(Ethylenediaminetetraacetic Acid)cerium(IV) [Ce^{IV}(EDTA)] Complexes with Dual Hydrophobic Binding Sites as Highly Efficient Catalysts for the Hydrolysis of Phosphodiesters

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β-Cyclodextrin (β-CD) derivatives **1** with an amino group at C(6), C(3), or C(2) were homogeneously linked together by an ethylenediaminetetraacetic acid (EDTA) bridge (*Scheme*). Coordination of the linker to metal ions and cooperation of the dual cavities of **3** in binding hydrophobic guests were properly demonstrated by NMR techniques and a fluorescence-based titration method, respectively. The hydrolysis of bis(4-nitrophenyl) phosphate (BNPP) in the presence of Ce^{IV} complexes of β-CD dimers **3** was tens of millionfold faster than that in the absence of the Ce^{IV} complexes. Hydrophobic binding of the β-CD cavities was estimated to contribute to the catalysis by a factor of up to 520, and the type of modified sugar unit and the bridging positions influenced this cooperation between the β-CD moieties and the catalytic metal center.

Introduction. – The hydrolysis of phosphodiesters is crucial in many important biochemical processes [1], and has, therefore, attracted increasing interest. During the last decades, enormous effort has been directed to the development of catalysts for this chemical transformation. Since metal ions are found to play a pivotal role in the active sites of many natural nucleases [2], it is not surprising that the majority of catalysts developed so far to catalyze the hydrolysis of phosphodiesters take advantage of metal ions [3]. Their effective mediation of substrate hydrolysis has marked the lanthanides [4], and especially Ce^{IV}, as entities for special attention [5]. However, formation and precipitation of cerium(IV) hydroxide gels usually occur above pH 4 and complicate kinetics studies of the Ce^{IV}-mediated hydrolysis of phosphodiesters under physiological condition. Complex formation of the Ce^{IV} ion offers a possibility to solve this problem. It has been reported that, in the presence of excess γ-cyclodextrin, the Ce^{IV} solutions remained homogeneous around pH 7 and could catalyze the hydrolysis of peptides and phosphates [6–7]. *Moss* and co-workers obtained homogeneous solutions under aqueous micellar conditions to accelerate the reaction of phosphodiester [8]. Ethylenediaminetetraacetic acid (EDTA) and its derivatives, which constitute one of the main classes of lanthanide receptors [9], can form a stable complex with Ce^{IV}. On the other hand, cyclodextrins are widely used to build receptors capable of binding varieties of hydrophobic substrates in aqueous solution [10]. By combining these two features, we synthesized EDTA-bridged β-cyclodextrin dimers **3**[2]) and found that the

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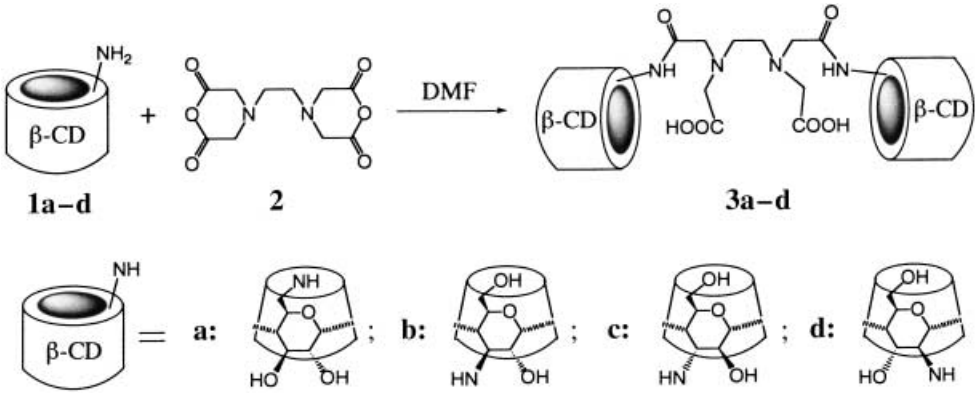
[2]) For convenience, the term 'β-cyclodextrin' or 'β-CD' is used for all cycloheptakis(1 → 4)-(glycosyl) derivatives, *i.e.*, for all cyclic oligosaccharides containing glucoside- or altroside-type sugar units.

hydrophobic binding sites greatly contribute to the efficient catalysis by the Ce^{IV} complexes of these β-cyclodextrin dimers in the hydrolysis of phosphodiester.

Results and Discussion. – *Synthesis.* The β-cyclodextrin dimers **3** have the EDTA linker attached to either the primary or secondary face, and the modified sugar unit of the β-cyclodextrin moieties is of either the glucoside or the altroside type. It is known that β-cyclodextrin has a rather rigid hydrophobic cavity, and its glucoside units adopt exclusively a $^4C_1$ conformation which can hardly flip to the $^1C_4$ conformation. However, an altroside unit needs only *ca.* 8 kcal · mol^{-1} to produce the $^4C_1 \rightleftarrows C_4$ flip, thus the cyclodextrin residues of dimers **3c** and **3d** are expected to have conformational flexibility. The synthetic route to the dimers **3a**–**d** is shown in the *Scheme*. The aminocyclodextrins **1a**–**d** were synthesized from β-cyclodextrin according to literature procedures [11]. Reactions of EDTA dianhydride **2** with a slight excess of the aminocyclodextrins **1a**–**d** were performed in dry DMF at room temperature. Subsequent separation of the reaction mixtures on a reversed-phase *Lobar* column provided **3a**–**d** in 70–90% yields. Their structures were established by spectroscopic means. In both the ^{1}H- and ^{13}C-NMR spectra, the signals related to the modified sugar units of **3c** and **3d** were obviously broader than those of **3a** and **3b**. Compound **3c** even hardly showed obvious peaks for C(4A) and C(5A) of the altroside unit at room temperature. This fact is indicative of the conformational flexibility of the cyclodextrin cavities of dimers **3c** and **3d**.

Scheme. *Synthesis and Structure of the EDTA-Bridged β-Cyclodextrin* (β-CD) *Dimers*[2]. The β-CD residues are composed of one modified sugar unit (see **a**–**d**) and six glucoside-type residues.



All β-cyclodextrin dimers **3a**–**d** demonstrated the expected molecular-ion peak at m/z 2523 in the FAB-MS. In the ^{1}H-NMR spectra, the EDTA moiety showed a broad *s* at 3.2–3.3 ppm for the CH_2CH_2 protons. Its CH_2CO protons overlapped with the cyclodextrin signals or appeared as *s* in the range 3.6–3.85 ppm. In the ^{1}H,^{13}C-COSY plots, one island was observed at *ca.* 3.25/52.4 ppm and two islands in the range 3.6–3.85/57–58 ppm for the CH_2CO residues. Two peaks always appeared at *ca.* 173.3 and 170 ppm for the carbonyl C-atoms. These facts are indicative of a C_2 symmetry. The ratio of the integration of the CH_2CH_2 *s* to that of all the anomeric protons (12 H–C(1) + 2 H–C(1A)) of the cyclodextrin moieties was usually *ca.* 1:3.5, consistent with the dimer structure. The normal shift patterns for the glucoside- and altroside-type units were basically demonstrated in both the ^{1}H- and ^{13}C-NMR spectra of all four compounds **3a**–**d**. Compared to the corresponding aminocyclodextrins, the N-bearing position displayed only a trivial shift for the C-atom, but a

great downfield shift (from 2.5–2.8 to *ca.* 3.7 or *ca.* 4.2 ppm) for the proton, which agrees well with acylation of the NH_2 group.

Metal-Complex Formation. It is reasonable to expect that the EDTA spacer of the β-CD dimer can act as multidentate ligand to chelate metal ions. Precipitation usually occurs in the aqueous solution of ceric(IV) nitrate above pH 4. However, the aqueous solution of **3** and $Ce(NH_4)_2(NO_3)_6$ in a 1:1 molar ratio remained homogeneous even at neutral pH, strongly suggesting complex formation between **3** and Ce^{IV}. In D_2O soln. buffered at pD 7 with 40 mM phosphate, **3a** demonstrated in the ^{1}H-NMR spectrum one broad *s* at 2.92 ppm for the CH_2CH_2 protons and another at 3.30 ppm for the CH_2CO protons of the EDTA spacer. Upon addition of 1 equiv. of $Ce(NH_4)_2(NO_3)_6$ to the NMR sample solution, the former *s* became very broad in the range 3–3.2 ppm, while the latter one was shifted downfield and overlapped with the β-CD signals.

Although these ^{1}H-NMR measurements afforded the direct evidence for complex formation, the solubility of the complex was not good enough to ensure the collection of ^{13}C- and 2D-NMR spectra required for the characterization of the complex. Fortunately, the complexes [Zn^{II} (**3**)] were more soluble and allowed us to do NMR measurement to probe the complex-formation ability. On addition of $Zn(NO_3)_2$ to the D_2O solution of **3c**, the broad *s* of the CH_2CH_2 protons was shifted from *ca.* 3.23 to *ca.* 2.86 ppm and split into two broad bands separated by 34 Hz, which implies that the two protonated (by the intramolecular COOH groups) amino groups deprotonate and coordinate to Zn^{2+} to form a five-membered ring which differentiates the geminal protons of the CH_2CH_2 moiety. Chelation is also indicated by the CH_2CO protons of the linker being shifted upfield by *ca.* 0.15 ppm. Upon complexation, the signals of the cyclodextrin moiety also demonstrated obvious changes. It is interesting to note that the broad peaks of H–C(3A) and H–C(5A) became sharp, and their splitting patterns became clear. The signals of the C(4A) and C(5A) atoms of the altroside units are clearly observed. These observations suggest that coordination of the linker significantly reduces the flexibility of the cyclodextrin cavity.

The H–C(5A) of the modified sugar unit demonstrated a 0.05 ppm downfield shift upon complexation, while H–C(2A) was shifted 0.05 ppm upfield. Even signals relating to other sugar units showed obvious shifts. One *d* at 4.93 ppm was shifted upfield to 4.89 ppm and overlapped with H–C(1A). Some of the signals in the range 3.88–3.67 ppm were shifted downfield and overlapped with H–C(4A).

Inclusion Complex Formation. The binding ability of dimers **3a–d** with the substrate bis(4-nitrophenyl) phosphate (BNPP) was investigated by UV titration. An association constant $K_a = 5.9 \cdot 10^4$ M^{-1} was obtained for the **3b** · BNPP binding, 78-fold larger than that of the β-CD · BNPP binding. The [Zn^{II} (**3b**)] complex also bound BNPP much tighter ($K_a = 5.1 \cdot 10^3$ M^{-1}) than β-CD. The UV changes of BNPP on binding with the other β-CD dimers or their Zn^{II} complexes were too small to allow determination of the binding constants. To investigate the inclusion complexation ability of these β-CD dimers, we employed the fluorescence guests sodium 6-{{4-[(4-aminophenyl)methyl]phenyl}amino}naphthalene-2-sulfonate (MON) and 6,6′-[methylenebis(4,1-phenyleneimino)]bis[naphthalene-2-sulfonate] (BIS). The two guests are almost fluorescence-silent in aqueous solution. When bound in the cyclodextrin cavity, they emit strongly at *ca.* 450 nm. By following the changes in fluorescence intensity upon addition of the host to the guest solution, the binding constants can be derived. As

shown in *Table 1*, all dimers formed 1:1 complexes with the guests MON and BIS, and the binding constants were larger than that of β-CD, indicating good cooperation of the two β-CD rings of **3** in binding the guests. Dimers **3a** and **3b** bound MON two orders of magnitudes stronger than β-cyclodextrin, whereas **3c** and **3d** showed only a slight enhancement relative to β-CD. The presence of the altroside units in **3c** and **3d** may account for this decrease in binding ability since it slightly distorts the hydrophobic cavity.

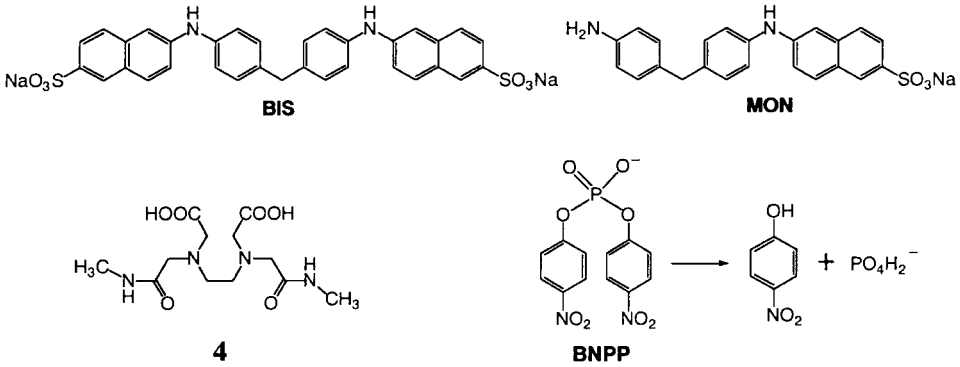


Table 1. *Binding Constants* [M^{-1}] *of* β*-CD and its Dimers* **3a**–**d** *with MON and BIS*[a])

	3a	**3b**	**3c**	**3d**	β-CD
MON	$7.59 \cdot 10^5$	$9.00 \cdot 10^5$	$3.10 \cdot 10^4$	$1.79 \cdot 10^5$	$9.35 \cdot 10^3$
BIS	$1.13 \cdot 10^6$	$1.08 \cdot 10^6$	$5.45 \cdot 10^5$	$5.46 \cdot 10^5$	not 1:1

[a]) Conditions: 0.05M carbonate buffer, pH 10.6, 0.2M KCl, 25°. The binding constants were measured by following the change in fluorescence intensity of the guests with the guest concentration fixed at $5.00 \cdot 10^{-6}$ M and those of the hosts varying in the range $9.0 \cdot 10^{-6} - 3.0 \cdot 10^{-5}$ M.

Catalytic Hydrolysis of Phosphodiester. The observation of the high acceleration of the hydrolysis of phosphates by La^{III} has aroused increasing interest in the use of lanthanides to promote the hydrolysis of phosphodiesters [12]. The apparent mechanistic feature of lanthanide-mediated hydrolysis of phosphates may involve a lanthanide cation functioning simultaneously as acid and base catalyst. As a *Lewis* acid, a lanthanide ion can interact with the oxide anion moiety of the phosphodiester substrate and lessen its negative charge; at the same time, it may deliver its coordinated hydroxide anion to attack the P-atom. Since the magnitude of charge on the metal ion directly influences the acidity of the bound water molecule, attention should be addressed to the Ce-atom, the only lanthanide with oxidation state +4 readily available. Here we focus on the nonoxidative hydrolysis of phosphodiester BNPP catalyzed by the Ce^{IV} complex of β-CD dimers **3a**–**d**.

The Ce^{IV} complexes were generated *in situ* by addition of stoichiometric amounts of $Ce(NH_4)_2(NO_3)_6$ to the EDTA ligands **3a**–**d**, and their catalysis of the hydrolysis of BNPP in HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) buffer solution (pH 7) at 25° was followed by the release of 4-nitrophenoxide at 400 nm. The reactions

were monitored over 4 half-lives. The observed pseudo-first-order rate constants are listed in *Table 2*. In the absence of metallocatalysts, BNPP is very stable at pH 7.0 and 25°, and decomposition by the buffer solution alone corresponds to a rate constant $k_{un} = 1.1 \cdot 10^{-11}\ s^{-1}$. However, when $2.75 \cdot 10^{-4}$ M [Ce^{IV}**3a**] was used, the k_{obs} increased to $6.17 \cdot 10^{-4}\ s^{-1}$, corresponding to a 56-millionfold acceleration. The Ce^{IV} complexes of the three other dimers **3b**–**d** also demonstrated comparable rate accelerations.

Table 2. *The Pseudo-First-Order Rate Constants* [s^{-1}] *for the Hydrolysis of BNPP*[a])

	Ce^{IV} complex with				
	3a	**3b**	**3c**	**3d**	**4** + β-CD
k_{obs}	$6.17 \cdot 10^{-4}$	$3.07 \cdot 10^{-4}$	$1.71 \cdot 10^{-4}$	$1.53 \cdot 10^{-4}$	$1.18 \cdot 10^{-6}$
Synergism[b])	520	260	145	130	1

[a]) [BNPP] = $2.00 \cdot 10^{-5}$ M, [dimer] = [Ce^{IV}] = $2.75 \cdot 10^{-4}$ M or [**4**] = 1/2 [β-CD] = [Ce^{IV}] = $2.75 \cdot 10^{-4}$ M. The kinetic measurements were performed at 25° by following the absorption change at 400 nm of the reaction solutions, which were buffered at pH 7.0 with 50 M HEPES, 0.2M KCl and remained clear during the measurements.

[b]) Synergism: $k_{obs}/(k_{obs})_{\mathbf{4}+\beta\text{-CD}}$

To verify the contribution of the cooperation of the hydrophobic binding sites and the Ce^{IV} complex, we prepared ligand **4** by replacing the cyclodextrin parts of **3** with nonbinding methyl groups, and used **4** in the control reaction. As is shown in *Table 2*, the control reaction with the mixture **4**/Ce^{IV}/β-CD (in a molar ratio of 1:1:2) as catalyst gave a k_{obs} of $1.18 \cdot 10^{-6}\ s^{-1}$, *i.e.*, a k_{obs} 520 times smaller than that observed with the catalyst [Ce^{IV} (**3a**)]. When ceric(IV) nitrate was used instead of the Ce^{IV} complexes, precipitation occurred, and the kinetic measurement could not be done. These data clearly demonstrate that covalent combination of a Ce^{IV} complex with cyclodextrin moieties can result in extensive cooperation of these functions in the cleavage of BNPP.

The dependence of the pseudo-first-order constants on the catalyst concentration was further investigated. For each catalyst, saturation of the substrate was observed (*Fig. 1*). Treatment of these kinetic data with the *Lineweaver-Burk* equation afforded the kinetics parameters (*Table 3*). All the catalysts showed a K_m around 0.1 mM, 2 orders of magnitude smaller than that for the hydrolysis of BNPP by ceric ion reported by *Moss* and *Ragunathan* [13]. The results indicate that the [Ce^{IV} (**3**)] complexes bind the substrate much stronger than the Ce^{4+} itself. Obviously, the hydrophobic cavities of the β-CD dimers enhance the binding of BNPP to the bound Ce^{4+}. Although all the catalysts bound BNPP with almost the same strength, their k_{cat} values were varied by several times and were 18- to 78-million times larger than k_{un}. The large enhancement of BNPP hydrolysis is surely related to the high charge/size ratio of the Ce^{4+} ion and the hydrophobic binding of the β-CD dimers. In *Fig. 2*, this cooperation is represented schematically. The high charge/size ratio intensifies the interaction of Ce^{4+} with the $P-O^-$ and acidifies bound H_2O, while the hydrophobic binding of the β-CD moieties directs the substrate close to the catalytic site, thus promoting the extremely strong transition-state (TS) binding demonstrated by the very smaller dissociation constant K_{TS}. The kinetic parameters suggest that the variation in bridging position and structure

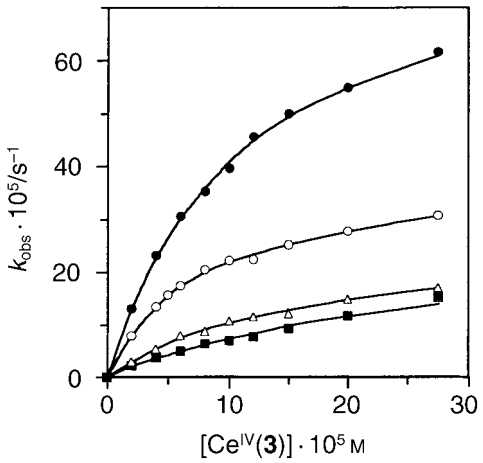


Fig. 1. *Saturation kinetics curves for the hydrolysis of BNPP by the Ce^{IV} complexes of* **3a** (●), **3b** (○), **3c** (△), and **3d** (■), *in 50 mM HEPES buffer solution* (pH 7) *at 25°*

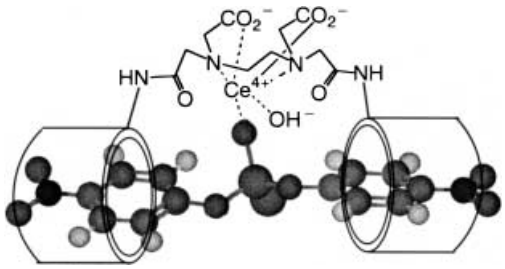


Fig. 2. *Schematic presentation for the cooperation of the hydrophobic binding with the catalytic functionality*

Table 3. Michaelis-Menten *Kinetics Parameters for the Hydrolysis of BNPP by [Ce^{IV}* (**3**)*]*

Ce^{IV} complex with:	k_{cat} [s^{-1}]	K_m [M]	k_{cat}/k_{un}	k_{cat}/K_m [$s^{-1} \cdot M^{-1}$]	K_{TS} [M]
3a	$8.62 \cdot 10^{-4}$	$1.11 \cdot 10^{-4}$	$7.84 \cdot 10^{7}$	7.75	$1.42 \cdot 10^{-12}$
3b	$3.92 \cdot 10^{-4}$	$7.80 \cdot 10^{-5}$	$3.57 \cdot 10^{7}$	5.03	$2.19 \cdot 10^{-12}$
3c	$2.85 \cdot 10^{-4}$	$1.74 \cdot 10^{-4}$	$2.59 \cdot 10^{7}$	1.63	$6.73 \cdot 10^{-12}$
3d	$1.97 \cdot 10^{-4}$	$1.68 \cdot 10^{-4}$	$1.79 \cdot 10^{7}$	1.17	$9.39 \cdot 10^{-12}$

of the modified sugar unit significantly affect the transition-state binding but not the ground-state binding, *i.e.*, they affect the cooperation in catalysis between the binding sites and catalytic center. The glucoside-type dimers bind in the transition state 3–6 times stronger than the altroside-type dimers. As a result, **3a** and **3b** showed a 3–7 times higher second-order-rate constants (k_{cat}/K_m) than **3c** and **3d** for the overall catalytic reaction.

Conclusions. – Two β-cyclodextrin moieties were linked by EDTA to produce the four β-cyclodextrin homo-dimers **3a**–**d**[2]). The bridge was attached either at C(6) and C(3) of a glucoside unit or to C(3) and C(2) of an altroside unit. Binding studies

indicated that the two cyclodextrin cavities cooperate well in binding ditopic hydrophobic guest molecules. NMR Investigations suggested that both amino N-atoms of the EDTA linker coordinate to Zn^{2+}, and a stable metal complex can be formed. The Ce^{4+} complexes of these β-cyclodextrin dimers, prepared *in situ* by mixing the ligands **3** with Ce^{4+} in a 1:1 molar ratio, demonstrated a dozens-of-millionfold rate enhancement in catalyzing the hydrolysis of bis(4-nitrophenyl) phosphate at neutral pH. The hydrophobic binding was estimated to contribute to the rate enhancement by a factor of up to 520 by careful comparison with the noninclusion reference ligand **4**. The catalysis followed *Michaelis-Menten* kinetics. All β-cyclodextrin dimers showed ground-state binding of the same strength. However, the transition-state binding, which was much stronger than the ground-state binding, differed by several times for different catalysts. As a result, the second-order rate constant (k_{cat}/K_m) of the dimer bridged at C(6) (**3a**) was 7 times that of **3d** for the overall catalytic reaction.This means that the type of modified sugar and its bridging site are obviously influencing the cooperation between the β-cyclodextrin cavities and the catalytic Ce^{4+} complex.

Experimental Part

General. Pyridine and DMF were dried over 4-Å molecular sieves. Other solvents and chemicals were of reagent grade and used as received from commercial sources. Reversed-phase column chromatography (CC): *Merck* prepacked *Lobar* column (*LiChroprep® RP-18*, size *B* or size *C*). UV/VIS Spectra: *Hitachi U-3000* spectrophotometer. ^{1}H- and ^{13}C-NMR Spectra: *Varian Unityplus-500* and *Jeol JNM-A-500* spectrometers; δ in ppm rel. to internal CH_3CN, J in Hz. FAB-MS: *Jeol JMSDX-303* spectrometer; in m/z.

Amino-β-cyclodextrins **1**. They were prepared according to literature procedures [11].

EDTA-Bridged β-Cyclodextrin Dimers: General Procedure. The amino-β-cyclodextrin **1** (1 mmol) and EDTA dianhydride **2** (=*4,4′-(ethane-1,2-diyl)bis[morpholine-2,6-dione]*) were dissolved in dry DMF (20 ml), and the mixture was stirred at r.t. for one day. H_2O (5 ml) was then added and the resultant mixture heated for 5 h at 80°. After evaporation, the residue was taken up in H_2O (500 ml) and filtered. The filtrate was then subjected to reversed-phase CC (gradient $H_2O \rightarrow 35\%$ H_2O/MeOH (1 l each)). The β-cyclodextrin dimer fractions were evaporated. Lyophilization of the residue afforded the desired product.

6A,6′A-(Ethane-1,2-diylbis{[(carboxymethyl)imino]-1-oxoethane-2,1-diylimino})bis[6A-deoxy-β-cyclodextrin] (**3a**): Yield 85%. R_f 0.20. ^{1}H-NMR (500 MHz, D_2O): 4.99–4.96 (*m*, 12 H–C(1), 2 H–C(1A)); 3.89–3.73 (*m*, 12 H–C(3), 12 H–C(5), 22 H–C(6), 2 H–C(3A), 2 H–C(5A), 2 CH_2COO); 3.71–3.66 (*m*, 2 H–C(6), 2 H–C(6A)); 3.62 (*s*, 2 CH_2CON); 3.59–3.54 (*m*, 12 H–C(2), 2 H–C(2A), 2 H–C(6A)); 3.51–3.44 (*m*, 12 H–C(4); 3.38 (*t*, J = 9.3, 2 H–C(4A)); 3.22 (*s*, CH_2CH_2). ^{13}C-NMR (125 MHz, D_2O): 173.0 (COO); 170.0 (CON); 102.6 (C(1), C(1A)); 83.6 (C(4A)); 82.1, 82.0 C(C(4)); 73.9, 73.6, 72.9, 72.8, 72.7 (C(2), C(3), C(5), C(2A), C(3A)); 70.7 (C(5A)); 61.2 (C(6)); 57.2 (CH_2COO); 57.0 (CH_2CON); 52.2 (CH_2CH_2); 40.8 (C(6A)). FAB-MS: 2523 ($[M+1]^+$).

3A,3′A-(Ethane-1,2-diylbis{[(carboxymethyl)imino]-1-oxoethane-2,1-diylimino})bis[3A-deoxy-β-cyclodextrin] (**3b**): Yield 71%. R_f 0.20. ^{1}H-NMR (500 MHz, D_2O): 5.03 (*d*, J = 3.63, 2 H–C(1A)); 5.00-4.98 (*m*, 10 H–C(1)); 4.86 (*d*, J = 3.63, 2 H–C(1)); 4.18 (*t*, J = 10.3, 2 H–C(3A)); 3.94–3.72 (*m*, 12 H–C(3), 12 H–C(5), 24 H–C(6), 2 H–C(5A), 4 H–C(6A), 4 NCH_2CO); 3.65 (*dd*, J = 3.63,10.3, 2 H–C(2A)); 3.62 (*t*, J = 10.3, 2 H–C(4A)); 3.57–3.44 (*m*, 12 H–C(2), 12 H–C(4)); 3.26 (*s*, CH_2CH_2). ^{13}C-NMR (125 MHz, D_2O): 173.5 (COO); 170.7 (CON); 102.7, 102.6, 102.4 (C(1)); 101.8 (C(1A)); 82.0, 81.9, 81.6 (C(4)); 79.0 (C(4A)); 73.9, 75.8, 73.2,73.0, 72.9, 72.7, 72.6, 72.4, 72.1 (C(2), C(3), C(5)); 73.5 (C(5A)); 71.2 (C(2A)); 61.3, 61.2 (C(6), C(6A)); 57.9 (CH_2COO); 57.3 (CH_2CON); 54.3 (C(3A)); 52.5 (CH_2CH_2). FAB-MS: 2523 ($[M+1]^+$).

3A,3′A-(Ethane-1,2-diylbis{[(carboxymethyl)imino]-1-oxoethane-2,1-diylimino})bis[3A-deoxy-A-monoaltro-β-cyclodextrin] (**3c**): Yield 80%. R_f 0.20. ^{1}H-NMR (500 MHz, D_2O): 5.04–4.95 (*m*, 10 H–C(1)); 4.93 (*d*, J = 3.89, 2 H–C(1)); 4.89 (*d*, J = 5.64, 2 H–C(1A)); 4.27 (br., 2 H–C(3A)); 4.09 (br., 2 H–C(5A)); 3.94 (br., 2 H–C(4A)); 3.88–3.67 (*m*, 12 H–C(3), 12 H–C(5), 24 H–C(6), 2 H–C(2A), 4 H–C(6A), 2 CH_2COO); 3.64 (br., 2 CH_2CON); 3.60–3.47 (*m*, 12 H–C(2), 12 H–C(4)); 3.30–3.14 (br., CH_2CH_2).

^{13}C-NMR (125 MHz, D_2O): 173.1 (COO); 170.2 (CON); 104.3 (C(1A)); 102.6, 102.3, 102.2, 102.0 (C(1)); 81.9, 81.7, 81.6, 80.8 (C(4)); *ca.* 78 (C(4A)); *ca.* 76 (C(5a)); 74.2, 74.1, 74.0, 73.9, 73.6, 73.2,73.0, 72.8, 72.7, 72.6, 72.4 (C(2), C(3), C(5)); 70.4 (C(2A)); 61.3, 61.1, 60.9 C(6), C(6A)); 57.6 (CH_2CON); 57.4 (CH_2COO); 52.3 (C(3A)); 52.1 (CH_2CH_2). FAB-MS: 2523 ($[M+1]^+$).

2A,2′A-(Ethane-1,2-diylbis{[(carboxymethyl)imino]-1-oxoethane-2,1-diylimino})bis[2A-deoxy-A-monoaltro-β-cyclodextrin] (**3d**): Yield 72%. R_f 0.20. ^{1}H-NMR (500 MHz, D_2O): 5.06 (d, $J=3.91$, 4 H−C(1)); 4.99–4.94 (m, 8 H−C(1), 2 H−C(1A)); 4.17 (m, 2 H−C(5A)); 4.10 (dd, $J=7.4$, 10.3, 2 H−C(2A)); 4.01–3.94 (m, 2 H−C(3), 2 H−C(4A)); 3.91–3.64 (m, 10 H−C(3), 12 H−C(5), 24 H−C(6), 2 H−C(3A), 4 H−C(6A), 4 CH_2CO); 3.60–3.46 (m, 12 H−C(2), 12 H−C(4)); 3.28 (s, CH_2CH_2). ^{13}C-NMR (125 MHz, D_2O): 173.5 (COO); 170.4 (CON); 102.7, 102.6, 102.5, 102.0, 101.8, 101.7 (C(1), C(1A)); 81.9, 81.6, 81.5, 81.3 (C(4)); 79.1 (C(4A)); 76.0 (C(5A)); 74.2, 74.1, 73.9, 73.8, 73.7, 73.6, 73.0, 72.9, 72.8, 72.7, 72.6, 72.5, 72.4 (C(2), C(3), C(5)); 68.5 (C(3A)); 61.3, 61.2, 61.1, 61.0 (C(6), C(6A)); 57.6 (CH_2COO); 57.4 (CH_2CON); 53.4 (C(2A)); 52.5 (CH_2CH_2). FAB-MS: 2523 ($[M+1]^+$).

Determination of Association Constants. In a quartz cell, 0.05M carbonate buffer (pH 10.6; 2 ml) containing 0.2M KCl and $1.0\cdot10^{-5}$ M fluorescent guest was incubated at 25°. To the cell were added aliquots of a host stock soln. consisting of 0.2M KCl, $1.0\cdot10^{-5}$ M fluorescent guest, and $1.0\cdot10^{-5}$ M β-cyclodextrin dimer **3** buffered at pH 10.6 with 0.05M carbonate, until saturation of the guest was reached. The fluorescence changes were followed under the condition of $\lambda_{ex}=315$ nm and slits ex/em = 5 nm/5 nm. A nonlinear least-squares approach was used to fit the titration curves to a 1:1 binding model, and association constants for the host-guest binding were derived thereby.

Kinetics. To a cuvette was added 50 mM HEPES buffer (pH 7.0) (1.913 ml) containing 0.2M KCl. Therein was injected an appropriate volume of 4.16 mM Ce^{4+} complex freshly prepared *in situ* by mixing the $Ce(NH_4)_2(NO_3)_6$ solution and a soln. of ligand **3** in pure H_2O. The soln. in the cuvette was adjusted to a total volume of 2.05 ml by adding pure H_2O and thermostated at 25°. After a couple of minutes equilibration time, 25 µl of 1.62 mM BNPP in pure H_2O was injected to initiate the hydrolysis. The release of 4-nitrophenol was monitored by following the absorbance change against time at 400 nm over 4 half-lives. All solns. remained clear during the time of kinetic measurements. The reactions followed the first-order kinetics law, and pseudo-first-order rate constants were estimated by nonlinear fitting of the kinetic curves with $r>0.997$.

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