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Supporting Information

Selenium-Containing Organic Nanoparticles as Silent Precursor for Ultra-Sensitive Thiol-Responsive Transmembrane Anion Transport

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Contents

1. Synthetic procedures and characterization data

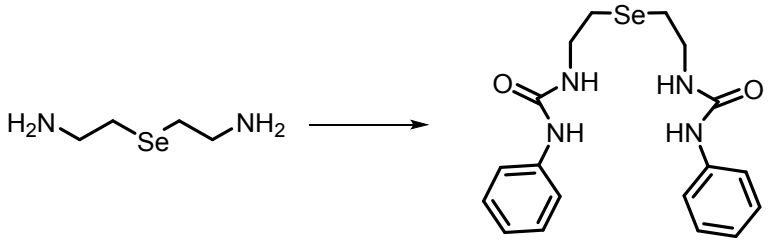
1.1 General Information

^{1}H, ^{13}C and ^{77}Se NMR spectra were recorded on a Bruker AVANCEIII500. Chemical shifts were referenced to DMSO-d6 residue (2.50 ppm for ^{1}H NMR, 39.5 ppm for ^{13}C NMR). Mass spectroscopy measurements were performed on a Bruker micro ToF-Q Ⅱ. Dynamic Light Scattering (DLS) experiments were carried out with Malvern Instrument Zetasizer Nano ZS equipped with a HE-Ne laser (633nm, 4mW) and an avalanche photodiode detector. Fluorescence spectra were recorded on a SHIMADZU RF–5301PC fluorophotometer. Transmission Electron Microscopy (TEM) was recorded on a JEM-2100F instrument with an accelerating voltage of 120 kV. Scanning Electron Microscopy (SEM) was recorded on a JEOL-JSM-6700F instrument. All reactions were monitored by thin layer chromatography (TLC) visualizing with ultraviolet light (UV), column chromatography purifications were carried out using silica gel.

Bis(2-aminoethyl) selenoether was synthesized according to previously reported method with minor modification.[1] Briefly, N-boc-bromoethylamine could be converted into bis(2-aminoethyl) selenoether by reacting with Na_2Se before deprotection of Boc. These bis-amino scaffolds were then reacted with appropriate aryl isothiocyanates to give desired products. Other materials were used as provided by supplier.

1.2 Synthesis of 1P, 1F, 1F2, 2P, 2F, 2F2

1,1'-(Selenobis(ethane-2,1-diyl))bis(3-phenylurea) (1P)



1P

Bis(2-Aminoethyl)selenide (0.6 mmol) and phenyl isocyanate (1.3 mmol) were suspended in anhydrous THF (5 mL) and stirred under N_2 atmosphere at room temperature for 16 hours. The mixture was then concentrated by evaporation under reduced pressure. The residue was washed with cold DCM and MeOH. The obtained solid was then dried under high vacuum to yield the product as yellow solid. Yield: 65%. ^{1}H NMR (DMSO-d6): δ 8.55 (s, 2H), 7.38 (d, J = 7.6 Hz, 4H), 7.21 (t, J = 7.9 Hz, 4H), 6.88 (t, J = 7.3 Hz, 2H), 6.30 (t, J = 5.7 Hz, 2H), 2.68 (t, J = 7.1 Hz, 4H). ^{13}C NMR (DMSO-d6): δ 155.1, 140.5, 128.7, 121.1, 117.72, 23.54. HRMS (ESI): calcd. for $C_{18}H_{22}N_4O_2Se$ $[M+H]^+$ 406.0908; found 406.0291.

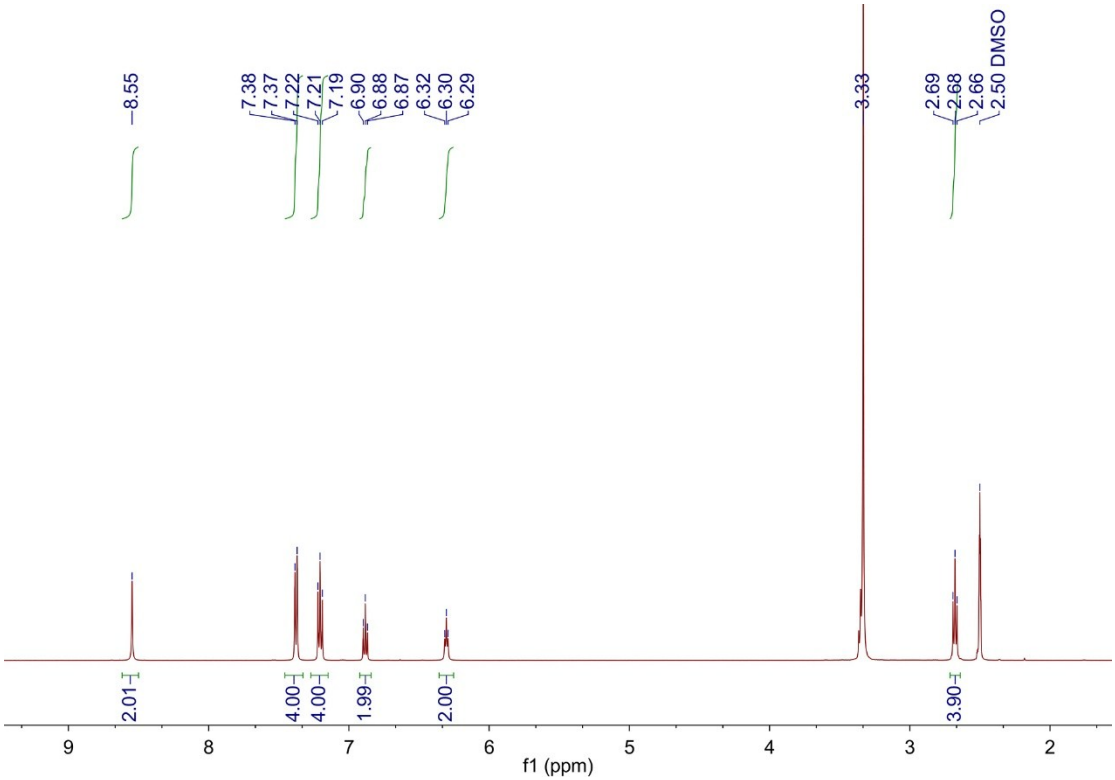


Figure S1. ^{1}H NMR spectrum of **1P** in DMSO-d6.

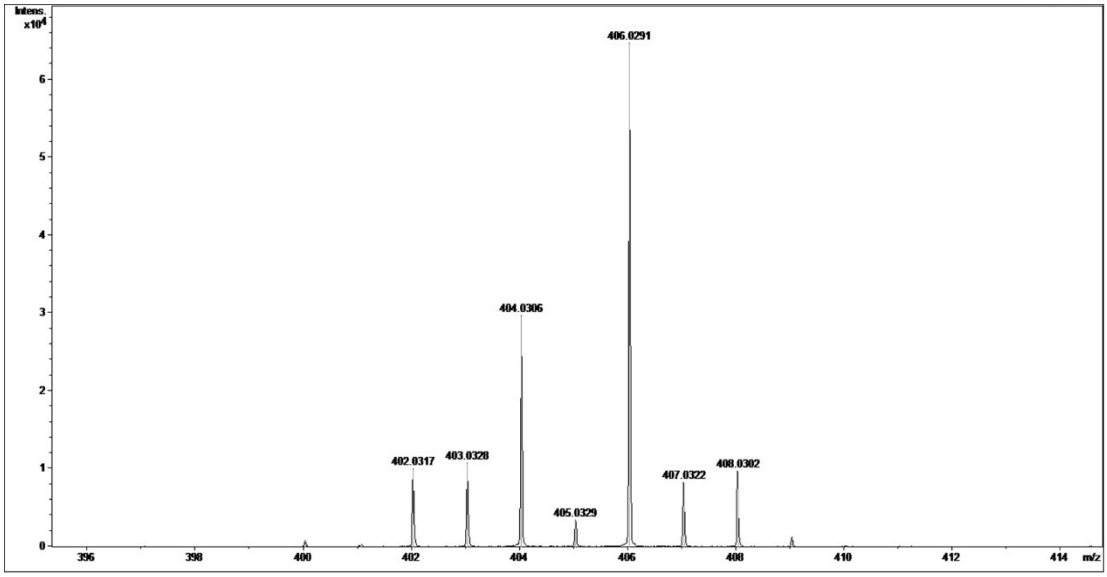
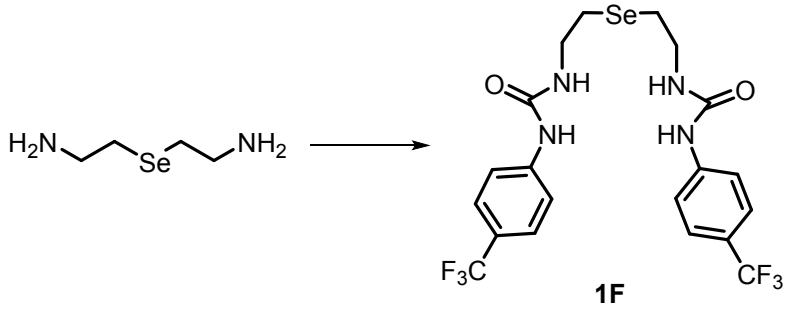


Figure S2. HR-MS spectrum of **1P**.

1,1'-(Selenobis(ethane-2,1-diyl))bis(3-(4-(trifluoromethyl)phenyl)urea) (1F)



Bis(2-Aminoethyl)selenide (0.8 mmol) and 4-(trifluoromethyl)phenyl isocyanate (1.64 mmol) were suspended in anhydrous THF (5 mL) and stirred under N_2 atmosphere at room temperature for 16 hours. The mixture was then concentrated by evaporation under reduced pressure. The residue was then purified by column chromatography on silica gel to yield the product as white solid. Yield: 70%. ^{1}H NMR (DMSO-d6): δ 9.03 (s, 2H), 7.57 (q, J = 8.9 Hz, 8H), 6.48 (t, J = 5.8 Hz, 2H), 2.69 (t, J = 7.1 Hz, 4H). ^{13}C NMR (DMSO-d6): δ 154.7, 144.2, 125.9, 123.6, 121.1, 177.3, 23.3. HRMS (ESI): calcd. for $C_{20}H_{20}F_6N_4O_2Se$ $[M+H]^+$ 543.0656; found $[M+H]^+$ 543.0682.

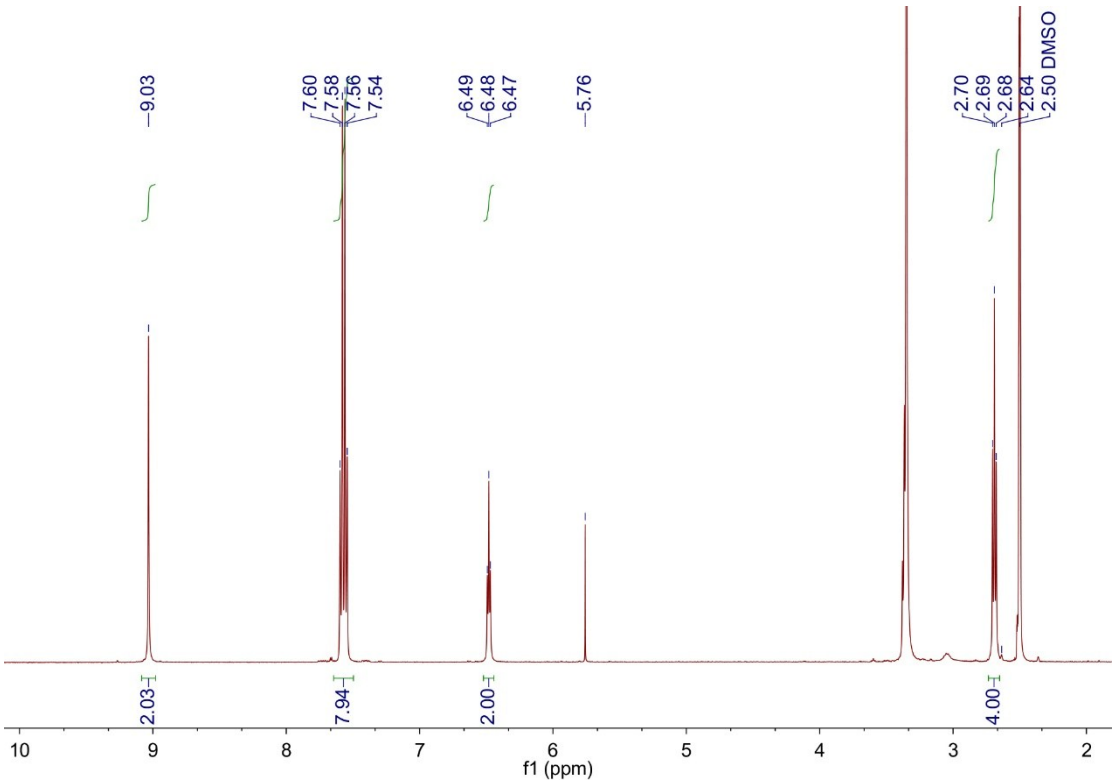


Figure S3. ^{1}H NMR spectrum of **1F** in DMSO-d6.

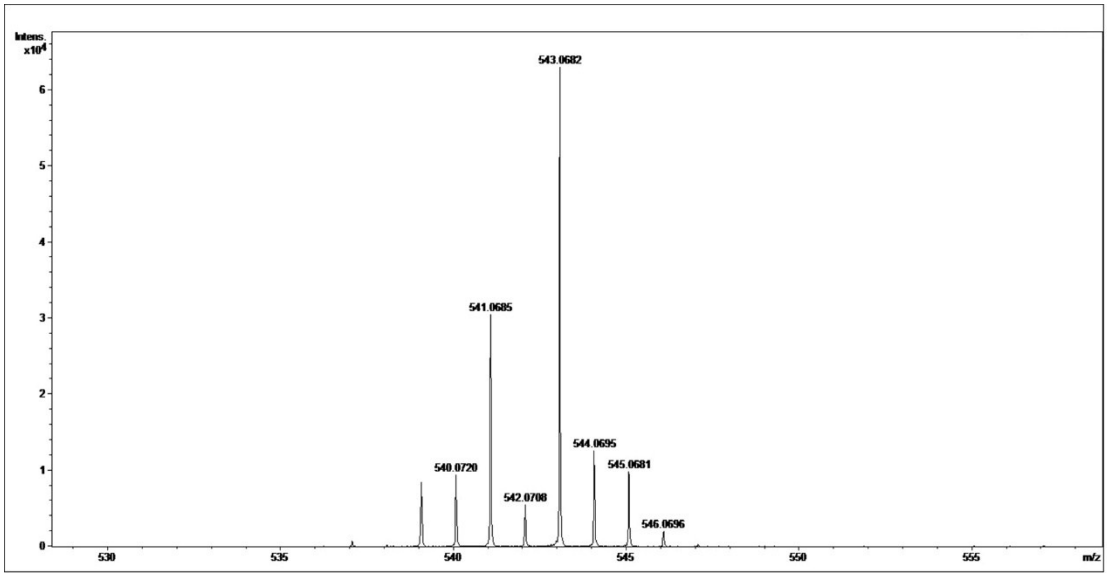
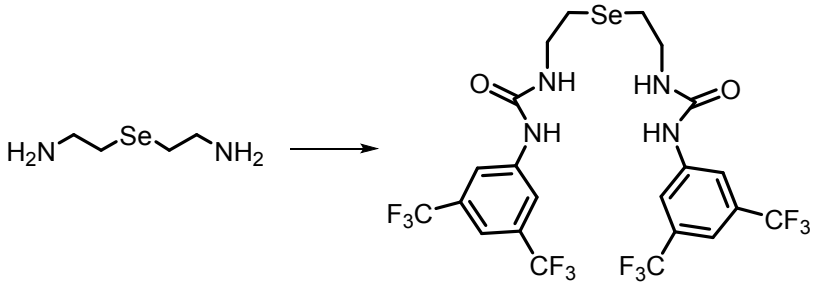


Figure S4. HR-MS spectrum of **1F**.

1,1'-(Selenobis(ethane-2,1-diyl))bis(3-(3,5-bis(trifluoromethyl)phenyl)urea) (1F2)



1F2

Bis(2-Aminoethyl)selenide (0.7 mmol) and 3,5-bis(trifluoromethyl)phenyl isocyanate (1.44 mmol) were suspended in anhydrous THF (5 mL) and stirred under N_2 atmosphere at room temperature for 16 hours. The mixture was then concentrated by evaporation under reduced pressure. The residue was then purified by column chromatography on silica gel to yield the product as white solid. Yield: 55%. ^{1}H NMR (DMSO-d6): δ 9.37 (s, 2H), 8.07 (s, 4H), 7.53 (s, 2H), 6.66 (t, J = 5.6 Hz, 2H), 2.70 (t, J = 7.1 Hz, 4H). ^{13}C NMR (DMSO-d6): δ 154.7, 142.5, 130.5, 124.5, 122.3, 117.2, 113.5, 23.2. HRMS (ESI): calcd. for $C_{22}H_{18}F_{12}N_4O_2Se$ $[M+H]^+$ 679.0403; found $[M+H]^+$ 679.0243.

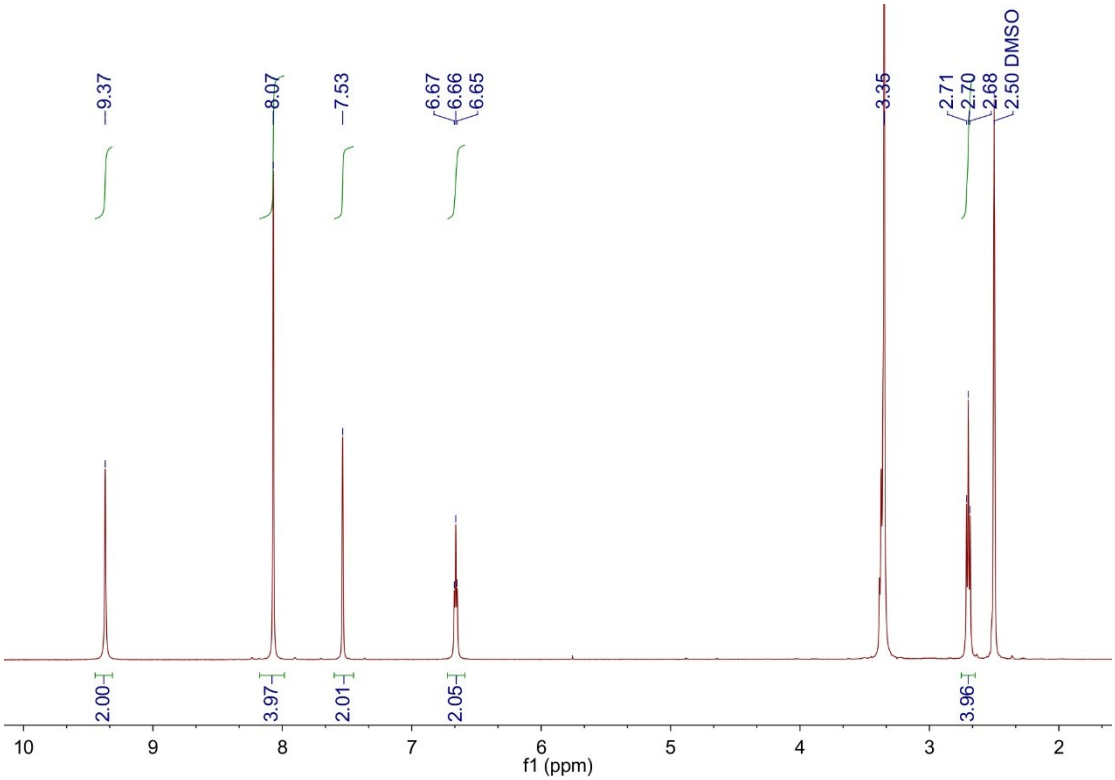


Figure S5. ^{1}H NMR spectrum of **1F2** in DMSO-d6.

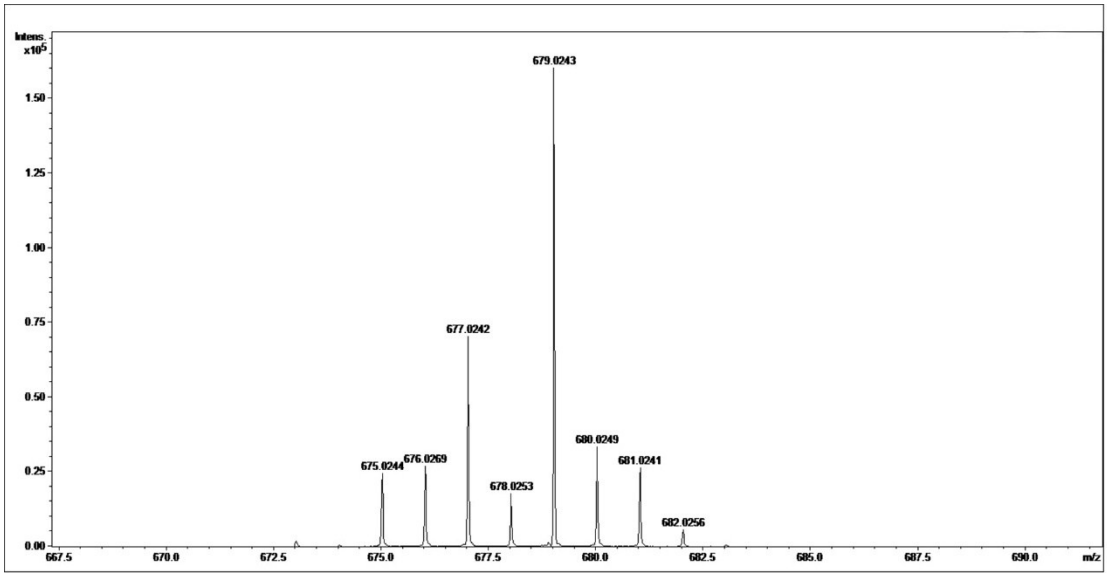
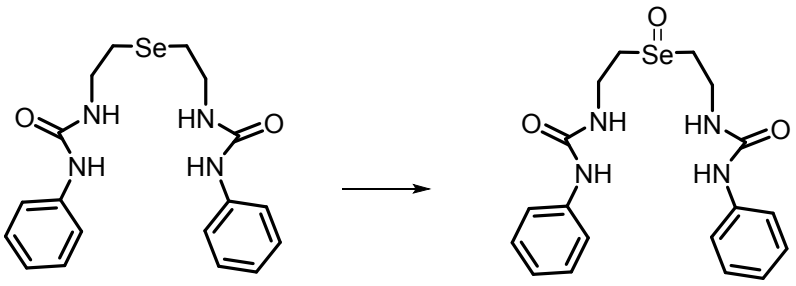


Figure S6. HR-MS spectrum of **1F2**.

1,1'-(Seleninylbis(ethane-2,1-diyl))bis(3-phenylurea) (2P)



1P **2P**

1P (0.5 mmol) was dissolved in a 1:1 solution of MeOH/CH_2Cl_2 (10 mL/mmol) and cooled to 0 °C. N-Chlorosuccinimide (0.6 mmol) was added, and the resulting mixture was stirred for 30 min at 0 °C. The solution was then diluted with an equal volume of CH_2Cl_2, and a 10% solution of NaOH was added (10 mL/mmol). The resulting mixture was stirred for 5 min, and the organic phase was separated, dried over $MgSO_4$, and concentrated. The crude selenoxide was then precipitation in water to get desired product. Yield: 90%. ^{1}H NMR (DMSO-d6): δ 8.73 (s, 2H), 7.39 (d, J = 7.7 Hz, 4H), 7.20 (t, J = 7.9 Hz, 4H), 6.88 (t, J = 7.3 Hz, 2H), 6.51 (t, J = 5.7 Hz, 2H), 3.65 – 3.45 (m, 4H), 3.18 – 3.04 (m, 2H), 2.93 – 2.79 (m, 2H). ^{13}C NMR (DMSO-d6): δ 155.4, 140.4, 128.7, 121.2, 117.9, 47.1, 34.1. HRMS (ESI): calcd. for $C_{18}H_{22}N_4O_3Se$ $[M+H]^+$ 423.0857; found $[M+H]^+$ 423.0927.

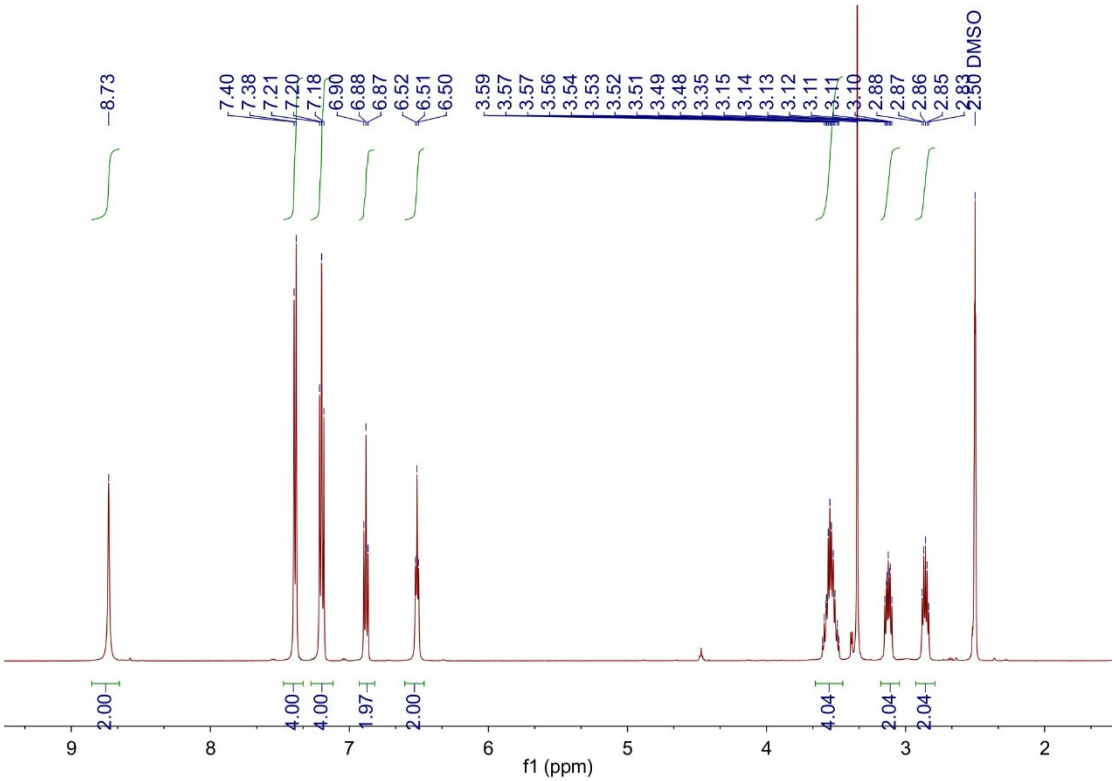


Figure S7. ^{1}H NMR spectrum of **2P** in DMSO-d6.

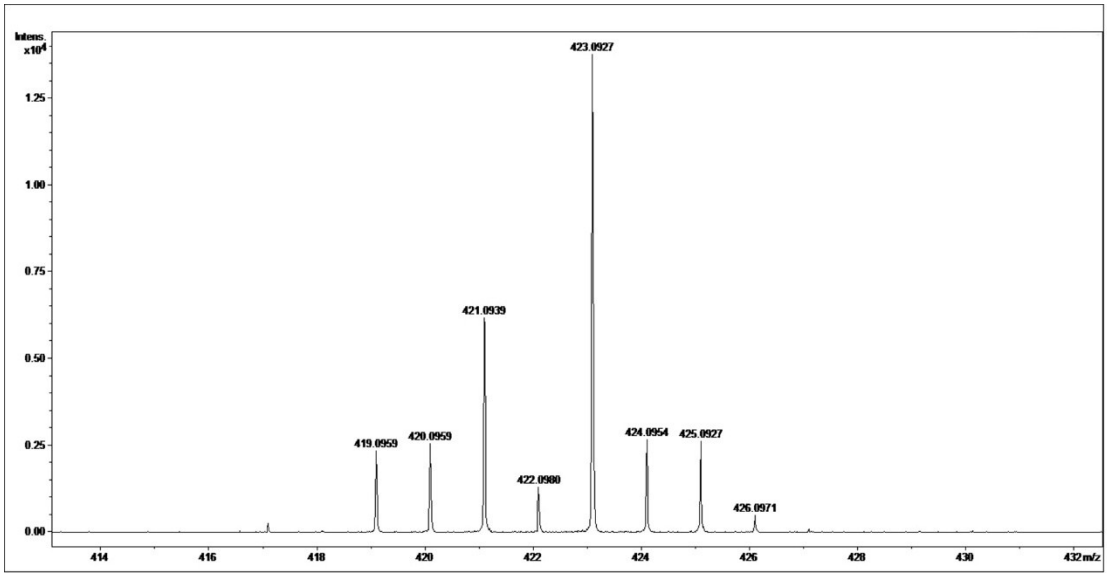
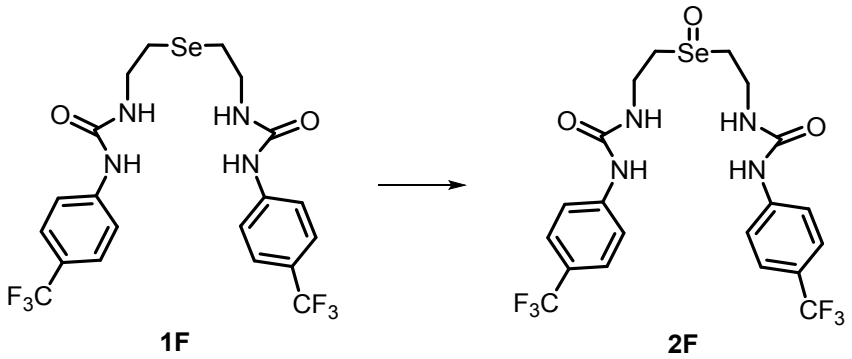


Figure S8. HR-MS spectrum of **2P**.

1,1'-(Seleninylbis(ethane-2,1-diyl))bis(3-(4-(trifluoromethyl)phenyl)urea) (2F)



1F (0.5 mmol) was dissolved in a 1:1 solution of MeOH/CH_2Cl_2 (10 mL/mmol) and cooled to 0 °C. N-Chlorosuccinimide (0.6 mmol) was added, and the resulting mixture was stirred for 30 min at 0 °C. The solution was then diluted with an equal volume of CH_2Cl_2, and a 10% solution of NaOH was added (10 mL/mmol). The resulting mixture was stirred for 5 min, and the organic phase was separated, dried over $MgSO_4$, and concentrated. The crude selenoxide was then precipitation in water to get desired product. Yield: 88%. ^{1}H NMR (DMSO-d6): δ 9.22 (s, 2H), 7.56 (dd, *J* = 31.3, 8.7 Hz, 8H), 6.70 (t, *J* = 5.7 Hz, 2H), 3.65 – 3.49 (m, 4H), 3.20 – 3.10 (m, 2H), 2.92 – 2.82 (m, 2H). ^{13}C NMR (DMSO-d6): δ 155.0, 144.1, 125.9, 123.5, 121.0, 177.4, 46.9, 39.5, 34.2. HRMS (ESI): calcd. for $C_{20}H_{20}F_6N_4O_3Se$ $[M+H]^+$: 559.0605; found $[M+H]^+$ 559.0673.

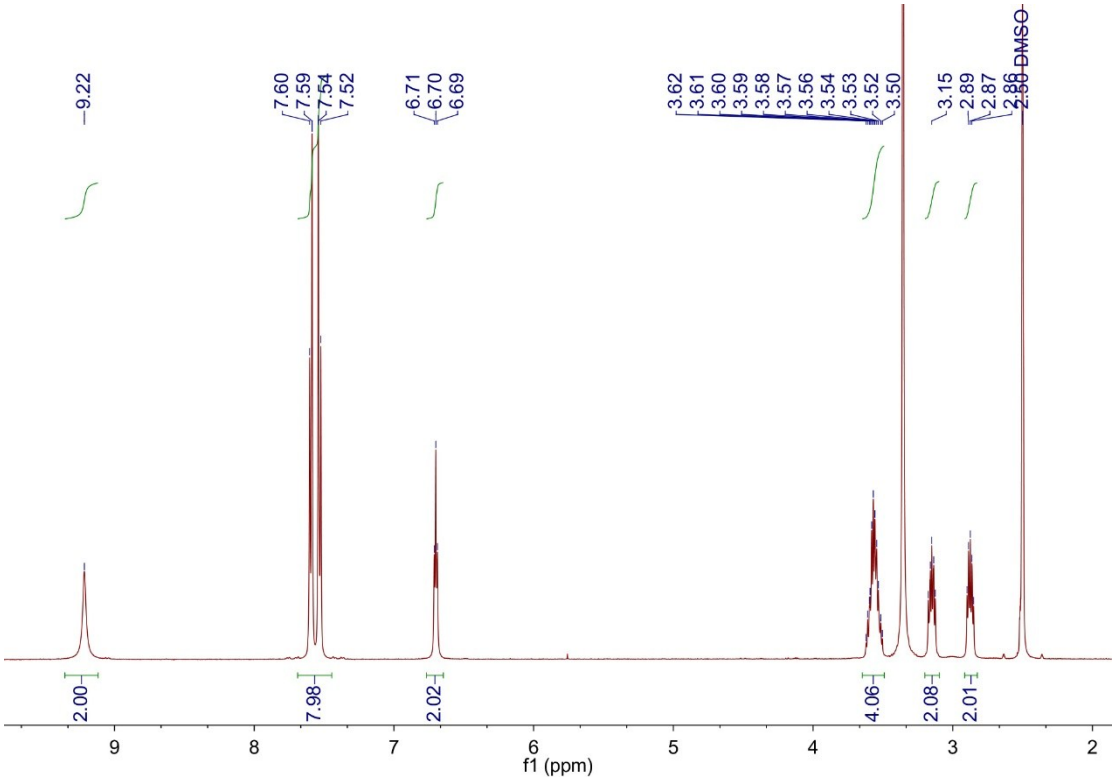


Figure S9. ^{1}H NMR spectrum of **2F** in DMSO-d6.

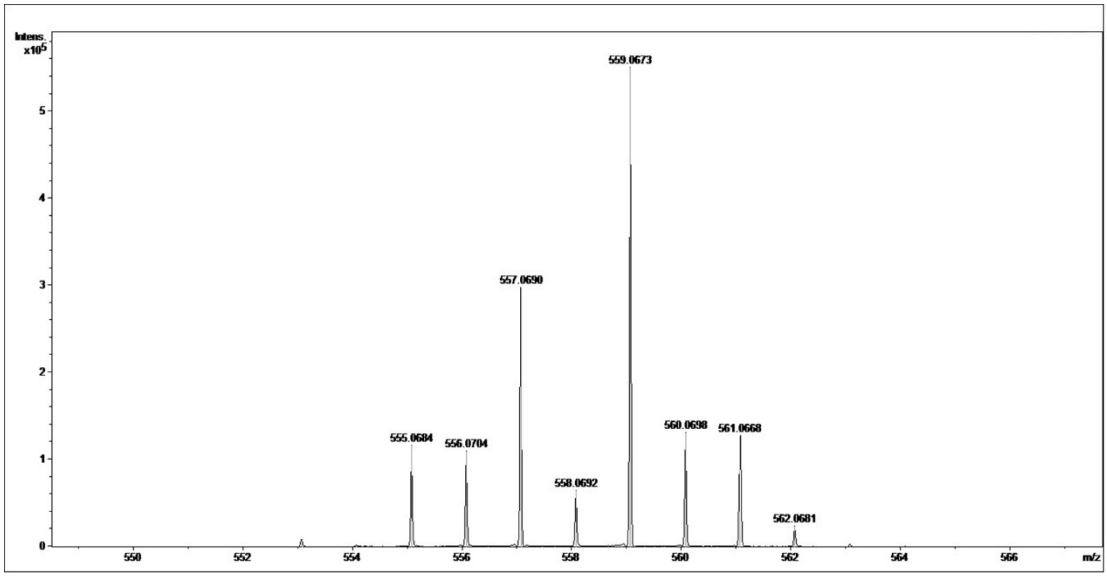
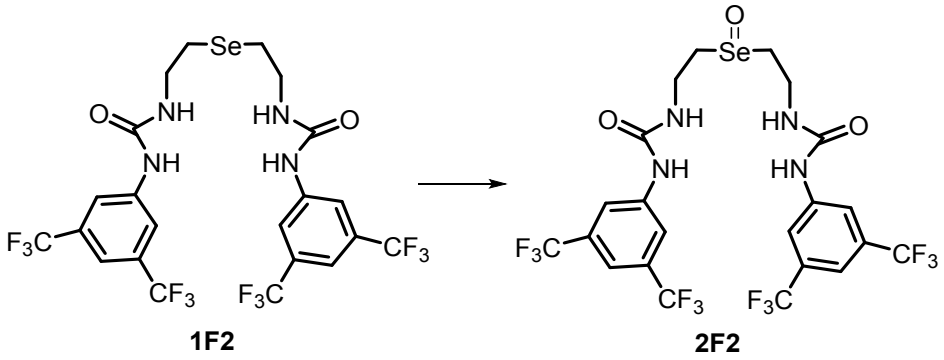


Figure S10. HR-MS spectrum of **2F**.

1,1'-(Seleninylbis(ethane-2,1-diyl))bis(3-(3,5-bis(trifluoromethyl)phenyl)urea) (2F2)



1F2 **2F2**

1F2 (0.5 mmol) was dissolved in a 1:1 solution of MeOH/CH_2Cl_2 (10 mL/mmol) and cooled to 0 °C. N-Chlorosuccinimide (0.6 mmol) was added, and the resulting mixture was stirred for 30 min at 0 °C. The solution was then diluted with an equal volume of CH_2Cl_2, and a 10% solution of NaOH was added (10 mL/mmol). The resulting mixture was stirred for 5 min, and the organic phase was separated, dried over $MgSO_4$, and concentrated. The crude selenoxide was then precipitation in water to get desired product. Yield: 64%. ^{1}H NMR (DMSO-d6): δ 9.59 (s, 2H), 8.07 (s, 4H), 7.52 (s, 2H), 6.93 (t, J = 5.2 Hz, 2H), 3.67 – 3.52 (m, 4H), 3.1 – 3.27 (m, 2H), 2.98 – 2.85 (m, 2H). ^{13}C NMR (DMSO-d6): δ 154.9, 142.5, 130.4, 124.4, 122.2, 117.2, 113.4, 46.5, 34.4. HRMS (ESI): calcd. for $C_{22}H_{18}F_{12}N_4O_3Se$ $[M+H]^+$ 695.0353; found $[M+H]^+$ 695.0200.

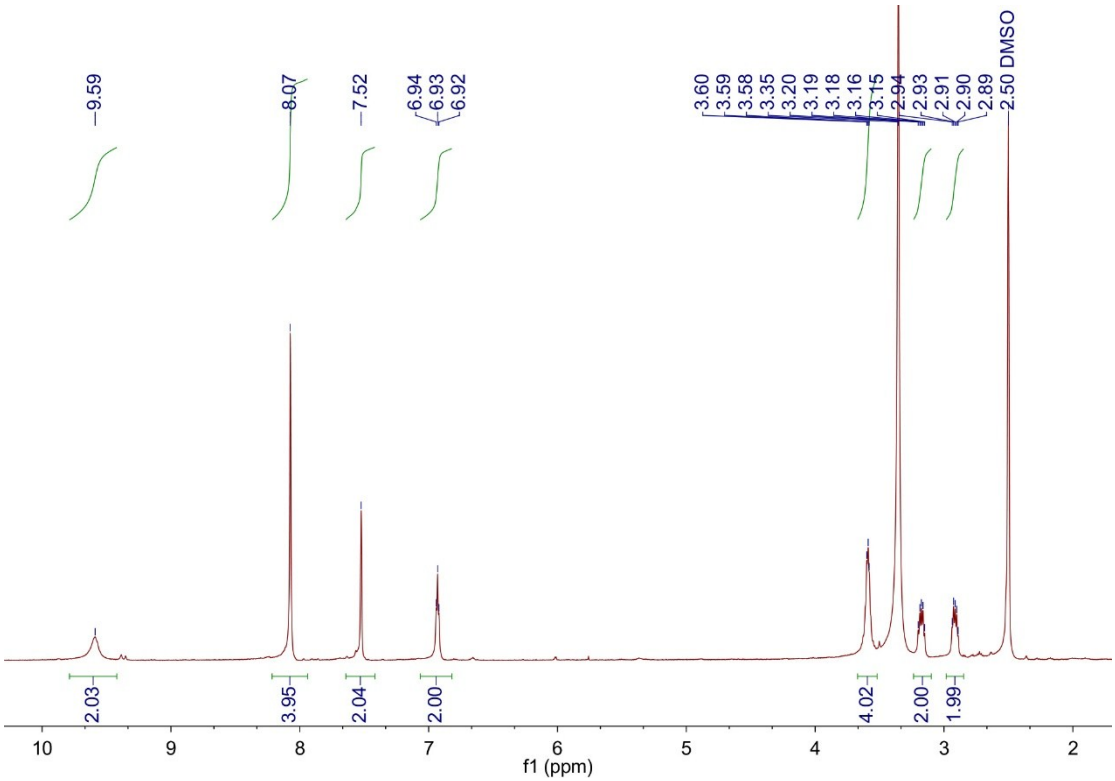


Figure S11. ^{1}H NMR spectrum of **2F2** in DMSO-d6.

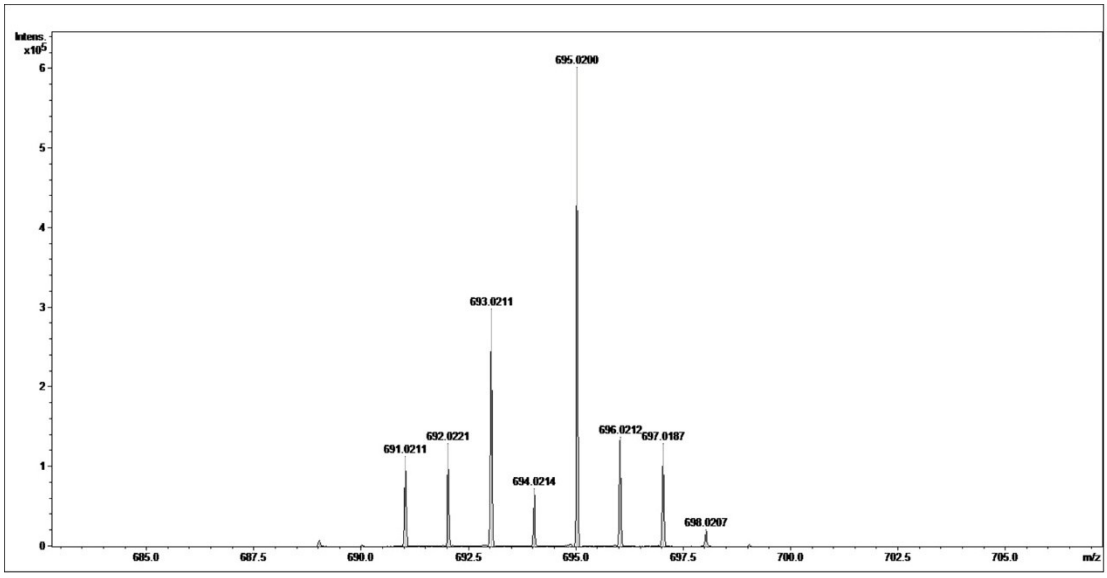


Figure S12. HR-MS spectrum of **2F2**.

1.5 HPLC measurements

To assess the hydrophobicity of receptors, their retention times were measured by reverse-phase HPLC.[2] The mobile phase was prepared with HCOOH, acetonitrile and water. Samples under test were prepared as DMSO solutions at a concentration of 0.1 mg/mL. Samples were injected (2 μL) directly onto an Agilent SB C-18 column (2.1 mm50 mm 1.8 μm particle size) and separated by using acetonitrile (50%) and HCOOH (0.1%) in water over 5 min. UV data were recorded at 19 - 600 nm, and mass spectra were recorded by using positive-ion electrospray ionization to assign retention times to respective receptors.

1.6 reduction spectra of 2F and 2F2

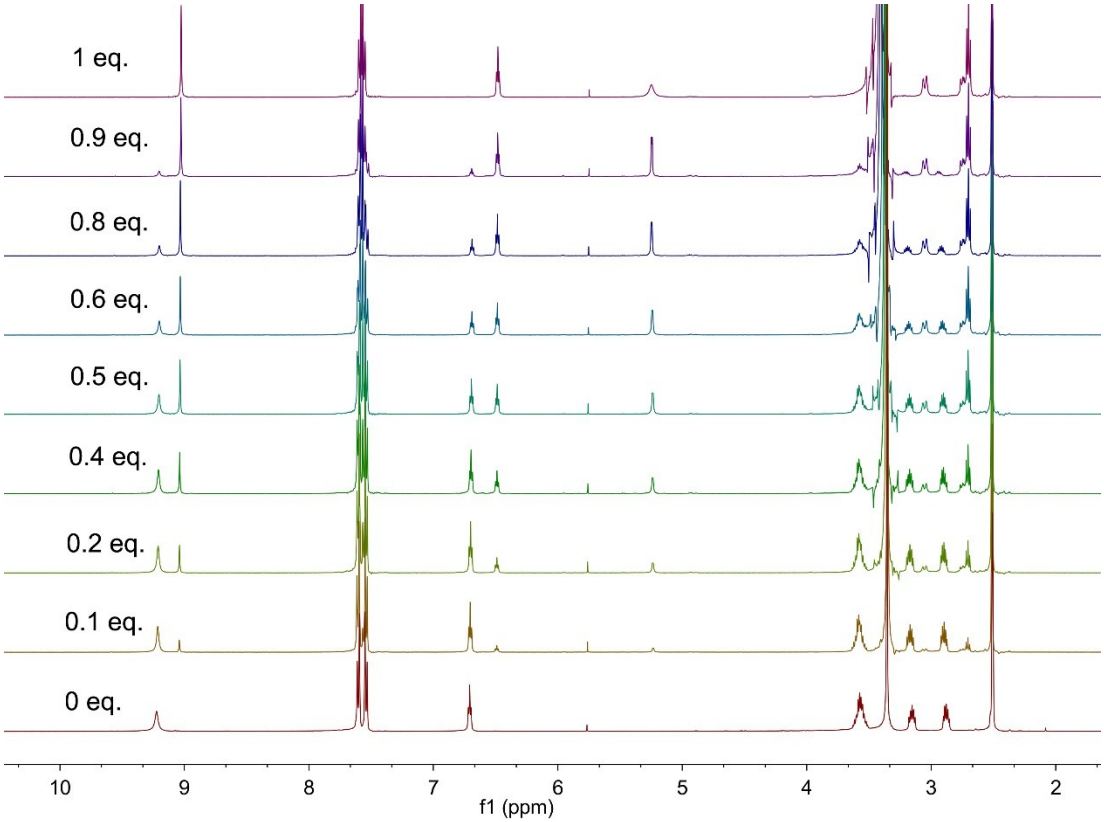


Figure S13. Stack plot of ^{1}H NMR (500 MHz, 298 K) spectra obtained from titration of **2F** (20 mM) against DTT in [D6]DMSO/0.5% water.

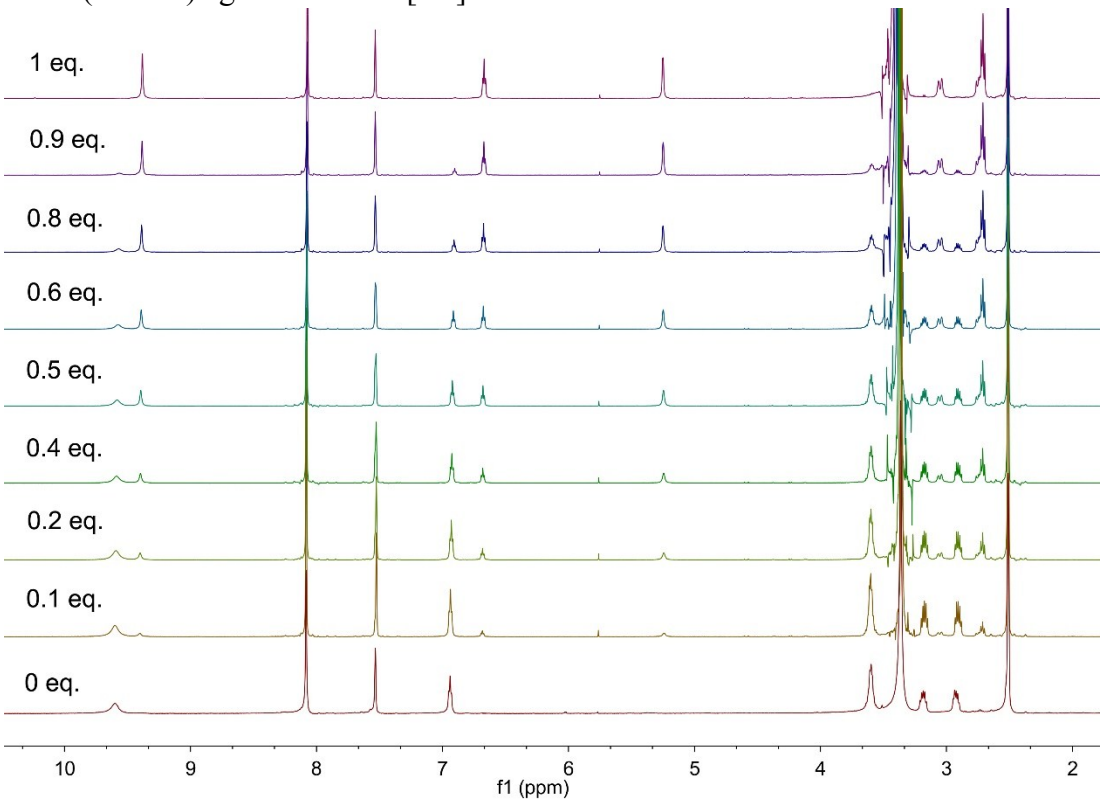


Figure S14. Stack plot of ^{1}H NMR (500 MHz, 298 K) spectra obtained from titration of **2F2** (20 mM) against DTT in [D6]DMSO/0.5% water.

2. Anion binding affinities

General procedure

Chloride binding affinities were investigated by using 1H NMR spectroscopic titration in DMSO-d6/5% H2O. TBAC (100 mm) in a solution of receptor (1 mm) in [D6]-DMSO/5% H2O was prepared as the guest solution. Receptor (1 mm) in [D6]DMSO/5% H2O was prepared as the host solution. To perform the titration experiment, aliquots of the guest and host solutions in a total volume of 500 µL were mixed (the volume of the guest solution was initially 0 µL, then 5 - 450 µL, and finally 500 µL). Binding constants were obtained by fitting the change in chemical shift of the thiourea NH signal to a 1:1 model by using the WinEQNMR 2 computer program.[3] In this study, both NH signals were considered.

Titration spectra and Fit Plot

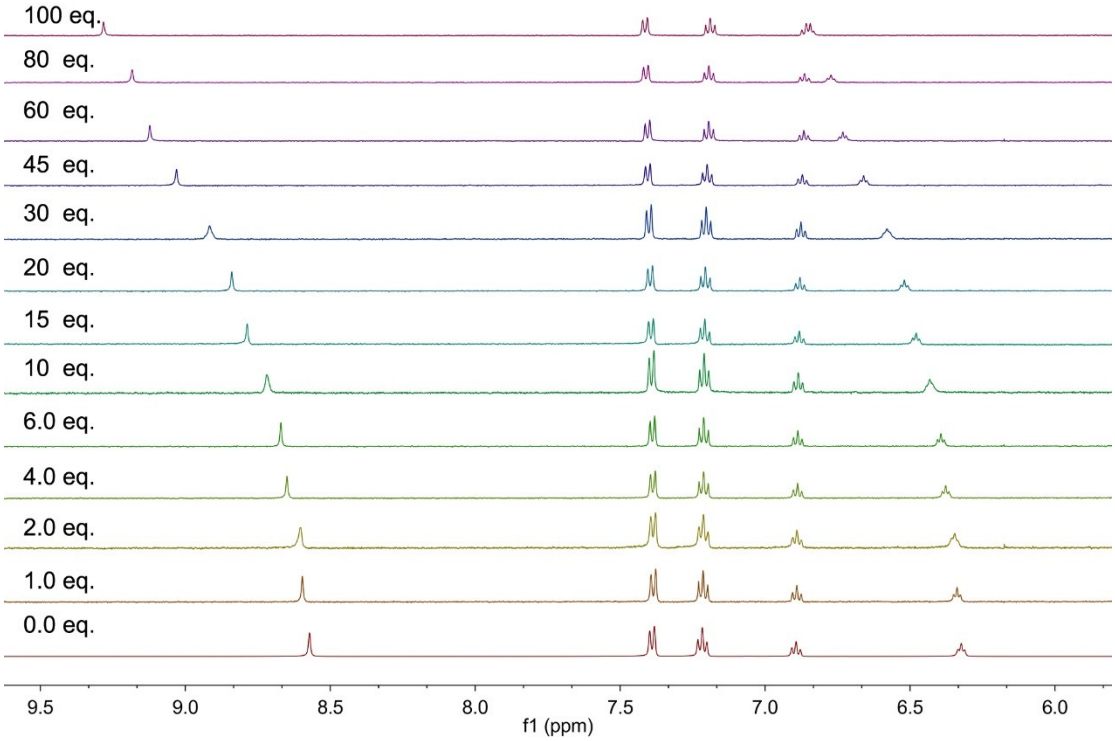


Figure S15. Stack plot of partial ^{1}H NMR spectra from titration of **1P** with TBACl.

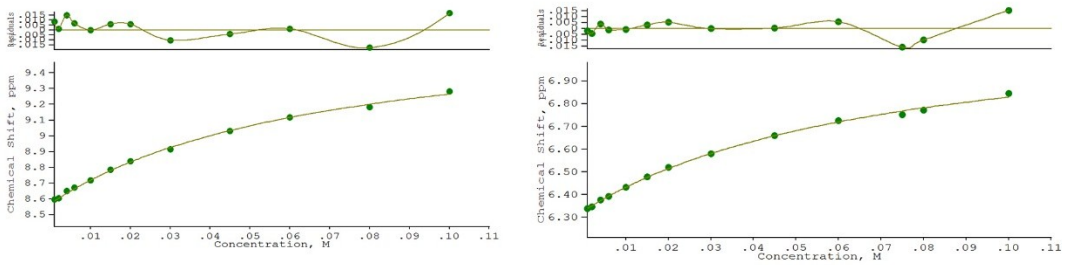


Figure S16. Fitplot for the ^{1}H NMR titration of **1P** with TBACl. (a) NH signal at δ = 8.55 ppm, Ka = 17 M^{-1}; (b) NH signal at δ = 6.30 ppm, Ka = 19 M^{-1}.

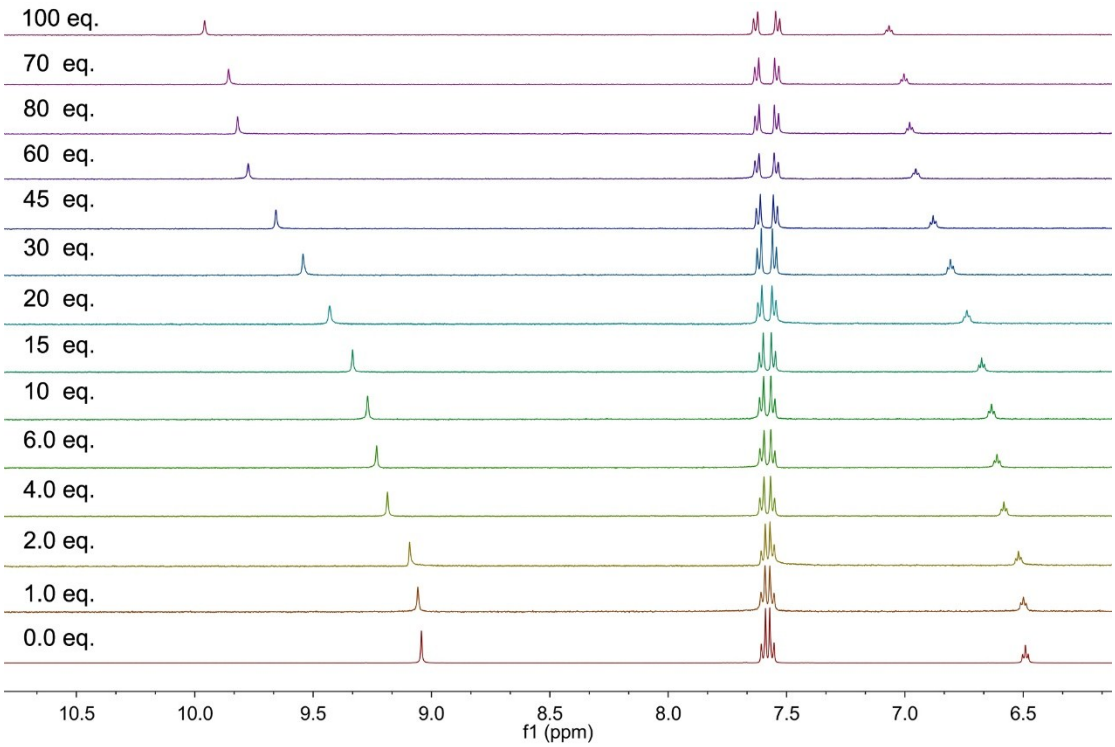


Figure S17. Stack plot of partial ^{1}H NMR spectra from titration of **1F** with TBACl.

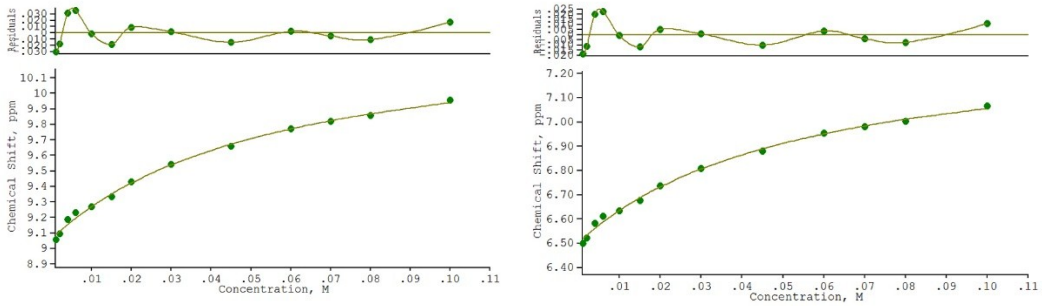


Figure S18. Fitplot for the ^{1}H NMR titration of **1F** with TBACl. (a) NH signal at δ = 9.03 ppm, Ka = 29 M^{-1}; (b) NH signal at δ = 6.48 ppm, Ka = 24 M^{-1}.

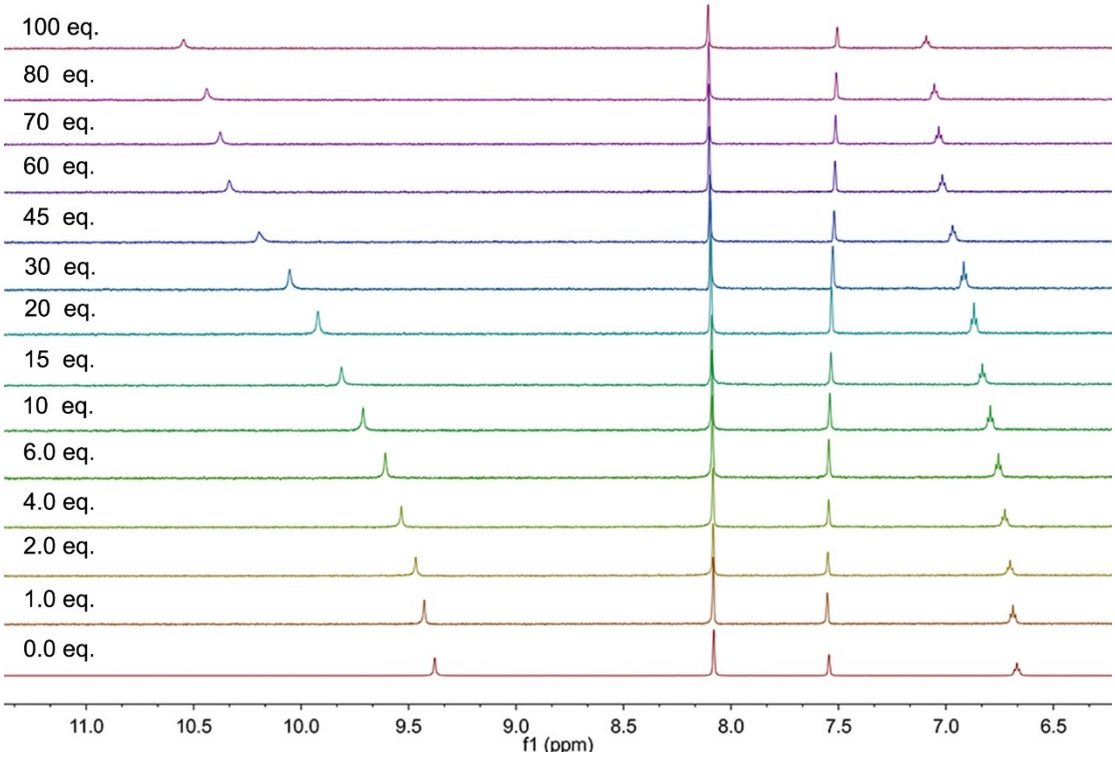


Figure S19. Stack plot of partial ^{1}H NMR spectra from titration of **1F2** with TBACl.

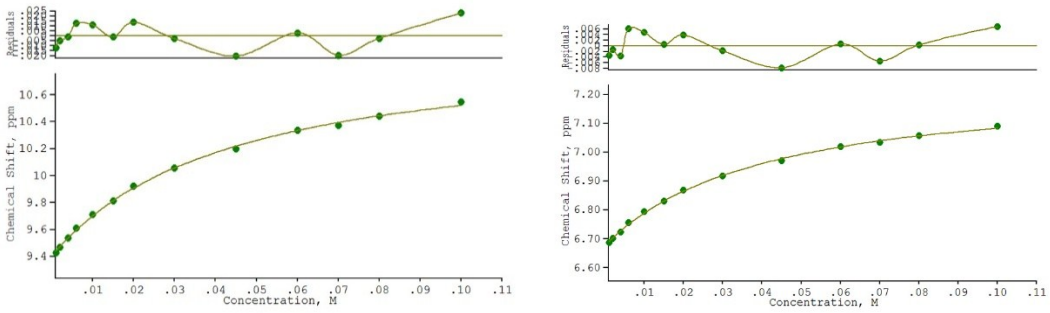


Figure S20. Fitplot for the ^{1}H NMR titration of **1F2** with TBACl. (a) NH signal at δ = 9.37 ppm, Ka = 38 M^{-1}; (b) NH signal at δ = 6.66 ppm, Ka = 40 M^{-1}.

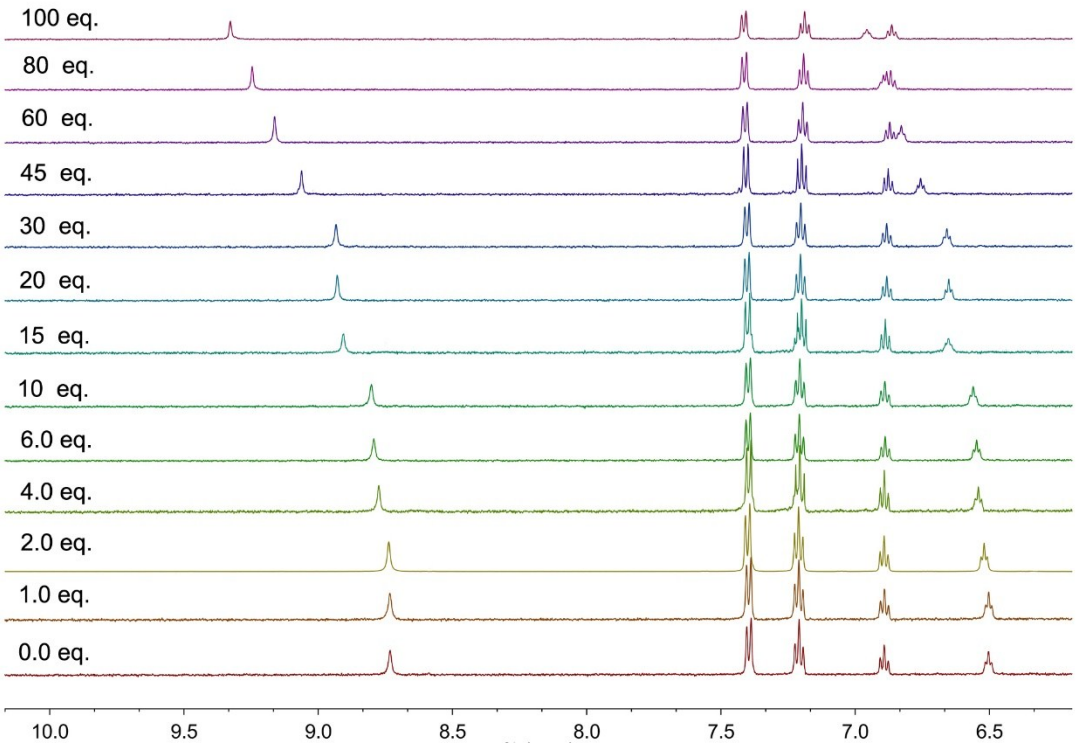


Figure S21. Stack plot of partial ^{1}H NMR spectra from titration of **2P** with TBACl.

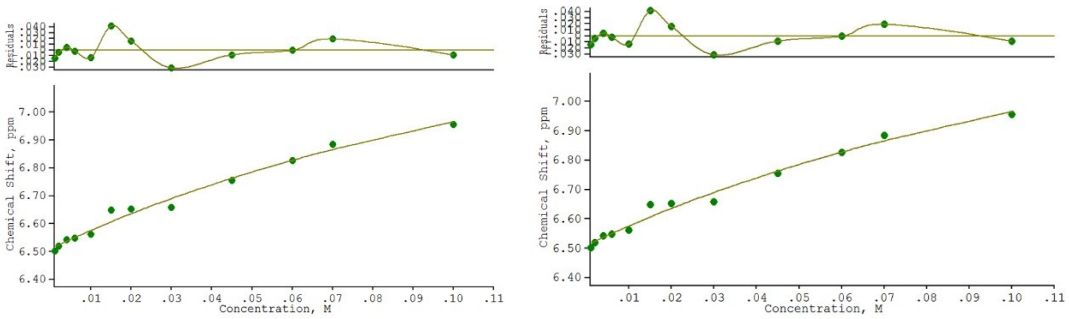


Figure S22. Fitplot for the ^{1}H NMR titration of **2P** with TBACl. (a) NH signal at δ = 8.73 ppm, Ka < 10 M^{-1}; (b) NH signal at δ = 6.51 ppm, Ka < 10 M^{-1}.

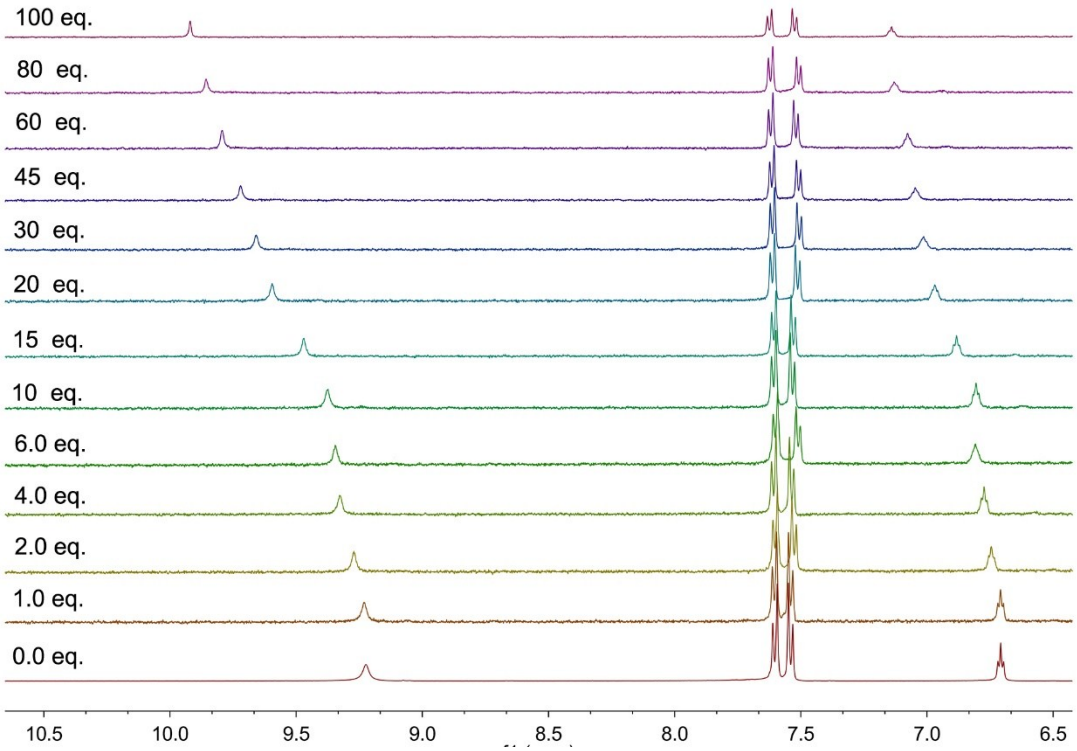


Figure S23. Stack plot of partial ^{1}H NMR spectra from titration of **2F** with TBACl.

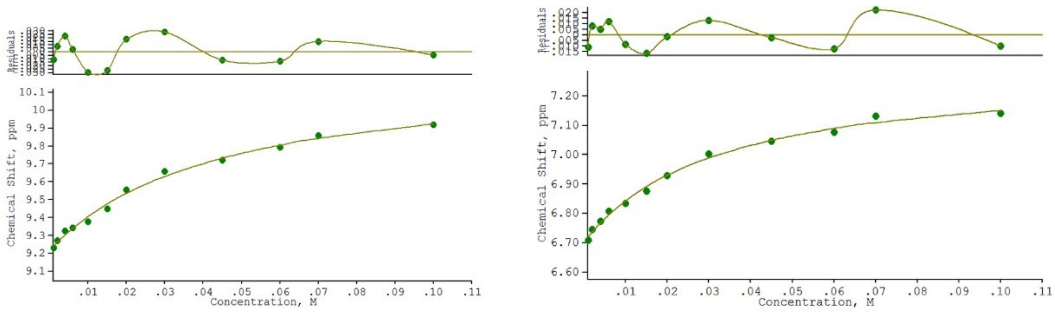


Figure S24. Fitplot for the ^{1}H NMR titration of **2F** with TBACl. (a) NH signal at δ = 9.22 ppm, Ka = 27 M^{-1}; (b) NH signal at δ = 6.70 ppm, Ka = 31 M^{-1}.

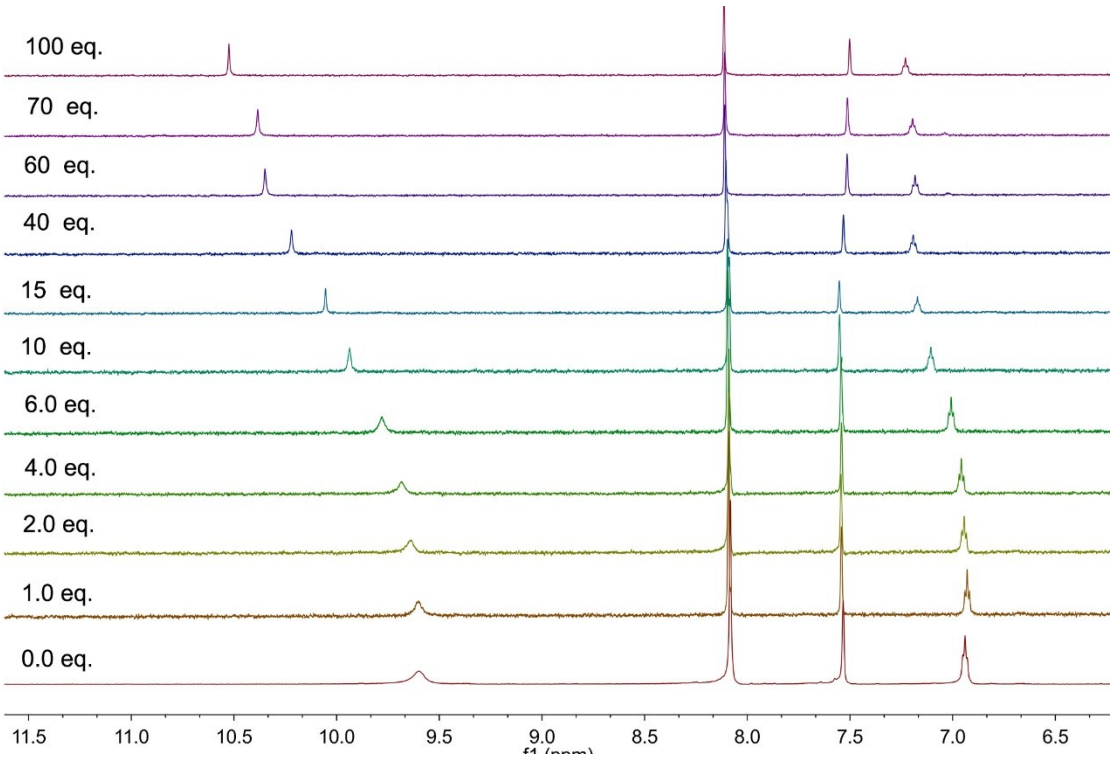


Figure S25. Stack plot of partial ^{1}H NMR spectra from titration of **2F2** with TBACl.

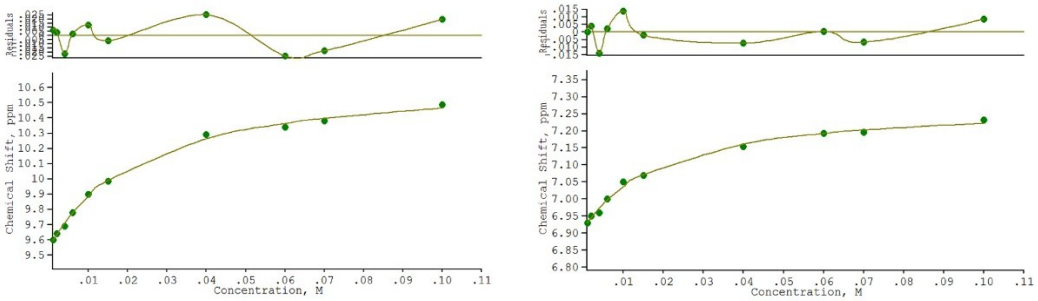


Figure S26. Fitplot for the ^{1}H NMR titration of **2F2** with TBACl. (a) NH signal at δ = 9.59 ppm, Ka = 36 M^{-1}; (b) NH signal at δ = 6.93 ppm, Ka = 44 M^{-1}.

3. Dynamic Light Scattering

The size of **NPP**, **NPF** and **NPF2** were measured using a Malvern Nano_S instrument (Malvern, U.K.) at room temperature.

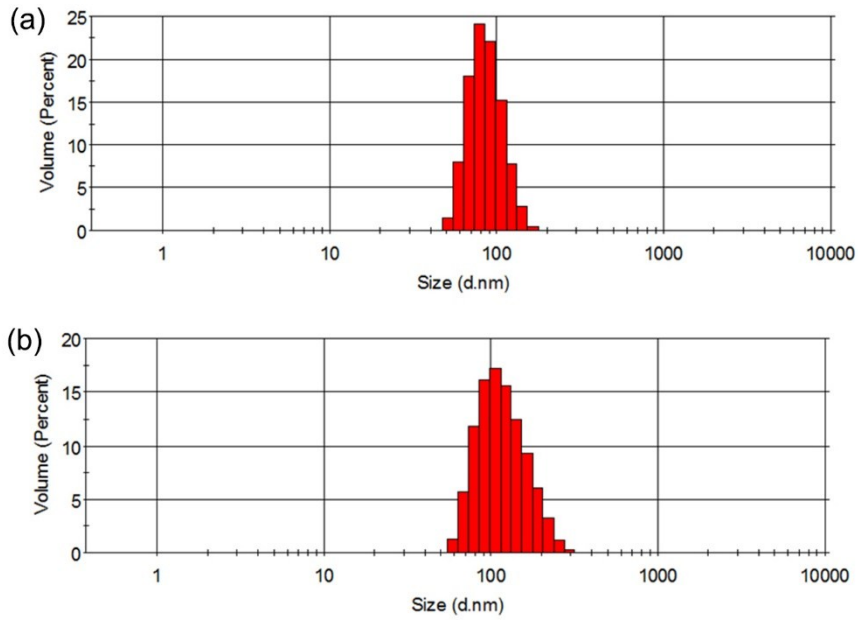


Figure S27. DLS results for (a) 0.10 mM **NPF** and (b) 0.10 mM **NPF2** in water.

4. SEM images of NPF and NPF2

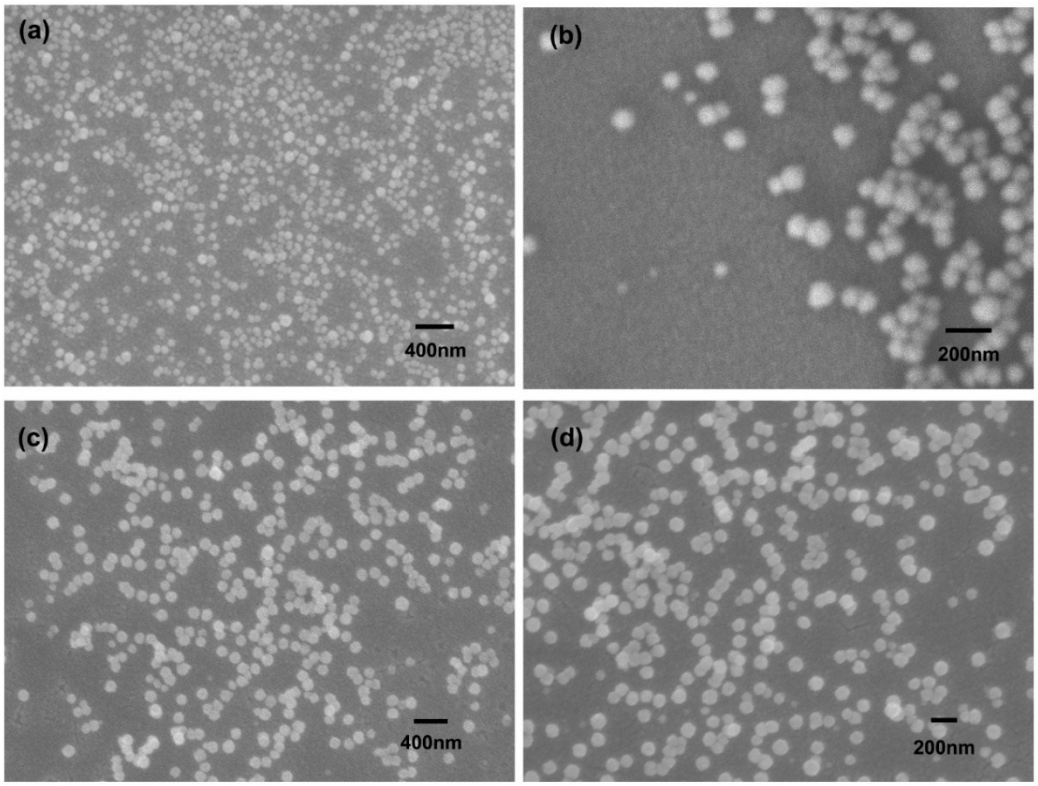


Figure S28. (a, b) SEM images of **NPF** (c, d) SEM images of **NPF2**

5. Calculations

Complexes of representative transporters **1F2**, **2F2** with chloride ion were subjected to theoretical caculation using *ab initio* methods by Gaussian 09 program (Hartree-Fock, 6-31+G* basis set, implemented within Spartan '06). It was found that **1F2** and **2F2** took very similar conformations to bind chloride ion, the "1, 5-position" distances are both 5.6 Å, and the hydrogen bonds are all 2.6 Å long.

6. Procedures for anion transport experiments

6.1 Cl^-/NO_3^- antiport

In the Cl^-/NO_3^- antiport experiment, a typical procedure is as follow:

9.0 mg EYPC (or 5.2 mg EYPC with 1.9 mg cholesterol) was first dissolved in 3 mL $CHCl_3$ and evaporated under reduced pressure. The formed thin film was then dried under high vacuum for 3 h. The lipid was hydrated with 200 mM $NaNO_3$ solution containing 1 mM lucigenin for 2 h at 40°C followed by 10 freeze-thaw cycles using liquid nitrogen and thermostat water bath. The resulted suspension was then extruded though 0.22 um polycarbonate membrane before purified by Sephadex G-75 to remove the extra vesicular dye using 200 mM $NaNO_3$ solution as elute. The obtained vesicles were kept under 4°C. Then 1.94ml 200 mM $NaNO_3$ solution containing 25 mM NaCl was added before putting 60 ul vesicle solution into a fluorimetric cell. The fluorescence emission was continuously monitored at 503 nm (excited at 372 nm). And then 10 ul methanol solution of the carrier or 10 ul water solution of the nanoparticles was added with gentle stirring. Finally, 10 ul of 50% Triton X-100 water solution was added to achieve completely lysis of the vesicles. Using E_0 and E_∞ represent the initial and final emission intensity, the collected data of fluorescence time course was normalized according to equation of $(E_0-E_t)/(E_0-E_\infty)$. The injection of additives part during the fluorescence experiment of the spectrum was subtracted for clarity.

6.2 Hill analysis

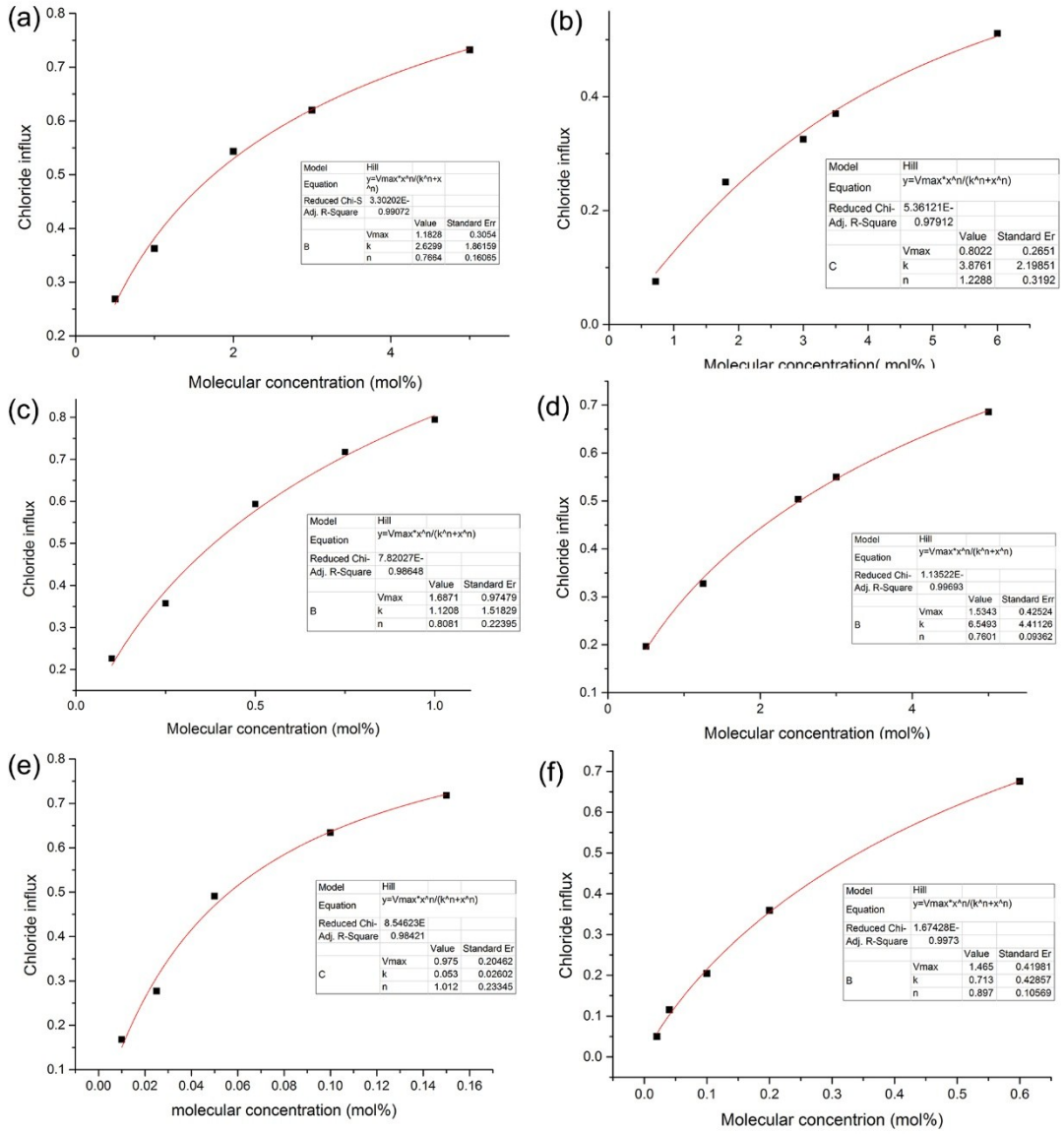


Figure S29. Hill plot of chloride transport promoted by varying concentrations of (a) **1P** (b) **2P** (c) **1F** (d) **2F** (e) **1F2** (f) **2F2** from unilamellar EYPC vesicles loaded with 1 mM lucigenin and 200 mM aqueous NaNO3. The vesicles were suspended in a solution containing 200 mM NaNO3 and 25 mM NaCl.

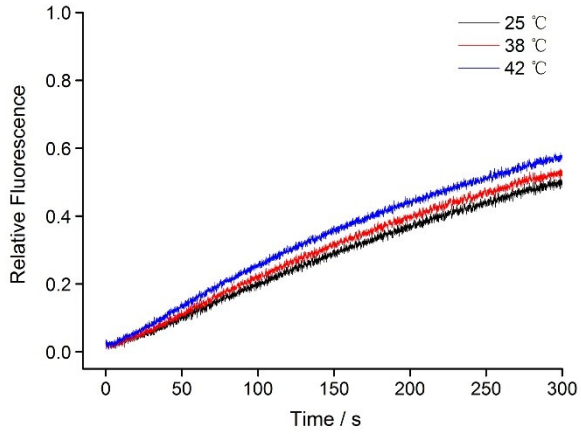


Figure S30. Turn-on chloride transport of **NPF2** (2 mol%) at different temperatures with 0.006 mM DTT from LUVs loaded with 1.0 mM lucigenin and 200 mM aqueous $NaNO_3$. The vesicles were suspended in a solution containing 200 mM $NaNO_3$ and 25 mM NaCl.

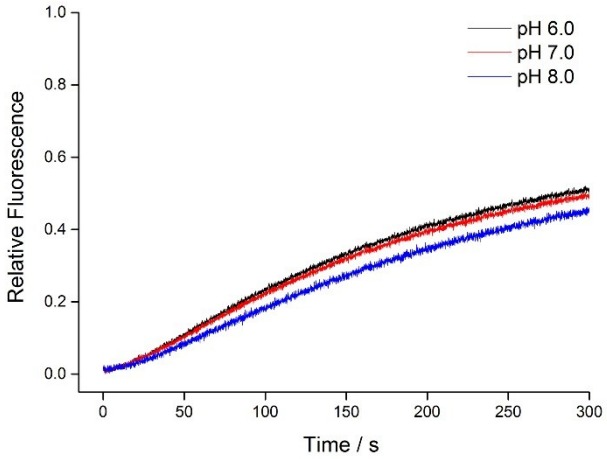


Figure S31. Turn-on chloride transport of **NPF2** (2 mol%) at different pH values with 0.006 mM DTT from LUVs loaded with 1.0 mM lucigenin and 200 mM aqueous $NaNO_3$. The vesicles were suspended in a solution containing 200 mM $NaNO_3$ and 25 mM NaCl.

6.3 Carboxyfluorescein (CF) assay

9.0 mg EYPC was first dissolved in 3.0 mL $CHCl_3$ and evaporated under reduced pressure. The formed thin film was then dried under high vacuum for 3 h. The lipid was hydrated with a solution of 100 mM NaCl buffered at pH 7.0 with 25 mM HEPES and CF (20 mM) for 2 h at 40°C followed by 10 freeze-thaw cycles using liquid nitrogen and thermostat water bath. The resulted suspension was then extruded though 0.22 um polycarbonate membrane before purified by Sephadex G-75 to remove the extra vesicular dye using the same solution without CF as elute. The obtained vesicles were kept under 4°C. Then 1.94 ml 100 mM NaCl solution buffered at pH 7.0 with 25 mM HEPES was added before putting 60 ul vesicle solution into a fluorimetric cell. The fluorescence emission was continuously monitored at 517 nm (excited at 492 nm). And then carrier, nanoparticles or GSH was added with gentle stirring. Finally, 10 ul of 50% Triton X-100 water solution was added to achieve completely lysis of the vesicles. Using E_0 and E_∞ represent the initial and final emission intensity, the collected data of fluorescence time course was normalized according to equation of $(E_0-E_t)/(E_0-E_\infty)$. The injection of additives part of the spectrum was subtracted for clarity. No CF transport was observed indicated that LUVs were intact with the transporter or nanoparticle.[4]

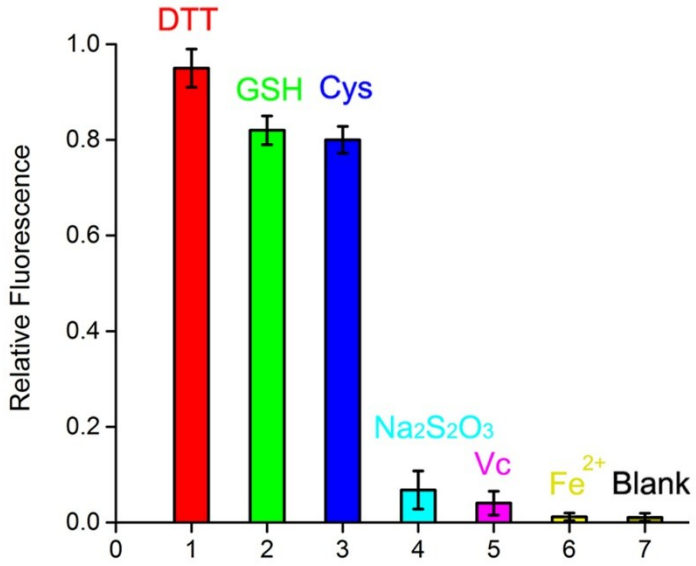


Figure S32. Comparison of in situ turn-on chloride transport of **NPF2** (2.0 mol%) by different reductants (0.10 mM) at 5 mins: 1, DTT; 2, GSH; 3, cysteine; 4, $Na_2S_2O_3$; 5, vitamin C; 6, Fe^{2+}; 7, blank

6.4 Detection of different amino acids

Amino acids for test were dissolved in water. The pH of the samples were carefully adjusted to 7.0 with 5.0 M NaOH solution. 10 ul of the sample (final concentration 5.0 nM, pure water was used as blank) was added to 1.94 ml 200 mM $NaNO_3$ solution with 2 mol% **NPF2**. After 30 minutes, 60 ul lucigenin-containing LUVs solution and 10 ul of 5.0 M NaCl solution was added. The fluorescence emission was continuously monitored at 503 nm (excited at 372 nm). Finally, 10 ul of 50% Triton X-100 water solution was added to achieve completely lysis of the vesicles. Relative fluorescence was obtained as mentioned above. For each data, the background fluorescence change was deducted.

6.5 UV spectra of disassembling process of nanoparticles

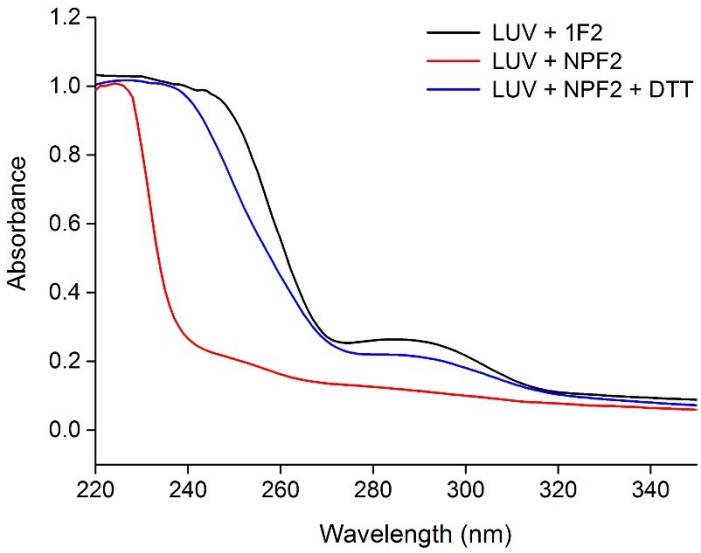


Figure S33. UV spectra of **1F2** (1.0 mol%), **NPF2** (1.0 mol%) or **NPF2** (1.0 mol%) with DTT (0.01 mM) in the presence of LUV.

7. ^{77} Se NMR experiment and spectra

43 mg of **1P** or 30 mg of **2P** were dissolved in 500 µl of DMSO-d6.

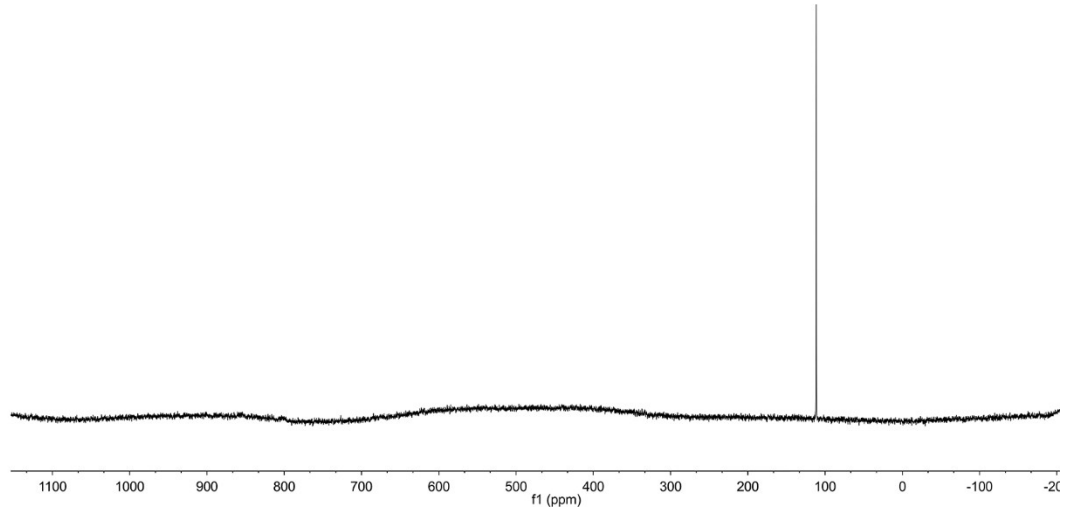


Figure S34. ^{77}Se NMR of selenide **1P**. (DMSO-d6, 95 MHz) δ= 111 ppm.

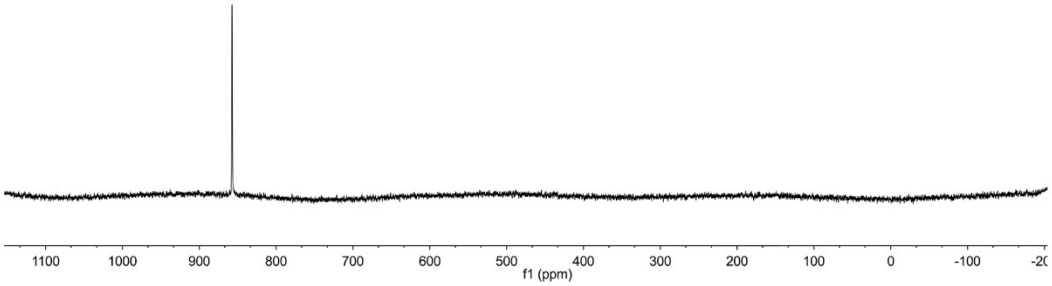


Figure S35. ^{77}Se NMR of selenoxide **2P**. (DMSO-d6, 95 MHz) δ= 857 ppm.

8. References

[1] M. D. Milton, S. Khan, J. D. Singh, V. Mishra, B. L. Khandelwal, *Tetrahedron Lett.* **2005**, 46, 755.

[2] H. Valkenier, C. J. E. Haynes, J. Herniman, P. A. Gale, A. P. Davis, *Chem. Sci.* **2014**, 5, 1128

[3] M. J. Hynes, *J. Chem. Soc., Dalton Trans.* **1993**, 311.

[4] S. Matile, N. Sakai, in *Analytical Methods in Supramolecular Chemistry*, (Ed. C. A. Schalley), Wiley -VCH, Weinheim, **2007**, pp. 381.