Supplementary Material (ESI) for Chemical Communications

Cotransport of H^+/Cl^- by a synthetic prodigiosin mimic

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Electronic Supplementary Information

Synthesis and Characterisation

Compound 2 5-Methyl-3,4-diphenyl-1H-pyrrole-2-carboxylic acid (1-methyl-1H-imidazol-2-ylmethyl)-amide

1-Methyl-2-aminomethylimidazole (260 mg, 2.4 mmol) was stirred with 2.0 M trimethylaluminium solution in hexane (3 ml, 6 mmol) in dichloromethane (40 ml) for 30 min. 5-Methyl-3,4-diphenyl-*1H*-pyrrole-2-carboxylic acid ethyl ester (365 mg, 1.2 mmol) was added to the solution and the reaction heated at reflux for 7 days. The reaction was quenched by the dropwise addition of dilute HCl solution and the reaction mixture extracted with dichloromethane solution (2 x 50 ml). The organic phase was reduced in *vacuo* and the residue purified by column chromatography eluting with dichloromethane/methanol (10:1 *v/v*) on silica gel affording compound **2** as a white solid (123 mg, 0.33 mmol, 27%).

^{1}H NMR 300 MHz in $CDCl_3$ δ (ppm): 2.36 (s, 3H, C*H*$_3$pyrrole), 3.61 (s, 3H, C*H*$_3$imidazole), 4.50 (d, 2H, *J* = 5.4, C*H*$_2$), 6.04 (t, 1H, *J* = 5.4, CON*H*) 6.79 (s, 1H, imidazole*H*), 6.85 (s, 1H, imidazole*H*), 6.99-7.28 (m, 10H, Ar*H*), 9.33 (s, br, 1H, N*H*-Pyrrole). ^{13}C NMR 75 MHz in $CDCl_3$ δ (ppm): 1.1, 12.3, 35.5, 121.5, 125.9, 127.6, 127.8, 128.0, 138.5, 129.0, 130.1, 130.5, 134.4, 134.6, 161.0. ES$^+$ HRMS: m/z: Calc. for $C_{23}H_{22}N_4O$ $[M]^+$: 371.1866; found 371.1869.

Compound 3 5-Methyl-3,4-diphenyl-1H-pyrrole-2-carboxylic acid pyridin-2-ylamide

2-Aminopyridine (3.08 g, 32.7 mmol) was stirred with 2.0 M trimethylaluminium solution in hexane (12 ml, 24 mmol) in dichloromethane (50 ml) for 30 min. 5-Methyl-

3,4-diphenyl-*1H*-pyrrole-2-carboxylic acid ethyl ester (1 g, 3.3 mmol) was added to the solution and the mixture was heated at reflux for 5 days. The reaction was quenched by the dropwise addition of dilute HCl solution and the reaction mixture extracted with dichloromethane solution (2 x 50 ml). The organic solution was reduced in *vacuo* and the residue was purified by column chromatography on silica gel eluting with dichloromethane/methanol (100:2 *v/v*). The solvent was removed and the residue was recrystallized from acetonitrile to yield compound **3** as a white solid (157 mg, 0.44 mmol, 13%).

^{1}H NMR 300 MHz in $CDCl_3$ δ (ppm): 2.40 (s, 3H, C*H*$_3$), 6.70-8.11 (m, 16H, Ar*H* and Pyr*H*), 7.98 (s, 1H, CON*H*Ar), 9.42 (s, br, 1H, N*H*-Pyrrole). ^{13}C NMR 75 MHz in DMSO-d_6 δ (ppm): 12.5, 114.2, 119.3, 126.1, 128.1, 128.3, 129.3, 130.2, 130.9, 134.1, 134.5, 137.9, 148.3, 151.7, 154.4. ES^+ mass spectrum: *m/z* (%): 354 (100) $[M+H]^+$, 707 (10) $[2M+H]^+$. ES^+ HRMS: m/z: Calc. for $C_{23}H_{20}N_3O$ $[M+H]^+$: 354.1601; found: 354.1599.

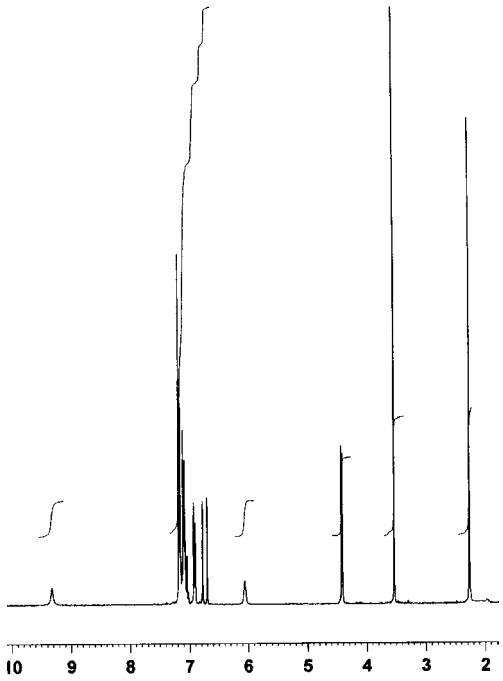

Figure S1 ^{1}H NMR of compound **2** in $CDCl_3$

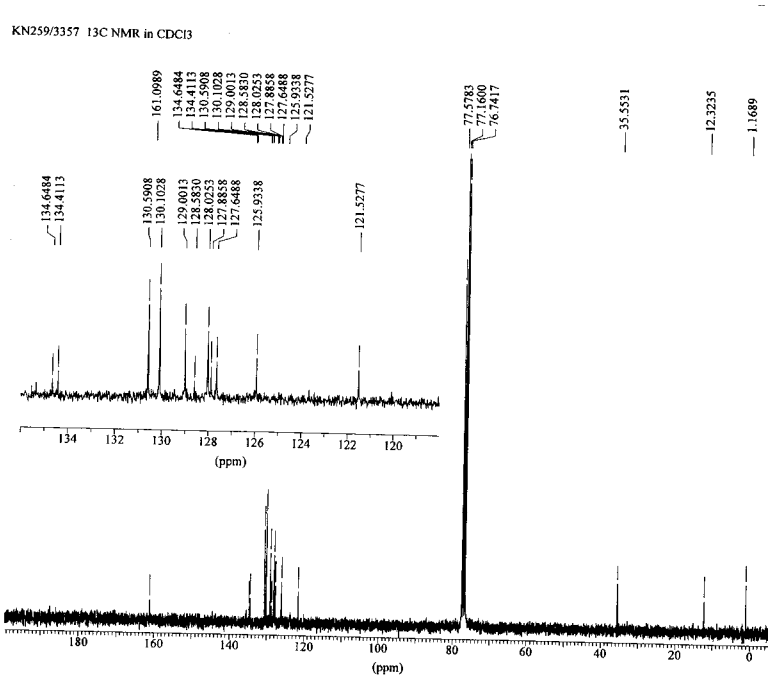

Figure S2 ^{13}C NMR of compound **2** in $CDCl_3$

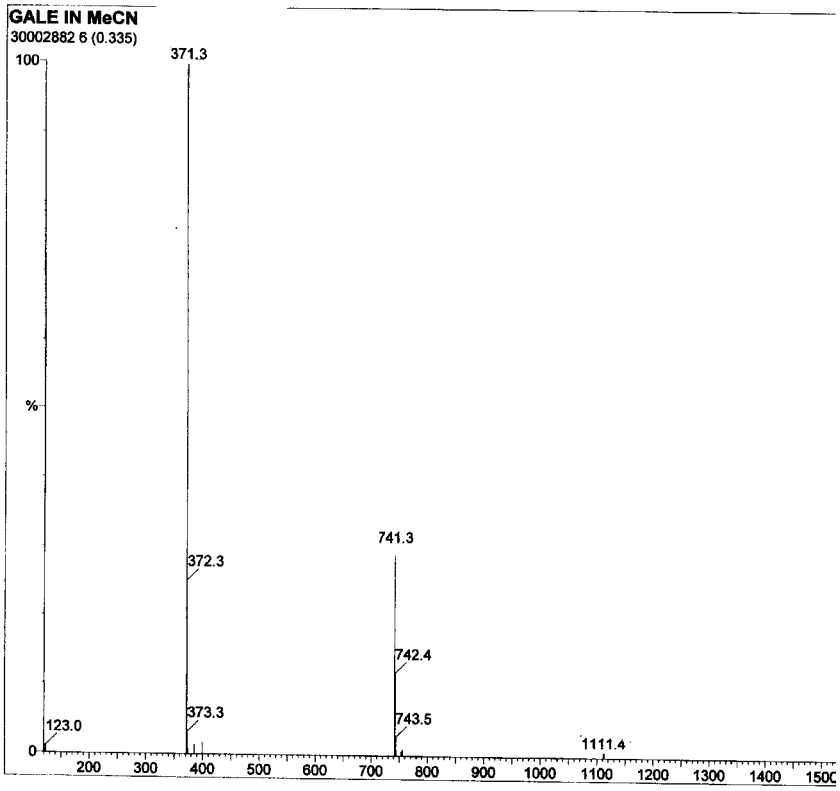

Figure S3 Positive ES MS of Compound **2**.

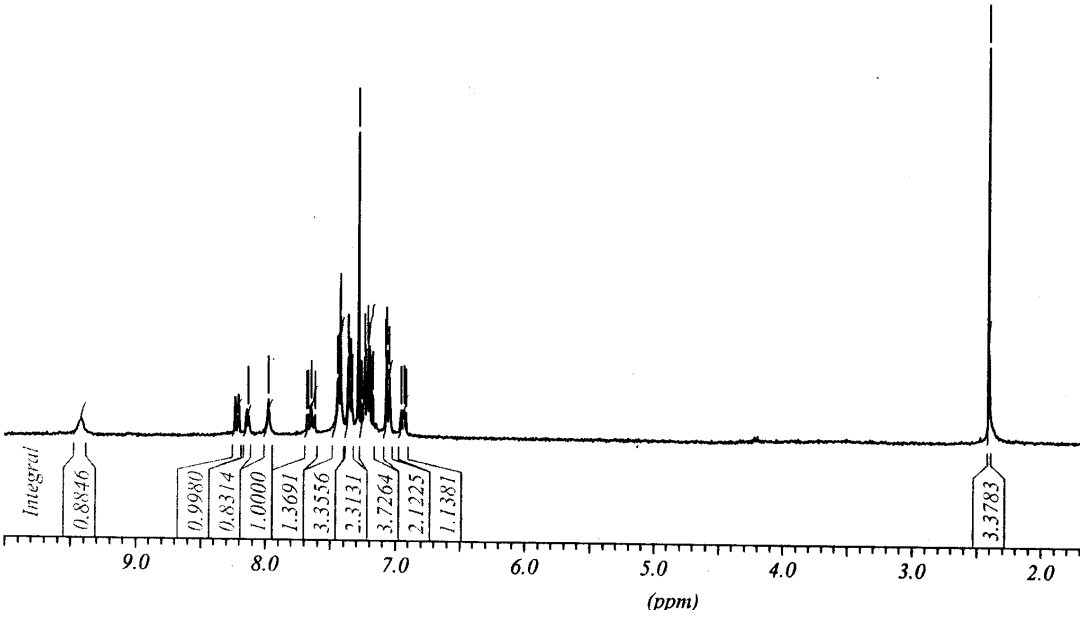

Figure S4 ^{1}H NMR of Compound **3** in $CDCl_3$

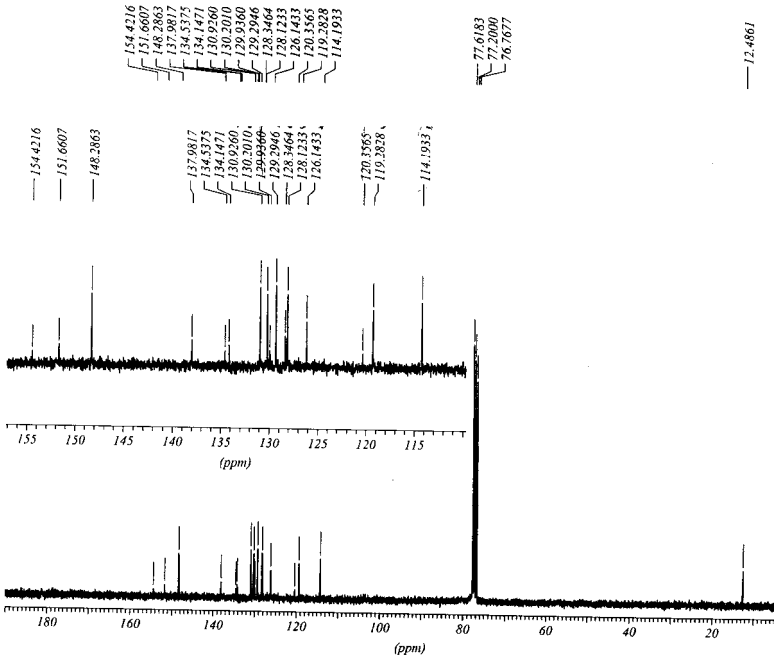

Figure S5 ^{13}C NMR of Compound **3** in $CDCl_3$

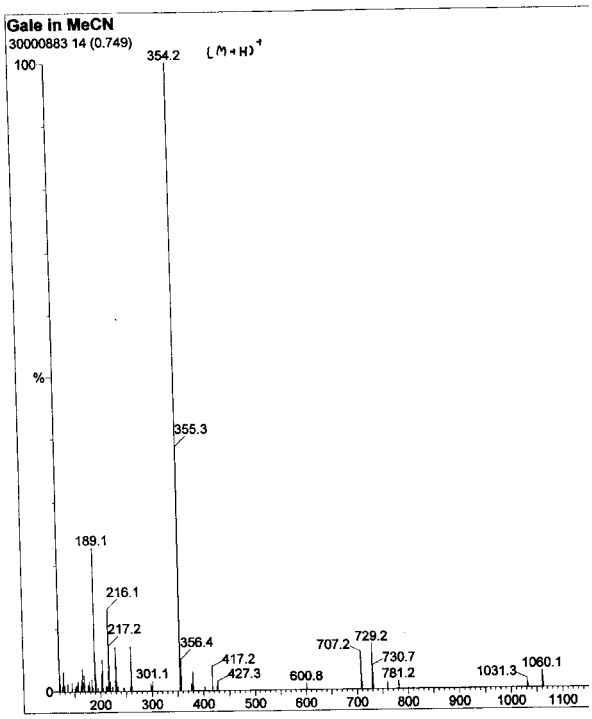

Figure S6 Positive ES MS of Compound **3**

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Preparation of Unilamellar Vesicles

A chloroform solution of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids) and cholesterol were combined in a 10 mL round bottom flask. The chloroform was removed using a rotary evaporator and the lipid film was dried under a high vacuum system for over an hour. Upon addition of 1.0 mL solution (500 mM NaCl and 5 mM citric acid, pH 4.0 or 7.2) and a Pyrex glass bead, the solution was vortexed to rehydrate the lipid film. The lipid solution was subject 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 vesicles were dialyzed for 10 hours to remove unencapsulated salts and/or fluorophores. Dialysis used an outside solution of 500 mM $NaNO_3$ and 5 mM citric acid at the same pH.

Chloride Transport Assay

Unilamellar vesicles (200 nm mean diameter) composed of POPC/cholesterol (7:3 molar ratio), and containing an inside solution of 500 mM NaCl and 5 mM citric acid, pH 4.0 or 7.2, and an outside solution of 500 mM $NaNO_3$ and 5 mM citric acid, at the same pH, were added to a solution of 500 mM $NaNO_3$ and 5 mM citric acid, pH 4.0 or 5 mM sodium phosphate, pH 7.2 for a final lipid concentration of 1 mM. When the The chloride release from vesicles upon addition of a compound (10 μM) was monitored using an Accumet chloride selective electrode for 20 minutes. The vesicles were lysed with detergent (polyoxyethylene (8) lauryl ether) to release all chloride ions.

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Chloride Transport Assay

Three different conditions were generated using unilamellar vesicles (200 nm mean diameter) composed of POPC/cholesterol (7:3 molar ratio). (a) inside vesicles, pH 4.0, outside vesicles, pH 4.0. Vesicles with inside solution of 500 mM NaCl and 5 mM citric acid, pH 4.0, and an outside solution of 500 mM $NaNO_3$ and 5 mM citric acid, pH 4.0, were diluted in a solution of 500 mM $NaNO_3$ and 5 mM citric acid, pH 4.0 for a final lipid concentration of 1 mM. (b) inside vesicles, pH 4.0, outside vesicles, pH 6.7. Vesicles with inside solution of 500 mM NaCl and 5 mM citric acid, pH 4.0, and an outside solution of 500 mM $NaNO_3$ and 5 mM citric acid, pH 4.0, were diluted in a solution of 500 mM $NaNO_3$ and 5 mM sodium phosphate, pH 7.2 for a final lipid concentration of 1 mM, and a final external pH of 6.7. (c) inside vesicles, pH 7.2, outside vesicles, pH 7.2. Vesicles with inside solution of 500 mM NaCl and 5 mM citric acid, pH 7.2, and an outside solution of 500 mM $NaNO_3$ and 5 mM citric acid, pH 7.2, were diluted in a solution of 500 mM $NaNO_3$ and 5 mM citric acid, pH 7.2 for a final lipid concentration of 1 mM.

pH Detection Assay

Oregon Green® 514 (10 μM), a pH sensitive fluorophore (purchased from Molecular Probes Inc) was encapsulated inside 200 nm mean diameter POPC/cholesterol (7:3 molar ratio) vesicles along with 500 mM NaCl and 5 mM citric acid, pH 4.0. Vesicles were dispersed in 500 mM $NaNO_3$ and 5 mM sodium phosphate, pH 7.2 for a final lipid concentration of 550 μM. The fluorescence of Oregon Green® 514 was monitored using 555 nm emission upon excitation at 510 nm with a Perkin Elmer luminescence

spectrometer. The fluorescence baseline was monitored for 200 seconds, upon which compounds **2** and **3** (8.8 μM) were added to the vesicle solution. The vesicles were lysed at 1000 seconds with detergent (polyoxyethylene (8) lauryl ether) to dissipate the gradient.