Supporting Information

A New Macrocycle Demonstrates Ditopic Recognition Properties

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Contents

1. General

Synthesis of dibromide **2** has been reported elsewhere.[1] All reagents were purchased from Novabiochem or Aldrich Chemical Company. Dimethylformamide (DMF) was stored over molecular sieves and degassed prior to use. Tetrahydrofuran (THF) was dried by distillation from sodium benzophenone ketyl. *t*-BuOH was dried with CaH_2, and then distilled. Other reagents are used as received. All reactions were run under a nitrogen atmosphere.

Chromatography (silica gel 60Å. 200-400 mesh; Natland International) was used for product purification. ^{1}H NMR spectra were recorded at 400 MHz or 500 MHz. MS analysis of the products was performed using the Electron Spray technique. Elemental analysis was performed by Atlantic Microlab Inc. Melting points are uncorrected.

2. Sythesis and characterization of compounds 1-6

Diacid 3

To a suspension of **2** (5.0 g, 9.3 mmol) in 80 mL pyridine was added CuCN (4.3 g, 46.5 mmol). The mixture was refluxed under nitrogen for 10 h. After this time, the solvent was removed under reduced pressure and the residue partitioned four times between chloroform and 10% aqueous ammonia solution. The combined organic layer was washed with water, dried with anhydrous Na_2SO_4 and the salts filtered off. The solvent was removed under reduced pressure and the residue was crystallized in acetone / hexanes to afford 3.8 g (96% yield) of the corresponding dicyanide as a white solid. mp 105-107 °C; ^{1}H NMR (acetone-d_6) δ 4.03 (m, 4H), 4.18 (m, 4H), 7.74 (m, 4H), 7.81 (d, J = 7.5 Hz, 2H), 7.95 (m, 3H); MS (ES^+) *m/z* (rel intens.) = 428.2 (100) $[M + H]^+$, 450.2 (35) $[M + Na]^+$; Anal. Calcd. for $C_{23}H_{17}N_5O_4$: C, 64.63; H, 4.01; Found: C, 64.37; H, 3.93.

A suspension of the dicyanide (5.0 g, 11.7 mmol) in 100 mL concentrated HCl solution was refluxed for 3 d. After this time, the solvent was removed under reduced pressure. The yellow residue was dissolved in 10% NaOH solution, and then acidified with concentrated HCl solution. The product precipitated and was collected by filtration to afford 4.0 g (90% yield) of **3** as a white solid. mp 133-135 °C; ^{1}H NMR (DMSO-d_6) δ 8.00 (t, J = 8.0 Hz, 2H), 8.27 (m, 4H), 8.38 (s, 3H), 13.5 (s, 2H); MS (ES^-) *m/z* (rel intens.) = 376.2 (100) $[M - H]^-$; Anal. Calcd. for $C_{19}H_{11}N_3O_6 \cdot 1/2H_2O$: C, 59.07; H, 3.11; Found: C, 58.70; H, 3.00.

Monoester 4

A solution of **3** (5 g, 13.3 mmol) in 80 mL saturated HCl / EtOH was stirred at rt for 1 d. The solvent was removed under reduced pressure and the residue partitioned three times between chloroform and 5% aqueous K_2CO_3 solution. The combined organic layer was dried with anhydrous Na_2SO_4 and the salts filtered off. Removal of the solvent under reduced pressure gave a yellow residue which was crystallized in acetone / hexanes to afford 4.3 g (75% yield) of the corresponding diester as a white solid. mp 69-71 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7.2 Hz, 6H), 4.46 (t, J = 6.8 Hz, 4H), 7.82 (t, J = 8.0 Hz, 2H), 7.81 (t, J = 8.0 Hz, 1H), 8.23 (d, J = 7.6 Hz, 2H), 8.26 (d, J = 7.6 Hz, 2H), 8.46 (d,

J = 7.6 Hz, 2H); MS (ES^+) *m/z* (rel intens.) = 434.3 (100) $[M + H]^+$; Anal. Calcd. for $C_{23}H_{19}N_3O_6 \cdot H_2O$: C, 61.47; H, 4.68; Found: C, 61.41; H, 4.69.

To a solution of the diester (4 g, 9.2 mmol) in 100 mL of 40% aqueous THF, was added dropwise a solution of NaOH (0.37 g, 9.2 mmol) in 10 mL water. The mixture was stirred at rt for 1 d. After this time, the mixture was partitioned between water and ethyl acetate, and the water phase was concentrated and acidified with concentrated HCl solution. The product **4** precipitated and was collected by filtration as a white solid. Yield: 65%. mp 120-122 °C; ^{1}H NMR (acetone-d_6) δ 1.34 (t, *J* = 7.2 Hz, 3H), 4.40 (t, *J* = 6.8 Hz, 2H), 8.07 (m, 2H), 8.21 (d, *J* = 7.6 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.29 (d, *J* = 7.6 Hz, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 8.43 (m, 3H). MS (ES^-) *m/z* (rel intens.) = 404.1 (100) $[M - H]^-$. Anal. Calcd. for $C_{21}H_{15}N_3O_6 \cdot 2HCl$: C, 52.74; H, 3.58; Found: C, 52.50; H, 3.61.

Amine 5

To a suspension of **4** (5.0 g, 12.3 mmol) in 80 mL dry *t*-BuOH at rt was added diphenylphosphoryl azide (DPPA) (2.9 mL, 13.5 mmol). Triethylamine (2.3 mL, 18.5 mmol) was then added. The mixture was stirred for 4 h, and then refluxed for 6 h. After this time, the solvent was removed under reduced pressure and the residue was partitioned between chloroform and 5% aqueous K_2CO_3 solution three times. The combined organic layer was dried with anhydrous Na_2SO_4 and the salts filtered off. Removal of the solvent under reduced pressure gave a yellow residue which was purified with flash chromatography (mobile phase: 30% ethyl acetate in hexanes) to afford 3.5 g (60% yield) of the corresponding Boc protected amine as a white solid. mp 83-85 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3H), 1.50 (s, 9H), 4.46 (t, *J* = 6.8 Hz, 2H), 7.33 (s, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.94 (t, *J* = 7.6 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 8.13 (t, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 8.31 (d, *J* = 8.0 Hz, 1H), 8.42 (d, *J* = 8.0 Hz, 1H). MS (ES^+) *m/z* (rel intens.) = 477.3 (100) $[M + H]^+$, 509.3 (80) $[M + MeOH + H]^+$; Anal. Calcd. for $C_{25}H_{24}N_4O_6$: C, 63.02; H, 5.08; Found: C, 63.13; H, 5.13.

The above product was dissolved in 10 mL CH_2Cl_2. To this solution was added 4 mL trifluoroacetic acid (TFA). The mixture was stirred at rt for 1 d. After this time, the solvent and TFA were removed under reduced pressure. The residue was partitioned three times between chloroform and 5% K_2CO_3 aqueous solution. The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was recrystallized in acetone / hexanes to give 2.8 g (90% yield) of **5** as a yellow solid. mp 78- 80 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3H), 4.46 (t, *J* = 6.8 Hz, 2H), 5.02 (s, 2H), 6.71 (d, *J* = 8.4 Hz, 1H), 7.37 (t, *J* = 8.4 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.99 (t, *J* = 8.0 Hz, 1H), 8.11 (t, *J* = 8.0 Hz, 1H), 8.27 (m, 3H), 8.41 (d, *J* = 7.6 Hz, 1H). MS (ES^+) *m/z* (rel intens.) = 377.2 (100) $[M + H]^+$; Anal. Calcd. for $C_{20}H_{16}N_4O_4$: C, 63.82; H, 4.28; Found: C, 63.90; H, 4.29.

Glycine derivative 6

To a solution of **5** (0.5 g, 1.33 mmol) in 2 mL DMF was added Boc-glycine-OH (0.35 g, 2.0 mmol), *O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluoro-

phosphate (HBTU) (0.76 g, 2.0 mmol) and *N*-hydroxybenzotriazole (HOBT) (0.27 g, 2.0 mmol). Triethylamine (0.5 mL, 4.0 mmol) was then added, and the mixture was stirred at 40 °C for 1 d. After this time, the mixture was partitioned three times between chloroform and 5% aqueous K_2CO_3 solution. The combined organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The product was purified with flash chromatography (mobile phase: 10% acetone in chloroform). Removal of the solvent under reduced pressure gave 0.43 g (60% yield) of the corresponding Boc protected amine as a white solid. mp 117- 119 °C; ^{1}H NMR (acetone-d_6) δ 1.35 (t, J = 7.2 Hz, 3H), 1.40 (s, 9H), 4.05 (d, J = 6.2 Hz, 2H), 4.42 (t, J = 6.8 Hz, 2H), 6.37 (s, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.83 (t, J = 8.4 Hz, 1H), 8.12 (t, J = 7.6 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.29 (d, J = 7.6 Hz, 1H), 8.32 (m, 2H), 8.41 (d, J = 7.6 Hz, 1H), 9.61 (s, 1H); MS (ES^+) *m/z* (rel intens.) = 534.3 (100) $[M + H]^+$, 556.3 (20) $[M + Na]^+$, 566.3 (30) $[M + MeOH + H]^+$; Anal. Calcd. for $C_{27}H_{27}N_5O_7 \cdot 1/2H_2O$: C, 59.89; H, 5.22; Found: C, 59.82; H, 5.58.

To a solution of the Boc protected amine (0.6 g, 1.1 mmol) in 30 mL of 40% aqueous THF was added dropwise a solution of NaOH (42 mg, 1.2 mmol) in 5 mL water. The mixture was stirred at rt for 1 d. After this time, the mixture was partitioned between water and ethyl acetate three times. The combined water phase was concentrated to ca. 20 mL and was poured into a flask containing 50 mL chloroform. The aq. layer was acidified with 1 eq. 10% HCl solution and the white precipitate immediately extracted into chloroform by shaking the mixture. The organic phase was dried and evaporated at reduced pressure to give acid derivative as a white solid. Yield: 85%. mp 122-124 °C; ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 9H), 3.80 (d, J = 6.2 Hz, 2H), 7.21 (s, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.86 (t, J = 8.4 Hz, 1H), 7.95 (m, 2H), 8.01 (d, J = 8.0 Hz, 1H), 8.30 (t, J = 8.0 Hz, 1H), 8.24 (m, 3H), 10.74 (s, 1H); MS (ES^+) *m/z* (rel intens.) = 504.2 (100) $[M - H]^-$; Anal. Calcd. for $C_{25}H_{23}N_5O_7 \cdot 1/2H_2O$: C, 58.48; H, 4.68; Found: C, 58.44; H, 4.58.

The above product was suspended in 10 mL water. The mixture was acidified with 2 mL concentrated HCl solution and stirred at rt until homogeneous (ca. 20 min). The solvent was then removed under reduced pressure to give yellowish crude product, which was then redissolved in 2 mL methanol, and precipitated with ether. The product was isolated by filtration as a white solid. Yield: 90%. mp 140-142 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 2H), 7.80 (d, J = 8.4 Hz, 1H), 7.88 (t, J = 8.4 Hz, 1H), 8.13 (m, 5H), 8.32 (d, J = 7.6 Hz, 1H), 8.36 (d, J = 7.6 Hz, 1H), 11.18 (s, 1H); MS (ES^+) *m/z* (rel intens.) = 404.2 (100) $[M - H]^-$; Anal. Calcd. for $C_{20}H_{15}N_5O_5 \cdot 2H_2O \cdot 2HCl$: C, 46.69; H, 4.08; Found: C, 46.57; H, 4.07.

Macrocycle 1

To a solution of **6** (0.2 g, 0.5 mmol) in 250 mL DMF was added triethylamine (0.25 mL, 2.0 mmol). A solution of DPPA (0.22 mL, 1.0 mmol) in 10 mL DMF was added dropwise over a period of 5 h. The mixture was stirred at rt for 2 d. Removal of the solvent under reduced pressure afforded a yellow residue that was partitioned three times between chloroform and water. The combined organic layer was dried and evaporated under reduced pressure. Flash chromatography (mobile phase: 20% acetone in chloroform) gave the crude product as a white solid, which was further purified by

washing the solid with acetone. Yield: 35%. mp 189-191 °C; ^{1}H NMR ($CDCl_3$) 4.46 (d, *J* = 5.2 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.89 (s, 1H), 8.03 (t, *J* = 8.0 Hz, 1H), 8.19 (t, *J* = 8.0 Hz, 1H), 8.26 (m, 3H), 8.41 (m, 2H); MS (ES^+) *m/z* (rel intens.) = 388.2 (100) $[M + H]^+$; 406.2 (50) $[M + H_2O + H]^+$; 420.2 (60) $[M + MeOH + H]^+$; Anal. Calcd. for $C_{20}H_{13}N_5O_4 \cdot H_2O$: C, 59.21; H, 3.70; Found: C, 59.09; H, 3.31.

3. 1D ^{1}H NMR, COSY and NOESY of macrocycle 1

The COSY and NOESY spectra of macrocycle **1** are shown in Figure S2 and S4 respectively. For a comparison, the same region of the corresponding 1D NMR is shown in Figure S3 and S5 respectively.

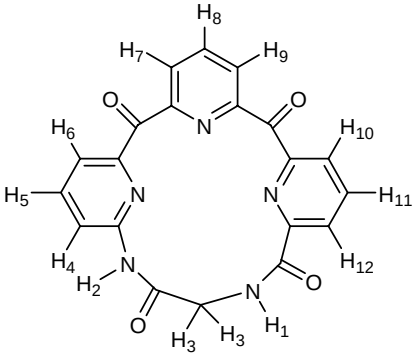


Figure S1 Macrocycle **1**

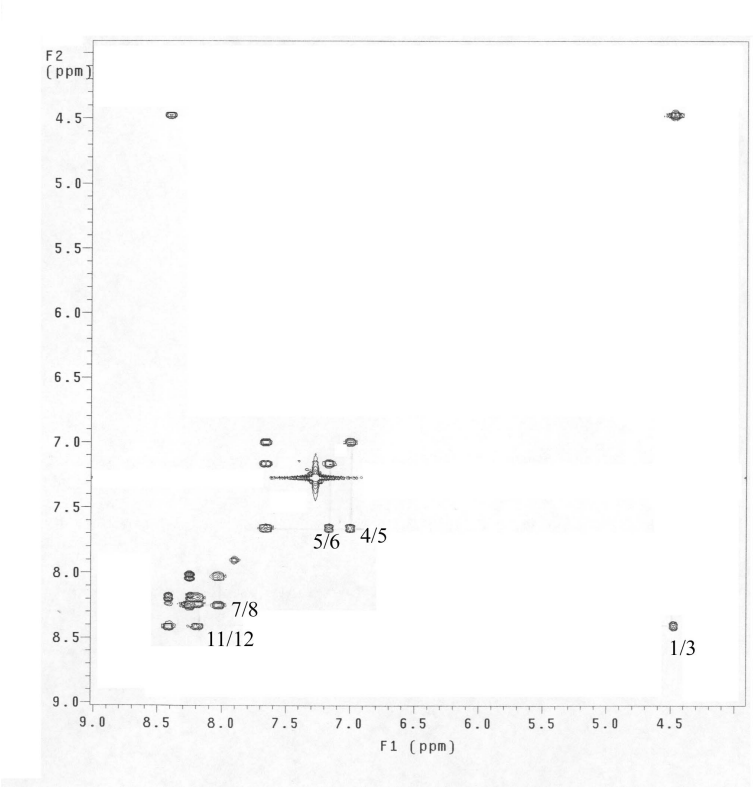


Figure S2 COSY spectrum of macrocycle **1** in $CDCl_3$.

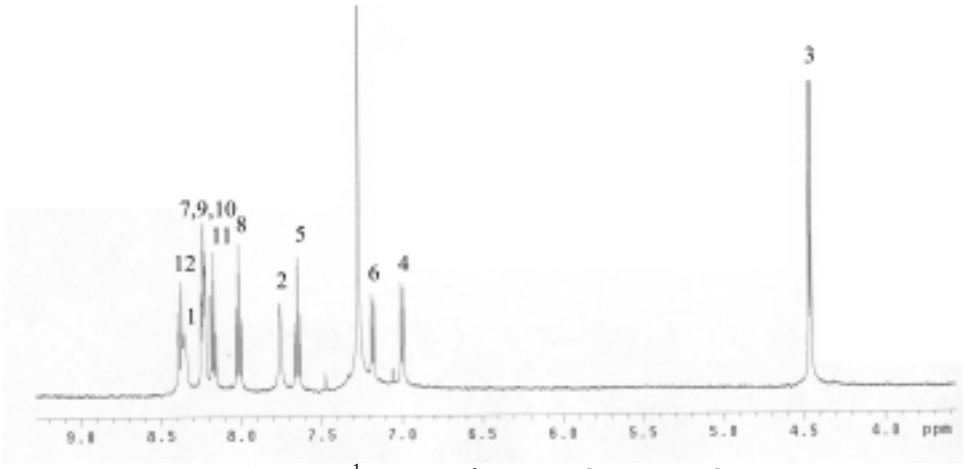


Figure S3 ^{1}H NMR of macrocycle **1** in $CDCl_3$.

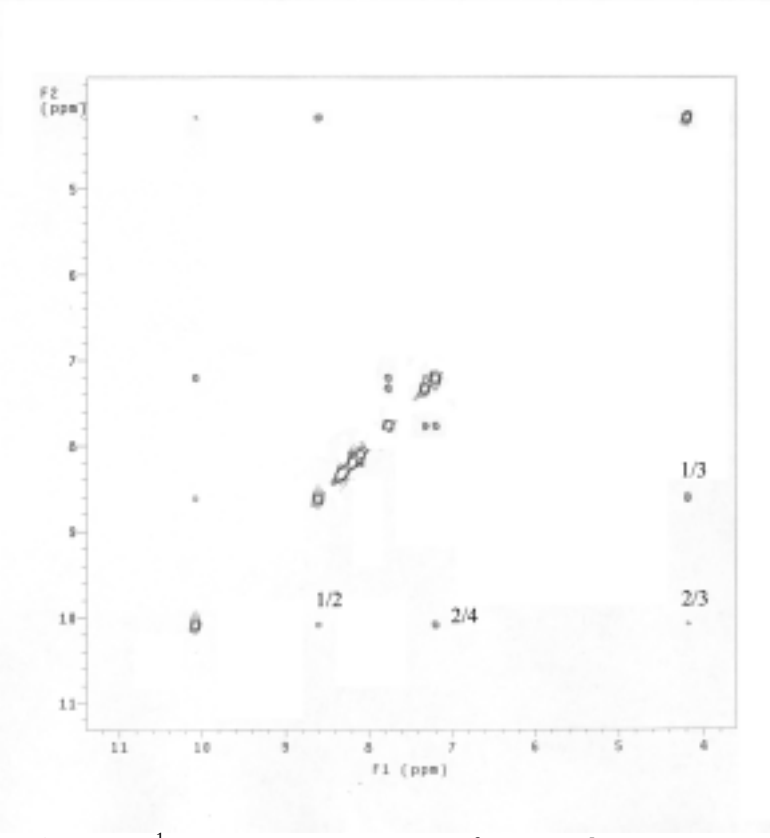


Figure S4 ^{1}H NMR NOESY spectrum of macrocycle **1** in DMSO.

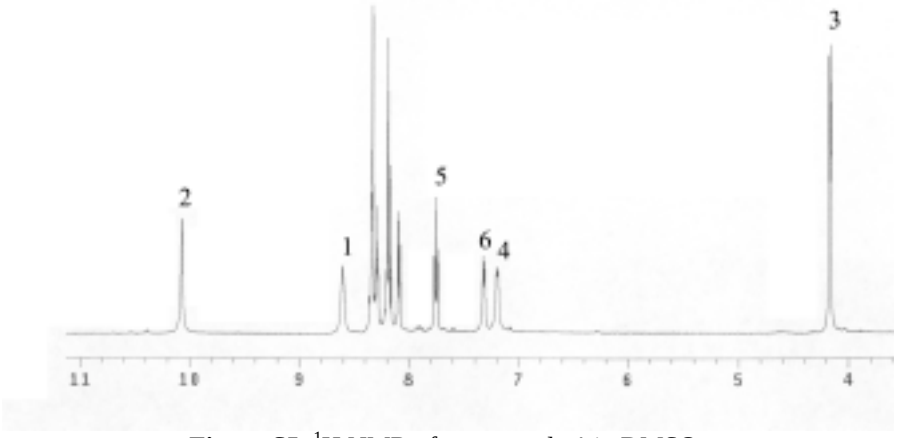
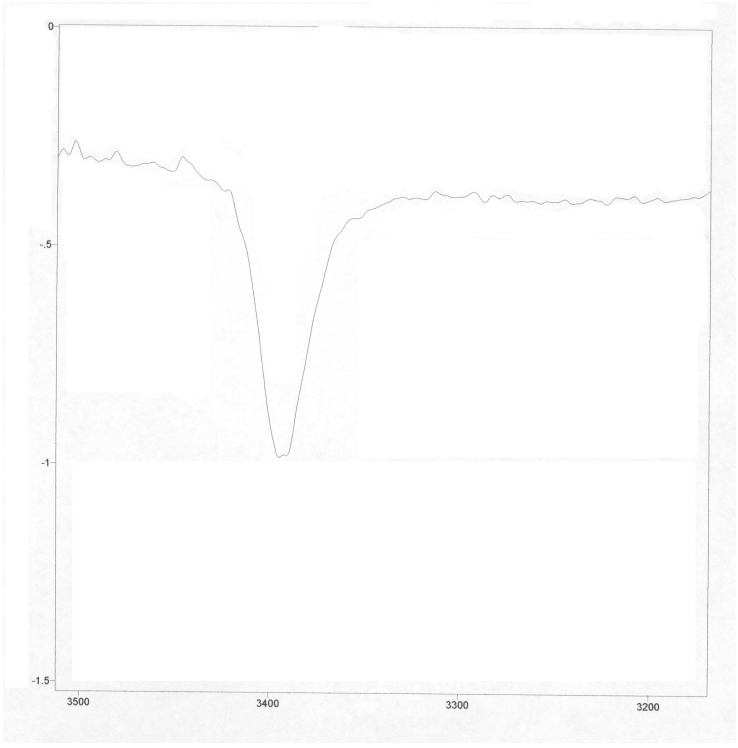


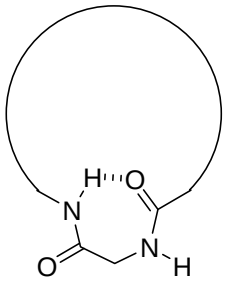
Figure S5 ^{1}H NMR of macrocycle **1** in DMSO.

4. IR of macrocycle 1

All IR spectra were obtained with a Perkin-Elmer 2000 spectrometer. Samples were run as $CDCl_3$ solutions at 1 mM concentration. The IR spectrum of macrocycle **1** at NH stretching region is shown in Figure S6. The absorption at 3390 cm^{-1} corresponds to hydrogen-bond free N-H stretch, [2] which indicates no γ turn in **1** (Figure S6b).



(a)



(b)

Figure S6. (a) NH stretching region of the FT-IR spectra of macrocycle **1** in $CDCl_3$; (b) Schematic representative of a γ turn.

5. Binding isotherms via ^{1}H NMR titrations

All quoted association constants were the average of three titrations. For each experiment, a 1 mM stock solution of macrocycle **1** in $CDCl_3$ was prepared. A 0.5 mL of the stock solution was then measured into an NMR tube and its spectra recorded (500 MHz NMR, 298 K). Small aliquots of guest solution prepared in the range of 20 mM-50 mM (high concentrations were used for the TBA salts) were measured into the tube and the spectra recorded after each addition. The chemical shift of proton H-2 (Figure S1) or H-5 (in cases when the peak of H-2 is too broad to measure) was used to generate the binding isotherm.

An iterative curve-fitting method using Origin 6.1 (Aston Scientific Ltd.) was used to generate the binding constants according to the following equation:

$$y = (D_{max}/(2/(K*x-1-K*Ht+((1-K*x+K*Ht)^2+4*K*x)^0.5)+1)) \qquad \text{(eq. S1)}$$

Where:
y: chemical shift
D_{max}: maximum chemical shift
K: binding constant
Ht: concentration of the host
x: total concentration of the guest

Representative binding isotherms are shown in Figure S7-10.

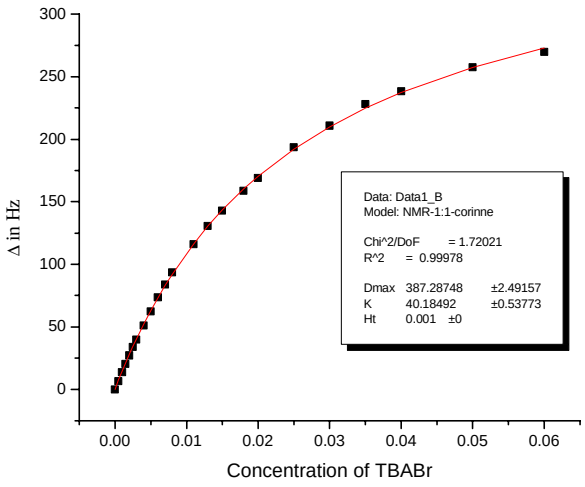


Figure S7. Binding isotherm for the complexation of macrocycle **1** and TBA-Br.

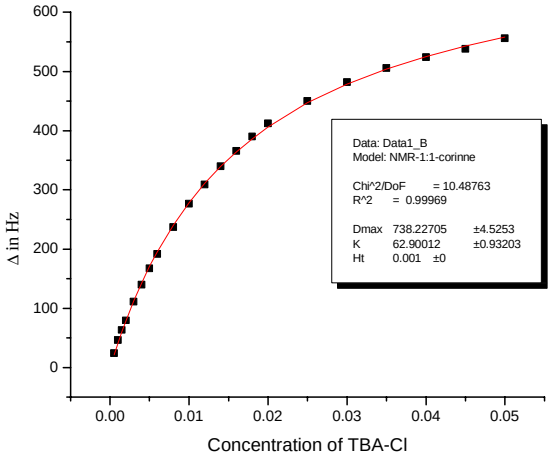


Figure S8. Binding isotherm for the complexation of macrocycle **1** and TBA-Cl

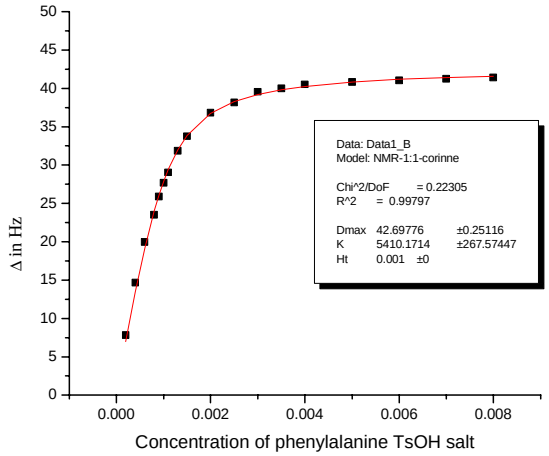


Figure S9. Binding isotherm for the complexation of macrocycle **1** and phenylalanine TsOH salt

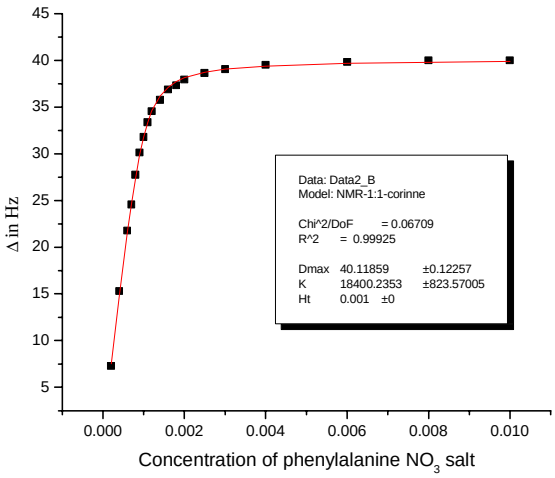


Figure S10. Binding isotherm for the complexation of macrocycle **1** and phenylalanine HNO_3 salt

6. Job's plots

Stock solutions (2 mM) of host and guest were prepared and were measured into NMR tubes with the following host:guest ratios: 10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8;1:9. ^{1}H NMR spectra of all these solution were recorded, and the chemical shifts of H-5 or H-2 were analyzed.

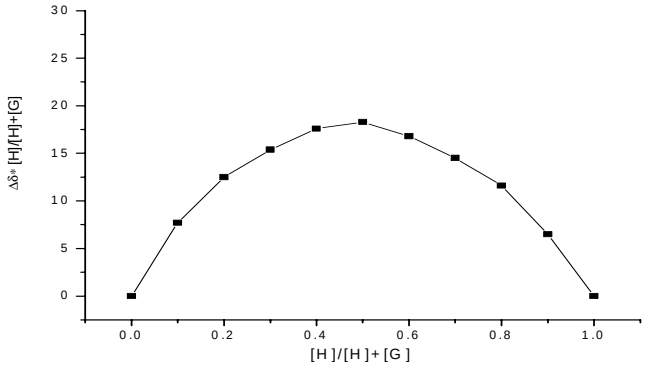


Figure S11. The Job's plot of macrocycle **1** with phenylalanine HBr salt. The chemical shifts of H-5 (see Figure S1) were measured.

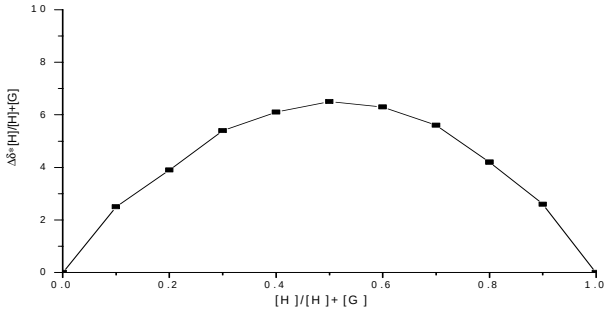


Figure S12. The Job's plot of macrocycle **1** with TBA-Br. The chemical shifts of H-2 (see Figure S1) were measured.

7. Computational calculations of free ligand 1, and its $MeNH_3^+F^-$ complex

Molecular mechanics calculations (MMFF94) were used to study the structure of the free ligand **1**. From a variety of starting points, the common conformer was the one shown in Figure S13. The central pyridine ring is calculated to be anti-parallel to the other two rings, presumably to minimize the dipole moment of the host. The conformation of the peptidic chain of the host resembles that suggested by spectroscopic analysis. Thus, there is no hydrogen bond between the *i*+1 N-H and the *i*-1 carbonyl, both N-H groups are proximal to the glycine methylene, and the *i*+1 N-H (aniline type nitrogen) is proximal to the H-atom *ortho* to it.

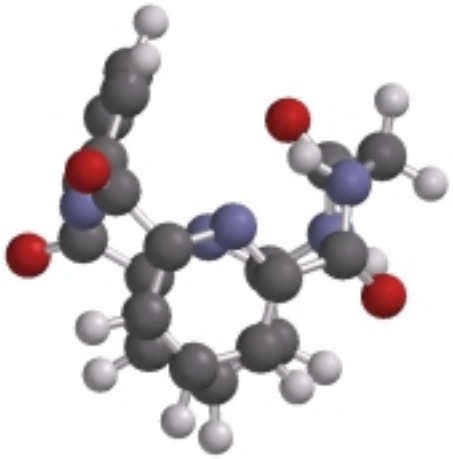
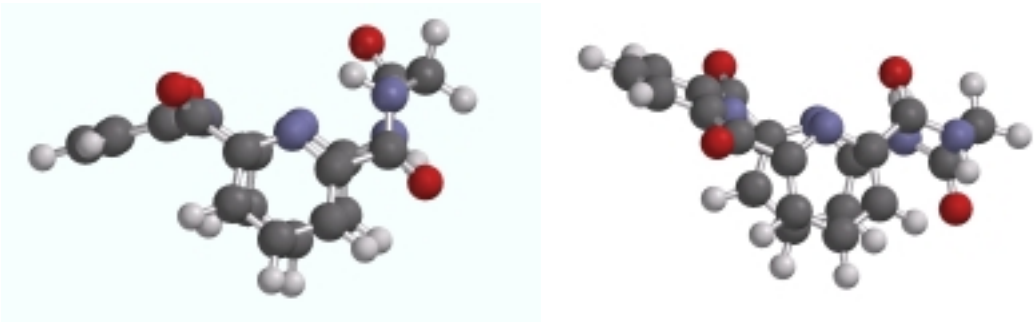


Figure S13. MMFF94 minimized structure of free host **1**.

Constraining the pyridine rings into a conformation suitable for ammonium ion binding resulted in two preferred conformations in which either the *i*, or *i*+1 N-H points towards the ammonium-binding pocket (Figure S14). Calculations suggest that it is the latter that is the lower in energy; at least in part because the pyridine rings can more readily twist with respect to one another. Note that the stability of this conformation does not appear to result from the formation of a γ-turn hydrogen bond. The distance between the *i*–1 carbonyl and *i*+1 N-H is calculated to be 2.669Å. Note also that this calculated

conformation is in agreement with the NMR data showing that it is the *i*+1 amide N-H signal that shifts the most upon salt binding.



a b

Figure S14. The two lowest energy conformations (MMFF94) of the host 1 where the pyridine rings are constrained in a binding conformation. a) *i*, N-H pointing towards the ammonium-binding pocket. b) *i*+1 N-H points towards the ammonium-binding pocket.

We used the lower energy conformation depicted in Figure S14b as a starting point for quantum mechanical calculations of the corresponding methyl ammonium fluoride complex. (To the author's knowledge, semi-empirical calculations do not yet handle well, charged hydrogen bonds such as N···H-N^+ interactions.) Fluoride was chosen as an anion to minimize computational time, and was hydrogen bonded to the *i*+1 amide N-H and positioned so that it may interact with the bound ammonium ion. Hatree-Fock calculations with a 6-31G** basis set gave the structure shown in Figure 2 (text) and Figure S15 (below). The host is calculated to move back towards its minimum conformation in the free state, while the guest is seen to partially decomplex. The distances between the three, pyridine nitrogens and the ammonium hydrogens in the structure are 1.913 Å, 2.962 Å and 3.465 Å. However in the structure, one *i*-1 carbonyl ammonium hydrogen distance is relatively short (2.000Å), as is one fluoride ammonium ion hydrogen distance (2.015 Å). Thus while two of the pyridine/ammonium hydrogen ions are calculated to weaken, the interaction between the two ions, as well as hydrogen bonding to the *i*-1 carbonyl are seem to compensate.

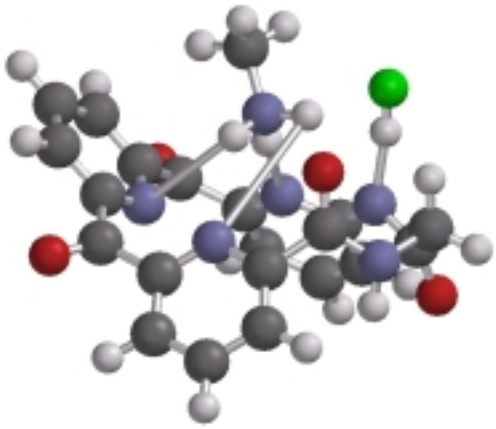


Figure S15: Hatree-Fock calculated structure of the **1**.MeNH4^{+}F^{-} complex (6-31G** basis set). The host is calculated to move back towards its minimum conformation in the free state, while the guest is seen to partially decomplex.

References:

(1) G. R. Newkome, A. Nayak, J. D. Sauer, P. K. Mattschei, S. F. Watkins, F. Fronczek and W. H. Benton, *J. Org. Chem*., 1979, **44**, 3816.

(2) S. Kubik, *J. Am. Chem. Soc.*, **1999**, 121, 5846.