Nucleobase-Functionalized β-Cyclodextrins. Preparation and Spectral Properties

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The nucleobase-functionalized β-cyclodextrins in which a nucleobase was linked to C-6 of β-cyclodextrin directly or through a flexible carbon chain were synthesized. Their structures were determined on the basis of the spectroscopic data and elemental analyses. Fast atom bombardment mass spectrometry was employed to determine the molecular weight of these nonvolatile compounds. The specific interaction of the nucleobase moiety with the cyclodextrin cavity, the influence of chain length on their inclusion abilities, and the pH-dependent conformational change of these compounds are discussed on the basis of analyses of these molecules by UV and circular dichroism spectra.

Cyclodextrins are naturally occurring doughnut-shaped molecules usually composed of six, seven, and eight D-glucose units (α-, β-, and γ-cyclodextrins, respectively) and are known to be able to form inclusion complexes with a wide variety of molecules. Due to these properties, cyclodextrins show in many reactions the enzyme-like activities such as reaction-rate enhancement, stereoselection, regioselection, and enantioselection.[1] Recently, chemical modification of cyclodextrins with various functional groups has been extensively investigated in order to make more effective enzyme models.[2]

The nucleosides are among the most biologically important compounds, comprising the monomeric units of DNA and RNA. The increasing recognition of various biological activities of the nucleosides has generated interest in the synthesis of cyclodextrin derivatives functionalized with the nitrogen bases of nucleic acids, which can be regarded as nucleoside analogues of cyclodextrins. In this paper, we report on the syntheses and spectral properties of the nucleobase-functionalized β-cyclodextrins, in which a nucleobase is linked to C-6 of β-cyclodextrin directly or through a flexible carbon chain (Chart I).[3] The specific interaction of the nucleobase moiety with the cyclodextrin cavity, the influence of chain length on their inclusion abilities, and the pH-dependent conformational change of these compounds are discussed on the basis of measurements of UV and circular dichroism (CD) spectra under various conditions.

Preparation. The β-cyclodextrin derivatives, Thy-C_3-β-CD (**6**), Ur-C_3-β-CD (**7**), Cyt-C_3-β-CD (**8**), and Ade-C_3-β-CD (**9**) (Chart I), were prepared by two different routes from the corresponding alkyl halides **1**, which were readily obtained by haloalkylation of the respective nucleobases (i.e., thymine, uracil, cytosine, and adenine) according to the procedure of Leonard et al.[4] (Scheme I). When 1-(3-bromopropyl)thymine (**1a**) was treated with 6-deoxy-6-mercapto-β-cyclodextrin (**4**), prepared from the corresponding tosylate **3**,[5,6] in aqueous 30% EtOH in the presence of K_2CO_3 at room temperature under nitrogen for 1 week, crystalline Thy-C_3-β-CD (**6**) was obtained in 23% yield. Similar reactions of **1b-d** with **4** afforded Ur-C_3-β-CD (**7**) (38%), Cyt-C_3-β-CD (**8**) (20%), and Ade-C_3-β-CD (**9**) (27%), respectively. As an alternative way, the bromides (or chlorides) **1a-d** were first converted to the corresponding thiols **2** by the standard method, purification of **2** being much easier than that of **4**. These

Scheme I

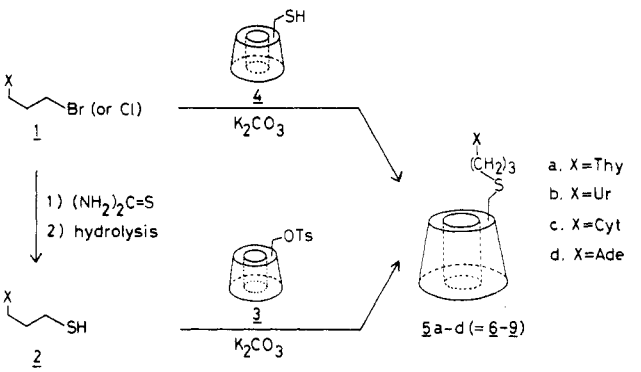

Chart I

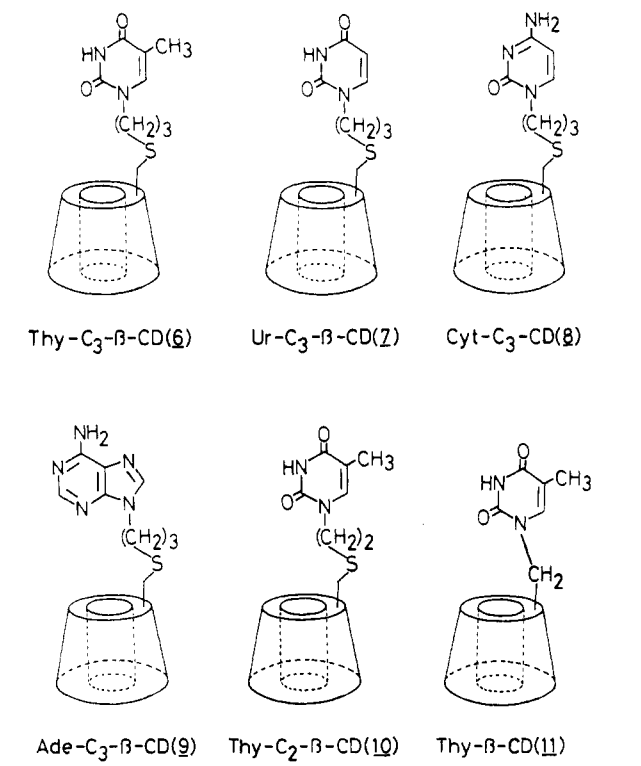

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Table I. Spectral Data of β-Cyclodextrin Derivatives

compound	IR, cm^{-1}	^1H NMR (D_2O), δ[a]	anal. calcd/found C	H	N	FAB MS
Thy-C_3-β-CD (6)	3400, 1690, 1670, 1640, 1160	1.83 (5 H, Thy-CH_3, -CH_2-), 2.52 (2 H, -SCH_2-), 2.90 (2 H, CD-CH_2S-), 3.20-4.10 (42 H, CD-C_2-C_6H, -NCH_2-), 4.96 (7 H, CD-C_1H), 7.23 (1 H, Thy-C_6H)	$C_{50}H_{80}N_2O_{36}S\cdot 9H_2O$ 4060 40.64	6.61 6.68	1.89 1.61	1317 $(M + H)^+$
Ur-C_3-β-CD (7)	3400, 1690, 1675, 1640, 1160	1.84 (2 H, -CH_2-), 2.94 (2 H, -SCH_2-), 2.91 (2 H, CD-CH_2S-), 3.20-4.00 (42 H, CD-C_2-C_6H, -NCH_2-), 4.93 (7 H, CD-C_1H), 5.71 (d, 1 H, Ur-C_5-H, J = 7.7 Hz), 7.45 (d, 1 H, Ur-C_6H, J = 7.7 Hz)	$C_{49}H_{78}N_2O_{36}S\cdot 10H_2O$ 39.68 39.66	6.68 6.68	1.89 1.69	1303 $(M + H)^+$
Cyt-C_3-β-CD (8)	3400, 1665, 1640, 1160	1.87 (2 H, -CH_2-), 2.47 (2 H, -SCH_2-), 2.86 (2 H, CD-CH_2S-), 3.20-4.00 (42 H, CD-C_2-C_6H, -NCH_2-), 4.92 (7 H, CD-C_1H), 5.85 (d, 1 H, Cyt-C_5H, J = 7.5 Hz), 7.83 (d, 1 H, Cyt-C_6H, J = 7.5 Hz)	$C_{49}H_{79}N_3O_{35}S\cdot 7H_2O$ 41.20 41.32	6.56 6.57	2.94 2.92	1302 $(M + H)^+$
Ade-C_3-β-CD (9)	3400, 1640, 1160	2.22 (4 H, -CH_2-, -SCH_2-), 2.85 (2 H, CD-CH_2S-), 3.05-4.02 (42 H, CD-C_2-C_6H, -NCH_2-), 4.90 (7 H, CD-C_1H), 7.96 8.15 (2 s, 2 H, Ade-C_2H, C_8H)	$C_{50}H_{79}N_5O_{34}S\cdot 8H_2O$ 41.10 41.04	6.43 6.37	4.71 4.75	1326 $(M + H)^+$
Thy-C_2-β-CD (10)	3400, 1690, 1670, 1640, 1160	1.78 (3 H, Thy-CH_3), 2.84 (4 H, -SCH_2-, CD-CH_2S-), 3.20-4.00 (42 H, CD-C_2-C_6H, -NCH_2-), 4.93 (7 H, CD-C_1H), 7.37 (1 H, Thy-C_6H)	$C_{49}H_{78}N_2O_{36}S\cdot 6H_2O$ 41.70 41.63	6.42 6.19	1.97 1.78	
Thy-β-CD (11)	3400, 1690, 1670, 1640, 1160	1.75 (3 H, Thy-CH_3), 3.20-4.00 (42 H, CD-C_2-C_6H, -NCH_2-), 4.97 (7 H, CD-C_1H), 7.36 (1 H, Thy-C_6H)	$C_{47}H_{74}N_2O_{36}\cdot 8H_2O$ 40.69 40.61	6.54 6.47	2.07 1.70	1243 $(M + H)^+$

[a] Center of the broad absorption. Internal standard: $(CH_3)_3SiCH_2CH_2CH_2SO_3Na$ (DSS).

Table II. Ultraviolet Maxima (nm) of β-Cyclodextrin Derivatives

compound	pH 1.0[a]	7.0[b]	9.18[b]	13.0[c]
Thy-C_3-β-CD (6)		273	272	272
Ur-C_3-β-CD (7)		266	269	266
Cyt-C_3-β-CD (8)	284	276	276	276
Ade-C_3-β-CD (9)	260	263	263	263
Thy-C_2-β-CD (10)		273	273	272
Thy-β-CD (11)		272	272	272

[a] 0.1 N HCl. [b] Phosphate buffer. [c] 0.1 N NaOH.

thiols were allowed to react with 6-*O*-tosyl-β-cyclodextrin (3) in aqueous 30% EtOH in the presence of K_2CO_3 at room temperature to give the products 6–9 in better yields (50–60%). Therefore, this route seems to be the better one, especially due to the high yield of product, easy purification, and the potential applicability to the multifunctionalization of cyclodextrins. Similarly, Thy-C_2-β-CD (10) (Chart I) was prepared from 1-(2-bromoethyl)thymine in a 43% yield via the latter route. On the other hand, Thy-β-CD (11) was obtained in a 50% yield by treatment of 3 with excess of thymine in dry Me_2SO in the presence of K_2CO_3. All these products were purified by either recrystallization or chromatography on Sephadex G-15, and their purity was checked by HPLC.

Structural Assignment. The spectral data (IR, ^1H NMR, MS) of the β-cyclodextrin derivatives 6–11 are summarized in Table I. It was readily determined that these products contain β-cyclodextrin and nucleobase moieties in a 1:1 ratio by ^1H NMR spectroscopy using the ratio of the characteristic signals of C-1 protons of β-cyclodextrin (at ca. 4.8 ppm) and aromatic protons of the nucleobase. The UV spectra of β-cyclodextrin derivatives 6–11 showed the similar pH dependences to those of the corresponding nucleosides (Table II),[7] confirming the site

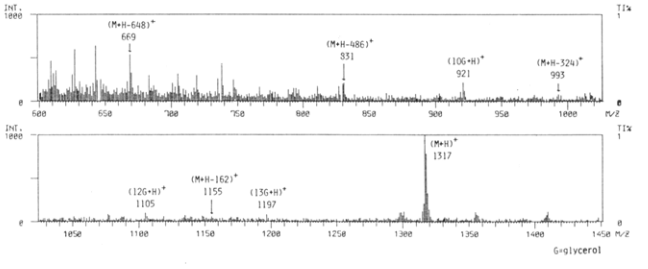

Figure 1. Positive-ion FAB mass spectrum of Thy-C_3-β-CD (6).

of alkylation on the nucleobase. Elemental analyses (Table I) show that these compounds in the solid state tend to involve several moles of water, the amount of which varies depending on the method of purification and the drying period. Therefore, the precise molecular weight of these samples needed to be determined. Conventional electron impact (EI) mass spectrometry and even field desorption (FD) mass spectrometry failed to detect molecular ions of these nonvolatile substances. However, the recently developed fast atom bombardment (FAB) mass spectrometry[8] has now been successfully employed in molecular weight determinations of β-cyclodextrin derivatives 6–11. The positive-ion FAB mass spectrum of Thy-C_3-β-CD (6) showed an intense molecular ion peak $(M + H)^+$ at m/z 1317 (Figure 1) as well as fragment ions at m/z 1155, 993, 831, and 669 with increasing intensities, which arise from subsequent losses of glucose units in the molecular ions. The similar fragmentation pattern for α- and β-cyclodextrins was observed by laser-assisted FD mass spectrometry.[9] Similarly, the intense molecular ion peaks $(M + H)^+$ of 7–11 were observed in their positive ion FAB mass spectra (see Table I).

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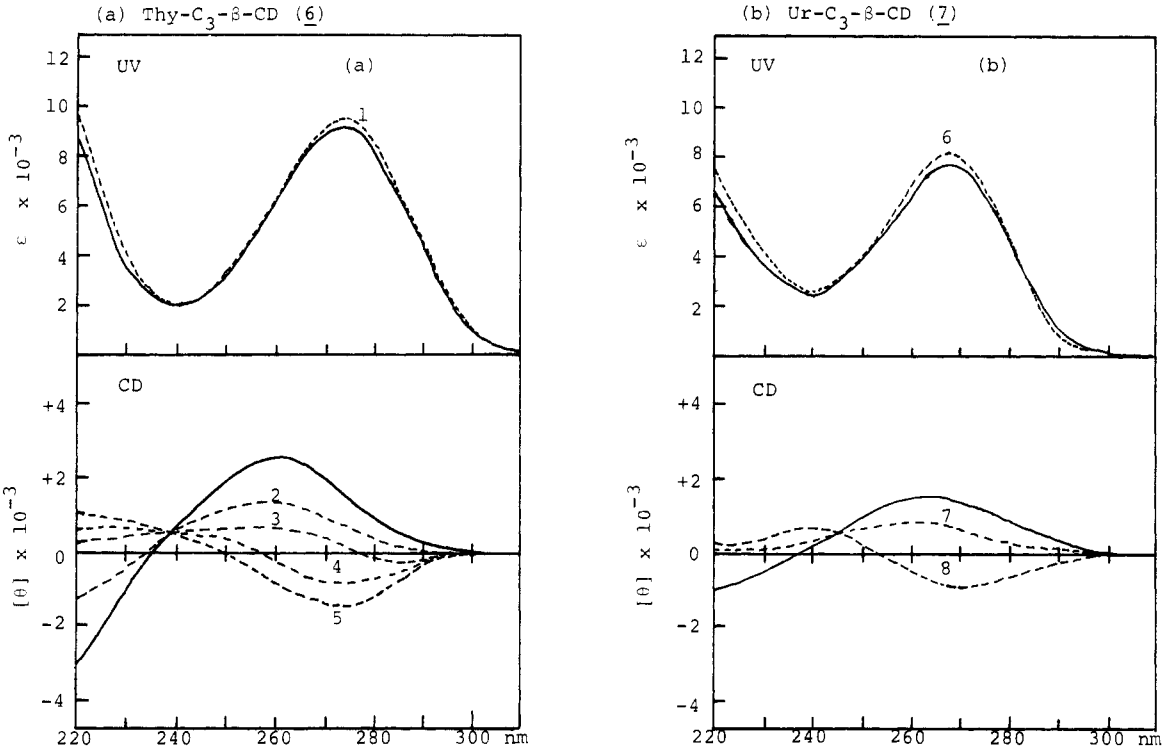

Figure 2. UV absorption and CD spectra of Thy-C_3-β-CD (**6**) (a) and Ur-C_3-β-CD (**7**) (b) in phosphate buffer (pH 9.18) at 25 °C (—); inclusion complex of **6** (and **7**) with Ad-COONa (---): 1 (3.21×10^{-4} M), 2 (1.52×10^{-4} M), 3 (2.03×10^{-4} M), 4 (2.53×10^{-4} M), 5 (5.02×10^{-4} M), 6 (3.26×10^{-4} M), 7 (1.56×10^{-4} M), 8 (5.92×10^{-4} M).

Results and Discussion

β-Cyclodextrins Linked to a Nucleobase through a Flexible Carbon Chain. The UV and CD spectra of β-cyclodextrin derivatives **6–9** are shown in Figures 2 and 3. The CD spectra at pH 9.18[10] of Thy-C_3-β-CD (**6**) and Ur-C_3-β-CD (**7**) are very similar in shape to those of the corresponding nucleosides, thymidine and uridine,[11] respectively, although slightly smaller in magnitude (Figure 2). In contrast, the CD spectra of Cyt-C_3-β-CD (**8**) and Ade-C_3-β-CD (**9**) are rather different from those of cytidine and adenosine[11] (Figure 3). The CD spectra of these compounds show a dramatic change upon the addition of the guest compound such as 1-adamantanecarboxylate. The magnitude of CD curve decreases linearly with increasing concentration of 1-adamantanecarboxylate as shown in Figures 2 and 3. Particularly, the complete reversal of the Cotton effect was observed for the pyrimidine base derivatives **6–8**. These results may be attributed to the conformational change upon guest inclusion.[6] In the case of Ade-C_3-β-CD (**9**), the larger magnitude of the CD curve (Figure 3b) compared with those of **6–8** suggests that the adenine moiety in **9** interacts with the cyclodextrin cavity more strongly than the pyrimidine base in **6–8**. This is also supported by the association constants of complex formation with 1-adamantanecarboxylate and *p*-nitrophenol by these β-cyclodextrin derivatives, which are determined by CD and UV spectroscopy (Tables III and IV). The value for **9** is smaller than those for **6-8** but still larger than that of β-cycldextrin, indicating that the adenine moeity of **9** is slightly included in the cyclodextrin cavity.

β-Cyclodextrin Linked Directly to the Nucleobase. In order to elucidate the effect of carbon chain, we have also studied the spectral properties of Thy-β-CD (**11**) and compared them with those of Thy-C_3-β-CD (**6**) and Thy-C_2-β-CD (**10**). Although the CD spectrum of Thy-β-CD (**11**) at pH 9.18 is very similar to those of Thy-C_3-β-CD (**6**) and Thy-C_2-β-CD (**10**) in shape, the magnitude of the

Table III. Association Constants of Guest Compounds by β-Cyclodextrin Derivatives[a]

host	guest	K_{assocn},[b] M^{-1}
Thy-C_3-β-CD (**6**)	1-Ad-COONa	3700 (3800)[c]
	p-nitrophenol	660
Ur-C_3-β-CD (**7**)	1-Ad-COONa	3000
Cyt-C_3-β-CD (**8**)	1-Ad-COONa	4700
Ade-C_3-β-CD (**9**)	1-Ad-COONa	1900
Thy-C_2-β-CD (**10**)	1-Ad-COONa	2800
	p-nitrophenol	560
Thy-β-CD (**11**)	1-Ad-COONa	8400 (8100)[c,d]
	p-nitrophenol	1700
β-cyclodextrin	1-Ad-COONa	625[e]
	p-nitrophenol	480[e]

[a] Unless otherwise noted, the constants were estimated on the basis of the UV spectral change. [b] In phosphate buffer (pH 9.18), 25 °C. [c] The constants were estimated on the basis the CD spectral change. [d] In borate buffer (pH 11.0), 25 °C. [e] Reported value (see ref 2a and 6).

Table IV. Association Constants of Guest Compounds by β-Cyclodextrin Derivatives in the Presence of Nucleobases[a,b]

host	guest	K_{assocn}, M^{-1}
6 + adenine	1-Ad-COONa	7700
	p-nitrophenol	1200
6 + adenosine	1-Ad-COONa	4900
6 + AMP	1-Ad-COONa	6500
6 + purine	1-Ad-COONa	2700
11 + adenosine	1-Ad-COONa	8900
9 + thymine	1-Ad-COONa	1500
9 + thymidine	1-Ad-COONa	1900

[a] Estimated on the basis of the UV spectral change. [b] In phosphate buffer (pH 9.18), 25 °C.

(10) The CD spectra of these compounds at pH 9.18 are essentially same as those at pH 7.0.

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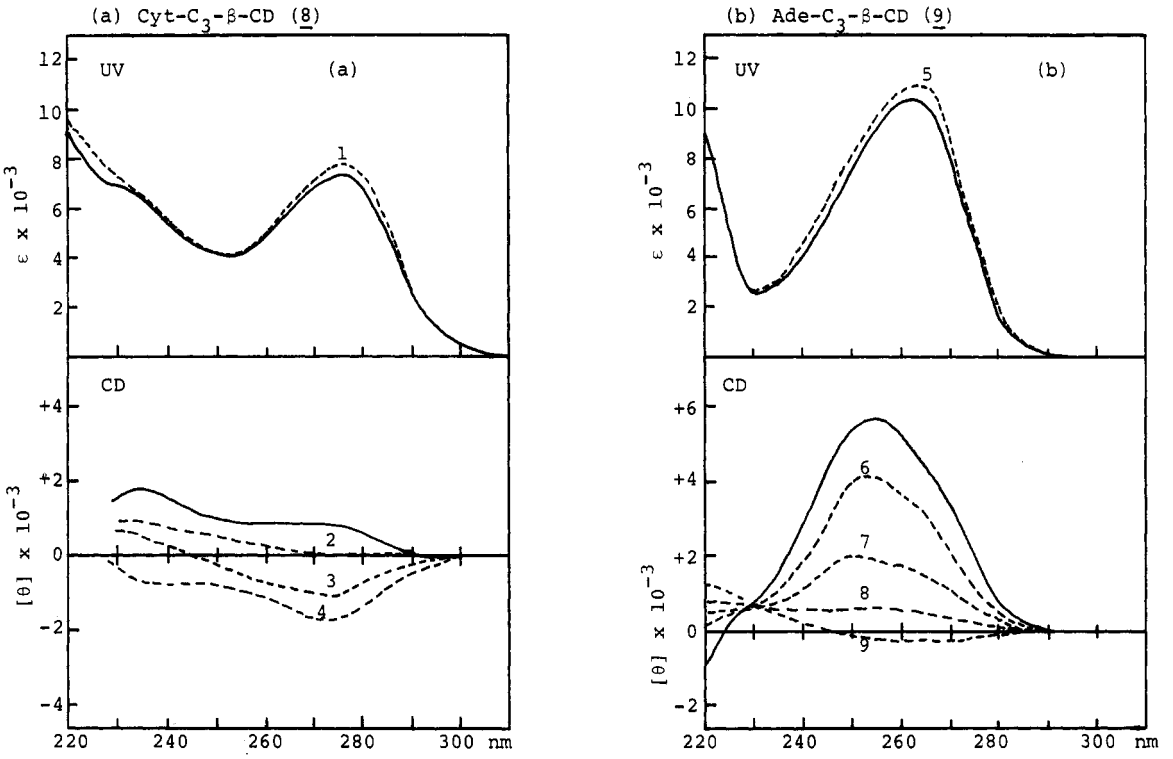

Figure 3. UV absorption and CD spectra of Cyt-C_3-β-CD (**8**) (a) and Ade-C_3-β-CD (**9**) (b) in phosphate buffer (pH 9.18) at 25 °C (—); inclusion complex of **8** (and **9**) with Ad-COONa (---); 1 (3.75×10^{-4} M), 2 (1.19×10^{-4} M), 3 (6.80×10^{-4} M), 4 (2.36×10^{-3} M), 5 (3.92×10^{-4} M), 6 (2.11×10^{-4} M), 7 (4.17×10^{-4} M), 8 (6.18×10^{-4} M), 9 (2.11×10^{-3} M).

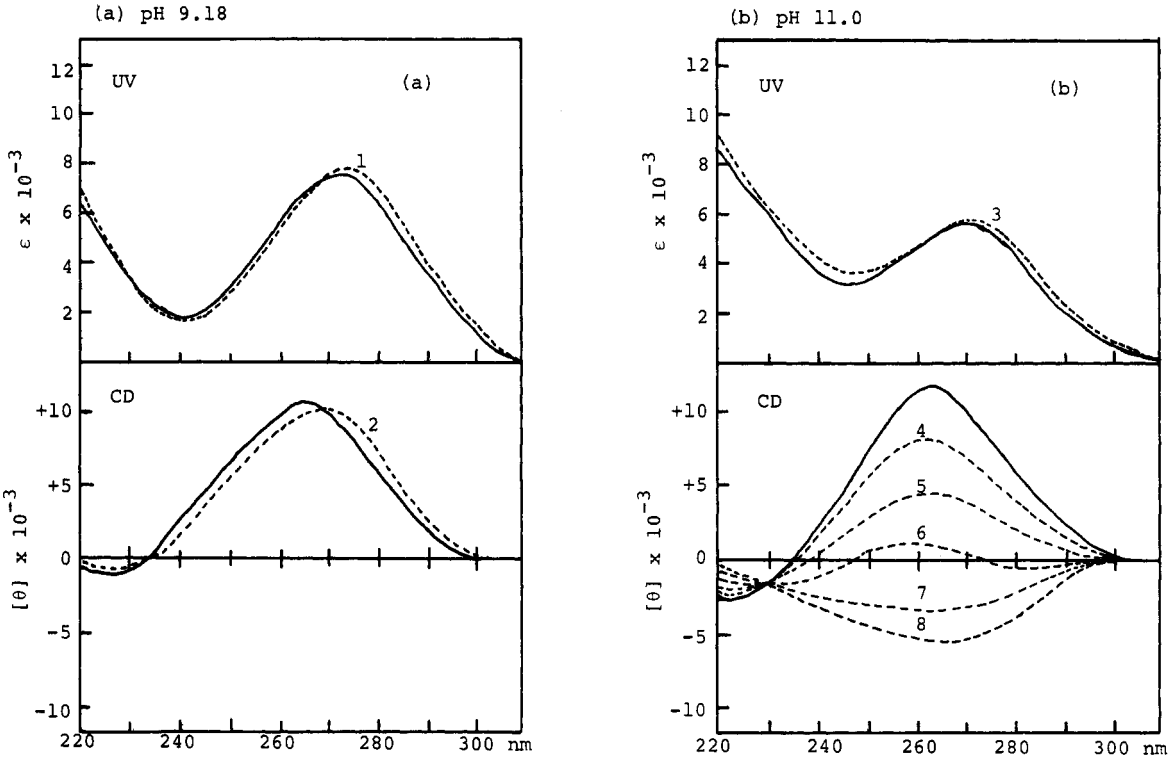

Figure 4. UV absorption and CD spectra of Thy-β-CD (**11**) (—) in phosphate buffer (pH 9.18) (a) and borate buffer (pH 11.0) (b) at 25 °C; inclusion complex of **11** with Ad-CONA (---): 1 (3.31×10^{-4} M), 2 (2.48×10^{-3} M), 3 (3.44×10^{-4} M), 4 (7.38×10^{-5} M), 5 (1.23×10^{-4} M), 6 (1.71×10^{-4} M), 7 (3.16×10^{-4} M), 8 (4.28×10^{-4} M).

positive band around 260 nm is remarkably increased (Figure 4), indicating the closer proximity of the β-cyclodextrin and thymine chromophore in **11**. Interestingly, in the presence of guest compound the CD spectrum of Thy-β-CD (**11**) shows a rather different pattern from that of Thy-C_3-β-CD (**6**); namely, addition of 1-adamantanecarboxylate causes no decreases in the CD spectrum of **11** (Figure 4a), while the similar red shift of the UV absorption band of **11** is observed as **6**. On the other hand, the CD spectrum of Thy-β-CD (**11**) at pH 11.0, which is almost same as that at pH 9.18, behaves differently in the presence of the guest compound. The CD spectra continuously changed on the addition of 1-adamantanecarboxylate, and finally the total reverse of the Cotton effect around 260 nm was observed as seen in the cases of **6**–**9** (Figure 4b). This unique pH effect on the CD spectral change of **11** by

the guest inclusion is noteworthy, since it seems to be closely related to the ease of hydrogen bonding between the thymine moiety and β-cyclodextrin. However, further studies will be needed for a decisive explanation for these results.

Finally, the association constants of these β-cyclodextrin derivatives and the guest compound (i.e., 1-adamantanecarboxylate) were measured in the presence of various nucleobases in order to clarify the effects of the "base-pairing". All these chemically modified β-cyclodexrins show some increase in their binding abilities compared with β-cyclodextrin (Table III). Furthermore, as Table IV indicates, the association constants of Thy-C_3-β-CD (**6**) in the presence of adenine, adenosine, and AMP are larger than that of **6**, while purine shows no such enhancing effect. *These results are indicative of the presence of thymine-adenine base pairing at the outside of the cyclodextrin cavity of 6*, which makes these compounds like capped cyclodextrin. In contrast, the association constant of Ade-C_3-β-CD (**9**) is not increased by thymine and thymidine. This can be explained by the less favorable interaction between the thymine bases and the adenine moiety of **9**, because the latter in **9** seems to be partially included in the cyclodextrin cavity as mentioned above.

In conclusion, it can be revealed by the CD spectral studies that the nucleobase-functionalized β-cyclodextrins **6–11** undergo the conformational changes on inclusion of the guest compound like 1-adamantanecarboxylate. In the case of Thy-C_3-β-CD (**6**), its binding ability can be imporved by adenine derivatives, probably due to the base-pairing between thymine and adenine. These results are also suggestive of a possibility of selective binding of the nucleobases by these β-cyclodextrin derivatives using the hydrogen bonding between the specific nucleobases such as thymine-adenine. Further attempts for appropriate capped β-cyclodextrin by the hydrogen-bonded nucleic acid base pairs are now in progress and will be presented in the near future.

Experimental Section

General Procedures. Melting points were measured with a Yanagimoto micro melting apparatus and are uncorrected. ^{1}H NMR spectra, IR spectra, UV spectra, and CD spectra were taken with a JEOL PS-100 spectrometer (or a Hitachi R-600 spectrometer), a JASCO DS-701G infrared spectrophotometer, a Hitachi EPS-3T spectrophotometer, and a JASCO 20C spectrometer, respectively. FAB mass spectra were obtained with a JEOL TMS-DX 300 spectrometer operating at 2-keV accelerating voltage. Samples were deposited in glycerol pastes onto a probe tip and irradiated by a beam of xenon atoms derived by neutralizing ions that had been accelerated through 6 keV in beam energy.

1-(3-Mercaptopropyl)thymine (2a). A solution of the bromide **1a** (247 mg, 1 mmol) and thiourea (91 mg, 1.2 equiv) in 95% EtOH (10 ml) was refluxed for 5 h in a nitrogen atmosphere. The solvent was removed at reduced pressure, and the solid thiouronium salt was treated with sodium hydroxide (50 mg) in water (10 ml) under reflux in a nitrogen atmosphere for 30 min. The solution was cooled, acidified to pH 3–4, and extracted with chloroform. The chloroform layer was dried over $MgSO_4$ and evaporated. The residue was chromatographed on a silica gel column using chloroform and methanol (100:1) to give a thiol **2a** (153 mg, 77%): mp 113–115 °C; IR ($CHCl_3$) 3380, 1695, 1685 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) 1.52 (t, 1 H, SH, J = 7.2 Hz, exchangeable by D_2O), 1.93 (d, 3 H, CH_3, J = 1.2 Hz), 2.09 (m, 2 H, C-CH_2-C), 2.58 (m, 2 H, C-CH_2-S), 3.86 (t, 2 H, N-CH_2-C, J = 6.6 Hz), 7.03 (d, 1 H, Thy-C_6H, J = 1.2 Hz), 9.23 (br s, 1 H, NH, exchangeable by D_2O).

1-(3-Mercaptopropyl)uracil (2b) was prepared from the bromide **1b** in the manner described above. Since this product is very unstable (air-sensitive), the crude product was used for the next reaction without further purifications: ^{1}H NMR (δ, $CDCl_3$) 1.54 (t, 1 H, SH, J = 7.4 Hz, exchangeable by D_2O), 2.01 (m, 2 H, C-CH_2-C), 2.55 (m, 2 H, C-CH_2-S), 3.89 (t, 2 H, N-CH_2-C, J = 6.6 Hz), 5.76 (d, 1 H, Ur-C_5H, J = 7.4 Hz), 7.21 (d, 1 H, Ur-C_6H, J = 7.4 Hz), 8.92 (br s, 1 H, NH, exchangeable by D_2O).

1-(3-Mercaptopropyl)cytosine (2c) was prepared from the bromide **1c** in the same manner in 63% yield: mp 193–196 °C; IR (Nujiol) 3340, 1670 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) 1.45 (t, 1 H, SH, J = 7.8 Hz, exchangeable by D_2O), 1.99 (m, 2 H, C-CH_2-C), 2.53 (m, 2 H, C-CH_2-S), 3.87 (t, 2 H, N-CH_2-C, J = 6.6 Hz), 5.70 (d, 1 H, Cyt-C_5H, J = 7.5 Hz), 7.19 (d, 1 H, Cyt-C_6H, J = 7.5 Hz).

9-(3-Mercaptopropyl)adenine (2d) was prepared from the bromide **1d** in the same manner in 65% yield: mp 137–140 °C; IR (Nujiol) 3250, 3100 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) 1.54 (t, 1 H, SH, J = 7.5 Hz, exchangeable by D_2O), 2.05–2.56 (m, 4 H, C-CH_2-C, C-CH_2-S), 4.34 (t, 2 H, N-CH_2-C, J = 6.3 Hz), 5.75 (br s, 2 H, NH_2, exchangeable by D_2O), 7.79, 8.33 (s, 1 H, Ade-C_2H, Ade-C_8H).

1-(2-Mercaptoethyl)thymine was prepared from the 1-(2-bromoethyl)thymine in the same manner in 90% yield: mp 164–166 °C; IR (Nujiol) 3360, 1695, 1665 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$) 1.42 (t, 1 H, SH, J = 8.9 Hz, exchangeable by D_2O), 1.93 (d, 3 H, CH_3, J = 1.1 Hz), 2.83 (m, 2 H, C-CH_2-S), 3.88 (t, 2 H, N-CH_2-C, J = 6.7 Hz)e, 7.07 (d, 1H, Thy-C_6H, J = 1.1 Hz), 8.88 (bs, 1 H, NH, exchangeable by D_2O).

6-Deoxy-6-[(3-(thym-1-yl)propyl)thio]-β-cyclodextrin (6). General Procedure. Method A. A solution of 6-deoxy-6-mercapto-β-cyclodextrin (**4**) (230 mg) and **1a** (100 mg) in 50 mL of degassed aqueous potassium carbonate (pH 9–10) containing 30% ethanol was stirred at room temperature under nitrogen. After 1 week, the solution was acidified to pH 3–4 by 10% HCl and then concentrated to 20 mL under the reduced pressure. Trichloroethylene (1 mL) was added to the solution, and the precipitate formed was collected. After evaporation of trichloroethylene in vacuo, the residue was chromatographed on a Sephadex G-15 column using water to give product **6** (61 mg, 23%). This procedure was used for the preparation of **7–9**. The chemical yields and physical constants are summarized in Table V. The spectral (IR, ^{1}H NMR, UV, MS) data and elemental analyses are given in Tables I and II.

Method B. To a solution of **2a** (100 mg) and potassium carbonate (70 mg) in degassed 30% ethanol (50 mL) was added 6-*O*-tosyl-β-cyclodextrin (**3**) (260 mg). The resulting solution was stirred at room temperature for 4 days under nitrogen. The reaction mixture was neutralized and concentrated to 20 mL under the reduced pressure. Trichloroethylene (1 mL) was added, and the precipitate formed was collected. After evaporation of trichloroethylene in vacuo, the residue was recrystallized from methanol–water, and the recrystallization was repeated to give a product **6** (152 mg, 58%). This procedure was used for preparation of **7–10**.

6-Deoxy-6-(thym-1-yl)-β-cyclodextrin (11). To a solution of thymine (250 mg) in dry Me_2SO were added 6-*O*-tosyl-β-cyclodextrin (**3**) (520 mg) and potassium carbonate (330 mg). The resulting suspension was heated at 70–80 °C for 5 h. The solvent was removed under reduced pressure, and the residue was chromatographed on a Sephadex G-15 column using water to give product **11** (223 mg, 43%): mp 274–280 °C dec. The spectral data are given in Table I.

Association Constants between β-Cyclodextrin Derivatives and Guest Molecules. The difference UV spectra were taken between β-cyclodextrin derivatives (1×10^{-4} M) alone and β-cyclodextrin derivatives (1×10^{-4} M) in the presence of 1-

Table V. Chemical Yields and Physical Constants of β-Cyclodextrin Derivatives

product	method	yield, %	mp, °C
6	A	23	282–288 dec
	B	58	
7	A	38	240–249 dec
	B	63	
8	A	20	231–239 dec
	B	50	
9	A	27	270–278 dec
	B	55	
10	B	50	277–283 dec

adamantanecarboxylate in phosphate buffer (pH 9.18) 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 1.27×10^{-4} M to 4.97×10^{-4} M. The association constants between β-cyclodextrin derivatives and *p*-nitrophenol (5×10^{-5} M) were also estimated by the difference spectra at 25 °C and pH 9.18, where β-cyclodextrin derivatives concentration range from 3.13×10^{-4} M to 1.51×10^{-3} M. The association constants in the presence of nucleobases were obtained in the same manner, where nucleobases concentration was same as the host concentration. The association constant between **6** (or **11**) and 1-adamantanecarboxylate was also estimated by means of CD spectra, where the concentrations of **6** (or **11**) and the carboxylate were 1.5×10^{-4} and 1.52×10^{-4} to 5.02×10^{-4} M (or 1.0×10^{-4} and 1.23×10^{-4} to 4.28×10^{-4} M), respectively. These spectral data were treated by the Benesi–Hildebrand method.

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Improved Synthesis and Electrophilic Bromination of Benzo[1,2-*c*:3,4-*c*']dithiophene. Charge-Transfer and Cycloaddition Reaction with Tetracyanoethylene

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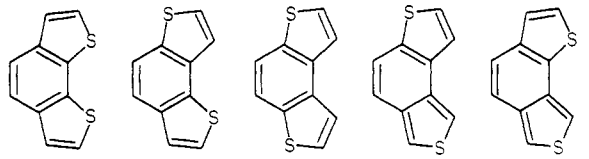
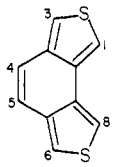
Benzo[1,2-*c*:3,4-*c*']dithiophene (**6**) is prepared in two steps and 47% overall yield from its tetrahydro precursor **7**. Bromination of **6** (NBS, AcOH) occurs in the thiophene moieties; preparation of the 1-Br, 3-Br, 1,3-Br_2, 1,3,6-Br_3, 1,3,6,8-Br_4, and 1,3,4,5,6-Br_5 derivatives of **6** is described. With tetracyanoethylene, **6** first forms a blue charge-transfer complex, which, on reflux in chloroform, is converted to the Diels–Alder type bis-adduct **16**.

All six isomeric benzodithiophenes **1–6** are known.[1]



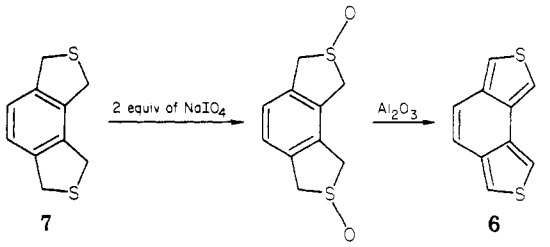
1 **2** **3** **4** **5**



6

Compounds **1–3**, with only benzo[*b*]thiophene moieties, are readily obtained in 50–70% yield. Compounds **4** and **5**, which each contain one benzo[*c*]thiophene moiety, are stable only in solution;[1] attempts to isolate them result in polymerization, presumably due to intermolecular Diels–Alder reactions analogous to those observed with other benzo[*c*]thiophenes.[2] Compound **6**, with two benzo[*c*]-thiophene moieties, on the other hand, is stable in pure form.[1,3,4] Previous syntheses gave relatively poor yields of **6**.[5] We describe here an improved synthesis of **6**. We also report on its bromination, as a typical electrophilic substitution reaction, and on its novel reaction with tetracyanoethylene.

Oxidation of tetrahydrobenzo[1,2-*c*:3,4-*c*']dithiophene (**7**)[6] with sodium periodate gave the disulfoxide **8** in 75% yield. Subsequent dehydration with neutral alumina[2] gave crystalline, pure **6**, mp 108–110 °C, in 63% yield. This



7 **8** **6**

route to **6** involves fewer steps than methods based on construction of the central, benzenoid ring.[1,3] Although it requires one more step than the direct dehydrogenation of **7**,[4] the overall yield is considerably better.

There are no previous studies on electrophilic substitution reactions of **6**. Bromination was selected as a typical example. Treatment of **6** at room temperature with 1 equiv of *N*-bromosuccinimide (NBS) in acetic acid gave two monobromo derivatives **9** and **10** and the dibromo derivative **11**. Compounds **9** and **10** were obtained as an approximately 1:1 mixture in 70% yield. They were separated from **11** (6%) by column chromatography, and **9** could be obtained pure by trituration of the **9**, **10** mixture with hexane.

The structures of **9–11** are based primarily on their NMR spectra, summarized in Table I. Several features identify **9** and **10**. First, both spectra show two strongly coupled "vinyl" protons (H_4, H_5), showing that bromination occurred in the thiophene moieties and not at the central double bond (contrast with phenanthrene). In **9**, both

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(2) Cava, M. P.; Pollack, N. M.; Mamer, O. A.; Mitchell, M. J. *J. Org. Chem.* **1971**, *36*, 3932 and references cited therein.
(3) MacDowell, D. W. H.; Maxwell, M. H. *J. Org. Chem.* **1970**, *35*, 799.
(4) Hart, H.; Sasaoka, M. *J. Am. Chem. Soc.* **1978**, *100*, 4326.
(5) The procedures in ref 1 and 3 are multistep, the starting materials are not readily available, and the yield in the final step is only 20%. The yield of **6** from **7** as reported in ref 4 was 35%.
(6) Giovannini, E.; Vuilleumier, H. *Helv. Chim. Acta* **1977**, *60*, 1452.

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