Electronic Supporting Information (ESI)

Simple isophthalamides/dipicolineamides as active transmembrane anion transporters

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1. STRUCTURES OF THE STUDIED COMPOUNDS

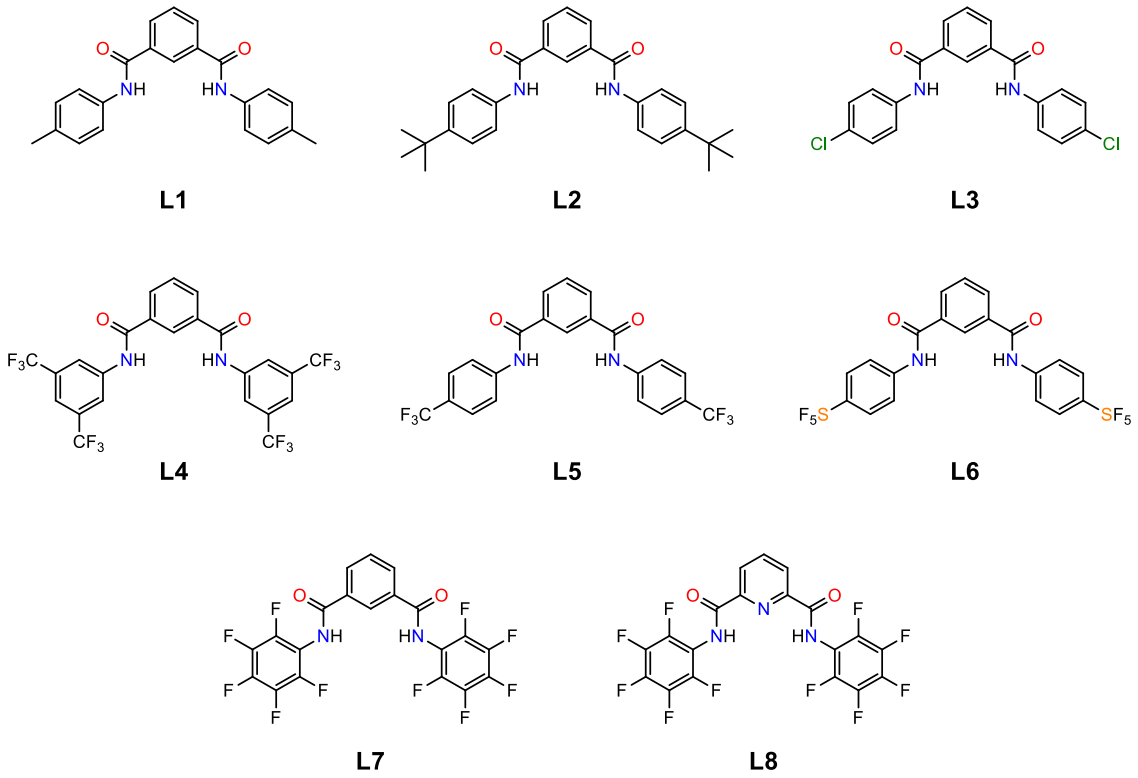


Figure S1. Structures of the studied compounds.

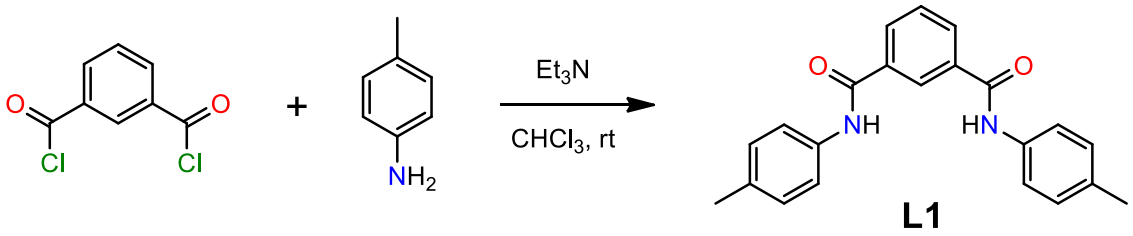
2. SYNTHESES AND CHARACTERIZATION DATA

2.1. General procedures and methods

Commercial reagents were employed as received without any further purification. NMR spectra were recorded at 298 K on a Varian Mercury-300 MHz spectrometer, employing DMSO-d_6 as solvent, with its residual signal 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 ionization source employed was electrospray in its positive mode. X-ray diffraction studies were carried out at 298 K on a Bruker Smart APEX CCD diffractometer.

2.2. Isophthalamides and dipicolineamides

2.2.1. *N^1,N^3-bis(p-tolyl)isophthalamide (L1)*



4-methylaniline (0.25 g, 2.3 mmol) and triethylamine (0.32 mL, 2.3 mmol) were mixed in chloroform (5 mL). Subsequently, small portions of isophthaloyl chloride (0.24 g, 1.2 mmol) were added to the mixture over a period of several minutes. A white precipitate was formed, and the mixture was stirred at room temperature for 12 hours. The solid was filtrated, washed with distilled water (3 × 10 mL) and dried *in vacuo*. On the other hand, the mother liquor was washed with a 1M HCl aqueous solution (3 × 10 mL). The organic phase was then dried with anhydrous sodium sulfate, filtered and the solvent removed under vacuum. The resulting white solid, together with the former precipitate, were analyzed by means of ^{1}H NMR spectroscopy and, after confirming their purity, they were combined (0.26 g, 63%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.34 (s, 2H), 8.52-8.48 (m, 1H), 8.12 (dd, J = 7.7, 1.6 Hz, 2H), 7.74-7.62 (m, 5H), 7.17 (d, J = 8.3 Hz, 4H), 2.29 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 164.9 (ArC), 136.5 (ArC), 135.2 (ArC), 132.8 (ArC), 130.5 (ArCH), 129.1 (ArCH), 128.6 (ArCH), 126.9 (ArCH), 120.4 (ArCH), 20.5 (CH_3). HR-MS (+ESI): found m/z 345.1609 ($[M+H]^+$), $[C_{22}H_{21}N_2O_2]^+$ requires m/z 345.1598 (monoisotopic mass).

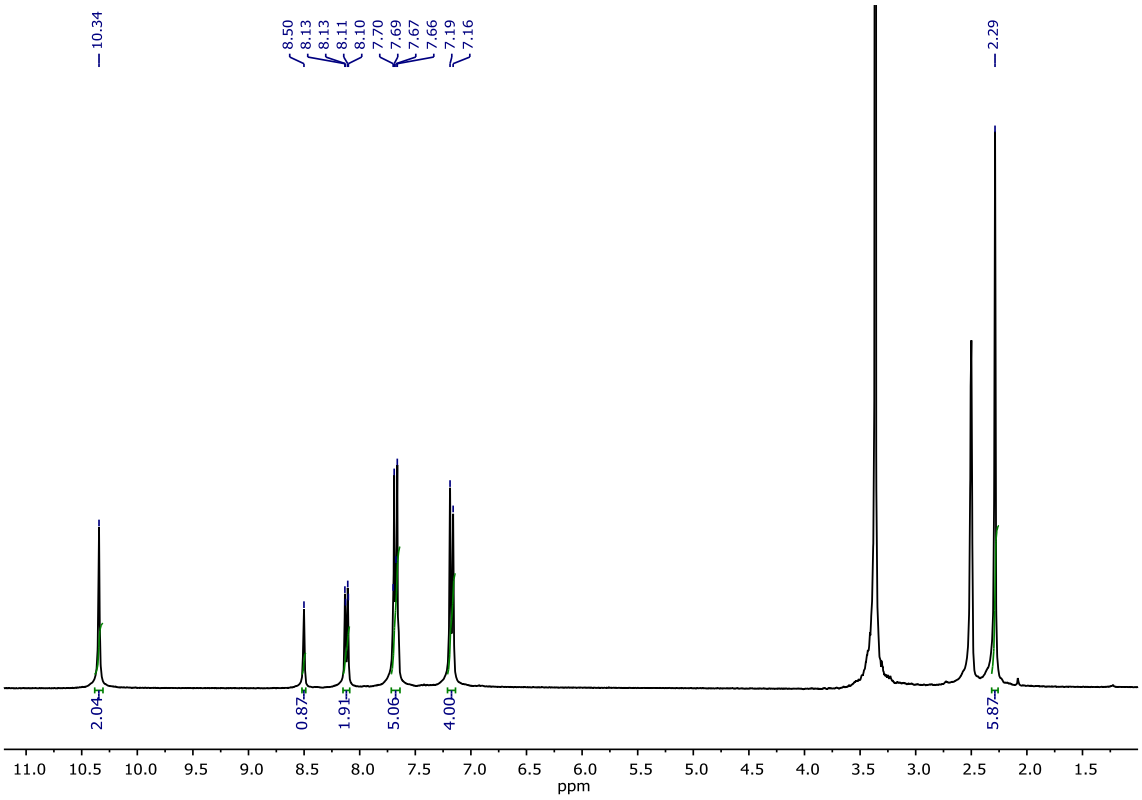


Figure S2. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L1**.

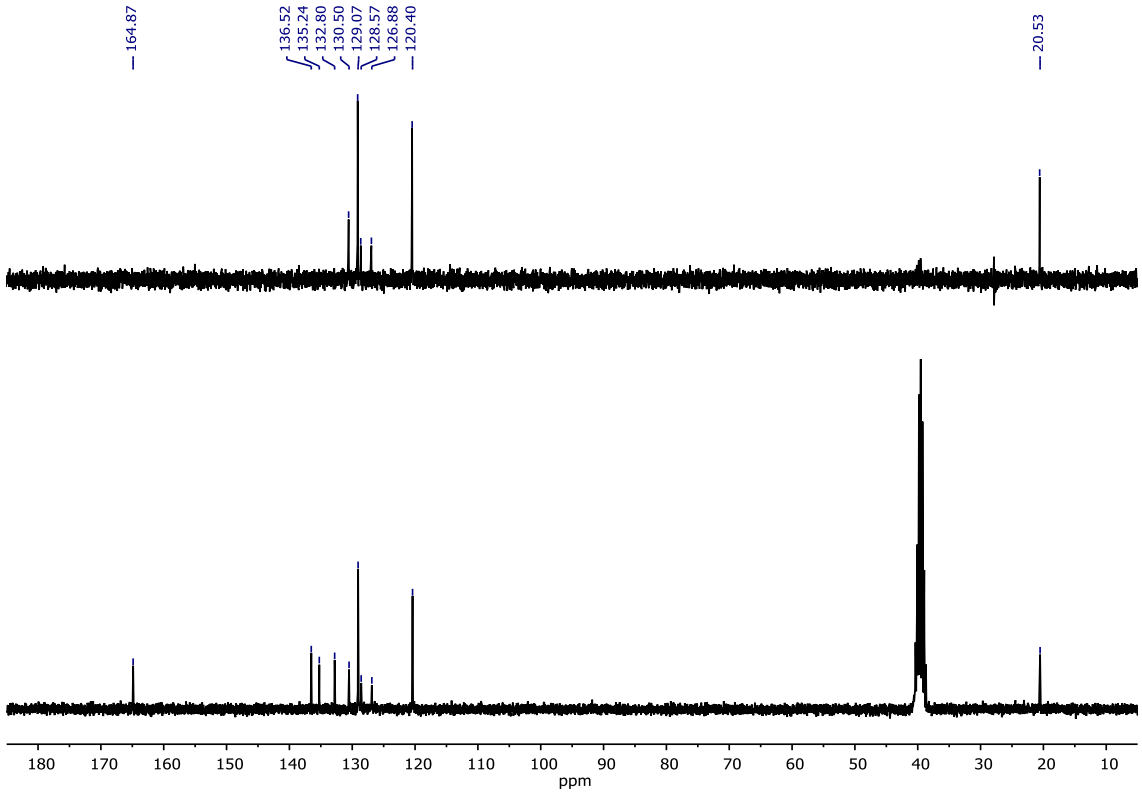


Figure S3. ^{13}C and DEPT NMR spectra (75 MHz, DMSO-d_6) for compound **L1**.

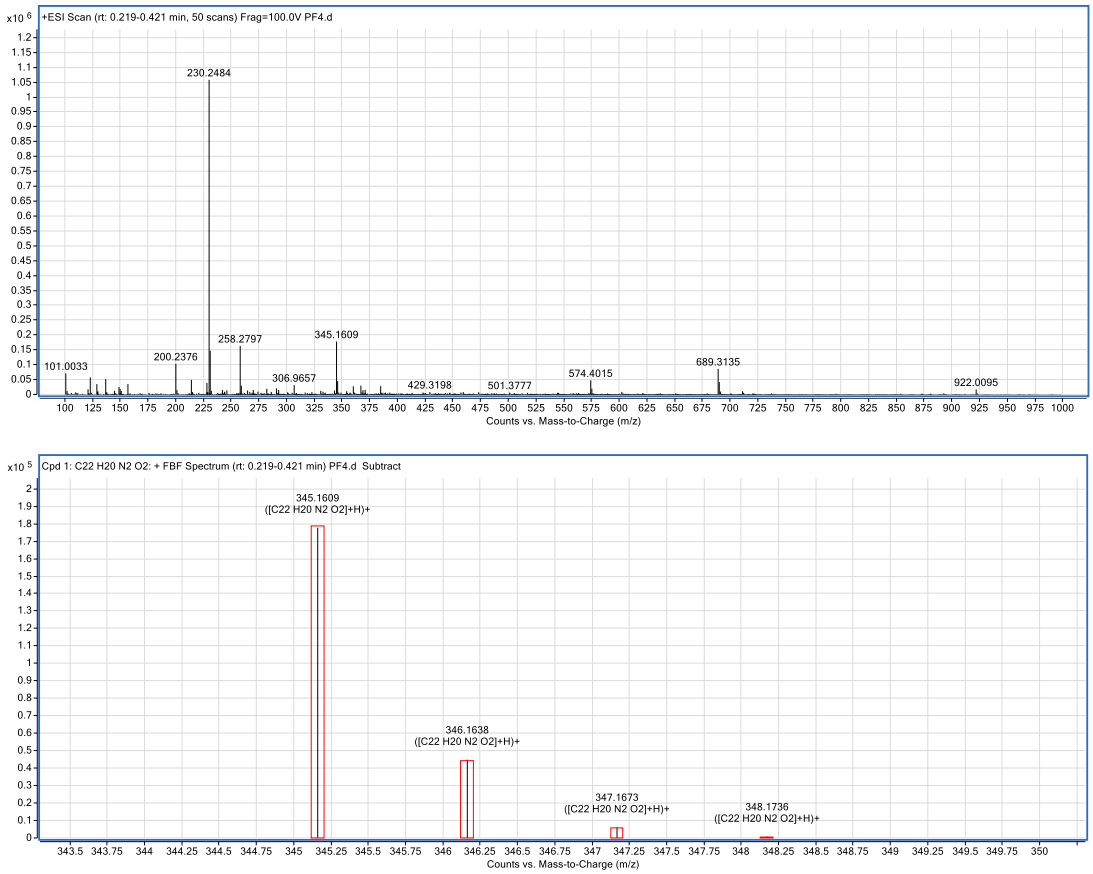
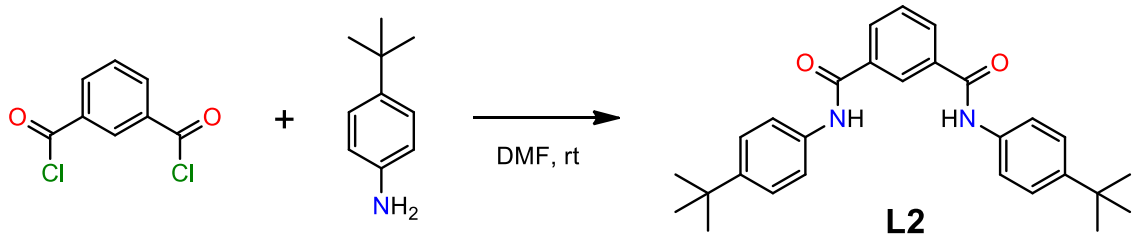


Figure S4. HR-MS (+ESI) spectrum for compound **L1**.

2.2.2. *N^1,N^3-bis(4-(tert-butyl)phenyl)isophthalamide (L2)*



4-*tert*-butylaniline (0.54 mL, 3.4 mmol) was dissolved in dimethylformamide (4 mL). Subsequently, small portions of isophthaloyl chloride (0.35 g, 1.7 mmol) were added to the solution over a period of several minutes. The mixture was stirred at room temperature for 30 minutes and subsequently it was poured into a beaker containing 12 mL of distilled water, which led to the formation of a white precipitate. This solid was filtrated, washed with water and recrystallized in an ethanol-water mixture (0.12 g, 16%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.36 (s, 2H), 8.51 (s, 1H), 8.13 (dd, J = 7.8, 1.4 Hz, 2H), 7.80-7.61 (m, 5H), 7.39 (d, J = 8.6 Hz, 4H), 1.29 (s, 18H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 164.9 (ArC), 146.2 (ArC), 136.5 (ArC), 135.3 (ArC), 130.6 (ArCH), 128.6 (ArCH), 127.0 (ArCH), 125.3 (ArCH), 120.2 (ArCH), 34.2 (C), 31.3 (CH_3). HR-MS (+ESI): found m/z 429.2534 ($[M+H]^+$), $[C_{28}H_{33}N_2O_2]^+$ requires m/z 429.2537 (monoisotopic mass).

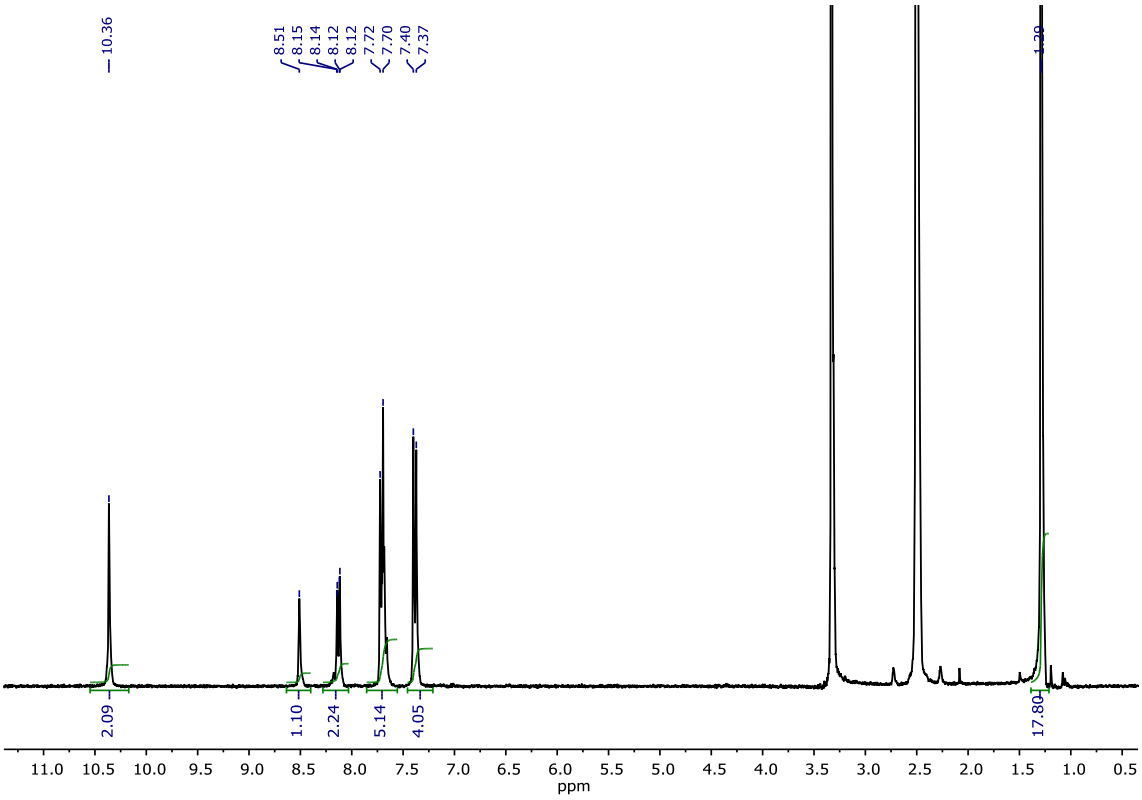


Figure S5. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L2**.

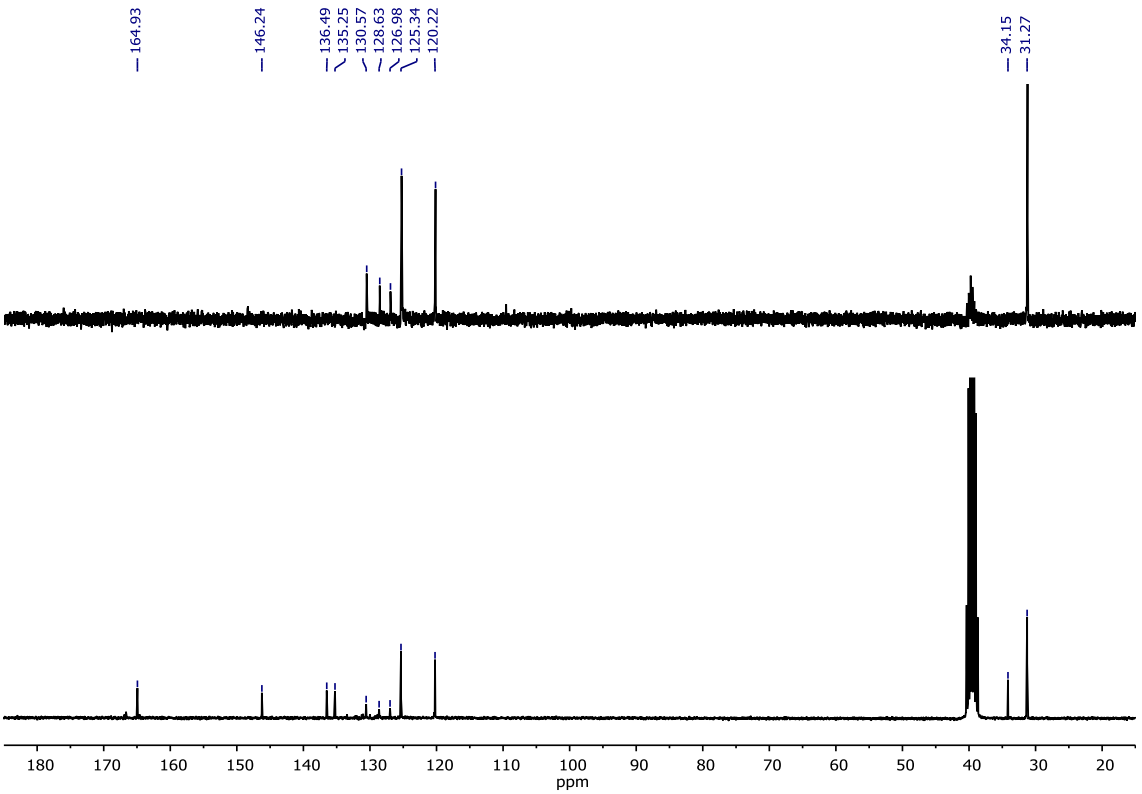


Figure S6. ^{13}C and DEPT NMR spectra (75 MHz, DMSO-d_6) for compound **L2**.

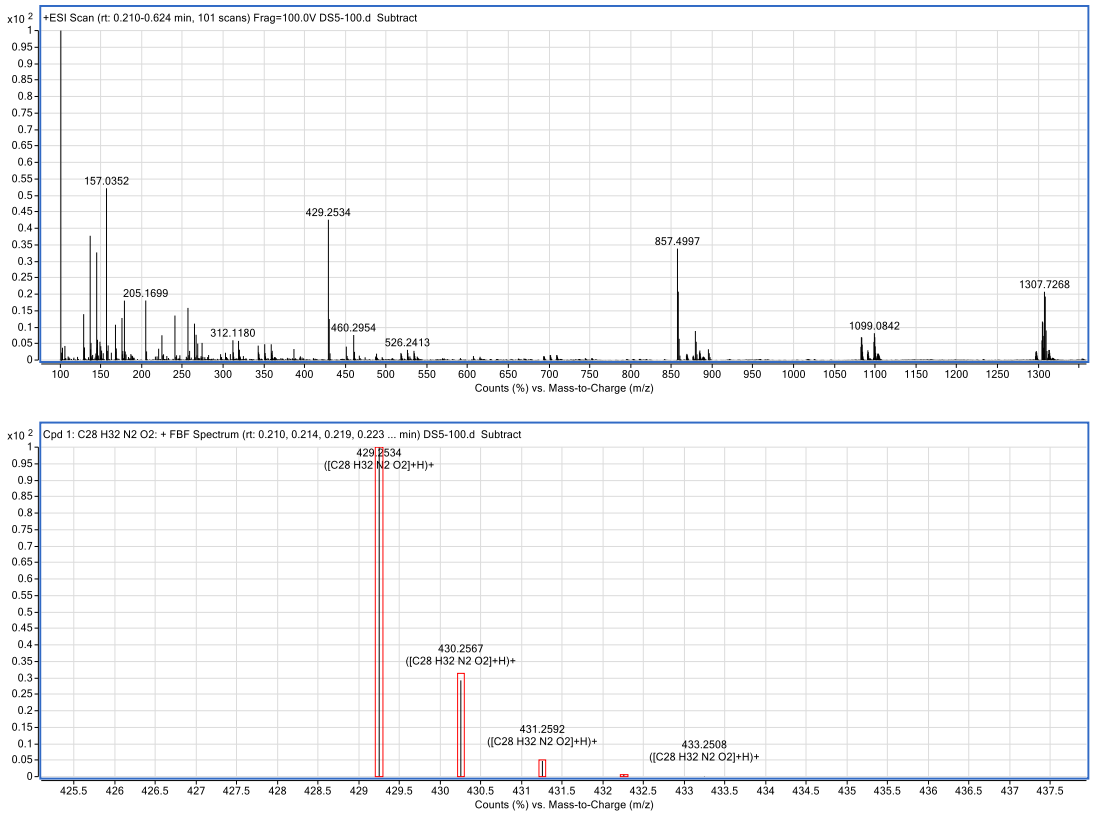
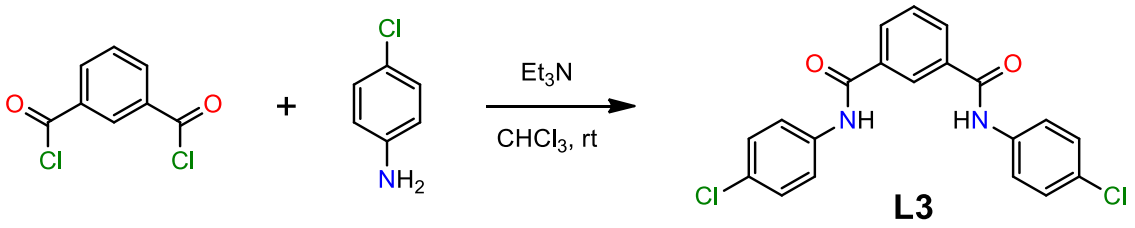


Figure S7. HR-MS (+ESI) spectrum for compound **L2**.

2.2.3. *N^1,N^3-bis(4-chlorophenyl)isophthalamide (L3)*



4-chloroaniline (0.31 g, 2.4 mmol) and triethylamine (0.33 mL, 2.4 mmol) were mixed in chloroform (5 mL). Subsequently, small portions of isophthaloyl chloride (0.25 g, 1.2 mmol) were added to the mixture over a period of several minutes. A white precipitate was formed, and the mixture was stirred at room temperature for 12 hours. The solid was filtrated, washed with distilled water (3 × 10 mL) and dried *in vacuo* (0.37 g, 77%). Slow evaporation of a solution of the compound in *n*-butanol provided colorless single crystals, suitable for X-ray diffraction analysis. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.57 (s, 2H), 8.53-8.50 (m, 1H), 8.19-8.13 (m, 2H), 7.90-7.82 (m, 4H), 7.75-7.66 (m, 1H), 7.47-7.39 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 165.1 (ArC), 138.0 (ArC), 135.0 (ArC), 130.8 (ArCH), 128.7 (ArCH), 128.6 (ArCH), 127.5 (ArC), 127.1 (ArCH), 121.9 (ArCH). HR-MS (+ESI): found *m/z* 385.0512 ($[M+H]^+$), $[C_{20}H_{15}Cl_2N_2O_2]^+$ requires *m/z* 385.0505 (monoisotopic mass).

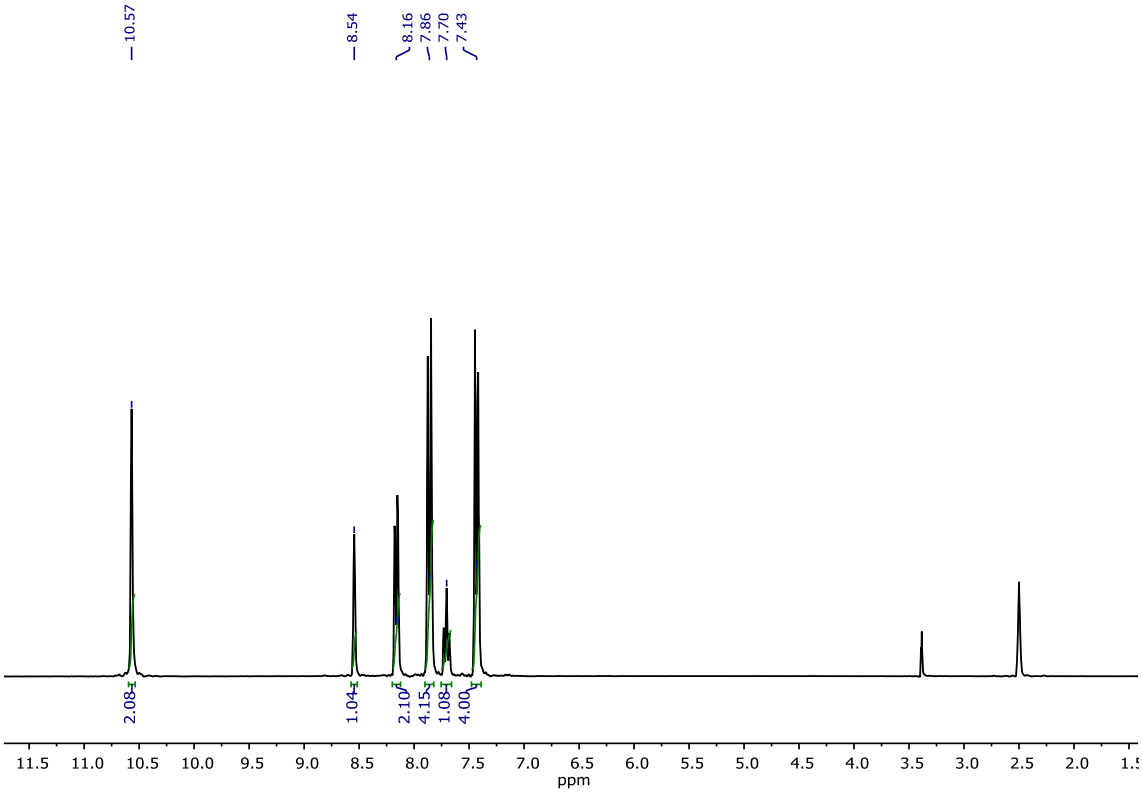


Figure S8. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L3**.

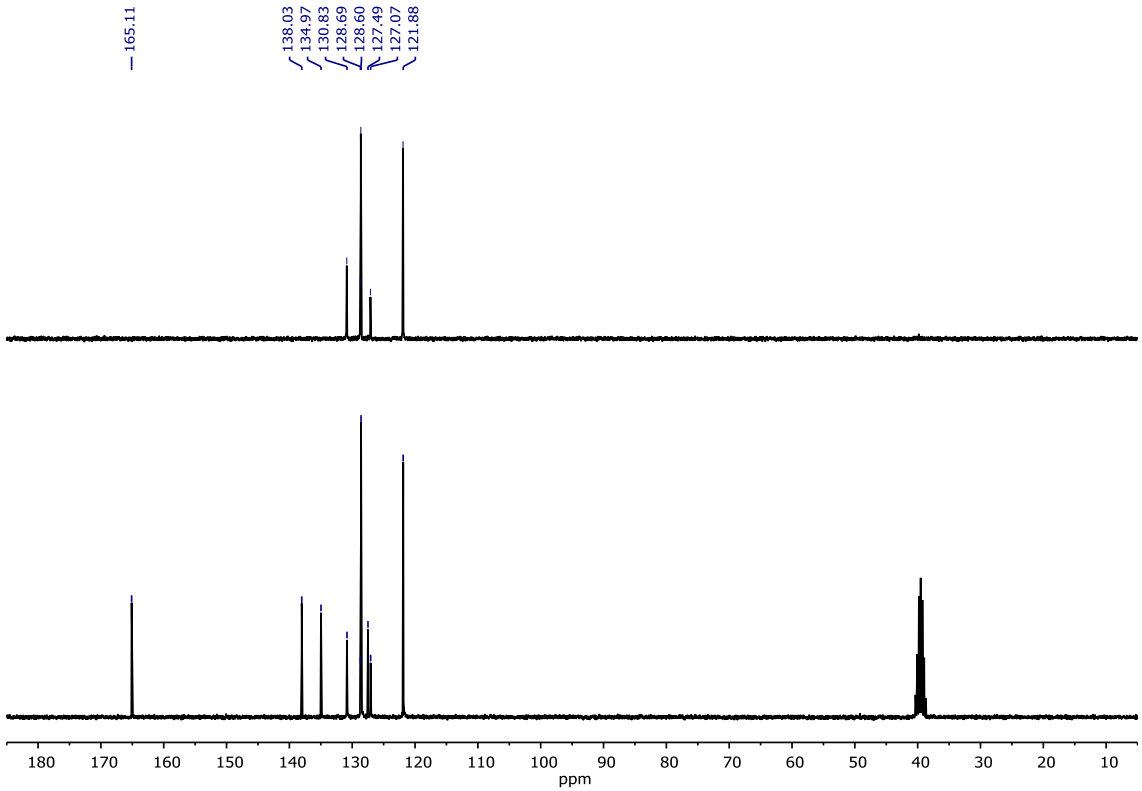


Figure S9. ^{13}C and DEPT NMR spectra (75 MHz, DMSO-d_6) for compound **L3**.

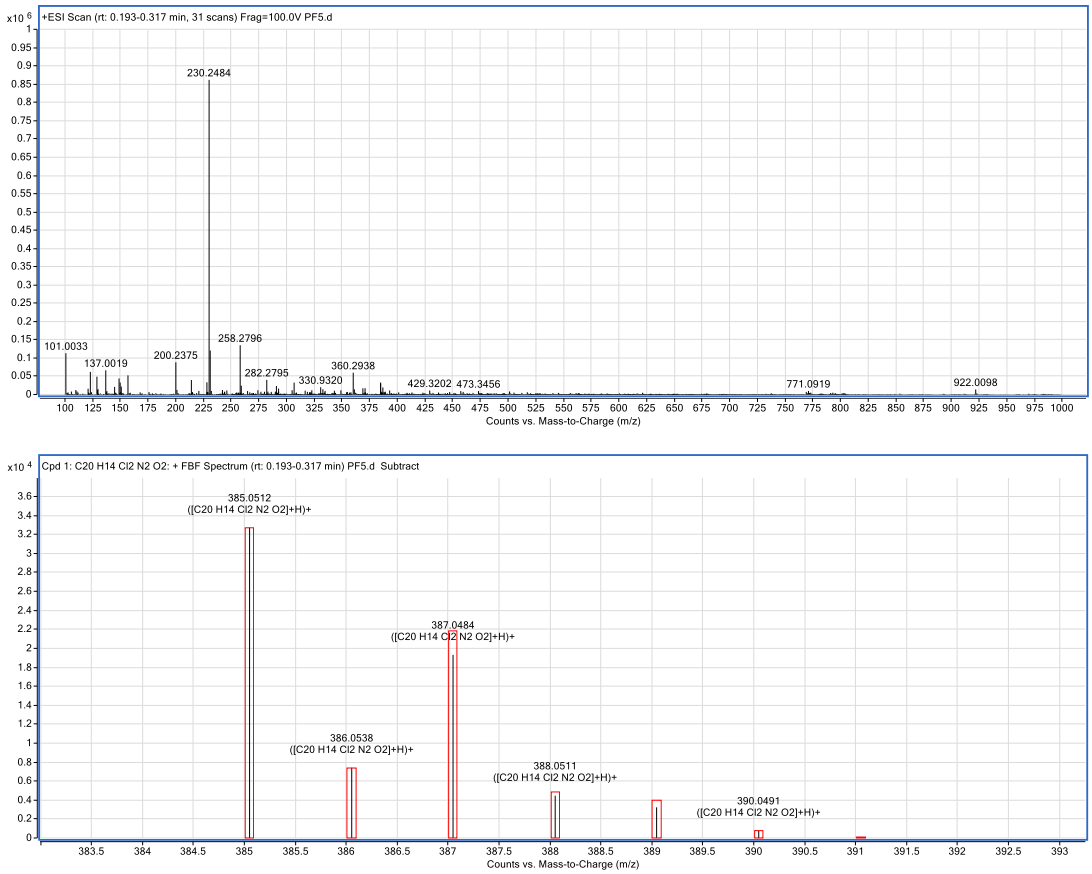
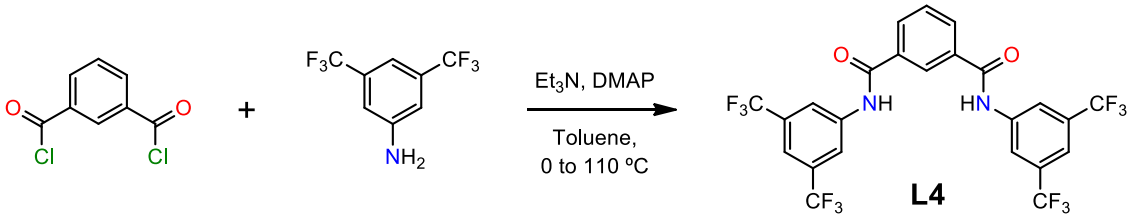


Figure S10. HR-MS (+ESI) spectrum for compound **L3**.

2.2.4. *N¹,N³-bis(3,5-bis(trifluoromethyl)phenyl)isophthalamide (L4)*



3,5-bis(trifluoromethyl)aniline (0.77 mL, 4.8 mmol), triethylamine (0.67 mL, 4.8 mmol) and 4-dimethylaminopyridine (0.59 g, 4.8 mmol) were mixed in toluene (25 mL). The mixture was stirred at 0 °C and small portions of isophthaloyl chloride (0.50 g, 2.4 mmol) were subsequently added over a period of several minutes. A white precipitate was formed and the reaction mixture was stirred at 110 °C for four days. Once room temperature was reached, distilled water (5 mL) was added and the resulting solution was transferred to a separating funnel. After adding toluene (25 mL) and distilled water (45 mL) and shaking, a white solid was formed. This solid was isolated by filtration, washed with distilled water (3 × 5 mL) and dried under vacuum. On the other hand, the organic phase was concentrated to dryness and ethyl acetate (150 mL) was added to the residue. The resulting suspension was washed with a 1M HCl aqueous solution (3 × 100 mL) and the organic phase was dried with anhydrous sodium sulfate, filtered and concentrated to dryness. Dichloromethane (10 mL) was added to the residue and the resulting white solid was filtrated, washed with the same solvent (4 × 10 mL) and dried *in vacuo*. After analyzing this solid and the former one by ^{1}H NMR spectroscopy it was concluded that both of them were contaminated, so they were combined, dissolved with ethyl acetate (100 mL) and this solution was washed with a saturated aqueous solution of sodium carbonate (3 × 100 mL). The organic phase was then dried with anhydrous sodium sulfate, filtered and the solvent removed under vacuum, leading to the desired compound (0.91 g, 63%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 11.01 (s_b, 2H), 8.64 (s_b, 1H), 8.54 (s, 4H), 8.23 (d, J = 7.7 Hz, 2H), 7.84-7.71 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 165.5 (ArC), 141.0 (ArC), 134.3 (ArC), 131.3 (ArCH), 130.7 (ArC, q, ^{2}J = 33 Hz), 129.0 (ArCH), 127.4 (ArCH), 123.3 (ArC, q, ^{1}J = 271 Hz), 119.9 (ArCH, q, ^{3}J = 3.0 Hz), 116.6 (ArCH, q, ^{3}J = 3.5 Hz). HR-MS (+ESI): found m/z 589.0784 ($[M+H]^+$), $[C_{24}H_{13}F_{12}N_2O_2]^+$ requires m/z 589.0780 (monoisotopic mass).

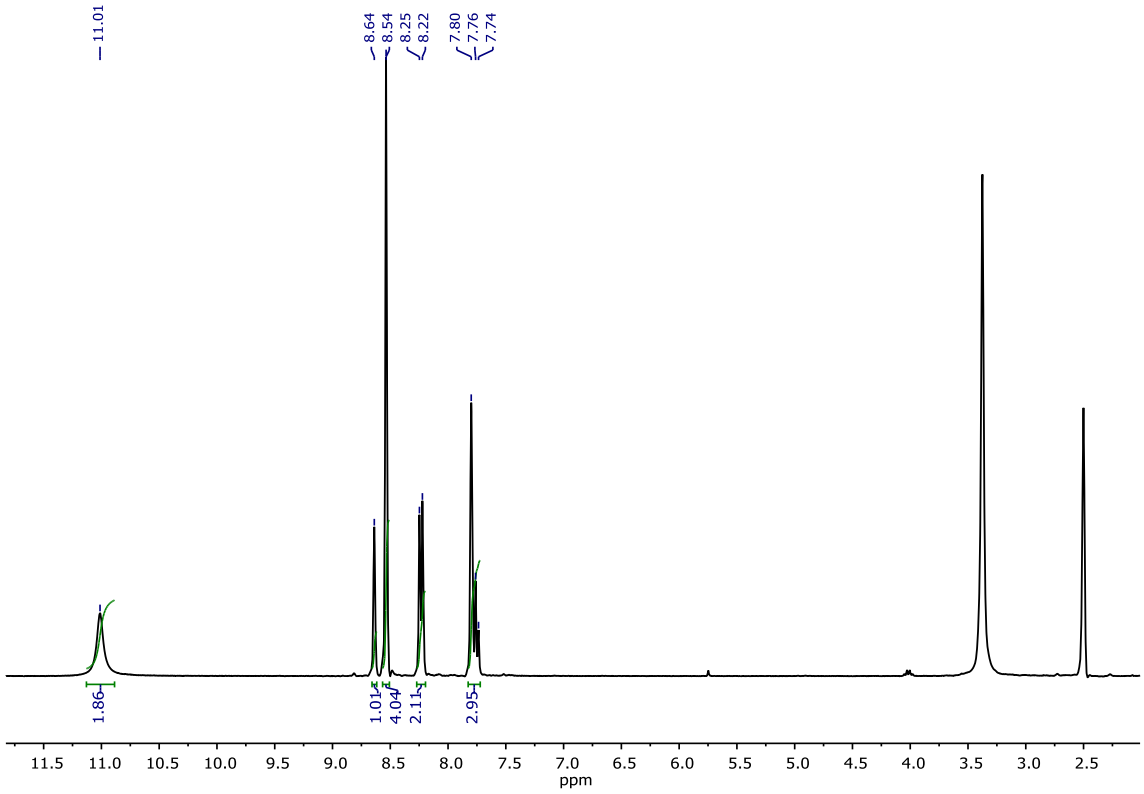


Figure S11. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L4**.

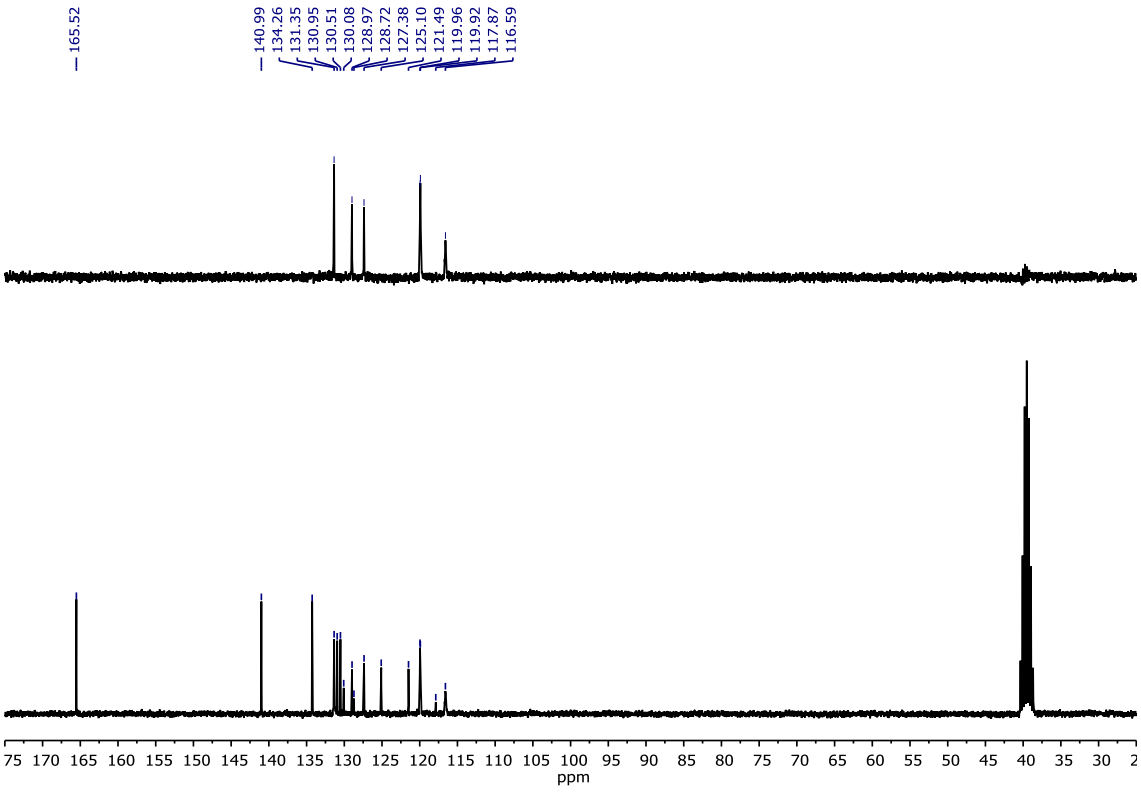


Figure S12. ^{13}C and DEPT NMR spectra (75 MHz, DMSO-d_6) for compound **L4**.

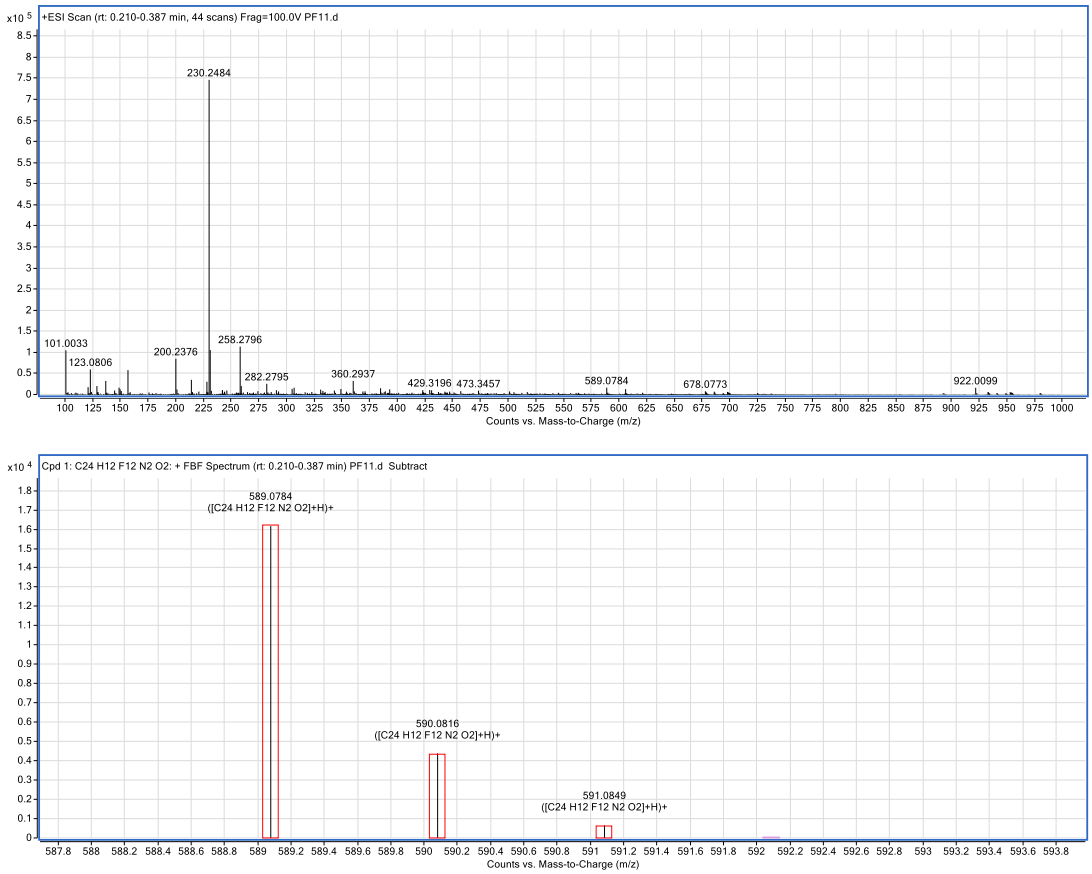
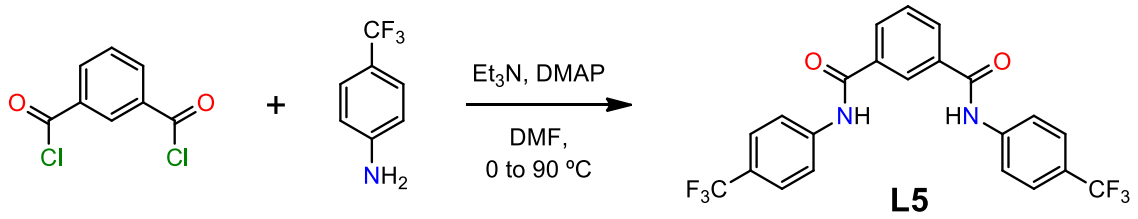


Figure S13. HR-MS (+ESI) spectrum for compound **L4**.

2.2.5. *N^1,N^3-bis(4-(trifluoromethyl)phenyl)isophthalamide (L5)*



4-trifluoromethylaniline (0.40 mL, 3.2 mmol), triethylamine (0.66 mL, 4.7 mmol) and 4-dimethylaminopyridine (0.38 g, 3.1 mmol) were mixed in dimethylformamide (20 mL). The resulting solution was cooled down to 0 °C with an ice-water bath and small portions of isophthaloyl chloride (0.32 g, 1.6 mmol) were subsequently added to the mixture over a period of several minutes. Once the addition was completed, the reaction mixture was stirred at 90 °C for 24 hours. After reaching room temperature, the mixture was poured into a beaker containing 100 mL of distilled water and this solution was extracted with ethyl acetate (4 × 35 mL). The combined organic phase was washed with brine (2 × 50 mL), distilled water (2 × 50 mL) and a 1M HCl aqueous solution (4 × 35 mL). Afterwards, it was dried with anhydrous sodium sulfate, filtered and concentrated to dryness. The orange residue was recrystallized in dichloromethane, leading to a powdery white solid, the desired product (0.11 g, 15%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.77 (s, 2H), 8.56 (s, 1H), 8.19 (d, J = 7.5 Hz, 2H), 8.04 (d, J = 7.5 Hz, 4H), 7.80-7.68 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 165.7 (ArC), 142.7 (ArC), 134.9 (ArC), 131.3 (ArCH), 129.0 (ArCH), 127.4 (ArCH), 126.1 (ArCH, q, ^{3}J = 3.6 Hz), 124.5 (ArC, q, ^{1}J = 271 Hz), 124.0 (ArC, q, ^{2}J = 31 Hz), 120.4 (ArCH). HR-MS (+ESI): found m/z 453.1029 ($[M+H]^+$), $[C_{22}H_{15}F_6N_2O_2]^+$ requires m/z 453.1032 (monoisotopic mass).

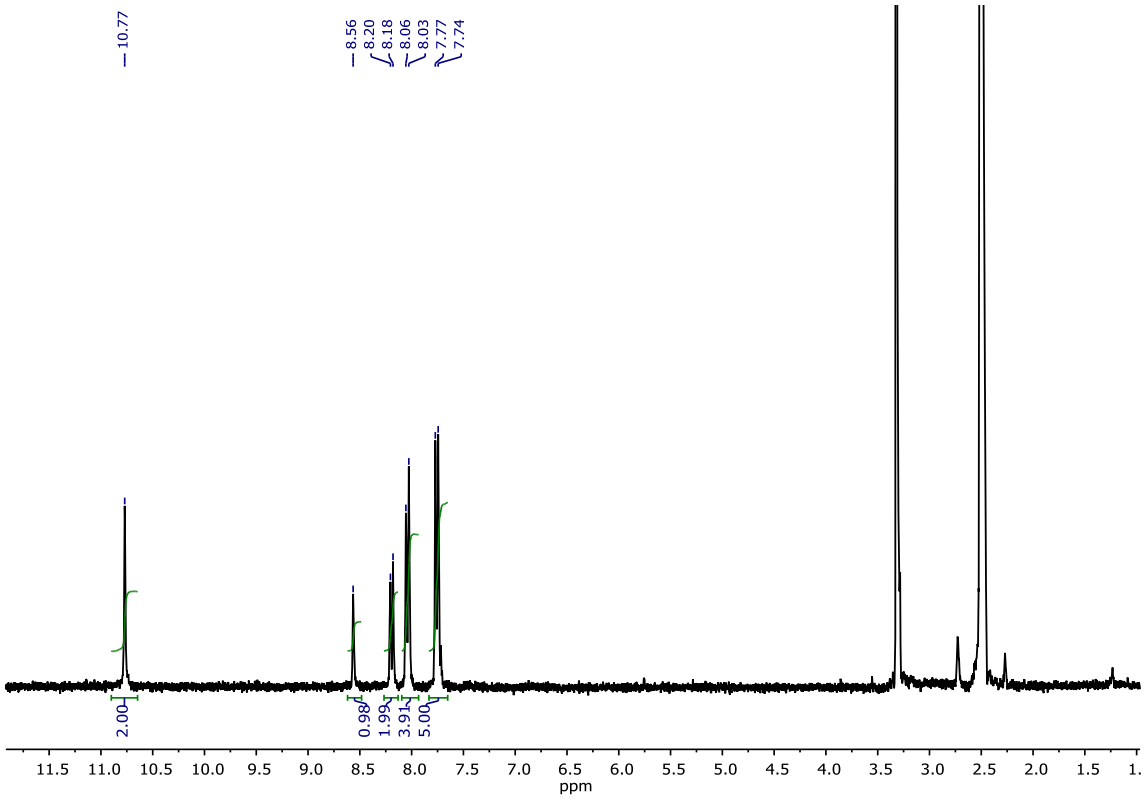


Figure S14. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L5**.

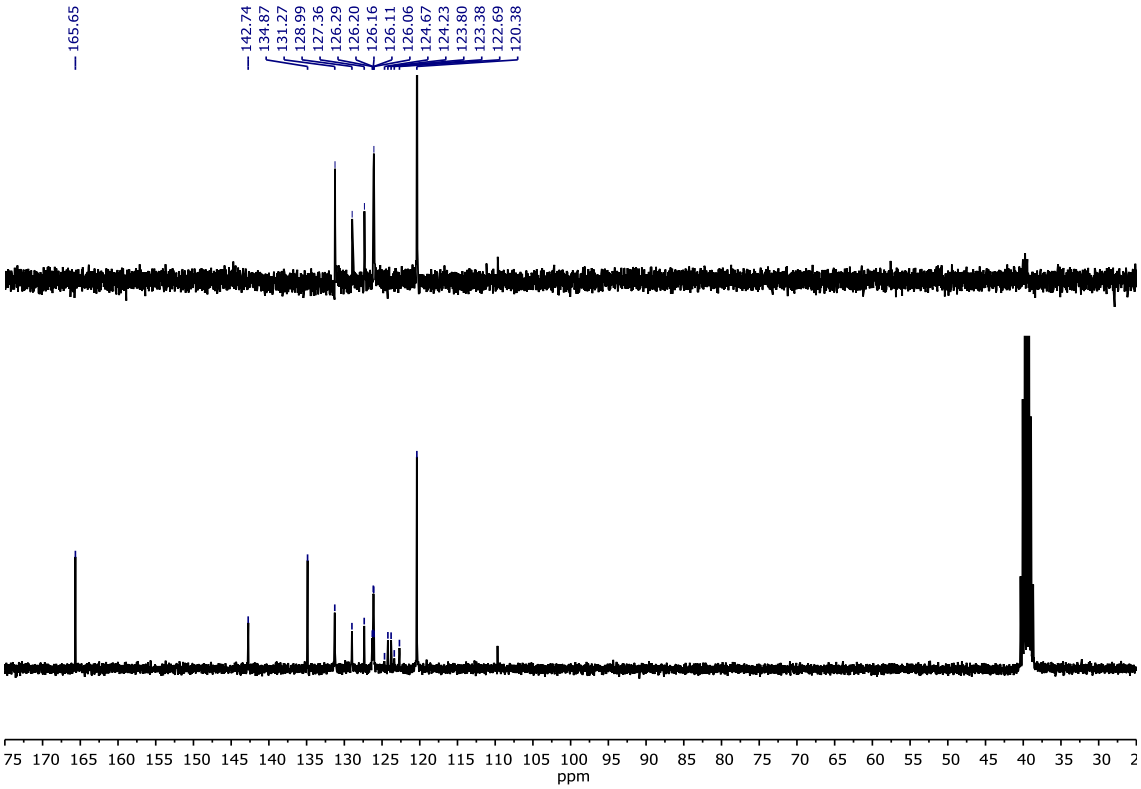


Figure S15. ^{13}C and DEPT NMR spectra (75 MHz, DMSO-d_6) for compound **L5**.

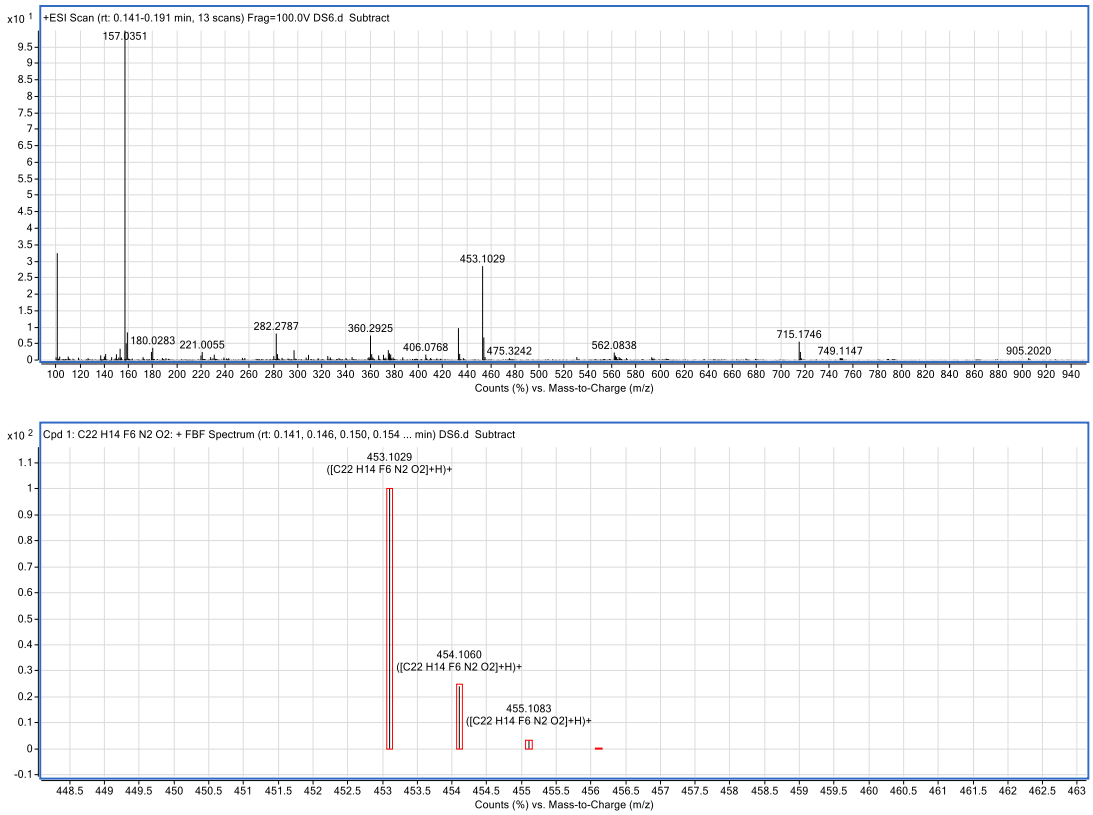
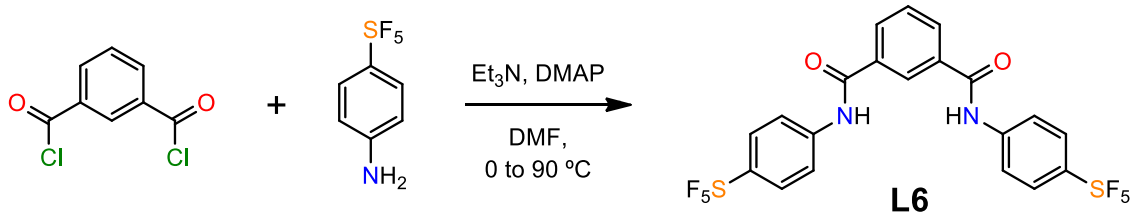


Figure S16. HR-MS (+ESI) spectrum for compound **L5**.

2.2.6. *N^1,N^3-bis(4-(pentafluorosulfanyl)phenyl)isophthalamide (L6)*



4-pentafluorosulfanylaniline (0.60 g, 2.7 mmol), triethylamine (0.52 mL, 3.7 mmol) and 4-dimethylaminopyridine (0.29 g, 2.4 mmol) were mixed in dimethylformamide (25 mL). The mixture was stirred at 0 °C and small portions of isophthaloyl chloride (0.21 g, 1.0 mmol) were subsequently added over a period of several minutes. The reaction mixture was stirred at 90 °C for three days. After reaching room temperature, the mixture was poured into a beaker containing 100 mL of distilled water and this solution was extracted with ethyl acetate (3 × 35 mL). The combined organic phase was washed with brine (2 × 50 mL), distilled water (2 × 50 mL), a 1M HCl aqueous solution (3 × 35 mL) and a saturated aqueous solution of sodium carbonate (3 × 35 mL). Afterwards, it was dried with anhydrous sodium sulfate, filtered and concentrated to dryness. The brown residue was purified by column chromatography, using silica gel as stationary phase and a hexane-ethyl acetate mixture (from 85:15 to 70:30) as eluent. The solvent was removed under vacuum, yielding a yellow solid, the desired product (0.07 g, 12%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.90 (s, 2H), 8.58 (s, 1H), 8.20 (d, J = 7.8 Hz, 2H), 8.04 (d, J = 9.0 Hz, 4H), 7.93 (d, J = 9.1 Hz, 4H), 7.74 (t, J = 7.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 165.6 (ArC), 147.7 (ArC, quint, ^{2}J = 16 Hz), 142.4 (ArC), 134.6 (ArC), 131.3 (ArCH), 128.9 (ArCH), 127.4 (ArCH), 126.8 (ArCH, quint, ^{3}J = 5.1 Hz), 120.0 (ArCH). HR-MS (+ESI): found m/z 569.0404 ($[M+H]^+$), $[C_{20}H_{15}F_{10}N_2O_2S_2]^+$ requires m/z 569.0410 (monoisotopic mass).

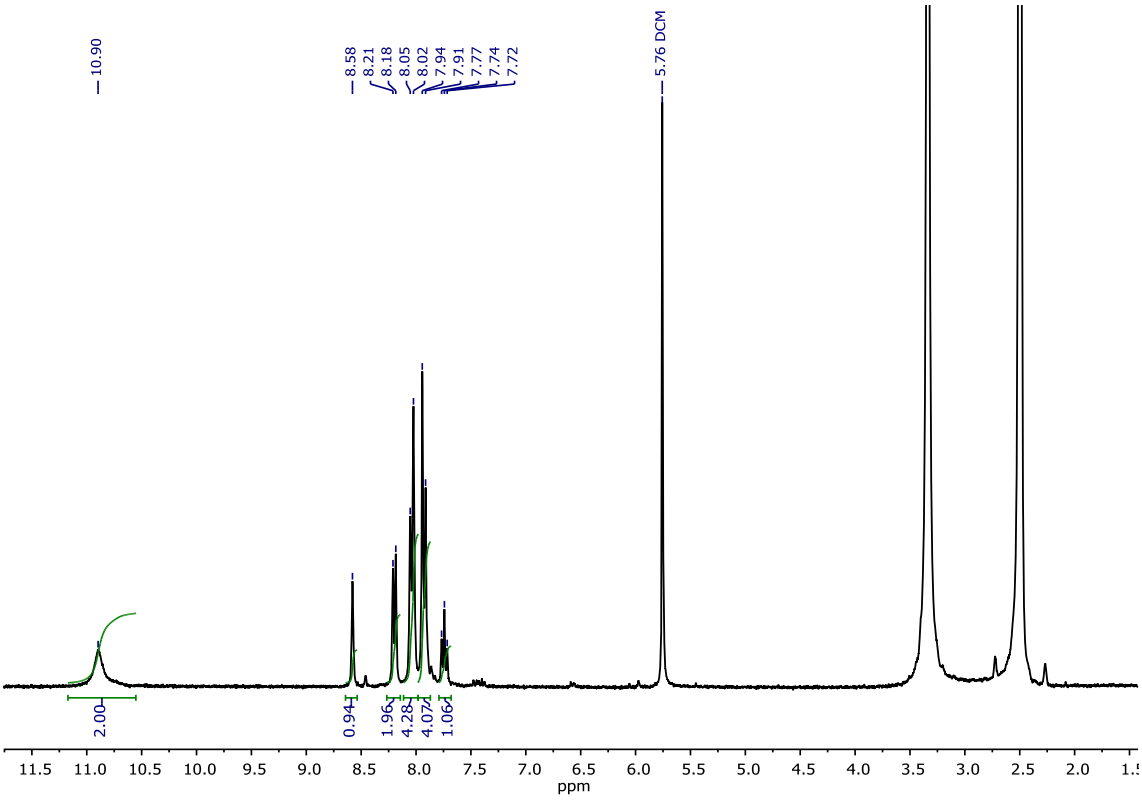


Figure S17. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L6**.

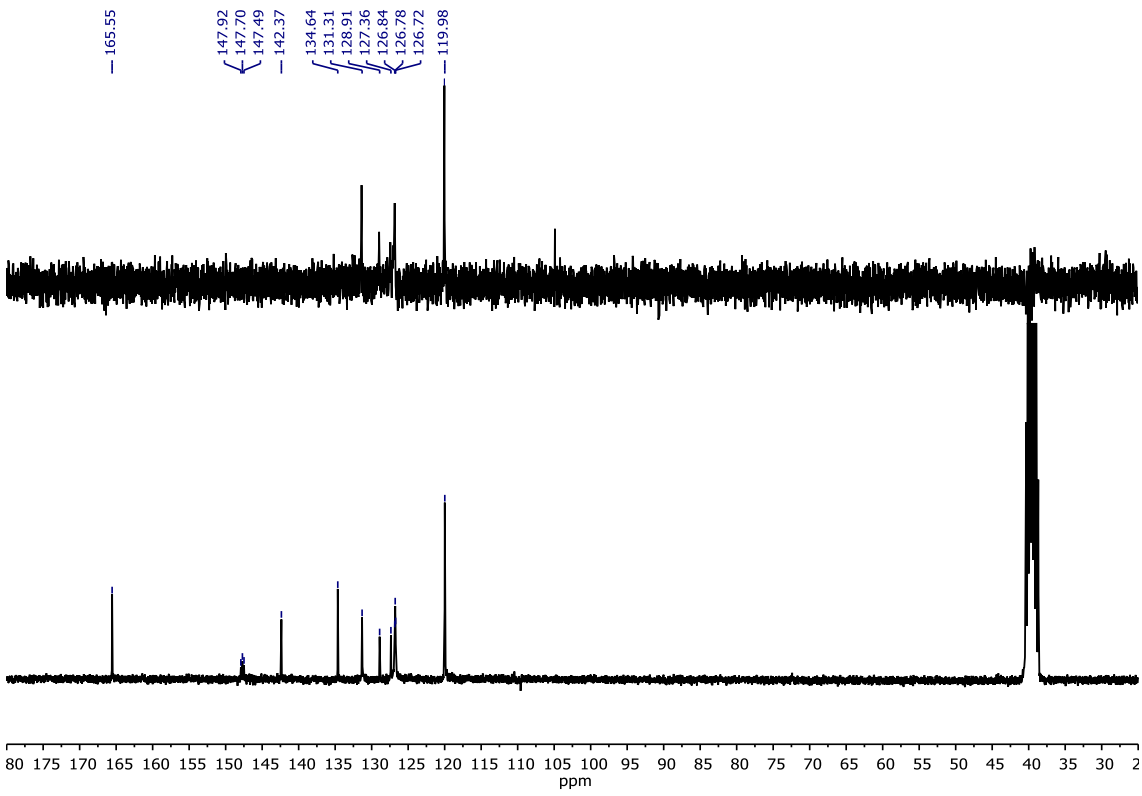


Figure S18. ^{13}C and DEPT NMR spectra (75 MHz, DMSO-d_6) for compound **L6**.

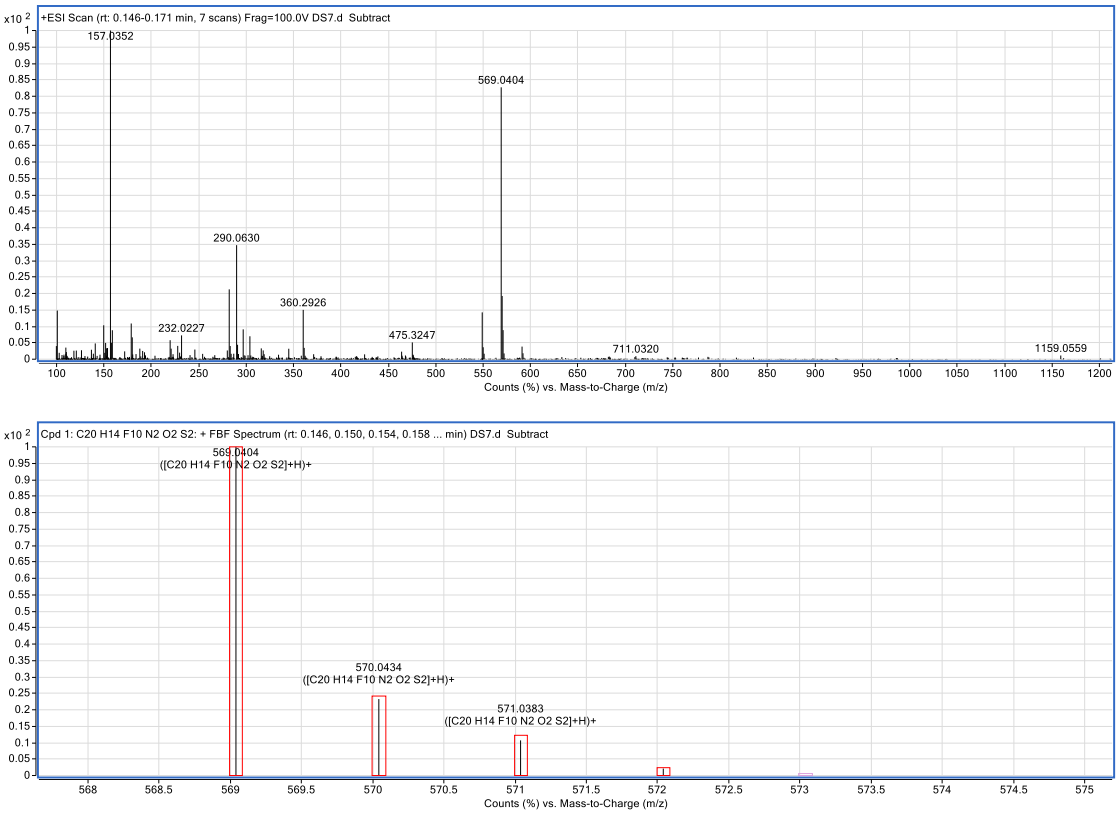
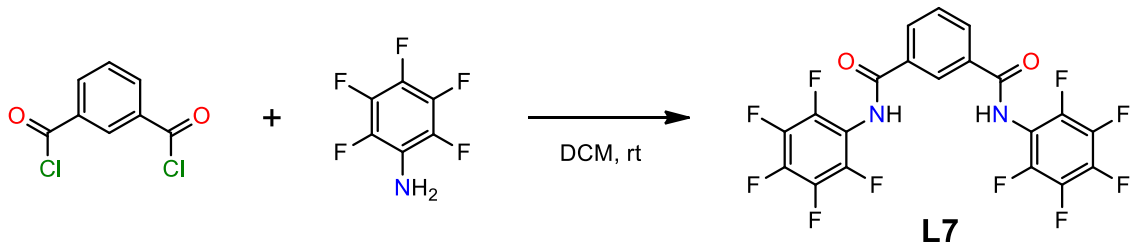


Figure S19. HR-MS (+ESI) spectrum for compound **L6**.

2.2.7. *N^1,N^3-bis(perfluorophenyl)isophthalamide (L7)*



To a solution of pentafluoroaniline (1.81 g, 9.8 mmol) in dichloromethane (10 mL) a solution of isophthaloyl chloride (0.60 g, 2.5 mmol) in 10 mL of the same solvent was added. The solution was stirred at room temperature for 72 h. The formation of a white precipitate was observed. The reaction mixture was diluted with further 20 mL of dichloromethane and washed with a 1M NaOH aqueous solution (3 × 10 mL). The white precipitate was dissolved. Subsequently, the organic phase was washed with a 1M HCl aqueous solution (3 × 10 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give the desired product as a white solid (1.09 g, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.82 (s, 2H), 8.63 (s, 1H), 8.26 (d, J = 8.0 Hz, 2H), 7.78 (t, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 168.0, 136.0, 134.9, 132.4, 130.9. HR-MS (+ESI): found m/z 497.0349 ([M+H]$^+$), $[C_{20}H_7F_{10}N_2O_2]^+$ requires m/z 497.0342 (monoisotopic mass).

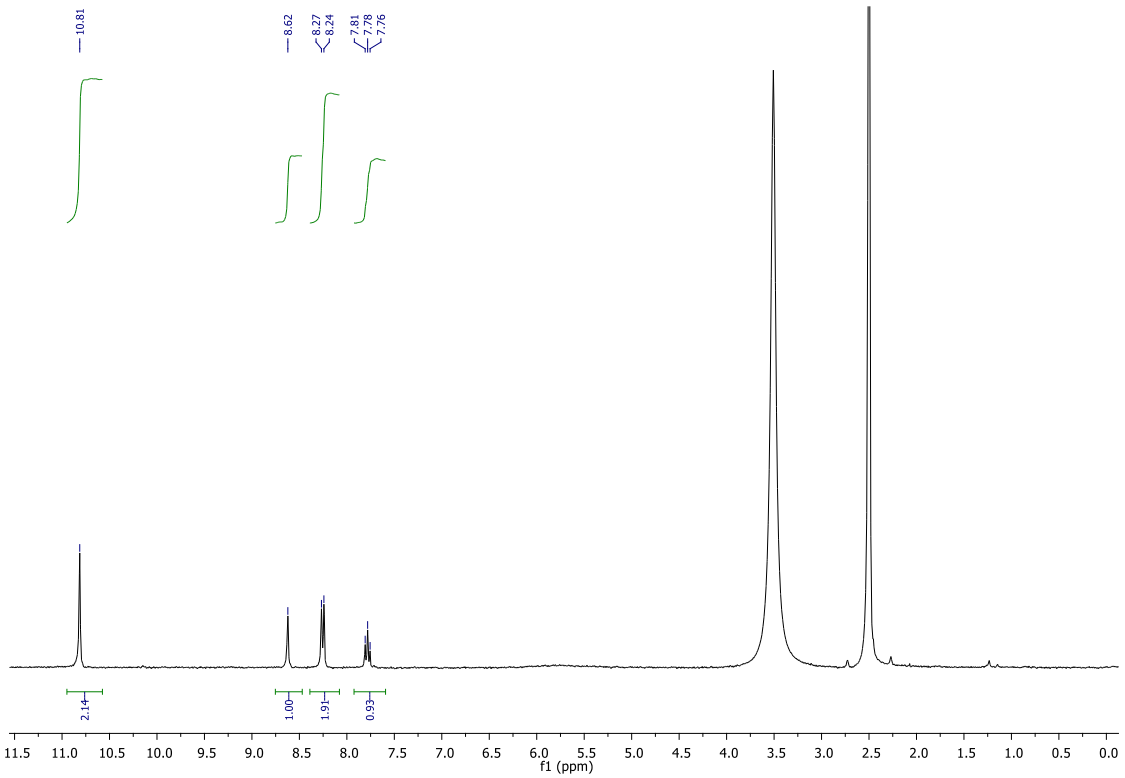


Figure S20. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L7**.

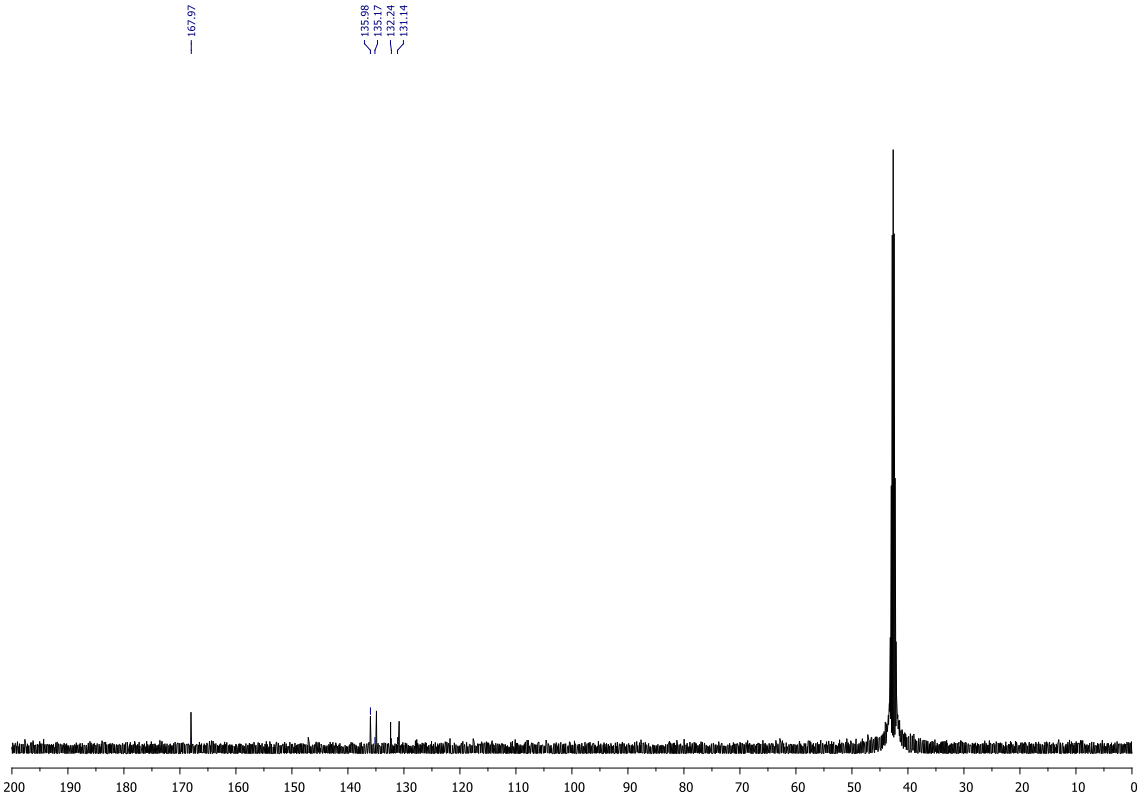


Figure S21. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) for compound **L7**.

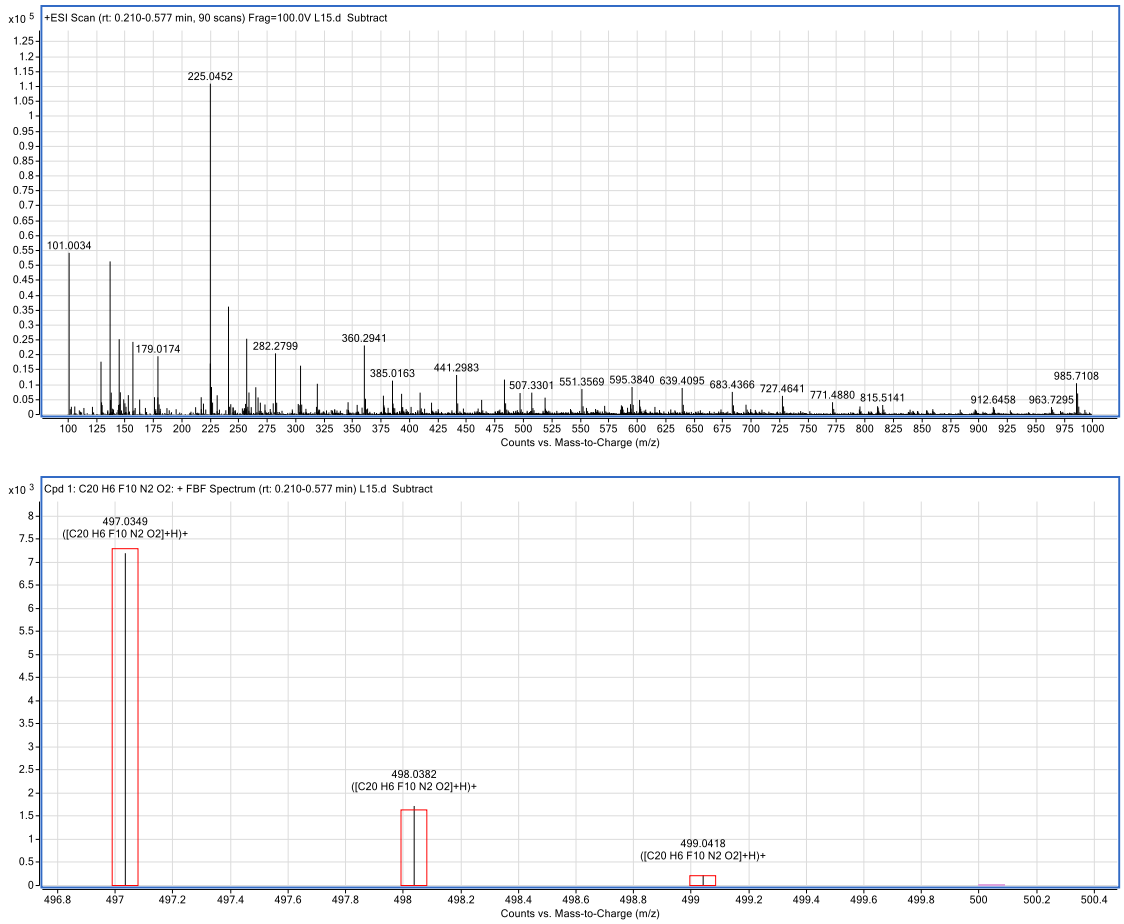
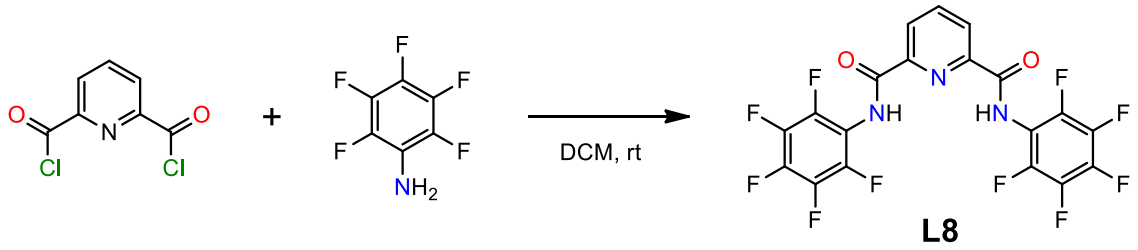


Figure S22. HR-MS (+ESI) spectrum for compound **L7**.

2.2.8. *N^2,N^6-bis(perfluorophenyl)pyridine-2,6-dicarboxamide (L8)*



L8

To a solution of pentafluoroaniline (1.80 g, 9.8 mmol) in dichloromethane (10 mL) a solution of pyridine-2,6-dicarbonyl dichloride (0.50 g, 2.5 mmol) in the same solvent (10 mL) was added. The solution was stirred at room temperature for 24 h. The formation of a pink precipitate was observed. The precipitate was filtered off and the filtrate concentrated to dryness to yield a pink oil which was purified by flash chromatography using silica gel as stationary phase and dichloromethane as eluent. The desired product was obtained as a white microcrystalline solid (0.91 g, 75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 11.09 (s, 2H), 8.50-8.30 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 165.1, 150.4, 143.7, 130.1. HR-MS (+ESI): found *m/z* 498.0295 ($[M+H]^+$), $[C_{19}H_6F_{10}N_3O_2]^+$ requires *m/z* 498.0295 (monoisotopic mass).

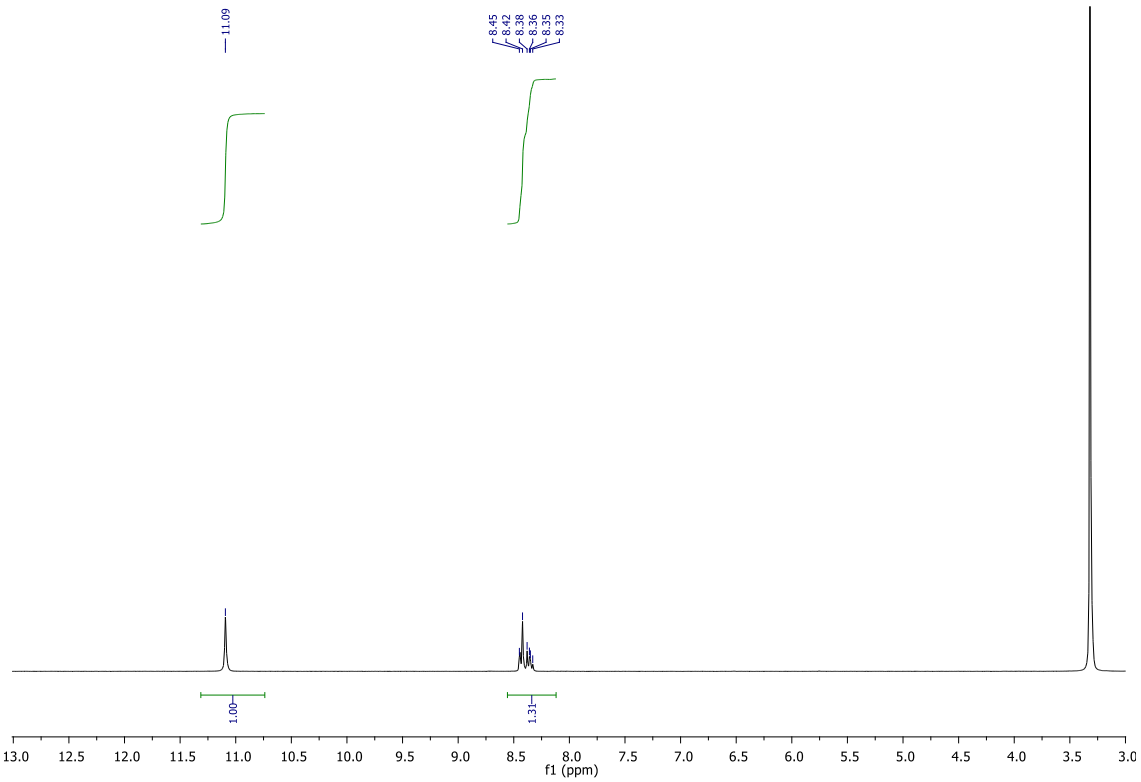


Figure S23. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **L8**.

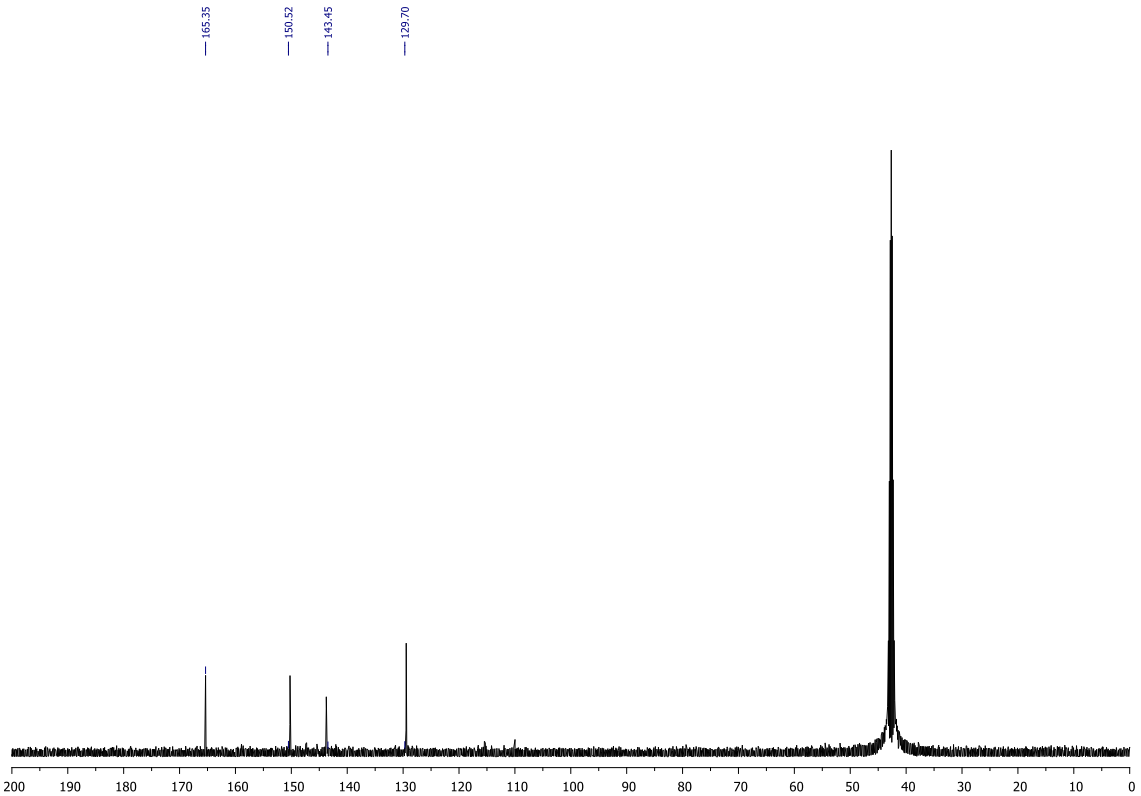


Figure S24. ^{13}C NMR spectrum (75 MHz, DMSO-d_6) for compound **L8**.

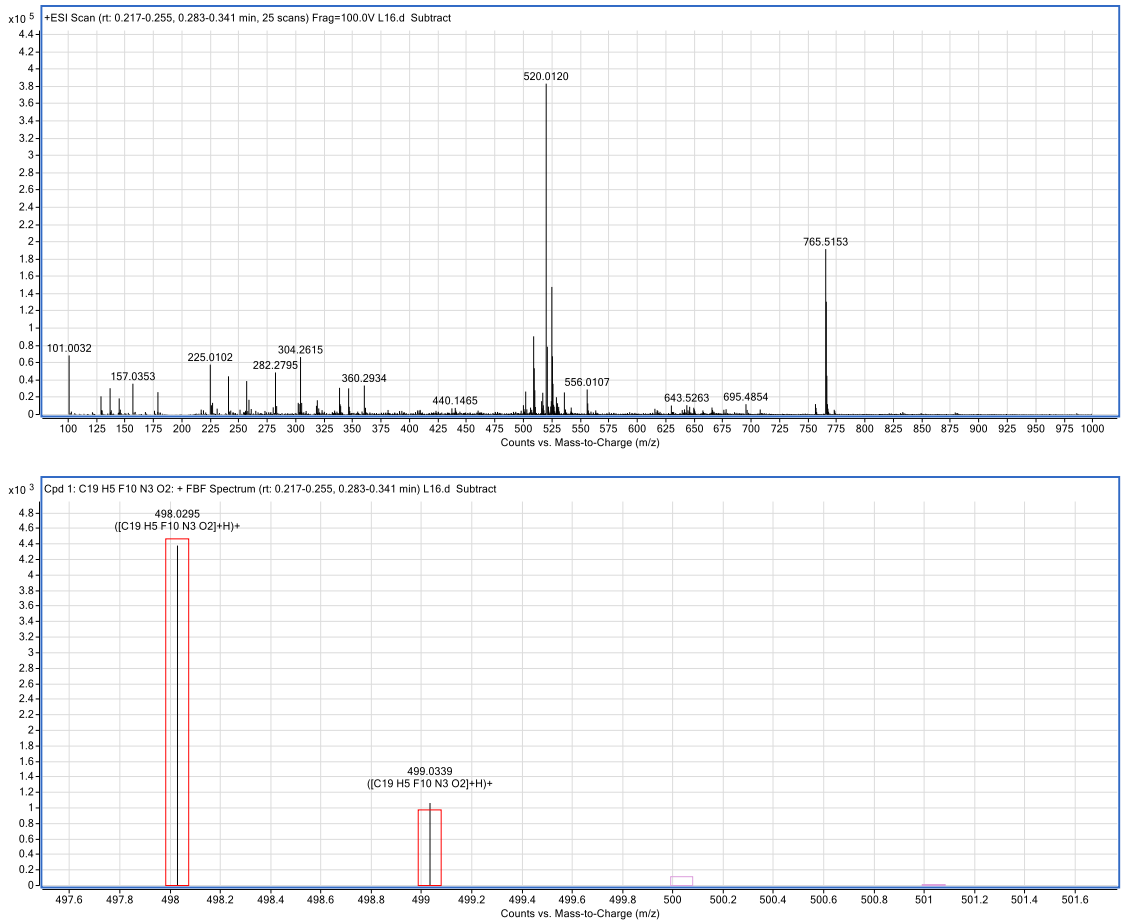


Figure S25. HR-MS (+ESI) spectrum for compound **L8**.

3. X-RAY DIFFRACTION STUDIES

*Solid state structure of compound **L3***

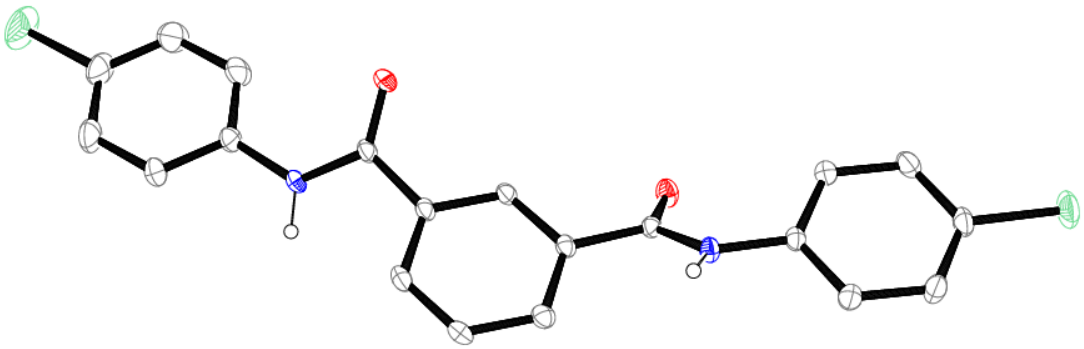


Figure S26. X-ray molecular structure of compound **L3**. Hydrogen atoms, except those involved in hydrogen-bonding interactions, are omitted for the sake of simplicity. The ORTEP plot is at the 30% probability level.

Table S1. Crystal data and refinement details for **L3**.

formula	$C_{20}H_{14}Cl_2N_2O_2$
MW	385.23
crystal system	Monoclinic
space group	*P* $2_1/c$
T/K	298(2)
a/Å	36.809(5)
b/Å	5.0601(7)
c/Å	9.7506(13)
α/deg	90
β/deg	95.567(2)
γ/deg	90
V/Å^3	1807.5(4)
F(000)	792
Z	4
λ, Å (MoKα)	0.71073
D_{calc}/g cm^{-3}	1.416
μ/mm^{-1}	0.376
θ range/deg	2.22 – 27.99
R_{int}	0.1635
reflections measured	19218
unique reflections	4093
reflections observed	3019
GOF on F^2	0.945
$R1^a$	0.0540
$wR2^b$	0.1375
Largest ≠ peak & hole/eÅ^{-3}	0.417 and -0.304

^{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 were obtained by slow evaporation of a solution of the isolated compound in *n*-butanol. Three dimensional X-ray data were collected on a BRUKER SMART APEX CCD diffractometer. Complex scattering factors were taken from the

program SHELXL-2016[1] running under the WinGX program system[2] as implemented on a Pentium® computer. The structure was solved with SIR92[3] and refined by full-matrix least-squares on F^2. All hydrogen atoms, except those corresponding to the NH fragments of the amide groups, which were refined freely in the last 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 summarized in **Table S1**.

1 SHELXL-2016: Sheldrick, G. M. *Acta Cryst*. **2008**, *A64*, 112-122.

2 WinGX: Farrugia, L. J. *J. Appl. Cryst*. **1999**, *32*, 837-838.

3 SIR92: Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Cryst.* **1994**, *27*, 435.

4. ^{1}H NMR TITRATIONS

4.1. Titration procedure and titration data fitting

2 mL of a DMSO-d_6 stock solution of the corresponding receptor (host) were prepared (0.01 or 0.005 M, depending on the receptor). From this solution, 1 mL was taken to prepare the solution of the titrating agent (guest; tetrabutylammonium chloride), thus avoiding the dilution effect. 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 microsyringe through the septum, the solution homogenized and the spectrum recorded. This process was repeated between 12 and 20 times.

For each ^{1}H NMR titration, the spectra were processed and the chemical shifts of the signal corresponding to the protons of the NH fragments of the amide groups were represented against the concentration of chloride in solution. The titration profiles were fitted satisfactorily to a 1:1 (L:Cl^-) binding model, L being the receptor. Association constants K_a were obtained employing the WinEQNMR software.[4]

[4] Hynes, M. J. *J. Chem. Soc. Dalton Trans.* **1993**, 311-312.

4.2. ^{1}H NMR titration spectra and fitted binding isotherms

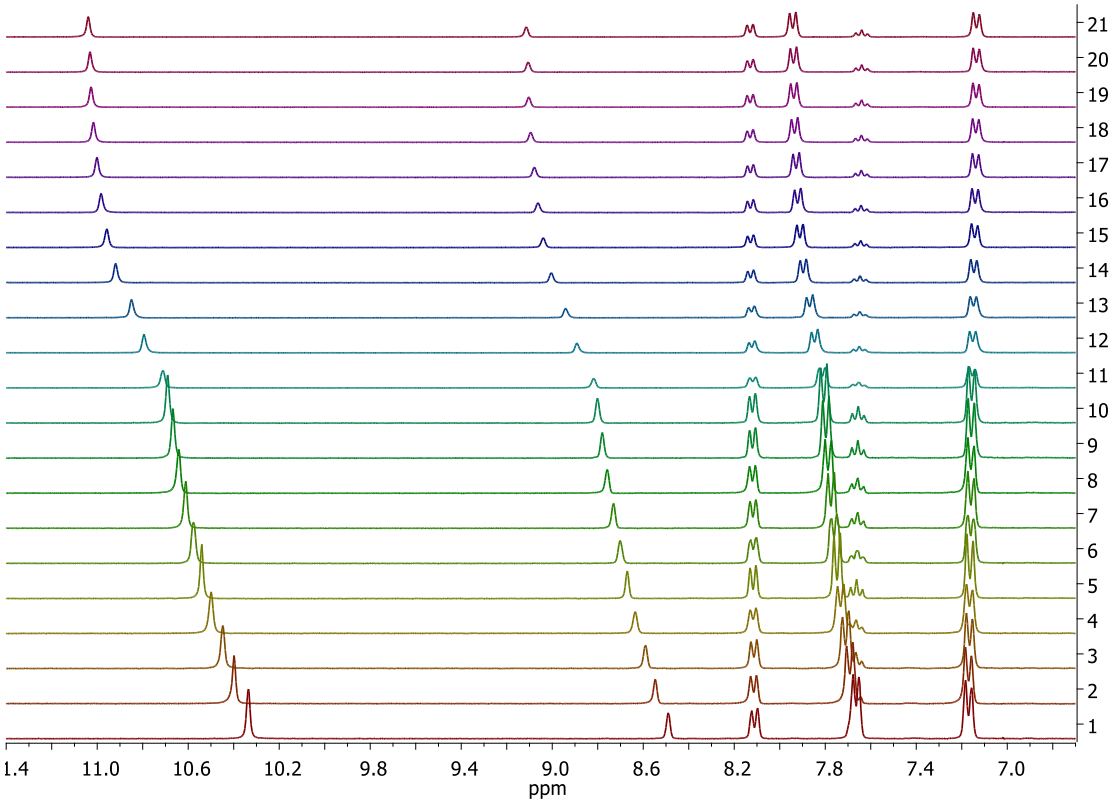


Figure S27. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl to a 0.01 M solution of **L1**.

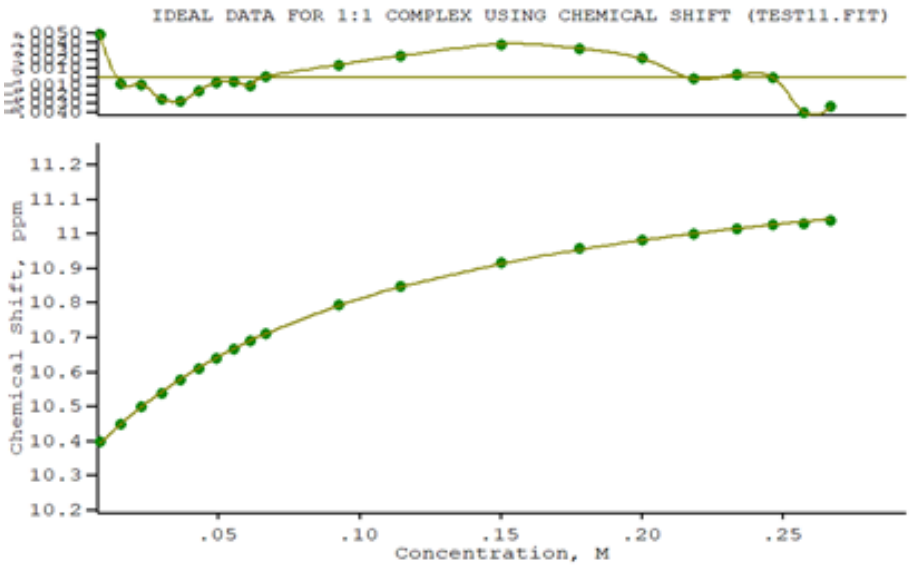


Figure S28. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **L1** with a 0.1 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 (L:Cl^{-}) binding model.

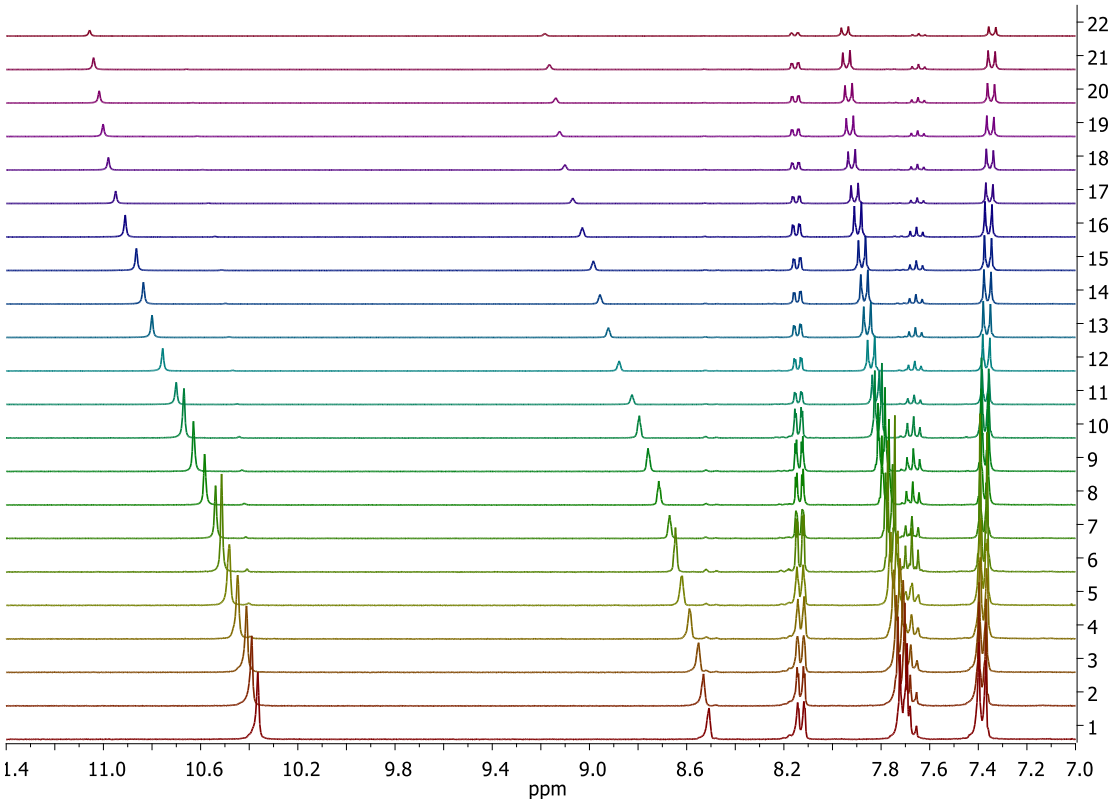


Figure S29. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl to a 0.01 M solution of **L2**.

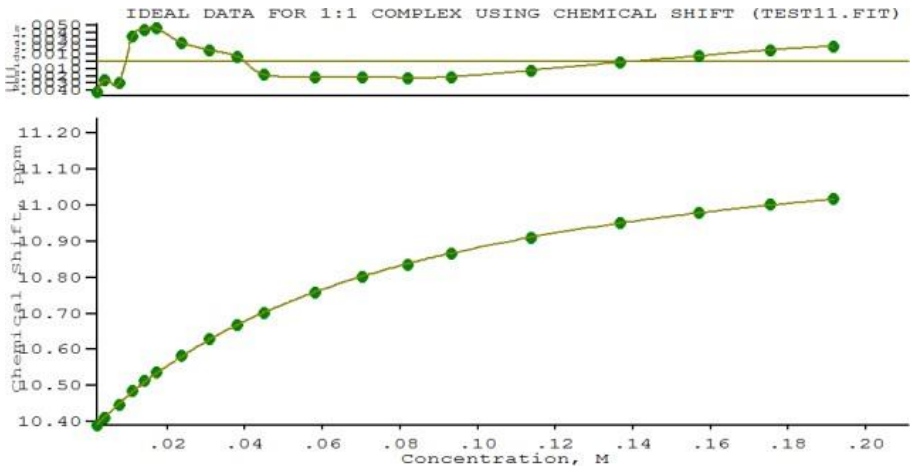


Figure S30. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **L2** with a 0.1 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 (L:Cl^-) binding model.

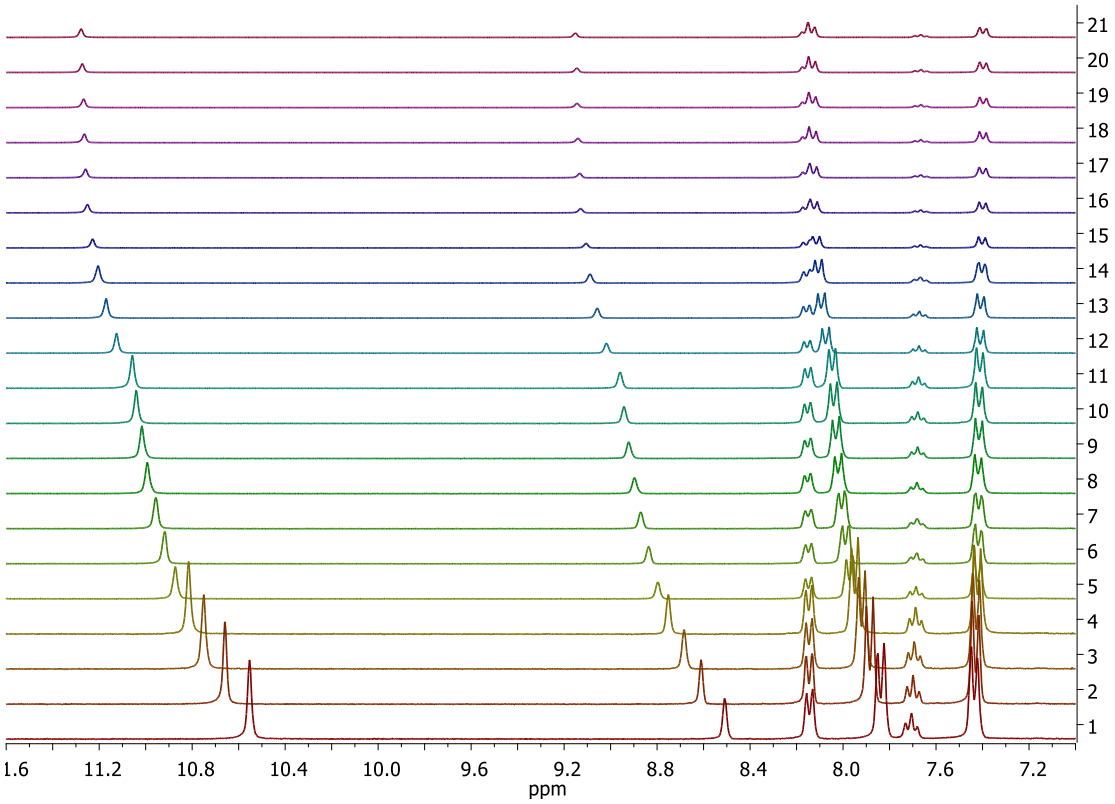


Figure S31. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl to a 0.01 M solution of **L3**.

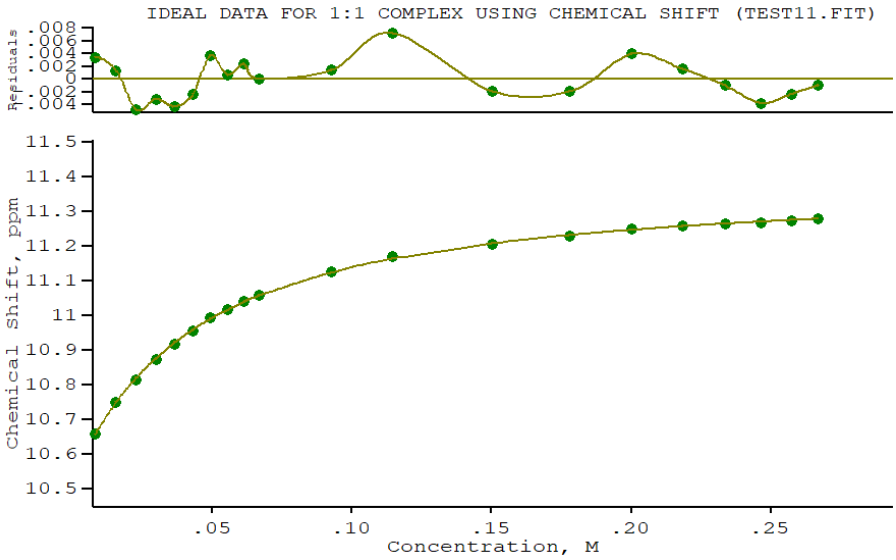


Figure S32. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **L3** with a 0.1 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 (L:Cl^{-}) binding model.

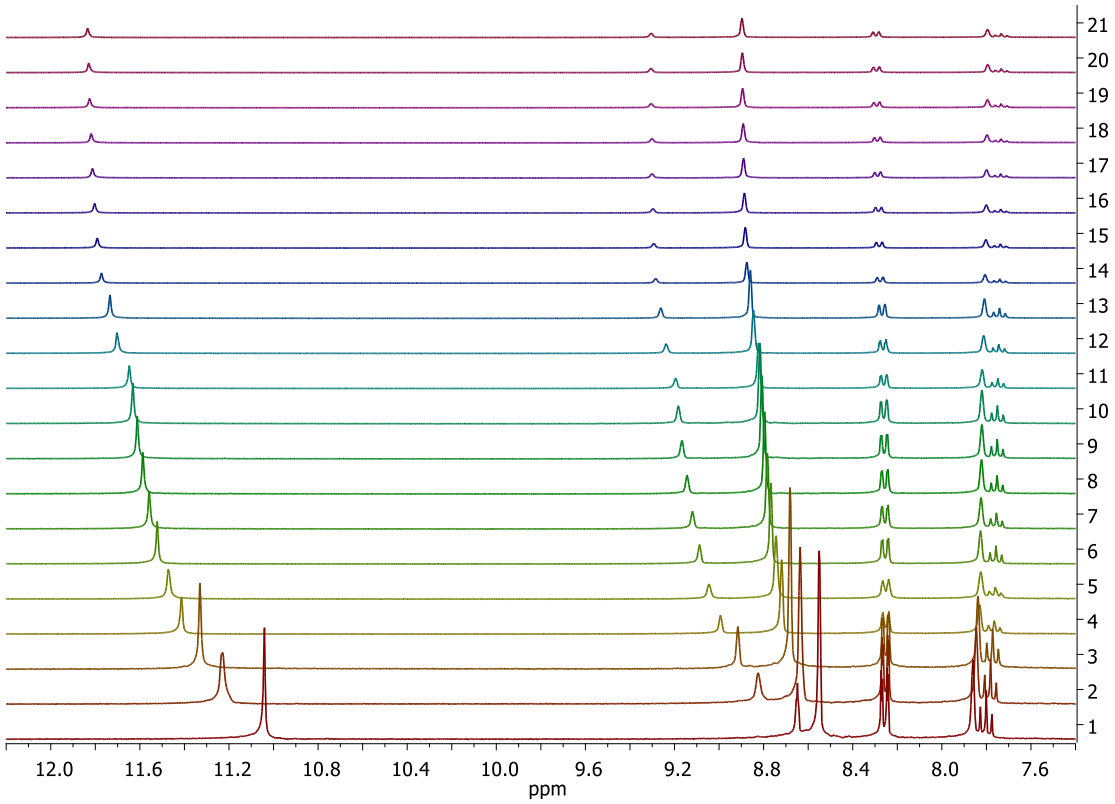


Figure S33. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl to a 0.01 M solution of **L4**.

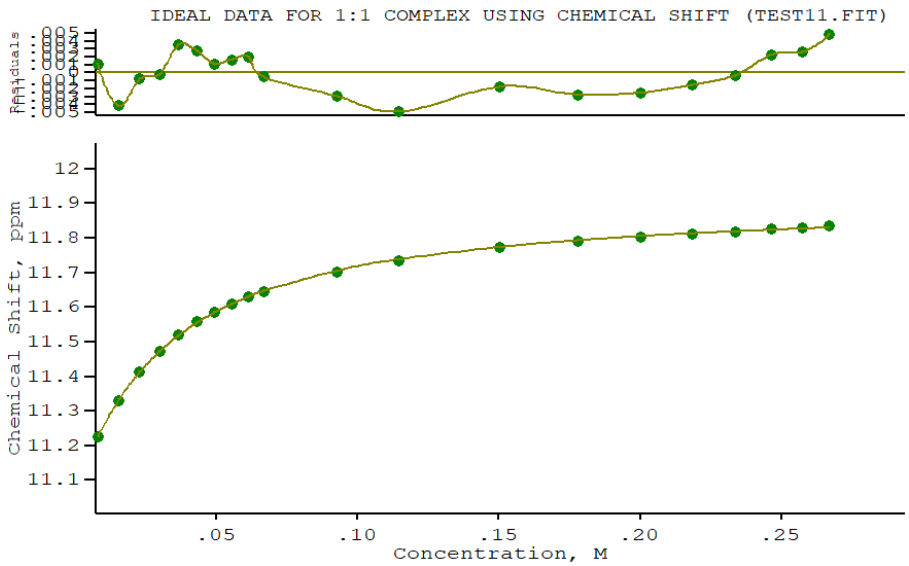


Figure S34. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **L4** with a 0.1 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 (L:Cl^-) binding model.

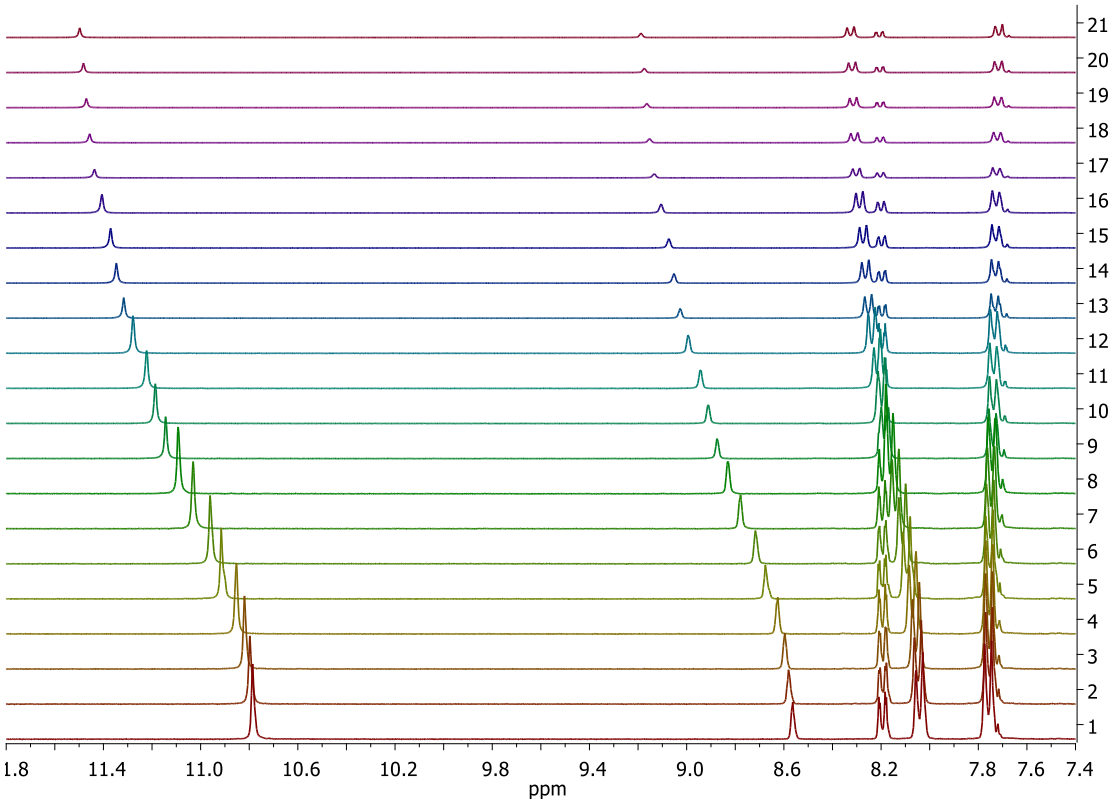


Figure S35. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl to a 0.01 M solution of **L5**.

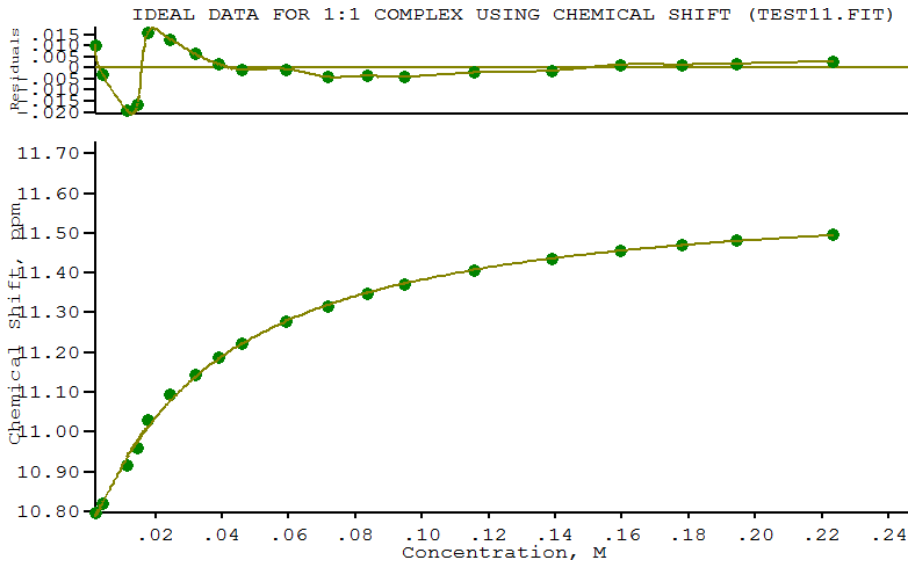


Figure S36. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **L5** with a 0.1 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 ($L:Cl^-$) binding model.

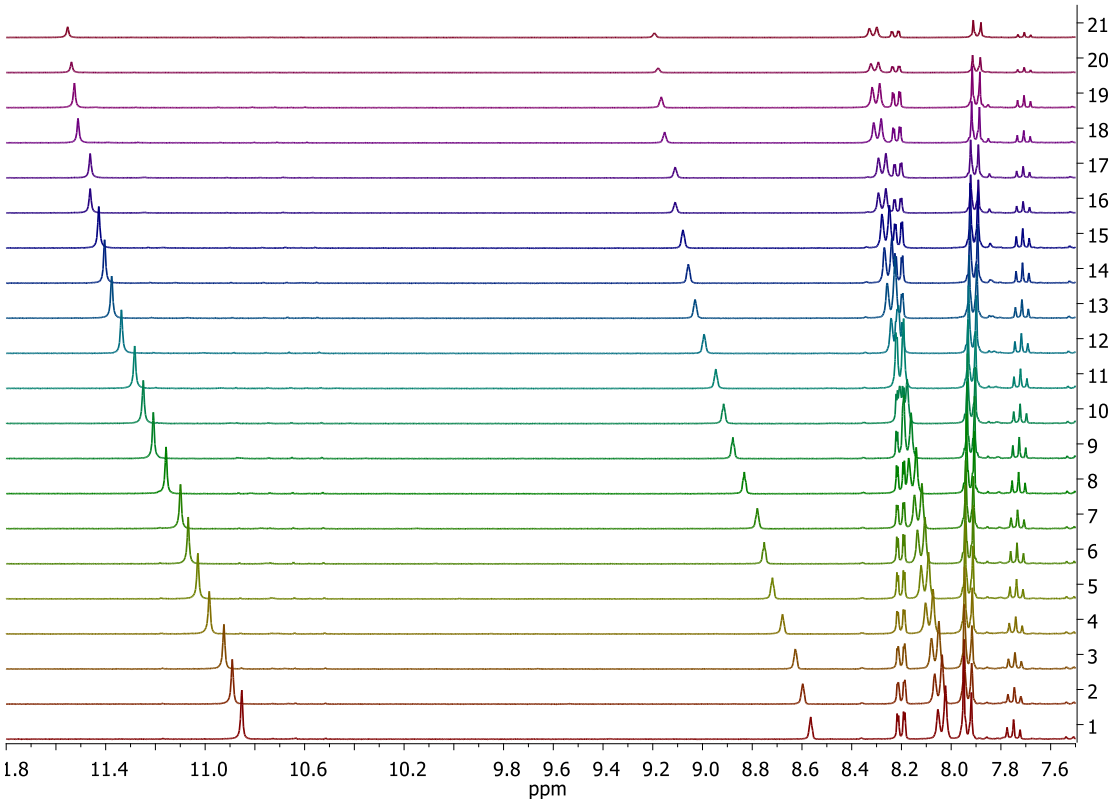


Figure S37. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.1 M solution of TBACl to a 0.01 M solution of **L6**.

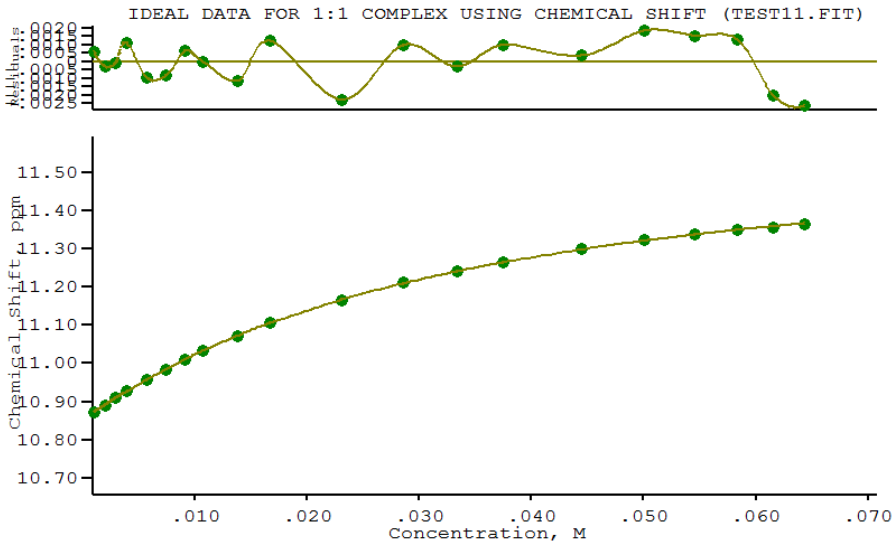


Figure S38. Fitted binding isotherm obtained for the titration of a 0.01 M solution of compound **L6** with a 0.1 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 (L:Cl^{-}) binding model.

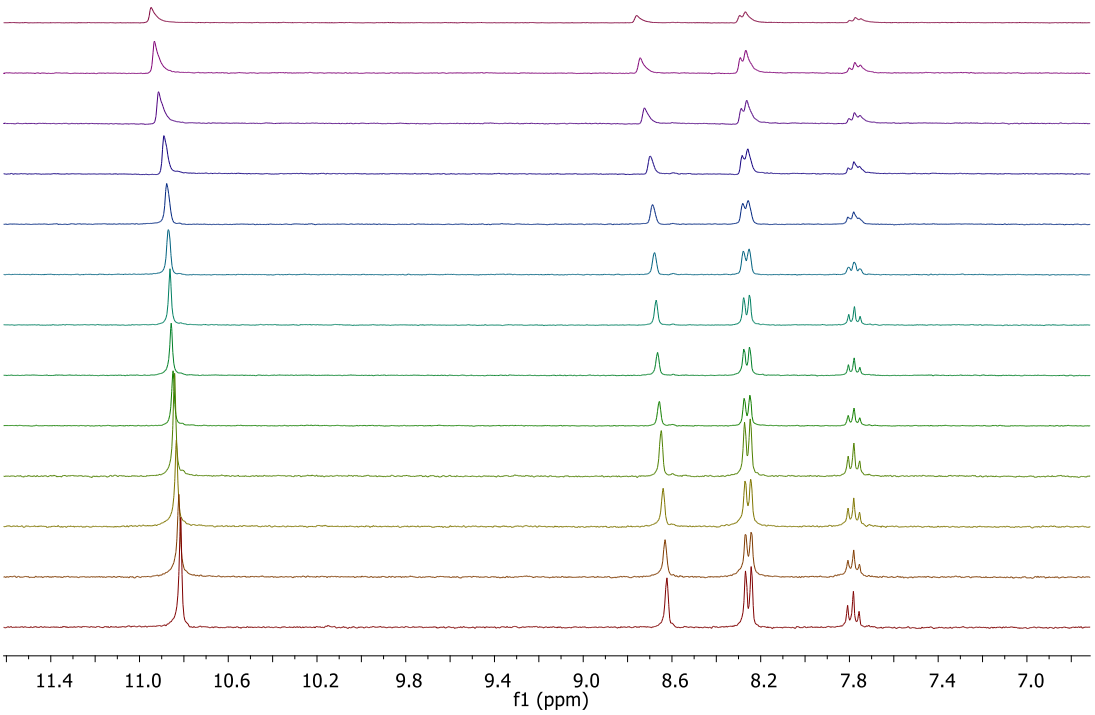


Figure S39. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.075 M solution of TBACl to a 0.005 M solution of **L7**.

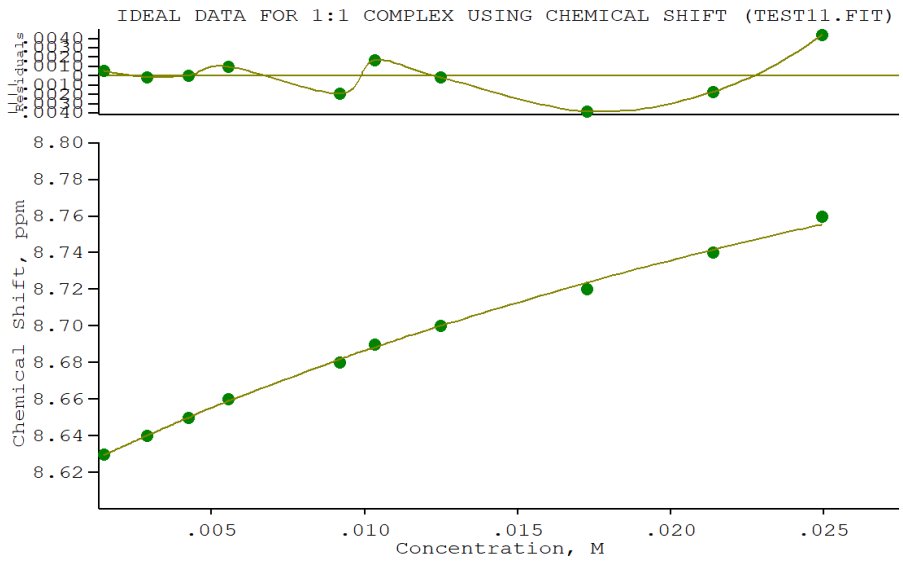


Figure S40. Fitted binding isotherm obtained for the titration of a 0.005 M solution of compound **L7** with a 0.075 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 ($L:Cl^{-}$) binding model.

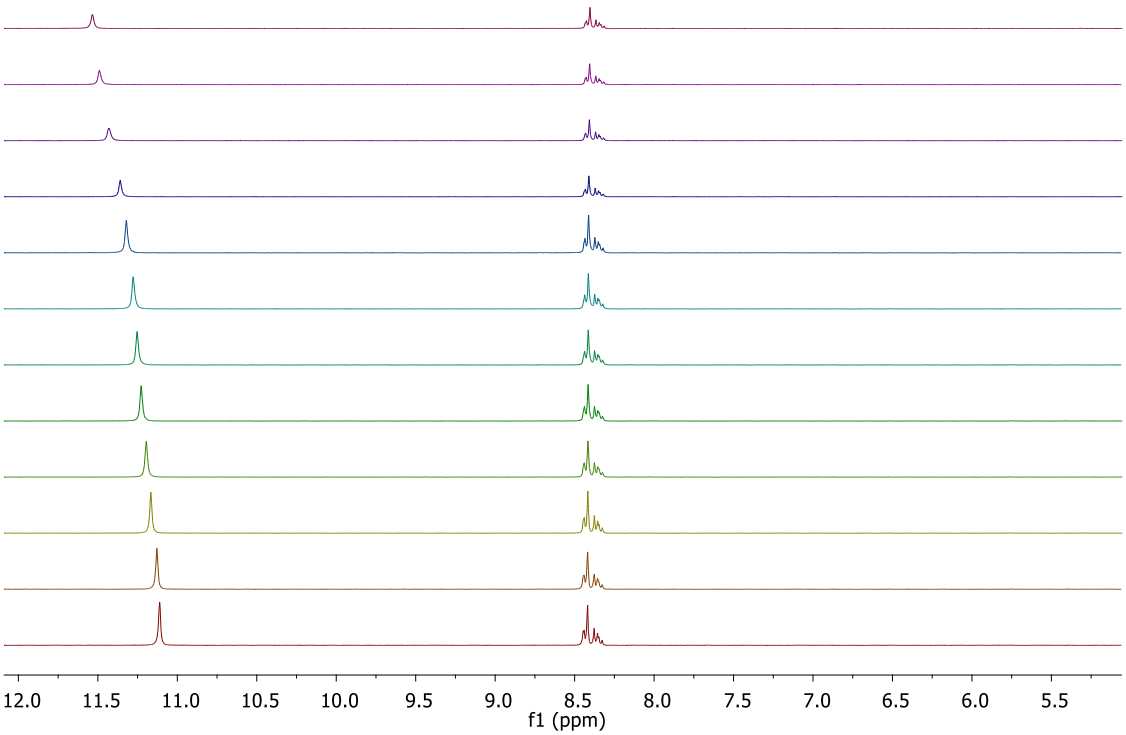


Figure S41. Excerpt of the ^{1}H NMR spectra (300 MHz, DMSO-d_6) obtained upon addition of different aliquots of a 0.075 M solution of TBACl to a 0.005 M solution of **L8**.

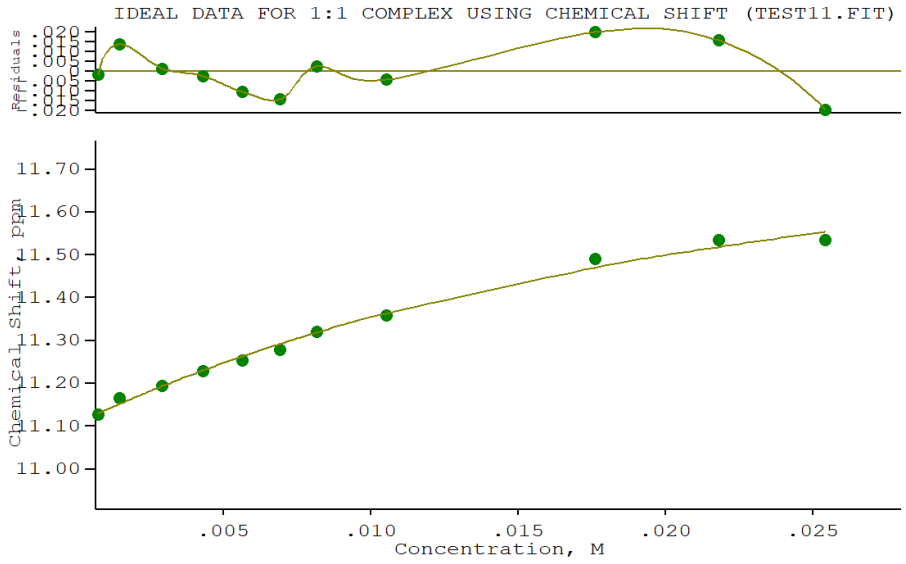


Figure S42. Fitted binding isotherm obtained for the titration of a 0.005 M solution of compound **L8** with a 0.075 M solution of TBACl (DMSO-d_6). The graph shows the change in chemical shift of the signal of the NH protons of the molecule, fitted to a 1:1 (L:Cl^{-}) binding model.

5. TRANSMEMBRANE ANION TRANSPORT EXPERIMENTS

5.1. Preparation of phospholipid vesicles

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocoline (POPC) (20 mg/mL) (Sigma Aldrich) was concentrated to dryness under reduced pressure and the resulting film was dried under high vacuum for, at least, two hours. The lipid film was rehydrated with 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, depending on the experiment). The resulting suspension was vortexed and subjected to nine freeze-thaw cycles and allowed to rest for 15 minutes at room temperature; subsequently, it was extruded twenty-nine times through a polycarbonate membrane (200 nm) employing a LiposoFast basic extruder (Avestin, Inc.). The resulting unilamellar vesicles were dialyzed 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. The vesicles stock solution was obtained by diluting to 10 mL with the external solution.

5.2. 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 was added at t = 60 s and the chloride released was monitored employing a chloride-selective electrode (HACH 9652C). Once the experiment was finished, a detergent (Triton-X, 10% dispersion in water, 20 µL) was added to lyse the vesicles and release all the encapsulated chloride. This value was considered 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) at t = 50 s, 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

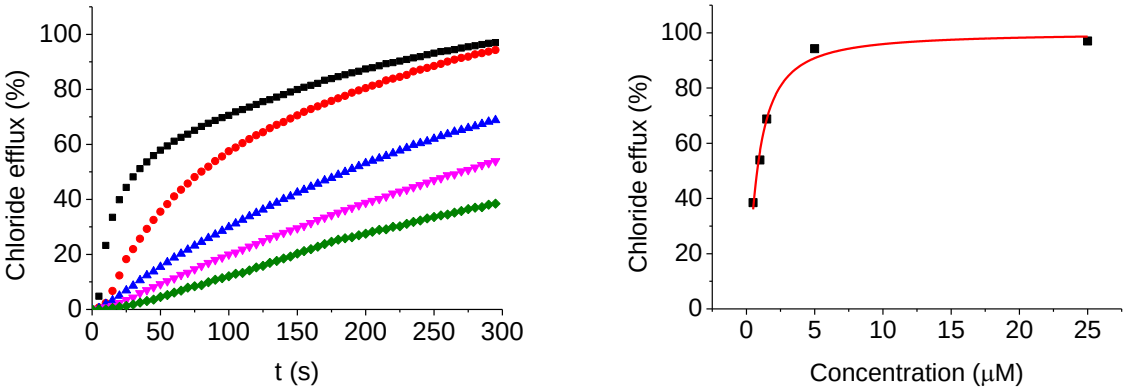


Figure S43. Left: chloride efflux promoted by **L3** at different concentrations (25 μM, black; 5 μM, red; 1.5 μM, blue; 1 μM, magenta; 0.5 μM, green) in unilamellar POPC liposomes. Vesicles were loaded with 489 mM NaCl buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. Each trace represents the average of at least three trials. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L3**. Data have been fitted with Hill equation (continuous line).

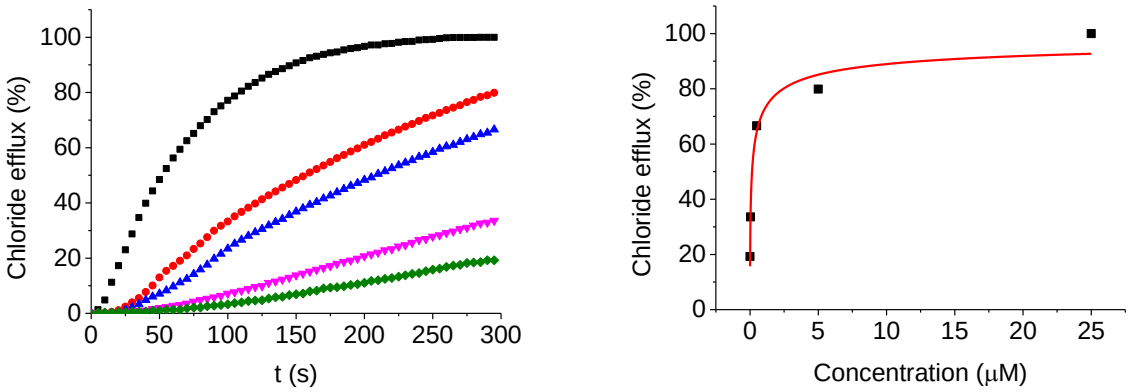


Figure S44. Left: chloride efflux promoted by **L4** at different concentrations (25 μM, black; 5 μM, red; 0.5 μM, blue; 0.05 μM, magenta; 0.005 μM, green) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. Each trace represents the average of at least three trials. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L4**. Data have been fitted with Hill equation (continuous line).

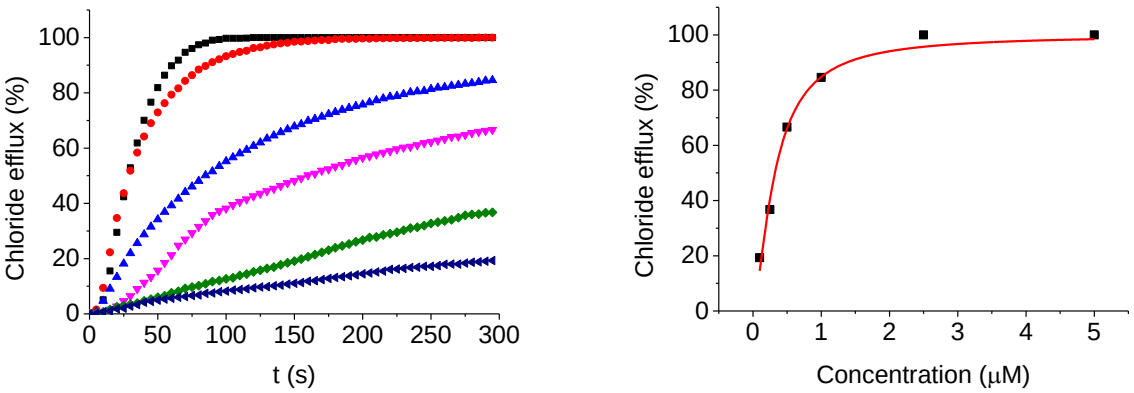


Figure S45. Left: chloride efflux promoted by **L5** at different concentrations (5 μM, black; 2.5 μM, red; 1 μM, light blue; 0.5 μM, magenta; 0.25 μM, green; 0.1 μM, dark blue) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. Each trace represents the average of at least three trials. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L5**. Data have been fitted with Hill equation (continuous line).

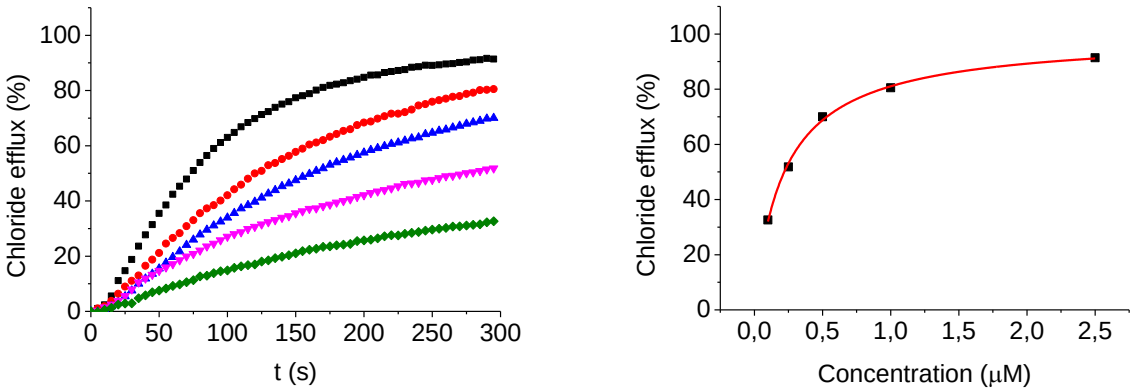


Figure S46. Left: chloride efflux promoted by **L6** at different concentrations (2.5 μM, black; 1 μM, red; 0.5 μM, blue; 0.25 μM, magenta; 0.1 μM, green) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. Each trace represents the average of at least three trials. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L6**. Data have been fitted with Hill equation (continuous line).

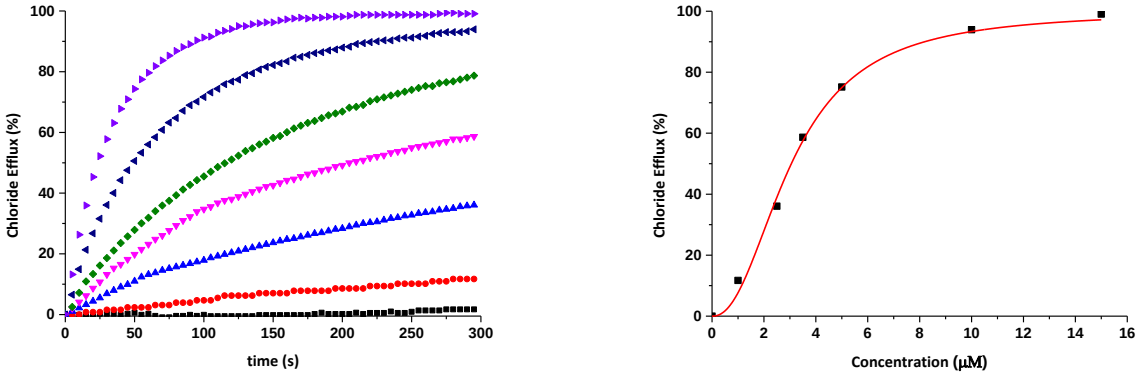


Figure S47. Left: chloride efflux promoted by **L7** at different concentrations (15 μM, violet; 10 μM, dark blue; 5 μM, green; 3.5 μM, magenta; 2.5 μM, light blue; 1 μM, red; 0.5 μM, black) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. Each trace represents the average of at least three trials. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L7**. Data have been fitted with Hill equation (continuous line).

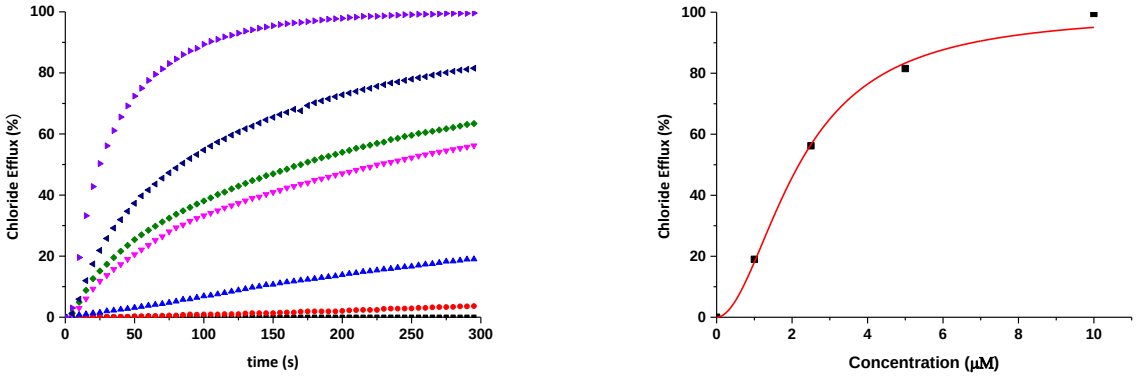


Figure S48. Left: chloride efflux promoted by **L8** at different concentrations (10 μM, violet; 5 μM, dark blue; 3.5 μM, green; 2.5 μM, magenta; 1 μM, light blue; 0.5 μM, red; DMSO (blank), black) in unilamellar POPC vesicles. Vesicles loaded with 489 mM NaCl were buffered at pH 7.2 with 5 mM phosphate and dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. Each trace represents the average of at least three trials. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L8**. Data have been fitted with Hill equation (continuous line).

Study of the Cl^-/HCO_3^- exchange

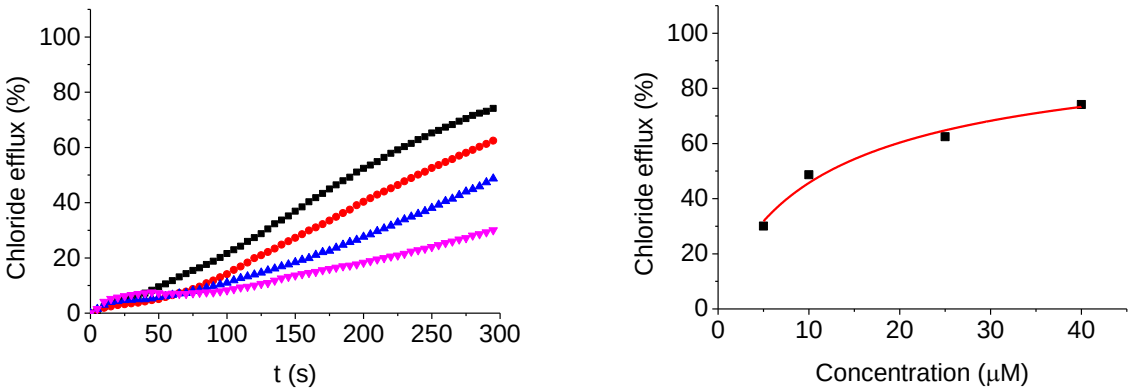


Figure S49. Left: chloride efflux promoted by **L4** at different concentrations (40 μM, black; 25 μM, red; 10 μM, blue; 5 μM, magenta) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L4**. Data have been fitted with Hill equation (continuous line).

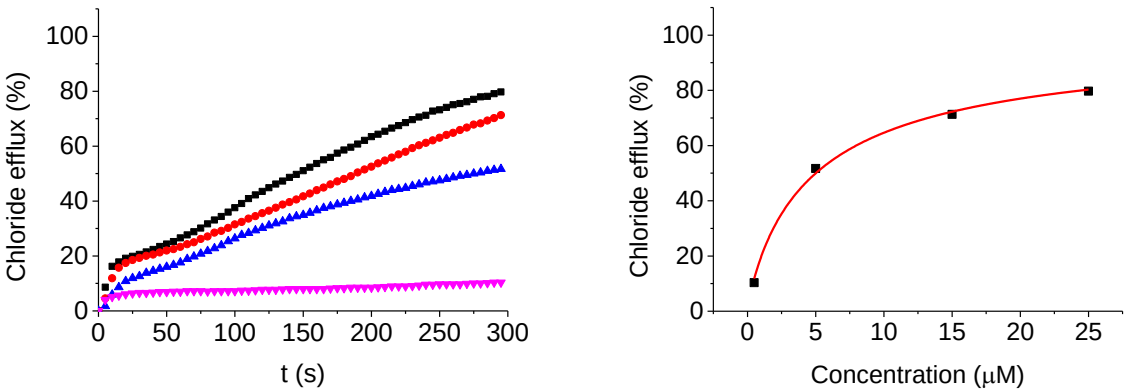


Figure S50. Left: chloride efflux promoted by **L5** at different concentrations (25 μM, black; 15 μM, red; 5 μM, blue; 0.5 μM, magenta) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L5**. Data have been fitted with Hill equation (continuous line).

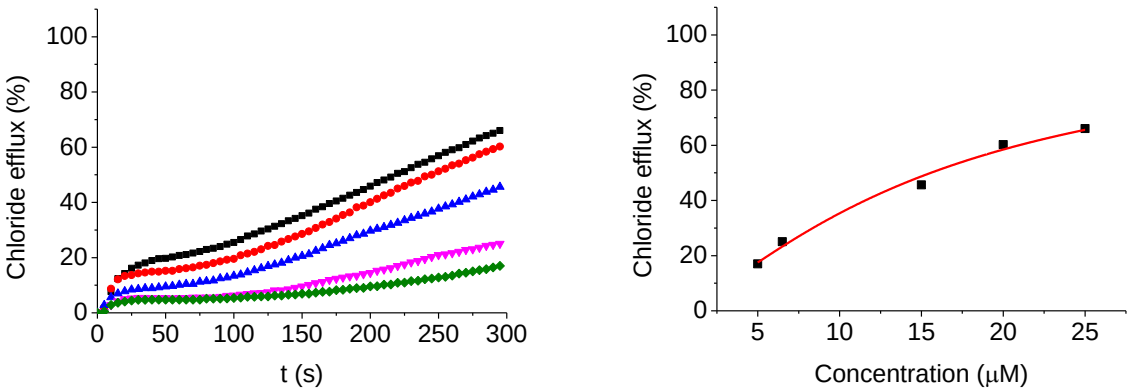


Figure S51. Left: chloride efflux promoted by **L6** at different concentrations (25 μM, black; 20 μM, red; 15 μM, blue; 6.5 μM, magenta; 5 μM, green) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, pH 7.2), were immersed in Na_2SO_4 (150 mM Na_2SO_4, 40 mM HCO_3^- and 20 mM phosphate buffer, pH 7.2). Each trace represents an average of at least three different experiments. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L6**. Data have been fitted with Hill equation (continuous line).

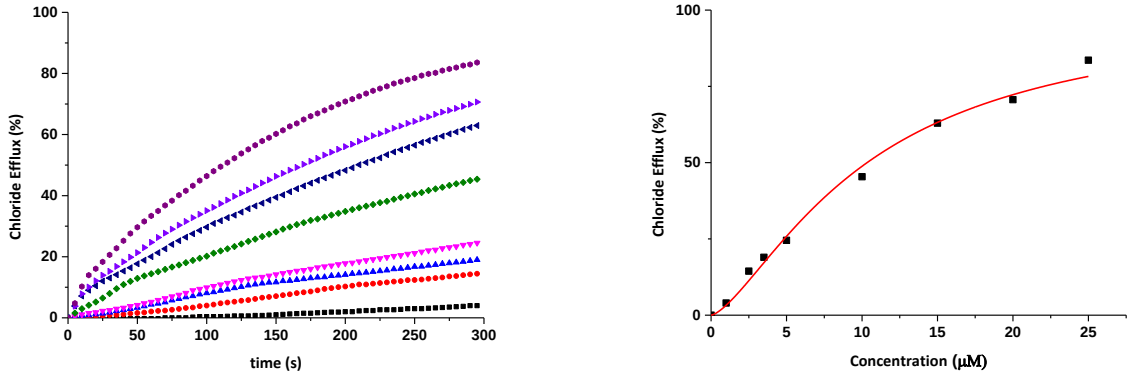


Figure S52. Left: chloride efflux promoted by **L7** at different concentrations (25 μM, purple; 20 μM, violet; 15 μM, dark blue; 10 μM, green; 5 μM, magenta; 3.5 μM, light blue; 2.5 μM, red; 1 μM, black) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, 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. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L7**. Data have been fitted with Hill equation (continuous line).

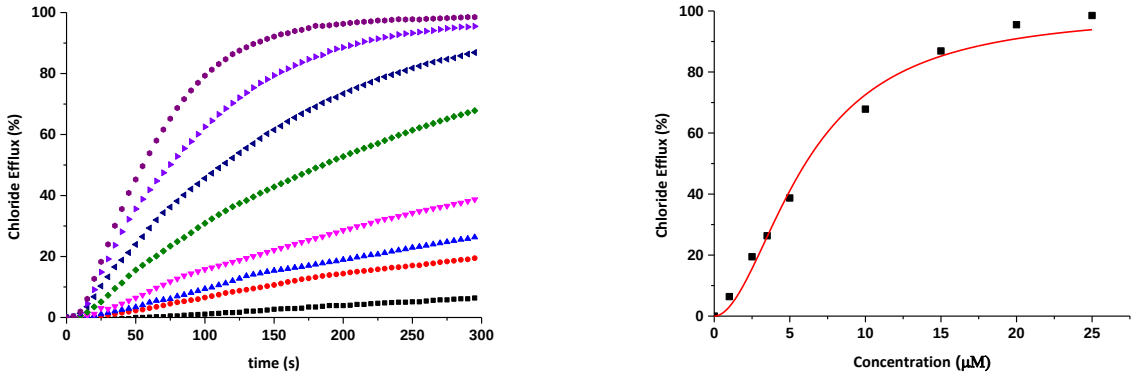


Figure S53. Left: chloride efflux promoted by **L8** at different concentrations (25 μM, purple; 20 μM, violet; 15 μM, dark blue; 10 μM, green; 5 μM, magenta; 3.5 μM, light blue; 2.5 μM, red; 1 μM, black) in unilamellar POPC vesicles. Vesicles, which contained NaCl (451 mM NaCl and 20 mM phosphate buffer, 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. Right: normalized chloride efflux at 300 s plotted against the concentration of compound **L8**. Data have been fitted with Hill equation (continuous line).

Table S2. Transport activities expressed as EC_{50} (μM) and Hill parameter for compounds **L1**-**L8**.

Compound	EC_{50} NO_3^-/Cl^-	Hill parameter, *n* NO_3^-/Cl^-	EC_{50} HCO_3^-/Cl^-	Hill parameter, *n* HCO_3^-/Cl^-	Lipophilicity (logP)[c]
L1	[a]	[a]	[a]	[a]	3.88
L2	[a]	[a]	[a]	[a]	5.70
L3	0.79	1.2 ± 0.1	[b]	[b]	4.77
L4	0.15	0.49 ± 0.07	12.24	0.85 ± 0.09	5.56
L5	0.32	1.5 ± 0.1	5.01	0.87 ± 0.05	4.73
L6	0.22	0.96 ± 0.03	15.57	1.36 ± 0.09	4.71
L7	3.05	2.2 ± 0.2	10.3	2.06 ± 0.08	4.35
L8	2.2	1.9 ± 0.2	6.03	1.9 ± 0.1	4.21

[a] No significant chloride efflux was detected with compound added up to 5%. [b] EC_{50} >> 8% (at 8% just 35% of encapsulated chloride is released). [c] Determined for the deprotonated form of the compounds through Virtual Computational Chemistry Laboratory.

5.3. Emission spectroscopy transport experiments

5.3.1. Carboxyfluorescein-based assays

POPC vesicles were loaded with a sodium chloride aqueous solution containing the fluorophore (451 mM NaCl and 20 mM phosphate buffer, 50 mM carboxyfluorescein, pH 7.2) and treated according to the procedure described in *Section 5.1*. After being subjected to extrusion, liposomes were suspended in a sodium sulphate aqueous solution (150 mM Na_2SO_4 and 20 mM phosphate buffer, pH 7.2). The unencapsulated carboxyfluorescein was removed by size-exclusion chromatography, using Sephadex G-25 as the stationary phase and the external solution as the mobile phase. Emission spectra were recorded on a Hitachi F-7000 spectrofluorometer and the assays were conducted in 1-cm disposable cells, the final POPC concentration in the cuvette being 0.05 mM and the total volume 2.5 mL. At t = 60 s the anion carrier was added and the emission changes were recorded during five minutes. At t = 360 s a pulse of the detergent Triton-X (10%) was added to lyse the vesicles and release all entrapped carboxyfluorescein.

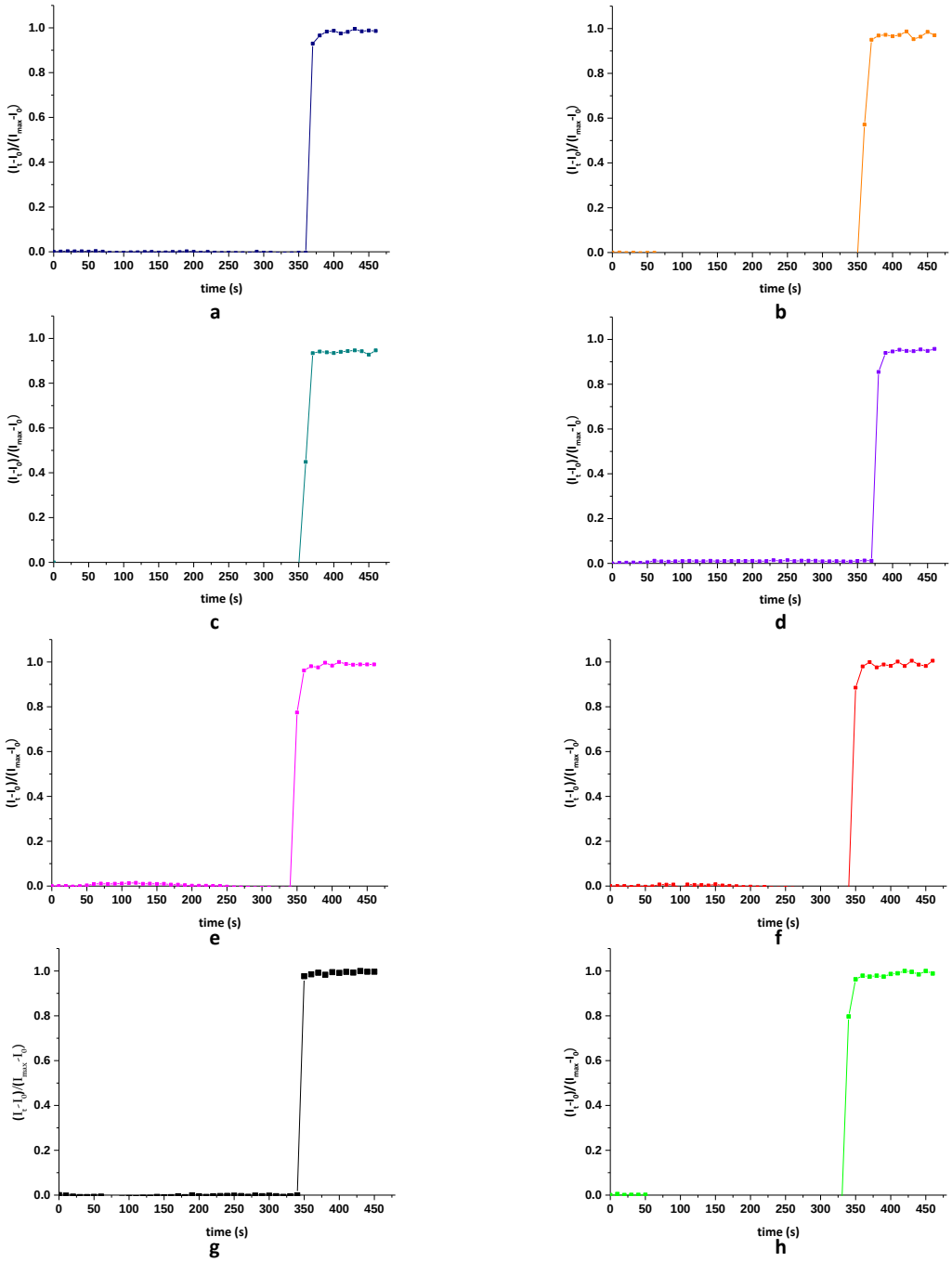


Figure S54. Carboxyfluorescein leakage observed upon addition of compounds **L1**-**L8** (a-h) to POPC vesicles (0.05 mM). Vesicles (loaded with 451 mM NaCl buffered at pH 7.2 with 20 mM phosphate, and containing 50 mM CF; I.S. 500 mM) were suspended in Na_2SO_4 (150 mM, buffered at pH 7.2 with 20 mM phosphate; I.S. 500 mM). At t = 60 s the anion carrier was added (1% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of two trials, performed with two different batches of vesicles.

5.3.2. HPTS-based assays

First of all, a calibration curve matching I_{460}/I_{403}, the relationship between the emission intensity at 460 nm and that at 403 nm (corresponding to the excitation wavelengths of the dye's deprotonated and protonated forms, respectively) of an HPTS aqueous solution (15 nM), prepared with a sodium chloride aqueous solution (126.2 mM NaCl and 10 mM phosphate buffer), and the pH was built. In order to do it, aliquots of a NaOH aqueous solution (0.5 M), prepared with a sodium chloride aqueous solution (126.2 mM NaCl and 10 mM phosphate buffer), were succesively added to the HPTS solution, and after each addition the emission spectra at 403 and 460 nm and the pH value of the solution were recorded. Data were fitted to an *S*-logistic model with OriginPro®, which provided an $R^2 > 0.99$.

Subsequently, 7:3 POPC:cholesterol vesicles were loaded with a sodium chloride aqueous solution containing the fluorophore (126.2 mM NaCl and 10 mM phosphate buffer, 10 μM HPTS, pH 7.2) and treated according to the procedure described in *Section 5.1*. The unencapsulated HPTS was removed by size-exclusion chromatography, using Sephadex G-25 as the stationary phase and the external solution as the mobile phase. The collected vesicles were suspended in a sodium nitrate aqueous solution (126.2 mM $NaNO_3$ and 10 mM phosphate buffer, pH 7.2) to a final volume of 10 mL. Emission spectra were recorded on a Hitachi F-7000 spectrofluorometer and the assays were performed in 1-cm disposable cells, the final POPC concentration in the cuvette being 0.5 mM and the total volume 2.5 mL. At t = 30 s a pulse of a sodium hydroxide aqueous solution (11 μL of a 0.5 M solution) was added to create a pH gradient, the final concentration of the base in the cuvette being 2.2 mM. At t = 60 s an aliquot of the corresponding receptor in DMSO (or DMSO, in the case of the blank) was added and the emission spectra at 403 and 460 nm were recorded for five more minutes. No detergent was added at the end of the experiments.

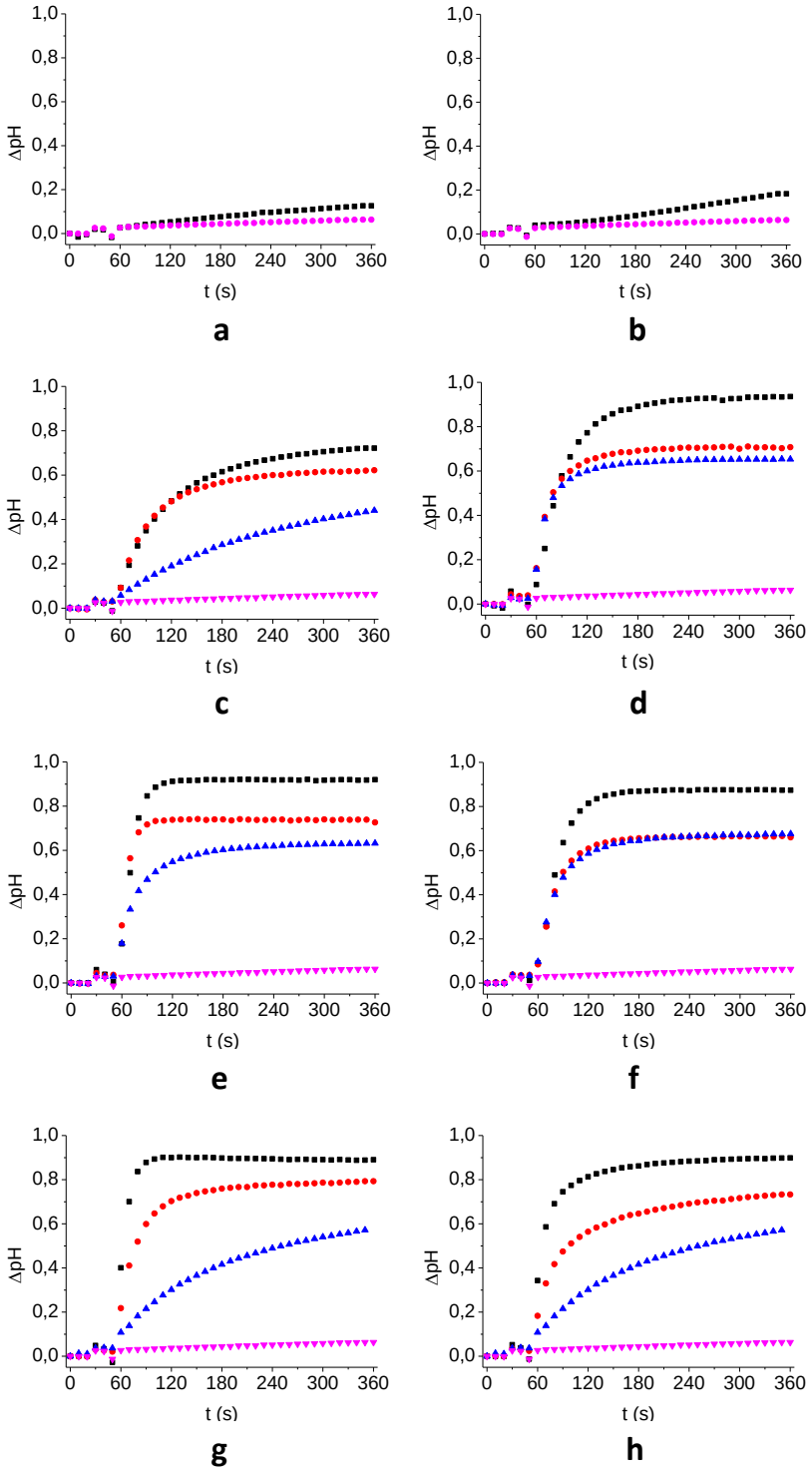


Figure S55. Variation of pH upon addition of compounds **L1**-**L8** (a-h) to 7:3 POPC:cholesterol vesicles (0.5 mM POPC). Vesicles (loaded with 126.2 mM NaCl buffered at pH 7.2 with 10 mM phosphate, and containing 10 µM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ aqueous solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM phosphate; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (11 µL, 0.5 M) was added, and at t = 60 s the anion carrier was added (1%, black; 0.5%, red; 0.2%, blue; blank, magenta). The blank is DMSO (10 µL). Each trace represents the average of at least three different trials, performed with at least two different batches of vesicles.