Stress-Responsive Rhodamine Bioconjugates for Membrane-Potential-Independent Mitochondrial Live-Cell Imaging and Tracking

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Table S1. Current state of the art (Steroid conjugate moieties for cellular imaging).

Important Sterol Probes	***Synthetic challenges***	***Optical Properties***	***Use***
Quinoline sterol conjugate	Multiple steps	Excitation laser used for cell imaging 403 nm	Anticancer agent turned into imaging agent, Endoplasmic reticulum imaging[1]
BODIPY Cholesterol Probes	9 steps or more	Excitation: ~580 nm Emission: ~610 nm Smaller Stokes' shift	Cholesterol transport in living cells[2] Membrane order determination in model membranes.
NBD cholesterol Probes	5 steps	Excitation: ~470 nm Emission: ~530 nm Smaller Stokes' shift	Membrane order determination using model membrane[3] ACAT activity in living cells.
Dansyl Cholesterol probes	5 steps	Excitation: ~350 nm Emission:~520 nm	Intracellular transport of cholesterol from plasma membrane[4]
Dansyl Cortisol	1 step	Absorption maxima: ~350 nm Emission maxima~500 nm[5]	core–shell-type molecularly imprinted polymer nanoparticles (MIP-NPs) for cortisol sensing[6]. No cellular studies reported.
Pyridinium cholesterol	3 steps	Excitation: ~450 nm Emission: ~600 nm Large Stokes' shift	Cell membrane imaging and also enables liposome interaction[7]
Current study	1 step	Excitation: 561 nm laser Emission: ~590 nm No bleed through in other channels	Mitochondrial imaging with mapping of stressed mitochondrial cells.

Solvents	RC1				RC2			
	λ_{abs} (nm)	λ_{emi} (nm)	Stokes' Shift (cm^{-1})	Φ_f	λ_{abs} (nm)	λ_{emi} (nm)	Stokes' Shift (cm^{-1})	Φ_f
Dioxane	564	587	695	0.20	562	588	787	0.37
DMF	562	589	816	0.41	563	589	784	0.47
THF	559	584	766	0.59	560	584	734	0.62
Acetonitrile	556	583	833	0.32	557	581	742	0.39
Methanol	555	580	777	0.42	556	578	685	0.53
DMSO	566	593	804	0.48	567	592	745	0.58
Water	560	585	763	0.006	561	593	962	0.29

Table S2. Photophysical properties of **RC1** and **RC2** in different solvents.

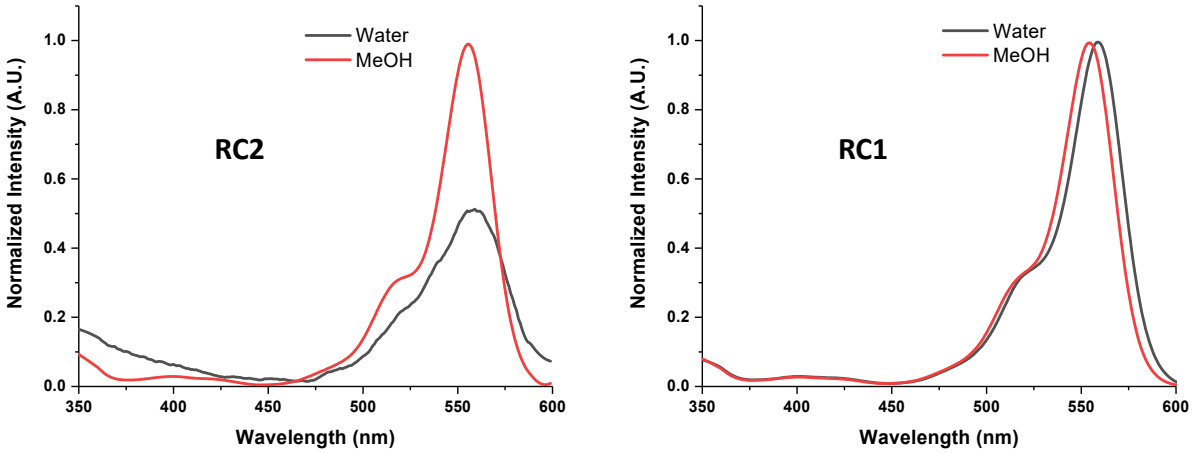

Figure S1. Excitation Spectra of **RC1** and **RC2** in water and methanol

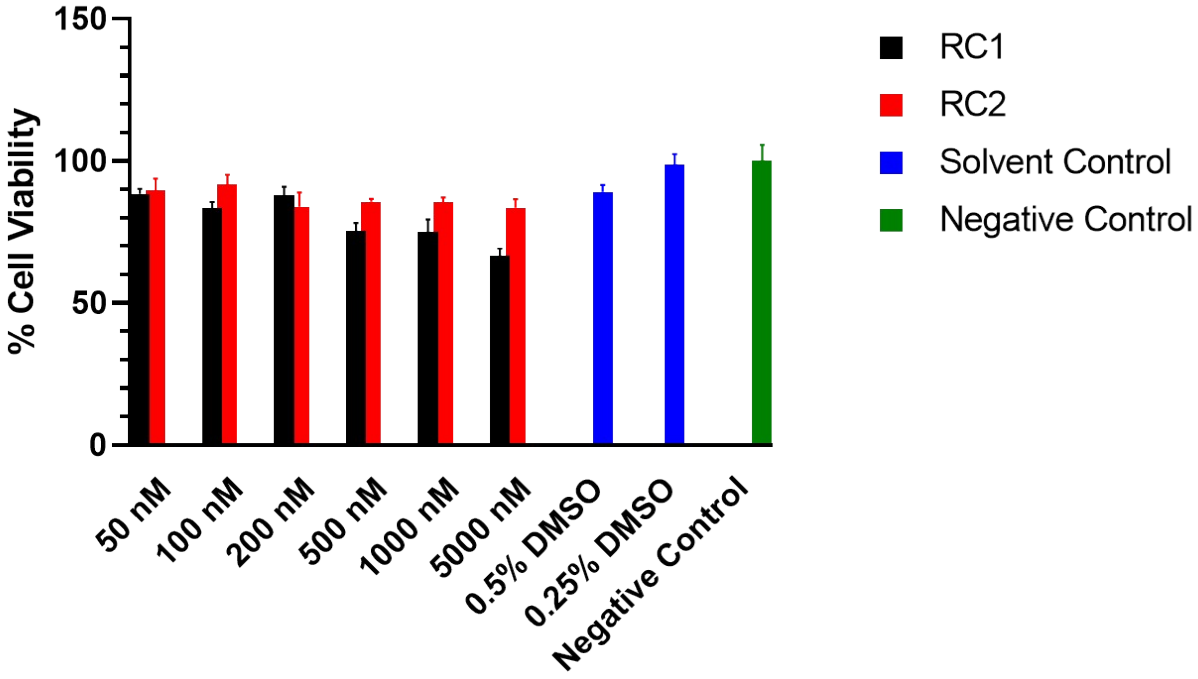

Figure S2. Cell cytotoxicity results of **RC1** and **RC2**

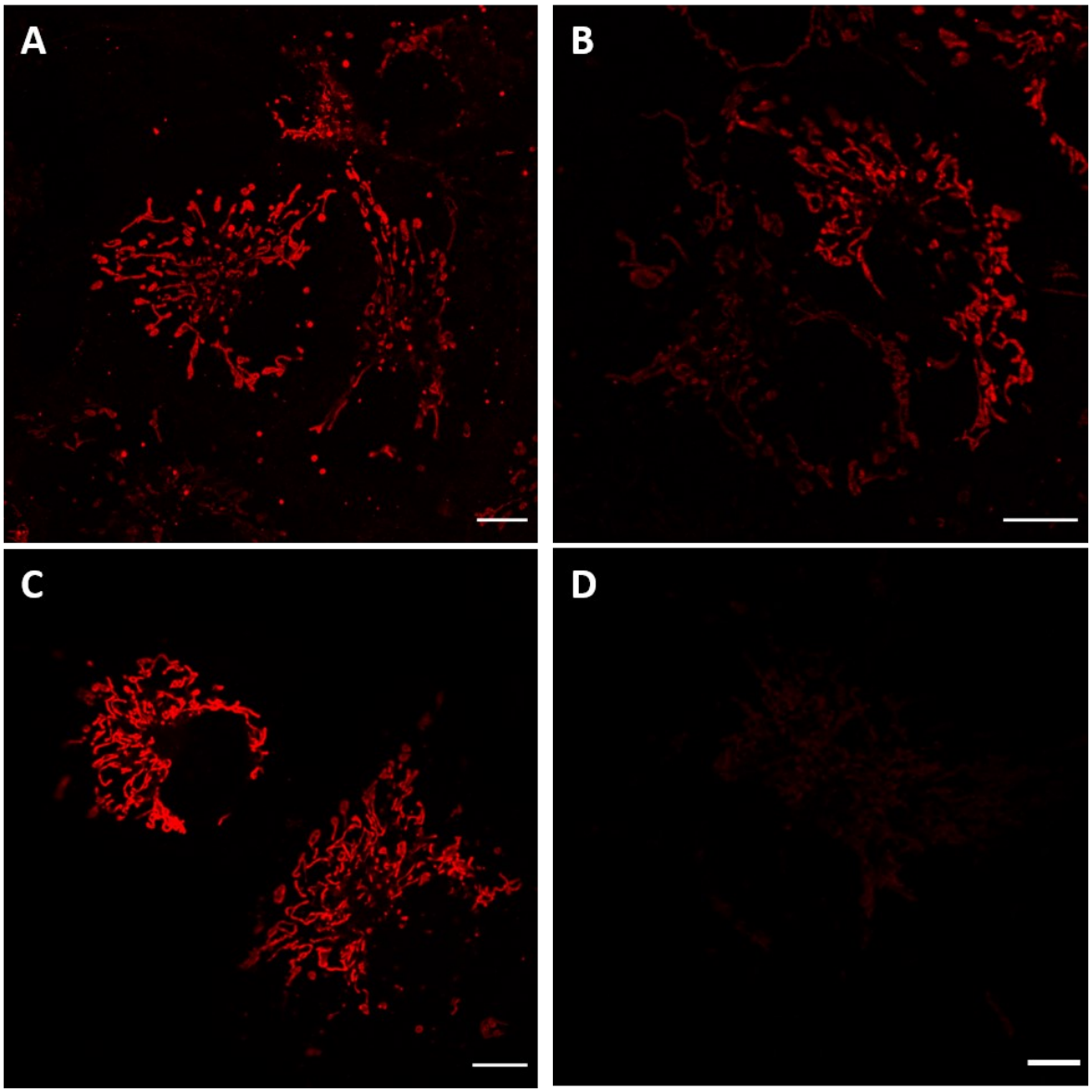

Figure S3. One colour images of serum stressed cells. **(A)** and **(C)** represent the serum-starved cells treated with **RC1** and **RC2,** respectively. **(B)** and **(D)** represent healthy cells treated with **RC1** and **RC2,** respectively. Scale factor: 10 µm

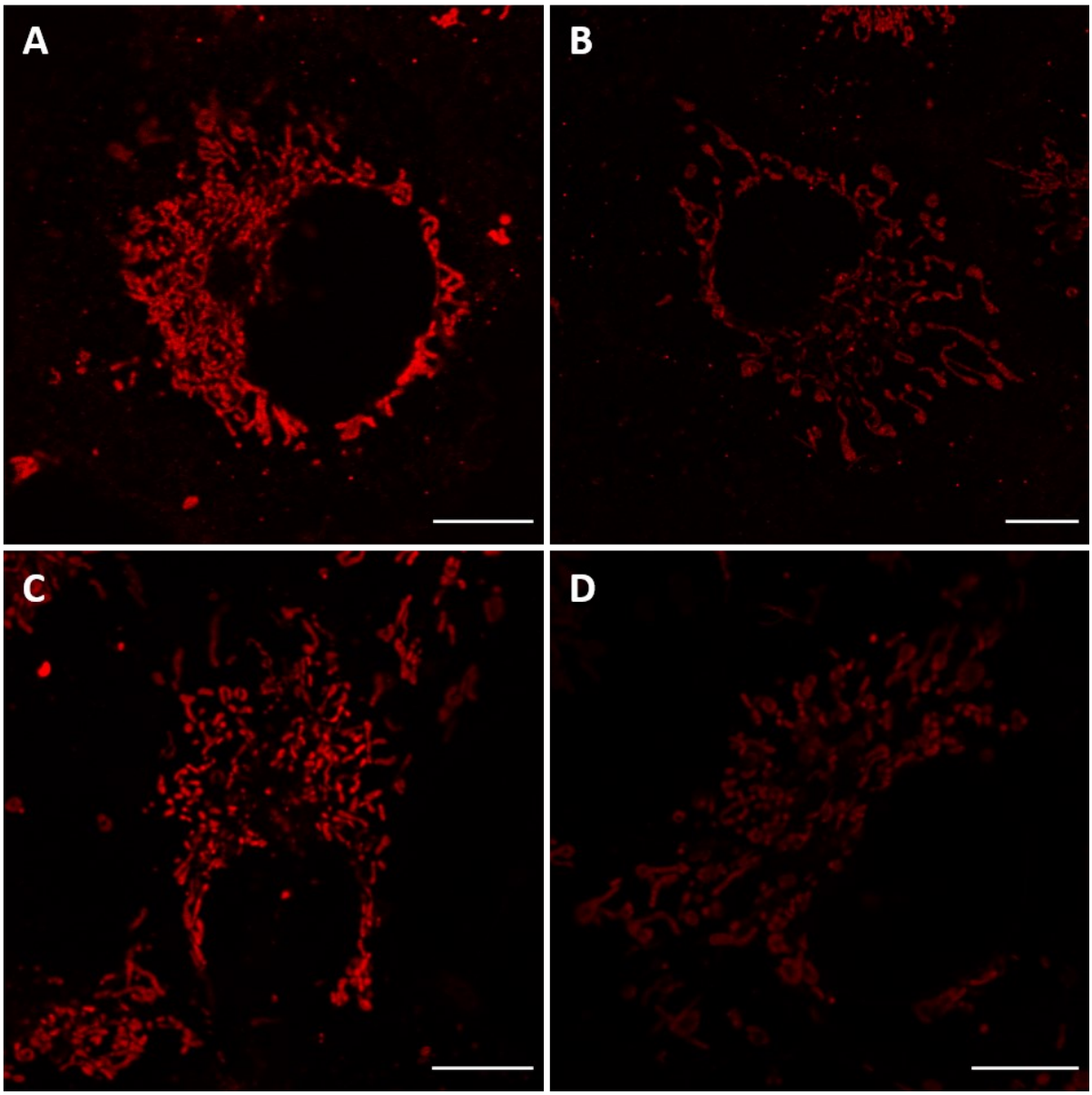

Figure S4. One colour image of oxidatively stressed cells. (A) and (C) represent the oxidative cells treated with RC1 and RC2, respectively. (B) and (D) represent healthy cells treated with RC1 and RC2, respectively. Scale factor: 10 μm

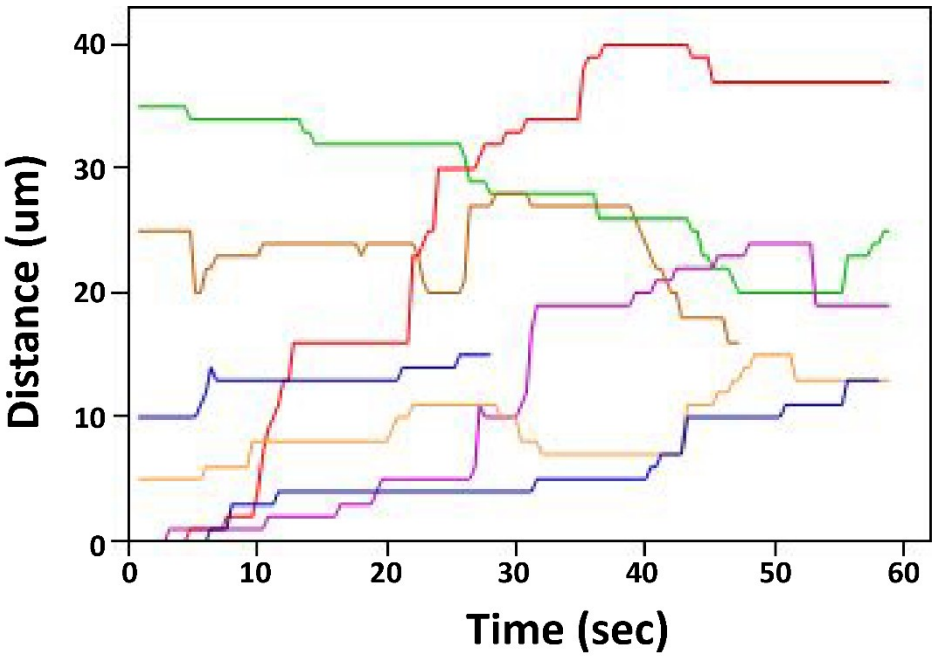

Figure S5. Representative tracks of typical bidirectional motion of mitochondria in cells labelled with **RC2**.

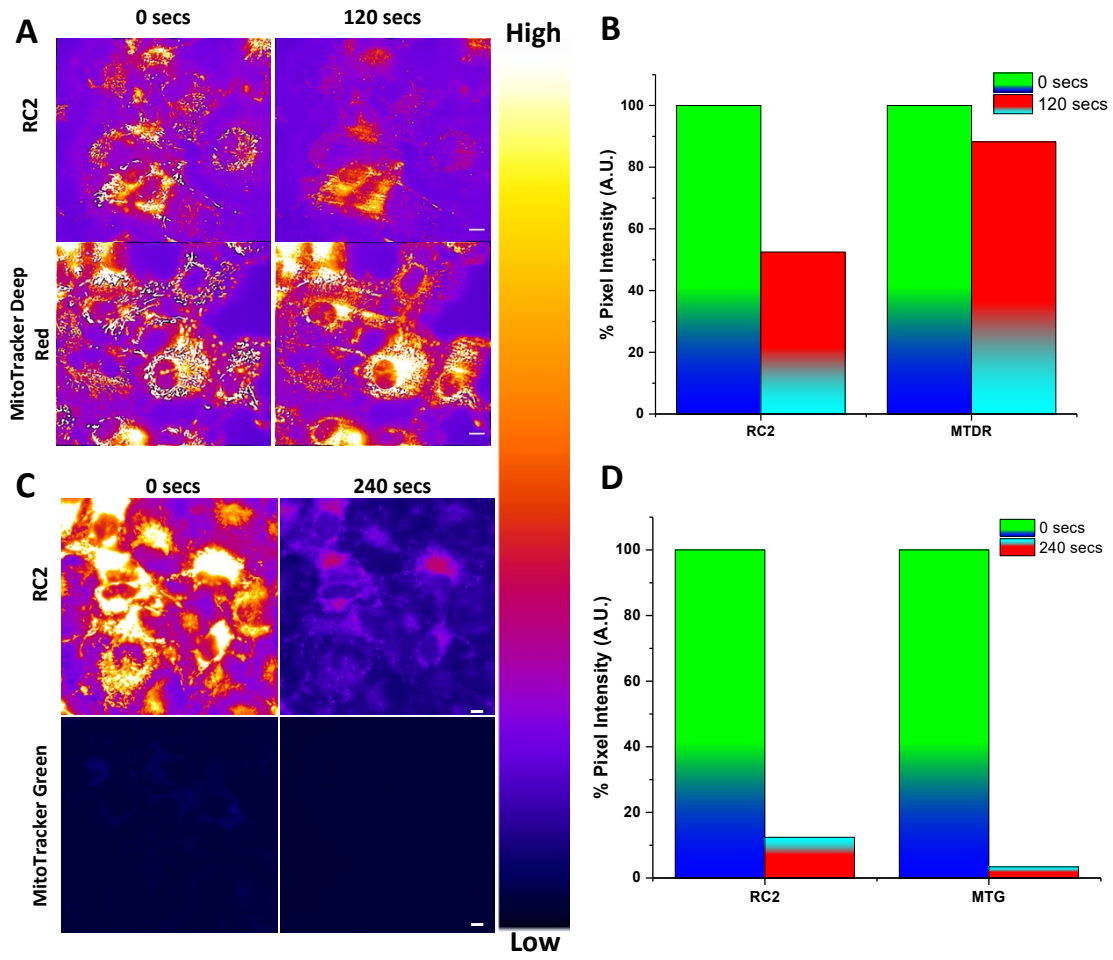

Figure S6. Comparative Brightness & Photostability Assay. (A) and (B) panels represent Cos-7 cells stained with RC2 and MitoTracker Deep red (MTDR); (C) and (D) panels represent Cos-7 cells stained with RC2 and MitoTracker green (MTG). Image intensities at 0 sec are considered as 100%-pixel intensity (for every probe channel) and comparative changes in pixel intensities are plotted to represent the photostability (B) & (D). scale bar: 10 µm

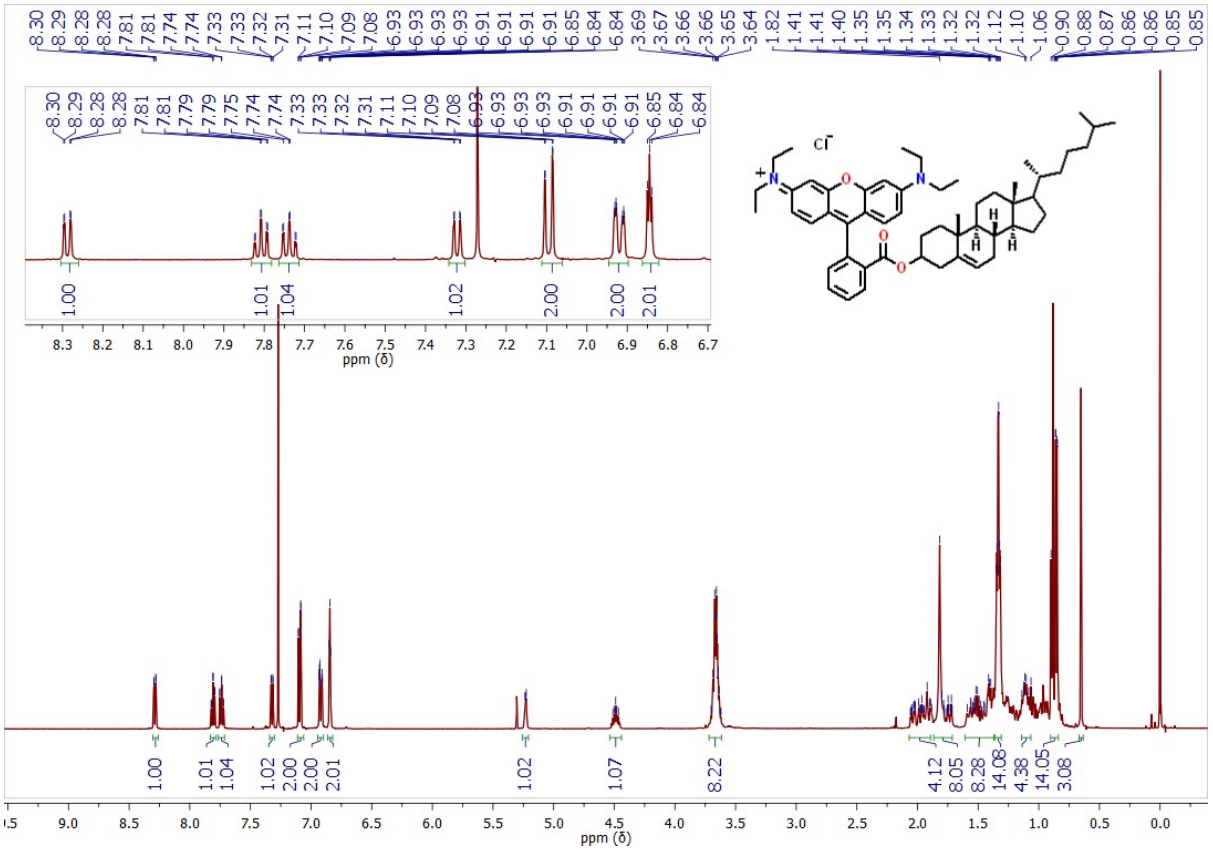

Figure S7. ^{1}H NMR of compound RC1 in $CDCl_3$.

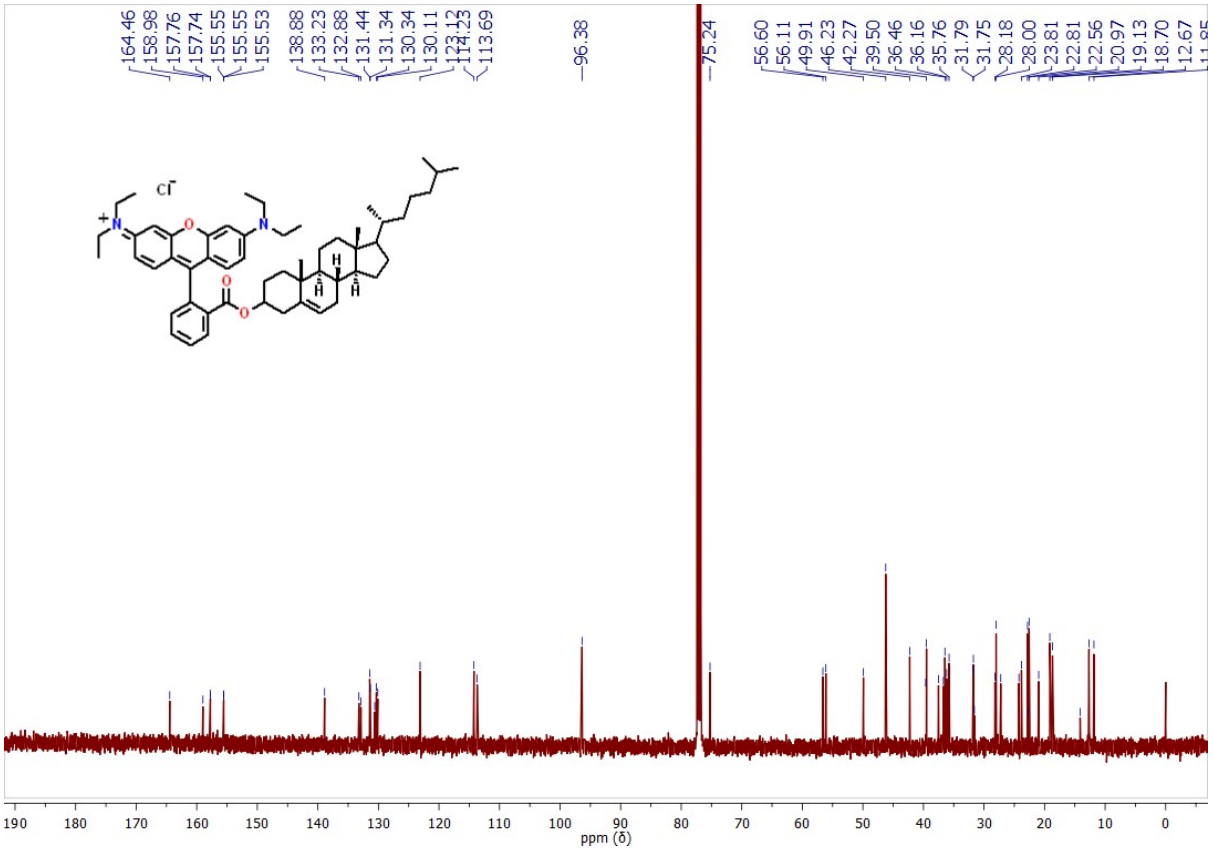

Figure S8. ^{13}C NMR of compound RC1 in $CDCl_3$.

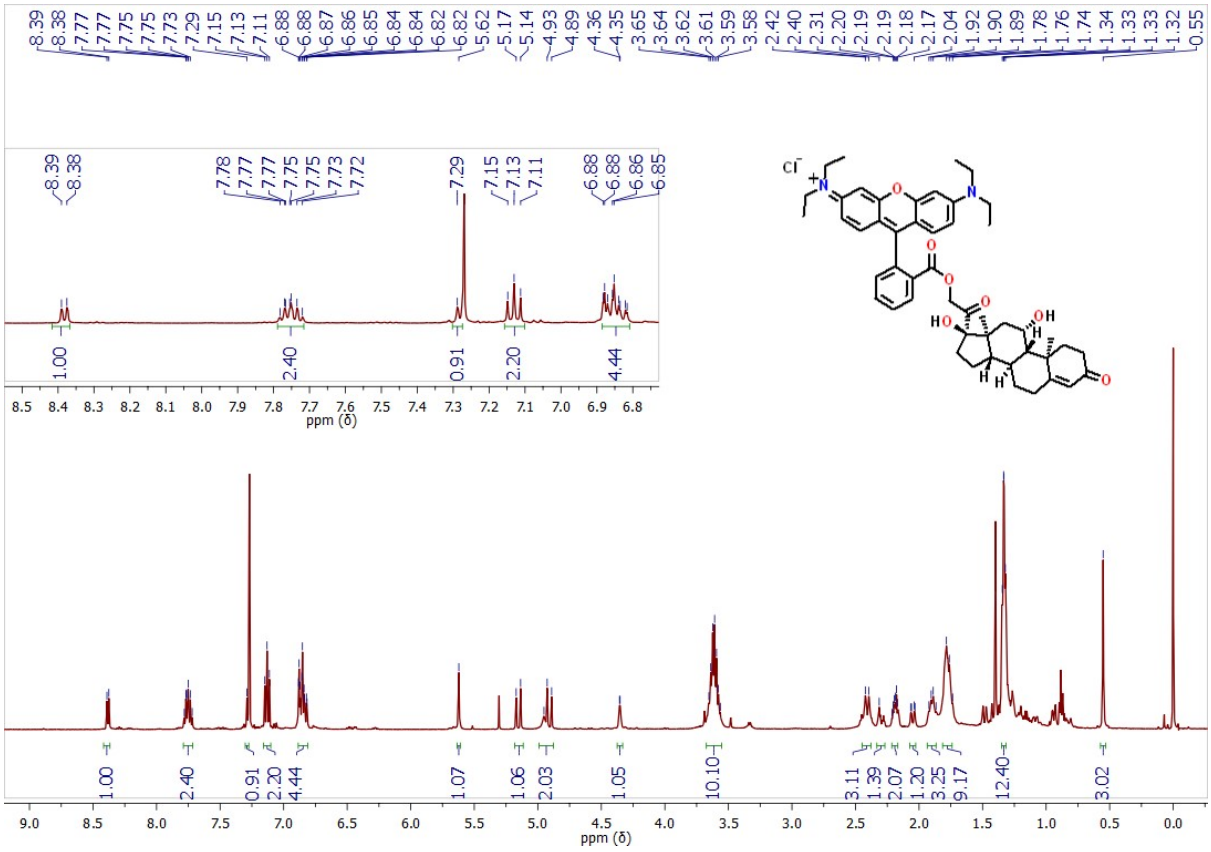

Figure S9. ^{1}H NMR of compound RC2 in $CDCl_3$.

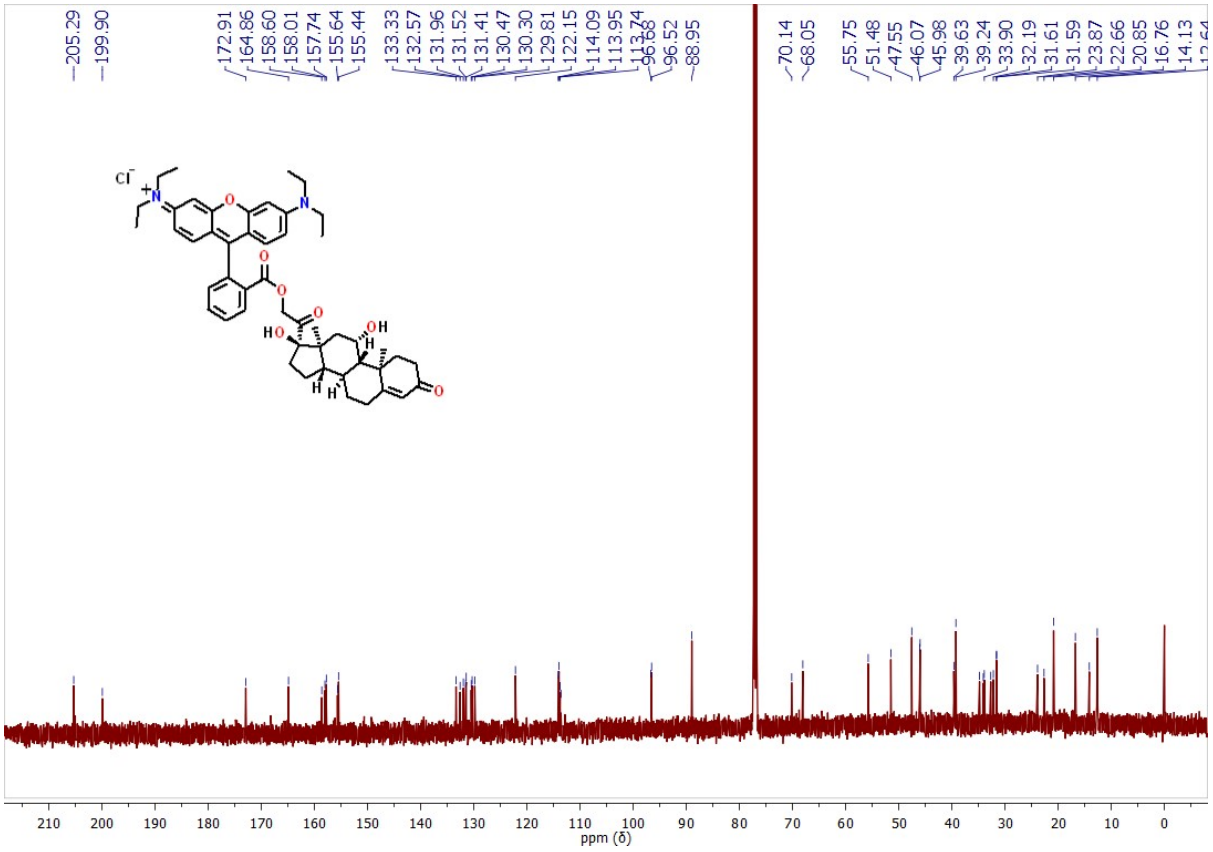

Figure S10. ^{13}C NMR of compound RC2 in $CDCl_3$.

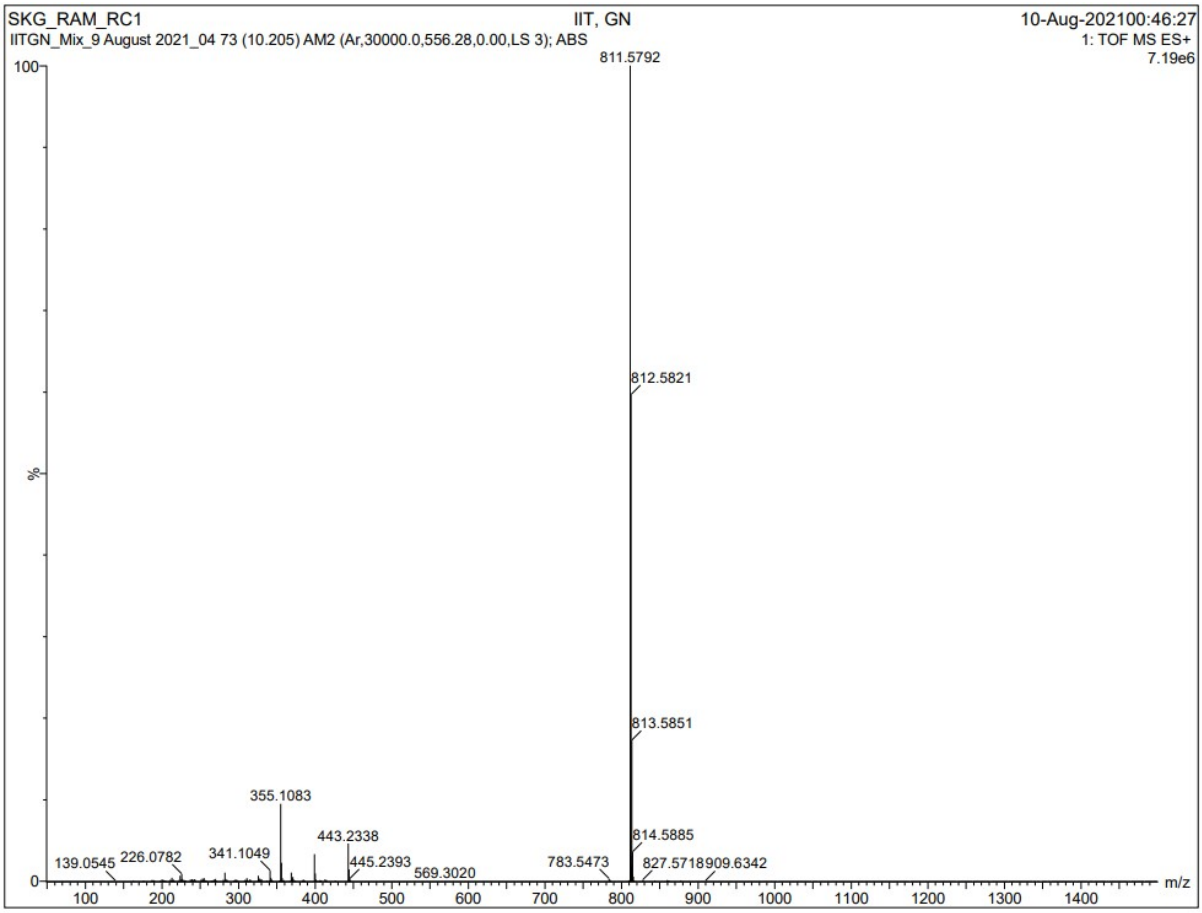

Figure S11. ESI mass profile of RC1 (RC1: HRMS (ESI) (m/z): [M]+ calcd. for $C_{55}H_{75}N_2O_3$, 811.5773, found, 811.5792, difference 1.9 ppm (0.0019).

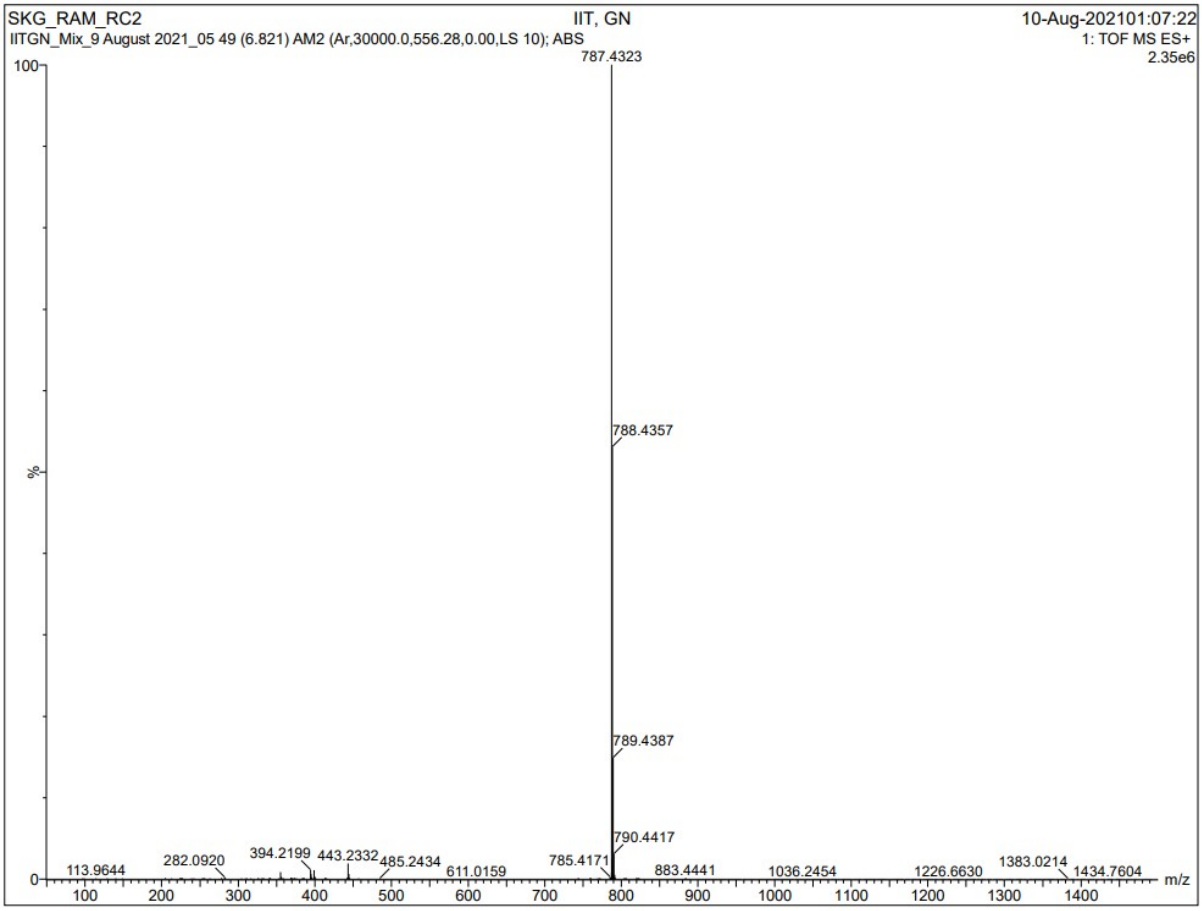

Figure S12. ESI mass profile of RC2 (RC2: HRMS (ESI) (m/z): [M]+ calcd. for $C_{49}H_{59}N_2O_7$, 787.4317, found, 787.4323, difference 0.6 ppm (0.0006).

References

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