Tris-ureas as transmembrane anion transporters

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1. Synthesis

All reactions were performed in oven-dried glassware under a slight positive pressure of nitrogen. ^{1}H-NMR (400 MHz, 500MHz) and ^{13}C NMR (100 MHz, 125MHz) spectra were determined on a Varian INOVA-400 spectrometer, and Varian INOVA-500 spectrometer. Chemical shifts for ^{1}H-NMR are reported in parts per million (ppm), calibrated to the residual solvent peak set, with coupling constants reported in Hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet. Chemical shifts for ^{13}C NMR are reported in ppm, relative to the central line of a septet at δ = 39.52 ppm for deuterio-dimethylsulfoxide. Infrared (IR) spectra were recorded on a NICOLET 5700 FT-IR spectrophotometer and reported in wavenumbers (cm^{-1}). Microanalytical data were obtained using a Fisons EA CHNS-O instrument (*T* = 1000 °C). Fluorescence spectra were recorded on a Cary Eclypse spectrofluorimeter. All solvents and starting materials were purchased from commercial sources where available. Proton NMR titrations were performed by adding aliquots of the putative anionic guest (as the TBA salt, 0.075 M) in a solution of the receptor (0.005M) in DMSO-d_6/0.5% water to a solution of the receptor (0.005M). Receptors **L^1** and **L^3** have already been reported in the literature.[1]

Synthesis of L^1
To a solution of *o*-nitrophenyl isocyanate (1.23 mmol; 0.20 g) in 15 ml of DCM was added a suspension of amine (0.41 mmol; 0.10 g) in 15 ml of DCM. The mixture of reaction was left stirring at reflux under N_2 atmosphere overnight. The precipitate formed was collect by filtration washed with hot THF and then dried over vacuum to give a yellow solid.
Yield 80.6% (0.33 mmol; 0.19 g); M.p. > 250°C; ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K): δ_{ArH} 6.90-7.30 (m, 6H), 7.47 (d, J = 7.0 Hz, 2H), 7.64 (t, J = 7.0 Hz, 2H), 7.72 (d, J = 7.0 Hz, 2H), 8.04 (d, J = 8.5 Hz, 2H), 8.22 (d, J = 8.0 Hz, 2H), 8.47 (s, 2H, NH), 9.16 (s, 2H,NH) 9.71 (s, 2H,NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{Ar} 122.27, 122.74, 123.33, 123.50, 125.01, 125.15, 125.27, 129.47, 132.53, 134.73, 134.82, 137.83 ; δ_{CO} 152.80, 153.50.

Synthesis of L^2
To a solution of *m*-nitrophenyl isocyanate (1.23 mmol; 0.20 g) in 15 ml of DCM was added a suspension of amine (0.41 mmol; 0.10 g) in 15 ml of DCM. The mixture of reaction was left stirring at reflux under N_2 atmosphere overnight. The precipitate formed was collect by filtration washed with hot THF and then dried over vacuum to give a yellow solid.
Yield 68.7% (0.28 mmol; 0.16 g); M.p. 240 °C; ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K): δ_{ArH} 7.05-7.16 (m, 2H), 7.48-7.55 (m, 8H), 7.58-7.65 (m, 2H), 7.70 (d, J = 5.0 Hz, 2H), 7.78 (d, J = 5.0 Hz, 2H), 8.23 (s, 2H), 8.48 (s, 2H, NH), 8.51 (s, 2H,NH) 9.67 (s, 2H,NH); ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{Ar} 112.01, 116.08, 124.05, 124.36, 129.96, 131.23, 141.21, 148.06; δ_{CO} 153.03, 154.26.

Synthesis of L^3
To a solution of *p*-nitrophenyl isocyanate (1.23 mmol; 0.20 g) in 15 ml of DCM was added a suspension of amine (0.41 mmol; 0.10 g) in 15 ml of DCM. The mixture of reaction was left stirring at reflux under N_2 atmosphere overnight. The precipitate formed was collect by filtration washed with hot THF and then dried over vacuum to give a yellow solid.

Yield 76.4% (0.32 mmol; 0.18 g); M.p. 250 °C; ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K): δ_{ArH} 7.19-7.14 (m, 4H), 7.50-7.61 (m, 4H), 7.66 (d, J = 9.0 Hz, 4H), 8.13 (d, J = 8.5 Hz, 4H), 8.30 (s, 2H, NH), 8.47 (s, 2H,NH), 9.86 (s, 2H,NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{Ar} 117.35, 124.30, 124.55, 125.04, 130.89, 131.38, 140.87, 146.51; δ_{CO} 152.62, 154.13.

Synthesis of 1,3bis(2(3(4(trifluoromethyl)phenyl)ureido)phenyl)urea L[4]

A solution of *p*-trifluorophenyl isocyanate (0.534 mmol, 0.1 g) and amine (0.267 mmol, 0.078g) in 30 ml of DCM was refluxed under N_2 atmosphere for 6h. The precipitate obtained was filtered off and dried under vacuum to give a white solid.

Yield 91% (0.150 g, 0.243 mmol); M.p. >250°C; ^{1}H-NMR (400 MHz, DMSO-d_6, 298 K): δ_{CH} 7.06-7.17 (m, 4H), 7.52-7.7 (m, 12H), 8.19 (s, NH, 2H), 8.47 (s, NH, 2H), 9.54 (s, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{C} 117.80, 121.50, 121.82, 124.23, 124.30, 124.37, 126.03, 131.12, 131.30, 143.61; δ_{CO} 152.95, 154.15. LRMS (ES$^-$) m/z: 615.0154 [M-H$^+$]$^-$.

Synthesis of 1,3-bis(2-(3-(3,5-bis(trifluoromethyl)phenyl)ureido) phenyl) urea L[5]

To a solution of 3,5-Bis(trifluoromethyl)phenyl isocyanate (1.44 mmol; 0.367 g) in 10 ml of DCM was added a suspension of amine (0.413 mmol; 0.1 g) in 20 ml of DCM. The mixture of reaction was left stirring at reflux under N_2 atmosphere overnight. The precipitate formed was collect by filtration washed with DCM and with hot MeOH and then dried over vacuum to give a white solid.

Yield 81% (0.332 mmol; 0.25 g); M.p. >250°C; ^{1}H-NMR (400 MHz, DMSO-d_6, 298 K): δ_{ArH} 7.12 (4H), 7.50-7.54 (m, 2H), 7.56 (s, 2H), 7.57-7.61 (m, 2H), 8.06 (s, 4H), 8.35 (s, 2H,NH), 8.49 (s, 2H,NH), 9.89 (s, 2H,NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{C} 114.11, 117.57, 121.90, 124.42, 124.48, 124.54, 124.61, 130.14, 130.47, 130.79, 131.12, 131.31, 142.01; δ_{CO} 152.99, 154.35. LRMS (ES$^-$) m/z: 751.0983 [M-H$^+$]$^-$.

Synthesis of 1,3-bis(2-(3-(4-fluorophenyl)ureido)phenyl)urea L[6]

To a solution of 4-Fluorophenyl isocyanate (1.24 mmol; 0.170 g) in 15 ml of DCM was added a suspension of amine (0.413 mmol; 0.1 g) in 15 ml of DCM. The mixture of reaction was left stirring at reflux under N_2 atmosphere overnight. The resulting precipitate was filtered off washed several times with DCM and with hot THF and then dried over vacuum to give a white solid.

Yield 70.3 % (0.29 mmol; 0.150 g); M.p. >250°C; ^{1}H-NMR (400 MHz, DMSO-d_6, 298 K): δ_{ArH} 7.05-7.15 (m, 8H), 7.40-7.50 (m, 4H), 7.50-7.62 (m, 4H), 8.07 (s, 2H, NH), 8.46 (s, 2H, NH), 9.14 (s, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{C} 115.11, 115.33, 119.82, 119.90, 124.09 (ArCF), 131.10, 131.47, 136.18, 153.23, 154.14; δ_{CO} 156.08, 158.45.
LRMS (ES$^-$) m/z: 515.1141 [M-H$^+$]$^-$.

Synthesis of 1,3-bis(2-(3-(p-tolyl)ureido)phenyl)urea L[7]

A suspension of amine (0.413 mmol; 0.1 g) in 20 ml of DCM was added to a solution of 4-Methylphenyl isocyanate (1.44 mmol; 0.192 g) in 10 ml of DCM. The mixture of reaction was left stirring at reflux under N_2 atmosphere overnight. The precipitate obtained was collect by filtration washed several times with DCM and with hot THF and then dried over vacuum to give a white solid.

Yield 63.3% (0.26 mmol; 0.133 g); M.p. >250°C; ^{1}H-NMR (400 MHz, DMSO-d_6, 298 K): δ_{ArH} 7.00-7.11 (m, 8H), 7.32 (d, J=5.0 Hz, 4H), 8.01 (s, 2H, NH), 8.43 (s, 2H, NH), 8.96 (s, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{C} 20.34; δ_{Ar} 118.27, 123.73, 123.90, 124.07, 124.19, 129.14, 130.53, 130.98, 131.56, 137.23; δ_{CO} 153.18, 154.10.
LRMS (ES$^-$) m/z: 507.0681 [M-H$^+$]$^-$.

Synthesis 1,3-bis(2-(3-(2-methoxyphenyl)ureido)phenyl)urea L[8]

A suspension of 2-Methoxyphenyl isocyanate (1.44 mmol, 0.215 g) and amine (0.413 mmol, 0.1g) in 25 ml of DCM was refluxed under N_2 atmosphere overnight. The precipitate obtained was filtered off and dried under vacuum to give a white solid.

Yield 62.7% (0.26 mmol; 0.140 g); M.p. 181°C; ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K): δ_{CH} 3.83; δ_{ArH} 6.82-7.10 (m, 10H); 7.55 (d, J=10.0 Hz, 2H) 7.68 (d, J=5.0 Hz, 2H), 8.10 (d, J=10.0 Hz, 2H), 8.42 (s, 2H, NH), 8.50 (s, 2H, NH), 8.70 (s, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_{C} 55.65, δ_{ArC} 110.68, 118.59, 120.47, 121.76, 123.41, 123.44, 123.92, 124.14, 128.74, 130.51, 131.71, 147.70; δ_{CO} 153.27, 153.57. LRMS (ES$^-$) m/z: 539.1984 [M-H$^+$]$^-$.

Synthesis of 1,3-bis(2-(3-(4-methoxyphenyl)ureido)phenyl)urea L[9]

To a solution of 4-Methoxyphenyl isocyanate (1.44 mmol, 0.215 g) in 10 ml of DCM was added a suspension of amine (0.413 mmol; 0.1 g) in 15 ml of DCM. The mixture of reaction was left stirring at reflux under N_2 atmosphere overnight. The precipitate formed was filtered off washed several times with DCM and dried over vacuum to give a white solid. Yield 67.2% (0.28 mmol; 0.150 g); M.p. 205°C; ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K): δ_{CH} 3.70; δ_{ArH} 6.84 (d, J=10.0 Hz, 4H), 7.00-7.11 (m, 4H), 7.34 (d, J=5.0 Hz, 4H), 7.54 (d, J=5.0 Hz, 4H), 7.60 (d, J=10, 4H), 7.98 (s, 2H, NH), 8.43 (s, 2H, NH), 8.89 (s, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6, 298 K) δ_C 55.14; δ_{ArC} 113.96, 119.97, 123.64, 123.83, 124.11, 124.26, 130.91, 131.74, 132.84, 153.32; δ_{CO} 154.14, 154.40. LRMS (ES$^-$) m/z: 539.3063 [M-H$^+$]$^-$.

2. Crystallizations

Suitable crystals were selected and data collected on a Rigaku AFC12 goniometer at 100K equipped with an enhanced sensitivity (HG) Saturn724+ detector mounted at the window of an FR-E-Superbright molybdenum anode generator with either VHF Varimax optics (70µm focus) for [**L^5**(Cl$^-$)](TBA$^+$) and [**L^4**(Cl$^-$)$_2$](TBA$^+$)$_2$ or HF Varimax optics (100µm focus) for [**L^5**(AcO$^-$)](TBA$^+$). Cell determination, data collection, data reduction, cell refinement and absorption correction were carried out using CrystalClear[i]. With the data reduction, cell refinement and absorption correction using CrystalClear[i]. Structure solution using either SHELXS[ii] or SUPERFLIP[iii] and refinement using SHELXL

[i] *CrystalClear- SM Expert 3.1 b27,* **2013** or *CrystalClear- SM Expert 2.1 b29,* **2013**, Rigaku
[ii] G. M. Shedrick, *Acta Cryst*, 2015, **C71**, 3-8
[iii] L. Palatinus, G. Chapuis, *J. Appl. Cryst*., 2007, **40**, 786

Table S1. Summary of the crystallization experiments in different solvents for the receptors **L^1**-**L^6**. Conditions yielding single crystals are indicated as (√)**.** (●) indicates an unsuccessful experiment and (-) is used to indicate "not applied" experimental conditions.

Receptor	Host	solvent								
		AcOEt	**MeOH**	**EtOH**	**MeOH/MeNO$_2$**	**THF /DMF**	**THF**	**MeNO$_2$**	**MeCN**	**DMSO**
L^1	-	-	-	-	-	-	-	-	-	●
L^1	**AcO$^-$**	-	-	-	-	●	-	-	-	●
L^1	**Cl$^-$**	-	-	●	-	●	-	-	●	●
L^1	**HCO$_3^-$**	-	-	-	-	●	-	-	-	●
L^1	**NO$_3^-$**	-	-	-	-	-	-	-	-	●
		-								
L^2	-	-	-	-	-	-	-	-	-	●
L^2	**Cl$^-$**	-	-	-	-	-	-	-	-	●
L^2	**HCO$_3^-$**	-	-	-	-	-	-	-	-	●
L^2	**NO$_3^-$**	-	-	-	-	-	-	-	-	●
		-								
L^3	-	-	-	-	-	-	-	-	-	●
L^3	**AcO$^-$**	-	-	-	-	-	√ **TBA$^+$AcO$^-$**	-	●	●
L^3	**Cl$^-$**	-	●	-	-	-	-	-	●	●
L^3	**HCO$_3^-$**	-	-	-	-	-	-	-	●	●
L^3	**NO$_3^-$**	-	-	-	-	-	-	-	-	●
		-								
L^4	-	-	-	-	-	-	-	-	-	●
L^4	**AcO$^-$**	√ **TBA$^+$AcO$^-$**	-	●	-	-	-	-	●	●
L^4	**Cl$^-$**	-	-	-	√ **[L^4(Cl)$_2$](TBA$^+$)$_2$ a**	-	-	-	√**[L^4(Cl)$_2$](TBA$^+$)$_2$ b**	●
L^4	**HCO$_3^-$**	-	-	●	-	●	-	●	●	●
L^4	**NO$_3^-$**	-	-	-	-	-	-	-	-	●
		-								
L^5	-	-	-	-	-	-	-	-	-	●

L^5	AcO^-	-	-	-	-	-	-	-	-	√ $[L^5(AcO^-)](TBA$
L^5	Cl^-	-	-	-	-	-	-	-	-	√ $[L^5(Cl^-)](TBA^+$
L^5	HCO_3^-	-	-	-	-	-	-	-	-	●
L^5	NO_3^-	-	-	-	-	-	-	-	-	●
		-								
L^6	-	-	-	-	-	-	-	-	-	●
L^6	AcO^-	-	-	-	-	-	-	-	-	●
L^6	Cl^-	-	-							●
L^6	HCO_3^-	-	-	-	-	-	-	-	-	●
L^6	NO_3^-	-	-	-	-	-	-	-	-	●
L^7	-	-	-	-	-	-	-	-	-	●
L^7	AcO^-	√ TBA^+AcO^-	-	●	-	-	-	-	-	●
L^7	Cl^-	-	-	●						●
L^7	HCO_3^-	-	-	-	-	-	-	●	●	●
L^7	NO_3^-	-	-	-	-	-	-	-	-	●
L^8	-	-	-	-	-	-	-	-	-	●
L^8	AcO^-	-	-							●
L^8	Cl^-	-	-	-	-	-	-	-	-	●
L^8	HCO_3^-	-	-	-	-	-	-	-	-	●
L^8	NO_3^-	-	-	-	-	-	-	-	-	●
L^9	-	-	-	-	-	-	-	-	-	●
L^9	AcO^-	-	-							●
L^9	Cl^-	-	-	-	-	-	-	-	-	●
L^9	HCO_3^-	-	-	-	-	-	-	-	-	●
L^9	NO_3^-	-	-	-	-	-	-	-	-	●

3. Single Crystal X-Ray diffractions

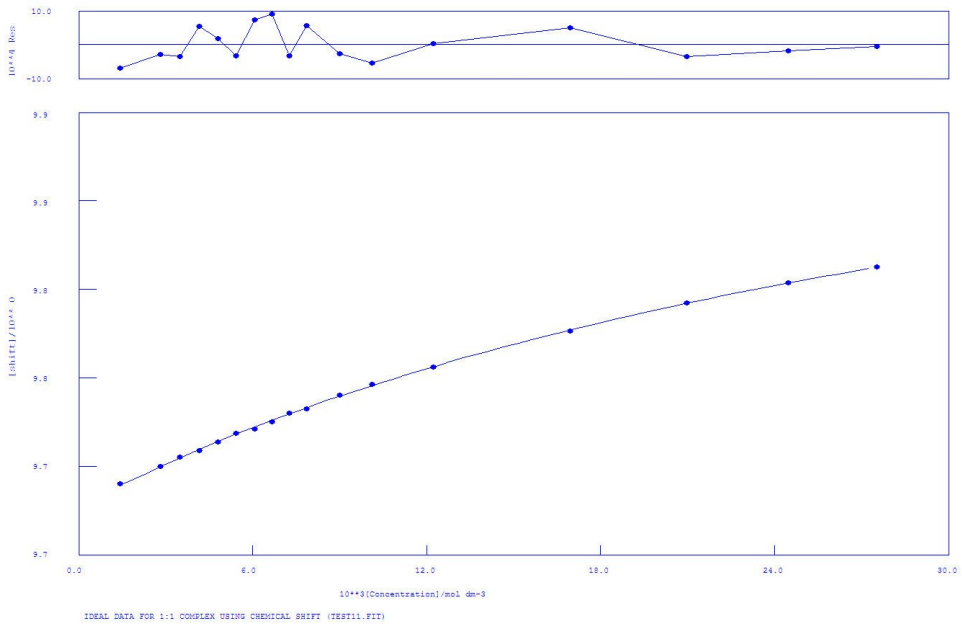
	$[L^4(Cl^-)_2](TBA^+)_2$	$[L^5(Cl^-)](TBA^+)$	$[L^5(AcO^-)](TBA^+)$
Empirical formula	$C_{61}H_{94}Cl_2F_6N_8O_3$	$C_{47}H_{56}Cl_1F_{12}N_7O_3$	$C_{49}H_{59}F_{12}N_7O_5$
Formula weight	1172.34	1030.44	1054.03
Crystal system	triclinic	triclinic	triclinic
Space group	*P*–1	*P*–1	*P*–1
a /Å	11.210(3)	12.919(5)	14.0834(10)
b /Å	11.448(3)	13.561(6)	14.4242(10)
c /Å	25.187(8)	14.982(6)	15.5069(11)
α / º	96.774(4)°	107.158(9)°	110.124(3)°
β / º	93.788(5)°	93.703(8)°	90.770(3)°
γ / º	96.805(5)°	90.818(6)°	114.879(3)°
V /Å^3	3176.6(16)	2501.2(18)	2636.6(3)
T / K	100(2)	150	100(2)
Crystal shape	Plate	Chunk	Plate
Crystal size / m^3	0.07 × 0.07 × 0.01 mm^3	0.06 × 0.05 × 0.04 mm^3	0.136 × 0.099 × 0.032 mm^3
Colour	colourless	colourless	colourless
Z	2	2	2
***θ* range for data collection**	2.075 – 27.514°	2.191 – 25.028°	2.453 – 27.543°
Index ranges	$-14 \le h \le 14$, $-14 \le k \le 14$,	$-15 \le h \le 15$, $-16 \le k \le 16$,	$-17 \le h \le 18$, $-18 \le k \le 18$,

	$-32 \le l \le 32$	$-15 \le l \le 17$	$-20 \le l \le 19$
Reflections collected	42312	27362	34533
Independent reflections	14541 [R_{int} = 0.1136]	8776 [R_{int} = 0.0962]	12056 [R_{int} = 0.0406]
Completeness	99.8 % (θ = 25.242°)	97.0 % (θ = 25.242°)	99.7 % (θ = 25.242°)
Absorption correction	Semi–empirical from equivalents	Semi–empirical from equivalents	Semi–empirical from equivalents
Max. and min. transmission	1.000 and 0.420	1.000 and 0.485	1.000 and 0.792
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	14541 / 80 / 767	8776 / 42 / 709	12056 / 0 / 687
Goodness-of-fit on F^2	1.005	1.009	1.030
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0779, $wR2$ = 0.1987	$R1$ = 0.0874, $wR2$ = 0.2085	$R1$ = 0.0447, $wR2$ = 0.1082
R indices (all data)	$R1$ = 0.1334, $wR2$ = 0.2332	$R1$ = 0.1510, $wR2$ = 0.2528	$R1$ = 0.0653, $wR2$ = 0.1174
Largest diff. peak and hole	0.733 and –0.337 e Å^{-3}	0.561 and –0.309 e Å^{-3}	0.374 and –0.289 e Å^{-3}

Table S3. Main intermolecular interactions [Å and °]..

Phase	D–H···A	d(D–H)	d(H···A)	d(D···A)	∠(DHA)	Symmetry
$[L^4(Cl^-)_2](TBA^+)_2$	N1—H1···Cl2	0.885(18)	2.36(2)	3.224(3)	165(3)	
	N2—H2···Cl1	0.878(18)	2.49(2)	3.313(3)	157(3)	
	N3—H3···Cl1	0.872(18)	2.48(3)	3.233(3)	146(3)	
	N4—H4···Cl2	0.872(18)	2.34(2)	3.183(3)	162(3)	
	N5—H5···Cl1	0.866(18)	2.51(2)	3.313(3)	154(3)	
	N6—H6···Cl1	0.862(18)	2.41(2)	3.223(3)	158(3)	-
$[L^5(Cl^-)](TBA^+)$	C10–H10···O2	0.95	2.28	2.844(6)	117.0	
	N1–H1···Cl1^{i}	0.875(19)	2.45(3)	3.265(4)	156(4)	–x+1,–y+1,–z+2
	N2–H2···Cl1	0.89(2)	2.75(3)	3.500(4)	144(4)	
	N3–H3A···Cl1	0.877(19)	2.33(2)	3.207(4)	175(4)	-
	N4–H4A···Cl1^{i}	0.867(19)	2.74(3)	3.518(4)	151(4)	–x+1,–y+1,–z+2
	N5–H5A···N4	0.89(2)	2.37(5)	2.757(5)	106(3)	-
	N5–H5A···Cl1	0.89(2)	2.56(3)	3.397(5)	158(4)	-
	N6–H6A···Cl1	0.879(19)	2.46(3)	3.250(4)	150(4)	
$[L^5(AcO^-)](TBA^+)$	N2–H2···N1	0.835(18)	2.342(18)	2.7476(18)	110.5(14)	-
	N2–H2···O61	0.835(18)	2.337(18)	3.0830(17)	149.0(16)	
	N3–H3A···O61	0.897(19)	1.887(19)	2.7791(17)	172.3(17)	
	N5–H5A···O62	0.874(19)	2.30(2)	3.0832(17)	149.6(16)	-
	N6–H6A···O62	0.837(18)	1.935(18)	2.7612(17)	168.5(17)	-
	N1–H1···O62	0.85(2)	1.99(2)	2.832(2)	173(2)	-
	N4–H4A···O61	0.87(2)	1.97(2)	2.827(2)	167.8(18)	-x, -y, 1-z

4. Proton NMR titration fitting

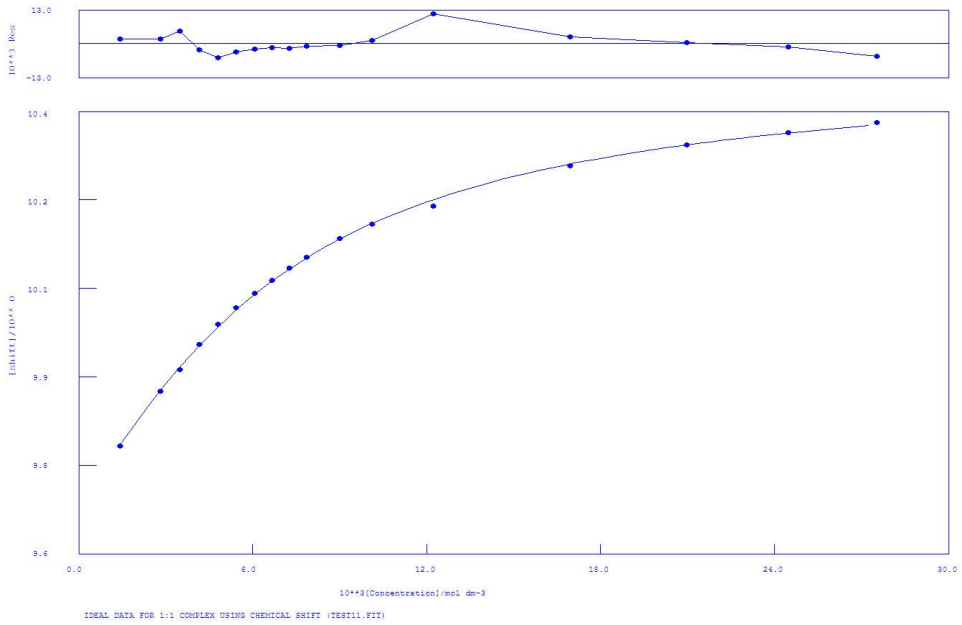


Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 16:58:18 on 02/17/2015

IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	3.17022E+01	2.000E-01	6.873E-01	3.531E+01	K1
2	1	9.72211E+00	2.000E-01	3.455E-04	5.035E+00	SHIFT M
3	1	9.96243E+00	1.000E+00	2.539E-03	2.321E+01	SHIFT ML

Figure S1. ^{1}H-NMR of **L^1** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 16:53:04 on 02/17/2015

IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
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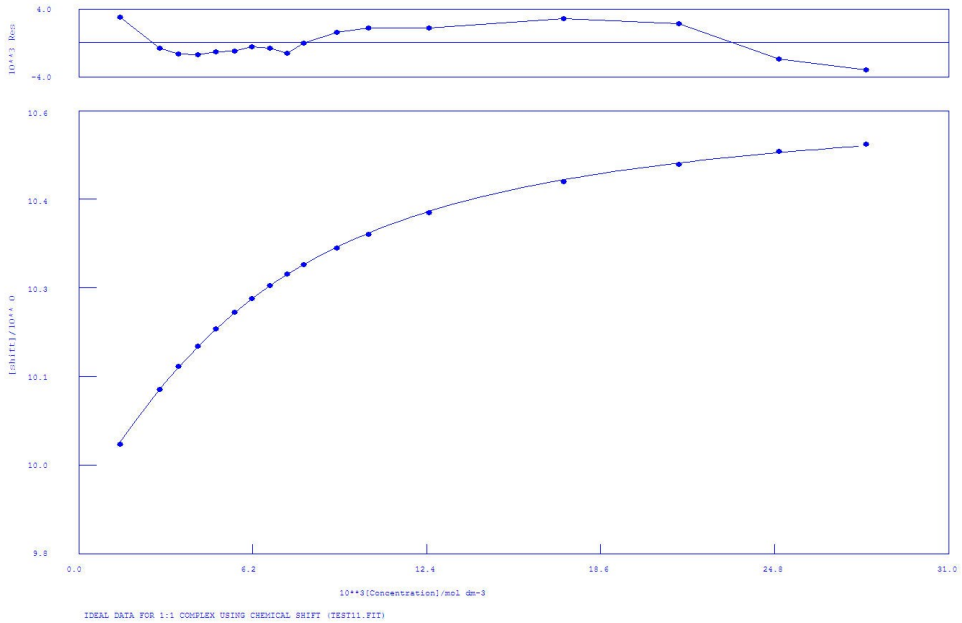
3	1	1.04912E+01	1.000E+00	6.073E-03	1.177E+01	SHIFT ML

Figure S2. ^{1}H-NMR of **L^2** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 16:41:10 on 02/17/2015

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Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
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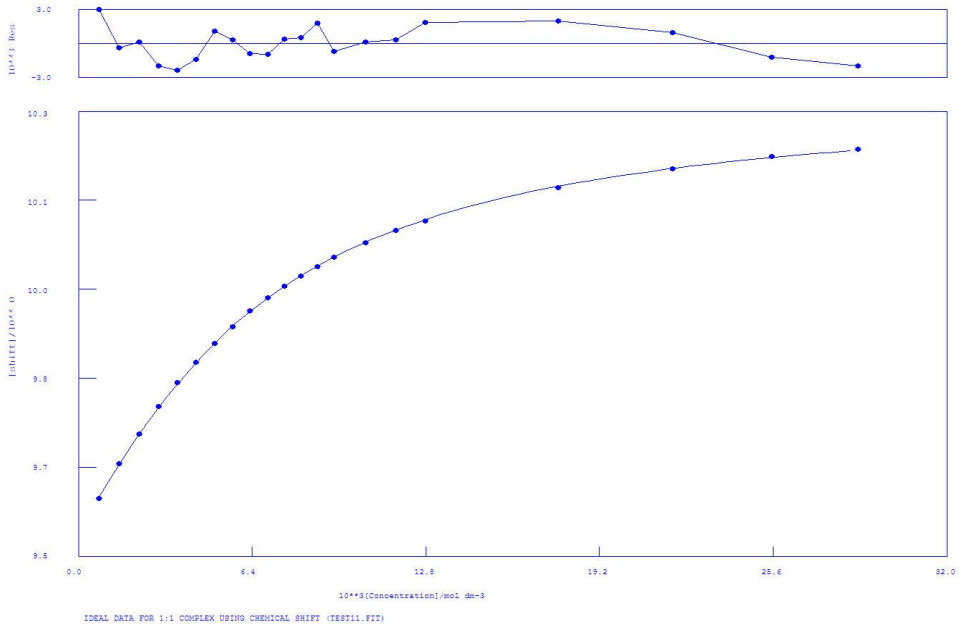
2	1	9.88391E+00	2.000E-01	2.225E-03	5.221E+00	SHIFT M
3	1	1.06373E+01	1.000E+00	2.620E-03	1.096E+01	SHIFT ML

Figure S3. ^{1}H-NMR of **L^{3}** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 17:48:04 on 01/09/2014

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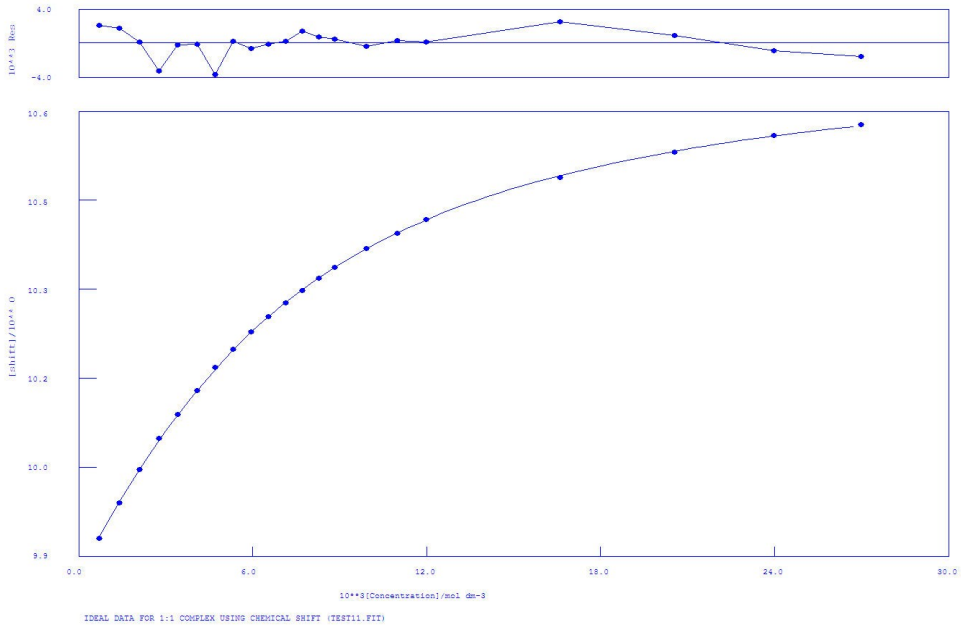
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.61563E+02	2.000E-01	2.742E+00	1.681E+01	K1
2	1	9.54024E+00	2.000E-01	1.182E-03	3.397E+00	SHIFT M
3	1	1.03369E+01	1.000E+00	1.915E-03	1.098E+01	SHIFT ML

Figure S4. ^{1}H-NMR of **L^{4}** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 19:42:53 on 02/06/2014

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Reaction: M + L = ML
FILE: TEST11.FIT
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File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
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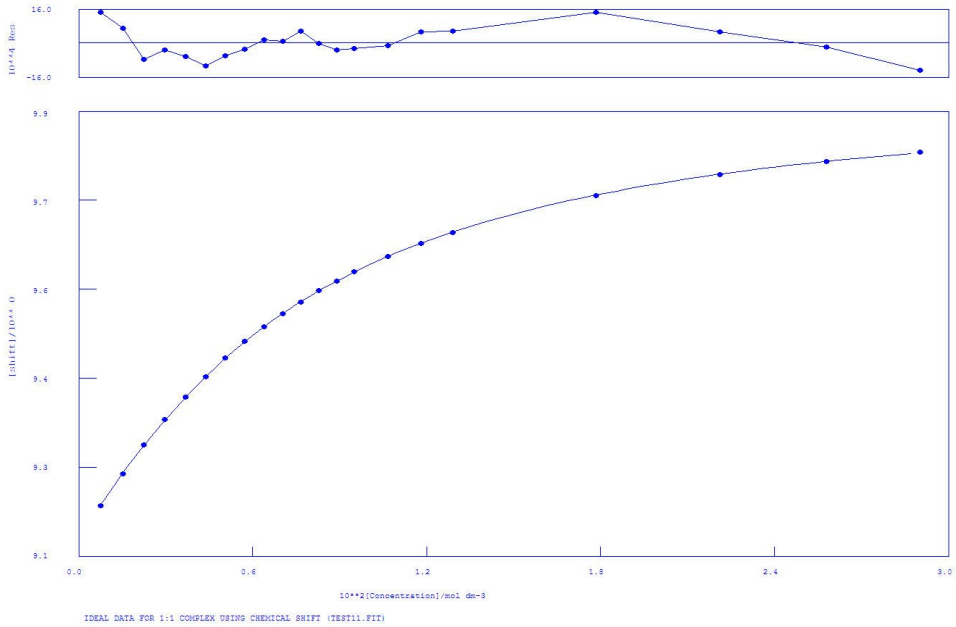
3	1	1.07138E+01	1.000E+00	1.704E-03	6.852E+00	SHIFT ML

Figure S5. ^{1}H-NMR of **L^{5}** with $TEAHCO_3$ in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
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Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.04939E+02	2.000E-01	9.658E-01	1.576E+01	K1
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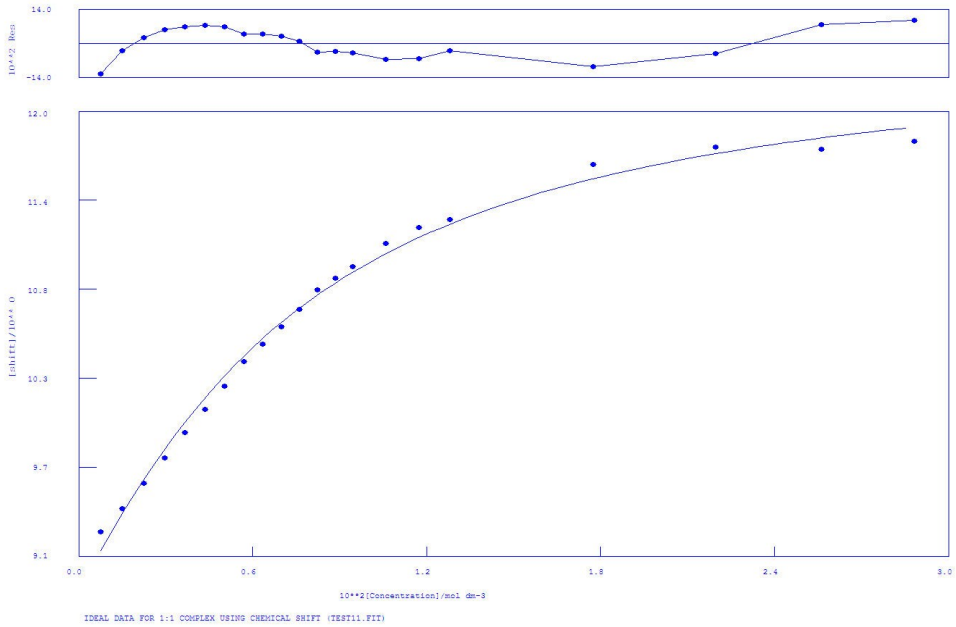
3	1	9.96097E+00	1.000E+00	1.013E-03	1.091E+01	SHIFT ML

Figure S6. ^{1}H-NMR of **L^{6}** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
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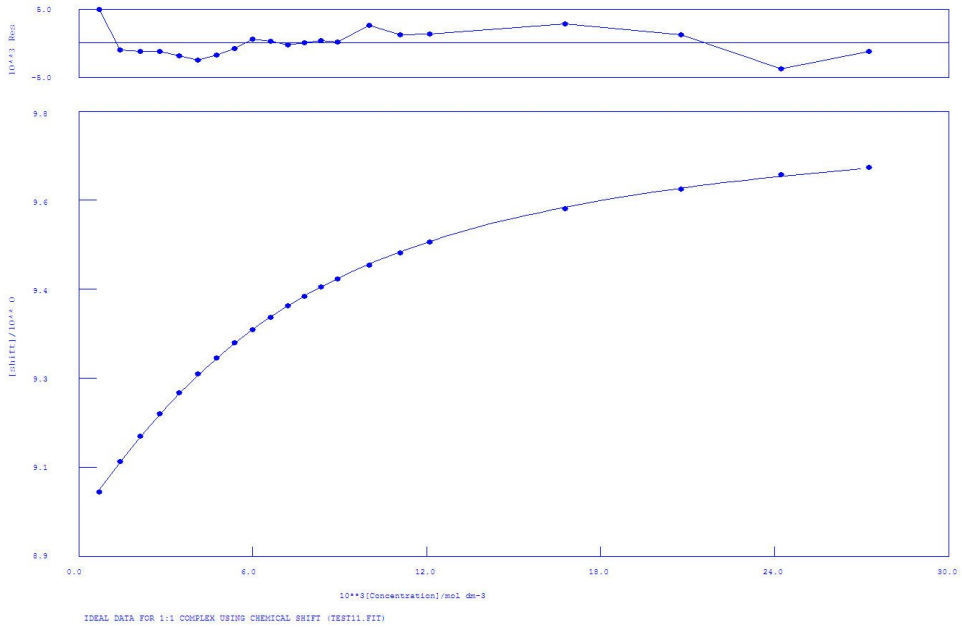
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.02716E+02	2.000E-01	2.129E+01	2.067E+01	K1
2	1	8.86368E+00	2.000E-01	5.171E-02	3.945E+00	SHIFT M
3	1	1.25001E+01	1.000E+00	9.678E-02	1.301E+01	SHIFT ML

Figure S7. ^{1}H-NMR of **L^{6}** with $TEAHCO_3$ in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.

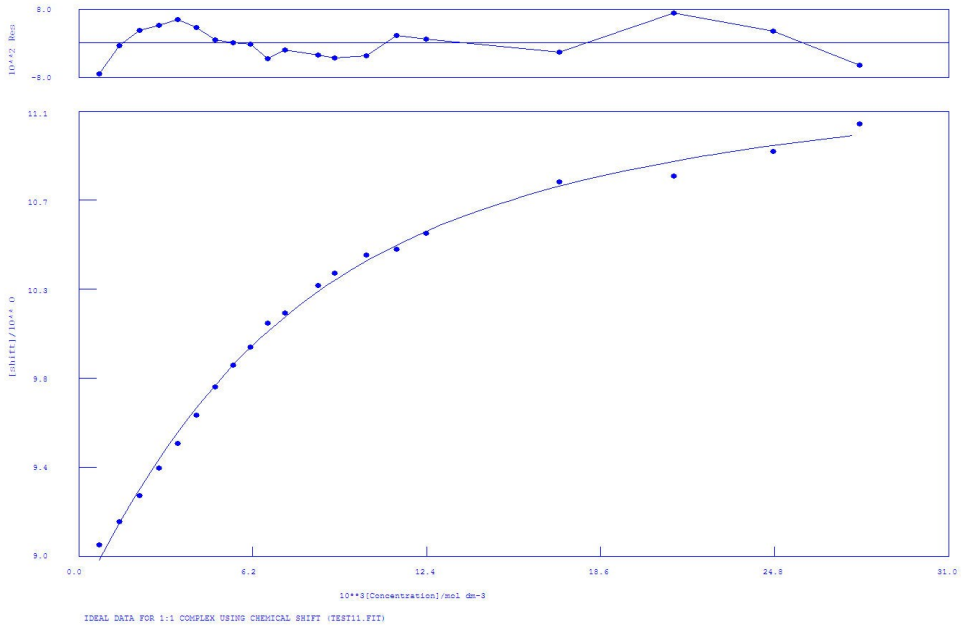


Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 16:56:34 on 01/09/2014

IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.26371E+02	2.000E-01	3.075E+00	1.713E+01	K1
2	1	8.97249E+00	2.000E-01	1.541E-03	3.524E+00	SHIFT M
3	1	9.82067E+00	1.000E+00	2.807E-03	1.107E+01	SHIFT ML

Figure S8. ^{1}H-NMR of **L^{7}** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.

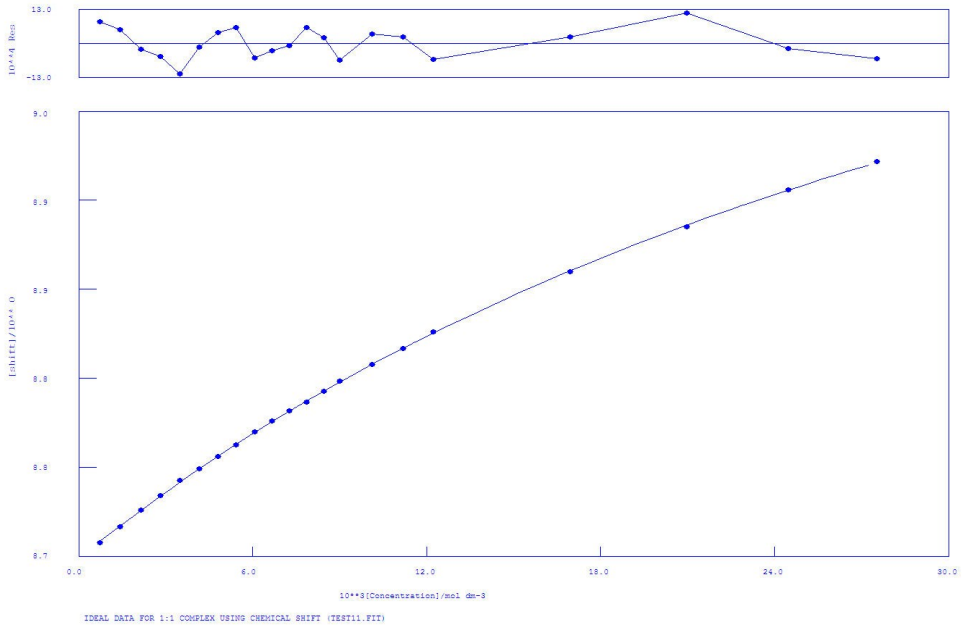


Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 17:04:09 on 01/09/2014

IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.20888E+02	2.000E-01	1.984E+01	1.954E+01	K1
2	1	8.78946E+00	2.000E-01	3.013E-02	3.647E+00	SHIFT M
3	1	1.14096E+01	1.000E+00	5.761E-02	1.260E+01	SHIFT ML

Figure S9. ^{1}H-NMR of **L^{7}** with $TEAHCO_3$ in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.

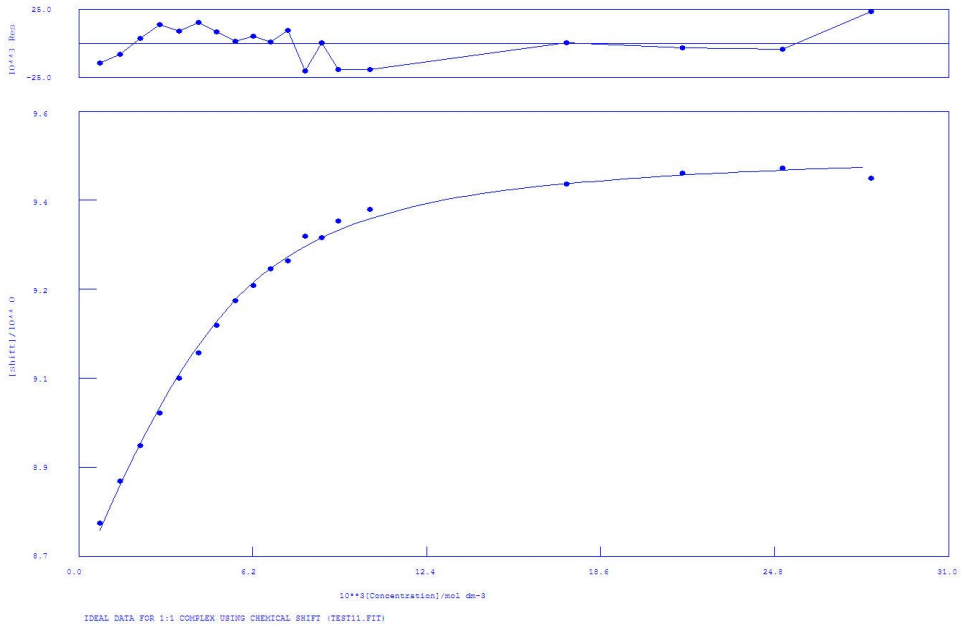


Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 17:10:32 on 01/09/2014

IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.80113E+01	2.000E-01	5.287E-01	1.527E+02	K1
2	1	8.69857E+00	2.000E-01	3.825E-04	5.741E+00	SHIFT M
3	1	9.33840E+00	1.000E+00	7.126E-03	1.205E+02	SHIFT ML

Figure S10. ^{1}H-NMR of **L^{8}** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 17:21:46 on 01/09/2014

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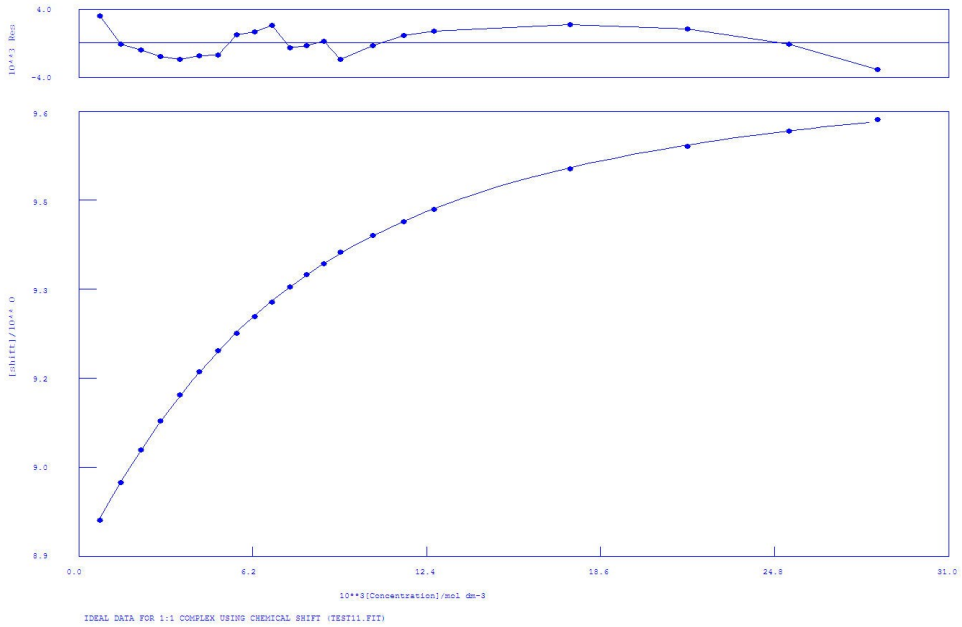
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	8.01729E+02	2.000E-01	6.472E+01	7.721E+00	K1
2	1	8.65338E+00	2.000E-01	9.999E-03	2.041E+00	SHIFT M
3	1	9.53084E+00	1.000E+00	1.042E-02	5.862E+00	SHIFT ML

Figure S11. ^{1}H-NMR of **L^{8}** with $TEAHCO_3$ in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.

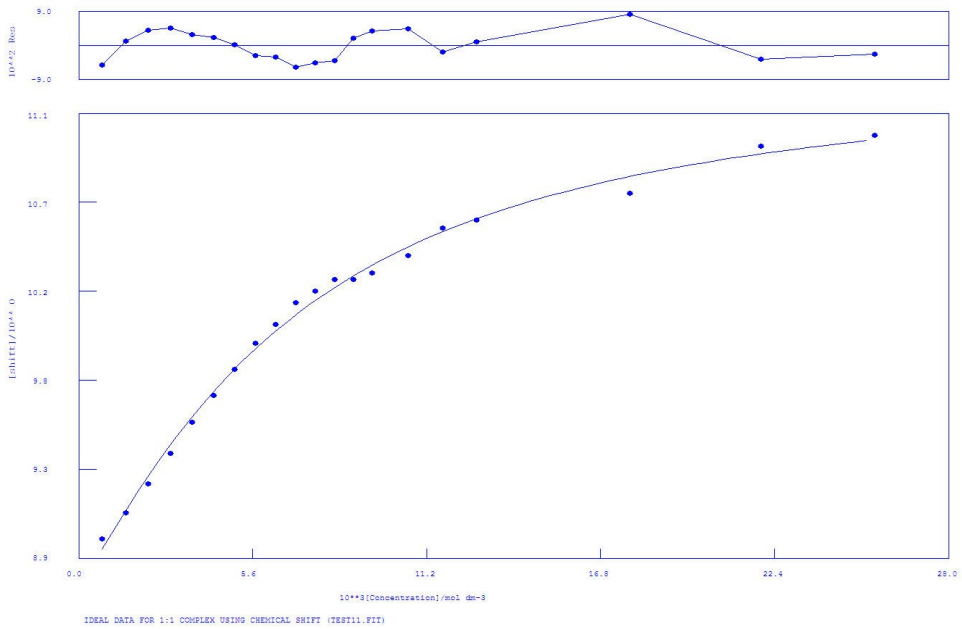


Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 17:37:12 on 01/09/2014

IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.25143E+02	2.000E-01	2.507E+00	1.560E+01	K1
2	1	8.89882E+00	2.000E-01	1.286E-03	3.501E+00	SHIFT M
3	1	9.70864E+00	1.000E+00	2.144E-03	9.930E+00	SHIFT ML

Figure S12. ^{1}H-NMR of **L^{9}** with TBACl in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.



Calculations by WinEQNMR Version 1.20 by Michael J. Hynes
Program run at 17:42:08 on 01/09/2014

IDEAL DATA FOR 1:1 COMPLEX USING CHEMICAL SHIFT (TEST11.FIT)
Reaction: M + L = ML
FILE: TEST11.FIT
IDEAL DATA: K1 = 63.091; DELTA M = 20.0; DELTA ML = 120.0
File prepared by M. J. Hynes, October 22 2000

NO.	A	PARAMETER	DELTA	ERROR	CONDITION	DESCRIPTION
1	1	2.39579E+02	2.000E-01	2.332E+01	2.334E+01	K1
2	1	8.73131E+00	2.000E-01	3.287E-02	3.760E+00	SHIFT M
3	1	1.14046E+01	1.000E+00	6.816E-02	1.561E+01	SHIFT ML

Figure S13. ^{1}H-NMR of **L^{9}** with $TEAHCO_3$ in DMSO-d_6/0.5%H_2O. The fitting has been obtained following the most downfield shifted NH proton.

5. Anion transport studies

Preparation of Vesicles

A lipid film of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesterol (0% or 30%) was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 2 hours. The lipid film was rehydrated by vortexing with an internal solution (489 mM NaCl, 5 mM phosphate buffer at pH 7.2). The lipid suspension was then subjected to nine freeze-thaw cycles and allowed to age for 30 min at room temperature before extruding 20 times through a 200 nm polycarbonate membrane. The resulting unilamellar vesicles were dialyzed against the external solution to remove unencapsulated NaCl salts. The vesicles were diluted to 5mL with the external solution to form a stock solution of lipid.

Samples for assay were prepared by diluting lipid stock solution to 5mL (using the external solution) to give a solution of 1mM lipid. Chloride efflux was monitored using a chloride selective electrode (Accumet). To initiate the experiment compounds were added as solutions in DMSO, to give a 1:50 compound to lipid ratio (2mol%). At the end of the experiment detergent (octaethylene glycol monododecyl ether) was added to allow the determination of 100% chloride efflux. Experiments were repeated in triplicate and all traces presented are the average of three trials. The chloride electrode was calibrated against sodium chloride solutions of known concentration.

Chloride Transport Assays

Unilamellar POPC vesicles containing NaCl, prepared as described above, were suspended in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule (10 mM) 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 octaethylene glycol monododecyl ether and a total chloride reading was taken at 7 min.

Bicarbonate Transport Assay

Unilamellar POPC vesicles containing 451 mM NaCl solution buffered to pH 7.2 with 20 mM sodium phosphate salts, prepared as described above, were suspended in 150 mM Na_2SO_4 solution buffered to pH 7.2 with sodium phosphate salts. The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule (10 mM) was added to start the experiment and chloride efflux was monitored using a chloride sensitive electrode. At 2 min, $NaHCO_3$ solution (1 M in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts) was added so that the outer solution contained 40 mM $NaHCO_3$. At 7 min, the vesicles were lysed with 50 µl of octaethylene glycol monododecyl ether and a total chloride reading was taken at 9 min.

Lucigenin Assay for Chloride/Sulphate Exchange

POPC vesicles were prepared as described above containing a NaCl solution (2 mM lucigenin, 100 mM NaCl, 20 mM phosphate buffer at pH 7.2). The lipid suspension was then subjected to nine freeze-thaw cycles and allowed to age for 30 min at room temperature before extruding 25 times through a 200 nm polycarbonate membrane. The unincorporated lucigenin was removed by size exclusion chromatography on a Sephadex G-25 column using a sodium chloride solution as eluent (100 mM NaCl, 20 mM phosphate buffer at pH 7.2).

Unilamellar POPC vesicles containing NaCl and lucigenin were suspended in a NaCl solution buffered to pH 7.2 with 20 mM sodium phosphate salts. The lipid concentration per sample was 0.5 mM. The internal chloride concentration could be monitored by the fluoresence of intravesicular lucigenin after excitation at 372 nm and recording the emission at 503 nm using a Varian Cary Eclipse Fluorescence Spectrophotometer. At t = 30 s, a pulse of Na_2SO_4 was added such that the final external SO_4^- concentration was 40 mM. After 60 s, a DMSO solution of the carrier molecule was added to start ion transport. After 300 s the vesicles were lysed with 30 µl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v).

HPTS Assay for HCl Co-transport

POPC vesicles were prepared as described above containing a NaCl solution (1 mM HPTS (8-hydroxypyrene-1,3,6-trisulphonic acid), 489 mM NaCl, 5 mM phosphate buffer at pH 7.2). The lipid suspension was then subjected to nine freeze-thaw cycles and allowed to age for 30 min at room temperature before extruding 25 times through a 200 nm polycarbonate membrane. The unincorporated HPTS was removed by size exclusion chromatography on a Sephadex G-25 column using a sodium sulfate solution as eluent (162 mM Na_2SO_4, 5 mM phosphate buffer at pH 7.2).

Unilamellar POPC vesicles containing NaCl, were suspended in a Na_2SO_4 solution buffered to pH 7.2 with sodium phosphate salts. The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule (10 mM) was added to start the experiment. The fluoresence of intravesicular HPTS was monitored by excitation at both 403 nm and 460 nm and recording the emission at 510 nm using a Varian Cary Eclipse Fluorescence Spectrophotometer. After 240 s the vesicles were lysed with 30 µl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v). The internal pH was obtained by fitting the data to the following equation[1]:

$$pH = \frac{-1}{1.796}\ln\left(\frac{4.2055}{I_{460\,nm}/I_{403\,nm} - 1}\right) + 7.6142$$

Cl^-/NO_3^- antiport studies

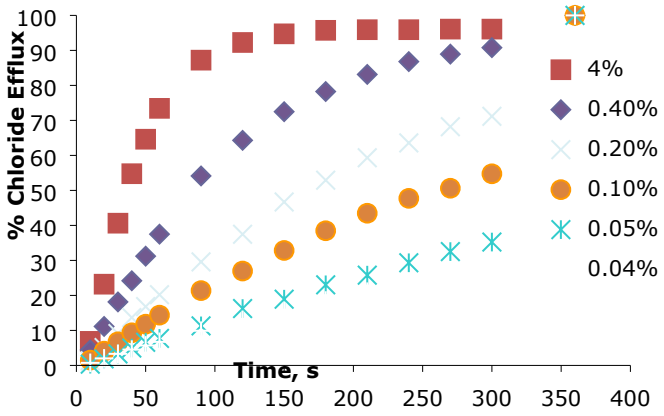


Figure S14. Chloride efflux promoted by a various concentration of compound **L²** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

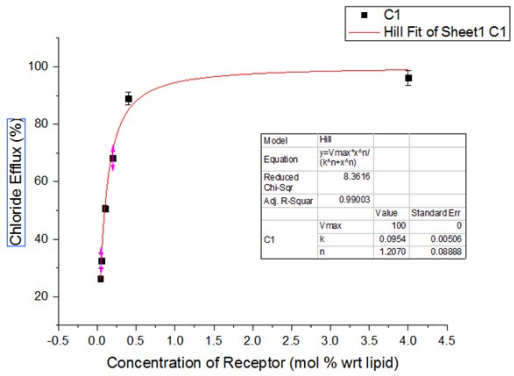


Figure S15. Hill plot of chloride efflux promoted by varying concentrations of compound **L²** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

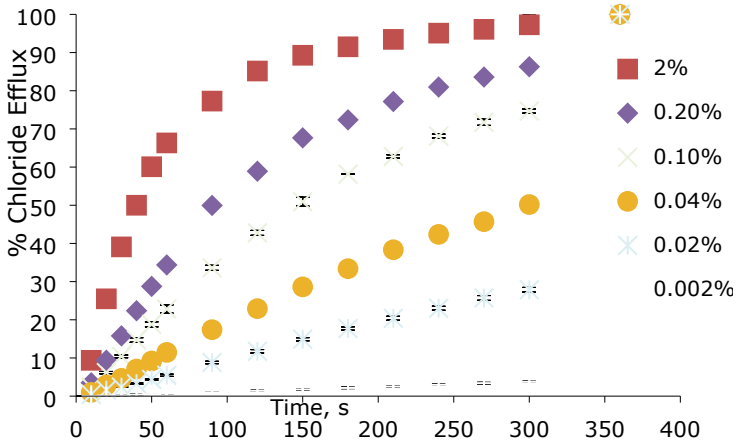


Figure S16. Chloride efflux promoted by a various concentration of compound **L³** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

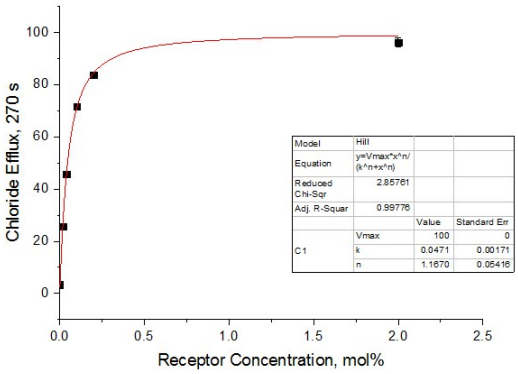


Figure S17. Hill plot of chloride efflux promoted by varying concentrations of compound **L³** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

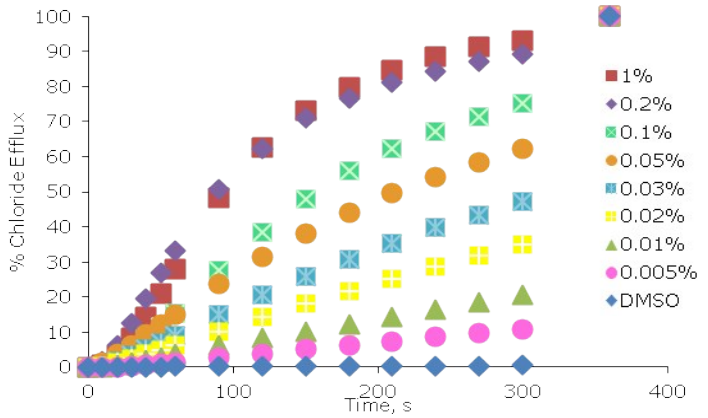


Figure S18. Chloride efflux promoted by a various concentration of compound **L^4** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

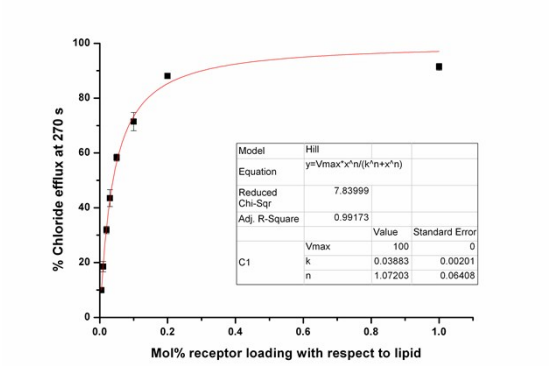


Figure S19. Hill plot of chloride efflux promoted by varying concentrations of compound **L^4** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

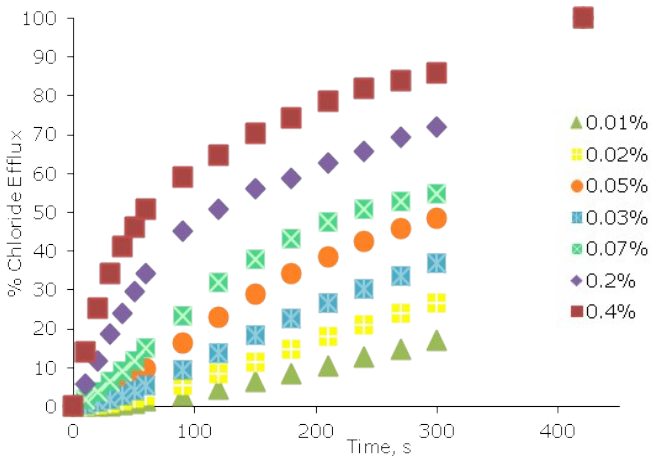


Figure S20. Chloride efflux promoted by a various concentration of compound **L**[5] from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

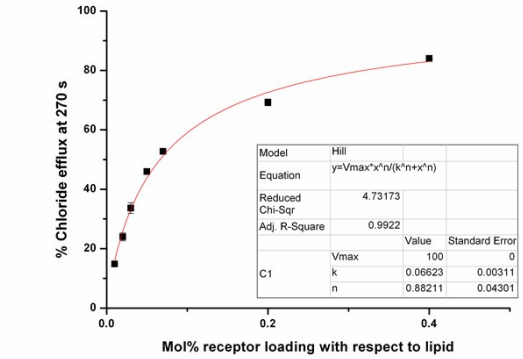


Figure S21. Hill plot of chloride efflux promoted by varying concentrations of compound **L**[5] from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

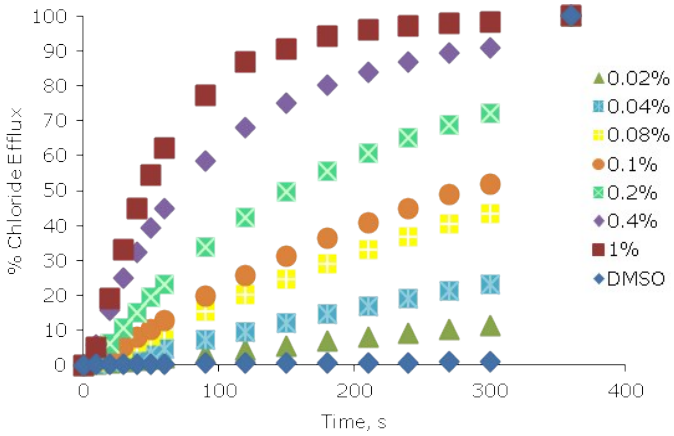


Figure S22. Chloride efflux promoted by a various concentration of compound **L**^{6} from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

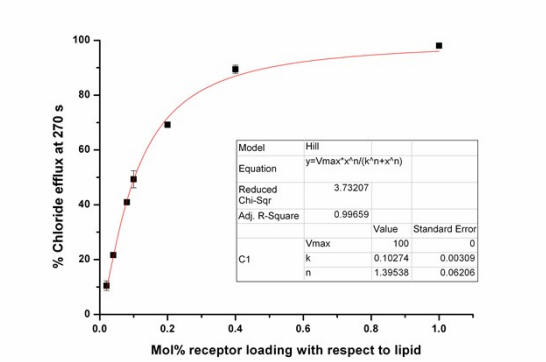


Figure S23. Hill plot of chloride efflux promoted by varying concentrations of compound **L**^{6} from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

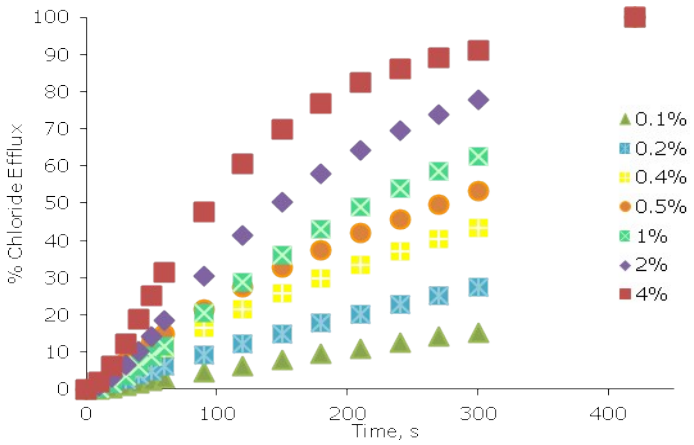


Figure S24. Chloride efflux promoted by a various concentration of compound **L**[7] from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

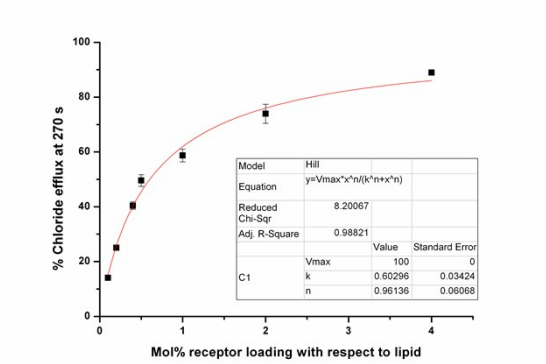


Figure S25. Hill plot of chloride efflux promoted by varying concentrations of compound **L**[7] from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

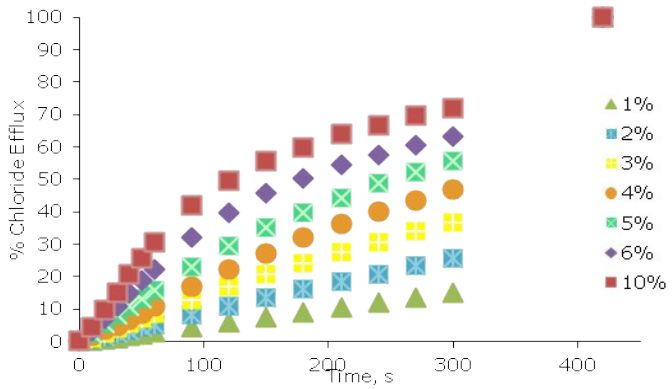


Figure S26. Chloride efflux promoted by a various concentration of compound **L[8]** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

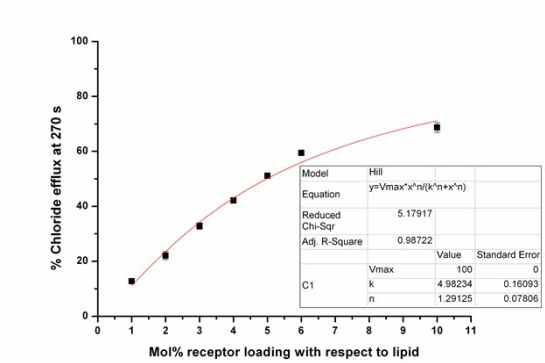


Figure S27. Hill plot of chloride efflux promoted by varying concentrations of compound **L[8]** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

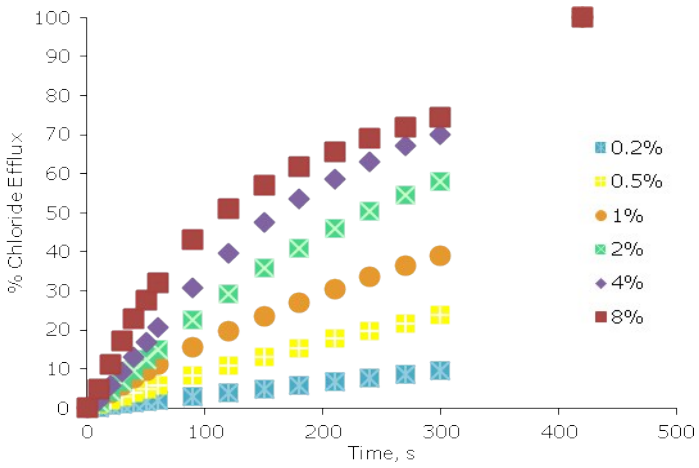


Figure S28. Chloride efflux promoted by a various concentration of compound **L[9]** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

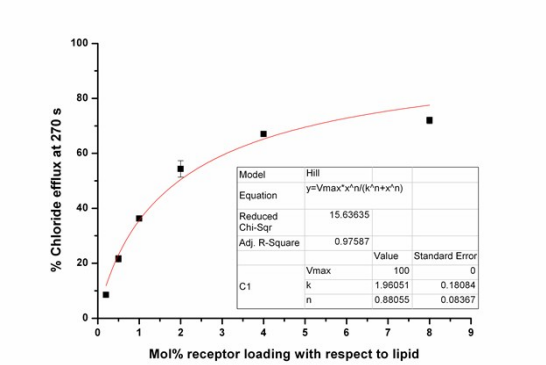


Figure S29. Hill plot of chloride efflux promoted by varying concentrations of compound **L[9]** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5mM sodium phosphate salts. Each point represents an average of 3 trials.

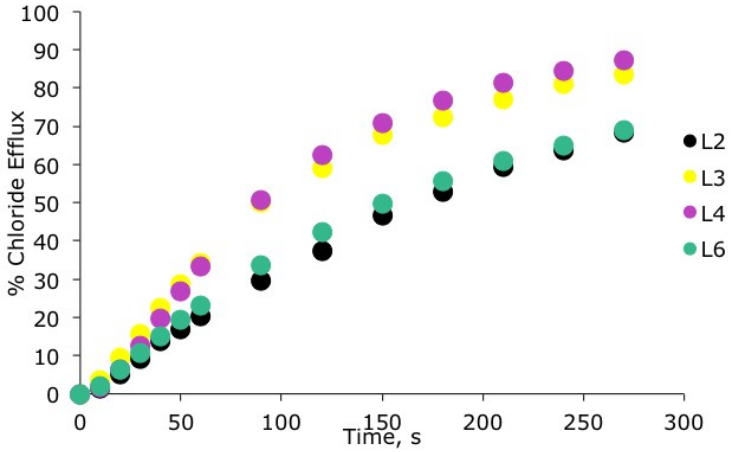


Figure S30. Comparison between the Cl^-/NO_3^- transport properties of **L2**, **L3**, **L4**, and **L6** (0.2 mol% carrier to lipid) from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

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Cl^-/HCO_3^- antiport studies

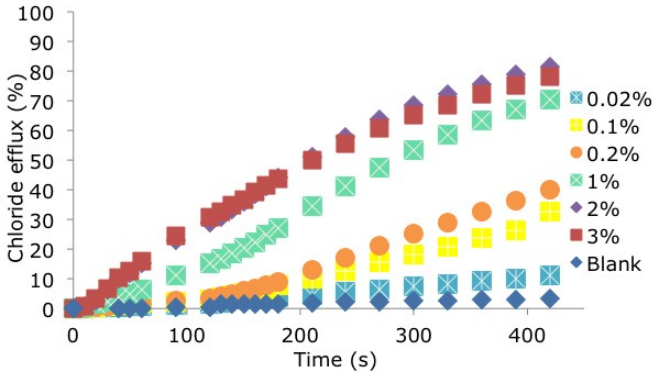

Figure S31. Chloride efflux promoted by various concentrations of **L^2** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

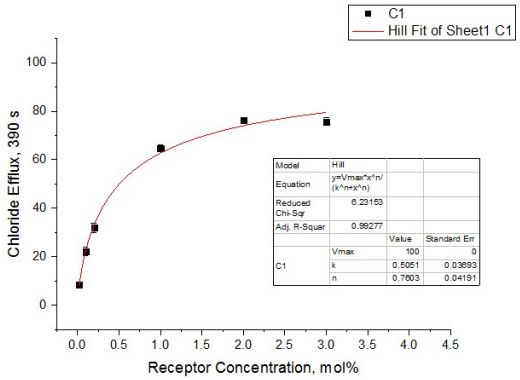

Figure S32. Hill plot of chloride efflux promoted varying concentrations of compound **L^2** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

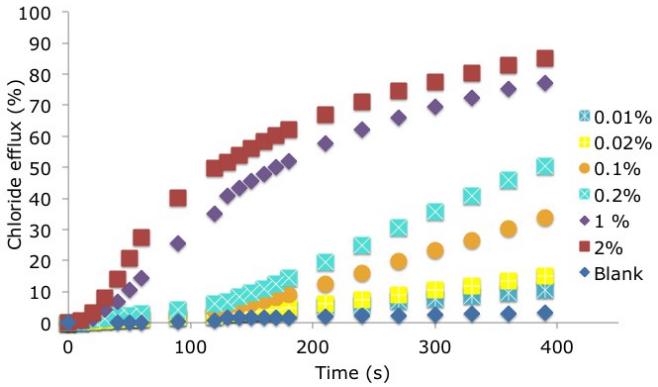

Figure S33. Chloride efflux promoted by various concentrations of **L³** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

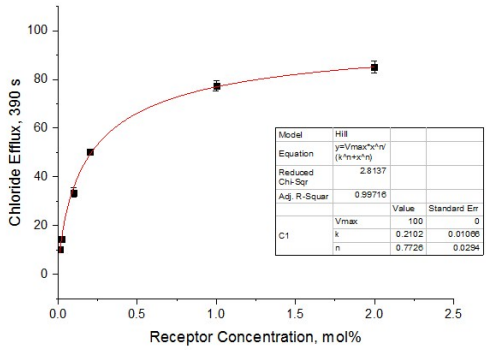

Figure S34. Hill plot of chloride efflux promoted varying concentrations of compound **L³** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

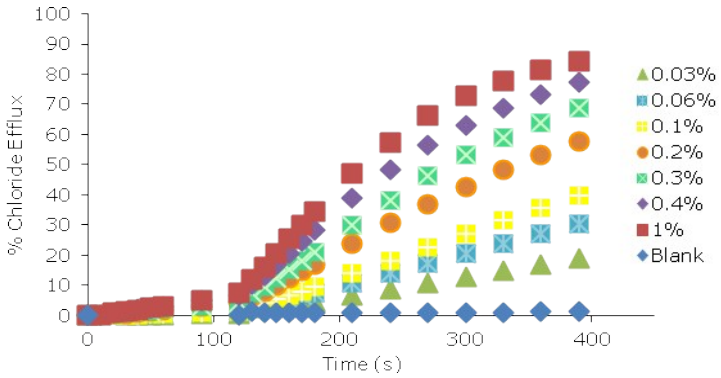

Figure S35. Chloride efflux promoted by various concentrations of **L[4]** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

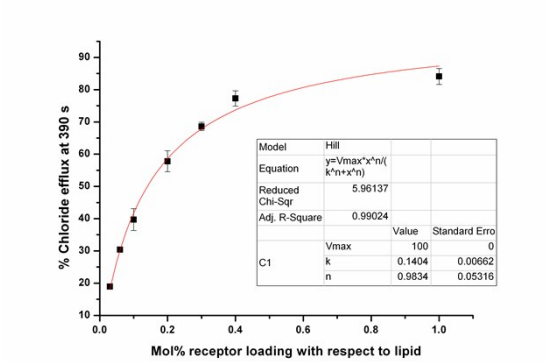

Figure S36. Hill plot of chloride efflux promoted varying concentrations of compound **L[4]** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

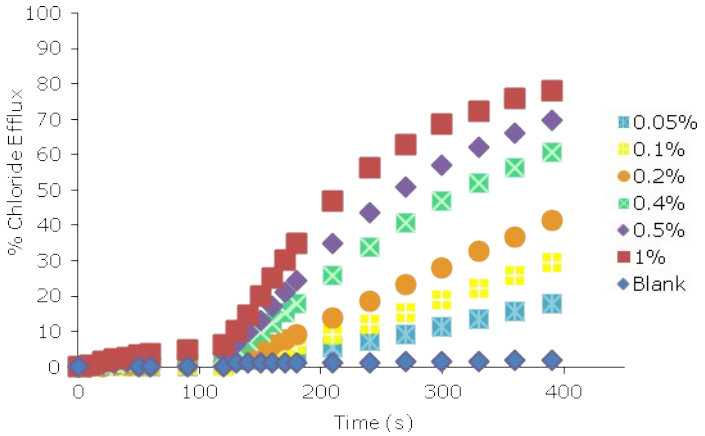

Figure S37. Chloride efflux promoted by various concentrations of **L[5]** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

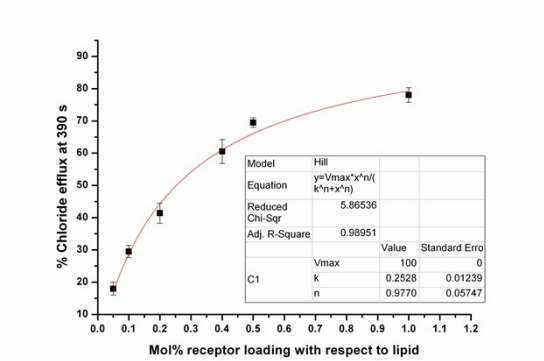

Figure S38. Hill plot of chloride efflux promoted varying concentrations of compound **L[5]** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

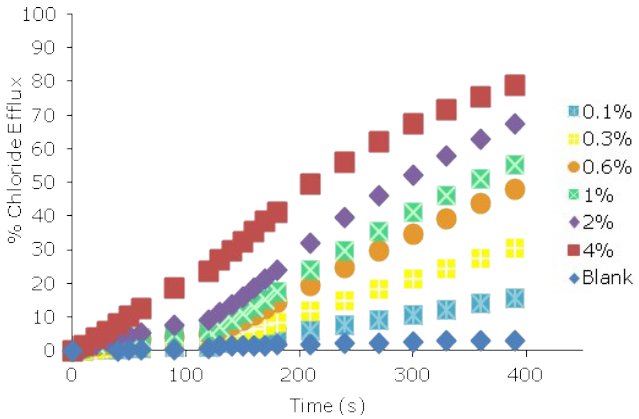

Figure S39. Chloride efflux promoted by various concentrations of **L⁶** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

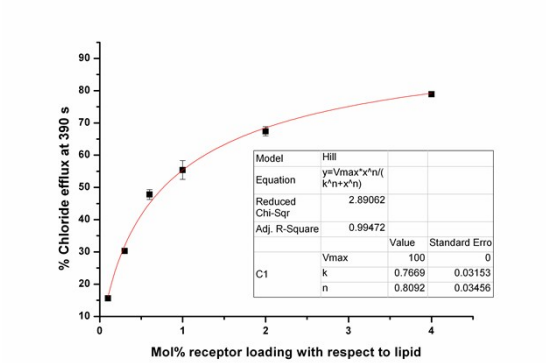

Figure S40. Hill plot of chloride efflux promoted varying concentrations of compound **L⁶** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

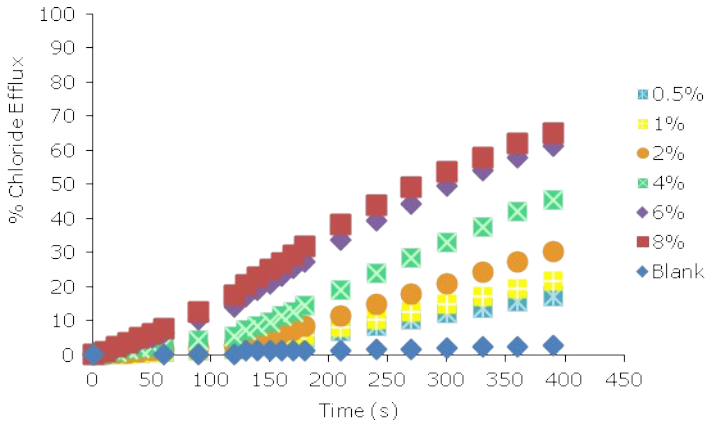

Figure S41. Chloride efflux promoted by various concentrations of **L[7]** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

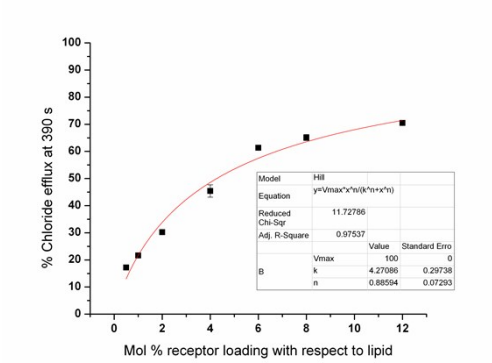

Figure S42. Hill plot of chloride efflux promoted varying concentrations of compound **L[7]** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

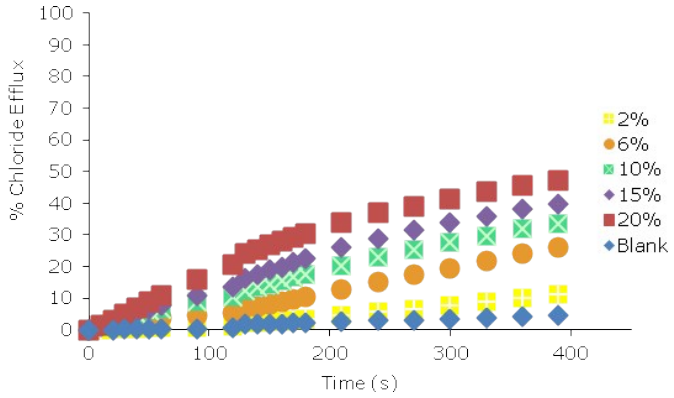

Figure S43. Chloride efflux promoted by various concentrations of **L[8]** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

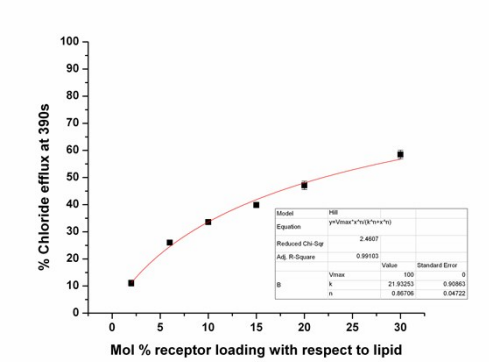

Figure S44. Hill plot of chloride efflux promoted varying concentrations of compound **L[8]** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

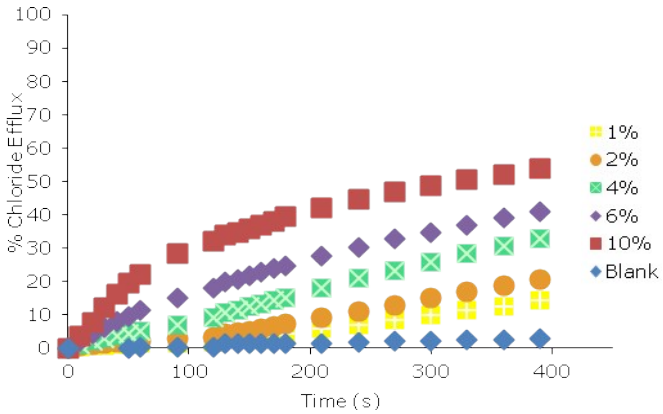

Figure S45. Chloride efflux promoted by various concentrations of **L[9]** from unilamellar POPC vesicles loaded with 451 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 150 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t. 120 s a solution of sodium bicarbonate was added such that the external concentration of bicarbonate was 40 mM. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

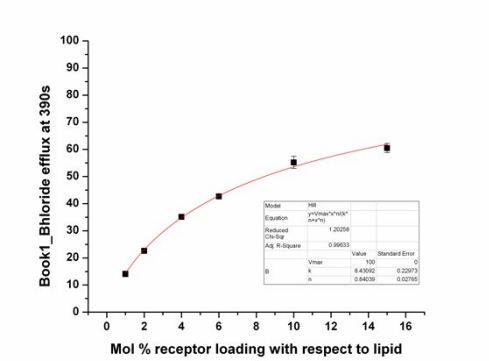

Figure S46. Hill plot of chloride efflux promoted varying concentrations of compound **L[9]** from unilamellar POPC vesicles loaded with 451mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts upon addition of a bicarbonate 'pulse', bringing the external concentration of bicarbonate to 40mM. The vesicles were dispersed in 150mM Na_2SO_4 buffered to pH 7.2 with 20mM sodium phosphate salts. Each point represents an average of 3 trials.

NaCl/CsCl symport studies

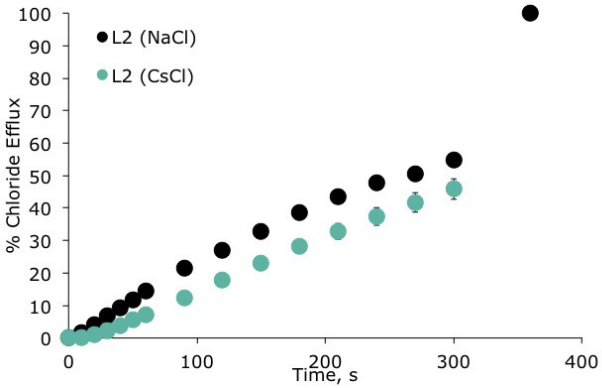

Figure S47. Chloride efflux promoted by a DMSO solution of compound **L²** (0.1 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl or 489mM CsCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

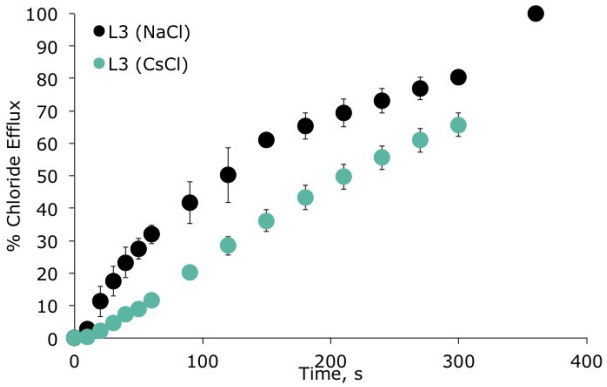

Figure S48. Chloride efflux promoted by a DMSO solution of compound **L²** (0.1 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl or 489mM CsCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

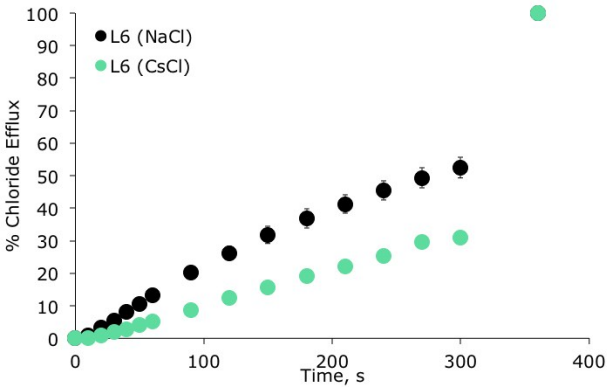

Figure S49. Chloride efflux promoted by a DMSO solution of compound **L⁶** (0.1 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl or 489mM CsCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the

experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

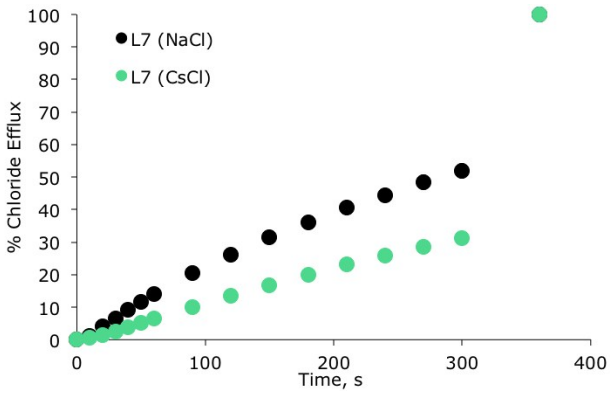

Figure S50. Chloride efflux promoted by a DMSO solution of compound **L**[7] (0.5 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl or 489mM CsCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

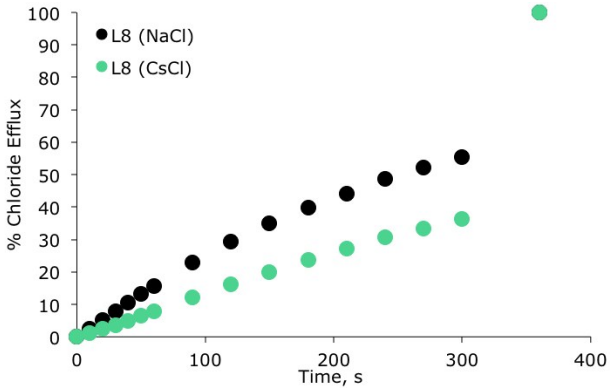

Figure S51. Chloride efflux promoted by a DMSO solution of compound **L**[8] (5 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl or 489mM CsCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

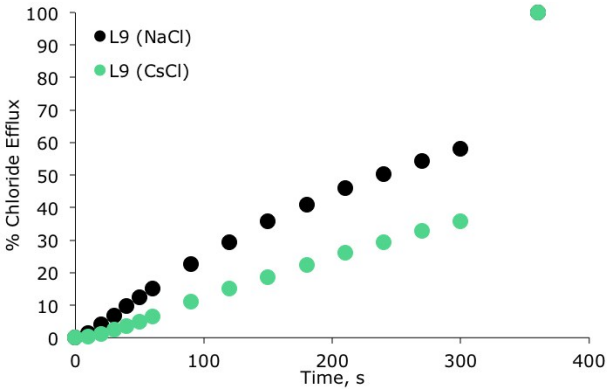

Figure S52. Chloride efflux promoted by a DMSO solution of compound **L**[9] (2 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl or 489mM CsCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

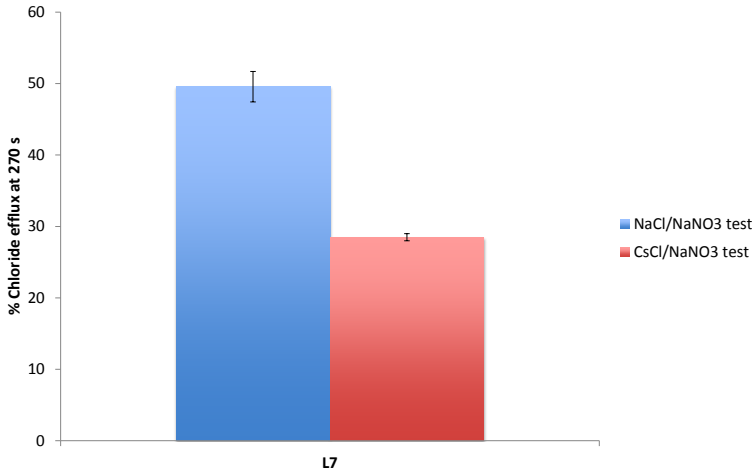

Figure S53 Percentage chloride efflux at 270 s mediated by **L[7]**, (0.5 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl (red) or 489mM CsCl (blue) buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts.

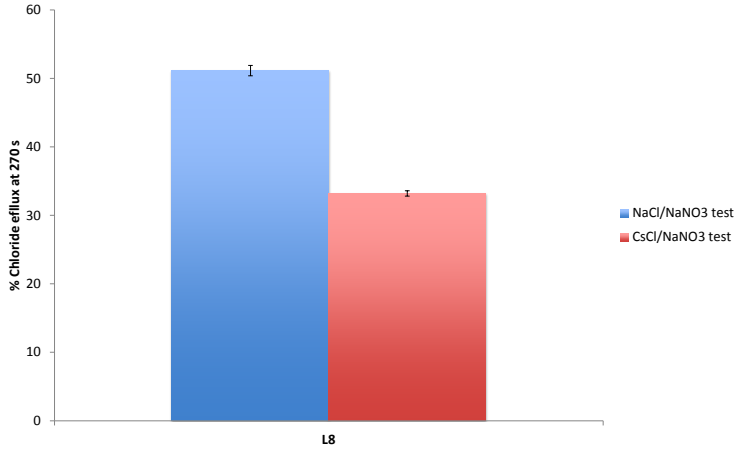

Figure S54 Percentage chloride efflux at 270 s mediated by **L[8]**, (5 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl (red) or 489mM CsCl (blue) buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts.

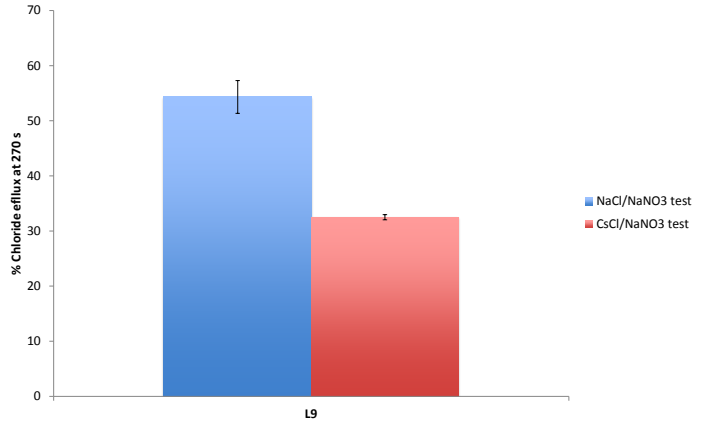

Figure S55 Percentage chloride efflux at 270 s mediated by **L[9]**, (2 mol% carrier to lipid) from unilamellar POPC vesicles loaded with either 489mM NaCl (red) or 489mM CsCl (blue) buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts.

Lucigenin Assay

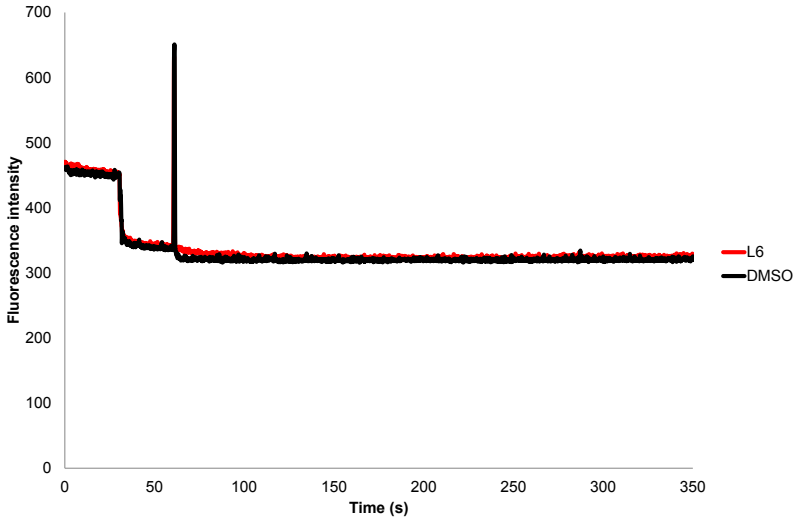

Figure S56. Lucigenin fluorescence intensity of unilamellar POPC vesicles loaded with 100 mM NaCl and 2mM lucigenin dye buffered to pH 7.2 with 20 mM sodium phosphate salts upon addition of compound **L⁶**. The vesicles were suspended in a solution containing 100 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. A sulphate pulse was added at t = 30 s such that the external concentration of sulphate was 40 mM. **L⁶** (2 mol%) were added as solutions in DMSO at t = 60 s. Experiments were repeated in triplicate and the traces represents an average of three trials.

HPTS Assay

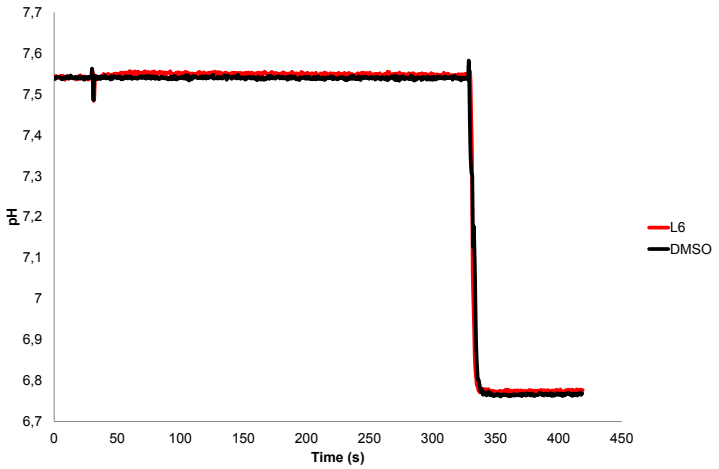

Figure S57 Change in the intravesicular pH promoted by 2 mol% of receptor **L⁶** from unilamellar POPC vesicles loaded with 489 mM NaCl and 1 mM HPTS buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in a solution containing 167 mM $NaSO_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end

of the experiment detergent was added to lyse the vesicles. Experiments were repeated in triplicate and the traces represents an average of three trials.

Cholesterol assays

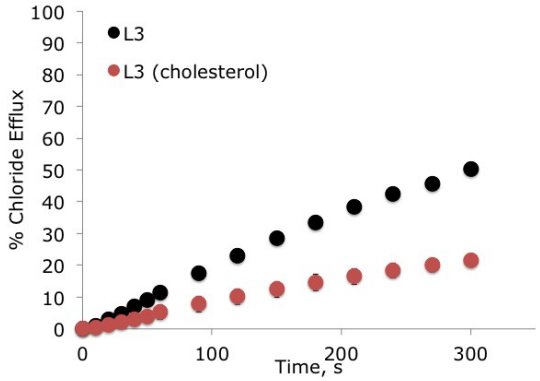

Figure S58. Chloride efflux promoted by a DMSO solution of compound **L[3]** (0.03 mol% carrier to lipid) from unilamellar vesicles comprising of either POPC or POPC/cholesterol (7:3 molar ratio), loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

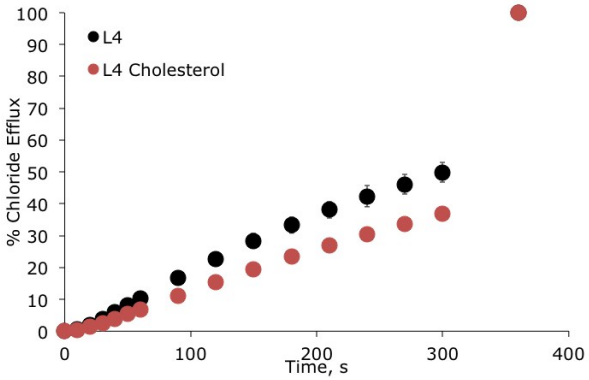

Figure S59. Chloride efflux promoted by a DMSO solution of compound **L[4]** (0.03 mol% carrier to lipid) from unilamellar vesicles comprising of either POPC or POPC/cholesterol (7:3 molar ratio), loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

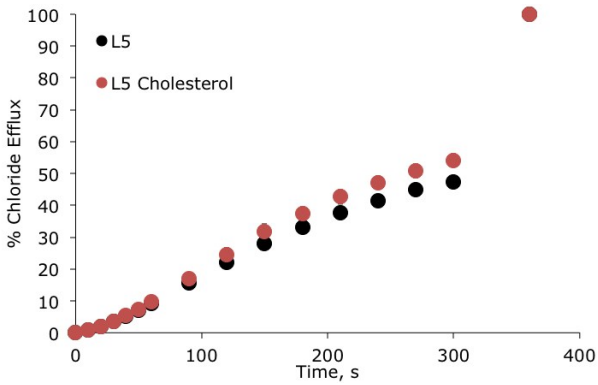

Figure S60. Chloride efflux promoted by a DMSO solution of compound **L[5]** (0.05 mol% carrier to lipid) from unilamellar

vesicles comprising of either POPC or POPC/cholesterol (7:3 molar ratio), loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

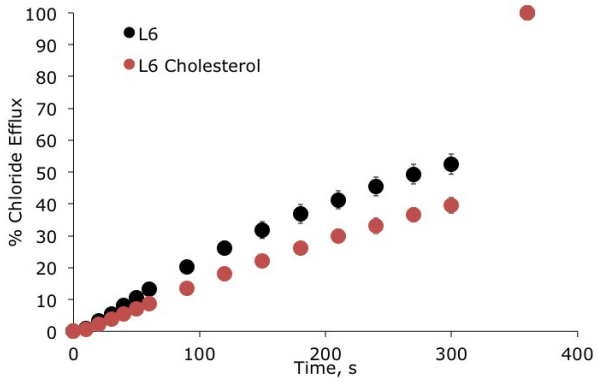

Figure S61. Chloride efflux promoted by a DMSO solution of compound **L[6]** (0.1 mol% carrier to lipid) from unilamellar vesicles comprising of either POPC or POPC/cholesterol (7:3 molar ratio), loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

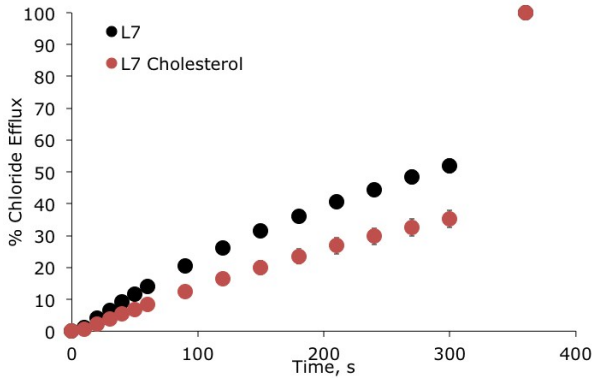

Figure S62. Chloride efflux promoted by a DMSO solution of compound **L[7]** (0.5 mol% carrier to lipid) from unilamellar vesicles comprising of either POPC or POPC/cholesterol (7:3 molar ratio), loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

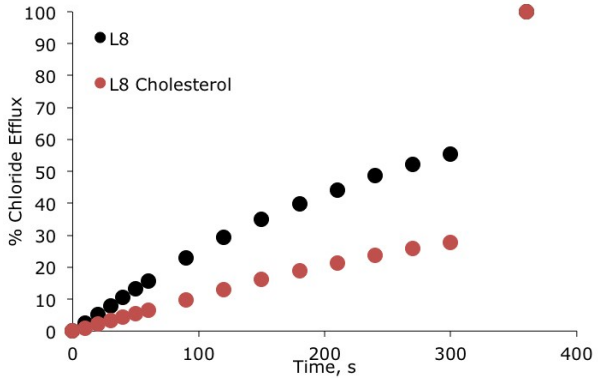

Figure S63. Chloride efflux promoted by a DMSO solution of compound **L[8]** (5 mol% carrier to lipid) from unilamellar vesicles comprising of either POPC or POPC/cholesterol (7:3 molar ratio), loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM

sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

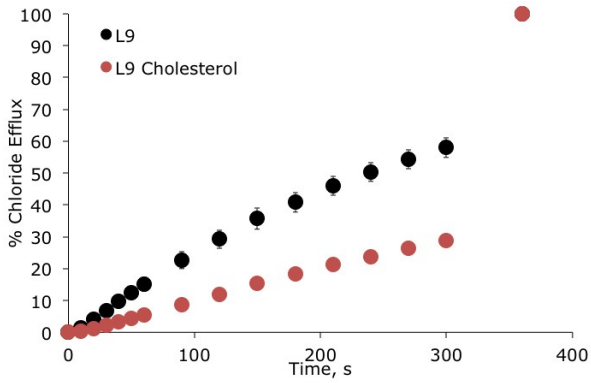

Figure S64. Chloride efflux promoted by a DMSO solution of compound **L[9]** (2 mol% carrier to lipid) from unilamellar vesicles comprising of either POPC or POPC/cholesterol (7:3 molar ratio), loaded with 489mM NaCl buffered to pH 7.2 with 5mM sodium phosphate salts. The vesicles were dispersed in 489mM $NaNO_3$ buffered to pH 7.2 with 5mM sodium phosphate salts. At the end of the experiment detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride efflux. Each point represents an average of three trials. DMSO was used as a control.

References

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[iii] L. Palatinus, G. Chapuis, *J. Appl. Cryst*., 2007, **40**, 786