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Hydrogen-Bond-Mediated Self-Assembly of 26-Membered Diaza Tetraester Crowns of 3,5-Disubstituted 1*H*-Pyrazole. Dimerization Study in the Solid State and in $CDCl_3$ Solution

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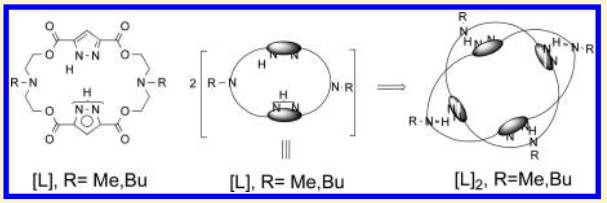
Supporting Information

ABSTRACT: By using an improved synthetic method reported earlier, the cyclic stannoxanes obtained from RN-diethanolamine (R = Me, Bu) and dibutyltin oxide have been reacted with 1*H*-pyrazole-3,5-dicarbonyl dichloride to afford 26-membered diaza tetraester crowns (**1**, R = Me; **3**, R = Bu) and 39-membered triaza hexaester crowns (**2**, R = Me; **4**, R = Bu). The new structures were identified from their analytical and spectroscopic (^{1}H and ^{13}C NMR, FAB-MS, and/or ESI-MS) data. Both diaza tetraester crowns (**1** and **3**), containing two 1*H*-pyrazole units, self-assemble into dimeric species through the formation of four hydrogen bonds involving the two NH pyrazole groups and the two tertiary amine groups of both crowns, as proved by X-ray crystallography and NMR analysis. Preliminary NMR, ESI-MS, MALDI-TOF-MS, and molecular modeling studies suggest that, in $CDCl_3$ solution, **1** interacts with ethyleneurea (ETU), affording 1:1, 2:1, and 2:2 **1**—ETU complexes.



INTRODUCTION

Hydrogen bonding is the favorite intermolecular force in self-assembling systems by virtue of its directionality, specificity, and biological relevance.[1,2] Whereas self-assembly may be taken as a simple collection and aggregation of components into a confined entity, self-organization is considered as the spontaneous but information-directed generation of organized functional structures under equilibrium conditions.[3] In such structures, hydrogen bonds exhibit typical grouplike behavior, and acting together they become much stronger (*cooperativity*). Fundamental studies on these phenomena have gradually developed a new discipline so-called "noncovalent synthesis", based on the reversible formation of multiple hydrogen bonds.[4]

Most natural building blocks, such as carbohydrates, amino acids, and nucleic acids, offer a rich source of H-bond donors and acceptors. Hydrogen-bonding interactions in DNA/RNA systems have recently inspired the use of such motifs to stabilize a range of synthetic structures, and it has led to the formation of a number of novel ensembles. Reference 5 collects several reviews covering this interesting topic.

In this respect, 1*H*-pyrazole can be an appropriate building block, since it has both hydrogen donor and acceptor sites in adjacent positions which can build up a self-complementary hydrogen-bonded assembly.[6] Elguero et al. have examined many 3,5-disubstituted NH-pyrazoles by the combined use of ^{13}C and/or ^{15}N-CP/MAS solid-state NMR spectroscopy and X-ray crystallography, showing that they self-assemble, forming a variety of hydrogen-bonded cyclic dimers, trimers, and tetramers.[7] On the basis of this principle, Ochsenfeld et al. have recently presented an elegant study about the insertion of pyrazole units in a peptide which associates intermolecularly, giving rise to nanorosette-type structures in water.[8] Low-temperature NMR experiments conducted in a freonic solvent have proved that the interaction of 3-methylpyrazole and 3-amino-5-methylpyrazole with the protected tripeptide L-Val-L-Val-L-Val-tBu gives rise to 2:2 complexes in which the peptides display an antiparallel association, with the pyrazole units symmetrically capping both sides.[9] Also, aminopyrazole—peptide hybrid ligands were shown to undergo a specific interaction with amyloid plaques developed in Alzheimer's disease.[10]

We have recently reported that the interaction of the sodium pyrazolate salt of diethyl 1*H*-pyrazole-3,5-dicarboxylate with (+)-amphethamine, β-phenethylamine, and homoveratrylamine hydrochlorides leads to interesting crystal structures in which the ammonium cations and pyrazolate anions are interconnected

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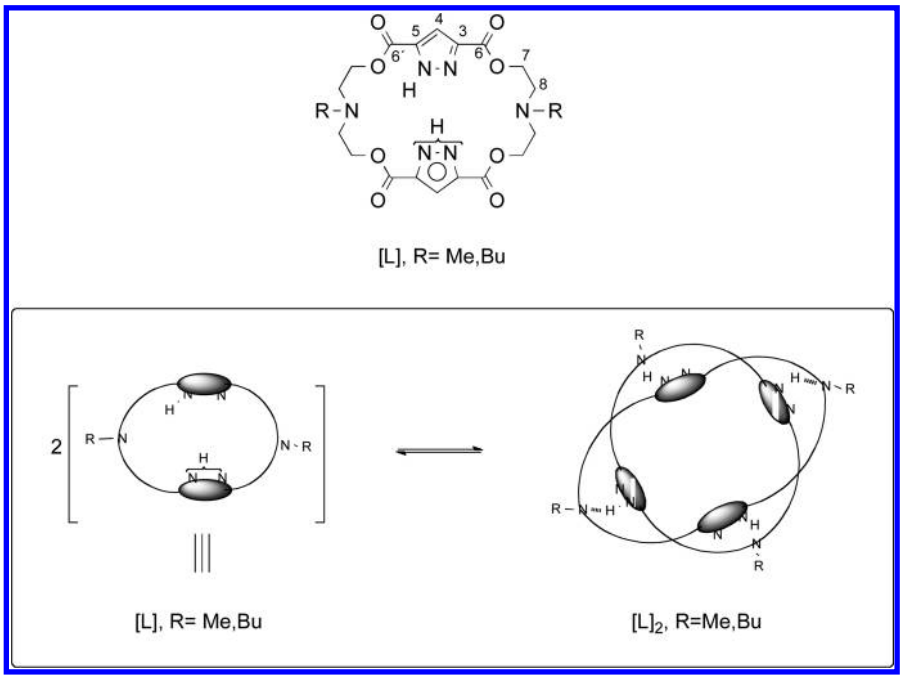
Chart 1



between them through extended hydrogen-bond networks, forming hetero double-helical structures.[11] However, studies on the reversible self-assembly of pyrazole-containing systems in aprotic organic solution are scarce.[12]

Here, we report the first examples of a hydrogen-bonded guided dimerization occurring in a macrocycle containing pyrazole units. The dimerization occurs both in the solid state and reversibly in chloroform solution by simultaneous self-assembly of four NH (pyrazole)····NR (tertiary amine) hydrogen bonds (see Chart 1).

Additionally, preliminary studies of the interaction of these systems with ethyleneurea (ETU) have been carried out in order to assess the behavior of these dimers in the presence of a complexing agent able to form competitive hydrogen bonds.

RESULTS AND DISCUSSION

Previously, some of us reported a synthetic procedure for the obtention of a 26-membered *N*-methyl-substituted macrocycle ([L], R = Me; Chart 1) containing two 1*H*-pyrazole units.[13] Now, by improving the above synthetic procedure as depicted in Scheme 1, the reaction of 1*H*-pyrazole-3,5-dicarbonyl dichloride with the cyclic stannoxane 2,2-dibutyl-6-methyl-6-aza-1,3-dioxa-2-stannacyclooctane has led to the isolation of the expected diaza tetraester crown **1** (MH^+ *m*/*z* 479) in 10% yield with a high degree of purity. In addition, in the same reaction a new triaza hexaester crown of larger size (39-membered) containing three 1*H*-pyrazole units **2** (MH^+ *m*/*z* 718) was isolated in low yield (1%) (Scheme 1).

Following a similar synthetic scheme, the reaction of the cyclic stannoxane obtained from *N*-butyldiethanolamine and dibutyltin oxide with 1*H*-pyrazole dichloride afforded two new 26- and 39-membered macrocycles with structures **3** (MH^+ *m*/*z* 563) and **4** (MH^+ *m*/*z* 844) in similar yields (10% and 1%, respectively) (Scheme 1). All these structures (**1**−**4**) were established on the basis of their analytical and spectroscopic data (FAB-MS and/or ESI-MS, ^{1}H and ^{13}C NMR). In both series, the two triaza hexaester crowns **2** and **4** were obtained as pure amorphous solids. However, crystallization of both diaza tetraester crowns **1** and **3** from acetonitrile afforded crystals suitable for X-ray diffraction studies, which demonstrated that, after crystallization, both compounds adopt the dimeric structure $\mathbf{1}'[L^1]_2$ (mp 214−216 °C) and $\mathbf{3}'[L^3]_2$ (mp 189−190 °C), respectively (see Chart 1 and Figures 1 and 2).

The asymmetric unit of **1**′ consists of a macrocyclic unit and one acetonitrile molecule. The macrocyclic unit displays 2-fold symmetry with both methyl groups and pyrazole units oriented toward the same side of the macrocyclic plane defined by the ether oxygen atoms and the tertiary nitrogens at the middle of the bridges (Figure 1).

The most interesting aspect of this structure is revealed when the asymmetric unit is grown in a search for hydrogen bond contacts. Each macrocycle is associated with another equivalent unit rotated by 90° through a four-hydrogen-bond network, forming dimers (Figure 1, left). The hydrogen bonds are established between the pyrazole NH groups as the hydrogen-bond donors and the central tertiary nitrogen atoms as the hydrogen-bond acceptors (N1···N3, 2.886 Å; N1−H···N3, 1.959 Å and 165.3°). The different dimers in the crystal structure do not show hydrogen bonds between them or with the acetonitrile molecules which are located in special positions (see Figure 2).

The crystal structure of compound **3**′ is analogous to that of compound **1**′, but in this case, the asymmetric unit shows two slightly different macrocyclic units (units A and B, Figure 1, right) and a water molecule. Each macrocycle presents 2-fold symmetry with an elongated ellipsoidal disposition analogous to that observed for compound **1**′. Again, the most interesting aspect of this crystal structure is the observation of hydrogen-bonded dimers on growing the asymmetric unit. Different from

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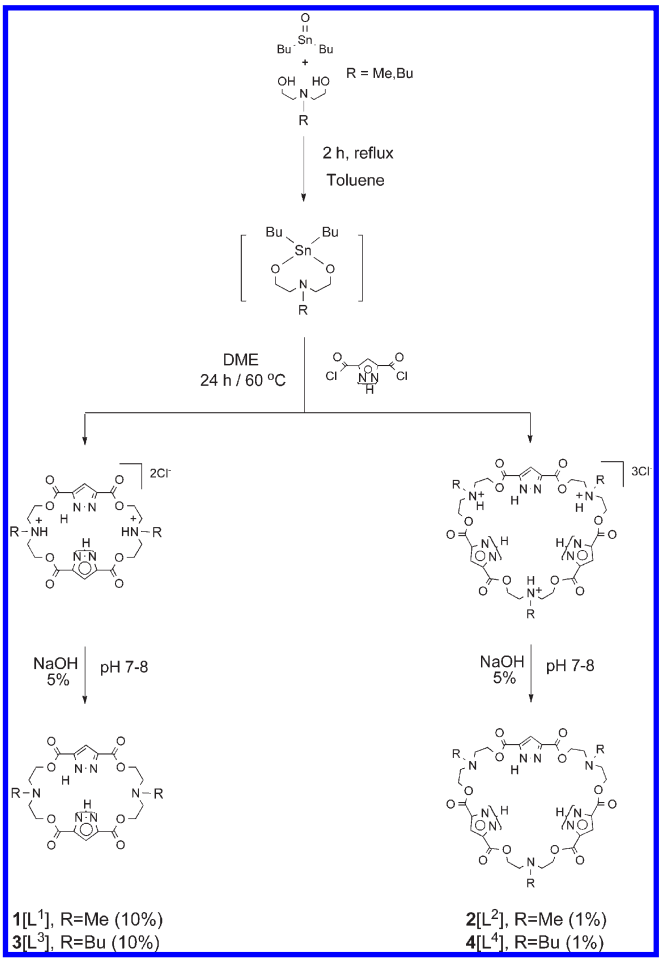
Scheme 1



the case for **1**′, here the dimers are formed between units A and B, preserving the same four-hydrogen-bond pattern which implies N−H pyrazole groups as hydrogen donors and tertiary nitrogen atoms at the middle of the chain as hydrogen bond acceptors (N1A···N3B, 2.866 Å; N1A−H1A···N3B, 2.058 Å and 156.7°; N1B···N3A, 2.933 Å; N1B−H1B···N3A, 2.097 Å and 164.3°). As pointed out for **1**′, also for **3**′ the different dimers do not show any hydrogen-bonding interaction between them.

^{1}H NMR spectra of **1** and **3** in $CDCl_3$ at 298 K showed the resonances of each compound split into two sets of peaks. In order to understand and assign those spectra, we carried out a series of variable-concentration experiments. The intensity of the peaks showed concentration dependence, suggesting the existence of an intermolecular dynamic equilibrium due to an autoassociation process. An additional EXSY experiment showing exchange correlation bands between monomer and dimer resonances corroborated this result. Figure 3 illustrates the dependence on concentration of the ^{1}H NMR spectra of compounds **1** and **3**.

For our compounds the dimerization process, 2 M = D, is in the slow exchange condition on the NMR time-scale. Therefore, separate bands are observed at characteristic frequencies for the monomer and dimer species, labeled as M and D in Figure 3.

Using appropriate equations (see Figure 1S in the Supporting Information), the dimerization constants for compounds **1** and **3** were calculated to be 53.9 ± 0.9 and 97.0 ± 1.3 M^{-1}, respectively.

The effect of temperature was evaluated by recording variable-temperature ^{1}H NMR spectra of a 0.01 M solution of compounds **1** and **3** in $CDCl_3$. Activation parameters of dimerization ($\Delta H^{\ddagger}$ = +20.2 ± 0.8 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −15.9 ± 2 cal mol^{-1} K^{-1} for compound **1** and $\Delta H^{\ddagger}$ = +18.4 ± 0.8 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −12.8 ± 2 cal mol^{-1} K^{-1} for compound **3**) were calculated from Eyring plots[14] (see Figure 2S in the Supporting Information).

Dimers **1**′ and **3**′ and monomers **1** and **3** show significant differences in their ^{13}C NMR spectra (see Table 1).

In **1** and **3**, the pyrazole carbon atoms C-3 and C-5, the carbonylic atoms C-6 and C-6′, and the pairs of methylene carbons C-7,7′ and C-8,8′ are magnetically equivalent due to the prototropic equilibrium of the pyrazole ring.[15] However, in **1**′ and **3**′ the NH···NR (R = Me, Bu) hydrogen bonds break the magnetic equivalence of the pyrazole environment, and consequently, the carbon atoms closer to the pyrazole sp^2 nitrogens C-3, C-6, C-7, and C-8 appear at lower field than C-5, C-6′, C-7′, and C-8′ carbon atoms, which lie at the side of the macrocycle where hydrogen bonding occurs.

To check the complexing ability of these dimer entities in the presence of substrates that can form competitive hydrogen bonds, we have performed NMR and MS studies of a system containing **1** and *N*,*N*′-ethyleneurea (ETU). As observed by ^{1}H NMR, it is clear that addition of increasing amounts of ETU to a 16.41 mM solution of **1** in $CDCl_3$ displaces the monomer−dimer equilibrium toward the monomer (Figure 3S in the Supporting Information). However, starting from a more concentrated solution of compound **1** in $CDCl_3$ (30 mM)—in which the dimer species is predominant—the ^{1}H and ^{13}C NMR spectra show that addition of 3 equiv of ETU is not enough to completely displace the monomer−dimer equilibrium to the monomer species. It was also noted that the NH(ETU) and CO(ETU) signals experience high-field shifts (0.10 and 0.11 ppm, respectively), which suggest that ETU is simultaneously breaking the NH···NR hydrogen bonds belonging to the dimer species and interacting with the monomer units of compound **1** (see Figure 4S and Table 1S in the Supporting Information). On the other hand, the analysis by electrospray spectroscopy (ESI-MS) (positive mode) of a 18.8 mM solution of **1** in chloroform after addition of 3 equiv of ETU showed peaks at *m*/*z* 587.2 (20%) and 1065.3 (0.3%) consistent with the formation of 1:1 and 2:1 **1**−ETU complexes, respectively. Further evidence for the formation of **1**−ETU complexes was also obtained from MALDI-TOF-MS. The spectra of compound **1** treated with 3 equiv of ETU using DCTB as the matrix in the absence and in the presence of NaI as cationizing agent (Figures 6SA and 6SB and Table 3S in the Supporting Information) In addition, the same spectra show significant peaks at *m*/*z* 1107.2, 1105.2, 1079.2, 1063.2, and 1039.2 corresponding to the partial loss of side chain fragments which suggest the formation of a 2:2 or 2:1 **1**−ETU complex.

The above data together with those obtained by molecular modeling studies suggest that new (ETU)CO···HN(Pz) and (ETU)NH···OC−Pz hydrogen bonds may be involved in the formation of 1:1, 2:1, and 2:2 **1**−ETU complexes (see Figure 4 and Figure 7S in the Supporting Information).

■ CONCLUSIONS

By improving a synthetic method previously reported by some of us,[13] the cyclic stannoxanes obtained from RN-diethanolamine (R = Me, Bu) and dibutyltin oxide react with 1*H*-pyrazole-

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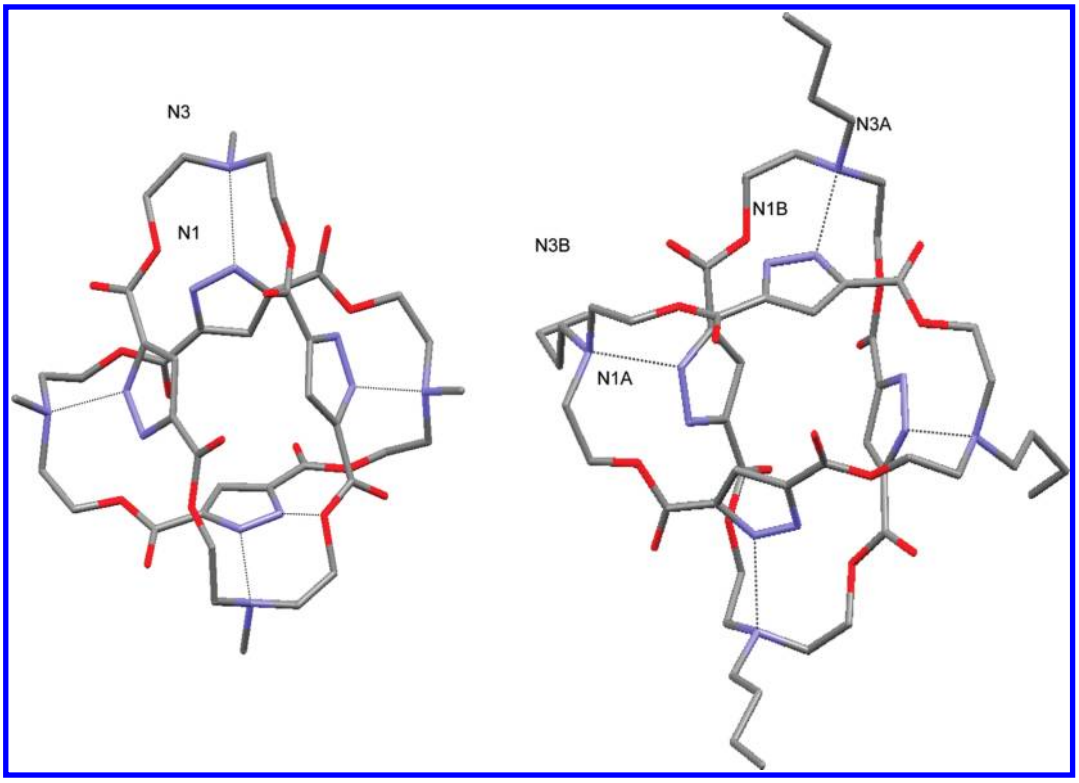

Figure 1. Hydrogen-bonded dimer formed in **1′** (left) and **3′** (right). In both dimers two molecules are linked by four hydrogen bonds involving N−H groups of pyrazole as donors and tertiary amine as the hydrogen bond acceptors. Hydrogen atoms are not included.

3,5-dicarbonyl dichloride to give 26-membered diaza tetraester crowns containing two 1*H*-pyrazole rings, **1**[L^1] (R = Me) and **3**[L^3] (R = Bu), in 10% yield. In the same reactions were also obtained triaza hexaester crowns of larger size (39 membered) containing three 1*H*-pyrazole rings, **2**[L^2] (R = Me) and **4**[L^4] (R = Bn), in 1% yield.

Crystallization of both 26-membered diaza tetraester crowns **1**[L^1] and **3**[L^3] from acetonitrile afforded crystals suitable for X-ray diffraction studies which demonstrated that, after crystallization, the 1*H*-pyrazole units self-assemble into the dimeric species **1′**[L^1]$_2$ and **3′**[L^3]$_2$, respectively. In both cases, the dimerization occurs through the formation of four hydrogen bonds involving the two NH pyrazole groups and the two tertiary amine groups of both crowns. A ^{1}H NMR study of **1** and **3** in $CDCl_3$ solution at variable concentration as well as EXSY experiments at 298 K showed the existence of an intermolecular dynamic equilibrium due to an autoassociation process; the dimerization constants for compounds **1** and **3** were calculated to be 53.9 ± 0.9 M^{-1} and 97.0 ± 1.3 M^{-1}, respectively. Activation parameters of dimerization ($\Delta H^{\ddagger}$ = +20.2 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −15.9 cal mol^{-1} K^{-1} for compound **1** and $\Delta H^{\ddagger}$ = +18.4 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −12.8 cal mol^{-1} K^{-1} for compound **3**) were calculated from Eyring plots.[14]

To check the complexing ability of these dimer entities in the presence of substrates that can form competitive hydrogen bonds, we performed NMR, MS, and molecular modeling studies of a system containing **1** and *N*,*N*′-ethyleneurea (ETU). NMR studies suggest that ETU simultaneously breaks the NH···NR hydrogen bonds belonging to the dimer species and interacts with the monomer units of compound **1**. The ESI-MS of a solution of **1** in chloroform after the addition of 3 equiv of ETU show peaks at *m*/*z* 587.2 and 1065.3, consistent with the formation of 1:1 and 2:1 **1**−ETU complexes; MALDI-TOF-MS measured for the solid state of compound **1** treated with 3 equiv of ETU using DCTB as matrix also shows a peak at *m*/*z* 1065.2, which is in agreement with the 2:1 **1**−ETU complex detected by ESI-MS. In addition, significant peaks corresponding to the partial loss of side chain fragments which suggest the formation of a 2:2 **1**−ETU complex were shown. Finally, molecular modeling studies suggest that new (ETU)CO···HN(Pz) and (ETU)NH···OC−Pz hydrogen bonds may be involved in the formation of 1:1, 2:1, and 2:2 **1**−ETU complexes.

The studies here presented provide the first crystallographically solved examples of hydrogen-bonded guided dimerization of pyrazole macrocycles. Work is now in progress to selectively isolate and evaluate the stability constants of 2:1 or 2:2 **1**−ETU complexes and other complexes obtained by interaction of different urea derivatives with the monomer−dimer species reported here.

EXPERIMENTAL SECTION

General Considerations. All reagents were of commercial quality from freshly opened containers. Thionyl chloride was freshly distilled prior to use. Pyrazole-3,5-dicarboxylic acid hydrate, dibutyltin oxide, *N*-methyldiethanolamine, *N*-butyldiethanolamine, and reagent-quality solvents were used without further purification. The organic reactions were monitored by thin-layer chromatography (TLC) on precoated aluminum sheets of silica gel 60 PF_{254}. Flash column chromatography was performed in the indicated solvent system on silica gel (particle size 0.040−0.063 mm). Melting points were determined with a hot-stage microscope. ^{1}H and ^{13}C NMR spectra were recorded with 300, 400, and

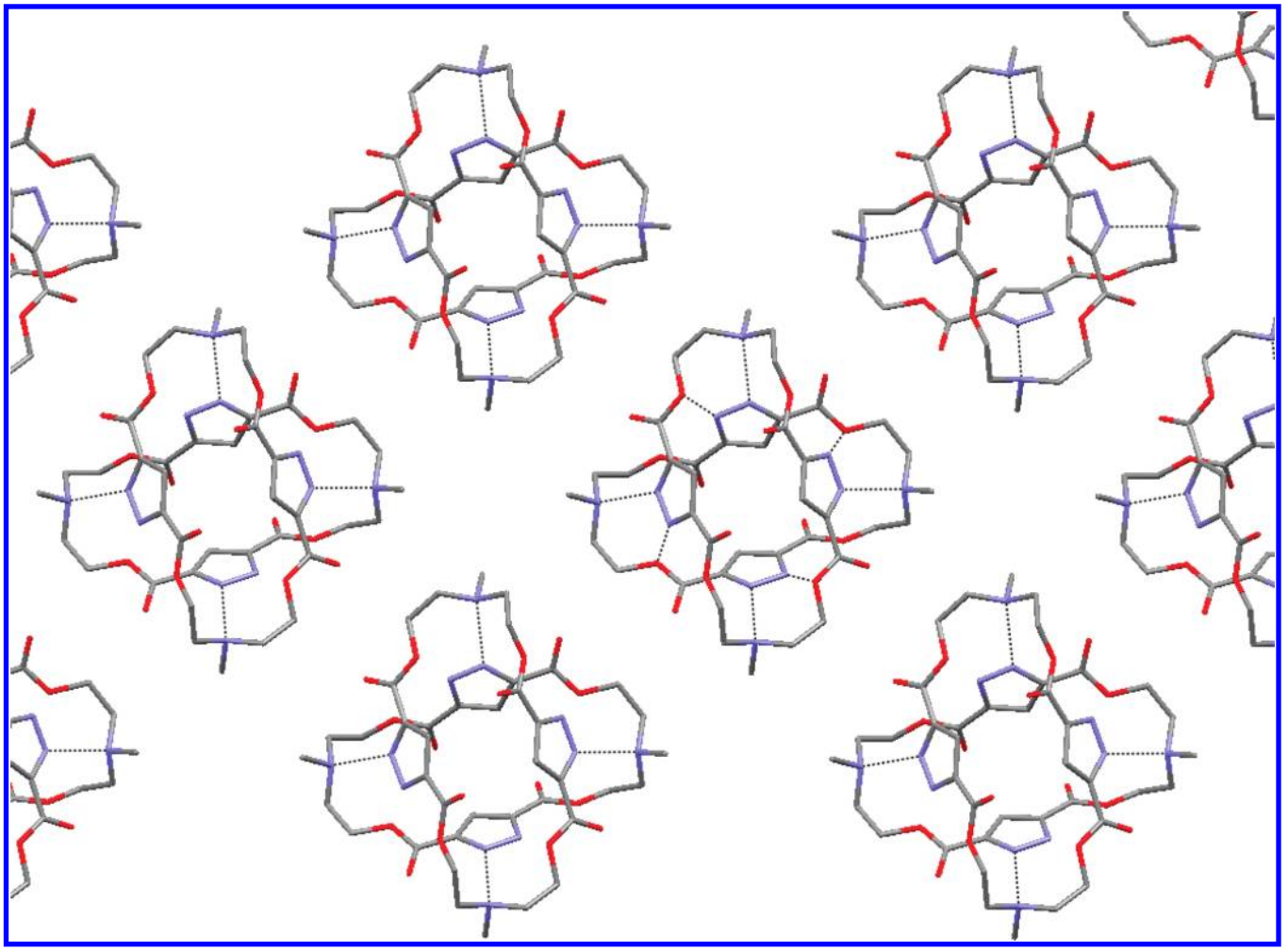

Figure 2. View of a part of the crystal packing of **1**′, showing the dimeric units.

500 MHz spectrometers at room temperature, employing DMSO-d_6 or $CDCl_3$ as solvent. The chemical shifts are reported in parts per million (ppm) from tetramethylsilane but were measured against the solvent signals. ^{1}H and ^{13}C assignments were performed on the basis of 2D-homonuclear (gCOSY, EXSY) and heteronuclear NMR experiments (gHSQC and gHMBC).

FAB mass spectra were measured using a *m*-nitrobenzyl alcohol (NBA) matrix.

1*H*-Pyrazole-3,5-dicarbonyl Dichloride. A suspension of 1*H*-pyrazole-3,5-dicarboxylic acid hydrate (2.00 g, 11.49 mmol) in freshly distilled thionyl chloride (350 mL) was heated at 140 °C for 2 h. The hot reaction mixture was filtered, and the resulting solution was evaporated to dryness to give the title compound (2.16 g, 11.22 mmol) as a white solid (mp 72−74 °C; lit.[16] mp 72−74 °C) in 98% yield.

Preparation of 26- and 39-Membered Aza Ester Crowns 1−4. The synthesis was performed in two reaction steps using dibutyltin oxide, *N*-methyl- or *N*-butyldiethanolamine and 1*H*-pyrazole-3,5-dicarbonyl dichloride as starting materials following the general procedure indicated above.

6-Substituted 2,2-Dibutyl-6-aza-1,3-dioxa-2-stannacyclooctane. To a solution of the corresponding N-substituted diethanolamine (11.49 mmol) in dry toluene (200 mL) was slowly added solid dibutyltin oxide (2.85 g, 11.49 mmol). The reaction mixture was refluxed for 2 h, and the water formed in the cyclocondensation was removed by azeotropic distillation. The resulting suspension was diluted into dry toluene (260 mL) and collected to be used in the following step.

Cyclization Reaction. The suspension obtained above was vigorously stirred and heated to 60 °C under argon. Then, a solution of 1*H*-pyrazole-3,5-dicarbonyl dichloride (2.21 g, 11.49 mmol) in dry dimethoxyethane (90 mL) was added dropwise over a period of 2 h. When the addition was complete, the reaction was allowed to proceed for 24 h at the same temperature (60 °C) and the mixture was then cooled to room temperature. The organic solvent was evaporated to dryness to give a solid material which was exhaustively extracted in a Soxhlet apparatus with boiling *n*-hexane until the tin salts were eliminated. The solid residue was suspended in water and the aqueous solution treated with 5% aqueous sodium hydroxide until pH 7−8 and extracted with chloroform. The organic layer was evaporated to dryness, affording a white solid, which was purified by flash column chromatography, with CH_2Cl_2−MeOH mixtures as eluents (100:1 to 10:1).

Methyl-Substituted Derivatives ***1****[L^1] and* ***2****[L^2]. 6,19-Dimethyl-3,9,16,22-tetraoxa-6,12,13,19,25,26-hexaazatricyclo[22.2.1.1^{11,14}]-octacosa-1(26),11(28),13,24(27)-tetraene-2,10,15,23-tetraone (****1****[L^1]).* The fraction with R_f = 0.48 (CH_2Cl_2−MeOH, 10:1) afforded the pure tetraester crown **1**[L^1] as a white solid (270 mg, 10%): mp 214−216 °C (MeCN). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 14.40 (s, 2H, NH), 6.91 (s, 2H, H-4), 4.33 (t, 8H, J = 4.7 Hz, 7-H), 2.74 (t, 8H, J = 4.7 Hz, H-8), 2.30 ppm (s, 6H, H-1″). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.4 (broad signal, CO-6,6′), not observed (C-3,5), 110.4 (C-4), 61.9 (C-7), 55.1 (C-8), 42.6 ppm (C-1″). FAB-MS (m/z (%)): 957 (5) [$2MH^+$], 479 (100) [MH^+]. Anal. Calcd for $C_{20}H_{26}N_6O_8$: C, 50.21; H, 5.48; N, 17.56. Found: C, 50.14; H, 5.40; N, 17.36.

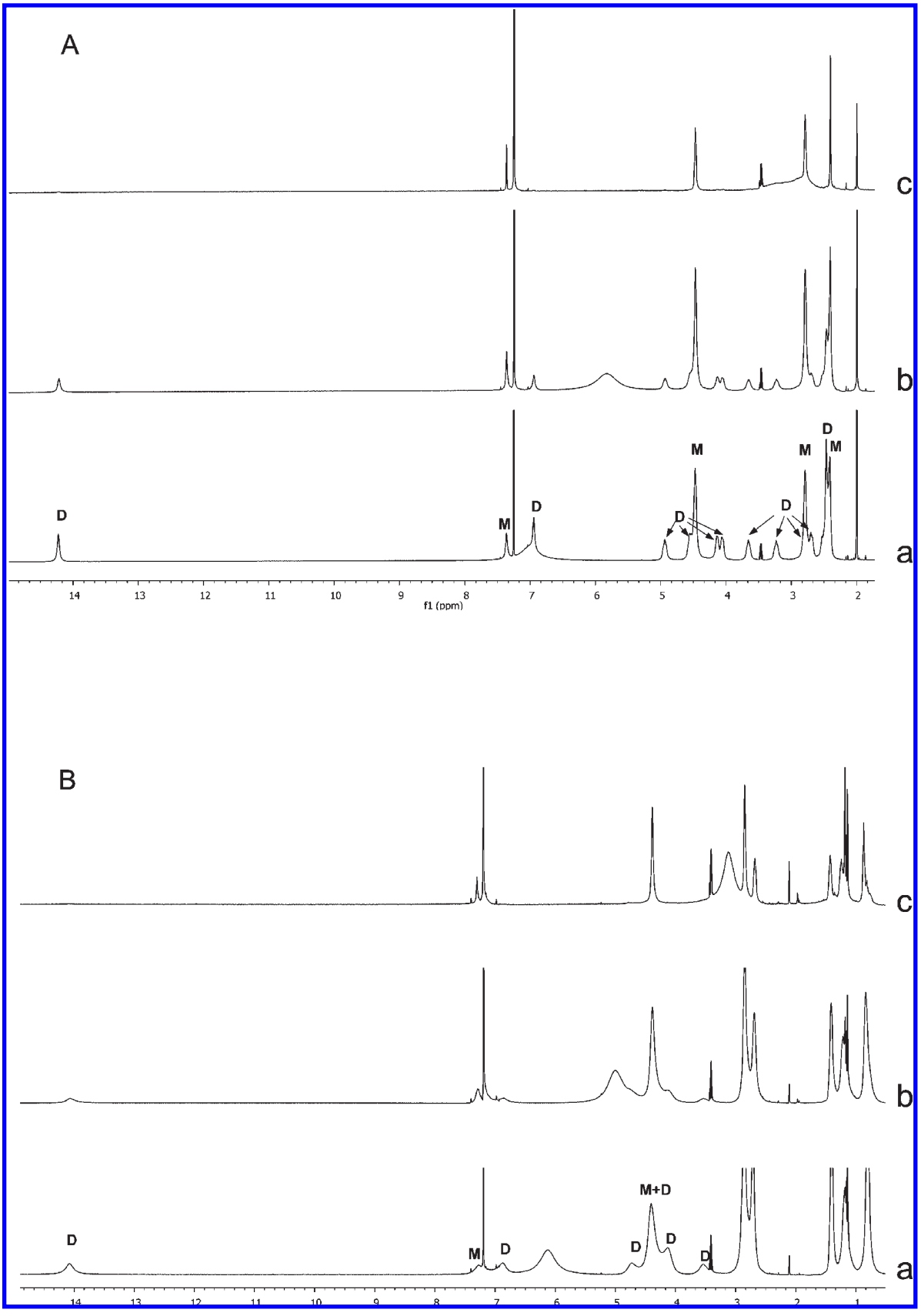

Figure 3. ^{1}H NMR spectra in $CDCl_3$ at different concentrations. (A) Compound **1**: (a) 1.944×10^{-2} M; (b) 0.748×10^{-2} M; (c) 0.335×10^{-2} M. (B) Compound **3**: (a) 1.002×10^{-2} M; (b) 0.385×10^{-2} M; (c) 0.209×10^{-2} M.

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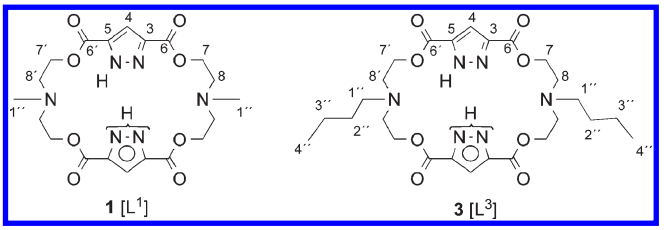
Table 1. Comparison of Monomer Species 1[L^1] and 3[L^3] ^{13}C NMR Chemical Shifts (δ (ppm)) with Those of Dimers 1′[L^1]$_2$ and 3′[L^3]$_2$ in $CDCl_3$ Solution



compd	CO-6,6′	C-3,5	C-4	C-7,7′	C-8,8′	C-1″	C-2″	C-3″	C-4″
1[L^1]	159.5	139.4	111.7	59.7	55.5	39.9			
1′[L^1]$_2$	162.7	143.0	110.5	62.9	55.9	44.4			
	160.0	136.0		62.3	54.2				
3[L_3]	159.8	139.5	111.6	60.1	51.3	50.8	25.0	20.7	14.0
3′[L_3]$_2$	162.9	142.9	110.3	64.0	54.0	55.5	25.3	20.6	13.9
	160.0	135.6		62.9	49.7				

*6,19,32-Trimethyl-3,9,16,22,29,35-hexaoxa-6,12,13,19,25,26,32,-38,39-nonaazatetracyclo[35.2.1.1^{11,14}.1^{24,27}]dotetraconta-1(39),1-1(41),13,24(42),26,37(40)-hexaene-2,10,15,23,28,36-hexaone (**2**[L^2]).* The fraction with R_f = 0.26 (CH_2Cl_2−MeOH, 10:1) afforded a small amount of the pure hexaester crown **2**[L^2] as a pure amorphous solid (30 mg, 1%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 14.58 (broad signal, 3H, NH), 6.82 (s, 3H, H-4), 4.31 (t, 12H, J = 5.2 Hz, H-7), 2.77 (t, 12H, J = 5.2 Hz, H-8), 2.30 ppm (s, 9H, H-1″). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 159.5 (CO-6,6′), 138.6 (broad signal, C-3,5), 110.3 (C-4), 62.1 (C-7), 55.0 (C-8), 42.0 ppm (C-1″). FAB-MS (m/z (%)): 718 (45) [MH^+], 479 (17) [$(2M/3 + 1)^+$], 240 (22) [$(M/3 + 1)^+$]. Anal. Calcd for $C_{30}H_{39}N_9O_{12}$: C, 50.21; H, 5.48; N, 17.56. Found: C, 50.07; H, 5.36; N, 17.33.

*Butyl-Substituted Derivatives **3**[L^3] and **4**[L^4]. 6,19-Dibutyl-3,9,-16,22-tetraoxa-6,12,13,19,25,26-hexaazatricyclo[22.2.1.1^{11,14}]octa-cosa-1(26),11(28),13,24(27)-tetraene-2,10,15,23-tetraone (**3**[L^3]).* The fraction with R_f = 0.54 (CH_2Cl_2−MeOH, 10:1) afforded the pure tetraester crown 3[L^3] as a white solid (310 mg, 10%): mp 189−190 °C (MeCN). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 14.46 (s, 2H, NH), 6.92 (s, 2H, H-4), 4.28 (t, 8H, J = 4.5 Hz, H-7), 2.78 (t, 8H, J = 4.5 Hz, H-8), 2.45 (t, 4H, J = 6.4 Hz, H-1″), 1.35 (m, 4H, H-2″), 1.26 (m, 4H, H-3″), 0.80 ppm (t, 6H, J = 7.3 Hz, H-4″). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 160.6 (very broad signal, CO-6), 158.90 (very broad signal, CO-6′), 143.0 (very broad signal, C-3), 134.6 (very broad signal, C-5), 110.5 (C-4), 62.67 (C-7), 53.7 (C-1″), 52.5 (C-8), 29.2 (C-2″), 19.7 (C-3″), 13.9 ppm (C-4″). FAB-MS (m/z (%)): 1125.3 (1.3) [$2MH^+$], 563.3 (100) [MH^+], 282 (23) [$(M/2 + 1)^+$]. ESI-MS (m/z (%)): 1147 (18) [2M + Na^+], 563.3 (100) [MH^+]. IR (KBr, cm^{-1}): 3436 (NH); 1732, 1719 (CO). Anal. Calcd for $C_{26}H_{38}N_6O_8$: C, 55.50; H, 6.81; N, 14.94. Found: C, 55.49; H, 6.69; N, 14.90.

*6,19,32-Tributyl-3,9,16,22,29,35-hexaoxa-6,12,13,19,25,26,32,38,39-nonaazatetracyclo[35.2.1.1^{11,14}.1^{24,27}]dotetraconta-1(39),11(41),-13,24(42),26,37(40)-hexaene-2,10,15,23,28,36-hexaone (**4**[L^4]).* The fraction with R_f = 0.48 (CH_2Cl_2−MeOH, 10:1) afforded a small amount of the pure hexaester crown 4[L^4] as a pure amorphous solid (25 mg, 1%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 14.43 (broad signal, 3H, NH), 6.90 (s, 3H, H-4), 4.27 (t, 12H, J = 4.6 Hz, H-7), 2.77 (t, 12H, J = 4.6 Hz, H-8), 2.50 (t, 4H, J = 6.7 Hz, H-1″), 1.32 (m, 6H, H-2″), 1.23 (m, 6H, H-3″), 0.78 ppm (t, 9H, J = 7.1 Hz, H-4″). ^{13}C NMR (DMSO-d_6. 100 MHz): δ 159.7 (CO-6,6′), 139.0 (C-3,5), 110.4 (C-4), 62.9 (C-7), 53.7 (C-8), 52.2 (C-1″), 29.1 (C-2″), 19.6 (C-3″), 13.7 ppm (C-4″). ESI-MS (m/z (%)): 844.4 (100) [MH^+]. IR (KBr, cm^{-1}): 3435 (NH); 1729 (CO). Anal. Calcd for $C_{39}H_{57}N_9O_{12}$: C, 55.50; H, 6.81; N, 14.94. Found: C, 55.32; H, 6.52; N, 14.78.

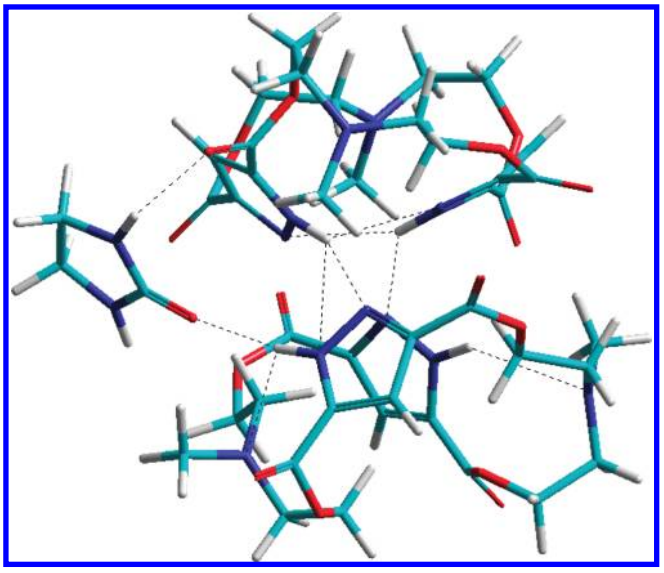


Figure 4. Calculated model for the 2:1 **1**−ETU complex.

K_{dim} Determination. In order to determine dimerization constants for compounds **1** and **3**, ^{1}H NMR dilution experiments were performed with the following procedure:[14] 0.5 mL samples of each compound in $CDCl_3$, at a known concentration (0.0194 M for **1** and 0.0100 M for **3**), were prepared. For each sample, aliquots of $CDCl_3$ were added successively to the NMR tube, and the ^{1}H NMR spectrum, at 298 K, was recorded after each addition. At least 20 different concentrations ranging from 0.004 to 0.019 M for **1** and from 0.002 to 0.010 M for **3** were used for the K_{dim} determination. Molar fractions were determined by integration of different pairs of monomer and dimer resonances to different concentrations. K_{dim} values were obtained from the slope of a linear-regression line, plotting $u/2(1 - u)^2$ against [H_0] (eq 5 in the Supporting Information). The effect of temperature was further evaluated for compounds **1** and **3**, by recording the ^{1}H NMR spectra of a 0.01 M solution at 25, 30, 35, 40, and 45 °C.

Heteroassociation Studies of Macrocycle 1 with *N,N′*-Ethyleneurea (ETU). *By Mass Spectrometry. Experimental Section.* Mass spectra recorded in $CHCl_3$ solution by electrospray ionization (ESI) in the positive ion detection mode were acquired using a commercial spectrometer with a QTOF hybrid analyzer. Those recorded by matrix assisted laser desorption ionization (MALDI-TOF) were recorded in the positive ion detection mode, using 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB)[17] as the matrix and sodium iodide as a cationizing agent.

MALDI Sample Preparation. DCTB matrix solution was made to a concentration of 10 mg mL^{-1} in dichloromethane (DCM), and the NaI solution was made to a concentration of 2 mg mL^{-1} in acetone. The sample solution was made as follows: to a 30 mM solution of compound **1** in $CHCl_3$ was added a 90 mM solution of ETU in $CHCl_3$. In a plastic snap-top lid sample vial, 5 μL of the sample solution was vortex-mixed with 20 μL of DCTB and with 0.5 μL of NaI solution. A 0.5 μL portion of the final mixture was spotted onto the sample plate and allowed to dry, leaving an opaque crystal layer.

By Molecular Modeling Calculations. Experimental Section. Molecular modeling studies were carried out using the AMBER[18] method implemented in the Hyperchem 7.5 package,[19] modified by the inclusion of appropriate parameters.[20] In each case, all possible complexes were built and optimized, taking into account the structures showing

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stabilizing interactions, such as additional hydrogen bonds, by use of the "simulated annealing" procedure carrying out molecular dynamics calculations at 400 K.

All calculations were carried out in vacuo with constant dielectric value. In the absence of explicit solvent molecules, a constant dielectric factor qualitatively simulates the presence of nonpolar solvents, as it takes into account the fact that the intermolecular electrostatic interactions should not die off appreciably with distance as in the gas phase. The relative binding energies of complexes under study were calculated by subtracting the energies of the macrocycle (as monomer or dimer) and *N*,*N*′-ethyleneurea (ETU) units from the minimum complex energy

$$E_c = E_{complex} - (E_{macrocycle} + E_{ETU})$$

for the 1:1 and 2:1 complexes and

$$E_c = E_{complex} - (E_{macrocycle} + 2E_{ETU})$$

for the 2:2 complex.

Starting structures for macrocycle monomer and dimer were built by using Hyperchem capabilities. Its geometry was minimized to a maximum energy gradient of 0.1 kcal $Å^{-1}$ mol^{-1} with the AMBER force field, using the Polak−Ribiere (conjugate gradient) minimizer, and the simulated annealing procedure was used to cover all conformational space. This geometry, in accordance with the X-ray structure, was always used in calculations of host/guest complexes. Different possible orientations of ETU units with respect to the macrocycle or its dimer were built by docking the structure of the ETU in the vicinity of the macrocycle and then minimizing the energy of the complex with no restraints.

X-ray Crystallographic Analysis of 1 and 3. Single crystals of **1** and **3** suitable for X-ray analysis were obtained by slow evaporation of CH_3CN solutions of the compounds. The data collection strategy was calculated with the program COLLECT.[21] Data reduction and cell refinement were performed with the programs HKL DENZO and SCALEPACK.[22] Data collection was performed at 293 K on a Nonius Kappa-CCD single-crystal diffractometer, using Mo Kα radiation (λ = 0.7173 Å). The crystal structure was solved by direct methods, using the program SIR-97.[23] Anisotropic least-squares refinement was carried out with SHELXL-97.[24]

Crystal Data for ***1′***: $C_{20}H_{26}N_6O_8 \cdot C_2H_3N$, M_r = 519.52, colorless, 0.20 × 0.15 × 0.10 mm^3, tetragonal, space group $I4_1/a$, $a = b$ = 13.1070(2) Å, c = 30.35107 Å, V = 5214.10(16) $Å^3$, Z = 8, R = 0.0979 and R_w = 0.2266 for all observed reflections (2784) and 168 parameters.

Crystal Data for ***3′***: $C_{26}H_{37}N_6O_8$, M_r = 569.62, colorless, 0.20 × 0.12 × 0.10 mm^3, monoclinic, space group $C2/c$, a = 15.8510(3) Å, b = 31.3650(10) Å, c = 13.622084) Å, β = 119.4050(10)°, V = 5899.9(3) $Å^3$, Z = 8, R = 0.0972, R_w = 0.2075 for all observed reflections (6471) and 366 parameters.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^1H and ^{13}C spectra of the new compounds **2**−**4**, NMR solution studies of compounds **1** and **3**, giving the thermodynamics for the dimerization constants of **1** and **3** in $CDCl_3$, experimental plots for obtaining the dimerization constants by ^1H NMR (Figure 1S), Eyring plots for compounds **1** and **3** (Figure 2S), hetero-association of Compound 1 with ETU by NMR spectroscopy. ^1H NMR spectra of a 16.41 mM solution of **1** in $CDCl_3$ with different concentrations of ETU (from 0.24 to 9.56 mM) (Figure 3S), a comparison of the ^1H NMR spectra of the predominant dimer species $\mathbf{1}'[L^1]_2$ (30 mM solution of **1** in $CDCl_3$) in the absence and in the presence of ETU (90 mM in $CDCl_3$) with that of the predominant monomer species $\mathbf{1}[L^1]$ (3.5 mM of **1** in $CDCl_3$) (Figure 4S), ^1H and ^{13}C NMR (δ (ppm)) data of the predominant dimer species $\mathbf{1}'[L^1]_2$ in the absence and in the presence of 3 equiv of ETU, and a comparison with ^1H and ^{13}C NMR data of the predominant species $\mathbf{1}[L^1]$ (Table 1S), mass spectroscopy studies giving ESI-MS of a **1**−ETU mixture in molar ratio 1:3 ([**1**] = 18.8 mM) measured in $CHCl_3$ solution (Figure 5S), most significant peaks of ESI-MS consistent with the formation of 1:1 or 2:1 **1**−ETU complexes (Table 2S), MALDI-TOF-MS data of a 30 mM sample of compound **1** in Cl_3CD after the addition of 3 equiv of ETU, spectra measured in the solid state by using DCTB as matrix in the absence (A) and in the presence of NaI (B) as cationizing agents (Figure 6S), and most significant peaks of MALDI-TOF-MS arising with partial lost of side chain fragments consistent with the formation of 2:2 or 2:1 **1**−ETU complexes (Table 3S), molecular modeling calculations detailing complexes with ETU (A, 1:1 **1**−ET; B, 2:1 **1**−ETU; C 2:2 **1**−ETU), (Figure 7S), and CIF files giving crystallographic data for **1′** and **3′**. This material is available free of charge via the Internet at http://pubs.acs.org.

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