70. Dendrophanes: Novel Steroid-Recognizing Dendritic Receptors

Preliminary Communication

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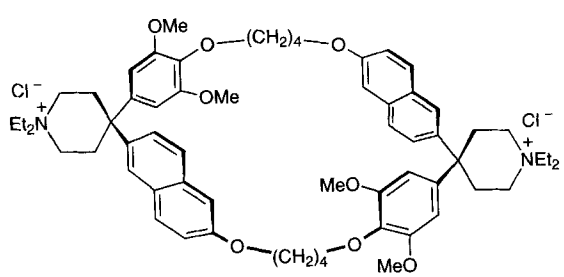
The H_2O-soluble *dendr*itic cyclo*phanes* (dendrophanes) **3–5** of first to third generation with molecular weights up to nearly 20 kD were synthesized, purified, and characterized. Cyclophane **2**, which served as the initiator core (generation zero), was prepared from tetrabromocyclophane **10** in a four-step sequence which involved as the first transformation a high-yielding, four-fold Pd(0)-catalyzed *Suzuki* cross-coupling reaction with 4-(benzyloxy)-phenyl-boronic acid to give **18**. The X-ray crystal-structure analysis of tetrabromocyclophane **10** displayed an open, nearly rectangular box with opposite aromatic walls being 8.3 and 11.4 Å apart and of suitable size for the incorporation of steroidal substrates. ^{1}H-NMR Binding titrations in borate-buffered D_2O/CD_3OD 1:1 showed that cyclophane-tetracarboxylate **2** forms 1:1 inclusion complexes with steroids (*Table 2*). Complexation was found to be enthalpically driven with higher binding affinities measured for the more apolar substrates. ^{1}H-NMR Titrations in the same solvent also provided clear evidence for core-selective complexation of testosterone (**21**) by the dendrophanes **3** (1st), **4** (2nd), and **5** (3rd generation) carrying up to 108 carboxylate surface groups. The stability of the corresponding 1:1 complexes was hardly affected by the size of the dendritic shell, although some generation-dependent conformational changes in the receptor cavity seemed to take place. Remarkably, host-guest exchange kinetics in all recognition processes were fast on the ^{1}H-NMR time scale.

Together with the naturally abundant cyclodextrins [1], cyclophanes do form the major part of synthetic receptors for inclusion complexation of apolar substrates [2]. Only a limited number of synthetic hosts are capable of steroid recognition [3] [4], a process of fundamental importance in biology [5]. Recent X-ray structural data for steroid-binding proteins, enzymes, and antibodies [6] revealed that natural receptors, similar to cyclophane hosts, prefer complexing the voluminous steroidal substrates in binding sites largely shaped by aromatic amino-acid side chains, thus taking advantage of favorable desolvation processes and apolar dispersion as well as polar CH···π interactions.



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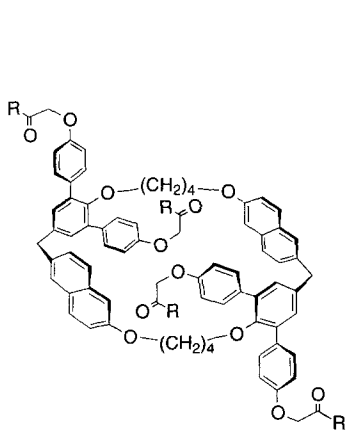
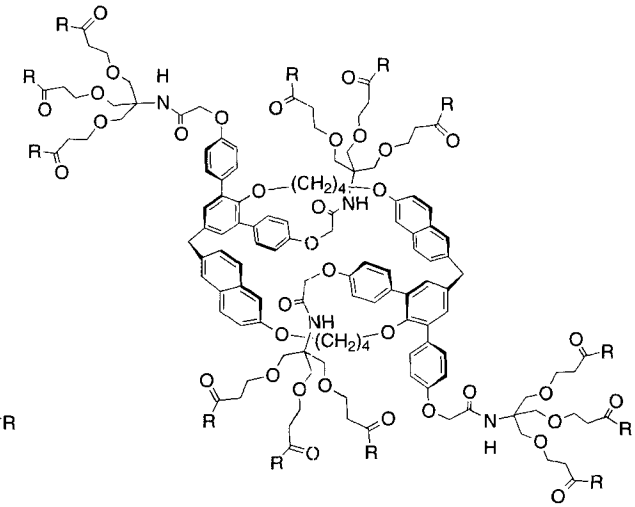
Cyclophane receptors such as **1**, prepared by bridging two naphthyl(phenyl)methane units, possess apolar, highly preorganized cavities and form stable 1:1 inclusion complexes with steroids in aqueous solutions [3a, d] [4a, b]. We became interested in attaching a protein-mimicking, surface-functionalized dendritic shell [7] to a similar cyclophane and to explore whether the newly constructed dendrophanes (*dendr*imer cyc*lophanes*) [8] would prefer specific, stoichiometric steroid complexation within the hydrophobic core over nonspecific, random incorporation of substrates within the dendritic branches [9]. Here we describe the synthesis of the novel core cyclophane **2** (generation zero), and of the H_2O-soluble dendrophanes **3**–**5** of first, second, and third generation, as well as preliminary ^{1}H-NMR studies demonstrating the steroid-binding properties of these macromolecules.



6 R = MeO
2 R = OH



7 R = MeO
3 R = OH
8 R = $(MeOOCCH_2CH_2OCH_2)_3CNH$
4 R = $(HOOCCH_2CH_2OCH_2)_3CNH$

The synthesis of compounds **2**–**9** started with the preparation of the novel tetrabromocyclophane **10** (*Scheme 1*)[1]). Grignard addition of silyl-protected **11**, prepared from 6-bromo-2-naphthol, to aldehyde **12** [10] yielded alcohol (±)-**13** which was reduced by catalytic hydrogenation [11] to the naphthyl(phenyl)methane derivative **14**. Regiospecific *ortho*-bromination at low temperature [12] gave phenol **15**, which was alkylated with 1,4-dichlorobutane to yield **16**. Removal of the silyl protecting group resulted in naphthol **17**, and macrocyclization of **17** gave the poorly soluble cyclophane **10**, which was prepared in multigram quantities.

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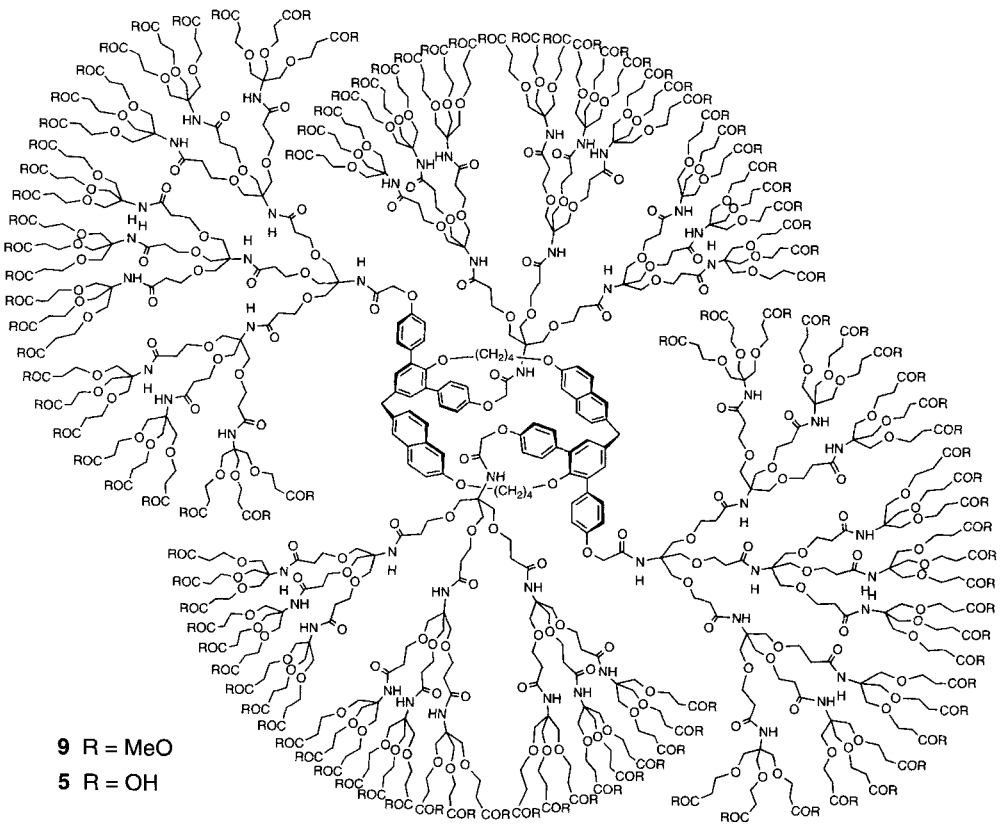
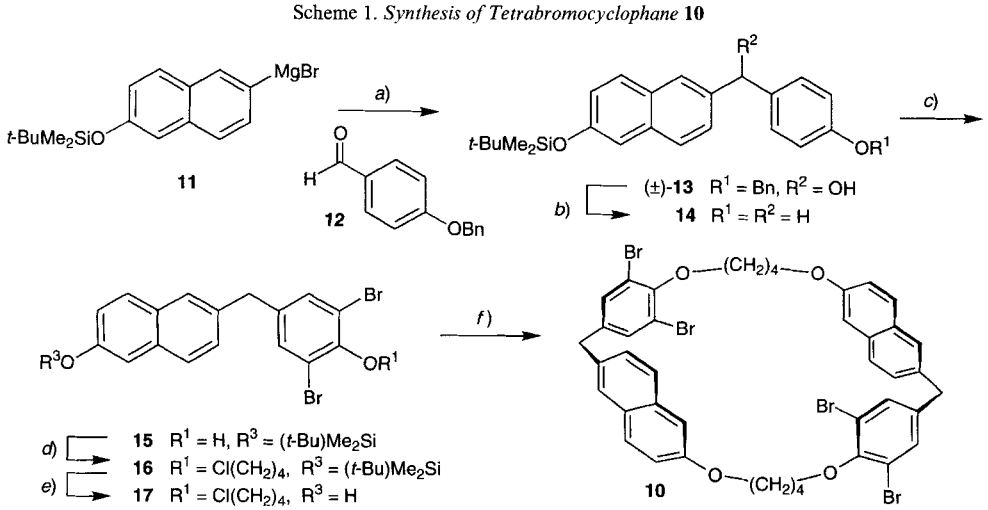
[1]) All new compounds were fully characterized spectroscopically (IR, ^{1}H- and ^{13}C-NMR, EI-, FAB-, or MALDI-TOF-MS) and by elemental analysis. The glassy dendrophanes **3**–**5** and **7**–**9** did not give correct elemental analyses due to solvent inclusion.



Scheme 1. *Synthesis of Tetrabromocyclophane* **10**



a) THF, 2 h. *b*) H_2, 10% Pd/C, MeOH, 6 d; 70% (from **11**). *c*) Br_2, *t*-$BuNH_2$, toluene, 4 h, −40°. *d*) $Cl(CH_2)_4Cl$, K_2CO_3, acetone, 2 d, reflux. *e*) Bu_4NF, THF, CH_2Cl_2, 10 min, 0°; 69% (from **14**). *f*) Cs_2CO_3, MeCN, 5 d, reflux, 17%.

Crystals of **10** were grown from toluene, and an X-ray crystal-structure analysis, the first one for a cyclophane made of two bridged naphthyl(phenyl)methane moieties, showed an open, nearly rectangular box of inversion symmetry with parallel, opposite arranged aromatic walls being 11.40 Å (C(1)···C(1a)) and 8.31 Å (C(11)···C(3a)) apart from each other (*Fig. 1*). Two molecules of toluene penetrate the cavity from opposite sides and, in an antiparallel offset arrangement, form π-π and CH···π interactions with the host. Typical contacts are observed between the toluene Me groups and the dibromophenylene moieties (*e.g.* C(24)···C(22a) = 3.70 Å), and between the toluene rings and the naphthalene protons (*e.g.* C(27)···H–C(10a) = 2.95 Å). The crystal packing of **10** revealed infinite molecular channels formed by stacking cyclophanes and occupied by toluene molecules as described[2]).

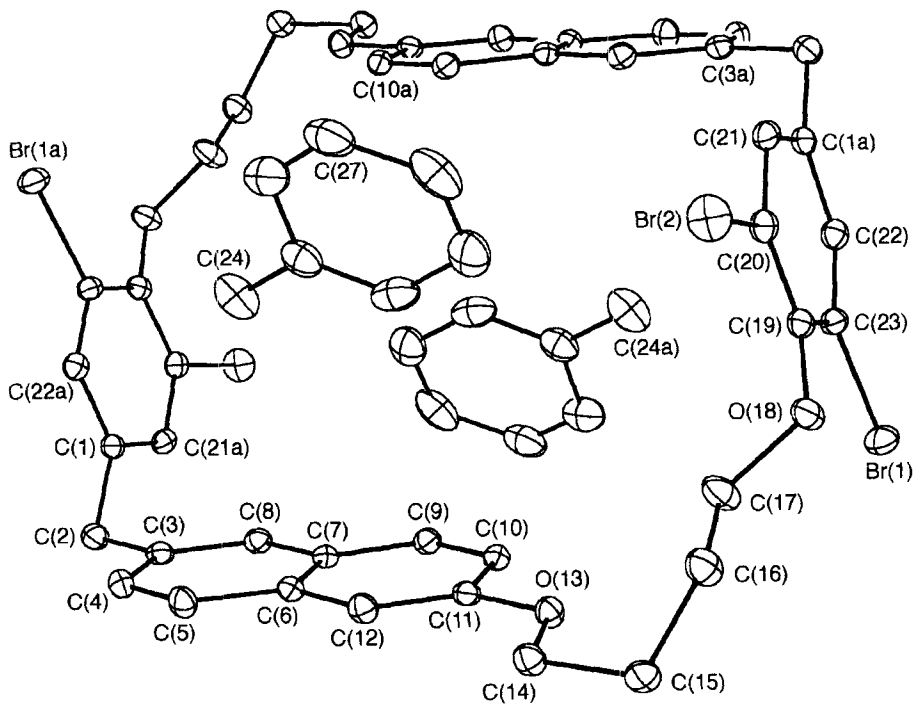


Fig. 1. *Molecular structure of* **10**. Arbitrary numbering; vibrational ellipsoids are shown at the 30% probability level.

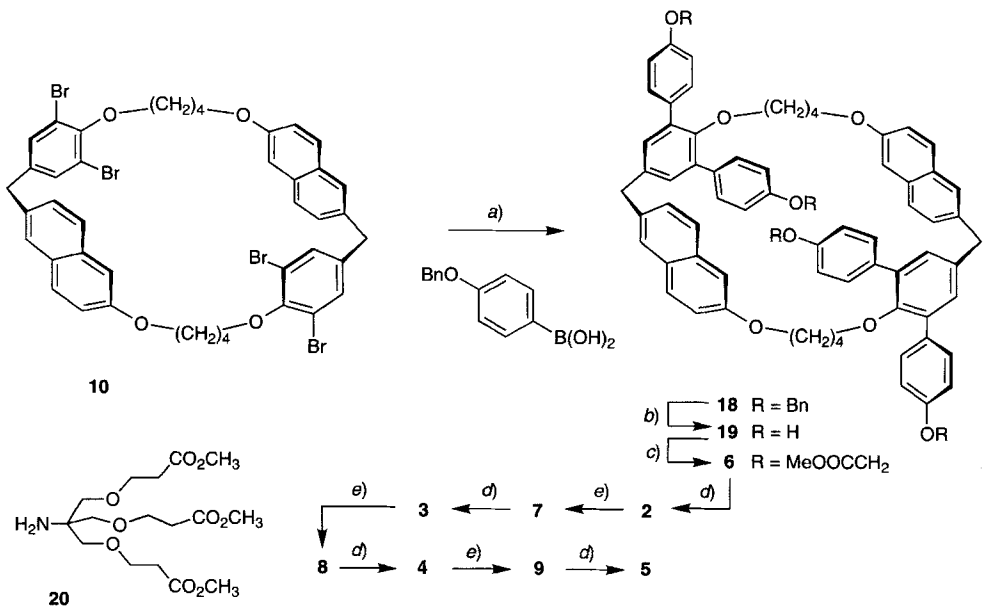
X-Ray Crystal-Structure Analysis of 18,37,40,44-Tetrabromo-11,16,30,35-tetraoxaheptacyclo[34.2.2.2^{17,20}.1^{3,7}.1^{16,10}.1^{22,26}.1^{25,29}]hexatetraconta-3,5,7(46),8,10(45),17,19,22,24,26(42),27,29(41),36,38,39,43-hexadecaene (**10**)·*2 Toluenes* ($1/2(C_{42}H_{36}Br_4O_4)\cdot(C_7H_7)$, M_r 554.3). Single crystals with linear dimensions of *ca.* 0.2 mm were grown from toluene over a period of 4 days. Triclinic space group $P\bar{1}$; $D_c = 1.51$ g cm^{-3}; $Z = 2$; $a = 10.029(2)$, $b = 10.520(3)$, $c = 12.590(3)$ Å; $\alpha = 78.26(2)$, $\beta = 80.42(2)$, $\gamma = 70.67(2)°$; $V = 1220$ Å^3; Nonius *CAD4* diffractometer; MoK_α radiation; $\lambda = 0.7107$ Å; $\theta \leq 27.0°$; $T = 230$ K. The structure was solved by direct methods and refined by full-matrix least-squares analysis (SHELXTL PLUS) using an isotropic extinction correction and an

[2]) A similar crystal-packing motif was also observed in the X-ray crystal structure of a 1:1 complex betweeen **10** and *p*-xylene and will be discussed in detail in an upcoming full paper by us (unpublished results).

exponentially modified weight factor $r = 5$ Å^2 (heavy atoms anisotropic, H-atoms isotropic, whereby H-positions are based on configurational considerations). $R(F) = 0.043$, $wR(F) = 0.050$ for 312 variables and 3702 observed reflections with $I > 2\sigma(I)$. Further details of the crystal-structure analyses of **10** are available on request from the Director of the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ (UK), on quoting the full journal citation.

Four-fold Pd(0)-catalyzed *Suzuki* cross-coupling [13] with 4-(benzyloxy)phenylboronic acid [14] gave **18** in high yield (77%) (*Scheme 2*). Hydrogenolysis to remove the benzyl protecting groups [15] provided tetraphenol **19** which was alkylated with methyl 2-bromoacetate in DMF to give tetraester **6**, and basic hydrolysis yielded the core cyclophane, tetraacid **2**. To construct the amidopolyether dendrophanes, the branching methodology introduced by *Newkome et al.* [16] was applied as described earlier [8] [17].

Scheme 2. *Synthesis of the Core Cyclophane* **2** *and the Water-Soluble Dendrophanes* **3**–**5**



a) [Pd(PPh$_3$)$_4$], toluene/EtOH/THF/H$_2$O, Na$_2$CO$_3$, 7 d, 80°; 77%. *b*) 10% Pd/C, (NH$_4$)HCO$_2$, THF, 30 min, reflux; 98%. *c*) BrCH$_2$CO$_2$Me, K$_2$CO$_3$, DMF, 2 d, 60°; 66%. *d*) LiOH, H$_2$O/THF/MeOH, 2 d, 25°; 99% (**2**); 99% (**3**); 94% (**4**); 90% (**5**). *e*) **20**, DCC (*N*,*N*′-dicyclohexylcarbodiimide), BtOH (1-hydroxy-1*H*-benzotriazol), THF; 3 d, 50°, 63% (**7**); 3 d, 25°, 92% (**8**); 3 d, 25°, 82% (**9**).

Tetraacid **2** was reacted with the branched monomer **20** [16] under classical peptide coupling conditions using *N*,*N*′-dicyclohexylcarbodiimide (DCC) [18] to give dodecaester **7**, and the ester groups were hydrolyzed leading to the H$_2$O-soluble dendrophane **3** of the first generation. Repetitive coupling and hydrolysis yielded the second-generation dendrophanes **8** and **4**, and, ultimately, the corresponding third-generation compounds **9** and **5**. The first-generation dodecaester **7** was crystalline and gave colorless needles

upon recrystallization from MeOH. All other dendrophanes were glassy compounds, and purification was best accomplished by repetitive preparative gel-permeation chromatography (GPC, *Biorad Biobeads SX-1* in toluene) at the stage of esters **8** and **9** to remove low-molecular-weight reagents and defect polymers. The corresponding H_2O-soluble carboxylates **3**–**5** were subsequently obtained in practically quantitative yields and not further purified[3]).

The purity of the dendrophanes **7**–**9** was confirmed by sharp GPC peaks (UV detection) as well as spectroscopically (*Table 1*). The ^{13}C-NMR spectra (125 MHz, 298 K) showed all and only the expected signals, fully resolved up to the second-generation dendrophanes. The ^{13}C-NMR spectrum of the third-generation ester **9** was consistent as well with the proposed structure and showed 33 of a total of 41 resonances. With the exception of two C-resonances belonging to the most inner part of the shell, all signals of the branches were found. The carbonyl C-atom resonances were resolved at δ 171.9

Table 1. *Selected Physical and Spectral Data of Dendrophanes* **7**–**9**[a])

7: Colorless, tender needles. M.p. 99.5–100.0° (MeOH). FT-IR ($CHCl_3$): 3006, 1736, 1679. ^{1}H-NMR (500 MHz, CD_2Cl_2): 1.45, 1.59 (2*m*, 8 H, $ArOCH_2(CH_2)_2CH_2$); 2.55 (*t*, *J* = 6.3, 24 H, $OCH_2CH_2CO_2Me$); 3.19, 3.41 (2*t*, *J* = 5.6, 8 H, $ArOCH_2(CH_2)_2CH_2$); 3.65 (*s*, 36 H, CO_2Me); 3.71 (*t*, *J* = 6.3, 24 H, $OCH_2CH_2CO_2Me$); 3.75 (*s*, 24 H, $NHC(CH_2OCH_2)_3$); 4.04 (*s*, 4 H, $ArCH_2Naph$); 4.43 (*s*, 8 H, $ArOCH_2CONH$); 6.71 (*d*, *J* = 2.3, 2 H, Naph); 6.86 (*s*, 4 H, zero-gen. NH); 6.88 (*dd*, *J* = 9.0, 2.3, 2 H, Naph); 7.00 (*d*, *J* = 8.9, 8 H, Ar); 7.16 (*s*, 4 H, Ar); 7.30 (*dd*, *J* = 8.5, 1.6, 2 H, Naph); 7.50 (*d*, *J* = 8.9, 8 H, Ar); 7.59 (*m*, 6 H, Naph). ^{13}C-NMR (125 MHz, CD_2Cl_2): 24.9; 25.3; 35.2; 42.0; 51.9; 60.1; 65.7; 67.3; 68.2; 69.5; 71.4; 106.6; 114.9; 119.4; 126.9; 127.3; 128.2; 129.1; 129.2; 130.5; 131.2; 133.0; 133.4; 135.7; 137.2; 138.1; 152.6; 157.12; 157.15; 168.0; 172.2. FAB-MS: 2655 (100, M^+, $^{13}C_2{}^{12}C_{136}H_{172}N_4O_{48}^+$; calc. 2655). Anal. calc. for $C_{138}H_{172}N_4O_{48}$ (2654.88): C 62.43, H 6.53; found: C 62.65, H 6.77.

8: Glassy compound. FT-IR ($CHCl_3$): 3005, 1733, 1672. ^{1}H-NMR (500 MHz, CD_2Cl_2): 1.43, 1.54 (2 br. *m*, 8 H, $ArOCH_2(CH_2)_2CH_2$); 2.41 (*t*, *J* = 6.4, 24 H, OCH_2CH_2CONH); 2.52 (*t*, *J* = 6.3, 72 H, $OCH_2CH_2CO_2Me$); 3.21, 3.37 (2 br. *m*, 8 H, $ArOCH_2(CH_2)_2CH_2$); 3.63–3.73 (*m*, 300 H, 1st- and 2nd-gen. $NHC(CH_2OCH_2)_3$, CO_2Me); 4.02 (br. *s*, 4 H, $ArCH_2Naph$); 4.47 (br. *s*, 8 H, $ArOCH_2CONH$); 6.14 (*s*, 12 H, 1st-gen. NH); 6.71 (br. *d*, *J* = 2.4, 2 H, Naph); 6.85 (*dd*, *J* = 8.9, 2.4, 2 H, Naph); 6.98 (*s*, 4 H, zero-gen. NH); 7.03 (*d*, *J* = 8.8, 8 H, Ar); 7.17 (*s*, 4 H, Ar); 7.31 (br. *d*, *J* ≈ 7, 2 H, Naph); 7.51 (*d*, *J* = 8.8, 8 H, Ar); 7.57 (*m*, 6 H, Naph). ^{13}C-NMR (125 MHz, CD_2Cl_2): 24.2; 24.9; 34.6; 37.2; 41.6; 51.4; 59.7; 59.8; 65.1; 66.8; 67.5; 67.6; 69.0; 69.1; 70.9; 106.1; 114.4; 118.9; 126.3; 126.9; 127.6; 128.6; 128.8; 130.0; 130.8; 132.5; 133.0; 135.3; 136.7; 137.5; 152.2; 156.6; 156.7; 167.4; 170.6; 171.9. MALDI-TOF-MS: 6846 (100, $[M + Na]^+$, $^{13}C_4C_{314}H_{472}N_{16}O_{144}\cdot Na^+$; calc. 6846).

9: Glassy compound. FT-IR ($CHCl_3$): 1732, 1670. ^{1}H-NMR (500 MHz, CD_2Cl_2): 1.35–1.45 (br. *s*, 8 H, $ArOCH_2(CH_2)_2CH_2$); 2.39 (br. *t*, *J* ≈ 7, 72 H, 2nd-gen. OCH_2CH_2CONH); 2.52 (br. *t*, *J* ≈ 7, 24 H, 1st-gen. OCH_2CH_2CONH); 2.54 (br. *t*, *J* ≈ 7, 216 H, $OCH_2CH_2CO_2Me$); 3.30–3.35 (2 br. *s*, 8 H, $ArOCH_2(CH_2)_2CH_2$); 3.63–3.73 (br. *m*, 948 H, 1st-, 2nd-, and 3rd-gen. $NHC(CH_2OCH_2)_3$, CO_2Me); 4.05 (br. *s*, 4 H, $ArCH_2Naph$); 4.47 (br. *s*, 8 H, $ArOCH_2CONH$); 6.23 (br. *s*, 36 H, 2nd-gen. NH); 6.44 (br. *s*, 12 H, 1st-gen. NH); 6.75–7.65 (*m*, 36 H, Naph, Ar, zero-gen. NH). ^{13}C-NMR (125 MHz, CD_2Cl_2)[b]): 34.6; 36.9; 37.0; 51.5; 59.7; 59.8; 66.7; 67.5; 67.8; 69.0; 69.1; 106.4; 114.8; 119.3; 126.5; 127.3; 127.9; 129.0; 129.3; 130.6; 131.2; 132.0; 133.1; 133.4; 135.8; 137.3; 152.7; 156.9; 157.0; 167.2; 170.5; 170.8; 171.9. MS (MALDI-TOF): 19325 (100, M^+, $^{13}C_{10}{}^{12}C_{848}H_{1372}N_{52}{}^{18}O_1{}^{16}O_{431}{}^+$; calc. 19327).

[a]) Matrix for MALDI-TOF-MS, α-cyano-4-hydroxycinnamic acid, and for FAB-MS, 3-nitrobenzyl alcohol.

[b]) 33 of a total of 41 C-resonances were found. Seven CH_2 groups (cyclophane, part of the 1st-gen. monomer unit) and one quaternary C-atom (1st-gen. branching) were not visible or buried. The aromatic C-atom resonances were very weak.

[3]) Minor losses during aqueous extractions reduced some yields to *ca.* 90%.

(108 C, COOMe), 170.8 (36 C, CONHR), 170.5 (12 C, CONHR), and 167.2 (4 C, CONHR). The corresponding ^{1}H-NMR spectra were consistent too, though much less informative compared to the ^{13}C-NMR spectra due to severe line broadening by dynamic effects at the higher generations.

Mass-spectral analysis (fast-atom-bombardment (FAB) or matrix-assisted laser-desorption-ionization time-of-flight (MALDI-TOF)) provided the M^+ or $[M + Na]^+$ signals for dendrophanes **3**, **4**, and **7**–**9**. The MALDI-TOF mass spectrum for the third-generation dendrophane **9** showed the well-resolved molecular ion peak at m/z 19325 as the base peak besides a typical fragmentation pattern resulting from partial loss of the dendritic branches. Dendrophanes **3**–**5**, **8**, and **9** tenaceously incorporated solvents that could not be removed completely even by drying at elevated temperatures (70°) for several days under high vacuum ($5 \cdot 10^{-4}$ Torr). Nevertheless, ^{1}H-NMR studies confirmed that organic solvents like toluene or CH_2Cl_2 in dendrophanes **7**–**9** could be reduced to less than 2 mol-%. On the other hand, even after drying at 100°/$5 \cdot 10^{-4}$ Torr for several days, the highly hygroscopic third-generation acid **5** proved to retain persistently more than 20 mol-% of H_2O, as determined by *Karl-Fischer* methodology [19].

Steroid recognition by the dendritic core cyclophane **2** and dendrophanes **3**–**5** was investigated by 500-MHz ^{1}H-NMR binding titrations in borate-buffered D_2O[4]) (pD 10.5)/CD_3OD 1:1 (v/v) at 298 K [20]. Association constants K_a were determined by nonlinear least-squares curve-fitting analysis [21] of the changes in chemical shift recorded for protons of the binding partner held at constant concentration during the titration[5]). We first studied the complexation properties of the novel cyclophane **2**. In titrations at constant testosterone (**21**) concentration, evaluation of the complexation-induced upfield shifts $\Delta\delta$ of the Me(19) and Me(18) resonances of the guest **21** (*Fig. 2*)

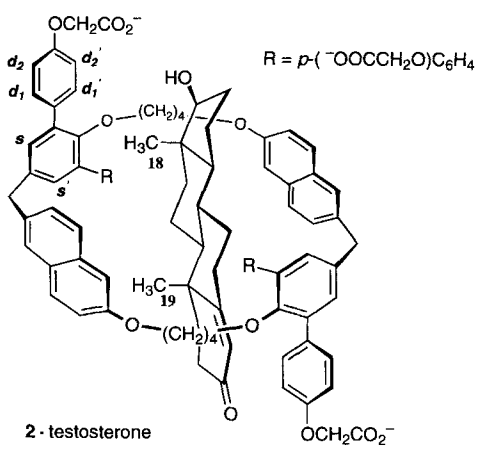


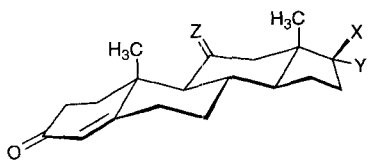
Fig. 2. *Schematic drawing of the axial inclusion complex of testosterone with cyclophane* **2**. Protons that were monitored during ^{1}H-NMR binding titrations are labeled.

[4]) High concentrations (0.1–0.5M) of borate buffer had to be used due to the multiple carboxylic-acid groups of the higher-generation dendrophanes.

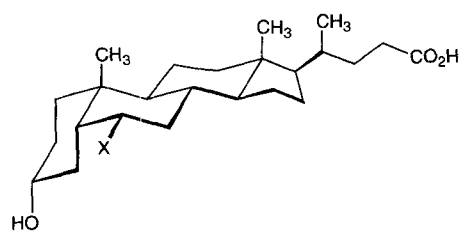
[5]) In a typical titration, one component was kept constant at 0.5 mM concentration, and the other was varied from 0.5 to 5.0 mM to reach 70–90% saturation.

yielded $K_a = 1350 \pm 150$ l·mol^{-1} for the formed 1:1 complex. The calculated saturation shifts $\Delta\delta_{sat}$ were −0.81 ppm for Me(19) and −0.24 ppm for Me(18). Inverse titrations at constant host concentration, in which the downfield shifts ($\Delta\delta_{sat}$ = +0.35 to +0.50 ppm) of the aromatic 1,1′:3′,1″-terphenyl resonances *s*, d_1, and d_2 (*Fig. 2*) were evaluated, gave an identical stability constant $K_a = 1300 \pm 100$ l·mol^{-1} (complexation free energy $\Delta G^0 = -4.2$ kcal mol^{-1}). Remarkably, after some initial broadening, the signals *s*, d_1, and d_2 of the host started to split into a total of six sharp signals ($s \neq s'$, $d_1 \neq d_1'$, and $d_2 \neq d_2'$) near saturation indicating that the barrier of rotation about the biphenyl-type axes in the 1,1′:3′,1″-terphenyl moieties of **2** becomes slow on the NMR time scale as a result of the axial inclusion of testosterone [3a, d].

Linear *van't Hoff* regression analysis ($r^2 = 0.99$) of variable-temperature ^{1}H-NMR titrations at 293, 300, 307, and 314 K showed that the complexation of testosterone (**21**) by receptor **2** at room temperature is enthalpically driven ($\Delta H^0 = -5.0$ kcal mol^{-1}; $T\Delta S^0 = -0.8$ kcal mol^{-1}) – to a lesser extent, though, than expected, compared to the



	X	Y	Z
21 testosterone	OH	H	H_2
22 progesterone	MeC(O)	H	H_2
23 cortisone	$HOCH_2C(O)$	OH	O
25 hydrocortisone	$HOCH_2C(O)$	OH	β-OH



	X
24 lithocholic acid	H
26 hyodeoxycholic acid	OH

Table 2. *Association Constants* K_a [1 mol^{-1}] *and Complexation Free Enthalpies* $\Delta G°$ [kcal mol^{-1}] *for Dendrophane Complexes in Borate-Buffered* D_2O (pD 10.5)/CD_3OD *1:1 (v/v) at 298 K.* Also shown are the calculated and, in parentheses, the maximum observed complexation-induced upfield shifts $\Delta\delta_{sat}$ and $\Delta\delta_{max\ obs}$, respectively, for the resonances of Me(19) and Me(18) of the bound steroid.

Host	Guest	K_a [1 mol^{-1}]	$\Delta G°$[a] [kcal mol^{-1}]	$\Delta\delta_{sat}$ ($\Delta\delta_{max\ obs}$)	
				Me(19)	Me(18)
2	**21**	1300	−4.2	[b]	
2	**22**	1520	−4.3	[b]	
2	**23**	380	−3.5	[b]	
2	**24**	270	−3.3	[b]	
2	**25**	80	−2.6	[b]	
2	**26**	40	−2.2	[b]	
2	**21**	1350	−4.3	−0.81 (−0.67)	−0.24 (−0.18)
3	**21**	700	−3.9	−0.97 (−0.74)	−0.25 (−0.19)
4	**21**	750	−3.9	−1.60 (−1.22)	−0.35 (−0.27)
5	**21**	1100	−4.2	−1.33 (−1.13)	−0.30 (−0.24)

[a]) Uncertainties in $\Delta G°$: ±0.1 kcal mol^{-1}. [b]) Host signals were followed ($\Delta\delta_{sat}$ = 0.16–0.50 ppm).

strongly enthalpically driven complexation of **21** by cyclophane **1** ($\Delta H^0 = -12.0$ kcal mol^{-1}, $T\Delta S^0 = -7.3$ kcal mol^{-1}, $\Delta G^0 = -4.7$ kcal mol^{-1}) [3d]. We explain this reduced enthalpic driving force for testosterone inclusion by **2**, as compared to **1**, by hydrophobic effects [22]; probably, a significantly higher degree of desolvation occurs upon substrate incorporation by the novel cyclophane **2** which contains a much deeper cavity than **1**.

Similar to **1** [3a,d], cyclophane **2** discriminates between steroids of different polarity: complexation strength decreases from progesterone (**22**), to testosterone (**21**), to cortisone (**23**), to lithocholic acid (**24**), to hydrocortisone (**25**), and to hyodeoxycholic acid (**26**; *Table 2*). The stability of the inclusion complexes is lowered by increasing steroid polarity and by electrostatic repulsion, if the substrates also possess carboxylate residues.

All three dendrophanes **3**–**5** formed 1:1 complexes with testosterone of comparable stability to that of core cyclophane **2**, indicating that the cyclophane binding site remains open and accessible within the dendritic shells (*Table 2*). The large complexation-induced changes in chemical shift observed for the steroidal methyl protons Me(19) ($\Delta\delta_{sat} = 0.97$–1.60 ppm) and Me(18) ($\Delta\delta_{sat} = 0.24$–0.35 ppm) in titrations at various dendrophane concentrations clearly demonstrate that the steroid binds in the cyclophane cavity rather than in nonspecific, fluctuating voids in the dendritic shell. Conspicuously, there is a large change in $\Delta\delta_{sat}$ of over 0.6 ppm for the Me(19) *s* when going from the first- to the second-generation dendrophane (*Table 2*), possibly induced by a different, generation-dependent complex geometry. Inverse titrations at constant dendrophane concentration further supported the data in *Table 2* and yielded stability constants $K_a = 1200$ and 800 l·mol^{-1} for the complexes formed between testosterone (**21**) and **3** or **4**, respectively.

Remarkably, the guest signals could be nicely followed in all ^{1}H-NMR binding titrations, although they increasingly broadened with increasing dendrophane generation. Apparently, the host-guest exchange kinetics are fast on the ^{1}H-NMR time scale, even in studies with the third-generation dendrophane **5**, in which the dendritic branches are densely packed in a globular layer of *ca.* 2 nm radius around the core. These unexpectedly fast host-guest exchange kinetics are in agreement with observations made previously for a family of arene-binding dendrophanes with a narrower apolar pocket [8]. To more precisely address the host-guest exchange kinetics, quantitative studies, based on fluorescence relaxation techniques [23], are now on their way.

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