Novel Water-Soluble β-Cyclodextrin−Calix[4]arene Couples as Fluorescent Sensor Molecules for the Detection of Neutral Analytes

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The synthesis and the host−guest chemistry of two novel fluorescent β-cyclodextrin−calix[4]arene couples **1** and **2** is described. The compounds were prepared by coupling a β-cyclodextrin linked with an aminoxylyl spacer with two different calix[4]arene building blocks each bearing a fluorophore. The fluorophores attached to **1** and **2** are the 2-naphthylamine and the dansyl moiety, respectively. The unsymmetric calix[4]arene functionalization was achieved by monoprotection of the 1,3-diformylcalix[4]arene. The host molecules **1** and **2** were used as fluorescent probes for several organic analytes in aqueous solution. **1** shows sensitivity for a series of neutral organic guest species, such as steroids, terpenes, and other natural products. In contrast, compound **2** does not show sensitivity for organic analytes in aqueous solution. The different behavior of the two fluorescent probes **1** and **2** is explained by the different strength of self-inclusion of each fluorphoric group into the β-cyclodextrin cavity.

Introduction

Host−guest complexation is one of the basic principles in the development of new devices for sensing of ions or neutral molecules. In the field of ion sensing, high sensitivity and selectivity have been achieved with a variety of ion receptor molecules applied in ion selective electrodes or field effect transistors.[1−3] However, for neutral molecules both selective recognition and its transduction are much more difficult and detection of neutral organic species is mainly based on mass sensitive or spectroscopic measurements.[4−10] A class of natural host compounds for organic molecules is the cyclodextrins, which are cyclic oligosaccharides consisting of 6, 7, or 8 glucose moieties.[11,12] They are water soluble and have the shape of a truncated cone with a hydrophobic cavity, which renders them capable of complexation of organic molecules. To transduce the complexation of cyclodextrins with guests into a physical signal, Ueno and others have prepared fluorescent cyclodextrins by appending a fluorophore to one of the hydroxyl groups of β- and γ-cyclodextrins.[5−9] Sensing is based on the competition for the hydrophobic cavity between a guest molecule and the covalently linked fluorophore resulting in a change in optical properties. Without appropriate guest species present, the fluorophore is partially residing in the hydrophobic cavity of the cyclodextrin, whereas the presence of organic analytes leads to decomplexation of the fluorophore and a concomitant decrease of its fluorescence quantum yield.[13]

The most important selectivity parameter for functionalized cyclodextrins is the size fit between host and guest. For the development of sensors for specific molecules, it is necessary to introduce further selectivity to the host−guest complexation characteristics of cyclodextrins by attaching further functional groups or building blocks. We have followed this approach by attaching functionalized calix[4]arenes to the cyclodextrin moiety. Calix[4]arenes are a class of well-known and frequently used building blocks in supramolecular chemistry that can serve as a molecular platform and offer eight positions for substitution, four each at the upper and the lower rims.[14,15] Their chemistry is rather well explored, and they are relatively easy to modify selectively.[16,17] Calix[4]arenes can be attached to surfaces via spacers on their lower rim which offers the opportunity for immobilization of sensing molecules.[18−20] In previous experiments, we

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(1) Cobben, P. L. H. M.; Egberink, R. J. M.; Bomer, J. G.; Bergveld, P.; Verboom, W.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1992**, *114*, 10573.
(2) Engbersen, J. F. J.; Reinhoudt, D. N. *Analysis* **1994**, *22*, M16.
(3) Antonisse, M. M. G.; Lugtenberg, R. J. W.; Egberink, R. J. M.; Engbersen, J. F. J.; Reinhoudt D. N. In *Chemosensors of Ion and Molecule Recognition*; Desvergne, J. P., Czarnik, A. W., Eds.; Kluwer Academic Publ.; The Netherlands, 1997; p 23.
(4) Diamond, D.; McKervey, M. H. *Chem. Soc. Rev.* **1996**, 15.
(5) Ueno, A. *Supramol. Sci.* **1996**, *3*, 31 and references cited herein.
(6) Ikeda, H.; Nakamura, M.; Ise, N.; Oguma, N.; Nakamura, A.; Ikeda, T.; Toda, F.; Ueno,A. *J. Am. Chem. Soc.* **1996**, *118*, 10980.
(7) Liu, Y.; Zhang, Y.-M.; Qi, A.-D.; Chen, R.-T.; Yamamoto, K.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1997**, *62*, 1826.
(8) Hamasaki, K.; Usui, S.; Ikeda, H.; Ueno, A. *Supramol. Chem.* **1997**, *8*, 125.
(9) Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartor, G. *J. Org. Chem.* **1997**, *62*, 6283.
(10) Dickert, F. L.; Bauer, P. A. *Adv. Mater.* **1991**, *3*, 436.
(11) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803.
(12) Szejtli, J. *Cyclodextrin Technology*, Kluwer Academic Publishers: Dordrecht, 1988.
(13) Corradini, R.; Dossena, A.; Marchelli, R.; Panagia, A.; Sartor, G.; Saviano, M.; Lombardi, A.; Pavone, V. *Chem. Eur. J.* **1996**, *2*, 373.
(14) Gutsche, C. D. *Calixarenes, Monographs in Supramolecular Chemistry*; Stoddart, J. F., Ed.; Royal Society of Chemistry: Cambridge, 1989; Vol. 1.
(15) Böhmer,V. *Calixarenes: A versatile Class of Macrocyclic Compounds*; Vicens, J., Böhmer, V., Eds.; Kluwer Academic Publishers: Dordrecht, 1991.
(16) Böhmer, V. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713.
(17) Takeshita, M.; Shinkai, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1088.
(18) Thoden van Velzen, E. U.; Engbersen, J. F. J.; de Lange, P. J.; Mahy, J. W.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 6853.
(19) Schierbaum, K.-D.; Weiss, T.; Thoden van Velzen, E. U.; Engbersen, J. F. J.; Reinhoudt, D. N.; Göpel, W. *Science* **1994**, *265*, 1413.

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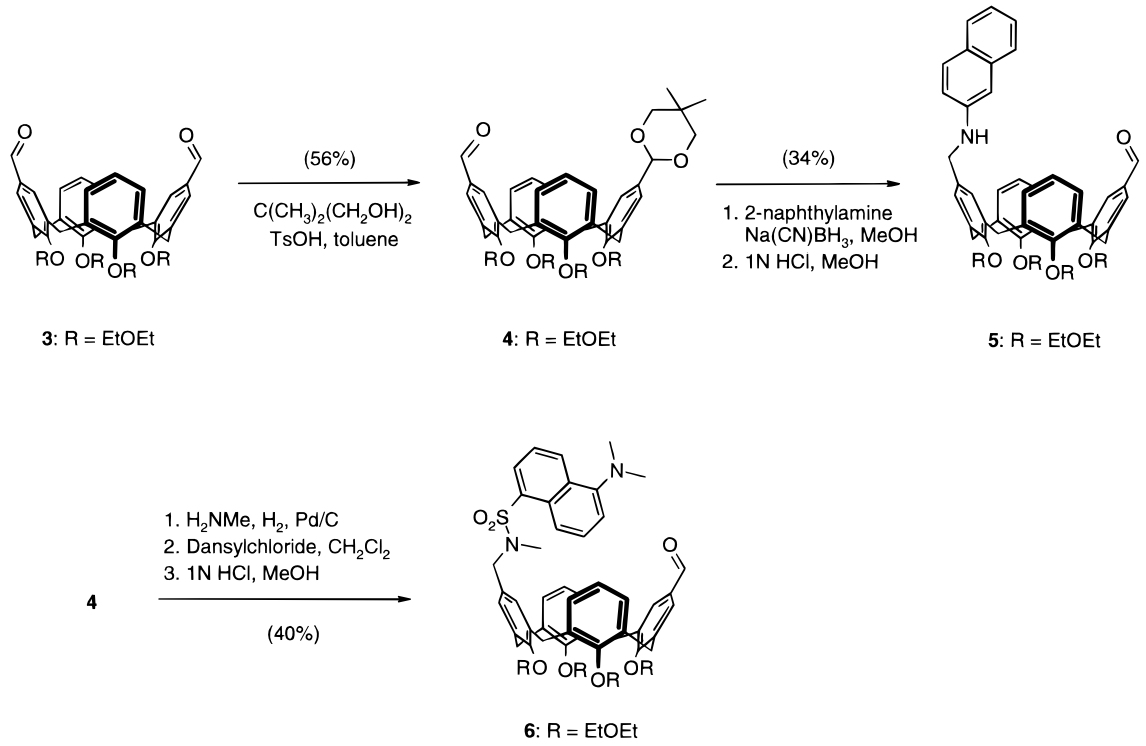
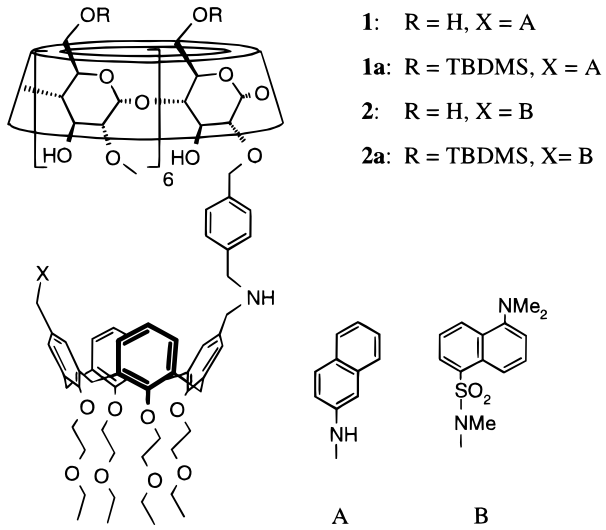
Scheme 1



have coupled β-cyclodextrin to calix[4]arene via a *p*- and an *o*-xylyl spacer.[21] Fluorescence titrations with these β-cyclodextrin−calix[4]arene couples gave up to 400-fold increased binding constants for the fluorescent probes 1-anilino-8-naphthalenesulfonate (ANS) and 2-*p*-toluidino-6-naphthalenesulfonate (TNS) in aqueous solution compared with native β-cyclodextrin.[22] This increased binding was attributed to an extension of the hydrophobic cavity of the cyclodextrin by the calix[4]arene moiety. That is, the calix[4]arene does not only offer positions for further modification but also improves the complexation characteristics. A two-component sensor system, consisting of an independent fluorophore as reporter group and the β-cyclodextrin−calix[4]arene couple as host molecule, was successfully tested in aqueous solution for its ability to respond on the addition of organic analytes.[22] The next step toward a realistic sensing system is to combine the optical reporter and the cyclodextrin−calix[4]arene host covalently in one molecule.

In this paper we report the synthesis and the host−guest chemistry of such novel cyclodextrin−calix[4]arene couples **1** and **2** (Chart 1) which have fluorophores covalently attached at the calix[4]arene moiety as a reporter group for guest inclusion.

Chart 1



Results and Discussion

Fluorescent β-Cyclodextrin−Calix[4]arene Couples. The β-cyclodextrin−calix[4]arene couples **1** and **2** were synthesized in a convergent way. The calix[4]arene building blocks **5** and **6** as well as the functionalized cyclodextrin derivative **7** were synthesized and coupled in one of the last reactions. The key step in the synthesis of the unsymmetrically substituted calix[4]arenes **5** and **6** is the monoprotection of bisaldehyde **3** (Scheme 1).

Compound **3** was obtained by Gross formylation of tetra(2-ethoxyethyl)calix[4]arene following a literature procedure.[23] Monoprotection of the bisaldehyde was achieved by reacting **3** with 1 equiv of 2,2-dimethyl-1,3-propanediol in refluxing toluene and TsOH as catalyst.[24] The yield of 56% for monoaldehyde−mono-1,3-dioxolane **4** was slightly higher than statistical, and 25% of the

(20) Huisman, B.-H.; Thoden van Velzen, E. U.; van Veggel, F. C. J. M.; Engbersen, J. F. J.; Reinhoudt, D. N. *Tetrahedron Lett.* **1995**, *36*, 3273.

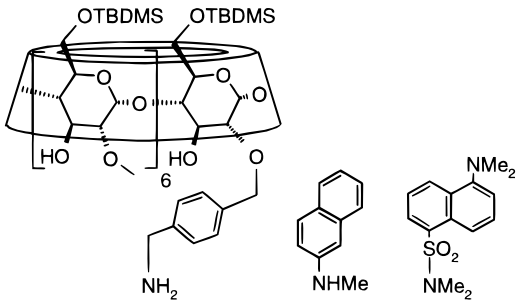
(21) van Dienst, E.; Snellink, B. H. M.; von Piekartz, I.; Grote Gansey, M. H. B.; Venema, F.; Feiters, M. C.; Nolte, R. J. M.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem.* **1995**, *60*, 6537.

(22) van Dienst, E.; Snellink, B. H. M.; von Piekartz, I.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Chem. Soc., Chem. Commun.* **1995**, 1151.

(23) Arduini, A.; Fanni, S.; Manfredi, G.; Pochini, A.; Ungaro, R.; Sicuri, A. R.; Ugozzoli, F. *J. Org. Chem.* **1995**, *60*, 1448.

(24) In our hands, reacting **3** simply with 1,3-propanediol as a protecting agent led to a redox reaction which did not result in the desired monoprotected product.

Chart 2



7 **8** **9**

starting compound **3** could be recovered. For the chromatographic workup, the silica column was pretreated with the eluent and 2 v/v% of triethylamine, as the 1,3-dioxolane unit of **4** is partly cleaved on acidic silica. The 2-naphthylamine moiety was attached onto **4** by formation of the Schiff base from **4** and 2-naphthylamine in dry methanol. Reduction with sodium cyanoborohydride and cleavage of the dioxolane protective group by addition of hydrochloric acid gave the fluorophore-appended calix[4]arene **5** in a yield of 34%. For connecting the dansyl (= 1-dimethylaminonaphthalene-5-sulfonyl) fluorophore to aldehyde **4**, a reductive amination with methylamine was performed. The resulting methylamine−dioxolane−calix[4]arene was used without further purification for the esterification with dansyl chloride. After in situ hydrolysis of the dioxolane protective group and flash chromatography, **6** was obtained in 40% yield. The cyclodextrin building block **7** (Chart 2) was prepared as previously reported from heptakis(6-*O*-TBDMS)-β-cyclodextrin and *p*-bromomethylbenzonitrile.[21] The overall yield for **7** starting from β-cyclodextrin was 26%. This lipophilic β-cyclodextrin building block with seven TBDMS groups allows an easy workup of the coupled products by standard silica gel chromatography. The combination of the building block **7** with the calixarenes **5** or **6**, respectively, was achieved by a reductive amination using sodium cyanoborohydride in methanol. After purification by column chromatography, **1a** and **2a** were obtained in 66% and 67% yields, respectively. The coupled products **1a** and **2a** were deprotected at the primary hydroxyls with silicium tetrafluoride in acetonitrile.[25] The final purification of **1** and **2** was achieved by dialysis in perbenzoylated cellulose tubes, resulting in **1** and **2** as colorless powders after freeze-drying in yields of 60% and 65%, respectively. The lack of symmetry both of the calix[4]arene and of the cyclodextrin moiety of the novel compounds renders their NMR spectra rather complicated. The signals for the protons of the cyclodextrin moiety coincide with the signals for the protons of the ethylethoxy chains and the methylene bridges in the calixarene parts of the molecules. Compounds **1** and **2** are soluble both in water and in polar organic solvents.[26]

Fluorescence Measurements. The maximum emission wavelength of an aqueous solution of the fluorescent probe **1** is at 408 nm, and for **2** it is at 538 nm. The

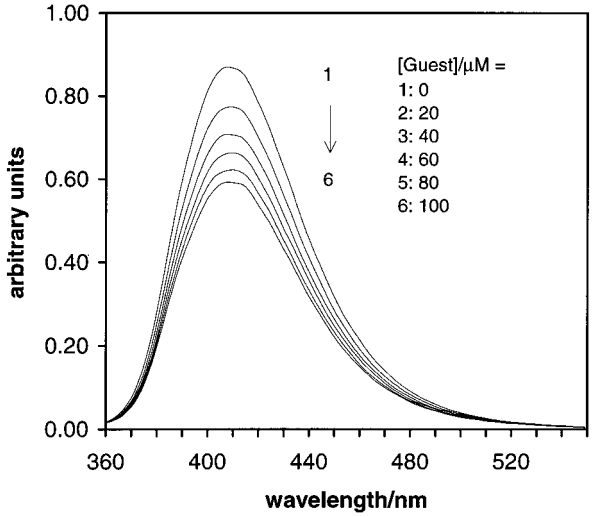


Figure 1. Fluorescence spectra of **1** in aqueous solution upon addition of guest = ethinyl-nortestosterone ([**1**] = 1.45 μM, λ_{ex} = 350 nm, λ_{em} = 408 nm).

reference compounds **8** and **9**, which resemble the two fluorophores that are appended at **1** and **2**, respectively, show maximum fluorescence emissions at 418 and 572 nm in aqueous solution. The blue shift of 10 and 34 nm for the attached fluorophores in **1** and **2** respectively, indicates that these are located in a less polar environment than the native fluorophores in water. The higher blue shift for the dansyl fluorophore compared to the naphthyl group is due to its higher sensitivity toward changes in the polarity of its environment.[27] The above observations support the idea that the fluorophores of compounds **1** and **2** are shielded from the aqueous environment and most probably are located intramolecularly in the cyclodextrin cavities.

Competition experiments provide further evidence for this assumption. The fluorescence intensity of an aqueous solution of **1** decreased upon addition of various guest species. In Figure 1, the changes in relative fluorescence intensity of **1** (1.45 μM) upon the addition of ethinyl-nortestosterone is shown. The excitation maximum in the absorption spectra does not shift, which excludes that a change in absorption is the reason for the decrease in fluorescence intensity. As water is known to quench fluorescence emission by radiationless decay, this implies that the 2-aminonaphthyl fluorophore in **1** becomes more exposed to water after addition of a guest.

An additional indication for this mechanism is the selectivity pattern of **1** for organic analytes. The selectivity of host molecules toward different analytes is generally expressed in terms of $\Delta I/I_0$ values for each guest species, where ΔI is the difference in the fluorescence intensity of the solution before and after addition of a guest species.[28] These values reflect binding constants K and intrinsic fluorescence I_∞ at the saturation point and are concentration dependent. To obtain comparable results, they are measured at saturation of complexation. The $\Delta I/I_0$ values for **1** and several organic guest molecules are shown in Figure 2. Sensing molecule **1** shows sensitivity for steroids, terpenes, and some of the other

(25) Corey, E. J.; Yi, K. Y. *Tetrahedron Lett.* **1992**, *33*, 2289.

(26) Compounds **1** and **2** dissolve in water up to approximately 2 mg/mL. They also form aggregates in aqueous solution, as was concluded from the soap-like appearance and line broadening in ^{1}H NMR. This phenomenon is under investigation at the moment.

(27) Lakowicz, J. R., Ed. *Principles of Fluorescence Spectroscopy*; Plenum Publishing: New York, 1983.

(28) Ueno, A.; Minato, S.; Suzuki, J.; Fukushima, M.; Ohkubo, M.; Osa, T.; Hamada, F.; Murai, K. *Chem. Lett.* **1990**, 605.

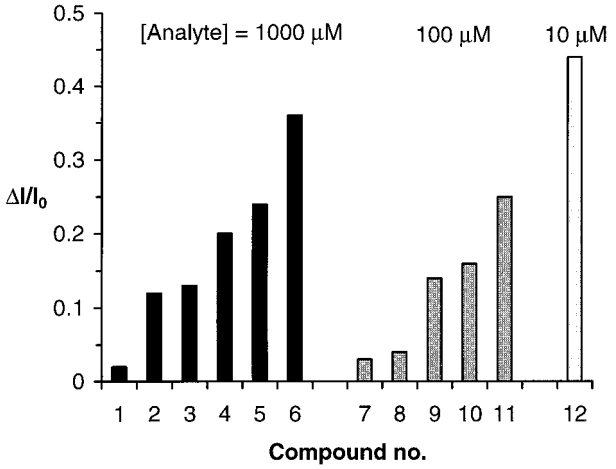


Figure 2. $\Delta I/I_0$ values for several organic analytes and host compound **1** ([**1**] = 1.45 μM): 1, Atropine; 2, *p-tert*-butylbenzoic acid; 3, *dl*-menthol; 4, skatol; 5, *dl*-carvone; 6, 1-adamantanol; 7, prednisolone; 8, cholic acid; 9, cholesterol; 10, corticosterone-acetate; 11, ethinyl-nortestosterone; 12, 1-adamantylbenzoate.

tested organic analytes. The sensitivity for steroids is approximately 10 times higher than that for terpenes. This selectivity within this series of analytes suggests that the cyclodextrin cavity is involved in the complexation process.[6]

Further quantification of the host−guest chemistry of **1** was achieved by fitting the data from fluorescence titrations of **1** to a Benesi−Hildebrandt type equation for 1:1 host−guest complexation (eq 1).[29] In this equation, ΔI denotes the change in fluorescence intensity of **1** upon addition of guest species, I_0 and I_∞ are the fluorescence intensities in the absence of guest and at the saturation point, respectively, and [G] refers to the total concentration of added analyte. The assumption implied in this equation, that the host concentration is much smaller than the concentration of added analyte, is valid under the conditions employed. In the case of a 1:1 host/guest stoichiometry, a plot of $1/\Delta I$ against 1/[G] should give a straight line from which the association constant K_{ass} is obtained by dividing the intercept with the ascent. For

$$\frac{1}{\Delta I} = \frac{1}{(I_0 - I_\infty)K_{\mathrm{Ass}}}\frac{1}{[\mathrm{G}]} + \frac{1}{(I_0 - I_\infty)} \qquad (1)$$

ethinyl-nortestosterone (norethindrone) and 1-adamantanol, a fluorescence titration was performed and the data were evaluated using eq 1. Straight lines were found for the plots of $1/\Delta I$ against 1/[G] indicating 1:1 complex stoichiometries. The resulting apparent association constant for the complex [**1** + norethindrone] (K = 10300 ± 1100 M^{-1}) was larger than the one for [**1** + adamantanol] (K = 1240 ± 120 M^{-1}). This is a remarkable difference compared to the association behavior of native β-cyclodextrin, which complexes 1-adamantanol (5900 M^{-1})[30] more strongly than norethindrone (940 M^{-1}).[31] This result suggests that the calix[4]arene moiety in **1** not only acts as a useful building block for future modifications but also changes the complexation properties of the novel sensing molecule compared to native β-cylodextrin by expanding the available hydrophobic cavity. More specifically, for steroid complexation it can provide an additional surface to enhance the hydrophobic interactions.[32]

In contrast to the behavior of compound **1**, aqueous solutions of compound **2**, having a dansyl group as a fluorophore, do not show a change in their fluorescence intensity upon the addition of guests. CPK models suggest a very strong, intramolecular inclusion of the dansyl group into the cyclodextrin cavity. Similar to the naphthyl group, the dansyl moiety can penetrate deeply into the cyclodextrin cavity, but the latter can also form strong hydrogen bonds between its sulfonamide group and the secondary hydroxyl groups of the cyclodextrin. Further evidence for the strong inclusion of the dansyl group is the large blue shift of the maximum fluorescence emission. Such a strongly included fluorophore can then hardly be expelled upon addition of organic guest species, explaining the insensitivity of **2** toward organic analytes. A similar behavior for a fluorophore appended to the secondary side of the natural β-cyclodextrin has been reported recently.[33]

Summary

In summary, we have shown that by a convergent approach different β-cyclodextrin−calix[4]arene host molecules with a fluorescent probe can be synthesized. The novel fluorescent sensing molecule **1** shows sensitivity for analytes such as steroids and terpenes. Its complexation properties are different compared to those of native β-cyclodextrin. This effect is attributed to the appended calixarene moiety which represents an enlargement of the cyclodextrin cavity. The similar compound **2** does not show sensitivity which is attributed to the strong inclusion of its fluorophore into the cyclodextrin cavity where it can hardly be expelled by external guest species. The difference in host−guest chemistry between compounds **1** and **2** demonstrates the importance of the right choice of the attached fluorophore. In our current synthetic efforts, we further use the possibilities for functionalization of the calix[4]arene moiety in order to optimize the binding properties of **1** and to enable attachment to surfaces.

Experimental Section

Materials and Methods. β-Cyclodextrin was kindly donated by Wacker-Chemie, München. All other 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. All reactions were carried out in an inert atmosphere. TLC was performed on aluminum sheets precoated with silica gel 60 F254 (E. 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 (E. Merck, 0.040−0.063 mm, 230−240 mesh). Compounds **3** and **7** were prepared according to literature procedures.[21,23] The reference fluorophore **8** was prepared from

(29) Connors, K. A. *Binding Constants*; Wiley & Sons: New York, 1987.
(30) Wang, Y.; Ikeda, T.; Ueno, A.; Toda, F. *Tetrahedron Lett.* **1993**, *31*, 4971.
(31) Sadlej-Sosnowska, N. *J. Incl. Phenom. Mol. Recogn. Chem.* **1997**, *27*, 31.
(32) Higler, I.; Timmerman, P.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1996**, *61*, 5920.
(33) Nelissen, H. F. M.; Venema, F.; Nolte, R. J. M. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2045.
(34) Perrin, D. D.; Armarego, W. F. L. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1989.

2-naphthylamine by monomethylation;[35] fluorophore **9** was prepared from dansyl chloride and dimethylamine.[36] Their analytical data were in accordance with literature data. For reasons of clarity, the name calix[4]arene was used instead of the official IUPAC name: pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]-octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecane. Melting points are uncorrected. For mass spectra recorded in the FAB mode, *m*-nitrobenzyl alcohol was used as a matrix; for MALDI-TOF, mass spectroscopy dihydroxybenzoic acid was used. ^{1}H NMR and ^{13}C NMR spectra were recorded at 250 and 63 MHz, respectively, and 297 K, unless otherwise stated. Residual solvent protons were used as internal standard. The preparation of the solutions for fluorescence spectroscopy was done using pure water (Millipore Q2) and a phosphate buffer (pH 7, $I = 0.02$). Fluorescence titrations were performed by adding aliquots (typically 10 μL) of the methanolic stock solution of the guest species to the aqueous host solution (3 mL). After each addition the fluorescence spectrum was recorded. The observed reduction in fluorescence intensity was corrected for the effect of pure methanol ($\Delta I < 3\%/100$ μL of methanol).

5-(5,5-Dimethyl-1,3-dioxolane-2-yl)-17-formyl-25,26,27,28-tetra(2-ethoxyethoxy)calix[4]arene (4). A solution of diformylcalix[4]arene **3** (2.0 g, 2.51 mmol), 2,2-dimethyl-1,3-propanediol (0.26 g, 2.51 mmol), and 10 mg of TsOH in 200 mL of toluene was refluxed for 24 h (Ott apparatus, molecular sieves (3 Å)). Subsequently, the solution was filtered over Na_2CO_3 and the solvent was evaporated. The crude reaction mixture was purified by chromatography (eluent: hexanes/ethyl acetate 2:1, column pretreated with hexanes/ethyl acetate 2:1 + triethylamine 2 v/v%) to give **4** as a thick, colorless oil in 56% yield (1.20 g, 1.40 mmol). A total of 25% of the starting material **3** could be recovered (0.50 g, 0.62 mmol): TLC $R_f = 0.60$ (hexanes/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$) δ 9.58 (s, 1 H), 7.18 (s, 2 H), 6.78 (s, 2 H), 6.42−6.46 (m, 6 H), 5.00 (s, 1 H), 4.51−4.37 (ABq, 4 H), 4.17−4.23 (m, 2 H), 4.02−3.97 (m, 6 H), 3.79−3.39 (m, 20 H), 3.18−3.07 (m, 4 H), 1.18−1.06 (m, 15 H), 0.69 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 191.7, 162.7, 157.1, 155.8, 136.6, 135.2, 134.6, 133.7, 132.4, 130.9, 130.2, 128.7, 128.0, 126.0, 122.6, 101.7, 73.5, 72.9, 69.6, 66.3, 30.8, 30.1, 23.1, 22.0, 15.3; HRMS (EI) *m*/*z* calcd for $C_{51}H_{66}O_{11}$ 854.4605, found 854.4636.

5-Formyl-17-(2′-naphthylaminomethyl)-25,26,27,28-tetra(2-ethoxyethoxy)calix[4]arene (5). A solution of monoprotected monoformylcalix[4]arene **4** (0.80 g, 0.94 mmol) and 2-naphthylamine (0.14 g, 1.0 mmol) in MeOH (50 mL) was stirred for 24 h at room temperature. Subsequently, Na(CN)BH_3 (80 mg) was added and the solution was stirred for another 24 h at room temperature. Then 1 N HCl was added (1 mL) and stirring was continued for 30 min. The solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (100 mL). The solution was washed with 2 N HCl and Na_2CO_3 solution (each 30 mL) and dried over Na_2CO_3. After evaporation of the solvent, the crude product was purified by chromatography (eluent: hexanes/ethyl acetate 2:1) to give **5** as a yellowish oil in 34% yield: TLC $R_f = 0.58$ (hexanes/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$) δ 9.64 (s, 1 H), 7.59−7.50 (m, 3 H), 7.29−7.22 (m, 1 H), 7.18 (s, 2 H), 7.13−7.07 (m, 1 H), 6.81−6.77 (m, 1 H), 6.69 (s, 1 H), 6.58 (s, 2 H), 6.52−6.42 (m, 6 H), 4.49 (ABq, 2 H, $J = 13.5$ Hz), 4.39 (ABq, 2 H, $J = 13.4$ Hz), 4.18−4.14 (m, 2 H), 4.04−4.02 (m, 6 H), 3.92 (s, 2 H), 3.76−3.73 (m, 8 H), 3.51−3.41 (m, 8 H), 3.16 (ABq, 2 H, $J = 13.5$ Hz), 3.05 (ABq, 2 H, $J = 13.7$ Hz), 1.64 (s(br), 1 H), 1.15−1.07 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 191.8, 162.6, 156.0, 155.8, 145.8, 136.5, 135.4, 134.8, 134.0, 132.5, 130.9, 130.2, 128.8, 128.1, 127.6, 126.1, 122.6, 121.8, 118.1, 104.9, 77.3, 73.6, 73.2, 69.7, 66.4, 48.1, 30.8, 15.3; MS (FAB) *m*/*z* calcd for $C_{56}H_{65}NO_9$ 895.5, found 895.7.

25,26,27,28-Tetra(2-ethoxyethoxy)-5-formyl-17-[(*N*-methyl)-5-dimethylaminonaphthylsulfonamidomethyl]calix[4]arene (6). Monoprotected diformylcalix[4]arene **4** (0.60 g, 0.70 mmol), 1 mL of a $MeNH_2$ solution in EtOH (8 M), and 60 mg of Pd/C (10%) were stirred in 50 mL of EtOH under an H_2 atmosphere for 24 h at room temperature. The solution was then filtered over Celite (E. Merck), and the solvent was removed in vacuo to give the intermediate monomethylaminomonoformylcalix[4]arene as a colorless oil. This crude intermediate product was reacted with 5-dimethylaminonaphthylsulfonyl chloride (dansyl chloride) (0.27 g, 1 mmol) in $CHCl_3/NEt_3$ (50 mL, 5:1) at room temperature for 1 d. For the deprotection of the acetal, the solvent was removed, the residue was dissolved in MeOH (50 mL), and 1 N HCl (1 mL) was added. After 1 h of stirring, the solvent was exchanged for CH_2Cl_2 and the solution was washed with Na_2CO_3 solution (30 mL) and dried over Na_2CO_3. After chromatography (eluent: hexanes/ethyl acetate 2:1), **6** was obtained as a greenish powder in 40% yield: mp 68−69 °C; TLC $R_f = 0.39$ (hexanes/ethyl acetate 1:1); ^{1}H NMR ($CDCl_3$) δ 9.61 (s, 1 H), 8.49 (d, 1 H, $J = 8.5$ Hz), 8.34 (d, 1 H, $J = 7$ Hz), 8.14 (d, 1 H, $J = 6.8$ Hz), 7.49 (q, 2 H), 7.20 (s, 2 H), 7.12 (d, 1 H, $J = 7.5$ Hz), 6.44−6.33 (m, 8 H), 4.48 (ABq, 2 H, $J = 12.5$ Hz), 4.35 (ABq, 2 H, $J = 13.3$ Hz), 4.18−4.14 (m, 2 H), 4.05−3.95, 3.77−3.71, 3.47−3.41 (m, 24 H), 3.15 (ABq, 2 H, $J = 13.5$ Hz), 2.95 (ABq, 2 H, $J = 13.0$ Hz), 2.82 (s, 6 H), 2.35 (s, 3 H), 1.16−1.06 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 191.6, 155.7, 136.5, 135.4, 134.5, 133.7, 130.9, 130.2, 128.4, 128.0, 122.7, 115.2, 73.3, 69.6, 66.4, 45.5, 33.2, 30.8, 15.3; MS (FAB) *m*/*z* calcd for $C_{59}H_{72}N_2O_{11}S$ 1016.49, found 1016.3. Anal. Calcd for $C_{59}H_{72}N_2O_{11}S$: C, 69.66; H, 7.13; N, 2.75. Found: C, 69.17; H, 7.13; N, 2.63.

Compound 1a. A solution of **7** (225 mg, 105 μmol) and **5** (90 mg, 100 μmol) in MeOH (10 mL) and THF (1 mL, for initial dissolving of **5**) was stirred for 6 h at room temperature. Then Na(CN)BH_3 (30 mg) and 1 drop of AcOH were added and the solution was stirred for another 24 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in $CHCl_3$ (50 mL). The solution was washed with 2 N HCl and a Na_2CO_3 solution (each 30 mL) and dried over Na_2CO_3. After evaporation of the solvent, the crude product was purified by chromatography (eluent: CH_2Cl_2/methanol 20:1) to give **1a** as a yellowish powder in 66% yield: mp 163−164 °C; TLC $R_f = 0.61$ (CH_2Cl_2/methanol 9:1); ^{1}H NMR ($CDCl_3$) δ 7.64−7.51 (m, 3 H), 7.33−7.11 (m, 6 H), 6.82−6.44 (m, 12 H), 5.25−4.65 (m, 15 H), 4.46 (d, 4 H, $J = 12.5$ Hz), 4.11−4.02, 3.82−3.80, 3.63−3.47 (m, 85 H), 3.16−3.06 (m, 10 H), 1.71 (s(br), 2 H), 1.20−1.13 (m, 12 H), 0.84 (s, 63 H), 0.00 (s, 42 H); ^{13}C NMR ($CDCl_3$) δ 155.8, 145.9, 135.6, 135.1, 134.4, 128.7, 128.0, 127.7, 127.4, 126.2, 125.9, 122.4, 121.9, 118.1, 101.5, 82.2, 77.2, 73.2, 71.7, 70.0, 66.4, 61.6, 60.1, 30.9, 25.9, 18.3, 15.3; MS (FAB) *m*/*z* calcd for $C_{154}H_{254}N_2O_{43}Si_7$ 3017.6, found 3040.4 ([M + Na]$^+$). Anal. Calcd for $C_{154}H_{254}N_2O_{43}Si_7$: C, 61.28; H, 8.48; N, 0.93. Found: C, 61.81; H, 8.49; N, 1.05.

Compound 2a. Compound **2a** was prepared by following the procedure as described for **1a** from compound **7** (212 mg, 99 μmol) and **6** (160 mg, 157 μmol). The crude product was purified by chromatography (eluent: CH_2Cl_2/methanol 20:1) to give **2a** as a slightly greenish powder in 67% yield: mp 162 °C; TLC $R_f = 0.58$ (CH_2Cl_2/methanol 9:1); ^{1}H NMR ($CDCl_3$) δ 8.54 (d, 1 H, $J = 8.5$ Hz), 8.40 (d, 1 H, $J = 8.5$ Hz), 8.18 (d, 1 H, $J = 7.3$ Hz), 7.57−7.46, 7.36−7.14 (m, 7 H), 6.75−6.21 (m, 10 H), 5.32−4.76 (m, 15 H), 4.44−4.35 (m, 4 H), 4.13−3.46 (m, 85 H), 3.16−3.13 (m, 10 H), 2.85 (s, 6 H), 2.48 (s, 3 H), 1.21−1.10 (m, 12 H), 0.84 (s, 63 H), 0.00 (s, 42 H); ^{13}C NMR ($CDCl_3$) δ 151.7, 135.7, 135.5, 134.2, 134.1, 130.3, 130.1, 128.6, 128.0, 127.7, 122.3, 120.0, 82.2, 77.3, 73.4, 73.1, 71.7, 69.6, 66.4, 61.6, 60.1, 45.4, 30.7, 25.9, 18.3, 15.3; MS (FAB) *m*/*z* calcd for $C_{157}H_{259}N_3O_{45}SSi_7$ 3136.6, found 3160.6 ([M + Na]$^+$). Anal. Calcd for $C_{157}H_{259}N_3O_{45}SSi_7$: C, 60.10; H, 8.26; N, 1.34; S, 1.02. Found: C, 60.02; H, 8.41; N, 1.41; S, 0.92.

Compound 1. A solution of **1a** (100 mg, 33 μmol) in CH_3CN/CH_2Cl_2 (each 20 mL) was stirred in an atmosphere of SiF_4 for 24 h. Then the solvents were removed in vacuo, and the residue was dissolved in water (50 mL). The pH of the solution was adjusted to 7 by adding a concentrated solution of Na_2CO_3. After two washings with hexanes (each 10 mL), the solution was filled into a dialysis tube (benzoylated cellulose tubing, Sigma) and was subjected to dialysis for 5 d. After

(35) Wessely, F.; Svoboda, W. *Monatsh.* **1951**, *82*, 621.
(36) Seiler, N.; Schmidt-Glenewinkel, T.; Schneider, H. H. *J. Chromatogr.* **1973**, *84*, 95.

freeze-drying of the dialyzed aqueous solution, **1** was obtained as a colorless powder in 60% yield: mp 205 °C (dec); TLC R_f = 0.16 (EtOAc/MeOH/H_2O 25:2:1); ^{1}H NMR (CD_3SOCD_3) δ 7.61−7.54 (m, 2 H), 7.45 (d, 1 H, J = 8.4 Hz), 7.36−7.22 (m, 6 H), 7.08−7.00 (m, 2 H), 6.92−6.89 (m, 2 H), 6.65−6.61 (m, 2 H), 6.38−6.23 (m, 6 H), 4.99−4.33 (m, 19 H), 3.91−2.96 (m, 104 H), 1.15−1.07 (m, 12 H); ^{13}C NMR (CD_3SOCD_3, 303 K) δ 127.1, 124.4, 120.9, 118.1, 102.4, 98.6, 95.3, 81.1, 78.2, 75.0, 72.7, 71.6, 68.1, 64.6, 59.2, 58.5, 56.1, 50.9, 45.8, 39.3, 29.5, 27.8, 12.0; MS (MALDI-TOF) *m*/*z* calcd for $C_{112}H_{156}N_2O_{43}$ 2218.4, found 2241.1 ([M + Na]$^+$), 2256.7 ([M + K]$^+$). Anal. Calcd for $C_{112}H_{156}N_2O_{43}\cdot 8H_2O$: C, 56.94; H, 7.34; N, 1.19. Found: C, 56.87; H, 7.02; N, 1.15.[37]

Compound 2. Compound **2** was prepared according to the procedure described for **1** from compound **2a** (90 mg, 29 μmol). Dialysis and freeze-drying yielded **2** as a colorless powder in 65% yield: mp 233 °C (dec); TLC R_f = 0.30 (EtOAc/MeOH/H_2O 25:2:1); ^{1}H NMR[38] (DMSO, 500 MHz, 303 K) δ 8.53 (d, 1 H, J = 13 Hz), 8.26 (d, 1 H, J = 12.5 Hz), 8.08 (d, 1 H, J = 12.5 Hz), 7.68−7.60, 7.38−7.18 (m, 7 H), 6.78−6.75, 6.56−6.50, 6.45−6.40, 6.36−6.33 (m, 10 H), 5.11−4.98, 4.96−4.81, 4.77−4.71, 4.17−3.92, 3.86−3.32 (m, 85 H), 3.19−3.13 (m, 6 H), 3.10 (d, 4 H, 13 Hz), 2.96 (d, 4 H, J = 13 Hz), 2.82 (s, 6 H), 2.54 (s, 3 H), 1.84−1.43 (m, 12 H); MS (MALDI-TOF) *m*/*z* calcd for $C_{115}H_{161}N_3O_{45}S$ 2337.9, found 2361.9 ([M + Na]$^+$), 2377.9 ([M + K]$^+$). Anal. Calcd for $C_{115}H_{161}N_3O_{45}S\cdot 5H_2O$: C, 56.90; H, 7.10; N, 1.73. Found: C, 56.97; H, 7.05; N, 1.74.

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(37) In general, elemental analysis of cyclodextrin derivatives reveals the presence of several equivalents of water, even when the compounds are dried vigourously.

(38) The signal at 2.54 ppm in the ^{1}H NMR spectrum overlaps with the solvent signal. Its exact position was deduced from a ROESY experiment. We were not able to obtain a ^{13}C NMR spectrum of sufficient intensity of compound **2** due to the low amount of compound available.