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Steroid–porphyrin conjugate for saccharide sensing in protic media †

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A new saccharide receptor in protic media has been designed and synthesized. The receptor combines advantages of steroids, which are responsible for saccharide binding, and of the porphyrin moiety acting as a signalling component of the molecule due to changes in UV-vis electronic spectra. The synthesis is based on condensation of steroid aldehyde with pyrrole to form the porphyrin unit with four protected steroid moieties. After deprotection, *meso*-substituted porphyrin contains 12-hydroxy groups on the steroidal part. The receptor is soluble in aqueous solutions and exhibits high complexation affinity towards saccharides. Because the receptor extensively aggregates in water, most of the experiments were performed in 50% aqueous 2-propanol where aggregation is significantly eliminated. Binding is evidenced by spectral changes in the Soret region of the receptor in UV-vis absorption spectra allowing the evaluation of the binding constants. Additional confirmation of binding is obtained using ^{1}H NMR, Raman and IR spectroscopies and the surface plasmon resonance technique. The receptor exhibits higher selectivity for oligosaccharides over monosaccharide. The results point to the importance of a combination of multiple binding *via* H-bonding and hydrophobic interactions.

Introduction

The development of molecular systems with tailored binding properties for selected substrates in polar protic media remains a challenge for supramolecular chemistry. Saccharides represent one of the most important selectors in biology responsible for intercellular recognition, differentiation, novel binding mechanisms,[1] (for a basic review see ref. 2), and immunity response. Several successful receptors for binding of O-alkyl saccharides in organic solvents[3] have been reported. These receptors are based on polypyridine macrocycles, spirobifluorene, polyaza-cleft compounds, macrocycles combining cholic acid binaphthol units, and calixresorcinols. In contrast, due to their limited solubility and strong competition with solvent molecules, only a few receptors for effective binding of saccharides in aqueous media have been reported to date.[4] X-Ray analyses of saccharide binding proteins revealed that effective mimics of carbohydrate selective binding should be based on a hydrophobic cavity with a high density of proper binding groups.[1,2] Usually, molecular association is made possible not by a single weak interaction but through the simultaneous cooperation of several weak interactions.

Our design of saccharide receptors for aqueous media is based on a three-dimensional hydrophobic cavity with appropriate functionalities; a prerequisite for cooperative multiple interactions where several binding modes are involved. Multiple cooperative binding of the saccharide OH– and CH–groups with groups of the multifunctional receptor occurs *via* H-bonding with the OH groups of steroids and hydrophobic interactions with the steroid moiety. The receptor is constructed using the building block strategy where the binding part (polyhydroxy-steroid derivatives) is combined with the signalling part — a porphyrin unit that gives not only specific UV-vis and fluorescence properties, but possibly contributes with additional binding site functionality. The flexibility of petals attached to the porphyrin *meso*-positions results in a molecular structure which can easily adopt a geometry suitable for saccharide binding. The receptor has potential applications in enantioselective transport over cell membranes[5] and as a multifunctional component for construction of molecular nanodevices and electrochemical sensors.[6] Members of our group have reported synthesis, behaviour and saccharide sensing in aqueous media by porphyrins bearing 1,1′-binaphthyl substituents in the *meso*-position[7] and phenylphosphonates,[8] by porphyrin-cryptand cyclic receptors[9] and by 1,1′-binaphthyl substituted resorcinol-methyl red conjugates.[10] While elegant receptors reported by Shinkai *et al.*[11] rely on covalent binding of saccharides by boronic acid derivatives, our receptors are based on non-covalent interactions (H-bonding, CH–π interactions) giving efficient complexation even in polar protic media.

Here, we present a *meso*-substituted steroid–porphyrin conjugate, soluble in polar protic media, bearing twelve hydroxyl groups as multiple binding centres. Selected saccharides were used as physico-chemical models for binding studies. The solvent mixtures used in this study eliminate extended aggregation of the sensor.

Results and discussion

Design of the receptor

Our recent papers[12] revealed that combining steroids with the porphyrin moiety provides a molecule of promising potential

† Electronic supplementary information (ESI) available: binding isotherms, absorbance changes in the Soret region of **6** upon addition of saccharides, and corresponding Job plots indicating the stoichiometry of the complexes for all studied saccharides; binding of octyl β-D-glucopyranoside in a $CHCl_3$–CH_3OH mixture (9 : 1) measured using UV-vis; blank experiments documenting absorbance changes after addition of saccharide. See http://www.rsc.org/suppdata/ob/b3/b302947f/

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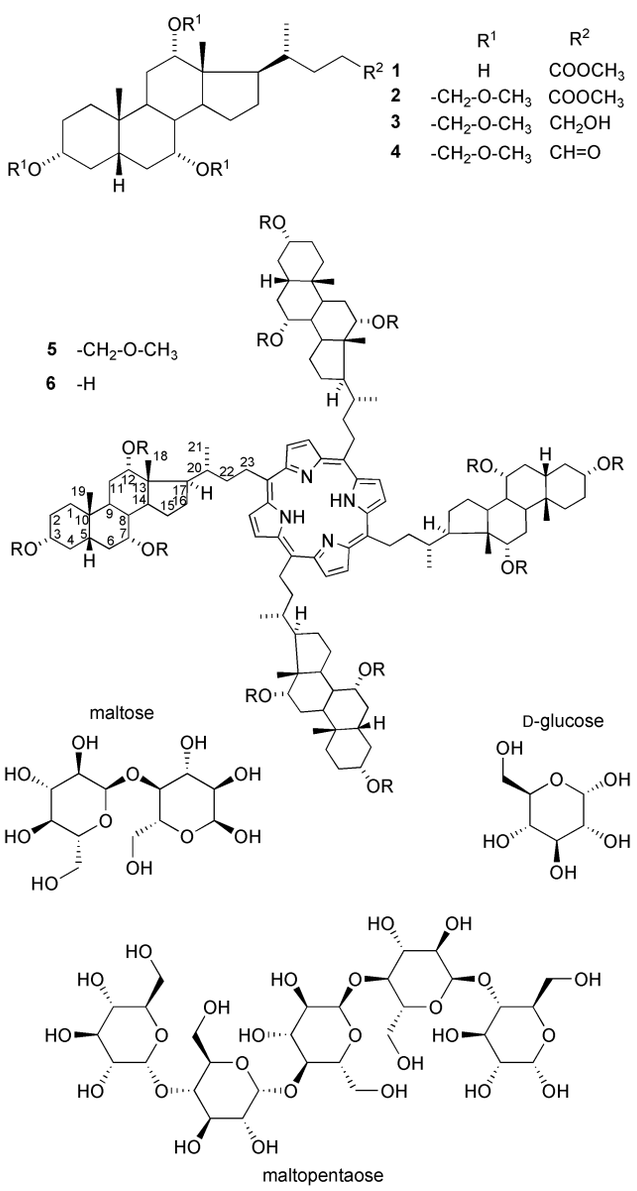
for saccharide recognition because the former is a binding part and the latter behaves as a signalling part of the molecule. The design of the receptor is based on the covalent attachment of the steroid moieties at 5,10,15,20-porphyrin positions *via* a short aliphatic chain. According to the MM2 calculations, steroids are flexible along the linkages with a rotational barrier of less than 4 kJ mol^{-1}. Furthermore, the calculations indicate that in the fully extended conformation the receptor is almost planar and has a length, measured between the most distant points, of about 40 Å (Scheme 1). The length of saccharides was estimated using the MM2 method to be 5.5 Å (D-glucose), 5.8 Å (maltose), 12.6 Å (maltotriose), 17.6 Å (maltotetraose) and 21.5 Å (maltopentaose) and the diameters obtained from space filling models are about 5.5 Å. In a polar solvent, steroid moieties are expected to fold into a cavity of comparable diameter to the size of the saccharide. In other words, the receptor is flexible and can bind suitable substrates under the induced fit principle. Specific advantages of this design are the high degree of preorganization and rigidity of the interacting steroid moieties, capable of hydrogen bonding interactions, solubility in polar solvents especially in water, and a chiral discrimination potential. The designed receptor consists of balanced polar and non-polar functions.



Scheme 1 Synthesis and molecular structures of the receptor **6** and some saccharides.

Synthesis of the receptor

In most reported cases incorporation of cholic acid to steroid-based receptors is achieved by using an amine or ester bonding.[13] Our approach starts from methyl cholate **1**, which was prepared according to a reported procedure[14] (Scheme 1). The starting compound **1** was treated with chloromethyl methyl ether yielding corresponding ether **2**, which was finally reduced by lithium aluminium hydride in THF to alcohol **3**. This compound was further oxidized to aldehyde **4** by a chromium(VI)-oxide–pyridine complex in dichloromethane at 0 °C. Aldehyde **4** was condensed with pyrrole in dichloromethane using boron trifluoride etherate as a catalyst to yield an intermediate, which was oxidized with tetrachloro-1,4-benzoquinone to afford protected tetrasteroidyl porphyrin **5**. Deprotection of **5** by hydrochloric acid in a dichloromethane–methanol mixture afforded the target steroid–porphyrin conjugate, receptor **6**.

Aggregation behaviour of the receptor

The receptor **6** is a hydrophobic porphyrin soluble in 2-propanol aqueous mixtures. In 2-propanol, the absorption spectrum was characterized by an intense Soret band at 417 nm and by four bands in the Q region at 521, 555, 602 and 662 nm. The lowest energy band at 662 nm corresponds to a transition to the first electronic excited singlet state. Two fluorescence emission peaks were located at 665 and 733 nm. In aqueous 2-propanol (5% v/v), the Soret band of porphyrin **6** was red-shifted by 7 nm, considerably broadened with a large hypochromicity. These spectral changes were accompanied by a strong decrease of the fluorescence emission intensity (Fig. 1a,c), which point to extensive porphyrin aggregation. So as to obtain more information on the molecular form of **6** we performed resonance light scattering experiments (RLS) because the amount of scattered light is directly proportional to the volume of particles and monomeric molecules and small oligomers show no enhanced scattering.[15,16] In 5% aqueous 2-propanol, intensive RLS profiles were recorded confirming the formation of extended aggregates of **6** (Fig. 2c). The RLS peak is centred at about 435 nm and is slightly red-shifted compared with the Soret band of **6** at 424 nm due to self-absorption of scattered light near the Soret maxima.

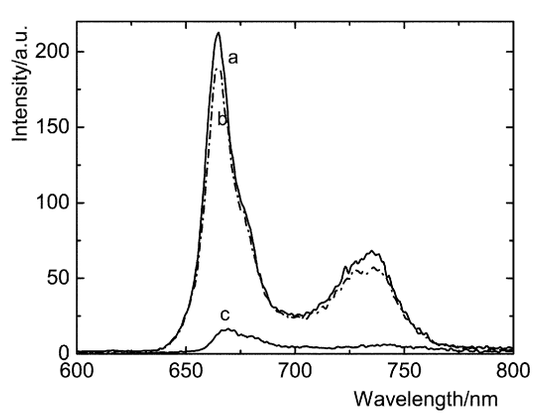


Fig. 1 Fluorescence emission spectra of optically matched solutions of porphyrin **6** at the excitation wavelength (about 520 nm) in 2-propanol (a), 50% (b) and 5% (c) aqueous 2-propanol.

The negative peak of **6** in 2-propanol results from self-absorption of light by the monomeric porphyrin (Fig. 2a). All described spectral features in 5% 2-propanol point to exciton coupling between neighboring porphyrin units due to the formation of extended self-aggregates. Since extended aggregates are poorly defined and many of the potential binding sites can be buried in the interior of the aggregate it is preferred to use solvent mixtures, in which the receptor is predominantly in the monomer form. We found that when using 50% aqueous

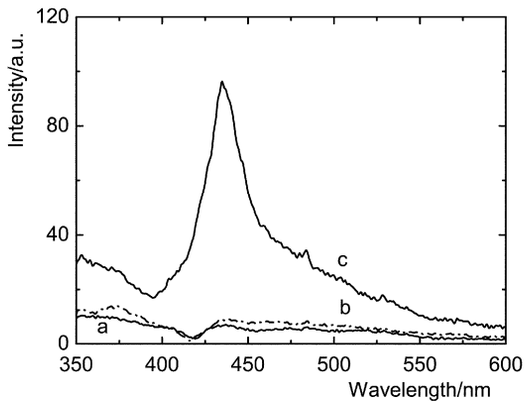


Fig. 2 Resonance light-scattering profiles of 5.6 μM **6** in 2-propanol (a), 50% (b) and 5% (c) aqueous 2-propanol.

2-propanol, the emission intensity of **6** is about the same as in neat 2-propanol (Fig. 1a,b) and no RLS scattering peak appears (Fig. 2b). There was still some hypochromicity of the Soret band indicating exciton coupling between porphyrin units in the excited S_2 states. No fluorescence quenching or negative peak on the RLS profile in 50% aqueous 2-propanol indicated that face-to-face dimers or extended aggregates can be excluded. It is reasonable to suppose interactions between porphyrin units and steroids on the intermolecular basis. This solvent mixture seems to be a good compromise between usage of aqueous solutions for molecular recognition of saccharides and defined molecular form of the receptor.

Complexation of saccharides

To determine if **6** binds saccharides, UV-vis absorption and fluorescence spectra of 5.6 μM **6** in 50% aqueous 2-propanol were recorded in the presence of saccharides (see ESI).† No fluorescence changes were observed. On the contrary adding maltotriose, maltotetraose and maltopentaose resulted in a well-defined and reproducible hypochromicity of the Soret band with a sharp saturation beyond three equivalents of saccharide (Figs. 3 and 4). Binding of **6** was further examined by the method of continuous variation to determine the overall stoichiometry. The intercepts were at mole fraction values of 0.50 corresponding to the formation of a 1 : 1 complex (Fig. 4, inset). Analysis of titration data by non-linear least-squares (Fig. 4) provided binding constants summarized in Table 1 allowing comparison of the binding efficiency.

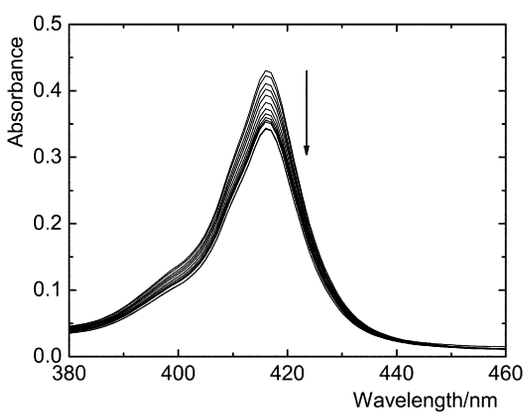


Fig. 3 UV-Vis titration of 5.6 μM **6** with maltopentaose in 50% 2-propanol. The arrow shows changes due to increasing concentration of maltopentaose.

Binding of D-glucose or maltose has a similar effect on the absorption spectrum of **6** as described above, however, total complexation of **6** needed a larger excess of saccharide. Interestingly, the Job plots gave evidence of the 2 : 1 stoichiometry

Table 1 Binding constants between **6** and saccharide[a]

Saccharide	K_b/M^{-1}
Maltotriose	$(1.4 \pm 0.2) \times 10^5$
Maltotetraose	$(4.3 \pm 0.5) \times 10^5$
Maltopentaose	$(7.6 \pm 1.2) \times 10^5$

[a] In water–2-propanol, 1 : 1 v/v at room temperature.

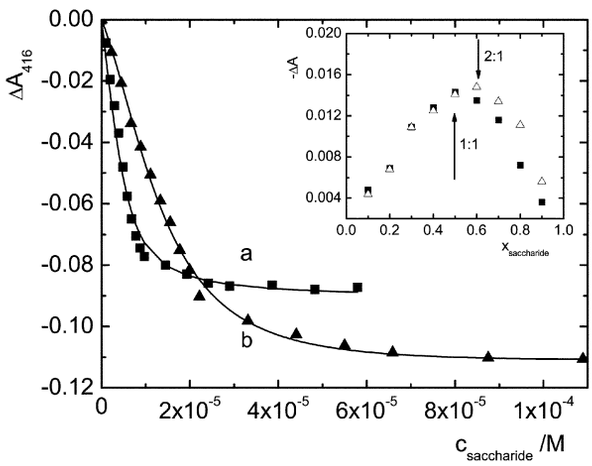


Fig. 4 Absorbance changes of 5.6 μM **6** upon addition of maltopentaose (a) and maltose (b) in 50% 2-propanol. Solid lines are the theoretical isotherms obtained by the least-squares fit to the experimental data. Inset: Job plots for binding maltopentaose (1 : 1) and maltose (2 : 1) calculated from absorbance changes at 416 nm. The sum of total concentrations of interacting components is constant (5.6 μM), *x* is the molar fraction of saccharide.

for a saccharide–porphyrin complex (Fig. 4, inset). In this case, however, the spectroscopic changes did not make it possible to differentiate 1 : 1 and 2 : 1 complex formation processes. The overall binding constants were 1.2×10^9 M^{-2} and 3.2×10^9 M^{-2} for D-glucose and maltose, respectively. The binding constants of 1 : 1 complexation were estimated to be in the range 10^3–10^4 M^{-1}.

From the results we see that increased saccharide size in the order D-glucose ≈ maltose < maltotriose < maltotetraose < maltopentaose leads to enhanced binding by receptor **6**. It seems that the receptor flexibility is a favourable factor for binding. In neat 2-propanol no binding occurs. It points to the importance of a combination of multiple binding *via* H-bonding and hydrophobic interactions that are effective in aqueous solutions.

To confirm that **6** binds saccharides, ^{1}H-NMR titration experiments were performed. Because of difficulties in analysing ^{1}H-NMR quantitatively due to receptor aggregation and limited solubility at concentrations needed (~10^{-3} M), the binding experiments were not performed in aqueous 2-propanol. Instead, we used chloroform–methanol mixtures for binding maltotriose and octyl β-D-glucopyranoside and DMSO for D-glucose. Nevertheless, the chemical shifts of the proton resonances of the steroidal part (the positions 18, 19 and 21) and of saccharide induced by increasing saccharide/**6** ratios can indicate the binding and involvement of the steroid moieties (Scheme 1). We performed simultaneously UV-vis titrations in order to compare NMR and UV-vis results in the same solvent system.

The experiment with maltotriose was performed in a $CDCl_3$–CD_3OD mixture (1 : 1 v/v) (Fig. 5). At a molar ratio of maltotriose/**6** of 0.25 small downfield chemical shifts were observed, probably due to porphyrin aggregation. Further additions of maltotriose to this solution lead to upfield shifts as more saccharide is bound. Significant upfield shifts were observed for the β-pyrrole proton resonance (0.045 ppm for 2 equiv. of

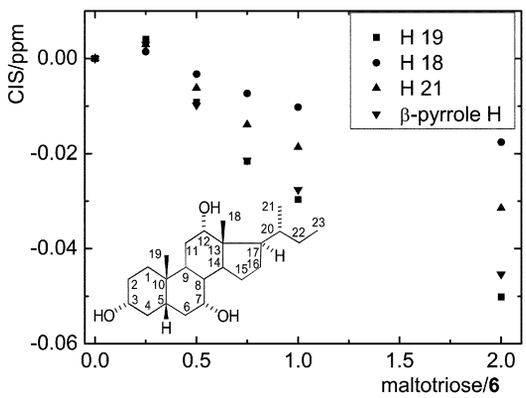


Fig. 5 Chemical induced shift of 5.7 mM **6** upon addition of maltotriose in $CDCl_3$–CD_3OD (1 : 1 v/v).

maltotriose) and for the methyl protons 19 (0.05 ppm), while the methyl protons 18 (0.02 ppm) and 21 (0.03 ppm) experienced less contact with maltotriose. The upfield shifts are common for guest binding and in our specific case it denotes an effect of a microenvironment of high electron density, due to the lone electron pairs of glycosidic oxygen atoms and oxygens of the pyranose cycle. This observation, together with the upfield shifts of the β-pyrrole resonances are a clear sign of binding at the steroidal moieties with maltotriose located over the porphyrin ring.

Because of complexity of the ^{1}H NMR spectra of the saccharides studied, the overlap of saccharide and steroid–porphyrin proton signals, and solubility/aggregation obstacles it was not possible to unambiguously assign the respective saccharide signals. For this reason we performed binding experiments with a model saccharide, octyl β-D-glucopyranoside, in a $CDCl_3$–CD_3OD mixture (9 : 1). This solvent mixture enabled simultaneous observation of the saccharide and receptor proton signals. Similarly to the above described ^{1}H NMR experiments we met solubility limitations, so we were not able to record a complete binding isotherm. The receptor proton resonances moved upfield as follows (two saccharide equivalents, $\delta/10^{-3}$ ppm): the methyl protons 18, 19 and 21 moved by 5.6, 3.4 and 3.8, respectively. Hydrogens at position 3 (11.6) and 7 (8.4) also moved upfield. The saccharide proton resonances are also shifted upfield (two saccharide equivalents, $\delta/10^{-3}$ ppm): H1 (6.0), H2 (12.8), H5 (14.4), H6a (3.0), H6b (6.2), H1a′ (5.8) and H1b′ (6.4). The upfield shifts of the presented proton resonances document that the receptor interacts with octyl β-D-glucopyranoside.

Though the used solvent system is far from our main aim to study a receptor working in aqueous media we were able to compare NMR and UV-vis results. The UV-vis spectra of the receptor **6** changed after addition of octyl β-D-glucopyranoside similarly to the above described changes (see ESI for details).† The binding constant was estimated to be 5.5×10^5 M^{-1}. The ^{1}H NMR and UV-vis results confirm that both methods are suitable to detect the saccharide complexation. Interestingly, weak binding of D-glucose was also observed in DMSO-d_6, a highly competitive solvent. At four equivalents of D-glucose, the methyl proton resonances moved upfield by 4.6, 2.4 and 5.2 ($\delta/10^{-3}$ ppm) at the positions 21, 18 and 19, respectively. In addition, a broadening of the OH resonances corroborated the importance of hydroxyl functions for the binding process. All presented NMR shifts were in the range of single Hz and were significant and reproducible. The NMR spectra of **6** (1.6–8.1 mM) in the absence of saccharide did not show any concentration dependent shifts of the proton resonances within experimental error of 0.1 Hz for methyls and 0.3 Hz for other hydrogens.

Further indications of the saccharide binding were obtained by vibrational spectroscopies. The Raman spectrum of octyl β-D-glucopyranoside in a thin film exhibited bands at 2887 and 2851 cm^{-1} (pyranoside C–H), which were shifted to 2860 and 2822 cm^{-1} in the complex. IR Spectroscopy revealed involvement of the OH groups in binding. The OH bands of octyl β-D-glucopyranoside at 3370 cm^{-1} and of **6** at 3390 cm^{-1} were shifted to 3295 cm^{-1} in the complex indicating effective hydrogen bonding. Also, the C–O band moved from 1078 to 1074 cm^{-1}.

The ability of the solid film of **6** to bind saccharides was confirmed by the surface plasmon resonance (SPR) technique. This technique is often used for monitoring receptor–ligand interactions, including sensing of organic pollutants.[17,18] SPR allows direct monitoring of binding of an analyte to a receptor by following changes in the refractive index at the sensor surface. The receptor **6** was deposited on a gold film of the optical chip, layered with an aqueous solution of 5 mM saccharide and incubated for 5 min at room temperature. A photodiode recorded the intensity of polarized light reflected from the gold surface and from the surface coated with the receptor–saccharide complex. The differences in refractive indices are corresponding SPR curves (Fig. 6). A response of the receptor film to saccharides was given by a significant shift of the angle of resonance after incubation with a saccharide solution (Fig. 6a,b). The binding is partially reversible as documented by washing the layer with water (Fig. 6c,d). Non-quantitative wash out of complexed saccharide from the receptor layer can be explained by the high stability of the complex. The control experiments with non-complexing 5,10,15,20-tetraphenylporphyrin instead of **6** gave no change in SPR curves after treatment with saccharide.[7] These results also confirm the binding and pave the way towards development of new sensors for saccharides.

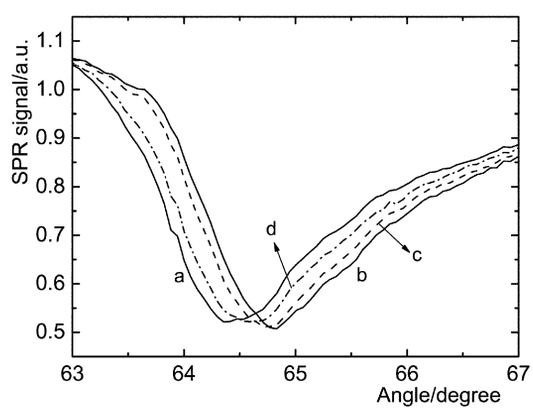


Fig. 6 SPR curves of **6** with D-glucose on a gold surface. Curves (a) and (b) represent **6** layered with water and 5 mM D-glucose in water, respectively. Curve (c) was recorded after first washing the layer of **6** (curve b) and curve (d) after washing the layer seven times.

Conclusions

We have described a convenient synthesis, spectral and binding properties of a novel steroid–porphyrin receptor. We have demonstrated that compound **6** bearing steroid functions forms complexes with saccharides in an aqueous solution and in the solid state. The mechanism of the binding involves hydrogen bonding between the steroidal part and saccharide, which is located over the porphyrin moiety. Porphyrin, a good chromophore, is a signalling part of the receptor and, hence, the binding can be followed by UV-vis changes. The receptor has higher selectivity for oligosaccharides over monosaccharide. The use of the SPR technique provides a powerful method to detect saccharides in solutions because saccharide is bound to the solid film of the receptor. This arrangement allows construction of new saccharide sensors.

Experimental

Materials and methods

D-Glucose (Aldrich), maltose monohydrate (Merck), maltotriose hydrate (Aldrich), maltotetraose (Fluka), maltopentaose (Fluka), octyl β-D-glucopyranoside (Fluka), and cholic acid (Sigma-Aldrich or Steraloids) were used as received. All solvents were purified by standard procedures. Melting points were determined on a Boëtius (Germany) micro melting point apparatus (Kofler block) and are uncorrected. Optical rotations were measured on an Opton polarimeter (Germany) at 25 °C. UV-Vis spectra were taken on a spectrophotometer Varian CARY400 SCAN.

Binding stoichiometries of saccharide/**6** complexes were obtained by the Job's method of continuous variations. The solutions of porphyrin **6** and saccharide were mixed to a standard volume while the sum of both concentrations was kept constant at 5.6 μM. Two sets of solutions with the same concentrations of **6** were prepared in the presence and absence of saccharide. Absorbance differences were recorded at 416 nm. The binding stoichiometry was obtained from the intercepts after linear least-squares fits to the left- and right-hand portions of Job's plots.

Titration experiments were performed in a 1 cm square quartz cell filled with 5.6 μM **6** in a water–2-propanol (1 : 1 v/v) or $CHCl_3$–CH_3OH mixture (9 : 1 v/v) at room temperature. A stock saccharide solution in 5.6 μM **6** was added stepwise in small increments. The binding isotherms were recorded at the Soret maximum of **6** and were analysed by non-linear least squares. For the stoichiometry 1 : 1 the hyperbolic relationship between the observed absorbance change ($\Delta A = A - A_o$) and the equilibrium free molar concentration of saccharide is as follows[19]

$$\Delta A = \Delta A_\infty K_b[\text{saccharide}]/(1 + K_b[\text{saccharide}])$$

where A_o is the absorbance in the absence of saccharide, A_∞ is the absorbance of a corresponding complex and $\Delta A_\infty = A_\infty - A_o$ The free molar concentration [saccharide] was expressed as a function of the added total concentration using the equation

$$[\text{saccharide}]_t = [\text{saccharide}] + P_t K_b[\text{saccharide}]/(1 + K_b[\text{saccharide}])$$

where P_t is the total molar concentration of **6**. For evaluation of 2 : 1 binding constants the equation for the binding isotherm 2 : 1 was applied.[19] All UV-vis results are from three independent experiments. The spectra of solutions did not show any changes even after standing 12 h at room temperature.

IR and Raman spectra were recorded on a FTIR spectrometer Nicolet Avatar 320 and FT NIR spectrometer Equinox 55/S equipped with a Raman accessory FRA 106/S (Bruker) and a Nd–YAG laser (1064 nm) (Coherent), respectively. The complex octyl β-D-glucopyranoside–**6** was measured at room temperature as a thin film prepared by mixing of respective components in 2-propanol and by slow evaporation of the solvent. ^{1}H NMR spectra were recorded using a Varian UNITY-200 (200.04 MHz for ^{1}H) or a UNITY-500 (499.8 MHz for ^{1}H) FT NMR spectrometer in $CDCl_3$ using Me_4Si as an internal standard at 300 K. The latter spectrometer was used for measurement of all binding experiments. The coupling constants are given in Hz. Mass spectra were recorded by means of a VG Analytical ZAB-EQ spectrometer. Resonance light-scattering experiments (RLS) were conducted using simultaneous scans of the excitation and emission monochromators through the range of 300–600 nm on a Perkin-Elmer LS 50B luminescence spectrophotometer. Surface plasmon resonance measurements (SPR) were performed on a Spreeta Experiments Kit (Texas Instruments, USA). Analytical TLC was performed on silica gel G (ICN Biomedicals) followed by spraying with concentrated sulfuric acid and heating. For flash column chromatography a silica gel 32–63, 60 Å (ICN Biomedicals or Silpearl, Kavalier) and for preparative TLC 200 × 200 mm plates (thickness 0.4 mm, silica gel G, ICN Biomedicals) were used. The software for the molecular mechanics (MM2) calculations was the Cambridge Software Chem3D 2000 package (6.0). Energy minimization of the isolated **6** was performed until the root-mean-squares of the reference gradient was below 0.100.

Synthesis

Methyl 3α,7α,12α-tri(methoxymethoxy)-5β-cholan-24-oate (2). To a stirred benzene solution (50 ml) of methyl cholate (**1**, 1.1 g, 2.6 mmol), chloromethyl methyl ether (2.52 g, 2.4 ml, 31.3 mmol) and *N*,*N*-diisopropylethylamine (4.04 g, 5.44 ml, 31.3 mmol) were added. The mixture was stirred for 10 days at room temperature. The solid was removed by filtration and washed twice with benzene (250 ml). The organic filtrates were combined and evaporated yielding 1.43 g (98%) of **2** in the form of a syrup. Found (%): C, 67.3; H, 9.9. $C_{31}H_{54}O_8$ requires C, 67.1; H, 9.8. ν_{max}/cm^{-1} ($CHCl_3$): 1736 (C=O); 1146, 1102, 1038, 913 (CH_3OCH_2O); δ_H (200 MHz, $CDCl_3$): 4.54–4.77 (6H, m, 3 × –O–C*H*$_2$–O–), 3.79 (1H, bt, C(12)*H*), 3.61 (1H, m, C(7)–*H*) 3.47 (3H, s, C*H*$_3$–OOC–), 3.40 (1H, m, C(3)*H*), 3.44, 3.39, 3.35 (9H, 3s, 3 × C*H*$_3$–O–C–), 0.96–2.37 (24H, m, steroid H), 0.98 (3H, d, *J* 6.10 Hz, C(21)*H*), 0.90 (3H, s, C(19)*H*), 0.69 (3H, s, C(18)*H*).

3α,7α,12α-Tri(methoxymethoxy)-5β-cholan-24-ol (3). Ester **2** (500 mg, 0.9 mmol) was dissolved in freshly distilled THF (70 ml) under argon. Lithium aluminium hydride (44 mg, 1.17 mmol) was added and the mixture was stirred under reflux for 12 h. The solvent was carefully evaporated to dryness and the residue was treated with 5% aqueous citric acid (50 ml). Then, ethyl acetate (50 ml) was added and the two phase system was vigorously stirred for 1 h. The organic layer was separated, then washed with water (3 × 50 ml), dried by anhydrous sodium sulfate and evaporated to yield **3** (439 mg, 92%). Mp 88–91 °C. Found (%): C, 68.75, H, 10.8. $C_{30}H_{54}O_7$ requires C, 68.6; H, 10.8. ν_{max}/cm^{-1} ($CHCl_3$): 3619, 3475 (OH); 1146, 1102, 1038, 911 (CH_3OCH_2O); δ_H (200 MHz, $CDCl_3$): 4.53–4.76 (6H, m, 3 × –O–C*H*$_2$–O–), 3.80 (1H, bt, C(12)*H*), 3.62 (3H, m, C(7)*H* and 2 × C(24)*H*), 3.39 (1H, m, C(3)*H*), 3.44, 3.39, 3.36 (9H, 3s, 3 × C*H*$_3$–O–), 0.98–2.39 (25H, m, steroid H), 0.97 (3H, d, *J* 6.4 Hz, C(21)*H*), 0.90 (3H, s, C(19)*H*), 0.69 (3H, s, C(18)*H*).

3α,7α,12α-Tri(methoxymethoxy)-5β-cholan-24-al (4). Pyridine (1.8 ml, 22 mmol) was added dropwise to a stirred suspension of chromium(VI) oxide (735 mg, 7.35 mmol) and anhydrous magnesium sulfate (1 g) in CH_2Cl_2 (20 ml) at 0 °C under argon. Stirring continued for 20 min at 0 °C. Then, a solution of alcohol **3** (530 mg, 1 mmol) in CH_2Cl_2 (5 ml) was added and the reaction mixture was stirred under argon at 0 °C for an additional 3 h. After dilution with ether (25 ml), the mixture was applied on a silica gel column (50 g). The product was eluted with a CH_2Cl_2–ether mixture (1 : 1, 150 ml), solvents were evaporated and residual pyridine was removed by co-evaporation with toluene. Aldehyde **4** (490 mg, 92%) was obtained as a syrup. Found (%): C, 68.7; H, 9.9. $C_{30}H_{52}O_7$ requires C, 68.7; H, 10.0. ν_{max}/cm^{-1} ($CHCl_3$): 2827, 2726, 1721 (CH=O); 1148, 1127, 1043, 1031, 912 (CH_3OCH_2O); δ_H (200 MHz, $CHCl_3$): 9.77 (1H, t, *J* 2.0 Hz, C(24)*H*), 4.58–4.80 (6H, m, 3 × –O–C*H*$_2$–O–), 3.80 (1H, bt, C(12)*H*), 3.62 (1H, m, C(7)*H*), 3.39 (1H, m, C(3)*H*), 3.44, 3.39, 3.36 (9H, 3s, 3 × C*H*$_3$–O–), 0.98–2.39 (25H, m, steroid H), 0.96 (3H, d, *J* 6.4, C(21)*H*), 0.90 (3H, s, C(19)*H*), 0.62 (s, 3H, C(18)H).

***meso*-Tetrakis[3α,7α,12α-tri(methoxymethoxy)-5β-cholan-24-yl]porphyrin (5).** Aldehyde **4** (200 mg, 0.38 mmol) was dissolved

in CH_2Cl_2 (38.5 ml) under nitrogen and pyrrole (26 mg, 0.39 mmol) and boron trifluoride etherate (18 mg, 0.125 mmol) were added. After 48 h tetrachloro-1,4-benzoquinone (70 mg, 0.28 mmol) was added and the mixture was refluxed for 1 h. After cooling the mixture was treated with 5% aqueous $NaHCO_3$ (40 ml), the organic layer was separated, washed with water (3 × 40 ml), dried by anhydrous sodium sulfate and evaporated. The residue was dissolved in CH_2Cl_2 and purified on a silica gel column (50 g) using a CH_2Cl_2–MeOH (9 : 1) mixture. It yielded 16 mg (7.3%) of a thick syrupy product **5**. Found (%): C, 71.1; H, 9.3; N, 2.75. $C_{136}H_{214}N_4O_{24}$ requires C, 71.4; H, 9.4; N, 2.45. $[\alpha]_D^{25}/10^{-1}$ deg cm^2 g^{-1} +78 (*c* 0.5 in CH_2Cl_2); ν_{max}/cm^{-1} ($CHCl_3$): 3320 (porphyrin); 1146, 1102, 1038, 913 (CH_3-OCH_2O); δ_H (500 MHz, $CDCl_3$): 9.48 (8H, m, pyrrole H), 4.4–5.2 (24H, m, 12 × O–C*H*$_2$–O), 3.54 (12H, bs, 4 × –O–C*H*$_3$), 3.36 (24H, bs, 8 × –O–C*H*$_3$), 3.2–4.1 (12H, m, steroid –C*H*–O), 0.86–2.36 (96H, m, steroid H), 0.79–0.92 (12 × 3H, m, steroid methyls), −2.7 (s, 2H, pyrrole N*H*); (FAB) *m*/*z*: 2289 (100%), 2245.5 (80), 2216 (75), 2198 (50); λ_{max}/nm (CH_2Cl_2), (ε/dm^3 mol^{-1} cm^{-1}): 419 (1.95 × 10^5).

***meso*-Tetrakis[3α,7α,12α-trihydroxy-5β-cholan-24-yl]porphyrin (6).** Porphyrin **5** (15 mg, 6,5 μmol) was treated with concentrated hydrochloric acid (15 μl) in a CH_2Cl_2–MeOH mixture (2 ml, 1 : 1) under stirring for 48 h at room temperature. The reaction mixture was evaporated to dryness and triethylamine (0.5 ml) was added. The residue was mixed with water (10 ml) and the sedimented solid was separated. Washing with water was repeated and the product was dried *in vacuo* at 50 °C. The crude product was dissolved in a CH_2Cl_2–MeOH mixture (1 ml, 9 : 1) and purified on a preparative TLC plate (20 × 20 cm) using CH_2Cl_2–MeOH (9 : 1) yielding 10 mg (87%) of an amorphous slightly hygroscopic solid **6**. Found (%): C, 76.3; H, 9.4; N, 3.0. $C_{112}H_{166}N_4O_{12}$ requires C, 76.4; H, 9.5; N, 3.2. ν_{max}/cm^{-1} ($CHCl_3$): 3428, 3265 (OH); 1076, 1043 (C–O); δ_H (500 MHz, DMSO-d_6): 9.6 (8H, bs, pyrrole H), 5.34 (4H, m, 4 × C(12)*H*), 5.05 (4H, bs, 4 × C(7)*H*), 4.79 (4H, bs, 4 × C(3)*H*), 3.48–4.28 (12H, m, 12 × O*H*),), 0.82–2.33 (96H, m, steroid H), 0.70–1.02 (12 × 3H, m, steroid methyls), −2.82 (2H, 2 × pyrrole N*H*); δ_H (500 MHz, DMSO-d_6–D_2O, 2 : 1 v/v): 9.45 (8H, bs, pyrrole H), 5.34 (4H, m, 4 × C(12)*H*), 5.05 (4H, bs, 4 × C(7)*H*), 4.79 (4H, bs, 4 × C(3)*H*), 0.6–1.0 (12 × 3H, m, angular C*H*$_3$); δ_H (500 MHz, $CDCl_3$–CD_3OD, 9 : 1 v/v): 9.43 (8H, bs, pyrrole H), 5.38 (4H, m, 4 × C(12)*H*), 5.08 (4H, bs, 4 × C(7)*H*), 4.80 (4H, bs, 4 × C(3)*H*), 3.05–4.20 (12H, m, O*H*), 0.78–2.35 (96 H, m, steroid H), 0.60–1.03 (12 × 3H, m, steroid methyls); (FAB) *m*/*z*: 1760.5 (100%), 1453.2 (6), 1411.1 (14); λ_{max}/nm (2-propanol), (ε/dm^3 mol^{-1} cm^{-1}): 417 (1.78 × 10^5), 521 (7.37 × 10^3), 555 (5.89 × 10^3), 602 (2.52 × 10^3), 662 (3.48 × 10^3).

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