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Electronic Supplementary Information (ESI) for:

Using membrane composition to fine-tune the pK_a of an optical liposome pH sensor

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Contents

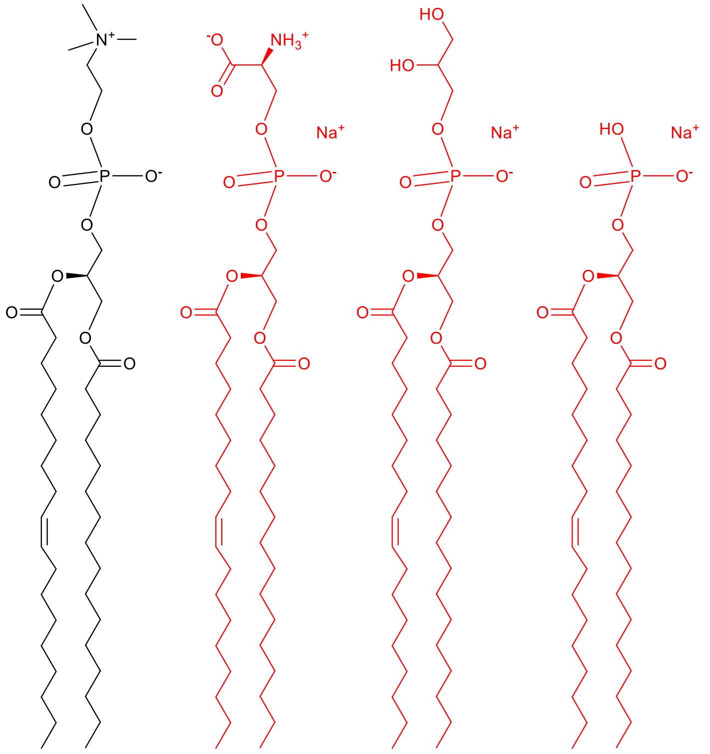
A. Supplemental Table S1 and Figures S1-S5

Table S1. Photophysical data for compounds **1-4** in acidified organic solvent[a] at 21°C

Probe	Absorption λ_{max} (nm)	Emission λ_{max} (nm)	Φ_F[b]
Dye **1**	648	668	0.08
Probe **2**	649	667	0.08
Dye **3**	643	669	0.13
Probe **4**	642	668	0.14

[a] Solvent = methanol containing 2 mM HCl.

[b] Value is based on five measurements for each compound, using both methylene blue (Φ_F = 0.02 in water)[S4] and bis[4-(dimethylamino)phenyl]squaraine (Φ_F = 0.70 in chloroform)[S5] as reference standards.



POPC POPS POPG POPA

Figure S1. Molecular structures of the lipids used in liposome formulations. Neutral lipids are shown in black and anionic lipids are shown in red.

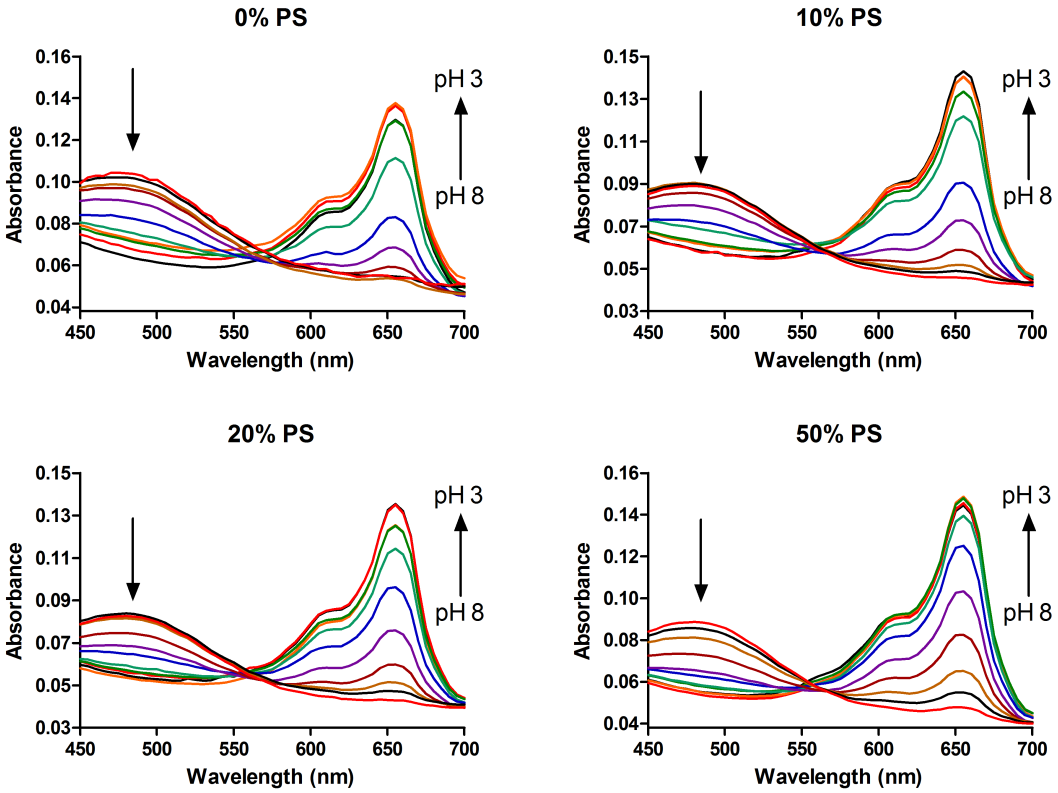


Figure S2. Full pH titration absorbance spectra for probe **2** in liposomes containing varying molar proportions of anionic phospholipid. Data corresponds to curves shown in Figure 2A.

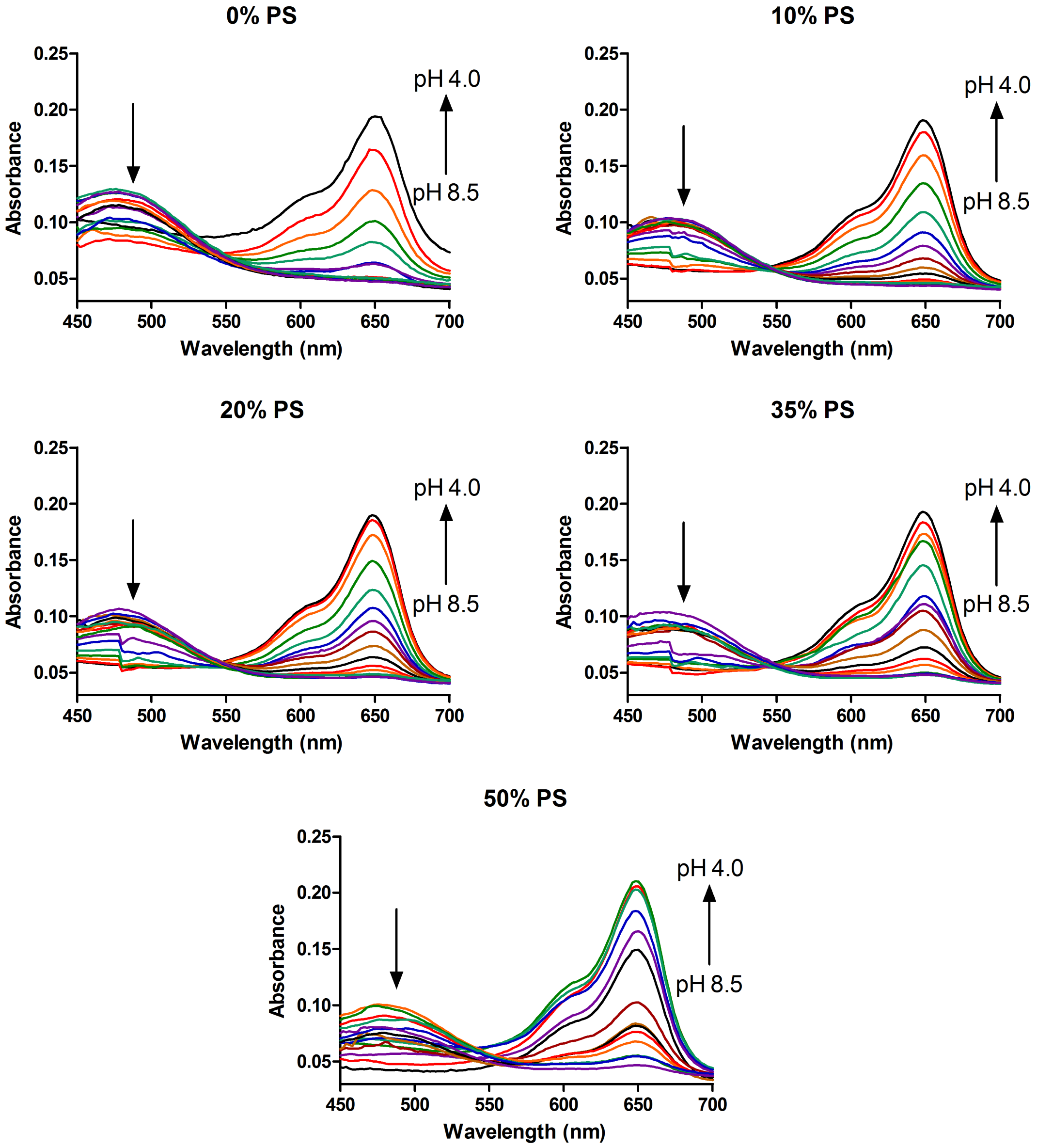


Figure S3. Full pH titration absorbance spectra for probe **4** in liposomes containing varying molar proportions of anionic phospholipid. Data corresponds to curves shown in Figure 2B.

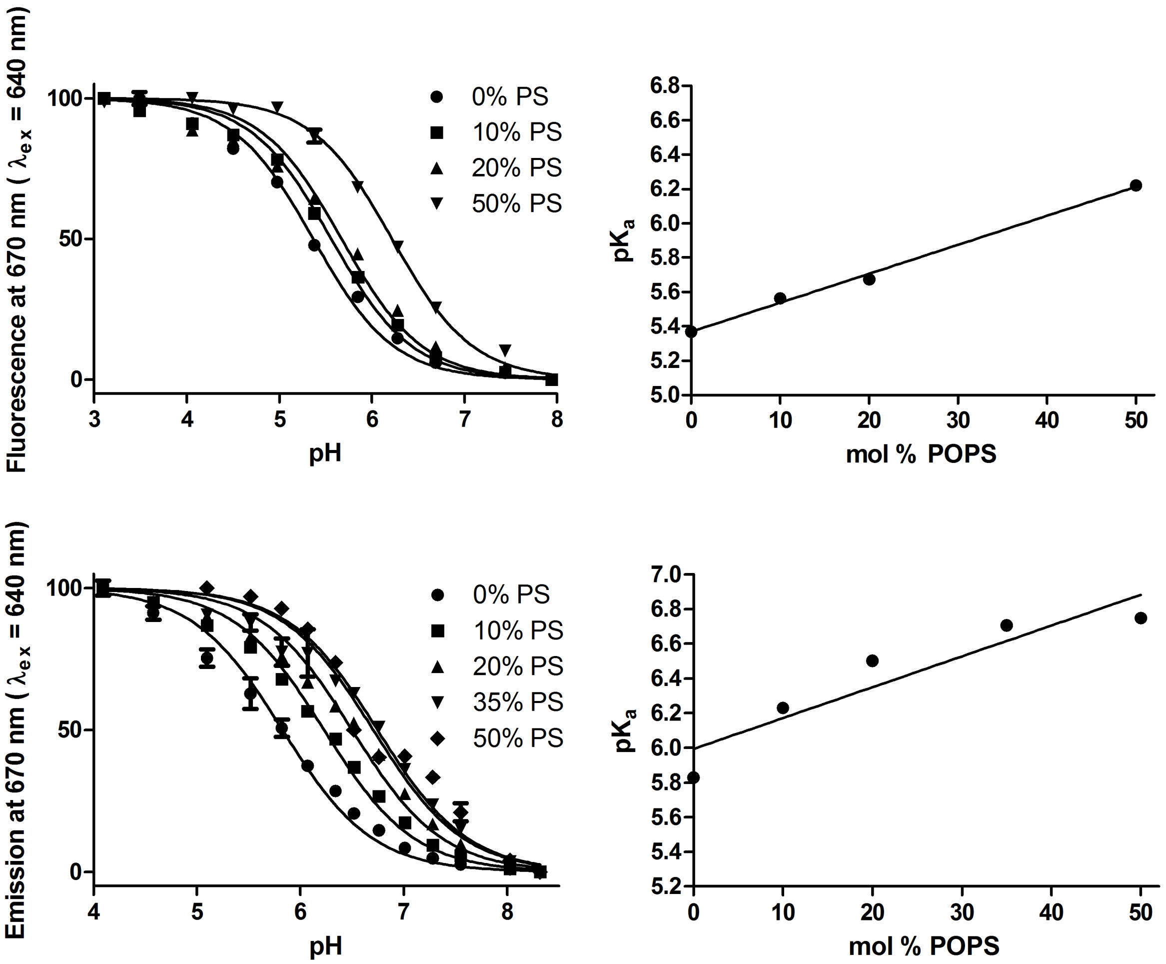


Figure S4. Fluorescence pH titration of probe 2 (*upper*) and probe 4 (*lower*) in the same liposome composition as shown in Figure 2 with a plot of sigmoidal curve fitting for each data set. The graphs on the right show computed pK_a as a function of mol % PS for each probe. Fluorescence excitation was at 640 nm, and emission was followed at 670 nm.

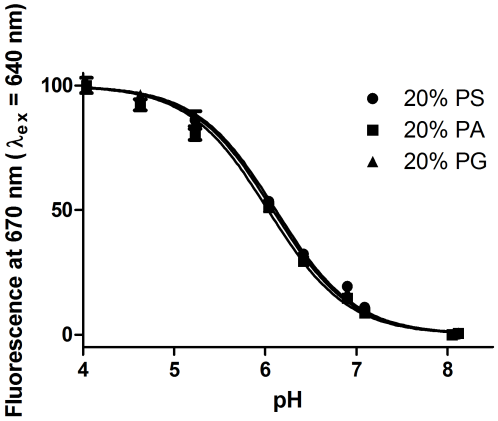
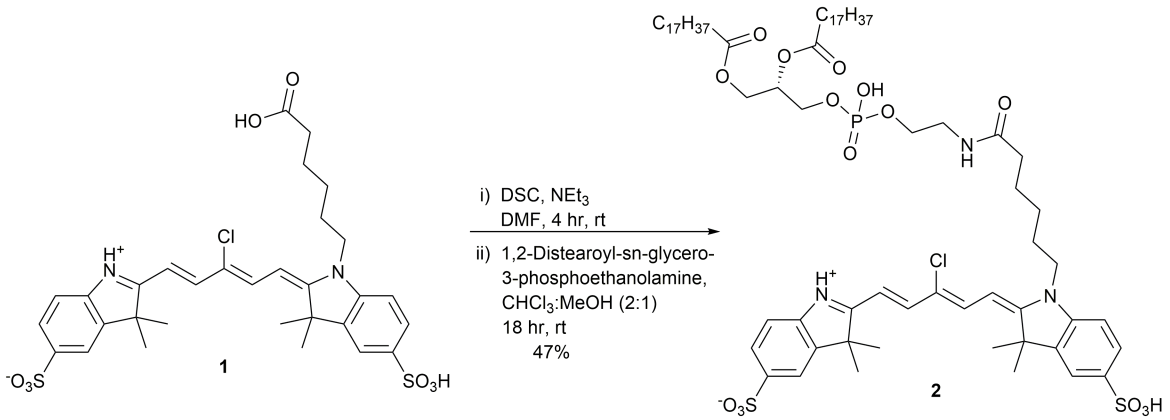


Figure S5. Fluorescence pH titration of probe **2** in the same liposome composition as shown in Figure 3 with a plot of sigmoidal curve fitting for each data set. Fluorescence excitation was at 640 nm, and emission was followed at 670 nm.

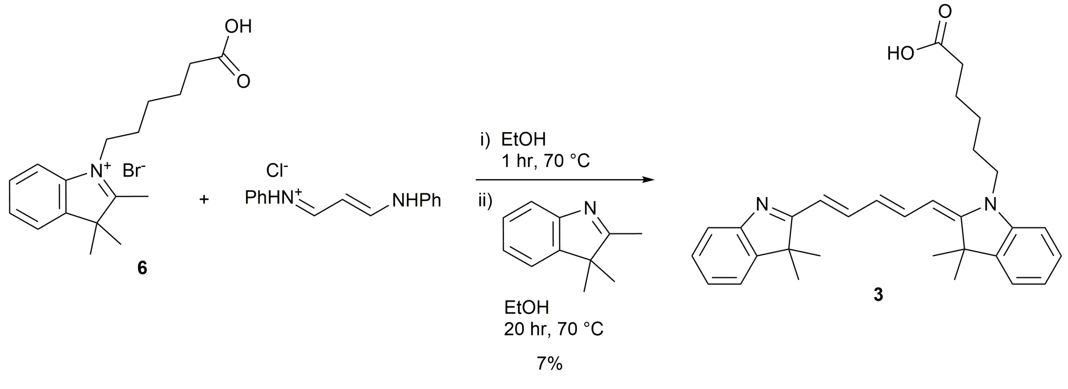
B. Synthesis

Synthesis of 2



*Compound **2***: Cyanine dye **1** was synthesized as previously described.[S2] To 16.2 mg (0.024 mmol) compound **1** in 1 mL DMF was added 9.5 mg (0.037 mmol) *N*,*N'*-disuccinimidyl carbonate and 31 µL (0.22 mmol) triethylamine. The mixture was stirred at room temperature for 4 hours to form the NHS ester. Upon completion (monitored by TLC), 28.6 mg (0.038 mmol) of DPSE was added in a solution of $CHCl_3$:MeOH:H_2O (65:35:8, 10 mL). This reaction was stirred overnight at room temperature. Solvents were evaporated and residue was purified using preparative TLC (SiO_2, 65:35:8 $CHCl_3$:MeOH:H_2O) to yield 15.7 mg (47%) of **2** as a blue solid. ^1H NMR (500 MHz, $CDCl_3$:CD_3OD:D_2O, 7:3:0.1) δ ppm 7.77 (m, 3 H), 7.68 (m, 1 H), 7.63 (m, 1 H), 7.59 (m, 1 H), 7.39 (s, 1 H), 7.32 (m, 1 H), 6.81 (m, 1 H), 6.45 (m, 1 H), 5.86 (m, 1 H), 5.14 (m, 1 H), 4.29 (m, 1 H), 4.03 (m, 6 H), 3.88 (m, 2 H), 3.78 (m, 3 H), 2.22 (d, *J*=7.1 Hz, 4 H), 2.09 (m, 2 H), 1.74 (m, 2 H), 1.54 (s, 12 H), 1.38 (s, 6 H), 1.19 (m, 62 H), 0.79 (t, *J*=6.8 Hz, 6 H). HRMS (ESI-TOF): $[M-2H]^{2-}$ calculated *m/z* for $C_{72}H_{113}ClN_3O_{15}PS_2$: 694.8525; measured 694.8504.

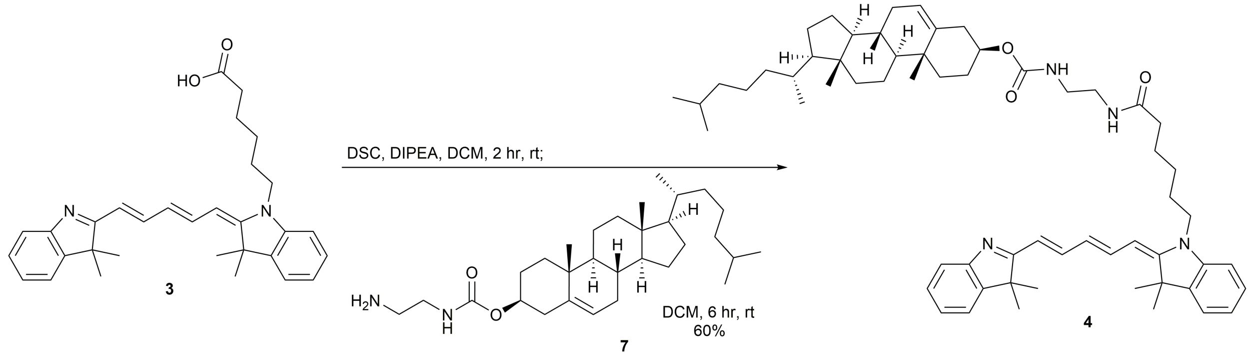
Synthesis of 3



*Compound **3***: Alkylated indolenine **6** and malonaldehyde bis(phenylimine) hydrochloride were both synthesized using published procedures.[S3] 178 mg (0.50 mmol) of **6** and 155 mg (0.60 mmol) of the malonaldehyde were dissolved in 8 mL ethanol and heated to 70 °C for 1 hour. The solution was cooled, 0.25 mL (1.6 mmol) of 1,3,3-trimethylindolenine (Alfa Aesar) was added and heating at 70 °C was resumed for 26 hours. Solvent was removed and product was purified using column chromatography (SiO_2, 1-4% water in acetonitrile, 0.1% acetic acid) to give 16.2 mg (7%) of **3** as a blue solid. ^1H NMR (600 MHz, CD_3OD): δ ppm 7.81 (t, *J*=12.9 Hz, 1 H), 7.65 (t, *J*=13.1 Hz, 1 H), 7.39 (d, *J*=7.3 Hz, 1 H), 7.30 (m, 4 H), 7.17 (m, 1 H), 7.04 (m, 2 H), 6.41 (t, *J*=12.2 Hz, 1 H), 6.18 (d, *J*=14.4 Hz, 1 H), 5.90 (d, *J*=12.9 Hz, 1 H), 3.89 (t, *J*=7.5 Hz, 2 H), 2.28 (t, *J*=7.2 Hz, 2 H), 1.77 (m, 2 H), 1.69 (m, 2 H), 1.63 (s, 6 H), 1.48 (s, 8 H). ^{13}C NMR (151 MHz, CD_3OD): δ ppm 180.9, 177.6, 165.9, 147.6, 147.2, 145.6, 143.3, 143.0, 139.8, 127.9, 127.8, 124.4, 123.6, 122.3, 121.6, 114.9, 108.5, 99.4, 50.6, 42.7, 34.5, 29.3, 26.9, 26.4, 26.1, 24.9, 24.2. HRMS (ESI-TOF): $[M + H]^+$ calculated *m/z* for $C_{31}H_{37}N_2O_2$: 469.2850; measured 469.2821.

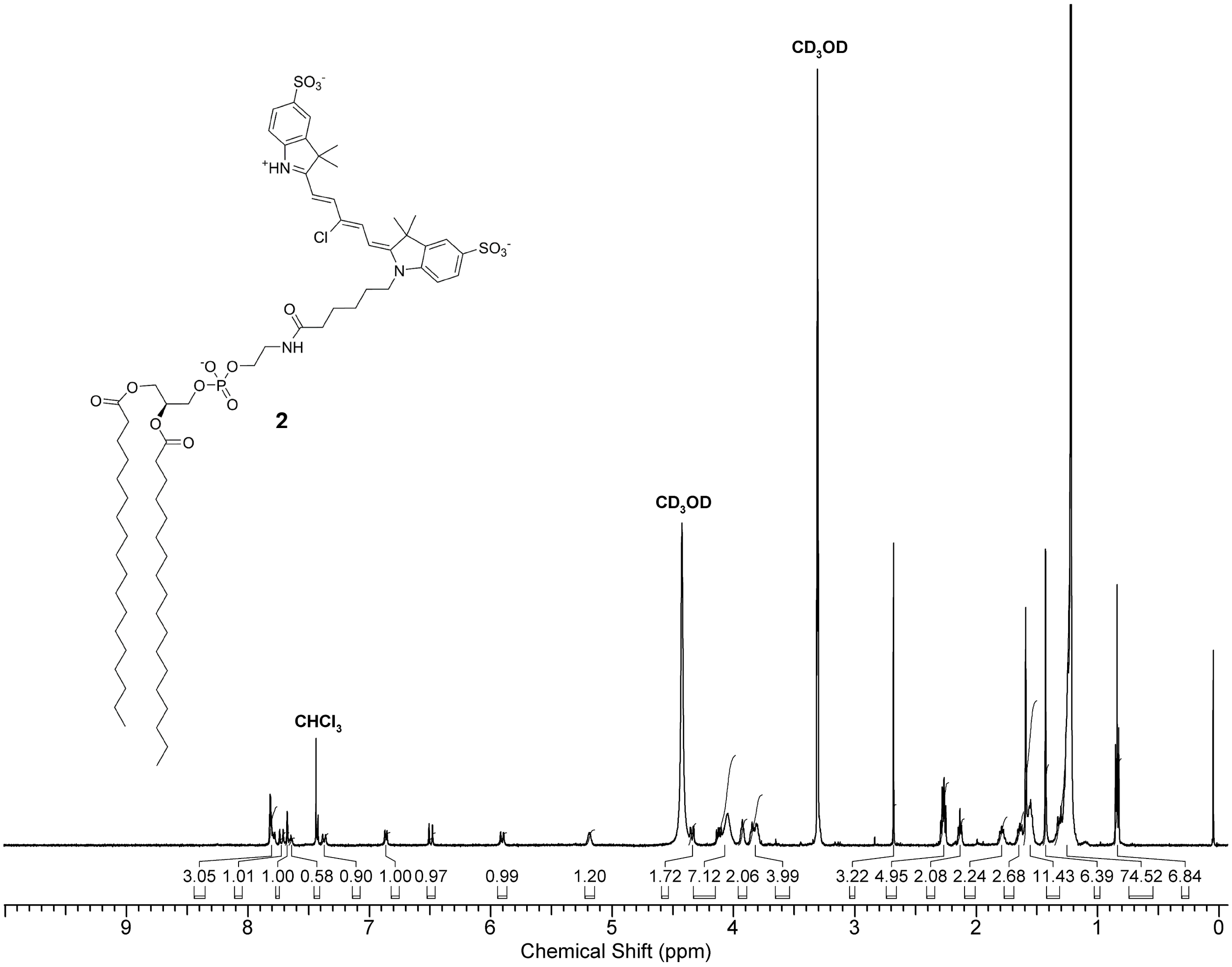
Synthesis of 4

*Compound **4***: *N,N′*-disuccinimidyl carbonate (9.8 mg, 0.038 mmol) and diisopropylethylamine (10 µL, 0.057 mmol) were added to a solution of cyanine **3** (12.3 mg, 0.026 mmol) in dichloromethane (2 mL). The solution was stirred at room temperature for 2 hours until TLC showed complete conversion to NHS ester. Cholesterol amine (**7**)[S4] (19.8 mg, 0.042 mmol) was added and stirred at room temperature for 6 hours until TLC showed complete consumption of NHS ester. Solvent was removed and the crude material was purified using column chromatography to give 15.4 mg (64%) of probe **4** as a dark red solid. ^1H NMR (500 MHz, DMSO-d_6): δ ppm 7.76 - 7.83 (m, 1 H), 7.63 (t, *J*=13.1 Hz, 1 H), 7.37 (d, *J*=7.8 Hz, 1 H), 7.40 (d, *J*=7.3 Hz, 2 H), 7.22 - 7.31 (m, 2 H), 7.10 - 7.19 (m, 2 H), 6.99 (t, *J*=5.1 Hz, 1 H), 6.77 - 6.89 (m, 2 H), 6.28 (d, *J*=15.2 Hz, 1 H), 6.21 (t, *J*=12.7 Hz, 1 H), 5.55 (d, *J*=11.0 Hz, 1 H), 5.30 (br. s., 1 H), 4.30 (t, *J*=11.2 Hz, 1 H), 3.65 (br. s., 2 H), 3.03 - 3.10 (m, 2 H), 2.95 - 3.03 (m, 2 H), 2.26 (d, *J*=13.0 Hz, 1 H), 2.18 (t, *J*=12.5 Hz, 1 H), 2.04 (t, *J*=7.1 Hz, 2 H), 1.83 - 1.98 (m, 2 H), 1.77 (t, *J*=9.2 Hz, 3 H), 1.41 - 1.63 (m, 18 H), 1.26 - 1.41 (m, 16 H), 1.23 (br. s., 3 H), 1.12 (br. s., 1 H), 0.91 - 1.08 (m, 10 H), 0.88 (d, *J*=6.1 Hz, 5 H), 0.84 (d, *J*=6.4 Hz, 8 H), 0.63 (s, 3 H). LC-HRMS (ESI-TOF): $[M + H]^+$ calculated *m/z* for $C_{61}H_{86}N_4O_3$: 923.6773; measured 923.6729.



C. Compound characterization

Compound **2**: ^{1}H NMR (500 MHz, 7:3:0.1 $CDCl_3$:CD_3OD:D_2O); ESI-HRMS (-ve) (*lower*)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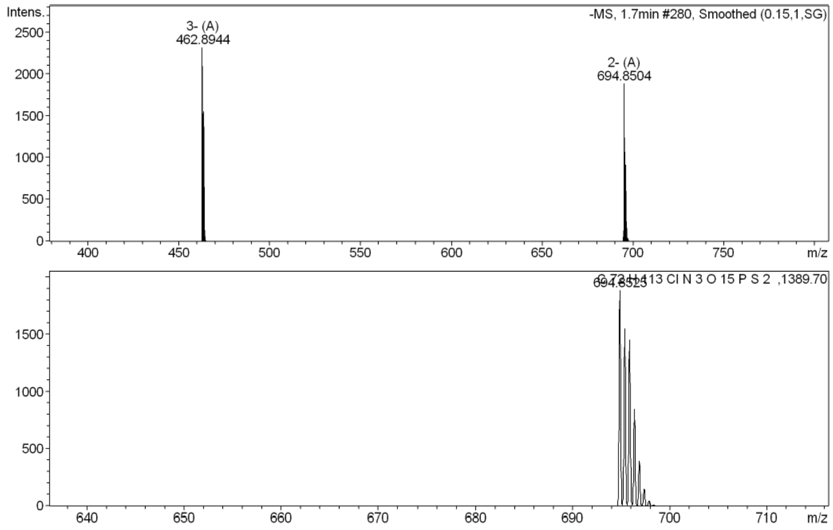
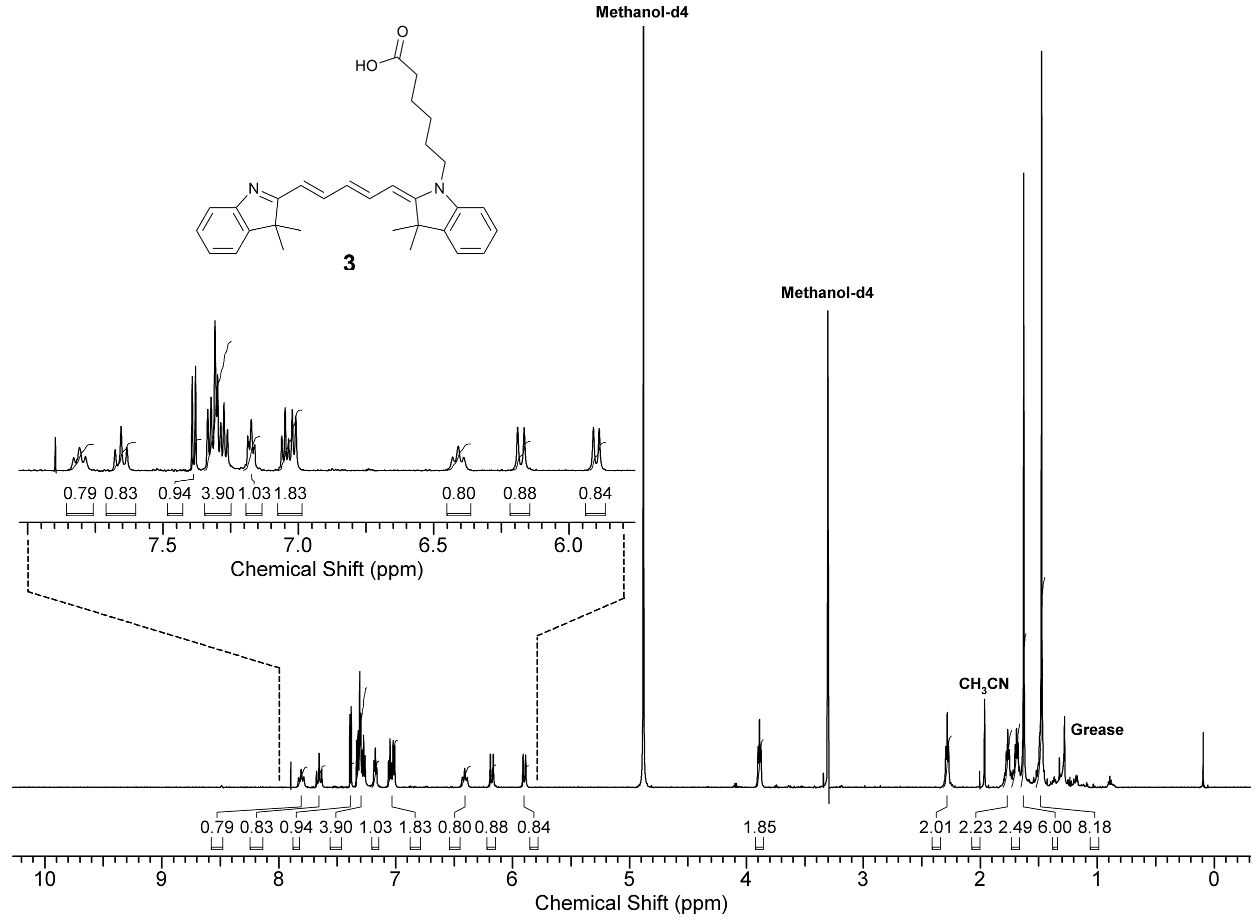


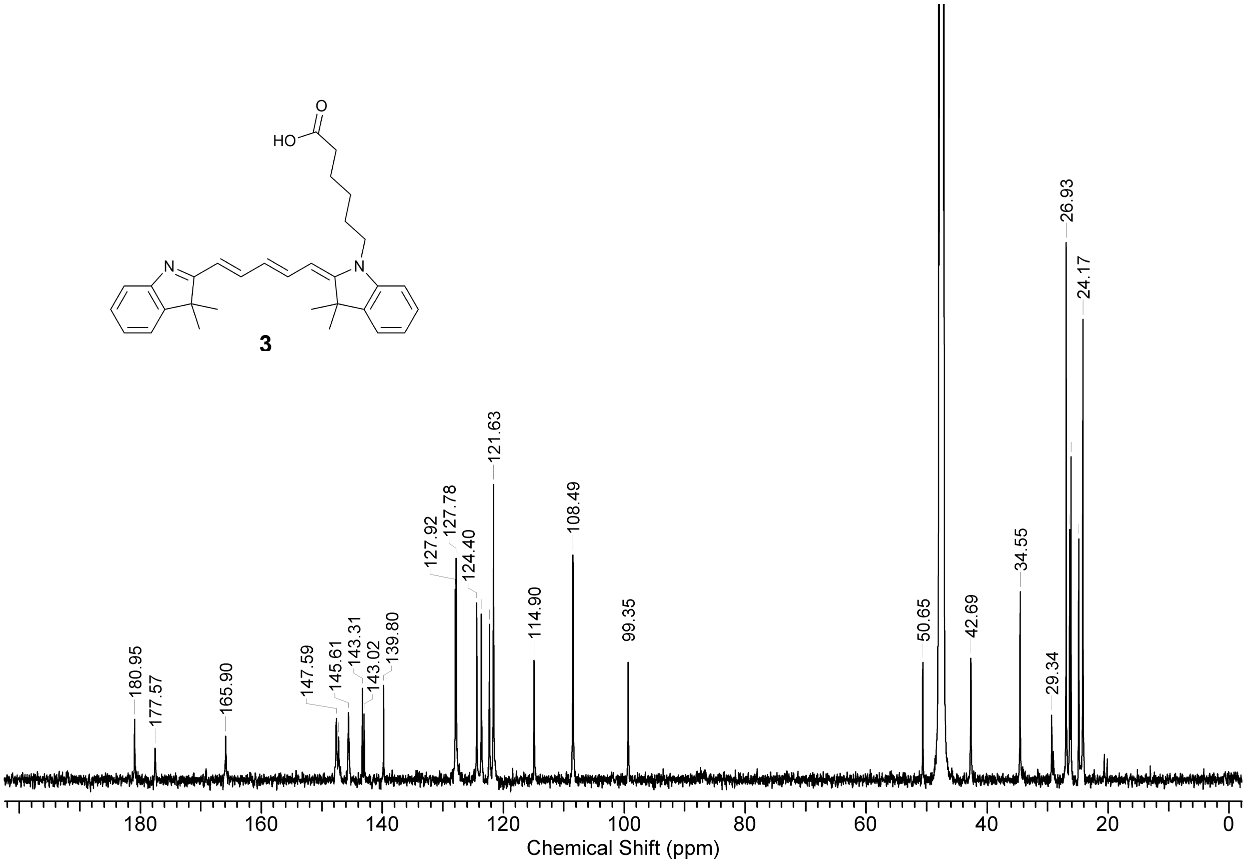
Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e^- Conf
694.8504	1	C 72 H 113 Cl N 3 O 15 P S 2	694.8525	3.1	1.2	18.0	ok	even

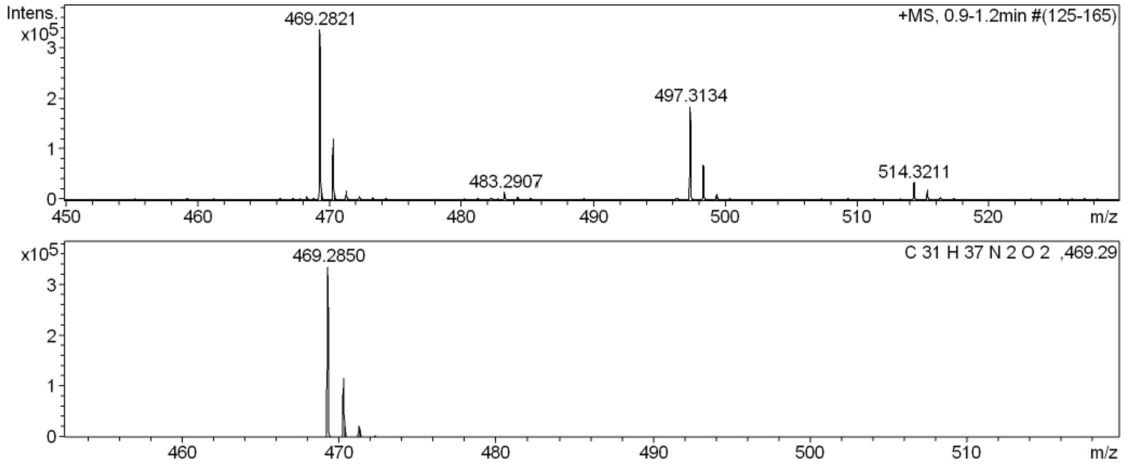
Compound **3**: ^{1}H NMR (600 MHz, CD_3OD)



Compound **3**: ^{13}C NMR (151 MHz, CD$_3$OD); ESI-HRMS (*lower*)

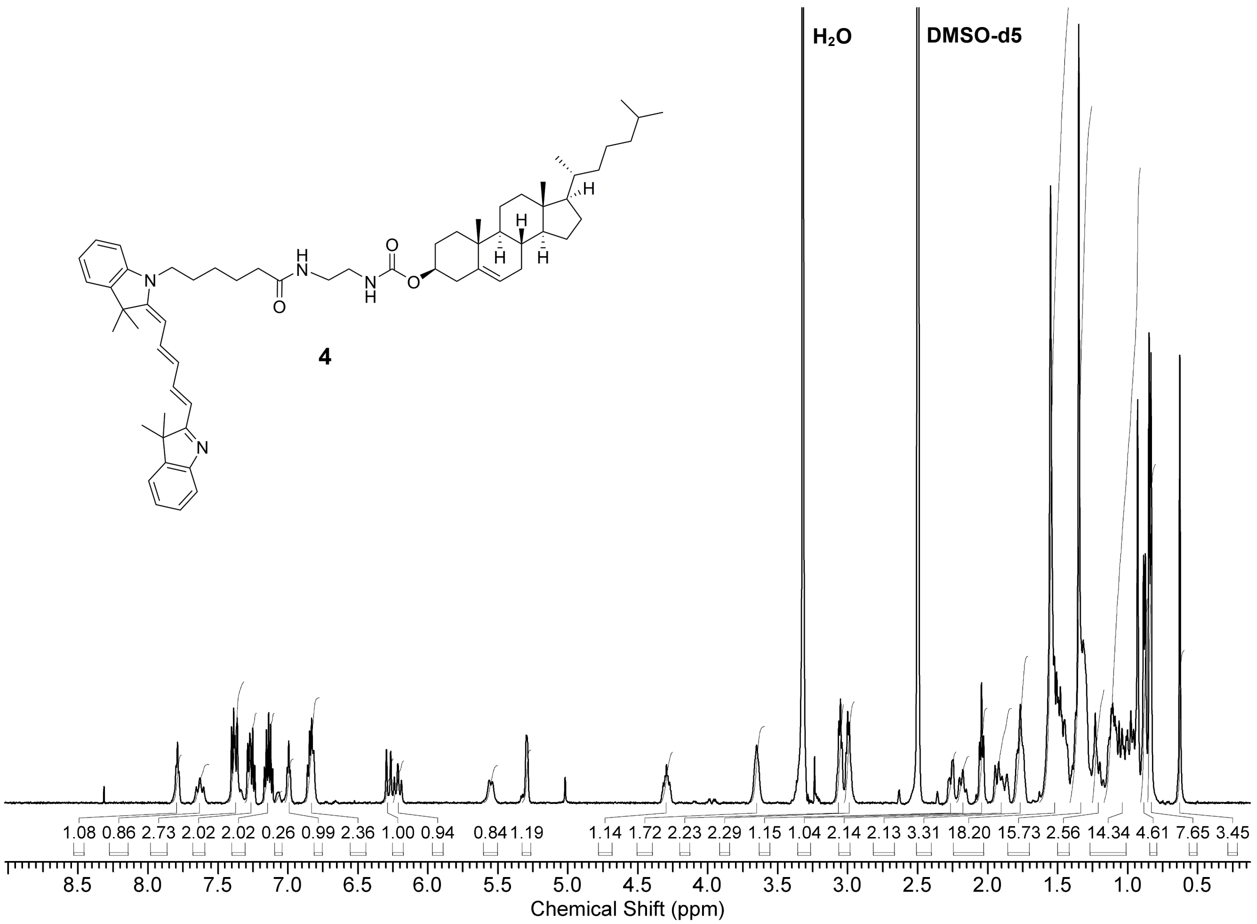
3

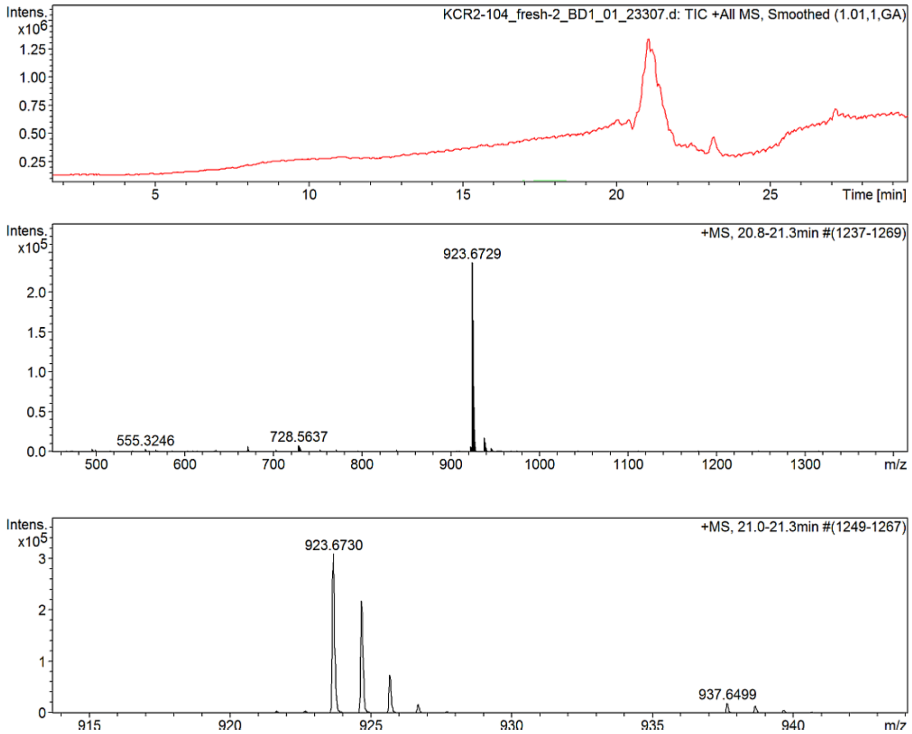




Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
469.2821	1	C 31 H 37 N 2 O 2	469.2850	6.0	7.0	14.5	ok	even

Compound **4**: ^{1}H NMR (500 MHz, CD_3OD); LC-HRMS (*lower*)





D. References

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