Supplementary Material (ESI) for Chemical Communications

Electronic Supporting information

Uncovering New Properties of Imidazolium Salts: Cl^- Transport and Supramolecular Control of the Transmembrane Activity

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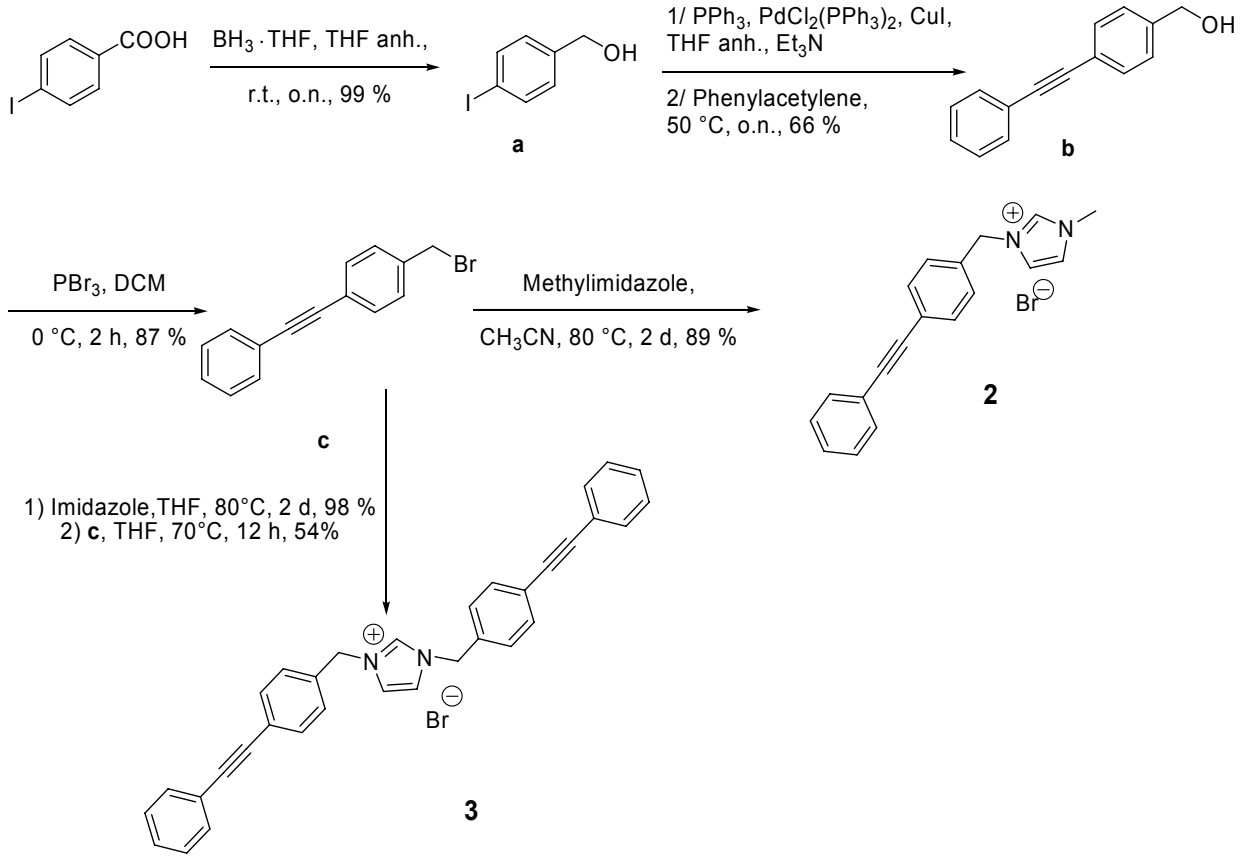
General Remarks.

4-iodobenzoïc acid, borane in tetrahydrofuran (BH_3 in THF), phosphorus tribromide (PBr_3), imidazole, methylimidazole, cucurbit[7]uril, α-cyclodextrin and Sephadex G-25 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 TSQ Quantum Ultra (Thermo Scientific) with accurate mass options instrument (Université de Montréal Mass Spectrometry Facility). Either protonated molecular ions $(M+H)^+$ or silver adducts $(M+Ag)^+$ were used for empirical formula confirmation. L-α-phosphatidylcholine was purchased from Avanti Polar Lipids. Liposome fluorimetric assays were recorded using a Varian Cary Eclipse Fluorescence spectrophotometer. NMR experiments were recorded on Advance 300 Bruker, at 300.13 and 75.49 MHz, respectively. Chemical shifts are given in ppm (δ) and measured relative to residual solvent. UV spectroscopy was performed using a Cary Bio 100 UV-visible spectrophotometer.

Synthesis



4-Iodobenzyl alcohol (a) : 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 saturated $NaHCO_3$ 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 (b) : To a carefully degassed solution of **a** (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 66 % 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 (c) : (4-Phenylethynyl)benzyl alcohol **b** (1.44 mmol) was dissolved in 5 mL of DCM. The mixture was kept 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 **c** as a white solid in 87 % 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.

1-Methyl-3-(4-phenylethynyl)benzyl imidazolium bromide (2)

Methylimidazole (29 µL, 0.36 mmol) was diluted in 10 mL of acetonitrile and (4-phenylethynyl)benzyl bromide **c** (97.6 mg, 0.36 mmol) was added to the solution. The solution was stirred 48 h at 80 °C and the solvant was removed in vacuuo to yield 110 mg of compound **2**. Yield 86 %. ^{1}H NMR (CD_3OD, 400MHz): δ=7.66 (d, J=1.9Hz, 1H), 7.63 (s, 1H), 7.61 (d, J=8.4 Hz, 2H), 7.52-7.54 (m, 2H), 7.45 (d, J=8.4Hz, 2H), 7.38-7.42 (m, 2H), 5.46 (s, 2H), 3.96 ppm (s, 3H). ^{13}C NMR (CD_3OD, 75 MHz): δ=134.7, 132.8, 132.0, 129.2, 129.0, 125.1, 124.8, 123.5, 123.2, 53.1, 35.9 ppm. HR-MS (ESI): m/z Calcd for $C_{19}H_{17}N_2$ $[M-Br]^+$: 273.1386, found 273.1387

(*N,N'*-diphenylethynylbenzyl)imidazolium bromide 3: 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 **c** is added to the mixture. The solution is stirred at room temperature for 12 h 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 2x 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 **c** are disolved in 10 mL of THF. The mixture is stirred 12 h at 70 °C and the resulting precipitate is filtered, washed with 10 mL of THF, and dried in vacuuo. 134 mg of **3** were obtained. Yield 54 %. ^{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). ^{13}C NMR (CD_3OD, 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

Preparation of liposomes for lucigenin-based assays. A stock solution of egg-yolk phosphatidylcholine (EYPC) in $CHCl_3$ (60 mg) was evaporated under reduced pressure on the water bath at *r.t.* to produce a thin film that was dried *in vaccuo* for 2 h at 35 °C. The lipid film was hydrated with 1 mL of 10 mM sodium phosphate containing 100 mM NaCl and 2 mM lucigenin.

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 placed on a vortex 3 times for 1 min to facilitate hydration. The cloudy solution was extruded with an Avanti High Pressure Mini-Extruder through a 100 nm polycarbonate membrane at least 20 times until the solution became transparent. A Sephadex G-25 column (15 cm x 1 cm) was used to remove the extravesicular dye. Each stock solution of liposomes was used that same day.

Lucigenin-based ion transport assays[13]. A 20 μL aliquot of the stock solution of EYPC liposomes was added to a cuvette containing 2 mL of a solution of NaCl and 10 mM phosphate buffer to obtain a 0.25-0.3 mM solution of phospholipid. The fluorescence of intravesicular dye was monitored by excitation at 372 nm and the emission was recorded at 503 nm. A 400 μL aliquot of a 0.25 mM solution of **1, 2** and **3** in MeOH was injected. At the end of the experiment, 10% aqueous Triton-X was injected to lyse the liposomes. The temperature was set to 25 ^{o}C. The resulting Cl^- concentration gradient across the lipid membrane was relieved by compound-mediated transmembrane transport of Cl^- from the liposomes, resulting in the increase of lucigenin fluorescence. Experiments were repeated in triplicate and all traces reported are the average of the three trials.

Lucigenin-based ion transport assays in the presence of α-CD and CB7. Intravesicular 100 mM NaCl, 10 mM phosphate buffer, extravesicular 100 mM $NaNO_3$, 10 mM phosphate buffer (pH 6.3), 25^{o}C. **3** (0.25 mM) was injected at t = 35s. **3** + 2 eq **CB7:** 100 μL of **CB7** (2 mM) was injected at t = 10s and 400 μL of 3 (0.25 mM) was injected at t = 35s. **3** + 2 eq **α-CD**: 100 μL of **α-CD** (2 mM) of was injected at t = 10s and 400 μL of **3** (0.25 mM) was injected at t = 35s.

Molecular modeling. All calculations were performed on a Windows® Vista platform. In order to assess the energy content for various molecules designed, semi-empirical quantum calculations were undertaken using the PM6 method in gas phase or in aqueous solution (MOPAC2009^{TM}; ©Stewart Computational Chemistry). Theoretical calculations were carried out at the restricted Hartree-Fock level (RHF) using the PM6 semi-empirical SCF-MO methods. All structures were optimized to a gradient inferior to 0.1 using the eigenvector following method. The MOPAC Cartesian coordinates were generated with OpenBabel 2.2.0 graphical interface (©Chris Morley) from the geometries obtained with ArgusLab UFF.

Formation of the inclusion complex of 3 with CB7: Job's Plot

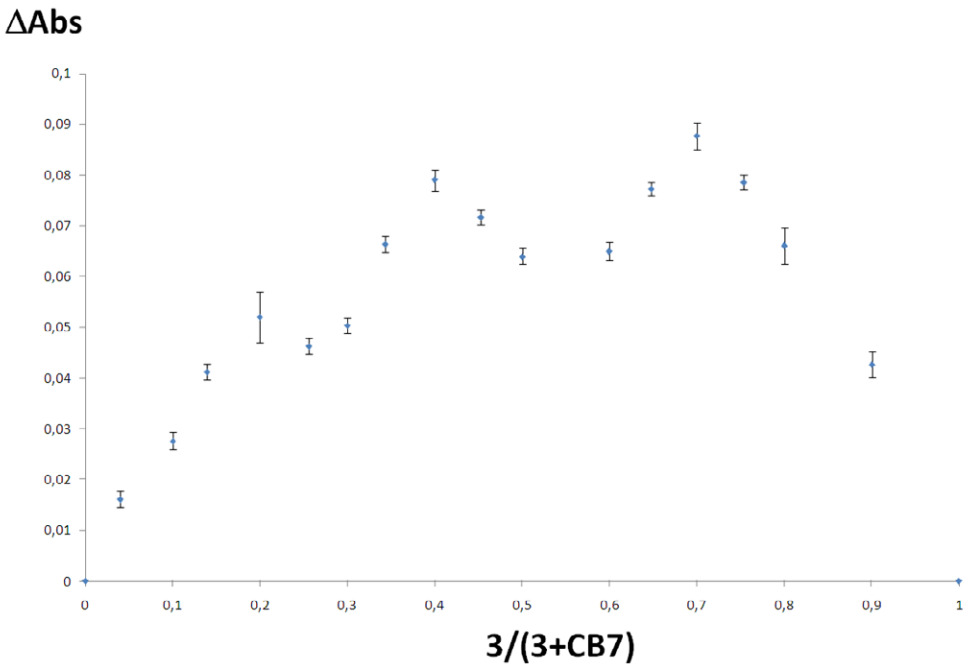


Figure S1: Continuous variation plot (Job's plot) derived from UV data for **3** in H_2O/MeOH with **CB7** at 280 nm. The UV study was made in triplicata.

Examination of the Job plot shows several slope changes, indicating the presence of multiple stoechiometry complexes. We previously observed the same behaviour of imidazolium salts when they form multiple complexes with different macrocycles (N. Noujeim, B. Jouvelet, A. R. Schmitzer *J. Phys. Chem. B*, **2009**, *113*, 16159–16168. L. Leclercq, A. R. Schmitzer *J. Phys. Chem. B*, **2008**, *112*, 11064–11070).

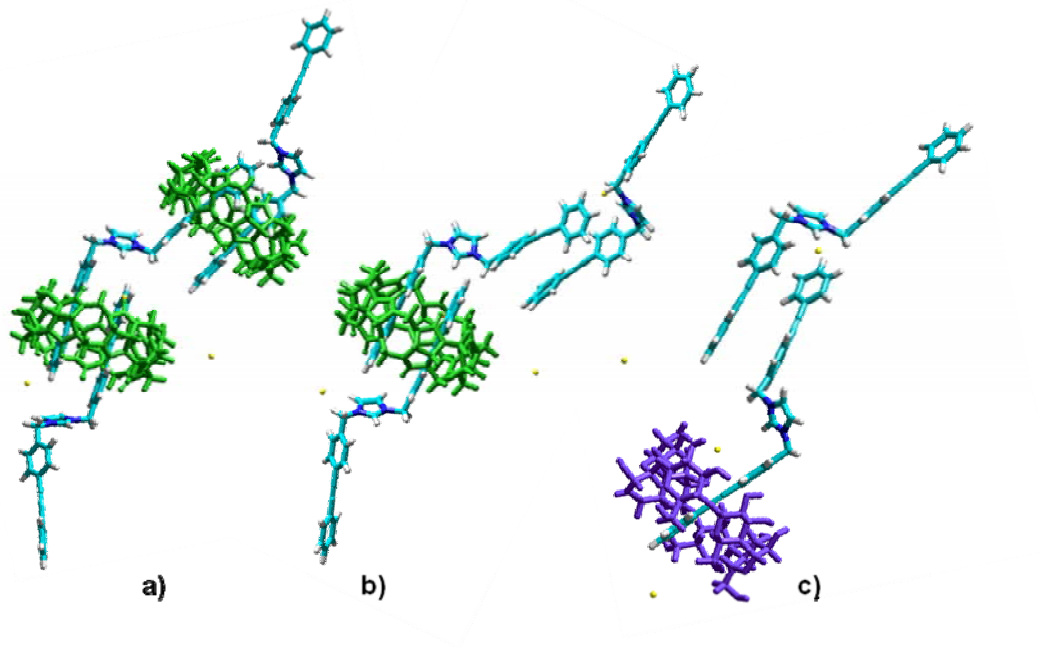


Figure S2: PM3 snapshots obtained by PM3 energy minimization: (*a*) and (*b*) **3** with **CB7**; c) **3** with **α-CD**

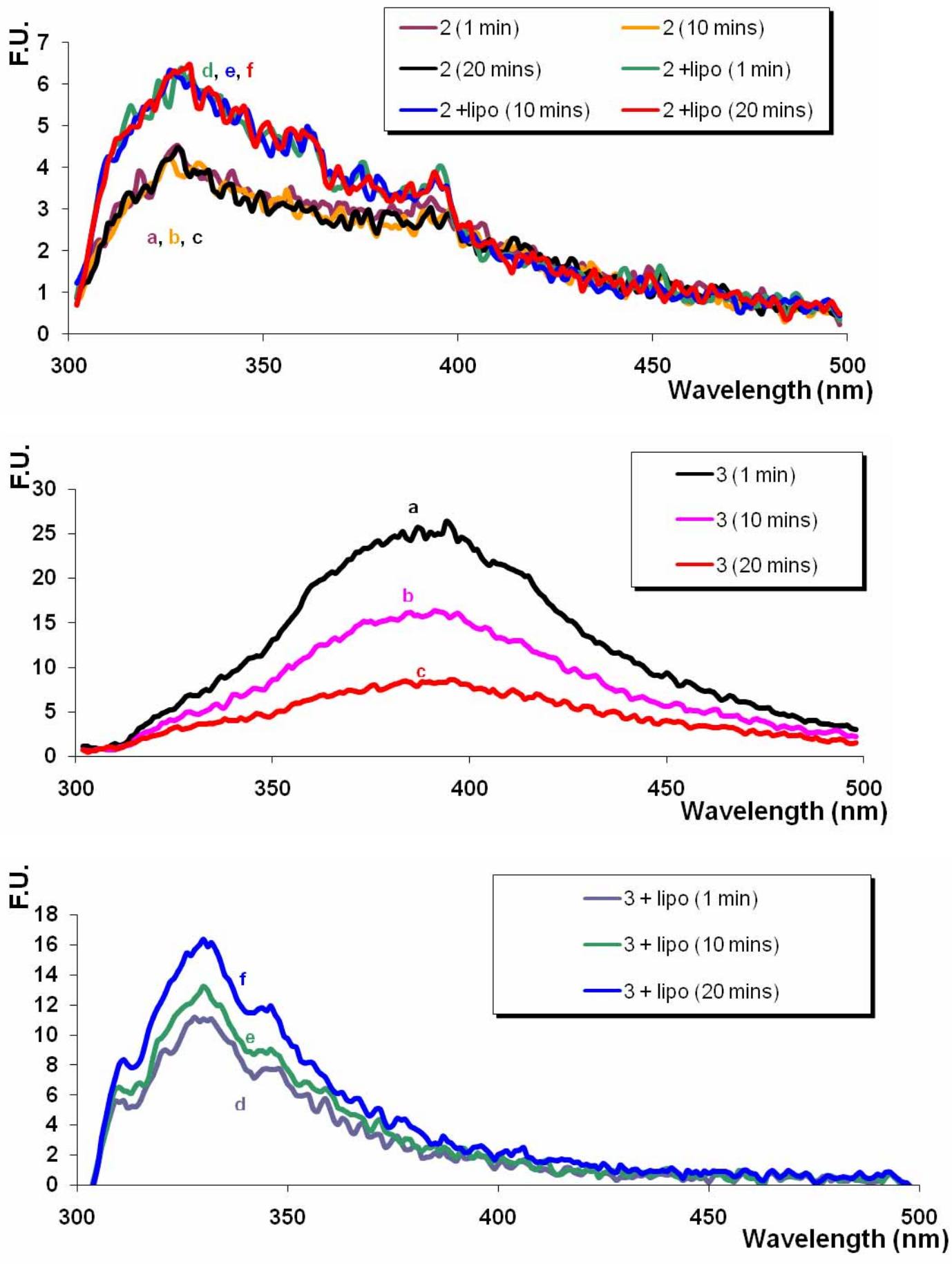


Figure S3: Fluorescence spectra of **2** and **3** in the absence and in the presence of liposomes.