Recognition of Steroids by Self-Assembled Monolayers of Calix[4]arene – Resorcin[4]arene Receptors

Arianna Friggeri, Frank C. J. M. van Veggel,* and David N. Reinhoudt*[a]

Abstract: Calix[4]arene – resorcin[4]-arene (2:1) receptor adsorbate **2** equipped with four didecyl sulfide groups self-assembles in monolayers (SAMs) on gold. These monolayers were characterized by contact angle measurements, polarized infrared external reflectance spectroscopy (PIERS), electrochemical capacitance and resistance measurements, and X-ray photoelectron spectroscopy (XPS). Interactions of a monolayer of **2** with steroid guests were investigated by surface plasmon resonance (SPR) spectroscopy. The results show that steroids interact more strongly with a monolayer of **2** than with a hydrophobic reference monolayer of octadecanethiol. There is virtually no detectable interaction with a hydrophilic reference monolayer of 11-mercaptoundecan-1-ol. Mixed monolayers of **2**/11-mercaptoundecan-1-ol and of **2**/decanethiol showed lower SPR responses with the steroids than the pure monolayer of **2**. Concentration-dependent experiments with prednisolone-21-acetate, corticosterone-21-acetate, and cortisone-21-acetate on the mixed monolayer showed Langmuir-type adsorption with affinity constants between 2.0×10^5 and $3.5 \times 10^5\,\text{M}^{-1}$.

Keywords: monolayers · steroids · supramolecular chemistry · surface analysis · surface plasmon resonance

Introduction

Steroids are present in all eukaryotic organisms and they are involved in a great variety of biologically important processes.[1, 2] The importance of this class of compounds has lead to a vast scientific interest in steroid – receptor interactions. Our understanding of the latter has been greatly improved by X-ray studies of biological steroid receptors.[3] From these studies, it appears that in general, hydrophobic desolvation and dispersion interactions are the major driving forces for the association process, as well as host – guest shape complementarity.[4]

The mimicry of molecular recognition of steroids by synthetic receptors is of current interest in supramolecular chemistry. Diederich et al. synthesized a cyclophane-based receptor capable of complexing cholesterol in water, with an association constant of $1.5 \times 10^5\,\text{M}^{-1}$.[5] Derivatized β- and γ-cyclodextrins have also been used to solubilize cholesterol in water[6] and as fluorescent receptor molecules for bile acids, respectively.[7] In our group, Higler et al.[8] recently showed that molecular platform **3** is able to complex steroids in $CDCl_3$. So far, recognition of steroid molecules with synthetic receptors has only been attempted in solution.[9] Self-assembled monolayers (SAMs) can be viewed as model systems for cell membranes.[10] In a recent publication Grazzini et al.[11] showed that steroids in some cases express their activity through receptor – steroid interactions *at the surface* of the cell membrane. Therefore, we decided to modify steroid receptor **3** into an adsorbate molecule capable of forming self-assembled monolayers on gold and to use such monolayers for the detection of steroids at the water interface. Previously, in our group, SAMs of receptor molecules have been successfully employed for sensor purposes. Resorcin[4]arene-based adsorbates (e.g. **1**) have been used for the detection of perchloroethylene from the gas phase[12] and to investigate the affinity of relatively small, neutral molecules to such receptor surfaces in aqueous solution.[13] Recently, we have shown that crown ether adsorbates can detect cations in aqueous solution.[14]

In this paper, we describe the synthesis of **2** and the interactions of several steroids with SAMs of **2**, as well as mixed SAMs of **2** with hydrophobic ($C_{10}H_{23}SH$) and hydrophilic ($HOC_{11}H_{22}SH$) thiols. Surface plasmon resonance (SPR) shows that the interactions of steroids with a monolayer of **2** are governed in part by the hydrophobic character of the guests, but probably also by the three-dimensional structure of the monolayer of **2**. Furthermore, we have determined affinity constants between 2.0×10^5 and $3.5 \times 10^5\,\text{M}^{-1}$ for the interactions of prednisolone-21-acetate, corti

[a] Prof. Dr. Ir. David N. Reinhoudt, Dr. Ir. Frank C. J. M. van Veggel, Drs. Arianna Friggeri
Supramolecular Chemistry and Technology
MESA Research Institute, University of Twente
P. O. Box 217, NL-7500AE Enschede (The Netherlands)
Fax: (+31) 53-4894-645
Email: smct@ct.utwente.nl

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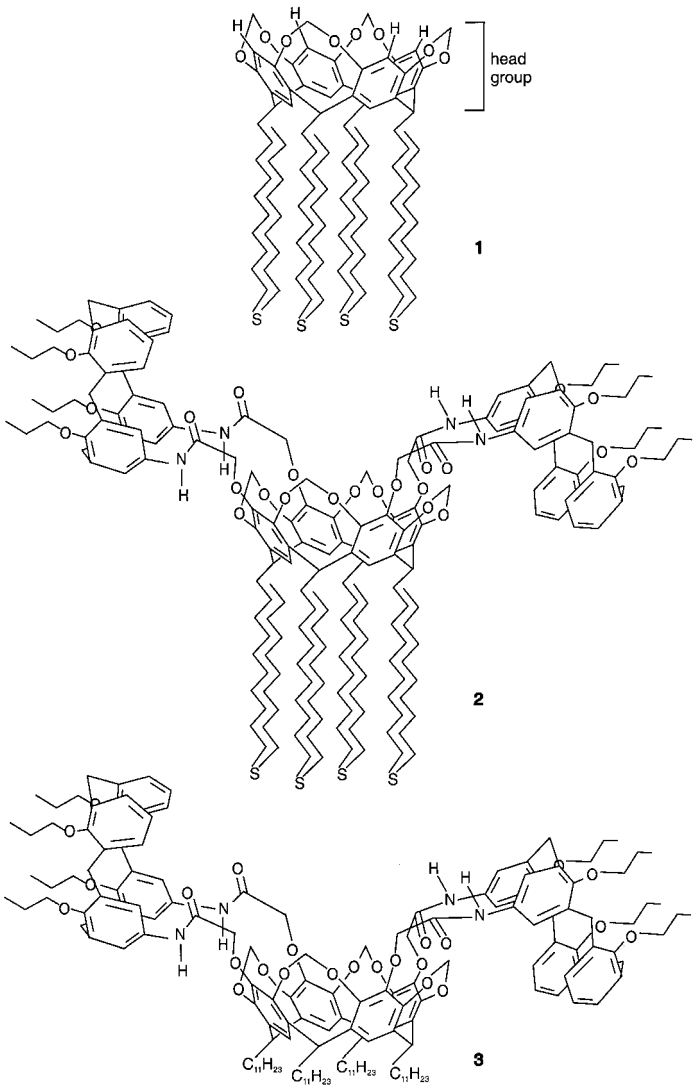

costerone-21-acetate,and cortisone-21-acetate and the **2**/$HOC_{11}H_{22}SH$ mixed monolayer. Previously, SPR has been applied to the study of antibody–antigene type interactions.[15] More recently, Whitesides et al.[16] used it to investigate monolayer-surfactant interactions.

Results and Discussion

For the synthesis of **2**, the hydroxy moieties in compound **4** were first protected with *tert*-butyldimethylsilyl (TBDMS) groups. Subsequently the terminal double bonds of the pendent alkyl chains were reacted with decanethiol in the presence of 9-borabicyclo[3.3.1]nonane (9-BBN) as a radical activator.[17] Compound **5** was deprotected in THF with $Bu_4N^+F^-$ and subsequently allowed to react with two equivalents of calix[4]arene **8**. The reaction produced **2** (*endo*–*exo*)[8] in 21 % yield, as the most abundant isomer (Scheme 1), the *endo*–*endo* and *exo*–*exo* isomers were isolated in 8 and 18 % yield, respectively. As previous studies in solution showed that steroids interact almost equally with all three isomers,[8] only the *endo*–*exo* isomer was used for monolayer formation.

SAMs of **2** were prepared as described in the experimental section and characterized by means of contact angle measurements, polarized infrared external reflectance spectroscopy (PIERS), electrochemical capacitance and resistance measurements, and by X-ray photoelectron spectroscopy (XPS). Contact angle measurements show that the layers present a hydrophobic interface ($\theta_a = 103 \pm 2^\circ$) and are relatively disordered ($\Delta\theta = 24^\circ$), when compared with a decanethiol monolayer or a cavitand (**1**) layer ($\Delta\theta = 16^\circ$).[13] The head group of **2** occupies a much larger area than that of the supporting alkyl sulfide chains. Therefore the disorder shown in the layer of **2** fully agrees with previous findings of our group,[18] that a well-packed ordered monolayer is obtained only when the area occupied by the head group is smaller than the area occupied by the anchoring alkyl chains. The infrared spectrum of the monolayer of **2** (Figure 1) shows several characteristic bands in the 3000–2800 cm^{-1} region, corresponding to the CH_2 and CH_3 stretching vibrations of the resorcinarene's decyl chains[19] and the propyl substituents at the lower rim of the calixarenes. A rather intense peak at 1466 cm^{-1} is most probably due to CH_2 bending vibrations. Furthermore the amide I and amide II bands are present at 1699 and 1543 cm^{-1}, respectively. The peak at 1319 cm^{-1} is tentatively assigned to the amide C–N stretching vibrations. The symmetric and asymmetric aromatic ether band is present at 1214 cm^{-1}.[20, 21] The monolayer of **2** was further characterized by electrochemical capacitance and resistance measurements. The capacitance ($C_{ml} = 3.11\ \mu F\,cm^{-2}$) is somewhat high, when compared with other resorcin[4]arene-based monolayers of slightly smaller adsorbate molecules.[19] The resistance ($R =$ 1690 Ω) is particularly low. The value of C_{ml} indicates that the thickness of the monolayer is smaller than when all the molecules are adsorbed with the alkyl moieties perpendicular to the gold surface. Together with the resistance this clearly shows that **2** forms relatively disordered monolayers, with probably quite a number of defects. Further experimental evidence of the actual presence of the desired molecule on the gold surface was provided by the XPS data. The results shown in Table 1 agree very well with the theoretical values.

A schematic representation of the two-channel SPR set-up used for the measurements of the interaction of the steroids with the monolayers is shown in Figure 2. Saturated aqueous solutions of the steroids were used due to the extremely low solubility of these molecules in water. The concentrations of the steroid solutions were determined by UV experiments. Furthermore, according to UV experiments with Reichardt's Dye, none of the steroid solutions showed aggregation of the solutes.[22] The changes in SPR angle ($\Delta\alpha$) caused by the addition of the solutions of steroids **I**–**X** are shown in Figure 3. Each of the steroids shows a characteristic behavior when in contact with this particular receptor surface. Cholic acid gives the highest response and cholesterol gives almost no response. This corresponds with the solubilities of the steroids in water, the former is the most and the latter is the least soluble of the steroids studied. However, for all the other steroids, there is no correlation between the $\Delta\alpha$ and the steroid concentrations of the solutions. The responses of the same steroids have been tested on hydrophobic ($C_{18}H_{37}SH$) and hydrophilic ($HOC_{11}H_{22}SH$) reference monolayers and are

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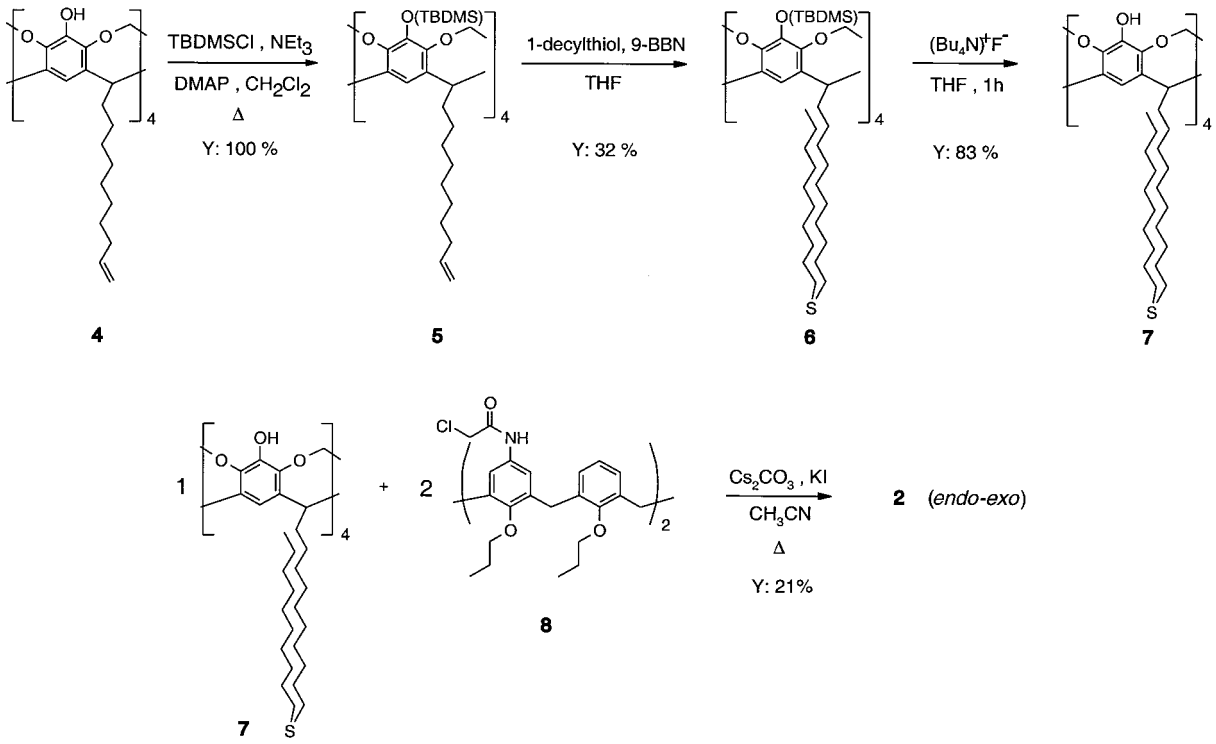

Scheme 1. Synthesis of **2** (*endo–exo*) receptor adsorbate. Y = yield.

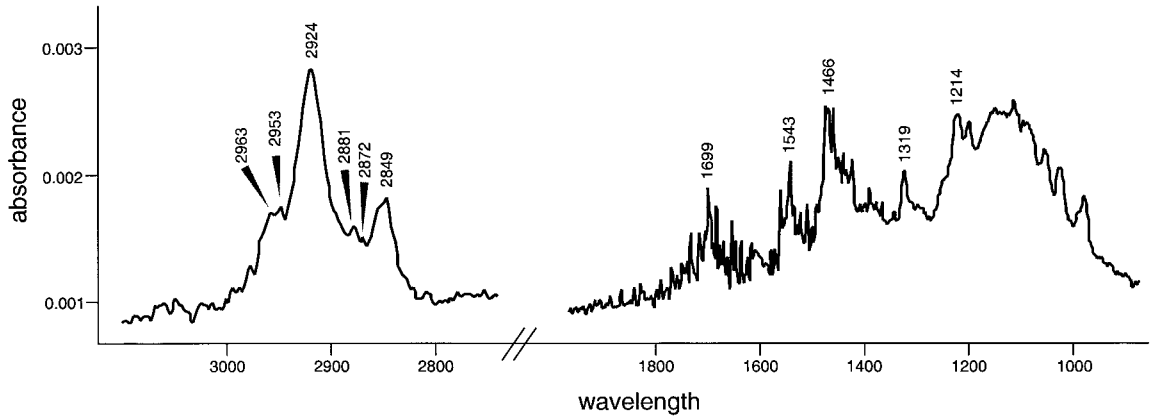

Figure 1. PIERS of **2** SAM on gold.

Table 1. XPS of adsorbate of **2**. Values are percentages corrected for Au.

	S	C	O	N
Experimental	1.5	84.4	12.8	1.3
Calculated	1.8	85.7	10.7	1.8

also shown in Figure 3. On the $C_{18}H_{37}SH$ monolayer all the steroids showed quite high $\Delta\alpha$ values, most probably due to the strong hydrophobic attraction between substrate and surface in water. The intensity of the responses correlates nicely with the solubilities of the guests. On the other hand, *there is no interaction between the steroids and the hydrophilic surface*. These results suggest that hydrophobic interactions may also contribute considerably to the $\Delta\alpha$ values obtained on the monolayer of **2**.

Mixed monolayers of **2** and $HOC_{11}H_{22}SH$ were prepared in order to obtain more densely packed two-component monolayers. In these monolayers, the voids between the molecules of **2** adsorbed on gold are patched with $HOC_{11}H_{22}SH$. Since the steroids only interact with the receptors **2** and not with monolayers of $HOC_{11}H_{22}SH$, we can safely exclude contribution to the SPR signal from the surrounding $HOC_{11}H_{22}SH$ molecules. The mixed **2**/$HOC_{11}H_{22}SH$ (**2**/HO-) layers were prepared by the immersion of a monolayer of **2** in an ethanolic solution of $HOC_{11}H_{22}SH$, followed by rinsing extensively with CH_2Cl_2, EtOH, and water. In order to assess the immersion time necessary for the $HOC_{11}H_{22}SH$ molecules to fill the voids in the monolayers of **2** completely, the substrates were taken out of the 11-mercaptoundecan-1-ol solution after 1 and 5 min. The electrochemical capacitance and resistance were compared with the values obtained for a $HOC_{11}H_{22}SH$

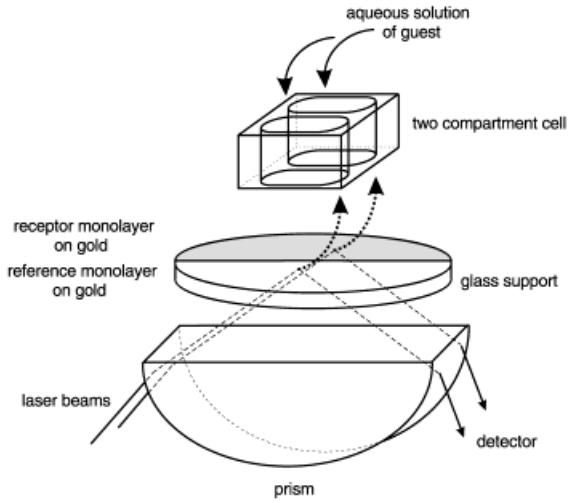

Figure 2. Schematic representation of the SPR measuring set-up.

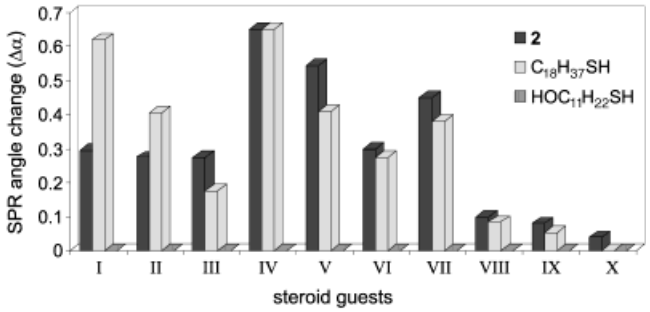

Figure 3. SPR responses of a monolayer of **2** (black), an octadecanethiol monolayer (light gray), and a mercaptoundecanol monolayer (dark gray) to **I**. prednisone (1.47×10^{-4})[a]; **II**. prednisolone-21-acetate (7.30×10^{-5}); **III**. norethindrone (3.34×10^{-5}); **IV**. cholic acid (6.85×10^{-4}); **V**. corticosterone-21-acetate (8.61×10^{-5}); **VI**. cortisone-21-acetate (5.47×10^{-5}); **VII**. corteolone (1.03×10^{-4}); **VIII**. estriol (6.23×10^{-5}); **IX**. α-estradiol (8.24×10^{-6}) and **X**. cholesterol (5.17×10^{-6}). [a]All concentrations are in mol L^{-1}.

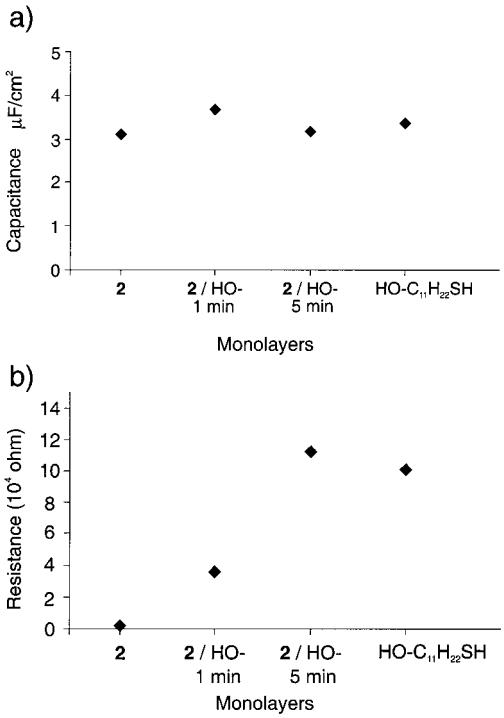

Figure 4. a) Electrochemical capacitance of a monolayer of **2**, **2**/HO- (1 and 5 min) mixed monolayers, and an 11-mercaptoundecan-1-ol monolayer. b) Electrochemical resistance of a monolayer of **2** ($R = 1.69 \times 10^3\ \Omega$), **2**/HO- (1 and 5 min) mixed monolayers ($R = 3.54 \times 10^4$ and $1.12 \times 10^5\ \Omega$), and an 11-mercaptoundecan-1-ol monolayer ($R = 1.01 \times 10^5\ \Omega$).

Table 2. Advancing (θ_a) and receding (θ_r) contact angles of water on monolayers of **2**, mixed **2** and mercaptoundecanol (1 and 5 min), and mercaptoundecanol.

	2	**2**/HO- (1 min.)	**2**/HO- (5 min.)	$HOC_{11}H_{22}SH$
θ_a	103 ± 2	88 ± 2	90 ± 2	< 20
θ_r	79 ± 1	51 ± 2	54 ± 2	< 20

monolayer. Figure 4a shows that the capacitance values for the pure monolayer of **2**, the mixed layers, and the pure $HOC_{11}H_{22}SH$ layer are all quite similar. However, the resistance measurements (Figure 4b), revealed that after a 1 min immersion time in the 11-mercaptoundecan-1-ol ethanolic solution, the layer had a resistance of 3.54×10^4 ohm and after 5 min of 1.12×10^5 ohm, very similar to the resistance of a $HOC_{11}H_{22}SH$ monolayer ($R = 1.01 \times 10^5$ ohm). Therefore, the two-component monolayer obtained after a 5 min immersion time in $HOC_{11}H_{22}SH$ was a well-packed layer with relatively few defect sites. Contact angle measurements and PIERS were used to evaluate the extent of adsorption of the 11-mercaptoundecan-1-ol molecules and the possible desorption of **2** within the 5 min immersion time in the $HOC_{11}H_{22}SH$ ethanolic solution. The contact angle results (Table 2) show a slight decrease in the advancing contact angle from the monolayer of **2** to the mixed layers, but even the latter remain relatively hydrophobic. This is a first indication that most of the molecules of **2** remain adsorbed on the gold surface. PIERS measurements show no significant increase in the intensities of the $\nu_a(CH_2)$ and $\nu_s(CH_2)$ absorption peaks (Figure 5a). Most probably the number of $HOC_{11}H_{22}SH$ molecules that intercalate into the **2:1** monolayer is quite small, and therefore they do not contribute significantly to the intensities of these absorption peaks. Moreover, when compared with the spectrum of the layer of **2**, in the mixed **2**/HO- layer the $\nu_s(CH_2)$ peak is shifted by 4 cm^{-1} to higher wavelengths. Apparently, in the mixed monolayers, the alkyl chains are in a more liquid-like state than in the layer of **2**.[23] Contact angle measurements also show that the disorder increases on going from a monolayer of **2** ($\Delta\theta = 24^\circ$) to the mixed layers ($\Delta\theta = 36 - 37^\circ$) (Table 2). For definite proof that desorption of **2** did not occur during the formation of the mixed monolayers, layers of **2** and perdeuterated decanethiol ($C_{10}D_{21}SH$) were prepared following the same procedure as for the **2**/HO- adsorbates. PIERS measurements show no decrease in the intensities of the $\nu(CH_2)$ and $\nu(CH_3)$ absorption peaks in the **2**/$C_{10}D_{21}SH$ (5 min) spectrum, but they do show the same shift to higher wavelengths of the $\nu_s(CH_2)$ band (Figure 5b).

The SPR responses of the **2**/HO- monolayer towards steroids **I**–**X** are given in Figure 6. For most steroids the response of the **2**/HO- (5 min) mixed monolayer is considerably lower than that of the monolayer of **2**. In general, it seems that decreasing the hydrophobic character of the monolayer of **2** by adding a hydrophilic component

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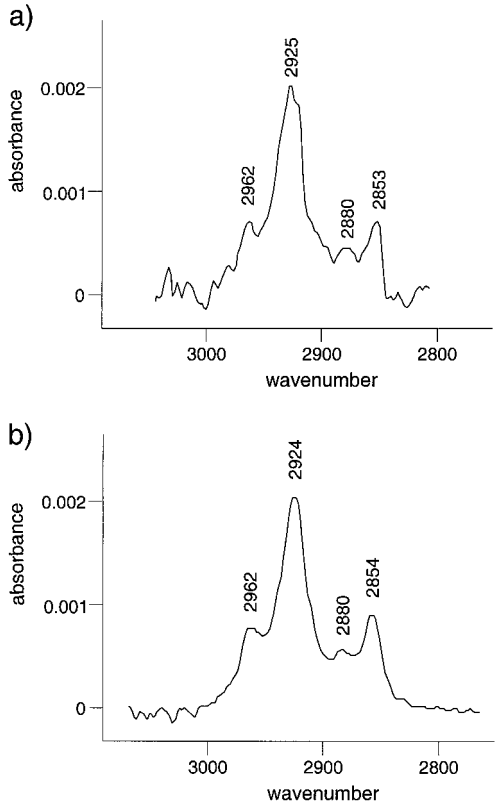

Figure 5. a) PIERS of **2**/HO- (5 min) mixed monolayer. (b) PIERS of **2**/$C_{10}D_{23}SH$ (5 min) mixed monolayer.

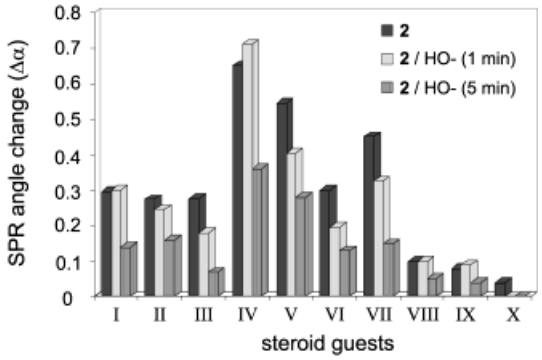

Figure 6. SPR responses of a monolayer of **2** (black), a **2**/HO- (1 min) monolayer (light gray), and a **2**/HO- (5 min) monolayer (dark gray) to: **I**. prednisone (1.47×10^{-4})[a]; **II**. prednisolone-21-acetate (7.30×10^{-5}); **III**. norethindrone (3.34×10^{-5}); **IV**. cholic acid (6.85×10^{-4}); **V**. corticosterone-21-acetate (8.61×10^{-5}); **VI**. cortisone-21-acetate (5.47×10^{-5}); **VII**. cortexolone (1.03×10^{-4}); **VIII**. estriol (6.23×10^{-5}); **IX**. β-estradiol (8.24×10^{-6}), and **X**. cholesterol (5.17×10^{-6}). [a]All concentrations are in mol L^{-1}.

$(HOC_{11}H_{22}SH)$, decreases the intensity of steroid responses. The same measurements were carried out with mixed monolayers of **2** and the hydrophobic decanethiol (Figure 7). Surprisingly, most of the responses to the layer **2** are still larger than for the **2**/CH_3- (5 min) mixed monolayer. Only guests **I**, **II**, **VIII** and **IX** are exceptions. This means that the hydrophobicity of the layer of **2** is not the only factor responsible for the observed steroid–receptor interactions. These results suggest that, for steroid sensing, the relatively disordered structure of a monolayer of **2** has an advantage in

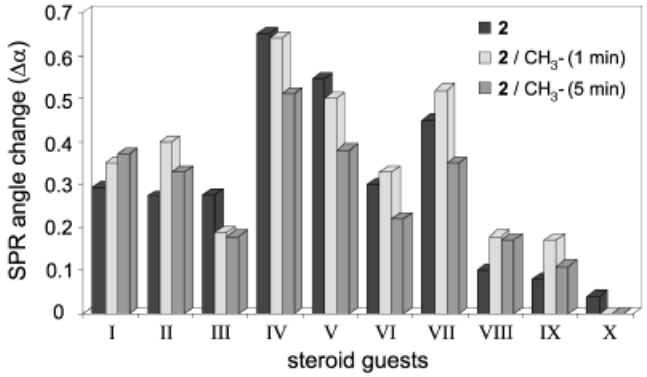

Figure 7. SPR responses of a monolayer of **2** (black), a **2**/CH_3- (1 min) monolayer (light gray), and a **2**/CH_3- (5 min) monolayer (dark gray) to **I**. prednisone (1.47×10^{-4})[a]; **II**. prednisolone-21-acetate (7.30×10^{-5}); **III**. norethindrone (3.34×10^{-5}); **IV**. cholic acid (6.85×10^{-4}); **V**. corticosterone-21-acetate (8.61×10^{-5}); **VI**. cortisone-21-acetate (5.47×10^{-5}); **VII**. cortexolone (1.03×10^{-4}); **VIII**. estriol (6.23×10^{-5}); **IX**. β-estradiol (8.24×10^{-6}), and **X**. cholesterol (5.17×10^{-6}). [a]All concentrations are in mol L^{-1}.

terms of sensitivity, as the presence of voids may favor the intercalation of guest molecules and/or render the monolayer structure more flexible and therefore better suited to accommodate guests.

We can assume two types of adsorption sites in the monolayer of **2** viz the receptors **2** and the spaces between them. The mixed monolayer has only the receptor sites of **2**. The nature of the interactions of steroids with such receptor sites was studied in more detail for prednisolone-21-acetate (**II**).[24] Aqueous solutions of **II** of different concentrations were added to both a monolayer of **2** and to a **2**/HO- (5 min) mixed monolayer (Figure 8). Assuming that the receptor–guest interaction is the prevalent one, both sets of points have been fitted to a Langmuir isotherm (Figure 9). In this we assume that maximum surface coverage is obtained when the layer is exposed to the saturated steroid solution. This seems to be a reasonable assumption as, for a saturated solution, the number of guest molecules per receptor molecule, calculated

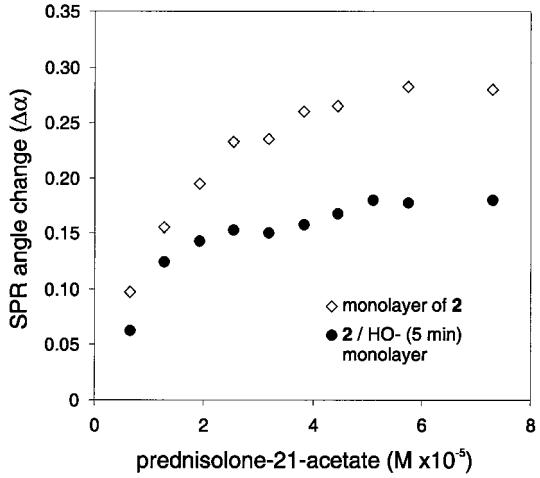

Figure 8. SPR angle changes measured upon addition of prednisolone-21-acetate on a monolayer of **2** (◇) and on a **2**/HO- (5 min) monolayer (●). Each point corresponds to the average value obtained from four measurements and has an error of 0.02 $\Delta\alpha$.

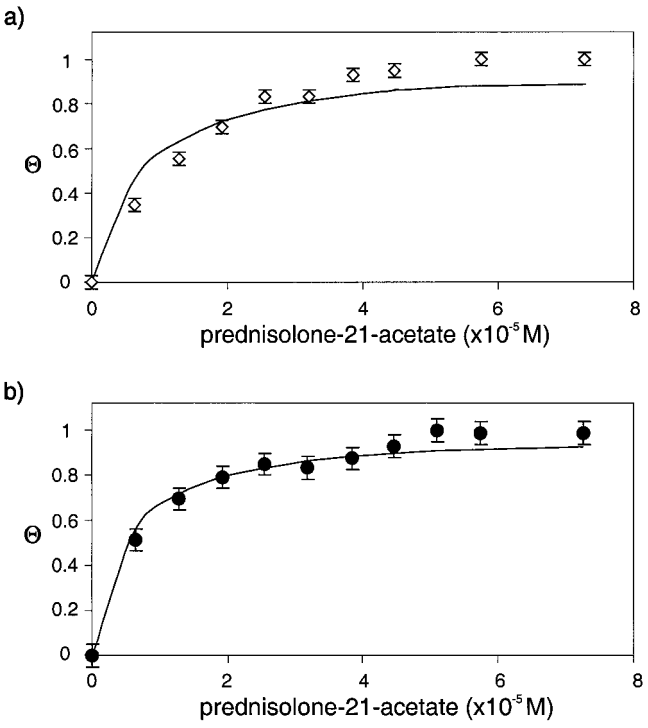

Figure 9. a) Fractional coverage (θ) of a monolayer of **2** as a function of the concentration ($\times 10^{-5}$ M) of prednisolone-21-acetate ($\diamond$). The line is the result of a Langmuir isotherm curve fitting calculation ($r^2 = 0.94$). b) Fractional coverage (θ) of a **2**/HO- (5 min) mixed monolayer as a function of the concentration ($\times 10^{-5}$ M) of prednisolone-21-acetate ($\bullet$). The line is the result of a Langmuir isotherm curve fitting calculation ($r^2 = 0.98$).

from SPR $\Delta\alpha$ values,[25] is at a maximum of four on the mixed monolayer and six on the layer of **2**. These numbers correspond to a complete coverage of the exposed surface area of the host,[26] in the case of the mixed monolayer, and support the guest intercalation assumption in the case of the monolayer of **2**. The possibility of multilayer formation can therefore be eliminated. The fact that for a saturated solution of guest, four molecules of guest interact with one host molecule is not consistent with the initial assumption that the receptor constitutes one type of adsorption sites. Apparently, the four guest molecules adsorb on different parts of the host, most probably each with a slightly different affinity constant, but such small differences cannot be distinguished by the measuring method employed. Therefore a Langmuir type behavior was observed. For the interaction of **II** with the **2**/HO- (5 min) mixed monolayer, a relatively good Langmuir isotherm curve fit could be obtained ($r^2 = 0.98$),[27, 28] and an affinity constant of $(2.00 \pm 0.20) \times 10^5\,\text{M}^{-1}$ was calculated. However, for the interaction of **II** with the monolayer of **2**, the measured points do not fit very accurately to the Langmuir isotherm ($r^2 = 0.94$), suggesting the occurrence of guest–guest interactions and/or the presence of different types of adsorption sites. The difference between the SPR response of **II** on the monolayer of **2** and on the **2**/HO- (5 min) mixed monolayer is shown in Figure 10. These points represent the interaction of the guest molecules with the voids between the receptor molecules, in the monolayer of **2**. The points fit well to a sigmoid curve ($r^2 = 0.99$) indicating that the

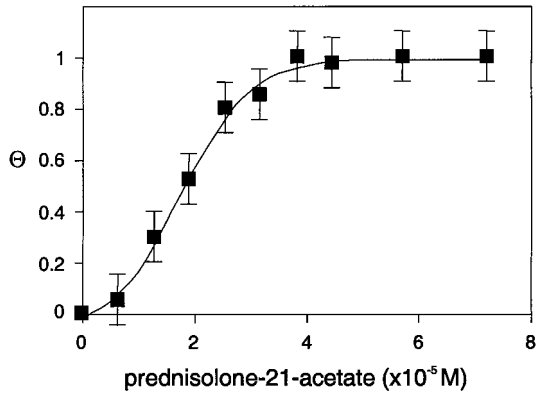

Figure 10. Difference between the SPR responses on the monolayer of **2** and on the **2**/HO- (5 min) mixed monolayer (■). The points have been fitted to a sigmoid curve ($r^2 = 0.99$).

interaction *between* adsorbed molecules of prednisolone-21-acetate, in the voids, is significant.[29]

The interactions of corticosterone-21-acetate (**V**) and cortisone-21-acetate (**VI**) with the **2**/HO- (5 min) mixed monolayer were also investigated, and similar curves to those shown in Figure 9, for guest **II**, were obtained. With the same assumptions as for **II**, affinity constants of $(2.15 \pm 0.38) \times 10^5\,\text{M}^{-1}$ and $(3.51 \pm 0.74) \times 10^5\,\text{M}^{-1}$ were calculated for guests **V** and **VI** respectively, on the **2**/HO- (5 min) monolayer.[30] Moreover, the SPR response of **V** (Figure 6) is more intense than for **II** and **VI** even though the affinity constants are very similar. This means that molecules of **V** may have a different position on the monolayer than those of **II** and **VI**, allowing a greater number of steroids to be present at the receptor surface.

Conclusion

In conclusion, we have shown that the recognition of steroids with synthetic receptor monolayers is possible. Monolayers of **2** have proven to be better receptor layers than simple hydrophobic (octadecanethiol) or hydrophilic ($HOC_{11}H_{22}SH$) interfaces. The higher responses obtained for a monolayer of **2** are most probably due to the overall structure of the layer: a combination of specific steroid receptors and voids which provides the receptors with space to adapt to the guest molecules, and permits guest intercalation between the receptor molecules. These are important aspects that should be taken into consideration in the design of new, more selective and sensitive synthetic receptor monolayers.

Experimental Section

Chemicals: THF was freshly distilled from Na/benzophenone before use. For synthetic purposes dichloromethane, ethyl acetate, and hexanes (petroleum ether isomer mixture with boiling point between 60 and 80 °C) were distilled from calcium chloride. All other reagents were used as received; p. a. grade solvents were used for monolayer preparation. All reactions were conducted under an argon atmosphere.

Gold substrates: Gold substrates were prepared by evaporating 47.5 nm of gold on a glass slide, 25 mm in diameter, with a 2 nm titanium layer for

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adhesion. Immediately before use, the substrates were cleaned with an oxygen plasma (10 min) and subsequently immersed in ethanol for 5 min to remove the oxide layer.[31]

Monolayer preparation: All glassware used to prepare monolayers was immersed in *piraña* solution. **Caution!**: *piraña* solution should be handled with care; it has been reported to detonate unexpectedly. Next, the glassware was rinsed with large amounts of high-purity water (Millipore). Monolayers of **2** were prepared by immersing the gold substrates, with minimal delay, in 0.1 mM adsorbate solutions (chloroform/ethanol, 1:1), which were then heated at 60 °C for 16 h.[19] The samples were then allowed to cool to room temperature before being taken out of the adsorbate solutions and rinsed thoroughly with dichloromethane, ethanol, and water (Millipore). Monolayers of octadecanethiol, 11-mercaptoundecan-1-ol, deuterated and nondeuterated decanethiol were prepared by immersing the gold substrates in the respective 1 mM ethanolic solutions for 16 h at room temperature, and were then subjected to the same rinsing procedure as described above. Mixed monolayers were prepared by immersing rinsed monolayers of **2** in 1 mM ethanolic solutions of decanethiol, deuterated decanethiol, or 11-mercaptoundecan-1-ol for either 1 or 5 min. The layers were then subjected to the same rinsing procedure as described above.

Surface plasmon resonance: For all SPR experiments, a two-channel vibrating-mirror angle scan set-up, based on the Kretschmann configuration,[32] was used. This set-up has previously been described by Kooyman et al.,[33] therefore a detailed description of the instrument will not be given in the present paper. It will suffice to say that light from a 2-mW HeNe laser (wavelength 632 nm) is directed onto the prism surface by means of a vibrating mirror and that the intensity of the reflected light is monitored by a large-area photodiode. Changes in plasmon angle ($\Delta\alpha$) can be determined with an accuracy of 0.002°. Figure 2 shows how it is possible to measure simultaneously the changes in SPR angle which occurs on receptor and reference monolayers, upon addition of a certain solution of guest, with the aid of a two-compartment cell appropriately placed on both adsorbates. Before each measurement, the two cell compartments were each filled with 800 mL of water. After stabilization of the SPR signal (≈30 minutes), 700 mL of water were removed from each compartment and replaced with 700 mL of a certain guest solution. Once the signal was recorded, before measurement of a second signal, the solution of the first guest was removed and repeated washings with water (700 mL ×3 or ×4) were carried out. SPR measurements were repeated four times for each monolayer-guest system. For every experiment saturated solutions of guests were employed: **I**. prednisone (1.47×10^{-4} M), **II**. prednisolone-21-acetate (7.30×10^{-5} M), **III**. norethindrone (3.34×10^{-5} M), **IV**. cholic acid (6.85×10^{-4} M), **V**. corticosterone-21-acetate (8.61×10^{-5} M), **VI**. cortisone-21-acetate (5.47×10^{-5} M), **VII**. cortexolone (1.03×10^{-4} M), **VIII**. estriol (6.23×10^{-5} M), **IX**. β-estradiol (8.24×10^{-6} M), and **X**. cholesterol (5.17×10^{-6} M), with the exception of the concentration dependence experiments carried out with prednisolone-21-acetate. The latter were also repeated four times on the layer of **2** and on the **2**/HO- (5 min) mixed layer.

Instrumentation: ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AC 250 spectrometer in $CDCl_3$ using traces of nondeuterated solvent as internal standard. FAB-mass spectra were obtained with a Finnigan MAT90 mass spectrometer. Determination of the concentrations of the saturated steroid solutions, and studies of steroid aggregation behavior were carried out by UV measurements on a Hewlett Packard 8452A diode array spectrophotometer. Polarized infrared external reflectance spectroscopy (PIERS) was performed on a Biorad FTS60A spectrophotometer at an angle of incidence of 87°, in a nitrogen-purged chamber. For each spectrum 1024 scans were carried out, with a 2 cm^{-1} resolution. A freshly prepared deuterated decanethiol monolayer was used as background spectrum. Contact angle (CA) measurements were carried out on a KRÜSS Contact Angle Measuring System G10. Measurements for a drop of water whose volume was gradually increased (advancing CA) and then decreased (receding CA) were repeated on three sites of the same sample. For each receptor monolayer three samples were measured, and the average values are reported. X-ray photoelectron spectroscopy (XPS) was performed on a VG Escalab 220i-XL with monochromatic $Al_{K\alpha}$ X-ray source. Electrochemical measurements were performed with an AUTOLAB PGSTAT10, in a home-made electrochemical cell equipped with a platinum counter electrode, a mercury sulfate reference electrode (+0.61 V_{NHE}), and a screw cap to position the gold working electrode. For capacitance measurements the cell was filled with K_2SO_4 electrolyte solution (50 mL, 0.1 M). Nitrogen was bubbled through the cell for at least 5 min before each measurement. Cyclic voltammograms were recorded between −0.3 and −0.1 V at scan rates of: 0.1, 0.2, and 0.5 $V\,s^{-1}$, and the capacitance was calculated from the voltammograms recorded at 0.2 $V\,s^{-1}$, at −0.2 V_{MSE}. The values reported are the average of measurements on three individual samples. Heterogeneous electron transfer (HET) and impedance measurements were carried out in the presence of K_2SO_4 (50 mL, 0.1 M), $K_3[Fe(CN)_6]$ (1 mM), and $K_4[Fe(CN)_6]$ (1 mM) electrolyte solution. HET cyclic voltammograms were recorded between 0 and −0.7 V_{MSE} with a scan rate of 0.1 $V\,s^{-1}$. Resistance values were obtained by analyzing the impedance spectra measured at −0.2 V, between 0.1 Hz and 10 KHz, with the Equivalent Circuit software package.[34] The system can be well described by a circuit consisting of a parallel resistance (R_{ml}) and a capacitance (C_{dl}) in series with a second resistance (R_{el}). Where R_{el} is the resistance of the electrolyte, R_{ml} is the resistance of the monolayer, and C_{dl} is the capacitance of the monolayer.[35, 36]

Surface area calculations: The exposed surface area of the host (283 $Å^2$) and the contact surface of the guest (74 $Å^2$) were estimated from CPK models, as well as from computer modeling images (Quanta/CHARMm, release July 1997, Connoly Surfaces). Considering a top view of **2**, the exposed surface area consists of the *endo*-calix[4]arene cavity and two of its lower rim propyl substituents, the resorcin[4]arene cavity, two aromatic rings of the *exo*-calix[4]arene and two of its propyl substituents, and the four amide moieties linking the two calix[4]arenes to the resorcin[4]arene. Maximum possible contact of the guest surface to the receptor surface was presumed as the measurements are carried out in water, and hydrophobic interactions between the steroids and the monolayer are highly favored.

Synthesis of 5: To a solution of **4** (0.61 g, 0.53 mmol) in CH_2Cl_2 (50 mL) was added TBDMS-Cl (1.60 g, 0.01 mol), triethylamine (1.40 mL, 0.01 mol), and a catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred at 40 °C for 48 h. After cooling the reaction mixture to room temperature the solution was washed with HCl (1 N, 50 mL), H_2O (3 × 50 mL), and brine (50 mL). The organic layer was dried over Na_2SO_4 and then concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, hexanes/EtOAc, 8:2). Pure product **5** was obtained as a colorless oil in 100% yield. ^{1}H NMR δ = 6.55 (s, 4H, ArH), 5.76–5.58 (m, 4H, RC*H*=CH_2), 5.57 and 4.15 (AB-q, J = 7.5 Hz, 8H, OCH_2O), 4.90–4.72 (m, 8H, RCH=CH_2), 4.53 (t, J = 7.1 Hz, 4H, ArCHAr), 2.12–1.97 (m, 8H, RCH_2C=C), 1.90 (q, J = 7.1 Hz, 8H, R$CH_2$$CHAr_2$), 1.35–1.05 (m, 48H, CH_2), 0.85 (s, 36H, *t*Bu), 0.02 (s, 24H, CH_3Si); ^{13}C NMR δ = 146.4, 140.8, 139.2, 111.7 (Ar), 138.3 (R*C*H=CH_2), 114.1 (RCH=*C*H_2), 98.8 (OCH_2O), 36.9 (ArCHAr), 33.8 (*C*H_2CH=CH_2), 29.8–28.9 (CH_2), 27.9 ((CH_3)$_3$*C*Si), 25.6 ((CH$_3$)$_3$CSi), −4.4 (CH_3Si); MS (FAB, NPOE): *m/z* (%): 1610.4 (50) [*M*+H]$^+$, 1553.3 (100) [*M* − *t*Bu].

Synthesis of 6: To a solution of **5** (0.33 g, 0.21 mmol) in THF (50 mL) at 0 °C was added decanethiol (0.43 mL, 2.10 mmol) and then 9-BBN (0.50 mL of a 0.5 M solution in THF). The reaction mixture was stirred for 1 h, during which it was allowed to warm to room temperature and subsequently stirred for 15 h at room temperature. The solvent was evaporated in vacuo and the crude product was purified by flash column chromatography (SiO_2, hexanes/EtOAc, 8:2). Product **6** was obtained as a colorless oil, in 32% yield. ^{1}H NMR δ = 6.52 (s, 4H, ArH), 5.56 and 4.16 (AB-q, J = 7.5 Hz, 8H, OCH_2O), 4.59–4.48 (t, J = 7.1 Hz, 4H, ArCHAr), 2.36 (t, J = 7.1 Hz, 16H, RCH_2S), 2.08–1.95 (m, 8H, R$CH_2$$CHAr_2$), 1.54–1.35 (m, 16H, C$H_2$$CH_2S$), 1.31–1.00 (m, 112H, CH_2), 0.82 (s, 36H, *t*Bu), 0.75 (t, J = 6.7 Hz, 12H, CH_2CH_3), 0 (s, 24H, CH_3Si); ^{13}C NMR δ = 146.4, 140.7, 138.3, 111.7 (Ar), 99.0 (OCH_2O), 36.9 (ArCHAr), 32.2–28.9 (CH_2), 27.8 ((CH_3)$_3$*C*Si), 25.5 ((CH_3)$_3$CSi), 22.6 (*C*$H_2$$CH_3$), 14.1 ($CH_2$*C*$H_3$), −4.4 ($CH_3Si$); MS (FAB, MB): *m/z* (%): 2305.6 (100) [*M*].

Synthesis of 7: A solution of **6** (0.12 g, 0.05 mmol) in THF (30 mL) was cooled to 0 °C, and tetrabutylammonium fluoride trihydrate (0.33 g, 1.04 mmol) was added. The reaction mixture was stirred for 1 h, during which it was allowed to warm to room temperature. The solvent was evaporated in vacuo and the residue was taken up in CH_2Cl_2 (50 mL), washed with H_2O (3 × 50 mL), and dried over Na_2SO_4. The crude product was purified by recrystallization from MeOH to afford **7** in 83% yield. M.p.: 158–161 °C; ^{1}H NMR δ = 6.63 (s, 4H, ArH), 5.39 (s, 4H, OH), 5.95 and 4.45 (AB-q, J = 7.5 Hz, 8H, OCH_2O), 4.69 (t, J = 7.1 Hz, 4H, ArCHAr), 2.50 (t, J = 7.1 Hz, 16H, RCH_2S), 2.25–2.09 (m, 8H, R$CH_2$$CHAr_2$), 1.65–1.48 (m, J = 7.1 Hz, 16H, R$CH_2$$CH_2S$), 1.48–1.20 (m, 112H, CH_2), 0.90 (t, J = 6.7 Hz, 12H, CH_2CH_3); ^{13}C NMR δ = 147.2,

142.0, 140.2, 139.5 (Ar), 99.6 (OCH_2O), 36.0 (ArCHAr), 32.2, 31.9, 29.7–28.9 (CH_2), 22.7 (CH_2CH_3), 14.1 (CH_3); MS (FAB, NBA): *m/z* (%): 1848.3 (100) $[M]^+$; $C_{112}H_{184}O_{12}S_4$ ($3CH_3OH$) (1849.3): calcd C 70.94, H 10.15, S 6.59; found C 70.97, H 9.97, S 6.44.

Synthesis of 2: To a suspension of **7** (0.06 g, 0.03 mmol) in CH_3CN (14 mL), Cs_2CO_3 (0.13 g, 0.65 mmol) and a catalytic amount of KI were added. The reaction mixture was brought to reflux temperature and a solution of **8** (0.05 g, 0.07 mmol) in CH_3CN (20 mL) was added over a period of 8 h. The reaction mixture was subsequently stirred for another 12 h. The solvent was then evaporated in vacuo and the residue was taken up in CH_2Cl_2 (20 mL), washed with HCl (1N, 20 mL) and H_2O (2×20 mL). The organic layer was dried over Na_2SO_4 and the crude product was purified by flash column chromatography (SiO_2, hexanes/EtOAc, 3:1). The **2** *endo–exo* isomer was obtained as a white powder, in 21% yield. M.p.: 103–105 °C; ^{1}H NMR (please note that square brackets indicate that the protons belong to the cavitand moiety of the molecule) $\delta = 8.66$ (s, 2H, NH), 8.27 (s, 2H, NH), 7.00 (d, $J = 2.45$ Hz, *o*-NHAr*H*), 6.82 (d, $J = 3.05$ Hz, 2H, *o*-NHAr*H*), 6.67–6.42 (m, 20H, ArH), 5.85 [d, $J = 7.00$ Hz, 2H, OCH_2O], 5.79 [d, $J = 6.7$ Hz, 2H, OCH_2O], 4.70 [t, $J = 7.6$ Hz, 2H, ArCHAr], 4.62–4.30 (m, 18H, ArCHAr, $OCH_2C(O)$, OCH_2O, $ArCH_2Ar$), 4.23, 4.17 (2d, $J = 15.3$ and 15.7 Hz, $OCH_2C(O)$), 3.80–3.70 (m, 16H, $OCH_2CH_2CH_3$), 3.14–2.97 (m, 8H, $ArCH_2Ar$), 2.46–2.39 [m, 16H, RCH_2S], 2.16–2.05 [m, 8H, RCH_2CHAr_2], 1.85 (m, 16H, $OCH_2CH_2CH_3$), 1.55–1.43 [m, 16H, CH_2CH_2S], 1.38–1.15 [m, 112H, CH_2], 0.93 (t, $J = 7.18$ Hz, 24H, $OCH_2CH_2CH_3$), 0.78 [t, $J = 6.7$ Hz, 12H, CH_3]; ^{13}C NMR $\delta = 165.5$ (CO); MS (FAB, NBA): *m/z* (%): 3213.1 (30) $[M - \text{propyl}]^+$, 3254.9 (100) $[M]^+$, 3276.8 (30) $[M+\text{Na}]^+$; $C_{200}H_{284}N_4O_{24}S_4$ (3254): calcd C 73.73, H 8.79, N 1.72, S 3.94; found C 73.19, H 8.40, N 1.70, S 3.50.

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