Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information

Receptors for sulfate that function across a wide pH range in mixed aqueous-DMSO media

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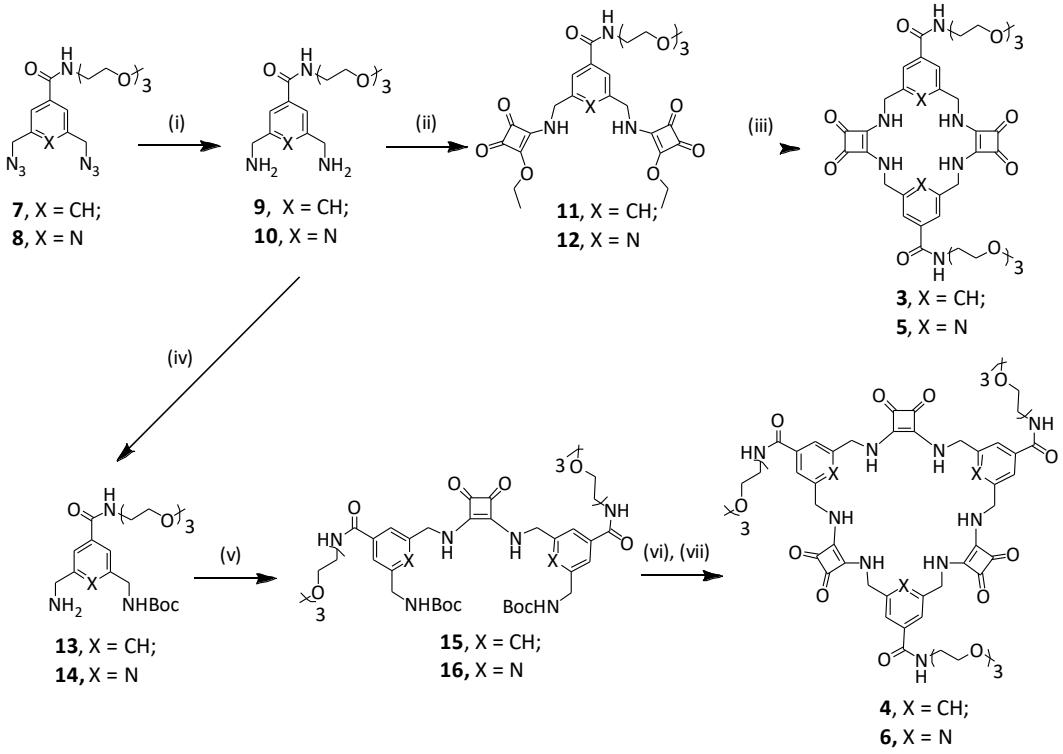
Contents

General experimental

^{1}H NMR spectra were recorded using a Bruker Avance III 600 spectrometer at a frequency of 600 MHz, Bruker Avance III 500 spectrometer at a frequency of 500 MHz or Bruker Avance III 400 spectrometer at a frequency of 400 MHz, and are reported as parts per million (ppm) with DMSO-*d*$_6$ (δ_H 2.50 ppm), C*D*Cl$_3$ (δ_H 7.26 ppm) or C*D*$_3$CN (δ_H 1.94 ppm) as an internal reference. The data are reported as chemical shift (δ), multiplicity (br = broad, s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, m = multiplet, br s = broad singlet), coupling constant (*J* Hz) and relative integral. ^{13}C NMR spectra were recorded using a Bruker Avance III 600 spectrometer at a frequency of 150 MHz, Bruker Avance III 500 spectrometer at a frequency of 125 MHz or Bruker Avance III 400 spectrometer at a frequency of 100.6 MHz and are reported as parts per million (ppm) with DMSO-*d*$_6$ (δ_C 39.52 ppm) or C*D*Cl$_3$ (δ_c 77.16 ppm) or C*D*$_3$CN (δ_c 1.32 and 118.26 ppm) as an internal reference. High-resolution ESI spectra were recorded on a Bruker BioApex Qe 7T Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR) with an Apollo Dual source, via syringe infusion. Analytical TLC was performed using precoated silica gel plates (Merck Kieselgel 60 F254). Tetrabutylammonium salts were used as supplied and were stored in a vacuum desiccator over silica drying beads and phosphorous pentoxide. Unless otherwise stated, all other reagents were commercially available and used as supplied.

Methyl 3,5-bis(azidomethyl)benzoate[1] and methyl 2,6-bis(bromomethyl)isonicotinate[2,3] were prepared according to literature methods.

Synthesis and characterization of novel compounds

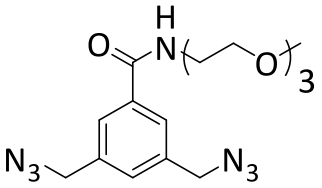


Scheme S1: Synthesis of the desired triethylene glycol monomethyl ether (TEG) appended MSQs **1** – **4**.

Conditions: (i) Ph_3P, H_2O, THF, RT, (**9**, 89%; **10**, --[a]); (ii) diethyl squarate, EtOH, RT, 16 hrs, (**11**, 68%; **12**, 76%); (iii) **7** or **8**, EtOH, RT, 16 hrs (**3**, 63%; **5**, 72%); (iv) Boc_2O, CH_2Cl_2, RT, 16 hrs, (**13**, 65%; **14**, --[a]); (v) diethyl squarate, EtOH, RT, 16 hrs, (**15**, 60%; **16**, 60%); (vi) TFA/CH_2Cl_2, RT, 2 hrs; (vii) **11** or **12**, EtOH, RT, 48 hrs (**4**, 42%; **6**, 50%).

[a] Due to the unstable nature of the amine **10**, compound was stored and characterised as the di(Boc) protected **17** or used immediately after synthesis.

3,5-Bis(azidomethyl)-*N*-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzamide (**7**)



Methyl 3,5-bis(azidomethyl)benzoate (150 mg, 0.609 mmol) was dissolved in THF (9 mL) then a solution of sodium hydroxide (24 mg, 0.609 mmol) in water (1 mL) was added and the reaction mixture was stirred overnight in the absence of light. The solvent was evaporated using a positive flow of $N_{2(g)}$, and the residue was re-dissolved in 2 mL of CH_2Cl_2. [HBTU]PF_6 (276 mg, 0.911 mmol, 1.2 eq.) and *i*Pr_2EtN (157 mg, 1.21 mmol, 2.0 eq.) were added to the solution and the mixture was stirred for 5 minutes. 2-(2-(2-Methoxyethoxy)ethoxy)ethan-1-amine (128 mg, 0.784 mmol, 1.3 eq.) was dissolved in 4 mL of CH_2Cl_2 and added dropwise (1 drop per second), then the reaction mixture was stirred for 24 h at room temperature. The solvent was removed *in vacuo,* and the mixture was purified using flash silica gel chromatography (3:1-9:1 EtOAc/hexane) to isolate the product **7** as a colorless oil (266 mg). Tetramethyl-urea by-product (δ_H 2.81 ppm) was present and could not be separated from the product; however, based on ^{1}H NMR evidence the yield is approximately quantitative (229 mg, >95%). An infrared spectrum was not collected for this reason. R_f = 0.48 in 3:1 EtOAc/hexane. **^{1}H NMR** (C*D*Cl_3, 400 MHz) δ_H 3.33 (s, 3 H), 3.55-3.53 (m, 2 H), 3.68 - 3.64 (m, 10 H), 4.44 (s, 4 H), 6.93 (m, 1 H), 7.42 (s, 1 H), 7.73 (s, 2 H); **^{13}C NMR** (C*D*Cl_3, 100 MHz, 298 K) δ_C 40.1, 54.3, 59.1, 70.2, 70.3, 70.5, 70.6, 71.9, 126.7, 130.5, 136.0, 136.9, 166.9; **HRMS** (ESI, MeOH) *m/z*: 400.1704 $[M+Na]^+$ calcd. for $C_{16}H_{23}N_7NaO_4^+$ 400.1704, 777.3515 $[2M+Na]^+$ calcd. for $C_{32}H_{46}N_{14}NaO_8^+$ 777.3521.

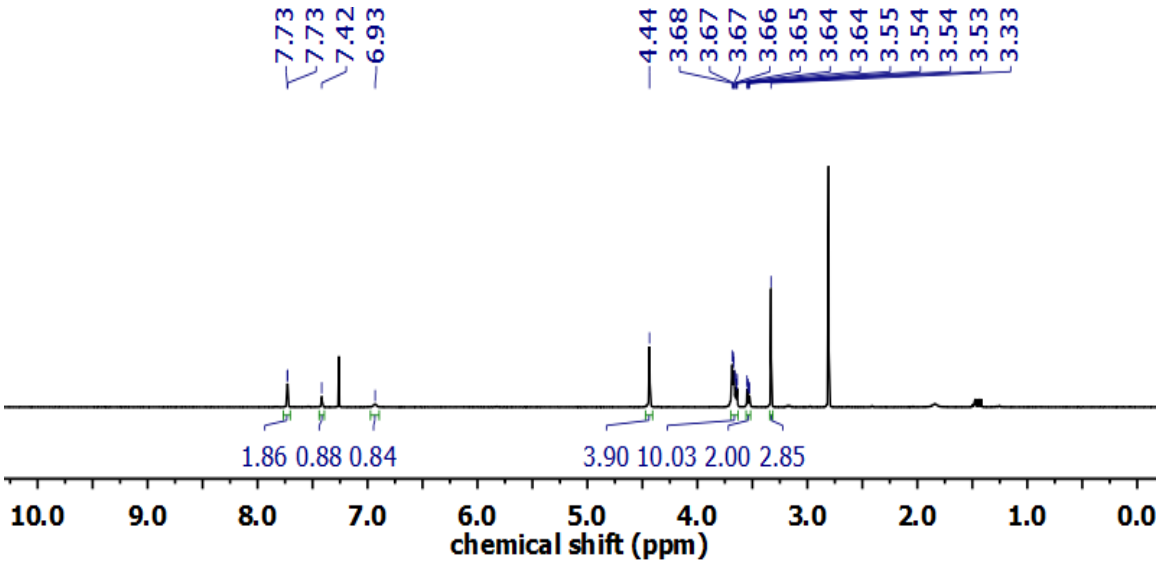


Figure S1. ^{1}H NMR of compound **7**.

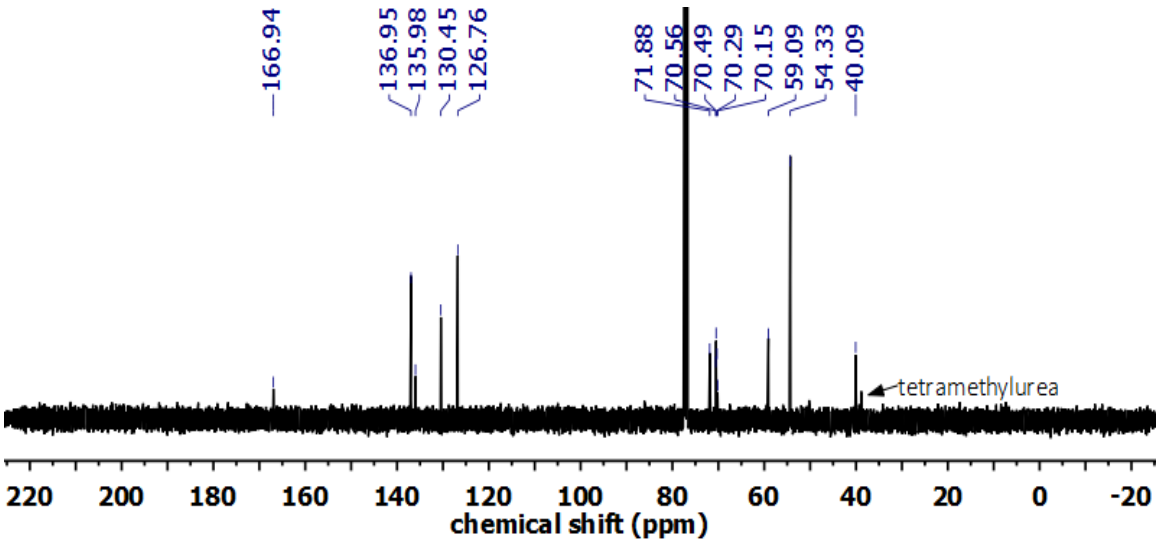
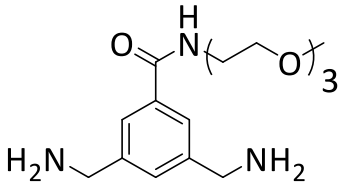


Figure S2. ^{13}C NMR of compound **7**.

3,5-Bis(aminomethyl)-*N*-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzamide (**9**)



Diazide **7** (115 mg, 0.305 mmol) was dissolved in THF (10 mL). Triphenylphosphine (493 mg, 1.88 mmol) was added and the reaction mixture was stirred in an unsealed flask for 2 h. Distilled water (2 mL) was added and the reaction was stirred in the dark for 18 h. The solvent was evaporated to dryness and the residue was purified using silica gel chromatography (4:1 CH_2Cl_2/CH_3OH – 4:1:0.01 CH_2Cl_2/CH_3OH/conc. $NH_4OH_{(aq)}$) and after removal of solvent from the appropriate fractions *in vacuo*, the product **9** was isolated as a colorless oil (88 mg, 89% yield). R_f = 0.2. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 2.22 (br. s, 4 H), 3.31 (s, 3 H), 3.52 - 3.50 (m, 2 H), 3.65 - 3.61 (m, 10 H), 3.89 (s, 4 H), 7.12 (m, 1 H), 7.39 (s, 2 H), 7.64 (s, 1 H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 39.9, 46.1, 59.0, 70.1, 70.3, 70.5, 70.6, 72.0, 124.7, 129.2, 135.3, 143.4, 167.7; **HRMS** (ESI, MeOH) *m/z*: 326.2070 $[M+H]^+$ calcd. for $C_{16}H_{28}N_3O_4^+$ 326.2074, 348.1890 $[M+Na]^+$ calcd. for $C_{16}H_{27}N_3NaO_4^+$ 348.1894, 364.1630 $[M+K]^+$ calcd. for $C_{16}H_{27}KN_3O_4^+$ 364.1633. **ν_{max} (film)/cm^{-1}:** 3351 (broad), 3294 (broad), 3068, 2871, 1640, 1598, 1542, 1452, 1350, 1308, 1247, 1199.

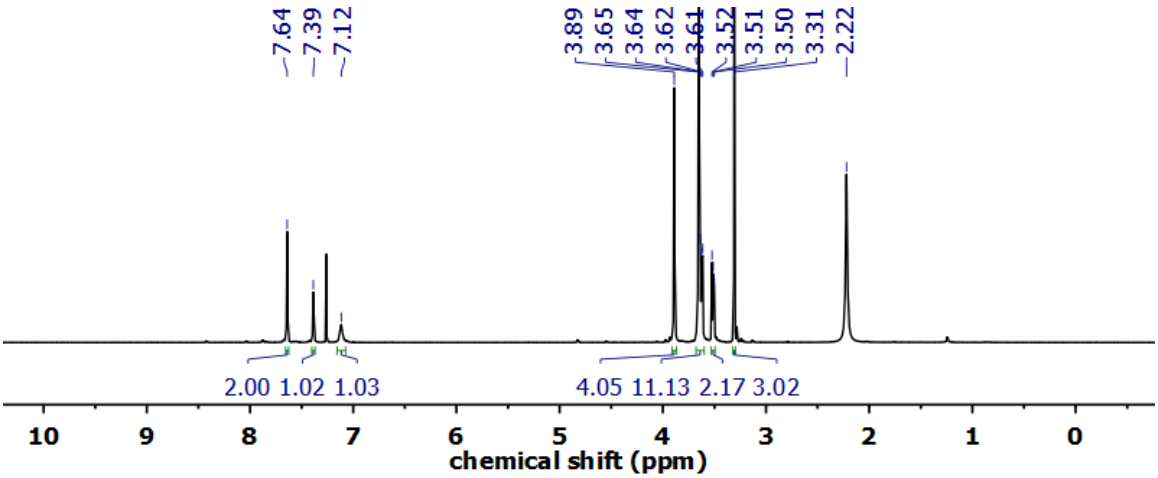


Figure S3. [1]H NMR of compound **9**.

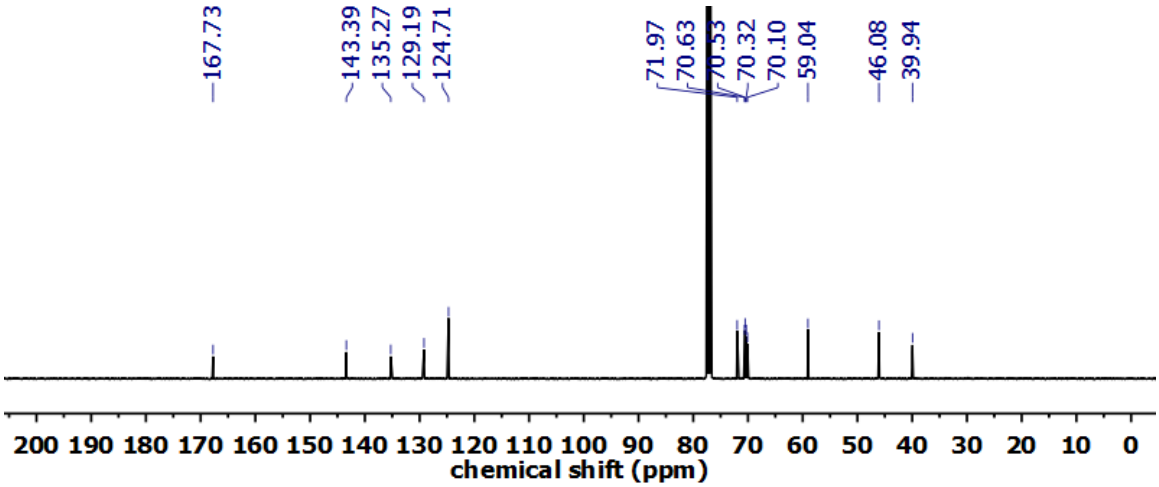
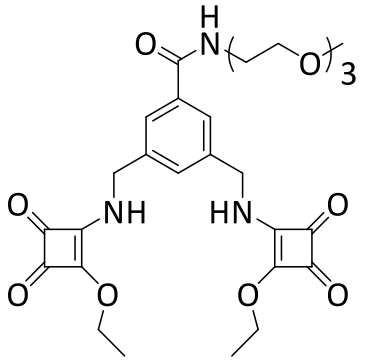


Figure S4. [13]C NMR of compound **9**.

3,5-Bis(((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)methyl)-*N*-(2-(2-(2-methoxyethoxy) ethoxy)ethyl)benzamide (**11**)



The diamine **9** (71 mg, 0.218 mmol), was dissolved in ethanol (5 mL) and a solution of diethyl squarate (82 mg, 0.480 mmol) in ethanol (5 mL) was added dropwise (1 drop per second) then the reaction mixture was stirred at room temperature for 19 hours. The solvent was removed under reduced pressure and the mixture was purified using silica gel column chromatography (9:1 CH_2Cl_2/CH_3OH) to isolate the product **11** as a colorless oil (85 mg, 68% yield). R_f = 0.6. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 1.49 - 1.36 (m, 6 H); 3.26 (s, 3 H), 3.52 - 3.47 (m, 2 H), 3.70 - 3.57 (m, 10 H), 4.90 - 4.54 (m, 8 H), 7.53 - 7.27 (m, 2 H), 7.70 (s, 2 H), 7.90 - 7.74 (m, 2 H); **^{13}C NMR** ($CDCl_3$, 100 MHz, 298 K) δ_C 16.0, 40.1, 48.2, 58.9, 69.8, 70.2, 70.3, 70.5, 70.7, 71.9, 126.3, 129.6, 130.3, 135.7, 138.5, 167.0, 172.7, 177.8, 183.4, 189.4; **HRMS** (ESI, MeOH) *m/z*: 596.2225 $[M+Na]^+$ calcd. for $C_{28}H_{35}N_3NaO_{10}^+$ 596.2215, 1169.4570 $[M+Na]^+$ calcd. for $C_{56}H_{70}N_6NaO_{20}^+$ 1169.4537. **ν_{max} (film)/cm^{-1}:** 3489 (broad), 3230 (broad), 2980, 2932, 2874, 1803, 1703, 1588, 1530, 1493, cm^{-1}.

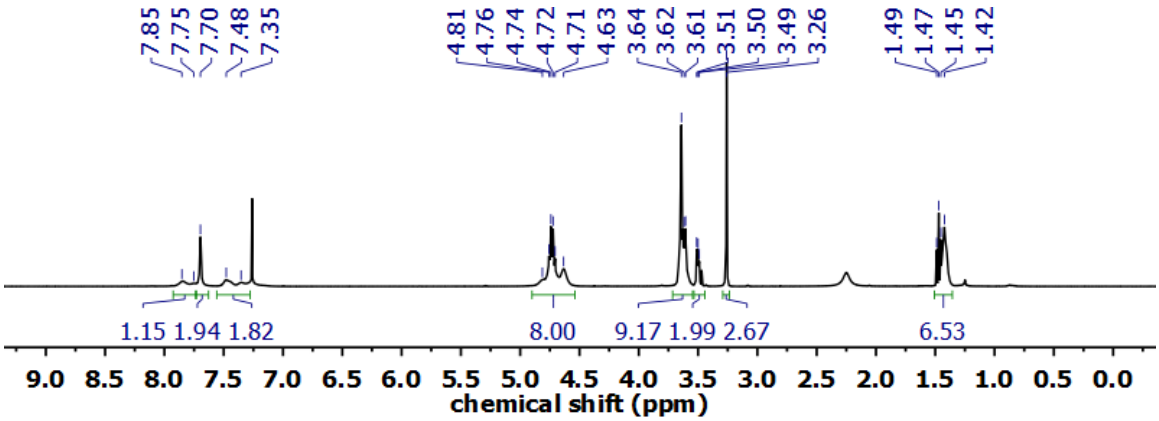


Figure S5. ^{1}H NMR of compound **11**.

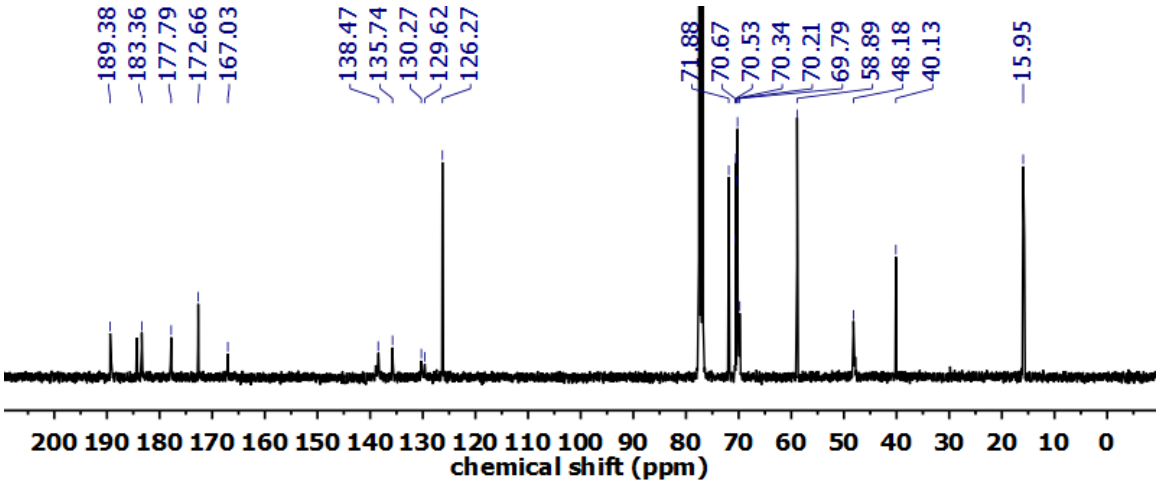
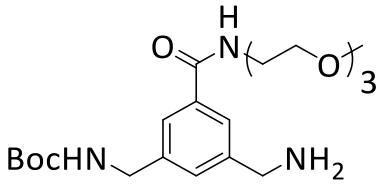


Figure S6. ^{13}C NMR of compound **11**.

Tert-butyl (3-(aminomethyl)-5-((2-(2-(2-methoxyethoxy)ethoxy)ethyl)carbamoyl)benzyl)carbamate (**13**)



Diamine **9** (129 mg, 0.396 mmol, 1 eq.) was dissolved in 10 mL methanol, and the solution was cooled to 0 °C. 1 eq. of aqueous HCl (389 μL, 1.02 mol L^{-1}) was added to 3 mL distilled water and the dilute solution was added dropwise to the diamine solution and stirred at 0 °C for half an hour. Boc_2O (87 mg, 0.396 mmol, 1 eq.) was dissolved in 10 mL methanol and added dropwise (1 drop per second) to the protonated amine solution at 0 °C. The reaction was stirred overnight, warming to room temperature. Et_3N (40 mg, 0.396 mmol, 1 eq.) was then added and the mixture was stirred for 2 hours. The solvent was removed and the residue purified using silica gel column chromatography (9:1 CH_2Cl_2/CH_3OH – 9:1:0.02 CH_2Cl_2/CH_3OH/conc. $NH_4OH_{(aq)}$ – 4:1:0.1 CH_2Cl_2/CH_3OH/conc. $NH_4OH_{(aq)}$) and after combining the appropriate fractions, the product **13** was isolated as a colorless oil (110 mg, 65% yield). R_f = 0.1 in 9:1 CH_2Cl_2/CH_3OH. **^{1}H NMR** (CD_3CN, 400 MHz) δ_H 1.40 (s, 9 H), 3.23 (s, 3 H), 3.25 (br. s, 2 H), 3.43 - 3.41 (m, 2 H), 3.47 (m, 2 H), 3.62 - 3.51 (m, 8 H), 3.89 (s, 2 H), 4.19 (s, 2 H), 6.22 (s, 1 H), 7.36 (s, 1 H), 7.56 (s, 1 H), 7.73 (s, 2 H); **^{13}C NMR** (CD_3CN, 100 MHz, 298 K) δ_C 28.7, 40.5, 44.5, 53.6, 58.9, 70.2, 70.9, 71.0, 72.5, 79.7, 126.1, 126.4, 130.6, 135.8, 141.7, 157.2, 168.2; **LRMS** (ESI, MeOH) *m/z*: 448.3 $[M+Na]^+$ calcd. for $C_{21}H_{35}N_3NaO_6^+$ 448.2, 464.3 $[M+K]^+$ calcd. for $C_{21}H_{35}KN_3O_6^+$ 464.2, 873.4 $[2M+Na]^+$ calcd. for $C_{42}H_{70}N_6NaO_{12}^+$ 873.5. **ν_{max} (film)/cm^{-1}:** 3308 (broad), 2924, 1708, 1645, 1538.

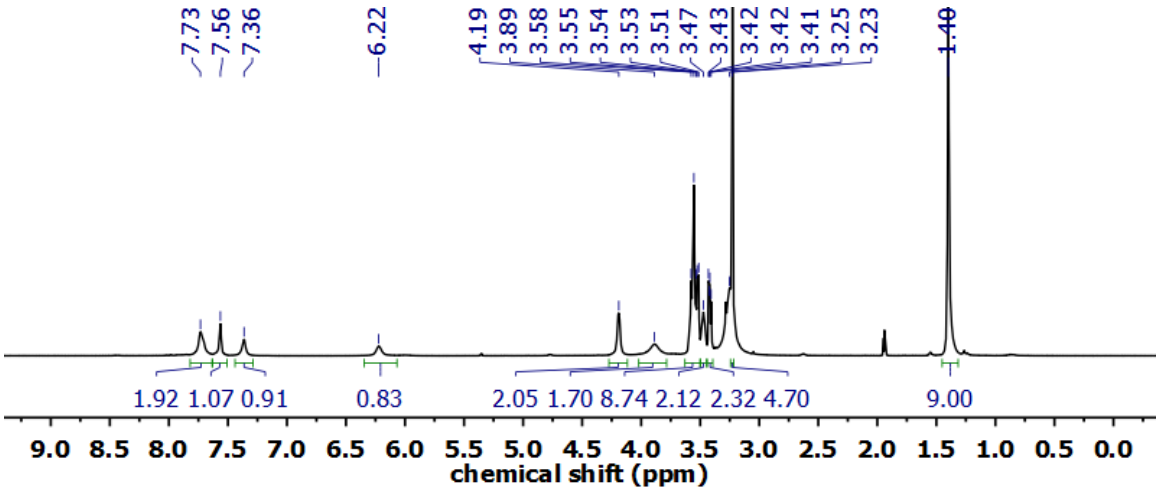


Figure S7. ^{1}H NMR of compound **13**.

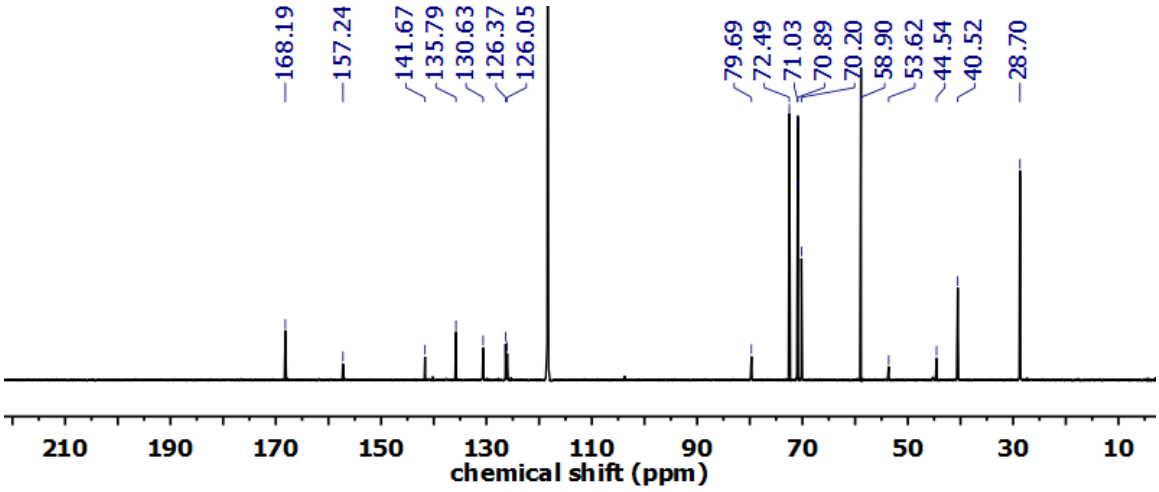
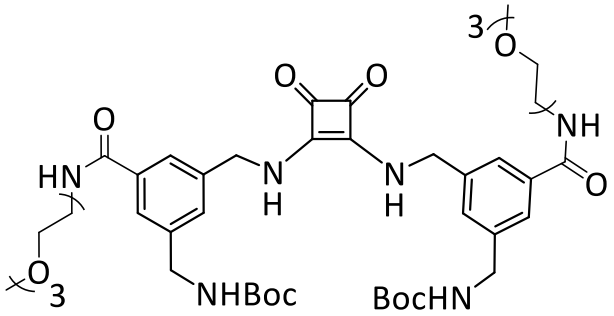


Figure S8. ^{13}C NMR of compound **13**.

Di-*tert*-butyl (((((3,4-dioxocyclobut-1-ene-1,2-diyl)bis(azanediyl))bis(methylene))bis(5-((2-(2-(2-methoxyethoxy)ethoxy)ethyl)carbamoyl)-3,1-phenylene))bis(methylene))dicarbamate (**15**)



MonoBoc-protected amine **13** (165 mg, 0.398 mmol) was dissolved in ethanol (5 mL) and a solution of diethyl squarate (23 mg, 0.136 mmol) in ethanol (5 mL) was added then the mixture was stirred for 2 days at room temperature. The solvent was removed under reduced pressure and the residue was purified using silica gel column chromatography (9:1 CH_2Cl_2/CH_3OH) and after isolating the appropriate fractions and solvent removal, the product **15** was isolated as a colorless oil (75 mg, 60% yield). R_f = 0.32. **^{1}H NMR (DMSO-*d*$_6$, 400 MHz)** δ_H 1.38 (s, 18 H), 3.21 (s, 3 H), 3.43 - 3.37 (m, 4 H), 3.56 - 3.47 (m, 16 H), 4.15 (d, *J* = 6 Hz, 4 H), 4.77 (s, 4 H), 7.33 (s, 2 H), 7.41 (t, *J* = 6 Hz, 2 H), 7.65 (s, 2 H), 7.67 (s, 2 H), 8.50 (t, J = 6 Hz, 2 H); **^{13}C NMR (DMSO-*d*$_6$, 100 MHz)** δ_C 28.2, 43.3, 46.7, 58.0, 68.9, 69.6, 69.7, 71.3, 77.9, 124.8, 125.1, 128.6, 134.9, 138.9, 140.8, 155.8, 166.1, 182.6; **HRMS** (ESI, MeOH) *m/z*: 951.4682 $[M+Na]^+$ calcd. for $C_{46}H_{68}N_6NaO_{14}^+$ 951.4686, 751.3641 $[M-2Boc+Na]^+$ calcd. for $C_{36}H_{52}N_6NaO_{10}^+$ 751.3637, 487.2291 $[M+2Na]^{2+}$ calcd. for $C_{46}H_{68}N_6Na_2O_{14}^{2+}$ 487.2289. **ν_{max} (film)/cm^{-1}:** 3229 (broad), 2980, 2931, 2873, 1802, 1702.

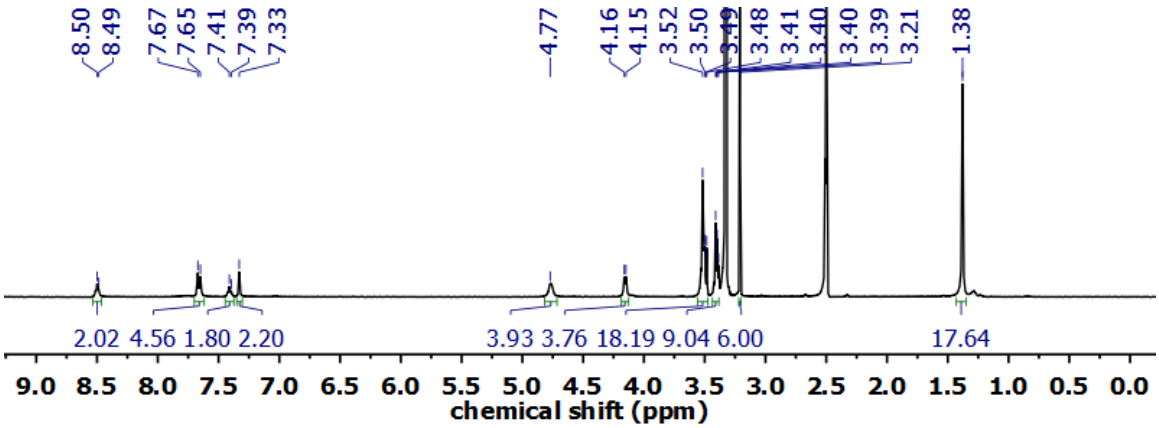


Figure S9. ^{1}H NMR of compound **15**.

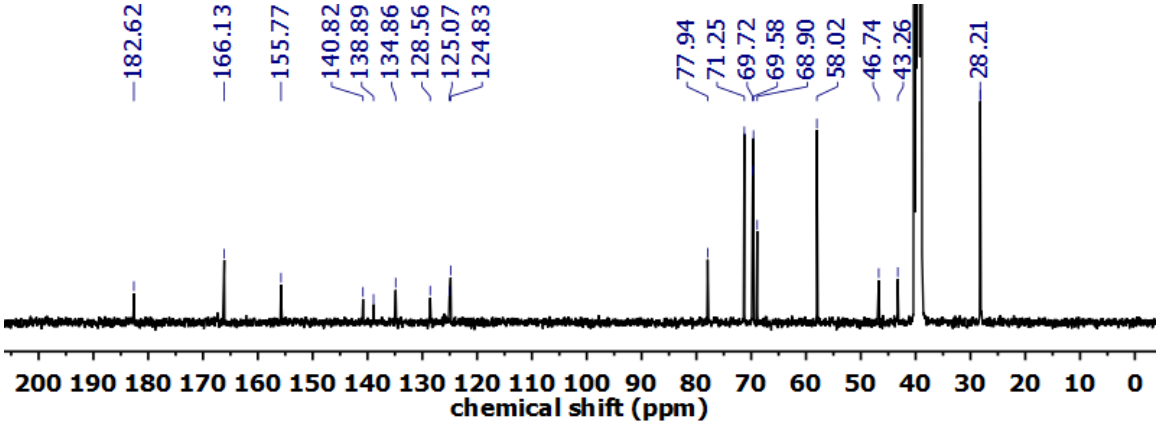
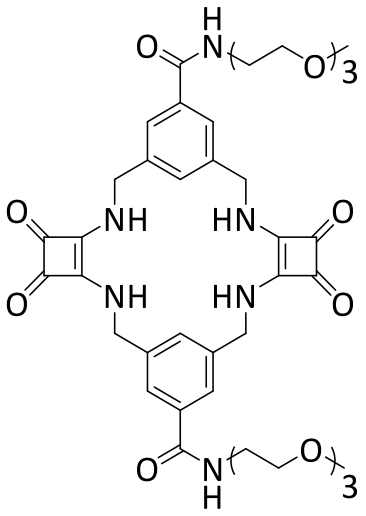


Figure S10. ^{13}C NMR of compound **15**.

[2]MSQ-**3**



Diamine **9** (48.2 mg, 0.15 mmol) was dissolved in EtOH (10 mL) then added to a solution of **11** (85.0 mg, 0.15 mmol) and Et_3N (0.3 mL) in EtOH (10 mL) and the mixture was stirred at room temperature for 16 hours. The resulting precipitate was collected by filtration and washed with cold 1:1 (v/v) EtOH/H_2O (3 × 3 mL) to yield compound [2]MSQ-**3** (76 mg, 63%) as a colorless solid. **M.p.** 216 – 224 °C (decomp.); **^{1}H NMR** (400 MHz, DMSO-d_6): 3.21 (s, 6 H), 3.38 – 3.60 (m, 24 H), 4.42 – 5.17 (m, 8 H), 7.18 – 7.53 (m, 2 H), 7.54 – 7.79 (m, 4 H), 7.97 (br s, 2 H), 8.56 (br s, 2 H); **^{13}C NMR** (100 MHz, DMSO-d_6): 46.6, 58.6, 69.3, 70.0, 70.1, 70.2, 71.7, 125.1, 135.0, 140.9, 166.3, 167.9, 182.9, 3 signals obscured or overlapped; **HRMS** (ESI, MeOH) calcd. for $C_{40}H_{50}N_6O_{12}Na$ $[M + Na]^+$ 829.3379, found 829.3379; **ν_{max} (film)/cm^{-1}:** 3276 (broad), 2921, 1670, 1628, 1597.

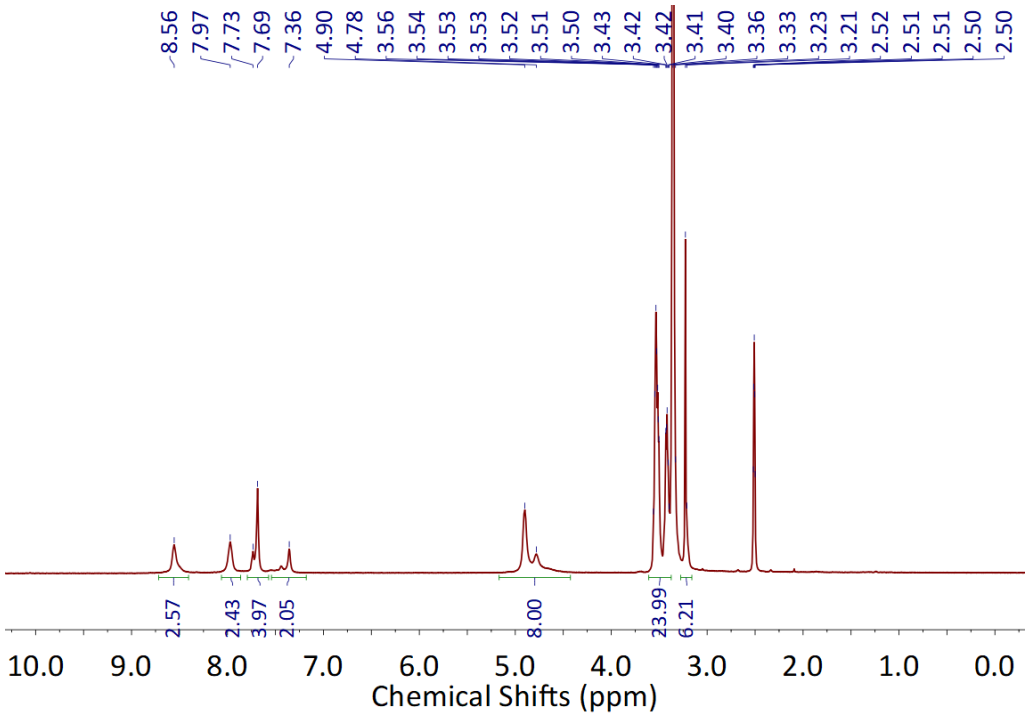


Figure S11. ^{1}H NMR of compound [2]MSQ-**3**.

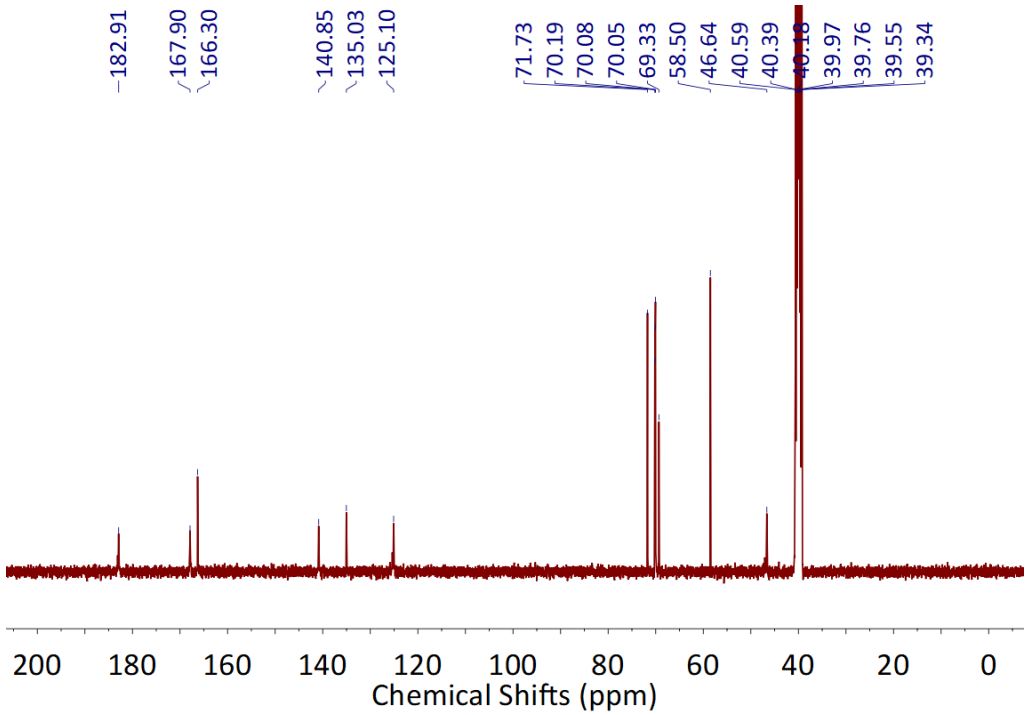
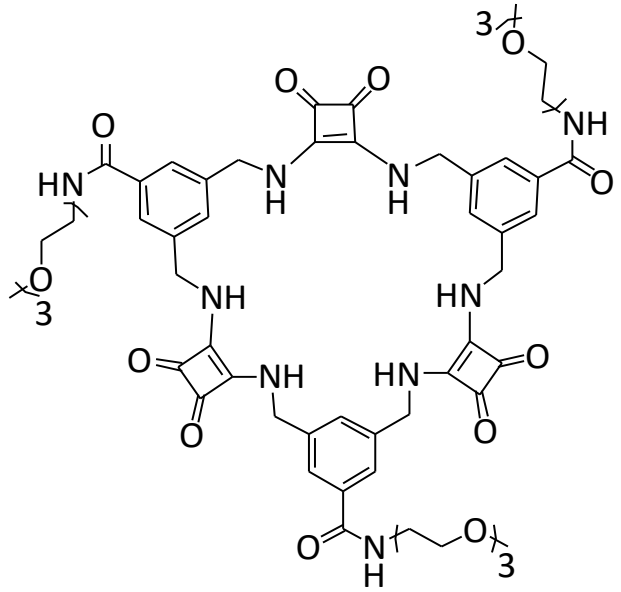


Figure S12. ^{13}C NMR of compound [2]MSQ-**3**.

[3]MSQ-**4**



Compound **15** (102.0 mg, 0.11 mmol) was dissolved in a solution of TFA/CH_2Cl_2 (1:1 v/v, 5 mL) before the reaction mixture was stirred at room temperature for 2 hours and then concentrated under reduced pressure. The resulting oil was then added to a solution of **11** (63.1 mg, 0.11 mmol) and Et_3N (0.5 mL) in EtOH (200 mL) and stirred at room temperature for 48 hrs. The solvent was removed under reduced pressure to give a yellow oil. Subjection of this material to flash silica gel chromatography (5/95 v/v methanol/dichloromethane elution) and concentration of the appropriate fractions (R_f 0.3) gave the compound [3]MSQ-**4** (56 mg, 42%) as a beige solid. **M.p.** 232 – 234 °C (decomp.); **^{1}H NMR** (600 MHz, DMSO-d_6): 3.22 (s, 9 H), 3.38 – 3.44 (m, 12 H), 3.45 – 3.68 (m, 24 H), 4.76 (s, 12 H), 7.44 (s, 3 H), 7.74 (br s, 12 H), 8.56 (t, J = 5.5 Hz, 3 H); **^{13}C NMR** (150 MHz, DMSO-d_6): 47.1, 58.5, 69.3, 70.0, 70.1, 70.2, 71.7, 126.3, 129.7, 135.7, 140.0, 166.4, 167.8, 183.1, 2 signals obscured or overlapped; **HRMS** (ESI, MeOH) calcd. for $C_{60}H_{75}N_9O_{18}Na_2$ [M + 2 Na]$^{2+}$/2 627.7507, found 627.7504; **ν_{max} (film)/cm^{-1}:** 3394 (broad), 3127, 1510, 1492.

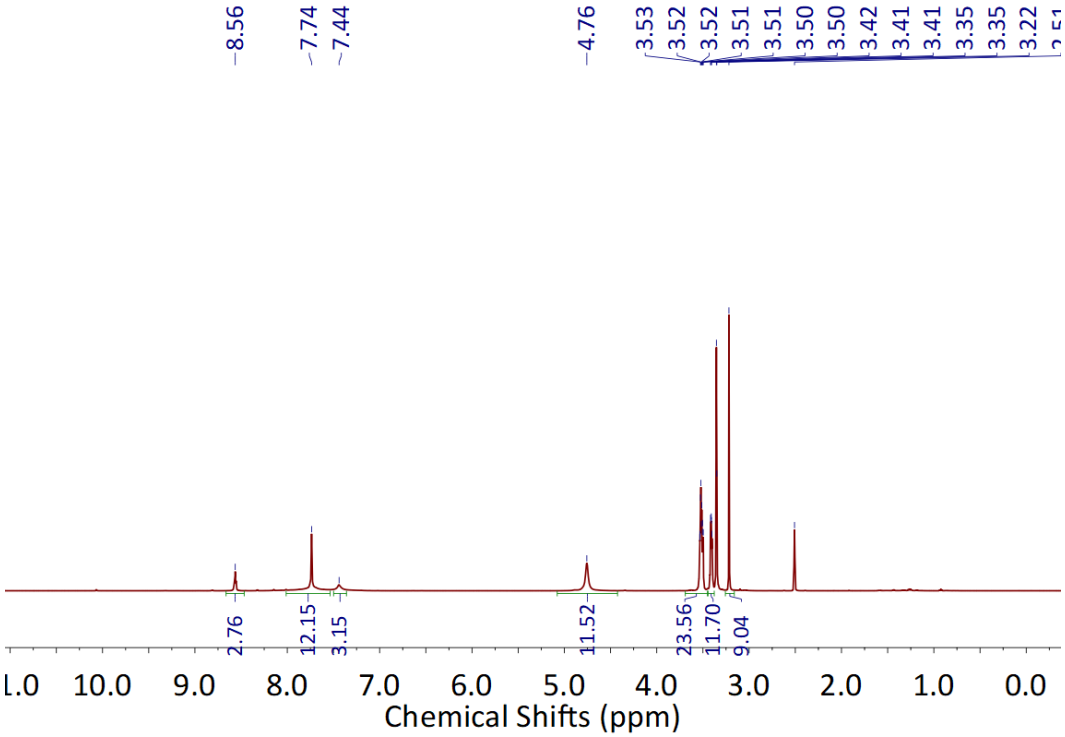


Figure S13. ^{1}H NMR of compound [3]MSQ-**4**.

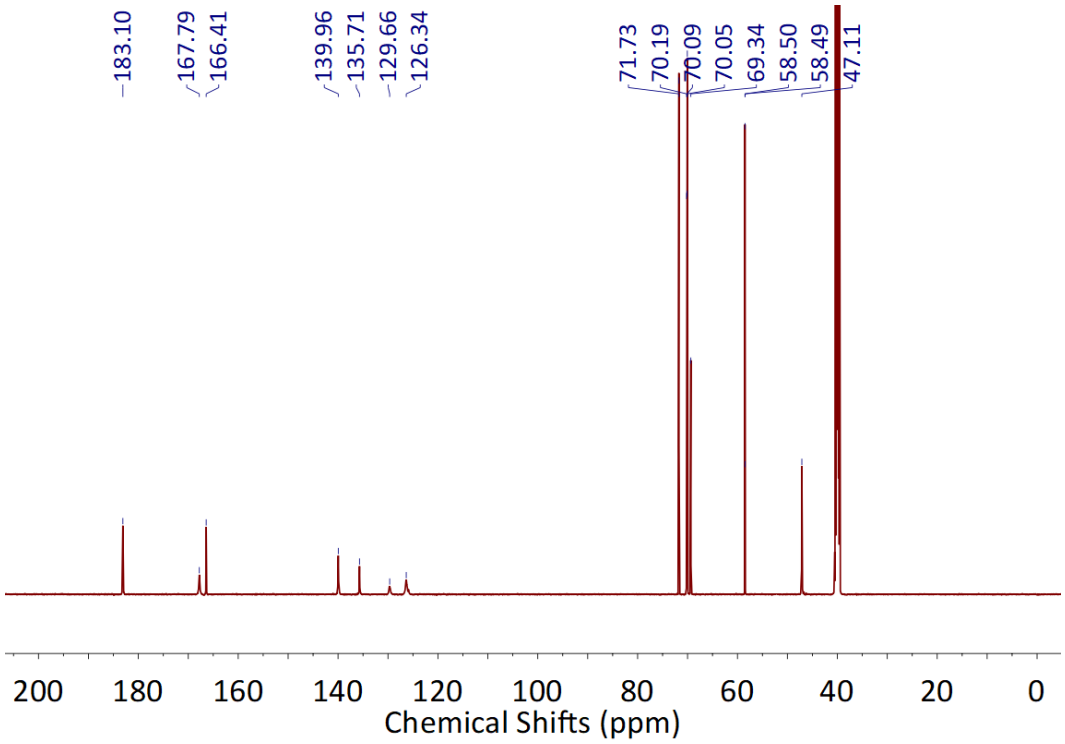
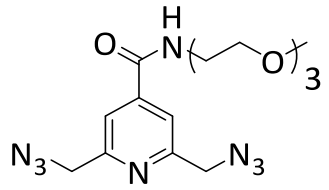


Figure S14. ^{13}C NMR of compound [3]MSQ-**4**.

2,6-Bis(azidomethyl)-*N*-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)isonicotinamide **(8)**



A solution of NaOH (61 mg, 1.5 mmol) in water (2 mL) was added to a solution of methyl 2,6-bis(azidomethyl)isonicotinate (371 mg, 1.5 mmol) in THF (15 mL). The resulting mixture was stirred for 16 hours at room temperature and then neutralized by additional of HCl (aq.) (1 M). The solvent was removed under reduced pressure to give a colorless solid which was dissolved in CH_2Cl_2 (10 mL) then HBTU (626 mg, 1.65 mmol), followed by DIPEA (213 mg, 1.65 mmol) were added. The mixture was stirred for 3 minutes and then 2-(2-(2-methoxyethoxy)ethoxy)ethan-1-amine (269 mg, 1.65 mmol) was added dropwise and the mixture was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure to give a yellow oil. Subjection of this material to flash silica gel chromatography (1:1 v/v ethyl acetate/hexane elution) and concentration of the appropriate fractions (R_f 0.4) gave the compound **8** (500 mg, 62%) as a beige oil. **^{1}H NMR** (400 MHz, C*D*Cl_3): 3.28 (s, 3 H), 3.52 (t, *J* = 5.0 Hz, 2 H), 3.62 – 3.67 (m, 8 H), 4.54 (s, 4 H), 4.50 (t, *J* = 5.0 Hz, 2 H), 7.26 (s, 1 H), 7.66 (s, 2 H); **^{13}C NMR** (100 MHz, C*D*Cl_3): 40.2, 55.2, 58.9, 69.5, 70.3 70.5, 70.6, 71.9, 118.7, 144.1, 156.9, 165.0; **HRMS** (ESI, MeOH) calcd. for $C_{15}H_{22}N_8O_4Na$ $[M + Na]^+$ 401.1654, found 401.1657; **ν_{max} (film)/cm^{-1}:** 3019 (broad), 2104, 1721, 1608, 1450.

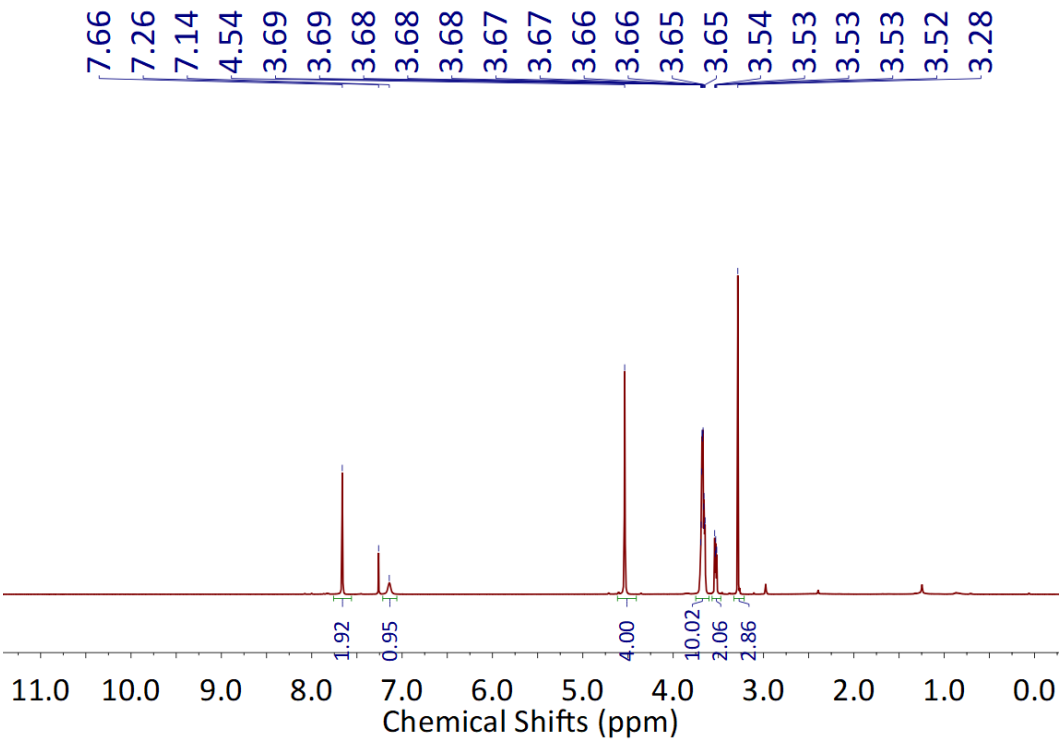


Figure S15. ^{1}H NMR of compound **8**.

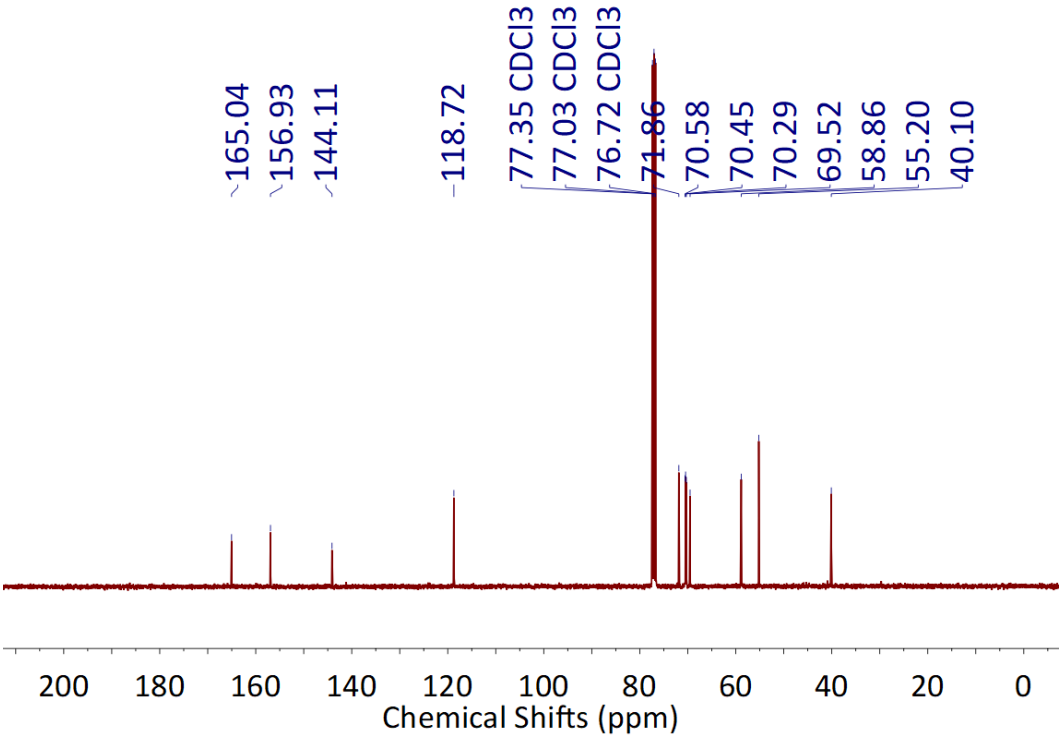
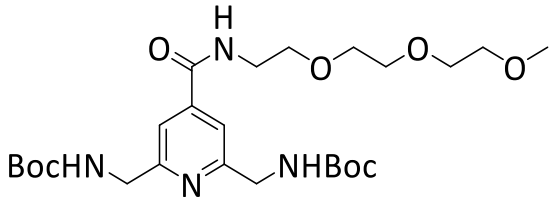


Figure S16. ^{13}C NMR of compound **8**.

Di-*tert*-butyl ((4-((2-(2-(2-methoxyethoxy)ethoxy)ethyl)carbamoyl)-pyridine- 2,6-diyl)bis(methylene)) -dicarbamate **(17)**



Ph_3P (144 mg, 0.55 mmol) was added to a solution of compound **8** (95 mg, 0.25 mmol) in THF (5 mL) and the resulting solution was stirred at room temperature for 2 hours, then 0.1 mL water was added and the mixture was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure to give a yellow oil. The resulting oil was then dissolved in CH_2Cl_2 (5 mL) and Boc_2O (120 mg, 0.55 mmol) was added at room temperature. The resulting mixture was stirred at room temperature for 16 hours. The solvent was then removed under reduced pressure to give a yellow oil. Subjection of this material to flash silica gel chromatography (5:95 v/v methanol/ dichloromethane elution) and concentration of the appropriate fractions (R_f 0.4) gave the compound **17** (116 mg, 88%) as a beige solid. **M.p.** 102 – 108 °C; **^{1}H NMR** (500 MHz, C*D*Cl_3): 1.49 (s, 18 H), 3.32 (s, 3 H), 3.35 – 3.61 (m, 2 H), 3.62 – 3.71 (m, 10 H), 4.52 (s, 2 H), 4.55 (s, 2 H), 5.63 (br s, 2 H), 7.55 (br s, 1 H), 7.64 (s, 2 H); **^{13}C NMR** (125 MHz, C*D*Cl_3): 28.4, 40.0, 45.6, 58.8, 69.7, 70.2, 70.4, 70.5, 71.8, 79.7, 117.7, 143.6, 156.0, 158.1, 165.6; **HRMS** (ESI, MeOH) calcd. for $C_{25}H_{42}N_4O_8Na$ $[M + Na]^+$ 549.2895, found 549.2896; **ν_{max} (film)/cm^{-1}:** 3019 (broad), 2104, 1721, 1608, 1450.

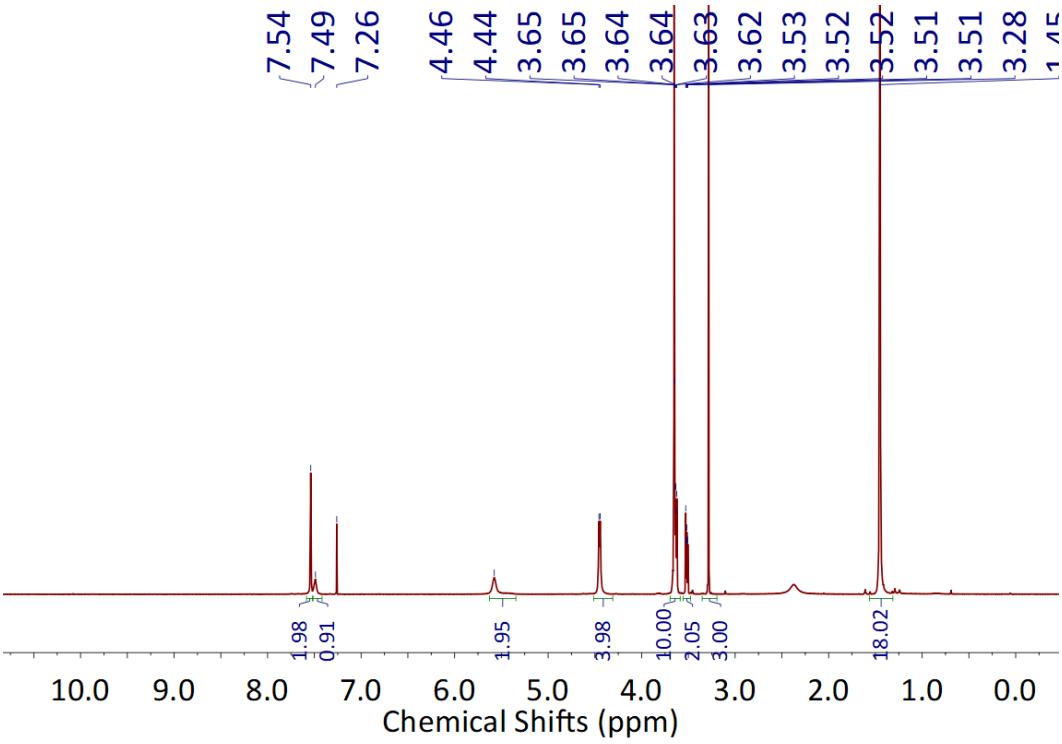


Figure S17. ^{1}H NMR of compound **S3**.

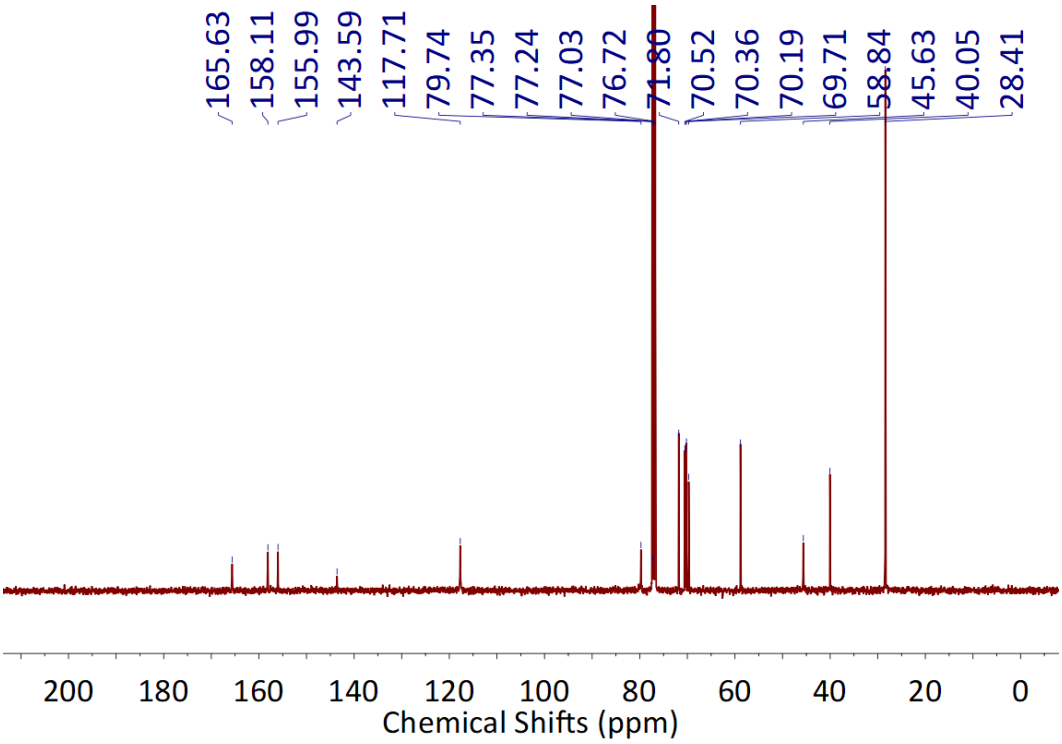
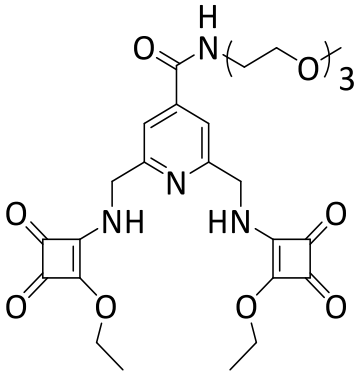


Figure S18. ^{13}C NMR of compound **S3**.

2,6-Bis(((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)methyl)-*N*-(2-(2-(2-methoxyethoxy)ethoxy) -ethyl)isonicotinamide **(12)**



A solution of **8** (110 mg, 0.29 mmol) in THF (15 ml) was added to a solution of Ph_3P (168 mg, 0.64 mmol) at room temperature for 2 hours and to the resulting mixture was added 1 ml water and the reaction mixture was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure to give a yellow oil, which was added to a solution of diethyl squarate (110 mg, 0.64 mmol) in EtOH (10 mL) at room temperature and stirred at room temperature for 24 hours. The solvent was removed under reduced pressure to give a yellow oil. Subjection of this material to flash silica gel chromatography (5/95 v/v methanol/dichloromethane elution) and concentration of the appropriate fractions (R_f 0.4) gave the compound **12** (125 mg, 76%) as a beige solid. **M.p.** 52 -58 °C; **^{1}H NMR** (500 MHz, C*D*Cl_3): 1.43 (t, *J* = 7.1 Hz, 6 H), 3.26 (s, 3 H), 3.46 – 3.54 (m, 2 H), 3.56 – 3.71 (m, 10 H), 4.75 – 5.05 (m, 8 H), 7.47 (s, 1 H), 7.64 (s, 2 H), 7.75 (br s, 2H); **^{13}C NMR** (125 MHz, C*D*Cl_3): 15.8, 40.1, 48.7, 58.7, 69.4, 69.8, 70.1, 70.2, 70.4, 71.8, 118.3, 144.0, 156.6, 164.9, 172.7, 177.9, 183.4, 189.1; **HRMS** (ESI, MeOH) calcd. for $C_{27}H_{34}N_4O_{10}Na$ $[M + Na]^+$ 597.2167, found 597.2169; **ν_{max} (film)/cm^{-1}:** 3345 (broad), 2900, 2021, 1736, 1207.

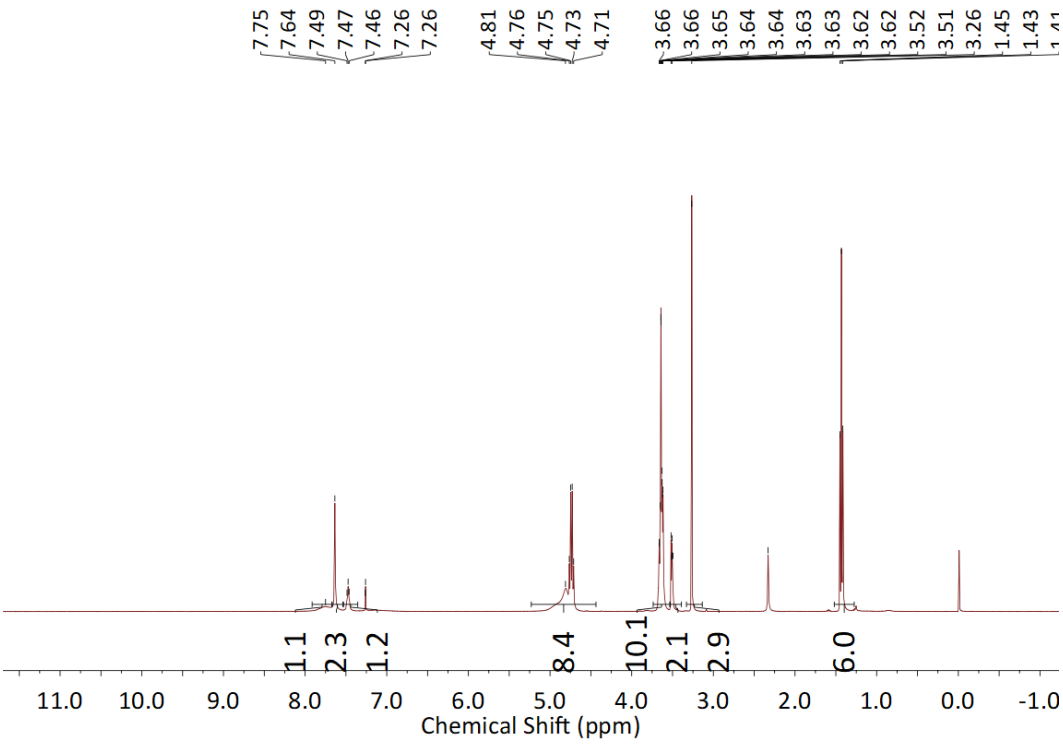


Figure S19. ^{1}H NMR of compound **12**.

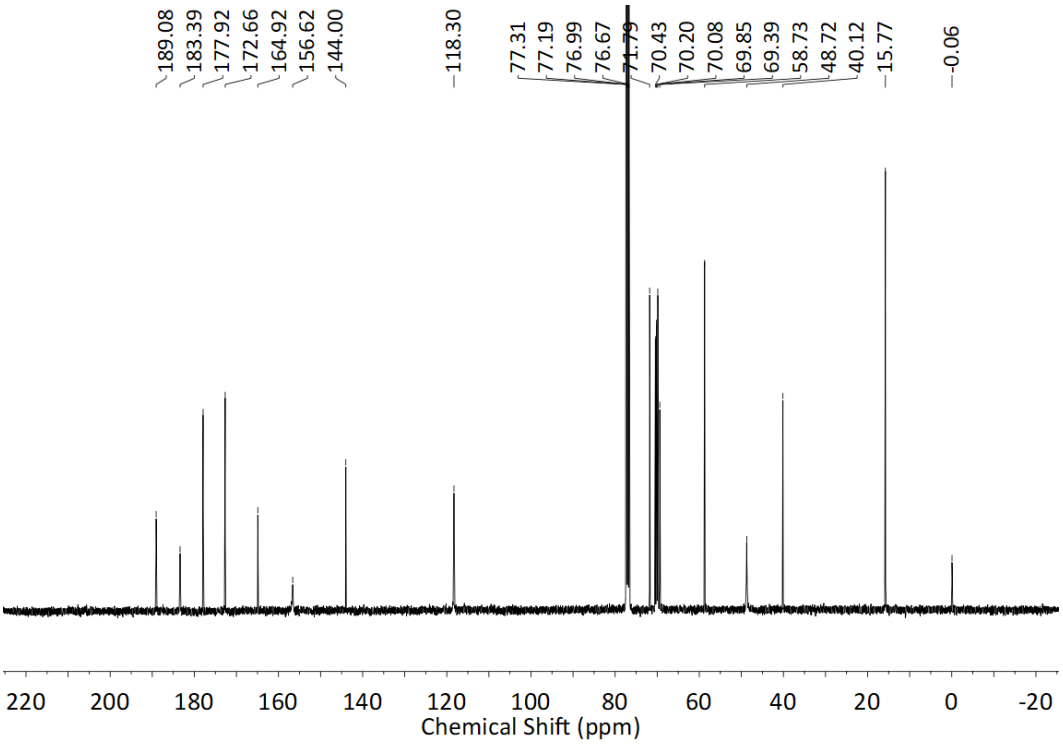
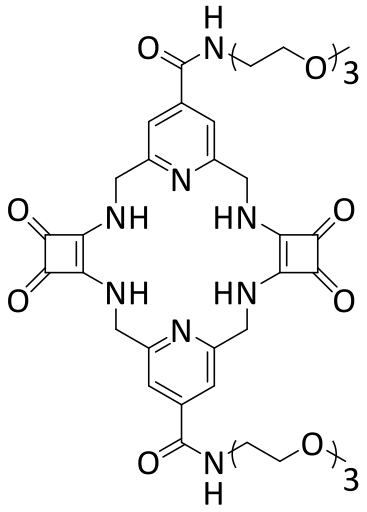


Figure S20. ^{13}C NMR of compound **12**.

[2]MSQ-**5**



Compound **17** (47 mg, 0.09 mmol) was dissolved in a solution of TFA/CH_2Cl_2 (1:1, v/v, 3 mL) and the reaction mixture was stirred at room temperature for 2 hours then concentrated under reduced pressure. The resulting oil was then added to a solution of **12** (52 mg, 0.09 mmol) and Et_3N (0.3 mL) in EtOH (10 mL) and stirred at room temperature for 16 hours. The resulting precipitate was collected by filtration and washed with EtOH (3 mL) then Et_2O (2×5 ml) to yield compound [2]MSQ-**5** (52 mg, 72%) as a colourless solid. **M.p.** 236 – 246 °C (decomp.); **^{1}H NMR** (500 MHz, DMSO-d_6): 3.32 (s, 6 H), 3.42 – 3.56 (m, 24 H), 5.06 (s, 8 H), 7.70 (s, 4 H), 8.20 (br s, 4 H), 8.81 (br s, 2 H); **^{13}C NMR** (125 MHz, DMSO-d_6): 58.5, 69.1, 70.12, 70.14, 70.2, 71.8, 118.5, 14.5, 156.4, 164.9, 167.6, 183.3, 2 signals obscured or overlapped; **HRMS** (ESI, MeOH) calcd. for $C_{38}H_{48}N_8O_{12}Na$ $[M + Na]^+$ 831.3284, found 831.3268; **ν_{max} (film)/cm^{-1}:** 3332 (broad), 2910, 2005, 1723, 1304.

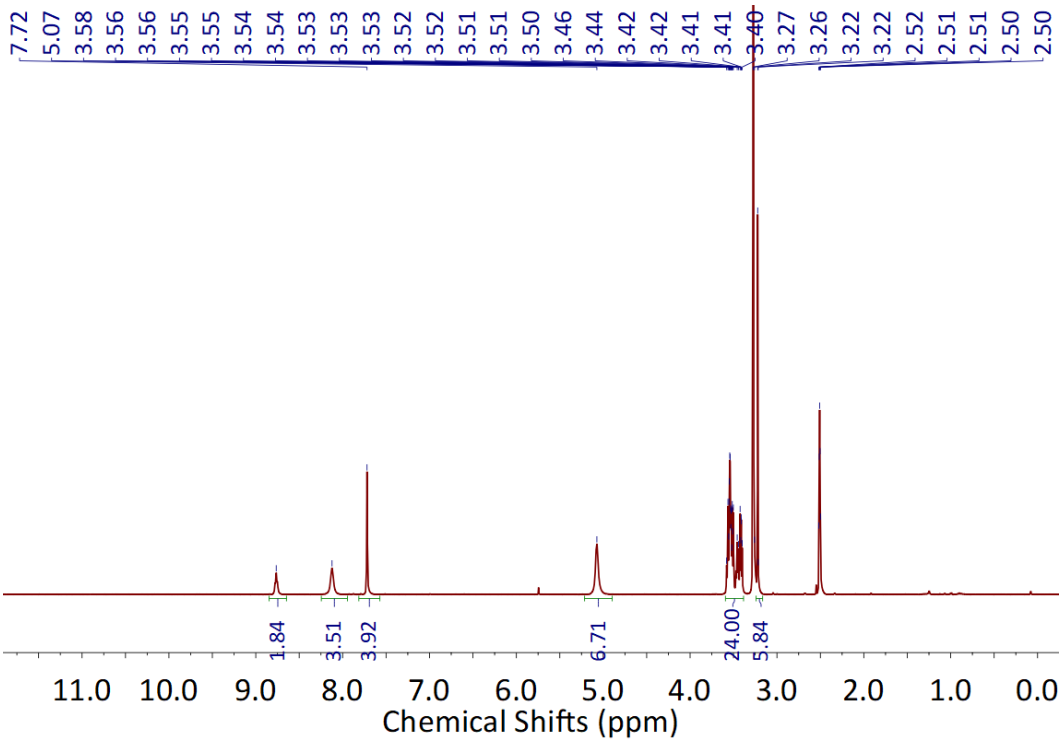


Figure S21. ^{1}H NMR of compound [2]MSQ-**5**.

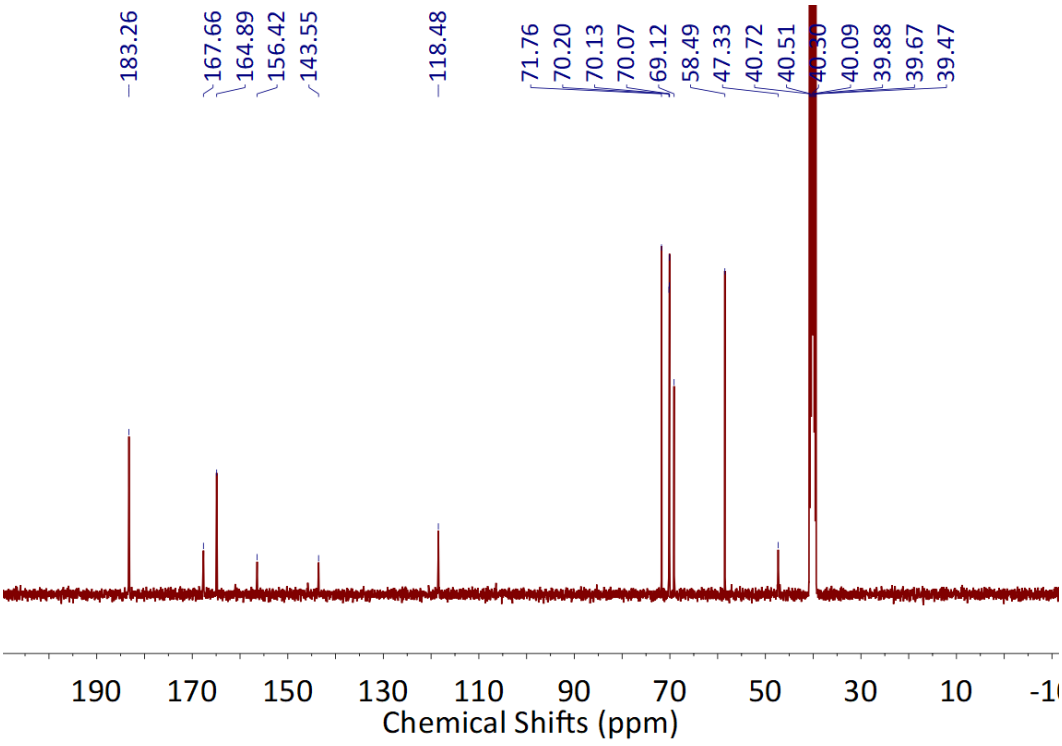
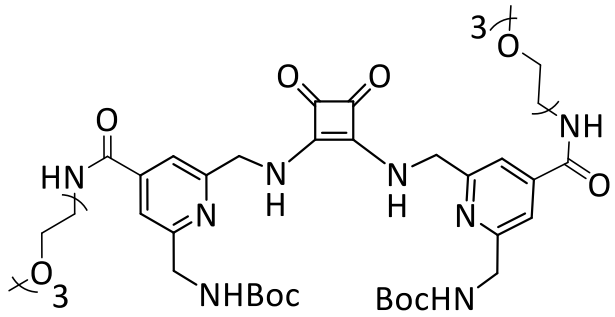


Figure S22. ^{13}C NMR of compound [2]MSQ-**5**.

Di-*tert*-butyl (((((3,4-dioxocyclobut-1-ene-1,2-diyl)bis(azanediyl))-bis(methylene))bis(4-((2-(2-(2-methoxyethoxy)ethoxy)ethyl)carbamoyl)pyridine-6,2-diyl))bis(methylene))dicarbamate **(16)**



Ph_3P (73 mg, 0.27 mmol) was added to a solution of compound **8** (48 mg, 0.13 mmol) in THF (3 mL) and the resulting solution was stirred at room temperature for 2 hours then 0.1 mL water was added and the mixture was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure to give a yellow oil, which was then added to a solution of Boc_2O (26 mg, 0.12 mmol) in CH_2Cl_2 (8 mL) at room temperature and the resulting mixture was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure to give a yellow oil. The subjection of this material to flash silica gel chromatography (0.2/7.8/92 v/v aqueous ammonia /methanol/dichloromethane elution) and concentration of the appropriate fractions (R_f 0.5) gave compound **14**. Due to the instability of compound **14**, it was dried in vacuum for 1 hour and then immediately added to a solution of diethyl squarate (11 mg, 0.065 mmol) and Et_3N (0.1 mL) in EtOH (5 mL) at room temperature. The resulting solution was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure to give a yellow oil. The subjection of this material to flash silica gel chromatography (5/95 v/v methanol/dichloromethane elution) and concentration of the appropriate fractions (R_f 0.4) gave the compound **16** (31 mg, 60%) as a beige oil. **^{1}H NMR** (500 MHz, C*D*Cl_3): 1.42 (s, 18 H), 3.34 (s, 6 H), 3.52 – 3.57 (m, 4 H), 3.59 – 3.64 (m, 20 H), 4.42 (s, 4 H), 4.83 (s, 4 H), 6.00 (s, 2 H), 7.53 (s, 4 H), 7.84 (br s, 4 H); **^{13}C NMR** (125 MHz, C*D*Cl_3): 28.4, 40.1, 45.7, 49.0, 58.8, 69.5, 70.1, 70.3, 70.4, 71.8, 79.8, 118.2, 143.7, 156.3, 157.3, 159.3, 165.7, 168.2, 183.2, 1 signal obscured or overlapped; **HRMS** (ESI, MeOH) calcd. for $C_{44}H_{66}N_8O_{14}Na$ $[M + Na]^+$ 953.4591, found 953.4602; **ν_{max} (film)/cm^{-1}:** 3321 (broad), 2219, 1697, 1601, 1420.

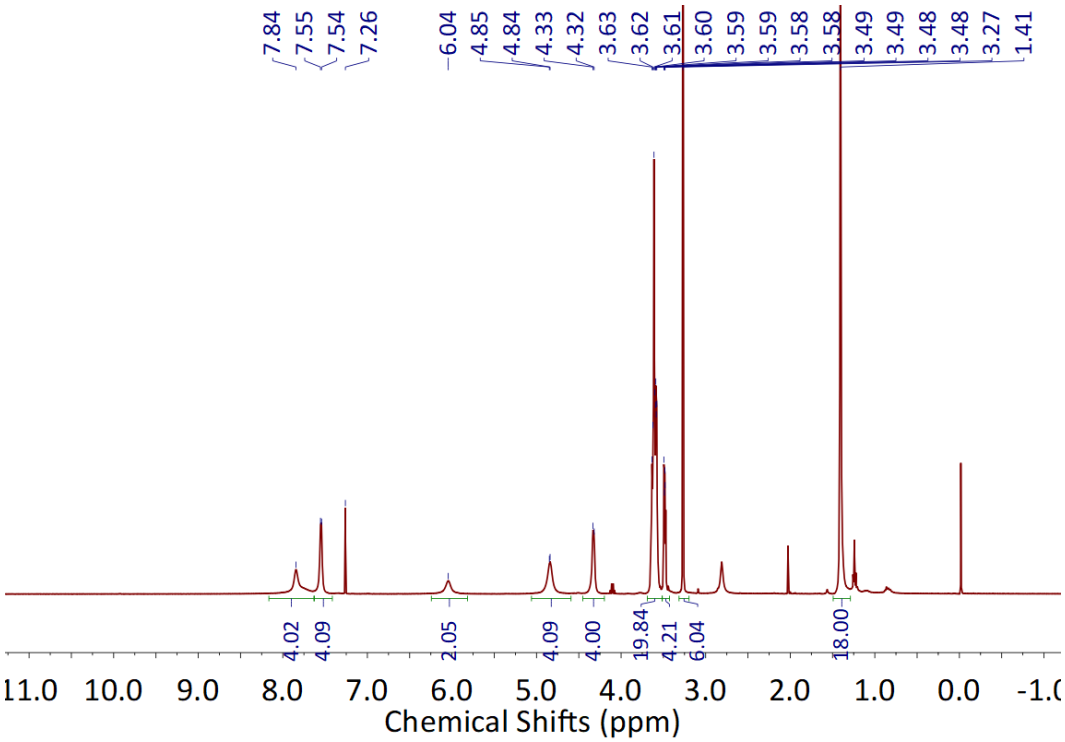


Figure S23. ^{1}H NMR of compound **16**.

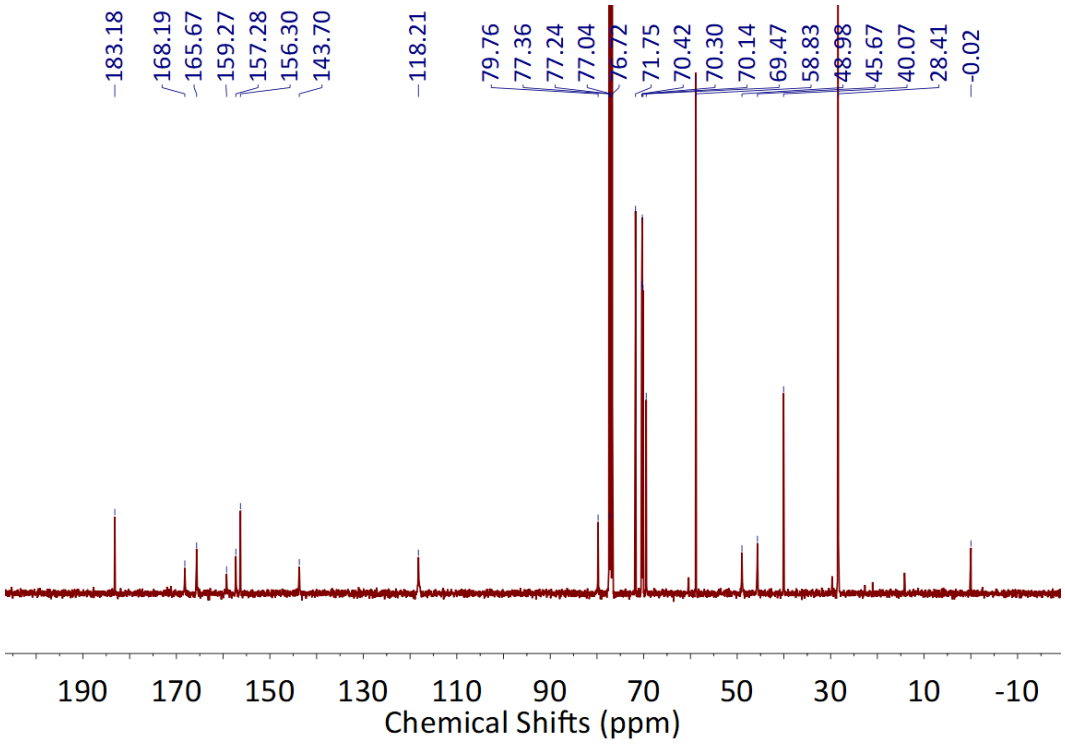
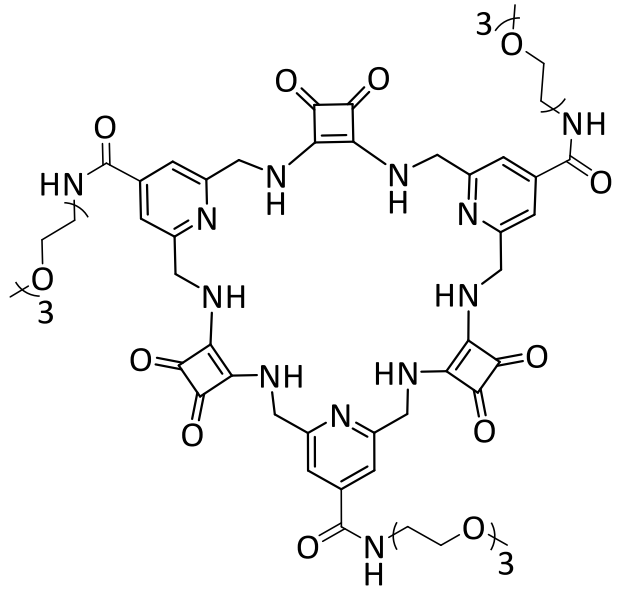


Figure S24. ^{13}C NMR of compound **16**.

[3]MSQ-**6**



Compound **16** (74 mg, 0.08 mmol) was dissolved in a solution of TFA/CH_2Cl_2 (1:1, v/v, 3 mL) and the reaction mixture was stirred at room temperature for 2 hours then concentrated under reduced pressure. The resulting oil was then added to a solution of **12** (46 mg, 0.08 mmol) and Et_3N (0.5 mL) in EtOH (20 mL) and stirred at room temperature for 48 hrs. The solvent was then removed under reduced pressure to give a yellow oil. Subjection of this material to flash silica gel chromatography (5/95 v/v methanol/dichloromethane elution) and concentration of the appropriate fractions (R_f 0.3) gave the compound [3]MSQ-**6** (48 mg, 50%) as a beige solid. **M.p.** 256 – 270 °C (decomp.); **^{1}H NMR** (500 MHz, DMSO-d_6): 3.22 (s, 9 H), 3.40 – 3.49 (m, 12 H), 3.51 – 3.63 (m, 24 H), 4.92 (s, 12 H), 7.74 (s, 6 H), 8.00 (br s, 6 H), 8.91 (s, 3 H); **^{13}C NMR** (125 MHz, DMSO-d_6): 48.8, 58.6, 69.3, 70.2, 70.3, 70.4, 71.9, 118.8, 143.9, 158.8, 165.1, 168.6, 183.5, 1 signal obscured or overlapped; **HRMS** (ESI, MeOH) calcd. for $C_{57}H_{72}N_{12}O_{18}Na$ $[M + Na]^+$ 1258.4872, found 1258.4877; **ν_{max} (film)/cm^{-1}:** 3420 (broad), 2857, 1994, 1756, 1286.

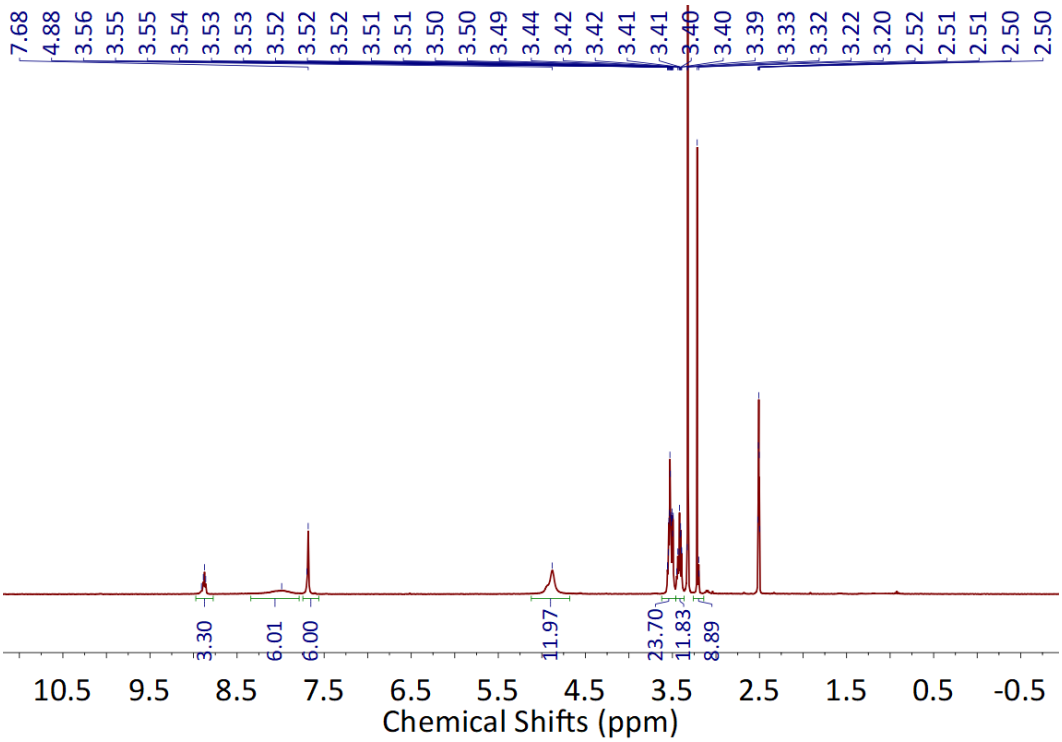


Figure S25. ^{1}H NMR of compound [3]MSQ-**6**.

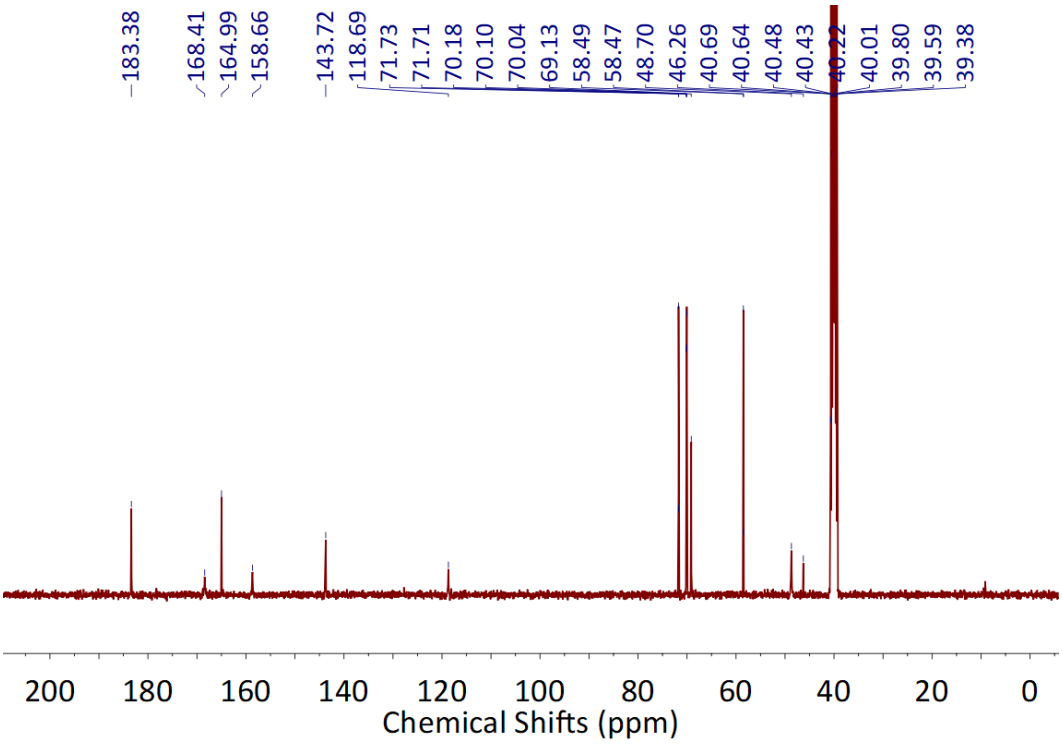


Figure S26. ^{13}C NMR of compound [3]MSQ-**6**.

^{1}H NMR Binding studies

Both salt and receptor were dried under high vacuum for 48 hours prior to use. A 0.3 – 1.0 mM stock solution of the receptor was accurately prepared in the stated solvents (Milli-Q H_2O and DMSO-d_6 mixtures) using a volumetric flask. Solutions of tetrabutylammonium (TBA) salts to be titrated were prepared in separate 2 mL vials, by addition of 200 μL of the required receptor solution to the pre-weighed TBA salts using auto pipettes. The concentration of anions solution was approximately 70 times that of the host. In each case, 550 μL of host solution in an NMR tube was titrated with aliquots of anion stock solution, and after each addition, the ^{1}H NMR spectrum was recorded on a Bruker Avance III 400 or Bruker Avance III 600 spectrometer after thorough mixing at 300 K. Typically, additions were performed in the following order: 10 × 1.5 μL, 2 × 7.5 μL, 4 × 14 μL (total 86 μL). Typically, a total of at least 12 equiv. of anion was added to the receptor solutions. Titrations were performed in triplicate to give K_a values. Non-linear curve fitting of the experimentally obtained titration isotherms (equivalents of anion versus chemical shift of NH, aromatic CH and methylene CH protons) using the program HypNMR2008 (Hyperquad®) enabled the calculation of association constants (K_a/M^{-1}) using a 1:1 global fits model.

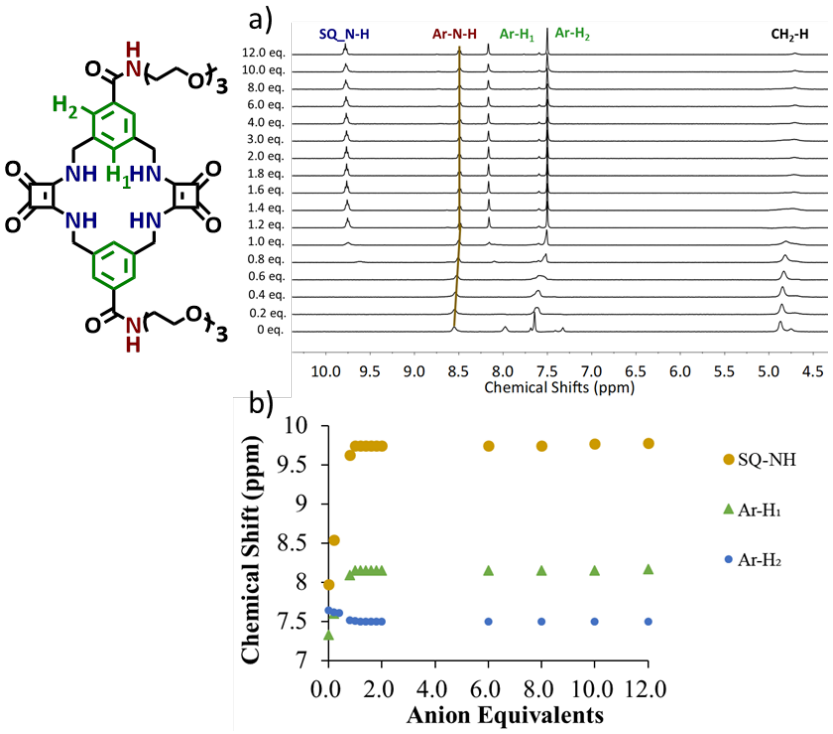


Figure S27. a) ^{1}H NMR titration; b) Binding isotherms of [2]MSQ-**3** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:9 (v/v) H_2O-DMSO-d_6 mixture. ($K_a > 10^4$ M^{-1})

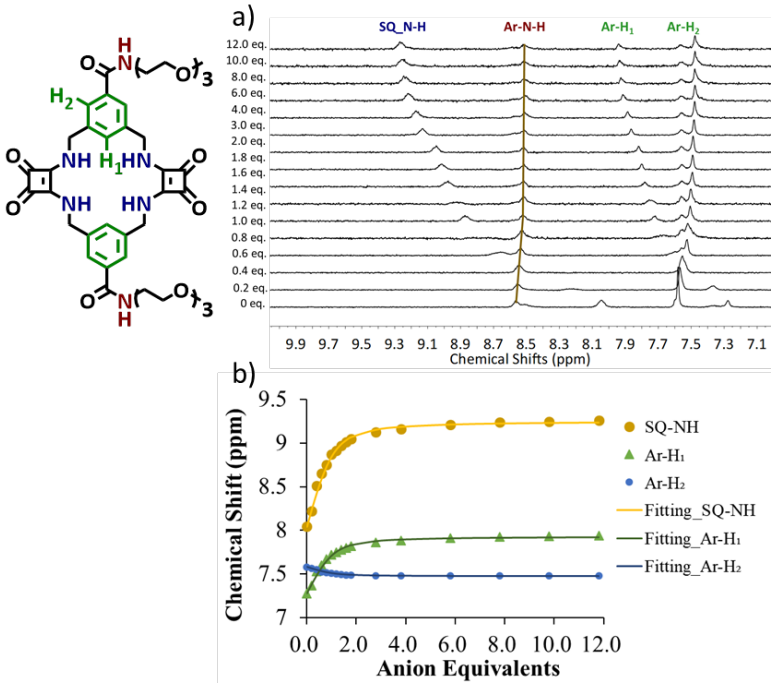


Figure S28. a) ^{1}H NMR titration; b) Binding isotherms of [2]MSQ-**3** (0.8 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:2 (v/v) H_2O-DMSO-d_6 mixture. (K_a = 3170 M^{-1})

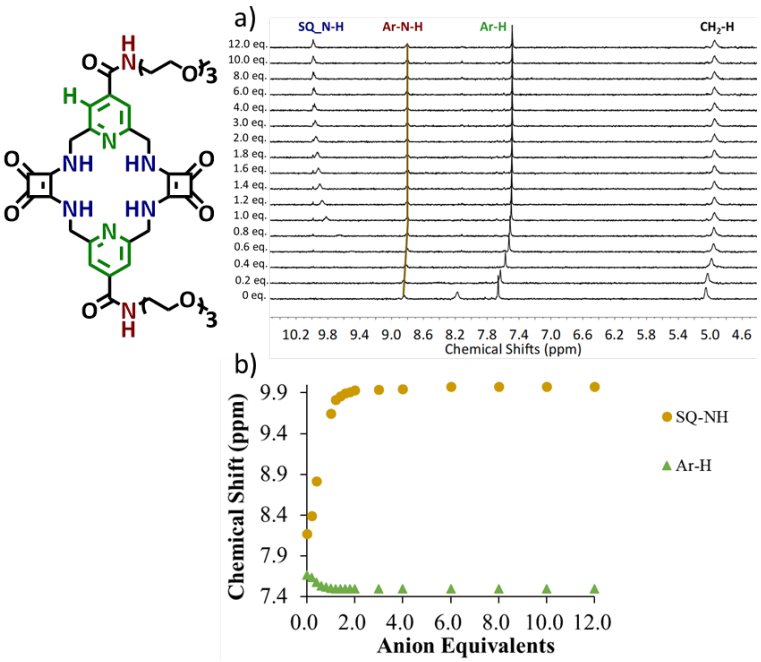


Figure S29. a) ^{1}H NMR titration; b) Binding isotherms of [2]MSQ-**5** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:9 (v/v) H_2O-DMSO-d_6 mixture. ($K_a > 10^4$ M^{-1})

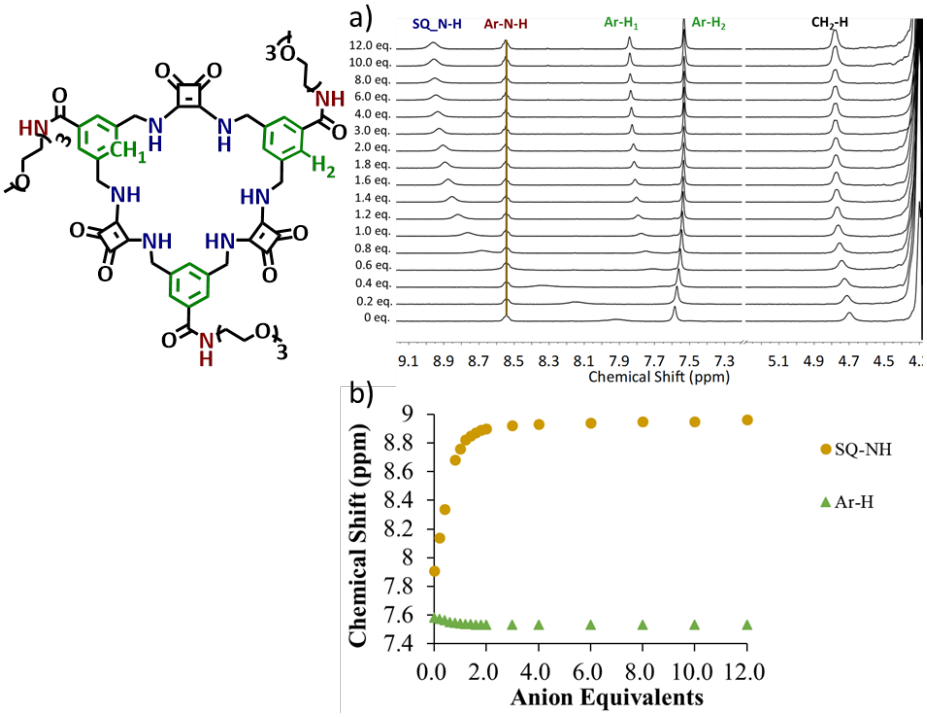


Figure S30. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**4** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:2 (v/v) H_2O-DMSO-d_6 mixture. ($K_a > 10^4$ M^{-1})

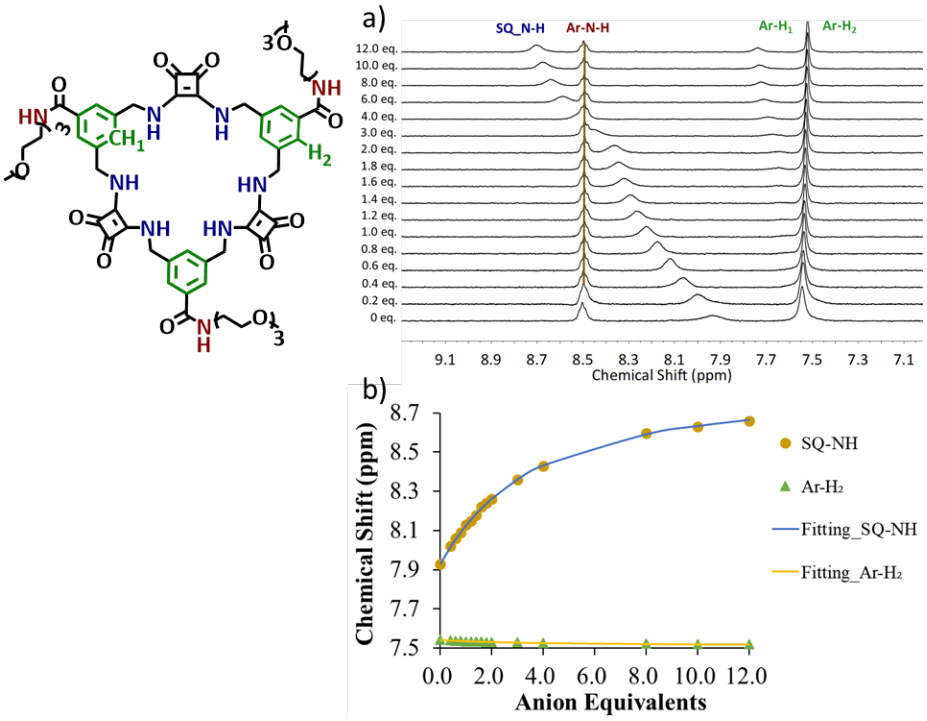


Figure S31. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**4** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture. (K_a = 1340 M^{-1})

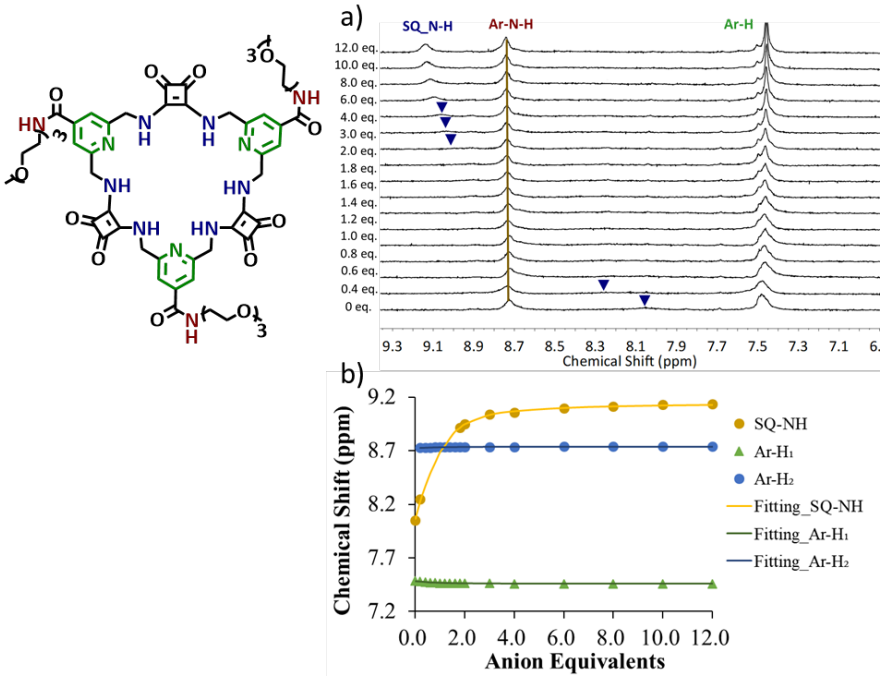


Figure S32. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**6** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture. (K_a = 4870 M^{-1}).

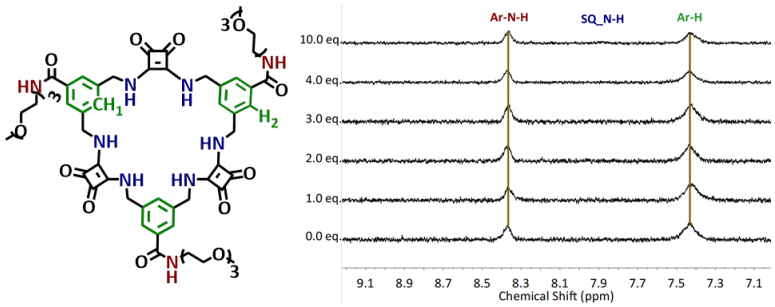


Figure S33. a) ^{1}H NMR titration of [3]MSQ-**4** (0.3 mM) with 0.0 – 10.0 equiv. $(TBA)_2SO_4$ in 97:3 (v/v) H_2O-DMSO-d_6 mixture. (No binding observed)

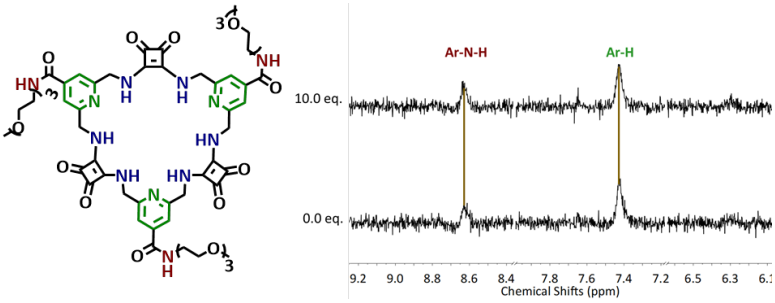


Figure S34. a) ^{1}H NMR titration of [3]MSQ-**6** (0.3 mM) with 0.0 – 10.0 equiv. $(TBA)_2SO_4$ in 97:3 (v/v) H_2O-DMSO-d_6 mixture. (No binding observed)

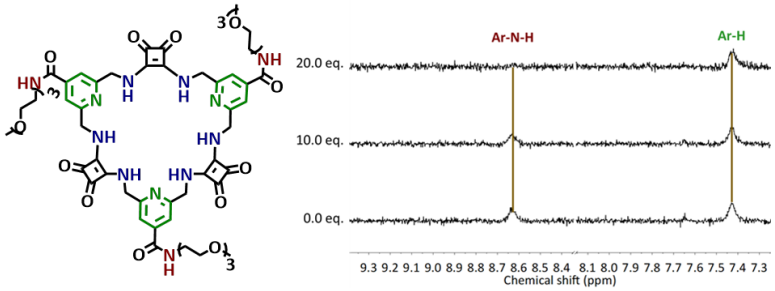


Figure S35. ^{1}H NMR titration of [3]MSQ-**6** (0.3 mM) with 0.0 – 20.0 equiv. $(TBA)_2SO_4$ in 97:3 (v/v) H_2O-DMSO-d_6 mixture, pH 3.2 (HNO_3). (No binding observed)

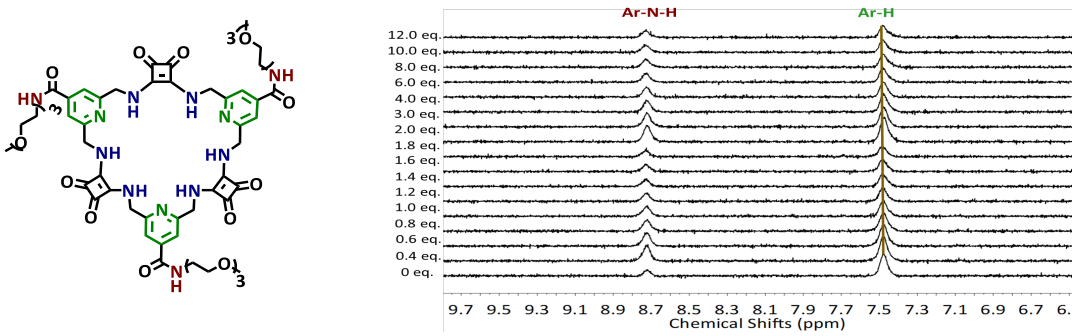


Figure S36. ^{1}H NMR titration of [3]MSQ-**6** (1.0 mM) with 0.0 – 12.0 equiv. $TBAH_2PO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture. (No binding observed)

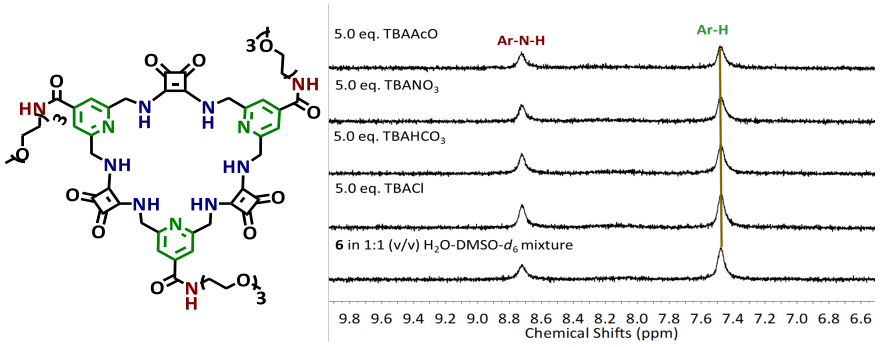


Figure S37. ^{1}H NMR screen of anion binding to [3]MSQ-**6** (1.0 mM) in 1:1 (v/v) H_2O-DMSO-d_6 mixture. From top, spectra obtained after addition of 5 equiv. each of TBAOAc, $TBANO_3$, $TBAHCO_3$, TBACl and bottom **6** only. (No binding observed)

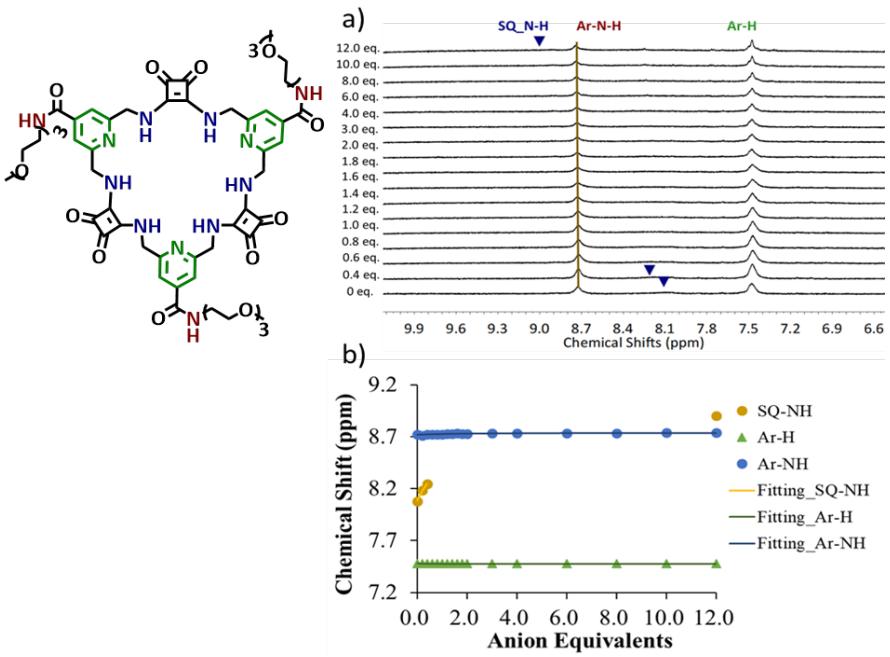


Figure S38. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**6** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SeO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture. (K_a = 950 M^{-1})

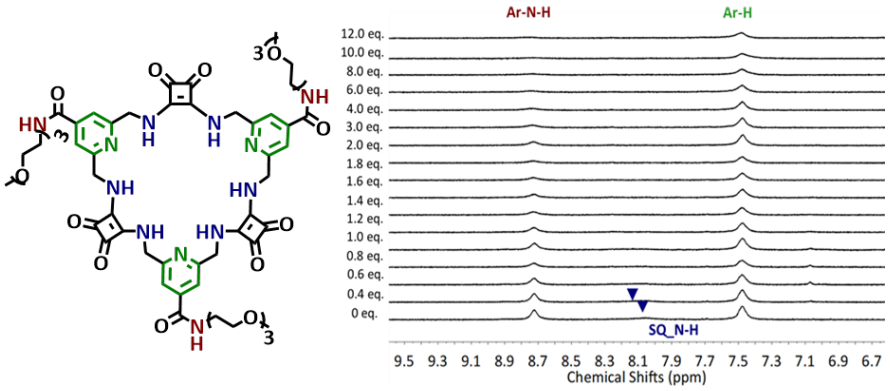


Figure S39. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**6** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2CrO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture. (K_a < 10 M^{-1})

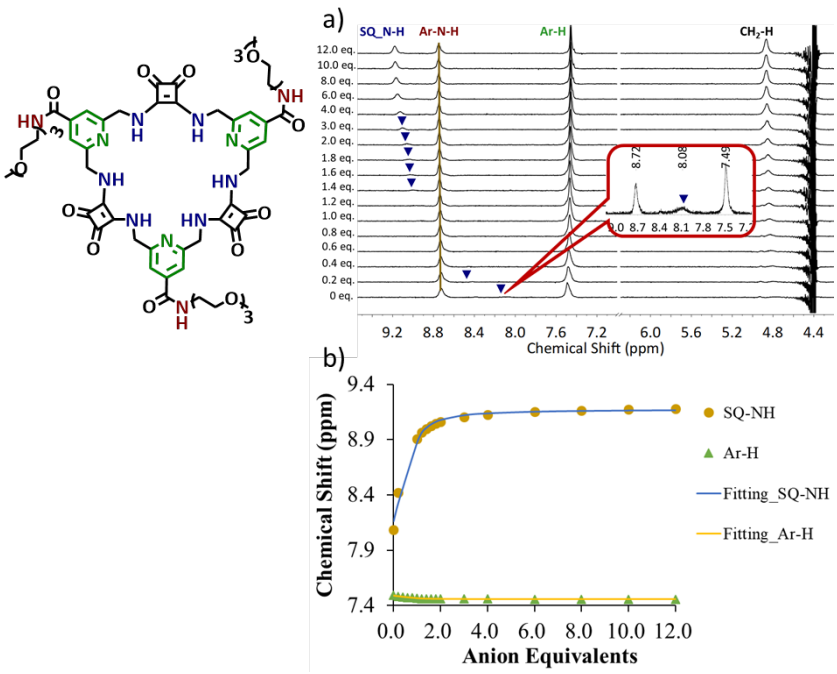


Figure S40. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**6** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture (pH 3.2) with 20 mM Tris buffer in the presence of 2.5 mM phosphates, 10 mM Cl^-, 250 mM NO_3^-. (K_a = 8910 M^{-1}).

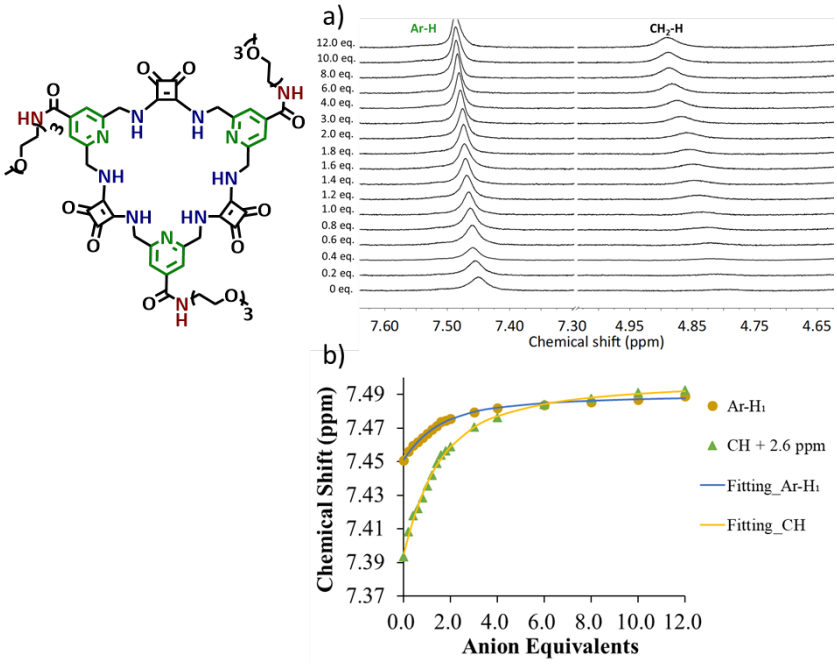


Figure S41. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**6** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture (pH 14.1) with 2.5 mM phosphates, 15 mM Cl^- , 250 mM NO_3^-. (K_a = 610 M^{-1})

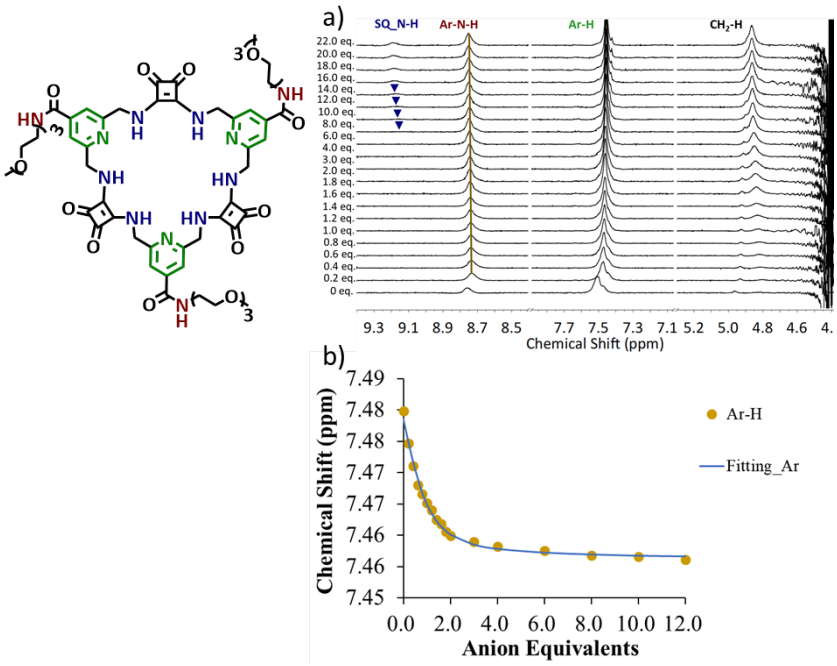


Figure S42. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**6** (1.0 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 1:1 (v/v) H_2O-DMSO-d_6 mixture (pH 7.4) with 20 mM Tris buffer with 1.5 mM $H_2PO_4^-/HPO_4^{2-}$, 106 mM Cl^- , 28 mM H_2CO_3/HCO_3^-. (K_a = 2490 M^{-1})

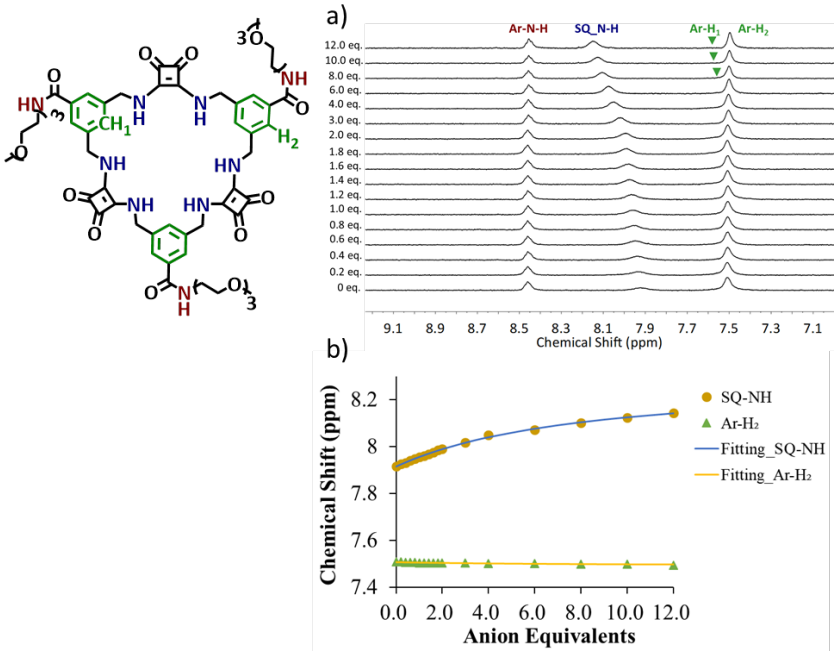


Figure S43. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**4** (0.8 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 2:1 (v/v) H_2O-DMSO-d_6 mixture. (K_a = 130 M^{-1})

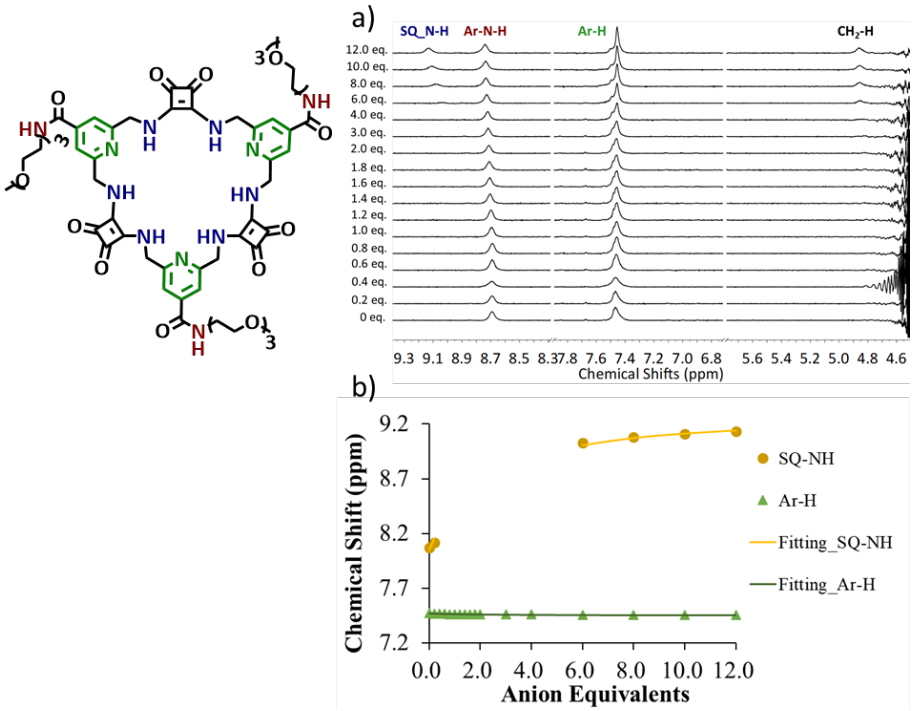


Figure S44. a) ^{1}H NMR titration; b) Binding isotherms of [3]MSQ-**6** (0.8 mM) with 0.0 – 12.0 equiv. $(TBA)_2SO_4$ in 2:1 (v/v) H_2O-DMSO-d_6 mixture. (K_a = 480 M^{-1}). As a reuslt of peak broadening the signal attibuted to the squaramide protons was not observed between additions of 0.2 to 6.0 equiv of $(TBA)_2SO_4$.

References:

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2. *EU, US Pat.,* CA2078585A1, DE59203209D1, EP0533131A1, EP0533131B1, 1992.
3. S. G. Gouin, M. Roger, N. Leygue, D. Deniaud, K. Julienne, E. Benoist, C. Picard, J. Kovensky and C. Galaup, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 2684-2688.