Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

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To cite this Article Liu, Yu , You, Chang-Cheng , Kunieda, Mieko , Nakamura, Asao , Wada, Takehiko and Inoue, Yoshihisa(2000) 'Molecular Recognition Studies on Supramolecular Systems. 29. Anilino- and *m*-Toluidino-β-Cyclodextrins: Structural and Conformational Analyses and Molecular Recognition of Aliphatic Alcohols', Supramolecular Chemistry, 12: 3, 299 — 316
To link to this Article: DOI: 10.1080/10610270008029451
URL: http://dx.doi.org/10.1080/10610270008029451

SUPRAMOLECULAR CHEMISTRY, Vol. 12, pp. 299-316
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Molecular Recognition Studies on Supramolecular Systems. 29. Anilino- and *m*-Toluidino-β-Cyclodextrins: Structural and Conformational Analyses and Molecular Recognition of Aliphatic Alcohols

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(Received March 09, 2000)

Mono(6-anilino-6-deoxy)-β-cyclodextrin **(1)** and mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** were synthesized and characterized. Circular dichroism and fluorescence spectral studies and fluorescence lifetime measurements have been performed to elucidate the conformations of **1** and **2** in aqueous buffer solution. 1-D and 2-D NMR spectra of **2** have been measured in D_2O to deduce its structure and detailed conformation in solution. From the circular dichroism, fluorescence, and NMR spectroscopic studies, it was revealed that the substituents appended to **1** and **2** penetrate into the cyclodextrin cavity forming a stable self-inclusion complex in aqueous solution, and also that the short linkage between the *m*-toluidino and cyclodextrin moieties makes the cyclodextrin ring of **2** deformed to some extent. The complex stability constants (K_S) of **1** and **2** for a series of aliphatic alcohols have been determined by using spectropolarimetric titrations in aqueous phosphate buffer solution (pH 7.20) at 25°C to elucidate the role of introduced substituents and the weak interactions involved in inclusion complexation by the modified cyclodextrins. The results obtained indicate that the van der Waals and hydrophobic interactions mainly contribute to the formation of complexes between the cyclodextrins and aliphatic alcohols, and the inclusion complexation process involves the induced-fit mechanism. Modified β-cyclodextrin **2** can recognize not only the size, shape, and hydrophobicity of the guest molecules, but also chiral guests, affording a moderate enantioselectivity of 1.55 for (+)/(-)-borneol.

Keywords: Modified cyclodextrins, 2D-NMR analyses, Inclusion complexation, Molecular recognition, Aliphatic alcohol

INTRODUCTION

Recently, much effort has been devoted to the synthesis of chemically modified cyclodextrins in order to enhance the original abilities of native cyclodextrins to recognize molecules, to assemble each other, and to catalyze reactions [1–5]. Ueno *et al.* reported the syntheses and molecular recognition behavior of a series of chromophore-appended cyclodextrins with various aliphatic alcohols [6–10], and found that self-inclusion of the introduced substituent plays

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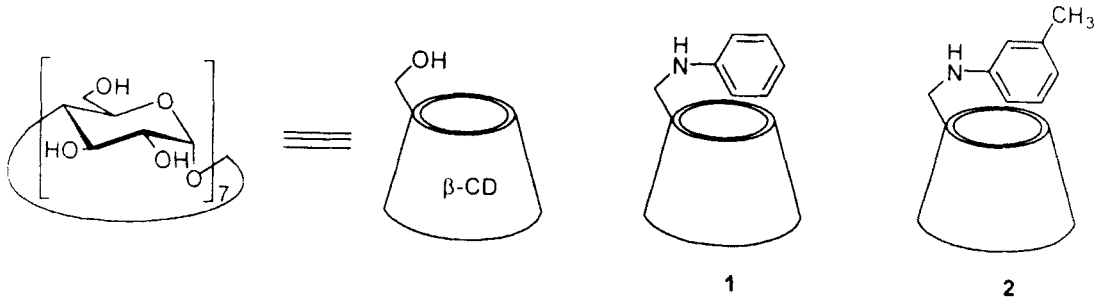

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CHART 1

an important role in determining the molecular and enantio selectivities of modified cyclodextrins. Breslow *et al.* designed and prepared various bridged bis(cyclodextrin)s, which showed dramatically enhanced molecular recognition abilities as compared to the parent cyclodextrins and were employed as antibody mimics [11–13]. In our previous works [14–16], we synthesized a variety of chemically modified cyclodextrins bearing pyridyl, arylseleno, quinolyl, tryptophan, and furfuryl moieties, and assessed their molecular recognition abilities with amino acids, aliphatic alcohols, and naphthalene derivatives as guest molecules. The results obtained indicate that the shape, size, and hydrophobicity of the substituent introduced dramatically alter the original molecular and enantio selectivities of native cyclodextrins, and several weak forces, including van der Waals, dipole-dipole, induced dipole-dipole, electrostatic, hydrophobic and hydrogen-bonding interactions, cooperatively contribute to the formation of host-guest complexes.

We have recently reported the syntheses of mono(6-anilino-6-deoxy)-β-cyclodextrin **(1)** [17] and mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** [18]. However, we have found that our previous sample of **2** contained an appreciable amount of the raw material, probably because the reaction period was not long enough to complete the reaction, although **1** could be obtained in satisfactory yield and purity under the same reaction conditions. In this paper, we wish to report the revised synthesis and purification of **2** as well as the elucidation of the conformations of **1** and **2** in aqueous buffer solution using circular dichroism (CD), steady-state and time-correlated fluorescence, and 1-D and 2-D NMR spectroscopy. Furthermore, the complex stability constants for **1** and **2** with various aliphatic alcohols are determined at 25 °C in phosphate buffer solution (pH 7.2) by spectropolarimetric titration. The spectral studies will reveal that the *m*-toluidino group introduced at the 6-position of **2** is deeply included in the cyclodextrin cavity forming a stable self-inclusion complex, and that the short linker between the substituent and cyclodextrin's rim causes a distortion of the cyclodextrin ring to some extent. Interestingly, the deeply self-included substituent is readily driven out of the cavity upon inclusion complexation of a guest molecule, accompanying recovery of the deformed cyclodextrin ring. Phenomenologically, this behavior is very similar to the induced-fit mechanism observed for the interaction between enzyme and substrate. Close examinations of the complex stability constants obtained indicate that the shape, size, and hydrophobicity of guests determine the stabilities of host-guest complexes, and the hydrophobic and van der Waals interactions are the major driving forces in molecular recognition of cyclodextrins.

RESULTS AND DISCUSSION

Induced Circular Dichroism Spectra

It is well known that inclusion complexation of achiral chromophoric guests by cyclodextrin induces circular dichroism (ICD) signal at the absorption transition band(s) of the chromophore. Hence, the ICD signal is taken as evidence for inclusion complexation and is often employed as a spectral tool for elucidating the location and orientation of guest in cyclodextrin cavity [19, 20]. The UV and CD spectra of mono(6-anilino-6-deoxy)-β-cyclodextrin **(1)** and mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** in phosphate buffer solution at 25 °C are shown in Figure 1. Modified β-cyclodextrins **1** and **2**, possessing analogous aromatic chromophores, afforded very similar UV and somewhat different CD spectra. Thus, the CD spectra exhibit two positive Cotton effect peaks of different relative intensity at the wavelengths corresponding to the ^1L_a and ^1L_b bands.

N-Methylaniline, when included in β-cyclodextrin, would be considered as a good reference compound for the anilino-, and probably toluidino-, β-cyclodextrins. However, as can be seen from Figure 2, the inclusion complex of *N*-methylaniline with β-cyclodextrin gave a distinctly different ICD spectrum, showing a weak negative Cotton effect ($\Delta\varepsilon = -0.106$) for the ^1L_b band at 300.8 nm and a moderate positive Cotton effect ($\Delta\varepsilon = +1.245$) for the ^1L_a band at 241.2 nm.

As to the orientation of guest molecule included in cyclodextrin, an empirical rule has been proposed by Shimizu *et al.* [19], Kajtár *et al.* [20, 21], and Harata [22]: if the transition moment of the guest chromophore is parallel to the axis of symmetry of cyclodextrin (that is, the axis of the cavity), then the sign of the ICD signal for that transition will be positive, whereas if the moment axis is aligned perpendicular to the cavity axis, the sign of ICD will be negative. According to this rule, we can properly predict that *N*-methylaniline is included into the β-cyclodextrin cavity in the axial direction (Figure 3a). However, this empirical rule does not work any more in rationalizing the two positive Cotton effect peaks observed for the hosts **1** and **2** (Figure 1).

In this aspect, Kodaka's proposal seems attractive, since he claims that the sign of ICD will be opposite to the expectation of the above rule, if the chromophore is located outside the cyclodextrin cavity [23]. Following Kodaka's proposal, we may assume that the phenyl group is located outside the cavity and the chromophore plane is aligned perpendicular to the cavity axis (Figure 3b). However, this prediction obviously conflicts with the experimental data obtained in the 2-D NMR and steady-state and time-correlated fluorescence spectral studies. At present, we simply point out that the linkage between the substituent and cyclodextrin may affect the ICD spectra of modified cyclodextrin, and the above two empirical rules are not always applicable to any type of modified cyclodextrins. Probably, the deformed cyclodextrin ring, as demonstrated by the 2-D NMR spectrum discussed below, is responsible for the unusual ICD spectra of **1** and **2**.

Fluorescence Spectra and Fluorescence Lifetimes

Aniline and *m*-toluidine are very sensitive to the local microenvironment and can be used as fluorescent probes. As shown in Figure 4, *N*-methylaniline shows relatively weak fluorescence (trace a) in aqueous buffer solution, but gives stronger fluorescence (trace b) with a hypsochromic shift in the presence of a 500-fold excess of β-cyclodextrin, as a result of inclusion complexation into the hydrophobic cyclodextrin cavity. This result is consistent with that reported by Hoshino *et al.* for aniline – β-cyclodextrin system [24]. Much enhanced and blue-shifted fluorescence was observed for the anilino- and *m*-toluidino-appended cyclodextrins (**1** and **2**) (Figure 4, traces C and d), indicating that the flu-

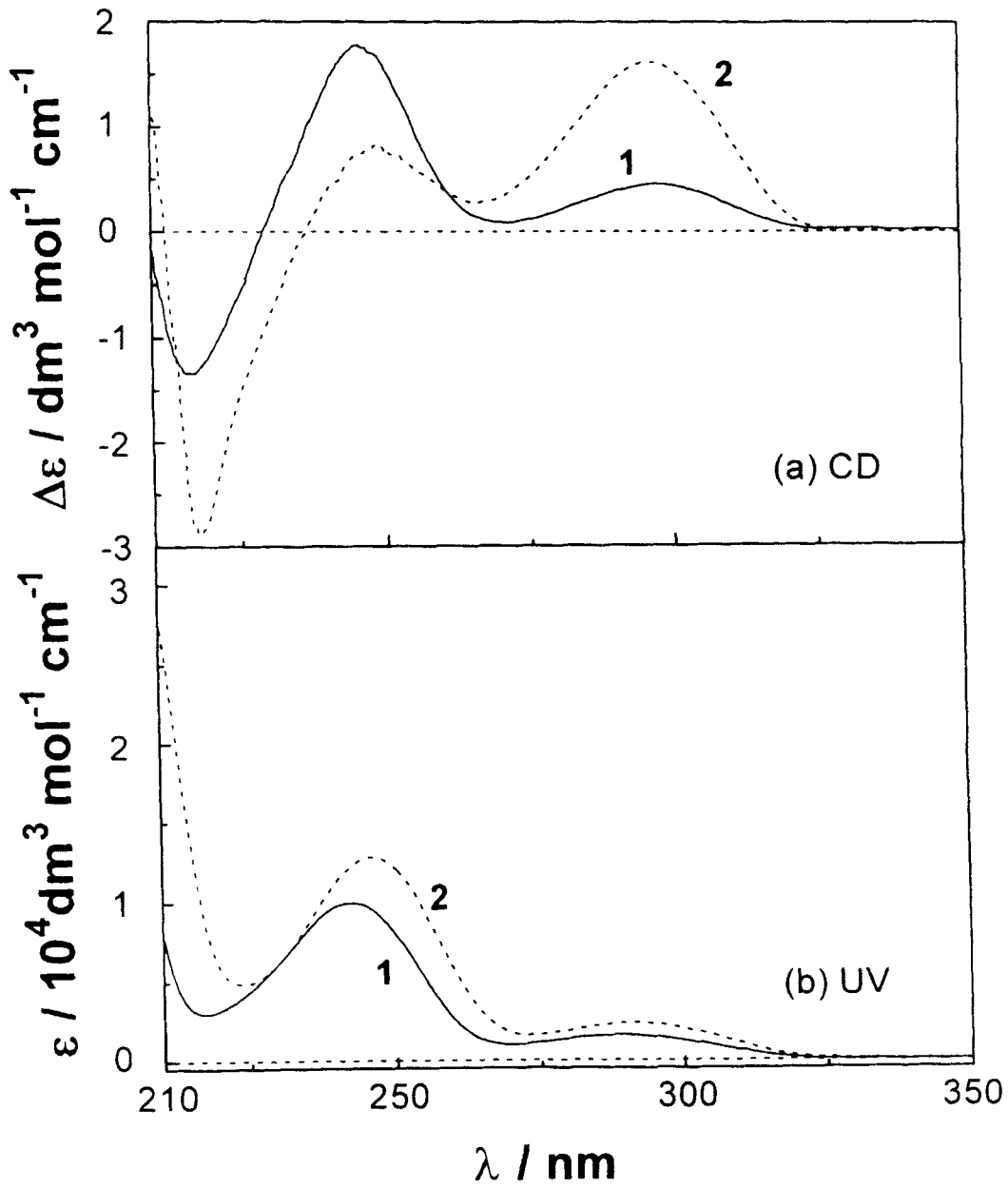

FIGURE 1 (a) Circular dichroism and (b) absorption spectra of mono(6-anilino-6-deoxy)-β-cyclodextrin **(1)** (0.1 mM) and mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** (0.1 mM) in phosphate buffer solution (pH 7.20) at 25 °C

orescent substituents were embedded deeply into the hydrophobic cavity. Furthermore, the fluorescence intensities of modified β-cyclodextrins **1** and **2** decreased dramatically upon addition of a guest, suggesting that the fluorophore was driven out of the cavity to the hydrophilic environment in bulk water.

In order to further investigate the conformational changes of **1** and **2** induced by guest inclusion in aqueous solution, the nanosecond

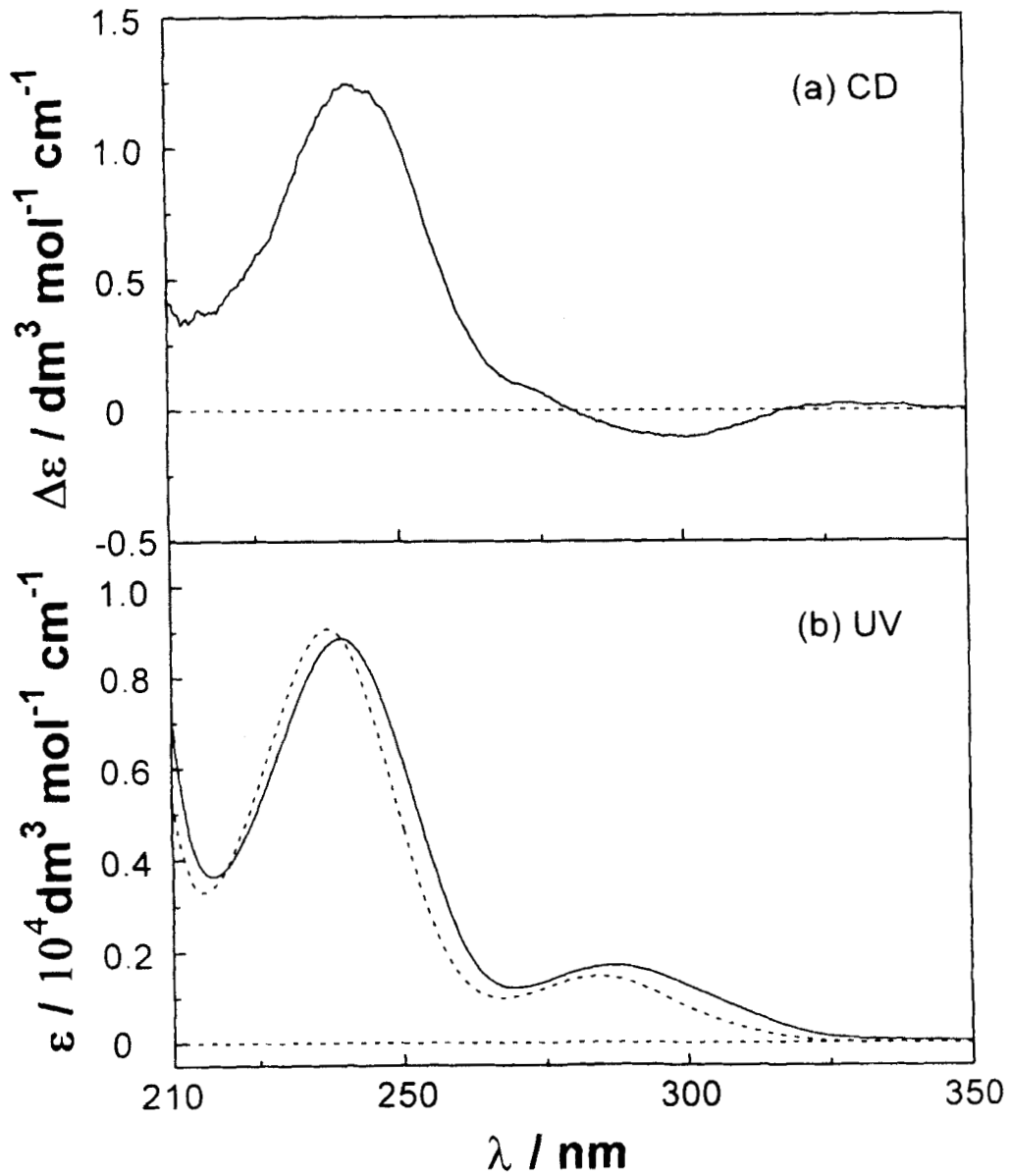

FIGURE 2 (a) Circular dichroism and (b) absorption spectra of *N*-methylaniline (0.12 mM) in the presence (solid line) and absence (dotted line) of β-cyclodextrin (13.3 mM), where the Δε values were calculated by assuming all of the *N*-methylaniline was complexed by β-cyclodextrin

time-resolved fluorescence experiments were carried out. Since the rates of complexation/decomplexation are much slower than the fluorescence lifetime, the decay profile of fluorescence intensity ($F(t)$) can be described as the sum of unimolecular decays of all fluorescing species existing in the solution:

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

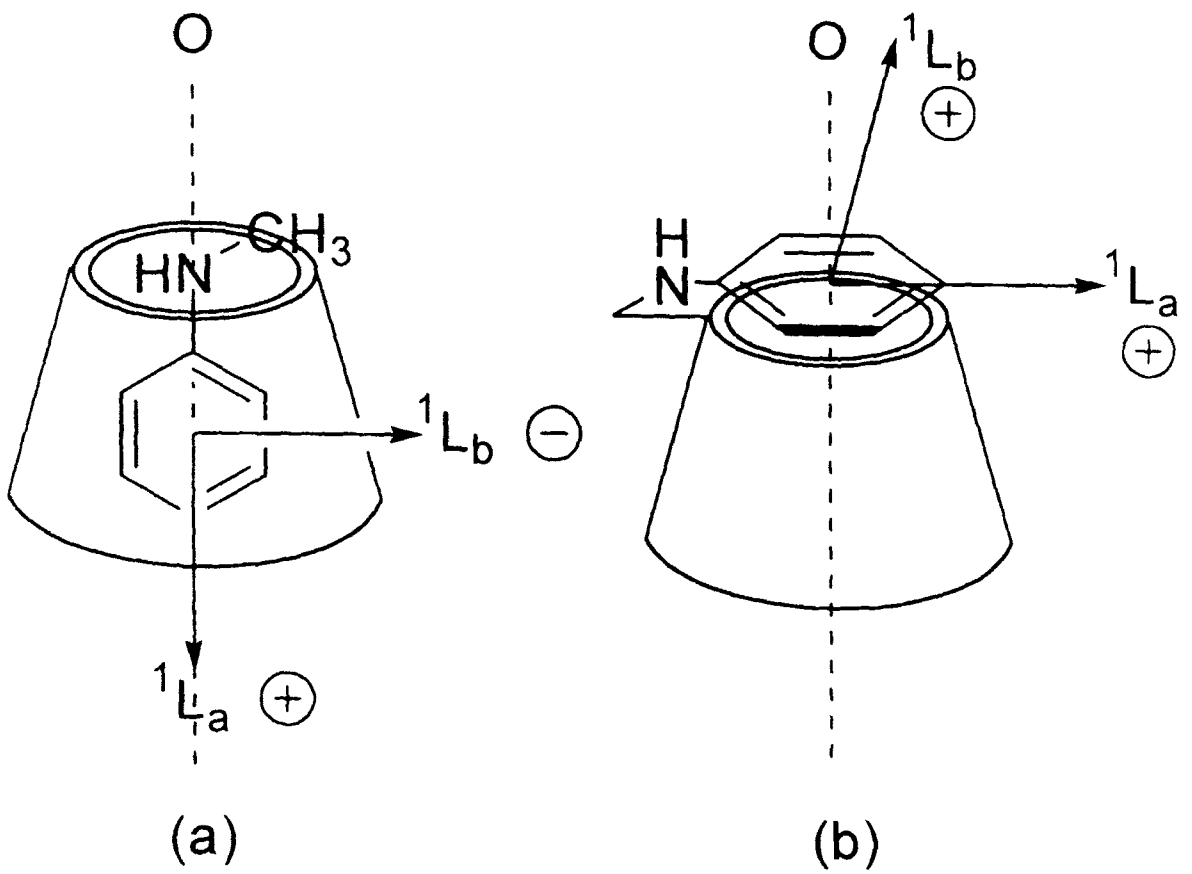

FIGURE 3 (a) The inclusion mode of *N*-methylaniline with β-cyclodextrin according to Kajtár's *empirical rule*; (b) The orientation of anilino group of **1** concluded from Kodaka's proposal

where A_i and τ_i represent the initial abundance and lifetime of the *i*'th species. The results are listed in Table I. The fluorescence decay profiles obtained with aqueous solutions (pH 7.2) of **1** and **2** in the absence of guest could be analyzed by a single exponential function to give the lifetime of 2.8 and 2.2 ns, respectively. These lifetimes coincide with that of *N*-methylaniline in hexane. It is thus indicated that only one fluorescing species exists in the aqueous solution, which is most probably the self-included anilino- or toluidinocyclodextrin. However, it is generally believed that there is a self-inclusion/exclusion equilibrium for the fluorophoric substituent introduced to cyclodextrin and therefore two lifetimes are often observed even in the absence of added guest [7, 15b, 25]. The single lifetimes observed in the present cases indicate that the self-inclusion of **1** and **2** is strong enough to reduce the contribution of the outside component to a negligible level. In the presence of added guest, the time profile of the fluorescence intensity of **1** and **2** obeys a double-exponential decay, affording two lifetimes (τ_S and τ_L) for a single solution. For both **1** and **2**, the longer lifetimes (τ_L = 2.7 and 2.2–2.4 ns, respectively) are essentially the same as those obtained in the absence of a guest (τ = 2.8 and 2.2 ns, respectively), while the shorter lifetimes (τ_S = 1.1 and 1.1–1.2 ns, respectively) are very close to those

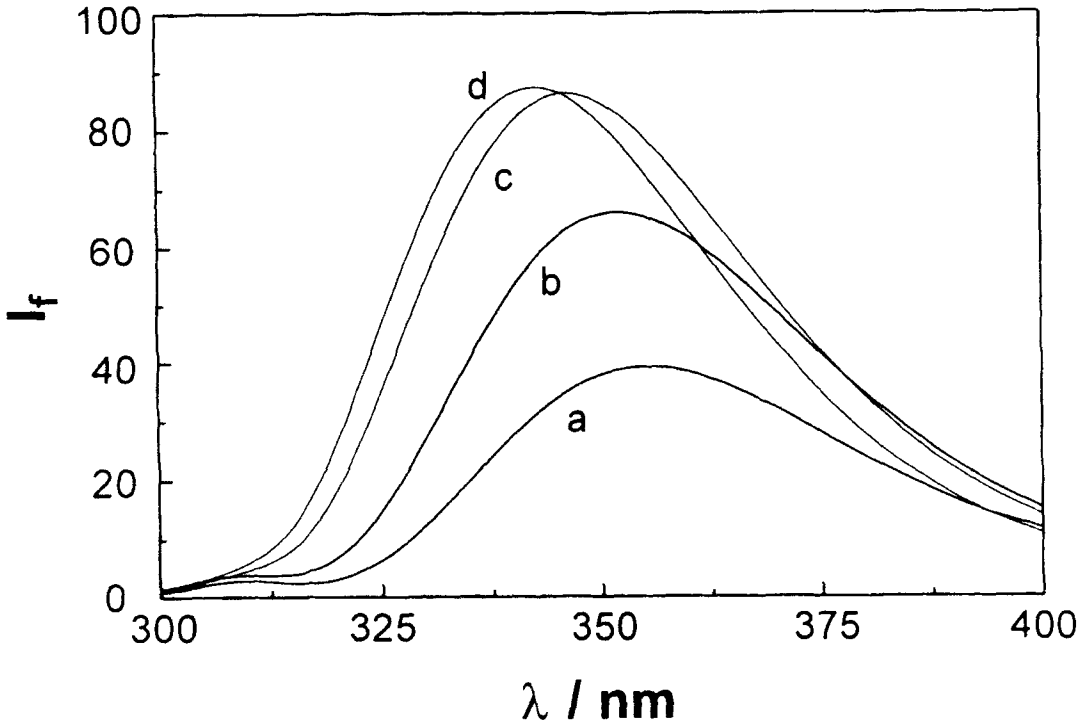

FIGURE 4 Fluorescence spectra of (a) *N*-methylaniline (10 μM), (b) *N*-methylaniline (10 μM) in the presence of β-cyclodextrin (5 mM), (c) mono(6-anilino-6-deoxy)-β-cyclodextrin **(1)** (10 μM), and (d) mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** (10 μM) in phosphate buffer solution (pH 7.20, 0.1 M) at 25 °C; the excitation wavelength was 280 nm

determined for *N*-methylaniline in aqueous solution (τ = 1.1 ns).

The fluorescence lifetimes of *N*-methylaniline, its complex with β-cyclodextrin, and **2** were also measured in aqueous solutions of different pH. As shown in Table I, *N*-methylaniline gave practically the same fluorescence lifetime (1.0–1.1 ns) over a pH range of 4–10, but the addition of β-cyclodextrin made the fluorescence decay double-exponential, irrespective of the pH employed. The short and long lifetimes obtained (1.0–1.1 and 2.8–3.7 ns) may be attributed to *N*-methylaniline in the bulk water and in the cyclodextrin cavity, respectively. In contrast, the toluidine-appended cyclodextrin **2** gave only one fluorescence lifetime (1.9–2.2 ns) at pH 7.2 and 10, which is attributable to the toluidine moiety included in the cavity. Only at pH 4.0, the fluorescence of **2** exhibited two-component decay (τ_S = 1.0 and τ_L = 2.2 ns). The lack of the short lifetime at higher pH clearly indicates that the toluidine moiety in **2** is strongly self-included in the cavity and therefore much more acidic condition (e.g. pH 4) is needed to observe the fast-decaying component which arises from the toluidine moiety located outside the cavity. We may conclude therefore that the self-included species is the major component of **1** and **2** in the aqueous buffer solution at pH 7.2. It is interesting to compare the longer lifetimes (τ_L) determined for anilinocyclodextrin (**1**) (2.8 ns) and *N*-methylaniline – cyclodextrin complex (3.7 ns) at pH 7.2. The appreciably shorter τ_L for **1** is readily interpreted by shallower penetration of the appended anilino group into the cavity as a result of the short linker.

TABLE I Fluorescence Lifetimes (τ) and Relative Quantum Yields (Φ) of Modified β-Cyclodextrins (**1** And **2**), Aniline, and *N*-Methylaniline in the Presence and Absence of Complex-Forming Additives in Aqueous and/or Hexane Solution at 25 °C

Compound	*solvent*	*pH*	*concentration/10^{-5} mol L^{-1}*	*additive*	*equivalent*	τ_s / *ns*	Φ_s / %	τ_1 / *ns*	Φ_1 / %	χ^2
1	H_2O	7.2	23.09	-	–	-	-	2.8	100	1.34
				1-heptanol	20	1.1	10	2.7	90	1.44
2	H_2O	4.0	12.7	-	–	1.0	16	2.2	84	1.12
		7.2	11.9	-	–	–	–	2.2	100	1.49
		10.0	5.75	-	–	-	-	1.9	100	1.27
		7.2	22.37	cyclohexanol	246	1.2	24	2.2	76	1.25
			10.08	(-)-borneol	8	1.1	37	2.4	63	1.14
			10.34	2-adamantanol	3	1.2	35	2.2.	65	1.39
Aniline	H_2O	7.2	27.16	-	-	0.8	100	-	-	1.18
				β-cyclodextrin	15	0.8	59	2.7	41	1.18
N-methylaniline	Hexane	–	17.73	-	-	-	-	2.2	100	1.35
	H_2O	4.0	115.7	-	-	1.0	100	-	-	1.42
				β-cyclodextrin	10	1.1	26	3.6	74	1.02
		7.2	23.8	-	–	1.1	100	-	-	1.43
				β-cyclodextrin	49	1.1	23	3.7	77	1.06
		10.0	329.4	–	–	1.1	100	–	–	1.54
				β-cyclodextrin	4	1.0	35	2.8	65	1.43

^{1}H NMR Spectral Studies of Conformation of Toluidinocyclodextrin (2)

NMR technique is one of the most powerful tools for structural and conformational analyses of modified cyclodextrins [26, 27]. From the circular dichroism and fluorescence spectral studies described above, it was revealed that the substituent of the modified cyclodextrin is strongly self-included in the cavity in the absence of guests. In order to elucidate more detailed structure of the self-inclusion complex, we performed the conformational and orientational analyses of **2** using 1-D and 2-D NMR techniques such as total correlation spectroscopy (TOCSY), correlated spectroscopy (COSY), and rotating frame nuclear Overhauser effect spectroscopy (ROESY).

In the structural analysis of cyclodextrin derivatives, TOCSY is particularly useful as a method to extract the spin system of D-glucose units from an overlapping spectrum region [27, 28]. In the present study, the heavily overlapped ^{1}H NMR signals of **2** could be fully assigned to seven sets of protons which belong to each D-glucose unit by using 1-D and 2-D TOCSY spectra. In principle, different correlation peaks can be observed by changing the mixing time used in 2-D TOCSY measurement. Thus, the correlation peaks of cyclodextrin's H2 and H3 with H1 appear with a short mixing time, while those of H2, H3, H5, and H6 with H1 are observed with longer mixing times. We therefore employed several different mixing times of 70, 120, 150, and 160 ms in order to assign all D-glucose protons in **2**. Figure 5 illustrates a typical

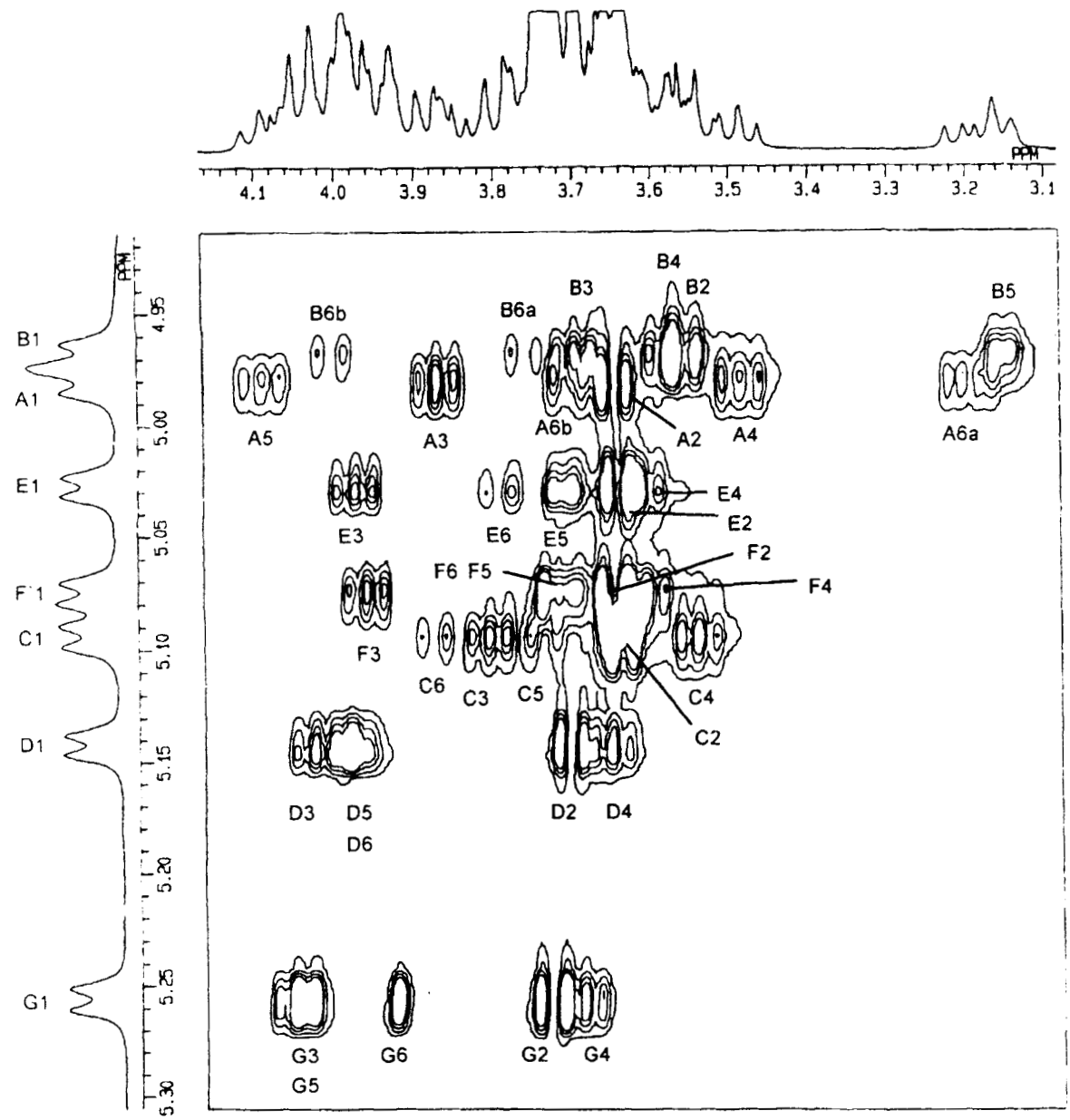

FIGURE 5 Part of the TOCSY spectrum of mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** (8 mM) in D_2O at 35 °C obtained with a mixing time of 160 ms

2-D TOCSY spectrum of **2** with a mixing time of 160 ms. A combination of the TOCSY and COSY spectra provided us with more exact assignment. However, for some glucose units, the cross peaks for H6 were not clear in the 2-D TOCSY spectra. In such a case 1-D TOCSY was used to assign the signal peaks for H6. By irradiating the peak for H5 in a 1-D TOCSY experiment with a mixing

time of 20 ms, all of the H6 peaks could be assigned.

Nuclear Overhauser effect spectroscopy (NOESY) is often used to find pairs of protons that are close in space (< 4Å) [29]. However, the ROESY technique is superior to NOESY when the correlation time approaches the inverse of the Larmor frequency of the protons to be observed, particularly in the case of medium-size molecules such as cyclodextrins [25, 27, 28]. Hence, we employed the ROESY technique to deduce the sequence of the D-glucose units in **2**. The distance between the H1 of one glucose unit and the H4 of the adjacent glucose is short enough to produce an NOE, which can be observed by ROESY, as shown in Figures 6a and 6b. The glucose unit, to which the *m*-toluidino moiety is introduced through C6, can be readily assigned on the basis of the largest up-field shift for H6a and H6b, and is defined as the glucose unit **A**. The H4 of the adjacent glucose unit **B** can be identified by its negative cross peak with the H1 resonance of the glucose unit **A** in the ROESY spectrum. In a similar manner, the sequence of all glucose units in **2** were successively determined. The result of the assignments is shown in Figure 7.

The ROESY spectra can also provide us with the detailed information concerning the orientation of the *m*-toluidino group in **2**. In accord with the schematic drawing shown in Figure 8a, the aromatic proton-7 was correlated with the glucose protons **G**5, **G**6, and **A**6b; the aromatic proton-8 was correlated with **E**3; the aromatic proton-9 was correlated with **D**5; the aromatic proton-10 was correlated with **B**5, **B**6a, and **B**6b; and the methyl protons were correlated with **F**3 and **F**5. From these observations, we can explicitly conclude that the *m*-toluidino group in **2** is obliquely included in the cyclodextrin cavity forming a well defined self-inclusion complex with a fairly fixed conformation and orientation, as illustrated in Figure 8b. The complicated, but well-dissolved, NMR spectrum of **2**, which makes the full assignment of all protons possible, may be attributed to the seriously deformed cyclodextrin ring, for which the very short linker group and the strong self-inclusion of the substituent are jointly responsible. In this context, it is interesting to note that the solubility of **2** in water is fairly higher than that of native β-cyclodextrin, since a deformed cyclodextrin ring is considered to facilitate the destruction of the hydrogen-bond network of the secondary side of cyclodextrin, enhancing the solubility.

Molecular Recognition by 1 and 2

In order to investigate quantitatively the complexation behavior of hosts **1** and **2**, spectropolarimetric titrations were performed with a series of aliphatic alcohols in aqueous phosphate buffer solution (pH 7.20) at 25 °C. As shown in Figure 9, stepwise addition of a known amount of a guest to a solution of **2** (0.1 mM) caused substantial decrease in Δε at 296 nm and moderate increase at 245 nm, indicating significant conformational changes of the *m*-toluidino moiety.

Assuming the 1:1 host:guest stoichiometry, the inclusion complexation equilibrium is expressed by eq 2.

$$H + G \overset{K_S}{\rightleftharpoons} H \cdot G \qquad (2)$$

The complex stability constant (K_S) can be determined from the analysis of the CD spectral changes upon addition of a guest (Figure 9), by using the non-linear least squares method according to the curve fitting eq 3 [30]:

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

where $[G]_0$ and $[H]_0$ refer to the total concentrations of the guest and host, and α the proportionality coefficient, which may be taken as a sensitivity factor for the change in Δε (or conformation) induced by guest inclusion [15c, 30]. For

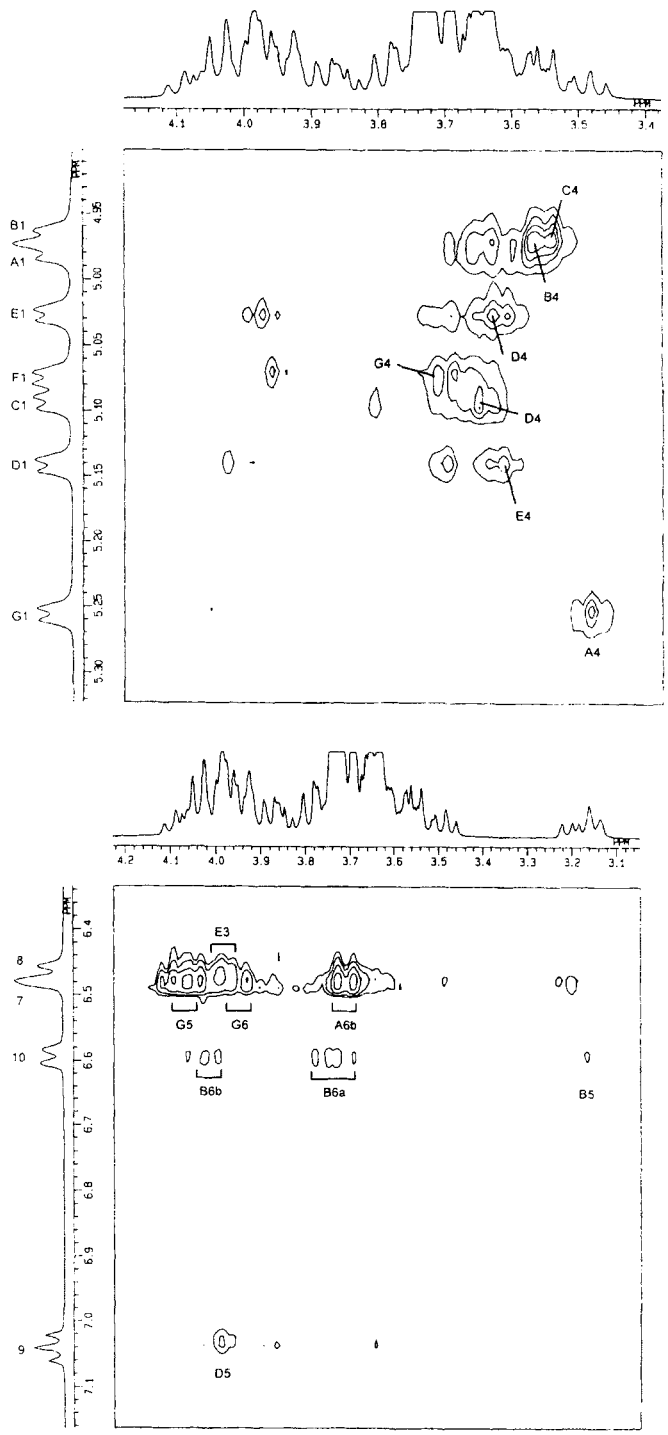

FIGURE 6 A part of the ROESY spectrum of mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** (8 mM) in D_2O at 35 °C obtained with a mixing time of 400 ms; (a) NOE correlation between the H1 and H4 protons of adjacent glucose units and (b) NOE correlation between the aromatic and glucose protons

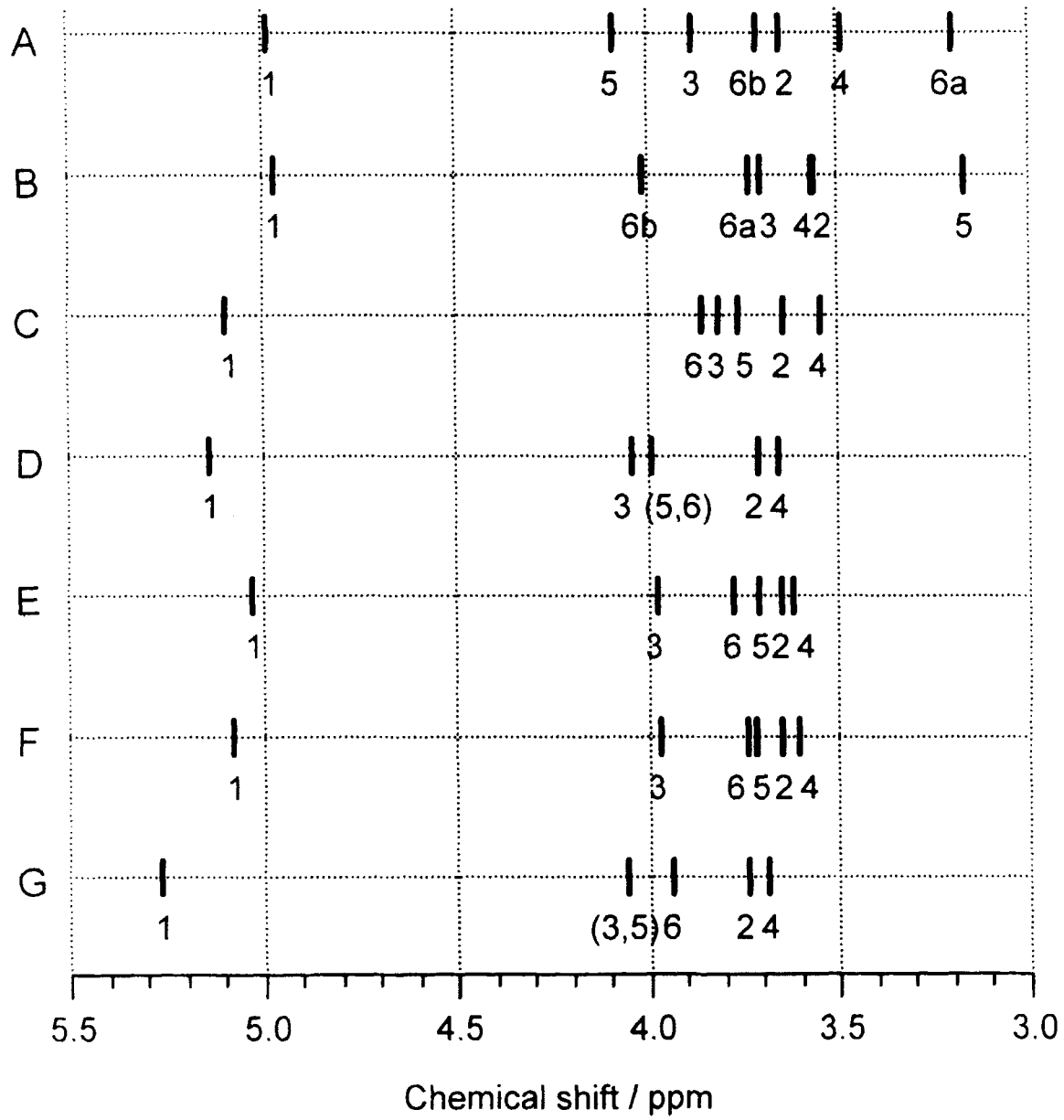

FIGURE 7 Full assignment of the glucose protons in mono[6-(*m*-toluidino)- 6-deoxy]-β-cyclodextrin (2) by NMR spectroscopy

each host examined, the plot of the change in Δε (ΔΔε) at 296 nm as a function of $[G]_0$ gave an excellent fit, verifying the validity of the 1:1 complex stoichiometry. Figure 10 illustrates representative results of the curve fitting for the complexation of 1-pentanol with **1** and **2**. The K_S values and the Gibbs free energy change of complex formation ($-\Delta G°$) are listed in Table II, along with the corresponding values reported for native β-cyclodextrin [31].

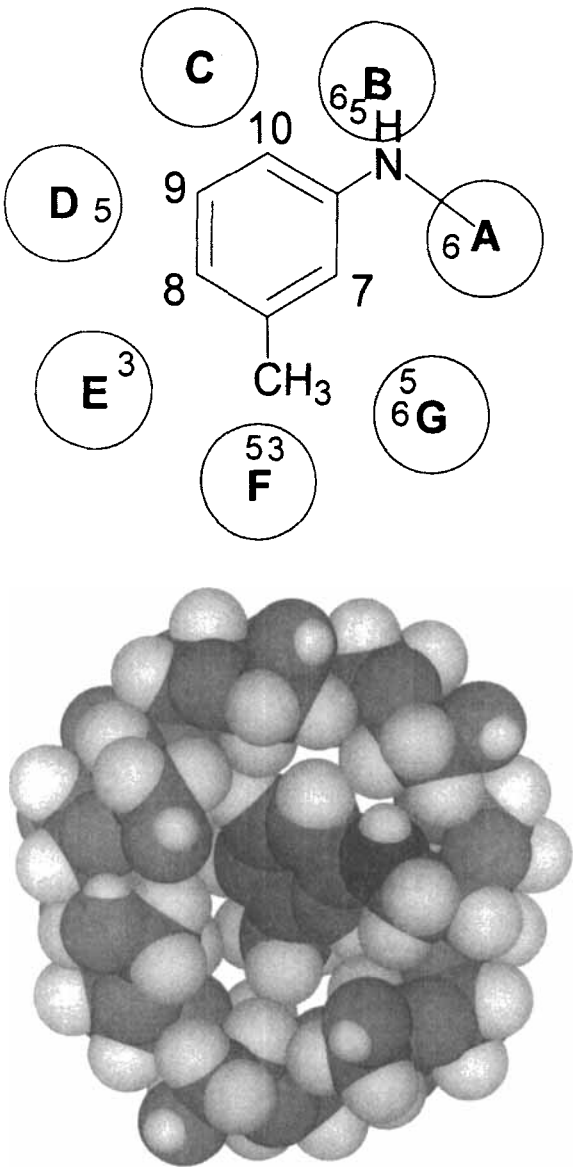

FIGURE 8 (a) The correlation between the protons of *m*-toluidino group and glucose protons; (b) Space-filling model of the self-inclusion form of **2**

As has been demonstrated in several cases [14, 15, 30, 32], an important feature of the inclusion complexation by cyclodextrin is that several weak forces cooperatively govern the stability of the host-guest complex. Therefore, it is crucial to elucidate the major force(s) which drive the formation of cyclodextrin complexes. As can be seen from Table II, hosts **1** and **2** are obviously better binders for 1-alkanols and cycloalkanols than native β-cyclodextrin, and **1** gives roughly 1.5 times higher K_S for most guests than those obtained with **2**, although 3-methyl-1-butanol is bound more strongly by **2** rather than **1**. For both hosts, the $-\Delta G°$ value increases linearly with increasing number of carbon atom (N_C) in the alkanol guest, affording the unit increment per methylene ($-d\Delta G°/dN_C$) of 2.5 kJ/mol for **1** and 2.1 kJ/mol for **2**. These values are somewhat smaller than that (3.1 kJ/mol) obtained with native β-cyclodextrin [31]. This suggests that the introduced substituent and/or the deformed cyclodextrin ring diminish the potential gains which could be obtained from the full van der Waals and hydrophobic interactions with extended methylene chain. However, the fact that the $-\Delta G°$ value steadily increases with increasing N_C means that the van der Waals and hydrophobic interactions are still the major driving forces for the inclusion complexation of modified β-cyclodextrins **1** and **2** with the alcohol guests.

The shape and rigidity of guest molecule also play an important role. Possessing the same number of carbons and the cyclic, bicyclic, and tricyclic skeletons, the three C_{10} alcohols, i.e. menthol, borneol, and adamantanol, display entirely different complexation behavior upon inclusion by **2**. It is well known that β-cyclodextrin, carrying a hydrophobic cavity of 262 Å^3, forms the most stable inclusion complexes with several adamantane derivatives [7, 15, 33]. Hence, it is not unexpected that 1- and 2-adamantanols afford the largest K_S in the order of 10^5, while (+)- and (-)-menthols give the lowest K_S around 3000. In this context, the fairly large K_S for (+)- and (-)-borneols ($6–10 \times 10^4$ M^{-1}) should rather be noted and may be attributed to the rigid, ball-like shape.

TABLE II Stability Constant (K_S) and Gibbs Free Energy Change (-ΔG°) for Inclusion Complexation of β-Cyclodextrin, Mono(6-anilino-6-deoxy)-β-cyclodextrin (**1**), and Mono[6-(*m*-toluidino)-6-deoxy]-β-Cyclodextrin (**2**) with Some Aliphatic Alcohols in Phosphate Buffer Solution (pH 7.20, 0.1 mol L^{-1}) at 25 °C

host	*guest*	K_S	*log* K_S	-ΔG°/*kJ mol*$^{-1}$	α
β-cyclo dextrin[a]	1-butanol	16	1.20	6.9	
	1-pentanol	63	1.81	10.3	
	1-hexanol	219	2.34	13.3	
	Cyclopentanol	174	2.24	12.8	
	Cyclohexanol	692	2.84	16.2	
1	1-butanol	84	1.92	11.0	1570
	1-pentanol	196	2.29	13.1	1570
	3-methyl-1-butanol	232	2.37	13.5	2960
	1-hexanol	454	2.66	15.2	1860
	1-heptanol	1870	3.27	18.7	1690
	Cyclopentanol	525	2.72	15.5	2840
	Cyclohexanol	1978	3.30	18.8	2810
2	1-butanol	67	1.83	10.4	4670
	1-pentanol	139	2.14	12.2	5280
	3-methyl-1-butanol	309	2.49	14.2	5610
	1-hexanol	315	2.50	14.3	4790
	1 -heptanol	821	2.91	16.6	4770
	Cyclopentanol	431	2.63	15.0	6640
	Cyclohexanol	1253	3.10	17.7	16000
	1-adamantanol	116330	5.07	28.9	84960
	2-adamantanol	194400	5.29	30.2	40150
	(*S*)-(+)-2-octanol	903	2.96	16.9	5370
	(*R*)-(-)-2-octanol	955	2.98	17.0	4670
	(+)-menthol	3420	3.53	20.2	33470
	(-)-menthol	3213	3.51	20.0	35080
	(+)-borneol	96800	4.99	28.5	42960
	(-)-borneol	62640	4.80	27.4	42090

a. Data taken from ref. 31.

The complexation behavior can also be discussed in terms of the sensitivity factor α (defined in eq 3), which is a quantitative measure of conformational changes induced by guest inclusion. Carrying a more hydrophobic substituent, toluidino-β-cyclodextrin **2** binds most of the guests examined more strongly with much larger α values (by a factor of 2–5) than anilino-β-cyclodextrin **1** does. Taking into account the substituent's original conformation determined above by the 2-D NMR studies, this result is reasonably accounted for by assuming that the

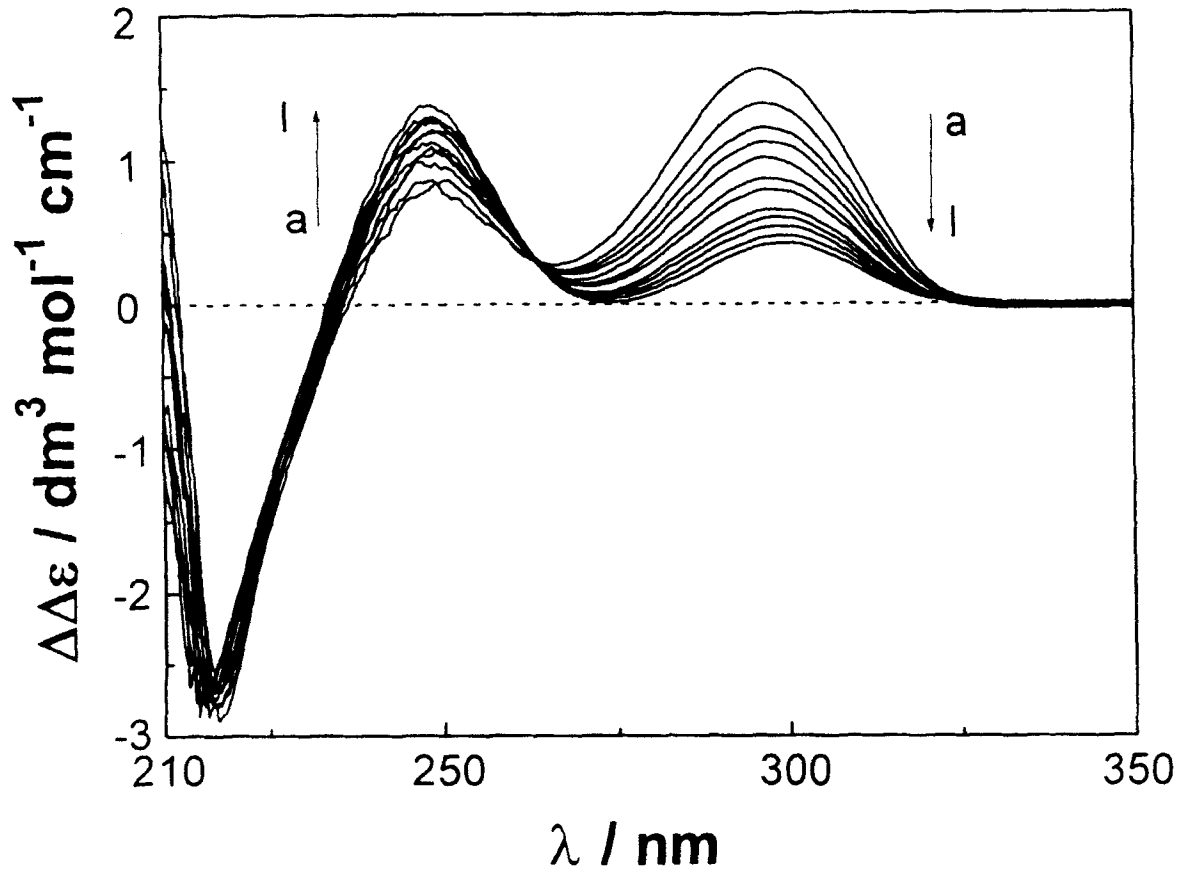

FIGURE 9 CD spectral changes of mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** (0.25 mM) in phosphate buffer solution at pH 7.20 upon addition of 1-pentanol of 0, 1.5, 3.1, 4.6, 6.1, 9.2, 12.3, 18.4, 24.5, 30.6, 45.9, and 61.3 mM (from a to l)

toluidino substituent in **2** is more deeply self-included in the cavity than the anilino in **1**, and therefore it suffers more drastic conformational changes upon guest inclusion. Upon inclusion by modified cyclodextrins, the guest should compete with the substituent for the cavity. Nonetheless, the binding constants obtained for the modified β-cyclodextrins are fairly larger than those for native β-cyclodextrin (Table II) [31, 34, 35], which would be rationalized by the induced fit process driven by the exclusion of the substituent and the concurrent recovery of the deformed cyclodextrin ring.

Chiral recognition is another point of interest in this study. In our previous studies, we have investigated the chiral recognition behavior of native and modified cyclodextrins toward chiral alcohols and amino acids, and found that native and most modified cyclodextrins prefer L-amino acids, although the enantioselectivities are not very high [16, 17, 36]. In the present case, **2** prefers the (+)-isomer of borneol with a moderate enantioselectivity of 1.55 for (+)/(-)-borneol. In contrast, the enantiomeric pairs of 2-octanol and menthol show poor chiral discrimination ($|K^-/K^+|$ or $|K^+/K^-| = 1.06$), as was the case with most cyclodextrin derivatives [30]. These results may indicate that the substituents introduced affect the chiral microenvironment to some extent, acting as a spacer to fix the

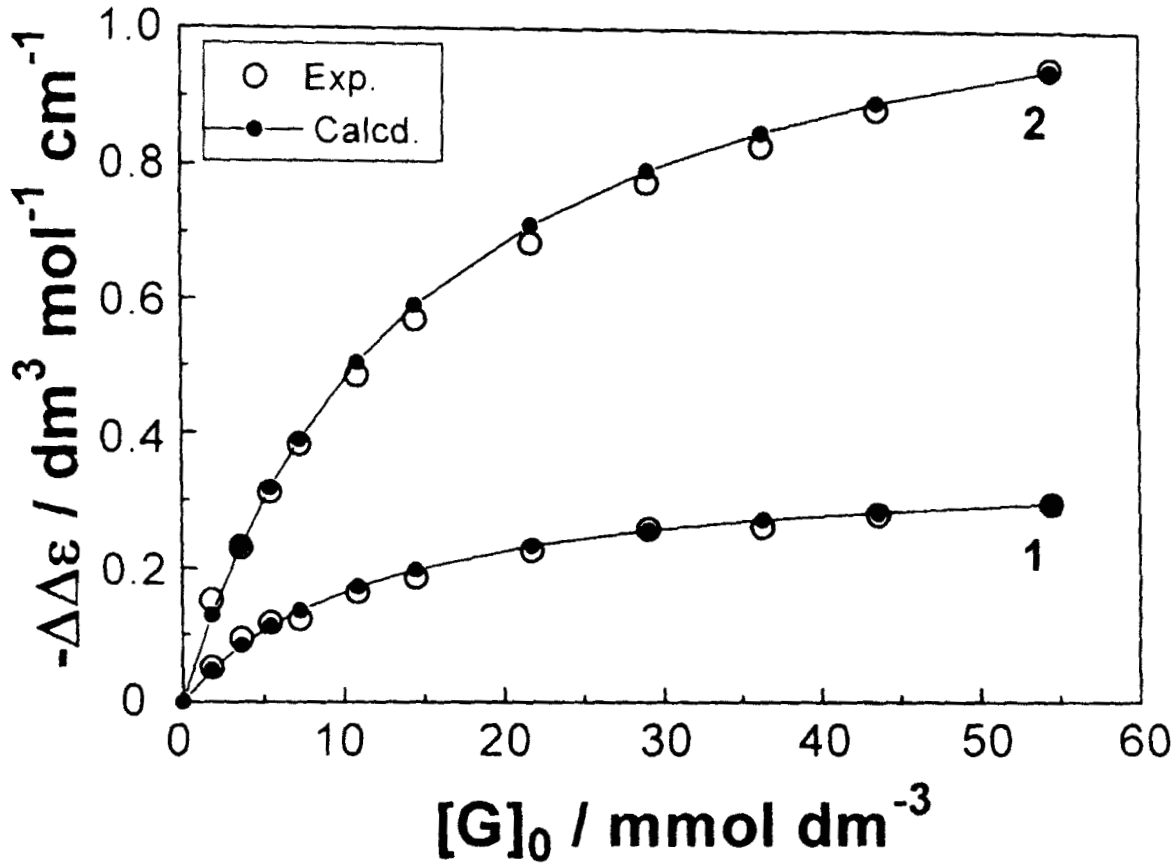

FIGURE 10 Curve-fitting analyses for complexations of 1-pentanol with hosts **1** and **2**

included guest, and contribute to enhance the chiral recognition especially for rigid guests such as borneol.

EXPERIMENTAL SECTION

General Procedures

Mass spectrum was obtained on a JEOL JMS-DX-303 instrument. Combustion analyses were performed on a Perkin-Elmer-240 instrument. IR and UV spectra were obtained on a Nicolet FT-IR 5DX and Shimadzu UV-2401PC spectrometer, respectively. Circular dichroism spectra were measured in a conventional quartz cell (light path 1 cm) on a JASCO J-720S spectropolarimeter equipped with a PTC-328WI temperature controller. Fluorescence spectra were recorded in a quartz cell maintained at 25 + 0.1 °C on a JASCO FP-750 spectrofluorometer.

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 the pulsed light source, and the excitation light was made monochromatic by a 10 cm monochrometer. 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 10000 were collected in each measurement. The accumulated signals were then processed and the lifetime determined by deconvolution with nonlinear least squares fit.

1-D and 2-D NMR spectra of **2** (8 mM) were recorded in D_2O at 35 °C on Bruker AM200 and JEOL EX-400 spectrometers, respectively. Two-dimensional total correlation spectroscopy (TOCSY) measurements were repeated with the mixing times of 70, 120, 150, and 160 ms. Rotating frame nuclear Overhauser effect spectroscopy (ROESY) measurements were performed with the mixing times of 400 and 450 ms.

Materials

All guest alcohols were commercially available and used without further purification. *N*-Methylaniline which was distilled from potassium hydroxide under a reduced pressure prior to use. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 M phosphate buffer solution of pH 7.2 for the spectral measurements. β-Cyclodextrin of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried *in vacuo* at 95 °C for 24 h prior to use. *N,N*-Dimethylformamide (DMF) was dried over calcium hydride for two days and then distilled under a reduced pressure prior to use. Mono(6-anilino-6-deoxy)-β-cyclodextrin **(1)** and mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin **(2)** were prepared by the reaction of mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin (6-OTs-β-CD) [37] with freshly distilled aniline and *m*-toluidine in DMF, respectively, basically according to the procedure described previously [17]. A typical procedure for the synthesis of **2** is described below.

Mono[6-(*m*-toluidino)-6-deoxy]-β-cyclodextrin (2)

Mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin (2.6 g, 2 mmol) and *m*-toluidine (10 mL) were reacted for 3 days in DMF (20 mL) at 85 °C with stirring under nitrogen atmosphere. The reaction mixture was evaporated *in vacuo* at 40 °C to dryness. The residue was dissolved in a minimum amount of hot water, and the resulting solution was added to acetone (200 mL) with stirring to give a gray precipitate. The above procedure was repeated twice. After drying, the combined product was purified by chromatography over Sephadex-G25 to give a crude sample of **2** in 50% yield. The crude sample was purified by preparative HPLC on a column of YMC-Pack Pro C18 (50 mm × 20 mm i.d.) by eluting with a solvent mixture of water and acetonitrile (90:10 by volume). UV (H_2O) λ_{max} (ε) 246.4 (12860), 291.4 nm (2381 M^{-1} cm^{-1}). MS (FAB/NaI) *m/z* 1224 (M^+-5H_2O). IR (KBr) ν 3364, 2909, 1716, 1625, 1542, 1412, 1361, 1338, 1307, 1233, 1147, 1071, 1018, 933, 841, 749 cm^{-1}. ^1H NMR (DMSO-d_6, 200 MHz, TMS, ppm) δ 2.1 (s, 3H), 3.1–3.8 (m, 42H), 4.3–4.6 (m, 6H), 4.6–4.9 (m, 7H), 5.0–5.2 (m, 1H), 5.6–5.9 (m, 14H), 6.2–6.4 (m, 3H,), 6.8–6.9 (t, 1H). $C_{49}H_{77}O_{34}N{\cdot}5H_2O$ (1314.2): calcd C 44.78, H 6.67, N 1.07; found C 44.63, H 6.75, N: 1.01.

SUPPLEMENTARY MATERIAL AVAILABLE

^1H NMR spectra of compound **2** (22 pages).

Acknowledgements

This work was supported by the National Outstanding Youth Fund (Grant No. 29625203), Natural Science Foundation of China (Grant No. 29992590–8 and 29972029), Trans-Century Qualified Personal Fund (Sun-Light Plan) of Tianjin Municipality, and Tianjin Natural Science Fund (Grant No. 993601311), which are gratefully acknowledged. We are grateful to Ms. Makiko Niki at the Inoue Photochirogenesis Project for her technical assistance.

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