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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

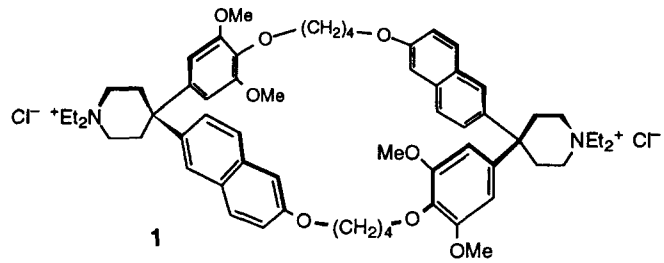
ABSTRACT *The synthesis, characterization, and steroid binding properties of two novel cyclophane receptors shaped by two naphthylphenylmethane spacers are reported. Cyclophane* ***1*** *forms inclusion complexes with bile acids, corticoids, and androgenic steroids in D_2O/CD_3OD 1:1. Specific functional 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,[6l,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 ^{1}H-NMR binding studies with a large variety of steroid substrates in aqueous solutions varying from D_2O/CD_3OD 1:1 to pure D_2O. 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.



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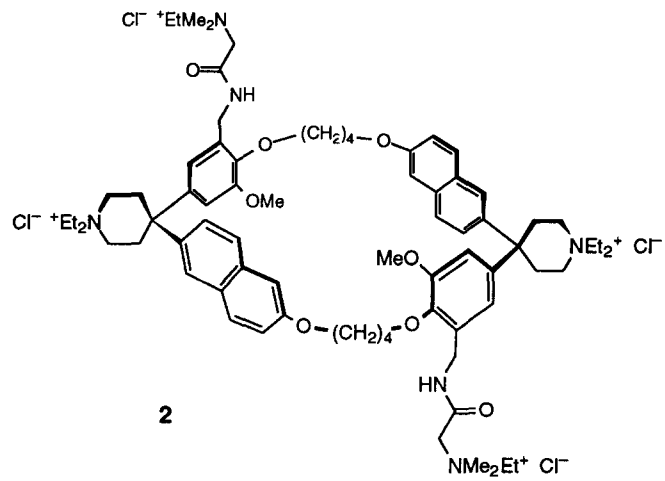

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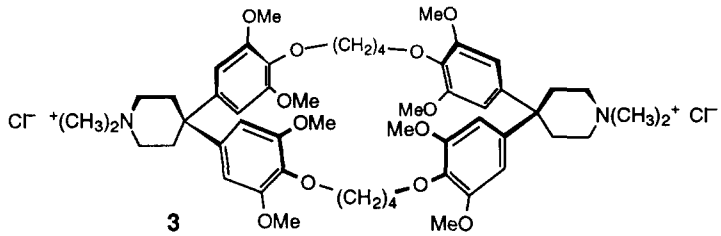
2. Design, Synthesis, and Solubility Properties of the Steroid Receptors 1 and 2. Tetraoxa[n.1.n.1]paracyclophanes such as **3** possess ideally sized apolar cavities for the tight inclusion complexation of flat aromatic guests. The distance between the two bridging O-atoms at one diphenylmethane unit 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**.



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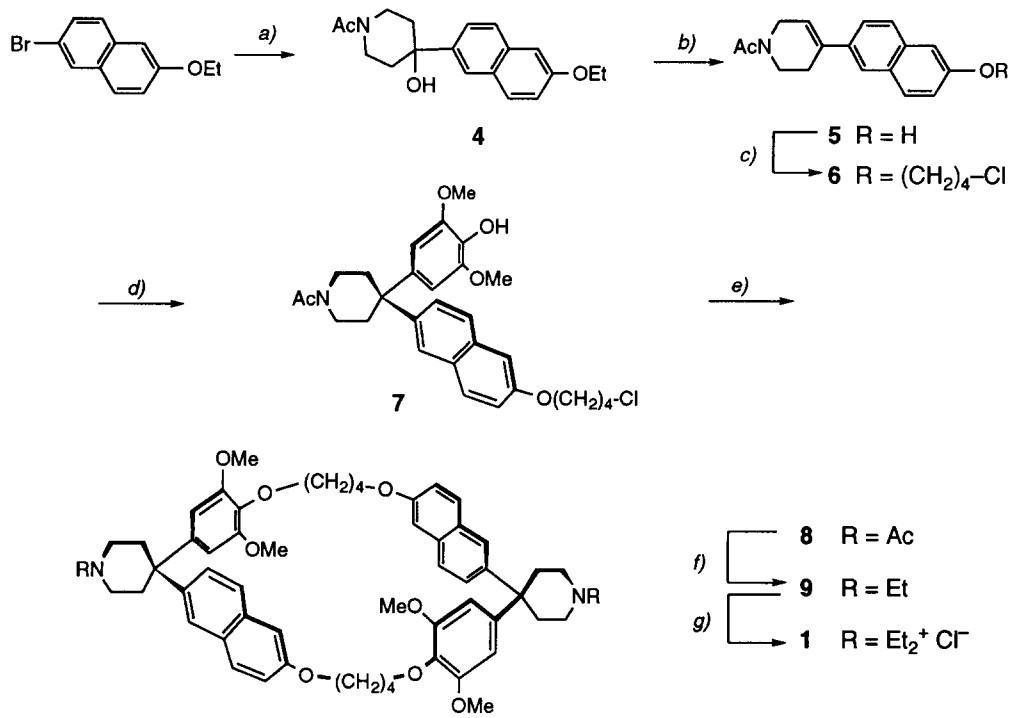
The synthesis of **1**, in a route which readily affords gram quantities, is shown in *Scheme 1*. The Grignard reagent prepared from 6-bromo-2-ethoxynaphthalene[20] was reacted with 1-acetyl-4-piperidone in tetrahydrofuran (THF) to afford the alcohol **4**. Treatment with an excess of BBr_3 effected 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 BF_3•Et_2O 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 D_2O/CD_3OD 1:1 at concentrations ≤ 6 x 10^{-3} M. No changes in ^1H NMR chemical shift indicative of aggregation[21] were observed in the concentration range from 8 x 10^{-4} M to 6 x 10^{-3} M, and the receptor concentration was kept in this range during subsequent binding studies.

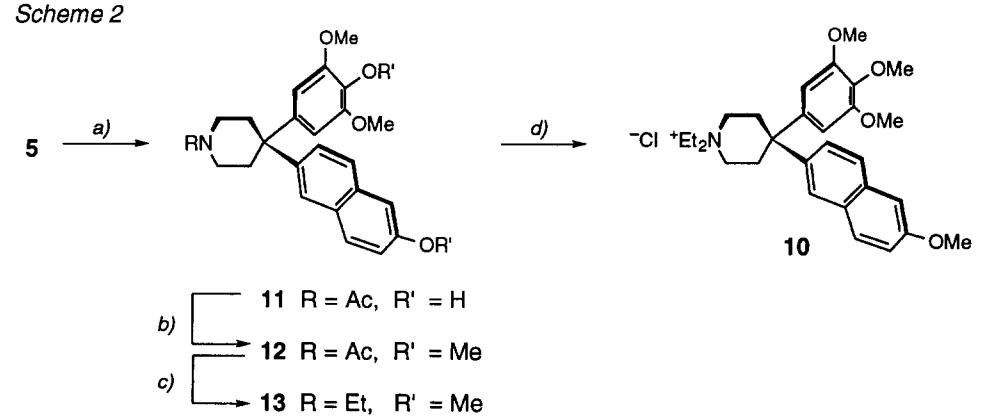
For the synthesis of cyclophane **2**, additional functionality needed to be introduced into the macrocyclic framework (*Scheme 3*). For this purpose, **6** was reacted with guaiacol to give **14**. Bromination with *N*-bromosuccinimide (NBS) at low temperature in the presence of base occurred selectively *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 BH_3•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**.

Scheme 1



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)* BF_3•Et_2O, 2,6-dimethoxyphenol, CH_2Cl_2, 20 °C, 5-8 d, 81%. *e)* Cs_2CO_3, DMF, 80 °C, 3 d, 25 %. *f)* BH_3•THF, reflux, 24 h, 60%. *g)* EtI, $CHCl_3$, 20 °C, 3.5 d, then ion exchange (Dowex, Cl^-), 93%.

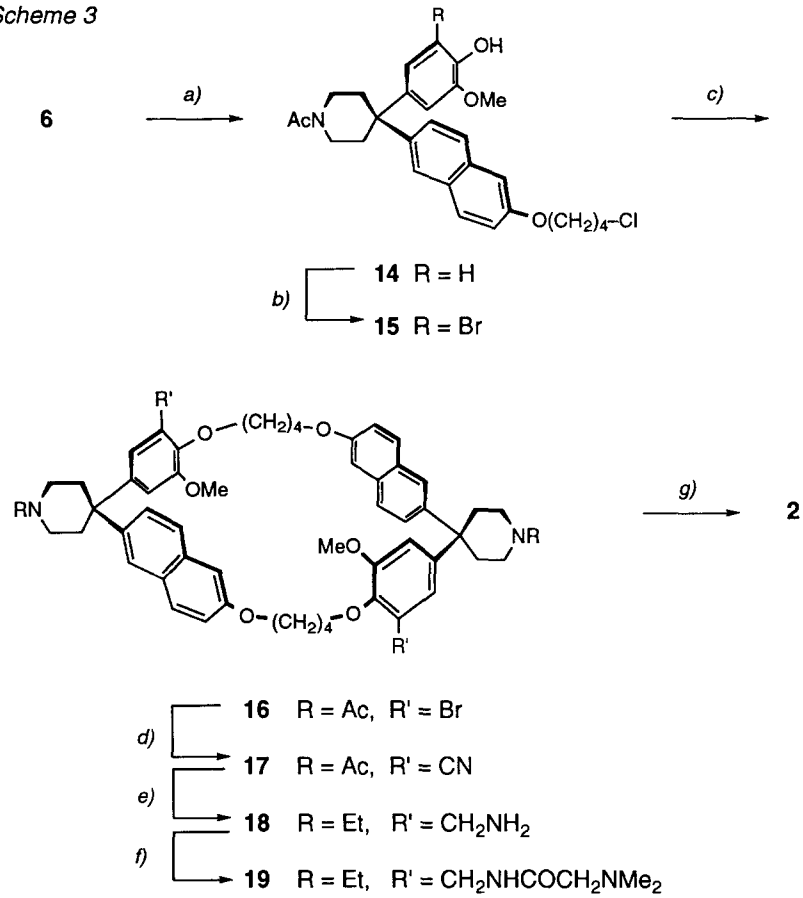
Scheme 2



a) BF_3•Et_2O, 2,6-dimethoxyphenol, nitrobenzene, 80 °C, 5 h, 48%. *b)* K_2CO_3, CH_3I, acetone, reflux, 14 h, 90%. *c)* BH_3•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 H_2O 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) BF_3•Et_2O, guaiacol, CH_2Cl_2, 20 °C, 9 d, 90%. *b)* NBS, CH_2Cl_2, CH_3OH, cat. NaH, − 50 °C, 8 h, 59%. *c)* Cs_2CO_3, CH_3CN, reflux, 3 d, 42%. *d)* CuCN, NMP, 190 °C, 14 h, 85%. *e)* BH_3•THF, reflux, 12 h, 95%. *f)* *N,N*-dimethylglycine, $(S(2\text{-pyr}))_2$, PPh_3, CH_2Cl_2, 20 °C, 16 h, 32%. *g)* EtI, $CHCl_3$, 20 °C, 4 d, then Dowex (Cl^-), 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 D_2O/CD_3OD 1:1 (v/v) at 293 K (*Table 1*), 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.

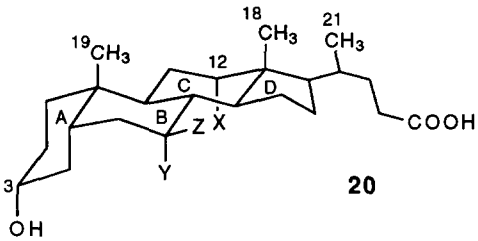
Table 1. Association constants K_a and binding free energies $-\Delta G^\circ$ for steroid complexes of cyclophane **1** in D_2O/CD_3OD 1:1 (v/v) at 293 K. The maximum observed upfield complexation-induced change in ^1H NMR chemical shift of the steroid methyl group protons $\Delta\delta_{max\ obs}$ and the change calculated for saturation binding $\Delta\delta_{sat}$ are shown.

Steroid	K_a [L mol^{-1}]	$-\Delta G^\circ$ [kcal mol^{-1}]	$\Delta\delta_{max\ obs}$ ($\Delta\delta_{sat}$)		
			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 Na_2CO_3.

High selectivity was observed in the complexation of bile acids. The complex of lithocholic acid (**20e**) is $\approx$ 2 kcal mol^{-1} more stable than the complex of deoxycholic acid (**20b**) which has an additional hydroxy group at C(12α). 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(12α), 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.



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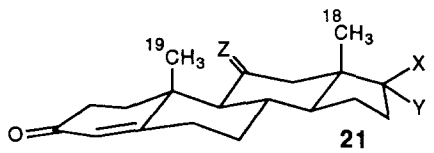
Steroid	X	Y	Z
a Cholic acid	OH	OH	H
b Deoxycholic acid	OH	H	H
c Chenodeoxycholic acid	H	OH	H
d Ursodeoxycholic acid	H	H	OH
e Lithocholic acid	H	H	H



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a Hydrocortisone	$COCH_2OH$	OH	αH,βOH
b Cortisone	$COCH_2OH$	OH	O
c Testosterone	OH	H	H_2

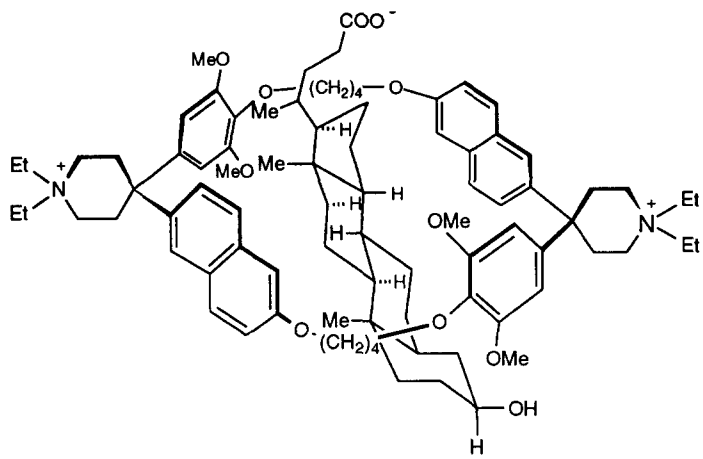


Figure 1: Schematic representation of the axial inclusion complex of lithocholic acid (**20e**)

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 L mol^{-1}, $-\Delta G^o$ = 2.77 kcal mol^{-1}) and camphor (K_a = 145 L mol^{-1}, $-\Delta G^o$ = 2.90 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 L mol^{-1}, $-\Delta G^o \approx$ 3.0 - 4.0 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 ^{1}H NMR binding titrations (*Table 2*) and evaluated the thermodynamic quantities ΔH^o and ΔS^o by van't Hoff analysis.

In D_2O/CD_3OD 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^o as a function of ΔS^o (*Fig. 2*).[24,30]

Table 2. Association constants K_a at various temperatures and binding free energies $-\Delta G^o$ (298 K) for 1:1 complexes of steroids and cyclophane **1** in D_2O/CD_3OD 1:1 and the thermodynamic quantities ΔH^o (kcal mol^{-1}) and ΔS^o (cal K^{-1} mol^{-1}) calculated from van't Hoff analysis.

Steroid	K_a (L mol^{-1}) [a]					$-\Delta G^o$	ΔH^o	ΔS^o
	298 K	303 K	308 K	313 K	318 K	kcal mol^{-1}	kcal mol^{-1}	cal K^{-1} mol^{-1}
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: ± 10%. [b] Solutions contain 0.01 M Na_2CO_3.

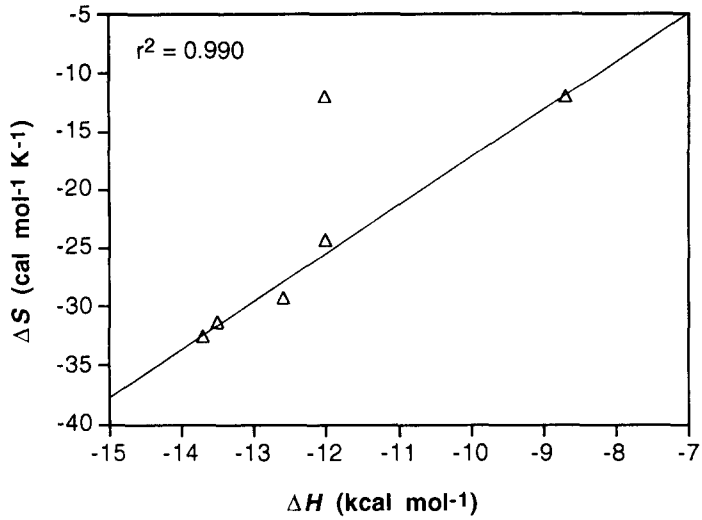


Figure 2. Isoequilibrium relationship between the changes in enthalpy and entropy for the complexation of steroids by **1** in D_2O/CD_3OD 1:1 (298 K, for data see *Table 2*).

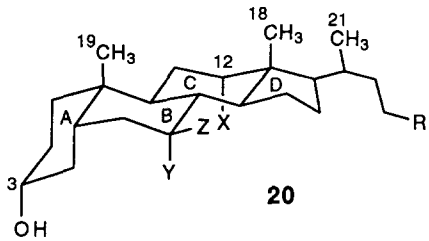
With its two additional cationic side chains, cyclophane **2** is highly soluble (≥ 10 mM) in pure D_2O, and this allowed us to perform steroid complexation studies in this environment. Unfortunately, most of the steroids are very insoluble in pure D_2O or their complexes with **2** precipitated out of solution, which limited the amount of studies that could be undertaken. A further limitation to quantitative ^1H NMR binding studies in homogenous D_2O-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 D_2O/CD_3OD 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 D_2O. The binding data with cyclophane **2** in pure D_2O 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 D_2O/CD_3OD 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.



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	X	Y	Z	R
f	H	OH	H	$CONHCH_2COO^-\ Na^+$
g	OH	H	OH	$CONH(CH_2)_3N^+(CH_3)_2-(CH_2)_3SO_3^-$

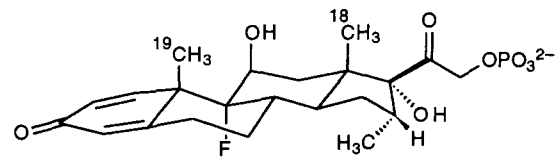

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Table 3. Association constants K_a and binding free energies $-\Delta G^o$ (298 K) for 1:1 complexes of steroids and cyclophanes **1** and **2** in D_2O and D_2O/CD_3OD mixtures (v/v). The maximum observed upfield complexation-induced change in ^1H NMR chemical shift of the steroid methyl group protons $\Delta\delta_{max\ obs}$ and the change calculated for saturation binding $\Delta\delta_{sat}$ are shown.

Run	Cyclo-phane	Steroid	Solvent	K_a [a] (L mol^{-1})	$-\Delta G^o$ (kcal mol^{-1})	$\Delta\delta_{max\ obs}$ ($\Delta\delta_{sat}$) CH_3 (19)	CH_3 (18)
1.	**2**	**21b**	D_2O/CD_3OD 1:1	310	3.40	0.61 (1.39)	0.13 (0.31)
2.			D_2O/CD_3OD 3:1	2260	4.58	[c]	0.18 (0.33)
3.			D_2O/CD_3OD 10:1	4960	5.04	[c]	0.22 (0.35)
4.			D_2O [b]	10000	5.5		
5.	**1**	**22**	D_2O/CD_3OD 1:1	660	3.85	0.45 (0.77)	0.14 (0.25)
6.	**2**	**22**	D_2O/CD_3OD 1:1	8040	5.33	[c]	0.24 (0.28)
7.	**2**	**22**	D_2O	24900	6.00	0.87 (1.57)	0.33 (0.41)
8.	**2**	**20f**	D_2O	37300	6.24	0.28 (0.35)	[c]
9.	**2**	**20g**	D_2O	no significant binding			

[a] Reproducibility in K_a ± 10%. [b] Determined by extrapolation from linear plot of $-\Delta G^o$ (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 D_2O/CD_3OD 1:1 to pure water. The complex

stability in pure D_2O ($K_a \approx 10^4$ L mol^{-1}, $-\Delta G^o$ = 5.5 kcal mol^{-1}) which, for solubility reasons could not be determined experimentally, was extrapolated from the linear plot of the binding free energy $-\Delta G^o$ 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 D_2O, 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 D_2O/CD_3OD 1:1 the complex of **2** with dexamethasone phosphate (**22**, K_a = 8040 L mol^{-1}, *run 6*) is 1.5 kcal mol^{-1} more stable than the corresponding complex of **1** (K_a = 660 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 D_2O to D_2O/CD_3OD 1:1, the stability of the complex of **2** with cortisone decreases by 2.1 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 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 D_2O/CD_3OD 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(12α) 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 D_2O/CD_3OD 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 D_2O and in D_2O/CD_3OD, 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). CH_2Cl_2 and CH_3CN were distilled from CaH_2 immediately prior to use. EtI was distilled at atmospheric pressure in a foil wrapped apparatus. $BF_3{\cdot}OEt_2$ was distilled from CaH_2 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 (^{13}C), 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 D_2O and 0.2-1.0 mM in D_2O/CD_3OD 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 CH_3-resonances ($\Delta\delta = \delta_{free} - \delta_{obs}$) was plotted against the host concentration, and quantitative binding numbers (K_a, $-\Delta G^o$, and $\Delta\delta_{sat}$) 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 $-\Delta G^o$ 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 H_2O/CH_3OH 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 D_2O/CD_3OD 1:1 (v/v) and studies with **2** were done in solvent mixtures varying from D_2O/CD_3OD 1:1 to pure D_2O. When guests bearing carboxylic acid groups were used, 0.01 M Na_2CO_3 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 I_2 were added to initiate the formation of the Grignard reagent. After a few minutes the yellow color of I_2 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. NH_4Cl (400 mL). The organic solvent was evaporated to give an aqueous slurry which was extracted with CH_2Cl_2 (1 L). The organic layer was washed with sat. aq. NaCl (2 x 1 L) and dried (Na_2SO_4). The residue obtained by evaporation of the solvent was washed with large portions of Et_2O and recrystallized from $(CH_3)_2CHOH$ to yield **4** (49 g, 50%) as a white powder. Analytical sample from toluene: m.p. 169-171 °C. IR ($CDCl_3$): 3580, 3200-3500 (O-H), 1620 (C=O). ^1H NMR (500 MHz, $CDCl_3$): 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 $C_{19}H_{23}NO_3$ (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). BBr_3 (57 mL, 0.60 mol) was slowly added at 20 °C to **4** (39.8 g, 0.127 mol) in CH_2Cl_2 (2 L), and the resulting green solution was refluxed for 2.5 h, allowed to cool to 20 °C, and quenched carefully with CH_3OH. Evaporation of the solvents gave a yellow slurry which was washed on a fritted glass funnel with generous portions of H_2O and Et_2O. 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, CD_3OD): 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*, $J \approx 3$, 2 H); 6.21 (br. *s*, 1 H); 7.04 (*dd*, J = 8.7, 2.5, 1 H); 7.06 (*d*, $J \approx 2.5$, 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^+, $C_{17}H_{17}NO_2$), 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 H_2O, sat. aq. NH_4Cl (100 mL) was added and the DMF removed *in vacuo*. The residue was suspended in CH_2Cl_2 (400 mL), the organic solution washed with sat. aq. NH_4Cl, dried (Na_2SO_4), and evaporated to give a viscous yellow oil. After removal of excess 1,4-dichlorobutane by destillation *in vacuo*, the crude product was filtered through a pad of SiO_2 (CH_2Cl_2/CH_3OH 95:5), the resulting solid washed with a small amount of Et_2O 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 ($CDCl_3$): 1625 (C=O). ^1H NMR (500 MHz, $CDCl_3$): 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^+, $C_{21}H_{24}ClNO_2$), 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 CH_2Cl_2 (175 mL) was added $BF_3{\cdot}Et_2O$ (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 CH_3OH (20 mL), then CH_2Cl_2 (300 mL), and H_2O (300 mL) was added. The organic phase was dried (Na_2SO_4) 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 SiO_2 (1 kg, CH_2Cl_2/CH_3OH 98:2) yielded **7** (32 g, 81%) as an off-white foam which was recrystallized from hexane/CH_2Cl_2 5:1 for analytical purposes: m.p. 139-141 °C. IR ($CDCl_3$): 3525 (O-H), 1620 (C=O). ^1H NMR (500 MHz, $CDCl_3$): 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 $C_{29}H_{34}ClNO_5$ (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}.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] (8). A mixture of **7** (20.5 g, 0.04 mol) and Cs_2CO_3 (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 CH_2Cl_2 (600 mL) and the solution washed with H_2O (2 x 400 mL), dried (Na_2SO_4), and evaporated to give a yellow-brown foam. Chromatography on SiO_2 (1 L, CH_2Cl_2/CH_3OH 97:3) afforded **8** (4.8 g, 25%) as a white solid. Analytical sample from EtOH/CH_2Cl_2 5:1: m.p. 308 °C (dec.). IR ($CDCl_3$): 1630 (C=O). ^1H NMR (500 MHz, $CDCl_3$): 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 $C_{58}H_{66}N_2O_{10}$ (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 BH_3·THF (52 mL, 52 mmol) was refluxed for 24 h, then quenched with CH_3OH and evaporated. The residue was refluxed for 1 h in abs. EtOH/conc. aq. H_2SO_4 7:3 (100 mL) and the solution neutralized with NaOH. The residue obtained after evaporation was partitioned between $CHCl_3$ (600 mL) and 1 N NaOH (600 mL). The aqueous layer was exhaustively extracted with $CHCl_3$ and the combined organic phases dried (Na_2SO_4) and evaporated, leaving a solid which was chromatographed on SiO_2 (600 mL, EtOAc/$CHCl_3$/NEt_3 55:37:8). The product was dissolved in hot $CHCl_3$ (300 mL) and the solution washed with 1 N NaOH (200 mL) and dried (Na_2SO_4). The solution was concentrated to 75 mL, and addition of Et_2O (800 mL) precipitated **9** (1.92 g, 60%) as a white crystalline solid. Analytical sample from CH_3OH/$CHCl_3$ 7:3: m.p. 248-250 °C. ^1H NMR (500 MHz, $CDCl_3$): 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 $C_{58}H_{70}N_2O_8$ (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 $CHCl_3$ (100 mL) was washed with 1 N NaOH (75 mL) and dried (Na_2SO_4). The drying agent was filtered off and washed with acid-free $CHCl_3$ (50 mL, prepared by passing through basic Al_2O_3, 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 CH_3OH (400 mL) and deionized H_2O (400 mL) passed through a column of anion exchange resin (Cl^-). Elution with CH_3OH/deionized H_2O 1:1 gave a white foam which was dried *in vacuo* and dissolved in CH_3OH (100 mL). The solution was concentrated to ≈ 70 mL, and addition of Et_2O 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, CD_3OD): 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 $C_{62}H_{80}Cl_2N_2O_8$·$8H_2O$ (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 BF_3·Et_2O (7.9 g, 0.056 mol), and the mixture was warmed to 80 °C for 5 h, then quenched at 20 °C with CH_3OH (5 mL) and H_2O (5 mL). Excess 2,6-dimethoxyphenol was distilled off *in vacuo* and the resulting brown oil triturated thoroughly with boiling Et_2O to give a grey solid which was adsorbed on SiO_2 (12 g) from acetone solution. Chromatography on SiO_2 (400 mL, $CHCl_3$/CH_3OH 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). ^{1}H NMR (500 MHz, CD_3COCD_3): 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*, $J \approx 2$, 1 H); 7.69 (*d*, J = 8.6, 1 H). MS (EI, 20 eV): 421 (M^+, 100). Anal. calc. for $C_{25}H_{27}NO_5$ (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), CH_3I (8 g, 0.06 mol) and K_2CO_3 (7 g, 0.05 mol) in acetone (100 mL) was refluxed overnight, filtered, and evaporated to give a thick oil. Dissolution in CH_2Cl_2 and filtration through a pad of SiO_2 (CH_2Cl_2/CH_3OH 95:5) gave **12** (0.50 g, 90%) as a clear-yellow-orange oil. IR ($CDCl_3$): 1700 (C=O). ^{1}H NMR (500 MHz, $CDCl_3$): 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*, $J \approx 2$, 1 H); 7.71 (*d*, J = 8.9, 1 H). FAB HR-MS: 450.2269 ([M + H]$^+$, $C_{27}H_{32}NO_5$), 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 BH_3·THF (10 mL, 10 mmol) in THF (100 mL) was refluxed for 6 h, quenched with CH_3OH, and evaporated. The resulting thick oil was refluxed for 2 h in abs. EtOH/conc. aq. H_2SO_4 96:4 (50 mL), after which the solution was neutralized with 2 N NaOH and evaporated. The residue was partitioned between $CHCl_3$ (200 mL) and 2 N NaOH (200 mL), the organic layer dried (Na_2SO_4), and evaporated. Chromatography on SiO_2 (100 mL, EtOAc/NEt_3 96:4) gave **13** (0.28 g, 58%) as a thick clear oil. ^{1}H NMR (500 MHz, $CDCl_3$): 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]$^+$, $C_{27}H_{34}NO_4$), 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 K_2CO_3 (≈ 5 mg) in $CHCl_3$ (10 mL) was stirred in the dark at 20 °C for 4 d, then diluted to 75 mL with $CHCl_3$. The mixture was washed with 1 N HCl (25 mL), dried (Na_2SO_4), and evaporated to give a thick oil which was dissolved in CH_3OH/H_2O 4:1 and run through an anion exchange column (Cl^-) with CH_3OH/H_2O as the eluent: 0.25 g (87%) of **10** which was recrystallized from Et_2O/$CHCl_3$ 4:1 to give a fluffy white powder: m.p. 136-139 °C. ^{1}H NMR (500 MHz, $CDCl_3$): 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*, $J \approx 9$, 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]$^+$, $C_{29}H_{38}NO_4$), 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 BF_3·Et_2O (166.4 g, 1.172 mol) in dry CH_2Cl_2 (450 mL) was stirred at 20 °C for 9 d. After addition of CH_3OH (150 mL) and H_2O (1 L), the organic layer was separated and washed with H_2O (2 x 1 L) and sat. aq. NaCl (1 x 1 L). The combined aqueous extracts were washed with CH_2Cl_2 (2 x 500 mL) and the combined organic layers dried (Na_2SO_4) and evaporated *in vacuo*. Excess guaiacol was removed

by vacuum destillation (pot temperature < 100 °C) and flash chromatography (4 kg SiO_2, CH_2Cl_2/hexane/Et_2O 2:1:1 followed by CH_2Cl_2/Et_2O 1:1) provided **14** (72.6 g, 90%) as a white foam. Analytical sample from hexane/CH_2Cl_2 5:1: m.p. 172-174 °C. IR (CCl_4): 3556 (O–H), 1651 (C=O). ^1H NMR (500 MHz, $CDCl_3$): 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 $C_{28}H_{32}ClNO_4$ (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 CH_2Cl_2/CH_3OH (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 H_2O (2 x 1 L) and sat. aq. NaCl (1 L). The combined aqueous layers were washed with CH_2Cl_2 (500 mL) and the combined organic layers dried (Na_2SO_4). Solvent removal *in vacuo* gave a brown foam which was filtered through a plug of flash SiO_2 (500 g, CH_2Cl_2/Et_2O 1:1). Flash chromatography (10 kg SiO_2, EtOAc followed by 10 kg SiO_2, CH_2Cl_2/CH_3OH 100:1.25) afforded **15** (49.8 g, 59%). Analytical sample from hexane/CH_2Cl_2 5:1: m.p. 103-105 °C. IR (CCl_4): 3542 (O–H), 1652 (C=O). ^1H NMR (360 MHz, $CDCl_3$): 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 $C_{28}H_{31}BrClNO_4$ (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}.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] (16). A solution of **15** (17.31 g, 0.031 mol) and Cs_2CO_3 (62.0 g, 0.190 mol) in CH_3CN (3.5 L) was heated to reflux for 3 d. Filtration and evaporation *in vacuo* was followed by dissolution of the crude product in CH_2Cl_2 (600 mL), and the organic solution was extracted with H_2O (2 x 600 mL) and sat. aq. NaCl (600 mL), dried (Na_2SO_4), and evaporated *in vacuo*. Flash chromatography on SiO_2 (1.2 kg, CH_2Cl_2/CH_3OH 100:1) afforded **16** (6.79 g, 42%) of which an analytical sample was recrystallized from toluene: m.p. > 300 °C (dec.). IR (CCl_4): 1631 (C=O). ^1H NMR (360 MHz, $CDCl_3$): 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 $C_{56}H_{60}Br_2N_2O_8$ (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]tetraoxa heptacyclo[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 CH_2Cl_2 (500 mL) and conc. aq. NH_4OH (300 mL) was added. After stirring at 20 °C for 3 h, the blue aqueous layer was separated and exhaustively extracted with CH_2Cl_2 (6 x 100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. Flash chromatography on SiO_2 (3 kg, CH_2Cl_2/CH_3OH 50:1) afforded **17** (6.58 g, 85%) as a white solid. Analytical sample from *p*-xylene/CH_2Cl_2 5:1: m.p. > 300 °C. IR ($CHCl_3$): 1632 (C=O). ^{1}H NMR (360 MHz, $CDCl_3$): 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 $C_{58}H_{60}N_4O_8$ (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]tetraoxahepta-cyclo[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 BH_3·THF (80 mL) was heated to reflux for 12 h, then carefully quenched with CH_3OH (100 mL). After evaporation *in vacuo*, EtOH/conc. aq. H_2SO_4 100:3 (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 CH_2Cl_2, the organic layers combined, washed with sat. aq. NaCl, dried (Na_2SO_4), 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, CH_2Cl_2, 20 °C, 5 h), chromatography (SiO_2, CH_2Cl_2/NEt_3 20:1), deprotection (CH_2Cl_2/CF_3COOH 1:1, 20 °C, 12 h), and recrystallization from hexane/CH_2Cl_2 10:1: m.p. 120 °C (dec.). IR (film): 3369 (NH_2). ^{1}H NMR (500 MHz, $CDCl_3$): 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). ^{13}C NMR (125 MHz, $CDCl_3$): 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]$^+$, $C_{58}H_{72}N_4O_6$), calc. 921.5530.

***N,N'*-[(1,1''-Diethyl-40',44'-dimethoxydispiro[piperidine-4,2'-[11,16,30,35]tetra oxaheptacyclo[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).** Triphenylposphine (0.356 g, 1.36 mmol), *N,N*-dimethylglycine (0.140 g, 1.36 mmol), NEt_3 (0.275 g, 2.72 mmol) and 2,2'-dithiodipyridine (0.298 g, 1.36 mmol) were added to CH_2Cl_2 (25 mL) followed by **18** (0.500 g, 0.54 mmol) in CH_2Cl_2, 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 CH_2Cl_2 (3 x 50 mL), combination of the organic

layers, washing with sat. aq. NaCl (100 mL), drying (Na_2SO_4) and evaporation *in vacuo* afforded a yellow oil. Flash chromatography on SiO_2 (75 g, CH_2Cl_2/NEt_3 20:1) provided **19** (0.190 g, 32%) which, for analytics, was crystallized from Et_2O/THF 10:1: m.p. 218-220 °C (dec.). IR ($CDCl_3$): 1662 (C=O). ^1H NMR (500 MHz, $CDCl_3$): 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]$^+$, $C_{66}H_{86}N_6O_8$), calc. 1091.6585. Anal. calc. for $C_{66}H_{86}N_6O_8$ (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.$2^{17,20}$.$1^{3,7}$.$1^{16,10}$.$1^{22,26}$.$1^{25,29}$]hexatetraconta[3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43]hexadecaene-21',4''-piperidinium]-tetrachloride (2). A solution of **19** (0.100 g, 0.092 mmol) in $CHCl_3$ was washed with 1 M aq. NaOH (10 mL) and dried (Na_2SO_4). 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 CH_3OH/deionized H_2O 1:1. The white product was recrystallized from Et_2O/CH_3OH 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, CD_3OD): 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*$^+$, $C_{74}H_{106}N_6O_8Cl_4$), calc. 1346.68. Analysis obtained on the tetraammonium iodide before ion exchange: calc. for $C_{74}H_{106}N_6O_8I_4 \cdot 3\ H_2O$ (1769.37): C 50.23, H 6.38, N 4.75; found: C 50.43, H 6.54, N 4.66.

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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.; Létoffé, 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) Casdorph, 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 (^{1}H 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.

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