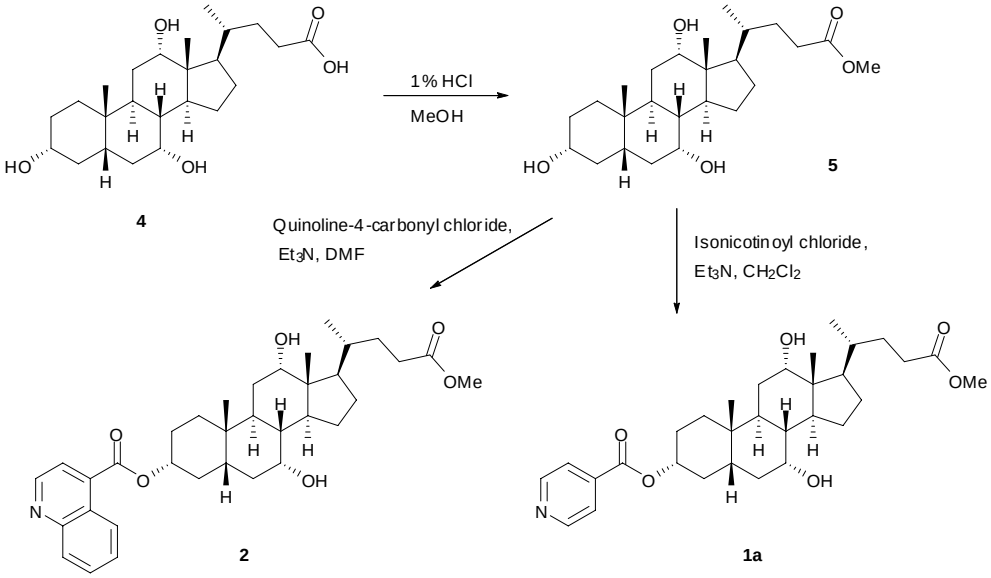
Supplementary Information

Palladium(II)-Gated Ion Channels

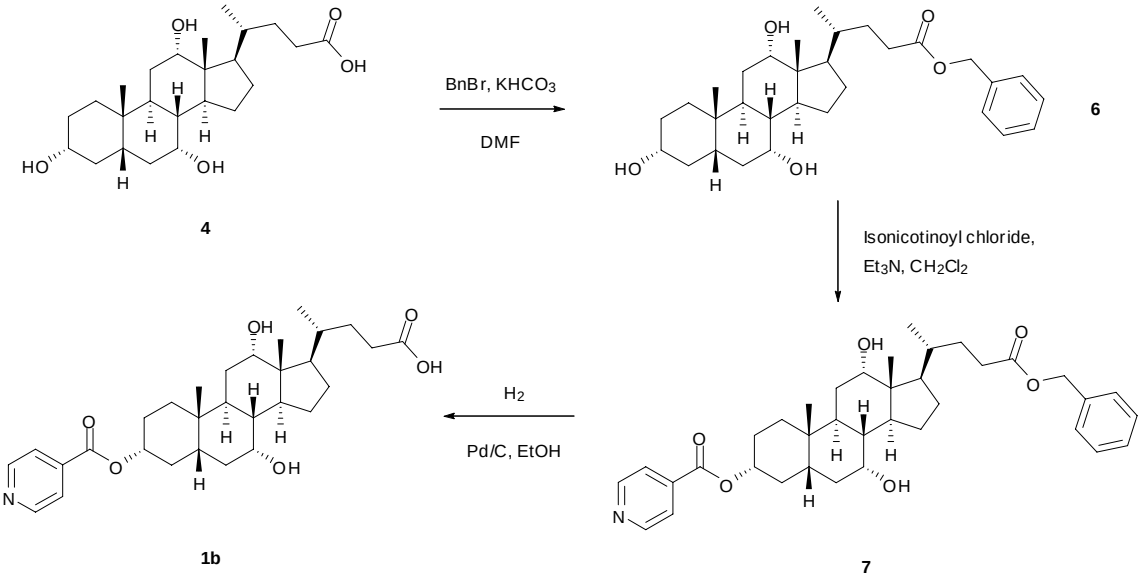
Craig P. Wilson and Simon J. Webb*

S1: Synthetic Details

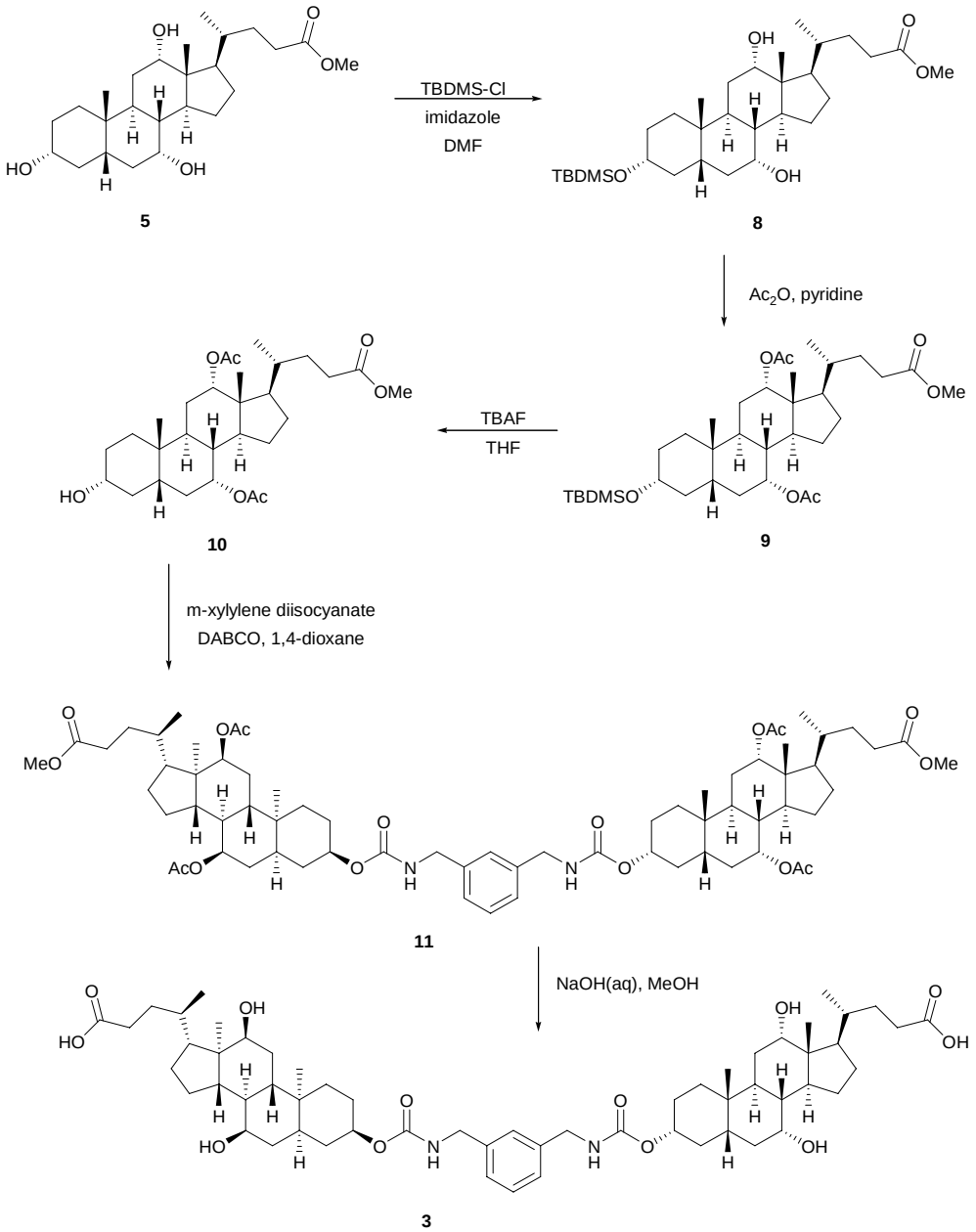
S1.1. Synthetic Schemes



Scheme 1: Synthetic route to compounds **1a** and **2**



Scheme 2: Synthetic route to compound **1b**.



5 **8** **9** **10** **11** **3**

Scheme 3: Improved synthetic route to Kobuke's channel forming compound **3**

S.1.2. Instrumentation

Unless otherwise indicated, reagents and solvents were obtained from commercial suppliers (Aldrich, Fluka or Acros). Anhydrous dichloromethane and pyridine were obtained by distillation from calcium hydride. Anhydrous *N*,*N*-dimethylformamide was obtained by drying over consecutive batches of 4Å molecular sieves. Anhydrous 1,4-dioxane was obtained by drying over fresh 4Å molecular sieves. Anhydrous tetrahydrofuran was obtained by distillation from sodium/benzophenone ketyl. Thin layer chromatography was carried out using Merck aluminium-backed F_{254} silica gel plates, and visualised under UV light (256 nm or 365 nm), or with alkaline aqueous potassium permanganate. Preparative column (flash) chromatography was carried out using commercially available normal phase silica gel.

NMR spectra were measured on Bruker DPX300, DPX400, or DPX500 instruments for solutions in $CDCl_3$, DMSO-d_6, or methanol-d_4, and were assigned with the aid of COSY, HMBC, HMQC, and DEPT spectra where appropriate. Coupling constants are given in Hz; multiplicities are given as singlet (s), doublet (d), triplet (t),

quartet (q), quintet (qn), or multiplet (m), with broad signals indicated using the abbreviation br. Electrospray mass spectra were measured on a Micromass LCT instrument using a Waters 2790 separations module with electrospray ionisation and TOF fragment detection. Samples were prepared using a 50:50:0.1 acetonitrile/water/formic acid solution. MALDI MS measurements were made on a Thermo Finnegan MAT95 XP instrument. Fluorescence spectroscopy was carried out on a Perkin-Elmer LS55 fluorimeter. Temperature control was attained using a Julabo F25-HE water circulator. UV-visible spectroscopy was carried out using a Jasco V-660 spectrophotometer with the temperature controlled by a Jasco EHC-716 Peltier.

S.1.3. Synthesis of compounds 1-11, and Pd(II) complexes of 1a and 2

Methyl cholate (5) (modified from literature preparation[S1,S2])

Cholic acid **4** (1 g, 2.45 mmol) was stirred for 18 hours at room temperature in 1 % hydrochloric acid in methanol (20 mL). This solution was then evaporated *in vacuo* to yield methyl cholate **5** as a white solid (0.85 g, 82 %). δ_H ($CDCl_3$, 400 MHz): 0.70 (s, 3H, $C^{18}H_3$), 0.91 (s, 3H, $C^{19}H_3$), 1.00 (d, 3H, J = 6Hz, $C^{21}H_3$), 1.10 – 2.45 (overlapping signals, 24H, steroid CHs/CH_2s), 3.51 (qn, 1H, J = 4Hz, C^3H-OH), 3.69 (s, 3H, CO_2CH_3), 3.78 (br s, 3H, RO*H*), 3.88 (m, 1H, C^7H-OH), 4.00 (m, 1H, C^{12}H-OH). δ_C ($CDCl_3$, 100 MHz): 12.9 ($C^{18}H_3$), 17.7 ($C^{21}H_3$), 22.9 ($C^{19}H_3$), 23.6 (CH_2), 26.8 (CH), 27.9 (CH_2), 28.5 (CH_2), 30.5 (CH_2), 31.3 (CH_2), 31.5 (CH_2), 34.9 (CH_2), 35.1 (C), 35.7 (CH) 35.7 (CH_2), 39.6 (CH_2), 39.8 (CH), 41.8 (CH), 42.1 (CH), 46.8 (CH), 47.4 (C), 52.0 (CO_2CH_3), 68.9 (C^7H-OH), 72.6 (C^3H-OH), 73.6 (C^{12}H-OH), 175.3 (C=O). MS (ES+): m/z 445, $[M+Na]^+$. HRMS for $C_{25}H_{42}O_5Na$: expected 445.2924, found 445.2921.

Methyl 3α-isonicotinoyl-7α,12α-dihydroxycholanate (1a)

To a stirred solution of methyl cholate **5** (100 mg, 0.237 mmol, 1 eq.) in dry dichloromethane (50 mL) was added isonicotinoyl chloride hydrochloride (42 mg, 0.237 mmol, 1 eq.) followed by triethylamine (66 μL, 4.74 mmol, 20 eq.). The mixture was stirred overnight, then diluted with water and extracted with dichloromethane twice. The combined organic extracts were dried ($MgSO_4$), filtered, and the filtrate evaporated to give the crude product. This was purified by flash chromatography (eluant: ethyl acetate) to give the title compound **1a** (R_f 0.56, EtOAc) as a white foam (69 mg, 55 % yield). δ_H ($CDCl_3$, 400 MHz): 0.82 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.09 (d, 3H, J = 6 Hz, $C^{21}H_3$), 1.18 – 2.70 (overlapping signals, 24H, steroid protons), 3.78 (s, 3H, CO_2CH_3), 4.00 (m, 1H, C^7 C*H*(OH)), 4.13 (m, 1H, C^{12} C*H*(OH)), 4.96 (m, 1H, C^3H(OH)), 7.97 (d, 2H, J = 6 Hz, pyridyl C*H*), 8.87 (d, 2H, J = 6 Hz, pyridyl C*H*). δ_C ($CDCl_3$, 100 MHz): 12.9 ($C^{18}H_3$), 17.7 ($C^{21}H_3$), 22.8 ($C^{19}H_3$), 23.6 (CH_2), 27.0 (CH_2), 27.0 (CH), 27.9 (CH_2), 28.7 (CH_2), 31.2 (CH_2), 31.5 (CH_2), 34.8 (CH_2), 35.1 (C), 35.2 (CH_2), 35.4 (CH_2), 35.7 (CH), 39.8 (CH), 41.6 (CH), 42.3 (CH), 46.9 (C), 47.6 (CH), 51.9 (CO_2CH_3), 68.6 (C^7H(OH)), 73.4 (C^{12}H(OH)), 76.4 (C^3H(OH)), 123.3 (ArCH), 138.6 (ArC), 150.8 (ArCH) 164.9 (pyridyl C=O), 175.2 (ester C=O). MS (ES+): m/z 528, $[M+H]^+$. HRMS for $C_{31}H_{46}O_6N$: expected 528.3320, found 528.3318. Elemental analysis for $C_{31}H_{45}O_6N.⅓C_6H_{14}$: expected C: 71.23%, H: 9.00%, N: 2.52%; found C: 71.21%, H: 8.55%, N: 2.63%.

Benzyl cholate (6) (modified from literature preparation;[S3] NMR spectra matched literature values[S4])

To a solution of cholic acid **4** (0.5 g, 1.22 mmol, 1 eq.) in dry DMF (10 mL) was added potassium hydrogen carbonate (0.134 g, 1.34 mmol, 1.1 eq.) and benzyl bromide (0.16 mL, 1.34 mmol, 1.1 eq.), and the solution stirred at room temperature for 24 hours. The reaction mixture was then diluted with ether to approximately 4-5 times the original volume, and then washed with water. The aqueous layer was then extracted with ether, and the combined ether extracts then finally washed with a saturated aqueous brine solution. The organic extracts were dried ($MgSO_4$), filtered, and the filtrate evaporated. The residue was then purified by flash chromatography (eluant: ethyl acetate) to give the title compound **6** as a white solid (0.35 g, 58 %). δ_H ($CDCl_3$, 300 MHz): 0.55 (s, 3H, CH_3), 0.79 (s, 3H, CH_3), 0.89 (d, 3H, J = 5.5Hz), 0.80 – 2.40 (several overlapping signals, 24H, steroid CHs and CH_2s), 3.33 (m, 1H, C^3HOH), 3.74 (overlapping signals, 4H, $C^{12}H$OH and 3 x RO*H*), 3.85 (br s, 1H, C^7HOH), 5.02 (d, 2H, J = 1.75 Hz, PhCH_2), 7.20 – 7.30 (m, 5H, Ar*H*). δ_C ($CDCl_3$, 75 MHz): 12.8 (CH_3), 17.7 (CH_3), 22.8 (CH_3), 23.6 (CH_2), 26.9 (CH), 27.9 (CH_2), 28.5 (CH_2), 30.6 (CH_2), 31.3 (CH_2), 31.7 (CH_2), 35.1 (C), 35.2 (CH_2), 35.7 (CH), 35.8 (CH_2), 39.7 (CH), 39.8 (CH_2), 41.8 (CH), 42.2 (CH), 46.8 (C), 47.4 (CH), 66.5 (PhCH_2), 68.8, 72.2, 73.5 (C^3, C^7 and C^{12} *C*H), 128.6, 128.9, 136.4 (Ar*C*), 174.6 (C=O). MS (ES+): m/z 521, $[M+Na]^+$. HRMS for $C_{31}H_{46}O_5Na$: expected 521.3237, found 521.3242.

Benzyl 3α-isonicotinoyl-7α,12α-dihydroxycholanate (7)

The reaction conditions for this preparation were identical to the conditions for the preparation of **1b** from methyl cholate, but using benzyl cholate (167 mg, 0.335 mmol). Purification was by flash chromatography (eluant: 2:1 (v/v) chloroform/ethyl acetate) to give the title compound **7** as a white foam (90 mg, 44 %). δ_H ($CDCl_3$, 400 MHz): 0.79 (s, 3H, $C^{18}H_3$), 1.04 (s, 3H, $C^{19}H_3$), 1.08 (d, 3H, *J* = 6 Hz, $C^{21}H_3$), 1.18 – 2.70 (overlapping signals, 24H, steroid protons), 3.98 (m, 1H, C^7H(OH)), 4.10 (m, 1H, C^{12}H(OH)), 4.95 (septet, 1H, *J* = 4 Hz, C_3H(O-isonicotinyl)), 5.21 (d, 2H, *J* = 3.2Hz, PhCH_2), 7.45 (br, 5H, Ar*H*), 7.98 (d, 2H, *J* = 5.2 Hz, pyridyl C*H*), 8.86 (d, 2H, *J* = 4.8 Hz, pyridyl C*H*). δ_C ($CDCl_3$, 100 MHz): 13.0 (C^{18} or C^{19} CH_3), 17.8 (C^{18} or C^{19} CH_3), 23.0 (C^{21} CH_3), 23.5 (CH_2), 27.0 (CH_2), 27.2 (CH), 27.8 (CH_2), 28.8 (CH_2), 31.2 (CH_2), 31.7 (CH_2), 34.6 (CH_2), 35.1 (C), 35.1 (CH_2), 35.2 (CH_2), 35.4 (CH), 40.0 (CH), 41.7 (CH), 42.5 (CH), 47.0 (CH), 47.7 (C), 66.6 (PhCH_2), 68.8 (C^3H), 73.1 (C^7H), 75.0 (C^{12}H), 123.7 (pyridyl ArCH), 128.7 (phenyl ArCH), 129.0 (phenyl ArCH), 136.0 (pyridyl ArC), 149.9 (pyridyl ArCH), 174.3 (2 x C=O). MS (ES+): m/z 604, $[M+H]^+$. Elemental analysis for $C_{37}H_{49}NO_6.½C_6H_{14}$: expected C: 74.27%, H: 8.73%, N: 2.17%; found C: 74.45%, H: 8.78%, N: 2.34%. HRMS for $C_{37}H_{50}NO_6$: expected 604.3638, found 604.3635.

3α-Isonicotinoyl-7α,12α-dihydroxycholanic acid (1b)

Benzyl ester **7** (90 mg, 0.15 mmol, 1 eq.) was dissolved in ethanol, and palladium-on-charcoal (8 mg, 0.05 eq.; wet, 10% wt. based on dry solid) added to the solution. The flask containing this suspension was evacuated, and then purged with nitrogen. This was repeated three times. The flask was evacuated once more, and then stirred under a hydrogen atmosphere overnight. Once TLC analysis indicated the reaction was complete, the reaction flask was evacuated and purged with nitrogen. The black suspension was then filtered through Celite™, and the filtrate evaporated to a white solid and triturated with dichloromethane to remove trace starting material impurities. The purified product was then suspended in water and lyophilised to give the title compound **1b** as a white powder (45 mg, 59 %). δ_H (MeOH-d_4, 300 MHz): 0.74 (s, 3H, CH_3), 0.97 (d, 3H, $C^{21}H_3$), 1.03 (s, 3H, CH_3), 1.00 – 3.45 (overlapping signals, 24H, steroid protons), 3.82 (m, 1H, $C^{12}H$(OH)), 3.99 (m, 1H, C^7H(OH)), 4.59 (br, 1H, C^3H(O-isonicotinoyl)), 7.93 (br s, 2H, pyridyl C*H*, *ortho* to C=O), 8.75 (br s, 2H, pyridyl C*H*, *meta* to C=O). δ_C (DMSO-d_6, 75 MHz): 12.6 (CH_3), 17.3 (CH_3), 22.6 (CH_3), 23.1 (CH_2), 24.9 (CH_2), 26.4 (CH), 26.5 (CH_2), 27.6 (CH_2), 28.7 (CH_2), 34.7 (CH_2), 31.2 (CH_2), 34.7 (C), 35.1 (CH_2), 35.3 (CH), 39.6 (CH), 41.4 (CH), 41.7 (CH), 42.5 (CH_2), 46.4 (*C*H), 47.8 (C), 71.1 (C^7H), 74.7 (C^{12}H), 76.3 (C^3H), 122.8 (pyridyl CH), 137.6 (pyridyl C), 151.1 (pyridyl CH), 172.9 (C=O), 175.4 (C=O). MS (ES+): m/z 514, $[M+H]^+$. Elemental analysis for $C_{30}H_{43}NO_6$: expected C: 70.15%, H: 8.44%, N: 2.73%; found C: 70.29%, H: 8.42%, N: 2.78%. HRMS for $C_{30}H_{44}NO_6$: expected 514.3169, found 514.3153.

Quinoline-4-carbonyl chloride

To a suspension of quinoline-4-carboxylic acid (400 mg, 2.3 mmol, 1 eq.) in dichloromethane was added oxalyl chloride (1.15 mL, 2M solution in dichloromethane, 2.3 mmol, 1 eq.) and 1-2 drops of dimethylformamide. This resulted in complete dissolution of the starting material to give a strongly coloured yellow solution. The reaction was stirred for 18 hours, after which time it had precipitated a yellow solid. On addition of further dichloromethane this solid dissolved, thus the reaction mixture was then evaporated *in vacuo* to give the title compound as a yellow-green solid (440 mg, 99 %). δ_H (400 MHz, DMSO-d_6): 7.96 (t, 1H, *J* = 8 Hz, quinoline 6-C*H*), 8.12 (t, 1H, *J* = 8 Hz, quinoline 7-C*H*), 8.25 (d, 1H, *J* = 5.2 Hz, quinoline 2-C*H*), 8.46 (d, 1H, *J* = 9 Hz, quinoline 8-C*H*), 8.76 (d, 1H, *J* = 9 Hz, quinoline 5-C*H*), 9.33 (d, 1H, *J* = 5.2 Hz, quinoline 3-C*H*). δ_C (100 MHz, DMSO-d_6): 122.4 (C^6), 124.7 (C), 125.9 (C^5), 128.4 (C^2), 130.0, 130.2 (C^7 and C^8), 136.3 (C), 148.8 (C), 150.8 (C^3), 167.9 (C=O).

Methyl 3α-(quinoline-4-carbonyl)-7α,12α-dihydroxycholanate (2)

To a solution of methyl cholate **5** (970 mg, 1.15 mmol, 1 eq.) in dimethylformamide (4 mL) was added the quinoline-4-carbonyl chloride (as a solution in 1 mL DMF, 1.15 mmol, 1 eq.), followed by triethylamine (320 µL, 2.3 mmol, 2 eq.). Addition of triethylamine resulted in the reaction mixture changing from a green solution to a pink suspension. After stirring for 20 hours, the reaction mixture was diluted with water and extracted twice with dichloromethane. The combined organic extracts were then dried ($MgSO_4$), filtered, and the filtrate evaporated to give the crude compound as an orange oil. This was purified by flash chromatography (eluant: 1:1 chloroform/ethyl acetate) to give the product **2** as a pale pink solid (380 mg, 29 %). δ_H (400 MHz, $CDCl_3$): 0.64 (s, 3H, C^{18}/C^{19} CH_3), 0.88 (s, 3H, C^{18}/C^{19} CH_3), 0.91 (d, 3H, *J* = 6Hz, $C^{21}H_3$), 1.05 – 2.55 (several overlapping

signals, 24H, steroid C*H*/CH_2), 3.60 (s, 3H, CO_2CH_3), 3.82 (m, 1H, C^7/C^{12} C*H*(OH)), 3.93 (m, 1H, C^7/C^{12} C*H*(OH)), 4.90 (m, 1H, C^3*H*(OH)), 7.58 (t, 1H, *J* = 7 Hz, quinoline 6-C*H*), 7.70 (t, 1H, *J* = 7 Hz, quinoline 7-C*H*), 7.82 (d, 1H, *J* = 4.5 Hz, quinoline 2-C*H*), 8.09 (d, 1H, *J* = 8.5 Hz, quinoline 8-C*H*), 8.68 (d, 1H, *J* = 8.5 Hz, quinoline 5-C*H*), 8.94 (d, 1H, *J* = 4.5 Hz, quinoline 3-C*H*). δ_C (100 MHz, $CDCl_3$): 13.0 (C^{18} or C^{19} CH_3), 17.8 (C^{21}), 23.0 (C^{18} or C^{19} CH_3), 23.5 (CH_2), 27.1 (CH_2), 27.2 (CH), 27.8 (CH_2), 28.7 (CH_2), 31.2 (CH_2), 31.4 (CH_2), 34.7 (CH_2), 35.1 (CH_2), 35.5 (CH), 35.6 (CH_2), 39.9 (CH), 41.7 (CH), 42.5 (CH), 47.0 (CH) 47.6 (C), 51.2 (CO_2CH_3), 68.6 (C^7 or C^{12} CH), 73.3 (C^7 or C^{12} CH), 76.6 (C^3H), 122.5 (quinoline C^6), 125.5 (quinoline C), 126.1 (quinoline C^5), 128.5 (quinoline C^2), 130.1, 130.3 (quinoline C^7 and C^8), 136.1 (quinoline C), 149.4 (quinoline C), 150.2 (quinoline C^3), 166.3 (quinoline C=O), 175.1 (ester C=O). The quaternary signal expected to appear at *ca.* 35.1 ppm cannot be seen due to overlapping signals but can be observed in the ^{1}H-^{13}C HMBC spectrum. Elemental Analysis for $C_{35}H_{48}NO_6.½H_2O$: expected C: 71.64%, H: 8.25%, N: 2.39%; found C: 71.77%, H: 8.14%, N: 2.20%. MS (ES+): m/z 578, $[M+H]^+$. HRMS for $C_{35}H_{48}NO_6$: expected 578.3482, found 578.3475.

Palladium Complex Pd(1a)$_2$Cl$_2$

To a solution of *trans*-bis(acetonitrile)dichloropalladium(II) (55 mg, 0.212 mmol, 1 eq.) in dry tetrahydrofuran (6 mL), was added a solution of **1a** (224 mg, 0.424 mmol, 2 eq.) in dry tetrahydrofuran (6 mL). The yellow solution was stirred at room temperature for 2 hours. Hexane was then added to the reaction mixture to precipitate the product. The product was isolated by Buchner filtration and dried in the oven at 140 ℃, to give the product as a yellow solid (70 mg, 27 %). δ_H (400 MHz, $CDCl_3$): 0.78 (s, 6H, C^{18}/C^{19} Me), 1.01 (s, 6H, C^{18}/C^{19} Me), 1.05 (d, 6H, *J* = 6Hz, $C^{21}H_3$), 1.10 – 2.70 (overlapping signals, 48H, steroid ring protons), 3.75 (s, 6H, CO_2CH_3), 3.94 (br m, 2H, C^7*H*(OH)), 4.08 (br m, 2H, C^{12}*H*(OH)), 4.91 (m, 2H, C^3*H*(OH)), 7.95 (d, 4H, *J* = 6.5 Hz, pyridyl C*H*), 9.04 (d, 4H, *J* = 6.5 Hz, pyridyl C*H*s). δ_C (100 MHz, $CDCl_3$): 13.0, 22.9 (C^{18}/C^{19} *C*H_3), 17.8 (C^{21} *C*H_3), 23.5 (CH_2), 26.9 (CH), 27.3 (CH_2), 27.8 (CH_2), 28.8 (CH_2), 31.3 (CH_2), 31.4 (CH_2), 34.6 (CH_2), 35.1 (CH_2), 35.1 (CH_2), 35.4 (C), 35.5 (CH), 39.9 (CH), 41.6 (CH), 42.5 (CH), 46.9 (CH), 47.6 (C), 52.0 (CO_2CH_3), 68.6 (*C*^{7}H), 73.3 (*C*^{12}H), 77.3 (*C*^{3}H, underneath solvent peak), 124.7 (pyridyl CH), 140.8 (pyridyl C), 154.4 (pyridyl CH), 163.2 (pyridyl C=O), 175.1 (ester C=O). Elemental Analysis for $C_{62}H_{90}Cl_2N_2O_{12}Pd.H_2O$: expected C: 59.54%, H: 7.41%, N: 2.24%, Cl: 5.67%; found C: 59.88%, H: 7.45%, N: 2.12%, Cl: 5.73%.

Palladium Complex Pd(2)$_2$Cl$_2$

To a solution of *trans*-bis(acetonitrile)dichloropalladium(II) (25 mg, 0.0964 mmol, 1 eq.) in dry tetrahydrofuran (3 mL), was added a solution of **2** (224 mg, 0.192 mmol, 2 eq.) in dry tetrahydrofuran (3 mL). This yellow solution was stirred at room temperature for 2 hours. Hexane was then added to the reaction mixture to precipitate the product. The product was isolated by Buchner filtration and dried in the oven at 140 ℃, to give the product as a yellow solid (36 mg, 28 %). δ_H (400 MHz, $CDCl_3$): 0.81 (s, 6H, C^{18}/C^{19} CH_3), 1.05 (s, 6H, C^{18}/C^{19} CH_3), 1.08 (d, 6H, *J* = 6Hz, $C^{21}H_3$), 1.20 – 2.76 (overlapping signals, 48H, steroid protons), 3.76 (s, 6H, CO_2CH_3), 3.98 (m, 2H, C^7*H*(OH)), 4.11 (m, 2H, C^{12}H(OH)), 5.07 (m, 2H, C^3*H*(OH)), 7.90 (t, 2H, *J* = 8 Hz, quinoline C^6*H*), 8.07 (d, 2H, *J* = 5.4 Hz, quinoline C^3*H*), 8.20 (br, 2H, quinoline C^7*H*), 8.87 (d, 2H, *J* = 8.4 Hz, quinoline C^8*H*), 9.65 (d, 2H, *J* = 5.4 Hz, quinoline C^2*H*), 10.20 (br, 2H, quinoline C^5*H*). δ_C (100 MHz, $CDCl_3$): 13.0 (C^{18}/C^{19} CH_3), 17.8 (C^{21} CH_3), 22.9 (C^{18}/C^{19} CH_3), 23.5 (2 x CH_2), 27.1 (CH), 27.2 (CH_2), 27.8 (CH_2), 28.9 (CH_2), 31.5 (CH_2), 31.7 (CH_2), 34.7 (CH_2), 35.1 (CH_2), 35.1 (C), 35.5 (CH), 39.9 (CH), 41.7 (CH), 42.5 (CH), 46.9 (C), 47.6 (CH), 52.0 (CO_2CH_3), 68.6 (C^7 or C^{12} CHs), 73.3 (C^7 or C^{12} CH), 77.7 (C^3H), 122.8 (ArCH), 122.9 (ArCH), 126.1 (ArC), 126.6 (ArCH), 127.1 (ArC), 129.8 (ArCH), 132.1 (ArCH), 138.9 (ArC), 154.6 (ArCH), 164.7 (C=O), 175.1 (C=O). Elemental Analysis for $C_{70}H_{94}Cl_2N_2O_{12}Pd.¼CDCl_3$: expected C: 61.92%, H: 6.97%, N: 2.06%, Cl: 7.15%; found C: 61.34%, H: 6.97%, N: 1.98%, Cl: 6.87%.

Platinum complex *cis*-Pt(1a)$_2$I$_2$ (adapted from literature procedure[S5])

A solution of potassium tetrachloroplatinate (25 mg, 60.2 µmol, 1 eq.) in water (40 µL) was warmed to 40 ℃. To this solution was added potassium iodide (59 mg, 361 µmol, 6 eq.). Upon heating the solution to 70 ℃, the solution changed from a red solution to a dark brown solution. Upon reaching 70 ℃, the solution was immediately cooled to room temperature and filtered to remove suspended particulates. To the filtrate solution was then added **1a** (63 mg, 120.4 µmol, 2 eq.). This immediately precipitated a yellow solid, which was isolated by vacuum filtration and washed with ethanol to give the product as a yellow solid (51 mg, 56 %). δ_H ($CDCl_3$, 300 MHz): 0.73 (s, 6H, C^{18} or C^{19} CH_3), 0.95 (s, 6H, C^{18} or C^{19} CH_3), 1.01 (d, 6H, *J* = 3.6 Hz, $C^{21}H_3$), 1.10 – 2.56 (overlapping signals, 48H), 3.70 (s, 6H, CO_2CH_3), 3.88 (m, 2H, C^7*H*), 4.02 (m, 2H, C^{12}H), 4.88 (br, 2H, C^3H), 7.88 (d, 4H, *J* = 6.5 Hz, pyridyl C*H*), 8.99 (d, 2H, *J* = 6.5 Hz, pyridyl C*H*), 9.05 (m, 1H, pyridyl C*H*), 9.13 (d, 1H, *J*

= 7 Hz, pyridyl C*H*). δ_C (75 MHz, $CDCl_3$): 12.6 (CH_3), 17.4 (CH_3), 22.6 (CH_3), 23.1 (CH_2), 26.6 (CH_2), 26.9 (CH), 28.5 (CH_2), 30.9 (CH_2), 31.1 (CH_2), 34.3 (CH_2), 34.7 (CH), 35.1 (C), 39.6 (CH), 41.2 (CH), 42.1 (CH), 46.6 (C), 47.3 (CH), 51.5 (CH_3, ester), 68.1 (CH, C^7 or C^{12}), 72.9 (CH, C^7 or C^{12}), 76.8 (CH, C^3), 124.6 (ArCH), 139.6 (ArC), 156.2 (ArCH), 162.8 (C=O, isonicotinoyl), 174.6 (C=O, methyl ester).

Methyl 3α-(*tert*-butyldimethylsilyl)oxy-7α,12α-dihydroxycholanate (8)

To a stirred solution of methyl cholate **5** (500 mg, 1.2 mmol, 1 eq.) in dry dimethylformamide was added *tert*-butyldimethylchlorosilane (0.85 g, 5.6 mmol, 4.7 eq.) and imidazole (1 g, 14.6 mmol, 12 eq.). The reaction mixture was stirred for 24 h, then the solvent removed by evaporation *in vacuo* to give the crude product as a clear oil, which crystallised upon standing overnight. This material was then recrystallised from di-isopropyl ether to give white crystals of compound **8** (380 mg, 60 %). δ_H (400 MHz, $CDCl_3$): 0.00 (s, 6H, $(CH_3)_2{}^tBuSi$), 0.64 (s, 3H, $C^{18}H_3$), 0.83 (s, 12H, $(CH_3)_3CSi$ and $C^{19}H_3$), 0.93 (d, 3H, $C^{21}H_3$), 1.00 – 2.40 (several overlapping signals, 24H), 3.43 (m, 1H, C^3H(OH)), 3.67 (s, 3H, CO_2CH_3), 3.84 (m, 1H, $C^{12}H$(OH)), 3.97 (m, 1H, C^7H(OH)). δ_C (100 MHz, $CDCl_3$): -4.2 ($(CH_3)_2{}^tBuSi$), 12.9 ($C^{18}H_3$), 17.7 ($C^{21}H_3$), 18.7 (TBDMS C), 23.0 ($C^{19}H_3$), 23.5 (CH_2), 26.4 ($(CH_3)_3Me_2Si$), 27.2 (CH), 27.8 (CH_2), 28.8 (CH_2), 31.3 (CH_2), 31.4 (CH_2), 31.4 (CH_2), 34.9 (C), 35.1 (CH_2), 35.5 (CH), 35.8 (CH_2), 40.0 (CH), 40.4 (CH_2), 41.9 (CH), 42.5 (CH), 46.9 (C), 47.5 (CH), 51.9 (CO_2CH_3), 68.7 (C^7H(OH)), 73.3 (C^{12}H(OH)), 175.1 (C=O). MS (ES+): m/z 537, $[M+H]^+$. HRMS for $C_{31}H_{60}O_5NSi$ ($[M+NH_4]^+$): expected 554.4235, found 552.4243.

Methyl 3α-(*tert*-butyldimethylsilyl)-7α,12α-diacetoxycholanate (9)

To a stirred solution of diol **8** (2.43 g, 4.53 mmol, 1 eq.) in pyridine (12 mL) was added acetic anhydride (4.3 mL, 45.3 mmol, 10 eq.). This solution was stirred at 40 ℃ for 3 days, under the protection of a $CaCl_2$ drying tube. The pyridine/acetic acid/acetic anhydride was removed by evaporation *in vacuo*. The resulting oil was then partitioned between chloroform and water, and the aqueous layer extracted with chloroform (2 x 50 mL). The combined organic extracts were then washed once with saturated aqueous sodium bicarbonate solution. The organic extracts were then dried ($MgSO_4$), filtered, and the filtrate evaporated *in vacuo* to yield compound **9** as a yellow oil which required no further purification (2.42 g, 86 %). δ_H ($CDCl_3$, 400 MHz): 0.09 (s, 6H, $^tBu(CH_3)_2Si$), 0.75 (s, 3H, CH_3), 0.83 (d, 3H, J = 6 Hz, $C^{21}H_3$), 0.91 (s, 12H, $(CH_3)_3CMe_2Si$ and CH_3), 0.95 – 2.43 (overlapping signals 24H, steroid protons), 2.10, 2.17 (acetyl CH_3), 3.46 (m, 1H, C^3H(OH)), 3.69 (s, 3H, CO_2CH_3), 4.90 (m, 1H, C^7 or C^{12} CH(OH)), 5.09 (m, 1H, C^7 or C^{12} CH(OH)). δ_C ($CDCl_3$, 100 MHz): -4.1 ($(CH_3)_2{}^tBuSi$), 12.6 ($C^{18}H_3$), 17.9 ($C^{21}H_3$), 18.8 (TBDMS C), 22.1 ($C^{19}H_3$), 23.0 (2 x acetyl CH_3), 23.1 (CH_2), 26.0 (CH_2), 26.3 (CH_2), 26.3 (^tBu, 3 x CH_3), 27.6 (CH_2), 29.5 (CH_2), 31.3 (CH_2), 31.5 (C), 31.8 (CH_2), 34.8 (CH), 35.0 (CH_2), 35.5 (CH), 38.2 (CH_2), 39.7 (CH), 41.7 (CH), 43.9 (CH), 45.5 (C), 47.7 (CH), 52.0 (CO_2CH_3), 71.3 (C^7H or C^{12}H), 73.4 (C^7H or C^{12}H), 75.9 (C^3H), 171.0 (acetyl C=O), 175.0 (ester C=O).

Methyl 3α-hydroxy-(7α,12α-diacetoxy) cholanate (10)

Compound **9** (2.42 g, 3.9 mmol, 1 eq.) was dissolved in tetrahydrofuran (36 mL) and treated with tetrabutylammonium fluoride (TBAF, 1.0 M solution in tetrahydrofuran, 3.9 mL, 3.9 mmol, 1 eq.). Upon addition of TBAF, the solution immediately took on a strong orange colour that faded in intensity over time. The reaction mixture was stirred overnight at room temperature. TLC analysis (1:1 (v/v) toluene/ethyl acetate) showed the presence of a small amount of starting material, so a further equivalent of TBAF was added. Upon completion of the reaction, the tetrahydrofuran was evaporated *in vacuo*, and the resulting residue diluted with chloroform. This solution was washed twice with water, then dried ($MgSO_4$), filtered, and the filtrate evaporated to give a yellow oil. This oil was purified by flash chromatography (eluant 1:1 toluene/ethyl acetate) to give product **10** as an off-white foam (1 g, 51 %). δ_H ($CDCl_3$, 400 MHz): 0.66 (s, 3H, CH_3), 0.73 (d, 3H, J = 6Hz, $C^{21}H_3$), 0.84 (s, 3H, CH_3), 0.90 – 2.32 (several overlapping signals, 24H), 2.01 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 3.42 (qn, 1H, J = 4Hz, C^3H-OH), 3.59 (s, 3H, CO_2CH_3), 4.82 (m, 1H, $C^{12}H$-OAc), 5.00 (m, 1H, C^7H-OAc). δ_C ($CDCl_3$, 100 MHz): 12.6 ($C^{18}H_3$), 17.9 ($C^{21}H_3$), 21.9 ($C^{19}H_3$), 22.0 (2 x acetyl CH_3), 22.9 (CH), 23.1 (CH_2), 26.0 (CH_2), 27.6 (CH_2), 29.3 (CH), 30.8 (CH_2), 31.1 (CH_2), 31.2 (CH_2), 31.7 (CH_2), 34.7 (C), 35.0 (CH), 35.2 (CH_2), 38.1 (CH), 39.0 (CH_2), 41.4 (CH), 43.8 (CH), 45.4 (C), 47.7 (CH), 51.9 (ester CH_3), 171.0 (acetyl C=O), 171.1 (acetyl C=O), 175.0 (ester C=O). MS (ES+): m/z 529 ($[M+Na]^+$).

Bis[(7α,12α-diacetoxy-24-methoxycarbonyl)-3-cholanyl] *N,N'*-xylylene dicarbamate (11)

meta-Xylylene diisocyanate (97 μL, 0.99 mmol, 0.5 eq.) and 1,4-diazabicyclo-[2.2.2]octane (DABCO) (44 mg, 0.396 mmol, 0.2 eq.) were added to a stirred solution of cholate **10** (1g, 1.98 mmol, 1 eq.) in dry 1,4-dioxane (26mL) at room temperature. The solution was then heated to 60 ℃ and stirred for 24 hours. After bein g allowed to cool, the reaction mixture was then diluted with chloroform. This solution was then washed with dilute hydrochloric acid, saturated aqueous sodium bicarbonate, and saturated aqueous brine solution. The organic extracts were dried ($MgSO_4$), filtered, and the filtrate evaporated *in vacuo* to give the crude product. This residue was purified by flash chromatography (eluant 1:1 (v/v) toluene/ethyl acetate, to give the title compound **11** (R_f 0.21) as a clear oil (360 mg, 30 %). δ_H ($CDCl_3$, 400 MHz): 0.75 (s, 6H, 2 x CH_3), 0.83 (d, 6H, *J* = 6.4 Hz, 2 x $C^{21}H_3$), 0.94 (s, 6H, 2 x CH_3), 1.06 – 2.40 (overlapping signals, 48H, steroid protons), 2.14 (s, 12H, 4 x acetyl CH_3), 3.69 (s, 6H, 2 x CO_2CH_3), 4.36 (d, 4H, *J* = 6 Hz, CH_2PhCH_2), 4.51 (m, 2H, C^3H(OH)), 4.93 (m, 2H, C^7 or C^{12} C*H*(OH)), 5.11 (m, 2H, C^7 or C^{12} C*H*(OH)), 7.21 – 7.35 (overlapping signals, 4H, Ar*H*). δ_C ($CDCl_3$, 100 MHz): 12.6 ($C^{18}H_3$), 17.9 ($C^{21}H_3$), 21.8 ($C^{19}H_3$), 22.1 (CH), 23.0 (CH), 23.2 (CH_2), 25.9 (CH_2), 27.6 (CH_2), 27.7 (CH_2), 29.2 (CH), 31.2 (CH_2), 31.3 (CH_2), 31.6 (CH_2), 34.7 (C), 35.0 (CH), 35.5 (CH_2), 38.1 (CH), 41.3 (CH), 43.7 (acetyl CH_3), 45.3 (CH_2), 45.4 (CH_2) 45.4 (C), 47.7 (acetyl CH_3), 52.0 (CO_2CH_3), 71.2 (C^7H), 75.2 ($C^{12}H$), 75.8 (C^3H), 124.5 (ArCH), 127.0 (ArCH), 128.6 (ArCH), 129.4 (ArCH), 139.5 (ArC), 156.6 (carbamate C=O), 170.8 (acetyl C=O), 170.9 (acetyl C=O), 175.0 (ester C=O). MS (MALDI, α matrix): 1223, $[M+Na]^+$. HRMS for $C_{68}H_{100}O_{16}N_2Na$, $[M+Na]^+$: expected 1223.6965, found 1223.6946.

Bis(7α,12α-dihydroxy-24-carboxyl-3-cholanyl) *N,N'*-xylylene dicarbamate (3) (NMR spectra matched literature values[S6])

To a stirred solution of dimethyl ester **11** (450 mg, 0.375 mmol, 1 eq.) in methanol (88 mL) was added 10% (w/v) aqueous sodium hydroxide (22 mL) and the mixture was stirred at reflux for 2 days. The solution was then acidified to pH 2-3 using 1M hydrochloric acid and the solvent evaporated *in vacuo*. The residue was then washed with chloroform and water until a white solid was obtained. After decantation of the supernatant, this solid was dissolved in methanol. Any undissolved solid was filtered off, and the filtrate evaporated *in vacuo* to give the crude product as an off-white solid. This was then purified by flash chromatography (eluant 7:1 (v/v) chloroform/methanol) to yield the title compound **3** as a tacky white solid. This product was then lyophilised to give a white powder (55 mg, 15 %). δ_H ($CDCl_3$, 400 MHz): 0.68 (s, 6H, 2 x CH_3), 0.90 (s, 6H, 2 x CH_3), 0.97 (d, 6H, *J* = 6 Hz, 2 x $C^{21}H_3$), 1.00 – 2.45 (overlapping signals, 48H, steroid protons), 3.76 (m, 2H, C^7 or C^{12} C*H*(OH)), 3.92 (m, 2H, C^7 or C^{12} C*H*(OH)), 4.21 (d, 4H, *J* = 5 Hz, CH_2PhCH_2), 4.36 (m, 2H, C^3H(OR)), 7.10 – 7.21 (m, 4H, Ar*H*). MS (MALDI, α matrix): 1027, $[M+Na]^+$. HRMS for $C_{58}H_{89}O_{12}N_2$, $[M+H]^+$; expected 1005.6410, obtained 1005.6419.

S2: Alkali metal ion transport assays

S.2.1. Preparation of large unilamellar vesicles

Egg yolk phosphatidylcholine (EYPC, 64 μmol) and cholesterol (16 μmol) were dissolved in spectroscopic grade chloroform and HPLC grade methanol. The chloroform/methanol mixture was evaporated *in vacuo* to give a thin film of lipid on the wall of the flask. To the thin film was then added 1.2 mL of MOPS buffer containing 100mM HPTS dye (20 mM MOPS corrected to pH 7.4 using sodium or potassium hydroxide as appropriate, and 100mM MCl where M = Na or K as appropriate). The thin film was then detached from the sides of the flask by vortex mixing. The turbid suspension of lipids was then extruded 19 times through an 800 nm polycarbonate membrane to give a suspension of large unilamellar vesicles (concentration of lipids = 67 mM). Excess dye was removed by gel permeation chromatography of 1 mL vesicle suspension on PD-10 SEC columns (Sephadex G-25) to give a stock vesicle solution of 3.5 mL (final concentration of lipids = 19 mM)

S.2.2. Procedure for HPTS fluorescent assay of metal ion transport

In a typical experiment, 100 μL of the resulting stock vesicle solution was diluted to 2 mL with fresh MOPS buffer. To this vesicle suspension was then added 20 μL of a 1 mM solution of **1a** (or similar compound) in tetrahydrofuran, followed by 20 μL of a 500 μM solution of $PdCl_2$ in methanol. The cuvette was then placed in the fluorimeter with slow stirring, and the fluorescence emission at 510 nm, resulting from simultaneous excitation at 405 nm and 460 nm, was monitored over a 45-minute time period. After three minutes (180 s) incubation at 25

℃, 13 μL of a 1 M $NaOH_{(aq)}$ solution was added, to provide the "base pulse". At forty minutes (2400 s), 40 μL of a 25 % v/v solution of Triton X-100 detergent in MOPS was added to lyse the vesicles. The change in the normalised ratio I_{460}/I_{405} with time gave the rate of M^+/H^+ exchange though the phospholipid bilayer.

S.2.3. Procedure for the determination of first order rate constants

The change in the normalised ratio I_{460}/I_{405} (henceforth referred to as I_{norm}) with time was iteratively fitted to first order kinetics using an equation of the general form:

$$I_{norm} = I_{\infty} - \exp(kt + c)$$

S.2.4. Sodium ion transport rates

For all sodium ion transport experiments, the HPTS assays were repeated several times and showed good reproducibility (Figure S1).

Data set	Rate constant / s^{-1}
Repeat 1 (●)	25.0×10^{-4}
Repeat 2 (●)	24.3×10^{-4}
Repeat 3 (●)	25.3×10^{-4}

Table S1: Fitted rate constants for the three different repetitions of the $PdCl_2$ + **1a** Na^+ transport experiment shown in Fig S1 (a).

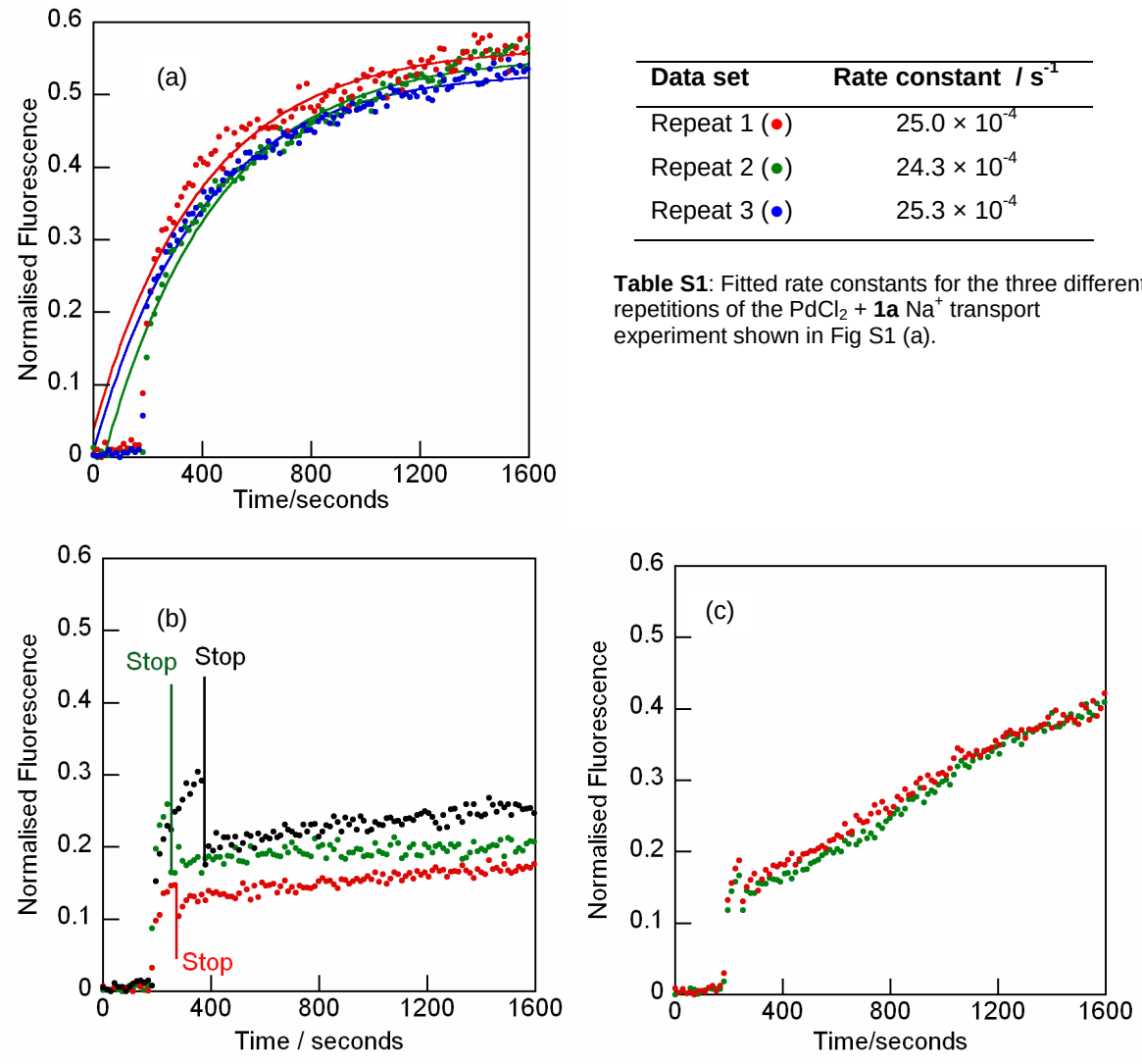


Figure S1:
(a) Change in HPTS fluorescence with time for three different repetitions of the $PdCl_2$ + **1a** sodium ion transport experiment;
(b) Repeats of the channel-closing experiments for the standard $PdCl_2$ + **1a** sodium ion transport experiment. The channels are closed after different time periods; 4 minutes (●, ●) or 6 minutes (●).
(c) Repetitions of the "closing and re-opening" experiments.

S.2.5. Potassium ion transport assays

For all potassium ion transport experiments, the HPTS assays were repeated several times and showed good reproducibility. Representative results obtained from applying the HPTS assay to the potassium transport series are shown in Figure S2 and calculated rate constants are given in the main article text (Table 1). The rates of the transport of sodium or potassium by $PdCl_2$ + **1a** or $PdCl_2$ + **1b** are compared in Figure S2 (b).

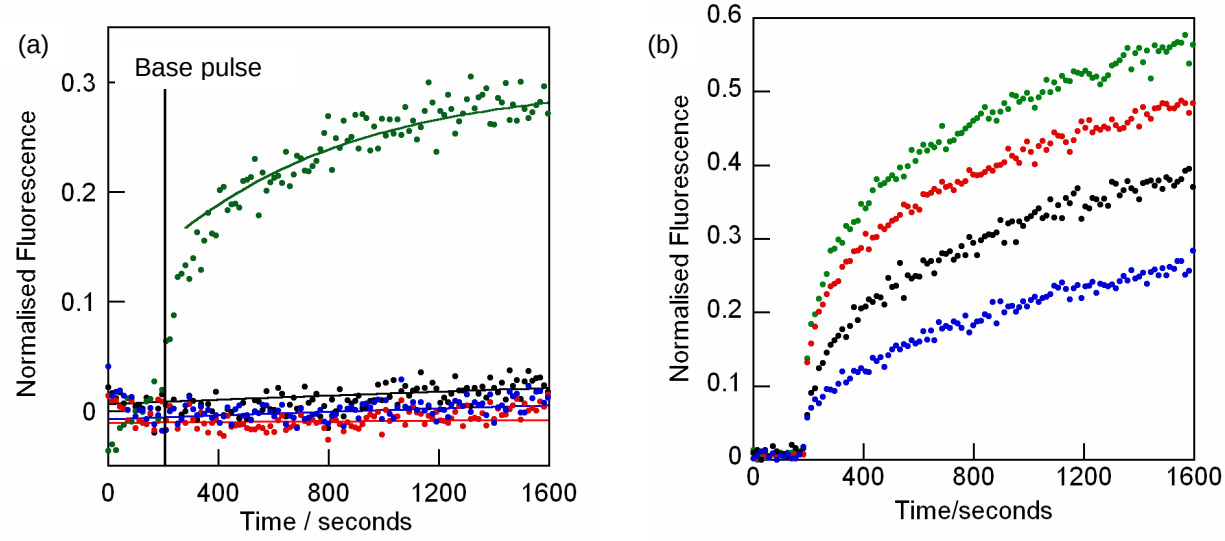


Figure S2:
(a) Change in fractional HPTS emission intensity after KOH addition (base pulse at 3 mins) to vesicles containing HPTS. Vesicles mixed with: $PdCl_2$ only (●); **1a** only (●); both **1a** and $PdCl_2$ (●); **3** only (●). The background rate has been subtracted as described in the manuscript;
(b) Change in fractional HPTS emission intensity after NaOH/KOH addition (base pulse at 3 mins) to vesicles containing encapsulated HPTS. Vesicles mixed with: **1a** and $PdCl_2$, Na^+ assay (●); **1b** and $PdCl_2$, Na^+ assay (●); **1a** and $PdCl_2$, K^+ assay (●); **1b** and $PdCl_2$, K^+ assay (●). These data are unsubtracted.

S.2.6. Sodium ion transport by $PdCl_2$ +1a, $PdCl_2$ +2, trans-$Pd(1a)_2Cl_2$ and cis-$Pt(1a)_2I_2$

The combination of $PdCl_2$ and **2** did not transport Na^+ more effectively than **2** only, although much like with **1a** and **1b**, a “burst phase” was observed.

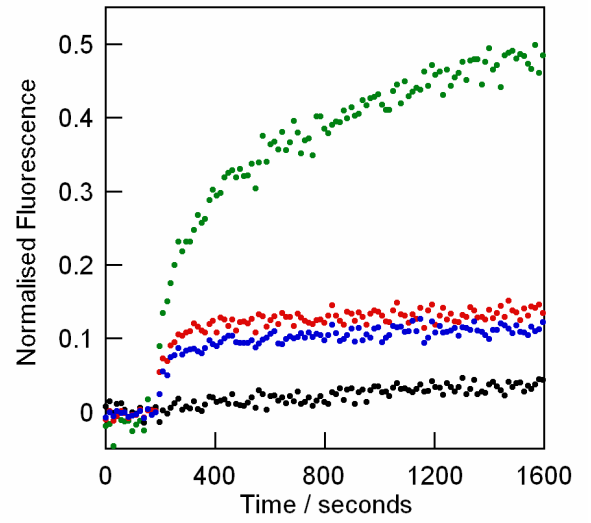


Figure S3: Change in fractional HPTS emission intensity after NaOH addition (base pulse at 3 mins) to vesicles containing encapsulated HPTS. Vesicles mixed with: **1a** and $PdCl_2$ (●); **2** and $PdCl_2$ (●); **2** only (●); $PdCl_2$ only (●). The background rate has been subtracted.

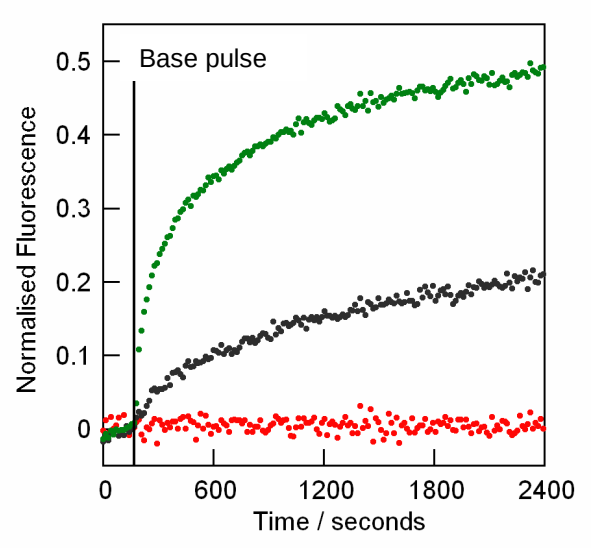


Figure S4: Change in fractional HPTS emission intensity after NaOH addition (base pulse at 3 mins) to vesicles containing encapsulated HPTS. Vesicles mixed with: **1a** and $PdCl_2$ (●); *trans*-Pd(**1a**)$_2$Cl$_2$ (●); *cis*-Pt(**1a**)$_2$I$_2$ (●). The background rate has been subtracted.

S.2.7. Sodium ion transport with the base pulse applied before the addition of $PdCl_2$ and 1a.

Reversing the sequence of addition of the base pulse and the channel-forming compounds **1a** and $PdCl_2$ revealed that transport through preformed channels was responsible for the base-pulse

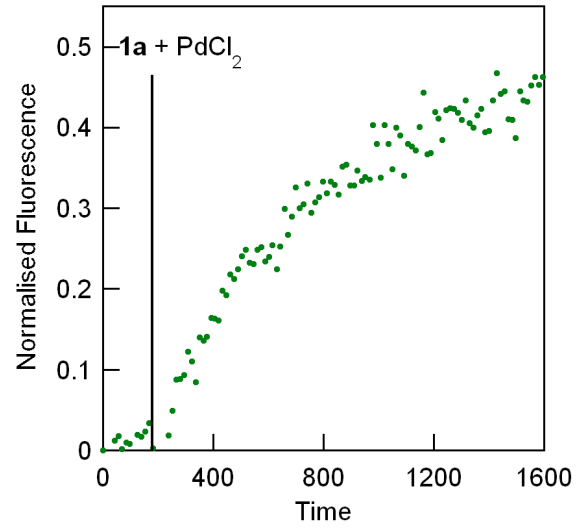


Figure S5: Change in fractional HPTS emission intensity after NaOH addition (base pulse at 0 mins) followed by the addition of **1a** + $PdCl_2$ (●) (3 mins); The background rate has been subtracted.

S3: Determination of Ionophoric Mechanism: Channel vs. Carrier

S.3.1. Sodium ion transport through ($PdCl_2$ + 1a) channels in DPPC vesicles.[S7]

Comparing the ability of $PdCl_2$ + **1a** to transport ions through DPPC membranes at 25 ℃ to transport rates through EYPC membranes at 25 ℃ was used to determine if the combination of $PdCl_2$ + **1a** formed ion channels or ion carriers in the bilayer. If the ionophoric system shows similar or better activity in gel-phase DPPC vesicles compared to fluid phase EYPC vesicles, then the ionophoric system forms a channel.[S7]
DPPC vesicles encapsulating HPTS were produced using the method detailed in S.2.1 above, but with extrusion carried out above the melting temperature of the DPPC membranes (42 ℃). The rates of Na^+ transport were measured at 25 ℃ as detailed in S.2.2 above. In addition, $PdCl_2$, **1a** and $PdCl_2$ + **1a** were added to DPPC vesicle suspensions at 25 ℃, the samples briefly warmed to 45 ℃ (30 s), then cooled back to 25 ℃. This was followed by a base pulse at 180 s.

The results of these studies closely matched our postulated mechanism. Addition of $PdCl_2$ + **1a** to DPPC vesicles at 25 ℃ gave no Na^+ transport because the components were immobile in the gel-phase DPPC membranes and could not combine to form an active Pd(**1a**)$_2$Cl$_2$ complex. However if the DPPC vesicles were briefly warmed to the fluid phase (30 s) and cooled back into the gel-phase before application of the base pulse, excellent rates of transport were observed as $PdCl_2$ and **1a** had combined to form active channels. These rates were much higher than those observed with EYPC vesicles, which suggests that $PdCl_2$ + **1a** channels are very active once formed and the subsequent formation of more channels in the membrane is rate-limiting; the subsequent formation of channels is now very slow in the gel-phase DPPC membranes.

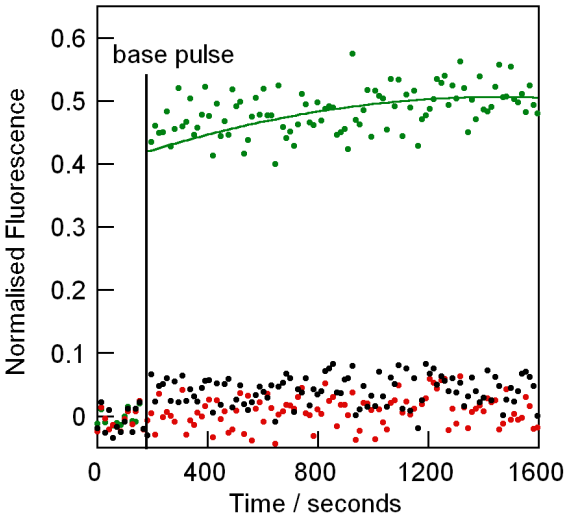


Figure S6: Normalised change in HPTS emission after NaOH addition to DPPC vesicles containing HPTS (base pulse at 180 s). Vesicles mixed with: $PdCl_2$ (●); **1a** (●); **1a** + $PdCl_2$ (●). The background rate has been subtracted. Curve fit is to guide the eye.

S.3.2 U-tube metal picrate transport experiments[S8]

A glass U-tube (14 mm internal diameter) was incubated in a water bath at 25 ℃. A chloroform solution of the potentially transporting species (1 mM, 10 mL) was placed in the U-tube to form the bottom layer. To one side of the U-tube was added a receiving phase of distilled water (5 mL), and to the other side was added a source phase of 0.01% metal picrate in MOPS buffer (5 mL, buffer corrected to pH 7.4, [picrate] = 436 μM). Addition of the source phase marked the start of the experiment. At the start, an aliquot (2 mL) was taken from the receiving phase and analysed for the presence of picrate by UV spectroscopy. After measurement, the sample was immediately replaced back in the U-tube. Measurements were then taken every two hours for ten hours. The chloroform phase was stirred at 300 rpm throughout the experiment to ensure efficient diffusion of any carrier-ion complex to the receiving phase.

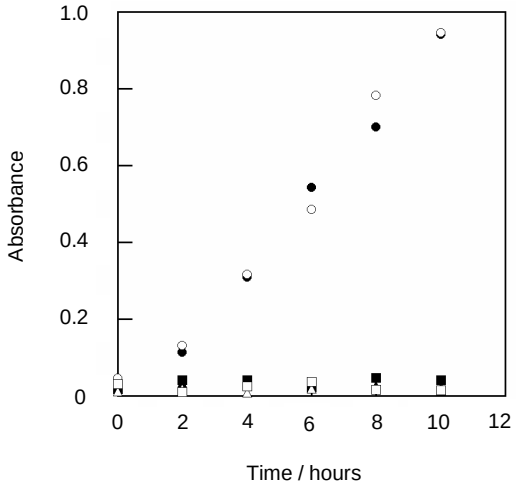


Figure S7: Plot of picrate absorbance in the receiving phase as a function of time. Filled shapes represent the Na^+ transport assay, empty shapes the K^+ transport assay. Dibenzo-18-crown-6 is represented by circles (●, ○), *trans*-Pd(**1a**)$_2$Cl$_2$ by triangles (▲, △), and **1a** by squares (■, □).

S4: Determination of Binding Constants

S.4.1. Fluorescence titrations of vesicle-bound 2 with $PdCl_2$

Egg yolk phosphatidylcholine (64 μmol) and cholesterol (16 μmol) were dissolved in spectroscopic grade chloroform and HPLC grade methanol. The chloroform/methanol mixture was evaporated *in vacuo* to give a thin film of lipid on the walls of the flask. To the thin film was then added 1.2 mL of MOPS buffer, buffered to pH 7.4. The thin film was then detached from the sides of the flask by vortexing. The turbid suspension of lipids was then extruded 19 times through an 800 nm polycarbonate membrane to give a suspension of large unilamellar vesicles.

These vesicles were then diluted to 3.5 mL to replicate the dilution effect of gel permeation chromatography. An aliquot of this stock solution (200 μL) was diluted to 1.96 mL with fresh MOPS buffer and 40 μL of a solution of 1 mM **2** in THF was then added to give a total volume of 2mL in the cuvette; this solution (**Solution A**) was similar to that used in the ion channel experiments (20 μM **2** vs. 10 μM **1a** in the latter). A second solution was made up with 200 μL vesicles, 40 μL of 1 mM **2** in THF, and 1760 μL of a 1.136 mM solution of $PdCl_2$ in MOPS buffer, which gave a 2 mL vesicle solution of [$PdCl_2$] = 1 mM (**Solution B**). The concentrations of **2** and the concentration of lipids were identical in both samples, at 20 μM and 2 mM respectively and twice their concentrations used in the ion transport experiments. A vesicle solution with [$PdCl_2$] = 4 mM (**Solution C**) was also created from a stock solution of 4.544 mM.

The titration was repeated three times using **Solutions B** and **C**. After addition of each $PdCl_2$ aliquot, fluorescence spectra were recorded immediately and after five minutes, and showed little change. The data obtained were fitted to a 1 + 2 binding isotherm (Pd + **2**) and non-linearly regressively fitted using Dynafit.[S9]

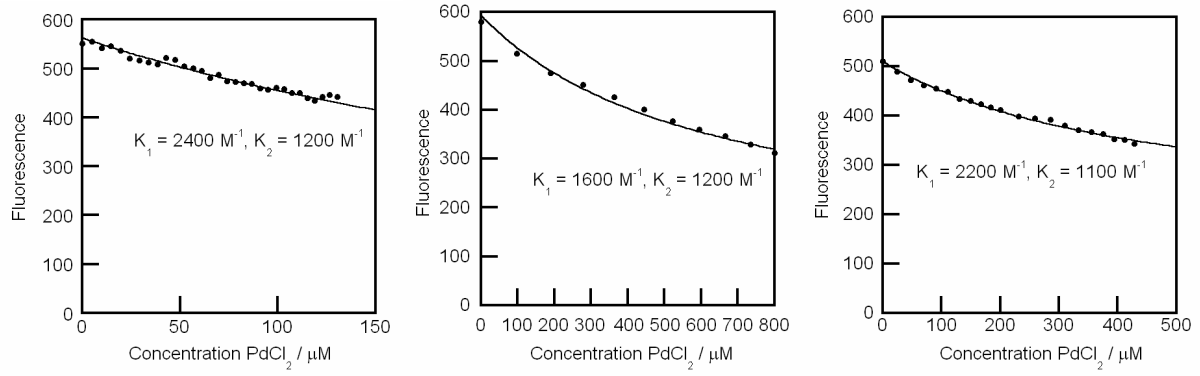


Figure S8: Calculated curve fits for the titration of membrane-bound **2** with $PdCl_2$.

S.4.2. NMR competition experiments

Samples of Pd(**1a**)$_2$$Cl_2$ or Pd(**2**)$_2$$Cl_2$ were dissolved in $CDCl_3$ (5 mg in 0.75 mL), then mixed with either one or two molar equivalents of **2** or **1a** respectively. ^{1}H NMR spectra were recorded at t = 0 h, then re-recorded at t = 24 h after equilibration. Excerpts of the spectra obtained are shown below, with the resonances due to the products labelled as follows: A = Pd(**1a**)$_2$$Cl_2$, B = **2**, C = Pd(**1a**)(**2**)Cl_2, D = **1a**, E = Pd(**2**)$_2$$Cl_2$.

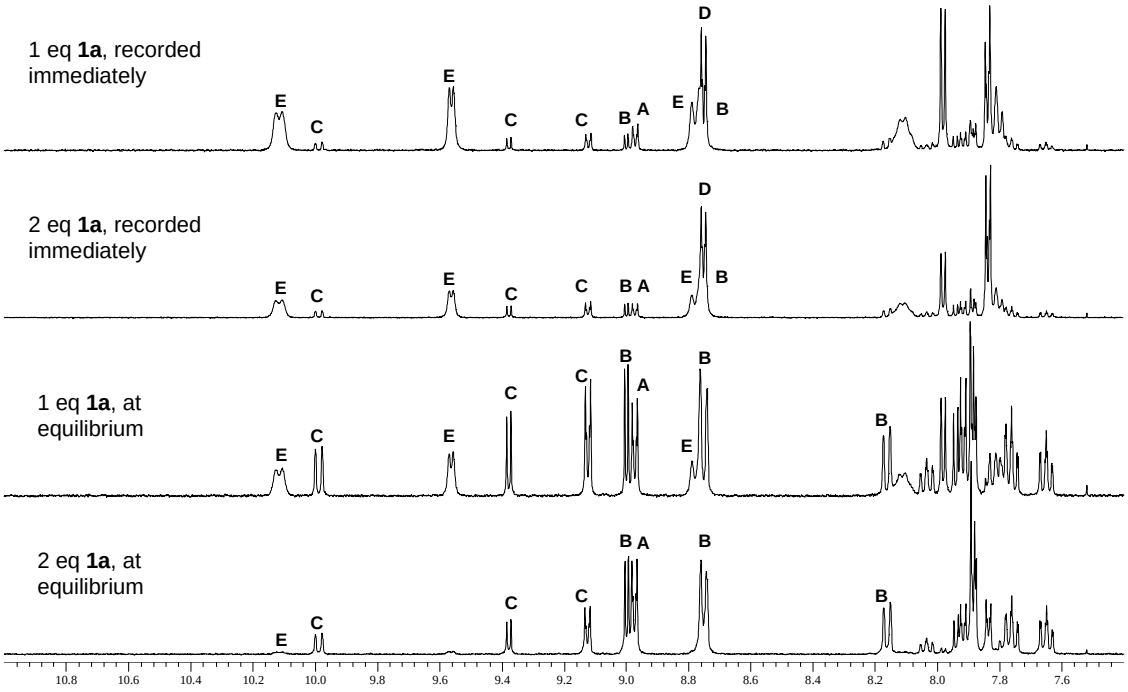


Figure S9: Addition of **1a** to complex Pd(**2**)$_2$Cl$_2$

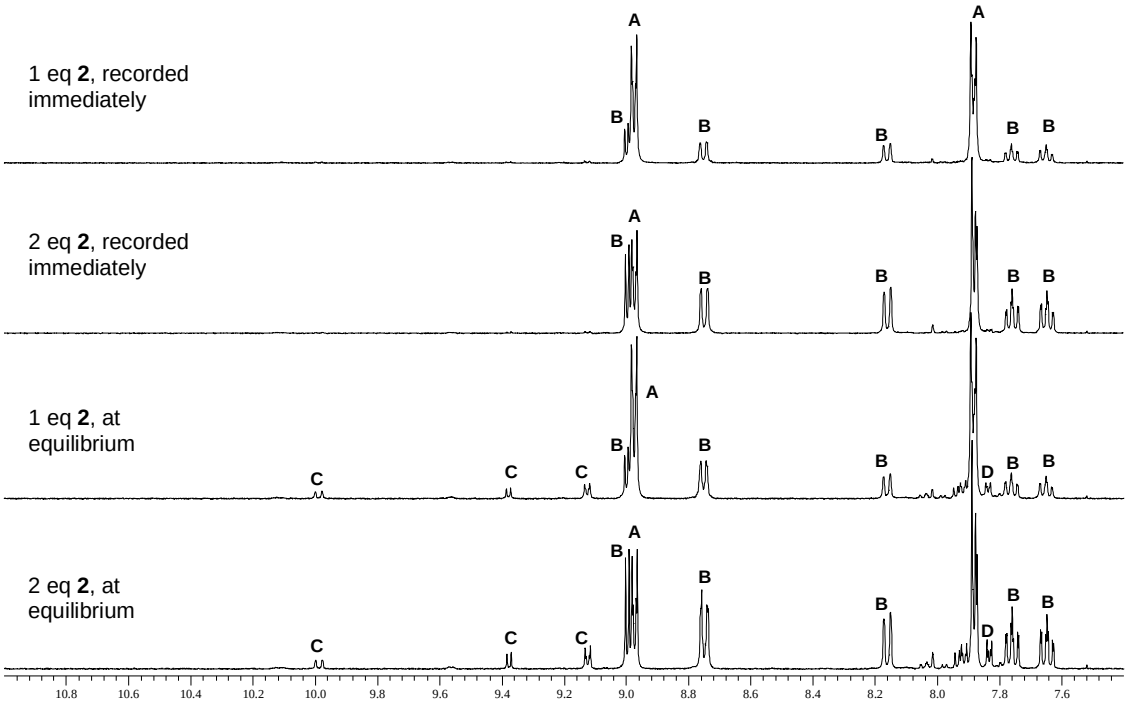


Figure S10: Addition of **2** to complex Pd(**1a**)$_2$Cl$_2$

The integrals were calculated relative to the total integral of the C^{3}H proton of all species in the system, which has been assigned an arbitrary integral of 1.

S.4.3. Analysis of data from NMR competition experiments

Important Equilibria and Assumptions

$$PdCl_2 + \mathbf{1a} \overset{K_1^{\mathbf{1a}}}{\rightleftharpoons} PdCl_2(\mathbf{1a}) \qquad PdCl_2 + \mathbf{2} \overset{K_1^{\mathbf{2}}}{\rightleftharpoons} PdCl_2(\mathbf{2})$$

$$PdCl_2(\mathbf{1a}) + \mathbf{1a} \overset{K_2^{\mathbf{1a}}}{\rightleftharpoons} PdCl_2(\mathbf{1a})_2 \qquad PdCl_2(\mathbf{2}) + \mathbf{2} \overset{K_2^{\mathbf{2}}}{\rightleftharpoons} PdCl_2(\mathbf{2})_2$$

$$PdCl_2(\mathbf{1a}) + \mathbf{2} \overset{K_2^{\mathbf{1a,2}}}{\rightleftharpoons} PdCl_2(\mathbf{2})(\mathbf{1a})$$

$$PdCl_2(\mathbf{2}) + \mathbf{1a} \overset{K_2^{\mathbf{2,1a}}}{\rightleftharpoons} PdCl_2(\mathbf{2})(\mathbf{1a})$$

Since the concentrations used in the competition experiments (~ 0.01 M) are too high for significant amounts of the species $PdCl_2$, $PdCl_2(\mathbf{1a})$ and $PdCl_2(\mathbf{2})$, the relevant equilibria are:

$$PdCl_2(\mathbf{1a})_2 + \mathbf{2} \overset{K_3}{\rightleftharpoons} PdCl_2(\mathbf{2})(\mathbf{1a}) + \mathbf{1a} \qquad K_3 = \frac{K_2^{\mathbf{1a,2}}}{K_2^{\mathbf{1a}}}$$

$$PdCl_2(\mathbf{2})(\mathbf{1a}) + \mathbf{2} \overset{K_4}{\rightleftharpoons} PdCl_2(\mathbf{2})_2 + \mathbf{1a} \qquad K_4 = \frac{K_2^{\mathbf{2}}}{K_2^{\mathbf{2,1a}}}$$

If we assume that the nature of the non-exchanging ligand has little effect on the position of the equilibrium (e.g. displacement of **2** by **1a** occurs to the same extent for $PdCl_2(\mathbf{2})(\mathbf{1a})$ and $PdCl_2(\mathbf{2})_2$, then:

$$K_3 \sim K_4 \sim \frac{K_2^{\mathbf{1a}}}{K_2^{\mathbf{2}}}$$

Equilibrium Equations

Let: $PdCl_2(\mathbf{1a})_2$ = A **2** = B $PdCl_2(\mathbf{2})(\mathbf{1a})$ = C **1a** = D $PdCl_2(\mathbf{2})_2$ = E

and the subscripts "o" denote starting concentrations and "e" denote equilibrium concentrations

$$K_3 = \frac{[PdCl_2(\mathbf{1a})(\mathbf{2})][\mathbf{1a}]}{[PdCl_2(\mathbf{1a})_2][\mathbf{2}]} \qquad \frac{[C]_e[D]_e}{[A]_e[B]_e}$$

$$K_4 = \frac{[PdCl_2(\mathbf{2})_2][\mathbf{1a}]}{[PdCl_2(\mathbf{1a})(\mathbf{2})][\mathbf{2}]} \qquad \frac{[E]_e[D]_e}{[C]_e[B]_e}$$

$$K_3K_4 \sim (K_3)^2 = \frac{[PdCl_2(\mathbf{2})_2][\mathbf{1a}]^2}{[PdCl_2(\mathbf{1a})_2][\mathbf{2}]^2} \qquad \frac{[E]_e[D]_e^{\,2}}{[A]_e[B]_e^{\,2}}$$

NMR Data

We can easily measure resonances in the aromatic region from species A, B, C, and E, but not D (both resonances are obscured). The integrals are referenced to the integral of the combined 3H resonances for all 5 species.

Reference Integral Integral of the 3- proton (1H) = $2[PdCl_2(\mathbf{2})_2] + 2[PdCl_2(\mathbf{1a})(\mathbf{2})] + 2[PdCl_2(\mathbf{1a})] + [\mathbf{2}] + [\mathbf{1a}]$

Integral of the 3- proton = $2[E]_e + 2[C]_e + 2([A]_o - [E]_e - [C]_e) + ([B]_o - [C] - 2[E]) + [D]$

Integral of the 3- proton = $2[A]_o + ([B]_o - [C] - 2[E]) + [D]$ and $[D] = [C] + 2[E]$

Integral of the 3- proton = $1 = 2[A]_o + [B]_o$

Product Integrals

Species A Integral of a pyridyl proton enviroment of $PdCl_2(\mathbf{1a})_2$ (4H) = $\dfrac{4[A]_e}{2[A]_o + [B]_o}$

If $[B]_o = 2[A]_o$ Integral A = $\dfrac{[A]_e}{[A]_o}$ If $[B]_o = [A]_o$ Integral A = $\dfrac{4[A]_e}{3[A]_o}$

Species B Integral of a quinolyl proton enviroment of **2** (H) = $\dfrac{[B]_e}{2[A]_o + [B]_o}$

If $[B]_o = 2[A]_o$ Integral B = $\dfrac{[B]_e}{4[A]_o}$ If $[B]_o = [A]_o$ Integral B = $\dfrac{[B]_e}{3[A]_o}$

Species C Integral of a quinolyl proton enviroment of $PdCl_2(\mathbf{2})(\mathbf{1a})$ (1H) = $\dfrac{[C]_e}{2[A]_o + [B]_o}$

If $[B]_o = 2[A]_o$ Integral C = $\dfrac{[C]_e}{4[A]_o}$ If $[B]_o = [A]_o$ Integral C = $\dfrac{[C]_e}{3[A]_o}$

Species E Integral of a quinolyl proton enviroment of $PdCl_2(\mathbf{2})_2$ (2H) = $\dfrac{2[E]_e}{2[A]_o + [B]_o}$

If $[B]_o = 2[A]_o$ Integral E = $\dfrac{[E]_e}{2[A]_o}$ If $[B]_o = [A]_o$ Integral E = $\dfrac{2[E]_e}{3[A]_o}$

Thus from the competition experiment mixing 1 eq. $PdCl_2(\mathbf{1a})_2$ with 2 eq. **2** i.e. $[A]_o = 2[B]_o$

therefore Integral C = 0.06, so $[C]_e = 0.24[A]_o$

Integral E < 0.01, so $[E]_e \sim 0[A]_o$

It is not necessary to know the values of all the integrals, since in terms of $[C]_e$ and $[E]_e$

$[A]_e = ([A]_o - [C]_e - [E]_e) = ([A]_o - 0.24[A]_e - 0[A]_e) = 0.76[A]_o$

$[B]_e = ([B]_o - [C]_e - 2[E]_e) = (2[A]_o - 0.24[A]_e - 0[A]_e) = 1.76\,[A]_o$ (measured value: $[B]_e = 1.74$

$[D]_e = ([C]_e + 2[E]_e) = 0.24[A]_o$

$$K_3 = \frac{[PdCl_2(\mathbf{1a})(\mathbf{2})][\mathbf{1a}]}{[PdCl_2(\mathbf{1a})_2][\mathbf{2}]} = \frac{[C]_e[D]_e}{[A]_e[B]_e} = \frac{(0.24[A]_o)(0.24[A]_o)}{(0.76[A]_o)(1.76[A]_o)} = 0.043$$

Therefore since $K_3 \sim \dfrac{K_2^{\mathbf{1a}}}{K_2^{\mathbf{2}}}$

and $K_2^{\mathbf{2}} \sim 1100\ M^{-1}$ for $PdCl_2$ to membrane-bound **2** (in a similar environment to $CDCl_3$),

then $K_2^{\mathbf{1a}} \sim 25500\ M^{-1}$ and by extension $K_1^{1a} \sim 51000\ M^{-1}$

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