Electronic Supplementary Information (ESI)

Small-molecule anion transporters display *in vitro* antimicrobial activity against clinically relevant bacterial strains

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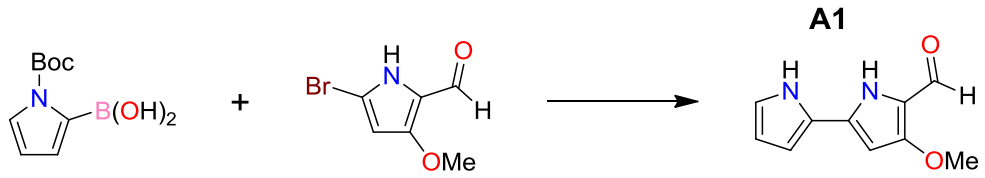
1. SYNTHESES AND CHARACTERISATION DATA

1.1. General procedures and methods

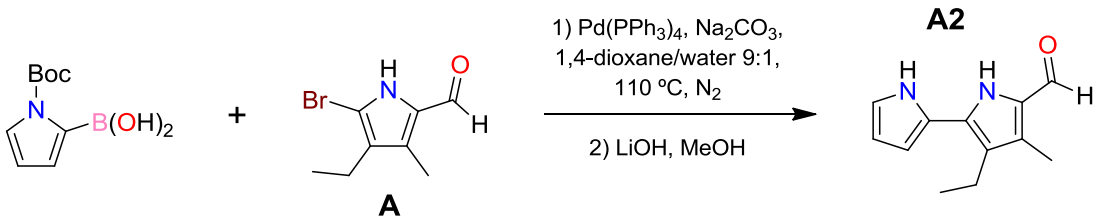
Commercial reagents were employed as received without any further purification. X-ray diffraction studies were carried out at 100 K on a Bruker Smart APEX CCD diffractometer. NMR spectra were recorded at 298 K on Varian Mercury-300 MHz and Varian Unity Inova-400 MHz, employing $CDCl_3$ or DMSO-d_6 as solvents, with their residual signals being used to reference the spectra. High-resolution mass spectra were performed on an Agilent 6545 Q-TOF mass spectrometer coupled to a 1260 Infinity liquid chromatographer from the same brand; the ionisation source employed was electrospray in its positive mode. ^{1}H NMR titrations were carried out in DMSO-d_6 solutions at 293 K and the resulting data were fitted with the Bindfit software.[1]

Synthesis and characterisation of compound A1



Precursor 5-bromo-3-methoxy-1*H*-pyrrole-2-carbaldehyde and compound **A1** were synthesised according to reported procedures.[2] The characterisation of both compounds can be found there.

Synthesis and characterisation of compound A2

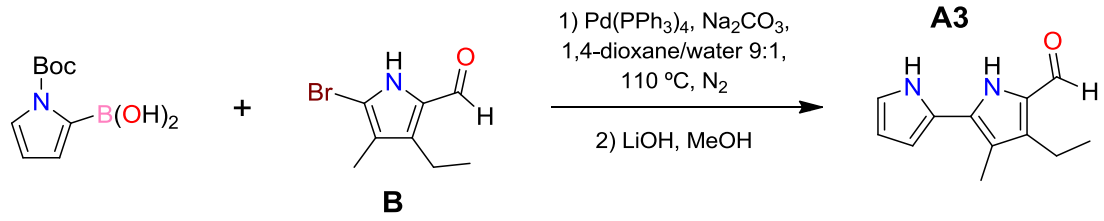


Compound **A** (1.78 g, 8.24 mmol), prepared according to the procedure that can be found in the literature,[3] and (1-(*tert*-butoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (2.64 g, 12.5 mmol, 1.52 equiv.) were dissolved in a 1,4-dioxane/water (9:1, *v*/*v*) mixture (50 mL), and nitrogen was passed through the resulting solution for 1 h. A 100 mL Schlenk flask was charged with a mixture of $Pd(PPh_3)_4$ (0.476 g, 0.412 mmol, 0.05 equiv.) and Na_2CO_3 (1.75 g, 16.5 mmol,

1 Thordarson, P. *Chem. Soc. Rev.* **2011**, *40*, 1305-1323.
2 Dairi, K.; Tripathy, S.; Attardo, G.; Lavallée, J.-F. *Tetrahedron Lett.* **2006**, *47*, 2605-2606.
3 Kancharla, P.; Reynolds, K. A. *Tetrahedron*, **2013**, *69*, 8375-8385.

2.00 equiv.) and purged with nitrogen for 15 min. The solution of compound **A** and boronic acid was added *via* syringe to the Schlenk flask and the resulting reaction mixture was stirred at 110 °C under a nitrogen atmosphere for 20 h. Upon cooling down to room temperature the mixture was diluted with water (100 mL), neutralised with 36% aqueous HCl solution to pH = 3 and extracted with ethyl acetate (3 × 50 mL). The extracts were combined, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure (and additionally using a Schlenk line to remove solvent residues) to give a sticky dark green oil. The residue was redissolved in tetrahydrofuran (25 mL), a suspension of LiOH (2.5 g, 0.10 mol) in methanol (20 mL) was added in a dropwise manner over a period of 3 min in the presence of nitrogen passing through the flask and the resulting mixture was stirred at room temperature for 1 h. The solvents were evaporated to dryness under reduced pressure, the residue was partially redissolved in ethyl acetate (100 mL) and the obtained suspension was washed with water (2 × 100 mL). The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and the ethyl acetate was evaporated to dryness under reduced pressure. The residue was purified by gravity column chromatography (length = 25 cm, diameter = 3 cm silica gel column filling, EtOAc/*n*-hexane, 0%/100% → 10%/90% → 15%/85% → 20%/80%, *v*/*v*) and the fractions containing compound **A2** were evaporated to dryness. The residue was washed with *n*-hexane (2 × 20 mL) and air-dried to afford compound **A2** as a green powder (1.06 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 11.66, 11.64 (2 overlapped bs, 2H, NH), 9.36 (s, 1H, CHO), 7.01-6.97 (m, 1H), 6.74-6.70 (m, 1H), 6.36-6.31 (m, 1H), 2.70 (q, J = 7.5 Hz, 2H), 2.33 (s, 3H), 1.20 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 174.1 (CHO), 136.0 (C_{Ar}), 134.2 (C_{Ar}), 128.0 (C_{Ar}), 125.0 (C_{Ar}), 123.5 (C_{Ar}), 120.5 (CH_{Ar}), 110.4 (CH_{Ar}), 110.1 (CH_{Ar}), 18.0 (CH_2), 14.0 (CH_3), 8.8 (CH_3). HR-MS (+ESI): found *m*/*z* 203.1175 ($[M+H]^+$), $[C_{12}H_{14}N_2OH]^+$ requires *m*/*z* 203.1179 (monoisotopic mass).

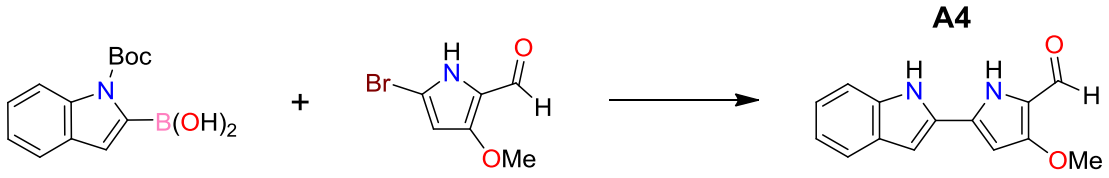
Synthesis and characterisation of compound A3



Compound **B** (1.46 g, 6.76 mmol), prepared according to the procedure that can be found in the literature,[3] and (1-(*tert*-butoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (2.14 g, 10.1 mmol, 1.49 equiv.) were dissolved in a 1,4-dioxane/water (9:1, *v*/*v*) mixture (50 mL), and nitrogen was passed through the resulting solution for 1 h. A 100 mL Schlenk flask was charged

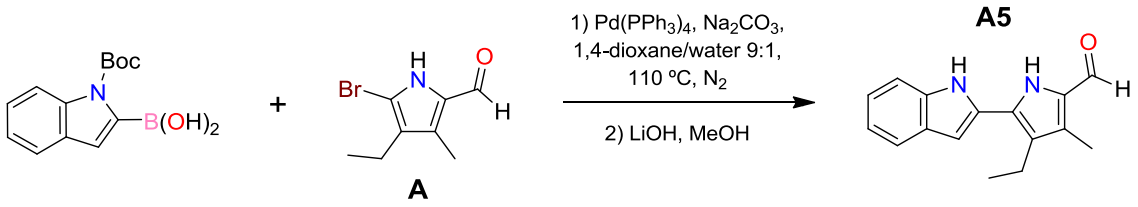
with a mixture of $Pd(PPh_3)_4$ (0.409 g, 0.354 mmol, 0.052 equiv.) and Na_2CO_3 (1.43 g, 13.5 mmol, 2.00 equiv.) and purged with nitrogen for 15 min. The solution of compound **B** and boronic acid was added *via* syringe to the Schlenk flask and the resulting reaction mixture was stirred at 110 °C under a nitrogen atmosphere for 17 h. Upon cooling down to room temperature the mixture was diluted with 1,4-dioxane (≈50 mL) and water (100 mL), neutralised with 36% aqueous HCl solution to pH = 3 and extracted with ethyl acetate (3 × 50 mL). The extracts were combined, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure (and additionally using a Schlenk line to remove solvent residues) to give a dark green solid. A suspension of LiOH (2.76 g, 0.115 mol) in methanol (50 mL) was added in a dropwise manner to a solution of the *N*-Boc derivative of compound **A3** in tetrahydrofuran (25 mL) over a period of 3 min and the resulting mixture was stirred at room temperature for 1 h. The solvents were evaporated to dryness under reduced pressure. Water (150 mL) was added and extraction with ethyl acetate (3 × 100 mL) was carried out. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure (solvent residues were removed using a Schlenk line). The residue was purified by gravity column chromatography (length = 26 cm, diameter = 4 cm silica gel column filling, EtOAc/*n*-hexane, 0%/100% → 5%/95% → 10%/90% → 15%/85% → 20%/80%, *v*/*v*), the fractions containing compound **A3** were evaporated to dryness, the residue was washed with *n*-hexane (2 x 20 mL) and air-dried to afford compound **A3** as green crystals (646 mg, 47%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 11.74 (bs, 2H, NH), 9.35 (s, 1H, CHO), 7.04-6.98 (m, 1H), 6.75-6.70 (m, 1H), 6.37-6.32 (m, 1H), 2.77 (q, J = 7.5 Hz, 2H), 2.23 (s, 3H), 1.24 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 173.8 (CHO), 143.2 (C_{Ar}), 135.0 (C_{Ar}), 127.0 (C_{Ar}), 124.0 (C_{Ar}), 120.6 (CH_{Ar}), 117.6 (C_{Ar}), 110.9 (CH_{Ar}), 110.0 (CH_{Ar}), 17.2 (CH_2), 16.9 (CH_3), 10.6 (CH_3). HR-MS (+ESI): found m/z 203.1176 ($[M+H]^+$), $[C_{12}H_{14}N_2OH]^+$ requires m/z 203.1179 (monoisotopic mass).

Synthesis and characterisation of compound A4



Precursor 5-bromo-3-methoxy-1*H*-pyrrole-2-carbaldehyde and compound **A4** were prepared according to what has been reported in the literature.[2,4] The characterisation of both compounds can be found there.

Synthesis and characterisation of compound A5

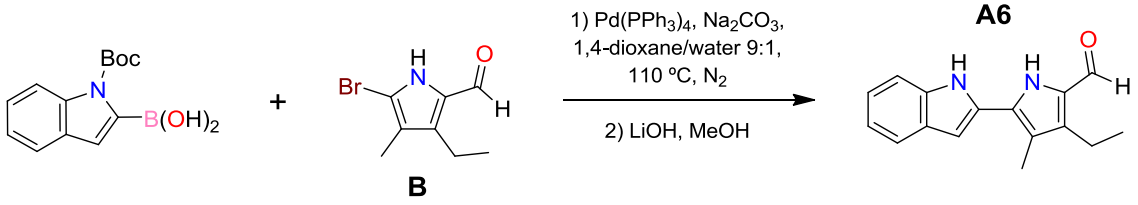


Compound **A** (2.30 g, 10.6 mmol), prepared according to the procedure that can be found in the literature,[3] and (1-(*tert*-butoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (4.16 g, 15.9 mmol, 1.50 equiv.) were dissolved in a 1,4-dioxane/water (9:1, *v*/*v*) mixture (50 mL), and nitrogen was passed through the resulting solution for 1 h. A 100 mL Schlenk flask was charged with a mixture of $Pd(PPh_3)_4$ (0.648 g, 0.561 mmol, 0.053 equiv.) and Na_2CO_3 (2.26 g, 21.3 mmol, 2.01 equiv.) and purged with nitrogen for 15 min. The solution of compound **A** and boronic acid was added *via* syringe to the Schlenk flask and the resulting reaction mixture was stirred at 110 °C under a nitrogen atmosphere for 19 h. Upon cooling down to room temperature the mixture was diluted with water (100 mL), neutralised with 36% aqueous HCl solution to pH = 3 and extracted with ethyl acetate (3 × 50 mL) (during the extraction partial precipitation of the *N*-Boc derivative was observed). The extracts were combined, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure (and additionally using a Schlenk line to remove solvent residues) to give a dark orange solid. A suspension of LiOH (3.0 g, 0.13 mol) in methanol (30 mL) was added in a dropwise manner to a suspension of the raw product in tetrahydrofuran (20 mL) over a period of 30 sec and the resulting mixture was stirred at room temperature for 2 h. The solvents were evaporated to dryness under reduced pressure, water (200 mL) was added to the residue and extraction with ethyl acetate (3 x 100 mL) was carried out. The combined organic extracts (dark orange-coloured) were dried over anhydrous Na_2SO_4, filtered and the solvent removed in a rotary evaporator. Analytically pure compound **A5** (a yellow fluffy powder) was obtained by heating a suspension of the crude product in *n*-hexane (100 mL) to its boiling point, filtration and air-drying. Yield: 848 mg, 32%. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 11.59 (s, 1H, NH), 11.28 (bs, 1H, NH), 9.63 (s, 1H, CHO), 7.61-7.55 (m, 1H), 7.45-7.38 (m, 1H), 7.17-7.10 (m, 1H), 7.06-6.99 (m, 1H), 6.81 (s, 1H),

[4] Daïri, K.; Yao, Y.; Faley, M.; Tripathy, S.; Rioux, E.; Billot, X.; Rabouin, D.; Gonzalez, G.; Lavallée, J.-F.; Attardo, G. *Org. Process Res. Dev.* **2007**, *11*, 1051-1054.

2.70 (q, *J* = 7.5 Hz, 2H), 2.31 (s, 3H), 1.14 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 177.2 (CHO), 136.1 (C_{Ar}), 130.4 (C_{Ar}), 129.2 (C_{Ar}), 128.8 (C_{Ar}), 128.5 (C_{Ar}), 128.3 (C_{Ar}), 125.1 (C_{Ar}), 122.1 (CH_{Ar}), 120.2 (CH_{Ar}), 119.6 (CH_{Ar}), 111.2 (CH_{Ar}), 100.9 (CH_{Ar}), 17.2 (CH_2), 14.3 (CH_3), 8.2 (CH_3). HR-MS (+ESI): found *m/z* 253.1336 ($[M+H]^+$), $[C_{16}H_{17}N_2O]^+$ requires *m/z* 253.1335 (monoisotopic mass).

Synthesis and characterisation of compound A6



Compound **B** (1.03 g, 4.77 mmol), prepared according to the procedure that can be found in the literature,[3] and (1-(*tert*-butoxycarbonyl)-1*H*-pyrrol-2-yl)boronic acid (2.49 g, 9.54 mmol, 2.00 equiv.) were dissolved in a 1,4-dioxane/water (9:1, *v*/*v*) mixture (50 mL), and nitrogen was passed through the resulting solution for 1 h. A 100 mL Schlenk flask was charged with a mixture of $Pd(PPh_3)_4$ (0.556 g, 0.481 mmol, 0.101 equiv.) and Na_2CO_3 (1.01 g, 9.53 mmol, 2.00 equiv.) and purged with nitrogen for 15 min. The solution of compound **B** and boronic acid was added *via* syringe to the Schlenk flask and the resulting reaction mixture was stirred at 110 °C under a nitrogen atmosphere for 21 h. Upon cooling down to room temperature the mixture was diluted with water (100 mL), neutralised with 36% aqueous HCl solution to pH = 3 and extracted with ethyl acetate (3 × 50 mL) (during the extraction partial precipitation of the *N*-Boc derivative was observed). The extracts were combined, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure (and additionally using a Schlenk line to remove solvent residues) to give an orange oil. A suspension of LiOH (2.6 g, 0.11 mol) in methanol (30 mL) was added in a dropwise manner to a suspension of the raw product in tetrahydrofuran (25 mL) over a period of 30 sec and the resulting mixture was stirred at 50 °C for 3 h. The mixture was poured into water (150 mL) and extraction with ethyl acetate (3 × 50 mL) was carried out. The combined organic extracts were evaporated to dryness under reduced pressure, the residue was partially redissolved in a mixture of *n*-hexane (50 mL) and ethyl acetate (25 mL), and the resulting suspension was heated to its boiling point. Analytically pure compound **A6** (two batches of the product) was filtered out (filtration of the hot mixture) and air-dried. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 11.85 (bs, 1H, NH), 11.52 (bs, 1H, NH), 9.63 (s, 1H, CHO), 7.61-7.53 (m, 1H), 7.46-7.38 (m, 1H), 7.18-7.08 (m, 1H), 7.08-6.98 (m, 1H), 6.81 (s, 1H), 2.76 (q, *J* = 7.5 Hz, 2H), 2.23 (s, 3H), 1.15 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz,

DMSO-d_6): δ (ppm) = 177.2 (CHO), 137.4 (C_{Ar}), 136.3 (C_{Ar}), 129.8 (C_{Ar}), 129.5 (C_{Ar}), 128.4 (C_{Ar}), 128.2 (C_{Ar}), 122.2 (CH_{Ar}), 120.3 (CH_{Ar}), 119.7 (CH_{Ar}), 117.8 (C_{Ar}), 111.4 (CH_{Ar}), 101.5 (CH_{Ar}), 25.2 (CH_2), 16.6 (CH_3), 10.1 (CH_3). HR-MS (+ESI): found *m/z* 253.1334 ($[M+H]^+$), $[C_{16}H_{16}N_2OH]^+$ requires *m/z* 253.1335 (monoisotopic mass).

1.2. Characterisation data

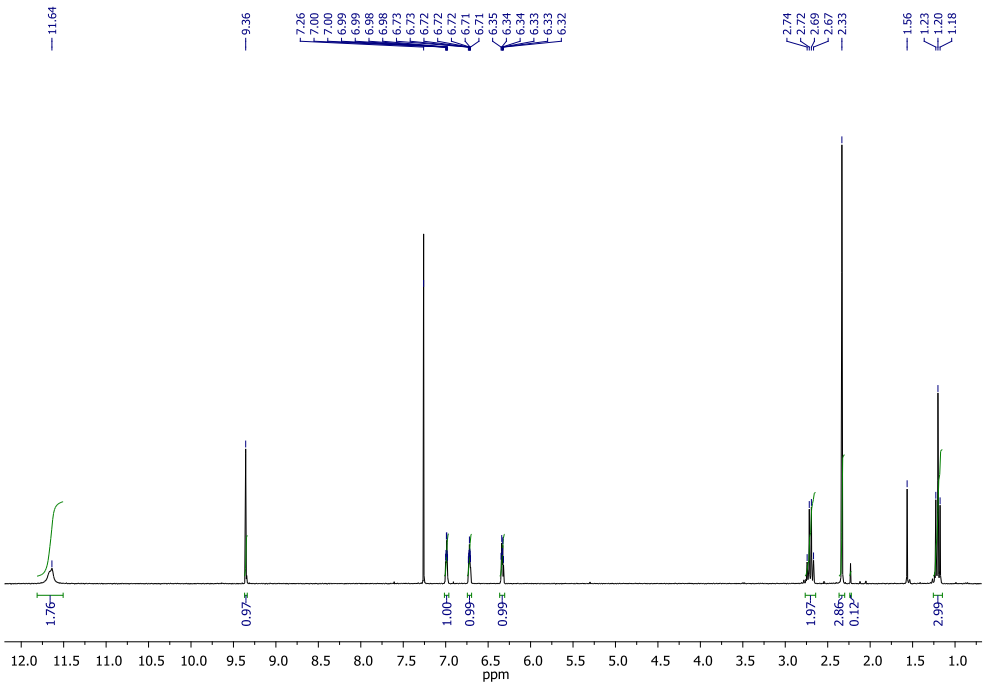


Figure S1. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **A2**.

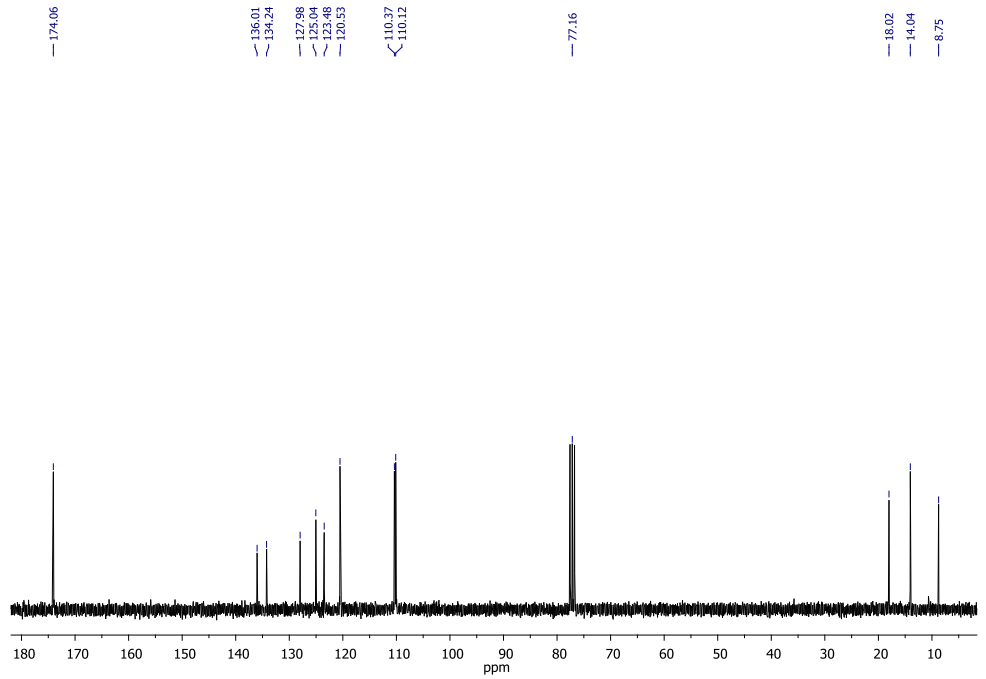


Figure S2. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) for compound **A2**.

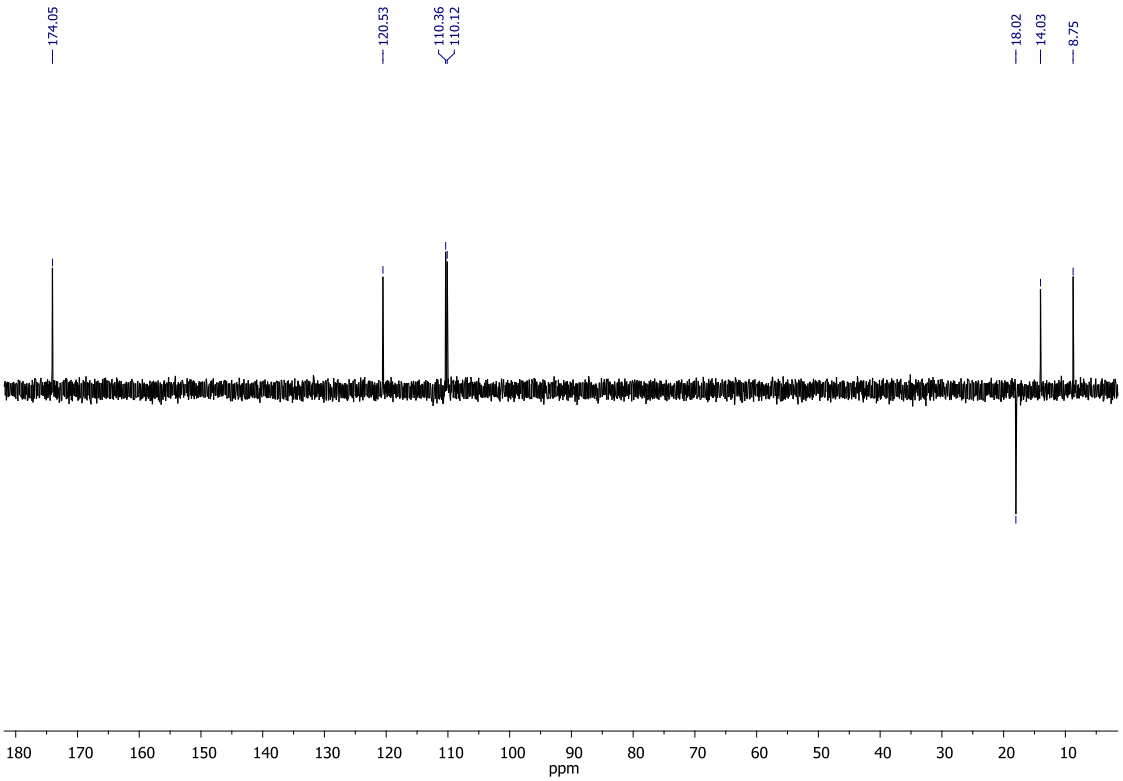


Figure S3. ^{13}C NMR dept135 spectrum (75 MHz, $CDCl_3$) for compound **A2**.

Figure S4. HR-MS (+ESI) spectrum for compound **A2**.

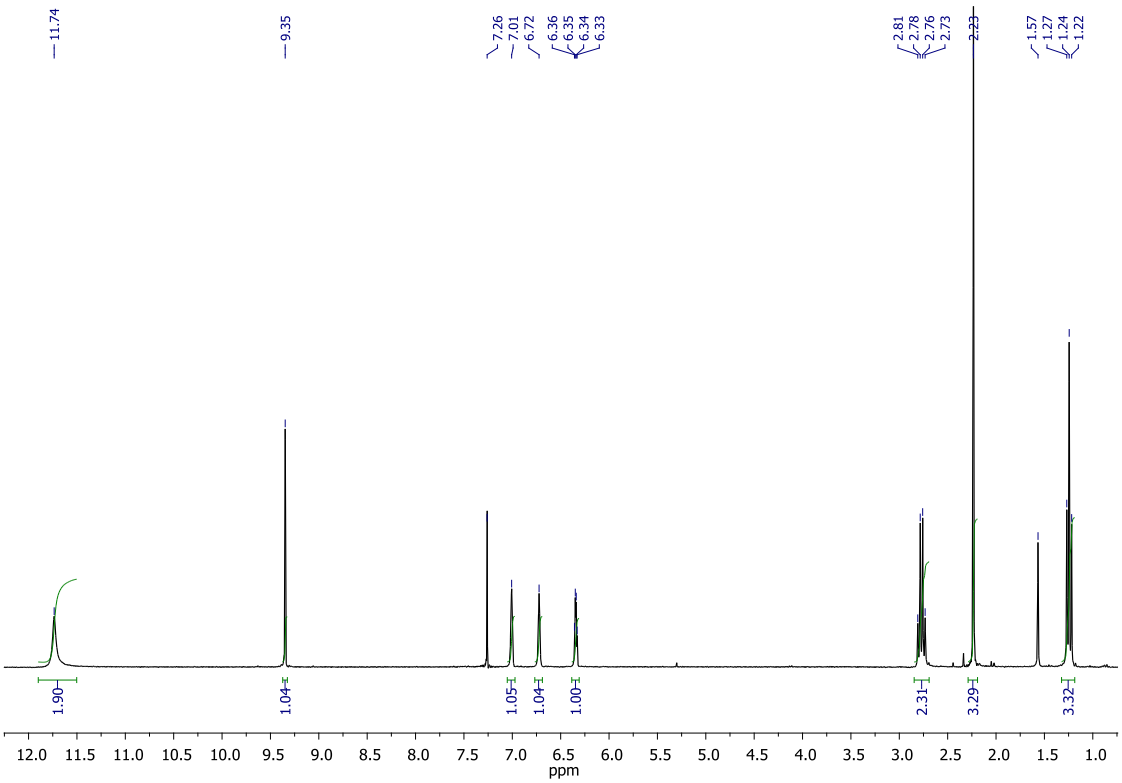


Figure S5. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **A3**.

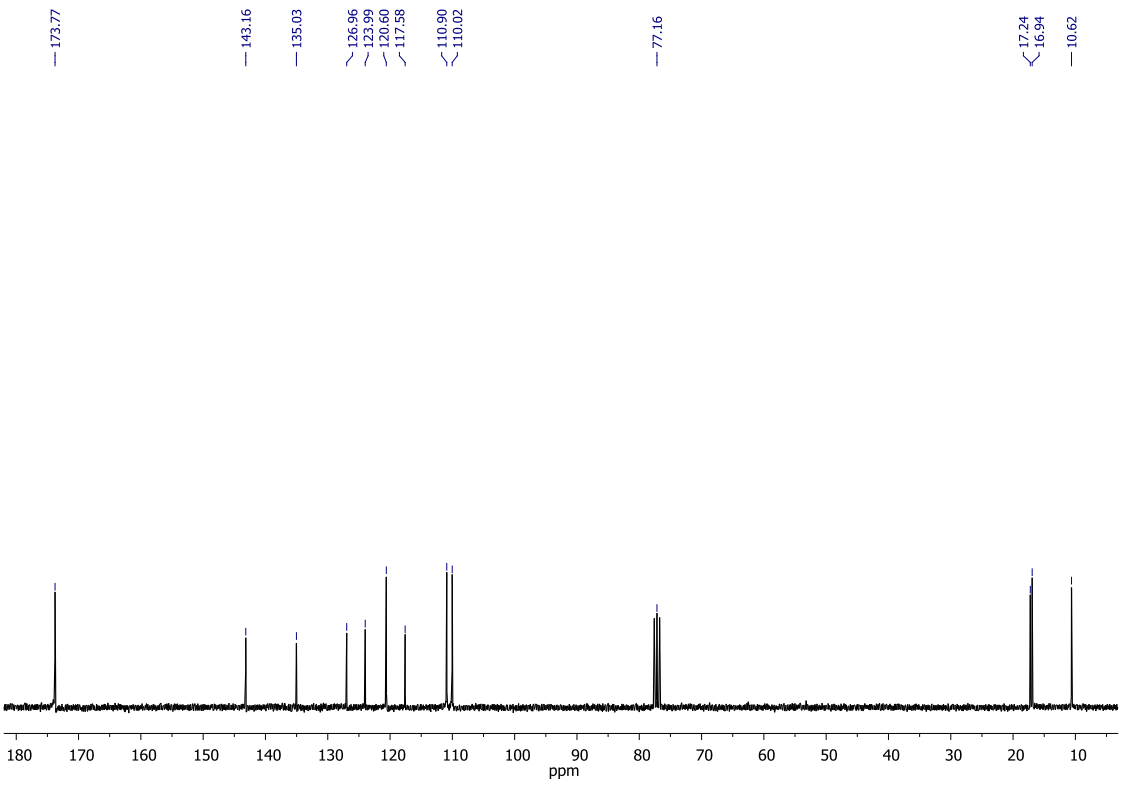


Figure S6. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) for compound **A3**.

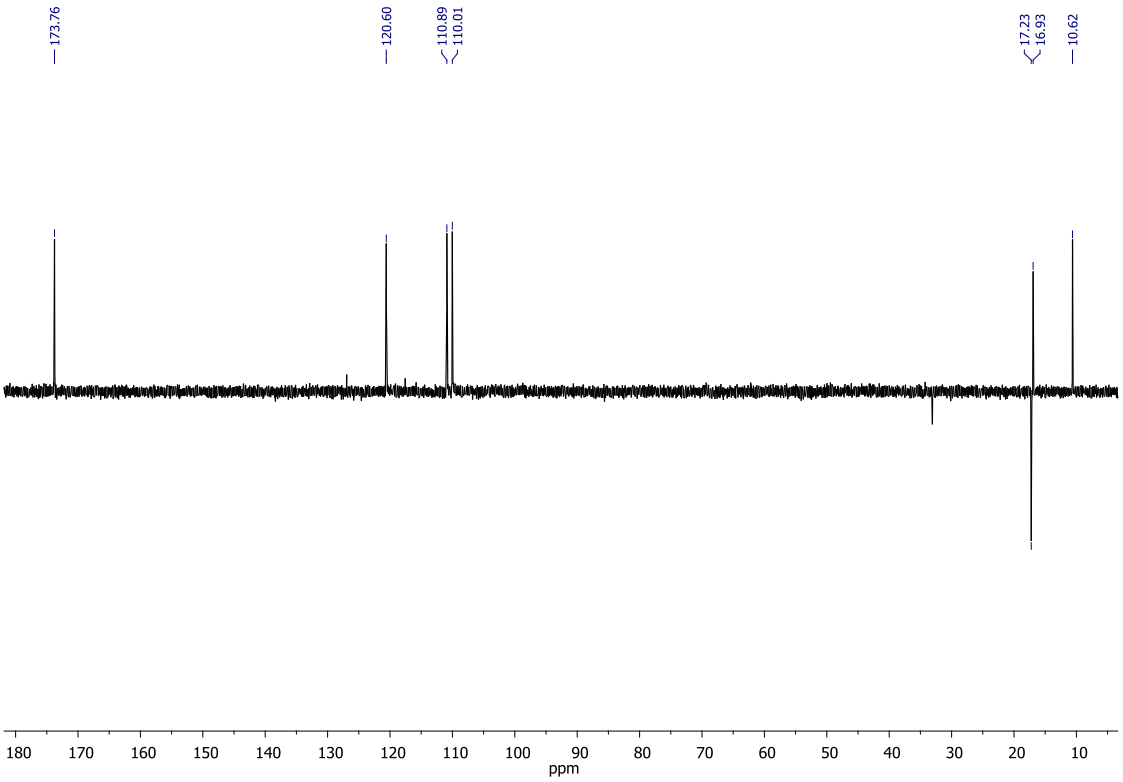


Figure S7. ^{13}C NMR dept135 spectrum (75 MHz, $CDCl_3$) for compound **A3**.

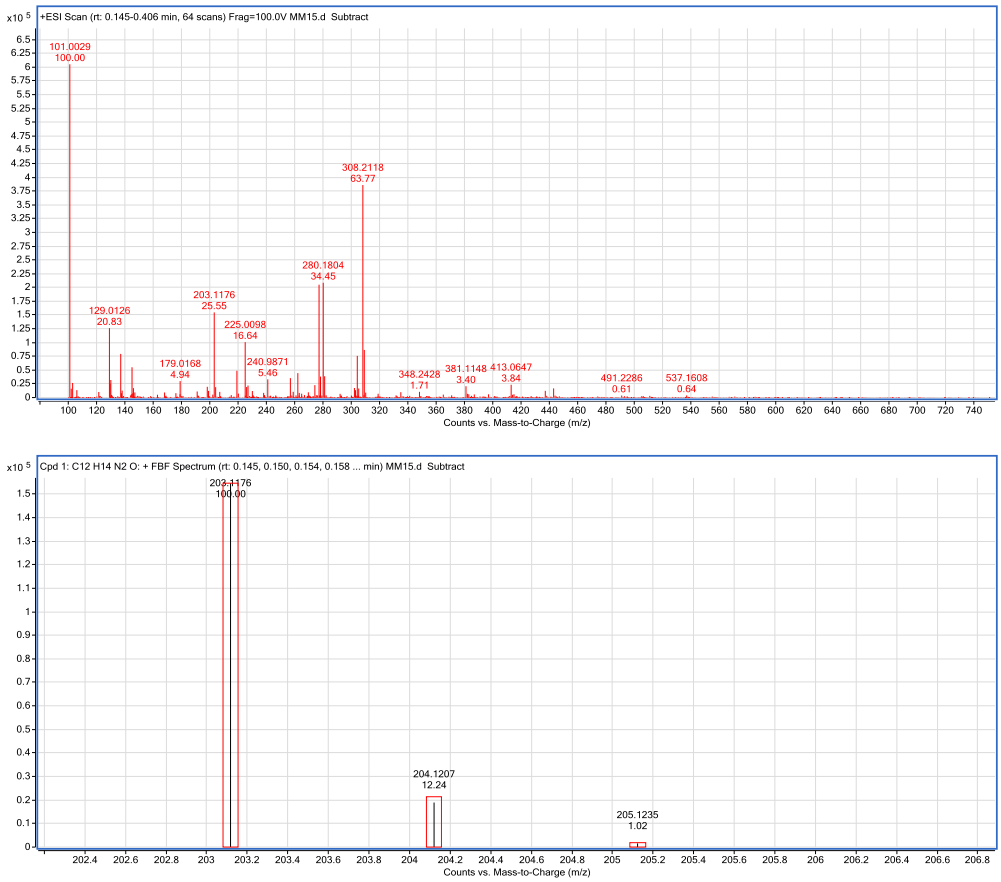


Figure S8. HR-MS (+ESI) spectrum for compound **A3**.

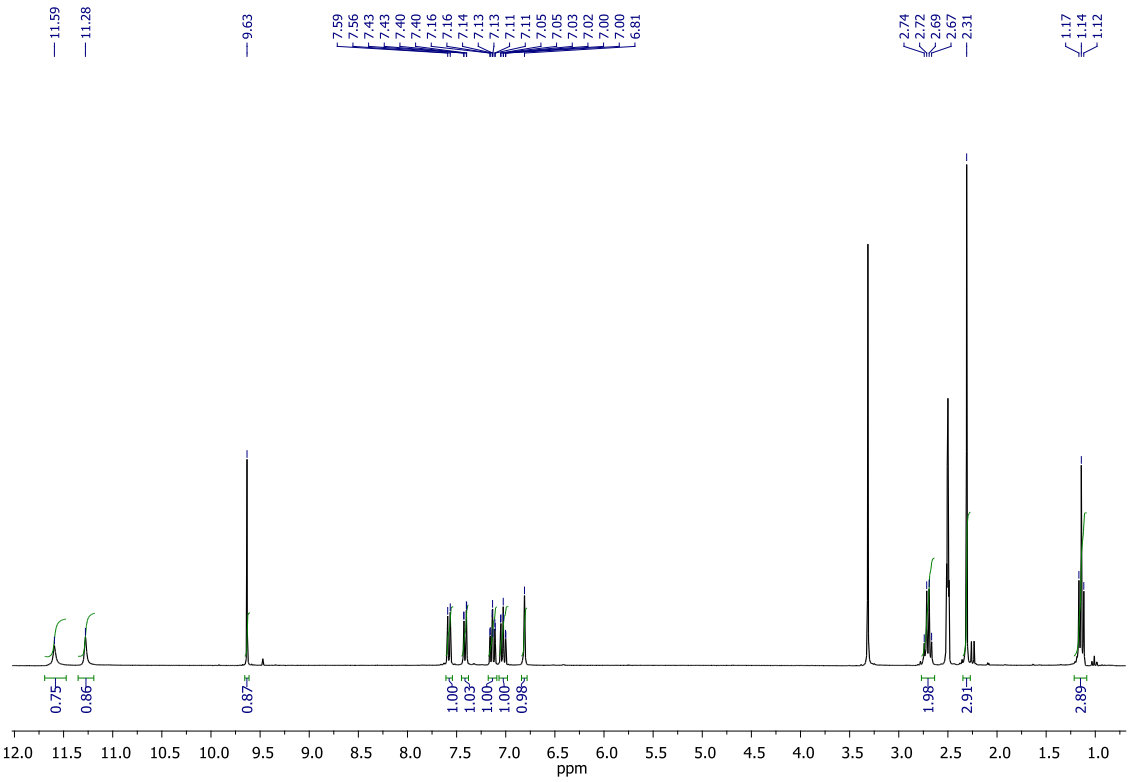


Figure S9. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **A5**.

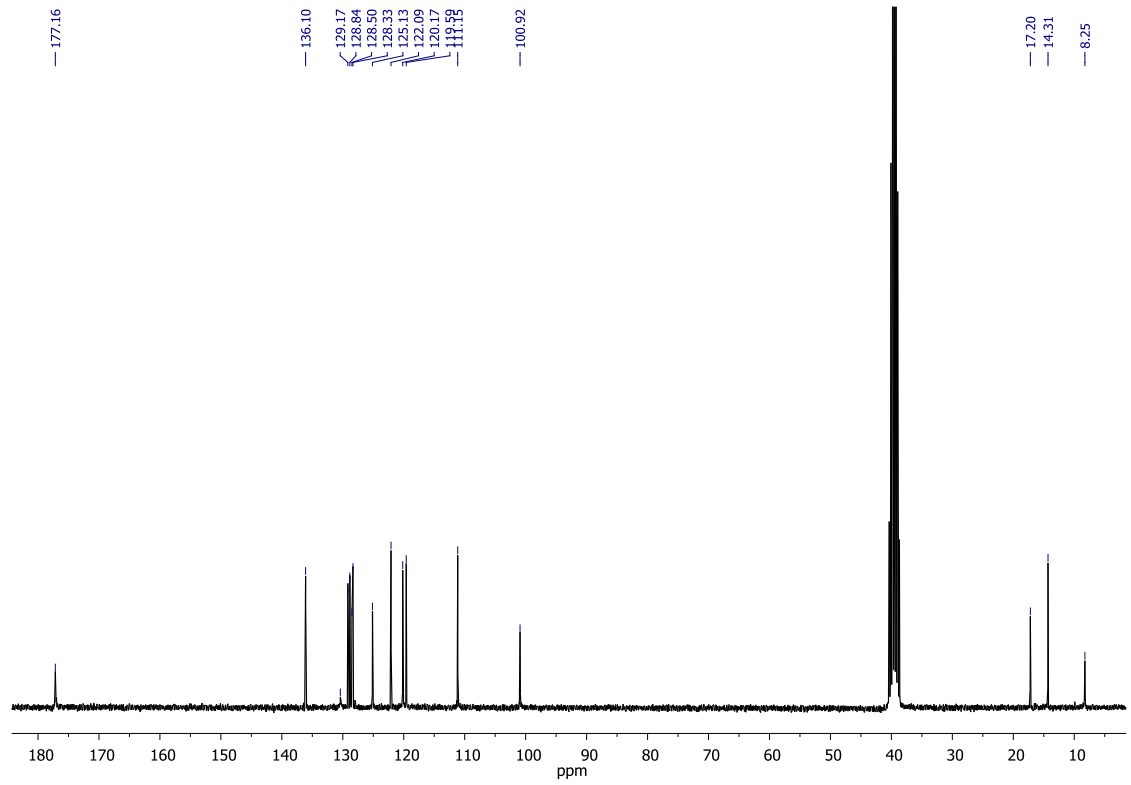


Figure S10. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) for compound **A5**.

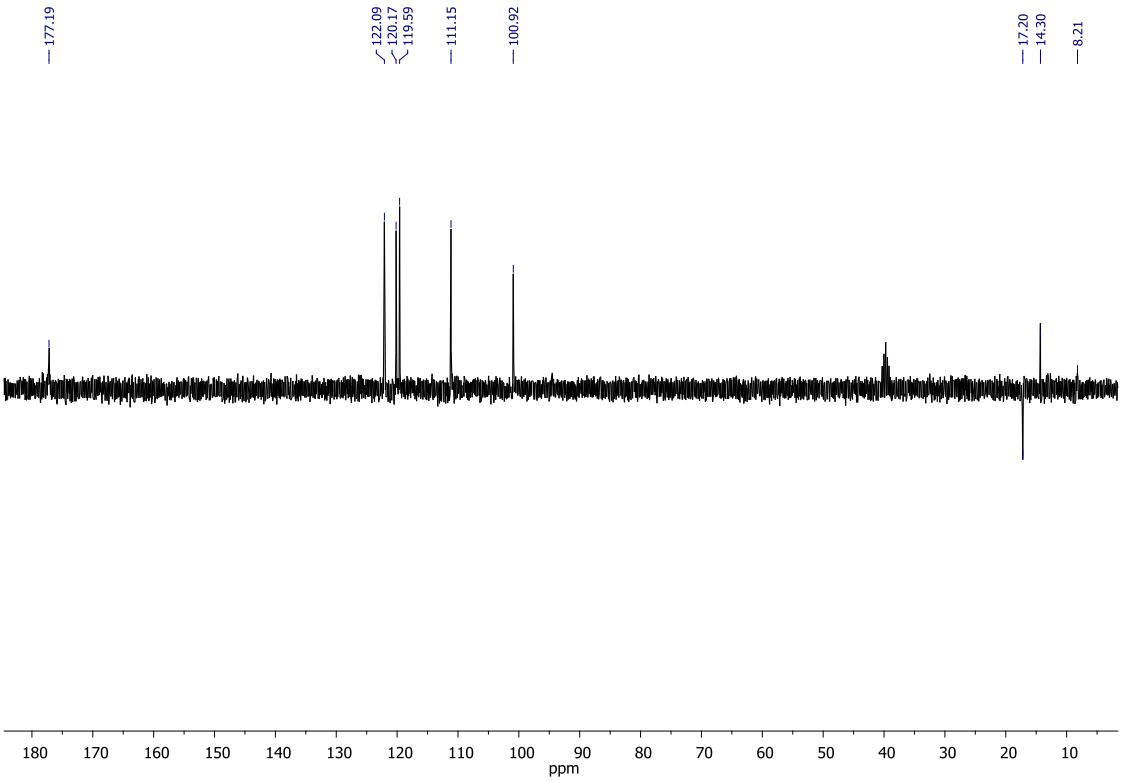


Figure S11. ^{13}C NMR dept135 spectrum (75 MHz, DMSO-d_6) for compound **A5**.

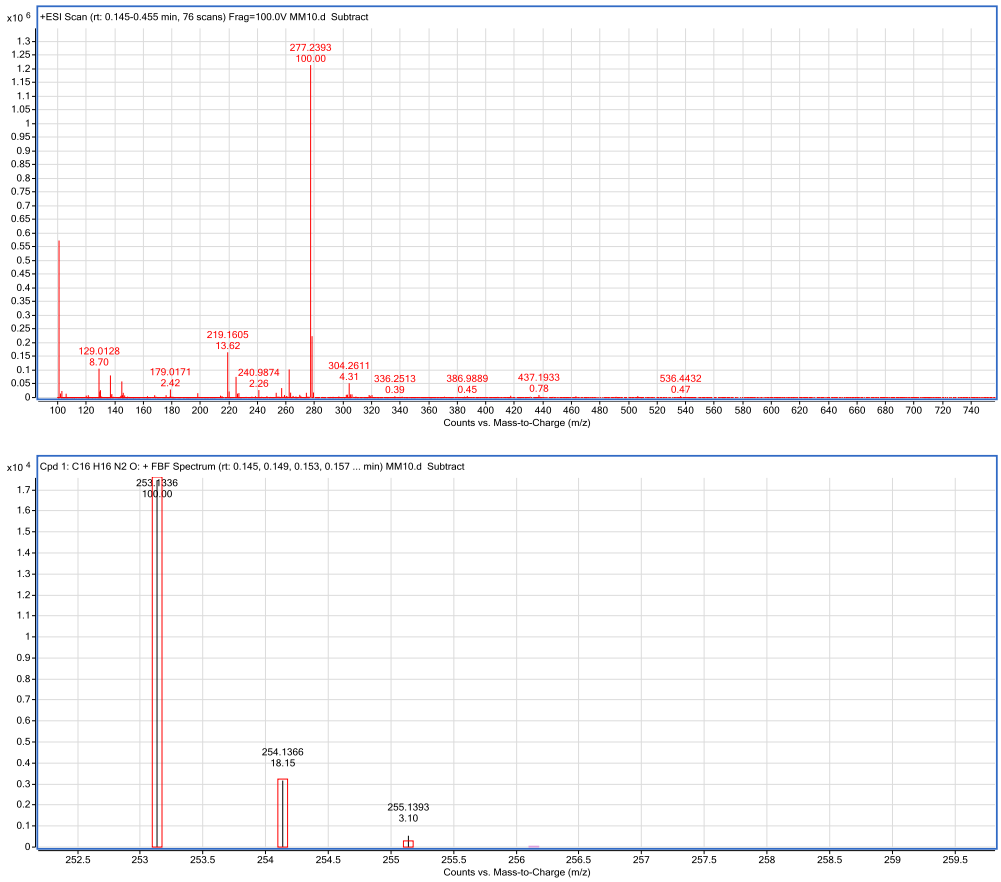


Figure S12. HR-MS (+ESI) spectrum for compound **A5**.

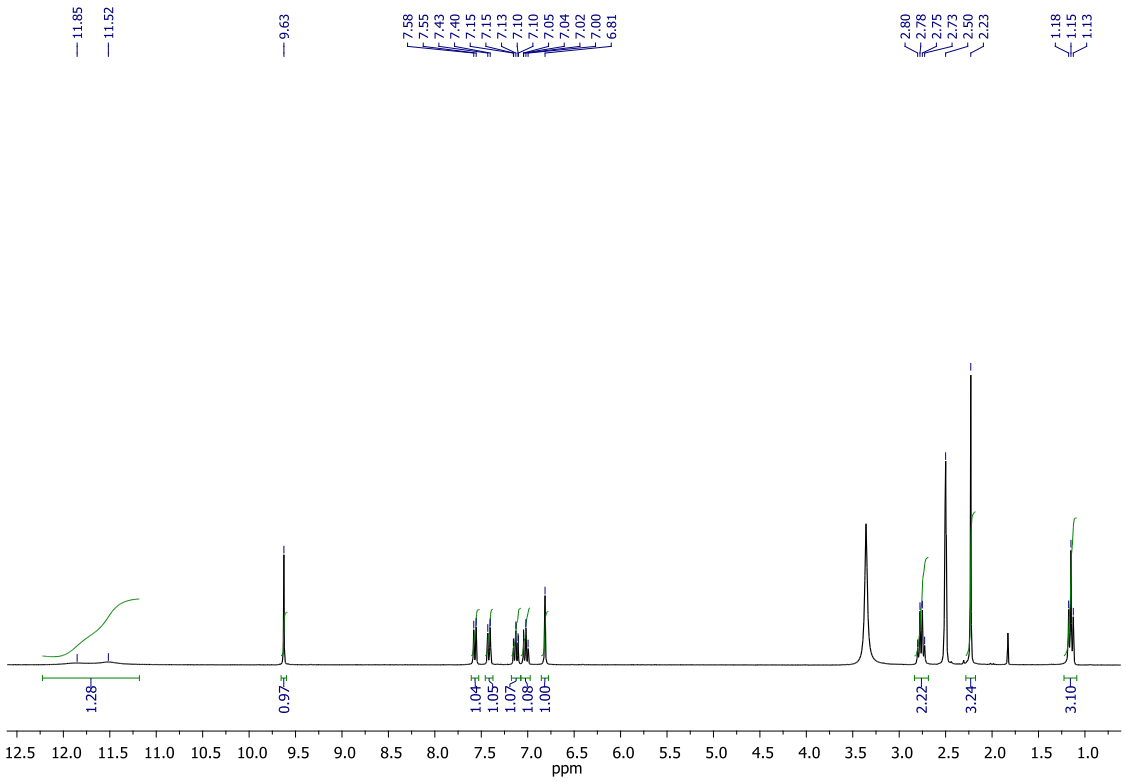


Figure S13. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **A6**.

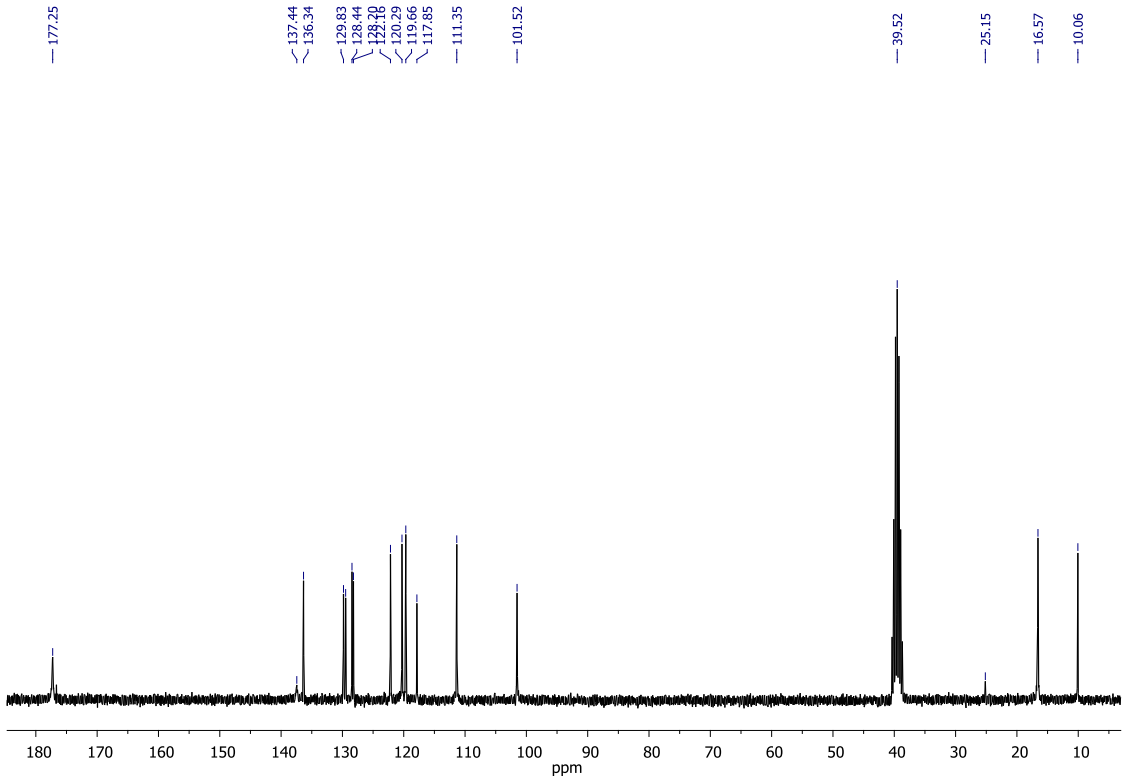


Figure S14. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) for compound **A6**.

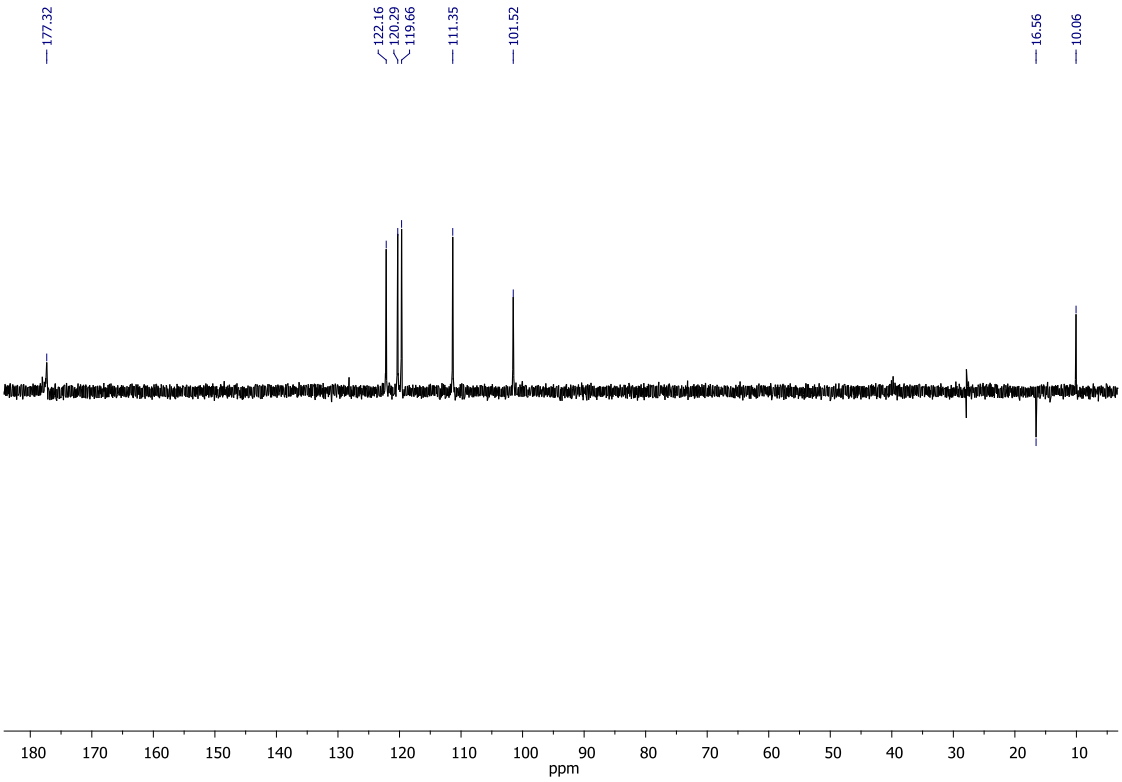


Figure S15. ^{13}C NMR dept135 spectrum (75 MHz, DMSO-d_6) for compound **A6**.

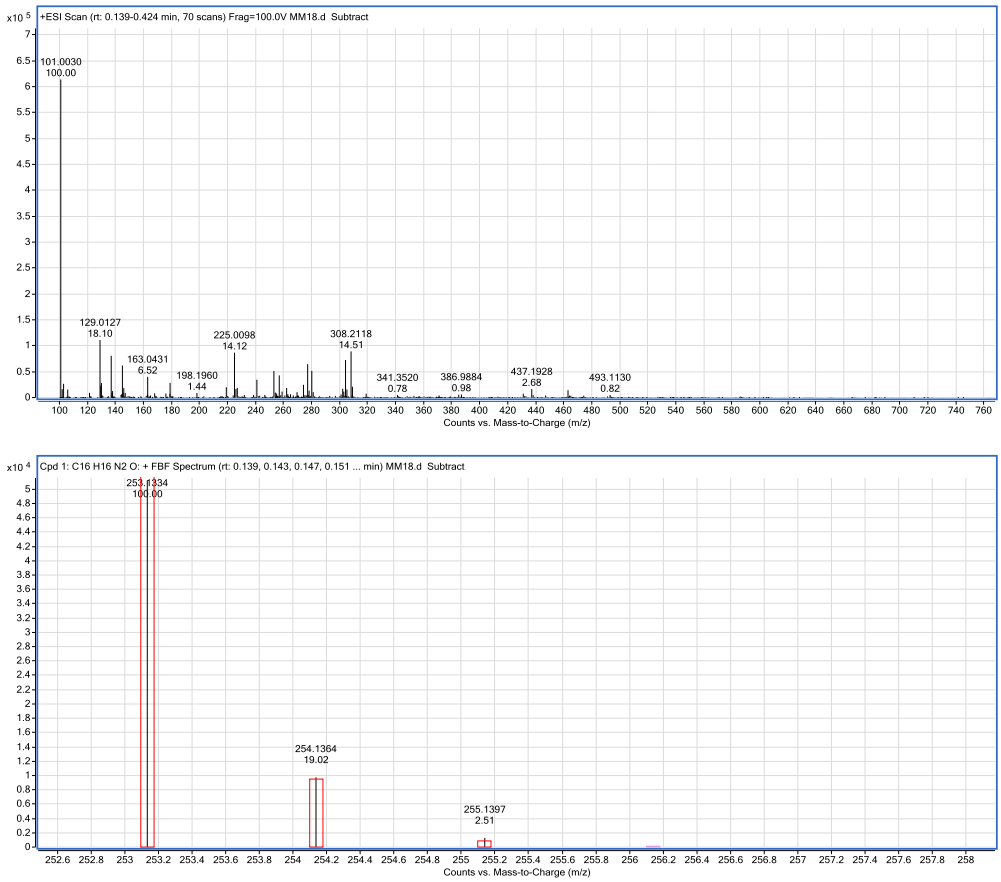


Figure S16. HR-MS (+ESI) spectrum for compound **A6**.

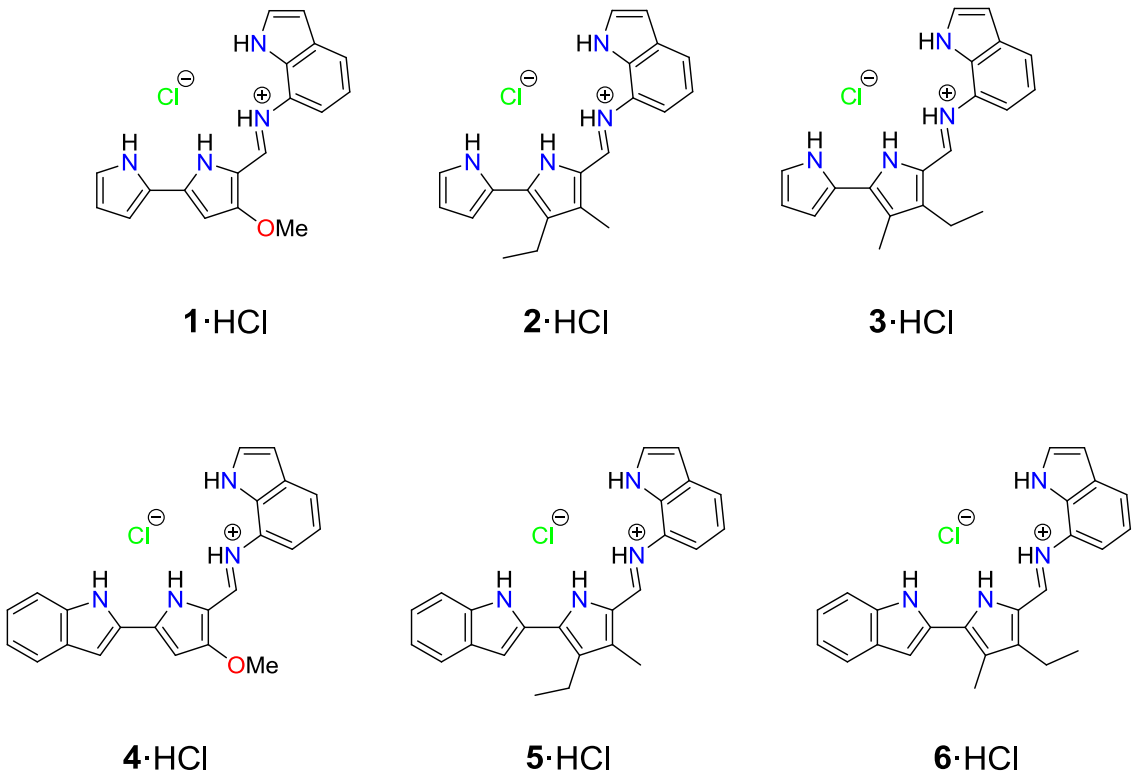
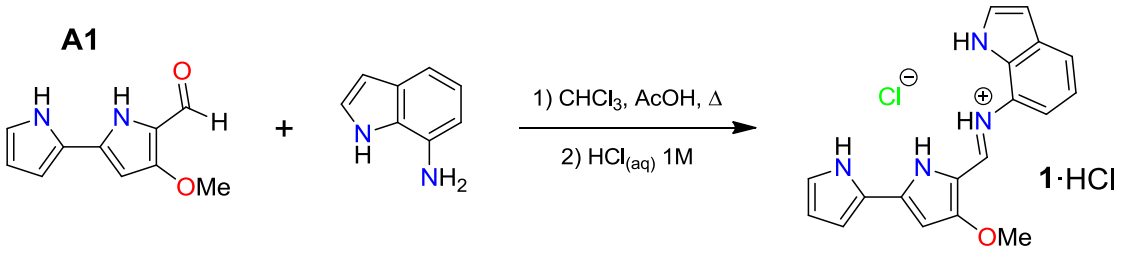


Figure S17. Structures of the studied compounds.

Synthesis and characterisation of compound 1·HCl



A mixture of aldehyde **A1** (100 mg, 0.53 mmol), 7-aminoindole (83 mg, 0.63 mmol) and glacial acetic acid (50 µL) in chloroform (10 mL) was heated to reflux for 24 hours. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (15 mL). The solution of the crude compound was washed with 1 M aqueous HCl solution (3 × 15 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **1**·HCl as a dark red crystalline powder (116 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.19 (s, 1H, NH), 10.98 (d, J = 13.9 Hz, 1H, NH), 10.86 (s, 1H, NH), 10.35 (s, 1H, NH), 7.79 (d, J = 14.0 Hz, 1H), 7.41-7.39 (m, 1H), 7.33-7.31 (m, 1H), 7.02-6.99 (m, 3H), 6.77 (m, 1H), 6.51 (m, 1H), 6.24 (m, 1H), 5.89 (s, 1H), 3.85 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 165.1 (C_{Ar}), 144.2 (C_{Ar}), 130.4 (C_{Ar}), 129.7 (imine CH), 127.2 (C_{Ar}), 126.0 (CH_{Ar}), 125.2 (CH_{Ar}), 123.5 (C_{Ar}), 122.4 (C_{Ar}), 119.8 (CH_{Ar}), 118.5 (CH_{Ar}), 115.1 (CH_{Ar}),

113.9 (C_{Ar}), 111.6 (CH_{Ar}), 105.8 (CH_{Ar}), 102.4 (CH_{Ar}), 92.2 (CH_{Ar}), 58.8 (CH_3). HR-MS (+ESI): found *m*/*z* 305.1392 ($[M+H]^+$), $[C_{18}H_{16}N_4OH]^+$ requires *m*/*z* 305.1397 (monoisotopic mass).

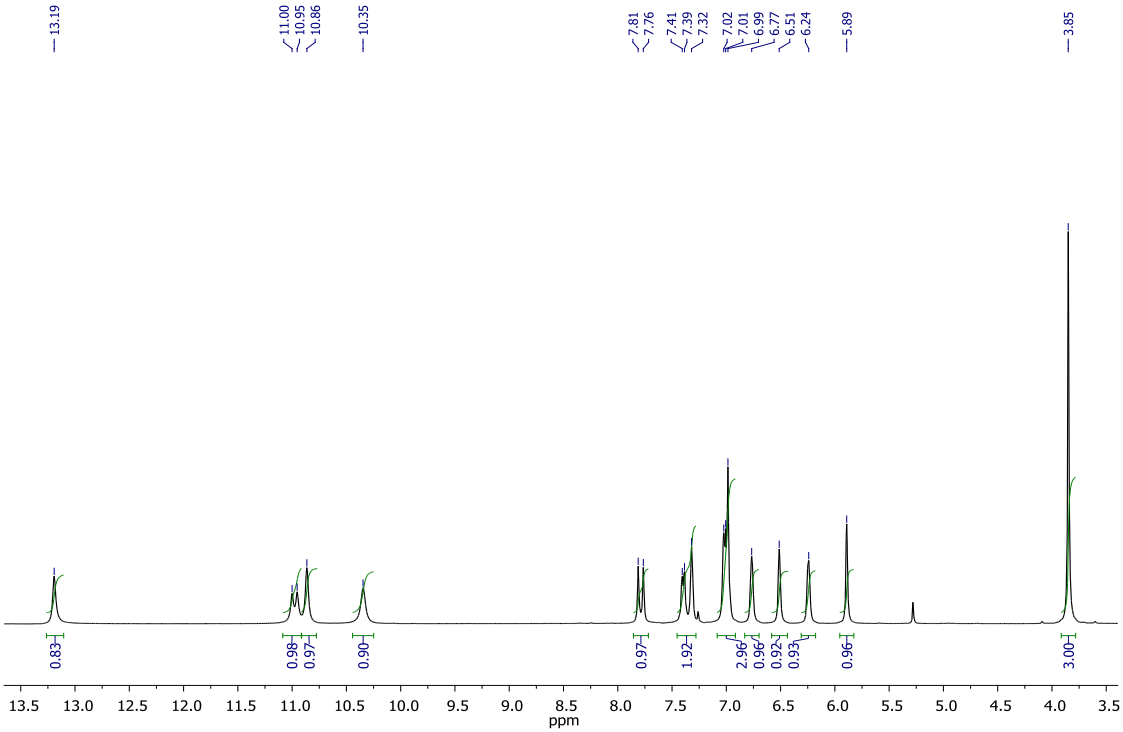


Figure S18. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **1**·HCl.

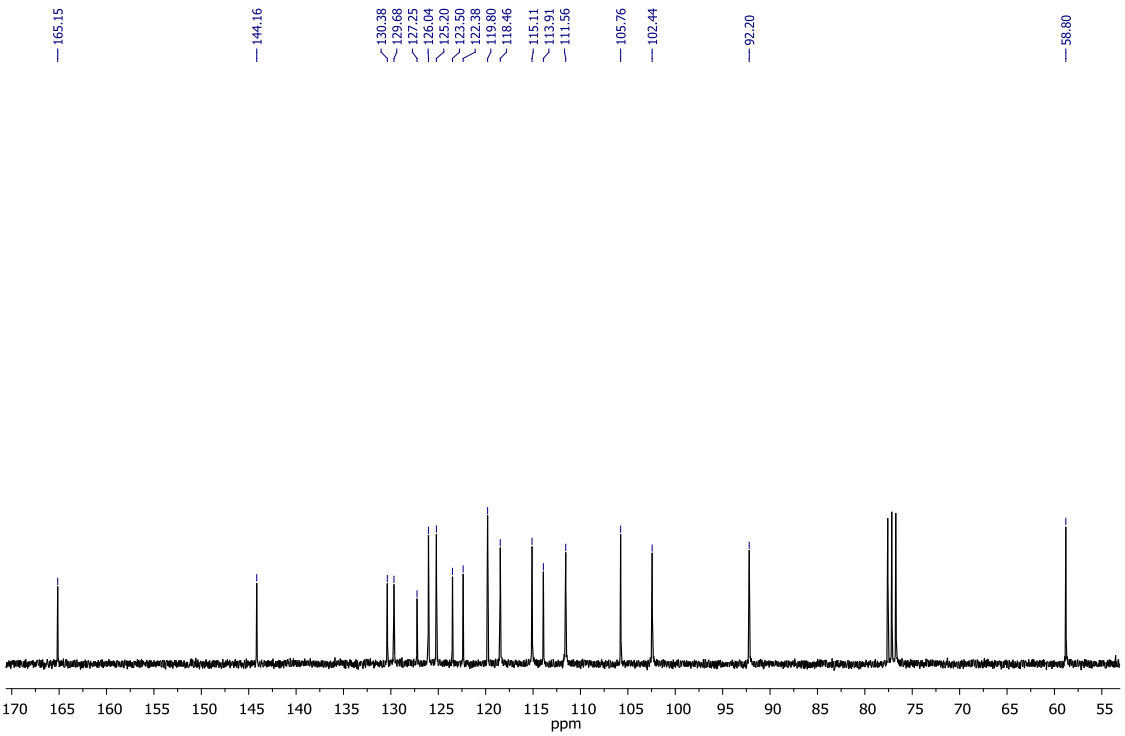


Figure S19. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) for compound **1**·HCl.

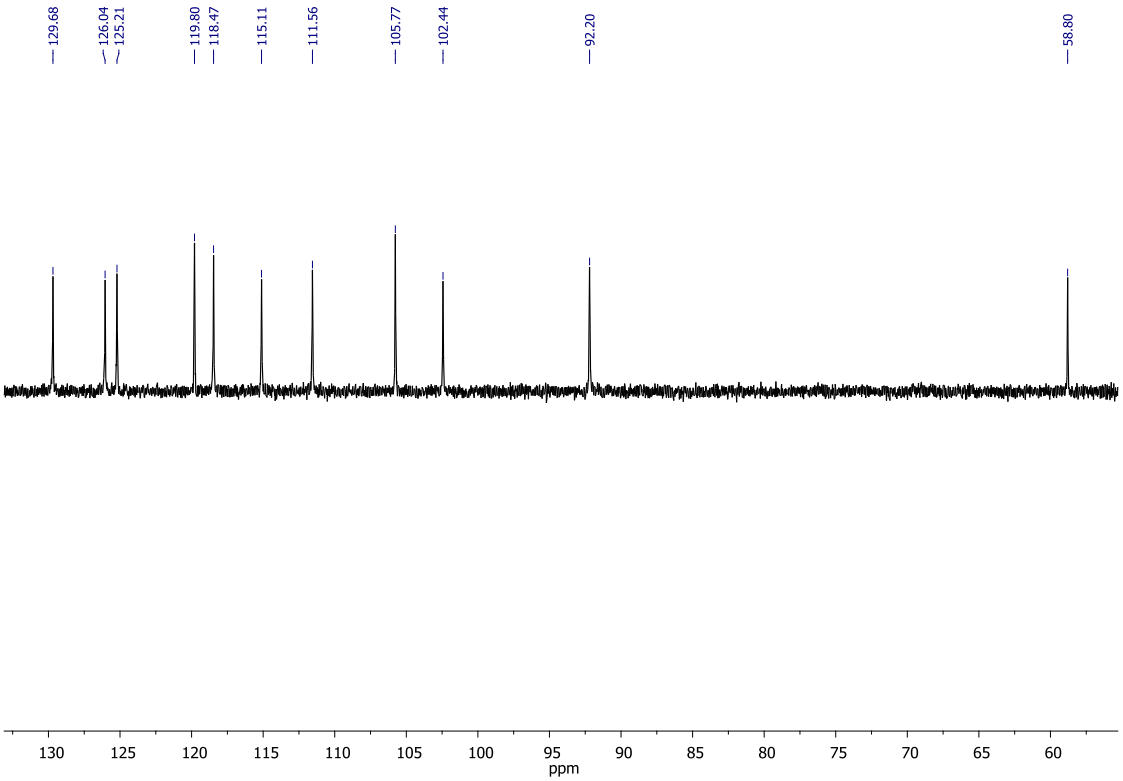


Figure S20. ^{13}C NMR dept135 spectrum (75 MHz, $CDCl_3$) for compound **1**·HCl.

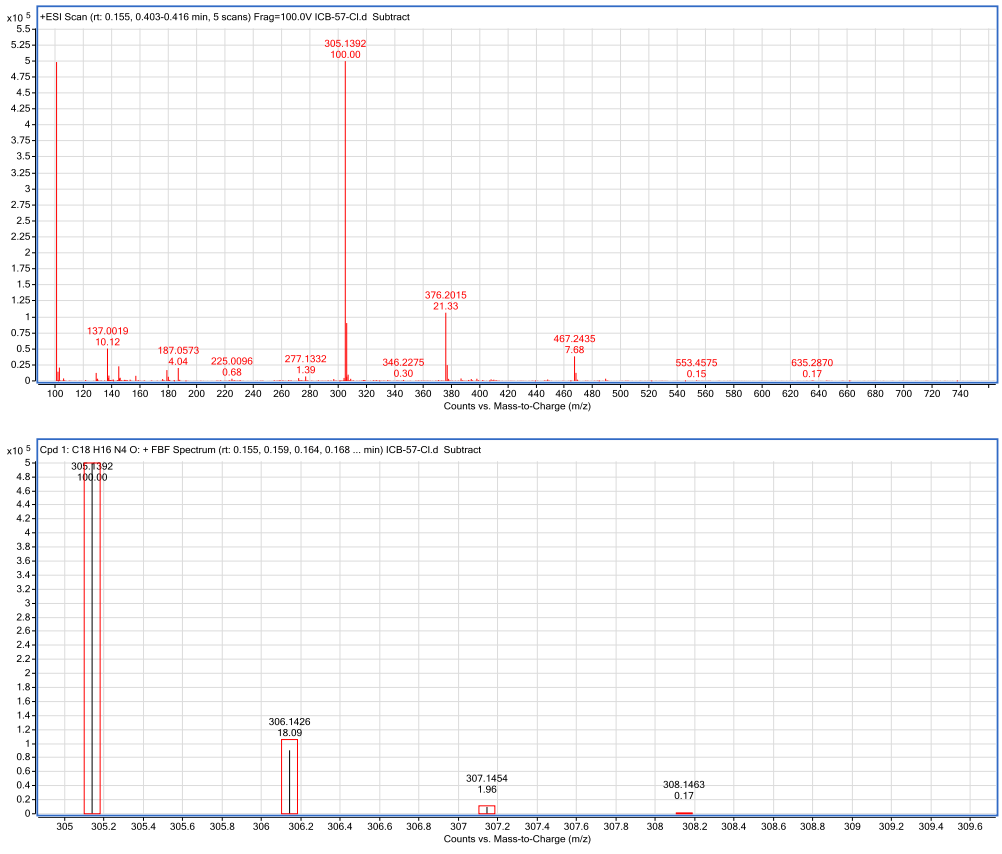
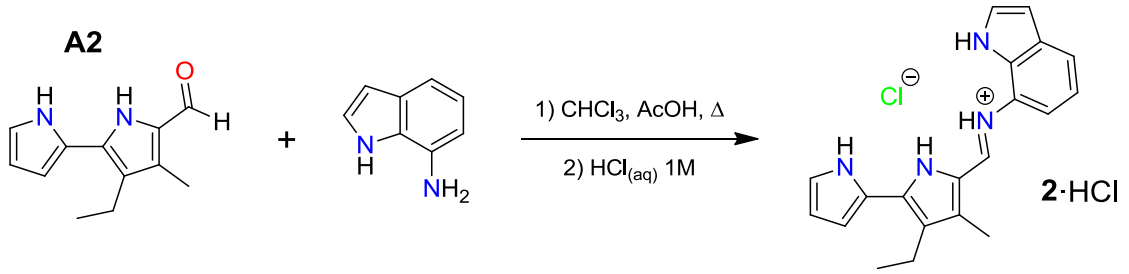


Figure S21. HR-MS (+ESI) spectrum for compound **1**·HCl.

Synthesis and characterisation of compound 2·HCl



A mixture of aldehyde **A2** (100 mg, 0.49 mmol), 7-aminoindole (78 mg, 0.59 mmol) and glacial acetic acid (50 µL) in chloroform (10 mL) was heated to reflux for 48 hours. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (15 mL). The solution of the crude compound was washed with 1 M aqueous HCl solution (3 × 15 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **2**·HCl as a red crystalline powder (145 mg, 83%). Slow evaporation of a solution of the compound in a chloroform-methanol mixture provided orange single crystals, suitable for X-ray diffraction analysis. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 13.13 (s, 1H, NH), 12.42 (m, 1H, NH), 11.54 (s, 1H, NH), 11.24 (s, 1H, NH), 8.60 (bs, 1H), 7.59-7.51 (m, 3H), 7.24 (s, 1H), 7.14-7.08 (m, 2H), 6.58-6.56 (m, 1H), 6.38-6.35 (m, 1H), 2.71 (q, J = 7.1 Hz, 2H), 2.37 (s, 3H), 1.08 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 142.8 (C_{Ar}), 140.7 (C_{Ar}), 137.9 (imine CH), 129.9 (C_{Ar}), 127.2 (C_{Ar}), 126.3 (CH_{Ar}), 124.6 (CH_{Ar}), 123.6 (C_{Ar}), 122.2 (C_{Ar}), 121.6 (C_{Ar}), 119.7 (CH_{Ar}), 119.0 (CH_{Ar}), 113.6 (CH_{Ar}), 110.8 (CH_{Ar}), 109.8 (CH_{Ar}), 102.3 (CH_{Ar}), 17.1 (CH_2), 14.2 (CH_3), 9.3 (CH_3). HR-MS (+ESI): found m/z 317.1759 ($[M+H]^+$), $[C_{20}H_{20}N_4H]^+$ requires m/z 317.1761 (monoisotopic mass).

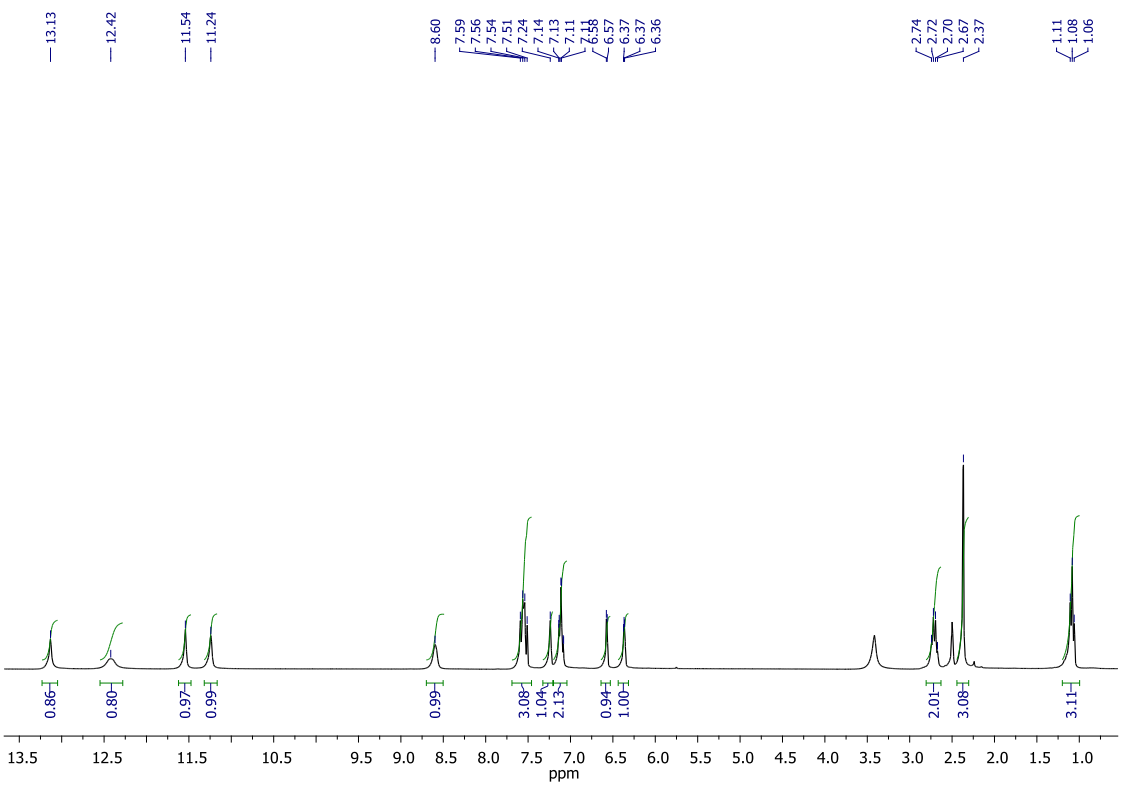


Figure S22. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **2**·HCl.

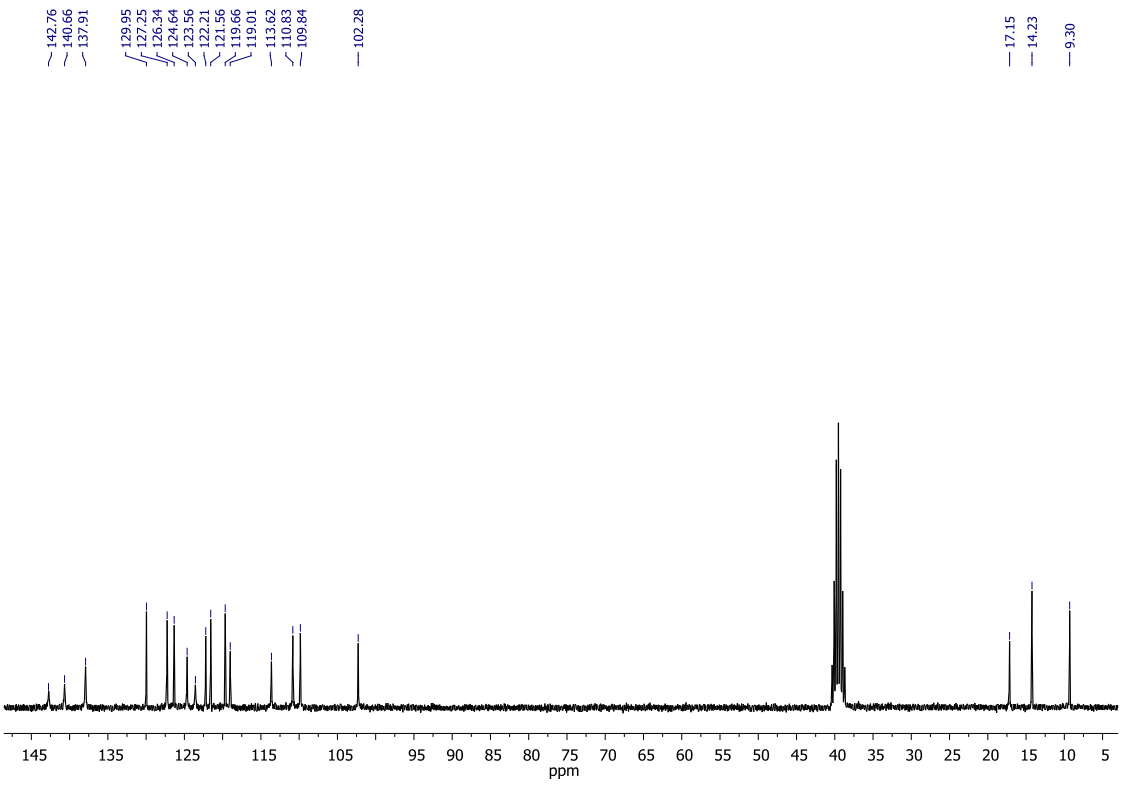


Figure S23. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) for compound **2**·HCl.

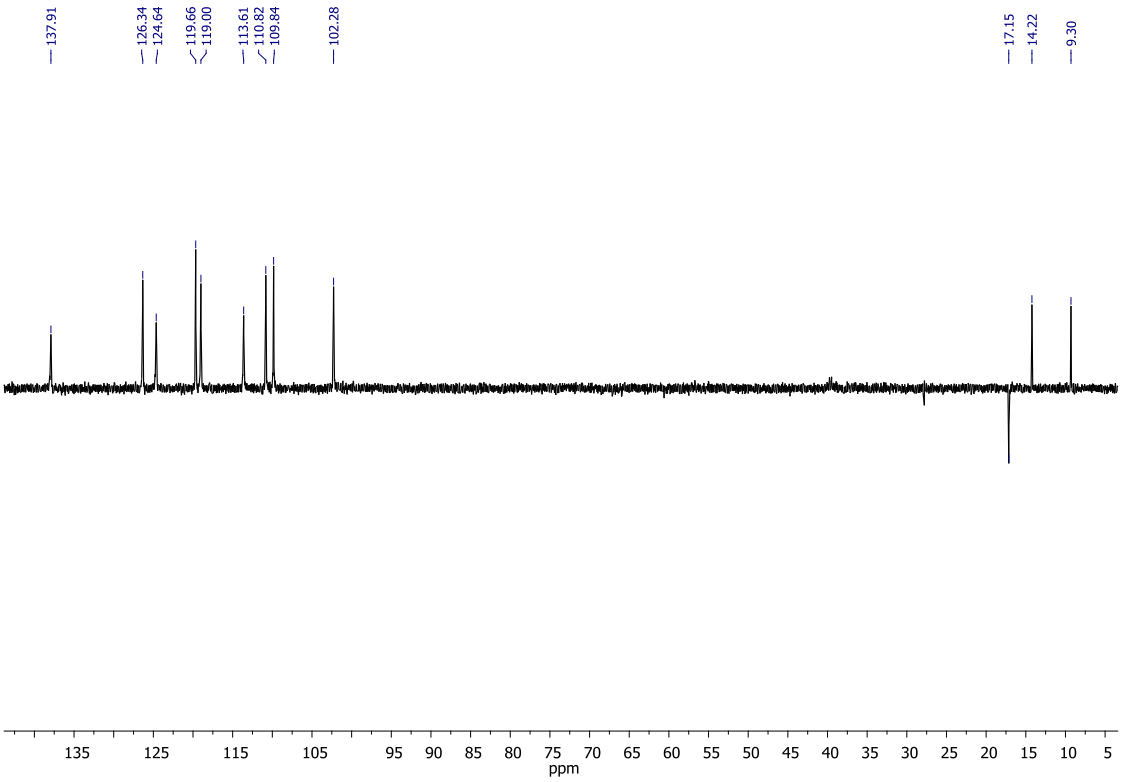


Figure S24. ^{13}C NMR dept135 spectrum (75 MHz, DMSO-d_6) for compound **2**·HCl.

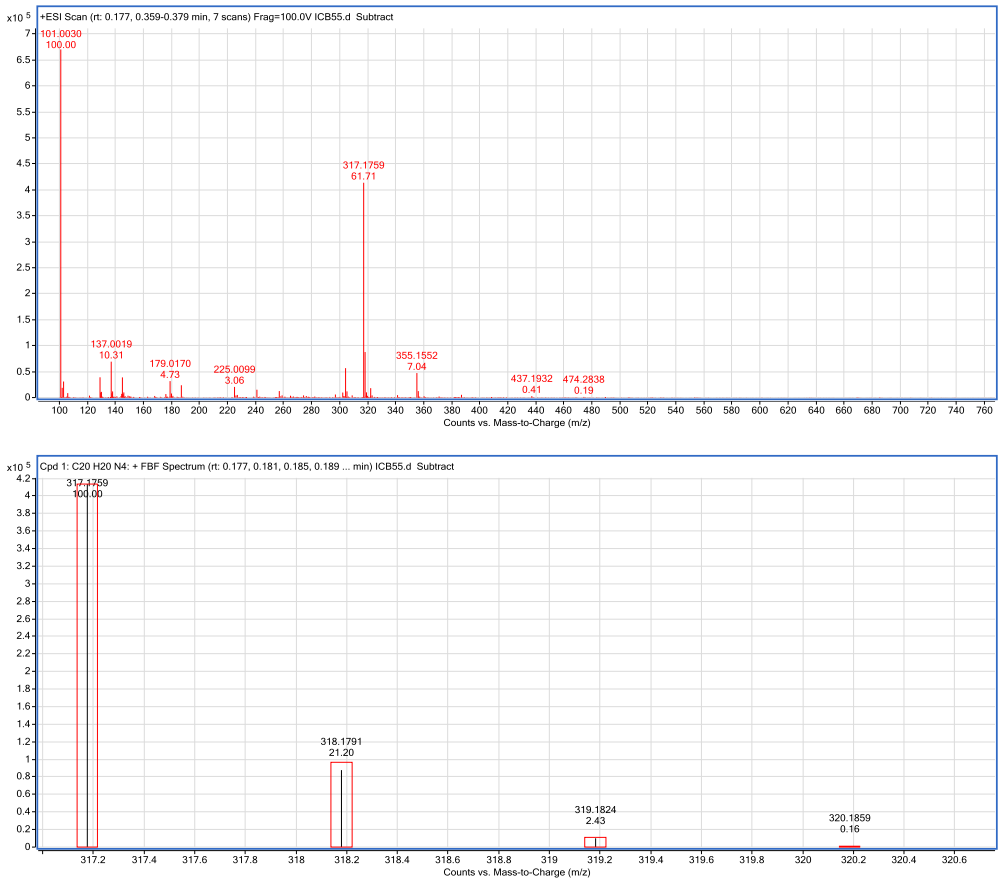
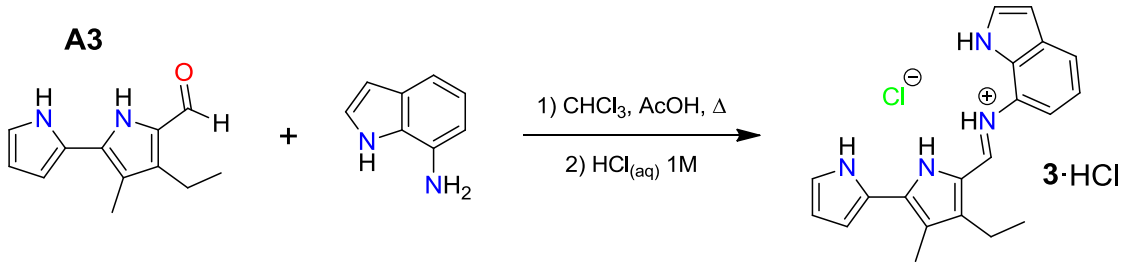


Figure S25. HR-MS (+ESI) spectrum for compound **2**·HCl.

Synthesis and characterisation of compound 3·HCl



A mixture of aldehyde **A3** (211 mg, 1.04 mmol), 7-aminoindole (153 mg, 1.16 mmol, 1.11 equiv.) and glacial acetic acid (50 µL) in chloroform (10 mL) was heated to reflux for 23 hours. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (30 mL). The solution of the crude compound was washed with 1 M aqueous HCl solution (3 × 20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **3**·HCl as a dark red non-crystalline solid (269 mg, 73%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 13.17 (s, 1H, NH), 12.44 (bs, 1H, NH), 11.55 (s, 1H, NH), 11.30 (s, 1H, NH), 8.60 (s, 1H), 7.68-7.49 (m, 3H), 7.30-7.21 (m, 1H), 7.19-7.07 (m, 2H), 6.62-6.54 (m, 1H), 6.42-6.33 (m, 1H), 2.84 (q, J = 7.5 Hz, 2H), 2.25 (s, 3H), 1.16 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 149.1 (C_{Ar}), 141.6 (C_{Ar}), 137.4 (imine CH), 129.9 (C_{Ar}), 127.3 (C_{Ar}), 126.3 (CH_{Ar}), 124.8 (CH_{Ar}), 123.6 (C_{Ar}), 121.9 (C_{Ar}), 121.3 (C_{Ar}), 120.3 (C_{Ar}), 119.7 (CH_{Ar}), 119.0 (CH_{Ar}), 113.8 (CH_{Ar}), 110.8 (CH_{Ar}), 109.8 (CH_{Ar}), 102.3 (CH_{Ar}), 17.0 (CH_2), 16.1 (CH_3), 10.0 (CH_3). HR-MS (+ESI): found m/z 317.1759 ($[M+H]^+$), $[C_{20}H_{20}N_4H]^+$ requires m/z 317.1761 (monoisotopic mass).

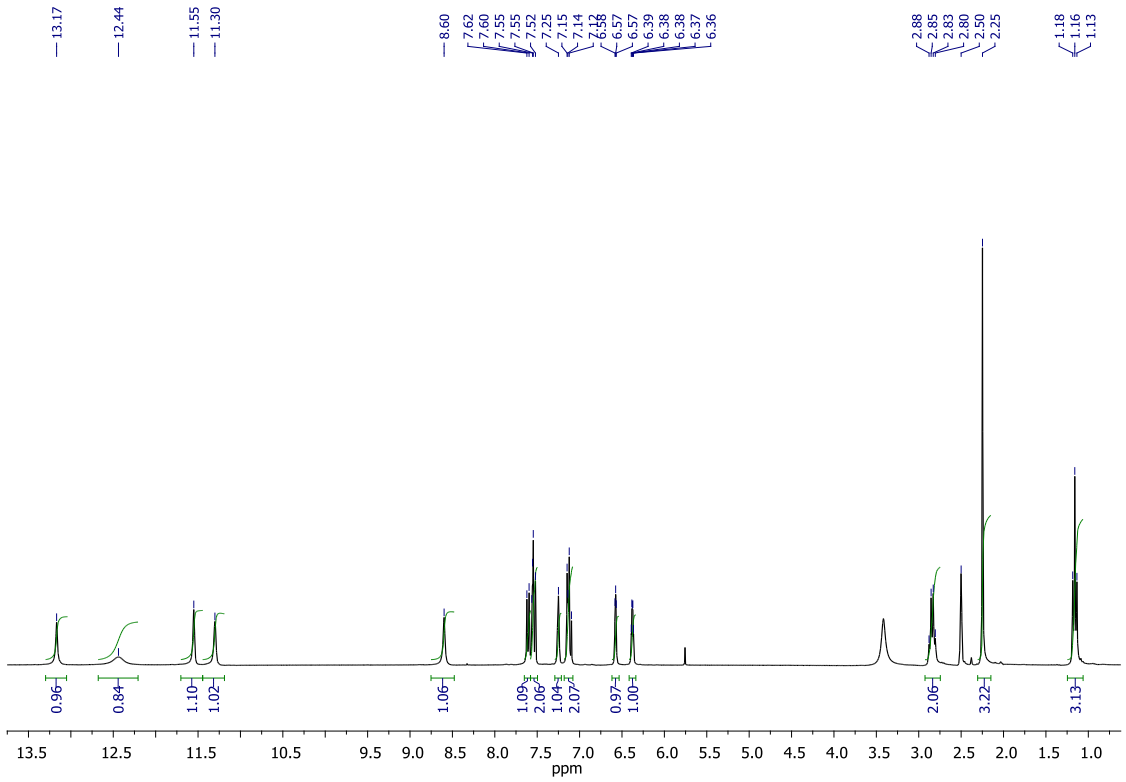


Figure S26. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **3**·HCl.

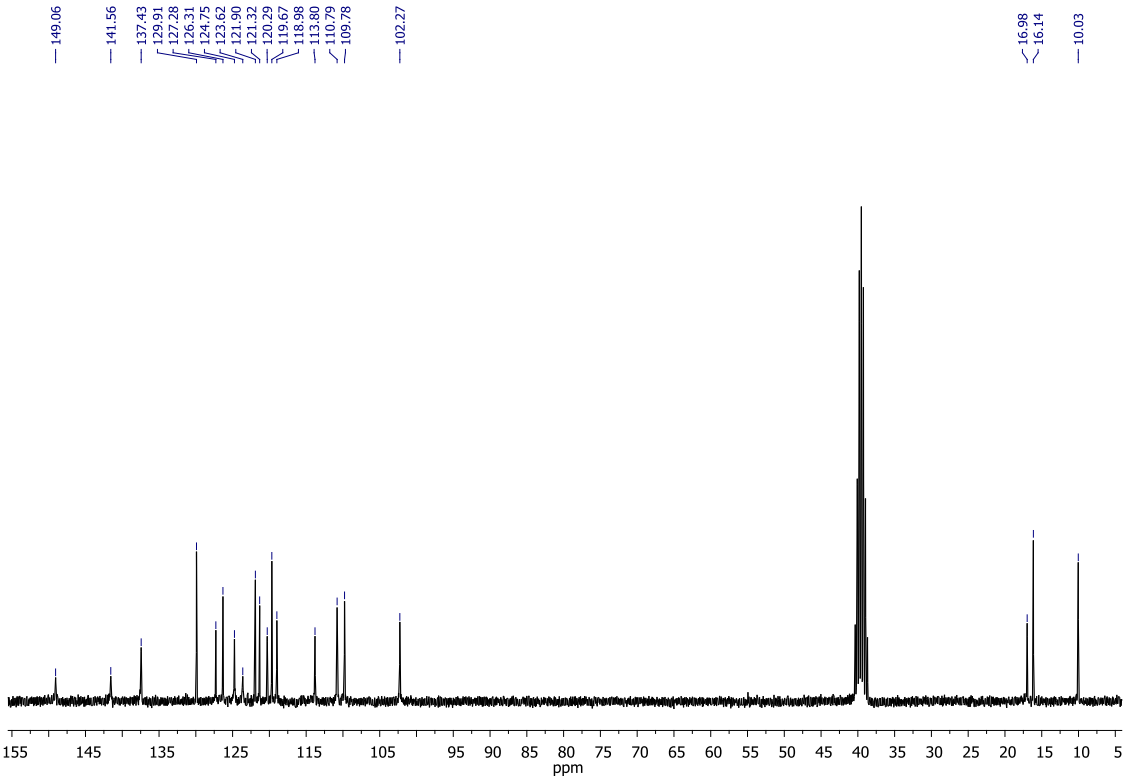


Figure S27. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) for compound **3**·HCl.

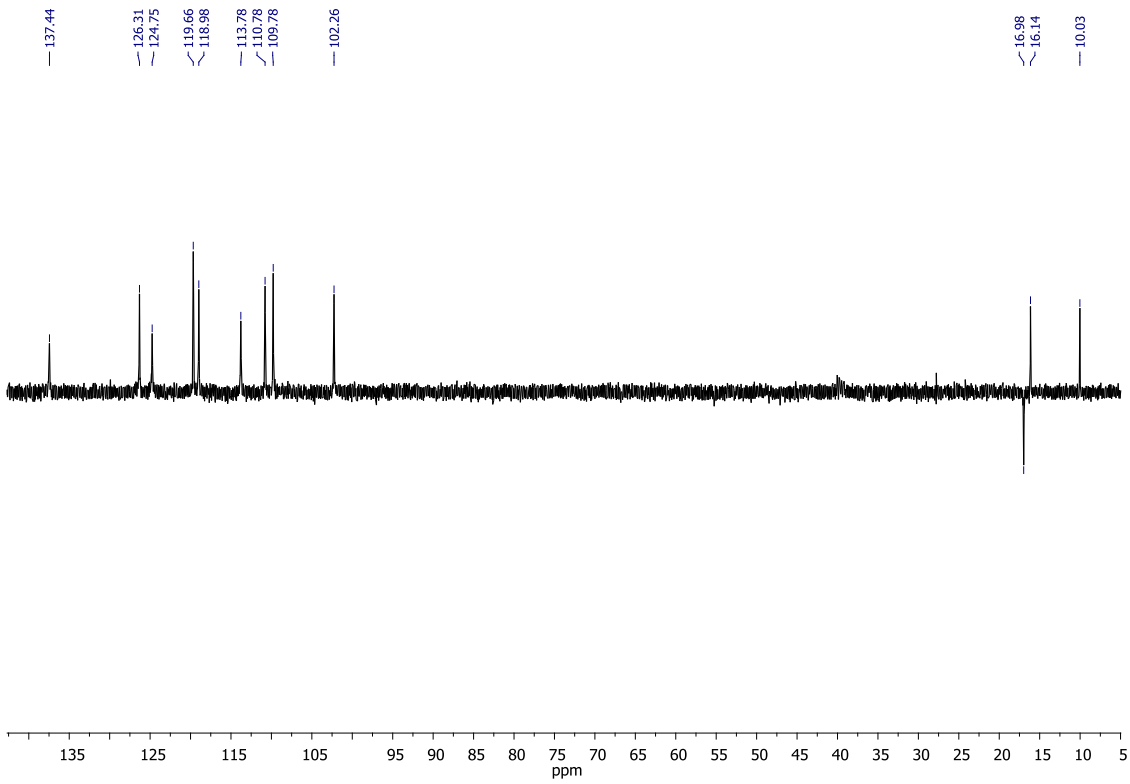


Figure S28. ^{13}C NMR dept135 spectrum (75 MHz, DMSO-d_6) for compound **3**·HCl.

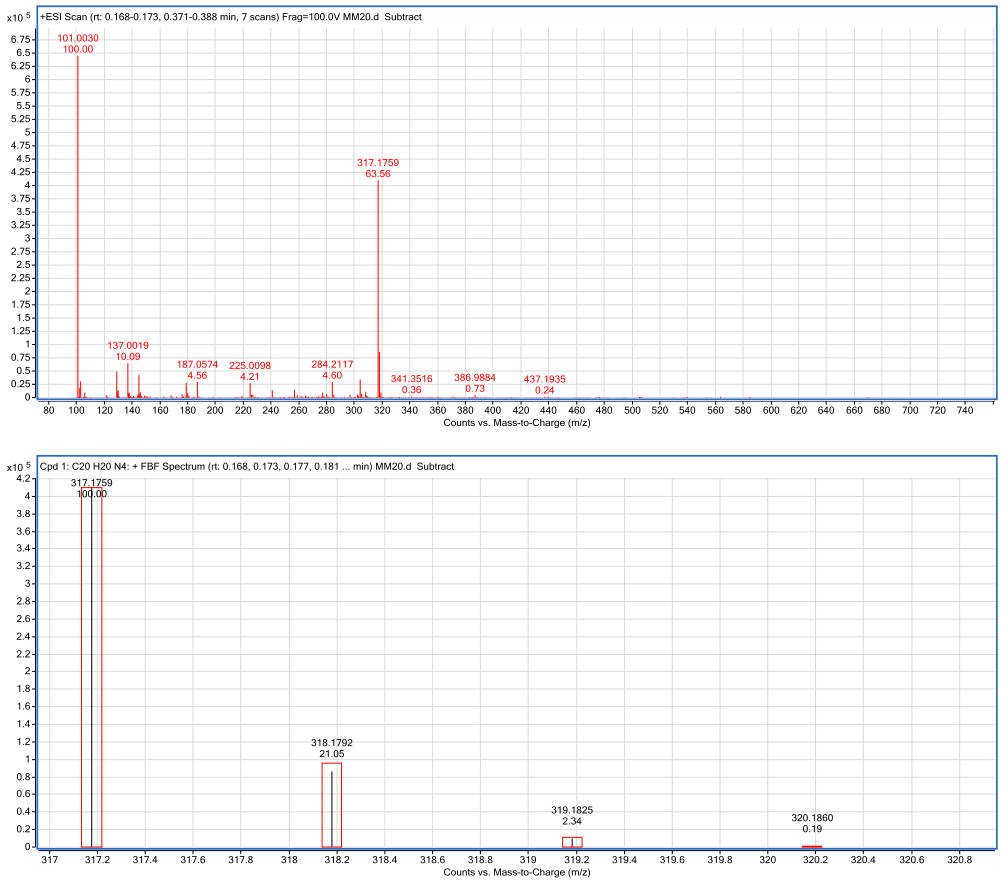
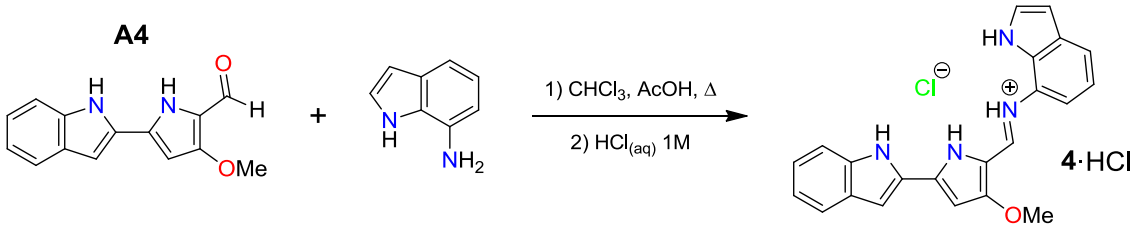


Figure S29. HR-MS (+ESI) spectrum for compound **3**·HCl.

Synthesis and characterisation of compound 4·HCl



A mixture of aldehyde **A4** (100 mg, 0.42 mmol), 7-aminoindole (60 mg, 0.46 mmol, 1.1 equiv.) and glacial acetic acid (50 µL) in chloroform (10 mL) was heated at 65 °C for 24 hours. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (20 mL). The solution of the crude compound was washed with 1 M aqueous HCl solution (3 × 15 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **4**·HCl as a purple crystalline solid (112 mg, 76%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 13.34 (s, 1H, NH), 12.64 (bs, 1H, NH), 12.07 (s, 1H), 11.49 (s, 1H), 8.51 (s, 1H), 7.70 (d, J = 7.9 Hz, 1H), 7.60-7.45 (m, 5H), 7.30-7.24 (m 1H), 7.15-7.07 (m, 2H), 6.83 (s, 1H), 6.61-6.57 (m, 1H), 4.08 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) = 138.1 (C_{Ar}), 136.0 (imine CH), 130.0 (C_{Ar}), 127.7 (C_{Ar}), 127.5 (C_{Ar}), 127.3 (C_{Ar}), 126.4 (CH_{Ar}), 124.4 (CH_{Ar}), 123.4 (C_{Ar}), 121.4 (CH_{Ar}), 120.6 (CH_{Ar}), 119.8 (CH_{Ar}), 119.1 (CH_{Ar}), 113.8 (ArC), 111.9 (CH_{Ar}), 109.8 (CH_{Ar}), 104.1 (CH_{Ar}), 102.3 (CH_{Ar}), 94.3 (CH_{Ar}), 59.1 (CH_3). HR-MS (+ESI): found m/z 355.1555 ($[M+H]^+$), $[C_{22}H_{19}N_4O]^+$ requires m/z 355.1553 (monoisotopic mass).

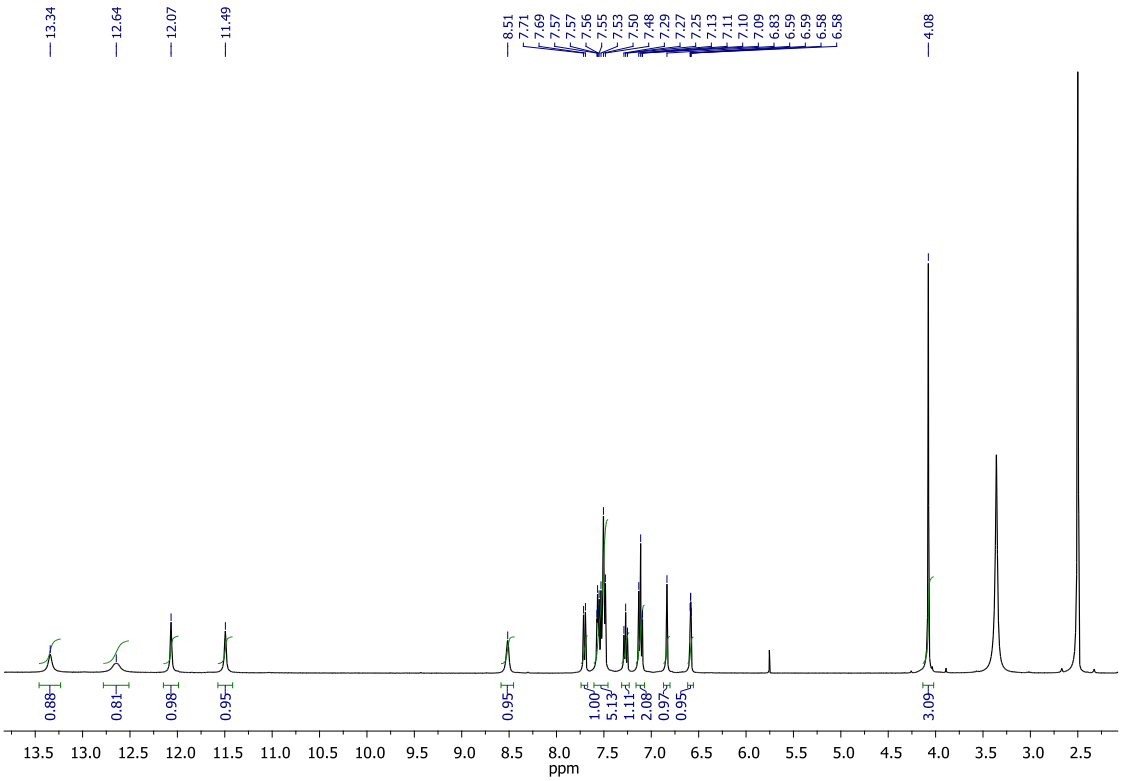


Figure S30. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) for compound **4**·HCl.

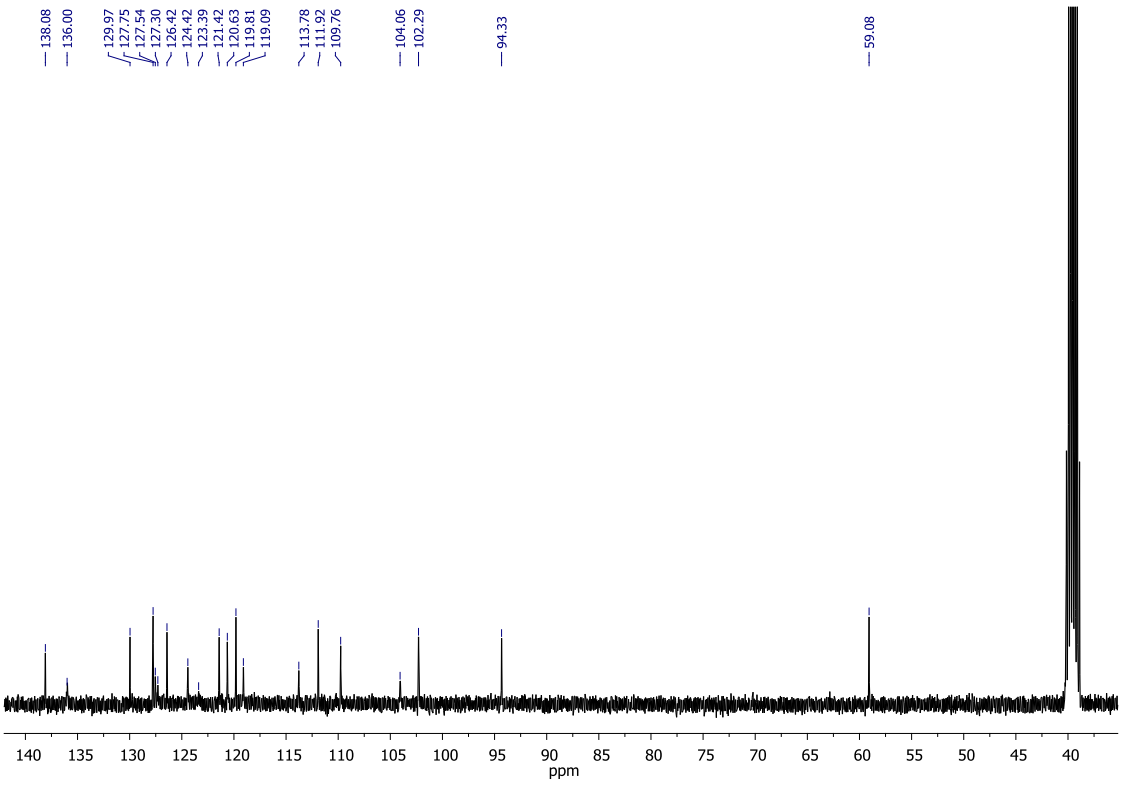


Figure S31. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) for compound **4**·HCl.

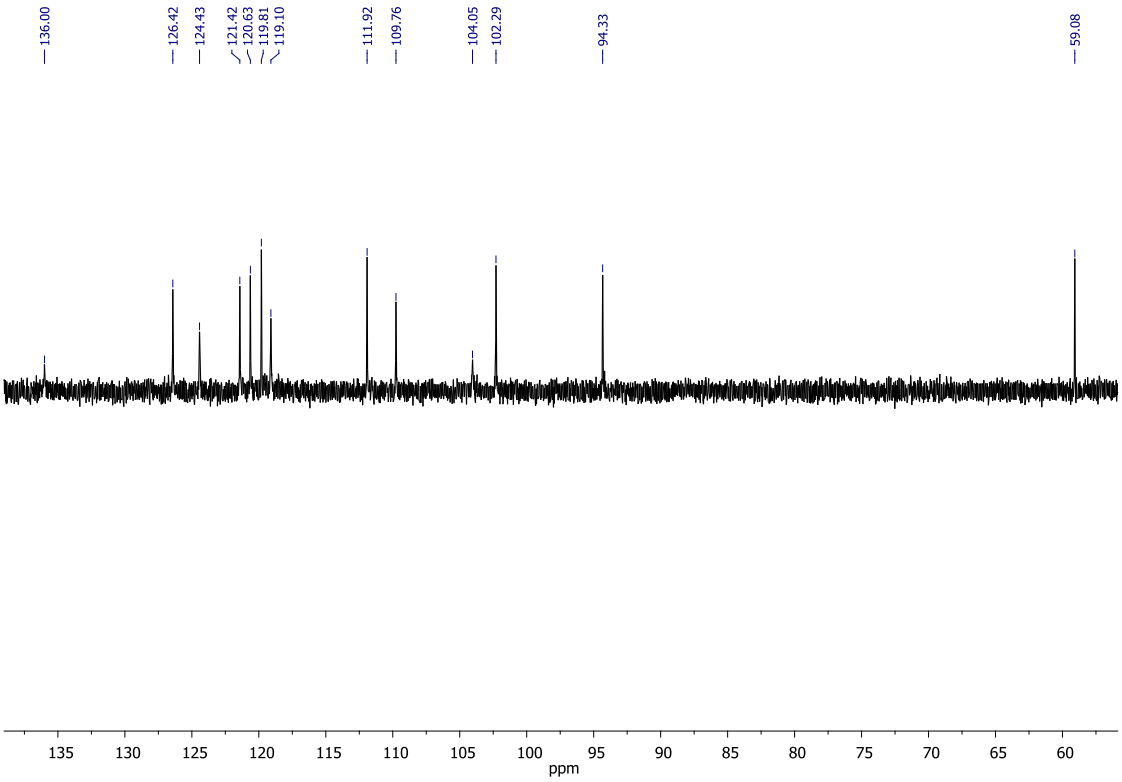


Figure S32. ^{13}C NMR dept135 spectrum (100 MHz, DMSO-d_6) for compound **4**·HCl.

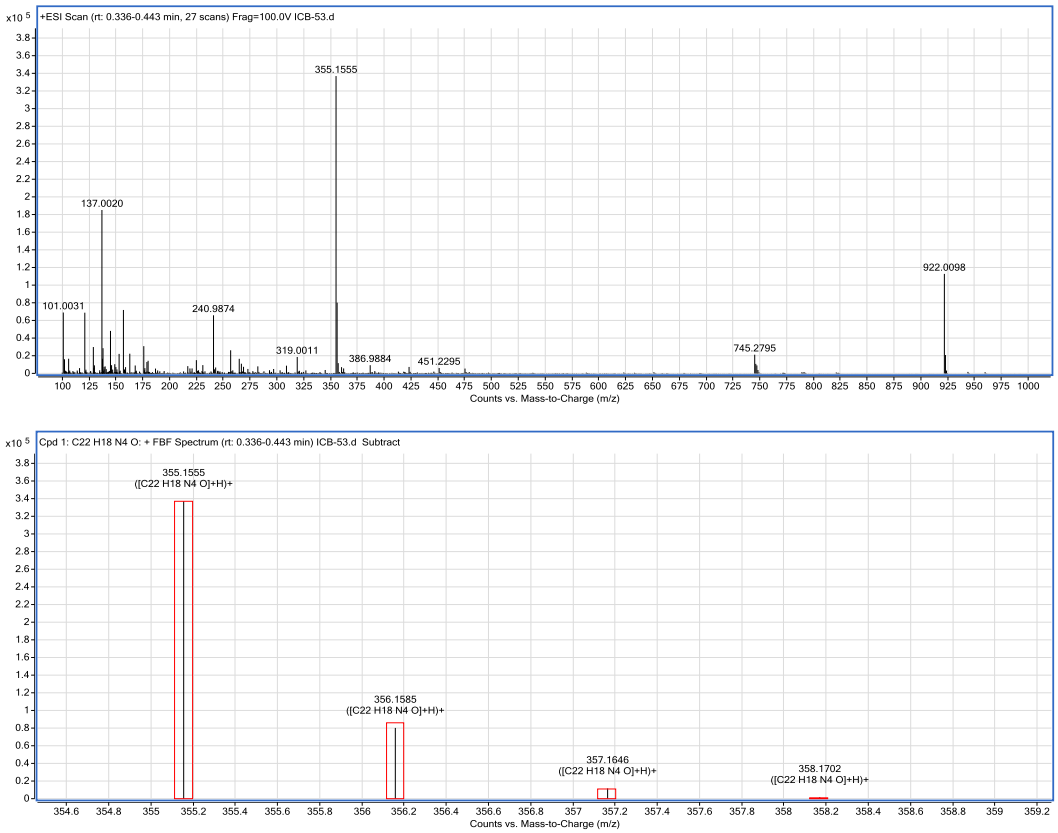
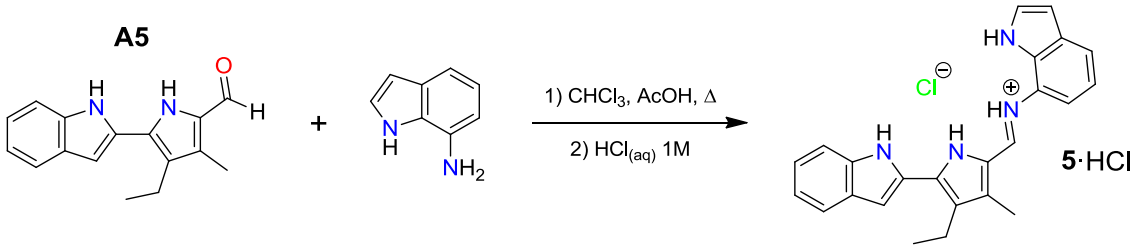


Figure S33. HR-MS (+ESI) spectrum for compound **4**·HCl.

Synthesis and characterisation of compound 5·HCl



A mixture of aldehyde **A5** (100 mg, 0.40 mmol), 7-aminoindole (63 mg, 0.47 mmol, 1.2 equiv.) and glacial acetic acid (50 µL) in chloroform (10 mL) was heated at 65 °C for 24 hours. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (30 mL). The solution of the crude compound was washed with 1 M aqueous HCl solution (3 × 20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **5**·HCl as a dark red non-crystalline solid (119 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.55 (s, 1H, NH), 11.92 (d, J = 14.4 Hz, 1H, NH), 11.04 (s, 1H, NH), 10.35 (s, 1H, NH), 7.96 (d, J = 14.5 Hz, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.38 (t, J = 2.7 Hz, 1H), 7.34-7.28 (m, 1H), 7.17-7.06 (m, 3H), 6.96 (t, J = 7.7 Hz, 1H), 6.58-6.52 (m, 1H), 2.66 (q, J = 7.6 Hz, 2H), 2.25 (s, 3H), 1.19 (t, J = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 141.3 (C_{Ar}), 141.2 (C_{Ar}), 137.8 (C_{Ar}), 134.4 (imine CH), 130.7 (C_{Ar}), 130.3 (C_{Ar}), 128.5 (C_{Ar}), 127.6 (C_{Ar}), 127.0 (C_{Ar}), 126.4 (CH_{Ar}), 125.5 (CH_{Ar}), 123.0 (C_{Ar}), 122.9 (C_{Ar}), 121.6 (CH_{Ar}), 121.0 (CH_{Ar}), 119.8 (CH_{Ar}), 112.2 (CH_{Ar}), 107.7 (CH_{Ar}), 106.7 (CH_{Ar}), 102.7 (CH_{Ar}), 18.3 (CH_2), 13.7 (CH_3), 9.6 (CH_3). HR-MS (+ESI): found m/z 367.1918 ($[M+H]^+$), $[C_{24}H_{23}N_4]^+$ requires m/z 367.1917 (monoisotopic mass).

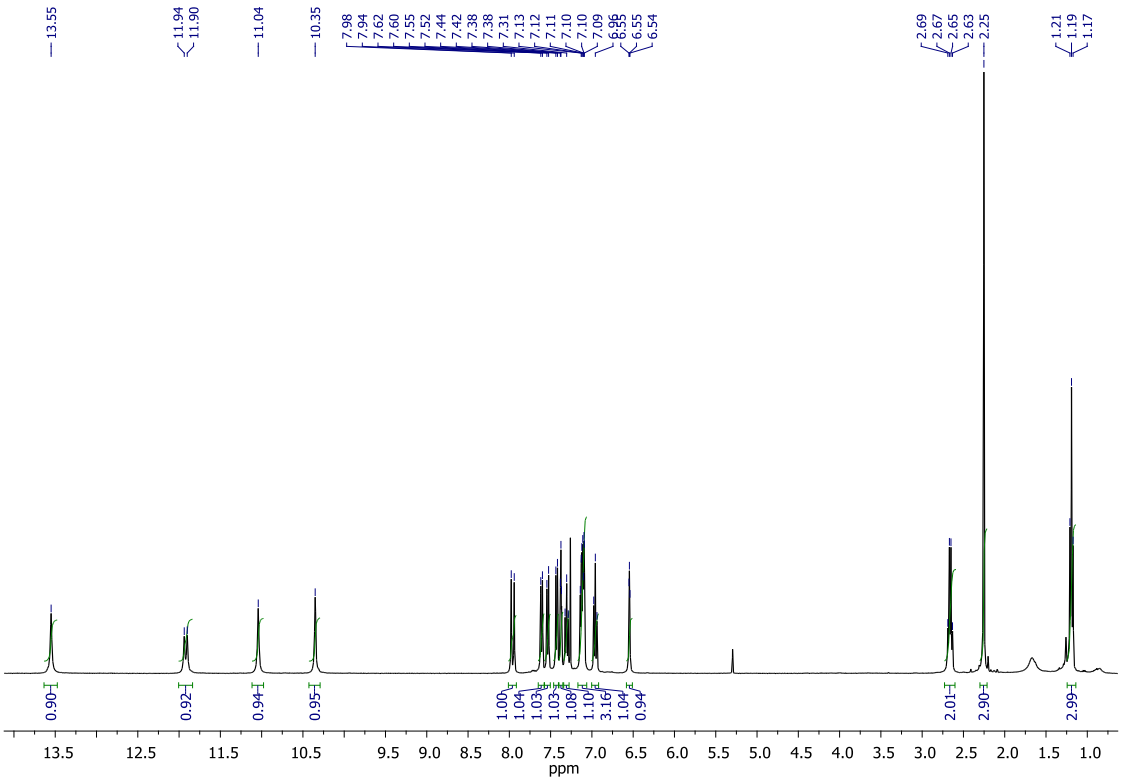


Figure S34. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **5**·HCl.

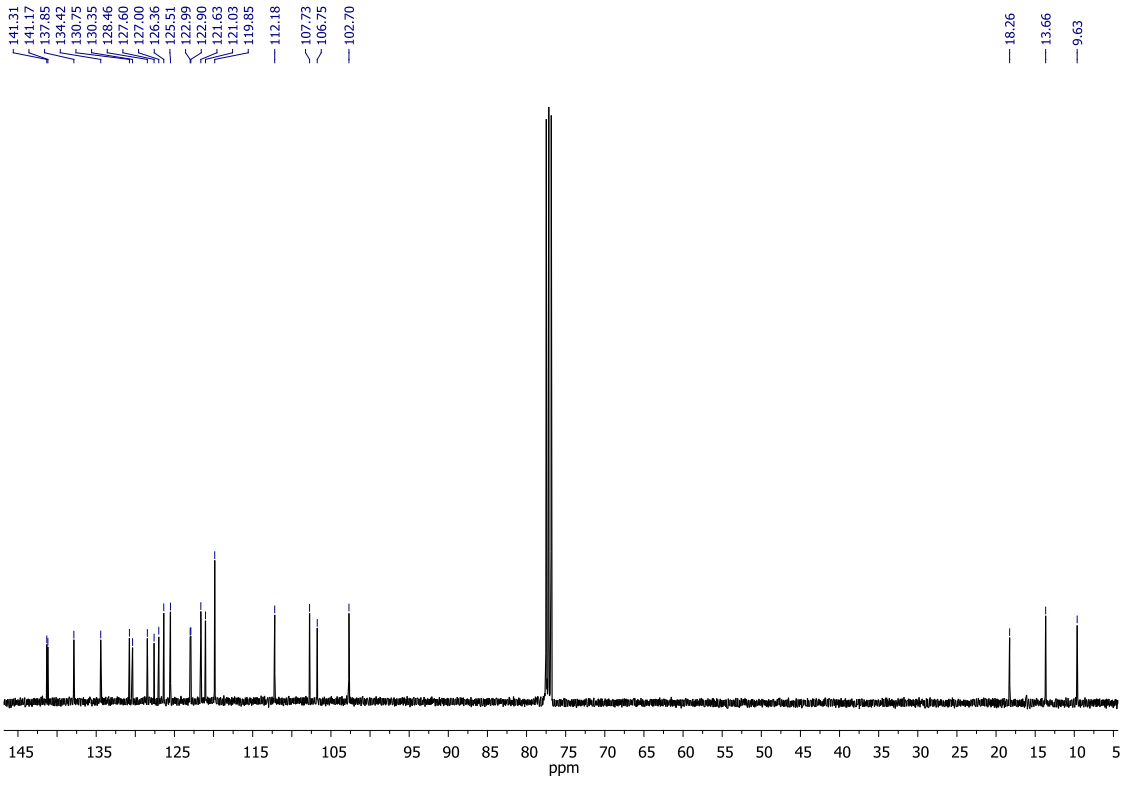


Figure S35. ^{13}C NMR spectrum (75 MHz, $CDCl_3$) for compound **5**·HCl.

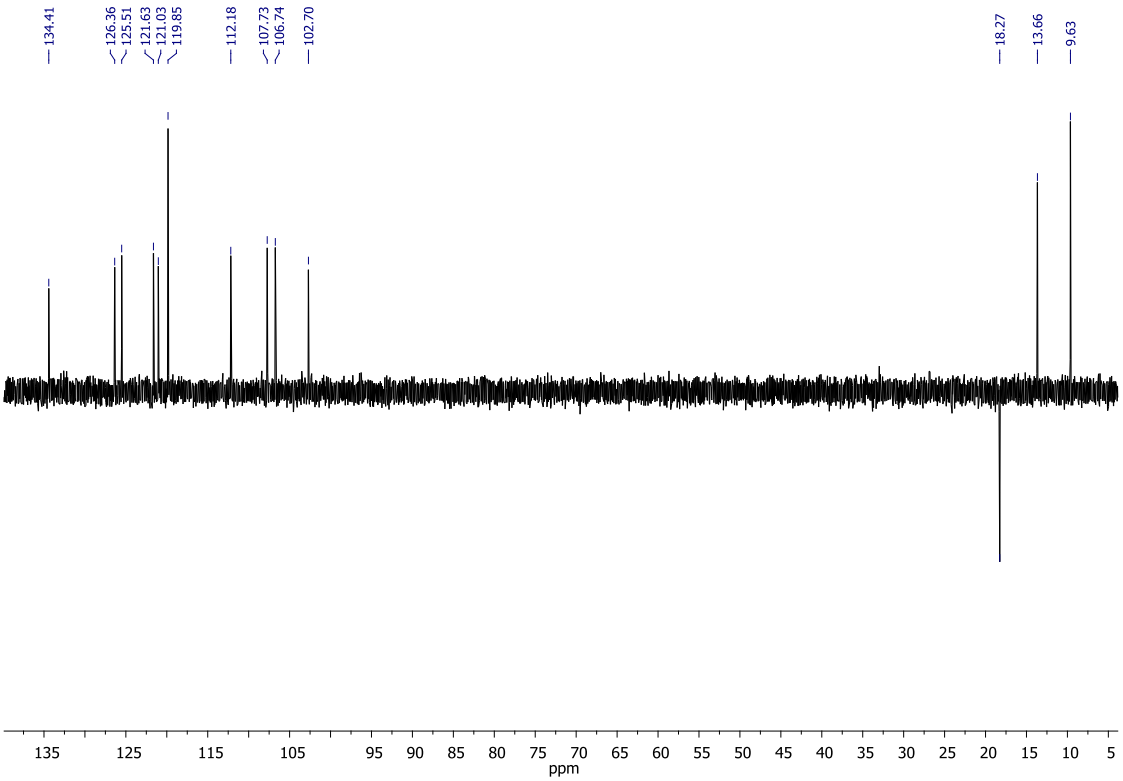


Figure S36. ^{13}C NMR dept135 spectrum (75 MHz, $CDCl_3$) for compound **5**·HCl.

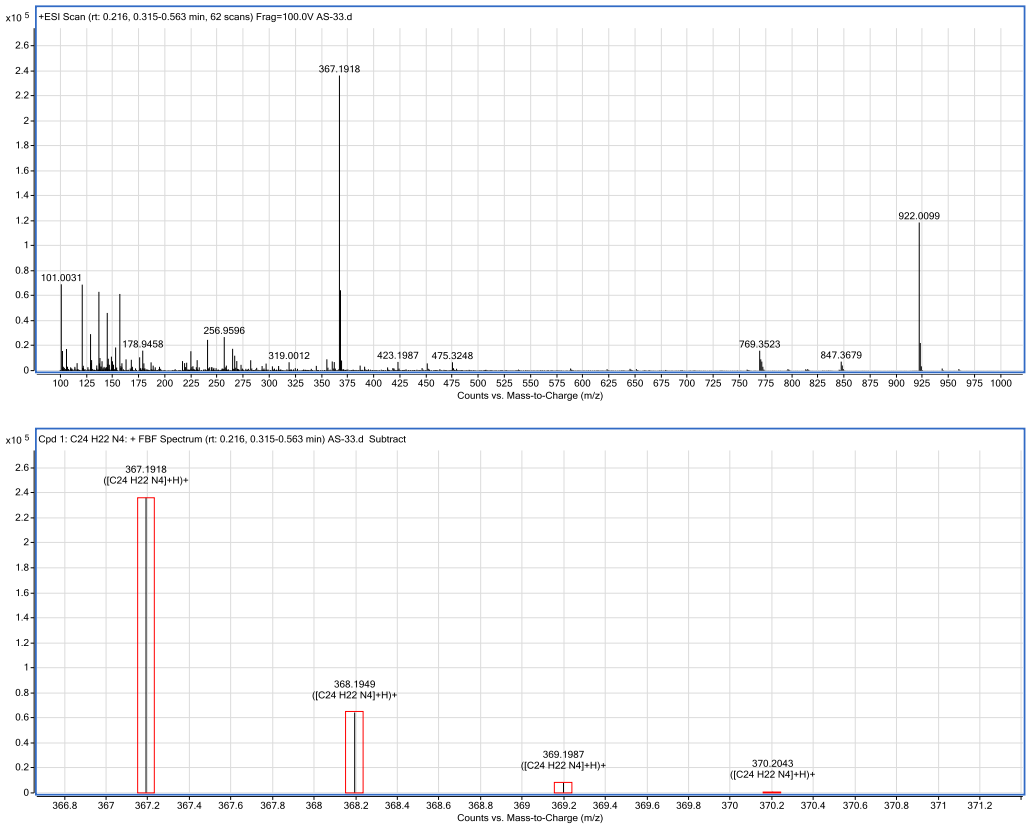
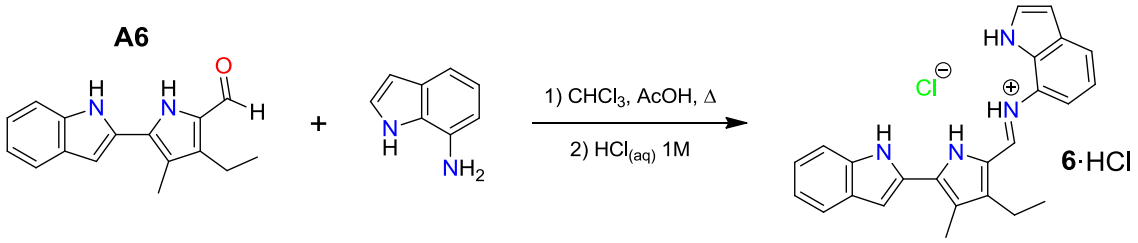


Figure S37. HR-MS (+ESI) spectrum for compound **5**·HCl.

Synthesis and characterisation of compound 6·HCl



A mixture of aldehyde **A6** (215 mg, 0.85 mmol), 7-aminoindole (125 mg, 0.94 mmol, 1.11 equiv.) and glacial acetic acid (50 µL) in chloroform (10 mL) was heated at 55 °C for 22 hours. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (30 mL). The solution of the crude compound was washed with 1 M aqueous HCl solution (3 × 20 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **6**·HCl as a dark red non-crystalline solid (219 mg, 64%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 13.27 (bs, 1H, NH), 11.59 (s, 1H, NH), 11.39 (s, 1H, NH), 8.77 (s, 1H), 7.72-7.48 (m, 5H), 7.32 (s, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.15 (t, J = 9.0 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H), 6.63-6.56 (m, 1H), 2.88 (q, J = 7.5 Hz, 2H), 2.35 (s, 3H), 1.19 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 141.1 (imine CH), 139.2 (C_{Ar}), 137.9 (C_{Ar}), 130.0 (C_{Ar}), 127.8 (C_{Ar}), 127.8 (C_{Ar}), 127.7 (C_{Ar}), 126.5 (CH_{Ar}), 123.9 (CH_{Ar}), 122.2 (C_{Ar}), 121.4 (C_{Ar}), 121.0 (CH_{Ar}), 120.4 (CH_{Ar}), 119.8 (CH_{Ar}), 119.6 (CH_{Ar}), 112.1 (CH_{Ar}), 110.9 (CH_{Ar}), 105.5 (CH_{Ar}), 102.3 (CH_{Ar}), 17.0 (CH_2), 16.1 (CH_3), 10.0 (CH_3). HR-MS (+ESI): found m/z 367.1916 ($[M+H]^+$), $[C_{24}H_{22}N_4H]^+$ requires m/z 367.1917 (monoisotopic mass).

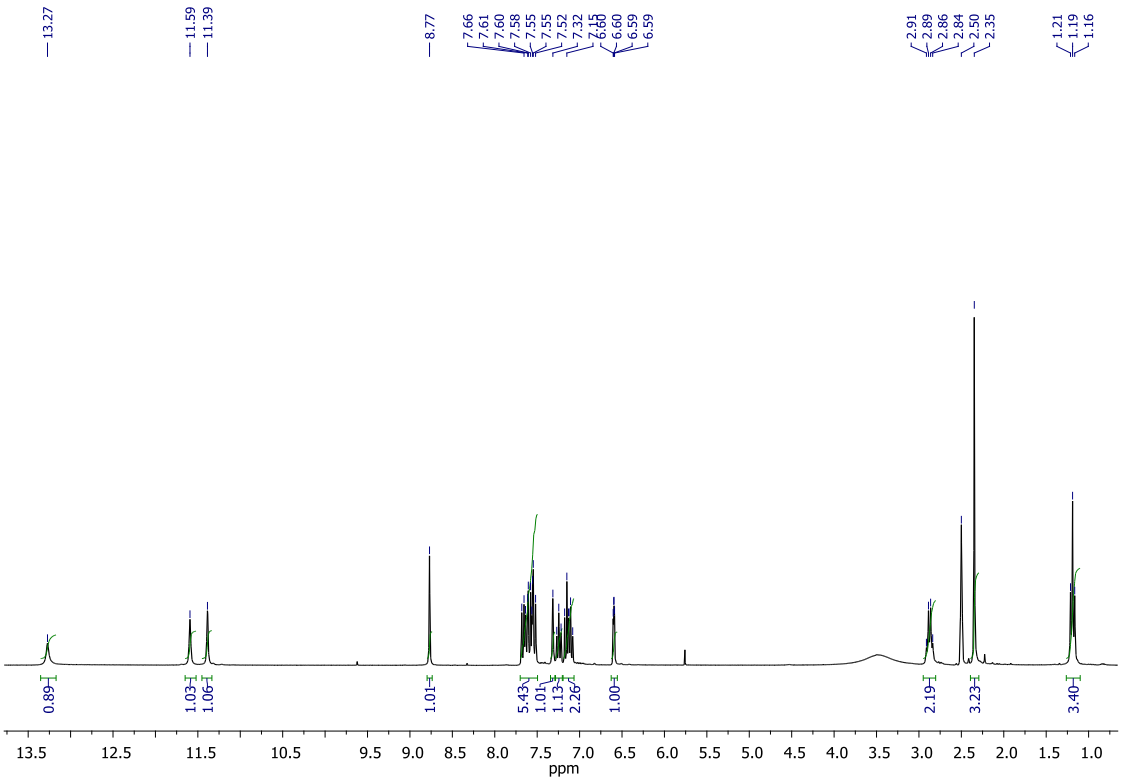


Figure S38. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **6**·HCl.

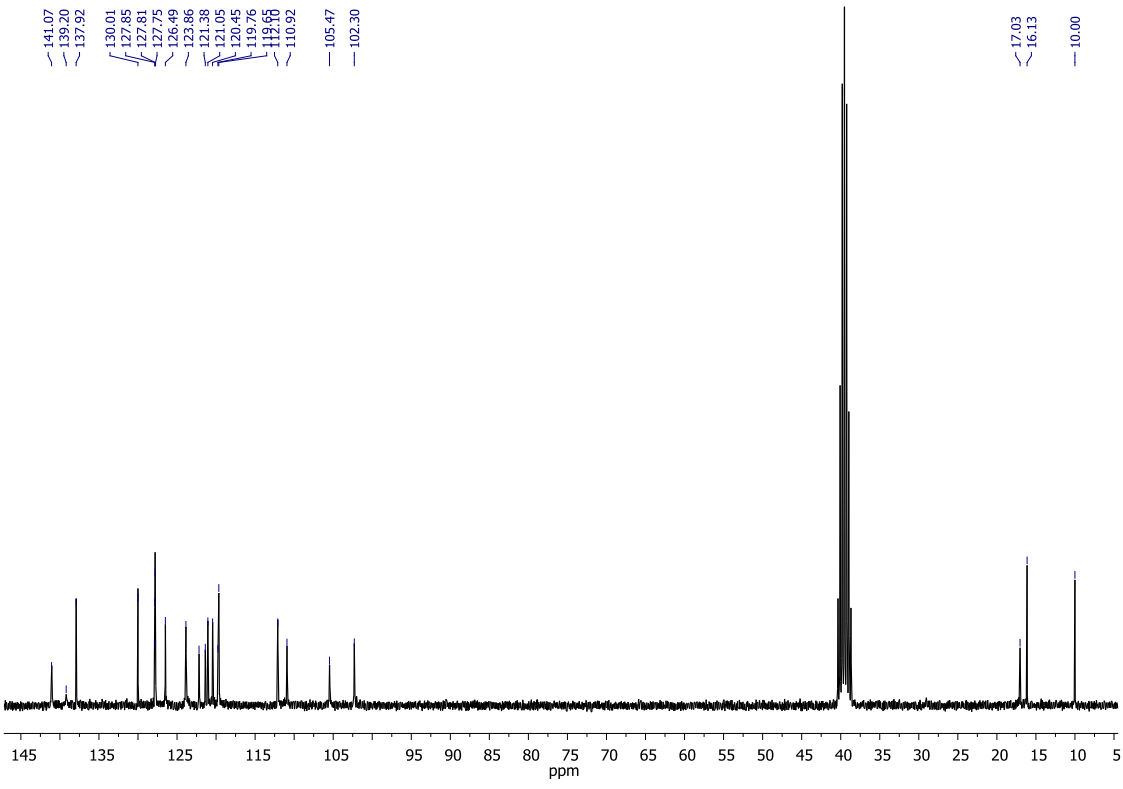


Figure S39. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) for compound **6**·HCl.

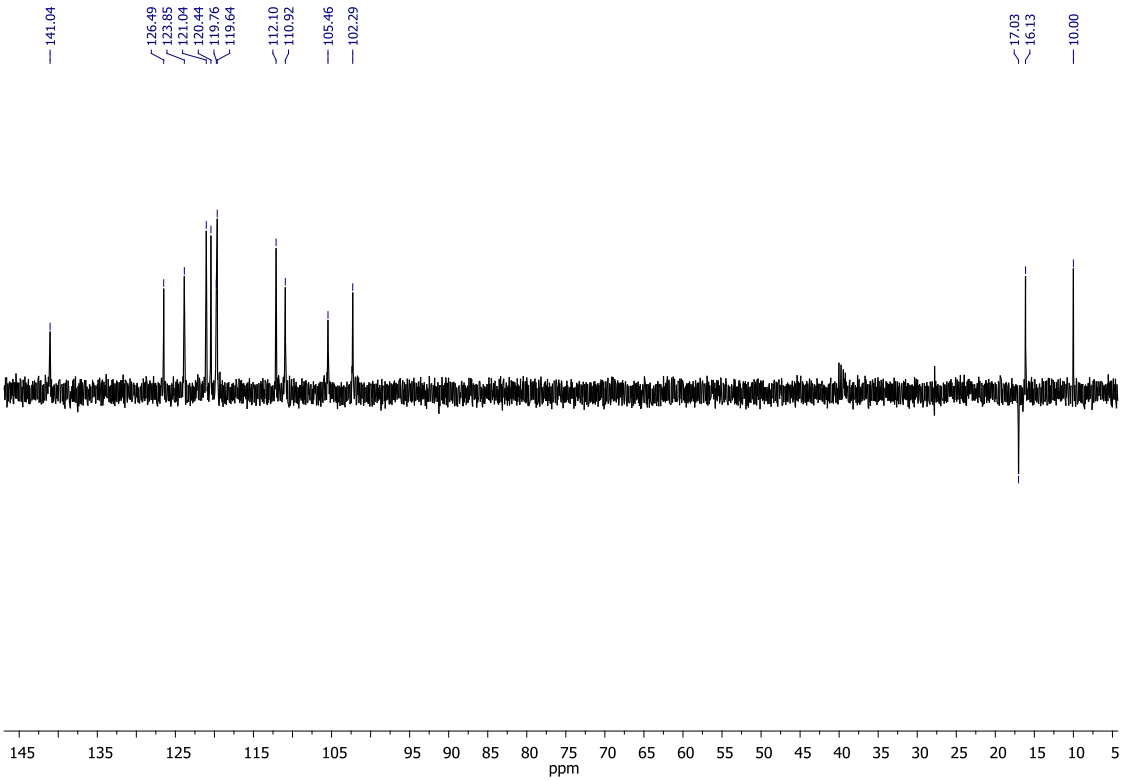


Figure S40. ^{13}C NMR dept135 spectrum (75 MHz, DMSO-d_6) for compound **6**·HCl.

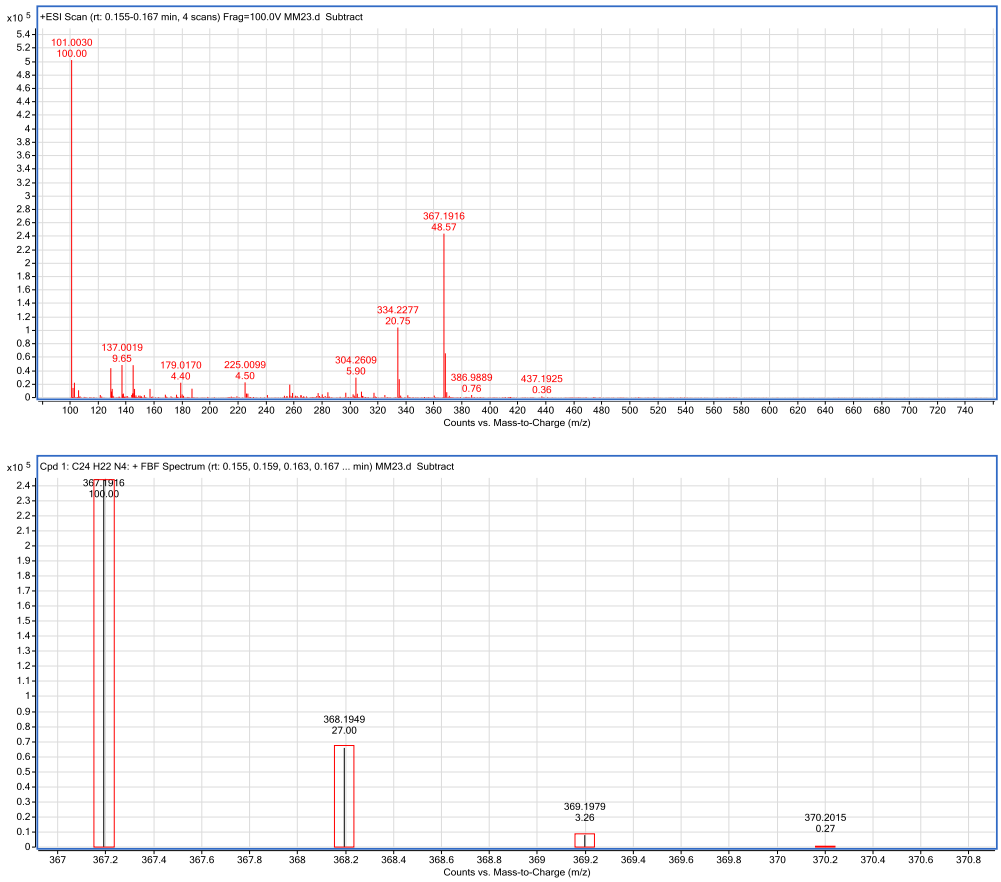


Figure S41. HR-MS (+ESI) spectrum for compound **6**·HCl.

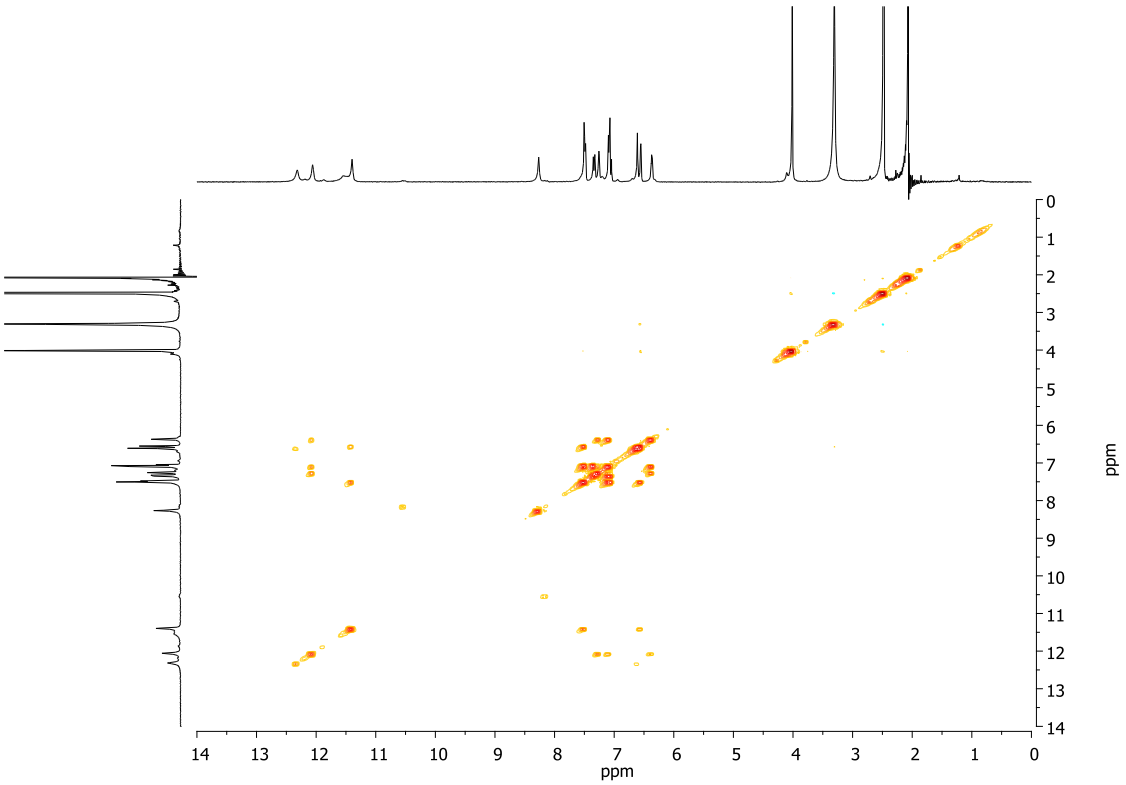


Figure S42. ^{1}H-^{1}H COSY spectrum (300 MHz, DMSO-d_6) for compound **1**·$HClO_4$.

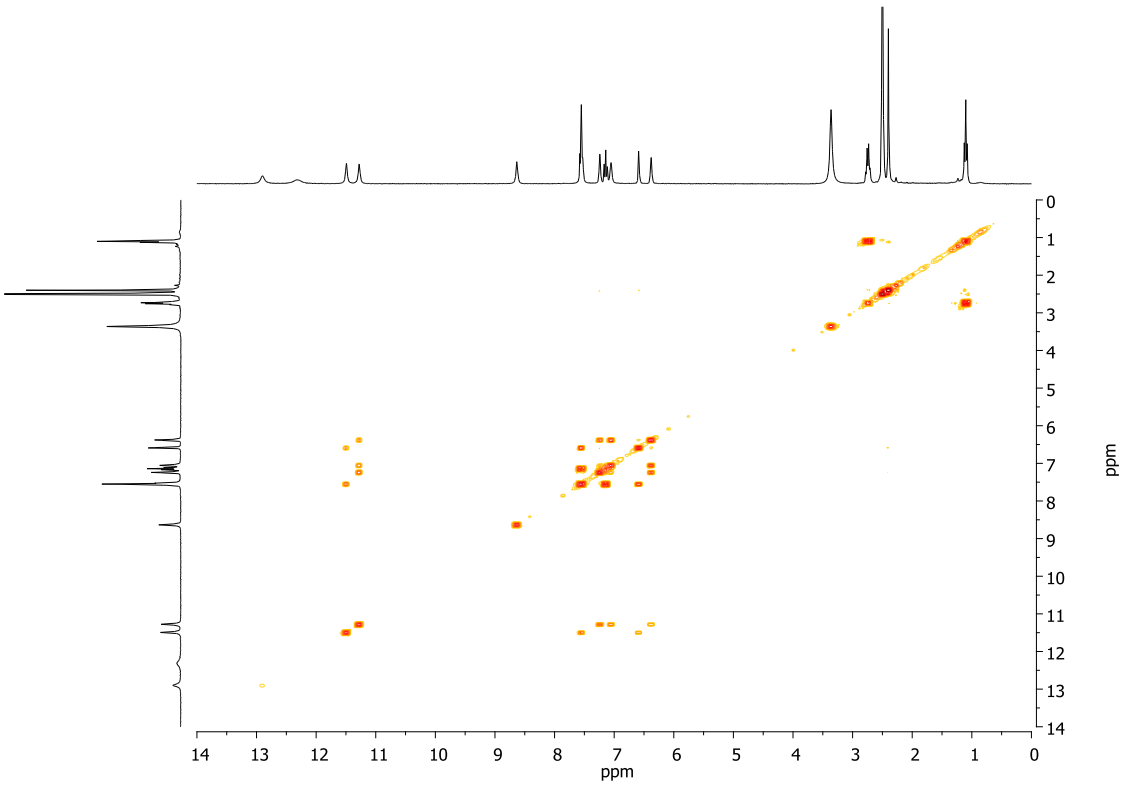


Figure S43. ^{1}H-^{1}H COSY spectrum (300 MHz, DMSO-d_6) for compound **2**·$HClO_4$.

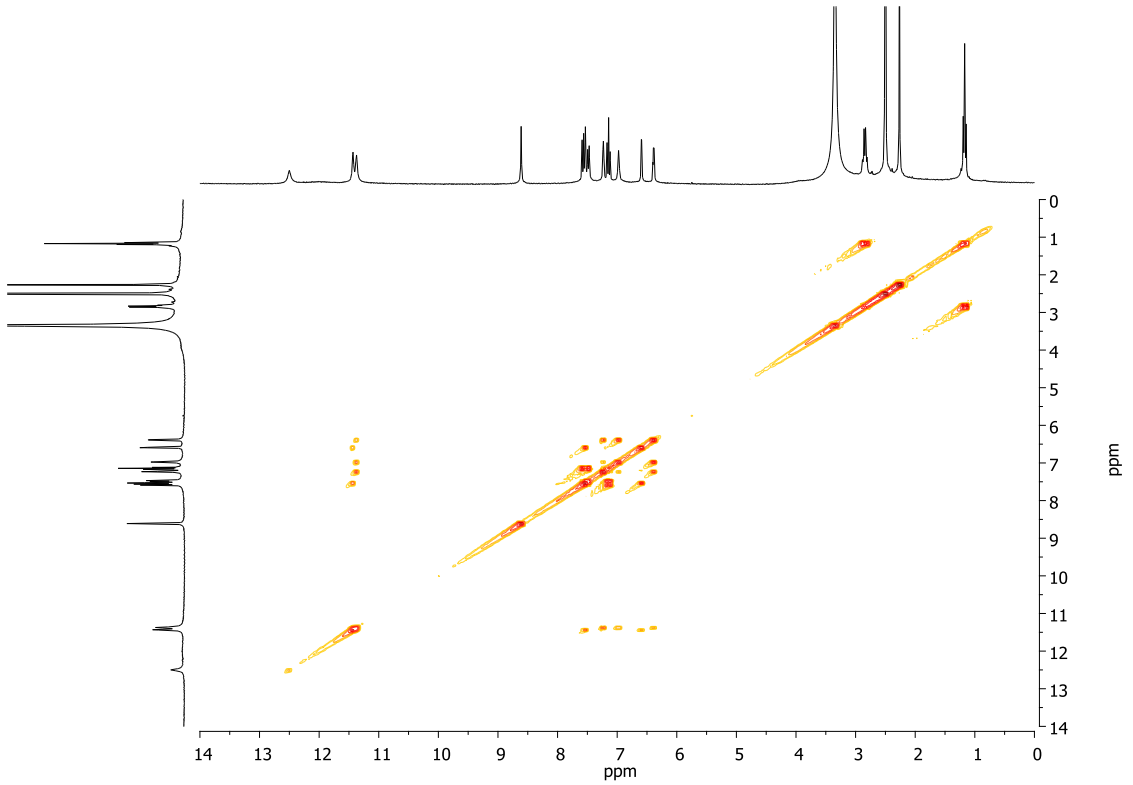


Figure S44. ^{1}H-^{1}H COSY spectrum (300 MHz, DMSO-d_6) for compound **3**·$HClO_4$.

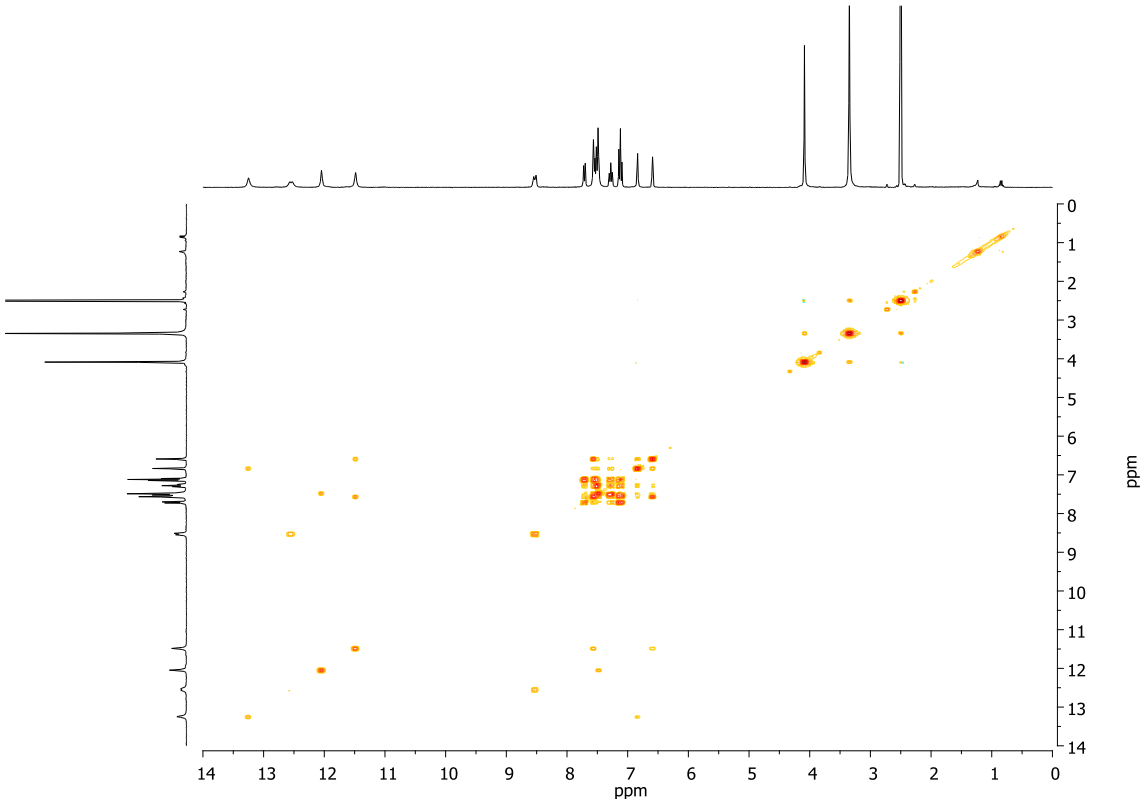


Figure S45. ^{1}H-^{1}H COSY spectrum (300 MHz, DMSO-d_6) for compound **4**·$HClO_4$.

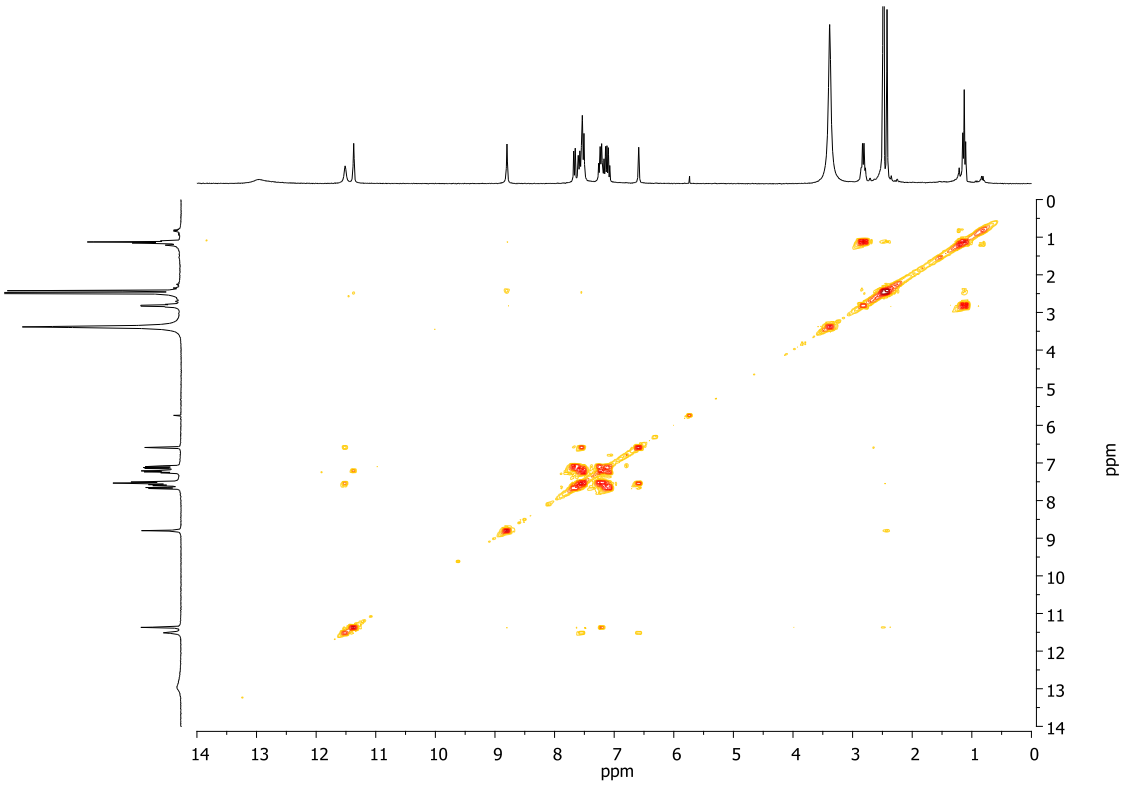


Figure S46. ^{1}H-^{1}H COSY spectrum (300 MHz, DMSO-d_6) for compound **5**·$HClO_4$.

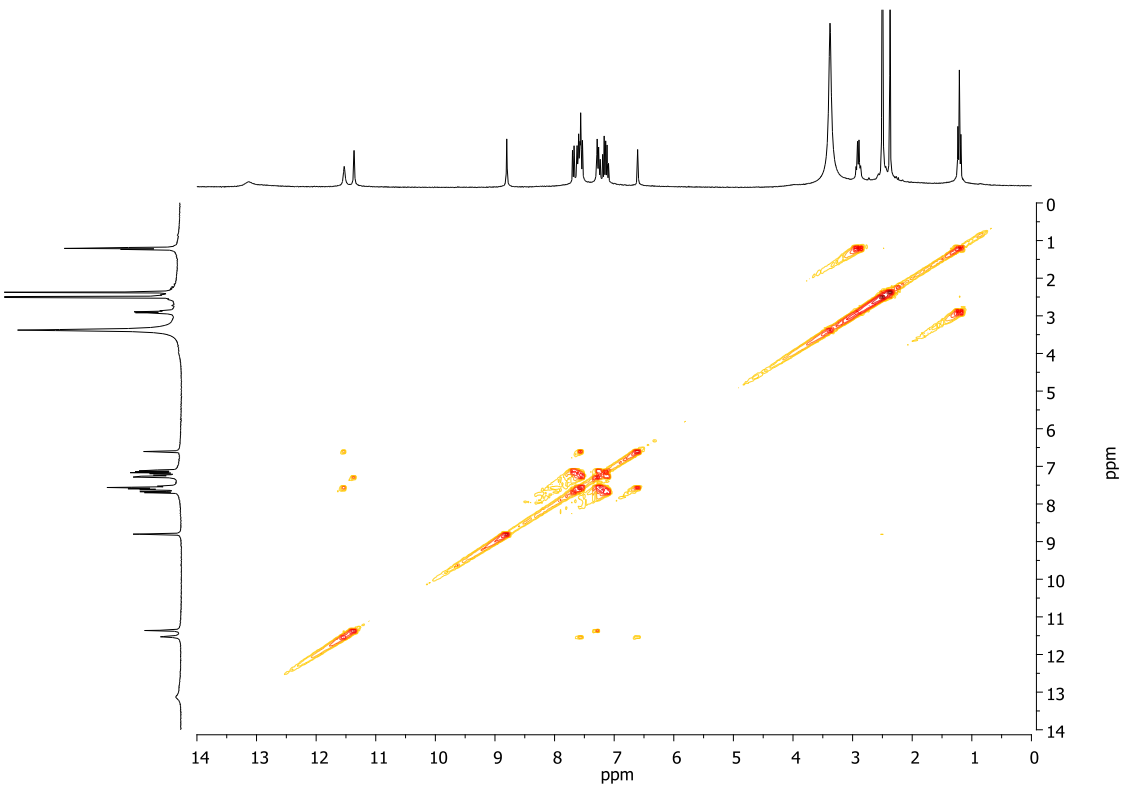


Figure S47. ^{1}H-^{1}H COSY spectrum (300 MHz, DMSO-d_6) for compound **6**·$HClO_4$.

2. X-RAY DIFFRACTION STUDIES

Solid state X-ray structure of compound 2·HCl

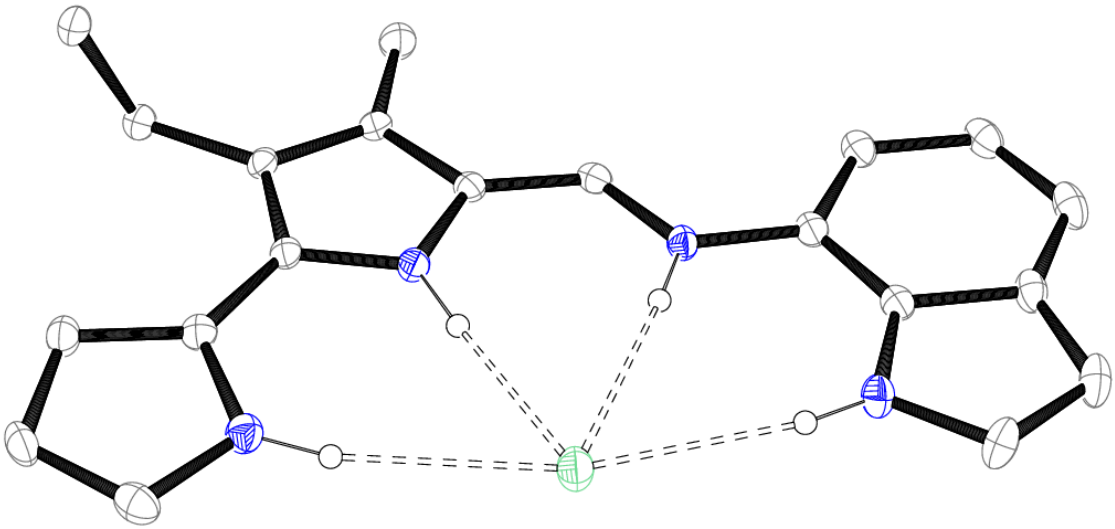


Figure S48. Solid state X-ray structure of compound **2**·HCl. Hydrogen atoms, except those involved in hydrogen-bonding interactions, are omitted for the sake of simplicity. The ORTEP plot is at the 30% probability level.

Table S1. Crystal data and refinement details for **2**·HCl.

formula	$C_{20}H_{21}ClN_4$
MW	352.86
crystal system	Monoclinic
space group	$P2_1/n$
T/K	100(2)
a/Å	8.463(3)
b/Å	23.161(8)
c/Å	9.168(3)
α/deg	90
β/deg	104.664(7)
γ/deg	90
V/Å^3	1738.4(1)
F(000)	744
Z	4
λ, Å (MoK_α)	0.71073
D_{calc}/g cm^{-3}	1.348
μ/mm^{-1}	0.230
θ range/deg	1.76–28.26
R_{int}	0.1022
reflections measured	19796
unique reflections	4016
reflections observed	2198
GOF on F^2	0.874
$R1^a$	0.0543
$wR2^b$	0.1086
Largest ≠ peak & hole/eÅ^{-3}	0.396 and -0.397

^{a} $R1 = \sum ||F_0| - |Fc|| / \sum |F_0|$. ^{b} $wR2$ (all data) $= \{\sum[w(|F_0|^2 - |Fc|^2)^2]/\sum[w(F_0^4)]\}^{1/2}$

Single crystals of compound **2**·HCl were obtained by slow evaporation of a solution of the isolated compound in a 1:1 chloroform-methanol mixture.

Three dimensional X-ray data were collected on a BRUKER SMART APEX CCD diffractometer. Complex scattering factors were taken from the program SHELX-2016[5] running under the WinGX program system[6] as implemented on a Pentium® computer. The structure was solved with SIR92[7] and refined by full-matrix least-squares on F^2. All hydrogen atoms, except those corresponding to the NH fragments of the imine function and the pyrrole and indole rings, which were refined freely in the final stages of refinement, were included in calculated positions and refined in riding mode. Refinement converged with anisotropic displacement parameters for all non-hydrogen atoms. Crystal data and details on data collection and refinement are summarised in **Table S1**.

[5] SHELX-2016: Sheldrick, G. M. *Acta Cryst*. **2008**, *A64*, 112-122.
[6] WinGX: Farrugia, L. J. *J. Appl. Cryst*. **1999**, *32*, 837-838.
[7] SIR92: Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Cryst.* **1994**, *27*, 435.

3. ^{1}H NMR TITRATIONS

3.1. Titration procedure and titration data fitting

Dichloromethane solutions of the hydrochloric salts of the six compounds were washed three times with a 1 M sodium hydroxide aqueous solution and, after that, they were rinsed, also three times, with a 1 M perchloric acid aqueous solution, yielding the corresponding hydroperchloric salts. 2 mL of a DMSO-d_6 stock solution of the corresponding tambjamine in its perchlorate form (host) were prepared (0.01 M). From this solution, 1 mL was taken to prepare the solution of the titrating agent (guest; the corresponding tetrabutylammonium –chloride or nitrate– or tetraethylammonium –bicarbonate– salt); in this way the dilution effect is avoided. 0.5 mL of the solution of the host were put into an NMR tube, which was capped with a septum, and the ^{1}H NMR spectrum was recorded. Subsequently, an aliquot of the solution of the guest was added with a proper microsyringe through the septum, the solution homogenised and the spectrum recorded. This process was repeated 20 times. For bicarbonate, an additional experiment was performed to confirm the deprotonation and coordination processes that take place: aliquots of a TBAOH solution were added (up to roughly 1 eq.), followed by the addition of aliquots of a $TEAHCO_3$ solution. For the dilution studies, compound **1** was used as model. 0.05 M DMSO-d_6 solutions of **1**·HCl and **1**·$HClO_4$ were diluted several times and the spectra recorded.

For each ^{1}H NMR titration, the signals of one, two or three NH protons of the molecules were monitored for changes in chemical shift, which provided several data sets that were employed in the determination of the equilibrium constants *K*. The fitting, performed with the Bindfit software,[1] takes into account all data sets at the same time, thus improving the quality of the non-linear curve fitting. Different binding models were tried to fit the data in the best possible way. In the case of the chloride adducts, the 1:1 (LH:Cl) binding model was attempted in the first place; however, the experimental data did not fit satisfactorily to this model for any of the six compounds. The 2:1 (LH:Cl) binding model, both fully cooperative and non-cooperative, was also tried, the latter being the one that best fitted to the data in all cases. Hence, this model was selected, and **Figs. S55-S66** display the fitted binding isotherms and the species distribution diagrams for the titrations of the perchlorate salts derived from the six tambjamines with TBACl. In this way, it was possible to calculate K_{11} with acceptable errors. For all the nitrate adducts the 1:1 (LH:NO_3) binding model was used, as it provided a complete fit of the data, and the K_a values were determined (**Figs. S73-S84**). Concerning the dilution studies, for both **1**·HCl and **1**·$HClO_4$ the data were fitted to the 1:1 binding model.

3.2. ^{1}H NMR titration spectra and fitted binding isotherms

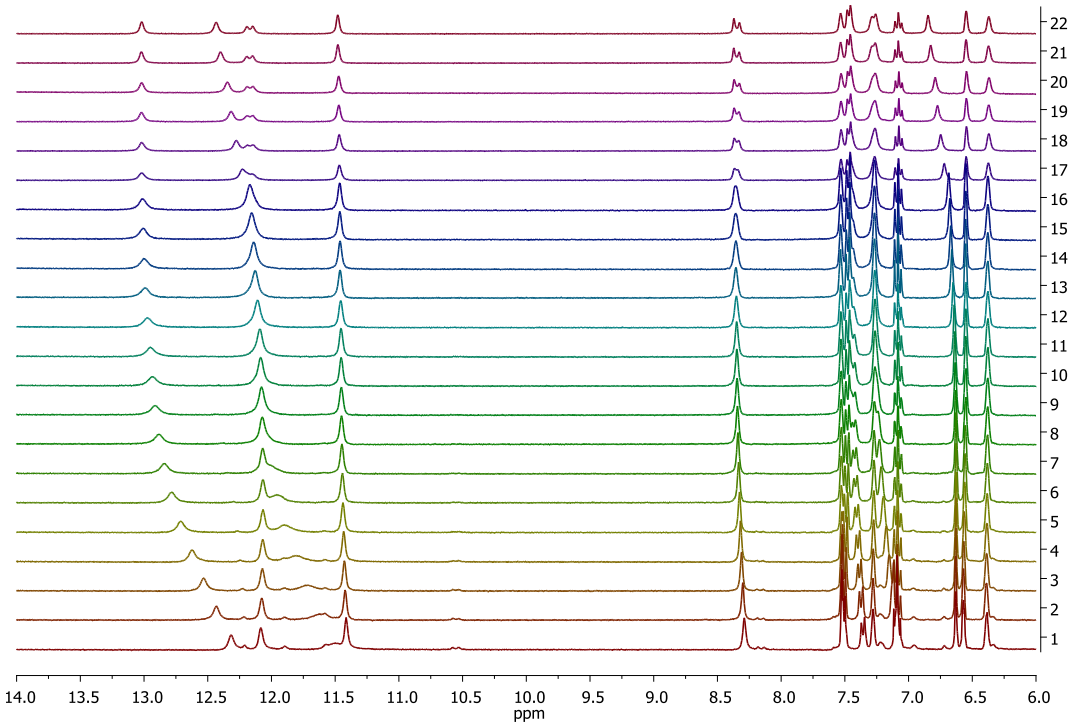


Figure S49. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl, prepared with a 0.01 M solution of **1**·$HClO_4$, to a 0.01 M solution of **1**·$HClO_4$.

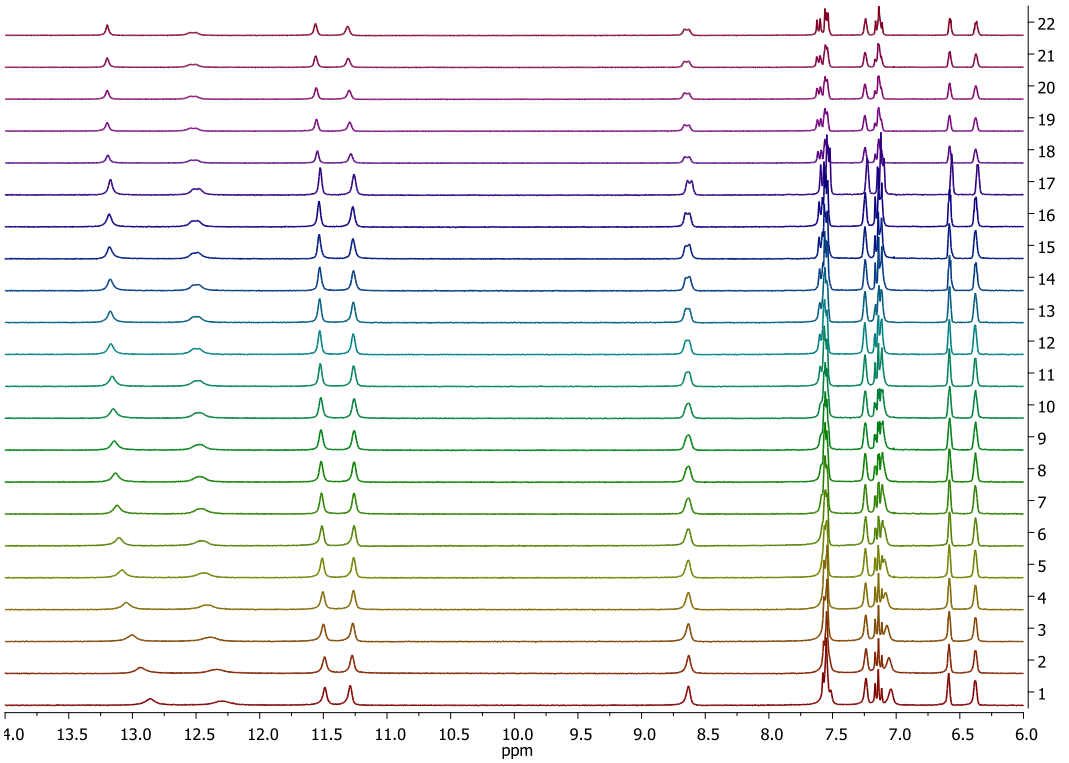


Figure S50. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl, prepared with a 0.01 M solution of **2**·$HClO_4$, to a 0.01 M solution of **2**·$HClO_4$.

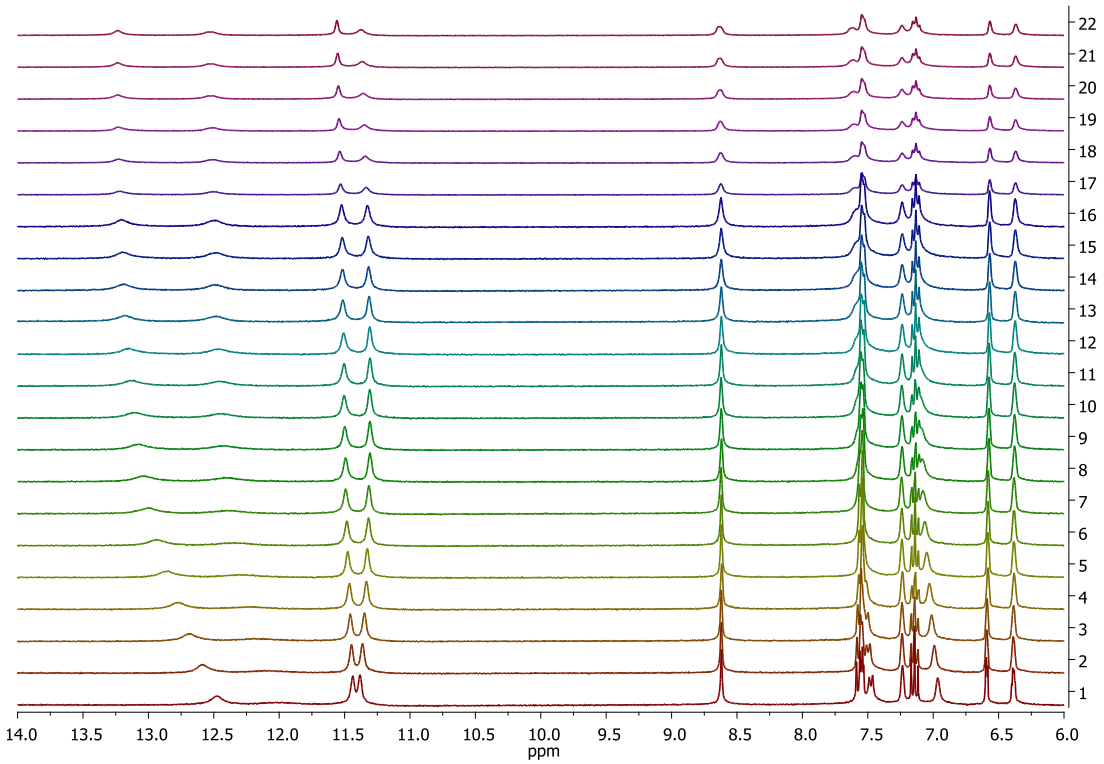


Figure S51. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl, prepared with a 0.01 M solution of **3**·$HClO_4$, to a 0.01 M solution of **3**·$HClO_4$.

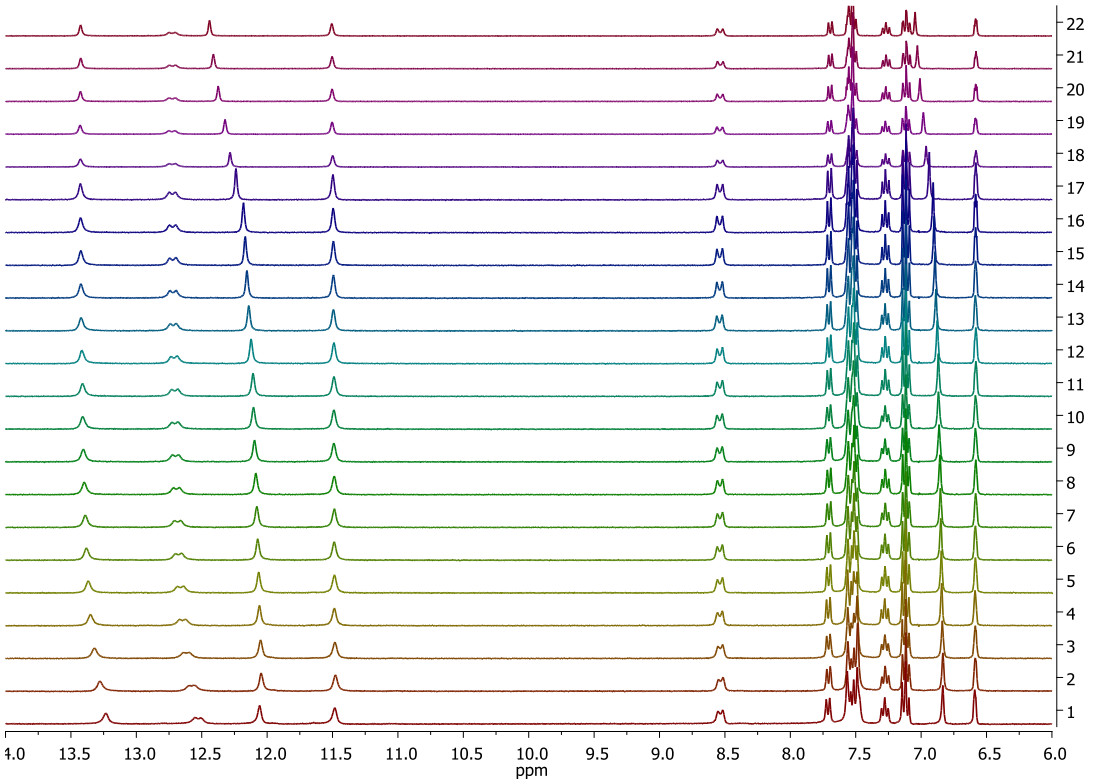


Figure S52. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl, prepared with a 0.01 M solution of **4**·$HClO_4$, to a 0.01 M solution of **4**·$HClO_4$.

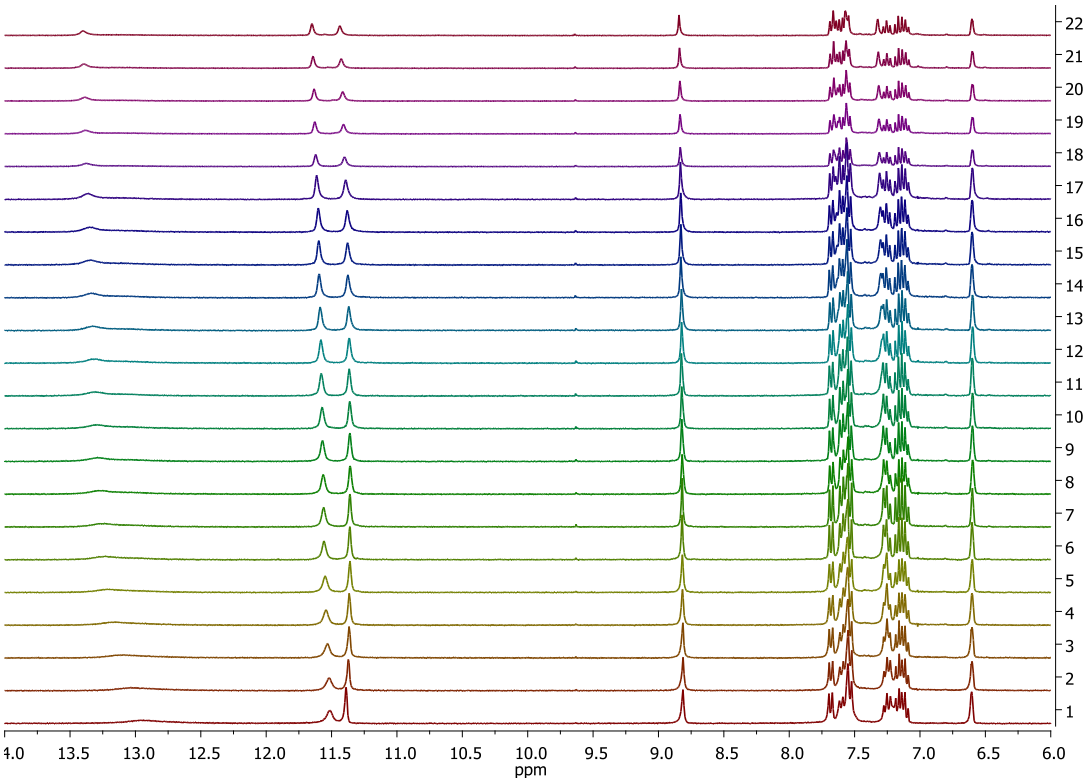


Figure S53. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl, prepared with a 0.01 M solution of **5**·$HClO_4$, to a 0.01 M solution of **5**·$HClO_4$.

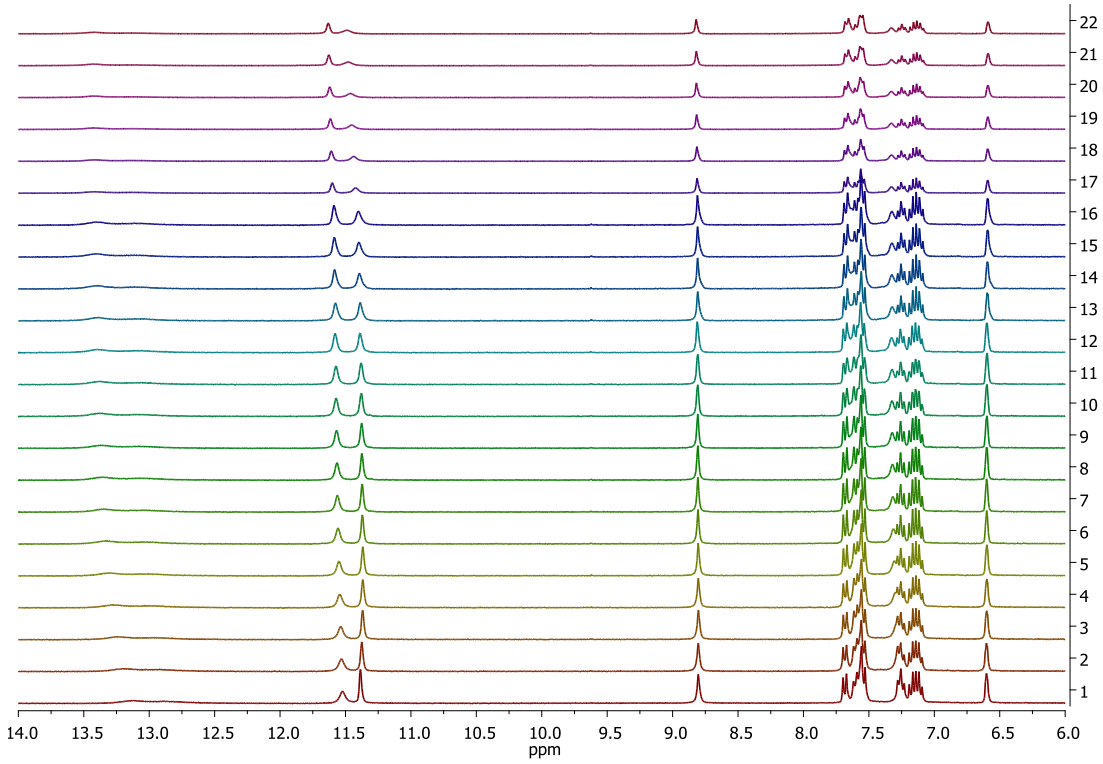


Figure S54. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl, prepared with a 0.01 M solution of **6**·$HClO_4$, to a 0.01 M solution of **6**·$HClO_4$.

Figure S55. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **1**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, fitted to the 2:1 (LH:Cl) non-cooperative binding model. K_{11} = 549±39 M^{-1}.

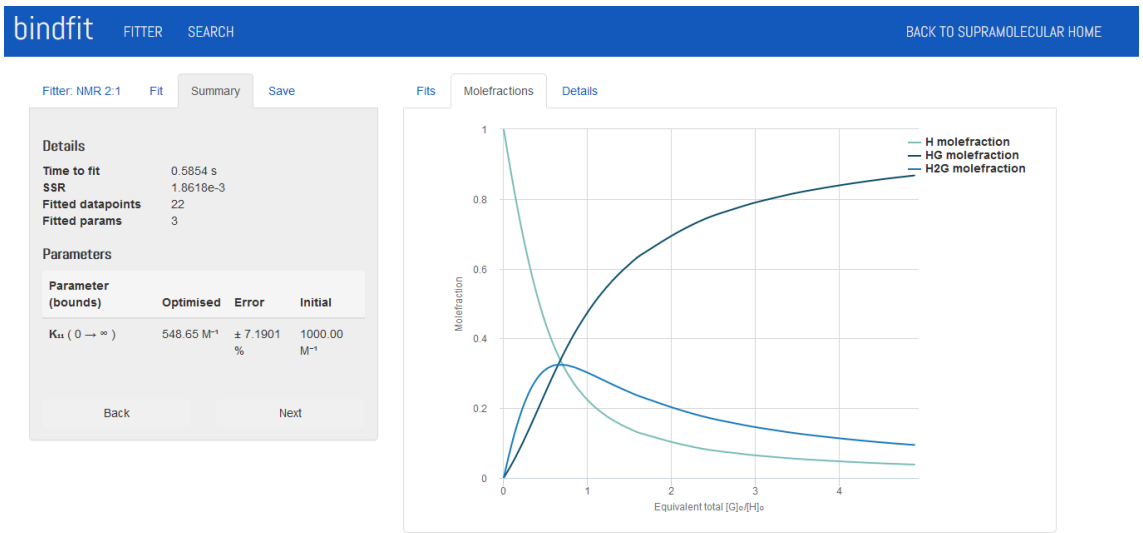


Figure S56. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **1**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the chloride anion.

Figure S57. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **2**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, fitted to the 2:1 (LH:Cl) non-cooperative binding model. K_{11} = 714±70 M^{-1}.

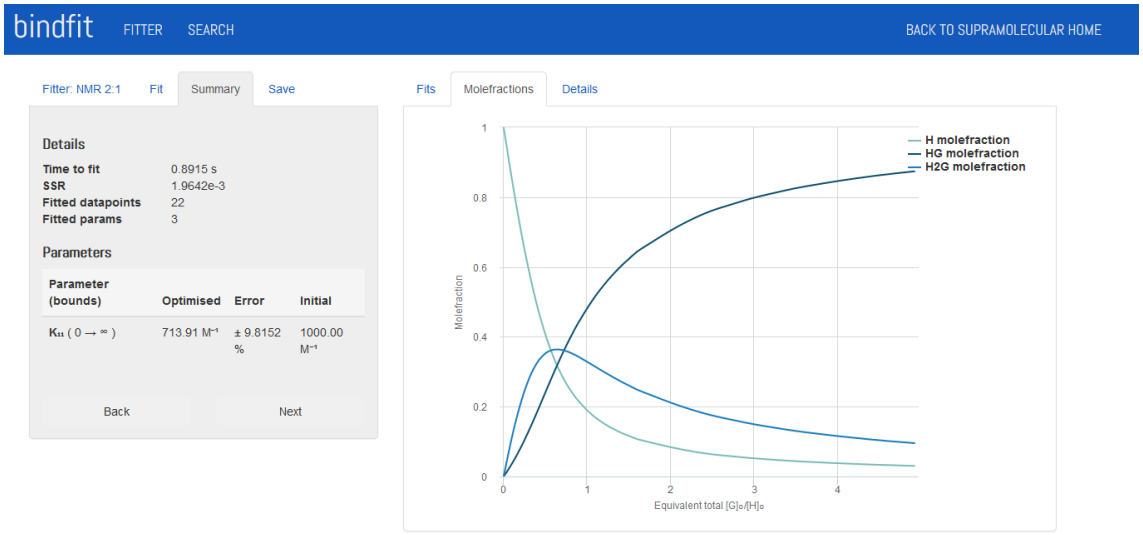


Figure S58. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **2**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the chloride anion.

Figure S59. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **3**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the changes in chemical shift of the signals of three of the NH protons of the molecule, fitted to the 2:1 (LH:Cl) non-cooperative binding model. K_{11} = 3626±528 M^{-1}.

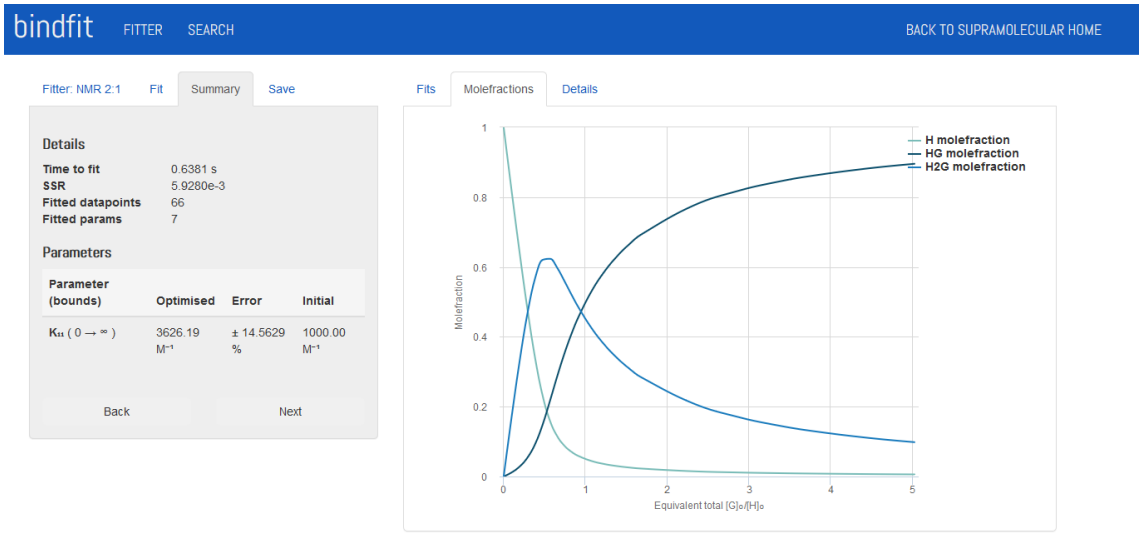


Figure S60. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **3**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the chloride anion.

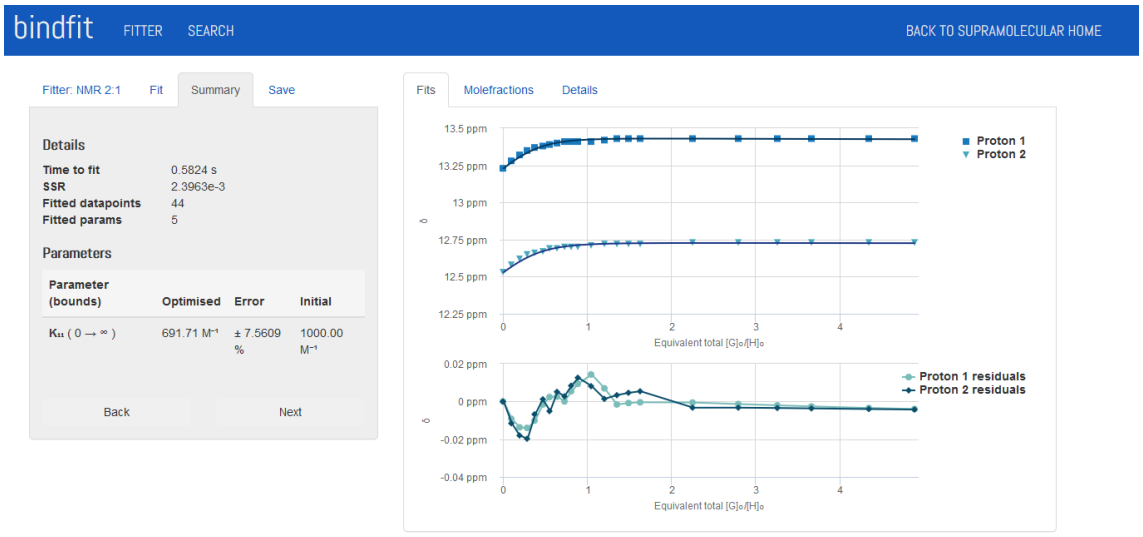


Figure S61. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **4**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the changes in chemical shift of the signals of two of the NH protons of the molecule, fitted to the 2:1 (LH:Cl) non-cooperative binding model. K_{11} = 692±52 M^{-1}.

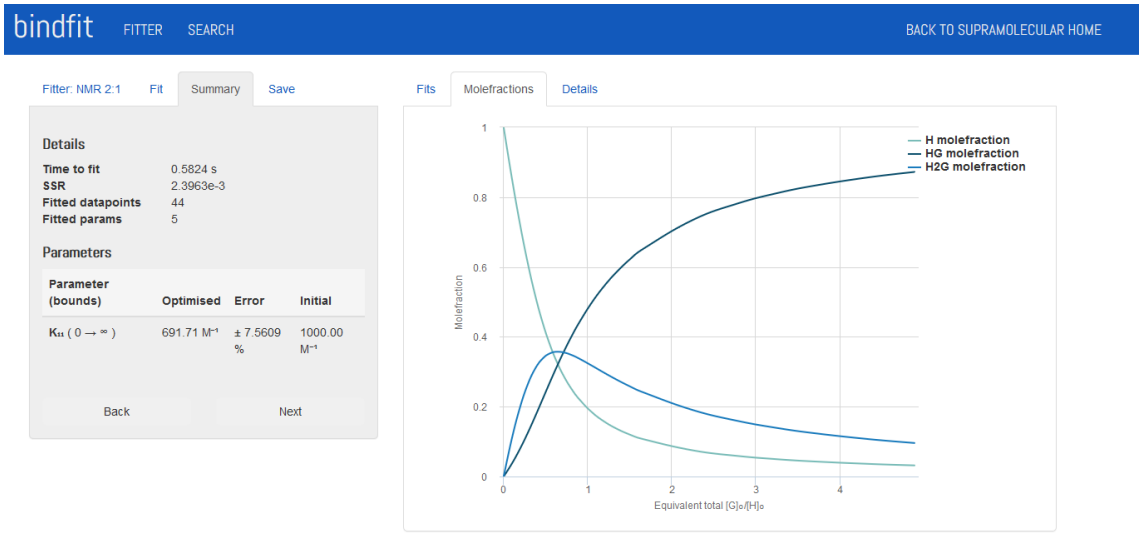


Figure S62. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **4**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the chloride anion.

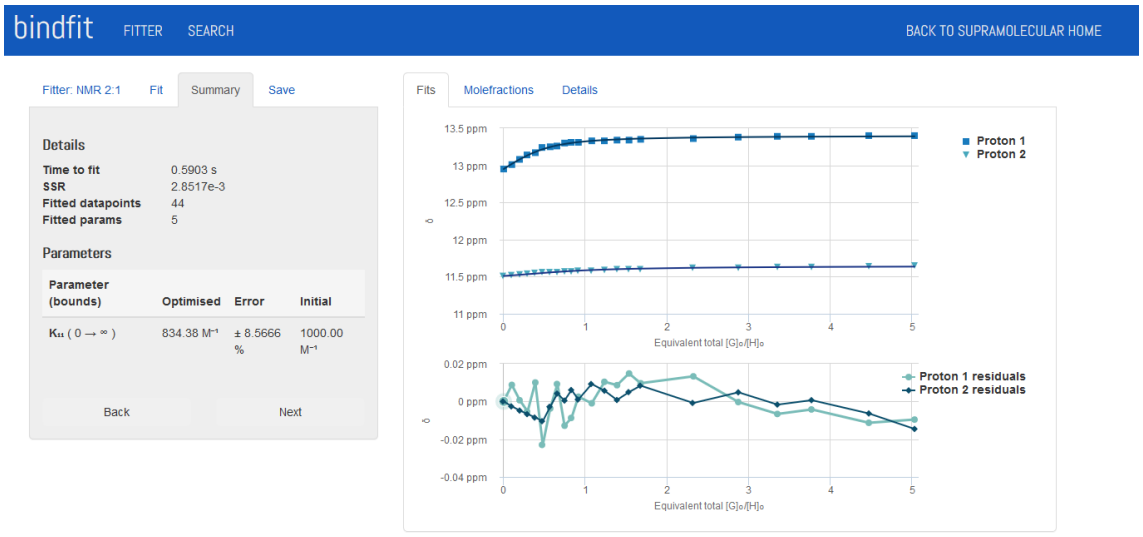


Figure S63. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **5**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the changes in chemical shift of the signals of two of the NH protons of the molecule, fitted to the 2:1 (LH:Cl) non-cooperative binding model. K_{11} = 834±71 M^{-1}.

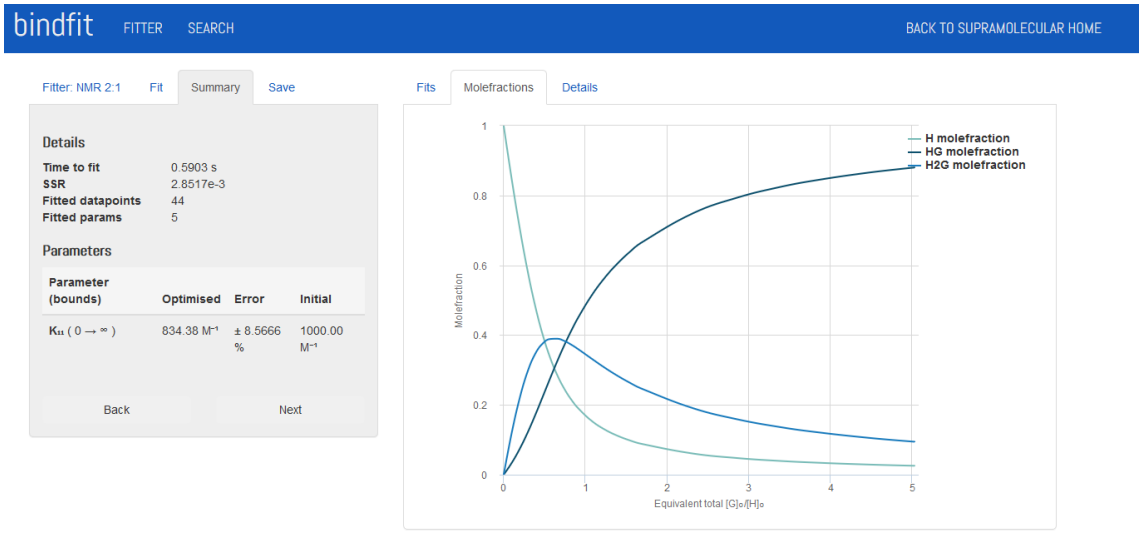


Figure S64. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **5**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the chloride anion.

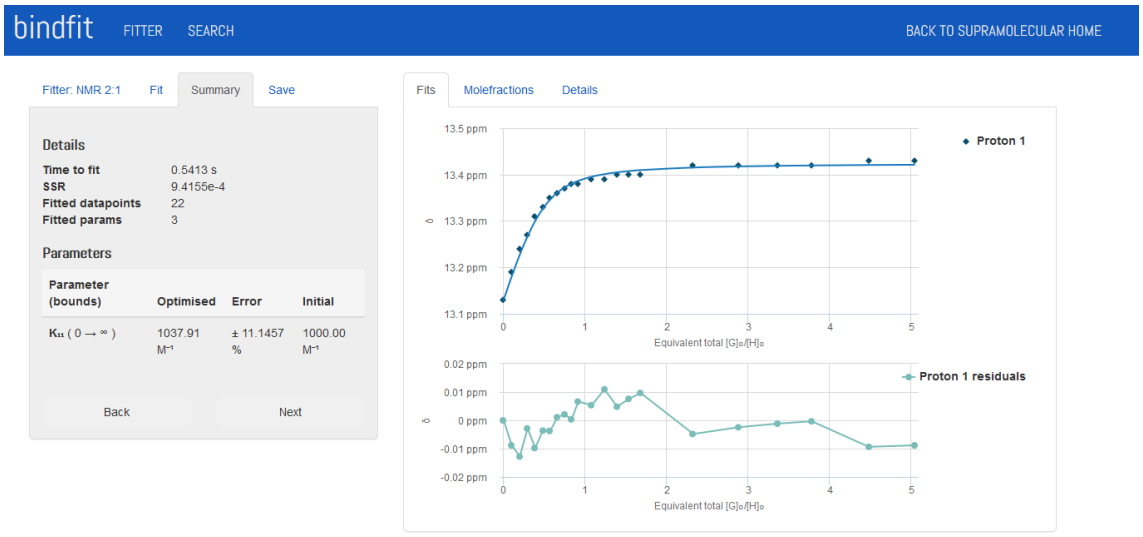


Figure S65. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **6**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, fitted to the 2:1 (LH:Cl) non-cooperative binding model. K_{11} = 1038±116 M^{-1}.

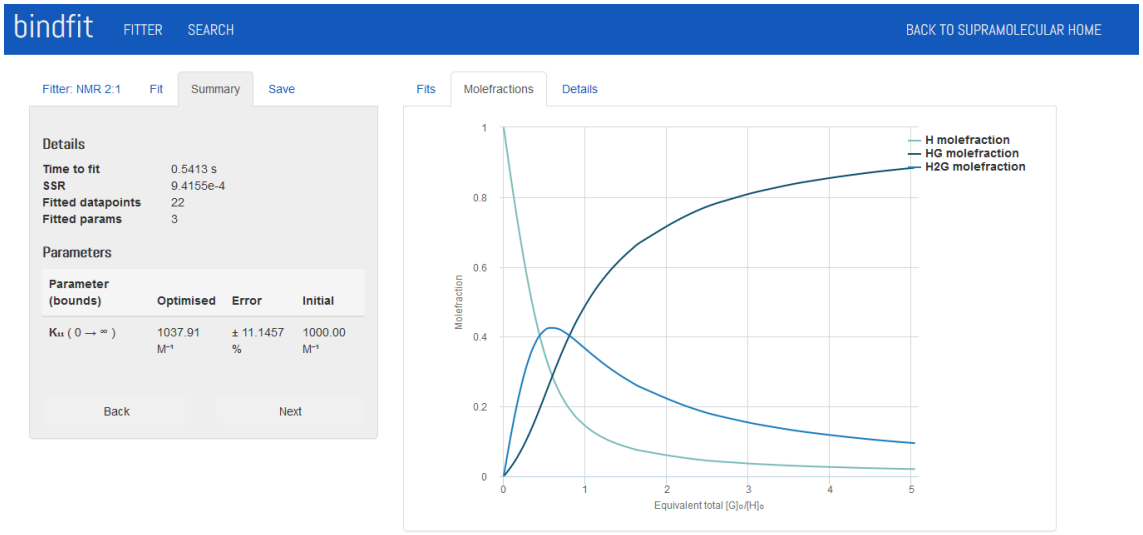


Figure S66. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **6**·$HClO_4$ with a 0.1 M solution of TBACl (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the chloride anion.

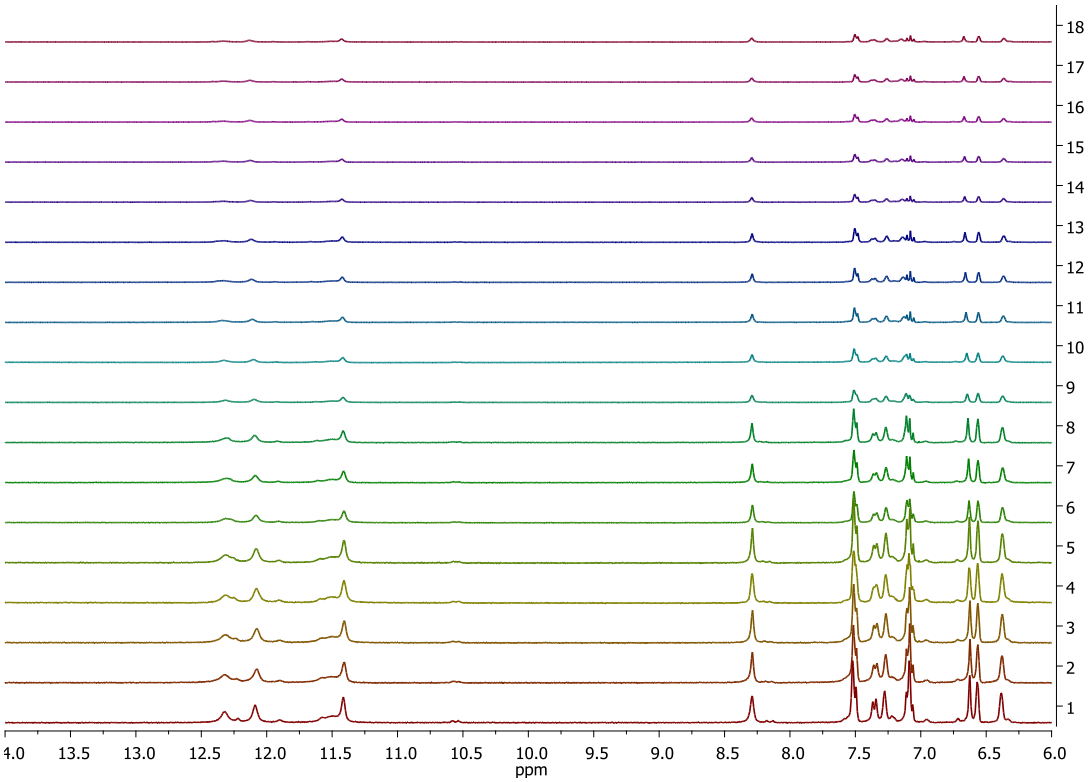


Figure S67. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.6 M solution of $TBANO_3$, prepared with a 0.01 M solution of **1**·$HClO_4$, to a 0.01 M solution of **1**·$HClO_4$.

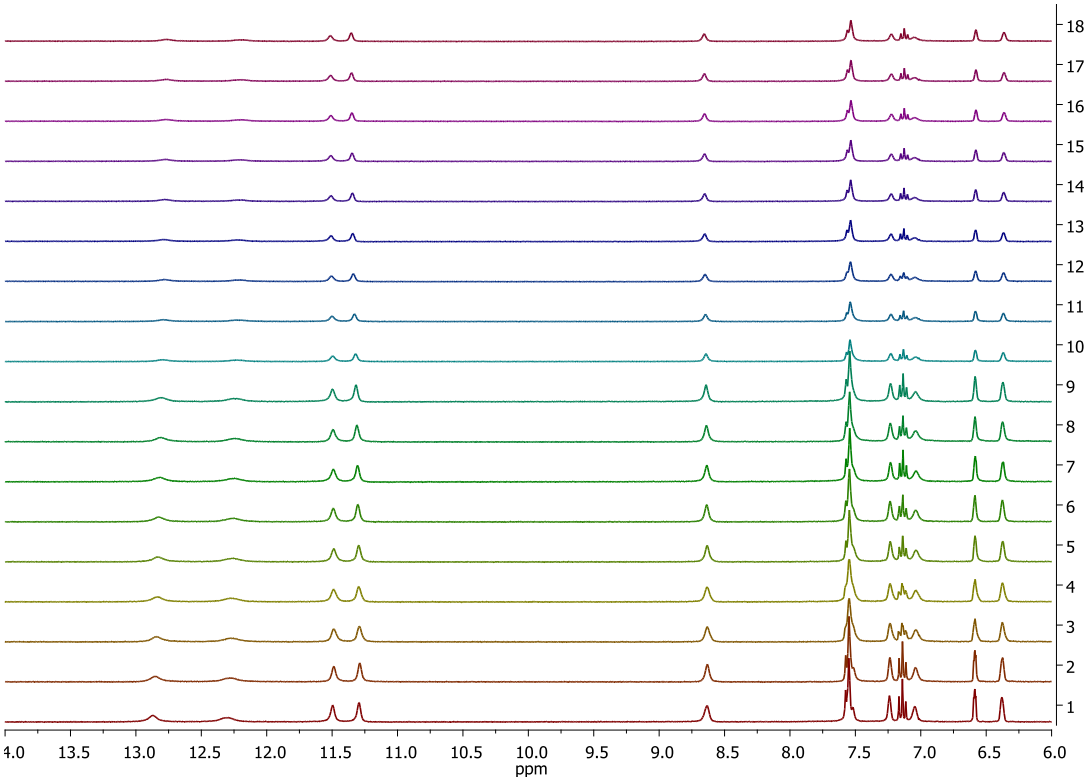


Figure S68. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.6 M solution of $TBANO_3$, prepared with a 0.01 M solution of **2**·$HClO_4$, to a 0.01 M solution of **2**·$HClO_4$.

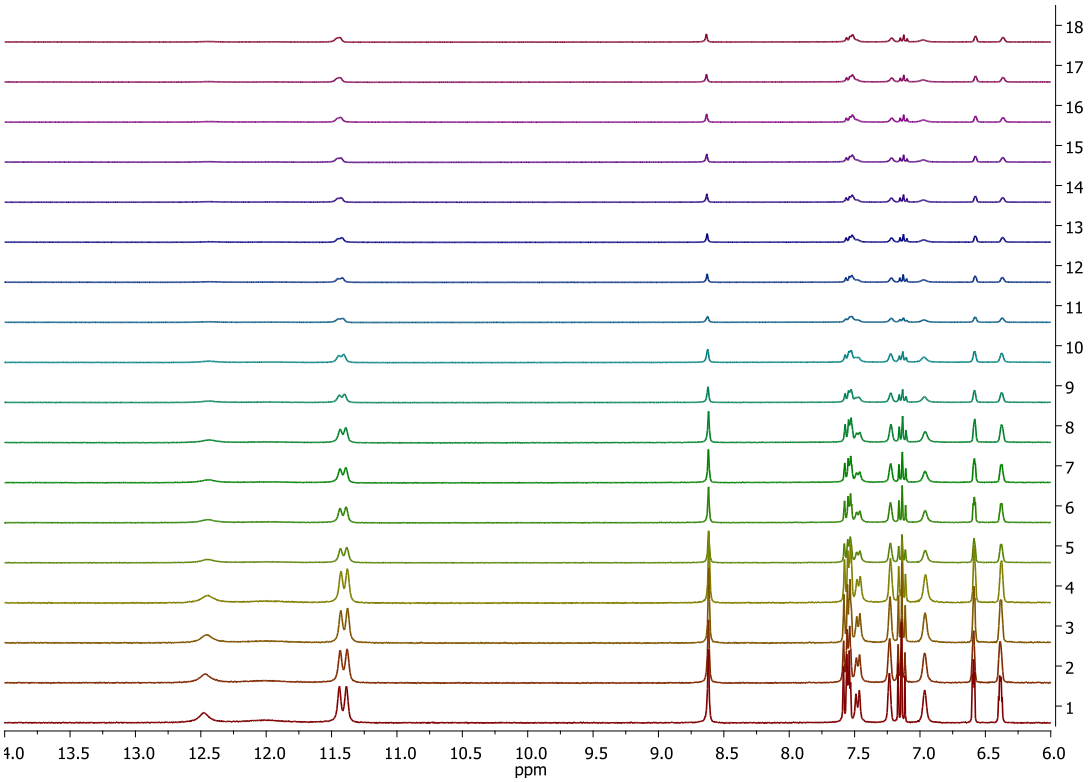


Figure S69. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.6 M solution of $TBANO_3$, prepared with a 0.01 M solution of **3**·$HClO_4$, to a 0.01 M solution of **3**·$HClO_4$.

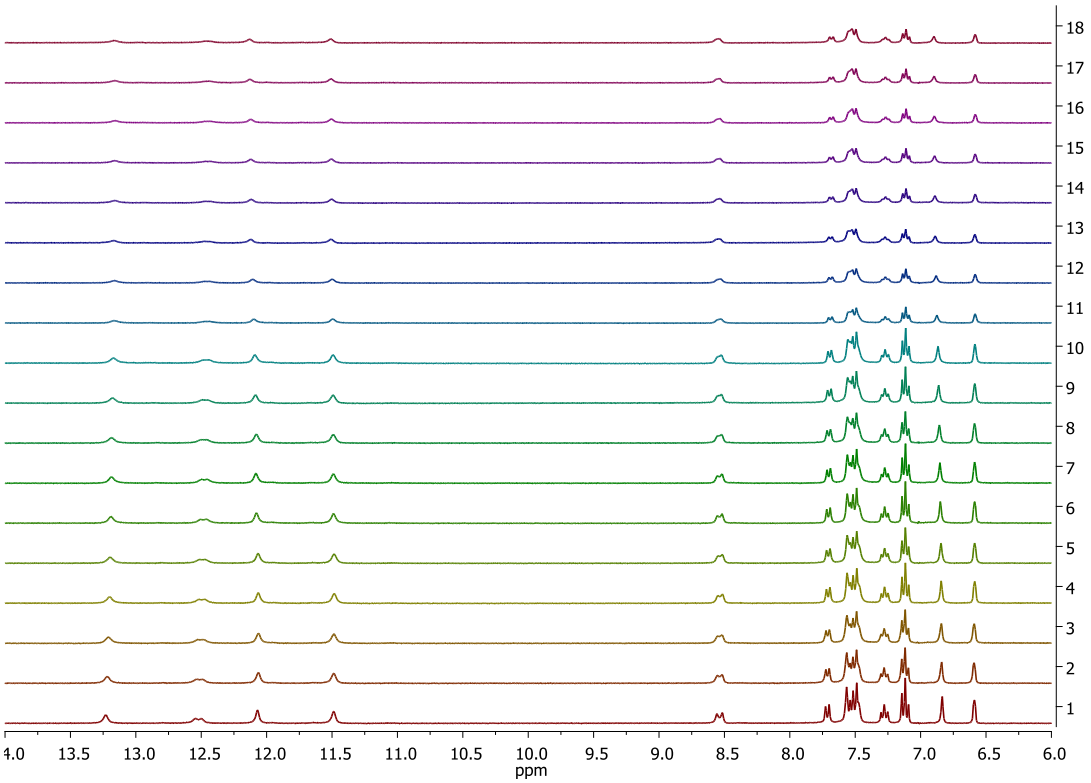


Figure S70. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.6 M solution of $TBANO_3$, prepared with a 0.01 M solution of **4**·$HClO_4$, to a 0.01 M solution of **4**·$HClO_4$.

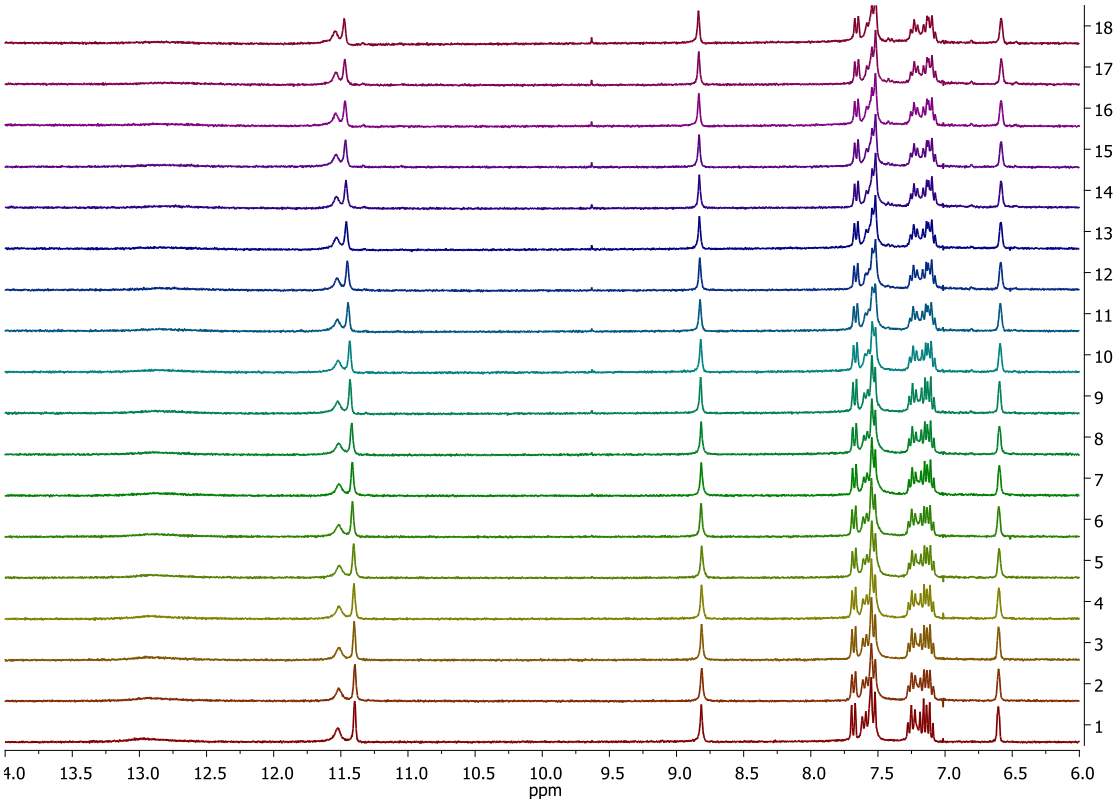


Figure S71. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.6 M solution of $TBANO_3$, prepared with a 0.01 M solution of **5**·$HClO_4$, to a 0.01 M solution of **5**·$HClO_4$.

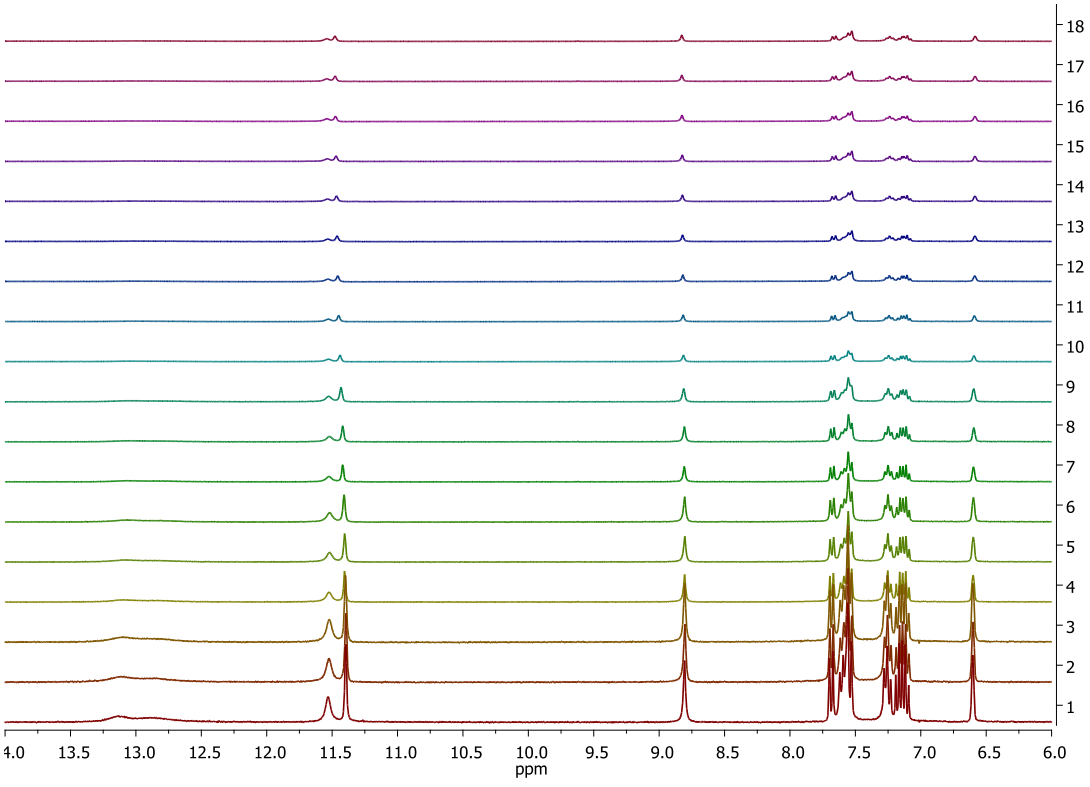


Figure S72. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.6 M solution of $TBANO_3$, prepared with a 0.01 M solution of **6**·$HClO_4$, to a 0.01 M solution of **6**·$HClO_4$.

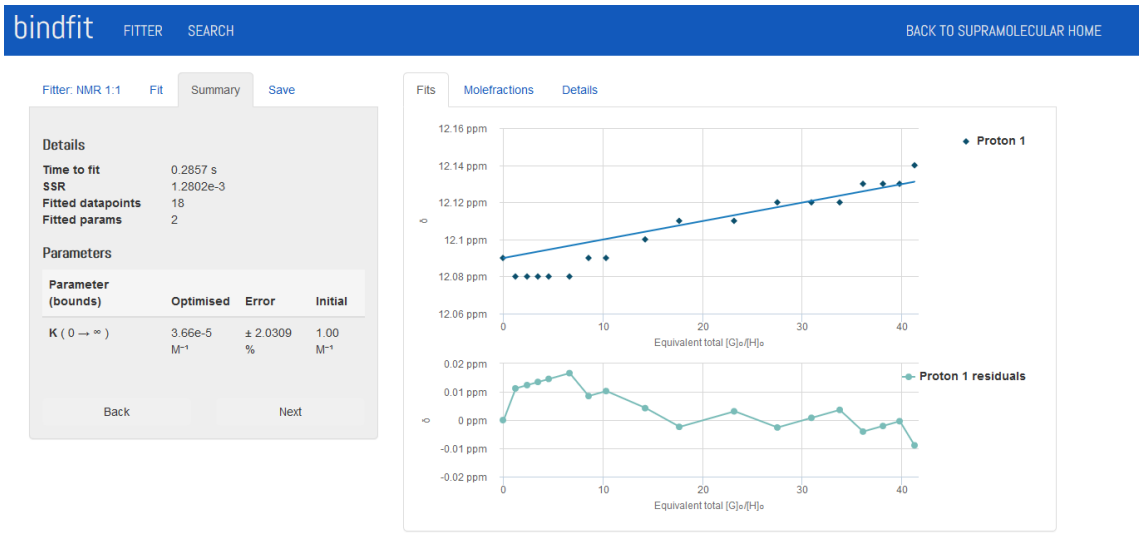


Figure S73. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **1**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, fitted to the 1:1 (LH:NO_3) binding model. K_a = (3.66±0.07)·10^{-5} M^{-1}.

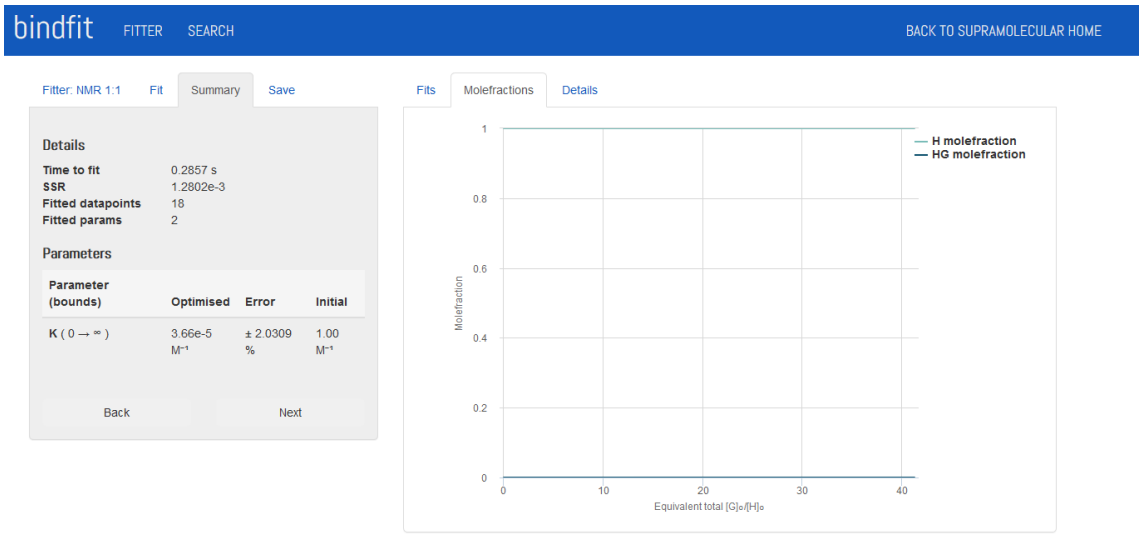


Figure S74. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **1**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the nitrate anion. The association constant K_a is so low that no variations are appreciated in the diagram.

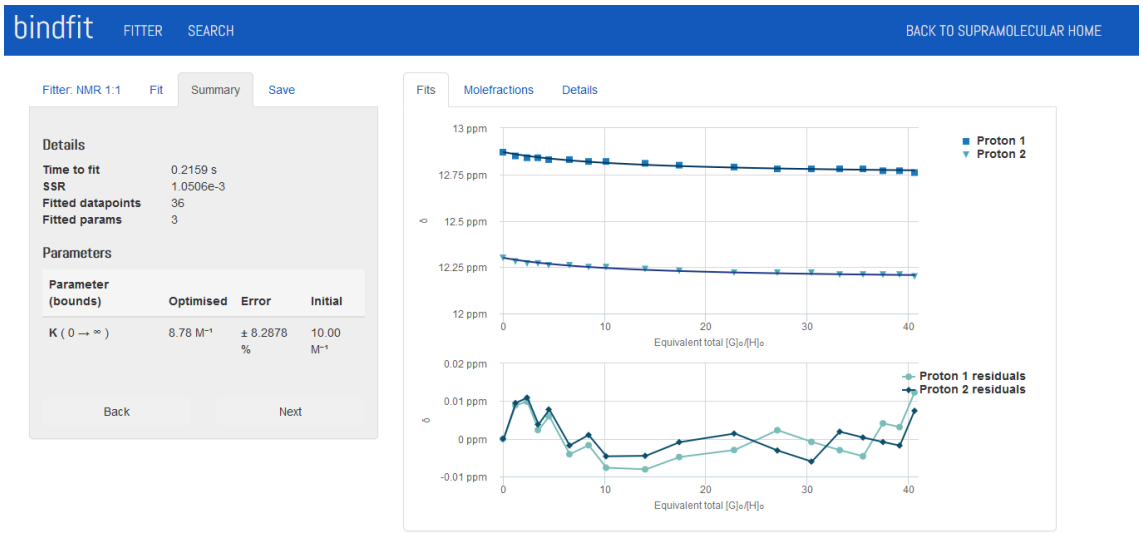


Figure S75. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **2**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the changes in chemical shift of the signals of two of the NH protons of the molecule, fitted to the 1:1 (LH:NO_3) binding model. K_a = 8.8±0.7 M^{-1}.

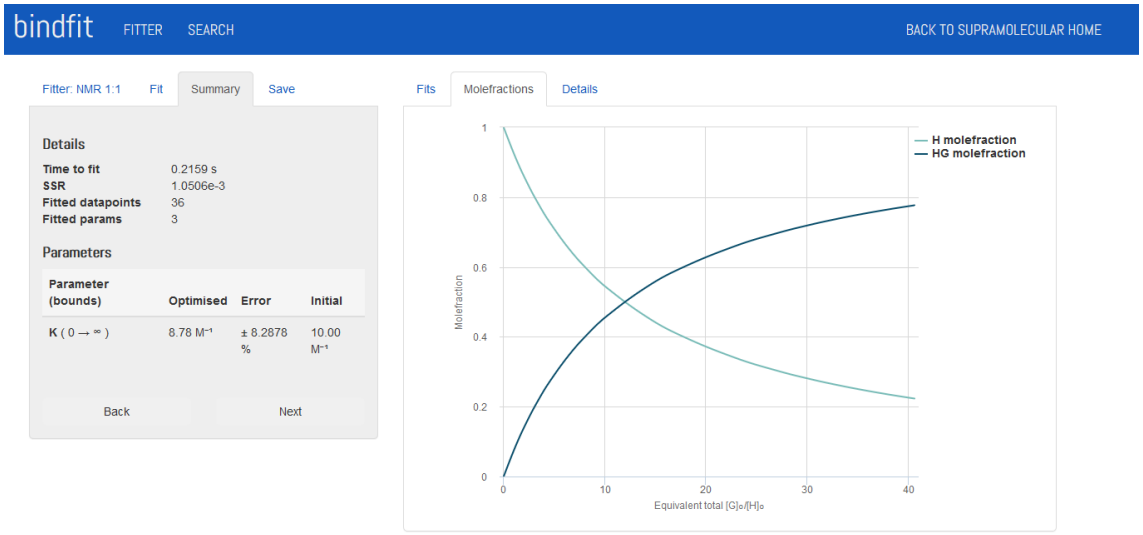


Figure S76. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **2**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the nitrate anion.

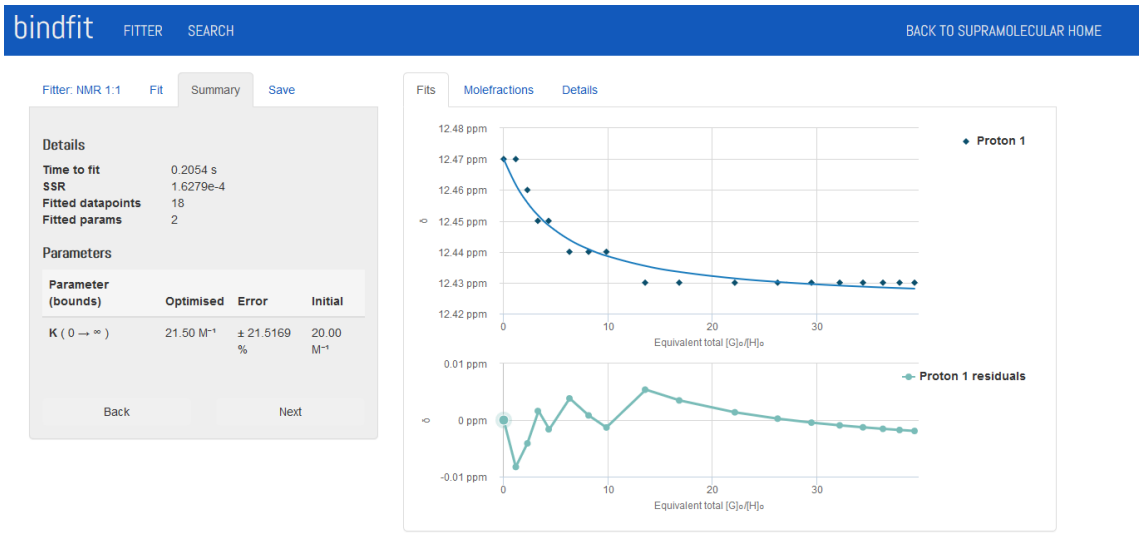


Figure S77. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **3**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, fitted to the 1:1 (LH:NO_3) binding model. K_a = 21.5±4.6 M^{-1}.

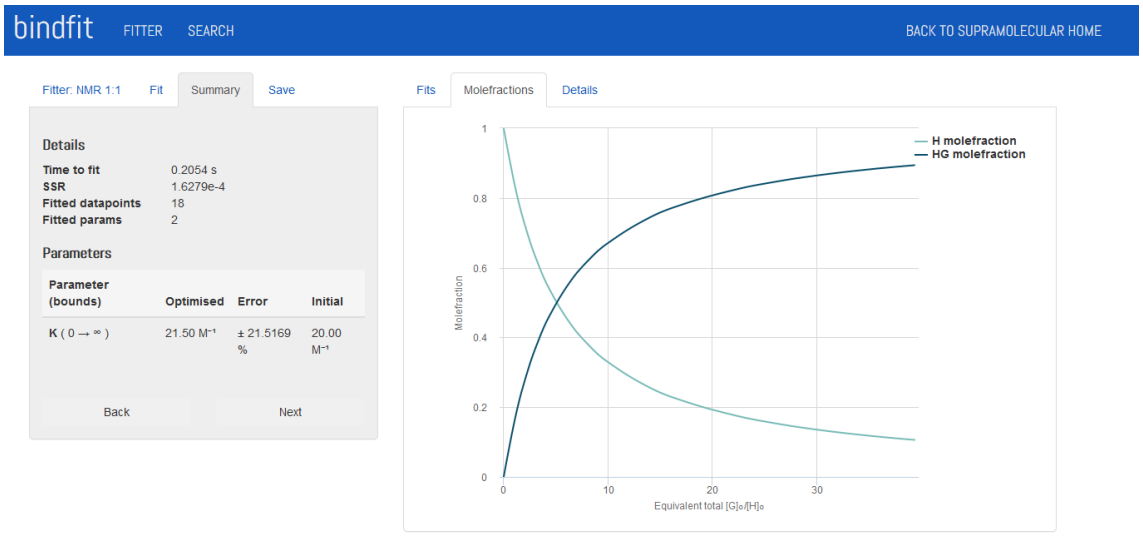


Figure S78. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **3**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the nitrate anion.

Figure S79. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **4**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, fitted to the 1:1 (LH:NO_3) binding model. K_a = 13.8±1.6 M^{-1}.

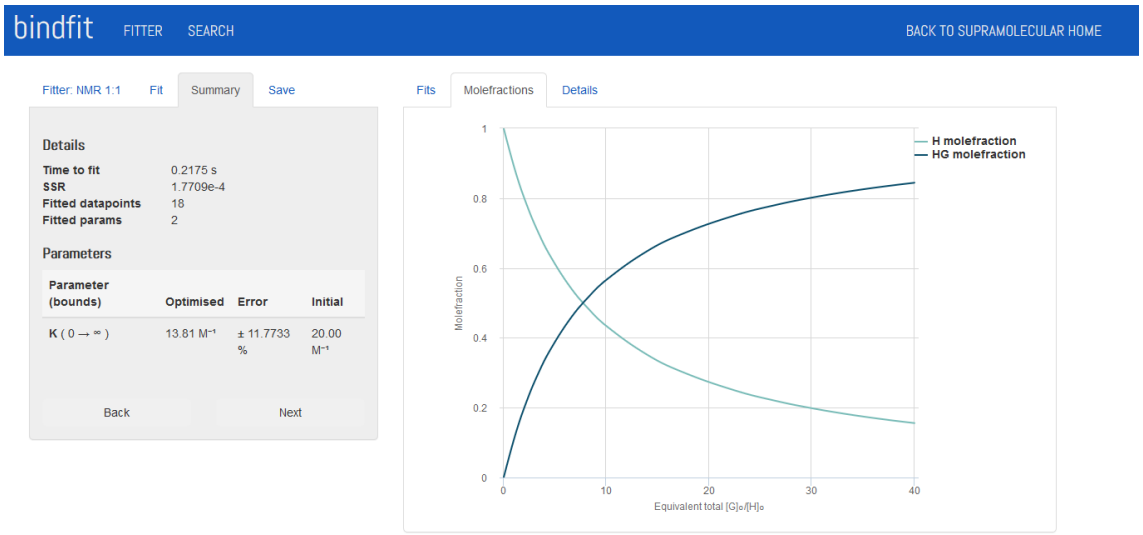


Figure S80. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **4**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the nitrate anion.

Figure S81. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **5**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, fitted to the 1:1 (LH:NO_3) binding model. K_a = (2.73±0.01)·10^{-5} M^{-1}.

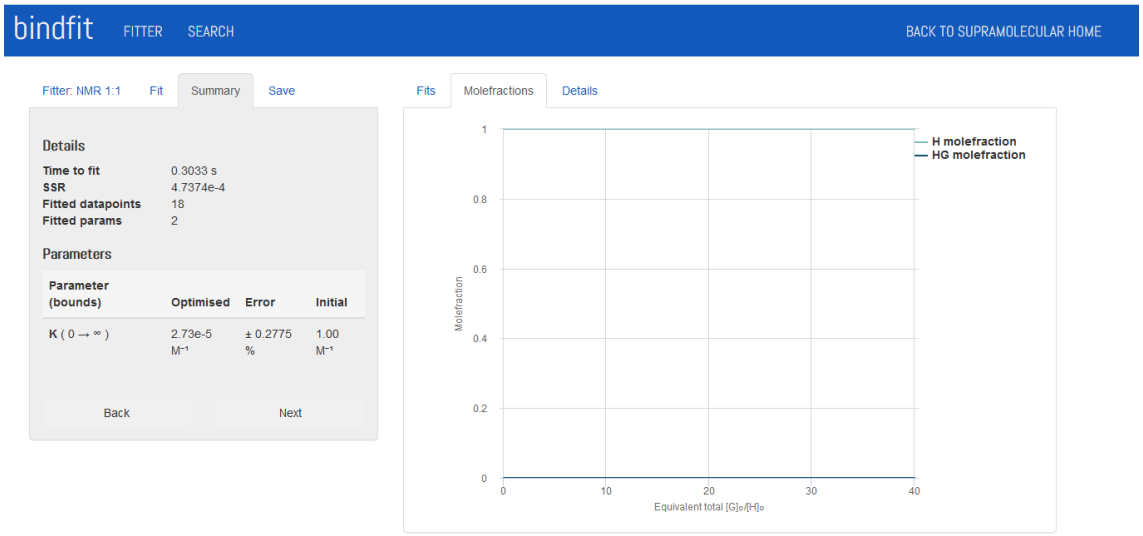


Figure S82. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **5**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the nitrate anion. The association constant K_a is so low that no variations are appreciated in the diagram.

Figure S83. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **6**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6). In order to avoid the dilution effect, the latter was prepared with the former. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, which was fitted to the 1:1 (LH:NO_3) binding model. K_a = 1.70±0.08 M^{-1}.

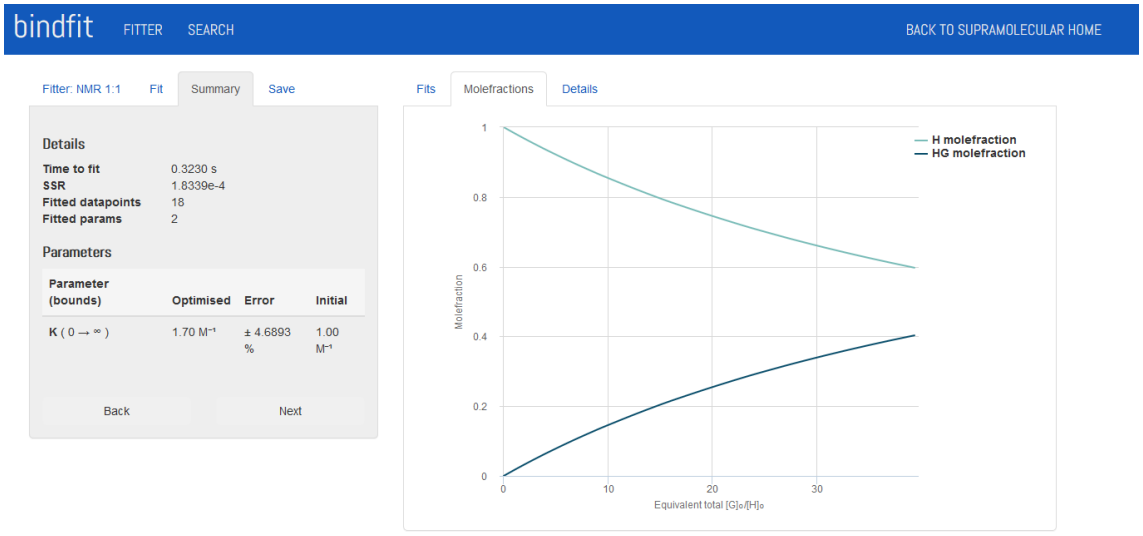


Figure S84. Species distribution diagram obtained for the titration of a 0.01 M solution of compound **6**·$HClO_4$ with a 0.6 M solution of $TBANO_3$ (DMSO-d_6), to show how the composition of the mixture changes over the course of the titration. *H* corresponds to the host, the protonated tambjamine, and *G* to the guest, the nitrate anion.

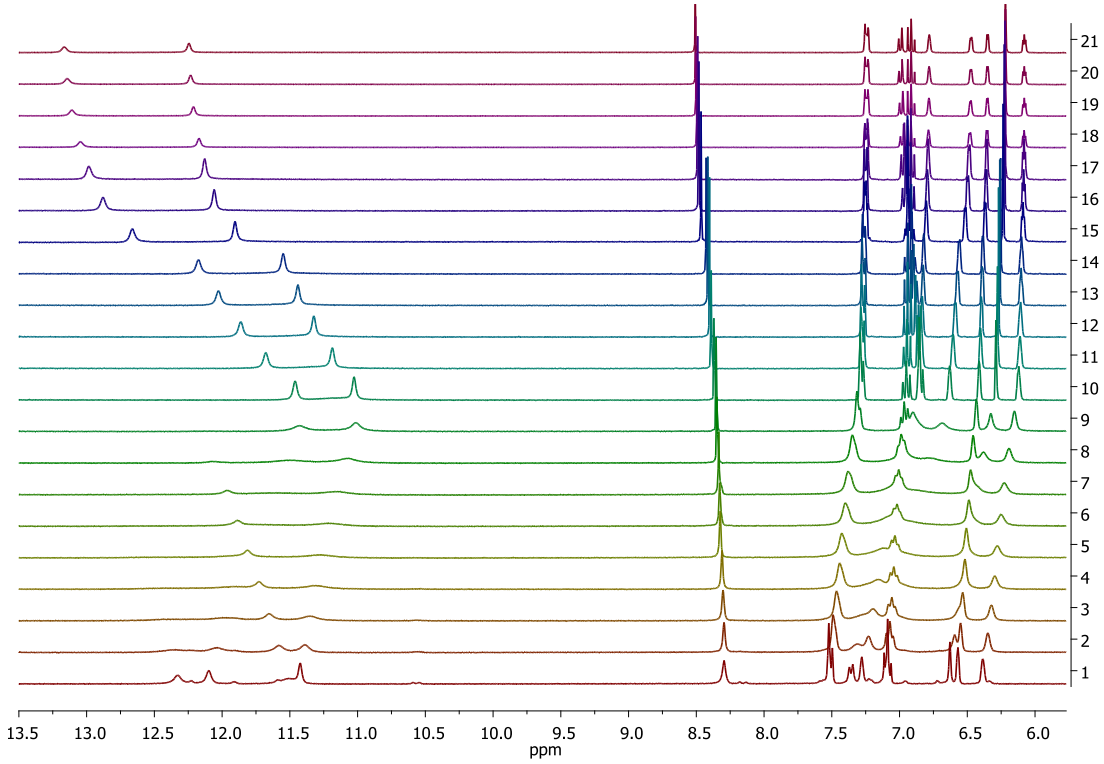


Figure S85. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.2 M solution of $TEAHCO_3$, prepared with a 0.01 M solution of **1**·$HClO_4$, to a 0.01 M solution of **1**·$HClO_4$.

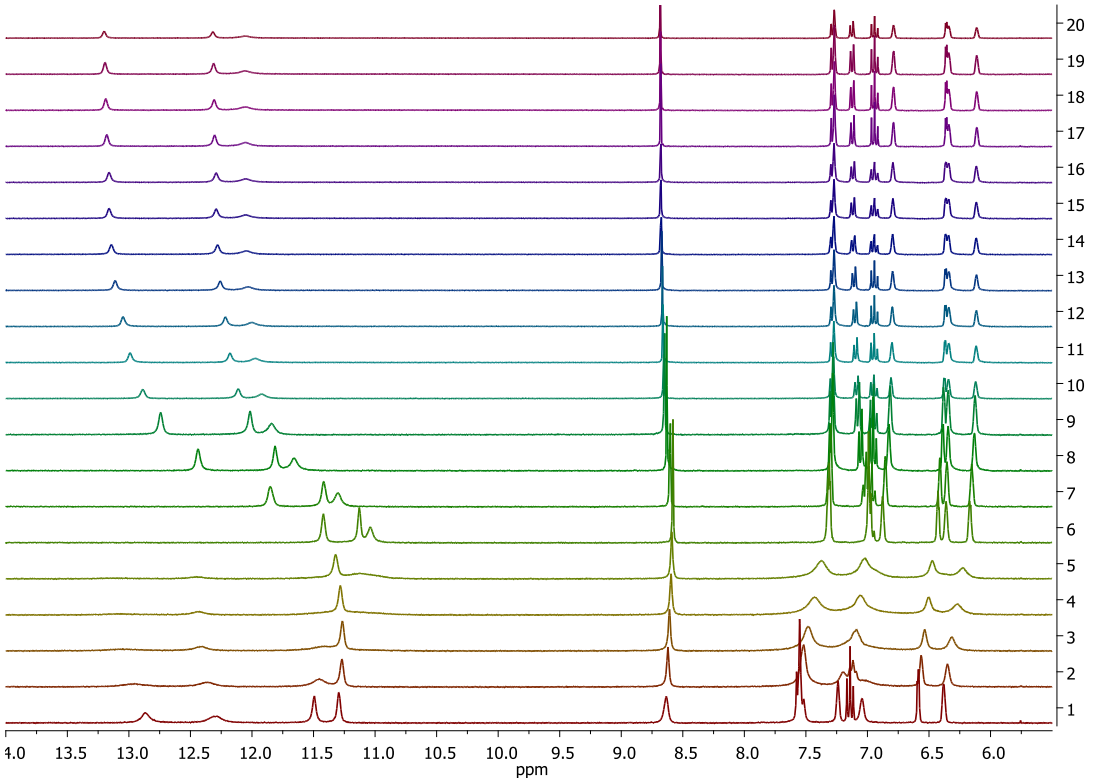


Figure S86. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.2 M solution of $TEAHCO_3$, prepared with a 0.01 M solution of **2**·$HClO_4$, to a 0.01 M solution of **2**·$HClO_4$.

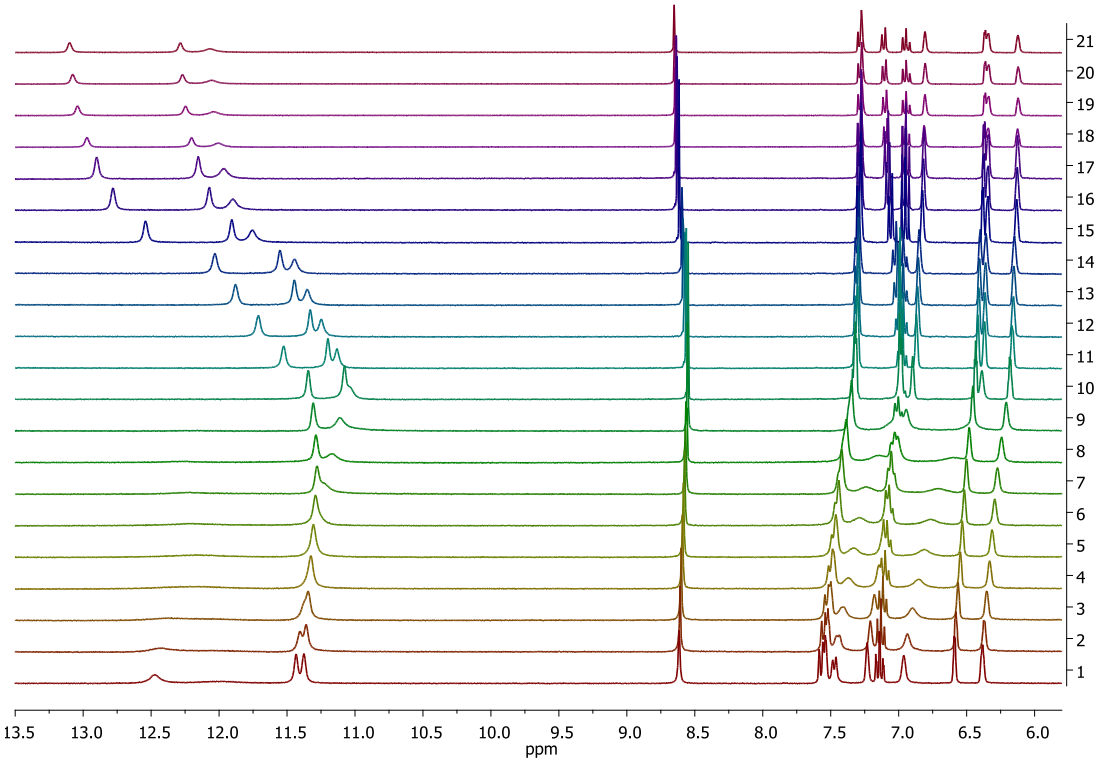


Figure S87. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.2 M solution of $TEAHCO_3$, prepared with a 0.01 M solution of **3**·$HClO_4$, to a 0.01 M solution of **3**·$HClO_4$.

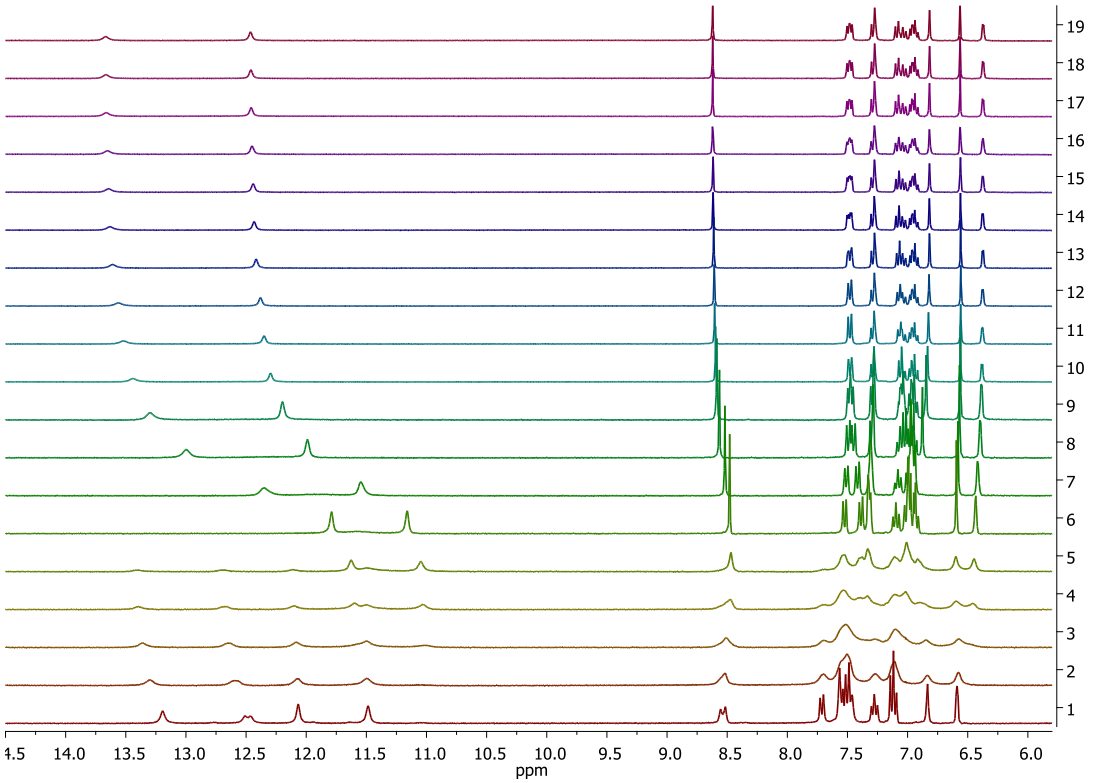


Figure S88. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.2 M solution of $TEAHCO_3$, prepared with a 0.01 M solution of **4**·$HClO_4$, to a 0.01 M solution of **4**·$HClO_4$.

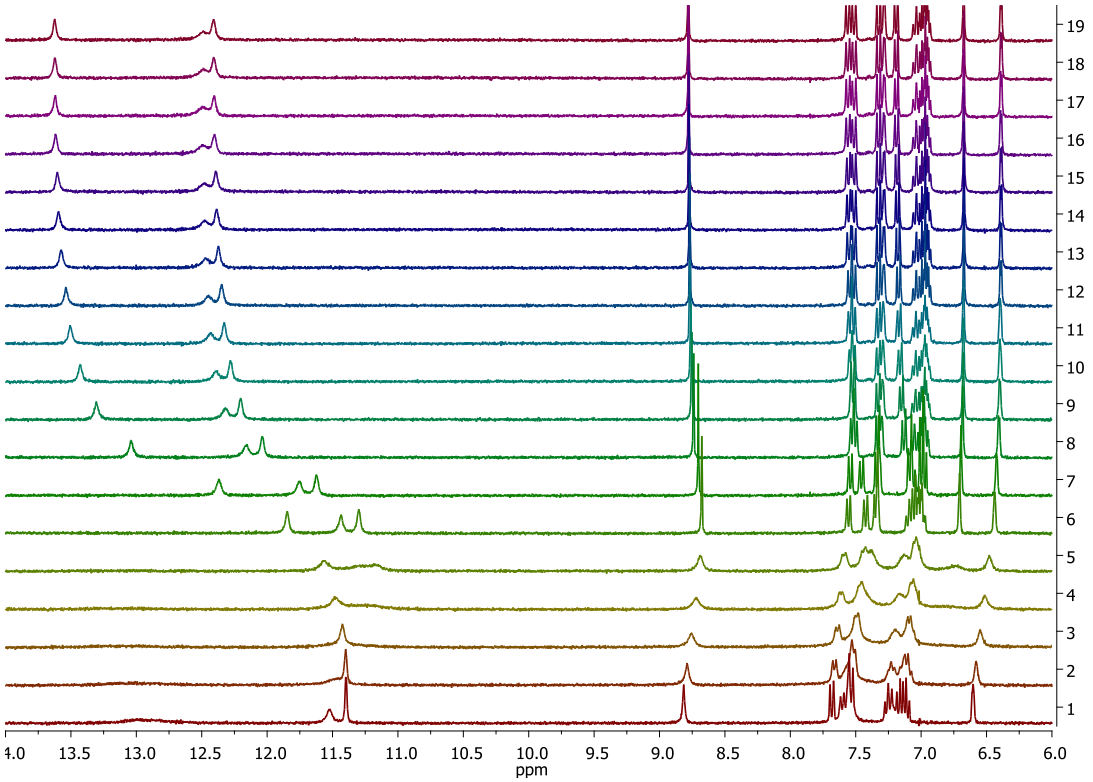


Figure S89. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.2 M solution of $TEAHCO_3$, prepared with a 0.01 M solution of **5**·$HClO_4$, to a 0.01 M solution of **5**·$HClO_4$.

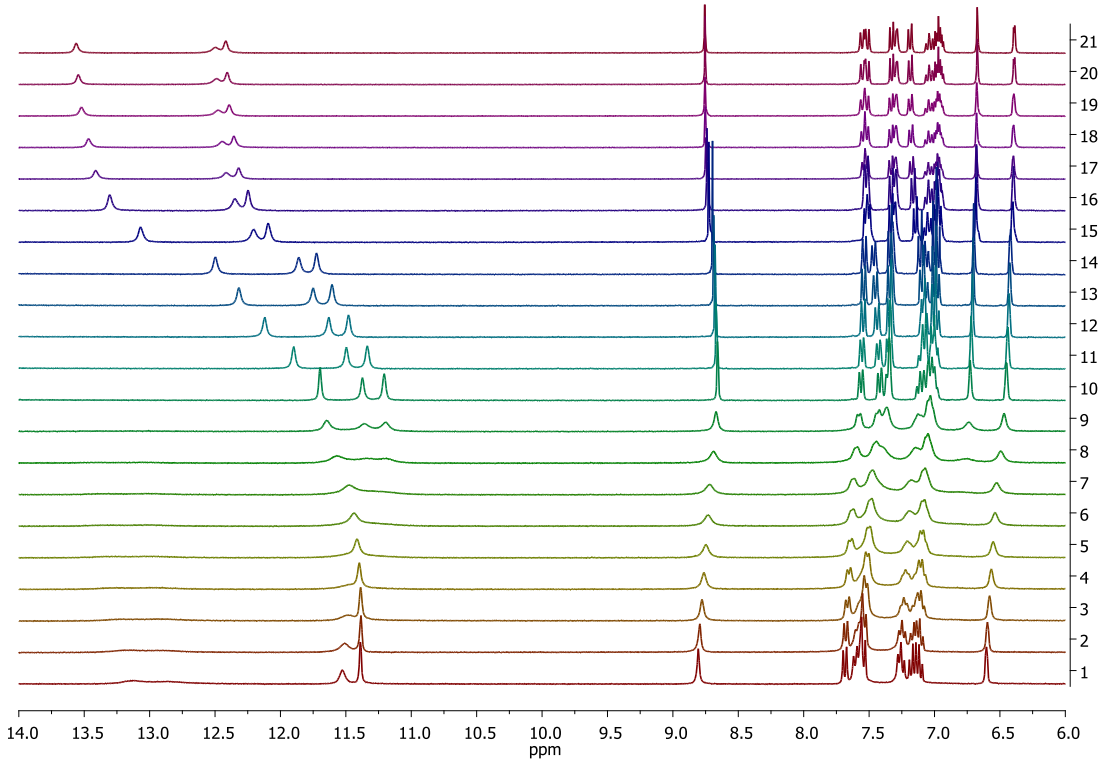


Figure S90. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.2 M solution of $TEAHCO_3$, prepared with a 0.01 M solution of **6**·$HClO_4$, to a 0.01 M solution of **6**·$HClO_4$.

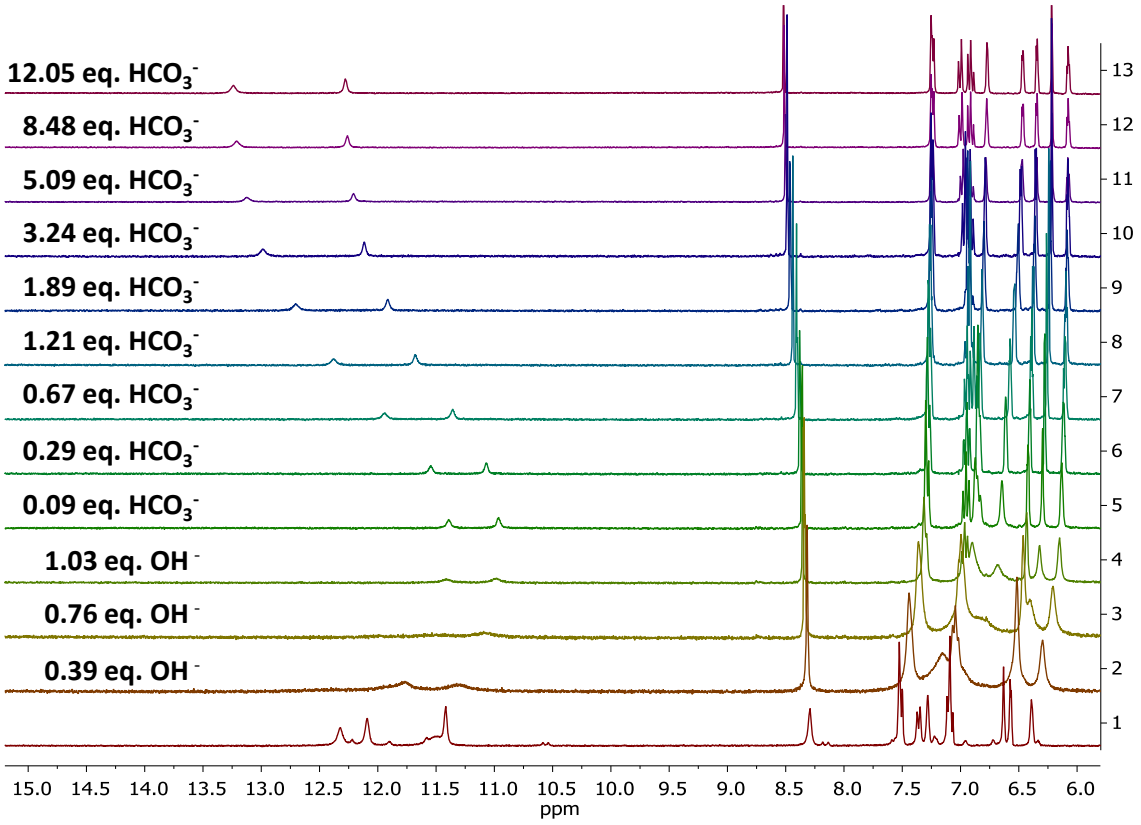


Figure S91. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of (a) a 0.2 M solution of TBAOH (up to 1.03 eq.) and (b) a 0.2 M solution of $TEAHCO_3$, prepared with a 0.01 M solution of **1**·$HClO_4$, to a 0.01 M solution of **1**·$HClO_4$. Upon addition of roughly 1 eq. of TBAOH deprotonation occurs and after that bicarbonate coordinates to the neutral tambjamine. This behaviour is similar to that observed when the titration is carried out only with the bicarbonate anion (**Fig. S85**).

3.3. Dilution studies

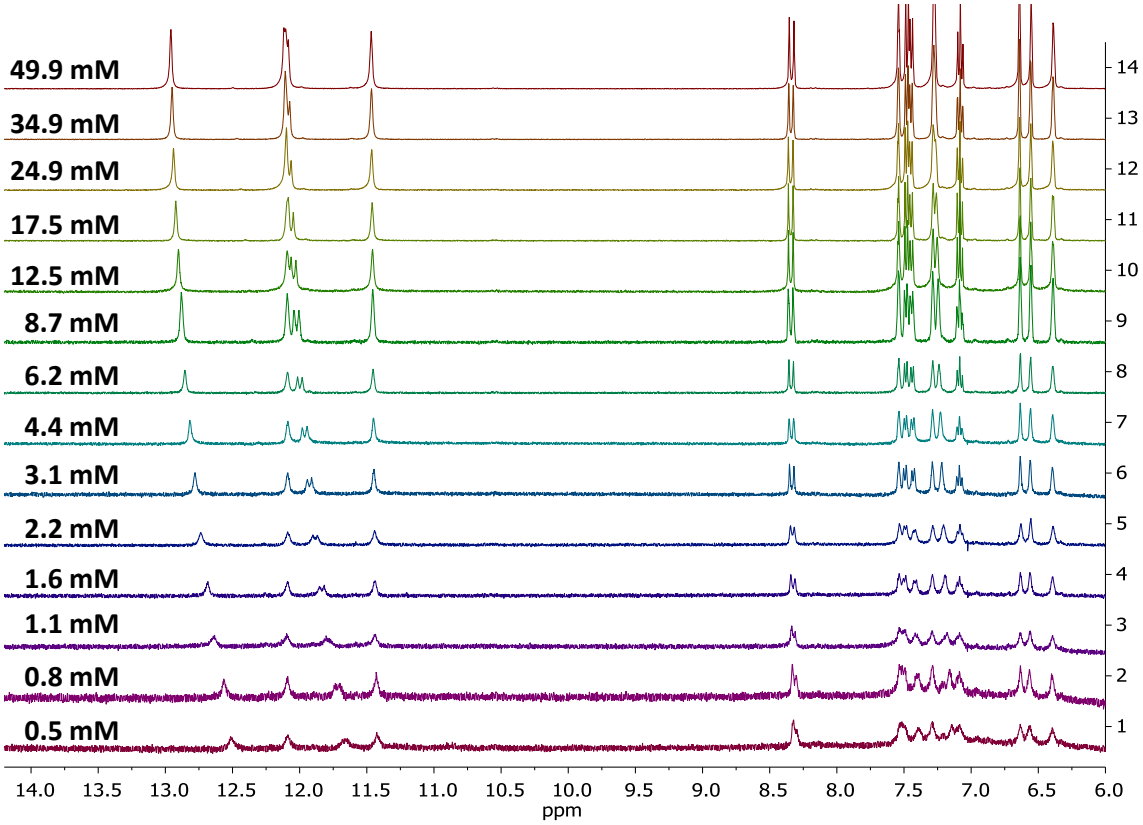


Figure S92. Excerpt of the ^{1}H NMR spectra (400 MHz, DMSO-d_6) obtained upon several dilutions of a 0.05 M solution of **1**·HCl.

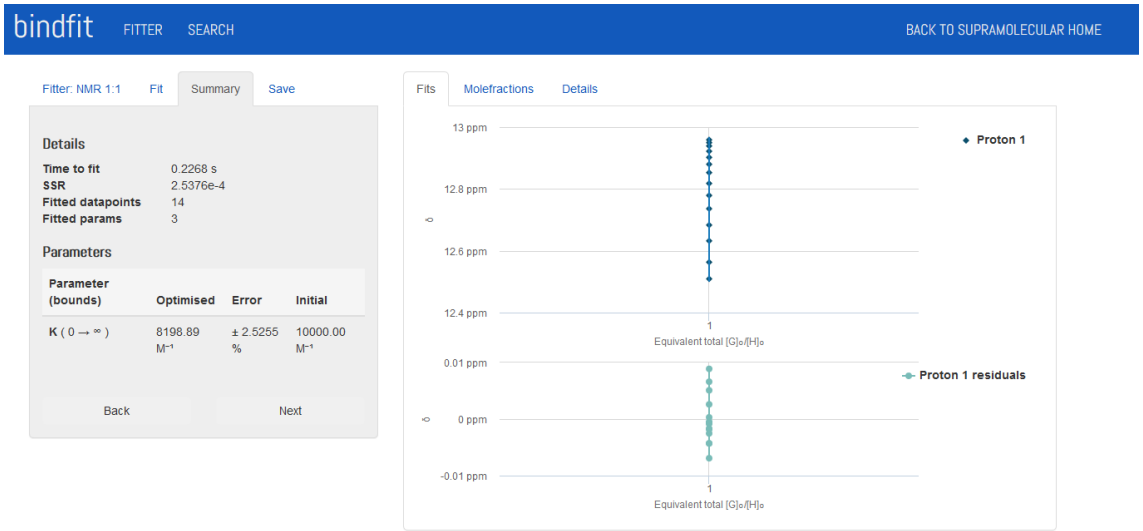


Figure S93. Fitted binding isotherm obtained for the dilution of a 0.05 M DMSO-d_6 solution of **1**·HCl. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, which was fitted to the 1:1 (LH:Cl) binding model. In this case *K* represents the equilibrium constant for the formation of the chloride adduct. K_a = 8199±207 M^{-1}.

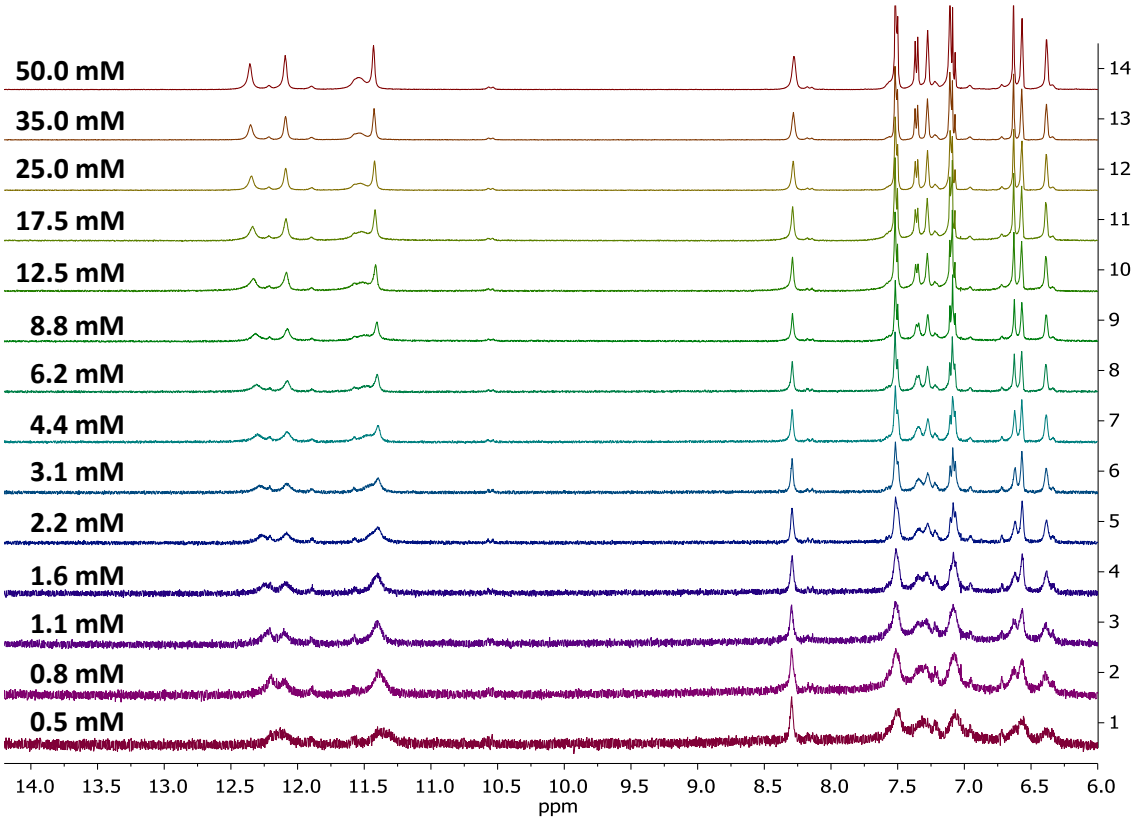


Figure S94. Excerpt of the ^{1}H NMR spectra (400 MHz, DMSO-d_6) obtained upon several dilutions of a 0.05 M solution of **1**·$HClO_4$.

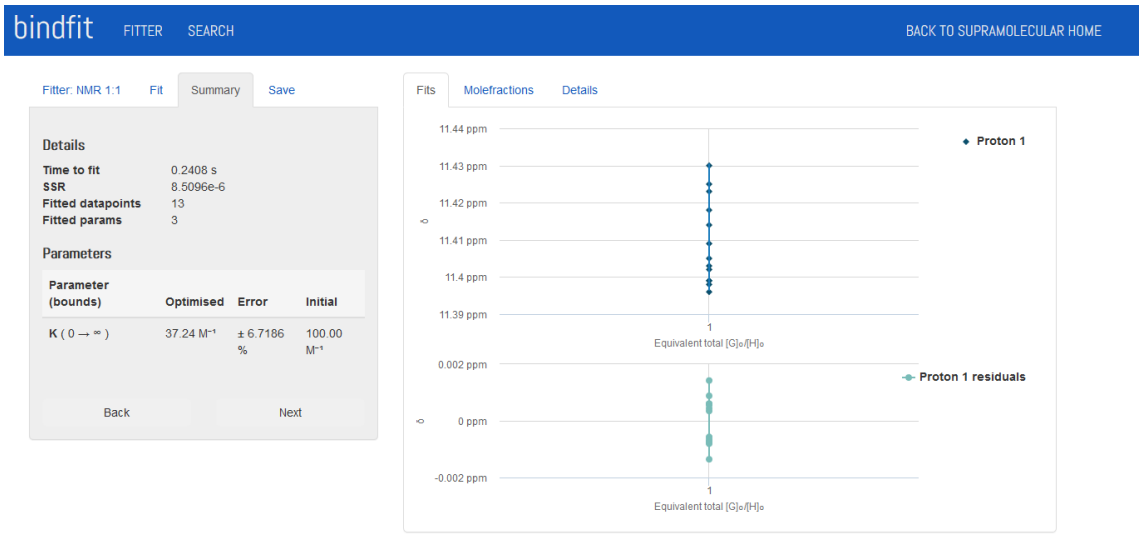


Figure S95. Fitted binding isotherm obtained for the dilution of a 0.05 M DMSO-d_6 solution of **1**·$HClO_4$. The graph shows the change in chemical shift of the signal of one of the NH protons of the molecule, which was fitted to the 1:1 (LH:ClO_4) binding model. In this case *K* represents the equilibrium constant for the formation of the perchlorate adduct. K_a = 37.2±2.5 M^{-1}. This value is in line with the low coordinating character of the perchlorate anion if compared with that of chloride.

4. TRANSMEMBRANE ANION TRANSPORT EXPERIMENTS

4.1. Preparation of phospholipid vesicles

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocoline (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 regarded 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.

Study of the Cl^-/NO_3^- exchange

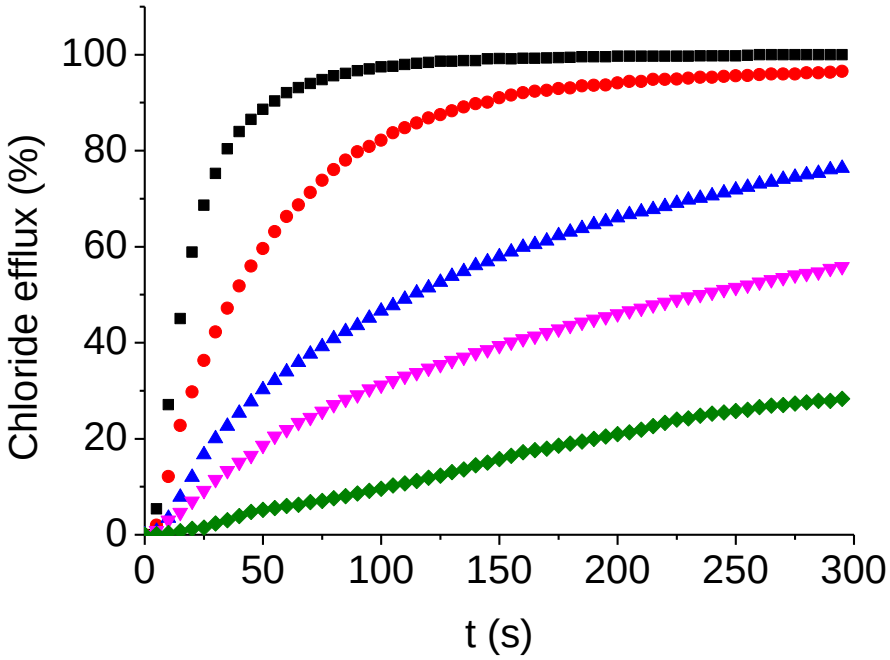


Figure S96. Chloride efflux promoted by **1**·HCl at different concentrations (5 μM, black; 0.25 μM, red; 0.05 μM, blue; 0.025 μM, magenta; 0.005 μM, green) in unilamellar POPC liposomes. Vesicles were loaded with 489 mM NaCl buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. Each trace represents the average of at least three trials.

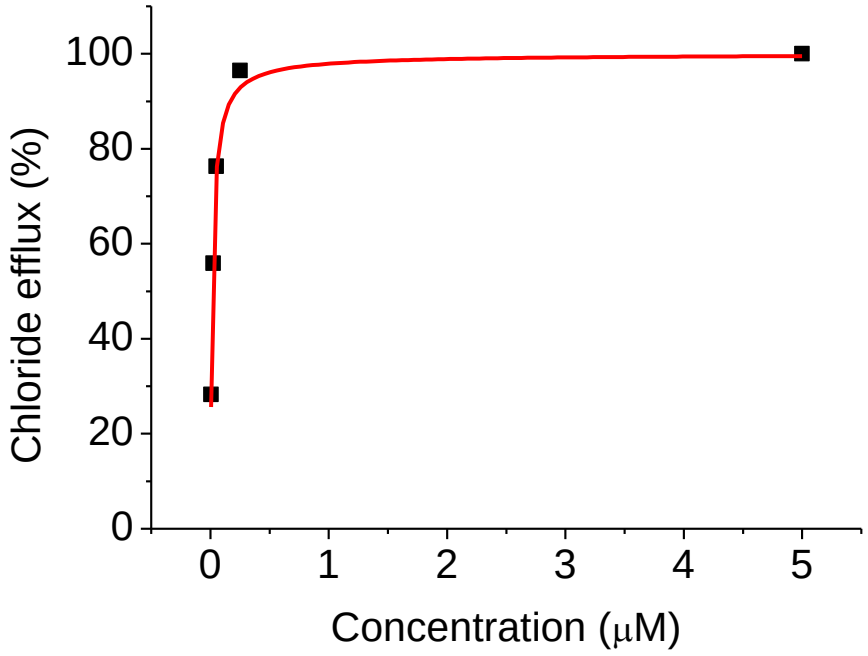


Figure S97. Normalised chloride efflux at 300 s plotted against the concentration of compound **1**·HCl. Data have been plotted with Hill equation (continuous line).

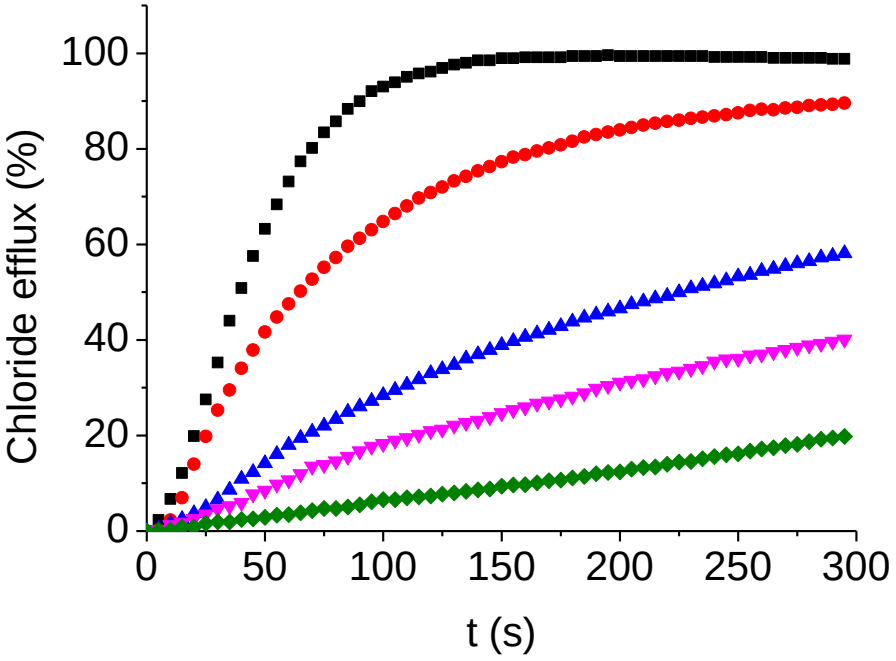


Figure S98. Chloride efflux promoted by **2**·HCl at different concentrations (5 μM, black; 0.25 μM, red; 0.05 μM, blue; 0.025 μM, magenta; 0.005 μM, green) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

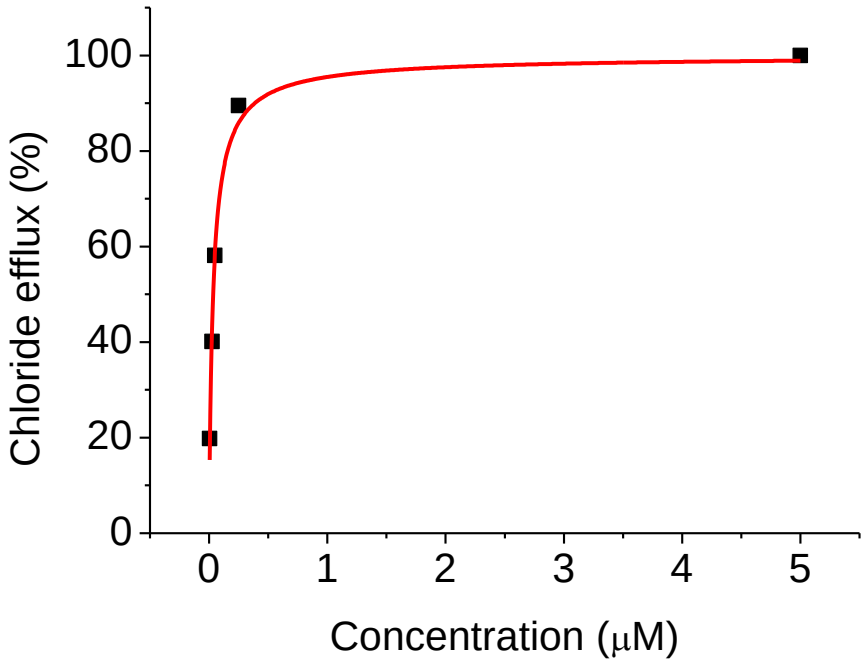


Figure S99. Normalised chloride efflux at 300 s plotted against the concentration of compound **2**·HCl. Data have been plotted with Hill equation (continuous line).

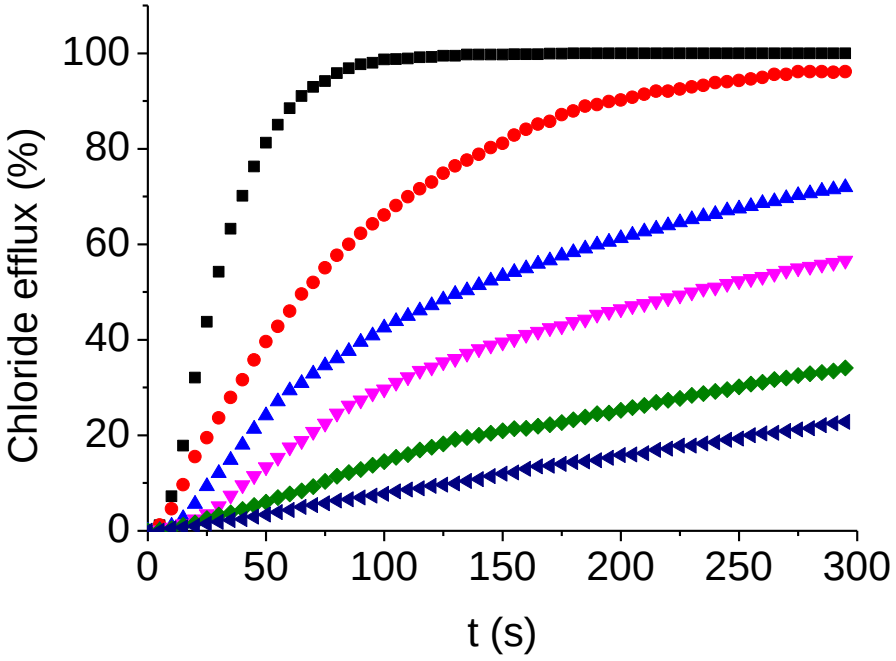


Figure S100. Chloride efflux promoted by **3**·HCl at different concentrations (5 μM, black; 0.25 μM, red; 0.05 μM, light blue; 0.025 μM, magenta; 0.015 μM, green; 0.005 μM, dark blue) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

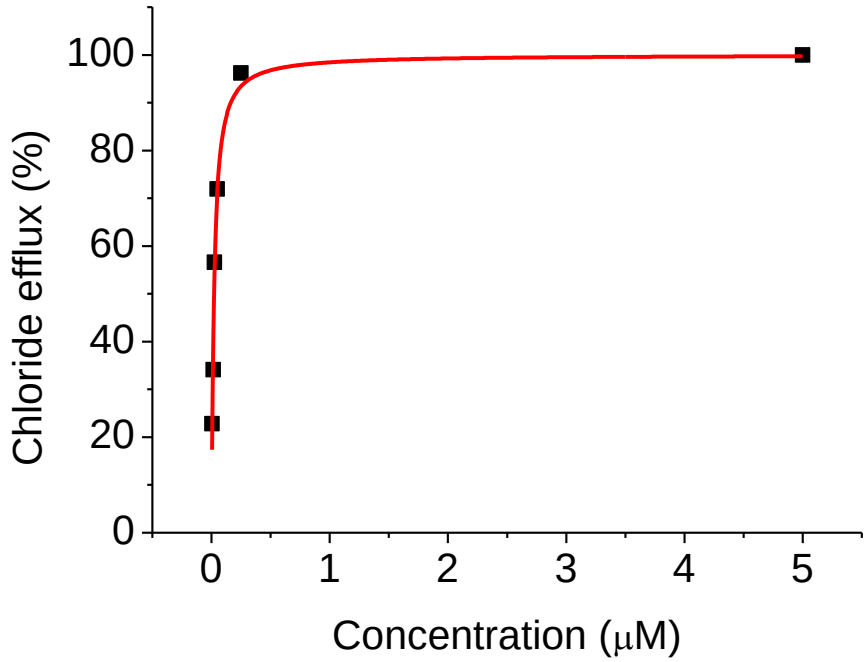


Figure S101. Normalised chloride efflux at 300 s plotted against the concentration of compound **3**·HCl. Data have been plotted with Hill equation (continuous line).

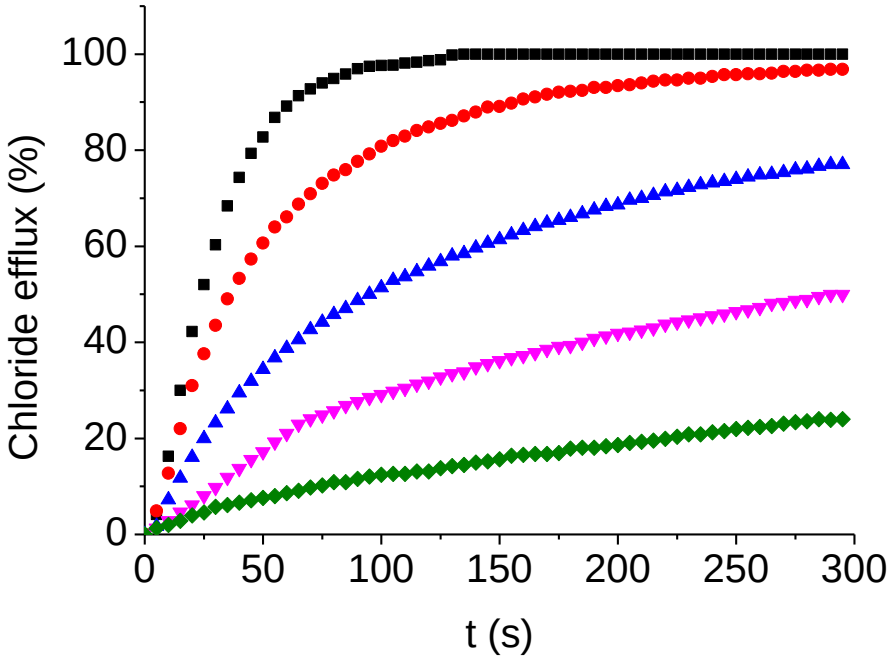


Figure S102. Chloride efflux promoted by **4**·HCl at different concentrations (2 μM, black; 0.25 μM, red; 0.1 μM, blue; 0.025 μM, magenta; 0.01 μM, green) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

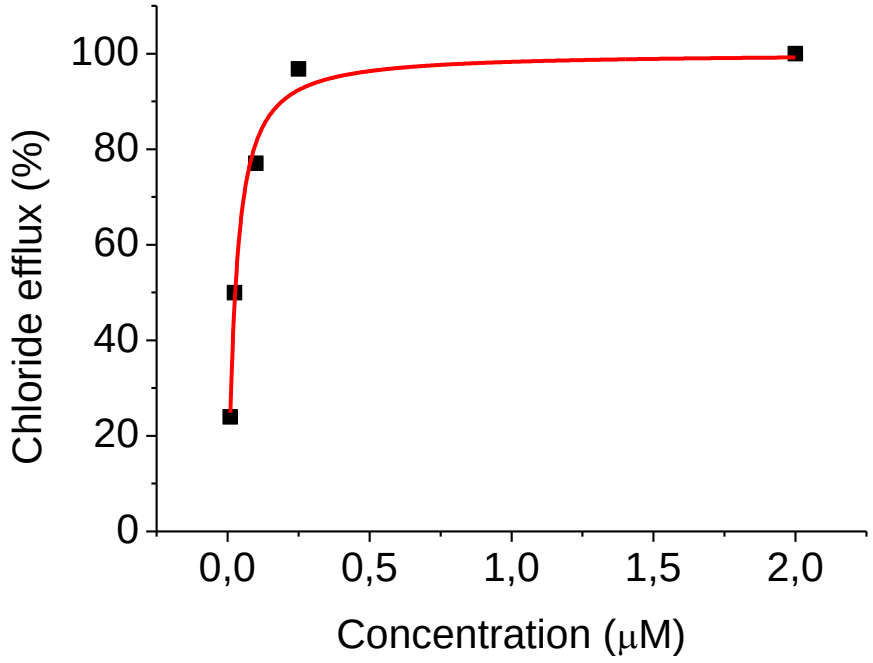


Figure S103. Normalised chloride efflux at 300 s plotted against the concentration of compound **4**·HCl. Data have been plotted with Hill equation (continuous line).

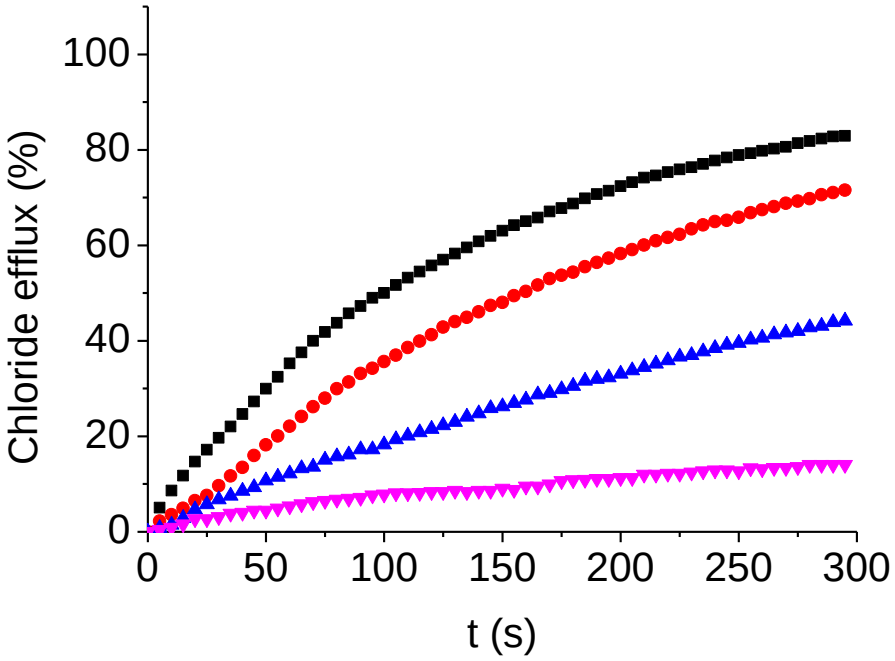


Figure S104. Chloride efflux promoted by **5**·HCl at different concentrations (10 μM, black; 5 μM, red; 0.5 μM, blue; 0.02 μM, magenta) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

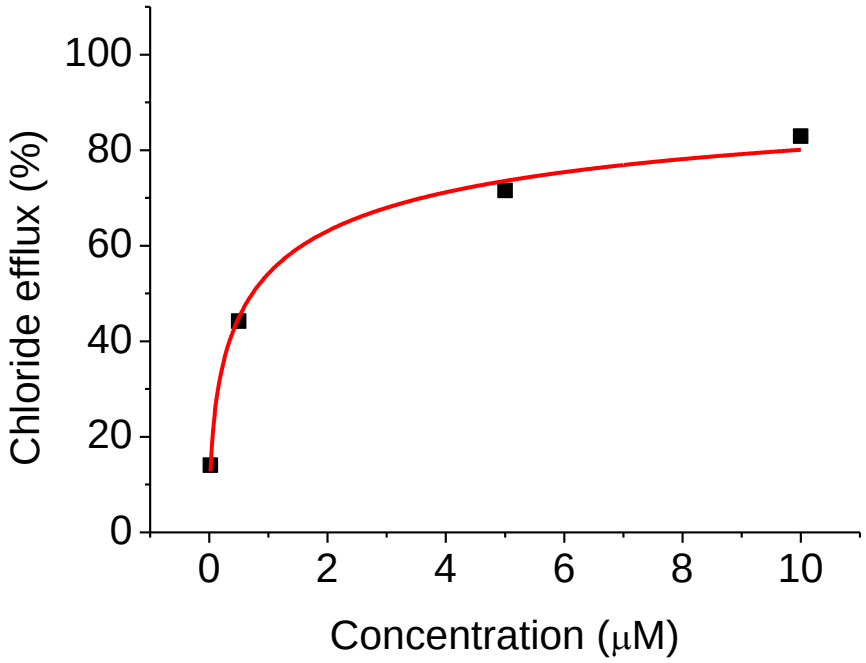


Figure S105. Normalised chloride efflux at 300 s plotted against the concentration of compound **5**·HCl. Data have been plotted with Hill equation (continuous line).

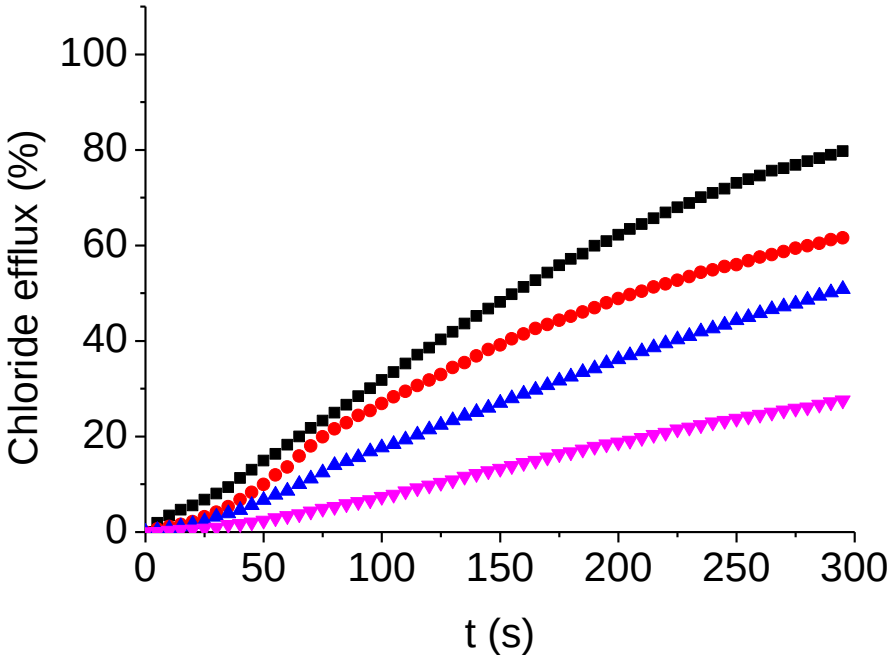


Figure S106. Chloride efflux promoted by **6**·HCl at different concentrations (10 μM, black; 2.5 μM, red; 1.5 μM, blue; 0.5 μM, magenta) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2. Each trace represents the average of at least three trials.

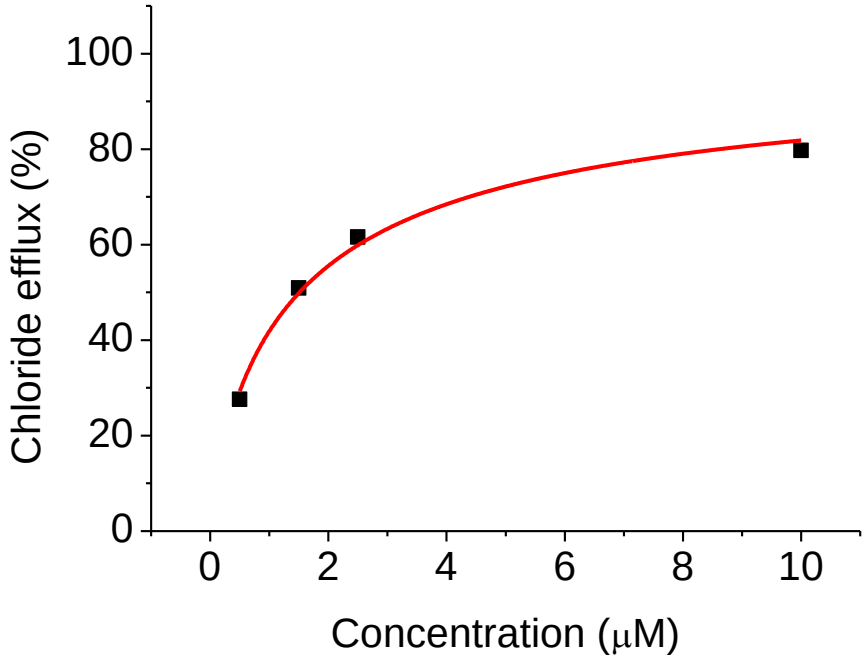


Figure S107. Normalised chloride efflux at 300 s plotted against the concentration of compound **6**·HCl. Data have been plotted with Hill equation (continuous line).

Study of the Cl^-/HCO_3^- exchange

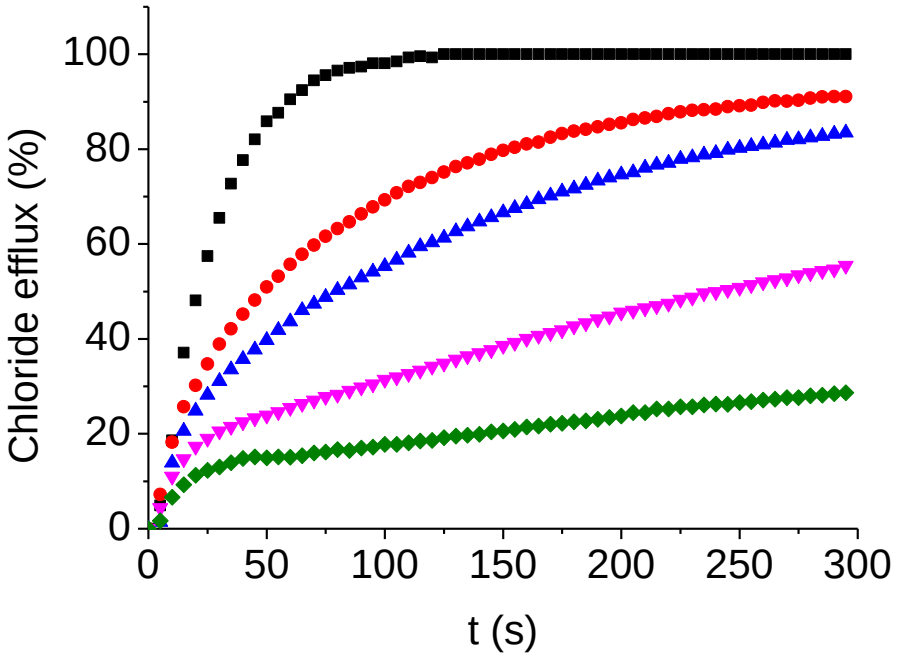


Figure S108. Chloride efflux promoted by **1**·HCl at different concentrations (5 μM, black; 1 μM, red; 0.5 μM, blue; 0.15 μM, magenta; 0.05 μM, green) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

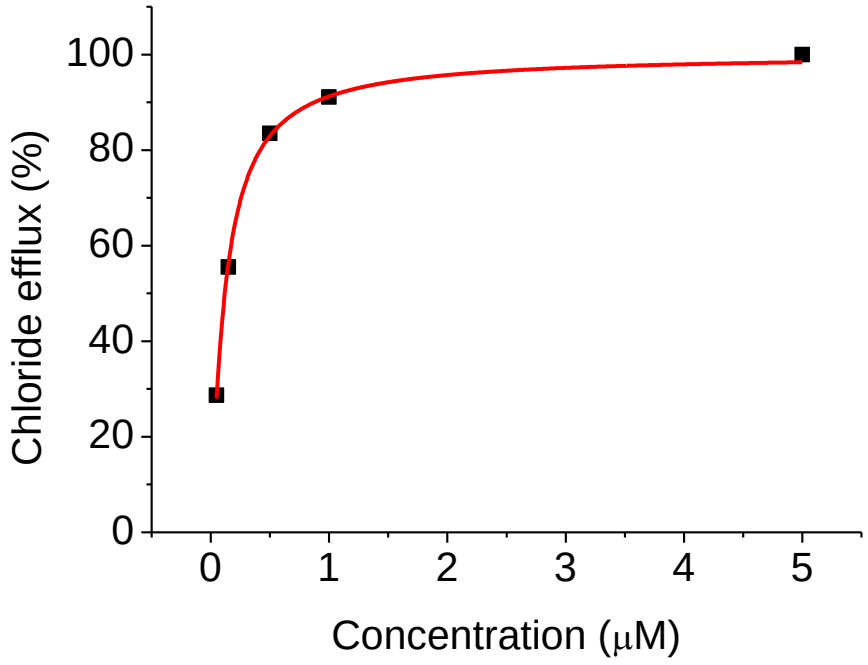


Figure S109. Normalised chloride efflux at 300 s plotted against the concentration of compound **1**·HCl. Data have been plotted with Hill equation (continuous line).

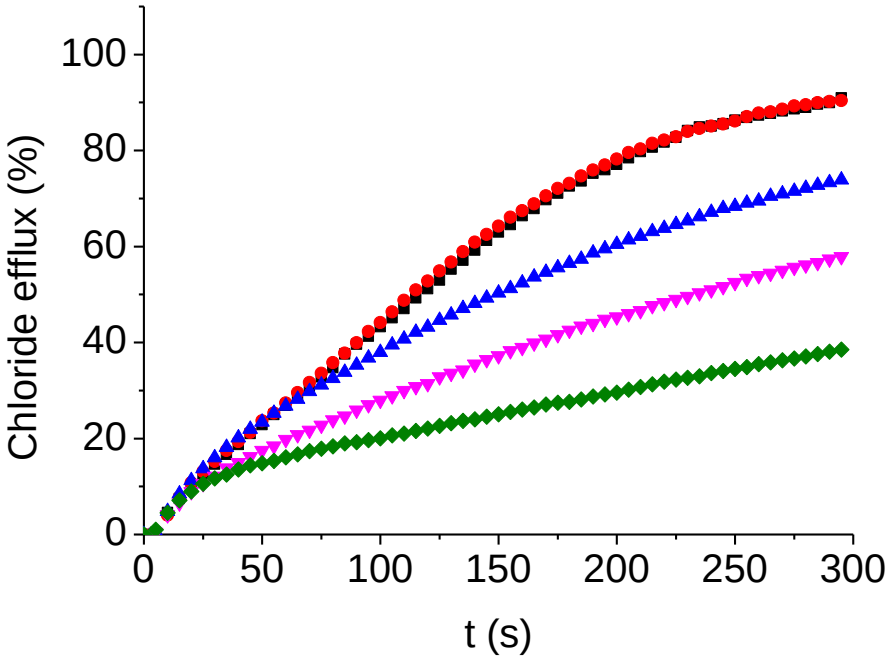


Figure S110. Chloride efflux promoted by **2**·HCl at different concentrations (10 μM, black; 7.5 μM, red; 2.5 μM, blue; 1.5 μM, magenta; 0.5 μM, green) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

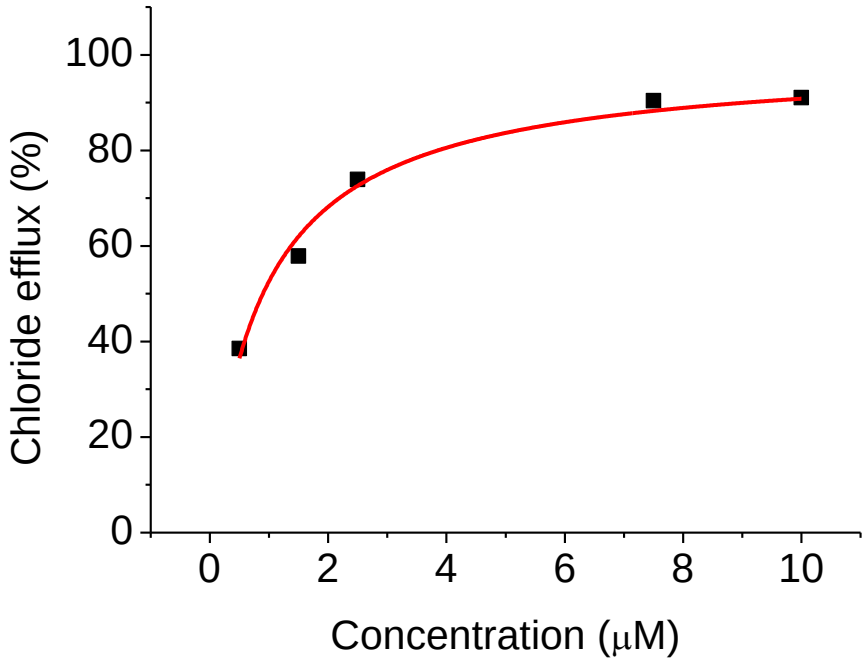


Figure S111. Normalised chloride efflux at 300 s plotted against the concentration of compound **2**·HCl. Data have been plotted with Hill equation (continuous line).

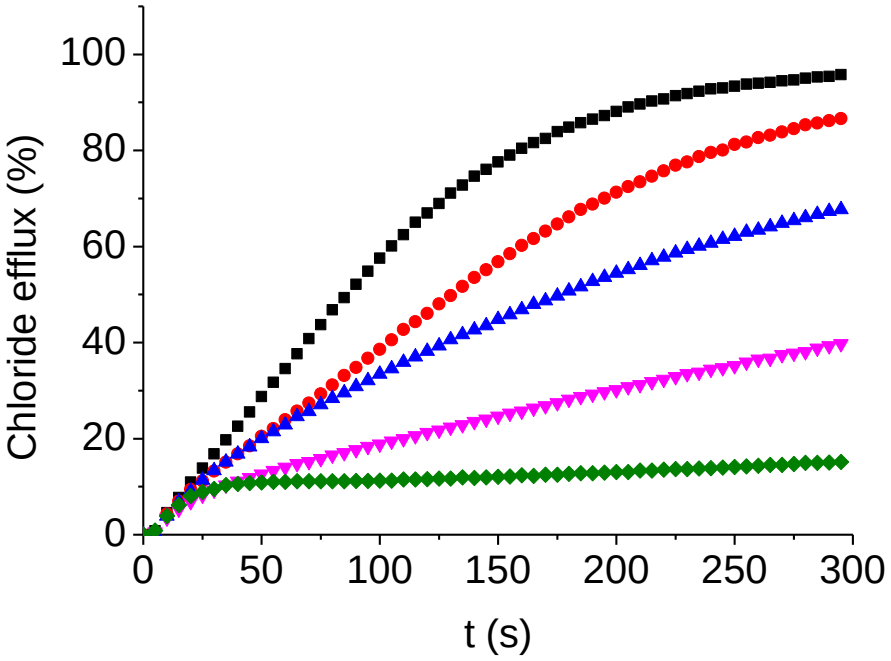


Figure S112. Chloride efflux promoted by **3**·HCl at different concentrations (10 μM, black; 5 μM, red; 1.5 μM, blue; 0.5 μM, magenta; 0.05 μM, green) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

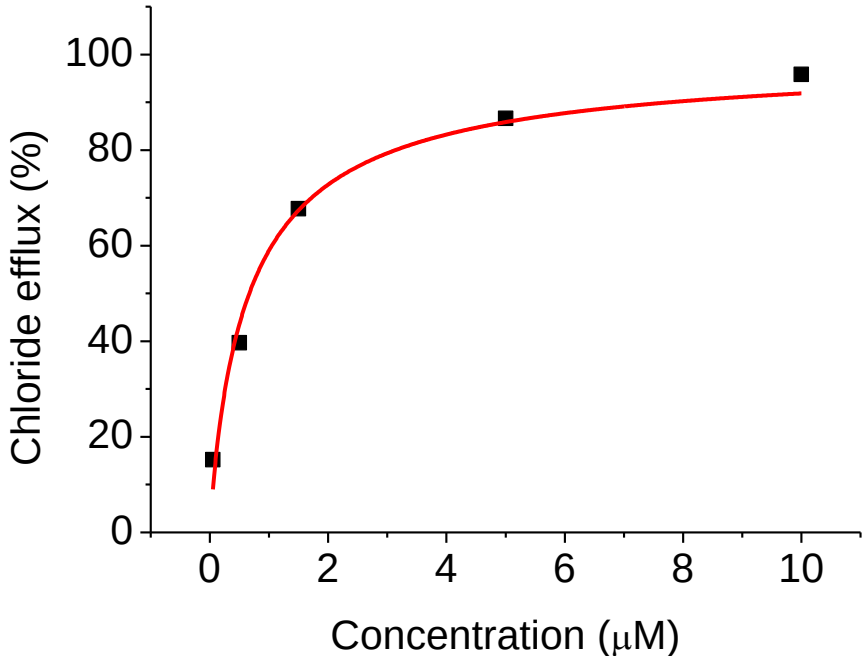


Figure S113. Normalised chloride efflux at 300 s plotted against the concentration of compound **3**·HCl. Data have been plotted with Hill equation (continuous line).

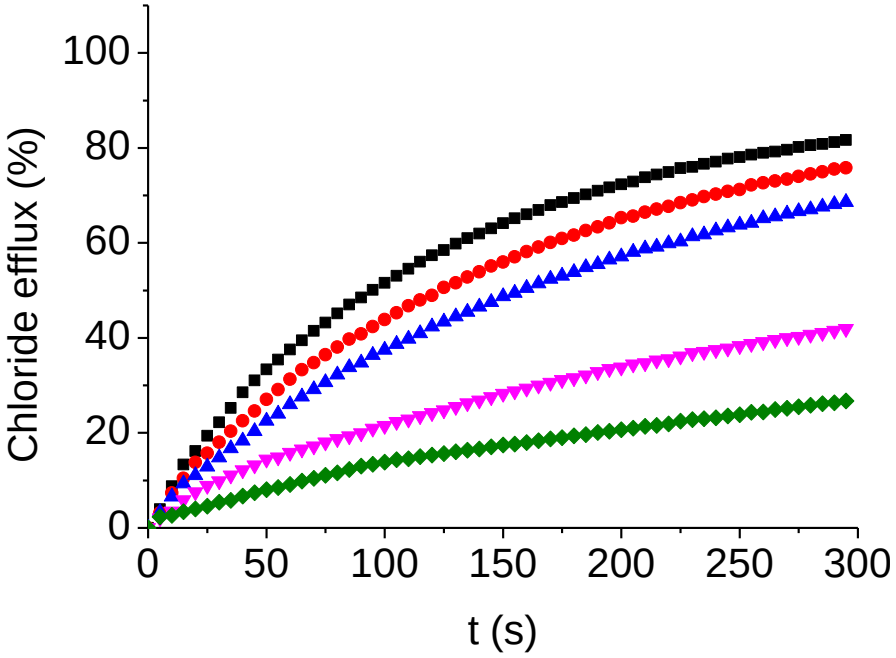


Figure S114. Chloride efflux promoted by **4**·HCl at different concentrations (2 μM, black; 1.5 μM, red; 1 μM, blue; 0.25 μM, magenta; 0.1 μM, green) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments.

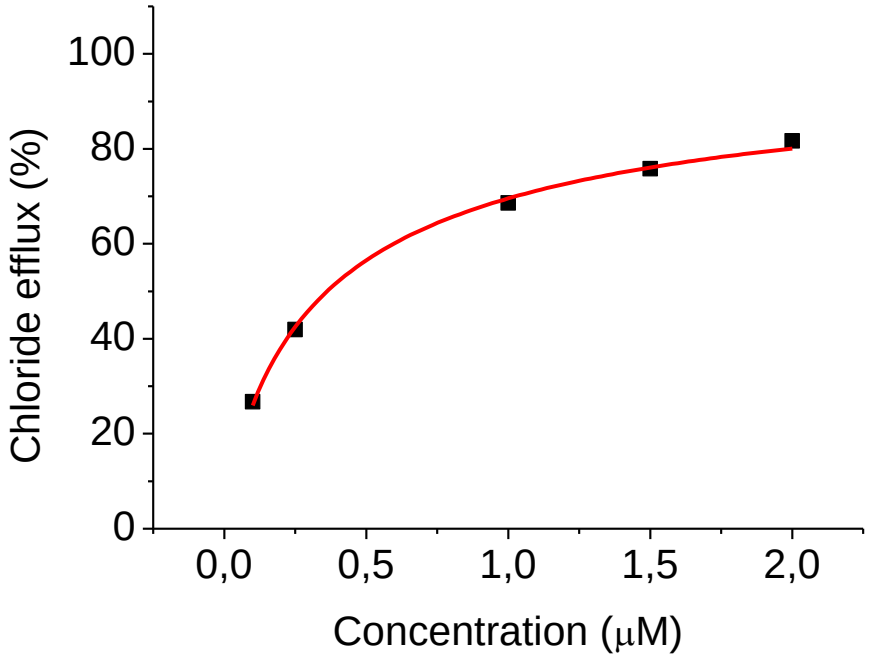


Figure S115. Normalised chloride efflux at 300 s plotted against the concentration of compound **4**·HCl. Data have been plotted with Hill equation (continuous line).

Table S2. Transport activities expressed as EC_{50} (nM) and Hill parameter for compounds (**1**-**6**)·HCl.

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)[c]
1·HCl	16 ± 2	0.9 ± 0.1	118 ± 4	1.10 ± 0.04	3.01
2·HCl	33 ± 4	0.9 ± 0.1	896 ± 79	0.95 ± 0.08	4.09
3·HCl	21 ± 2	1.1 ± 0.1	666 ± 103	0.9 ± 0.1	4.09
4·HCl	27 ± 2	1.1 ± 0.1	362 ± 13	0.81 ± 0.02	4.18
5·HCl	730 ± 102	0.53 ± 0.04	[a]	[a]	5.27
6·HCl	1511 ± 106	0.80 ± 0.06	[b]	[b]	5.27

[a,b] In both cases no EC_{50} value could be determined due to precipitation of the compound at high concentrations (in the case of **5**·HCl the maximum amount of chloride detected at 15 μM is roughly 25%, whereas when the concentration of **6**·HCl is 20 μM this amount is, approximately, 40%). [c] Determined for the deprotonated form of the compounds through Virtual Computational Chemistry Laboratory.

5. ANTIBACTERIAL ACTIVITY ON BACTERIAL STRAINS AND CLINICAL ISOLATES

5.1. Bacterial strains and culture conditions

The bacterial strains *Acinetobacter baumannii* ATCC 17978, *Pseudomonas aeruginosa* PAO1, *Staphylococcus aureus* CECT 5190 (methicillin resistant) and *Enterococcus faecium* CECT 5253 (vancomycin resistant) were used in this study. *A. baumannii* and *P. aeruginosa* strains were kept at 37 ^oC in Mueller-Hinton (MH) broth or agar, while *S. aureus* and *E. faecium* were maintained in Tryptic Soy (TS) broth or agar at 37 ^oC.

To test the efficacy of compound **1**·HCl in clinical strains, ten Gram-negative and seven Gram-positive strains were used. Gram-negative strains: one XDR clinical isolate of *K. pneumoniae* (Kp1; producing CTX-M and OXA-48, only susceptible to amikacin, fosfomycin and colistin), five MDR clinical isolates of *E. coli* with different resistance profiles (EC1, EC2 and EC3; isolates harbouring an Extended Spectrum Beta-Lactamase (E.S.B.L), EC4; isolate harbouring a VIM carbapenemase and EC5; isolate harbouring an OXA-48) and two XDR clinical isolates of *A. baumannii* (AbI1; isolate harbouring a NDM-2 and an OXA-51, only susceptible to colistin and tigecycline (ST- 103) and Ab4249; isolate harbouring an OXA-51, OXA-24 and hiperproduction of AmpC, only susceptible to amikacin, colistin and tigecycline (ST-24)) were used. ATCC *E. coli* 25922 and ATCC *A. baumannii* 19606 were employed as quality control for the Gram-negative strains. Gram-positive strains: four clinical strains of *S. aureus*; two methicillin-sensitive *S. aureus* (MSSA15 and MSSA16) and two methicillin-resistant *S. aureus* (MRSA15 and MRSA16); and two *S. epidermidis* clinical strains (SE14 and SE94). ATCC *S. aureus* 29213 was used as quality control for the Gram-positive strains.

5.2. Determination of minimal inhibitory concentrations (MICs)

Susceptibility profiles were determined by broth microdilution according to CLSI criteria.[8] In brief, serial dilutions of the compounds were prepared in Mueller Hinton Broth ranging from 100 µM to 3.125 µM (1:2 dilutions) in 96-well plates. All strains were adjusted to 0.5 McFarland in water, and diluted 1:20 in MHB. 5 µL of the bacterial dilution were added to each well, and the plates were incubated 18-20 hours. Wells with MHB alone and MHB without compounds inoculated with bacteria were used as negative and positive growth controls, respectively. MIC was defined as the lowest concentration at which no growth was observed. The MICs reported are the mean values from at least three independent replicates.

To test the antimicrobial efficacy of compound **1**·HCl in clinical isolates, the MIC values were determined by the broth microdilution method according to EUCAST guidelines.[9] Cells from the subculture were suspended in Mueller Hinton Broth (MHB; Becton Dickinson, Le Pont de Clarx, France) to reach a turbidity of 0.5 in the McFarland scale ($1.5 \cdot 10^8$ colony-forming units (cfu)/mL) and subsequently the inocula was adjusted to the desired concentration. Different concentrations of the compounds used were prepared with MHB (1:2 dilutions). The concentration range employed was 50 - 0.0976 µM. Solutions of compound **1**·HCl and the inocula were put in a 96-well microtiter plate, except in the last two columns corresponding to the sterility control: in one of them, inocula without compound **1**·HCl was put and, in the other one, culture medium. After that, plates were incubated at 37 ^{o}C for 24 h. Efficacy was defined as the lowest concentration of antibiotic at which no turbidity of the bacterial culture was appreciated.

5.3. Growth curves

The influence of compound **1**·HCl on bacterial growth was monitored using a Synergy HT microplate reader (BioTek Instruments, Inc.). Compound **1**·HCl was selected due to the inhibitory effects showed in MIC determinations both in Gram-negative (*A. baumannii*) and Gram-positive (*S. aureus* and *E. faecium*) bacteria. Cultures of *A. baumannii* ATCC 17978, *S. aureus* CECT 5190 and *E. faecium* CECT 5253 were first grown in the appropriate liquid growth medium overnight at 37 ^{o}C with shaking. Cultures were then diluted 1:100 in medium alone (as control) and in medium supplemented with different concentrations of compound **1**·HCl. MICs

[8] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: 17th informational supplement M07–A9*, Clinical and Laboratory Standards Institute, Wayne, PA, **2012**.

[9] The European Committee on Antimicrobial Susceptibility Testing, *Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method*, v 3.0, **2013**. Available at: http://www.eucast.org (last accessed December 27, 2016).

and values below MIC were tested for each strain. The growth rate was monitored every 10 minutes at 600 nm in 24-well plates for 24 hours. Each growth condition was tested in triplicate.

5.4 Bactericidal assay

After a MIC assay, all the samples were homogenized, and aliquots of 2 µL representative of each condition were seeded in Mueller-Hinton agar plates, incubated for 24 hours, and checked for the presence of bacterial growth. Additionally, the whole content of the MICs samples were seeded in agar plates and checked for bacterial growth after 24 hour incubation (data not shown). Results showed that in *A. baumannii* ATCC17978 the MIC of compound **1** HCl presented bactericidal effect.

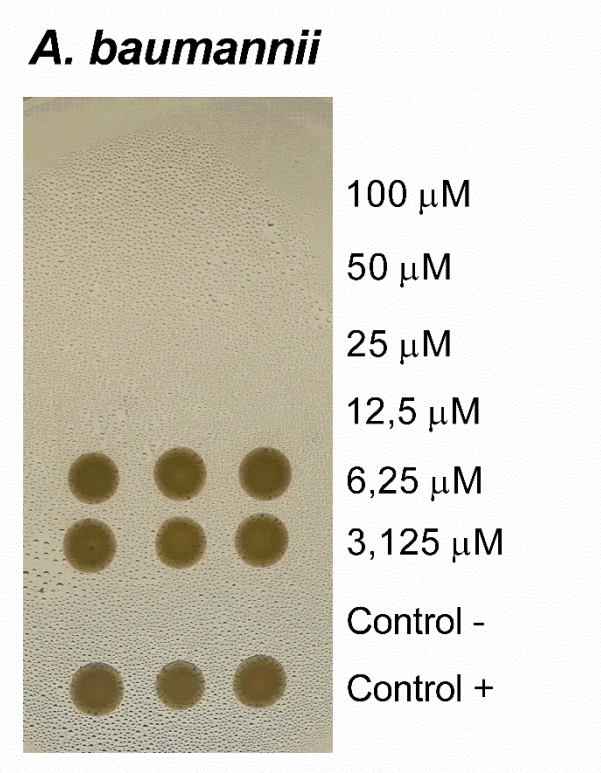


Figure S116. Bactericidal activity of compound **1** HCl in *A. baumannii* ATCC17978.

6. HEMOCOMPATIBILITY OF COMPOUND 1·HCl

6.1. Measurement of hemolytic activity

Hemolytic activity was tested in erythrocytes obtained from rat blood. Fresh rat blood contained in a 3-mL EDTA tube was centrifuged for 15 min at 1700 rpm to obtain the red cells, which were washed twice with a saline solution (NaCl 150 mM) and resuspended in Dulbecco's phosphate-buffered saline (DPBS) using the same initial volume of blood. Tubes containing serial dilutions of compound **1**·HCl in a final volume of 1 mL of DPBS were prepared, and 25 µL of erythrocyte suspension were added to each tube. DPBS alone and DPBS with 20% of Triton X-100 were used as negative and positive controls, respectively. After 1 hour of incubation at 37 ^{o}C with gentle shaking, tubes were centrifuged for 15 min at 1700 rpm, and 200 µL of supernatant from each tube were carefully transferred to a 96-well plate. The release of hemoglobin was monitored measuring the optical density at 570 nm. The hemolysis percentage was calculated using the following formula:

$$\% \text{ hemolysis} = \frac{ODs - OD0}{OD100 - OD0} \times 100$$

where ODs is the optical density (OD) of the sample; OD0 is the OD of the negative control and OD100 is the OD of the positive control. Data are the mean of three independent replicates.

6.2. Hemagglutination assay

Hemagglutination activity of compound **1**·HCl was studied following the protocol described by Nayak *et al.* with some modifications.[10] In brief, different dilutions of the **1**·HCl solution in DPBS were prepared in a 96-well polystyrene plate with round bottom (100 µL per well). 100 µL of the erythrocyte suspension were diluted in 10 mL of DPBS, and 100 µL of this diluted erythrocyte suspension were subsequently added to each well. Erythrocytes added to DPBS alone were used as control. The plate was incubated for 2 hours at 37 ^{o}C in order to observe visually the formation of button-like structures caused by the erythrocytes agglutination.

[10] Nayak, D.; Kumari, M.; Rajachandar, S.; Ashe, S.; Thathapudi, N. C.; Nayak, B. *ACS Appl. Mater. Interfaces*, **2016**, *8* (42), 28538-28553.