Squaramide-Based Ion Pair Receptors Can Facilitate Transmembrane Transport of KCl and Zwitterions Including Highly Polar Amino Acids

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Supporting Information

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S1. General information

Unless specifically indicated, all chemicals and reagents used in this study were purchased from commercial sources and used as received. If necessary, purification of products was performed using column chromatography on silica gel (Merck Kieselgel 60, 230-400 mesh) with mixtures of chloroform/methanol. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck Kieselgel 60 F254). ^{1}H and ^{13}C NMR spectra used in the characterization of products were recorded on Bruker Avance 300 MHz spectrometer. Two-dimensional NMR spectra (ROESY, COSY and HSQC) were recorded on a Bruker Avance III HD 500 MHz. In each case, the spectra were calibrated to the residual solvent resonances. Multiplets were assigned as s (singlet), d (doublet), and m (multiplet). The HRMS data were obtained on a Quattro LC Micromass or Shimadzu LCMS-IT-TOF unit.

S2. Synthetic procedures and characterization

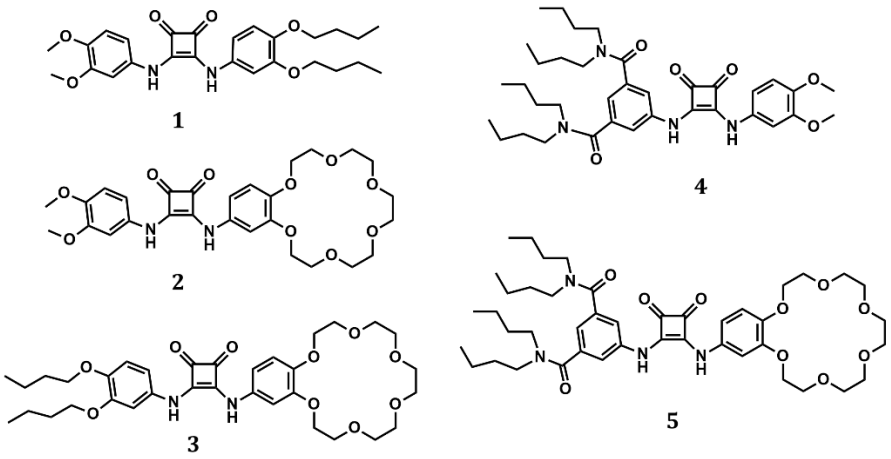
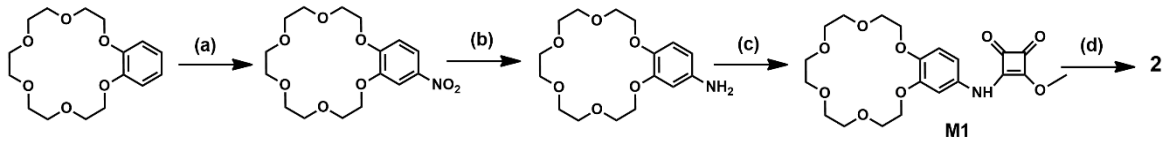


Figure S1. Structure of receptors **1**-**5**.

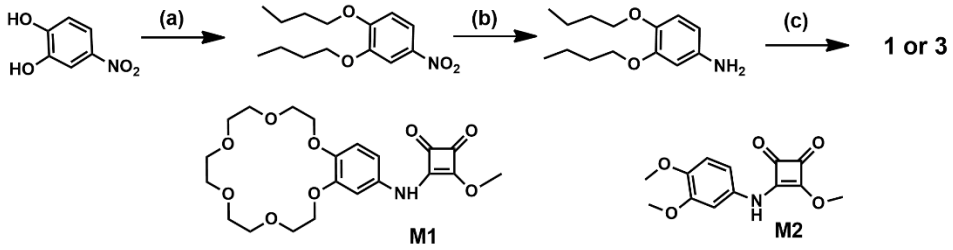


Scheme S1. Synthesis of receptor **2.** Reagents and conditions: (a) chloroform, HNO_3/CH_3COOH (2:1), 0°C to room temperature, overnight, yield 95%; (b) H_2, Pd/C, MeOH/THF, 12 h, room temperature, yield 98%; (c) 3,4-dimethoxy-3-cyclobutene-1,2-dione, MeOH, room temperature, overnight, yield 68%; (d) 3,4-dimethoxyaniline, TEA, MeOH, 24 h, room temperature, yield 79% for receptor **2**.

Preparation of receptor 2.

Compound **M1** was synthesized according to the literature procedure with small modifications starting from benzo-18-crown-6 ether.[S1] To a solution of compound **M1** (0.5 g, 1.14 mmol) in MeOH (15 ml) was added 3,4-dimethoxyaniline (0.17 g, 1.14 mmol) and the mixture was stirred for 24 h at room temperature. Then the reaction mixture was filtered and the collected solid material was washed several times with methanol. The obtained off-white solid was dried *in vacuo* to give receptor **2** (0.5 g, 0.9 mmol, 79 % yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.71 (s, 2H), 7.34 (s, 2H), 7.03-6.80 (m, 4H), 4.18-4.00 (m, 4H), 3.87-3.67 (m, 10H), 3.65-3.47 (m, 12H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.72, 165.44, 149.79, 149.22, 145.58, 144.89, 132.77, 132.71, 114.47, 113.08, 110.64, 110.48, 105.28, 104.28, 70.29, 69.25, 69.08, 68.99, 68.52, 56.31, 55.95. HRMS (ESI): calcd for $C_{28}H_{34}N_2O_{10}Na$ [M+ Na]$^+$: 581.21057, found: 581.20993.



Scheme S2. Synthesis of receptors **1** and **3.** Reagents and conditions: (a) 2 equiv. 1-iodobutane, K_2CO_3, acetone, reflux, 48 h, yield 78%; (b) EtOH, $SnCl_2 \times 2H_2O$, 70°C, 2h, 98%; (c) compound **M1** or **M2**, TEA, MeOH, 24 h, room temperature, yield 81% and 69% for **1** and **3**, respectively.

Preparation of compound M2.

To a solution of 3,4-dimethoxy-3-cyclobutene-1,2-dione (1.0 g, 7.04 mmol) in MeOH (25 ml) was added dropwise over a period of 10 min 3,4-dimethoxy aniline (1.0 g, 6.54 mmol) in MeOH (10 mL) at room temperature. After being stirred for 24 h, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography (2 % methanol in chloroform) to give compound **M2** as a yellow solid (1.6 g, 6.08 mmol, 93 % yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 10.63 (s, 1H), 7.08 (s, 1H), 7.90-7.70 (m, 2H), 4.37 (s, 3H), 3.82-3.64 (d, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 183.88, 178.65, 169.31, 149.39, 146.20, 131.75, 112.59, 111.95, 105.28, 60.86, 56.19, 55.88, 49.05. HRMS (ESI): calcd for $C_{13}H_{13}NO_5$ [M+ Na]$^+$: 286.06859, found: 286.06876.

Preparation of 1,2-dibutoxy-4-nitrobenzene.

To a solution of the 4-nitrocatechol (1.2 g, 7.7 mmol) in acetone were added 1-iodobutane (1.84 ml, 16.7 mmol) and K_2CO_3 (6.42 g, 46.5 mmol), the mixture was stirred for 48 h in reflux. Next, the solvent was evaporated, residual oil was dissolved in CH_2Cl_2 (50 mL), washed with distilled water three times, and then with 0.5 molar HCl solution, dried with $MgSO_4$, evaporated and dried *in vacuo*. The title compound was obtained as a yellow oil (1.6 g, 6 mmol, 78% yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95-7.80 (m, 1H), 7.75-7.65 (m, 1H), 7.20-7.10 (d, 1H), 4.25-3.95 (m, 4H), 1.85-1.65 (m, 4H), 1.55-1.35 (m, 4H), 1.00-0.85 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.86, 148.53, 140.90, 118.07, 112.25, 108.04, 69.02, 68.93, 30.99, 30.91, 19.13, 19.09, 14.09, 14.06. HRMS (ESI): calcd for $C_{14}H_{22}NO_4$[M+ H]$^+$: 268.15433, found: 268.15392.

Preparation of receptor 1.

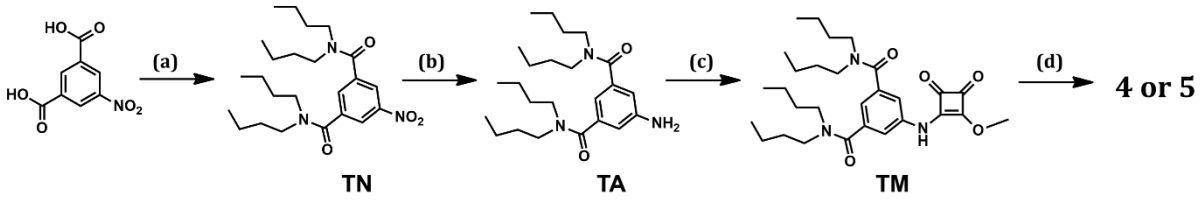
To a degassed solution of 1,2-dibutoxy-4-nitrobenzene (0.6 g, 2.24 mmol) in 20 mL of a THF/MeOH mixture (1:4) was added 10 mg of 10% Pd/C. The reaction mixture was kept under an H_2 atmosphere (balloon pressure) at room temperature overnight. Then, the catalyst was removed by filtration through a pad of Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to give the crude product in quantitative yield (0.52 g). The obtained 3,4-dibutoxyaniline was used in the next step without further purification. To the solution of 3,4-dibutoxyaniline (0.52 g, 2.20 mmol) in methanol (10 ml) was added **M2** (0.58 g, 2.20 mmol) at room temperature, and TEA (0.61 mL, 4.40 mmol). After being stirred for 48 h, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography (1 % methanol in chloroform) to give receptor **1** as a yellow solid (1.0 g, 1.79 mmol, 81 % yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.80-9.60 (d, 2H), 7.45-7.25 (m, 2H), 7.02-6.90 (m, 2H), 6.90-6.75 (m, 2H), 4.05-3.95 (m, 2H), 3.95-3.87 (m, 2H), 3.79 (s, 3H), 3.74 (s, 3H), 1.80-1.60 (m, 4H), 1.55-1.35 (m, 4H), 1.00-0,85 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.70, 165.41, 149.78, 145.56, 145.15, 132.95, 132.70, 115.54, 113.08, 110.76, 110.65, 110.46, 110.38, 105.69, 104.26, 69.17, 68.44, 56.30, 55.95, 31.41, 31.16, 19.21, 14.17, 14.14. HRMS (ESI): calcd for $C_{26}H_{32}N_2O_6Na$ [M+ Na]$^+$: 491.21526, found: 491.21532.

Preparation of receptor 3.

To the solution of 3,4-dibutoxyaniline (0.27 g, 1.14 mmol) in methanol (10 mL) was added **M1** (0.5 g, 1.14 mmol) at room temperature, and triethylamine (0.31 mL, 2.20 mmol). After being stirred for 24 h, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography (5 % methanol in chloroform) to give receptor **3** as a dark green solid (0.51 g, 0.79 mmol, 69 % yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 10.50-10.30 (d, 2H), 7.50-7.35 (m, 2H), 7.05-6.85 (m, 4H), 4.20-4.05 (m, 4H), 4.05-3.95 (m, 2H), 3.95-3.87 (m, 2H), 3.85-3.70 (m, 4H), 3.70-3.45 (m, 12H), 1.80-1.60 (m, 4H), 1.55-1.35 (m, 4H), 1.05-0.85 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.43, 165.53, 165.45, 149.76, 148.61, 145.09, 144.37, 133.18, 132.91, 115.54, 113.30, 110.56, 110.38, 105.75, 105.58, 104.52, 70.00-67.79 (m), 31.43, 31.17, 19.22, 14.16. HRMS (ESI): calcd for $C_{34}H_{46}N_2O_{10}$ [M - H$^+$]$^-$: 641.30797, found: 641.30506.



Scheme S3. Synthesis of receptors **4** and **5.** [a] Reagents and conditions: (a) oxalyl chloride, DMF, 0°C, Ar, dry CH_2Cl_2; 2 equiv. dibutylamine, TEA, 24 h; yield 70%; (b) EtOH, $SnCl_2{\times}2H_2O$, 70°C, 2h, 96%; (c) 3,4-dimethoxy-3-cyclobutene-1,2-dione, MeOH, room temperature, overnight, 65%; (d) 3,4-dimethoxyaniline or 4-aminobenzo-18-crown-6 ether, MeOH, TEA, 24 h, room temperature, yield 70% and 78% for **4** and **5**, respectively.

Preparation of compound TN.

A solution of 5-nitroisophtalic acid (3.0 g, 14.2 mmol) in 20 ml anhydrous dichloromethane was placed under an argon atmosphere and the solution cooled to 2°C in an ice bath. Oxalyl chloride (2.68 mL, 31.3 mmol) and dimethylformamide (41 mg, 0.57 mmol) were added. When the mixture was stirred at room temperature for 2 h, a solution of dibutylamine (4.8 mL, 28.5 mmol) and triethylamine (5.0 mL, 35.9 mmol) in anhydrous dichloromethane (20 mL) was added dropwise. After being stirred for 24 h at room temperature, the reaction mixture was diluted with 20 ml dichloromethane and shaken with 20 ml 0.5 M HCl. The organic phase was separated and treated with solution $NaHCO_3$, dried over anhydrous $MgSO_4$. The solvent was then removed and purified by silica gel column chromatography (chloroform) to give compound **TN** as a dark yellow oil (4.3 g, 9.94 mmol, 70 % yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (s, 2H), 7.75 (s, 1H), 3.55-3.35 (m, 4H), 3.20-3.05 (m, 4H), 1.70-1.25 (m, 12H), 1.15-1.00 (m, 4H), 1.00-0.85 (m, 6H), 0.80-0.60 (m, 6). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.80, 148.06, 139.46, 130.78, 121.97, 48.65, 44.56, 30.67, 29.60, 20.19, 19.60, 14.24, 13.81. HRMS (ESI): calcd for $C_{24}H_{40}N_3O_4$ [M+]$^+$: 434.30133, found: 434.30118.

Preparation of compound TA.

A flask equipped with a stirring bar containing a solution of compound **TN** (2.0 g, 4.6 mmol) in 20 ml dry ethanol was placed in an oil bath at 70°C under an argon atmosphere and $SnCl_2{\cdot}2H_2O$ (2.1 g, 9.3 mmol) was added. The mixture was further stirred for 1 h and then allowed to cool down and then poured into ice. The pH is made basic to 10 by the addition of 5% aqueous NaOH and the resulting basic mixture is kept one hour under stirring. The aqueous mixture was extracted four times with ethyl acetate, the organic phases were collected and washed with brine and dried over anhydrous $MgSO_4$. The solvent was then removed under reduced pressure to give a yellow oil (1.77g, 4.4 mmol, 96% yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 6.50 (s, 2H), 6.25 (s, 1H), 5.44 (s, 2H), 3.45-3.35 (m, 4H), 3.30-3.05 (m, 4H), 1.60-1.30 (m, 8H), 1.40-1.20 (m, 4H), 1.20-1.00 (m, 4H), 1.00-0.85 (m, 6H), 0.85-0.60 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.78, 149.22, 138.49, 112.10, 111.19, 48.34, 43.87, 30.75, 29.67, 20.11, 19.73, 14.25, 13.91. HRMS (ESI): calcd for $C_{24}H_{42}N_3O_2$ [M+]$^+$: 404.32715, found: 404.32698.

Preparation of compound TM.

To the solution of amine **TA** (1.89 g, 4.7 mmol) in methanol were added 3,4-dimethoxy-3-cyclobutene-1,2-dione (0.67 g, 4.7 mmol) and triethylamine (0.69 mL, 5 mmol) at room temperature. After being stirred for 24 h, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography (5% methanol in chloroform) to give compound **TM** as a dark yellow solid (1.54 g, 3.0 mmol, 65% yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (s, 1H), 7.38 (s, 2H), 6.92 (s, 1H), 4.39 (s, 3H), 3.75-3.30 (m, 4H), 3.25-2.95 (m, 4H), 1.7-1.20 (m, 12H), 1.15-0.95 (m, 4H), 0.95-0.80 (m, 6H), 0.80-0.55 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 188.14, 184.69, 184.57, 179.62, 169.49, 138.96, 138.62, 119.76, 117.72, 61.10, 48.39, 44.12, 30.61, 29.65, 20.08, 19.66, 14.25, 13.81. HRMS (ESI): calcd for $C_{29}H_{44}N_3O_5$[M+]+:514.32755, found: 514.32725.

Preparation of receptor 4.

To the solution of 3,4-dibutoxyaniline (0.15 g, 1.0 mmol) in methanol (10 mL) was added compound **TM** (0.51 g, 1.0 mmol) at room temperature, and TEA (0.27 mL, 2.0 mmol). After being stirred for 24 h, the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (5 % methanol in chloroform) to give receptor **4** as an off-white solid (0.44 g, 0.70 mmol, 70 % yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.94 (s, 1H), 9.79 (s, 1H), 7.47 (s, 2H), 7.30 (s, 1H), 7.00-6.80 (m, 3H), 3.90-3.65 (d, 6H), 3.55-3.35 (m, 4H), 3.30-3.05 (m, 4H), 1.65-1.25 (m, 12H), 1.20-1.00 (m, 4H), 1.00-0.85 (m, 6H), 0.85-0.60 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.76, 181.46, 169.56, 166.26, 165.12, 149.77, 145.79, 139.31, 139.15, 132.37, 118.88, 116.79, 112.93, 110.70, 104.38, 56.21, 55.90, 48.47, 44.14, 30.67, 29.67, 20.10, 19.70, 14.24, 13.85. HRMS (ESI): calcd for $C_{36}H_{51}N_4O_6Na$ [M+ Na]$^+$: 635.38032, found: 635.37995.

Preparation of receptor 5.

To the solution of compound **M1** (0.44 g, 1.0 mmol) in methanol (10 mL) was added compound **TM** (0.51 g, 1.0 mmol) at room temperature, and triethylamine (0.27 mL, 2.0 mmol). After being stirred for 24 h, the reaction mixture was concentrated and the residue was purified by silica gel column chromatography (5% methanol in chloroform) to give receptor **5** as a yellow solid (0.63 g, 0.78 mmol, 78 % yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.93 (s, 1H), 9.78 (s, 1H), 7.46 (s, 2H), 7.27 (s, 1H), 7.05-6.90 (m, 1H), 6.90-6.75 (m, 2H), 4.20-4.00 (m, 4H), 3.85-3.65 (m, 4H), 3.65-3.48 (m, 12H), 3.48-3.35 (m, 4H), 3.30-3.10 (m, 4H), 1.70-1.20 (m, 12H), 1.15-1.00 (m, 4H), 1.00-0.85 (m, 6H), 0.80-0.60 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 182.83, 181.54, 169.56, 166.30, 165.18, 149.17, 145.14, 139.27, 139.17, 132.39, 118.89, 116.81, 114.37, 110.97, 105.51, 70.8-70.0 (m), 69.4-68.8 (m), 68.52, 48.47, 44.14, 30.66, 29.67, 20.09, 19.69, 14.29, 13.88. HRMS (ESI): calcd for $C_{44}H_{64}N_4O_{10}Na$ [M+ Na]$^+$: 831.45146, found: 831.45162.

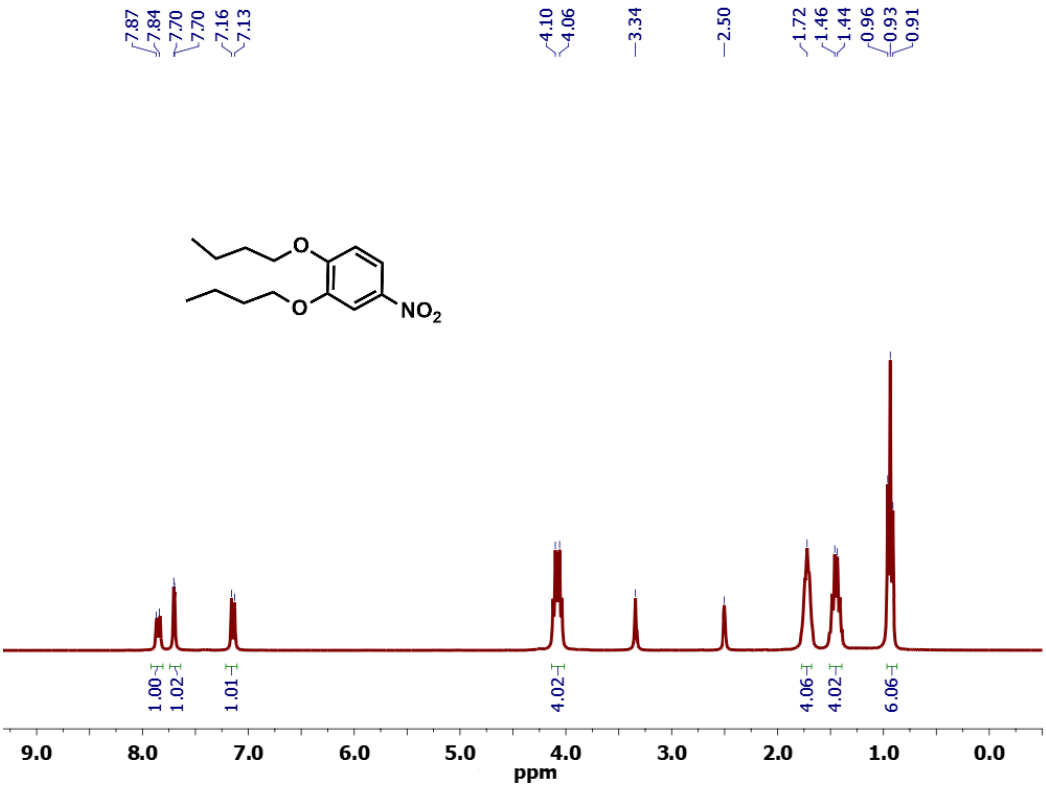


Figure S2. ^{1}H NMR spectrum of 1,2-dibutoxy-4-nitrobenzene in DMSO-d_6.

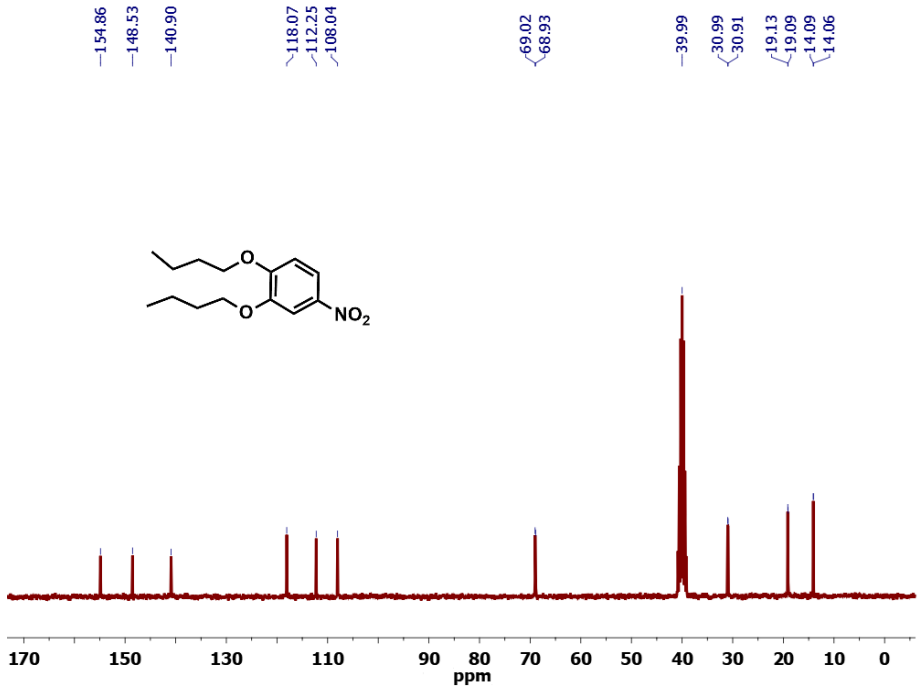


Figure S3. ^{13}C NMR spectrum of 1,2-dibutoxy-4-nitrobenzene in DMSO-d_6.

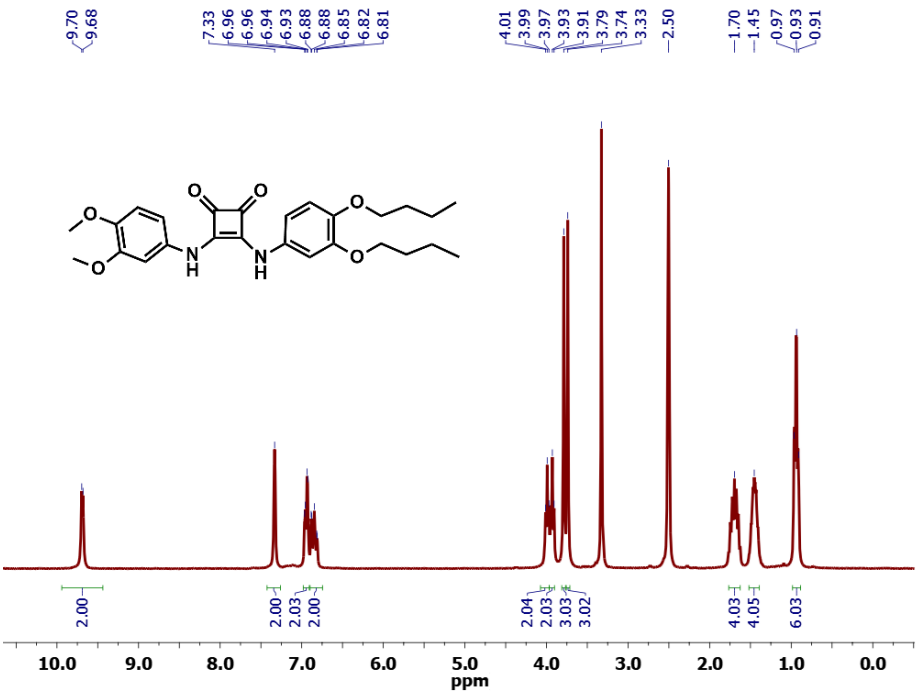


Figure S4. ^{1}H NMR spectrum of receptor **1** in DMSO-d_6.

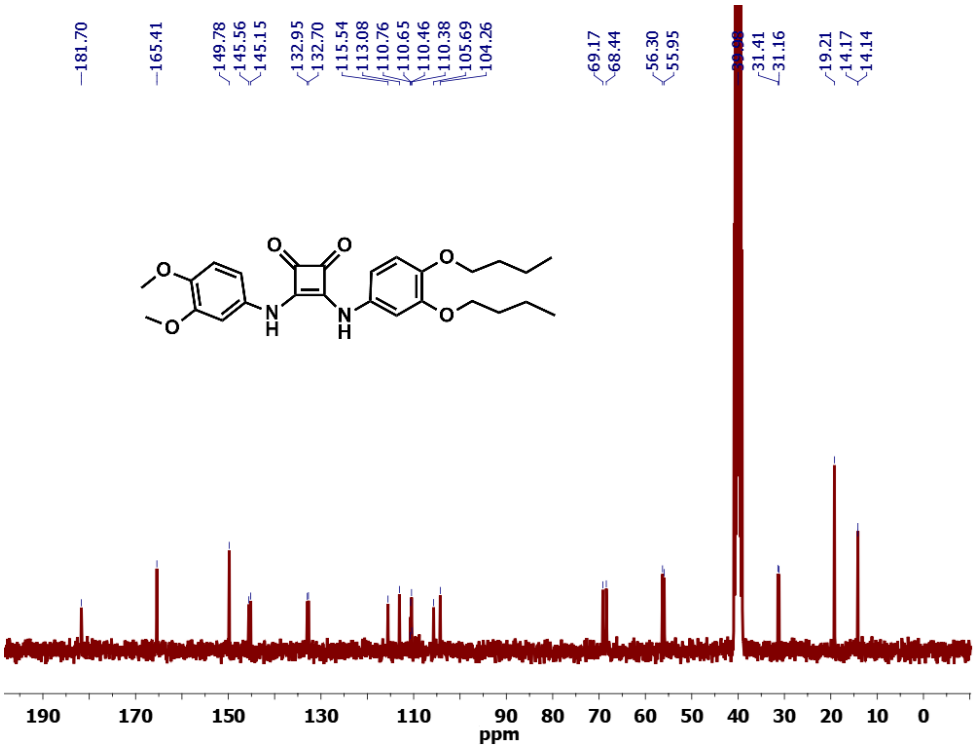


Figure S5. ^{13}C NMR spectrum of receptor **1** in DMSO-d_6.

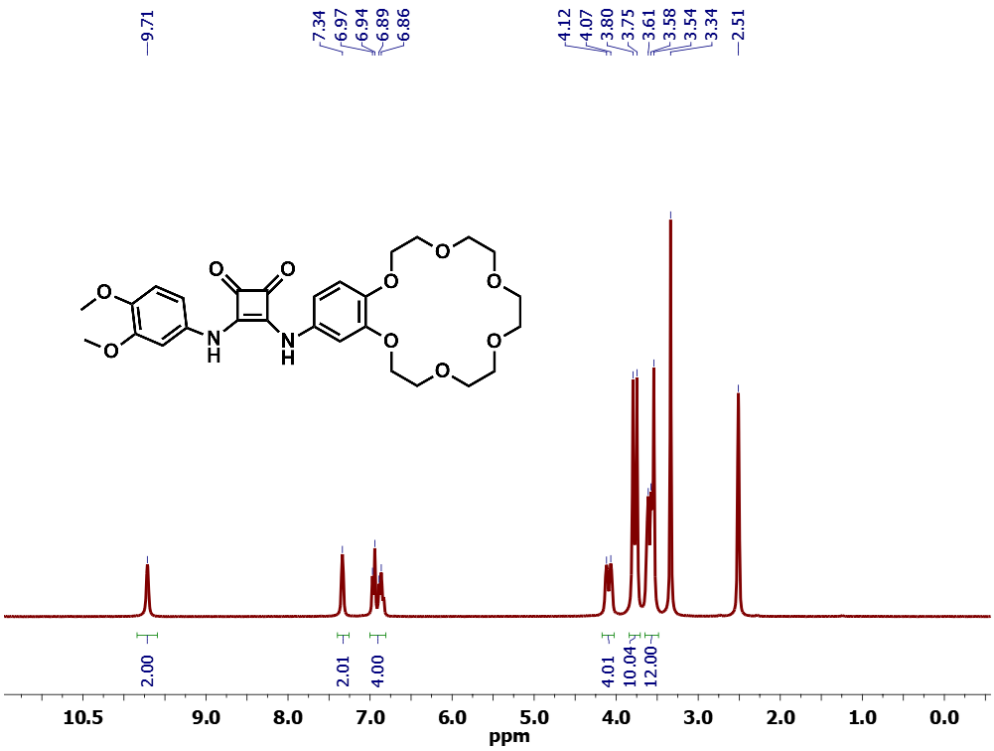


Figure S6. ^{1}H NMR spectrum of receptor **2** in DMSO-d_6.

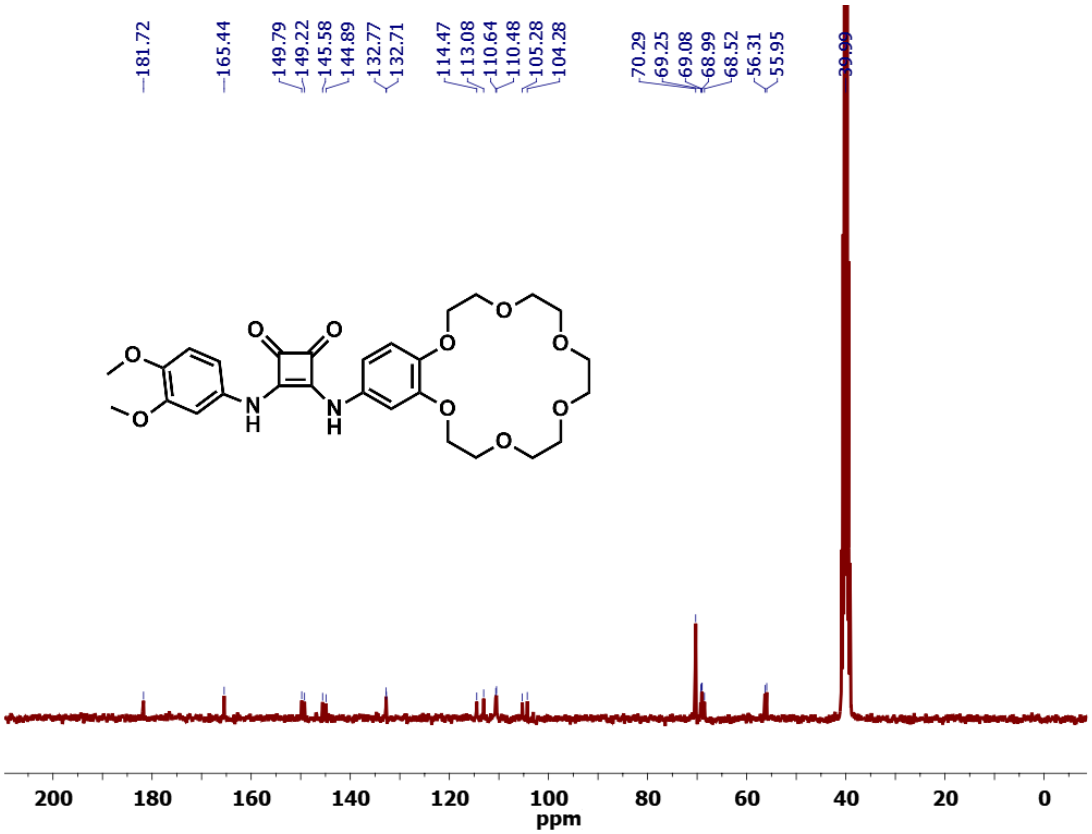


Figure S7. ^{13}C NMR spectrum of receptor **2** in DMSO-d_6.

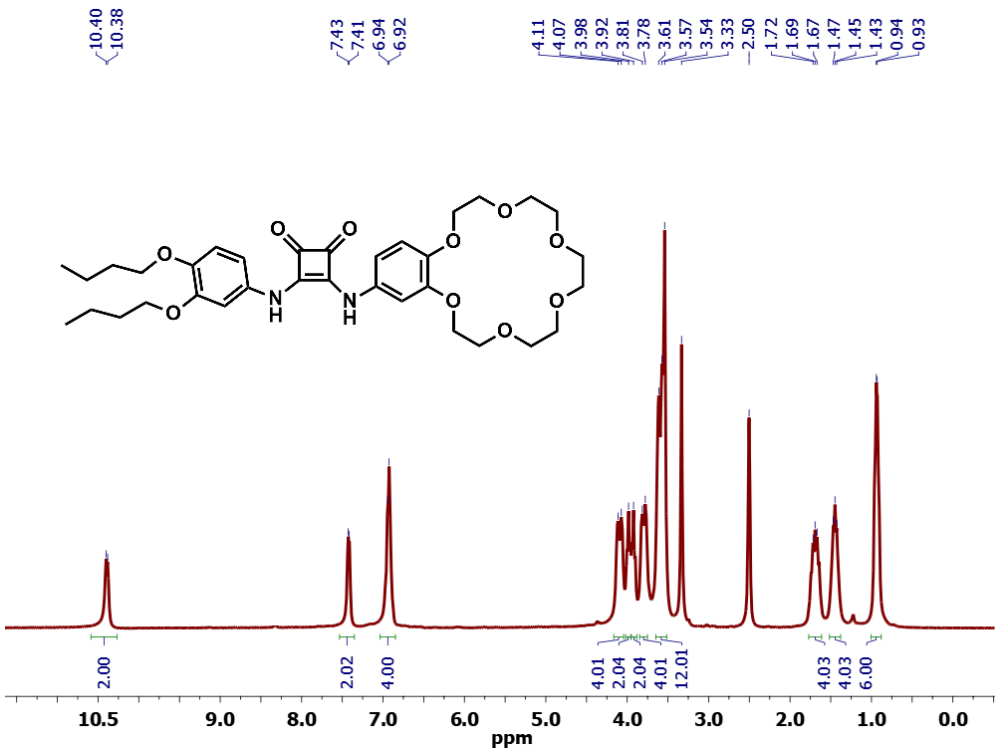


Figure S8. ^{1}H NMR spectrum of receptor **3** in DMSO-d_6.

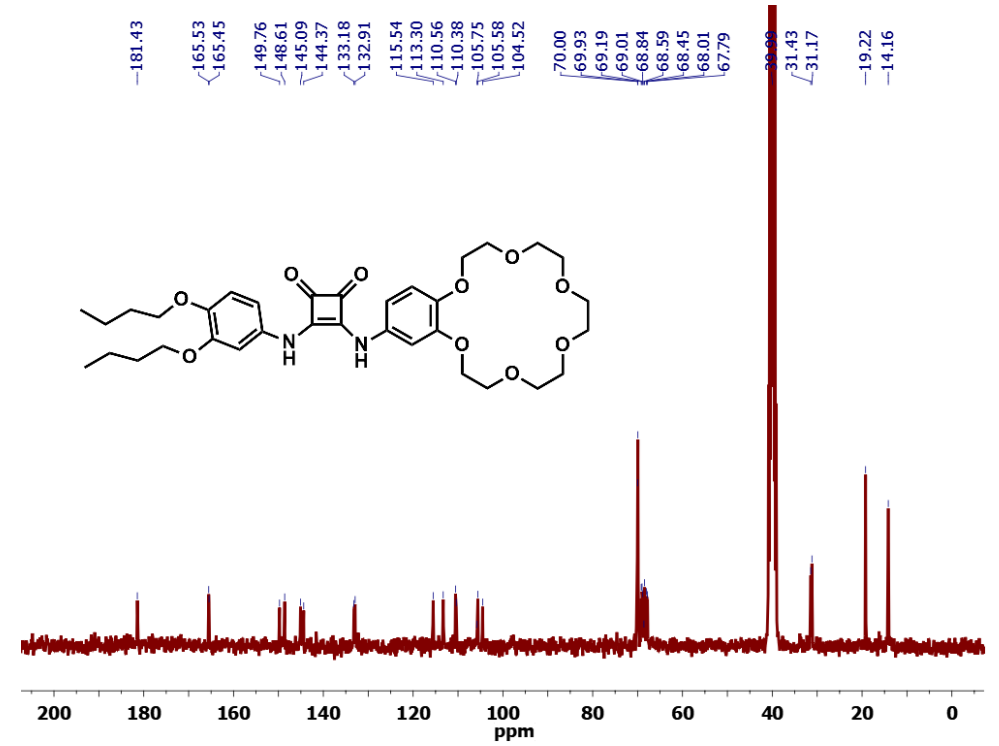


Figure S9. ^{13}C NMR spectrum of receptor **3** in DMSO-d_6.

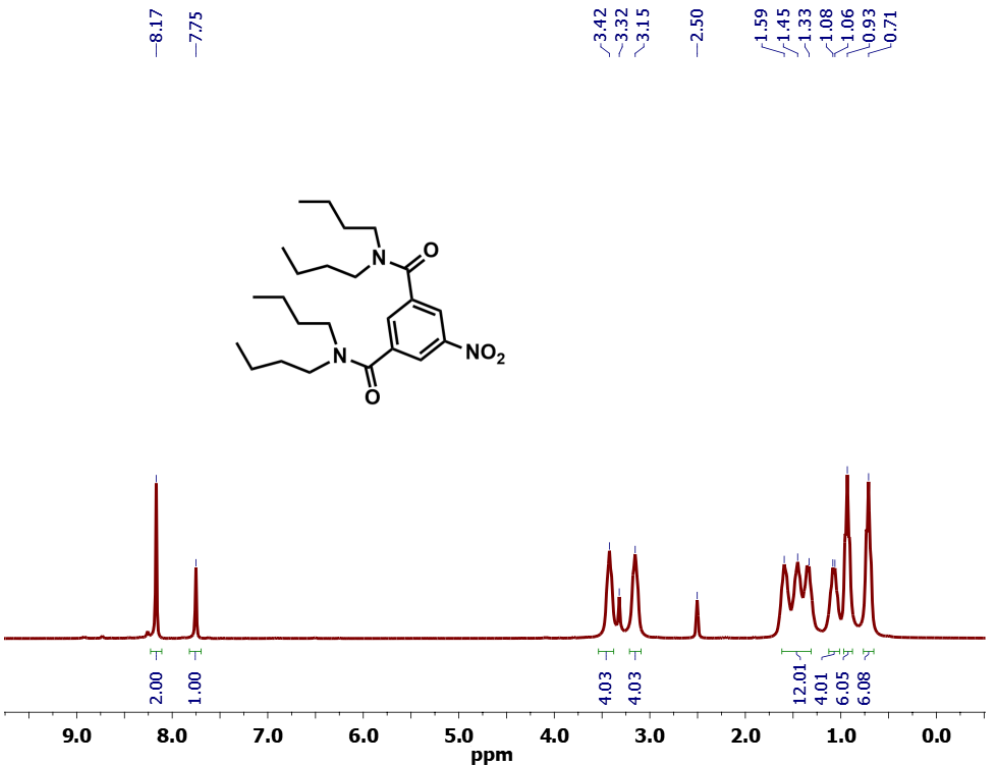


Figure S10. ^{1}H NMR spectrum of compound TN in DMSO-d_6.

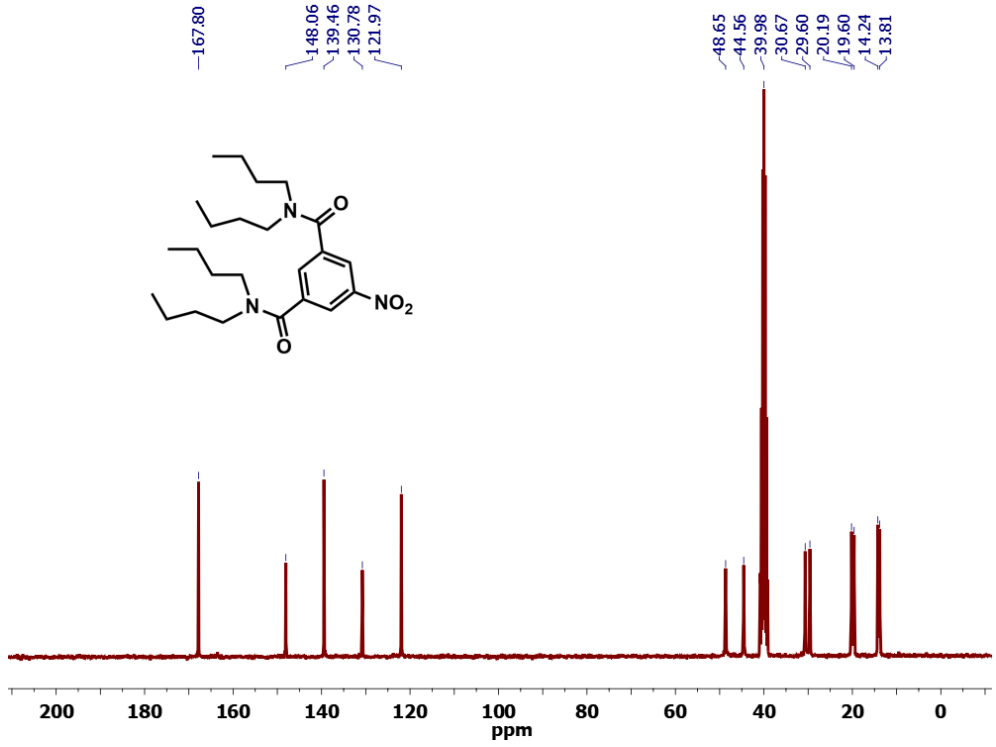


Figure S11. ^{13}C NMR spectrum of compound TM in DMSO-d_6.

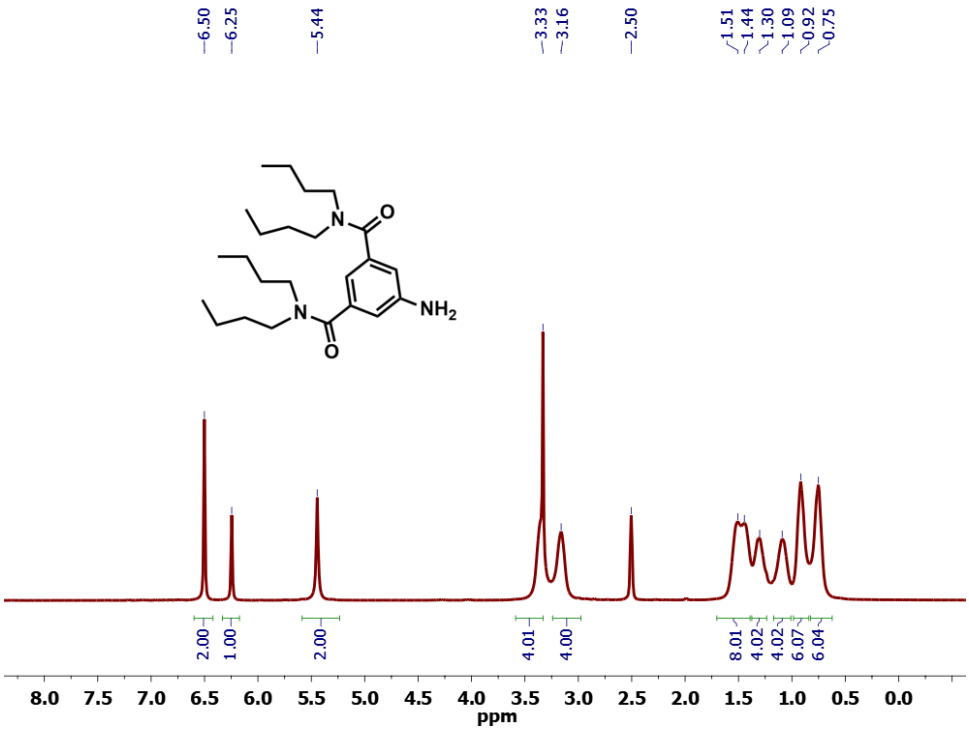


Figure S12. ^{1}H NMR spectrum of compound TA in DMSO-d_6.

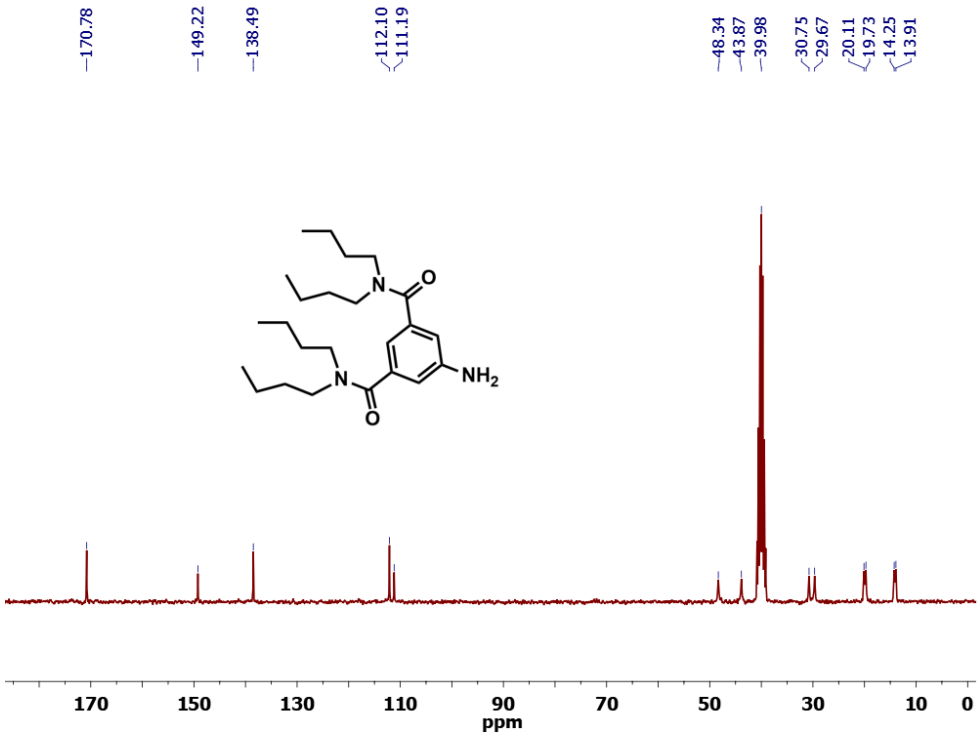


Figure S13. ^{13}C NMR spectrum of compound TA in DMSO-d_6.

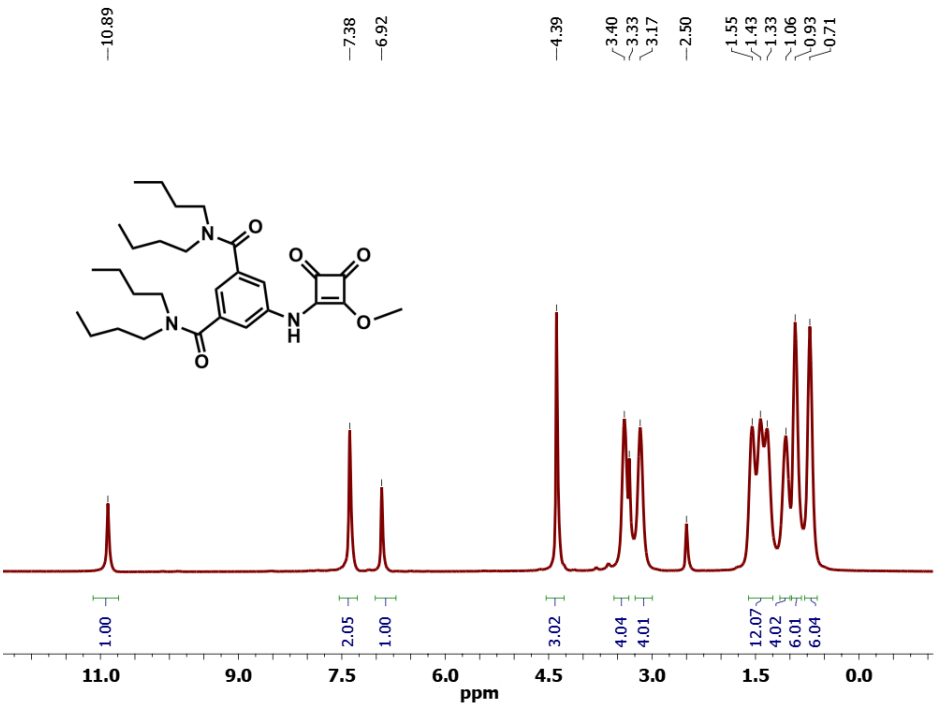


Figure S14. ^{1}H NMR spectrum of compound TM in DMSO-d_6.

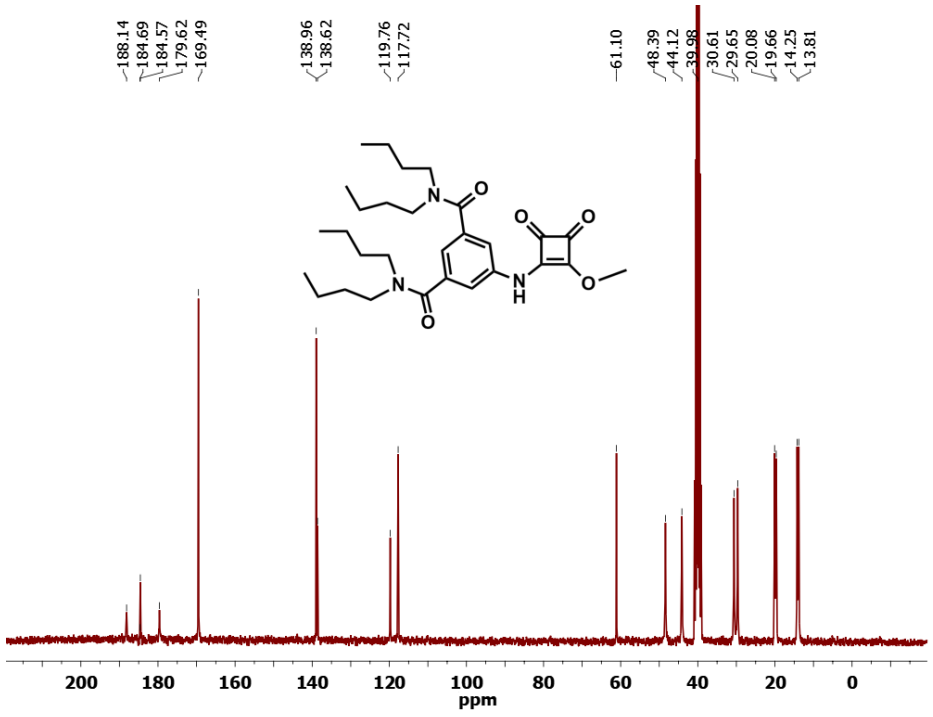


Figure S15. ^{13}C NMR spectrum of compound TM in DMSO-d_6.

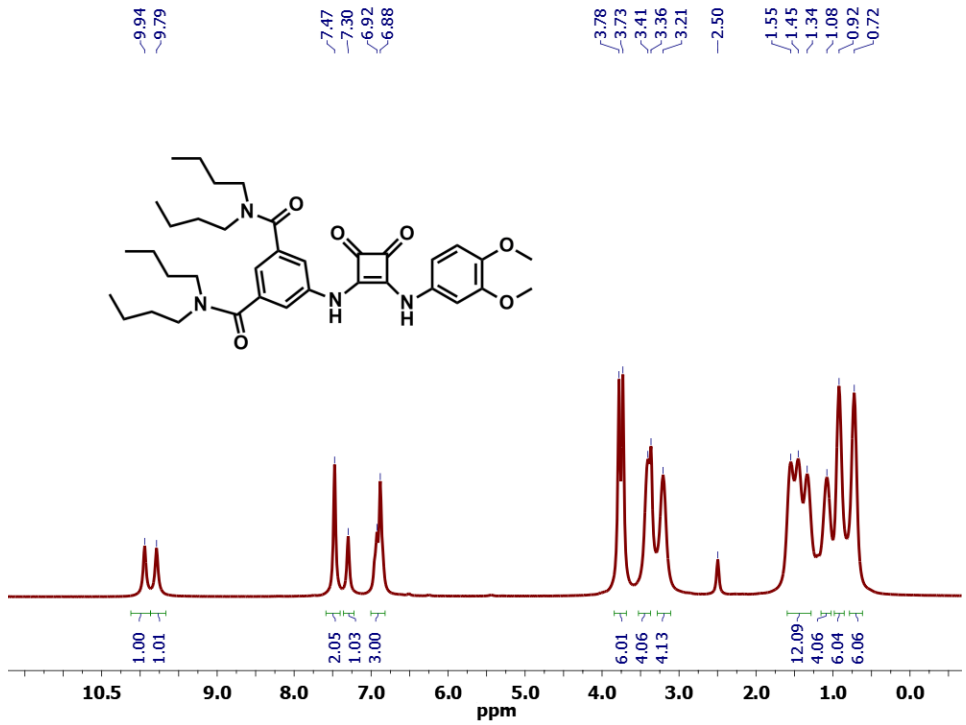


Figure S16. ^{1}H NMR spectrum of receptor **4** in DMSO-d_6.

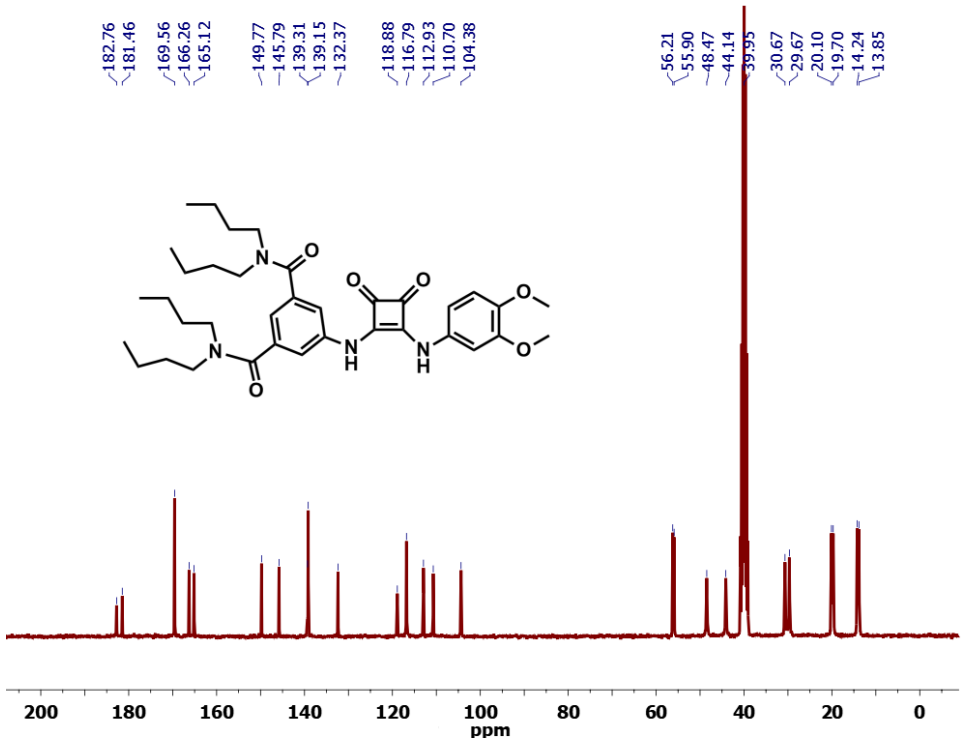


Figure S17. ^{13}C NMR spectrum of receptor **4** in DMSO-d_6.

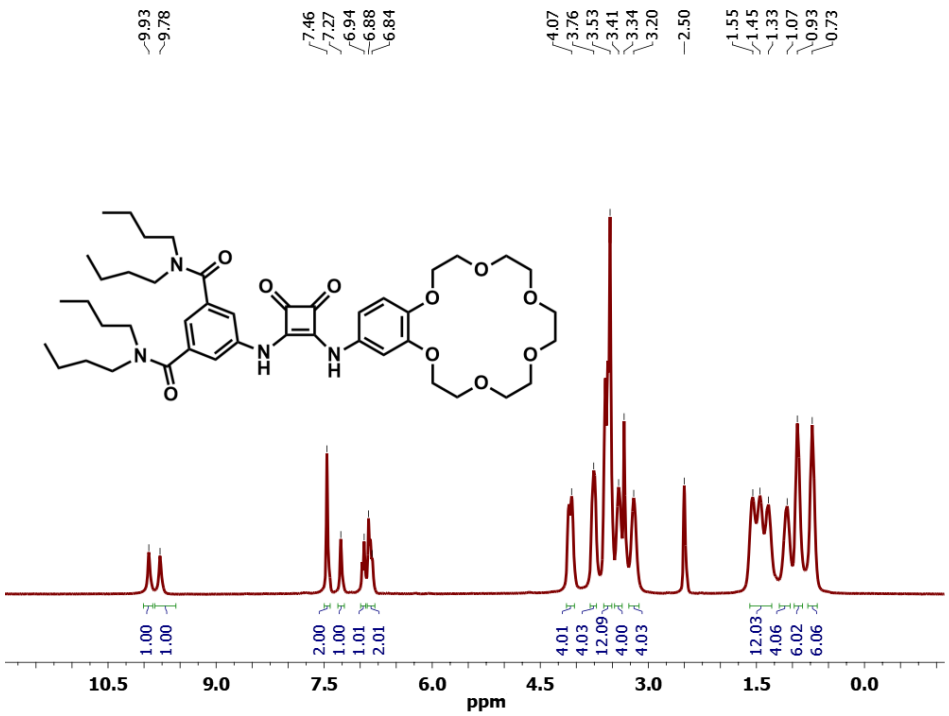


Figure S18. ^{1}H NMR spectrum of receptor **5** in DMSO-d_6.

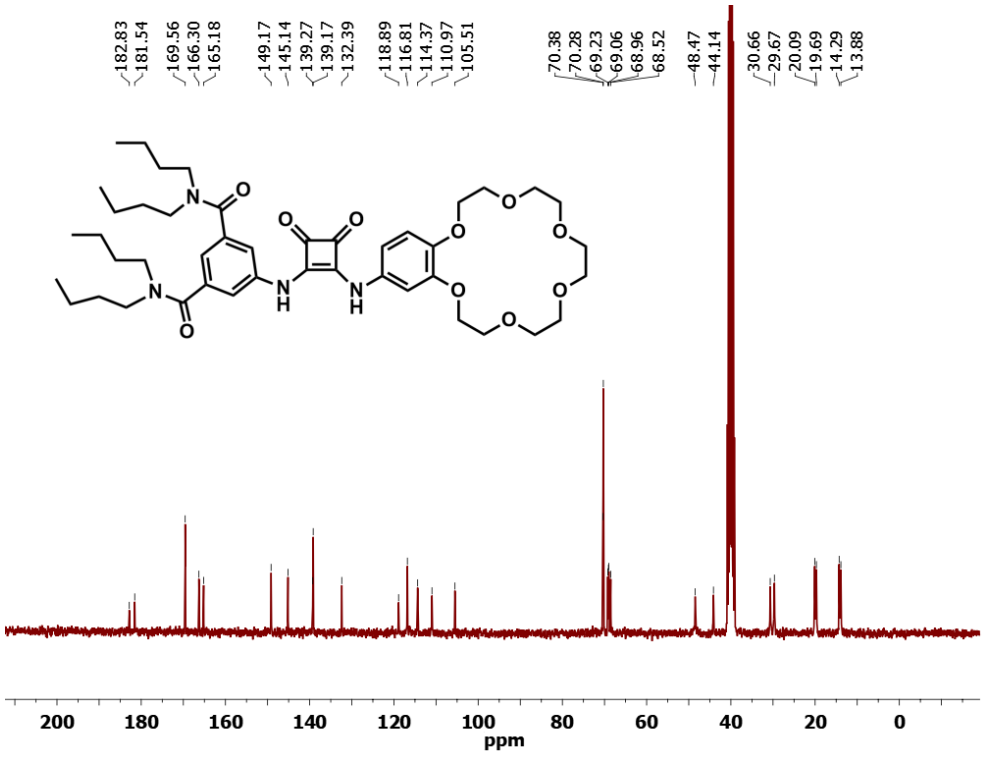


Figure S19. ^{13}C NMR spectrum of receptor **5** in DMSO-d_6.

S3. UV-Vis titrations

UV–vis titrations: experiments were performed in CH_3CN solution at 298 K using a Hitachi U-2910 spectrophotometer. To a 10 mm cuvette was added 2.5 mL of freshly prepared solution of studied receptor ([**1**] = 2.0×10^{-5} M, [**3**] = 2.6×10^{-5} M, [**4**] = 2.0×10^{-5} M, [**5**] = 3.0×10^{-5} M), and in case of ion-pair binding studies, 1 mol equivalent of cation (KPF_6 or $NaClO_4$) was added before titrations. Small aliquots of ca. 1.5×10^{-3} M TBAX solution containing suitable the receptor at the same concentration as in cuvette were added and a spectrum was acquired after each addition. The resulting titration data were analyzed using BindFit (v0.5), available online at http://supramolecular.org. Each titration was carried out in triplicate. Reported values are calculated as weighted arithmetic mean, where the weights were the errors obtained for each value separately. The given uncertainty of the association constants is the largest of the variance (external or internal).

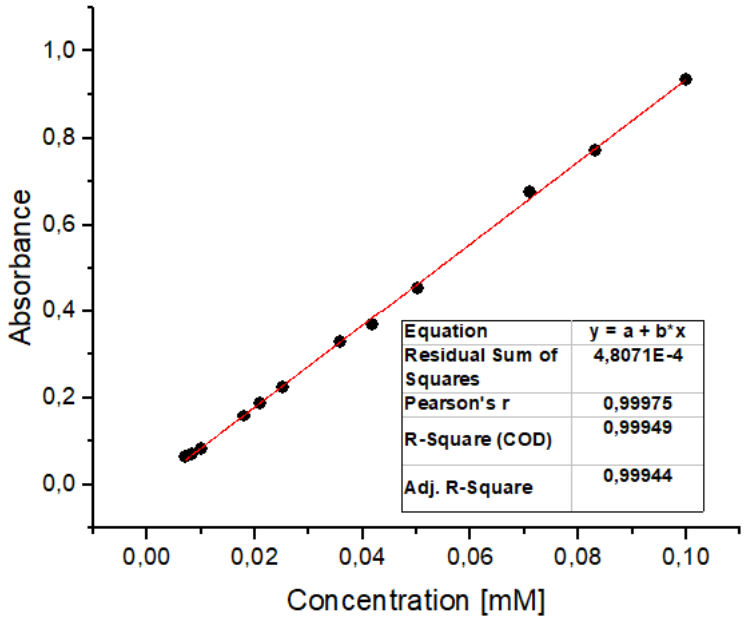


Figure S20. Typical dilution curve of receptor **5** (360 nm).

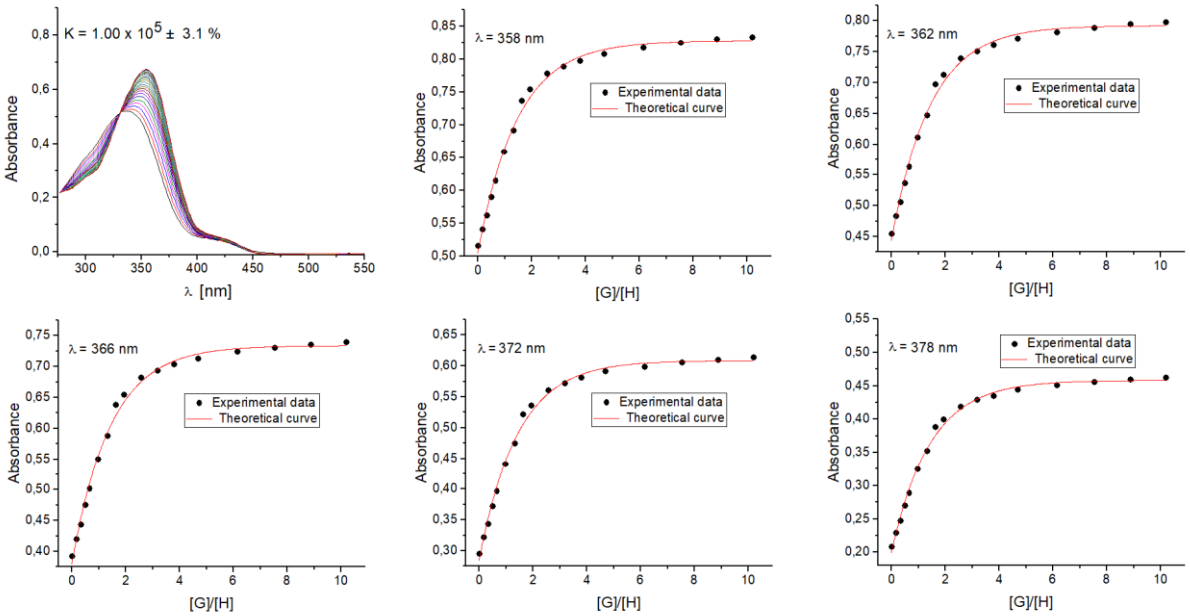


Figure S21. UV-Vis titration of receptor **1** with TBACl in CH_3CN and selected binding isotherms.

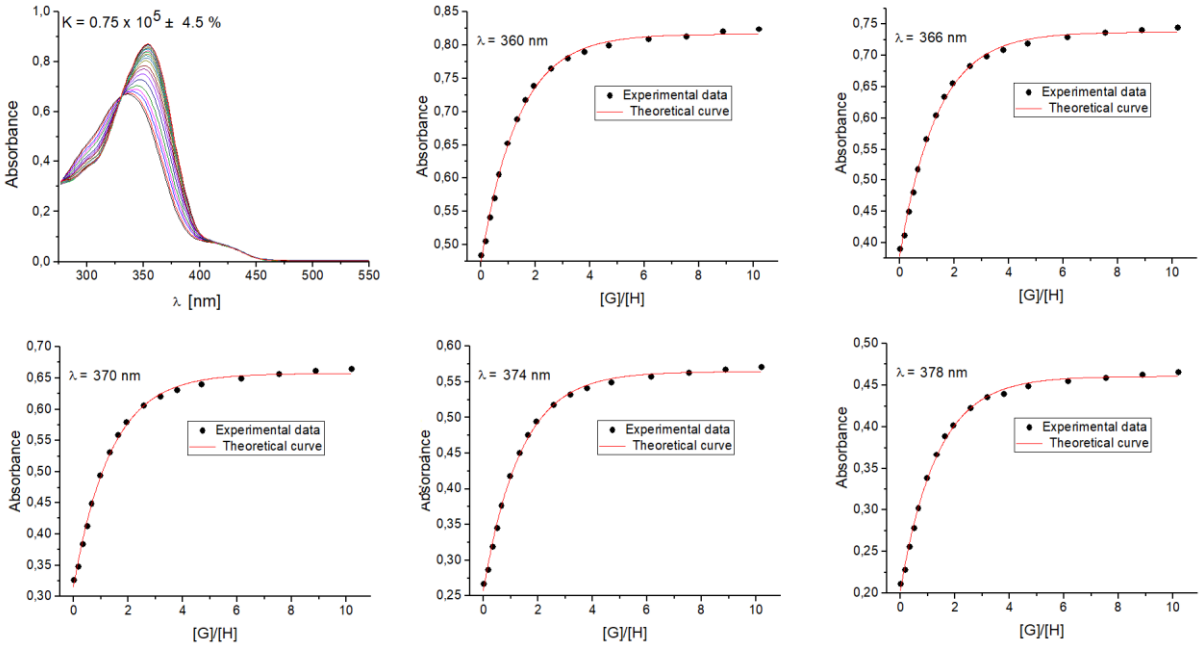


Figure S22. UV-Vis titration of receptor **1** with TBACl in the presence of 1 equivalent of KPF_6 in CH_3CN and selected binding isotherms.

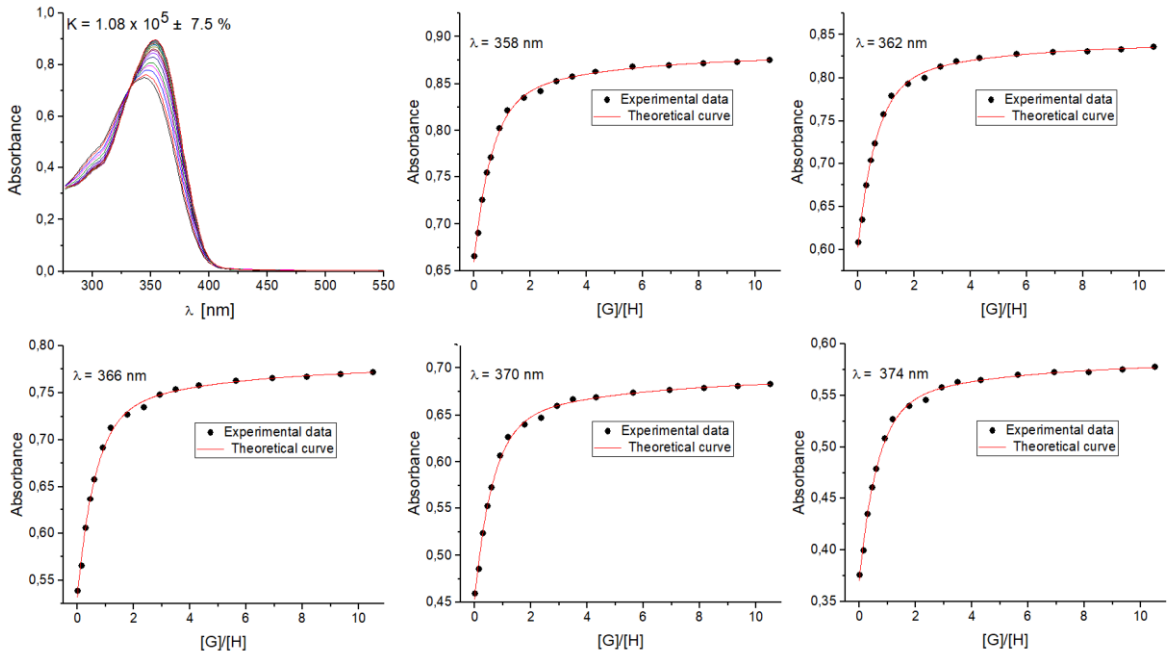


Figure S23. UV-Vis titration of receptor **3** with TBACl in CH_3CN and selected binding isotherms.

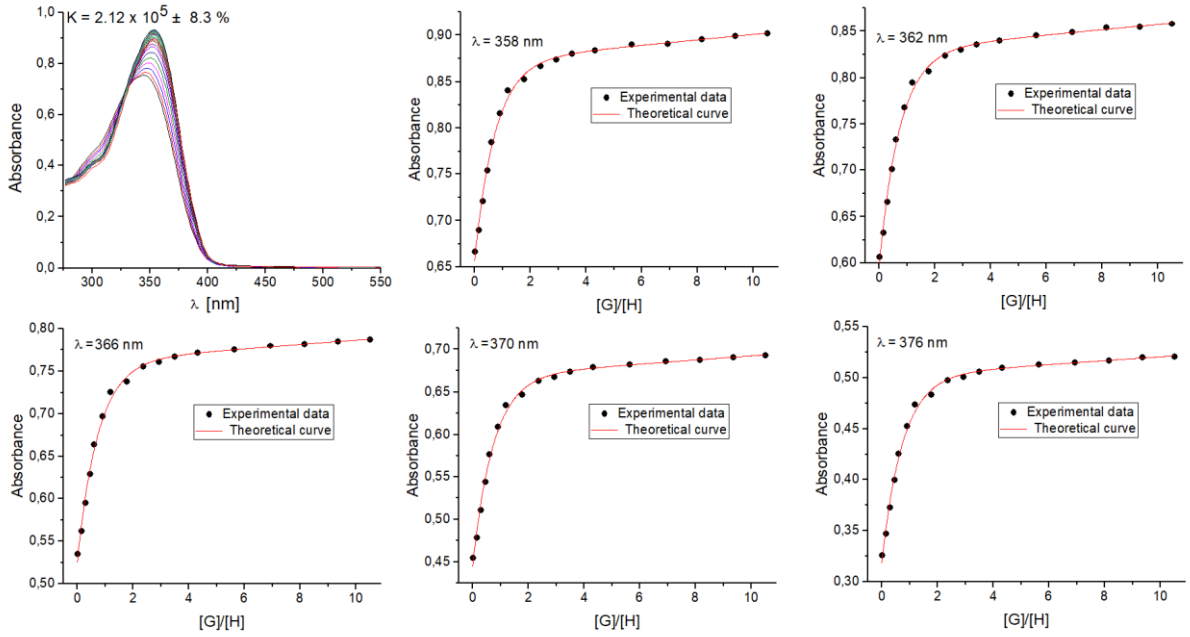


Figure S24. UV-Vis titration of receptor **3** with TBACl in the presence of 1 equivalent of KPF_6 in CH_3CN and selected binding isotherms.

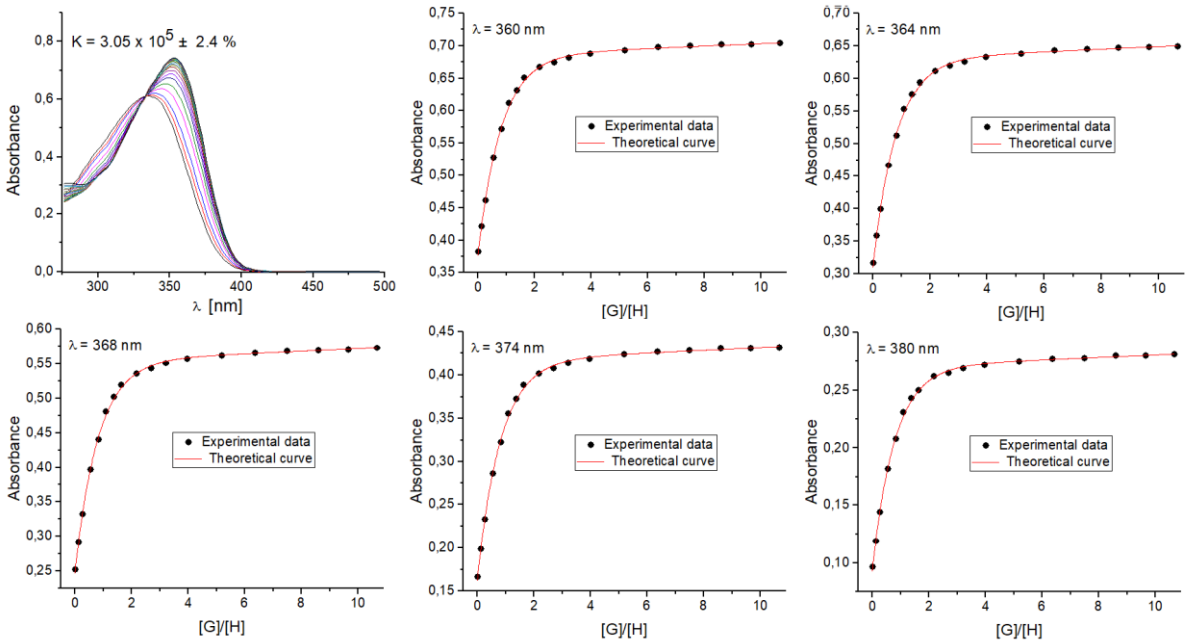


Figure S25. UV-Vis titration of receptor **4** with TBACl in CH_3CN and selected binding isotherms.

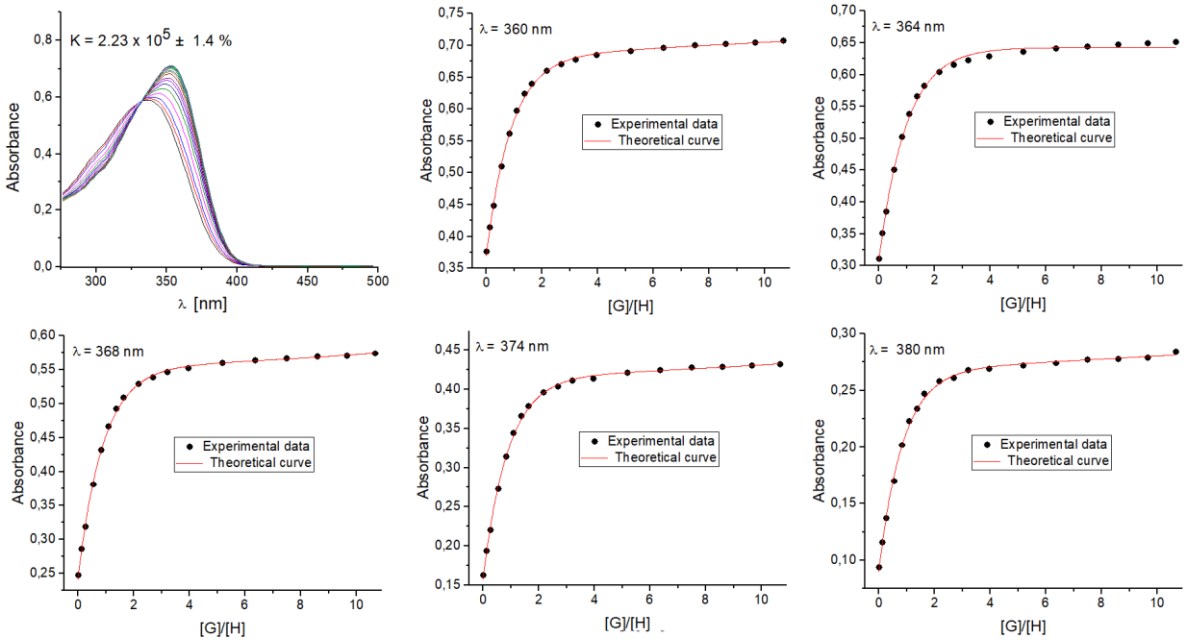


Figure S26. UV-Vis titration of receptor **4** with TBACl in the presence of 1 equivalent of KPF_6 in CH_3CN and selected binding isotherms.

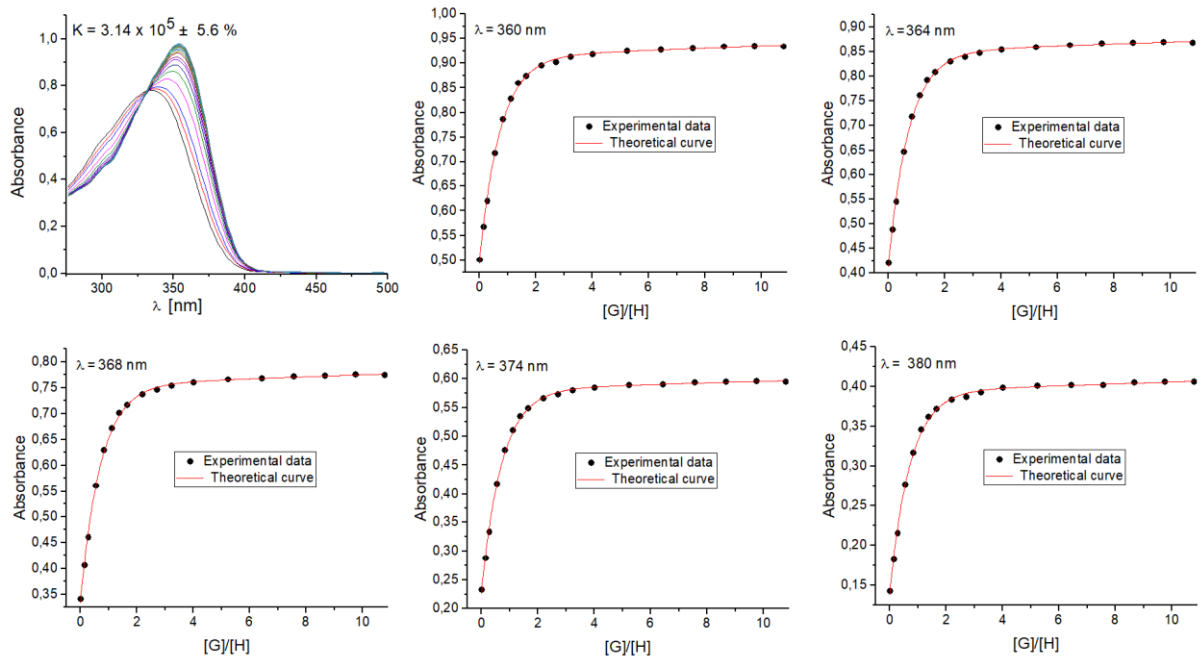


Figure S27. UV-Vis titration of receptor **5** with TBACl in CH_3CN and selected binding isotherms.

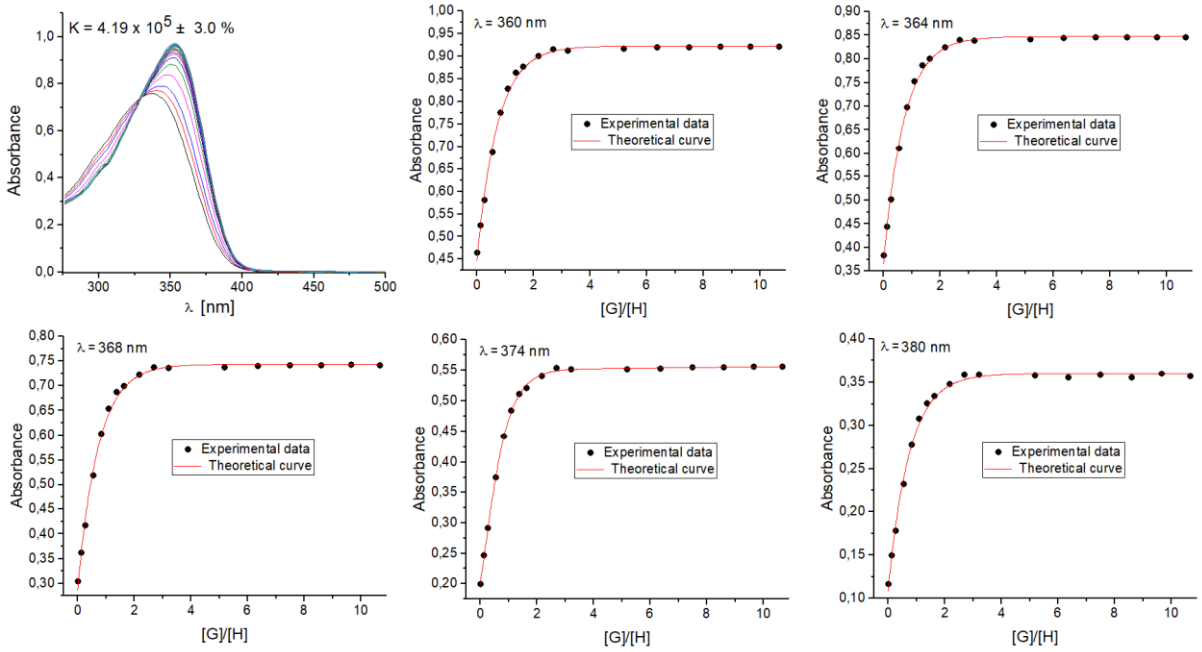


Figure S28. UV-Vis titration of receptor **5** with TBACl in the presence of 1 equivalent of $NaClO_4$ in CH_3CN and selected binding isotherms.

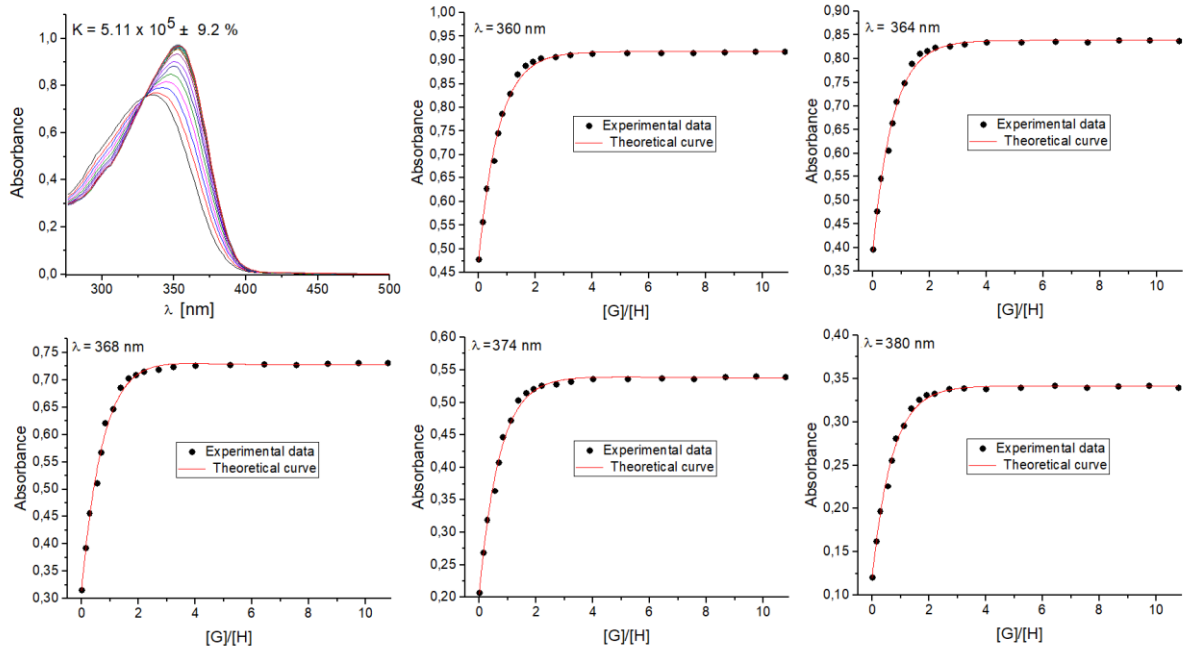


Figure S29. UV-Vis titration of receptor **5** with TBACl in the presence of 1 equivalent of KPF_6 in CH_3CN and selected binding isotherms.

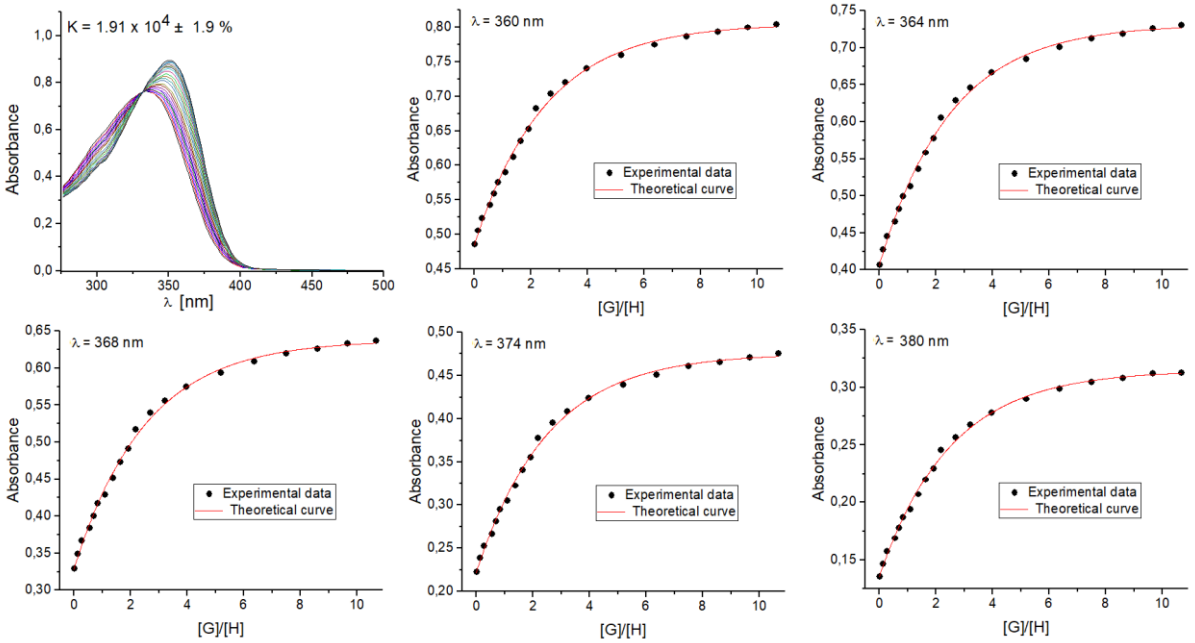


Figure S30. UV-Vis titration of receptor **5** with TBABr in CH_3CN and selected binding isotherms.

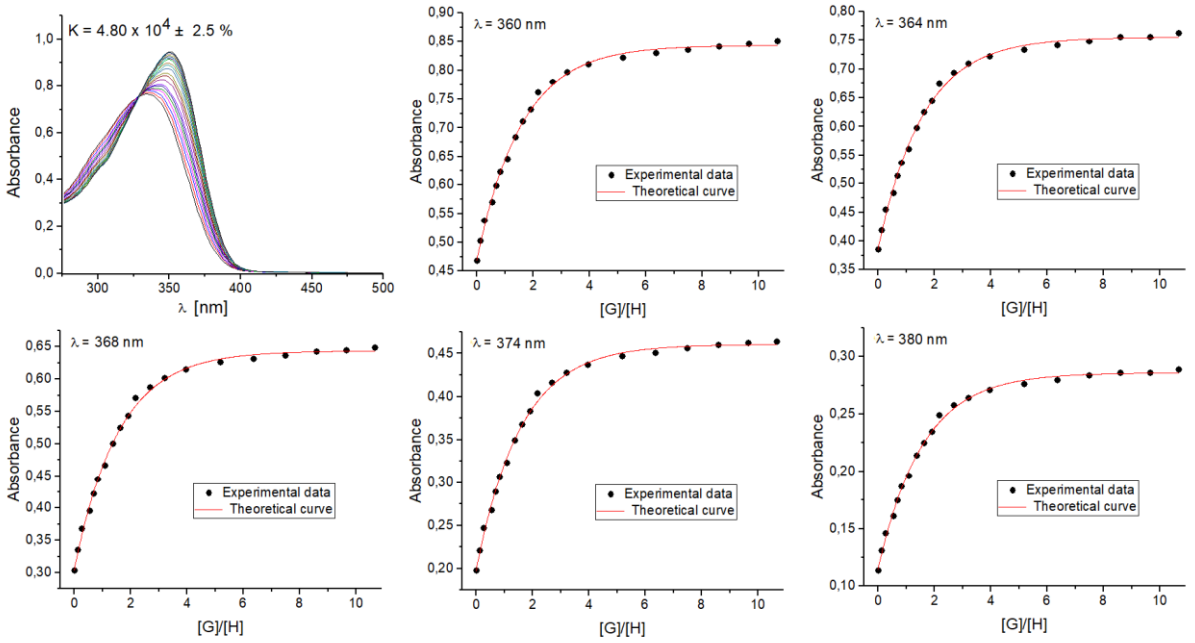


Figure S31. UV-Vis titration of receptor **5** with TBABr in the presence of 1 equivalent of KPF_6 in CH_3CN and selected binding isotherms.

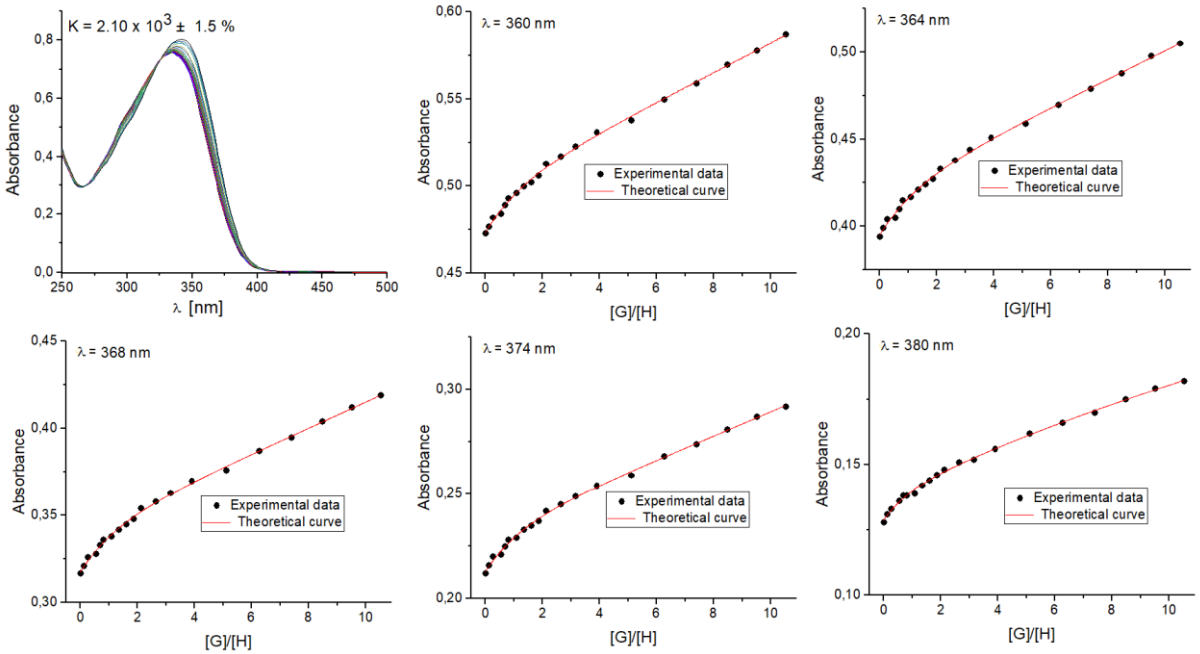


Figure S32. UV-Vis titration of receptor **5** with $TBANO_3$ in CH_3CN and selected binding isotherms.

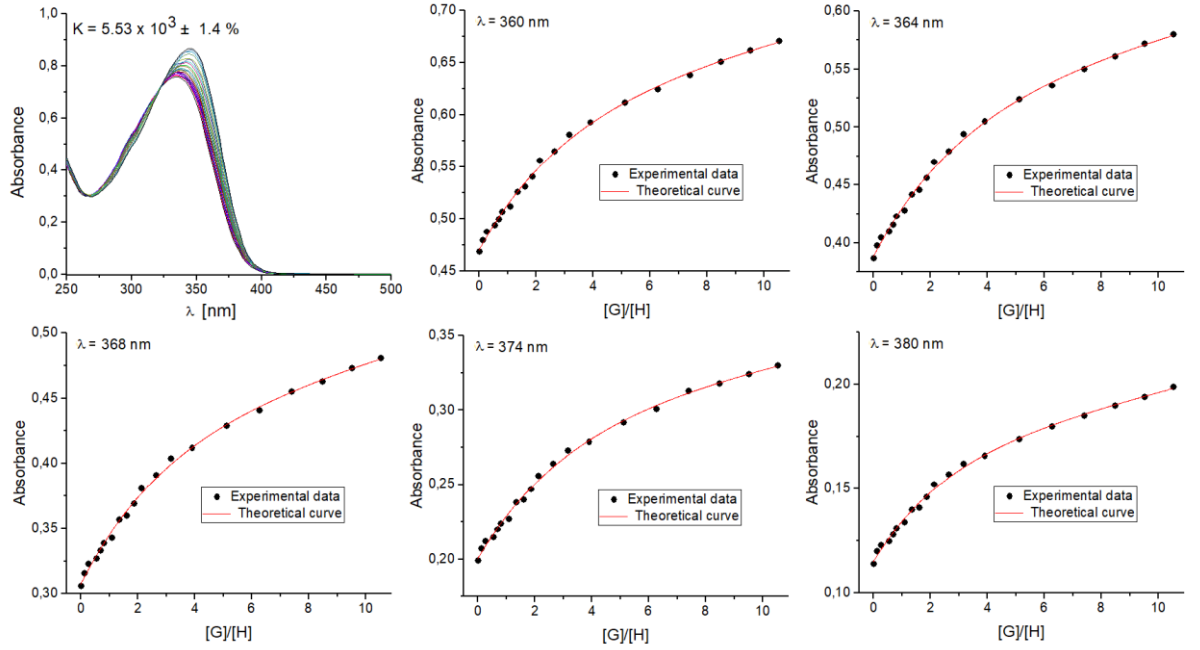


Figure S33. UV-Vis titration of receptor **5** with $TBANO_3$ in the presence of 1 equivalent of KPF_6 in CH_3CN and selected binding isotherms.

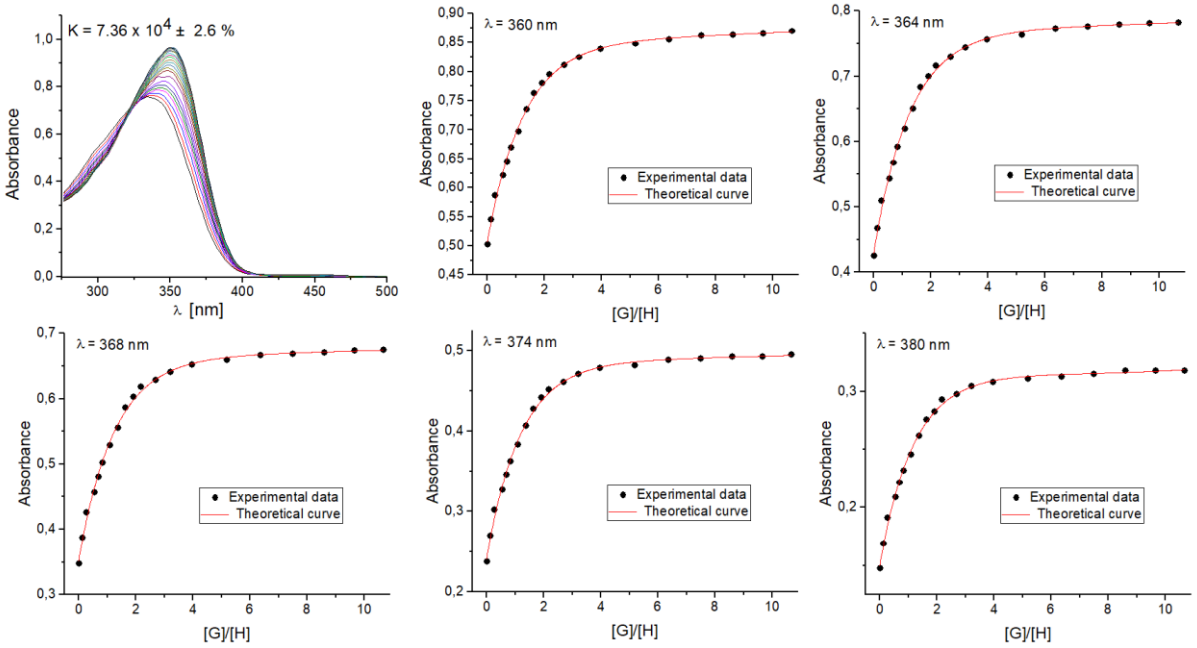


Figure S34. UV-Vis titration of receptor **5** with $TBANO_2$ in CH_3CN and selected binding isotherms.

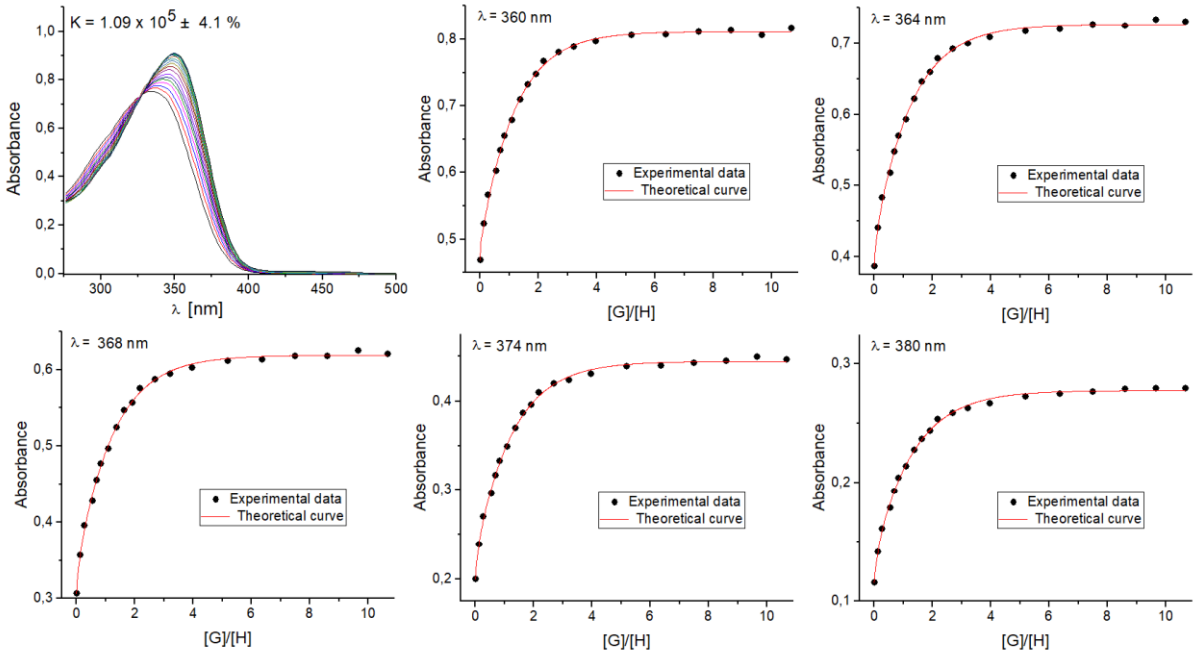


Figure S35. UV-Vis titration of receptor **5** with $TBANO_2$ in the presence of 1 equivalent of KPF_6 in CH_3CN and selected binding isotherms.

S4. NMR titrations

The ^1H NMR titrations were conducted at 298 K in DMSO-d_6. In each case, 500 µL of freshly prepared 3.0 mM solution of **2** or **5** was added to a 5 mm NMR tube. In the case of ion pair titrations, the receptors were firstly pretreated with one equivalent of KPF_6. Then small aliquots of solution of TBAX, containing receptor at constant concentration, were added and a spectrum was acquired after each addition. The resulting titration data were analyzed using BindFit (v0.5), available online at http://supramolecular.org.

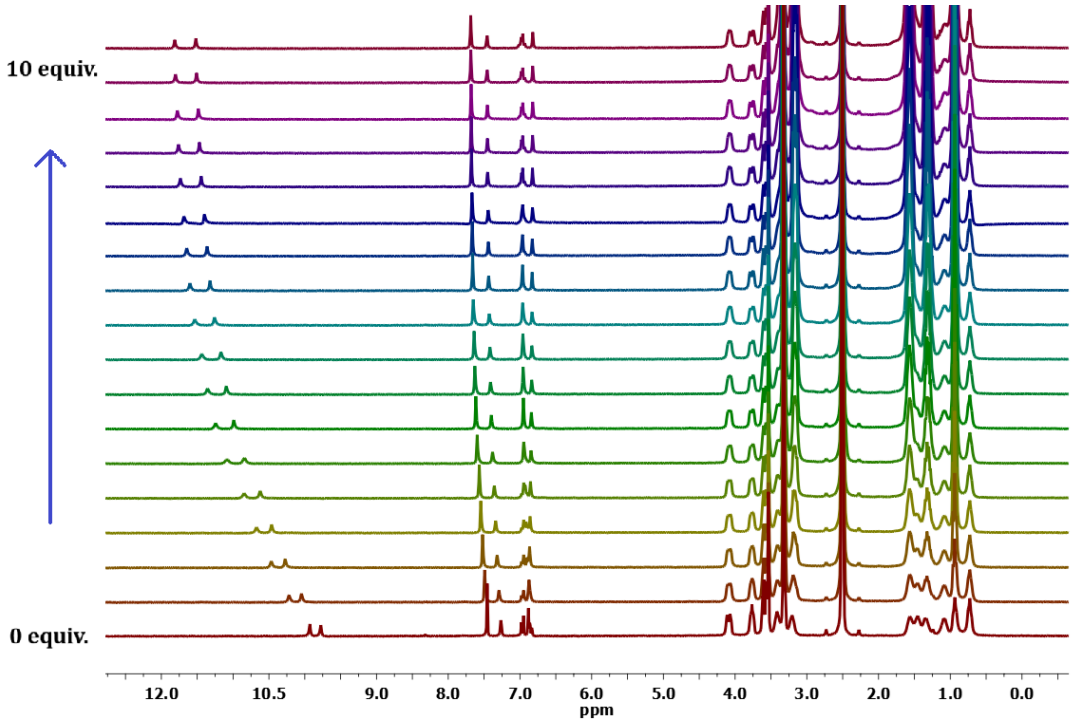


Figure S36. ^{1}HNMR spectra recorded upon titration of **5** in DMSO-d_6 with TBACl.

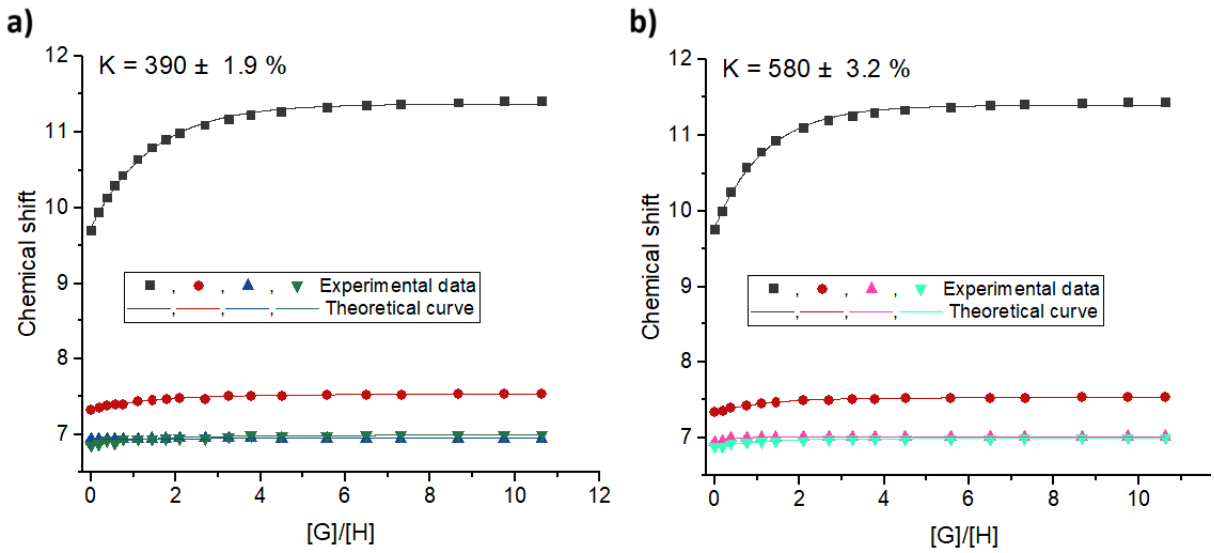


Figure S37. ^{1}H NMR titration binding isotherms of **2** in DMSO-d_6 (a) upon addition of increasing amounts of TBACl, (b) in the presence of 1 equivalent of KPF_6.

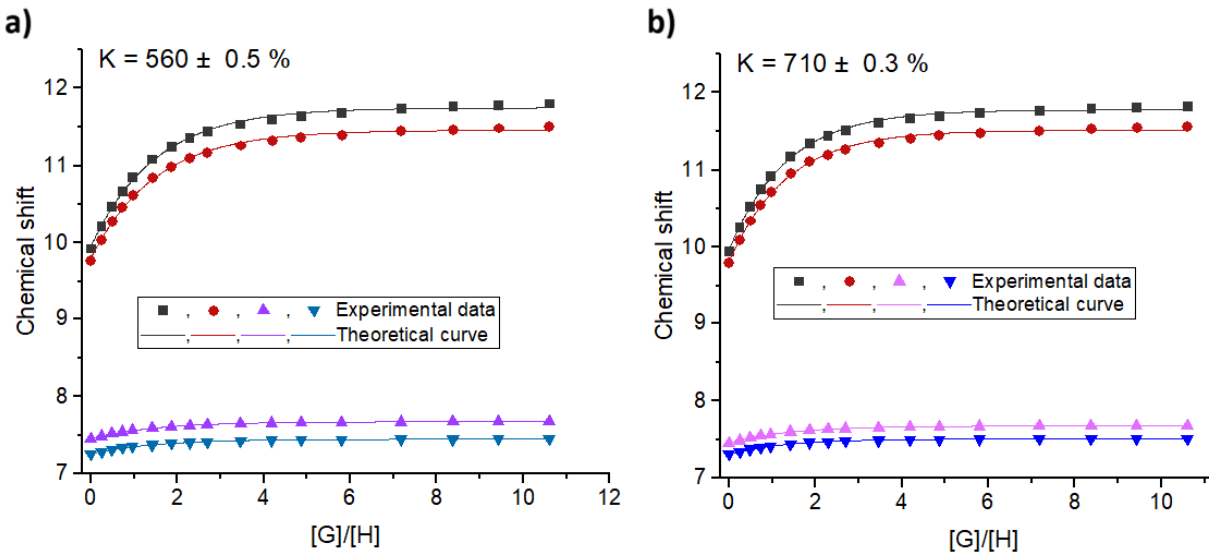


Figure S38. ^{1}H NMR titration binding isotherms of **5** in DMSO-d_6 (a) upon addition of increasing amounts of TBACl, (b) in the presence of 1 equivalent of KPF_6.

S5. Crystal data

The X-ray measurement of **2** was performed at 130.0(5) K on a Bruker D8 Venture PhotonII diffractometer equipped with a TRIUMPH monochromator and a MoKα fine focus sealed tube (λ = 0.71073 Å). A total of 2584 frames were collected with Bruker APEX3 program.[S2] The frames were integrated with the Bruker SAINT software package[S3] using a narrow-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 42472 reflections to a maximum ϑ angle of 25.05° (0.84 Å resolution), of which 5240 were independent (average redundancy 8.105, completeness = 99.8%, R_{int} = 6.05%, R_{sig} = 3.20%) and 4661 (88.95%) were greater than $2\sigma(F^2)$. The final cell constants of a = 7.2681(8) Å, b = 10.3927(10) Å, c = 19.5823(19) Å, α = 89.272(3)°, β = 87.184(4)°, γ = 87.050(4)°, V = 1475.3(3) Å^3, are based upon the refinement of the XYZ-centroids of 9747 reflections above 20 $\sigma(I)$ with 5.695° < 2ϑ < 51.16°. Data were corrected for absorption effects using the Multi-Scan method.[S4] The ratio of minimum to maximum apparent transmission was 0.804. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.555 and 0.975.

The structure was solved and refined using SHELXTL Software Package[S5,S6] using the space group $P\bar{1}$, with Z = 2 for the formula unit, $C_{28}H_{34}BrK_{0.89}N_2Na_{0.11}O_{10}$. The final anisotropic full-matrix least-squares refinement on F^2 with 438 variables converged at $R1$ = 4.84%, for the observed data and $wR2$ = 10.78% for all data. The goodness-of-fit was 1.212. The largest peak in the final difference electron density synthesis was 0.521 e$^-$/Å^3 and the largest hole was -0.431 e$^-$/Å^3 with an RMS deviation of 0.067 e$^-$/Å^3. On the basis of the final model, the calculated density was 1.521 g/cm^3 and F(000), 698 e$^-$. The details concerning the crystal data and structural parameters of **2** are collected in Table S1.

The structure is disordered and contains non-integer number of K^+ and Na^+ species with refined occupancy yielding 0.886(2):0.067(2):0.045(1) for K^+ and two alternative sites of Na^+ moieties respectively. In the structure Br^- anions are also disordered and are located at three possible sites with refined occupancy equal 0.841(1):0.114(2):0.045(1). Disorder of the anion is associated with the disorder of the squaramide moiety located at two alternative sites with refined occupancy yielding 0.955(1):0.045(1). To preserve reasonable geometry of the disordered moieties a number of distance, angle and ADP restraints were used. All ordered and major component disordered (occupancy > 50%) non-H atoms were refined anisotropically. Most of the hydrogen atoms were placed in calculated positions and refined within the riding model with isotropic ADPs set to be either 1.2 or 1.5 times larger than U_{eq} of the corresponding heavy atom. Two amide H atoms engaged in hydrogen bonds were fully refined including their isotropic temperature factors. Molecular graphics were prepared using the program Mercury 2020.2.0.[S7,S8] Thermal ellipsoid parameters are presented at a 50% probability level in Figure S39.

Table S1. Data collection and structure refinement parameters for **2**.

Formula	$C_{28}H_{34}BrK_{0.89}N_2Na_{0.11}O_{10}$ corresponding to: $C_{28}H_{34}N_2O_{10}$ + 0.89 × KBr + 0.11 × NaBr
M_x/ g·mol^{-1}	675.73
T/ K	130.5(5)
λ/ Å	0.71073
Crystal size	0.016 × 0.115 × 0.422 mm
Space group	$P\bar{1}$
Unit cell dimensions	a = 7.2681(8) Å α = 89.272(3)° b = 10.3927(10) Å β = 87.184(4)° c = 19.5823(19) Å γ = 87.050(4)°
V/ Å^3, Z	1475.3(3), 2
D_x/ g·cm^{-3}	1.521
μ/ mm^{-1}	1.579
F(000)	698
ϑ_{min}, ϑ_{max}	2.81°, 25.05°
Index ranges	$-8 \le h \le 8$, $-12 \le k \le 12$, $-23 \le l \le 23$
Reflections collected/ independent	42472/ 5240 (R_{int} = 0.0605)
Completeness	99.8%
Absorption correction	Multi-Scan
T_{max}, T_{min}	0.975, 0.555
Refinement method	Full-matrix LSQ on F^2
Data / restraints / parameters	5240 / 52 / 438
GOF on F^2	1.212
Final R indices	4661 data; $I>2\sigma(I)$ $R1$ = 0.0484, $wR2$ = 0.1048 all data $R1$ = 0.0557, $wR2$ = 0.1078
$\Delta\rho_{max}$, $\Delta\rho_{min}$	0.521 e·Å^{-3}, -0.431 e·Å^{-3}

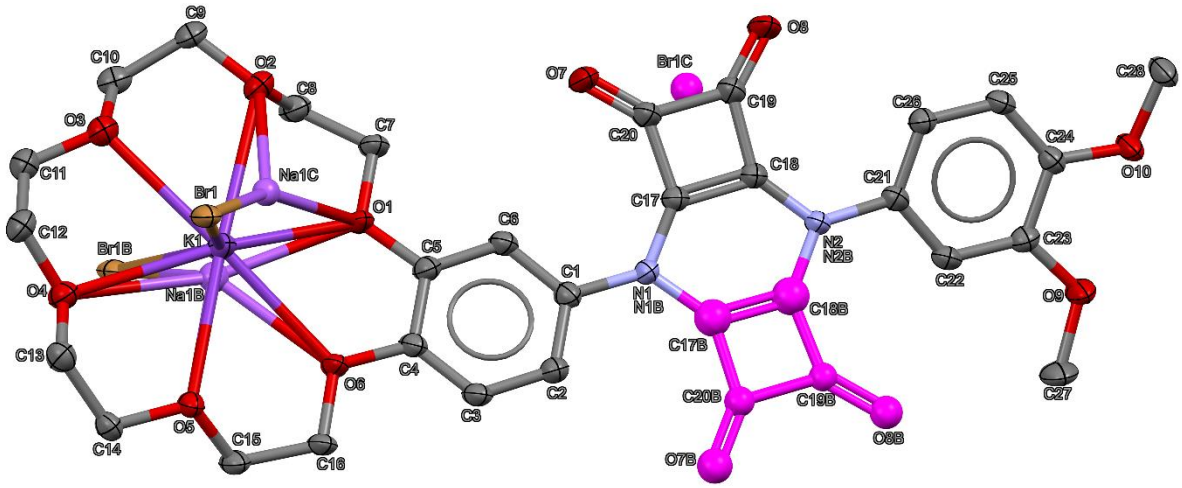


Figure S39. Thermal ellipsoid plot of the **2** at 50% probability level together with numbering scheme, hydrogen atoms omitted for clarity.

S6. 2D NMR spectra

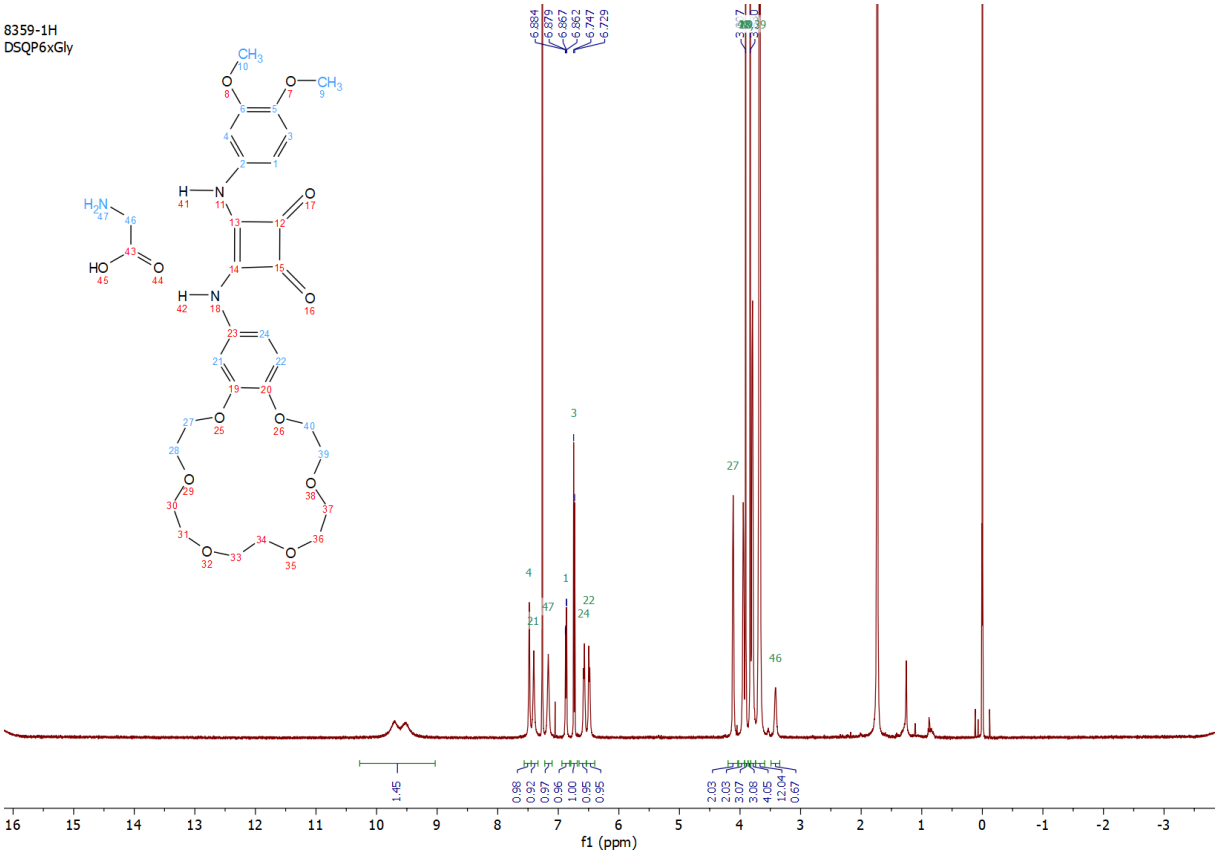


Figure S40. ^{1}H NMR spectrum of Gly×**2** in $CDCl_3$.

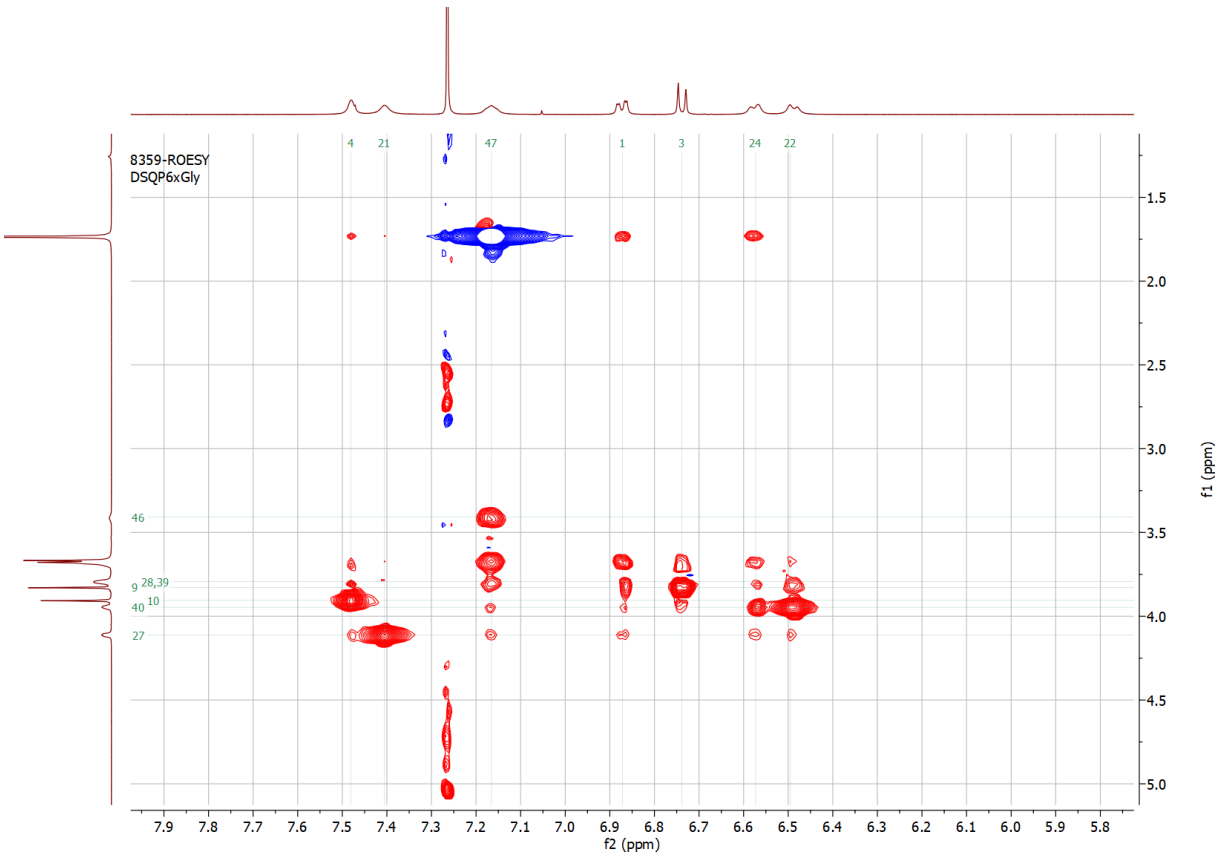


Figure S41. ROESY NMR spectrum of Gly×**2** in $CDCl_3$.

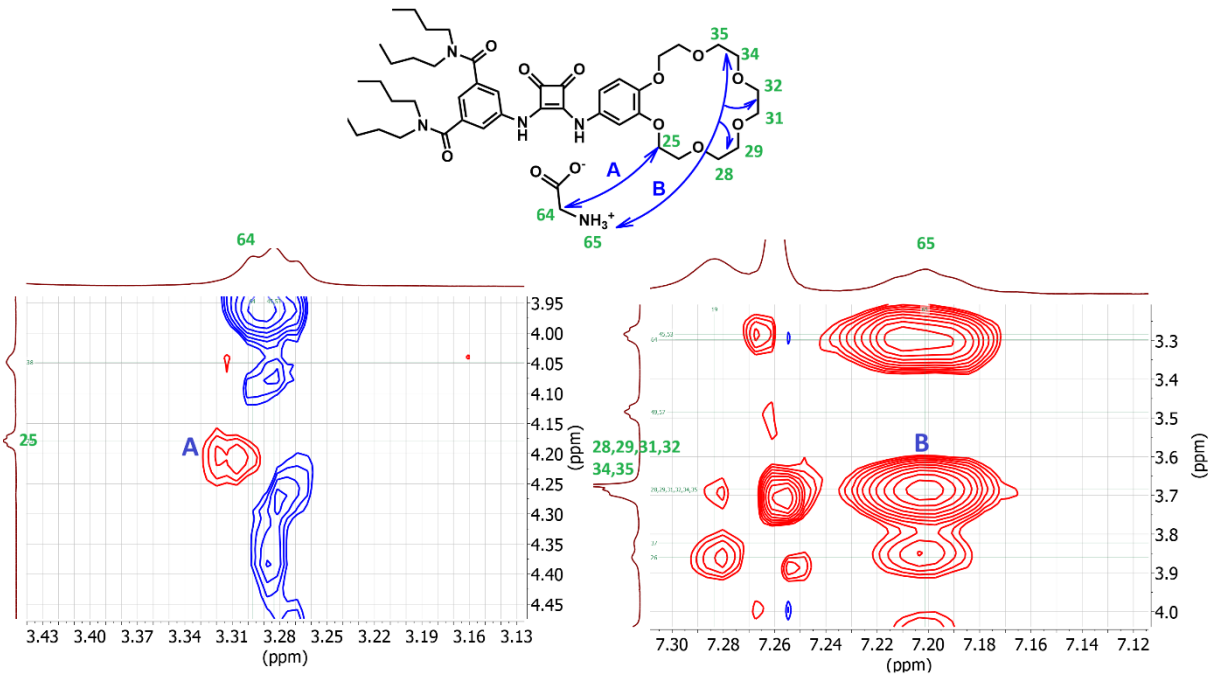


Figure S42. Interaction scheme of **5** with the Gly molecule confirmed by ROESY and partial ROESY spectra of interactions A, B.

S7. Transmembrane transport

S7.1. KCl transport assay

Liposome (LUV) preparation: POPC was obtained from Avanti Polar Lipids, Inc. (catalog# 850457C) and cholesterol was obtained from VWR Life Science (MDL# MFCD00003646). A combination of 70% POPC and 30% cholesterol was used to mimic mammalian membrane composition. The lipids were stored as a solution in chloroform at -20°C (28.63 mM POPC and 12.27 mM cholesterol for a 7:3 mixture). For each experiment, a lipid film was formed in a round bottom flask from this chloroform solution under reduced pressure using a rotary evaporator and further dried under vacuum for at least 4 hours. The lipid film was vortexed with a solution of 496 mM KCl buffered to pH 7.4 with 10 mM HEPES. The resultant lipid suspension was then subjected to 9 cycles of freeze-thaw where the lipid was alternatingly submersed in liquid nitrogen (freeze) and thawed in mildly warm water (thaw). After the freeze-thaw cycles, the lipid suspension was allowed to equilibrate for 30 min at room temperature. Then, the lipid suspension is extruded 27 times through a 200 nm polycarbonate membrane (Nucleopore™) using an extrusion device (Avanti Polar Lipids, Inc). To remove any unincorporated KCl the liposome suspension was subjected to dialysis (Spectra/Por® 2 Membrane, MWCO 12-14 kD) in an external buffer (167 mM Na_2SO_4, 10 mM HEPES, pH 7.4) for at least 2 hours.

Data collection and work-up: After dialysis, the obtained LUVs were diluted in the external buffer to obtain a lipid concentration of 1 mM. The sample was stirred at 350 rpm for 1 minute to equilibrate the sample suspension. A DMSO solution (18.75 µL) of the test compound was added to achieve a concentration of 5 mol% with respect to lipid, and either the chloride efflux was monitored using a chloride ion selective electrode or the potassium efflux was monitored using a potassium ion selective electrode. After 5 minutes of transport, the vesicles were lysed with 50 µL of 10% Triton X-100 and a final reading was taken at 6 min. The electrode readings were converted to external chloride or potassium concentrations [X] using a standard calibration according to the manual of the ion selective electrode (Nernst equation). The initial concentration value, $[X]_0$ (after 1 minute equilibration) was set at 0% efflux and the final concentration, $[X]_{final}$ (at 6 minutes) was set as 100% efflux while all other data points, $[X]$ were converted to percentages using the equation below. The results are given in the main text.

$$\%\ efflux = \frac{[X] - [X]_0}{[X]_{final} - [X]_0} \times 100\%$$

S7.2. Cu-calcein assay for amino acids

Liposome (LUV) preparation: POPC was obtained from Avanti Polar Lipids, Inc. (catalog# 850457C) and cholesterol was obtained from VWR Life Science (MDL# MFCD00003646). A combination of 70% POPC and 30% cholesterol was used to mimic mammalian membrane composition. The lipids were stored as a solution in chloroform at -20°C (28.63 mM POPC and 12.27 mM cholesterol for a 7:3 mixture). For each experiment, a lipid film was formed in a round bottom flask from this chloroform solution under reduced pressure using a rotary evaporator and further dried under vacuum for at least 4 hours. The lipid film was vortexed with a solution containing Cu^{2+} and fluorescent dye (pH 7.4, 20 mM HEPES, 100 mM Na_2SO_4, 0.2 mM $CuSO_4$, 0.2 mM calcein). The resultant lipid suspension was then subjected to 9 cycles of freeze-thaw where the lipid was alternatingly submersed in liquid nitrogen (freeze) and thawed in mildly warm water (thaw). After the freeze-thaw cycles, the lipid suspension was allowed to equilibrate for 30 min at room temperature. Then, the lipid suspension is extruded 27 times through a 200 nm polycarbonate membrane (Nucleopore™) using an extrusion device (Avanti Polar Lipids, Inc). The unbound copper ions and calcein dye were separated from the LUVs using size exclusion chromatography (Sephadex G-50 column with eluent: 20 mM HEPES, 100 mM Na_2SO_4, pH 7.4).

Amino acid transport: For each experiment, a stock solution of the LUV solution was added to an external solution containing copper ions (pH 7.4, 20 mM HEPES, 100 mM Na_2SO_4, 0.2 mM $CuSO_4$) to achieve a final concentration of lipids of 1 mM. 2mL of this solution was transferred to a glass cuvette with a stir bar and placed in an Agilent Cary Eclipse fluorescence spectrophotometer and kinetic fluorescence emission (λ_{ex} = 495 nm, λ_{em} = 515 nm) was recorded over 10 min. 30 s after the start of the experiment, 60 µL of a 1 M amino acid solution (pH 7.4) was added to obtain the final concentration 30 mM amino acid. 30 s later, a stock DMSO solution of transporter was added to achieve 5 mol% transporter with respect to the lipid. At the end of the experiment (8 min) 50 µL of 10% (v/v) Triton X-100 solution was added to disrupt the LUVs and fluorescence was measured for another 2 minutes. The results are shown in Figure S43.

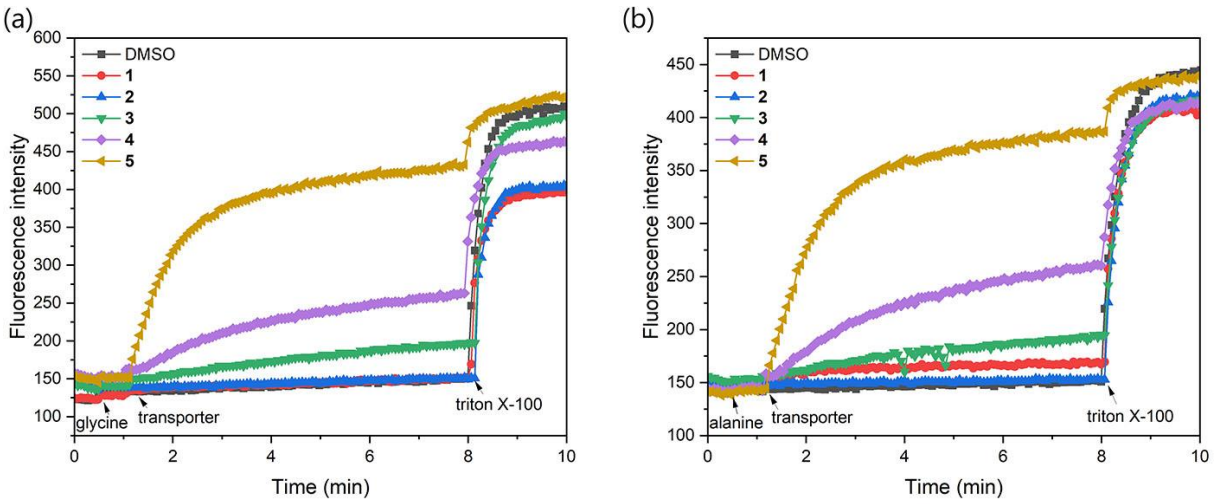


Figure S43. Putative amino acid transport across 200 nm LUVs (7:3 POPC:cholesterol) induced by compounds **1-5** measured using the Cu^{2+}-calcein assay. 1 mM LUVs containing Cu^{2+}-calcein buffer(pH 7.4, 20 mM HEPES, 100 mM Na_2SO_4, 0.2 mM $CuSO_4$, 0.2 mM calcein) were suspended in an external buffer (pH 7.4, 20 mM HEPES, 100 mM Na_2SO_4, 0.2 mM $CuSO_4$,) and the fluorescence intensity was measured for 10 minutes (λ_{ex} = 495 nm, λ_{em} = 515 nm). At *t* = 0.5 min 30 mM amino acid was added, at *t* = 1 min transporter was added (5 mol% with respect to lipid), and at *t* = 8 min Triton X-100 was added. DMSO was used as a negative control. (a) glycine transport. (b) alanine transport.

Potential interference fom Cu^{2+} transport: As noted by the original paper by Gale and co-workers,[S9] an increase in fluorescence using the Cu^{2+}-calcein assay could be due to amino acid transport, Cu^{2+} transport or a combination of both. To check for the possibility of Cu^{2+} transport, we preformed the following control experiment: liposomes were prepared as described above. For each experiment, the LUV stock solution was diluted in a copper-free external solution (pH 7.4, 20 mM HEPES, 100 mM Na_2SO_4) to achieve a final lipid concentration of 1 mM. 2mL of this solution was transferred to a glass cuvette with stir bar and placed in an Agilent Cary Eclipse fluorescence spectrophotometer and kinetic fluorescence emission (λ_{ex} = 495 nm, λ_{em} = 515 nm) was recorded over 10 min. 30 s after the start of the experiment, 20 µL of a 20 mM $CuSO_4$ solution was added to obtain a final concentration 0.2 mM $CuSO_4$. 30 s later, a stock DMSO solution of transporter was added to achieve 5 mol% transporter with respect to the lipid. A run using 8-hydroxyquinoline was used as a positive control (8-hydroxyquinoline is a known Cu^{2+} transporter). At the end of the experiment (8 min) 50 µL of 10% (v/v) Triton X-100 solution was added to disrupt the LUVs and fluorescence was measured for another 2 minutes. The results are

shown in Figure S44. Although the addition of Cu^{2+} (before addition of transporter) already leads to a change in fluorescence due to equilibration of the system, it is clear that compound **5** accelerates this equilibration and the addition point can be clearly seen (similar to 8-hydroxyquinoline, but **5** is not as active as 8-hydroxyquinoline). This suggests that compound **5** might be able to transport Cu^{2+} to some extent and that the Cu^{2+}-calcein assay should not be used to assess the amino acid transport facilitated by **5**.

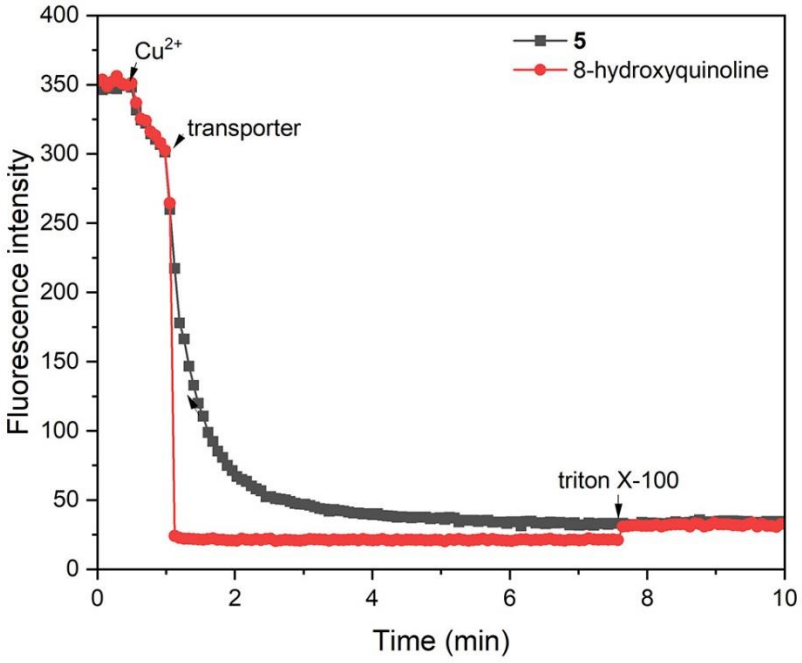


Figure S44. Putative Cu^{2+} transport across 200 nm LUVs (7:3 POPC:cholesterol) induced by compounds **5** and 8-hydroxyquinoline. 1 mM LUVs containing Cu^{2+}-calcein buffer(pH 7.4, 20 mM HEPES, 100 mM Na_2SO_4, 0.2 mM $CuSO_4$, 0.2 mM calcein) were suspended in an external copper-free buffer (pH 7.4, 20 mM HEPES, 100 mM Na_2SO_4) and the fluorescence intensity was measured for 10 minutes (λ_{ex} = 495 nm, λ_{em} = 515 nm). At *t* = 0.5 min 0.2 mM $CuSO_4$ was added, at *t* = 1 min transporter was added (5 mol% with respect to lipid), and at *t* = 8 min Triton X-100 was added.

S7.3. ^{13}C NMR assay

A solution of POPC in chloroform (freshly deacidified by passing through a pad of activated basic alumina) was prepared in a 5 ml round-bottom flask was evaporated and the lipid film formed was dried under vacuum for at least 8 h. The lipid film was hydrated with 9:1 (v/v) H_2O/D_2O of a buffered solution (100 mM $NaNO_3$, 20 mM HEPES, pH 7.4) by sonication for 30 s and vortexing for 5 min to facilitate vesicle formation to a lipid concentration of 30 mM. The lipid solution was subjected to 18 freeze/thaw cycles and vortexed after every 3 cycles for 30 s to facilitate hydration. It was then extruded 35 times through a 1.0 µm polycarbonate membrane to obtain a concentrated lipid stock solution. For each ^{13}C NMR transport experiment, the lipid stock solution prepared as stated above (300 µL) was added to an external Gly-1-^{13}C solution (300 µL, pH 7.4, 20 mM HEPES, 100 mM $NaNO_3$, 50 mM Gly-1-^{13}C). DMSO solutions of **4** or **5** (10%mol) or DMSO as control was added to this solution. The total volume of DMSO added for each sample was fixed at 30 µL. The resulting solution was stirred at room temperature for 10 min. An aqueous solution of $MnSO_4$ (30 µL, 0.5 mM) and D_2O (67 µL) was then added, and the sample was subject to ^{13}C NMR measurement. Acquisition parameters: frequency – 100.6 MHz; relaxation delay – 2 s; number of scans – 1024; temperature – 298 K, total acquisition time – ca. 1h.

S7.4. Fluorescamine assay

The assay was adapted from the original report by Chen *et al.*[S10]

Liposome (LUV) preparation: POPC was obtained from Avanti Polar Lipids, Inc. (catalog# 850457C) and cholesterol was obtained from VWR Life Science (MDL# MFCD00003646). A combination of 70% POPC and 30% cholesterol was used to mimic mammalian membrane composition. The lipids were stored as a solution in chloroform at -20°C (28.63 mM POPC and 12.27 mM cholesterol for a 7:3 mixture). For each experiment, a lipid film was formed in a round bottom flask from this chloroform solution under reduced

pressure using a rotary evaporator and further dried under vacuum for at least 4 hours. The lipid film was vortexed with the internal solution (50 mM HEPES, 250 mM amino acid, pH 7.4) until all lipid was suspended. The resultant lipid suspension was then subjected to 9 cycles of freeze-thaw where the lipid was alternatingly submersed in liquid nitrogen (freeze) and thawed in mildly warm water (thaw). After the freeze-thaw cycles, the lipid suspension was allowed to equilibrate for 30 min at room temperature. Then, the lipid suspension is extruded 27 times through a 200 nm polycarbonate membrane (Nucleopore™) using an extrusion device (Avanti Polar Lipids, Inc). To remove any unincorporated amino acid the liposome suspension was subjected to dialysis (Spectra/Por® 2 Membrane, MWCO 12-14 kD) in an external buffer (50 mM HEPES, pH 7.4) for approximately 18 hours. *Note: In the case of arginine, the internal solution had to be adjusted to pH 7.4 using HCl, and the external solution was therefore adjusted to contain the same amount of NaCl to avoid a chloride gradient. For all other amino acids tested, there was no need to adjust the pH from the HEPES buffer with HCl.*

Amino acid transport: For each experiment, the stock LUV solution was diluted in external buffer (50 mM HEPES, pH 7.4) to achieve a final lipid concentration of 10 mM. 2.5 mL of this lipid solution was transferred to a new dialysis tubing with MWCO 3.5kD, and either 250 µL of neat DMSO or 250 µL of a 5 mM DMSO solution of compound **5** (resulting in 5 mol% final concentration) was added to the LUVs inside the dialysis tubing and hand-mixed with a pipette. The dialysis tubing was placed immediately inside a beaker containing 120 mL of external solution and the solution was stirred at 250 rpm. At regular time intervals (0, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes), 1 mL of the external solution was removed to determine the external amino acid concentration (see below). After 60 minutes, 250 µL of 10% Triton X-100 was added to the inside of the dialysis tubing to lyse the LUVs and dialysis was continued for another 60 minutes. At the end, a final 1 mL aliquot was removed from the external solution to determine the theoretical maximum external amino acid concentration.

Determining external amino acid concentration and % efflux: For each of the 1 mL aliquots that were removed during the amino acid transport procedure, was added 2 mL borate buffer (pH 9) and 1 mL fluorescamine solution (1 mg fluorescamine in 5 mL acetone). The solutions were centrifuged for 30 seconds at 2000 rpm using an Eppendorf Centrifuge 5810, followed by 30 seconds of shaking at 800 rpm using a VWR Thermal Shake Touch at room temperature. 150 µL of these solutions were then transferred to a 96-well plate and the fluorescence intensity was measured using a BioTek Cytation 5 plate reader (λ_{ex} = 390 nm, λ_{em} = 480 nm). Each day, a calibration curve was also generated using stock solutions with known amino acid concentration that were subjected to the same fluorescamine procedure. This calibration curve was then used to convert the fluorescence intensity readings to amino acid concentration [AA], and the % efflux can then be calculated suing the following equation (where *[AA]* is the amino acid concentration at any given time, $[AA]_0$ is the amino concentration at the beginning of the experiment, and $[AA]_{final}$ is the amino acid concentration 60 minutes after the addition of Triton X-100):

$$\%\, efflux = \frac{[AA] - [AA]_0}{[AA]_{final} - [AA]_0} \cdot 100\%$$

S7.5. Calcein leakage assay

Liposome (LUV) preparation: POPC was obtained from Avanti Polar Lipids, Inc. (catalog# 850457C) and cholesterol was obtained from VWR Life Science (MDL# MFCD00003646). A combination of 70% POPC and 30% cholesterol was used to mimic mammalian membrane composition. The lipids were stored as a solution in chloroform at -20°C (28.63 mM POPC and 12.27 mM cholesterol for a 7:3 mixture). For each experiment, a lipid film was formed in a round bottom flask from this chloroform solution under reduced pressure using a rotary evaporator and further dried under vacuum for at least 4 hours. The lipid film was vortexed with the internal solution (70 mM calcein, 10 mM Tris-HCl, 150 mM NaCl, pH 7.4) until all lipid was suspended. The resultant lipid suspension was then subjected to 9 cycles of freeze-thaw where the lipid was alternatingly submersed in liquid nitrogen (freeze) and thawed in mildly warm water (thaw). After the freeze-thaw cycles, the lipid suspension was allowed to equilibrate for 30 min at room temperature. Then, the lipid suspension was extruded 27 times through a 200 nm polycarbonate membrane (Nucleopore™) using an extrusion device (Avanti Polar Lipids, Inc). The unincorporated calcein dye was separated from the LUVs using size exclusion chromatography (Sephadex G-50 column with eluent: 10 mM Tris-HCl, 150 mM NaCl, pH 7.4).

Calcein leakage: For each experiment, the stock LUV solution was diluted in external buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) to achieve a final lipid concentration of 10 µM. 3 mL of this solution was transferred to a glass cuvette with stir bar and placed in an Agilent Cary Eclipse fluorescence spectrophotometer and kinetic fluorescence emission (λ_{ex} = 490 nm, λ_{em} = 520 nm) was recorded over 14 min. Approximately 1 minute after the start of the experiment, 30 µL of a stock DMSO solution of transporter was added to achieve 5 mol% transporter with respect to the lipid. At the end of the experiment (11 min) 30 µL of 10% (v/v) Triton X-100 solution was added to disrupt the LUVs and fluorescence was measured for another 3 minutes. The fluorescence intensities *F* were converted to % calcein leakage using the following equation (whereby *F* is the fluorescence intensity at a given time, F_0 is the fluorescence intensity at the beginning of the experiment (before addition transporter) and F_{final} is the fluorescence intensity at the end of the experiment (after the addition of Trion X-100)):

$$\%\ calcein\ leakage = \frac{F - F_0}{F_{final} - F_0} \cdot 100\%$$

The results are shown in Figure S44. A small increase in fluorescence is observed for some compounds, albeit with large errors. Presumably, this is the result of optical interference between the yellow squaramide-based compounds and the yellow calcein dye. Additional leakage assays were performed to confirm this hypothesis (see below). Furthermore, most 'leakage' is seen for compound **1**, which does not show any activity in any other assay, suggesting the observation is most likely optical interference. Nevertheless, even if the observed results suggest a small amount of calcein leakage, it cannot explain the 100% amino acid efflux observed in the other experiments. It is therefore more likely that the observed amino acid efflux is due to 'true' transport involving non-covalent interaction between the transporter and amino acids than due to membrane disruption.

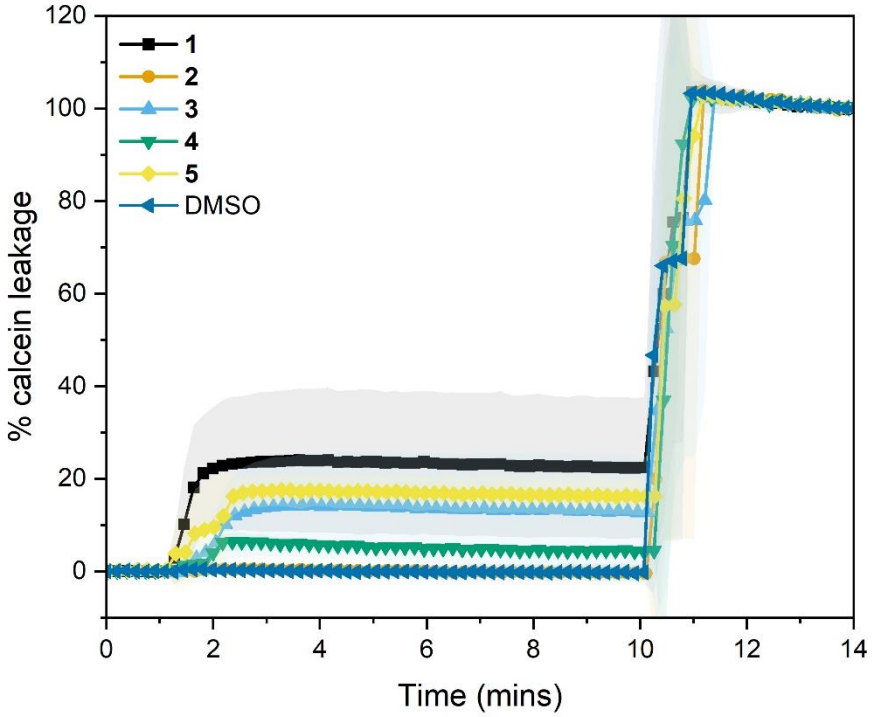


Figure S45. Potential calcein leakage from 200 nm LUVs (7:3 POPC: cholesterol) induced by compounds **1**-**5**. 10 μM LUVs containing calcein buffer (70 mM calcein, 10 mM Tris-HCl, 150 mM NaCl, pH 7.4) were suspended in an external calcein-free buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) and the fluorescence intensity was measured for 14 minutes (λ_{ex} = 490 nm, λ_{em} = 520 nm). At *t* = 1 min transporter was added (5 mol% with respect to lipid), and at *t* = 11 min Triton X-100 was added. DMSO was used as a negative control. Results are the average of 3 independent repeats and shaded arrows indicate standard deviations.

S7.6. Dextran leakage assay

We used a modified Dextran leakage assay.[S11]

Liposome (LUV) preparation: FITC labeled dextran (FITC-Dextran) with an average molecular weight of 3,000-5,000 was purchased from Sigma-Aldrich (catalog# FD4-250MG). POPC was obtained from Avanti Polar Lipids, Inc. (catalog# 850457C) and cholesterol was obtained from VWR Life Science (MDL# MFCD00003646). A combination of 70% POPC and 30% cholesterol was used to mimic mammalian membrane composition. The lipids were stored as a solution in chloroform at -20°C (28.63 mM POPC and 12.27 mM cholesterol for a 7:3 mixture). For each experiment, a lipid film was formed in a round bottom flask from this chloroform solution under reduced pressure using a rotary evaporator and further dried under vacuum for at least 4 hours. The lipid film was vortexed with the internal solution (18.5 mM FITC-Dextran, 50 mM HEPES, 150 mM NaCl, pH 7.4) until all lipid was suspended. The resultant lipid suspension was then subjected to 9 cycles of freeze-thaw where the lipid was alternatingly submersed in liquid nitrogen (freeze) and thawed in mildly warm water (thaw). After the freeze-thaw cycles, the lipid suspension was allowed to equilibrate for 30 min at room temperature. Then, the lipid suspension was extruded 27 times through a 200 nm polycarbonate membrane (Nucleopore™) using an extrusion device (Avanti Polar Lipids, Inc). The unincorporated FITC-Dextran was separated from the LUVs using size exclusion chromatography (Sephadex G-50 column with eluent: 50 mM HEPES, 150 mM NaCl, pH 7.4).

Dextran leakage: For each experiment, the stock LUV solution was diluted in external buffer (50 mM HEPES, 150 mM NaCl, pH 7.4) to achieve a final lipid concentration of 10 µM. 3 mL of this solution was transferred to a glass cuvette with a stir bar and placed in an Agilent Cary Eclipse fluorescence spectrophotometer and kinetic fluorescence emission (λ_{ex} = 480 nm, λ_{em} = 518 nm) was recorded over 14 min. Approximately 1 minute after the start of the experiment, 30 µL of a stock DMSO solution of transporter was added to achieve 5 mol% transporter with respect to the lipid. At the end of the experiment (11 min) 30 µL of 10% (v/v) Triton X-100 solution was added to disrupt the LUVs and fluorescence was measured for another 3 minutes. The results are shown in Figure S45 and show no evidence of leakage.

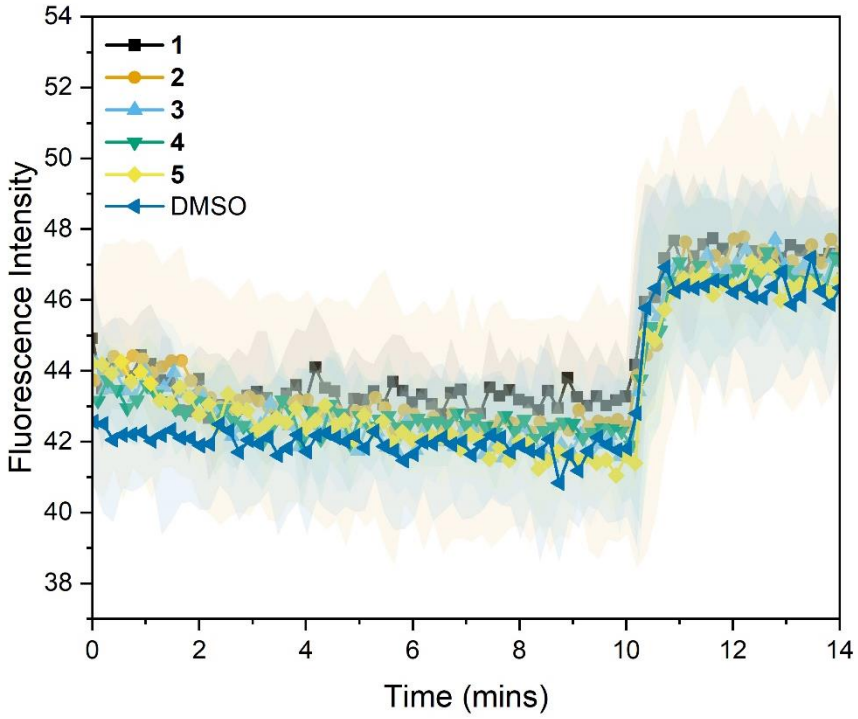


Figure S46. Potential FITC-Dextran leakage from 200 nm LUVs (7:3 POPC: cholesterol) induced by compounds **1**-**5**. 10 µM LUVs containing FITC-Dextran buffer (18.5 mM FITC-Dextran, 50 mM HEPES, 150 mM NaCl, pH 7.4) were suspended in an external buffer (50 mM HEPES, 150 mM NaCl, pH 7.4) and the fluorescence intensity was measured for 14 minutes (λ_{ex} = 480 nm, λ_{em} = 518 nm). At *t* = 1 min transporter was added (5 mol% with respect to lipid), and at *t* = 11 min Triton X-100 was added. DMSO was used as a negative control. Results are the average of 3 independent repeats and shaded arrows indicate standard deviations.

S7.7. DLS experiments

Liposome (LUV) preparation: POPC was obtained from Avanti Polar Lipids, Inc. (catalog# 850457C) and cholesterol was obtained from VWR Life Science (MDL# MFCD00003646). A combination of 70% POPC and 30% cholesterol was used to mimic mammalian membrane composition. The lipids were stored as a solution in chloroform at -20°C (28.63 mM POPC and 12.27 mM cholesterol for a 7:3 mixture). For each experiment, a lipid film was formed in a round bottom flask from this chloroform solution under reduced pressure using a rotary evaporator and further dried under vacuum for at least 4 hours. The lipid film was vortexed with the internal solution (50 mM HEPES, 250 mM glycine, pH 7.4) until all lipid was suspended. The resultant lipid suspension was then subjected to 9 cycles of freeze-thaw where the lipid was alternatingly submersed in liquid nitrogen (freeze) and thawed in mildly warm water (thaw). After the freeze-thaw cycles, the lipid suspension was allowed to equilibrate for 30 min at room temperature. Then, the lipid suspension was extruded 27 times through a 200 nm polycarbonate membrane (Nucleopore™) using an extrusion device (Avanti Polar Lipids, Inc). To remove any unincorporated amino acid the liposome suspension was subjected to dialysis (Spectra/Por® 2 Membrane, MWCO 12-14 kD) in external buffer (50 mM HEPES, pH 7.4) for approximately 18 hours.

DLS experiment: The stock LUV solution was diluted in an external buffer (50 mM HEPES, pH 7.4) to achieve a final lipid concentration of 10 mM. 2.5 mL of this lipid solution was transferred to a new dialysis tubing with MWCO 3.5kD, and either 250 µL of neat DMSO or 250 µL of a 5 mM DMSO solution of compound **5** (resulting in 5 mol% final concentration) was added to the LUVs inside the dialysis tubing and hand-mixed with a pipette. The dialysis tubing was placed immediately inside a beaker containing 120 mL of external

solution and the solution was stirred at 250 rpm. After 60 minutes, the LUV solution inside the dialysis tubing was removed and diluted to a final concentration of 10 µM lipid and the dynamic light scattering spectrum of this solution was measured using a NanoBrook Omni (Brookhaven Instruments) particle size analyzer. The results are shown in Figure S46. No difference in liposome size is seen between DMSO and compound **5** containing LUVs, and the maximum particle size is around 200 nm, as expected from the extrusion. This indicates that compound **5** does not affect the integrity of the LUVs and functions as a pure transporter.

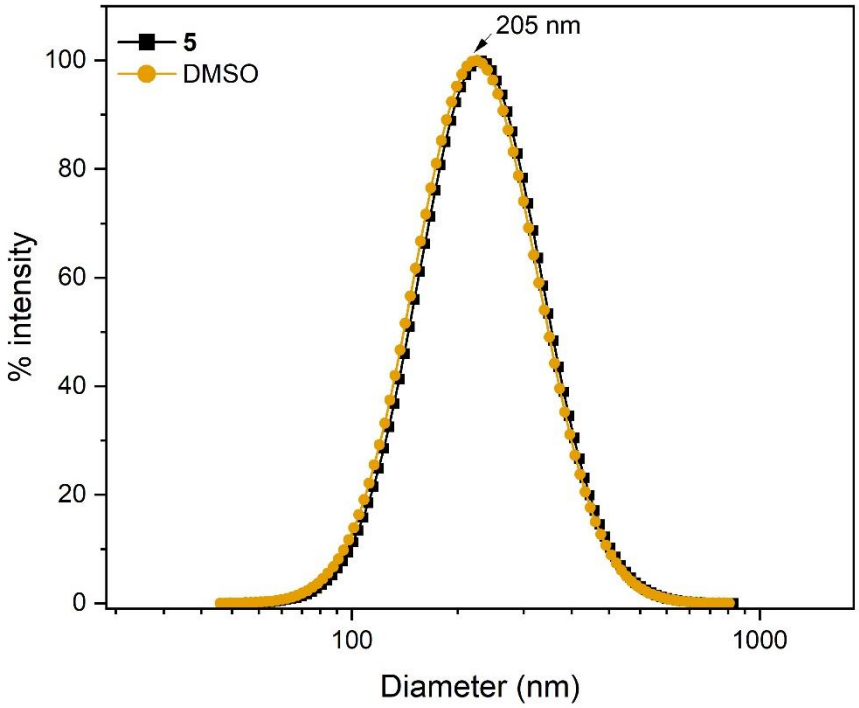


Figure S47. DLS spectra of 200 nm LUVs (7:3 POPC:cholesterol) LUVs containing glycine buffer (50 mM HEPES, 250 mM glycine, pH 7.4) and suspended in an external buffer (50 mM HEPES, pH 7.4) that were incubated for 60 minutes with either DMSO or compound **5** (5 mol% with respect to lipid).

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