



0040-4020(94)00905-8

STEROID COMPLEXATION BY CYCLOPHANE RECEPTORS IN AQUEOUS SOLUTION: SUBSTRATE SELECTIVITY, ENTHALPIC DRIVING FORCE FOR CAVITY INCLUSION, AND ENTHALPY- ENTROPY COMPENSATION

Blake R. Peterson, Peter Wallimann, Daniel R. Carcanague, and
François Diederich*
Laboratorium für Organische Chemie
Universitätstrasse 16, ETH-Zentrum, 8092 Zürich, Switzerland

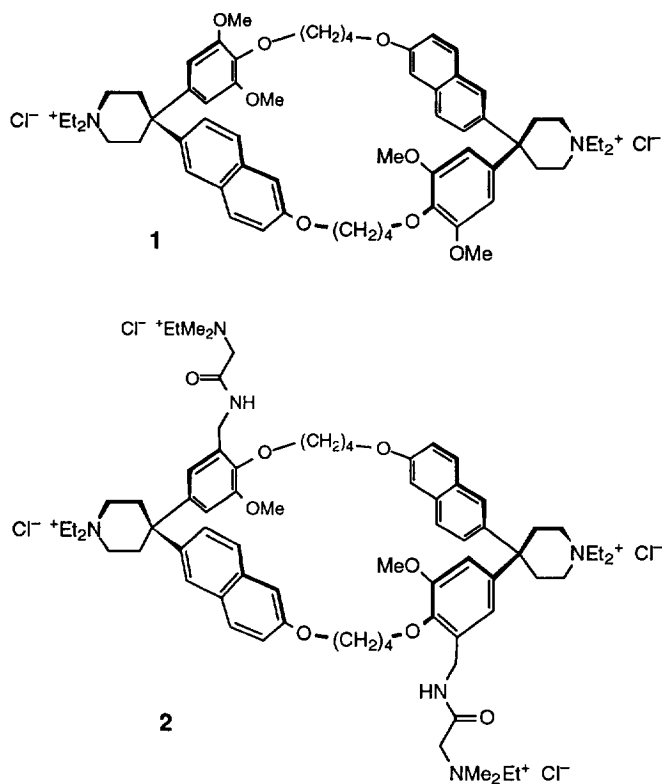
ABSTRACT *The synthesis, characterization, and steroid binding properties of two novel cyclophane receptors shaped by two naphthylphenylmethane spacers are reported. Cyclophane 1 forms inclusion complexes with bile acids, corticoids, and androgenic steroids in D₂O/CD₃OD 1:1. Specific functional group solvation effects generate high binding selectivity in the series of structurally similar bile acid derivatives: the complex of lithocholic acid is ≈ 2 kcal/mol more stable than the complex of deoxycholic acid. Steroid complexation by 1 is enthalpically driven, and complexation thermodynamics follows a strong enthalpy-entropy compensation relationship. Cyclophane 2 with 4 quaternary ammonium centers shows a much higher non-aggregated water-solubility than 1 with its two quaternary centers and forms stable steroid inclusion complexes in pure water. Complexes of anionic steroids with 2 are stabilized by both apolar interactions and ion pairing.*

1. INTRODUCTION

Water-soluble cyclophanes^[1,2] with large apolar cavities represent, besides the cyclodextrins,^[3] the major class of receptors capable of complexation of organic solutes in aqueous solution. Whereas the majority of molecular recognition studies with cyclophane receptors in the past addressed the binding of aromatic substrates,^[1,2] complexation of aliphatic guests has lately received increasing attention.^[4,5] Selective inclusion complexation of steroids has been investigated with both the cyclodextrins^[3,6,7] and a few spacious cyclophanes.^[8-13] Efficient, selective steroid complexation by synthetic receptors in aqueous solution may lead to interesting applications in medicine. Such complexes could be utilized for the delivery of insoluble steroidal drugs and offer alternatives for the formulation of these compounds. Binding studies with cyclodextrins have already shown improved steroid formulation,^[6,7a] steroid solubility enhancements,^[6c,e,k,l] and improvements of the hydrolytic stability^[6b,e,j] of cardiac glycosides such as digitoxin as a result of inclusion complexation. However, cyclodextrins generally give poor substrate selectivity and the stoichiometry of the formed complexes varies greatly.^[6f,g] A receptor with a high cholesterol affinity might be useful for the dissolution of cholesterol deposits in atherosclerotic plaque.^[13,14] With selective bile acid binders, a reduction of low-density lipoprotein (LDL), the plasma cholesterol transport protein whose concentration levels are directly related to the development of atherosclerosis, could be achieved.^[15] The liver takes up most of the plasma cholesterol via LDL receptor-

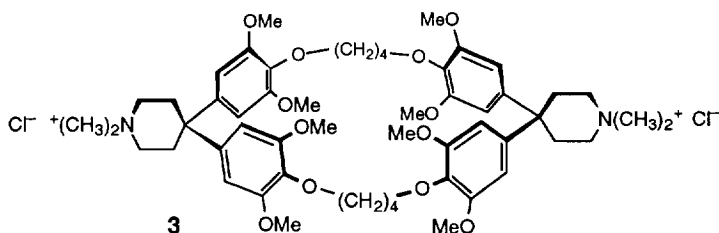
mediated endocytosis and converts it into bile acids and steroid hormones. The bile acids are secreted into the upper intestine, where they promote dietary fat absorption through emulsification, and are subsequently recycled by the liver. If bile acid recycling is interrupted, the liver takes up more plasma cholesterol for conversion into bile acids and this is achieved by the production of more LDL receptors. The recycling of bile acids can be reduced and, hence, the LDL-associated plasma cholesterol levels in patients lowered by the uptake of large quantities of bile acid binding cationic resins like cholestyramine (dose up to 12 g per day).^[16,17] Efficient, selective molecular bile acid receptors should be able to accomplish the same task at a much lower dose.

Prior to this study, no systematic investigation of steroid complexation was reported and the factors determining the stability and selectivity of steroid complexes, in addition to the usually cited hydrophobic binding effect, were not known. To improve the understanding of steroid recognition by synthetic and biological receptors, we prepared the two cyclophanes **1** and **2** which contain cavities of sufficient size for the inclusion of steroids. Here we report on the synthesis of these two receptors and ¹H-NMR binding studies with a large variety of steroid substrates in aqueous solutions varying from D₂O/CD₃OD 1:1 to pure D₂O. These studies show remarkable binding strength and selectivity and demonstrate that complexation of steroids in aqueous environment is enthalpically controlled while exhibiting a strong enthalpy-entropy compensation.



2. Design, Synthesis, and Solubility Properties of the Steroid Receptors 1 and 2.

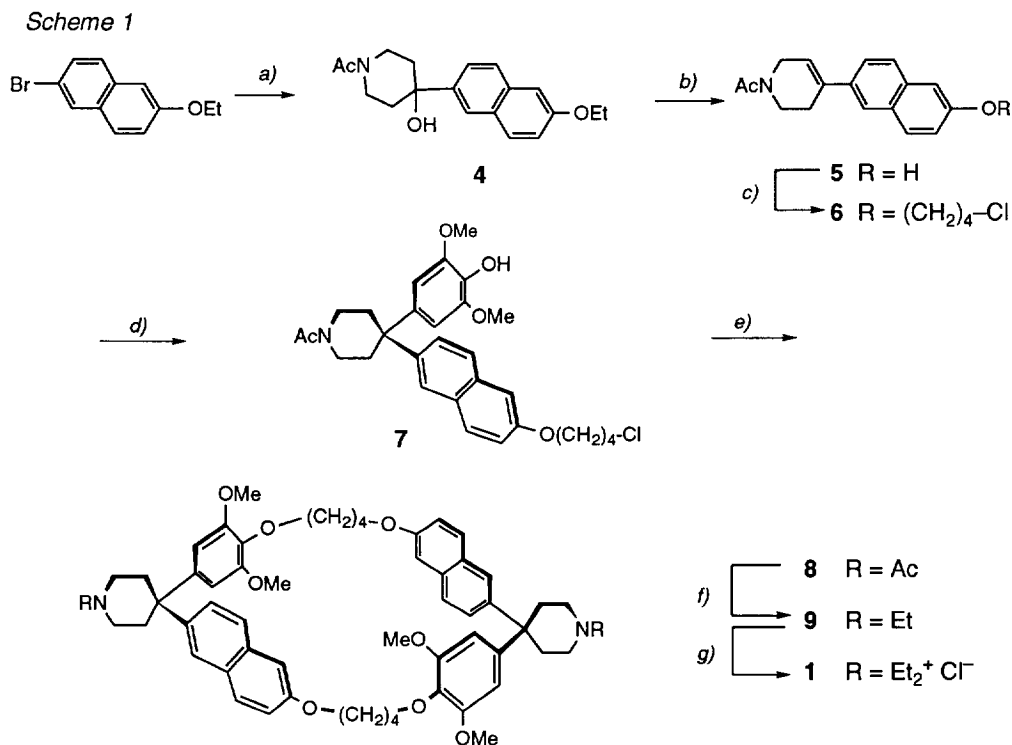
Tetraoxa[n.1.n.1]paracyclophanes such as **3** possess ideally sized apolar cavities for the tight inclusion complexation of flat aromatic guests. The distance between the two bridging O-atoms at one diphenylmethane unit in **3**, which defines the cavity width, is 8.41 Å (X-ray analysis).^[18] To shape a more spacious cavity suitable for steroids, naphthylphenylmethane units^[9] were incorporated into the new receptors **1** and **2**. At calculated values between 10.4 and 11.0 Å, depending on the cyclophane conformers,^[19] the distance between the bridging O-atoms at the naphthylphenylmethane units in **1** and **2** is considerably larger than the corresponding O...O distance in **3**.



The synthesis of **1**, in a route which readily affords gram quantities, is shown in *Scheme 1*. The Grignard reagent prepared from 6-bromo-2-ethoxynaphthalene^[20] was reacted with 1-acetyl-4-piperidone in tetrahydrofuran (THF) to afford the alcohol **4**. Treatment with an excess of BBr_3 effected both dehydration and ether cleavage to give **5**. Alkylation with 1,4-dichlorobutane afforded **6** which was reacted at 20 °C with 2,6-dimethoxyphenol in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ to yield the cyclization component **7**. When heat was applied to the latter reaction to force it to completion, partial cleavage of the 4-chlorobutyl naphthyl ether was observed. Cyclization to macrocycle **8** was effected by treatment of **7** with Cs_2CO_3 in dimethylformamide (DMF). Reduction of **8** to the diamine **9** and quaternization followed by ion exchange (Cl^-) gave receptor **1**. As a control for binding studies to demonstrate the importance of cavity inclusion, the acyclic compound **10** was prepared following a similar route *via* **11** → **12** → **13** → **10** (*Scheme 2*).

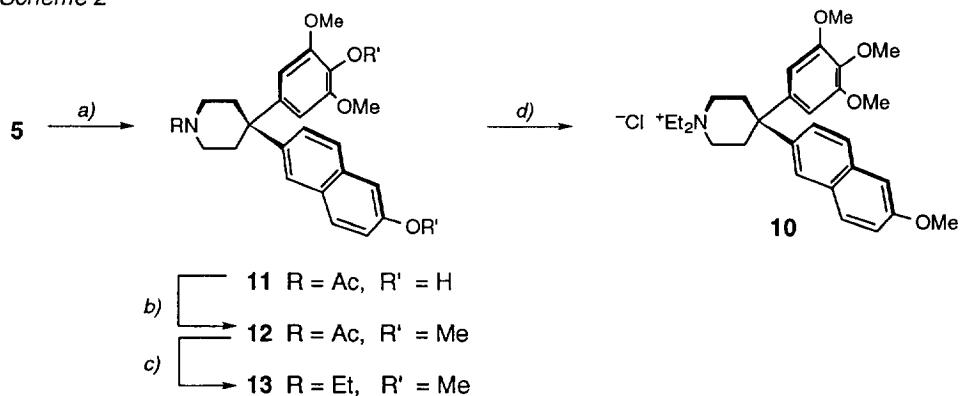
Cyclophane **1** is soluble without aggregation in $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ 1:1 at concentrations $\leq 6 \times 10^{-3}$ M. No changes in ^1H NMR chemical shift indicative of aggregation^[21] were observed in the concentration range from 8×10^{-4} M to 6×10^{-3} M, and the receptor concentration was kept in this range during subsequent binding studies.

For the synthesis of cyclophane **2**, additional functionality needed to be introduced into the macrocyclic framework (*Scheme 3*). For this purpose, **6** was reacted with guaiacol to give **14**. Bromination with *N*-bromosuccinimide (NBS) at low temperature in the presence of base occurred selectively *ortho* to the phenolic HO-group yielding **15** and left the reactive naphthalene moiety unchanged. Following cyclization of **15** to **16** (Cs_2CO_3 , CH_3CN), the Br-substituents were transformed into cyano groups by heating **16** with CuCN in *N*-methylpyrrolidone (NMP) to 190 °C. The dinitrile **17** was subsequently reduced with $\text{BH}_3 \cdot \text{THF}$ to the diamine **18** which was reacted with *N,N*-dimethylglycine in the presence of PPh_3 and 2,2'-dithiodipyridine^[22] to afford diamide **19**. Quaternization with EtI followed by ion exchange (Cl^-) afforded the target receptor **2**.



a) Mg , THF, 1-acetyl-4-piperidone, 20 °C, 2 h, 50%. b) BBr_3 , CH_2Cl_2 , reflux, 2.5 h. c) NaH , DMF, 1,4-dichlorobutane, 20 °C, 12 h, 65% (steps b) and c)). d) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 2,6-dimethoxyphenol, CH_2Cl_2 , 20 °C, 5-8 d, 81%. e) Cs_2CO_3 , DMF, 80 °C, 3 d, 25%. f) $\text{BH}_3 \cdot \text{THF}$, reflux, 24 h, 60%. g) EtI , CHCl_3 , 20 °C, 3.5 d, then ion exchange (Dowex, Cl^-), 93%.

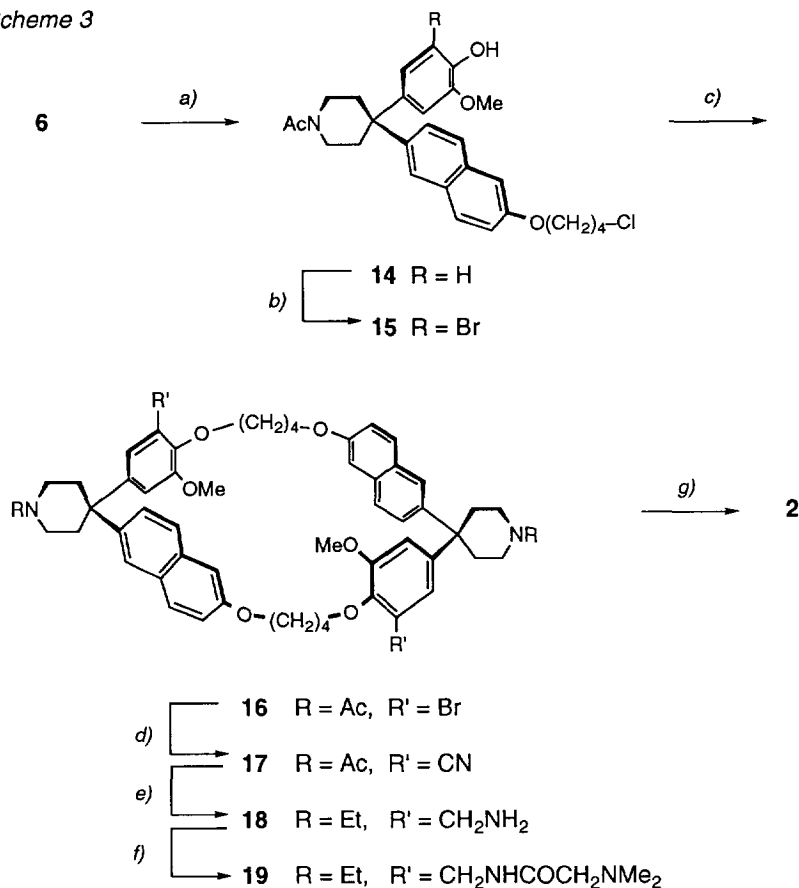
Scheme 2



a) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 2,6-dimethoxyphenol, nitrobenzene, 80 °C, 5 h, 48%. b) K_2CO_3 , CH_3I , acetone, reflux, 14 h, 90%. c) $\text{BH}_3 \cdot \text{THF}$, reflux, 6 h, 58%. d) EtI , CHCl_3 , 20 °C, 4 d, then ion exchange (Dowex, Cl^-), 87%.

With its four quaternary ammonium ions, receptor **2** is freely soluble in H₂O up to $c \geq 10$ mM. The analysis of the ¹H NMR chemical shifts of the ethyldimethylammonium group protons did not show any indication for self-complexation of the onium groups as a result of a cation- π -effect.^[23] Presumably, the cavity is too large for this undesirable interaction to occur.

Scheme 3



a) BF₃•Et₂O, guaiacol, CH₂Cl₂, 20 °C, 9 d, 90%. b) NBS, CH₂Cl₂, CH₃OH, cat. NaH, -50 °C, 8 h, 59%. c) Cs₂CO₃, CH₃CN, reflux, 3 d, 42%. d) CuCN, NMP, 190 °C, 14 h, 85%. e) BH₃•THF, reflux, 12 h, 95%. f) *N,N*-dimethylglycine, (S(2-pyr))₂, PPh₃, CH₂Cl₂, 20 °C, 16 h, 32%. g) EtI, CHCl₃, 20 °C, 4 d, then Dowex (CF), 63%.

3. Steroid Complexation Studies.

Steroid complexation by **1** and **2** was investigated in 500 MHz ¹H NMR binding titrations in which the complexation-induced changes in chemical shift of the isolated signals of the steroid methyl groups were monitored and evaluated. Bile acids, corticoids, and androgenic steroids form stable complexes with **1** in D₂O/CD₃OD 1:1 (v/v) at 293 K (Table I), in which the substrates are included axially, with free axial

rotation, as schematically depicted in *Fig. 1*. This geometry allows the highly solvated functional groups of the steroids at C(3) in ring A and at C(17) in ring D to orient into the solution.

Table 1. Association constants K_a and binding free energies $-\Delta G^\circ$ for steroid complexes of cyclophane **1** in D₂O/CD₃OD 1:1 (v/v) at 293 K. The maximum observed upfield complexation-induced change in ¹H NMR chemical shift of the steroid methyl group protons $\Delta\delta_{\max \text{ obs}}$ and the change calculated for saturation binding $\Delta\delta_{\text{sat}}$ are shown.

Steroid	K_a [L mol ⁻¹]	$-\Delta G^\circ$ [kcal mol ⁻¹]	$\Delta\delta_{\max \text{ obs}}$ ($\Delta\delta_{\text{sat}}$)		
			CH ₃ (19)	CH ₃ (18)	CH ₃ (21)
20a [a]	145	2.90	0.32 (0.73)	0.22 (0.56)	0.09 (0.25)
20b [a]	250	3.21	0.43 (0.76)	0.34 (0.66)	0.18 (0.39)
20c [a]	810	3.91	0.37 (0.47)	1.12 (1.40)	0.69 (0.89)
20d [a]	1750	4.35	0.27 (0.30)	1.34 (1.49)	1.09 (1.23)
20e [a]	7075	5.18	0.54 (0.55)	1.44 (1.49)	0.88 (0.90)
21a	1095	4.08	1.19 (1.44)	0.19 (0.26)	
21b	1510	4.26	1.27 (1.48)	0.26 (0.30)	
21c	3545	4.76	1.39 (1.48)	0.41 (0.43)	

[a] Solutions contain 0.01 M Na₂CO₃.

High selectivity was observed in the complexation of bile acids. The complex of lithocholic acid (**20e**) is ≈ 2 kcal mol⁻¹ more stable than the complex of deoxycholic acid (**20b**) which has an additional hydroxy group at C(12 α). The observed differential upfield complexation shifts of the three methyl group resonances (*Table 1*) suggest that the rings C and D of lithocholic acid are preferentially encapsulated by **1**. This generates a large number of favorable contacts between the apolar surfaces of these rings and the cavity walls (*Fig. 1*). A similar orientation of deoxycholic acid in the cavity would require considerable desolvation of the hydroxy group at C(12 α), since it would be located deeply inside the apolar cavity. Apparently, this is too costly, and inclusion occurs in a different orientation to minimize the energetically unfavorable desolvation of the hydroxy group. The shifts of the methyl resonances of **20b** indicate that deoxycholic acid is preferentially encapsulated with ring B which positions the hydroxy group more outside the cavity.

The presence of polar, highly solvated groups at the central rings B and C of the steroid skeleton generally affects both geometry and stability of the inclusion complexes formed with **1**. Bile acids **20a-d** with strongly solvated HO-groups at these rings bind less well than lithocholic acid. Similarly, in the series **21a-c**, testosterone (**21c**) forms a more stable complex than the corticoids **21a,b** with polar functional groups at ring C. The observed shifts of the steroid methyl resonances in the complexes suggest that **20a,b** are preferentially encapsulated with ring B, **20c-e** with rings C and D, and **21a-c** with rings A and B. It seems as if interactions of the unsaturated enone system in ring A of **21a-c** with the electron-rich aromatic cavity walls of **1** are particularly favorable.

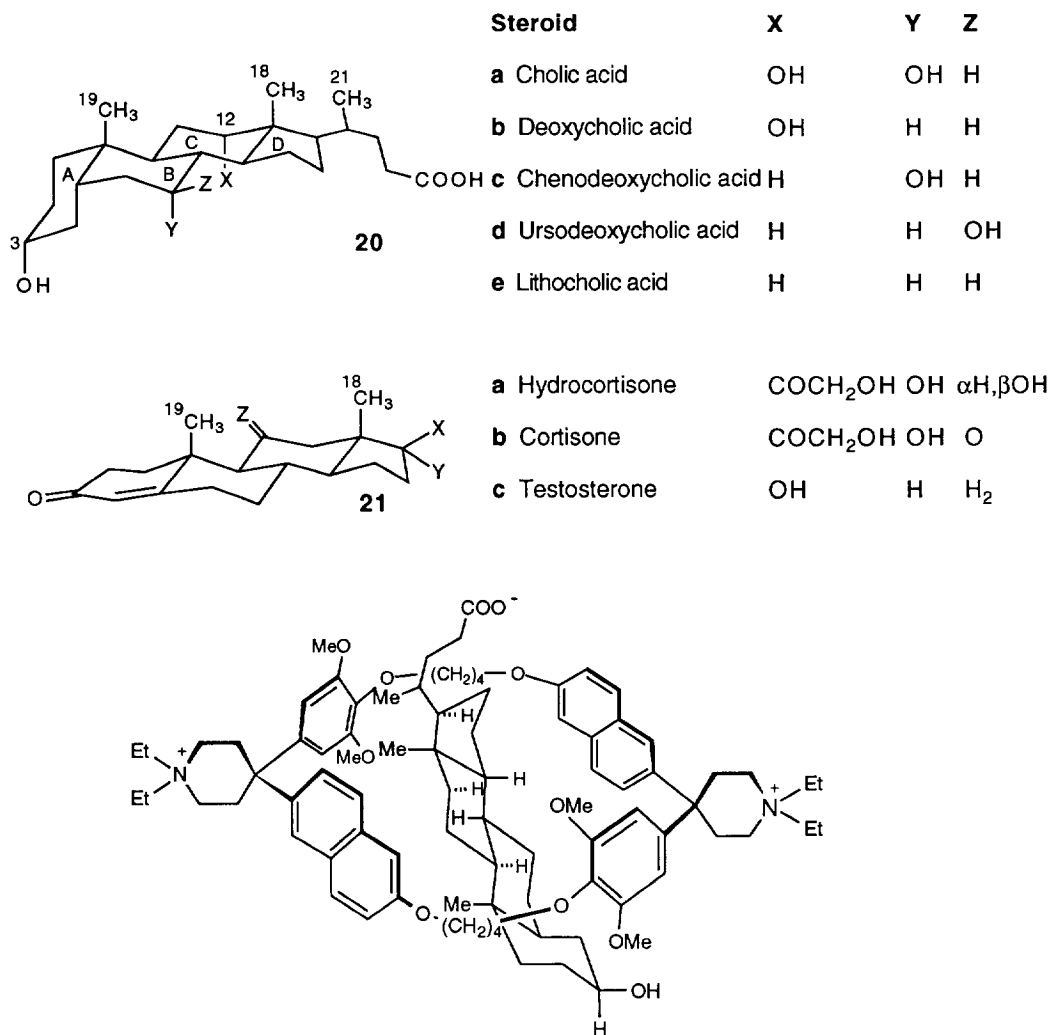


Figure 1: Schematic representation of the axial inclusion complex of lithocholic acid (**20e**)

The acyclic compound **10** failed to show any binding of bile acids. This demonstrates that apolar interactions and desolvation as a result of cavity inclusion are the driving forces for complexation by **1** and that ion pairing between the piperidinium centers of the host and the carboxylate ions of these steroids is not a significant binding interaction.

Cyclophane **1** is highly selective for steroids. The complexation of smaller alicyclic guests like 1-adamantaneacetic acid ($K_a = 115 \text{ L mol}^{-1}$, $-\Delta G^\circ = 2.77 \text{ kcal mol}^{-1}$) and camphor ($K_a = 145 \text{ L mol}^{-1}$, $-\Delta G^\circ = 2.90 \text{ kcal mol}^{-1}$) is weaker because these guests are too small to fill the large cavity of **1**. Stable 1:1 inclusion complexes ($K_a \approx 200 - 850 \text{ L mol}^{-1}$, $-\Delta G^\circ \approx 3.0 - 4.0 \text{ kcal mol}^{-1}$) are also formed with [m.n]paracyclophanes.^[8] In these complexes, the two phenyl rings of the guest stack with the two

trialkoxybenzene rings of the host and undergo edge-to-face interactions with the two naphthalene rings of **1**.

In previous work we showed that the tight inclusion complexation of aromatic substrates in aqueous solutions by cyclophane receptors like **3** is entropically unfavorable and strongly enthalpically driven.^[18,24] These thermodynamic characteristics differ entirely from those measured for loose apolar association processes such as membrane and micelle formation^[25] which are characterized by small enthalpic changes and favorable entropic terms. We explained the enthalpic driving force for tight cyclophane-arene inclusion complexation in water with a strong gain in solvent cohesive interactions and in dispersion interactions.^[26] Since these complexes are also stabilized by particularly strong attractive aromatic-aromatic host-guest interactions, both of the π - π and edge-to-face type,^[27] it remained unclear whether enthalpically driven complexation is a general characteristics of tight apolar binding in aqueous solution or whether it is rather limited to the specific case of arene complexation. To answer this question, we studied the complexation of steroids in variable temperature ¹H NMR binding titrations (*Table 2*) and evaluated the thermodynamic quantities ΔH° and ΔS° by van't Hoff analysis.

In D₂O/CD₃OD 1:1, the stability of the steroid complexes of **1** decreases considerably with increasing temperature and the van't Hoff plots proved to be perfectly linear indicating that changes in the heat capacity were insignificant in the considered temperature interval of 20 K. *Table 2* clearly shows that steroid complexation in the aqueous solvent mixture is driven by a strong change in enthalpy which is partially compensated by an unfavorable change in entropy. We take this as evidence that all tight apolar binding processes in aromatic binding pockets or cavities, whether involving aromatic or alicyclic substrates, are enthalpically driven in aqueous solutions.^[28,29] The observed compensatory effect of the entropic on the enthalpic change constitutes a perfect isoequilibrium relationship ($r^2 = 0.99$) as evidenced by the linearity of the plot of ΔH° as a function of ΔS° (*Fig. 2*).^[24,30]

Table 2. Association constants K_a at various temperatures and binding free energies $-\Delta G^\circ$ (298 K) for 1:1 complexes of steroids and cyclophane **1** in D₂O/CD₃OD 1:1 and the thermodynamic quantities ΔH° (kcal mol⁻¹) and ΔS° (cal K⁻¹ mol⁻¹) calculated from van't Hoff analysis.

Steroid	K_a (L mol ⁻¹) [a]					$-\Delta G^\circ$ kcal mol ⁻¹	ΔH° kcal mol ⁻¹	ΔS° cal K ⁻¹ mol ⁻¹
	298 K	303 K	308 K	313 K	318 K			
20e [b]	5300	4270	3410	2720	2060	5.08	- 8.7	- 12.0
21c	2600	1860	1310	1000	720	4.66	- 12.0	- 24.4
20d [b]	1000	670	480	340	240	4.09	- 13.5	- 31.4
21b	890	590	410	290	210	4.02	- 13.7	- 32.5
21a	630	435	340	220	165	3.82	- 12.6	- 29.3

[a] Reproducibility in K_a : $\pm 10\%$. [b] Solutions contain 0.01 M Na₂CO₃.

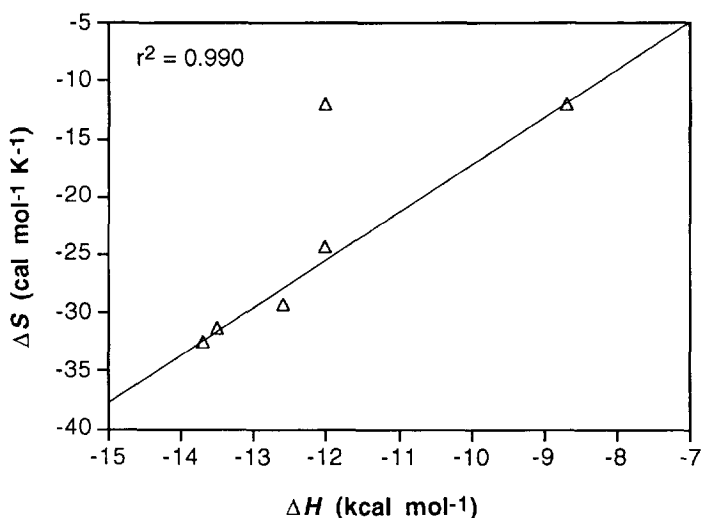


Figure 2. Isoequilibrium relationship between the changes in enthalpy and entropy for the complexation of steroids by **1** in D₂O/CD₃OD 1:1 (298 K, for data see Table 2).

With its two additional cationic side chains, cyclophane **2** is highly soluble (≥ 10 mM) in pure D₂O, and this allowed us to perform steroid complexation studies in this environment. Unfortunately, most of the steroids are very insoluble in pure D₂O or their complexes with **2** precipitated out of solution, which limited the amount of studies that could be undertaken. A further limitation to quantitative ¹H NMR binding studies in homogenous D₂O-solution was given by slow exchange kinetics. Steroids like lithocholic acid (**20e**) and ursodeoxycholic acid (**20d**) form highly stable complexes which shifts the decomplexation kinetics onto the NMR time scale, leading to extremely broadened signals which could not be evaluated. An additional problem represents the self-aggregation of many steroids in pure water.^[31]

Table 3 shows the results of ¹H NMR complexation studies of cyclophane **2** with steroids that are soluble and non-aggregating^[32] in pure water or binary methanolic mixtures with high water content. Cortisone (**21b**) was sufficiently soluble for use in binding studies in D₂O/CD₃OD 10:1, whereas the complexation of the bile acid derivatives **20f** (sodium glycochenodeoxycholate) and **20g** (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) and of the dianionic steroid **22** (disodium dexamethasone-21-phosphate) could be studied in pure D₂O. The binding data with cyclophane **2** in pure D₂O were compared to those in less polar methanolic mixtures to evaluate the relative importance of ion pairing interactions for the stability of the anionic steroid complexes. Comparisons with cyclophane **1** were made to evaluate the effect of the charged side chains in **2** on the apolar binding capacity of the cyclophane cavity.

The results shown in Table 3 allow one to draw the following conclusions:

i) The comparison of the cortisone complexes formed by cyclophanes **1** ($K_a = 890$ L mol⁻¹, Table 2) and **2** ($K_a = 310$ L mol⁻¹, run 1, Table 3) in D₂O/CD₃OD 1:1 shows that receptor **1** binds this neutral

steroid better by a factor of 3. This observation can be explained by the more favorable solvation and the reduced lipophilicity of the cavity of **2** due to the proximity of the two charged side chains.

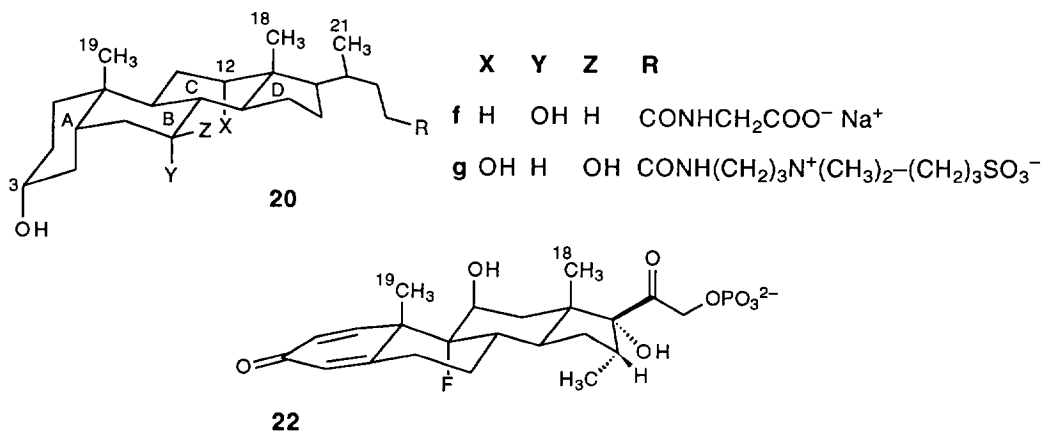


Table 3. Association constants K_a and binding free energies $-\Delta G^\circ$ (298 K) for 1:1 complexes of steroids and cyclophanes **1** and **2** in D₂O and D₂O/CD₃OD mixtures (v/v). The maximum observed upfield complexation-induced change in ¹H NMR chemical shift of the steroid methyl group protons $\Delta\delta_{\max \text{ obs}}$ and the change calculated for saturation binding $\Delta\delta_{\text{sat}}$ are shown.

Run	Cyclo- phane	Steroid	Solvent	K_a [a] (L mol ⁻¹)	$-\Delta G^\circ$ (kcal mol ⁻¹)	$\Delta\delta_{\max \text{ obs}}$ (CH ₃ (19))	$\Delta\delta_{\text{sat}}$ (CH ₃ (18))
1.	2	21 b	D ₂ O/CD ₃ OD 1:1	310	3.40	0.61 (1.39)	0.13 (0.31)
2.			D ₂ O/CD ₃ OD 3:1	2260	4.58	[c]	0.18 (0.33)
3.			D ₂ O/CD ₃ OD 10:1	4960	5.04	[c]	0.22 (0.35)
4.			D ₂ O [b]	10000	5.5		
5.	1	22	D ₂ O/CD ₃ OD 1:1	660	3.85	0.45 (0.77)	0.14 (0.25)
6.	2	22	D ₂ O/CD ₃ OD 1:1	8040	5.33	[c]	0.24 (0.28)
7.	2	22	D ₂ O	24900	6.00	0.87 (1.57)	0.33 (0.41)
8.	2	20f	D ₂ O	37300	6.24	0.28 (0.35)	[c]
9.	2	20g	D ₂ O	no significant binding			

[a] Reproducibility in $K_a \pm 10\%$. [b] Determined by extrapolation from linear plot of $-\Delta G^\circ$ (runs 1-3) against E_T for the solvent mixtures of runs 1-3. [c] Due to large $\delta\delta$ values, the decomplexation occurs on the NMR time scale and the signals are very broad and cannot be evaluated.

ii) Cortisone binding studies with cyclophane **2** in aqueous solutions of varying methanol content (runs 1-4) underline the promoting power of water for apolar complexation: Complexation strength increases by more than 2 kcal mol⁻¹ upon passing from D₂O/CD₃OD 1:1 to pure water. The complex

stability in pure D₂O ($K_a \approx 10^4 \text{ L mol}^{-1}$, $-\Delta G^\circ = 5.5 \text{ kcal mol}^{-1}$) which, for solubility reasons could not be determined experimentally, was extrapolated from the linear plot of the binding free energy $-\Delta G^\circ$ of runs 1-3 against the E_T value^[33,34] of the solvent mixtures used in these runs. Strong linear free energy relationships between empirical solvent parameters and the free energy for apolar complexation in binary aqueous solvent mixtures are well known.^[26,27a,34]

iii) In both binary solvent mixtures and pure D₂O, steroids **20f** and **22** with anionic side chains form particularly stable complexes with cyclophane **2** (runs 6-8), despite one HO-group on either ring B and C, respectively. Whereas cyclophane **1** is the better receptor for neutral steroids, cyclophane **2** with its two cationic side arms is the stronger binder of the anionic derivatives. In D₂O/CD₃OD 1:1 the complex of **2** with dexamethasone phosphate (**22**, $K_a = 8040 \text{ L mol}^{-1}$, run 6) is $1.5 \text{ kcal mol}^{-1}$ more stable than the corresponding complex of **1** ($K_a = 660 \text{ L mol}^{-1}$, run 5). The complexes between **2** and anionic steroids clearly are stabilized by additional ion pairing interactions between the charged center of the substrate and the cationic side chains.^[35] That ion pairing is a significant stabilizing interaction in addition to apolar binding is also indicated by the following comparison: Upon changing from pure D₂O to D₂O/CD₃OD 1:1, the stability of the complex of **2** with cortisone decreases by $2.1 \text{ kcal mol}^{-1}$ (runs 1 and 4). In contrast, the same change in solvent polarity decreases the stability of the complex with anionic **22** only by $0.7 \text{ kcal mol}^{-1}$ (runs 6 and 7), since the strengthening ion pairing interaction in the methanolic solution compensates for the decrease in apolar interactions.

iv) No significant complexation in millimolar concentration ranges by **2** was observed with the zwitterionic steroid **20g**. Unfavorable changes in solvation of two HO-groups at rings B and C upon inclusion complexation should reduce the association strength in addition to possible electrostatic repulsion between the onium centers of the substrate and those of the side arms of **2**.

4. Conclusion

The cyclophane receptors **1** and **2**, which are shaped by two naphthylphenylmethane units, are capable of selective steroid recognition in aqueous solution. Smaller alicyclic substrates as well as flat aromatic guests do not bind strongly in their large cavities. Steroids are included axially into the cavity of **1** and the complexes are mainly stabilized through apolar interactions and desolvation forces. High substrate selectivity among the bile acids results from functional group desolvation upon cavity inclusion: in D₂O/CD₃OD 1:1 lithocholic acid (**20e**), which does not bear polar groups on rings B and C, forms a complex with **1** that is 2 kcal mol^{-1} more stable than the complex of deoxycholic acid (**20b**) which possesses a HO-group at C(12 α) of ring C.

Important new information on the nature of the (hydrophobic) forces^[36] for tight apolar complexation in aqueous solution was obtained. The van't Hoff analysis of variable temperature ¹H NMR binding titration data showed that steroid complexation by cyclophane **1** in D₂O/CD₃OD 1:1 is enthalpically driven with a strong isoequilibrium relationship between the favorable enthalpic and the partially compensating unfavorable entropic term. All tight cavity inclusion by cyclophane receptors, whether involving aromatic or alicyclic substrates, seems to be enthalpically driven. It still remains to be determined, to what extent specific interactions of the substrates with the aromatic cavity walls of the cyclophane receptors contribute to this enthalpic driving force for tight apolar complexation.

Cyclophane **2** forms less stable complexes with neutral steroids than **1** since its two polar side arms reduce the lipophilicity of its cavity binding site. In contrast, anionic steroids form particularly stable complexes with **2** both in pure D₂O and in D₂O/CD₃OD, since the quaternary centers in its side arms undergo efficient ion pairing interactions with the anionic centers of the cavity bound steroids.

The present study demonstrates that cyclophanes, unlike cyclodextrins, may show high binding selectivity among structurally related steroids in aqueous solution. These receptors now await exploration in steroid formulation and delivery as well as in other desirable health-related applications such as bile acid depletion with the aim of increasing the number of LDL receptors in the liver.

EXPERIMENTAL SECTION

General. Reagents used were reagent grade chemicals. Steroids for binding studies were purchased from Aldrich, Fluka, or Sigma and were used without further purification. All reactions were performed under an argon atmosphere unless otherwise noted. THF was freshly distilled from sodium benzophenone ketyl. DMF was dried by storage for at least 3 d over basic alumina (Merck, act. I). CH₂Cl₂ and CH₃CN were distilled from CaH₂ immediately prior to use. EtI was distilled at atmospheric pressure in a foil wrapped apparatus. BF₃·OEt₂ was distilled from CaH₂ at 10 Torr. 2,6-Dimethoxyphenol was crystallized from hexanes and stored in the dark. Silica gel (230-400 mesh, 0.040-0.063 mm) was purchased from E. Merck. Alkyl ammonium iodides were converted to the chlorides by passing an aqueous solution through Dowex 18X-400 strongly basic anion exchange resin, prepared by thoroughly rinsing sequentially with water, 1 N aq. NaOH, water until neutral, 1 N aq. HCl, then water again until neutral. Millipore filtered water was used exclusively for all operations with ionic compounds. Evaporation and concentration *in vacuo* was done at water aspirator pressure, drying *in vacuo* at 10⁻² Torr. Melting points are uncorrected. NMR spectra (TMS reference) were obtained at either 125.6 MHz (¹³C), 360 MHz (¹H) or 500 MHz (¹H) at 300 K if not stated otherwise. MS: (*m/z*, %). Fast atom bombardment spectra (FAB MS) were determined in *m*-nitrobenzyl alcohol as the matrix. Elemental analyses were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich, by Spang Microanalytical Laboratories (Eagle Harbor, MI), or by Desert Analytics (Tucson, AZ). CAS Registry Services provided the names for the macrocyclic compounds.

Complexation Studies. All ¹H NMR binding titrations at fast host-guest exchange were performed on a Bruker 500 MHz spectrometer thermostated to ± 0.1 K accuracy. The steroid concentrations were kept constant (0.1 - 0.2 mM in D₂O and 0.2-1.0 mM in D₂O/CD₃OD mixtures) and the host concentrations varied to ensure a range of 20-90% saturation binding. The complexation-induced change in chemical shift values of the steroid CH₃-resonances ($\Delta\delta = \delta_{\text{free}} - \delta_{\text{obs}}$) was plotted against the host concentration, and quantitative binding numbers (K_a , $-\Delta G^\circ$, and $\Delta\delta_{\text{sat}}$) were obtained with the non-linear least-squares curve-fitting program Associate V1.5 by Blake R. Peterson, ETH Zürich. The reproducibility of K_a -values was ± 10%. The reported K_a and $-\Delta G^\circ$ values are averages of those calculated from all methyl group protons of the steroids that could be monitored during the titration. Prior to use, cyclophanes **1** and **2** were passed over an anion exchange resin with H₂O/CH₃OH 1:1 as eluent, then dried

for 24 h at 90 °C/0.1 Torr and stored moisture-free. All binding studies with cyclophane **1** were done in D₂O/CD₃OD 1:1 (v/v) and studies with **2** were done in solvent mixtures varying from D₂O/CD₃OD 1:1 to pure D₂O. When guests bearing carboxylic acid groups were used, 0.01 M Na₂CO₃ was added to ensure complete ionization to the carboxylate ions. For NMR sample preparation, stock solutions of host and guest were obtained by weighing the compounds on a Mettler AT20 microbalance into analytical micro glass vials and adding these together with the NMR solvent into 5 mL volumetric flasks. Prior to use, the stock solutions were sonicated to ensure complete dissolution of the components. Aliquots of the stock solutions were pipetted into NMR tubes by using Gilson Pipetman 200 or 1000 µL micropipettes, and solvent was added to give a constant volume between 0.70 and 1.00 mL. Before recording spectra, the samples were carefully mixed by shaking.

1-Acetyl-4-hydroxy-4-[2-(6-ethoxy)naphthyl]piperidine (4). A solution of 2-bromo-6-ethoxynaphthalene^[20] (6.0 g, 0.025 mol) in THF (50 mL) was added to magnesium turnings (10.3 g, 0.42 mol), and a few crystals of I₂ were added to initiate the formation of the Grignard reagent. After a few minutes the yellow color of I₂ disappeared and the remainder of the bromide (92.3 g, 0.37 mol) in THF (1.5 L) was added slowly. The dark green solution was warmed to reflux for 10 min and allowed to cool to 20 °C over 1 h. A solution of 1-acetyl-4-piperidone (46.5 g, 0.33 mol) in THF (600 mL) was added over 10 min to give a yellow suspension. The mixture was stirred at 20 °C for 2 h, then quenched with sat. aq. NH₄Cl (400 mL). The organic solvent was evaporated to give an aqueous slurry which was extracted with CH₂Cl₂ (1 L). The organic layer was washed with sat. aq. NaCl (2 x 1 L) and dried (Na₂SO₄). The residue obtained by evaporation of the solvent was washed with large portions of Et₂O and recrystallized from (CH₃)₂CHOH to yield **4** (49 g, 50%) as a white powder. Analytical sample from toluene: m.p. 169-171 °C. IR (CDCl₃): 3580, 3200-3500 (O-H), 1620 (C=O). ¹H NMR (500 MHz, CDCl₃): 1.48 (*t*, *J* = 7.0, 3 H); 1.90 and 1.93 (2 x *ddd*, *J* = 13.7, 5.3, 2.5, 2 H); 2.05 and 2.13 (2 x *ddd*, *J* = 13.1, 12.6, 4.5, 2 H); 2.15 (*s*, 3 H); 3.15 and 3.65 (2 x *ddd*, *J* = 13.0, 12.9, 2.9, 2 H); 3.75 and 4.61 (2 x *ddd*, *J* = 13.2, 4.5, 2.5, 2 H); 4.15 (*q*, *J* = 7.0, 2 H); 7.12 (*d*, *J* = 2.5, 1 H); 7.16 (*dd*, *J* = 8.7, 2.5, 1 H); 7.53 (*dd*, *J* = 8.7, 1.9, 1 H); 7.73 (*d*, *J* = 8.7, 2 H); 7.82 (*d*, *J* = 1.9, 1 H). MS (EI, 20 eV): 313 (*M*⁺, 100). Anal. calc. for C₁₉H₂₃NO₃ (313.4): C 72.82, H 7.40, N 4.47; found: C 72.76, H 7.42, N 4.50.

1-Acetyl-4-[2-(6-hydroxy)naphthyl]-3,4-dehydropiperidine (5). BBr₃ (57 mL, 0.60 mol) was slowly added at 20 °C to **4** (39.8 g, 0.127 mol) in CH₂Cl₂ (2 L), and the resulting green solution was refluxed for 2.5 h, allowed to cool to 20 °C, and quenched carefully with CH₃OH. Evaporation of the solvents gave a yellow slurry which was washed on a fritted glass funnel with generous portions of H₂O and Et₂O. Drying *in vacuo* gave crude **5** which was used without further purification in the next reaction. Analytical sample from toluene: m.p. 193 °C (dec.). IR (KBr): 1630 (C=O). ¹H NMR (500 MHz, CD₃OD): 2.15 and 2.19 (2 x *s*, 3 H); 2.65 and 2.73 (br. 2 x *m*, 2 H); 3.77 and 3.83 (2 x *t*, *J* = 5.8, 2 H); 4.22 and 4.24 (2 x *q*, *J* = 3, 2 H); 6.21 (br. *s*, 1 H); 7.04 (*dd*, *J* = 8.7, 2.5, 1 H); 7.06 (*d*, *J* = 2.5, 1 H); 7.54 (*d*, *J* = 8.7, 1 H); 7.59 (*d*, *J* = 8.7, 1 H); 7.70 (*d*, *J* = 8.7, 1 H); 7.72 (br. *d*, 1 H). HR-MS (EI, 20 eV): 267.1274 (*M*⁺, C₁₇H₁₇NO₂), calc. 267.1259.

1-Acetyl-4-[2-[6-(4-chlorobutoxy)]naphthyl]-3,4-dehydropiperidine (6). A NaH dispersion (60%, 13.2 g, 0.33 mol) was washed with petroleum ether (2 x 100 mL) and suspended in

DMF (200 mL). A solution of crude **5** (40 g, 0.15 mol) in DMF (500 mL) was added dropwise followed by 1,4-dichlorobutane (203 g, 1.60 mol). After stirring for 12 h at 20 °C and quenching with H₂O, sat. aq. NH₄Cl (100 mL) was added and the DMF removed *in vacuo*. The residue was suspended in CH₂Cl₂ (400 mL), the organic solution washed with sat. aq. NH₄Cl, dried (Na₂SO₄), and evaporated to give a viscous yellow oil. After removal of excess 1,4-dichlorobutane by distillation *in vacuo*, the crude product was filtered through a pad of SiO₂ (CH₂Cl₂/CH₃OH 95:5), the resulting solid washed with a small amount of Et₂O and dried *in vacuo* to give **6** (31 g, 65% starting from **4**) as a white powder. Analytical sample from abs. EtOH: m.p. 89-94 °C. IR (CDCl₃): 1625 (C=O). ¹H NMR (500 MHz, CDCl₃): 2.03 (br. *m*, 4 H); 2.16 and 2.20 (2 x *s*, 3 H); 2.66 and 2.71 (br. 2 x *m*, 2H); 3.66 (*t*, *J* = 6.1, 2 H); 3.71 and 3.87 (2 x *t*, *J* = 5.7, 2 H); 4.12 (*t*, *J* = 5.6, 2 H); 4.18 and 4.29 (br. 2 x *q*, 2 H); 6.13 and 6.20 (br. 2 x *m*, 1 H); 7.10 (*d*, *J* = 2.3, 1 H); 7.13 (*ddd*, *J* = 8.8, 2.3, 1.9, 1 H); 7.53 (*dd*, *J* = 8.8, 1.9, 1 H); 7.68-7.73 (*m*, 3 H). HR-MS (EI, 20 eV): 357.1481 (*M*⁺, C₂₁H₂₄ClNO₂), calc. 357.1495.

1-Acetyl-4-{2-[6-(4-chlorobutoxy)]naphthyl}-4-(4-hydroxy-3,5-dimethoxyphenyl) piperidine (7). To a solution of **6** (27.7 g, 0.077 mol) and 2,6-dimethoxyphenol (60 g, 0.39 mol) in CH₂Cl₂ (175 mL) was added BF₃·Et₂O (83.3 g, 0.59 mol), and the resulting dark solution was stirred at 20 °C until the starting material was consumed (5 - 8 d). The reaction mixture was quenched with CH₃OH (20 mL), then CH₂Cl₂ (300 mL), and H₂O (300 mL) was added. The organic phase was dried (Na₂SO₄) and evaporated to give a thick brown oil from which excess 2,6-dimethylphenol was removed by distillation *in vacuo* at a bath temperature ≤130 °C. Chromatography on SiO₂ (1 kg, CH₂Cl₂/CH₃OH 98:2) yielded **7** (32 g, 81%) as an off-white foam which was recrystallized from hexane/CH₂Cl₂ 5:1 for analytical purposes: m.p. 139-141 °C. IR (CDCl₃): 3525 (O-H), 1620 (C=O). ¹H NMR (500 MHz, CDCl₃): 2.02 (*m*, 4 H); 2.11 (*s*, 3 H); 2.36-2.55 (br. 2 x *m*, 4 H); 3.53-3.60 (br. *m*, 4 H); 3.64 (*t*, *J* = 5.6, 2 H); 3.80 (*s*, 6 H); 4.10 (*t*, *J* = 5.5, 2 H); 5.43 (*s*, 1 H); 6.47 (*s*, 2 H); 7.07 (*d*, *J* = 2.5, 1 H); 7.14 (*dd*, *J* = 8.9, 2.5, 1 H); 7.25 (*dd*, *J* = 8.7, 1.9, 1 H); 7.63 (*d*, *J* = 8.7, 1 H); 7.64 (*s*, 1 H); 7.70 (*d*, *J* = 8.9, 1 H). MS (EI, 20 eV): 511 (*M*⁺, 100). Anal. calc. for C₂₉H₃₄ClNO₅ (512.05): C 68.03, H 6.69, N 2.74; found: C 68.18, H 6.74, N 2.68.

1,1''-Diacetyl-18',37',40',44'-tetramethoxy-dispiro[piperidine-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.13,7.16,10.1^{22,26}.1^{25,29}]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21'4''-piperidine] (8). A mixture of **7** (20.5 g, 0.04 mol) and Cs₂CO₃ (46 g, 0.14 mol) in DMF (2 L) was stirred at 80 °C for 3 d. After filtration through celite, the solvent was removed *in vacuo*, the residue dissolved in CH₂Cl₂ (600 mL) and the solution washed with H₂O (2 x 400 mL), dried (Na₂SO₄), and evaporated to give a yellow-brown foam. Chromatography on SiO₂ (1 L, CH₂Cl₂/CH₃OH 97:3) afforded **8** (4.8 g, 25%) as a white solid. Analytical sample from EtOH/CH₂Cl₂ 5:1: m.p. 308 °C (dec.). IR (CDCl₃): 1630 (C=O). ¹H NMR (500 MHz, CDCl₃): 1.91 and 2.06 (br. 2 x *m*, 8 H); 2.09 (*s*, 6 H); 2.26-2.38 and 2.41-2.55 (br. 2 x *m*, 8 H); 3.41-3.54, 3.61 and 3.94 (br. 3 x *m*, 8 H); 3.65 (*s*, 12 H); 3.95 (*t*, *J* = 6.1, 4 H); 4.13 (*t*, *J* = 6.5, 4 H); 6.34 (*s*, 4 H); 6.98 (*d*, *J* = 2.5, 2 H); 7.08 (*dd*, *J* = 8.9, 2.5, 2 H); 7.13 (*dd*, *J* = 8.7, 1.9, 2 H); 7.51 (*d*, *J* = 8.7, 2 H); 7.63 (*d*, *J* = 8.9, 2 H); 7.64 (*s*, 2 H). FAB MS: 951 (*M*⁺). Anal. calc. for C₅₈H₆₆N₂O₁₀ (951.18): C 73.24, H 6.99, N 2.95; found: C 72.97, H 7.06, N 2.97.

1,1''-Diethyl-18',37',40',44'-tetramethoxy-dispiro[piperidine-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.1^{3,7}.1^{6,10}.1^{22,26}.1^{25,29}]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21'4''-piperidine] (9). A solution of **8** (3.30 g, 3.46 mmol) in THF (100 mL) and 1 M BH₃·THF (52 mL, 52 mmol) was refluxed for 24 h, then quenched with CH₃OH and evaporated. The residue was refluxed for 1 h in abs. EtOH/conc. aq. H₂SO₄ 7:3 (100 mL) and the solution neutralized with NaOH. The residue obtained after evaporation was partitioned between CHCl₃ (600 mL) and 1 N NaOH (600 mL). The aqueous layer was exhaustively extracted with CHCl₃ and the combined organic phases dried (Na₂SO₄) and evaporated, leaving a solid which was chromatographed on SiO₂ (600 mL, EtOAc/CHCl₃/NEt₃ 55:37:8). The product was dissolved in hot CHCl₃ (300 mL) and the solution washed with 1 N NaOH (200 mL) and dried (Na₂SO₄). The solution was concentrated to 75 mL, and addition of Et₂O (800 mL) precipitated **9** (1.92 g, 60%) as a white crystalline solid. Analytical sample from CH₃OH/CHCl₃ 7:3: m.p. 248-250 °C. ¹H NMR (500 MHz, CDCl₃): 1.06 (br. *t*, 6 H); 1.90 and 2.05 (br. 2 x *m*, 8 H); 2.25-2.71 (*m*, 20 H); 3.61 (*s*, 12 H); 3.96 (*t*, *J* = 6.2, 4 H); 4.13 (*t*, *J* = 6.5, 4 H); 6.36 (*s*, 4 H); 6.98 (*d*, *J* ≈ 2, 2 H); 7.06 (*dd*, *J* = 8.9, 2.4, 2 H); 7.16 (*dd*, *J* = 8.7, ≈ 2, 2 H); 7.50 (*d*, *J* = 8.7, 2 H); 7.62 (*d*, *J* = 8.9, 2 H); 7.65 (br. *s*, 2 H). FAB MS: 923 (*M*⁺). Anal. calc. for C₅₈H₇₀N₂O₈ (923.21): C 75.46, H 7.64, N 3.03; found: C 75.24, H 7.78, N 3.03.

1,1,1'',1''-Tetraethyl-18',37',40',44'-tetramethoxy-dispiro[piperidinium-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.1^{3,7}.1^{6,10}.1^{22,26}.1^{25,29}]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21'4''-piperidinium] dichloride (1). A solution of **9** (0.92 g, 1.0 mmol) in CHCl₃ (100 mL) was washed with 1 N NaOH (75 mL) and dried (Na₂SO₄). The drying agent was filtered off and washed with acid-free CHCl₃ (50 mL, prepared by passing through basic Al₂O₃, act. I). Ethyl iodide (40 mL) was added, and the solution stirred in the dark at 20 °C for 3.5 d. The white suspension was concentrated and the solution obtained by addition of CH₃OH (400 mL) and deionized H₂O (400 mL) passed through a column of anion exchange resin (Cl⁻). Elution with CH₃OH/deionized H₂O 1:1 gave a white foam which was dried *in vacuo* and dissolved in CH₃OH (100 mL). The solution was concentrated to ≈ 70 mL, and addition of Et₂O gave a solid precipitate which was dried *in vacuo* at 78 °C to yield **1** (0.97 g, 93%) as a hygroscopic white solid: m.p. 258-260 °C (dec.). ¹H NMR (500 MHz, CD₃OD): 1.19-1.29 (2 x *t*, *J* ≈ 7, 12 H); 1.84 and 2.00 (2 x *q*, *J* = 6.5, 8 H); 2.80 (br. *s*, 8 H); 3.29-3.33 (br. 2 x *m*, 16 H); 3.71 (*s*, 12 H); 3.97 (*t*, *J* = 6.5, 4 H); 4.10 (*t*, *J* = 6.5, 4 H); 6.58 (*s*, 4 H); 7.02-7.05 (*m*, 4 H); 7.26 (*dd*, *J* = 8.9, 1.5, 2 H); 7.54 (*d*, *J* = 8.9, 2 H); 7.70 (*d*, *J* = 8.9, 2 H); 7.82 (*s*, 2 H). FAB MS: 1015 ([*M* - Cl]⁺, 30); 951 ([*M* - 2 Cl - Et]⁺, 100). Anal. calc. for C₆₂H₈₀Cl₂N₂O₈·8H₂O (1196.35): C 62.25, H 8.09, N 2.34; found: C 62.05, H 7.12, N 2.45.

1-Acetyl-4-[2-(6-hydroxy)naphthyl]-4-(4-hydroxy-3,5-dimethoxyphenyl)piperidine (11). To a suspension of 2,6-dimethoxyphenol (5.04 g, 0.033 mol) and **5** (2.77 g, 0.010 mol) in nitrobenzene (10 mL) was added BF₃·Et₂O (7.9 g, 0.056 mol), and the mixture was warmed to 80 °C for 5 h, then quenched at 20 °C with CH₃OH (5 mL) and H₂O (5 mL). Excess 2,6-dimethoxyphenol was distilled off *in vacuo* and the resulting brown oil triturated thoroughly with boiling Et₂O to give a grey solid which was adsorbed on SiO₂ (12 g) from acetone solution. Chromatography on SiO₂ (400 mL, CHCl₃/CH₃OH 95:5) gave **11** (2.05 g, 48%) as a yellow-orange foam. Analytical sample from toluene:

m.p. 243-245 °C. IR (KBr): 1600 (C=O). ¹H NMR (500 MHz, CD₃COCD₃): 2.12 (*s*, 3 H); 2.35-2.50 (br. 2 x *m*, 4 H); 3.54-3.70 (br. 2 x *m*, 4 H); 3.80 (*s*, 6 H); 6.47 (*s*, 2 H); 7.10 (*s*, 1 H); 7.11 (*dd*, *J* = 8.6, 2.5, 1 H); 7.20 (*dd*, *J* = 8.9, 2.5, 1 H); 7.57 (*d*, *J* = 8.9, 1 H); 7.60 (*d*, *J* = 2, 1 H); 7.69 (*d*, *J* = 8.6, 1 H). MS (EI, 20 eV): 421 (*M*⁺, 100). Anal. calc. for C₂₅H₂₇NO₅ (421.49): C 71.24, H 6.46, N 3.32; found: C 71.34, H 6.44, N 3.33.

1-Acetyl-4-[2-(6-methoxy)naphthyl]-4-(3,4,5-trimethoxyphenyl)piperidine (12). A mixture of **11** (0.52 g, 1.20 mmol), CH₃I (8 g, 0.06 mol) and K₂CO₃ (7 g, 0.05 mol) in acetone (100 mL) was refluxed overnight, filtered, and evaporated to give a thick oil. Dissolution in CH₂Cl₂ and filtration through a pad of SiO₂ (CH₂Cl₂/CH₃OH 95:5) gave **12** (0.50 g, 90%) as a clear-yellow-orange oil. IR (CDCl₃): 1700 (C=O). ¹H NMR (500 MHz, CDCl₃): 2.18 (*s*, 3 H); 2.30-2.60 (br. 2 x *m*, 4 H); 3.50-3.60 (br. *m*, 4 H); 3.77 (*s*, 6 H); 3.80 (*s*, 3 H); 3.91 (*s*, 3 H); 6.47 (*s*, 2 H); 7.09 (*d*, *J* = 2.5, 1 H); 7.15 (*dd*, *J* = 8.9, 2.5, 1 H); 7.28 (*dd*, *J* = 8.7, 1.9, 1 H); 7.66 (*d*, *J* = 8.7, 1 H); 7.67 (*d*, *J* = 2, 1 H); 7.71 (*d*, *J* = 8.9, 1 H). FAB HR-MS: 450.2269 ([*M* + H]⁺, C₂₇H₃₂NO₅), calc. 450.2280.

1-Ethyl-4-[2-(6-methoxy)naphthyl]-4-(3,4,5-trimethoxyphenyl)piperidine (13). A solution of **12** (0.50 g, 1.10 mmol) and 1 M BH₃·THF (10 mL, 10 mmol) in THF (100 mL) was refluxed for 6 h, quenched with CH₃OH, and evaporated. The resulting thick oil was refluxed for 2 h in abs. EtOH/conc. aq. H₂SO₄ 96:4 (50 mL), after which the solution was neutralized with 2 N NaOH and evaporated. The residue was partitioned between CHCl₃ (200 mL) and 2 N NaOH (200 mL), the organic layer dried (Na₂SO₄), and evaporated. Chromatography on SiO₂ (100 mL, EtOAc/NEt₃ 96:4) gave **13** (0.28 g, 58%) as a thick clear oil. ¹H NMR (500 MHz, CDCl₃): 1.07 (*t*, *J* = 7.2, 3 H); 2.34 (*q*, *J* = 7.2, 2 H); 2.39-2.70 (*m*, 8 H); 3.74 (*s*, 6 H); 3.81 (*s*, 3 H); 3.91 (*s*, 3 H); 6.48 (*s*, 2 H); 7.09 (*d*, *J* = 2.5, 1 H); 7.14 (*dd*, *J* = 8.9, 2.5, 1 H); 7.31 (*dd*, *J* = 8.7, 1.9, 1 H); 7.65 (*d*, *J* = 8.7, 1 H); 7.69 (*s*, 1 H); 7.71 (*d*, *J* = 8.9, 1 H). FAB HR-MS: 436.2494 ([*M* + H]⁺, C₂₇H₃₄NO₄), calc. 436.2488.

1,1-Diethyl-4-[2-(6-methoxy)naphthyl]-4-(3,4,5-trimethoxyphenyl)piperidinium chloride (10). A solution of **13** (0.25 g, 0.57 mmol), EtI (10 mL) and K₂CO₃ (≈ 5 mg) in CHCl₃ (10 mL) was stirred in the dark at 20 °C for 4 d, then diluted to 75 mL with CHCl₃. The mixture was washed with 1 N HCl (25 mL), dried (Na₂SO₄), and evaporated to give a thick oil which was dissolved in CH₃OH/H₂O 4:1 and run through an anion exchange column (Cl⁻) with CH₃OH/H₂O as the eluent: 0.25 g (87%) of **10** which was recrystallized from Et₂O/CHCl₃ 4:1 to give a fluffy white powder: m.p. 136-139 °C. ¹H NMR (500 MHz, CDCl₃): 1.36 and 1.42 (2 x *t*, *J* = 7.2, 6 H); 2.75-2.90 (br. *m*, 4 H); 3.50-3.80 (br. 2 x *m*, 8 H); 3.80 (*s*, 3 H); 3.81 (*s*, 6 H); 3.91 (*s*, 3 H); 6.47 (*s*, 2 H); 7.09 (*d*, *J* = 2.5, 1 H); 7.19 (*dd*, *J* = 8.9, 2.5, 1 H); 7.25 (*dd*, *J* = 9, 1.9, 1 H); 7.69 (*s*, 1 H); 7.70 (*d*, *J* = 8.9, 1 H); 7.75 (*d*, *J* = 8.9, 1 H). FAB HR-MS: 464.2783 ([*M* - Cl]⁺, C₂₉H₃₈NO₄), calc. 464.2801.

1-Acetyl-4-[2-[6-(4-chlorobutoxy)]naphthyl]-4-(4-hydroxy-3-methoxyphenyl)piperidine (14). A solution of **6** (59.8 g, 0.167 mol), guaiacol (124.7 g, 1.005 mol) and freshly distilled BF₃·Et₂O (166.4 g, 1.172 mol) in dry CH₂Cl₂ (450 mL) was stirred at 20 °C for 9 d. After addition of CH₃OH (150 mL) and H₂O (1 L), the organic layer was separated and washed with H₂O (2 x 1 L) and sat. aq. NaCl (1 x 1 L). The combined aqueous extracts were washed with CH₂Cl₂ (2 x 500 mL) and the combined organic layers dried (Na₂SO₄) and evaporated *in vacuo*. Excess guaiacol was removed

by vacuum distillation (pot temperature < 100 °C) and flash chromatography (4 kg SiO₂, CH₂Cl₂/hexane/Et₂O 2:1:1 followed by CH₂Cl₂/Et₂O 1:1) provided **14** (72.6 g, 90%) as a white foam. Analytical sample from hexane/CH₂Cl₂ 5:1: m.p. 172-174 °C. IR (CCl₄): 3556 (O–H), 1651 (C=O). ¹H NMR (500 MHz, CDCl₃): 2.00 (*m*, 4 H); 2.10 (*s*, 3 H); 2.36-2.50 (br. 2 x *m*, 4 H); 3.49-3.74 (br. 2 x *m*, 4 H); 3.64 (*t*, *J* = 6.1, 2 H); 3.77 (*s*, 3 H); 4.09 (*t*, *J* = 5.5, 2 H); 5.76 (br. *s*, 1 H); 6.71 (*d*, *J* = 2.1, 1 H); 6.79 (*dd*, *J* = 8.4, 2.1, 1 H); 6.84 (*d*, *J* = 8.4, 1 H); 7.06 (*d*, *J* = 2.5, 1 H); 7.13 (*dd*, *J* = 9.0, 2.5, 1 H); 7.24 (*dd*, *J* = 8.8, 1.9, 1 H); 7.62 (*d*, *J* = 8.8, 1 H); 7.64 (*s*, 1 H); 7.69 (*d*, *J* = 9.0, 1 H). MS (EI, 70 eV): 481.2 (*M*⁺, 100). Anal. calc. for C₂₈H₃₂ClNO₄ (482.02): C 69.77, H 6.69, N 2.91, O 13.28, Cl 7.36; found: C 69.80, H 6.61, N 2.85, O 13.09, Cl 7.25.

1-Acetyl-4-{2-[6-(4-chlorobutoxy)]naphthyl}-4-(3-bromo-4-hydroxy-5-methoxy phenyl)piperidine (15). A solution of **14** (72.6 g, 0.151 mol) in dry CH₂Cl₂/CH₃OH (1.8 L, 100:1) was cooled to - 50 °C with a cryostat in an acetone bath. A catalytic amount of NaH (60% dispersion in oil, 100 mg) was added followed by recrystallized NBS (26.81 g, 0.151 mol) and the solution stirred at - 50 °C until all NBS was dissolved (8 h). After warming to 20 °C, water (1 L) was added and the organic layer separated and washed with H₂O (2 x 1 L) and sat. aq. NaCl (1 L). The combined aqueous layers were washed with CH₂Cl₂ (500 mL) and the combined organic layers dried (Na₂SO₄). Solvent removal *in vacuo* gave a brown foam which was filtered through a plug of flash SiO₂ (500 g, CH₂Cl₂/Et₂O 1:1). Flash chromatography (10 kg SiO₂, EtOAc followed by 10 kg SiO₂, CH₂Cl₂/CH₃OH 100:1.25) afforded **15** (49.8 g, 59%). Analytical sample from hexane/CH₂Cl₂ 5:1: m.p. 103-105 °C. IR (CCl₄): 3542 (O–H), 1652 (C=O). ¹H NMR (360 MHz, CDCl₃): 2.01 (*m*, 4 H); 2.10 (*s*, 3 H); 2.33-2.48 (br. 2 x *m*, 4 H); 3.50-3.79 (br. 2 x *m*, 4 H); 3.66 (*t*, *J* = 6.2, 2 H); 3.77 (*s*, 3 H); 4.10 (*t*, *J* = 5.6, 2 H); 5.95 (br. *s*, 1 H); 6.63 (*d*, *J* = 2.1, 1 H); 7.02 (*d*, *J* = 2.1, 1 H); 7.08 (*d*, *J* = 2.5, 1 H); 7.15 (*dd*, *J* = 8.9, 2.5, 1 H); 7.22 (*dd*, *J* = 8.8, 1.9, 1 H); 7.64 (*d*, *J* = 8.8, 1 H); 7.65 (*s*, 1 H); 7.71 (*d*, *J* = 8.9, 1 H). MS (EI, 70 eV): 561.1 (*M*⁺). Anal. calc. for C₂₈H₃₁BrClNO₄ (560.92): C 59.96, H 5.57, N 2.50, O 11.41, Cl 6.32, Br 14.25; found: C 59.87, H 5.52, N 2.41, O 11.47, Cl 6.54, Br 14.24.

1,1''-Diacetyl-18,37'-dibromo-40',44'-dimethoxy-dispiro[piperidine-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.13,7.16,10.122,26.125,29]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21',4''-piperidine] (16). A solution of **15** (17.31 g, 0.031 mol) and Cs₂CO₃ (62.0 g, 0.190 mol) in CH₃CN (3.5 L) was heated to reflux for 3 d. Filtration and evaporation *in vacuo* was followed by dissolution of the crude product in CH₂Cl₂ (600 mL), and the organic solution was extracted with H₂O (2 x 600 mL) and sat. aq. NaCl (600 mL), dried (Na₂SO₄), and evaporated *in vacuo*. Flash chromatography on SiO₂ (1.2 kg, CH₂Cl₂/CH₃OH 100:1) afforded **16** (6.79 g, 42%) of which an analytical sample was recrystallized from toluene: m.p. > 300 °C (dec.). IR (CCl₄): 1631 (C=O). ¹H NMR (360 MHz, CDCl₃): 1.99 (*m*, 8 H); 2.08 (*s*, 6 H); 2.28-2.47 (br. 2 x *m*, 8 H); 3.43-3.89 (br. 2 x *m*, 8 H); 3.62 (*s*, 6 H); 3.97 (*t*, *J* = 5.8, 4 H); 4.17 (*t*, *J* = 6.4, 4 H); 6.55 (*d*, *J* = 2.0, 2 H); 6.99 (2 x *s*, 4 H); 7.09 (2 x *dd*, *J* = 8.9, 2.0, 4 H); 7.52 (*d*, *J* = 8.9, 2 H); 7.62 (*s*, 2 H); 7.63 (*d*, *J* = 8.9, 2 H). FAB MS: 1049.4 ([*M* + H]⁺). Anal. calc. for C₅₆H₆₀Br₂N₂O₈ (1048.91): C 64.13, H 5.77, N 2.67, O 12.20, Br 15.24; found: C 64.06, H 5.90, N 2.74, O 12.42, Br 14.92.

1,1''-Diacetyl-40',44'-dimethoxy-dispiro[piperidine-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.1^{3,7}.1^{6,10}.1^{22,26}.1^{25,29}]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21',4''-piperidine]-18',37'-dicarbonitrile (17). A solution of **16** (8.64 g, 8.24 mmol) and CuCN (1.55 g, 17.3 mmol) in *N*-methylpyrrolidone (45 mL) was heated to 190 °C for 14 h. After cooling, the reaction mixture was poured into CH₂Cl₂ (500 mL) and conc. aq. NH₄OH (300 mL) was added. After stirring at 20 °C for 3 h, the blue aqueous layer was separated and exhaustively extracted with CH₂Cl₂ (6 x 100 mL). The combined organic layers were dried (Na₂SO₄) and evaporated *in vacuo*. Flash chromatography on SiO₂ (3 kg, CH₂Cl₂/CH₃OH 50:1) afforded **17** (6.58 g, 85%) as a white solid. Analytical sample from *p*-xylene/CH₂Cl₂ 5:1: m.p. > 300 °C. IR (CHCl₃): 1632 (C=O). ¹H NMR (360 MHz, CDCl₃): 1.98 (*m*, 8 H); 2.09 (*s*, 6 H); 2.27-2.53 (*br. 2 x m*, 8 H); 3.41-3.99 (*br. 2 x m*, 8 H); 3.69 (*s*, 6 H); 4.16 (*br. m*, 8 H); 6.88 (*s*, 2 H); 6.93 (*s*, 2 H); 7.03 (*s*, 2 H); 7.05 (*dd*, *J* = 8.9, 1.7, 2 H); 7.11 (*dd*, *J* = 9.0, 2.4, 2 H); 7.57 (*d*, *J* = 9.0, 2 H); 7.64 (*s*, 2 H); 7.65 (*d*, *J* = 8.9, 2 H). FAB MS: 941.6 ([*M* + H]⁺). Anal. calc. for C₅₈H₆₀N₄O₈ (941.15): C 74.02, H 6.43, N 5.95; found: C 73.49, H 6.63, N 5.72.

1,1''-Diethyl-40',44'-dimethoxy-dispiro[piperidine-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.1^{3,7}.1^{6,10}.1^{22,26}.1^{25,29}]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21',4''-piperidine]-18',37'-dimethanamine (18). A solution of **17** (2.00 g, 2.13 mmol) and 1 M BH₃·THF (80 mL) was heated to reflux for 12 h, then carefully quenched with CH₃OH (100 mL). After evaporation *in vacuo*, EtOH/conc. aq. H₂SO₄ 100:3 (100 mL) was added and the mixture refluxed for 1 h. After cooling, 1 M aq. NaOH was added until basic to litmus and the ethanol removed *in vacuo*. The remaining aqueous slurry was extracted exhaustively with CH₂Cl₂, the organic layers combined, washed with sat. aq. NaCl, dried (Na₂SO₄), and evaporated *in vacuo* to give crude **18** (1.9 g, 95%) which was used in subsequent reactions without further purification. Analytical samples were obtained by BOC-protection (di-*t*-butyldicarbonate, CH₂Cl₂, 20 °C, 5 h), chromatography (SiO₂, CH₂Cl₂/NEt₃ 20:1), deprotection (CH₂Cl₂/CF₃COOH 1:1, 20 °C, 12 h), and recrystallization from hexane/CH₂Cl₂ 10:1: m.p. 120 °C (*dec.*). IR (film): 3369 (NH₂). ¹H NMR (500 MHz, CDCl₃): 1.04 (*t*, *J* = 7.1, 6 H); 1.69 (*br. s*, 4 H); 1.95 (*q*, *J* = 6.5, 4 H); 2.04 (*q*, *J* = 6.5, 4 H); 2.33 (*q*, *J* = 7.1, 4 H); 2.45-2.67 (*br. m*, 16 H); 3.60 (*s*, 6 H); 3.68 (*s*, 4 H); 3.96 (*t*, *J* = 6.3, 4 H); 4.13 (*t*, *J* = 6.3, 4 H); 6.56 (*s*, 2 H); 6.73 (*s*, 2 H); 6.97 (*s*, 2 H); 7.07 (*dd*, *J* = 9.0, 1.5, 2 H); 7.13 (*dd*, *J* = 8.8, 1.8, 2 H); 7.48 (*d*, *J* = 8.8, 2 H); 7.62 (*d*, *J* = 9.0, 2 H); 7.64 (*s*, 2 H). ¹³C NMR (125 MHz, CDCl₃): 12.08; 25.20; 25.88; 36.06; 42.66; 44.50; 50.23; 52.41; 55.66; 66.63; 71.75; 106.21; 110.68; 119.00; 119.84; 124.87; 126.71; 126.84; 128.59; 129.34; 132.59; 136.47; 141.80; 143.74; 144.03; 151.99; 156.84. FAB HR-MS: 921.6643 ([*M* + H]⁺, C₅₈H₇₂N₄O₆), calc. 921.5530.

***N,N'*-[(1,1''-Diethyl-40',44'-dimethoxydispiro[piperidine-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.1^{3,7}.1^{6,10}.1^{22,26}.1^{25,29}]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21',4''-piperidine]-18',37'-diyl)bis(methylene)]bis[2-dimethylamino)-acetamide (19).** Triphenylphosphine (0.356 g, 1.36 mmol), *N,N*-dimethylglycine (0.140 g, 1.36 mmol), NEt₃ (0.275 g, 2.72 mmol) and 2,2'-dithiodipyridine (0.298 g, 1.36 mmol) were added to CH₂Cl₂ (25 mL) followed by **18** (0.500 g, 0.54 mmol) in CH₂Cl₂, and the mixture was stirred at 20 °C for 16 h. Addition of 1 M aq. NaOH (50 mL), phase separation, extraction of the aqueous phase with CH₂Cl₂ (3 x 50 mL), combination of the organic

layers, washing with sat. aq. NaCl (100 mL), drying (Na₂SO₄) and evaporation *in vacuo* afforded a yellow oil. Flash chromatography on SiO₂ (75 g, CH₂Cl₂/NEt₃ 20:1) provided **19** (0.190 g, 32%) which, for analytics, was crystallized from Et₂O/THF 10:1: m.p. 218-220 °C (dec.). IR (CDCl₃): 1662 (C=O). ¹H NMR (500 MHz, CDCl₃): 1.05 (*t*, *J* = 7.2, 6 H); 1.95 (*m*, *J* = 6.4, 4 H); 2.03 (*m*, *J* = 6.5, 4 H); 2.06 (*s*, 12 H); 2.32 (*q*, *J* = 7.2, 4 H); 2.42-2.62 (br. 3 x *m*, 16 H); 2.81 (*s*, 4 H); 3.62 (*s*, 6 H); 3.95 (*t*, *J* = 6.4, 4 H); 4.12 (*t*, *J* = 6.5, 4 H); 4.37 (*d*, *J* = 5.8, 4 H); 6.59 (*d*, *J* = 2.0, 2 H); 6.74 (*d*, *J* = 2.0, 2 H); 6.96 (*d*, *J* = 2.4, 2 H); 7.05 (*dd*, *J* = 8.9, 2.5, 2 H); 7.11 (*dd*, *J* = 8.7, 1.7, 2 H); 7.30 (*t*, *J* = 5.8, 2 H); 7.47 (*d*, *J* = 8.7, 2 H); 7.61 (*d*, *J* = 8.9, 2 H); 7.62 (*s*, 2 H). FAB HR-MS: 1091.6643 ([*M* + H]⁺, C₆₆H₈₆N₆O₈), calc. 1091.6585. Anal. calc. for C₆₆H₈₆N₆O₈ (1091.46): C 72.63, H 7.94, N 7.70; found: C 72.77, H 7.83, N 7.64.

1,1,1'',1''-Tetraethyl-18',37'-[[[(ethyldimethylammonio)acetyl]amino]methyl]-40',44'-dimethoxy-dispiro[piperidine-4,2'-[11,16,30,35]tetraoxaheptacyclo[34.2.2.2^{17,20}.1^{3,7}.1^{6,10}.1^{22,26}.1^{25,29}]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21',4''-piperidinium]-tetrachloride (2). A solution of **19** (0.100 g, 0.092 mmol) in CHCl₃ was washed with 1 M aq. NaOH (10 mL) and dried (Na₂SO₄). Freshly distilled EtI (10 mL) was added and the mixture stirred in the dark at 20 °C for 4 d. Removal of the solvent *in vacuo* provided a white powder which was eluted through an anion exchange column (Cl⁻) with CH₃OH/deionized H₂O 1:1. The white product was recrystallized from Et₂O/CH₃OH 1:20, and drying *in vacuo* at 75 °C afforded **2** (0.078 g, 63%): m.p. 250 °C (dec.). IR (KBr): 1676 (C=O). ¹H NMR (500 MHz, CD₃OD): 1.05-1.43 (br. 2 x *m*, 18 H); 2.00-2.10 (2 x *m*, 8 H); 2.65-3.86 (br. 5 x *m*, 44 H); 3.85 (*s*, 6 H); 4.09 (br. *s*, 4 H); 4.25 (br. *s*, 4 H); 4.39 (br. *s*, 4 H); 6.90-7.00 (*m*, 4 H); 7.16 (br. *s*, 4 H); 7.31 (br. *s*, 2 H); 7.63 (br. *s*, 2 H); 7.84 (br. *s*, 2 H); 7.95 (br. *s*, 2 H). MS (MALDI-TOF): 1346.05 (*M*⁺, C₇₄H₁₀₆N₆O₈Cl₄), calc. 1346.68. Analysis obtained on the tetraammonium iodide before ion exchange: calc. for C₇₄H₁₀₆N₆O₈I₄·3 H₂O (1769.37): C 50.23, H 6.38, N 4.75; found: C 50.43, H 6.54, N 4.66.

Acknowledgement. This work was initially funded by the U.S. National Science Foundation and the U.S. Office of Naval Research and subsequently by ETH Zürich and Hoffmann-LaRoche. Seed funding for steroid complexation studies was provided by a Merck Sharp & Dohme Career Development Award. We thank Professor W. L. Jorgensen (Yale University) for valuable discussions and his continuing interest in this study.

REFERENCES

- [1] Diederich, F. *Cyclophanes*, Royal Society of Chemistry: Cambridge, 1991.
- [2] Seel, C.; Vögtle, F. *Angew. Chem.* **1992**, *104*, 542-563; *ibid. Int. Ed.* **1992**, *31*, 528-549.
- [3] Szejtli, J. *Cyclodextrin Technology*, Kluwer: Dordrecht, 1988.
- [4] Franke, J.; Vögtle, F. *Angew. Chem.* **1985**, *97*, 224-225; *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 219-221.
- [5] Kearney, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 9907-9919.

- [6] a) Kempfle, M. A.; Müller, R. F.; Palluk, R.; Winkler, H. A. *Biochim. Biophys. Acta* **1987**, *923*, 83-87; b) Yoshida, A.; Yamamoto, M.; Hirayama, F.; Uekama, K. *Chem. Pharm. Bull.* **1988**, *36*, 4075-4080; c) Králová, K.; Mitterhauszerová, L. *Pharmazie* **1989**, *44* (H.9), 623-625; d) Shimada, K.; Komine, Y.; Oe, T. *J. Liq. Chromatogr.* **1989**, *12*, 491-500; e) Loftsson, T.; Ólafsdóttir, B. J.; Fridriksdóttir, H. *Acta Pharm. Nord.* **1990**, *2*, 303-312; f) Liu, F.; Kildsig, D. O.; Mitra, A. K. *Pharm. Res.* **1990**, *7*, 869-873; g) Djedaïni, F.; Perly, B. *J. Pharm. Sci.* **1991**, *80*, 1157-1161; h) Claudy, P.; Létouffé, J. M.; Germain, P.; Bastide, J. P.; Bayol, A.; Blasquez, S.; Rao, R. C.; Gonzalez, B. *J. Therm. Anal.* **1991**, *37*, 2497-2506; i) Liu, F.; Kildsig, D. O.; Mitra, A. K. *Drug Dev. Ind. Pharm.* **1992**, *18*(15), 1599-1612; j) Albers, E.; Müller, B. W. *J. Pharm. Sci.* **1992**, *81*, 756-761; k) De Caprio, J.; Yun, J.; Javitt, N. B. *J. Lip. Res.* **1992**, *33*, 441-443; l) Chun, I. K.; Yun, D. S. *Int. J. Pharm.* **1993**, *96*, 91-103; m) Wang, Y.; Ikeda, T.; Ueno, A.; Toda, F. *Tetrahedron Lett.* **1993**, *34*, 4971-4974.
- [7] a) Pitha, J.; Anaissie, E. J.; Uekama, K. *J. Pharm. Sci.* **1987**, *76*, 788-790; b) Pitha, J.; Hoshino, T. *Int. J. Pharm.* **1992**, *80*, 243-251; c) Pitha, J.; Anand, R. *Antiviral Chem. Chemother.* **1993**, *4*, 65-66.
- [8] For a preliminary communication of parts of this work, see: Carcanague, D. R.; Diederich, F. *Angew. Chem.* **1990**, *102*, 836-838; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 769-771.
- [9] Kawakami, H.; Yoshino, O.; Odashima, K.; Koga, K. *Chem. Pharm. Bull.* **1985**, *33*, 5610-5613.
- [10] Kumar, S.; Schneider, H.-J. *J. Chem. Soc. Perkin Trans. 2*, **1989**, 245-250.
- [11] Murakami, Y.; Hayashida, O.; Ito, T.; Hisaeda, Y. *Chem. Letters* **1992**, 497-500.
- [12] Wilcox, C. S.; Webb, T. H.; Zawacki, F. J.; Glagovich, N.; Suh, H. *Supramol. Chem.* **1993**, *1*, 129-137.
- [13] Peterson, B. R.; Diederich, F. *Nature*, submitted.
- [14] Ross, R. *Nature* **1993**, *362*, 801-809.
- [15] a) Brown, M. S.; Goldstein, J. L. *Science* **1986**, *232*, 34-47; b) Brown, M. S.; Goldstein, J. L. *Sci. Am.* **1984**, *251*(5), 52-60.
- [16] a) Casdorff, H. R. in *Lipid Pharmacology*, Vol. 2(2), Paoletti, R., Glueck, C. J. (eds.), Academic Press: New York, 1976, pp 222-256; b) Subbiah, M. T. R.; Yunker, R. L.; Rymaszewski, Z.; Kottke, B. A.; Bale, L. K. *Biochim. Biophys. Acta* **1987**, *920*, 251-258.
- [17] Inhibition of endogenous cholesterol biosynthesis with HMG CoA-reductase inhibitors: Endo, A. *J. Med. Chem.* **1985**, *28*, 401-405.
- [18] Ferguson, S. B.; Seward, E. M.; Diederich, F.; Sanford, E. M.; Chou, A.; Inocencio-Szweda, P.; Knobler, C. B. *J. Org. Chem.* **1988**, *53*, 5593-5595.
- [19] MacroModel V4.0; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440-467. In a Monte Carlo search (AMBER*) in vacuo, 1000 conformations of **2** were generated.
- [20] Huisgen, R.; Sorge, G. *Liebigs Ann. Chem.* **1950**, *566*, 162-184.
- [21] Diederich, F. *Angew. Chem.* **1988**, *100*, 372-396; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 362-386.
- [22] Mukayama, T.; Matsueda, R.; Suzuki, M. *Tetrahedron Lett.* **1970**, 1901-1904.
- [23] Petti, M. A.; Shepodd, T. J.; Barrans, R. E., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1988**, *110*, 6825-6840.

- [24] a) Diederich, F.; Smithrud, D. B.; Sanford, E. M.; Wyman, T. B.; Ferguson, S. B.; Carcanague, D. R.; Chao, I.; Houk, K. N. *Acta Chem. Scand.* **1992**, *46*, 205-215. b) Smithrud, D. B.; Wyman, T. B.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5420-5426.
- [25] Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley: New York, 1980, 2nd. ed.
- [26] a) Smithrud, D. B.; Diederich, F. *J. Am. Chem. Soc.* **1990**, *112*, 339-343. b) Smithrud, D. B.; Sanford, E. M.; Chao, I.; Ferguson, S. B.; Carcanague, D. R.; Evanseck, J. D.; Houk, K. N.; Diederich, F. *Pure Appl. Chem.* **1990**, *62*, 2227-2236.
- [27] a) Ferguson, S. B.; Sanford, E. M.; Seward, E. M.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5410-5419; b) Klebe, G.; Diederich, F. *Philos. Trans. R. Soc. London A.* **1993**, *345*, 37-48.
- [28] Cromwell, W. C.; Byström, K.; Eftink, M. R. *J. Phys. Chem.* **1985**, *89*, 326-332.
- [29] Ref. [1], Chapter 7, pp 246-263.
- [30] a) Lumry, R.; Rajender, S. *Biopolymers* **1970**, *9*, 1125-1227; b) Searle, M. S., Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 10690-10697; c) Inoue, Y.; Liu, Y.; Tong, L.-H.; Shen, B.-J.; Jin, D.-S. *J. Am. Chem. Soc.* **1993**, *115*, 10637-10644.
- [31] Steroid self-aggregation: Haberland, M. E., Reynolds, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2313-2316.
- [32] Critical aggregation concentrations (cac): **22** does not aggregate up to 50 mM (¹H NMR) and **20g** has a cac of 8 mM (Hjelmeland, L. M.; Nebert, D. W.; Osborne, J. C., Jr. *Anal. Biochem.* **1983**, *130*, 72-82.
- [33] Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, VCH: Weinheim, 1988, 2. ed., pp 359-384.
- [34] Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442-6448.
- [35] a) Schneider, H.-J. *Angew. Chem.* **1991**, *103*, 1419-1439; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1417-1436; b) Schneider, H.-J.; Theis, I. *Angew. Chem.* **1989**, *101*, 757-759; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 753-755.
- [36] Engberts, J. B. F. N.; Blokzijl, W. *Angew. Chem.* **1993**, *105*, 1610-1648; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1545-1579.

(Received 15 March 1994)