A Calix[4]arene Ureidopeptide Dimer Self-Assembled through Two Superposed Hydrogen Bond Arrays

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Abstract: Dimerization of calix[4]arene ureidopeptides is demonstrated for the first time. Two calix[4]arenes tetrasubstituted in the upper rim with -NHCONH^L^LeuNHC$_8$H$_{17}$ (**1**) and -NHCONH^L^Leu^D^Leu-OMe (**2**) were prepared and studied by NMR, circular dicroism, and gel permeation chromatography. Compound **2** self-assembles through urea−urea hydrogen bonds, as well as by an additional set of hydrogen bonds provided by the peptide side chains, with participation of the ester carbonyls. The absence of such group in **1** causes the monomer structure to be favored in this case.

Introduction

Simple fragments geometrically and functionally complementary may self-assemble to form structures with designed cavities or networks. This association is mediated by noncovalent complementary interactions such as hydrogen bonds, hydrophobic forces, and metal−ligand bonding.[1,2]

The dimerization of calix[4]arenes containing urea substituents (Figure 1) at the upper rim has been extensively studied.[3] Dimerization of such ureidocalixarenes is only possible through the urea groups. Molecules of the size of benzene or cubane can be accommodated into the resulting cavity.[3] It seems likely that use of additional sets of hydrogen bonds could modify the self-assembly process, giving raise to larger cavities. One possibility is to employ scaffolds found in protein secondary structures, such as α-helices or antiparallel β-strands. Peptide chains adopt an extended conformation with the carbonyl and NH groups of each residue located at alternate sides of the backbone in β-strands. In this arrangement, lateral chains extend alternatively above and below the mean plane defined by the resulting hydrogen-bonded sheet. A significant effort has been devoted to the preparation of artificial stable β-sheets in recent years.[4] Specifically, a chemical model of a protein β-sheet dimer where urea groups and peptides combine has been developed by Nowick.[5] Recently, Rebek et al. have reported the synthesis and association properties of a series of ureido calix[4]arenes *N*-linked to α-amino acids.[3g,k] In these examples, urea−urea self-assembly takes place exclusively with isoleucine and valine, other amino acids giving raise to association only with simple phenylureidocalix[4]arenes (heterodimers). The effect of extended peptide chains on the dimerization of ureidocalix[4]-arenes has never been investigated before.

Here we describe the synthesis and association behavior of calix[4]arenes (**1**), having four leucines (octylamide at the C-terminus, both enantiomers prepared) linked to the urea groups at the upper rim, and **2**, with four dipeptide ^L^Leu-^D^Leu-OMe chains on top of the ureas. Two types of self-assembled dimers could be envisaged for these compounds, based on careful CPK models inspection. On one hand, a classical urea−urea dimer may result, with the chains extending away from the seam of hydrogen bonds provided by the urea functions (Figure 2a). In this arrangement, lateral chains lie close enough to mutually interact through a new set of hydrogen bonds that could stabilize the dimeric structure. Alternatively, the formation of urea−amide hydrogen bonds would result in a larger cavity, through an antiparallel β-sheet arrangement (Figure 2b). Alternation of L- and D-configurations would result in assemblies with all the bulky side chains be located outside the cavities, leaving more space for suitable guests to be encapsulated. In principle, such a dimerization mode should be enthalpically favored for calixarenes containing longer chains than **2**, as the number of intermolecular hydrogen bonds would increase. However, the entropy penalty associated with the long, flexible chains could turn into a failure for the dimers to form. The examples shown

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(1) For review articles, see: (a) Philp, D.; Stoddart, J. F *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1154−1196. (b) Rebek, J., Jr. *Chem. Soc. Rev.* **1996**, *25*, 255−264. (c) Conn, M. M.; Rebek, J., Jr. *Chem. Rev.* **1997**, *97*, 1647−1668. (d) de Mendoza, J. *Chem. Eur. J.* **1998**, *4*, 1373−1377.

(2) For designs based on metal coordination, see: (a) Stang, P. J. *Chem. Eur. J.* **1998**, *4*, 19−27. (b) Takeda, N.; Umemoto, K.; Yamaguchi, K.; Fujita, M. *Nature* **1999**, *398*, 794−796. (c) Olenyuk, B.; Whiteford, J. A.; Fechtenkötter, A.; Stang, P. J. *Nature* **1999**, *398*, 796−799.

(3) For self-assembled ureido calix[4]arene dimers, see: (a) Shimizu, K. D.; Rebek, J., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12403−12407. (b) Hamann, B. C.; Shimizu, K. D.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1326−1329. (c) Castellano, R. K.; Rudkevich, D. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1996**, *118*, 10002−10003. (d) Mogck, O.; Böhmer, V.; Vogt, W. *Tetrahedron* **1996**, *52*, 8489−8496. (e) Mogck, O.; Paulus, E. F.; Böhmer, V.; Thondorf, I.; Vogt, W. *Chem. Commun.* **1996**, 2533−2534. (f) Mogck, O.; Pons, M.; Böhmer, V.; Vogt, W. *J. Am. Chem. Soc.* **1997**, *119*, 5706−5712. (g) Castellano, R. K.; Kim, B. H.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1997**, *119*, 12671−12672. (h) Castellano, R. K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 3657−3663. (i) Brody, M. S.; Schalley, C. A.; Rudkevich, D. M.; Rebek, J., Jr. *Angew. Chem., Int. Ed.* **1999**, *38*, 1640−1644. (j) Schalley, C. A.; Castellano, R. K.; Brody, M. S.; Rudkevich, D. M.; Siuzdak, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 4568−4579. (k) Castellano, R. K.; Nuckolls, C.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1999**, *121*, 11156−11163. (l) Rebek, J., Jr. *Chem. Commun.* **2000**, 637−643. (m) Vysotsky, M. O.; Thondorf, I.; Böhmer, V. *Angew. Chem. Int. Ed.* **2000**, *39*, 1264−1267. For self-assembled ureido calix[6]arene dimers, see: (n) González, J. J.; Ferdani, R.; Albertini, E.; Blasco, J. M.; Arduini, A.; Pochini, A.; Prados, P.; de Mendoza, J. *Chem. Eur. J.* **2000**, *6*, 73−80.

(4) (a) Nowick, J. S.; Smith, E. M.; Pairish, M. *Chem. Soc. Rev.* **1996**, 401−415. (b) Smith, C. K.; Regan, L. *Acc. Chem. Res.* **1997**, *30*, 153−161. (c) Gellman, S. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 717−725. (d) Nowick, J. S. *Acc. Chem. Res.* **1999**, *32*, 287−296.

(5) (a) Nowick, J. S.; Mahrus, S.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 1066−1072. (b) Nowick, J. S.; Tsai, J. H.; Bui, Q-Ch. D.; Maitra, S. *J. Am. Chem. Soc.* **1999**, *121*, 8409−8410 and references therein.

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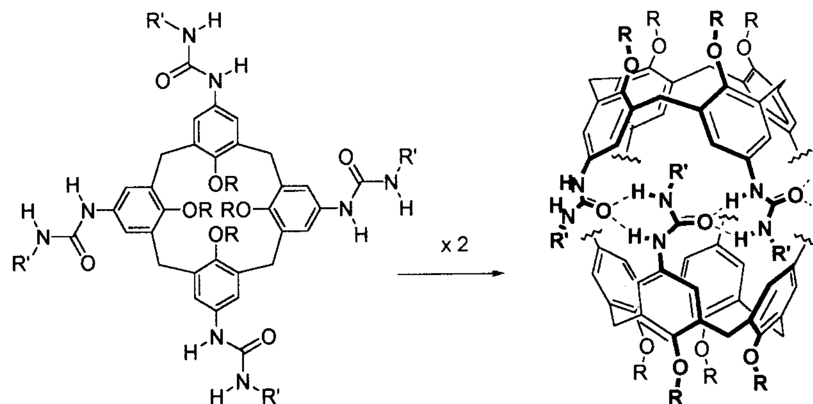


Figure 1. Dimerization of ureido calix[4]arenes.

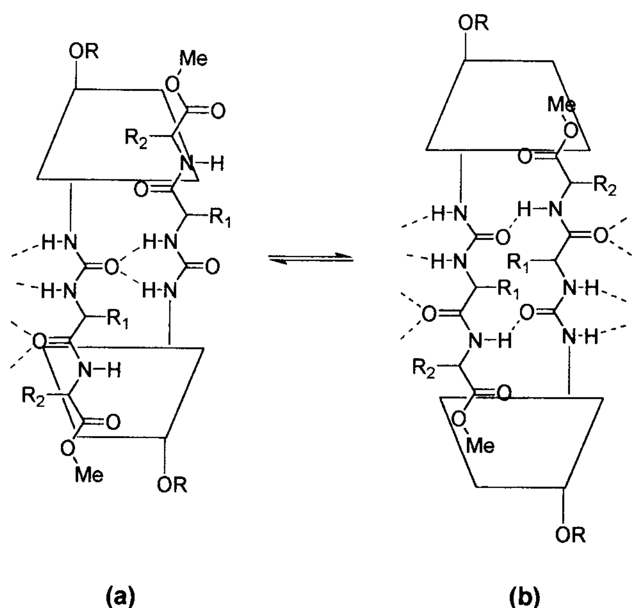


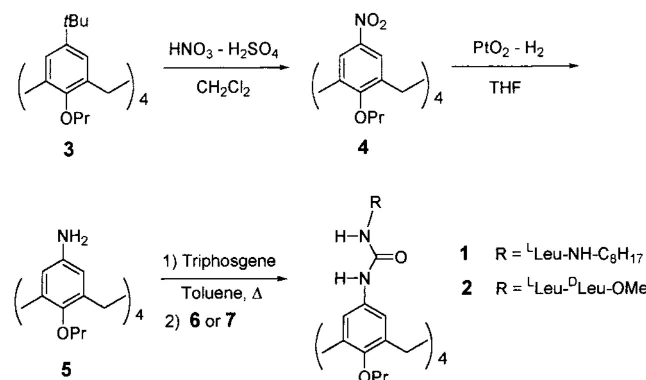
Figure 2. Schematic representation of the two dimerization modes of ureidodipeptide calixarenes. (a) Urea–urea dimerization. (b) Urea–amide dimerization.

here should therefore be considered as the minimalist examples where both modes of self-assembly could operate. CPK inspection revealed that other arrangements, such as those resulting from changes in the urea conformations or from rotations about the chains are unlikely. In such cases, due to forced twists, loss of hydrogen bond contacts or steric hindrance, some of the bulky leucyl chains would be forced to occupy simultaneously the interior of the cavities.

Results and Discussion

Synthesis. Compounds **1** and **2** were synthesized in three steps from calix[4]arene (**3**),[6] fixed into a cone conformation by the presence of four *O*-propyl groups at the lower rim (Scheme 1). The *ipso*-nitration of **3** using an improved modification of the previously described method[7] produced tetranitro derivative **4** in 82% yield. Reduction of the nitro groups with H_2/PtO_2 in THF gave aminocalix[4]arene (**5**)[8] in a 95% yield. Finally, compound **1** was prepared in a 25% yield by reaction of **5** with triphosgene in toluene followed by in situ treatment of the resulting isocyanate with leucyloctylamide (**6**). Reaction of the isocyanate with H-^{L}Leu-^{D}Leu-OMe. HCl (**7**) obtained by desprotection of Boc-^{L}Leu-^{D}Leu-OMe[9] afforded **2** in a 24% yield.

Scheme 1



Self-Assembly. Leucyl Amide 1. Tetramide **1** was found to be monomeric under all experimental conditions explored. In correlation with a monomer weight (calcd 1725) a single peak at 1532 was observed by gel permeation chromatography (GPC) in chloroform.[10] No peaks for the dimer nor for higher order aggregates were seen by mass spectrometry (MALDI-TOF).

The number and pattern of the ^{1}H NMR signals in polar competitive solvents, such as DMSO-d_6, methanol-d_4 or THF-d_8 corresponded to that expected for monomeric tetrasubstituted calix[4]arenes of C_4 symmetry: two signals for the aromatic protons and three NH protons and a AX system for the methylene bridges (Figure 3a).[11] ^{13}C NMR spectra were also in agreement with a C_4-symmetrical cone conformation, as indicated by the chemical shifts of the methylene bridges.[12]

In apolar solvents, such as $CDCl_3$, $CDCl_2CDCl_2$, C_6D_6, toluene-d_8, *p*-xylene-d_{10}, or mesitylene-d_{12}, compound **1** showed a double set of signals for each group of protons: two substituted aromatic rings, six NH protons, two AX systems for the methylene bridges and two different *O*-propyl substituents (Figure 3b). A NH signal was shifted downfield, indicating that

(6) Iwamoto, K.; Araki, K.; Shinkai, S. *J. Org. Chem.* **1991**, *56*, 4955–4962.

(7) Verboom, W.; Durie, A.; Egberink, R. J. M.; Asfari, Z.; Reinhoudt, D. N. *J. Org. Chem.* **1992**, *57*, 1313–1316.

(8) van Wageningen, A. M. A.; Snip, E.; Verboom, W.; Reinhoudt, D. N.; Boerrigter, H. *Liebigs Ann. Rec.* **1997**, 2235–2245.

(9) Nitecki, D. E.; Halpern, B.; Estley, J. W. *J. Org. Chem.* **1968**, *33*, 684–689.

(10) GPC measurements were made in chloroform, using polystyrenes (mol. masses between 990 and 9750 Da) as standards. Due to the different shapes and physicochemical properties of polystyrenes and calix[4]arenes, the calibration was corrected with seven calixarenes of known molecular masses (see Supporting Information).

(11) Sansone, F.; Barboso, S.; Casnati, A.; Fabbi, M.; Pochini, A.; Ugozzoli, F.; Ungaro, R. *Eur. J. Org. Chem.* **1998**, 897–905.

(12) Jaime, C.; de Mendoza, J.; Prados, P.; Nieto, P. M.; Sánchez, C. *J. Org. Chem.* **1991**, *56*, 3372–3376.

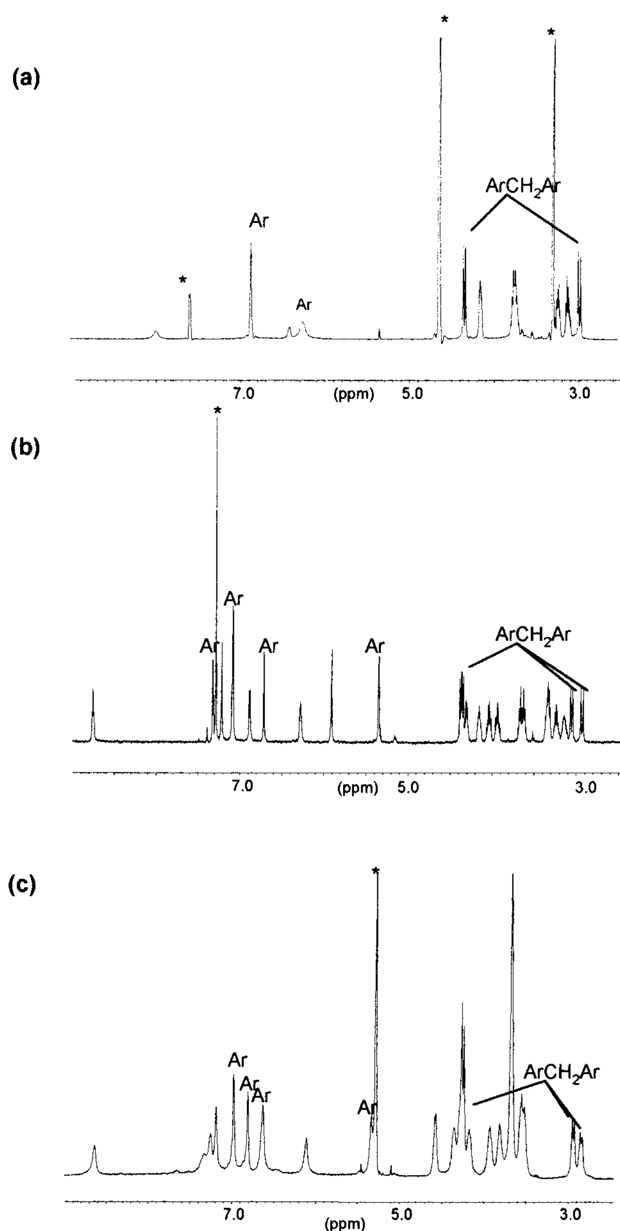


Figure 3. 500 MHz ^{1}H NMR spectra of calixarenes **1** and **2**. (a) Spectrum of **1** in CH_3OH-d_4 + $CDCl_3$ (1:1), 25 °C. (b) Spectrum of **1** in $CDCl_3$, 25 °C. (c) Spectrum of **2** in CD_2Cl_2, -10 °C. Asterisks indicate solvent signals.

strong hydrogen bonding in these solvents was present. No changes in the spectrum were discernible upon dilution, in agreement with a monomer in which the C_4-symmetry has been broken by intramolecular hydrogen bonds.

No changes in the ^{1}H NMR spectrum were noticeable upon mixing equimolecular amounts of L- and D-enantiomers of **1** (D-enantiomer: $[\alpha]^{25}_{D} = +53°$). Thus, a *meso*-heterodimer was apparently not formed.

Rebek et al. have previously described that a related ureido calix[4]arene containing four leucine ester residues does not dimerize, but gives raise to heterodimers in the presence of suitable partners (e.g., phenylureidocalix[4]arenes).[3g,k] In contrast, no heterodimers were observed for **1** under similar conditions. Likely, intramolecular hydrogen bonds that are absent in the related ester could operate in amide **1**, promoting the chains to fold back and preventing dimerization or heterodimerization. In agreement with this assumption, ROE through-space contacts between aromatic protons with methylene protons of the octyl group, and the isopropyl group of the leucine side chain were observed in *p*-xylene-d_{10} and $CDCl_3$ (see Supporting Information).

Dileucyl Ester 2. The dimerization of **2** in apolar solution was supported by GPC in chloroform. Two peaks were observed under the same conditions employed for **1**: a major peak corresponding to the monomer (found = 1437, calcd = 1789) and an additional minor peak accounting for a dimer **2.2** (found = 2659, calcd = 3578).[10]

The pattern of the ^{1}H NMR signals for **2** in C_6D_6, toluene-d_8 and *p*-xylene-d_{10} at room temperature and in CD_2Cl_2 at −10 °C was similar to that found for **1** in apolar solvents (Figure 3c). In mesitylene-d_{12}, CD_2Cl_2, $CDCl_3$, and $CDCl_2CDCl_2$ at room temperature spectra were broad and poorly defined. In *p*-xylene-d_{10}, coalescence of all signals was observed at 363 K. A $\Delta G^{\ddagger} = 16.5$ kcal mol^{-1} for the transition from dimer to monomer was calculated by monitoring changes of methylene signals in the range 303−393 K.

For this compound concentration-dependent chemical shifts for the NH protons (in CD_2Cl_2 or toluene-d_8) allowed the determination of the dimerization constant by dilution experiments ($K_a = 20$ M^{-1} in CD_2Cl_2 at 298 K).[13] From measurements at different temperatures, Van't Hoff plots gave $\Delta H° = -12$ kcal mol^{-1} and $\Delta S° = -38$ cal K^{-1} mol^{-1} for the dimerization process.

A substantially larger dimerization constant ($K_a = 5100$ M^{-1}) was found ($\Delta H° = -17$ kcal mol^{-1} and $\Delta S° = -40$ cal K^{-1} mol^{-1}) in toluene-d_8 (298 K). The higher value for $\Delta H°$ in toluene could simply be interpreted as a result of stronger hydrogen bonds in the less polar solvent. The high negative $\Delta S°$ values measured in both solvents account for reduction of conformational freedom during dimerization.

Structure of Dimer 2.2. The formation of a well-defined structure for **2.2** was further assessed by circular dicroism experiments. An intense chirality transfer ($[\theta] = -80$ cm^2 deg dmol^{-1}) from the amino acid side chains to the calixarene chromophore was observed at 260 nm at 5×10^{-4} M in chloroform. A similar chirality transfer has been reported for heterodimers of other chiral tetrasubstituted calixarenes,[3k] but it was not observed for **1**.

COSY and ROESY spectra were registered for **2.2** in toluene-d_8 (323 K) and CD_2Cl_2 (263 K). In both cases cone structures were confirmed by ROE contacts between aromatic and equatorial methylene protons, whereas the *O*-propyl substituent showed only contacts with the corresponding axial protons. The *syn* conformation of the urea group was demonstrated by contacts observed between the two urea NH protons. ROEs between the aromatic protons with the methyl ester group, and with the leucine isopropyl side chain were observed.

Further experiments were necessary to discriminate between the two possible structures for the dimer that were compatible with the NMR spectra (Figure 4). A COSY experiment was used to identify aromatic protons in the same ring (A−a, B−b) while the ROESY spectra showed two incompatible cross-relaxation cross-peaks A−a, B−b and a−b, A−B. Also exchange peaks between A−b and a−B protons were observed. Therefore, one of the cross-relaxation cross-peaks pairs should not correspond to real contacts, but could be explained by

(13) ^{1}H NMR titrations were carried out in *p*-xylene-d_{10} and CD_2Cl_2. Association constants were determined with a nonlinear regression fitting program using the concentration-dependent shifts of a NH amide group between 5×10^{-2}−5×10^{-4} M. The set of observed shifts (δ_{obs}) for each titration experiment was adjusted to the equation: $\delta_{obs} = \delta_{Dimer} + \{(\delta_{Monomer} - \delta_{Dimer}) \times [(-1 + (1 + 8K_aC)^{1/2}]/(4K_aC)\}$ where C corresponds to the set of concentrations and K_a, $\delta_{Monomer}$ and δ_{Dimer} are the calculated parameters.

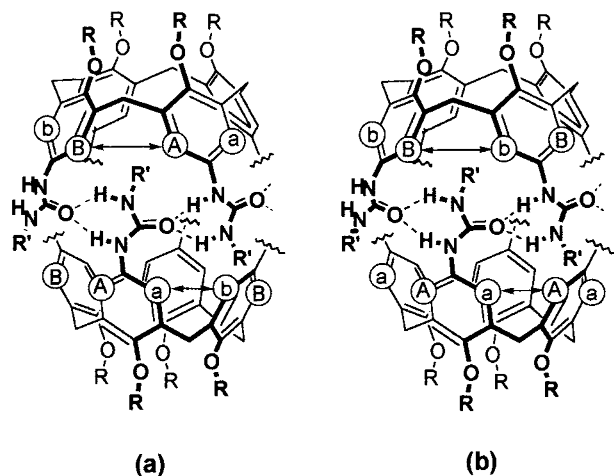


Figure 4. Two possible origins for ROE contacts between the aromatic protons. (a) Dimer of C_2-symmetry. (b) Dimer of C_4-symmetry.

Table 1. Intensity of ROE Signals with Respect to the $ArCH_2Ar$ Signal[a]

	temperature (K)	ROE a−A	ROE a−b	ratio a−b/a−A
1	363	0.192	0.197	1.03
	343	0.116	0.273	2.3
	323	0.104	0.499	4.8
2	283	0.072	0.059	0.82
	263	0.132	0.103	0.78
	243	0.360	0.190	0.520

[a] For a−A and a−b label definitions, see Figure 4.

exchange peaks. If the real ROEs correspond to a−b, A−B contacts both a monomer or a dimer with C_2-symmetry could be possible (Figure 4a). If the a−A and B−b peaks were the real ROEs, only a dimer formed by two calixarenes with different peptide conformations could explain the spectra (Figure 4b). Similar pattern of signals is observed in ROESY spectra of compound **1** with the same problem of simultaneously incompatible cross-relaxation cross-peaks pairs.

True and false ROEs could be distinguished by ROESY experiments run at different temperatures, since the intensity of cross-peaks due to exchange decrease simultaneously with the temperature.[14] Thus, ROESY of **1** (a monomer) in *p*-xylene-d_{10} and **2** in CD_2Cl_2 were registered at different temperatures. Cross-peak signals were integrated and the ratios between cross-peaks a−b and a−A were measured (Table 1). Since this ratio for **1** increased when the temperature decreases the true ROE were thus a−b and A−B, indicating that the calixarene has a C_2-symmetry. On the contrary, this ratio for **2** decreases as the temperature was lowered, indicating that ROE signal a−b is now due to exchange between A−b protons. Thus, a−A and B−b were the real cross-relaxation cross-peak (Figure 4b) and, therefore, the structure of **2** in apolar solvents must be a dimer formed by two C_4-symmetry calixarene subunits. The observed pattern should correspond to the C_4-symmetry of the dimer as a whole.

Considering the above symmetry patterns, an antiparallel β-sheet arrangement of the upper-rim chains through urea−amide hydrogen bonds (Figure 2b) could be ruled out for a dimer **2.2** with C_4-symmetry for each calixarene subunit (overall D_4-symmetry), because in such a structure both calixarenes should be identical. This is inconsistent with the observance of two different sets of signals for each calixarene. Similarly, an urea−urea hydrogen-bonded array without participation of the amino acid residues (Figure 2a) would not account for the experimental findings.

However, in an urea−urea dimer, the residues could establish additional intermolecular contacts, "shaking hands" between the amide NH donors of one subunit and the ester carbonyl acceptors of the other. Two different structures in a dynamic equilibrium can be proposed for dimer **2.2** considering the two possible *plus (P)* and *minus (M)* arrangements of the ureas[15] and the *syn* or *anti* orientations of each dipeptide backbones with respect to the urea carbonyl. These structures have been named *PsMa* (Figure 5a,b) and *PaMs* (Figure 5c,d). Other combinations (e.g., *PsMs*, *PsPa*) cannot dimerize with a full use of the hydrogen bonding potential. Although molecular mechanics and molecular dynamics calculations indicate that *PaMs* is more stable than *PsMa* in vacuo,[16] the experimentally observed ROE contacts are compatible with both conformations. In both optimized structures the peptide chains adopt an almost extended conformation. This agrees with the measured $^3J(CH_\alpha-NH)$ coupling constants (~6.5 Hz in *p*-xylene-d_{10}). For comparison, the model compound PhNHCONH-^{L}Leu-^{D}Leu-OMe (**8**) was synthesized. For this compound, with an extended conformation, dilution sensitive intermolecular hydrogen bonds were observed in $CDCl_3$, the coupling constant [$^3J(CH_\alpha-NH) = 8.2$ Hz] accounting for a canonical β-sheet arrangement.

Conclusions

Dimerization of ureidocalix[4]arenes is quite sensitive to the steric hindrance of the side chains of the substituents linked to the ureas. Predictably, compounds **1** and **2** should encounter difficulties to dimerize, considering that the chains are not long enough to allow stabilization through antiparallel β-sheets and that bulky leucine groups are directly attached to the ureas. This was indeed the case for octylamide **1**, which was found to be a monomer under all the experimental conditions investigated. However, compound **2**, with a leucyl ester instead of an octyl chain, is a classical urea−urea dimer, endowed with an additional seam of peripheral hydrogen bonds, caused by lateral chains interacting. Thus, the presence of the ester carbonyl weak hydrogen bond acceptors in **2** provides the necessary additional enthalpic binding energy to overcome the entropy penalty associated with the dimerization process.

Experiments are currently underway to have a further insight on the chances of longer peptide chains attached to ureidocalix[4]arenes to undergo self-assembly through extended arrays of hydrogen bonds in antiparallel β-sheets.

Experimental Section

General. Solvents were dried before use by standard methods. Purification was performed with silica gel plates (Merck 60 PF_{254} containing $CaSO_4$) using the chromatotron technique. Analytical thin-layer chromatography was carried out on silica gel plates (SiO_2, MN Alugram SIL G/UV_{254}). ^{1}H and ^{13}C NMR spectra were recorded on

(14) (a) Wagner, G.; Bodenhausen, G.; Müller, N.; Rance, M.; Sorensen, O.; Ernst, K. Wüthrich, R. R. *J. Am. Chem. Soc.* **1985**, *107*, 6440−6446. (b) Pons, M.; Klchkov, V., submitted for publication.

(15) *P* and *M* conformations were defined for the clockwise or counterclockwise arrangement of the urea carbonyls, respectively, if the calixarene is seen from the lower rim.

(16) , INSIGHT-II 2.3.0/Discover packages were employed. Standard potentials and atomic charges, as provided by the AMBER force field, were employed without modifications. Calculations were performed in vacuo, with a dielectric constant $\epsilon = 4$, and the initial structures were slowly relaxed by 300 steepest descent iterations, followed by full optimization with enough conjugate gradients iterations to reach a energy RMS gradient of less than 0.001 kcal mol^{-1} Å^{-1}. Minimized structures were subjected to a molecular dynamic protocol through 500 ps (50 ps heating to equilibration at 300 K). Coordinates were saved each 500 fs. Average coordinates taken for each 50 ps were minimized again obtaining in all the cases superimposable structures that therefore may be considered as a global minimum under the simulation conditions.

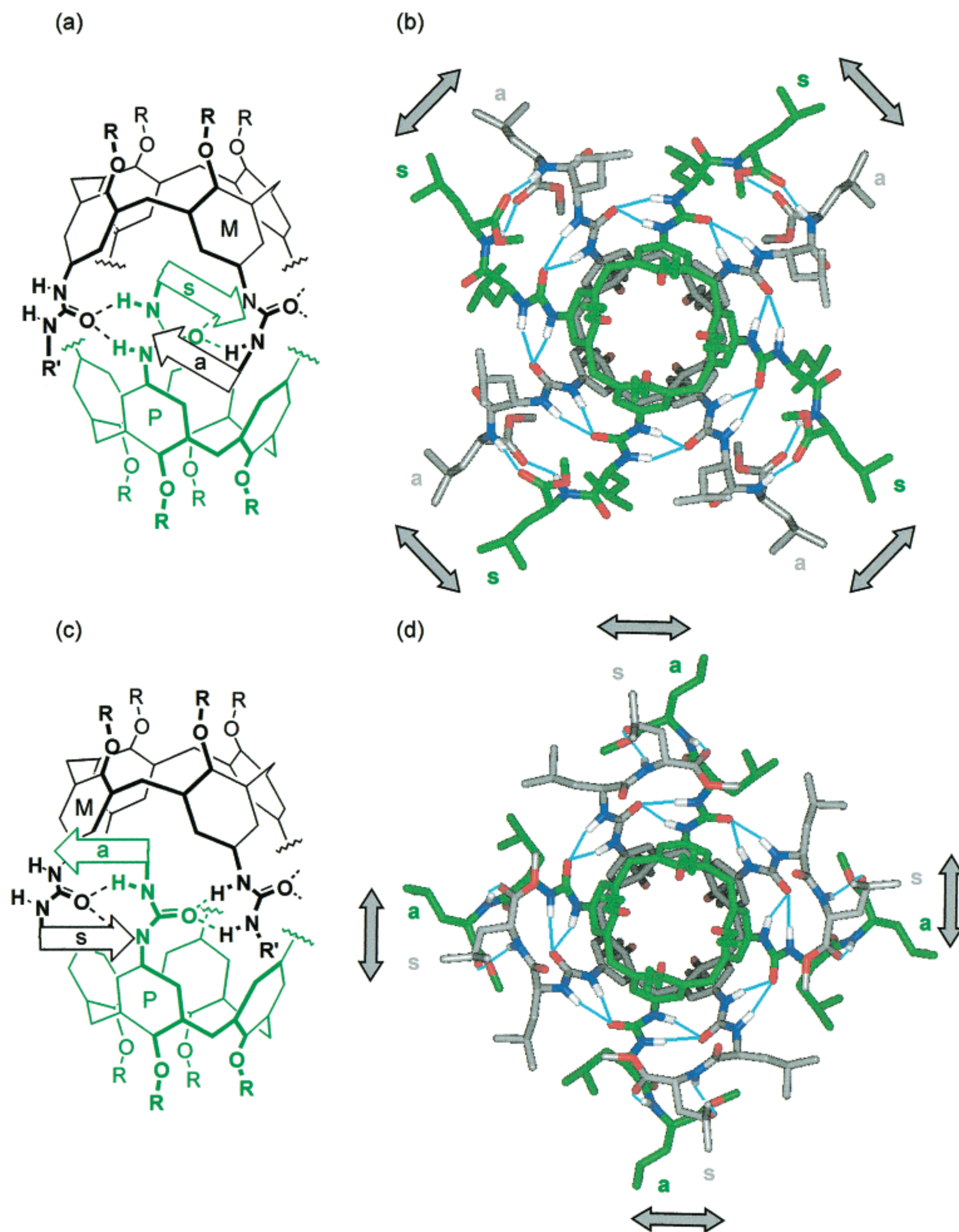


Figure 5. Optimized structures of **2.2** dimers. (a−b) Schematic side view and bottom projection of the *PsMa* conformation. (c−d) Schematic side view and bottom projection of the *PaMs* conformation. For clarity, only hydrogens participating in hydrogen bonds have been represented. For details on the calculations, see ref 16.

Bruker AMX-300 and DRX-500 spectrometers. Chemical shifts are reported as δ values in ppm from the solvent residual peak. Mass spectra were performed with a VG AutoSpec instrument using a FAB$^+$ technique (NBA: *m*-nitrobenzyl alcohol). Elemental analyses were obtained with a Perkin-Elmer 2400 CHN analyzer. Melting points were obtained on a Gallenkamp apparatus. Specific rotations were measured with a Perkin-Elmer 241 MC polarimeter.

5,11,17,23-Tetranitro-25,26,27,28-tetrapropoxycalix[4]arene (4). To a vigorously stirred solution of calixarene **3** (1.0 g, 1.18 mmol) in dry CH_2Cl_2 (20 mL) a [H_2SO_4−HNO_3 (65%) (1:1), 11.8 mmol] mixture was added under argon at room temperature. After stirring for 14 h, the reaction was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was triturated with MeOH to give pure **4** as a yellow solid (743 mg, 82%): mp >300 °C (lit.[7] mp >300 °C); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.55 (s, 8H, ArH), 4.52 (AB system, J = 14.1 Hz, 4H, $ArCH_2Ar$), 3.96 (t, J = 7.0 Hz, 8H, CH_2), 3.40 (AB system, J = 14.1 Hz, 4H, $ArCH_2Ar$), 1.90 (m, 8H, CH_2), 1.01 (t, J = 7.0 Hz, 12H, CH_3); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$, 25 °C, DEPT): δ = 161.7, 142.8, 135.4 (ArC), 123.9 (ArCH), 77.7 (OCH_2), 31.1 ($ArCH_2Ar$), 23.2 (CH_2), 10.0 (CH_3).

5,11,17,23-Tetraamino-25,26,27,28-tetrapropoxycalix[4]arene (5). A suspension of **4** (500 mg, 0.77 mmol) and PtO_2 (0.46 mmol) in THF (25 mL) was stirred under H_2 (1 atm) for 18 h. The reaction mixture was filtered through Celite and evaporated to give **5** as a pale yellow solid (393 mg, 95%): mp 134−137 °C (lit.[8] mp 234−236 °C); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.07 (s, 8H, ArH), 4.30 (AB system, J = 13 Hz, 4H, $ArCH_2Ar$), 3.72 (t, J = 7.6 Hz, 8H, CH_2), 3.05 (br s, 8H, NH_2), 2.91 (AB system, J = 13 Hz, 4H, $ArCH_2Ar$), 1.84 (m, 8H, CH_2), 0.94 (t, J = 7.6 Hz, 12H, CH_3); $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$, 25 °C, DEPT): δ = 150.0, 139.7, 135.6 (ArC), 115.8 (ArCH), 76.5 (OCH_2), 30.9 ($ArCH_2Ar$), 23.0 (CH_2), 10.2 (CH_3); LRMS (FAB) calcd for $C_{40}H_{52}N_4O_4$ $(M)^+$ 652.4, found 652.4.

Leucine Octylamide (6). To a solution of Boc-Leu-OSu (1.53 mmol) in anhydrous THF (8 mL) was added octylamine (1.83 mmol). The mixture was stirred under argon at room temperature for 4 h. The solid was separated by filtration. The solution was diluted with AcOEt and washed with HCl (1 N) and water. Evaporation of the dried ($MgSO_4$)

solvent in vacuo afforded an oil (Boc-Leu-NHC_8H_{17}). The oil was stirred in HCl/AcOEt (3 N, 5 mL) for 30 min, and the solvent was evaporated to give **6** as an oil (412 mg, 89%). $[\alpha]^{25}_D$ (c = 0.1, CH_3OH) = +14°; ^{1}H NMR (300 MHz, CH_3OH-d_4, 25 °C): δ = 3.85 (m, CH_α), 3.21 (m, 1H, NCH_2), 3.11 (m, 1H, NCH_2), 1.65 (m, 2H), 1.47 (m, 1H), 1.25 (m, 12H), 0.93 (m, 6H, CH_3), 0.83 (t, J = 7.2 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (75 MHz, CH_3OH-d_4, 25 °C, DEPT): δ = 170.4 (CO), 53.1 (CH), 41.7, 40.6, 32.9, 30.3, 30.1 (CH_2), 27.9 (CH), 23.6 (CH_2), 23.0, 22.6, 14.5 (CH_3); LRMS (FAB) calcd for $C_{14}H_{31}N_2O$ $[M + H]^+$ 243.2, found 243.3.

L-Leucyl-D-leucine Methyl Ester Hydrochloride (7). A solution of H-^{D}Leu-OMe•HCl (1.52 mmol) and $NaHCO_3$ (1.52 mmol) in water (4 mL) was added to a stirred solution of Boc-^{L}Leu-OSu (1.52 mmol) in anhydrous THF (8 mL). The mixture was stirred for 10 h, diluted with ether, washed with water, and dried. Evaporation of solvent afforded a white solid [Boc-^{L}Leu-^{D}Leu-OMe, $[\alpha]^{25}_D$ (c = 1, CH_3OH) = −2.6°, lit.[9] $[\alpha]^{25}_{546}$ (c = 0.01 g/mL, CH_3OH) = −2.2° deg dm^{-1} $(g/cm^3)^{-1}$] The solid was stirred in HCl/AcOEt (3 N, 5 mL) for 30 min. The solvent was evaporated to give **7** as a pale yellow solid (370 mg, 95%): mp 50−53 °C; $[\alpha]^{25}_D$ (c = 0.1, CH_3OH) = +42°; ^{1}H NMR (300 MHz, CH_3OH-d_4, 25 °C): δ = 4.37 (m, 1H, CH_α), 3.94 (m, 1H, CH_α), 3.65 (s, 3H, OCH_3), 1.71−1.55 (m, 6H), 0.92 (m, 9H, CH_3), 0.84 (d, J = 6.4 Hz, 3H, CH_3); $^{13}C\{^1H\}$ NMR (75 MHz, CH_3OH-d_4, 25 °C, DEPT): δ = 173.9, 170.6 (CO), 52.7, 52.0 (CH, OCH_3), 41.2, 40.6 (CH_2), 25.6, 25.1 (CH), 23.0, 22.7, 22.2, 21.2 (CH_3); LRMS (FAB) calcd for $C_{13}H_{27}N_2O_3$ $[M + H]^+$ 259.2, found 259.2.

***N*-Phenylaminocarbonyl-L-leucyl-D-leucine Methyl Ester (8).** To a solution of **7** (0.5 mmol) and diisopropylethylamine (0.5 mmol) in CH_2Cl_2 (4 mL) phenylisocyanate (0.5 mmol) was added. The mixture was stirred under argon at room temperature for 4 h. The solution was diluted with CH_2Cl_2 and washed with water. Evaporation of dried (Na_2SO_4) solvent in vacuo affored a residue which was purified on the chromatotron [CH_2Cl_2:MeOH (50:1)] to give pure **8** as a white solid (102 mg, 54%): mp 158−160 °C; $[\alpha]^{25}_D$ (c = 0.1, $CHCl_3$) = −48°; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.03 (d, J = 8.2 Hz, 1H, NH), 7.85 (s, 1H, NH), 7.10 (m, 3H, ArH, NH), 6.98 (t, J = 7.6 Hz, 2H, ArH), 6.82 (m, 1H, ArH), 4.64 (m, 2H, CH_α), 3.43 (s, 3H, OCH_3), 1.83 (m, 1H), 1.71−1.52 (m, 4H), 0.98 (d, J = 6.5 Hz, 6H, CH_3), 0.90 (d, J = 6.5 Hz, 6H, CH_3); $^{13}C\{^1H\}$ NMR (75 MHz, $CHCl_3$, 25 °C, DEPT): δ = 175.0, 172.9, 156.2 (CO), 139.1 (ArC), 128.5, 122.2, 119.5 (ArCH), 52.4, 52.0, 50.7 (CH, OCH_3), 41.9, 40.0 (CH_2), 24.9, 24.8 (CH), 23.0, 22.9, 22.1, 21.5 (CH_3); HRMS (FAB) calcd for $C_{20}H_{32}N_3O_4$ $[M + H]^+$ 378.2398, found 378.2398.

General Procedure for the Preparation of Ureidopeptides. A solution of **5** (100 mg, 0.15 mmol) in toluene (3 mL) was treated with a solution of triphosgene (0.2 mmol) in toluene (3 mL) at 110 °C for 2 h. The mixture was cooled to 30 °C and then treated with a solution of the corresponding peptide (0.66 mmol) and diisopropylethylamine (0.66 mmol) in toluene (3 mL). After 18 h under the same conditions, the solvent was evaporated, and the residue was taken in CH_2Cl_2 (15 mL), washed with water, dried (Na_2SO_4), and evaporated to dryness.

Compound 1. The crude residue was purified on the chromatotron [CH_2Cl_2:MeOH(25:1)] to afford pure **1** as a white solid (65 mg, 25%): mp 248−250 °C; $[\alpha]^{25}_D$ [c = 0.05, CH_3OH + $CHCl_3$ (1:1)] = −54°; ^{1}H NMR [300 MHz, CH_3OH-d_4 + $CDCl_3$ (1:1), 25 °C]: δ = 7.90 (br s, 4H, NH), 6.82 (s, 4H, ArH), 6.42 (br s, 4H, NH), 6.16 (br s, 4H, ArH), 4.26 (AB system, J = 13.3 Hz, 4H, $ArCH_2Ar$), 4.07 (m, 4H, CH_α), 3.67 (m, 8H, OCH_2), 3.16 (m, 4H, NCH_2), 3.06 (m, 4H, NCH_2), 2.89 (AB system, J = 13.3 Hz, 4H, $ArCH_2Ar$), 1.76 (m, 8H), 1.63 (m, 4H), 1.45−1.37 (m, 16H), 1.34−1.21 (m, 40H), 0.87 (t, J = 7.3 Hz, 12H, CH_3), 0.83−0.74 (m, 36H); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 8.75 (t, J = 5.5 Hz, 2H, NH), 7.32 (d, J = 2.3 Hz, 2H, ArH), 7.22 (s, 2H, NH), 7.10 (m, 4H, ArH, NH), 6.88 (d, J = 7.3 Hz, 2H, NH), 6.71 (d, J = 2.3 Hz, 2H, ArH), 6.27 (br s, 2H, NH), 5.90 (s, 2H, NH), 5.34 (d, J = 2.3 Hz, 2H, ArH), 4.37 (AB system, J = 13.2 Hz, 2H, $ArCH_2Ar$), 4.35 (AB system, J = 13.2 Hz, 2H, $ArCH_2Ar$), 4.30 (m, 2H, CH_α), 4.15 (m, 2H, CH_α), 4.03 (m, 2H, OCH_2), 3.92 (m, 2H, OCH_2), 3.69−3.59 (m, 4H, OCH_2), 3.35 (m, 4H, NCH_2), 3.24 (m, 2H, NCH_2), 3.14 (m, 2H, NCH_2), 3.05, (AB system, J = 13.3 Hz, 2H, $ArCH_2Ar$), 2.93 (AB system, J = 13.3 Hz, 2H, $ArCH_2Ar$), 1.98−1.88 (m, 6H), 1.86−1.80 (m, 6H), 1.78−1.73 (m, 2H), 1.68−1.64 (m, 2H), 1.61−1.51 (m, 6H), 1.41−1.39 (m, 4H), 1.38−1.28 (m, 42H), 1.11 (t, J = 7.4 Hz, 6H, CH_3), 0.97−0.10 (m, 36H, CH_3), 0.08 (d, J = 12.5 Hz, 6H, CH_3); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$, 25 °C, DEPT): δ = 177.1, 174.6, 157.8, 156.0 (CO), 155.2, 149.6, 138.7, 137.3, 134.7, 132.4, 131.6, 130.9 (ArC), 125.0, 124.6, 118.8, 115.0 (ArCH), 76.9, 76.5 (OCH_2), 54.3, 53.2 (CH), 40.7, 39.7, 39.6, 39.4 (CH_2), 31.84 ($ArCH_2Ar$), 31.8 (CH_2), 30.7 ($ArCH_2Ar$), 29.4, 29.3, 29.2, 29.1, 27.4, 27.0 (CH_2), 24.9, 24.5 (CH), 23.5 (CH_2), 23.1, 22.8 (CH_3), 22.6 (CH_2), 21.9, 21.3, 14.0, 10.8, 9.7 (CH_3); LRMS (FAB) calcd for $C_{100}H_{165}N_{12}O_{12}$ $[M + H]^+$ 1726.3, found 1726.5. Anal. Calcd for $C_{100}H_{164}N_{12}O_{12}$: C 69.65; H 9.58; N 9.74. Found: C 69.77; H 9.89; N 9.86.

Compound 2. The crude product was purified by the chromatotron technique [CH_2Cl_2:MeOH (25:1)] to afford pure **2** as a white solid (63 mg, 24%): mp 212−214 °C; $[\alpha]^{25}_D$ (c = 0.1, $CHCl_3$) = −91°; ^{1}H NMR (500 MHz, THF-d_8, 25 °C, monomer): δ = 8.40 (br s, 4H, NH), 7.11 (s, 4H, ArH), 6.98 (s, 4H, ArH), 6.76 (d, J = 6.5 Hz, 4H, NH), 6.35 (br s, 4H, NH), 4.47 (m, 4H, CH_α), 4.40 (m, 4H, CH_α), 4.34 (AB system, J = 13.0 Hz, 4H, $ArCH_2Ar$), 3.81 (m, 4H, OCH_2), 3.72 (m, 4H, OCH_2), 3.69 (s, 12H, OCH_3), 2.95 (AB system, J = 13.0 Hz, 4H, $ArCH_2Ar$), 1.89 (m, 8H, CH_2), 1.77−1.58 (m, 20H), 1.40 (m, 4H, CH), 0.98 (t, J = 7.4 Hz, 12H, CH_3), 0.95 (d, J = 6.6 Hz, 12H, CH_3), 0.93 (d, J = 6.4 Hz, 12H, CH_3), 0.91 (d, J = 6.3 Hz, 12H, CH_3), 0.85 (d, J = 6.6 Hz, 12H, CH_3); $^{13}C\{^1H\}$ NMR (75 MHz, CH_3OH-d_4, 25 °C, DEPT): δ = 176.4, 174.5, 157.7 (CO), 153.8, 136.4, 134.2 (ArC), 121.8, 121.2 (ArCH), 77.9 (OCH_2), 53.5, 52.8, 52.3 (CH, OCH_3), 42.9, 41.4 (CH_2), 32.2 ($ArCH_2Ar$), 26.05, 26.01 (CH), 24.3 (CH_2), 23.6, 23.4, 22.1, 21.8, 10.8 (CH_3); ^{1}H NMR (500 MHz, CD_2Cl_2, −10 °C, dimer): δ = 8.59 (br s, 4H, NH), 7.24 (br s, 4H, NH), 7.19 (s, 4H, ArH), 7.14 (br s, 4H, NH), 6.98 (s, 4H, ArH), 6.79 (s, 4H, ArH), 6.63 (br s, 8H, NH), 5.97 (br s, 4H, NH), 5.30 (s, 4H, ArH), 4.58 (m, 4H, CH_α), 4.36 (m, 4H, CH_α), 4.24 (m, 12H, CH_α, $ArCH_2Ar$), 4.13 (m, 4H, CH_α), 3.92 (m, 4H, OCH_2), 3.82 (m, 4H, OCH_2), 3.67 (m, 24H, OCH_3), 3.56 (m, 4H, OCH_2), 3.53 (m, 4H, OCH_2), 2.94 (AB system, J = 13.1 Hz, 4H, $ArCH_2Ar$), 2.83 (AB system, J = 12.5 Hz, 4H, $ArCH_2Ar$), 1.82−1.72 (m, 16H, CH_2), 1.70−1.18 (m, 48H), 1.03 (t, J = 7.2 Hz, 12H, CH_3), 0.95−0.74 (m, 96H, CH_3), 0.64 (m, 12H, CH_3); $^{13}C\{^1H\}$ NMR (125 MHz, p-xylene-d_{10}, 25 °C, HMQC): δ = 119.4, 116.6 (ArCH), 77.3, 77.0 (OCH_2), 55.2, 53.7, 52.2 (CH), 52.0, 51.95 (OCH_3), 51.94 (CH), 42.6, 42.5, 41.6 (CH_2), 32.7, 31.8 ($ArCH_2Ar$), 30.6 (CH_2), 25.8, 25.7, 24.4, 23.9 (CH), 23.81, 23.8, 22.6, 22.4, 14.7, 11.5, 10.5 (CH_3); HRMS (FAB) calcd for $C_{96}H_{148}N_{12}O_{20}Na$ $[M + Na]^+$ 1812.0830, found 1812.0837. Anal. Calcd for $C_{96}H_{148}N_{12}O_{20}.CH_3OH$: C 63.93, H 8.41, N 9.22. Found: C 63.67, H 8.90, N 8.86.

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Supporting Information Available: Gel permeation chromatograms (GPC), COSY, and ROESY spectra, and CD spectra of **1** and **2**; variable concentration and variable temperature ^{1}H NMR spectra of **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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