Electronic Supplementary Material (ESI) for Chemical Science
This journal is © The Royal Society of Chemistry 2014

Supplementary Information:

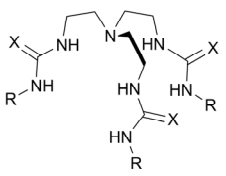
Synthetic transporters for sulfate: a new method for the direct detection of lipid bilayer sulfate transport

Nathalie Busschaert,[a] Louise E. Karagiannidis,[a] Marco Wenzel,[‡a] Cally J. E. Haynes,[a] Neil J. Wells,[a] Philip G. Young,[b] Damjan Makuc,[c,d] Janez Plavec,[c,d,e] Katrina A. Jolliffe*[b] and Philip A. Gale*[a]*

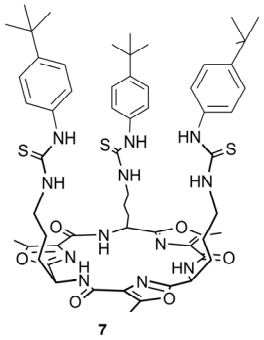
[a] Chemistry, University of Southampton, Southampton, SO17 1BJ, UK; [b] School of Chemistry (F11), The University of Sydney, 2006 NSW, Australia; [c] Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia; [d] EN-FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia; [e] Faculty of Chemistry and Chemical Technology, University of Ljubljana, SI-1000 Ljubljana, Slovenia; [‡] Present address: Fraunhofer IKTS, 01217, Dresden, Germany; Corresponding authors: janez.plavec@ki.si; kate.jolliffe@sydney.edu.au; philip.gale@soton.ac.uk

Electronic Supplementary Material (ESI) for Chemical Science
This journal is © The Royal Society of Chemistry 2014

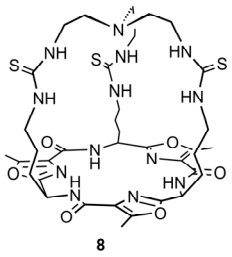
S1. OVERVIEW OF COMPOUNDS



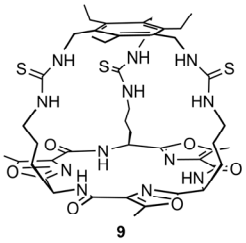
1 X=O R=C_6H_5
2 X=S R=C_6H_5
3 X=O R=C_6F_5
4 X=S R=C_6F_5
5 X=O R=*m*-$C_6H_3(CF_3)_2$
6 X=S R=*m*-$C_6H_3(CF_3)_2$



7



8



9

S2. EXPERIMENTAL PROCEDURES – VESICLE ASSAYS

These procedures describe typical membrane transport tests as referred to in the article. Internal and external solutions can vary (see caption of figures). Chloride concentrations during transport experiments were determined using an *Accumet* chloride-selective electrode. POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) was supplied by *Genzyme* 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*. NMR labeled $NaH^{13}CO_3$ and $Na_2{}^{33}SO_4$ were purchased from ISOTEC (Sigma-Aldrich).

S2.1 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 metal chloride (MCl) salt solution (489 mM MCl, 5 mM phosphate buffer at pH 7.2). The lipid suspension was then subjected to nine freeze-thaw cycles, where the suspension was alternatingly allowed to freeze 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 MCl salts.

This journal is © The Royal Society of Chemistry 2014

S2.2 Chloride/Nitrate Transport Assay

Unilamellar POPC (with 0 % or 30 % cholesterol) 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.

S2.3 Chloride/Bicarbonate Transport Assay

Unilamellar POPC vesicles containing 450 mM NaCl solution buffered to pH 7.2 with 20 mM sodium phosphate salts, prepared as described above, were suspended in the external medium consisting of a 162 mM Na_2SO_4 solution buffered to pH 7.2 with sodium phosphate salts (20 mM buffer). The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule was added to start the experiment and chloride efflux was monitored using a chloride sensitive electrode. At 2 min, a $NaHCO_3$ solution (1 M in 162 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 polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) and a total chloride reading was taken at 9 min. The initial value was set at 0 % chloride efflux and the final chloride reading was set as 100 % chloride efflux. All other data points were calibrated to these points.

S2.4 Chloride/Sulfate Transport Assay (ISE)

Unilamellar POPC vesicles containing 450 mM NaCl solution buffered to pH 7.2 with 20 mM sodium phosphate salts, prepared as described above, were suspended in the external medium consisting of a 162 mM Na_2SO_4 solution buffered to pH 7.2 with sodium phosphate salts (20 mM buffer). The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule was added to start the experiment and 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 was set as 100 %

This journal is © The Royal Society of Chemistry 2014

chloride efflux. All other data points were calibrated to these points.

S2.5 HPTS assays (HCl co-transport)

A lipid film of POPC was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing with 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, prepared as described above, 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]:

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

S2.6 Lucigenin assays (Cl^-/SO_4^{2-} antiport)

A lipid film of POPC was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing with 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).

This journal is © The Royal Society of Chemistry 2014

Unilamellar POPC vesicles containing NaCl and lucigenin, prepared as described above, 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 stock salt solution was added so that the outer concentration contained 40 mM of the new salt (stock solutions: 1 M $NaNO_3$, 1 M NaCl or 0.5 M Na_2SO_4). After 60 s, a methanol 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).

S2.7 Calcein assay (vesicle rupture)

A lipid film was rehydrated by vortexing with a NaCl solution (100 mM calcein, 450 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 calcein was removed by size exclusion chromatography on a Sephadex G-50 column using a sodium sulfate solution as eluent (162 mM Na_2SO_4, 20 mM phosphate buffer at pH 7.2).

Unilamellar POPC vesicles containing NaCl and calcein, prepared as described above, were suspended in a Na_2SO_4 solution buffered to pH 7.2 with 20 mM sodium phosphate salts. The lipid concentration per sample was 1 mM. The fluorescence emission of the encapsulated calcein at 520 nm after excitation at 490 nm was recorded continuously for a minimum of 16 hours using a Varian Cary Eclipse Fluorescence Spectrophotometer. At t = 30 s, a solution of the carrier molecule in DMSO (4 mol% w.r.t. lipid) was added to start ion transport. At the end of the experiment the vesicles were lysed with 30 µl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v). The fractional calcein release (FR) was calculated as follows:

$$FR = \frac{I_t - I_0}{I_{max} - I_0}$$

Where: I_t = fluorescence intensity at time t

I_0 = fluorescence intensity at time 0

I_{max} = fluorescence intensity after addition of detergent

This journal is © The Royal Society of Chemistry 2014

S2.8 ^{13}C NMR Anion Transport Assay

A lipid film of POPC was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing with a $NaH^{13}CO_3$ solution (155 mM $NaH^{13}CO_3$, 20 mM phosphate buffer at pH 7.4) and subjected to 6 freeze/thaw cycles. The mixture was placed on a vortexer every 3 cycles for 30s to facilitate hydration. The cloudy solution was then extruded 35 times through a 5.0 μm polycarbonate membrane. In order to exchange external $NaH^{13}CO_3$ with Na_2SO_4, this solution was placed in dialysis tubing and stirred overnight in a Na_2SO_4 solution (20 mM phosphate buffer and 50 mM Na_2SO_4 at pH 7.4).

^{13}C NMR spectra were acquired on a Agilent Technologies VNMRS 600 MHz spectrometer equipped with ^{13}C–^{31}P broadband probe. Sample temperature was set at 300 K. The ^{13}C frequency was set to 150.80 MHz, spectral width was 38 kHz, the number of data points 32K. The spectra were recorded using 90-degree pulse, relaxation delay was 200 ms and number of scans 1600. Each experiment consisted of several steps:

(i) 3.5 mL of POPC liposomes (20 mM POPC) were prepared as described above, containing 155 mM $NaH^{13}CO_3$ buffered to pH 7.4 with 20 mM phosphate buffer, dispersed in 50 mM Na_2SO_4 buffered to pH 7.4 with 20 mM phosphate buffer. An initial ^{13}C NMR spectrum of 600 μL of this liposome solution was acquired (540 μL stock lipid and 60 μL D_2O to obtain a final 9:1 H_2O:D_2O mix).

(ii) A NaCl pulse was added to the remaining liposome solution (2.9 mL), resulting in final extravesicular concentrations of 100 mM NaCl and the ^{13}C NMR of this liposome mixture was taken (540 μL stock lipid and 60 μL D_2O to obtain a final 9:1 H_2O:D_2O mix). This step was omitted when testing for sulfate/bicarbonate exchange.

(iii) The remaining liposome solution was divided in aliquots of 600 μL and to each aliquot was added a DMSO solution of the putative transporter (24 μL of a 20 mM DMSO solution was added to obtain 4 mol% transporter to lipid ratio) and stirred for 1 hour at room temperature. The ^{13}C NMR of this liposome mixture was then taken (540 μL stock lipid and 60 μL D_2O to obtain a final 9:1 H_2O:D_2O mix).

(iv) To the NMR tube was then added 10 μL of a 100 mM $MnCl_2$ solution or a 100 mM $MnSO_4{\cdot}4H_2O$ solution when testing for sulfate/bicarbonate exchange (to obtain a final concentration of 1.6 mM Mn^{2+} or 1.6 % Mn^{2+}:Cl^- ratio) and the ^{13}C NMR was taken.

(v) Detergent was then added to the NMR tube (100 μL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v)) and shaken and the final ^{13}C NMR was obtained.

This journal is © The Royal Society of Chemistry 2014

S2.9 ^{33}S NMR Anion Transport Assay (external Mn^{2+})

A lipid film of POPC was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing with a $Na_2{}^{33}SO_4$ solution (162 mM $Na_2{}^{33}SO_4$, 20 mM phosphate buffer at pH 7.2) and subjected to 6 freeze/thaw cycles. The mixture was placed on a vortexer every 3 cycles for 30 s to facilitate hydration. The cloudy solution was then extruded 35 times through a 5.0 µm polycarbonate membrane. In order to exchange external $Na_2{}^{33}SO_4$ with NaCl, this solution was placed in dialysis tubing and stirred overnight in a NaCl solution (20 mM phosphate buffer and 450 mM NaCl at pH 7.2).

^{33}S NMR spectra were acquired on Agilent Technologies VNMRS 600 MHz NMR spectrometer equipped with heteronuclear broadband probe that could be tuned to the resonance frequency of 46.028 MHz. ^{33}S NMR chemical shifts were referenced with respect to 1 mM $Na_2{}^{33}SO_4$ (δ 0.0 ppm). The spectral width was 4 kHz and the number of data points was 4K. The spectra were recorded using a simple 90-degree pulse acquire sequence with relaxation delay of 250 ms. Typically 3200 scans were acquired to obtain a reliable sample signature and favourable S/N ratio. Sample temperature was set at 300 K.

Each experiment consisted of several steps:

(i) 3.0 mL of POPC liposomes (20 mM POPC) were prepared as described above, containing 162 mM $Na_2{}^{33}SO_4$ in 20 mM phosphate buffer at pH 7.2, dispersed in 450 mM NaCl buffered to pH 7.2 with 20 mM phosphate buffer. 25 µL of a 100 mM $MnCl_2$ solution was added (to obtain 0.5 mol% Mn^{2+}:$^{33}SO_4{}^{2-}$ ratio). An initial ^{33}S NMR spectrum of 600 µL of this liposome solution was acquired (540 µL stock lipid and 60 µL D_2O to obtain a final 9:1 H_2O:D_2O mix).

(ii) The remaining liposome solution was divided in aliquots of 600 µL and to each aliquot was added a DMSO solution of the putative transporter (24 µL of a 20 mM DMSO solution was added to obtain 4 mol% transporter to lipid ratio) and stirred for 2 hours at room temperature. The ^{33}S NMR of this liposome mixture was then taken (540 µL stock lipid and 60 µL D_2O to obtain a final 9:1 H_2O:D_2O mix).

(iii) Detergent was then added to the NMR tube (100 µL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v)) and shaken and the final ^{13}C NMR was obtained.

This journal is © The Royal Society of Chemistry 2014

S2.10 ^{33}S NMR Anion Transport Assay (internal Mn^{2+} or Fe^{3+})

A lipid film of POPC was formed from a chloroform solution under reduced pressure and dried under vacuum for at least 6 hours. The lipid film was rehydrated by vortexing with a $Na_2{}^{33}SO_4$ solution (162 mM $Na_2{}^{33}SO_4$, 20 mM phosphate buffer at pH 7.2, containing either 0.8 mM $MnSO_4.4H_2O$ (0.5 mol% Mn^{2+}:$^{33}SO_4{}^{2-}$ ratio) or 4 mM $Fe_2(SO_4)_3$ (5 mol% Mn^{2+}:$^{33}SO_4{}^{2-}$ ratio)) and subjected to 6 freeze/thaw cycles. The mixture was placed on a vortexer every 3 cycles for 30 s to facilitate hydration. The cloudy solution was then extruded 35 times through a 5.0 μm polycarbonate membrane. In order to exchange external $Na_2{}^{33}SO_4$ with NaCl, this solution was placed in dialysis tubing and stirred overnight in a NaCl solution (20 mM phosphate buffer and 450 mM NaCl at pH 7.2).

^{33}S NMR spectra were acquired on Agilent Technologies VNMRS 600 MHz spectrometer equipped with ^{15}N–^{107}Ag broadband probe. Sample temperature was set at 300 K. ^{33}S NMR chemical shifts were referenced with respect to 1 mM $Na_2{}^{33}SO_4$ (δ 0.0 ppm). The ^{33}S frequency was set to 46.028 MHz, spectral width was 4 kHz, the number of data points 4K. The spectra were recorded using 90-degree pulse, relaxation delay was 250 ms and number of scans 3200. Each experiment consisted of several steps:

(i) 3.0 mL of POPC liposomes (20 mM POPC) were prepared as described above, containing 162 mM $Na_2{}^{33}SO_4$ in 20 mM phosphate buffer at pH 7.2 with either 0.8 mM $MnSO_4.4H_2O$ (0.5 mol% Mn^{2+}:$^{33}SO_4{}^{2-}$ ratio) or 4 mM $Fe_2(SO_4)_3$ (5 mol% Mn^{2+}:$^{33}SO_4{}^{2-}$ ratio), and dispersed in 450 mM NaCl buffered to pH 7.2 with 20 mM phosphate buffer. An initial ^{33}S NMR spectrum of 600 μL of this liposome solution was acquired (540 μL stock lipid and 60 μL D_2O to obtain a final 9:1 H_2O:D_2O mix).

(ii) The remaining liposome solution was divided in aliquots of 600 μL and to each aliquot was added a DMSO solution of the putative transporter (24 μL of a 20 mM DMSO solution was added to obtain 4 mol% transporter to lipid ratio) and stirred for 2 hours at room temperature. The ^{33}S NMR of this liposome mixture was then taken (540 μL stock lipid and 60 μL D_2O to obtain a final 9:1 H_2O:D_2O mix).

(iii) Detergent was then added to the NMR tube (100 μL of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v)) and shaken and the final ^{13}C NMR was obtained.

This journal is © The Royal Society of Chemistry 2014

S3. CHLORIDE, NITRATE AND BICARBONATE TRANSPORT BY 7-9

S3.1. Chloride/nitrate transport

The chloride/nitrate transport ability of **1-6** have been reported elsewhere.[1,2] Here the chloride/nitrate transport ability of **7-9** are shown with error bars representing standard deviations.

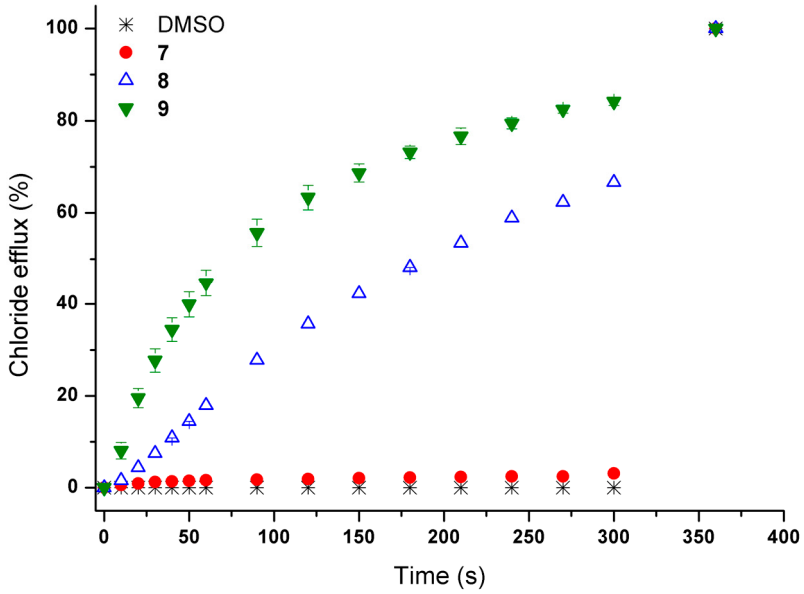


Fig S1. Chloride efflux promoted by **7-9** (4 mol% carrier to lipid) from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the electrode to 100 % chloride efflux. Each point represents the average of three trials and errors bars represent standard deviations. DMSO was used as control.

S3.2. Cholesterol test – evidence of mobile carrier mechanism

Vesicles were prepared in the same way as described above (see sections S2.1 and S2.2), but the lipid consisted of a 7:3 mixture of POPC and cholesterol. Cholesterol is believed to increase the viscosity of the membrane, hence slowing down diffusion in the lipid bilayer. This effect should be more pronounced in the case of a mobile carrier mechanism. The cholesterol test for **1-6** has been reported elsewhere.[1,2] Here the cholesterol test of **8** and **9** are shown with error bars representing standard deviations.

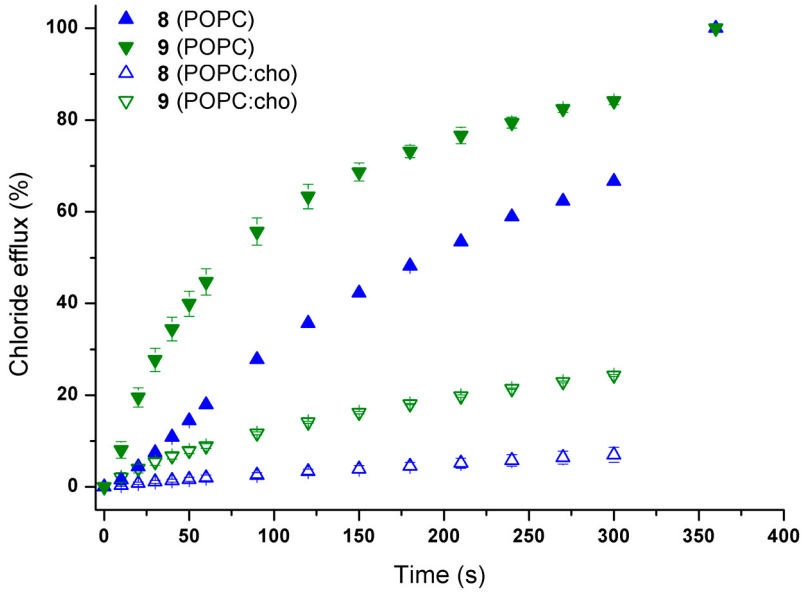


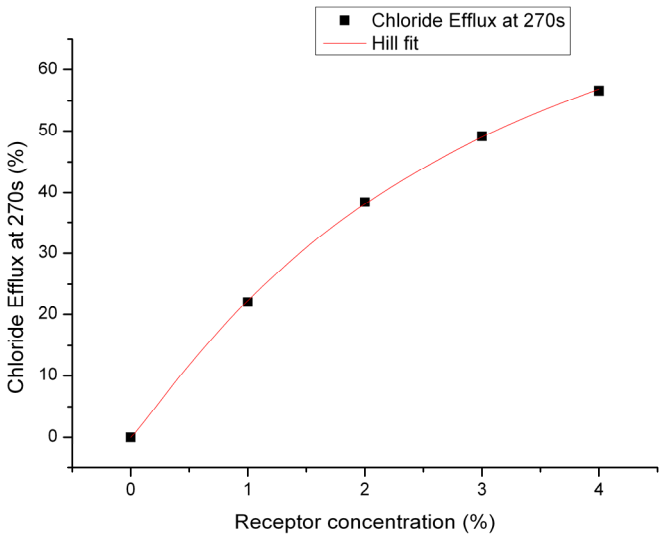
Fig. S2 Chloride efflux promoted by 4 mol% of receptor **8** and **9** from unilamellar POPC vesicles (closed symbols) and unilamellar POPC/cholesterol (7:3) vesicles (open symbols), loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mM $NaNO_3$ 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 electrode to 100 % chloride efflux. Each point represents the average of three trials.

S3.3. Hill Plots – EC_{50} values and evidence of mobile carrier mechanism

The chloride/nitrate transport assays were performed as described above for various concentrations of carrier for **8** and **9**, Hill plots of **1-6** have been reported elsewhere.[2] The chloride efflux (%) 270 s after the addition of carrier (chloride/nitrate) was plotted as a function of the carrier concentration. Data points were fitted to the Hill equation:

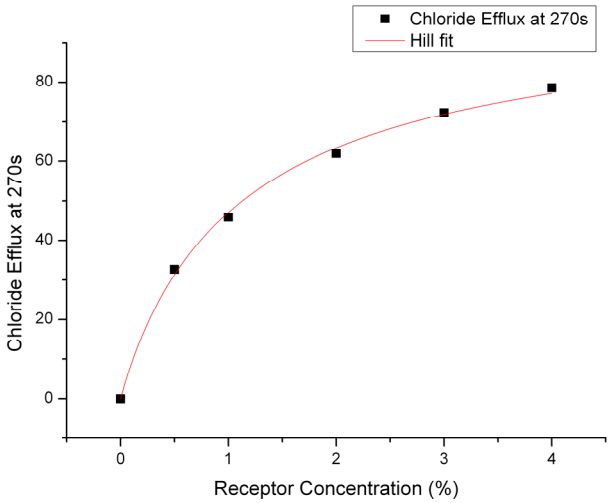
$$y = V_{\max} \frac{x^n}{k^n + x^n}$$

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 (usually 100%), n is the Hill coefficient and k is the carrier concentration needed to reach $V_{max}/2$. From the Hill plot $EC_{50,\,270s}$ values were calculated, defined as the carrier concentration (mol% carrier to lipid) needed to obtain 50 % chloride efflux after 270 s.



Model	Hill		
Equation	y=Vmax*x^n/(k^n+x^n)		
Reduced C	0.13773		
Adj. R-Squa	0.99939		
		Value	Standard Er
Chloride Eff	Vmax	100	0
Chloride Eff	k	3.11166	0.02551
Chloride Eff	n	1.10125	0.01784

Fig. S3 Hill plot for chloride release mediated by receptor **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. Chloride efflux was measured at 270 s.



Model	Hill		
Equation	y=Vmax*x^n/(k^n+x^n)		
Reduced C	2.47987		
Adj. R-Squa	0.99306		
		Value	Standard Er
Chloride Eff	Vmax	100	0
Chloride Eff	k	1.12957	0.04093
Chloride Eff	n	0.96266	0.04546

Fig. S4 Hill plot for chloride release mediated by receptor **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. Chloride efflux was measured at 270 s.

S3.4. Bicarbonate test using electrode – evidence of antiport mechanism

The chloride/bicarbonate transport ability of **1-6** have been reported elsewhere.[1,2] Here the chloride/bicarbonate transport ability of **7-9** are shown with error bars representing standard deviations.

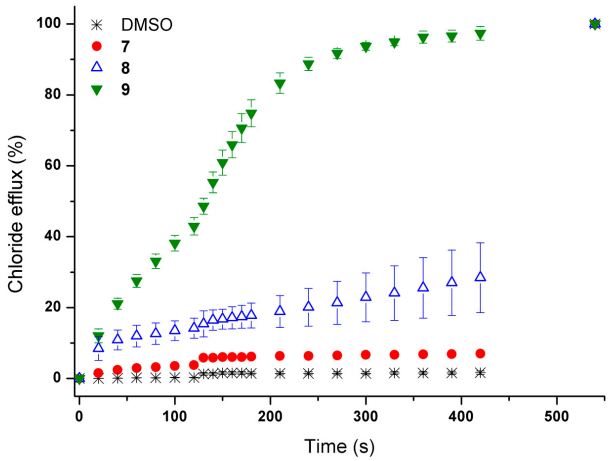


Fig S5. Chloride efflux promoted by **7-9** (4 mol% carrier to lipid) from unilamellar POPC vesicles loaded with 450 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a solution of $NaHCO_3$ was added to give a 40 mM external concentration. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the electrode to 100 % chloride efflux. Each point represents the average of minimum three trials and errors bars represent standard deviations. DMSO was used as control.

S3.5. Bicarbonate test using ^{13}C NMR – evidence of antiport mechanism

The bicarbonate transport ability of **1-2** using ^{13}C NMR has been reported elsewhere.[1] Here the chloride/bicarbonate and sulfate/bicarbonate transport ability of **3-9** are shown (see section S2.8). NMR spectra were processed using ACD Labs' 1D NMR Processor 12.01, the *fid* files were first submitted to Fourier transformation with backward linear prediction and subsequently automatically baseline corrected. All spectra are shown in absolute intensities.

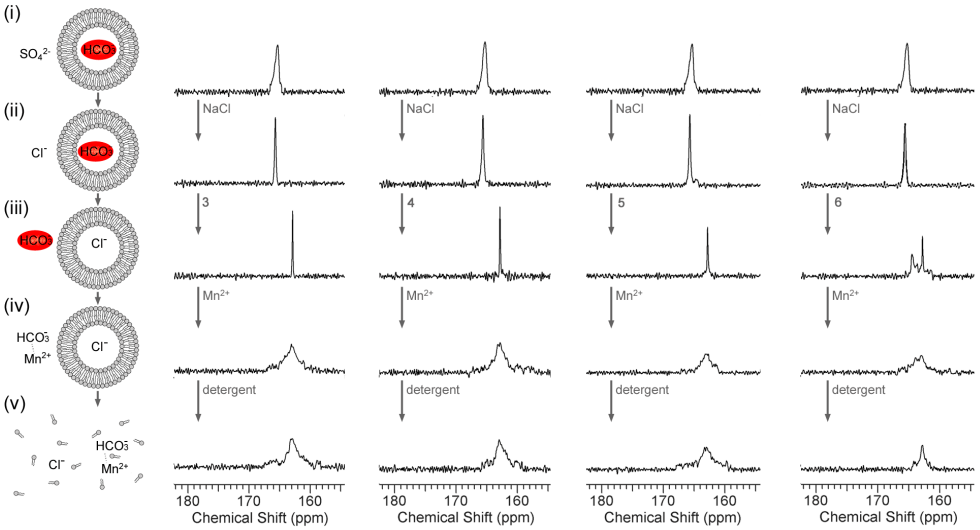


Fig S6. ^{13}C NMR experiments to detect bicarbonate/chloride transport by **3-6**. All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity. ^{13}C NMR spectra of (i) POPC vesicles loaded with 155 mM $NaH^{13}CO_3$, 20 mM phosphate buffer, pH 7.4, and dispersed in 50 mM Na_2SO_4, 20 mM phosphate buffer, pH 7.4; (ii) after the addition of the 100 mM NaCl pulse; (iii) after 1 hour incubation with transporter (4 mol% transporter to lipid); (iv) after the addition of $MnCl_2$ (1.5 mol% Mn^{2+}:Cl^- ratio); (v) after the addition of detergent

This journal is © The Royal Society of Chemistry 2014

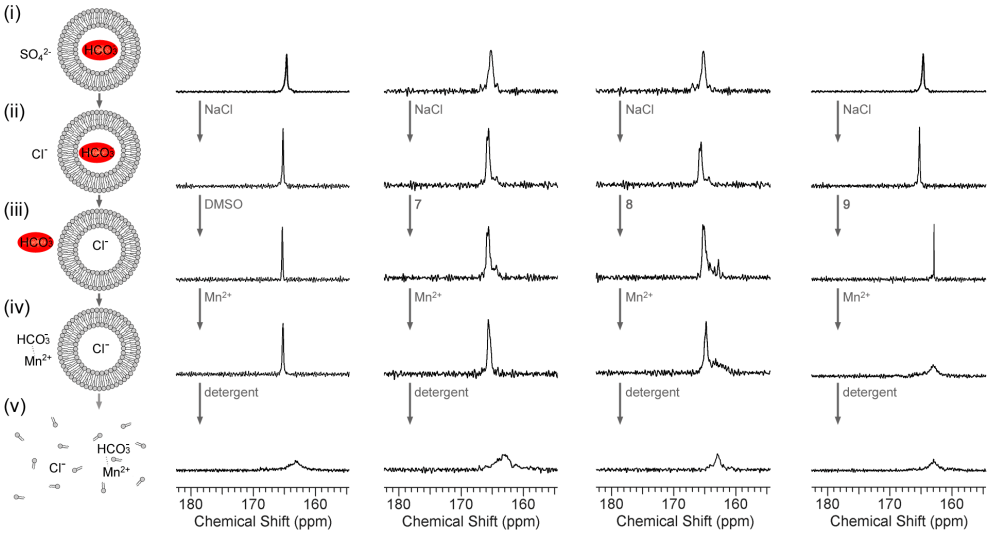


Fig S7. ^{13}C NMR experiments to detect bicarbonate/chloride transport by **7-9** and DMSO as control. All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity. ^{13}C NMR spectra of (i) POPC vesicles loaded with 155 mM $NaH^{13}CO_3$, 20 mM phosphate buffer, pH 7.4, and dispersed in 50 mM Na_2SO_4, 20 mM phosphate buffer, pH 7.4; (ii) after the addition of the 100 mM NaCl pulse; (iii) after 1 hour incubation with transporter or DMSO (4 mol% transporter to lipid); (iv) after the addition of $MnCl_2$ (1.5 mol% Mn^{2+}:Cl^- ratio); (v) after the addition of detergent

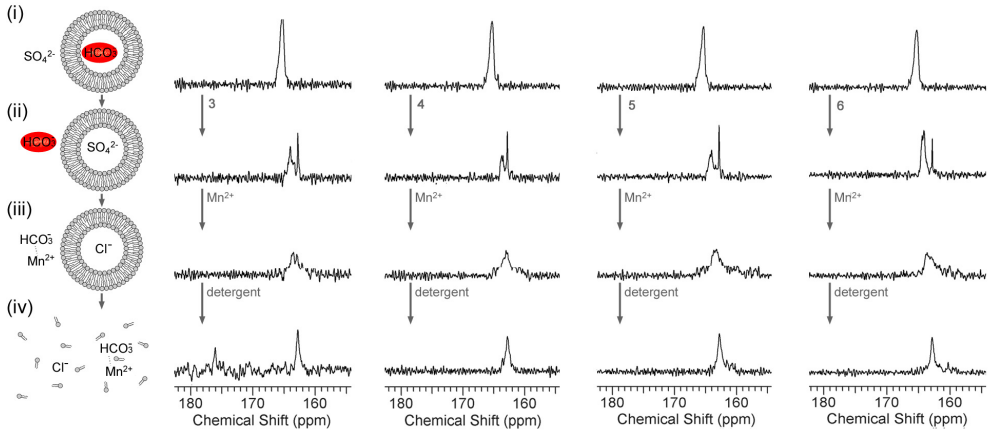


Fig S8. ^{13}C NMR experiments to detect bicarbonate/sulfate transport by **3-6**. All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity. ^{13}C NMR spectra of (i) POPC vesicles loaded with 155 mM $NaH^{13}CO_3$, 20 mM phosphate buffer, pH 7.4, and dispersed in 50 mM Na_2SO_4, 20 mM phosphate buffer, pH 7.4; (ii) after 1 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of $MnCl_2$ (1.5 mol% Mn^{2+}:Cl^- ratio); (iv) after the addition of detergent

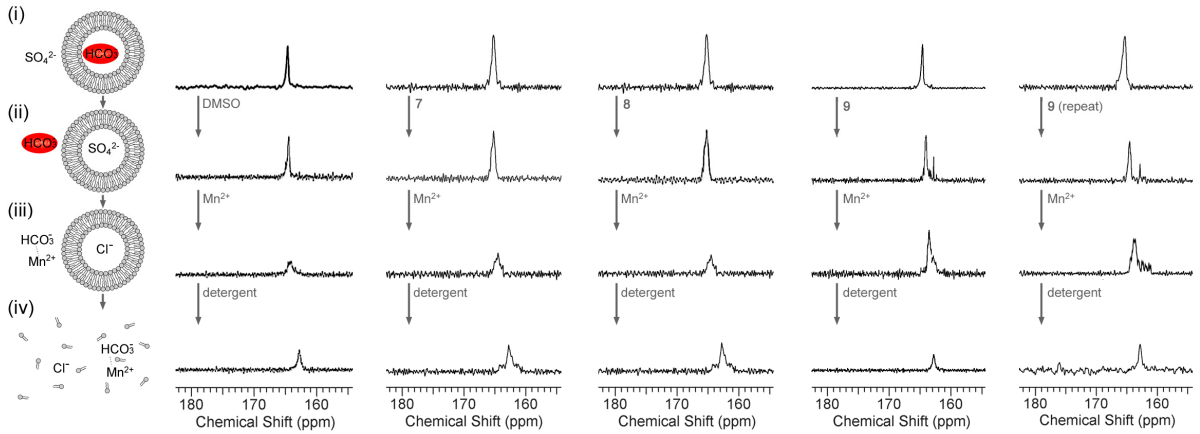


Fig S8. ^{13}C NMR experiments to detect bicarbonate/sulfate transport by **7-9** and DMSO as control. All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity. ^{13}C NMR spectra of (i) POPC vesicles loaded with 155 mM $NaH^{13}CO_3$, 20 mM phosphate buffer, pH 7.4, and dispersed in 50 mM Na_2SO_4, 20 mM phosphate buffer, pH 7.4; (ii) after 1 hour incubation with transporter or DMSO (4 mol% transporter to lipid); (iii) after the addition of $MnCl_2$ (1.5 mol% Mn^{2+}:Cl^- ratio); (iv) after the addition of detergent

S4. INDIRECT EVIDENCE FOR SULFATE TRANSPORT BY 1-9

S4.1. Chloride/sulfate transport using electrode

Here we report the chloride transport by **1-9** in the presence of external sufate (fig4 in manuscript). Error bars represent standard deviations.

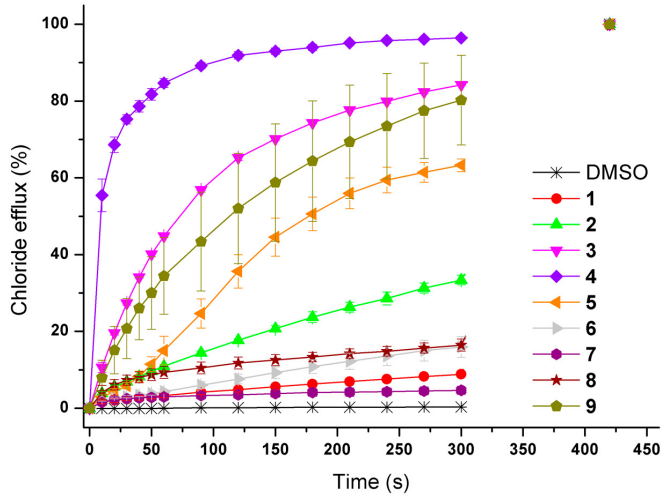


Fig S9. Chloride efflux promoted by **1-9** (4 mol% carrier to lipid) from unilamellar POPC vesicles loaded with 450 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the electrode to 100 % chloride efflux. Each point represents the average of minimum three trials and error bars represent standard deviations. DMSO was used as control.

This journal is © The Royal Society of Chemistry 2014

S4.2. Changing countercation – evidence for antiport mechanism

To test whether the symport of metal/chloride is possible (transport of ion pair), the vesicle studies described above in section S4.1 were repeated with various metal counterions (Na^+, K^+ and Cs^+). However, no significant differences in transport ability were found, making symport an unlikely event.

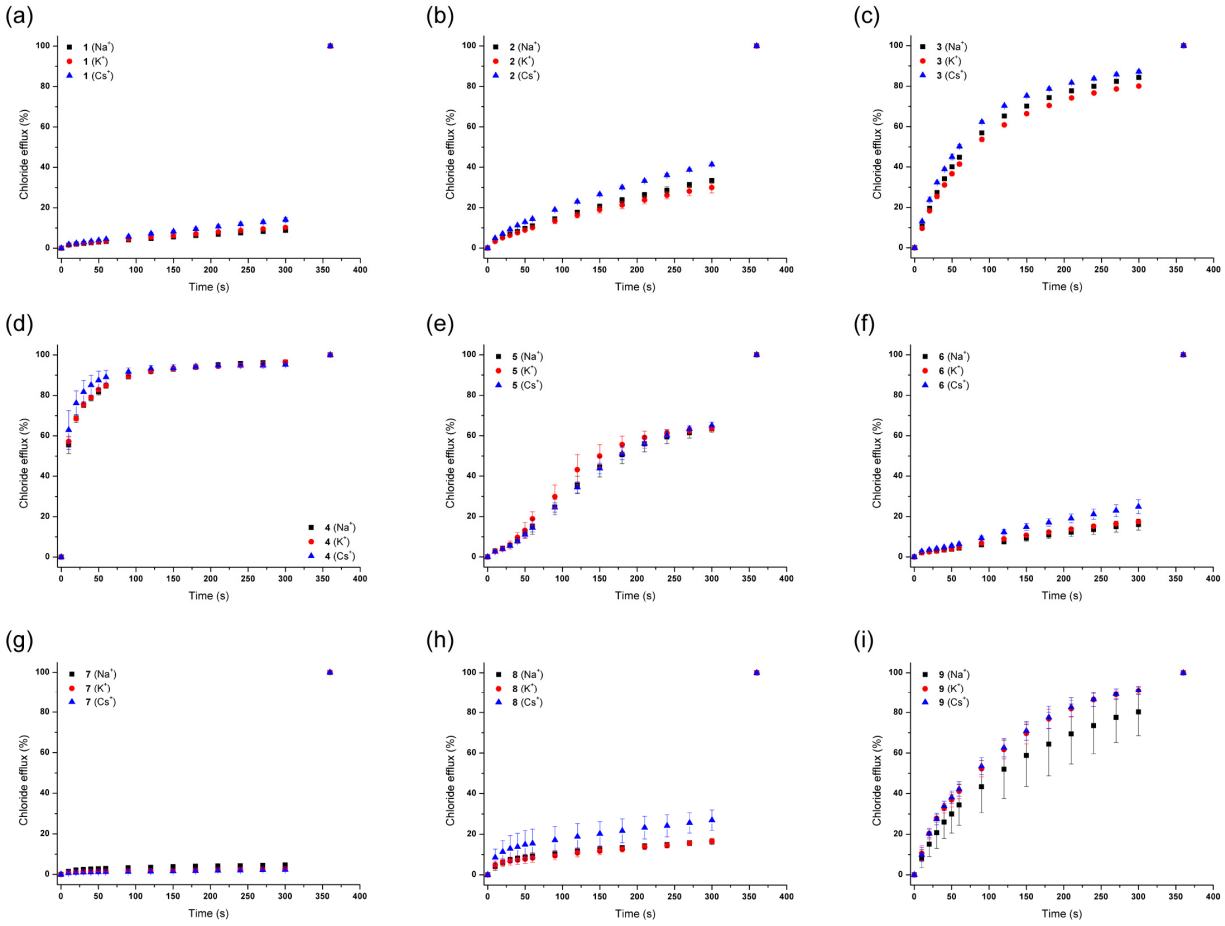


Fig S10. Chloride efflux promoted by **1**-**9** (4 mol% carrier to lipid) from unilamellar POPC vesicles loaded with 450 mM NaCl, KCl or CsCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the electrode to 100 % chloride efflux. Each point represents the average of minimum three trials and error bars represent standard deviations. DMSO was used as control. NaCl/Na_2SO_4 in black, KCl/Na_2SO_4 in red and CsCl/Na_2SO_4 in blue. (a) 4 mol% **1**; (b) 4 mol% **2**; (c) 4 mol% **3**; (d) 4 mol% **4**; (e) 4 mol% **5**; (f) 4 mol% **6**; (g) 4 mol% **7**; (h) 4 mol% **8**; (i) 4 mol% **9**.

S4.3. Calcein leakage test

Calcein is a large and highly charged fluorescent dye that self-quenches at high concentration. Thus, when calcein is encapsulated inside vesicles the fluorescence intensity is initially low and increases when it leaks out of the vesicles or when the vesicles are destroyed. This calcein leakage test was performed on vesicles containing NaCl and dispersed in Na_2SO_4 as described in section S2.7 and the result is shown in figure S11. No sign of leakage was observed.

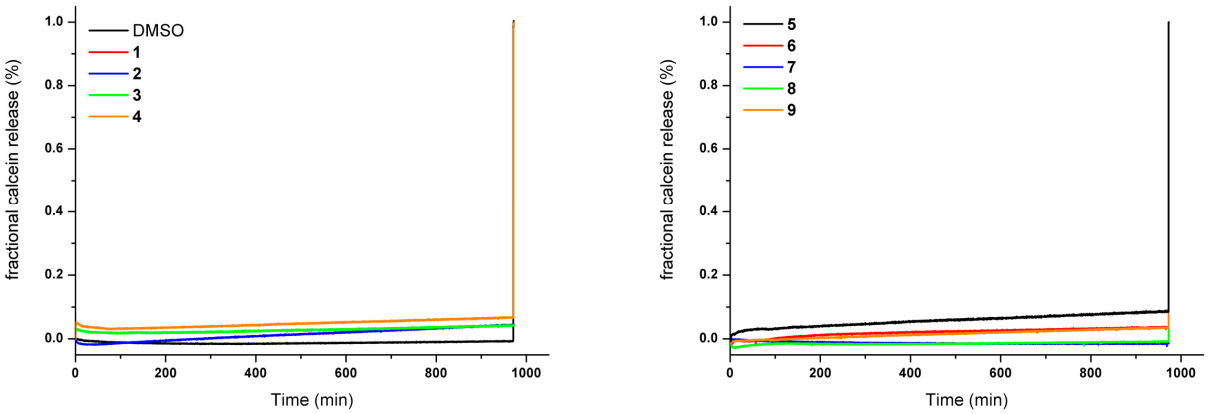


Fig. S11 Calcein leakage by **1-9** (4 mol% carrier to lipid) from unilamellar POPC vesicles loaded with 100 mM calcein and 450 mM NaCl, buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 15 s, a DMSO solution of the transporter was added to start the experiment. At the end of the experiment (± 18 hours), detergent was added to lyse the vesicles. The results are shown as % calcein leaked from the vesicles.

S4.4. HPTS assay – monitoring internal pH

HPTS was used to monitor the internal pH of the vesicles during transport, as described in section S2.5. This test has been reported for compounds **1-6** elswhere[2] and is now given in figure S12 for **8** and **9**.

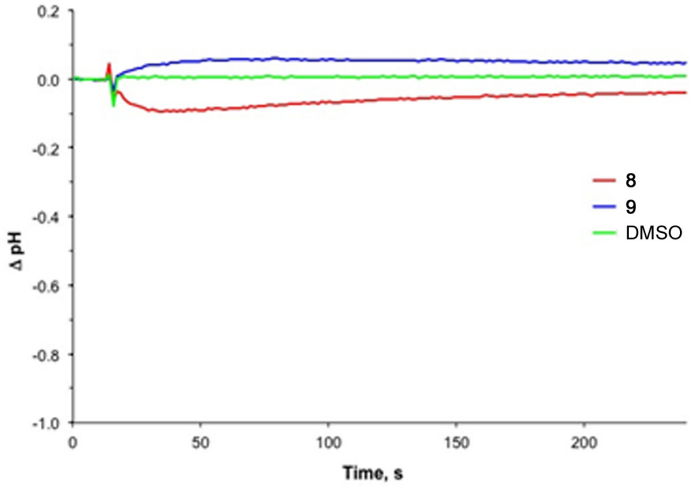


Fig. S12 Intravesicular pH change promoted by **8** and **9** (4 mol% carrier to lipid) from unilamellar POPC vesicles loaded with 1 mM HPTS and 489 mM NaCl, buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 5 mM sodium phosphate salts. At t = 15 s, a DMSO solution of the transporter was added to start the experiment. At the end of the experiment (t = 250 s), detergent was added to lyse the vesicles. Each point represents the average of three trials.

S4.5. Chloride/sulfate transport using lucigenin

In this experiment, there is no chloride gradient or pH gradient and HCl co-transport becomes very unlikely. The transport mechanism that can occur is antiport of chloride with the anion that was spiked into the external solution at t = 30 s. Increase in fluorescence indicates that lucigenin is no longer quenched by chloride and hence that chloride is transported out of the vesicle. This test has been reported for compounds **3**-**6** elswhere[2] and is now given in figure S13 for **8** and **9**.

(a) compound **8** (b) compound **9**

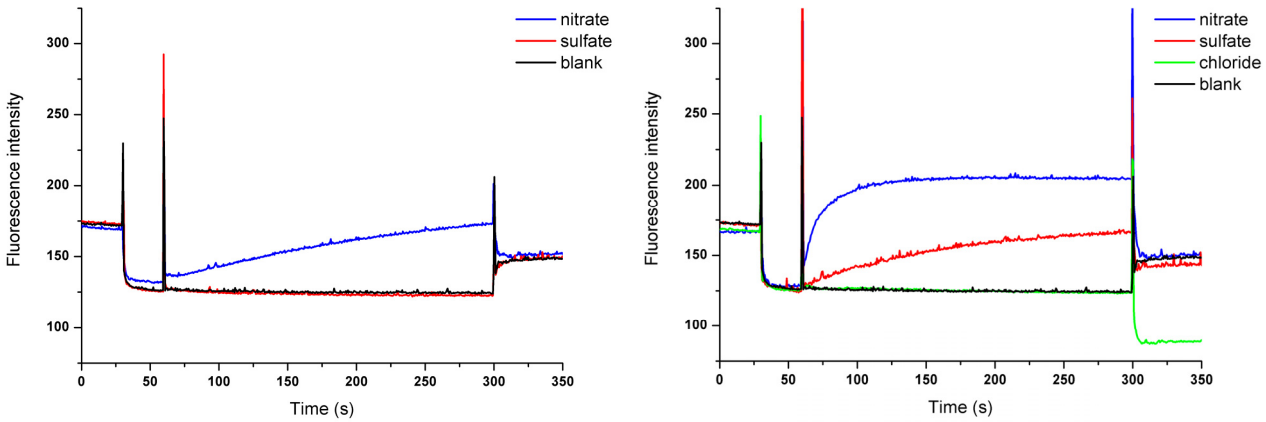


Fig. S13 Unilamellar POPC vesicles were loaded with 100 mM NaCl and 2 mM lucigenin buffered to pH 7.2 with 20 mM sodium phosphate salts and dispersed in a 100 mM NaCl solution (buffered to pH 7.2). At t = 30 s, a solution of the appropriate anion was added (final concentration of 40 mM $NaNO_3$ or 40 mM Na_2SO_4). At t = 60 s, a methanol solution of the transporter **8** (a) or **9** (b) (4 mol% carrier to lipid) was added. At the end of the experiment (240 s), detergent was added to lyse the vesicles. The blank measurement refers to the addition of Na_2SO_4, followed by the addition of methanol. Each point represents the average of three trials.

S5. DIRECT EVIDENCE FOR SULFATE TRANSPORT BY 1-9 USING ^{33}S NMR

S5.1. ^{33}S NMR spectra with external Mn^{2+}

In these tests, vesicles are prepared containing ^{33}S labeled Na_2SO_4 and dispersed in a NaCl solution containing 0.5 mol% $MnCl_2$ (with respect to $^{33}SO_4^{2-}$), as described in section S2.9. A small amount of transporter is added as a DMSO solution and the mixture was allowed to incubate for 2 hours at room temperature to allow transport. A decrease of the ^{33}S peak indicates sulfate transport. NMR spectra were processed using ACD Labs' 1D NMR Processor 12.01, the *fid* files were first submitted to Fourier transformation with backward linear prediction and subsequently automatically baseline corrected. All spectra are shown in absolute intensities. The results are shown in figures S14-S16.

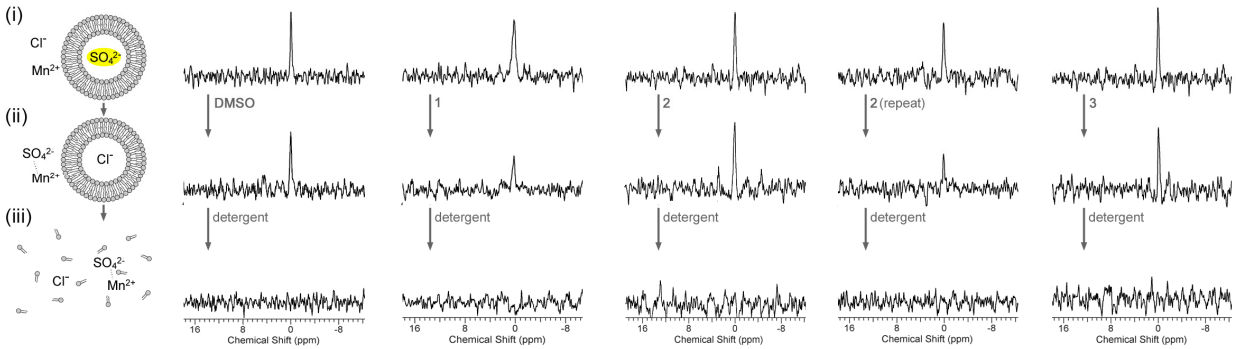


Fig S14. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate Cl^-/SO_4^{2-} antiport (external Mn^{2+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: DMSO, **1**, **2**, **2** (repeat) and **3**). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$, 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl with $MnCl_2$ (0.5 mol% Mn^{2+}:$^{33}SO_4^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

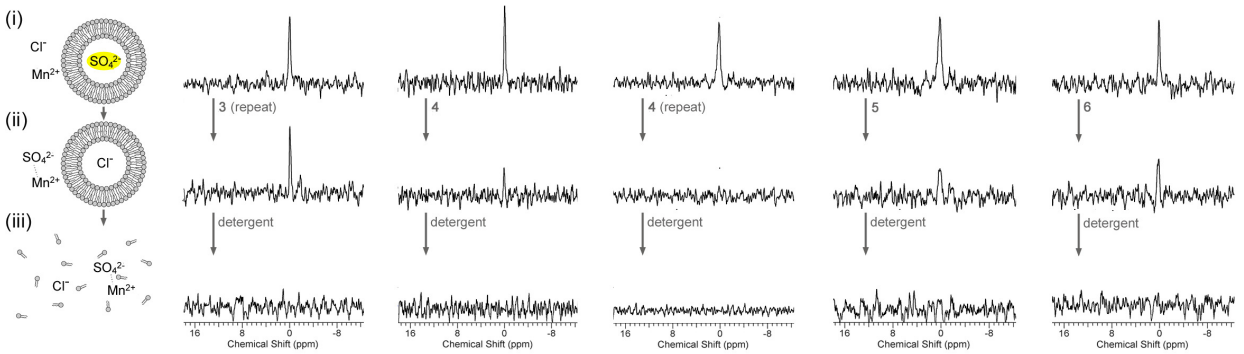


Fig S15. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate Cl^-/SO_4^{2-} antiport (external Mn^{2+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: **3** (repeat), **4**, **4** (repeat), **5** and **6**). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$, 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl with $MnCl_2$ (0.5 mol% Mn^{2+}:$^{33}SO_4^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

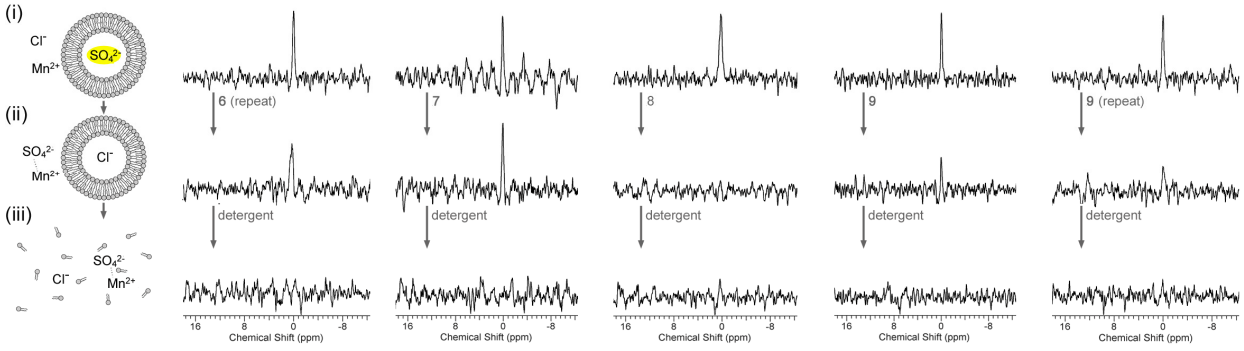


Fig S16. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate Cl^-/SO_4^{2-} antiport (external Mn^{2+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: **6** (repeat), **7**, **8**, **9** and **9** (repeat)). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$, 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl with $MnCl_2$ (0.5 mol% Mn^{2+}:$^{33}SO_4^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

This journal is © The Royal Society of Chemistry 2014

S5.2. ^{33}S NMR spectra with internal Mn^{2+}

In these experiments, vesicles are prepared containing $Na_2{}^{33}SO_4$ and $MnSO_4$ (0.5 mol% Mn^{2+} with respect to $^{33}SO_4{}^{2-}$) and dispersed in a NaCl solution, as described in section S2.10. A small amount of transporter is added as a DMSO solution and the mixture was allowed to incubate for 2 hours at room temperature to allow transport. The appearance of a new ^{33}S peak indicates sulfate transport. NMR spectra were processed using ACD Labs' 1D NMR Processor 12.01, the *fid* files were first submitted to Fourier transformation with backward linear prediction and subsequently automatically baseline corrected. All spectra are shown in absolute intensities. The results are shown in figures S17-S19.

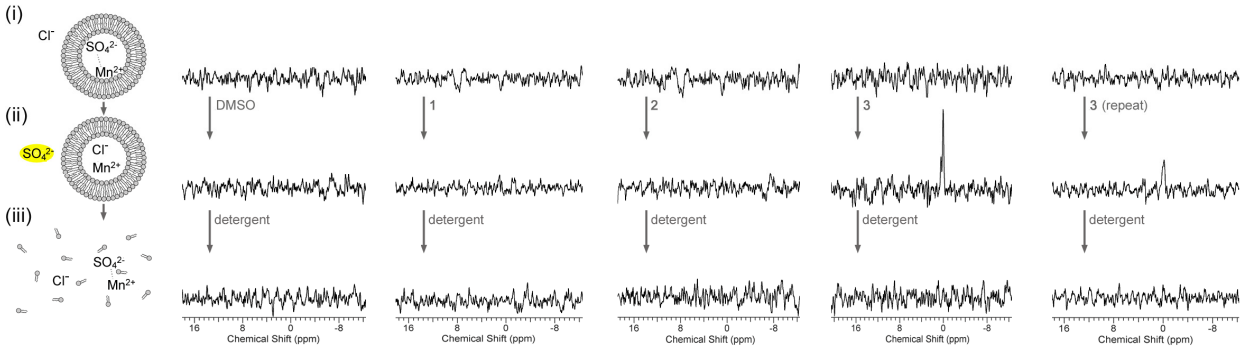


Fig S17. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate $Cl^-/SO_4{}^{2-}$ antiport (internal Mn^{2+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: DMSO, **1**, **2**, **3** and **3** (repeat)). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$ with $MnSO_4$ (0.5 mol% Mn^{2+}:$^{33}SO_4{}^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl, 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

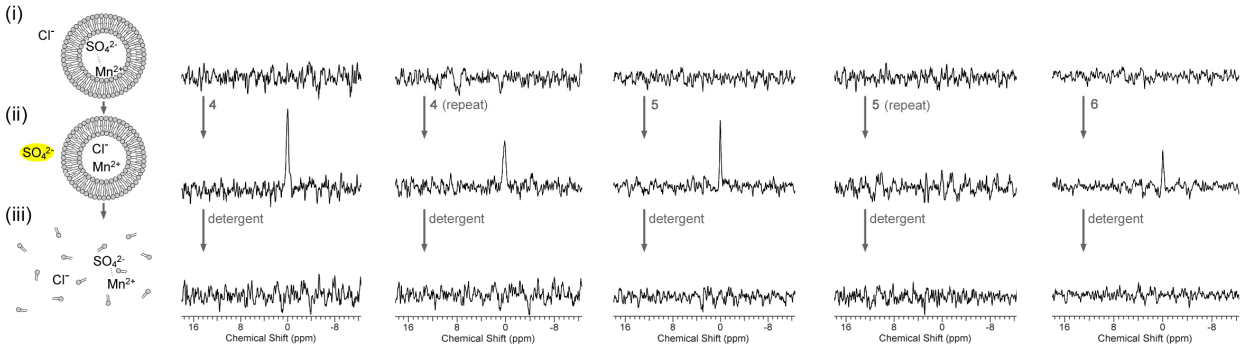


Fig S18. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate $Cl^-/SO_4{}^{2-}$ antiport (internal Mn^{2+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: **4**, **4** (repeat), **5**, **5** (repeat) and **6**). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$ with $MnSO_4$ (0.5 mol% Mn^{2+}:$^{33}SO_4{}^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl, 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

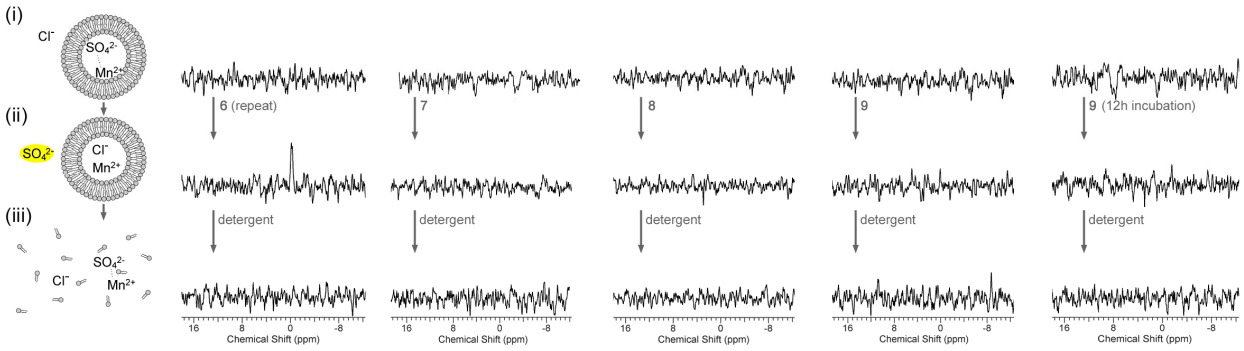


Fig S19. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate Cl^-/SO_4^{2-} antiport (internal Mn^{2+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: **6** (repeat), **7**, **8**, **9** and **9** (12h incubation)). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$ with $MnSO_4$ (0.5 mol% Mn^{2+}:$^{33}SO_4^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl, 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

S5.3. ^{33}S NMR spectra with internal Fe^{3+}

In these experiments, vesicles are prepared containing Na_2SO_4 and $Fe_2(SO_4)_3$ (5 mol% Fe^{3+} with respect to $^{33}SO_4^{2-}$) and dispersed in a NaCl solution, as described in section S2.10. A small amount of transporter is added as a DMSO solution and the mixture was allowed to incubate for 2 hours at room temperature to allow transport. The appearance of a new ^{33}S peak indicates sulfate transport. NMR spectra were processed using ACD Labs' 1D NMR Processor 12.01, the *fid* files were first submitted to Fourier transformation with backward linear prediction and subsequently automatically baseline corrected. All spectra are shown in absolute intensities. The results are shown in figures S20-S22.

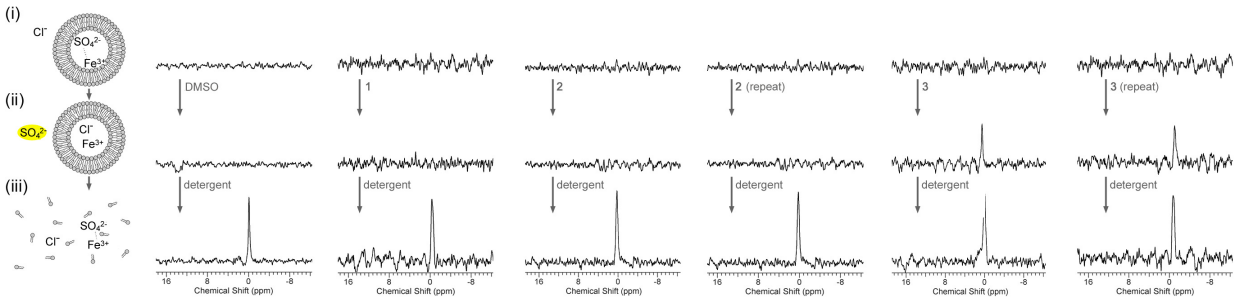


Fig S20. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate Cl^-/SO_4^{2-} antiport (internal Fe^{3+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to righ: DMSO, **1**, **2**, **2** (repeat), **3** and **3** (repeat)). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$ with $Fe_2(SO_4)_3$ (5 mol% Fe^{3+}:$^{33}SO_4^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl, 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

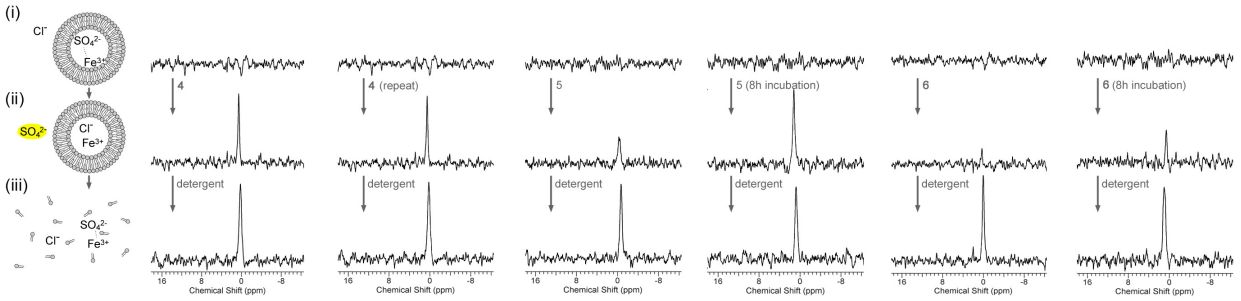


Fig S21. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate Cl^-/SO_4^{2-} antiport (internal Fe^{3+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: **4**, **4** (repeat), **5**, **5** (8h incubation), **6** and **6** (8h incubation)). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$ with $Fe_2(SO_4)_3$ (5 mol% Fe^{3+}:$^{33}SO_4^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl, 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

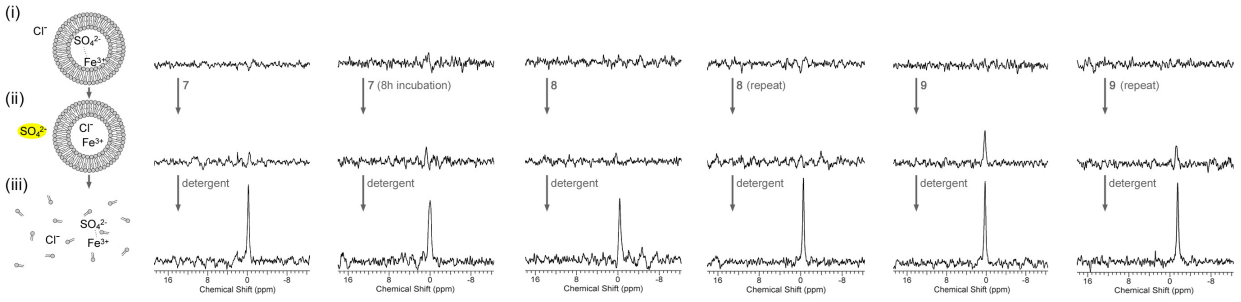


Fig S22. ^{33}S NMR experiments indicating that some of the receptors are able to facilitate Cl^-/SO_4^{2-} antiport (internal Fe^{3+}). All spectra were recorded in 9:1 water:D_2O solutions and are shown at absolute intensity (from left to right: **7**, **7** (8h incubation), **8**, **8** (repeat), **9** and **9** (repeat)). ^{33}S NMR spectra of (i) the POPC vesicles loaded with 162 mM $Na_2{}^{33}SO_4$ with $Fe_2(SO_4)_3$ (5 mol% Fe^{3+}:$^{33}SO_4^{2-}$ ratio), 20 mM phosphate buffer, pH 7.2, and dispersed in 450 mM NaCl, 20 mM phosphate buffer, pH 7.2; (ii) after 2 hour incubation with transporter (4 mol% transporter to lipid); (iii) after the addition of detergent to lyse all vesicles.

S5.4. Determination of Fe^{3+} precipitate

It was observed that upon addition of $Fe_2(SO_4)_3$ to a phosphate buffered $Na_2{}^{33}SO_4$ solution (preparation of internal solution for ^{33}S NMR experiments), a small amount of pale yellow precipitate formed. Wet chemistry tests were used in order to investigate whether Fe^{3+} ions were still present in solution – a requirement for signal broadening in the ^{33}S NMR experiments – using the following procedure (the result is shown in figure S23):

- Step (a): A Na_2SO_4 solution was prepared (162 mM, buffered to pH 7.2 using 20 mM sodium phosphate salts).
- Step (b): An $Fe_2(SO_4)_3$ solution (179 mM) was prepared.
- Step (c): $Fe_2(SO_4)_3$ solution (b) was added to the Na_2SO_4 solution, so that 5.0 mol% or 7.5 mol% Fe^{3+} ions were added with respect to SO_4^{2-} ions from Na_2SO_4. This resulted in the formation of the previously observed yellow precipitate.

This journal is © The Royal Society of Chemistry 2014

- Step (d): The resultant precipitate (c) was centrifuged for 17 minutes at 4000 rpm, giving a clear supernatant liquid.
- Step (e): 5 drops of 1 M NaOH was added to the supernatant liquid from step (d), resulting in a pale yellow solution – indicative of the production of $Fe(OH)_3$.
- Step (f): 5 drops of 1 M NaSCN was added to the supernatant liquid from step (d), resulting in the observation of an orange/red solution – indicative of the production of $Fe(SCN)_3$.

Hence, the overall Fe(III) concentration contained within the vesicles is < 5 %. Tests have shown that despite precipitation of $FePO_4$ there is still a sufficient amount of Fe(III) present in solution to broaden the ^{33}S NMR signal during step (i) in the ^{33}S NMR experiments. Upon lysing the vesicles with detergent a sharp ^{33}S signal is observed. This is most likely the result of the presence of excess phosphate in the external buffer which removes all remaining Fe^{3+} from solution and no peak broadening is seen in step (iii) of the ^{33}S NMR experiment.

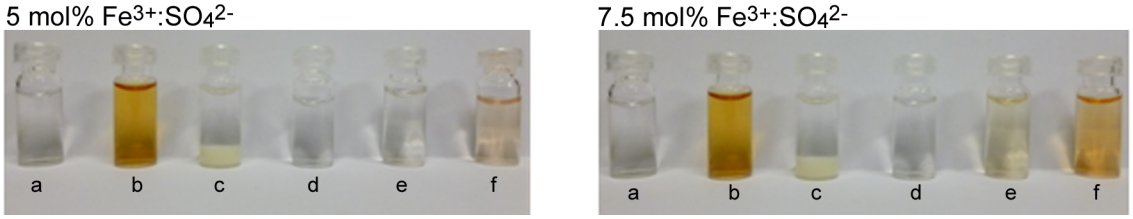


Fig S23. Wet chemistry experiments to see if there is Fe^{3+} present in the internal solution of the vesicles tested with ^{33}S NMR. (left) a ratio of 5 mol% Fe^{3+}:SO_4^{2-} was used, (right) a ratio of 7.5 mol% Fe^{3+}:SO_4^{2-} was used. The letters in the figure refers to the steps as described in the procedure above.

The precipitate that was formed upon addition of detergent during the NMR experiments, was collected by centrifuging at 4000 rpm. The supernatant was removed and the precipitate was washed three times with MilliQ water and finally dried overnight in high vacuum. The obtained solid was characterised using SEM-EDX. The SEM image was taken on a FEI XL30ESEM (environmental scanning electron microscope) and X-ray analysis was carried out with a Thermo NSS7 EDX system, using a Thermo Ultradry silicon drift X-ray detector. Four random areas were chosen from the original SEM image (figure S24) and an elemental determination was performed on each area using EDX (figure S25). It can be seen in Figure S25 and Tables S1-S4 that the major atomic composition in the precipitate is O, P and Fe – indicating that the precipitate is mainly $FePO_4$.

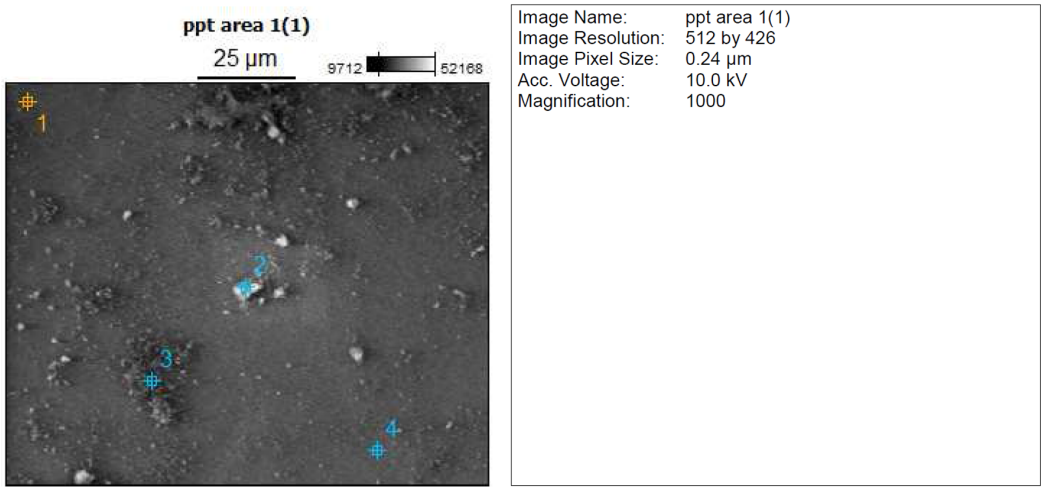


Fig S24. SEM image of the precipitate formed during the ^{33}S NMR experiments with internal Fe^{3+}. The numbers indicate the areas that were analysed using EDX (Fig S25).

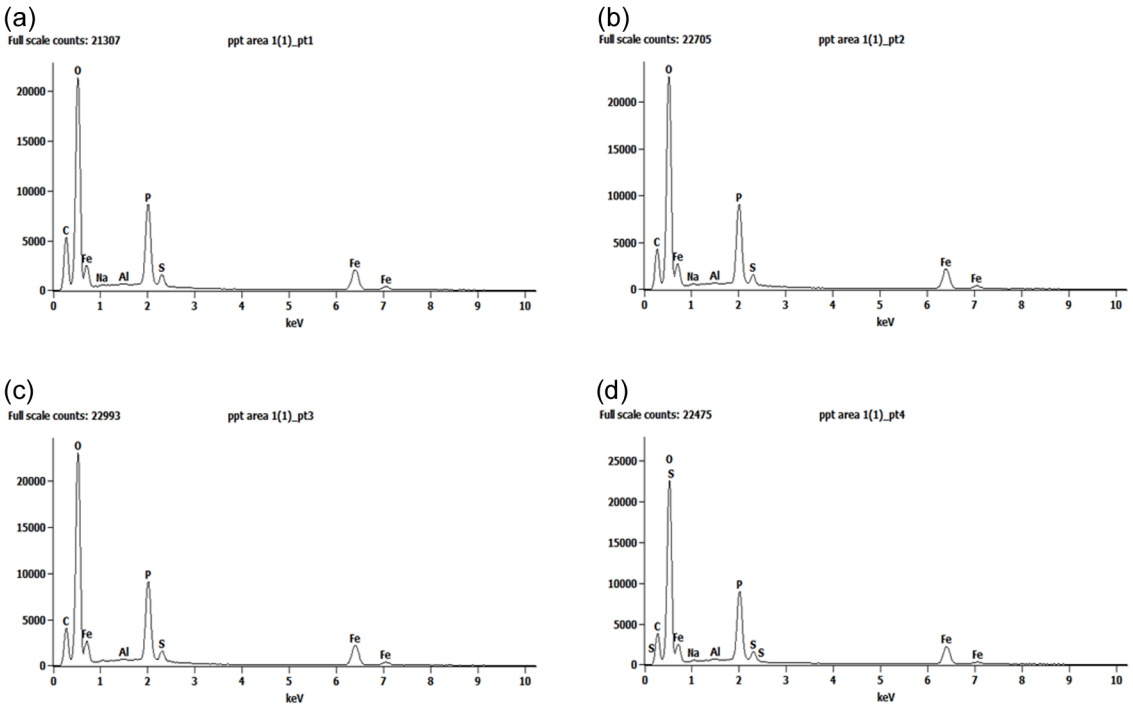


Fig S25. EDX profile of the precipitate formed during the ^{33}S NMR experiments with internal Fe^{3+}. EDX of (a) area 1 in Fig S24; (b) area 2 in Fig S24; (c) area 3 in Fig S24; (d) area 4 in Fig S24.

Table S1 – Results of the EDX analysis on various areas of the precipitate formed during the ^{33}S NMR experiments with internal Fe^{3+}: Weight %

	C-K	*O-K*	*Na-K*	*Al-K*	*P-K*	*S-K*	*Fe-K*
ppt area 1(1)_pt1	*26.37*	*45.07*	*0.18*	*0.08*	*9.17*	*1.47*	*17.67*
ppt area 1(1)_pt2	*22.84*	*46.49*	*0.20*	*0.06*	*9.96*	*1.49*	*18.95*
ppt area 1(1)_pt3	*21.84*	*46.85*		*0.10*	*10.13*	*1.59*	*19.49*
ppt area 1(1)_pt4	*21.34*	*46.62*	*0.19*	*0.10*	*10.23*	*1.59*	*19.91*

This journal is © The Royal Society of Chemistry 2014

Table S2 – Results of the EDX analysis on various areas of the precipitate formed during the ^{33}S NMR experiments with internal Fe^{3+}: Weight % Error (+/- 1 Sigma)

	C-K	O-K	Na-K	Al-K	P-K	S-K	Fe-K
ppt area 1(1)_pt1	*+/-0.18*	*+/-0.24*	*+/-0.02*	*+/-0.01*	*+/-0.07*	*+/-0.03*	*+/-0.18*
ppt area 1(1)_pt2	*+/-0.17*	*+/-0.25*	*+/-0.02*	*+/-0.01*	*+/-0.08*	*+/-0.03*	*+/-0.18*
ppt area 1(1)_pt3	*+/-0.17*	*+/-0.25*		*+/-0.02*	*+/-0.08*	*+/-0.03*	*+/-0.19*
ppt area 1(1)_pt4	*+/-0.17*	*+/-0.25*	*+/-0.03*	*+/-0.02*	*+/-0.08*	*+/-0.03*	*+/-0.19*

Table S3 – Results of the EDX analysis on various areas of the precipitate formed during the ^{33}S NMR experiments with internal Fe^{3+}: Normalized Wt. %

	C-K	O-K	Na-K	Al-K	P-K	S-K	Fe-K
ppt area 1(1)_pt1	*26.37*	*45.07*	*0.18*	*0.08*	*9.17*	*1.47*	*17.67*
ppt area 1(1)_pt2	*22.84*	*46.49*	*0.20*	*0.06*	*9.96*	*1.49*	*18.95*
ppt area 1(1)_pt3	*21.84*	*46.85*		*0.10*	*10.13*	*1.59*	*19.49*
ppt area 1(1)_pt4	*21.34*	*46.62*	*0.19*	*0.10*	*10.23*	*1.59*	*19.91*

Table S4 – Results of the EDX analysis on various areas of the precipitate formed during the ^{33}S NMR experiments with internal Fe^{3+}: Atom %

	C-K	O-K	Na-K	Al-K	P-K	S-K	Fe-K
ppt area 1(1)_pt1	*38.65*	*49.58*	*0.13*	*0.05*	*5.21*	*0.81*	*5.57*
ppt area 1(1)_pt2	*34.41*	*52.58*	*0.16*	*0.04*	*5.82*	*0.84*	*6.14*
ppt area 1(1)_pt3	*33.21*	*53.47*		*0.07*	*5.97*	*0.90*	*6.37*
ppt area 1(1)_pt4	*32.67*	*53.56*	*0.16*	*0.07*	*6.07*	*0.91*	*6.55*

S6. REFERENCES

1 N. Busschaert, P. A. Gale, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis and J. W. A. Harrell, *Chem. Commun.*, 2010, **46**, 6252.

2 N. Busschaert, M. Wenzel, M. E. Light, P. Iglesias-Hernández, R. Pérez-Tomás and P. A. Gale, *J. Am. Chem. Soc.*, 2011, **133**, 14136.