Electronic Supplementary Information

Phase partitioning, solvent-switchable BODIPY probes for high contrast cellular imaging and FCS.

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1. Multinuclear NMR and Additional Characterisation

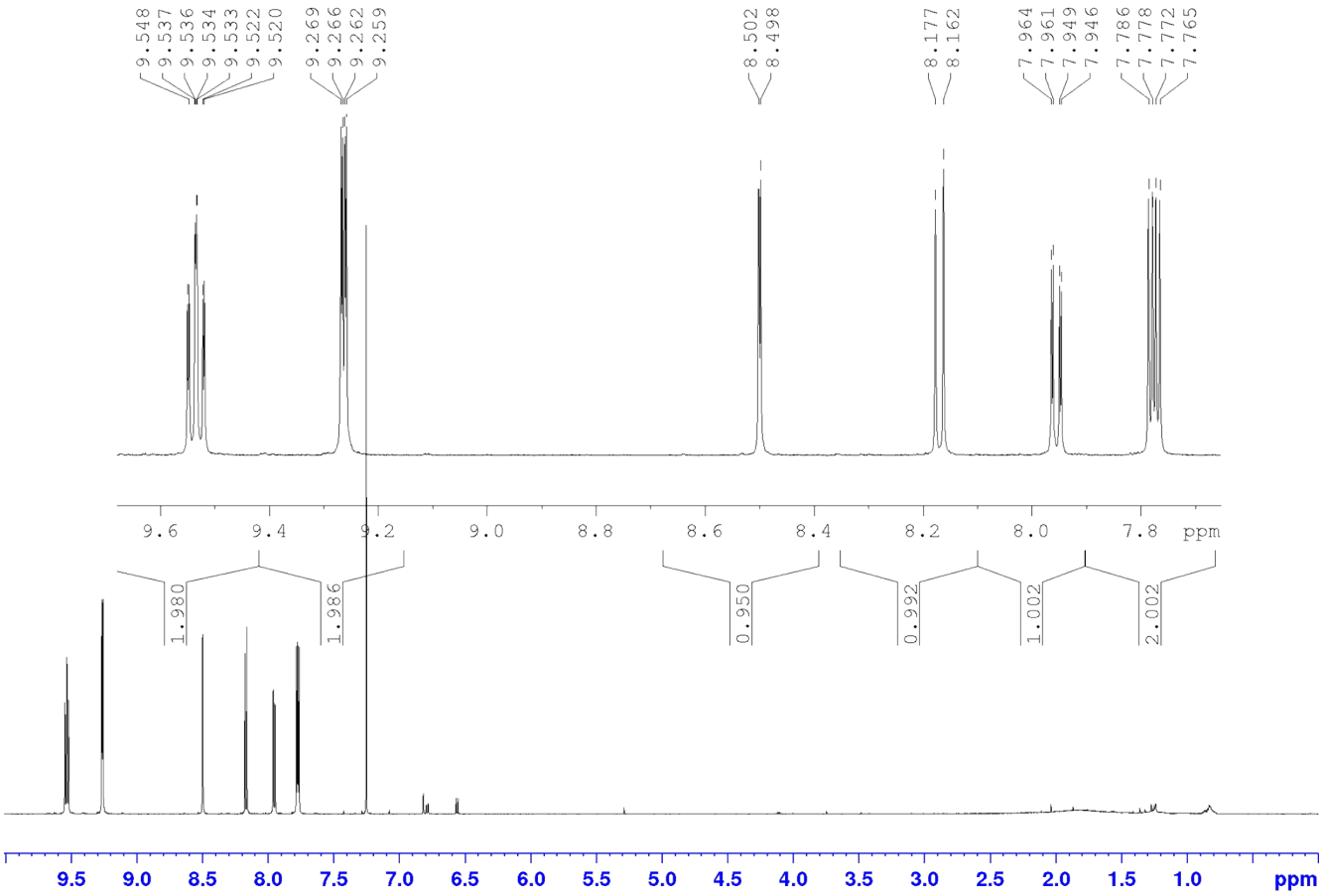

Figure S1 ^{1}H NMR (600 MHz) of **(1)** in $CDCl_3$

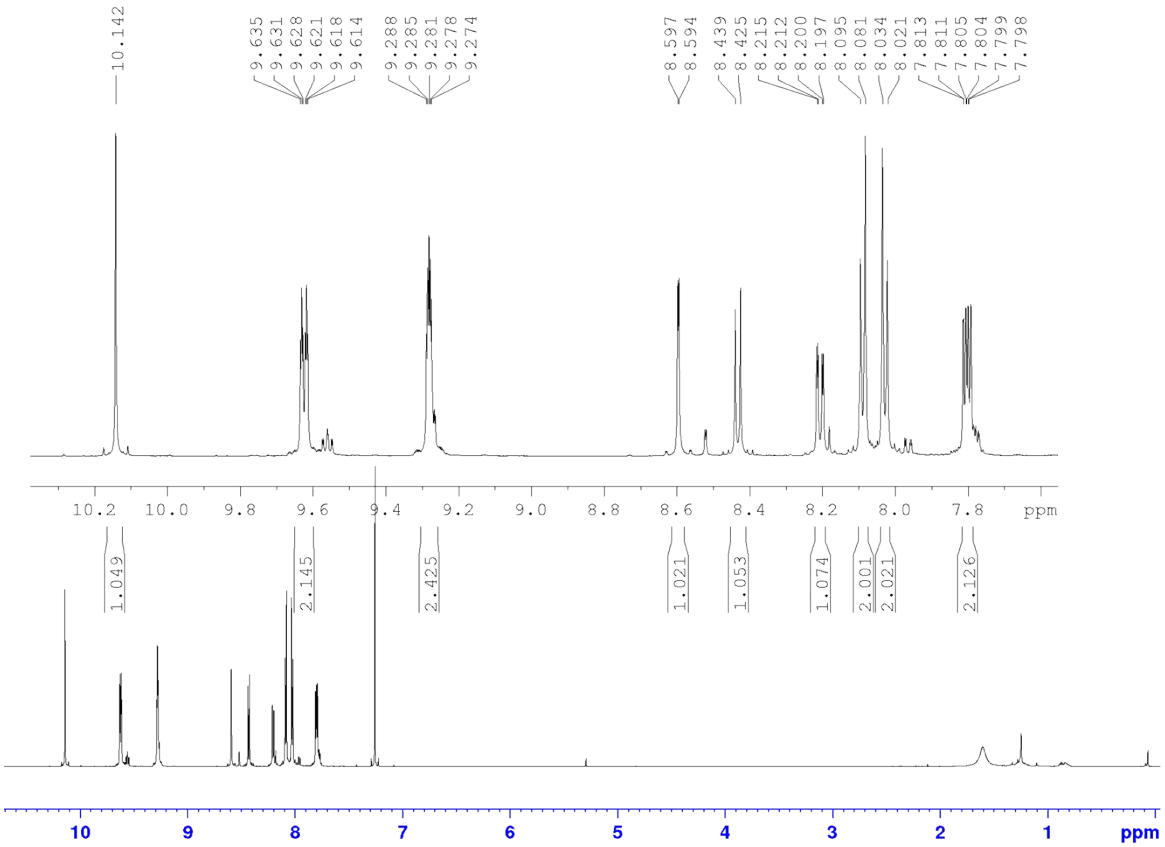

Figure S2 ^{1}H NMR (600 MHz) of **(2)** in $CDCl_3$

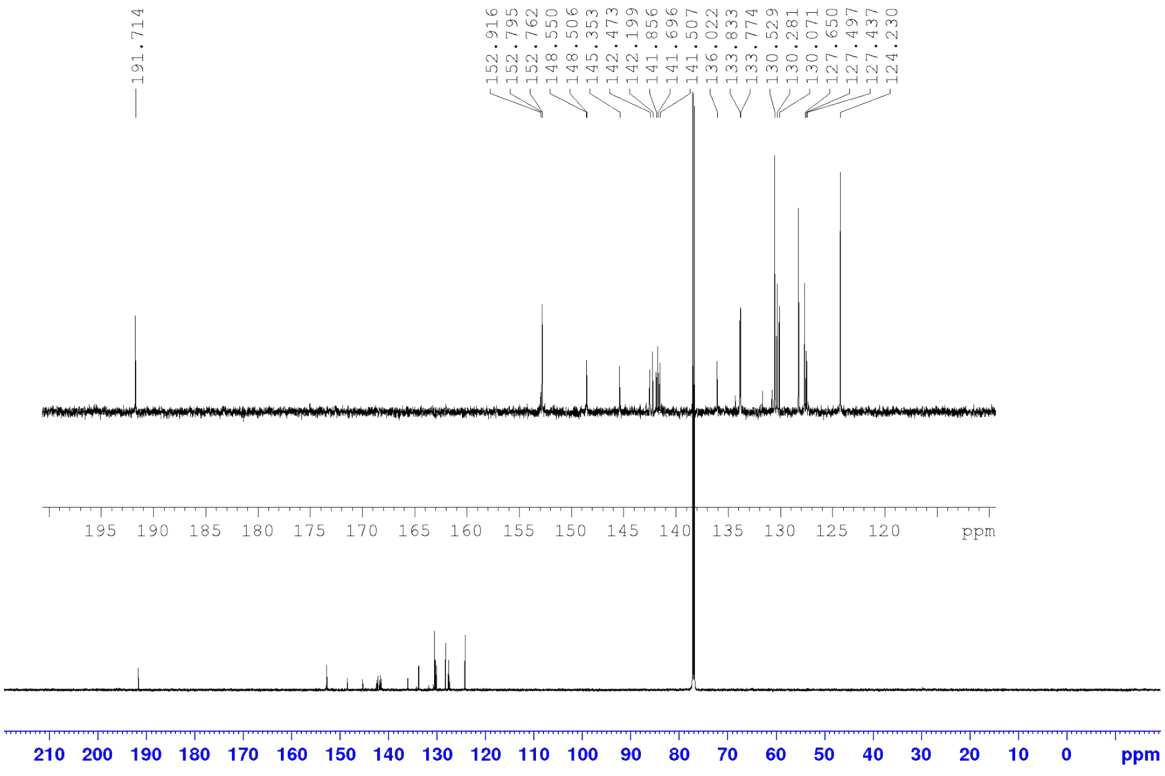

Figure S3 ^{13}C NMR (150 MHz) of **(2)** in $CDCl_3$

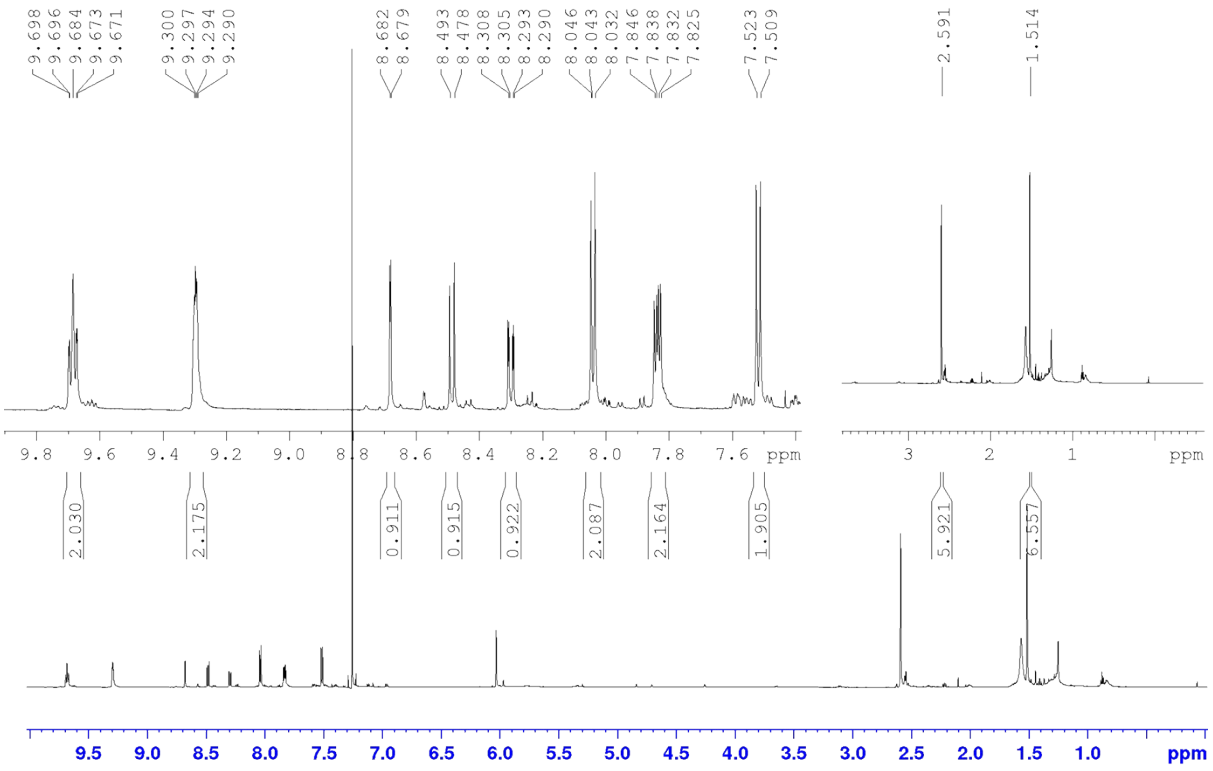

Figure S4 ^{1}H NMR (600 MHz) of **(3)** in $CDCl_3$

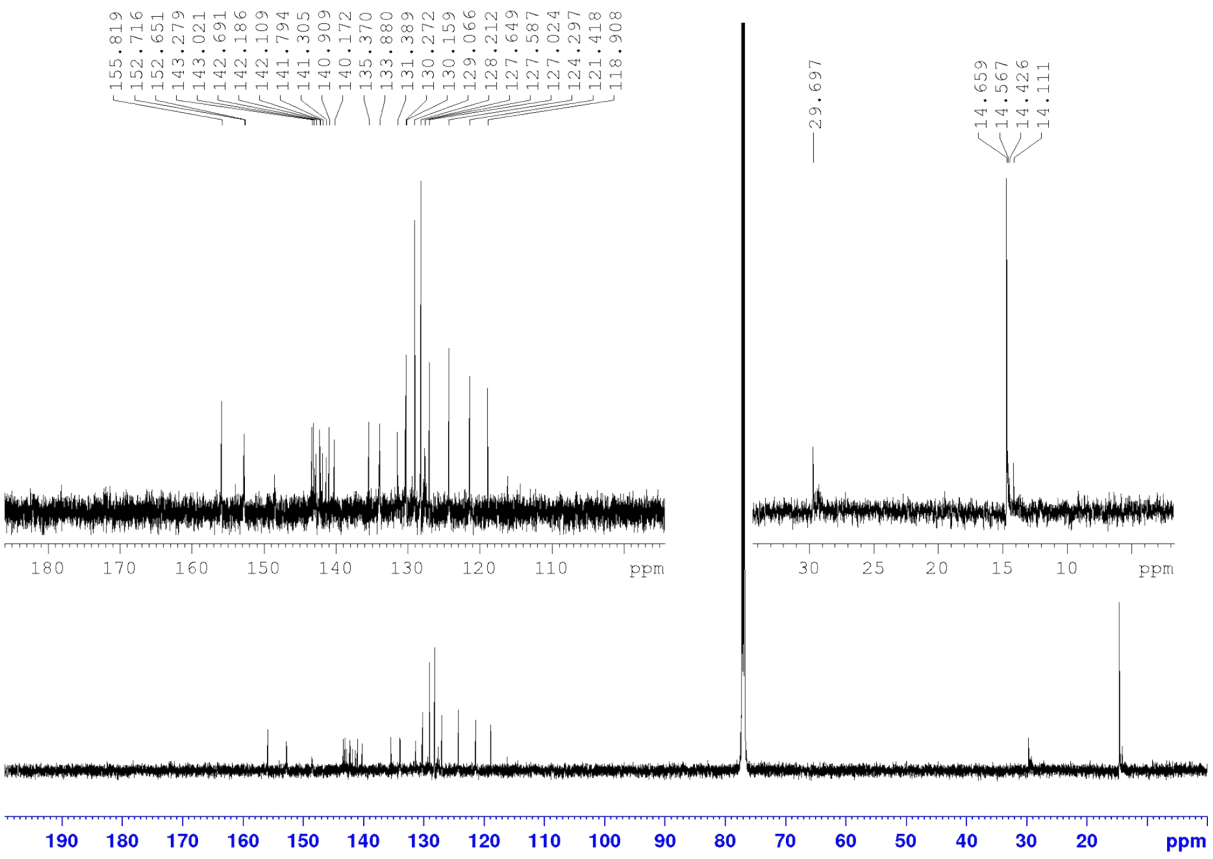

Figure S5 ^{13}C NMR (150 MHz) of **(3)** in $CDCl_3$

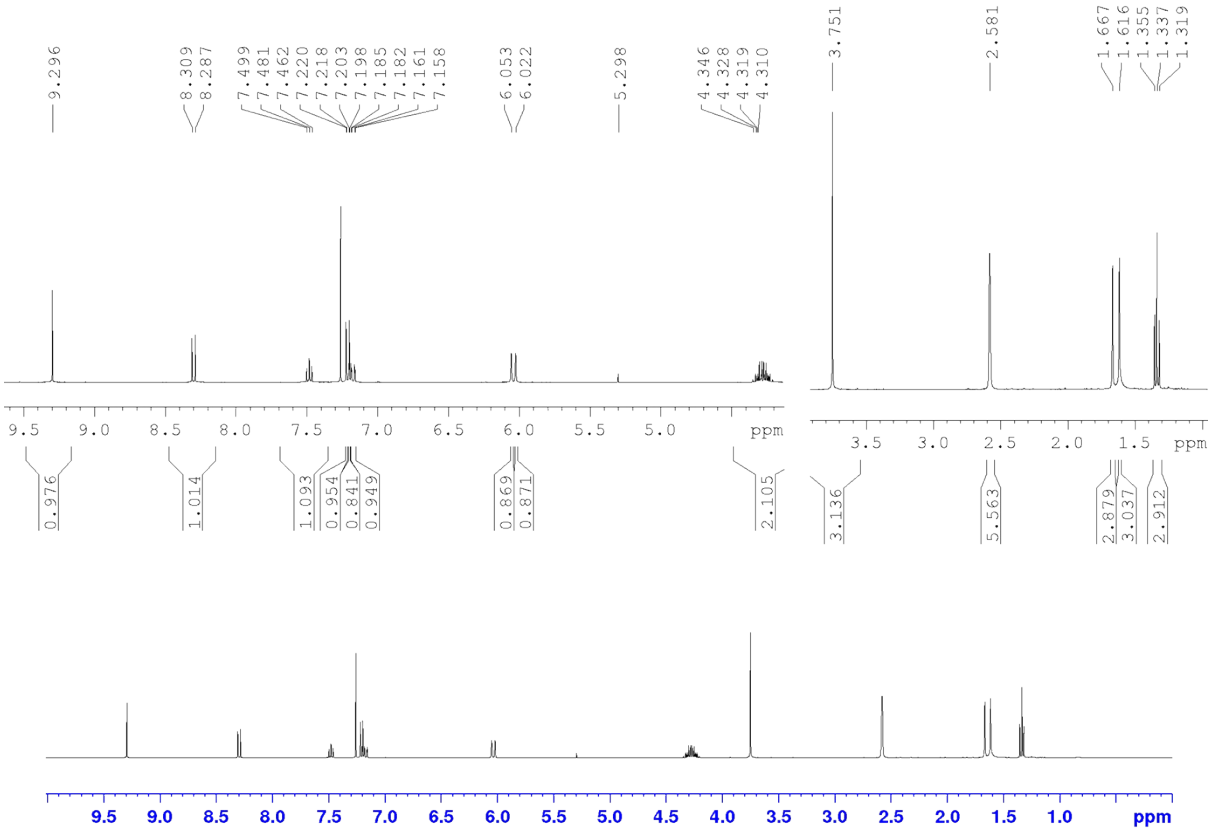

Figure S6 ^{1}H NMR (600 MHz) of **(4)** in $CDCl_3$

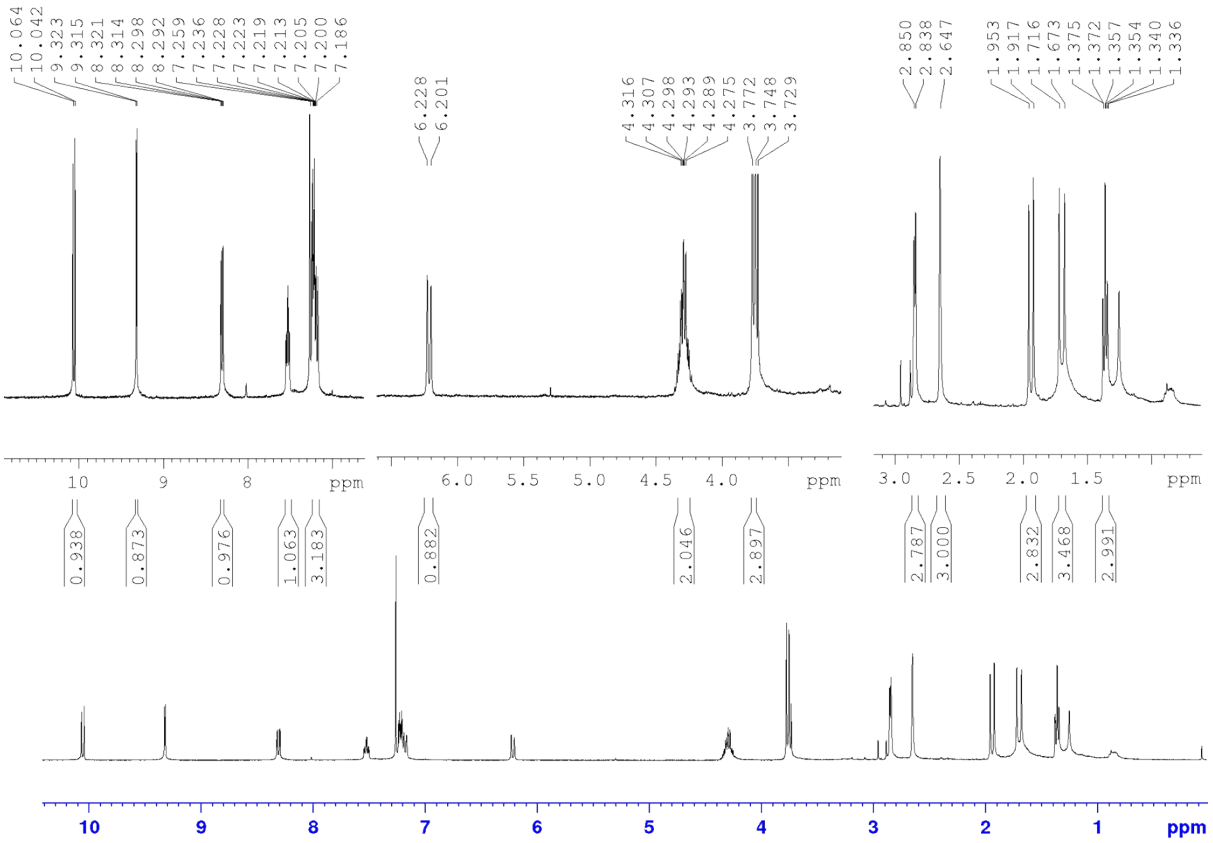

Figure S7 ^{1}H NMR (600 MHz) of **(5)** in $CDCl_3$

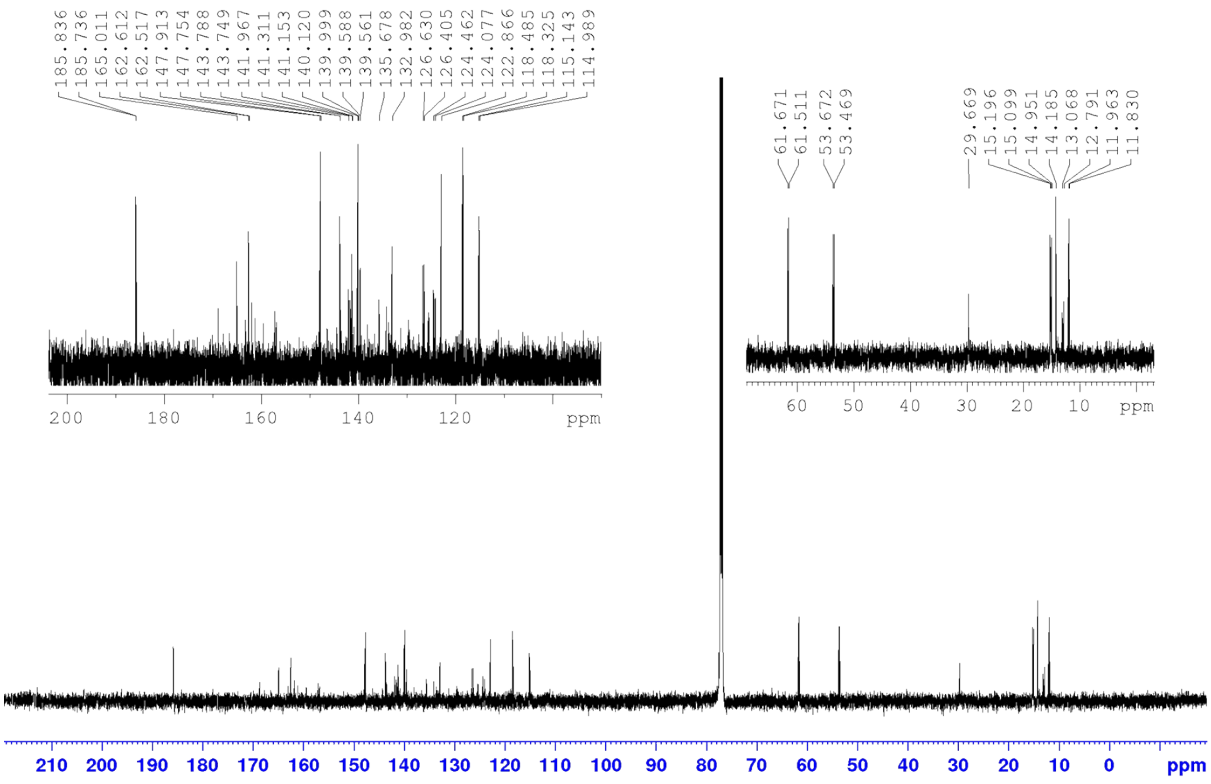

Figure S8 ^{13}C NMR (150 MHz) of **(5)** in $CDCl_3$

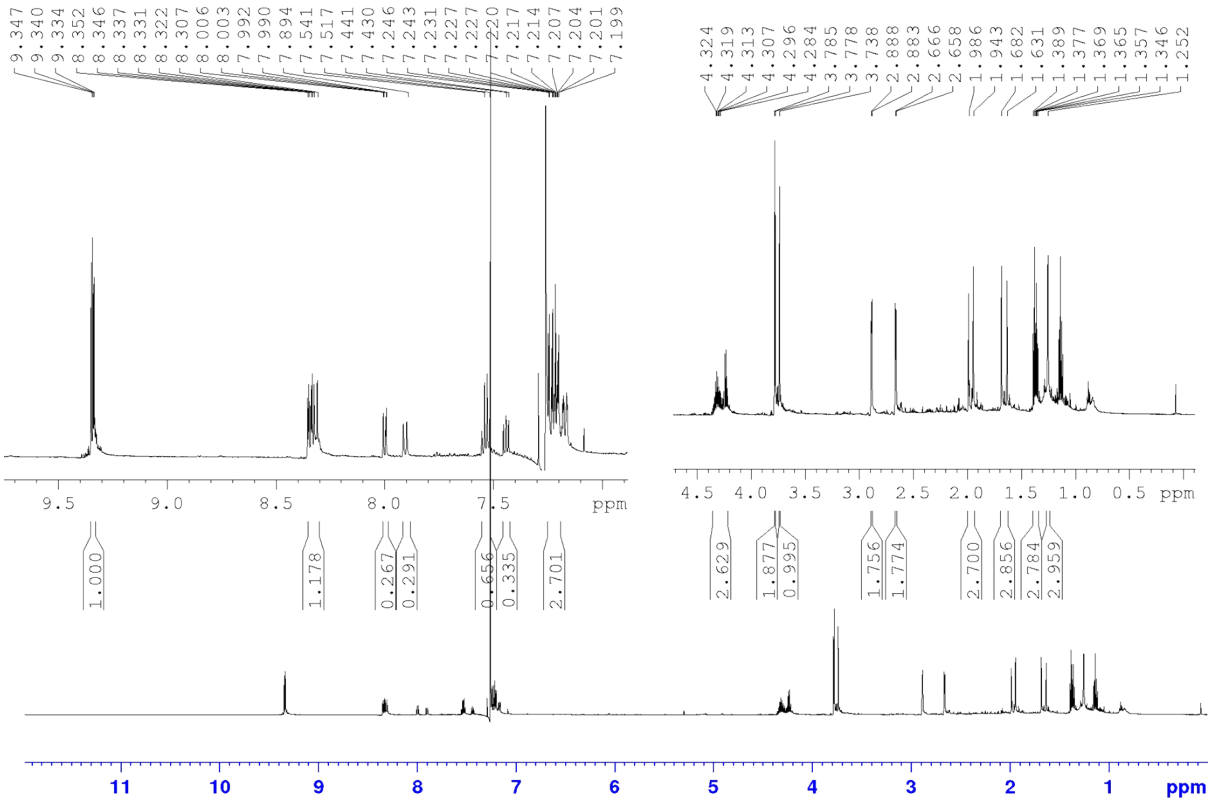

Figure S9 ^{1}H NMR (600 MHz) of **(6)** in $CDCl_3$

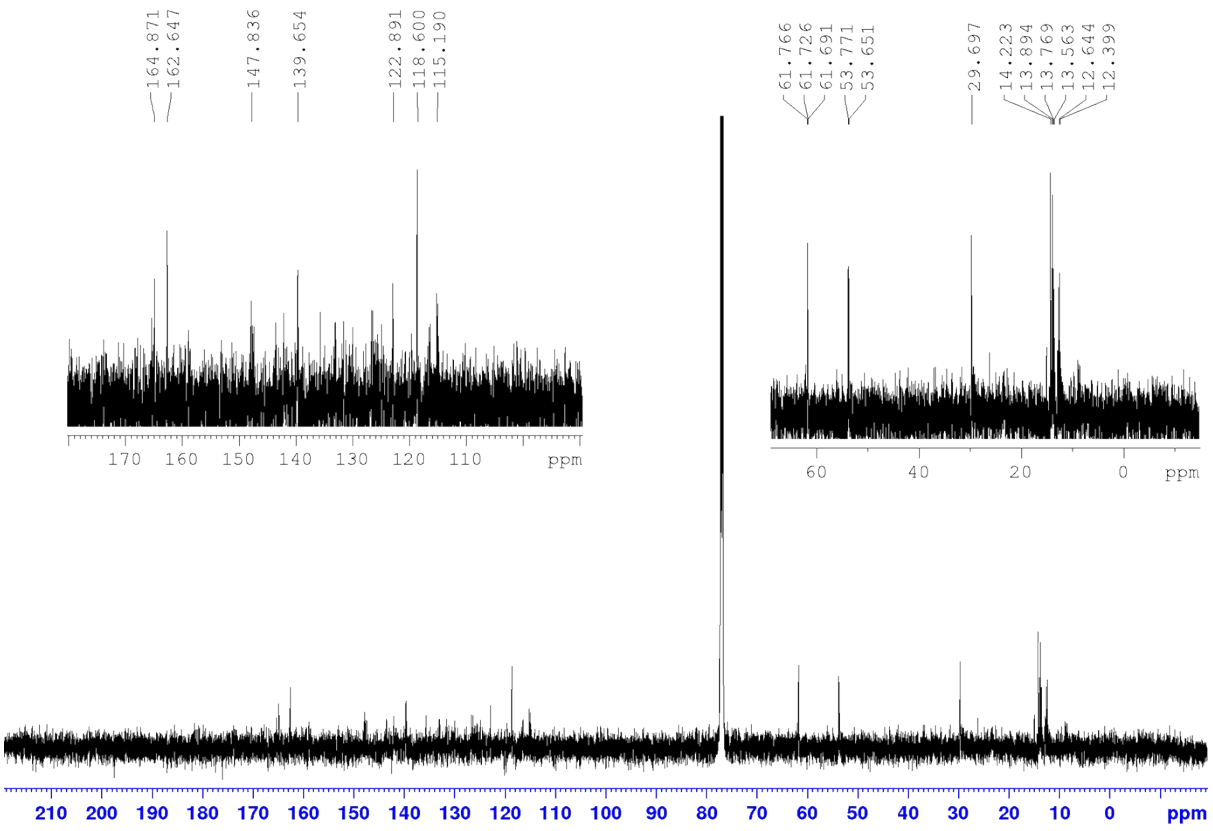

Figure S10 ^{13}C NMR (150 MHz) of **(6)** in $CDCl_3$

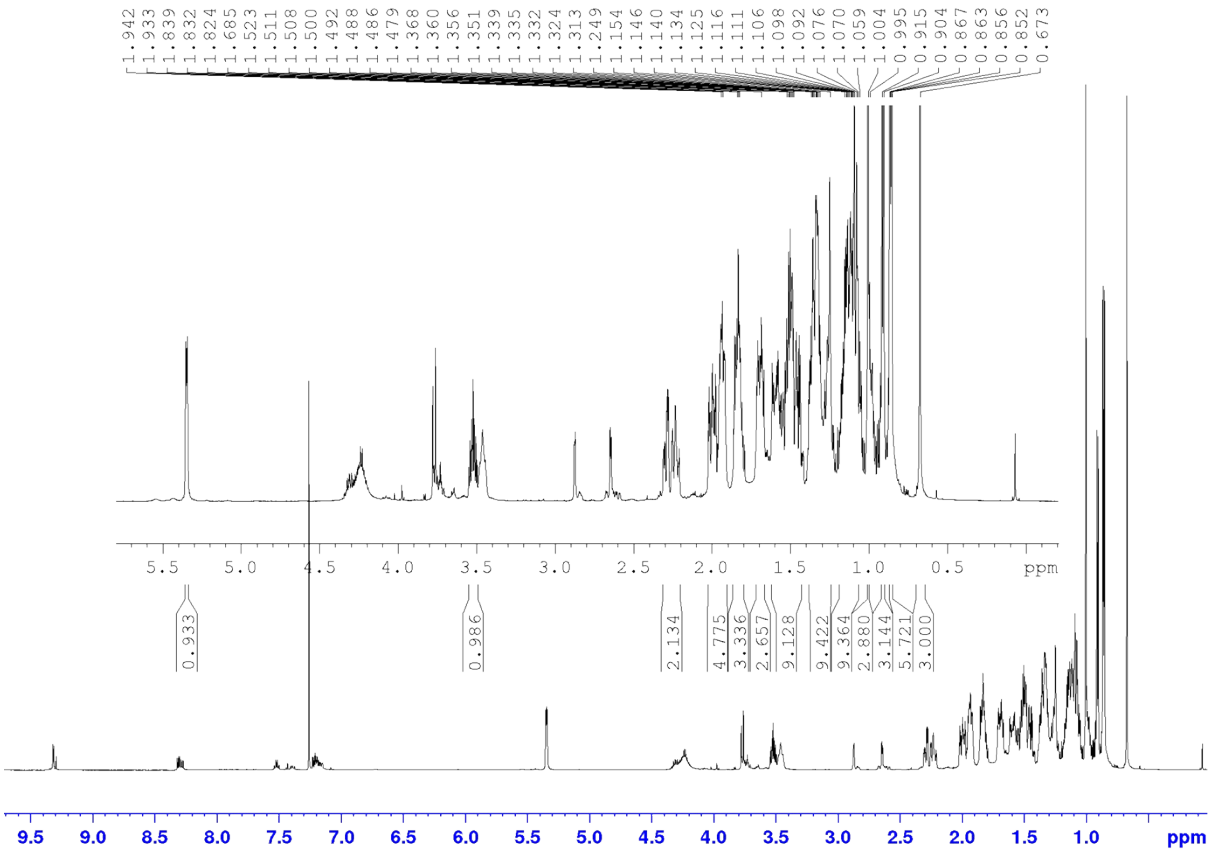

Figure S11 ^{1}H NMR (600 MHz) of **(7)** in $CDCl_3$. Inset Cholesterol peaks accounting for 57H

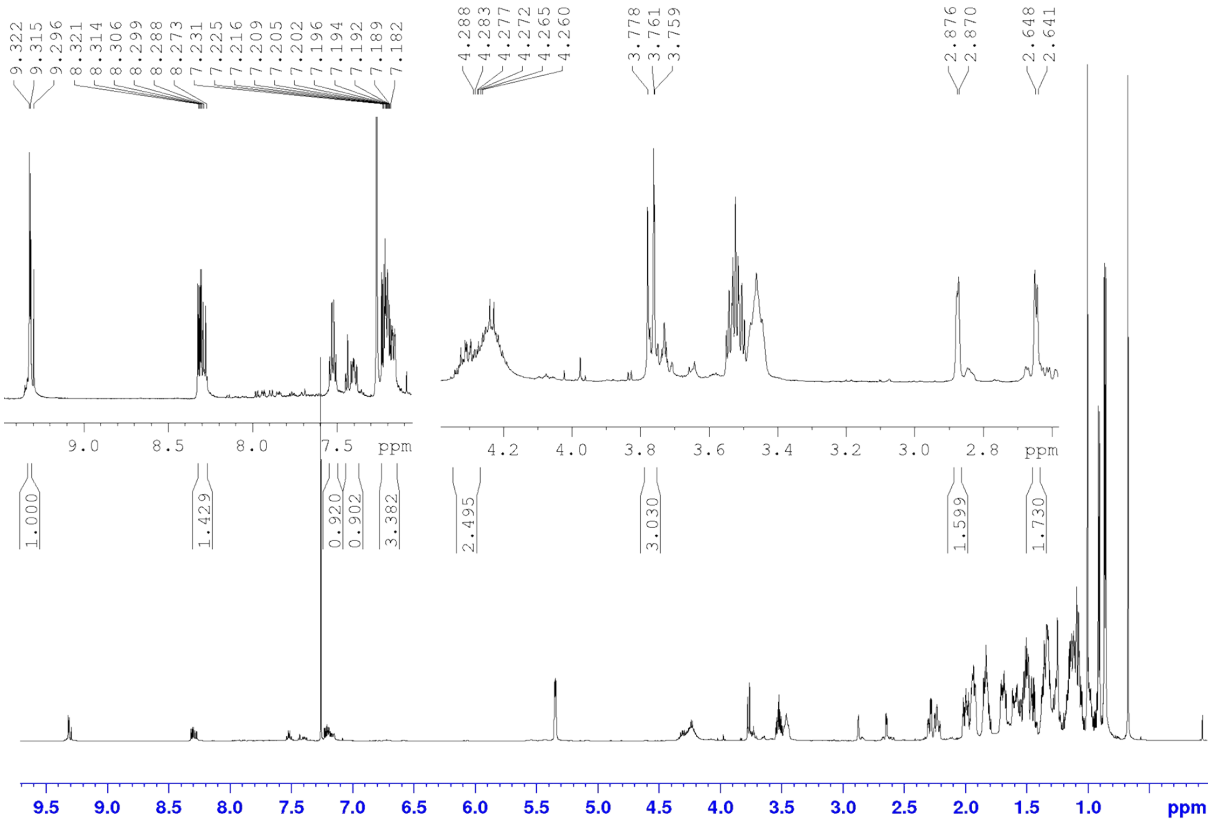

Figure S12 ^{1}H NMR (600 MHz) of **(7)** in $CDCl_3$. Inset Naphthyridyl peaks accounting for 15H

2. Additional Photophysics

For Dppz-Ar-BODIPY an investigation into the effect of water on the luminescent emission was carried out. A 10 µM solution of Dppz-Ar-BODIPY in acetonitrile was prepared. 3 mL of this solution was taken and the luminescent emission of Dppz-Ar-BODIPY was obtained in acetonitrile. Water was titrated into the sample, agitated and left to stand. Following this the emission spectrum was obtained. This was repeated in 30 µL titrations until 10 % of the sample volume had been added. The results showed that a 50 % decrease in emission intensity was observed upon adding 300 µL of water.

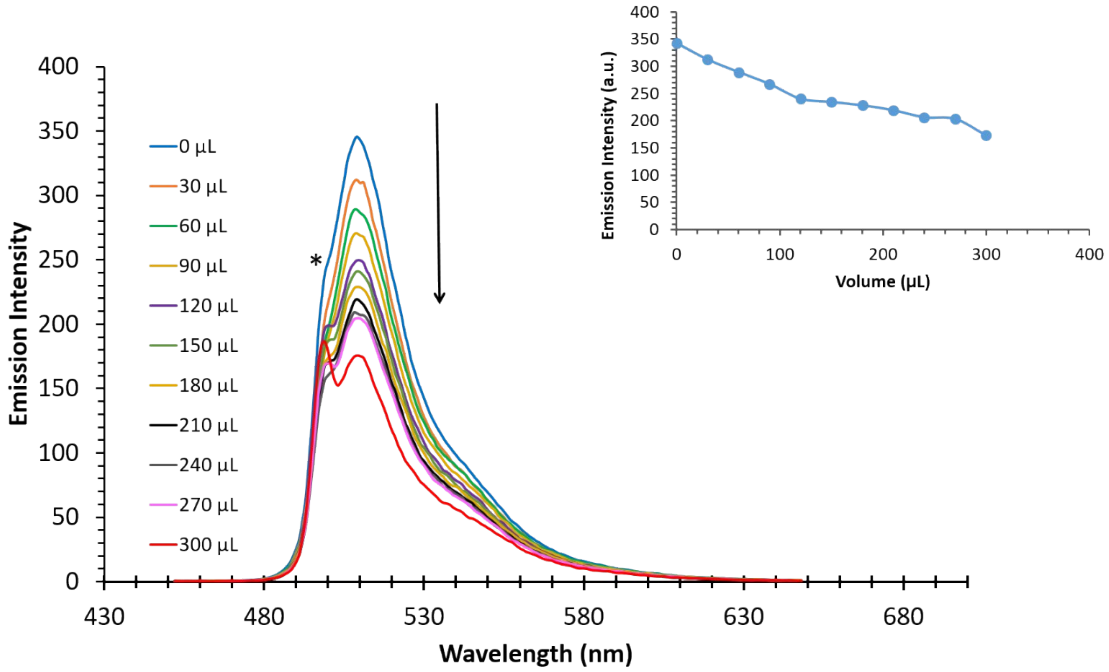

Figure S13 Emission spectra of Dppz-Ar-BODIPY **(3)** acetonitrile 5 mL (λex 501 nm, 10 µM, slit widths 2.5 nm). A total volume of 300 µL of water was titrated, resulting in a volume change of 6 %, shown in the graph inset. * indicates contribution from the excitation source.

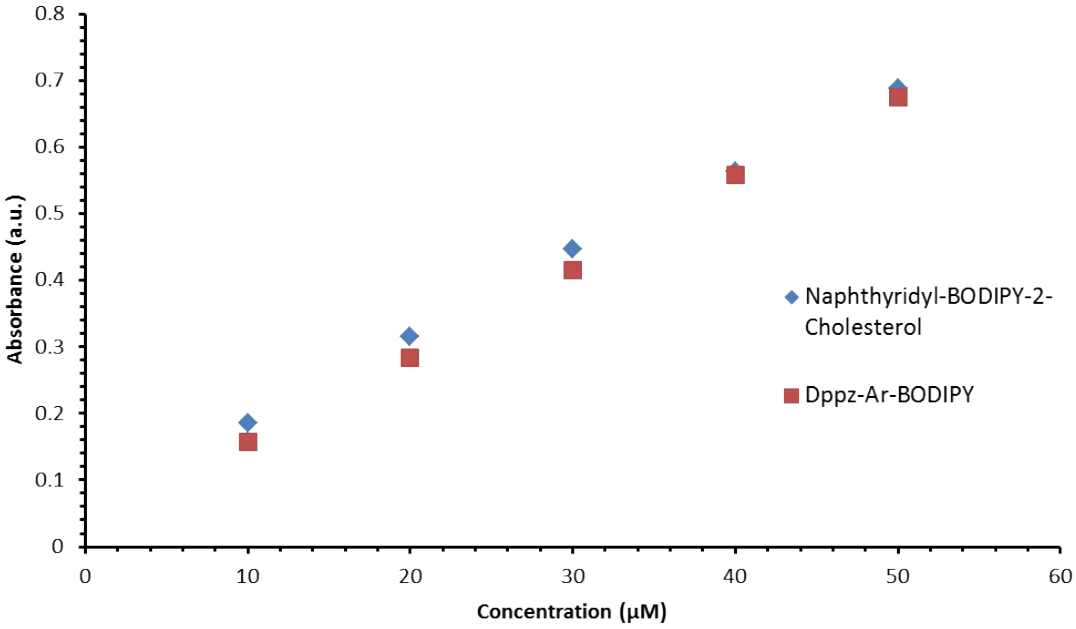

Figure S14 Concentration dependence study of Dppz-Ar-BODIPY **(3)** and Naphthyridyl-BODIPY-2-Cholesterol **(7)** in acetonitrile.

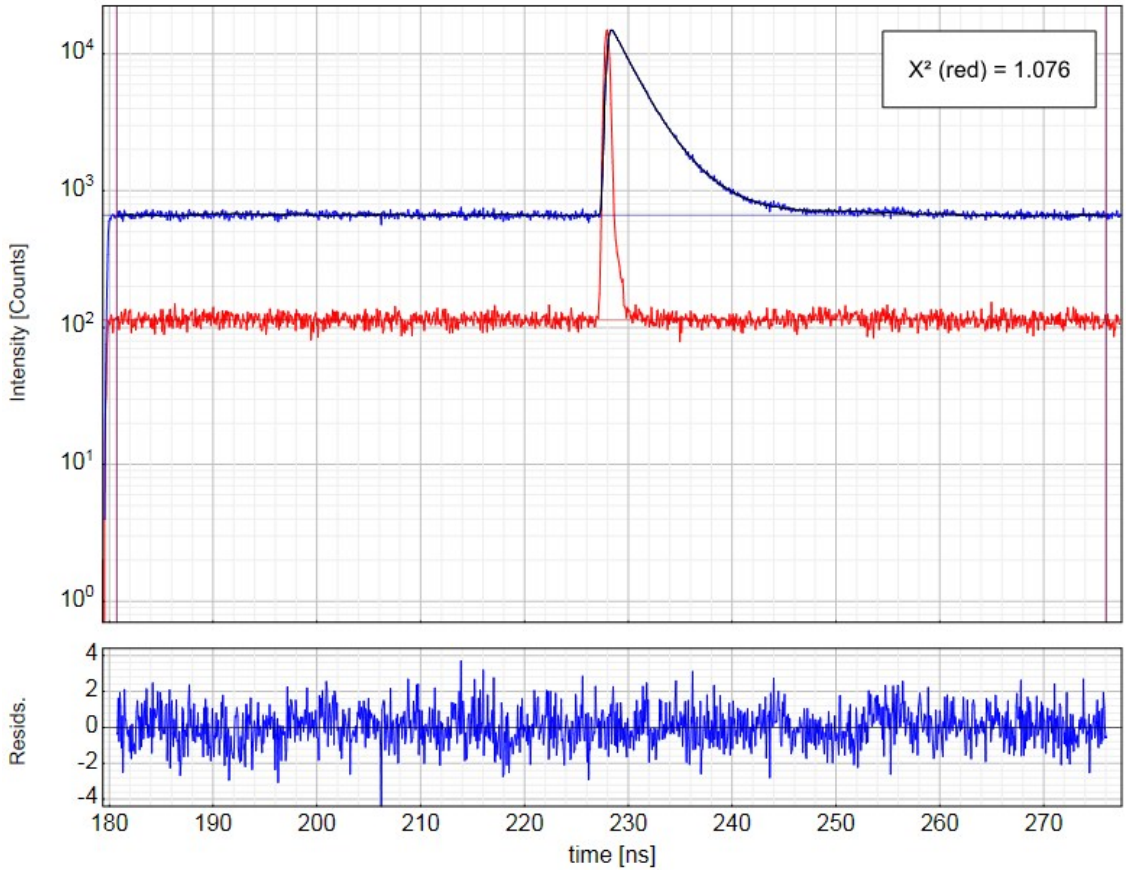

Figure S15 Time correlated Single Photon counting trace for Dppz-Ar-BODIPY **(3)** in Chloroform (10 µM) with IRF (red) at room temperature.

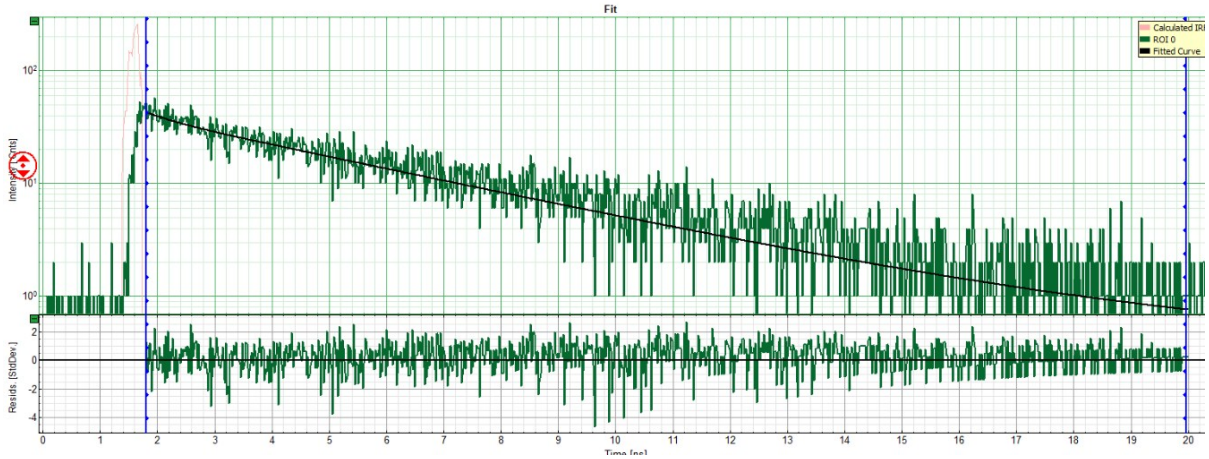

Figure S16 Fluorescent lifetime imaging decay for Naphthyridyl-BODIPY-2-Cholesterol **(7)** in GUVs DOPC/BSM/Chol 4:4:2 mol % (10 µM)

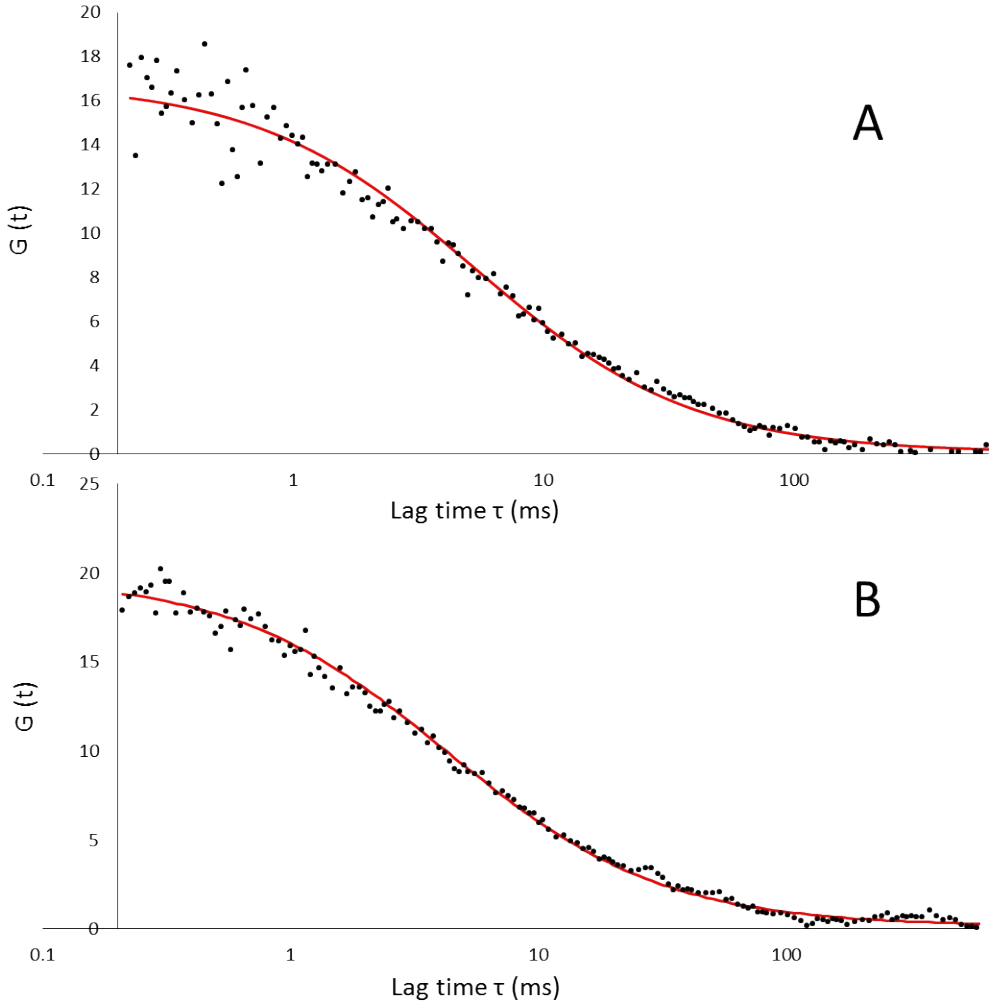

Figure S17 A - Fluorescent correlation spectroscopy autocorrelation function for Dppz-Ar-BODIPY in GUVs DOPC/BSM/Chol 4:4:2 mol % at concentration 0.001 mol % at room temperature. B

Naphthyridyl-BODIPY-2-Cholesterol **(7)** in GUVs DOPC/BSM/Chol 4:4:2 mol % at concentration 0.001 mol % at room temperature.

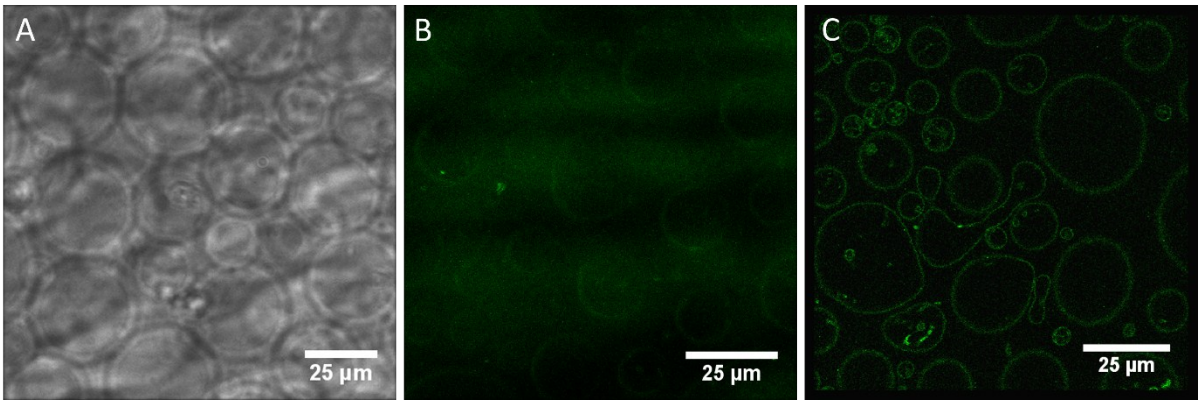

Figure S18 Uptake of Dppz-Ar-BODIPY in GUVs of composition DOPC 100 %. Dppz-Ar-BODIPY in $CHCl_3$ was added to a solution of GUVs in glucose 230 mM to give a final concentration of 10 µM. Uptake was monitored over a 105-minute period using confocal microscope and images after A) 60, B) 75, and C) 105 minutes.

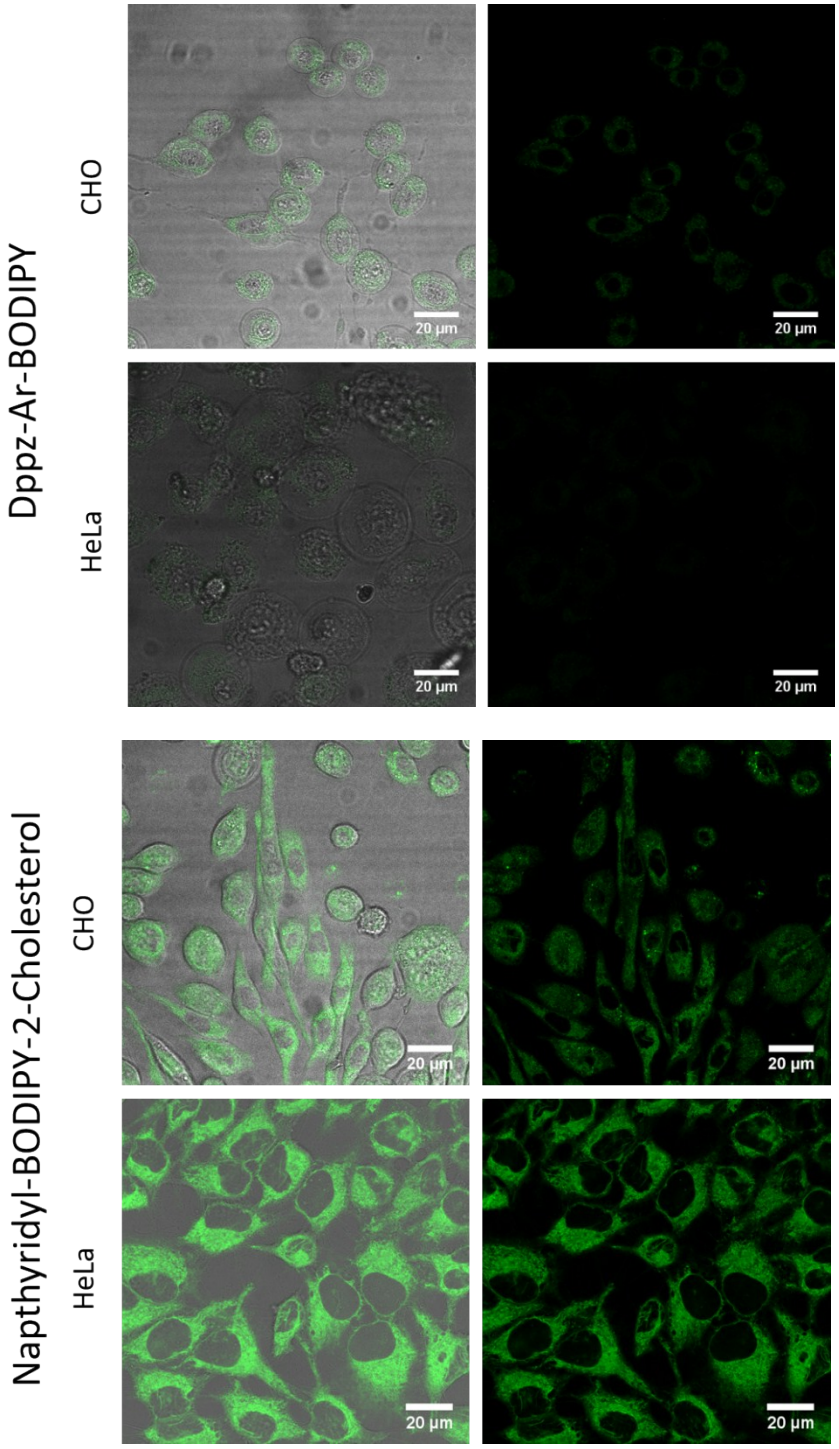

Figure S19 Confocal imaging of CHO and HeLa cells incubated with 5 µM Dppz-Ar-BODIPY and Napthyridyl-BODIPY-2-Cholesterol compound at 4°C for 24 h, showing the overlay of the BODIPY and background channels (column 1) and the BODIPY channel only (column 2).

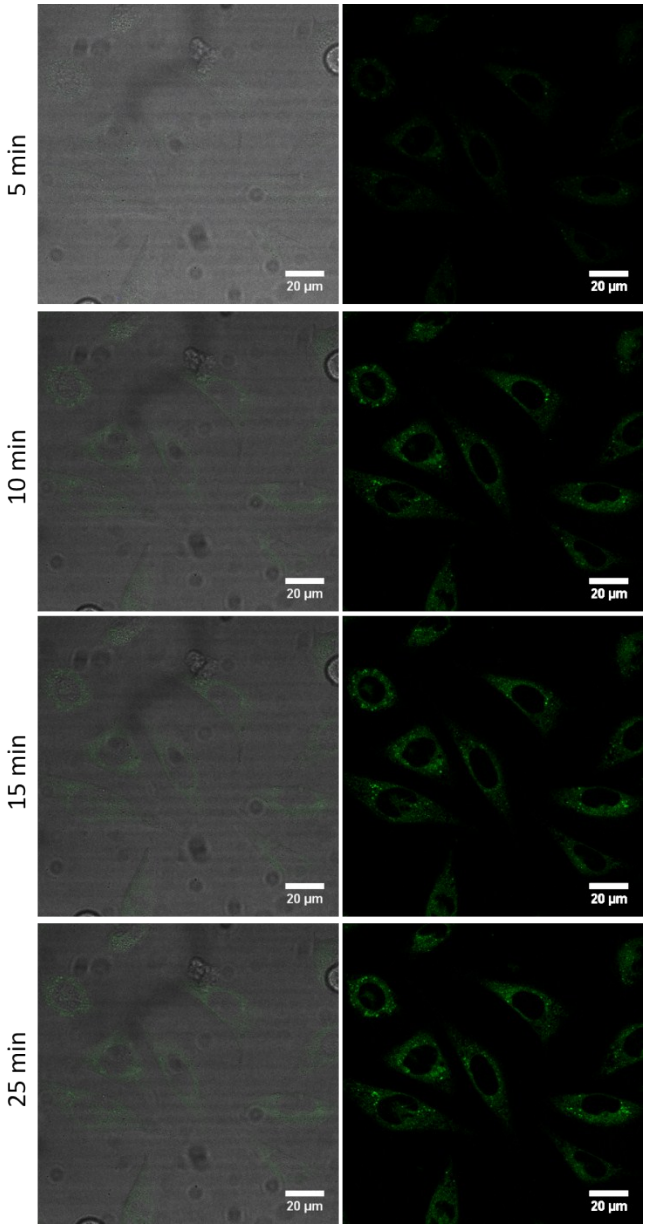

Figure S20 Live uptake of Dppz-Ar-BODIPY in live CHO cells. 5 µM of Dppz-Ar-BODIPY in cell media was added to CHO cells, and the uptake was monitored over 25 minutes using confocal microscopy, showing the overlay channels (column 1) and the emission of the BODIPY channel only (column 2).

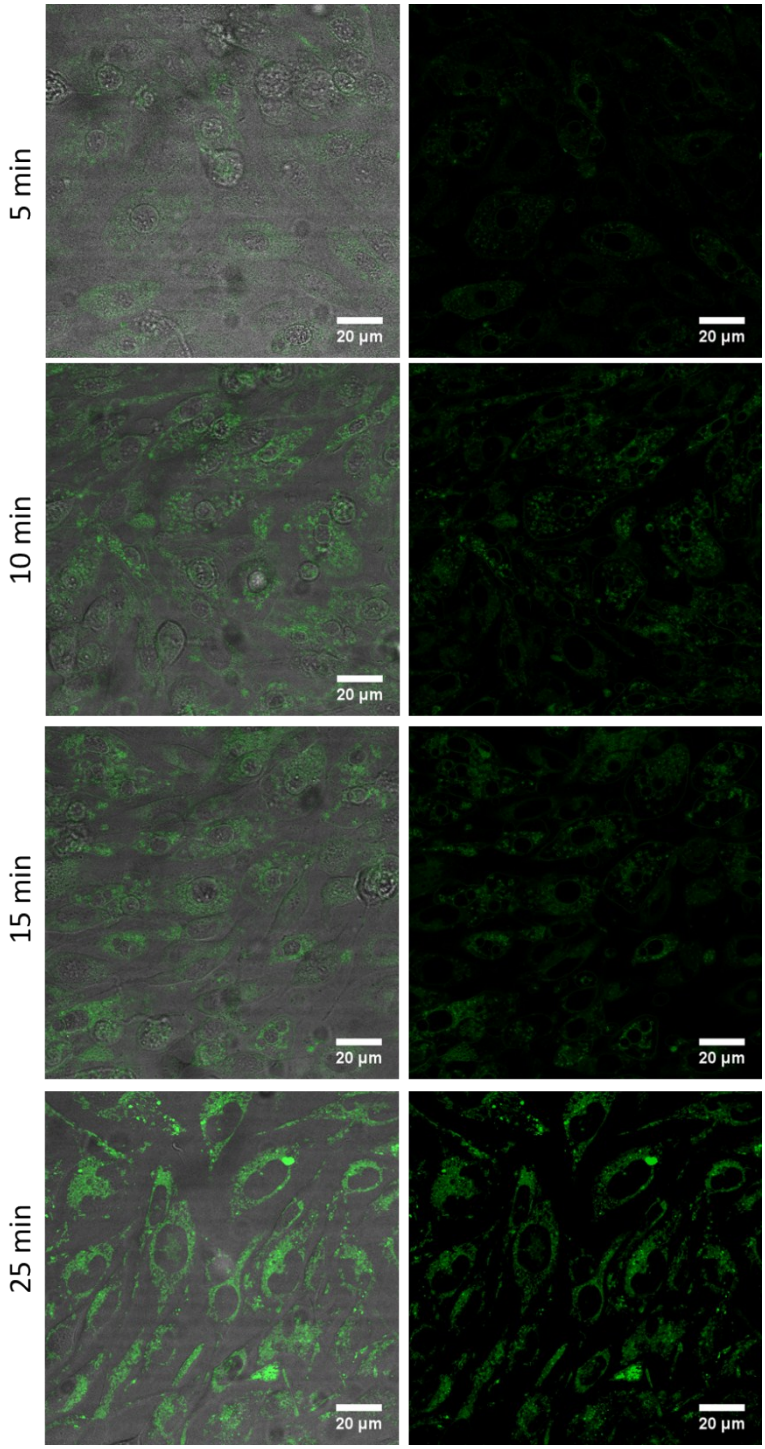

Figure S21 Live uptake of Dppz-Ar-BODIPY in HeLa cells. 5 µM of Dppz-Ar-BODIPY in cell media was added to HeLa cells, and the uptake was monitored over 25 minutes using confocal microscopy, showing the overlay channels (column 1) and the emission of the BODIPY channel only (column 2).

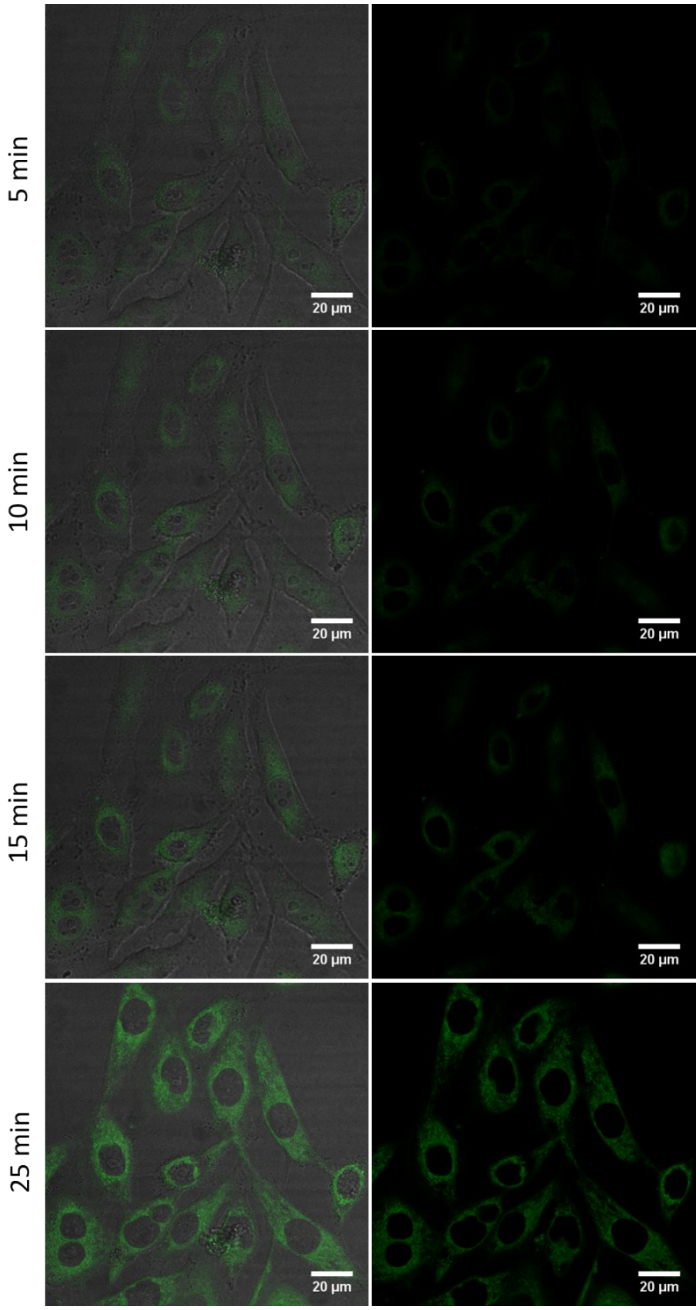

Figure S22 Live uptake of Napthyridyl-BODIPY-2-Cholesterol in HeLa cells. 5 µM of Napthyridyl-BODIPY-2-Cholesterol in cell media was added to HeLa cells, and the uptake was monitored over 25 minutes using confocal microscopy, showing the overlay channels (column 1) and the emission of the BODIPY channel only (column 2).

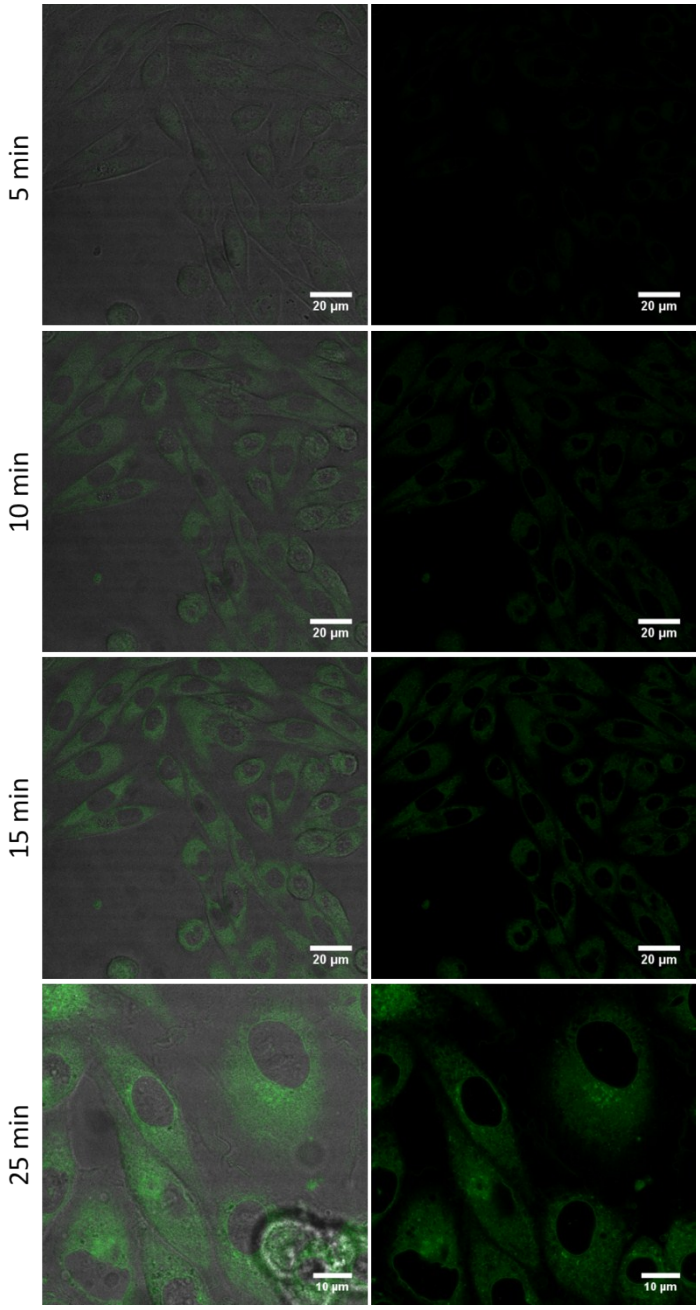

Figure S23 Live uptake of Napthyridyl-BODIPY-2-Cholesterol in CHO cells. 5 µM of Napthyridyl-BODIPY-2-Cholesterol in cell media was added to CHO cells, and the uptake was monitored over 25 minutes using confocal microscopy, showing the overlay channels (column 1) and the emission of the BODIPY channel only (column 2).

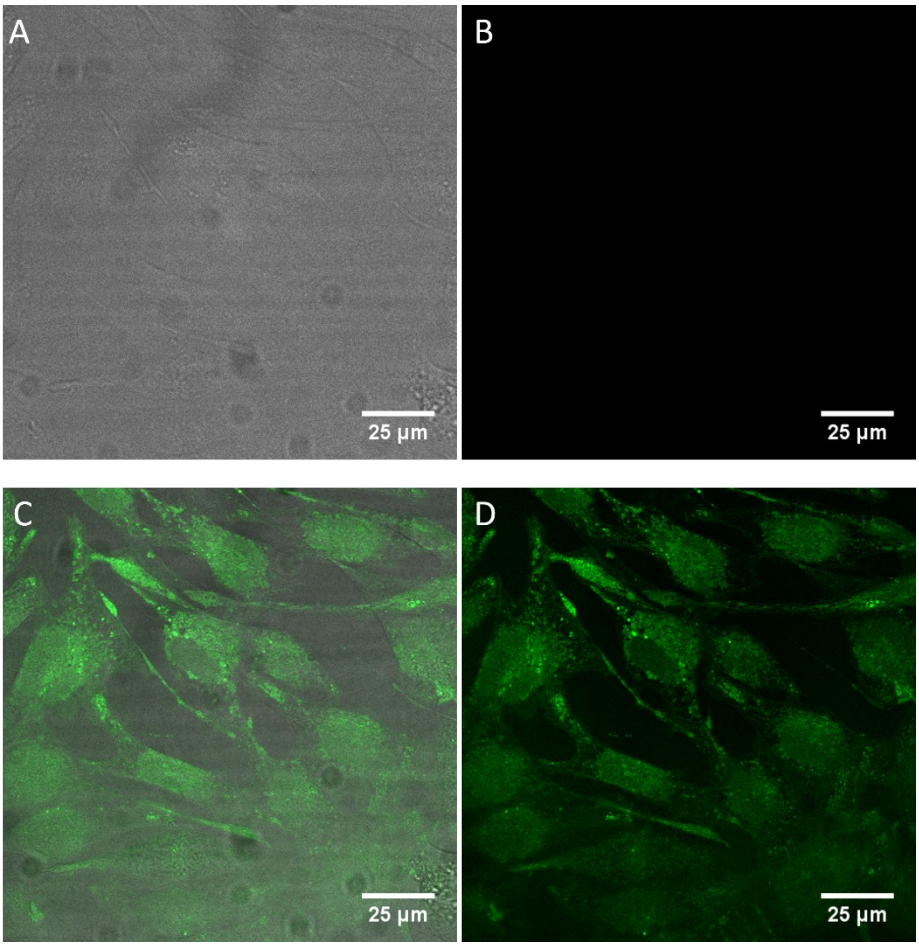

Figure S24 Control of Nile Red imaging settings without Nile Red present. To ensure there was no cross talk between Napthyridyl-BODIPY-2-Cholesterol and Nile Red for the colocalisation studies, HeLa cells stained with Napthyridyl-BODIPY-2-Cholesterol (5 µM) were imaged using the Nile Red imaging settings, without any Nile Red present in the sample. The cells (A) showed no emission from the Nile Red channel (B). C and D represent the Napthyridyl-BODIPY-2-Cholesterol imaging settings to show that Napthyridyl-BODIPY-2-Cholesterol was present in the sample.