

A synergistic approach to anion antiport

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ELECTRONIC SUPPLEMENTARY INFORMATION

Vesicle Studies

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (22.32 mg/mL) (Genzyme) was evaporated under reduced pressure to give a thin film. The lipid film was dried under high vacuum for at least 2 hours and rehydrated with sodium chloride solution (489mM NaCl buffered to pH 7.2 with 20mM sodium phosphate salts) by vortexing. The lipid suspension was then subjected to nine freeze-thaw cycles and twenty-nine extrusions through a 200 nm polycarbonate nucleopore membrane using a LiposoFast Basic extruder (Avestin, Inc.) to obtain unilamellar vesicles. The liposomes underwent dialysis in sodium sulphate solution (167mM sodium sulphate buffered to pH 7.2 with 20mM sodium phosphate salts) for 2 hrs. The vesicles were diluted to 5mL with a solution of 167mM sodium sulphate, buffered to pH 7.2 with 20mM sodium phosphate salts to form a stock solution of lipid.

Samples for assay were prepared by diluting lipid stock solution to 5mL (using 167mM sodium sulphate, buffered to pH 7.2 with 20mM sodium phosphate salts) to give a solution of 1mM lipid. Chloride efflux was monitored using a chloride selective electrode (Accumet). Compounds were added at t=0s as solutions in DMSO, to give a 1:50 compound to lipid ratio (2mol%), except for compound **5** which was added at a 1:30,000 compound to lipid ratio (0.03mol%). In all cases the total volume of solution added was 20µL. At t=60s a bicarbonate ‘pulse’ was introduced to the system such that the concentration of sodium bicarbonate in the external solution was 40mM. The experiment was run for a further 10mins. At t=660s detergent (octaethylene glycol monododecyl ether) was added to allow the determination of 100% chloride efflux. Control experiments were performed using the same procedure, but without the sodium

bicarbonate pulse at $t=60$ s. 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 a known concentration.

Bicarbonate Pulse Experiments

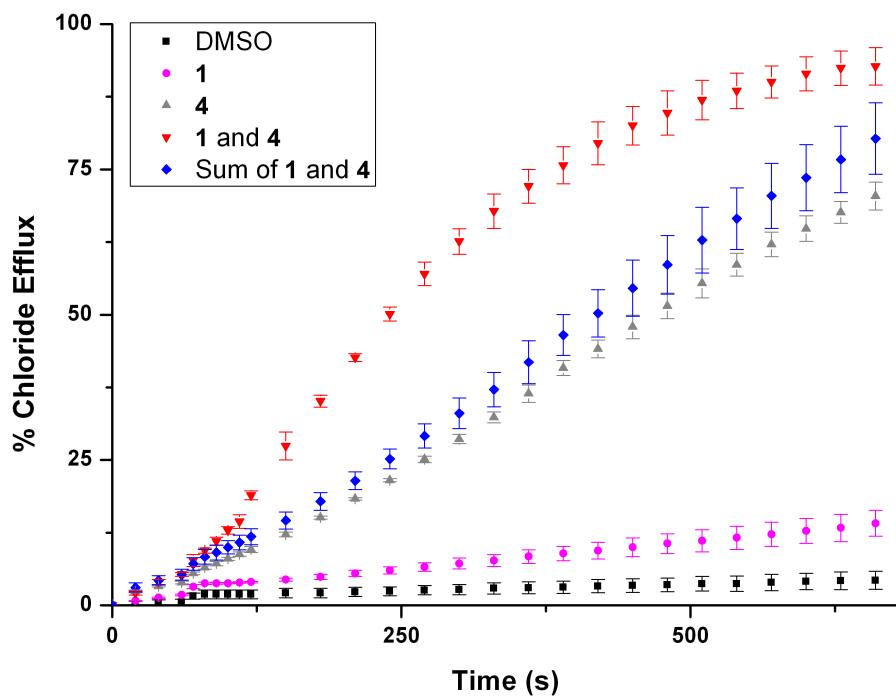


Figure S1 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **1** (2mol%), **4** (2mol%) and both **1** (2mol%) and **4** (2mol%). The corrected sum of **1** (2mol%) and **4** (2mol%) is shown for comparison. A sodium bicarbonate pulse was added at $t=60$ s such that the external bicarbonate concentration was 40mM. Each point represents an average of three trials.

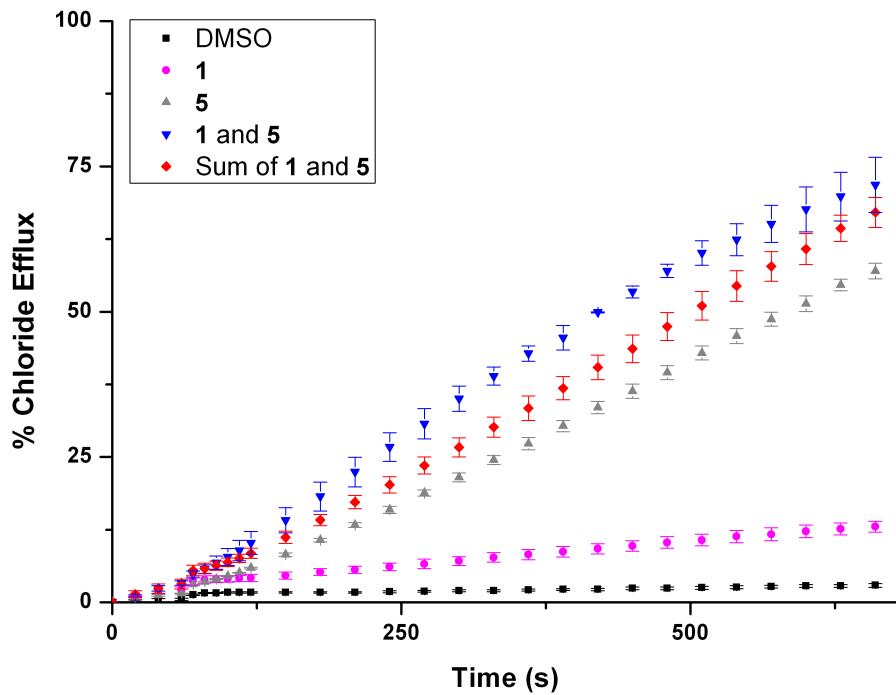


Figure S2 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **1** (2mol%), **5** (0.03mol%) and both **1** (2mol%) and **5** (0.03mol%). The corrected sum of **1** (2mol%) and **5** (0.03mol%) is shown for comparison. A sodium bicarbonate pulse was added at $t=60$ s such that the external bicarbonate concentration was 40mM. Each point represents an average of three trials.

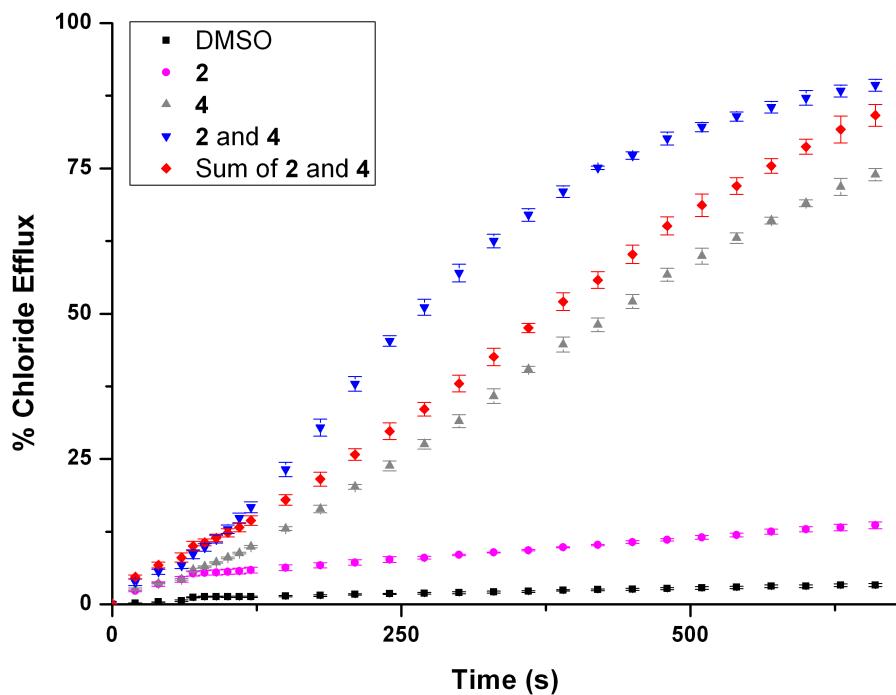


Figure S3 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **2** (2mol%), **4** (2mol%) and both **2** (2mol%) and **4** (2mol%). The corrected sum of **2** (2mol%) and **4** (2mol%) is shown for comparison. A sodium bicarbonate pulse was added at $t=60$ s such that the external bicarbonate concentration was 40mM. Each point represents an average of three trials.

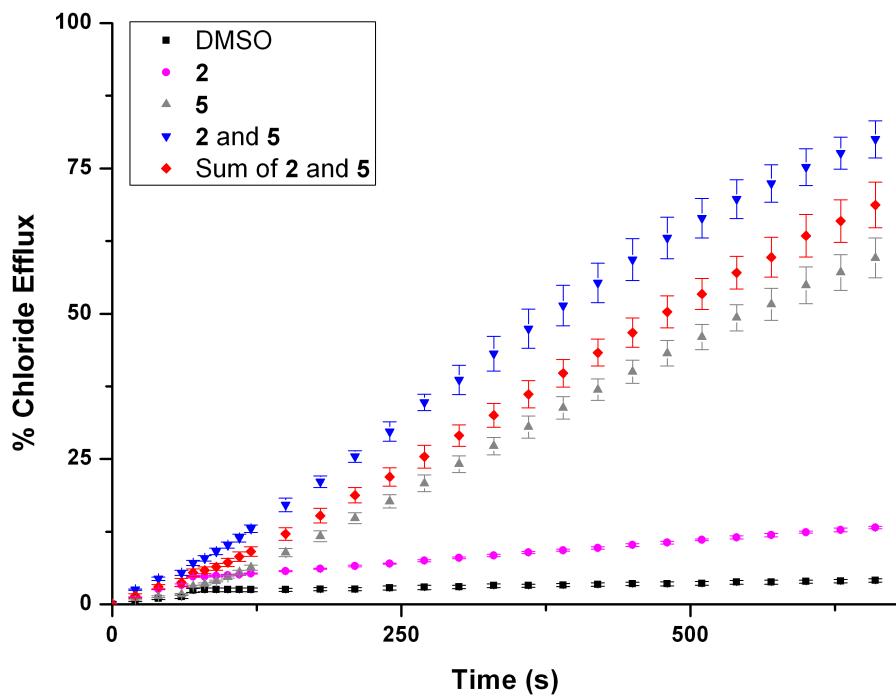


Figure S4 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **2** (2mol%), **5** (0.03mol%) and both **2** (2mol%) and **5** (0.03mol%). The corrected sum of **2** (2mol%) and **5** (0.03mol%) is shown for comparison. A sodium bicarbonate pulse was added at $t=60$ s such that the external bicarbonate concentration was 40mM. Each point represents an average of three trials.

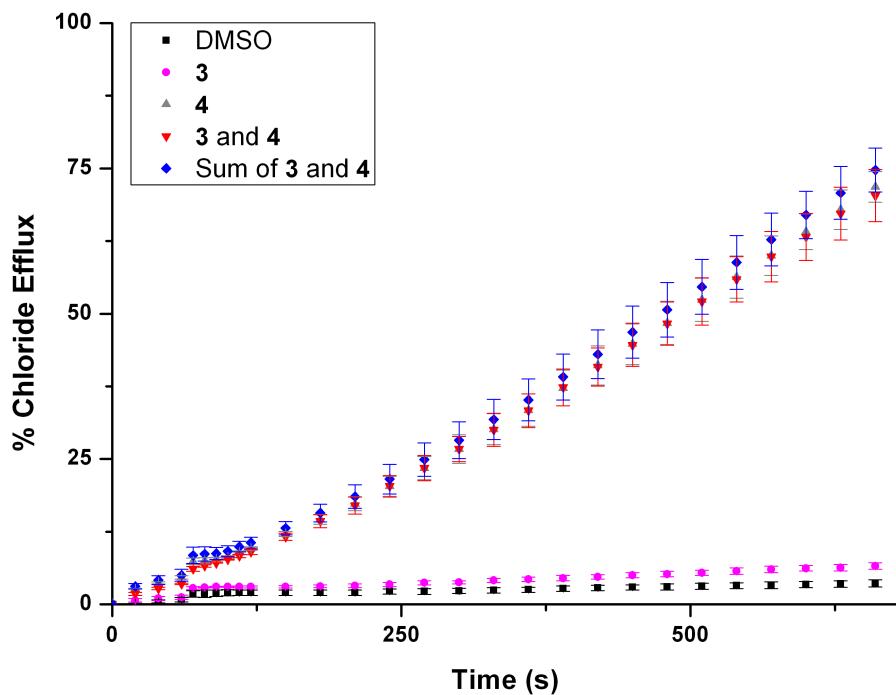


Figure S5 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **3** (2mol%), **4** (2mol%) and both **3** (2mol%) and **4** (2mol%). The corrected sum of **3** (2mol%) and **4** (2mol%) is shown for comparison. A sodium bicarbonate pulse was added at t=60s such that the external bicarbonate concentration was 40mM. Each point represents an average of three trials.

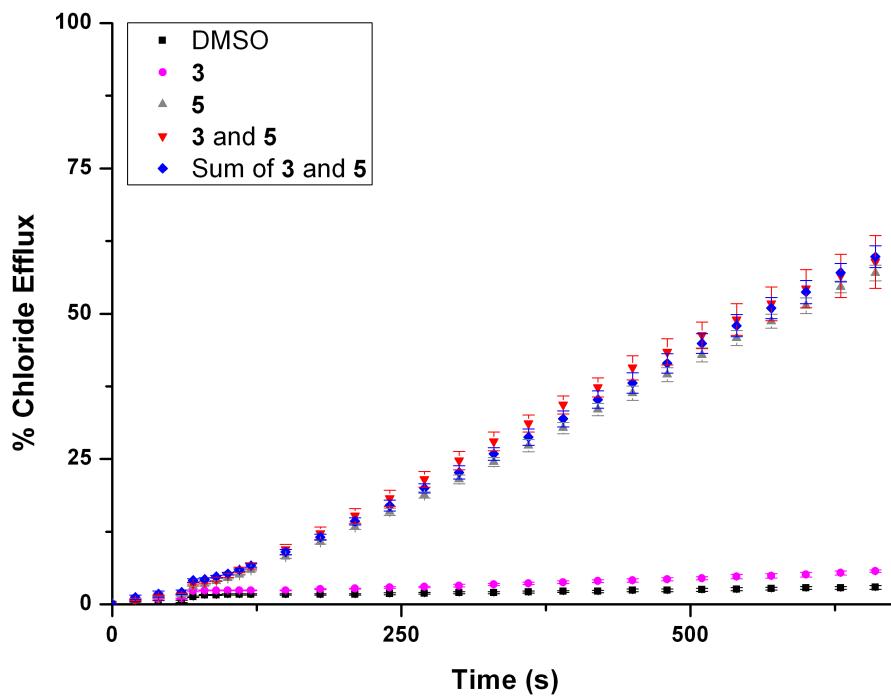


Figure S6 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **3** (2mol%), **5** (0.03mol%) and both **3** (2mol%) and **5** (0.03mol%). The corrected sum of **3** (2mol%) and **5** (0.03mol%) is shown for comparison. A sodium bicarbonate pulse was added at $t=60$ s such that the external bicarbonate concentration was 40mM. Each point represents an average of three trials.

Control Experiments

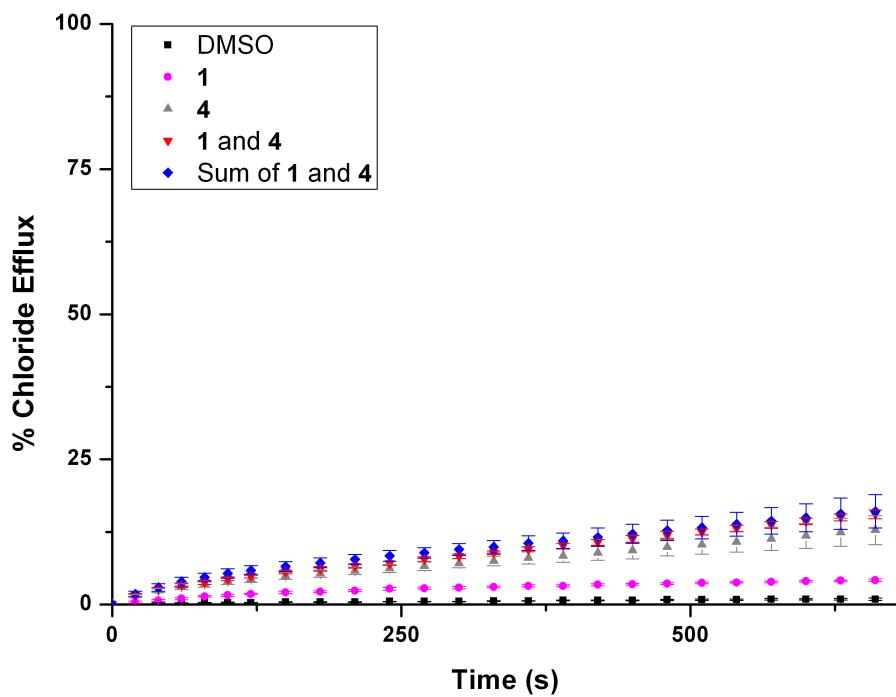


Figure S7 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **1** (2mol%), **4** (2mol%) and both **1** (2mol%) and **4** (2mol%). The corrected sum of **1** (2mol%) and **4** (2mol%) is shown for comparison. Each point represents an average of three trials.

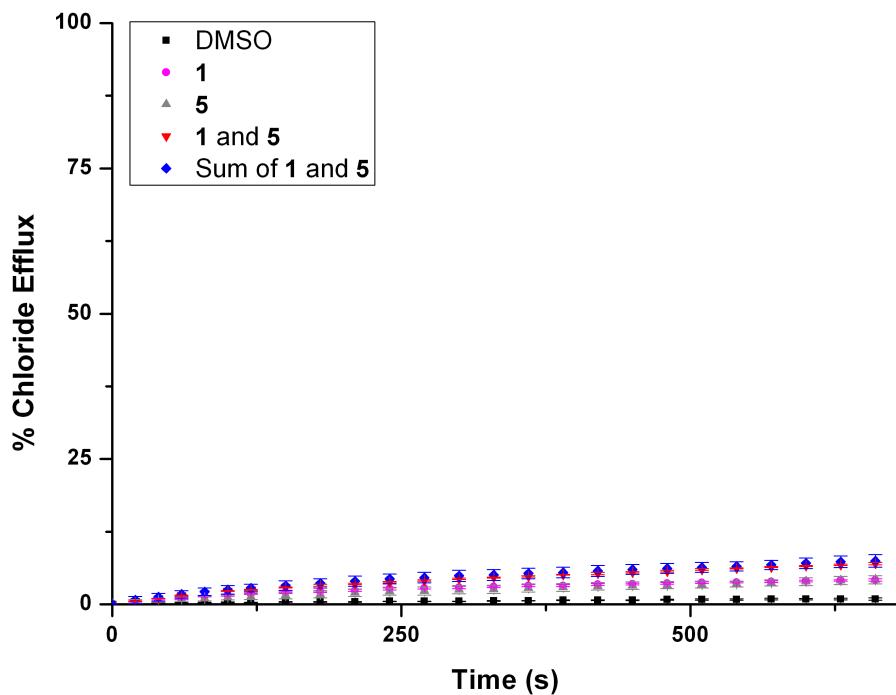


Figure S8 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **1** (2mol%), **5** (0.03mol%) and both **1** (2mol%) and **5** (0.03mol%). The corrected sum of **1** (2mol%) and **5** (0.03mol%) is shown for comparison. Each point represents an average of three trials.

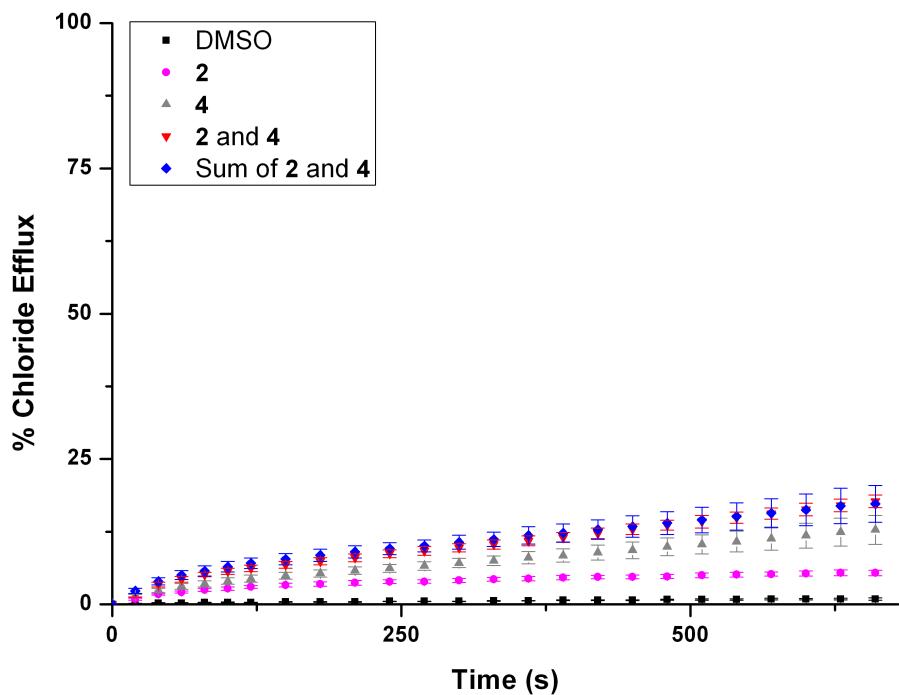


Figure S9 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **2** (2mol%), **4** (2mol%) and both **2** (2mol%) and **4** (2mol%). The corrected sum of **2** (2mol%) and **4** (2mol%) is shown for comparison. Each point represents an average of three trials.

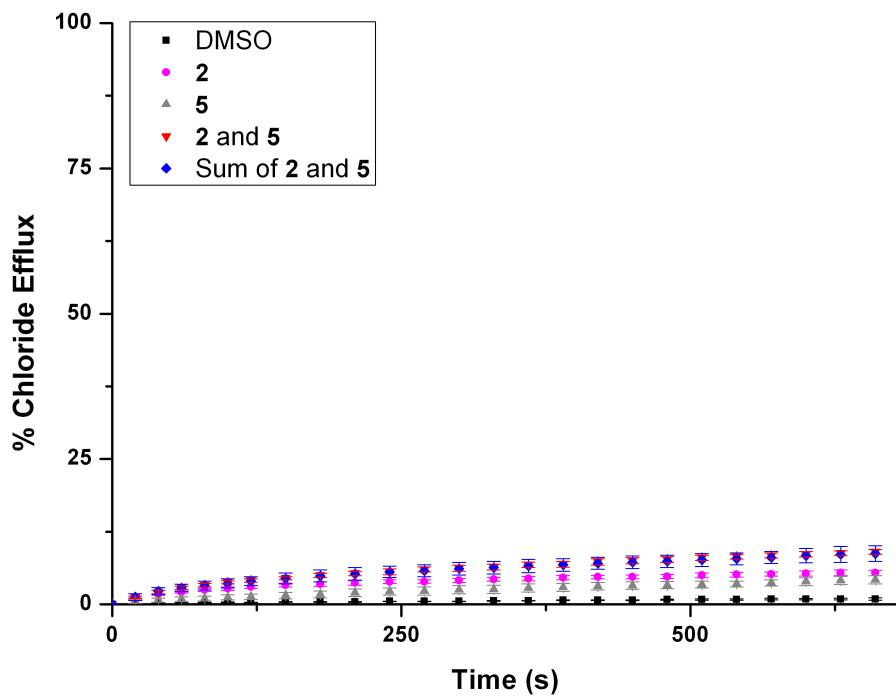


Figure S10 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **2** (2mol%), **5** (0.03mol%) and both **2** (2mol%) and **5** (0.03mol%). The corrected sum of **2** (2mol%) and **5** (0.03mol%) is shown for comparison. Each point represents an average of three trials.

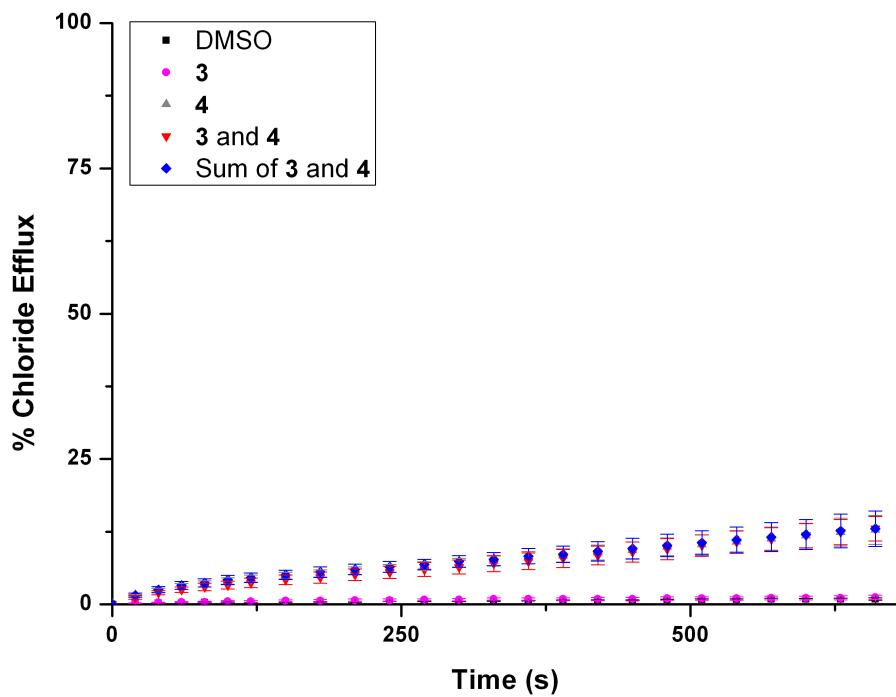


Figure S11 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **3** (2mol%), **4** (2mol%) and both **3** (2mol%) and **4** (2mol%). The corrected sum of **3** (2mol%) and **4** (2mol%) is shown for comparison. Each point represents an average of three trials.

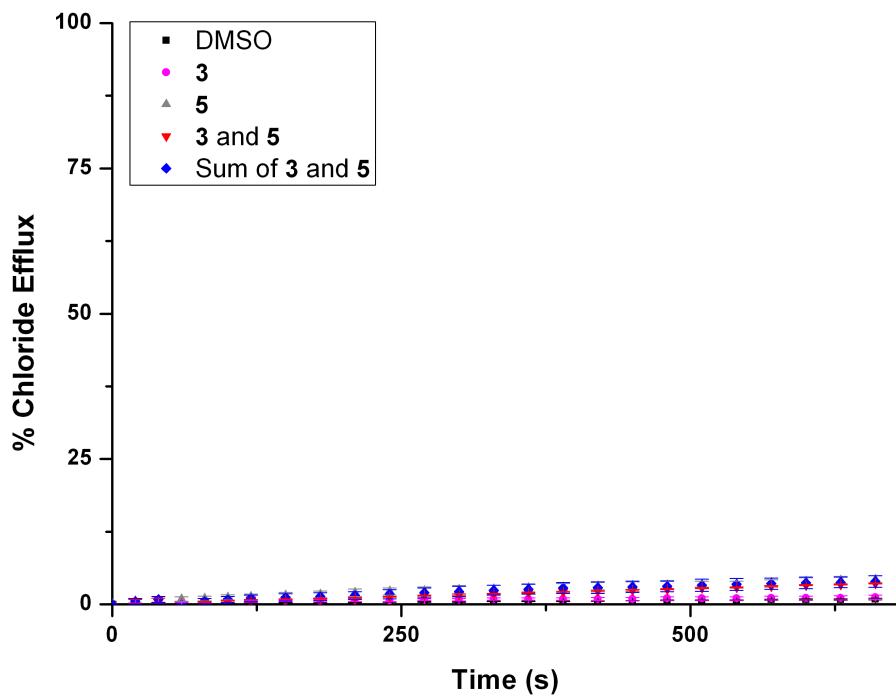


Figure S12 Chloride efflux from unilamellar POPC vesicles containing 489mM sodium chloride, buffered to pH 7.2 with 20mM sodium phosphate salts and suspended in 167mM sodium sulfate, buffered to pH 7.2 with 20mM sodium phosphate salts, upon addition of DMSO solutions of **3** (2mol%), **5** (0.03mol%) and both **3** (2mol%) and **5** (0.03mol%). The corrected sum of **3** (2mol%) and **5** (0.03mol%) is shown for comparison. Each point represents an average of three trials.