Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Polyfunctionalized macrocycles demonstrate enantioselective and ditopic binding properties

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Contents

1. General

Amine **4** has been previously reported (reference 6 of text). All reagents were purchased from Novabiochem or Aldrich Chemical Company. Dimethylformamide (DMF) was stored over molecular sieves and degassed prior to use. All reactions were carried out 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 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. Synthesis and characterization of compounds 2-9

Boc-phenylalanine 5

To a solution of **4** (0.5 g, 1.33 mmol) in 2 mL DMF was added Boc-phenylalanine-OH (0.35 g, 2.0 mmol), HBTU (0.76 g, 2.0 mmol) and 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 and evaporated at reduced pressure. The product was isolated by flash chromatography (mobile phase: 6% acetone in chloroform). Removal of the solvent under reduced pressure gave 0.56 g (60% yield) of **5** as a white solid. mp 115-117 °C; ^{1}H NMR (acetone-d_6) 1.31 (s, 9H), 1.34 (t, J = 7.2 Hz, 3H), 3.28 (m, 2H), 4.40 (t, J = 6.80 Hz, 2H), 4.67 (m, 1H), 6.29 (d, J = 8.0 Hz, 1H), 7.20 (t, J = 7.5 Hz, 1H), 7.26 (t, J = 7.5 Hz, 2H), 7.34 (d, J = 7.5 Hz, 2H), 7.78 (d, J = 7.5 Hz, 1H), 7.83 (t, J = 7.5 Hz, 1H), 8.09 (t, J = 8.0 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.35 (d, J = 7.5 Hz, 1H), 8.38 (t, J = 6.0 Hz, 1H), 8.42 (d, J = 7.0 Hz, 1H), 9.77 (s, 1H); MS (ES$^+$) *m/z* (rel intens.) = 624.2 (100) $[M + H]^+$; 656.2 (20) $[M + MeOH + H]^+$; Anal. Calcd. for $C_{34}H_{33}N_5O_7 \cdot H_2O$: C, 63.71; H, 5.51; Found: C, 63.90; H, 5.81.

Boc-Serine 6

To a solution of **4** (0.5 g, 1.33 mmol) in 2 mL DMF was added Boc-serine(*t*-Bu)-OH (0.34 g, 2.0 mmol), HBTU (0.76 g, 2.0 mmol) and 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 and evaporated at reduced pressure. The product was isolated by flash chromatography (mobile phase: 6% acetone in chloroform). Removal of the solvent under reduced pressure gave 0.43 g (65% yield) of **6** as a white solid. mp 110-111 °C; ^{1}H NMR (acetone-d_6) 1.13 (s, 9H), 1.35 (t, J = 7.2 Hz, 3H), 1.41 (s, 9H), 3.81 (m, 2H), 4.43 (m, 3H), 6.16 (s, 1H), 7.80 (d, J = 7.0 Hz, 1H), 7.88 (t, J = 7.5 Hz, 1H), 8.11 (t, J = 8.0 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 7.5 Hz, 1H), 8.31 (d, J = 8.0 Hz, 1H), 8.36 (t, J = 8.0 Hz, 2H), 8.44 (t, J = 8.0 Hz, 1H), 9.67 (s, 1H); MS (ES$^+$) *m/z* (rel intens.) = 620.2 (100) $[M + H]^+$; Anal. Calcd. for $C_{32}H_{37}N_5O_8$: C, 62.02; H, 6.02; Found: C, 61.79; H, 6.16.

Phenylalanine free amine 7

To a solution of **5** (0.75 g, 1.2 mmol) in 30 mL 30% 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, and extracted three times with water. 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 120-121 °C; ^{1}H NMR (DMSO-d_6) 1.28 (s, 9H), 3.02 (m, 2H), 4.45 (m, 1H), 7.16 (t, J = 7.6 Hz, 1H), 7.25 (t, J = 7.2 Hz, 2H), 7.36 (d, J = 7.6 Hz, 2H), 7.73 (d, J = 7.6 Hz, 1H), 7.86 (t, J = 8.0 Hz, 1H), 8.10 (t, J = 7.6 Hz, 1H), 8.20 (d, J = 6.8 Hz, 2H), 8.29 (m, 2H), 8.36 (m, 2H), 11.0 (s, 1H); MS (ES$^-$) *m/z* (rel intens.) = 594.3 (100) [M - H]$^-$; Anal. Calcd. for $C_{32}H_{29}N_5O_7 \cdot H_2O$: C, 62.70; H, 5.06; Found: C, 62.97; H, 5.05.

To a suspension of the above product (0.5 g, 0.99 mmol) in 10 mL water was added 2 mL of concentrated HCl solution. The mixture was stirred at rt until homogeneous (ca. 20 min). The solvent was removed under reduced pressure. The crude product was dissolved in methanol, and precipitated with ether. The product **7** was isolated by filtration as a white solid. Yield: 90%. mp 140-141 °C; ^{1}H NMR (DMSO-d_6) 3.17 (m, 2H), 4.31 (m, 1H), 7.30 (m, 5H), 7.81 (d, J = 7.2 Hz, 1H), 7.87 (d, J = 7.6 Hz, 1H), 8.13 (t, J = 7.6 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.23 (m, 2H), 8.34 (m, 3H), 8.39 (s, 2H), 11.29 (s, 1H); MS (ES$^-$) *m/z* (rel intens.) = 494.2 (100) [M - H]$^-$; Anal. Calcd. for $C_{27}H_{21}N_5O_5 \cdot 3HCl \cdot H_2O$: C, 52.13; H, 4.17; Found: C, 52.41; H, 4.00.

Serine free amine 8

To a solution of **6** (0.6 g, 1.0 mmol) in 30 mL 30% aqueous THF was added dropwise a solution of NaOH (40 mg, 1.0 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, and extracted three times with water. 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 the acid derivative as a white solid. Yield: 85%. mp 116-117 °C; ^{1}H NMR (DMSO-d_6) 1.05 (s, 9H), 1.36 (s, 9H), 3.54 (m, 2H), 4.35 (m, 1H), 6.83 (d, J = 8.4 Hz, 1H), 7.82 (t, J = 8.0 Hz, 2H), 7.93 (d, J = 7.6 Hz, 1H), 7.97 (t, J = 8.0 Hz, 2H), 8.25 (m, 3H), 8.30 (t, J = 7.6 Hz, 1H), 10.74 (s, 1H); MS (ES$^-$) *m/z* (rel intens.) = 590.3 (100) [M - H]$^-$; Anal. Calcd. for $C_{30}H_{33}N_5O_8 \cdot 1/2H_2O$: C, 59.99; H, 5.71; Found: C, 60.16; H, 5.68.

To a suspension of the above product (0.5 g, 0.99 mmol) in 50 mL saturated HCl / ethyl acetate was sonicated and stirred at rt for 24 h. The product **8** was isolated by filtration as a white solid. Yield: 85%. mp 135-136 °C; ^{1}H NMR (DMSO-d_6) 1.09 (s, 9H), 4.12 (m, 2H), 5.52 (m, 1H), 7.80 (t, J = 7.6 Hz, 1H), 7.89 (t, J = 7.6 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 8.11 (t, J = 8.0 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.24 (m, 3H), 8.35

(m, 2H), 11.26 (s, 1H), 13.76 (s, 1H); MS (ES^-) *m/z* (rel intens.) = 490.3 (100) $[M - H]^-$; Anal. Calcd. for $C_{25}H_{25}N_5O_6 \cdot 3HCl \cdot H_2O$: C, 49.36; H, 4.56; Found: C, 49.26; H, 4.88.

Macrocycle 2

To a solution of **7** (0.26 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 drop-wise over a period of 5 h. The mixture was stirred at rt for 2 d. Removal of the solvent under reduced pressure afforded a yellowish residue that was partitioned between chloroform and water. The organic layer was dried and evaporated under reduced pressure. Flash chromatography (mobile phase: 10% acetone in chloroform) gave crude product **2** as a white solid, which was purified by washing the solid with 1:1 mixture of hexanes/acetone. Yield: 38%. mp 187-188 °C; ^{1}H NMR (acetonitrile-d_6) 3.60 (m, 2H), 4.92 (m, 1H), 7.20 (m, 5H), 7.42 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.84 (t, J = 8.0 Hz, 1H), 8.20 (m, 3H), 8.28 (d, J = 8.0 Hz, 1H), 8.40 (s, 3H), 8.63 (s, 1H), 8.68 (d, J = 6.8 Hz, 1H), 9.78 (s, 1H); MS (ES^+) *m/z* (rel intens.) = 478.2 (60) $[M + H]^+$; 496.2 (100) $[M + H_2O + H]^+$; 510.2 (30) $[M + MeOH + H]^+$; Anal. Calcd. for $C_{27}H_{19}N_5O_4 \cdot H_2O$: C, 64.41; H, 4.37; Found: C, 64.45; H, 4.06.

Macrocycle 9

To a solution of **8** (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 drop-wise over a period of 5 h. The mixture was stirred at rt for 2 d. Removal of the solvent under reduced pressure afforded a yellowish residue that was partitioned between chloroform and water. The organic layer was dried and evaporated under reduced pressure. Flash chromatography (mobile phase: 6% acetone in chloroform) gave crude product **9** as a white solid, which was purified by washing the solid with 1:1 mixture of hexanes / acetone. Yield: 35%. mp 180-182 °C; ^{1}H NMR (acetone-d_6) 1.12 (s, 9H), 4.00 (m, 2H), 4.66 (m, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 8.15 (m, 2H), 8.18 (t, J = 7.6 Hz, 1H), 8.35 (m, 2H), 8.50 (d, J = 8.0 Hz, 1H), 9.02 (s, 1H); MS (ES^+) *m/z* (rel intens.) = 474.3 (100) $[M + H]^+$; 492.3 (80) $[M + H_2O + H]^+$; Anal. Calcd. for $C_{25}H_{23}N_5O_5 \cdot H_2O$: C, 62.24; H, 5.01; Found: C, 62.63; H, 4.87.

Macrocycle 3

A solution of **9** (50 mg) in 4 mL formic acid was stirred at rt for 12 h. After this time, the solvent was removed at reduced pressure. The residue was dissolved in 5 mL acetone, basified with 20 mL 2% K_2CO_3 solution. The mixture was extracted with chloroform 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 white solid that was crystallized in acetone/hexanes to give 65% of **3** as white solid. mp > 250 °C; ^{1}H NMR (acetone-d_6) 4.12 (m, 2H), 4.37 (s, 1H), 4.73 (m, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.86 (t, J = 8.4 Hz, 1H), 8.10 (d, J = 7.6 Hz, 1H), 8.14 (d, J = 7.6 Hz, 1H), 8.20 (m, 2H), 8.37 (t, J = 6.4 Hz, 1H), 8.40 (d, J = 6.8 Hz, 1H), 8.63 (d, J

= 6.8 Hz, 1H), 9.10 (s, 1H); MS (ES^+) *m/z* (rel intens.) = 436.2 (100) $[M + H_2O + H]^+$; Anal. Calcd. for $C_{21}H_{15}N_5O_5$: C, 60.43; H, 3.62; Found: C, 60.59; H, 3.61.

3. ^{1}H NMR NOESY spectra of the macrocycles

The NOESY spectra of macrocycle **2** and **3** are shown in Figure S2 and S4 respectively. For a comparison, the corresponding 1D NMR of macrocycle **2** and **3** are shown in Figure S3 and S5 respectively.

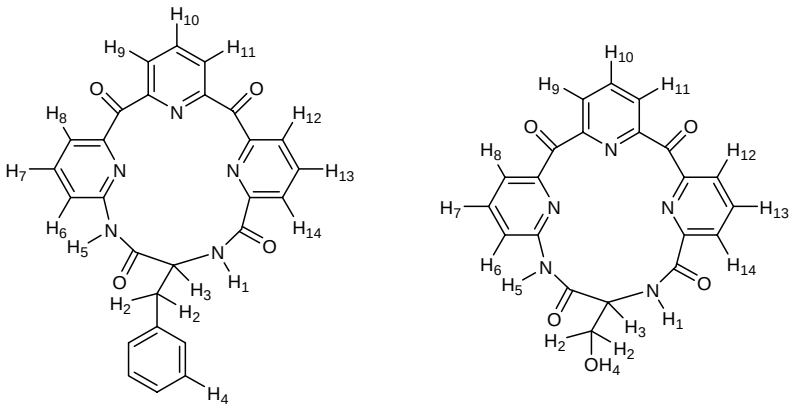

Figure S1. The structures of macrocycle **2** and **3**.

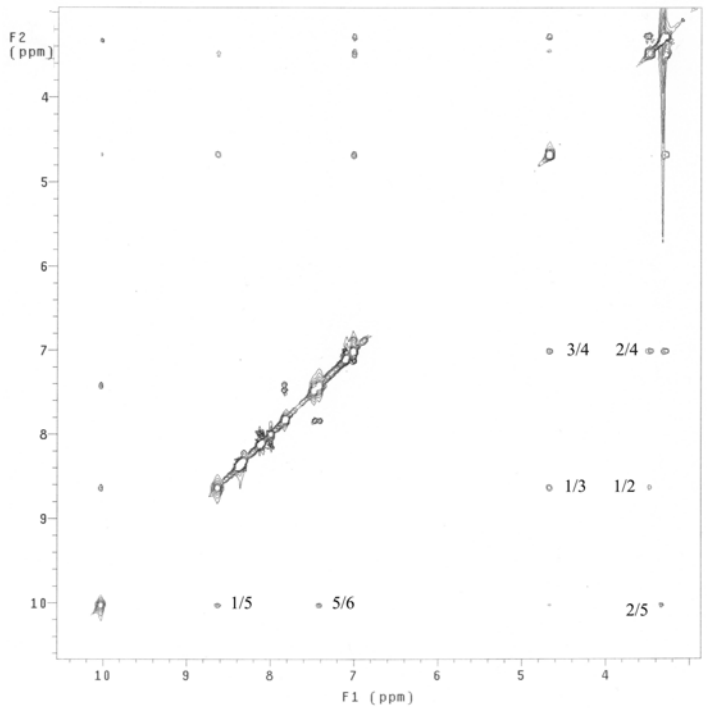

Figure S2 ^{1}H NMR NOESY spectrum of macrocycle **2** in DMSO-d_3.

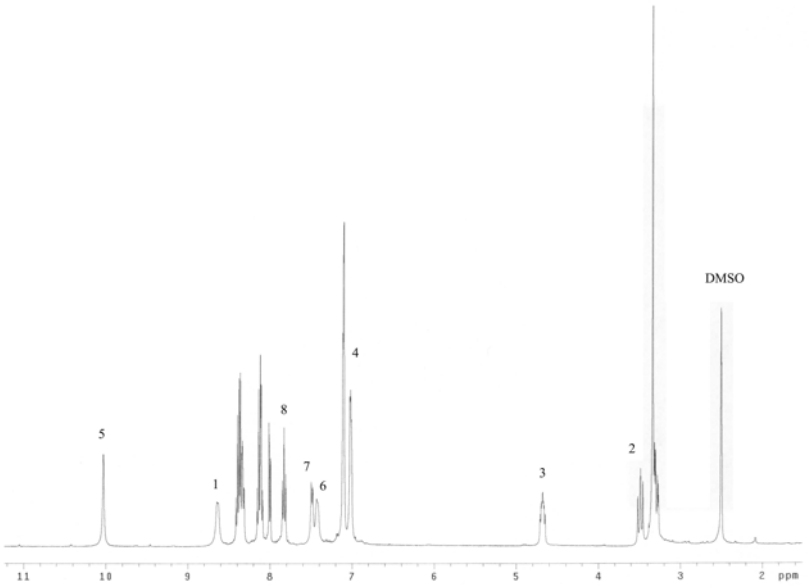

Figure S3 ^{1}H NMR of macrocycle **2** in DMSO-d_3.

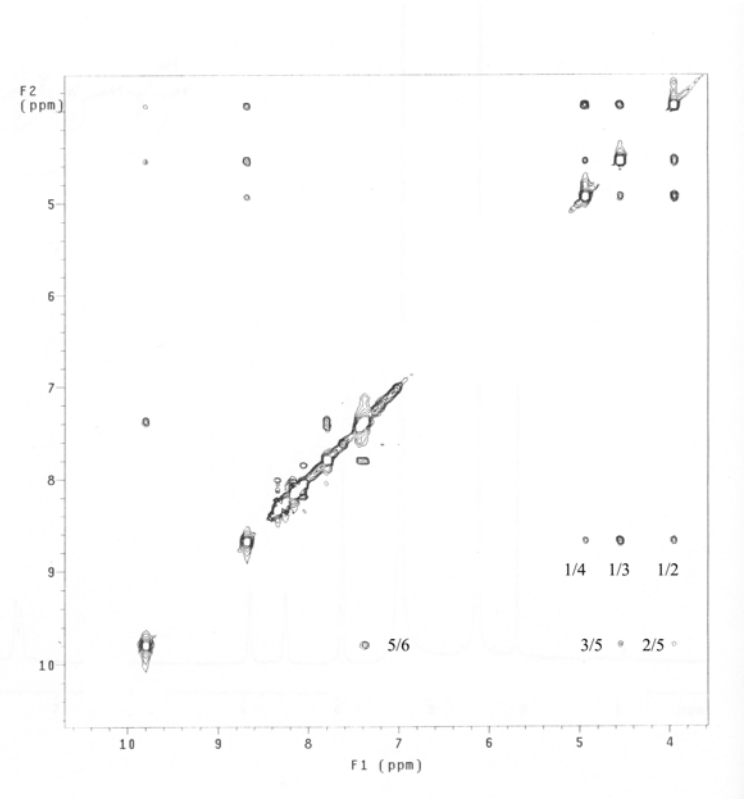

Figure S4 ^{1}H NMR NOESY spectrum of macrocycle **3** in DMSO-d_3.

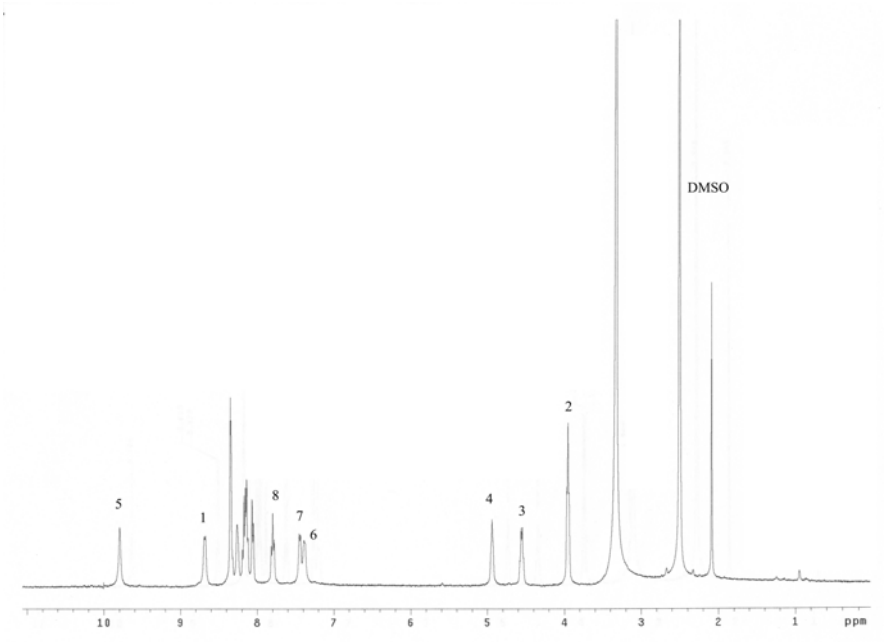

Figure S5 ^{1}H NMR of macrocycle **3** in DMSO-d_3.

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

All quoted association constants were the average of three titrations with the error within 10%. All titrations were carried out in a solvent system of 40% $CD_3CN/CDCl_3$. For each experiment, a 5 mM stock solution of macrocycle was prepared. A 0.1 mL of the stock solution was then measured into an NMR tube and the solution diluted to 1 mM. The spectrum was then recorded (500 MHz NMR, 298 K). Small aliquots of guest solution prepared in 20 mM were measured into the tube and the spectra recorded after each addition. The chemical shift of the proton H_7 (Figure S1) was used to generate the binding isotherm. Binding constant was calculated using an iterative curve-fitting method according to the equation previously described (reference 6 or text). Representative binding isotherms are shown in Figure S6-9.

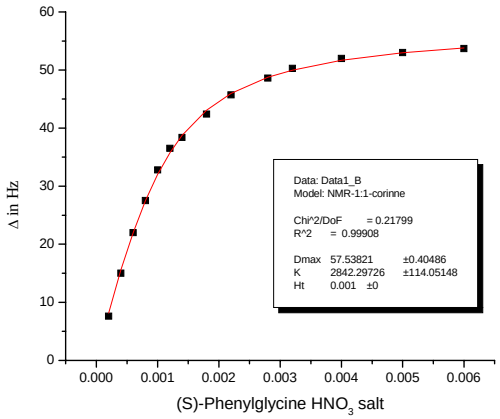

Figure S6. Binding isotherm for the complexation of macrocycle **2** and S-phenylglycine HNO_3 salt.

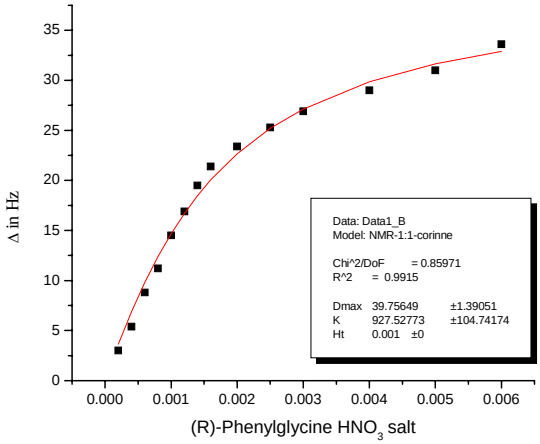

Figure S7. Binding isotherm for the complexation of macrocycle **2** and R-phenylglycine HNO_3 salt.

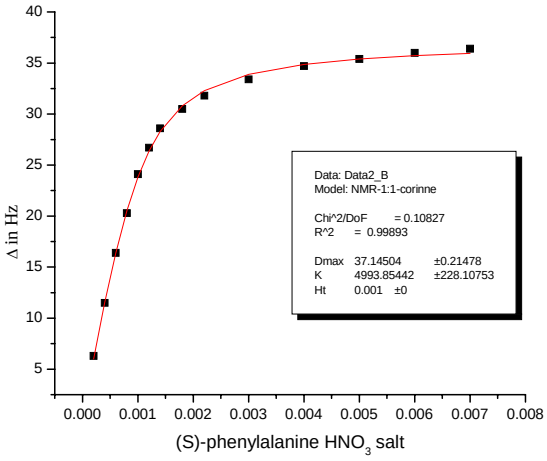

Figure S8. Binding isotherm for the complexation of macrocycle **2** and S-phenylalanine HNO_3 salt.

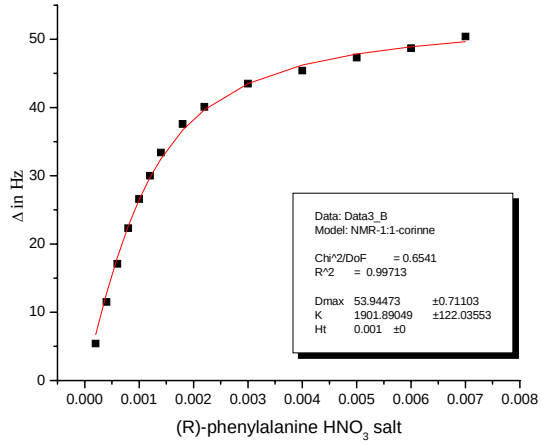

Figure S9. Binding isotherm for the complexation of macrocycle **2** and R-phenylalanine HNO_3 salt.

6. Job's plot

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_7 were analyzed.

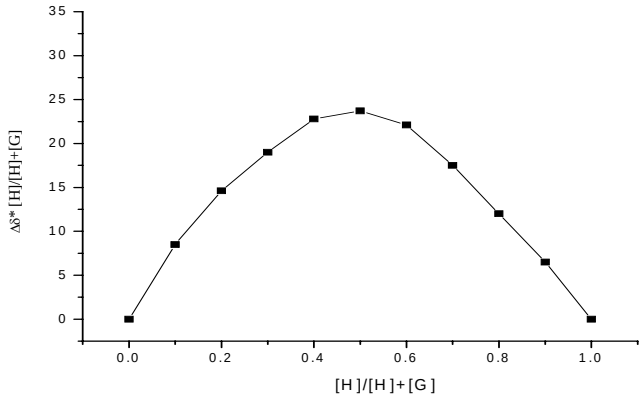

Figure S10. The job's plot of macrocycle **2** with S-phenylalanine HNO_3 salt.