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Cystinophanes, a Novel Family of Aromatic-Bridged Cystine Cyclic Peptides: Synthesis, Crystal Structure, Molecular Recognition, and Conformational Studies

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Abstract: A novel family of aromatic-bridged cystine cyclic peptides (cystinophanes) has been synthesized by a single-step procedure involving condensation of 1,3 aromatic (Ph or Pyr unit) dicarbonyl dichloride with either the simple L-cystine dimethyl ester to provide cystinophanes of 26-, 39-, and 52-membered rings through 2+2, 3+3, and 4+4 cyclization, respectively, or with cystine bis-peptides (H_2N-Xaa-Cyst-Xaa-NH_2) leading to a variety of 1+1 cystine-based peptidocyclophanes. ^{1}H NMR and CD studies have shown these cystinophanes to adopt a β-turn-like structure in solution. X-ray crystal structure of a representative member (**3a**) containing two aromatic rings has shown a collapsed ring conformation with a near parallel face-to-face orientation of aromatic rings—a feature also suggested by NMR studies. The propensity of cystinocyclophanes to adopt β-turn-type conformation is attributed to the presence of S−S linkage and the need to maintain a near orthogonal value of its torsion angle. The potential of cystinophanes to serve as artificial receptors in molecular recognition and host−guest complexation studies has been demonstrated with 26-membered, pyridine-bridged macrocycle **3b**, which binds (^{1}H NMR) to a number of 1,ω-alkane dicarboxylic acids [$(CH_2)_n(COOH)_2$, n = 1, ..., 4] and shows maximum affinity ($K_{assoc} = 3.69 \times 10^2$ M^{-1}) and selectivity for glutaric acid (n = 3) dicarboxylate. Crystal parameters for **3a** are as follows: $C_{32}H_{36}N_4O_{12}S_4 \cdot H_2O \cdot 2C_4H_8O_2$, space group $P2_12_12_1$ with a = 11.748(1) Å, b = 17.317(1) Å, and c = 24.306(2) Å.

Introduction

Modification of peptides by introducing conformational constraints in the backbone is an area of intense current interest.[1] In recent years, particular interest has been shown in the design of peptidomimetics that may adopt a bioactive β-turn-type conformation.[2] A wide spectrum of molecular frameworks ranging from simple glucose to more complex polycyclic steroids has been used as scaffoldings to fix the conformation of, otherwise flexible, linear peptides in a well-defined secondary structure.[3]

Cyclic peptides[4] have a built-in conformational restraint and offer attractive alternatives to linear peptidomimetics in designing simple models for studying biological interactions. The conformational flexibility of a cyclic peptide can be further

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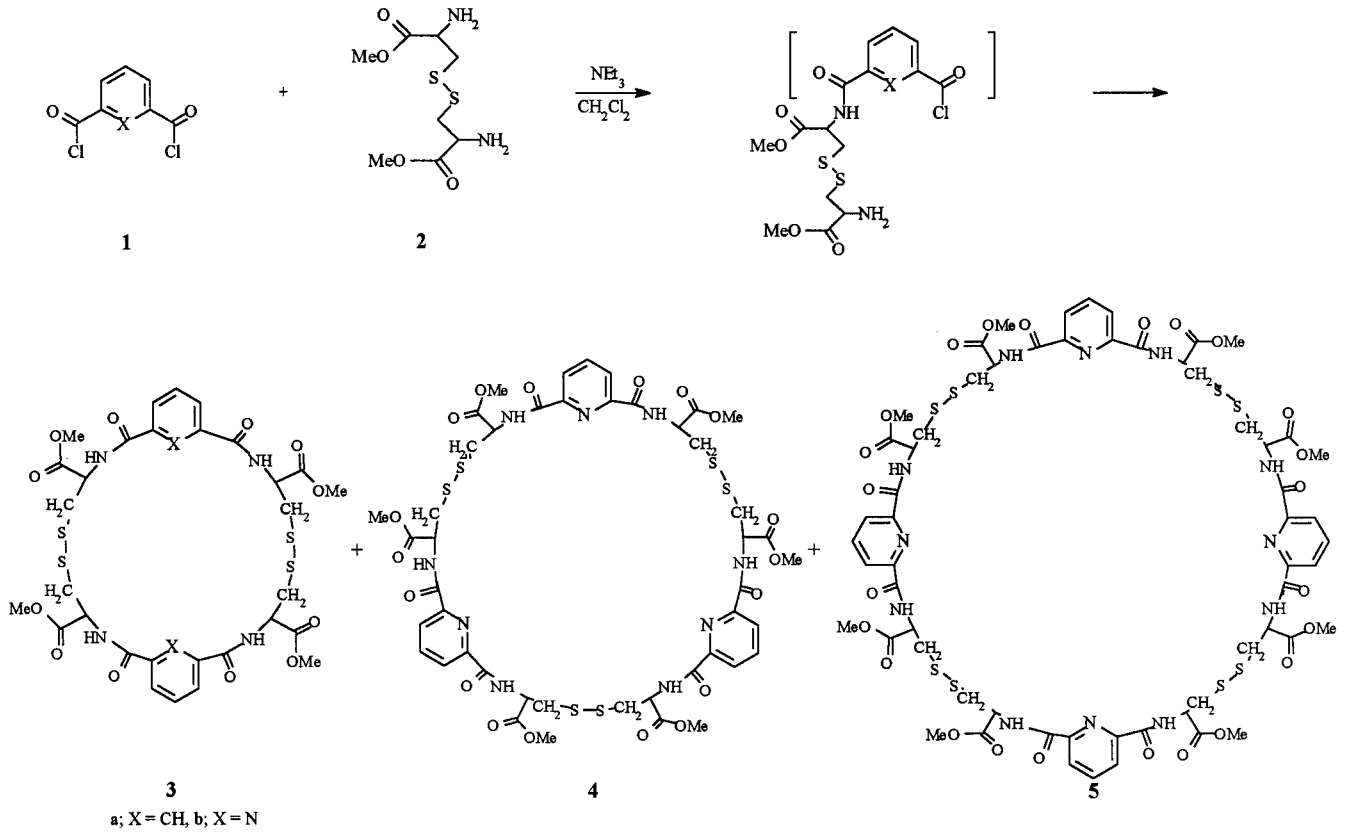
Scheme 1. One-Step Condensation of 1,3 aromatic (X = N or CH) Dicarbonyl Dichlorides and Cystine Dimethyl Ester To Give Cystino Cyclophanes



restricted by incorporating β-amino acids,[5] D-amino acids,[6] disulfide linkages,[7] or semirigid aromatic units[8] into the cyclic backbone.

Introduction of multiple constraints, for example, a combination of S−S linkages and rigid aromatic units, into the cyclic backbone was considered an exciting possibility by us to design cyclic peptides that may show a locked-in, well-defined secondary structure and serve as useful models for studying biological interactions.

We provide herein the first illustration of this concept, report on the construction of a novel family of aromatic-bridged cystine cyclic peptides with the general structure cyclo(Ar-CONH-Cyst-NHCO−)$_n$ and cyclo (Ar-CONH-Xaa-Cyst-Xaa-NHCO−) (Ar = Ph or Pyr; Cyst = L-cystine dimethyl ester; n = 2, 3, 4, and Xaa = amino acid X), and demonstrate by ^{1}H NMR and crystallographic studies that in solution, as well as in the solid state, these peptides prefer to adopt a β-turn-like structure with near-parallel, face-to-face orientation of aromatic units. The novel macrocyclic peptides named as cystinophanes represent the first examples of chiral cyclophanes containing cystine in their cyclic framework.

The present strategy is flexible with respect to the nature of the aromatic (Ar) unit and the amino acid (Xaa) and apart from providing a new class of S−S containing peptidocyclophanes, useful as conformationally constrained models for studying structure−activity relationships in biological interactions or in selective host−guest chemistry, as demonstrated here with pyridine-bridged cystinophane **3b**, which serves as an effective host for a number of 1,ω-alkane dicarboxylates with maximum affinity and selectivity for glutaric acid substrates. The design should also lead to attractive models with an extensive π network of multiple aromatic rings useful for studying noncovalent aromatic $\pi-\pi$ interactions that are known to play an important role in stabilizing protein folding patterns.[9] An additional attractive feature is the presence of built-in handles (as protected COOH groups) that can be ligated via peptide

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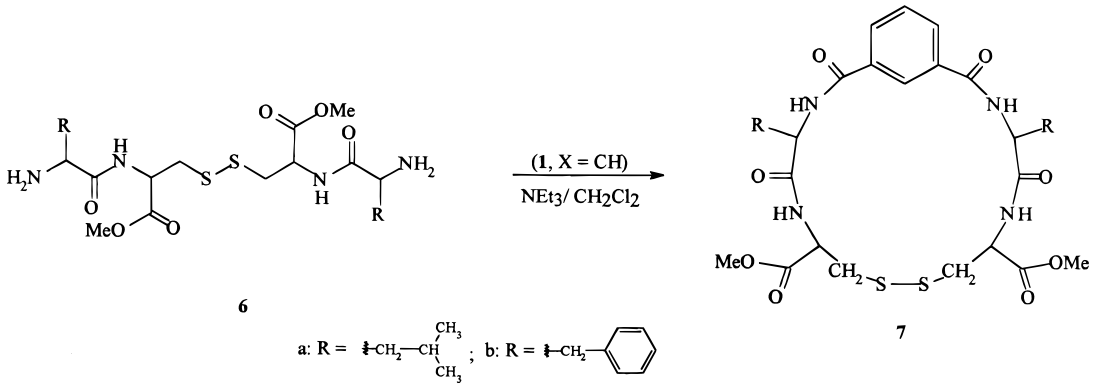
Scheme 2. One-Step Synthesis of Peptido Cyclophanes



chemistry to a variety of subunits providing attractive models for novel artificial protein design[10] or peptidomimetic drugs.[11]

Discussion and Results

The single-step synthetic strategy for cystinophanes involves the condensation of 1,3 aromatic (Ph or Pyr) dicarbonyl dichloride either with the simple L-cystine dimethyl ester (Scheme 1) to provide cystinophanes of 26-, 39-, and 52-membered rings through 2+2, 3+3, and 4+4 cyclization, respectively, or with cystine bis-peptides (H_2N-Xaa-Cyst-Xaa-NH_2; Xaa = amino acid X) leading to a variety of 1+1 cystine-based peptidocyclophanes[12] (Scheme 2).

Thus, treatment of L-cystine dimethyl ester with isophthaloyl dichloride in the presence of triethylamine under high dilution conditions afforded the 26-membered 2+2 macrocycle **3a** in 52% yield. Under similar conditions 2,6 pyridine dicarbonyl dichloride yielded a mixture of three products with similar TLC behavior which were separated by chromatography on silica gel with $CHCl_3$/MeOH (95:5) as eluent. The products isolated in yields of 51% (**3b**), 12% (**4**), and 4% (**5**) were fully characterized (Experimental Section).

A noteworthy feature in the cyclization reaction of cystine dimethyl ester with aromatic dicarbonyl dichlorides (Scheme 1) was the formation of 2+2 macrocycles **3a** and **3b** as the major product (>50% yield), as also observed earlier by us[13] in the reaction of cystine dimethyl ester with 1,3-adamantanedicarbonyl dichloride under similar experimental conditions. This may be because the 13-membered 1+1 macrocycle is much too constrained to accommodate the rigid aromatic (Ph or Pyr) or nonaromatic (adamantane) template.[13] This notion was supported by the fact that 19-membered 1+1 macrocycles **7a** and **7b** were the exclusive products isolated from the reaction of chain-extended cystine-bispeptides **6a** and **6b**, respectively, with isophthaloyl dichloride (Scheme 2). In principle, the ring size of the cyclophanes can be controlled by adjusting the length of the cystinyl bispeptide.[14]

A comparison of 26-membered [2+2] cyclization products of cystine dimethyl ester with aromatic (Ph or Pyr) and nonaromatic (Adm) templates has brought out interesting features. Thus, while the aromatic-bridged cystinophanes clearly exhibited the presence of β-turn-type features in their structure (vide infra), the adamantane analogue was practically devoid of any such characteristics (ROESY NMR submitted as Supporting Information). Of the three analogues, only the pyridine-bridged macrocycle **3b** had all its amide NHs locked in an intramolecular hydrogen bonding (NH···N ring)—a unique feature attributed to the receptor properties of **3b** for dicarboxylic acids.

The adamantane analogue was found to be relatively more soluble in nonpolar solvents and showed the capability of penetrating the lipid bilayer membranes very effectively.[13] In contrast, the aromatic-bridged cystinophanes exhibited practically no affinity for interaction with lipid bilayers.

The solution state conformation of cystinophanes **3**−**7** was examined by ^{1}H NMR, FT-IR, and CD studies. The presence of only a single set of resonances for the cystine and isophthaloyl units in the ^{1}H NMR spectrum of **3a** indicated the highly symmetrical nature of the macrocycle. In the ROESY NMR spectrum, strong cross-peaks were observed between NH and the aromatic singlet and aromatic doublet, indicating anti orientation of isophthaloyl carbonyls (**A**). A particularly diagnostic feature that signifies the formation of a β-turn-type structure in peptides was the presence of a strong cyst NH-C$^\beta$H$_2$ cross-peak in the ROESY spectrum of **3a**. The interaction of NH with only one C$^\beta$H$_2$ and a strong cross-peak between NH and C$^\alpha$H were additional noteworthy features in the spectrum (Figure 1). Further support for a β-turn-like conformation was provided by the presence of a prominent broad positive band at 229 nm in the CD spectrum of **3a** (Supporting Information). Interestingly, although ^{1}H NMR VT (variable temperature) studies (conducted in DMSO over the temperature range 293−363 K, Supporting Information) showed relatively high-temperature coefficients ($d\delta/dT = -5.28$ ppb/K) for amide NHs, thus ruling out any intramolecular hydrogen bonding in **3a**, presence of a strong (concentration independent) band at

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(13) 2+2 cyclization product was also found to be the major product in the reaction of cystine dimethyl ester with adamantane template (Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B.; Sukhaswami, M. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1105).

(14) Cystinyl pentapeptides [(Xaa)$_2$-Cyst-(Xaa)$_2$] afforded 25-membered macrocyclic peptides as the exclusive products through 1+1 cyclization with template dichlorides (Ranganathan, D.; Haridas, V. Unpublished results).

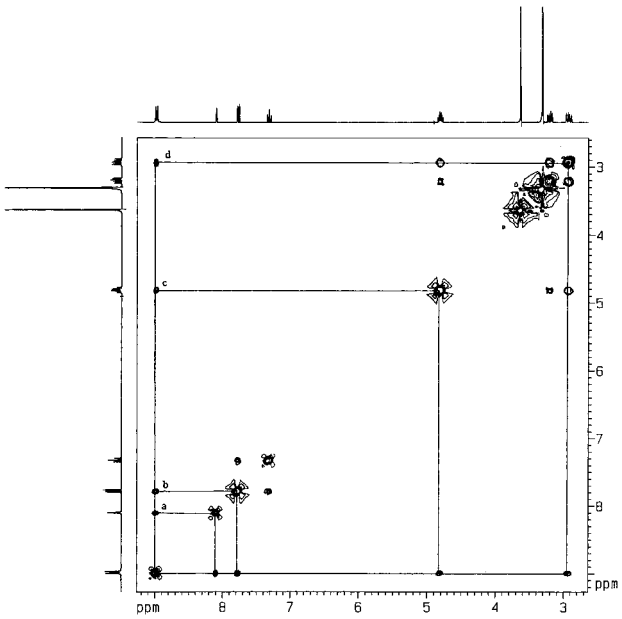


Figure 1. 300 MHz ROESY spectrum of **3a** in DMSO. The prominent ROE cross-peaks cyst NH-Ar(s), Cyst NH-Ar(d), Cyst NH-Cyst C$^{\alpha}$H, Cyst NH-Cyst C$^{\beta}$H$_2$ are denoted by the letters a, b, c, and d, respectively, in parentheses. Ar(s) and Ar(d) refer to aromatic singlet and doublet, respectively.

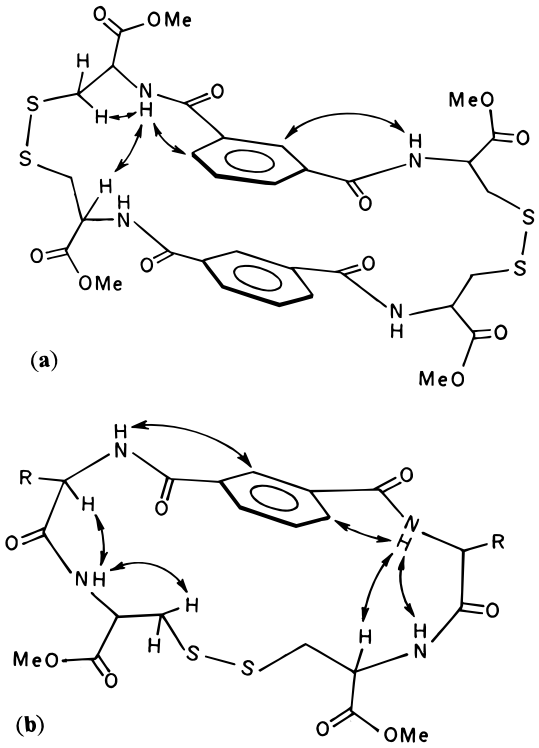


Figure 2. (a) Solution conformation of **3a**. NOE interactions [NH-Ar(s); NH-Ar(d); NH-C$^{\alpha}$H; NH-C$^{\beta}$H$_2$] are indicated by double-headed arrows. (b) Solution conformation of **7a,b**. NOE interactions in **7a** [Leu NH-Ar(s); Leu NH-Ar(d); Leu NH-Cyst NH; Leu NH-Cyst C$^{\alpha}$H; Cyst NH-Leu C$^{\alpha}$H; Cyst NH-Cyst C$^{\beta}$H$_2$] and in **7b** [Phe NH-Ar(s); Phe NH-Ar(d); Phe NH-Cyst C$^{\alpha}$H; Cyst NH-Phe C$^{\alpha}$H; Cyst NH-Cyst C$^{\beta}$H$_2$] are indicated by double-headed arrows.

3378 cm^{-1} in FT-IR ($CHCl_3$, 298 K) indicated internal amide to ester hydrogen bonding. On the basis of the above data, the solution structure of **3a** can be represented as shown in Figure 2a.

The cyclic oligomers of Pyr-bridged cystine macrocycles **3b**, **4**, and **5** on the other hand showed low-field NHs (downfield shifted by ~1.2 ppm) and relatively low dδ/dT values (~ −1 to −2 ppb/K), suggesting involvement of amide NHs in intramolecular hydrogen bonding (**B**). The intramolecular NH···N hydrogen bonding in macrocycles **3b**, **4**, and **5** was also supported by FT-IR and by the syn orientation of amide NHs as indicated by the absence of any cross-peaks between amide NHs and aromatic protons in their ROESY NMR spectra. The presence of only one set of resonances for Pyr and Cyst units at almost identical chemical shifts in **3b**, **4**, and **5** suggested similar conformation for these macrocycles. Like their isophthaloyl analogue **3a**, the Pyr-bridged macrocycles also showed a prominent broad positive band at ~217 nm in their CD spectra (TFE) (Supporting Information) indicating a β-turn-type conformation.

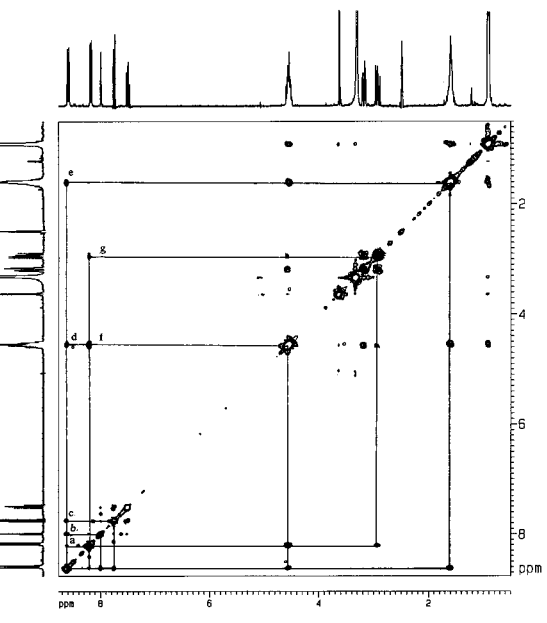
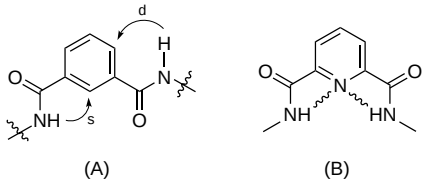


Figure 3. 300 MHz ROESY spectrum of **7a** in DMSO-d_6. The prominent ROE cross-peaks Leu NH-Cyst NH, Leu NH-Ar (s), Leu NH-Ar (d), Leu NH-Cyst C$^{\alpha}$H, Leu NH-Leu C$^{\beta}$H$_2$, Cyst NH-Leu C$^{\alpha}$H, and Cyst NH-Cyst C$^{\beta}$H$_2$ are denoted by the letters a, b, c, d, e, f, and g, respectively, in parentheses.



The Leu- and Phe-containing cystinophanes **7a** and **7b** (Scheme 2) showed striking similarity with each other and with **3a** with respect to conformation in DMSO. Thus, the ROESY spectra of both **7a** and **7b** exhibited strong cross-peaks between Leu/Phe-NH and aromatic singlet Ar(s) and aromatic doublet Ar(d) indicating anti orientation of amide carbonyls. The other significant ROESY cross-peaks in **7a** (Figure 3) suggesting β-turn features were Cyst NH-Leu NH, Cyst NH-Leu/Phe C$^{\alpha}$H, Cyst C$^{\alpha}$H-Leu/Phe NH, and Cyst NH-C$^{\beta}$H$_2$. VT studies (carried out with **7a** and **7b** over the temperature range 293−363 K in DMSO) showed the absence of any solvent shielded NHs (dδ/dT > −5 ppb/K). The presence of a prominent negative band at ~227 nm in the CD spectrum (Supporting Information) of

7a is consistent[16] with a β-turn-type conformation. On the basis of the above data, the solution state structure of cystinophanes **7a** and **7b** can be represented as shown in Figure 2b. The propensity of cyclophanes to adopt a β-turn-like structure may be attributed to the presence of S−S linkage and the need to maintain a near orthogonal value for the S−S dihedral angle. This notion was supported by the observation that replacement of cystine residue in **3a**/**3b** with a serine or a tyrosine unit led to cyclophanes totally devoid of β-turn features.[15]

The presence of intramolecular NH···N (ring) hydrogen bonds positioning the four amide NH groups into the interior of the ring suggested that the pyridine-bridged cystinophane **3b** may serve as a tailor-made receptor for dicarboxylic acids.[17]

The binding of **3b** with a range of tetrabutylammonium[18] (TBA) salts of 1,ω-alkane dicarboxylic acids [$(CH_2)_n(COOH)_2$; $n = 1$, ..., 4] and α-ketoglutaric acid (an important intermediate in Kreb's cycle) was examined by ^{1}H NMR studies. Addition of 1 equiv of the dicarboxylate guest to a $CDCl_3$ solution of **3b** resulted in considerable downfield shift of the host amide protons (Supporting Information). Interestingly, the NH shift (∼0.4 ppm) in the glutaric acid complex was found to be maximum and almost double the value (∼0.2 ppm) observed for succinic and adipic acids at the same (1:1) concentration. The α-ketoglutaric acid showed a similar profile of binding as glutaric acid.

With use of the NMR titration method,[19] the association constant (K_a) for glutaric acid bis-TBA salt was measured as 3.69×10^2 M^{-1}. The proposed bis-bidentate or tetrahydrogen-bonded structure (Figure 4) for the glutarate complex is supported by the maximum NH shift at a mole ratio of 1:1. The observed spectral changes in the C$^\alpha$ and C$^\beta$ methylenes of the glutarate guest in the ^{1}H NMR of 1:1 complex (Supporting Information) are also consistent with the proposed structure.

Suitable crystals for X-ray diffraction could only be obtained for **3a**. The solid-state structure of cystinophane **3a** is shown in Figure 5. Some important features of the crystal structure are the following: (i) all trans amide bonds, (ii) anti-arrangement of isophthaloyl carbonyls, and (iii) all ester groups oriented outward. The collapsed ring structure of **3a** with a curved architecture (Figure 6) has a cavity 3.6 Å across (phenyl plane to phenyl plane) and 12.8 Å long (S2−S4). The two meta-linked aromatic rings in **3a** display intramolecular face-to-face orientation. The angle between the planes of the two aromatic rings is 0.7°, very close to parallel (Figure 7). The distances of 4.726 and 4.298 Å between the two pairs of C$^\alpha$ atoms (C$^\alpha_{1A}$−C$^{\alpha'}_{2A}$ and C$^\alpha_{3A}$−C$^{\alpha'}_{4A}$, respectively) indicate turn features. The torsion angles for S1−S2 and S3−S4 are +96° and +92°, respectively. Table 1 presents all the torsion angles in **3a**.

There is no internal hydrogen bonding in **3a** in the solid state. However, molecules of the cyclophane **3a** are connected to each other by intermolecular hydrogen bonds through H_2O as the bridge into infinite chains parallel to the *a* axis of the cell (Figures 7 and 8). Interestingly, six molecules of **3a** form a cavity large enough to encapsulate two ethyl acetate molecules. The cavities are shown in Figure 8 in which the ethyl acetate molecules were omitted for clarity. The cavities are bounded, for example, by S1f, S2f, N1e, S4h, and S3h. The carbonyl oxygen of one ethyl acetate molecule is the receptor for a hydrogen bond from N1H moieties (N1e in Figure 8, for example), Table 2.

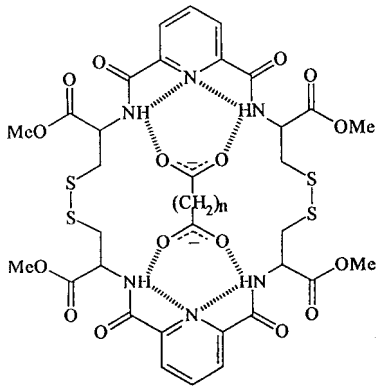


Figure 4. The proposed tetrahydrogen-bonded structure for the molecular recognition complex of receptor **3b** and glutaric acid (n = 3) bis-TBA salt.

Table 1. Torsional Angles in the Crystal (deg)

angle		value	angle		value
C2P−C1P−C0′1−N1		17	C8P−C9P−C0′3−N3		12
C1P−C0′1−N1−C1A	ω_{01}	−179	C9P−C0′3−N3−C3A	ω_{03}	−175
C0′1−N1−C1A−C1′	ϕ_1	−100	C0′3−N3−C3A−C3′	ϕ_3	−73
N1−C1A−C1′−O1M	ψ_1	55	N3−C3A−C3′−O3M	ψ_3	145
C1A−C1′−O1M−C1M	ω_1	178	C3A−C3′−O3M−C3M	ω_3	178
N1−C1A−C1B−S1	χ_{11}	−159	N3−C3A−C3B−S3	χ_{31}	−171
C1A−C1B−S1−S2	χ_{12}	65	C3A−C3B−S3−S4	χ_{32}	46
C1B−S1−S2−C2B		96	C3B−S3−S4−C4B		92
C8P−C7P−C0′2−N2		−150	C2P−C3P−C0′4−N4		−164
C7P−C0′2−N2−C2A	ω_{02}	−178	C3P−C0′4−N4−C′4A	ω_{04}	174
C0′2−N2−C2A−C2′	ϕ_2	−97	C0′4−N4−C4A−C4′	ϕ_4	−84
N2−C2A−C2′−O2M	ψ_2	−2	N4−C4A−C4′−O4M	ψ_4	175
C2A−C2′−O2M−C2M	ω_2	−172	C4A−C4′−O4M−C4M	ω_4	−179
N2−C2A−C2B−S2	χ_{21}	−62	N4−C4A−C4B−S4	χ_{41}	−49
C2A−C2B−S2−S1	χ_{22}	−65	C4A−C4B−S4−S3	χ_{42}	−69

Table 2. Hydrogen Bonds in the Crystal of **3a**

type	donor	acceptor	D−A (Å)	H−A (Å)
pept.−solv.	N1	O1s[a]	3.004	2.14
intermol.	N2	O3[b]	3.038	2.15
intermol.	N3	O4[c]	3.036	2.17
pept.−solv.	N4	W1[d]	2.896	2.11
solv.−pept.	W1	O02	2.916	
solv.−pept.	W1	O04	2.717	

[a] Carbonyl oxygen of ethyl acetate. [b] Symmetry equivalent of reported coordinates at $-x$, $^1/_2 + y$, $^3/_2 - z$. [c] Symmetry equivalent of reported coordinatesat $-^1/_2 + x$, $^1/_2 - y$, $2 - z$. [d] Symmetry equivalent of reported coordinates at $^1/_2 + x$, $^1/_2 - y$, $2 - z$.

(15) Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B. Unpublished results.

(16) Woody, R. W. In *Peptides: Analysis, Synthesis, Biology*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: New York, 1985; Vol. 7, pp 16−115.

(17) Although several examples of acyclic receptors for dicarboxylic acids are reported (for example, see: Hosseini, M. W.; Lehn, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 3525. Breslow, R.; Rajagopalan, R.; Schwartz, J. *J. Am. Chem. Soc.* **1981**, *103*, 2905. Kimura, E.; Sakonaka, A.; Yatsanami, T.; Kodami, M. *J. Am. Chem. Soc.* **1981**, *103*, 3041. Rebek, J., Jr.; Nemeth, D.; Ballester, P.; Lin, F. T. *J. Am. Chem. Soc.* **1987**, *109*, 3474. Tanaka, Y.; Kato, Y.; Aoyama, Y. *J. Am. Chem. Soc.* **1990**, *112*, 2807. Garcio-Tellado, F.; Geib, S. J.; Goswami, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 9265. Geib, S. J.; Vincent, C.; Fan, E.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 119. Karle, I. L.; Ranganathan, D.; Haridas, V. *J. Am. Chem. Soc.* **1997**, *119*, 2777), the reports on the cyclic receptors for dicarboxylic acids are still scarce (Garcia-Tellado, F.; Goswami, S.; Chang, S.-K.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 7393).

(18) Carboxylates being charged hydrogen-bond acceptors offer an additional advantage and exhibit increasing binding strength with amide NHs. (Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072. Fan, E.; Van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369.)

(19) The association constant (K_a) was obtained by using the following equation: $K_{assoc} = \alpha/[(1 - \alpha)([G] - \alpha[H])]$, where $\alpha = (\delta - \delta_0)/(\delta_{max} - \delta_0)$, δ_0 is the initial chemical shift (host alone), δ is the chemical shift at each titration point, and δ_{max} is the chemical shift when the receptor is entirely bound (Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072 and references therein).

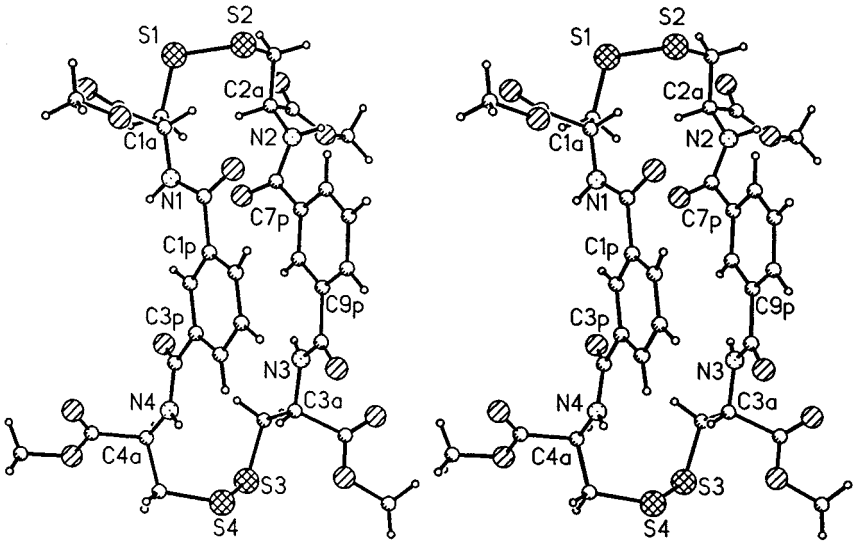


Figure 5. The conformation of **3a** in the crystal, shown in a stereodiagram.

The solution conformation of **3a** arrived at from the ^{1}H NMR data shows good agreement with the solid-state structure. The parallel face-to-face orientation of the two meta-linked phenyl rings was also indicated in the ^{1}H NMR of **3a** by noticeable upfield shift (~0.15 ppm) of the aromatic proton meta to the amide carbonyls as compared to the model compounds **7a** and **7b** containing only one phenyl ring. The alternate possibility of edge-to-face conformer is ruled out since the upfield shift is relatively smaller than expected for the aromatic proton in edge-to-face orientation and the aromatic proton ortho to both the substituents (appearing as a sharp singlet in ^{1}H NMR) shows almost the same δ value as in model compounds **7a** and **7b**.

Conclusion. A simple one-step strategy for the synthesis of aromatic-bridged cystine macrocycles (cystinophanes) is described. The ring size can be controlled through 2+2, 3+3, and 4+4 cyclizations. The design also permits the incorporation of a variety of amino acids into the ring as illustrated with the preparation of cyclophanes **7a** and **7b**. ^{1}H NMR studies have shown that these cystinophanes tend to adopt a β-turn-like structure in solution. CD studies have supported the NMR results. A near-parallel face-to-face orientation of the two phenyl rings was also shown by modest upfield shift of the aromatic protons in **3a**. These results were confirmed by single-crystal X-ray studies on **3a** which showed a collapsed ring conformation with near-parallel orientation of the two phenyl rings. The macrocyclic rings of **3a** are connected to each other through H_2O bridges. Six molecules of **3a** aggregate to form a cavity large enough to encapsulate two molecules of ethyl acetate. The β-turn features of cystinophanes are noteworthy and may be due to the presence of S−S linkage, which needs to maintain a near orthogonal value of its torsion angle. The promise of pyridine-bridged cystinophanes as artificial receptors for dicarboxylic acids has been demonstrated with 26-membered macrocycle **3b**. The cystinophane **3b** with all the four amide NHs locked in an intramolecular NH···N (ring) hydrogen bond and pointing into the center of the cavity serves as an effective host for bis-TBA salts of 1,ω-alkane dicarboxylic acids with maximum affinity ($K_{assoc} = 3.69 \times 10^2$ M^{-1}) and selectivity for glutaric acid substrates.

Experimental Section

All amino acids used were of L-configuration. Melting points are uncorrected. Optical rotations were measured with an automatic JASCO digital polarimeter; concentrations are given in gram/100 mL.

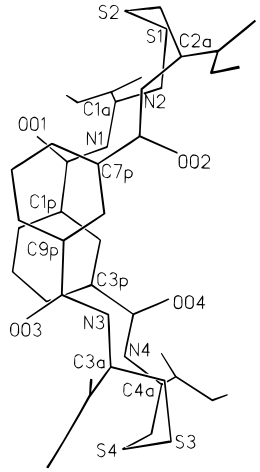


Figure 6. Side view of **3a** showing the curvature of the molecule. The stacked aromatic rings are parallel to each other.

IR spectra were recorded on Perkin-Elmer 580/1600 and 882/1600 FT spectrometers either in chloroform solution or as KBr pellets, and prominent peaks are expressed in reciprocal centimeters. The circular dichroism (CD) spectra were recorded in a JASCO J20 spectropolarimeter in quartz cells of 1 mm path length at 25° C. ^{1}H NMR spectra were obtained on a Bruker WM-400 spectrometer. The chemical shifts are recorded in δ, with TMS at 0.00 as internal reference. FAB mass spectra were recorded on a JEOL SX-120/DA-6000 instrument with *m*-nitrobenzyl alcohol as the matrix. Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100−200 mesh) columns which were generally made from a slurry in hexane or ethyl acetate or chlorofom. Reactions were monitored wherever possible by TLC.

Preparation of Cystinophanes Listed in Schemes 1 and 2. (1) General Procedure. **(a) Preparation of Cystino-Bispeptides Boc-Xaa-Cyst-Xaa-Boc (Xaa = Leu or Phe, Cyst = Cystine Dimethyl Ester).** A well-stirred and ice-cooled solution of cystine dimethyl ester (generated in situ from 5 mmol of cystine dimethyl ester dihydrochloride and 10 mmol of triethylamine in ~50 mL of dry CH_2Cl_2) was admixed with the activated ester of Boc-Xaa (prepared separately from 10 mmol, each, of Boc-Xaa, DCC, and *N*-hydroxysuccinimide in ~ 50 mL of dry CH_2Cl_2). The combined reaction mixture was stirred at room temperature for 24 h, the precipitated DC urea filtered, and the filtrate was washed with 20 mL each of ice-cold 1 N H_2SO_4, water, and bicarbonate solution, dried (anhydrous $MgSO_4$), and evaporated in vacuo. The residue was purified on a short column of silica gel with ethyl acetate/hexane as eluents to give cystino-bispeptides **6a** and **6b** in ~60−65% yields.

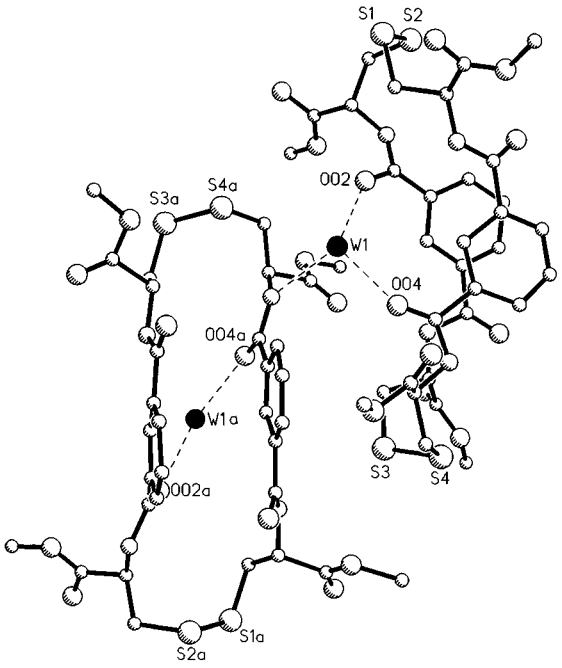


Figure 7. The hydrogen bond environment about the water molecule W1 and the symmetry related W1a. The cystinophane molecules are connected into an infinite chain by the water molecules. The orientation of **3a** on the left shows the parallel planes near which most of the backbone atoms lie.

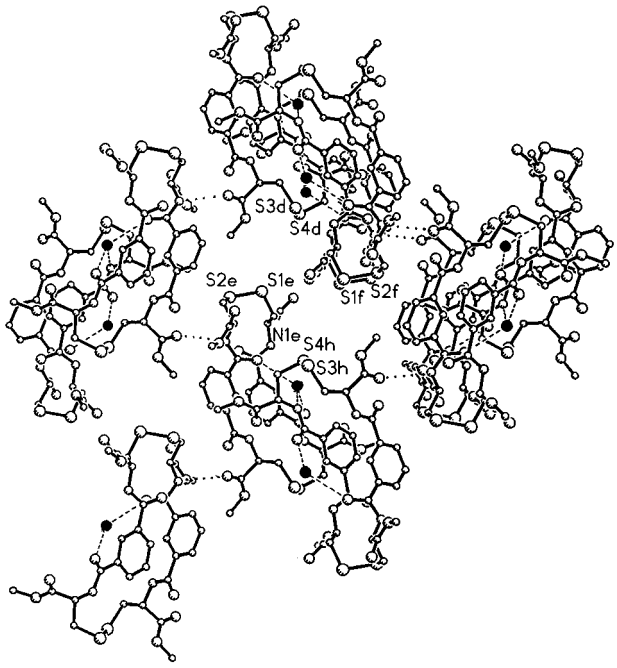


Figure 8. Projection down the *a* axis of the cystinophane crystal. Hydrogen bonds involving water molecules (darkened) are indicated by dashed lines. Dotted lines indicate N2H···O3 hydrogen bonds between the infinite chains of molecules. Ethyl acetate molecules have been omitted for clarity. They reside in the channels represented by the open spaces such as between S1f, S2f and S3h, S4h.

(b) Condensation of Cystine Dimethyl Ester or N-Deprotected Cystino Bispeptides with 1,3 Aromatic Dicarbonyl Dichlorides: Preparation of Cystinophanes 3a,b, 4, 5, and 7a,b. A solution of 1,3 aromatic dicarbonyl dichloride (**1**, X = CH or N) (5 mmol) in dry CH_2Cl_2 (30 mL) was added dropwise (0.5 h) to a well-stirred and ice-cooled solution of cystine dimethyl ester (generated in situ at 0 °C from 5 mmol of cystine dimethyl ester dihydrochloride and 10 mmol of triethylamine in 50 mL of dry CH_2Cl_2) or cystino bispeptide dimethyl ester (5 mmol, prepared by N-deprotection of the bis-Boc derivative with 25% TFA in 15 mL of dry CH_2Cl_2, followed by neutralization with saturated sodium carbonate, extraction with CH_2Cl_2, and drying of the organic layer with anhydrous $MgSO_4$). The reaction mixture was further diluted with ~150 mL of dry CH_2Cl_2 and left stirred at room temperature for 12 h, and washed with 20 mL, each, of ice-cold 2 N H_2SO_4, water and bicarbonate solution, and the organic extract was dried (anhydrous $MgSO_4$) and evaporated in vacuo. The residue was chromatographed on a column of silica gel and products eluted with $CHCl_3$/MeOH (95:5) eluent.

(2). Selected Data of Cystinophanes. 3a: Yield 52%; mp 132−134 °C; $[\alpha]^{26}_D$ −36.33 (*c* 1.94, $CHCl_3$); IR (KBr) 3385, 3072, 2959, 1750, 1680, 1671 (sh), 1648, 1586 (sh), 1556, 1538, 1515, 1481, 1442 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.88 (4H, dd, J = 5.1, 8.7 Hz), 3.19 (4H, dd, J = 6.3, 7.5 Hz), 3.59 (12H, s), 4.74 (4H, m), 7.31 (2H, t, J = 7.8 Hz), 7.71 (4H, d, J = 7.8 Hz), 7.95 (2H, s), 8.99 (4H, d, J = 7.5 Hz); FAB-MS *m*/*z* (%) 797 (95) $[M + H]^+$.

3b: Yield 51%; mp 102−104 °C; $[\alpha]^{26}_D$ +177.92 (*c* 4.0, $CHCl_3$); IR (KBr) 3404, 2959, 1751, 1689, 1559 (sh), 1528, 1443, 1354 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.31 (4H, dd, J = 4.5, 15 Hz), 3.51 (4H, dd, J = 4.5, 14.4 Hz), 3.73 (12H, s), 5.09 (4H, m), 8.08 (2H, t, J = 7.5 Hz), 8.37 (4H, d, J = 7.8 Hz), 8.82 (4H, d, J = 8.1 Hz); FAB-MS *m*/*z* (%) (100) 799 $[M + H]^+$.

4: Yield 12%; semisolid; $[\alpha]^{26}_D$ −28.80 (*c* 1.70, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 3.27 (12H, m), 3.72 (18H, brs), 4.97 (6H, m), 8.04 (3H, t, J = 7.5 Hz), 8.36 (6H, d, J = 7.8 Hz), 8.84 (6H, d, J = 5.1 Hz); IR (KBr) 3361, 2962, 2934, 1751, 1681, 1559 (sh), 1524, 1444, 1355 cm^{-1}; FAB-MS *m*/*z* (%) 1198 (100) $[(M + H)^+]$, 799 (80) $[M^+ - \mathbf{3b} + H]$.

5: Yield 4%; mp 136−138 °C; $[\alpha]_D^{26}$ −65.29 (*c* 1.1, $CHCl_3$); IR (KBr) 3360, 2959, 1749, 1681, 1534, 1444, 1355 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.29 (16H, m), 3.71 (24H, brs), 4.97 (8H, m), 8.03 (4H, t, J = 7.5 Hz), 8.33 (8H, d, J = 7.5 Hz), 8.88 (8H, d, J = 8.1 Hz); FAB-MS *m*/*z* (%) 1597 (60) $[M + H]^+$, 1198 (43) $[M^+ - \mathbf{3b} + H]$, 799 (100) $[M^+ - 2 \times \mathbf{3b} + H]$.

6a: Yield 62%; mp 71−72 °C; $[\alpha]^{26}_D$ +26.32 (*c* 4.40, $CHCl_3$); IR (KBr) 3332, 2967, 1751, 1718, 1687, 1661, 1533, 1514 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 0.93 (12H, d, J = 5 Hz), 1.46 (18H, m), 1.67 (6H, m), 3.18 (4H, m), 3.77 (6H, s), 4.25 (2H, m), 4.88 (2H, m), 5.39 (2H, d, J = 7.5 Hz), 7.62 (2H, brd); FAB-MS *m*/*z* (%) 717 (8) $[M + Na]^+$, 695 (20) $[M + H]^+$, 595 (44) $[M - Boc + H]^+$, 495 (100) $[M - 2 \times Boc + H]^+$.

6b: Yield 65%; mp 119−121 °C; IR (KBr) 3354, 2986, 2937, 1744, 1694, 1671, 1534 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.42 (18H, s), 3.18 (8H, m), 3.81 (6H, s), 4.63 (2H, m), 4.92 (2H, m), 5.54 (2H, d, J = 7.5 Hz), 7.06−7.55 (12H, m); FAB-MS *m*/*z* (%) 763 (16) $[M + H]^+$, 663 (62) $[M - Boc + H]^+$, 563 (100) $[M - 2 \times Boc + H]^+$.

7a: Yield 42%; mp 254−256 °C; $[\alpha]^{26}_D$ −147.02 (*c* 3.85, $CHCl_3$); IR (KBr) 3292, 2964, 1749, 1650, 1534 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 0.90 (12H, m), 1.62 (6H, m), 2.93 (2H, dd, J = 9.9, 12 Hz), 3.18 (2H, dd, J = 4.2, 13.8 Hz), 3.63 (6H, s), 4.55 (4H, m), 7.49 (1H, t, J = 7.8 Hz), 7.75 (2H, d, J = 7.8 Hz), 7.99 (1H, s), 8.18 (2H, d, J = 7.8 Hz), 8.59 (2H, d, J = 8.7 Hz); FAB-MS *m*/*z* (%) 625 (88) $[M + H]^+$.

7b: Yield 45%; mp 216−218 °C; $[\alpha]_D^{26}$ −191.60 (*c* 0.57, $CHCl_3$/MeOH; 3:1); IR (KBr) 3291, 2941, 2684, 1744, 1732, 1674, 1653, 1537, 1442 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) 2.92 (4H, t, J = 9.6 Hz), 3.13 (4H, m), 3.62 (6H, s), 4.65 (4H, m), 7.18−7.28 (10H, m), 7.46 (1H, t, J = 7.5 Hz), 7.59 (2H, d, J = 7.5 Hz), 7.99 (1H, s), 8.18 (2H, d, J = 7.5 Hz), 8.87 (2H, d, J = 8.7 Hz); FAB-MS *m*/*z* (%) 693 (100) $[M + H]^+$.

Preparation of Tetrabutylammonium Salts of 1,ω-Alkane Dicarboxylic Acids.[19] To a stirred solution of the dicarboxylic acid (1 mmol) in dry methanol (~2 mL) was added 2 equiv of a 0.1 N solution of tetrabutylammonium hydroxide in methanol/toluene (SRL) in one portion. The resulting mixture after 2 h of stirring at room temperature was subjected to vacuum and the thick syrupy residue was dried for 24 h over P_2O_5 in a vacuum desiccator. The dried bis-TBA salts were directly used for ^1H NMR titration experiments.

^1H NMR Titration of 3b with the Bis-TBA Salt of Glutaric Acid in $CDCl_3$. An initial NMR spectrum of the solution of **3b** (8 mg/0.5

mL of $CDCl_3$; 0.02 M) was taken, and the initial chemical shift (δ_0) of the amide NHs was determined to be 8.825 ppm. The solution of the bis-TBA salt of glutaric acid (123 mg in 1.0 mL, 0.2 M) was then added, initially in 10 μL portions, and the chemical shift (δ) of the amide NH recorded after each addition. After ~2 equiv (100 μL) of guest had been added, the aliquot amount for each subsequent addition was increased to 20, 30, 50, 100, and 200 μL until a total of 500 μL was added. The chemical shift (δ_{max}) of the amide proton at saturation point (~1:1 molar ratio) was 9.332 ppm. The association constant (K_a) was then determined by using the equation mentioned under ref 19.

(a) X-ray Diffraction Analysis. The crystallographic parameters for the crystal of **3a** are the following: $C_{32}H_{36}N_4O_{12}S_4 \cdot H_2O \cdot 2C_4H_8O_2$, space group $P2_12_12_1$ with $a = 11.748(1)$ Å, $b = 17.317(1)$ Å, and $c = 24.306(2)$ Å, $V = 4944.6(7)$ Å^3, $Z = 4$, $d_{calc} = 1.329$ g/cm^3, and $F_{ooo} = 2080$. The crystal habit was indistinct, approximately a prism, with dimensions of 0.45 × 0.80 × 0.53 mm. X-ray data were collected on an automated Siemens diffractometer in the $\theta/2\theta$ mode, constant scan speed of 10 deg/s, 1° scan width and $2\theta_{max} = 115°$ with Cu Kα radiation ($\lambda = 1.54178$ Å). The crystal was covered with microscope immersion oil immediately after removal from mother liquor to prevent escape of solvent cocrystallized with **3a** during data collection. Three check reflections, monitored after every 97 measurements, indicated a fairly smooth decrease in intensity, down to ~83% at the end of the data collection. The measured intensities were corrected for the decay of scattering power.

The structure determination was straightforward with direct methods as programmed in SHELXTL, Version 4.2 (Siemens Analytical X-ray Instrument Co., Madison, WI). Two cocrystallized ethyl acetate molecules were located in difference maps after several cycles of least-squares refinement on the parameters for the principal molecule and water (W1). The water molecule was bound tightly by hydrogen bonds and had a relatively low thermal factor value ($U_{eq} = 0.09$) while the ethyl acetate molecules were rather mobile with U_{eq} values ranging from 0.18 to 0.48. Full-matrix anisotropic least squares were performed with the hydrogen atoms placed in ideal positions and allowed to ride with the C or N atoms to which each was bonded. The number of parameters refined was 586, using 2451 data measured $>4\sigma(F)$ out of a toal of 3745 independent data measured. The final R factor was 10.6% for the data measured $>4\sigma$.

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Supporting Information Available: CD spectra of **3a** (Figure 1S), **4** and **5** (Figure 2S), and **7a** (Figure 3S) in TFE; ROESY NMR of **3b** (Figure 4S), adamantane analogue of **3b** (Figure 5S); ^{1}H NMR (binding studies of succinic, glutaric, and adipic acids with **3b**; Figures 6S to 20S); Structural details, including coordinates, bond lengths, bond angles, anisotropic thermal parameters and coordinates of hydrogen atoms for **3a** (31 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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