Electronic Supplementary Information (ESI)

Transmembrane anion transport promoted by thioamides

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Contents

1 General information

1.1 Materials

All solvents and reagents were commercially available and used as received unless otherwise stated.

ACROS: Lawesson's reagent 99%;

Sigma-Aldrich: tetrabutylammonium chloride for ion pair chromatography, ≥99.0%;

Euriso-top: DMSO-d_6 + 0.03%TMS v/v (>99.8% D).

TLC was carried out on Merck silica gel 60 F_{254} plates.

Preparative chromatography was done manually on Merck silica gel 60 (230 – 400 mesh) or with the aid of Teledyne ISCO CombiFlash instrument using RediSep normal-phase silica flash columns.

1.2 Instruments and methods

Nuclear magnetic resonance (NMR) spectroscopy

The NMR spectra were recorded using Agilent NMR (^{1}H: 400 MHz, ^{13}C: 100 MHz) or Bruker AM-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) spectrometers at ambient temperature in DMSO-d_6. The chemical shifts, δ, are reported in parts per million (ppm) and coupling constants, J, are given in hertz (Hz). The NMR spectra were referenced to the solvent residual signal (^{1}H: δ_{DMSO} = 2.500 ppm, $\delta_{chloroform}$ = 7.260 ppm, ^{13}C: δ_{DMSO} = 39.50 ppm). Data are reported as follows: chemical shift, multiplicity (s – singlet, bs – broad singlet, d – doublet, t – triplet, dd- doublet of doublets, dt – doublet of triplets, etc.), coupling constant and integration.

Mass spectrometry

The ESI-MS spectra were obtained using Mariner (ESI TOF) or API 365 (ESI 3Q) mass spectrometers with methanol as the spray solvent.

Elemental analysis

Elemental analysis was performed using a CHN analyzer Vario EL III.

Melting points determination

The melting points are uncorrected.

X-ray data collection and refinement

Good quality single-crystal of **3T**×TBACl was selected for the X-ray diffraction experiment at T = 100(2) K. Diffraction data were collected on the Agilent Technologies SuperNova Dual Source diffractometer with Cu$K\alpha$ radiation (λ = 1.54184 Å) using CrysAlis RED software.[1] The analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid,[2] implemented in SCALE3 ABSPACK scaling algorithm, was applied.[3] The structural determination procedure was carried out using the SHELX package.[4] The structure was solved with direct methods and then successive least-square refinement was carried out based on the full-matrix least-squares method on F^2 using the SHELXL program.[5] All H-atoms linked to the N-atoms were located on a Fourier difference map and refined as riding with U_{iso}(H) = 1.2U_{eq}(N). The N–N bond lengths were

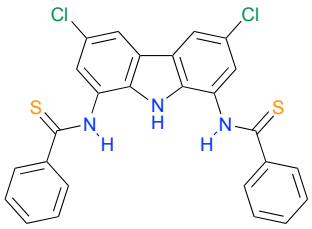
restrained to 0.87 Å. Other H-atoms were positioned geometrically, with C–H equal to 0.93, 0.96 and 0.97 for the aromatic, methyl and methylene H-atoms, respectively, and constrained to ride on their parent atoms with U_{iso}(H) = xU_{eq}(C), where x = 1.2 for the aromatic and methylene H-atoms, and 1.5 for the methyl-H-atoms.

The CCDC 2027226 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

The data collection was accomplished at the Core Facility for Crystallographic and Biophysical research. The „Core facility for crystallographic and biophysical research to support the development of medicinal products" project is carried out within the TEAM-TECH Core Facility programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

2 Synthetic procedures

Preparation of **1T**



$C_{26}H_{17}Cl_2N_3S_2$ = 506.47

A 50 ml, 2-neck, round-bottomed flask was dried for 10 min with a stream of hot air (*ca.* 500°C) from heat-gun and cooled down to rt in a desiccator. Then the flask was charged with diamide **1A** (238 mg, 0.502 mmol), Lawesson's reagent (444 mg, 1.10 mmol) and a stir bar. Then the flask was equipped with a reflux condenser connected to a Schlenk line and its side neck was closed with a stopper. Air was removed by three pump/thaw cycles and then dry THF (30 ml) was added in a stream of argon through the side neck. The reaction mixture was intensively stirred and refluxed overnight. After this time the mixture was cooled down and 2.0 g of silica gel was added. Solvents were evaporated on a rotary evaporator and the solid residue was dried under high vacuum. Then the solid residue was suspended in hexane (40 ml) and put on top of a chromatographic column made of 110 g of silica suspended in $CHCl_3$. The column was eluted with $CHCl_3$ (*ca.* 500 ml), $CHCl_3$: CH_3CO_2Et = 40 : 1 (*ca.* 500 ml) and $CHCl_3$: CH_3CO_2Et = 30 : 1 until the desired product was completely washed out. Fractions containing pure product were combined and evaporated on a rotary evaporator yielding 293 mg (98.0%) of yellow solid.

M.p.: decomposition at 301°C

^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.69 (s, 2H, thioamide NHs), 11.23 (s, 1H, carbazole NH), 8.37 (bs, 2H, carbazole CH-4/5), 8.03 (d, J = 7.6 Hz, 4H, phenyl CH-*orto*), 7.77 (bs, 2H, carbazole CH-2/7), 7.56 (t, *J* = 7.3 Hz, 2H, phenyl CH-*para*), 7.48 (t, *J* = 7.5 Hz, 4H, phenyl CH-*meta*).

^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 199.19, 141.09, 133.99, 131.30, 127.98, 125.66, 124.62, 124.46, 122.98, 119.23.

Elemental analysis: calcd. for $C_{26}H_{17}Cl_2N_3S_2$: C, 61.66; H, 3.38; N, 8.30, found: C, 61.56; H, 3.52; N, 8.27.

HR MS (TOF MS ES$^-$) m/z calcd. for $C_{26}H_{16}Cl_2N_3S_2^-$: 504.0163 found: 504.0168.

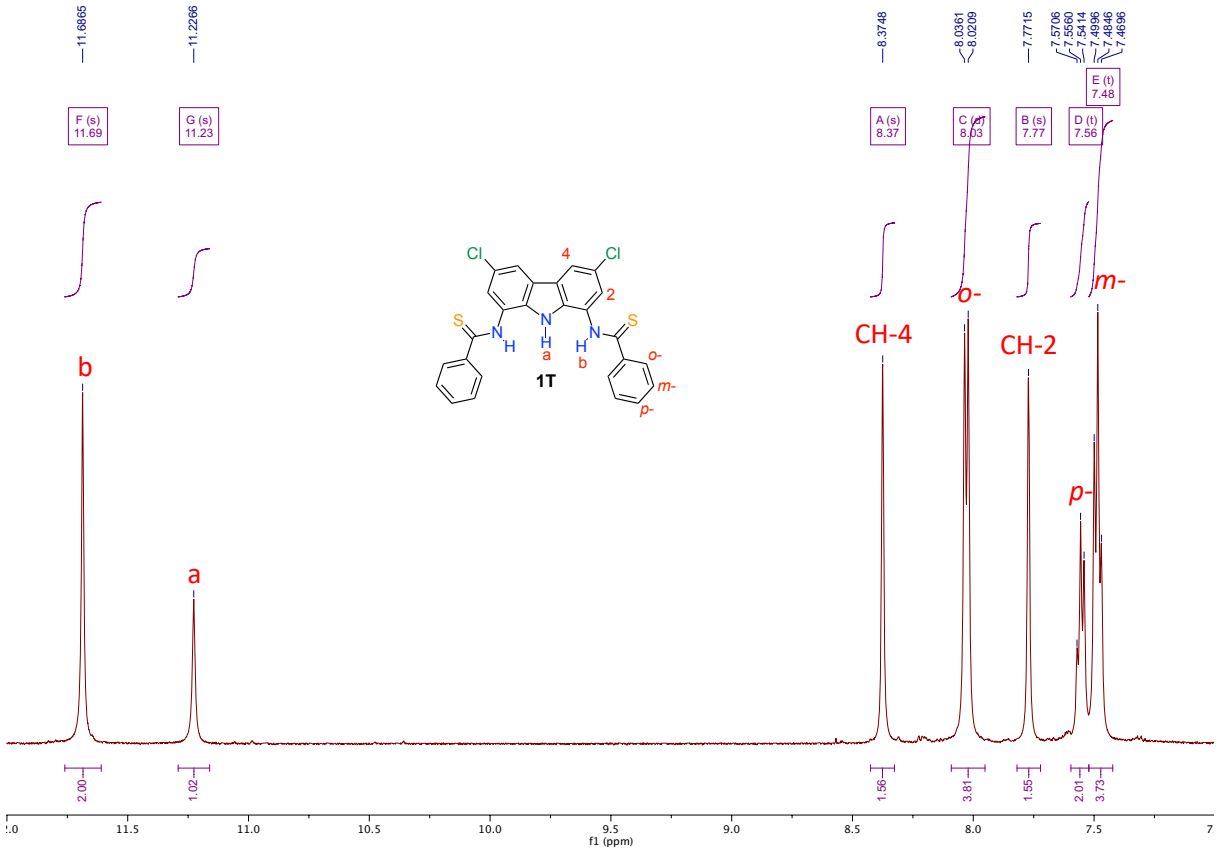


Figure S1. ^{1}H NMR spectrum of **1T** in DMSO-d_6.

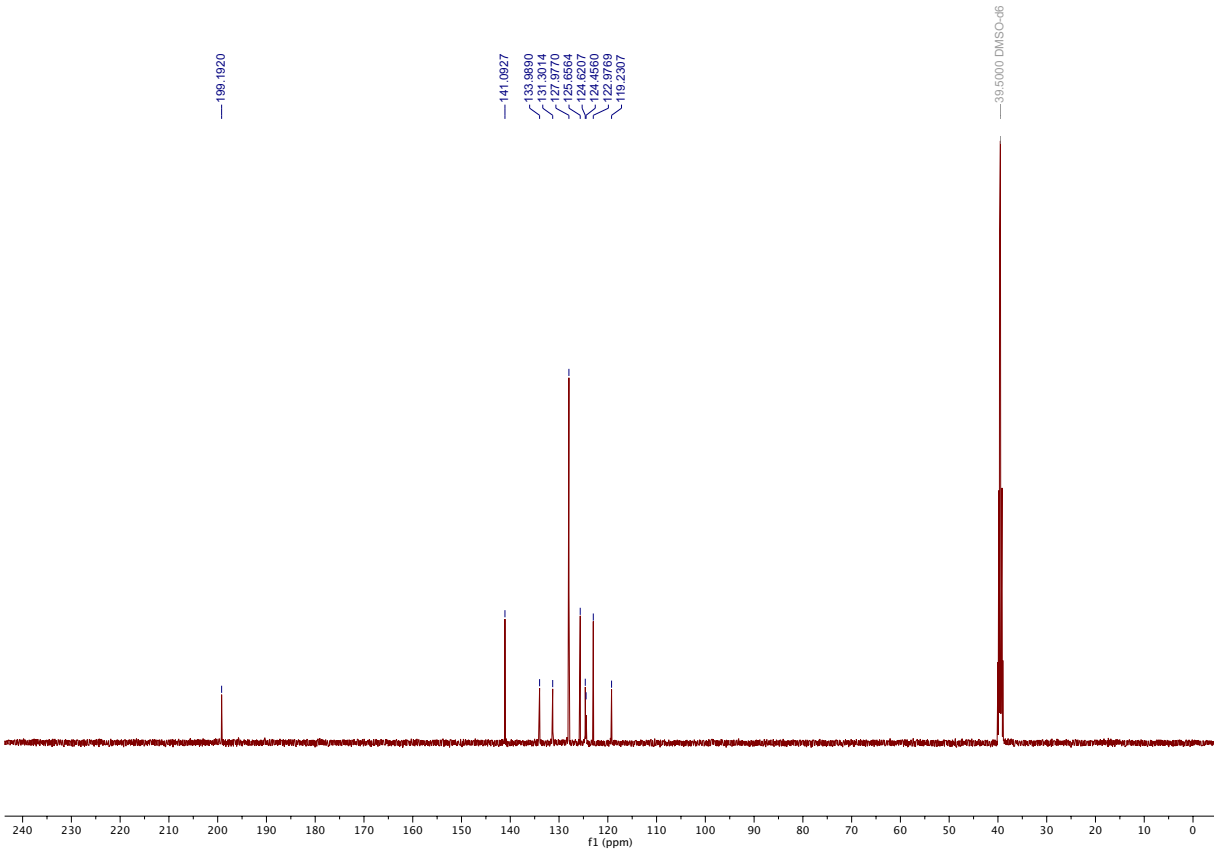
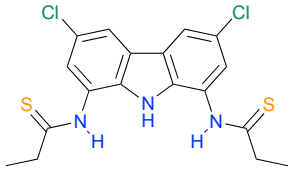


Figure S2. ^{13}C NMR spectrum of **1T** in DMSO-d_6.

Preparation of **2T**



$C_{18}H_{17}Cl_2N_3S_2$ = 410.38

A 50 ml, 2-neck, round-bottomed flask was dried for 10 min with a stream of hot air (*ca.* 500°C) from heat-gun and cooled down to rt in a desiccator. Then the flask was charged with diamide **2A** (96 mg, 0.25 mmol), Lawesson's reagent (221 mg, 0.546 mmol) and a stir bar. Then the flask was equipped with a reflux condenser connected to a Schlenk line and its side neck was closed with a stopper. Air was removed by three pump/thaw cycles and then dry THF (35 ml) was added in a stream of argon through the side neck. The reaction mixture was intensively stirred and refluxed for 48 h. After this time the mixture was cooled down and 2.3 g of silica gel was added. Solvents were evaporated on a rotary evaporator and the solid residue was dried under high vacuum. Then the solid residue was suspended in hexane (40 ml) and put on top of a chromatographic column made of 100 g of silica suspended in $CHCl_3$. The column was eluted with $CHCl_3$ (ca. 250 ml), $CHCl_3$: CH_3CO_2Et = 24 : 1 (*ca*. 250 ml), $CHCl_3$: CH_3CO_2Et = 47 : 3 (*ca*. 250 ml), 23 : 2 (*ca*. 250 ml), 9 : 1 (*ca*. 250 ml), 22 : 3 (*ca.* 1000 ml) and 43 : 7 until the desired product was completely washed out. Fractions containing pure product were combined and evaporated on a rotary evaporator yielding 83 mg (79.7 %) of yellow solid.

M.p.: decomposition at 270°C;

^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.53 (s, 2H, thioamide NH); 10.84 (s, 1H, carbazole NH); 8.31 (d, *J* = 2.0 Hz, 2H, carbazole CH); 7.75 (d, *J* = 2.0 Hz, 2H, carbazole CH); 2.88 (q, *J* = 7.5 Hz, 4H, methylene CH_2); 1.35 (t, *J* = 7.5 Hz, 6H, methyl CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 208.02, 133.48, 124.93, 124.47, 123.81, 122.99, 119.04, 38.84, 13.92.

Elemental analysis: calcd. for $C_{18}H_{17}Cl_2N_3S_2$: C, 52.68; H, 4.18; N, 10.24, found: C, 52.64; H, 4.35; N, 10.09.

HR MS (TOF MS ES$^-$) m/z calculated for $C_{18}H_{16}Cl_2N_3S_2^-$: 408.0163 found: 408.0161.

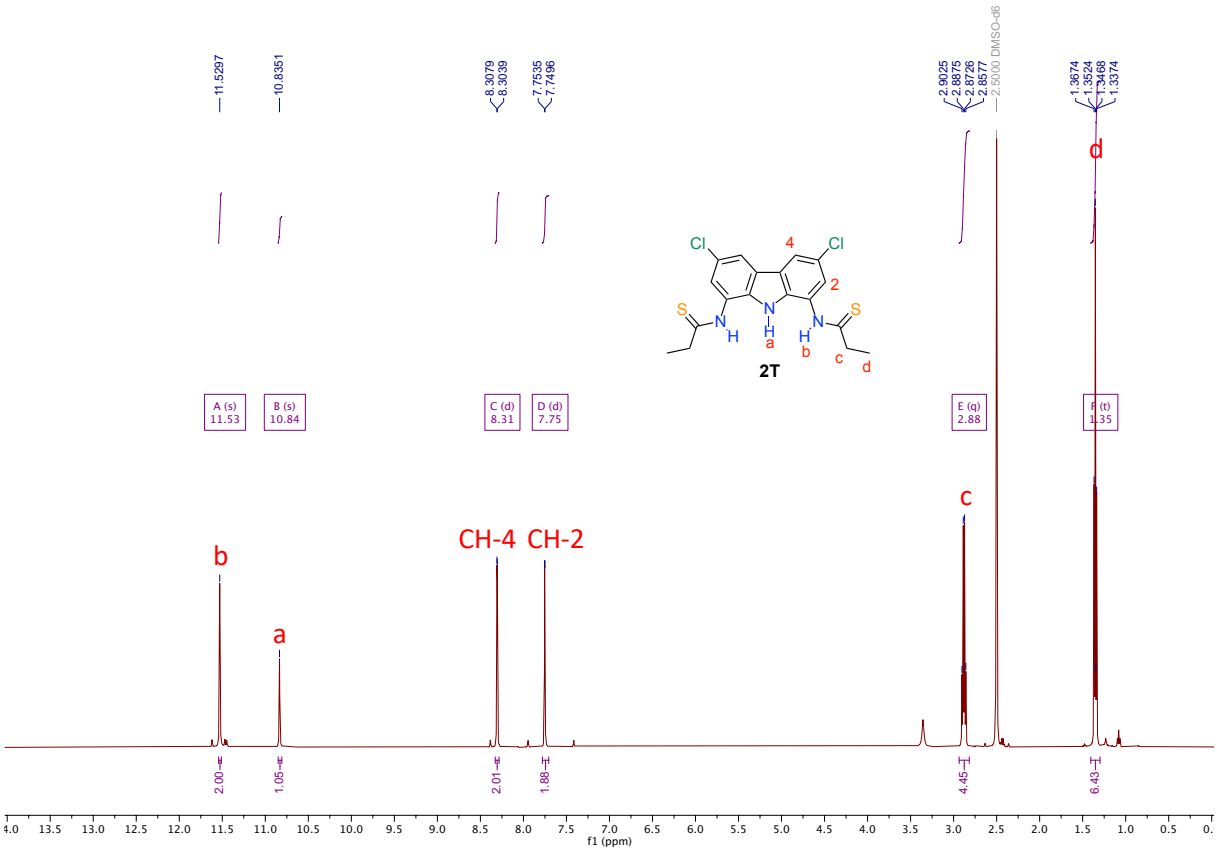


Figure S3. ^{1}H NMR spectrum of **2T** in DMSO-d_6.

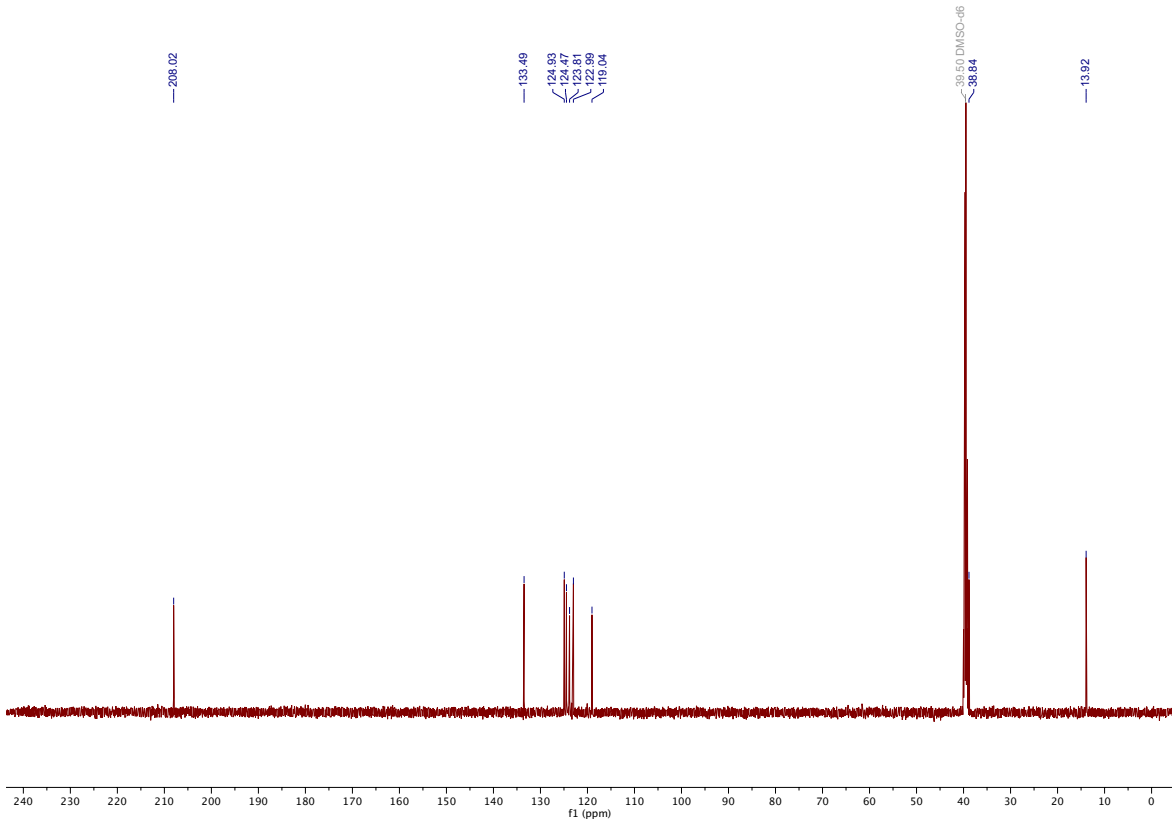
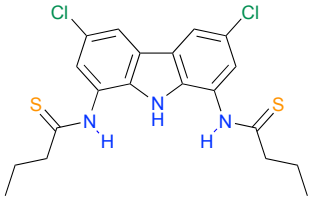


Figure S4. ^{13}C NMR spectrum of **2T** in DMSO-d_6.

Preparation of **3T**



$C_{20}H_{21}Cl_2N_3S_2$ = 438.44

A 50 ml, 2-neck, round-bottomed flask was dried for 10 min with a stream of hot air (*ca.* 500°C) from heat-gun and cooled down to rt in a desiccator. Then the flask was charged with diamide **3A** (102 mg, 0.251 mmol), Lawesson's reagent (221 mg, 0.546 mmol) and a stir bar. Then the flask was equipped with a reflux condenser connected to a Schlenk line and its side neck was closed with a stopper. Air was removed by three pump/thaw cycles and then dry THF (20 ml) was added in a stream of argon through the side neck. The reaction mixture was intensively stirred and refluxed for 72 h. After this time the mixture was cooled down and 2.4 g of silica gel was added. Solvents were evaporated on a rotary evaporator and the solid residue was dried under high vacuum. Then the solid residue was suspended in hexane (40 ml) and put on top of a chromatographic column made of 100 g of silica suspended in $CHCl_3$. The column was eluted with $CHCl_3$ (*ca.* 500 ml), $CHCl_3$: CH_3CO_2Et = 22 : 1 (*ca.* 250 ml), $CHCl_3$: CH_3CO_2Et = 9 : 1 (*ca.* 250 ml) and $CHCl_3$: CH_3CO_2Et = 4 : 1 until the desired product was completely washed out. Fractions containing pure product were combined and evaporated on a rotary evaporator yielding 85 mg (80.2%) of yellow solid.

M.p.: decomposition at 282°C.

^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.56 (s, 2H, thioamide NH), 10.64 (s, 1H, carbazole NH), 8.31 (d, *J* = 1.9 Hz, 2H, carbazole CH), 7.71 (d, *J* = 2.0 Hz, 2H, carbazole CH), 2.85 (t, 4H, *J* = 6.0 Hz, CH_2C=S), 1.93 – 1.81 (m, 4H, CH_2), 1.02 (t, *J* = 7.4 Hz, 6H, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 206.45, 133.45, 124.90, 124.54, 123.78, 123.06, 119.12, 47.69, 22.61, 13.27.

Elemental analysis: calcd. for $C_{20}H_{21}Cl_2N_3S_2$: C, 54.79; H, 4.83; N, 9.58, found: C, 54.88; H, 5.06; N, 9.47.

HR MS (TOF MS ES$^-$) m/z calcd. for $C_{20}H_{20}Cl_2N_3S_2^-$: 436.0476 found: 436.0472.

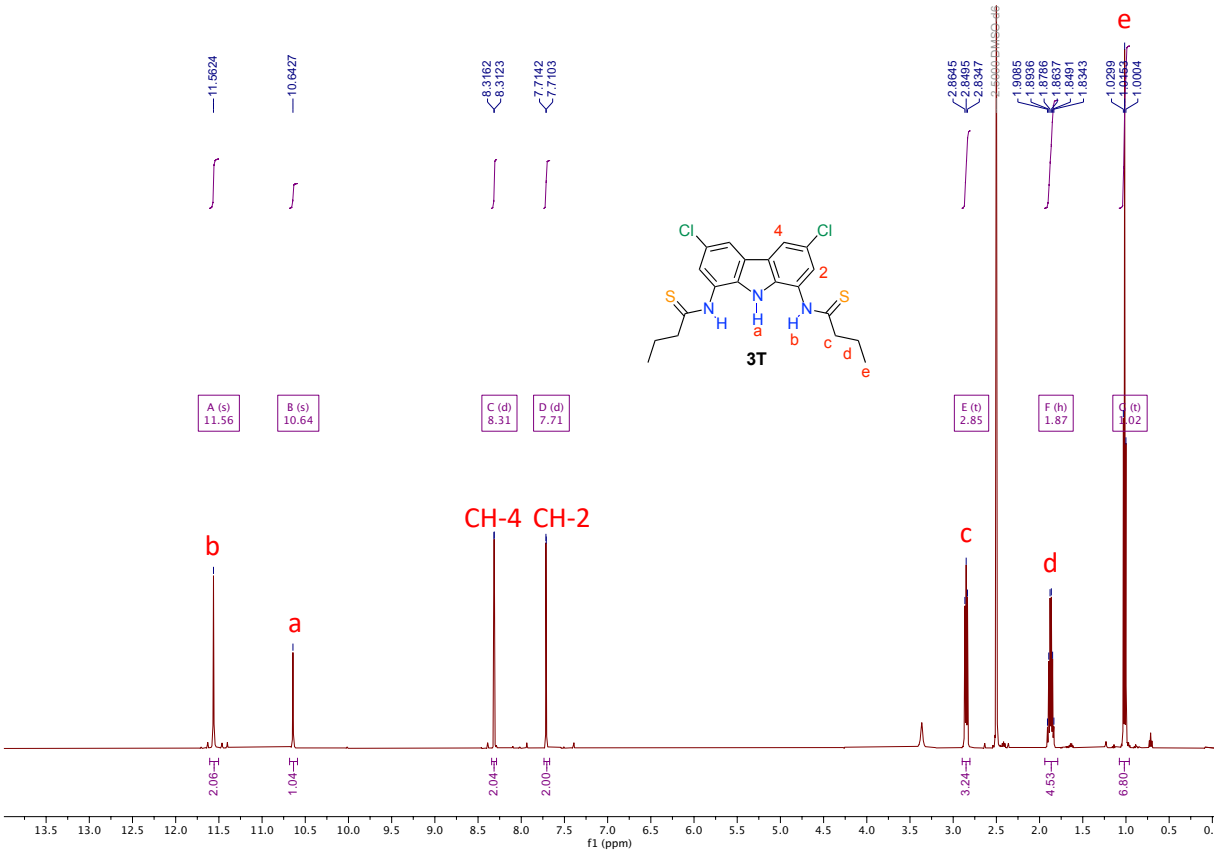


Figure S5. ^{1}H NMR spectrum of **3T** in DMSO-d_6.

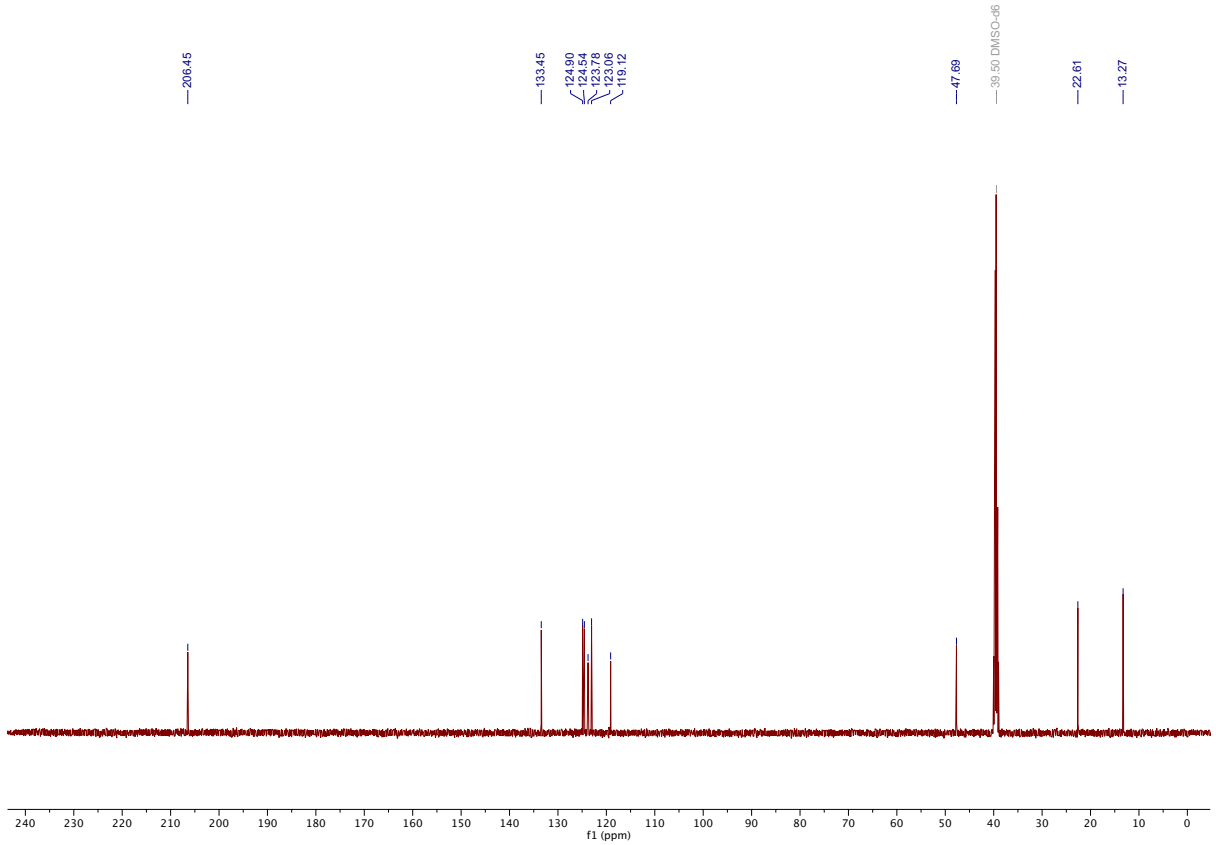
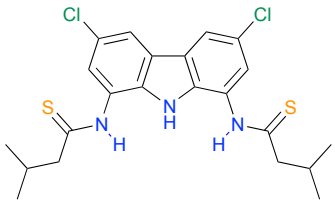


Figure S6. ^{13}C NMR spectrum of **3T** in DMSO-d_6.

Preparation of **4T**



$C_{22}H_{25}Cl_2N_3S_2$ = 466.49

A 50 ml, 2-neck, round-bottomed flask was dried for 10 min with a stream of hot air (*ca.* 500°C) from heat-gun and cooled down to rt in a desiccator. Then the flask was charged with diamide **4A** (108 mg, 0.25 mmol), Lawesson's reagent (222 mg, 0.549 mmol) and a stir bar. Then the flask was equipped with a reflux condenser connected to a Schlenk line and its side neck was closed with a stopper. Air was removed by three pump/thaw cycles and then dry dichloroethane (20 ml) was added in a stream of argon through the side neck. The reaction mixture was intensively stirred and refluxed for 72 h. After this time the mixture was cooled down and 2 g of silica gel was added. Solvents were evaporated on a rotary evaporator and the solid residue was dried under high vacuum. Then the pre-loaded mixture was separated by chromatography on 40 g cartridge using CombiFlash instrument. The column was eluted with the flow rate of 8 mL/min with $CHCl_3$ (15 min), $CHCl_3$: CH_3CO_2Et gradient from 0% to 5% over 15 min, $CHCl_3$: CH_3CO_2Et = 19 : 1 (120 min). Fractions containing pure product were combined and evaporated on a rotary evaporator yielding 75 mg (64.7%) of yellow solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.57 (s, 2H, thioamide NH), 10.46 (s, 1H, carbazole NH), 8.33 (d, J = 1.5 Hz, 2H, carbazole CH-4), 7.66 (d, J = 1.5 Hz, 2H, , carbazole CH-2), 2.75 (d, J = 7.2 Hz, 4H, CH_2), 2.40 – 2.25 (m, 2H, CH), 1.03 (d, J = 6.6 Hz, 12H, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO} 205.56, 133.46, 124.89, 124.66, 123.79, 123.15, 119.22, 54.88, 39.50, 29.07, 21.96.

Elemental analysis: calcd. for $C_{22}H_{25}Cl_2N_3S_2$: C, 56.64; H, 5.40; N, 9.01, found: C, 56.75; H, 5.54; N, 8.94.

HR MS (TOF MS ES$^-$) m/z calcd. for $C_{22}H_{24}Cl_2N_3S_2^-$: 464.0789 found: 464.0786.

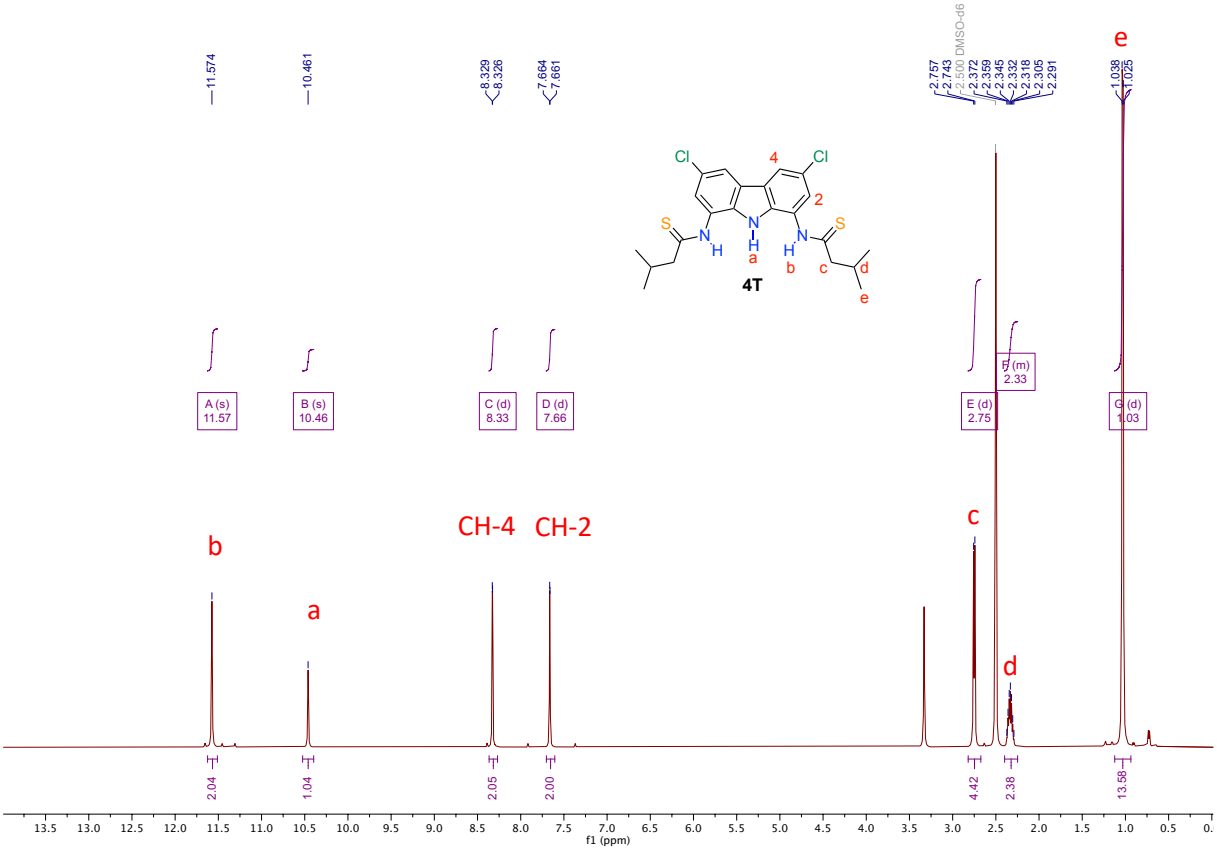


Figure S7. ^{1}H NMR spectrum of **4T** in DMSO-d_6.

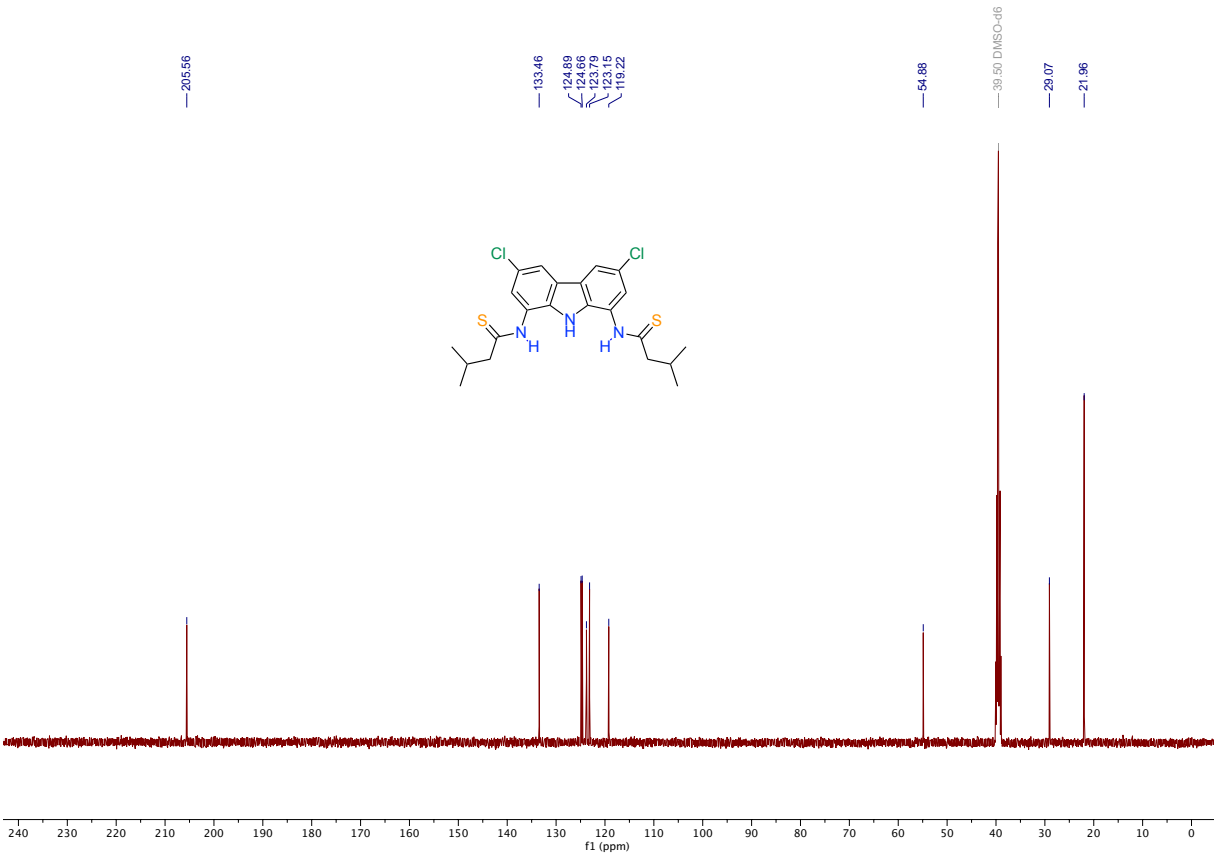


Figure S8. ^{13}C NMR spectrum of **4T** in DMSO-d_6.

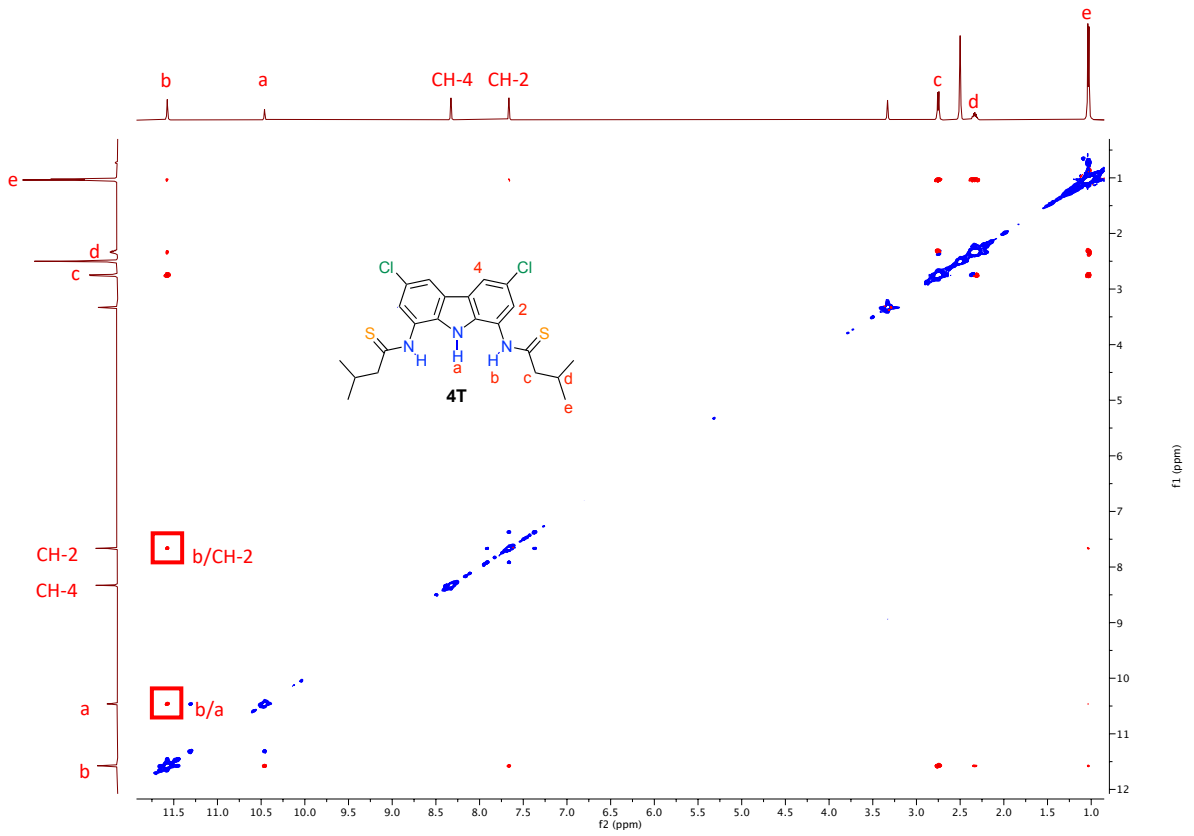
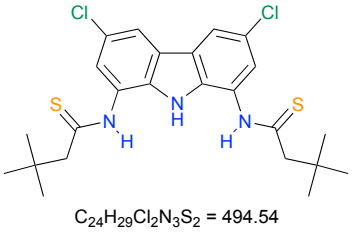


Figure S9. ^{1}H ROESY spectrum of **4T** in DMSO-d_6.

Preparation of **5T**



$C_{24}H_{29}Cl_2N_3S_2$ = 494.54

A 50 ml, 2-neck, round-bottomed flask was dried for 10 min with a stream of hot air (*ca.* 500°C) from heat-gun and cooled down to rt in a desiccator. Then the flask was charged with diamide **5A** (116 mg, 0.251 mmol), Lawesson's reagent (222 mg, 0.549 mmol) and a stir bar. Then the flask was equipped with a reflux condenser connected to a Schlenk line and its side neck was closed with a stopper. Air was removed by three pump/thaw cycles and then dry dichloroethane (20 ml) was added in a stream of argon through the side neck. The reaction mixture was intensively stirred and refluxed for 72 h. After this time the mixture was cooled down and 2.0 g of silica gel was added. Solvents were evaporated on a rotary evaporator and the solid residue was dried under high vacuum. Then the solid residue was suspended in hexane (40 ml) and put on top of a chromatographic column made of 110 g of silica gel suspended in $CHCl_3$. The column was eluted with $CHCl_3$ (*ca.* 500 ml), $CHCl_3$: EA = 25 : 1 (*ca.* 250 ml), 25 : 1 (*ca.* 250 ml), 22 : 1 (*ca.* 250 ml), 19 : 1 (*ca.* 250 ml) until the desired product was completely washed out. Fractions containing pure product were combined and evaporated on a rotary evaporator yielding 118 mg (95.1 %) of yellow solid.

M.p.: decomposition at 278°C.

^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.43 (s; 2H; thioamide NH); 10.30 (s; 1H; carbazole NH); 8.33 (d; *J* = 2.0 Hz; 2H; carbazole CH-4/5); 7.59 (d; *J* = 2.0 Hz; 2H; carbazole CH-2/7); 2.85 (s; 4H; methylene CH_2); 1.14 (s; 18H; *t*-butyl CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 203.50, 133.43, 125.04, 124.75, 123.89, 123.17, 119.18, 59.12, 32.02, 29.77.

Elemental analysis: calcd. for $C_{24}H_{29}Cl_2N_3S_2$: C, 58.29; H, 5.91; N, 8.50, found: C, 58.37; H, 6.06; N, 8.25.

HR MS (TOF MS ES$^-$) m/z calcd. for $C_{24}H_{28}Cl_2N_3S_2^-$: 492.1102; found: 492.1113.

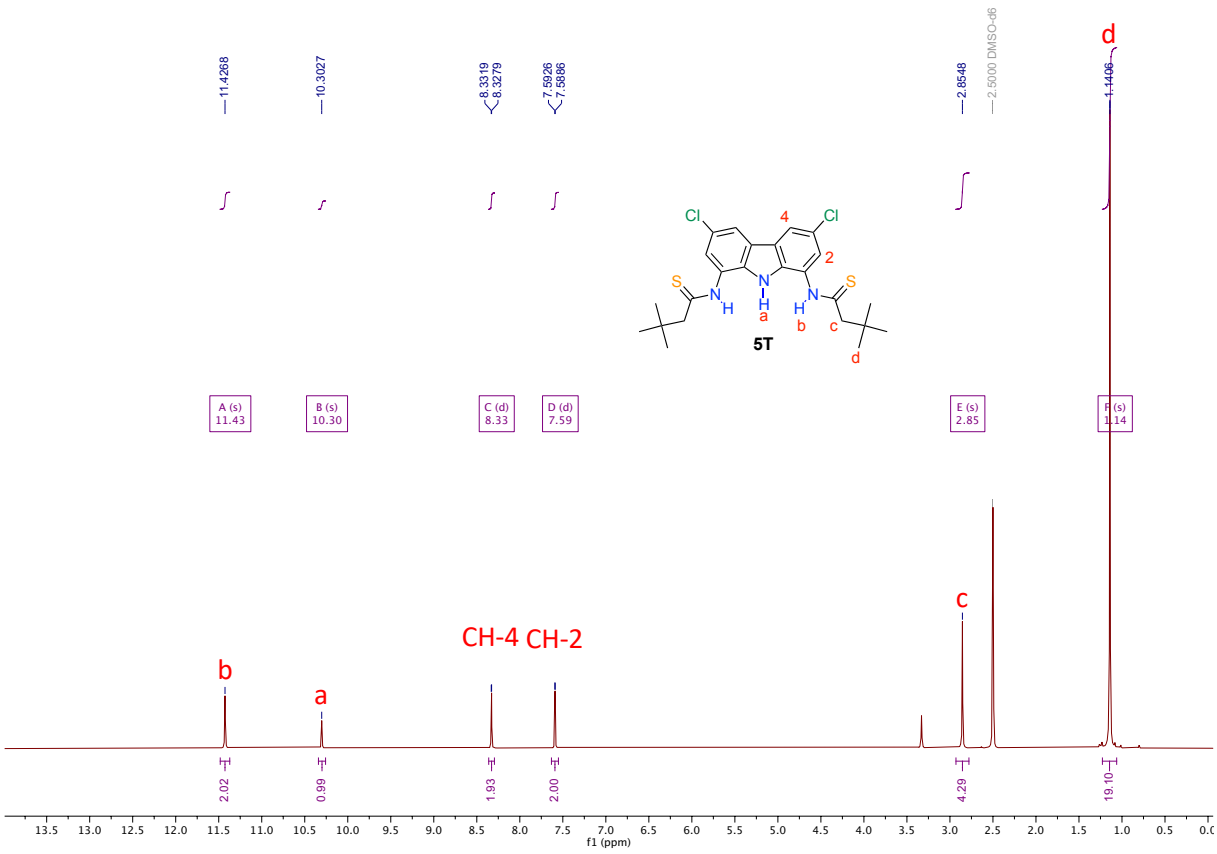


Figure S10. ^{1}H NMR spectrum of **5T** in DMSO-d_6.

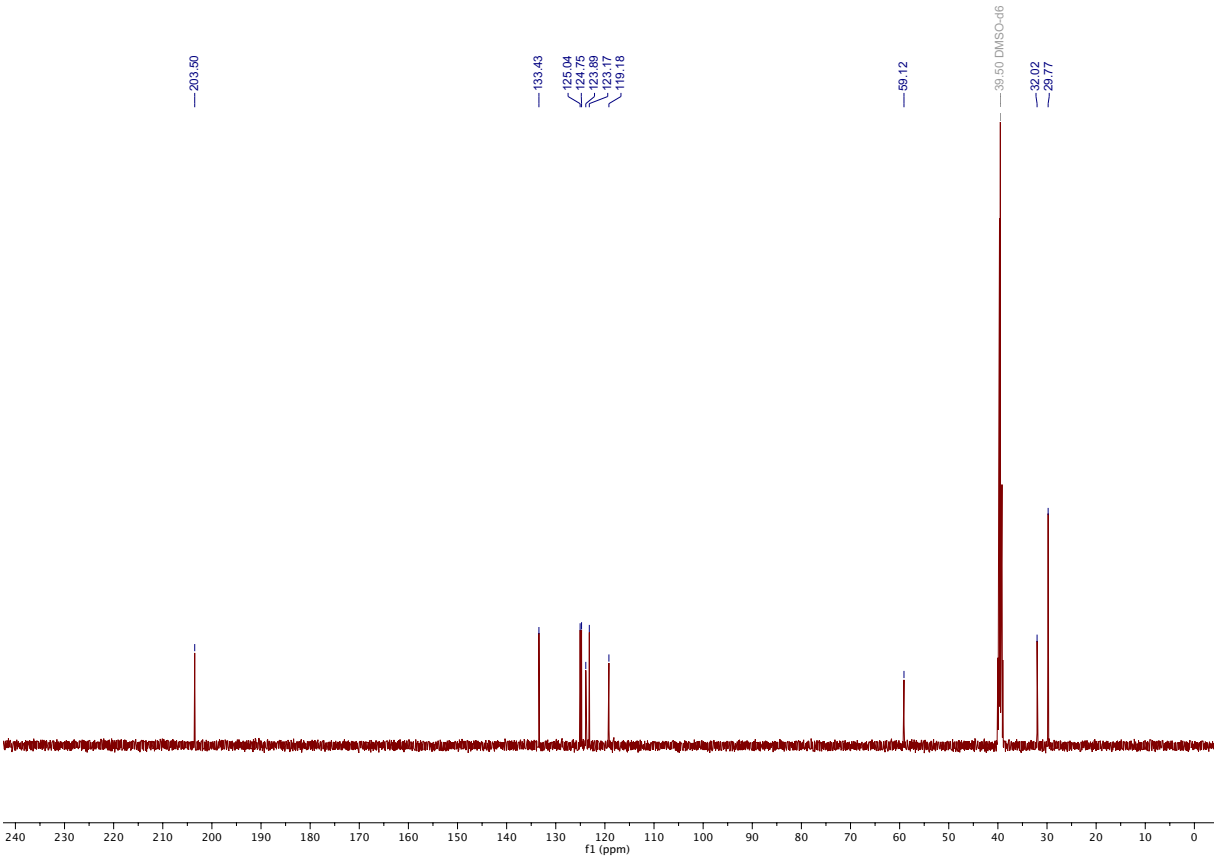
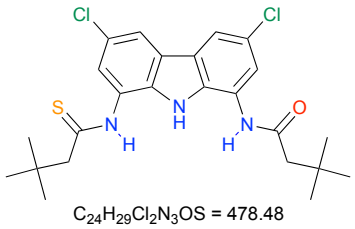


Figure S11. ^{13}C NMR spectrum of **5T** in DMSO-d_6.

Preparation of **5AT**



$C_{24}H_{29}Cl_2N_3OS$ = 478.48

A 50 ml, 2-neck, round-bottomed flask was dried for 10 min with a stream of hot air (*ca.* 500°C) from heat-gun and cooled down to rt in a desiccator. Then the flask was charged with diamide **5A** (116 mg, 0.25 mmol), Lawesson's reagent (101 mg, 0.25 mmol) and a stir bar. Then the flask was equipped with a reflux condenser connected to a Schlenk line and its side neck was closed with a stopper. Air was removed by three pump/thaw cycles and then dry dichloroethane (20 ml) was added in a stream of argon through the side neck. The reaction mixture was intensively stirred and refluxed for 72 h. After this time the mixture was cooled down and 2 g of silica gel was added. Solvents were evaporated on a rotary evaporator and the solid residue was dried under high vacuum. Then the pre-loaded mixture was separated by repeated chromatography on CombiFlash instrument. Preliminary separation was achieved on 40 g cartridge using flow rate of 8 mL / min and the following eluents $CHCl_3$: CH_3CO_2Et = 19 : 1 (120 min), $CHCl_3$: CH_3CO_2Et gradient from 5 to 10% over 15 min and finally $CHCl_3$: CH_3CO_2Et = 9 : 1 (120 min). All fractions containing the desired monothioamide were combined and purified once again on two combined 12 g Gold cartridges using $CHCl_3$: CH_3CO_2Et gradient from 0 to 5% over 240 min and $CHCl_3$: CH_3CO_2Et 5% (60 min). Fractions containing pure product were combined and evaporated on a rotary evaporator yielding 20 mg (16.7 %) of yellow solid.

^{1}H NMR (DMSO-d_6) δ_{DMSO}: 11.55 (s, 1H, thioamides NH), 10.56 (s, 1H, carbazole NH), 10.00 (s, 1H, amide NH), 8.30 (d, *J* = 1.8 Hz, 1H, carbazole CH-5), 8.12 (d, *J* = 1.8 Hz, 1H, carbazole CH-4), 7.90 (d, *J* = 1.8 Hz, 1H, carbazole CH-2), 7.41 (d, *J* = 1.6 Hz, 1H, carbazole CH-7), 2.88 (s, 2H, CH_2C=S), 2.35 (s, 2H, CH_2C=O), 1.16 (s, 9H, *t*-Bu on the thioamide side), 1.08 (s, 9H, *t*-Bu on the amide side);

^{13}C NMR (DMSO-d_6) δ_{DMSO}: 203.85, 170.57, 133.40, 129.89, 125.06, 124.64, 124.59, 124.24, 124.00, 123.73, 122.96, 119.26, 117.74, 115.92, 58.90, 49.20, 32.07, 30.89, 29.78, 29.59.

HR MS (TOF MS ES$^-$): m/z calcd. for $C_{24}H_{28}Cl_2N_3OS^-$: 476.1330, found: 476.1331;

Elemental Analysis calcd. for $C_{24}H_{29}Cl_2N_3OS$: C, 60.24; H, 6.11; N, 8.78; found: C, 60.61; H, 6.39; N, 8.39.

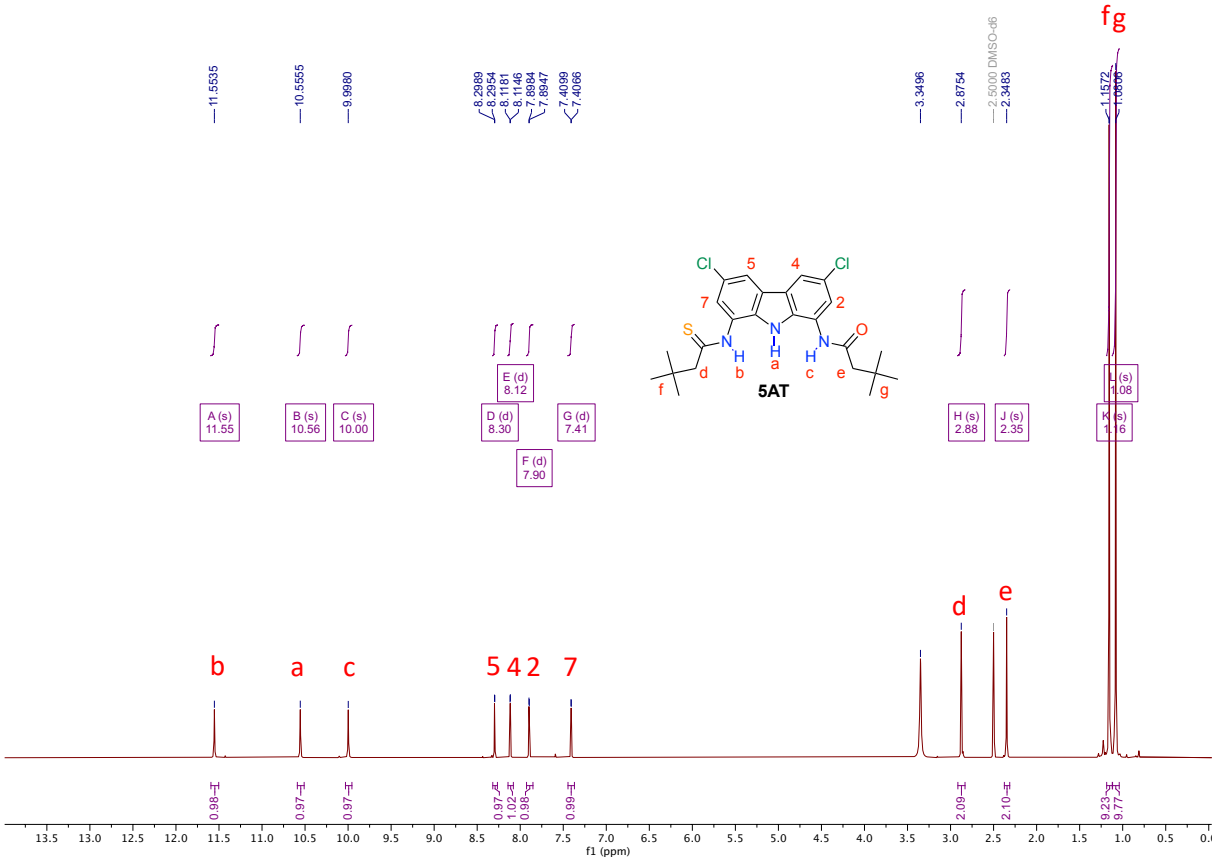


Figure S12. ^{1}H NMR spectrum of **5AT** in DMSO-d_6.

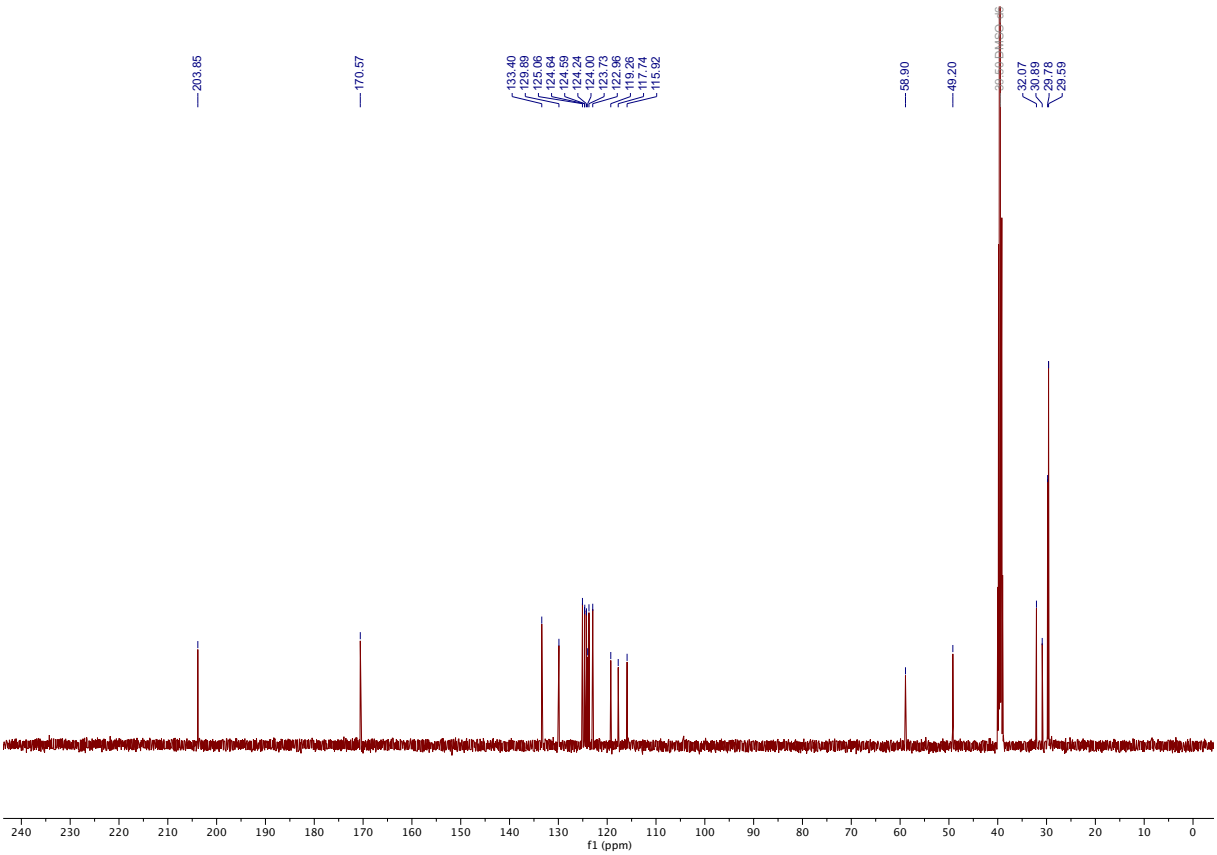


Figure S13. ^{13}C NMR spectrum of **5AT** in DMSO-d_6.

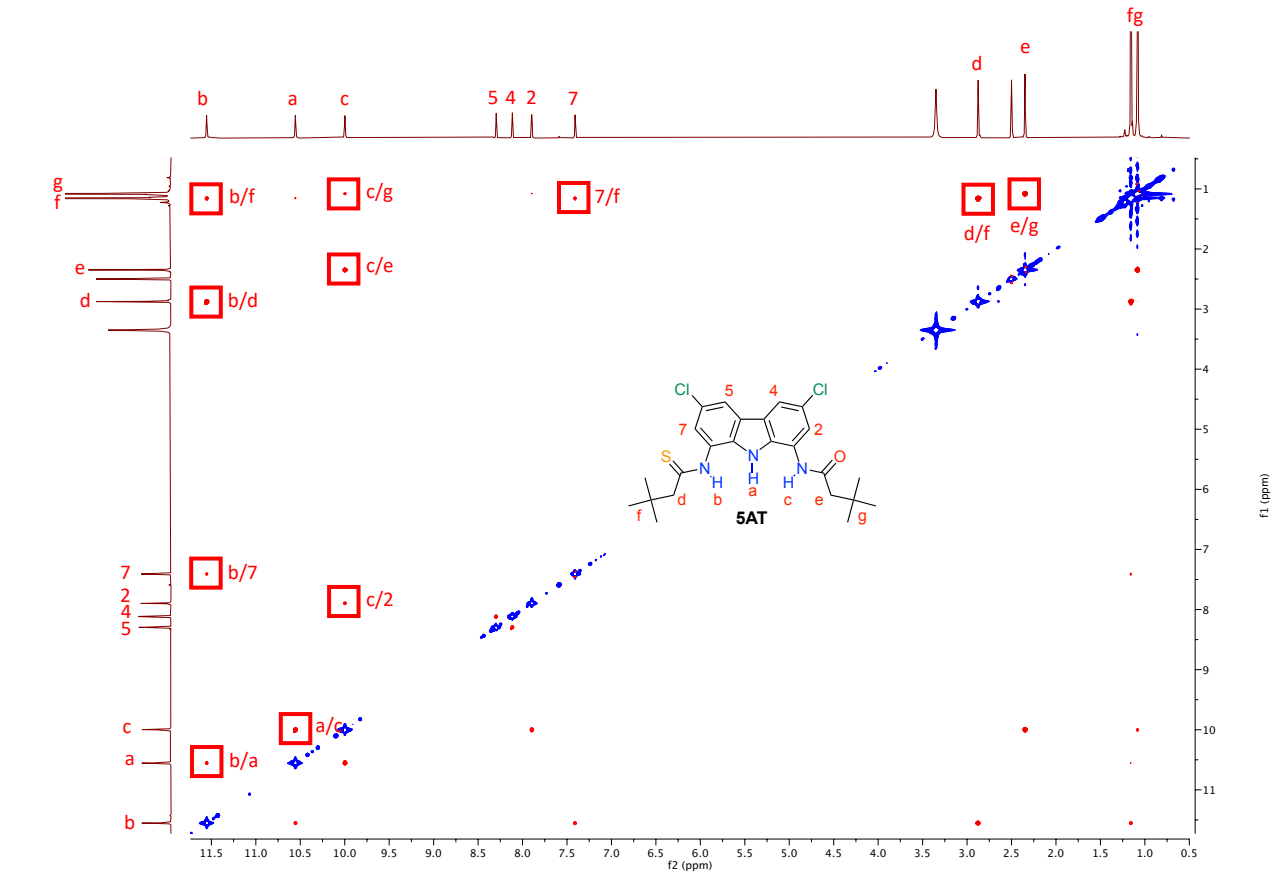


Figure S14. ^{1}H ROESY spectrum of **5AT** in DMSO-d_6.

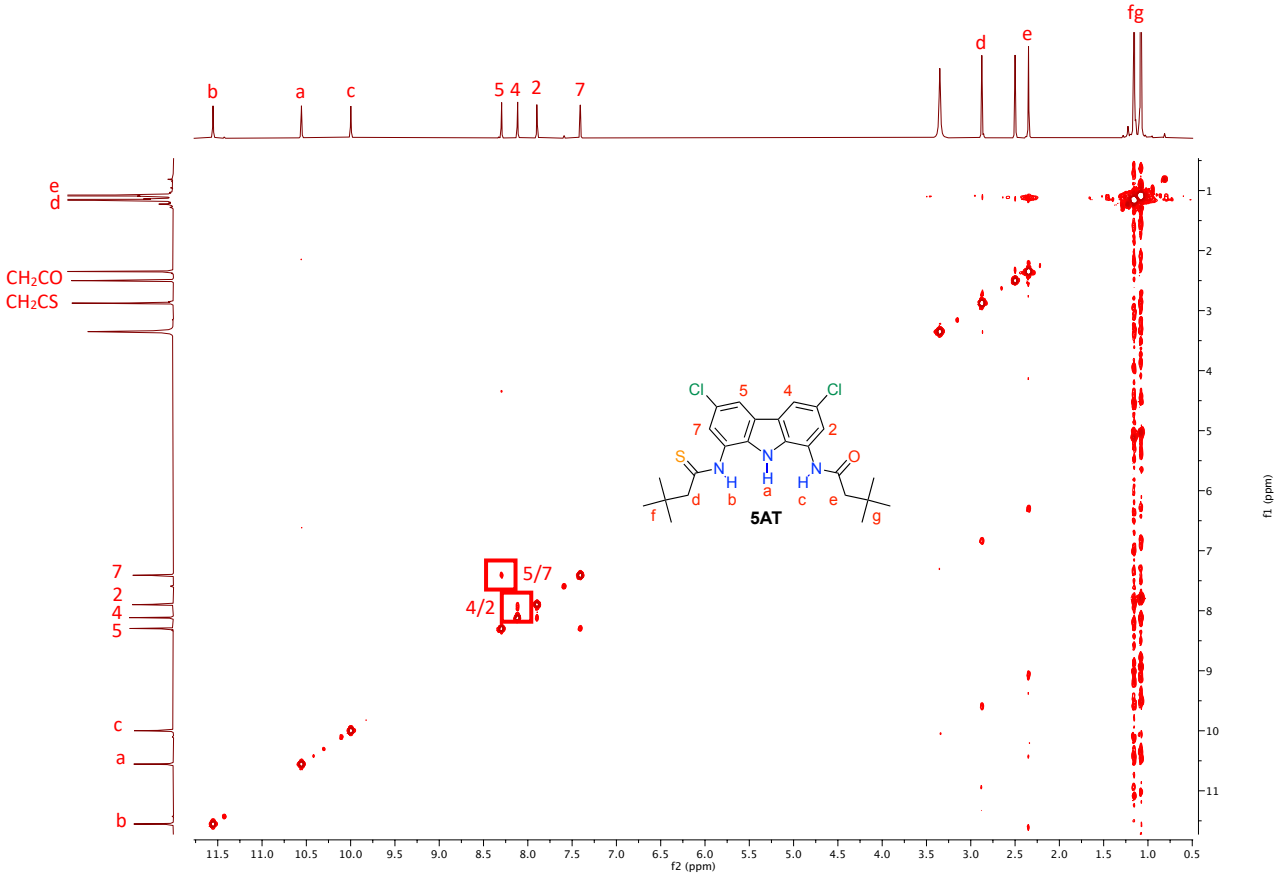
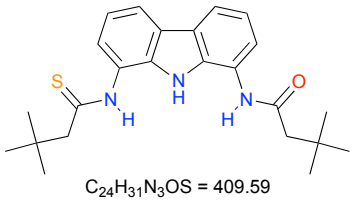


Figure S15. ^{1}H COSY spectrum of **5AT** in DMSO-d_6.

Preparation of **6AT**



$C_{24}H_{31}N_3OS$ = 409.59

Monothioamide **6AT** has been obtained as a side product from the synthesis of **6T**. After purification by column chromatography 83 mg (40.3%) of **6AT** was obtained.

M.p.: decomposition above 282°C.

^{1}H NMR (500 MHz, DMSO-d_6) δ_{DMSO}: 11.47 (s; 1H; thioamide NH); 10.06 (s; 1H; carbazole NH); 9.95 (s; 1H; amide NH); 8.08 (d, J = 7.5 Hz, 1H; CH-5 on the thioamide side); 7.94 (d, J = 7.7 Hz, 1H; CH-4 on the amide side); 7.61 (dd; J_1 = 7.8, J_2 = 1.0 Hz; 1H; CH-2 on the amide side); 7.34 (d, J = 7.7, 1H; CH-7 on the thioamide side); 7.22 (t; J = 7.7 Hz; 1H; CH-6 on the thioamide side); 7.17 (t; J = 7.8 Hz; 1H; CH-3 on the amide side); 2.87 (s; 2H; methylene CH_2 from the thioamide arm); 2.34 (s; 2H; methylene CH_2 from the amide arm); 1.17 (s; 9H; *t*-butyl CH_3 from the thioamide arm); 1.10 (s; 9H; *t*-butyl from the amide arm).

^{13}C NMR (126 MHz, DMSO-d_6) δ_{DMSO}: 202.76, 170.19, 134.29, 131.48, 124.60, 124.47, 124.10, 123.61, 123.40, 119.43, 119.07, 119.05, 118.28, 116.28, 58.89, 49.13, 31.91, 30.84, 29.85, 29.68.

Elemental analysis: calcd. for $C_{24}H_{31}N_3OS$: C, 70.38; H, 7.63; N, 10.26, found: C, 70.07; H, 7.61; N, 10.05.

HR MS (TOF MS ES$^-$) m/z calcd. for $C_{24}H_{30}N_3OS^-$: 408.2110 found: 408.2112.

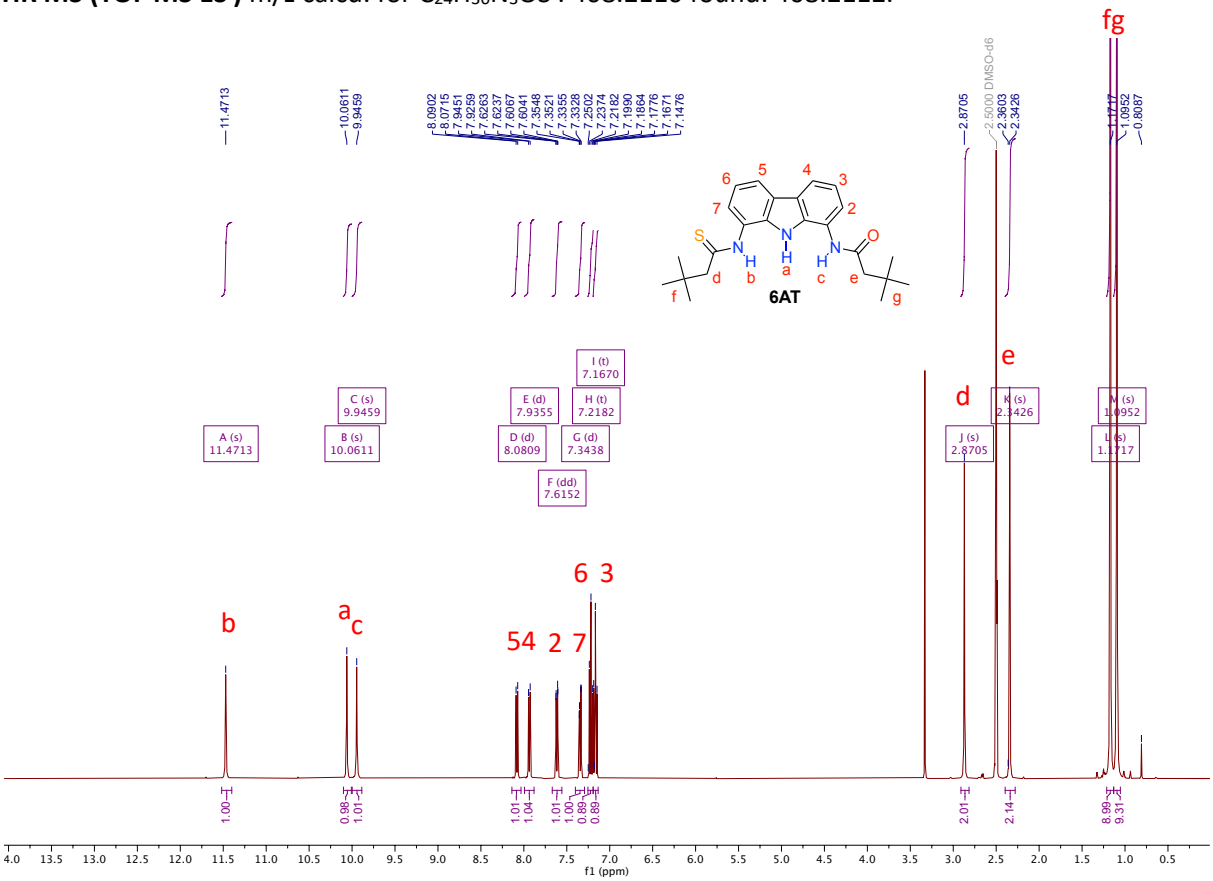


Figure S16. ^{1}H NMR spectrum of **6AT** in DMSO-d_6.

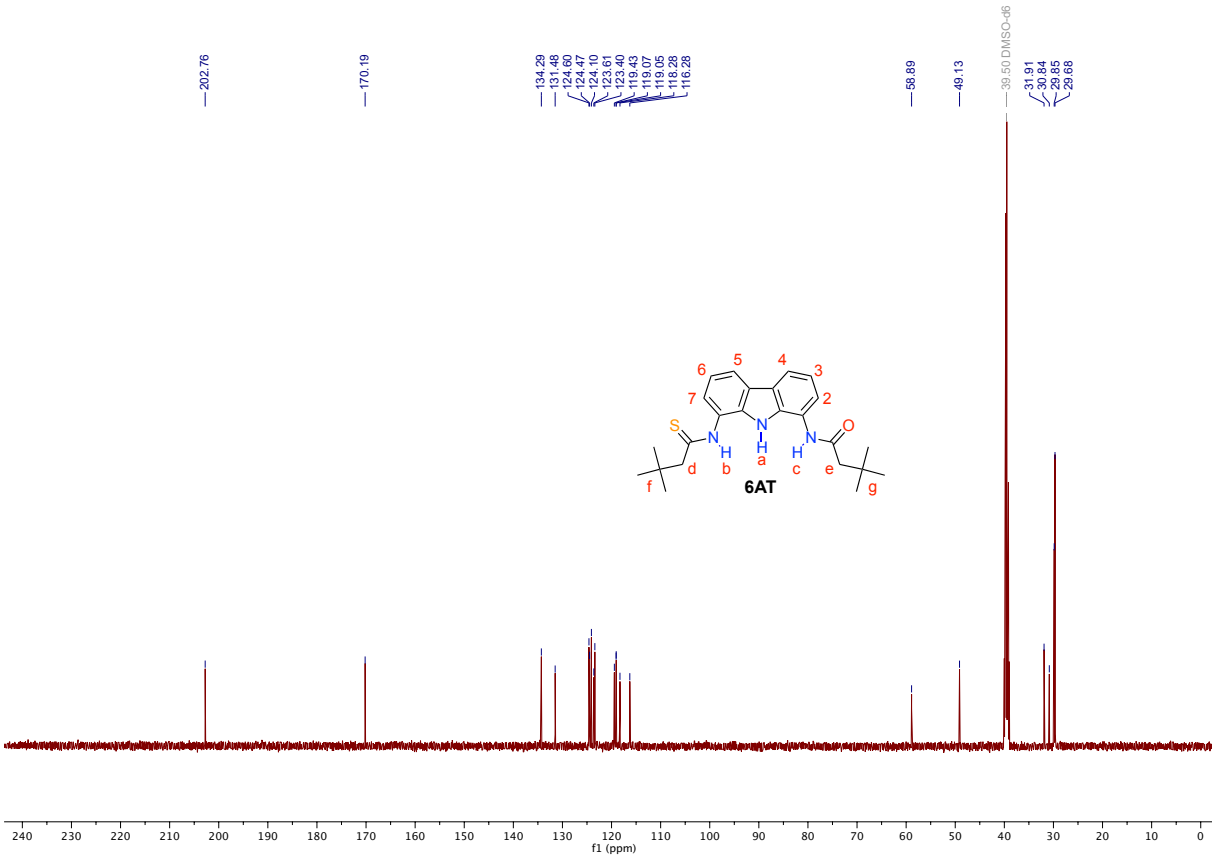


Figure S17. ^{13}C NMR spectrum of **6AT** in DMSO-d_6.

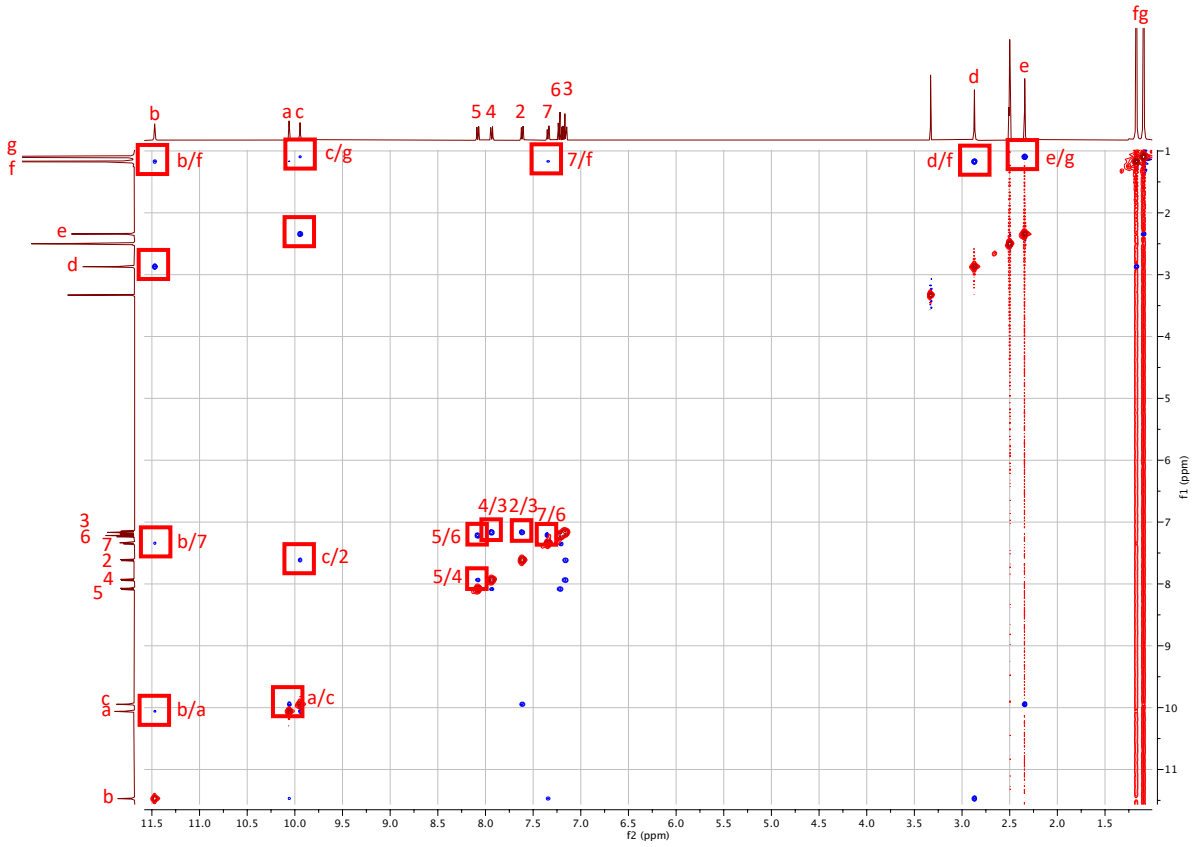


Figure S18. ^{1}H ROESY spectrum of **6AT** in DMSO-d_6.

3 Binding studies

3.1 Materials

Tetrabutylammonium chloride was obtained from Sigma-Aldrich and was used as received.

DMSO-d_6 (99.8% isotopic purity, containing less than 0.02% water) was obtained from Eurisotop in septum-sealed vials.

DMSO-d_6/H_2O mixtures were obtained using distilled H_2O and their concentrations are expressed as weight-weight percentage.

3.2 General procedure for ^{1}H NMR titrations

All the reagents were weighted separately on a Mettler Toledo Excellence XA105DU analytical balance (readability 0.01 mg) in screw-capped vials sealed with Teflon-covered septa. All the solvent/solution manipulations were done using gas-tight Hamilton glass syringes. Titrants were prepared by dissolving appropriate salts in the solution of the receptor in order to avoid dilution of the receptor during titration. Titrations were performed in screw-capped NMR tubes sealed with Teflon-covered septa, by adding aliquots of the titrant solution to the receptor solution and recording ^{1}H NMR spectra after each addition. The NMR spectra were measured on Agilent 400 MHz spectrometer.

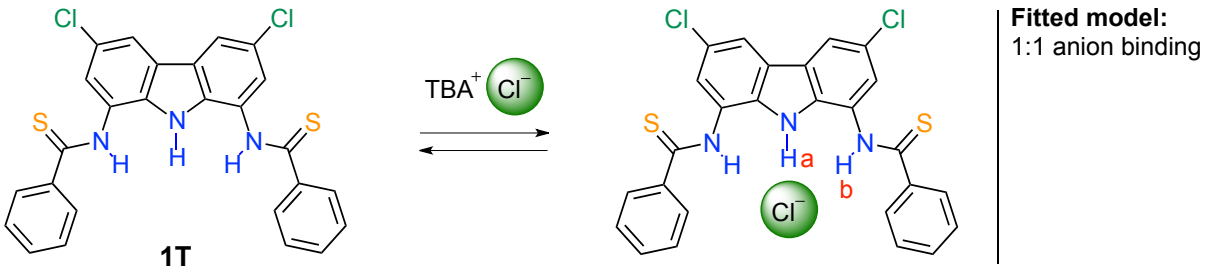
Typical ^{1}H NMR titration procedure. To a solution of host (600 µl, typically 0.005 M or 0.01 M) in a septum-sealed screw-cap NMR tube appropriate aliquots of titrant (typically 15 times more concentrated than the host solution, dissolved in the solution of host to avoid dilution) were added with a 25 µl gas-tight microsyringe. Association constants were calculated from changes in chemical shifts of most affected protons of the ligands, as indicated in each case below.

3.3 Data fitting

The ^{1}H NMR titration data were fitted with BindFit software.[6,7] Association constants $ß_{1:1}$ and chemical shifts of 1:1 complexes were set as free parameters for fitting, whereas chemical shifts of free ligands were constrained to be equal to experimentally measured values. Association constants derived from independent experiments were averaged using arithmetic mean.

3.4 ^{1}H NMR titration of 0.005 M solution of receptor **1T** in DMSO-d_6/0.5% H_2O with 0.15 M solution of TBA^+Cl^- (dissolved in the solution of receptor 1T).

Titration of **1T** with TBACl produced very weak changes in the ^{1}H NMR chemical shifts of all protons. The changes for protons **a** nad **b** were the most pronounced and were therefore simultaneously fitted to the 1: 1 model using BindFit software.



Scheme 1. Chloride binding to **1T**. Chemical shifts of the indicated protons were used for fitting.

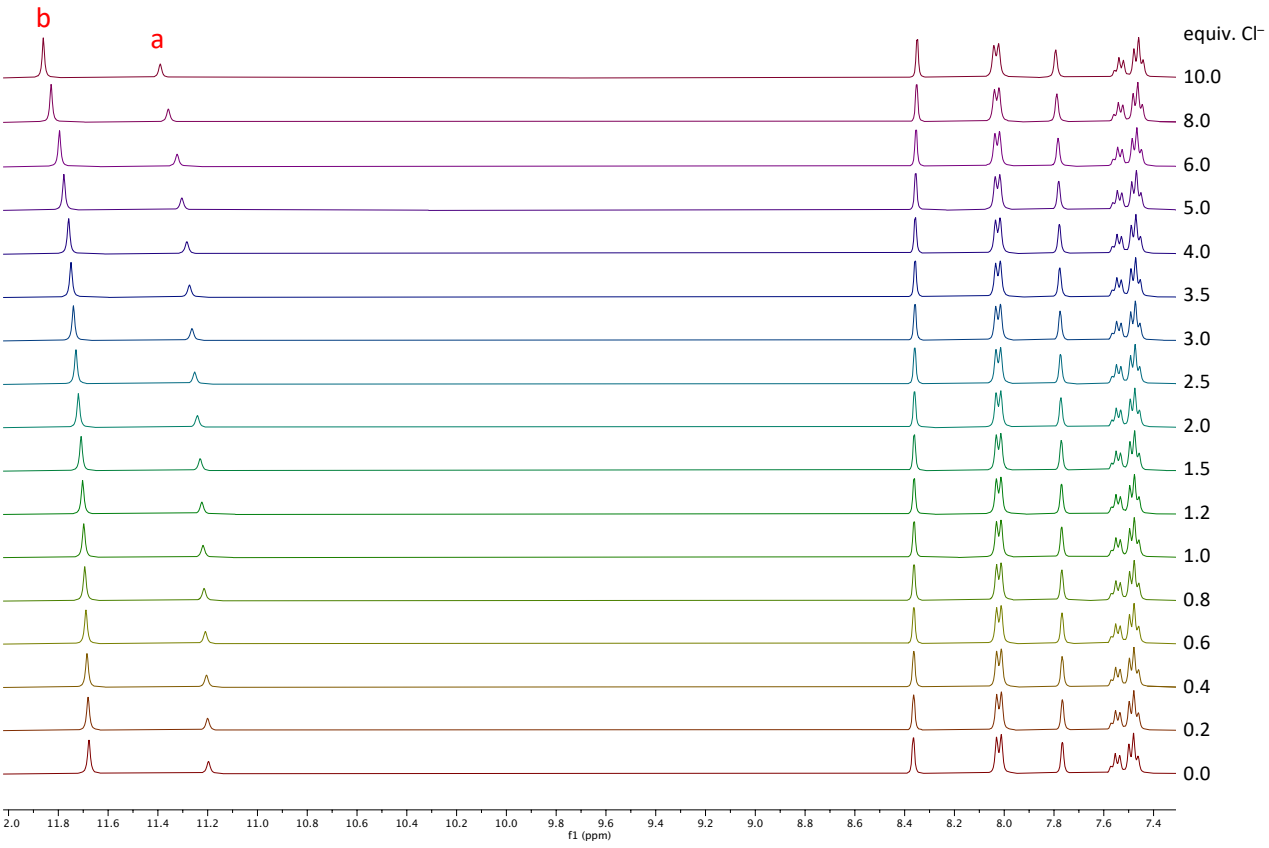


Figure S19. ^{1}H NMR titration of **1T** (0.005 M) with TBACl. The marked peaks were used for fitting.

Raw data:

Added volume of titrant solution [µL]	Equivalents of TBA^+Cl^-	Chemical Shift [ppm]	
		$NH_{(thioamide)}$	$NH_{(carbazole)}$
0.0	0.00	11.6801	11.2081
4.0	0.20	11.6847	11.2131
8.0	0.39	11.6892	11.2175
12.0	0.59	11.6934	11.2225
16.5	0.80	11.6983	11.2278
20.5	0.99	11.7024	11.2320
25.0	1.20	11.7071	11.2370
31.5	1.50	11.7136	11.2438
43.0	2.01	11.7244	11.2553
54.5	2.50	11.7348	11.2663
66.5	2.99	11.7448	11.2769
79.0	3.49	11.7548	11.2874
92.0	3.99	11.7646	11.2978
120.0	5.00	11.7837	11.3177
150.0	6.00	11.8019	11.3365
218.0	8.00	11.8358	11.3715
300.0	10.0	11.8673	11.4030

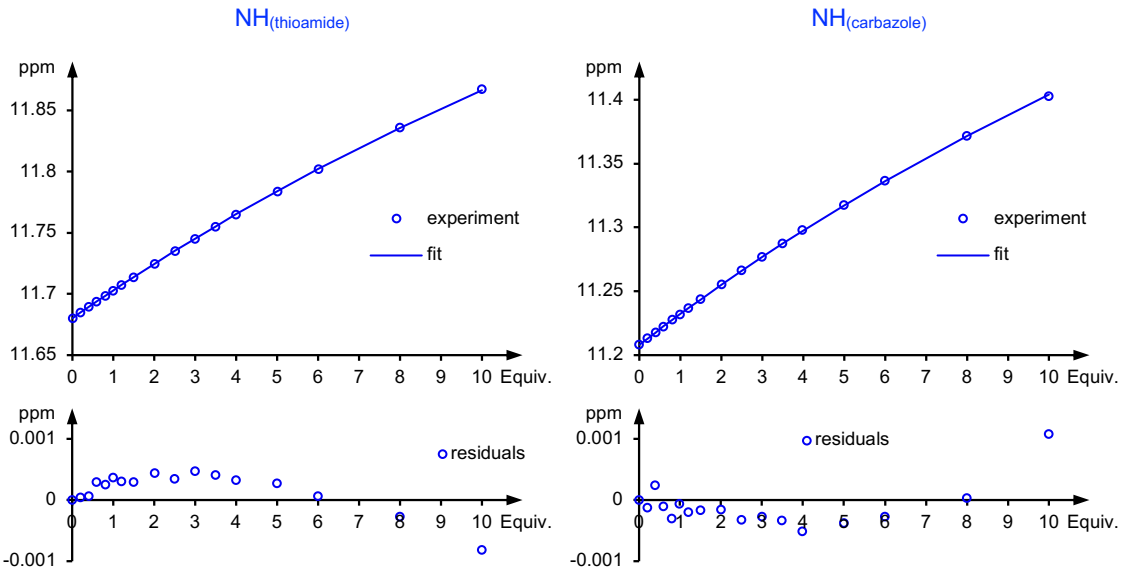


Figure S20. Data points and fitting curves for ^1H NMR titration of **1T** (0.005 M in DMSO-d_6/0.5% H_2O) with TBACl.

a) Binding constant K derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

$K = 5.42\ M^{-1} \pm 0.19\ M^{-1}$

b) Chemical shifts derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

	$NH_{(thioamide)}$	$NH_{(carbazole)}$
Receptor **1T** [ppm]	11.6801	11.2081
1T × TBA^+Cl^- [ppm]	12.5695	12.1433

c) Binding constant K derived from independent experiment repeated according to the same methodology:

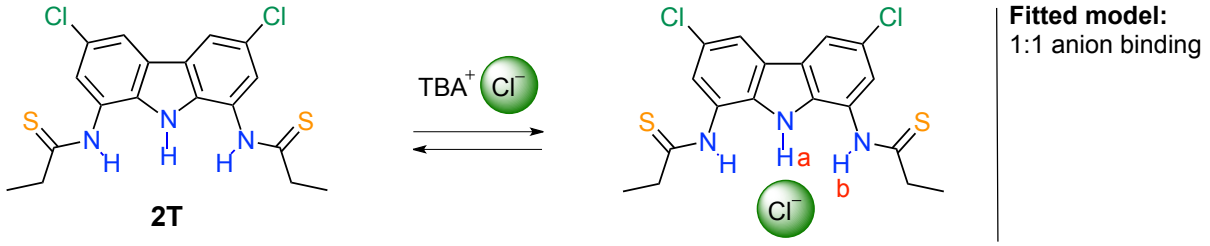
$$\mathbf{K = 4.64\ M^{-1} \pm 0.17\ M^{-1}}$$

d) Binding constant averaged from the two experiments:

$$\mathbf{K = 5.03\ M^{-1}, reported\ as < 10\ M^{-1}}$$

3.5 ^{1}H NMR titration of 0.005 M solution of receptor **2T** in DMSO-d_6/0.5% H_2O with 0.15 M solution of TBA^+Cl^- (dissolved in the solution of receptor **2T**).

Titration of **2T** with TBACl produced the most significant changes in the ^{1}H NMR chemical shifts of the carbazole NH and the thioamide NHs. The data sets for these two signals were therefore simultaneously fitted to the 1: 1 model using Bindfit software.



Scheme 2. Chloride binding to **2T**.

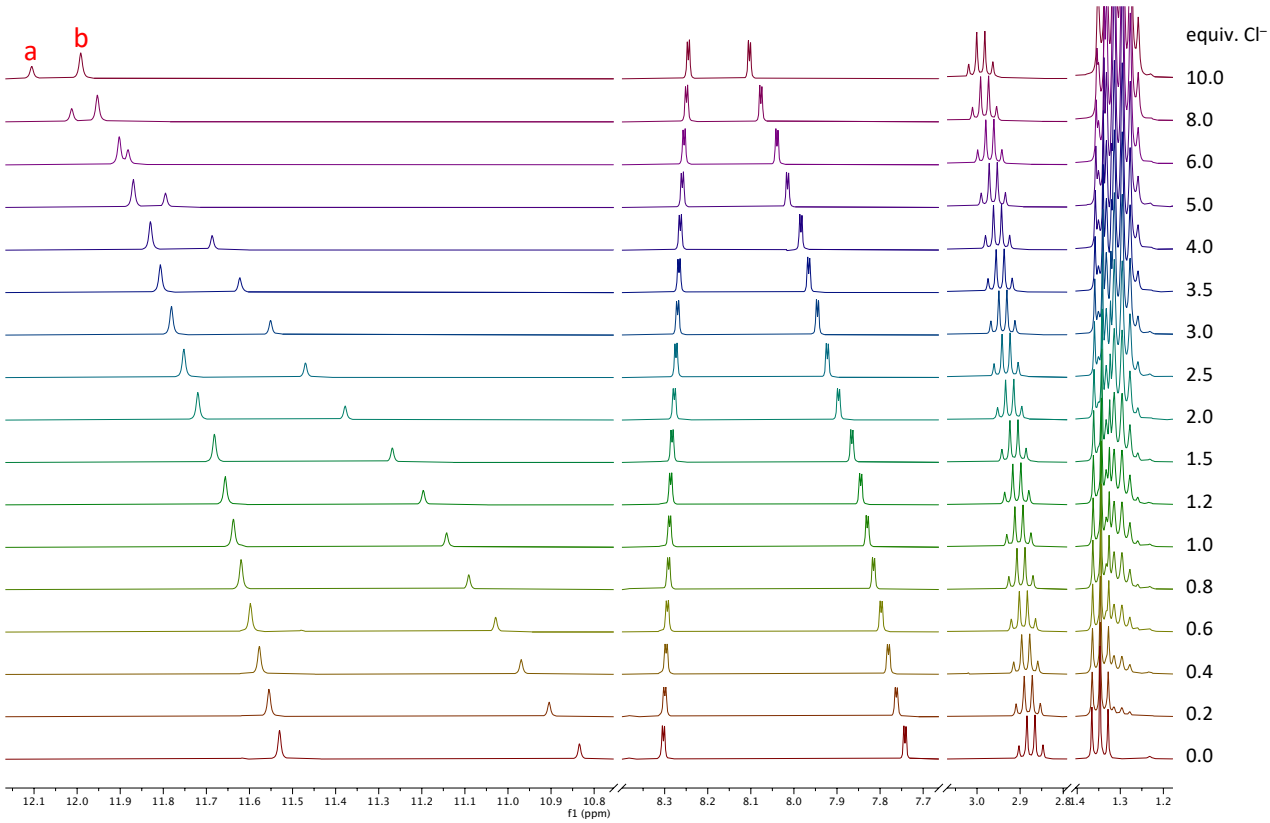


Figure S21. ^{1}H NMR titration of **2T** (0.005 M) with TBACl.

Raw data

Added volume of titrant solution [µL]	Equivalents of TBA^+Cl^-	Chemical Shift [ppm]	
		$NH_{(thioamide)}$	$NH_{(carbazole)}$
0.0	0.00	11.5302	10.8343
4.0	0.20	11.5546	10.9044
8.0	0.39	11.5772	10.9692
12.0	0.59	11.5977	11.0285
16.5	0.80	11.6194	11.091
20.5	0.99	11.6374	11.1425
25.0	1.20	11.6562	11.1966
31.5	1.50	11.6814	11.2685
43.0	2.01	11.7198	11.3779
54.5	2.50	11.7524	11.4699
66.5	2.99	11.7811	11.5511
79.0	3.49	11.8068	11.6226
92.0	3.99	11.8299	11.6855
120.0	5.00	11.8695	11.7949
150.0	6.00	11.9021	11.8818
218.0	8.00	11.9529	12.0124
300.0	10.0	11.9914	12.1053

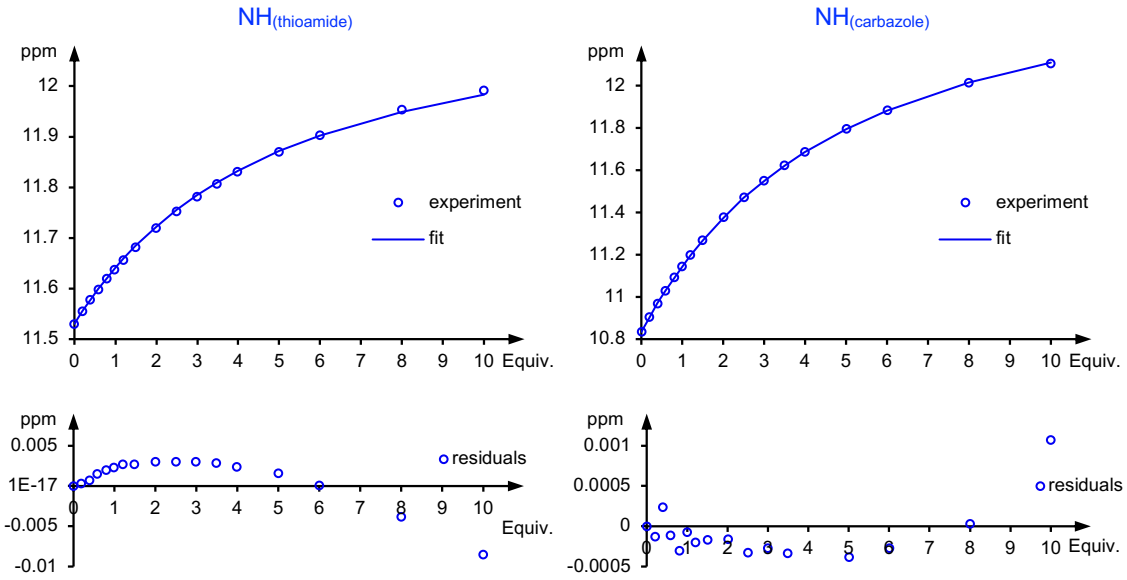


Figure S22. Data points and fitting curves for ^1H NMR titration of **2T** (0.005 M in DMSO-d_6/0.5% H_2O) with TBACl.

a) Binding constant K derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

$K = 49.2 \pm 0.4\ M^{-1}$

b) Chemical shifts derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

	$NH_{(thioamide)}$	$NH_{(carbazole)}$
Receptor **2T** [ppm]	11.5302	10.8343
2T × TBA^+Cl^- [ppm]	12.1806	12.6656

c) Binding constant K derived from independent experiment repeated according to the same methodology:

concentration of host: 0.005M

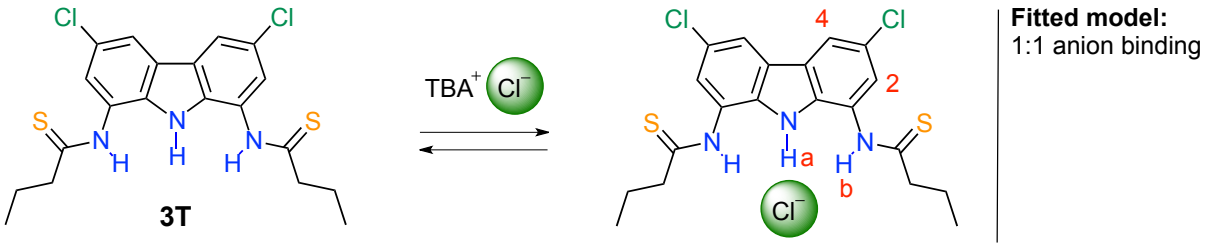
$K = 49.0 \pm 0.4\ M^{-1}$

d) Binding constant averaged from the two experiments:

$K = 49.1\ M^{-1}$

3.6 ^{1}H NMR titration of 0.005 M solution of receptor **3T** in DMSO-d_6/0.5% H_2O with 0.15 M solution of TBA^+Cl^- (dissolved in the solution of receptor **3T**).

Titration of **3T** with TBACl produced the most significant changes in the ^{1}H NMR chemical shifts of the carbazole NH and the thioamide NHs. The data for these two signals were simultaneously fitted to the 1: 1 model using Bindfit software.



Scheme 3. Chloride binding to **3T**.

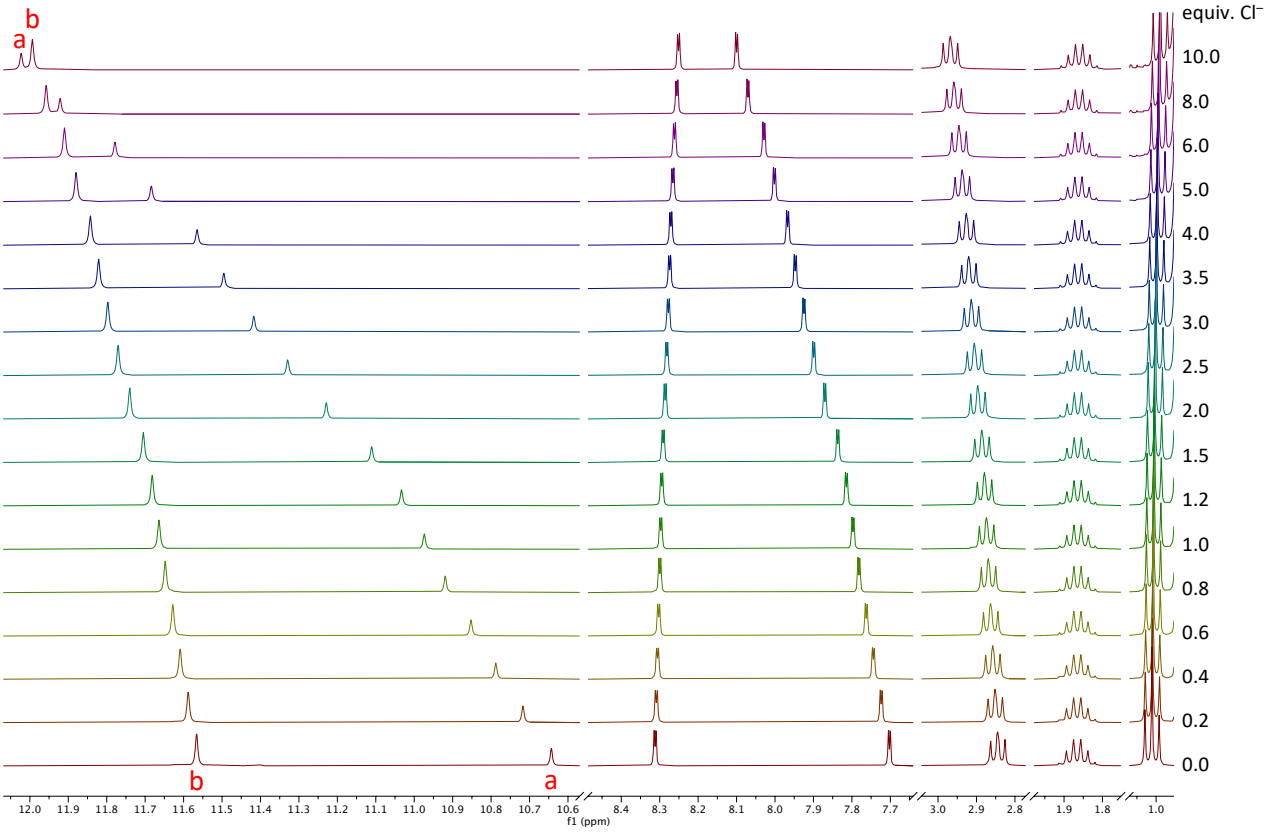


Figure S23. ^{1}H NMR titration of **3T** (0.005 M) with TBACl.

Raw data

Added volume of titrant solution [µL]	Equivalents of TBA^+Cl^-	Chemical Shift [ppm]	
		$NH_{(thioamide)}$	$NH_{(carbazole)}$
0.0	0.00	11.5663	10.6433
4.0	0.20	11.5879	10.7170
8.0	0.39	11.6088	10.7876
12.0	0.59	11.6280	10.8523
16.5	0.80	11.6479	10.9193
20.5	0.99	11.6640	10.9738
25.0	1.20	11.6816	11.0329
31.5	1.50	11.7048	11.1107
43.0	2.01	11.7401	11.2288
54.5	2.50	11.7704	11.3292
66.5	2.99	11.7973	11.4174
79.0	3.49	11.8210	11.4956
92.0	3.99	11.8425	11.5649
120.0	5.00	11.8796	11.6839
150.0	6.00	11.9100	11.7785
218.0	8.00	11.9573	11.9211
300.0	10.0	11.9933	12.0224

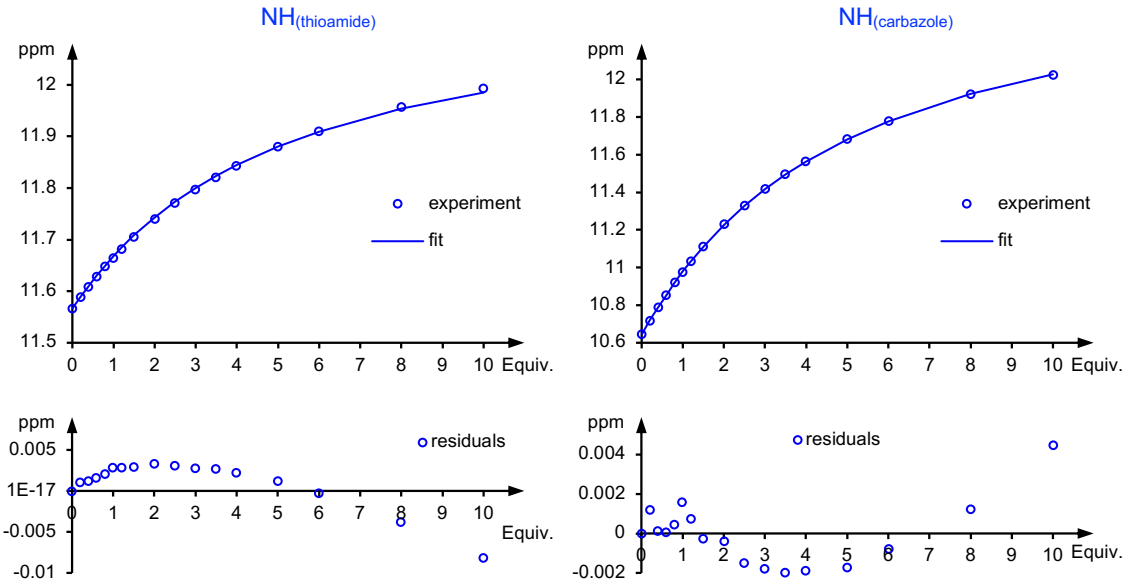


Figure S24. Data points and fitting curves for ^1H NMR titration of **3T** (0.005 M in DMSO-d_6/0.5% H_2O) with TBACl.

a) Binding constant K derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

$K = 48.3 \pm 0.4\ M^{-1}$

b) Chemical shifts derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

	$NH_{(thioamide)}$	$NH_{(carbazole)}$
Receptor **3T** [ppm]	11.5663	10.6433
3T × TBA^+Cl^- [ppm]	12.1715	12.6425

c) Binding constant K derived from independent experiment repeated according to the same methodology:

concentration of host: 0.005M

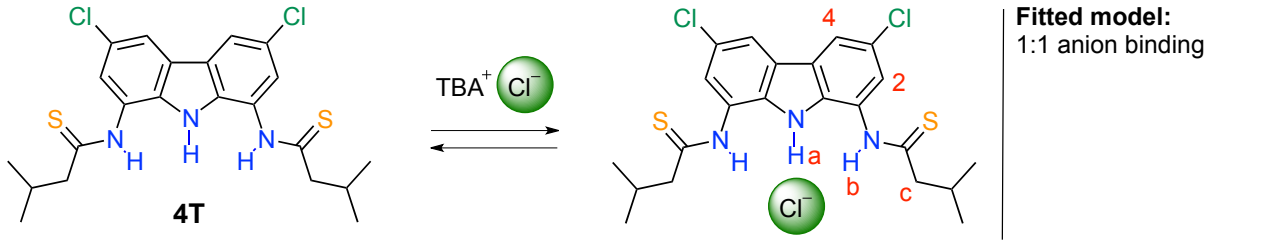
$K = 50.9 \pm 0.3\ M^{-1}$

d) Binding constant averaged from the two experiments:

$K = 49.6\ M^{-1}$

3.7 ^{1}H NMR titration of 0.01 M solution of receptor **4T** in DMSO-d_6/0.5% H_2O with 0.15 M solution of TBA^+Cl^- (dissolved in the solution of receptor **4T**).

Titration of **4T** with TBACl produced the most significant changes in the ^{1}H NMR chemical shifts of the carbazole NH, thioamide NHs and carbazole CH-2 protons. However, due to severe broadening, the carbazole NH signal was not suitable for binding constant determination. Therefore, the data for the thioamide NH and carbazole CH-2 were suplemented by chemical shifts of methylene protons **c** and all three simultaneously fitted to the 1: 1 model using Bindfit software.



Scheme S4. Chloride binding to **4T**.

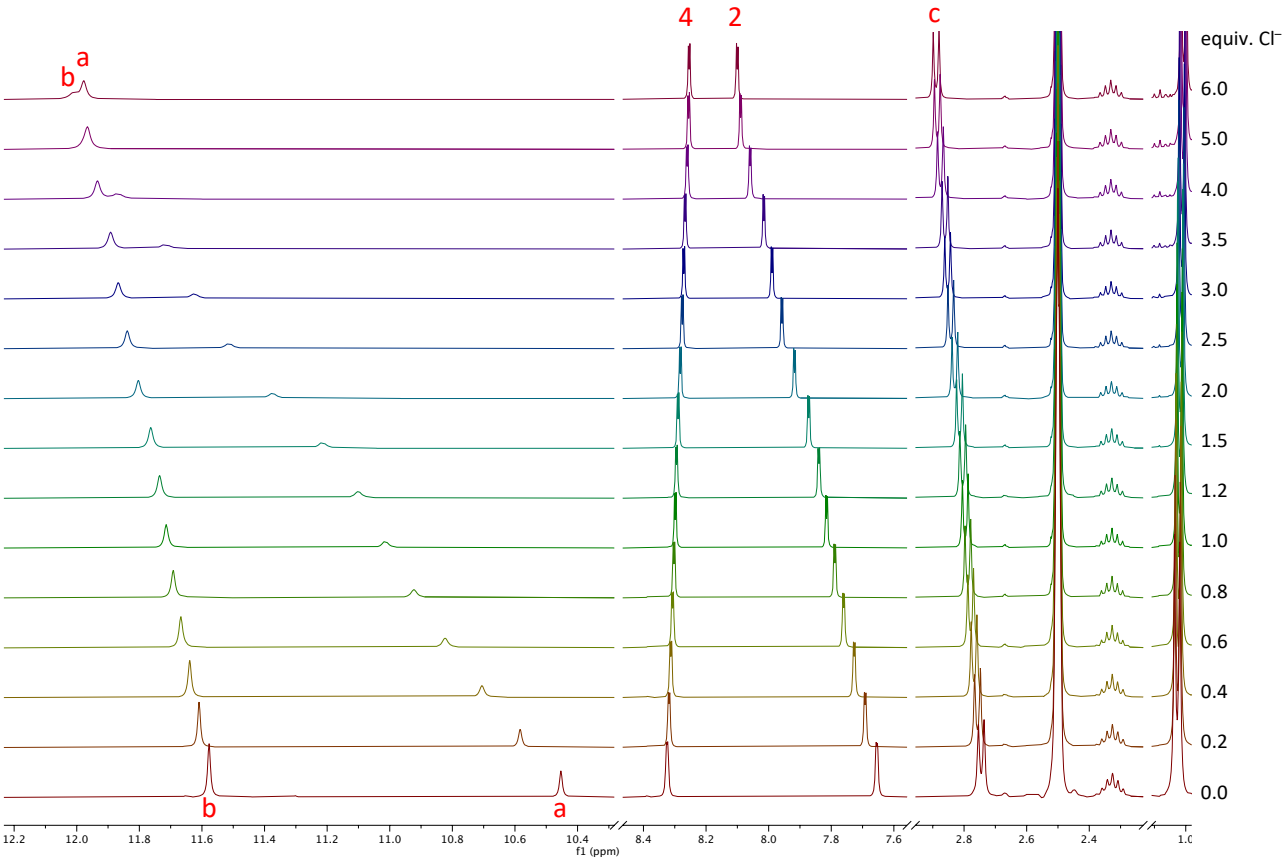


Figure S25. ^{1}H NMR titration of **4T** (0.01 M) with TBACl.

Raw data

Added volume of titrant solution [µL]	Equivalents of TBA^+Cl^-	Chemical Shift [ppm]		
		$NH_{(thioamide)}$	CH-2	$CH(CH_3)_2$
0.00	0.00	11.5783	7.6544	2.7449
8.00	0.20	11.6137	7.6942	2.7572
16.25	0.40	11.6447	7.7303	2.7683
25.00	0.60	11.6731	7.7642	2.7791
33.75	0.80	11.6972	7.7932	2.7880
42.75	1.00	11.7190	7.8196	2.7964
52.25	1.20	11.7405	7.8446	2.8041
67.25	1.51	11.7697	7.8781	2.8148
92.25	2.00	11.8080	7.9229	2.8290
120.50	2.50	11.8437	7.9609	2.8413
150.50	3.01	11.8700	7.9913	2.8511
183.00	3.51	11.8923	8.0164	2.8592
218.75	4.01	11.9132	8.0379	2.8663
300.50	5.01	11.9456	8.0715	2.8774
400.50	6.00	11.9805	8.0968	2.8860
575.50	7.34	12.0084	8.1211	2.8945

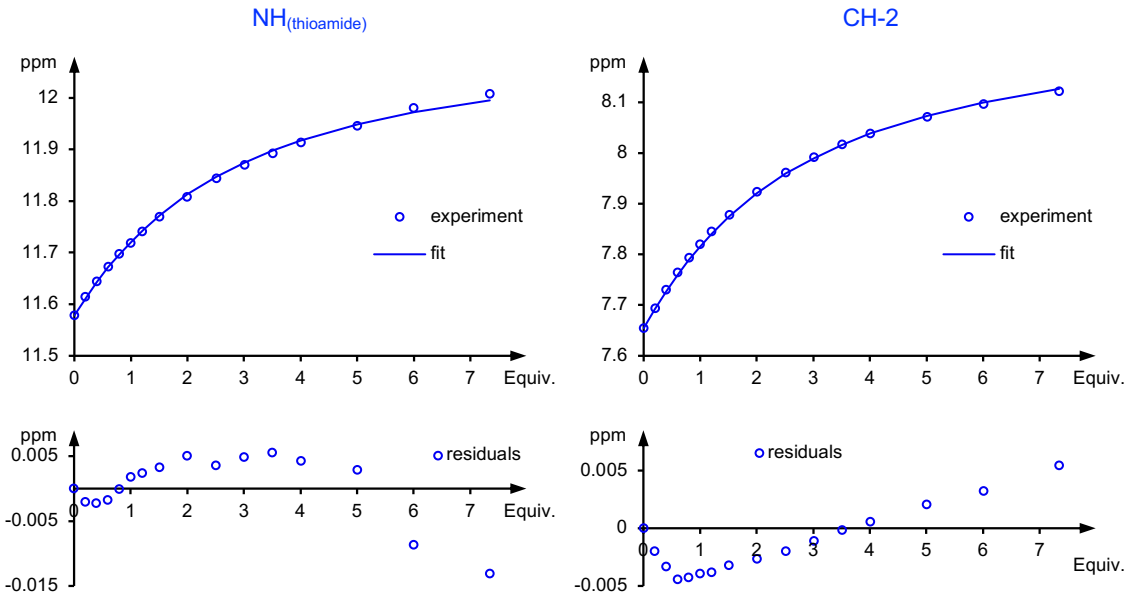


Figure S26. Data points and fitting curves for ^{1}H NMR titration of **4T** (0.01 M in DMSO-d_6/0.5% H_2O) with TBACl.

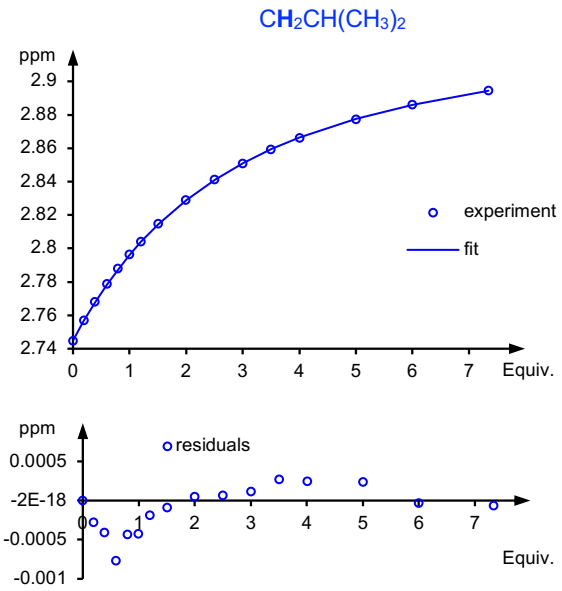


Figure S27. Data points and fitting curves for ^{1}H NMR titration of **4T** (0.01 M in DMSO-d_6/0.5% H_2O) with TBACl.

a) Binding constant K derived from simultaneous fitting of 1:1 model to the three selected protons using BindFit:

K = 47.1 ± 1.21 M^{-1}

b) Chemical shifts derived from simultaneous fitting of 1:1 model to the three selected protons using BindFit:

	$NH_{(thioamide)}$	CH-2	$CH(CH_3)_2$
Receptor **4T** [ppm]	11.5783	7.6543	2.7449
4T × TBA^+Cl^- [ppm]	12.1296	8.2786	2.9425

c) Binding constant K derived from independent experiment repeated according to the same methodology:

concentration of host: 0.01 M

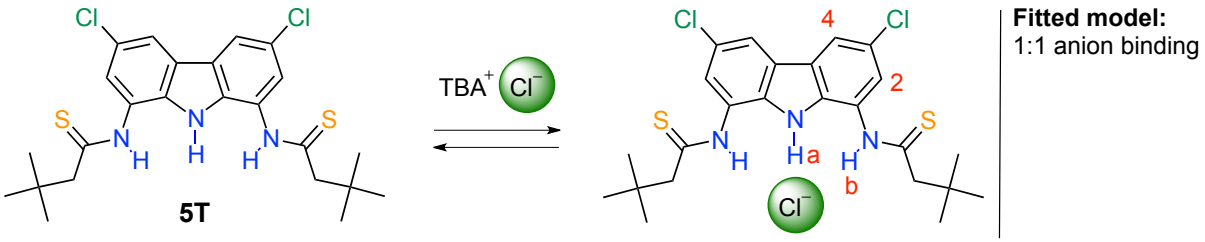
K = 44.1 ± 0.5 M^{-1}

d) Binding constant averaged from the two experiments:

K = 45.6 M^{-1}

3.8 ^{1}H NMR titration of 0.005 M solution of receptor **5T** in DMSO-d_6/0.5% H_2O with 0.15 M solution of TBA^+Cl^- (dissolved in the solution of receptor **5T**).

Titration of **5T** with TBACl produced significant changes in the ^{1}H NMR chemical shifts of the carbazole NH and the thioamide NHs. The data for these two signals were simultaneously fitted to the 1: 1 model using BindFit software.



Scheme 5. Chloride binding to **5T**.

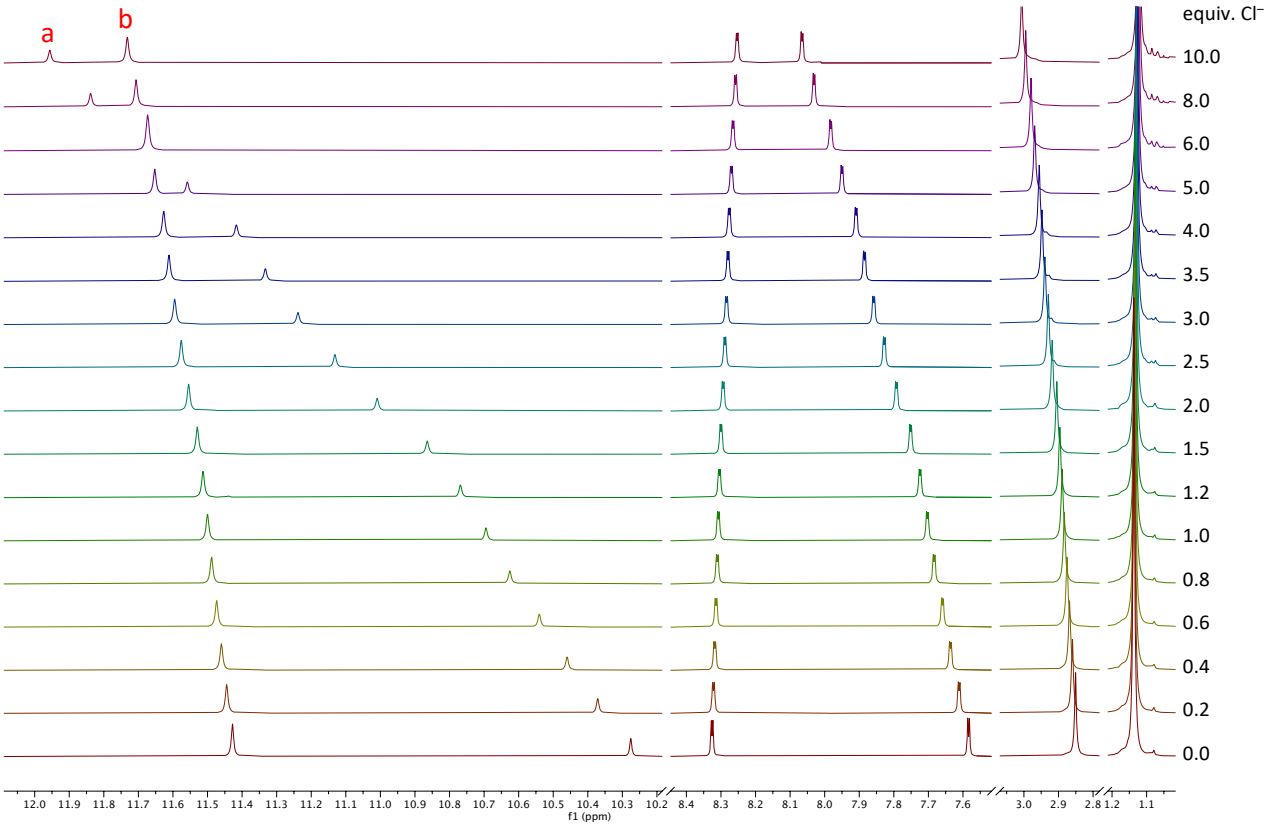


Figure S28. ^{1}H NMR titration of **5T** (0.005 M) with TBACl. The marked peaks were used for fitting.

Raw data

Added volume of titrant solution [µL]	Equivalents of TBA^+Cl^-	Chemical Shift [ppm]	
		$NH_{(thioamide)}$	$NH_{(carbazole)}$
0.0	0.00	11.4281	10.2759
4.0	0.20	11.4444	10.3712
8.0	0.39	11.4596	10.4595
12.0	0.59	11.4733	10.5400
16.5	0.80	11.4880	10.6251
20.5	0.99	11.4999	10.6945
25.0	1.20	11.5121	10.7683
31.5	1.50	11.5292	10.8641
43.0	2.01	11.5544	11.0091
54.5	2.50	11.5758	11.1313
66.5	2.99	11.5946	11.2381
79.0	3.49	11.6114	11.3326
92.0	3.99	11.6264	11.4164
120.0	5.00	11.6525	11.5583
150.0	6.00	11.6729	11.6729
218.0	8.00	11.7064	11.8379
300.0	10.0	11.7315	11.9557

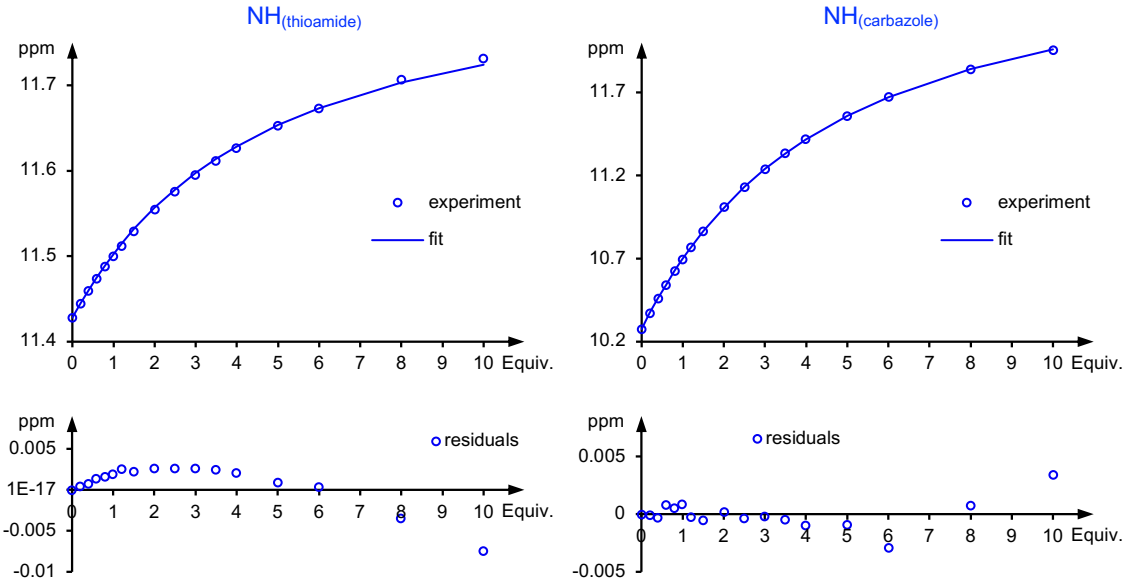


Figure S29. Data points and fitting curve for ^1H NMR titration of **5T** (0.005 M in DMSO-d_6/0.5% H_2O) with TBACl.

a) Binding constant K derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

$K = 52.9 \pm 0.3\ M^{-1}$

b) Chemical shifts derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

	$NH_{(thioamide)}$	$NH_{(carbazole)}$
Receptor **5T** [ppm]	11.4281	10.2759
5T × TBA^+Cl^- [ppm]	11.8446	12.6446

c) Binding constant K derived from independent experiment repeated according to the same methodology:

concentration of host: 0.005M

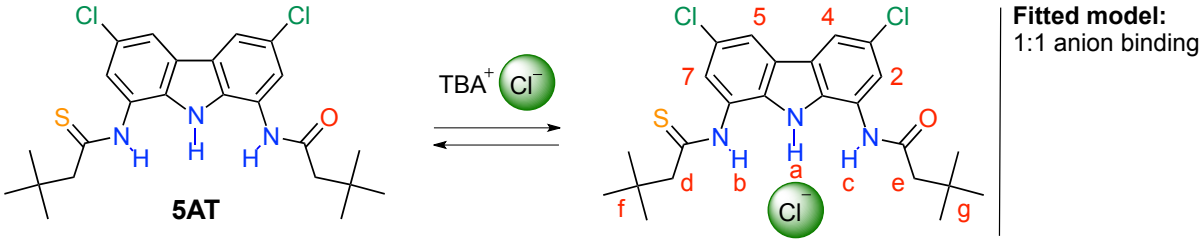
$K = 51.6 \pm 0.3\ M^{-1}$

d) Binding constant averaged from the two experiments:

$K = 52.3\ M^{-1}$

3.9 ^{1}H NMR titration of 0.005 M solution of receptor **5AT** in DMSO-d_6/0.5% H_2O with 0.15 M solution of TBA^+Cl^- (dissolved in the solution of receptor **5AT**).

Titration of **5AT** with TBACl produced significant changes (>0.3ppm) in the ^{1}H NMR chemical shifts of 4 protons: carbazole NH, amide NH, carbazole CH-2 and carbazole CH-7. Remarkably, the thioamide proton NH shifted upfield, unlike in di(thioamides), and to a relatively weak extent. Nevertheless, all three NH protons directly involved in the binding event were used for binding constant determination and simultaneously fitted to the 1: 1 model using BindFit software.



Scheme 6. Chloride binding to **5AT**.

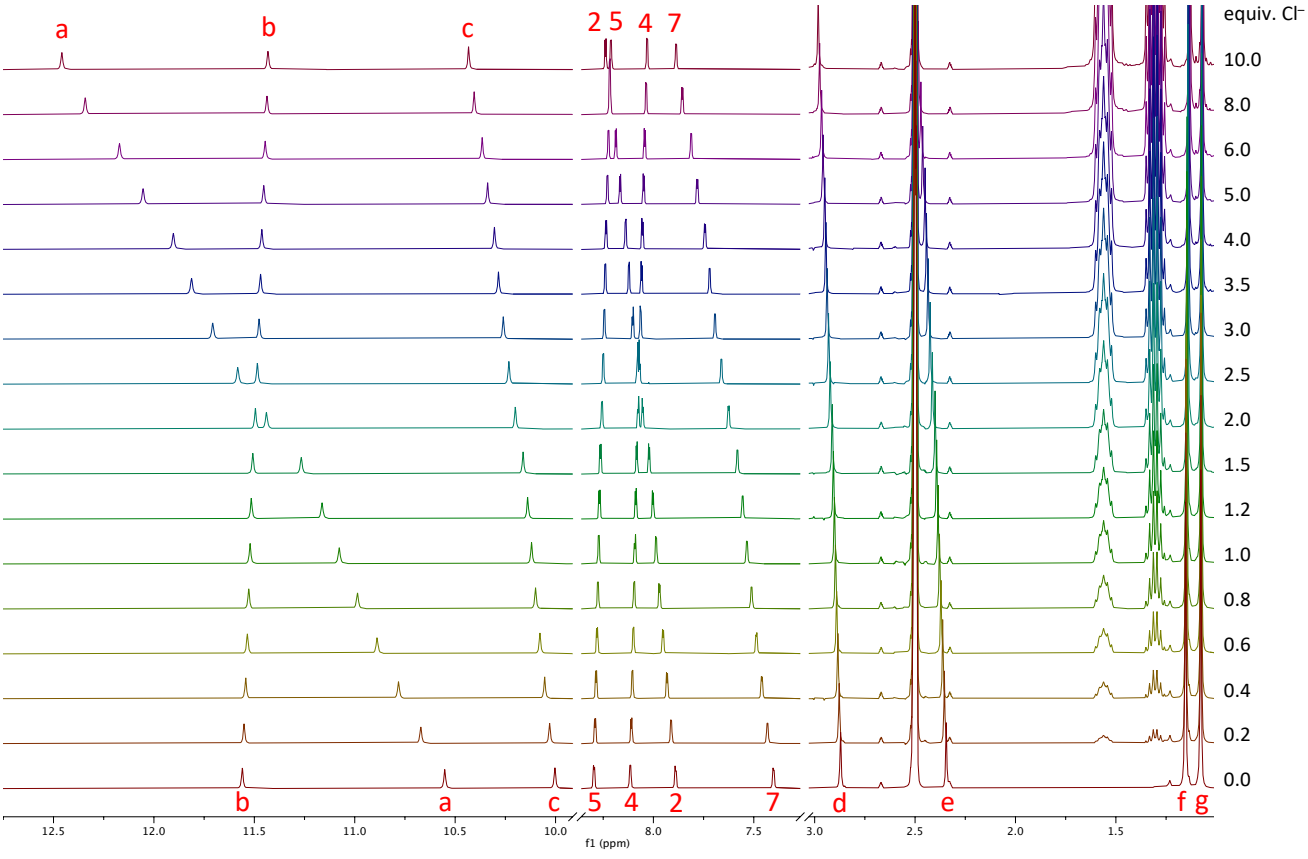


Figure S30. ^{1}H NMR titration of **5AT** (0.005 M) with TBACl.

Raw data

Added volume of titrant solution [µL]	Equivalents of TBA^+Cl^-	Chemical Shift [ppm]		
		$NH_{(thioamide)}$	$NH_{(carbazole)}$	$NH_{(amide)}$
0.0	0.00	11.5609	10.5538	10.0039
4.0	0.20	11.5519	10.6757	10.0311
8.0	0.39	11.5436	10.7876	10.0559
12.25	0.60	11.5352	10.8981	10.0804
16.5	0.80	11.5282	10.9974	10.1025
20.75	1.00	11.5216	11.0885	10.1226
25.0	1.20	11.5154	11.1723	10.1415
31.5	1.50	11.5073	11.2882	10.1672
42.75	2.00	11.4948	11.4566	10.2048
54.5	2.50	11.4845	11.6021	10.2369
67.25	3.02	11.4759	11.7293	10.2656
79.25	3.50	11.4686	11.8292	10.2877
92.25	4.00	11.4626	11.9197	10.3082
120.0	5.00	11.4526	12.0696	10.3417
150.0	6.00	11.4454	12.1866	10.3682
218.25	8.00	11.4361	12.3546	10.4069
300.0	10.00	11.4312	12.4694	10.4342

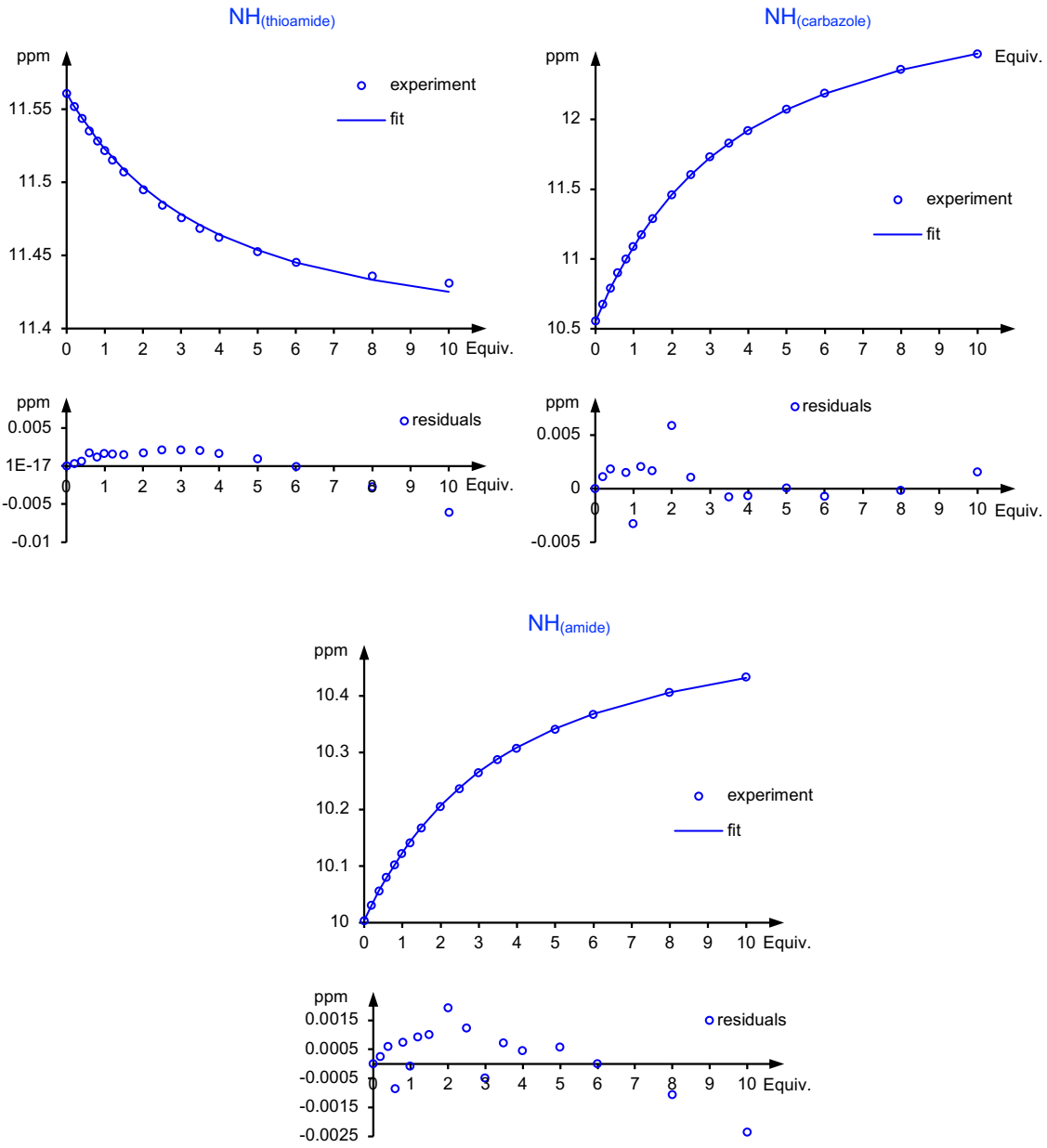


Figure S31. Data points and fitting curves for ^{1}H NMR titration of **5AT** (0.005 M in DMSO-d_6/0.5% H_2O) with TBACl.

a) Binding constant K derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

K = 68.3 ± 0.2 M^{-1}

b) Chemical shifts derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

	$NH_{(thioamide)}$	$NH_{(carbazole)}$	$NH_{(amide)}$
Receptor **5AT** [ppm]	11.5609	10.5538	10.0039
5AT × TBA^+Cl^- [ppm]	11.3821	13.0784	10.5674

c) Binding constant K derived from independent experiment repeated according to the same methodology:

concentration of host: 0.005M

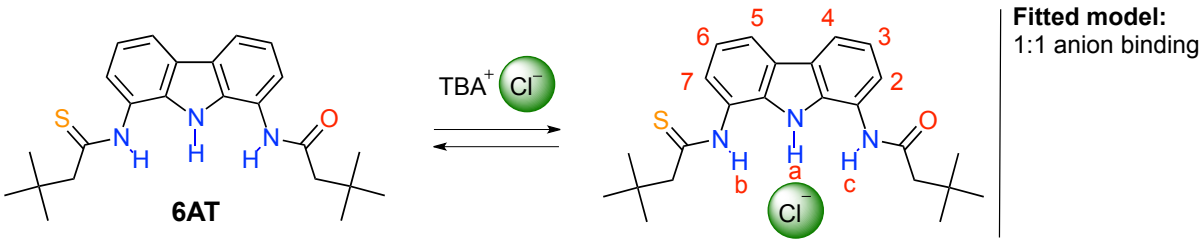
$K = 65.7 \pm 0.3\ M^{-1}$

d) Binding constant averaged from the two experiments:

$K = 67.0\ M^{-1}$

3.10 ^{1}H NMR titration of 0.005 M solution of receptor **6AT** in DMSO-d_6/0.5% H_2O with 0.15 M solution of TBA^+Cl^- (dissolved in the solution of receptor **6AT**).

Titration of **6AT** with TBACl produced significant changes (>0.3ppm) in the ^{1}H NMR chemical shifts of only 1 NH proton and 2 CH protons: carbazole NH, CH-2 and CH-7. Remarkably, the thioamide proton NH shifted slightly upfield, like in the mono(thioamide) **5AT** and unlike in di(thioamides). Nevertheless, all three NH protons directly involved in the binding event were used for binding constant determination and to this end simultaneously fitted to the 1: 1 model using BindFit software.



Scheme 7. Chloride binding to **6AT**.

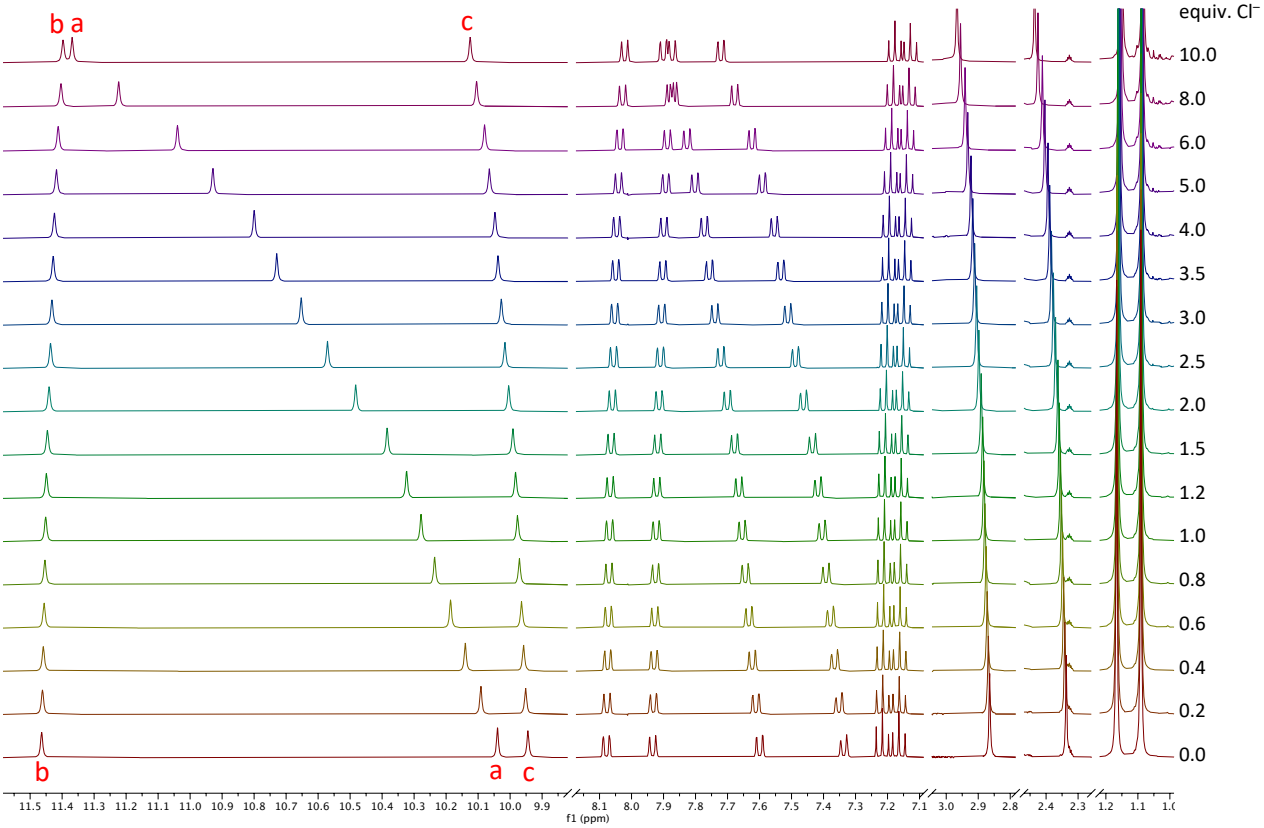


Figure S32. ^{1}H NMR titration of **6AT** (0.005 M) with TBACl. The marked peaks were used for fitting.

Raw data

Added volume of titrant solution [µL]	Equivalents of TBA^+Cl^-	Chemical Shift [ppm]		
		$NH_{(thioamide)}$	$NH_{(carbazole)}$	$NH_{(amide)}$
0.0	0.00	11.4639	10.0393	9.9441
4.0	0.20	11.4609	10.0910	9.9510
8.0	0.39	11.4585	10.1398	9.9578
12.0	0.59	11.4561	10.1859	9.9640
16.5	0.80	11.4534	10.2355	9.9707
20.5	0.99	11.4512	10.2781	9.9761
25.0	1.20	11.4489	10.3232	9.9826
31.5	1.50	11.4455	10.3839	9.9908
43.0	2.01	11.4403	10.4821	10.0042
54.5	2.50	11.4358	10.5705	10.0160
66.5	2.99	11.4314	10.6526	10.0271
79.0	3.49	11.4277	10.7290	10.0375
92.0	3.99	11.424	10.7995	10.0470
120.0	5.00	11.4174	10.9285	10.0644
150.0	6.00	11.4119	11.0391	10.0794
218.0	8.00	11.4032	11.2228	10.1045
300.0	10.0	11.3966	11.3681	10.1244

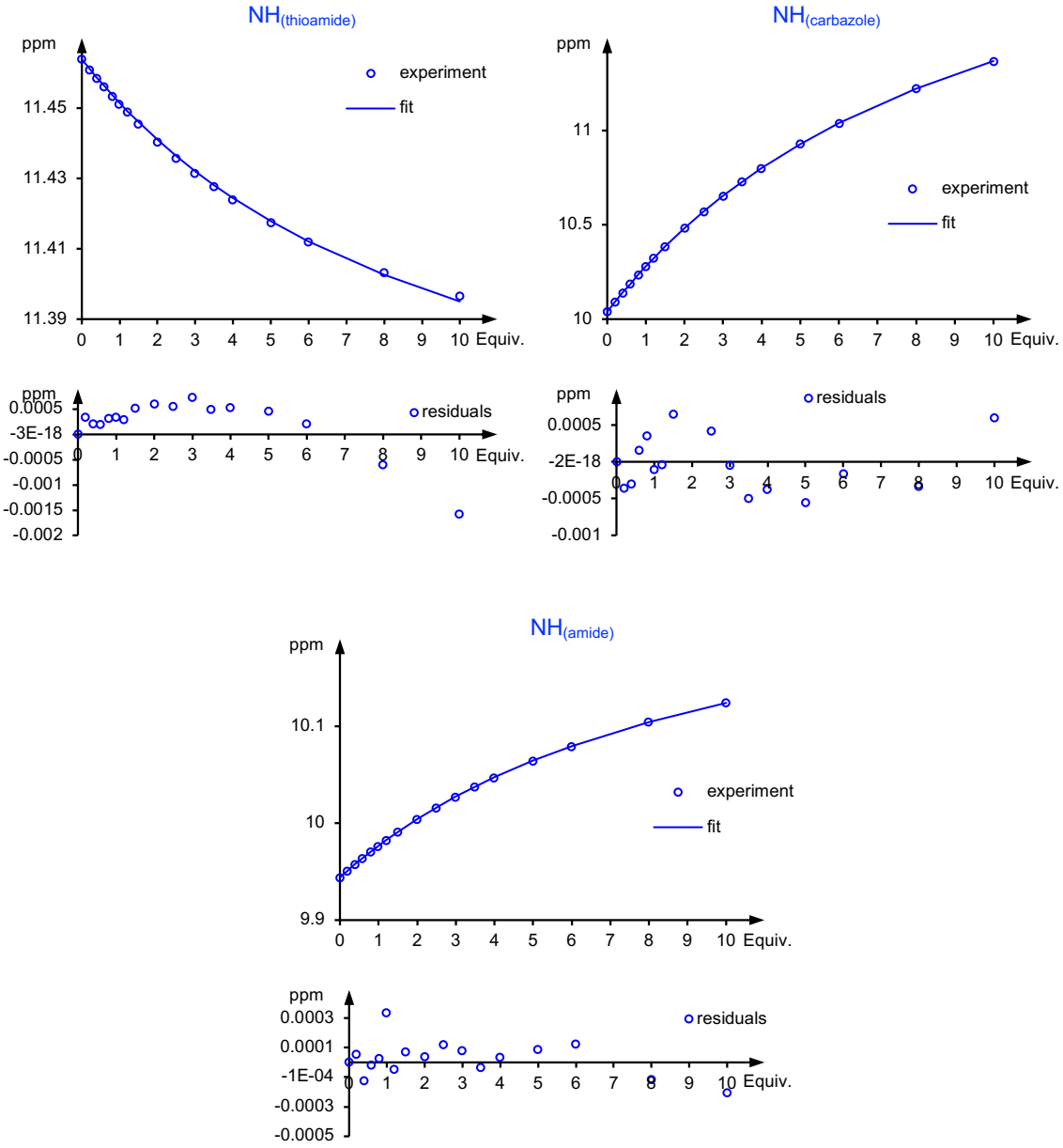


Figure S33. Data points and fitting curve for ^{1}H NMR titration of **6AT** (0.005 M in DMSO-d_6/0.5% H_2O) with TBACl.

a) Binding constant K derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

K = 23.1 ± 0.1 M^{-1}

b) Chemical shifts derived from simultaneous fitting of 1:1 model to the two selected protons using BindFit:

	$NH_{(thioamide)}$	$NH_{(carbazole)}$	$NH_{(amide)}$
Receptor **6AT** [ppm]	11.4639	10.0393	9.9441
6AT × TBA^+Cl^- [ppm]	11.3320	12.5856	10.2891

c) Binding constant K derived from independent experiment repeated according to the same methodology:

concentration of host: 0.005M

$K = 23.4 \pm 0.04\ M^{-1}$

d) Binding constant averaged from the two experiments:

$K = 23.3\ M^{-1}$

4 Transmembrane anion transport experiments

4.1 Preparation of phospholipid vesicles

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (20 mg/mL) (Sigma Aldrich) was evaporated to dryness using a rotary evaporator and the resulting film was dried under high vacuum for, at least, two hours. A sodium chloride aqueous solution (489 mM and 5 mM phosphate buffer, pH 7.2, or 451 mM and 20 mM phosphate buffer, pH 7.2) was added to rehydrate the lipid film. The resulting suspension was vortexed and subjected to nine freeze-thaw cycles; subsequently, it was extruded through a polycarbonate membrane (200 nm) employing a LiposoFast basic extruder (Avestin, Inc.). The resulting unilamellar vesicles were dialysed against a sodium nitrate (489 mM and 5 mM phosphate buffer, pH 7.2) or a sodium sulphate (150 mM and 20 mM phosphate buffer, pH 7.2) aqueous solutions, to remove unencapsulated chloride.

4.2 ISE transport experiments

Unilamellar vesicles (average diameter: 200 nm) made of POPC and containing a sodium chloride aqueous solution (489 mM and 5 mM phosphate buffer, pH 7.2, for chloride/nitrate exchange assays, or 451 mM and 20 mM phosphate buffer, pH 7.2, for chloride/bicarbonate exchange assays) were suspended in a sodium nitrate (489 mM and 5 mM phosphate buffer, pH 7.2) or a sodium sulphate (150 mM and 20 mM phosphate buffer, pH 7.2) aqueous solution, respectively, the final lipid concentration being 0.5 mM and the final volume 5 mL. A solution of the carrier in DMSO, usually 5 µL to avoid the influence of the organic solvent during the experiments, was added, and the chloride released was monitored employing a chloride-selective electrode (HACH 9652C). Once the experiment was finished, a surfactant (Triton-X, 10% dispersion in water, 20 µL) was added to lyse the vesicles and release all the encapsulated chloride. This value was taken as 100% release and used as such. For the chloride/bicarbonate exchange assays, a sodium bicarbonate aqueous solution was added to the vesicles suspended in the sodium sulphate one (150 mM and 20 mM phosphate buffer, pH 7.2), the final bicarbonate concentration during the experiment being 40 mM. The chloride efflux was monitored for another five minutes, until the vesicles were lysed with the surfactant.

4.3 Study of the Cl^-/NO_3^- exchange

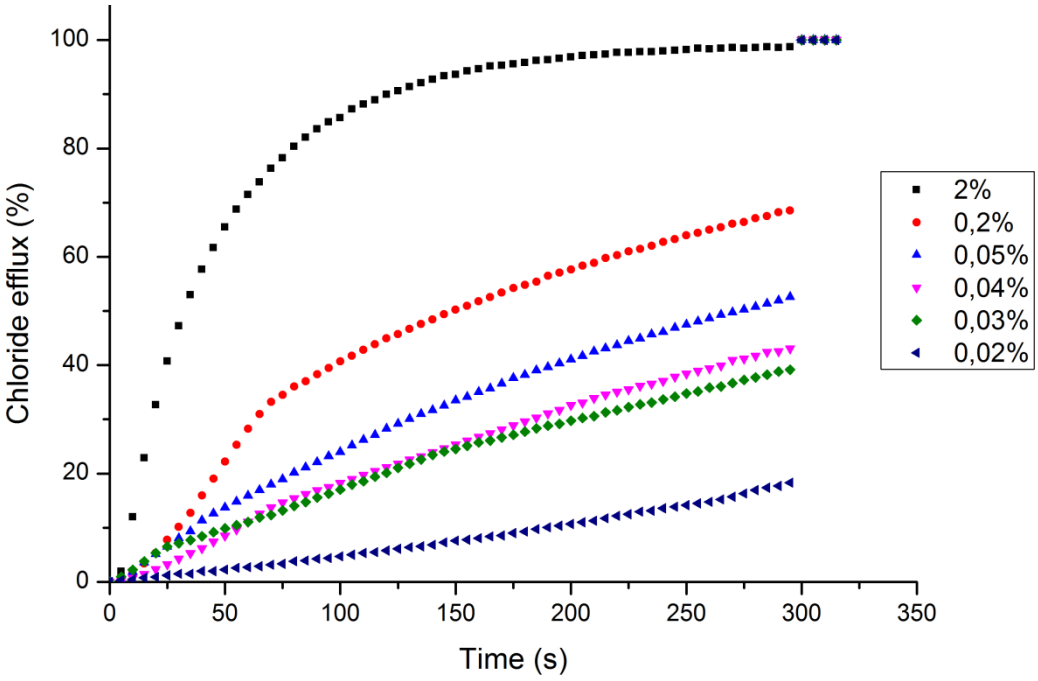


Figure S34. Chloride efflux promoted by **2T** at different concentrations (2% - 0.02% carrier to POPC molar ratio, 10 - 0.1 µM) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

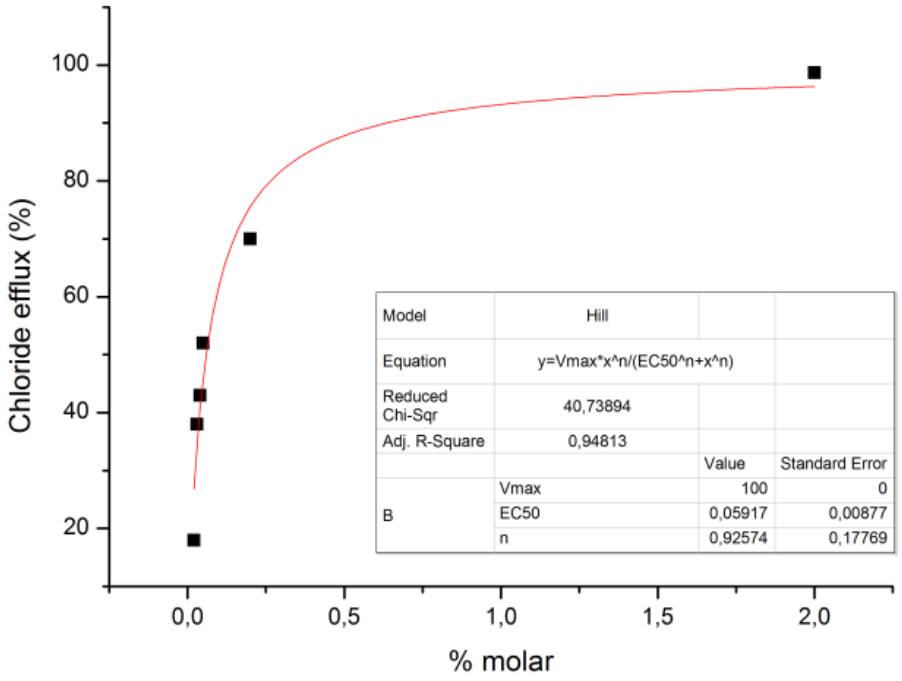


Figure S355. Normalised chloride efflux at 300 s plotted against the concentration of compound **2T**. Data have been plotted with Hill equation fitting curve (continuous line).

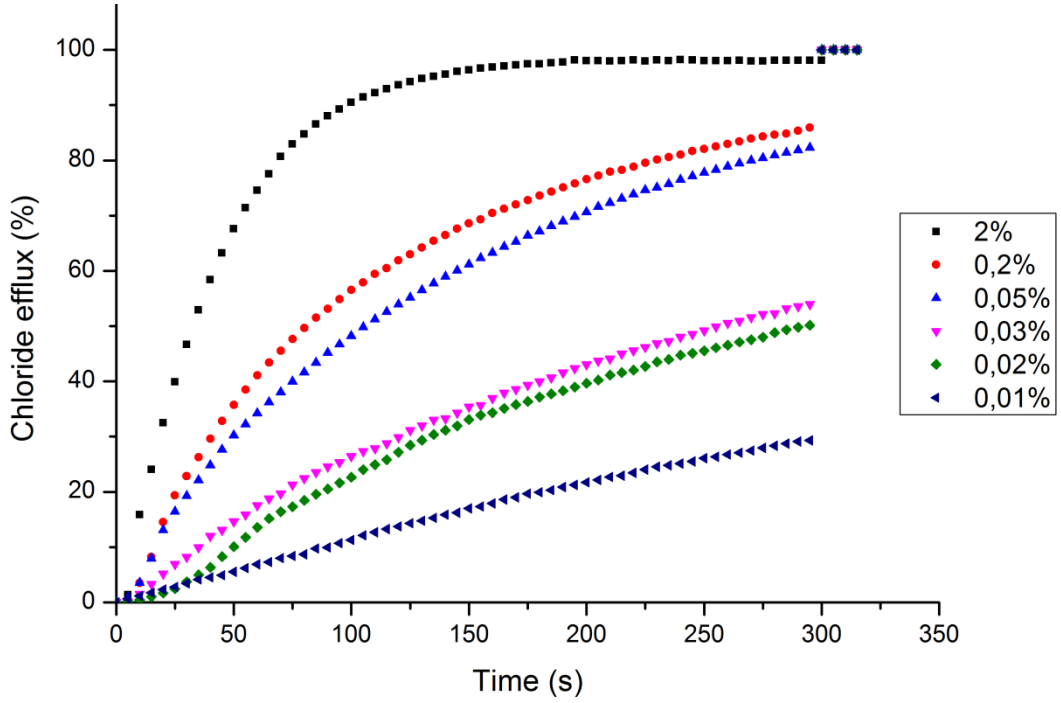


Figure S36. Chloride efflux promoted by **3T** at different concentrations (2% - 0.01% carrier to POPC molar ratio, 10 - 0.05 µM) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

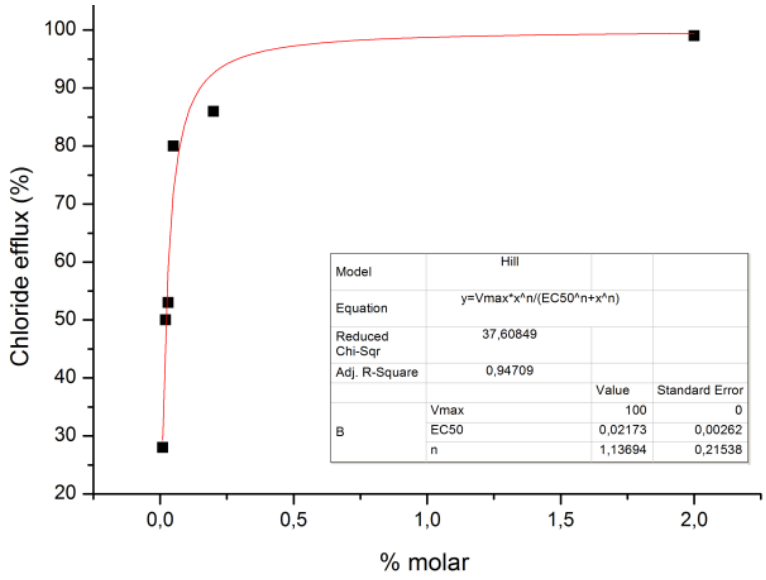


Figure S37. Normalised chloride efflux at 300 s plotted against the concentration of compound **3T**. Data have been plotted with Hill equation (continuous line).

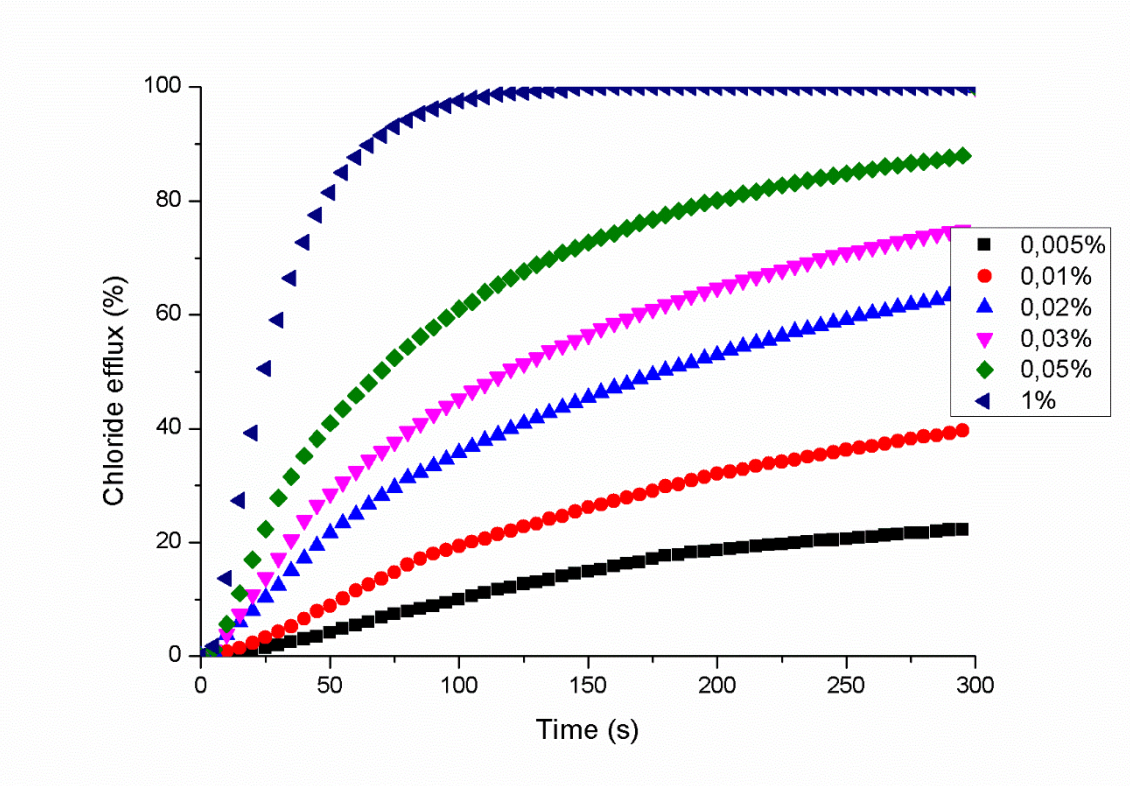


Figure S38. Chloride efflux promoted by **4T** at different concentrations (1% - 0.005% carrier to POPC molar ratio, 5 - 0.025 μM) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

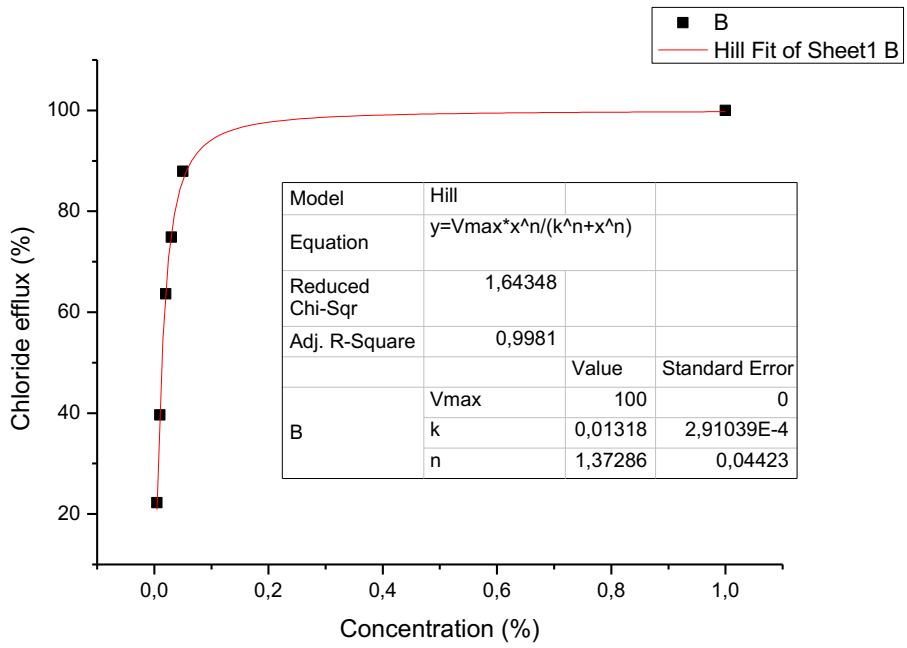


Figure S39. Normalised chloride efflux at 300 s plotted against the concentration of compound **4T**. Data have been plotted with Hill equation (continuous line).

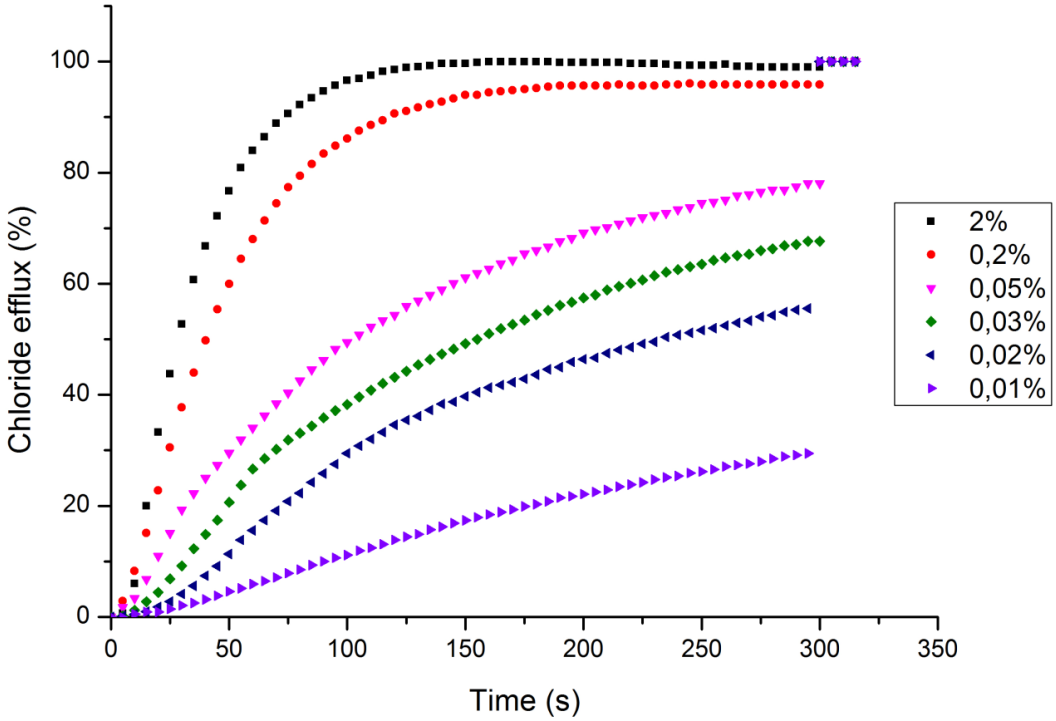


Figure S40. Chloride efflux promoted by **5T** at different concentrations (2% - 0.01% carrier to POPC molar ratio, 10 - 0.05 µM) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

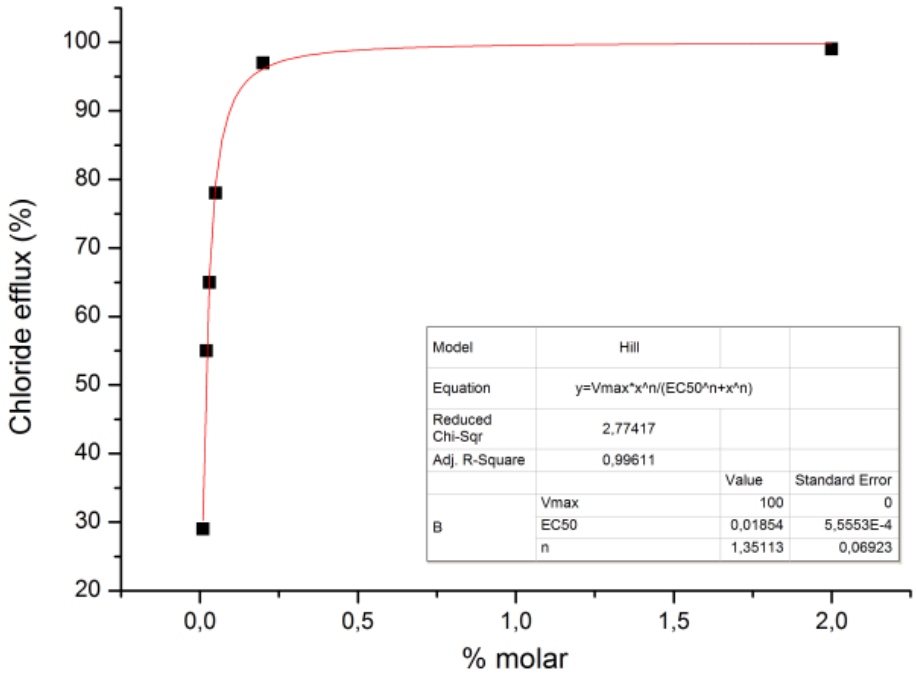


Figure S41. Normalised chloride efflux at 300 s plotted against the concentration of compound **5T**. Data have been plotted with Hill equation (continuous line).

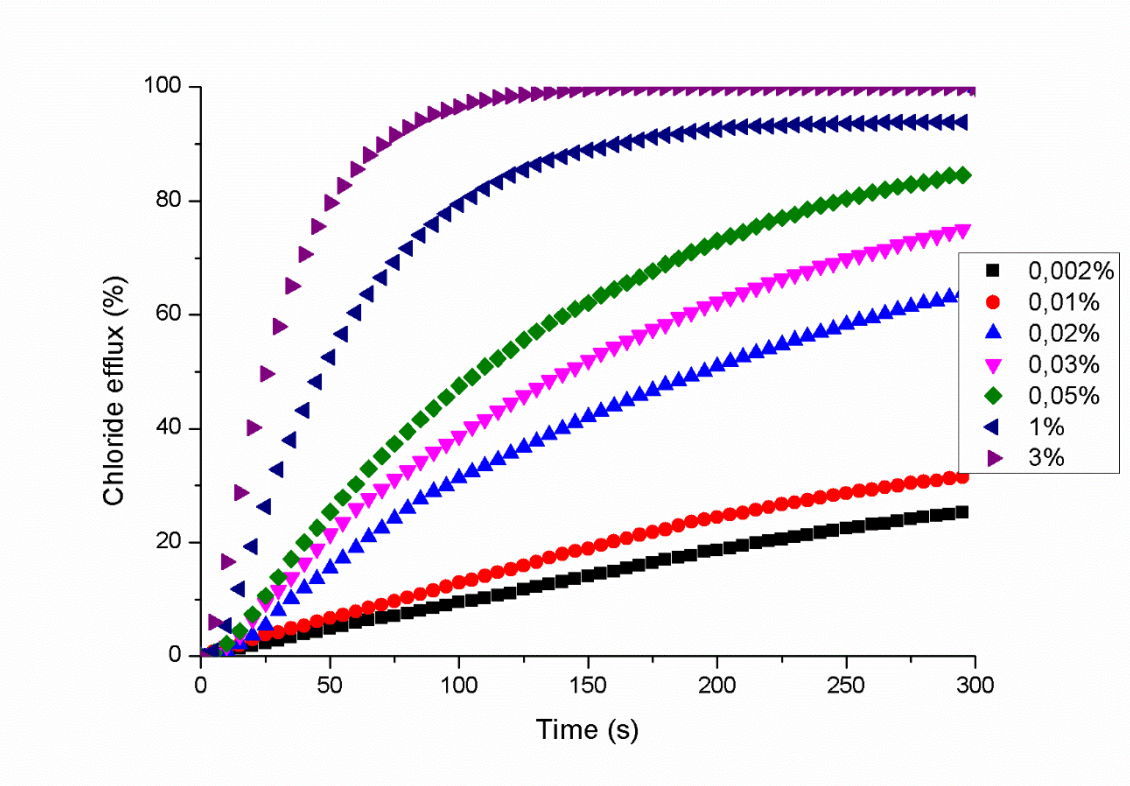


Figure S42. Chloride efflux promoted by **5AT** at different concentrations (3% - 0.002% carrier to POPC molar ratio, 15 - 0.05 µM) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

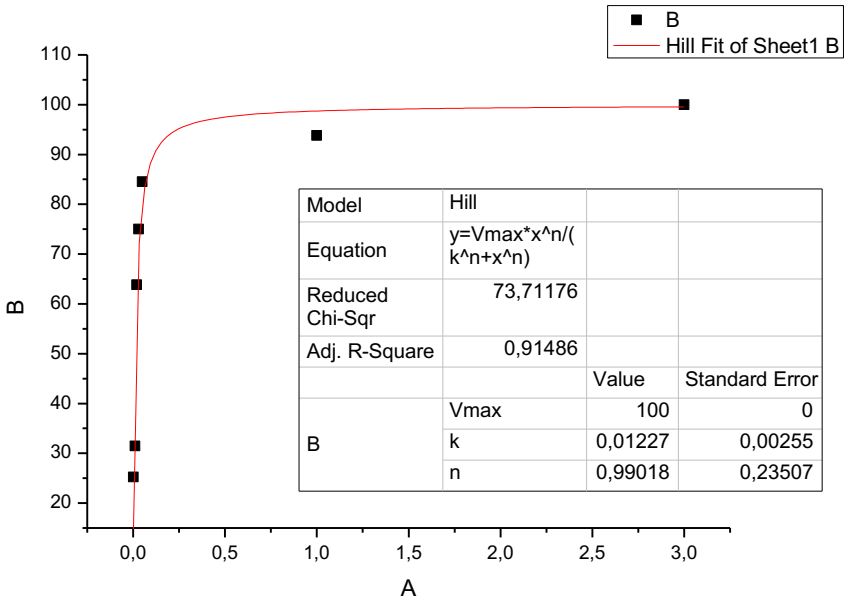


Figure S43. Normalised chloride efflux at 300 s plotted against the concentration of compound **5AT.** Data have been plotted with Hill equation (continuous line).

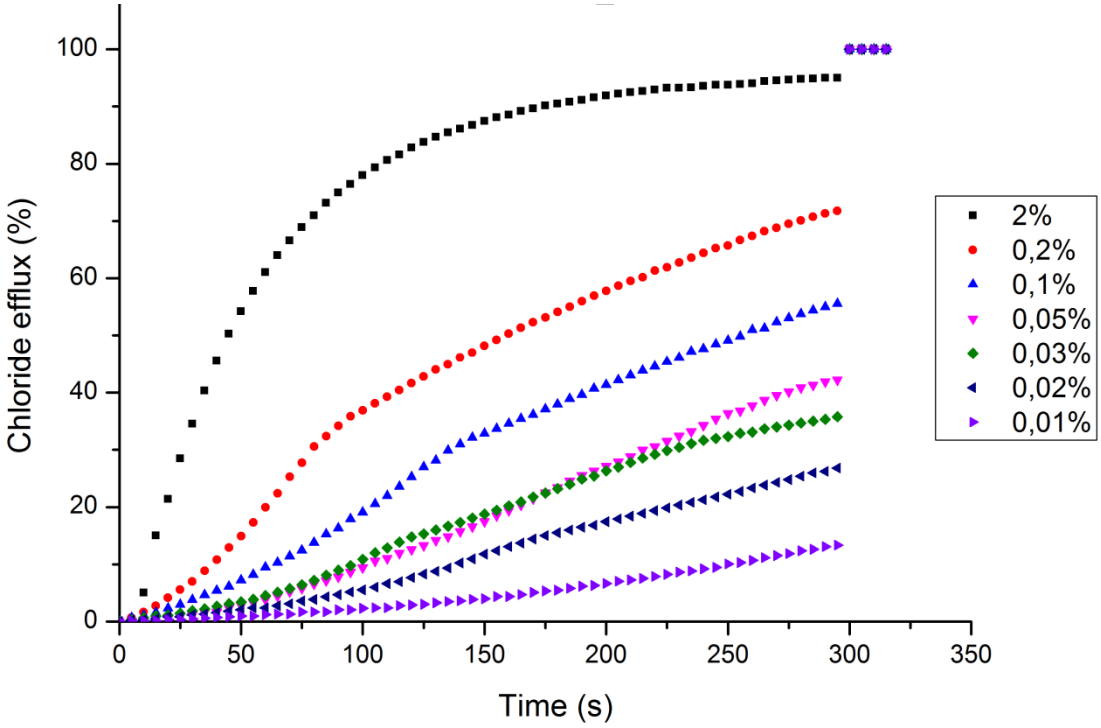


Figure S44. Chloride efflux promoted by **6AT** at different concentrations (2% - 0.01% carrier to POPC molar ratio, 10 - 0.05 µM) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

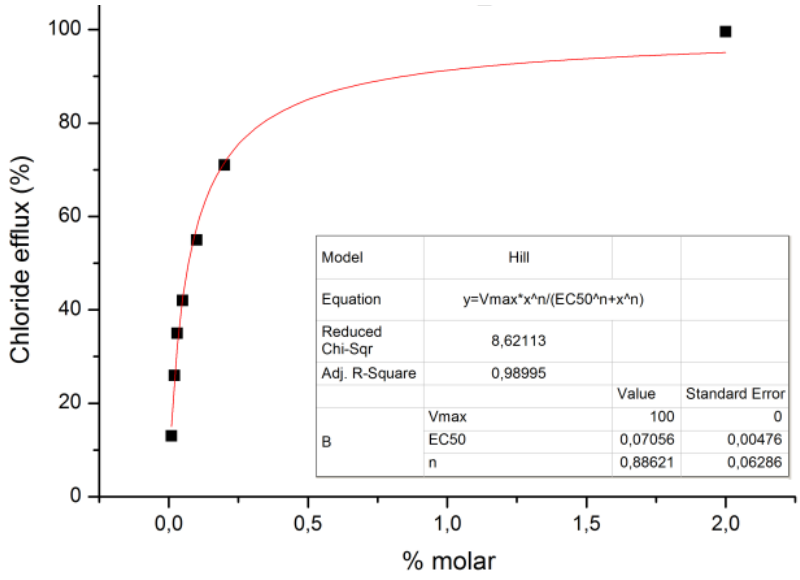


Figure S45. Normalised chloride efflux at 300 s plotted against the concentration of compound **6AT.** Data have been plotted with Hill equation (continuous line).

4.4 Study of the Cl^-/HCO_3^- exchange

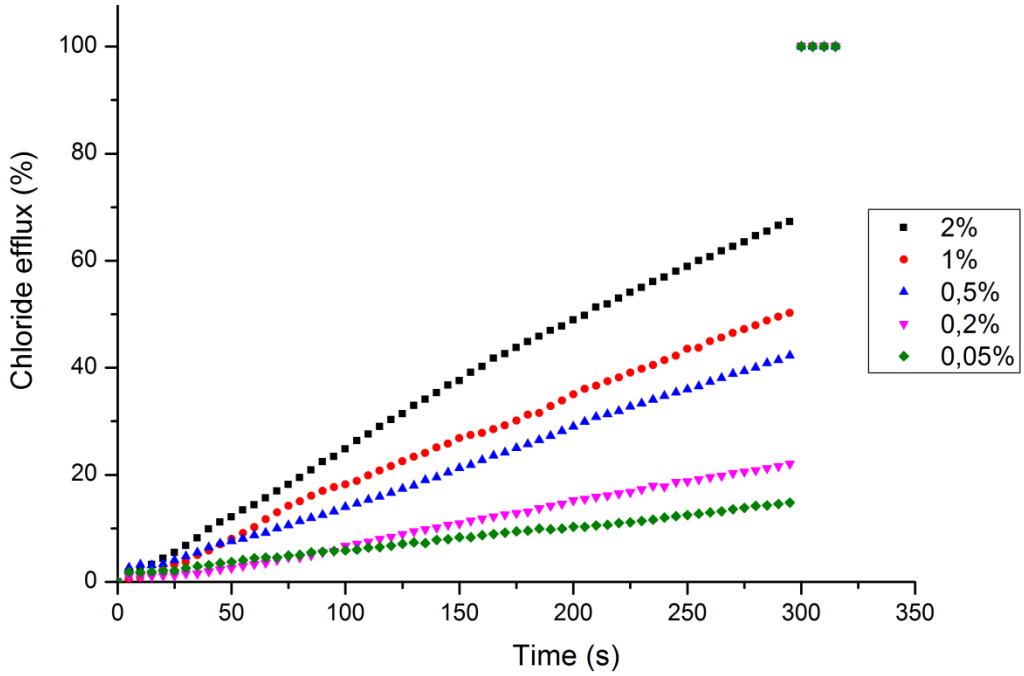


Figure S46. Chloride efflux promoted by **2T** at different concentrations (2% - 0.05% carrier to POPC molar ratio, 10 - 0.25 µM) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

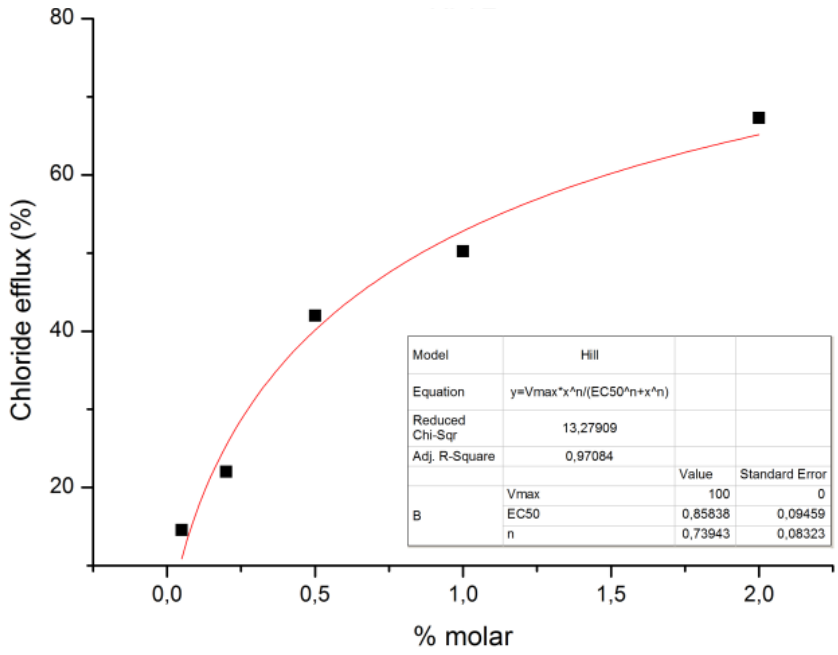


Figure S47. Normalised chloride efflux at 300 s plotted against the concentration of compound **2T**. Data have been plotted with Hill equation (continuous line).

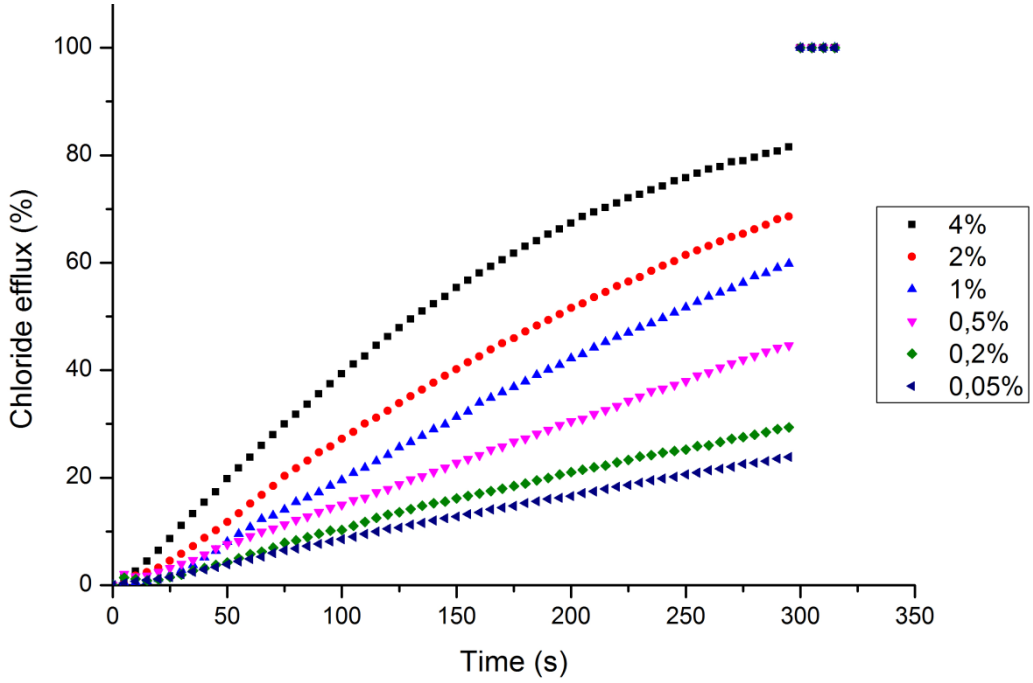


Figure S48. Chloride efflux promoted by **3T** at different concentrations (4% - 0.05% carrier to POPC molar ratio, 20 - 0.25 μM) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

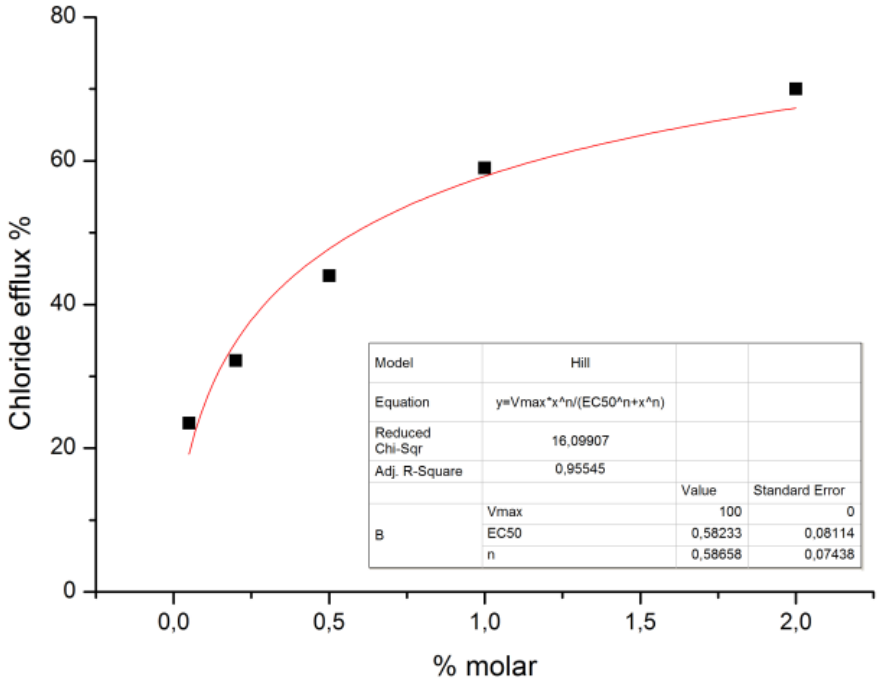


Figure S49. Normalised chloride efflux at 300 s plotted against the concentration of compound **3T**. Data have been plotted with Hill equation (continuous line).

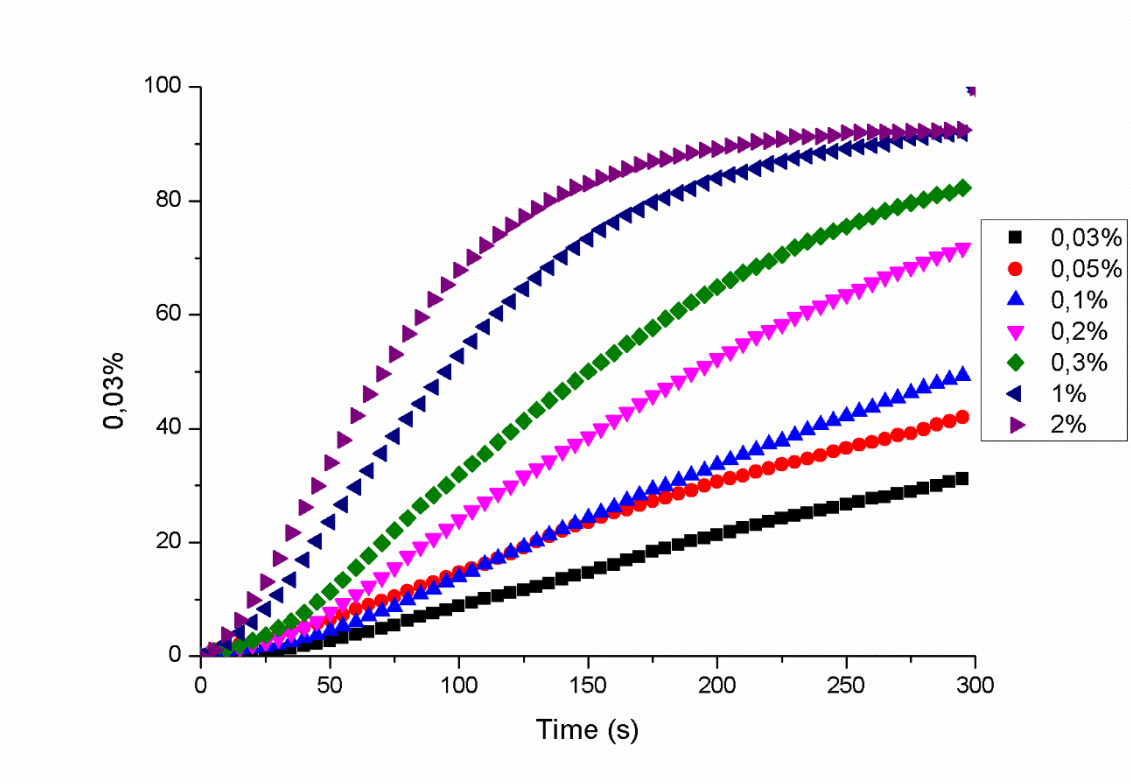


Figure S50. Chloride efflux promoted by **4T** at different concentrations (2% - 0.03% carrier to POPC molar ratio, 10 - 0.15 μM) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

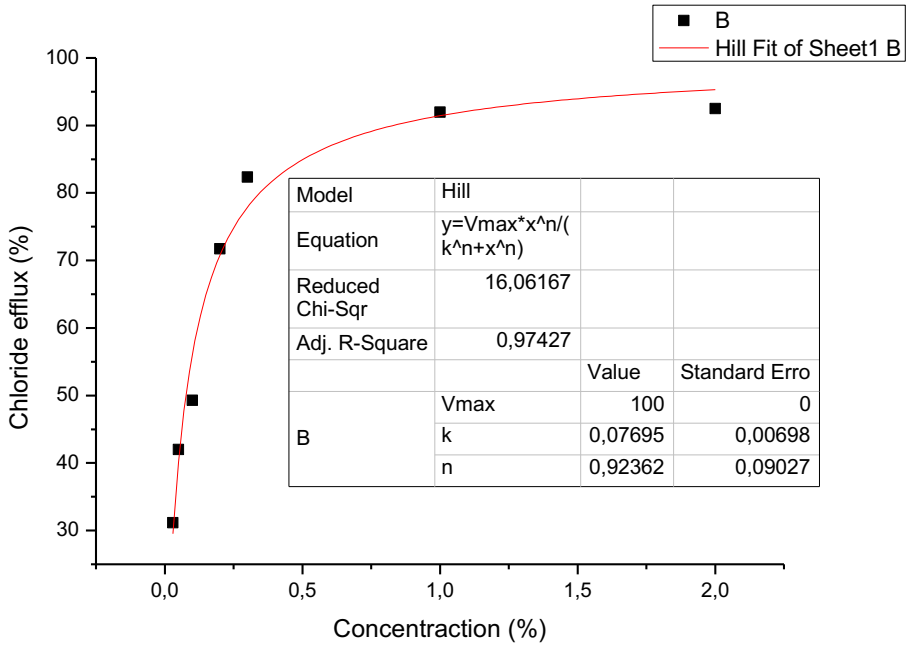


Figure S51. Normalised chloride efflux at 300 s plotted against the concentration of compound **4T**. Data have been plotted with Hill equation (continuous line).

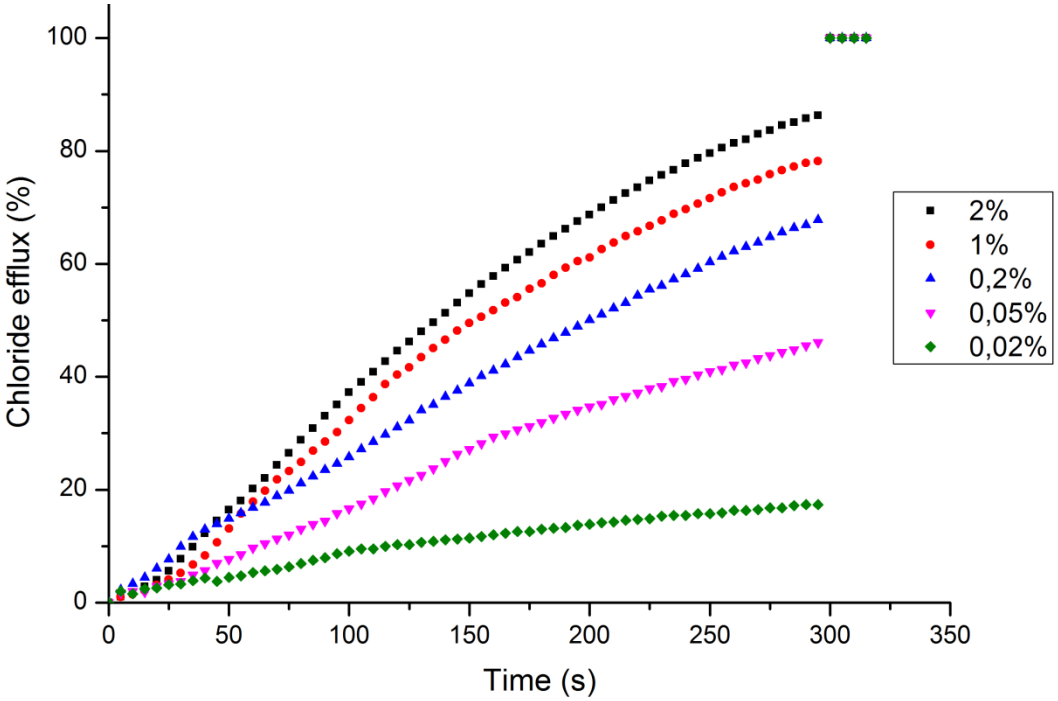


Figure S52. Chloride efflux promoted by **5T** at different concentrations (2% - 0.02% carrier to POPC molar ratio, 10 - 0.1 µM) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

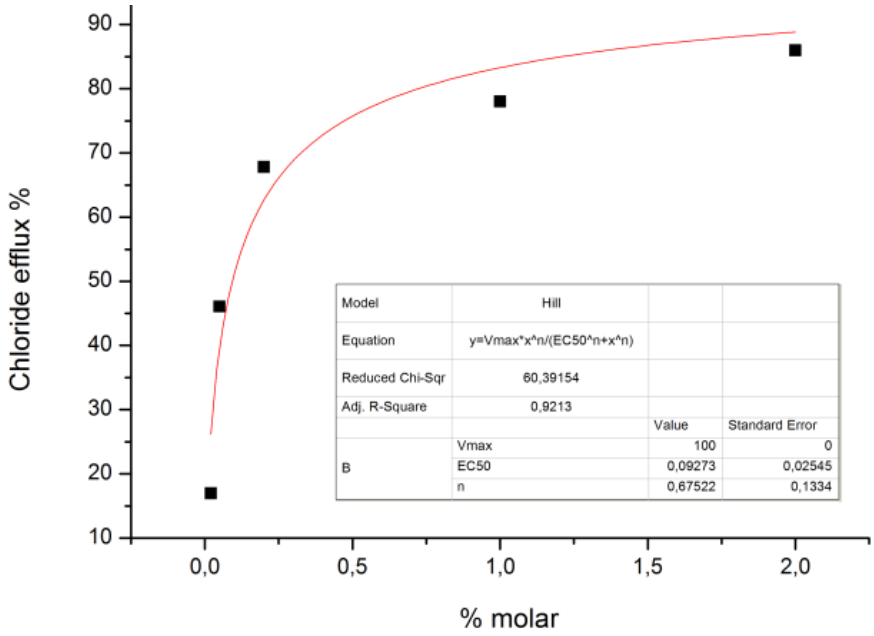


Figure S53. Normalised chloride efflux at 300 s plotted against the concentration of compound **5T**. Data have been plotted with Hill equation (continuous line).

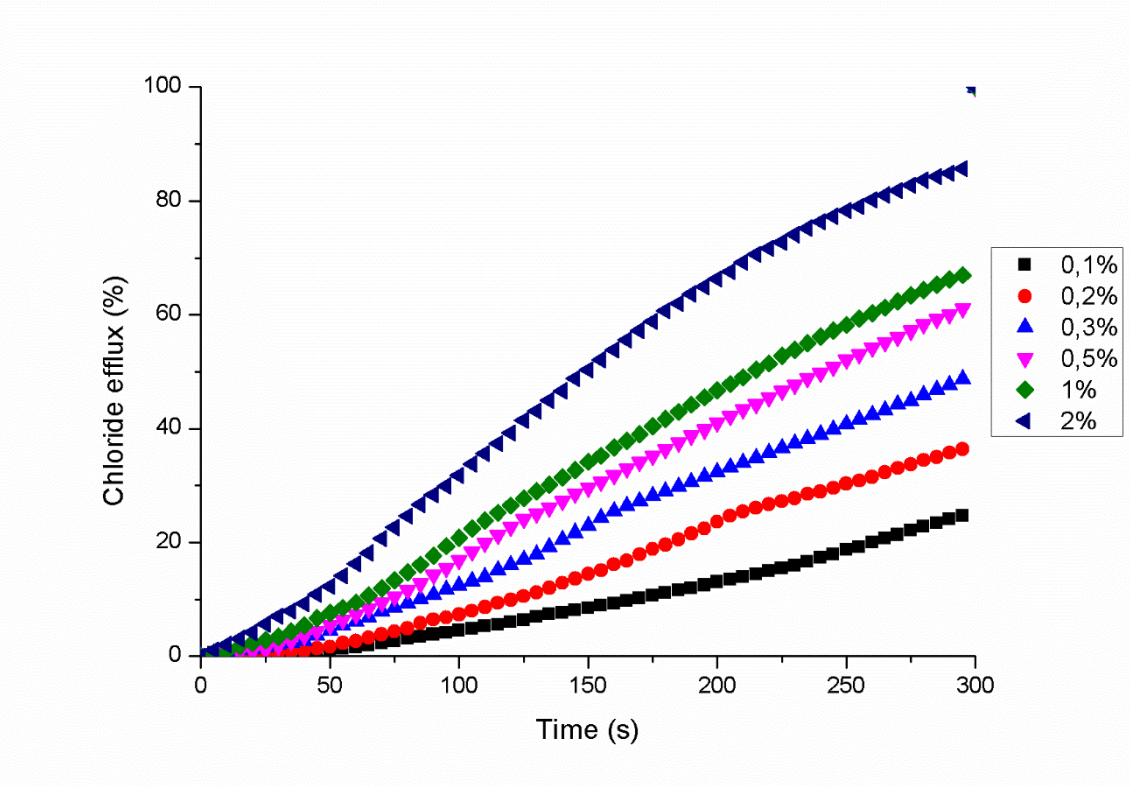


Figure S54. Chloride efflux promoted by **5AT** at different concentrations (2% - 0.1% carrier to POPC molar ratio, 10 - 0.5 µM) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

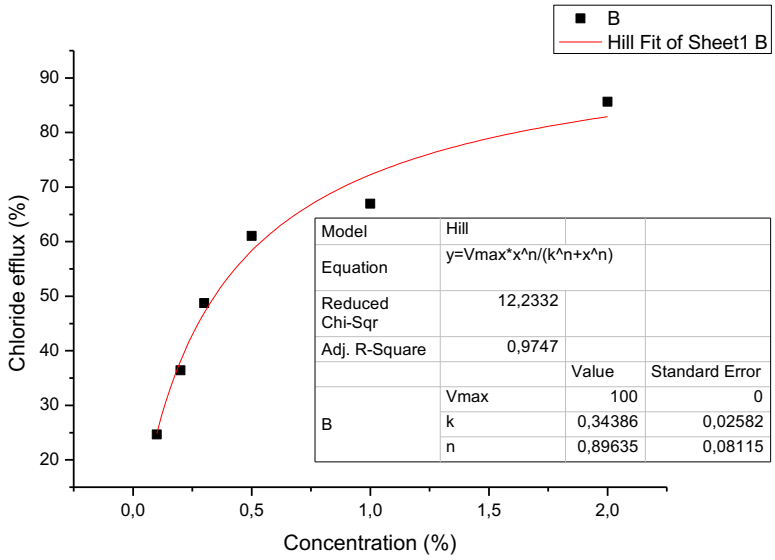


Figure S55. Normalised chloride efflux at 300 s plotted against the concentration of compound **5AT**. Data have been plotted with Hill equation (continuous line).

4.5 Study of the Cl^-/SO_4^{2-} exchange

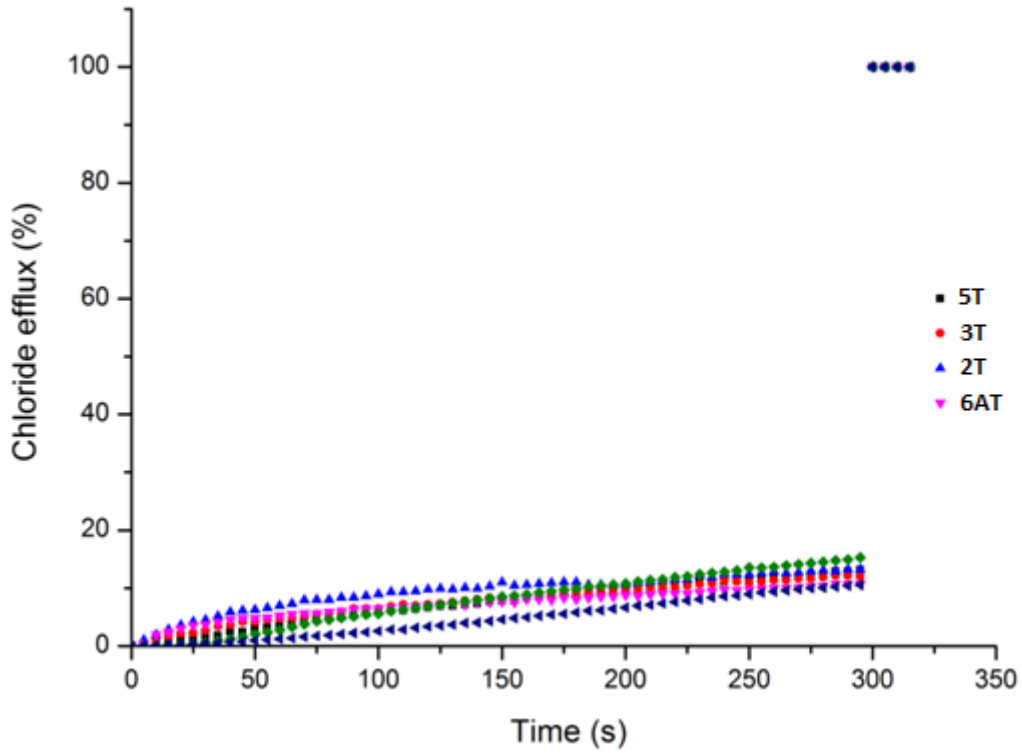


Figure S56. Chloride efflux promoted by 5T, 3T, 2T and 6AT (10 µM , 2 % mol carrier to lipid concentration) in unilamellar POPC vesicles. Vesicles loaded with 451 mM NaCl buffered at pH 7.2 with 20 mM phosphate dispersed in 150 mM Na_2SO_4 buffered at pH 7.2. Each trace represents the average of at least three trials

Table S1. Transport activities expressed as EC_{50} (nM) and Hill parameter for compounds (**1A**-**6AT**).

Compound	EC_{50} (nM) NO_3^-/Cl^-	Hill parameter, *n* NO_3^-/Cl^-	EC_{50} (nM) HCO_3^-/Cl^-	Hill parameter, *n* HCO_3^-/Cl^-	Lipophilicity (logP)[a]
1A	-[b]	-	-[b]	-	5.74
1T	-[b]	-	-[b]	-	6.25
2A	-[b]	-	-[b]	-	3.98
2T	297	0.93	4290	0.74	5.13
3A	-[b]	-	-[b]	-	4.41
3T	107	1.14	2910	0.59	5.67
4A	184[c]	0.725[c]	-[b]	-	4.58
4T	66	1.37	385	0.92	5.86
5A	-[b]	-	-[b]	-	5.08
5AT	61	0.99	1719	0.90	5.98
5T	93	1.35	465	0.67	6.44
6AT	353	0.88	-[b]	-	5.48

[a] Determined using ALOGPS 2.1 software.[8] [b] The compound was not active enough to calculate EC_{50} value. [c] Values from reference[9]

5 X-ray measurements

5.1 General procedure for crystallizations

X-ray quality single crystals of **3T**×TBACl were grown by slow diffusion of pentane into a solution of **3T** and TBACl (slight excess) in 1,2-dichloroethane.

5.2 X-ray structure

Table S2. Crystal data and structure refinement for **3T**×TBACl

Identification code	**3T**×TBACl
Empirical formula	$C_{36}H_{57}Cl_3N_4S_2$
Formula weight	716.32
Temperature/K	100(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	12.06169(12)
b/Å	15.09891(15)
c/Å	21.2481(2)
α/°	90
β/°	99.4856(10)
γ/°	90
Volume/Å^3	3816.77(7)
Z	4
ρ_{calc}g/cm^3	1.247
μ/mm^{-1}	3.419
F(000)	1536.0
Crystal size/mm^3	0.36 × 0.23 × 0.08
Radiation	Cu$K\alpha$ (λ = 1.54184)
2Θ range for data collection/°	7.216 to 134.156
Index ranges	$-14 \le h \le 14$, $-18 \le k \le 18$, $-25 \le l \le 25$
Reflections collected	51923
Independent reflections	6805 [R_{int} = 0.0359, R_{sigma} = 0.0173]
Data/restraints/parameters	6805/3/421
Goodness-of-fit on F^2	1.063
Final R indexes [I>=2σ (I)]	R_1 = 0.0419, wR_2 = 0.1179
Final R indexes [all data]	R_1 = 0.0457, wR_2 = 0.1228
Largest diff. peak/hole / e Å^{-3}	1.39/-0.57

Table S3. Bond lengths for **3T**×TBACl

Atom	**Atom**	**Length/Å**	**Atom**	**Atom**	**Length/Å**
C(1)	C(2)	1.404(3)	C(15)	C(16)	1.519(4)
C(1)	C(6)	1.411(3)	C(17)	C(18)	1.521(3)
C(1)	N(1)	1.374(3)	C(17)	N(3)	1.345(3)
C(2)	C(3)	1.385(3)	C(17)	S(2)	1.648(2)
C(2)	N(2)	1.420(3)	C(18)	C(19)	1.518(3)
C(3)	C(4)	1.403(3)	C(19)	C(20)	1.526(4)
C(4)	C(5)	1.384(3)	C(21)	C(22)	1.522(3)
C(4)	Cl(1)	1.7472(19)	C(21)	N(33)	1.520(2)
C(5)	C(6)	1.400(3)	C(22)	C(23)	1.532(3)
C(6)	C(7)	1.452(3)	C(23)	C(24)	1.522(3)
C(7)	C(8)	1.391(3)	C(25)	C(26)	1.521(3)
C(7)	C(12)	1.410(3)	C(25)	N(33)	1.520(3)

C(8)	C(9)	1.388(3)	C(26)	C(27)	1.548(3)
C(9)	C(10)	1.400(3)	C(27)	C(28)	1.496(5)
C(9)	Cl(2)	1.746(2)	C(29)	C(30)	1.526(3)
C(10)	C(11)	1.385(3)	C(29)	N(33)	1.522(2)
C(11)	C(12)	1.399(3)	C(30)	C(31)	1.535(3)
C(11)	N(3)	1.421(3)	C(31)	C(32)	1.513(4)
C(12)	N(1)	1.379(3)	C(33)	C(34)	1.521(3)
C(13)	C(14)	1.522(3)	C(33)	N(33)	1.525(2)
C(13)	N(2)	1.346(3)	C(34)	C(35)	1.527(3)
C(13)	S(1)	1.656(2)	C(35)	C(36)	1.525(3)
C(14)	C(15)	1.521(3)			

Table S4. Values of valence angles for **3T**×TBACl

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C(2)	C(1)	C(6)	121.86(18)	N(2)	C(13)	S(1)	124.83(16)
N(1)	C(1)	C(2)	127.65(18)	C(15)	C(14)	C(13)	114.7(2)
N(1)	C(1)	C(6)	110.48(17)	C(16)	C(15)	C(14)	112.5(2)
C(1)	C(2)	N(2)	117.97(17)	C(18)	C(17)	S(2)	124.58(16)
C(3)	C(2)	C(1)	118.09(18)	N(3)	C(17)	C(18)	111.36(18)
C(3)	C(2)	N(2)	123.90(18)	N(3)	C(17)	S(2)	124.03(17)
C(2)	C(3)	C(4)	119.23(18)	C(19)	C(18)	C(17)	118.73(19)
C(3)	C(4)	Cl(1)	117.34(15)	C(18)	C(19)	C(20)	114.5(2)
C(5)	C(4)	C(3)	123.95(18)	C(1)	N(1)	C(12)	107.57(17)
C(5)	C(4)	Cl(1)	118.68(15)	C(13)	N(2)	C(2)	127.71(17)
C(4)	C(5)	C(6)	116.79(18)	C(17)	N(3)	C(11)	129.68(18)
C(1)	C(6)	C(7)	105.75(17)	N(33)	C(21)	C(22)	114.34(16)
C(5)	C(6)	C(1)	120.08(18)	C(21)	C(22)	C(23)	111.62(17)
C(5)	C(6)	C(7)	134.17(19)	C(24)	C(23)	C(22)	111.52(19)
C(8)	C(7)	C(6)	134.05(19)	N(33)	C(25)	C(26)	116.14(17)
C(8)	C(7)	C(12)	120.04(18)	C(25)	C(26)	C(27)	109.1(2)
C(12)	C(7)	C(6)	105.90(18)	C(28)	C(27)	C(26)	114.5(3)
C(9)	C(8)	C(7)	116.94(19)	N(33)	C(29)	C(30)	115.63(16)
C(8)	C(9)	C(10)	123.6(2)	C(29)	C(30)	C(31)	109.07(17)
C(8)	C(9)	Cl(2)	118.32(17)	C(32)	C(31)	C(30)	111.7(2)
C(10)	C(9)	Cl(2)	118.03(16)	C(34)	C(33)	N(33)	116.58(16)
C(11)	C(10)	C(9)	119.45(18)	C(33)	C(34)	C(35)	109.01(16)
C(10)	C(11)	C(12)	117.83(19)	C(36)	C(35)	C(34)	111.69(17)
C(10)	C(11)	N(3)	124.79(18)	C(21)	N(33)	C(29)	106.61(14)
C(12)	C(11)	N(3)	117.14(18)	C(21)	N(33)	C(33)	111.28(15)
C(11)	C(12)	C(7)	122.10(19)	C(25)	N(33)	C(21)	111.41(15)
N(1)	C(12)	C(7)	110.26(17)	C(25)	N(33)	C(29)	111.54(15)
N(1)	C(12)	C(11)	127.64(19)	C(25)	N(33)	C(33)	105.77(15)
C(14)	C(13)	S(1)	121.83(16)	C(29)	N(33)	C(33)	110.30(15)
N(2)	C(13)	C(14)	113.34(18)				

Table S5. Values of torsion angles for **3T**×TBACl

A	B	C	D	Angle/˚	A	B	C	D	Angle/˚
C(1)	C(2)	C(3)	C(4)	0.7(3)	C(17)	C(18)	C(19)	C(20)	76.3(3)
C(1)	C(2)	N(2)	C(13)	-136.6(2)	C(18)	C(17)	N(3)	C(11)	174.52(19)
C(1)	C(6)	C(7)	C(8)	-178.7(2)	Cl(1)	C(4)	C(5)	C(6)	178.21(14)
C(1)	C(6)	C(7)	C(12)	0.1(2)	Cl(2)	C(9)	C(10)	C(11)	178.94(15)
C(2)	C(1)	C(6)	C(5)	0.3(3)	N(1)	C(1)	C(2)	C(3)	-179.27(18)
C(2)	C(1)	C(6)	C(7)	179.72(17)	N(1)	C(1)	C(2)	N(2)	2.9(3)
C(2)	C(1)	N(1)	C(12)	-178.99(18)	N(1)	C(1)	C(6)	C(5)	179.10(17)

C(2)	C(3)	C(4)	C(5)	-0.3(3)	N(1)	C(1)	C(6)	C(7)	-1.4(2)
C(2)	C(3)	C(4)	Cl(1)	-178.62(15)	N(2)	C(2)	C(3)	C(4)	178.33(18)
C(3)	C(2)	N(2)	C(13)	45.7(3)	N(2)	C(13)	C(14)	C(15)	-135.8(2)
C(3)	C(4)	C(5)	C(6)	-0.1(3)	N(3)	C(11)	C(12)	C(7)	174.19(17)
C(4)	C(5)	C(6)	C(1)	0.1(3)	N(3)	C(11)	C(12)	N(1)	-5.8(3)
C(4)	C(5)	C(6)	C(7)	-179.2(2)	N(3)	C(17)	C(18)	C(19)	173.28(19)
C(5)	C(6)	C(7)	C(8)	0.6(4)	S(1)	C(13)	C(14)	C(15)	44.6(3)
C(5)	C(6)	C(7)	C(12)	179.4(2)	S(1)	C(13)	N(2)	C(2)	2.4(3)
C(6)	C(1)	C(2)	C(3)	-0.6(3)	S(2)	C(17)	C(18)	C(19)	-4.7(3)
C(6)	C(1)	C(2)	N(2)	-178.44(17)	S(2)	C(17)	N(3)	C(11)	-7.5(3)
C(6)	C(1)	N(1)	C(12)	2.2(2)	C(21)	C(22)	C(23)	C(24)	177.08(19)
C(6)	C(7)	C(8)	C(9)	179.0(2)	C(22)	C(21)	N(33)	C(25)	56.4(2)
C(6)	C(7)	C(12)	C(11)	-178.69(17)	C(22)	C(21)	N(33)	C(29)	178.36(17)
C(6)	C(7)	C(12)	N(1)	1.3(2)	C(22)	C(21)	N(33)	C(33)	-61.3(2)
C(7)	C(8)	C(9)	C(10)	-0.8(3)	C(25)	C(26)	C(27)	C(28)	-64.7(3)
C(7)	C(8)	C(9)	Cl(2)	-179.13(15)	C(26)	C(25)	N(33)	C(21)	59.7(2)
C(7)	C(12)	N(1)	C(1)	-2.2(2)	C(26)	C(25)	N(33)	C(29)	-59.3(2)
C(8)	C(7)	C(12)	C(11)	0.3(3)	C(26)	C(25)	N(33)	C(33)	-179.27(18)
C(8)	C(7)	C(12)	N(1)	-179.71(17)	C(29)	C(30)	C(31)	C(32)	82.5(2)
C(8)	C(9)	C(10)	C(11)	0.6(3)	C(30)	C(29)	N(33)	C(21)	-167.31(17)
C(9)	C(10)	C(11)	C(12)	0.1(3)	C(30)	C(29)	N(33)	C(25)	-45.5(2)
C(9)	C(10)	C(11)	N(3)	-174.18(19)	C(30)	C(29)	N(33)	C(33)	71.7(2)
C(10)	C(11)	C(12)	C(7)	-0.5(3)	C(33)	C(34)	C(35)	C(36)	-174.22(17)
C(10)	C(11)	C(12)	N(1)	179.51(18)	C(34)	C(33)	N(33)	C(21)	-46.0(2)
C(10)	C(11)	N(3)	C(17)	-44.3(3)	C(34)	C(33)	N(33)	C(25)	-167.18(17)
C(11)	C(12)	N(1)	C(1)	177.79(19)	C(34)	C(33)	N(33)	C(29)	72.1(2)
C(12)	C(7)	C(8)	C(9)	0.3(3)	N(33)	C(21)	C(22)	C(23)	157.61(18)
C(12)	C(11)	N(3)	C(17)	141.4(2)	N(33)	C(25)	C(26)	C(27)	175.3(2)
C(13)	C(14)	C(15)	C(16)	61.1(3)	N(33)	C(29)	C(30)	C(31)	-167.80(17)
C(14)	C(13)	N(2)	C(2)	-177.23(19)	N(33)	C(33)	C(34)	C(35)	-179.48(16)

6 Literature references

[1] CrysAlis CCD and CrysAlis RED, Oxford Diffraction, Oxford Diffraction Ltd: Yarnton, 2008

[2] R. C. Clark, J. S. Reid, *Acta Cryst. Sect. A* **1995**, *51*, 887–897

[3] CrysAlis CCD and CrysAlis RED, Oxford Diffraction, Oxford Diffraction Ltd: Yarnton, 2008.

[4] G. M. Sheldrick, *Acta Cryst. Sect. A* **2008**, *64*, 112–122.

[5] G. M. Sheldrick, *Acta Cryst. Sect. A* **2008**, *64*, 112–122.

[6] http://supramolecular.org

[7] a) P. Thordarson, *Chem. Soc. Rev.*, **2011**, *40*, 1305-1323; b) D. B. Hibbert, P. Thordarson, *Chem. Commun.*, **2016**, *52*, 12792-12805.

[8] Igor V. Tetko, Vsevolod Y. Tanchuk, *J. Chem. Inf. Comput. Sci.*, **2002**, *42*, 1136-1145.

[9] K. M. Bąk, K. Chabuda, H. Montes, R. Quesada and M. J. Chmielewski, *Org. Biomol. Chem.*, **2018**, *16*, 5188-5196.