Electronic Supplementary Information (ESI)

Formulation and evaluation of anion transporters in nanostructured lipid carriers

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1. STRUCTURES OF THE STUDIED COMPOUNDS

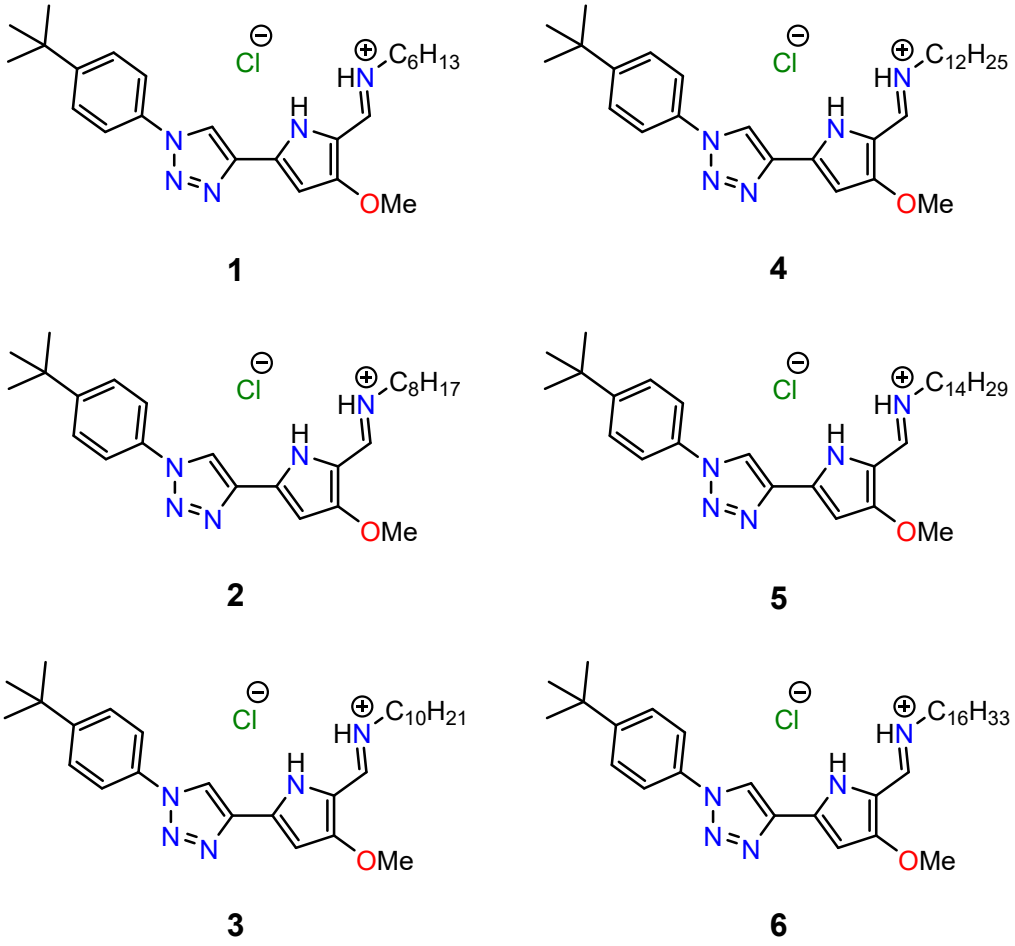


Chart 1. Structures of the studied compounds.

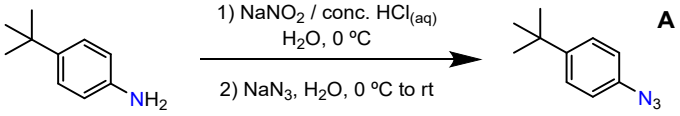
2. SYNTHESES AND CHARACTERISATION DATA

2.1. General procedures and methods

Commercial reagents were employed as received without any further purification. ^{1}H, ^{13}C and DEPT NMR spectra of precursors, intermediates and final compounds were recorded at 25 °C on a Varian Mercury-300 MHz spectrometer (for ^{1}H NMR titration experiments a Varian Unity Inova-400 MHz spectrometer was employed), using $CDCl_3$ or DMSO-d_6 as solvents, with their residual signals being used to reference the spectra. High-resolution mass spectra were recorded on an Agilent 6545 Q-TOF mass spectrometer coupled to a 1260 Infinity liquid chromatographer from the same brand; the ionisation source employed was electrospray in its positive mode. Absorption and emission spectra were recorded on Hitachi U-3900 and F-7000 spectrophotometers, respectively.

2.2. Precursors and intermediates

2.2.1. Compound A



4-(*Tert*-butyl)aniline (2.1 mL, 13.0 mmol) was mixed with a concentrated HCl aqueous solution (20 mL) and the obtained mixture was stirred vigorously at 0 °C (ice bath) for 15 min. Note 1: the aniline was not dissolved. A solution of sodium nitrite (1.37 g, 19.9 mmol, 1.5 equiv.) in water (15 mL) was added in a dropwise manner over a period of 5 min. Note 2: formation of a yellow solution was observed. The mixture was stirred at 0 °C for an additional 30 min. A solution of sodium azide (1.74 g, 26.8 mmol, 2.1 equiv.) in water (15 mL) was added dropwise at 0 °C within 5 min and the resulting reaction mixture was stirred vigorously at room temperature for a further 3 h. Note 3: addition of the sodium azide solution resulted in the disappearance of the yellow colour. The mixture was extracted with ethyl acetate (3 × 35 mL), the extracts were combined, washed with water (1 × 50 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to give compound **A** as a yellow liquid (1.7 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.43-7.35 (m, 2H), 7.02-6.95 (m, 2H), 1.33 (s, 9H). ^{13}C NMR {DEPT-135} (75 MHz, $CDCl_3$): δ (ppm) = 148.1 (ArC), 137.2 (ArC), 126.8 (ArCH), 118.7 (ArCH), 34.6 (C), 31.5 (CH_3). No satisfactory HR-MS (+ESI) analysis result was obtained for this compound.

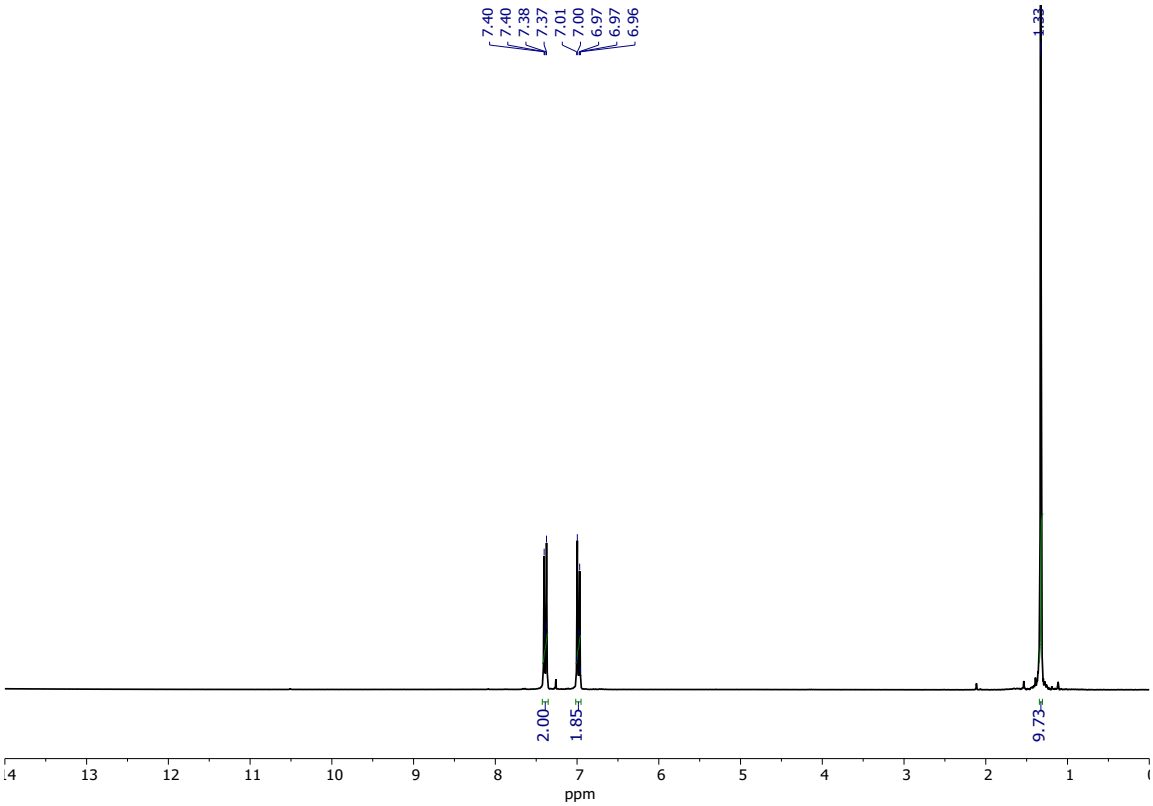


Figure S1. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **A**.

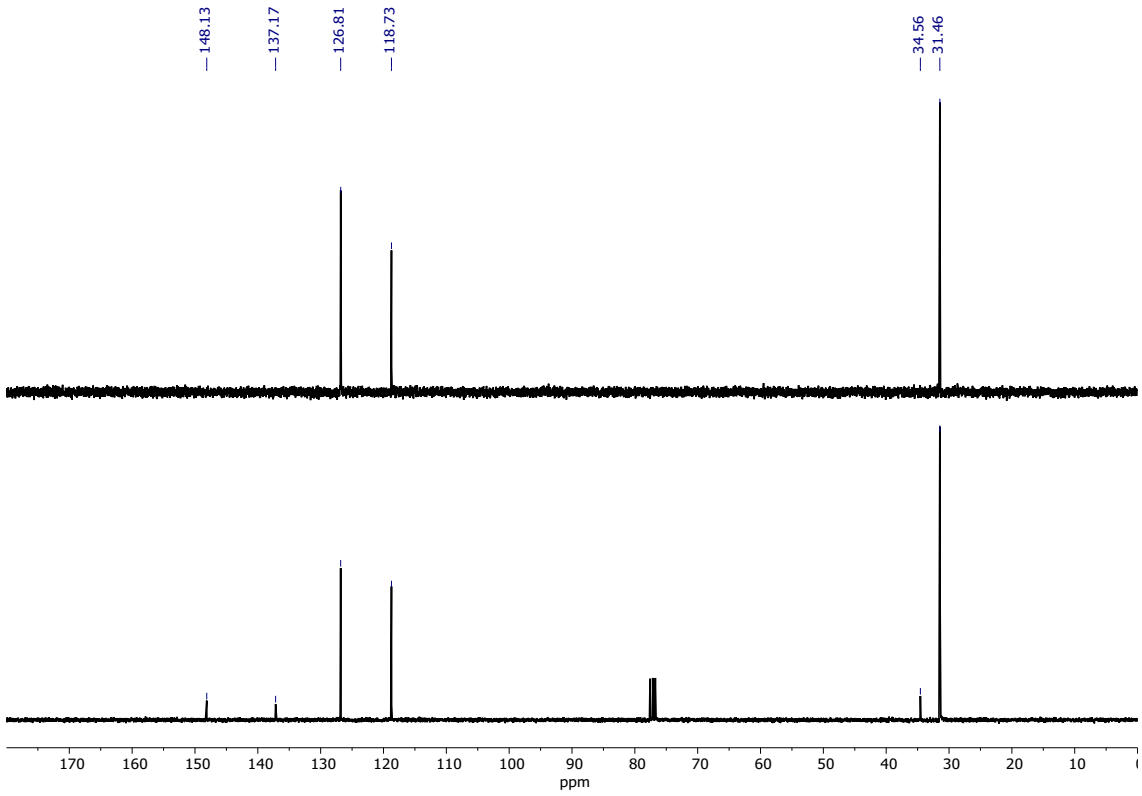
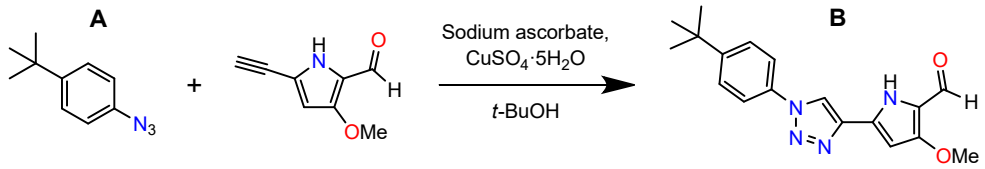


Figure S2. ^{13}C and DEPT-135 NMR spectra (75 MHz, $CDCl_3$) for compound **A**.

2.2.2. Compound B



5-ethynyl-3-methoxy-1*H*-pyrrole-2-carbaldehyde[1] (1.47 g, 9.86 mmol) and compound **A** (1.71 g, 9.76 mmol) were dissolved partially in *tert*-butanol (20 mL) in a 250-mL Schlenk tube. A solution of (+)-sodium L-ascorbate (389 mg, 1.96 mmol, 0.2 equiv.) in water (10 mL) and a solution of $CuSO_4 \cdot 5H_2O$ (243 mg, 0.97 mmol, 0.1 equiv.) in water (10 mL) were subsequently added to the resulting suspension. Note: a gradual change of colour from dark brown to pale brown was observed upon addition of the $CuSO_4$ solution. The tube was closed with a stopper and the reaction mixture was stirred at room temperature for 18 h. The content of the flask was poured into water (100 mL) and the precipitate was filtered out and washed with water (3 × 30 mL) and diethyl ether (1 × 30 mL). NMR analysis revealed that compound **B** was still contaminated with compound **A**, so diethyl ether (roughly 50 mL) was added to the product and the resulting suspension stirred at room temperature for 15 min. The solid was filtered out and air-dried to give compound **B**, now pure, as a brown non-crystalline solid (1.96 g, 62%). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.08 (s, 1H, NH), 9.47 (s, 1H, CHO), 9.09 (s, 1H, triazole CH), 7.77 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 6.53 (d, J = 2.4 Hz, 1H), 3.90 (s, 3H), 1.34 (s, 9H). ^{13}C NMR {DEPT-135} (75 MHz, DMSO-d_6): δ (ppm) = 173.9 (CHO), 157.8 (ArC), 151.8 (ArC), 140.2 (ArC), 134.0 (ArC), 129.4 (ArC), 126.9 (ArCH), 120.1 (ArCH), 119.9 (ArCH), 118.7 (ArC), 93.7 (ArCH), 58.1 (CH_3), 34.6 (C), 31.0 (CH_3). HR-MS (+ESI): found m/z 325.1644 ($[M+H]^+$), $[C_{18}H_{21}N_4O_2]^+$ requires m/z 325.1665 (monoisotopic mass).

[1] E. Hernando, V. Capurro, C. Cossu, M. Fiore, M. García-Valverde, V. Soto-Cerrato, R. Pérez-Tomás, O. Moran, O. Zegarra-Moran and R. Quesada, *Sci. Rep.*, 2018, **8**, 2608.

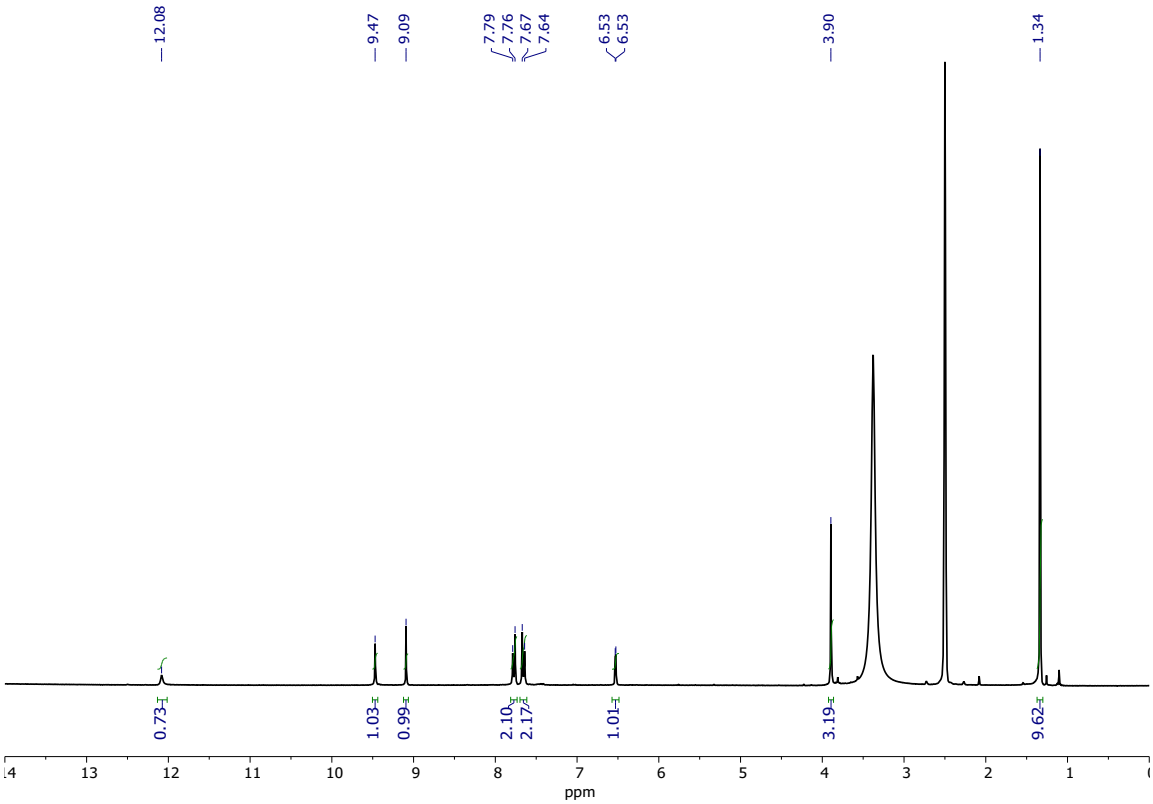


Figure S3. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for compound **B**.

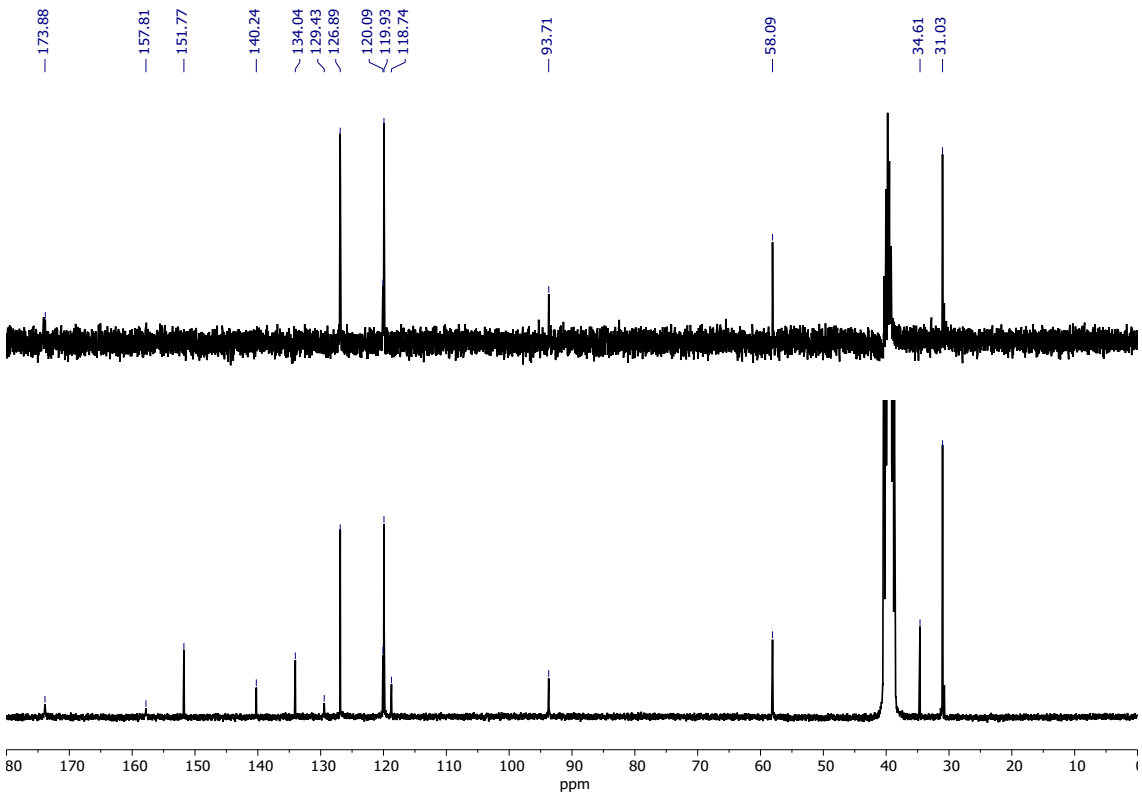


Figure S4. ^{13}C and DEPT-135 NMR spectra (75 MHz, DMSO-d_6) for compound **B**.

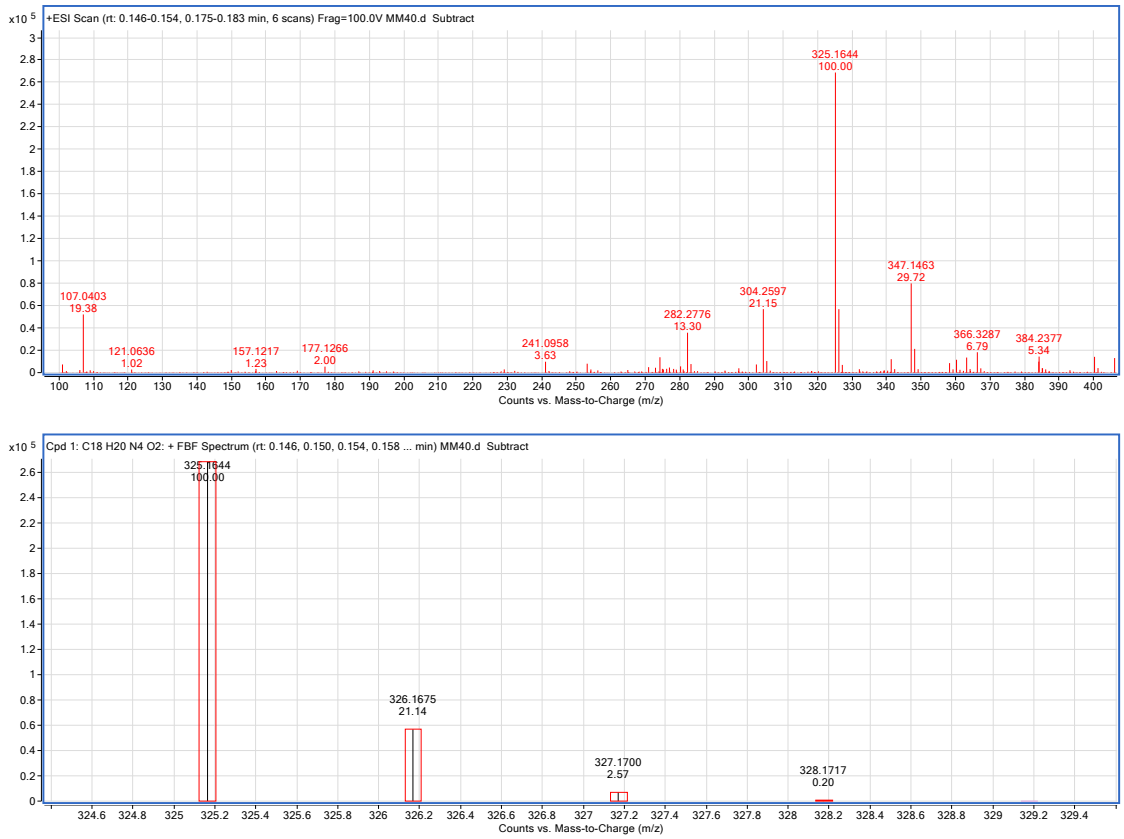
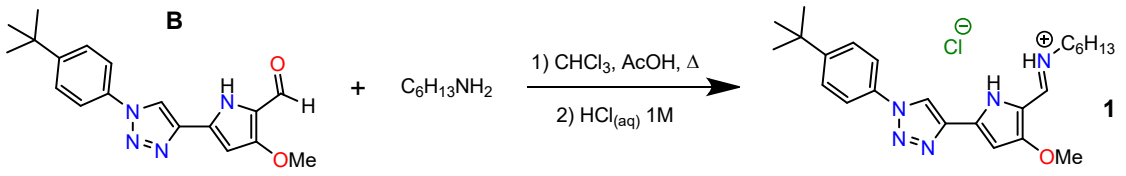


Figure S5. HR-MS (+ESI) spectrum for compound **B**.

2.3. Click-tambjamines

2.3.1. Compound 1



A mixture of aldehyde **B** (100 mg, 0.31 mmol), *n*-hexylamine (122 µL, 0.92 mmol, 3.0 equiv.) and glacial acetic acid (100 µL) in chloroform (15 mL) was stirred at 65 °C for 16 h. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (15 mL). The solution of the crude compound was washed with a 1 M HCl aqueous solution (3 × 15 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **1** as a brown non-crystalline solid (106 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.82 (s, 1H, pyrrole NH), 11.36-11.01 (m, 1H, imine NH), 9.07 (s, 1H, triazole CH), 7.74 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 15.2 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 6.57 (d, J = 1.7 Hz, 1H), 3.98 (s, 3H), 3.56 (q, J = 6.4 Hz, 2H), 1.81 (quint, J = 7.2 Hz, 2H), 1.50-1.16 (m, 15H), 0.94-0.84 (m, 3H). ^{13}C NMR {DEPT-135} (75 MHz, $CDCl_3$): δ (ppm) = 163.3 (ArC), 152.7 (ArC), 144.0 (imine CH), 139.5 (ArC), 139.1 (ArC), 134.0 (ArC), 126.7 (ArCH), 121.4 (ArCH), 120.5 (ArCH), 111.3 (ArC), 93.5 (ArCH), 58.7 (CH_3), 51.6 (CH_2), 34.8 (C), 31.2 (CH_3), 31.2 (CH_3), 29.9 (CH_2), 26.2 (CH_2), 22.4 (CH_2), 14.0 (CH_3). HR-MS (+ESI): found m/z 408.2767 ($[M+H]^+$), $[C_{24}H_{34}N_5O]^+$ requires m/z 408.2763 (monoisotopic mass).

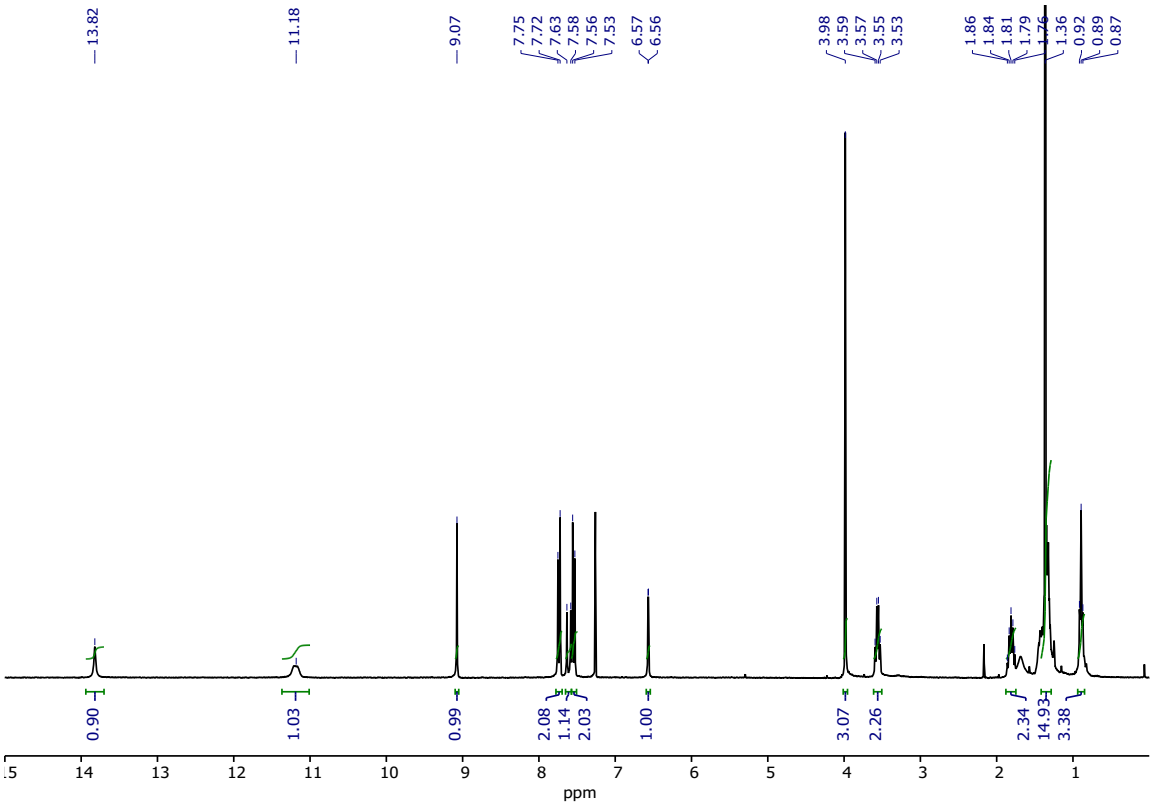


Figure S6. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **1**.

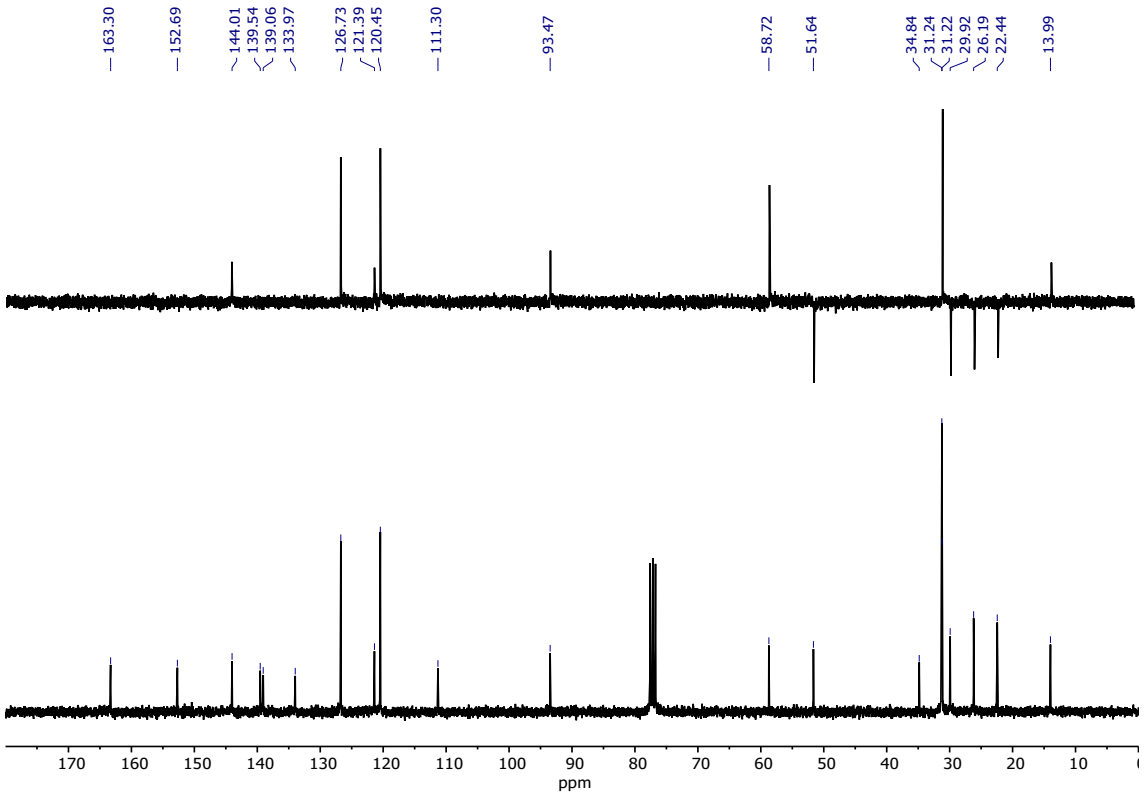


Figure S7. ^{13}C and DEPT-135 NMR spectra (75 MHz, $CDCl_3$) for compound **1**.

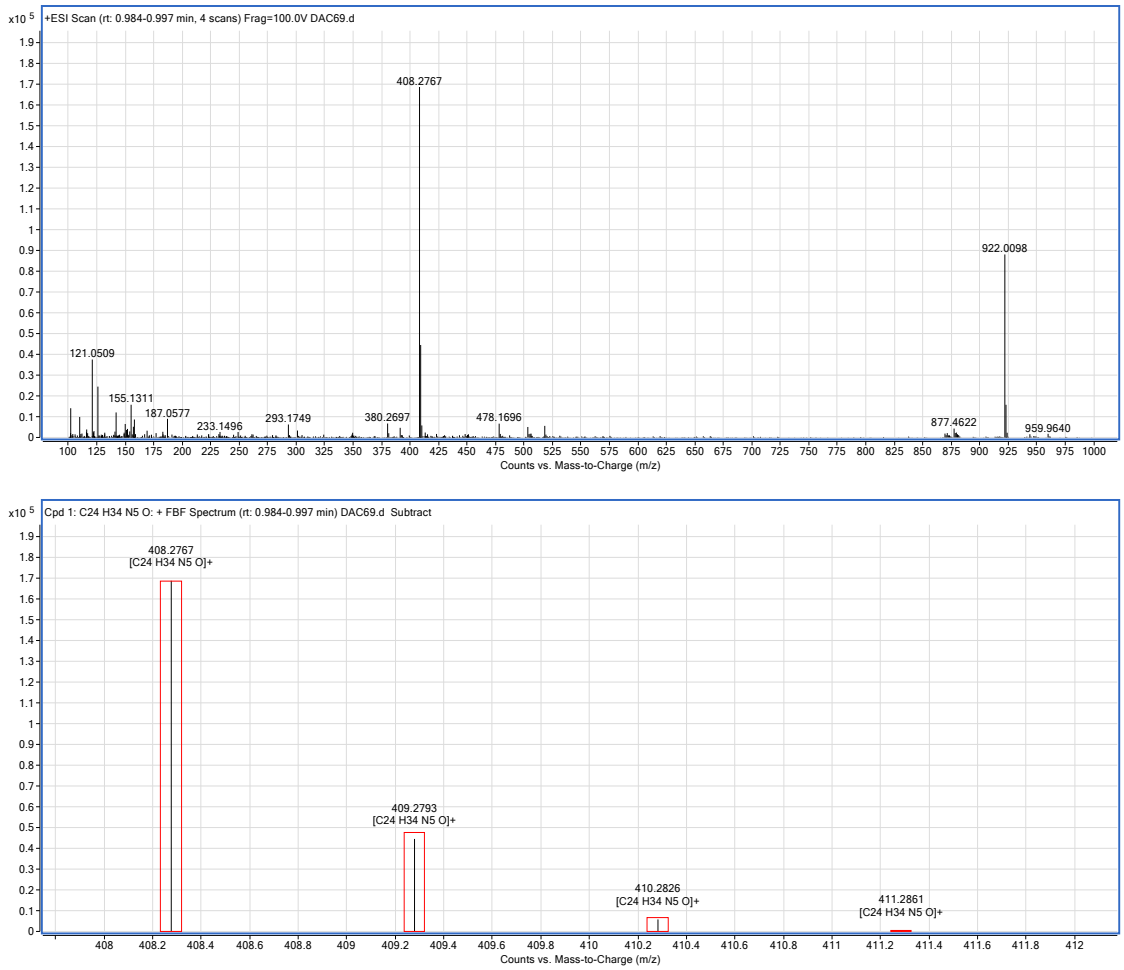
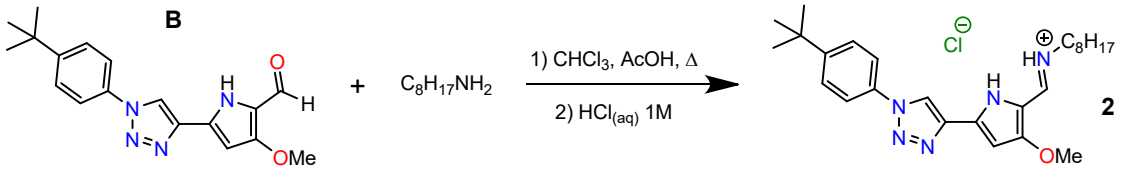


Figure S8. HR-MS (+ESI) spectrum for compound **1**.

2.3.2. Compound 2



A mixture of aldehyde **B** (236 mg, 0.73 mmol), *n*-octylamine (240 µL, 1.45 mmol, 2.0 equiv.) and glacial acetic acid (50 µL) in chloroform (15 mL) was stirred at 60 °C for 9 h. Upon cooling to room temperature the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (30 mL). The solution of the crude compound was washed with a 1 M HCl aqueous solution (3 × 20 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **2** as a brown non-crystalline solid (176 mg, 51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.80 (s, 1H, pyrrole NH), 11.35-10.99 (m, 1H, imine NH), 9.07 (s, 1H, triazole CH), 7.74 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 15.3 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 6.57 (d, J = 1.8 Hz, 1H), 3.99 (s, 3H), 3.56 (q, J = 6.4 Hz, 2H), 1.81 (quint, J = 7.4 Hz, 2H), 1.48-1.18 (m, 19H), 0.92-0.82 (m, 3H). ^{13}C NMR {DEPT-135} (75 MHz, $CDCl_3$): δ (ppm) = 163.4 (ArC), 152.8 (ArC), 144.0 (imine CH), 139.6 (ArC), 139.3 (ArC), 134.1 (ArC), 126.8 (ArCH), 121.5 (ArCH), 120.6 (ArCH), 111.4 (ArC), 93.6 (ArCH), 58.8 (CH_3), 51.7 (CH_2), 34.9 (C), 31.8 (CH_2), 31.3 (CH_3), 30.1 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 26.6 (CH_2), 22.7 (CH_2), 14.2 (CH_3). HR-MS (+ESI): found m/z 436.3074 ($[M+H]^+$), $[C_{26}H_{38}N_5O]^+$ requires m/z 436.3076 (monoisotopic mass).

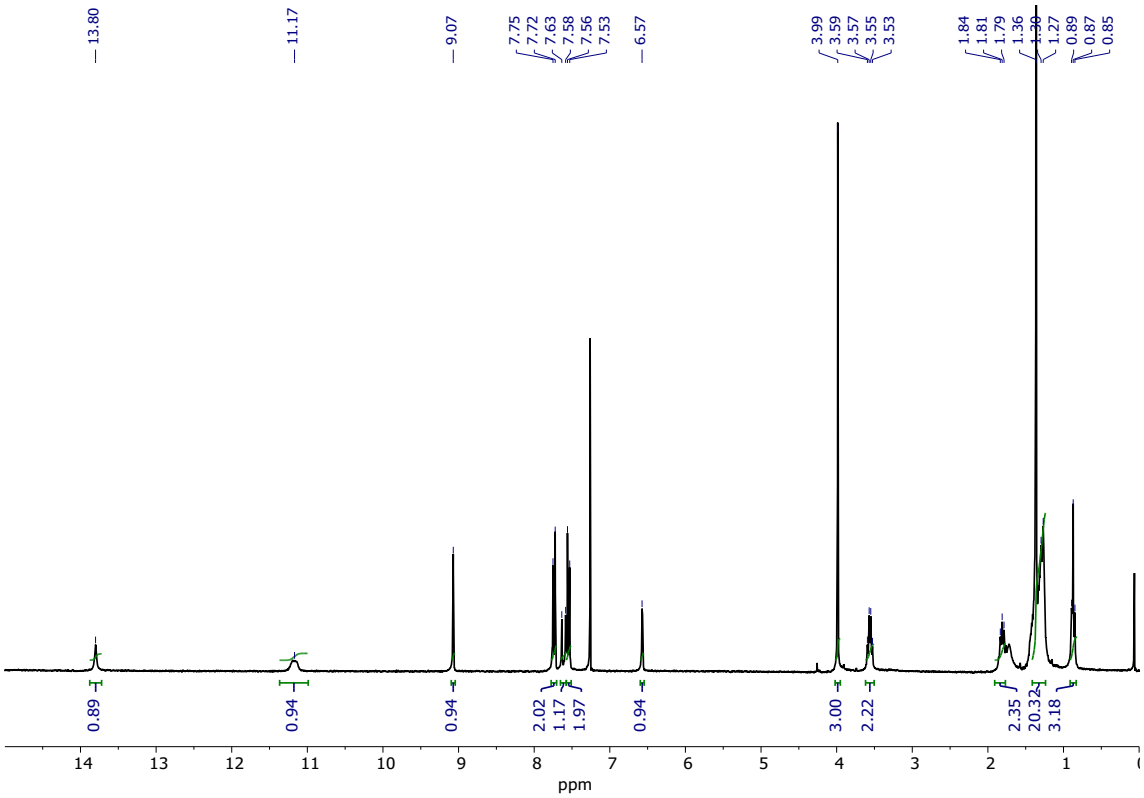


Figure S9. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **2**.

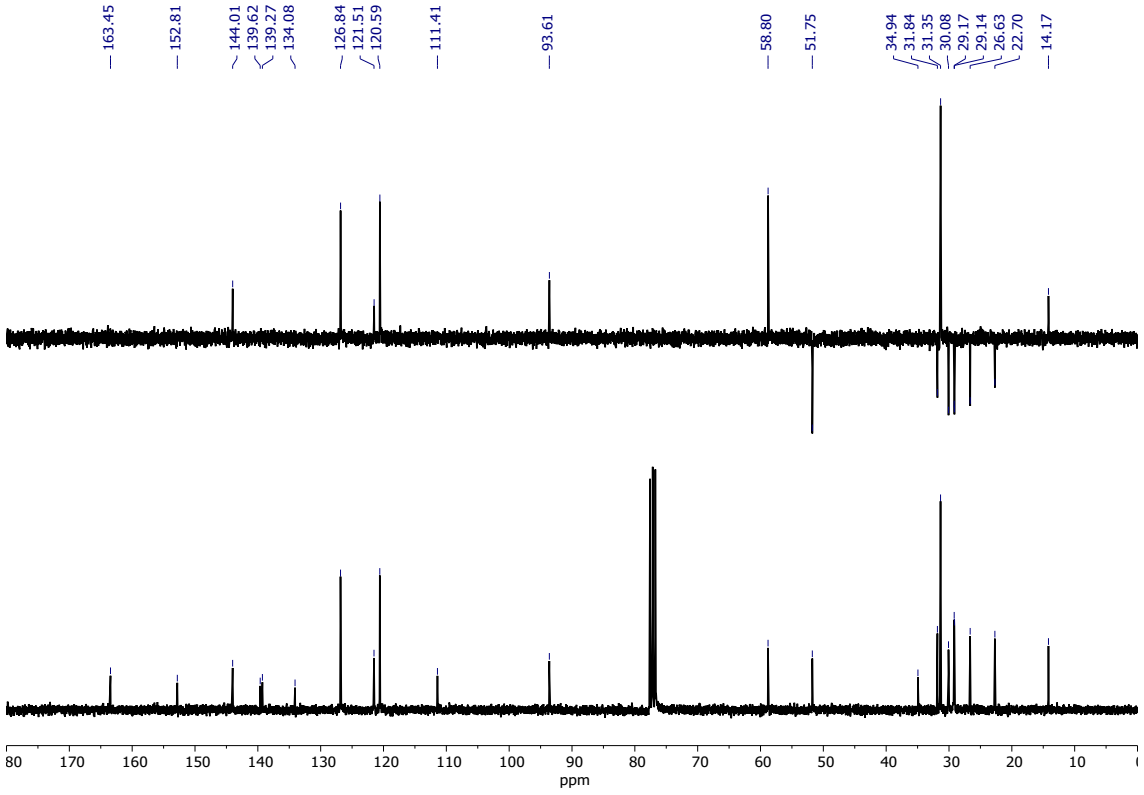


Figure S10. ^{13}C and DEPT-135 NMR spectra (75 MHz, $CDCl_3$) for compound **2**.

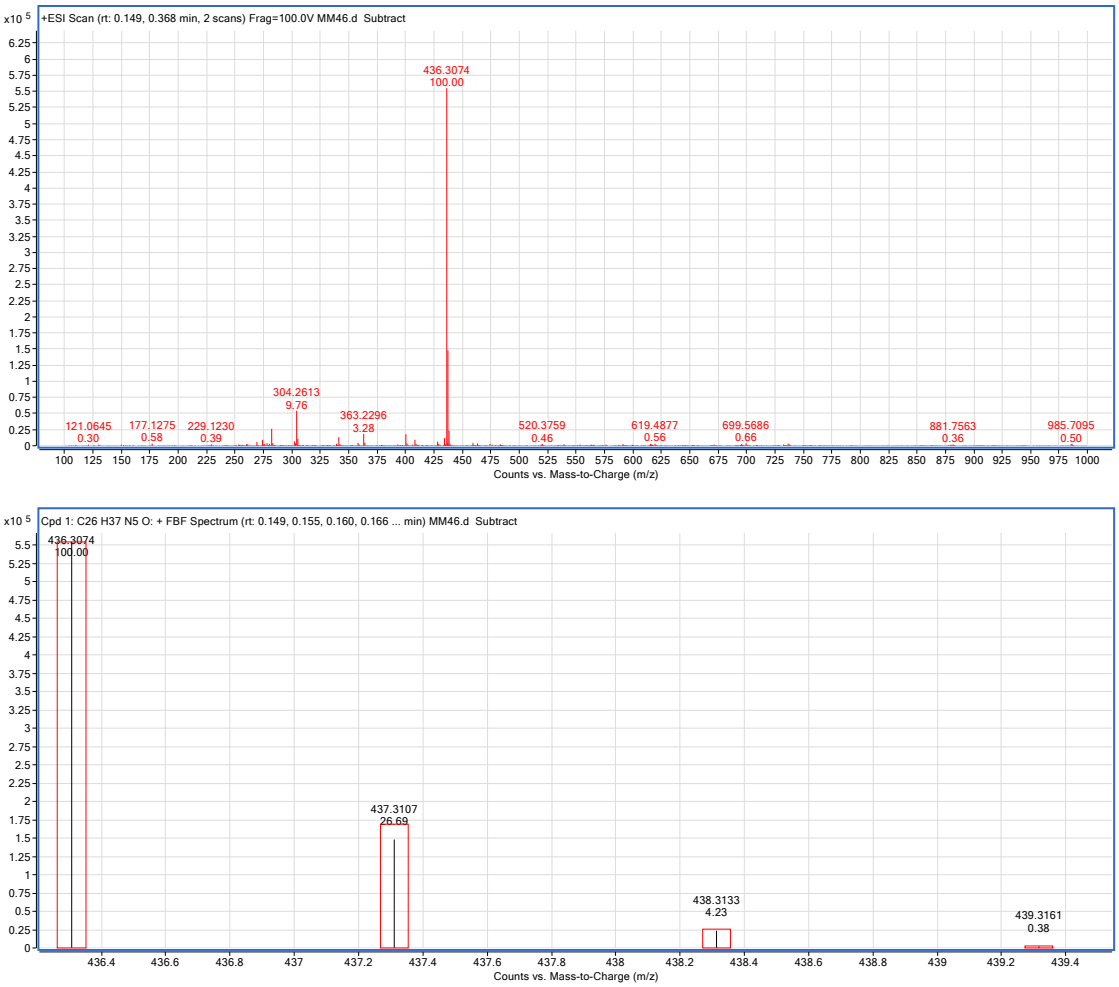
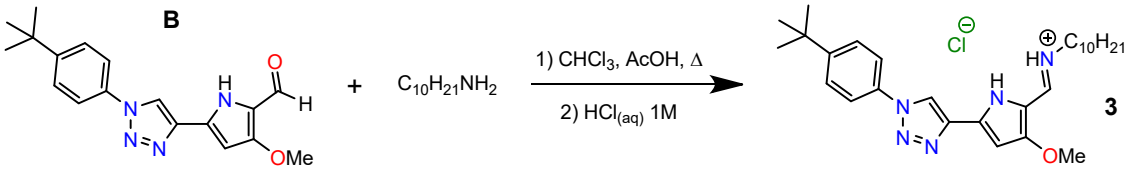


Figure S11. HR-MS (+ESI) spectrum for compound **2**.

2.3.3. Compound 3



A mixture of aldehyde **B** (203 mg, 0.63 mmol), *n*-decylamine (143 µL, 0.73 mmol, 1.2 equiv.) and glacial acetic acid (100 µL) in chloroform (15 mL) was stirred at 65 °C for 17 h. Upon cooling to room temperature, the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (15 mL). The solution of the crude compound was washed with a 1 M HCl aqueous solution (5 × 20 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure. The residue was recrystallised from a mixture of dichloromethane and *n*-hexane to give compound **3** as a brown waxy solid (210 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.57 (s, 1H, pyrrole NH), 10.95 (s_b, 1H, imine NH), 8.93 (s, 1H, triazole CH), 7.84-7.15 (m, 5H), 6.34 (s, 1H), 3.74 (s, 3H), 3.43 (s_b, 2H), 1.63 (s_b, 2H), 1.43-0.90 (m, 23H), 0.78-0.58 (m, 3H). ^{13}C NMR {DEPT-135} (75 MHz, $CDCl_3$): δ (ppm) = 162.8 (ArC), 152.3 (ArC), 144.0 (imine CH), 139.2 (ArC), 138.3 (ArC), 133.6 (ArC), 126.4 (ArCH), 120.9 (ArCH), 119.9 (ArCH), 110.9 (ArC), 93.0 (ArCH), 58.4 (CH_3), 51.3 (CH_2), 34.5 (C), 31.5 (CH_2), 30.9 (CH_3), 29.6 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.8 (CH_2), 26.2 (CH_2), 22.3 (CH_2), 13.8 (CH_3). HR-MS (+ESI): found *m/z* 464.3384 ($[M+H]^+$), $[C_{28}H_{42}N_5O]^+$ requires *m/z* 464.3389 (monoisotopic mass).

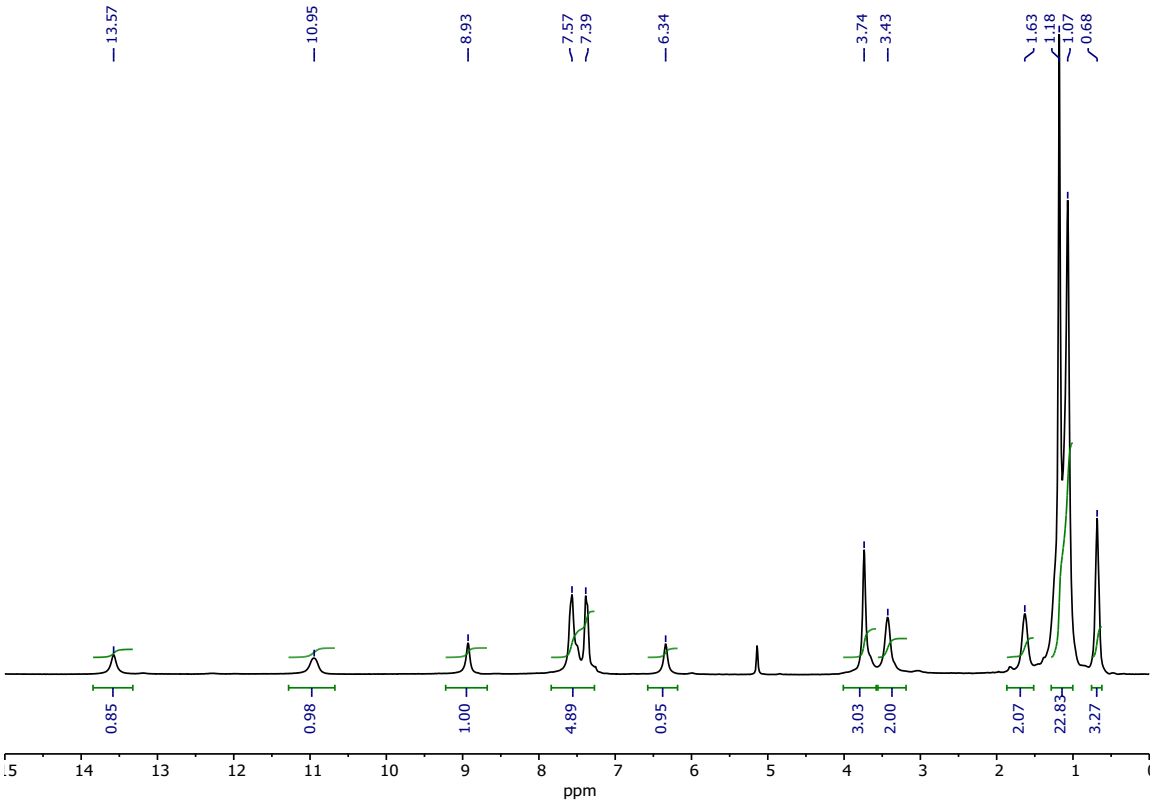


Figure S12. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **3**.

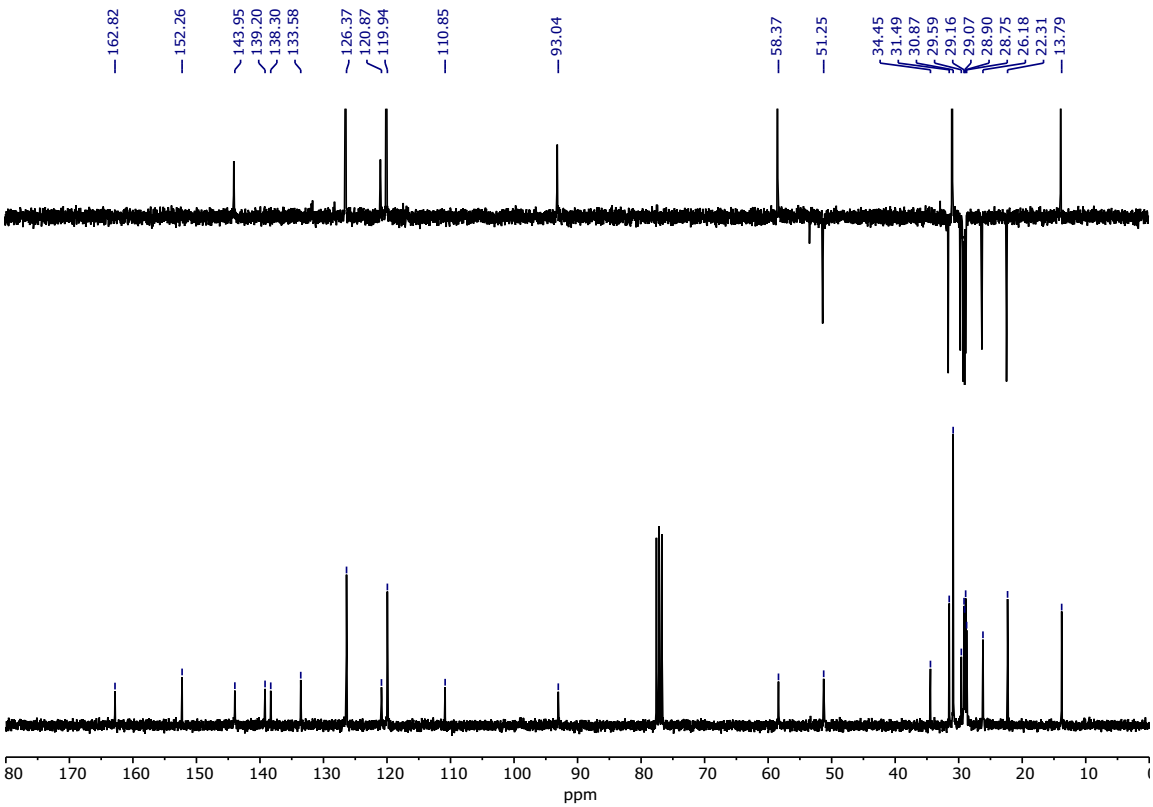


Figure S13. ^{13}C and DEPT-135 NMR spectra (75 MHz, $CDCl_3$) for compound **3**.

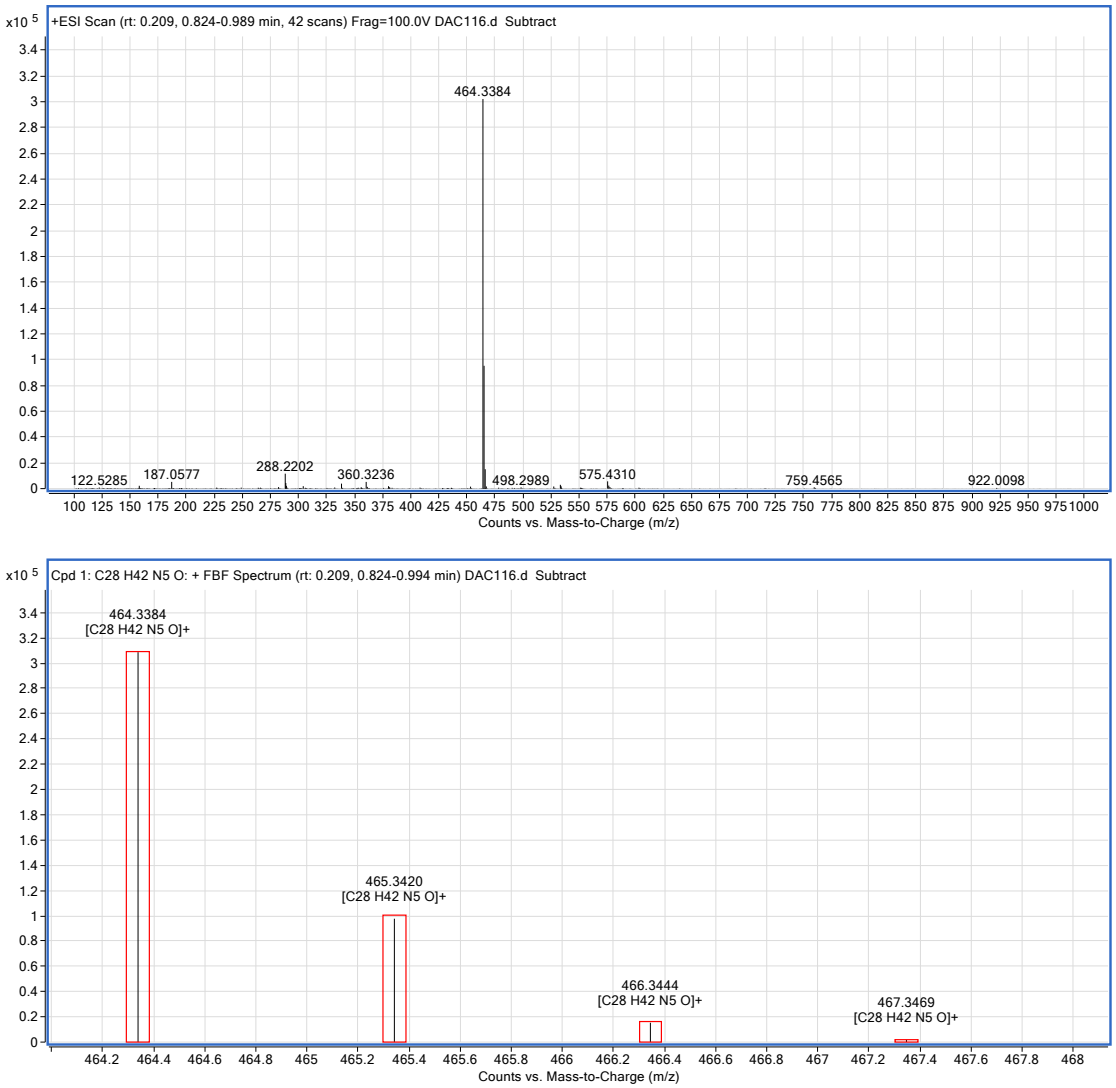
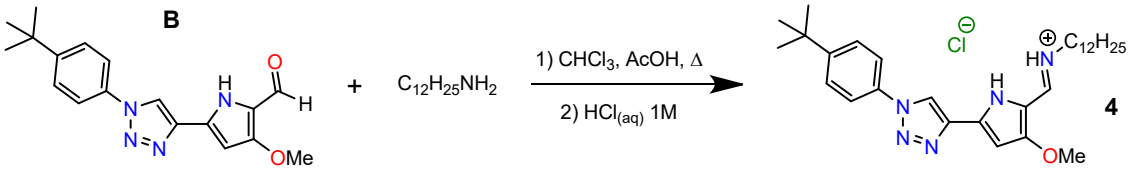


Figure S14. HR-MS (+ESI) spectrum for compound **3**.

2.3.4. Compound 4



A mixture of aldehyde **B** (102 mg, 0.31 mmol), *n*-dodecylamine (87 mg, 0.46 mmol, 1.5 equiv.) and glacial acetic acid (100 µL) in chloroform (15 mL) was stirred at 65 °C for 21 h. Upon cooling to room temperature, the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (15 mL). The solution of the crude compound was washed with a 1 M HCl aqueous solution (5 x 60 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure to give compound **4** as a brown waxy solid (126 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.79 (s, 1H, pyrrole NH), 11.34-11.00 (m, 1H, imine NH), 9.06 (s, 1H, triazole CH), 7.72 (d, J = 8.7 Hz, 2H), 7.60 (d, J = 15.2 Hz, 1H), 7.53 (d, J = 8.7 Hz, 2H), 6.57-6.51 (m, 1H), 3.96 (s, 3H), 3.54 (q, J = 6.5 Hz, 2H), 1.79 (quint, J = 7.1 Hz, 2H), 1.48-1.12 (m, 27H), 0.91-0.79 (m, 3H). ^{13}C NMR {DEPT-135} (75 MHz, $CDCl_3$): δ (ppm) = 163.1 (ArC), 152.5 (ArC), 144.0 (imine CH), 139.4 (ArC), 138.7 (ArC), 133.8 (ArC), 126.6 (ArCH), 121.2 (ArCH), 120.2 (ArCH), 111.1 (ArC), 93.3 (ArCH), 58.6 (CH_3), 51.5 (CH_2), 34.7 (C), 31.8 (CH_2), 31.1 (CH_3), 29.8 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 26.4 (CH_2), 22.5 (CH_2), 14.0 (CH_3). HR-MS (+ESI): found m/z 492.3704 ($[M+H]^+$), $[C_{30}H_{46}N_5O]^+$ requires m/z 492.3702 (monoisotopic mass).

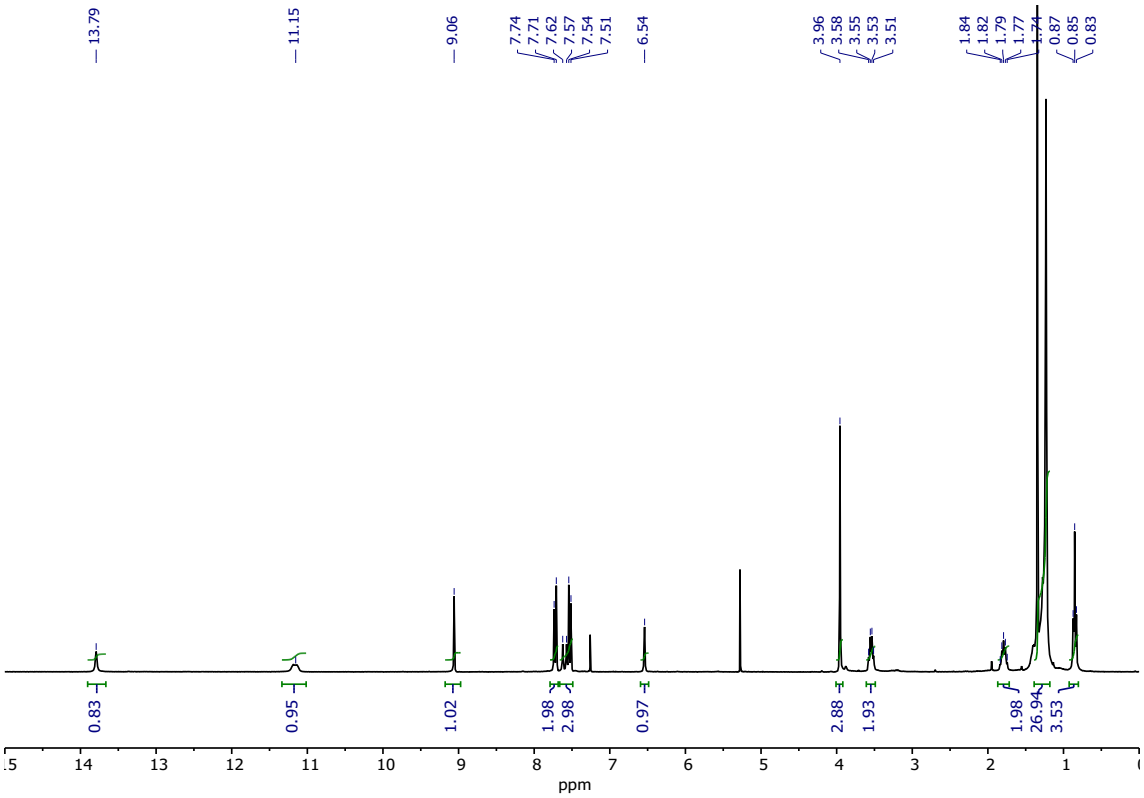


Figure S15. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **4**.

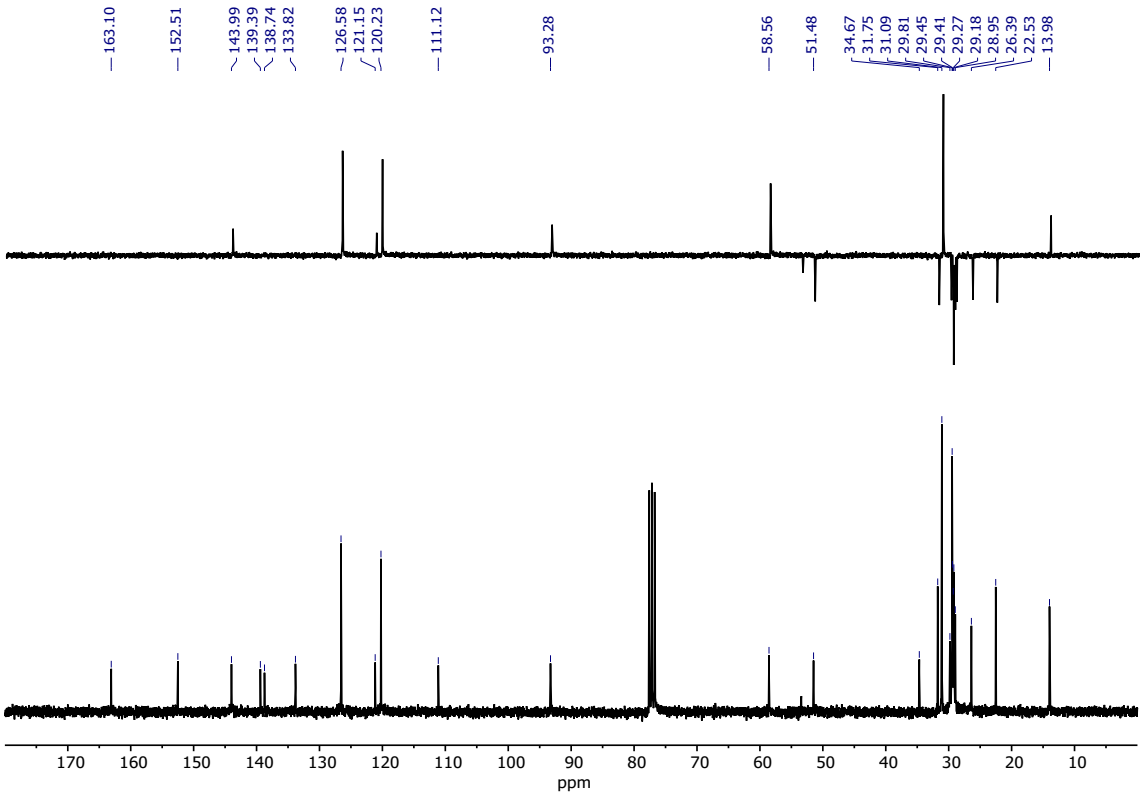


Figure S16. ^{13}C and DEPT-135 NMR spectra (75 MHz, $CDCl_3$) for compound **4**.

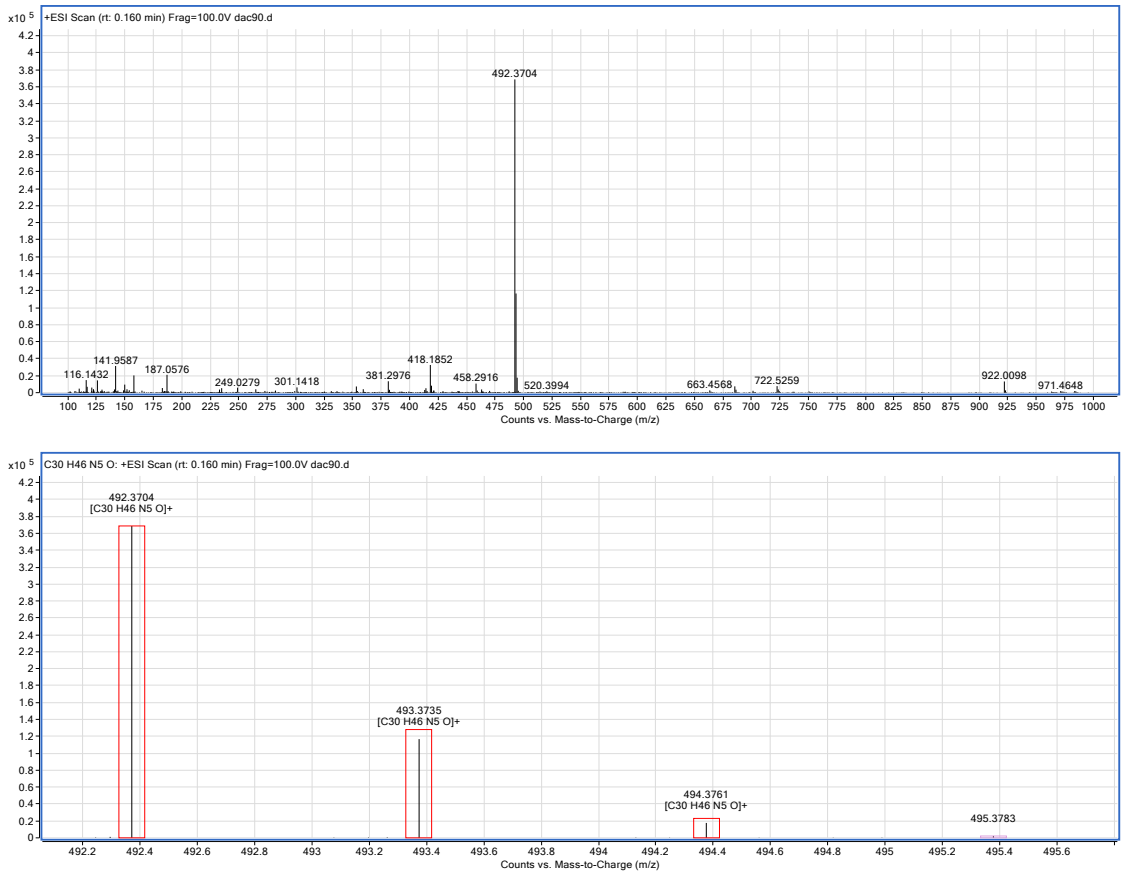
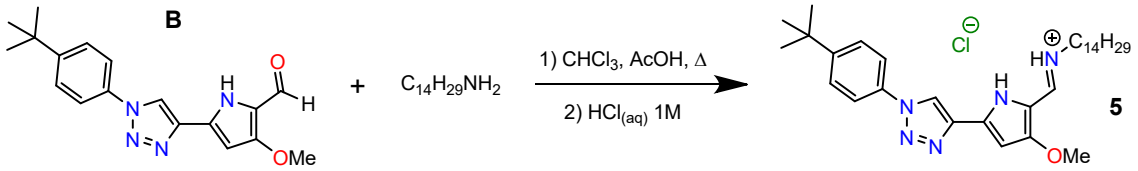


Figure S17. HR-MS (+ESI) spectrum for compound **4**.

2.3.5. Compound 5



A mixture of aldehyde **B** (103 mg, 0.32 mmol), *n*-tetradecylamine (79 mg, 0.36 mmol, 1.2 equiv.) and glacial acetic acid (100 µL) in chloroform (15 mL) was stirred at 65 °C for 18 h. Upon cooling to room temperature, the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (15 mL). The solution of the crude compound was washed with a 1 M HCl aqueous solution (5 × 150 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure to give compound **5** as a brown waxy solid (119 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.66 (s, 1H, pyrrole NH), 11.26-10.81 (m, 1H, imine NH), 8.99 (s, 1H, triazole CH), 7.64 (d, *J* = 7.9 Hz, 2H), 7.55 (d, *J* = 15.2 Hz, 1H), 7.44 (d, *J* = 7.7 Hz, 2H), 6.42 (s, 1H), 3.82 (s, 3H), 3.57-3.38 (m, 2H), 1.81-1.58 (m, 2H), 1.44-0.98 (m, 31H), 0.86-0.66 (m, 3H). ^{13}C NMR {DEPT-135} (75 MHz, $CDCl_3$): δ (ppm) = 163.1 (ArC), 152.5 (ArC), 144.0 (imine CH), 139.4 (ArC), 138.7 (ArC), 133.8 (ArC), 126.6 (ArCH), 121.1 (ArCH), 120.2 (ArCH), 111.1 (ArC), 93.3 (ArCH), 58.6 (CH_3), 51.5 (CH_2), 34.7 (C), 31.8 (CH_2), 31.1 (CH_3), 29.8 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 26.4 (CH_2), 22.5 (CH_2), 14.0 (CH_3). HR-MS (+ESI): found *m/z* 520.4023 ($[M+H]^+$), $[C_{32}H_{50}N_5O]^+$ requires *m/z* 520.4015 (monoisotopic mass).

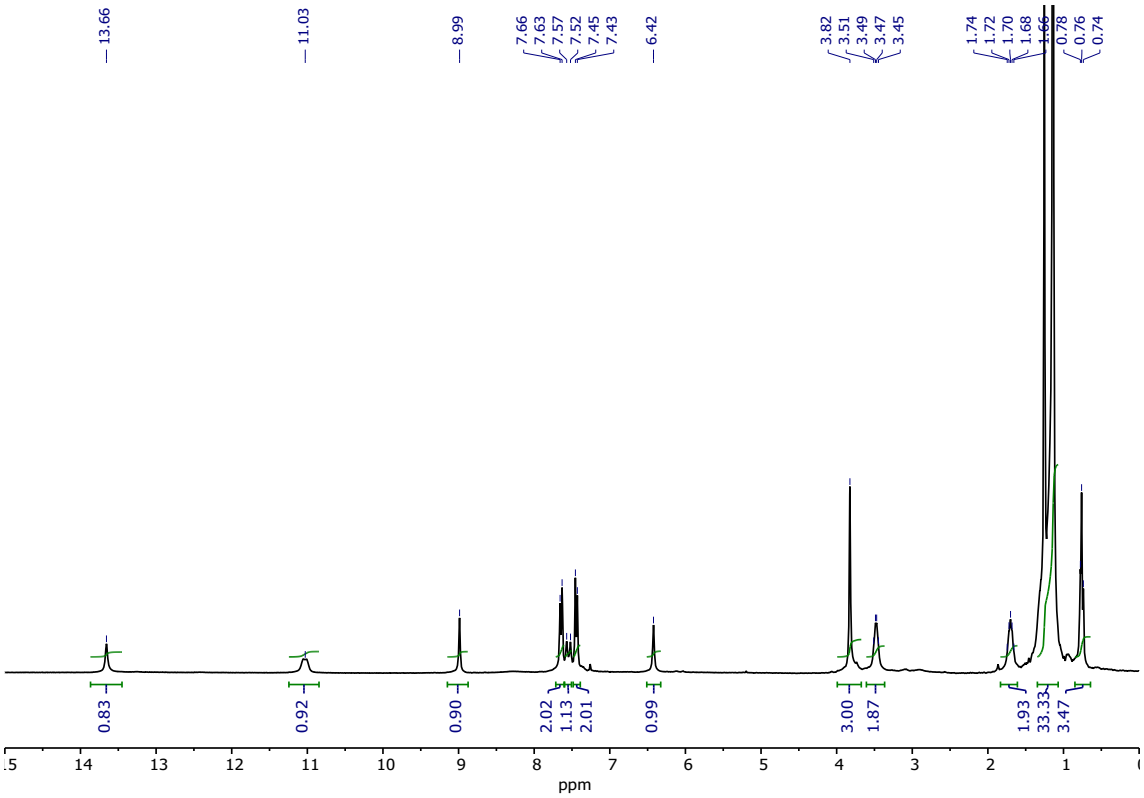


Figure S18. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **5**.

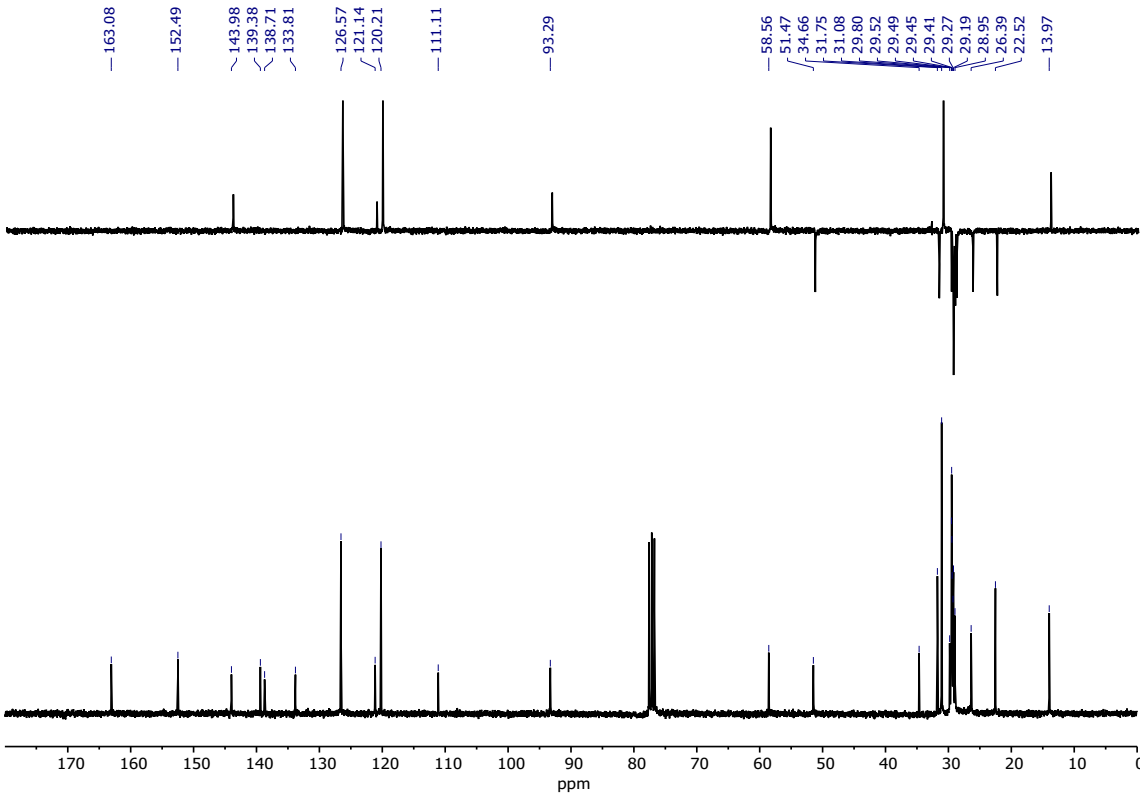


Figure S19. ^{13}C and DEPT-135 NMR spectra (75 MHz, $CDCl_3$) for compound **5**.

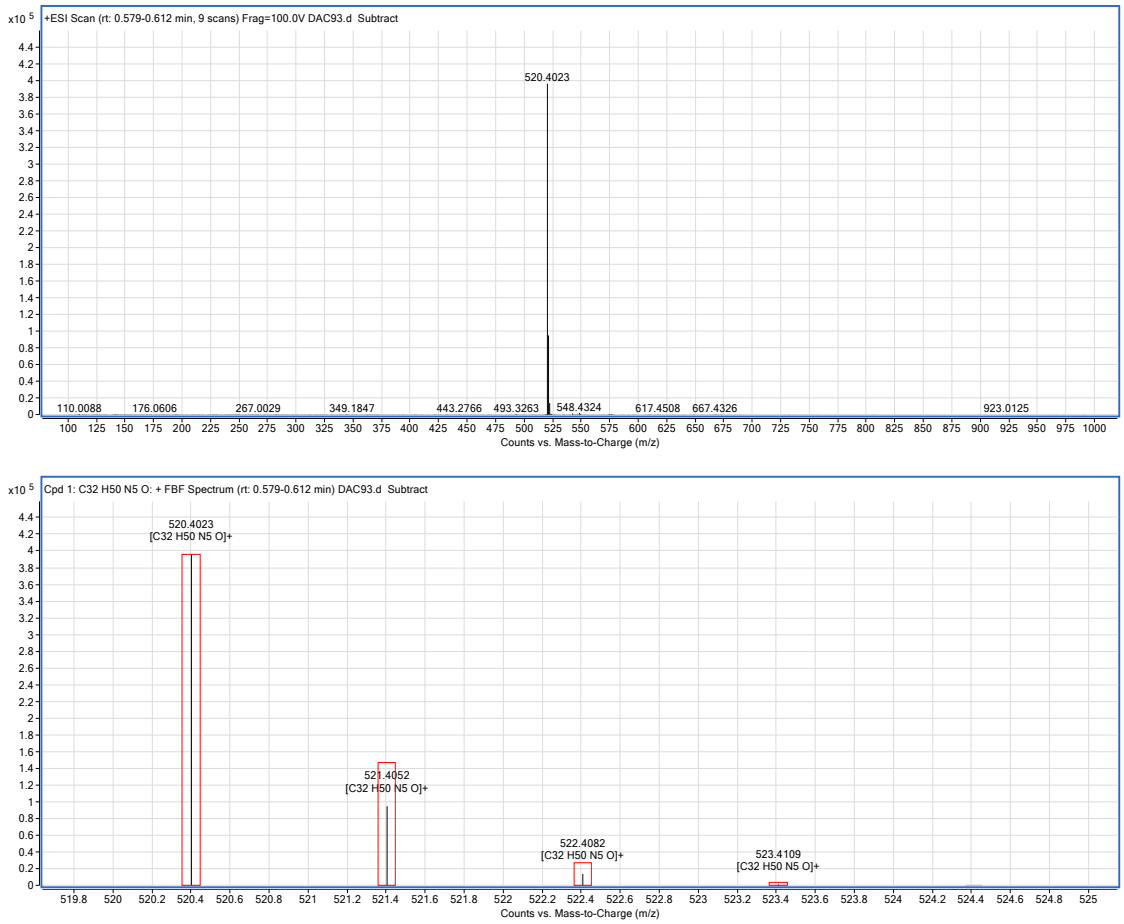
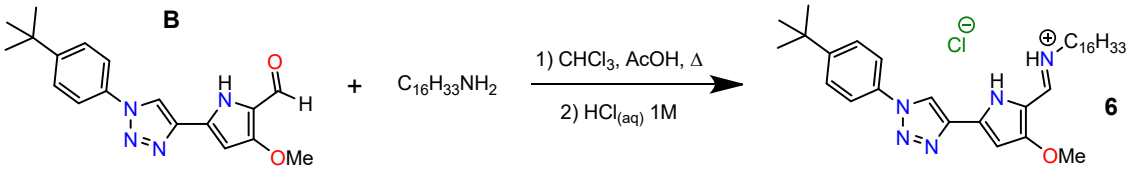


Figure S20. HR-MS (+ESI) spectrum for compound **5**.

2.3.6. Compound 6



A mixture of aldehyde **B** (101 mg, 0.31 mmol), *n*-hexadecylamine (94 mg, 0.34 mmol, 1.1 equiv.) and glacial acetic acid (100 µL) in chloroform (15 mL) was stirred at 65 °C for 21 h. Upon cooling to room temperature, the chloroform was evaporated under reduced pressure and the residue was redissolved in dichloromethane (15 mL). The solution of the crude compound was washed with a 1 M HCl aqueous solution (5 × 150 mL), dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure to give compound **6** as a brown waxy solid (119 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 13.67 (s, 1H, pyrrole NH), 11.36-10.78 (m, 1H, imine NH), 9.00 (s, 1H, triazole CH), 7.66 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 15.1 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 6.44 (s, 1H), 3.84 (s, 3H), 3.58-3.40 (m, 2H), 1.85-1.60 (m, 2H), 1.42-1.07 (m, 35H), 0.85-0.70 (m, 3H). ^{13}C NMR {DEPT-135} (75 MHz, $CDCl_3$): δ (ppm) = 163.1 (ArC), 152.5 (ArC), 144.0 (imine CH), 139.4 (ArC), 138.8 (ArC), 133.8 (ArC), 126.6 (ArCH), 121.2 (ArCH), 120.2 (ArCH), 111.1 (ArC), 93.3 (ArCH), 58.6 (CH_3), 51.5 (CH_2), 34.7 (C), 31.8 (CH_2), 31.1 (CH_3), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 26.4 (CH_2), 22.6 (CH_2), 14.0 (CH_3). HR-MS (+ESI): found *m/z* 548.4324 ($[M+H]^+$), $[C_{34}H_{54}N_5O]^+$ requires *m/z* 548.4328 (monoisotopic mass).

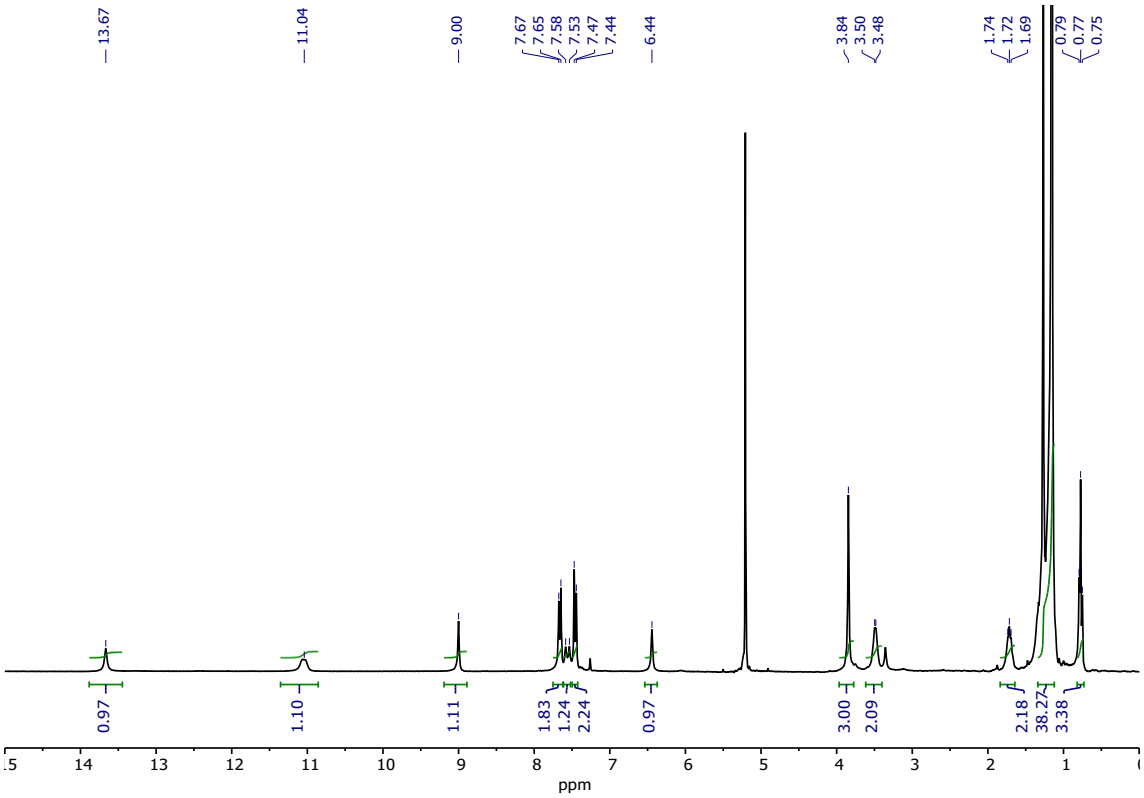


Figure S21. ^{1}H NMR spectrum (300 MHz, $CDCl_3$) for compound **6**.

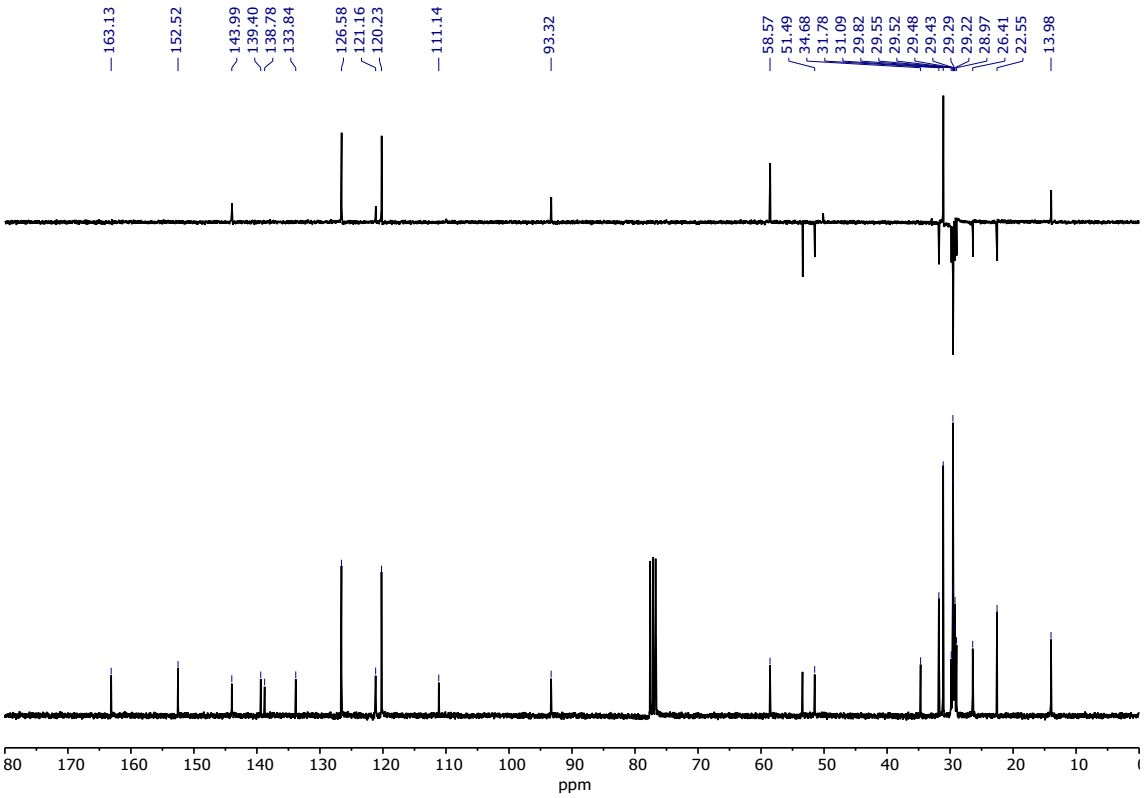


Figure S22. ^{13}C and DEPT-135 NMR spectra (75 MHz, $CDCl_3$) for compound **6**.

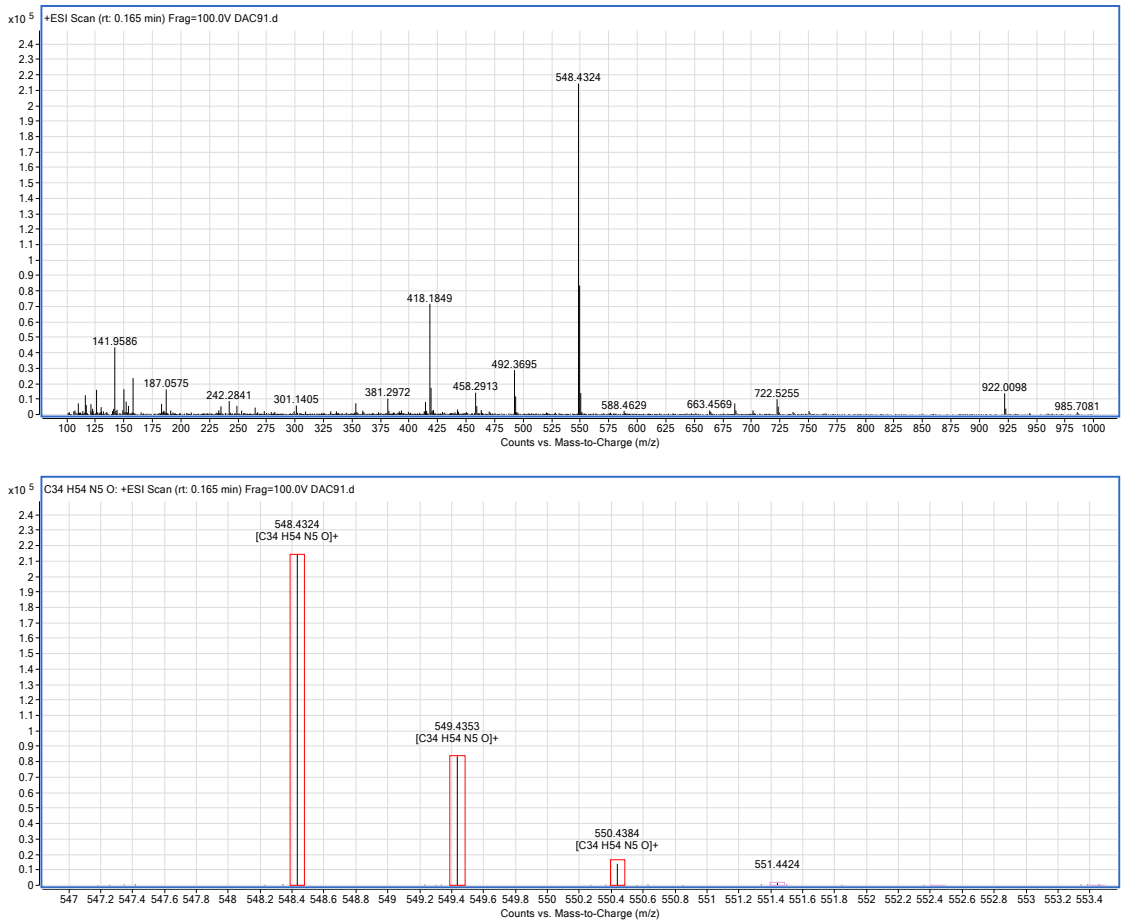


Figure S23. HR-MS (+ESI) spectrum for compound **6**.

3. ABSORPTION AND EMISSION SPECTRA OF THE CLICK-TAMBJAMINES

Absorption spectra

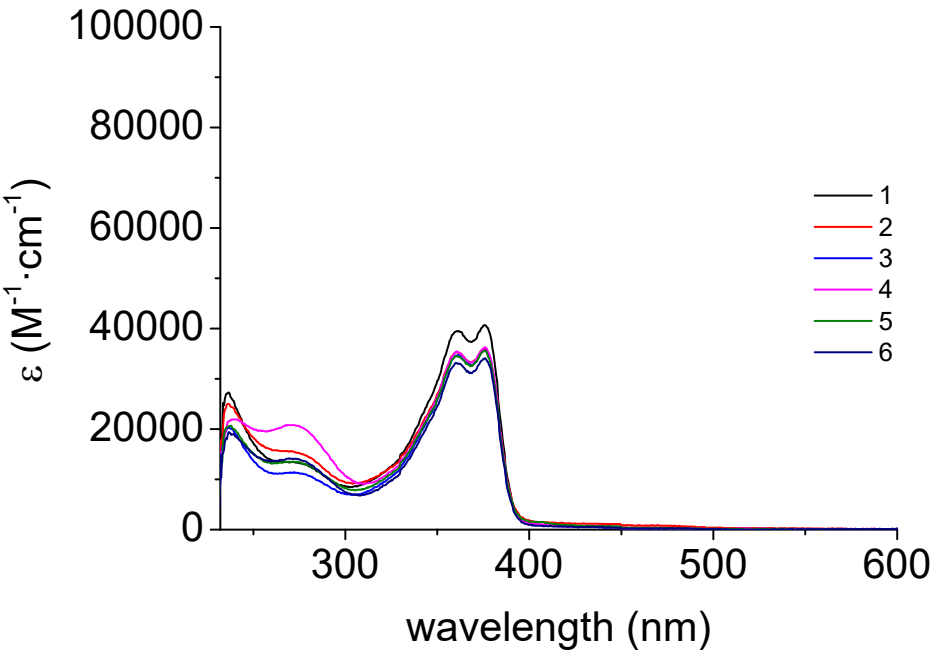


Figure S24. Absorption spectra recorded for 10^{-5} M solutions of the pure compounds in chloroform.

Emission spectra

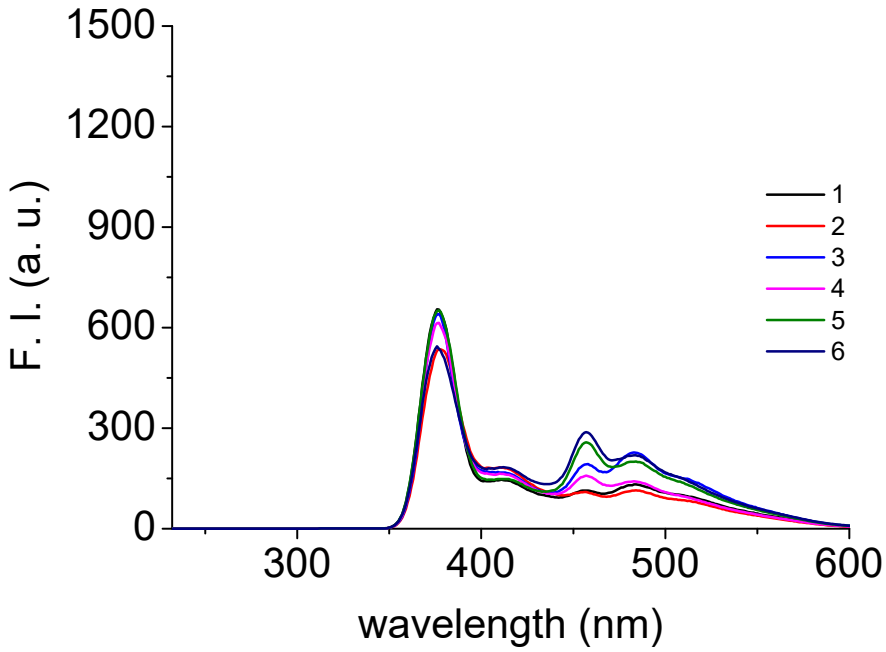


Figure S25. Emission spectra recorded for 10^{-5} M solutions of the pure compounds in chloroform. Each sample was excited at the wavelength of the maximum of its absorption spectrum (376 nm in all cases). EX Slit = 20.0 nm; EM Slit = 10.0 nm.

4. DETERMINATION OF ASSOCIATION CONSTANTS FROM ^{1}H NMR

4.1. Procedure and data fitting

800 µL of a 50 mM solution of the corresponding compound in a 9:1 $CDCl_3$/DMSO-d_6 mixture were prepared and the ^{1}H NMR spectrum of this solution was recorded. Aliquots of this solution were taken to prepare 35 and 25 mM solutions of the compound, whose spectra were recorded. From the former 17.5, 8.75, 4.38, 2.19, 1.09 and 0.55 mM solutions were prepared, while from the latter 12.5, 6.25, 3.13, 1.56 and 0.78 mM solutions were prepared, and their spectra were recorded. All spectra were registered at 298 K.

For each ^{1}H NMR stack plot, the signals of the protons of the imine and pyrrole NH groups of the molecules were monitored for changes in chemical shift (in the case of **1**, only the former was considered), which provided several data sets that were employed in the determination of association constants K_a. Fitting was performed with the Bindfit software.[2] In all cases data were fitted satisfactorily to the 1:1 (LH:Cl) binding model, LH being the protonated receptor and Cl the chloride anion. The results are summarised in **Table S1**.

Table S1. Association constants K_a (M^{-1}) calculated for the chloride adducts derived from the studied compounds (9:1 $CDCl_3$:DMSO-d_6, 298 K).

Compound	K_a
1	1235 ± 162
2	1229 ± 103
3	1258 ± 101
4	1290 ± 88
5	1480 ± 180
6	1344 ± 127

[2] (a) Thordarson, P. *Chem. Soc. Rev.*, 2011, **40**, 1305-1323; (b) www.supramolecular.org (last access: October 2020).

4.2. ^{1}H NMR spectra and fitted binding isotherms

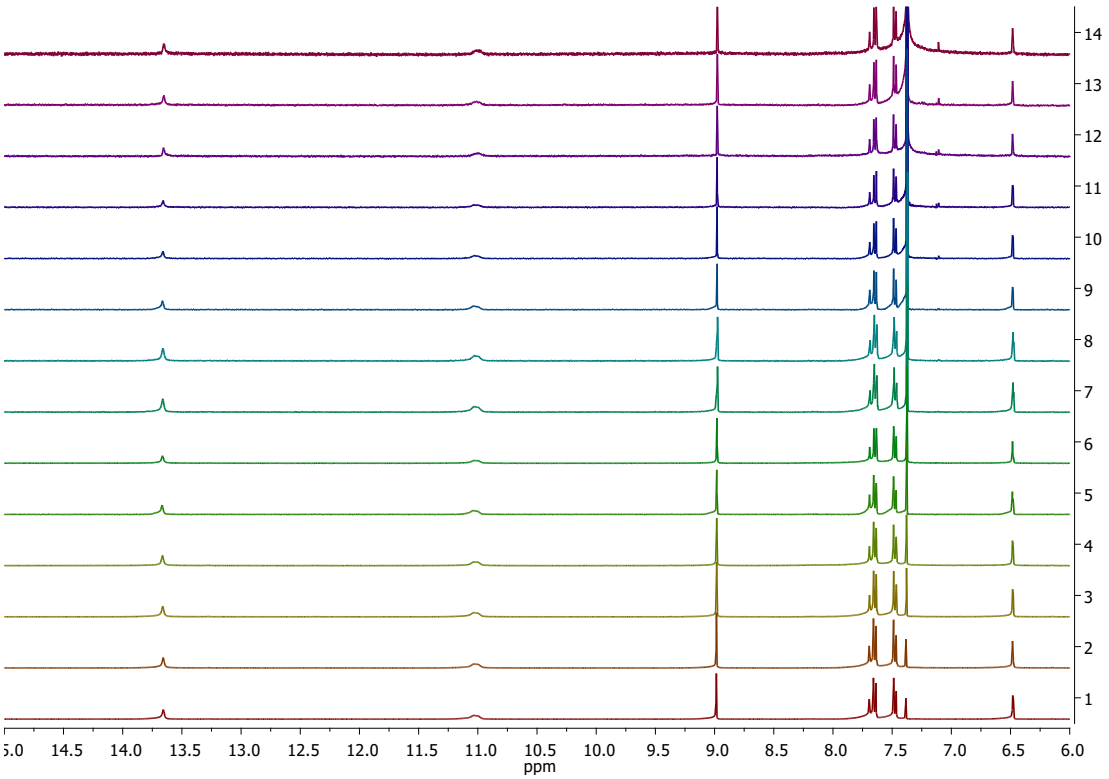


Figure S26. Excerpt of the ^{1}H NMR spectra (400 MHz, 9:1 $CDCl_3$:DMSO-d_6, 298 K) recorded for different solutions of compound **1**, from the highest (spectrum 1, 50 mM) to the lowest (spectrum 14, 0.55 mM) concentration.

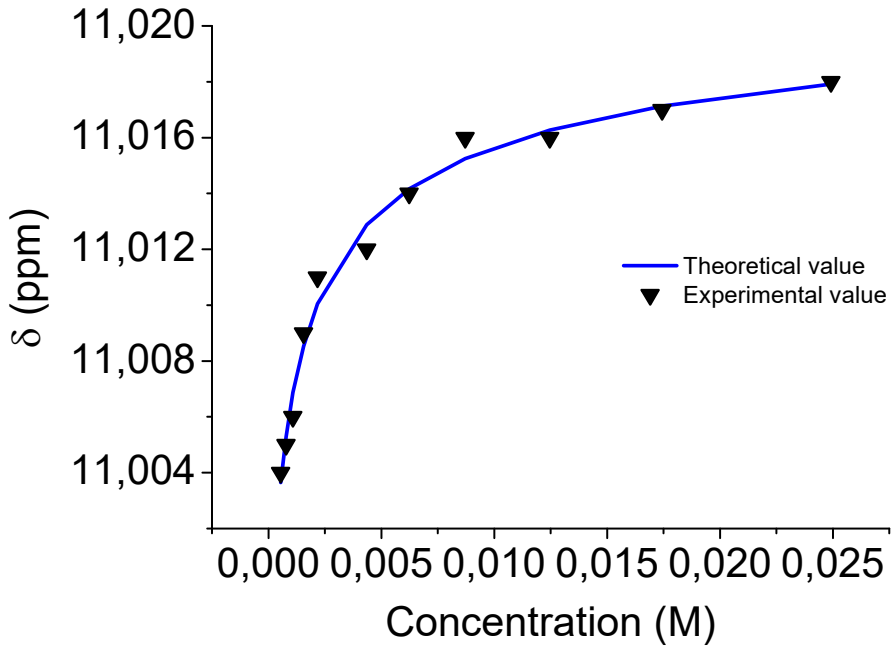


Figure S27. Fitted binding isotherm obtained for compound **1** (9:1 $CDCl_3$:DMSO-d_6, 298 K). The graph shows the change in chemical shift of the signal due to the imine NH proton, fitted to the 1:1 (LH:Cl) binding model. K_a = 1235 ± 162 M^{-1}.

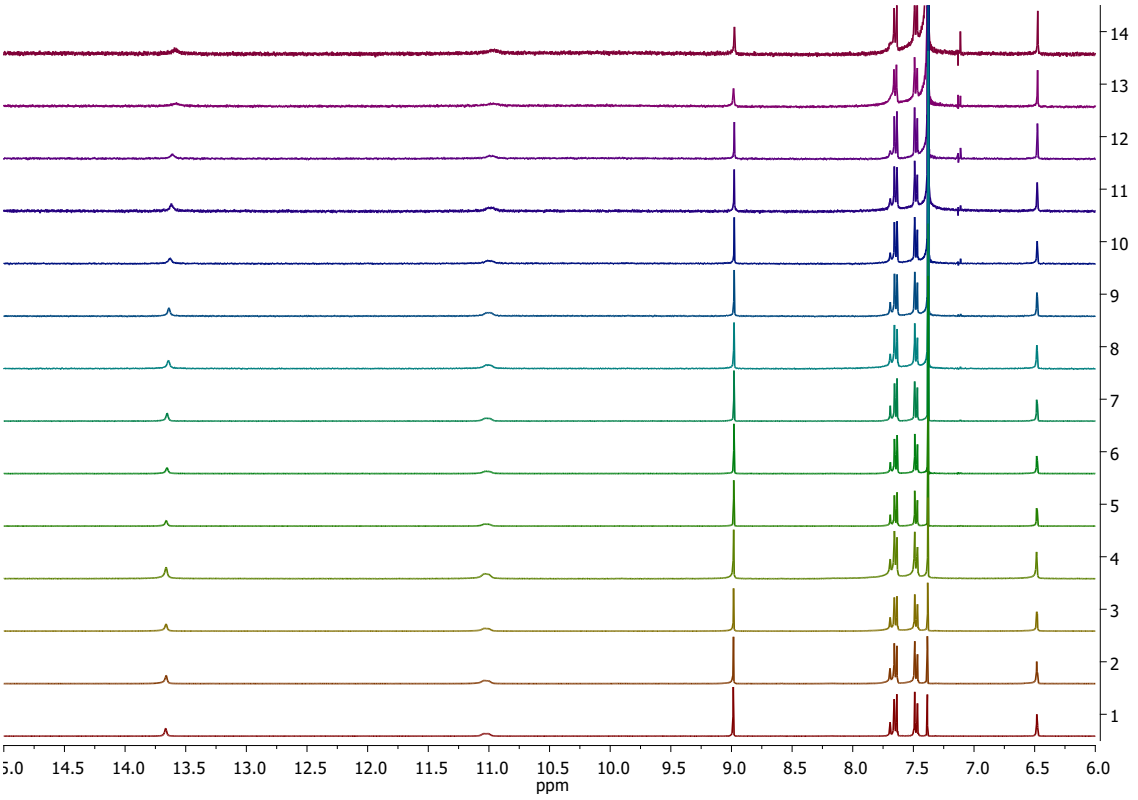


Figure S28. Excerpt of the ^{1}H NMR spectra (400 MHz, 9:1 $CDCl_3$:DMSO-d_6, 298 K) recorded for different solutions of compound **2**, from the highest (spectrum 1, 50 mM) to the lowest (spectrum 14, 0.55 mM) concentration.

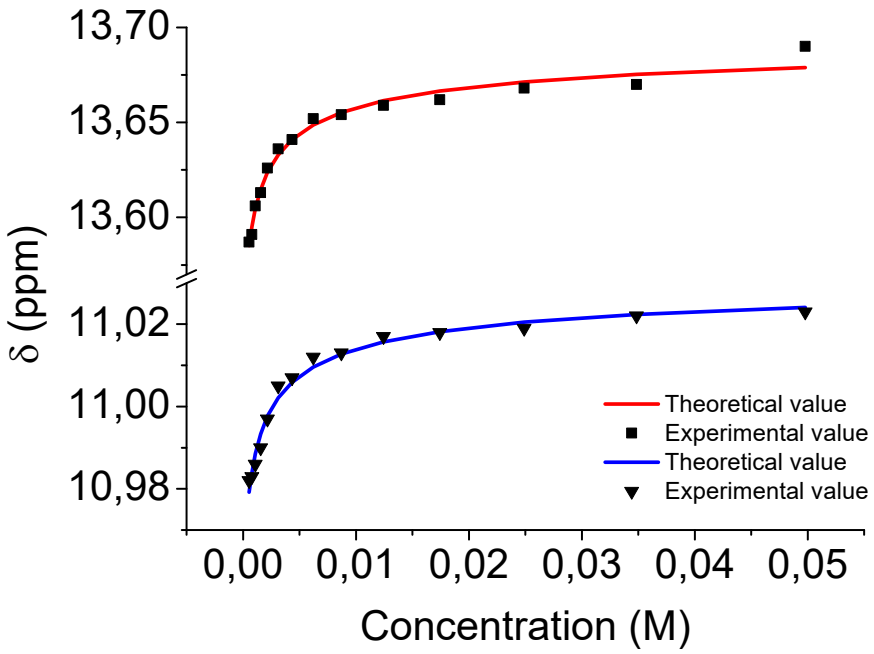


Figure S29. Fitted binding isotherm obtained for compound **2** (9:1 $CDCl_3$:DMSO-d_6, 298 K). The graph shows the change in chemical shift of the signals due to the imine (blue) and pyrrole (red) NH protons, fitted to the 1:1 (LH:Cl) binding model. K_a = 1229 ± 103 M^{-1}.

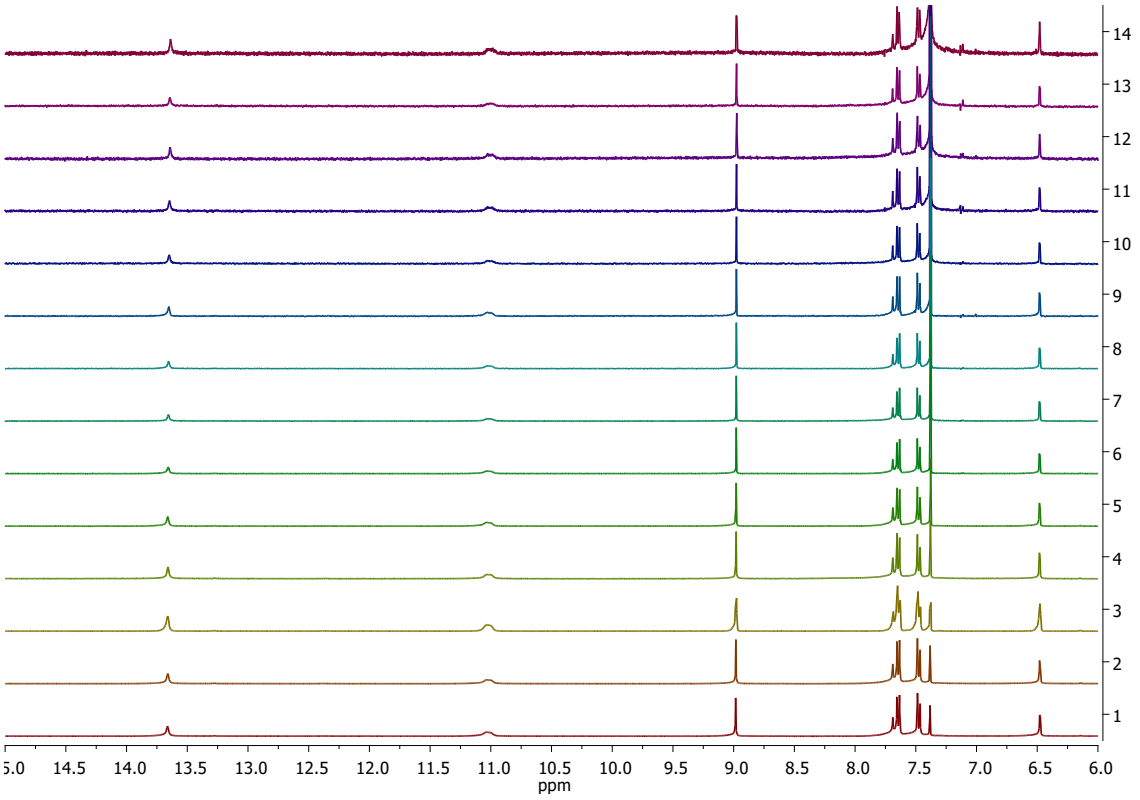


Figure S30. Excerpt of the ^{1}H NMR spectra (400 MHz, 9:1 $CDCl_3$:DMSO-d_6, 298 K) recorded for different solutions of compound **3**, from the highest (spectrum 1, 50 mM) to the lowest (spectrum 14, 0.55 mM) concentration.

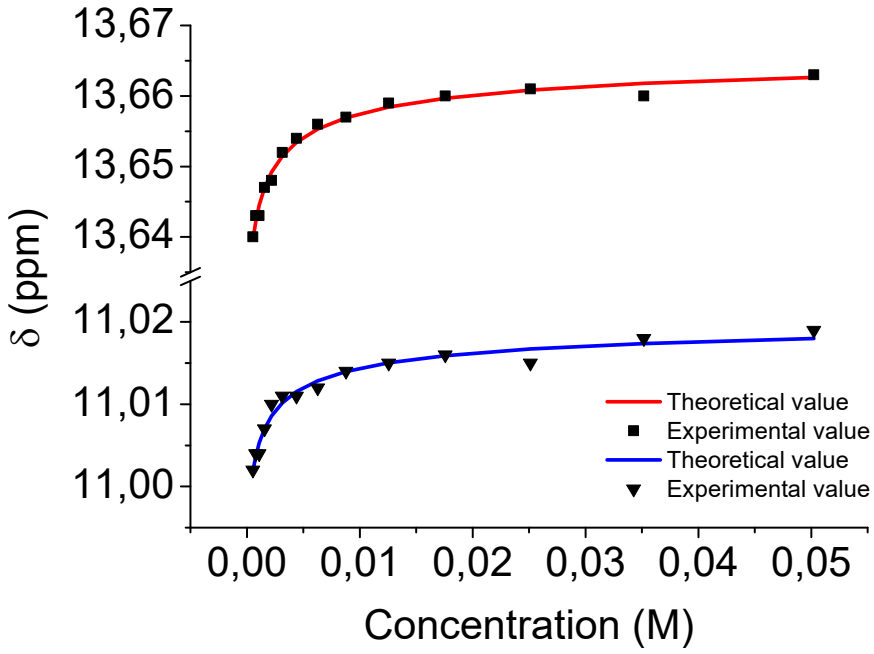


Figure S31. Fitted binding isotherm obtained for compound **3** (9:1 $CDCl_3$:DMSO-d_6, 298 K). The graph shows the change in chemical shift of the signals due to the imine (blue) and pyrrole (red) NH protons, fitted to the 1:1 (LH:Cl) binding model. K_a = 1258 ± 101 M^{-1}.

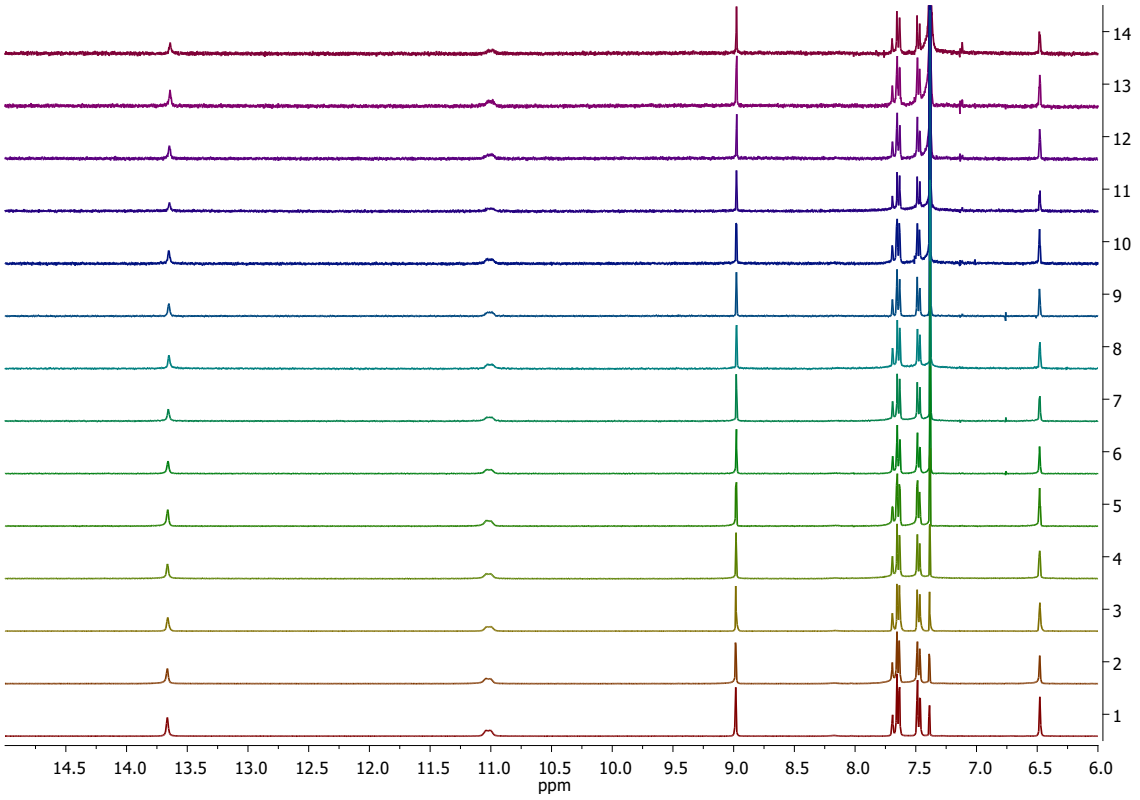


Figure S32. Excerpt of the ^{1}H NMR spectra (400 MHz, 9:1 $CDCl_3$:DMSO-d_6, 298 K) recorded for different solutions of compound **4**, from the highest (spectrum 1, 50 mM) to the lowest (spectrum 14, 0.55 mM) concentration.

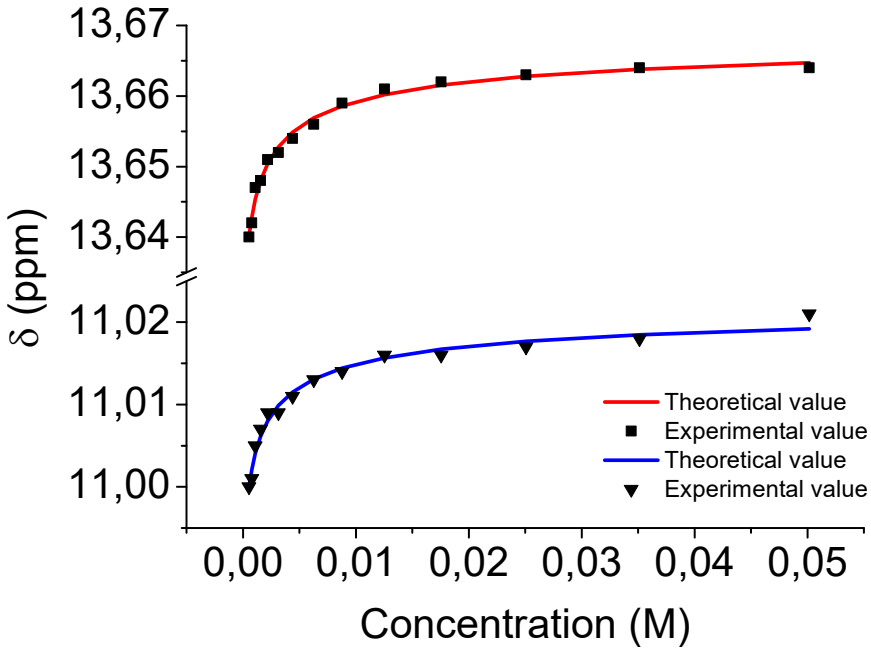


Figure S33. Fitted binding isotherm obtained for compound **4** (9:1 $CDCl_3$:DMSO-d_6, 298 K). The graph shows the change in chemical shift of the signals due to the imine (blue) and pyrrole (red) NH protons, fitted to the 1:1 (LH:Cl) binding model. K_a = 1290 ± 88 M^{-1}.

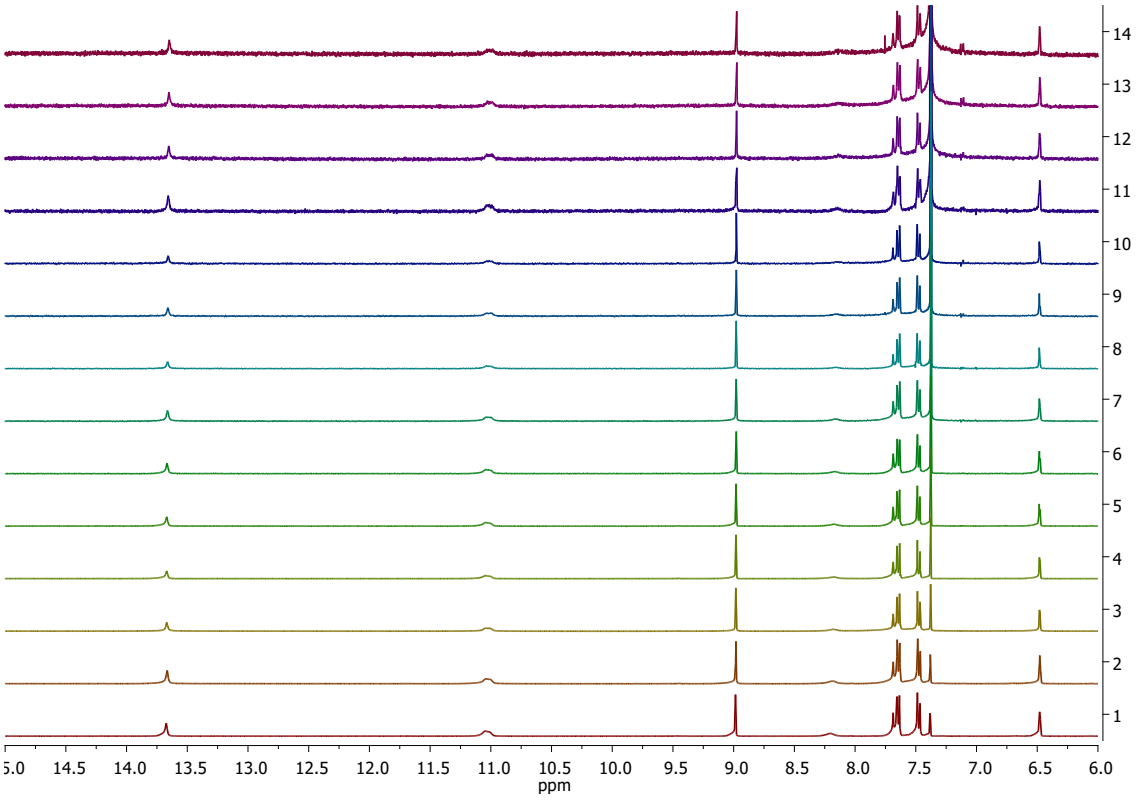


Figure S34. Excerpt of the ^{1}H NMR spectra (400 MHz, 9:1 $CDCl_3$:DMSO-d_6, 298 K) recorded for different solutions of compound **5**, from the highest (spectrum 1, 50 mM) to the lowest (spectrum 14, 0.55 mM) concentration.

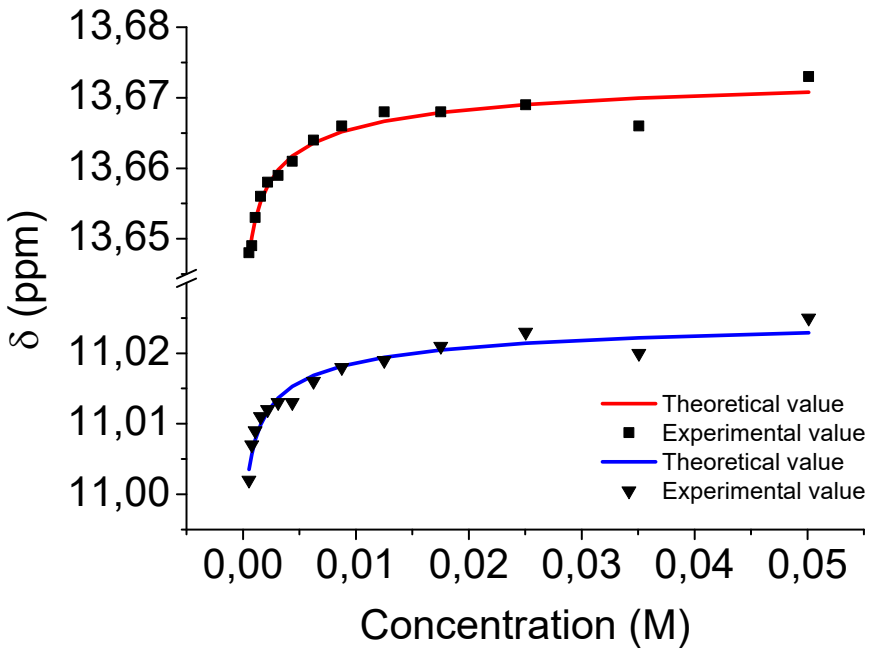


Figure S35. Fitted binding isotherm obtained for compound **5** (9:1 $CDCl_3$:DMSO-d_6, 298 K). The graph shows the change in chemical shift of the signals due to the imine (blue) and pyrrole (red) NH protons, fitted to the 1:1 (LH:Cl) binding model. K_a = 1480 ± 180 M^{-1}.

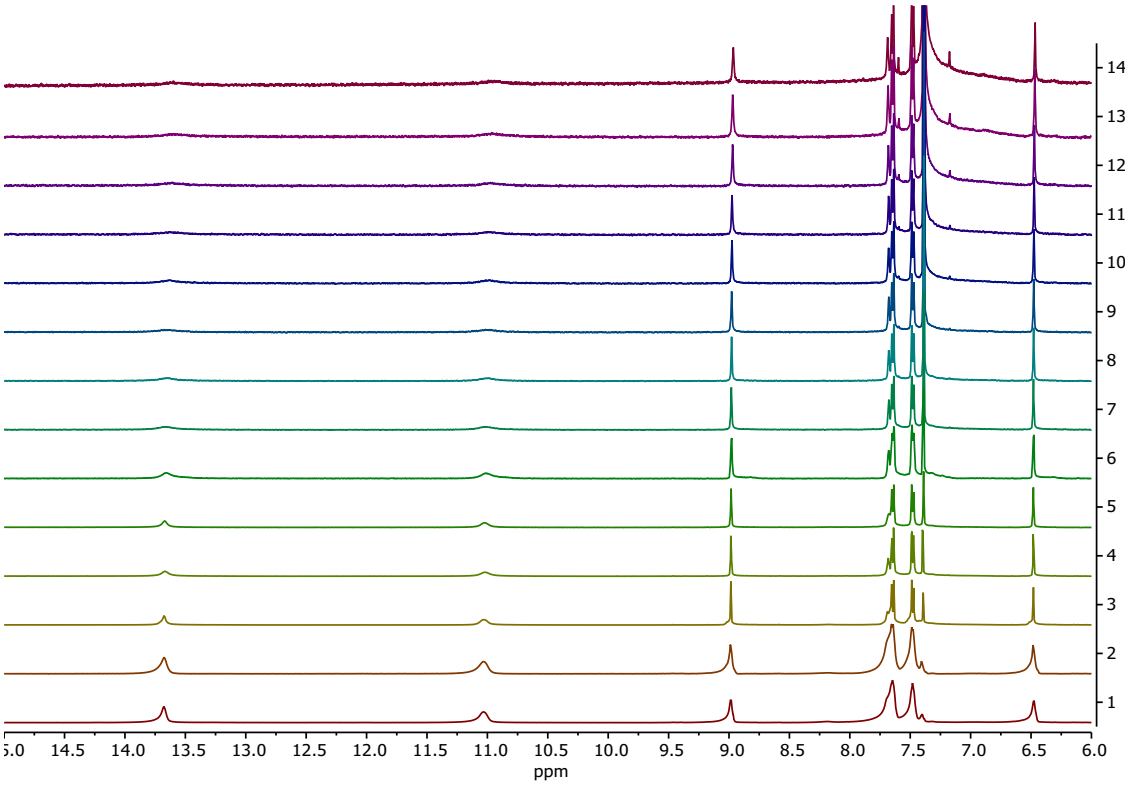


Figure S36. Excerpt of the ^{1}H NMR spectra (400 MHz, 9:1 $CDCl_3$:DMSO-d_6, 298 K) recorded for different solutions of compound **6**, from the highest (spectrum 1, 50 mM) to the lowest (spectrum 14, 0.55 mM) concentration.

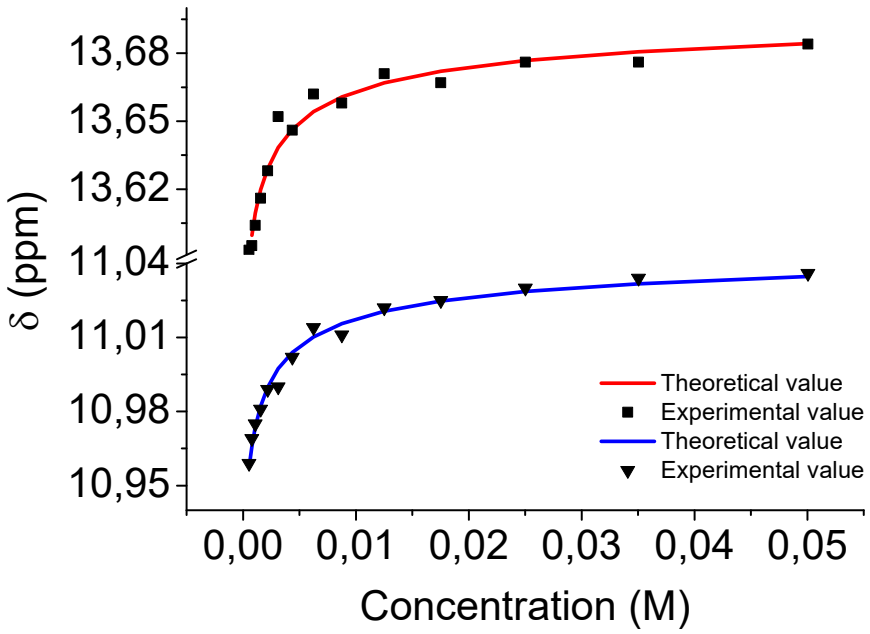


Figure S37. Fitted binding isotherm obtained for compound **6** (9:1 $CDCl_3$:DMSO-d_6, 298 K). The graph shows the change in chemical shift of the signals due to the imine (blue) and pyrrole (red) NH protons, fitted to the 1:1 (LH:Cl) binding model. K_a = 1344 ± 127 M^{-1}.

5. PREPARATION AND CHARACTERISATION OF NLCs

5.1. Preparation

Firstly, the components of the formulations were weighed. On one hand, the ingredients of the lipid phase: 10 mg of the click-tambjamine, 1000 mg of Precirol® ATO 5 (Gattefosé, Spain) and 100 mg of Miglyol® 812 N (F) (Cremer Oleo Division, Germany). On the other hand, those of the aqueous phase: 100 mg of Poloxamer 188 (Corning, USA) and 200 mg of Tween® 80 (Panreac Química, Spain). In addition, 150 mg of trehalose (Acros Organics, Belgium) were weighed and 15 mL of Milli-Q® Type 1 ultrapure water were taken.

The protocol to prepare these formulations is as follows: the components of the lipid phase (the click-tambjamine, Precirol® ATO 5 and Miglyol® 812 N (F)) were put together in a beaker. Poloxamer 188 and Tween® 80 were combined in another beaker and Milli-Q® Type 1 ultrapure water (15 mL) was added to dissolve them; this step was performed with stirring (gentle) in order to ease the dissolution of the surfactants. Once dissolved, both phases were warmed separately in a water bath at 70 °C. In this way, lipids melt, which allows the dispersion of the drug (in this case, a click-tambjamine), and the aqueous phase is maintained at the same temperature of the lipid phase. When lipids were completely melted, the aqueous phase was poured on the lipid phase and the resulting emulsion was sonicated at 50 W for 30 seconds; in this step it is important to spin the beaker gently to create a uniform emulsion. Subsequently, the emulsion was cooled down to room temperature while stirring for 15 minutes, and then it was left in the fridge and allowed to rest for 2-3 hours, to permit the formation of nanoparticles. Trehalose, a cryo-protecting agent, was dissolved in Milli-Q® Type 1 ultrapure water (2 mL) and added to the emulsion while stirring. Then, this emulsion was transferred to vials (4 mL per vial) and the content lyophilised to obtain the powdery formulations. Lyophilisation was performed in a Telstar® LyoBeta lyophiliser following this programme (**Table S2**):

Table S2. Lyophilisation conditions of the formulations.

Process	Temperature (°C)	Pressure (mbar)	Time (hours)
Freezing	-50	–	1
Freezing	-50	–	2
Chamber vacuum	–	0.2	–
Primary drying	-50	0.2	5
Primary drying	20	0.2	1
Primary drying	20	0.2	6
Secondary drying	20	–	24

5.2. Characterisation

First, nanoparticles were defined for size and dispersion (SPAN) by means of NS300 Nanosight (Malvern-Panalytical Ltd, Spain) based on nanoparticle tracking analysis (NTA). Initially, 100 nm polystyrene nanoparticle size calibration standard (Malvern Panalytical Ltd, Spain) was measured. ±10% of this stated size range was deemed acceptable after 5, 60 second recordings. Next, 2 mg of each nanoparticle were diluted in 1 mL of Milli-Q® Type 1 ultrapure water (Wasserlab, QA03DP30GR model) and sonicated at 37 °C in an Ultrasonic Cleaner (ATU®, Spain) at 50 W for 30 seconds. Later, the nanoparticles were diluted in Milli-Q® Type 1 ultrapure water until 20-100 particles per frame were observed and then measured with a minimum of 5, 60 second recordings with manual settings used for the camera level in all samples and a detection threshold of 10-17 for the NLC-**1**, threshold of 12 for the NLC-**2**, threshold of 9 for the NLC-**3**, threshold of 18 for the NLC-**4**, threshold of 18 for the NLC-**5** and threshold of 9 for the NLC-**6**.

Zeta potential was measured using the Zetasizer Nano Z (Malvern-Panalytical Ltd, Spain). Firstly, -40 mV zeta potential transfer standard (ZTS1240 Malvern Panalytical Ltd, Spain) was measured. ±5,8 mV of the stated zeta potential was deemed acceptable after 3 measurements of 12 runs. Next, 2 mg of each nanoparticle were diluted in 1 mL of Milli-Q® Type 1 ultrapure water (Wasserlab, QA03DP30GR model), sonicated at 37 °C in an Ultrasonic Cleaner (ATU®, Spain) at 50 W for 30 seconds, and measured for surface zeta potential using a minimum of 3 measurements of 12 runs. The parameters used are as follows: equilibrium times of 120 seconds, delay between measurement times of 20 seconds, cuvette DTS1070 (Malvern-Panalytical Ltd, Spain), automatic attenuation and voltage selection, and auto-mode analysis model. Each measurement met the quality criteria for average count rate, phase plot and frequency plot.

For nanoparticle morphology study, transmission electron microscopy (TEM) analysis was performed under negative staining (**Figure S38**). Thus, 2 µL of each sample were adhered for 1 minute on carbon-coated grids (Agar S160-3 grids) previously hydrophilised by glow discharge (Leica ACE200, 30 s at 10 mA). After removing excess liquid by blotting with filter paper, grids were negatively stained using 1% uranyl acetate for 1 minute. Samples were visualised in a JEOL JEM 1400 Plus transmission electron microscope operated at 100 kV and images were acquired with an integrated Hamamatsu Flash sCMOS digital camera.

TEM images showed nearly oval small particles. It becomes possible to observe the shape, structure and differences of the sample surface, confirming that the particles presented

a rough surface. The specific morpholgy, surface area and diffusion paths, offers a controlled release of the encapsulated active substances (**1**, **2**, **3**, **4**, **5** and **6**). Also in the NLCs, excess of cryoprotector could be observed as small crystals in all the samples. As TEM images revealed, NLC-**1** and NLC-**2** tended to form small aggregates.

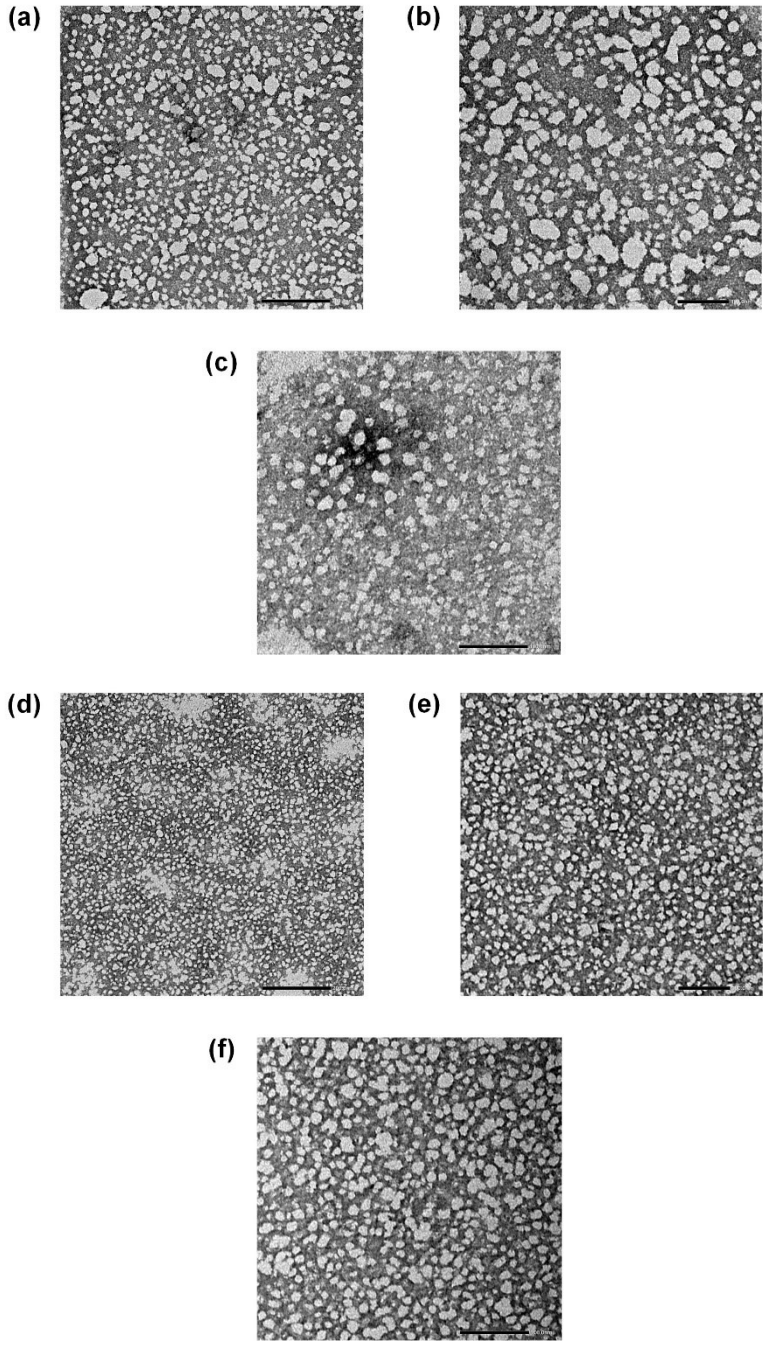


Figure S38. TEM photographs taken for the formulations of compounds **1** [(a) 40x, (b) 60x, (c) 80x] and **2** [(d) 40x, (e) 60x, (f) 80x].

6. DETERMINATION OF THE CONCENTRATION OF THE CLICK-TAMBJAMINES IN THE NLCs

6.1. Absorbance-concentration relationships (Lambert-Beer law)

In order to calculate the concentration of the six compounds in the formulations, UV-Vis spectrophotometry was employed. First of all, the relationship between absorbance and concentration was determined for each pure compound. Thus, 10^{-3} M stock solutions of the click-tambjamines in a 1:1 chloroform:methanol mixture were prepared in 10-mL volumetric flasks and, from this solution, six diluted solutions: $3\cdot10^{-5}$, $2.5\cdot10^{-5}$, $2\cdot10^{-5}$, $1.5\cdot10^{-5}$, $1\cdot10^{-5}$ and $0.5\cdot10^{-5}$ M. The spectra of these solutions were recorded at 293 K in an Hitachi U-3900 spectrophotometer, and the calibration straight lines at λ = 342 nm, the wavelength that provided the best fittings, obtained (see **Figures S39** to **S50**). Since the optical path of the quartz cuvettes is 1 cm, the slope of the lines corresponds to the molar extinction coefficient of the compounds ($M^{-1}\cdot cm^{-1}$). The equations of the calibration straight lines and the coefficients of determination (R^2) of the fittings are displayed in **Table S3**.

Table S3. Equations of the calibration straight lines obtained for the studied compounds and coefficients of determination (R^2).

Compound	Equation of the calibration straight line	Coefficient of determination (R^2)
1	A = 29752·[**1**]	0.9999
2	A = 28220·[**2**]	0.9996
3	A = 28292·[**3**]	0.9997
4	A = 30149·[**4**]	0.9993
5	A = 24363·[**5**]	0.995
6	A = 26300·[**6**]	0.9981

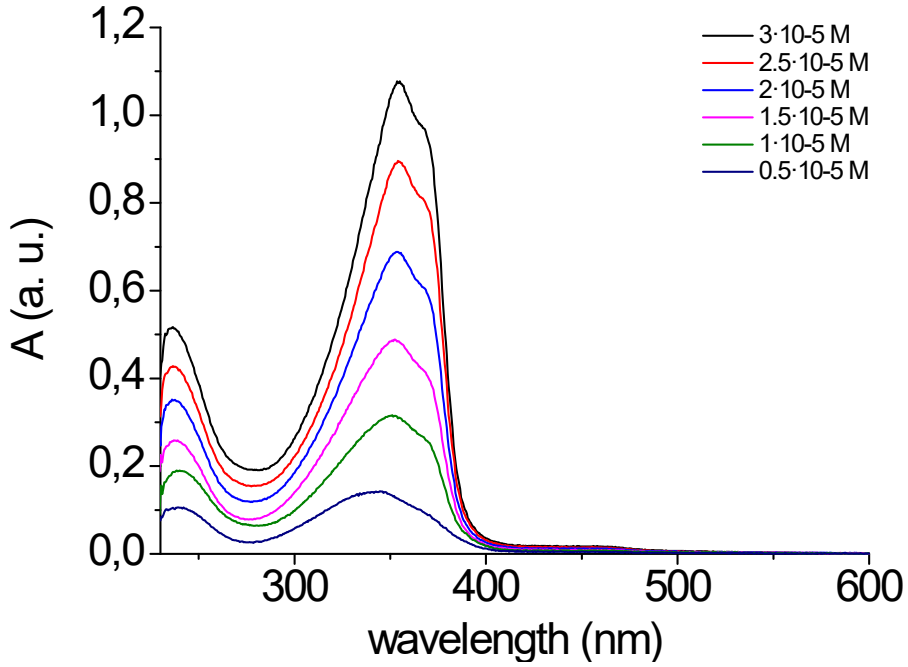


Figure S39. Spectra recorded for solutions of pure compound **1** of different concentrations in a 1:1 chloroform:methanol mixture.

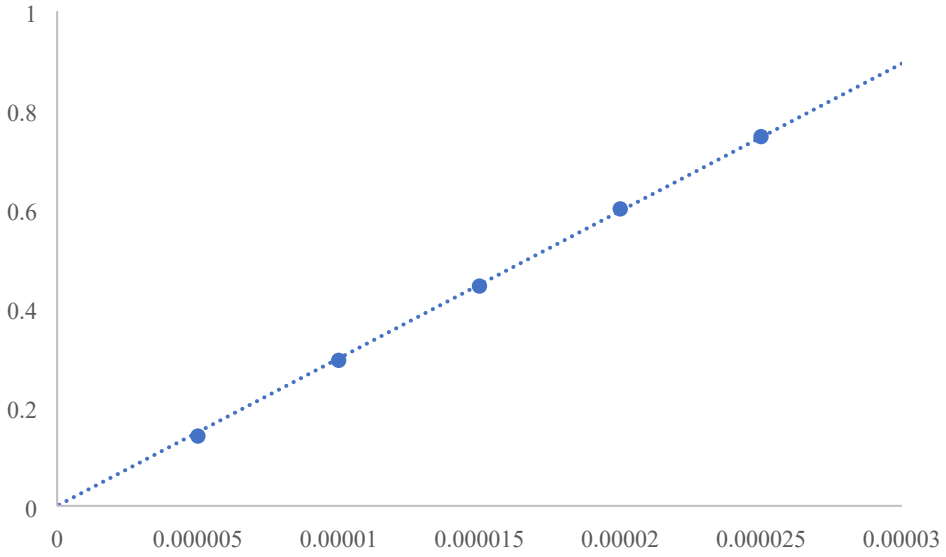


Figure S40. Calibration line (Lambert-Beer law) obtained for pure compound **1** at λ = 342 nm.

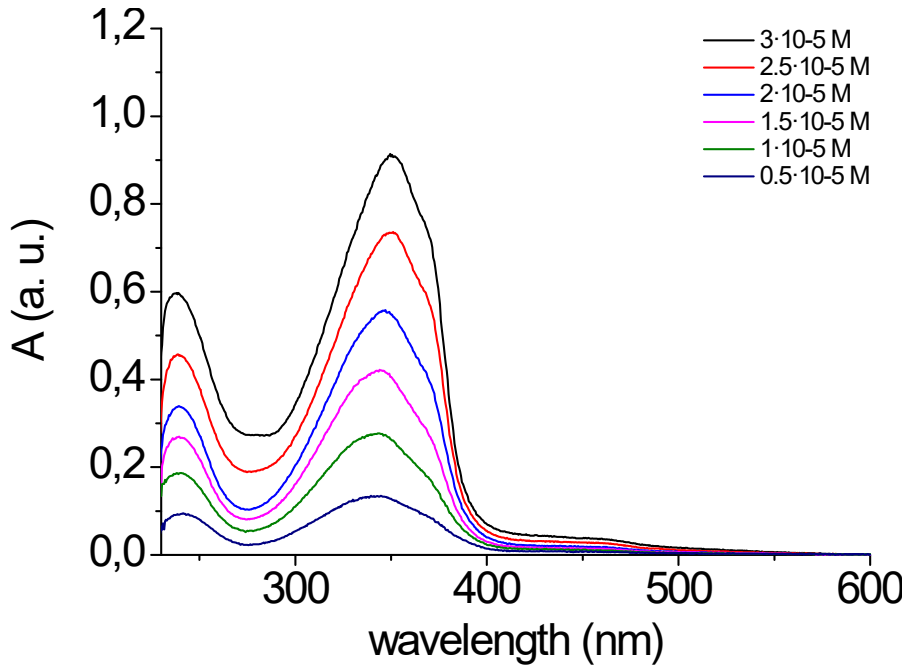


Figure S41. Spectra recorded for solutions of pure compound **2** of different concentrations in a 1:1 chloroform:methanol mixture.

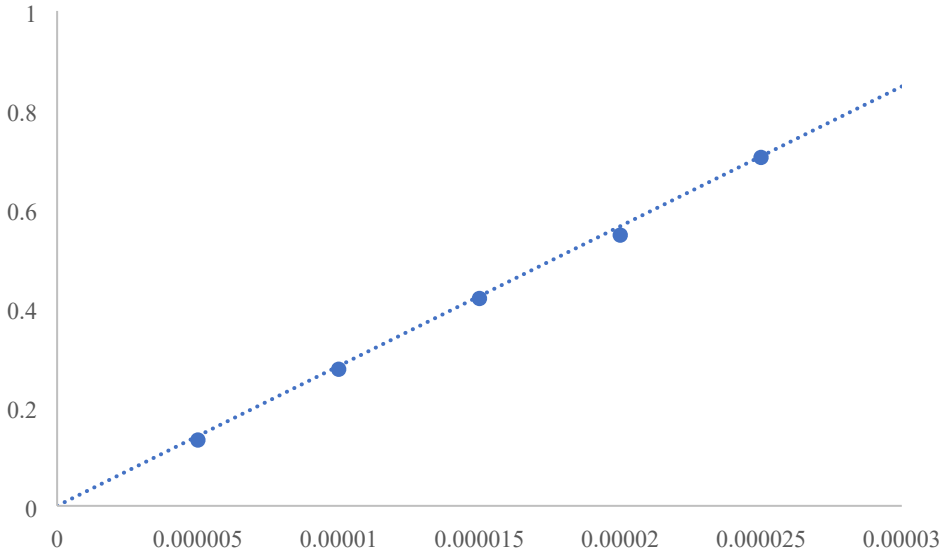


Figure S42. Calibration line (Lambert-Beer law) obtained for pure compound **2** at λ = 342 nm.

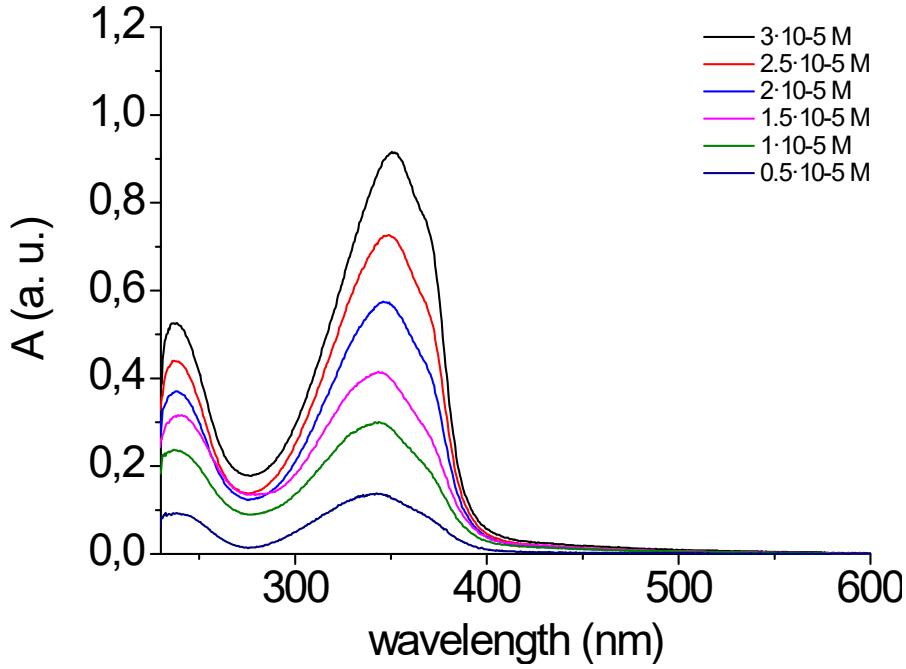


Figure S43. Spectra recorded for solutions of pure compound **3** of different concentrations in a 1:1 chloroform:methanol mixture.

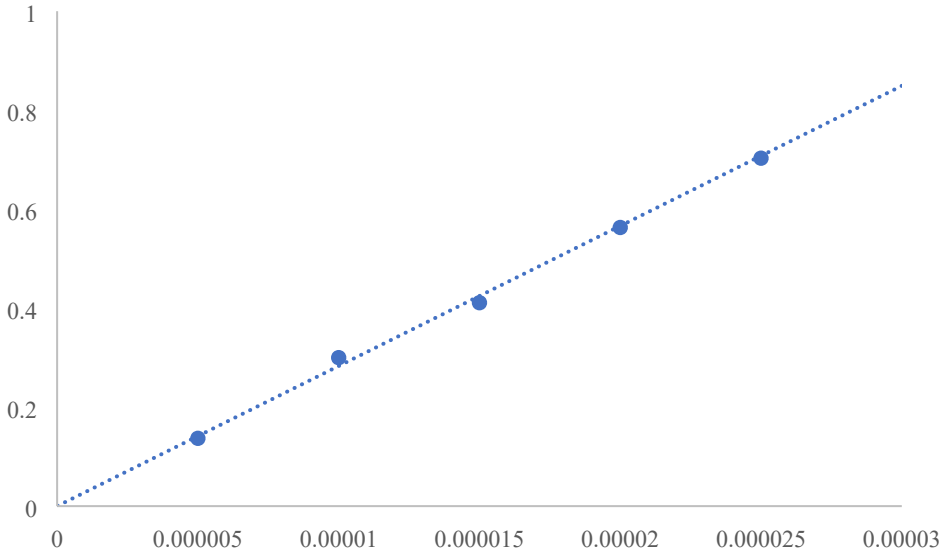


Figure S44. Calibration line (Lambert-Beer law) obtained for pure compound **3** at λ = 342 nm.

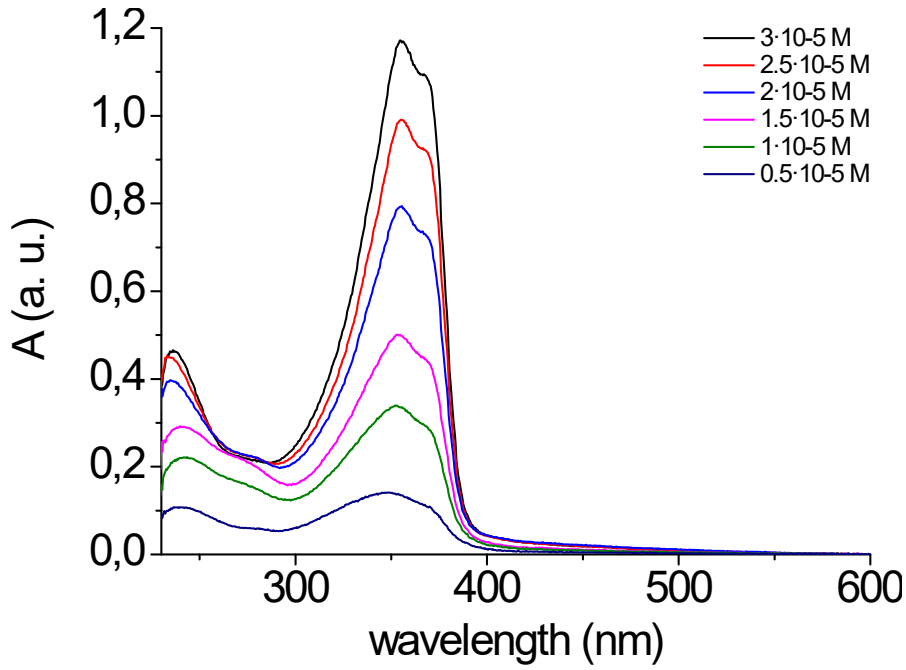


Figure S45. Spectra recorded for solutions of pure compound **4** of different concentrations in a 1:1 chloroform:methanol mixture.

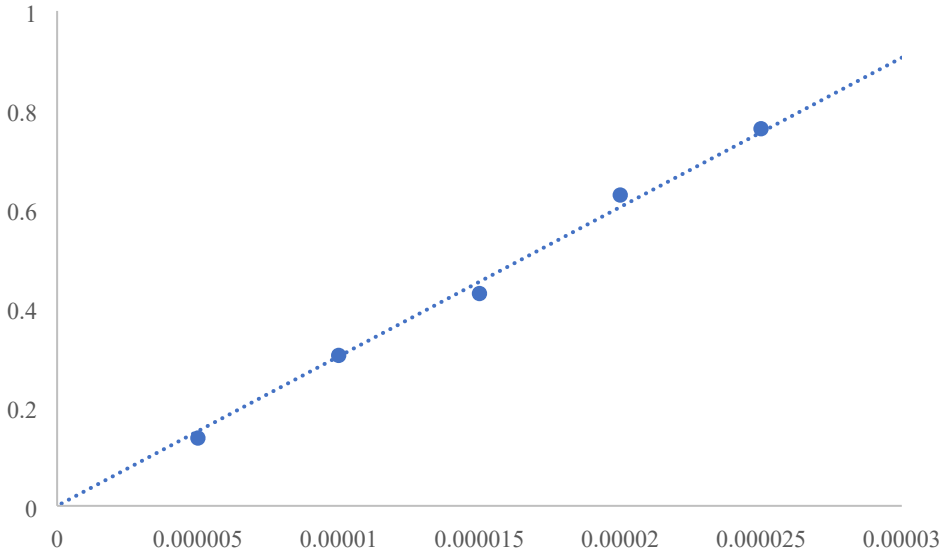


Figure S46. Calibration line (Lambert-Beer law) obtained for pure compound **4** at λ = 342 nm.

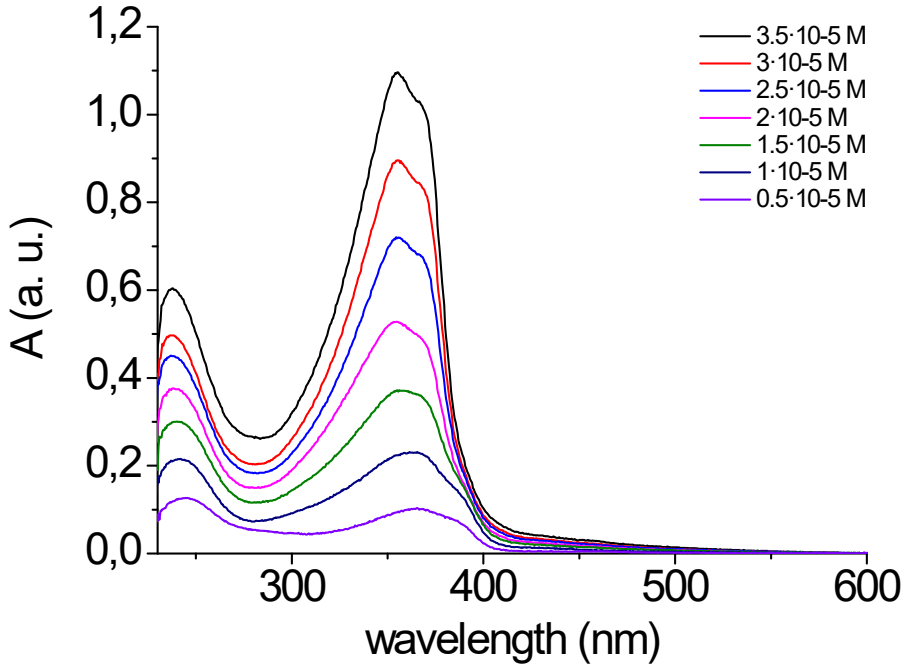


Figure S47. Spectra recorded for solutions of pure compound **5** of different concentrations in a 1:1 chloroform:methanol mixture.

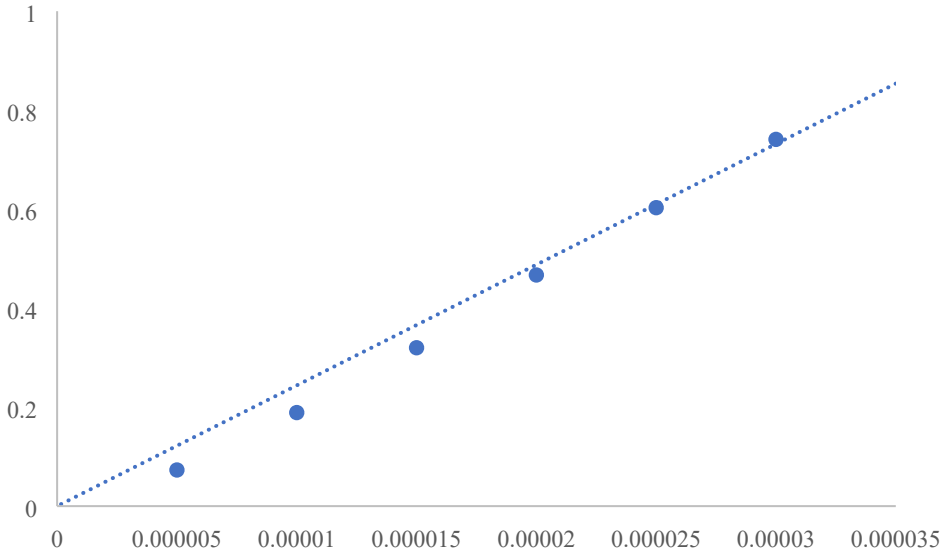


Figure S48. Calibration line (Lambert-Beer law) obtained for pure compound **5** at λ = 342 nm.

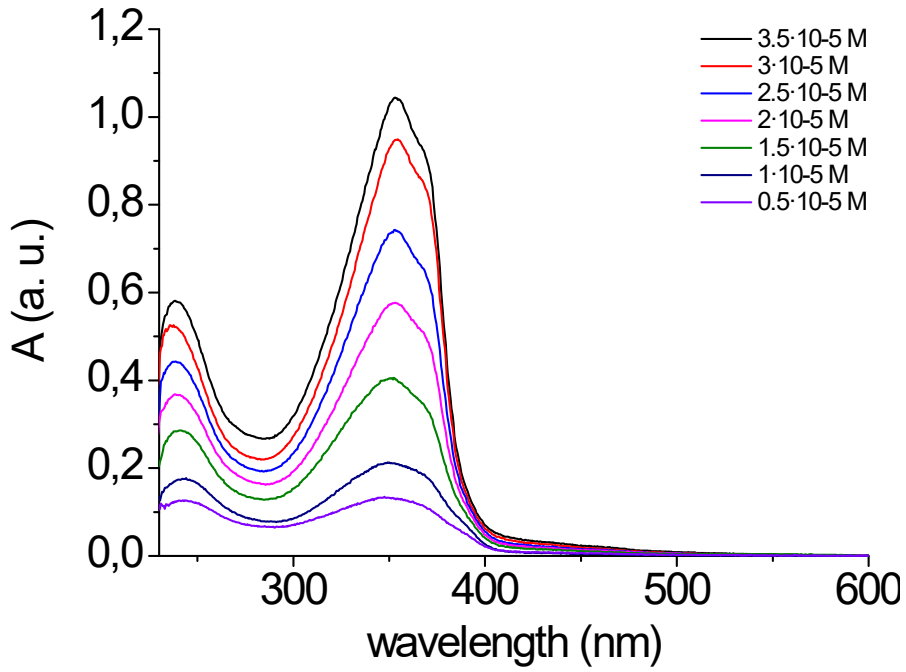


Figure S49. Spectra recorded for solutions of pure compound **6** of different concentrations in a 1:1 chloroform:methanol mixture.

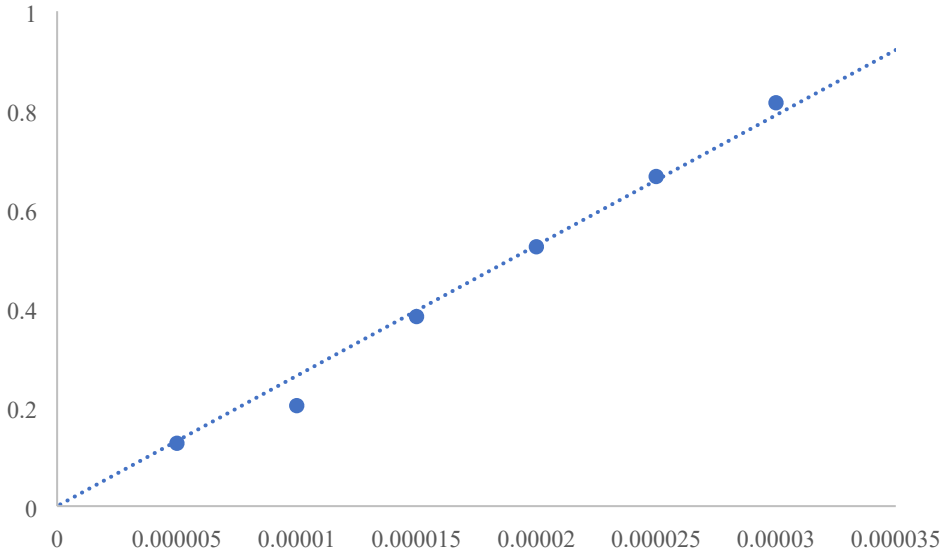


Figure S50. Calibration line (Lambert-Beer law) obtained for pure compound **6** at λ = 342 nm.

6.2. Concentration of the compounds in the formulations

To determine the concentration of the studied compounds in the samples, firstly aliquots of the six formulations (15.0 mg of each) were taken and fully dissolved in a 1:1 chloroform:methanol mixture, employing 10-mL volumetric flasks. Secondly, the spectrum of each solution was recorded at 293 K in an Hitachi U-3900 spectrophotometer. This procedure was performed thrice in order to check the homogeneity of the samples; in the case of the blank, consisting of nanoparticles containing no compound, the protocol was similar. Once the spectra were recorded, the mean spectrum of the compound in each formulation was calculated (**Figure S51**). The spectrum of the blank solution does not show any bands in the 300-400 nm region, where the click-tambjamines strongly absorb.

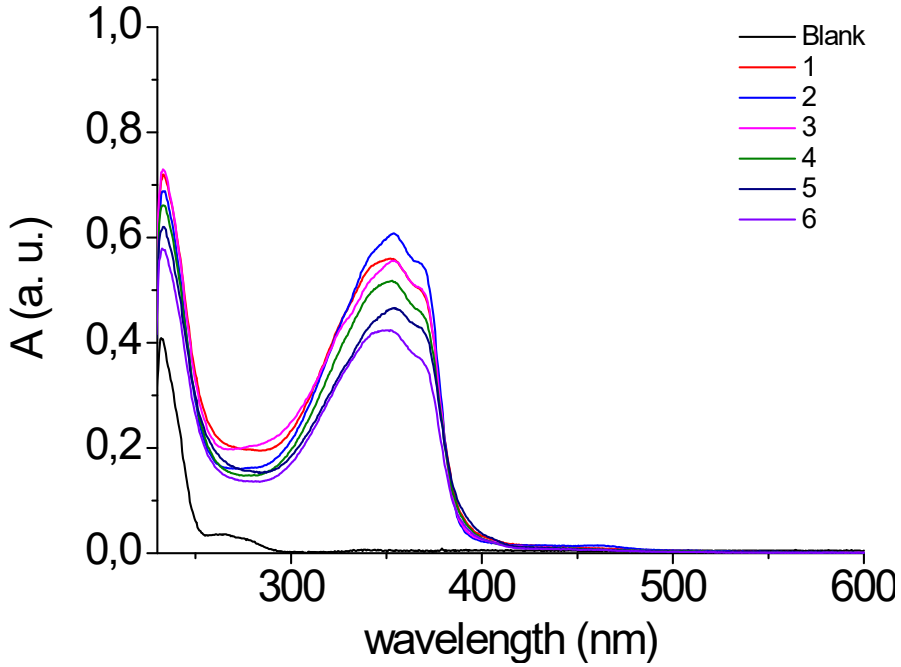


Figure S51. Mean spectra obtained for solutions of the six formulations (1:1 chloroform:methanol mixture) and the blank. Each spectrum corresponds to the average of three spectra recorded from different aliquots of each sample.

Taking into consideration the equations of the calibration straight lines obtained previously (**Table S3**) and the absorbance of the compound contained in each formulation, it is possible to determine the concentration of the compounds in the samples. For instance, in the case of compound **1**:

The equation of the calibration straight line is:

$$A = 29752 \cdot [1]$$

The mean absorbance at λ = 342 nm is A = 0.546. So, replacing in this equation:

$$0.546 = 29752 \cdot [1]$$

$$[1] = 1.83 \cdot 10^{-5}\ M$$

Since all the aliquots taken from the sample (15.0 mg) were dissolved in 10 mL of a 1:1 chloroform:methanol mixture:

$$\frac{1.83 \cdot 10^{-5}\ mol\ compound\ 1}{1\ L\ solution} \cdot \frac{444.01\ g\ compound\ 1}{1\ mol\ compound\ 1} \cdot \frac{10^{6}\ ug\ compound\ 1}{1\ g\ compound\ 1} \cdot \frac{1\ L\ solution}{10^{3}\ mL\ solution} \cdot \frac{10\ mL\ solution}{15.0\ mg\ formulation} =$$

$$\frac{5.43\ ug\ compound\ 1}{mg\ formulation}$$

Given that the mass of compound employed to prepare each formulation is known, and therefore the theoretical concentration of the click-tambjamines in the samples, it is possible to calculate the encapsulation efficacy. Following with the example of compound **1**, its theoretical concentration is 6.36 µg/mg. So:

$$\%\ Encapsulation\ efficacy = \frac{Experimental\ concentration}{Theoretical\ concentration} \cdot 100 = \frac{5.43\ ug/mg}{6.36\ ug/mg} \cdot 100 = 85\%$$

If the same procedure is followed for the rest of compounds, **Table S4** is generated:

Table S4. Experimental and theoretical concentrations of the compounds in the formulations and encapsulation efficacies.

Compound	Experimental concentration (µg/mg)	Theoretical concentration (µg/mg)	Encapsulation efficacy (%)
1	5.43	6.36	85
2	6.33	6.92	91
3	6.13	6.67	92
4	5.74	6.33	91
5	6.63	6.92	96
6	6.20	6.57	94

7. TRANSMEMBRANE ANION TRANSPORT EXPERIMENTS IN VESICLES

7.1. Preparation of phospholipid vesicles

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocoline (POPC) (20 mg/mL) (Sigma Aldrich) (or a 7:3 POPC:cholesterol mixture, in the case of HPTS-based assays) was evaporated *in vacuo* using a rotary evaporator and the resulting film was dried under high vacuum for, at least, 2 h. Different aqueous solutions were used to rehydrate the lipid film: (a) ISE assays: 489 mM NaCl, 5 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/NO_3^- exchange experiments, or 451 mM NaCl, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/HCO_3^- exchange experiments; (b) Carboxyfluorescein-based assays: 451 mM NaCl, 20 mM NaH_2PO_4, 50 mM CF, I.S. 500 mM, pH 7.2; (c) HPTS-based assays: 126.2 mM $NaNO_3$, 10 mM NaH_2PO_4, 1 mM HPTS, I.S. 150 mM, pH 7.2. The resulting suspension was vortexed and subjected to nine freeze-thaw cycles; subsequently, it was extruded twenty-nine times through a polycarbonate membrane (200 nm) employing a LiposoFast basic extruder (Avestin, Inc.). The resulting unilamellar vesicles were: (a) ISE assays: dialysed against a $NaNO_3$ aqueous solution (489 mM $NaNO_3$, 5 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/NO_3^- exchange experiments) or a Na_2SO_4 aqueous solution (150 mM Na_2SO_4, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/HCO_3^- exchange experiments) to remove the unencapsulated chloride; (b) Carboxyfluorescein-based assays: subjected to size-exclusion chromatography, using Sephadex G-25 as the stationary phase and a Na_2SO_4 aqueous solution (150 mM Na_2SO_4, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2) as the mobile phase, to remove the unencapsulated carboxyfluorescein; (c) HPTS-based assays: subjected to size-exclusion chromatography, using Sephadex G-25 as the stationary phase and the inner solution without HPTS (126.2 mM $NaNO_3$, 10 mM NaH_2PO_4, I.S. 150 mM, pH 7.2) as the mobile phase, to remove the unencapsulated HPTS. Vesicles were collected in a 10-mL volumetric flask, using either the external solution (ISE and carboxyfluorescein-based assays) or the inner one without the probe (HPTS-based assays) to bring the suspension to the desired volume.

7.2. ISE transport experiments

Unilamellar vesicles (mean diameter: 200 nm) made of POPC and containing a NaCl aqueous solution (489 mM NaCl, 5 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/NO_3^- exchange experiments, or 451 mM NaCl, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/HCO_3^- exchange experiments) were dispersed in a $NaNO_3$ aqueous solution (489 mM $NaNO_3$, 5 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/NO_3^- exchange experiments) or a Na_2SO_4 aqueous solution (150

mM Na_2SO_4, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for Cl^-/HCO_3^- exchange experiments). A certain volume of a solution of the free compound in DMSO or of a suspension of the formulation in the external solution was added at t = 0 s, and the chloride released monitored for 300 s with a chloride-selective electrode (HACH 9652C). At t = 300 s a surfactant (Triton-X, 20% dispersion in water, 20 µL) was added to lyse the vesicles and release all the encapsulated chloride. This value was considered as 100% release and used as such. Regarding the Cl^-/HCO_3^- exchange assays, a 500 mM $NaHCO_3$ aqueous solution prepared with the Na_2SO_4 one (150 mM Na_2SO_4, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2) was added at t = -10 s to the vesicles suspension, the HCO_3^- concentration during the experiment being 40 mM. The rest of the procedure is similar to that described before. In all cases the final lipid concentration during the assays was 0.5 mM and the final volume 5 mL.

Concerning the solutions of the free compounds, different stock solutions were prepared, depending on their solubility in DMSO (10 mM for **1**, **2** and **3**; 2 mM for **4** and **5** and 1 mM for **6**). From these solutions or the corresponding dilutions less than 40 µL were taken to avoid the influence of the organic solvent during the experiments. In the case of the suspensions of the formulations, some calculations were performed to determine the volume of stock suspension that was required to get a certain concentration of the compound during the experiment. The concentration of each compound in the formulations, determined by means of UV-Vis spectrophotometry (*Section 6.2*), was used to carry out these calculations. In all cases 15.0 mg of each formulation were suspended in 5 mL of the external solution (489 mM $NaNO_3$, 5 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for the Cl^-/NO_3^- exchange assays, or 150 mM Na_2SO_4, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2, for the Cl^-/HCO_3^- exchange assays). In order to ensure the homogeneity of the suspensions these were sonicated and vortexed alternatily. At this stage the suspensions were ready to be used.

Given that the concentration of the compounds in the formulations is known (see *Section 6.2*), as it is the amount of sample taken (15.0 mg) and the volume of the external solution employed to prepare the suspensions (5 mL), it is possible to determine their concentration in such suspensions. For instance, in the case of compound **1**:

$$\frac{5.43\ ug\ compound\ 1}{1\ mg\ formulation}\cdot\frac{15.0\ mg\ formulation}{5\ mL\ suspension}\cdot\frac{1\ g\ compound\ 1}{10^6\ ug\ compound\ 1}\cdot\frac{1\ mol\ compound\ 1}{444.01\ g\ compound\ 1}\cdot\frac{10^3\ mL\ suspension}{1\ L\ suspension} =$$

$$3.67\cdot10^{-5}\ \text{M compound } \mathbf{1} = 0.0367\ \text{mM compound } \mathbf{1}$$

Since the final volume of the experiments is also known (5 mL), the volume of stock suspension that has to be taken in order to obtain different concentrations of the compounds during the experiments can be calculated. Following with the example of compound **1**, if a 1% mol carrier to lipid concentration (5 µM, $5{\cdot}10^{-3}$ mM) is to be obtained:

$$0.0367\ mM{\cdot}x\ mL = 5{\cdot}10^{-3}\ mM{\cdot}5\ mL$$

$$x = 0.681\ mL = 681\ uL$$

In order to run ISE experiments 3 mL of a 20 mg/mL solution of POPC in chloroform were employed:

$$3\ mL\ POPC\ solution{\cdot}\frac{20\ mg\ POPC}{1\ mL\ POPC\ solution}{\cdot}\frac{1\ g\ POPC}{10^3\ mg\ POPC}{\cdot}\frac{1\ mol\ POPC}{760\ g\ POPC} = 7.89{\cdot}10^{-5}\ mol\ POPC$$

Since the volume of the POPC stock solution is 10 mL, the POPC concentration in this solution would be:

$$[POPC]_{stock} = \frac{7.89{\cdot}10^{-5}\ mol\ POPC}{0.01\ L\ solution} = 7.89{\cdot}10^{-3}\ M\ POPC = 7.89\ mM\ POPC$$

As commented above, the POPC concentration during the experiments is 0.5 mM. So, the volume of stock solution that has to be taken to fulfil these conditions would be:

$$7.89\ mM{\cdot}x\ mL = 0.5\ mM{\cdot}5\ mL$$

$x = 0.317\ mL = 317\ uL$

Considering the final volume of the experiments (5 mL), the volume taken from the POPC stock solution and the different volumes taken from the stock suspension of the formulations, a table can be generated (for Cl^-/HCO_3^- exchange assays, the volume of the $NaHCO_3$ solution that has to be added, 400 µL, needs to be considered too). Since for certain compounds (especially the most active ones) some concentrations are quite low, dilutions of the stock suspension had to be prepared. Following with **1**:

[1]	**V external solution (µL)**	**V POPC stock solution (µL)**	**V formulation suspension (µL)**
1% (5 µM)	4002	317	681
0.1% (0.5 µM)	4615	317	68.1
0.03% (0.15 µM)	4479	317	204
0.02% (0.1 µM)	4547	317	136
0.01% (0.05 µM)	4615	317	68.1
0.005% (0.025 µM)	4342	317	341

In this case, for the first two experiments (5 and 0.5 µM of compound **1**) the stock suspension (0.0367 mM) was employed; for the following three (0.15, 0.1 and 0.05 µM of

compound **1**) a ten times more diluted suspension (0.00367 mM) was prepared and used; for the last one (0.025 μM of compound **1**), a hundred times more diluted suspension (0.000367 mM) was employed. The homogeneity of the diluted suspensions was ensured by sonicating and vortexing once. The stock suspensions were used up to three days after their preparation and stored in the fridge, but the diluted ones were prepared on a daily basis.

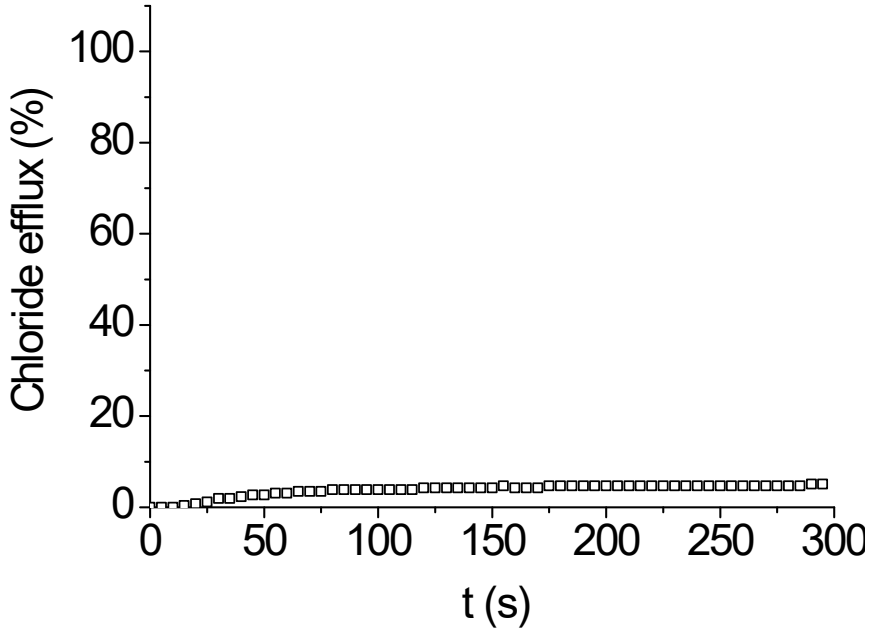


Figure S52. Chloride efflux promoted by the blank (lipid nanoparticles containing no compound) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. The trace represents the average of at least three trials, performed with at least three batches of vesicles.

The blank consisted of 15.0 mg of lipid nanoparticles containing no compound suspended in 5 mL of the corresponding external solution. This suspension was prepared as described for the formulations (*vide supra*) and, as these, used up to three days after its preparation and kept in the fridge. Different volumes of this suspension were tried, and in all cases the chloride efflux was lower than 5%. As an example, this is shown in **Figure S52** for experiments involving the Cl^-/NO_3^- exchange.

Considering the data shown in this section, as those displayed in *Section 5.1*, it is possible to determine the total mass of lipids in a typical ISE experiment. Firstly, the mass of POPC in 317 μL of the vesicles stock solution would be:

$$317\ \mu L\ POPC\ solution \cdot \frac{1\ L\ POPC\ solution}{10^6\ \mu L\ POPC\ solution} \cdot \frac{7.89 \cdot 10^{-3}\ mol\ POPC}{1\ L\ POPC\ solution} \cdot \frac{760\ g\ POPC}{1\ mol\ POPC} \cdot \frac{10^3\ mg\ POPC}{1\ g\ POPC} = 1.90\ mg\ POPC$$

The proportion of lipids in the assayed formulations, excluding the carrier, amounts to 70.5% (1100 mg –1000 mg of Precirol and 100 mg of mygliol– in 1560 mg of formulation; see *Section 5.1*). So, the mass of lipids in 15.0 mg of formulation would be:

$$15.0\ mg\ formulation \cdot 0.705 = 10.5769\ mg\ lipids$$

To get 1% (5 µM) concentration of compound **1** in an ISE assay, 681 µL of the stock suspension of the formulation (total volume = 5 mL) had to be taken. So:

$$0.681\ mL\ suspension \cdot \frac{10.5769\ mg\ lipids}{5\ mL\ suspension} = 1.44\ mg\ lipids$$

Therefore, the total mass of lipids during an ISE assay performed in these conditions would be:

$$Total\ mass\ of\ lipids = 1.90\ mg\ POPC + 1.44\ mg\ lipids\ (NLCs) = 3.34\ mg\ lipids$$

So, 56.9% of the lipids come from POPC and 43.1% from NLCs.

A similar calculation can be performed for a less active transporter; for instance, compound **6**. To get 1% (5 µM) concentration of this compound in the experiment, 785 µL of the stock suspension of the formulation (total volume = 5 mL) had to be taken. So:

$$0.785\ mL\ suspension \cdot \frac{10.5769\ mg\ lipids}{5\ mL\ suspension} = 1.66\ mg\ lipids$$

Consequently, the total mass of lipids during an ISE assay performed in these conditions would be:

$$Total\ mass\ of\ lipids = 1.90\ mg\ POPC + 1.66\ mg\ lipids\ (NLCs) = 3.56\ mg\ lipids$$

In this case, 53.4% of the lipids come from POPC and 46.6% from NLCs.

Table S5. Transport activities (Cl^-/NO_3^- exchange) expressed as EC_{50} (nM) and Hill parameter (*n*) for the studied compounds, both free and formulated, and their calculated lipophilicities.

	Free		Formulated		Lipophilicity
Compound	**EC_{50}**	***n***	**EC_{50}**	***n***	**logP** [e]
1	25 ± 2	1.06 ± 0.08	87 ± 4	1.24 ± 0.08	5.57
2	29 ± 3	1.0 ± 0.1	74 ± 4	0.93 ± 0.05	6.56
3	71 ± 5	0.60 ± 0.02	85 ± 7	1.02 ± 0.07	7.18
4	2795 ± 68	0.96 ± 0.03	183 ± 12	0.65 ± 0.03	7.99
5	– [a]	– [a]	– [b]	– [b]	8.81
6	– [c]	– [c]	– [d]	– [d]	9.60

[a] These values could not be determined reliably, since 50% chloride efflux was not reached, not even at the highest concentration tried (15 µM). Therefore, in this case EC_{50} > 15 µM.

[b] These values could not be determined reliably. However, at the highest concentration tried (5 µM), chloride efflux is close to 50%, so $EC_{50} \approx 5$ µM.

[c] These values could not be determined reliably. When [**6**] = 5 μM, chloride efflux is 31%. Higher concentrations were not tried given the low solubility of the compound in DMSO.

[d] These values could not be determined realiably, since 50% chloride efflux was not reached, not even at the highest concentration tried (10 μM).

[e] Average logP determined for the deprotonated form of the compound through Virtual Computational Chemistry Laboratory (VCCLAB).

Table S6. Transport activities (Cl^-/HCO_3^- exchange) expressed as EC_{50} (nM) and Hill parameter (*n*) for the studied compounds, both free and formulated, and their calculated lipophilicities.

	Free		Formulated		Lipophilicity
Compound	**EC_{50}**	***n***	**EC_{50}**	***n***	**logP** [d]
1	201 ± 26	1.1 ± 0.2	640 ± 72	1.0 ± 0.1	5.57
2	155 ± 16	0.84 ± 0.07	706 ± 78	0.9 ± 0.1	6.56
3	8420 ± 536	0.49 ± 0.03	1041 ± 23	0.75 ± 0.01	7.18
4	– [a]	– [a]	– [b]	– [b]	7.99
5	– [c]	– [c]	– [c]	– [c]	8.81
6	– [c]	– [c]	– [c]	– [c]	9.60

[a] These values could not be determined reliably, since 50% chloride efflux was not reached, not even at the highest concentration tried (15 μM). Therefore, in this case EC_{50} > 15 μM.

[b] These values could not be determined reliably, since 50% chloride efflux was not reached, not even at the highest concentration tried (10 μM). Therefore, in this case EC_{50} > 10 μM.

[c] These values could not be determined reliably, since 50% chloride efflux was not reached.

[d] Average logP determined for the deprotonated form of the compound through Virtual Computational Chemistry Laboratory (VCCLAB).

Cl^-/NO_3^- exchange assays

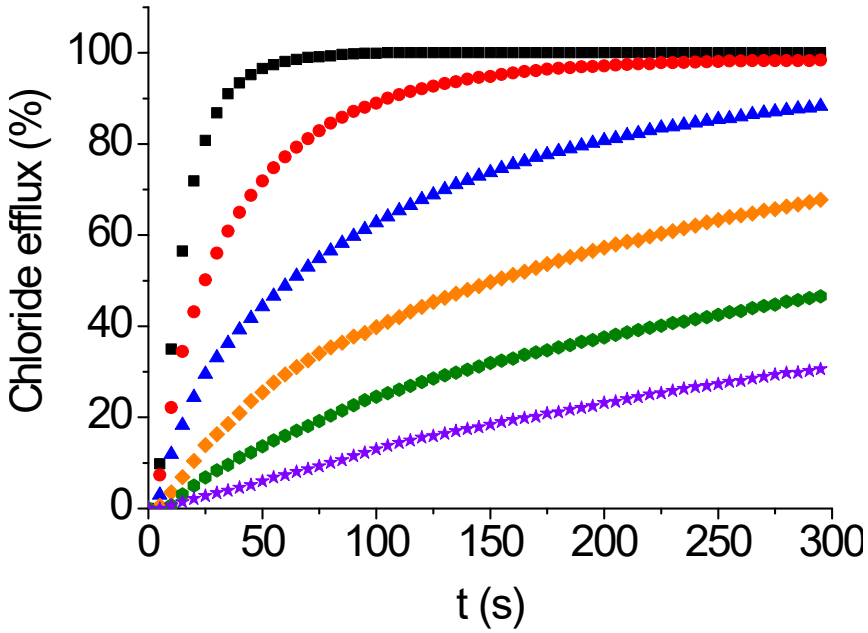


Figure S53. Chloride efflux promoted by free **1** (5 μM, black; 0.5 μM, red; 0.15 μM, blue; 0.05 μM, orange; 0.025 μM, green; 0.01 μM, purple) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

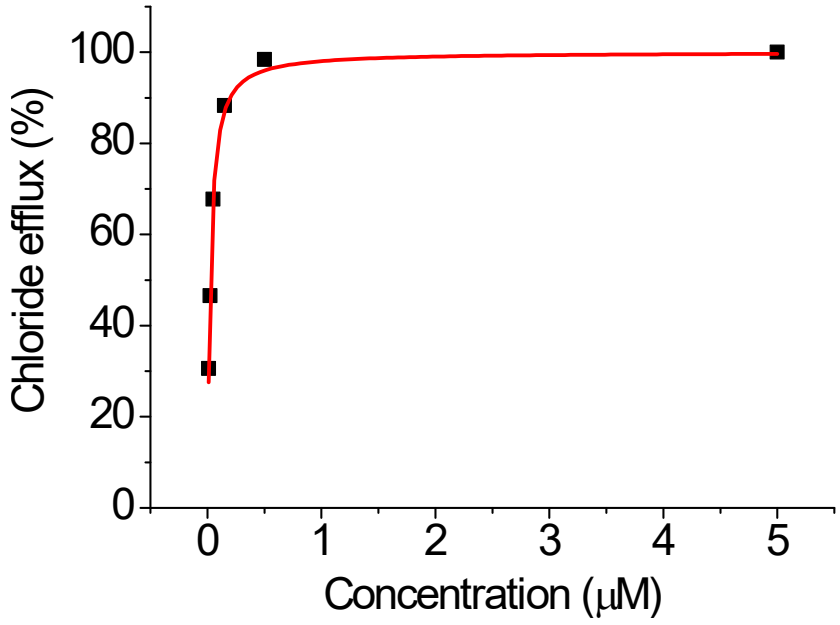


Figure S54. Hill analysis for free **1**.

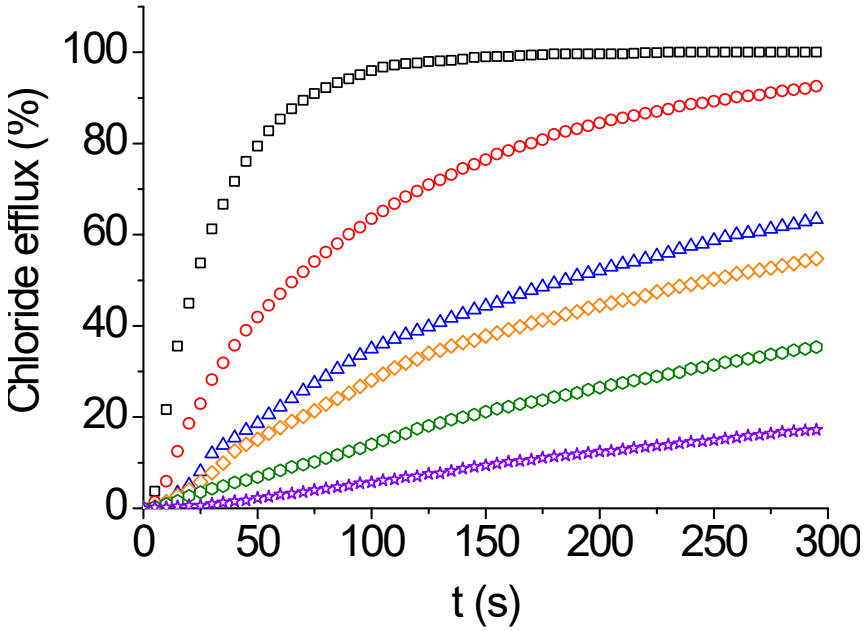


Figure S55. Chloride efflux promoted by encapsulated **1** (5 μM, black; 0.5 μM, red; 0.15 μM, blue; 0.1 μM, orange; 0.05 μM, green; 0.025 μM, purple) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

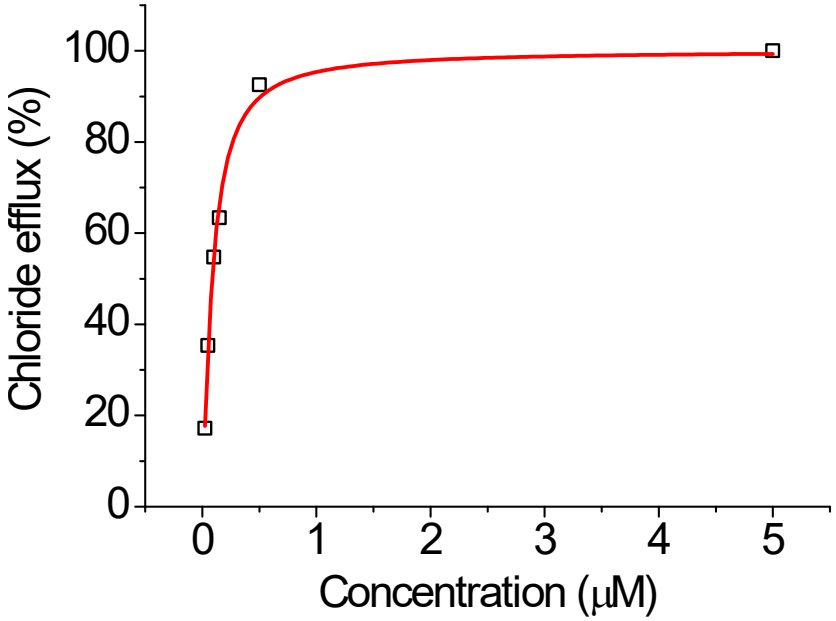


Figure S56. Hill analysis for encapsulated **1**.

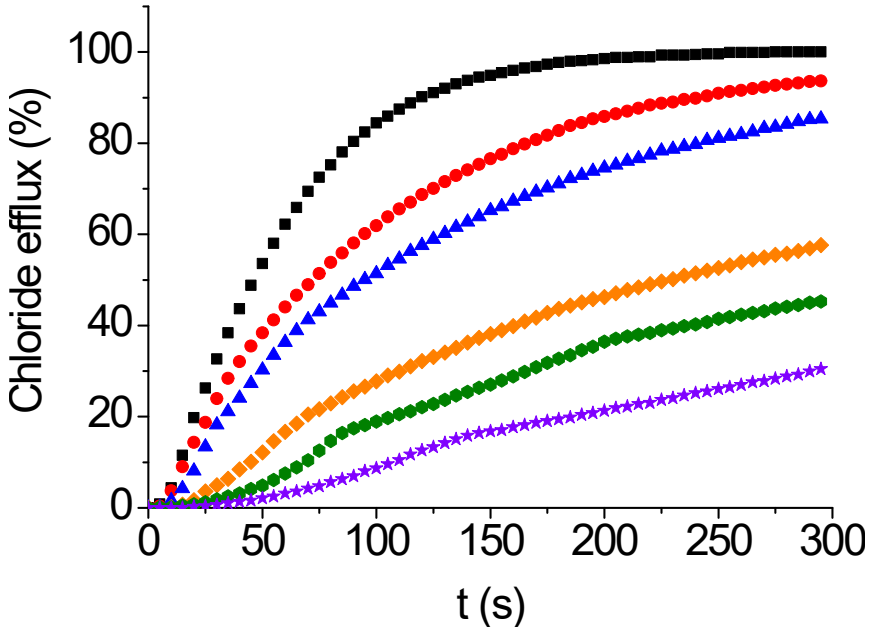


Figure S57. Chloride efflux promoted by free **2** (5 μM, black; 0.25 μM, red; 0.15 μM, blue; 0.05 μM, orange; 0.025 μM, green; 0.01 μM, purple) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

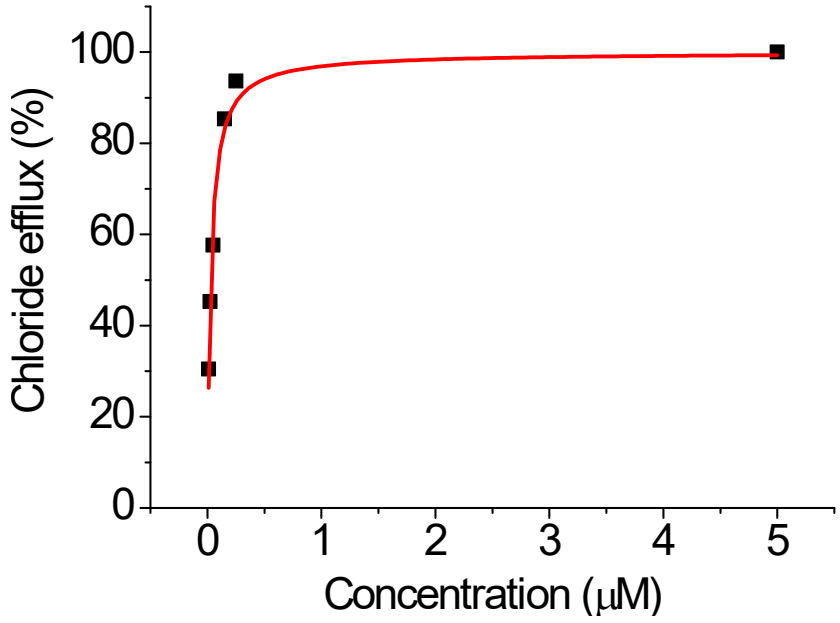


Figure S58. Hill analysis for free **2**.

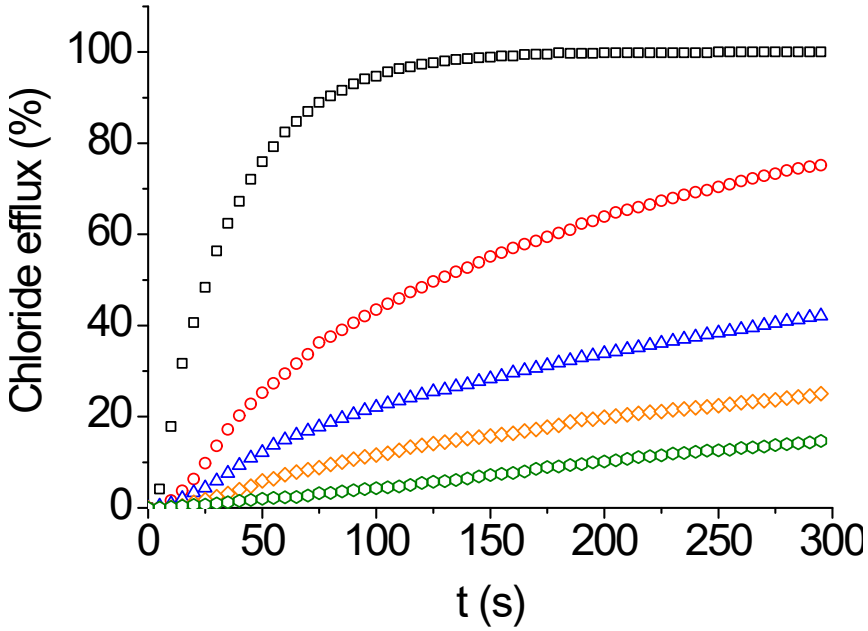


Figure S59. Chloride efflux promoted by encapsulated **2** (5 μM, black; 0.25 μM, red; 0.05 μM, blue; 0.025 μM, orange; 0.01 μM, green) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

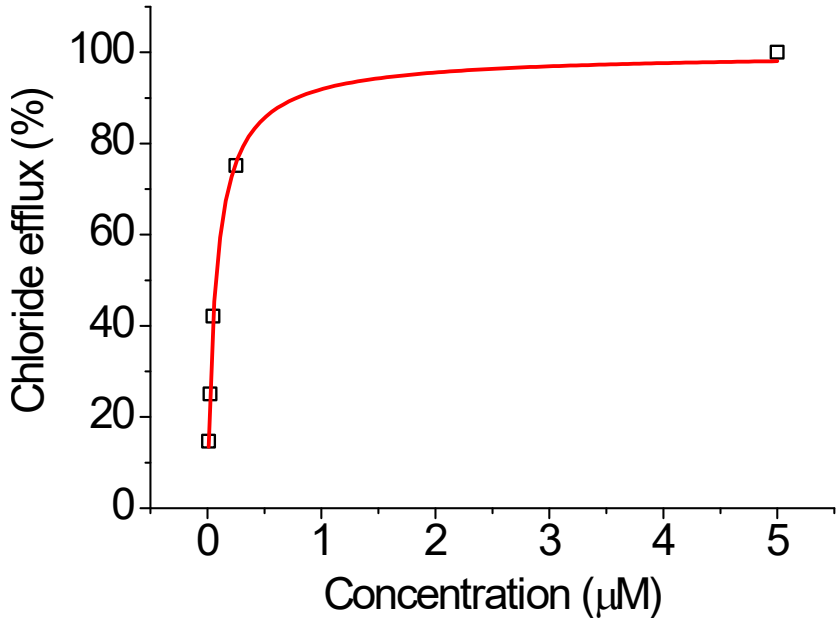


Figure S60. Hill analysis for encapsulated **2**.

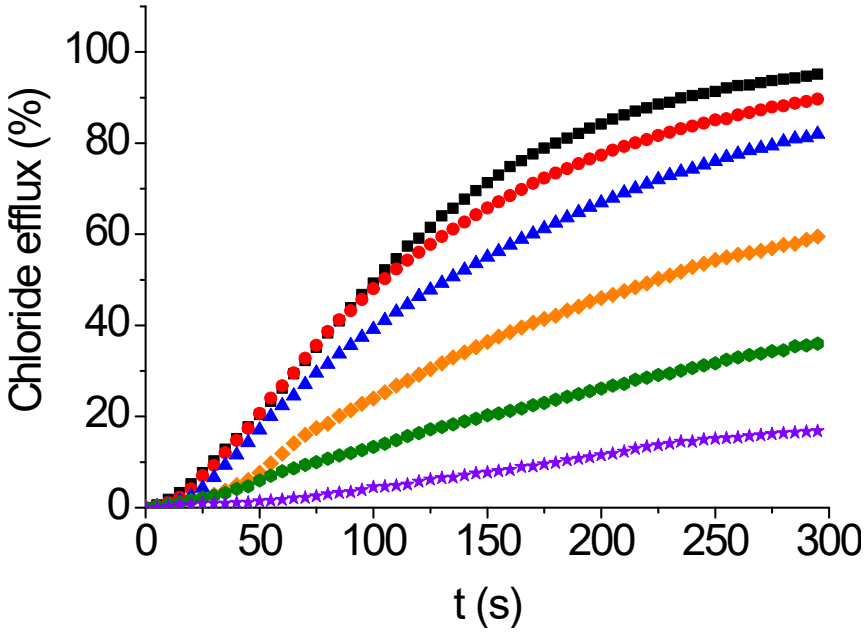


Figure S61. Chloride efflux promoted by free **3** (5 μM, black; 2.5 μM, red; 1 μM, blue; 0.15 μM, orange; 0.025 μM, green; 0.005 μM, purple) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

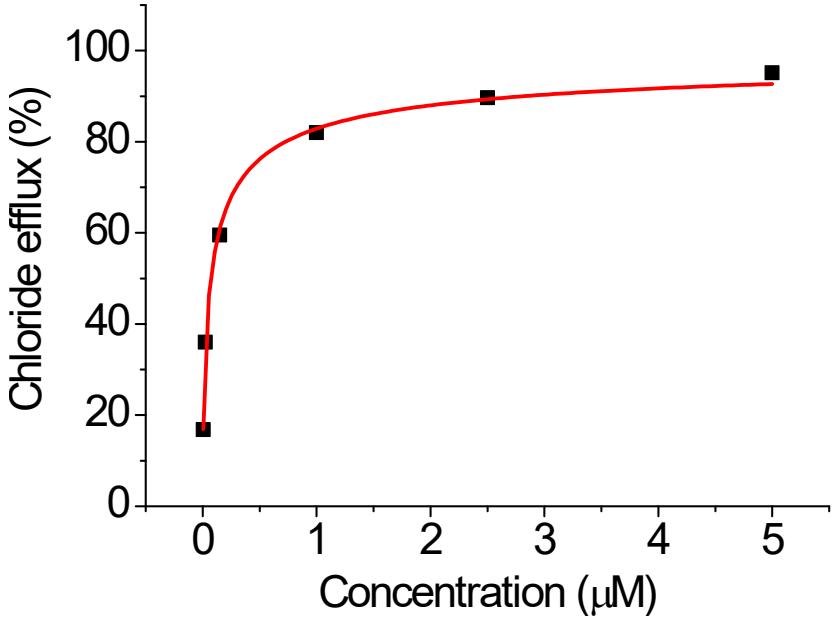


Figure S62. Hill analysis for free **3**.

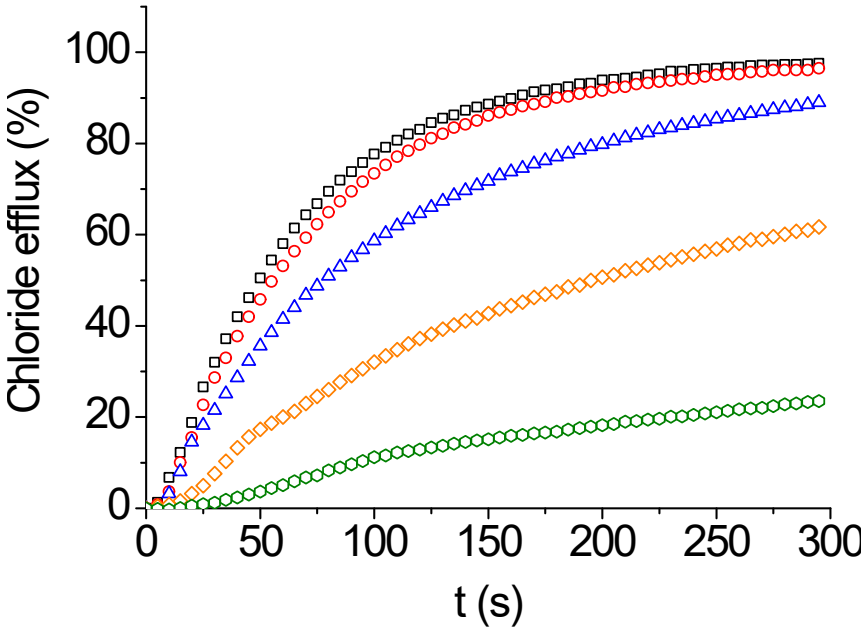


Figure S63. Chloride efflux promoted by encapsulated **3** (5 μM, black; 2.5 μM, red; 0.5 μM, blue; 0.15 μM, orange; 0.025 μM, green) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

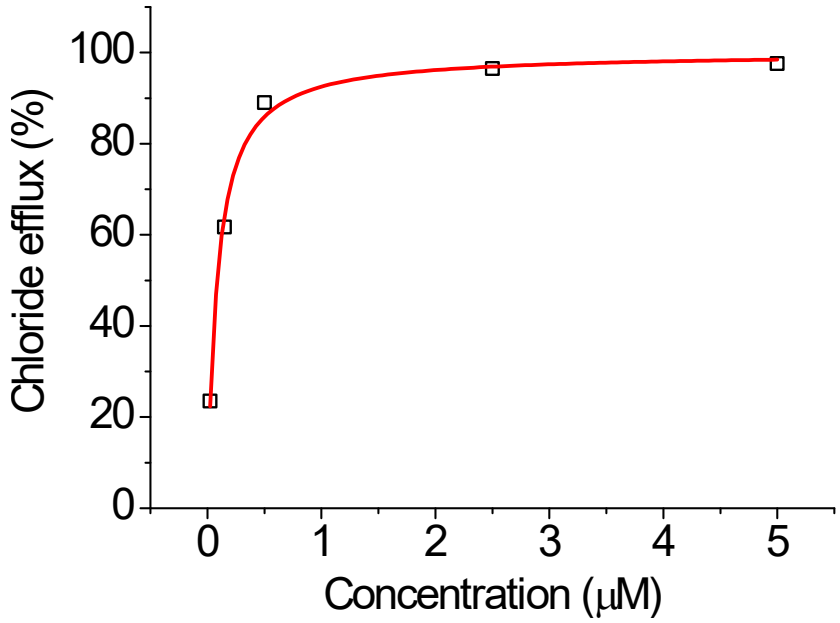


Figure S64. Hill analysis for encapsulated **3**.

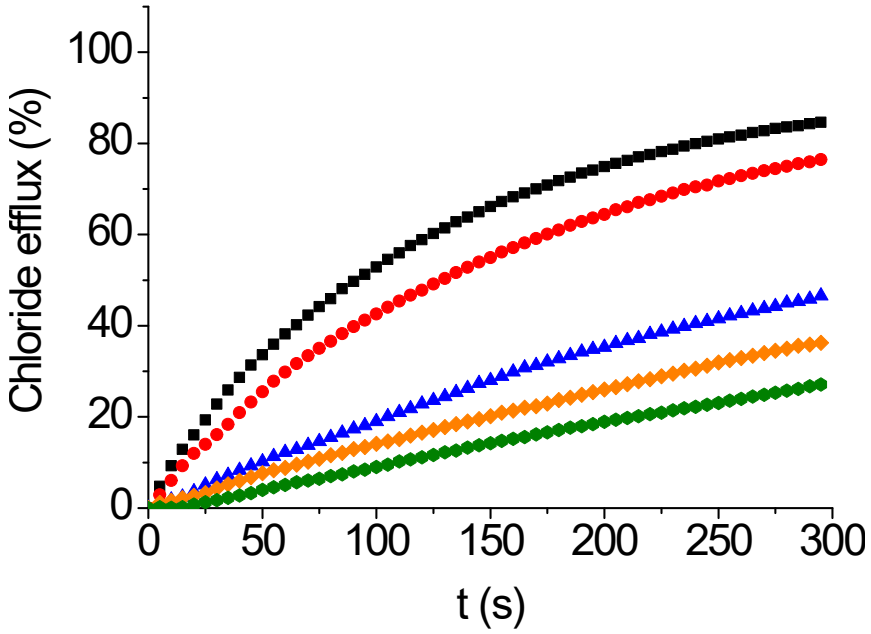


Figure S65. Chloride efflux promoted by free **4** (15 μM, black; 10 μM, red; 2.5 μM, blue; 1.5 μM, orange; 1 μM, green) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

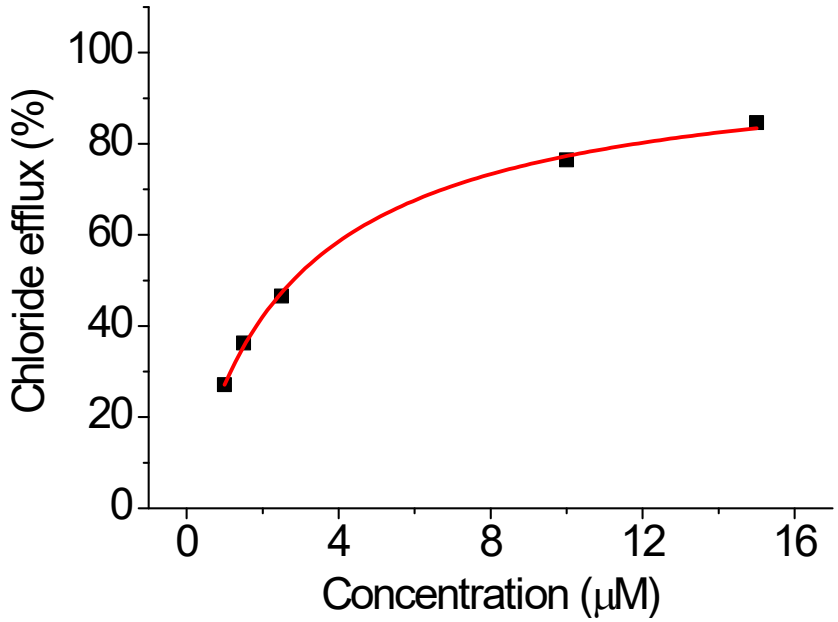


Figure S66. Hill analysis for free **4**.

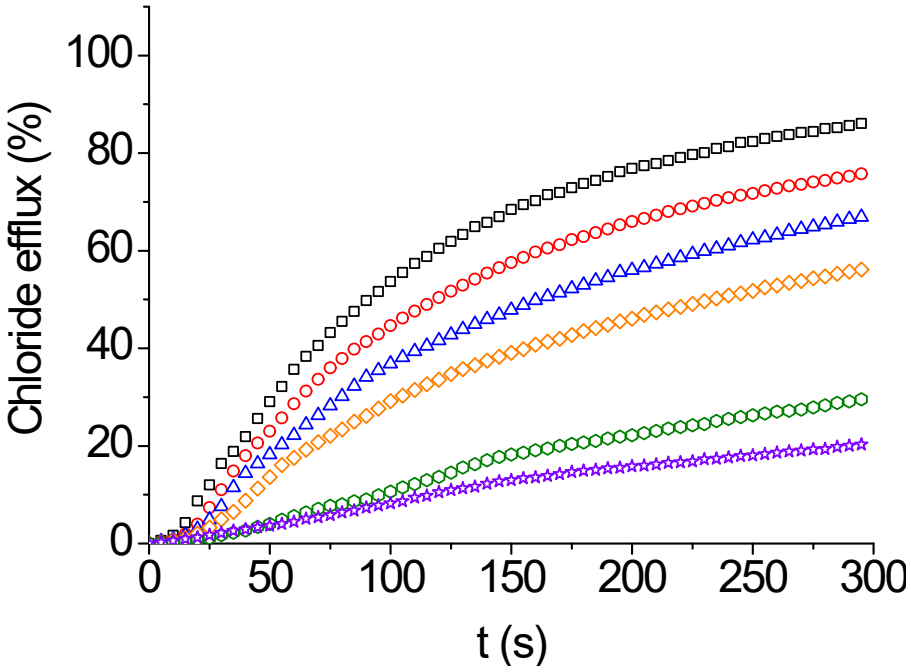


Figure S67. Chloride efflux promoted by encapsulated **4** (5 μM, black; 1 μM, red; 0.5 μM, blue; 0.25 μM, orange; 0.05 μM, green; 0.025 μM, purple) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

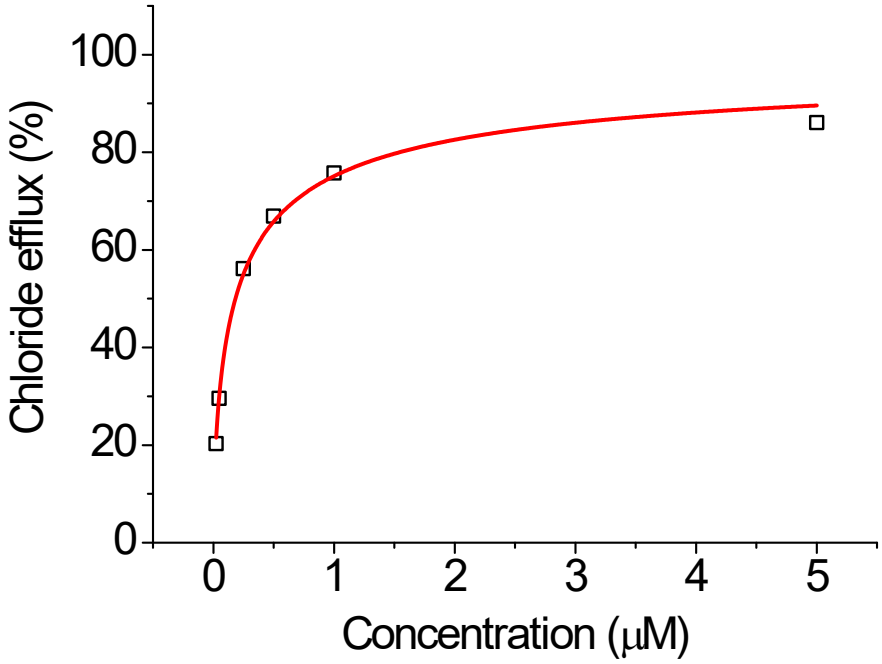


Figure S68. Hill analysis for encapsulated **4**.

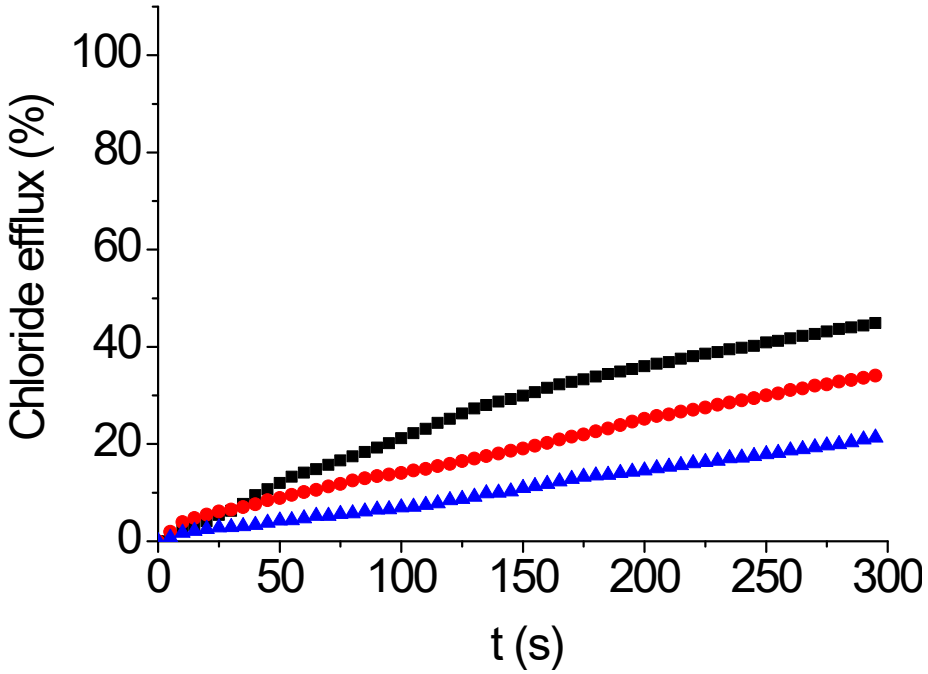


Figure S69. Chloride efflux promoted by free **5** (15 µM, black; 10 µM, red; 5 µM, blue) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

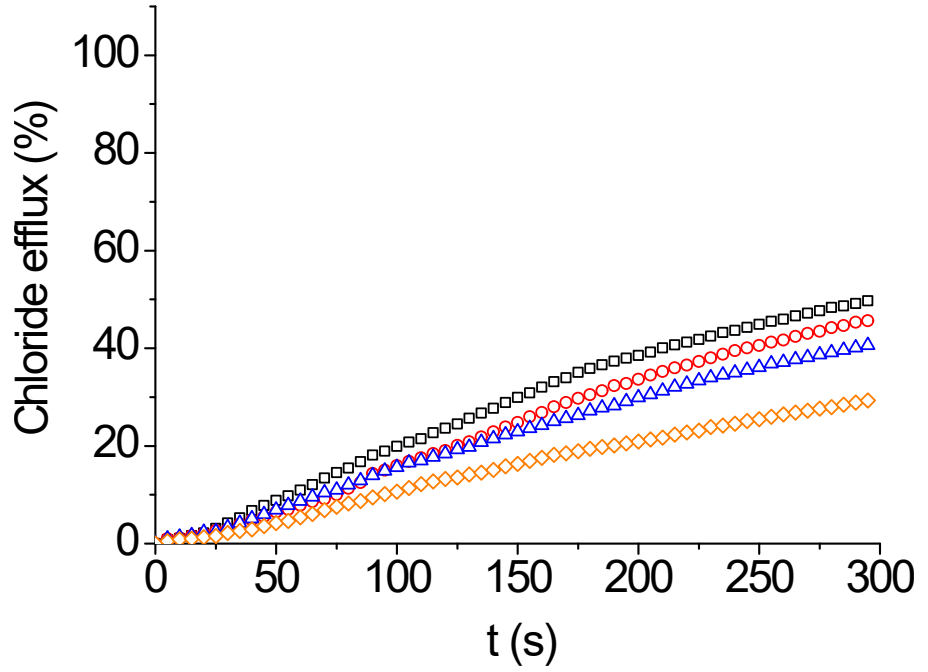


Figure S70. Chloride efflux promoted by encapsulated **5** (5 µM, black; 2.5 µM, red; 1 µM, blue; 0.25 µM, orange) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

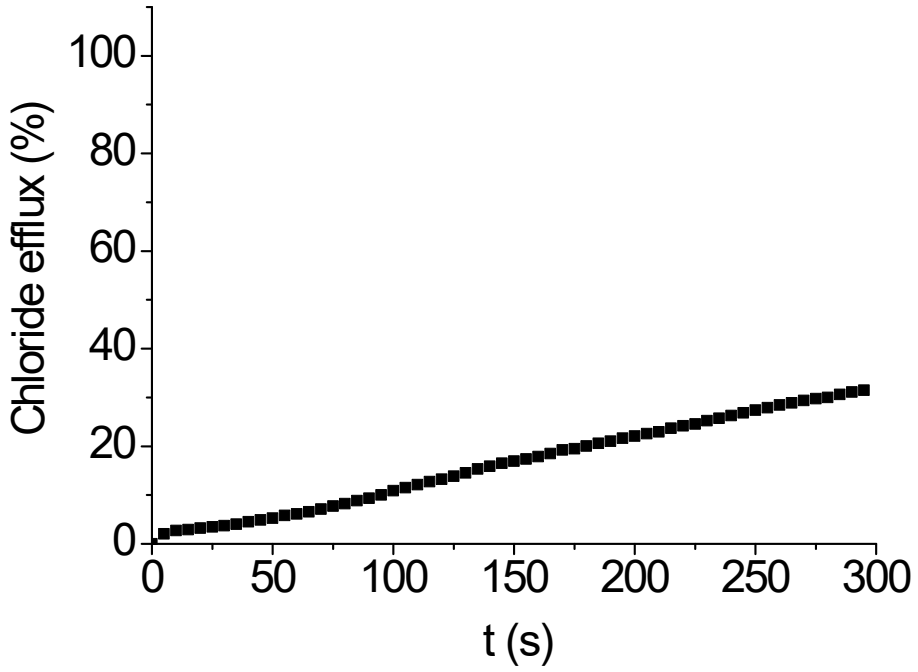


Figure S71. Chloride efflux promoted by free **6** (5 μM, black) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. The trace represents the average of at least three trials, performed with at least three batches of vesicles.

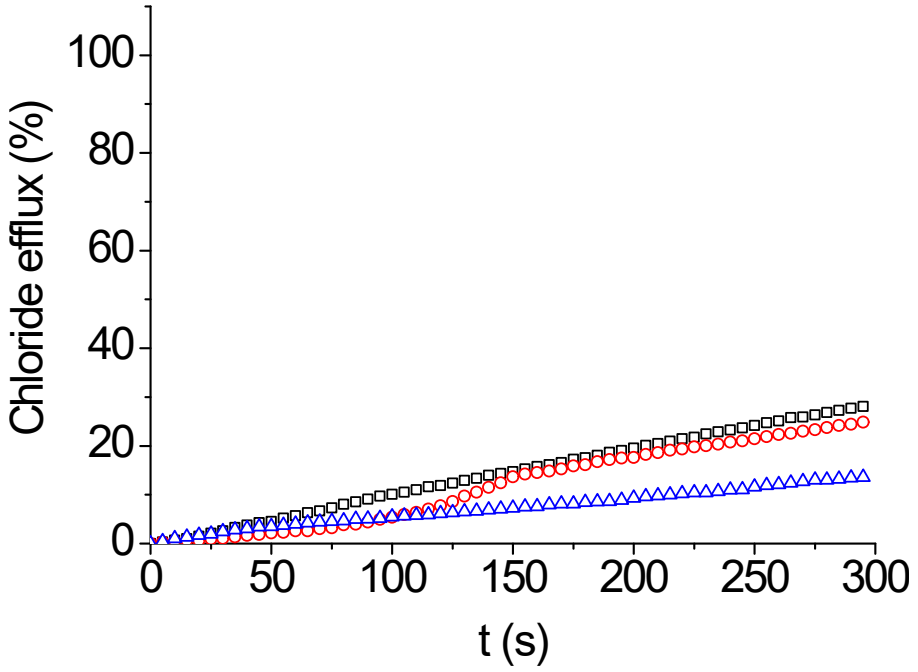


Figure S72. Chloride efflux promoted by encapsulated **6** (10 μM, black; 5 μM, red; 0.5 μM, blue) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at pH 7.2 with 5 mM NaH_2PO_4 and dispersed in a 489 mM $NaNO_3$ solution buffered at pH 7.2. Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

Cl^-/HCO_3^- exchange assays

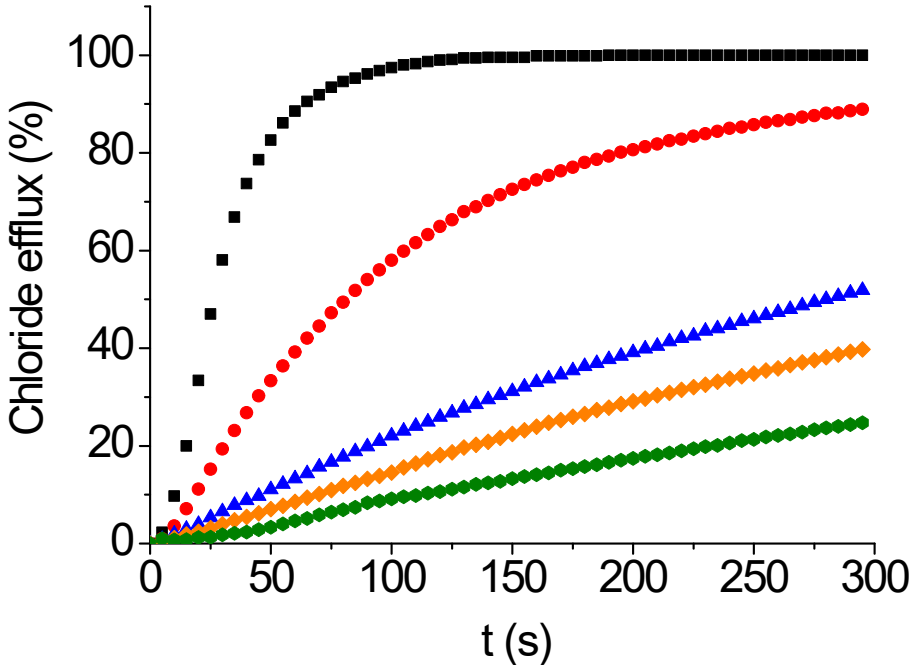


Figure S73. Chloride efflux promoted by free **1** (5 μM, black; 1 μM, red; 0.25 μM, blue; 0.15 μM, orange; 0.05 μM, green) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

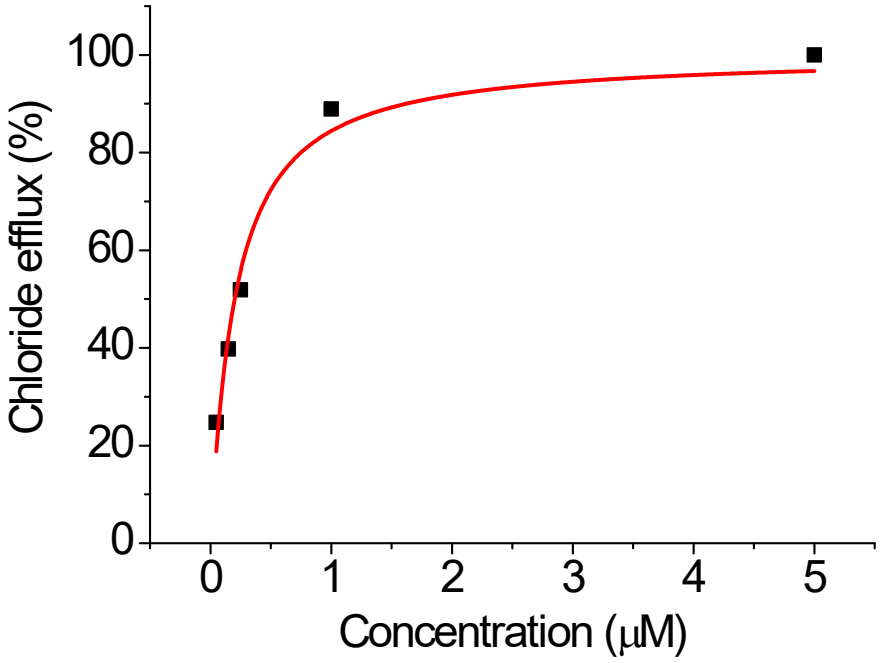


Figure S74. Hill analysis for free **1**.

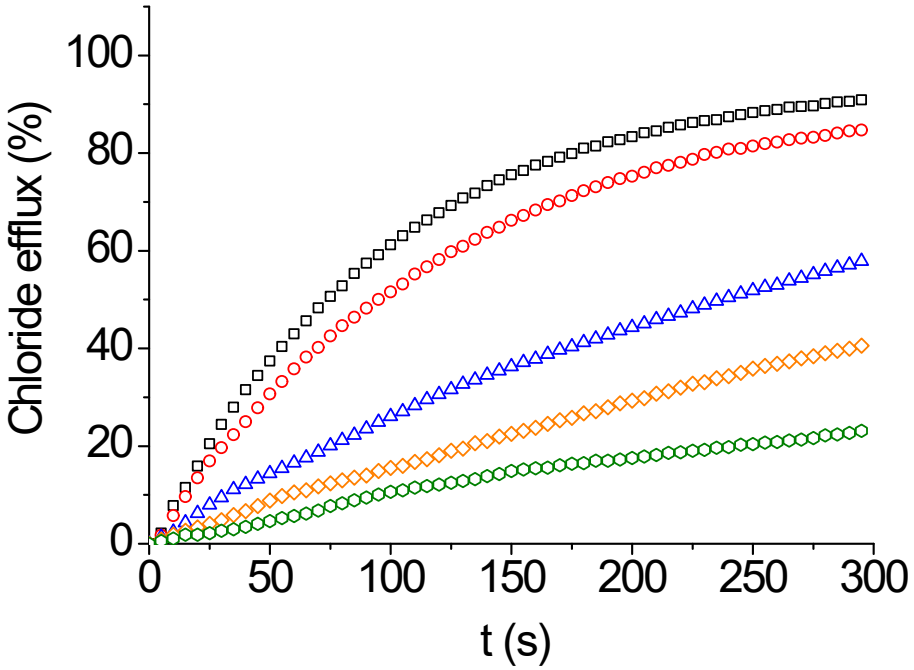


Figure S75. Chloride efflux promoted by encapsulated **1** (5 μM, black; 2.5 μM, red; 1 μM, blue; 0.5 μM, orange; 0.15 μM, green) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

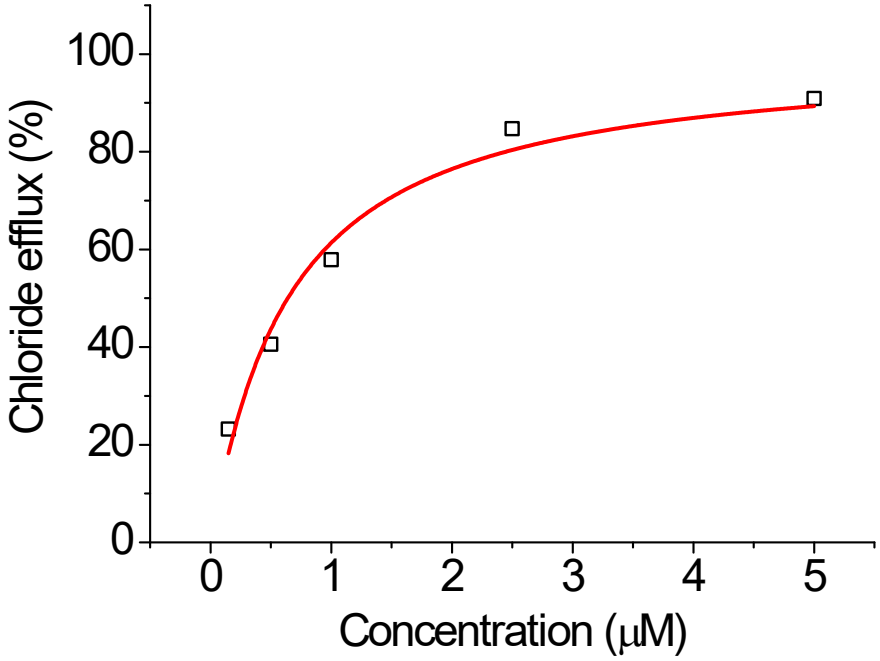


Figure S76. Hill analysis for encapsulated **1**.

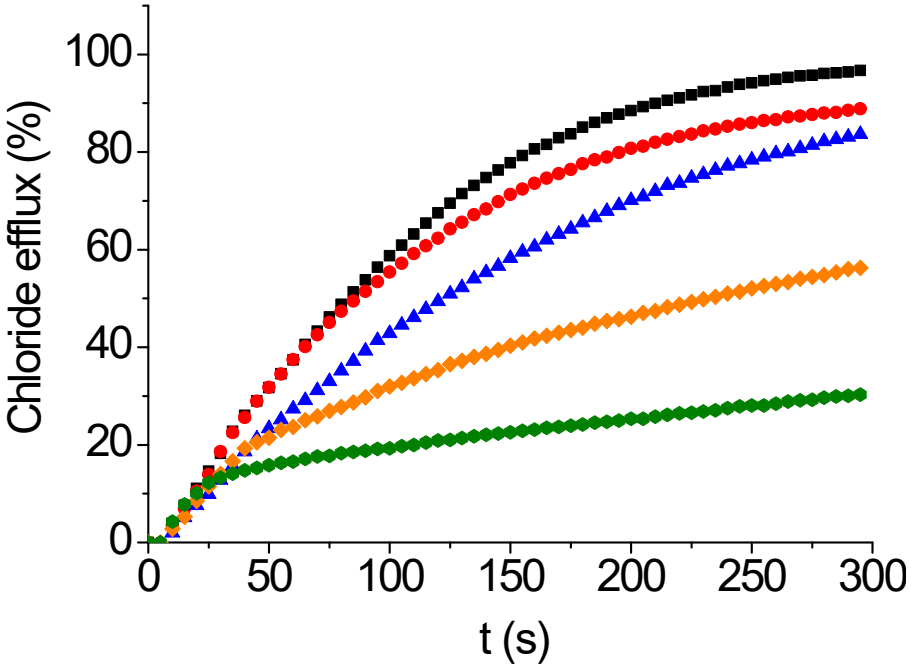


Figure S77. Chloride efflux promoted by free **2** (5 μM, black; 1.5 μM, red; 1 μM, blue; 0.25 μM, orange; 0.05 μM, green) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

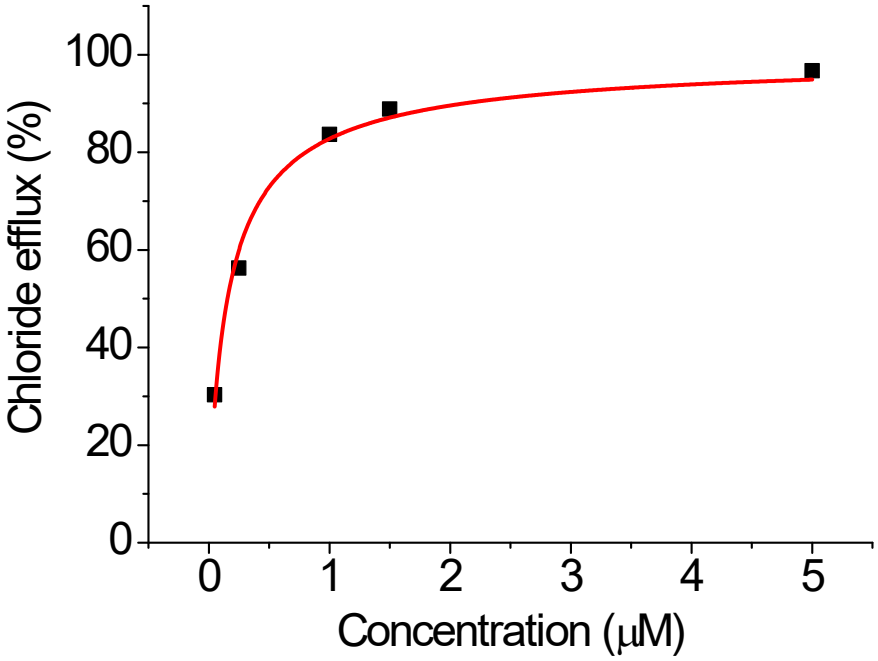


Figure S78. Hill analysis for free **2**.

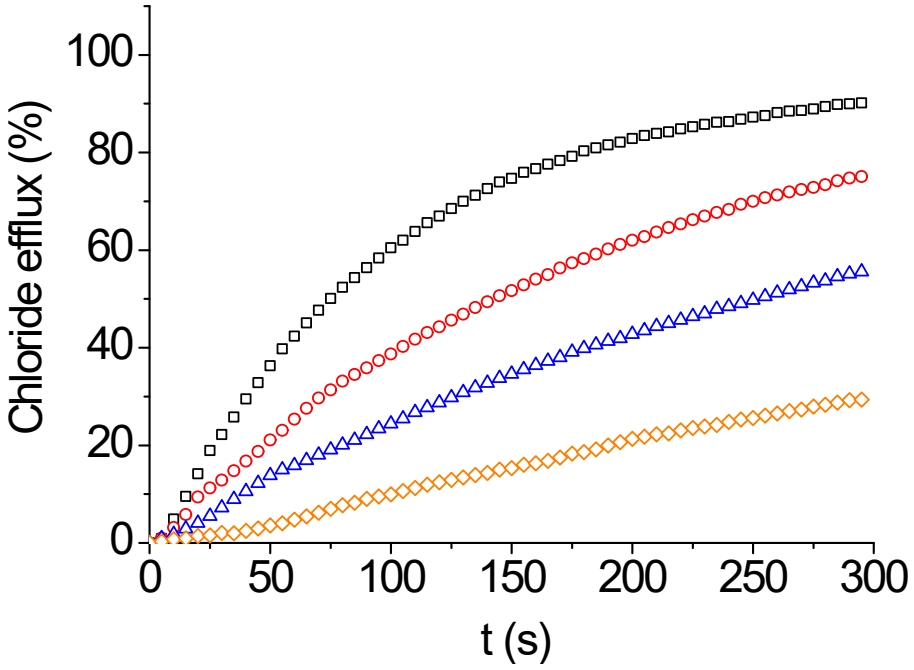


Figure S79. Chloride efflux promoted by encapsulated **2** (5 μM, black; 2.5 μM, red; 1 μM, blue; 0.25 μM, orange) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

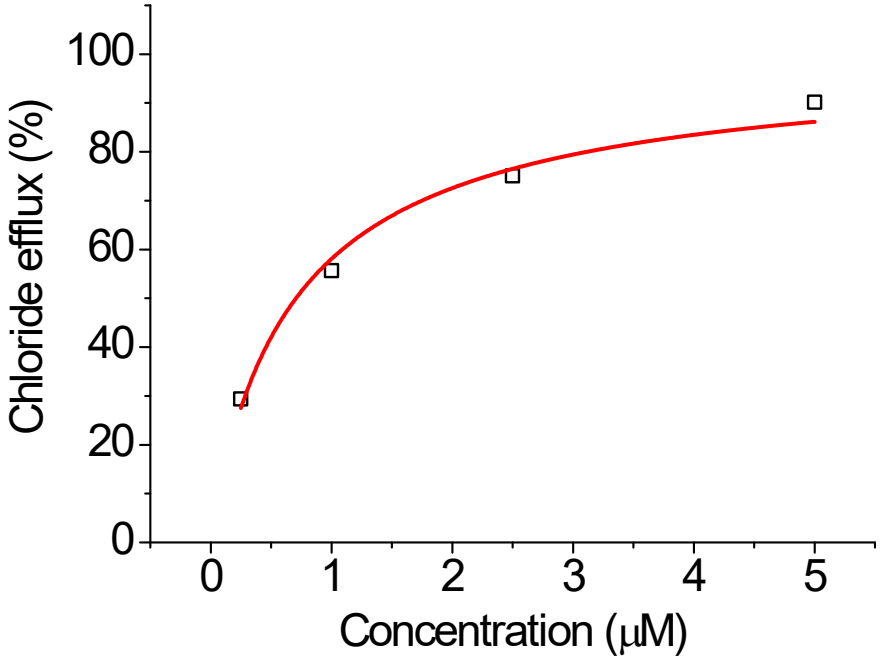


Figure S80. Hill analysis for encapsulated **2**.

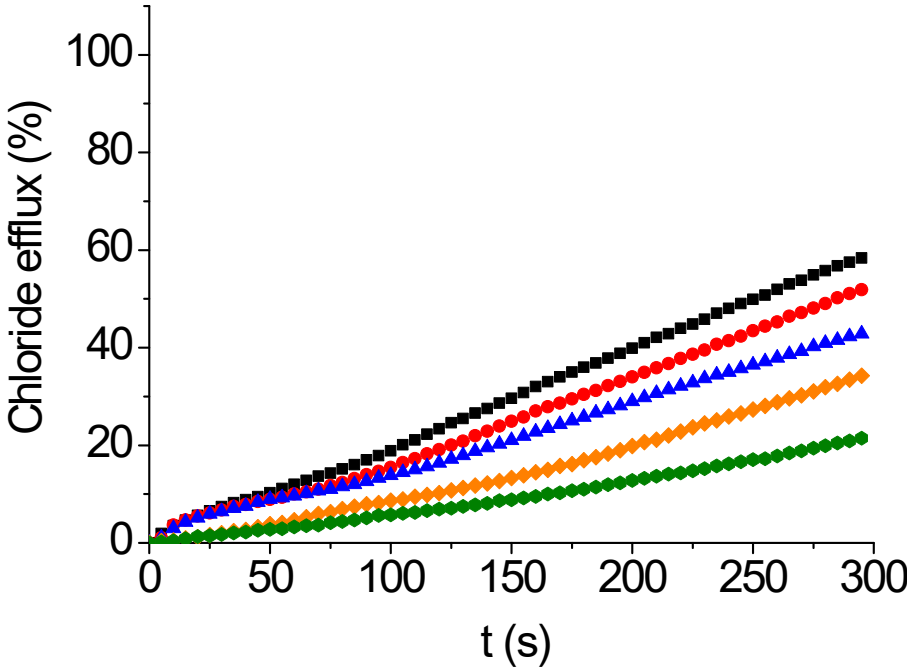


Figure S81. Chloride efflux promoted by free **3** (15 μM, black; 10 μM, red; 5 μM, blue; 2.5 μM, orange; 0.5 μM, green) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

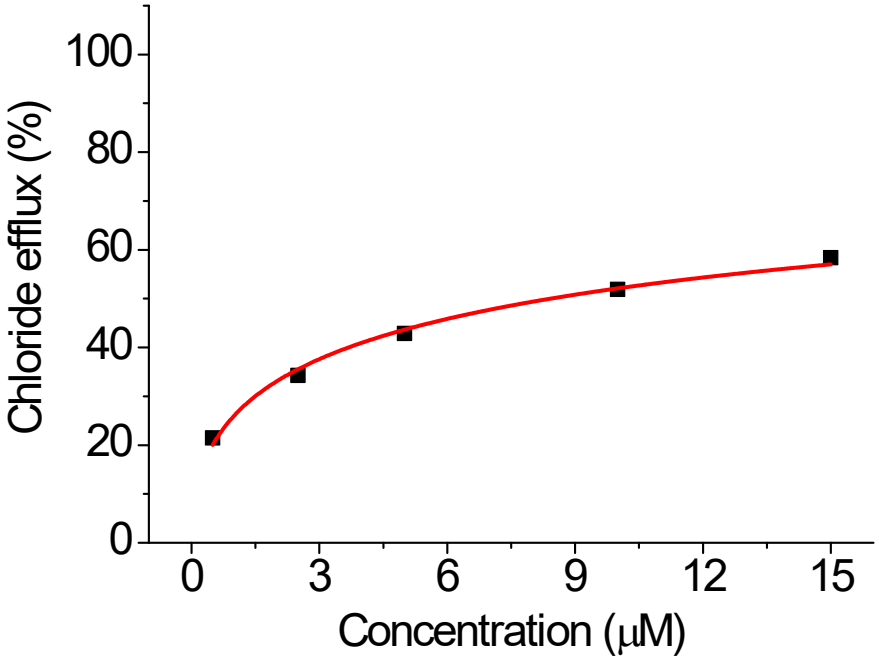


Figure S82. Hill analysis for free **3**.

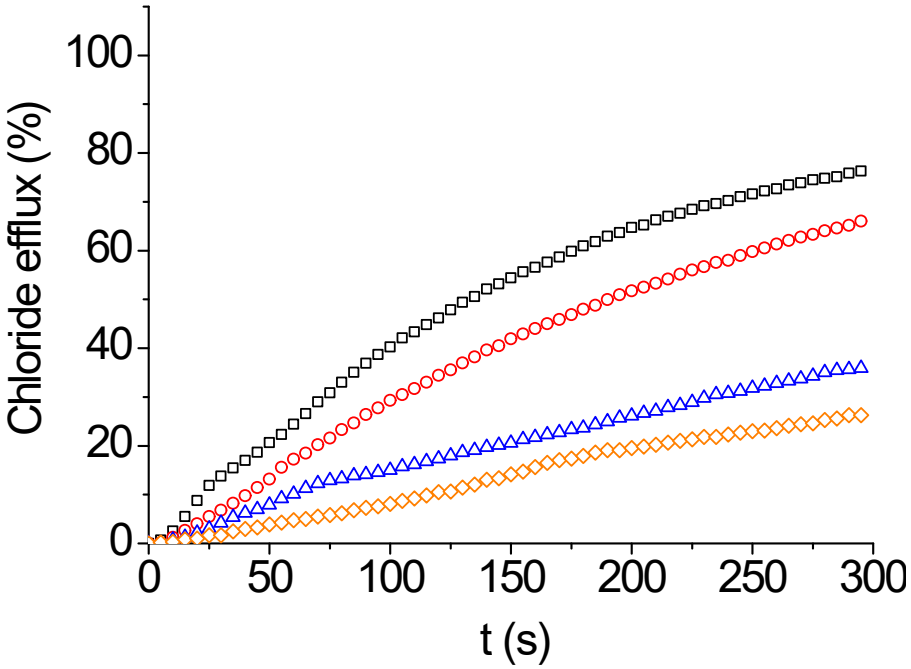


Figure S83. Chloride efflux promoted by encapsulated **3** (5 μM, black; 2.5 μM, red; 0.5 μM, blue; 0.25 μM, orange) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

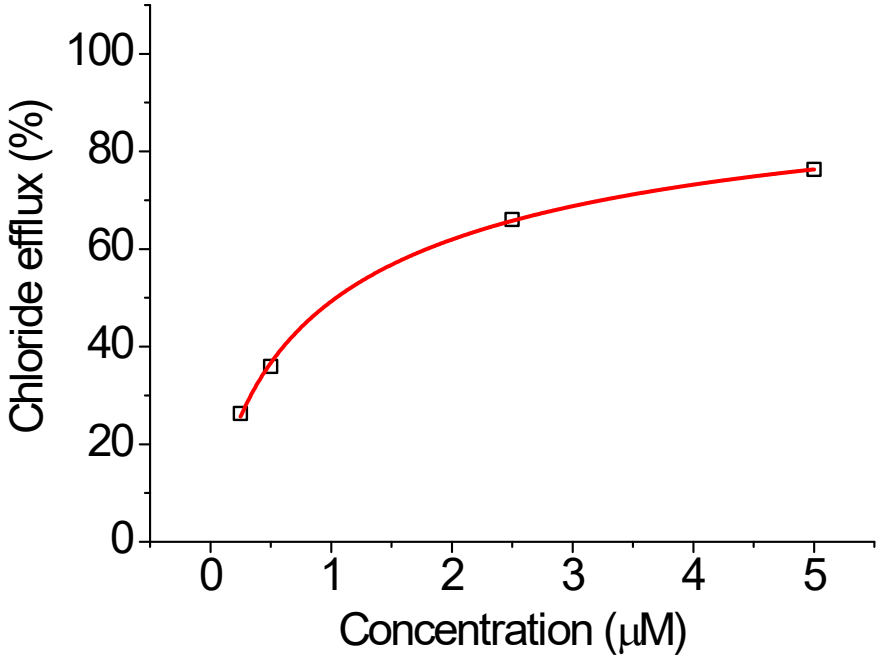


Figure S84. Hill analysis for encapsulated **3**.

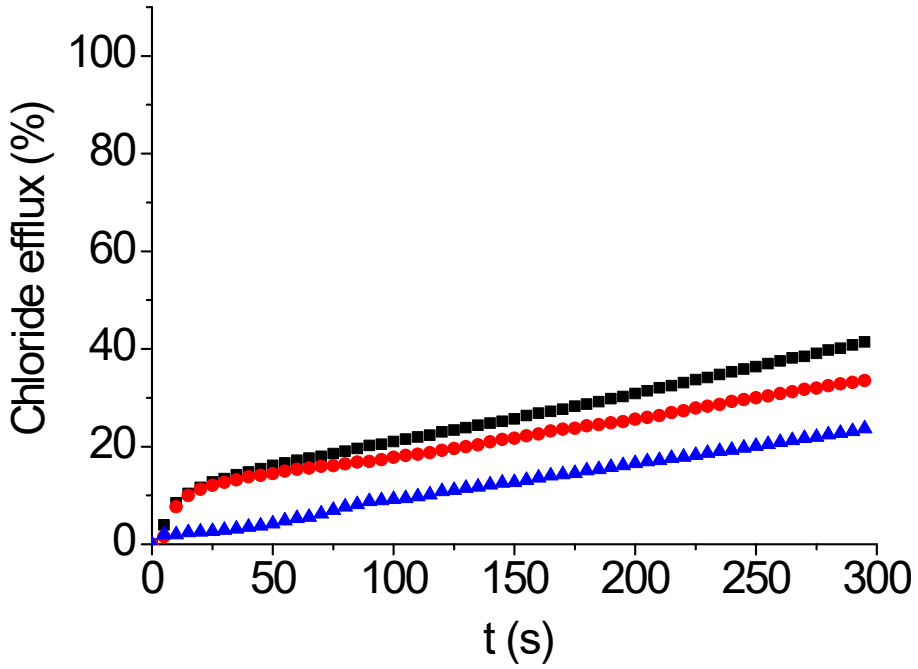


Figure S85. Chloride efflux promoted by free **4** (15 µM, black; 10 µM, red; 5 µM, blue) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

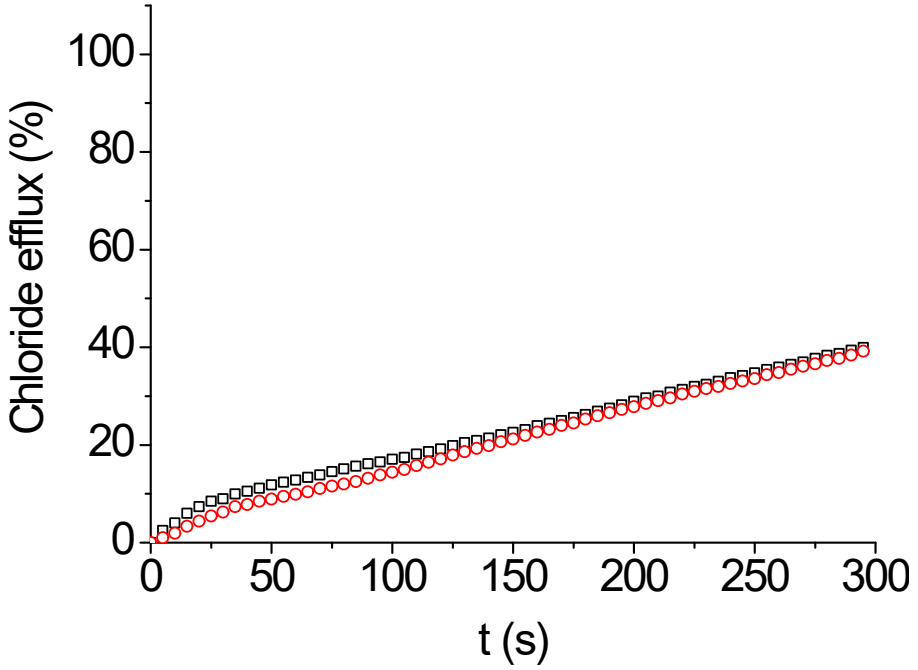


Figure S86. Chloride efflux promoted by encapsulated **4** (10 µM, black; 5 µM, red) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

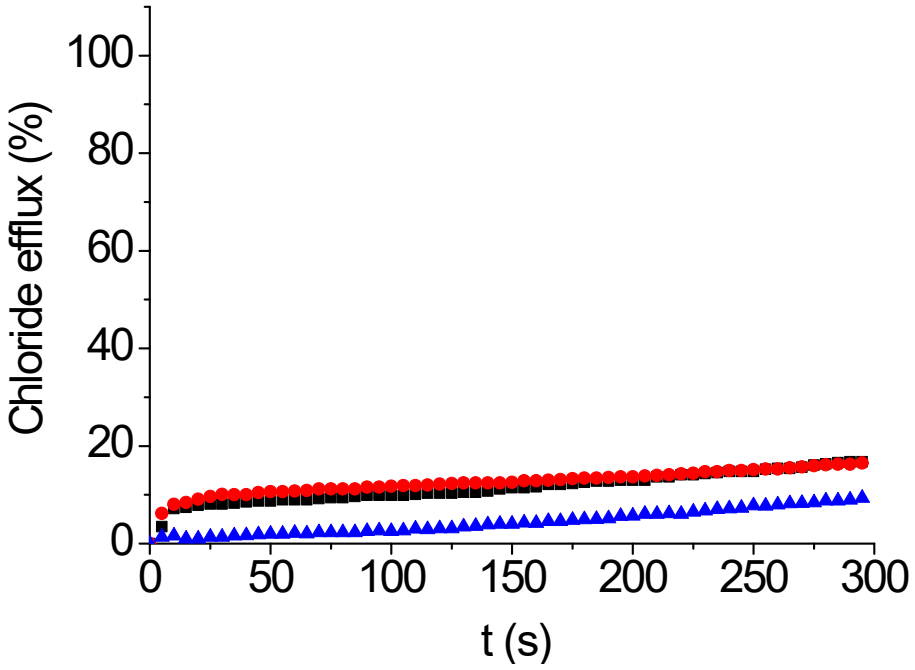


Figure S87. Chloride efflux promoted by free **5** (15 μM, black; 10 μM, red; 5 μM, blue) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

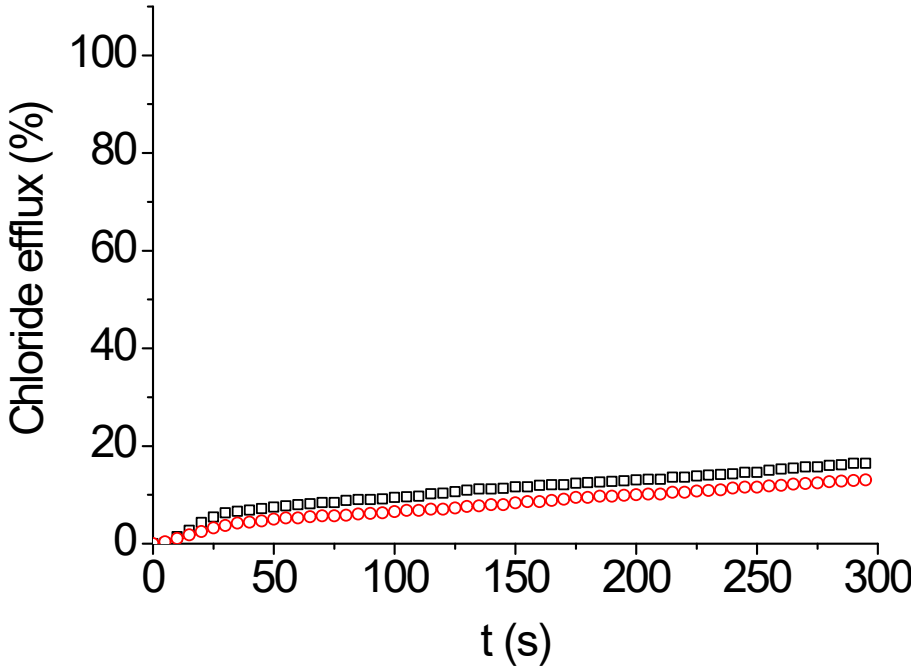


Figure S88. Chloride efflux promoted by encapsulated **5** (10 μM, black; 5 μM, red) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

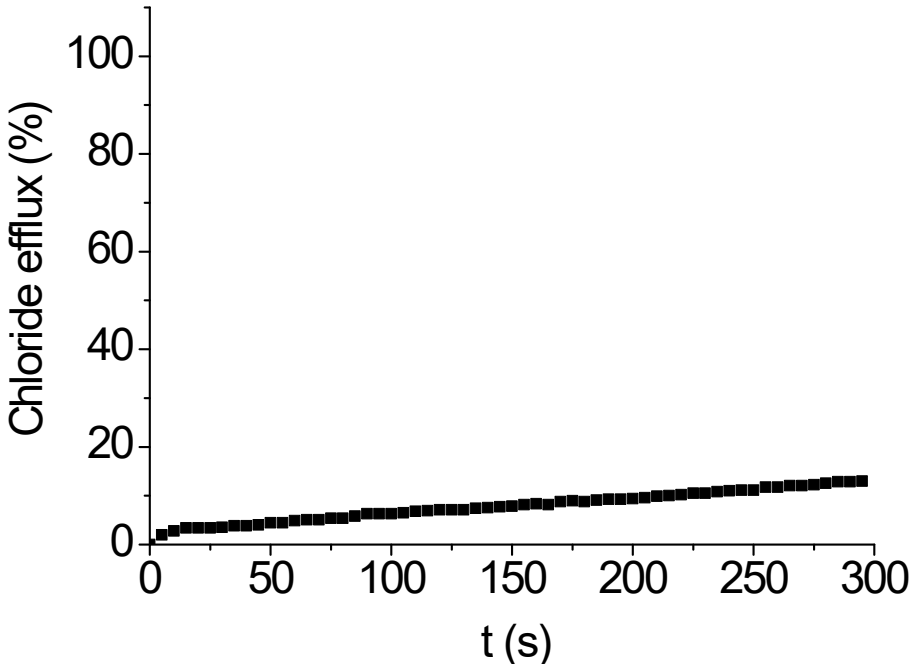


Figure S89. Chloride efflux promoted by free **6** (5 μM, black) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

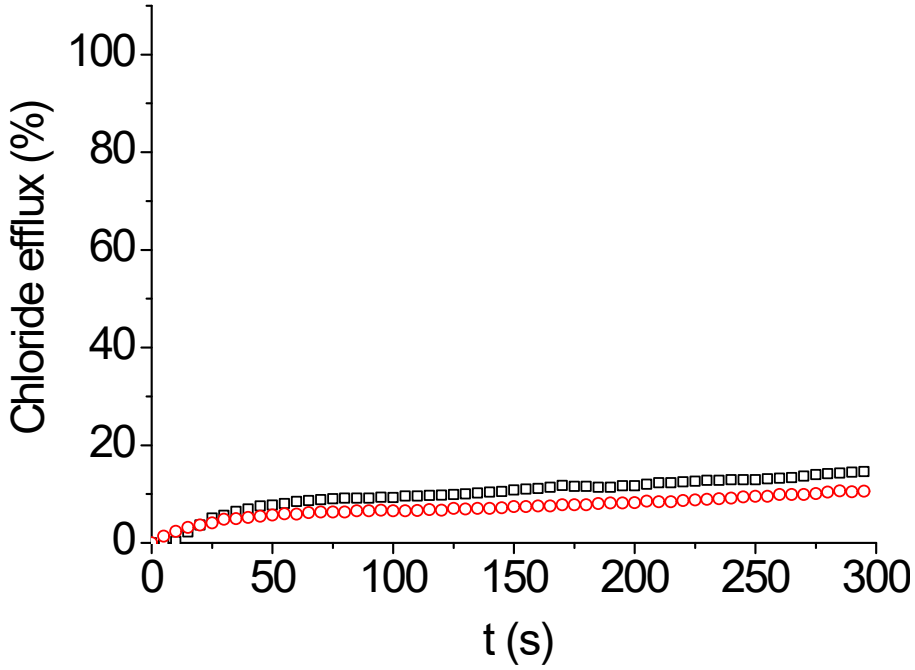


Figure S90. Chloride efflux promoted by encapsulated **6** (10 μM, black; 5 μM, red) in unilamellar POPC vesicles. Vesicles, loaded with a NaCl solution (451 mM NaCl and 20 mM NaH_2PO_4, pH 7.2), were immersed in a Na_2SO_4 solution (150 mM Na_2SO_4, 40 mM $NaHCO_3$ and 20 mM NaH_2PO_4, pH 7.2). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

7.3. Emission spectroscopy transport experiments

7.3.1. Carboxyfluorescein-based assays

Vesicles made of POPC were loaded with a NaCl aqueous solution (451 mM NaCl, 20 mM NaH_2PO_4, 50 mM CF, I.S. 500 mM, pH 7.2) and treated according to the procedure described in *Section 7.1*. The experiments were performed in 1-cm disposable cells, the final POPC concentration in the cuvette being 0.05 mM and the total volume 2.5 mL. At t = 60 s an aliquot of a solution of the free compound in DMSO or of a suspension of the formulation in the external solution was added, and emission changes were recorded for 300 s. At t = 360 s a pulse of a detergent (Triton-X, 20% dispersion in water, 20 µL) was added to lyse the vesicles and free all the entrapped CF. The obtained emission value was regarded as 100% release and used to normalise the data.

In all cases the concentration of compound with respect to that of POPC was 0.5% (0.25 µM of compound, either free or encapsulated, in the cuvette). In order to prepare the stock suspensions of the formulations the protocol followed was similar to that described in *Section 7.2*. In this case, the suspensions were prepared with the Na_2SO_4 aqueous solution (150 mM Na_2SO_4, 20 mM NaH_2PO_4, I.S. 500 mM, pH 7.2). The calculations are similar to those reported previously, taking into consideration that the POPC concentration in the cell is now 0.05 mM and the volume of the experiments is 2.5 mL. By the same token, the blank consisted of either DMSO (6.25 µL), for assays involving the free compounds, or a suspension of 15.0 mg of lipid nanoparticles containing no compound in 5 mL of the external solution (20 µL), for experiments involving the formulations.

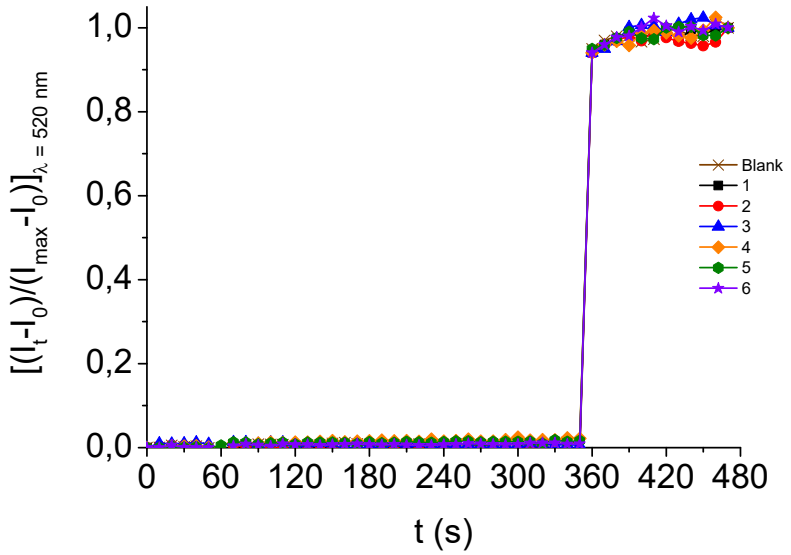


Figure S91. Carboxyfluorescein leakage observed upon addition of the free compounds to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the anion carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. The blank is DMSO (6.25 μL). Each trace represents the average of six trials, performed with three batches of vesicles.

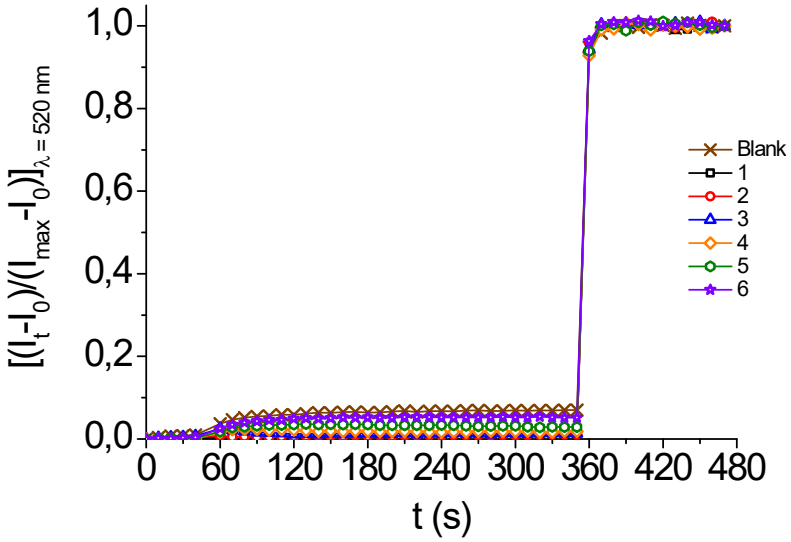


Figure S92. Carboxyfluorescein leakage observed upon addition of the encapsulated compounds to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the formulation containing the carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. The blank consists of a suspension of lipid nanoparticles containing no compound (20 μL). Each trace represents the average of six trials, performed with three batches of vesicles.

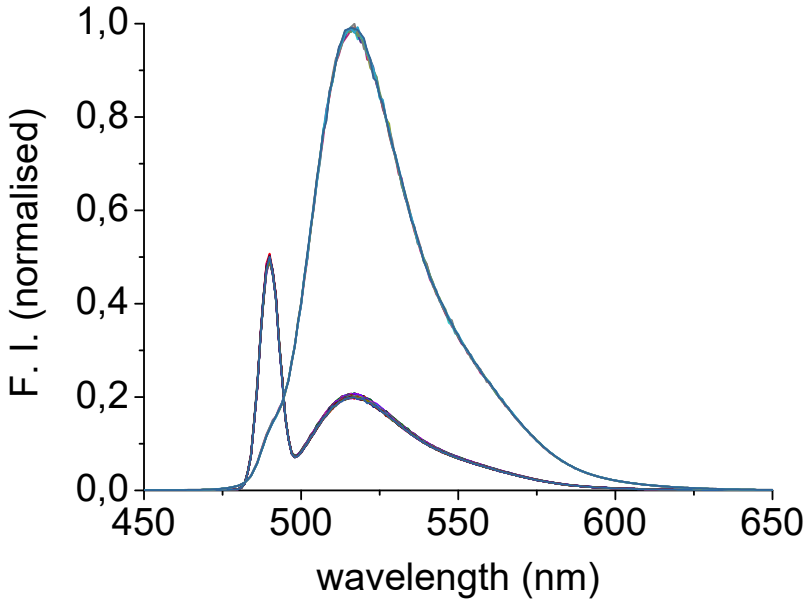


Figure S93. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of free **1** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the anion carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

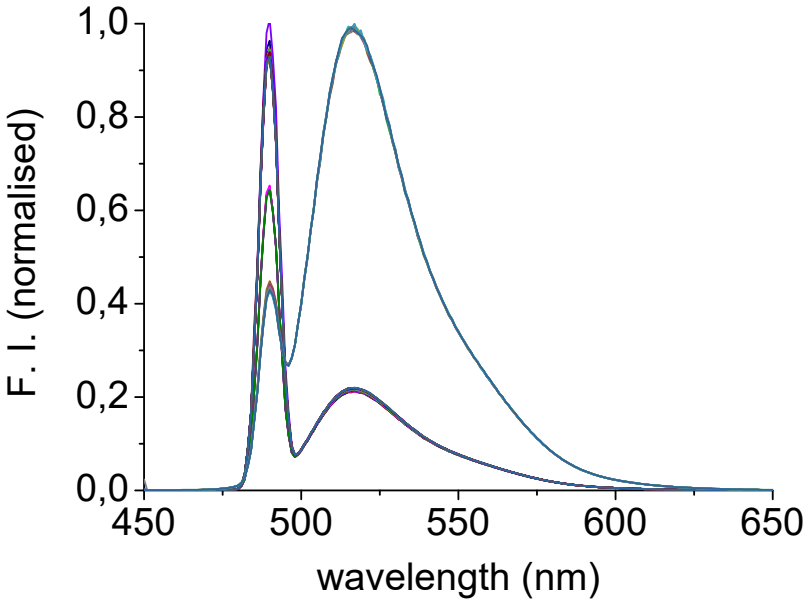


Figure S94. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of encapsulated **1** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the formulation containing the carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

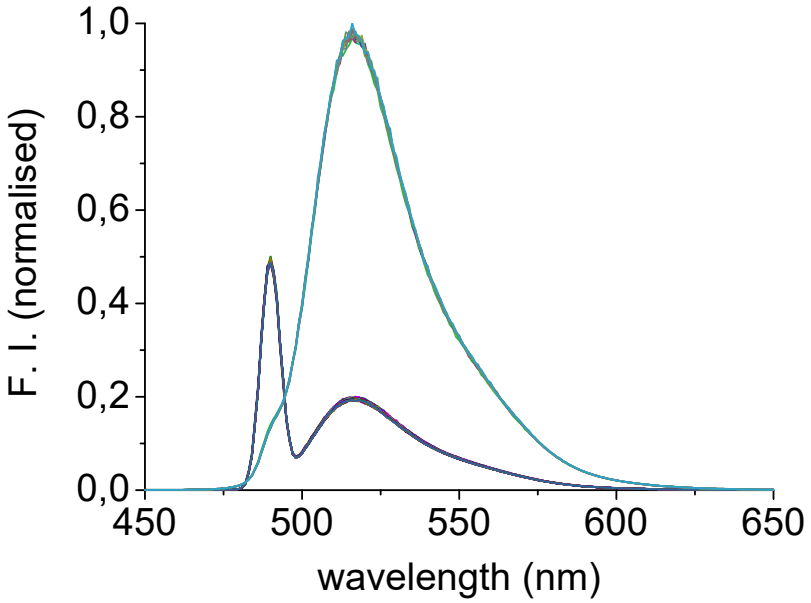


Figure S95. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of free **2** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the anion carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

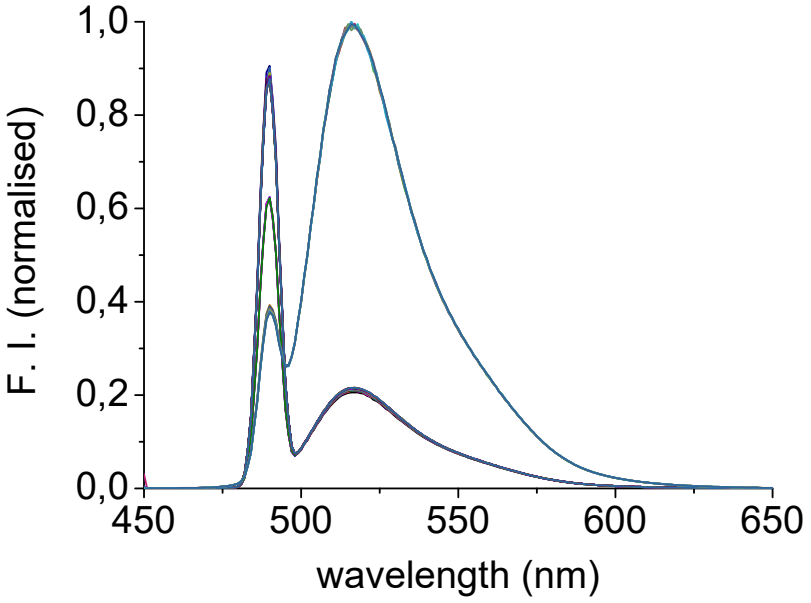


Figure S96. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of encapsulated **2** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the formulation containing the carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

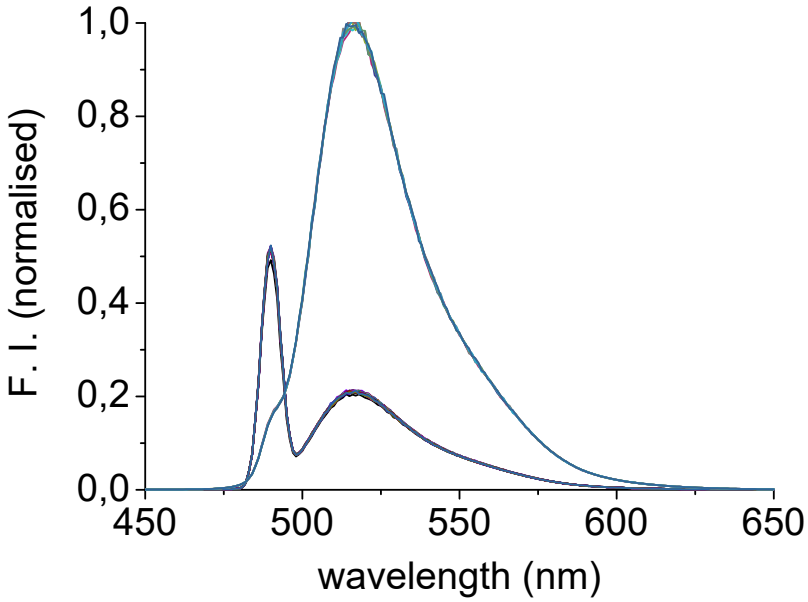


Figure S97. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of free **3** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the anion carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

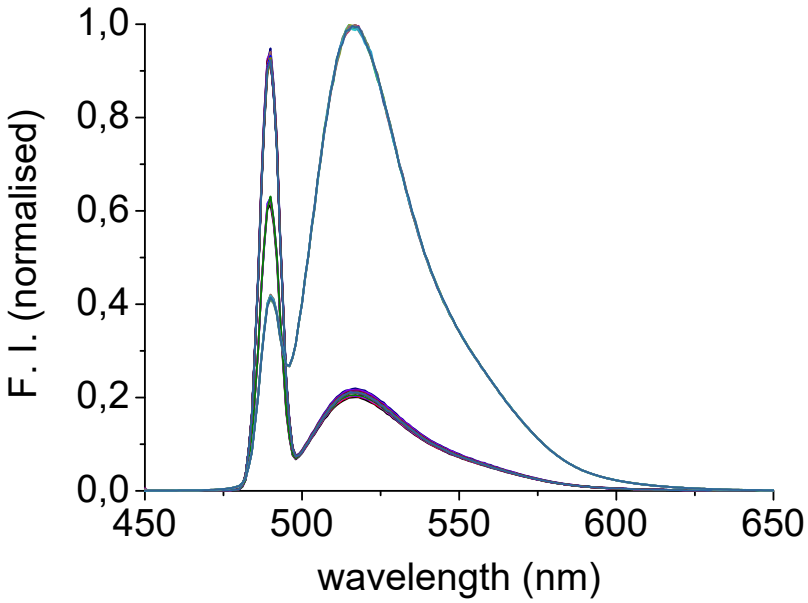


Figure S98. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of encapsulated **3** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the formulation containing the carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

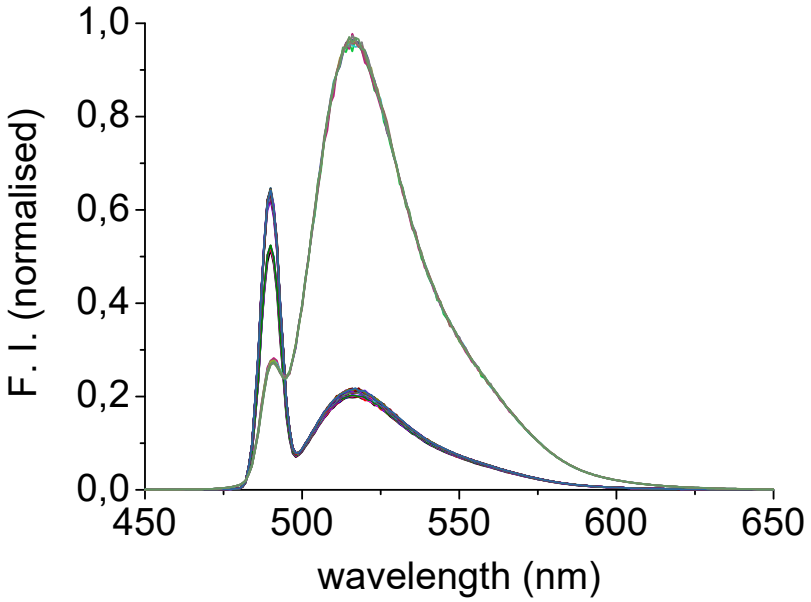


Figure S99. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of free **4** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the anion carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

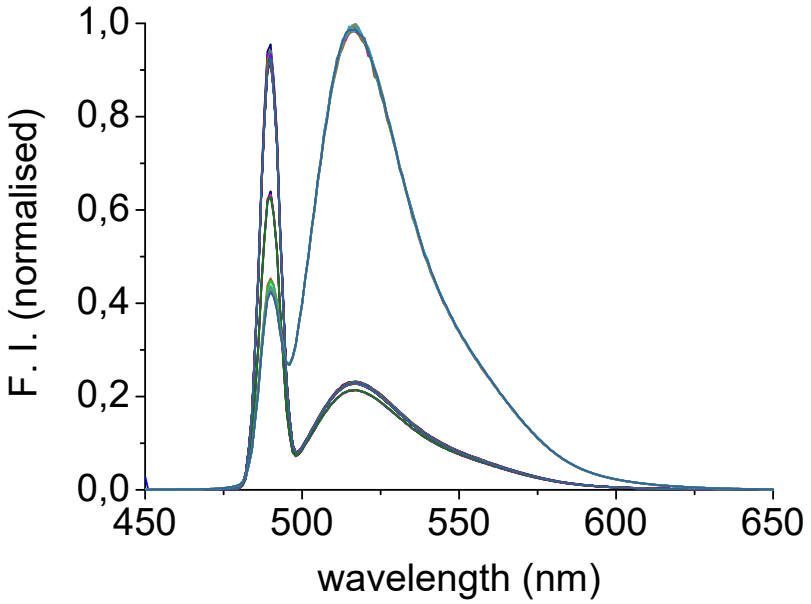


Figure S100. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of encapsulated **4** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the formulation containing the carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

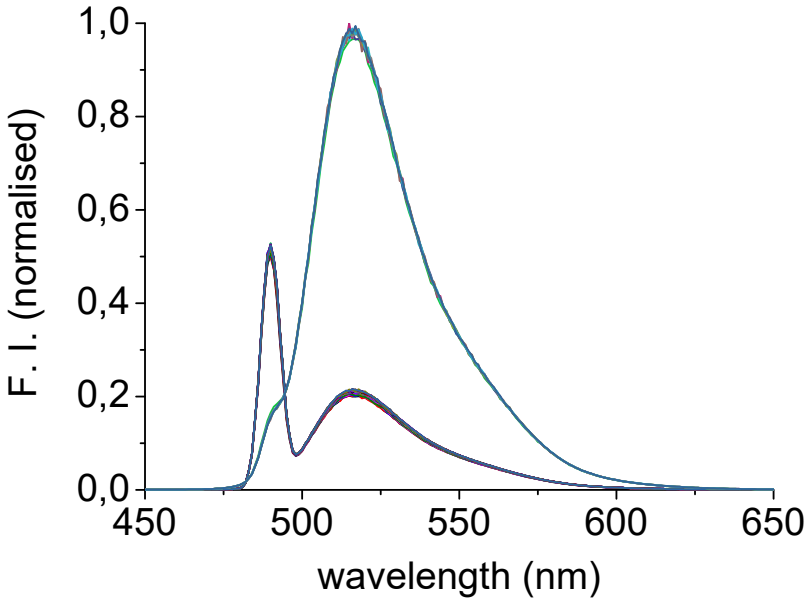


Figure S101. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of free **5** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the anion carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

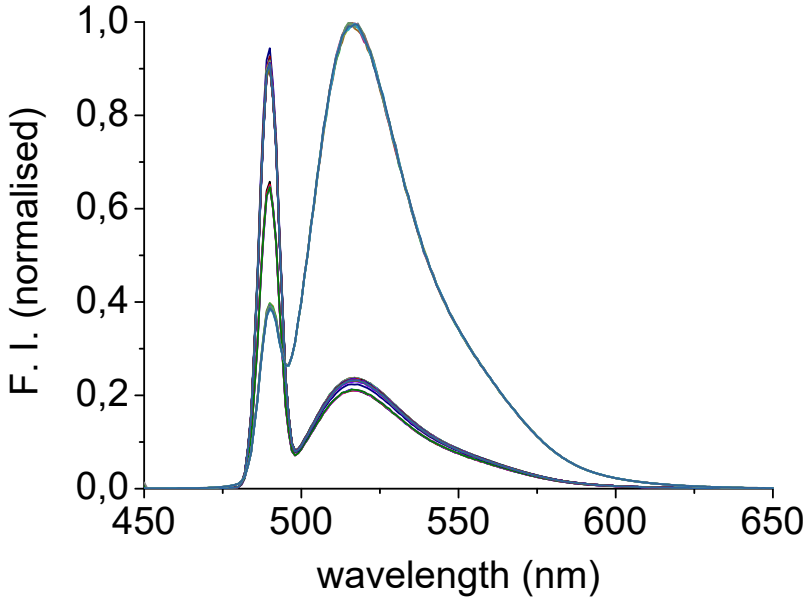


Figure S102. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of encapsulated **5** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the formulation containing the carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

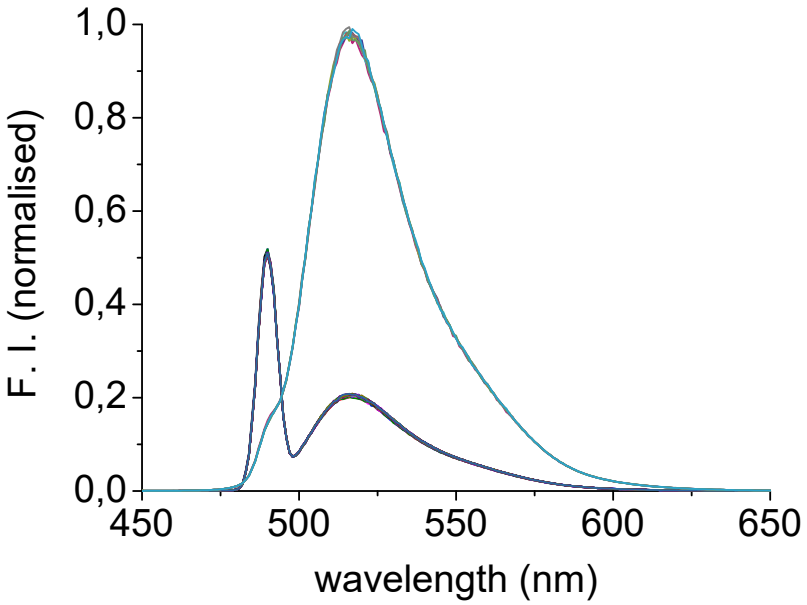


Figure S103. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of free **6** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the anion carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

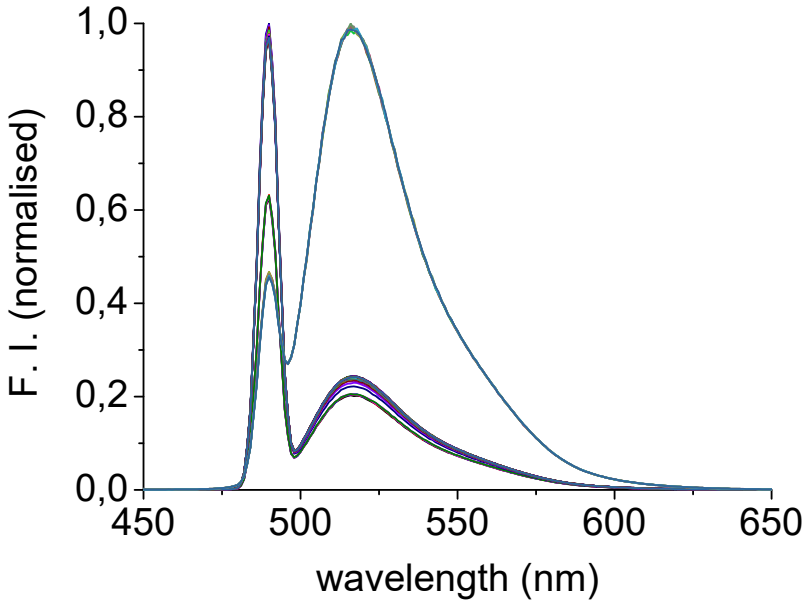


Figure S104. Carboxyfluorescein normalised fluorescence intensity recorded upon addition of encapsulated **6** to POPC vesicles (0.05 mM). Vesicles (loaded with a 451 mM NaCl solution buffered at pH 7.2 with 20 mM NaH_2PO_4, and containing 50 mM CF; I.S. 500 mM) were suspended in a Na_2SO_4 solution (150 mM Na_2SO_4, buffered at pH 7.2 with 20 mM NaH_2PO_4; I.S. 500 mM). At t = 60 s the formulation containing the carrier was added (0.5% mol carrier to lipid), while at t = 360 s the detergent (20 μL) was added. Each trace represents the average of six trials, performed with three batches of vesicles.

7.3.2. HPTS-based assays

7:3 POPC:cholesterol vesicles were loaded with a $NaNO_3$ aqueous solution (126.2 mM $NaNO_3$, 10 mM NaH_2PO_4, 1 mM HPTS, I.S. 150 mM, pH 7.2) and treated according to the procedure described in *Section 7.1*. The experiments were performed in 1-cm disposable cells, the final POPC concentration in the cuvette being 0.5 mM. At t = 30 s a pulse of a NaOH aqueous solution (20 μL of a 0.5 M solution) was added to get a final concentration of 4 mM of the base in the cuvette; at t = 60 s an aliquot of a solution of the anion carrier in DMSO or of a suspension of the formulation containing the anion carrier was added; and at t = 360 s a pulse of a detergent (Triton-X, 20% dispersion in water, 20 μL) was added to lyse the vesicles and release all the entrapped HPTS; the experiment finished when a stable reading was obtained. Along the assay, the emission intensities collected at 510 nm when exciting the sample at 460 (the excitation wavelength of the probe's deprotonated form) and 403 nm (the excitation wavelength of the probe's protonated form) were recorded every 10 s (I_{460} and I_{403}, respectively), which provided a series of I_{460}/I_{403} ratios that were normalised by referring them to that obtained after adding the surfactant.

The concentration of compound with respect to that of POPC was 1, 0.1, 0.01 and 0.001% (5, 0.5, 0.05 and 0.005 μM of compound, respectively, either free or encapsulated, in the cuvette). In order to prepare the stock suspensions of the formulations the protocol followed was similar to that described in *Section 7.2*. In this case, the suspension was prepared with the inner solution without the probe (126.2 mM $NaNO_3$, 10 mM NaH_2PO_4, I.S. 150 mM, pH 7.2). The calculations are similar to those reported previously, taking into consideration that the POPC concentration in the cell is 0.5 mM and the volume of the experiments is 2.5 mL. The blank consisted of either DMSO (6.25 μL), for assays involving the free compounds, or a suspension of 15.0 mg of lipid nanoparticles containing no compound in 5 mL of the external solution (38 μL), for experiments involving the formulations.

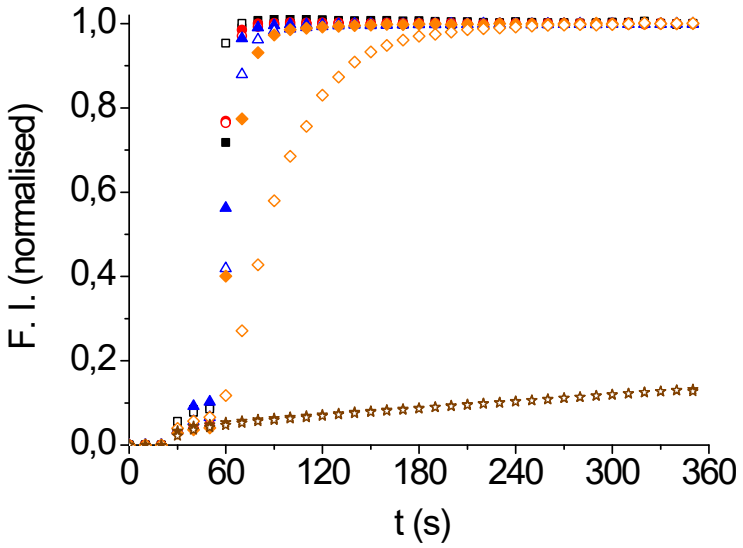


Figure S105. Emission changes induced by free (filled symbol) and encapsulated (hollow symbol) **1** (5 μM, black; 0.5 μM, red; 0.05 μM, blue; 0.005 μM, orange; blank, brown) in 7:3 POPC:cholesterol vesicles (0.5 mM POPC). Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 μL) or lipid nanoparticles containing no compound (38 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

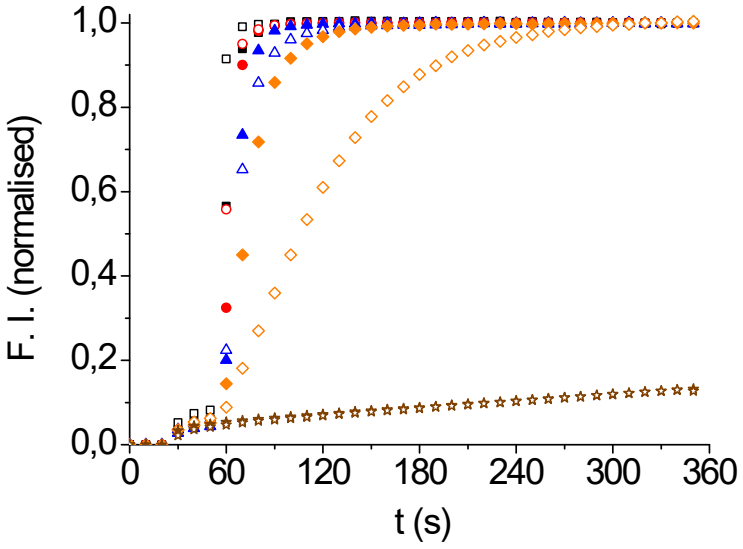


Figure S106. Emission changes induced by free (filled symbol) and encapsulated (hollow symbol) **2** (5 μM, black; 0.5 μM, red; 0.05 μM, blue; 0.005 μM, orange; blank, brown) in 7:3 POPC:cholesterol vesicles (0.5 mM POPC). Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 μL) or lipid nanoparticles containing no compound (38 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

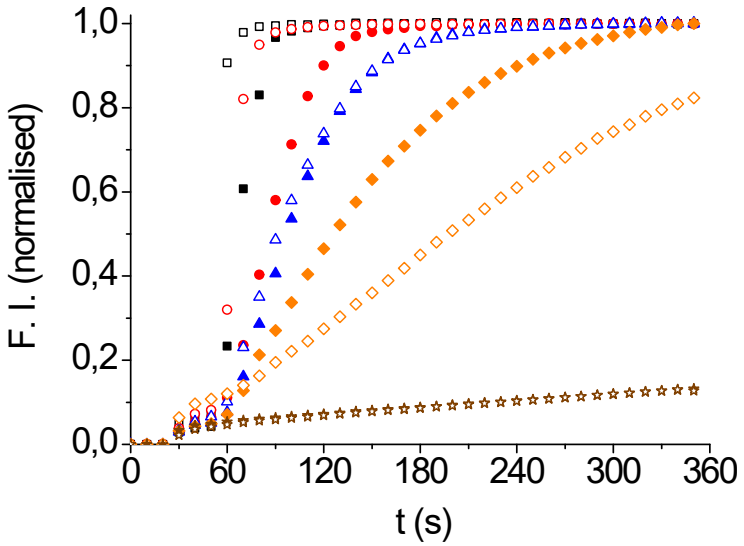


Figure S107. Emission changes induced by free (filled symbol) and encapsulated (hollow symbol) **3** (5 μM, black; 0.5 μM, red; 0.05 μM, blue; 0.005 μM, orange; blank, brown) in 7:3 POPC:cholesterol vesicles (0.5 mM POPC). Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 μL) or lipid nanoparticles containing no compound (38 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

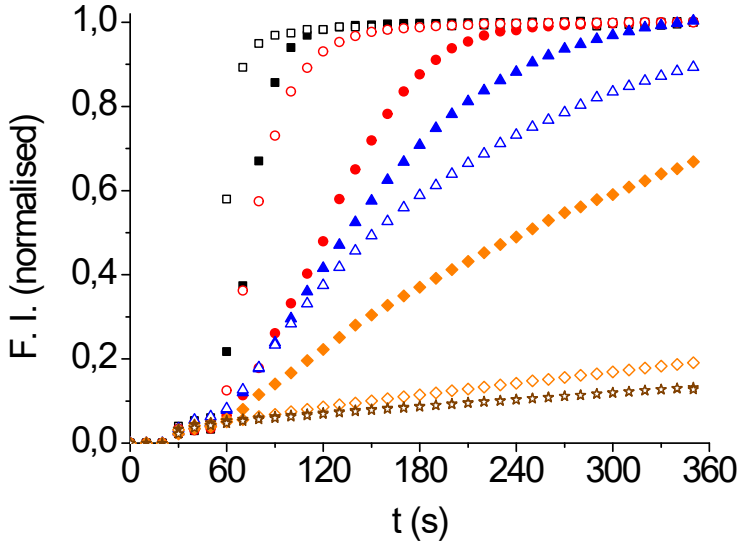


Figure S108. Emission changes induced by free (filled symbol) and encapsulated (hollow symbol) **4** (5 μM, black; 0.5 μM, red; 0.05 μM, blue; 0.005 μM, orange; blank, brown) in 7:3 POPC:cholesterol vesicles (0.5 mM POPC). Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 μL) or lipid nanoparticles containing no compound (38 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

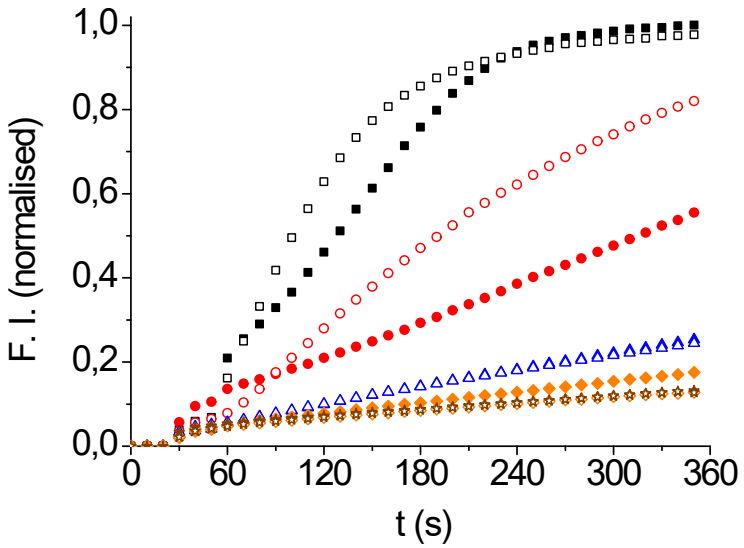


Figure S109. Emission changes induced by free (filled symbol) and encapsulated (hollow symbol) **5** (5 µM, black; 0.5 µM, red; 0.05 µM, blue; 0.005 µM, orange; blank, brown) in 7:3 POPC:cholesterol vesicles (0.5 mM POPC). Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 µL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 µL) or lipid nanoparticles containing no compound (38 µL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

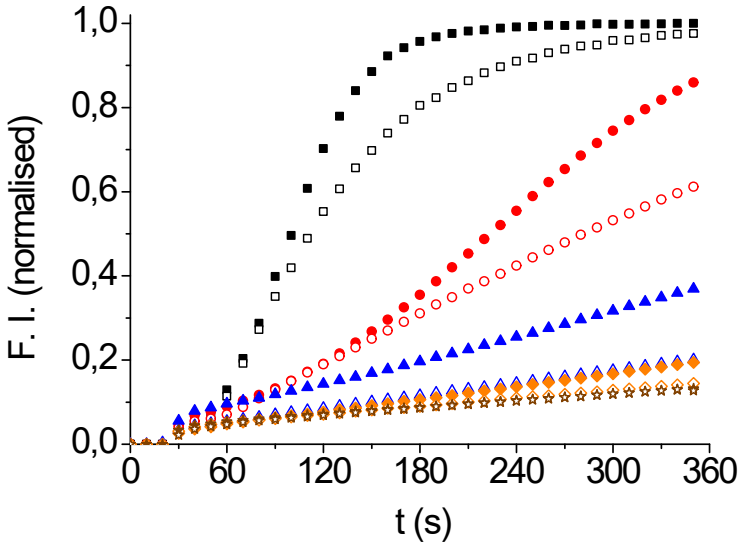


Figure S110. Emission changes induced by free (filled symbol) and encapsulated (hollow symbol) **6** (5 µM, black; 0.5 µM, red; 0.05 µM, blue; 0.005 µM, orange; blank, brown) in 7:3 POPC:cholesterol vesicles (0.5 mM POPC). Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 µL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 µL) or lipid nanoparticles containing no compound (38 µL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

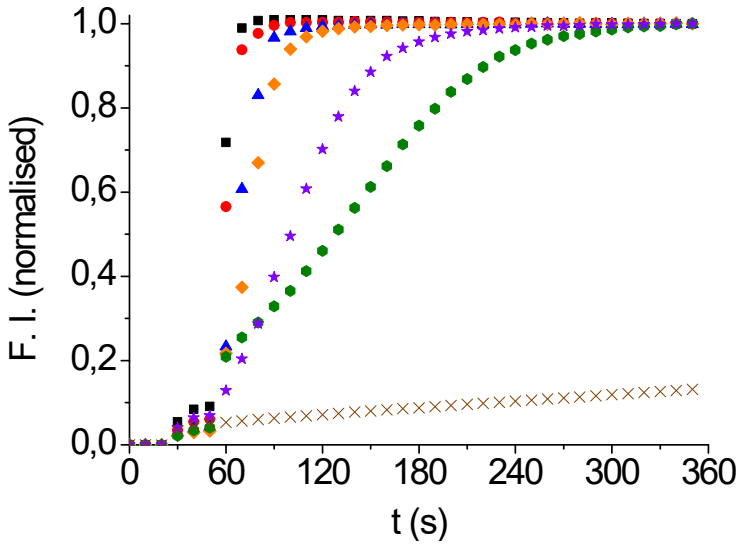


Figure S111. Emission changes induced by the free compounds (5 µM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 µL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 µL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

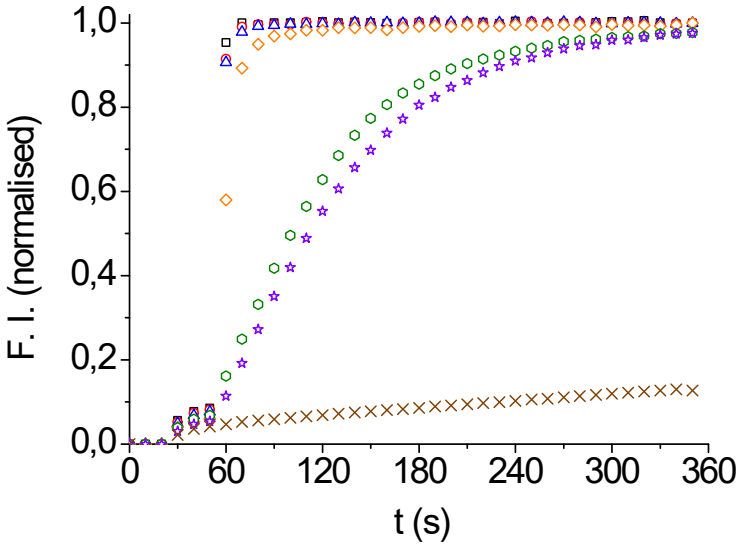


Figure S112. Emission changes induced by the encapsulated compounds (5 µM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 µL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank consists of lipid nanoparticles containing no compound (38 µL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

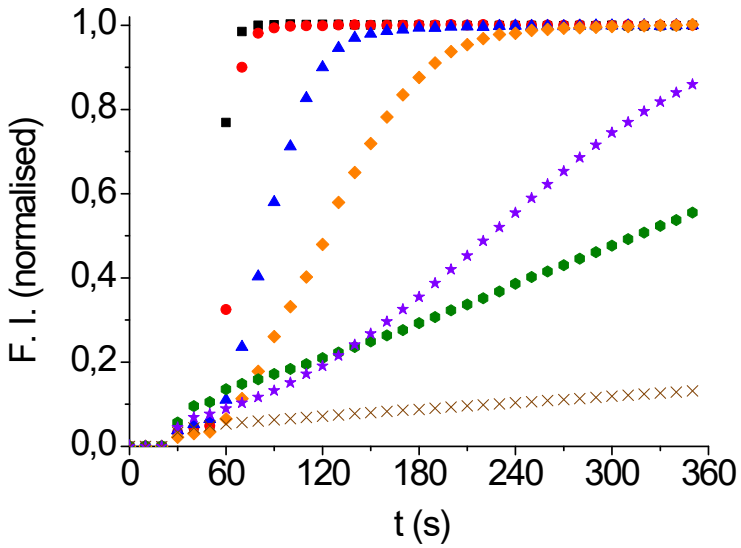


Figure S113. Emission changes induced by the free compounds (0.5 μM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

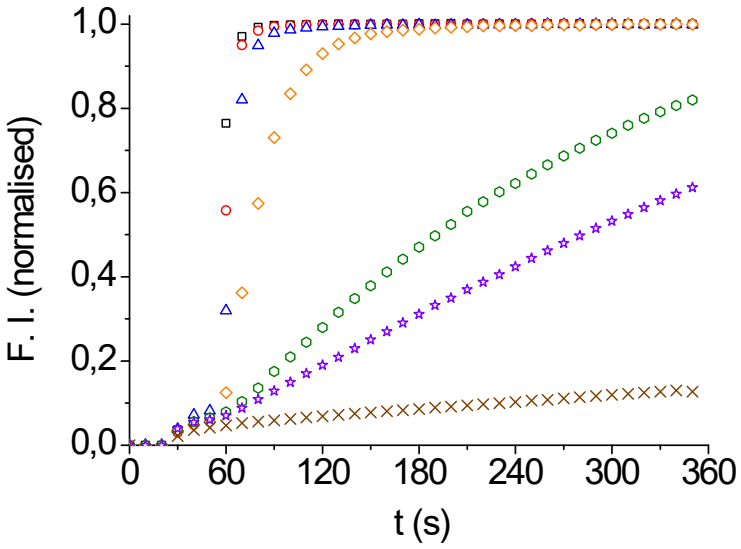


Figure S114. Emission changes induced by the encapsulated compounds (0.5 μM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank consists of lipid nanoparticles containing no compound (38 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

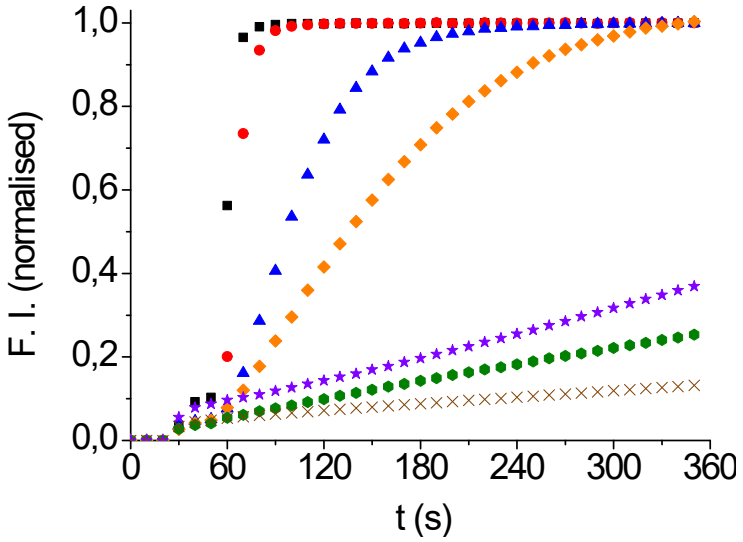


Figure S115. Emission changes induced by the free compounds (0.05 μM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

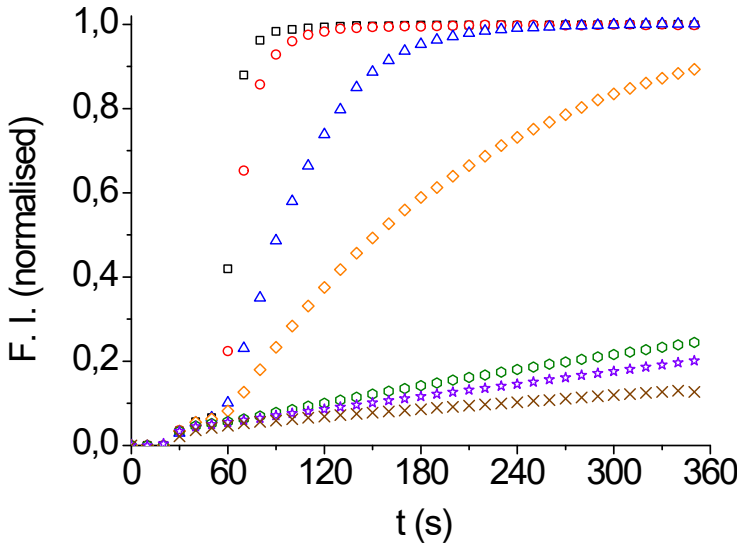


Figure S116. Emission changes induced by the encapsulated compounds (0.05 μM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 μL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank consists of lipid nanoparticles containing no compound (38 μL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

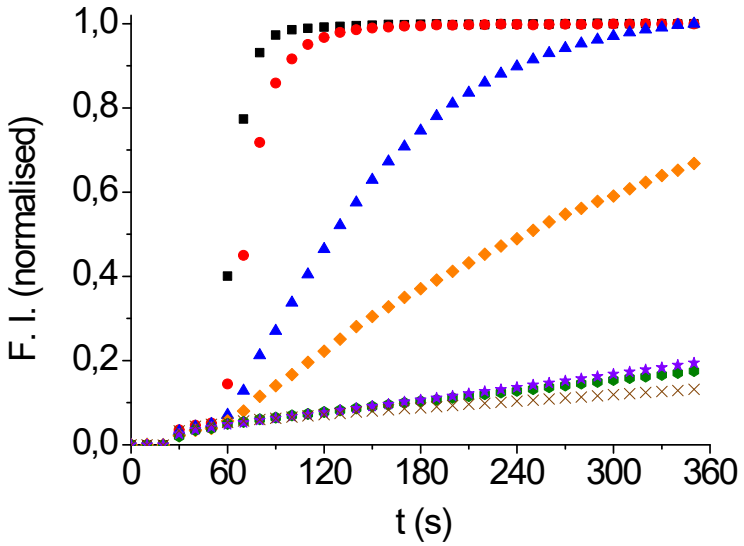


Figure S117. Emission changes induced by the free compounds (0.005 µM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 µL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank is DMSO (6.25 µL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

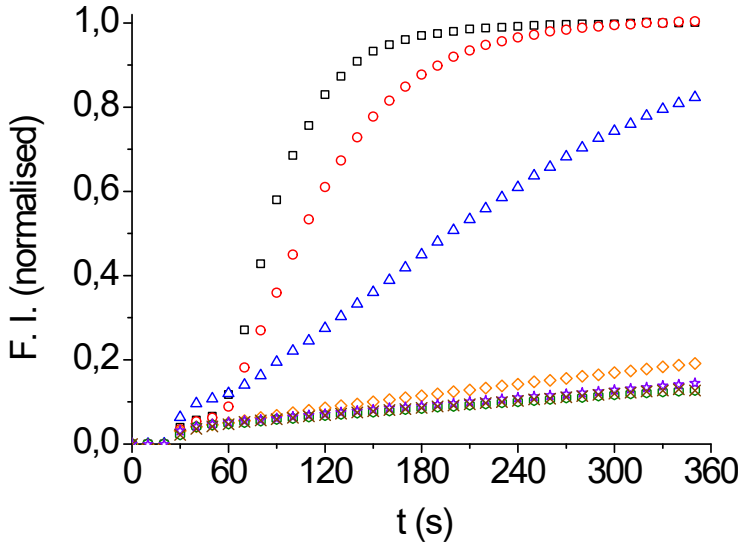


Figure S118. Emission changes induced by the encapsulated compounds (0.005 µM) (**1**, black; **2**, red; **3**, blue; **4**, orange; **5**, green; **6**, purple; blank, brown) in 7:3 POPC:cholesterol vesicles. Vesicles (loaded with a 126.2 mM $NaNO_3$ solution buffered at pH 7.2 with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a $NaNO_3$ solution (126.2 mM $NaNO_3$ buffered at pH 7.2 with 10 mM NaH_2PO_4; I.S. 150 mM). At t = 30 s an aliquot of a NaOH solution (20 µL, 0.5 M) was added, and at t = 60 s the anion carrier was added. The blank consists of lipid nanoparticles containing no compound (38 µL). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.