Cyclodextrin Dimers as Receptor Molecules for Steroid Sensors

Menno R. de Jong, Johan F. J. Engbersen, Jurriaan Huskens,* and David N. Reinhoudt*[a]

Abstract: The dansyl-modified dimer **9** complexes strongly with the steroidal bile salts. Relative to native β-cyclodextrin, the binding of cholate (**1a**) and deoxycholate (**1b**) salts is especially enhanced. These steroids bind exclusively in a 1:1 fashion. For other bile salts (**1c**–**1e**) both 1:1 and 1:2 complexes were observed with stabilities similar to those of native β-cyclodextrin. This indicates that only one cavity is used, with a small contribution from the second. The difference is attributed to the absence of a 12-hydroxy group in the second group of steroids. Comparison with a dimer that lacks the dansyl moiety (**6**) shows that this group especially hinders the cooperative binding of **1a** and **1b**. The smaller interference in the binding of the other steroids indicates that self-inclusion of the dansyl moiety hardly occurs. This weak self-inclusion is supported by fluorescence studies. The dansyl fluorescence of dimer **9** is less blue-shifted than that of other known dansyl-appended cyclodextrin derivatives; this is indicative of a more polar micro-environment. Addition of guests causes a change in fluorescence intensity.

Keywords: calorimetry · cyclodextrins · fluorescence spectroscopy · sensors · steroids

Introduction

Host–guest complexation is an important principle in the development of sensor devices for the detection of ions and neutral molecules. Ions can be detected selectively and sensitively by using receptor molecules in ion selective electrodes or field-effect transistors.[1–3] Recognition and signal transduction are much more difficult for organic species. The detection of organic species is usually based on spectroscopic or mass sensitive methods.

A well-known class of host compounds for organic molecules is the naturally occurring cyclodextrins, cyclic oligosaccharides that consist of six, seven (β-cyclodextrin, Figure 1), or eight glucose moieties.[4, 5] These water-soluble molecules, which are shaped like a truncated cone, possess a hydrophobic cavity that enables the complexation of organic guests in aqueous solution. The possibility of using cyclodextrins for sensor purposes, by appending a fluorescent receptor molecule, has been studied by Ueno[6] and others.[7, 8] In this type of system, the generation of a signal is based on the competition for the cyclodextrin cavity between the covalently attached fluorophore and an added guest. In the absence of a guest, the fluorophore partially resides inside the cavity. Addition of a guest leads to decomplexation of the detector moiety and to a concomitant decrease in fluorescence quantum yield.[9] The selectivity of the sensing molecules resembles that found for native β-cyclodextrin. 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 constants for binding to the receptor compared with binding to β-cyclodextrin.

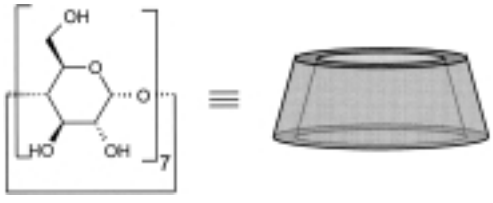

Figure 1. Structure of β-cyclodextrin.

We want to increase the selectivity of cyclodextrin-based sensing molecules toward larger guest molecules. This can be achieved by extending the hydrophobic cavity.[10] In a previous paper, we reported on the use of fluorophore-appended cyclodextrin-calix[4]arene couples as sensing molecules with an enlarged cavity.[11] Their sensing behavior, however, depended heavily on the aggregation state of the hosts and the host–guest complexes.[12] Ueno and co-workers have used biomolecules as large caps for cyclodextrin-based sensing molecules. Thus, cyclodextrin derivatives with monensin moieties,[13] or biotin-modified cyclodextrins that interact with avidin,[14] were investigated as receptors with an enlarged hydrophobic cavity.

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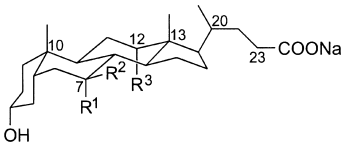
Another way to create a larger cavity is to couple two cyclodextrins to give dimers.[15–17] Covalently coupled cyclodextrins form very strong complexes with appropriate guests.[18–20] Hitherto there has only been one example of a fluorophore-modified cyclodextrin dimer.[21] In this dimer the cyclodextrins are connected by their small, primary sides.

In this paper, we report the synthesis, steroid complexation, and sensing properties of a fluorescent β-cyclodextrin dimer in which the cyclodextrins are connected through their secondary sides. This molecule has enhanced water solubility compared with the previously reported β-cyclodextrin-calixarene couples; this prevents aggregation phenomena from having an influence on the sensing behavior of the host molecules. Cooperation between the two cavities should be more likely when they are connected on the secondary side rather than on the primary side.[22]

Results and Discussion

A class of steroids whose interaction with β-cyclodextrin and derivatives has been well studied is that of the bile acids (Figure 2).[22–28] They are surfactant-like molecules that play a role in the metabolism and excretion of cholesterol in mammals.[29]

They have a characteristic structure, with a side chain at C17, methyl groups at C10, C13, and C20, and a carboxylic acid at C23 of the steroid skeleton. They differ in the number and position of hydroxy groups at C3, C7, and C12. The thermodynamics[25] and kinetics[26] of the inclusion of some bile acid salts into native β-cyclodextrin have been studied by NMR spectroscopy. The thermodynamics of inclusion[30] and the effect of the presence of cyclodextrins on the thermodynamics of micelle formation[27] have been studied by microcalorimetry. The complex geometry of β-cyclodextrin with **1d**,[22] **1a**, and **1b**[28] was studied by ROESY measurements. The aliphatic side chain of the steroid is shown to enter 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 co-workers[28] also studied the formation of complexes between β-cyclodextrin dimers connected at the primary side and **1a** and **1b**. For **1b**, the formation of an oligomeric structure is proposed.



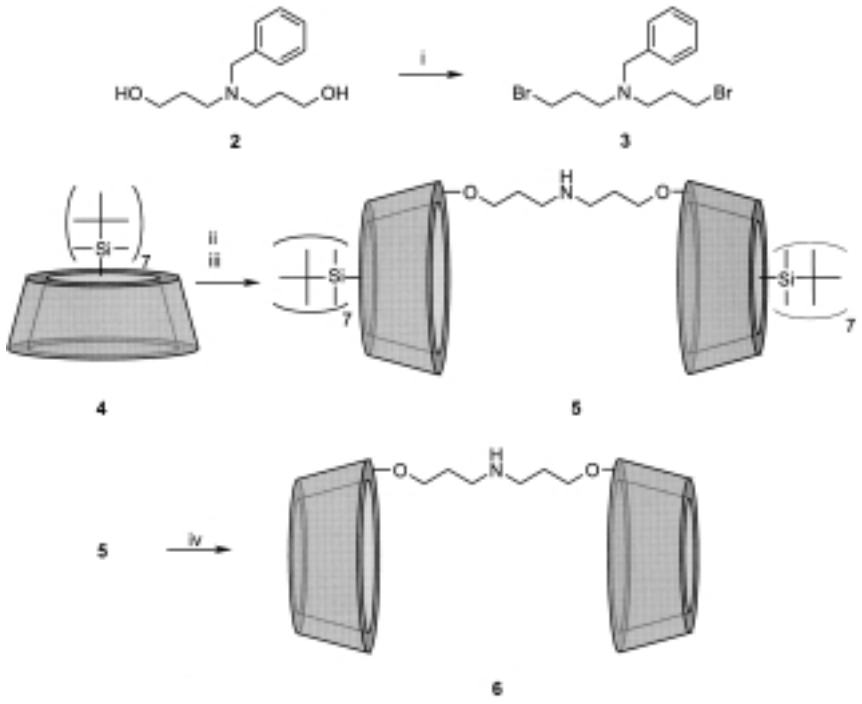
	Steroid	R^1	R^2	R^3
1a	Cholate	OH	H	OH
1b	Deoxycholate	H	H	OH
1c	Chenodeoxycholate	OH	H	H
1d	Ursodeoxycholate	H	OH	H
1e	Lithocholate	H	H	H

Figure 2. Structures of bile salts.

Synthesis: Our strategy for the preparation of cyclodextrin dimers connected through their secondary sides involved the use of β-cyclodextrin that is protected at the primary side with *tert*-butyldimethylsilyl (TBDMS) groups. The dipropylamino-β-cyclodextrin dimer **6** was obtained by deprotonating an excess of heptakis(6-*O*-TBDMS)-β-cyclodextrin **4** with lithium hydride and subsequent reaction with *N*-benzylbis(3-bromopropyl)amine **3** (Scheme 1). This procedure is known to yield cyclodextrin derivatives modified at the C2 position,[8, 10] because the C2 hydroxy group is more acidic than the C3 hydroxy group.[31] The relative simplicity of the ^{1}H and ^{13}C spectra confirms the C_2 symmetry of the dimer and, thus, the twofold C2 substitution.

Because **4** and the *N*-benzyldipropylamino β-cyclodextrin dimer co-eluted during the chromatographic purification, the benzyl group was directly removed by catalytic reduction, to give the pure TBDMS-protected dimer **5**. This was deprotected by using tetrabutylammonium fluoride in THF to give the desired product **6**.

The spacer **8** for the fluorescent dimer **9** (Scheme 2) was synthesized by treating bis(3-hydroxypropyl)amine **7** first with one equivalent of dansyl chloride and subsequently with



Scheme 1. Synthesis of dimer **6**. i) HBr. ii) LiH, **3**. iii) Pd/C, NH_4HCOO. iv) Tetrabutylammonium fluoride.

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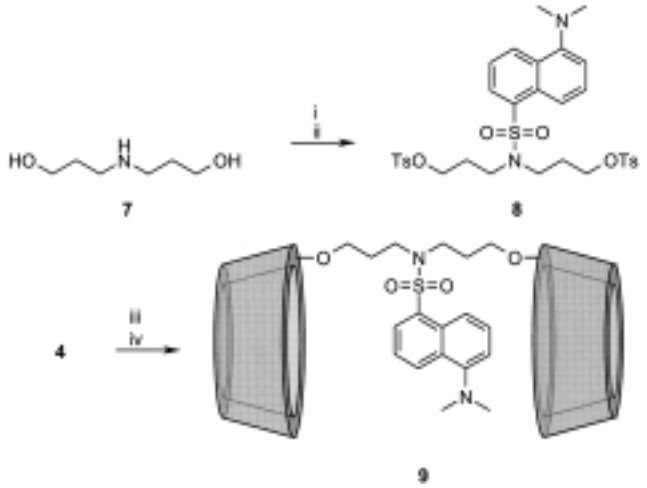

Scheme 2. Synthesis of dimer **9**. i) DansCl, TEA. ii) TsCl, pyridine. iii) LiH, **8**. iv) Tetrabutylammonium fluoride.

two equivalents of tosyl chloride. The cyclodextrin dimer was obtained by treatment of **8** with deprotonated TBDMS-β-cyclodextrin **4** followed by deprotection with tetrabutylammonium fluoride in THF. All compounds were satisfactorily characterized by NMR spectroscopy and FAB-MS.

Complexation of bile acid salts in β-cyclodextrin: The complexation and sensing behavior of **9**, as studied by fluorescence spectroscopy, appeared to be complicated (see below). Therefore, we employed the more universal technique of microcalorimetry to study the binding stoichiometry and thermodynamics. These data were compared with data for **6** in order to elucidate the underlying binding mechanism and the role of the fluorophore. The contribution of a single cavity to the binding thermodynamics of the steroids was assessed by comparison with data for native β-cyclodextrin.

Dilution experiments of the bile salts proved that the titrations with β-cyclodextrin, **6**, and **9** were performed below the critical micelle concentration (cmc) of the bile salts. The titrations of steroids with β-cyclodextrin showed enthalpograms typical of 1:1 complex formation. The thermodynamic data are listed in Table 1.

Cholate (**1a**) and deoxycholate (**1b**) have far lower stability constants than the other steroids. Although there is little agreement in the literature on the exact values for the association constants of bile salts in β-cyclodextrin, this trend usually holds.[23, 25, 27, 28] It is thought that the hydroxy group at the C12 carbon atom of **1a** and **1b** (Figure 2) prevents deep inclusion of the steroid in the cyclodextrin cavity.[25]

Table 1. Thermodynamic parameters (298 K) for the interaction between β-cyclodextrin and bile salts.

Steroid	K [M^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]
1a	4.1×10^3	-4.9	-6.0	-1.1
1b	3.6×10^3	-4.8	-7.0	-2.2
1c	1.8×10^5	-7.1	-7.4	-0.3
1d	7.8×10^5	-8.0	-9.2	-1.2
1e	1.9×10^6	-8.5	-9.7	-1.2

Complexation of bile acid salts in cyclodextrin dimer 6: The complex formation of this dimer was studied by titration of a steroid solution against a solution of dimer **6** in water. The titration data for **1a** and **1b** followed a 1:1 binding model (Figure 3a). However, the binding of chenodeoxycholate (**1c**), ursodeoxycholate (**1d**), and lithocholate (**1e**) to the dimer could only be fitted to a sequential 1:2 binding model (Figure 3b).

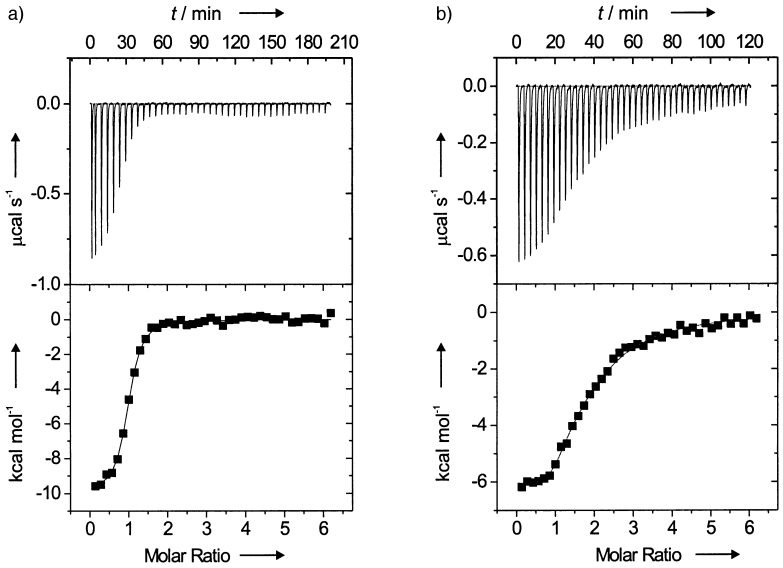

Figure 3. Calorimetric titration of the binding of a) **1a** and b) **1b** to dimer **6**. Upper halves: raw data for sequential 7 µL injections of guest (4.0×10^{-4} M) into host (1.4×10^{-5} M). Lower halves: the integrated heat pulse data corrected for dilution controls (squares) and fit (solid line) to the appropriate binding model.

From Table 2 it can be seen that the formation of 1:1 or 1:2 complexes coincides with the presence or absence of the C12 hydroxy group. Compounds **1a** and **1b**, which have this group, are both bound by the dimers in a 1:1 fashion. The complex stabilities were increased by a factor of 70 and 700, respectively, relative to binding in native β-cyclodextrin (Table 1).

These two steroids can only be partially included in a cyclodextrin cavity, because the C12 hydroxy group prevents deeper protrusion through the cavity.[25] Consequently, a large part of the hydrophobic skeleton is still available for complexation by a second cavity. A large fraction of the increase in binding strength relative to that of native β-cyclodextrin is accounted for by a more favorable entropy component. The loss of conformational flexibility for the cyclodextrin dimer, when both cavities cooperate in the binding of a single guest, is apparently compensated for

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Table 2. Thermodynamic parameters (298 K) for the interaction between dimer **6** and bile salts.

Steroid	K_1 [M^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]	K_2 [M^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]
1a	2.8×10^5	−7.4	−5.3	2.1	–	–	–	–
1b	2.4×10^6	−8.7	−9.4	−0.6	–	–	–	–
1c	5.2×10^6	−9.1	−6.0	3.0	1.4×10^5	−7.0	−7.2	−0.2
1d	3.6×10^6	−8.9	−11.7	−2.7	1.0×10^5	−6.8	−8.4	−1.6
1e	8.9×10^6	−9.4	−9.4	0.0	1.4×10^6	−8.3	−9.0	−0.6

by the release of a large amount of water from the cavity to the bulk.[32]

In the case of the other steroids, deep inclusion into a single β-cyclodextrin unit is possible, leaving the other cavity available for the complexation of a second guest molecule. "Sequential binding" describes the binding behavior accurately, as apparently, the two binding sites in the dimer do not behave as identical, independent binding sites. The binding of a steroid in one cavity influences the binding in the other. The increase of K_1 relative to that found for native β-cyclodextrin indicates a small contribution of the second β-cyclodextrin in the binding of the first steroid molecule. The second steroid has to compete with the first; this leads to the large difference between K_1 and K_2 observed for **1c** and **1d**. The difference between K_1 and K_2 for **1c** is caused by a large positive ΔS upon binding of the first steroid. This suggests that this steroid causes efficient dehydration of both cavities (like **1a**). The ΔH value is not as favorable for **1c** as it is for **1d** and **1e**; this indicates that the fit in the cavity is less tight for **1c** than it is for these last two steroids.[32] In contrast, **1d** shows a stronger and better fit in the dimer than in β-cyclodextrin, as shown by a more favorable enthalpy, and a concomitant greater rigidity, as shown by the decrease in entropy.

Lithocholate **1e** exhibits almost independent binding of a second steroid; this is reflected in the small difference between K_1 and K_2. Moreover, the thermodynamic parameters for both binding events are almost identical to those for the binding to native β-cyclodextrin.

Complexation of bile acid salts in dimer 9: Titrations with **9** as the host, performed under the same conditions as for **6**, revealed a decrease in binding strength of roughly an order of magnitude for **1a** and **1b** bound to **9** when compared with their binding to **6** (Table 3).

This decrease is mainly caused by an unfavorable change in the binding entropy relative to that found with **6**. The binding enthalpy remains almost unchanged.

For the other steroids, the magnitudes of K_1 and K_2 are less affected by the presence of the dansyl group in between the two cavities. However, distinct but opposite changes in binding enthalpy and entropy were observed in nearly all cases.

The small effect of the presence of the dansyl group on K_2 for **1c**, **1d**, and **1e** indicates that the fluorophore does not penetrate deeply into one of the cavities. If it were deep inside, one would expect a reduction of K_2 due to competition between the steroid and the dansyl moiety for the second cavity.

The larger effect of the dansyl moiety on the association constants for **1a** and **1b**, for which the cooperation of both cavities is necessary in order to obtain strong binding, indicates that the fluorophore hinders this cooperation. This notion is in agreement with the mainly entropic nature of the decrease in complex stability for these steroids.

The disturbance of the cooperation in the case of the other steroids is less pronounced, as these steroids are strongly bound in one cavity, and the second cavity offers only a moderate improvement of the binding strength. If a dansyl group is added to this system, the binding strengths of these steroids are still enhanced compared with those with native β-cyclodextrin. The changes in binding entropy and enthalpy indicate that the interactions responsible for the increase in binding strength, relative to that found for native β-cyclodextrin, are not the same as those in dimer **6**. Hydrophobic interactions between the steroid and the fluorophore may play a role.

The calorimetric studies showed that cyclodextrin cavities connected at the secondary side can cooperate in the binding of bile salts. Thus, they differ from previously reported dimers that were connected through the primary side of the cyclodextrins.[21],[28] These did not show the formation of strong 1:1 complexes with **1a** and **1b**, which is in accordance with the different binding behavior suggested here.

Steady state fluorescence measurements with dimer 9: The sensing properties of dimer **9** were studied using fluorescence spectroscopy. The maximum emission wavelength for an aqueous solution of **9** is at 540 nm. The blue-shift relative to *N*,*N*-dimethyl dansylamide, which was reported to have an emission maximum at 572 nm,[11] indicates that the dansyl

Table 3. Thermodynamic parameters (298 K) for the interaction between dimer **9** and bile salts.

Steroid [M^{-1}]	K_1 [kcal mol^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [M^{-1}]	K_2 [kcal mol^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$
1a	3.6×10^4	−6.2	−5.1	1.1	–	–	–	–
1b	1.9×10^5	−7.2	−8.9	−1.7	–	–	–	–
1c	2.2×10^6	−8.6	−8.0	0.7	1.8×10^5	−7.1	−9.1	−1.9
1d	3.1×10^6	−8.8	−10.8	−1.9	2.0×10^5	−7.2	−8.6	−1.4
1e	1.6×10^6	−8.4	−11.7	−3.2	7.6×10^5	−8.0	−11.1	−3.1

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group in the dimer is located in a less polar environment than water. Previously reported dansyl-modified β-cyclodextrin derivatives show larger blue-shifts, down to 520 nm.[8] This is further evidence for the weak inclusion of the dansyl moiety of **9** by the cyclodextrin derivatives cavities.

When well-known guests for β-cyclodextrin, such as adamantanecarboxylate, adamantanamine, and 4-*tert*-butylbenzoate were added to an aqueous solution of **9**, negligible changes in fluorescence intensity were observed. In contrast, addition of the bile salts led to a fluorescence response. In Figure 4a, fluorescence titration curves for the addition of **1a**, **1b**, **1c**, and **1d** to **9** are shown.

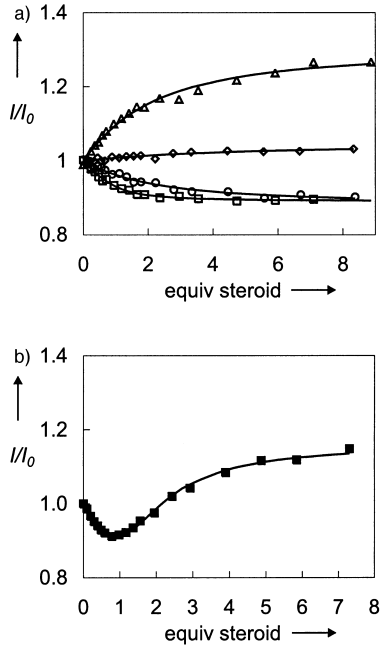

Figure 4. Normalized fluorescence intensity changes of **9** (1.5 μM) at 540 nm as a function of the concentration of bile salts a): **1a** (◇), **1b** (○), **1c** (△), and **1d** (□), b): **1e** (■).

To our surprise, some guest molecules caused an increase in fluorescence intensity. For sensor molecules based on a single dansyl-modified β-cyclodextrin cavity, the addition of guests was always found to lead to a decrease in fluorescence intensity. The different sensing behavior of the dimer probably reflects the shallow inclusion of the dansyl moiety. Interaction of the fluorophore with the hydrophobic steroid skeleton may also improve the shielding from the surrounding water, thereby increasing the fluorescence intensity. The interaction of the hydroxy groups in the steroid may play a role in the quenching.

An interesting observation is that an increase in fluorescence intensity coincides with a positive entropic contribution to the binding strength. Possibly, efficient cavity dehydration (positive entropy) destroys a solvent shell in the vicinity of the complex[32] and this could lead to a more hydrophobic environment for the fluorophore.

The cooperatively bound **1b** is detected by **9** with comparable selectivity to **1d**. In contrast, both monomeric β-cyclodextrin receptors[13, 14, 33] and the dimer, which is connected through the primary side[21] and lacks the possibility of cooperative binding of bile salts, show a strong preference for **1d**.

The data from these titrations were fitted to a 1:1 binding model. Independent Job's plot analyses confirmed the presence of 1:1 complexes for **1b** and **1c**. It is remarkable that the titrations with **1c** and **1d** could be well fitted to a 1:1 binding model, as the binding constants obtained by 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. The calculated values for the association constants are shown in Table 4. They are similar to the K_1 values found by calorimetric titrations.

Table 4. 1:1 binding constants and sensitivities ([**9**] = 1.3 μM, [Guest] = 6.5 μM) for the interaction between dimer **9** and bile salts, as obtained by fluorescence spectroscopy.

Steroid	K_1 [M^{-1}]	K_2 [M^{-1}]	I/I_0
1a	1.2×10^5	–	1.03
1b	5.8×10^5	–	0.91
1c	6.5×10^5	–	1.22
1d	2.5×10^6	–	0.89
1e	6.2×10^6	1.0×10^6	1.11

Only in the titration of **1e** with **9** is the presence of complexes of higher stoichiometry shown by fluorescence. With up to one equivalent of guest the fluorescence intensity decreases, after one equivalent, it starts to increase again (Figure 4b). The data from this titration could be fitted to a 1:2 binding model (Table 4) and the K values obtained are similar to those found by calorimetry.

Conclusion

The cyclodextrin dimers **6** and **9** are very efficient hosts for the complexation of steroids. The stoichiometry of a given steroid – cyclodextrin-dimer complex depends on the complex geometry of that steroid with native β-cyclodextrin. The fluorescence response of dimer **9** on a given steroid is markedly different from that of monomeric sensing molecules. 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. In fact, the looser fit of the reporter dansyl group and the effect of the guest structure on the fluorescence intensity observed here, may lead to sensing molecules with little competition from the reporter and to more guest-sensitive reporters. In addition, optimization of this receptor molecule type may lead to sensors with "on" rather than "off" signaling.

Experimental Section

Materials and methods: β-Cyclodextrin was kindly donated by Wacker-Chemie, München. All chemicals were used as received, unless otherwise stated. Solvents were purified according to standard laboratory methods.[34] Solvents for fluorescence spectroscopy were of analytical grade. Thin-layer

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chromatography was performed on aluminum sheets precoated with silica gel 60 F254 (Merck). The cyclodextrin spots were visualized by dipping the sheets in 5% sulfuric acid in ethanol followed by heating. Chromatographic separations were performed on silica gel 60 (Merck, 0.040–0.063 mm, 230–240 mesh). *N*-benzylbis(3-hydroxypropyl)amine **2**,[35] bis(3-hydroxypropyl)amine (**7**),[35] and TBDMS protected β-cyclodextrin (**4**)[10] were prepared according to literature procedures.

Melting points are uncorrected. Mass spectra were recorded with a Finnigan MAT 90 spectrometer with *m*-nitrobenzyl alcohol/*o*-nitrophenyloctyl ether 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 $CHCl_3$ ($\delta = 7.25$), or HDO ($\delta = 4.65$). ^{13}C chemical shifts (63 or 75 MHz) are given relative to $CDCl_3$. Calorimetric titrations were performed at 25 °C by using a Microcal VP-ITC titration microcalorimeter. Sample solutions were prepared with NaOH (1 mM) in pure water (Millipore Q2). Titrations were performed by adding aliquots of a guest solution to the host solution. The titrations were analyzed with a least-squares-curve fitting procedure. Control experiments involved addition of guest to NaOH (1 mM) solution and addition of NaOH (1 mM) to a host solution. Fluorescence measurements were performed on an Edinburgh SF 900 spectrometer. Sample solutions were prepared with a phosphate buffer (pH 7, $I = 0.02$) in pure water (Millipore Q2). Fluorescence titrations were performed by adding aliquots of guest in host solution to the pure host solution and vice versa. After each addition the fluorescence spectrum was recorded.

***N*-Benzylbis(3-bromopropyl)amine (3)**: *N*-benzylbis(3-hydroxy)propylamine (**2**, 0.35 g, 1.6 mmol) was heated under reflux in concentrated HBr (6 mL) for 36 h. The solution was neutralized with NaOH (1 mM), after which CH_2Cl_2 was added. The organic layer was washed with water and brine, and dried over $MgSO_4$ to obtain **3** as a yellowish oil in 91% yield. ^{1}H NMR ($CDCl_3$): $\delta = 7.37–7.27$ (m, 5H), 3.58 (s, 2H), 3.47 (t, $J = 6.5$ Hz, 4H), 2.60 (t, $J = 6.6$ Hz, 4H), 2.08–1.99 (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta =$ 139.3, 128.7, 128.3, 127.0, 58.8, 52.0, 31.8, 30.5; MS (FAB): *m*/*z* calcd for $[MH]^+$ 348.0; found 348.1.

TBDMS-protected dipropylaminocyclodextrin dimer 5: LiH (30 mg, 3.7 mmol) was added to a solution of dried (100 °C, 0.1 mbar, 5 h) **4** (6.0 g, 3.1 mmol) in dry THF (50 mL). The mixture was stirred for one hour at room temperature and then for one week under reflux. During the first three days, **2** (0.53 g, 1.5 mmol) was added in portions. The solvent was removed in vacuo, and chloroform was added. The solution was washed with water (2 ×) and brine, and dried over $MgSO_4$. After removal of the solvent, the crude product was purified by column chromatography (ethyl acetate/ethanol/water 100:2:1), after which a mixture of **4** and benzyl protected cyclodextrin dimer was obtained. A solution of this mixture (1.0 g) and ammoniumformate (2.0 g) in EtOH (150 mL) was heated under reflux for 2 h in the presence of a catalytic amount of 10% Pd/C. The catalyst was removed by filtration through Celite, and the solvent was removed in vacuo. The product was purified by repeated column chromatography (ethyl acetate/ethanol/water 10:2:1, CH_2Cl_2/MeOH 85:15) to obtain **5** as a white powder in 5% yield (two steps). ^{1}H NMR ($CDCl_3$/CD_3OD): $\delta = 4.88–4.85$ (m, 14H), 4.27–3.35 (m, 92H), 2.01–1.88 (m, 4H), 1.05–0.84 (m, 126H), 0.04–0.00 (m, 84H); ^{13}C NMR ($CDCl_3$/CD_3OD): $\delta = 102.5–101.3$, 82.1–79.8, 73.6–72.2, 61.9–61.5, 26.0, 25.9, 25.8, 18.5, 18.3, 18.2, 1.0, −5.0, −5.1, −5.2, −5.3, −5.4; MS (FAB): *m*/*z* calcd for $[M+Na]^+$ 3986; found 3988.6.

Dipropylaminocyclodextrin dimer 6: TBDMS-protected dimer **5** (123 mg, 0.031 mmol) was dissolved in THF (10 mL). After addition of a solution of tetrabutylammonium fluoride in THF (1 M, 1 mL), the solution was refluxed overnight. The solvent was removed in vacuo, and the residue dissolved in water. After washing with hexane (3 ×), ions were removed over amberlite ion-exchange resin. After freeze-drying, the dimer was obtained as a white powder in 50% yield. ^{1}H NMR (D_2O): $\delta = 5.11–5.10$ (m, 2H), 4.97–4.96 (m, 12H), 3.98–3.37 (m, 88H), 3.14–3.08 (m, 4H), 1.93–1.85 (m, 4H); ^{13}C NMR (D_2O): $\delta = 104.8–102.0$, 84.0–82.8, 75.6–75.4, 74.5–74.0, 71.7, 62.8, 48.1, 28.9; MS (FAB): *m*/*z* calcd for $[M+Na]^+$ 2388.8; found 2388.9.

***N*,*N*-bis(3-hydropropyl)dansylamide 10**: Dansyl chloride (1.0 g, 3.7 mmol) was added to a solution of dipropanolamine (0.60 g, 4.5 mmol) and triethylamine (1.5 g, 15 mmol) in CH_2Cl_2 (20 mL). The solution was stirred at room temperature for 1 h. The organic layer was washed with a saturated aqueous solution of NH_4Cl, water, and brine, and dried over $MgSO_4$. After evaporation of the solvent, **10** was obtained as a greenish oil in 96% yield. ^{1}H NMR ($CDCl_3$): $\delta = 8.53$ (d, $J = 8.7$ Hz, 1H), 8.29 (d, $J = 8.4$ Hz, 1H), 8.09 (d, $J = 7.2$ Hz, 1H), 7.58–7.48 (m, 2H), 7.18 (d, $J = 7.8$ Hz, 1H), 3.62 (t, 4H), 3.45 (t, 4H), 288 (s, 6H), 1.79–1.71 (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta =$ 151.7, 134.9, 130.3, 130.0, 129.9, 128.8, 128.2, 123.1, 119.2, 115.2, 59.1, 45.3, 44.9, 31.2; MS (FAB): *m*/*z* calcd for $[M+H]^+$ 367.2, found 367.1.

***N*,*N*-bis(3-tosyloxypropyl)dansylamide 8**: A solution of tosyl chloride (4.0 g, 21 mmol) in pyridine (20 mL) was added to a solution of **10** (1.3 g, 3.5 mmol) in pyridine (20 mL) at 0 °C. The solution was stirred at this temperature for 3 h. The mixture was shaken for 5 min with ice water and then acidified (pH 2) by using concentrated HCl. The product was extracted with ethyl acetate. The organic layer was washed with water, NaOH (1 M), water, and brine, and dried over $MgSO_4$. After evaporation of the solvent, the crude product was purified by column chromatography (ethyl acetate/hexanes 1:1) to give **9** as a greenish oil in 67% yield. ^{1}H NMR ($CDCl_3$): $\delta = 8.55$ (d, $J = 8.4$ Hz, 1H), 8.18 (d, $J = 9$ Hz, 1H), 8.10 (d, $J = 7.2$ Hz, 1H), 7.73 (d, $J = 8.1$ Hz, 4H), 7.53–7.47 (m, 2H), 7.34 (d, $J = 8.4$ Hz, 4H), 7.19 (d, $J = 7.5$ Hz, 1H), 3.93 (t, $J = 6$ Hz, 4H), 3.26 (t, $J = 7.2$ Hz, 4H), 2.89 (s, 6H), 2.45 (s, 6H), 1.87–1.78 (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 151.9$, 145.0, 134.2, 132.7, 130.7, 129.9, 128.3, 127.9, 123.1, 119.0, 115.3, 67.6, 45.4, 44.8, 44.5, 28.1, 21.6; MS (FAB): *m*/*z* calcd for $[M+H]^+$ 674.2; found 674. 2.

TBDMS-protected fluorescent cyclodextrin dimer 11: LiH (3 mg, 0.37 mmol) was added to a solution of dried (100 °C, 0.1 mbar, 5 h) TBDMS-protected β-cyclodextrin **4** (0.50 g, 0.26 mmol) in THF (50 mL). The mixture was stirred for 1 h at room temperature and then for 2 h under reflux. A solution of **8** (68 mg, 0.10 mmol) in THF (5 mL) was added and heating under reflux was continued for 5 days. The solvent was removed in vacuo and chloroform was added. The solution was washed with HCl (1 M), water, and brine, and dried over $MgSO_4$. After removal of the solvent and purification by column chromatography (ethyl acetate/ethanol/water 100:2:1) the product was obtained as a light green powder in 22% yield. ^{1}H NMR ($CDCl_3$/CD_3OD): $\delta = 8.51$ (d, $J = 8.4$ Hz, 1H), 8.24 (d, $J = 8.4$ Hz, 1H), 8.13 (d, $J = 7.5$ Hz, 1H), 7.55–7.45 (m, 2H) 7.15 (d, $J = 7.8$ Hz, 1H), 4.93–4.88 (m, 14H), 4.18–3.03 (m, 92H), 2.86 (s, 6H), 2.23–2.17 (m, 4H), 0.90–0.83 (m, 126H), 0.07–0.00 (m, 84H); ^{13}C NMR ($CDCl_3$/CD_3OD): $\delta = 151.7$, 135.4, 130.1, 130.0, 129.4, 127.9, 123.0, 119.7, 115.1, 102.4, 102.1, 101.9, 101.5, 99.1, 83.1, 82.0, 81.9, 81.8, 81.7, 80.9, 80.8, 79.5, 77.2, 73.6, 73.3, 73.2, 73.1, 72.5, 72.2, 72.0, 70.8, 62.5, 61.6, 61.1, 45.4, 43.9, 29.7, 28.1, 25.9, 25.7, 18.4, 18.3, 18.1, 1.0, −5.3, −5.3; MS (FAB): *m*/*z* calcd for $[M+Na]^+$ 4219.1; found 4221.6.

Fluorescent cyclodextrin dimer 9: TBDMS protected dimer **11** (140 mg, 0.034 mmol) was dissolved in THF (10 mL). After addition of a solution of tetrabutylammonium fluoride in THF (1 M, 1 mL), the solution was refluxed overnight. The solvent was removed in vacuo, and the residue dissolved in water. After washing with hexane (3 ×), salts were removed over amberlite ion-exchange resin. After freeze-drying, the dimer was obtained as a yellowish powder in 76% yield. ^{1}H NMR (D_2O): $\delta = 8.52$ (d, $J = 7.8$ Hz, 1H), 8.21–8.17 (m, 2H), 7.70–7.65 (m, 2H) 7.23 (d, $J = 7.5$ Hz, 1H), 5.01–4.91 (m, 14H), 3.96–3.27 (m, 92H), 2.79 (s, 6H), 1.88–1.65 (m, 4H); ^{13}C NMR (D_2O): $\delta = 153.9$, 135.6, 133.0, 132.4, 131.8, 129.9, 104.4, 84.4–83.6, 75.6–71.7, 62.7, 48.0, 30.6; MS (FAB): *m*/*z* calcd for $[M+H]^+$ 2599.9; found 2600.8.

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