Cooperative Multipoint Recognition of Organic Dyes by Bis(β-cyclodextrin)s with 2,2′-Bipyridine-4,4′-dicarboxy Tethers**

Yu Liu,*[a] Yong Chen,[a] Bin Li,[a] Takehiko Wada,[b] and Yoshihisa Inoue*[b]

Abstract: A series of novel 6,6′-bis(β-cyclodextrin)s linked by 2,2′-bipyridine-4,4′-dicarboxy tethers; that is, 2,2′-bipyridine-4,4′-dicarboxy-bridged bis(6-*O*-β-cyclodextrin) (**2**) and *N*,*N*′-bis(2-aminoethyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged (**3**), *N*,*N*′-bis(5-amino-3-azapentyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged (**4**) and *N*,*N*′-bis(8-amino-3,6-diazaoctyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-cyclodextrin) (**5**), has been synthesized as cooperative multipoint-recognition receptor models. The inclusion complexation behavior of **2**–**5** with organic dyes; that is, ammonium 8-anilino-1-naphthalenesulfonate, Brilliant Green, Methyl Orange, Acridine Red, and Rhodamine B, has been investigated in aqueous phosphate buffer solutions (pH 7.20) at 25 °C by means of ultraviolet, fluorescence, and circular dichroism spectrometry as well as by fluorescence lifetime measurements. The spectral titrations gave the complex stability constants (K_S) and Gibbs' free energy changes (ΔG°) for the inclusion complexation of **2**–**5** with the organic dyes and other thermodynamic parameters (ΔH° and ΔS°) for the inclusion complexation of **2**–**4** with the fluorescent dyes Acridine Red and Rhodamine B. Bis(β-cyclodextrin)s **2**–**5** displayed higher binding abilities toward most of the examined dye molecules than native β-cyclodextrin **1**; this is discussed from the viewpoints of the size/shape-fit concept, the induced-fit interaction, and cooperative, multipoint recognition by the bridging chain and the dual hydrophobic cavities. Thermodynamically, the inclusion complexation of **2**–**4** with Acridine Red is totally enthalpy driven with a negative or minor positive entropic contribution, but the inclusion complexation with Rhodamine B is mainly entropy-driven with a mostly positive, but occasionally negative, enthalpic contribution; in some cases this determines the complex stability.

Keywords: cyclodextrins · dyes/pigments · molecular recognition · supramolecular chemistry · thermodynamics

Introduction

It is well known that bridged bis(β-cyclodextrin)s with simple tethers show significantly enhanced molecular binding abilities toward a variety of guests in comparison with native β-cyclodextrin,[1–4] and therefore they provide an excellent model system for mimicking the substrate-specific interaction of enzymes.[5, 6] Indeed, with bis(β-cyclodextrin)s the orientation and separation of the two cyclodextrin moieties in a single molecule can be adjusted by altering the conformation of the bridging chain; this gives rise to the most effectively stabilized "sandwich" complex in aqueous solution. Hence, a number of dimeric β-cyclodextrins with considerable structural diversity have recently been synthesized in order to elucidate the recognition mechanism as controlled by the simultaneous operation of the available weak interactions and to gain insights into the factors governing the inclusion complexation behavior of bis(β-cyclodextrin)s.[7–12] Thermodynamic studies on the molecular recognition of organic and inorganic guests have concentrated mostly on native[13, 14] and

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Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/chemistry/or from the author. ICD spectrum of Methyl Orange (MO) in the absence and presence of β-cyclodextrin or bis(β-cyclodextrin)s; the method for calculating the thermodynamic parameters and stability constants (K_s) for 1:1 inclusion complexation of Acridine Red (AR) and Rhodamine B (RB) with β-cyclodextrin and bis(β-cyclodextrin)s **2**–**4**; typical plots of log K_S versus $1/T$ for the fluorometric titrations of **2**, **3**, and **4** with Acridine Red.

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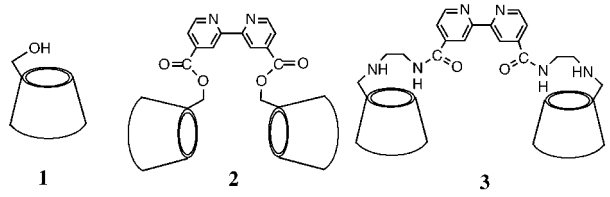
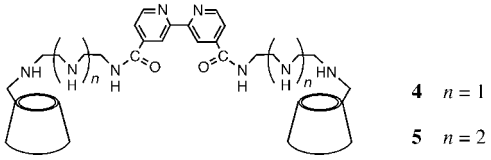
modified mono(β-cyclodextrin)s,[15] with only a limited amount of effort hitherto devoted to the molecular recognition thermodynamics of β-cyclodextrin dimers.[1]

We have reported the inclusion complexation thermodynamics of modified mono(β-cyclodextrin)s with several guest families such as amino acids,[16, 17] naphthalene derivatives, and other aromatic compounds.[18, 19] More recently, we have demonstrated that several organoselenium-bridged bis(β-cyclodextrin)s give much higher stability constants for some fluorescent dyes than those obtained with native β-cyclodextrin, and also that the transition metal complexes of these bis(β-cyclodextrin)s bind the guests more strongly.[20] In the present study, we wish to report the inclusion complexation behavior of a series of newly synthesized bis(β-cyclodextrin)s linked by 2,2′-bipyridine-4,4′-dicarboxy tethers with some representative organic dyes of different structures, and discuss their complexation thermodynamics with guest dyes such as Acridine Red and Rhodamine B. It is of particular interest to investigate thermodynamically the molecular recognition behavior of bis(β-cyclodextrin)s toward representative guests from the viewpoint of the size/shape–fit concept, and the role of the cooperative weak interactions working between the host and the guest.

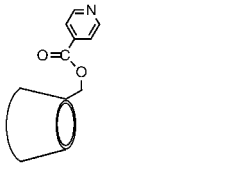


1 **2** **3**



4 $n = 1$

5 $n = 2$



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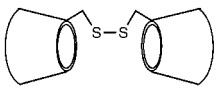

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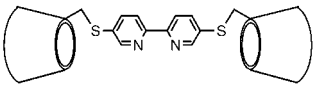

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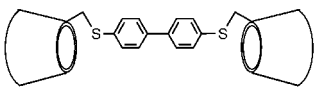

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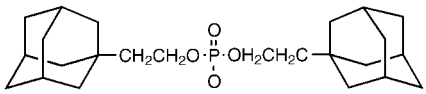

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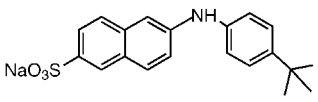

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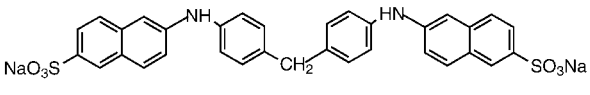

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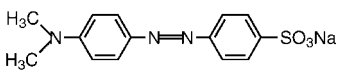
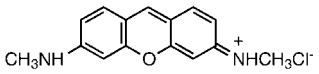
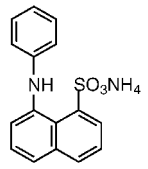


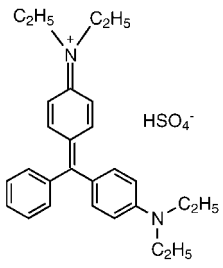
Methyl Orange



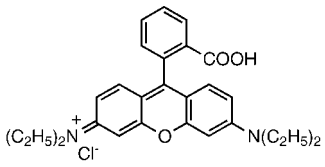
Acridine Red



ANS



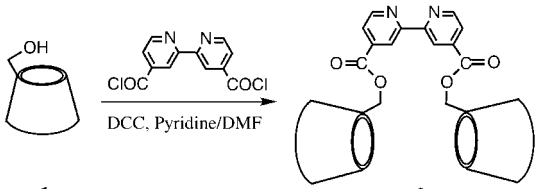
Brilliant Green



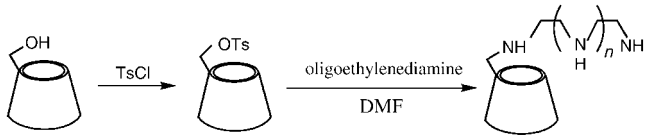
Rhodamine B

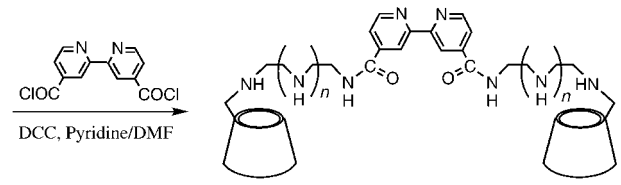
Results and Discussion

Synthesis: As illustrated in Scheme 1, the bipyridinedicarboxylate-bridged bis(β-cyclodextrin) **2** was synthesized in moderate yield by the reaction of 2,2′-bipyridine-4,4′-dicarboxylic



1 **2**





3 $n=0$

4 $n=1$

5 $n=2$

Scheme 1. Syntheses of bis(β-cyclodextrin)s **2**–**5**.

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dichloride and β-cyclodextrin, while bis(β-cyclodextrin)s **3**–**5** were synthesized from the corresponding mono[6-oligo(ethylenediamino)-6-deoxy]-β-cyclodextrin. In the latter route, mono(6-*O*-*p*-toluenesulfonyl)-β-cyclodextrin, prepared by the reaction of β-cyclodextrin with *p*-toluenesulfonyl chloride in aqueous alkaline solution,[21] was converted to mono-[6-oligo(ethylenediamino)-6-deoxy]-β-cyclodextrins in 72% yield by heating it in an excess amount of oligoethylenediamine at 70 °C for 7 h.[22] Subsequent reactions of these precursors in DMF with 2,2′-bipyridine-4,4′-dicarboxylic dichloride in dry pyridine containing dicyclohexylcarbodiimide gave the corresponding bis(β-cyclodextrin)s **3**–**5**. Caution should be exercised to keep the mixture anhydrous and at low temperature during the reaction, particularly at the initial stage, for a smooth and clean reaction without undesirable product(s).

Circular dichroism spectra: In order to obtain information about the original conformation of the β-cyclodextrin dimers with a chromophoric bipyridinedicarboxy tether in dilute aqueous solution, the circular dichroism (CD) spectra of **2**–**5** were taken at a concentration of 1×10^{-4} mol dm^{-3} in aqueous buffer solution at pH 7.20. As can be seen from Figure 1, all of the bis(β-cyclodextrin)s display weak, but

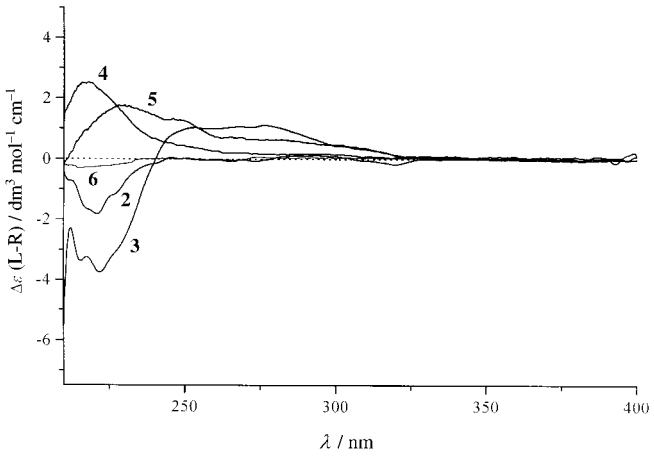

Figure 1. Circular dichroism spectra of bis(β-cyclodextrin)s **2**–**6** (1×10^{-4} mol dm^{-3}) in aqueous buffer solution (pH 7.20) at 25 °C.

appreciable, positive Cotton-effect peaks at 270-300 nm for the ^1L_b transition of the bipyridine chromophore; the $\Delta\varepsilon_{max}$ values are $+0.126$ dm^3 mol^{-1} cm^{-1} at 294 nm for **2**, $+1.112$ dm^3 mol^{-1} cm^{-1} at 276 nm for **3**, $+0.18$ dm^3 mol^{-1} cm^{-1} at 295 nm for **4**, and $+0.63$ dm^3 mol^{-1} cm^{-1} at 281 nm for **5**. On the other hand, the sign, shape, and magnitude of the induced circular dichroism (ICD) observed for the ^1L_a transition (210–230 nm) of the bipyridine chromophore in **2**–**5** are distinctly different from each other; the $\Delta\varepsilon$ values are -1.83 dm^3 mol^{-1} cm^{-1} at 221 nm for **2**, -3.76 dm^3 mol^{-1} cm^{-1} at 222 nm for **3**, $+2.52$ dm^3 mol^{-1} cm^{-1} at 218 nm for **4**, and $+1.52$ dm^3 mol^{-1} cm^{-1} at 229 nm for **5**. In comparison, the ICD spectra of the reference compound, mono(6-*O*-4-pyridinecarboxy)-β-cyclodextrin (**6**) showed a negative Cotton-effect peak for the ^1L_a band at 215 nm ($\Delta\varepsilon = -0.31$ dm^3 mol^{-1} cm^{-1}) and a small positive peak for the ^1L_b band at 275 nm ($\Delta\varepsilon = +0.067$ dm^3 mol^{-1} cm^{-1}); this can be attributed to the pyridine moiety's being shallowly included in the hydrophobic cavity of β-cyclodextrin.[23] From a comparison of the weak ICD spectra obtained for bis(β-cyclodextrin)s **2**–**5** with that of the reference **6**, we deduce that the bipyridine chromophore in **4** and **5** is not embedded in the cavity, while the chromophore in **2** and **3** is either perched on the edge of the cavity or shallowly penetrating into the cavity. Hence, the ^1L_a transition of **2** and **3** is deduced to be located in the negative region of the sector rule proposed by Kajtar,[24] Harata,[25] and Kodaka et al.,[26] while the weak, positive Cotton effect observed for the ^1L_b band of **4** and **5** would be better analyzed as an ordinary CD induced by the two distant chiral cyclodextrin moieties, although a shallow binding or perching model is not rigorously ruled out.

Fluorescence lifetime: Besides the information obtained from the ICD spectra, the fluorescence lifetime measurements provided us with more direct information about the microenvironmental changes around the fluorophore interacting with β-cyclodextrin dimers. In the present study, we performed nanosecond time-correlated fluorescence measurements with 8-anilino-1-naphthalenesulfonate (ANS) in an aqueous buffer solution (pH 7.20) in the presence and absence of bis(β-cyclodextrin)s **2**–**5** in order to assess the microenvironmental polarity around the included ANS.

Since the rates of complexation/decomplexation are much slower than that of the fluorescence decay, the decay profile of the fluorescence intensity ($F(t)$) can be described as the sum of the multiple, unimolecular decays for all the fluorescing species present in the solution [Equation (1)]:

$$F(t) = \Sigma A_i \exp(-t/\tau_i) \quad (i = 1,2,\ldots) \tag{1}$$

in which A_i and τ_i represent the initial abundance and lifetime of the *i*th fluorescing species. The fluorescence decay curve for ANS was perfectly fitted to a single exponential function in the absence of the host, and to a linear combination of two exponential functions in the presence of the β-cyclodextrin dimers. The fluorescence lifetimes (τ) and relative quantum yields (Φ) obtained with ANS in the presence and absence of bis(β-cyclodextrin)s are summarized in Table 1. The longer lifetimes (τ_L) in the presence of bis(β-cyclodextrin)s clearly indicate that the environment around ANS molecule is more hydrophobic in the cavity than in the bulk water.[12, 20, 27] Furthermore, the two-component decay

Table 1. Short and long fluorescence lifetimes (τ_S and τ_L) and relative quantum yields (Φ_S and Φ_L) of 8-anilino-1-naphthalenesulfonate (ANS) in the presence and absence of native β-cyclodextrin **1** and bis(β-cyclodextrin)s **2**–**5** in aqueous buffer solution (pH 7.20) at 25 °C.

ANS [μM]	Host	[Host]/[ANS]	τ_S [ns]	Φ_S [%]	τ_L [ns]	Φ_L [%]
500	none		0.4	100		
250	**1**	10	1.5	67.6	3.2	32.4
10	**2**	20	0.5	89.3	9.5	10.7
10	**3**	20	0.8	85.7	8.8	14.3
10	**4**	20	1.5	64.7	10.4	35.3
10	**5**	20	1.0	76.6	9.7	23.4

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indicates that the ANS molecule is located in two different environments, one of which is polar and the other nonpolar. Judging from the two different lifetimes (τ_S and τ_L) observed for ANS in the presence of bis(β-cyclodextrin)s, we infer that the short- and long-lived fluorescing species are assigned to the free and included ANS, respectively. It is noted that although the short lifetimes (τ_S) are essentially the same (1.0 ± 0.4 ns) for mono- and bis(β-cyclodextrin)s, distinctly different long lifetimes (τ_L) are obtained for mono- (3.2 ns) and bis(β-cyclodextrin)s (8.8–10.4 ns); this reflects the critical difference in microenvironmental hydrophobicity of the host cavities, or the cavity assembly formed upon complexation. It is also interesting to note that all of the bis(β-cyclodextrin)s gave only slightly different τ_L's (9.6 ± 0.6 ns) regardless of the tether length; this probably indicates the induced formation of a cavity assembly, which presents a larger more hydrophobic pseudocavity, upon cooperative binding of the large guests with two cyclodextrin moieties.

Spectroscopic titrations: For a more qualitative assessment of the inclusion complexation behavior of bis(β-cyclodextrin)s **2**–**5**, spectrophotometric experiments with Brilliant Green (BG), Methyl Orange (MO), Acridine Red (AR), and Rhodamine B (RB) were performed at 25 °C in aqueous phosphate buffer solution (pH 7.20) by using absorption, fluorescence, and/or circular dichroism spectroscopy. As exemplified in Figure 2, the fluorescence intensity of AR was significantly enhanced upon stepwise addition of bis(β-cyclodextrin) **4**.

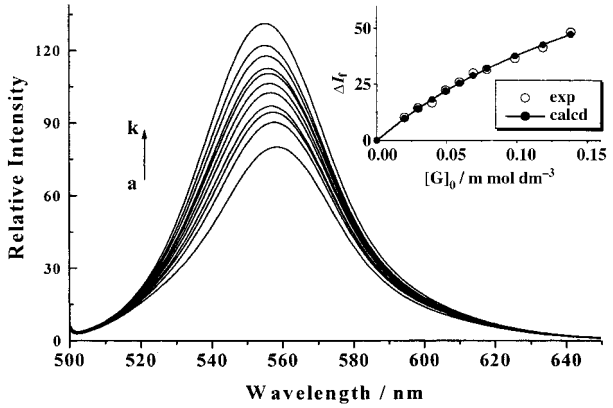

Figure 2. Fluorescence spectral changes of Acridine Red (4.7×10^{-6} mol dm^{-3}) and, inset: the nonlinear least-squares analysis of the differential intensity (ΔI_f) to calculate the complex stability constant (K_S) upon addition of from 0 (a) to 145×10^{-6} mol dm^{-3} (k) bis(β-cyclodextrin) **4** in aqueous buffer solution (pH 7.20). Ex = 490 nm, Em_0 = 558 nm (Em_0 = the original Em of Acridine Red, at which wavelength the ΔI_f is measured), width = 5 nm.

For stoichiometric 1:1 complexation, where the two β-cyclodextrin moieties in bis(β-cyclodextrin) are treated as a single unit, the inclusion complexation of guest (G) with host (H) is expressed by Equation (2).

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

The complex stability constant (K_S)[28] can be calculated from the analysis of the sequential changes in fluorescence intensity (ΔI_f) at varying host concentrations by using a nonlinear least-squares method according to the curve fitting Equation (3).[13]

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

Here $[G]_0$ and $[H]_0$ refer to the initial concentrations of the organic dye and bis(β-cyclodextrin), respectively, and α is the proportionality coefficient, which may be taken as a sensitivity factor for the fluorescence change upon complexation. For each host examined, the ΔI_f values were plotted as a function of $[G]_0$ to give excellent fits; this validated the 1:1 stoichiometry assumed above. In repeated measurements, the K_S values were reproducible within an error of $\pm 5\%$. The K_S values obtained are listed in Table 2, along with the free energy change of complex formation ($-\Delta G^\circ$).

Table 2. Complex stability constant (K_S) and Gibbs' free energy changes ($-\Delta G^\circ$) for 1:1 inclusion complexation of organic dyes with β-cyclodextrin **1** and bis(β-cyclodextrin)s **2**–**5** in aqueous buffer solution (pH 7.20) at 25 °C.

Host	Guest	K_S [M^{-1}]	log K_S	$-\Delta G^\circ$ [kJ mol^{-1}]	Method[a]
1	BG	2190	3.34	19.1	UVV
	MO	3560	3.55	20.3	CD
	AR	2630	3.42	19.5	FL
	RB	4240	3.63	20.7	FL
2	BG	3880	3.59	20.5	UVV
	MO	22700	4.36	24.9	CD
	AR	29400	4.47	25.5	FL
	RB	26700	4.43	25.2	FL
3	BG	8140	3.91	22.3	UVV
	MO	5330	3.73	21.3	CD
	AR	2680	3.43	19.6	FL
	RB	9830	3.99	22.8	FL
4	BG	6850	3.84	21.9	UVV
	MO	13300	4.12	23.5	CD
	AR	4130	3.62	20.6	FL
	RB	4700	3.67	21.0	FL
5	BG	3890	3.59	20.5	UVV
	MO	41600	4.62	26.4	CD
	AR	14300	4.15	23.7	FL
	RB	13100	4.12	23.5	FL

[a] UVV: Ultraviolet/Visible, CD: circular dichroism, FL: fluorescence.

It is interesting to note that RB displays the opposite fluorescence behavior upon inclusion complexation by the short-tethered and long-tethered bis(β-cyclodextrin)s. The fluorescence intensity of RB was significantly enhanced upon addition of the short-tethered **2** or **3**, but was decreased by adding the long-tethered **4** or **5**. The enhanced fluorescence upon complexation with **2** or **3** is a natural consequence of the inclusion of the fluorescent acid form of RB into the pseudocavity. However, the decreased fluorescence intensity upon inclusion of RB by **4** or **5** is somewhat unexpected, although the fluorescence intensity of RB is known to decrease upon inclusion with native β-cyclodextrin.[29] It is likely that the linker groups of **4** and **5** are too long to form well-defined pseudocavities; this in turn leads to a weaker cooperative size-matching complexation with RB. In addition,

the polyamine chains of **4** and **5** would predominantly interact with the lactone form of included RB through electrostatic, hydrogen-bonding, and/or electron transfer interactions, which are inevitably accompanied by the quenching of RB fluorescence.

Molecular binding ability and selectivity: It is well documented that, among the several possible weak interactions contributing to the complexation of organic guests with cyclodextrins, the most crucial contributions are made by the van der Waals and hydrophobic interactions, both of which are related to the size/shape matching between the guest and the host cavity. Other intermolecular interactions, such as hydrogen bonding, can also contribute to the inclusion complexation behavior of cyclodextrins to some extent. In this context, it is reasonable that the bis(β-cyclodextrin)s give more or less higher K_S's that critically depend on the guest shape. Even the highest molecular selectivity among the four guests employed is as low as 1.9 for native β-cyclodextrin **1** toward the RB/BG pair, but is much enhanced to 7.6 for **2** toward the AR/BG pair, 3.7 for **3** toward the RB/AR pair, 3.2 for **4** toward the MO/AR pair, and 10.7 for **5** toward the MO/BG pair. It is noteworthy that bis(β-cyclodextrin)s not only promote the guest binding in most cases, but also enhance the guest selectivity through multipoint recognition and fine tuning of the orientation of both the ditopic host and tether conformations to the structure and functionality of the guest.

The importance of guest structure is more clearly demonstrated by comparing the bis(β-cyclodextrin) effect for each guest. The bis(β-cyclodextrin) host that gives the highest enhancement for each guest dye (with the observed enhancement factors shown in the parentheses) is: **3** ($\times$3.7) for BG, **5** ($\times$11.7) for MO, **2** ($\times$11.2) for AR, and **2** ($\times$6.3) for RB. From a comparison of the enhancement factors, we may conclude that linear guests such MO and AR, rather than the triangular BG or T-shaped RB, are able to fully enjoy the cooperative multipoint binding of bis(β-cyclodextrin)s with the selectivity exhibiting more than 10-fold enhancement.

It is also interesting to compare the "host selectivity" sequence obtained for each guest dye. The K_S value for the complexation of each dye by native **1** and bis(β-cyclodextrin)s **2**–**5** increases in the order:

BG: $\mathbf{1} < \mathbf{2} \leq \mathbf{5} < \mathbf{4} < \mathbf{3}$
MO: $\mathbf{1} < \mathbf{3} < \mathbf{4} < \mathbf{2} < \mathbf{5}$
AR: $\mathbf{1} \leq \mathbf{3} < \mathbf{4} < \mathbf{5} < \mathbf{2}$
RB: $\mathbf{1} < \mathbf{4} < \mathbf{3} < \mathbf{5} < \mathbf{2}$

The triangular guest BG is better bound by **3** and **4**, which have moderate-length tethers, than by **2** and **5**; whereas the linear or T-shaped guests MO, AR and RB are better bound by the short-tethered **2** or the long-tethered **5**. One of the possible reasons for these contrasting host-selectivity sequences may be the guest shape, since the distance between the two cavities in host **2** is too short to appropriately accommodate the triangular BG, but is better fitted to the linear or T-shaped guests. On the other hand, the strong binding of the linear and T-shaped guests by the long-tethered **5** is not anticipated from the entropic point of view, since sandwich complexation by long-tethered bis(β-cyclodextrin)s inherently leads to a large decrease of conformational freedom. However, this unfavorable conformational freezing may be compensated for by extensive desolvation of the oligo(ethylenediamino) tether group upon complexation; this is supported by the complex thermodynamic study described below. In this context, the electrostatic and/or hydrogen-bonding interactions between the long-tethered **5** and the charged guests with multiple hydrogen-bonding groups may be jointly responsible for the strong complexation of **5** with the linear and T-shaped guests.

Another interesting point is that, although AR and RB, which contain analogous tricyclic fragments, display close K_s values for **2**, **4**, and **5**; bis(β-cyclodextrin) **3** shows the lowest K_s value for AR and the highest molecular selectivity for the RB/AR pair among the dimeric hosts examined. This may be attributed to the fact that the tether length and the relative rigidity of the bridged chain in **3** are unsuitable for the binding of the linear guest; this will inevitably result in low stability constants for linear guests with **3**. However, the formation of a sandwich complex between RB and host **3** supports an additional binding effect that compensates for the unfavorable association of the tricyclic fragment in RB with **3**; this consequently leads to stronger binding of RB with **3**. In the complexation of these guests with dual hosts, the two cyclodextrin moieties of **4** and **5** can manage to get closer to each other than those of **3** through the adjustment of the more flexible, longer tether chain. This would rationalize the comparable K_s's for AR and RB upon complexation with **4** and **5**, and the pronounced discrimination of these two guests by host **3**.

Thermodynamic parameters: In order to quantitatively investigate the cooperative binding behavior of bis(β-cyclodextrin)s from the thermodynamic viewpoint, fluorometric titrations of AR and RB with native β-cyclodextrin **1** and bis(β-cyclodextrin)s **2**–**4** were performed at several temperatures ranging from 25.0 to 40.0 °C to give the complex stability constants K_S at different temperatures. The thermodynamic parameters ΔG°, ΔH°, and $T\Delta S^\circ$ for each host–guest combination are listed in Table 3, along with the relevant values reported by Zhang and Breslow.[1]

Table 3. Thermodynamic parameters for 1:1 inclusion complexation of guest molecules with β-cyclodextrin **1** and bis(β-cyclodextrin)s **2**–**4** and **7**–**9** in aqueous solution at 25 °C.

Host	Guest	$-\Delta G^\circ$ [kJ mol^{-1}]	$-\Delta H^\circ$ [kJ mol^{-1}]	$T\Delta S^\circ$ [kJ mol^{-1}]	Ref.
1	AR	19.5	32.0	−12.5	[a]
	RB	20.7	−40.8	61.5	[a]
2	AR	25.5	32.8	−7.3	[a]
	RB	25.2	9.4	15.8	[a]
3	AR	19.6	30.7	−11.1	[a]
	RB	22.8	−27.1	49.9	[a]
4	AR	20.6	19.3	1.3	[a]
	RB	21.0	−92.4	113.4	[a]
7	**10**	41.3	67.5	−26.2	[b]
8	**10**	40.2	60.4	−20.2	[b]
9	**10**	36.1	62.2	−26.1	[b]
7	**11**	37.4	65.5	−28.1	[b]
8	**12**	43.0	89.5	−46.5	[b]

[a] This work. [b] Ref. [1].

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In order to visualize and compare the molecular binding behavior of native β-cyclodextrin **1** and bis(β-cyclodextrin)s **2**–**4**, the thermodynamic quantities obtained for the complexation of AR and RB with **1**–**4** are plotted in Figure 3.

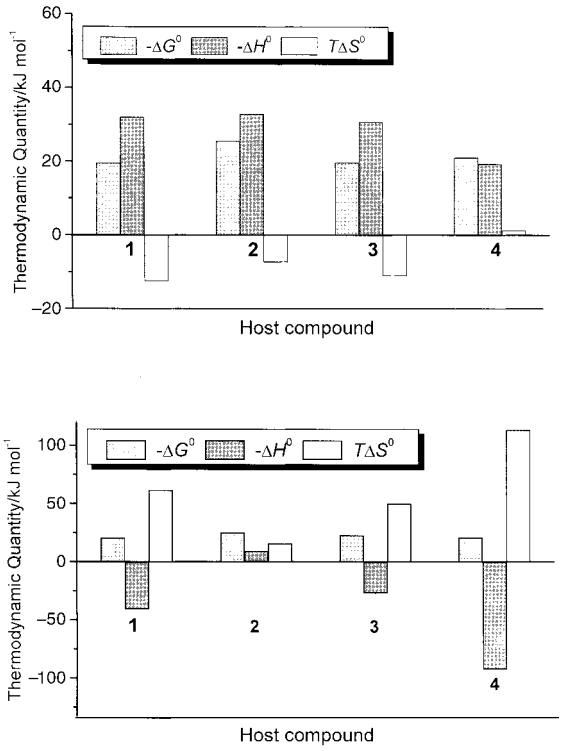

Figure 3. Free energy ($-\Delta G^\circ$), enthalpy ($-\Delta H^\circ$), and entropy changes ($T\Delta S^\circ$) for the inclusion complexation of top: Acridine Red and bottom: Rhodamine B with β-cyclodextrin and bis(β-cyclodextrin)s **2**–**4** in aqueous buffer solution (pH 7.20) at 25 °C.

Firstly, as can be readily recognized from Table 3 and Figure 3, AR and RB show dramatic contrasts in thermodynamic behavior upon complexation with native β-cyclodextrin **1**. Although the complex stabilities observed for native **1** with AR and RB are comparable with each other ($\Delta G^\circ = -19.5$ and -20.7 kJ mol^{-1}, respectively), the complexation of AR is exclusively driven by enthalpy ($\Delta H^\circ = -32.0$ kJ mol^{-1}) with a moderate entropic loss ($T\Delta S^\circ = -12.5$ kJ mol^{-1}), while that of RB is entirely driven by the large entropic gain ($T\Delta S^\circ = 61.5$ kJ mol^{-1}) which overwhelms the significant endothermic enthalpy change ($\Delta H^\circ = 40.8$ kJ mol^{-1}). Since the association process, which leads to the loss of conformational freedom, is inherently accompanied by entropic loss, as is the case with the complexation of AR, the large entropic gain observed for RB is not expected for a host–guest association. In view of the structural features of RB compared with those of AR, the extra desolvation arising from the lactonization of the heavily hydrated benzoate moiety of RB and/or from the desolvation of the oligo(ethylenediamino) tether upon inclusion complexation would be responsible for this unusually large positive entropy change.

As expected, the use of bis(β-cyclodextrin)s **2**–**4** enhances the original complex stabilities of both AR and RB compared with **1**, while preserving the same thermodynamic characteristics, that is, that the binding of AR is driven by enthalpy, whereas the complexation of RB is driven by entropy. Interestingly, the enhanced binding of AR by **2**–**4** is accomplished not by a further increase in the originally favorable enthalpic gain ($-\Delta H^\circ$) or by a strengthening of the van der Waals or hydrophobic interactions, but by a reduction in the large entropic loss ($T\Delta S^\circ$) for **1** (-12.5 kJ mol^{-1}) to less negative or even positive values for **2**–**4** (-7.3, -11.1, and $+1.3$ kJ mol^{-1}, respectively), for which more extensive desolvation of both RB and bis(β-cyclodextrin) upon complexation would be responsible. In contrast, the stronger binding of RB by **2** or **3** ($\Delta G^\circ = -25.2$ and -22.8 kJ mol^{-1}, respectively) than by **1** ($\Delta G^\circ = -20.7$ kJ mol^{-1}) is achieved by the exothermic or less endothermic enthalpy changes ($\Delta H^\circ = -9.4$ and 27.1 kJ mol^{-1} for **2** and **3**, respectively, versus 40.8 kJ mol^{-1} for **1**). Meanwhile, the much increased entropic gain ($T\Delta S^\circ = 113.4$ kJ mol^{-1}) for **4** is almost canceled by the simultaneously increased enthalpic loss ($\Delta H^\circ = 92.4$ kJ mol^{-1}); this affords an only slightly more stable RB complex with **4** ($\Delta G^\circ = -21.0$ kJ mol^{-1}) than with **1** ($\Delta G^\circ = -20.7$ kJ mol^{-1}).

A comparison of the thermodynamic parameters for the bis(β-cyclodextrin) series is also intriguing. As discussed above, the two guest dyes AR and RB, which possess a similar core structure but have different molecular sizes/shapes and substituents, display contrasting thermodynamic behavior upon complexation with the bis(β-cyclodextrin)s. As stated above, we have found enthalpy-driven complexation and entropy-governed enhancement in the case of AR, but entropy-driven complexation and enthalpy-governed enhancement in the case of RB. However, a close examination of Figure 3 leads us to the general trends of the thermodynamic parameters that are common to both AR and RB. Beyond the apparent differences in sign and magnitude of the ΔH° and $T\Delta S^\circ$ values for **2** to **4**, the enthalpic gain ($-\Delta H^\circ$) continuously becomes smaller upon extension of the tether from **2** to **4**, whereas the entropic gain ($T\Delta S^\circ$) shifts to the positive (favorable) side; this compensates for the enthalpic loss caused by extending the tether (except for the **3**–AR host–guest pair). The smaller enthalpic gains for the longer-tethered hosts may be ascribed to the difficulty for such hosts in forming a good hydrophobic pseudocavity and to the greater contribution of entropic gain that would arise from the more extensive desolvation of the tether upon sandwich complexation.

Enthalpy–entropy compensation: We have previously demonstrated that the enthalpy and entropy changes obtained for the inclusion complexation of various guests with native α- to γ-cyclodextrins are mutually compensatory; this gives a linear ΔH° versus $T\Delta S^\circ$ plot.[13] We have also proposed that the slope ($\alpha = 0.79-0.97$) and intercept ($T\Delta S_0 = 8-15$ kJ mol^{-1}) of the compensation plot can be used as quantitative measures of the conformational changes and the extent of induced desolvation upon complex formation.[12–15] The relatively steep slopes of 0.79–0.97, obtained with α- to γ-cyclodextrins,[15] mean that only 3–23% of the enthalpic gains from complex formation are reflected in the free energy change or complex stability. This is probably due to the global reorgan-

ization of the original hydrogen-bonding network in cyclodextrins upon inclusion complexation. The compensatory enthalpy–entropy relationship for a wide variety of modified mono(β-cyclodextrin)s has also been reported,[15, 18] and gives a steepr slope ($\alpha = 0.99$) and larger intercept ($T\Delta S_0 =$ 17 kJ mol^{-1}).

In the present study, the $T\Delta S^\circ$ values are plotted against the ΔH° values by using the thermodynamic parameters obtained in this work and the data reported by Zhang and Breslow[1] for bis(β-cyclodextrin)s of different types. As can be seen from Figure 4, the ΔH° against $T\Delta S^\circ$ plot gives an excellent straight

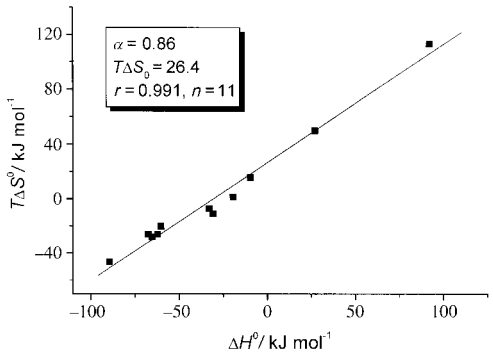

Figure 4. Enthalpy–entropy compensation plot for inclusion complexation of bis(β-cyclodextrin)s with various guests at 25 °C; see Supporting Information for the original data.

line with a correlation coefficient of 0.99, although the available data points are fairly limited ($n = 11$) and completely different host–guest combinations have been employed. The slope, α, and the intercept, $T\Delta S_0$, values for bis(β-cyclodextrin)s, as well as the corresponding values for natural cyclodextrins and modified mono(β-cyclodextrin)s from our previous reports,[15, 18] are summarized in Table 4. Unexpectedly, the slope for bis(β-cyclodextrin)s ($\alpha = 0.86$) is appreciably smaller than that for modified mono(β-cyclodextrin)s ($\alpha = 0.99$), but rather closer to that for native β-cyclodextrin ($\alpha = 0.80$).[15] On the other hand, bis(β-cyclodextrin)s show a much larger intercept ($T\Delta S_0 = 26$ kJ mol^{-1}) than those for native β-cyclodextrin ($T\Delta S_0 = 11$ kJ mol^{-1}) and modified mono(β-cyclodextrin)s ($T\Delta S_0 = 17$ kJ mol^{-1}). Theses results indicate that bis(β-cyclodextrin)s experience moderate conformational changes and extensive desolvation upon inclusion complexation—the latter may account for the fact that, despite the enthalpic destabilization of up to 92.4 kJ mol^{-1}, bis(β-cyclodextrin) **4** can bind RB solely as a consequence of the extremely large entropic gain ($T\Delta S^\circ = 113.4$ kJ mol^{-1}) that arises from the extensive desolvation of the long oligo(ethylenediamine) tether.

Table 4. Slope (α) and intercept ($T\Delta S_0$) of the ΔH° versus $T\Delta S^\circ$ plots for 1:1 inclusion complexation of native and modified cyclodextrins and bis(β-cyclodextrin)s in homogeneous solution.

Host	n[a]	α	$T\Delta S_0$ [kJ mol^{-1}]	Ref.
α-cyclodextrin	524	0.79	8	[b]
β-cyclodextrin	488	0.80	11	[b]
γ-cyclodextrin	58	0.97	15	[b]
modified cyclodextrins	128	0.99	17	[b]
bis(β-cyclodextrin)s	11	0.86	26	[c]

[a] Number of data sets used in the $\Delta H^\circ - T\Delta S^\circ$ analysis. [b] Ref. [15]. [c] This work.

Although the analysis was done by using a limited number of data, we have gained clear and sensible insights into the factors and mechanism governing the inclusion complexation of bis(β-cyclodextrin)s in the present case. Work concerned with extending the reliability of these thermodynamic data is currently in progress.

Experimental Section

Apparatus: Mass spectra were obtained by using a JEOL JMS-DX-303 instrument. ^{1}H NMR spectra were recorded on a Bruker AC–P200 instrument at 200 MHz with tetramethylsilane as internal reference. Infrared and ultraviolet spectra were recorded on Shimadzu IR-435 and Shimadzu UV-2401/PC instruments, respectively. Elemental analysis was performed on a Perkin–Elmer 2400C instrument. Fluorescence spectra were measured in a conventional quartz cell ($10 \times 10 \times 45$ mm) at 25 °C on a JASCO FP-750 spectrometer equipped with a temperature controller and with excitation and emission slits of 5 nm width. Circular dichroism (CD) spectra were recorded at 25 °C in a conventional quartz cell ($10 \times 10 \times 45$ mm) on a JASCO J-720S spectropolarimeter equipped with a PTC-348WI temperature controller to keep the sample temperature constant. Fluorescence lifetimes were determined by the time-correlated single-photon-counting method by 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 nm 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 for each measurement. The accumulated signals were then processed, and the lifetimes were determined by deconvolution by using the nonlinear least-squares-fit method.

Materials: Ammonium 8-anilino-1-naphthalenesulfonate (ANS), Brilliant Green (BG), and Methyl Orange (MO) were purchased from Wako Pure Chemical Industries, Ltd. Acridine Red (AR) was purchased from Chroma-Gesellschaft Schmid & Co. Rhodamine B (RB) was purchased from the Tianjin Chemical Reagent Plant. All chemicals were reagent grade and were used without further purification, unless noted otherwise. β-Cyclodextrin (**1**) of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried in vacuo for 12 h at 100 °C. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for two days and distilled under reduced pressure prior to use. Pyridine was refluxed over calcium hydride for 8 h and distilled prior to use. 2,2′-Bipyridine-4,4′-dicarboxylic dichloride was prepared according to the procedure reported by Evers et al.[30] Mono[6-oligo(ethylenediamino)-6-deoxy]-β-cyclodextrins were prepared according to the procedures reported by Harada et al.[31] Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, de-ionized water to make a 0.10 M phosphate buffer solution of pH 7.20 that was used in the spectral measurements.

Synthesis of 2,2′-bipyridine-4,4′-dicarboxy-bridged bis(6-*O*-β-cyclodextrin) (2): 2,2′-Bipyridine-4,4′-dicarboxylic dichloride (0.28 g, 1.0 mmol) was dissolved in dry pyridine (30 mL) containing dicyclohexylcarbodiimide (0.7 g, 34 mmol). Dry β-cyclodextrin (2.30 g, 2.0 mmol) in DMF (20 mL) was added to the solution at room temperature under a nitrogen atmosphere, and the resultant mixture was stirred for 18 h in an ice bath. The solution was allowed to warm up and was stirred for an additional two days at room temperature until no more precipitation was deposited. Then the precipitate was removed by filtration and the filtrate was evaporated under reduced pressure to dryness. The residue was dissolved in water, and then acetone was added to the solution to give a light red precipitate. The crude product obtained after drying was purified by column chromatography over Sephadex G-25 with distilled de-ionized water as the eluent to give a pure sample as a slightly reddish solid. Yield: 0.8 g, 0.3 mmol, 30%;

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m.p. ca. 180 °C (dec); ^{1}H NMR (200 MHz, D_2O, TMS): $\delta = 3.19 - 3.92$ (m, 85 H), 5.06 (m, 14 H), 7.99 (m, 2 H), 8.55(m, 2 H), 8.82 (m, 2 H); IR (KBr): $\tilde{\nu} = 3358.0$, 2907.8, 1725.8, 1703.0, 1646.9, 1397.1, 1371.1, 1327.7, 1235.1, 1140.1, 1071.9, 1023.1, 941.1, 841.9, 747.7, 573.5, 541.3 cm^{-1}; UV/Vis (water) λ_{max} (ε) = 302.3 nm (6185 mol^{-1} dm^3 cm^{-1}); MS: *m/z*: 2477 $[M+H]^+$; elemental analysis calcd (%) for $C_{96}H_{144}O_{72}N_2 \cdot 12H_2O$ (2694.3): C 42.80, H 6.29, N 1.04; found: C 42.53, H 6.20, N 1.00.

Synthesis of *N,N′*-bis(2-aminoethyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-cyclodextrin) (3): Bis(β-cyclodextrin) **3** was prepared in 20 % yield from 2,2′-bipyridine-4,4′-dicarboxylic dichloride and mono[6-(2-aminoethylamino)-6-deoxy]-β-cyclodextrin as a bright red solid according to the procedures described above. **3**. M.p. ca. 180 °C (dec); ^{1}H NMR (200 MHz, [D_6]DMSO, TMS): $\delta = 2.8 - 4.0$ (m, 92 H), 4.8 (m, 14 H), 8.0 (m, 2 H), 8.7 – 8.9 (m, 4 H); IR (KBr): $\tilde{\nu} = 3380.6$, 2930.2, 1650.3, 1549.5, 1370.5, 1241.6, 1155.8, 1081.2, 1031.2, 944.8, 857.6, 756.4, 606.2, 578.4, 530.1 cm^{-1}; UV/Vis (water): λ_{max} (ε) = 294.0 nm (10128 mol^{-1} dm^3 cm^{-1}); MS: *m/z*: 2561 $[M+H]^+$; elemental analysis calcd (%) for $C_{100}H_{156}O_{70}N_6 \cdot 8H_2O$ (2706.5): C 44.38, H 6.41, N 3.11; found: C 44.52, H 6.50, N 3.31.

Synthesis of *N,N′*-bis(5-amino-3-azapentyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-cyclodextrin) (4): Bis(β-cyclodextrin) **4** was prepared in 20 % yield from 2,2′-bipyridine-4,4′-dicarboxylic dichloride and mono[6-(5-amino-3-azapentylamino)-6-deoxy]-β-cyclodextrin as a yellowish solid according to procedures similar to those employed in the synthesis of **2**. M.p. ca. 180 °C (dec); ^{1}H NMR (200 MHz, [D_6]DMSO, TMS): $\delta = 2.6 - 3.0$ (m, 8 H), 3.2 – 3.9 (m, 92 H), 4.8 (m, 14 H), 8.0 (m, 2 H), 8.7 – 8.9 (m, 4 H); IR (KBr): $\tilde{\nu} = 3391.4$, 2930.7, 1654.8, 1549.5, 1411.1, 1368.3, 1241.9, 1155.9, 1080.5, 1031.1, 944.5, 857.1, 756.0, 705.9, 608.1, 578.9, 531.2 cm^{-1}; UV/Vis (water): λ_{max} (ε) = 293.0 nm (5366 mol^{-1} dm^3 cm^{-1}); MS: *m/z*: 2647 $[M+H]^+$; elemental analysis calcd (%) for $C_{104}H_{166}O_{70}N_8 \cdot 12H_2O$ (2864.6): C 43.61, H 6.69, N 3.91; found: C 43.70, H 7.02, N 3.83.

Synthesis of *N,N′*-bis(8-amino-3,6-diazaoctyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-cyclodextrin) (5): Bis(β-cyclodextrin) **5** was similarly prepared in 18 % yield from 2,2′-bipyridine-4,4′-dicarboxylic dichloride and mono[6-(8-amino-3,6-diazaoctylamino)-6-deoxy]-β-cyclodextrin as a yellowish solid. M.p. ca. 180 °C (dec); ^{1}H NMR (200 MHz, [D_6]DMSO, TMS): $\delta = 2.6 - 2.9$ (m, 16 H), 3.1 – 4.0 (m, 92 H), 4.9 (m, 14 H), 7.9 (m, 2 H), 8.8 (m, 4 H); IR (KBr): $\tilde{\nu} = 3413.7$, 2929.3, 1640.9, 1549.4, 1371.4, 1241.7, 1155.8, 1080.2, 1030.9, 944.7, 855.5, 755.7, 686.9, 578.1, 533.5 cm^{-1}; UV/Vis (water): λ_{max} (ε) = 292.6 nm (12308 mol^{-1} dm^3 cm^{-1}); MS: *m/z*: 2733 $[M+H]^+$; elemental analysis calcd (%) for $C_{108}H_{176}O_{70}N_{10} \cdot 5H_2O$ (2824.7): C 45.92, H 6.64, N 4.96; found: C 45.97, H 6.73, N 4.83.

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