Nucleobase Difunctionalized β-Cyclodextrins. Preparation and Spectral Observation of the Base Stacking and the Hydrogen-Bonded Nucleic Acid Base Pair

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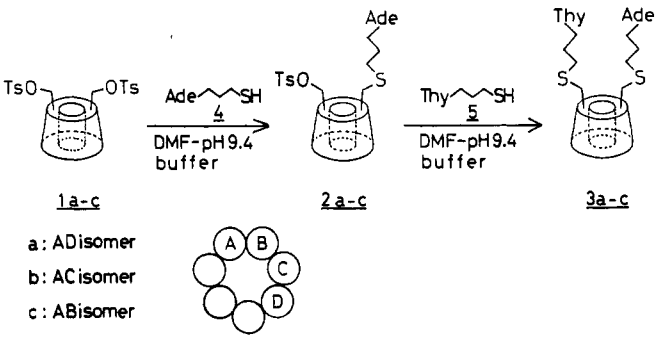
Syntheses and characterization of difunctionalized β-cyclodextrins (**3**, **6**, and **7**) are described, in which two kinds of nucleobases (adenine and thymine) are attached to the C-6 positions of β-cyclodextrin through flexible carbon chains. Specific base–base interactions and the pH control of binding abilities of these compounds are discussed on the basis of measurements of ^{1}H NMR, UV, and circular dichroism spectra.

The nucleosides, which are composed of sugars (ribose or deoxyribose) and nitrogen heterocycles (purine and pyrimidine bases), are monomeric units of DNA and RNA. It is these nucleobases which carry genetic information and play an important role in various biological functions of nucleic acids via their noncovalent bonding interactions such as hydrogen bonds and electrostatic bonds.[1] For example, in the double-stranded structure of DNA and RNA, adenine (guanine) and thymine or uracil (cytosine) regularly occur in pairs due to the specific hydrogen bonds.[2] This double-stranded structure is also stabilized by the stacking of nucleobases which is another type of base–base interaction. The temperature and pH are important factors for the regulation of such base pairing.[3] On the basis of these factors, we initiated studies of the preparation and characterization of nucleobase-functionalized cyclodextrins,[4] naturally occurring doughnut-shaped polyglucopyranoses,[5] and investigation of their chemical behavior. Of special interest was the design and synthesis of cyclodextrins functionalized through the flexible chain with two different kinds of nucleobases, which might exhibit base–base interactions over (or inside) the cavity. Conformational changes produced by these specific base–base interactions might affect the binding abilities of the cyclodextrin and be controllable by environmental conditions such as pH.

This paper describes the preparation and characterization of β-cyclodextrins which are functionalized doubly with a complementary nucleobase pair (adenine and thymine). The influence of the base–base interactions on the binding ability of these flexibly capped β-cyclodextrins is discussed on the basis of measurement of the ^{1}H NMR, UV, and circular dichroism (CD) spectra under various conditions. A remarkable pH dependence of the binding abilities observed for the adenine–thymine-functionalized cyclodextrins demonstrates a unique on–off switched capping mechanism due to the specific hydrogen bonds.[6]

Preparation. For the specific activation of two hydroxyl groups of β-cyclodextrin (β-CD), Tabushi and coworkers have devised a transannular disulfonate-capping method[7] which was applied to the syntheses of various difunctionalized β-CDs as potential enzyme mimics.[1,7,8] Recently, Fujita and co-workers have developed a highly effective method for the preparation and separation of all isomers of β-CD bis(tosylates) [AD (**1a**), AC (**1b**), and AB isomer (**1c**)] by means of reversed phase chromatography.[9] Utilizing these ditosylates, the specific difunctionalization of β-cyclodextrin with two different kinds of nucleobases on flexible chains (**3a–c**; Chart I) has been accomplished

Scheme I



by sequential treatment with 9-(3-mercaptopropyl)adenine (**4**)[4] and 1-(3-mercaptopropyl)thymine (**5**)[4] (Scheme I). Thus, when a solution of AD bis(tosylate) **1a** in pH 9.4 buffer was allowed to react with 2.2 equiv of **4** in dimethylformamide at room temperature for 2 days, monosubstituted **2a** and disubstituted **6a** were obtained in 36% and 16% yields, respectively, in addition to unreacted **1a** (24%). Treatment of **2a** with an excess of **5** under the same conditions gave the desired Thy,Ade-C_3-β-CD (**3a**) in 71% yield. Compounds **3b,c** were similarly prepared from **1b,c** via **2b,c** in 25–30% overall yields, respectively. These compounds were purified by the column chromatography (LiChroprep Rp-8, H_2O–CH_3CN). As shown in Figure 1, the two diastereomers of **2c** (i.e., AB and BA isomers; **2c′** and **2c″**) could be isolated in pure form, whereas the separation of the diastereomers **2a,b** and **3a,b** was not realized.

Difunctionalized β-CDs which had identical nucleobases, such as Ade,Ade-C_3-β-CD (**6a–c**) and Thy,Thy-C_3-β-CD (**7a–c**), were readily prepared by a similar treatment of the corresponding ditosylates **1a–c** with an excess of **4** and **5**, respectively.

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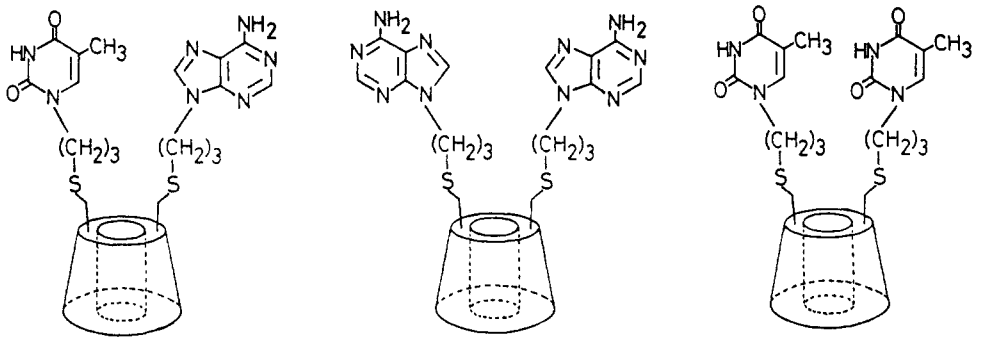
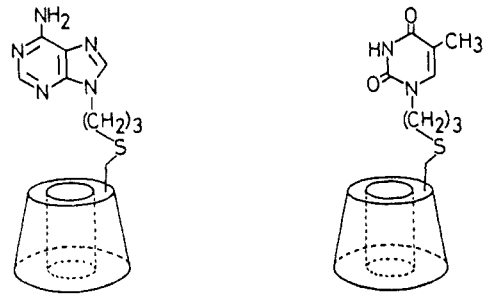
Chart I



Thy,Ade-C_3-β-CD (3a-c) Ade,Ade-C_3-β-CD (6a-c) Thy,Thy-C_3-β-CD (7a-c)



Ade-C_3-β-CD (8) Thy-C_3-β-CD (9)

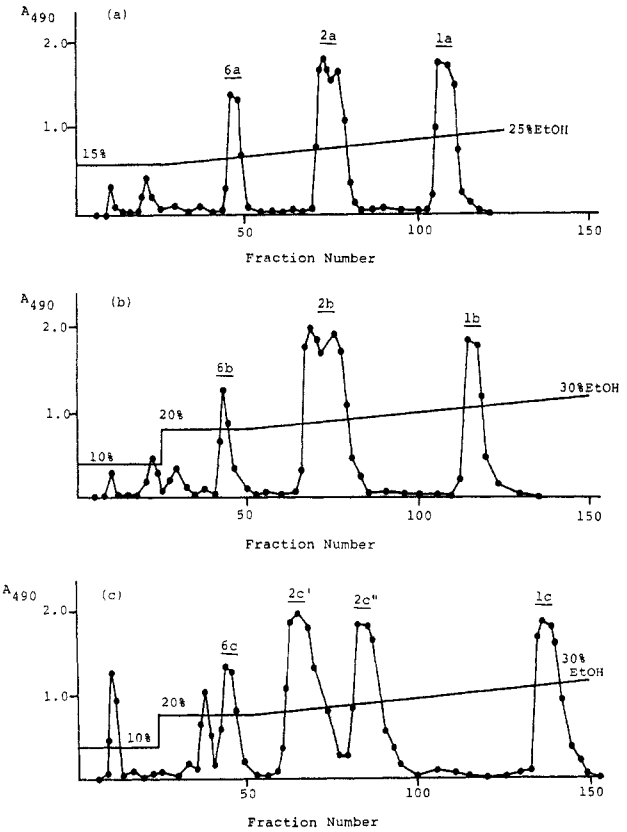


Figure 1. Chromatography of the mixture obtained from the reaction of **1a–c** with **4** on a Lobar LiChroprep Rp-8 (25 × 300 mm) column. The sample contained 200–500 mg of the mixture. Fractions of 10 mL each were collected at flow rates of 1.0–2.0 mL/min. (a) AD isomer. (b) AC isomer. (c) AB isomer. A_{490}: phenol–sulfuric acid method.[25]

Structural determinations of these compounds were based upon elemental analyses, IR, UV, and ^{1}H NMR spectra, and fast atom bombardment (FAB) mass spectrometry, which showed the correct molecular ion peaks for these nonvolatile molecules.

Table I. ^{1}H NMR Chemical Shifts of Nucleobase Moiety

compd	chemical shift (δ, D_2O)[a]			
	Thy-CH_3	Thy-C_6H	Ade-C_2H	Ade-C_8H
3a	1.80	7.39	7.99	8.19
			8.01	
3b	1.80	7.37	8.03	8.18
			8.04	
3c′	1.79	7.33	8.00	8.16
3c″	1.80	7.38	8.03	8.19
6a			7.87	8.22
			7.96	
6b			7.91	8.18
			8.02	
6c			7.97	8.16
7a	1.77	7.27		
7b	1.78	7.29		
7c	1.78	7.28		
		7.32		

[a] Internal standard: $(CH_3)_3SiCH_2CH_2CH_2SO_3Na$ (DSS).

Results and Discussion

^{1}H NMR Spectral Properties. The ^{1}H NMR spectra of nucleobase-functionalized β-cyclodextrins **3a–c**, **6a–c**, and **7a–c** displayed the characteristic signals of the nucleobase moieties in addition to those of β-cyclodextrin (Table I). In the case of Thy,Ade-C_3-β-CD (**3**), the adenine C_2-protons of AD (**3a**) and AC isomers (**3b**) appeared as two separate signals. This can be attributed to a diastereomeric mixture of **3a** and **3b** since the isolated AB isomers **3c′** and **3c″** exhibited corresponding signals as sharp 2 H singlets at δ 8.00 and 8.03, respectively.

In Figure 2, the chemical shifts of the adenine ring protons (C_2- and C_8-protons) of Ade,Ade-C_3-β-CD (**6a–c**) as well as monofunctionalized Ade-C_3-β-CD (**8**)[4] are shown. In compounds **6a** (AD) and **6b** (AC), the two C_2-protons

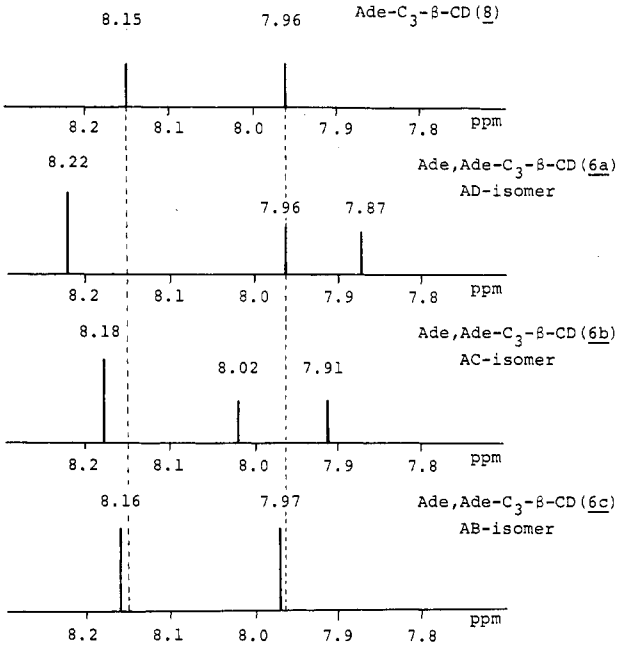


Figure 2. ^{1}H NMR chemical shifts of the adenine moiety for compounds **6a–c** and **8**.

appeared to be magnetically nonequivalent, while the C_8-protons appeared as a sharp 2 H singlet. Presumably the observation of two signals for the former is due to mutual anisotropic effects of the aromatic rings or some inclusion effects.[10] In contrast, compound **6c** (AB) displayed a singlet (each 2 H) for both C_2- (δ 7.97) and C_8-protons (δ 8.16), which indicates that two adenine moieties lie in the same environment in **6c**. Furthermore, the chemical shifts of both C_2- and C_8-protons of **6c** are very similar to those of **8**, but those of **6a** and **6b** are remarkably divergent. These results also suggest the presence of two magnetically nonequivalent adenine rings in the AD and AC isomers (**6a** and **6b**) due to some adenine–adenine interaction.

CD Spectral Properties. The circular dichroism (CD) induced by interaction of a chiral molecule like β-cyclodextrin with aromatic chromophores is known to provide important structural information.[11,12] The CD spectra of Ade,Ade-C_3-β-CD (**6a–c**) as well as their inclusion complexes are presented in Figure 3. The spectrum of each isomer (AD, AC, AB) of **6** shows a similar pattern with two positive bands at approximately 260 and 270 (small) nm, which is of larger magnitude than the reported CD curve of Ade-C_3-β-CD (**8**).[4] However, the spectra exhibit markedly different changes upon the addition of the guest compound 1-adamantanecarboxylate (Figure 3). Since the spectral change can be attributed to the conformational change upon guest inclusion,[13] the very pronounced change for the AD isomer (**6a**) is noteworthy. The complete reversal of the Cotton effect may be indicative of a change of slope of base plane to the C_7 axis of the β-cyclodextrin,[11,12,14] like an "induced fit" in enzymes.[15]

In contrast, the CD spectra of Thy,Thy-C_3-β-CD (**7a–c**) are markedly different for each isomer, although all isomers give a very similar spectra in the presence of the guest compound (Figure 4). This indicates some regulation of the conformational flexibility upon guest inclusion.

The CD spectra of Thy,Ade-C_3-β-CD (**3a–c**) are shown in Figure 5. Interestingly, the spectra of AD (**3a**) and AC isomers (**3b**), which reveal a large positive band around 260 nm, are very similar to that of RNA,[16] which suggests a similar thymine–adenine interaction in these compounds. For the AB isomer (**3c**), which is the only case that two diastereomers (**3c′** and **3c″**) can be isolated, each diastereomer gives a completely different spectrum; while **3c′** shows a large positive band, **3c″** gives a split CD curve (Figure 5(c)). However, the calculated average spectra for **3c′** and **3c″** are very similar to those for the AD and AC isomers (Figure 6). This indicates that corresponding spectral differences may be expected for each isomer of the inseparable diastereomixtures of **3a** (AD) and **3b** (AC). The remarkable spectral change of **3a–c** upon the inclusion of the guest compound may be attributed to the induced change of the base plane slope.[17]

Binding Abilities. The UV spectra of the nucleobase-difunctionalized β-cyclodextrins show the characteristic absorption bands at 260–270 nm due to the corresponding nucleobase moieties (Experimental Section). On the basis of the UV spectral change, the association constants (K_{assn}) of sodium 1-adamantanecarboxylate (1-Ad-COONa) and methyl orange (MO) with these β-CD derivatives were measured at pH 7.0 and 11.0. The results are summarized in Table II. All of the difunctionalized β-CDs show a slight increase in their binding abilities at pH 7.0 compared with monofunctionalized Ade-C_3-β-CD (**8**)[4] or Thy-C_3-β-CD (**9**)[4] except for **6c**, which suggests some kind of capping effect[5,7] arising from base–base interaction. Furthermore, Table II reveals an interesting pH dependency on the binding abilities for Thy,Ade-C_3-β-CD (**3**). The association constants for **3a** and especially **3b** were markedly lower at pH 11.0 and comparable with that of **8**, although the association constants for other compounds including **3c** were essentially unaffected by the change to a higher pH. The measurements of K_{assn} of **3b** and 1-Ad-COONa as a function of pH revealed a remarkable change between pH 9.0 and 10.0 (Figure 7). This is in good accordance with the pK_a of the N-3 proton of thymine base (i.e., thymidine pK_a = 9.8).[18] Therefore, the enhancement of K_{assn} for **3a** and **3b** at neutral pH compared with **8** can be attributed to hydrogen-bond formation between the thymine and adenine moieties which may form a sort of cap above the cyclodextrin cavity[5] (Figure 8). Additional support for this proposal was provided by a remarkable enhancement of the effect in dimethylformamide (DMF), in which the hydrogen bond is known to be stronger than in aqueous media.[19] As shown in Table III, K_{assn} of **3b** is almost 30 times greater than that of **8** in DMF (even 11 times greater than that of β-cyclodextrin). These results indicate that the effect of adenine–thymine base pairing on the cyclodextrin cavity is geometrically most favorable in **3b**, as represented schematically in Figure 8. While the adenine moiety in a monofunctionalized β-CD is prone to interact with the cavity to form a "shallow floor",[4] the effective formation of specific hy-

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Table II. Association Constants of Sodium 1-Adamantanecarboxylate, Methyl Orange (MO), and Sodium *p*-Nitrophenoxide (PNPNa) with β-Cyclodextrin Derivatives[a]

host	guest	K_{assn}(× 10^3, M^{-1})[b] pH 7	pH 11	K_{assn}(pH 7)/K_{assn}(pH 11)
Ade-C_3-β-CD (**8**)	1-Ad-COONa	2.7	2.4	1.13
	PNPNa	*c,d*	*d*	
Thy-C_3-β-CD (**9**)	1-Ad-COONa	3.7	3.5	1.06
	MO	5.2	4.8	1.08
	PNPNa	0.70[c]	0.73	0.96
Thy,Ade-C_3-β-CD (AD) (**3a**)	1-Ad-COONa	5.7	2.3	2.48
Thy,Ade-C_3-β-CD (AC) (**3b**)	1-Ad-COONa	9.6	2.3	4.17
	MO	4.0	1.2	3.33
	PNPNa	0.74[c]	*d*	
Thy,Ade-C_3-β-CD (AB) (**3c′**)	1-Ad-COONa	5.0	4.8	1.04
Thy,Ade-C_3-β-CD (BA) (**3c″**)	1-Ad-COONa	4.1	3.5	1.17
Ade,Ade-C_3-β-CD (AD) (**6a**)	1-Ad-COONa	3.6	3.6	1.00
Ade,Ade-C_3-β-CD (AC) (**6b**)	1-Ad-COONa	4.4	4.1	1.07
Ade,Ade-C_3-β-CD (AB) (**6c**)	1-Ad-COONa	2.3	2.1	1.10
Thy,Thy-C_3-β-CD (AD) (**7a**)	1-Ad-COONa	8.8	*d*	
	MO	8.0	9.5	0.84
Thy,Thy-C_3-β-CD (AC) (**7b**)	1-Ad-COONa	9.9	*d*	
	MO	7.5	7.6	0.99
Thy,Thy-C_3-β-CD (AB) (**7c**)	1-Ad-COONa	8.0	*d*	
	MO	8.2	9.4	0.87

[a] The constants were estimated on the basis of the UV spectral change; error estimates ±5%. [b] 0.05 M phosphate buffer (pH 7.00) and 0.05 M borate buffer (pH 11.0), 25 °C. [c] 0.05 M borate buffer (pH 8.5), 25 °C. [d] The association constant could not be estimated due to a very small change in the absorbance spectra.

Table III. Association Constants of Sodium *p*-Nitrophenoxide (PNPNa) with β-Cyclodextrin Derivatives[a]

host	guest	K_{assn}[b]
β-CD	PNPNa	400[c]
Ade-C_3-β-CD (**8**)	PNPNa	140
Thy,Ade-C_3-β-CD (AC) (**3b**)	PNPNa	4600

[a] The constants were estimated on the basis of the UV spectral change; error estimates ±5%. [b] In DMF at 25 °C. [c] Reported value (see ref 20).

drogen bonds between adenine and thymine in difunctionalized **3a,b** might prevent such adenine-cavity interaction and instead form the "deep floor" found in the capped cyclodextrins,[5,7] which would produce an enhancement of their binding abilities. Accordingly, the decrease of association constants for **3a,b** at the higher pH can be attributed to a conformational change which results from hydrogen-bond rupture (see Figure 8).

In contrast, the symmetrically difunctionalized compounds (**6** and **7**) showed no such pH dependency. In Ade,Ade-C_3-β-CD (**6**), the slight increment of K_{assn} observed for the AD (**6a**) and AC isomers (**6b**) may result from a much weaker interaction, base-stacking, which interferes with the adenine-cavity interaction[4] and results in a kind of capping effect (Figure 9, C), while the AB isomer (**6c**) geometrically disfavors the base-stacking and exhibits a K_{assn} similar to that of Ade-C_3-β-CD (**8**). On the other hand, the enhancement of K_{assn} for Thy,Thy-C_3-β-CD (**7**) may be attributed to an extended conformation which expands the hydrophobic region above the cavity upon inclusion of the guest molecules (as shown in Figure 9, A), since the interaction between thymine and the cavity is known to be weaker than that of adenine.[4]

Reactions with Chloroacetaldehyde. The reaction of the adenine derivatives with chloroacetaldehyde is known to lead to the fluorescent products (ε-adenine derivatives),[21] which have been utilized in fluorescent labeling experiments of DNA and RNA,[22,23] and in the detection of adenine residues free from base-pairing.[24] To examine the degree of base-pairing in the nucleobase-difunctionalized β-CDs, the adenine derivatives (**3a**, **3b**, **3c′**, and **8**) as well as adenosine itself were treated with chloroacetaldehyde at 25 °C (pH 6.4), and the reactions were followed by fluorescence spectroscopy which showed an intense band at 410 nm (excitation at 330 nm) (Figure 10). In all cases, the fluorescence band increased with the time and reached a stationary state within 60 h (Figure 11). Although the differences are generally small, the fluorescence intensities decreased in the order (**3c′ > 3a > 3b**) which is the reverse of their binding abilities at pH 7 (Table II). This suggests that the reduced reactivity of **3b** with chloroacetaldehyde may result from hydrogen bonding within the substrate.

Conclusion. Three different nucleobase-difunctionalized β-cyclodextrins Thy,Ade-C_3-β-CD (**3**), Ade,Ade-C_3-β-CD (**6**), and Thy,Thy-C_3-β-CD (**7**) have been synthesized. On the basis of their spectral properties and measurements of their binding abilities, the most plausible conformations for these difunctionalized β-cyclodextrins (in the order of decreasing binding abilities) are shown in Figure 9. In the case of Thy,Ade-C_3-β-CD (**3a** and **3b**), thymine–adenine interaction via hydrogen bonds (*complementary nucleobase pair*) plays an important role for the enhancement of their binding ability, which can be "switched on" and "switched off" by changing the pH (Figure 8). This is the first example of environmental control for conformational changes of β-cyclodextrin derivatives and is reminiscent of the denaturation of DNA.[3]

Experimental Section

General Procedures. Melting points were measured with a Yanagimoto micro melting apparatus and are uncorrected. ^1H NMR spectra, IR spectra, UV spectra, fluorescence spectra, and

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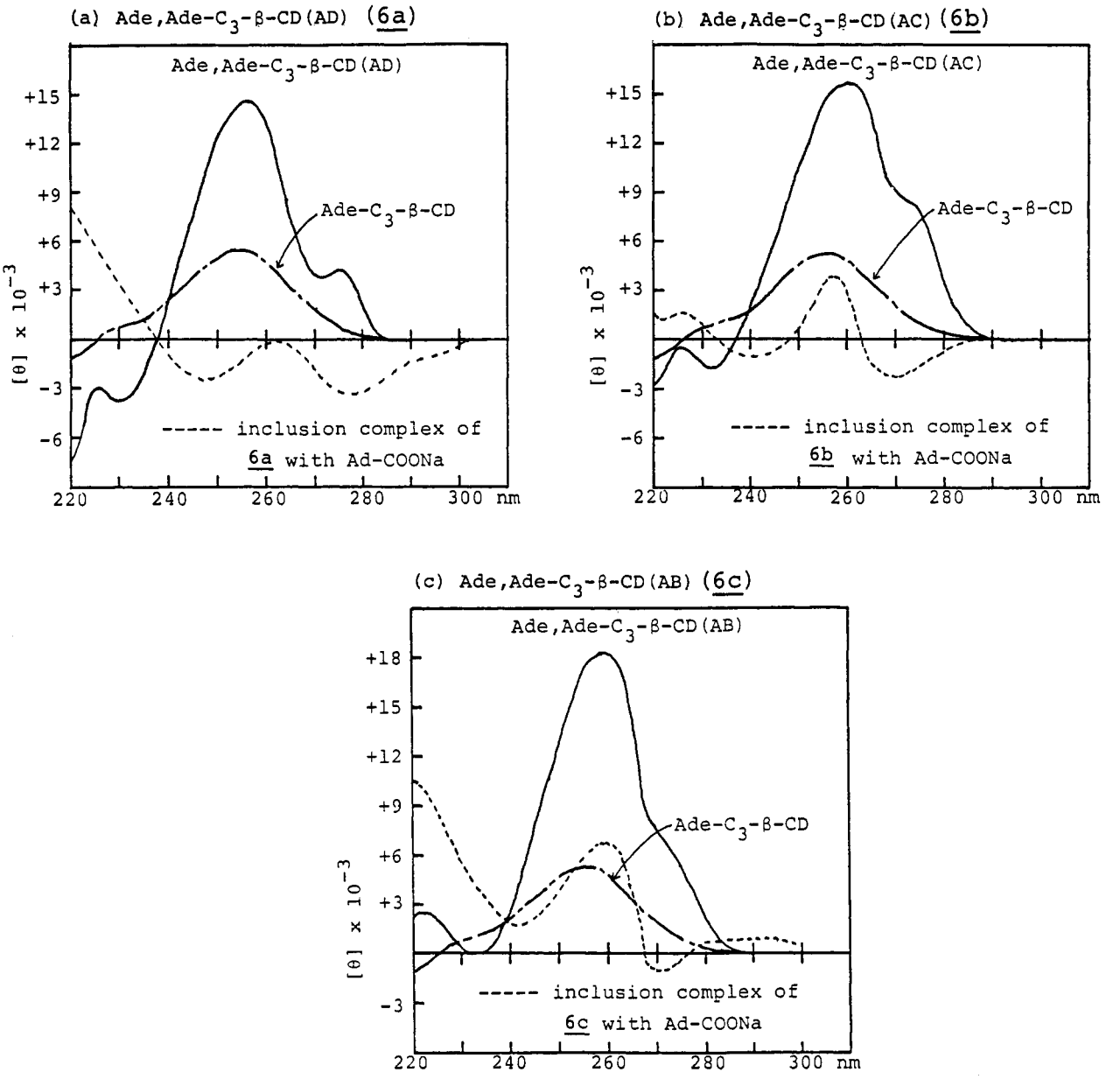

Figure 3. CD spectra of Ade,Ade-C_3-β-CD ((a) AD isomer; (b) AC isomer; (c) AB isomer) in phosphate buffer (pH 7.0) at 25 °C, of the inclusion complexes of **6a–c** with Ad-COONa (2.5×10^{-3} M), and of Ade-C_3-β-CD (8).

CD spectra were taken with a JEOL PS-100 spectrometer, a JASCO A-102 infrared spectrophotometer, a Hitachi Model 323 spectrophotometer, a Hitachi Model 650-60 fluorescence spectrophotometer, and a JASCO 40S spectrometer, respectively. FAB mass spectra were obtained with a JEOL TMS-DX 300 spectrometer operating at 1.5-keV accelerating voltage.

6,6′-Dideoxy-6-(tosyloxy)-6′-[(3-(aden-9-yl)propyl)thio]-β-cyclodextrin (2a–c). Compound 2a. 6A6D-Dideoxybis(tosyloxy)-β-cyclodextrin (**1a**) (220 mg, 1.5×10^{-4} mol) and 9-(3-mercaptopropyl)adenine (**4**) (70 mg, 2.2 equiv) were dissolved in DMF (3 mL). To this solution was added Na_2CO_3–$NaHCO_3$ buffer (pH 9.4, 7 mL), and the resulting solution was stirred at room temperature for 2 days under nitrogen. The reaction mixture was neutralized by 1 N HCl and unreacted **4** was extracted with $CHCl_3$–MeOH (10:1). After the water layer was evaporated in vacuo, the residue was chromatographed on a reversed phase column (Lobar column LiChroprep Rp-8, Merck Ltd., 25 × 300 mm) using H_2O–EtOH (a stepwise followed by a linear gradient elution was applied (see Figure 1)) to give product **2a** (80 mg, 36%): colorless powder; mp 247–251 °C dec; IR (KBr) 3350, 1640, 1180, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 2.07 (2 H, $-CCH_2C-$), 2.39 (2 H, $-SCH_2C-$), 2.45 (3 H, $PhCH_3$), 2.89 (2 H, CD-CH_2S-), 3.10–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 5.02 (7 H, CDC_1H), 7.39 and 7.63 (2 d, 4 H, Ar H), 8.10 (s, 1 H, Ade-C_2H), 8.17 (s, 1 H, Ade-C_8H); FAB MS 1480 $(M + H)^+$. Anal. Calcd for $C_{57}H_{85}N_5O_{36}S_2 \cdot 5H_2O$: C, 44.44; H, 6.01; N, 4.39. Found: C, 44.29; H, 5.71; N, 4.37.

Compound 2b was prepared from **1b** and **4** in the manner described above in 36% yield: colorless powder; mp 241–245 °C dec; IR (KBr) 3350, 1640, 1180, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 2.09 (2 H, $-CCH_2C-$), 2.46 (5 H, $-SCH_2C-$, $PhCH_3$), 2.89 (2 H, CD-CH_2S-), 3.10–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 4.97 (7 H, CD-C_1H), 7.47 and 7.74 (2 d, 4 H, Ar H), 8.05 (s, 1 H, Ade-C_2H), 8.21 (s, 1 H, Ade-C_8H); FAB MS 1480 $(M + H)^+$. Anal. Calcd for $C_{57}H_{85}N_5O_{36}S_2 \cdot 5H_2O$: C, 44.44; H, 6.01; N, 4.39. Found: C, 44.36; H, 5.83; N, 4.25.

Compounds 2c′ and 2c″ were prepared from **1c** and **4** in the same manner in 20% and 17% yield, respectively. **2c′**: colorless powder; mp 248–250 °C dec; IR (KBr) 3350, 1640, 1180, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 2.10 (2 H, $-CCH_2C-$), 2.42 (2 H, $-SCH_2C-$), 2.51 (3 H, $PhCH_3$), 2.91 (2 H, CD-CD_2S-), 3910–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 5.01 (7 H, CD-C_1H), 7.47 and 7.69 (2 d, 4 H, Ar H), 8.14 (s, 1 H, Ade-C_2H), 8.22 (s, 1 H, Ade-C_8H); FAB MS 1480 $(M + H)^+$. Anal. Calcd for $C_{57}H_{85}N_5O_{36}S_2 \cdot 4H_2O$: C, 44.95; H, 5.95; N, 4.44. Found: C, 44.71; H, 5.78; N, 4.38. **2c″**: colorless powder; mp 246–249 °C dec; IR (KBr) 3350, 1640, 1180, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 2.08 (2 H, $-CCH_2C-$), 2.40 (3 H, $-SCH_2C-$), 2.47 (3 H, $PhCH_3$), 2.90 (2 H, CD-CH_2S-), 3.10–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 5.01 (7 H, CD-C_1H), 7.47 and 7.74 (2 d, 4 H, Ar H), 8.09 (s, 1 H, Ade-C_2H), 8.23 (s, 1 H, Ade-C_8H); FAB MS 1480 $(M + H)^+$. Anal. Calcd for $C_{57}H_{85}N_5O_{36}S_2 \cdot 4H_2O$:

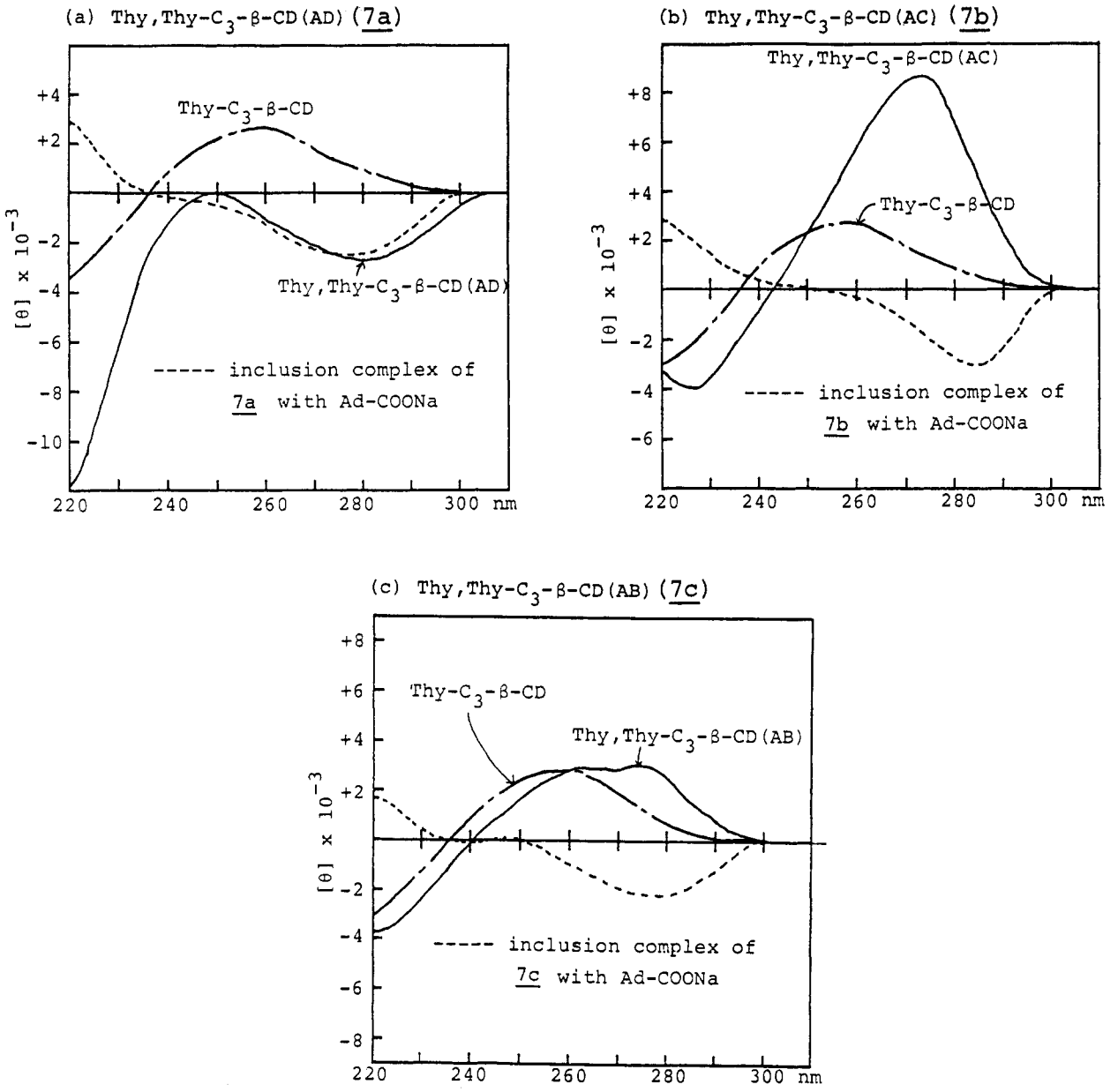

Figure 4. CD spectra of Thy,Thy-C_3-β-CD ((a) AD isomer; (b) AC isomer; (c) AB isomer) in phosphate buffer (pH 7.0) at 25 °C, of the inclusion complexes of **7a–c** with Ad-COONa (2.5×10^{-3} M), and of Thy-C_3-β-CD (**9**).

C, 44.95; H, 5.95; N, 4.44. Found: C, 44.73; H, 5.79; N, 4.35.

6,6′-Dideoxy-6-[(3-(thym-1-yl)propyl)thio]-6′-[(3-(aden-9-yl)propyl)thio]-β-cyclodextrin (3a–c). Compound 3a. 2a (80 mg) and 1-(3-mercaptopropyl)thymine (**5**) (200 mg) were dissolved in DMF (5 mL). To this solution was added Na_2CO_3–$NaHCO_3$ buffer (pH 9.4, 10 mL), and the resulting solution was stirred at room temperature under nitrogen. After 1 week, the reaction mixture was neutralized by 1 N HCl and excess **5** was extracted with $CHCl_3$. After the water layer was evaporated in vacuo, the residue was chromatographed on a reversed phase column (Lobar column LiChroprep Rp-8, Merck Ltd., 25 × 300 mm) using H_2O–EtOH to give product **3a** (70 mg, 71%): colorless powder; mp 260–265 °C dec; IR (KBr) 3350, 1690, 1670, 1640, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 1.80 (3 H, Thy-CH_3), 1.92, 2.09 (4 H, $-CCH_2C-$), 2.51 (4 H, $-SCH_2C-$), 2.85 (4 H, CD-CH_2S-), 3.10–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 4.98 (7 H, CD-C_1H), 7.39 (s, 1 H, Thy-C_6H), 7.99, 8.01 (1 H, Ade-C_2H), 8.19 (s, 1 H, Ade-C_8H): UV (pH 7.0 phosphate buffer) λ_{max} 265 nm (ε 17 200); FAB MS 1508 (M + H)$^+$. Anal. Calcd for $C_{58}H_{89}N_7O_{35}S_2 \cdot 6H_2O$: C, 43.09; H, 6.30; N, 6.07. Found: C, 43.02; H, 6.18; N, 5.99.

Compound 3b was prepared from **2b** and **5** in the same manner in 84% yield: colorless powder; mp 260–263 °C dec; IR (KBr) 3350, 1690, 1670, 1640, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 1.80 (3 H, Thy-CH_3), 1.95, 2.04 (4 H, $-CCH_2C-$), 2.51 (4 H, $-SCH_2C-$), 2.85 (4 H, CD-CH_2S-), 3.10–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 4.96 (7 H, CD-C_1H), 7.37 (s, 1 H, Thy-C_6H), 8.03, 8.04 (1 H, Ade-C_2H), 8.18 (s, 1 H, Ade-C_8H); UV (pH 7.0 phosphate buffer) λ_{max} 265 nm (ε 17 000); FAB MS 1508 (M + H)$^+$. Anal. Calcd for $C_{58}H_{89}N_7O_{35}S_2 \cdot 6H_2O$: C, 43.09; H, 6.30; N, 6.07. Found: C, 43.01; H, 6.22; N, 5.83.

Compounds 3c′ and 3c″ were prepared from **2c′** (or **2c″**) and **5** in the same manner in 63% and 64% yield, respectively. **3c′**: colorless powder; mp 249–251 °C dec; IR (KBr) 3350, 1690, 1670, 1640, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 1.79 (3 H, Thy-CH_3), 1.93, 2.09 (4 H, $-CCH_2C-$), 2.53 (4 H, $-SCH_2C-$), 2.86 (4 H, CD-CH_2SC-), 3.10–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 5.00 (7 H, CD-C_1H), 7.33 (s, 1 H, Thy-C_6H), 8.00 (s, 1 H, Ade-C_2H), 8.16 (s, 1 H, Ade-C_8H); UV (pH 7.0 phosphate buffer) λ_{max} 265 nm (ε 15 700); FAB MS 1508 (M + H)$^+$. Anal. Calcd for $C_{58}H_{89}N_7O_{35}S_2 \cdot 5H_2O$: C, 43.58; H, 6.24; N, 6.13. Found: C, 43.32; H, 6.09; N, 5.75. **3c″**: colorless powder; mp 236–239 °C dec; IR (KBr) 3350, 1690, 1670, 1640, 1160 cm^{-1}; ^1H NMR (δ, D_2O) 1.80 (3 H, Thy-CH_3), 1.92, 2.08 (4 H, $-CCH_2C-$), 2.52 (4 H, $-SCH_2-$), 2.86 (4 H, CD-CH_2S-), 3.10–4.00 (42 H, CD-C_2C_6H, $-NCH_2C-$), 4.96 (7 H, CD-C_1H), 7.38 (s, 1 H, Thy-C_6H), 8.03 (s, 1 H, Ade-C_2H), 8.19 (s, 1 H, Ade-C_8H); UV (pH 7.0 phosphate buffer) λ_{max} 265 nm (ε 16 300); FAB MS 1508 (M + H)$^+$. Anal. Calcd for $C_{58}H_{89}N_7O_{35}S_2 \cdot 5H_2O$: C, 43.58; H, 6.24; N, 6.13. Found: C, 43.46; H, 6.12; N, 6.04.

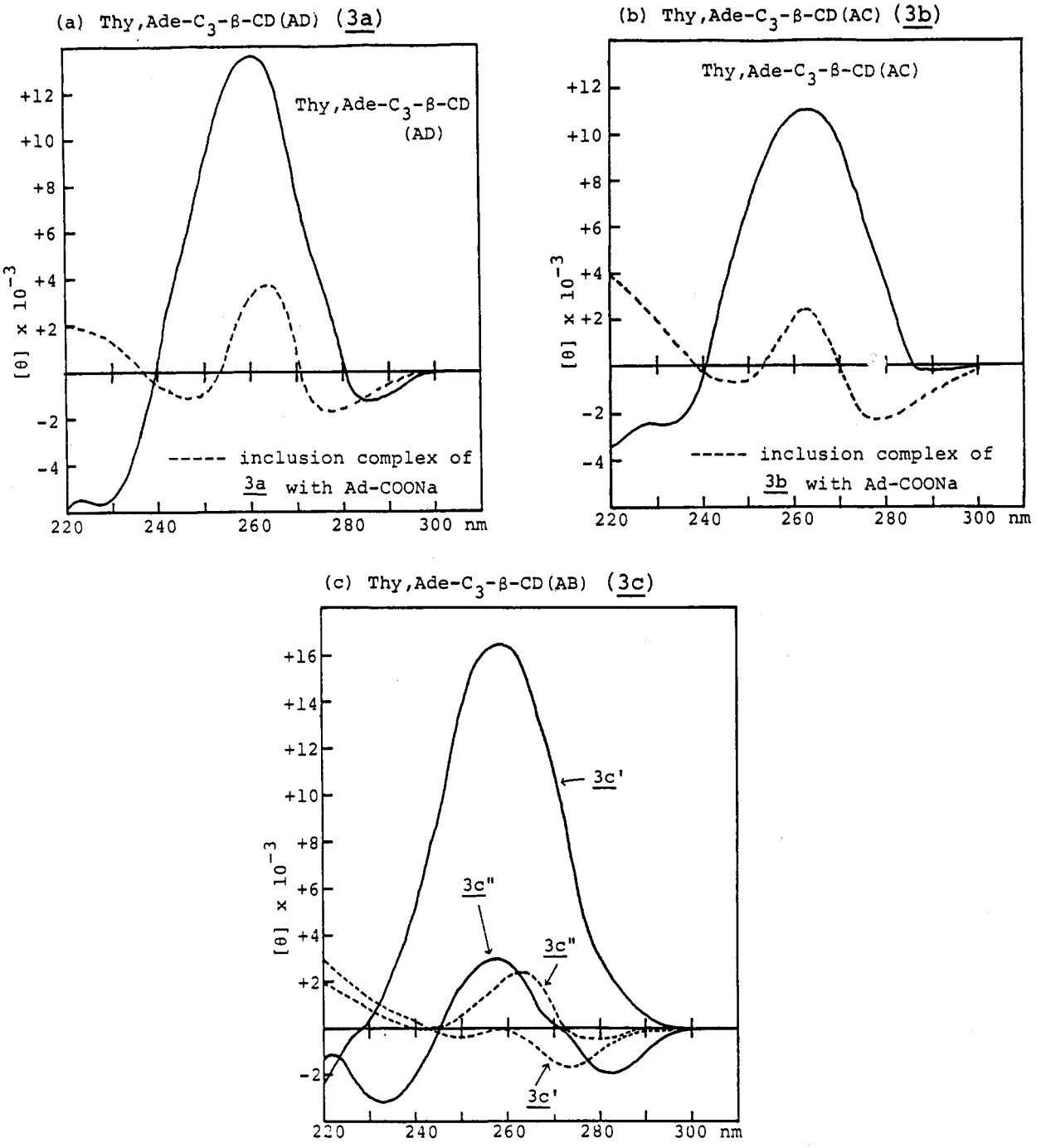

Figure 5. CD spectra of Thy,Ade-C_3-β-CD ((a) AD isomer; (b) AC isomer; (c) AC isomer) in phosphate buffer (pH 7.0) at 25 °C and of the inclusion complexes of **3a–c** with Ad-COONa (2.5×10^{-3} M).

6,6′-Dideoxy-6,6′-bis[(3-(thym-1-yl)propyl)thio]-β-cyclodextrin (7a–c). Compound 7a: 6A6D-Dideoxybis(tosyloxy)-β-cyclodextrin (**1a**) (120 mg) and **5** (200 mg) were dissolved in DMF (5 mL). To this solution was added Na_2CO_3–$NaHCO_3$ buffer (pH 9.4, 15 mL), and the resulting solution was stirred at 50 °C under nitrogen. After 2 days, the reaction mixture was neutralized by 1 N HCl and excess **5** was extracted with $CHCl_3$. After the water layer was evaporated in vacuo, the residue was chromatographed on a reversed phase column (Lobar column LiChroprep Rp-8, Merck Ltd., 25 × 300 mm) using H_2O–EtOH to give product **7a** (61 mg, 49%): colorless powder; mp 268–271 °C dec; ^{1}H NMR (δ, D_2O) 1.77 (10 H, Thy-CH_3, –CCH_2C–), 2.49 (4 H, –SCH_2C–), 2.83 (4 H, CD-CH_2S–), 3.10–4.00 (42 H, CD-C_2C_6H, –NCH_2C–), 4.97 (7 H, CD-C_1H), 7.27 (s, 2 H, Thy-C_6H); UV (pH 7.0 phosphate buffer) λ_{max} 273 nm (ε 18 800); FAB MS 1499 $(M + H)^+$. Anal. Calcd for $C_{58}H_{90}N_4O_{37}S_2 \cdot 7H_2O$: C, 42.85; H, 6.45; N, 3.45. Found: C, 42.84; H, 6.40; N, 3.47.

Compound 7b was prepared from **1b** and **5** in the same manner in 58% yield: colorless powder; mp 274–276 °C dec; ^{1}H NMR (δ, D_2O) 1.78 (10 H, Thy-CH_3, –CCH_2C–), 2.46 (4 H, –SCH_2C–), 2.85 (4 H, CD-CH_2S–), 3.10–4.00 (42 H, CD-C_2C_6H, –NCH_2C–), 4.93 (7 H, CD-C_1H), 7.29 (s, 2 H, Thy-C_6H); UV (pH 7.0 phosphate buffer) λ_{max} 273 nm (ε 19 000); FAB MS 1499 $(M + H)^+$. Anal. Calcd for $C_{58}H_{90}N_4O_{37}S_2 \cdot 7H_2O$: C, 42.85; H, 6.45; N, 3.45. Found: C, 42.78; H, 6.41; N, 3.35.

Compound 7c was prepared from **1c** and **5** in the same manner in 41% yield: colorless powder; mp 250–253 °C dec; ^{1}H NMR (δ, D_2O) 1.78 (10 H, Thy-CH_3, –CCH_2C–), 2.51 (4 H, –SCH_2C–), 2.86 (4 H, CD-CH_2S–), 3.10–4.00 (42 H, CD-C_2C_6H, –NCH_2C–), 4.96 (7 H, CD-C_1H), 7.28, 7.32 (2 H, Thy-C_6H); UV (pH 7.0 phosphate buffer) λ_{max} 273 nm (ε 18 300); FAB MS 1499 $(M + H)^+$. Anal. Calcd for $C_{58}H_{90}N_4O_{37}S_2 \cdot 7H_2O$: C, 42.85; H, 6.45; N, 3.45. Found: C, 42.78; H, 6.35; N, 3.53.

6,6′-Dideoxy-6,6′-bis[(3-(aden-9-yl)propyl)thio]-β-cyclodextrin (6a–c) were prepared from **1a–c** and **4** in the same manner; compounds **6a–c** were obtained in 52%, 59%, and 48% yield, respectively. **6a**: colorless powder; mp 275–278 °C dec; ^{1}H NMR (δ, D_2O) 2.08 (4 H, –CCH_2C–), 2.40 (4 H, –SCH_2C–),

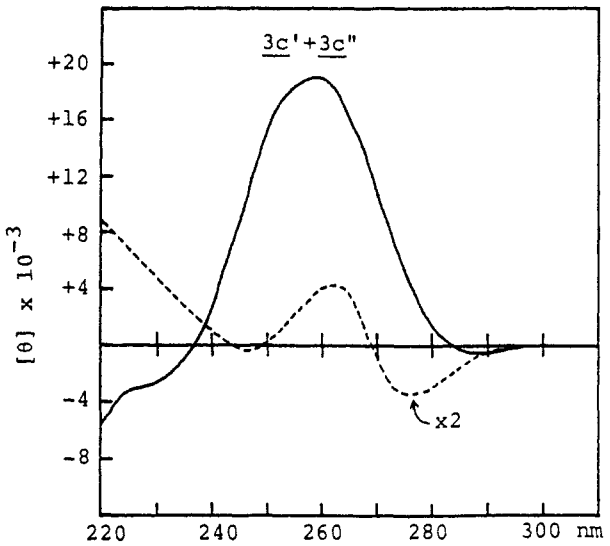

Figure 6. Calculated CD spectra from the spectra of **3c′** and **3c″** (—) and from the inclusion complexes of **3c′** (and **3c″**) with Ad-COONa (---).

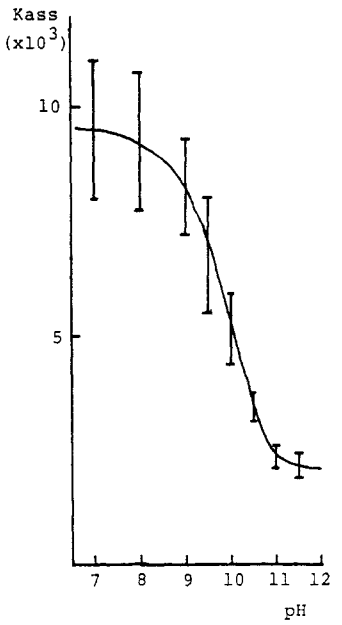

Figure 7. pH dependence of the association constants of sodium 1-adamantanecarboxylate with Thy,Ade-C_3-β-CD (AC, **3b**) at 25 °C.

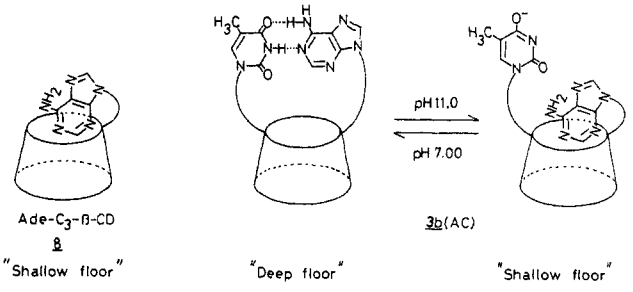

Figure 8.

2.82 (4 H, CD-CH_2S–), 3.10–4.00 (42 H, CD-C_2C_6H, –NCH_2C–), 5.04 (7 H, CD-C_1H), 7.87 (s, 1 H, Ade-C_2H), 7.96 (s, 1 H, Ade-C_2H), 8.22 (s, 2 H, Ade-C_8H); UV (pH 7.0 phosphate buffer) λ_{max} 262 nm (ε 21 300); FAB MS 1517 $(M + H)^+$. Anal. Calcd for $C_{58}H_{88}N_{10}O_{33}S_2 \cdot 6H_2O$: C, 42.85; H, 6.20; N, 8.62. Found: C, 42.50; H, 6.06; N, 8.44. **6b**: colorless powder; mp 268–272 °C dec; ^1H NMR (δ, D_2O) 2.05 (4 H, –CCH_2C–), 2.43 (4 H, –SCH_2C–), 2.82 (4 H, CD-CH_2S–), 3.10–4.00 (42 H, CD-C_2C_6H, –NCH_2C–), 4.96 (7 H, CD-C_1H), 7.91 (s, 1 H, Ade-C_2H), 8.02 (s, 1 H, Ade-C_2H), 8.18 (s, 2 H, Ade-C_8H); UV (pH 7.0 phosphate buffer) λ_{max} 262 nm (ε 24 800); FAB MS 1517 $(M + H)^+$. Anal. Calcd for $C_{58}H_{88}N_{10}O_{33}S_2 \cdot 5H_2O$: C, 43.33; H, 6.15; N, 8.71. Found: C, 43.13; H, 5.94; N, 8.83. **6c**: colorless powder; mp 271–275 °C dec; ^1H NMR (δ, D_2O) 2.08 (4 H, –CCH_2C–), 2.40 (4 H, –SCH_2C–), 2.81 (4 H, CD-CH_2S–), 3.10–4.00 (42 H, CD-C_2C_6H, –NCH_2C–), 4.98 (7 H, CD-C_1H), 7.97 (s, 2 H, Ade-C_2H), 8.16 (s, 2 H, Ade-C_8H); UV (pH 7.0 phosphate buffer) λ_{max} 262 nm (ε 26 700); FAB MS 1517 $(M + H)^+$. Anal. Calcd for $C_{58}H_{88}N_{10}S_2 \cdot 7H_2O$: C, 42.38; H, 6.26; N, 8.52. Found: C, 42.16; H, 6.15; N, 8.48.

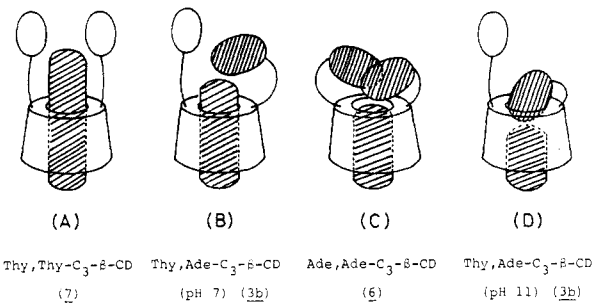

Figure 9.

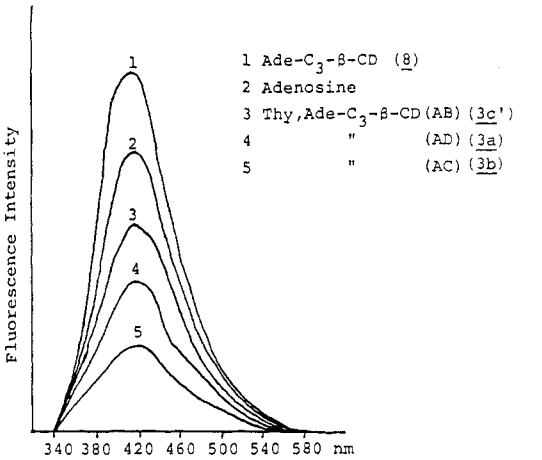

Figure 10. Fluorescence spectra of the reaction mixture of adenine derivatives and chloroacetaldehyde. Excitation was at 330 nm.

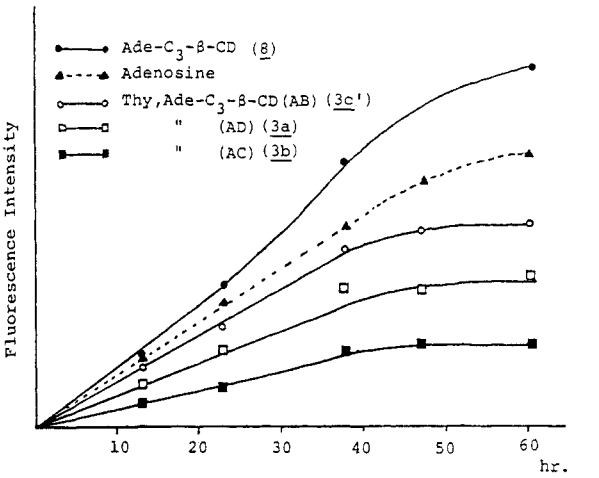

Figure 11. Fluorescence intensity at 410 nm vs. time of incubation (pH 6.4, 25 °C) for the reaction mixture of adenine derivatives and chloroacetaldehyde.

Association Constants between β-Cyclodextrin Derivatives and Guest Molecules. The difference UV spectra were taken between β-cyclodextrin derivatives (5.0×10^{-5} M) alone and β-cyclodextrin derivatives (5.0×10^{-5} M) in the presence of sodium 1-adamantanecarboxylate in phosphate buffer (pH 7.0) and borate

buffer (pH 11.0) solution at 25 °C. The reciprocal of the difference absorbance around 260–280 nm was plotted against the reciprocal of the guest concentration. From the slope and the intercept, the association constant was obtained. The 1-adamantanecarboxylate concentration ranges from 1.0×10^{-4} M to 7.5×10^{-4} M. The association constants between β-cyclodextrin derivatives and methyl orange (5×10^{-5} M) or sodium *p*-nitrophenoxide (5×10^{-5} M) were also estimated by the difference spectra at 25 °C, where substituted β-cyclodextrin concentration range from 1.0×10^{-4} M to 1.0×10^{-3} M. The association constants in various pH were estimated in the same manner at 25 °C in Na_2CO_3–$NaHCO_3$ buffer (pH 8.0–11.5) solution. These spectral data were treated by the Benesi–Hildebrand method as previously reported.[4]

Reaction of Chloroacetaldehyde with Adenine Derivatives. The mixture of chloroacetaldehyde (1×10^{-2} M) with adenine derivatives (1×10^{-5} M) in phosphate buffer (pH 6.4) solution was incubated at 25 °C. The reaction was followed by a fluorescence measurement at 410 nm (at 13–60 h after the start of incubation).

Acknowledgment. We are grateful to Dr. K. Fujita (Fukuyama University) for the private communication of preparation, separation, and identification of **1a–c** and the valuable discussion.

Photochemistry of Polyhaloarenes. 4. Phototransformations of Perchloro-*o*-phenoxyphenol in Basic Media

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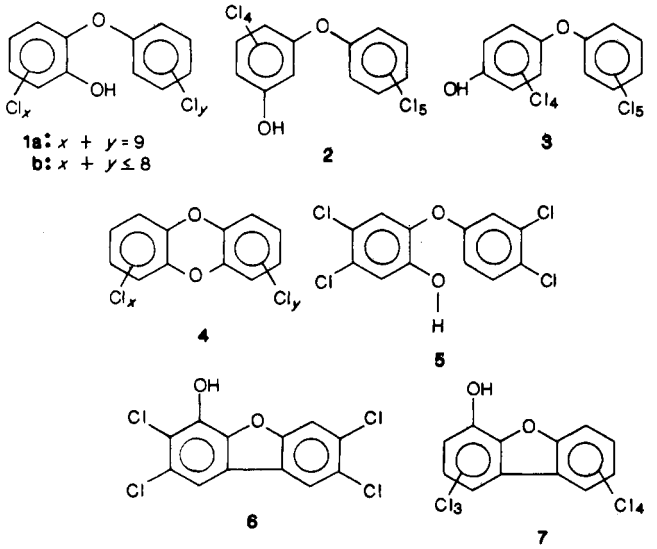
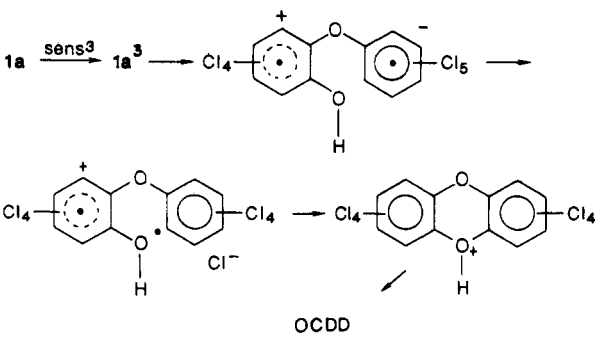
Irradiation of the sodium salt of the conjugate base of perchloro-*o*-phenoxyphenol (PreD$^-$Na$^+$) in methanol (300 nm) in the presence of sensitizer *m*-methoxyacetophenone generates ether cleavage products and mono- and di-dechlorination with no cyclization to OCDD. In the presence of sensitizer and excess triethylamine, irradiation of perchloro-*o*-phenoxyphenol leads to OCDD as a major product with ether cleavage and dechlorination products representing important reaction pathways. Photodecomposition of the conjugate base of perchloro-*o*-phenoxyphenol in methanol reveals a small amount of cyclization, while irradiation in methanol in the presence of a 10-fold excess of triethylamine increases the quantum yield for cyclization 17-fold. The photolytic transformations of the conjugate base of perchloro-*o*-phenoxyphenol in the presence of excess triethylamine are dependent upon solvent polarity with the quantum yield for cyclization increasing strongly in methanol or water/acetonitrile (70:30) relative to that in dibutyl ether. These results are interpreted in terms of electron transfer to PreD$^-$ to form a radical dianion.

Our interest in the photochemical transformations of perchloro-*o*-, *m*-, and *p*-phenoxyphenol (**1a**, **2**, and **3**) is stimulated by the fact that these species are important contaminants in commercial pentachlorophenol,[1] absorb in the sunlight spectral range (near 300 nm), and possess the structural potential for conversion to polychlorodibenzodioxins **4**, which would include the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin substrate (TCDD),[2] mimics of TCDD (**5** and **6**),[3] and closely related analogues.



Scheme I



Our earlier studies[4,5] revealed that direct irradiation of perchloro-*o*-phenoxyphenol (**1a**) (300 nm, cyclohexane) results in (a) ether cleavage forming polychlorobenzenes, phenols and catechol and (b) reductive dechlorination generating polychloro-*o*-phenoxyphenols **1b**, while sensitized photodecomposition (acetone; *m*-methoxyacetophenone in cyclohexane) results in either predominant (83.6%) or substantial (32%) cyclization to polychlorodibenzodioxins **4** and hydroxyheptachlorodibenzofuran (**7**).

The cyclization pathway in acetone was enhanced by 85% in the presence of an electron-transfer reagent, triethylamine. Since the C–Cl bond energy should be about 95 kcal/mol,[6] while the triplet state energy is 72 kcal/mol

(1) Deinzer, M.; Lamberton, J.; Griffin, D.; Miller, T. *Biomed. Mass Spectrom.* **1978**, *5*, 566–571.
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