166. Dendrophanes: Water-Soluble Dendritic Receptors as Models for Buried Recognition Sites in Globular Proteins

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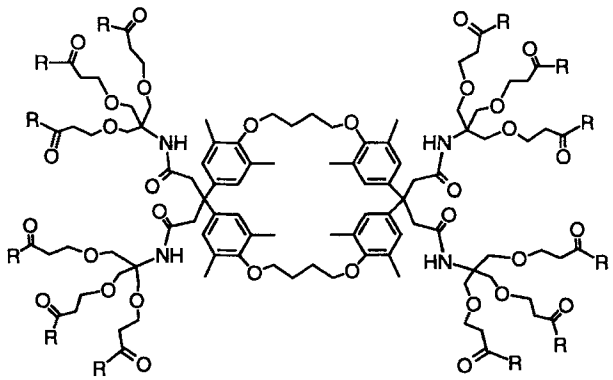
Water-soluble *dendr*itic cyclo*phanes* (*dendrophanes*) of first (**1**, **4**), second (**2**, **5**), and third generation (**3**, **6**) with poly(ether amide) branching and 12, 36, and 108 terminal carboxylate groups, respectively, were prepared by divergent synthesis, and their molecular recognition properties in aqueous solutions were investigated. Dendrophanes **1**–**3** incorporate as the initiator core a tetraoxa[6.1.6.1]paracyclophane **7** with a suitably sized cavity for inclusion complexation of benzene or naphthalene derivatives. The initiator core in **4**–**6** is the [6.1.6.1]cyclophane **8** shaped by two naphthyl(phenyl)methane units with a cavity suitable for steroid incorporation. The syntheses of **1**–**6** involved sequential peptide coupling to monomer **9**, followed by ester hydrolysis (*Schemes 1* and *4*). Purification by gel-permeation chromatography (GPC; *Fig. 3*) and full spectral characterization were accomplished at the stage of the intermediate poly(methyl carboxylates) **10**–**12** and **23**–**25**, respectively. The third-generation 108-ester **25** was also independently prepared by a semi-convergent synthetic strategy, starting from **4** (*Scheme 5*). All dendrophanes with terminal ester groups were obtained in pure form according to the ^{13}C-NMR spectral criterion (*Figs. 1* and *5*). The MALDI-TOF mass spectra of the third-generation derivative **25** (mol. wt. 19328 D) displayed the molecular ion as base peak, accompanied by a series of ions $[M - n(1041 \pm 7)]^+$, tentatively assigned as characteristic fragment ions of the poly(ether amide) cascade. A similar fragmentation pattern was also observed in the spectra of other higher-generation poly(ether amide) dendrimers. Attempts to prepare monodisperse fourth-generation dendrophanes by divergent synthesis failed. ^{1}H-NMR and fluorescence binding titrations in basic aqueous buffer solutions showed that dendrophanes **1**–**3** complexed benzene and naphthalene derivatives, whereas **4**–**6** bound the steroid testosterone. Complexation occurred exclusively at the cavity-binding site of the central cyclophane core rather than in fluctuating voids in the dendritic branches, and the association strength was similar to that of the complexes formed by the initiator cores **7** and **8**, respectively (*Tables 1* and *3*). Fluorescence titrations with 6-(*p*-toluidino)naphthalene-2-sulfonate as fluorescent probe in aqueous buffer showed that the micropolarity at the cyclophane core in dendrophanes **1**–**3** becomes increasingly reduced with increasing size and density of the dendritic superstructure; the polarity at the core of the third-generation compound **3** is similar to that of EtOH (*Table 2*). Host-guest exchange kinetics were remarkably fast and, except for receptor **3**, the stabilities of all dendrophane complexes could be evaluated by ^{1}H-NMR titrations. The rapid complexation-decomplexation kinetics are explained by the specific attachment of the dendritic wedges to large, nanometer-sized cyclophane initiator cores, which generates apertures in the surrounding dendritic superstructure.

1. Introduction. – Dendrimer technology [1] provides a unique tool for creating synthetic models of globular proteins containing buried recognition and/or catalytic sites. Recently, we reported the synthesis of water-soluble dendritic iron porphyrins as functional mimics of globular electron-transfer heme proteins [2]. Electrochemical investigations in aqueous solution showed that the reduced polarity imposed by the densely packed dendritic branches around the electroactive core destabilized the more charged Fe^{III} state relative to Fe^{II}, and, as a result, the redox potential of the Fe^{III}/Fe^{II} couple was strongly shifted to a more positive value. Thus, the dendritic superstructure nicely mimics a major function of the peptidic shell around the buried iron heme in electron-transfer

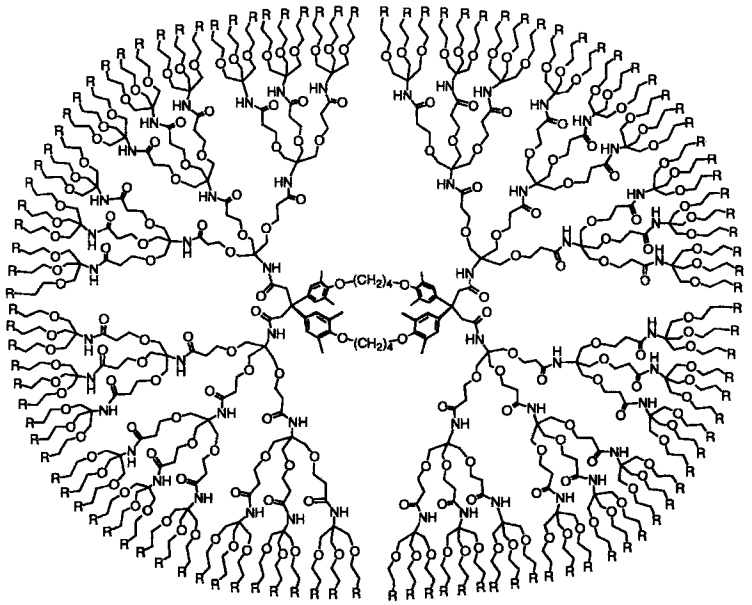
proteins such as cytochrome-*c* by reducing the micropolarity around the electrophore and, thereby, modifying its electrochemical behavior [3].

Following these investigations, we became interested in developing dendritic model systems for apolar binding sites that are deeply buried within globular proteins [4]. Specifically, we wished to investigate the influence of the shielding superstructure on kinetics and thermodynamics of inclusion complexation by cyclophane receptors [5]. Although a few dendritic receptors have been prepared [6–10], specific complexation has either not been achieved or not been thoroughly studied. To reach this objective, we prepared the *dendrophanes* (*dendr*itic cyclo*phanes*) **1**–**3** [5a] and **4**–**6** [5b], the first members of a new class of dendritic receptors for arenes and steroids, containing the cyclophanes **7** and **8** as initiator cores [11]. The latter were functionalized with dendritic wedges containing water solubility providing surface groups.

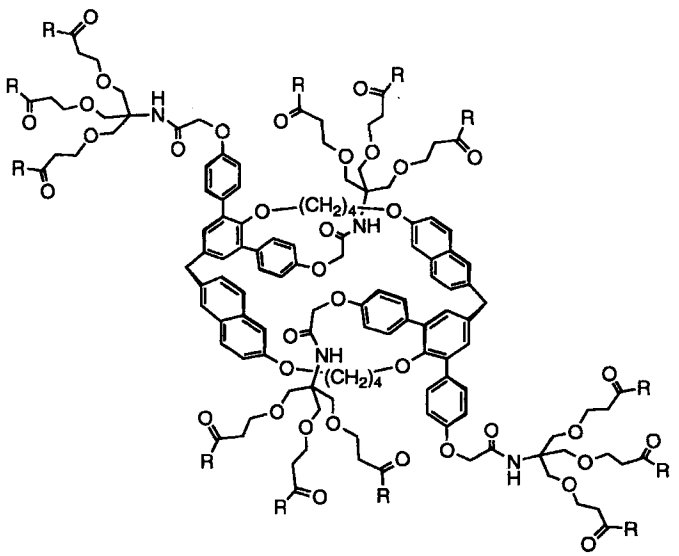


1 R = OH
10 R = OMe
2 R = NHC($CH_2OCH_2CH_2COOH$)$_3$
11 R = NHC($CH_2OCH_2CH_2COOMe$)$_3$

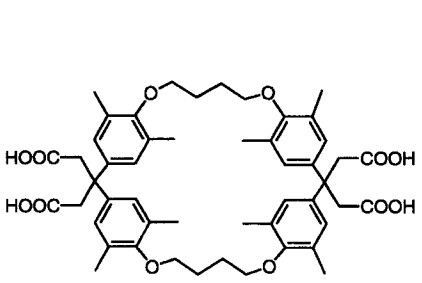
In studies with **1**–**6**, we intended to explore *i*) whether the well-defined cyclophane recognition site at the center of the dendrimer remains open and effective at higher dendritic generations, or whether hydrophobic collapse causes the dendritic branches to occupy and inhibit the binding site, *ii*) how the polarity of the binding site changes with increasing dendritic branching and shielding from solvent, and *iii*) how the kinetics of inclusion complexation is influenced by the increased branching. Here, we describe the synthesis of **1**–**6** by divergent [1] and, in the case of third-generation dendrophane **6**, also by a semi-convergent method [7] and provide NMR-spectral evidence for the high degree of purity of the compounds possessing molecular weights of *ca.* 2000 to 20000 D. The mass-spectral characterization of the poly(ether amide) cascades, in particular of the compounds with molecular weights above 10000 D will be discussed in greater detail since such information is lacking in the literature [12]. We subsequently report thermodynamic and kinetic binding studies with the dendrophanes and show that their central cyclophane cores are active receptor sites for flat aromatic substrates (**1**–**3**) or for steroids (**4**–**6**) with basically no competition occurring from nonspecific guest incorporation within the surrounding dendritic branches.



3 R = COOH
12 R = COOMe
13 R = $CONHC(CH_2OCH_2CH_2COOMe)_3$
14 R = $CONHC(CH_2OCH_2CH_2COOH)_3$



4 R = OH
23 R = OMe
5 R = $NHC(CH_2OCH_2CH_2CO_2H)_3$
24 R = $NHC(CH_2OCH_2CH_2CO_2Me)_3$



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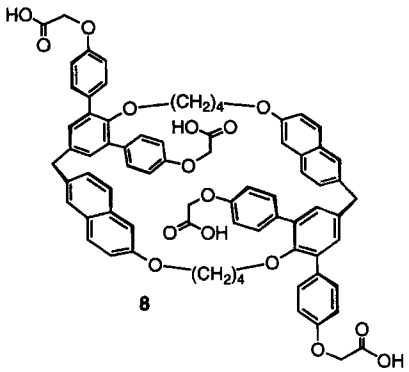

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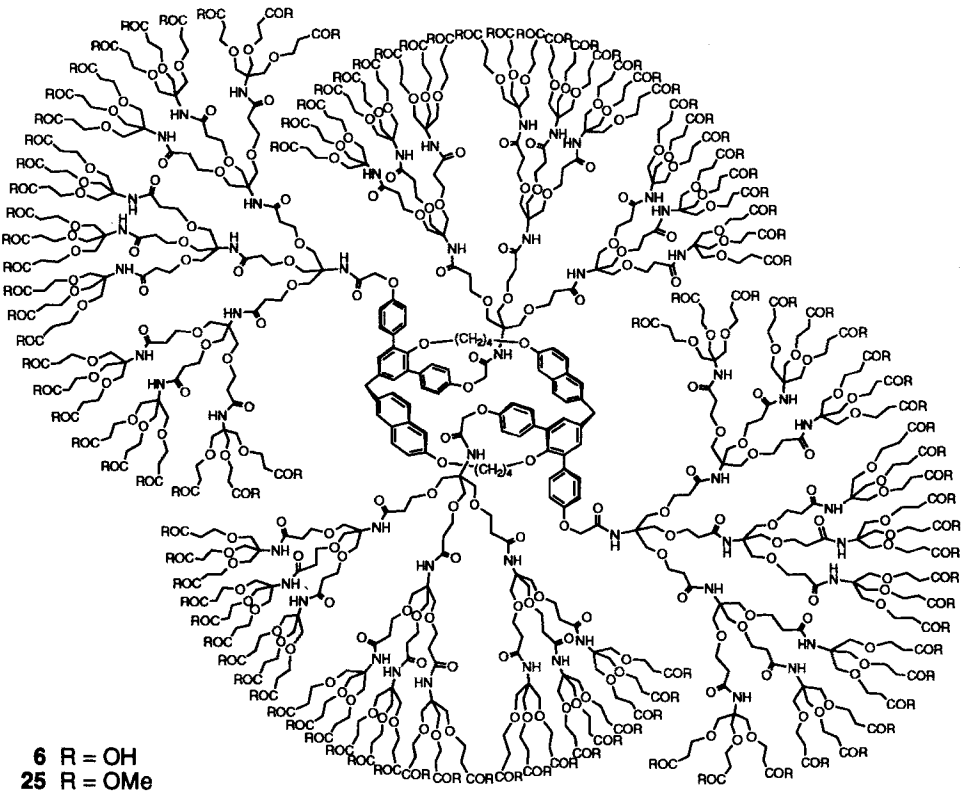
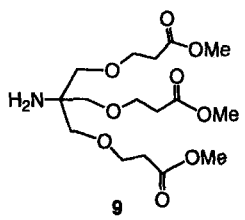


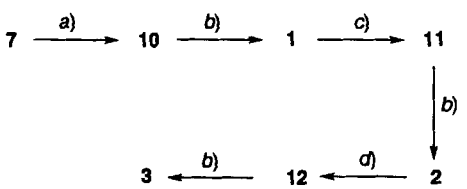
6 R = OH
25 R = OMe
26 R = $NHC(CH_2OCH_2CH_2COOMe)_3$
27 R = $NHC(CH_2OCH_2CH_2COOH)_3$

2. Results and Discussion. – 2.1. *Divergent Synthesis of the Arene-Binding Dendrophanes* **1**–**3**. The poly(ether amide) cascade introduced by *Newkome et al.* [13] was applied for the preparation of the dendrophanes **1**–**3** of first to third generation (*Scheme 1*). Stirring a solution of the initiator core **7** with a large excess of monomer **9** and dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxy-1*H*-benzotriazole

(BtOH) in THF afforded the first-generation derivative [G-1]-12-ester **10** as a colorless viscous oil which crystallized slowly upon standing. In the convenient short nomenclature used, the number in square brackets identifies the generation and is followed by the number of terminal functional groups [14]. By slow diffusion of hexane into a toluene solution of **10**, crystals suitable for X-ray structural analysis were obtained. The crystal structure [5a] of **10**, one of the very few known solid-state structures of dendrimers [15], showed an open rectangular cavity suitable for the incorporation of flat arenes. High structural disorder was observed at the ends of the dendritic branches which suggests that, in general, solving the solid-state structures of higher-generation dendrimers with flexible branches will be quite unlikely [15a–c] [16]. Although the dendritic branches in the solid-state structure start folding back onto the tetraoxa[6.1.6.1]cyclophane skeleton, the dendritic shell in **10** is not yet sufficiently extended to fully surround the binding site. According to computer models [17], a densely packed, full encapsulation of the recognition site occurs only at the stage of the third- or higher-generation derivatives. In the crystal packing, molecules of **10** form stacks generating channels which extend across the macrocyclic cavities [11].

Scheme 1. *Divergent Synthesis of Dendrophanes* **1**–**3**





a) **9**, DCC, BtOH, THF, 3 d, 50°; 60%. *b*) LiOH, THF/MeOH/H_2O, 2 d, r.t.; 80% (**1**), 90% (**2**), not determined (**3**). *c*) **9**, DCC, BtOH, THF, 3 d, r.t., 45%. *d*) DCC, BtOH, THF, 2 d, 45°; 52%.

Hydrolysis of **10** (LiOH, THF/MeOH/H_2O) followed by acidification afforded [G-1]-12-acid **1** as a colorless, hygroscopic foam which was quite insoluble in most organic solvents but could be dissolved in Me_2SO or basic aqueous solution. The second-generation derivative, [G-2]-36-ester **11**, was readily obtained in 45% yield as a viscous oil by coupling **1** with monomer **9** followed by preparative gel-permeation chromatography (GPC, *Biorad Biobeads SX-1*, PhMe). Its ^1H- and ^{13}C-NMR spectra ($CDCl_3$) displayed all expected resonances, and the matrix-assisted laser-desorption-ionization (MALDI-TOF) mass spectrum depicted with equal intensity the $[M + Na]^+$ and the $[M + K]^+$ ions as base peaks at m/z 6488 and 6508, respectively, beside one very

minor peak at m/z 5444 ($[M - 1022]^+$). In view of the very high purity of **11** according to the ^{1}H- and ^{13}C-NMR criteria, the latter ion most probably results from a mass-spectrometric fragmentation of yet unknown origin. Hydrolysis (LiOH, THF/MeOH/H_2O) followed by acidification afforded [G-2]-36-acid **2** in 90% yield which was purified from inorganic salts by dialysis using a dialysis tube made from benzylated cellulose. The ^{1}H-NMR spectrum of **2** in D_2O depicted all expected resonances, whereas 17 out of the 20 expected resonances were observed in the ^{13}C-NMR spectrum.

Peptide coupling between **2** and monomer **9** followed by GPC purification (PhMe) yielded the third-generation [G-3]-108-ester **12** with 108 terminal ester groups as a colorless, highly viscous oil in 52% yield. Due to slow molecular tumbling motion, the ^{1}H-NMR spectrum ($CDCl_3$) showed broadened, partially overlapping signals; nevertheless, most resonances of the first-, second-, and third-generation branches were visible in the intensity ratio of 1:3:9, respectively. The high purity of the compound was nicely evidenced by the ^{13}C-NMR spectrum ($CDCl_3$) which displayed 20 of the 26 resonances expected for a D_{2h}-symmetrical compound (*Fig. 1*). No impurity was observed except for residual PhMe from the GPC purification, which was tenaciously retained by the dendrophane and could not be removed even at high vacuum ($5 \cdot 10^{-5}$ Torr). Incorporation of larger amounts of solvent in higher-generation dendrimers is generally observed; in this respect, the synthetic macromolecules resemble the natural proteins which also include large amounts of solvent.

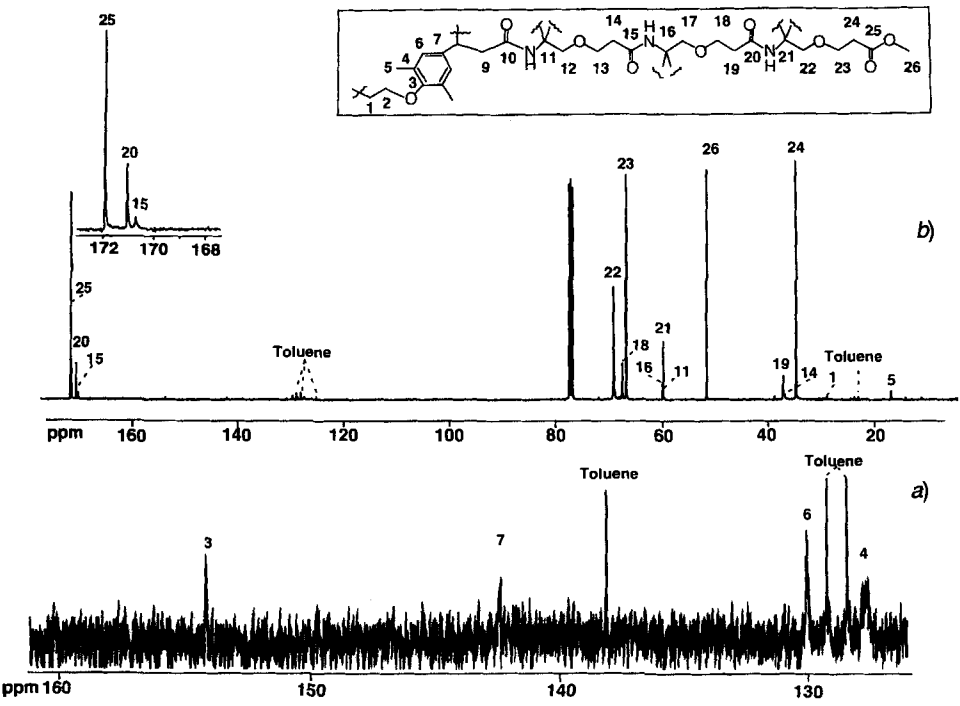


Fig. 1. a) *Aromatic and* b) *aliphatic region of the* ^{13}*C-NMR spectrum* (125 MHz, $CDCl_3$) *of [G-3]-108-ester* **12**. Of the expected 26 resonances assigned to the dendrophane, 20 were visible together with 5 resonances of trapped toluene. The insert on the upper left shows the C=O resonances. Arbitrary numbering.

Mass-spectrometric analysis of **12** ($^{12}C_{825}{}^{13}C_9H_{1368}N_{52}{}^{18}O^{16}O_{427}$ requires 18971) proved to be very challenging, and a survey of the literature [12] showed that mass spectra of poly(ether amide) dendrimers of this size have not been published. All attempts to detect the molecular ion by electron-spray-ionization (ESI) mass spectra remained unsuccessful. Finally, the MALDI-TOF mass spectrum depicted in *Fig. 2* was recorded in a reproducible way in α-cyano-4-hydroxycinnamic acid (CCA; 2-cyano-3-(4-hydroxyphenyl)prop-2-enoic acid) as the matrix. It depicts the molecular ion (*m/z* 18958) as the base peak besides a series of fragment ions, *e.g.*, at *m/z* 17937 ($[M - 1034]^+$), 15850 ($[M - 3122]^+$), 14521 ($[M - 4451]^+$), and 4556. In the GPC, the sharp and symmetrical peaks of dendrophanes of different generations, which strongly differ in their molecular weights, are nicely separated (*Fig. 3*). It can, therefore, be excluded that the peak at *m/z* 4556 corresponds to a lower-molecular-weight dendritic impurity with severe defects in the branching due to incomplete conversion in the growth process during peptide coupling. The origin of the higher molecular weight ions in the *m/z* range between 14500 and 18958 remains unclear at present; however, in view of the ^{13}C-NMR purity of **12**, we assign them to mass spectrometrically generated fragment ions rather than to impurities such as dendrophanes with defects in the dendritic shell.

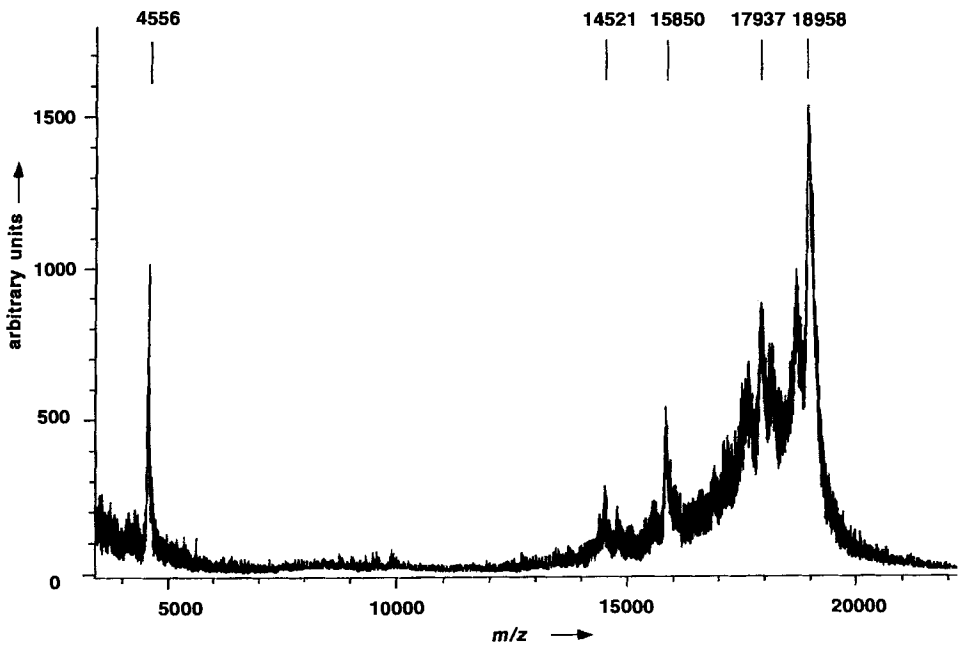


Fig. 2. *MALDI-TOF Mass spectrum of* **12** *recorded in a CCA matrix*

Hydrolysis (LiOH, THF/MeOH/H_2O) afforded the third-generation dendrophane **3**, which, after workup and drying, was used without further purification in the complexation studies. Attempts were subsequently made to prepare the fourth-generation derivatives [G-4]-324-ester **13** and [G-4]-324-acid **14** with 324 terminal functional groups. The coupling reaction between [G-3]-108-acid **3** and monomer **9** provided a product which

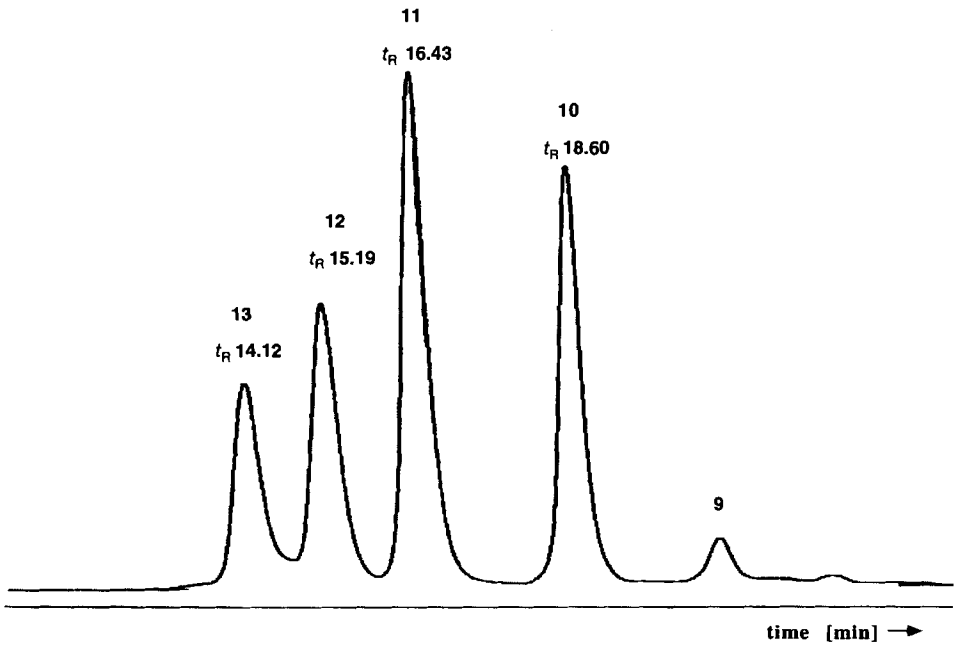


Fig. 3. *Analytical GPC* (THF) *of a mixture of monomer* **9**, *[G-1]-12-ester* **10**, *[G-2]-36-ester* **11**, *[G-3]-108-ester* **12**, *and the product distribution obtained in attempts to prepare [G-4]-324-ester* **13**. Flow rate 0.5–1.0 ml min^{-1}; detection at 254 nm.

was purified by GPC, followed by chromatography on SiO_2 (CH_2Cl_2/MeOH 97:3). The analysis of the resulting material, which was isolated as a highly viscous colorless oil in 35% yield, by both ^1H- and ^{13}C-NMR spectroscopy remained inconclusive with respect to identity and homogeneity of the isolated material: only strongly broadened peaks of the higher-generation branching were observed in both spectra. A series of MALDI-TOF mass spectra of **13** in various matrices (sinapic acid, anthracene-1,8,9-triol (dithranol), and 2,4,6-trihydroxyacetophenone) eventually provided very similar results showing clearly that the coupling between [G-3]-108-acid **3** and **9** to give the [G-4]-324-ester **13** ($^{12}C_{2425}{}^{13}C_{29}H_{4068}N_{160}{}^{16}O_{1290}{}^{18}O_2$ requires 56488) had not gone to completion. All spectra (*Fig. 4*) showed a very broad signal expanding from *m/z* 56000 to 38000 with a maximum around *m/z* 48400, which means that 70 to 108 peptidic couplings had taken place at the 108 terminal COOH residues of **3**; this in return corresponds to a degree of conversion between 65 and 100%. The polydisperse [G-4]-324-ester **13** was subsequently hydrolyzed with LiOH in MeOH/H_2O to afford the polydisperse [G-4]-324-acid **14** which was used for complexation studies in form of its poly(lithium carboxylate).

To investigate the possibility of nonspecific guest incorporation within the branches of poly(ether amide) cascades, the [G-3]-81-acid **15**, lacking the cyclophane recognition site, was prepared starting from the tris(acyl halide) **16** as the initiator core *via* the sequence **16** → [G-1]-9-ester **17** → [G-1]-9-acid **18** → [G-2]-27-ester **19** → [G-2]-27-acid **20** → [G-3]-81-ester **21** → [G-3]-81-acid **15** (*Scheme 2*). Similarly to the dendrophane series, purification and characterization was mainly performed at the stage of the dendritic esters, which were all isolated as colorless, highly viscous oils. After GPC

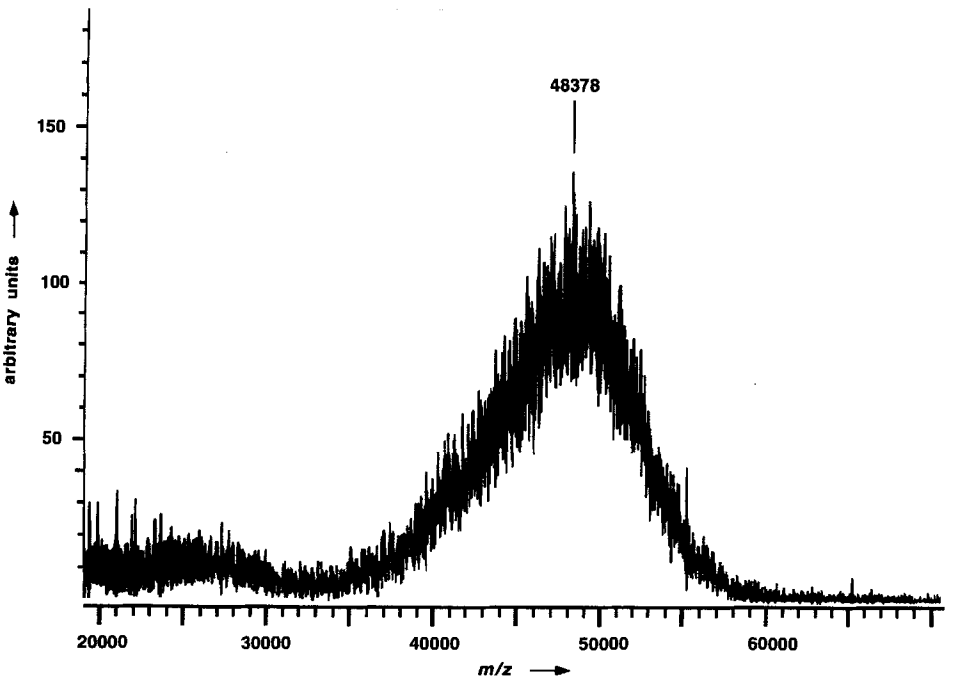


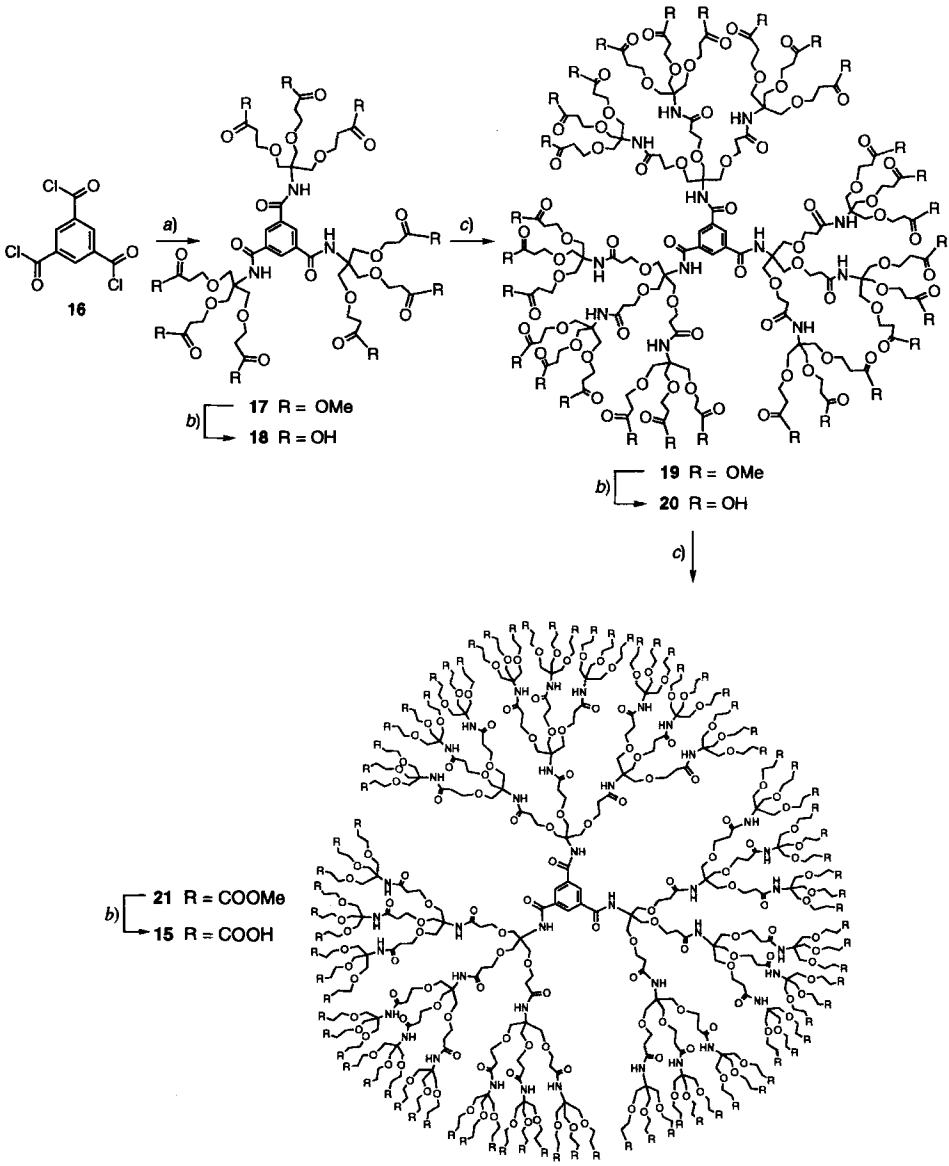
Fig. 4. *MALDI-TOF Mass spectrum in a sinapic-acid matrix, demonstrating the polydispersity of [G-4]-324-ester* **13**

purification and chromatography on SiO_2 (CH_2Cl_2/MeOH 95:5), the ^{13}C-NMR spectrum ($CDCl_3$) of **21** showed 12 of the expected 19 resonances, and no impurity peaks were observed. Besides the molecular ion M^+ at *m/z* 13822 ($^{12}C_{589}{}^{13}C_8H_{1014}N_{39}O_{318}$ requires 13826), the MALDI-TOF mass spectrum (CCA) of **21** depicted a fragmentation sequence, which seems to be characteristic for higher-generation poly(ether amide) cascades (see *Sect. 2.2* below). Intense peaks were observed in intervals of *ca.* 1041 mass units at *m/z* 12779 ($[M - 1047]^+$), 11737 ($[M - 2089]^+$), 10695 ($[M - 3131]^+$), 9652 ($[M - 4174]^+$), and 8610 ($[M - 5216]^+$). Hydrolysis of **21** (LiOH, MeOH/H_2O) gave the poly(lithium carboxylate) corresponding to **15** which was used in fluorimetric binding assays.

For comparison, dendrophane **22** was also prepared as an arene-binding receptor with non-ionizable peripheral groups, which, on the basis of its 12 terminal triethylene glycol monomethyl ether residues, is soluble in H_2O (*Scheme 3*).

2.2. *Divergent Synthesis of the Steroid-Binding Dendrophanes* **4**–**6**. The synthesis of the steroid-binding dendrophanes followed the divergent protocol described above for the arene-binding analogs (*Scheme 4*). The ester derivatives **23**–**25** of first to third generation, which were purified and fully characterized, all gave sharp, symmetrical, and well-separated GPC peaks (PhMe) similar to those shown in *Fig. 3*. Coupling of monomer **9** to the initiator core **8** provided the [G-1]-12-ester **23** in form of up to 0.5 cm long, colorless needles. In the FAB mass spectrum (NOBA), the MH^+ ion appeared as base peak together with the $[M + Na]^+$ peak at 2677 (38%) and a peak at *m/z* 2551 (51%) resulting from the fragmentation of a dendritic branch with elimination of an

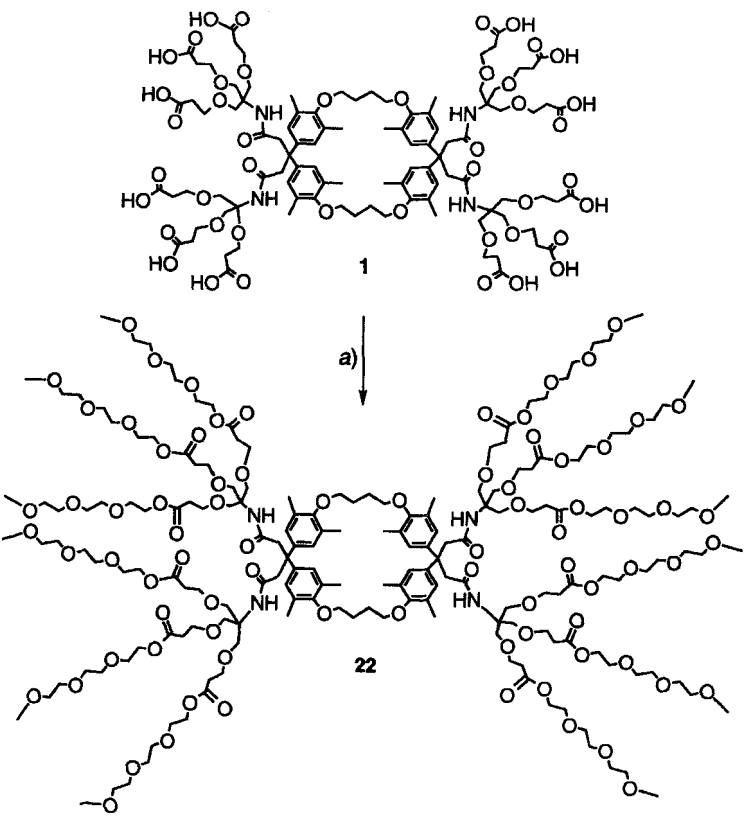
Scheme 2. *Divergent Synthesis of Compound [G-3]-81-Acid* **15**



a) **9**, Et_3N, CH_2Cl_2, 14 h, r.t.; 50%. *b*) LiOH, THF/MeOH/H_2O, 2 d, r.t.; *ca.* 89 (**18**), 85% (**20**), not determined (**15**). *c*) **9**, DCC, BtOH, THF, 2 d; 53% (**19**), 43% (**21**).

$[OCH_2CH_2CO_2Me]^+$ fragment. The MALDI-TOF mass spectrum, on the other hand, showed the $[M + Na]^+$ ion as base peak besides weaker peaks for the molecular ion at *m/z* 2654, the $[M + K]^+$ ion at *m/z* 2693, and the fragment ion at *m/z* 2551. Hydrolysis of **23** afforded the [G-1]-12-acid **4**, whose ^{13}C-NMR spectrum depicted all expected

Scheme 3. *Synthesis of the Dendrophane [G-1]-12-Ester* **22** *with Neutral Water-Solubility Providing Surface Groups*

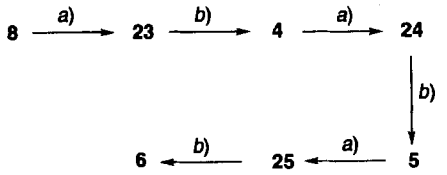


a) Triethylene glycol monomethyl ether, DCC, BtOH, THF, 2 d; 92%.

30 resonances. Traces of the solvents THF and MeOH from the hydrolysis reaction were still visible even after drying for 3 d at $70°/5 \cdot 10^{-4}$ Torr. The FAB mass spectrum (NOBA) of **4** showed the MH^+ peak as base peak at m/z 2487 besides the fragment ions $[M - OCH_2CH_2OMe]^+$ at m/z 2397 (35%) and $[M - C[CH_2O(CH_2)_2CO_2H]_3]^+$ at m/z 2168. The ^{1}H-NMR spectra of **4** depicted solvent-dependent dynamic effects. Presumably as a result of the atropisomerism about the 1,1′:3′,1″-terphenyl moieties [11], the spectra in a 1:1 mixture of borate buffer (pD 10.5) in D_2O and CD_3OD, or in pure $(CD_3)_2SO$ showed the presence of one major and at least one minor conformer, whereas the spectrum recorded in pure borate buffer depicted only broad humps of *ca.* 100 Hz width. We take this as evidence for the aggregation of **4** in the pure buffer, despite the twelve charged COO^- residues.

Coupling **4** with **9**, followed by GPC purification and drying (3 d, $70°/5 \cdot 10^{-5}$ Torr) afforded the [G-2]-36-ester **24** as a colorless glass in 92% yield, which corresponds to a yield of 99.3% for each of the 12 coupling steps. The ^{1}H-NMR spectrum ($CDCl_3$) was consistent with the proposed structure with dynamic conformational effects again generating two (or more) sets of aromatic resonances of different intensity. In contrast, the

Scheme 4. *Divergent Synthesis of Dendrophanes* **4**–**6**



a) **9**, DCC, BtOH, THF, 4 d, 50°, 62% (**23**); 3 d, r.t., 92% (**24**); 3 d, r.t., 82% (**25**). *b*) LiOH, THF/MeOH/H_2O, 2–3 d, r.t.; 99% (**4**), 94% (**5**), 90% (**6**).

^{13}C-NMR spectrum was highly conclusive and depicted all expected 36 highly resolved resonances and complete absence of any impurity peaks. The MALDI-TOF spectrum displayed the $[M + Na]^+$ ion as base peak in addition to a single minor peak at *m/z* 5802 ($[M - 1044]^+$) resulting from the characteristic fragmentation of higher molecular weight poly(ether amide) cascades already mentioned in *Sect. 2.1*. Hydrolysis of **24** provided [G-2]-36-acid **5** in 94% yield. The resonances in both ^{1}H- and ^{13}C-NMR spectra (basic borate buffer solutions) were strongly broadened as a result of dynamic effects; however, the absence of the Me-ester resonances clearly confirmed complete hydrolysis.

The third-generation compound [G-3]-108-ester **25** (mol. wt. 19328 D) was obtained from **5** in 82% yield after GPC purification, which corresponds to an average yield of 99.4% in each of the 36 coupling steps. Clearly, only extremely high-yielding reactions, such as the well-established peptide coupling with DCC/BtOH [18], can be used to produce higher-generation, high-molecular-weight dendrimers such as **25** in sufficient purity. According to the ^{1}H-NMR spectrum (CD_2Cl_2), less than 2% solvent was retained in the glassy solid obtained after vigorous drying at 70°/5 · 10^{-5} Torr. The spectrum displayed all expected resonances, although some lines were quite broad, which prevented an accurate integration. Again, the ^{13}C-NMR spectrum (125 MHz, $CDCl_3$) was highly conclusive; it demonstrated the high purity of **25** and depicted 33 of the 41 expected resonances (*Fig. 5*). The six CH_2 groups of the cyclophane (C(11), C(16) to C(19), C(24); *Fig. 5*, arbitrary numbering) were not visible even after 12000 scans; in contrast, all aromatic resonances appeared, although some were broadened due to dynamic effects. All resonances of the dendritic shell were visible with the exception of C(26) and C(27) in the first-generation branching, which are presumably masked by stronger signals from higher-generation C-atoms. The four C=O resonances, with expected relative intensities of 108, 36, 12, and 4, respectively, are particularly well-separated; they appear at 172.3 (C(40)), 171.2 (C(35)), 170.9 (C(30)), and 167.6 (C(25)) ppm. The MALDI-TOF spectrum (CCA matrix, *Fig. 6*) displayed the M^+ ion as the base peak at *m/z* 19325 ($^{12}C_{848}{}^{13}C_{10}H_{1372}N_{52}{}^{16}O_{432}{}^{18}O$ requires 19329) besides the characteristic series of fragment ions $[M - n(1041 \pm 7)]^+$ with $n = 1-5$. Depending on the conditions of the mass-spectrometric experiment (nature of matrix and solvent, probe preparation, laser flux, use of the reflectron), the number of these fragment ions (up to 8 were observed) as well as their relative intensities varied substantially [19a,b]. Also, depending on the mass-spectrometric conditions, the sodium complex of the molecular ion M^+ was observed as the base peak at *m/z* 19353 ($^{12}C_{848}{}^{13}C_{10}H_{1372}N_{52}Na^{16}O_{432}{}^{18}O$ requires 19351.7). The degree of spectral resolution also varied strongly.

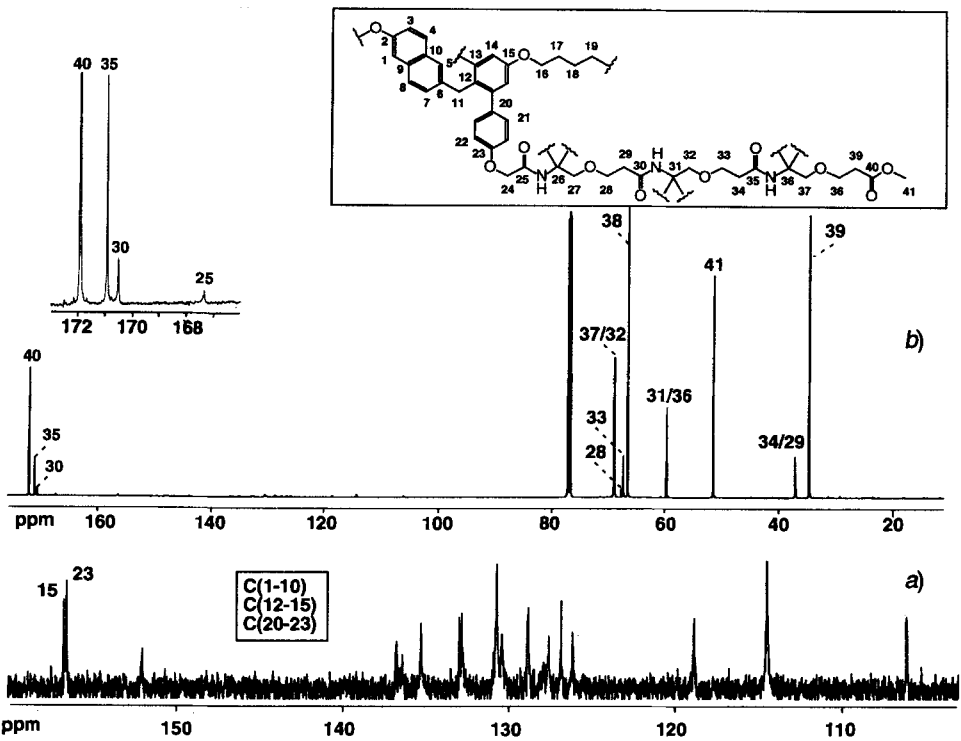


Fig. 5. a) *Aromatic and* b) *aliphatic region of the* ^{13}*C-NMR spectrum* (125 MHz, $CDCl_3$) *of [G-3]-108-ester* **25**. The insert on the upper left shows the four C=O resonances. Arbitrary numbering.

A more extensive analysis of the mass-spectral data was undertaken to clarify the origin of the $[M - n(1041 \pm 7)]^+$ ions and to exclude the possibility that these peaks assigned as fragment ions resulted from defects in the dendritic branching rather than from mass-spectral fragmentation [19]. This analysis was, however, not conclusive; at this stage, the ^{13}C-NMR purity of the third-generation dendrimer **25** and its synthetic lower-generation precursors provides the best (indirect) support for their assignment as fragment rather than as impurity ions. Impressive chemical evidence for the high purity of **25**, prepared by divergent synthesis, was later obtained (*Sect. 2.3*) by the semi-convergent synthesis of this third-generation compound: the samples independently prepared by both methods showed identical spectral properties.

Basic hydrolysis of **25** gave the [G-3]-108-acid **6** as an extremely hygroscopic glassy compound in 90% yield, which was used in the steroid-binding studies without further purification. Attempts to prepare the fourth-generation dendrophane [G-4]-324-ester **26** ($C_{2478}H_{4072}N_{160}O_{1296}$ requires 56844) gave only a polydisperse mixture of incompletely coupled products after GPC purification. The MALDI-TOF mass spectrum of **26** depicted a broad, unresolved band extending from *m/z* 39000 to 57000, similar to the one recorded for the polydisperse [G-4]-324-ester **13** (*Fig. 4*, *Sect. 2.1*); between 75 and 108 COOH groups of **6** had reacted with monomer **9** under the DCC/BtOH coupling conditions. Hydrolysis gave the water-soluble polydisperse [G-4]-324 acid **27** which was used without purification as poly(lithium carboxylate) in complexation studies.

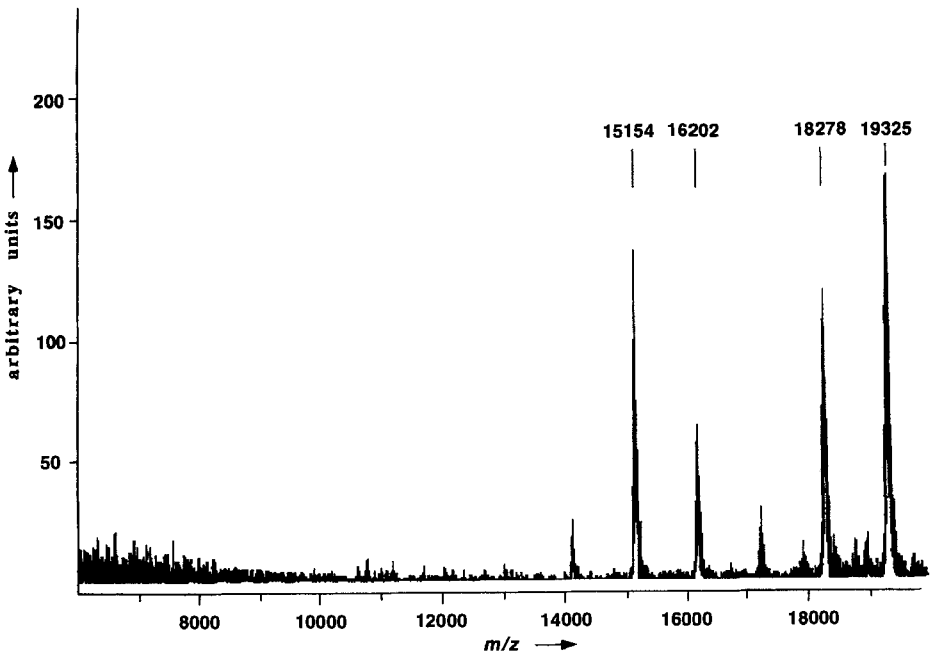
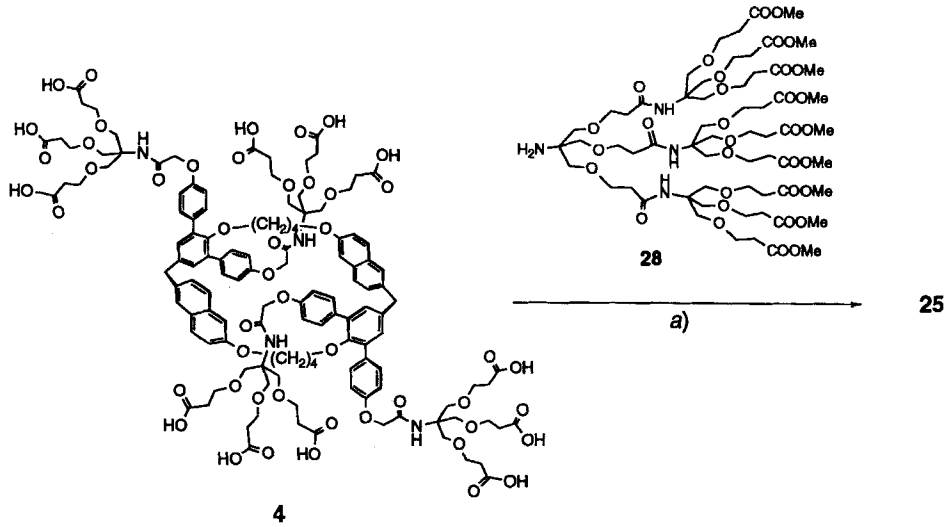


Fig. 6. *MALDI-TOF Mass spectrum of* **25** *recorded in a CCA matrix*

2.3. *Semi-Convergent Synthesis of [G-3]-108-Ester* **25**. To further prove the high purity of the third-generation dendrophane **25** prepared by divergent synthesis (*Sect. 2.2*), this compound was also produced in a semi-convergent route by coupling (DCC/BtOH) the first-generation 12-acid **4** to the dendritic wedge **28** [20] (*Scheme 5*), two analytically pure components. This semi-convergent route to **25** involves only a 12-fold coupling (in contrast to the 36-fold coupling from **5** to **25** in the divergent synthesis). Therefore, the probability of incomplete conversion and resulting defects in the dendritic branching was strongly reduced. GPC Purification followed by drying ($70°/5 \cdot 10^{-5}$ Torr) afforded a compound which, according to the spectral data (^{1}H- and ^{13}C-NMR, MALDI-TOF-MS), was identical to the dendrophane **25** obtained by divergent synthesis. This result demonstrates that third-generation poly(ether amide) dendrimers such as **13** and **25** with 108 surface ester groups can be produced in high purity by divergent synthesis; it also provides additional support to the assignment of the $[M - n(1041 \pm 7)]^+$ peaks in the MALDI-TOF-MS of **25** as fragment ions.

2.4. *Host-Guest Complexation Studies*. 2.4.1. *Complexation of Arenes by Dendrophanes* **1**–**3**. The complexation properties of dendrophanes **1**–**3** were first investigated at 300 K by 500-MHz ^{1}H-NMR titrations in 0.066M phosphate buffer in D_2O (pD 8.4) in the presence of small quantities of organic solvent required to ensure solubility of the guests **29**–**33** (see *Fig. 7*). The high hygroscopicity of the dendrophanes as well as their tendency to incorporate various amounts of solvent made it difficult to accurately prepare stock solutions of the host by weighing. Therefore, concentrations of dendrophanes in titration solutions were best determined by ^{1}H-NMR integration relative to the guest signals taken as internal standards. Titrations at either constant guest or host

Scheme 5. *Semi-Convergent Synthesis of [G-3]-108-Ester* **25**



a) DCC, BtOH, THF/DMF, 3 d, r.t.; 62%.

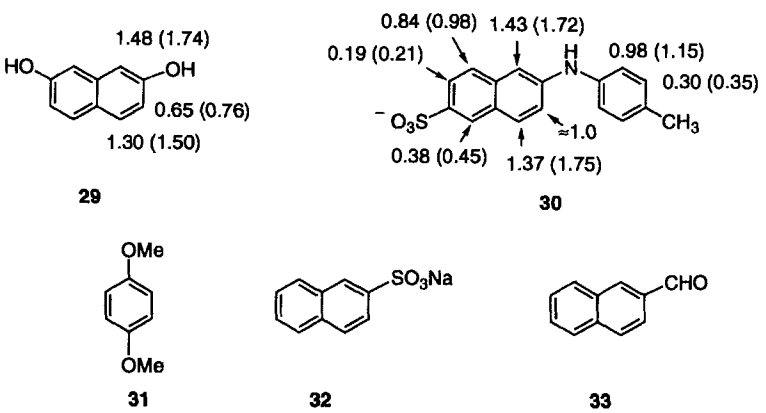


Fig. 7. *Guests in the binding titrations with dendrophanes* **1**–**3**. Shown are the maximum observed upfield shifts ($\Delta\delta_{max\ obs}$ in ppm) and, in parenthesis, upfield shifts calculated for saturation binding ($\Delta\delta_{sat}$) of protons of **29** and **30** in the ^{1}H-NMR spectra of complexes of dendrophane **1**. In a soln. with [**2**] = 1.14 mM and [**29**] = 0.48 mM in D_2O buffer/$(CD_3)_2SO$ 93.2:6.8, the following upfield shifts of the naphthalene protons were observed: 0.74 (H–C(1)), 0.43 (H–C(3)), and 0.80 (H–C(4) ppm).

concentration were evaluated by nonlinear least-squares curve-fitting [21] and yielded similar results. Both naphthalene-2,7-diol (**29**) and 6-(*p*-toluidino)naphthalene-2-sulfonate (TNS; **30**) formed 1:1 complexes with **1** and **2** revealing association constants K_a in the order of 10^3 to 10^4 l mol^{-1} (*Table 1*). The complex stabilities were thus similar to those measured for complexes of the non-dendritic core cyclophane **7** [11].

The complexation-induced changes in ^{1}H-NMR chemical shifts observed for protons of both cyclophane and substrate produced unambiguous evidence for specific inclusion

Table 1. *Association Constants* K_a [1 mol^{-1}] *and Complexation Free Enthalpies* $\Delta G°$ [kcal mol^{-1}] *for Dendrophane Complexes in 0.066M Phosphate Buffer in* D_2O (pD 8.4, NMR) *or* H_2O (pH 8.0, Fluorescence) *at 300 K*

Dendrophane	Guest	K_a [1 mol^{-1}]	$\Delta G°$ [kcal mol^{-1}][a])
^{1}H-NMR Titrations[b]):			
7	**29**[c])	4300	−5.0
1	**29**[c])	1800	−4.4
2	**29**[c])	1700	−4.3
22	**29**[d])	930	−4.0
1	**30**[e])	8000	−5.3
2	**30**[e])	2200	−4.6
1	**31**[c])	370	−3.5
1	**32**[c])	230	−3.2
1	**33**[f])	1170	−4.2
Fluorescence Titrations[g])			
1	**30**	10500	−5.5
2	**30**	8000	−5.3
3	**30**	5500	−5.1

[a]) Uncertainties in $\Delta G°$: ± 0.1 kcal mol^{-1}. [b]) Concentration of the component held constant: 0.5–1.0 mM: concentration of the variable component: 0.25–2.5 mM. [c]) In D_2O buffer/$(CD_3)_2SO$ 97.3:2.7. [d]) In pure D_2O buffer. [e]) In D_2O buffer/CD_3OD 85:15. [f]) In D_2O buffer/CD_3OD 1:1. [g]) In pure aqueous phosphate buffer, [**30**] = $1 \cdot 10^{-5}$ M, [dendrophane] = 10^{-5} to $6 \cdot 10^{-4}$ M.

complexation in the cavity of the cyclophane core. In titrations with **1** and **2** at constant host concentration, downfield shifts of 0.20 to 0.26 ppm (at *ca.* 80% saturation binding) for the *s* of the eight aromatic cyclophane H-atoms were measured; such changes in chemical shift are characteristic for [*n*.1.*n*.1]paracyclophane-arene complexation. In titrations at constant guest concentration, the guest protons of **29** and **30** (TNS) were strongly and differentially shifted to higher field (*Fig. 7*). These changes in ^{1}H-NMR chemical shift would not be observed in case of nonspecific guest incorporation within the dendritic branches. With increasing degree of branching, the host-guest exchange rates were significantly lowered as indicated by the line broadening of the ^{1}H-NMR resonances of the guest at comparable complexation strength. Titrations with [G-1]-12-acid **1** gave sharp, resolved signals, whereas, in titrations with the second-generation dendrophane **2**, the peaks were already strongly broadened. Titrations with [G-3]-108-acid **3** no longer displayed resolved signals at all, a finding attributed to slow host-guest exchange processes on the ^{1}H-NMR time scale.

The water-soluble dendrophane **22** with triethylene glycol monomethyl ether end groups also formed a 1:1 complex with the naphthalenediol **29** in pure D_2O buffer; however, its stability was significantly reduced with respect to that of the complex with the corresponding [G-1]-12-ester **1** (*Table 1*). This could indicate that the triethylene glycol monomethyl ether groups of **22**, which are of reduced polarity compared to the poly(ether amide) branches in **1**, partially occupy the binding cavity. As an additional difference, the host-guest exchange kinetics in the complexation by **22** is slowed down compared to the binding by **1**, as indicated by strong broadening of the guest resonances of **29** held constant during the titration.

Demonstration of inclusion complexation by the third-generation dendrophane **3** was obtained in fluorescence titrations with the fluorescence probe TNS (**30**) [22a,b] [23] in aqueous phosphate buffer (0.066M, pH 8.0) at 300 K. These titrations were also evaluated by nonlinear least-squares fitting and provided association constants in satisfactory agreement with those obtained by the ^{1}H-NMR studies. Upon changing from **1** to **2** and to **3**, the stability of the formed 1:1 complexes only varied slightly (ΔG° between -5.5 and -5.1 kcal mol^{-1}; *Table 1*). While the dendrophanes did not give any indication for formation of TNS complexes other than of 1:1 stoichiometry, the core cyclophane **7** in both fluorescence and ^{1}H-NMR titrations demonstrated a strong tendency for formation of host-guest complexes with 2:1 stoichiometry in which the two apolar moieties of the substrate are separately included in two cavity binding sites [11]. The dendritic branches in **1**–**3** apparently prevent an on-top-stacking of two cyclophanes, needed for a 2:1 host-guest complexation mode. Fluorescence titrations were also performed with the polydisperse [G-4]-324-acid **14**, and an association constant $K_a \approx 2000$ l mol^{-1} ($\Delta G^\circ = 4.5$ kcal mol^{-1}) was estimated for the 1:1 complex with TNS. Thus, even on the fourth-generation stage, the binding cavity remains accessible and open to the guest.

TNS (**30**) is known as a fluorescent probe whose emission intensity and maximum are highly dependent on solvent polarity [23]. Fluorescence titrations with TNS suggested that the cyclophane binding site becomes more apolar with increasing dendritic generation number. The maximum of the fluorescence emission of TNS was sequentially blue-shifted when changing from the complex of the core cyclophane **7** to those of dendrophanes **1**–**3** and **14** (*Table 2*). In addition, the fluorescence intensity strongly increased (up to 300-fold) in this series. The data show that the dendritic shell influences the micropolarity [2] [24] of the dendrophane binding sites and reduces it in aqueous solution to a polarity corresponding closely to that of EtOH. Interestingly, the change from third- to fourth-generation dendrophane no longer shifted the emission maximum of bound TNS (*Table 1*). This observation is in agreement with previous computer modeling [17] indicating that full dendritic encapsulation of the core binding site would occur at the stage of the third generation.

To investigate a geometrically undefined incorporation of substrates in the dendritic branches, fluorescence titrations were also attempted with the [G-3]-81-acid **15** which does not contain a specific recognition site. However, the bathochromic shift of the emission maximum and the emission intensity of **30** were so weak that no significant incorporation of the fluorophore in the dendrimer could be detected. Both fluorescence

Table 2. *Emission Maxima* (λ_{max}) *of TNS* (**30**; $c = 10$ μM) *in Aqueous Phosphate Buffer* (pH 8.0) *Bound in the Cyclophane Cavity of Different Dendrophanes* ($c = 0.25$ mM; $\lambda_{exc} = 360$ nm, $T = 300$ K). The emission maxima of TNS in selected protic solvents are given for comparison [23a].

Environment	λ_{max} [nm]	Environment	λ_{max} [nm]
[G-0]-4-Acid **7**	*ca.* 450[a])	[G-4]-324-Acid **14** (polydisperse)	432
[G-1]-12-Acid **1**	443	H_2O	*ca.* 500
[G-2]-36-Acid **2**	435	MeOH	443
[G-3]-108-Acid **3**	432	EtOH	429

[a]) Value measured in the sections of binding titrations where 1:1 association was predominant.

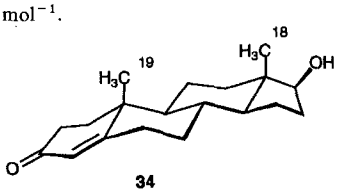
and ^{1}H-NMR titration data (*Fig. 7*) thus suggest that dendrophane recognition is restricted to the initiator core rather than to the dendritic shell.

2.4.2. *Steroid Complexation by Dendrophanes* **4**–**6**. Testosterone (**34**) recognition by the dendrophanes **4**–**6** was investigated by 500-MHz ^{1}H-NMR binding titrations in borate-buffered [25] D_2O (pD 10.5)/CD_3OD 1:1 at 298 K (*Table 3*). High concentrations (0.1–0.5M) of borate buffer were used due to the multiple carboxylic-acid groups of the higher-generation dendrophanes. Preliminary studies had indicated that the measured association constants were strongly dependent on the buffer concentration and the pD of the solution; whether this is due to aggregation effects or to changes in their hydrodynamic volumes [26] remains unclear. Titrations with dendrophane **4** and **5** were executed either at constant guest or constant host concentration; titrations with the higher-generation derivative **6** were only performed at constant guest concentration. In a typical titration, the constant concentration was 0.5 mM and the variable one changed between 0.5 and 5.0 mM which permitted to reach 70 to 90% saturation binding.

Table 3. *Association Constants* K_a *and Complexation Free Enthalpies* $\Delta G°$ *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 parenthesis, the maximum observed complexation-induced upfield shifts, $\Delta\delta_{sat}$ and $\Delta\delta_{max\ obs}$, for the resonances of Me(19) and Me(18) of the bound steroid held at constant concentration.

Host	Guest	K_a [1 mol^{-1}]	$\Delta G°$[a]) [kcal mol^{-1}]	$\Delta\delta_{sat}$ ($\Delta\delta_{max\ obs}$)	
				Me(19)	Me(18)
8	**34**	1350	−4.3	−0.81 (−0.67)	−0.24 (−0.18)
4	**34**	700[b])	−3.9	−0.97 (−0.74)	−0.25 (−0.19)
5	**34**	750[b])	−3.9	−1.60 (−1.22)	−0.35 (−0.27)
6	**34**	1100	−4.2	−1.33 (−1.13)	−0.30 (−0.24)

[a]) Uncertainties in $\Delta G°$: ± 0.1 kcal mol^{-1}. [b]) Inverse titration at constant dendrophane concentration gives K_a = 1200 (**4** · **34**) and 800 (**5** · **34**) 1 mol^{-1}.



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The three dendrophanes **4**–**6** of first to third generation and the core cyclophane **8** [5b] [11] formed 1:1 inclusion complexes of comparable stability ($\Delta G°$ between −3.9 and −4.3 kcal mol^{-1}) with testosterone (**34**) in basic D_2O/CD_3OD 1:1, indicating that the cyclophane binding site remains open and accessible. The steroid binds axially in the cyclophane cavity rather than in nonspecific, fluctuating voids in the dendritic shell. This is clearly evidenced by the large complexation-induced changes in chemical shift observed for the steroidal Me(19) and Me(18) resonances in titrations at constant guest concentration (*Table 3*). Cavity inclusion was also apparent from the downfield shifts of the three terphenyl resonances ($\Delta\delta_{sat}$ = + 0.35 to + 0.51 ppm) and the O–CH_2CON resonance ($\Delta\delta_{sat}$ = + 0.33 ppm) as well as from the upfield shift of the naphthyl(phenyl)methane CH_2 signal ($\Delta\delta_{sat}$ = − 0.16 ppm) of [G-1]-12-acid **4** in the ^{1}H-NMR

binding titration at constant dendrophane concentration. 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 3*), possibly induced by a different, generation-dependent complex geometry. ^{1}H-NMR Titration studies with the polydisperse [G-4]-324-acid **27** were problematic, and meaningful titration curves could not be obtained.

In contrast to the arene binding studies with dendrophanes **1**–**3**, the guest signals could be nicely followed in all ^{1}H-NMR titrations with dendrophanes **4**–**6**, although they increasingly broadened with increasing dendrophane generation. Apparently, the host-guest exchange kinetics are fast on the ^{1}H-NMR time scale (first-order decomplexation rate constant $k_{decompl} > 10^2$–10^3 s^{-1}) even in studies with the third-generation dendrophane **6**, in which the dendritic branches are densely packed in a globular layer of *ca.* 2 nm radius around the core. The faster exchange kinetics in the steroid-binding dendrophane series **4**–**6**, as compared to the arene-binding series **1**–**3**, is probably due to the less dense dendritic superstructure as a result of the larger initiator core **8** as compared to **7**. Steroid-receptor exchange kinetics have been measured in water by fluorescence relaxation kinetics [27]. In collaboration with *M. A. Kempfle*, the first-order decomplexation rate constants in 0.2M aqueous borate buffer (pD 10.5) of the fluorescent steroids 6,7,8,14-tetradehydrotestosterone and 4,6,8(14)-ergostatrien-3-one bound to [G-3]-108-acid **6** were determined as $1.5 \cdot 10^4$ and $3.4 \cdot 10^3$ s^{-1}, respectively [28].

3. Conclusions. – With the two series of first- to third-generation dendrophanes **1**–**3** and **4**–**6** bearing 12, 36, and 108 terminal carboxy groups, respectively, the first water-soluble dendrimers with a defined active recognition site at the central core were prepared and their molecular recognition properties investigated. The target molecules were obtained by hydrolysis of the corresponding poly(methyl carboxylates) **10**–**12** and **23**–**25**, respectively. The first-generation derivatives **10** and **23** were crystalline solids, and [G-1]-12-ester **10** was characterized by X-ray crystallography. The second- and third-generation compounds **11** and **24** ([G-2]), and **12** and **25** ([G-3]) were prepared by divergent synthesis using peptide-coupling methodology, subsequently purified by GPC, and subjected to extensive spectral characterization. They were all obtained in pure form according to the ^{13}C-NMR spectral criterion. The MALDI-TOF mass spectra of the third-generation derivative **25** (mol. wt. 19328 D) displayed the molecular ions as base peak, accompanied by a series of ions $[M - n(1041 \pm 7)]^+$ which we tentatively assign as characteristic fragment ions of the poly(ether amide) cascade. A similar fragmentation pattern was also observed in the spectra of other higher poly(ether amide) dendrimers such as **21**. The high purity of **25**, prepared by the divergent route, was confirmed by its independent synthesis following a semi-convergent strategy: the spectral properties of samples of **25** resulting from either divergent or semi-convergent synthesis were found to be identical. Whereas pure material was obtained up to the third generation, attempts to prepare the monodisperse fourth-generation dendrophanes [G-4]-328-esters **13** and **27** having molecular weights above 56000 D failed. According to the MALDI-TOF mass spectra, only a polydisperse mixture of incompletely coupled products was isolated after GPC purification. These findings clearly demonstrate the limits of divergent dendrimer synthesis; we believe that it will be very difficult to prepare poly(ether amide) dendrimers larger than the third-generation dendrophanes **12** and **25** in pure form using the divergent synthesis strategy.

Dendrophanes **1**–**3** form 1:1 host-guest complexes with benzene and naphthalene derivatives in basic aqueous buffer containing small amounts of organic cosolvent, whereas **4**–**6** bind testosterone in basic aqueous buffer/MeOH 1:1. A combination of ^{1}H-NMR and fluorescence binding titrations provided the following, unprecedented results.

i) The structurally well-defined cyclophane recognition sites at the center of the dendrophanes remain open and effective at all dendritic generations studied. Hydrophobic collapse of the dendritic branches with inhibitory occupation of the cavity binding site probably occurs to some extent in water but does not affect binding strongly. Thus, the dendrophanes form inclusion complexes of similar stability to those formed by the initiator core cyclophanes **7** and **8**, respectively. In all complexes, the substrates are exclusively located in the central cyclophane cavities, and nonspecific incorporation into fluctuating voids in the dendritic shell is negligible.

ii) Fluorescence titrations with the fluorescent probe TNS (**30**) demonstrated that the micropolarity around the binding cavity in the series **1**–**3** became significantly reduced with increasing dendritic superstructure. The micropolarity at the center of the third-generation dendrophane **3** in water is comparable to that of EtOH. It does not become further reduced upon changing to the polydisperse fourth-generation derivative [G-4]-324-acid **14** which shows that full encapsulation of the central cyclophane cavity is established at the third-generation stage. However, despite the more lipophilic character of the binding site in dendrophanes as compared to cyclophanes, complexation strength did not increase.

iii) The host-guest exchange kinetics observed for all dendrophanes is remarkably fast: ^{1}H-NMR binding titrations, which rely on fast host-guest exchange ($k_{decompl} > 10^2$–10^3 s^{-1}), were possible with all dendrophanes, except with **3** which showed slow host-guest exchanges on the NMR time scale. The fast host-guest exchange kinetics with **6** was confirmed by fluorescence relaxation measurements with fluorescent steroidal substrates [28]. This result is in sharp contrast to the findings by *Meijer* and coworkers [8], who observed substrate encapsulation for hours and days in their poly(propylene imine) dendrimers. These different findings can readily be explained: The *Meijer* dendrimers have a tight, densely packed superstructure diverging from a small initiator core. In contrast, the four dendritic wedges in **1**–**6** are attached to large, nanometer-sized cyclophane cores which produces apertures through which substrates can rapidly enter or leave the binding cavity. Furthermore, the *Meijer* dendrimers possess both strongly H-bonding and sterically encumbering surface groups which generate tight substrate encapsulation at the interior, whereas the carboxylates at the dendrophane surfaces will not densely pack for electrostatic reasons.

The effect of the size of the central core on the host-guest exchange kinetics is nicely visible in the comparison between the third-generation dendrophanes **3** and **6**. Whereas **3**, with its smaller cyclophane core, shows slow arene-binding kinetics on the NMR time scale, compound **6**, with its larger initiator core, complexes the much bulkier steroidal substrates with fast exchange kinetics on that time scale.

iv) The reduced micropolarity [2] at the central cyclophane core and the fast host-guest exchange kinetics make H_2O-soluble dendrophanes attractive targets as catalytically active mimics of globular enzymes. Such *catalytic dendrophanes*, with catalytic cyclophane initiator cores [29] that are shielded from the aqueous solution by dendritic

superstructure, are now under construction, targeting the acceleration of reactions which particularly benefit from a reduced environmental polarity.

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Experimental Part

General. See [11]. BtOH (1-hydroxybenzotriazol) from *Fluka* containing 10–15% H_2O was used without further purification. Prep. gel permeation chromatography (GPC): *Biobeads S-XI* from *Biorad*, eluent PhMe; detection at 238 nm by UV detector from *Knauer*; the polymer was dispersed in PhMe overnight and filled into a glass column (85 × 7 cm); for loading, the dendrophanes were dissolved in a minimum amount of PhMe. Anal. GPC: *TSKgel-G-3000-HXL* column (30 × 0.78 cm, separation range 1000–100000 D) from *TosoHaas*. Dialysis: dialysis tube made from benzylated cellulose from *Sigma*. Drying under high vacuum (h.v.): $5 \cdot 10^{-5}$ Torr. Fluorescence titrations: *Spex-Fluorolog-1680* 0.22 m double spectrometer, 450-W Xe lamp. Explicit ^{1}H-NMR peak assignments for the lower-generation dendrophanes were reported in [5] and are not repeated here. For ^{1}H-NMR binding titrations and the preparation of D_2O buffers, see [11]. MALDI-TOF-MS (*m/z* (%)): *Bruker Reflex* spectrometer with 2-(4-hydroxyphenylazo)benzoic acid (HABA), sinapic acid, or α-cyano-4-hydroxycinnamic acid (CCA) as matrix; positive-ion mode. In general, samples were prepared by mixing 1 µl of a soln. of the dendrophane (*ca.* 1 mg ml^{-1}) in CH_2Cl_2 with 1 µl of a soln. of the matrix (0.1M) in MeCN/EtOH/H_2O 50:45:5. A 1 µl sample was then deposited on the probe tip, dried under mild vacuum, and analyzed. For MALDI-TOF and FAB mass spectra of dendrimers, the experimentally observed highest peak in the molecular-ion cluster is reported followed in parenthesis by the calculated isotopic molecular formula. Cascade molecules were named by Dr. *M. V. Kisakürek, Helv. Chim. Acta.*

Dodecamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI}-{(5,14,20,29,32,33,36,37-Octamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2,17,17-tetrayl)tetrakis{[(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}dodecakis[propanoate] (**10**). A soln. of **7** [11] (0.4 g, 0.47 mmol), **9** (2.6 g, 9.50 mmol), BtOH (0.226 g, 1.97 mmol), and DCC (0.8 g, 3.88 mmol) in THF (40 ml) was stirred for 1 h at r.t. and then heated to 50°. DCC (0.8 g, 3.88 mmol) was added, and the mixture was stirred for 3 d at 50°. Evaporation yielded an oil which was dissolved in PhMe and filtered to remove the formed *N,N'*-dicyclohexylurea. After evaporation, GPC yielded **10** (0.60 g, 60%) as a thick oil which crystallized upon standing. Crystals for X-ray analysis were obtained by diffusion of hexane into a soln. of **10** in PhMe. M.p. 96–97° (MeOH). IR ($CHCl_3$): 3404*w*, 3005*m*, 2953*m*, 2877*w*, 1734*s*, 1672*m*, 1647*m*, 1519*w*, 1438*s*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.85–1.95 (*m*, 8 H); 2.10 (*s*, 24 H); 2.51 (*t*, *J* = 6.4, 24 H); 3.06 (*s*, 8 H); 3.45 (*s*, 24 H); 3.59 (*t*, *J* = 6.4, 24 H); 3.67 (*s*, 36 H); 3.75–3.85 (*m*, 8 H); 6.43 (*s*, 4 H); 6.61 (*s*, 8 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 16.38; 26.71; 34.41; 45.26; 46.65; 51.28; 59.23; 66.33; 68.47; 71.78; 127.42; 129.34; 141.74; 153.52; 171.21; 171.63. FAB-MS: 2299.1 (100, MH^+; calc. for $^{12}C_{113}{}^{13}C_1H_{169}N_4O_{44}$: 2298.1). X-Ray: see [5a].

3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI}-{(5,14,20,29,32,33,36,37-Octamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2,17,17-tetrayl)tetrakis{[(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}dodecakis[propanoic Acid] (**1**). A soln. of **10** (0.143 g, 0.062 mmol) in THF/MeOH 1:2 (6 ml) and LiOH · H_2O (300 mg, 7.15 mmol) in H_2O (4 ml) were stirred at r.t. for 2 d. After evaporation, H_2O was added, the aq. soln. washed with $CHCl_3$ and acidified to pH 1 with conc. aq. HCl soln. The aq. mixture was extracted with AcOEt (5 ×), the combined org. phase evaporated, and traces of AcOH and H_2O were removed by distillation with heptane and THF. Drying at r.t./h.v. gave **1** (120 mg, 80%). Colorless hygroscopic foam. IR (KBr): 3400*m* (br.), 2925*m*, 1715*s*, 1644*m*, 1198*m*, 1110*s*. ^{1}H-NMR (500 MHz, (D_8)THF): 1.80–1.90 (*m*, 8 H); 2.01 (*s*, 24 H); 2.35 (*t*, *J* = 6.5, 24 H); 3.06 (br. *s*, 8 H); 3.35 (*s*, 24 H); 3.45 (*t*, *J* = 6.5, 24 H); 3.70–3.80 (*m*, 8 H); 6.60 (*s*, 8 H); 6.75 (br. *s*, 4 H). ^{13}C-NMR (125 MHz, (D_8)THF): 17.13; 27.68; 35.19; 45.78; 48.18; 60.84; 69.78; 72.78; 129.01; 130.18; 143.17; 155.19; 172.69; 173.05 (1 resonance overlapped by THF peaks). FAB-MS: 2152 ([M + Na]$^+$), 2130 (M^+; calc. for $^{12}C_{101}{}^{13}C_1H_{144}N_4O_{44}$: 2129.9).

Hexatriacontamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV}-{(5,14,20,29,32,33,36,37-Octamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2-17,17-tetrayl)tetrakis{[(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}hexatriacontakis[propanoate] (**11**).

A soln. of **1** (207 mg, 0.097 mmol), **9** (1.3 g, 3.4 mmol), BtOH (0.37 g, 2.7 mmol), and DCC (0.7 g, 3.40 mmol) was stirred at r.t. for 3 d, after which the suspension was evaporated. The residual oil was dissolved in PhMe, the soln. filtered and evaporated and the residue purified by GPC and dried at 40°/h.v.: **11** (0.268 g, 45%). Viscous oil. IR ($CHCl_3$): 3425*w*, 3370*w*, 3003*m*, 2953*m*, 2877*m*, 1732*s*, 1670*m*, 1517*m*, 1112*s*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.85–1.95 (*m*, 8 H); 2.08 (*s*, 24 H); 2.33 (*t*, *J* = 6.7, 24 H); 2.50 (*t*, *J* = 6.3, 72 H); 3.11 (br. *s*, 8 H); 3.39 (*s*, 24 H); 3.55 (*t*, *J* = 6.3, 24 H); 3.60–3.72 (*m*, 252 H); 3.75–3.83 (*m*, 8 H); 6.17 (*s*, 12 H); 6.45 (*s*, 4 H); 6.62 (*s*, 8 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 16.74; 26.97; 34.71; 37.15; 44.97; 46.93; 51.63; 59.63; 59.80; 66.74; 67.51; 68.80; 69.10; 72.10; 127.76; 129.67; 142.21; 153.81; 170.92; 171.54; 172.01. MALDI-TOF-MS (HABA): 6508 (100, $[M + K]^+$), 6488 (100, $[M + Na]^+$; calc. for $^{13}C_3{}^{12}C_{291}H_{468}N_{16}O_{140}$: 6489), 5444.

3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV}-{(5,14,20,29,32,33,36,37-Octamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2-17,17-tetrayl)tetrakis{[(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}hexatriacontakis[propanoic Acid] (**2**). A mixture of **11** (180 mg, 0.03 mmol) and 1M aq. LiOH (4 ml, 4 mmol) in H_2O/MeOH/THF 2:2:1 (8 ml) was stirred at r.t. for 3 d. After evaporation of the org. cosolvents, a few ml of H_2O were added to the residue, and the soln. was transferred into a dialysis tube. After dialysis against H_2O (2–3 l) for 24 h, evaporation and drying at 40°/h.v. yielded **2** (150 mg, 90%). Colorless powder. ^{1}H-NMR (400 MHz, D_2O): 2.00–2.10 (*m*, 8 H); 2.15 (br. *s*, 24 H); 2.40–2.60 (*m*, 96 H); 3.30–3.80 (*m*, 200 H); 3.98–4.02 (*m*, 8 H); 6.81 (*s*, 8 H). ^{13}C-NMR (100 MHz, D_2O): 18.80; 28.27; 40.38; 47.06; 50.09; 60.61; 62.89; 69.23; 71.43; 75.11; 130.82; 132.99; 144.69; 155.89; 171.12; 176.5; 182.91 (17 out of 20 expected resonances).

Octahecamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV},3^{XXXVI},3^{XXXVII},3^{XXXVIII},3^{XXXIX},3^{XL},3^{XLI},3^{XLII},3^{XLIII},3^{XLIV},3^{XLV},3^{XLVI},3^{XLVII},3^{XLVIII},3^{XLIX},3^{L},3^{LI},3^{LII},3^{LIII},3^{LIV},3^{LV},3^{LVI},3^{LVII},3^{LVIII},3^{LIX},3^{LX},3^{LXI},3^{LXII},3^{LXIII},3^{LXIV},3^{LXV},3^{LXVI},3^{LXVII},3^{LXVIII},3^{LXIX},3^{LXX},3^{LXXI},3^{LXXII},3^{LXXIII},3^{LXXIV},3^{LXXV},3^{LXXVI},3^{LXXVII},3^{LXXVIII},3^{LXXIX},3^{LXXX},3^{LXXXI},3^{LXXXII},3^{LXXXIII},3^{LXXXIV},3^{LXXXV},3^{LXXXVI},3^{LXXXVII},3^{LXXXVIII},3^{LXXXIX},3^{XC},3^{XCI},3^{XCII},3^{XCIII},3^{XCIV},3^{XCV},3^{XCVI},3^{XCVII},3^{XCVIII},3^{XCIX},3^{C},3^{CI},3^{CII},3^{CIII},3^{CIV},3^{CV},3^{CVI},3^{CVII}-{(5,14,20,29,32,33,36,37-Octamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2-17,17-tetrayl)tetrakis{[(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris{(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}}octaheca[propanoate] (**12**). A mixture of **2** (120 mg, 0.02 mmol), **9** (1.2 g, 3.16 mmol), BtOH (0.22 g, 1.6 mmol), and DCC (0.67 g, 1.77 mmol) in THF (10 ml) was stirred at 45° for 2 d, while additional DCC (0.5 g, 1.31 mmol) was added after 24 h. After evaporation, PhMe was added, and the mixture was filtered. Evaporation of the filtrate followed by GPC and drying at 40°/h.v. afforded **12** (0.20 g, 52%). Viscous clear oil. IR ($CHCl_3$): 2953*w*, 1732*s*, 1670*m*, 1111*m*. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.85–1.95 (*m*, 8 H); 2.08 (*s*, 24 H); 2.37 (*t*, *J* ≈ 5, 72 H); 2.50 (*t*, *J* = 6.5, 216 H); 2.78–2.85 (*m*, 24 H); 3.0 (br. *s*, 8 H); 3.39 (*s*, 24 H); 3.42–3.47 (*m*, 24 H); 3.60–3.70 (*m*, 900 H); 3.78–3.84 (*m*, 8 H); 6.3 (br. *s*, 52 H); 6.54 (*s*, 8 H). ^{13}C-NMR (125 MHz, $CDCl_3$): 16.65; 26.50; 34.64; 36.73; 37.03; 51.60; 59.10; 59.79; 59.92; 66.70; 67.46; 69.00; 72.10; 127.5 (br.); 129.67; 142.21; 153.83; 170.64; 171.00; 171.99 (20 out of 26 expected resonances). MALDI-TOF-MS (CCA): 18958 (100, M^+; calc. for $^{12}C_{825}{}^{13}C_9H_{1368}N_{52}{}^{16}O_{427}{}^{18}O_1$: 18971), 17937, 15850, 14521, 4556.

3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV},3^{XXXVI},3^{XXXVII},3^{XXXVIII},3^{XXXIX},3^{XL},3^{XLI},3^{XLII},3^{XLIII},3^{XLIV},3^{XLV},3^{XLVI},3^{XLVII},3^{XLVIII},3^{XLIX},3^{L},3^{LI},3^{LII},3^{LIII},3^{LIV},3^{LV},3^{LVI},3^{LVII},3^{LVIII},3^{LIX},3^{LX},3^{LXI},3^{LXII},3^{LXIII},3^{LXIV},3^{LXV},3^{LXVI},3^{LXVII},3^{LXVIII},3^{LXIX},3^{LXX},3^{LXXI},3^{LXXII},3^{LXXIII},3^{LXXIV},3^{LXXV},3^{LXXVI},3^{LXXVII},3^{LXXVIII},3^{LXXIX},3^{LXXX},3^{LXXXI},3^{LXXXII},3^{LXXXIII},3^{LXXXIV},3^{LXXXV},3^{LXXXVI},3^{LXXXVII},3^{LXXXVIII},3^{LXXXIX},3^{XC},3^{XCI},3^{XCII},3^{XCIII},3^{XCIV},3^{XCV},3^{XCVI},3^{XCVII},3^{XCVIII},3^{XCIX},3^{C},3^{CI},3^{CII},3^{CIII},3^{CIV},3^{CV},3^{CVI},3^{CVII}-{(5,14,20,29,32,33,36,37-Octamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2-17,17-tetrayl)tetrakis{[(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}}octaheca[propanoic Acid] (**3**). A mixture of **12** (128 mg, 0.0067 mmol) and 1M aq. LiOH (0.4 ml, 0.4 mmol) in THF/MeOH 1:1 (4 ml) was stirred at r.t for 3 d. Workup as described for **2** provided **3** which was used in the fluorimetric binding studies.

[G-4]-324-Ester **13** *and [G-4]-324-Acid* **14** (Polydisperse mixtures). A soln. of **3** (50 mg, 0.0029 mmol), **9** (1.2 g, 3.16 mmol), DCC (0.9 g, 4.40 mmol), and BtOH (0.37 g, 2.7 mmol) was stirred in THF (10 ml) for 3 d at r.t. Workup as described for **11** followed by chromatography (SiO_2, $CHCl_3$/MeOH 95:5) afforded **13** (0.056 g, 35%). Viscous clear oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.90–2.00 (br. *m*, *ca.* 70 H); 2.30–2.40 (br. *m*, *ca.* 165 H);

2.45–2.70 (br. *m*, *ca.* 400 H); 3.70–3.80 (br. *m*, *ca.* 1700 H); 6.53, 6.60 (2 br. *s*, *ca.* 100 H). MALDI-TOF-MS (sinapic acid): 38000–56000 with a maximum around 48300 (calc. for $^{12}C_{2425}{}^{13}C_{29}H_{4068}N_{160}{}^{16}O_{1290}{}^{18}O_2$: 56488).

A mixture of **13** (58 mg, 0.001 mmol), 1M aq. LiOH (0.4 ml, 0.4 mmol), and MeOH (2 ml) was stirred at r.t. for 3 d. The crude poly(lithium carboxylate) of **14** obtained by evaporation and drying at r.t./h.v. was used without further purification for fluorimetric binding studies.

Nonamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII}-{(Benzene-1,3,5-triyl)tris{[(carbonylimino)(methanetetrayl)]-tris(methyleneoxy)}}nonakis[propanoate] (**17**). To a soln. of **16** (2.1 g, 7.9 mmol) in CH_2Cl_2 (20 ml), **9** (7.7 g, 20 mmol) and Et_3N (3 ml) were added at 0°. After stirring for 14 h at r.t., the solvent was evaporated and the residual thick oil was dissolved in AcOEt. Washing (H_2O), drying ($MgSO_4$), evaporation, and chromatography (SiO_2, CH_2Cl_2/MeOH 95:5) gave **17** (5.2 g, 50%). Thick colorless oil. IR ($CHCl_3$): 3620*w*, 3019*m*, 1734*m*, 1667*m*, 1516*m*, 438*m*, 1206*s*. ^{1}H-NMR (200 MHz, $CDCl_3$): 2.53 (*t*, *J* = 6.2, 18 H); 3.60 (*s*, 27 H); 3.69 (*t*, *J* = 6.2, 18 H); 3.79 (*s*, 18 H); 6.72 (*s*, 3 H); 8.23 (*s*, 3 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 34.42; 51.37; 60.23; 66.46; 68.70; 128.27; 135.56; 166.00; 171.70. FAB-MS: 1294.6 (100, M^+).

Heptacosamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI}-{(Benzene-1,3,5-triyl)tris{[(carbonylimino)(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}heptacosakis[propanoate] (**19**). A soln. of **17** (0.66 g, 0.51 mmol) in THF/MeOH 1:2 (6 ml) together with LiOH · H_2O (90 mg, 2.15 mmol) in H_2O (4 ml) was stirred at r.t. for 2 d. Workup as described for **1** afforded [G-1]-9-acid **18** as a colorless foam which was used without further purification for the subsequent transformation. Crude **18** (*ca.* 0.6 g, *ca.* 0.51 mmol), **9** (2.63 g, 6.9 mmol), DCC (1.7 g, 5.7 mmol), and BtOH (1.42 g, 6.89 mmol) in THF (15 ml) were stirred at r.t. for 3 d. Workup as described for **11** afforded **19** (1.20 g, 53%). Thick oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.37 (*t*, *J* = 6.6, 18 H); 2.49 (*t*, *J* = 6.6, 54 H); 3.55–3.65 (*m*, 207 H); 3.77 (br. *s*, 18 H); 6.15 (br. *s*, 9 H); 6.80 (br. *s*, 3 H); 8.21 (*s*, 3 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 34.67; 37.19; 51.60; 59.77; 66.70; 67.60; 68.86; 69.04; 135.64; 170.78; 171.90; 171.98 (12 out of 14 expected resonances). MALDI-TOF-MS (CCA): 4440.3 (100, $[M + Na]^+$; calc. for $^{12}C_{190}{}^{13}C_2H_{312}N_{12}NaO_{102}$: 4442.9), 4077, 3729, 3382.

Henoctacontamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV},3^{XXXVI},3^{XXXVII},3^{XXXVIII},3^{XXXIX},3^{XL},3^{XLI},3^{XLII},3^{XLIII},3^{XLIV},3^{XLV},3^{XLVI},3^{XLVII},3^{XLVIII},3^{XLIX},3^{L},3^{LI},3^{LII},3^{LIII},3^{LIV},3^{LV},3^{LVI},3^{LVII},3^{LVIII},3^{LIX},3^{LX},3^{LXI},3^{LXII},3^{LXIII},3^{LXIV},3^{LXV},3^{LXVI},3^{LXVII},3^{LXVIII},3^{LXIX},3^{LXX},3^{LXXI},3^{LXXII},3^{LXXIII},3^{LXXIV},3^{LXXV},3^{LXXVI},3^{LXXVII},3^{LXXVIII},3^{LXXIX},3^{LXXX}-{(Benzene-1,3,5-triyl)tris{[(carbonylimino)(methanetetrayl)]tris-{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris{(methyleneoxy)(1-oxopropane-3,1-diyl)-imino(methanetetrayl)]tris(methyleneoxy)}}}}henoctacontakis[propanoate] (**21**). A soln. of **19** (1.0 g, 0.226 mmol) and 1M aq. LiOH (9.1 ml, 9.1 mmol) in THF/MeOH 1:3 (12 ml) was stirred at r.t. for 2 d. Workup as described for **1** afforded **20** (0.78 g, 85%) as a colorless foam which was used for the subsequent transformation. ^{1}H-NMR (200 MHz, CD_3OD): 2.52 (*t*, *J* = 6.2, 72 H); 3.25–3.35 (*m*, 9 H); 3.65–3.75 (*m*, 126 H); 3.85 (*s*, 18 H); 8.30 (*s*, 3 H).

A mixture of **20** (0.29 g, 0.072 mmol), **9** (1.1 g, 2.9 mmol), DCC (1.7 g, 5.7 mmol), and BtOH (0.26 g, 1.9 mmol) in THF (7 ml) was reacted at r.t. for 3 d. Workup as described for **11**, followed by chromatography (SiO_2, CH_2Cl_2/MeOH 95:5) afforded **21** (0.43 g, 43%). Thick colorless oil which eventually turned into a plastic solid. IR ($CHCl_3$): 3367*w*, 2945*w*, 2877*w*, 1735*s*, 1669*s*, 1517*m*, 1111*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.30–2.40 (*m*, 54 H); 2.50 (*t*, *J* = 6.6, 180 H); 3.5–3.7 (*m*, 711 H); 6.2 (br. *s*, 27 H); 6.75, 6.85 (2 br. *s*, 12 H); 8.2 (*s*, 3 H) (27 third-generation NH not visible). ^{13}C-NMR (100 MHz, $CDCl_3$): 34.66; 37.09; 37.10; 51.45; 51.60; 53.46; 59.77; 59.90; 66.71; 67.50; 69.03; 69.03; 170.40; 170.89; 171.89 (15 out of 19 expected resonances). MALDI-TOF-MS (CCA): 13822 (M^+, calc. for $^{12}C_{589}{}^{13}C_8H_{1014}N_{39}O_{318}$: 13826), 12779, 11737, 10695, 9652, 8610 ($[M - n(1041 \pm 7)]^+$, $n = 1-5$).

3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV},3^{XXXVI},3^{XXXVII},3^{XXXVIII},3^{XXXIX},3^{XL},3^{XLI},3^{XLII},3^{XLIII},3^{XLIV},3^{XLV},3^{XLVI},3^{XLVII},3^{XLVIII},3^{XLIX},3^{L},3^{LI},3^{LII},3^{LIII},3^{LIV},3^{LV},3^{LVI},3^{LVII},3^{LVIII},3^{LIX},3^{LX},3^{LXI},3^{LXII},3^{LXIII},3^{LXIV},3^{LXV},3^{LXVI},3^{LXVII},3^{LXVIII},3^{LXIX},3^{LXX},3^{LXXI},3^{LXXII},3^{LXXIII},3^{LXXIV},3^{LXXV},3^{LXXVI},3^{LXXVII},3^{LXXVIII},3^{LXXIX},3^{LXXX}-{(Benzene-1,3,5-triyl)tris{[(carbonylimino)(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris{(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyl-eneoxy)}}}}henoctacontakis[propanoic Acid] (**15**). A soln. of **21** (50 mg, 0.0036 mmol) and 1M aq. LiOH (0.44 ml, 0.44 mmol) in MeOH (2 ml) was stirred at r.t. for 2 d. The crude poly(lithium carboxylate) salt of **15** obtained by evaporation of the solvent was used directly in the fluorimetric binding assay.

Dodecakis[2-(2-methoxyethoxy)ethoxy)ethyl]3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI}-{(5,14,20,29,32,33,36,37-Octamethyl-7,12,22,27-tetraoxapentacyclo[26.2.2.2^{3,6}.2^{13,16}.2^{18,21}]octatriaconta-3,5,13,15,18,20,28,30,31,33,35,37-dodecaene-2,2,17,17-tetrayl)tetrakis{[(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}dodecakis[propanoate] (**22**). A mixture of **1** (95 mg, 0.045 mmol), triethylene glycol monomethyl ether (4 ml, 3.90 g, 24 mmol), DCC (0.45 g, 2.1 mmol), and BtOH (0.15 mmol, 1 mmol) in THF (3 ml) was stirred for 3 d at r.t. Workup as described for **11** followed by chromatography (SiO_2, CH_2Cl_2/MeOH 92.5:7.5) afforded **22** (0.16 g, 92%). Clear oil. IR ($CHCl_3$): 3001*m*, 2880*m*, 1734*s*, 1667*m*, 1650*m*, 1107*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.88–1.93 (*m*, 8 H); 2.09 (*s*, 24 H); 2.55 (*t*, *J* = 6.3, 24 H); 3.07 (br. *s*, 8 H); 3.35 (*s*, 36 H); 3.41 (br. *s*, 24 H); 3.55–3.75 (*m*, 144 H); 3.78–3.82 (*m*, 8 H); 4.20 (*t*, *J* = 4.8, 24 H); 6.42 (br. *s*, 4 H); 6.59 (*s*, 8 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 16.25; 26.56; 34.03; 46.50; 51.14; 58.57; 59.14; 63.10; 66.15; 68.41; 68.629, 68.63 (2×); 70.09 (2×); 71.49, 127.9; 129.23; 141.61; 153.39; 170.98; 171.10. FAB-MS: 3884.4 (100, M^+; calc. for $^{12}C_{184}{}^{13}C_2H_{312}N_4O_{80}$: 3884.0).

Dodecamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI}-{(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-18,37,40,44-tetrayl)tetrakis{[(4,1-phenylene)oxy(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}dodecakis[propanoate] (**23**). A suspension of **8** (1.87 g, 1.55 mmol), **9** (6.90 g, 18.2 mmol), DCC (4.13 g, 20.0 mmol), and BtOH (530 mg, 3.30 mmol) was stirred at 50° for 4 d. After evaporation, the residue was taken up in CH_2Cl_2 (50 ml), and all solids were removed by filtration. The filtrate was evaporated, and the remaining yellow oil (14 g) was chromatographed (SiO_2 (480 g), CH_2Cl_2/AcOMe 1:1 → 1:2). The product was precipitated from cold MeOH and subsequently recrystallized from MeOH: **23** (2.56 g, 62%). Colorless, tender needles. R_f 0.28 (SiO_2, CH_2Cl_2/AcOMe 1:1). M.p. 99.5–100.0° (MeOH). IR ($CHCl_3$): 3404*w*, 3006*w*, 2953*w*, 2871*w*, 1736*s*, 1679*m*, 1607*m*, 1532*m*, 1509*s*, 1439*m*, 1386*m*, 1179*m*, 1144*m*, 1072*m*, 1005*w*, 949*w*, 850*w*, 836*w*. ^{1}H-NMR (500 MHz, CD_2Cl_2): 1.43–1.48, 1.57–1.62 (2 *m*, 8 H); 2.55 (*t*, *J* = 6.3, 24 H); 3.19, 3.41 (2 *t*, *J* = 5.6, 8 H); 3.65 (*s*, 36 H); 3.71 (*t*, *J* = 6.3, 24 H); 3.75 (*s*, 24 H); 4.04 (*s*, 4 H); 4.43 (*s*, 8 H); 6.71 (*d*, *J* = 2.3, 2 H); 6.86 (*s*, 4 H); 6.88 (*dd*, *J* = 9.0, 2.3, 2 H); 7.00 (*d*, *J* = 8.9, 8 H); 7.16 (*s*, 4 H); 7.32 (*dd*, *J* = 8.5, 1.6, 2 H); 7.50 (*d*, *J* = 8.9, 8 H); 7.59 (*m*, 6 H). ^{13}C-NMR (125 MHz, CD_2Cl_2): 24.85; 25.29; 35.16; 42.03; 51.87; 60.10; 65.70; 67.27; 68.20; 69.54; 71.38; 106.58; 114.88; 119.44; 126.85; 127.34; 128.20; 129.10; 129.25; 130.53; 131.21; 132.97; 133.44; 135.72; 137.15; 138.11; 152.58; 157.12; 157.16; 167.96; 172.19. FAB-MS: 2677 (38, $[M + Na]^+$), 2655 (100, MH^+; calc. for $^{12}C_{137}{}^{13}CH_{172}N_4O_{48}$: 2654.1). Anal. calc. for $C_{138}H_{172}N_4O_{48}$ (2654.9): C 62.43, H 6.53; found: C 62.65, H 6.77.

3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI}-{(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-18,37,40,42-tetrayl)tetrakis{[(4,1-phenylene)oxy(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}dodecakis[propanoic Acid] (**4**). A soln. of **23** (400 mg, 0.15 mmol) and 1M aq. LiOH (8.0 ml, 8.0 mmol) in THF/MeOH 5:1 (24 ml) was stirred at r.t. for 3 d, after which the mixture was acidified under ice-cooling with aq. 1N HCl (8.5 ml; sat. with NaCl). The org. solvents were evaporated at r.t./10^{-2} Torr leaving a gluey solid which was extracted with AcOMe (25 ml). The aq. phase was extracted again with AcOMe, and the combined org. phases were dried (Na_2SO_3), filtered, and evaporated to yield **4** (390 mg, 99%) after drying at 50°/h.v. for 24 h. Colorless foam. ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): 1.25–1.35, 1.40–1.50 (2*m*, 8 H); 2.42 (*t*, *J* = 6.3, 24 H); 2.98–3.08, 3.30–3.40 (2*m*, 8 H); 3.57 (*t*, *J* = 6.3, 24 H); 3.59 (*s*, 24 H); 3.98 (*s*, 4 H); 4.48 (*s*, 8 H); 6.76 (br. *s*, 2 H); 6.85 (br. *d*, *J* = 8.4, 2 H); 6.97 (*d*, *J* = 8.6, 8 H); 7.11 (*s*, 4 H); 7.20 (*s*, 4 H); 7.30 (br. *d*, *J* = 9.0, 2 H); 7.37 (*d*, *J* = 8.6, 8 H); 7.62 (*d*, *J* = 8.4, 2 H); 7.63 (*d*, *J* = 9.0, 2 H); 7.66 (br. *s*, 2 H). ^{13}C-NMR (125 MHz, CD_3OD): 24.32; 25.04; 34.35; 40.37; 59.90; 66.22; 66.76; 66.82; 68.71; 71.37; 106.55; 114.01; 118.84; 126.91; 127.04; 128.13; 128.56; 128.94; 129.81; 130.19; 132.34; 133.24; 135.07; 136.01; 137.83; 151.59; 156.41; 156.48; 169.02; 173.88. FAB-MS: 2487 (100, MH^+), 2397 (35, $[M - C_3H_5O_3]^+$), 2168 (56, $[M + H - C_{13}H_{21}O_9]^+$).

Hexatriacontamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV}-{(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-18,37,40,44-tetrayl)tetrakis{[(4,1-phenylene)oxy(1-oxoethane-2,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}hexatriacontakis[propanoate] (**24**). To a soln. of **4** (390 mg, 0.15 mmol), BtOH (140 mg, 0.90 mmol), and **9** (2.00 g, 5.27 mmol) in THF (10 ml), a soln. of DCC (1.10 g, 5.33 mmol) in THF (2 ml) was added dropwise at 4°, and the mixture was stirred at r.t. for 3 d. After evaporation, the residue was taken up in PhMe, filtered, and purified by GPC to give a viscous oil which was dried at 70°/h.v. for 3 d: **24** (943 mg, 92%). Glassy compound. IR ($CHCl_3$): 3005*w*, 2953*w*, 2882*w*, 1733*s*, 1672*m*, 1605*w*, 1508*m*, 1436*m*, 1364*m*, 1108*s*, 1072*m*, 1015*w*, 846*w*. ^{1}H-NMR (500 MHz, CD_2Cl_2): 1.40–1.48, 1.52–1.62 (2 br. *m*, 8 H); 2.41 (*t*, *J* = 6.4, 24 H); 2.52 (*t*, *J* = 6.3, 72 H); 3.05–

3.15, 3.33–3.40 (2 br. *m*, 8 H); 3.45–3.73 (*m*, 300 H); 4.02 (br. *s*, 4 H); 4.47 (br. *s*, 8 H); 6.14 (*s*, 12 H); 6.71 (br. *d*, $J = 2.4$, 2 H); 6.85 (*dd*, $J = 8.9$, 2.4, 2 H); 6.98 (*s*, 4 H); 7.03 (*d*, $J = 8.8$, 8 H); 7.17 (*s*, 4 H); 7.31 (br. *d*, $J \approx 8$, 2 H); 7.51 (*d*, $J = 8.8$, 8 H); 7.57 (*m*, 6 H). ^{13}C-NMR (125 MHz, CD_2Cl_2): 24.24; 24.87; 34.66; 37.16; 41.61; 51.40; 59.61; 59.80; 65.14; 66.76; 67.51; 67.59; 68.96; 69.07; 70.91; 106.05; 114.39; 118.93; 126.28; 126.88; 127.61; 128.60; 128.77; 130.04; 130.77; 132.45; 133.04; 135.30; 136.72; 137.50; 152.20; 156.63; 156.74; 167.37; 170.69; 171.86. MALDI-TOF-MS (CCA): 6846 (100, $[M + Na]^+$; calc. for $^{13}C_4C_{314}H_{472}N_{16}O_{144} \cdot Na$: 6846.0), 5802 (32).

3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV}-{(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-18,37,40,44-tetrayl)tetrakis{[(4,1-phenylene)oxy(1-oxoethane-2,1-diyl)imino(methanetetrayl)]-tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}hexatriacontakis[propanoic Acid] (**5**). A soln. of **24** (449 mg, 0.066 mmol) and 1N aq. LiOH (8 ml, 8 mmol) in THF/MeOH 1:1 (18 ml) was stirred at r.t. for 48 h and then acidified with 1N aq. HCl (8.5 ml; sat. with NaCl). The org. solvents were evaporated leaving a white solid which was extracted with AcOMe (2 × 25 ml). The combined org. phases were dried (Na_2SO_4) and evaporated leaving a hygroscopic foam which was dried at 70°/h.v. to constant weight: **5** (389 mg, 94%). Colorless glass. MALDI-TOF-MS (CCA): 6341 (100, $[M + Na]^+$; calc. for $^{12}C_{279}$ $^{13}C_3H_{400}N_{16}NaO_{144}$: 6340.4), 6022 (33), 5384 (23), 5064 (80).

Octahecamethyl 3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV},3^{XXXVI},3^{XXXVII},3^{XXXVIII},3^{XXXIX},3^{XL},3^{XLI},3^{XLII},3^{XLIII},3^{XLIV},3^{XLV},3^{XLVI},3^{XLVII},3^{XLVIII},3^{XLIX},3^{L},3^{LI},3^{LII},3^{LIII},3^{LIV},3^{LV},3^{LVI},3^{LVII},3^{LVIII},3^{LIX},3^{LX},3^{LXI},3^{LXII},3^{LXIII},3^{LXIV},3^{LXV},3^{LXVI},3^{LXVII},3^{LXVIII},3^{LXIX},3^{LXX},3^{LXXI},3^{LXXII},3^{LXXIII},3^{LXXIV},3^{LXXV},3^{LXXVI},3^{LXXVII},3^{LXXVIII},3^{LXXIX},3^{LXXX},3^{LXXXI},3^{LXXXII},3^{LXXXIII},3^{LXXXIV},3^{LXXXV},3^{LXXXVI},3^{LXXXVII},3^{LXXXVIII},3^{LXXXIX},3^{XC},3^{XCI},3^{XCII},3^{XCIII},3^{XCIV},3^{XCV},3^{XCVI},3^{XCVII},3^{XCVIII},3^{XCIX},3^{C},3^{CI},3^{CII},3^{CIII},3^{CIV},3^{CV},3^{CVI},3^{CVII},3^{CVIII}-{(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-18,37,40,44-tetrayl)tetrakis{[(4,1-phenylene)oxy(1-oxoethane-2,1-diyl)-imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)-(1-oxo-propane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}}octaheca[propanoate] (**25**). *Method A* (divergent synthesis): To **5** (132 mg, 0.021 mmol), **9** (858 mg, 2.26 mmol), and BtOH (59 mg, 0.38 mmol) in THF (7 ml), a soln. of DCC (466 mg, 2.26 mmol) in THF (2 ml) was added under ice-cooling, and the mixture was stirred for 3 d at r.t. After evaporation, the residue was taken up in PhMe, filtered, purified by GPC, and dried at 70°/h.v. for 3 d: **25** (331 mg. 82%). Glassy compound. IR ($CHCl_3$): 2955*w*, 2886*w*, 1733*s*, 1672*m*, 1605*w*, 1508*w*, 1434*w*, 1365*m*, 1105*m*. ^{1}H-NMR (500 MHz, CD_2Cl_2): 1.35–1.45 (*m*, 8 H); 2.39 (br. *t*, $J \approx 7$, 72 H); 2.52 (br. *t*, $J \approx 7$, 24 H); 2.54 (br. *t*, $J \approx 7$, 216 H); 3.30–3.35 (*m*, 8 H); 3.63–3.73 (br. *m*, 948 H); 4.05 (br. *s*, 4 H); 4.47 (br. *s*, 8 H); 6.23 (br. *s*, 36 H); 6.44 (br. *s*, 12 H); 6.65–7.75 (*m*, 36 H). ^{13}C-NMR (125 MHz, CD_2Cl_2): 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 (33 out of 41 expected resonances). MALDI-TOF-MS (CCA): 19325 (100, M^+; calc. for $^{12}C_{848}$ $^{13}C_{10}H_{1372}N_{52}$ $^{16}O_{431}$ ^{18}O: 19329), 18278 (70), 17236 (16), 16202 (39), 15154 (94), 14117 (14).

Method B (semi-convergent synthesis): Solns. of **28** (2.15 g, 1.51 mmol) [20] in THF (8 ml) and DCC (0.343 g, 1.66 mmol) in THF (4 ml) were added successively at 0° under N_2 to a stirred soln. of BtOH (0.12 g, 0.88 mmol) and **4** (0.106 g, 0.042 mmol) in THF/DMF 4:1 (5 ml). After stirring for 3 d at r.t. and then for 15 min to 50°, the mixture was diluted with CH_2Cl_2, filtered through a plug of *Celite* (AcOEt/hexane 1:1), washed with 10% aq. HCl soln., sat. aq. $NaHCO_3$ soln., and H_2O, dried ($MgSO_4$), and evaporated. GPC followed by drying for 2 d at 70°/10^{-1} Torr and 1 d at 70°/h.v. afforded **25** (0.510 g, 62%).

3,3^{I},3^{II},3^{III},3^{IV},3^{V},3^{VI},3^{VII},3^{VIII},3^{IX},3^{X},3^{XI},3^{XII},3^{XIII},3^{XIV},3^{XV},3^{XVI},3^{XVII},3^{XVIII},3^{XIX},3^{XX},3^{XXI},3^{XXII},3^{XXIII},3^{XXIV},3^{XXV},3^{XXVI},3^{XXVII},3^{XXVIII},3^{XXIX},3^{XXX},3^{XXXI},3^{XXXII},3^{XXXIII},3^{XXXIV},3^{XXXV},3^{XXXVI},3^{XXXVII},3^{XXXVIII},3^{XXXIX},3^{XL},3^{XLI},3^{XLII},3^{XLIII},3^{XLIV},3^{XLV},3^{XLVI},3^{XLVII},3^{XLVIII},3^{XLIX},3^{L},3^{LI},3^{LII},3^{LIII},3^{LIV},3^{LV},3^{LVI},3^{LVII},3^{LVIII},3^{LIX},3^{LX},3^{LXI},3^{LXII},3^{LXIII},3^{LXIV},3^{LXV},3^{LXVI},3^{LXVII},3^{LXVIII},3^{LXIX},3^{LXX},3^{LXXI},3^{LXXII},3^{LXXIII},3^{LXXIV},3^{LXXV},3^{LXXVI},3^{LXXVII},3^{LXXVIII},3^{LXXIX},3^{LXXX},3^{LXXXI},3^{LXXXII},3^{LXXXIII},3^{LXXXIV},3^{LXXXV},3^{LXXXVI},3^{LXXXVII},3^{LXXXVIII},3^{LXXXIX},3^{XC},3^{XCI},3^{XCII},3^{XCIII},3^{XCIV},3^{XCV},3^{XCVI},3^{XCVII},3^{XCVIII},3^{XCIX},3^{C},3^{CI},3^{CII},3^{CIII},3^{CIV},3^{CV},3^{CVI},3^{CVII},3^{CVIII}-{(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-18,37,40,44-tetrayl)tetrakis{[(4,1-phenylene)oxy(1-oxoethane-2,1-diyl)imino(methanetetrayl)]-tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris{[(methyleneoxy)(1-oxopropane-3,1-diyl)imino(methanetetrayl)]tris(methyleneoxy)}}}}octaheca[propanoic Acid] (**6**). A soln. of **25** (331 mg, 0.017 mmol) and 1N aq. LiOH (4 ml, 4 mmol) in THF/MeOH 1:1 (6 ml) was stirred at r.t. for 2 d, then acidified

under ice-cooling with 1N aq. HCl (4.1 ml; sat. with NaCl). Evaporation of the org. solvents at r.t./h.v. left a waxy product which was extracted with AcOMe/THF 1:1 (2 × 10 ml). Evaporation of the combined org. phases and drying at 70°/h.v. to constant weight yielded **6** (274 mg, 90%). Yellowish glassy compound.

[G-4]-324-Ester **26** *and [G-4]-324-Acid* **27** (Polydisperse mixtures). To a stirred soln. of **6** (200 mg, 0.011 mmol) and BtOH (80 mg, 0.52 mmol) in THF (30 ml), **9** (1.40 g, 3.69 mmol) in THF was added at 50°, followed by DCC (740 mg, 3.60 mmol) in THF (5 ml), and the mixture was stirred for 3 d at r.t. Additional **9** (1.00 g, 2.63 mmol) in THF (5 ml) and DCC (300 mg, 1.45 mmol) in THF (5 ml) were added, and stirring was continued for 3 more d. After evaporation, the residue was taken up in PhMe and the soln. filtered and evaporated. GPC followed by drying for 3 d at 70°/h.v. yielded **26** (498 mg, *ca.* 78%). MALDI-TOF-MS (CCA): 37000–57000. The [G-4]-324-ester **26** (490 mg, 0.0086 mmol) together with 1N LiOH (4.0 ml, 4.0 mmol) in MeOH (4 ml) was stirred for 6 d at r.t. Evaporation at r.t. in h.v. yielded the poly(lithium carboxylate) of **27** which was used without further purification in ^{1}H-NMR binding assays.

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