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Synthesis of Novel β-cyclodextrin Derivatives Bearing a 1-naphthyloxamino-oligo(ethyleneamino) Moiety and their Inclusion Complexation with some Fluorescent Dyes

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Two novel β-cyclodextrin derivatives bearing a (1-naphthyloxamino)-ethyleneamino (4) or (1-naphthyloxamino)-diethylenediamino (5) moiety have been synthesized by a convenient method in 34% and 30% yields, respectively. Examinations of the circular dichroism (CD) spectra and fluorescence lifetime revealed that the naphthyloxamino-oligo (ethyleneamino) moiety tethered to β-cyclodextrin is not deeply embedded in the hydrophobic cavity of β-cyclodextrin itself even in the absence of a guest. The inclusion complexation behavior of 4 and 5 with some fluorescent dyes, i.e. ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium 2-(*p*-toluidinyl)naphthalenesulfonate (TNS), Acridine Red (AR) and Rhodamine B (RhB), was assessed in aqueous phosphate buffer solution (pH 7.2) at 25°C by fluorometric titration to give the complex stability constants (K_S) and Gibbs free energy changes (ΔG^0) for the stoichiometric 1:1 inclusion complexation with the fluorescent dyes. The results obtained indicate that the naphthyloxamino-oligo(ethyleneamino) moiety attached to the β-cyclodextrin (1) can alter not only the original molecular binding-ability of the parent β-cyclodextrin, but also the molecular selectivity through the microenvironment changes of cyclodextrin cavity, which are discussed from the viewpoints of the size/shape-fit concept and the stereochemical complementary relationship between host cyclodextrin and model substrate.

Keywords: Modified cyclodextrins; Dyes; Inclusion complexation; Molecular recognition

INTRODUCTION

The chemical modified β-cyclodextrins, which are tethered by some simple functional groups, have been designed and synthesized to enhance the original molecular binding ability and selectivity of parent β-cyclodextrins, through the induced-fit interaction and the complementary geometrical relationship between the host and guest/substrate [1–7]. Consequently, a great deal of effort has been devoted to the synthesis of a wide variety of cyclodextrin derivatives with nucleophilic or electrophilic substituents attached to the primary side of cyclodextrin in order to examine their molecular recognition ability [7–10], and to gain insights into the factors governing the inclusion complexation phenomena of guest/substrate by the modified cyclodextrins. We have recently reported that the syntheses and molecular recognition of a series of modified cyclodextrins bearing an aromatic group and found the modified β-cyclodextrins tethered by one chromogenic aromatic moiety as a spectra probe can recognize not only the size/shape of guest molecules, but also the molecular chirality, giving fairly good enantioselectivity [11–13]. More recently, we have revealed that the interaction between some fluorescent dyes and β-cyclodextrin derivatives [14] and found the position and type of the substituent introduced to β-cyclodextrin are critical functions upon the inclusion complexation with dye molecules, which can be elucidated in terms of the conformational, electrostatic, hydrogen-bonding, and hydrophobic effects. Unfortunately, the effects of the tethered chain length between the chromophore group and cyclodextrin upon the inclusion complexation behavior have scarcely been investigated systematically so far.

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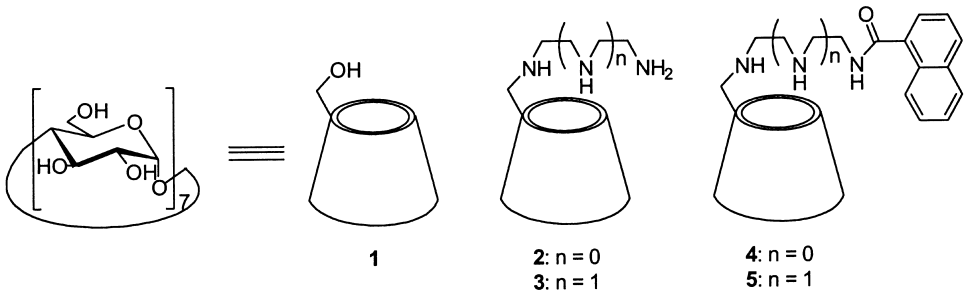


CHART 1

In the present study, we wish to report the results of our study on the synthesis of novel mono-[6-(1-naphthyloxamino)-oligo(ethyleneamino)-6-deoxy]-β-cyclodextrins **4** and **5**, shown in Chart 1, and their molecular recognition behavior with some dyes. The complex stability constants (K_S) and Gibbs free energy changes ($-\Delta G^0$) for 1:1 inclusion complexation of the modified β-cyclodextrins **4** and **5** with the selected fluorescent dyes (Chart 2) were determined at 25°C by the fluorometric titration technique in aqueous phosphate buffer solution (pH 7.2). Furthermore, the complexation behavior of the cyclodextrin derivatives **4** and **5** were also analyzed according to the changes in the circular dichroism (CD) spectra and fluorescence lifetime measurement. The results obtained for the inclusion complexation with host compounds **4** and **5**, together with those for parent β-cyclodextrin **1** and its derivative **3** [15], will serve our further understanding of the molecular recognition by cyclodextrins and chemical modified cyclodextrins. It is of our special interest to elucidate the effects of the tethered chain length between the chromophore and cyclodextrin upon inclusion complexation behavior with model substrates.

EXPERIMENTAL SECTION

General Procedure

Mass spectra were obtained by using a JEOC JMS-DX-303 instrument. ^{1}H NMR spectra were recorded on a Bruker AC-P200 instrument at 200 MHz in D_2O solution. Infrared and ultraviolet spectra were recorded on Bio-Rad FTS 135 and Shimadzu UV-2401/pc instruments, respectively. Elemental analyses were performed on a Perkin–Elmer 2400C instrument.

Materials

Ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium 2-(*p*-toluidinyl) naphthalene-sulfonate (TNS), and Rhodamine B (RhB) were purchased from Tokyo Kasei. Acridine Red (AR) was purchased from Chroma-Gesellschaft Schmid. All chemicals were reagent grade and used without further purification unless noted otherwise. β-cyclodextrin of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried *in vacuo* at

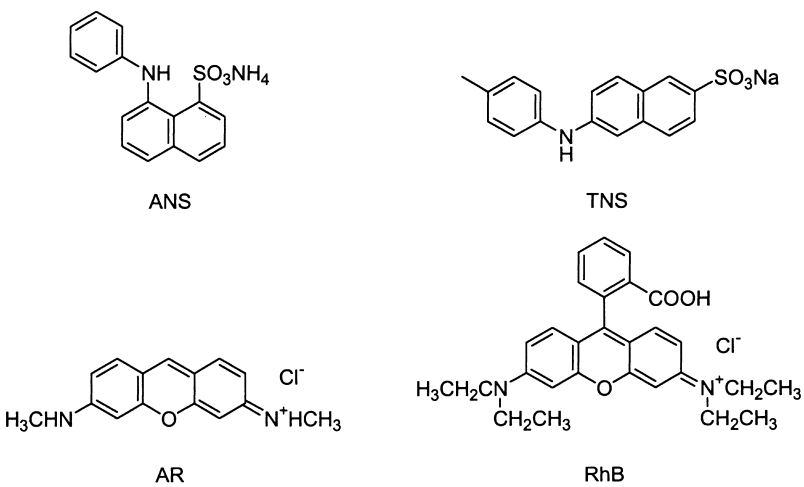


CHART 2

95°C for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and distilled under reduced pressure prior to use. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 mol dm^{-3} phosphate buffer solution of pH 7.20, which was used in the spectral measurement.

Synthesis of Mono[6-(1-naphthyloxamino)ethyleneamino-6-deoxyl-β-cyclodextrin (4)

Mono(6-ethylenediamino-6-deoxy)-β-cyclodextrin [16] was prepared by the reaction of ethylenediamine and mono[6-*O*-(*p*-tolylsulfonyl)]-β-cyclodextrin, which was synthesized by the reaction of β-cyclodextrin with *p*-toluenesulfonyl chloride in water [17]. The mono(6-ethylenediamino-6-deoxy)-β-cyclodextrin (0.5 g, 0.4 mmol) was added to the solution of 1-naphthoic acid (80 mg, 0.46 mmol) in dry DMF (30 ml) at 70°C with stirring for 24 h under N_2, in the presence of a little of molecular sieve as catalyst. The reaction mixture was evaporated under reduced pressure to dryness. The residue was dissolved in water and then poured into acetone to give an orange precipitate. After drying, the orange precipitate was purified by the column chromatography on Sephadex G-25 with the elution of distilled, deionized water two times, and then dried *in vacuo* to give a pure sample in 34% yield. ^{1}H NMR (D_2O, TMS, ppm) δ 7.3–8.5 (m, 7H), 4.98 (s, 7H), 3.0–4.0 (m). ^{13}C NMR (D_2O, TMS, ppm) δ 164.5, 136.0, 133.0, 129.7, 128.2, 126.8, 126.3, 125.9, 125.6, 102., 81.2, 77.0, 73.2, 72.2, 71.9, 66.5, 60.4, 49.0, 44.5, 39.0. $C_{55}H_{82}O_{35}N_2{\cdot}7H_2O$ (1457): Calcd C 45.33, H 6.64, N 1.92; found C 45.44, H 6.60, N 1.83. UV/Vis (water) λ_{max} (ε) 281.5 (3630), 224 nm (14125 $dm^3\,mol^{-1}\,cm^{-1}$). FT-IR (KBr) ν 3317, 2929, 1664, 1556, 1407, 1370, 1241, 1153, 1078, 1031, 994, 885 cm^{-1}. MS (FAB) *m*/*z* 1332 (M^++H-7H_2O).

Synthesis of Mono [6-(1-naphthyloxamino)diethylenediamino-6-deoxyl-β-cyclodextrin (5)

Compound **5** was synthesized by the reaction of mono[6-(diethylenetriamino)-6-deoxy]-β-cyclodextrin with 1-naphthoic acid in dry *N*,*N*-dimethylformamide according to a similar procedure described above for **4** in 30% yield. ^{1}H NMR (D_2O, TMS, ppm) δ 7.4–8.4 (m, 7H), 4.96 (s, 7H), 3.0–4.0 (m). ^{13}C NMR (D_2O, TMS, ppm) δ 164.6, 137.0, 134.0, 129.1, 127.8, 126.4, 126.1, 125.6, 125.4, 101.9, 81.1, 77.0, 73.1, 72.1, 71.9, 64.0, 60.3, 49.3, 47.2, 46.1, 35.7. $C_{57}H_{87}O_{35}N_3{\cdot}7H_2O$(1500): Calcd C 45.63, H 6.79, N 2.80; found C 45.34, H 6.67, N 2.67. UV/Vis (water) λ_{max} (ε) 282 (6166), 227 nm (13804 $dm^3\,mol^{-1}\,cm^{-1}$). FT-IR (KBr) ν 3317, 2930, 1662, 1564, 1407, 1370, 1242, 1153, 1078, 1031, 944, 854, cm^{-1}. MS (FAB) *m*/*z* 1375 (M^++H-7H_2O).

Measurement

Fluorescence spectra were measured in a conventional quartz cell (10mm × 10mm × 45 mm) at 25°C on a JASCO FP-750 spectrometer with the excitation and emission slits of 5 nm width. CD spectra were recorded in a conventional quartz cell (10mm × 10mm × 45 mm) on a JASCO J-720S spectropolarimeter equipped with a PTC-348WI temperature controller to keep the temperature at 25°C. Fluorescence lifetimes were determined by the time-correlated single-photon-counting method using a HORIBA NAES-550 instrument with a time resolution of 0.5 ns. A self-oscillating discharge lamp filled with hydrogen gas was employed as pulsed light source, and the excitation light was made monochromatic by a 10 cm monochromator. The emission from the sample was passed through an appropriate filter (Toshiba UV-33) placed before the detector unit in order to eliminate scattered excitation light. Maximum counts of up to 10,000 were collected for each measurement. The accumulated signal were then processed and the lifetime determined by deconvolution with nonlinear least-squares fit.

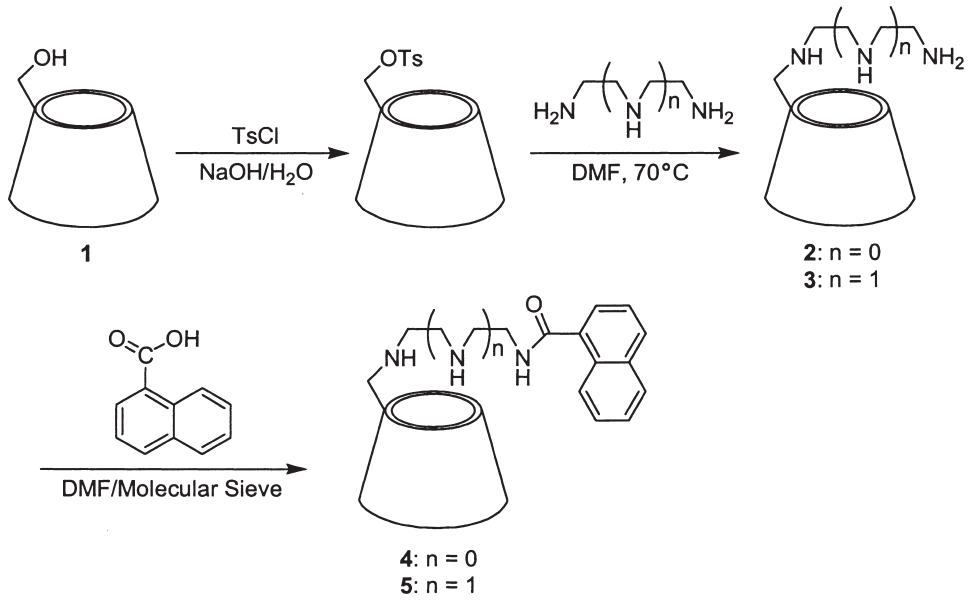
RESULTS AND DISCUSSION

Synthesis

Naphthyloxamino-oligo(ethyleneamino)-β-cyclodextrins, **4** and **5** were synthesized in satisfactory yields according to Scheme 1.

Circular Dichroism Spectra

As can be seen from Fig. 1, the induced circular dichroism (ICD) spectrum of modified β-cyclodextrins, **4** and **5** in buffer solution (pH 7.20) showed a weak positive Cotton effect peaks for ^1B_b at 225 nm ($\Delta\varepsilon = 0.12\,dm^3\,mol^{-1}\,cm^{-1}$ for **4**, $\Delta\varepsilon = 1.10\,dm^3\,mol^{-1}\,cm^{-1}$ for **5**) and weak negative Cotton peaks for ^1L_a at 273.8, 274.8 nm ($\Delta\varepsilon = -0.16\,dm^3\,mol^{-1}\,cm^{-1}$ for **4**, $\Delta\varepsilon = 0.13\,dm^3\,mol^{-1}\,cm^{-1}$ for **5**) for **4** and **5**, respectively. As compared with β-cyclodextrin derivatives **4** and **5**, β-cyclodextrin naphthoate shows a stronger positive Cotton effect peak and a stronger negative Cotton effect peak [18]. These results indicate that the relatively Cotton effect peak of naphthalene-modified β-cyclodextrins tethered by a simple chain becomes weaker with the increasing chain length between the naphthalene ring and the parent



SCHEME 1

β-cyclodextrin, which may be caused by the naphthyl far away from the rim of cyclodextrin cavity. According to the sector rule proposed by Kajtar [19] and Harata *et al.*'s empirical rule [20–22], the Cotton effect observed for the ^1B_b bands and the ^1L_a bands indicates that the naphtyl moiety is not deeply embedded into hydrophobic cavity of cyclodextrin itself, which may favor inclusion complexation with the relatively large guest molecules, such as ANS and RhB, etc. Simultaneous examination of each sample solution by CD spectrometry did not show any significant change upon addition of the guests, that also accounts for the naphtyl moiety which did not penetrate into the hydrophobic cavity of β-cyclodextrin. Therefore, we selected fluorescent dye as a probe to investigate the inclusion behavior of the compounds **4** and **5** by using fluorometric titration.

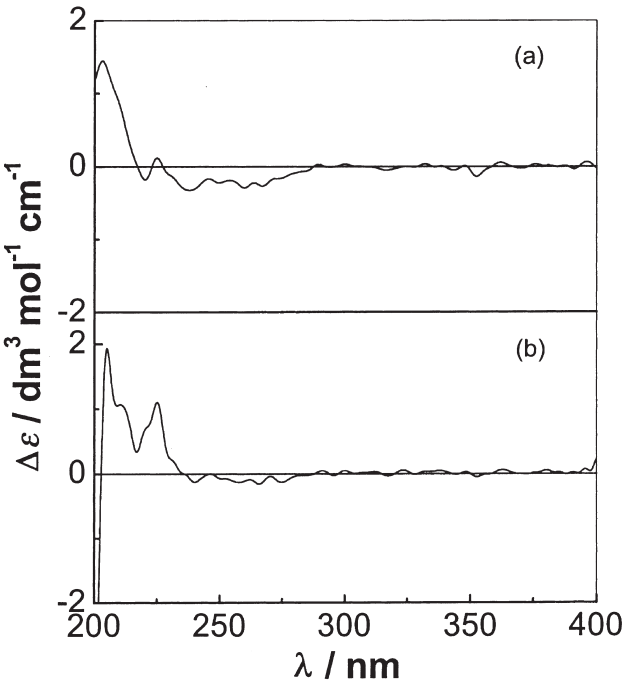


FIGURE 1 Circular dichroism spectra of compounds **4** (a) and **5** (b) (5×10^{-5} mol dm^{-3}) in pH 7.2 phosphate buffer solution at 25°C.

It is well known that the adamantane-ethanol is a rigid molecule, and its size is fit for the cavity of β-cyclodextrin very well, for this reason, there is strong interaction between adamantane-ethanol and β-cyclodextrin. Therefore, when adamantane-ethanol is included into the cavity of **4** or **5**, the side arm of cyclodextrin derivative, if it is originally self-included in cavity, must be expelled out of the cavity, leading to the decrease of fluorescence relative intensity. Thus, we measured the fluorescence of host **4** and **5** with 1-adamantane-ethanol whose concentration is 70 times more than that of hosts, **4** and **5**. The results show clearly that the relative intensity of **4** and **5** shows not any significant change upon the addition of 1-adamantane-ethanol, which indicates that the naphtyl moiety did not penetrate into the hydrophobic cavity of β-cyclodextrin.

Fluorescence Lifetime

In general, the inclusion complexation of fluorescent dyes by cyclodextrin hosts not only induces the fluorescence enhancement and peak shifts [23] but also leads to significantly elongated fluorescence lifetimes in the hydrophobic environment, as demonstrated by Bright [24] and Reinsborough

TABLE I Fluorescence lifetimes (τ) and relative quantum yields (Φ) of ANS in absence and presence of modified β-cyclodextrins in aqueous phosphate buffer solution (pH 7.20, 0.1 mol dm^{-3})

Guest	*Concentration* (μmol dm^{-3})	*Host*	*Equiv.*	τ_S (ns)	Φ_S (%)	τ_L (ns)	Φ_L (%)	χ^2	*Ref.*
ANS	500	None		0.4	100			1.46	[26]
	250	**1**	10	1.5	67.6	3.2	32.4	1.24	[26]
	10	**4**	24	2.9	73.6	10.9	26.4	1.48	This work
	10	**5**	20	2.8	58.1	10.1	41.9	1.56	This work

[25]. In this work, we performed the nanosecond time-resolved fluorescence experiments with ANS in aqueous buffer solution (pH 7.20) in presence or absence of β-cyclodextrin **1**, and its derivatives **4** and **5** in order to assess the micro-environment polarity around the included ANS.

Since the rate of complexation/decomplexation is much slower than that of the fluorescence decay, the decay profile of fluorescence intensity ($F(t)$) can be described as the sum of unimolecular decays for all fluorescing species present in solution:

$$F(t) = \sum A_i \exp(-t/\tau_i)\,(i = 1, 2, \ldots) \qquad (1)$$

where A_i and τ_i represent the initial abundance and lifetime of the ith species. In the absence of the host, the decay curve for ANS in buffer solution was perfectly fitted to a single-exponential function, otherwise, the decay profile of ANS in presence of β-cyclodextrin or its derivatives could be analyzed only by a linear combination of two exponential functions. The short and long fluorescence lifetimes (τ_s and τ_l) and relative quantum yields (Φ) observed for ANS in the presence of β-cyclodextrin **1**, compounds **4**, and **5** are summarized in Table I. The elongated lifetimes in the presence of hosts show that the environment around the ANS molecule is more hydrophobic than the bulk water. The two-component decay indicates that the ANS is located in two different environments. However, the shorter lifetimes ($\tau_s = 1.5$–2.9 ns) in the presence of hosts do not agree with the original lifetime (0.4 ns) of ANS in buffer. Then, the two lifetimes (τ_s and τ_l) observed in the presence of the host should originate from two different fluorescing states [26]. For compounds **4** and **5**, fluorescence lifetimes observed for ANS in the presence of **4** and **5** are similar to that for ANS in the present of the native β-cyclodextrin, there are a short lifetime and a long lifetime too, and they are all longer than that for ANS in the present of the native β-cyclodextrin. This shows that the naphthalene ring at the end of the oligo(ethyleneamino) spacer tethered to β-cyclodextrin can increase the micro-environment hydrophobicity of β-cyclodextrin inclusion with guest. Furthermore, this result can also persuade that the substituents do not enter in the cavity, which is consistent with that of the circular dichroism spectra measurement.

Excimer Formation of Modified Cyclodextrins

In the previously study of Ueno *et al.* [27], it is found that the modified cyclodextrin bearing a naphthalene ring at end of a spacer (6-deoxy-6-[14-(2-napthoxy)-3,6,9,12-(tetraoxa)-1-amino]-β-cyclodextrin) tends to form an association dimer in higher concentration solution and exhibits a new broad emission at longer wavelength region. In contrast, sharply with results reported by Ueno, the fluorescence spectra of modified β-cyclodextrins **4**–**5** possessing similar structure do not display new emission with increasing concentration, and the relative intensity of naphthalene emission only increases with increasing the concentration. As can be seen from Fig. 2, the results of fluorescence spectra clearly indicate that modified β-cyclodextrin **4** cannot form association dimer. This is reasonable, since the hydrophobic naphthalene part of β-naphthalene moiety is embedded into the cavity of β-cyclodextrin derivative in the longitudinal direction, which consequently produce strong van der Waals and hydrophobic interactions. By contrast, α-naphthalene moieties of **4** and **5** can only form very shallow longitudinal penetration due to the steric hindrance of the α-group, which leads to the relatively weak hydrophobic interaction between tethered moiety and cavity of β-cyclodextrin derivative [30]. These

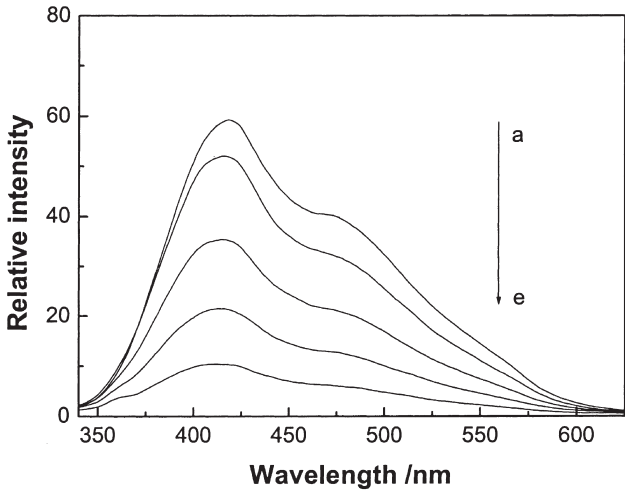


FIGURE 2 Fluorescence spectra of **4** at its various concentrations. The excitation wavelength was 320 nm. The host concentrations: 5.18, 2.07, 1.04, 0.52, and 0.21 mmol dm^{-3} (from *a* to *e*).

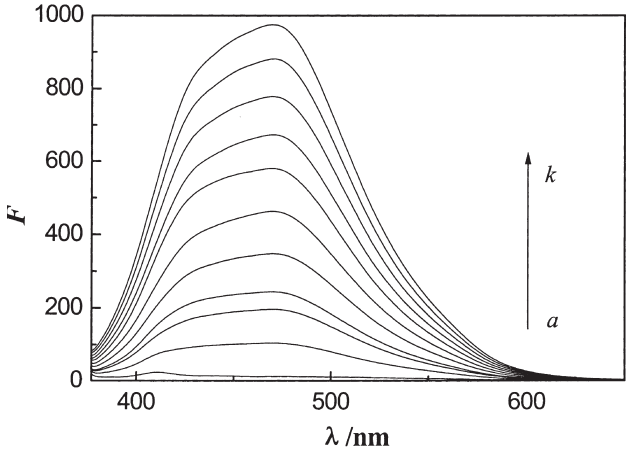


FIGURE 3 Fluorescence spectra of ANS ($1.7\,\mu mol\,dm^{-3}$) in the absence and presence of compound **5** at 25°C in aqueous buffer solution (pH 7.20). The excitation wavelength was 360 nm. The host concentration: 0, 17.7, 34.2, 44.3, 66.4, 88.5, 110.7, 132.8, 155, 177.1, and $199.2\,\mu mol\,dm^{-3}$ (from *a* to *k*).

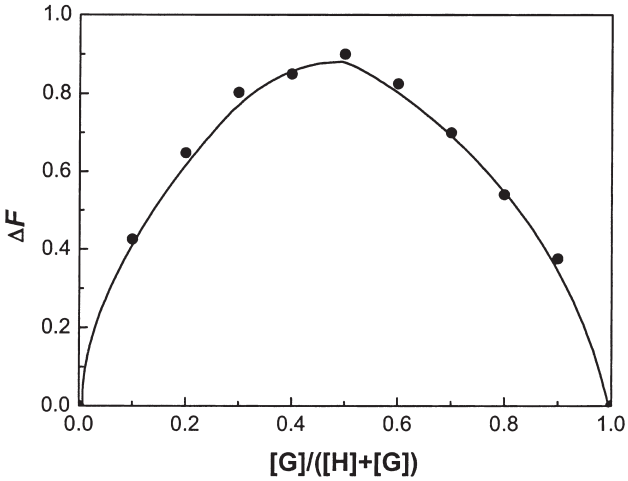


FIGURE 4 A Job plot of the complexation of compound **4** with TNS in aqueous solution. ($[G] + [H] = 25\,\mu mol\,dm^{-3}$).

results show that the link-position of functional group effects strongly on the excimer formation and self-assemble of modified β-cyclodextrins.

Spectrofluormetric Titration

In the fluormetric titration experiments, the fluorescence intensity of dyes gradually increased with increasing host concentration (Fig. 3), indicating the modified β-cyclodextrins with guest dyes to form the inclusion complexes. On the other hand, spectrofluormetric measurements have been performed to explore also the 1:1 complex stoichiometry upon inclusion complexation with β-cyclodextrin derivatives **4** and **5** in aqueous solution. A representative Job's plot for the complexation of compound **4** with TNS is shown in Fig. 4.

For a 1:1 stoichiometry, the inclusion complexation of dye guest (**G**) with host β-cyclodextrin derivative (**H**) is expressed by Eq. (2):

$$H + G \overset{K_S}{\rightleftharpoons} H{\cdot}G \tag{2}$$

The complex stability constant (K_S) can be determined using a non-linear least squares method according to the curve-fitting Eq. (3):

$$\Delta F = \{\alpha([H]_0 + [G]_0 + 1/K_S - \sqrt{\alpha^2([H]_0 + [G]_0 + 1/K_S)^2 - 4\alpha^2[H]_0[G]_0}\}/2 \tag{3}$$

where $[H]_0$ and $[G]_0$ denote the initial concentrations of host and guest, respectively. ΔF denotes the spectral change upon addition of host, where $\Delta F = F$ (with host) $- F$ (without host). The proportionality coefficient α is taken as a sensitivity factor for the spectral change related to the system and the instrument [28,29]. For each host compound examined, the plot of ΔF as a function of $[H]_0$ gives an excellent fit, verifying the validity of the 1:1 complex stoichiometry assumed above. Figure 5 illustrates the typical curve-fitting plots for the titration. There are no serious diversions between the experimental and calculated data, indicating 1:1 complexation only throughout the concentration range of host. When repeated measurements were made, the K_S value was reproducible within an error of ±5%, which corresponds to an estimated error of $0.15\,kJ\,mol^{-1}$ in the free energy of complexation (ΔG^0). The stability constant (K_S) of inclusion complexation of **4** and **5** with a series of guests are listed in Table II, along with Gibbs free energy change of complex formation ($-\Delta G^0$). For comparison purposes, the complex stability constants reported for β-cyclodextrin **1** and its derivative **3** are also listed in Table II.

Molecular Binding Ability

As can be seen from Table II, the oligo(ethylenediamino) or the naphthylox-amino-oligo(ethyleneamino) moiety tethered to β-cyclodextrin gives the modified β-cyclodextrins **3**–**5**, which alters not only the original molecular binding ability of parent β-cyclodextrin but also the molecular selectivity. Therefore, the functional sidearms perching on the edge of β-cyclodextrin cavity are considered to play an important role in determining how the guest molecule fit into the host cavity according to the sidearm's size, dipole, charge, hydrophobic, and functional group. In order to visualize the inclusion complexation behavior of modified β-cyclodextrins with some fluorescent dyes, the changing profiles of free energy change ($-\Delta G^0$) upon complexation with compounds **1** and **3**–**5** are shown in Fig. 6. Molecular binding ability of β-cyclodextrin and its derivatives for some dyes will be discussed below in terms of the

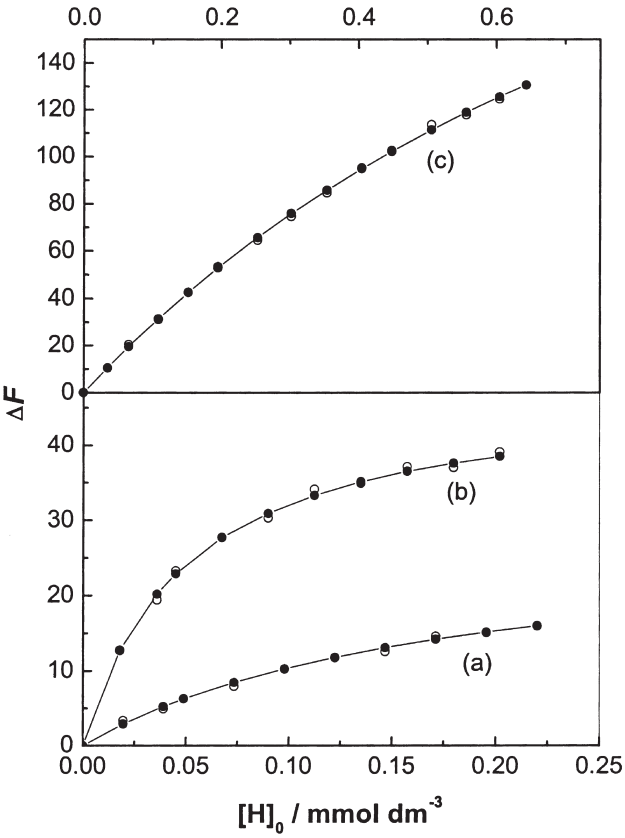


FIGURE 5 Curve-fitting analyses of fluorescence spectral titration of (a) AR with compound **5**, (b) RhB with compound **4**, (c) TNS with compound **5** in aqueous buffer solution at pH 7.20. Differential fluorescence intensity ΔF (hollow circle) was fitted to the theoretical value (solid circle) calculated for the stoichiometric 1:1 complexation.

size/shape-fit concept and the stereochemical complementary relationship between host and guest.

As can be seen from Table II and Fig. 6, the binding constants for inclusion complexation of host compounds (**1**, **3**–**5**) with TNS are roughly 2–38 times of magnitude larger than that with ANS, and the free energy changes ($-\Delta G^0$) increase from 16.83 to 20.6 kJ/mol. This seems reasonable, since the examination with Corey–Pauling–Koltum (CPK) space-fitting molecular models indicated that the hydrophobic naphthalene part of TNS can be embedded deeply into the cavity of β-cyclodextrin in the longitudinal direction. While the naphthalene moiety of ANS cannot penetrate in the longitudinal or lateral direction, only the anilino group can be embedded into the cavity. In the previous study, we have also demonstrated that the naphthalene ring prefers to penetrate into the cavity of β-cyclodextrin in the longitudinal direction [30], and showed the highest molecule selectivity up to 93 between 2-naphthalene-sulfonate and 1-naphthalenesulfonate. However, a close examination of the inclusion complexation of β-cyclodextrin (**1**) and its derivatives (**3**–**5**) reveals that the complex stability sequence for ANS, i.e. $\mathbf{4} > \mathbf{5} > \mathbf{3} > \mathbf{1}$, does not coincide with that for TNS, i.e. $\mathbf{1} > \mathbf{4} > \mathbf{3} > \mathbf{5}$. It is noted that the molecular binding ability of host compound is highly sensitive to the sidearm length attached to β-cyclodextrin. Possessing higher structural flexibility as compared with **1** and **3**, and **4** can adjust the orientation of aromatic moiety attached to the primary side of β-cyclodextrin and increase the micro-environmental hydrophobicity giving more tight inclusion complex with guest ANS. However, the modified β-cyclodextrin **5** carrying a sidearm of the longest chain does not give the most stability inclusion complex with ANS, probably due to the self-included hydrophobic sidearm in host **5**. Therefore, the enhanced chain length of sidearm attached to β-cyclodextrin increase not only the hydrophobic interactions but also the steric hindrance for ANS accommodated in the β-cyclodextrin cavity.

As can be seen from Table II and Fig. 6, a tendency analogues to ANS is seen in the inclusion phenomenon and the order of the binding ability of AR with β-cyclodextrin **1** and its derivatives **4** and **5**. The larger difference in the binding ability of host

TABLE II Stability constant (K_S) and Gibbs free energy change ($-\Delta G^0$) for the inclusion complexation of β-cyclodextrin **1** and its derivatives **3**–**5** with guest dyes at 25°C (pH 7.20 phosphate buffer)

Host	*Guest*	K_S	Log K_s	$-\Delta G^0$ (kJ mol^{-1})	*Ref.*
1	ANS	103	2.01	11.5	[15]
	TNS	4000	3.60	20.6	[15]
	AR	2630	3.42	19.5	[31]
	RhB	4300	3.63	20.73	[14]
3	ANS	266	2.42	13.8	[15]
	TNS	2870	3.46	19.7	[15]
	AR	974 ± 3	2.99	17.06 ± 0.01	This work
	RhB	3210 ± 53	3.51	20.01 ± 0.04	This work
4	ANS	518 ± 5	2.71	15.5 ± 0.03	This work
	TNS	3270 ± 67	3.51	20.06 ± 0.05	This work
	AR	5680 ± 94	3.75	21.43 ± 0.04	This work
	RhB	22000 ± 430	4.34	24.79 ± 0.05	This work
5	ANS	443 ± 8	2.65	15.10 ± 0.04	This work
	TNS	890 ± 12	2.95	16.83 ± 0.03	This work
	AR	4180 ± 73	3.62	20.67 ± 0.04	This work
	RhB	72200 ± 810	4.85	27.73 ± 0.03	This work

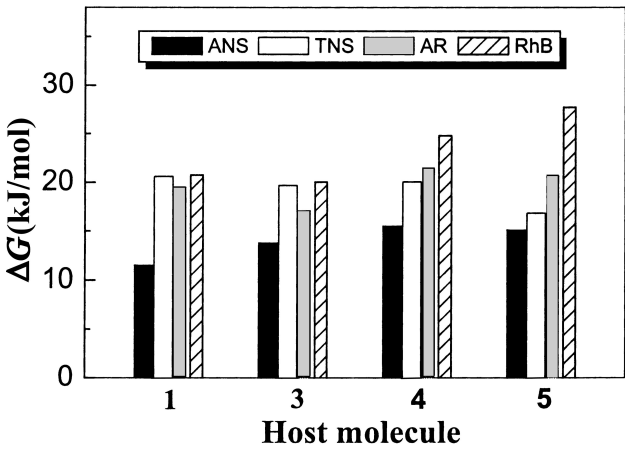


FIGURE 6 Gibbs free energy changes ($-\Delta G^0$) for the inclusion of complexation of β-cyclodextrin and its derivatives with guests at 25°C (pH 7.20 phosphate buffer).

compounds **4** and **5** for ANS and AR may be attributed to guest molecule's size and shape. It is noted that the stability constants (K_S) and the free energy changes ($-\Delta G^0$) of all guest with host **4** are much larger than that with **5** except for guest RhB. These results indicate that the microstructural change of the host molecule apparently governs the complexation phenomena to some extent besides the size and shape of guest molecule. It is interesting that the free energy change ($-\Delta G^0$) of inclusion complexation β-cyclodextrin **1** and its derivatives **4** and **5** with RhB are larger than that of ANS, TNS, and AR. Possessing the bulkiness and large steric hindrance group on the xanthene residue, the RhB molecule has only a small part to be embedded into the hydrophobic cavity of β-cyclodextrin according to examinations with CPK models. Thus, the higher complex stability ($\log K_S = 3.63–4.85$) among the four fluorescent dyes indicates that the lactonic form of RhB participates in the inclusion complexation with β-cyclodextrin **1** and its derivatives **4** and **5**, as we proposed [14]. It is significant that the binding ability of RhB molecule by modified β-cyclodextrin increases with the enhanced sidearm length tethered to β-cyclodextrin, i.e. **5** > **4** > **3** > **1**. The stability constant (K_S) of host **5** with RhB is greater by a factor of 16.8 than that with the parent β-cyclodextrin. Here, it is considered that the hydrophobic interactions enhanced by sidearm attached to β-cyclodextrin play a crucial role in the enhanced binding ability of the parent β-cyclodextrin for RhB. On the other hand, though the modified β-cyclodextrin **3**–**5** reduce the binding ability for TNS, their molecular selectivities are enhanced for AR/TNS and RhB/TNS. Host compound **5** shows the highest molecular selectivities up to 163 for RhB/ANS. These results indicate that the hydrophobic sidearm attached to β-cyclodextrin can not only alter the recognition ability for the guest molecule's size/shape but also the molecular selectivity.

Effect of PH on Molecular Recognition

As can be seen for Table III, the pH value of solution can strongly influence the binding ability of modified β-cyclodextrins for ANS. When the pH values of solution changed from 2 to 11, the stability constants of compound **5** for ANS correspondingly increase from 223 to 410 in acid solution (pH = 2–5), and decrease from 428 to 252 in basic solution (pH = 8–11), respectively. This may be attributed to the electrostatic exclusion and/or intermolecular hydrogen bond between host and guest. The aminos both in sidearm of **5** and in ANS molecule may bear some positive charge in acid solution due to protonation, therefore, the electrostatic exclusion interaction between host and guest will be strengthened with pH decreasing, leading the complex stability to decrease. On the other hand, hydroxide ion can also form intermolecular hydrogen bond with guest molecule, so the intermolecular hydrogen bond interaction between compound **5** and ANS will decrease with increasing pH, giving low complex stability. These results reveal the importance of the pH value of solution, which further indicate that electrostatic interaction and hydrogen bond play important roles in the determining the complex stability.

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TABLE III Stability constant (K_S) and Gibbs free energy change ($-\Delta G^0$) for the inclusion complexation of β-cyclodextrin derivatives (**5**) with guest dyes at 25°C

Host	*Guest*	K_S	$\log K_S$	$-\Delta G^0$ (kJ mol^{-1})	*pH*	*Salinity* (mol dm^{-3})	*Ref.*
5	ANS	223 ± 4	2.35	13.40 ± 0.03	2	Modulated by HCl	This work
	ANS	312 ± 5	2.49	14.24 ± 0.02	3	Modulated by HCl	This work
	ANS	410 ± 11	2.61	14.91 ± 0.04	5	Modulated by HCl	This work
	ANS	428 ± 9	2.63	15.02 ± 0.04	8	Modulated by NaOH	This work
	ANS	304 ± 3	2.48	14.17 ± 0.01	10	Modulated by NaOH	This work
	ANS	252 ± 8	2.40	13.71 ± 0.04	11	Modulated by NaOH	This work

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