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FLEXIBLY CAPPED β-CYCLODEXTRINS BY THE HYDROGEN-BONDED NUCLEIC ACID BASE PAIR. THE pH-CONTROL OF BINDING ABILITY BY AN ON-OFF-SWITCHED CAPPING

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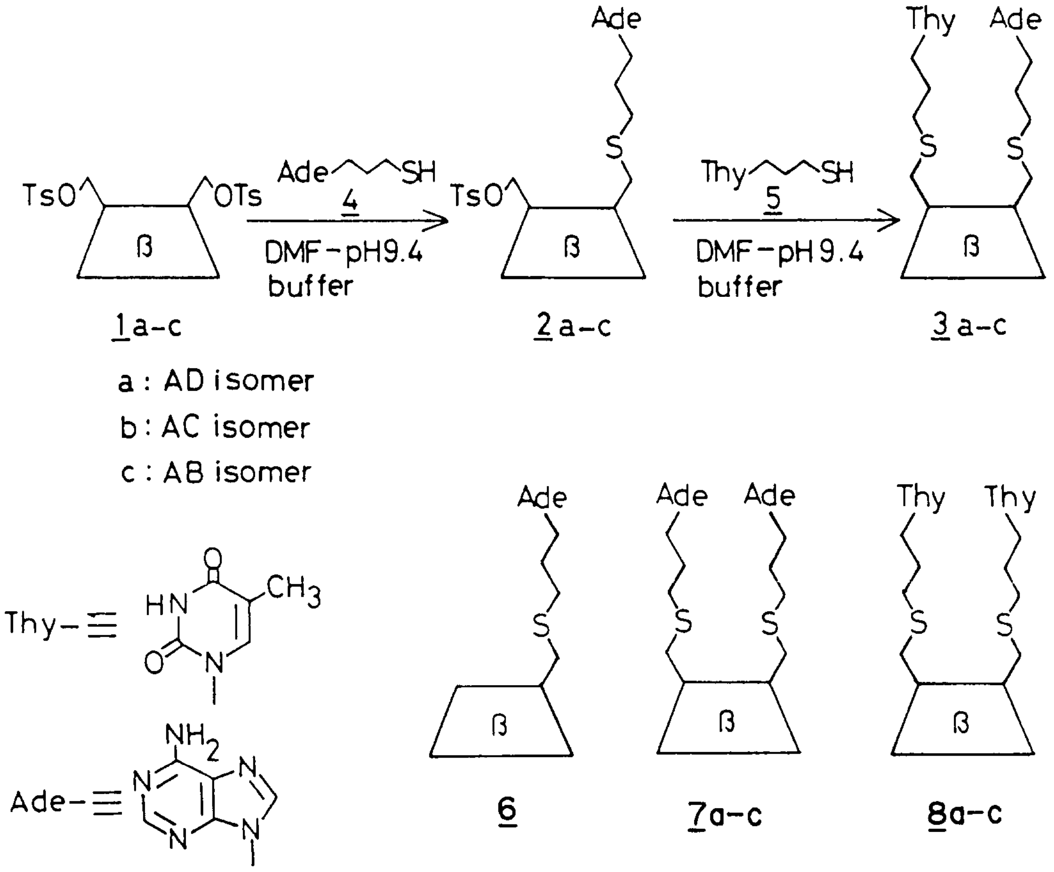
Abstract: Preparation and characterization of flexibly capped β-cyclodextrins (3a-c) by the hydrogen bonded nucleobase pair (i.e., adenine-thymine) are described.

The nucleosides are among the most biologically important substances, comprising the monomeric units of DNA and RNA. In the double-stranded structure of DNA and RNA, adenine (guanine) is always paired with thymine (cytosine) by the specific hydrogen bonds. Such base pairing is believed to be of fundamental importance for the replication of nucleic acids and the storage of the genetic information.[1] Furthermore, temperature and pH conditions are known to be important factors for the regulation of these base pairings.[2] On the other hand, chemical modification of cyclodextrins with various functional groups has been extensively investigated in order to make more effective models for enzyme action.[3] It seems to be especially interesting to design the cyclodextrins whose binding ability may be freely controlled by the environmental conditions like pH.

We now report the preparation and characterization of the flexibly capped β-cyclodextrins (β-CD)[4] by the complementary nucleobase pair (adenine-thymine) via the specific hydrogen bonding which can be "switched on" and "switched off" by changing the pH. The double functionalization of β-CD was accomplished by the sequential treatments of ditosylated β-CDs (AD-, AC-, and AB-isomers; 1a-c)[5] with 9-(3-mercaptopropyl)adenine (4)[6] and 1-(3-mercaptopropyl)thymine (5)[6]. When a pH-9.4 buffer solution of AD-ditosylate 1a was allowed to react with 2.2 equiv of 4 in DMF at room temperature for 2 days, mono-substituted 2a[7] was obtained in 36% yield. Then treatment of 2a with excess of 5 under the same conditions gave the desired 3a[7] in 71% yield. Compounds 3b,c were similarly prepared from 1b,c via 2b,c in 25-30% overall yields, respectively. All these compounds were purified by chromatography (Lichroprep Rp-8, H_2O-EtOH) and the purity was checked by HPLC (Finepack SIL C-18, H_2O-CH_3CN).[7] Structural determination of 3a-c was made on the basis of the elemental analyses, IR, UV and ^1H

NMR spectra, and fast atom bombardment (FAB) mass spectrometry which showed for each an intense molecular ion peak $(M+H)^+$ at m/z 1508. Similarly, compounds functionalized doubly with the same nucleobases such as Ade,Ade-C_3-β-CD (7a-c) and Thy,Thy-C_3-β-CD (8a-c) were prepared by treatment of 1a-c with excess of 4 and 5, respectively.[8]



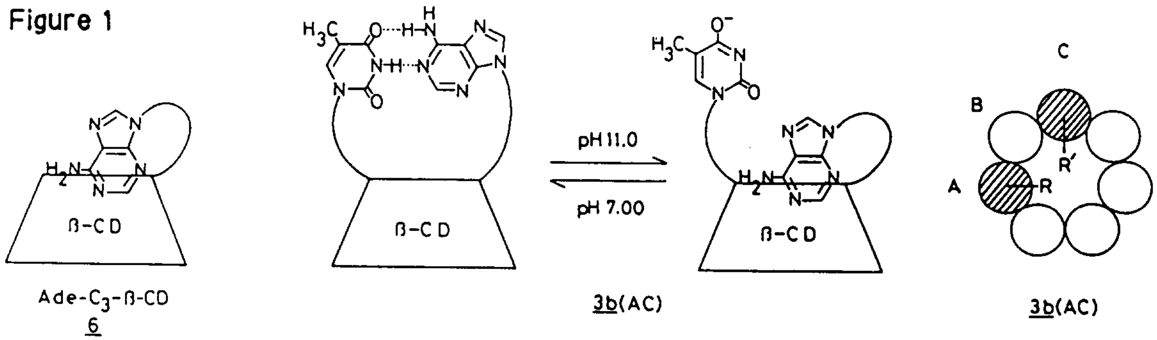
The association constants of 3a-c and 1-adamantanecarboxylate were measured at pH 7.0 and 11.0 by UV spectroscopy. As shown in Table 1, all of 3a-c show increase in their binding abilities compared with the mono-adenine-functionalized β-CD (Ade-C_3-β-CD; 6)[6] at the neutral condition (pH 7.0). Especially, the increment for 3a (2.1-fold) and 3b (3.6-fold) is noteworthy. Interestingly, the association constants of 3a and 3b were markedly decreased at pH 11.0 and no improvement in their binding abilities became appreciable compared with that of 6, while the association constants of 3c (3c' and 3c") and 6 were essentially invariable even at this higher pH. In addition, the association constants for the doubly functionalized compounds 7a-c and 8a-c were also almost invariable at pH 7.0 and pH 11.0. The remarkable pH-dependency of the association constant for 3b possibly indicates the most preferred formation of adenine-thymine base paring over the cyclodextrin cavity in 3b as shown schematically in Figure 1. It was previously revealed that in a series of nucleobase-functionalized β-CDs the adenine moiety is prone to interact with the cavity more strongly than the thymine base to form a "shallow floor".[6] In the doubly functionalized 3, however, the formation of the specific hydrogen bonds

Table I. Association Constans of Sodium 1-Adamantanecarboxylate (1-Ad-COONa) and Methyl Orange (MO) with β-Cyclodextrin Derivatives[a]

host	guest	Kass ($\times 10^3$, M^{-1})[b] pH 7	pH 11	Kass (pH 7) / Kass (pH 11)
3a (AD)	1-Ad-COONa	5.7	2.3	2.48
3b (AC)	1-Ad-COONa	9.6	2.3	4.17
	MO	4.0	1.2	3.33
3c' (AB)[c]	1-Ad-COONa	5.0	4.8	1.04
3c" (BA)[c]	1-Ad-COONa	4.1	3.5	1.17
6	1-Ad-COONa	2.7	2.4	1.13
7a (AD)	1-Ad-COONa	3.6	3.6	1.00
7b (AC)	1-Ad-COONa	4.4	4.1	1.07
7c (AB)	1-Ad-COONa	2.3	2.1	1.10
8a (AD)	1-Ad-COONa	8.8	—[d]	
	MO	8.0	9.5	0.84
8b (AC)	1-Ad-COONa	9.9	—[d]	
	MO	7.5	7.6	0.99
8c (AB)	1-Ad-COONa	8.0	—[d]	
	MO	8.2	9.4	0.87
Thy-C_3-β-CD (9)	1-Ad-COONa	3.7	3.5	1.06
	MO	5.2	4.8	1.08

[a]1-Ad-COONa: [Host] = 5.0×10^{-5} M, [Guest] = 1.0×10^{-1} ~ 7.5×10^{-4} M, Kass were estimated by the difference UV spectra of the host. MO: [Host] = 1.0×10^{-4} ~ 1.0×10^{-3} M, [Guest] = 5.0×10^{-5} M, Kass were estimated by the difference UV spectra of the guest. Error estimates ±5%. [b]0.05 M phosphate buffer (pH 11.0), 25 °C. [c]Diastereomers (see ref. 7). [d]The association constant could no be exactly estimated because of very small change in the absorbance spectra.

Figure 1



between adenine and thymine might prevent such adenine-cavity interaction and instead make a "deep floor" as seen in the capped cyclodextrins,[3] causing some increment of binding ability of 3a and 3b at neutral pH. Accordingly, the decrease of the association constants of 3a,b at pH 11.0 may be attributed to the conformational change of 3 (to those similar to that of 6) due to the hydrogen bond breaking at this higher pH. These results indicate that the binding ability of β-cyclodextrins is possibly controlled by the pH change using the biologically important complementary nucleic acid base-pairing.

The order of the binding ability observed for these doubly functionalized β-CD (AC-isomers) is, therefore, 8b≥3b(pH 7)>7b>3b(pH 11). Although compound 8 showed no pH-dpendency, its association constants were the largest of doubly functionalized compounds, probably due to the base stacking effects. Since the interaction between thymine and the cavity is known to be weaker than that of adenine,[6] 8 seems to take the extended conformation with expanding the hydrophobic space above the cavity.

Further investigations on the nucleobase-functionalized cyclodextrins targeting drug delivery are now in progress.

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References and Notes

(1) Lehninger, A. L."Principle of Biochemistry"; Chapter 27, p 793, Worth Publishers, Inc: New York, 1982.

(2) Lown, J. W. Acc. Chem. Res. 1982, 15, 381.

(3) (a) Emert, J.; Breslow, R. J. Am. Chem. Soc. 1975, 97, 670. (b) Tabushi, I.; Shimokawa, M.; Shimizu, N.; Shirakawa, H.; Fujita, K. J. Am. Chem. Soc. 1976, 98, 7855. (c) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. J. Am. Chem. Soc. 1977, 99, 7100. (d) Ueno, A.; Yoshimura, H.; Saka, R.; Osa, T. J. Am. Chem. Soc. 1979, 101, 2779. (e) Breslow, R.; Bovery, P.; Hersh, C. L. J. Am. Chem. Soc. 1980, 102, 2115. (f) Croft, A. P.; Bartsch, R. A. Tetrahedron, 1983, 39, 1417.

(4) For the capped-cyclodextrin family, see Tabushi, I. Acc. Chem. Res. 1982, 15, 66.

(5) Fujita, K.; Matsunaga, A.; Imoto, T. Tetrahedron Lett., 1984, 25, 5533.

(6) Nagai, K.; Hayakawa, K.; Kanematsu, K. J. Org. Chem. 1984, 49, 1022.

(7) The separation of each diastereomer of 2a,b or 3a,b has not been succeeded at the present stage, whereas the two diastereomers (i.e., AB- and BA-isomers) of 2c and 3c (3c', 3c") could be isolated in a pure form.

(8) Satisfactory IR, UV, ^{1}H NMR, FAB MS spectra as well as elemental analyses were obtained for all new compounds.

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