Electronic Supporting Information for

Lipophilic balance – a new design principle for transmembrane anion carriers

Hennie Valkenier,[a] Cally J. E. Haynes,[b] Julie Herniman,[b] Philip A. Gale,[b] and Anthony P. Davis[a]

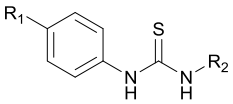
a. School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK.
E-mail: anthony.davis@bristol.ac.uk
b. Chemistry, University of Southampton, Southampton, SO17 1BJ, UK.
E-mail: philip.gale@soton.ac.uk

Contents

1. Synthesis of novel compounds 10c, 10d, 10f, 10g, and 10h

General

^{1}H NMR (300 MHz) and ^{13}C{^{1}H} NMR (75 MHz) spectra were determined on a Bruker AV300 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) and calibrated to the residual solvent peak in DMSO-*d*$_6$ (δ = 2.50 (^{1}H) and 39.51 ppm (^{13}C)) or $CDCl_3$ (δ = 7.26 (^{1}H)). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Matterson Satellite (ATR) and are reported in wavenumbers (cm^{-1}). High resolution electron spray (ES) mass spectra were recorded on a Bruker maXis ESI. All mass spectra are reported as *m/z* (relative intensity). Melting points were determined by a Barnstead Electrothermal 9100 melting point apparatus and were not corrected. Dry pyridine was obtained by distillation over KOH pellets. Dry DCM was obtained by distillation over CaH_2. Receptors **10a**, **10b**, and **10e** have been previously reported.[1]



10a R_1 = H, R_2 = hexyl
10b R_1 = ethyl, R_2 = hexyl
10c R_1 = H, R_2 = undecyl
10d R_1 = ethyl, R_2 = nonyl
10e R_1 = pentyl, R_2 = hexyl
10f R_1 = octyl, R_2 = propyl
10g R_1 = nonyl, R_2 = ethyl
10h R_1 = pentyl, R_2 = cyclohexyl

1-phenyl-3-undecylthiourea 10c

Undecylamine (0.500 g, 2.92 mmol) was dissolved in 5 mL DCM and phenyl isothiocyanate (0.395 g, 2.92 mmol) was added. The reaction was stirred overnight at room temperature under N_2. The solvent was removed under reduced pressure and the resulting residue was recrystallised from DCM/ hexane (50:50) to give the product as a white solid.

Yield: 724 mg (81 %); M_p = 62-64 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$, 298 K): δ = 9.41 (br. s, 1H, NH), 7.70 (br. s, 1H, NH), 7.40 (m, 2H, aryl-H), 7.32 (m, 2H, aryl-H), 7.09 (m, 1H, aryl-H), 3.44 (m, 2H CH_2), 1.52 (m, 2H, CH_2), 1.25 (m, 16H, 8 x CH_2), 0.86 (m, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-*d*$_6$, 298 K): δ = 180.2 (C=S), 139.3 (quaternary aryl C), 128.5 (aryl CH), 123.9 (aryl CH), 122.9 (aryl C-H), 43.8 (CH_2), 31.3 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.4 (CH_2), 26.4 (CH_2), 22.1 (CH_2), 13.9 (CH_3); HRMS (ES) for $[M+H]^+$ $C_{18}H_{31}N_2S$: *m/z* = 307.2208 (calculated), 307.2205 (experimental), Δδ(ppm) = -0.8; IR (solid): ν = 3220, 3040, 3000, 2930, 2920, 2850, 1600.

1-(4-ethylphenyl)-3-nonylthiourea 10d

Nonylamine (0.418 g, 2.92 mmol) was dissolved in 5 mL DCM and 4-ethylphenyl isothiocyanate (0.477 g, 2.92 mmol) was added. The reaction was stirred overnight at room temperature under N_2. The solvent was removed under reduced pressure and the resulting residue was purified by flash column chromatography on silica eluting with ethyl acetate/ hexane (50:50) to give the product as a white solid.

Yield: 765 mg (85 %); M_p = 81-84 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$, 298 K): δ = 9.32 (br. s, 1H, NH), 7.60 (br. s, 1H, NH), 7.27 (m, 2H, aryl-H), 7.14 (m, 2H, aryl-H), 3.43 (m, 2H, CH_2), 2.57 (overlapping with residual DMSO peak, q, ^{3}J(H, H) = 7.4 Hz, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.26 (m, 12H, 6 x CH_2), 1.17 (t, ^{3}J(H, H) = 7.7 Hz, 3H, CH_3), 0.86 (m, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-*d*$_6$, 298 K): δ = 180.2 (C=S), 139.6 (quaternary aryl C), 136.8 (quaternary aryl C), 127.8 (aryl CH), 123.3 (aryl CH), 43.8 (CH_2), 31.3 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 27.6 (CH_2), 26.4 (CH_2), 22.1 (CH_2), 15.6 (CH_3), 13.9 (CH_3); HRMS (ES) for $[M+H]^+$ $C_{18}H_{31}N_2S$: *m/z* = 307.2208 (calculated), 307.2208 (experimental), Δδ(ppm) = -1.8; IR (solid): ν = 3220, 3030, 2970, 2930, 2920, 2850, 1590.

1-(4-octylphenyl)-3-propylthiourea 10f

4-octylaniline (0.180 g, 0.88 mmol) was dissolved in 1 mL DCM and propyl isothiocyanate (0.09 g, 0.88 mmol) was added. The reaction was stirred at room temperature overnight under N_2. The solvent was removed under reduced pressure and the resulting residue was purified by flash column chromatography on SCX-2 modified silica (Biotage) eluting with MeOH to give the product as a white solid.

Yield: 145 mg (54 %); M_p = 56-58 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 298 K): δ = 9.32 (s, 1H, NH), 7.62 (s, 1H, NH), 7.27 (d, ^3J(H, H) = 8.4 Hz, 2H, aryl-H), 7.12 (d, ^3J(H, H) = 8.4 Hz, 2H, aryl-H), ~3.39 (overlapping with H_2O peak, 2H, CH_2), ~2.52 (overlapping with residual DMSO peak, 2H, CH_2), 1.54 (m, 4H, 2 x CH_2), 1.26 (m, 10H, 5 x CH_2), 0.86 (m, 6H, 2 x CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, 298 K): δ = 180.3 (C=S), 138.3 (quaternary aryl C), 136.8 (quaternary aryl C), 128.4 (aryl CH), 123.3 (aryl CH), 45.6 (CH_2), 34.6 (CH_2), 31.3 (CH_2), 31 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 22.1 (CH_2), 21.8 (CH_2), 13.9 (CH_3), 11.4 (CH_3); HRMS (ES) for $[M+H]^+$ $C_{18}H_{31}N_2S$: *m/z* = 307.2208 (calculated), 307.2200 (experimental), Δδ(ppm) = 0.9; IR (solid): ν = 3220, 3080, 3060, 2940, 2920, 2850, 1600.

1-ethyl-3-(4-nonylphenyl)thiourea 10g

4-nonylaniline (0.192 g, 0.88 mmol) was dissolved in 1 mL DCM and ethyl isothiocyanate (0.08 g, 0.88 mmol) was added. The reaction was stirred at room temperature overnight under N_2. The solvent was removed under reduced pressure and the resulting residue was purified by flash column chromatography on SCX-2 modified silica (Biotage) eluting with MeOH to give the product as a white solid.

Yield: 167 mg (62 %); M_p = 58-60 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 298 K): δ = 9.29 (br. s, 1H, NH), 7.58 (br. s, 1H, NH), 7.22 (m, 2H, aryl-H), 7.09 (m, 2H, aryl-H), 3.44 (overlapping with H_2O peak, m, 2H, CH_2), ~2.49 (overlapping with residual DMSO peak, m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.23 (m, 12H, 6 x CH_2), 1.07 (t, ^3J(H, H) = 7.1 Hz, 3H, CH_3), 0.83 (m, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, 298 K): δ = 180.0 (C=S), 138.3 (quaternary aryl C), 136.7 (quaternary aryl C), 128.4 (aryl CH), 123.4 (aryl CH), 34.6 (CH_2), 31.3 (CH_2), 31.0 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 22.1 (CH_2), 14.2 (CH_3), 14.0 (CH_3); HRMS for $[M+H]^+$ $C_{18}H_{31}N_2S$: *m/z* = 307.2208 (calculated), 307.2200 (experimental), Δδ(ppm) = 0.7; IR (solid): ν = 3220, 3120, 3040, 2940, 2920, 2850, 1600.

1-cyclohexyl-3-(4-pentylphenyl)thiourea 10h

Cyclohexyl isothiocyanate (0.412 g, 2.92 mmol) was dissolved in dry pyridine (3 mL) and 4-pentylaniline (0.477 g, 2.92 mmol) was added. The reaction was stirred at room temperature under N_2 for 4 hours. The pyridine was removed under reduced pressure and the resulting residue was purified by flash column chromatography on silica eluting with DCM/ MeOH (99:1), followed by flash column chromatography on SCX-2 modified silica (Biotage) eluting with MeOH to give the product as a white solid.

Yield: 284 mg (32 %); M_p = 86-88 °C; ^{1}H NMR (300 MHz, DMSO-d_6, 298 K): δ = 9.18 (s, 1H, NH), 7.43 (d, ^3J(H, H) = 7.7 Hz, 1H, NH), 7.27 (d, ^3J(H, H) = 8.1 Hz, 2H, aryl-H), 7.06 (d, ^3J(H, H) = 8.4 Hz, 2H, aryl-H), 4.03 (br. s, 1H, (NH)-C*H*-(CH_2)$_2$), ~2.47 (overlapping with residual DMSO peak, 2H, CH_2), 1.85 (m, 2H, CH_2), 1.45- 1.68 (m, 5H, aliphatic 5x CH), 1.21 (m, 9H, 9 x aliphatic CH), 0.81 (t, ^3J(H, H) = 6.8 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6, 298 K): δ = 179.2 (C=S), 138.0 (quaternary aryl C), 137.1 (quaternary aryl C), 128.2 (aryl CH), 123.0 (aryl CH), 52.1 (CH), 34.6 (CH_2), 31.9 (CH_2), 30.9 (CH_2), 30.7 (CH_2), 25.2 (CH_2), 24.5 (CH_2), 22.0 (CH_2), 13.9 (CH_3).; HRMS (ES) for $[M+H]^+$ $C_{18}H_{31}N_2S$: *m/z* = 305.2051 (calculated), 305.2042 (experimental) , Δδ(ppm) = 1.3; IR (solid): ν = 3220, 3090, 3050, 3030, 2940, 2920, 2850, 1600.

2. NMR spectra of 10c, 10d, 10f, 10g, and 10h. (recorded in DMSO-d_6)

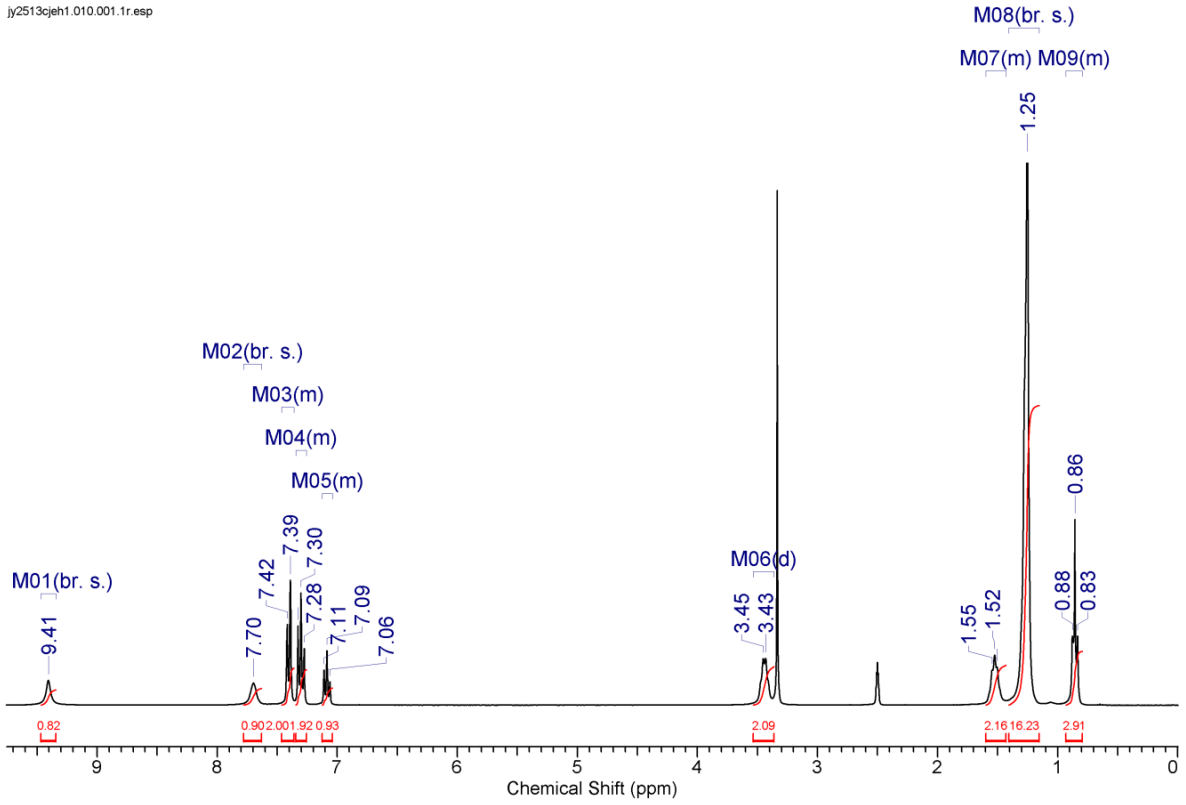


Figure S1. ^{1}H NMR spectrum of receptor **10c**.

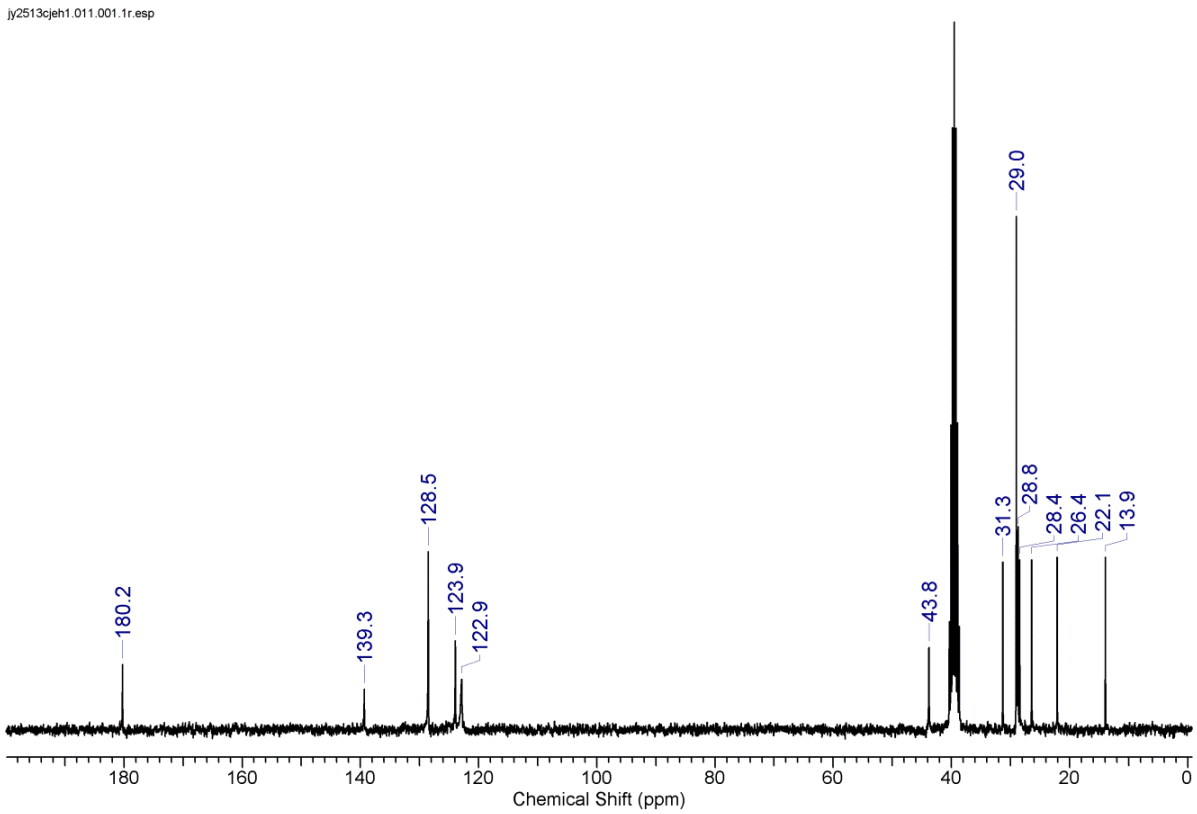


Figure S2. Full ^{13}C NMR spectrum of receptor **10c**.

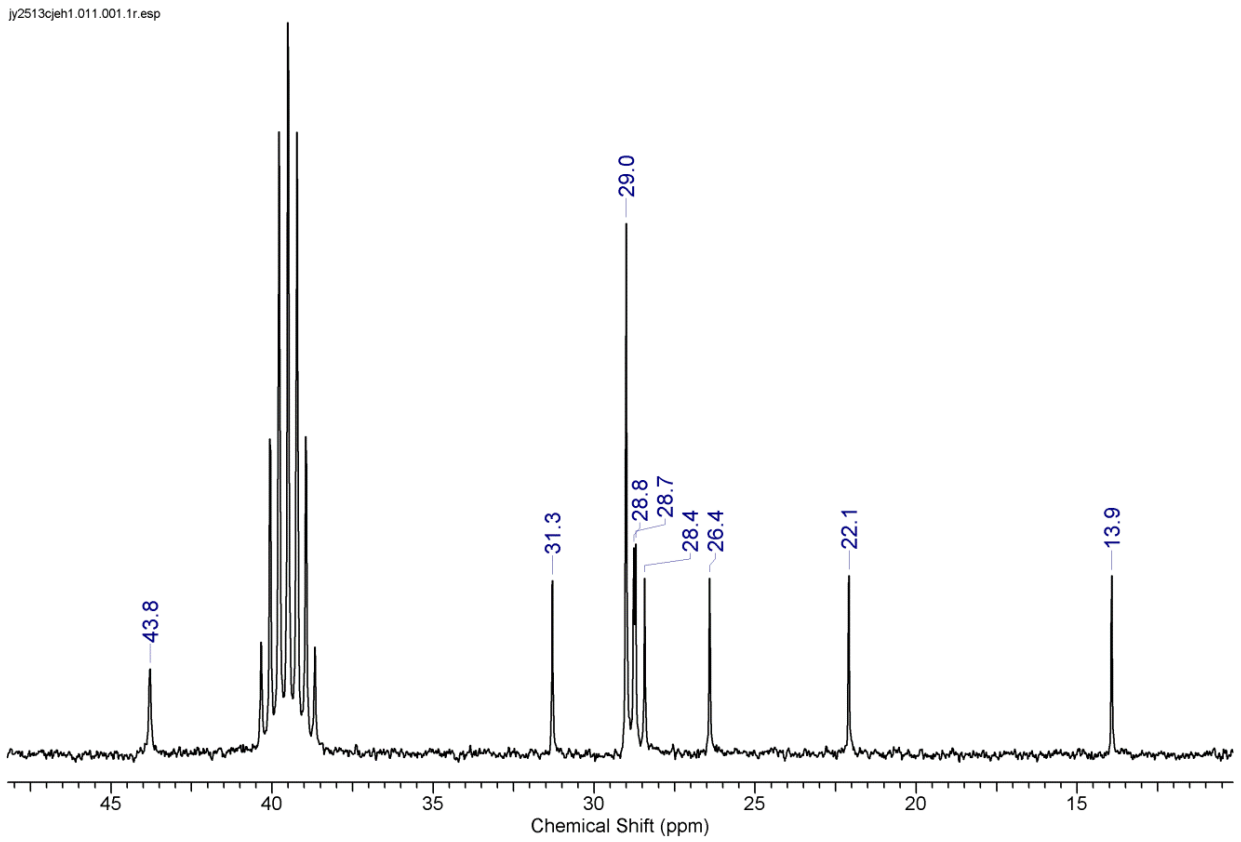


Figure S3. The aliphatic region of the ^{13}C NMR spectrum of receptor **10c**.

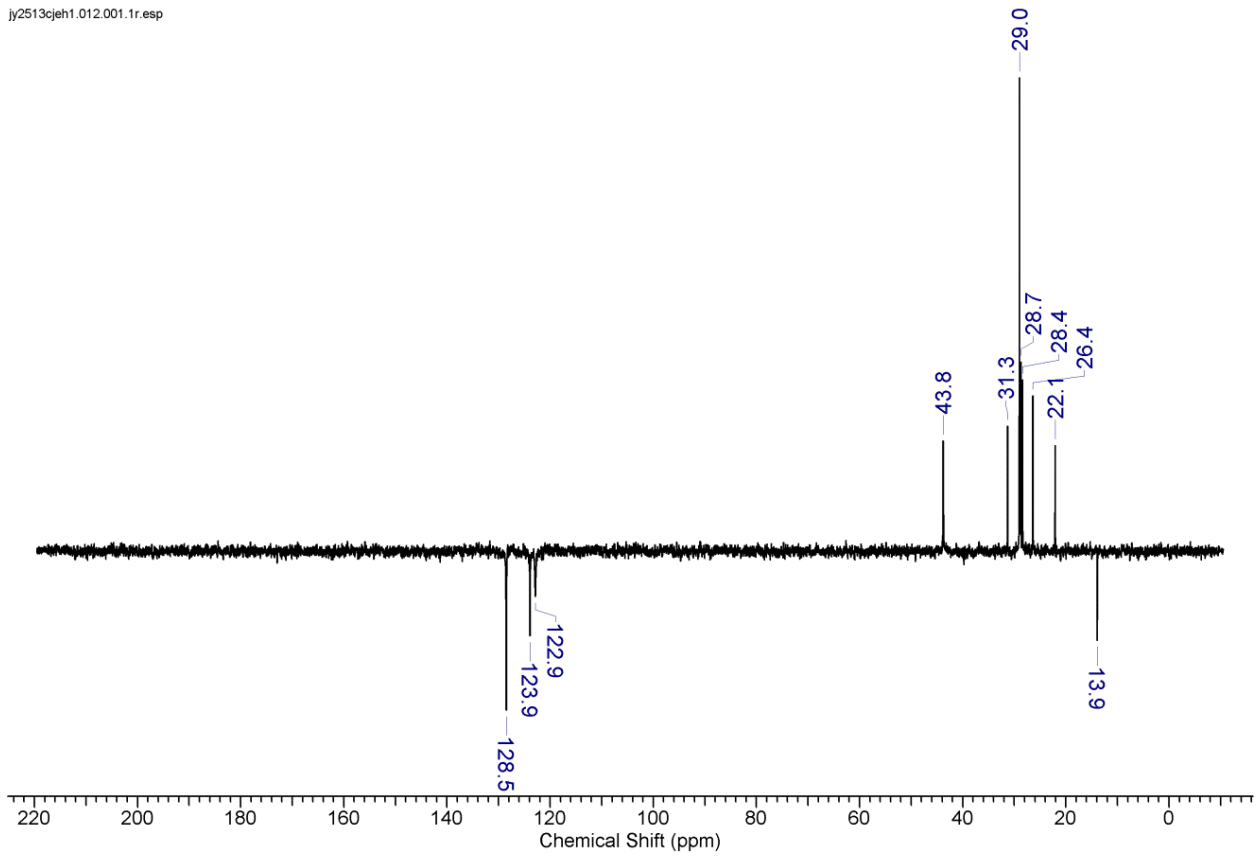


Figure S4. ^{13}C DEPT spectrum of receptor **10c**.

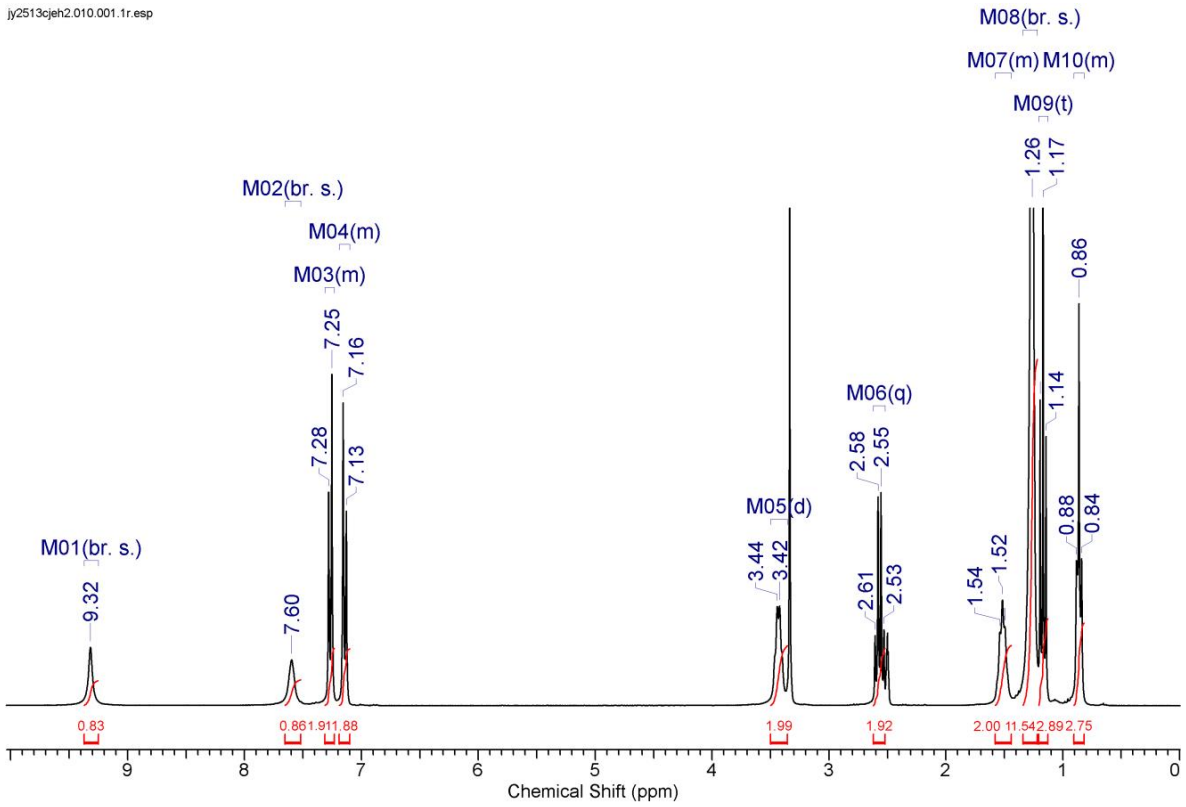


Figure S5. ^{1}H NMR spectrum of receptor **10d**.

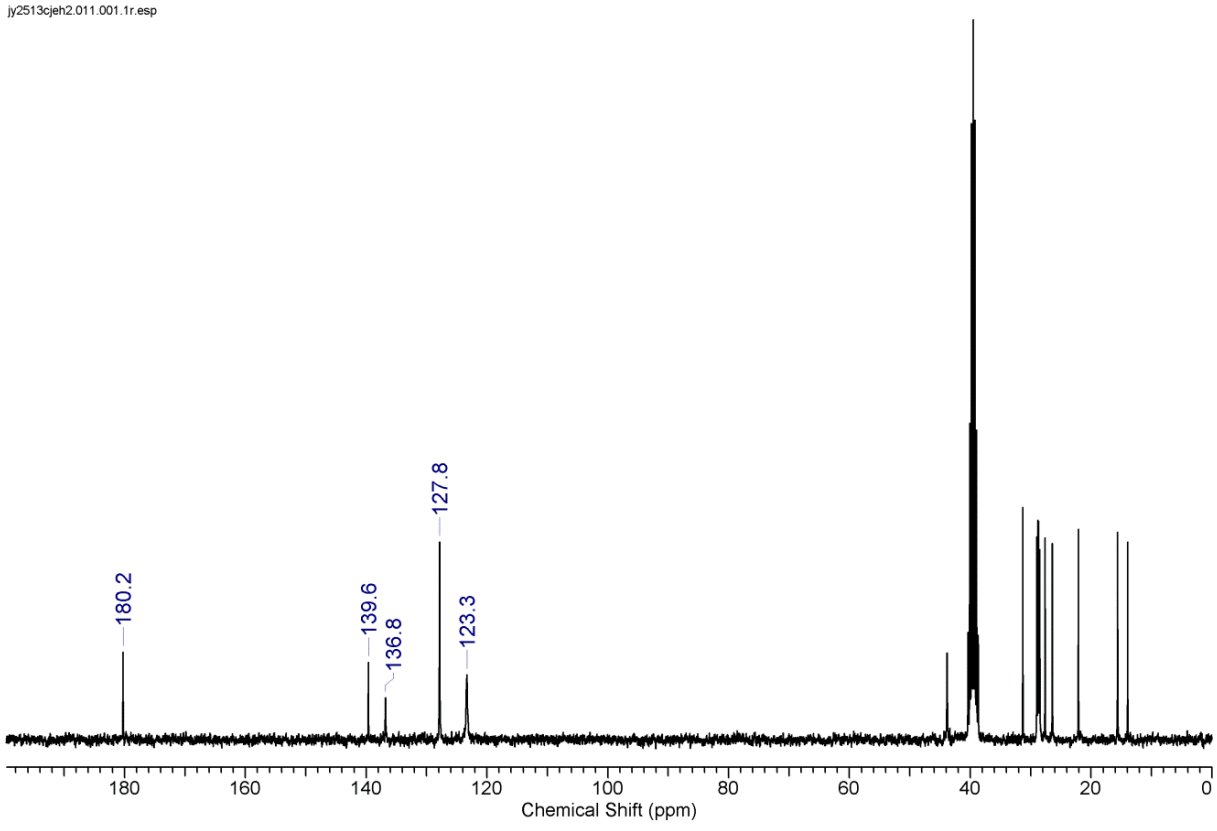


Figure S6. Full ^{13}C NMR spectrum of receptor **10d**.

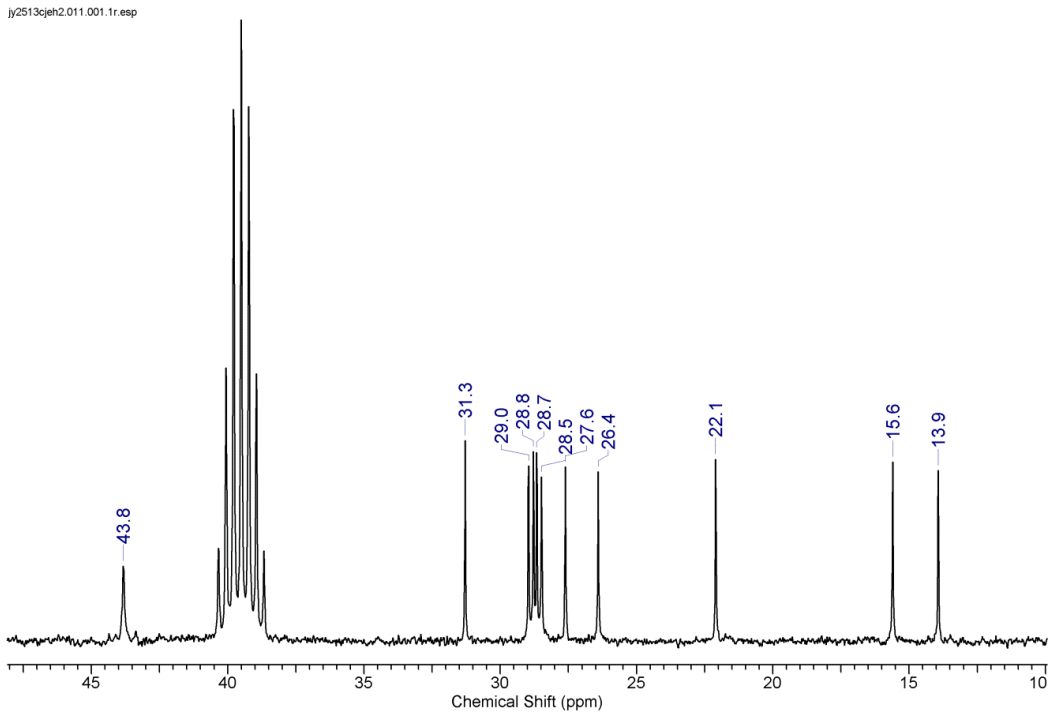


Figure S7. The aliphatic region of the ^{13}C NMR spectrum of receptor **10d**.

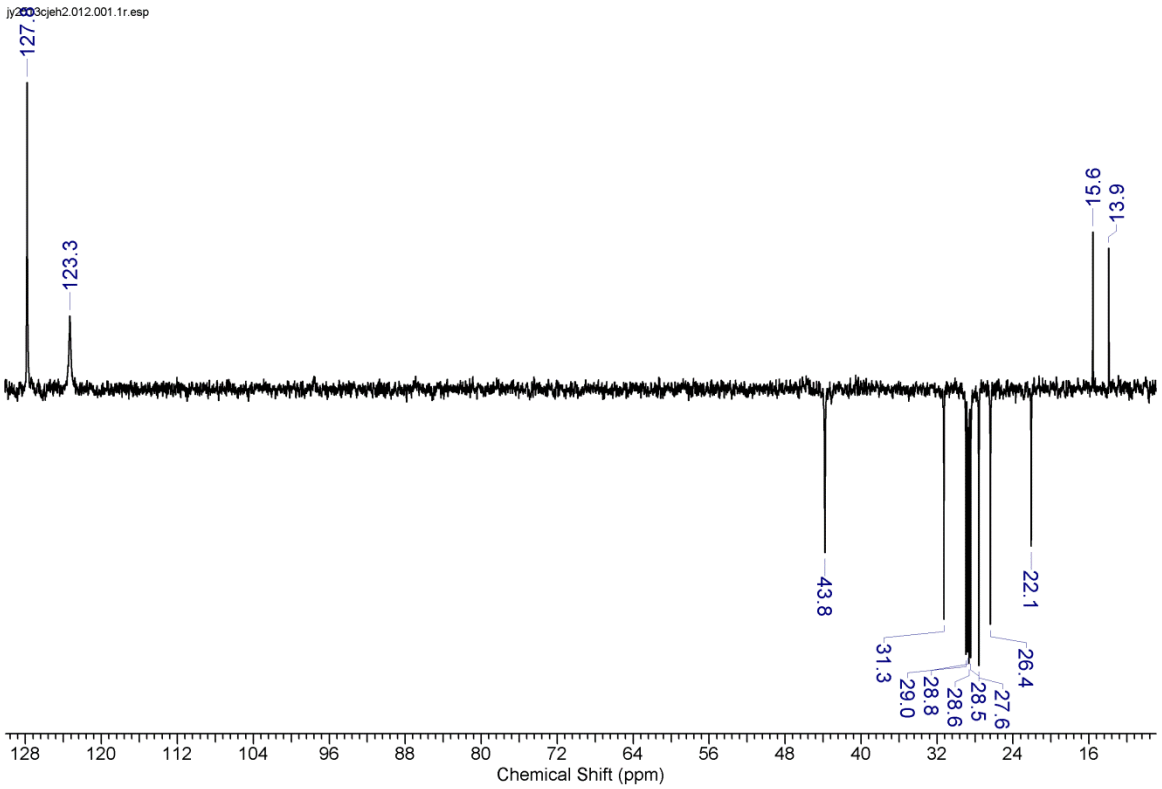


Figure S8. ^{13}C DEPT spectrum of receptor **10d**.

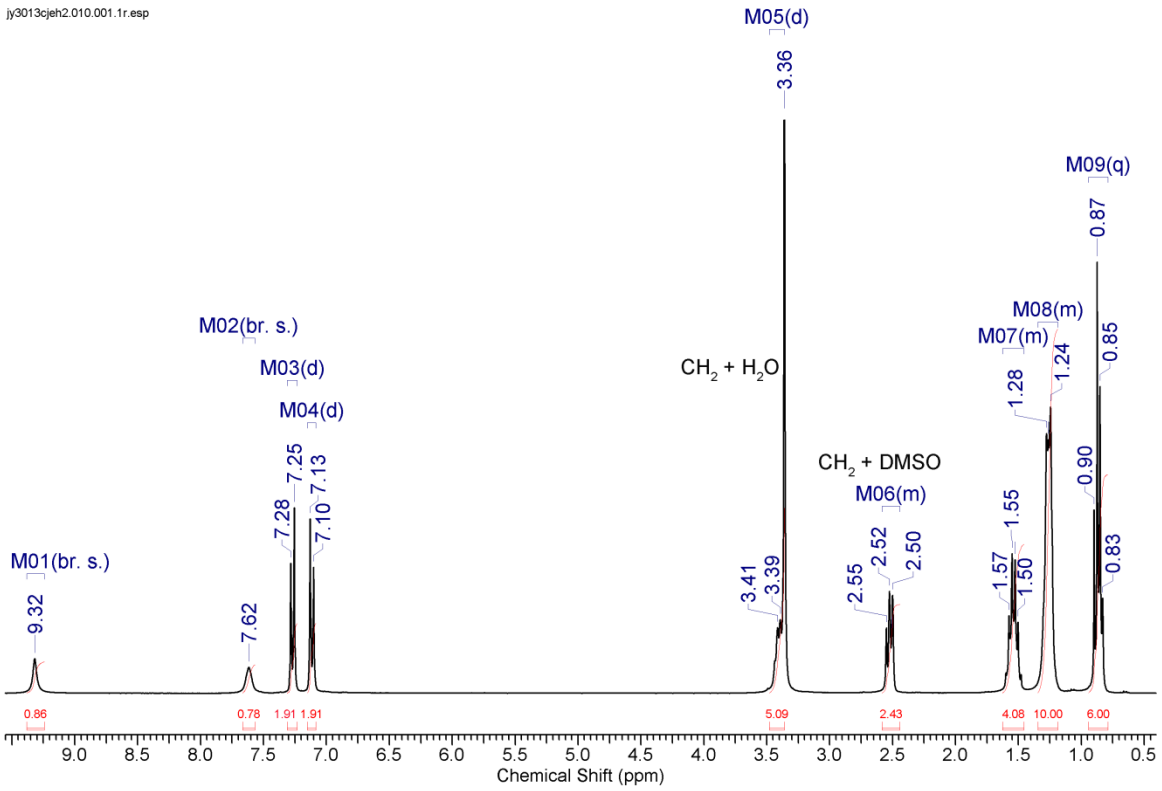


Figure S9. ^{1}H NMR spectrum of receptor **10f**.

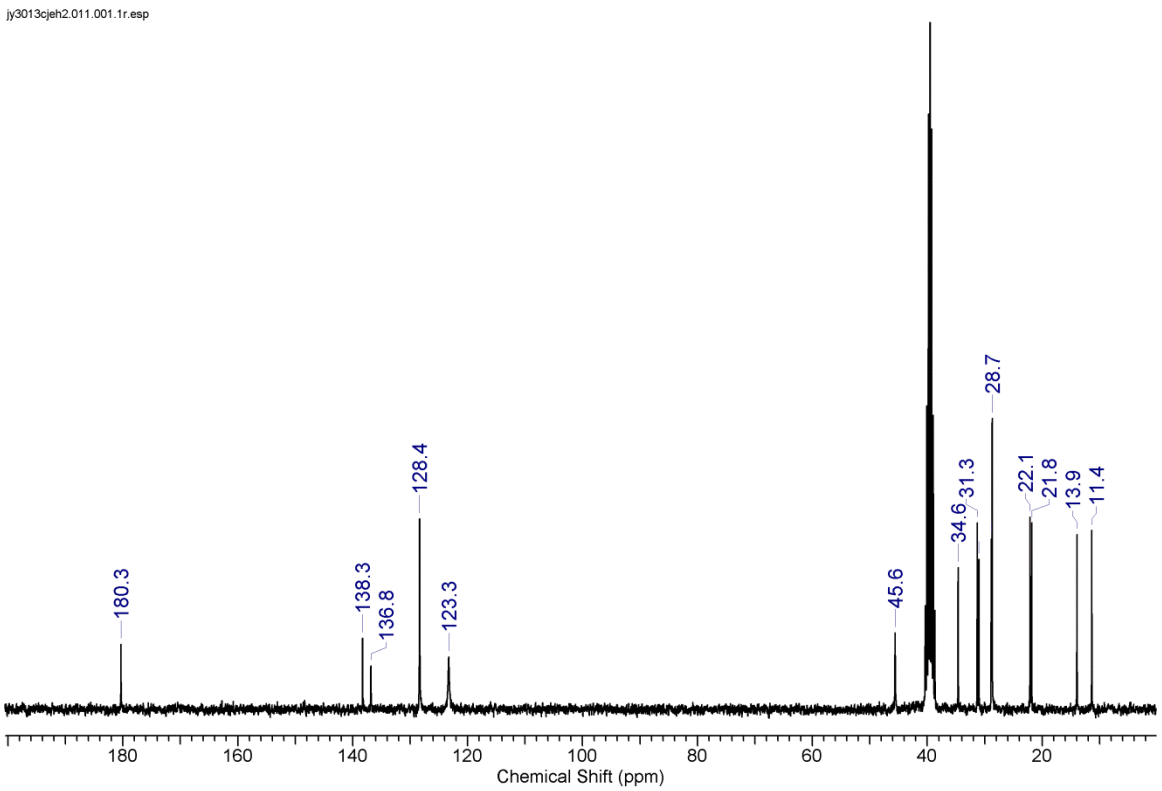


Figure S10. Full ^{13}C NMR spectrum of receptor **10f**.

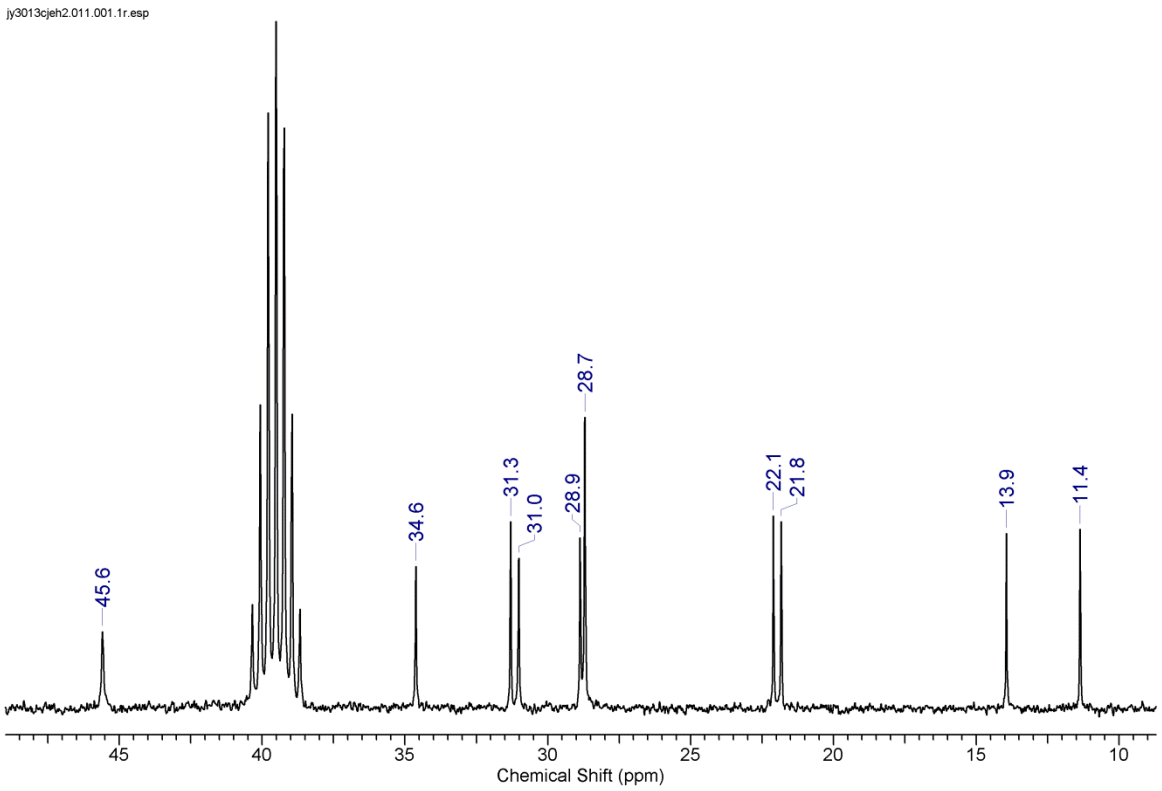


Figure S11. The aliphatic region of the ^{13}C NMR spectrum of receptor **10f**.

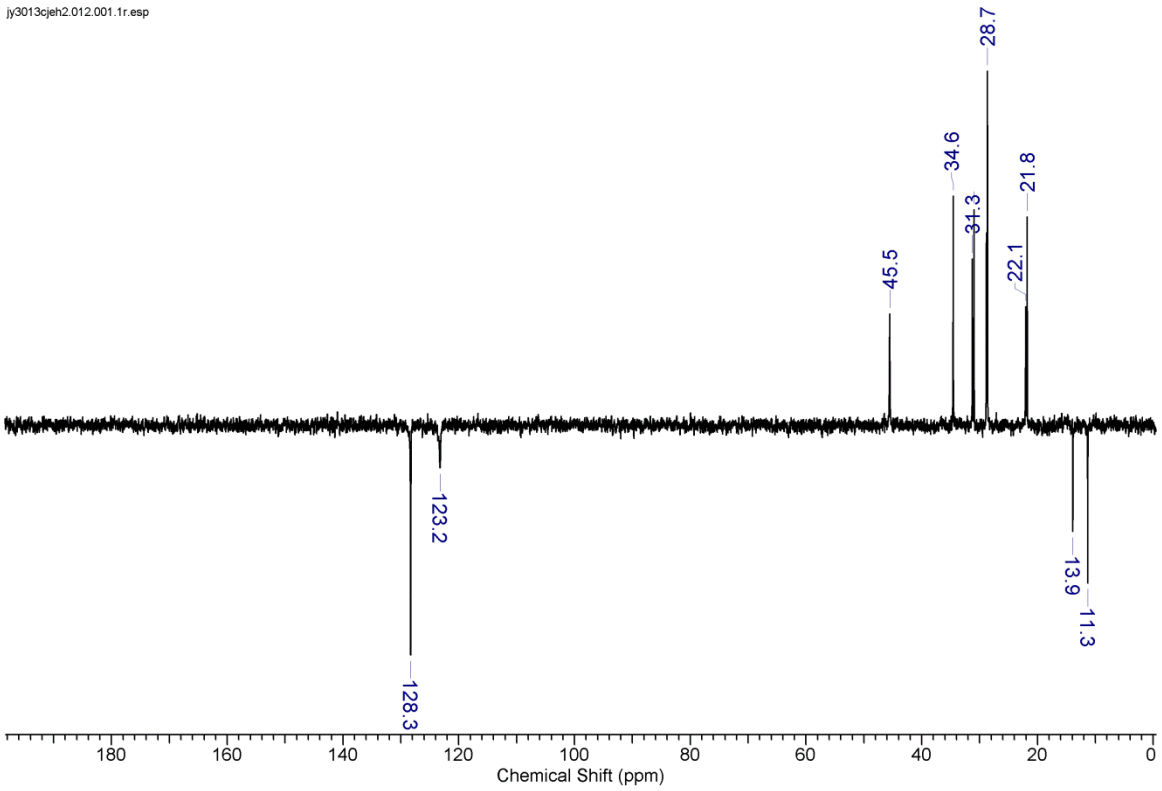


Figure S12. ^{13}C DEPT spectrum of receptor **10f**.

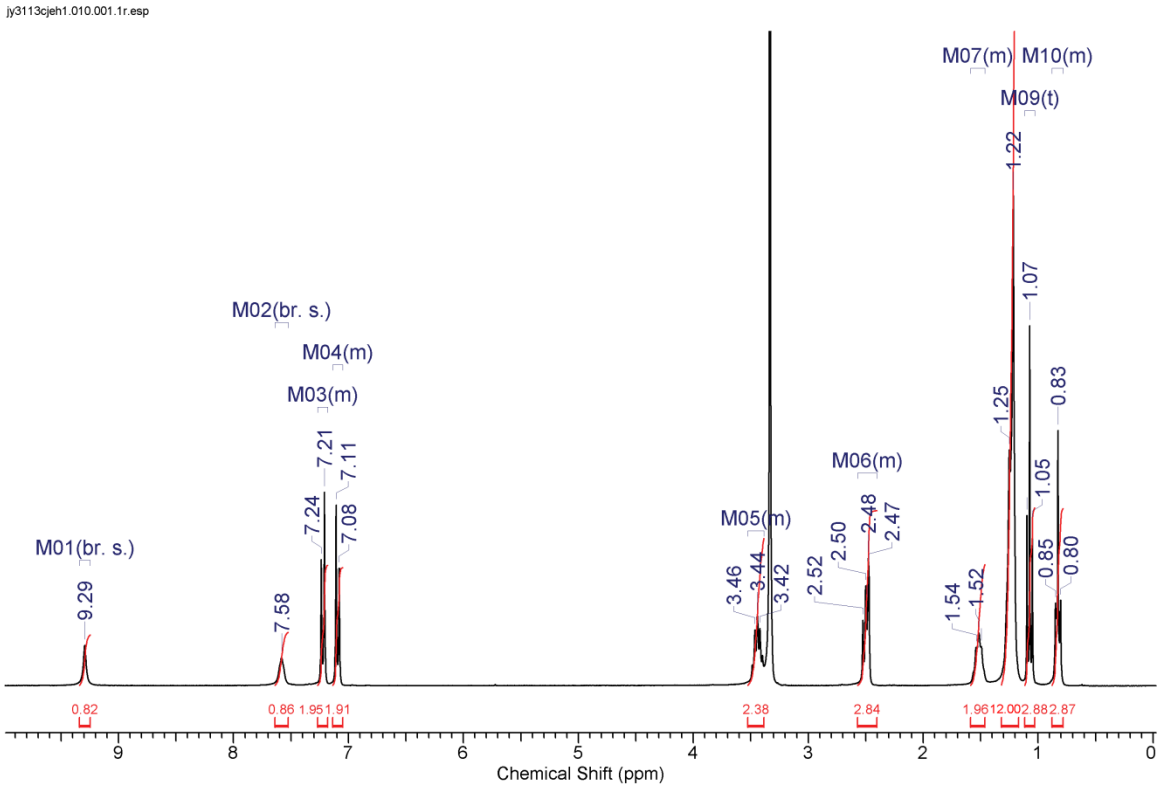


Figure S13. ^{1}H NMR spectrum of receptor **10g**.

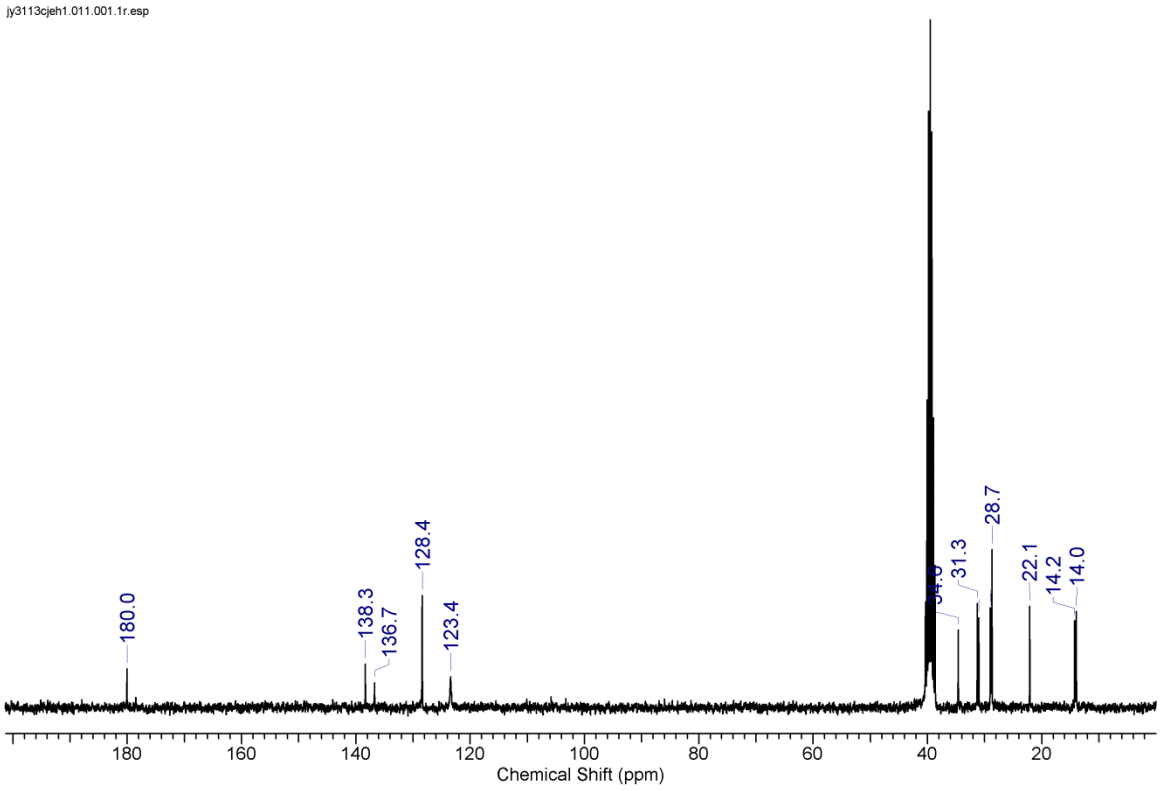


Figure S14. Full ^{13}C NMR spectrum of receptor **10g**.

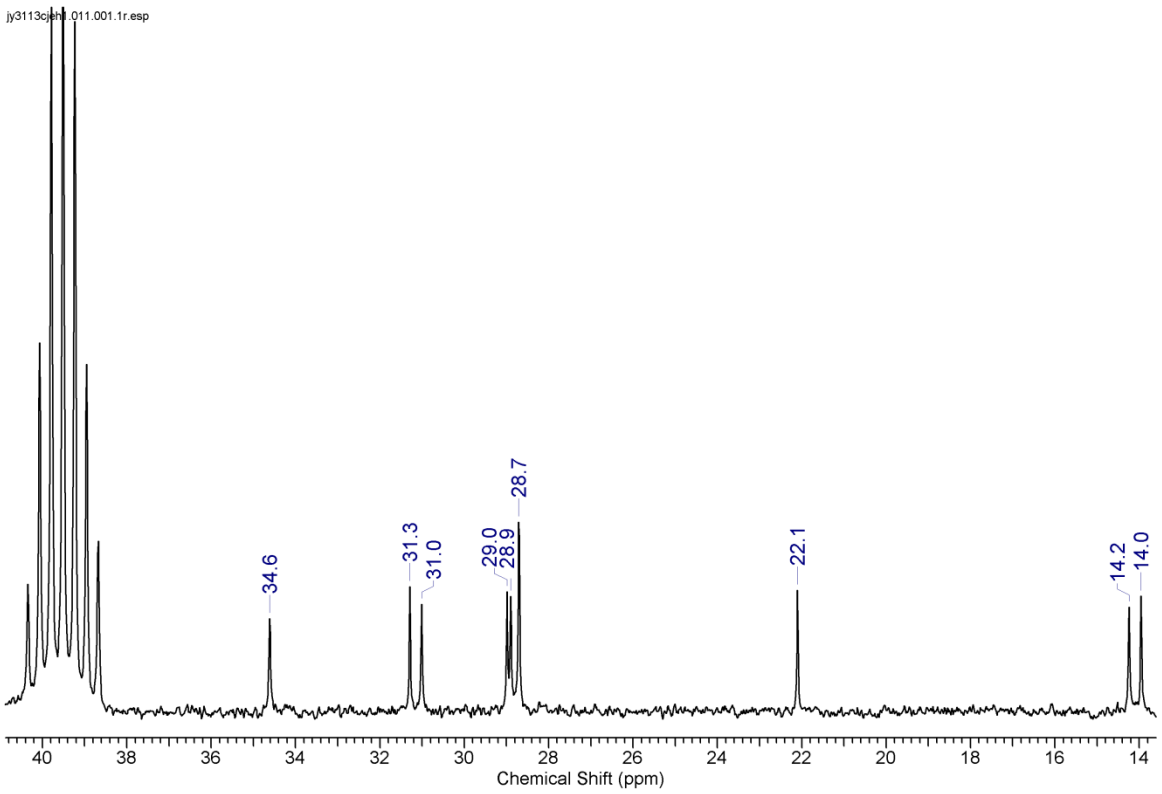


Figure S15. The aliphatic region of the ^{13}C NMR spectrum of receptor **10g**.

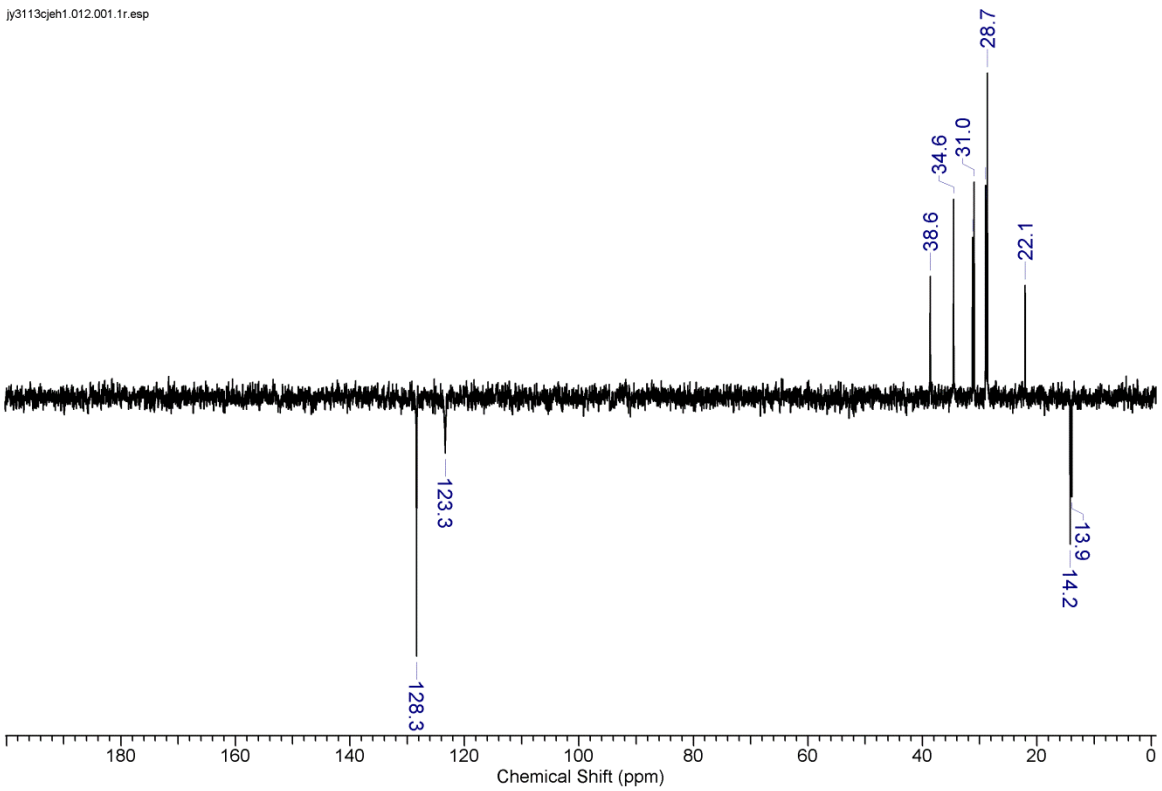


Figure S16. ^{13}C DEPT spectrum of receptor **10g**.

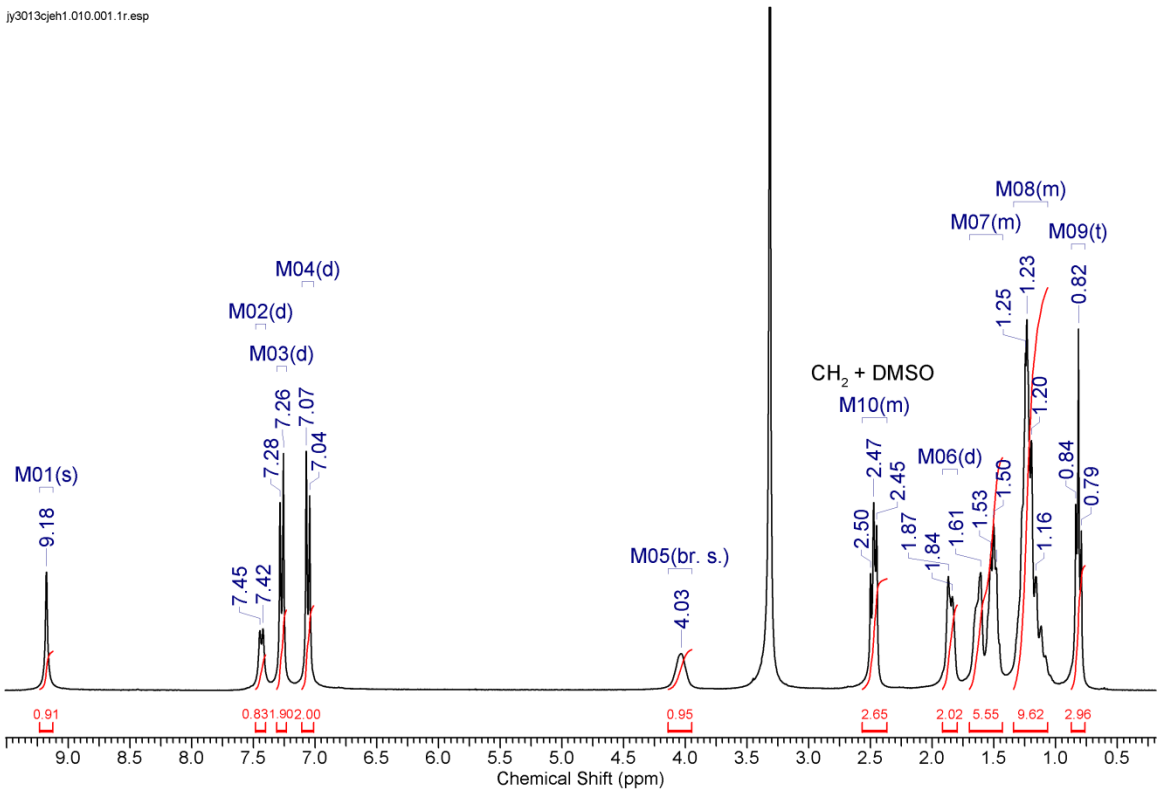


Figure S17. ^{1}H NMR spectrum of receptor **10h**.

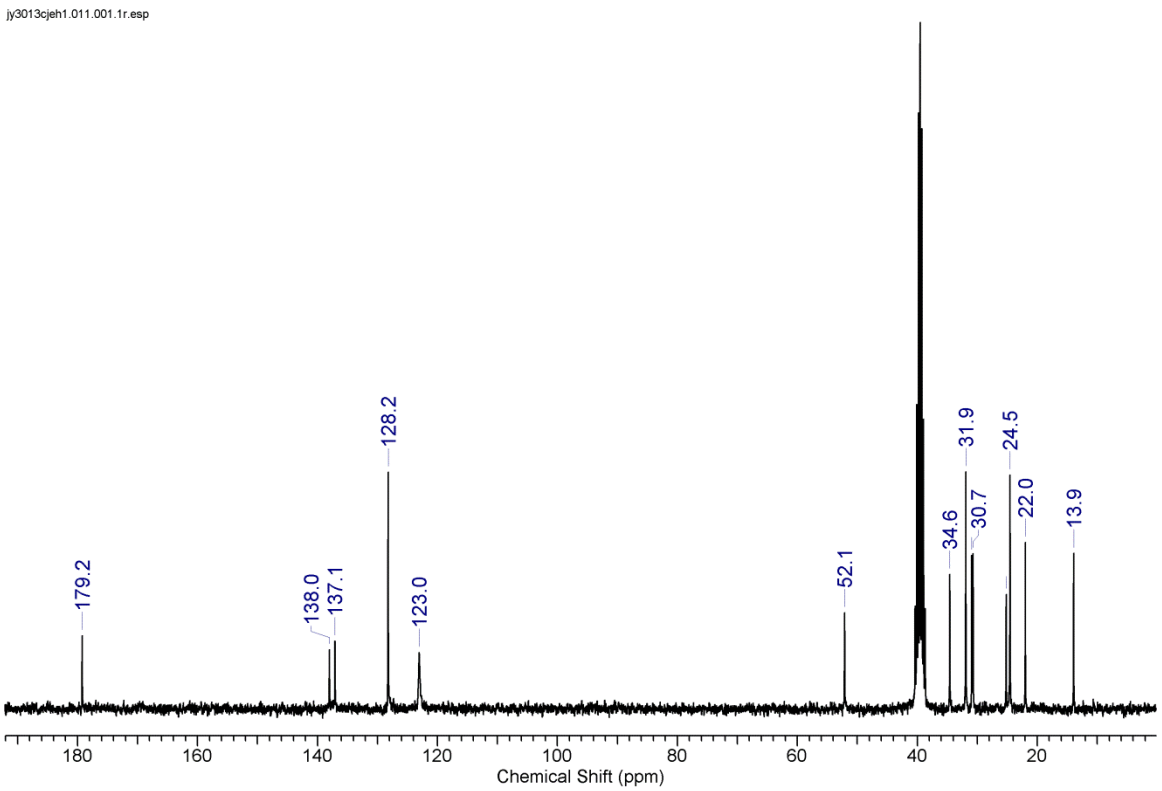


Figure S18. Full ^{13}C NMR spectrum of receptor **10h**.

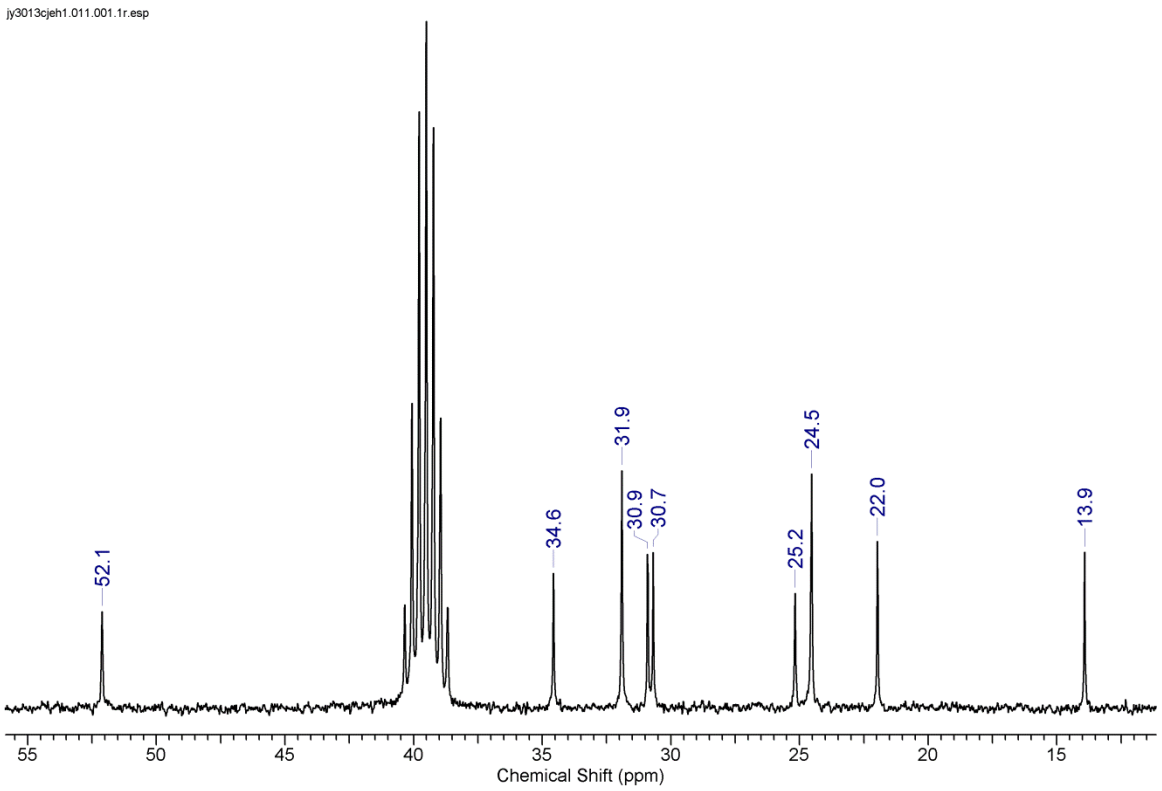


Figure S19. The aliphatic region of the ^{13}C NMR spectrum of receptor **10h**.

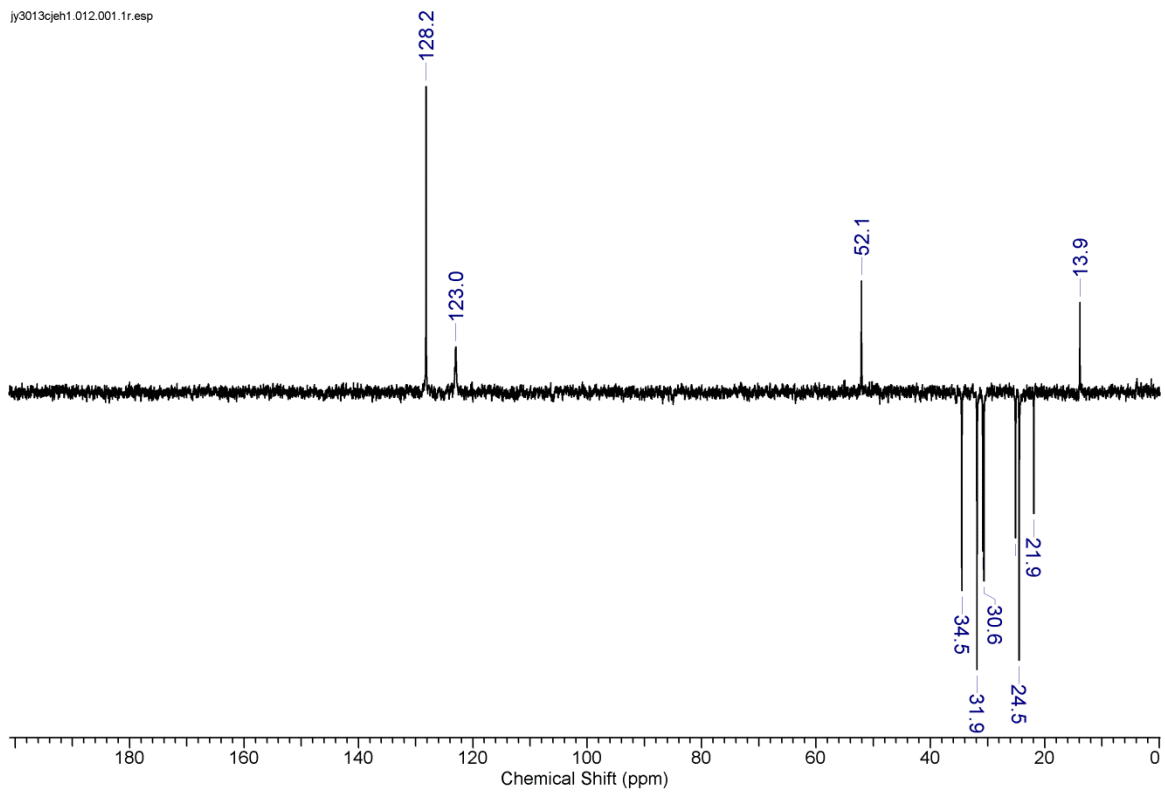


Figure S20. ^{13}C DEPT spectrum of receptor **10h**.

3. NMR binding studies

Experimental procedure

NMR titrations were performed by the addition of aliquots of tetrabutylammonium chloride (TBACl) (0.15 M) in a solution of the receptor (0.01 M) in DMSO-d_6/0.5% H_2O or $CDCl_3$ to a 0.01 M solution of the receptor in the same solvent. Both salt and receptor were dried under high vacuum prior to use. ^{1}H NMR spectra were recorded on a Bruker AV300 spectrometer and calibrated to the residual solvent peak in DMSO-d_6 (δ = 2.50 ppm) or $CDCl_3$ (δ = 7.26 ppm). In each case the change in chemical shift of the aromatic adjacent thiourea NH was studied. The WinEQNMR 2 computer program[2] was used to fit the data to a 1:1 binding model.

Binding analysis plots for ^{1}H NMR titrations

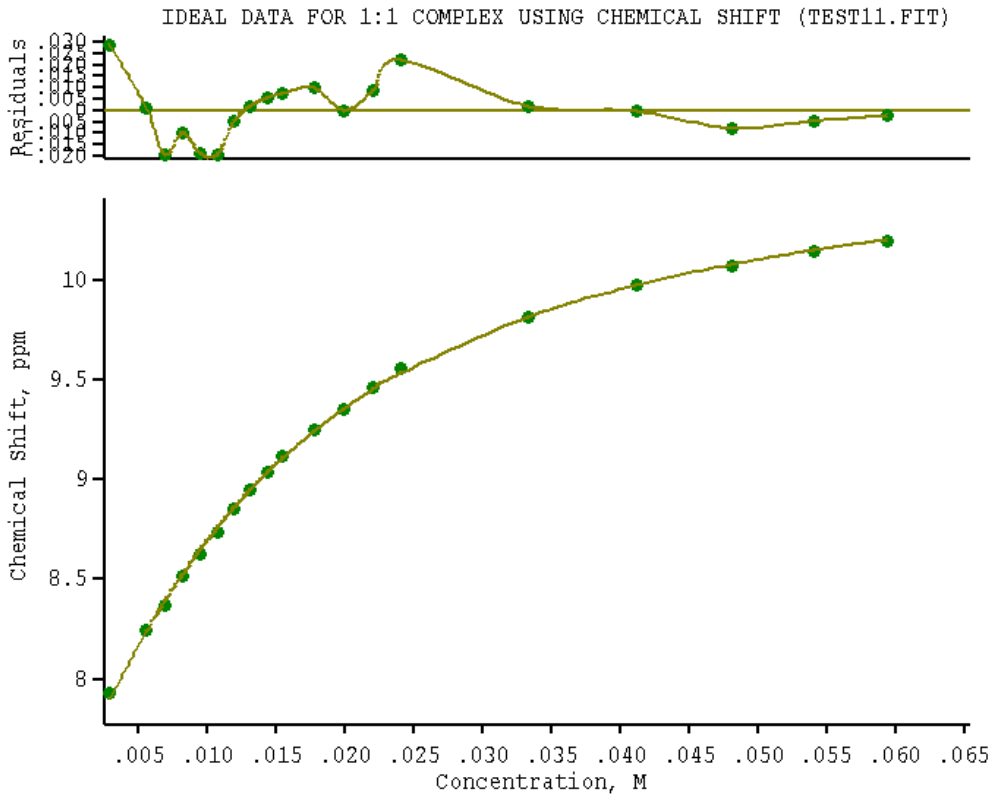


Figure S21. Receptor **10a** + TBACl in $CDCl_3$. K_a = 93 (±2.6) M^{-1}.

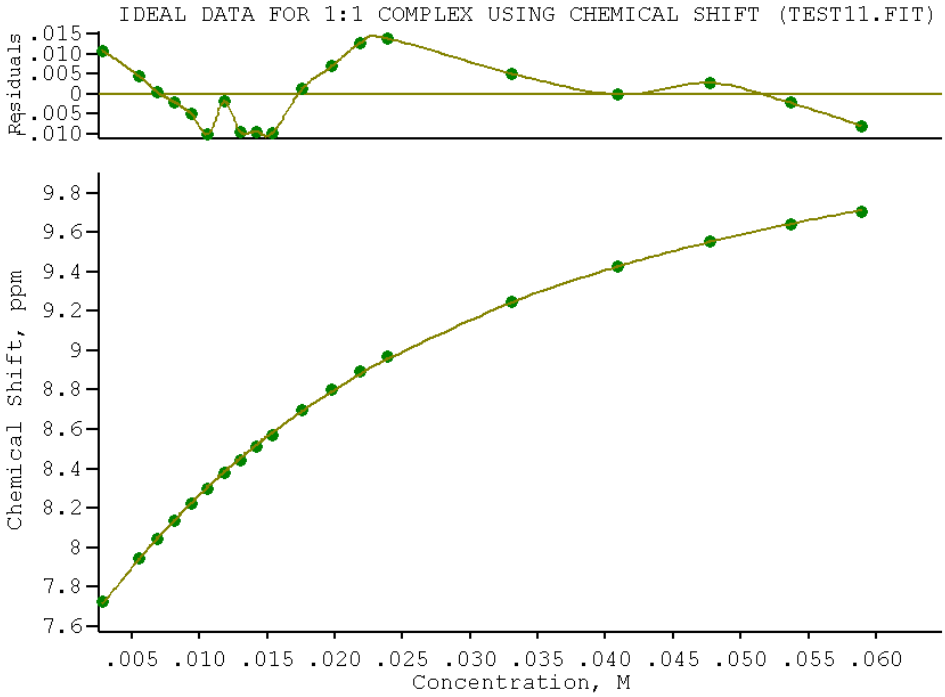


Figure S22. Receptor **10b** + TBACl in $CDCl_3$. K_a = 47 (±1.1) M^{-1}.

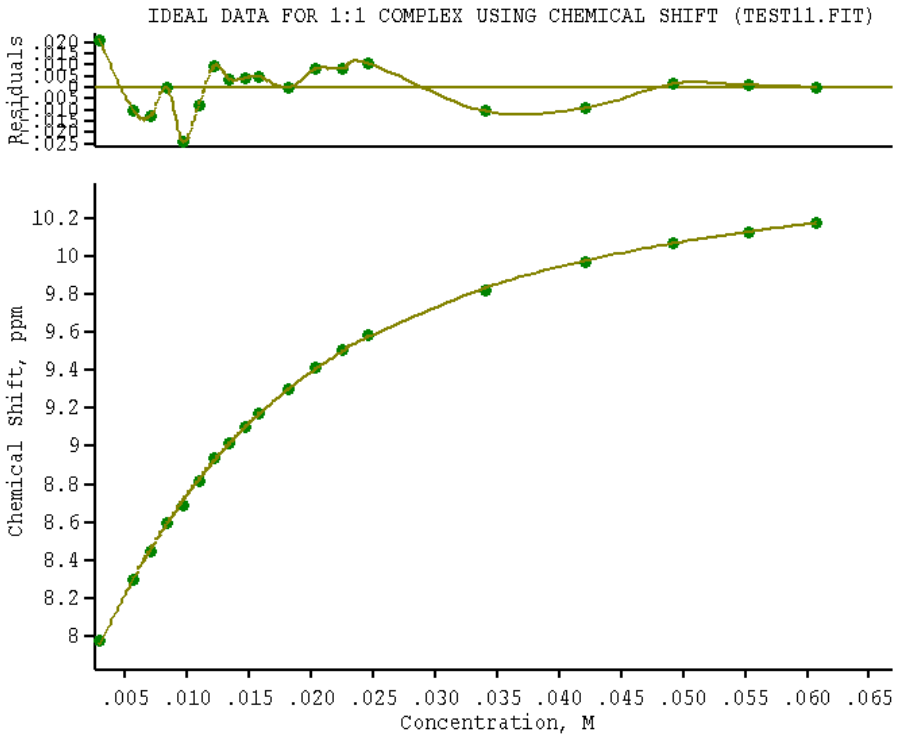


Figure S23. Receptor **10c** + TBACl in $CDCl_3$. K_a = 107 (±2.5) M^{-1}.

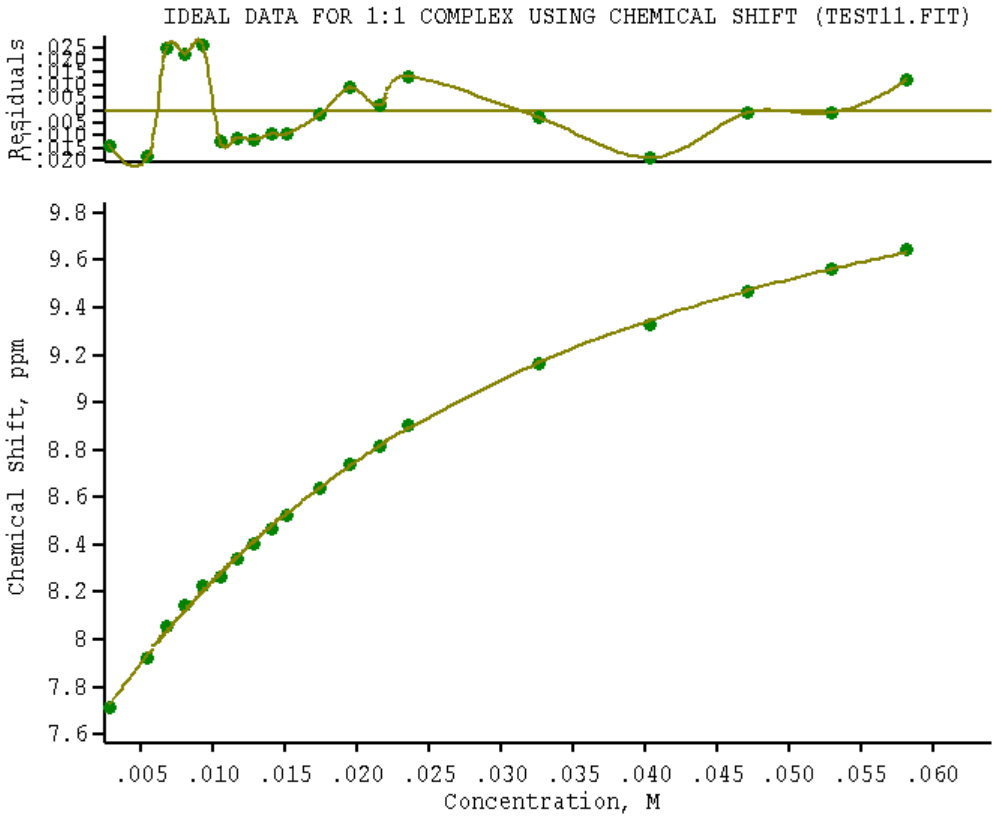


Figure S24. Receptor **10d** + TBACl in $CDCl_3$. K_a = 44 (±2) M^{-1}.

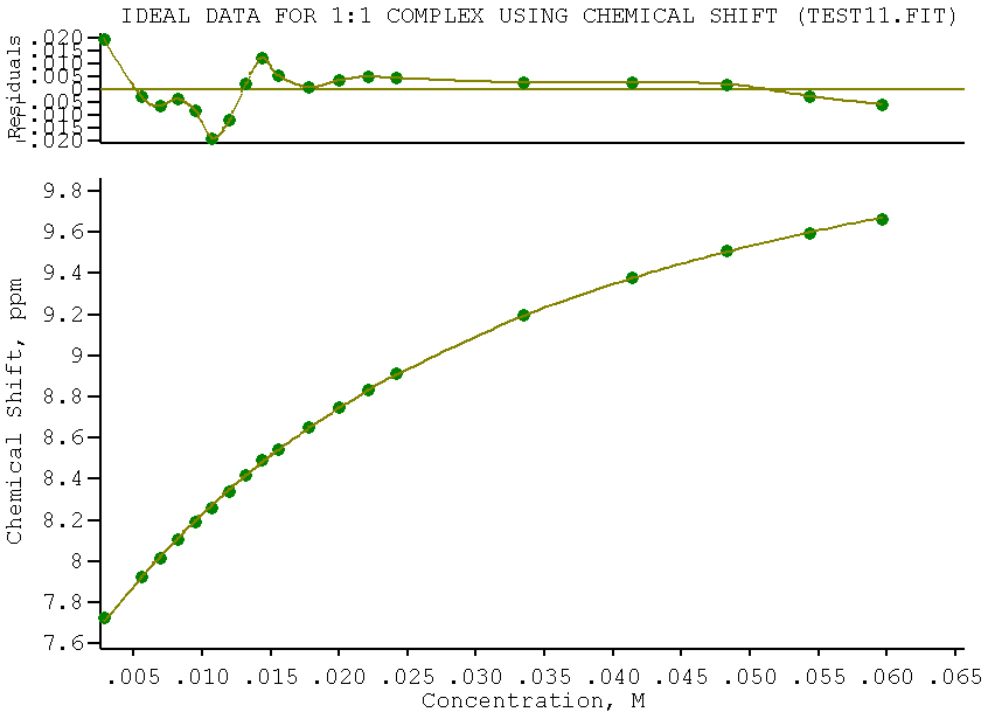


Figure S25. Receptor **10e** + TBACl in $CDCl_3$. K_a = 42 (±1.1) M^{-1}.

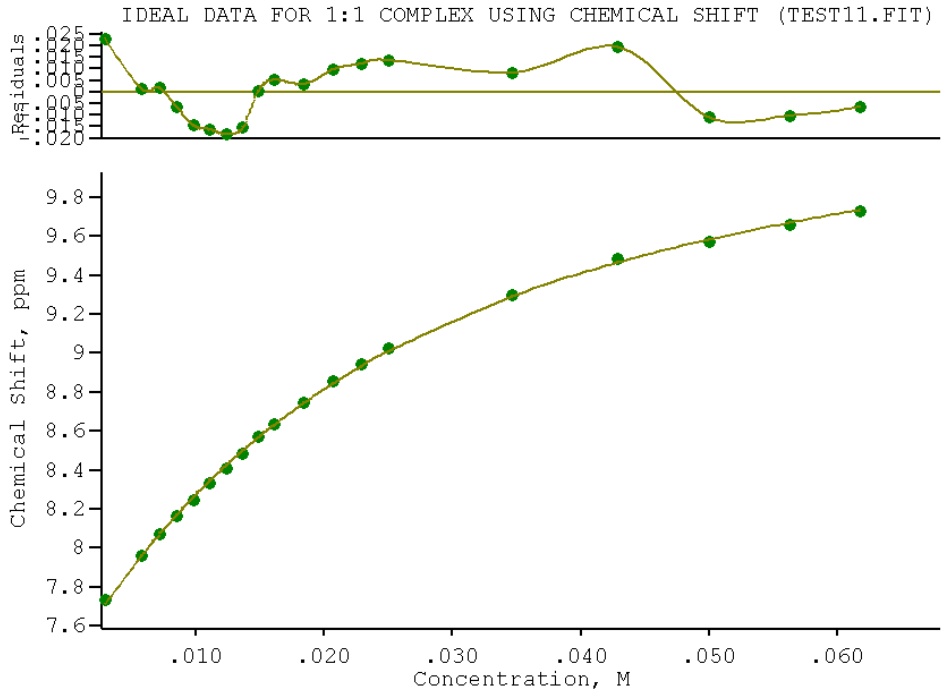


Figure S26. Receptor **10f** + TBACl in $CDCl_3$. K_a = 52 (±1.8) M^{-1}.

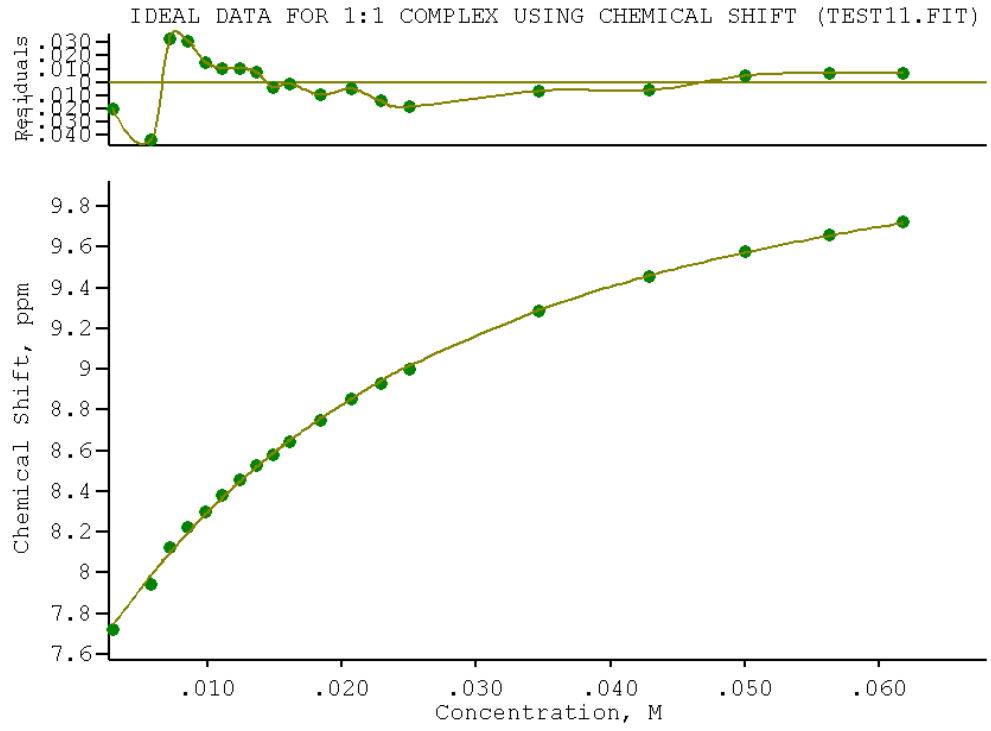


Figure S27. Receptor **10g** + TBACl in $CDCl_3$. K_a = 53 (±2.7) M^{-1}.

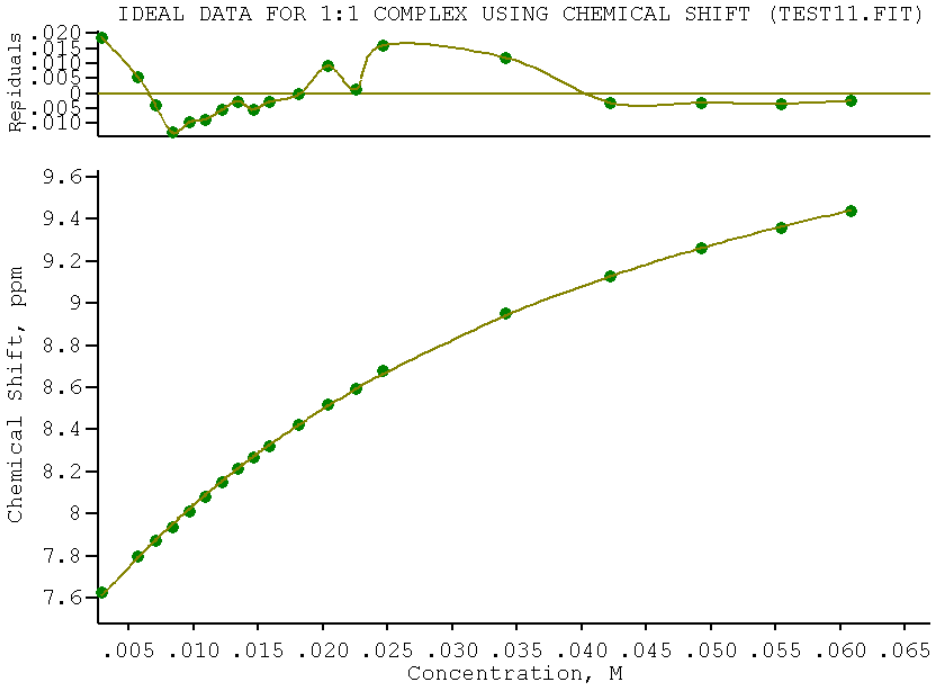


Figure S28. Receptor **10h** + TBACl in $CDCl_3$. K_a = 30 (±0.9) M^{-1}.

4. Measurement of chloride transport - lucigenin method

General procedure for transport measurements (receptor:lipid = 1:250)

Chloroform was deacidified by running it through a column of basic alumina. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC, 4.20 µmol) and cholesterol (1.80 µmol) as solutions of about 10 mM in deacidified chloroform were combined in a 5 mL round bottom flask and 24 nmol thiourea compound was added as solution in methanol. The solutions were mixed and the solvents were evaporated under a flow of nitrogen. The remaining residue was dried at vacuum for 1 hour, after which the residue was hydrated with 0.5 mL of 0.8 mM 10,10'-dimethyl-9,9'-biacridinium nitrate (lucigenin) solution in 225 mM $NaNO_3$ in Millipore water. The resulting suspension was sonicated for 30 seconds and stirred for 1 hour. The mixture was then frozen and thawed 10 times to break down multilamellar vesicles. The size distribution of the vesicles was narrowed by extrusion through a membrane with 200 nm pores (LipoFast) 29 times after which the vesicles were separated from free lucigenin by a size exclusion column (Sephadex G-50) eluted with 225 mM $NaNO_3$ in Millipore water. The collected vesicles were diluted with 225 mM $NaNO_3$ in Millipore water to 15.00 mL, yielding a solution of vesicles with 0.40 mM lipid concentration and a 1:250 mM thiourea to lipid ratio.

3.00 mL of this vesicle solution was placed in a quartz cuvette and the fluorescence intensity at 535 nm upon excitation at 450 nm was measured as function of the time at 25˚C using a PerkinElmer LS45 fluorescence spectrometer. 75.0 µL of 1.0 M NaCl in 225 mM $NaNO_3$ in Millipore water was added and the fluorescence intensity was measured for another 14 minutes. Fluorescence data were collected for four of these runs on each solution of vesicles. The plateau (before addition of chloride) and the vertical drop (the first 2.5 seconds after chloride addition, due to quenching of external lucigenin) were removed. Next the data are normalised: all fluorescence values (F) were divided the fluorescence value before addition of chloride (F_0). These normalised traces were averaged and are plotted in Figures 3a, 4a, and S29.

The data (F/F_0 as function of the time, t) were fitted by a single exponential decay function ($F/F_0 = a + be^{ct}$) and a double exponential decay function ($F/F_0 = y_0 + ae^{-bt} + ce^{-dt}$) using Origin 8.5.1. The initial rate (I) was obtained from the tangent to the double exponential decay fit at $t=0$ by differentiation to give $I = a{\cdot}b + c{\cdot}\,d$. The half-life was obtained from the fit parameters of the single exponential decay fit $t_{1/2} = ln\ 2 / c$.

Test for leaching of transporter from vesicle membranes - general procedure

The method described above was used to prepare a solution of vesicles (15 mL, 0.40 mM in lipid) with the thiourea preincorporated at a 1:250 receptor to lipid ratio. A portion of this solution (7.5 mL) was diluted to 15 mL, giving a solution 0.20 mM in lipid. A portion of the latter solution (5 mL) was then diluted further to 10 mL, giving a solution 0.10 mM in lipid. For all dilutions 225 mM $NaNO_3$ in Millipore water was used. Fluorescence data upon addition of NaCl were collected for two runs at 0.40 mM, for three runs at 0.20 mM, and for 3 runs at 0.10 mM. The obtained data were processed and averaged as described before for the regular transport experiments. Fluorescence decay curves are shown in Figures 3b, 3c and S30.

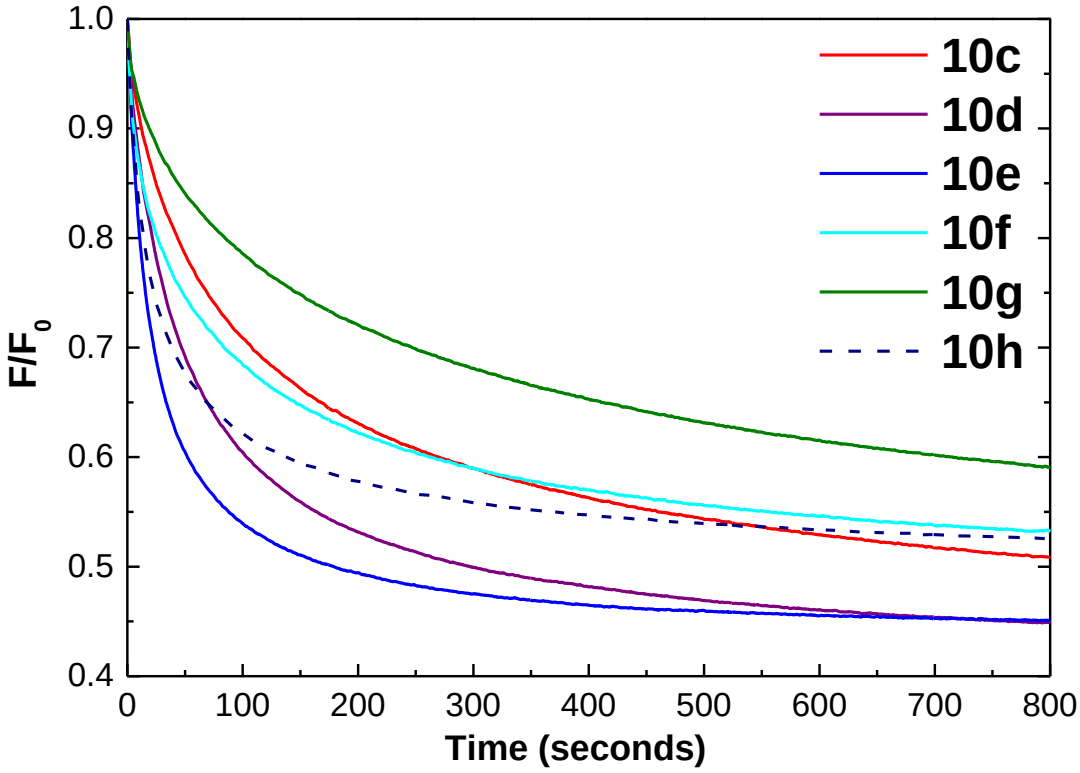


Figure S29. Normalised and averaged fluorescence transport traces of compounds **10c-h**.

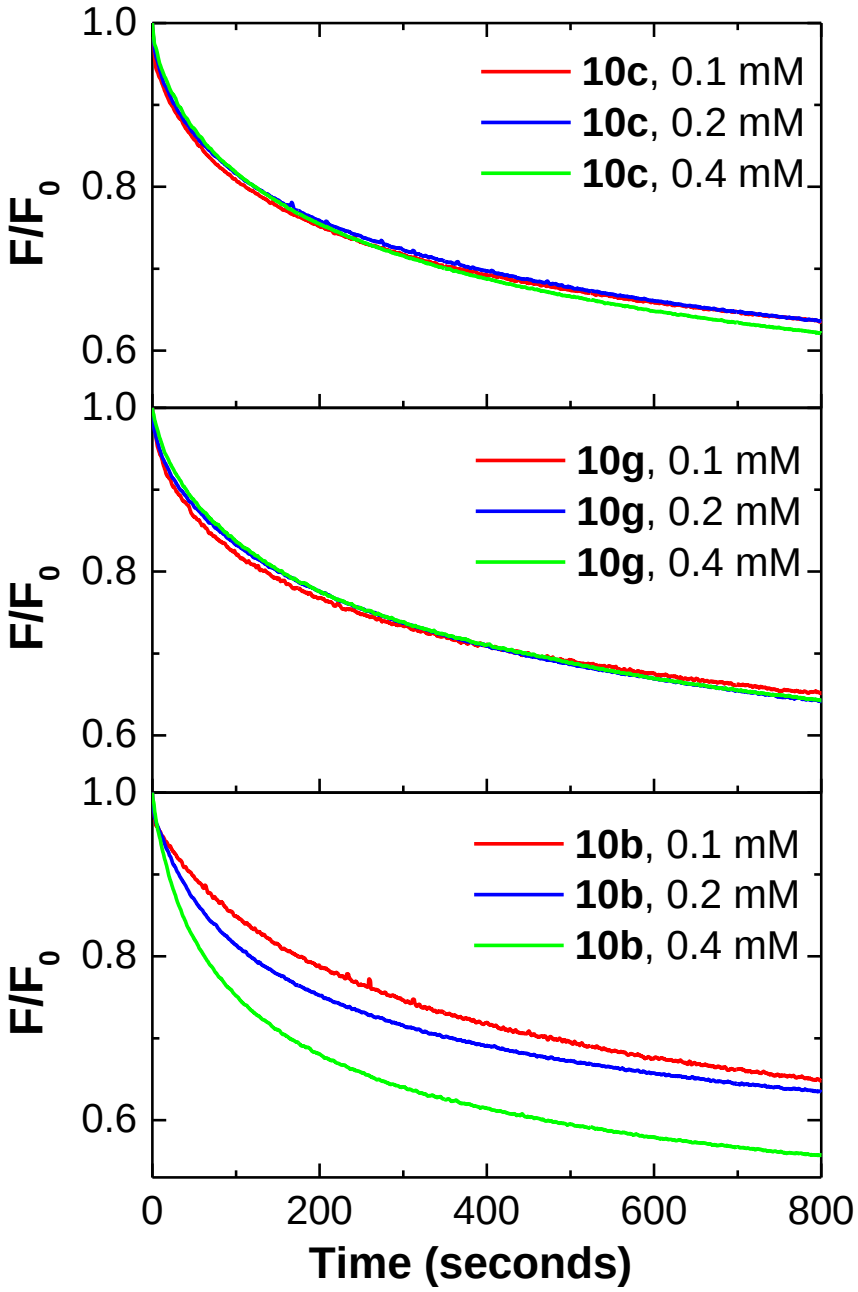


Figure S30. Test for leaching of compounds **10c**, **10g**, and **10b** (all at transporter to lipid ratio 1:250). The compounds **10c** and **10g** with 11 aliphatic carbon atoms do not show leaching, whereas compound **10b** with 8 aliphatic carbon atoms does leach signficantly from the vesicle membranes.

5. Measurement of chloride transport - ISE method

General

Chloride concentrations during transport experiments were determined using a Cole-Parmer chloride-selective electrode. POPC (1-palmitoyl-2-oleoyl-sn-glycero-3- phosphocholine) was supplied by Corden-Pharma and was stored at –20°C as a solution in chloroform (1 g POPC in 35 mL chloroform). Polyoxyethylene(8)lauryl ether was used as detergent and was supplied by TCI.

Preparation of Vesicles

A lipid film of POPC was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 4 hours. The lipid film was rehydrated by vortexing with a NaCl salt solution (489 mM NaCl, 5 mM phosphate buffer at pH 7.2). The lipid suspension was then subjected to nine freeze-thaw cycles, where the suspension was alternatingly frozen in a liquid nitrogen bath, followed by thawing in a water bath. The lipid suspension was allowed to age for 30 min at room temperature and was subsequently extruded 25 times through a 200 nm polycarbonate membrane. The resulting unilamellar vesicles were dialyzed against the external medium to remove unencapsulated NaCl salts.

Chloride/Nitrate Transport Assay

Unilamellar POPC vesicles containing NaCl, prepared as described above, were suspended in the external medium consisting of a 489 mM $NaNO_3$ solution buffered to pH 7.2 with sodium phosphate salts (5 mM buffer). The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule was added to start the experiment and the chloride efflux was monitored using a chloride sensitive electrode. At 5 min, the vesicles were lysed with 50 µl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO *v*/*v*) and a total chloride reading was taken at 7 min. The initial value was set at 0 % chloride efflux and the final chloride reading (at 7 minutes) was set as 100 % chloride efflux. All other data points were calibrated to these points.

Hill plots

During the Hill plots the chloride/nitrate transport assays were performed as described above for various concentrations of carrier. The chloride efflux (%) 270 s after the addition of carrier was plotted as a function of the carrier concentration. Data points were fitted to the Hill equation using Origin 8.1:

$$y = V_{max}\frac{x^n}{k^n + x^n}$$

where y is the chloride efflux at 270 s (%) and x is the carrier concentration (mol% carrier to lipid). V_{max}, k and n are the parameters to be fitted. V_{max} is the maximum efflux possible (this was fixed to 100%, as this is physically the maximum chloride efflux possible), n is the Hill coefficient and k is the carrier concentration needed to reach $V_{max}/2$ (when V_{max} is fixed to 100%, k equals EC_{50}). From the Hill plot it is therefore possible to directly obtain $EC_{50,270s}$ values, defined as the carrier concentration (molar % carrier to lipid) needed to obtain 50 % chloride efflux after 270 s. Within each Hill plot repeat, the chloride efflux for every concentration was monitored 3 times to ensure stability of the data during that repeat. Figures S36-S39 show all of the obtained Hill plots (including the results of the fit). Receptor **10g** was judged too inactive to perform a full Hill analysis.

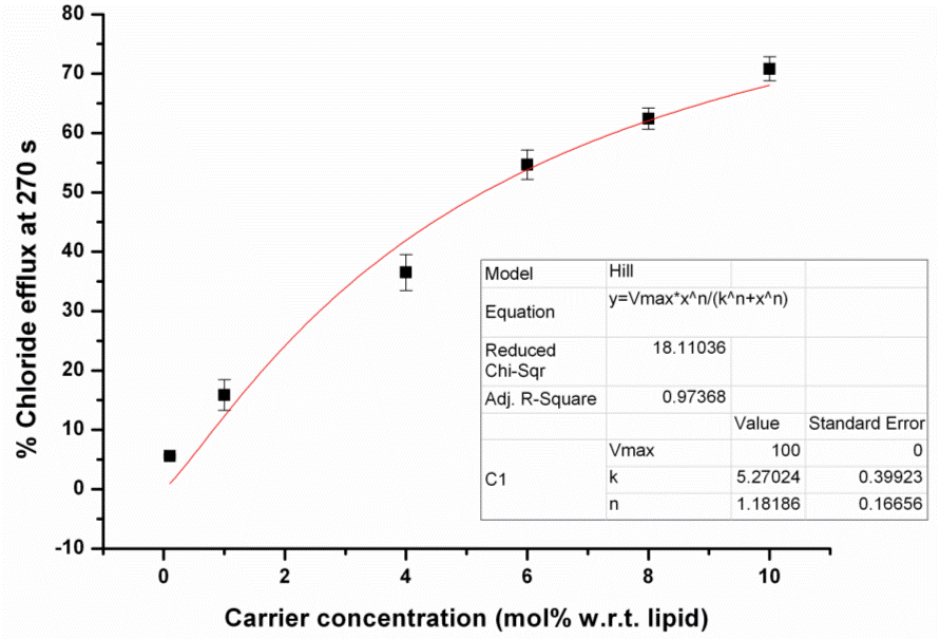


Figure S31. Hill plot for Cl^-/NO_3^- antiport by receptor **10c**.

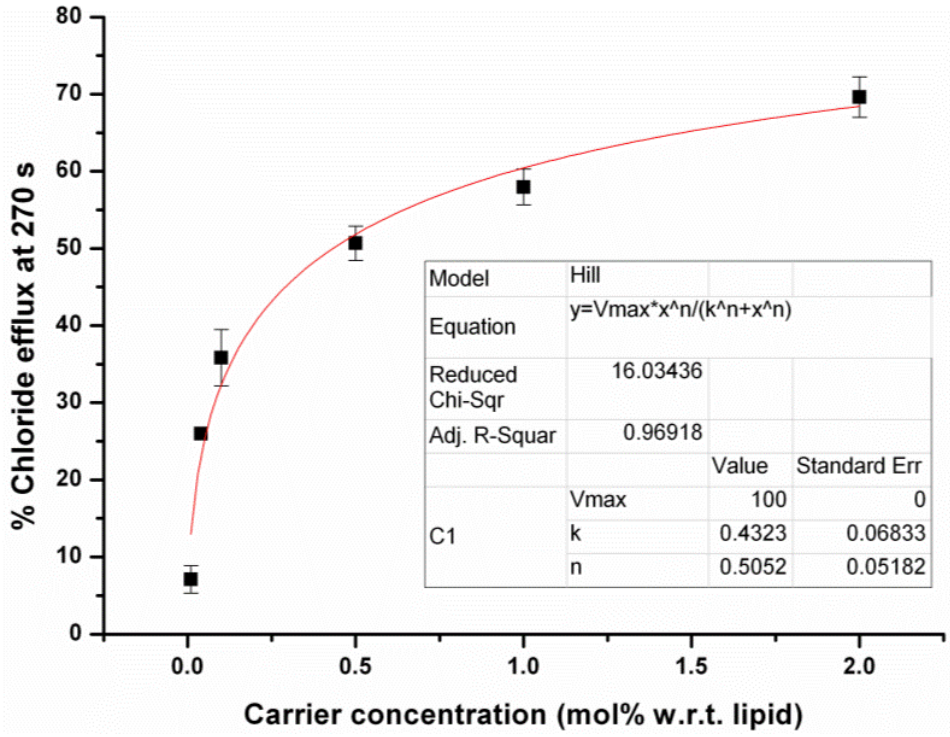


Figure S32. Hill plot for Cl^-/NO_3^- antiport by receptor **10d**.

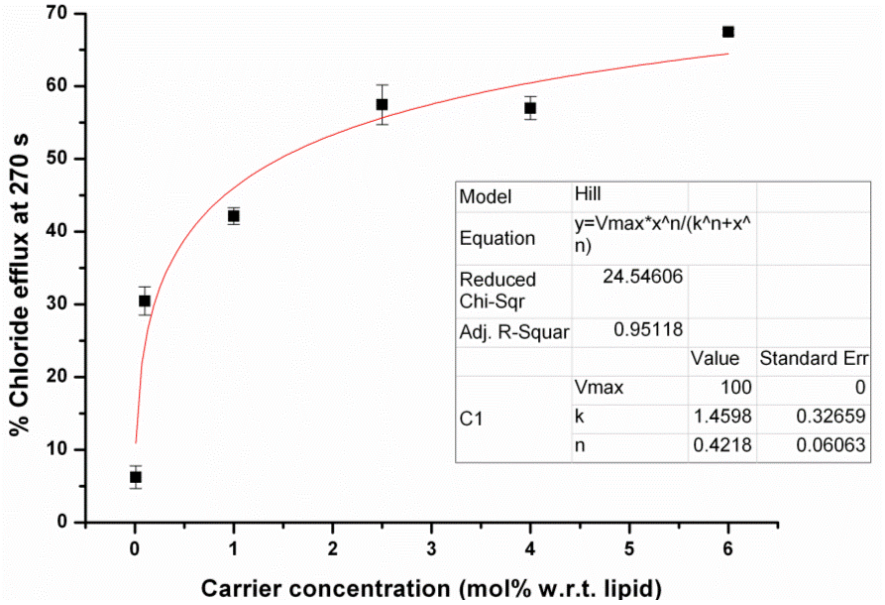


Figure S33. Hill plot for Cl^-/NO_3^- antiport by receptor **10f**.

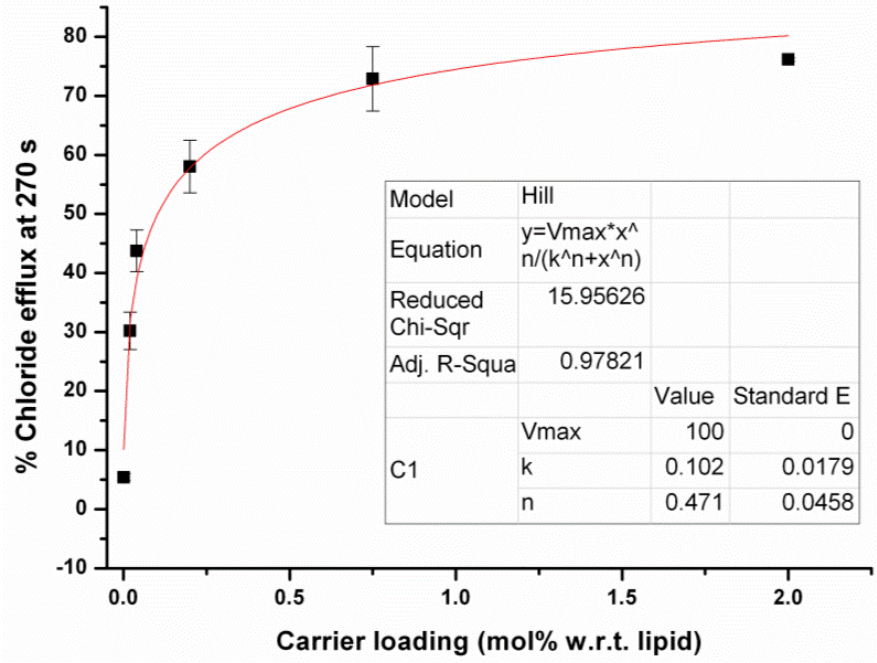


Figure S34. Hill plot for Cl^-/NO_3^- antiport by receptor **10h**.

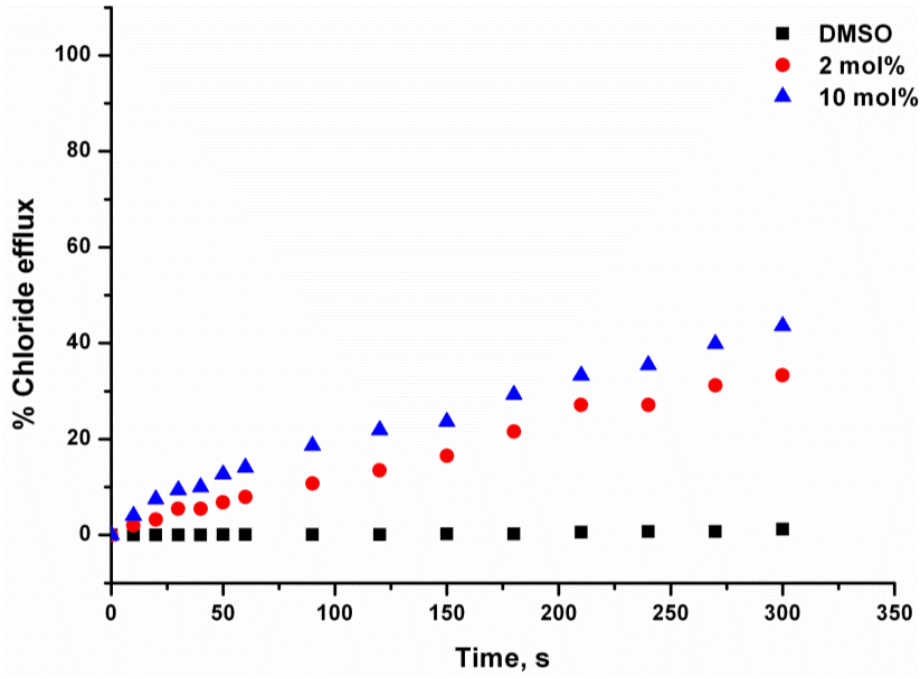


Figure S35. Cl^-/NO_3^- antiport by receptor **10g**. At 10 mol% carrier concentration less than 50 % of the internal chloride is released after 270 s, therefore $EC_{50\ 270s} > 10$ mol%.

6. Reversed phase HPLC measurements and logP calculations

The lipophilicities of transporters **10a-h** were assessed by measuring retention times on reversed phase HPLC. The mobile phase was prepared with LC-MS grade methanol and water (Fisher Scientific UK, Loughborough, UK), each contaning 0.1% HCOOH. Samples for injection were prepared as solutions in LC-MS grade methanol at a concentration of 2 μg/mL. HPLC separations were performed using a Dionex Ultimate® 3000 UHPLC (Thermo Scientific, Hemel Hempstead, UK). Samples were injected (2 μL) directly onto a Kinetex C18 Column (50 mm X 2.1 mm 1.7 μm particle size; Phenomenex, Torrance, CA, USA) thermostatically controlled at 40°C. The separation was achieved using 50% methanol in water for 2 minutes followed by a linear gradient to 100% methanol over 12 minutes then returning to 50 % methanol for 2 minutes at a flow rate of 0.3 mL/min. UV data were recorded at 254 nm and mass spectra were recorded using a Maxis™ ESI-ToF mass spectrometer (Bruker Daltonics, Bremen, Germany) using positive ion electrospray ionisation (120-1500 *m/z*) in order to assign retention times to the respective receptors.

For comparison, calculated logP (cLogP) values were also obtained using FieldView 2.0.2 and the VCC labs online calculator ALOGPS 2.1. Gale and co-workers have previously reported cLogP values calculated using Fieldview,[3] although their recent QSAR study indicated that values calculated using this model had somewhat poor correlation with experimental retention times.[1c] In that study the best correlation with values that could be readily calculated using free software was observed with the average logP value calculated with VCC labs.

The retention times and both sets of cLogP values are collected in the Table below. As expected, receptors **10a** and **10b** are less lipophilic than all of the receptors containing 11 aliphatic carbon atoms, and as such they have shorter retention times and lower logP values in both models. Notably, across the series **10c-g** there is little variation in retention time or calculated logP values. Hence, as expected, the lipophilicity of this series does not alter significantly. Receptor **10h** (containing a cyclohexyl substituent) seems to have a slightly shorter retention time and slightly lower calculated logP values, indicating that it is slightly less lipophilic than the rest of the receptors with 11 alipatic carbon atoms.

Receptor	Total # aliphatic C atoms	Retention time (min)	cLogP (FieldView 2.0.2[i])	Average logP (VCC labs[ii])
10a	6	6.6	3.55	3.64
10b	8	9.1	4.12	4.54
10c	11	11.9	5.50	6.14
10d	11	11.7	5.29	6.05
10e	11	11.7	5.29	5.95
10f	11	11.9	5.29	6.08
10g	11	12.0	5.29	6.05
10h	11	11.1	5.04	5.49

[i] FieldView 2.0.2 (TorchV10Lite) is available as freeware at http://www.cresset-group.com, Cresset BMD.

[ii] VCCLAB, Virtual Computational Chemistry Laboratory and ALOGPS 2.1, http://www.vcclab.org (accessed August 19th, 2013); VCCLAB 2005.

7. References

1. a) M. Wenzel, M. E. Light, A. P. Davis and P. A. Gale, *Chem. Commun.*, 2011, **47**, 7641-7643; b) S. J. Moore, M. Wenzel, M. E. Light, R. Morley, S. J. Bradberry, P. Gomez-Iglesias, V. Soto-Cerrato, R. Perez-Tomas and P. A. Gale, *Chem. Sci.*, 2012, DOI: 10.1039/c1032sc20551c; c) N. Busschaert, S. J. Bradberry, M. Wenzel, C. J. E. Haynes, J. R. Hiscock, I. L. Kirby, L. E. Karagiannidis, S. J. Moore, N. J. Wells, J. Herniman, G. J. Langley, P. N. Horton, M. E. Light, I. Marques, P. J. Costa, V. Félix, J. G. Frey and P. A. Gale, *Chem. Sci.*, 2013, DOI: 10.1039/c1033sc51023a.
2. M. J. Hynes, *J. Chem. Soc. Dalton Trans.*, 1993, 311-312.
3. a) S. J. Moore, C. J. E. Haynes, J. González, J. L. sutton, S. J. Brooks, M. E. Light, J. Herniman, G. J. Langley, V. Soto-Cerrato, R. Pérez-Tomás, I. Marques, P. J. Costa, V. Félix and P. A. Gale, *Chem. Sci.*, 2013, **4**, 103-117; b) S. J. Moore, M. Wenzel, M. E. Light, R. Morley, S. J. Bradberry, P. Gómez-Iglesias, V. Soto-Cerrato, R. Pérez-Tomás and P. A. Gale, *Chem. Sci.*, 2013, **3**, 2501-2509.