

Binding and Catalytic Properties of 2-O- and 3-O-Permethylated Cyclodextrins

Takuya Nagata, Keisuke Yoshikiyo, Yoshihisa Matsui, and Tatsuyuki Yamamoto*

Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu, Matsue 690-8504

Received June 23, 2008; E-mail: tyamamot@life.shimane-u.ac.jp

Hexakis(3-*O*-methyl)-α-cyclodextrin (**3α**) bound to *m*- and *p*-nitrophenolate ions more strongly, whereas hexakis(2-*O*-methyl)-α-cyclodextrin (**2α**) bound less strongly than native α-cyclodextrin. ROESY spectra showed that the 3-*O*-methyl groups of **3α** interact with the guest protons, whereas 2-*O*-methyl groups of **2α** do not. **3α** accelerated and **2α** decelerated the cleavage of *m*-nitrophenyl acetate in an alkaline solution, suggesting that the C(2)–OH of α-cyclodextrin is more catalytic than the C(3)–OH. However, the catalytic effect of **3α** was much smaller than that of native α-cyclodextrin. Loss of hydrogen bonding between the C(3)–OH and C(2)–OH by 3-O-permethylation is responsible for the small catalytic effects of **3α**. Similar results were obtained for β-cyclodextrin analogs.

Cyclodextrins (CDs) are cyclic oligomers composed of six (α-CD), seven (β-CD), or more α-D-glucopyranose units and stereoselectively catalyze the cleavage of phenyl esters in alkaline solutions.[1] The reaction proceeds via the prior inclusion of ester within the CD cavity, followed by the nucleophilic attack of the alkoxide ion derived from a secondary hydroxy group of CD to form the phenolate ion and acylated CD. However, there has been controversy as to whether the C(2)–OH or the C(3)–OH of CD is ionized and attacks the ester carbonyl first. Bender et al. inferred at first that the C(3)–OH appears to be ionized.[1b] Later, they suggested that the C(2)–OH is responsible for the accelerated release of phenol.[1c] Breslow et al.[2] found both C(2)- and C(3)-acylated compounds in the reaction mixtures of aryl esters with β-CD in alkaline solutions. They suggested that the alkoxide ion derived from the C(2)–OH of β-CD attacks the substrates first, followed by equilibration of sugar esters with neighboring hydroxy groups under basic conditions.[2b] On the other hand, Bergeron and Burton[3] inferred that the C(2)–OH and C(3)–OH do not differ substantially in catalytic activity. Subsequent computer simulation[4] of phenyl ester cleavage by β-CD has indicated that the alkoxide ion derived from the C(2)–OH is more inclined to the cavity interior than that derived from the C(3)–OH, and only the reaction path leading to C(2)-acylation shows an activation barrier lower than that for the corresponding reference reaction in water. Furthermore, ^{1}H NMR studies[5] of CDs in aqueous solutions showed that the C(3)–OH groups are hydrogen donors and the C(2)–OH groups are the acceptors in the formation of hydrogen bonds between them. These findings suggest that the alkoxide ion derived from the C(2)–OH attacks ester first. On the other hand, Fukudome et al.[6] showed that C(3)–monothio-CDs are much more effective than C(2)–monothio-CDs in promoting acyl transfer, suggesting that the C(3)–OH is more reactive than the C(2)–OH.

The present work deals with the catalytic effects of hexakis-(2-*O*-methyl)-α-CD (**2α**), hexakis(3-*O*-methyl)-α-CD (**3α**), and their β-CD analogs, heptakis(2-*O*-methyl)-β-CD (**2β**) and heptakis(3-*O*-methyl)-β-CD (**3β**), on the cleavage of 3- and 4-nitrophenyl acetates (*m*-NPA and *p*-NPA, respectively), together with their complexation with 3- and 4-nitrophenols (*m*-NP and *p*-NP, respectively), in an alkaline solution. It is expected that the obtained results will afford some insight into the role of the C(2)– and C(3)–OHs of CDs in acyl-transfer reactions. Procedures for the selective permethylation at the C(2)– and C(3)–OHs of CDs have already been developed by Ashton et al.[7] and Bergeron, Meeley, and co-worker[8] respectively.

Results and Discussion

Binding Properties of the Permethylated CDs. Binding constants (K_a) were determined by UV–vis spectrophotometry for complexation of the permethylated CDs and the corresponding native CDs with *p*-NP and *m*-NP in a carbonate buffer solution (pH 10.60) at 298 K (Table 1). Changes in absorbance of *m*-NP with the addition of β-CD, **2β**, and **3β** were too small to determine the K_a values for these systems. However, ^{1}H NMR signals of *m*-NP in D_2O containing 0.1 mol dm^{-3} Na_2CO_3 were appreciably changed with the addition of these hosts, and the K_a values for these systems were determined by NMR shift titration and are also shown in Table 1. Interestingly, the K_a value for a **3α**–*p*-NP system was remarkably larger than that for a native α-CD–*p*-NP system by a factor of about 8, whereas that for a **2α**–*p*-NP system was smaller than that for an α-CD–*p*-NP system. A similar tendency was found for the corresponding β-CD–*p*-NP and α-CD–*m*-NP systems. The K_a value for a **3β**–*m*-NP system was also larger than that of a **2β**–*m*-NP system, though it was slightly smaller than that of a native β-CD–*m*-NP system. These results indicate that the 3-*O*-methyl groups of **3α** and **3β** contribute to the stabilization of inclusion complexes with *m*-NP and *p*-NP, whereas the 2-*O*-methyl groups of **2α** and **2β** do not. This presumption was confirmed by ^{1}H NMR spectroscopy.

Figure 1 shows the ROESY (rotating-frame nuclear Overhauser enhancement spectroscopy) spectra of mixtures of

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Table 1. Binding Constants (K_a), Differences ($\Delta\varepsilon$) in Molar Absorbance between Complexed and Free Guests, and Wavelength (λ) Observed for Inclusion Complexes of Native and Permethylated CDs with Nitrophenolates in a Carbonate Buffer (pH 10.60) at 298 K, Together with K_a Values Determined by ^{1}H NMR Shift Titration in 0.1 mol dm^{-3} Na_2CO_3/D_2O at 298 K

Host	K_a/mol^{-1} dm^3	$\Delta\varepsilon$/mmol^{-1} dm^3 cm^{-1}	λ/nm
		p-NP	
α-CD	1860	3.96	417
2α	1420	5.84	419
3α	14200	6.78	419
β-CD	683	2.22	420
2β	33	2.37	420
3β	1080	4.06	419
		m-NP	
α-CD	233	0.507	450
2α	107	0.556	448
3α	434	0.623	453
β-CD[a)]	161		
2β[a)]	33		
3β[a)]	138		

a) The K_a values were determined by ^{1}H NMR shift titration in 0.1 mol dm^{-3} Na_2CO_3.

α-CD, **2α**, and **3α** with *p*-NP and *m*-NP in alkaline D_2O solutions. For a native α-CD–*p*-NP system (Figure 1a), the *ortho*-H (2,6-H in the figure) of *p*-NP gave a cross-peak with the C(3)–H of the host, and the *meta*-H (3,5-H in the figure), cross-peaks with both the C(3)– and C(5)–Hs. Similar cross-peaks were found in the case of a **3α**–*p*-NP system (Figure 1c). These results suggest that the *p*-NP ion deeply penetrates into the CD cavities of α-CD and **3α** in such a manner that the nitro group locates in the vicinity of the C(5)–H. On the other hand, the *ortho*- and *meta*-Hs of *p*-NP gave cross-peaks only with the C(3)–H of **2α** and no cross-peak with the C(5)–H (Figure 1b), suggesting that *p*-NP is included within the cavity of **2α** less deeply than the cases of α-CD and **3α**. Interestingly, the 2-*O*-methyl protons of **2α** gave no cross peaks with the *p*-NP protons, suggesting that 2-*O*-methyl groups of **2α** are far apart from the *p*-NP protons in an inclusion complex. In contrast, the 3-*O*-methyl protons of **3α** gave a clear cross-peak with the *ortho*-H of *p*-NP, indicating that the 3-*O*-methyl groups of **3α** are in close contact with the *ortho*-H of *p*-NP. Presumably, 2-*O*-methyl groups of **2α** are directed toward the outside of the cavity, whereas 3-*O*-methyl groups of **3α** are inclined to the cavity interior. Possible structures of the inclusion complexes of *p*-NP with **2α** and **3α** are illustrated in Figures 2a and 2b, respectively. The structure of the α-CD–*p*-NP complex should be similar to that of the **3α**–*p*NP complex. van der Waals interactions between the 3-*O*-methyl groups of **3α** and the *ortho*-H of *p*-NP, as well as an increase in hydrophobicity of the α-CD cavity by 3-O-permethylation, should be responsible for the observed large K_a values for a **3α**–NP system. 2-*O*-Methyl groups of **2α**, directed to the outside of the cavity, are not available for strengthening such van der Waals and hydrophobic interactions. The ROESY spectrum of native α-CD–*m*-NP is shown in Figure 1d. The 2-H and 4-H adjacent to the nitro group of *m*-NP gave strong cross-peaks with the C(3)–H of α-CD, together with weak cross-peaks between the 5-H and the C(3)–H and between the 2-H and the C(5)–H, suggesting that the nitro group of *m*-NP also locates in the vicinity of the C(5)–Hs. Similar results were obtained in the case of the **3α**–*m*-NP system (Figure 1f). That is to say, the system gave strong cross-peaks between the 2-H and 4-H of *m*-NP and the C(3)–H of **3α**, together with weak cross-peaks between the 2-H and 4-H of *m*-NP and the C(5)–H of **3α**, suggesting that the nitro group of *m*-NP locates in the vicinity of the C(5)–H of **3α**. However, the inclusion of *m*-NP within the cavity of **3α** is shallower than that of the **3α**–*p*-NP or α-CD–*m*-NP system, since the 5-H of *m*-NP gave no cross-peak with the C(3)–H of **3α**. In addition, all the protons of *m*-NP gave strong or weak cross-peaks with the 3-*O*-methyl protons of **3α**, suggesting that the 3-*O*-methyl groups are inclined to the cavity interior and take part in complexation. On the other hand, the **2α**–*m*-NP system gave 2 cross-peaks between the 2-H and 4-H of *m*-NP and the C(3)–H of **2α**, together with a very weak cross-peak between the 2-H of *m*-NP and the C(5)–Hs of **2α** (Figure 1e). No cross-peak was observed between any protons of *m*-NP and 2-*O*-methyl protons of **2α**. These results indicate that *m*-NP is shallowly included within the cavity of **2α** and also that 2-*O*-methyl groups of **2α** are directed toward the outside of the cavity, as was observed for the **2α**–*p*-NP system. Possible structures of the inclusion complexes of *m*-NP with **2α** and **3α** are illustrated in Figures 2c and 2d, respectively.

Catalytic Properties of the Permethylated CDs. Effects of the permethylated CDs and native CDs on the rate of cleavage of *m*-NPA and *p*-NPA were spectrophotometrically examined in a carbonate buffer (pH 10.60) at 298 K. Figure 3 shows, for an example, the effects of **2α** and **3α** on the cleavage of *m*-NPA. Interestingly, **2α** retarded and **3α** accelerated the cleavage. Such results were analyzed by a nonlinear least-squares curve-fitting analysis of changes in rate constants (k_{obsd}) with the host concentrations (c_0), based upon an assumption of 1:1 complexation,[1a] to give the binding (K_a) and rate constants (k_c) for examined CD–NPA complexes. The obtained curves (as shown by solid lines in Figure 3) were satisfactorily fitted to the data. The obtained K_a values and ratios (k_c/k_{un}) of k_c to the rate constants (k_{un}) in the absence of CDs are summarized in Table 2. The K_a and k_c/k_{un} values for α- and β-CD complexes with *m*-NPA and *p*-NPA were roughly equal to those reported by VanEtten et al.[1a] However, the obtained K_a values for the nitrophenyl acetates were very different from those for the corresponding nitrophenolate ions. Similar large differences in the K_a values between various phenyl acetates and the corresponding phenolates have been reported thus far.[1c] The difference are brought about by difference in the chemical properties and molecular structures in phenyl acetate and the corresponding phenolates. Not only **3α** but also **3β** accelerated the cleavage of *m*-NPA, suggesting that the alkoxide ion derived from the C(2)–OH of **3α** and **3β** are catalytically active. However, the k_c/k_{un} values for **3α** and **3β** were much smaller than those for native α- and β-CDs, respectively, though the K_a values were similar to each other. It is known that the hydrogens of C(3)–OH groups of native CDs

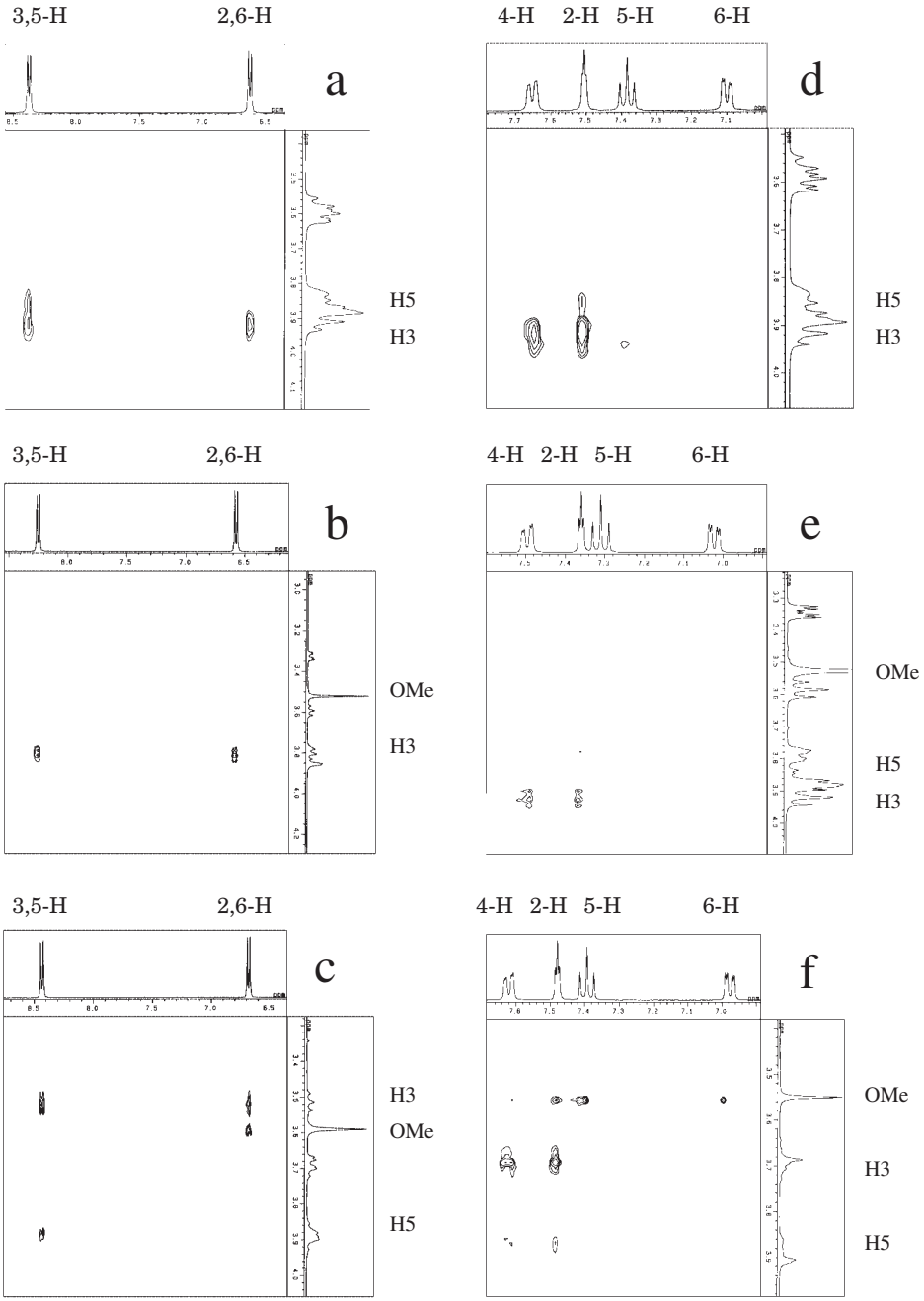

Figure 1. ROESY spectra of *p*-NP or *m*-NP in the presence of α-CD, **2α**, or **3α** in D_2O containing 0.1 mol dm^{-3} Na_2CO_3 at 298 K. (a) 10.2 mmol dm^{-3} *p*-NP + 42.0 mmol dm^{-3} α-CD, (b) 10.1 mmol dm^{-3} *p*-NP + 13.8 mmol dm^{-3} **2α**, (c) 10.1 mmol dm^{-3} *p*-NP + 12.9 mmol dm^{-3} **3α**, (d) 10.2 mmol dm^{-3} *m*-NP + 31.6 mmol dm^{-3} α-CD, (e) 10.2 mmol dm^{-3} *m*-NP + 16.9 mmol dm^{-3} **2α**, (f) 10.2 mmol dm^{-3} *m*-NP + 13.4 mmol dm^{-3} **3α**.

are hydrogen bonded to the oxygens of the C(2)–OH groups of adjacent glucopyranose units not only in the crystal state[9] but also in an aqueous solution,[5] and the hydrogen bonds stabilize the alkoxide ion formed by the acid dissociation of a CD hydroxy group[1b] to give a relatively low pK_a value (pK_a = 12.1,[1b] 12.33[10] for α-CD). Since the C(3)–OH groups are hydrogen donors in native CDs,[5] permethylation of the C(3)–OH groups breaks up such hydrogen bonding to destabilize the alkoxide ion derived from the C(2)–OH. As a result the pK_a values of **3α** and **3β** increase and the reactivities of their C(2)–OHs decrease. This is one of reasons why the k_c/k_{un} values for **3α** and **3β** are much smaller than those for native α- and β-CDs. Different reasoning is also possible: The permethylation will bring about a change in structure of the CD cavity, and the geometries for inclusion complexes of **3α** and **3β** will differ from those of native α- and β-CDs, respectively.[3] In fact, the ROESY spectra described in the previous section showed that the 3-*O*-methyl groups of **3α** are inclined to the cavity interior, whereas the C(3)–OH groups of native α-CD are directed to the oxygens of the C(2)–OH groups of adjacent glucopyranose units.[5] Thus, the distance between the catalytic site (the alkoxide ion of host) and the reaction site (the carbonyl carbon

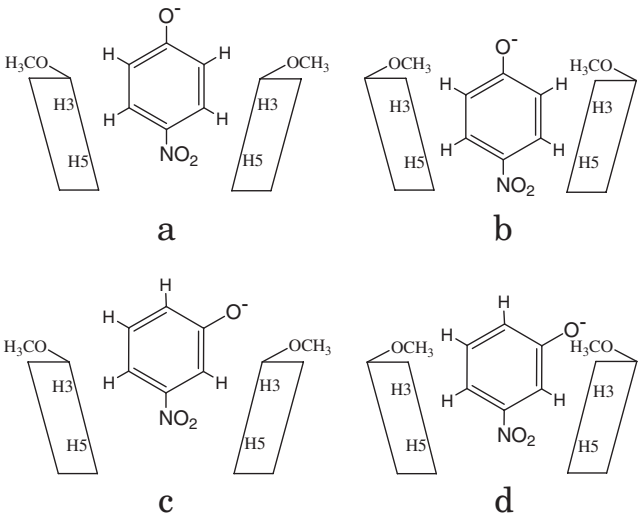

Figure 2. Possible structures of the inclusion complexes of **2α** and **3α** with *p*-NP and *m*-NP in alkaline solutions. (a) **2α**–*p*-NP complex, (b) **3α**–*p*-NP complex, (c) **2α**–*m*-NP complex, (d) **3α**–*m*-NP complex.

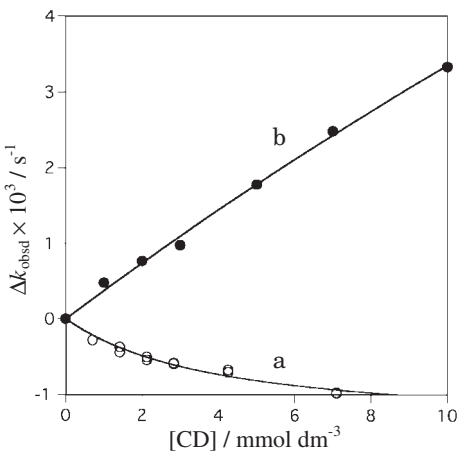

Figure 3. Plots of the difference (Δk_{obsd}) between the rates of the cleavage of *m*-NPA in the presence (k_{obsd}) and in the absence (k_{un}) of the permethylated CDs vs. CD concentration in a carbonate buffer (pH 10.60) at 298 K. Permethylated CDs added are **2α** (a) and **3α** (b), respectively.

of guest) in an inclusion complex will be changed by the permethylation, so that the activation energy of the reaction is changed. At the present stage of investigation, it is difficult to judge which is true or both. However, binding constants K_a for the *m*-NP complexes with **3α** and **3β** were not very different from those for the corresponding complex with native α- and β-CDs, respectively (Table 1), and the K_a values for *m*-NPA with **3α** and **3β** were also not very different from those for the corresponding complex with native α- and β-CDs, respectively (Table 2), suggesting that the change in structure of the CD cavity with 3-O-permethylation is not so large. Hence, it is more plausible that a decrease in reactivities of the C(2)–OH with 3-O-permethylation is mainly responsible for the observed low catalytic effects of **3α** and **3β** compared with those of native α- and β-CDs.

Table 2. Equilibrium (K_a) and Kinetic (k_c/k_{un})[a] Parameters for Complexes of Native and Permethylated CDs with Nitrophenyl Acetates in a Carbonate Buffer (pH 10.60) at 298 K

Host	*p*-NPA		*m*-NPA	
	K_a /mol^{-1} dm^3	k_c/k_{un}	K_a /mol^{-1} dm^3	k_c/k_{un}
α-CD	120	2.9	17	420
2α	30	0.41	280	0.49
3α	140	0.66	13	9.8
β-CD	160	9.3	40	260
2β	250	0.68	180	0.46
3β	50	5.1	47	9.5

a) $k_{un} = (5.00 \pm 0.05) \times 10^{-3}$ s^{-1} (*p*-NPA) and $(3.03 \pm 0.05) \times 10^{-3}$ s^{-1} (*m*-NPA).

On the contrary to **3α** and **3β**, **2α** and **2β** retarded the cleavage of *m*-NPA. As mentioned above, the C(3)–OH of native CD forms a hydrogen bond with the C(2)–OH. Similar formation of hydrogen bonds will be possible, even after permethylation at the C(2)–OH groups. If the C(3)–OH groups are stabilized by hydrogen bonding, the acid dissociation of the C(3)–OH to form the reactive alkoxide ion will be suppressed, and the C(3)–OH groups become catalytically inert. Furthermore, the nucleophilic attack of hydroxide ion in bulk solution on the substrate included in the cavity of **2α** or **2β** will be retarded by steric hindrance. As shown in Table 2, the K_a values for *m*-NPA complexes with **2α** and **2β** are significantly larger than those for *m*-NPA complexes with **3α** and **3β**, respectively, suggesting that *m*-NPA is more deeply included within the cavities of **2α** and **2β** than within those of **3α** and **3β** to be effectively protected from nucleophilic attack of hydroxide ion in bulk solution.

Similar results were obtained for *p*-NPA systems, except **3α**. Thus, the cleavage of *p*-NPA was decelerated by **2α** and **2β**, whereas accelerated by **3β**. Unexpectedly, **3α** decelerated the cleavage of *p*-NPA, though it accelerated the cleavage of *m*-NPA. The K_a value for a **3α**–*p*-NPA complex was about 10 times larger than that for a **3α**–*m*-NPA complex. In this connection, the K_a value for a **3α**–*p*-NP complex was also about 8 times larger than that for a native α-CD–*p*-NP complex (Table 1). Thus, *p*-NPA is so deeply and so rigidly trapped within the **3α** cavity that the included *p*-NPA has difficulty reaching the transition-state binding[11] in which the alkoxide ion derived from the C(2)–OH is linked to the acyl carbon. In contrast, **3β** accelerated the cleavage of *p*-NPA. The cavity of **3β** is larger that of **3α**, and *p*-NPA included within the **3β** cavity will have more freedom of motion and can more easily reach the transition state than that in **3α**.

In conclusion, hexakis(3-*O*-methyl)-α-CD (**3α**) accelerated the cleavage of *m*-NPA, whereas hexakis(2-*O*-methyl)-α-CD (**2α**) decelerated the reaction in alkaline solution, suggesting that the C(2)–OH of α-CD is more catalytic than the C(3)–OH. Similar results were observed for their β-CD analogs. However, the catalytic effects of **3α** and **3β** were much smaller than those of native α- and β-CDs, respectively. Loss of hydrogen bonding between the C(3)–OH and C(2)–OH by 3-O-permethylation are responsible for the small catalytic effects of **3α** and **3β**.

Experimental

Materials. The α- and β-CDs were supplied by Ensuiko Sugar Refining Co., Ltd. They were dried overnight in vacuo at 110 °C. *t*-Butylchlorodimethylsilane was purchased from Shin-Etsu Chemical Co., Ltd. Sodium hydride (50–72% in oil), tetrabutylammonium fluoride, 4-(*N*,*N*-dimethylamino)pyridine, and allyl bromide were purchased from Wako Pure Chemical Industries, Ltd. Methyl iodide and potassium *t*-butoxide were purchased from Tokyo Kasei Kogyo Co., Ltd., and Kanto Chemical Co., Inc., respectively. Silica gel 60 (Merck, 0.040–0.063 mm), Sephadex G-25 (Amersham Biosciences), and octadecyl-bonded silica gel (Organo Co.) were used for column chromatography. Organic solvents and other reagents were also commercially available.

Apparatus. The UV–vis spectra were recorded using a Shimadzu UV-2100 UV/Vis spectrophotometer equipped with a temperature-controlled cell holder. The NMR spectra were recorded on a JEOL Model JNM-A400 FT NMR spectrometer (400 MHz) with a sample tube of 5.0 mm diameter at 298 K. The phase-sensitive ROESY spectra were acquired with a mixing time of 1000 ms and 512 × 256 data points, followed by zero-filling, for the inclusion complexes of α-CD and permethylated CDs with *m*-NP and *p*-NP in D_2O containing 0.1 mol dm^{-3} Na_2CO_3. Methanol (δ 3.343[12]) was used as an internal reference for ^{1}H NMR measurements in D_2O.

Preparation of 2α and 2β. Hexakis(2-*O*-methyl)-α-CD (**2α**) was prepared according to a procedure reported by Ashton et al.[7] In a typical run, α-CD (9.92 g, 10.2 mmol) was allowed to react with *t*-butylchlorodimethylsilane (28.00 g, 185.8 mmol) in a solution of *N*,*N*-dimethylformamide (DMF, 100 mL) and pyridine (60 mL) containing a trace amount of 4-(*N*,*N*-dimethylamino)pyridine (21 mg) at 100 °C under nitrogen for 18 h. The reaction mixture was concentrated by evaporation, and the residue was partitioned between water (150 mL) and CH_2Cl_2 (150 mL). The organic layer was washed with $KHSO_4$ (0.5 mol dm^{-3}, 150 mL), dried over $CaSO_4$, and concentrated by evaporation. The concentrate was chromatographed on a SiO_2 column with CH_2Cl_2 as an eluent to give hexakis[2,6-bis(*O*-*t*-butyldimethylsilyl)]-α-CD (**1**, 17.95 g, 7.66 mmol, 75% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.038 (s, 18H), 0.043 (s, 18H), 0.14 (s, 18H), 0.15 (s, 18H), 0.88 (s, 54H), 0.91 (s, 54H), 3.48 (t, J = 9.3 Hz, 6H), 3.55 (dd, J = 2.9, 9.8 Hz, 6H), 3.70 (d, J = 11.2 Hz, 6H), 3.73 (d, J = 12.0 Hz, 6H), 3.95 (dd, J = 2.7, 11.2 Hz, 6H), 3.99 (t, J = 9.3 Hz, 6H), 4.37 (s, 6H), 4.79 (d, J = 2.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.2, −4.7, −4.6, 18.4, 18.8, 25.9, 26.2, 62.2, 72.0, 72.3, 74.6, 82.5, 102.6.

Sodium hydride (4.40 g) was added to a solution of **1** (4.65 g, 1.98 mmol) in tetrahydrofuran (THF, 120 mL) with cooling by ice-water under N_2 bubbling. To the solution, methyl iodide (6.5 mL) was added and stirred overnight. Methanol (15 mL) was added to the reaction mixture to decompose remaining sodium hydride. The reaction mixture was concentrated by evaporation, and the residue was partitioned between water (130 mL) and CH_2Cl_2 (250 mL). The organic layer was washed with water (100 mL) and aqueous NaCl solutions (2 × 100 mL), dried over $CaSO_4$, and concentrated by evaporation. The concentrate was chromatographed with a SiO_2 column and hexane/acetone (99:1, v/v) as an eluent to give hexakis[2-*O*-methyl-3,6-bis(*O*-*t*-butyldimethylsilyl)]-α-CD (**2**, 3.57 g, 1.47 mmol, 74% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.03 (s, 36H), 0.10 (s, 18H), 0.12 (s, 18H), 0.89 (s, 54H), 0.90 (s, 54H), 2.94 (d, J = 7.1 Hz, 6H), 3.32 (s, 18H), 3.66 (br, 12H), 3.88 (br, 6H), 4.11 (br, 12H), 5.17 (br, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.1, −4.8, −3.8, 18.3, 18.5, 26.0, 26.3, 57.5, 62.6, 73.3, 81.2, 95.3.

2 (2.52 g, 1.04 mmol) was dissolved in a THF solution containing tetrabutylammonium fluoride (1 mol dm^{-3}, 16 mL), and the resulting solution was refluxed overnight. The reaction mixture was concentrated by evaporation, and the residue was dissolved in water (7 mL). The aqueous solution was washed with CH_2Cl_2 (5 × 30 mL) to remove the tetrabutylammonium salt, and chromatographed with an octadecyl-bonded silica gel column and aqueous methanol (20, 40, and 60% methanol) as eluants to give **2α** (0.56 g, 0.53 mmol, 51%): ^{1}H NMR (400 MHz, D_2O): δ 3.36 (dd, J = 3.4, 10.2 Hz, 6H), 3.54 (s, 18H), 3.57 (t, J = 9.3 Hz, 6H), 3.78 (m, 6H), 3.86 (dd, J = 4.3, 12.6 Hz, 6H), 3.89 (dd, J = 1.8, 12.6 Hz, 6H), 4.00 (t, J = 10.0 Hz, 6H), 5.24 (d, J = 3.4 Hz, 6H); ^{13}C NMR (100 MHz, D_2O): δ 61.4, 63.1, 74.4, 74.7, 83.6, 84.5, 101.4.

2β was prepared in a similar manner: ^{1}H NMR (400 MHz, D_2O): δ 3.38 (dd, J = 3.4, 10.0 Hz, 7H), 3.56 (s, 21H), 3.61 (t, J = 9.5 Hz, 7H), 3.81 (m, 7H), 3.86 (d, J = 3.2 Hz, 14H), 3.99 (t, J = 9.5 Hz, 7H), 5.26 (d, J = 3.7 Hz, 7H); ^{13}C NMR (100 MHz, D_2O): δ 61.8, 62.9, 74.0, 74.7, 83.8, 84.0, 101.7.

Preparation of 3α and 3β. Hexakis(3-*O*-methyl)-α-CD (**3α**) was prepared according to a procedure reported by Bergeron et al.[8] In a typical run, α-CD (6.04 g, 6.21 mmol) was allowed to react with a large excess of allyl bromide (70 g, 0.58 mol) in a mixture of dimethyl sulfoxide (DMSO, 100 mL) and DMF (100 mL) containing barium oxide (30 g, 0.2 mol) and barium hydroxide octahydrate (30 g, 0.095 mol) at room temperature under N_2 for 3 days. The reaction mixture was treated with an aqueous ammonia solution (25%, 50 mL), and precipitates were filtered off. The filtrate was concentrated under vacuum. The residue was applied to a SiO_2 column and eluted with chloroform/ethyl acetate solutions (chloroform:ethyl acetate = 10:0, 9:1, and 8:2 by volume). Hexakis(2,6-di-*O*-allyl)-α-CD (**3**, 3.79 g, 2.61 mmol, 42% yield) was obtained from an eluate of 8:2 chloroform/ethyl acetate: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.45 (dd, J = 3.2, 9.8 Hz, 6H), 3.55 (t, J = 9.3 Hz, 6H), 3.70 (d, J = 2.7 Hz, 12H), 3.86 (d, J = 10.0 Hz, 6H), 4.00–4.08 (m, 12H), 4.12 (t, J = 9.3 Hz, 6H), 4.22 (dd, J = 7.0, 12.6 Hz, 6H), 4.44 (dd, J = 5.4, 12.7 Hz, 6H), 4.72 (s, 6H), 4.92 (d, J = 3.4 Hz, 6H), 5.20 (dd, J = 10.5, 15.4 Hz, 12H), 5.28 (dd, J = 13.7, 15.9 Hz, 12H), 5.93 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 68.8, 70.4, 72.6, 73.2, 73.6, 78.6, 83.5, 101.4, 117.3, 118.7, 134.2, 134.8.

The allyl ether **3** (3.50 g, 2.41 mmol) was dissolved in DMF (100 mL). Sodium hydride (2.63 g) was added to the solution at room temperature under N_2. To the mixture, a solution of methyl iodide (8.56 g, 60 mmol) in DMF (30 mL) was slowly added and allowed to stand overnight. After the addition of water (10 mL), the reaction mixture was taken up in chloroform (500 mL) and washed 5 times with 100 mL of water. The organic layer was dried over $CaSO_4$, filtrated, and concentrated under vacuum to give a crude product of hexakis(2,6-di-*O*-allyl-3-*O*-methyl)-α-CD (**4**, 5.37 g): ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.31 (dd, J = 3.1, 9.5 Hz, 6H), 3.57 (t, J = 9.3 Hz, 6H), 3.62 (t, J = 9.3 Hz, 6H), 3.63 (s, 18H), 3.71 (d, J = 9.8 Hz, 6H), 3.78–3.84 (m, 12H), 4.00 (dd, J = 5.4, 12.9 Hz, 6H), 4.06–4.10 (m, 12H), 4.27 (dd, J = 5.6, 12.9 Hz, 6H), 4.98 (d, J = 3.2 Hz, 6H), 5.13–5.17 (m, 12H), 5.23–5.33 (m, 12H), 5.94 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 62.1, 69.0, 71.2, 71.4, 72.3, 80.0, 81.4, 82.4, 100.9, 116.4, 117.0, 135.1, 135.6.

The crude compound **4** (5.37 g) was allowed to react with potassium *t*-butoxide (5.40 g) in DMSO (60 mL) at 100 °C for 6 h under nitrogen. The reactants were taken up in 400 mL of diethyl

ether and washed with water (5 × 100 mL). The ether layer was dried over $CaSO_4$, filtered, and evaporated to give a crude product of hexakis(3-*O*-methyl-2,6-di-*O*-prop-1-enyl)-α-CD (**5**, 4.12 g): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56 (d, $J = 6.6$ Hz, 18H), 1.64 (d, $J = 6.8$ Hz, 18H), 3.50–3.63 (m, 18H), 3.65 (s, 18H), 3.74 (t, $J = 3.5$ Hz, 6H), 3.89 (d, $J = 10.5$ Hz, 12H), 4.18 (dd, $J = 4.0$, 11.8 Hz, 6H), 4.39 (m, 12H), 5.13 (d, $J = 3.4$ Hz, 6H), 5.93 (d, $J = 4.6$ Hz, 6H), 6.09 (d, $J = 4.6$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.4, 9.5, 41.0, 61.6, 70.8, 71.3, 80.2, 81.6, 99.1, 101.1, 101.4, 145.3, 145.8.

The crude product **5** (4.12 g) was allowed to react with mercuric oxide (2.22 g) and mercuric chloride (2.22 g) in 50 mL of water and 25 mL of acetone by shaking for 10 min at room temperature. The mixture was centrifuged, and the supernatant was evaporated in vacuo. The residue was dissolved in 100 mL of water and washed with diethyl ether (3 × 50 mL). The aqueous layer was evaporated in vacuo, and the residue was applied to a Sephadex G-25 column. Fractions containing polysaccharide were combined and concentrated under vacuum to give **3α** (0.80 g, 0.76 mmol, 32% yield based on **3**): ^{1}H NMR (400 MHz, D_2O): δ 3.66 (s, 18H), 3.71 (t, $J = 9.3$ Hz, 6H), 3.72 (dd, $J = 2.7$, 10.0 Hz, 6H), 3.82 (t, $J = 10.0$ Hz, 6H), 3.87 (s, 6H), 3.88 (s, 12H), 5.04 (d, $J = 3.4$ Hz, 6H); ^{13}C NMR (100 MHz, D_2O): δ 61.6, 63.2, 74.0, 74.9, 80.9, 84.9, 103.6.

3β was prepared in a similar manner: ^{1}H NMR (400 MHz, D_2O): δ 3.67–3.80 (m, 28H), 3.69 (s, 21H), 3.88 (s, 14H), 5.08 (d, $J = 2.7$ Hz, 7H); ^{13}C NMR (100 MHz, D_2O): δ 62.3, 63.0, 74.6, 74.7, 80.0, 85.2, 103.4.

Spectrophotometric Determination of Binding Constants for *m*-NP and *p*-NP Complexes with CDs. The binding constants (K_a) for the complexation of *m*-NP and *p*-NP with CDs were determined by UV–vis spectroscopy in a sodium carbonate buffer (pH 10.60) at 298 K. The concentrations of *m*-NP and *p*-NP were 0.207 and 0.051 mmol dm^{-3}, respectively. In a typical run, 2.00 mL of base solutions were pipetted into a pair of 1.00-cm quartz cells, one of which was used as a reference cell and the other, as a sample cell. After thermal equilibrium at 298 K had been reached, aliquots of a CD solution containing *m*-NP or *p*-NP were added stepwise to the sample cell. In each step, a UV–vis spectrum was recorded from 600 to 300 nm. The complexation was observed at a wavelength where the largest change in absorbance arose (448–453 nm for *m*-NP and 417–420 nm for *p*-NP). The K_a values were determined by a nonlinear least-squares curve-fitting analysis of changes in the absorbance with the host concentration, based upon an assumption of 1:1 complexation. The thus-calculated curves were well-fitted to the observed data with correlation coefficient greater than 0.999.

Determination of Binding Constants for *m*-NP Complexes with β-CDs by Means of NMR Shift Titration. The K_a values for the complexation of *m*-NP with β-CD, **2β**, and **3β** were determined by ^{1}H NMR shift titration in D_2O containing 0.1 mol dm^{-3} Na_2CO_3 at 298 K. ^{1}H NMR spectra were recorded for sample solutions containing 4.08 mmol dm^{-3} *m*-NP and various concentrations of β-CD, **2β**, or **3β**. 4-H, 2-H, and 5-H of *m*-NP showed the largest changes in chemical shift with the addition of β-CD, **2β**, and **3β**, respectively. The K_a values were determined by a nonlinear least-squares curve-fitting analysis of changes in the chemical shift with the host concentration, based upon an assumption of 1:1 complexation. The thus-calculated curves were well-fitted to the observed data with correlation coefficient greater than 0.999.

Kinetics. The rate of ester cleavage was measured by following the appearance of the absorption of the corresponding phenoxide anion at 390 nm for *m*-NPA or at 410 nm for *p*-NPA in a sodium carbonate buffer (pH 10.60) at 298 K. In a typical run, 2.00 mL of base solutions containing CDs were pipetted into a pair of 1.00-cm quartz cells, one of which was used as a reference cell and the other, as a sample cell. After thermal equilibrium at 298 K had been reached, 50 μL of 20 mmol dm^{-3} *m*-NPA in methanol or 10 μL of 20 mmol dm^{-3} *p*-NPA in methanol was added to the sample cell, and the change in absorbance was followed. The results were treated by the least-squares curve-fitting analysis according to the ordinary first-order rate equation. All the reactions examined obeyed good first-order kinetics with respect to substrate in both the absence and presence of CDs. The results were analyzed by a nonlinear least-squares curve-fitting analysis of changes in the rate constants with the host concentrations, based upon an assumption of 1:1 complexation,[1a] to give the binding and rate constants for CD–NPA complexes.

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