at 23 °C in 0.05 M HEPES buffer solutions at pH 7.0. [e]CD refers to β-cyclodextrin. [f]Cu^{2+} ions from copper(II) perchlorate to give final concentration of 0.5 mM.

Table 3. Transphosphorylation reactions of *bis*-(*p*-nitrophenyl)carbonate at pH 7.0.[a]

Catalyst	Conc. (mM)	$10^3 k_{obs}$, h^{-1}	k_{obs}/k_{buffer}	Half-life (h)	% Complete[e]
---[b]	0.5	.000069	1.0	~1 x 10^7	3
Cu^{++}	0.5	---	---	---	32
CD[c]	1.0	---	---	---	21
CD/Cu^{++}[c]	1.0	---	---	---	39
en[d]	0.5	1.6	24000	420	95
en/Cu^{++}[d]	0.5	---	---	---	42
3	0.5	6.1	90000	110	100
3	1.0	9.2	130000	75	96
3/Cu^{++}	0.5	3.3	47000	210	96
3/Cu^{++}	1.0	1.1	15000	650	90
6	0.5	4.7	68000	150	78
6/Cu^{++}	0.5	2.2	32000	310	100
2	0.5	---	---	---	9
2/Cu^{++}	0.5	---	---	---	22
5	0.5	---	---	---	4
5/Cu^{++}	0.5	---	---	---	34

[a]Solutions were 100 μM in *bis*-(*p*-nitrophenyl)phosphate. Reactions were carried out at 23 °C in 0.05 M HEPES buffer solutions at pH 7.0. Cu^{2+} is from copper(II) triflate to give final concentration of 0.5 mM. [b]Extrapolated from rate measurement at 100 °C, S = –25.5 e.u. in Kirby, A. J.; Younas, J. *J. Chem. Soc. B* **1970**, 510. [c]CD refers to β-cyclodextrin. [d]en refers to ethylene diamine. [e]Calculated based on expected complete reaction after 2000 h.

Experimental Section

General

Microanalyses were carried out at Atlantic Microlab, Inc. (Norcross, GA). Mass spectra were obtained by use of a Kratos-30 mass spectrometer. FT–NMR spectra were obtained at 500 MHz or 250 MHz. Most of the chemicals used in this study were obtained from Aldrich Chemical (Milwaukee, WI). Biological buffers (HEPES, bis-tris propane) were purchased from Sigma Chemical (St. Louis, MO). 6-Monotosyl-β-cyclodextrin (**1**) was prepared as described previously.[8] Thin layer chromatography was performed on aluminum backed silica gel plates that were visualized using a *p*-anisaldehyde:methanol:acetic acid:sulfuric acid solution (1:200:20:10) stain followed by heating to produce a blue to black colored spot. The best solvent system for TLC of these amino cyclodextrin compounds was a solution of *n*-butanol:methanol: water:concentrated ammonium hydroxide (4:3:2:3). β-Cyclodextrin exhibited an R_f = 0.28 while the tosylate derivative ran slightly ahead at R_f = 0.35 under these conditions.

Kinetic method

The hydrolysis reactions were followed by measuring the absorbance change at 398 nm for the reactions that produce *p*-nitrophenoxide. The formation of *p*-nitroaniline was followed at 400 nm. Reactions were monitored to > 95 % completion for the acetate and carbonate, and the amide and phosphate hydrolysis were monitored for > 3.5 half-lives when possible; an endpoint was calculated based on the *p*-nitrophenoxide extinction coefficient. 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.

Mono-(6-amino-6-deoxy)-β-cyclodextrin (2)

β-Cyclodextrin (75.0 g, 66 mmol) was stirred in pyridine (500 mL). Tosyl chloride (39.4 g, 210 mmol) was added portionwise with cooling as needed to keep near rt, and the resulting solution was stirred for 20 h. Water (50 mL) was added and stirred for 1 h. The solution was concentrated *in vacuo* to give a glassy solid that was dissolved in hot water (500 mL) and allowed to cool overnight in the refrigerator. Filtration yielded a white solid that was dissolved in concentrated ammonium hydroxide (500 mL). The flask was sealed with a septum, and the solution was stirred for 4 days at rt. Concentration *in vacuo* afforded a white solid that was dissolved in water (200 mL). Application of this solution to a Sephadex-CM column (25 cm long x 6 cm diameter) was performed by pipette, and the column was eluted using a 0.05 M to 0.5 M aqueous ammonium bicarbonate buffer gradient. Fractions (22 mL) were collected until 4 L of buffer had been eluted. Spotting of every fifth tube on silica gel aluminum-backed plates was performed, and the cyclodextrin containing fractions were pooled, concentrated *in vacuo*, and lyophilized to afford **2** as a fluffy white solid (9.55 g, 13 %): R_f 0.26; ^{1}H NMR (D_2O) δ 2.6–3.0 (br m, 2.1H, N-CH_2), 3.3–3.9 (m, 40H, CD-*H*), 4.9 (br m, 7H, anomeric H); ^{13}C-NMR (DMSO-d_6) δ 42.0, 60.2, 72.4, 72.6, 73.3, 81.8, 81.9, 83.3, 102.3; FAB mass spectrum, m/e 1131.49 $(M+1)^+$. Anal. Calcd for $C_{42}H_{71}O_{34}N$ + $6H_2O$: C, 40.61; H, 6.74; N, 1.13. Found: C, 40.49; H, 6.46; N. 1 .11.

Mono-(6-aminoethylamino-6-deoxy)-β-cyclodextrin (3)

Mono-(6-tosyl)-β-cyclodextrin (0.543 g, 0.42 mmol) was stirred in neat ethylenediamine (5 mL, 75 mmol) in a sealed pressure tube heated on an oil bath at 75 °C for 6 h. The solution was concentrated *in vacuo* to dryness and dissolved in water (2 mL), which was added dropwise to absolute ethanol (100 mL), and the resulting white solid was collected by vacuum filtration. Application of an aqueous solution of this solid (100 mL) to a Sephadex-CM column (25 cm long x 6 cm diameter) was performed via pipette. The column was eluted using a 0.05 M to 0.5 M aqueous ammonium bicarbonate buffer gradient. Fractions (22 mL) were collected until 4 L of buffer had been eluted. Spotting of every fifth tube on aluminum-backed silica gel plates was performed, and the cyclodextrin containing fractions 50 to 80 were pooled, concentrated *in vacuo*, and lyophilized to afford **3** as a fluffy white solid (0.201 g, 40 %): R_f 0.20; ^{1}H NMR (D_2O) δ 2.6–3.0 (br m, 6.3H, N-CH_2), 3.3–3.9 (m, 42.9H, CD-*H*), 4.9 (br m, 7H, anomeric H), ^{13}C-NMR (D_2O) δ 42.3, 52.0, 52.6, 63.1, 73.2, 74.6, 74.9, 75.9, 83.7, 83.9, 86.3, 104.3, 104.6. FAB mass spectrum, m/e 1177.43 $(M^+ + 1)$. Anal. Calcd for $C_{44}H_{76}O_{34}N_2$ + 8.0 H_2O: C, 40.00, H, 7.02, N, 2.12. Found: C, 40.01; H, 6.55; N, 2.08.

Mono-(6-(N,N-dimethyl)-aminoethylamino-6-deoxy)-β-cyclodextrin (4)

Mono-(6-tosyl)-β-cyclodextrin (1.21 g, 0.93 mmol) was stirred in *N,N*-dimethylethylenediamine (10 mL, 100 mmol) in a sealed pressure tube heated on an oil bath at 75 °C for 20 h. The solution was concentrated *in vacuo* to dryness and dissolved in water (3 mL). The aqueous solution was added dropwise to isopropyl alcohol (100 mL), and the solid was collected by vacuum filtration. Application of an aqueous solution of this solid (100 mL) to a Sephadex-CM column (25 cm long x 6 cm diameter) was performed via pipette. The column was eluted using a 0.05 M to 0.5 M aqueous ammonium bicarbonate buffer gradient. Fractions (22 mL) were collected until 4 L of buffer had been eluted. Spotting of every fifth tube on aluminum-backed silica gel plates was performed, and the cyclodextrin containing fractions 41 to 55 were pooled, concentrated *in vacuo*, and lyophilized to afford a fluffy white solid. Recrystallization from water afforded clear needles (0.478 g, 43 %): R_f 0.19, ^{1}H NMR (D_2O) δ 2.2 (s, 6H, N-CH_3), 2.3–2.5 (m, 2H, N-CH_2), 2.6–2.8 (m, 3H, N-CH_2), 3.0 (br d, 1H, CD-CH_2-N), 3.4 (t, 1H, CD-*H*), 3.3–3.9 (m, 37H, CD-*H*), 4.9 (br m, 7H, anomeric H), ^{13}C-NMR (DMSO-d_6) δ 45.3 (q), 46.9 (t), 49.4 (t), 58.9 (t), 60.0 (t), 70.6 (d), 72.1 (d), 72.5 (d), 73.1 (d), 81.66 (d), 83.6 (d), 101.8 (d), 102.0 (d), 102.2 (d), FAB mass spectrum, m/e 1205.52 $(M^+ + 1)$. Anal. Calcd for

$C_{46}H_{80}O_{34}N_2$ + 6.0 H_2O: C, 42.08; H, 7.06, N, 2.13. Found: C, 42.07, H, 6.75; N, 2.08.

Mono-(6-(N,N,N'-trimethyl)-aminoethylamino-6-deoxy)-β-cyclodextrin (5)

Mono-(6-tosyl)-β-cyclodextrin (1.03 g, 0.79 mmol) was stirred in neat *N,N,N'*-trimethylethylenediamine (10 mL, 90 mmol) in a sealed pressure tube and heated on an oil bath at 75 °C for 30 h. The solution was concentrated *in vacuo* to dryness and dissolved in water (3 mL). The aqueous solution was added dropwise to absolute ethanol (100 mL), and the resulting white solid was collected by vacuum filtration. Application of an aqueous solution of this solid (150 mL) to a Sephadex-CM column (25 cm long x 6 cm diameter) was performed via pipette. The column was eluted using a 0.05 M to 0.5 M aqueous ammonium bicarbonate buffer gradient. Fractions (22 mL) were collected until 4 L of buffer had been eluted. Spotting of every fifth tube on aluminum-backed silica gel plates was performed, and the cyclodextrin containing fractions 40 to 70 were pooled, concentrated *in vacuo,* and lyophilized to afford a fluffy white solid. Recrystallization from water afforded clear needles (0.534 g, 55 %): R_f 0.19; ^{1}H NMR (D_2O) δ 2.0 (s, 6H, CH_2-N-CH_3), 2.1 (s, 3H, CD-N-CH_3), 2.2–2.8 (br m, 6H, N-CH_2), 3.2 (br t, 1H, CD-*H*), 3.3–3.9 (m, 39H, CD-*H*), 4.9 (br m, 7H, anomeric H); ^{13}C NMR (DMSO-d_6) δ 43.3, 45.4, 55.9, 57.0, 58.4, 59.9, 70.3, 72.1, 72.4, 73.0, 73.4, 81.2, 81.5, 84.1, 101.6, 102.0. FAB mass spectrum, m/e 1219.55 (M^++1). Anal. Calcd for $C_{47}H_{82}O_{34}N_2$ + 10.0 H_2O: C, 40.34; H, 7.34; N, 2.00. Found: C, 40.13; H, 7.24; N, 1.95.

N,N'-*Bis-6-(6-deoxy-β-cyclodextrinyl)ethylenediamine (6)*

Compound **3** (0.402 g, 0.34 mmol) and **1** (1.00 g, 0.77 mmol) were dissolved in DMF (3 mL) with K_2CO_3 (0.400 g) in a sealed pressure tube, and the reaction was heated in an oil bath at 80 °C for 19 h. The resulting solution was gravity filtered into acetone (1000 mL), and the precipitate (1.1 g) was collected by suction filtration. Application of an aqueous solution of this solid (100 mL) to a Sephadex-CM column (25 cm long x 6 cm diameter) was performed via pipette. The column was eluted using a 0.05 M to 0.5 M aqueous ammonium bicarbonate buffer gradient. Fractions (22 mL) were collected until 4 L of buffer had been eluted. Spotting of every fifth tube on aluminum-backed silica gel plates was performed, and the cyclodextrin containing fractions 45 to 50 were pooled, concentrated *in vacuo,* and lyophilized to afford a fluffy white solid (0.111 g, 34 %): R_f 0.09; ^{1}H NMR (D_2O) δ 2.2 (s, 6H, CH_2-N), 2.6 (br t, 1H, N-CH_2-CD), 2.9 (br m, 2H, N-CH_2-CD), 3.3 (br t, 2H, CD-*H*), 3.3–3.9 (m, 80H, CD-*H*), 4.9 (br m, 14H, anomeric H); ^{13}C-NMR (D_2O) δ 15.6, 60.0, 61.2, 70.0, 72.8, 73.0, 73.7, 73.9, 81.2, 82.0, 84.8, 102.0, 102.8. FAB mass spectrum, m/e 2293.83 (M^++1, 3.6 %); 1162.42 (M^+-**2**, 100 %).

N-*(p-Nitrophenyl-p-tert-butylbenzamide (PNPTBA)*

tert-Butylbenzoic acid (5.00 g, 0.029 mol) was dissolved in anhydrous benzene (125 mL) and stirred at reflux with thionyl chloride (15 mL) for 1 h. The solution was concentrated *in vacuo*, redissolved in anhydrous benzene (50 mL), and concentrated to dryness. The solid was dissolved in anhydrous benzene (50 mL) with dry pyridine (3 mL). A solution of 4-nitroaniline (4.27 g, 0.031 mol) in benzene (50 mL) was added to the stirred solution and warmed at reflux for 18 h. The solution was concentrated to dryness *in vacuo* to afford an orange solid that was dissolved in chloroform (125 mL), washed with pH 8 water (2 x 75 mL), pH 5 water (2 x 75 mL) and brine (2 x 100 mL). The organic layer was dried over sodium sulfate and concentrated to dryness. Recrystallization from benzene (90 mL) afforded white crystals (6.16 g; 74 %) mp 159–161 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9H, *t*Bu), 7.51 (d, 2H, Ar*H*), 7.8 (dd, 1H, Ar*H*), 8.2 (br s, 1H, N*H*), 8.23 (d, 2H, Ar*H*), ^{13}C NMR (DMSO-d_6) δ 31.1, 35.1, 119.4, 125.1, 126.0, 127.0, 131.0, 143.5, 143.9, 156.4, 165.8; ElMS, m/e 298.1 (M^+, 28 %), 161.4 (M^+-NO_2-C_6H_4-NH, 100 %). Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.32; H, 6.10; N, 9.34.

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