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## **Supplementary Information**

# Anion transport by ortho-phenylene bis-ureas across cell and vesicle membranes

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#### 1. Synthesis and Characterisation

#### **1.1. General Considerations**

All commercially available materials were used without further purification. DCM was dried by passing through a modified Grubbs system<sup>1</sup> manufactured by Anhydrous Engineering. Anhydrous DMF was purchased from Alfa Aesar and stored over molecular sieves. Reactions were monitored by TLC using Merck silica gel 60 F<sub>254</sub> TLC plates. Spots were visualised under ultraviolet light (254 nm) and by staining with KMnO<sub>4</sub> or ninhydrin. Flash column chromatography was performed on silica gel (technical grade, 40-63 μm particle size).

<sup>1</sup>H, <sup>13</sup>C and <sup>19</sup>F NMR spectra were recorded on Bruker Avance III HD 500 (carbon sensitive), Bruker Ascend 400, JEOL ECS 400 or JEOL ECS 300 spectrometers. Chemical shifts ( $\delta$ /ppm) are referenced relative to residual solvent signals in the deuterated solvent. Data are reported as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, p = pentet, hept = heptet, m = multiplet, br = broad.

Infrared (IR) spectra were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer, with signal intensities reported as follows: w = weak, m = medium, s = strong, br = broad.

Mass spectrometry was performed on a Bruker micrOTOF II spectrometer using electrospray ionisation (positive ion mode).

#### **1.2. Synthetic Procedures**

#### 4b: 1,1'-(4,5-dichloro-1,2-phenylene)bis(3-(3,5-bis(trifluoromethyl)phenyl)urea)



To a solution of 4,5-dichloro-*o*-phenylenediamine (50 mg, 0.282 mmol) in anhydrous DCM (10 mL) was added 3,5-bis(trifluoromethyl)phenyl isocyanate (196  $\mu$ L, 1.13 mmol) and the resultant solution refluxed under N<sub>2</sub> for 18.5 h. After this time, a precipitate had formed which was filtered, washed with DCM and dried under high vacuum to give the title compound as an off-white solid (165 mg, 85%). *R*<sub>f</sub> 0.61 (1:1 EtOAc–hexane). <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>)  $\delta$  9.12 (br s, 2H, **H6**), 8.20 (br s, 2H, **H4**), 8.15 (s, 4H, **H8**), 7.95 (s, 2H, **H2**), 7.63 (s, 2H, **H10**). <sup>13</sup>C NMR (126 MHz, acetone-*d*<sub>6</sub>)  $\delta$  153.8 (**C5**), 142.4 (**C7**), 132.5 (q, *J* =

33.1 Hz, **C9**), 132.2 (**C3**), 128.4 (**C1**), 126.9 (**C2**), 124.4 (q, *J* = 272.0 Hz, **C11**), 119.4 (q, *J* = 4.1 Hz, **C8**), 116.1 (hept, *J* = 3.9 Hz, **C10**). <sup>19</sup>F NMR (377 MHz, acetone-*d*<sub>6</sub>) δ -63.6 (s, 12F, 11-C**F**<sub>3</sub>). IR (neat) *v*/cm<sup>-1</sup> 3314 (br w, N–H), 3024 (w, C–H), 2973 (w, C–H), 1738 (br m), 1658 (br m), 1575 (br m), 1470 (m), 1373 (s), 1274 (s), 1173 (m), 1124 (s). HRMS (ESI) *m/z* calcd for  $C_{24}H_{12}Cl_2F_{12}N_4NaO_2$  [M+Na]<sup>+</sup> 709.0038, found 709.0021.

#### 4c: 1,1'-(4-(trifluoromethyl)-1,2-phenylene)bis(3-(3,5-bis(trifluoromethyl)phenyl)urea)



To a suspension of 3,4-diaminobenzotrifluoride (50 mg, 0.284 mmol) in anhydrous DCM (5 mL) was added 3,5-bis(trifluoromethyl)phenyl isocyanate (196  $\mu$ L, 1.14 mmol) and the resultant brown suspension left to reflux under N<sub>2</sub> for 3 h. The reaction mixture was allowed to cool to r.t. and the resultant precipitate was filtered, washed with DCM (x10) and dried under high vacuum to give a pale brown solid. This was purified by flash column chromatography (SiO<sub>2</sub>; 1 $\rightarrow$ 10% MeOH in DCM) to give the title compound as an off-white solid (59 mg, 30%). *R*<sub>f</sub> 0.10 (1:99 MeOH–DCM). <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>)  $\delta$  9.18 (br s, 2H, **H7** & **H18**), 8.38 (br s, 1H, **H16**), 8.24 (br s, 1H, **H5**), 8.17 (s, 4H, **H9** & **H20**), 8.02 – 7.99 (m, 2H, **H3** & **H14**), 7.64 (s, 2H, **H12** & **H23**), 7.55 (dd, *J* = 8.6, 2.2 Hz, 1H, **H13**). <sup>13</sup>C NMR (126 MHz, acetone-*d*<sub>6</sub>)  $\delta$  154.0 (**C17**), 153.6 (**C6**), 142.6 (**C8** & **C19**), 136.4 (**C15**), 132.6 (q, *J* = 33.0 Hz, **C10** & **C21**), 131.8 (**C4**), 126.9 (q, *J* = 32.6 Hz, **C2**), 125.5 (**C14**), 125.1 (q, *J* = 271.0 Hz, **C1**), 124.4 (q, *J* = 271.9 Hz, **C11** & **C22**), 123.3 (**C3**), 123.1 (q, *J* = 4.1 Hz, **C13**), 119.3 (**C9** & **C20**), 116.2 – 115.9 (m, **C12** & **C23**). <sup>19</sup>F NMR (377 MHz, acetone-*d*<sub>6</sub>)  $\delta$  -62.56 (s, 3F, 1-C**F**<sub>3</sub>), -63.55 (s, 6F, C**F**<sub>3</sub>), -63.56 (s, 6F, C**F**<sub>3</sub>), IR (neat) *v*/cm<sup>-1</sup> 3392 (br w, N–H), 3269 (br w, N–H), 3097 (w), 3011 (w, C–H), 1652 (m), 1471 (m), 1383 (m), 1277 (s), 1170 (m), 1125 (s). HRMS (ESI) *m/z* calcd for C<sub>25</sub>H<sub>13</sub>F<sub>15</sub>N<sub>4</sub>NaO<sub>2</sub> [M+Na]<sup>+</sup> 709.0691, found 709.0711.

4d: methyl 3,4-bis(3-(3,5-bis(trifluoromethyl)phenyl)ureido)benzoate



To a solution of methyl 3,4-diaminobenzoate (50 mg, 0.301 mmol) in anhydrous DCM (5 mL) was added 3,5-bis(trifluoromethyl)phenyl isocyanate (208 µL, 1.20 mmol) and the resultant cream suspension left to reflux under N<sub>2</sub> for 3 h. The reaction mixture was allowed to cool to r.t. and the resultant precipitate was filtered, washed with DCM (x10) and dried under high vacuum to give a white solid (147 mg).<sup>+</sup> This was suspended in anhydrous DCM (10 mL) and further 3,5-bis(trifluoromethyl)phenyl isocyanate (100 µL, 0.579 mmol) was added and the resultant white suspension left to reflux gently for 5 h. The reaction mixture was allowed to cool to r.t. and the resultant precipitate was filtered, washed with DCM (x10) and dried under high vacuum to give the title compound as a white solid (140 mg, 69%).  $R_{\rm f}$  0.48 (1:1 EtOAc-hexane). <sup>1</sup>H NMR (500 MHz, acetone-d<sub>6</sub>) δ 9.18 (br s, 1H, **H19**), 9.11 (br s, 1H, **H8**), 8.42 (br s, 1H, H17), 8.18 (s, 2H, H10), 8.17 (d, J = 1.9 Hz, 1H, H4), 8.15 (s, 2H, H21), 8.12 (br s, 1H, H6), 8.01 (d, J = 8.5 Hz, 1H, H15), 7.87 (dd, J = 8.6, 2.0 Hz, 1H, H14), 7.62 (br d, 2H, H13 & H24) 3.86 (s, 3H, H1). <sup>13</sup>C NMR (126 MHz, acetone-d<sub>6</sub>) δ 166.5 (C2), 154.5 (C7), 153.4 (C18), 142.7 (C9), 142.5 (C20), 138.3 (C16), 132.5 (q, J = 33.0 Hz, C11 & C22), 130.0 (C5), 128.4 (C4), 128.1 (C14), 126.8 (C3), 124.4 (q, J = 271.9 Hz, C12 & C23), 123.7 (C15), 119.3 (C10 & C21), 116.0 (C13 & C24), 52.3 (C1). <sup>19</sup>F NMR (283 MHz, acetone-d<sub>6</sub>) δ -63.54 (s, 6F, CF<sub>3</sub>), -63.55 (s, 6F, CF<sub>3</sub>). IR (neat) v/cm<sup>-1</sup> 3675 (br w), 3317 (br w, N–H), 2988 (m, C–H), 2973 (m, C-H), 2901 (m, C-H), 1731 (w, C=O ester), 1649 (w), 1577 (m), 1473 (m), 1383 (s), 1274 (s), 1124 (s), 1066 (s). HRMS (ESI) *m/z* calcd for C<sub>26</sub>H<sub>16</sub>F<sub>12</sub>N<sub>4</sub>NaO<sub>4</sub> [M+Na]<sup>+</sup> 699.0872, found 699.0867.

<sup>&</sup>lt;sup>+</sup> The solid was found to be impure by TLC and NMR analysis (mono-urea was observed). During the reaction it was found that a precipitate was forming on the sides of the flask. This would inhibit the efficient reaction of the mono-urea with the isocyanate. Thus more DCM was used (10 mL) and the suspension was refluxed *gently* to ensure that all reactants remained in the solvent, maximising the likelihood of the isocyanate coming into contact with the mono-urea.

4e: ethyl 3,4-bis(3-(3,5-bis(trifluoromethyl)phenyl)ureido)benzoate



To a solution of ethyl 3,4-diaminobenzoate (50 mg, 0.277 mmol) in anhydrous DCM (5 mL) was added 3,5-bis(trifluoromethyl)phenyl isocyanate (192  $\mu$ L, 1.11 mmol) and the resultant pale brown suspension left to reflux under N<sub>2</sub> for 3 h. The reaction mixture was allowed to cool to r.t. and the resultant precipitate was filtered, washed with DCM (x10) and dried under high vacuum to give the title compound as an off-white solid (172 mg, 90%). *R*<sub>f</sub> 0.66 (1:1 EtOAc–hexane). <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>)  $\delta$  9.18 (br s, 1H, **H20**), 9.10 (br s, 1H, **H9**), 8.42 (br s, 1H, **H18**), 8.18 (br s, 2H, **H11**), 8.16 – 8.14 (m, 3H, **H5** & **H22**), 8.12 (br s, 1H, **H7**), 8.02 (d, *J* = 8.5 Hz, 1H, **H16**), 7.88 (dd, *J* = 8.5, 2.0 Hz, 1H, **H15**), 7.63 (br s, 2H, **H14** & **H25**), 4.34 (q, *J* = 7.1 Hz, 2H, **H2**), 1.35 (t, *J* = 7.1 Hz, 3H, **H1**). <sup>13</sup>C NMR (126 MHz, acetone-*d*<sub>6</sub>)  $\delta$  166.0 (**C3**), 154.5 (**C8**), 153.4 (**C19**), 142.7 (**C10**), 142.5 (**C21**) 138.4 (**C17**), 132.5 (q, *J* = 33.0 Hz, **C12** & **C23**), 130.0 (**C6**), 128.4 (**C5**), 128.1 (**C15**), 127.2 (**C4**), 124.4 (q, *J* = 271.5 Hz, **C13** & **C24**) 123.7 (**C16**), 119.3 (**C11** & **C22**), 116.0 (m, **C14** & **C25**), 61.5 (**C2**), 14.6 (**C1**). <sup>19</sup>F NMR (283 MHz, acetone-*d*<sub>6</sub>)  $\delta$  -63.54 (s, 6F, **CF**<sub>3</sub>), -63.55 (s, 6F, **CF**<sub>3</sub>). IR (neat) v/cm<sup>-1</sup> 3675 (br w), 3323 (br w, N–H), 2988 (m, C–H), 2976 (m, C–H), 2901 (m, C–H), 1710 (w, C=O ester), 1667 (w), 1575 (w), 1472 (w), 138.2 (m), 1274 (m), 1066 (s). HRMS (ESI) *m/z* calcd for C<sub>27</sub>H<sub>18</sub>F<sub>12</sub>N<sub>4</sub>NaO<sub>4</sub> [M+Na]<sup>+</sup> 713.1029, found 713.1031.

#### hexyl 3,4-diaminobenzoate



To a mixture of 3,4-diaminobenzoic acid (100 mg, 0.66 mmol) and  $K_2CO_3$  (91 mg, 0.66 mmol) was added anhydrous DMF (10 mL) and the resultant brown suspension stirred at 160 °C under N<sub>2</sub> for 10 min. The reaction mixture was allowed to cool to r.t. and 1-iodohexane (97  $\mu$ L, 0.66 mmol) was then added. The brown suspension was stirred at 70 °C for 18 h and then allowed to cool to r.t. before partitioning between H<sub>2</sub>O (20 mL) and EtOAc (60 mL). The organic phase was washed with H<sub>2</sub>O (20 mL), brine (20 mL), dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo* to give a brown oil. This was purified by flash column chromatography (SiO<sub>2</sub>; 1:9  $\rightarrow$  1:1 EtOAc–hexane) to give the title compound as a beige solid (49 mg, 32%). *R*<sub>f</sub> 0.34 (1:1 EtOAc–hexane). <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>)  $\delta$  7.34 (d, *J* = 1.9 Hz, 1H, **H13**), 7.27 (dd, *J* = 8.1, 2.0 Hz, 1H, **H9**), 6.66 (d, *J* = 8.1 Hz, 1H, **H10**), 4.76 (br s, 2H, **H15**), 4.21 (br s, 2H, **H14**), 4.18 (t, *J* = 6.6 Hz, 2H, **H6**), 1.70 (p, *J* = 6.8 Hz, 2H, **H5**), 1.43 (p, *J* = 7.1 Hz, 2H, **H4**), 1.38–1.30 (m, 4H, **H2** & **H3**), 0.89 (t, *J* = 7.5 Hz, 3H, **H1**). <sup>13</sup>C NMR (126 MHz, acetone-*d*<sub>6</sub>)  $\delta$  167.3 (**C7**), 141.6 (**C11**), 134.8 (**C12**), 122.2 (**C9**), 120.5 (**C8**), 117.2 (**C13**), 114.4 (**C10**), 64.4 (**C6**), 32.3 (**C3**), 29.6 (**C5**)<sup>‡</sup>, 26.5 (**C4**), 23.3 (**C2**), 14.3 (**C1**). IR (neat) *v*/cm<sup>-1</sup> 3675 (br w), 3387 (w, N–H), 3325 (w, N–H), 3186 (w, N–H), 2956 (m, C–H), 2928 (m, C–H), 1683 (s, C=O), 1626 (m), 1589 (m), 1445 (m), 1313 (m), 1294 (s), 1226 (s), 1109 (m). HRMS (ESI) *m/z* calcd for C<sub>13</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 237.1598, found 237.1595.

#### 4f: hexyl 3,4-bis(3-(3,5-bis(trifluoromethyl)phenyl)ureido)benzoate



To a solution of hexyl 3,4-diaminobenzoate (20 mg, 0.08 mmol) in anhydrous DCM (5 mL) was added 3,5-bis(trifluoromethyl)phenyl isocyanate (59  $\mu$ L, 0.34 mmol) and the resultant yellow solution left to reflux under N<sub>2</sub> for 4.5 h. The reaction mixture was allowed to cool to r.t. and then concentrated *in vacuo* to give a brown suspension which was purified by flash column chromatography (SiO<sub>2</sub>; 1:99 MeOH–DCM). The fractions containing the title compound were combined and concentrated *in vacuo* to give an off-white solid (65 mg). This was further purified by triturating the solid with cold DCM and drying under high vacuum to give the title compound as an off-white solid (57 mg, 90%). *R*<sub>f</sub> 0.21 (1:4 EtOAc–hexane). <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>)  $\delta$  9.24 (br s, 1H, **H16**), 9.14 (br s, 1H, **H24**), 8.45 (br s, 1H, **H14**), 8.20 – 8.17 (m, 3H, **H9 & H26**), 8.16 (s, **H18**), 8.15 (br s, 1H, **H22**), 8.02 (d, *J* = 8.6 Hz, 1H, **H12**), 7.89 (dd, *J* = 8.5, 2.0 Hz, 2H, **H11**), 7.65 – 7.62 (m, 2H, **H21 & H29**), 4.30 (t, *J* = 6.6 Hz, 2H, **H6**), 1.79 – 1.73 (m, 2H, **H5**), 1.51 – 1.42 (m, 2H, **H4**), 1.40 – 1.30 (m, 4H, **H2 & H3**), 0.89 (t, *J* = 7.1 Hz, 3H, **H1**). <sup>13</sup>C NMR (126 MHz, acetone-*d*<sub>6</sub>)  $\delta$  166.1 (**C7**), 154.3 (**C23**), 153.4 (**C15**), 142.8 (**C25**), 142.5 (**C17**), 138.2 (**C13**),

<sup>&</sup>lt;sup>‡</sup> Assigned using HSQC/HMBC (overlaps with solvent peak).

132.5 (q, J = 33.0 Hz, **C19** & **C27**), 130.1 (**C10**), 128.3 (**C9**), 128.0 (**C11**), 127.1 (**C8**), 124.4 (q, J = 271.0 Hz, **C20** & **C28**), 123.7 (**C12**), 119.3 (**C18** & **C26**), 116.3 – 115.6 (m, **C21** & **C29**), 65.6 (**C6**), 32.2 (**C3**), 29.6 (**C5**)<sup>‡</sup>, 26.4 (**C4**), 23.2 (**C2**), 14.3 (**C1**). <sup>19</sup>F NMR (283 MHz, acetone- $d_6$ )  $\delta$  -63.54 (s, 6F, C**F**<sub>3</sub>), -63.55 (s, 6F, C**F**<sub>3</sub>). IR (neat)  $v/\text{cm}^{-1}$  3675 (br w), 3320 (br w, N–H), 2988 (m, C–H), 2971 (m, C–H), 2903 (m, C–H), 1725 (w, C=O ester), 1652 (m), 1574 (m), 1472 (m), 1382 (s), 1274 (s), 1173 (s), 1125 (s), 1056 (s). HRMS (ESI) m/z calcd for C<sub>31</sub>H<sub>26</sub>F<sub>12</sub>N<sub>4</sub>NaO<sub>4</sub> [M+Na]<sup>+</sup> 769.1655, found 769.1679.

#### octyl 3,4-diaminobenzoate



This compound was prepared according to a literature procedure<sup>2</sup> with minor modifications. To a 5 mL round bottom flask was added Al<sub>2</sub>O<sub>3</sub> (acidic, activated, 310 mg, 3 mmol) and MeSO<sub>3</sub>H (1 mL, 15 mmol) and the resultant white suspension stirred at r.t. for 5 min. 3,4-diaminobenzoic acid (100 mg, 0.66 mmol) was added to the suspension followed by 1-octanol (103 µL, 0.66 mmol) and the resultant dark brown suspension stirred at 80 °C under N₂ for 3.5 h. The reaction mixture was allowed to cool to r.t. and then poured into H<sub>2</sub>O (20 mL) and extracted with CHCl<sub>3</sub> (50 mL). The organic layer was washed with NaHCO<sub>3</sub> (2 x 20 mL), dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo* to give a beige solid. This was purified by flash column chromatography (SiO<sub>2</sub>; 1:99 MeOH–DCM) to give the title compound as an off-white solid (73 mg, 42%). R<sub>f</sub> 0.35 (1:1 EtOAc-hexane). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.47 (dd, J = 8.1, 1.9 Hz, 1H, H11), 7.41 (d, J = 1.9 Hz, 1H, H14), 6.68 (d, J = 8.1 Hz, 1H, H12), 4.24 (t, J = 6.7 Hz, 2H, H8), 3.55 (br s, 4H, H16 & H17), 1.73 (p, J = 6.9 Hz, 2H, H7), 1.46 – 1.38 (m, 2H, H6), 1.38 – 1.21 (m, 8H, H2, H3, H4 & H5), 0.88 (t, J = 6.8 Hz, 3H, H1). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  167.1 (**C9**), 140.4 (**C13**), 133.2 (**C15**), 123.4 (**C11**), 121.8 (C10), 118.5 (C14), 115.1 (C12), 64.8 (C8), 32.0 (C3), 29.44 (C5), 29.37 (C4), 29.0 (C7), 26.2 (C6), 22.8 (C2), 14.3 (C1). IR (neat) v/cm<sup>-1</sup> 3440 (br w, N-H), 3388 (br w, N-H), 3327 (br w, N-H), 3187 (br w, N–H), 2954 (m, C–H), 2920 (m, C–H), 2852 (m, C–H), 1683 (s, C=O), 1626 (m), 1589 (m), 1445 (m), 1312 (s), 1294 (s), 1228 (s), 1111 (m). HRMS (ESI) *m*/*z* calcd for C<sub>15</sub>H<sub>25</sub>N<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 265.1911, found 265.1916.

<sup>&</sup>lt;sup>‡</sup> Assigned using HSQC/HMBC (overlaps with solvent peak).

4g: octyl 3,4-bis(3-(3,5-bis(trifluoromethyl)phenyl)ureido)benzoate



To a solution of octyl 3,4-diaminobenzoate (20 mg, 0.08 mmol) in anhydrous DCM (5 mL) was added 3,5-bis(trifluoromethyl)phenyl isocyanate (52  $\mu$ L, 0.30 mmol) and the resultant pale yellow solution left to reflux under N<sub>2</sub> for 6 h. The reaction mixture was allowed to cool to r.t. and the resultant precipitate filtered, washed with cold DCM (x5) and dried under high vacuum to give the title compound as a white solid (51 mg, 88%). R<sub>f</sub> 0.20 (1:4 EtOAc-hexane). <sup>1</sup>H NMR (400 MHz, acetone-d<sub>6</sub>) δ 9.20 (br s, 1H, H18), 9.10 (br s, 1H, H26), 8.43 (br s, 1H, H16), 8.19 (s, 2H, H28), 8.18 (d, J = 1.9 Hz, 1H, H14), 8.16 (s, 2H, H20), 8.12 (br s, 1H, H24), 8.02 (d, J = 8.6 Hz, 1H, H11), 7.89 (dd, J = 8.5, 2.0 Hz, 1H, H10), 7.63 (br s, 2H, H23) & H30), 4.31 (t, J = 6.6 Hz, 2H, H8), 1.82 – 1.72 (m, 2H, H7), 1.47 (p, J = 6.6 Hz, 2H, H6), 1.42 – 1.25 (m, 8H, H2, H3, H4 & H5), 0.87 (t, J = 7.0 Hz, 3H, H1). <sup>13</sup>C NMR (126 MHz, acetone- $d_6$ )  $\delta$  166.1 (C9), 154.4 (C25), 153.4 (C17), 142.8 (C27), 142.6 (C19), 138.3 (C12), 132.5 (q, J = 33.0 Hz, C21 & C29), 130.1 (C15), 128.3 (C14), 128.0 (C10), 127.2 (C13), 124.4 (q, J = 271.0 Hz, C22 & C31) 123.7 (C11), 119.3 (C20 & C28), 116.1 – 115.8 (m, C23 & C30), 65.6 (C8), 32.5 (C3), 29.9 (C5)<sup>+</sup>, 29.8 (C4)<sup>+</sup>, 29.4 (C7)<sup>+</sup>, 26.8 (C6), 23.3 (C2), 14.3 (C1). <sup>19</sup>F NMR (470 MHz, acetone- $d_6$ )  $\delta$  -63.64 (s, 6F, CF<sub>3</sub>), -63.65 (s, 6F, CF<sub>3</sub>). IR (neat) v/cm<sup>-1</sup> 3317 (br w, N-H), 2964 (w, C-H), 2932 (w, C-H), 2860 (w, C-H), 1726 (w, C=O ester), 1645 (m), 1574 (m), 1472 (m), 1383 (m), 1274 (s), 1173 (m), 1126 (s). HRMS (ESI) *m/z* calcd for C<sub>33</sub>H<sub>30</sub>F<sub>12</sub>N<sub>4</sub>NaO<sub>4</sub> [M+Na]<sup>+</sup> 797.1968, found 797.2000.

<sup>&</sup>lt;sup>‡</sup> Assigned using HSQC/HMBC (overlaps with solvent peak).



**Figure S1.** <sup>1</sup>H NMR spectrum of **4b** in acetone-*d*<sub>6</sub>.





**Figure S3.** <sup>1</sup>H NMR spectrum of **4c** in acetone- $d_6$ .



**Figure S4.** <sup>13</sup>C NMR spectrum of **4c** in acetone- $d_6$ .



**Figure S5.** <sup>1</sup>H NMR spectrum of **4d** in acetone- $d_6$ .



Figure S6. <sup>13</sup>C NMR spectrum of 4d in acetone- $d_6$ .

S12



**Figure S7.** <sup>1</sup>H NMR spectrum of **4e** in acetone- $d_6$ .



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**Figure S9.** <sup>1</sup>H NMR spectrum of **hexyl 3,4-diaminobenzoate** in acetone-*d*<sub>6</sub>.



Figure S10. <sup>13</sup>C NMR spectrum of hexyl 3,4-diaminobenzoate in acetone-*d*<sub>6</sub>.



**Figure S11.** <sup>1</sup>H NMR spectrum of **4f** in acetone-*d*<sub>6</sub>.



Figure S12. <sup>13</sup>C NMR spectrum of 4f in acetone- $d_6$ .



Figure S13. <sup>1</sup>H NMR spectrum of octyl 3,4-diaminobenzoate in CDCl<sub>3</sub>.



Figure S14. <sup>13</sup>C NMR spectrum of octyl 3,4-diaminobenzoate in CDCl<sub>3</sub>.



**Figure S15.** <sup>1</sup>H NMR spectrum of **4g** in acetone- $d_6$ .



**Figure S16.** <sup>13</sup>C NMR spectrum of **4g** in acetone- $d_6$ .

#### 2. Determination of Chloride Binding Affinities through <sup>1</sup>H NMR Titrations

#### 2.1. Method

Binding constants were measured by titrating n-Bu<sub>4</sub>N<sup>+</sup>Cl<sup>-</sup> into a solution of the host in DMSO- $d_6/0.5\%$ H<sub>2</sub>O at 298 K. A stock solution of the host (~2 mM) in Millipore water (10 µL) and DMSO- $d_6$  (1.99 mL) was prepared. A stock solution of the guest was prepared by dissolving n-Bu<sub>4</sub>N<sup>+</sup>Cl<sup>-</sup> (previously dried under high vacuum) in the aforementioned stock solution of host (1 mL). The concentration of the guest stock solution was varied over different experiments. The host solution (500 µL) was transferred to an NMR tube and an initial NMR spectrum recorded (Varian VNMRS 500 spectrometer). Thereafter, aliquots of the guest solution were added to the NMR tube and a spectrum recorded after each addition.

The shift of two NH signals were followed and the 2x 18–20 measured data points fitted to a 1:2 (host:guest) binding model (Nelder-Mead method) using the Bindfit v0.5 applet (available as freeware from Supramolecular.org).<sup>++</sup> The binding constants obtained are reported in the main text (Table 1) and the fits to the most downfield NH signal are shown in Figures S18, S21, S24, S27, S30, S33 and S36.

As mentioned in the main text, binding stoichiometries were determined through residual distribution analysis, using the approach outlined by Jurczak<sup>3</sup> and Thordarson.<sup>4,5</sup> Here the titration data from each experiment were fitted to all reasonable binding models (1:1, 1:2 and 2:1) and the residual distribution plots visually compared (see Figures S19, S22, S25, S28, S31, S34 and S37). For all receptors tested, a regular, sinusoidal distribution of the residuals was observed when fitting to either 1:1 or 2:1 (host:guest) models, indicating that neither of these models are correct. However, when data were fitted to a 1:2 (host:guest) model, a stochastic distribution of residuals with low amplitude was observed in all cases, indicating that this is the most likely mode of binding.

<sup>&</sup>lt;sup>++</sup> Accessed on 20 October 2017.



#### 2.2. NMR Stackplots, Binding Curves and Residual Distribution Plots

**Figure S17.** Downfield region of the <sup>1</sup>H NMR spectra for the titration of  $Bu_4N^+Cl^-$  into **4a** in DMSO-d<sub>6</sub>/0.5% H<sub>2</sub>O at 298 K.



**Figure S18.** Observed binding curve (N*H*; starting at 9.82 ppm) and calculated fit (1:2 binding model) for **4a** when titrated against  $Bu_4N^+Cl^-$  in DMSO- $d_6/0.5\%$  H<sub>2</sub>O at 298 K.



**Figure S19.** Residual distribution plots for **4a** when titration data were fit to 1:1 (top), 1:2 (middle) and 2:1 (bottom) binding models. Proton 1 (N*H*; starting at 9.82 ppm) and proton 2 (N*H*; starting at 8.39 ppm) were monitored.



11.0 10.9 10.8 10.7 10.6 10.5 10.4 10.3 10.2 10.1 10.0 9.9 9.8 9.7 9.6 9.5 9.4 9.3 9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 f1 (ppm)

**Figure S20.** Downfield region of the <sup>1</sup>H NMR spectra for the titration of  $Bu_4N^+Cl^-$  into **4b** in DMSO-d<sub>6</sub>/0.5% H<sub>2</sub>O at 298 K.



**Figure S21.** Observed binding curve (N*H*; starting at 9.86 ppm) and calculated fit (1:2 binding model) for **4b** when titrated against  $Bu_4N^+Cl^-$  in DMSO- $d_6/0.5\%$  H<sub>2</sub>O at 298 K.



**Figure S22.** Residual distribution plots for **4b** when titration data were fit to 1:1 (top), 1:2 (middle) and 2:1 (bottom) binding models. Proton 1 (N*H*; starting at 9.86 ppm) and proton 2 (N*H*; starting at 8.47 ppm) were monitored.



**Figure S23.** Downfield region of the <sup>1</sup>H NMR spectra for the titration of  $Bu_4N^+Cl^-$  into **4c** in DMSO-d<sub>6</sub>/0.5% H<sub>2</sub>O at 298 K.



**Figure S24.** Observed binding curve (N*H*; starting at 9.94 ppm) and calculated fit (1:2 binding model) for **4c** when titrated against  $Bu_4N^+Cl^-$  in DMSO- $d_6/0.5\%$  H<sub>2</sub>O at 298 K.



**Figure S25.** Residual distribution plots for **4c** when titration data were fit to 1:1 (top), 1:2 (middle) and 2:1 (bottom) binding models. Proton 1 (N*H*; starting at 9.94 ppm) and proton 2 (N*H*; starting at 8.60 ppm) were monitored.



**Figure S26.** Downfield region of the <sup>1</sup>H NMR spectra for the titration of  $Bu_4N^+Cl^-$  into **4d** in DMSO-d<sub>6</sub>/0.5% H<sub>2</sub>O at 298 K.



**Figure S27.** Observed binding curve (N*H*; starting at 9.99 ppm) and calculated fit (1:2 binding model) for **4d** when titrated against  $Bu_4N^+Cl^-$  in DMSO- $d_6/0.5\%$  H<sub>2</sub>O at 298 K.



**Figure S28.** Residual distribution plots for **4d** when titration data were fit to 1:1 (top), 1:2 (middle) and 2:1 (bottom) binding models. Proton 1 (N*H*; starting at 9.99 ppm) and proton 2 (N*H*; starting at 8.60 ppm) were monitored.



**Figure S29.** Downfield region of the <sup>1</sup>H NMR spectra for the titration of  $Bu_4N^+Cl^-$  into **4e** in DMSO-d<sub>6</sub>/0.5% H<sub>2</sub>O at 298 K.



**Figure S30.** Observed binding curve (N*H*; starting at 9.99 ppm) and calculated fit (1:2 binding model) for **4e** when titrated against  $Bu_4N^+Cl^-$  in DMSO- $d_6/0.5\%$  H<sub>2</sub>O at 298 K.



**Figure S31.** Residual distribution plots for **4e** when titration data were fit to 1:1 (top), 1:2 (middle) and 2:1 (bottom) binding models. Proton 1 (N*H*; starting at 9.99 ppm) and proton 2 (N*H*; starting at 8.58 ppm) were monitored.



**Figure S32.** Downfield region of the <sup>1</sup>H NMR spectra for the titration of  $Bu_4N^+Cl^-$  into **4f** in DMSO-d<sub>6</sub>/0.5% H<sub>2</sub>O at 298 K.



**Figure S33.** Observed binding curve (N*H*; starting at 9.99 ppm) and calculated fit (1:2 binding model) for **4f** when titrated against  $Bu_4N^+Cl^-$  in DMSO- $d_6/0.5\%$  H<sub>2</sub>O at 298 K.



**Figure S34.** Residual distribution plots for **4f** when titration data were fit to 1:1 (top), 1:2 (middle) and 2:1 (bottom) binding models. Proton 1 (N*H*; starting at 9.99 ppm) and proton 2 (N*H*; starting at 8.59 ppm) were monitored.



**Figure S35.** Downfield region of the <sup>1</sup>H NMR spectra for the titration of  $Bu_4N^+Cl^-$  into **4g** in DMSO-d<sub>6</sub>/0.5% H<sub>2</sub>O at 298 K.



**Figure S36.** Observed binding curve (N*H*; starting at 9.99 ppm) and calculated fit (1:2 binding model) for **4g** when titrated against  $Bu_4N^+Cl^-$  in DMSO- $d_6/0.5\%$  H<sub>2</sub>O at 298 K.



**Figure S37.** Residual distribution plots for **4g** when titration data were fit to 1:1 (top), 1:2 (middle) and 2:1 (bottom) binding models. Proton 1 (N*H*; starting at 9.99 ppm) and proton 2 (N*H*; starting at 8.59 ppm) were monitored.

#### 3. Transport Studies

#### **3.1.** General Considerations

1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was obtained from Avanti<sup>®</sup> Polar Lipids, Inc. Cholesterol was obtained from Aldrich Chem. Co. Deacidified CHCl<sub>3</sub> was obtained by passing CHCl<sub>3</sub> through a column of activated basic alumina. All aqueous solutions were prepared using deionised water that had been passed through a Millipore filtration system.

Transport studies were conducted using a PerkinElmer LS 45 fluorescence spectrometer at 298 K, exciting the sample at 450 nm and measuring the emission at 535 nm. Samples were stirred vigorously during the experiment. Quartz Suprasil<sup>®</sup> cuvettes (4 windows, 10 mm pathlength) were used for all optical measurements.

#### 3.2. Measuring Intrinsic Activity: The Lucigenin Method

Stock solutions of POPC (~15 mM) and cholesterol (~8 mM) in deacidified CHCl<sub>3</sub> were prepared. A stock solution of the transporter (~35  $\mu$ M) in either methanol or acetone (both HPLC grade) was prepared.

Solutions of POPC (4.20 µmol) and cholesterol (1.80 µmol) were added to a 5 mL round bottom flask (7:3 ratio) followed by a solution of the transporter (0.24 nmol) to give a 1:25k transporter to lipid loading. The volatile solvents were removed under a stream of nitrogen and the resultant lipid film dried under high vacuum (1 h). To the flask was added a solution of lucigenin (0.8 mM, 500 µL) in aqueous NaNO<sub>3</sub> (225 mM) and the mixture was sonicated (30 s). The resultant yellow suspension was stirred vigorously (1 h) and then subjected to ten freeze-thaw cycles (flask placed in liquid nitrogen and then in warm water). The vesicle suspension was then transferred to a sizing apparatus and extruded through a polycarbonate membrane (200 nm pores) twenty-nine times. The resized vesicles were loaded onto a size exclusion column (packed with Sephadex<sup>®</sup> G-50 resin) and eluted with aqueous NaNO<sub>3</sub> solution (225 mM) to separate the vesicles from external lucigenin. The first cloudy band was collected and diluted with aqueous NaNO<sub>3</sub> solution (225 mM) to give a total volume of 15 mL (0.4 mM total lipid concentration).

To a cuvette equipped with a magnetic stirrer was added the suspension of vesicles (3 mL) and placed inside the fluorescence spectrometer. After the signal had stabilised (~90 s), the measurement was initiated. After 30 s, a solution of NaCl (1 M, 75  $\mu$ L) in aqueous NaNO<sub>3</sub> (225 mM) was added to the cuvette and the measurement allowed to run for 11 min. This process was repeated a further three times.

Data were processed using Origin 9 (Academic): for each run, the initial plateau (before the addition of chloride) was removed. The data were then normalised by dividing all fluorescence values (F) by the maximum fluorescence value ( $F_0$ ) for the individual runs. From the mean average of these data, the vertical drop (the first 1-2.5 s after chloride addition due to quenching of external lucigenin) was removed. The data were then normalised to give a final trace of relative fluorescence ( $F/F_0$ ) versus time.

#### 3.3. Quantification of Transport Rates

As described in our previous publication,<sup>6</sup> the inverse of the normalised fluorescence trace ( $F_0/F$ ) is directly proportional to the concentration of chloride inside the vesicles (according to the Stern-Volmer equation). Thus  $F_0/F$  was used in the quantification of transport rates.

Initial rates (*I*) were determined by fitting the  $F_0/F$  curve (0-500 s) to a double exponential decay function:

$$F_0/F = y - ae^{-bt} - ce^{-dt}$$

Differentiating at t = 0 allows for l to be calculated:

$$I = (a \times b) + (c \times d)$$

#### 3.4. Dose-Response Studies on Bis-urea 4a

The activity of **4a** was measured at six additional transporter to lipid loadings (1:2500, 1:40k, 1:80k, 1:250k, 1:500k and 1:1000k) using the methodology described in Section 3.2. When experiments were conducted at loadings of 1:40k and below, a more dilute stock solution (~350 nM) of transporter **4a** was used. All processed fluorescence decay traces are shown in Figure S38. Activity is clearly observable at even the lowest transporter to lipid loading of 1:1000k, where there is either 1 or 0 transporter molecules per LUV.<sup>6</sup> This indicates that **4a** is able to facilitate Cl<sup>-</sup>/NO<sub>3</sub><sup>-</sup> exchange as a single molecule. We thus conclude that **4a** is an exceptionally potent anionophore.



**Figure S38.** Chloride transport mediated by **4a** in 200 nm POPC/cholesterol (7:3) LUVs when preincorporated at various transporter to lipid loadings.

From the inverse of these traces, the initial rate (*I*) of transport was calculated and plotted as a function of the amount of transporter present inside the LUVs (Figure S39). The linear dose-response relationship observed suggests that **4a** mediates anion transport by acting as a monomeric mobile carrier, as opposed to forming species that are not 1:1 (host:guest) complexes such as dimers or channels. A mobile carrier mechanism has also previously been demonstrated for other members in the OPBU series.<sup>7,8</sup>



**Figure S39.** Plot of the initial rate of transport induced by **4a** as a function of its concentration inside LUVs.

#### 3.5. Leaching Studies on Bis-urea 4d

To determine whether **4d** resides exclusively within the membrane, or if an equilibrium exists between **4d** in the membrane and the aqueous phase, a leaching study was performed.

Lipid films were prepared by combining solutions of POPC (7  $\mu$ mol), cholesterol (3  $\mu$ mol) and **4d** (0.4 nmol) to give a 1:25k transporter to lipid loading. Thereafter the procedure as outlined in Section 3.2 was followed to form LUVs (~200 nm). After size exclusion chromatography, the collected vesicles were diluted to a volume of 10 mL with aqueous NaNO<sub>3</sub> (225 mM) to give a lipid concentration of 1.0 mM. Successive dilutions provided suspensions with lipid concentrations of 0.4 mM, 0.2 mM and 0.1 mM. All vesicles suspensions were stored on ice until required. The experiment was commenced by the addition of NaCl (1 M, 75  $\mu$ L) in aqueous NaNO<sub>3</sub> (225 mM) to 3 mL of the vesicle suspension inside the cuvette. For each lipid concentration, 2-3 replicate experiments were performed and the averaged fluorescence decay traces are shown in Figure S40.



**Figure S40.** Chloride transport mediated by **4d** in 200 nm POPC/cholesterol (7:3) LUVs as measured using the lucigenin method. The lipid concentration of the vesicle suspension was varied from 0.4 to 0.1 mM.

If an anionophore were to leach from the membrane, then dilution would lead to more of the anionophore partitioning into the aqueous phase leading to a lower concentration in the membrane. This would result in an attenuation in observed activity.<sup>9</sup>

From Figure S40, it appears that the traces at all lipid concentrations are almost superimposable; thus dilution does not significantly affect the activity of **4d**. We therefore conclude that **4d** is not capable of leaching and that the lower activity observed for this anionophore compared to more lipophilic esters in the OPBU series is not due to it being present in lower concentrations in the membrane.

#### 3.6. Deliverability Studies

LUVs were prepared in the same manner as described in Section 3.2, except that the anionophore was not added to the initial lipid mixture. After size exclusion chromatography, the collected vesicles were diluted with aqueous NaNO<sub>3</sub> (225 mM) to give a suspension with a notional lipid concentration of 0.4 mM and 3 mL of this was transferred to a cuvette. A solution of the anionophore (9.6  $\mu$ M, 5  $\mu$ L) in HPLC grade acetone was externally added to the cuvette by rapid plunger action of a 10  $\mu$ L syringe with the tip of the needle positioned just above the stirrer. Assuming the notional lipid concentration of 0.4 mM, this would give a transporter to lipid loading of 1:25k. NaCl (1 M, 75  $\mu$ L) in aqueous NaNO<sub>3</sub> (225 mM) was added 5 min after the addition of anionophore and the measurement allowed to run for a further 11 min. This process was repeated twice more, and data were processed as before. The corresponding F/F<sub>0</sub> traces are shown in Figures S41–S44.

Deliverability (D) was calculated by dividing the initial rate (I) of transport from this experiment by that measured when the anionophore was preincorporated. Values obtained for D are reported in Table 1 of the main text.



**Figure S41.** Chloride transport mediated by **4a** when the anionophore was either preincorporated in the membrane (continuous line) or added externally as solution in acetone to vesicles without anionophore (dashed line). The notional transporter to lipid loading was 1:25k in both experiments.



**Figure S42.** Chloride transport mediated by **4b** and **4c** when the anionophore was either preincorporated in the membrane (continuous lines) or added externally as solution in acetone to vesicles without anionophore (dashed lines). The notional transporter to lipid loading was 1:25k in all experiments.



**Figure S43.** Chloride transport mediated by **4d** and **4e** when the anionophore was either preincorporated in the membrane (continuous lines) or added externally as solution in acetone to vesicles without anionophore (dashed lines). The notional transporter to lipid loading was 1:25k in all experiments.



**Figure S44.** Chloride transport mediated by **4f** and **4g** when the anionophore was either preincorporated in the membrane (continuous lines) or added externally as solution in acetone to vesicles without anionophore (dashed lines). The notional transporter to lipid loading was 1:25k in all experiments.

#### 3.7. Measuring Transport Activity in YFP-FRT Cells

#### Cell culture

Fischer rat thyroid cells expressing the yellow fluorescent protein YFP-H148Q/I152L (YFP-FRT cells) were a generous gift of A.S. Verkman (University of California, San Francisco). They were cultured in Coon's modified Ham's F12 medium supplemented with 10% fetal bovine serum, 100 U mL<sup>-1</sup> penicillin, 100 mg mL<sup>-1</sup> streptomycin, 2 mM glutamine and the selection agent G418 (0.5 mg mL<sup>-1</sup>).

#### **Anionophore solutions**

Stock solutions of the anionophore (5–10 mM) were prepared in DMSO. Immediately before use, the solution was diluted with phosphate buffered saline (PBS) to achieve a final concentration of 50  $\mu$ M. By itself, the vehicle (DMSO) had no effect on YFP fluorescence.

#### Microplate reader

Analysis was performed using a FLUOstar OMEGA microplate reader (BMG LABTECH Ltd., Aylesbury, UK) equipped with syringe pump and excitation ( $500 \pm 10 \text{ nm}$ ) and emission ( $535 \pm 15 \text{ nm}$ ) filters. Cells were seeded in a 96-well plate and grown in the culture medium at 37 °C in an atmosphere of 5% CO<sub>2</sub> for 3 to 4 days until 90% confluence. Cells were washed twice with PBS solution before incubating with 40 µL of PBS containing either the test anionophore ( $50 \mu$ M) or vehicle (0.5-1% v/v DMSO) for 10 min. Fluorescence was measured 2 s before and 12 s after injection of 110 µL PBS iodide solution (containing

100 mM l<sup>-</sup>) into each well. Data are presented as means  $\pm$  SEM (n = 16-32 from at least four independent experiments). Representative time courses of cell fluorescence are shown in Figure S45.



**Figure S45.** Anion transport mediated by OPBUs (50  $\mu$ M) in YFP–FRT cells. Values of cell fluorescence were normalised to the fluorescence intensity at the time point when I<sup>-</sup> was injected into the wells. Addition of I<sup>-</sup> at t = 2 s is marked by a vertical decrease in fluorescence emission, possibly due to the increase in depth of the supernatant solution.

The rate of fluorescence quenching induced by the anionophore was calculated by fitting the final 12 s of the trace to a first order exponential function. Fluorescence quenching induced by the vehicle was subtracted from the measurements. The results are reported in Table 1 and Figure 5 of the main text.

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