Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

SUPPORTING INFORMATION

Anion Structure-Activity Relationship of Imidazolium-based Synthetic Transporters

Claude-Rosny Elie,[a] Mathieu Charbonneau[b] and Andreea R. Schmitzer*[a]

Département de Chimie, Université de Montréal

CP 6128 succursalle centre ville 2900 Edouard Montpetit H3C3J&, Montréal, Québec, Canada

Experimental section.

General. 4-iodobenzoïc acid, borane in tetrahydorfuran (BH_3 in THF), phosphorus tribromide (PBr_3) were obtained from Aldrich. Tetrahydrofuran (THF), dichloromethane (DCM), acetonitrile (CH_3CN), hexane and ethyl acetate (EtOAc) were purchased from EMD. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer at 300 and 75 MHz, respectively, in the indicated solvent. Chemical shifts are reported in ppm with internal reference to TMS. High-resolution mass spectra (HRMS) were recorded on a LC-MSD-Tof instrument from Agilent technologies in positive electrospray mode in general. Either protonated molecular ions $(M+H)^+$ or silver adducts $(M+Ag)^+$ were used for empirical formula confirmation. NMR spectra, for the complexation studies, were recorded on a Bruker spectrometer at 300 MHz. Phospholipids used to prepare liposomes were purchased from Avanti Polar Lipids. Size-exclusion chromatography was performed using Sephadex G-25. Fluorescent dyes HPTS and Lucigenin were purchased from Fluka and Molecular Probes, respectively. Liposome fluorimetric assays were recorded using a Varian Cary Eclipse Fluorescence spectrophotometer.

Synthesis. The synthesis and characterization of compound **1** were previously reported[11]. The compounds **2**-**4** have been synthesized by a counter ion change of compound **1** (Scheme 3). The (*N,N'*-Diphenylethynylbenzyl)imidazolium bromide salt (**1)** has been synthesized from imidazole using (4-phenylethynyl)benzyl bromide (**d)** in THF at 70°C during 12 hours (Scheme 2). The (4-phenylethynyl)benzyl bromide (**c)** has been obtained from 4-iodobenzoïc acid (**3a**) reduced to 4-iodobenzyl alcohol (**b**) using BH_3 in THF overnight at room temperature, then to react with benzylacetylene using PPh_3, $PdCl_2(PPh_3)_2$, CuI in dry THF with Et_3N overnight at 50 °C (Scheme 1).

4-Iodobenzyl alcohol (b) : 4-Iodobenzoïc acid (0.02 mol) diluted in 40 mL THF was added to 40 mL of a solution of BH_3 in THF 1.0 M and the mixture was stirred overnight at room temperature. The reaction was quenched with 100 mL of HCl 2 N and extracted with 3 × 140 mL of DCM. The combined organic layers

were washed with 2 × 80 mL of $NaHCO_3$ saturated then 2 × 80 mL of Brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the pure product as a white solid in a 99 % isolated yield. Mp 68 – 70 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, 2H, J = 8.2 Hz), 7.06 (d, 2H, J = 8.0 Hz), 4.58 (s, 2H), 2.04 (b, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.3, 137.4, 128.7, 92.9, 64.4. HRMS (ESI) calcd for $C_7H_7AgIO^+$ $[M+Ag]^+$: 340.8587, found 340.8591.

(4-Phenylethynyl)benzyl alcohol (c) : To a carefully degassed solution of 4-iodobenzyl alcohol **b** (4.3 mmol), PPh_3 (0.085 mmol), and $PdCl_2(PPh_3)_2$ (0.026 mmol) in 10 mL of dry THF and 5 mL of dry triethylamine was added CuI (0.085 mmol). The mixture was degassed for 5 min and a solution of phenylacetylene (4.3 mmol) in 2 mL of dry THF was added dropwise. The reaction was stirred overnight at 50 °C under nitrogen atmosphere. The mixture was added to 50 mL of ice water and the organic phase was recovered, dried over $MgSO_4$. The solvent was removed under reduced pressure to give the pure product as a white solid in 99 % isolated yield. Mp. 118 – 120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54-7.50 (m, 4H), 7.37-7.24 (m, 5H), 4.66 (s, 2H), 2.00 (b, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.9, 131.7, 131.5, 128.3, 128.2, 126.7, 123.1, 122.3, 89.3, 89.1, 64.8. HRMS (ESI) calcd for $C_{15}H_{13}O^+$ $[M+H]^+$: 209.0961, found 209.0969.

(4-Phenylethynyl)benzyl bromide (d) : (4-Phenylethynyl)benzyl alcohol (**c**) (1.44 mmol) was dissolved in 5 mL of DCM. The mixture was put at 0 °C and phosphorus tribromide was added dropwise. Then the mixture was stirred 2 hours at 0 °C and the solvent was removed under reduced pressure. The product was purified by flash chromatography (Hexane/EtOAc, 60:40) to afford the compound **d** as a white solid in 100 % isolated yield. Mp 94 – 96 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54-7.48 (m, 4H), 7.37-7.24 (m, 5H), 4.48 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.6, 131.9, 131.5, 129.0, 128.3, 128.2, 123.3, 122.9, 90.2, 88.8, 32.9. HRMS (ESI) calcd for $C_{15}H_{12}Br^+$ $[M+H]^+$: 271.0117, found 271.0109.

(*N,N'*-Diphenylethynylbenzyl)imidazolium bromide (1) : 27.5 mg (0.40 mmol) of imidazole and 37.8 mg of sodium hydride (1.57 mmol) are diluted in 10 mL of THF. After 5 minutes at room temperature, 100 mg (0.40 mmol) of (4-

phenylethynyl)benzyl bromide **d** is added to the mixture. The solution is stirred at room temperature for 12 hrs and the solvent is removed in vacuuo. 20 mL of water and 30 mL of DCM are added to the crude product. The organic phase is separated, washed with 2 x 20 mL of water and then dried on $MgSO_4$. The solvent is removed in vacuuo to yield 100 mg of the intermediate (4-Phényléthynyl)benzylimidazole. This compound is then used without further purification: 100 mg (0.47 mmol) of (4-phenylethynyl)benzylimidazole and 127 mg (0.47 mmol) of (4-phenylethynyl)benzyl bromide **d** are disolved in 10 mL of THF. The mixture is stirred 12 hrs at 70°C and the resulting precipitate is filtered, washed with 10 mL of THF, and dried in vacuuo. 134 mg of **1** were obtained. Yield 67 %. ^{1}H NMR (CD_3OD, 400MHz): *δ*=7.71 (s, 2H), 7.62 (d, *J*=8.3 Hz, 4H), 7.51-7.56 (m, 4H), 7.46 (d, *J*=8.3 Hz, 4H), 7.37-7.42 (m, 6H), 7.38-7.42 (m, 2H), 5.48 ppm (s, 4H). 13C NMR (CD3OD, 75 MHz): *δ*=134.6, 132.8, 132.0, 129.3, 129.2, 129.0, 125.2, 123.7, 123.5, 123.2, 90.9, 88.5, 53.3 ppm. HR-MS (ESI): m/z Calcd for $C_{33}H_{25}N_2$ [M-Br]+: 449.2012, found 449.2017.

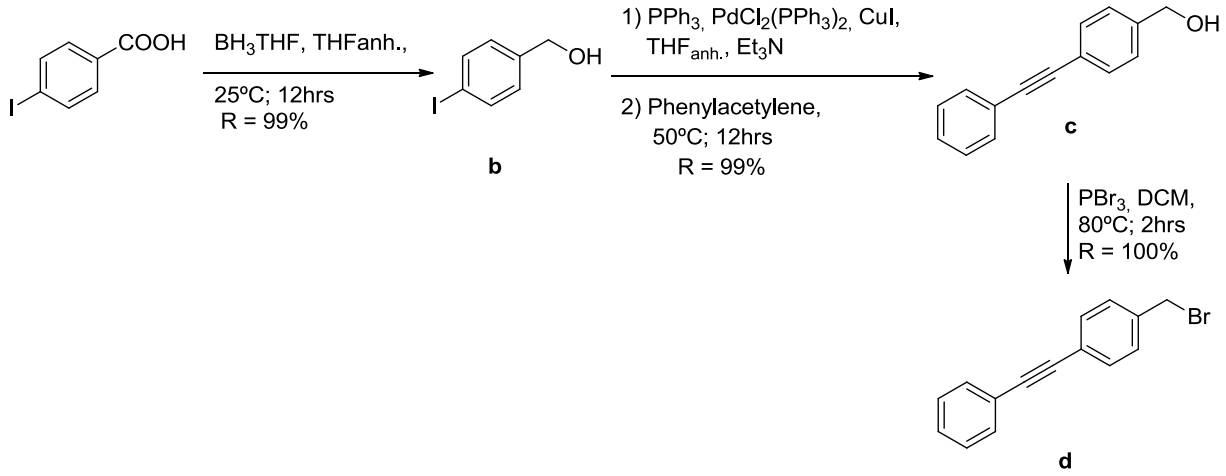
(*N,N'*-Diphenylethynylbenzyl)imidazolium Tetrafluoroborate (2) : 100 mg (0,19 mmoles) of (N,N'-Diphenylethynylbenzyl)imidazolium boride are dissolved in a mixture of MeOH/ACN (50 :50). Then, 32 mg (0,29 mmoles) of $NaBF_4$ are added to 10 mL of methanol before being poured onto the solution containing the imidazolium salt. The resulting mixture is then brought to 80°C and stirred with a magnetic stir bar during 12 hours. After evaporating the solvent under reduced pressure, 40mL of distilled water are added to the powder obtained. The mixture is heated to 100ºC for 12 hours under magnetic stirring, and then filtered on a fritted glass. The raw product is then dried at 80°C for 2 hours, in order to obtain 96 mg of a white powder (95% yield).^{1}H NMR (400 MHz, CHLOROFORM-*d*) □ ppm 9.40 (s, 1 H), 7.87 (d, *J*=1.41 Hz, 2 H), 7.62 - 7.66 (m, 4 H), 7.53 - 7.59 (m, 4 H), 7.43 - 7.50 (m, 10 H), 5.49 (s, 4 H).

(*N,N'*-Diphenylethynylbenzyl)imidazolium Hexafluorophosphate (3) : 100 mg (0,19 mmoles) of (N,N'-Diphenylethynylbenzyl)imidazolium boride are dissolved in a mixture of MeOH/ACN (50 :50). Then, 46 mg (0,29 mmoles) of NH_4PF_6 are added to 10 mL of methanol before being poured onto the solution containing the

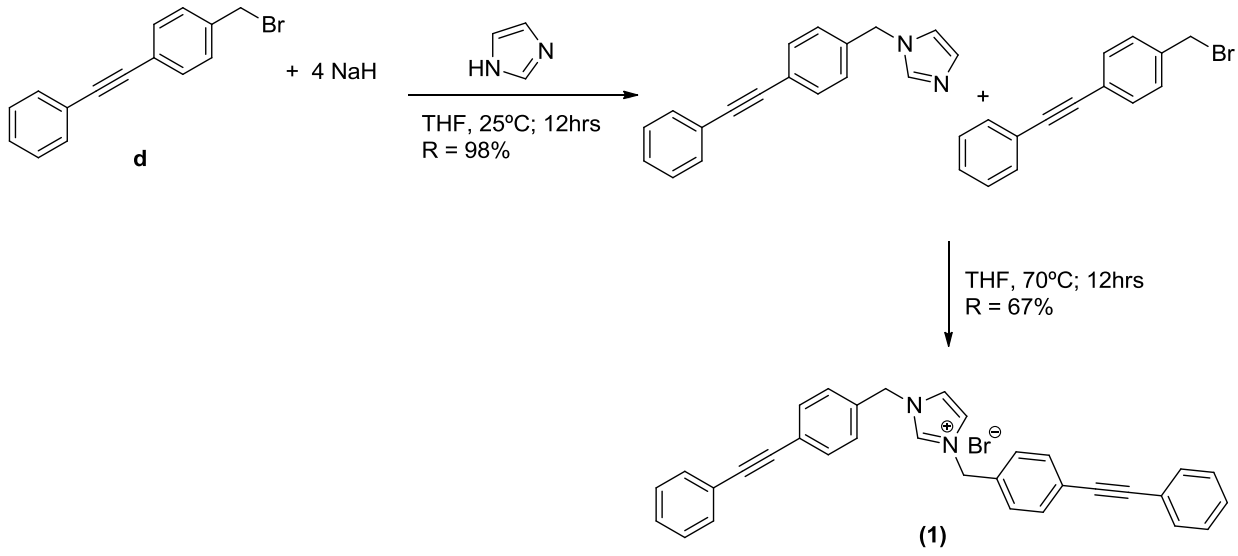
imidazolium salt. The resulting mixture is then brought to 80°C and stirred with a magnetic stir bar during 12 hours. After evaporating the solvent under reduced pressure, 40mL of distilled water are added to the powder obtained. The mixture is heated to 100ºC for 12 hours under magnetic stirring, and then filtered on a fritted glass. The raw product is then dried at 80°C for 2 hours, in order to obtain 113 mg of a white powder (99% yield).^{1}H NMR (400 MHz, CHLOROFORM-*d*) □ ppm 9.40 (s, 1 H), 7.87 (d, *J*=1.41 Hz, 2 H), 7.62 - 7.66 (m, 4 H), 7.53 - 7.59 (m, 4 H), 7.43 - 7.50 (m, 10 H), 5.49 (s, 4 H).

(*N,N'*-Diphenylethynylbenzyl)imidazolium Bistrifluorométhaneesulfonimide (4) : 100 mg (0,19 mmoles) of (N,N'-Diphenylethynylbenzyl)imidazolium boride are dissolved in a mixture of MeOH/ACN (50 :50). Then, 83 mg (0,29 mmoles) of $LiNTf_2$ are added to 10 mL of methanol before being poured onto the solution containing the imidazolium salt. The resulting mixture is then brought to 80°C and stirred with a magnetic stir bar during 12 hours. After evaporating the solvent under reduced pressure, 40mL of distilled water are added to the powder obtained. The mixture is heated to 100ºC for 12 hours under magnetic stirring, and then filtered on a fritted glass. The raw product is then dried at 80°C for 2 hours, in order to obtain 138 mg of a white powder (99% yield).^{1}H NMR (400 MHz, CHLOROFORM-d) □ ppm 9.40 (s, 1 H), 7.87 (d, J=1.41 Hz, 2 H), 7.62 - 7.66 (m, 4 H), 7.53 - 7.59 (m, 4 H), 7.43 - 7.50 (m, 10 H), 5.49 (s, 4 H).

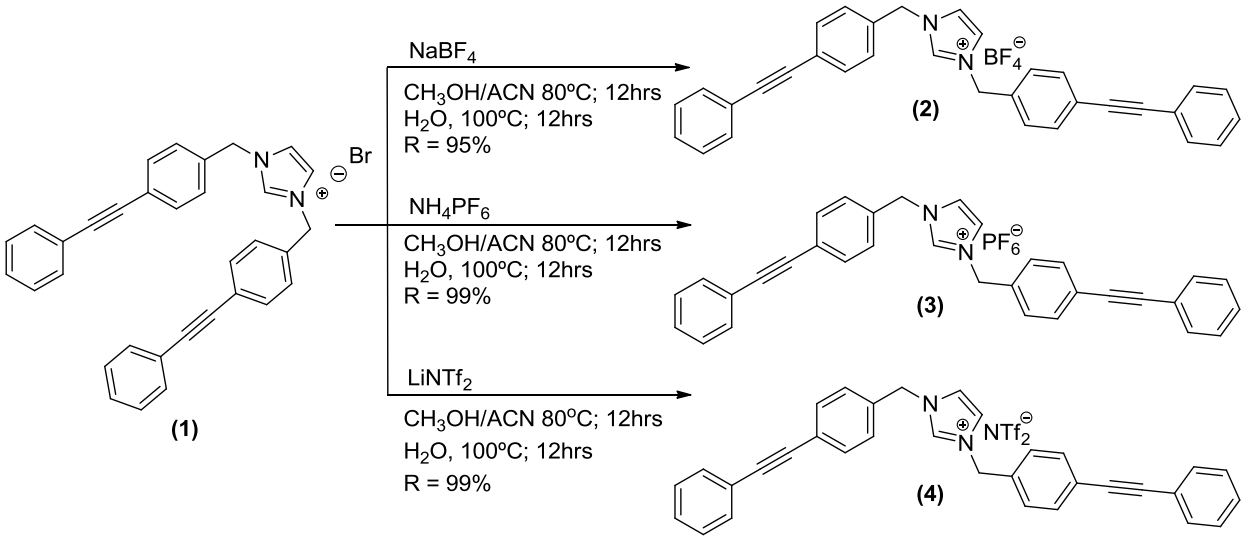
Scheme 1. : Synthesis of (4-Phenylethynyl)benzyl bromide



Scheme 2 : Synthesis of (*N,N'*-Diphenylethynylbenzyl)imidazolium bromide (**1**)



Scheme 3. : Counter anion change of **1** in order to obtain compounds **2-4**



Preparation of EYPC liposomes for Lucigenin-based assays. A stock solution of egg-yolk phosphatidylcholine (EYPC) in $CHCl_3$ (100 mg) was evaporated under reduced pressure on the water bath rt to produce a thin film that was dried *in vacuo* for 2 h at 35 ^{o}C. This lipid film was hydrated with 1 mL of 10 mM sodium phosphate (pH =6,4) containing sodium chloride, [NaCl] = 100 mM, and 2 mM Lucigenin[1]. Freeze/thaw cycles were repeated at least 10 times until no solid particles were visible. The frozen solution was warmed to 30-35 ^{o}C before every freeze cycle. The mixture was also placed on a vortexer 3 times for 1 min to facilitate hydration. The cloudy solution was extruded through a 100 nm polycarbonate membrane at least 20 times until the solution was transparent. This solution was passed down a Sephadex G-25 column (15 cm x 1 cm) to remove extravesicular lucigenin dye. The eluant was free of lucigenin and contained 10 mM phosphate buffer and 75 mM Na_2SO_4. The 2,6 mL of solution isolated from gel filtration was 50 mM in lipid, assuming all EYPC was incorporated into the liposomes. Each stock solution of liposomes was used that same day for any ion transport assays.

Preparation of DPPC liposomes for Lucigenin-based assays. DPPC lipid (50 mg) was dissolved in 5 mL of a chloroform/methanol mixture (5 % MeOH), and the resulting solution was then evaporated under reduced pressure at 45^{o}C to produce a thin film that was then dried *in vacuo* for 2 h. The lipid film was hydrated with 1mL of 10 mM sodium phosphate containing sodium chloride, [NaCl] = 100 mM, and 2 mM Lucigenin. After 10
freeze/thaw cycles (thawing, and then warming to 45^{o}C) the liposomes were extruded through a 100 nm polycarbonate membrane 21 times at temperature between 45-55^{o}C
(fluid state lipid). This solution was passed down a Sephadex G-25 column (15 cm x 1 cm) to remove extravesicular lucigenin dye. The eluant was free of lucigenin and contained 10 mM phosphate buffer and 75 mM Na_2SO_4. The 2,6 mL of solution isolated from gel filtration was 26,2 mM in lipid, assuming all DPPC was incorporated into the liposomes. Each stock solution of liposomes was used that same day for any ion transport assays.

Lucigenin-based ion transport assays. A 20 µL aliquot of the stock solution of liposomes was added to a cuvette containing 2 mL of a solution of salt $NaNO_3$ and 10 mM phosphate buffer to give a 0.25 – 0.3 mM solution of phospholipid. The fluorescence of intravesicular dye was monitored by excitation at 372 nm and the emission at 503 nm was recorded. For assays in EYPC liposomes, some time within the first 100 s of the experiment, an 400 µL aliquot of a 0,25 mM solution of imidazoliums **1, 2, 3** and **4** in MeOH was injected to give a solution that was less than 0,025 mM in imidazolium. At the end of the experiment, 10% aqueous Triton-X was injected to lyse the liposomes. The temperature was set to 37 ^{o}C. For kinetic analysis in EYPC liposomes, some time within the first 100 s of the experiment, nine 400 µL aliquots of 0,0215 mM to 0,75 mM solutions of imidazolium **4** in MeOH was injected to give solutions that were 0,004 mM to 0,123 mM in imidazolium respectively. The temperature was set to 37 ^{o}C. For assays in DPPC liposomes, some time within the first 100 s of the experiment, an 400 µL aliquot of a 0,25 mM solution of imidazolium **4** in MeOH was injected to give a solution that was less than 0,025 mM in imidazolium. The temperature was set to 25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C, 45^{o}C successively.

Preparation of liposomes for HPTS-based assays. A stock solution of egg-yolk phosphatidylcholine (EYPC) in $CHCl_3$ (100 mg) was evaporated on the water bath rt under reduced pressure to produce a thin film that was dried *in vacuo* for 2 h at 35 ^{o}C. This lipid film was hydrated with 1 mL of 10 mM sodium phosphate containing sodium perchlorate, [$NaClO_4$] = 100 mM, and 0.1 mM HPTS. Freeze/thaw cycles were repeated at least 10 times until no solid particles were visible. The frozen solution was warmed to 30-35 ^{o}C before every freeze cycle. The mixture was also placed on a vortexer 3 times for 1 min to facilitate hydration. The cloudy solution was extruded through a 100 nm polycarbonate membrane at least 20 times until the solution was transparent. This solution was passed down a Sephadex G-25 column (11 cm x 1 cm) to remove extravesicular HPTS dye. The eluant was identical to the solution used to hydrate the EYPC films except that it was free of HPTS. The 2,6 mL of solution isolated from gel filtration was 50 mM in lipid, assuming all EYPC was incorporated into the liposomes. Each stock solution of liposomes was used that same day for any ion transport assays.

HPTS-based ion transport assays. This procedure describes the typical ion transport assay. A 20 µL aliquot of the stock solution of EYPC liposomes was added to a cuvette containing 2 mL of a solution of salt $NaNO_3$, NaCl or $NaSO_4$ and 10 mM phosphate buffer to give a 0.25–0.3 mM solution of phospholipid. The fluorescence of intravesicular HPTS was monitored by excitation at both 403 nm and 460 nm and the emission at 510 nm was recorded. Some time within the first 5 min of the experiment, a 70-µL aliquot of a 1,5 mM solution of imidazolium **4** was injected to give a solution that was 0,0525 mM in amphiphile. At the end of the experiment (25 min), 10% aqueous Triton-X was injected to lyse the liposomes. The temperature was set to 37^{o}C.

Initial rate and the rate constant calculations

Fluorescence assays were run at three different III/EYPC ratios: 1/10, 1/50 and 1/100. The initial rate were determined at t=50s when the transporter is injected, following the Stern-Volmer equation[20].

$$\left(\frac{F_0}{F}\right) = 1 + K_{SV}[Cl^-]$$

with F_0 : maximum fluorescence intensity in absence of quencher

F : fluorescence intensity

K_{SV} : Stern-Volmer constant, here $K_{SV} = 142M^{-1}$

$[Cl^-]$: intravesicular chloride concentration

From this equation, we can deduce:

At t=0, $$-\left(\frac{d[Cl^-]}{dt}\right) = \left(\frac{F_0}{K_{SV}}\right) \bullet \left(\frac{1}{F^2}\right) \bullet \left(\frac{dF}{dt}\right)_{t=0} = V_0$$

With V_0 : initial rate

According to equation 4 (see the article), the initial rate of ion flow (V_0) is expected to have a dependence on the pseudo first ordre constant (k_{obsd}) and $[Cl^-]$ the total initial intravesicular chloride concentration (48mM).

$$V_0 = k_{obsd}[Cl^-]_{t=0}$$

Table S1. Determination of V_0 and k_{obds} at different 4/EYPC ratios

mol% 4/EYPC	[4] (mM)	(dF/dt) (s^{-1})	V_0 (mM/s)	V_0 average (mM/s)	K_{obsd} (s^{-1})	Standard deviation
1,0	0,004	0,019	0,242	0,192	0,004	9,45E-04
		0,014	0,181			
		0,012	0,153			
1,7	0,007	0,024	0,310	0,353	0,007	1,42E-03
		0,034	0,431			
		0,025	0,317			
3,4	0,014	0,040	0,509	0,641	0,013	3,32E-03
		0,047	0,598			
		0,064	0,818			
6,9	0,028	0,158	2,017	1,664	0,035	6,57E-03
		0,122	1,562			
		0,110	1,411			
8,6	0,035	0,105	1,344	1,535	0,032	4,07E-03
		0,136	1,734			
		0,119	1,526			
14,0	0,057	0,178	2,278	2,480	0,052	4,58E-03
		0,212	2,714			
		0,192	2,448			
18,0	0,074	0,299	3,822	3,279	0,068	9,82E-03
		0,233	2,975			
		0,238	3,041			
25,0	0,102	0,540	6,901	5,577	0,116	3,66E-02
		0,281	3,584			
		0,489	6,247			
30,0	0,123	0,442	5,642	5,363	0,112	2,29E-02
		0,493	6,295			
		0,325	4,151			

Table S2. Determination of activity of 4 at different 4/EYPC ratios

mol% 4/EYPC	Log(mol% 4/EYPC)	% of Rmax at 250 s	Average % of Rmax at 250 s	Standard deviation
1,0	0,0000	23,98	24,32	0,48
		24,87		
		24,12		
1,7	0,2304	35,14	37,64	2,98
		40,93		
		36,85		
3,4	0,5315	48,21	51,39	3,82
		50,34		
		55,62		
6,9	0,8388	90,14	85,55	5,20
		86,60		
		79,91		
8,6	0,9345	87,29	86,50	2,69
		83,51		
		88,71		
14,0	1,1461	92,51	90,17	3,30
		87,84		
		91,77		
18,0	1,2553	100,20	97,42	2,64
		97,11		
		94,94		
25,0	1,3979	100,00	99,23	0,71
		98,60		
		99,09		
30,0	1,4771	97,06	99,11	2,75
		102,23		
		98,04		

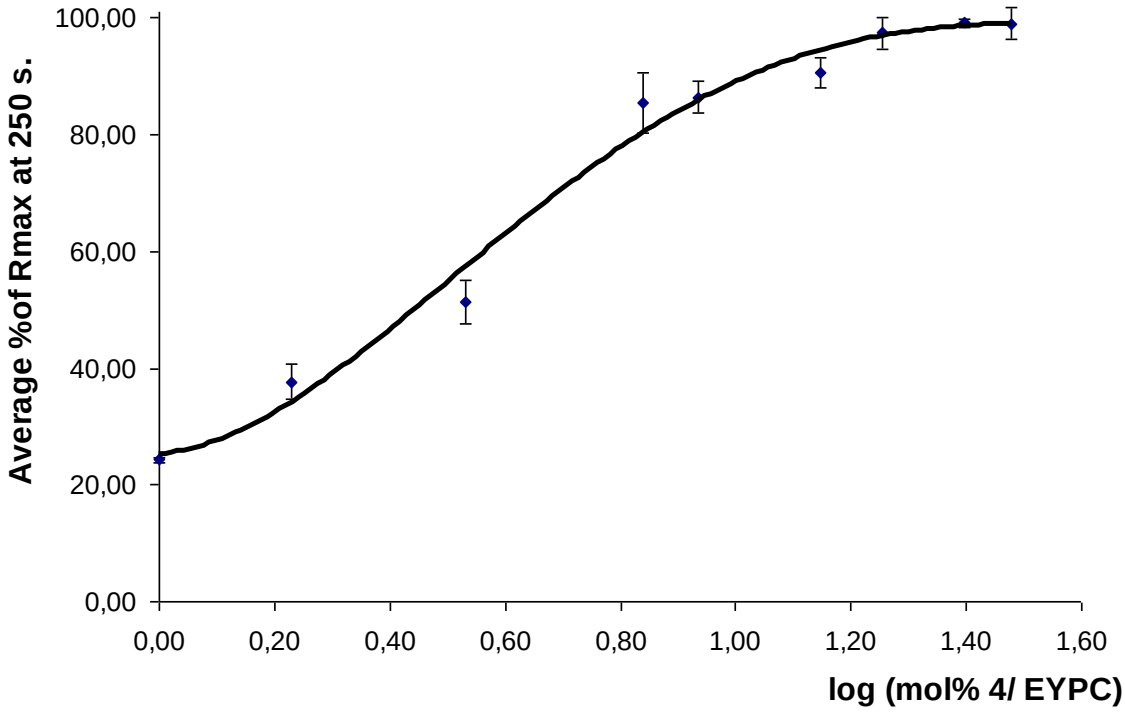


Figure S1_ Dose response curve for determination of the EC_{50} for imidazolium **4**. The data at each mol% is the average of 3 runs, with the standard deviation. The Log(mol% **4**/EYPC) that provokes a response half way between the baseline (24,32%) and maximum response (99,23%) has a value of 0,75. This corresponds to a mol% **4**/EYPC of 5,74%.