JOC The Journal of Organic Chemistry

Note

pubs.acs.org/joc

Anion-Induced Dimerization in *p*-Squaramidocalix[4]arene Derivatives

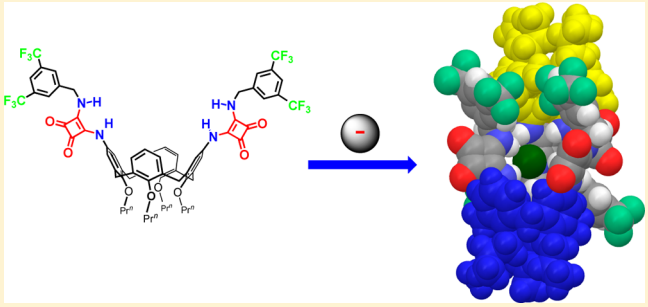
Carmine Gaeta,*[,†] Carmen Talotta,[†] Paolo Della Sala,[†] Luigi Margarucci,[‡] Agostino Casapullo,[‡] and Placido Neri[†]

[†]Dipartimento di Chimica e Biologia, Università di Salerno, Via Giovanni Paolo II 132, I-84084 Fisciano (Salerno), Italy

[‡]Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II 132, I-84084 Fisciano (Salerno), Italy

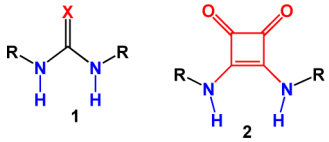
Supporting Information

ABSTRACT: Spherical anions induce the dimerization of calix[4]arene derivatives **3** and **4** bearing squaramide moieties at the *exo* rim (*p*-squaramidocalixarenes). ^{1}H NMR titration experiments showed that unlike the distal isomer **3**, proximal *p*-squaramidocalixarene **4** is also able to form dimeric complexes with trigonal-planar anions.



The development of efficient artificial receptors for the selective binding of biologically or environmentally important anionic species has been the object of an extensive research effort in the past decade.[1] Among the possible artificial anionic hosts, electroneutral species have been particularly investigated because of their ability to recognize anions over a large interval of pH.[2] The most common electroneutral anionic hosts generally contain polarized N−H fragments of amide,[3] urea/thiourea[4] (**1a**/**1b**; Chart 1) or pyrrole groups,[5] and very often display remarkable host properties.

Chart 1



In the last years, the squaramide subunit[6] **2** (Chart 1) has been considered as a novel H-bond-donating group that can coordinate anionic[7] guests because of its ability to establish bifurcated hydrogen bonds like urea/thiourea groups do. With respect to urea/thiourea groups, recent reports have shown that the squaramide ring presents structural and electronic diversities that make it superior as a H-bond-donating group.[8]

The calix[4]arene macrocycle[9] is greatly used as a molecular scaffold in the design of artificial anion receptors because of its ready availability and easy chemical modification, which allows the introduction of appropriate functional groups to create a suitable complementarity with anionic[10] guests. Thus, increasing attention has been devoted to the synthesis of hybrid calixarene hosts bearing electroneutral H-bond-donor units such as amide[11] and urea groups,[12] which often display remarkable recognition properties. In regard to hybrid calixarene−squaramide hosts, Jiang and co-workers[13] very recently reported the first examples of calix[4]arene-based receptors in which two squaramide moieties were introduced at the *endo* (or lower) rim. These hybrid hosts were able to recognize and sense $H_2PO_4^-$, AcO^-, and F^- anions.

As an alternative to functionalization of the the *endo* rim, the introduction of functionalities at the *exo* (or upper) rim of the calixarene skeleton plays a special role because it is considered the best way to exploit the preformed calixarene cavity in recognition processes evocating the use of calixarenes as enzyme mimics, as already precognized by Gutsche in his early papers.[14] Unexpectedly, no examples of hybrid *exo*-rim-linked calix[4]arene−squaramide hosts have been reported to date. This observation prompted us to investigate the introduction of squaramide moieties at the *exo* rim of the calix[4]arene macrocycle and to study the recognition properties of these novel *p*-squaramidocalix[4]arene derivatives **3** and **4** (Scheme 1) toward anionic guests.

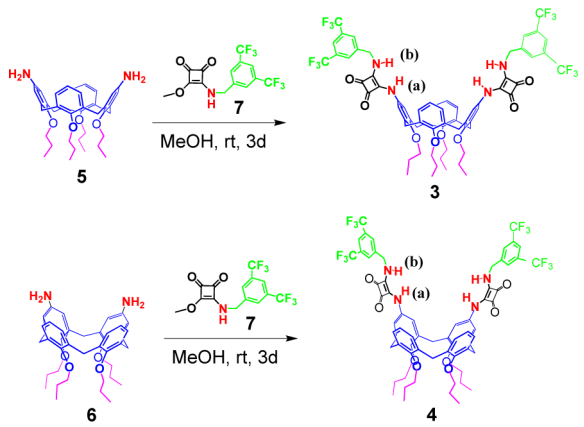
The synthesis of **3** and **4** is outlined in Scheme 1. In particular, the known precursors 5,17-diaminocalix[4]arene (**5**) and 5,11-diaminocalix[4]arene (**6**), obtained according to the procedure of Reinhoudt and co-workers[15] (see Scheme S1),[16] were coupled with squaramido ester **7** in MeOH as the solvent to give **3** and **4** in 30% and 35% yield, respectively.

The structures of regioisomeric *p*-squaramidocalix[4]arenes **3** and **4** were readily confirmed by spectral analysis.[16] In

Received: February 22, 2014
Published: March 19, 2014

   dx.doi.org/10.1021/jo500422u | J. Org. Chem. 2014, 79, 3704−3708

Scheme 1. Synthesis of *p*-Squaramidocalix[4]arene Derivatives 3 and 4



particular, the presence of a pseudomolecular ion peak at m/z 1265 (MH^+) in their ESI(+) mass spectra confirmed the molecular formula, while the ^1H and ^{13}C NMR spectra (Figures S1−S3) and the 2D COSY NMR spectra (Figures S4−S6) confirmed the distal and proximal disubstitutions in **3** and **4**, respectively.[16,17]

The ability of *p*-squaramidocalix[4]arene **3** to bind anionic guests was studied by standard ^1H NMR titrations[18] in which the host concentration was kept constant while the guest concentration was varied (Table 1). DMSO-d_6 containing 0.5% D_2O was used as the solvent in order to minimize the effect of atmospheric water absorption during the titrations.

The addition of spherical bromide anions, in the form of the tetrabutylammonium salt (TBABr), to the solution of receptor **3** caused downfield shifts of the NMR signals of both the NH_b and NH_a squaramide protons. This indicated that these groups were engaged in H-bonding interactions with the anionic guest with a fast complexation equilibrium. Surprisingly, a Job plot for **3** and Br^- (Figure S7)[16] showed a maximum at a mole fraction of 0.67,[18] thus indicating a 2:1 calixarene/bromide binding stoichiometry. The formation of the dimeric $Br^-\cdot(\mathbf{3})_2$ complex was also evidenced by the ESI(−) mass spectrum (Figure 1),

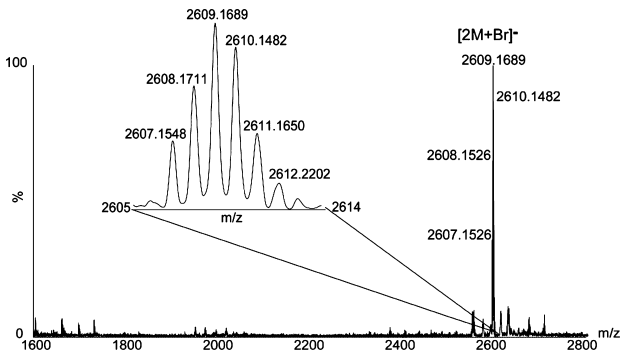


Figure 1. ESI(−) mass spectrum of a 1:1 mixture of **3** and Br^- in $CHCl_3$.

which evidenced a sharp isotope pattern centered at m/z 2609.17 $[2M + Br]^-$ in good agreement with the dimeric complex. The binding constants were estimated by NMR titrations carried out with a 1 mM solution of **3** using 0.1−4.0 mM TBABr at 25 °C. Nonlinear least-squares fitting for the NH_a proton was performed using the EQNMR program.[19] The $K_{1:1}$ and $K_{2:1}$ values are 500 and 230 M^{-1}, respectively, with $K_{1:1} > K_{2:1}$ (see Table 1).

An insight into the structure of the dimeric $Br^-\cdot(\mathbf{3})_2$ complex was obtained by molecular mechanics calculations using the MacroModel 9.0 program[20] with the optimized potentials for liquid simulations (OPLS) force field and H_2O as the solvent. In the structure with the lowest OPLS energy (Figure 2a), the calixarene macrocycle is locked in the cone conformation by the four propyl groups at the lower rim. The molecular model suggests that the stabilization of the dimeric $Br^-\cdot(\mathbf{3})_2$ complex is obtained by H-bonding interactions between the squaramide NHs and the bromide anion. In particular, two squaramidocalixarene molecules (Figure 2b) can assume a relative orientation that brings the two squaramide rings of two different calixarenes into approximately the same plane, promoting their square-planar coordination around the bromide anion. A comparable dimerization induced by a spherical anion was previously reported by Lhoták and co-workers[21] for a calix[4]arene derivative bearing proximally located ureido moieties at the *exo* rim.

Similar results were obtained for the chloride anion. In particular, the formation of the dimeric $Cl^-\cdot(\mathbf{3})_2$ complex was evidenced by the ESI(−) mass spectrum and by Job plot analysis, which showed a 2:1 binding stoichiometry. In this case also, $K_{1:1}$ (325 M^{-1}) was larger than $K_{2:1}$ (70 M^{-1}).

Thus, we can conclude here that spherical anions such as chloride and bromide induce dimerization of calix[4]arene **3** 1,3-difunctionalized at the *exo* rim with squaramide rings. It is likely that the dimerization process is thermodynamically driven by the favorable square-planar coordination of two squaramide rings of different calixarenes around at the spherical anion.

Interestingly, to the best of our knowledge, these are the first examples in the literature in which spherical anions induce a dimerization of squaramide-based hosts. Previously, Costa and co-workers[22] reported the dimerization of a squaramide-based host in a protic solvent induced by the tetrahedral sulfate anion.

Unlike distal *p*-squaramidocalix[4]arene **3**, the proximal regioisomer **4** showed good solubility in $CDCl_3$. Thus, its binding properties (Figures S8−S10) were studied in $CDCl_3$ containing 1% DMSO-d_6 and 0.5% D_2O.[16] Interestingly, Job plots of **4** and spherical guests such as Br^- and Cl^- showed maxima at a mole fraction of 0.67 in each case (Figure S9b,c).[16] These results indicate that host **4** also binds spherical anions with a 2:1 stoichiometry, forming dimeric complexes. The formation of the $Br^-\cdot(\mathbf{4})_2$ complex was also evidenced by the ESI(−) mass spectrum of **4** in the presence of 1 equiv of Br^- (Figure S11, top),[16] which evidenced the presence of a sharp pattern centered at m/z 2609 $[2M + Br]^-$ corresponding to the dimeric complex. Analogously, the ESI(−) mass spectrum obtained for a mixture of **4** and Cl^- (Figure S11, bottom)[16] corroborated the presence of the dimeric complex $Cl^-\cdot(\mathbf{4})_2$. Titrations of **4** with bromide and chloride anions (Figures S8−S10) gave binding isotherms for the squaramide NH_a proton, in which a downfield shift was observed until a plateau was reached at about 1 equiv of anion (Figure S9a). The titration chemical shift data were analyzed by nonlinear least-squares fitting procedures using the EQNMR program[19] to give the association constants reported in Table 1. In particular, a total association constant $K_{tot} = K_{1:1}K_{2:1} = (9.1 \pm 0.2) \times 10^7$ M^{-2} (Table 1) was determined for the $Cl^-\cdot(\mathbf{4})_2$ complex, a value significantly higher than that observed for the bromide-induced dimerization of **4** [$K_{tot} = (2.2 \pm 0.2) \times 10^6$ M^{-2}].

dx.doi.org/10.1021/jo500422u | J. Org. Chem. 2014, 79, 3704−3708

Table 1. Complexation Constants ($K_{1:1}$ and $K_{2:1}$) of Receptors 3[a] and 4[b] toward Chloride, Bromide, Benzoate, and Nitrate Anions As Determined by ^{1}H NMR Titrations (400 MHz) at 298 K

		Cl^-	Br^-	$PhCOO^-$	NO_3^-
3[a]	$K_{1:1}$	325 ± 20	500 ± 65	–[c]	–[c]
	$K_{2:1}$	70 ± 30	230 ± 40		
4[b]	$K_{1:1}$	(3.6 ± 0.4) × 10^4	(3.10 ± 0.34) × 10^3	(2.47 ± 0.37) × 10^3	(1.25 ± 0.2) × 10^3
	$K_{2:1}$	(2.54 ± 0.2) × 10^3	720 ± 30	102 ± 12	78 ± 10

[a] ^{1}H NMR titrations were carried out in DMSO-d_6/0.5% D_2O. [b] ^{1}H NMR titrations were carried out in $CDCl_3$/1% DMSO-d_6/0.5% D_2O. [c] No changes in the ^{1}H NMR spectra were observed.

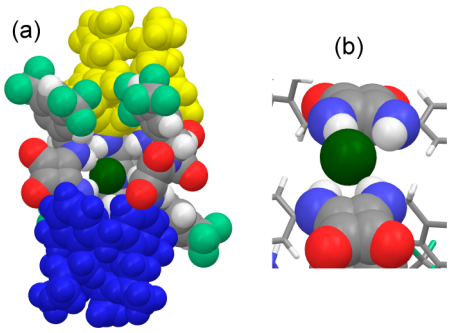


Figure 2. (a) CPK model of the energy-minimized structure of the $Br^-\cdot(3)_2$ complex (OPLS force field, H_2O, GB/SA solvent model). (b) View of the square-planar coordination of the two squaramide rings around the Br^- anion (green).

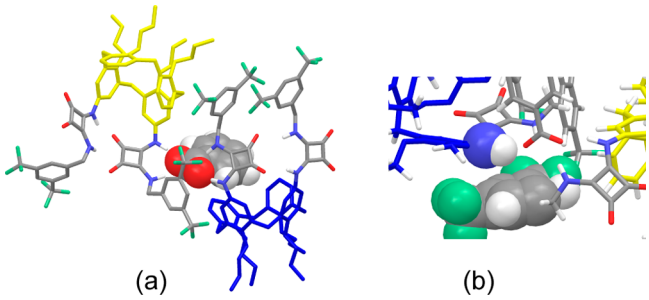


Figure 3. (a) Model of the energy-minimized structure of the $PhCOO^-\cdot(4)_2$ complex. (b) View of the NH_a disposition above the aromatic 3,5-trifluoromethylphenyl ring.

The addition of trigonal-planar anions such as nitrate and benzoate, in the form of their tetrabutylammonium salts, to solutions of 1,3-disubstituted *p*-squaramidocalix[4]arene receptor **3** did not cause significant shifts of the ^{1}H NMR signals of the host, indicating that no appreciable interactions were established between distal regioisomer **3** and the benzoate or nitrate anion. In contrast to **3**, the proximal regioisomer **4** exhibited good binding abilities toward trigonal-planar anionic guests. In fact, the addition of benzoate (Figure S12, top) or nitrate (Figure S12, bottom),[16] in the form of the TBA salt, to the solution of **4** caused a shift of both squaramide NH_b and NH_a signals in the ^{1}H NMR spectrum. In particular, a close inspection of the binding isotherms in Figure S12[16] revealed an initial upfield shift of the NH_a proton up to the addition of 0.5 equiv of anionic substrate. Above 0.5 equiv, the direction of the shift changed to downfield. As previously reported by Hamilton,[24] these type of complexation induced shifts (CISs) are due to two complexation equilibria. In the first one, at the beginning of the titration, the macrocycle is in excess with respect to the anion and the prevalent species is the dimeric complex $X^-\cdot(4)_2$. In the second one, as the anion concentration increases, the equilibrium shifts to the monomeric $X^-\cdot$**4** complex and reaches the saturation point toward the end of the titration. Also in these cases, nonlinear fitting of the titration data using the EQNMR program[19] gave the following association constants (Table 1): $K_{1:1}$ = (2.47 ± 0.37) × 10^3 and $K_{2:1}$ = 102 ± 12 for benzoate; $K_{1:1}$ = (1.25 ± 0.17) × 10^3 and $K_{2:1}$ = 78 ± 10 for nitrate. Regarding the dimeric $PhCOO^-\cdot(4)_2$ complex, molecular mechanics calculations (MacroModel 9.0 program, AMBER force field, $CHCl_3$ as the solvent) revealed a structure (Figure 3a) that is slightly different with respect to that observed for the dimeric $Br^-\cdot(3)_2$ complex. In particular, the benzoate ring is almost perpendicular to the two squaramide planes, with the oxygen atoms of the carboxylate group engaged in four H-bonds with the NH groups of two squaramide units of two different calixarene caps. Close inspection of the lowest-energy structure of the $PhCOO^-\cdot(4)_2$ complex (Figure 3b) revealed that the NH_a squaramide protons lie directly above the aromatic ring of the trifluoromethylphenyl moieties (Figure 3b). This accounts for the previously mentioned initial upfield shift of the NH_a protons in the titration of **4** with $PhCOO^-$ (Figure S12 top).

The surprising differences in the binding behavior toward trigonal-planar anions of the seemingly similar regioisomeric receptors **3** and **4** can be explained in terms of a balance between inter- and intramolecular H-bonds.[23] In fact, the more symmetrical distal isomer **3** has a strong tendency to give intermolecular H-bonding interactions, as evidenced by its aggregating tendency in the less polar $CDCl_3$ solvent, which reduces its binding affinities. On the other hand, the less symmetrical proximal isomer **4** is less prone to give intermolecular H-bonding interactions, as evidenced by its higher solubility in $CDCl_3$, leading to intramolecular H-bonds (Figure S13),[16,23] which favor both the 1:1 and 2:1 complexation with trigonal-planar anions. This indicates that intramolecular H-bonding interactions can be advantageous for receptor efficiency.[23]

In conclusion, we have here reported the anion-induced dimerization of *p*-squaramidocalix[4]arene derivatives **3** and **4**. The stabilization of the dimeric architectures was brought by the square-planar coordination of two squaramide rings around the anionic guests to give H-bonding interactions between squaramide NH protons and the anion. ^{1}H NMR titration experiments showed that both distal and proximal *p*-squaramidocalixarenes **3** and **4**, respectively, are able to form dimeric capsules in the presence of spherical anions such as chloride and bromide. However, significant complexation of trigonal-planar benzoate and nitrate anions was observed only with the proximal isomer **4**, which also forms stable dimeric $X^-\cdot(4)_2$ complexes, probably because of favorable intramolecular H-bonding interactions and greater complementarity with respect to the distal isomer **3**. To the best of our knowledge,

dx.doi.org/10.1021/jo500422u | J. Org. Chem. 2014, 79, 3704−3708

the above cases are the first examples in which a spherical anion induces dimerization of a squaramide-based receptor. The above-mentioned favorable intramolecular H-bonding interactions can be considered a new and interesting approach to optimize and tune the recognition properties of molecular receptors.

EXPERIMENTAL SECTION

General Information. High-resolution ESI-MS spectra were performed on a Q-ToF mass spectrometer equipped with an electrospray ion source. Each compound was analyzed by direct infusion using 80:18:2 CH_2Cl_2/CH_3OH/HCOOH as the solvent. The instrument was calibrated using [Glu1]-Fibrinopeptide B. All of the chemicals were reagent-grade and used without further purification. Anhydrous solvents were used as purchased from the supplier. When necessary, compounds were dried in vacuo over $CaCl_2$. Reaction temperatures were measured externally. Derivatives **5**,[15] **6**,[15] and **7**[25] were synthesized according to literature procedures. NMR spectra were recorded on a 400 MHz spectrometer; chemical shifts are reported relative to the residual solvent peak. One-dimensional ^1H and ^{13}C spectra, COSY-45, and NOESY were used for NMR peak assignment. COSY-45 spectra were recorded using a relaxation delay of 2 s with 30 scans and 170 increments of 2048 points each. NOESY spectra were recorded in phase-sensitive mode using a mixing time (t_m) of 200 ms. Monte Carlo conformational searches (10 000 steps) were performed with the MacroModel 9.0/Maestro 4.1 program[20] using the OPLS force field and H_2O or $CHCl_3$ solvent (GB/SA model). The starting structures were built on the basis of the cone conformation adopted by the tetrapropoxycalix[4]arene skeleton.

General Procedure for the Synthesis of *p*-Squaramidocalix[4]arene Derivatives 3 and 4. The appropriate aminocalix[4]arene derivative **5** or **6**[15] (0.10 g, 0.16 mmol) was dissolved in dry methanol (5 mL), and then derivative **7**[25] (0.25 g. 0.71 mmol) was added. The reaction mixture was stirred at room temperature for 48 h, filtered, and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with 1 N HCl and H_2O. The organic phase was dried on Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel.

Derivative ***3***. CH_2Cl_2/MeOH (9:1 v/v), white solid, 0.061 g, 30% yield. ESI(+) MS: *m*/*z* 1265 (MH^+). ^1H NMR (DMSO-d_6, 400 MHz, 298 K): δ 0.94 (t, $OCH_2CH_2CH_3$, J = 6.9 Hz, 6H), 1.02 (t, $OCH_2CH_2CH_3$, J = 6.9 Hz, 6H), 1.85−1.92 (m, $OCH_2CH_2CH_3$, 8H), 3.12 and 4.32 (AX, $ArCH_2Ar$, J = 12.8 Hz, 8H), 3.69 (br t, $OCH_2CH_2CH_3$, 4H), 3.84 (br t, $OCH_2CH_2CH_3$, 4H), 4.97 (s, OCH_2, 4H), 6.40 (br t, ArH, 2H), 6.48 (br d, ArH, 4H), 6.96 (br s, ArH, 4H), 7.94 (br s, NH, 2H), 8.05 (s, ArH, 2H), 8.11 (s, ArH, 4H), 9.45 (s, NH, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz, 298 K): δ 10.1, 10.5, 22.7, 22.9, 30.4, 46.2, 76.4, 76.6, 118.7, 121.5, 122.0, 124.7, 127.8, 128.7, 130.4, 130.7, 132.8, 133.6, 136.3, 142.6, 152.8, 155.3, 164.3, 168.3, 180.7, 183.9. Anal. Calcd for $C_{66}H_{60}F_{12}N_4O_8$: C, 62.66; H, 4.78. Found: C, 62.75; H, 4.69.

Derivative ***4***. CH_2Cl_2/MeOH (9:1 v/v), white solid, 0.071 g, 35% yield. ESI(+) MS: *m*/*z* 1265 (MH^+). ^1H NMR ($CDCl_3$/DMSO-d_6, 99:1 v/v, 400 MHz, 298 K): δ 0.93 (overlapped, $OCH_2CH_2CH_3$, 6H), 1.83 (overlapped, $OCH_2CH_2CH_3$, 8H), 3.06 and 4.29 (AX, $ArCH_2Ar$, J = 12.8 Hz, 2H), 3.10 and 4.32 (AX, $ArCH_2Ar$, J = 13.6 Hz, 4H), 3.16 and 4.33 (AX, $ArCH_2Ar$, J = 12.8 Hz, 2H), 3.75 (overlapped, $OCH_2CH_2CH_3$, 4H), 4.98 (s, OCH_2, 4H), 6.37 (broad, ArH, 2H), 6.51 (broad, ArH, 2H), 6.66 (broad, ArH, 2H), 7.73 (broad, ArH, 2H), 7.77 (broad, ArH, 2H), 7.79 (overlapped, ArH, 6H), 8.06 (br s, NH, 2H), 9.11 (s, NH, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz, 298 K): δ 10.2, 22.7, 22.8, 30.2, 30.4, 30.6, 46.0, 76.2, 76.3, 79.1, 121.2, 121.5, 121.6, 121.9, 124.6, 127.8, 127.9, 128.5, 130.3, 130.6, 132.6, 134.1, 134.3, 134.6, 134.9, 135.4, 142.3, 152.3, 156.1, 164.2, 168.2, 180.9, 183.4. Anal. Calcd for $C_{66}H_{60}F_{12}N_4O_8$: C, 62.66; H, 4.78. Found: C, 62.78; H, 4.68.

Determination of Association Constants by ^1H NMR Titrations. ^1H NMR titrations were performed at 298 K. The host concentration was kept constant while the guest concentration was varied [e.g., 1 mM host (**3** or **4**) using 0.1−2.5 mM TBAX]. In all cases, the signals of the host were followed and the data were analyzed by nonlinear regression analysis.[19]

Job Plot Experiments. Complexation stoichiometries were determined from Job plots using ^1H NMR spectroscopy.[18] Stock solutions of host (5 mM) and guest (5 mM) in $CDCl_3$ were prepared. Ten NMR spectra were obtained in the following volume ratios (host/guest): 100:400, 150:350, 200:300, 250:250, 300:200, 350:150, 40:100, 450:50, 500:0 (μL/μL). The chemical shift of NH squaramide protons was recorded for each sample, and the corresponding concentration of the complex was determined for each sample. The Job plot was obtained by plotting the complex concentration as a function of the mole fraction of the host.

ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR and 2D COSY spectra of derivatives **3** and **4**, NMR titration data, and detailed results of the molecular mechanics calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: cgaeta@unisa.it.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Italian MIUR (PRIN 20109Z2XRJ_006) for financial support.

DEDICATION

This paper is dedicated to Professor A. Zambelli on the occasion of his 80th birthday.

REFERENCES

(1) (a) Sessler, J. L.; Gale, P. A.; Cho, W.-S. *Anion Receptor Chemistry*; Royal Society of Chemistry: Cambridge, U.K., 2006. (b) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 191−221. (c) Steed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 506−519. (d) Katayev, E. A.; Ustynyuk, Y. A.; Sessler, J. L. *Coord. Chem. Rev.* **2006**, *250*, 3004−3037.

(2) (a) Gale, P. A. In *Encyclopedia of Supramolecular Chemistry*; Atwood, J. L., Steed, J. W., Eds.; Marcel Dekker: New York, 2004; p 3141. (b) Amendola, V.; Fabbrizzi, L.; Mosca, L. *Chem. Soc. Rev.* **2010**, *39*, 3889−3915. (c) Dydio, P.; Lichosyt, D.; Jurczak, J. *Chem. Soc. Rev.* **2011**, *40*, 2971−2985.

(3) (a) Choi, K.; Hamilton, A. D. *J. Am. Chem. Soc.* **2003**, *125*, 10241−10249. (b) Kang, S. O.; Llinares, J. M.; Powell, D.; VanderVelde, D.; Bowman-James, K. *J. Am. Chem. Soc.* **2003**, *125*, 10152−10153. (c) Otto, S.; Kubik, S. *J. Am. Chem. Soc.* **2003**, *125*, 7804−7805. (d) Bondy, C. R.; Loeb, S. J. *Coord. Chem. Rev.* **2003**, *240*, 77−99.

(4) (a) Smith, P. J.; Reddington, M. V.; Wilcox, C. S. *Tetrahedron Lett.* **1992**, *33*, 6085−6088. (b) Fan, E.; van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369−370. (c) Evans, A. J.; Matthews, S. E.; Cowley, A. R.; Beer, P. D. *Dalton Trans.* **2003**, 4644−4650. (d) Miyaji, H.; Collinson, S. R.; Prokes, I.; Tucker, J. H. R. *Chem. Commun.* **2003**, 64−65. (e) Takemoto, Y. *Org. Biomol. Chem.* **2005**, *3*, 4299−4306.

(5) (a) Gale, P. A.; Sessler, J. L.; Král, V.; Lynch, V. *J. Am. Chem. Soc.* **1996**, *118*, 5140−5141. (b) Cafeo, G.; Kohnke, F. H.; LaTorre, G. L.; White, A. J. P.; Williams, D. J. *Chem. Commun.* **2000**, 1207−1208. (c) Anzenbacher, P., Jr.; Try, A. C.; Miyaji, H.; Jursíková, K.; Lynch, V. M.; Marquez, M.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 10268−10272. (d) Sessler, J. L.; Anzenbacher, P., Jr.; Jursíková, K.; Miyaji, H.;

Genge, J. W.; Tvermoes, N. A.; Allen, W. E.; Shriver, J. A.; Gale, P. A.; Král, V. *Pure Appl. Chem.* **1998**, *70*, 2401−2408. (e) Camiolo, S.; Gale, P. A. *Chem. Commun.* **2000**, 1129−1130.

(6) (a) Prohens, R.; Tomás, S.; Morey, J.; Deyá, P. M.; Ballester, P.; Costa, A. *Tetrahedron Lett.* **1998**, *39*, 1063−1066. (b) Prohens, R.; Martorell, G.; Ballester, P.; Costa, A. *Chem. Commun.* **2001**, 1456−1457. (c) Prohens, R.; Rotger, M. C.; Piña, M. N.; Deyá, P. M.; Morey, J.; Ballester, P.; Costa, A. *Tetrahedron Lett.* **2001**, *42*, 4933−4936. (d) Amendola, V.; Bergamaschi, G.; Boiocchi, M.; Fabbrizzi, L.; Milani, M. *Chem.—Eur. J.* **2010**, *16*, 4368−4380. (e) Amendola, V.; Fabbrizzi, L.; Mosca, L.; Schmidtchen, F.-P. *Chem.—Eur. J.* **2011**, *17*, 5972−5981. (f) Alemán, J.; Parra, A.; Jiang, H.; Jørgensen, K. A. *Chem.—Eur. J.* **2011**, *17*, 6890−6899.

(7) (a) Jin, C.; Zhang, M.; Wu, L.; Guan, Y.; Pan, Y.; Jiang, J.; Lin, C.; Wang, L. *Chem. Commun.* **2013**, *49*, 2025−2027. (b) Rostami, A.; Wei, C. J.; Guárin, G.; Taylor, M. S. *Angew. Chem., Int. Ed.* **2011**, *50*, 2059−2062.

(8) (a) Garau, C.; Frontera, A.; Ballester, P.; Quiñonero, D.; Costa, A.; Deyá, P. M. *Eur. J. Org. Chem.* **2005**, 179−183. (b) Quiñonero, D.; Frontera, A.; Suñer, G. A.; Morey, J.; Costa, A.; Ballester, P.; Deyá, P. M. *Chem. Phys. Lett.* **2000**, *326*, 247−254. (c) Quiñonero, D.; Prohens, R.; Garau, C.; Frontera, A.; Ballester, P.; Costa, A.; Deyá, P. M. *Chem. Phys. Lett.* **2002**, *351*, 115−120. (d) Neus Piña, M.; Rotger, M. C.; Costa, A.; Ballester, P.; Deyá, P. M. *Tetrahedron Lett.* **2004**, *45*, 3749−3752. (e) Okino, T.; Hoashi, Y.; Furukawa, T.; Xu, X. N.; Takemoto, Y. *J. Am. Chem. Soc.* **2005**, *127*, 119−125. (f) Malerich, J. P.; Hagihara, K.; Rawal, V. H. *J. Am. Chem. Soc.* **2008**, *130*, 14416−14417.

(9) For general reviews of calix[*n*]arenes, see: (a) Böhmer, V. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713−745. (b) Gutsche, C. D. *Calixarenes Revisited*; Royal Society of Chemistry: Cambridge, U.K., 1998. (c) *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Eds.; Kluwer: Dordrecht, The Netherlands, 2001. (d) Böhmer, V. In *The Chemistry of Phenols*; Rappoport, Z., Ed.; Wiley: Chichester, U.K., 2003; Chapter 19. (e) *Calixarenes in the Nanoworld*; Vicens, J., Harrowfield, J., Eds.; Springer: Dordrecht, The Netherlands, 2007.

(10) (a) Edwards, N. Y.; Anthony, P. L. *Supramol. Chem.* **2013**, *25*, 446−463. (b) Matthews, S. E.; Beer, P. D. In *Calixarenes 2001*; Asfari, Z., Böhmer, V., Harrowfield, J., Vicens, J., Eds.; Kluwer: Dordrecht, The Netherlands, 2001; pp 421−439. (c) Lhoták, P. *Top. Curr. Chem.* **2005**, *255*, 65−95. (d) Matthews, S. E.; Beer, P. D. *Supramol. Chem.* **2005**, *17*, 411−435.

(11) (a) Flídrová, K.; Tkadlecová, M.; Lang, K.; Lhoták, P. *Tetrahedron Lett.* **2012**, *53*, 678−680. (b) Kim, H. J.; Kim, S. K.; Lee, J. Y.; Kim, J. S. *J. Org. Chem.* **2006**, *71*, 6611−6616. (c) Troisi, F.; Russo, A.; Gaeta, C.; Bifulco, G.; Neri, P. *Tetrahedron Lett.* **2007**, *48*, 7986−7989. (d) Troisi, F.; Gaeta, C.; Pierro, T.; Neri, P. *Tetrahedron Lett.* **2009**, *50*, 5113−5115.

(12) (a) Budka, J.; Lhoták, P.; Michlová, V.; Stibor, I. *Tetrahedron Lett.* **2001**, *42*, 1583−1586. (b) Dudič, M.; Lhoták, P.; Stibor, I.; Lang, K.; Prošková, P. *Org. Lett.* **2003**, *5*, 149−152. (c) Quinlan, E.; Matthews, S. E.; Gunnlaugsson, T. *Tetrahedron Lett.* **2006**, *47*, 9333−9338. (d) Yakovenko, A. V.; Boyko, I. V.; Kalchenko, V. I.; Baldini, L.; Casnati, A.; Sansone, F.; Ungaro, R. *J. Org. Chem.* **2007**, *72*, 3223−3231.

(13) Jin, C.; Zhang, M.; Deng, C.; Guan, Y.; Gong, J.; Zhu, D.; Pan, Y.; Jiang, J.; Wang, L. *Tetrahedron Lett.* **2013**, *54*, 796−801.

(14) (a) Gutsche, C. D. *Acc. Chem. Res.* **1983**, *16*, 161−170. For recent reports in which the calixarene cavity was exploited in recognition processes, see: (b) Gaeta, C.; Talotta, C.; Farina, F.; Camalli, M.; Campi, G.; Neri, P. *Chem.—Eur. J.* **2012**, *18*, 1219−1230. (c) Gaeta, C.; Talotta, C.; Farina, F.; Teixeira, F. A.; Marcos, P. M.; Ascenso, J. R.; Neri, P. *J. Org. Chem.* **2012**, *77*, 10285−10293. (d) Moerkerke, S.; Ménand, M.; Jabin, I. *Chem.—Eur. J.* **2010**, *16*, 11712−11719. (e) Coquière, D.; de la Lande, A.; Martì, S.; Parisel, O.; Prangé, T.; Reinaud, O. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10449−10454.

(15) Kelderman, E.; Verboom, W.; Engbersen, J. F. J.; Reinhoudt, D. N.; Heesink, G. J. T.; van Hulst, N. F.; Derhaeg, L.; Persoons, A. *Angew. Chem., Int. Ed. Engl.* **1992**, *8*, 1075−1077.

(16) See the Supporting Information for further details.

(17) In accordance with the C_{2v}-symmetric structure of distal regioisomer **3**, its ^{1}H NMR spectrum (Figure S1 top) (400 MHz, 298 K, DMSO-d_6) showed one AX system at 4.32 and 3.12 ppm (J = 12.8 Hz; see the COSY spectrum in Figure S4) for the four equivalent $ArCH_2Ar$ groups. The proximal functionalization of regioisomer **4** was readily proved by three AX systems for $ArCH_2Ar$ protons at 3.06/4.29 (2H), 3.10/4.32 (4H), and 3.16/4.33 ppm (2H)[16] in its ^{1}H NMR spectrum (Figure S1 bottom; also see the COSY spectrum in Figure S6) (400 MHz, $CDCl_3$/1%-DMSO-d_6, 298 K). The NH_b and NH_a (see Scheme 1) squaramide protons of both **3** and **4** were assigned on the basis of 2D NOESY spectra.

(18) Hirose, K. In *Analytical Methods in Supramolecular Chemistry*; Schalley, C. A., Ed.; Wiley-VCH: Weinheim, Germany, 2007; Chapter 2, pp 17−54.

(19) Hynes, M. J. *J. Chem. Soc., Dalton Trans.* **1993**, 311−312.

(20) *Macromodel*, version 9.0; Schrödinger, LLC: New York, 2005. Mohamadi, F.; Richards, N. G.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(21) Lang, K.; Cuřínová, P.; Dudič, M.; Prošková, P.; Stibor, I.; Šťastný, V.; Lhoták, P. *Tetrahedron Lett.* **2005**, *46*, 4469−4472.

(22) Piña, M. N.; Rotger, C.; Soberats, B.; Ballester, P.; Deyá, P. M.; Costa, A. *Chem. Commun.* **2007**, 963−965.

(23) We thank one of the unknown referees for important suggestions in this regard. For some examples of advantageous intramolecular H-bonds for recognition efficiency, see: (a) Gale, P. A. *Chem. Commun.* **2011**, *47*, 82−86. (b) Gale, P. A. *Acc. Chem. Res.* **2011**, *44*, 216−226. (c) Ramalingam, V.; Domaradzki, M. E.; Jang, S.; Muthyala, R. S. *Org. Lett.* **2008**, *10*, 3315−3318.

(24) Choi, K. A.; Hamilton, D. *J. Am. Chem. Soc.* **2003**, *125*, 10241−10249.

(25) Malerich, J. P.; Hagihara, K.; Rawal, V. H. *J. Am. Chem. Soc.* **2008**, *130*, 14416−14417.