Complexation Properties of Preorganized Receptor Molecules for Large, Neutral Guests

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Received March 10, 1997


Receptor molecules with a preorganized cavity were synthesized by combining two resorcin[4]arene and one calix[4]-arene building block. Based on predictions from a computational study which matches hosts and guests, several steroids, sugar derivatives, and alkaloids were selected for complexation studies. Experimentally the complexation of such guests by these receptor molecules was proven by 1H-NMR spectroscopy (association constants of 1.0–6.0 × 10^2 M^{-1} in CDCl_3). One of these receptors with four hydroxyl groups forms a dimeric capsule (K_{assoc} = 11 M^{-1} in CDCl_3). Evidence for this dimerization comes from 1H-NMR, FAB MS spectroscopy, and molecular mechanics and dynamics calculations.

Nowadays a large variety of synthetic hosts for relatively small guest species e.g. cations, anions, and small neutral molecules are described in the literature. In most cases the general strategy focuses on the complementarity of functional groups in receptor and guest; this approach is always guest-directed and requires for each individual guest a new synthetic pathway. With increasing size and structural complexity of the guest species, this approach becomes increasingly demanding in terms of design and the number of steps in the synthesis of a complementary host (or receptor) molecule.

An alternative approach to the synthesis of such receptor molecules is based on the proper combination of (different) molecular building blocks to which functional groups can be attached, giving rise to molecules with large well-defined cavities and hydrophobic surfaces. Most likely, the complexation of large guest molecules requires preorganized host molecules because otherwise the entropy might be too high to form an appropriate cavity. On the other hand, some flexibility gives the host molecules the possibility for structural adaptation that might be necessary for complexation. Computational methods for matching the shape of host and guest are nowadays available[1].

More or less planar building blocks that have recently been used in the design of receptor molecules[2] for the complexation of small neutral molecules and spherical anions are porphyrins[3] and steroids[4]. In our own work we use mainly non-planar building blocks that have a molecular cavity and a variety of functional groups e.g. calix[4]arenes[5] and resorcin[4]arenes[6]. Based on this strategy, we have synthesized different classes of large receptor molecules by combining calix[4]arenes with β-cyclodextrins[7], porphyrins[8], or resorcin[4]arenes. Calix[4]arenes and resorcin[4]arenes were combined in different ways[9]. In a 1:1 ratio a calix[4]arene and a resorcin[4]arene derivative form a carcerand that encapsulates amidines, sulfoxides, or ketones[9]. The combination of two calix[4]arenes and two resorcin[4]arenes yields a molecule that has a large, rigid cavity of nanosize dimensions[10]. A systematic search for suitable guests using the computer simulation program DOCK[11] revealed among others a good fit for different steroids, aromatic compounds, sugar derivatives, and fused ring systems. Unfortunately, in CDCl_3 solution complexation could not be detected probably because the cavity is too well solvated. Therefore, we have synthesized the more flexible combination of two upper rim 1,2-di-functionalized calix[4]arenes coupled to one resorcin[4]arene (1, 2, and 3, see Scheme 1). These compounds complex steroids (corticosteroids) in non-aqueous media with association constants of 0.9–9.5 × 10^3 M^{-1} in CDCl_3[12].

In this paper we describe the synthesis of molecules 4 and 5 (see Scheme 2) that are constructed by the combination of two resorcin[4]arene with one calix[4]arene building block. Since resorcin[4]arenes are known to have a more rigid cavity[13] than calix[4]arenes we expected molecules 4 and 5 to have better complexation properties than receptor molecules 1, 2, and 3. The complexation of a variety of steroids, carbohydrates, and alkaloids by these receptor molecules is described.

Results and Discussion 
Synthesis of 1:2 Receptor Molecules 4a–e

Previously, we described that two calix[4]arenes can be combined with one resorcin[4]arene in one step to give the 2:1 receptor molecules 1, 2, and 3[10]. However, the combination of two resorcin[4]arenes and one calix[4]arene is not possible in one step because when a tetrafunctionalized calix[4]arene is used, the flexibility of this building block
will lead to formation of a mixture of different products\[^{[9]}\].
Consequently, for the synthesis of the 1:2 coupled products
4 and 5 we first prepared 1:1 endo calix-resorcinarenes 6
(see Scheme 3) and coupled these with a second resorcin[4]-
aren. For this coupling it is necessary to have chlorocetamido
functionalities at the 3- and 4-positions of the calix[4]-
aren. We have previously reported the synthesis of 1:1 endo
6a (R^1 = pht, R^2 = H)\[^{[12]}\] and 6d (R^1 = NHCl(O)CH_2Cl,
R^2 = Si(CH_3)2C(CH_3)3)\[^{[9]}\]. The phthalimido\[^{[4]}\] groups in 6a
give the possibility of further functionalization.

Deprotection of the masked amino groups in 1:1 endo 6a
(R^1 = pht, R^2 = H) by reaction with hydrazine gave 1:1
endo 6b (R^1 = NH_2, R^2 = H), which was acylated by reaction
with α-chloroacetyl chloride to give 1:1 endo 6c [R^1 =
NHCl(O)CH_2Cl, R^2 = H] in 76% overall yield. The latter
was performed in the absence of base to prevent acylation
of the hydroxyl groups. Reaction between 1:1 endo 6c and
tetrahydroxycavitand 7 was performed in CH_3CN in a 1:3
ratio to suppress the intramolecular reaction leading to the
carcerands\[^{[10]}\]. From \(^1\)H-NMR and FAB MS spectroscopy
of the crude reaction mixture we can conclude that no carcerands or any exo-coupled products are formed. The position
of the amide hydrogen signal can be used to assign the
stereochemistry of the coupled products. A signal at lower
field (δ = 8.7–9.8) corresponds to an endo-coupled product,
whereas an exo-coupled amide gives rise to a signal at
higher field (δ = 8.0–8.3)\[^{[9]}\]. After purification by column
chromatography, exclusively the 1:2 endo–endo 4a (R^1 =
R^2 = H) was isolated in 31% yield. The absence of the 1:2
endo–exo isomer 5a might be explained by a favorable intramolecular interaction of the hydroxyl groups during formation
of the second bond between the 1:1 endo isomer and the second resorcin[4]arene, exclusively leading to the
1:2 endo–endo isomer 4a. This result is in agreement with the
effect of polar substituents on the stereochmistry of the
1:1 products as described in our previous paper\[^{[12]}\].

We were unable to detect 1:2 coupled products in the crude
reaction mixture of the reaction between silylated 1:1 endo 6d
[R^1 = NHCl(O)CH_2Cl, R^2 = Si(CH_3)2C(CH_3)3] and tetra-
hydroxycavitand 7, performed under the same conditions as
described above for the non-silylated 1:1 endo isomer 6c. In order to investigate whether this is due to the absence of the hydroxyl groups in 6d or steric hindrance by the silyl groups, the dipropyl 1:1 endo 6e (R' = pt, R'' = C3H7) was synthesized by alkylation of 1:1 endo 6a (R' = pt, R'' = H) with n-propyl iodide. Deprotection of the phthalimido groups with hydrazine gave 1:1 endo 6f (R' = NH2, R'' = C3H7), which was subsequently acylated to afford 1:1 endo 6g (R' = NHCOCH2Cl, R'' = C3H7) in 79% overall yield. Reaction between 6g and tetrahydroxyxavitand 7 was performed under the same reaction conditions as described above and after purification exclusively the 1:2 endo–endo isomer 4b (R' = H, R'' = C3H7) was isolated in 56% yield. The absence of 1:2 endo–exo isomer 5b (R' = H, R'' = C3H7) is in line with the results of the analogous reactions (vide supra). The formation of 4b shows that the hydroxyl groups in the 1:1 starting material are not necessary for the formation of 1:2 coupled products, but the large silyl groups in 1:1 endo 6d [R' = NHCOCH2Cl, R'' = Si(CH3)2C(CH3)2] prevent this formation by steric hindrance.

As a reference compound for the complexation studies, 1:2 endo–endo 4c (R' = R'' = C3H7) was synthesized in quantitative yield by alkylation of 1:2 endo–endo 4a (R' = R'' = H) with n-propyl iodide.

Characterization of 1:2 endo–endo 4a

In the FAB MS spectra of 1:2 endo–endo 4a (R' = R'' = H) in addition to the parent peak for the monomer (m/z 3246.9, 100%) a signal is present at m/z 6492.9 (7%) pointing to dimer formation; no higher oligomers could be detected. The 1H-NMR spectra of solutions of 1:2 endo–endo 4a (R' = R'' = H) in CDCl3 show a concentration dependency of the amide hydrogen signal, of the two aromatic hydrogen signals ortho to the amide group, of the hydroxyl hydrogen signal, and of the doublet for the methylene-bridge hydrogens (see Figure 1). Upon dilution one of the aromatic hydrogen signals (at δ = 6.5) is shifted downfield, while all the others are shifted upward.

Figure 1. 1H-NMR spectra of 1:2 endo–endo 4a (R' = R'' = H) in CDCl3 at 30°C at concentrations of 28.6 mM and 0.5 mM; (1) amide hydrogen signal, (2a) and (2b) aromatic hydrogen signals ortho to the amide group, (3) hydroxyl hydrogen signal, and (4) doublet of the methylene-bridge hydrogen atoms.

Dilution of a solution of tetrapropyl 1:2 endo–endo 4c (R' = R'' = C3H7) did not show concentration dependence. This suggests self-assembly phenomena involving the amide and the hydroxyl groups. From a dilution experiment of 4a in CDCl3 (see Figure 2) a dimerization constant Kdim of 11 ± 2 m−1 was determined[19].

Figure 2. 1H-NMR dilution curve of the amide hydrogen signal of 1:2 endo–endo 4a (R' = R'' = H) in CDCl3 at 25°C.

Unfortunately, the monomer and the dimer could not be observed separately by 1H-NMR spectroscopy at low tem-
perature since cooling leads to broadening and splitting of the amide hydrogen signal caused by hindered rotation around the amide bridge.

CPK molecular models showed that a dimeric capsule can be formed in which the two monomers of 4a are connected by eight hydrogen bonds between the hydroxyl groups and the amide bridges. Such a structure is in agreement with the shifts observed in the $^1$H-NMR spectra as shown in Figure 1.

Molecular modelling in the gas phase shows that an empty dimer in the shape of a tennis ball is not stable; the molecules collapse to fill the large cavity. Incorporation of up to nine chloroform molecules in the cavity leads to a closed dimer which does not collapse. When ten chloroform molecules are placed inside the cavity the dimer opens and releases one of the chloroform molecules. The molecular dynamics simulations of receptor 4a ($R^1 = R^2 = H$) with eight chloroform molecules in the cavity in a cubic 40 Å periodic box of OPLS CHCl$_3$ [119] showed that the dimer is stable during the 500 ps trajectory [119], held together by eight hydrogen bonds with eight solvent molecules inside (see Figure 3). These hydrogen bonds are occasionally broken and reformed during the dynamics study. The eight chloroform molecules which are completely shielded from the exterior cannot leave the tennis ball unless the monomers separate (see Figure 3). The chloroform molecules positioned in the resorcin[4]arenes point with a chloro atom in the cavity while those in the calix[4]arenes point with a hydrogen atom in the cavity [119]. Dynamics studies (during 100 ps) of a dimer containing nine chloroform molecules showed that in this case the dimer opens to release one of the solvent molecules.

**Complexation Studies with Receptors 1:2 endo-endo 4a (R$^1 = R^2 = H$) and 4c (R$^1 = R^2 = C_6H$_4)***

The association constants for complexes of receptor 1:2 endo-endo 4a ($R^1 = R^2 = H$) cannot be determined directly because the probes are concentration dependent. The complexation scheme consists of two equilibria, viz. the dimerization equilibrium:

$$H + H \Leftrightarrow H_2$$

and the complexation equilibrium:

$$H + G \Leftrightarrow HG$$

where $[H_2]$ is the dimer, $[H]$ the monomer, $[G]$ the guest, and $[HG]$ the complex concentration. If these equilibria are fast on the NMR time scale the observed chemical shift of the host can be described by equation 3:

$$\delta_{obs} = f_{\text{mono}} \times \delta_{\text{mono}} + f_{\text{dimer}} \times \delta_{\text{dimer}} + f_{\text{comp}} \times \delta_{\text{comp}}$$

In this equation, $f_{\text{mono}}$, $f_{\text{dimer}}$ and $f_{\text{comp}}$ are the molar fractions of host present as monomer, dimer, and complex, respectively.

From the dilution experiment of the host alone, the dimerization constant $K_{\text{dimer}}$ the shift of the monomer $\delta_{\text{mono}}$ and the shift of the dimer $\delta_{\text{dimer}}$ were obtained. These values were used in the calculation of the association constants of the complexes with receptor 4a [119].

Following our previous complexation studies with the receptors 1, 2, and 3, we first studied the affinity of 1:2 endo-endo isomer 4a ($R^1 = R^2 = H$) for corticosteroids. As described above this class of compounds gave a good fit in the DOCK search applied for the molecule containing two calix[4]arenes and two resorcin[4]arenes. Corticosteroids are hormonal steroids used against rheumatoid arthritis.
asthma, and other inflammatory diseases, and have all an oxygen function at \( C_1 \)\(^{20}\).

The complexation studies revealed that the presence of an acetate group at \( C_{21} \) and two hydroxyl groups at \( C_{11} \) and \( C_{17} \) are necessary for complexation by 1, 2, and 3 in CDCl\(_3\). For this reason, the complexation behavior of 1:2 endo--endo isomer 4a (\( R^1 = R^2 = H \)) was first studied with prednisolone 21-acetate (8), dexamethasone acetate (9), and hydrocortisone acetate (or cortisone acetate) (10) (see Scheme 4). Upon addition of these steroids to a solution of 4a in CDCl\(_3\) at 25°C, the absorptions of the amide hydrogen, both aromatic hydrogen signals ortho to the amide group, and the doublet for the bridge methylene hydrogen atoms (at \( \delta = 9.5, 7.5, 6.5, \) and 4.3, respectively) in the \(^1\)H-NMR spectrum of 4a split into two signals of equal intensity as a result of the chirality of the guest\(^{21}\), and several signals shift. The \(^1\)H-NMR spectra of the complexation of hydrocortisone acetate (10) by receptor 4a are given in Figure 4. The \( K_{\text{assoc}} \) values of complexes 4a·8, 4a·9, and 4a·10 in CDCl\(_3\) at 25°C are ca. 3.0 \( \times 10^3 \) \( \text{m}^{-1} \) (see entries 1–3 in Table 1).

To determine whether the same three functionalities in the steroid (acetate group at \( C_{21} \) and two hydroxyl groups at \( C_{11} \) and \( C_{17} \)) are crucial also in this case for complexation by 4a, several related steroid molecules were studied. Addition of fluocinolone acetonide acetate (11), containing a protected hydroxyl group at \( C_{17} \), to a solution of host 4a resulted in splitting of the amide hydrogen signal, one of the aromatic hydrogen signals ortho to the amide group, another aromatic hydrogen signal, and the doublet for the bridge methylene hydrogen atoms (at \( \delta = 9.5, 7.5, 6.9, \) and 4.3, respectively) in the \(^1\)H-NMR spectrum. Surprisingly, the \( K_{\text{assoc}} \) value of 6.0 \( \times 10^3 \) \( \text{m}^{-1} \) (entry 4, Table 1) is higher than that of complexes 4a·8, 4a·9, and 4a·10. Addition of corticosterone acetate (12), lacking the hydroxyl group at \( C_{17} \), also resulted in splitting of the amide hydrogen signal and one of the aromatic hydrogen signals ortho to the amide group; the \( K_{\text{assoc}} \) value is 1.5 \( \times 10^2 \) \( \text{m}^{-1} \) (entry 5, Table 1). Addition of prednisolone (13), lacking the acetate group at \( C_{21} \), cortisone acetate (14), having a keto function instead of a hydroxyl group at \( C_{11} \), and prednisone (15), without the acetate group and with the keto function, to a solution of receptor 4a in CDCl\(_3\) did not give rise to any significant change of guest or host signals. Other steroids that are not complexed by 4a were cholesteryl acetate, cholic acid methyl ester, dehydroisoandrosterone acetate, 16-dehydroprogrenolone acetate, and nothandrene. These results reveal the necessity of the acetate group and the hydroxyl group at \( C_{17} \) for complexation. Obviously, the hydroxyl group at \( C_{17} \) is not a prerequisite. To the best of our knowledge complexation of steroids has only been reported for receptors 1 to 3 in CDCl\(_3\)\(^{12}\), and in aqueous systems using cyclophanes\(^{22}\), cycloextrins\(^{23}\), and resorcin[4]arenes\(^{24}\).

The DOCK search revealed, besides for corticosteroids, also a good fit for large polycycles, aromatic compounds,
Figure 4. 1H-NMR spectra in CDCl₃ at 30°C of (a) hydrocortisone acetate (10), 3.0×10⁻² M, (b) 1:2 endo-endo 4a (R¹ = R² = H), 0.5 mM, and (c) 1:1 of mixture 4a and 10 (both 2.5×10⁻⁴ M); (1) amide hydrogen signal, (2a) and (2b) aromatic hydrogen signals ortho to the amide group, (3) hydroxyl hydrogen signal, and (4) doublet of the methylene-bridge hydrogen atoms.

Table 1. Results of complexation studies[25]

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<tr>
<th>Entry</th>
<th>Host</th>
<th>Guest</th>
<th>$K_{solv}$ [M⁻¹]</th>
<th>$ΔG_{sol}$ [kJ mol⁻¹]</th>
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[25] Determined at 25°C in CDCl₃; estimated error in $ΔG_{sol}$ is 10%.

and fused ring systems. To investigate whether these are complexed by receptor 4a, a variety of compounds was examined. Addition of acridine, adamantane, l-adamantan carboxylic acid, l-adamantol, β-borneol, caffeine, chiral D, cinnamyl acetate, dibenzo-24-crown-8, anthracene-9,10-dimethyl ether, phenyl acetate, and tetakis(4-methoxyphenyl)porphyrin did not give any significant change of guest or host signals.

However, upon addition of the alkaloid[25] quinidine (16) the amide hydrogen signal and one of the aromatic hydrogen signals ortho to the amide group split and upon addition of quinine (17) one of the aromatic hydrogen signals ortho to the amide group split and the amide hydrogen signal shifted. The $K_{solv}$ values were determined to be 2.0×10⁻² and 1.2×10⁻⁵ M⁻¹, respectively (entries 6 and 7, Table 1)[26]. Upon addition of cinchonidine (18) and cinchonine (19), both missing the methoxy group at the 6'-position, the amide hydrogen signal and both the aromatic hydrogen signals ortho to the amide group split while the guest signals around 8.0 ppm shifted. The $K_{solv}$ values were determined to be 4.0×10⁻² and 3.0×10⁻² M⁻¹, respectively (entries 8 and 9, Table 1).

In addition to steroids, another class of natural compounds which was found in the DOCK search are sugar derivatives. However, no extraction was observed in S-L extraction experiments with L-fucose, maltose, and d-sucrose in CDCl₃. Since the complexation studies with the steroids (vide supra) showed that the acetate functionality is important for complexation by receptor 4a and for solubility reasons, we continued with acetylated sugar derivatives. Addition of β-D-ribofuranose tetraacetate (20) to a solution of receptor 4a in CDCl₃ resulted in splitting of the other aromatic hydrogen signal ortho to the amide group and a shift of the amide hydrogen signal and one of the aromatic hydrogen signals; the $K_{solv}$ value is 3.0×10⁻⁵ M⁻¹ (entry 10, Table 1). Inouye et al. complexed methyl β-D-ribofuranoside by polypyridine-macrocycles with association constants up to 5×10⁻³ M⁻¹ in CDCl₃[27]. Upon addition of the pyranoses β-D-galactose pentaacetate, β-D-glucose pentaacetate (21), and acetobromoglucose (22) no significant changes were observed in the 1H-NMR spectra for signals of host or guest. Therefore, we conclude that 4a is selective for acetylated five-membered sugar derivatives.

Upon addition of triacetyl adenosine (23), having an additional adenine base compared with β-D-ribofuranose tetraacetate (20), to a solution of 1:2 isomer 4c in CDCl₃, the amide hydrogen signal and both aromatic hydrogen signals ortho to the amide group shifted. The $K_{solv}$ value of 2.0×10⁻³ M⁻¹ (entry 11, Table 1), is comparable to that of β-D-ribofuranose tetraacetate (20). However, addition of triacetyl inosine (24), triacetyl uridine (25), and isopropyl adenine to a solution of 4a in CDCl₃ did not give any significant changes, although only the bases in 24 and 25 differ from that of 23.

In order to determine what functionalities in receptor molecule 4a are important for the complexation, several control experiments were performed. To study the role of the four hydroxyl groups of 1:2 endo-endo 4a, the complexation properties of tetracetylpropyl 1:2 endo-endo 4e (R¹ = R² = R³ = CₘH₇) were determined for the guest which did form complexes with 1:2 isomer 4a (R¹ = R² = H). Upon addition of prednisolone acetate (8) and hydrocortisone acetate (10) to a solution of 4c in CDCl₃, the amide hydrogen signal and both aromatic hydrogen signals ortho to the
amide group split, while upon addition of dexamethasone acetate (9) and fluorocinolone acetonide acetate (11) only the first split. For the latter two guests the shift was too small to determine the association constants (entries 13 and 15, Table 1); for complexes 4c, 8 and 4c, 10 the $K_{assoc}$ values were $2.0 \times 10^2$ and $2.5 \times 10^2$ M$^{-1}$, respectively (entries 12 and 14, Table 1). In this case Job plots of the titration experiments with host 4c could be made, because tetrapropylyl 4c does not form a dimer in solution, which proved the 1:1 stoichiometry of the complexes. Upon addition of corticosterone acetate (12), quinidine (16), quinine (17), cinchonidine (18), cinchonine (19), $\beta$-dibrofarinose tetraacetate (20), and triacetyl adenosine (23) no significant shifts were observed for either host or guest signals. For these last seven compounds the presence of the hydroxyl groups in 4a ($R^1 = R^2 = H$) is a prerequisite for complexation. In the case of steroids 9 and 11 the complexation is significantly lower for receptor 4c ($R^1 = R^2 = C_3H_7$), while in case of steroids 8 and 10 the association constants are more or less similar.

From the complexation studies with receptors 1, 2, and 3 we know that the combination of amide groups and a large hydrophobic surface is sufficient for complexation of corticosteroids.[12] To investigate whether this is also the case for complexation of guest molecules 16 to 20, and 23, control experiments were performed with 2:1 endo - exo 2a ($R^1 = ptl$). Upon addition of all six guests to solutions of 2a in CDCl$_3$ no significant changes were observed for host or guest signals.

Conclusion

New receptor molecules 1:2 endo - exo 4a ($R^1 = R^2 = H$), 4b ($R^1 = H, R^2 = C_3H_7$), and 4c ($R^1 = R^2 = C_3H_7$) were synthesized following a host-directed approach, by combining two resorcin[4]arenes via one calix[4]arene. The stereochemistry of these receptor molecules is influenced by intramolecular interactions leading exclusively to endo - endo isomers.

Receptor molecule 4a ($R^1 = R^2 = H$) forms a spherical dimeric assembly, which is held together by eight hydrogen bonds between the hydroxyl groups and the amide bridges. Molecular dynamics simulations (300 ps) showed that this dimer is stable in chloroform and that the cavity is large enough to accommodate eight molecules of chloroform, which are completely shielded from the bulk chloroform. According to $^1H$-NMR spectroscopy, receptors 4a and 4c selectively complex corticosteroids, sugar derivatives, and alkaloids with association constants of 1.0 - 6.0 $\times 10^2$ M$^{-1}$ in CDCl$_3$. For the complexation of corticosteroids it is necessary that the host has an acetate moiety at C$_{17}$ and a hydroxyl group at C$_{11}$, while the hydroxyl groups of the receptor molecule are not essential. Receptor molecule 4a is selective for acetylated five-membered sugars and quinine derivatives, in which cases the hydroxyl groups of the receptor are crucial for complexation.

We have demonstrated that this new approach to combine the building blocks calix[4]arenes and resorcin[4]arenes, leads to receptor molecules with large hydrophobic surfaces which are very useful for the complexation of large, neutral organic molecules.

Experimental Section

Melting points were determined with a Reichert melting point apparatus and are uncorrected. - NMR: Bruker AC250 (1'H NMR: 250 MHz) or a Varian Unity 400 (1'H NMR: 400 MHz) spectrometer in CDCl$_3$. Residual solvent protons were used as internal standard and chemical shifts are given in ppm relative to tetramethysilane (TMS). - FAB MS: Finnigan MAT 90 spectrometer using m-nitrobenzyl alcohol (NBA) as a matrix. - All solvents were purified by standard procedures. Petroleum ether (PE) refers to the fraction with b.p. 60 - 80°C. All other chemicals were analytically pure and were used without further purification. All reactions were carried out under argon. The presence of solvent in the analytical samples was confirmed by $^1H$-NMR spectroscopy.

- Flash chromatography was performed on silica gel (SiO$_2$, E. Merck, 0.040 - 0.063 mm, 230 - 240 mesh). - Preparative thin-layer chromatography (TLC) was performed using precoated silica plates (E. Merck, Kieselgel 60 F$_{254}$, 2 mm). For dropwise additions a perufser was used. Tetrahydroxycitound 7971 and 1:1 isomers 6a[12] and 6d[10] were obtained following published procedures.

$41,59$-Diamino-1,9,25-dihydroxy-14,30,62,63-tetrapropoxy-1,47,49,57-tetrahexacyl-16H,22H,28H,34H-13,31,51,53-dimethano-2,46S,54,4,15,11,17,21,23,27,29,33-hexamethano-1,8,47,49-H-1,3-benzodioxacin[9',8',7:5,13-benzodioxacin[9,10-d][1,3]-dioxacin[4,5-l],1,3,6,9,33-benzotetraoxadiazacyclooctatetra-9,35(10H,36H)idine [1:1 endo, $R^1 = H_2$, $R^2 = H$] (6b): A mixture of 1:1 endo 6a ($R^1 = ptl$, $R^2 = H$) (0.57 g, 0.26 mmol) and NH$_2$NH$_2$ - H$_2$O (1.5 ml, 30.9 mmol) in EtOH/THF (80/40 ml) was refluxed overnight. The solvent was removed in vacuo to leave the residue dissolved in CH$_2$Cl$_2$ (75 ml), washed with 1 N HCl (25 ml), with H$_2$O (2 x 25 ml), with brine (25 ml), and dried with Na$_2$SO$_4$. The solvent was removed in vacuo to give the crude product which was used without further purification. Yield 0.39 g (78%). - $^1H$ NMR: $\delta = 9.07$ (s, 2H, NE), 2.40, 6.84, 6.53, 6.49, 6.60, 5.95 (s, 12H, ArH), 5.58 (m, 6H, OCH$_3$O, OH), 4.71 - 4.11 (m, 16H, ArCH$_2$Ar, OCH$_2$O), ArCHAr, OCH$_2$O, 3.8 - 3.6 (m, 12H, OCH$_2$CH$_2$CH$_2$NH$_2$). 3.13, 29.71, 2.80 (part of ABq, $\delta_{2H,2H}$ = 12.9 Hz, 3H, ArCH, ArH, 2.2 - 2.0 (m, 8H, CH$_2$(CH$_2$)$_2$CH$_2$O, 1.9 - 1.7 (m, 8H, OCH$_2$(CH$_2$)$_2$), 1.3 - 1.1 (m, 72H, CH$_2$(CH$_2$)$_2$CH$_3$), 0.91 (t, $\delta_{3H,3H}$ = 7.4 Hz, 12H, OCH$_2$CH$_2$), 0.8 - 0.7 (m, 12H, CH$_2$(CH$_2$)$_2$CH$_3$). - MS FAB; m/z: 1950.3 ($M^+$, C$_{63}$H$_{56}$N$_{20}$O$_{18}$ (1950.2)).

$41,59$-Bis(2-chloroacetamido)-19,25-dihydroxy-14,30,62,63-tetrapropoxy-1,47,49,57-tetrahexacyl-16H,22H,28H,34H-13,31,51,53-dimethano-2,46S,54,4,15,11,17,21,23,27,29,33-hexamethano-1,8,47,49-H-1,3-benzodioxacin[9',8',7:5,13-benzodioxacin[9,10-d][1,3]-dioxacin[4,5-l],1,3,6,9,33-benzotetraoxadiazacyclooctatetra-9,35(10H,36H)idine [1:1 endo, $R^1 = NHCO(O)CH_2Cl$, $R^2 = H$] (6c): A mixture of 1:1 endo 6b ($R^1 = NH_2$, $R^2 = H$) (0.39 g, 0.20 mmol), CI(O)OCI(O)CH$_2$ (0.16 mol, 2.01 mmol) in CH$_2$Cl$_2$ (65 ml) was stirred at room temp. for 5 h. The solution was washed with 1 N HCl (2 x 10 ml), H$_2$O (10 ml), 1 N NaOH (10 ml), H$_2$O (2 x 10 ml), brine (10 ml), and dried (Na$_2$SO$_4$). The solvent was removed in vacuo to give the pure product. Yield 0.41 g (97%), m.p. 259 - 262°C. - $^1H$ NMR: $\delta = 9.28$ (s, 2H, NHCO(O)CH$_2$, 7.85 (s, 2H, NHCO(O)CH$_2$), 7.37, 6.97, 6.47 (d, $\delta_{J(H,H)} = 2.3$, 6H, ArH), 6.84, 6.55 (s, 6H, ArH), 6.1 - 5.7 (m, 6H, OCH$_2$O, OH), 4.7 - 3.7 (m, 28H, ArCH$_2$Ar, OCH$_2$O), ArCHAr, OCH$_2$O
OCH₃CH₂CH₃, C(O)CH₂Cl), 3.2–3.0 (m, 4H, Ar-CH₂Ar), 2.2–2.0 [m, 8H. CH₂(OCH₂CH₂)₂], 1.9–1.7 (m, 8H, OCH₂CH₂CH₃), 1.4–1.1 [m, 72H, CH₂(OCH₂CH₂)₂CH₃], 1.0–0.9 (m, 12H, OCH₂CH₂CH₂Cl), 0.8–0.7 [m, 12H, CH₂(OCH₂CH₂)Cl] – 13C{¹H} NMR: δ = 166.96, 163.69 (s, C=O) – MS FAB; m/z: 2126.5 [M⁺ + Na (2126.1)] – C₅₆H₇₆O₇N₁O₂ – 3.0 H₂O (2157.7); calc'd: C 69.03, H 8.03, N 2.60;

41.59-Diphthalimido-14,19,25,30,62,63-hexapropoxy-1,47,49,57-tetraundecyl-16H,22H,28H,34H-13,31:31,31:55-dimethane-2,46,3:45,11:15,17,21,25,27,29,33-hexamethano-1,8H,47H,49H-1,3benzodioxacin9,8',4,5',11,13,13benzodioxacin9,8,4,5,13dioxacin4,5,13,14,15,36,9,33[1benzotrazaxadiazacyclooctatriacene-9,35(10H,36H)Cl] (1:1 endo, R¹ = ClF, R² = C₂H₅) (66): A mixture of 1:1 endo 6a (R¹ = ClF, R² = H) (0.3 g, 0.14 mmol), n-Pr(0.65 ml, 6.15 mmol) and K₂CO₃ (0.9 g, 6.51 mmol) in DMA (50 ml) was stirred overnight at 60°C. The solvent was removed in vacuo and the residue was dissolved in CHCl₃ (50 ml), washed with 1 N HCl (25 ml), with H₂O (2 × 25 ml), with brine (25 ml), and dried with Na₂SO₄. The solvent was removed in vacuo to give the crude product which was purified by column chromatography (SiO₂, EtOAc/EtPE, 30:70).

 Yield 0.26 g (83%), m.p. 176–178°C – 1H NMR: δ = 9.86 (s, 2H, NH), 7.8–7.6 (m, 8H, phArH), 7.36, 6.92, 6.74 (s, 6H, ArH), 6.8–6.7 (m, 6H, ArH), 6.56 [d, 3J(H,H) = 7.1 Hz, 1H, OCH₃], 5.98 (m, 3H, OCH₂Cl), 4.8–4.6 [m, 14H, Ar-CH₂Ar], OCH₂Cl (4.02 [part of ArBq, 3J(H,H) = 15.7 Hz, 2H, OCH₂C(O)O]), 3.9–3.8 (m, 12H, OCH₂CH₂CH₃), 3.3–3.2 (m, 4H, Ar-CH₂Ar), 2.3–2.1 [m, 8H, CH₂(0CH₂CH₃)₂], 2.0–1.9, 1.7–1.6 (m, 12H, OCH₂CH₂CH₃), 1.4–1.1 [m, 72H, CH₂(OCH₂CH₂)₂CH₃], 1.1–0.9 (m, 18H, OCH₂CH₂CH₃), 0.9–0.8 [m, 12H, CH₂(OCH₂CH₂)₂CH₃] – 13C{¹H} NMR: δ = 167, 162.16, 153.30 (s, C=O) – MS FAB; m/z: 2294.8 [M⁺ + Na (2294.3)] – C₁₅₉H₁₇₇O₇N₂O₂ – 2.5 H₂O (2313.0); calc'd: C 73.67, H 7.93, N 2.42; found C 73.62, H 7.84, N 2.45.

1:2 endo-end, R¹ = H, R² = H (4a): To a refluxing mixture of tetrahydroxybenzanth 7 (0.69 g, 0.57 mmol), C₆H₅CO₂H (0.25 g, 0.76) and KI (catalytic amount) in CH₂CN (125 ml) was added a solution of 1:1 endo 6a [R¹ = NHCO(0CH₂CH₃), R² = H] (0.40 g, 0.13 mmol) in THF (40 ml) over 8 h and the reaction mixture was stirred at reflux temp. for 8 h. The solvent was removed in vacuo and the residue was dissolved in CHCl₃ (150 ml), washed with 1 N HCl (50 ml), H₂O (50 ml), brine (50 ml), and dried with Na₂SO₄. The solvent was removed in vacuo to give the crude product which was purified by column chromatography (SiO₂, EtOAc/EtPE, 30:70).

 Yield 0.19 g (31%), m.p. 217–218°C – 1H NMR: δ = 9.48 (s, 4H, NH), 7.52, 6.51, 6.41, 6.51 (s, 16H, ArH), 6.1–5.9 (m, 12H, H₂O, OCH₃), 4.8–4.4 [m, 24H, Ar-CH₂Ar, OCH₂(OCH₂CH₃)], Ar-CH₂Ar, OCH₂Cl] 4.27 [part of ArBq, 3J(H,H) = 15.9 Hz, 4H, OCH₂C(O)O], 3.9–3.7 (m, 8H, OCH₂CH₂CH₃), 3.5, 5.3, 5.16 (part of ArBq, 3J(H,H) = 12.8 Hz, 4H, Ar-CH₂Ar), 2.3–2.0 [m, 16H, CH₂(OCH₂CH₃)₂CH₃], 2.0–1.9 (m, 8H, OCH₂CH₂CH₃), 1.5–1.1 [m, 14H, CH₂OCH₂CH₂CH₃], 1.02 [m, 3J(H,H) = 7.1 Hz, 12H, OCH₂CH₂CH₃], 0.9–0.8 [m, 24H, CH₂(OCH₂CH₂)₂CH₃] – 13C{¹H} NMR: δ = 166.47 (s, C=O) – MS FAB; m/z: 3246.9 [M⁺ (3246.0)] – C₁₅₉H₁₇₇O₇N₂ – H₂O (3266.4); calc'd: C 73.54, H 8.58, N 1.72; found C 73.33, H 8.67, N 1.57.

1:2 endo-end, R¹ = H, R² = C₂H₅ (4b): To a refluxing mixture of tetrahydroxybenzanth 7 (0.12 g, 0.95 mmol), C₆H₅CO₂H (0.06, 0.49 mmol), and KI (catalytic amount) in CH₂CN (50 ml) was added a solution of 1:1 endo 6a [R¹ = NHCO(0CH₂CH₃), R² = C₂H₅] (0.07 g, 0.03 mmol) in THF (10 ml) over 8 h and the reaction mixture was subsequently stirred at reflux temp. for 8 h. The solvent was removed in vacuo and the residue was dissolved in CHCl₃ (50 ml), washed with 1 N HCl (25 ml), H₂O (25 ml), brine (25 ml), and dried with Na₂SO₄. The solvent was removed in vacuo to give the crude product which was purified by preparative TLC (EtOAc/PE, 20:80), and trituration with EtOH. Yield 0.06 g (50%), m.p. 216–218°C – 1H NMR: δ = 9.62, 9.37 (s, 4H, NH), 7.47, 7.42, 6.58, 6.79, 6.69, 6.54, 6.47 (s, 16H, ArH), 6.1–5.9 (m, 10H, H₂O, OCH₃), 4.8–4.3 [m, 24H, Ar-CH₂Ar, OCH₂(OCH₂CH₃), Ar-CH₂Ar, OCH₂Cl] 4.22 [part of ArBq, 3J(H,H) = 15.9 Hz, 4H, OCH₂C(O)O], 3.9–3.6 (m, 12H, OCH₂CH₂CH₃), 3.5–3.1 (m, 4H, Ar-CH₂Ar), 2.3–2.0 (m, 16H, CH₂(OCH₂CH₂)₂CH₃), 2.0–1.9, 1.8–1.5 (m, 12H, OCH₂CH₂CH₃), 1.4–1.1 (m, 24H, CH₂(OCH₂CH₂)₂CH₃). – MS FAB: m/z: 2540.9 [M⁺ + Na (2540.3)] – C₁₅₉H₁₇₇NaO₇₅ – 3 H₂O (3386.6); calc'd C 73.06, H 8.75, N 1.65; found C 72.77, H 8.68, N 1.96.

1:2 endo-endo, R^1 = R^2 = OC_6H_5 (4c): A mixture of 1:2 endo-endo 4a (R^1 = R^2 = H) (0.14 g, 0.04 mmol), Prl (0.2 ml, 2.05 mmol), and K_2CO_3 (0.3 g, 2.17 mmol) in DMA (25 ml) was stirred at 60°C for 5 h. The solvent was removed in vacuo and the residue was dissolved in CHCl_3 (50 ml), washed with 1 M HCl (25 ml), H_2O (2 × 25 ml), brine (25 ml), and dried with NaSO_4. The solvent was removed in vacuo to give the crude product which was triturated with MeOH. Yield 0.14 g (95%), m.p. 158–160°C. – 'H NMR: δ = 9.49 (s, 4H, NH), 7.46, 6.48 [d, 3(JH/H) = 2 Hz, 8H, ArH], 6.88, 6.79 (s, 8H, ArH), 0.65, 5.88 [s, 6(JH/H) = 7.2 Hz, 8H, OCH_3], 4.8–4.3 [m, 24H, ArCH_2Ar, OCH_2(O)Ar, ArCHRAr, OCH(O)], 4.20 [part of A9, 3(JH/H) = 15.9 Hz, 4H, OCH(O)], 3.9–3.7 (m, 16H, OCH_2CH_2CH_3), 3.14 [part of A9, 3(JH/H) = 8.4 Hz, 4H, ArCH_2Ar, 2.3–2.0 (m, 16H, CH_2(CHOH)_2CH_3), 2.0–1.9, 1.9–1.7 (m, 16H, OCH_2CH_2CH_3), 1.5–1.1 (m, 144H, CH_2(CHOH)_2CH_3), 1.1–0.9 (m, 24H, OCH_2CH_2CH_3), 0.9–0.8 (m, 24H, CH_2(CHOH)_2CH_3). – 13C NMR: δ = 166.32 (s, C=O), – MS FAB: m/z 3415.6 [M+ (3416.2)], – C_{24}H_{57}O_{13} • 2H_2O (3452.8): calcd. C 73.75, H 8.87, N 1.62, found C 73.58, H 8.93, N 1.72.

Determination Association Constants: The association constants were determined by mixing 5 mm solutions of host and guest in CDC_6H_5 in nine different ratios (1.9–9.1) and monitoring the chemical shift. Because all the guest proton signals are obscured by host proton signals if a large excess of the host is present, these could not be used as a probe. The host amide proton signals on the other hand, shift and even split up upon the addition of steroid guests, and could be used as a probe. – The K_{assoc} values for complexes 4c • 8 and 4c • 10 were obtained with a nonlinear two-parameter fit of the chemical shift of the complex and the association constant [26]. The K_{assoc} values for the complexes with receptor 4a were determined with a two parameter fit of the chemical shift of the complex and the association constant based on the complexation model given by equations 1 and 2. In this fitting procedure, the characteristics of the dimerization equilibrium (equation 1) were used as fixed values.

Molecular Mechanics and Dynamics Simulations: Initial structures as well as visualizations were carried out with Quanta 4.1[20]. The MM and MD calculations were run with CHARMM 22.0 as implemented in the Quanta/CHARM package, and 23.0, respectively [20]. Parameters were taken from Quanta 4.1 and point charges were calculated with the charge template option in Quanta. Small “excess” charge was smoothed to non-polar hydrogens and carbons. Molecular mechanics simulations were performed in the gas phase with an empty dimer of 4a (R^1 = R^2 = H)[20], or one filled with eight, nine, or ten chloroform molecules. The starting structures for the MD simulations were minimized by ABN (adopted basis set Newton-Raphson) until the RMS of the energy gradient was < 0.01 kcal mol^{-1} Å^{-1}. No cutoff of the non-bonded interactions was applied. A constant dielectric constant and an ε of 1 were used.

Details of the MD simulations are as follows. The minimized dimers, filled with eight and nine molecules of chloroform, were dissolved in a cubic chloroform box of 40.2718 Å dimensions, filled with 484 OPLS CHCl_3[17] to give the correct density at 25°C. Solvent molecules that overlap with the complexes were removed (based on heavy atom interatomic distances ≤ 2.35 Å). Full periodic boundary conditions were imposed. Before running the MD simulation the system was minimized by 100 steps steepest descent followed by ABNR until the RMS on the energy gradient was ≤ 0.01 kcal mol^{-1} Å^{-1} or a maximum of 1000 steps was reached. During the simulation the non-bonded list was updated every 20 time steps with a cutoff of 12 Å. The van-der-Waals interactions were treated with the switch function between 9 and 11 Å, whereas the short range potential was applied to the electrostatic interactions (cut-off 11 Å). A constant dielectric constant and an ε of 1 were used. The system was heated to 300 K in 5 ps, followed by 10 ps of equilibration with scaling of velocities within a temp. window of ± 10°C. After equilibration no scaling of velocities was applied (no systematic deviation from 300 K was observed). The production phase consisted of 500 ps, and coordinates were saved every 200 time steps. For the numerical integration the velocity/leapfrog algorithm was applied. The SHAKE algorithm [23] on bonds involving hydrogen was used, allowing a time step of 1 fs.

Throughout this paper the abbreviation "pht" is used in the text and charts for a phthalimido group.

Calculation according to: J. Hornak, B. Dreux, *Helv. Chim. Acta* 1984, 67, 754–764. Dilution experiments were performed in duplo in the concentration range between 1.2 and 29.9 mM. All the probes gave the same dimerization constant.


During the 500 ps simulation, the dimeric species has diffused approximately 1 Å, which shows that in principle the time scale was long enough for the separation of the monomers.

We assume 1:1 complexes between 1,2-endo-endo 4a (R1 = R2 = H) and the guests although no Job plots could be made because of the dimerization. Fitting according to a discussion scheme with a dimeric complex [H2Cl] did not give satisfying results.


The chirality of the guest makes the overall complex chiral. Due to fast exchange between the free host and the complex on the

H-NMR chemical shift time scale, only two signals are observed for the four amide hydrogen atoms.


Quina was bought from Molecular Simulations Inc., Burlington, MA, USA.


A model for 4a was used with CH4 moieties attached to the remaining four residues instead of CH3H2.