Selective steroid recognition by a partially bridged resorcin[4]arene cavitand†

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The partially bridged resorcin[4]arene cavitand 1 featuring a cleft-shaped recognition site formed by two *anti*-quinoxaline bridges and four convergent HO-groups was prepared in three steps and characterised by X-ray crystallography; cavitand 1 was found to be a selective receptor for steroidal substrates in $CDCl_3$, with the best binding observed for steroids with a flat A-ring and two H-bonding sites on rings A and C/D.

While the inhibition of biological steroid receptors is an important target in contemporary medicinal chemistry[1] and a large amount of X-ray structural information on protein–steroid complexes has become available,[2] the number of studies with synthetic receptors, aimed at further deciphering principles of steroid recognition, is remarkably limited.[3] Artificial steroid receptors investigated in solution in the past include modified cyclodextrins and resorcin-[4]arenes as well as water-soluble cyclophanes. Here, we report the steroid recognition properties of **1** (Fig. 1), a representative of a recently introduced family of partially bridged resorcin[4]arene cavitands.[4,5] Whereas host–guest complexation by fully-bridged resorcin[4]arene-based cavitands[6] and self-assembled capsules[7] has been intensively investigated, molecular recognition studies with partially-bridged cavitands featuring distinct, cleft-type binding sites have not been reported.[8]

Cavitand **1** was conveniently prepared in three steps (see ESI for experimental details) from resorcinol and dodecanal, following a previously described protocol.[5] Crystals suitable for X-ray analysis were obtained by slow evaporation of a solution in acetone.‡ The structure reveals a preorganised cleft-type binding site, shaped by the two *anti*-oriented quinoxaline rings, into which the four free phenolic HO-groups converge (Fig. 1). The aperture of the cleft is 8.5 Å wide which should allow the incorporation of flat steroids with unsaturated rings.[9] The crystal packing displays a highly organised layered structure consisting of dimers of **1** (Fig. 2). Layers are separated into two domains: one with the intercalated undecyl chains and the other consisting of the resorcin[4]arene headgroups in a head-to-head arrangement. The dimers are stabilised by π–π stacking interactions between intercalating quinoxaline flaps at intermolecular distances between 3.46 and 4.55 Å. This dimerisation of **1** is also seen in $CDCl_3$ solution (see below). The layers are further stabilised by short H-bonds (d(O···O) = 2.67 and 2.68 Å) between two included acetone molecules and HO-groups of each cavitand.

A comprehensive complexation study, including 14 steroidal substrates, was conducted. ^{1}H NMR binding titrations at constant receptor concentration were performed at 298 K to determine the association constants K_a [M^{-1}] of the complexes formed by **1** in $CDCl_3$.[10] The K_a values (Table 1) were corrected for the dimerisation of **1** (K_{dim} = 152 M^{-1}) as well as of the steroids; dimerisation constants were obtained from ^{1}H NMR dilution experiments (see ESI). 1 : 1 Binding stoichiometries were ascertained by Job plot analysis. In all ^{1}H NMR experiments, the complexation-induced downfield shift of the HO-protons of

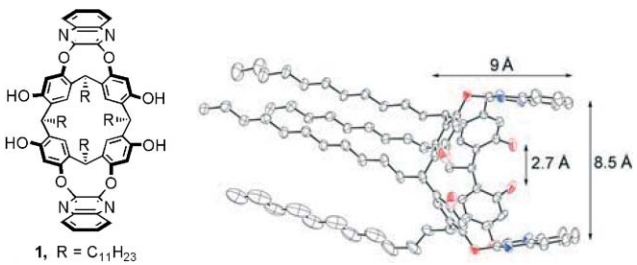

Fig. 1 Structure of receptor **1** and dimensions of the cleft-type binding site as measured from X-ray crystal structure analysis.

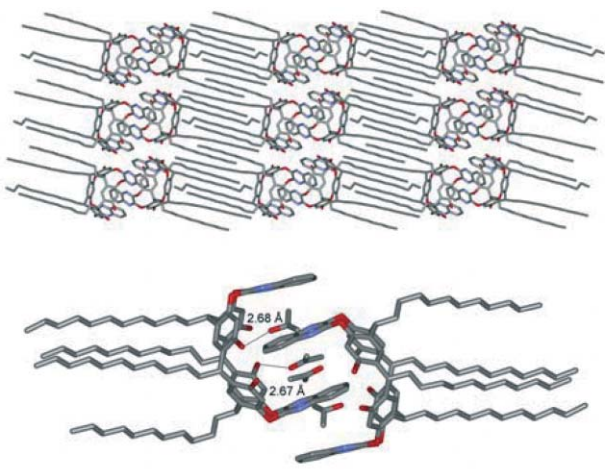

Fig. 2 (a) X-ray crystal structure showing the layered packing of **1**, and (b) head-to-head arrangement of **1** into π–π-stacking dimers and two acetone molecules H-bonded to each cavitand.

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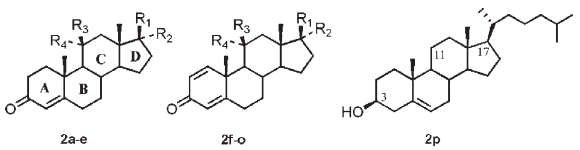
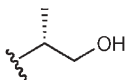
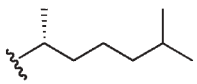
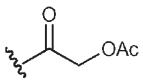
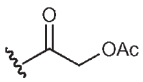
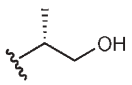
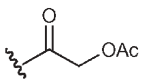
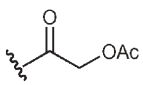
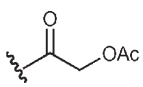
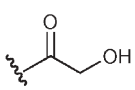
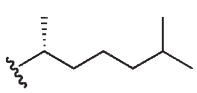
† Electronic supplementary information (ESI) available: protocols for the synthesis of **1**, crystal structure data for **1**, description of ^{1}H-NMR binding titrations, determination of dimerisation constants and Job plot analysis. See DOI: 10.1039/b509990k

Table 1 Association constants K_a [M^{-1}] from 500 MHz ^{1}H NMR titrations ($CDCl_3$, 298 K) for 1 : 1 complexes between **1** and steroids **2a–p**



Steroid	R_1	R_2	R_3	R_4	K_a [M^{-1}][a]
2a		H	H	H	43
2b		H	H	H	74
2c		H	H	H	n.d.[b]
2d		OH	OH	H	595
2e		OH	O	—	34
2f		H	H	H	111
2g	OH	H	H	H	104
2h	H	OH	H	H	536[c]
2i	O	—	H	H	105
2l		OH	H	H	668
2m		OH	O	—	458
2n		OH	OH	H	738
2o		OH	O	—	226
2p		H	H	H	n.d.[b]

[a] Titrations were performed at constant receptor concentration. K_a values are corrected for the dimerisation of **1** and the steroids. Reproducibility of K_a values ±10%. [b] Very weak binding. [c] Reproducibility ±15%.

receptor **1** was monitored and used to evaluate the association constants (see ESI).

The most important results obtained in the binding studies are summarized in the following:

i) Binding affinity is strongly affected by the shape of the tetracyclic steroidal core. Whereas fully saturated steroids are hardly bound at all, the association strength increases with a flattening of the A ring (*e.g.* see the complexation of **2m** *vs.* **2e**). According to molecular modeling,[11] the steroidal A-ring fully penetrates the 8.5 Å wide cleft of **1**. The steric host–guest complementarity becomes improved when the A-ring is changed from a ketone to an enone, and to a dienone.

ii) Complexation is also affected by the size of the side-chain at C(17): a long terpenoid side chain as in **2c** and **2p** leads to poor complexation while shorter side-chains (**2a,b,d–f,l–o**) or no side chain at all (**2g,i**) favour stronger binding. We tentatively propose that **2c** and **2p** may adopt different binding geometries, with their lipophilic rings B–D and the terpenoid side-chain interacting with the four undecyl legs of **1** outside the cleft site.

iii) The presence of a carbonyl group at C(3) in ring A is a prerequisite for measurable binding affinities: this group undergoes H-bonding to a pair of HO-groups in the resorcin[4]arene skeleton as shown by the docking simulation in Fig. 3.

iv) Binding affinity is strongly enhanced upon introduction of additional H-bonding functionality, such as an HO-group, directly at either C(11) in the C-ring or C(17) in the D-ring. Location of the second H-bonding group seems to be more preferential on the D-ring than on the C-ring: the steroids forming the most stable complexes all have an HO-group directly attached to C(17), such as **2d,h,l–n**.

v) The orientation of the HO-group at C(17) of the steroidal substrate affects binding affinity in a substantial way: whereas **2g** with a β-HO-group only gives a K_a-value of 104 M^{-1}, diastereoisomer **2h**, with an α-HO-group, yields K_a = 536 M^{-1}. Computer docking simulations indicate that this diastereoselectivity is caused by the facial non-equivalency of the steroid skeleton (Fig. 3). For steric reasons, the β-face with the two Me-groups tends to orient towards the entrance of the cleft, which prevents favourable interactions between the β-OH-group at C(17) of **2g** and the HO-groups on **1**. In the complex of **2h**, with the opposite configuration at C(17), the O···O distance is < 3 Å, thereby allowing efficient H-bonding.

In conclusion, we have shown that the readily available, cleft-shaped compound **1** is an efficient and selective receptor for steroids in $CDCl_3$. Structure–activity relationships show that stable complex formation requires (i) a flat steroidal A-ring, (ii) a carbonyl group at C(3) and (iii) a second H-bonding residue directly attached to C(11) or C(17) of the steroidal skeleton. Targeting other classes of substrates, such as carbohydrates, we currently address further elaboration of the H-bonding sites converging into the cleft as well as the introduction of additional recognition sites at the rim of the quinoxaline flaps.

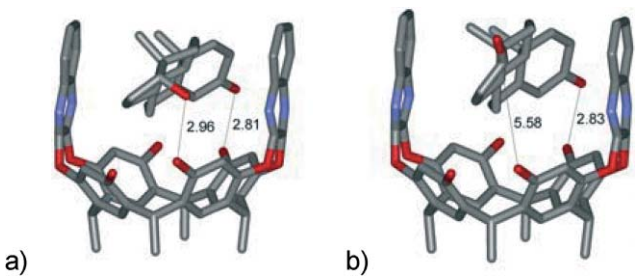


Fig. 3 Results of the docking studies for the **1** **2h** (a) and the **1** **2g** (b) complexes, showing distances between the O-atom involved in H-bonding.[11] Hydrogen atoms are removed for clarity.

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Notes and references

‡ Crystallographic data, collected at 220(2) K: $(C_{88}H_{116}N_4O_8)\cdot 2(C_3H_6O)$; 1474.00 g/mol; triclinic; space group P (no. 2), $D_c = 1.128$ g cm^{-3}, $Z = 2$, $a = 11.8400(2)$ Å, $b = 13.8058(2)$ Å, $c = 28.6689(4)$ Å, $\alpha = 78.641(1)$, $\beta = 80.066(1)$, $\gamma = 72.126(1)^\circ$, $V = 4341.04(11)$ Å^3. *Bruker–Nonius Kappa-CCD* diffractometer, Mo$K\alpha$ radiation, $\lambda = 0.7107$ Å, $\mu = 0.072$ mm^{-1}. Numbers of measured and unique reflections are 18839 and 11913, respectively ($R_{int} = 0.025$). Final $R(F) = 0.082$, w$R(F^2) = 0.212$ for 959 parameters and 8241 reflections with $I > 2\sigma(I)$ and $2.82 < \theta < 22.99^\circ$ (corresponding R-values based on all 11913 reflections are 0.116 and 0.239 respectively). All non-H atoms apart from those of one acetone molecule were refined anisotropically, H-atoms isotropically, whereby H-positions are based on stereochemical considerations. One of the two acetone solvent molecules exhibits static and dynamic disorder, which could not be resolved. CCDC 277163. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b509990k

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