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N-Linked Peptidocalix[4]arene Bisureas as Enantioselective Receptors for Amino Acid Derivatives

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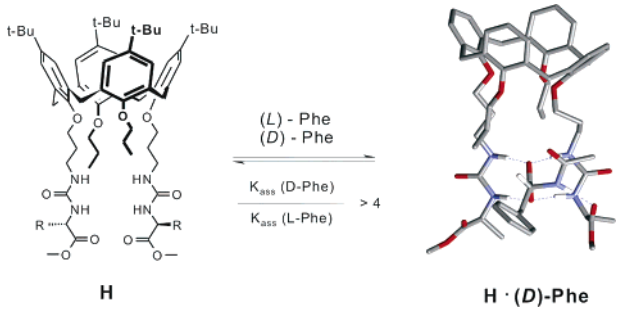

Bisurea calix[4]arenes **1** and **2** possessing L-amino acid moieties at the lower rim were synthesized by reaction of the methyl esters of glycine, L-alanine, or L-isoleucine with the appropriate isocyanate (**12** or **13**), obtained with a safe and efficient Curtius rearrangement from the corresponding carboxylic acid derivatives. The conformational properties of the ligands **1** and **2** were investigated by means of a combined NMR and molecular modeling study which evidences that they are deeply influenced by strong intramolecular H-bonds between the urea NH groups and the vicinal phenolic oxygen atoms or the opposite urea C═O group. Complexation studies performed by ESI-MS and NMR spectroscopy in acetone solution show that the binding ability of these bisurea hosts decreases by increasing the side chain size of the amino acid. Host **2b** has a remarkable binding ability for the *N*-acetyl-D-phenylalaninate anion with an interesting enantioselectivity (K^{D}_{ass}/K^{L}_{ass} = 4.14), which is explained on the basis of a three-point interaction mode of binding.

Introduction

Anion recognition is an important process in nature, being involved in the catalytic activity of enzymes, in the transfer of genetic information, and in ion transport through membrane channels.[1,2] Anion complexation is also quite important in chemical technology,[3−5] where relevant topics are anion sensing[5] and the template effect in organic synthesis.[6] Several macrocycles have been used for the design and synthesis of efficient and selective anion receptors,[3,4,7] and among them, a special place is occupied by calixarenes, cavity-shaped compounds which offer the possibility of a three-dimensional organization of binding groups.[8,9] In the field of anion recognition, a topic

† National Academy of Sciences of Ukraine.
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(1) Kubik, S.; Reyheller, C.; Stuwe, S. *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *52*, 137−187.
(2) Miller, C. *Nature* **2006**, *440*, 484−489.
(3) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 487−516.
(4) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 191−221.
(5) *Anion Sensing*; Stibor, I., Ed.; Topics in Current Chemistry 255; Springer: Berlin, 2005.
(6) Meshcheryakov, D.; Böhmer, V.; Bolte, M.; Hubscher-Bruder, W.; Arnaud-Neu, F.; Herschbach, H.; Van Dorsselaer, A.; Thondorf, I.; Mogelin, W. *Angew. Chem., Int. Ed.* **2006**, *45*, 1648−1652.
(7) Stibor, I.; Zlatuskova, P. Chiral Recognition of Anions. In *Anion Sensing*; Stibor, I., Ed.; Topics in Current Chemistry 255; Springer: Berlin, 2005; pp 31−63.
(8) Casnati, A.; Sansone, F.; Ungaro, R. Calixarene Receptors in Ion Recognition and Sensing. In *Advances in Supramolecular Chemistry*; Gokel, G. W., Ed.; Cerberus Press Inc.: South Miami, 2004; Vol. 9, pp 165−218.
(9) Lhotak, P. Anion receptors based on calixarenes. In *Anion Sensing*; Stibor, I., Ed.; Topics in Current Chemistry 255; Springer: Berlin, 2005; pp 65−95.

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of particular interest is the enantioselective recognition of chiral substrates, since it is well-known that the chemical properties and biological activity of organic species are strongly dependent on stereochemistry. Therefore, some efforts have been devoted to the design and synthesis of receptors for chiral recognition of anions.[7] Several chiral calixarenes have been synthesized[10−17] and used for the enantioselective recognition of chiral organic species, but only a few of them have shown chiral recognition of anions. We have previously reported[18] that upper rim, bridged peptidocalix[4]arenes, which were designed as selective receptors for aromatic carboxylates, have a slight preference in the recognition of *N*-acylamino acid carboxylates of the D-series. (Thio)urea groups are known to strongly interact with anionic species through hydrogen bonding,[3−5] but only a few synthetic receptors, incorporating these binding units for the recognition of chiral anions, are known. One example, containing both thiourea and chiral amide binding units, has shown interesting chiral discrimination properties toward the enantiomers of α-phenylglycinate and mandelate anions, with a selectivity factor of up to 4.8.[19] In this paper we report the synthesis and conformational and anion binding properties of a novel type of lower rim, urea-functionalized chiral calix[4]arenes, some of them showing interesting enantioselective recognition of chiral carboxylate anions.

Results and Discussion

Synthesis and Conformational Properties of the Ligands. (Thio)urea-functionalized calixarenes, which have already shown remarkable recognition[8,20] and self-assembly[21] properties, are usually prepared from aminocalixarenes and the proper iso(thio)-cyanates. An alternative, but less explored route would be via iso(thio)cyanatocalixarenes,[22,23] which are quite attractive synthetic intermediates,[24] but suffer from the drawback of being usually prepared from aminocalixarenes and highly toxic (thio)-phosgene.

Therefore, we developed an alternative, more general, and safer procedure for the synthesis of isocyanatocalixarenes which exploits the Curtius rearrangement of acylazides obtained from the easily available calixarene carboxylic acids.[25] With this synthetic route, we prepared calix[4]arenes **1** and **2** (Figure 1) functionalized at the lower rim with two urea binding groups separated from the macrocycle by propylene spacers. The urea groups bear methyl esters of α-amino acids (glycine (**1a**, **2a**), alanine (**1b**, **2b**), and isoleucine (**1c**, **2c**)) directly linked through their N-terminal end (N-linked peptidocalixarenes).

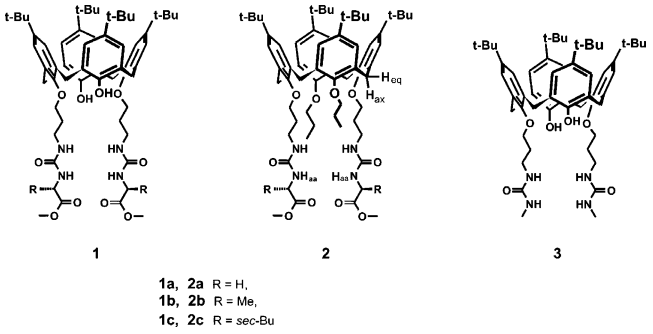
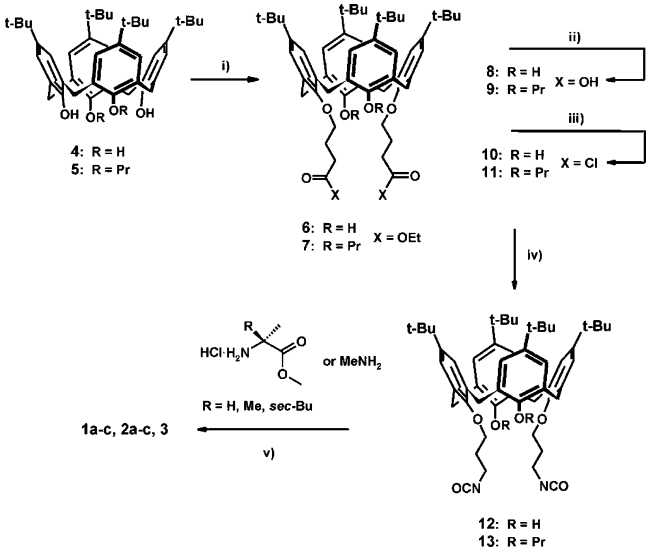


FIGURE 1. Structures of calix[4]arene ureas.

SCHEME 1. Synthesis of N-Linked Peptidocalix[4]arene Bisurea Receptors[a]



[a] Conditions and reagents: (i) (a) for **4**: acetonitrile, K_2CO_3, ethyl 4-bromobutyrate; (b) for **5**: DMF, NaH, ethyl 4-bromobutyrate; (ii) EtOH: H_2O = 1:1 (v/v), KOH; (iii) CH_2Cl_2, oxalyl chloride; (iv) benzene, azidotrimethylsilane, Bu_4NI(cat.); (v) CH_2Cl_2, triethylamine.

The full synthetic pathway is reported in Scheme 1. The diesters **6** and **7** were synthesized by alkylation[26−28] of tetrahydroxycalix[4]arene **4** and dipropoxycalix[4]arene **5**,[29] respectively, with ethyl 4-bromobutyrate. After hydrolysis in a water−ethanol medium with potassium hydroxide and further treatment with oxalyl chloride in dichloromethane, calix[4]arene acid chlorides **10** and **11** were obtained. The acid chlorides were then transformed into the corresponding isocyanates by means

(10) Vysotsky, M.; Schmidt, C.; Böhmer, V. Chirality in Calixarenes and Calixarene Assemblies. In *Advances in Supramolecular Chemistry*; Gokel, G. W., Ed.; JAI Press: Greenwich, CT, 2000; Vol. 7, pp 139−233.
(11) Ludwig, R. *Microchim. Acta* **2005**, *152*, 1−19.
(12) Grady, T.; Harris, S. J.; Smyth, M. R.; Diamond, D.; Hailey, P. *Anal. Chem.* **1996**, *68*, 3775−3782.
(13) Pinkhassik, E.; Stibor, I.; Casnati, A.; Ungaro, R. *J. Org. Chem.* **1997**, *62*, 8654−8659.
(14) Zheng, Y. S.; Zhang, C. *Org. Lett.* **2004**, *6*, 1189−1192.
(15) Erdemir, S.; Tabakci, M.; Yilmaz, M. *Tetrahedron: Asymmetry* **2006**, *17*, 1258−1263.
(16) Kocabas, E.; Karakucuk, A.; Sirit, A.; Yilmaz, M. *Tetrahedron: Asymmetry* **2006**, *17*, 1514−1520.
(17) Karakucuk, A.; Durmaz, M. S. A.; Yilmaz, M.; Demirel, A. *Tetrahedron: Asymmetry* **2006**, *17*, 1963−1968.
(18) Sansone, F.; Baldini, L.; Casnati, A.; Lazzarotto, M.; Ugozzoli, F.; Ungaro, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4842−4847.
(19) Qing, G. Y.; He, Y. B.; Zhao, Y.; Hu, C. G.; Liu, S. Y.; Yang, X. *Eur. J. Org. Chem.* **2006**, 1574−1580.
(20) Casnati, A.; Sansone, F.; Ungaro, R. *Acc. Chem. Res.* **2003**, *36*, 246−254.
(21) Bogdan, A.; Rudzevich, Y.; Vysotsky, M. O.; Bohmer, V. *Chem. Commun.* **2006**, 2941−2952 and references therein.
(22) Santoyo, G. F.; Torres, P. A.; Barria, C. S. *Eur. J. Org. Chem.* **2000**, 3587−3593.
(23) Sansone, F.; Chierici, E.; Casnati, A.; Ungaro, R. *Org. Biomol. Chem.* **2003**, *1*, 1802−1809.
(24) van Wageningen, A. M. A.; Snip, E.; Verboom, W.; Reinhoudt, D. N.; Boerrigter, H. *Liebigs Ann./Recl.* **1997**, 2235−2245.
(25) Boyko, V. I.; Yakovenko, A. V.; Tsymbal, I. F.; Kalchenko, V. I. *Mendeleev Commun.* **2006**, 24−26.
(26) Cobben, P. L. H. M.; Egberink, R. J. M.; Bomer, J. G.; Bergveld, P.; Verboom, W.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1992**, *114*, 10573−10582.
(27) Bitter, I.; Grun, A.; Toth, G.; Balazs, B.; Horvath, G.; Toke, L. *Tetrahedron* **1998**, *54*, 3857−3870.
(28) Webber, P. R. A.; Cowley, A.; Drew, M. G. B.; Beer, P. D. *Chem.−Eur. J.* **2003**, *9*, 2439−2446.
(29) Iwamoto, K.; Araki, K.; Shinkai, S. *Tetrahedron* **1991**, *47*, 4325−4342.

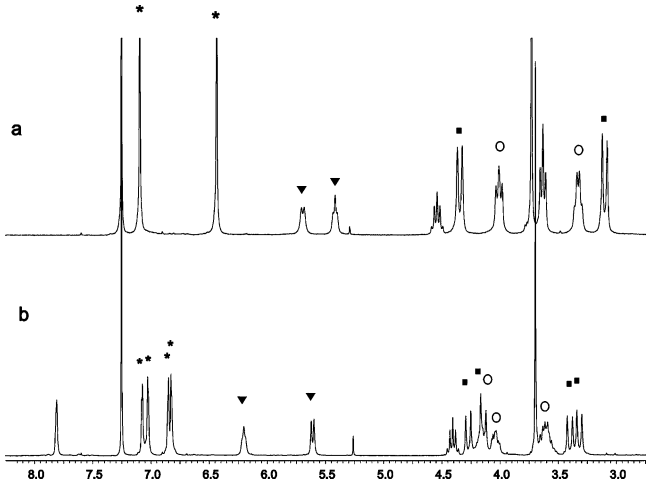


FIGURE 2. Expansions of the ^{1}H NMR spectra ($CDCl_3$, 298 K, 300 MHz) of (a) compound **2b** and (b) compound **1b** (*, ArH; ▼, NH; ■, $ArCH_2Ar$; ○, $OCH_2CH_2CH_2N$).

of a modified Curtius reaction with azidotrimethylsilane in benzene under reflux and in the presence of tetrabutylammonium iodide. Isocyanates **12** and **13** were recrystallized from acetonitrile (53−61% overall yields). Urea calix[4]arenes **1a**−**c**, **2a**−**c**, and **3** were synthesized in 70−94% yields by reacting the isocyanates **12** and **13** with methyl esters of L-amino acids (as hydrochloride salts) or methylamine in dichloromethane (DCM) and in the presence of triethylamine (except for **3**). Relatively pure materials were isolated by simple washing of the organic phase with water. Analytically pure compounds were obtained by crystallization from acetonitrile (**1b**, **2b**) or an acetone/hexane mixture (**1a**, **2a**, **1c**, **2c**, and **3**).

The structure of the synthesized ureas **1**−**3** was proved by ^{1}H and ^{13}C NMR and mass spectra. The ^{1}H NMR spectrum of **2b** (see Figure 2a and the Supporting Information, Figure S2) shows the expected splitting pattern for the methylene bridges, aromatic rings, and alkyl spacers, corresponding to a calixarene having C_{2v} symmetry. Compound **2b** is present, both in $CDCl_3$ and in acetone-d_6 (Table 1), in a *flattened cone* conformation as proved by the large $\Delta\delta$ (Figure 2a) between the signals of the two sets of aromatic protons ($\Delta\delta = 0.66$ and 0.56 ppm in the two solvents, respectively) and *tert*-butyl groups ($\Delta\delta = 0.52$ and 0.41 ppm, respectively), while in the more polar DMSO-d_6 a more *symmetrical cone* is present ($\Delta\delta = 0.035$ ppm for aromatic protons and $\Delta\delta = 0.024$ for *tert*-butyl groups). A similar pattern was also observed for compounds **2a** and **2c**.

On the contrary, the spectra of dihydroxycalixarenes **1b** (see Table 1) and **1c** have an unusual splitting pattern. For instance, the ^{1}H NMR of **1b** in $CDCl_3$ solution (Figure 2b) reveals a splitting of the signals of the methylene bridge and aromatic and propylene spacer protons. The equatorial and axial protons of the bridges show two pairs of doublets in the regions 3.30−3.42 and 4.08−4.30 ppm, the aromatic protons give rise to four doublets at 6.83, 6.86, 7.03, and 7.08 ppm, and the methylene protons of the spacers appear as multiplets (see Table 1 and Figure 2b). This lack of symmetry in the spectrum suggests all the protons of the groups mentioned above are diastereotopic. Also the ^{13}C spectra (see the Supporting Information) of **1b** and **2b** have different sets of signals corresponding to the aromatic units (12 signals for **1b** and only 8 for **2b**).

Interestingly, the ^{1}H NMR spectra in $CDCl_3$ of derivatives **1a** and **3**, lacking the chiral centers, reflect a C_{2v} symmetry of the macrocycle similarly to those for **2a**−**c**. These data suggest that the presence of the chiral centers is not sufficient to explain the particular behavior of **1b** and **1c**. Thus, we undertook a series of experiments to clarify why this diastereotopicity is so evident in dihydroxy compounds **1b**,**c** and not observed in the propyloxy derivatives **2b**,**c** containing the same L-amino acid units.

Replacement of $CDCl_3$ with acetonitrile-d_3, acetone-d_6, or DMSO-d_6 changes the ^{1}H NMR spectra of **1b** and **1c** significantly (see the Supporting Information, Figure S1), evidencing the loss of diastereotopicity. In fact, the methylene bridges essentially appear as two doublets, the signals of the aromatic protons consist of two singlets very close each to the other, and those of the methylene protons of the propylene spacers appear as a set of three multiplets. Thus, the behavior observed in $CDCl_3$ solution for both compounds **1b** and **1c** is clearly due to the formation of hydrogen bonds which could be either intramolecular or intermolecular, in the latter case causing the formation of hydrogen-bonded aggregates.[30] Dilution experiments with **1b** in $CDCl_3$ show that the ^{1}H NMR spectra do not change significantly in the 5×10^{-2} to 4×10^{-4} M concentration range, ruling out the presence of *self*-assembly phenomena. Moreover, the ^{1}H NMR spectrum of a 1:1 mixture of compounds **1b** and **2b** (10^{-3} M) shows the exact superimposition of the spectra of the two single compounds, excluding the formation of (hetero)dimers. In addition, DOSY experiments[31] carried out in $CDCl_3$ (7×10^{-3} M) allowed the determination of rather similar diffusion coefficients D for **1b** and **2b**, confirming that they are present as monomers. In fact, the hydrodynamic radii, calculated from the Stokes−Einstein equation (1) and equal to 4.1 and 5.5 Å for **1b** and **2b**, respectively, are in reasonable agreement with the estimated values (6.5 and 7.5 Å) of the modeled conformations (vide infra).

$$D = k_B T/(6\pi\eta r_H) \qquad (1)$$

Comparative measurements of the temperature coefficient ($\Delta\delta/\Delta T$) for the NH groups in $CDCl_3$ were performed for **1b** and **2b**. Upon increasing the temperature in the range 240−328 K, the signals of the N*H*CH_2 groups are shifted upfield in a similar manner (Figure 3). Temperature coefficients $\Delta\delta_{NH}/\Delta T$ are −3.9 and −3.7 ppb/K for compounds **1b** and **2b**, respectively, providing the evidence that hydrogen bonds are strong and intramolecular in both compounds.[32] 2D NOESY experiments on **1b** show a strong cross-peak between the urea NH proton (N*H*CH_2) and the axial protons of the methylene bridge (CH_{ax}), whereas this correlation is completely absent for **2b**. An average distance of 2.61 Å between the N*H*CH_2 and the CH_{ax} protons in **1b** was determined according to the equation

$$d_{NHCH_2-CH_{ax}} = d_{NH-NH}(V_{NH-NH}/V_{NHCH_2-CH_{ax}})^{1/6} \qquad (2)$$

where d is the distance, $V_{NHCH_2-CH_{ax}}$ is the volume of the N*H*CH_2−C*H*$_{ax}$ cross-peak, and V_{NH-NH} is the volume of the N*H*−N*H* cross-peak.

A conformational search on compound **1b**, carried out with Spartan'04 (MM force field), gave as the lowest energy conformer the molecular structure reported in Figure 4, where the urea NH groups are hydrogen bonded to the phenolic oxygen

(30) Rincon, A. M.; Prados, P.; de Mendoza, J. *Eur. J. Org. Chem.* **2002**, 640−644.
(31) Cohen, Y.; Avram, L.; Frish, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 520−554.
(32) Cierpicki, T.; Otlewski, J. *J. Biomol. NMR* **2001**, *21*, 249−261.

TABLE 1. ^{1}H NMR Chemical Shifts ($CDCl_3$, 298 K) of Selected Protons of Compounds 1a, 1b, 2b, and 3

compd	CH	OCH_2 spacer	CH_2N spacer	CH_2 spacer	H_{eq}	H_{ax}	ArH	ArH	NH_{aa}	$NHCH_2$
1b	4.41	4.17	3.61[a]	2.28	3.42	4.30	7.08	6.83	5.62 (d)	6.20 (t)
		4.04		2.20	3.34	4.17	7.03	6.85		
2b	4.55	4.01	3.34	2.34	3.13	4.37	7.10	6.44	5.73 (d)	5.45 (t)
1a		4.09	3.67	2.23	3.39	4.24	7.05	6.86	5.69 (t)	6.11 (t)
3		4.08	3.66	2.16	3.37	4.17	7.08	6.83	5.03 (br s)[b]	5.85 (t)

[a] Broad multiplet. [b] Signal of the $NHCH_3$ proton.

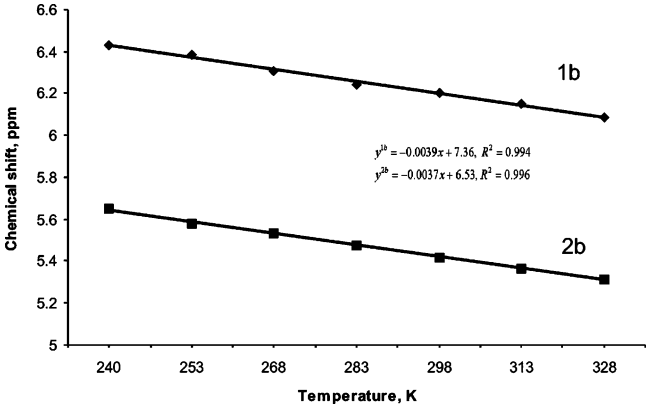


FIGURE 3. 3. Dependence of the chemical shift of $NHCH_2$ protons on the temperature.

atoms.[33] This conformation shows an average $NHCH_2-CH_{ax}$ distance of 2.2 Å, in nice agreement with the 2D NOESY data. Two diastereomeric conformations, one having a clockwise (Figure 4b) and the other a counterclockwise orientation of the hydrogen-bonding network, can be assumed. This results in a rigidification of the calixarene skeleton, which amplifies the differences of chemical shifts observed for diasterotopic nuclei. In more polar solvents such as acetone-d_6 or DMSO-d_6, due to the breaking of the hydrogen bonds, the spectra are simplified since the diastereotopic aromatic and propylene spacer hydrogen nuclei degenerate.

A conformational search on compound **2b** resulted in a lowest energy structure (Figure 5a) with the classical bifurcate hydrogen-bonding pattern, where the NH hydrogen atoms of one arm bind the urea C═O group of the opposite chain. At higher energy we found several other conformers having intramolecular hydrogen bonds (e.g., Figure 5b), but among the first 100 conformers no structure was found where the two urea arms are folded like in the lowest energy conformer of compound **1b**. This is partly due to the steric hindrance given by the propoxy groups, but also to the lower charge density present on the propoxy oxygen atoms of **2b** with respect to the hydrogen-bonded phenolic OH groups of **1b**.

The absence of a rigidifying intramolecular hydrogen-bonding network among the vicinal aromatic nuclei in **2b**, therefore, prevents the formation of diastereomeric structures, and as a consequence the ^{1}H NMR spectrum is characterized by a simple set of signals.

Anion Binding Properties of the Calix[4]arene Ureas. To preliminarily test the binding properties of the synthesized ligands, we first measured the ESI-MS spectra (negative mode) of compound **2c** (in water/acetone/CH_2Cl_2) in the presence of an equivalent amount of tetrabutylammonium chloride or *N*-acetylphenylalaninate (*N*-Ac-Phe-COO^-) salt. Interestingly, only the peaks of the 1:1 host/guest complex (at 1223.4 and 1394.7 amu), and no peak for the free host or the 1:2 or 2:1 species, are present. The stoichiometry of the complexes was also determined, for compounds **2b** and **2c**, using the Job plot method[34] with ^{1}H NMR experiments in $CDCl_3$ (Figure 6). In both cases only experiments with chloride anions have been performed, since the affinity for this anion is higher. The Job analysis reveals nice and symmetrical bell-shaped curves centered at a 0.5 molar ratio, thus confirming the presence only of the 1:1 host/guest complex.

We studied the ability of ureas **1b**, **2b**, and **2c** to bind anions and to recognize chiral anions by the NMR−CIS technique. In all cases, the complexes are kinetically labile on the ^{1}H NMR time scale in the studied conditions ($CDCl_3$ or acetone-d_6). All anionic guests were used as tetrabutylammonium salts. To avoid high dilution of the sample during the titrations, small aliquots of a concentrated (0.1−0.15 M) salt solution were added to the host solution (0.01 M). To estimate the binding constants (K_{ass}), nonlinear regression analysis (Figure 7) was performed with the Gauss−Newton−Marquardt method, using the Hyp-NMR2004[35,36] tools.

As is shown in Table 2, the anion binding to urea receptors **1b**, **2b**, and **2c** is rather weak in $CDCl_3$, especially if compared with binding of similar ligands reported in the literature.[37] This is clearly due to the formation of the strong intramolecular hydrogen bonding in this solvent (vide supra).[38]

Interestingly, as observed in other cases,[18] the association constants of the receptors **1** and **2** are remarkably higher in acetone-d_6 than in $CDCl_3$. In acetone-d_6 all the receptors have a rather good affinity for the anions tested. In particular, ligand **2b** effectively binds the *N*-acetylphenylalaninate anion, showing selectivity for the D- over the L-isomer. The enantioselective discrimination seems to reach a maximum when there is an optimal matching between the steric bulkiness of the guest and that of the amino acid side chain present in the host. In fact, the highest value of D/L selectivity is observed for the complexes of alanine-functionalized receptors with phenylalaninate. With less bulky guests, such as alaninate, discrimination between the

(33) Although at low resolution, the X-ray crystal structure of a C-linked (*N*-tosylalaninamido)peptidocalix[4]arene, was recently reported (ref 38) and showed a similar H-bonding pattern of the amide chains.

(34) Conners, K. A. *Binding Constants*; John Wiley & Sons: New York, 1987.
(35) Frassineti, C.; Ghelli, S.; Gans, P.; Sabatini, A.; Moruzzi, M. S.; Vacca, A. *Anal. Biochem.* **1995**, *231*, 374−382.
(36) Frassineti, C.; Alderighi, L.; Gans, P.; Sabatini, A.; Vacca, A.; Ghelli, S. *Anal. Bioanal. Chem.* **2003**, *376*, 1041−1052.
(37) Scheerder, J.; Fochi, M.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem.* **1994**, *59*, 7815−7820.
(38) Ben Sdira, S.; Felix, C. P.; Giudicelli, M. B. A.; Seigle-Ferrand, P. F.; Perrin, M.; Lamartine, R. J. *J. Org. Chem.* **2003**, *68*, 6632−6638.
(39) Tsukube, H.; Furuta, H.; Odani, A.; Takeda, Y.; Kudo, Y.; Inoue, Y.; Liu, Y.; Sakamoto, H.; Kimura, K. Determination of stability constants [in supramolecular chemistry]. In *Comprehensive Supramolecular Chemistry*, 8th ed.; Davies, J. E. D., Ripmeester, J. A., Eds.; Elsevier: Oxford, 1996; pp 425−482.

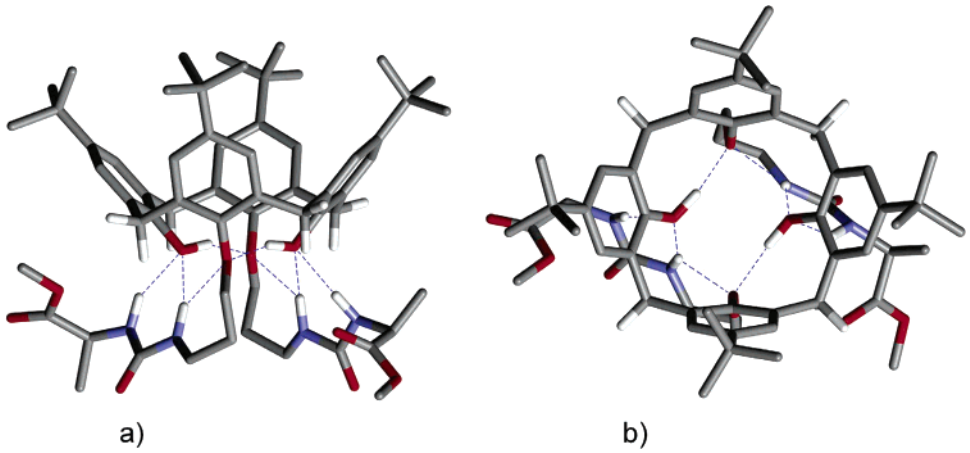


FIGURE 4. Side (a) and top (b) views of the molecular model of **1b** (hydrogen bonds in dashed blue lines).

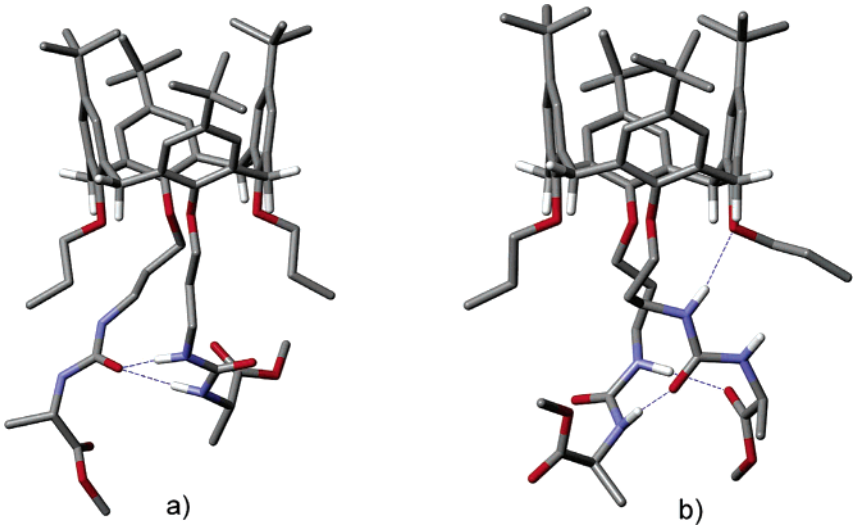


FIGURE 5. Lateral views of the two modeled conformers of compound **2b**: (a) minimum energy conformer (E_{rel} = 0 kcal/mol) and (b) a higher energy structure (E_{rel} = 4.2 kcal/mol) (hydrogen bonds in dashed blue lines).

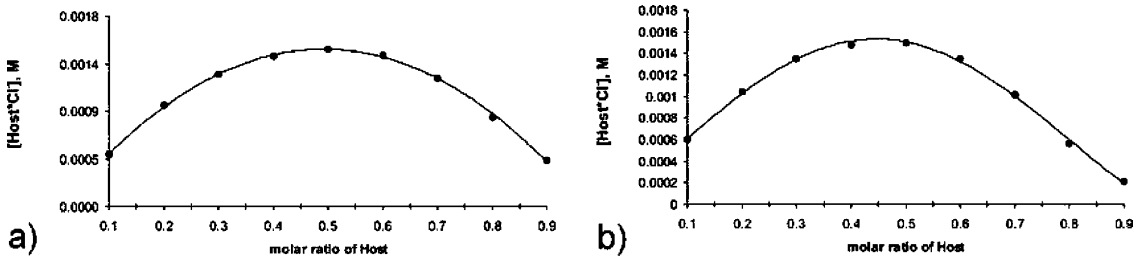


FIGURE 6. Job plots for complexes of **2b** (a) and **2c** (b) with tetrabutylammonium chloride ($CDCl_3$, 298 K), monitoring the N*H*CH_2 protons.

L- and D-forms practically vanishes. On the other hand, the presence of the bulky *sec*-butyl groups of isoleucine in the receptor **2c** significantly decreases its ability to bind anions and to recognize the D- over the L-form of the guest. Dihydroxy receptor **1b** is slightly less efficient than the dipropoxy analogue **2b**, but enantioselectivity for D-*N*-acetylphenylalaninate remains very similar.

To gain more insight into the origin of the observed enantioselectivity, we carried out some modeling studies on the **2b**·L-*N*-Ac-Phe-COO^- and **2b**·D-*N*-Ac-Phe-COO^- complexes, since we could not grow crystals of the complexes suitable for X-ray diffraction. *tert*-Butyl groups at the upper rim were omitted due to important reduction in the calculation times. The two energy minima obtained for the diastereomeric complexes are shown in Figure 8. Interestingly, in both of these structures the carboxylate group of the guest is linked to the urea NH groups through four bifurcated hydrogen bonds (donor−acceptor distances d[NH···O] = 2.78−2.84 Å). A second binding interaction is given by a hydrogen bond between the amide NH group of the guest and the ester C═O group (d[NH···O] = 2.87 and 2.88 Å). The two diastereomeric complexes differ in stability by the value ΔE_{calcd} = 1.2 kcal/mol, very close to that found by NMR titration (ΔE_{exptl} = 1.4 kcal/mol). The analysis of the molecular electrostatic potential indicates that the **2b**·L-*N*-Ac-

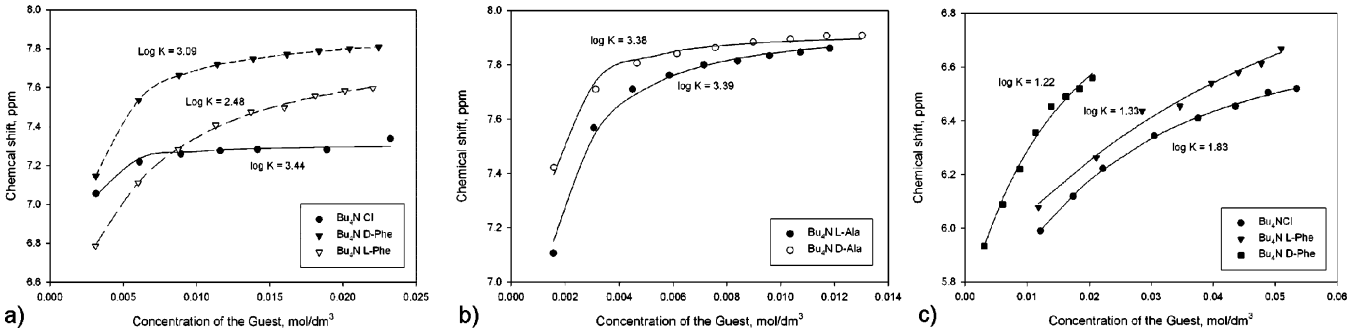


FIGURE 7. Calculated (solid lines) and experimental (symbols) chemical shifts of host **2b** protons upon varying the guest concentration (T = 298 K): (a, b) in acetone-d_6, N*H*CH$_2$ signals, (c) in CDCl$_3$, N*H*CH signals.

TABLE 2. Association Constants (K_{ass}, dm^3·mol^{-1}) and Free Energies of Formation ($\Delta G°$, kcal/mol) of Calixarene Complexes, 298 K (Errors within 10%)

host	guest[a]	$K_{ass}^{CDCl_3}$	$\Delta G°_{ass}^{,CDCl_3}$	$K_{ass}^{acetone-d_6}$	$\Delta G°_{ass}^{,acetone-d_6}$	D/L selectivity[b]
1b	Cl^-	14	−2.0	220	−4.1	3.55
	L-*N*-Ac-Phe-COO$^-$	20	−2.3	160	−3.9	
	D-*N*-Ac-Phe-COO$^-$	17	−2.2	570	−4.8	
2b	Cl^-	68	−3.2	2730	−6.1	4.14
	L-*N*-Ac-Phe-COO$^-$	21	−2.3	300	−4.4	
	D-*N*-Ac-Phe-COO$^-$	17	−2.1	1250	−5.5	
	L-*N*-Ac-Ala-COO$^-$	nd[c]	nd	2460	−5.9	0.98
	D-*N*-Ac-Ala-COO$^-$	nd	nd	2400	−5.9	
2c	Cl^-	46	−2.9	75[d]	−3.3	1.96
	L-*N*-Ac-Phe-COO$^-$	20	−2.3	40[d]	−2.8	
	D-*N*-Ac-Phe-COO$^-$	18	−2.2	80[d]	−3.4	

[a] As tetrabutylammonium salts. [b] The D/L selectivity was determined as the ratio of the corresponding binding constants.[39] [c] nd = not determined. [d] Acetone-d_6/CDCl$_3$ 3/1 (v/v).

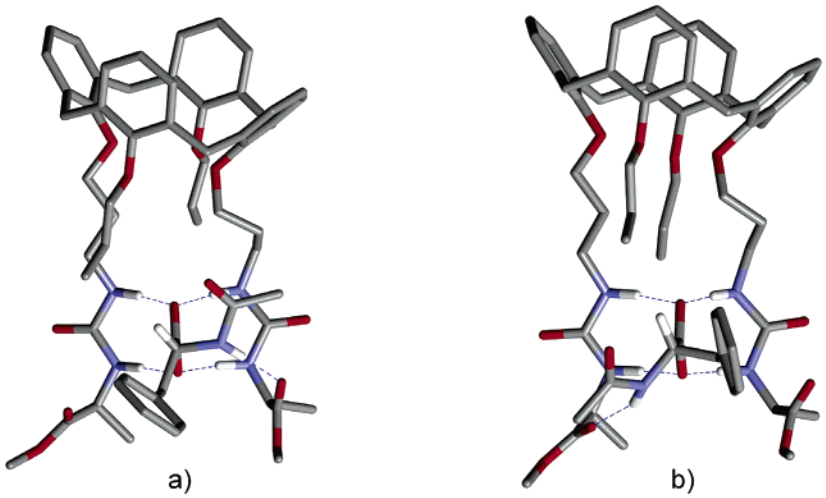


FIGURE 8. Energy minima of the complexes between calixarene receptors **2b** (*t*-Bu groups omitted) and (a) D-*N*-Ac-Phe-COO$^-$ or (b) L-*N*-Ac-Phe-COO$^-$.

Phe-COO$^-$ complex is sterically more crowded than the complex with the D-amino acid salt. In particular, in the L-Phe complex, a repulsion might originate between the phenyl moiety of the guest and the carboxymethyl group of one of the alanines of the host. The system itself seems however to be at the top of its possibility since, when the phenyl group of Phe is replaced by the less bulky methyl group (*N*-Ac-Ala-COO$^-$), the stability of the complexes **2b**·*N*-Ac-Ala-COO$^-$ is not affected (the five hydrogen bonds are still present) but the third interaction point is lost and no discrimination is observed. The absence of propyl groups on the receptor **1b** slightly lowers the crowdedness of the recognition site and consequently the enantioselective discrimination. On the other hand, an increase of the crowdedness in the host does not produce a positive effect and does not increase the chiral discrimination. In fact, the substitution of the alanine with the isoleucine moiety in the receptor arms (cf. receptor **2c**) causes a drop in both the stability of the complexes and the enantioselectivity, probably because the NH···O═C ester hydrogen bond is lost.

Conclusions

Lower rim bisurea calix[4]arene-based receptors **1** and **2** having amino acid methyl ester moieties linked through the

amino group (N-linked peptidocalix[4]arenes) were obtained by means of nucleophilic addition reactions of amino acid esters with isocyanatocalix[4]arenes **12** and **13**. The latter compounds were obtained by means of the Curtius rearrangement of acylazides. This rearrangement, seldom exploited in calixarene chemistry, is general and very efficient under mild conditions and affords important isocyanate intermediates to be used for the conjugation of different type of amines and the preparation of variably functionalized ureas. The final receptors **1** and **2** have in $CDCl_3$ solution the NH ureidic protons involved in strong intramolecular H-bonds. The conformational properties of the ligands were studied in detail by NMR spectroscopy and molecular modeling. It emerged that the presence of propoxy groups in ligand **2** deeply affects the H-bonding of the urea binding groups. However, since in both compounds **1** and **2** these H-bonds are strong and intramolecular, anion binding constants in apolar solvents are strongly depressed. However, because of the breaking of these H-bonds, in polar media, such as acetone, ligands **1** and **2** strongly bind anions. Association constants remarkably depend on the steric hindrance of the amino acid side chain present on the guest molecule. Ligand **2b**, having alanine residues close to the urea binding groups, shows a remarkable ability to recognize D-*N*-acetylphenylalaninate from the corresponding L-isomer. In terms of association constants it binds the D-form 4 times more strongly than the L-form. On the basis of molecular modeling studies of the host−guest complexes, a model, based on a three-point interaction mode of binding (two attractive and one repulsive), has been proposed to explain the observed enantioselectivity.

Experimental Section

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(ethoxycarbonylpropoxy)-26,28-dihydroxycalix[4]arene (6). To a suspension of tetra-*tert*-butylcalix[4]arene **4** (2.5 g, 3.85 mmol) and potassium carbonate (0.665 g, 4.81 mmol) in dry acetonitrile (65 mL) was added ethyl 4-bromobutyrate (3.15 g, 16.2 mmol), and the reaction mixture was refluxed for 17 h. After cooling, the solvent was removed and the colorless suspension was quenched with HCl (2 M, 30 mL) and extracted with dichloromethane (2 × 40 mL). The organic layer was washed with water (2 × 40 mL) and brine (50 mL) and then dried over anhydrous Na_2SO_4. Evaporation of the solvent resulted in a yellow oil, which was dissolved in ethanol (25 mL) and left to cool while the diester precipitated as colorless crystals. Yield: 2.57 g (76%). Mp: 173.7−174.9 °C. ^{1}H NMR ($CDCl_3$): δ 1.00 and 1.27 (18H each, 2s), 1.21 (6H, t, J = 6.0 Hz), 2.31 (4H, m), 2.86 (4H, t, J = 6.0 Hz), 3.34 (4H, d, J = 12.9 Hz), 4.04 (4H, t, J = 6.0 Hz), 4.15 (4H, q, J = 6.0 Hz), 4.27 (4H, d, J = 12.9 Hz), 6.85 and 7.04 (4H each, 2s), 7.65 (2H, s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 14.2, 25.4, 30.6, 31.7, 31.0, 31.6, 33.7, 33.9, 60.3, 75.0, 125.0, 125.5, 127.5, 132.6, 141.3, 146.9, 149.5, 150.7, 173.4. ESI-MS: *m*/*z* 899.6 ([M + Na]$^+$, 100). Anal. Calcd for $C_{56}H_{76}O_8$: C, 76.68; H, 8.42. Found: C, 76.24; H, 8.50.

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(ethoxycarbonylpropoxy)-26,28-dipropoxycalix[4]arene (7). To a suspension of 60% sodium hydride in oil (0.44 g, 10.9 mmol) in dry DMF (25 mL) was added dipropoxycalix[4]arene **5** (1.0 g, 1.36 mmol). After 50 min ethyl 4-bromobutyrate (2.12 g, 10.9 mmol) was added, and the reaction mixture was stirred at room temperature for 18 h. Then the reaction mixture was quenched with water (50 mL), acidified with HCl (6 M, 25 mL), and extracted with CH_2Cl_2 (60 mL). The organic layer was washed with a saturated solution of ammonium chloride (2 × 80 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to leave **7** as a yellow oil, which was then washed with hexane and then acetonitrile. Crystals (low melting) were obtained after continuous drying in vacuo at 60 °C. Yield: 1.24 g, 95%. ^{1}H NMR ($CDCl_3$): δ 0.99 (6H, t, J = 7.5 Hz) 1.05 and 1.10 (18H each, 2s), 1.27 (6H, t, J = 7.1 Hz), 2.02 (4H, m, J = 7.5 Hz), 2.32 (4H, m, J = 7.2 Hz), 2.51 (4H, m, J = 7.2 Hz), 3.15 (4H, d, J = 12.3 Hz), 3.81 (4H, t, J = 7.5 Hz), 3.91 (4H, t, J = 7.2 Hz) 4.17 (4H, q, J = 7.1 Hz), 4.41 (4H, d, J = 12.3 Hz), 6.74 and 6.81 (4H each, 2s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 10.3, 14.3, 23.3, 25.6, 29.7, 31.0, 31.2, 31.4, 33.7, 33.8, 60.2, 74.1, 77.04, 124.9, 125.0, 133.5, 133.8, 144.2, 144.5, 153.39, 153.40, 173.4. ESI-MS: *m*/*z* 983.89 ([M + Na]$^+$, 100). Anal. Calcd for $C_{62}H_{88}O_8$: C, 77.46; H, 9.23. Found: C, 77.71; H, 9.54.

General Procedure for the Synthesis of Diacids 8 and 9. To a solution of KOH (20-fold excess per group) in a water−ethanol mixture (1:3, 65 mL/mmol) was added the ester, and the reaction mixture was refluxed for 6 h. Then ethanol and part of the water were removed under reduced pressure. The residue was taken up into CH_2Cl_2, and HCl (6 M) was added. After 50 min of vigorous stirring the organic layer was separated, washed with saturated solution of ammonium chloride, dried over anhydrous Na_2SO_4, and evaporated to yield the appropriate acid as colorless crystals (**8**) or a foam (**9**).

Data for 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(hydroxycarbonylpropoxy)-26,28-dihydroxycalix[4]arene (8). Crystallized from ethanol. Yield: 89%. Mp: 260−261 °C. ^{1}H NMR (acetone-d_6): δ 1.01 and 1.23 (18H each, 2s), 2.35 (4H, m, J = 6.5 Hz), 2.47 (4H, t, J = 6.5 Hz), 3.42 (4H, d, J = 12.5 Hz), 4.076 (4H, t, J = 6.5 Hz), 4.31 (4H, d, J = 12.5 Hz), 7.05 and 7.11 (4H each, 2s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 24.9, 31.4, 31.0, 31.6, 32.3, 33.7, 33.9, 76.1, 125.1, 125.4, 127.6, 132.3, 141.3, 146.8, 149.8, 150.6, 180.2. ESI-MS: *m*/*z* 919.6 ([M − H]$^-$, 100). Anal. Calcd for $C_{52}H_{68}O_8 \cdot H_2O$: C, 74.43; H, 8.41. Found: C, 74.36; H, 8.07.

Data for 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(hydroxycarbonylpropoxy)-26,28-dipropoxycalix[4]arene (9). Crystallized from acetonitrile. Yield: 88%. Mp: 205−206 °C. ^{1}H NMR ($CDCl_3$): δ 0.84 (18H, s), 1.04 (6H, t, J = 7.5 Hz), 1.32 (18H, s), 1.96 (4H, m, J = 7.3 Hz), 2.48 (8H, m), 3.16 (4H, d, J = 12.5 Hz), 3.69 (4H, t, J = 7.3 Hz), 4.02 (4H, br t), 4.41 (4H, d, J = 12.5 Hz), 6.49 and 7.08 (4H each, 2s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 10.5, 23.4, 24.9, 31.0, 31.1, 31.6, 31.4, 33.5, 33.9, 73.8, 77.6, 124.4, 125.4, 132.0, 135.4, 143.9, 144.9, 152.5, 154.1, 180.8. ESI-MS: *m*/*z* 927.6 ([M + Na]$^+$, 100). Anal. Calcd for $C_{58}H_{80}O_8$: C, 76.95; H, 8.91. Found: C, 76.52; H, 9.29.

General Procedure for the Synthesis of Dichlorides 10 and 11. To a mixture of compound **8** or **9** in dry dichloromethane (60 mL/mmol) were added oxalyl chloride (highly toxic, corrosive) (8-fold excess per group) and a catalytic amount of DMF. The reaction mixture was stirred at ambient temperature for 15 h. Then the solvent and the oxalyl chloride were removed under reduced pressure. The resulting brown residue was dissolved in a petroleum ether/DCM mixture, and a dark oil separated from the mother liquid. Then the solvent was removed, and the resulting crude product was recrystallized from acetonitrile and dried in vacuum.

Data for 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(chlorocarbonylpropoxy)-26,28-dihydroxycalix[4]arene (10). Yield: 90%. Mp: 212.4−212.8 °C. ^{1}H NMR ($CDCl_3$): δ 0.98 and 1.27 (18H each, 2s), 2.37 (4H, m, J = 6.3 Hz), 3.36 (4H, d, J = 12.9 Hz), 3.54 (4H, t, J = 7.0 Hz), 4.03 (4H, t, J = 5.8 Hz), 4.19 (4H, d, J = 12.9 Hz), 6.84 and 7.05 (4H each, 2s), 7.47 (2H, s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 25.7, 30.9, 31.57, 31.6, 33.8, 34.0, 43.7, 73.8, 125.1, 125.6, 127.4, 132.7, 142.1, 147.7, 148.9, 150.2, 173.8. Anal. Calcd for $C_{52}H_{66}Cl_2O_6$: C, 72.79; H, 7.75. Found: C, 72.89; H, 7.71.

Data for 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(chlorocarbonylpropoxy)-26,28-dipropoxycalix[4]arene (11). Yield: 85%. Mp: 156−158 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (18H, s), 1.03 (6H, t, J = 7.6 Hz), 1.25 (18H, s), 1.94 (4H, m, J = 7.5 Hz), 2.44 (4H, m, J = 7.4 Hz), 3.15 (8H, m), 3.76 (4H, t, J = 7.5 Hz), 3.97 (4H, t, J = 7.4 Hz), 4.37 (4H, d, J = 12.6 Hz), 6.61 and 6.99 (4H each, 2s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 10.4, 23.4, 25.6, 31.0, 31.2, 31.5, 33.6, 33.9, 44.1, 72.7, 77.3, 124.7, 125.3, 132.5, 134.6, 144.2, 145.1,

152.6, 153.4, 173.7. Anal. Calcd for $C_{58}H_{78}Cl_2O_6$: C, 73.94; H, 8.34. Found: C, 73.90; H, 8.54.

General Synthesis of Isocyanates 12 and 13. To a solution of the corresponding acid chloride **10** or **11** in benzene (60 mL/mmol) was added azidotrimethylsilane (highly toxic!) (1.5 equiv per group) at 80 °C. The reaction mixture was refluxed for 1.5 h. Then an extra portion (30%) of azidotrimethylsilane was added, and the reaction mixture was refluxed for 30 min. The solvent was then removed under reduced pressure, and the resulting yellow residue was recrystallized from acetonitrile. The resulting crystals were dried in vacuum.

Data for 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(isocyanatopropoxy)-26,28-dihydroxycalix[4]arene (12). Yield: 87%. Mp: 269.5−271.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.01 and 1.27 (18H each, 2s), 2.26 (4H, m, J = 6.0 Hz), 3.38 (4H, d, J = 12.9 Hz), 3.94 (4H, t, J = 6 Hz), 4.07 (4H, t, J = 6 Hz), 4.22 (4H, d, J = 12.9 Hz), 6.87 and 7.05 (4H each, 2s), 7.59 (2H, s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 31.0, 31.8, 31.1, 31.6, 33.8, 33.9, 39.7, 72.3, 125.1, 125.7, 127.5, 132.8, 142.0, 147.7, 149.1, 150.2. ESI-MS: m/z 837.7 ([M + Na]$^+$, 100). IR (film): $\tilde{\nu}$ 2263 cm^{-1}. Anal. Calcd for $C_{52}H_{66}N_2O_6 \cdot CH_3CN \cdot H_2O$: C, 74.19; H, 8.19; N, 4.81. Found: C, 74.39; H, 8.03; N, 4.80.

Data for 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(isocyanatopropoxy)-26,28-dipropoxycalix[4]arene (13). To remove the solvent, after evaporation of CH_2Cl_2, the resulting foam was dissolved in hexane and evaporated. Yield: 86%. Mp: 127.6−128 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (18H, s), 0.99 (6H, t, J = 7.6 Hz), 1.26 (18H, s), 1.94 (4H, m, J = 7.5 Hz), 2.32 (4H, m, J = 7.4 Hz), 3.18 (4H, d, J = 12.6 Hz), 3.60 (4H, t, J = 7.5 Hz), 3.74 (4H, t, J = 7.4 Hz), 4.03 (4H, t, J = 7.5 Hz), 4.36 (4H, d, J = 12.6 Hz), 6.63 and 6.95 (4H each, 2s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 10.3, 23.4, 30.9, 31.2, 31.5, 31.6, 33.6, 33.9, 40.4, 71.6, 77.4, 124.7, 125.3, 132.6, 134.4, 144.2, 144.9 152.7, 153.4. ESI-MS: m/z 921.5 ([M + Na]$^+$, 100). IR (film): ν 2266 cm^{-1}. Anal. Calcd for $C_{58}H_{78}N_2O_6$: C, 77.47; H, 8.74; N, 3.12. Found: C, 77.41; H, 9.16; N, 2.67.

General Synthesis of Diurea Derivatives 1a−c, 2a−c, and 3. To a solution of the amino acid methyl ester hydrochloride (1% excess per isocyanate group) in dry dichloromethane (50 mL/mmol) containing triethylamine (2% excess per isocyanate group) (for diurea **3**, to an emulsion of aqueous methylamine, 2.5 mol/group, in dichloromethane, 50 mL/mmol) was added a solution of the proper isocyanate **12** or **13** in CH_2Cl_2. The reaction mixture was stirred at room temperature for 10 h, and then water was added. After 1 h of vigorous stirring, the organic layer was separated, washed with water, dried over anhydrous Na_2SO_4, and evaporated. The resulting residue was then recrystallized from acetonitrile (**1b**, **2b**) or an acetone/hexane mixture (**1a**, **2a**, **1c**, **2c**, and **3**).

Data for Compound 1a. Yield: 70%. Mp: 136−137 °C. ^{1}H NMR ($CDCl_3$): δ 1.00 and 1.27 (18H each, 2s), 2.23 (4H, m, J = 5.9 Hz), 3.39 (4H, d, J = 13.0 Hz), 3.67−3.70 (10H, m), 3.97 (4H, d, J = 5.6 Hz), 4.09 (4H, t, J = 5.6 Hz), 4.24 (4H, d, J = 13.0 Hz), 5.69 (2H, t, J = 5.6 Hz), 6.11 (2H, t, J = 5.6 Hz), 6.87 and 7.06 (4H each, 2s), 7.82 (2H, s). ^{13}C NMR ($CDCl_3$): δ 29.9, 31.0, 31.6, 31.9, 33.8, 34.0, 38.4, 42.0, 52.0, 75.1, 125.2, 125.7, 127.8, 132.5, 142.4, 147.3, 149.4, 149.7, 158.8, 171.9. ESI-MS: m/z 1015.7 ([M + Na]$^+$, 100). Anal. Calcd for $C_{58}H_{80}N_4O_{10} \cdot H_2O$: C, 68.88; H, 8.17; N, 5.54. Found: C, 68.21; H, 7.73; N, 5.61.

Data for Compound 2a. Yield: 88%. Mp: 169−170 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 and 1.33 (18H each, 2s), 0.98 (6H, t, J = 6.0 Hz), 1.86 (4H, m, J = 6.1 Hz), 2.35 (4H, m, J = 5.9 Hz), 3.13 (4H, d, J = 13.5 Hz), 3.32 (4H, m, J = 6.0 Hz), 3.63 (4H, t, J = 6.0 Hz), 3.73 (6H, s), 4.03 (8H, m), 4.37 (4H, d, J = 13.5 Hz), 5.61 (2H, t, J = 5.7 Hz), 5.74 (2H, br t), 6.43 and 7.11 (4H each, 2s). ^{13}C NMR ($CDCl_3$): δ 10.5, 23.4, 30.9, 31.1, 31.7, 31.4, 33.5, 34.0, 37.5, 41.9, 52.0, 72.4, 77.6, 124.3, 125.4, 131.8, 135.7 143.8, 144.8, 152.3, 154.3, 158.5, 171.9. ESI-MS: m/z 1099.8 ([M + Na]$^+$, 100). Anal. Calcd for $C_{64}H_{92}N_4O_{10} \cdot 2H_2O$: C, 69.04; H, 8.69; N, 5.03. Found: C, 69.30; H, 8.23; N, 5.32.

Data for Compound 1b. Yield: 90%. Mp: 136−140 °C. ^{1}H NMR ($CDCl_3$): δ 0.98 and 1.26 (18H each, 2s, *t*-Bu) 1.12 (6H, d, J = 7.2 Hz, CH_3(Ala)), 2.20 and 2.28 (2H each, 2m, CH_2CH$_2$N), 3.34 and 3.42 (2H each, 2d, J = 13.2 Hz, eq-ArCH_2Ar), 3.61 (4H, br m, CH_2N), 3.70 (6H, s, OCH_3), 4.04 and 4.17 (2H each, 2m, CH_2), 4.17 and 4.30 (2H each, 2d, J = 13.2 Hz, ax-ArCH_2Ar), 4.41 (2H, m, −C*H*(NH)), 5.62 (2H, d, J = 6.9 Hz, NH), 6.20 (2H, br t, NH), 6.83, 6.85, 7.03, and 7.08 (2H each, 4d, J = 2.2 Hz, ArH), 7.81 (2H, s, ArOH). ^{13}C NMR ($CDCl_3$): δ 18.1 (CH_3), 29.5 (CH_2), 29.5 (CH_2), 30.9 and 31.6 (C(*C*H$_3$)), 32.2 (Ar*C*H$_2$Ar), 33.8 and 33.9 (*C*(CH$_3$)), 39.1 (CH$_2$NH spacer), 48.5 (CH$_2$NH-Ala), 52.1 (OCH_3), 75.9 (OCH_2), 125.1, 125.4, 125.6, 126.0, 127.4, 128.2, 132.0, 132.6, 142.7, 147.4, 149.3, 149.4 (Ar), 158.2 (NC═O), 175.0 (OC═O). ESI-MS: m/z 1043.6 ([M + Na]$^+$, 100). Anal. Calcd for $C_{60}H_{84}N_4O_{10} \cdot H_2O$: C, 69.34; H, 8.34; N, 5.39. Found: C, 69.60; H, 8.43; N, 5.01.

Data for Compound 2b. Yield: 88%. Mp: 182−183 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 and 1.33 (18H each, 2s), 0.99 (6H, t, J = 7.4 Hz), 1.42 (6H, d, J = 7.4 Hz), 1.89 (4H, m), 2.34 (4H, m), 3.13 (4H, d, J = 12.5 Hz), 3.34 (4H, m), 3.64 (4H, t, J = 7.4 Hz), 3.73 (6H, s), 4.01 (4H, m), 4.37 (4H, d, J = 12.5 Hz), 4.55 (2H, m), 5.45 (2H, t, J = 5.6 Hz), 5.73 (2H, d, J = 6.9 Hz), 6.44 and 7.10 (4H each, 2s). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 10.6, 18.8, 23.4, 31.0, 31.1, 31.1, 31.7, 33.5, 34.0, 37.3, 48.7, 52.0, 72.5, 77.6, 124.3, 125.4, 131.8, 135.7, 143.8, 144.8, 152.3, 154.3, 158.1, 174.7. ESI-MS: m/z 1127.8 ([M + Na]$^+$, 100). Anal. Calcd for $C_{66}H_{96}N_4O_{10}$: C, 71,71; H, 8.75; N, 5.07. Found: C, 71.77; H, 8.87; N, 4.80.

Data for Compound 1c. Yield: 83%. Mp: 75−77 °C (glass). ^{1}H NMR ($CDCl_3$): δ 0.66 (6H, t, J = 6.8 Hz), 0.72 (6H, d, J = 6.8 Hz), 1.03 and 1.25 (18H each, 2s), 1.6 (4H, m), 2.19 (4H, m), 2.32 (2H, m) 3.34 and 3.46 (2H each, 2d, J = 12.8 Hz), 3.55−3.66 (4H, m), 3.69 (6H, s), 4.02 and 4.24 (2H each, 2m), 4.24 and 4.39 (2H each, 2d, J = 12.8 Hz), 4.43 (2H, m), 5.63 (2H, d, J = 8.7 Hz), 6.44 (2H, b t), 6.88, 6.94, 7.00, and 7.09 (2H each, 4d, J = 2.2 Hz), 8.27 (2H, s). ^{13}C NMR ($CDCl_3$): δ 11.5, 15.3, 24.5, 29.2, 31.0, 31.6, 32.5, 33.7, 34.0, 37.6, 39.5, 51.6, 57.2, 76.4, 125.1, 125.5, 125.6, 126.2, 127.4, 128.3, 132.1, 132.9, 142.8, 144.5, 149.2, 149.3, 158.6, 174.0. ESI-MS: m/z 1127.8 ([M + Na]$^+$, 100). Anal. Calcd for $C_{66}H_{96}N_4O_{10} \cdot H_2O$: C, 70.56; H, 8.79; N, 4.99. Found: C, 70.27; H, 8.56; N, 5.08.

Data for Compound 2c. Yield: 89%. Mp: 238−239 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 (18H, s, *t*-Bu), 0.91 (6H, t, J = 7.3 Hz, CH_3(Ile)), 0.95 (6H, d, J = 6.7 Hz, CH_3(Ile)), 0.99 (6H, t, J = 7.3 Hz, CH_3) 1.19 (2H, m, CH_2(CH$_3$)), 1.33 (18H, s, *t*-Bu), 1.43 (2H, m, CH_2(CH$_3$)), 1.86 (6H, 2m, CH$_2$(Pr) + C*H*(CH$_3$)), 2.35 (4H, m, CH_2), 3.12 (4H, d, J = 12.5 Hz, eq-ArCH_2Ar), 3.35 (4H, m, CH_2), 3.63 (4H, t, J = 7.3 Hz, CH_2), 3.71 (6H, s, OCH_3), 4.02 (4H, m, CH_2), 4.37 (4H, d, J = 12.5 Hz, ax-ArCH_2Ar), 4.53 (2H, m, C*H*(NH)), 5.43 (2H, br t, NH), 6.65 (2H, d, J = 8.2 Hz, NH), 6.44 and 7.099 (4H each, 2s, ArH). ^{13}C NMR ($CDCl_3$): δ 10.6 (OCH$_2$CH$_2$*C*H$_3$), 11.6 (*C*H$_3$CH$_2$(Ile)), 15.5 (*C*H$_3$CH(Ile)) 23.4 (OCH$_2$*C*H$_2$CH$_3$), 25.0 (CH$_3$*C*H$_2$(Ile)), 30.9 (Ar*C*H$_2$Ar), 31.0 (CH$_2$), 31.1 and 31. 7 (C(*C*H$_3$)), 33.5 and 34.1 (*C*(CH$_3$)), 37.2 (CH$_2$NH), 38.1 (CH$_3$*C*H(Ile)), 51.7 (OCH_3), 57.3 (CHNH(Ile)), 72.5 (O*C*H$_2$CH$_2$CH$_2$N), 77.6 (OCH$_2$CH$_2$CH$_3$), 124.3, 125.4, 131.8, 135.7, 143.8, 144.9, 152.3, 154.3 (Ar), 158.3 (NC═O), 173.7 (OC═O). ESI-MS: m/z 1211.8 ([M + Na]$^+$, 100). Anal. Calcd for $C_{72}H_{108}N_4O_{10}$: C, 72.69; H, 9.15; N, 4.7. Found: C, 72.31; H, 8.96; N, 4.81.

Data for Compound 3. Yield: 94%. Mp: 144−145 °C. ^{1}H NMR ($CDCl_3$): δ 0.98 (18H, s), 1.29 (18H, s), 2.16 (4H, m), 2.78 (6H, d, J = 3.3 Hz), 3.40 (4H, d, J = 13.2 Hz), 3.66 (4H, q, J = 7.8 Hz), 4.07 (4H, t, J = 6.0 Hz), 4.19 (4H, d, J = 13.2 Hz), 5.18 (2H, q, J = 4.7 Hz), 5.79 (2H, t, J = 4 Hz), 6.85 and 7.08 (4H each, 2s). ^{13}C NMR ($CDCl_3$): δ 26.9, 30.3, 30.9, 31.5, 31.7, 33.8, 34.0, 38.2, 75.2, 125.2, 125.7, 127.8, 132.6, 142.7, 147.7, 149.3, 149.5, 159.9. ESI-MS: m/z 899.7 ([M + Na]$^+$, 100). Anal. Calcd for $C_{54}H_{76}N_4O_6 \cdot 2.5H_2O$: C, 70.33; H, 8.85; N, 6.07. Found: C, 70.57; H, 8.45; N, 6.09.

Molecular Modeling. The conformational analysis of ligands **1b** and **2b** was carried out with the classical molecular mechanics force field (MMFF) and using the Monte Carlo method to randomly sample the conformational space. The complexes of the calixarene ligand **2b** have been obtained starting from the optimized geometry of the receptors, placing the carboxylate anion at a close distance from the urea NH protons, and leaving the system free to relax without constraints again using the molecular mechanics force field. The conformational analysis of these complexes allowed the selection of a few minimum conformations, whose energy was calculated by semiempirical methods at the PM3 level. All calculations were performed using Spartan 04[40] on a Pentium IV PC at 2.5 GHz.

(40) *SPARTAN 04*, release 1.01; Wavefunction, Inc.: Irvine, CA, 2004; http://www.wavefun.com.

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Supporting Information Available: 1D and 2D NMR spectra of the urea ligands and general experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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