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Complexation and Sensing Behavior of Dansyl-appended Cyclodextrins and Cyclodextrin Dimers with Bile Salts

MENNO R. DE JONG[a], PATRICK BERTHAULT[b], ARIE VAN HOEK[c], ANTONIE J.W.G. VISSER[d], JURRIAAN HUSKENS[a,*] and DAVID N. REINHOUDT[a]

[a]*Laboratory of Supramolecular Chemistry and Technology, MESA+ Research Institute, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands;* [b]*CEA Saclay, DSM/DRECAM/SCM, F91191 Gif sur Yvette, France;* [c]*Laboratory of Biophysics, Wageningen University, MicroSpectroscopy Centre, PO Box 8128, 6700 ET Wageningen, The Netherlands;* [d]*Laboratory of Biochemistry, MicroSpectroscopy Centre, Wageningen University, PO Box 8128, 6700 ET Wageningen, The Netherlands*

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The dansyl-modified cyclodextrin derivatives 2 and 3 form complexes with the steroidal bile salts. The selectivity of the monomeric derivative 3 is similar to that of native β-cyclodextrin. All binding constants with 3 are lowered compared to β-cyclodextrin because of the competition for the cavity between the steroids and the dansyl moiety. Cholate (4a) and deoxycholate (4b) form weak complexes, the other bile salts (4c–e) are complexed far more strongly. The difference is attributed to the absence of a 12-hydroxy group in the latter steroids. Data for dimer 2 reveal strongly enhanced binding of 4a and 4b and only slightly stronger complexes with the other steroids. Due to the low binding affinity of 3 for 4a and 4b, this receptor could not be used for their detection by fluorescence spectroscopy. Steroids 4c–e showed a decrease in fluorescence intensity. The detection of all steroids 4a–e was possible using 2. The fluorescence intensity of the dimer increased or decreased, depending on which steroid was added.

Keywords: Cyclodextrin; Fluorescence; Dansyl group; Binding constants

INTRODUCTION

The naturally occurring cyclodextrins, cyclic oligosaccharides consisting of six, seven (β-cyclodextrin **1**, Fig. 1), or eight glucose moieties, are well-known host compounds for organic molecules [1,2]. These water-soluble molecules shaped like a truncated cone possess a hydrophobic cavity, which enables the complexation of organic guests in aqueous solution.

The use of cyclodextrins for sensor purposes by appending a fluorescent receptor molecule has been studied by Ueno [3] and others [4,5]. In this system, the generation of a signal is based on the competition between the covalently-attached fluorophore and a guest for the cyclodextrin cavity. In the absence of a guest, the fluorophore partially resides in the cavity, but addition of a guest leads to decomplexation of the fluorophore and to a concomitant decrease in fluorescence quantum yield [6]. The sensitivity of this system is limited by the competition between complexation of the guest and self-inclusion of the fluorophore in the cyclodextrin cavity. This usually results in decreased binding to the modified cyclodextrin compared to the native β-cyclodextrin.

Our aim is to increase the selectivity of cyclodextrin-based sensing molecules toward larger guest molecules [7–9]. This is often achieved by coupling two cyclodextrins [10,11]. These cyclodextrin dimers have improved selectivity for elongated guests [12–16]. Recently, we reported the synthesis and complexation behavior of dansyl-appended cyclodextrin dimer **2** (Chart 1) and that of an analogue lacking the dansyl group [17].

Contrary to the dimers in which the cyclodextrins are connected by their small primary sides [18,19], they are capable of cooperative binding of steroids. The sensing behavior of **2** with steroids, however, appeared to be complicated [17].

*Corresponding author. Fax: +31-53-4894645. E-mail: smct@ct.utwente.nl

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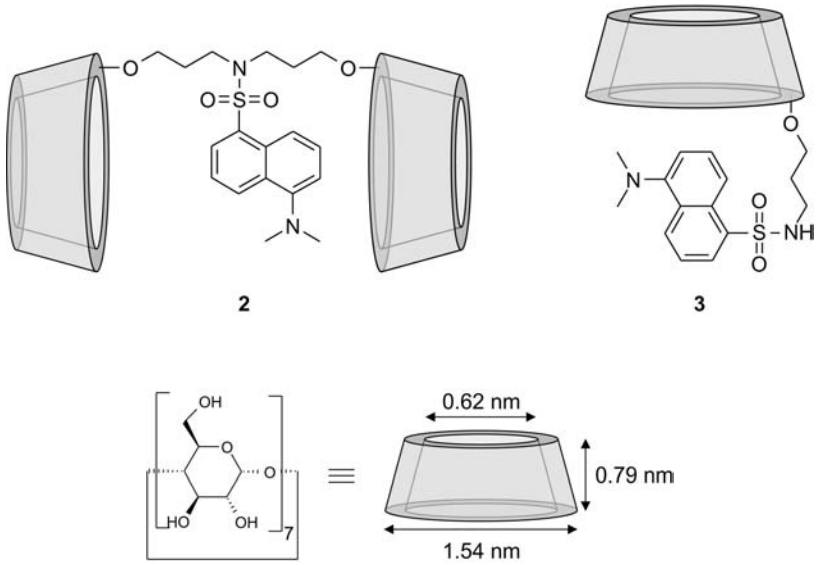


FIGURE 1 The structure of β-cyclodextrin **1**.

In this paper, we compare the complexation and sensing behavior of the newly synthesized cyclodextrin derivative **3**† with steroids with that of **1** and **2**. We have used calorimetry, steady state fluorescence spectroscopy, and fluorescence lifetime spectroscopy. We performed high resolution NMR experiments with **2** in order to obtain a better understanding of the factors influencing the fluorescence responses of cyclodextrin dimers.

RESULTS AND DISCUSSION

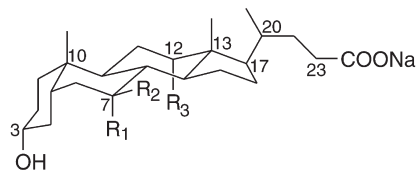
Calorimetry

We have studied the binding of **1**–**3** with bile salts **4a**–**e** (Fig. 2), [20] surfactant-like molecules that play a role in the metabolism and excretion of cholesterol in mammals. Their interaction with β-cyclodextrin and derivatives has been well documented [21–26]. They have a side chain at C-17, methyl groups at C-10, C-13, and C-20, and a carboxylic acid at C-23 of the steroid skeleton. They only differ in the number and position of hydroxyl groups at C-3, C-7, and C-12. The complex geometry of β-cyclodextrin (**1**) with **4a**, **4b**, [19] and **4d** [21] was studied by ROESY. The aliphatic side chain of the steroid enters the cyclodextrin from the secondary side. Additional interactions of the two steroid rings closest to the side chain with the interior of the cyclodextrin were observed. This leaves part of the steroid skeleton available for interaction with a second cyclodextrin. Tato and coworkers [19] studied the formation of complexes between primary side-connected β-cyclodextrin dimers and **4a** and **4b**. For **4b**, the formation of an oligomeric structure was proposed.

We employed microcalorimetry to study the binding stoichiometry and thermodynamics of **4a**–**e** to cyclodextrin derivatives **1**–**3**. The contribution of an extra cavity to the binding thermodynamics of the steroids was assessed by comparison of the data for **2** to the data for **1** and **3**.

Dilution experiments of the bile salts proved that the titrations with **1**–**3** were performed below the critical micelle concentration (cmc) of the bile salts. Titrations of **4a** and **4b** with **3** revealed very weak binding of these steroids. The steroids and **3** were not soluble enough in water to determine the binding constants accurately. Titrations of **4c**–**e** with **3** were possible. The enthalpograms show that the binding of these guests is enthalpy-driven, indicating a good fit in the cavity. The titration data were fitted to a 1:1 binding model (Table I).

Comparison of the data for **3** to those for **1** (Table I) show that also with native β-cyclodextrin, cholate (**4a**) and deoxycholate (**4b**) form complexes that have far lower stability constants than with the other steroids. The hydroxyl group at C-12 of **4a** and **4b**



	Steroid	R_1	R_2	R_3
4a	Cholate	OH	H	OH
4b	Deoxycholate	H	H	OH
4c	Chenodeoxycholate	OH	H	H
4d	Ursodeoxycholate	H	OH	H
4e	Lithocholate	H	H	H

FIGURE 2 The structure of the bile salts.

TABLE I Thermodynamic parameters (298 K) for the interaction between **1** or **3** and bile salts, as obtained by microcalorimetry

Steroid	**1***				**3**			
	K†	ΔG^0‡	ΔH^0‡	$T\Delta S^0$‡	K†	ΔG^0‡	ΔH^0‡	$T\Delta S^0$‡
4a	4.1×10^3	−4.9	−6.0	−1.1	–	–	–	–
4b	3.6×10^3	−4.8	−7.0	−2.2	–	–	–	–
4c	1.8×10^5	−7.1	−7.4	−0.3	3.0×10^4	−6.1	−7.6	−1.5
4d	7.8×10^5	−8.0	−9.2	−1.2	9.5×10^4	−6.8	−8.6	−1.8
4e	1.9×10^6	−8.5	−9.7	−1.2	6.1×10^5	−7.9	−10.0	−2.1

*Taken from Ref. [17]. †Values given in M^{-1}; $K = [HG]/[H][G]$. ‡Values given in kcal mol^{-1}.

(Fig. 2) might prevent deep inclusion of the steroid in the cyclodextrin cavity, which has a negative effect on the complex stability [24]. The binding strengths of steroids **4a–e** to **3** are considerably lower compared to **1**, probably due to the competition between the dansyl moiety and the steroid for the cavity. This decrease is mainly caused by a less favorable entropy term; the binding enthalpy remains virtually unchanged. Most likely, the dansyl group is complexed weakly into the cavity, which is mainly entropy-driven, so that replacement by the steroid causes a more unfavorable entropy change than in the case of **1**.

The thermodynamic data for the interaction between dimer **2** (Table II) and the steroids reveal that the presence or absence of the C-12 hydroxyl group is important for the binding, as already observed for the monomeric derivatives (Table I). Steroids **4a** and **4b**, which have this group, are bound by **2** in a 1:1 fashion. These two steroids can only be partially included in a cyclodextrin cavity, because the C-12 hydroxyl group prevents deeper protrusion through the cavity [24]. Consequently, a large part of the hydrophobic skeleton is still available for complexation by a second cavity. As is the case for steroids **4c–e**, the complexation of which by **3** is measurable (Table I), a decrease in the binding strength of roughly an order of magnitude for binding of **4a** and **4b** to **2** was observed when compared to the binding of the same steroids in a dimer lacking the dansyl group [17]. This decrease is mainly caused by an unfavorable change in the binding entropy. The binding enthalpy remains almost unchanged, again suggesting a weak, entropy-driven self-inclusion of the dansyl group.

In the case of **4c**, **4d**, and **4e**, deep inclusion into a single β-cyclodextrin unit is possible, and therefore the other cavity is still available. This results in complexation of a second guest molecule. Interestingly, unlike for the 1:1 complexes discussed so far, the binding enthalpy and entropy, both change compared with binding in a dimer lacking the dansyl group [17]. Apparently, in the 2:1 complexes, the presence of the dansyl in **2** leads to more changes in the way the two guests interact with the host and each other than just the competition of the dansyl with the steroids for the cavity. Possibly, hydrophobic interactions between the dansyl and the guests contribute as well.

The calorimetric studies have shown, that secondary side-connected cyclodextrin cavities can cooperate in the binding of bile salts, enabling the complexation of steroids that do not bind to the monomeric dansyl-appended cyclodextrin **3**. Thus, they differ from previously reported dimers that were connected via the primary side of the cyclodextrins [18,19]. These did not show the formation of strong 1:1 complexes with **4a** and **4b**. This different binding behavior can probably be attributed to the geometry of β-cyclodextrin complexes with the steroids as known from NMR, which shows that part of the hydrophobic steroid skeleton protrudes from the secondary side [19].

Steady State Fluorescence

In aqueous solution, **3** has an emission maximum at 555 nm. The blue-shift relative to *N*,*N*-dimethyl dansylamide ($\lambda_{max} = 572$ nm) [8] indicates that the dansyl moiety is in a less polar environment than water, consistent with its partial self-inclusion. Previously reported dansyl-modified β-cyclodextrin derivatives show larger blue-shifts, down to

TABLE II Stepwise thermodynamic parameters (298 K) for the interaction between dimer **2** and bile salts.*

Steroid	K_1†	ΔG^0‡	ΔH^0‡	$T\Delta S^0$‡	K_2†	ΔG^0‡	$\Delta H°$‡	$T\Delta S^0$‡
4a	3.6×10^4	−6.2	−5.1	1.1	–	–	–	–
4b	1.9×10^5	−7.2	−8.9	−1.7	–	–	–	–
4c	2.2×10^6	−8.6	−8.0	0.7	1.8×10^5	−7.1	−9.1	−1.9
4d	3.1×10^6	−8.8	−10.8	−1.9	2.0×10^5	−7.2	−8.6	−1.4
4e	1.6×10^6	−8.4	−11.7	−3.2	7.6×10^5	−8.0	−11.1	−3.1

*Taken from Ref. [17]. †Values given in M^{-1}; $K_1 = [HG]/[H][G]$, $K_2 = [HG_2]/[HG][G]$. ‡Values given in kcal mol^{-1}.

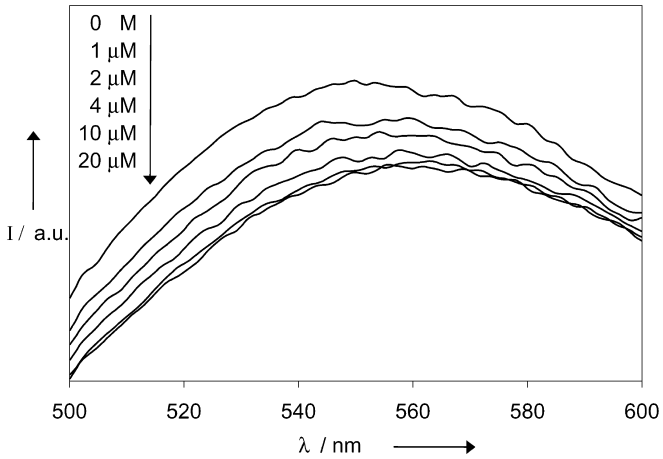


FIGURE 3 Fluorescence decrease upon the addition of **4e** to an aqueous solution of **3** (2.7 μM).

520 nm.† The moderate effect of the dansyl group on the binding constants of **2** and **3**, determined by calorimetry, indicates only weak inclusion of the dansyl moiety into the cyclodextrin cavity.

Addition of steroids that bind strongly to receptor molecule **3** gave rise to a decrease in fluorescence intensity and a concomitant increase of λ_{max} (Fig. 3). This is attributed to the more hydrophilic environment the dansyl experiences after its expulsion from the cavity by a guest.

The fluorescence of **3** did not decrease upon titration with cholate **4a** and deoxycholate **4b** (Fig. 4). Thus, no binding constants could be obtained. For **4c–e**, the changes in fluorescence intensity were large enough to allow determination of the binding constants (Table III).

The thermodynamic data obtained by calorimetry and fluorescence spectroscopy are in reasonable agreement. Both techniques indicate only weak or no interaction between the **3** and bile salts **4a** and **4b**. The binding constants obtained by fluorescence for **4c–e** are slightly larger than those found by microcalorimetry, but the selectivity order is the same. The most hydrophobic steroid **4e** binds strongest to **3**, **4c** has the weakest interaction. This order directly reflects the binding strengths of these steroids in the native β-cyclodextrin.

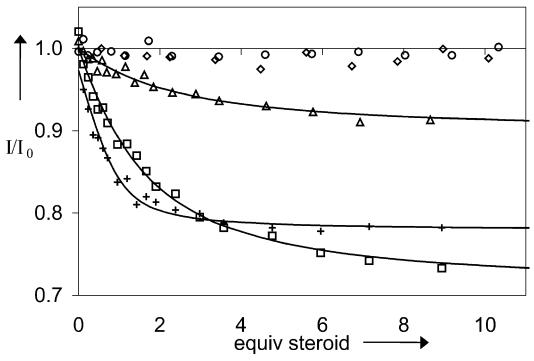


FIGURE 4 Normalized fluorescence intensity changes of **3** (2.7 μM) at 555 nm as a function of the concentration of bile salts. (a): **4a** (◇), **4b** (○), **4c** (△), **4d** (□), and **4e** (+).

The maximum emission wavelength of dimer **2** is at 540 nm. The blue-shift relative to **3** indicates that the dansyl group experiences more shielding from the environment in the dimer than in the monomer. Comparison to the previously reported dansyl-modified β-cyclodextrin derivatives,† however, indicates that the dansyl moiety is still relatively poorly shielded by the cyclodextrin cavities of **2**.

Addition of a 10-fold excess of steroids **4a–e** to a micromolar solution of **2** resulted in a fluorescence response (Fig. 5). The addition of bile salts did not always result in the decrease in fluorescence intensity expected when the dansyl moiety is expelled from the cavities. In some cases, a considerable increase in the fluorescence was observed. This complicated sensing behavior of **2** is not only observed for bile salts, as shown by the responses of some additional steroids **5–8** (Fig. 5).

For sensor molecules based on a single dansyl-modified β-cyclodextrin cavity such as **3** and the sensor molecules reported by Ueno, addition of guests was always found to lead to a decrease in fluorescence intensity. Especially the remarkable

TABLE III Binding constants and maximum intensity changes at 555 nm for the interaction between **3** and bile salts, as obtained by fluorescence spectroscopy

	K (M^{-1})	$I/I_{0,\,max}$
4c	2.1×10^5	0.90
4d	3.5×10^5	0.70
4e	2.2×10^6	0.80

†A closely related dansyl-appended cyclodextrin derivative with an ethyl rather than a propyl spacer has been prepared previously, see Ref. [5].

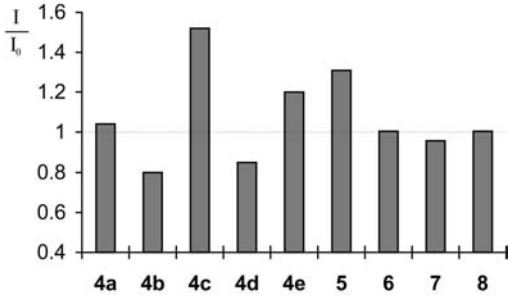


FIGURE 5 Fluorescence response of dimer **2** on the binding of steroidal guests; **5**: cortexolone, **6**: prednisolone, **7**: prednisone, **8**: corticosterone-21-acetate.

difference in fluorescence response between **2** and **3** indicates that the increase in fluorescence intensity caused by some guest molecules is solely due to the presence of the second cavity. Apparently, the position between the cyclodextrins which the dansyl moiety occupies after its expulsion from the cavities by the steroid (**4a**) or steroids (**4c**) is more hydrophobic than the cavity itself, possibly because of the proximity of the steroid(s). The interaction of the hydroxyl groups in the steroid may play a role in the quenching processes.

The cooperatively bound **4b** is detected with comparable selectivity by **2** as **4d**. In contrast, the monomeric β-cyclodextrin receptor **3** (see above) and a dimer which is connected via the primary side [18], which lack the possibility of cooperative binding of bile salts, show a strong preference for **4d**.

Remarkably, the data from fluorescence titrations fitted well to a 1:1 binding model for all steroids except **4e**. Independent Job's plot analyses confirmed the presence of 1:1 complex for **4b** and **4c**. It is remarkable that the titrations with **4c** and **4d** could be fitted well to a 1:1 binding model, as the binding constants obtained using calorimetry imply the presence of a substantial amount of 1:2 complex at the concentrations used. Apparently, inclusion of a second steroid does not lead to an additional change in fluorescence intensity upon binding of the second steroid. The calculated values for the association constants are shown in Table IV. The values for **4a**–**d** are similar to the K_1 values found by the calorimetric titrations.

Only in the titration of **4e** with **2**, the presence of complexes of higher stoichiometry is shown by fluorescence. Up to one equivalent of guest, the fluorescence intensity decreases; after one equivalent, it starts to increase. The data of this titration could be fitted to a 1:2 binding model (Table IV) and the K values obtained are similar to those found by calorimetry.

TABLE IV Stepwise binding constants for the interaction between dimer **2** and steroidal guests, as obtained by fluorescence spectroscopy

	K_1 (M^{-1})*	K_2 (M^{-1})*	I/I_0, max
4a	1.2×10^5	–	1.04
4b	5.8×10^5	–	0.80
4c	6.5×10^5	–	1.52
4d	2.5×10^6	–	0.85
4e	6.2×10^6	1.0×10^6	0.80/1.20
5	2.1×10^5	–	1.31
6	1.3×10^4	–	1.01
7	5.9×10^4	–	0.96
8	1.0×10^5	–	1.01

* $K_1 = [HG]/[H][G]$, $K_2 = [HG_2]/[HG][G]$

Fluorescence Lifetimes

The dansyl groups of fluorescent cyclodextrin derivatives in aqueous solution are usually flexible [27]. Conformations with the fluorophore inside the cavity as well as outside the cavity are possible in most cases. The interconversion between the two occurs on the timescale of nanoseconds. Analysis of the fluorescence decay can give information on the conformation of the fluorophore. Usually, the relative fluorescence intensity I at time t of a dansyl-appended cyclodextrin can be fitted to a bi-exponential decay function (Eq. (1)).

$$I_t = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} \quad (1)$$

The longer lifetime component is thought to originate from the conformation with an included fluorophore, and the shorter one from the conformation with the fluorophore in the bulk. Addition of a guest usually causes a decrease of the fraction of the long-lifetime component. This is attributed to an increase in the amount of time the dansyl spends outside the cavity as a result of competition with the added guest for the cavity (Fig. 6).

The fluorescence of the sensor molecules **2** and **3** showed bi-exponential decay curves. In both the cases, the fractions of the longer lifetime component, attributed to a dansyl moiety that is shielded from the aqueous environment, were relatively small (Table V). Moreover, compared to other dansyl-appended cyclodextrin derivatives, the long lifetime is relatively short. This is consistent with the data

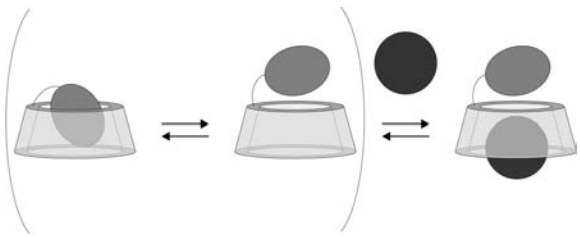


FIGURE 6 Conformational equilibria in solution and guest-induced conformational change commonly used to explain the fluorescence lifetime behavior of cyclodextrin derivatives.

TABLE V Fluorescence lifetimes (τ_1 and τ_2) and normalized amplitudes (A_1 and A_2) of **3** and **2** (host concentration: 10 μM) in the absence and presence of steroids (100 or 50 μM in the case of **3** or **2**, respectively), as obtained from fits of experimental fluorescence decay data to Eq. (1)

Host	Guest	A_1	τ_1 (ns)	A_2	τ_2 (ns)
3	–	0.9	7.3	0.1	11.8
	4a	0.7	6.6	0.3	10.0
	4b	0.7	6.8	0.3	10.2
	4c	0.6	5.0	0.4	9.1
	4d	0.8	4.2	0.2	9.3
	4e	0.4	5.8	0.6	8.8
2	–	0.6	6.1	0.4	10.2
	4a	0.5	5.7	0.5	12.6
	4b	0.6	4.4	0.4	10.6
	4c	0.4	6.7	0.6	14.7
	4d	0.6	5.0	0.4	9.5
	4e	0.5	5.6	0.5	10.0

from calorimetry and steady state fluorescence which suggested rather weak self-inclusion of the dansyl moiety.

The decrease in the fluorescence of **3** upon the addition of **4c**–**e** seems to be caused mainly by a decrease in the fluorescence lifetimes of both components (Table V). This contrasts most fluorescent cyclodextrin sensors reported in literature, where the decrease in the fluorescence is caused by a decrease in the fraction of the long-lifetime component of the fluorescence. The difference between **3** and the dansyl-appended cyclodextrin derivatives whose dynamic fluorescence behavior has been studied before, is that the dansyl is appended on the secondary side rather than on the primary side. As the hydrophobic region of the steroidal guest also protrudes from the secondary side, the new lifetimes may possibly be attributed to two species with more or less shielding the dansyl from the aqueous environment by the complex of cyclodextrin and steroid (Fig. 7).

The decrease in the fluorescence intensity of **2** upon addition of **4b** and **4d** are again caused by changes in the lifetimes rather than by a decrease of the fraction of the long-lifetime component. For **4c**, which caused a large increase in the fluorescence of **2**, both the contribution of the long-lived component and the lifetime value itself increased markedly. This is consistent with the notion of shielding of the dansyl from the environment by the steroid rather than the cyclodextrin after complexation.

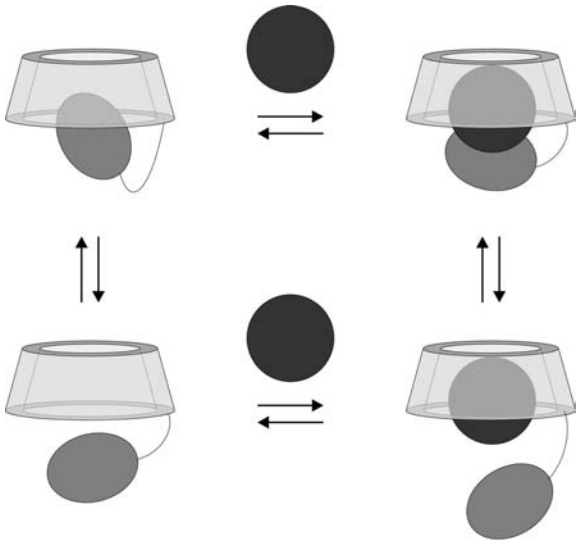


FIGURE 7 Possible conformational equilibria for a cyclodextrin with the fluorophore appended at the secondary side.

High-resolution NMR on Cyclodextrin Dimer 2

To investigate whether interactions between the steroids and the dansyl moiety play a role in the fluorescence response of **2** on steroids, further information on the geometry of cyclodextrin dimer **2** and its complexes with steroids was obtained by high-resolution NMR experiments. The 800 MHz ^{1}H NMR spectrum of **2** in D_2O at 37°C shows seven signals for the anomeric protons (Fig. 8a). This indicates that the two cavities of the dimer are equivalent, probably due to a fast exchange of the dansyl between the two cavities. The dansyl resonances are partially overlapping.

After the addition of **4b**, which forms a 1:1 host–guest complex with **2**, the H-1 resonances become less well dispersed (Fig. 8b). All dansyl protons shift and become more resolved. Addition of an excess of **4d**, which forms a 1:2 complex in which the steroids are included much deeper in the cavities, causes more pronounced shifts of the dansyl resonances and even less dispersed resonances of the anomeric protons (Fig. 8c).

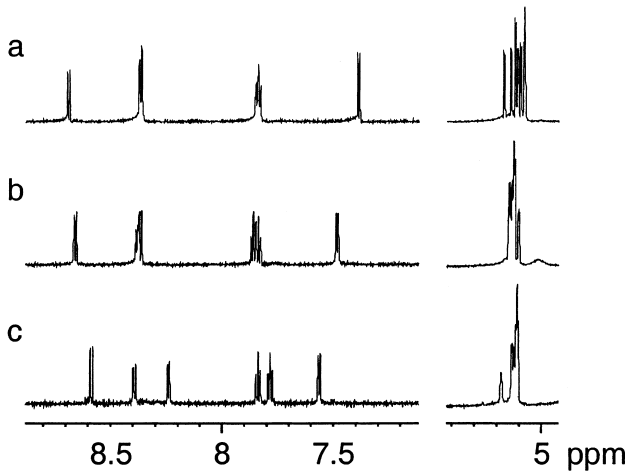


FIGURE 8 Parts of the ^{1}H NMR spectra of **2** in D_2O at 37°C in the absence (a), or presence of **4b** (b), or **4d** (c). The shifts around 8 ppm correspond to protons from the dansyl moiety, those around 5 ppm from the cyclodextrin anomeric protons.

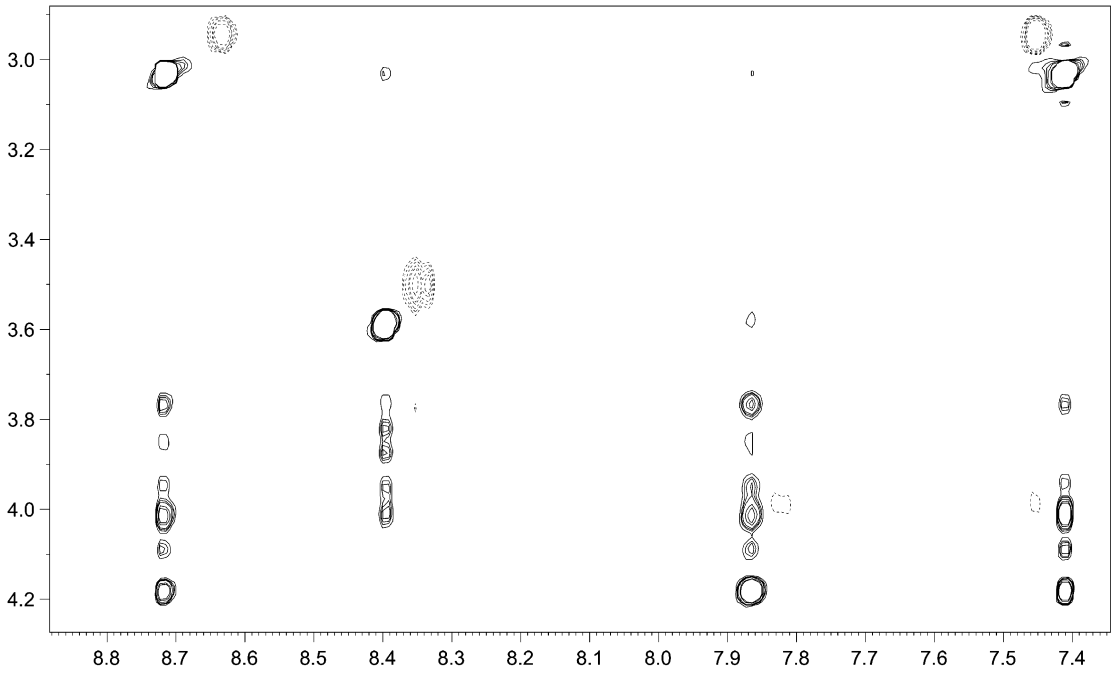


FIGURE 9 2D ROESY NMR spectrum of **2** in the absence (—), or in the presence of **4b** (---), or **4d** (. . .). Horizontal: dansyl region. Vertical: cyclodextrin region of the spectrum.

Two-dimensional off-resonance ROESY experiments [28] with an angle between the spin-lock field and the static field of 54.7° show through-space interactions between the dansyl protons and the protons in the interior of the cavity in the free dimer. Addition of **4b** causes these to diminish, and addition of **4d** causes them to disappear almost completely (dansyl protons, Fig. 9). Only through-space connectivities between the dansyl and the spacer protons at 3.0 and 3.6 ppm and between the steroid and the interior of the cavity are observed.

The NMR data agree well with partial self-inclusion of the dansyl moiety in the absence of the guest and expulsion of the dansyl from the cavity by the guest. For **4b** and **4d**, the dansyl does not stay very close to the guests in the complex, as evidenced by the absence of NOE between the steroid protons and the dansyl protons.

CONCLUSIONS

Cyclodextrin dimers such as **2** are efficient hosts for the complexation of various steroids. The stoichiometry of a given steroid-cyclodextrin dimer complex depends partly on the complex geometry of that steroid with native β-cyclodextrin. Still, the fluorescence response of dimer **2** on a given steroid has changed markedly when compared to the monomeric sensing molecule **3**. Steroids not detectable by **3** are detected by **2**. In addition, reduced shielding of the dansyl after its expulsion from the cavity by an external guest does not seem to be the only mechanism responsible for the generation of the signal. The fluorescence intensity may increase or decrease upon addition of a guest, depending on very subtle variations in complex geometry. Thus, cyclodextrin dimers are interesting hosts for the creation of sensing molecules. Optimization of this receptor molecule type may lead to sensors with *on* rather than *off* signaling. This can be attributed to the proper positioning of the fluorescent reporter group at the secondary side and to a shielding interaction of the analyte with the reporter group.

EXPERIMENTAL SECTION

Mono-2-*O*-(3′-aminopropyl)-heptakis-6-*O*-(*tert*-butyldimethylsilyl)-β-cyclodextrin [29] and dimer **2** [17] were prepared according to literature procedures. Mass spectra were recorded with a Finnigan MAT 90 spectrometer using NBA/NPOE as a matrix. NMR spectra were recorded at 25°C using a Bruker AC 250 and a Varian Inova 300 spectrometer. ^{1}H NMR chemical shifts (250 or 300 MHz) are given relative to residual $CHCl_3$ (7.25 ppm), or HDO (4.65 ppm). ^{13}C chemical shifts (63 or 75 MHz) are given relative to $CDCl_3$ (77 ppm). ^{1}H NMR spectra at 800 MHz have been performed on a BRUKER Avance DRX800 spectrometer, equipped with a 5 mm-(^{1}H, ^{13}C, ^{15}N) inverse probehead with three axes static field gradients. Calorimetric titrations were performed at 25°C using a Microcal VP-ITC titration microcalorimeter. Sample solutions were prepared using 1 mM NaOH in pure water (Millipore

Q2). Titrations were performed by adding aliquots of a guest solution to the host solution. The titrations were analyzed using least squares curve fitting procedure. Control experiments involved addition of guest to 1 mM NaOH solution and addition of 1 mM NaOH to a host solution. Fluorescence measurements were performed on an Edinburgh SF 900 spectrometer. Sample solutions were prepared using a phosphate buffer (pH 7, $I = 0.02$) in pure water (Millipore Q2). Fluorescence titrations were performed by adding aliquots of the guest in host solution to the pure host solution and vice versa, thus keeping the host concentration constant. After each addition, the fluorescence spectrum was recorded.

Time-resolved fluorescence measurements were carried out using mode-locked continuous wave lasers for excitation and time-correlated photon counting as detection technique. The experimental setup has been described in detail before [30–34]. The repetition rate of excitation pulses was 951 kHz, the wavelength 343 nm, the duration about 4 ps full width at half maximum (FWHM) and the pulse energy in the tens of pJ range. By applying a computer-controlled sheet type polarizer, fluorescence decays were detected polarized, parallel and perpendicular to the polarization direction of the excitation light. A cutoff filter (KV 408) combined with an interference filter (Balzers K55) were used for the rejection of scattered excitation light and a Hamamatsu R3809U-50 microchannel plate photomultiplier for observing fluorescence at a single photon level. Decay analysis was performed using a least squares method as described in Ref. [34].

Mono-2-*O*-[3′-(5″-dimethylaminonaphthalene-1″-sulfonamido)-propyl]-β-cyclodextrin 3

Dansyl chloride (220 mg, 82 μmol) and triethylamine (100 mg, 1 mmol) were added to a solution of mono-2-*O*-(3′-aminopropyl)-heptakis-6-*O*-(*tert*-butyldimethylsilyl)-β-cyclodextrin (100 mg, 50 μmol) in dichloromethane (10 ml). The solution was stirred at room temperature for 15 h. The reaction mixture was washed twice with 1 M HCl, water, a saturated solution of $NaHCO_3$, water, and brine, dried ($MgSO_4$). After evaporation of the solvent and purification by column chromatography (eluent EtOAc/EtOH/H_2O, 50:2:1) TBDMS-protected **3** was obtained as a light yellow solid in 58% yield (63 mg, 29 μmol). ^{1}H NMR ($CDCl_3/CD_3OD$) δ 8.49 (d, 1 H, $J = 8.4$ Hz), 8.37 (d, 1 H, $J = 8.7$ Hz), 8.19 (d, 1 H, $J = 6.6$ Hz), 7.55–7.49 (m, 2 H) 7.12 (d, 1 H, $J = 7.2$ Hz), 5.03–4.82 (m, 7 H), 4.15–3.28 (m, 46 H), 2.85 (s, 6 H), 1.65–1.61 (m, 2 H), 0.92–0.84 (m, 63 H), 0.06–0.01 (m, 42 H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 151.8, 135.5, 129.9, 129.1, 128.1, 123.3, 119.3, 115.0, 102.4, 102.0, 101.8, 101.1, 99.4, 82.8, 82.0, 80.4, 79.2, 76.6, 73.6, 72.5, 72.3, 72.0, 79.7, 62.8, 61.6, 61.3, 45.4, 26.0, 25.9, 25.8, 18.5, 18.3, −5.1, −5.2; MS (FAB) m/z calcd. for $C_{99}H_{186}N_2O_{37}SSi_7$ 2223.1, found 2246.9 $[M + Na]^+$.

TBDMS-protected **3** (58 mg, 27 μmol) was dissolved in TFA. After 5 min at room temperature, the TFA was removed *in vacuo*. Methanol was added and evaporated three times to remove residual TFA. The product was dissolved in water and washed three times with hexanes. After lyophilization, **3** was obtained as a light yellow powder in 81% yield (29 mg, 22 μmol). ^{1}H NMR (D_2O) δ 8.55 (d, 1 H, $J = 8.4$ Hz), 8.35 (d, 1 H, $J = 8.4$ Hz), 8.28 (d, 1 H, $J = 7.5$ Hz), 7.74–7.67 (m, 2 H) 7.22 (d, 1 H, $J = 7.8$ Hz), 5.10–4.82 (m, 7 H), 3.97–3.04 (m, 46 H), 2.79 (s, 6 H), 1.55–1.33 (m, 2 H); MS (FAB) m/z calcd. for $C_{57}H_{88}N_2O_{37}S$ 1424.5, found 1425.5 ($[M + H]^+$).

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