

## Nile Red Derivatives for Dual-Channel Organelle Imaging and LPS Detection

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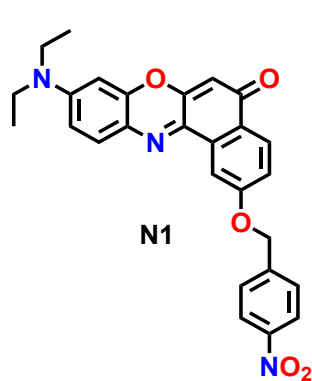
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### Supporting information

Table of Contents	
	Synthetic procedure and characterization details:
<b>S1</b>	Absorption spectrum of <b>N2</b> and <b>N3</b>
<b>S2</b>	Emission spectrum of <b>N2</b> and <b>N3</b>
<b>S3</b>	LPS interaction study with <b>N2</b> and <b>N3</b>
<b>S4</b>	MTT assay of <b>N1</b> , <b>N2</b> and <b>N3</b> in <b>COS-7 cells</b>
<b>S5</b>	Multiple cellular imaging of <b>N1</b> and <b>N2</b> in <b>COS-7 cells</b>
<b>S6</b>	Cellular imaging of <b>N3</b> at 488 nm and 561 nm
<b>VIDEO-S1</b>	Photoirradiation studies of <b>N1</b> , <b>COS-7 cells (Video)</b>
<b>VIDEO-S2</b>	Photoirradiation studies of <b>N1</b> , <b>HeLa cells (Video)</b>
<b>VIDEO S2 &amp; S4</b>	Photoirradiation studies of <b>N2</b> and <b>N3</b> in <b>COS-7 cells (Video)</b>
<b>S10</b>	<sup>1</sup> H and <sup>13</sup> C NMR & Mass of <b>N1</b>
<b>S11</b>	<sup>1</sup> H and <sup>13</sup> C NMR & Mass of <b>N2</b>
<b>S12</b>	<sup>1</sup> H and <sup>13</sup> C NMR & Mass of <b>N2</b>

### Synthetic procedure and characterization details: N1, N2 and N3

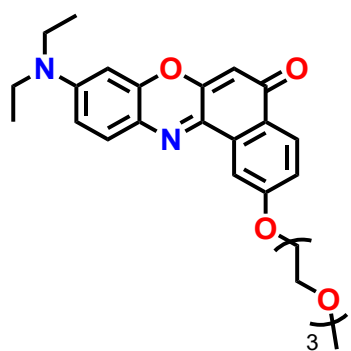
A solution of compound-2 [(9-(diethylamino)-2-hydroxy-5H-benzo[a]phenoxazin-5-one) (0.2 mmol) in anhydrous DMF was cooled to 0 °C. To this, potassium carbonate was added and the mixture was stirred for 10 minutes. Subsequently, 4-nitrobenzyl bromide (5 equiv, 1 mmol, **N1**), diethylene glycol 2-bromoethyl methyl ether (1 mmol, **N2**), and 1-bromododecane (1 mmol, **N3**) were added dropwise at ambient temperature under a nitrogen atmosphere. The reaction mixture was then heated to 70 °C and stirred for three hours. The reaction progress was monitored using Thin Layer Chromatography (TLC). Upon completion, the mixture was poured onto crushed ice and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography using DCM/hexane (15:85) as the eluent, affording the desired compound. The structural characterization details from <sup>1</sup>H, <sup>13</sup>C, and HRMS are provided in the supporting information.



**N1: 9-(diethylamino)-2-((4-nitrobenzyl)oxy)-5H-benzo[a]phenoxazin-5-one**

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.28 (d, *J* = 8.7 Hz, 2H), 8.02 (dd, *J* = 16.4, 5.6 Hz, 1H), 7.79 (d, *J* = 8.6 Hz, 2H), 7.57 (d, *J* = 9.1 Hz, 1H), 7.32 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.78 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.59 (s, 1H), 6.16 (s, 1H), 5.76 (s, 1H), 5.44 (s, 2H), 3.47 (dd, *J* = 14.1, 7.0 Hz, 4H), 1.16 (td, *J* = 7.1, 3.8 Hz, 6H). <sup>13</sup>C NMR (125

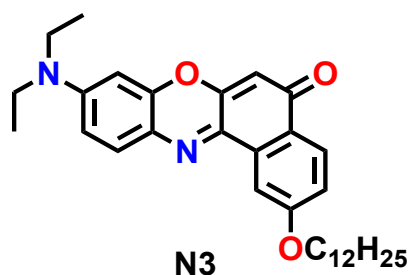
MHz, DMSO) δ 181.7, 160.9, 152.2 151.3, 147.5, 146.9, 144.9, 138.4, 134.0, 131.3, 128.7, 127.7, 125.7, 124.4, 124.0, 118.4, 118.4, 110.5, 107.3, 104.5, 96.4, 68.8, 55.3, 44.9, 12.9. Mass HRMS: Actual mass (M+H)= 470.1716, Observed mass (M+H)= 470.1729, mass error= 2.76 ppm



**N2: 9-(diethylamino)-2-(2-(2-methoxyethoxy)ethoxy)-5H-benzo[a]phenoxazin-5-one**

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.02 (d, *J* = 8.7 Hz, 1H), 7.93 (d, *J* = 2.6 Hz, 1H), 7.59 (d, *J* = 9.1 Hz, 1H), 7.26 (dd, *J* = 8.7, 2.6 Hz, 1H), 6.80 (dd, *J* = 9.2, 2.7 Hz, 1H), 6.62 (d, *J* = 2.7

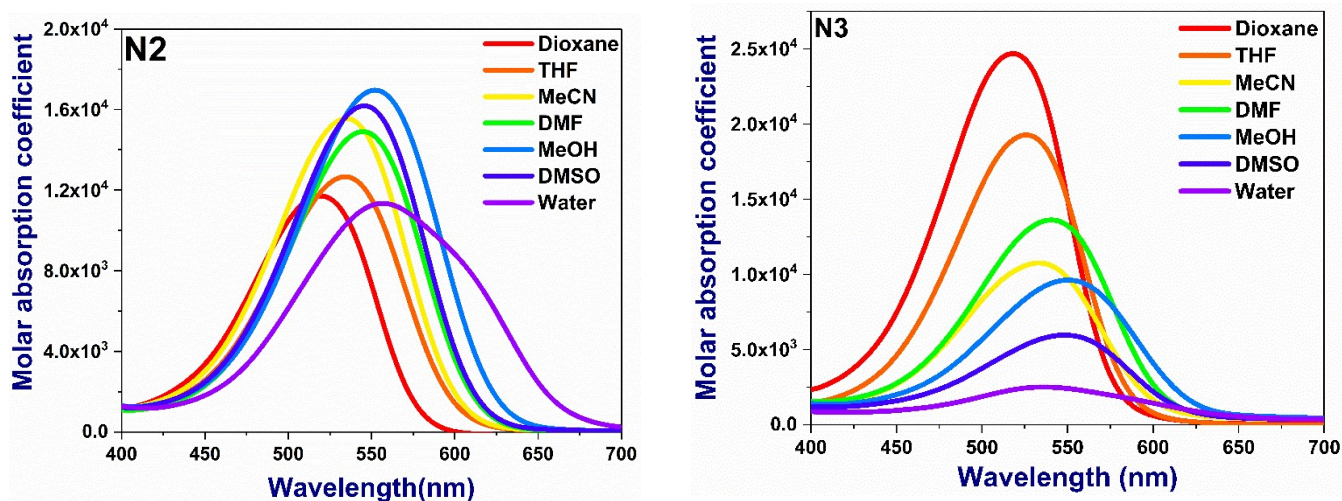
Hz, 1H), 6.17 (s, 1H), 4.28 (t,  $J = 7.3$ , 2H), 3.82 (t,  $J = 4.5$ , 2H), 3.63 (dd,  $J = 6.0, 3.5$  Hz, 2H), 3.57 – 3.55 (m, 2H), 3.53 (dt,  $J = 5.9, 3.2$  Hz, 3H), 3.51 – 3.47 (m, 3H), 3.45 – 3.41 (m, 2H), 3.23 (s, 3H), 1.16 (t,  $J = 7.0$  Hz, 7H).  **$^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ )**  $\delta$  181.8, 161.5, 152.1, 151.2, 146.9, 138.6, 134.0, 131.4, 127.7, 125.4, 124.4, 118.4, 110.5, 106.7, 104.5, 96.4, 71.7, 70.4, 70.2, 70.0, 69.3, 68.1, 58.5, 44.9, 12.9. **Mass** HRMS: Actual mass ( $M+H$ )= 481.2338, Observed mass ( $M+H$ )= 481.2347, mass error= 1.87 ppm.



**N3:** *9-(diethylamino)-2-(dodecyloxy)-5H-benzo[a]phenoxazin-5-one*

**$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )**  $\delta$  8.22 (d,  $J = 8.7$  Hz, 1H), 8.06 (d,  $J = 2.5$  Hz, 1H), 7.62 (d,  $J = 9.0$  Hz, 1H), 7.17 (dd,  $J = 8.7, 2.6$  Hz, 1H), 6.66 (dd,  $J = 9.1, 2.7$  Hz, 1H), 6.47 (d,  $J = 2.7$  Hz, 1H), 6.31 (s, 1H), 4.17 (t,  $J = 6.5$  Hz, 3H), 3.48 (q,  $J = 7.1$  Hz, 4H), 1.86 (t,  $J = 7.8$ , 2H), 1.51 (t,  $J = 7.7$  Hz, 3H), 1.27 (t,  $J = 7.1$  Hz, 18H), 0.88 (t,  $J = 7.0$  Hz, 6H).  **$^{13}\text{C}$  NMR (125 MHz, DMSO)**  $\delta$  183.3, 161.9, 151.8, 150.9, 146.7, 131.0, 127.7, 125.5, 118.3, 109.4, 106.6, 105.3, 105.3, 96.3, 45.0, 45.0, 31.9, 29.6, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 26.0, 22.7. **Mass** HRMS: Actual mass ( $M+H$ )= 503.3273 Observed mass ( $M+H$ )= 503.3271, mass error= -0.39 ppm.

**Fig S1.** Absorption spectra of compounds N2 & N3



**Fig S2:** Emission spectrum of compounds N2 & N3 in different solvents

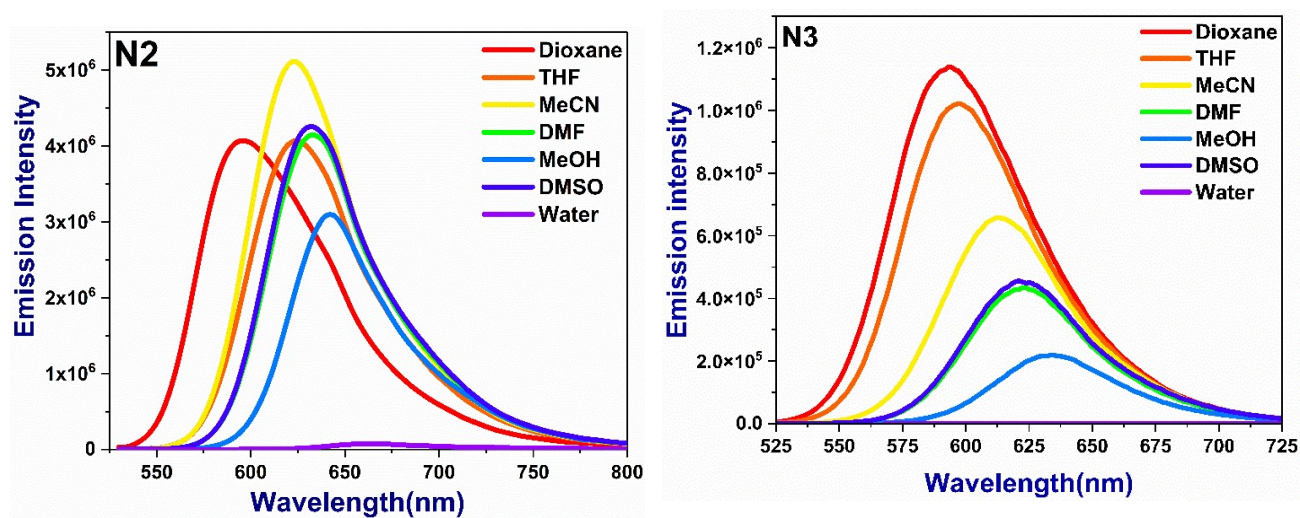


Fig S3: The effect of added LPS on the emission behavior of N2 and N3

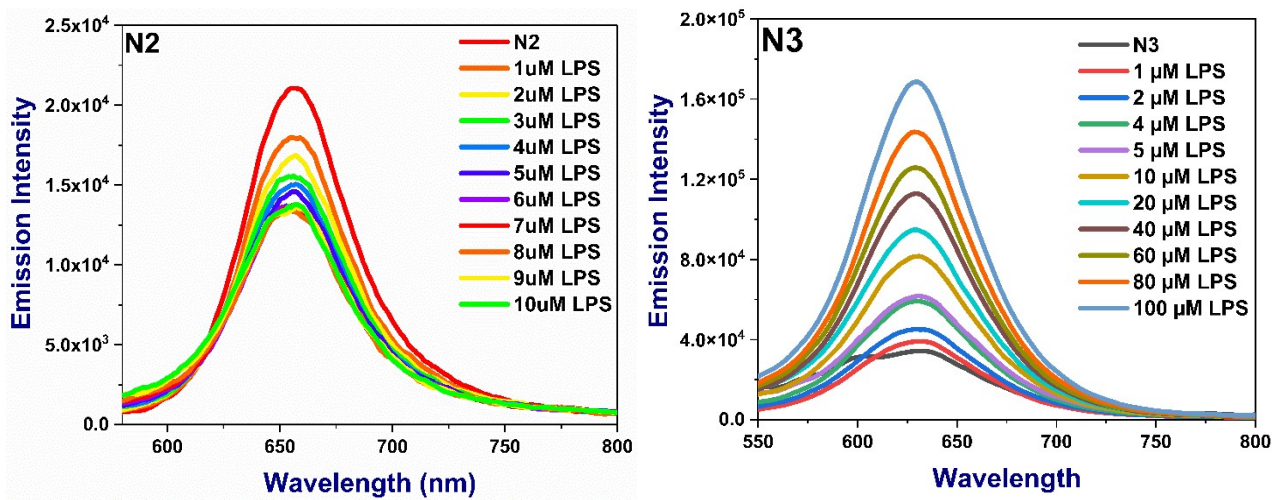


Fig S4: MTT assay of N1, N2 and N3 in COS-7 cells

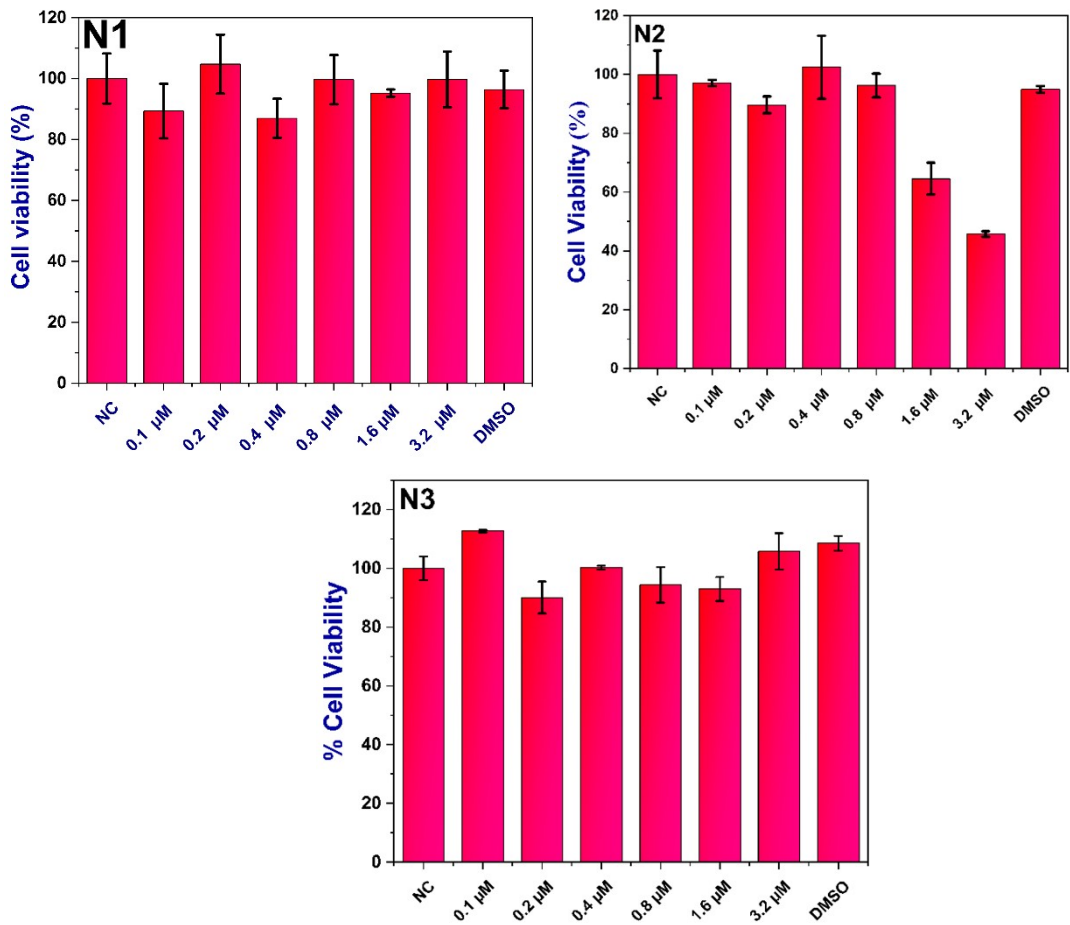


Fig S5: Multiple cellular imaging of **N1** and **N2** in COS-7 cells. Imaging of **N1** and **N2** in COS-7 cells (image represents multiple cells). Concentration: (**N1**= 0.5  $\mu$ M, **N2**= 0.5  $\mu$ M),  
*\*Live cell imaging condition: 37 °C, 5% CO<sub>2</sub>; Scale bar=10  $\mu$ m.*

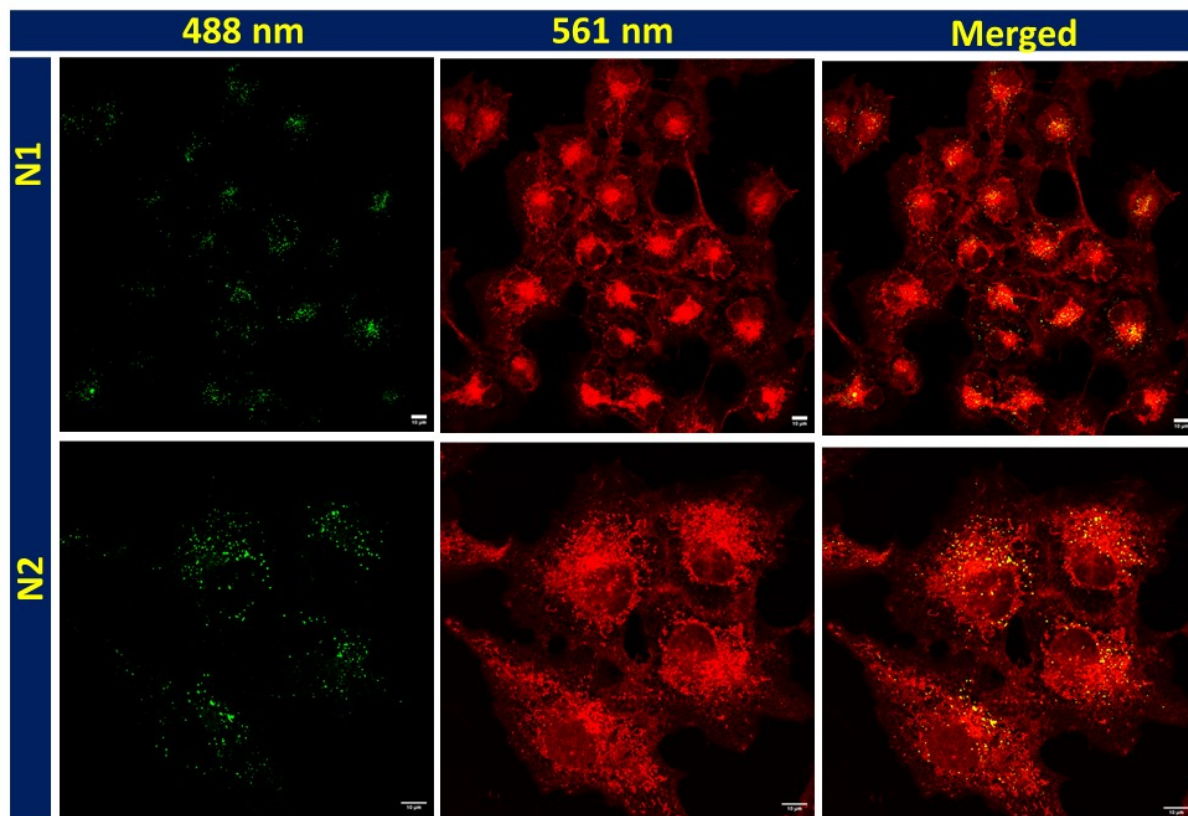


Fig S6: Cellular imaging of **N3** at 488 nm and 561 nm. **N3** unlike others does not show any imaging at 488 nm while we can see lysosomes at 561nm.

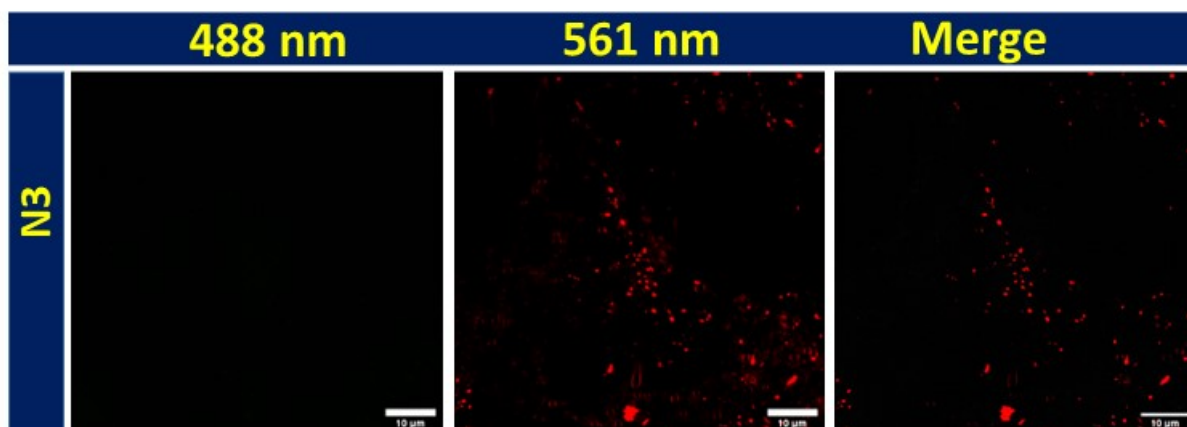
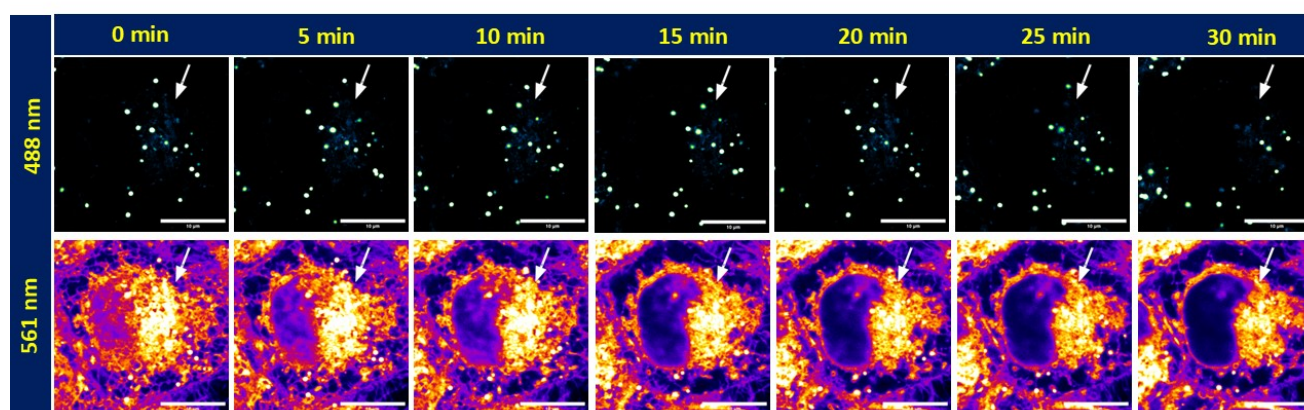


Fig S7: Photostability studies of **N2** and **N3** in COS-7 cells



Video S1: Video showing continuous photoirradiation studies of **N1**, COS-7 cells (Video attached as additional information)

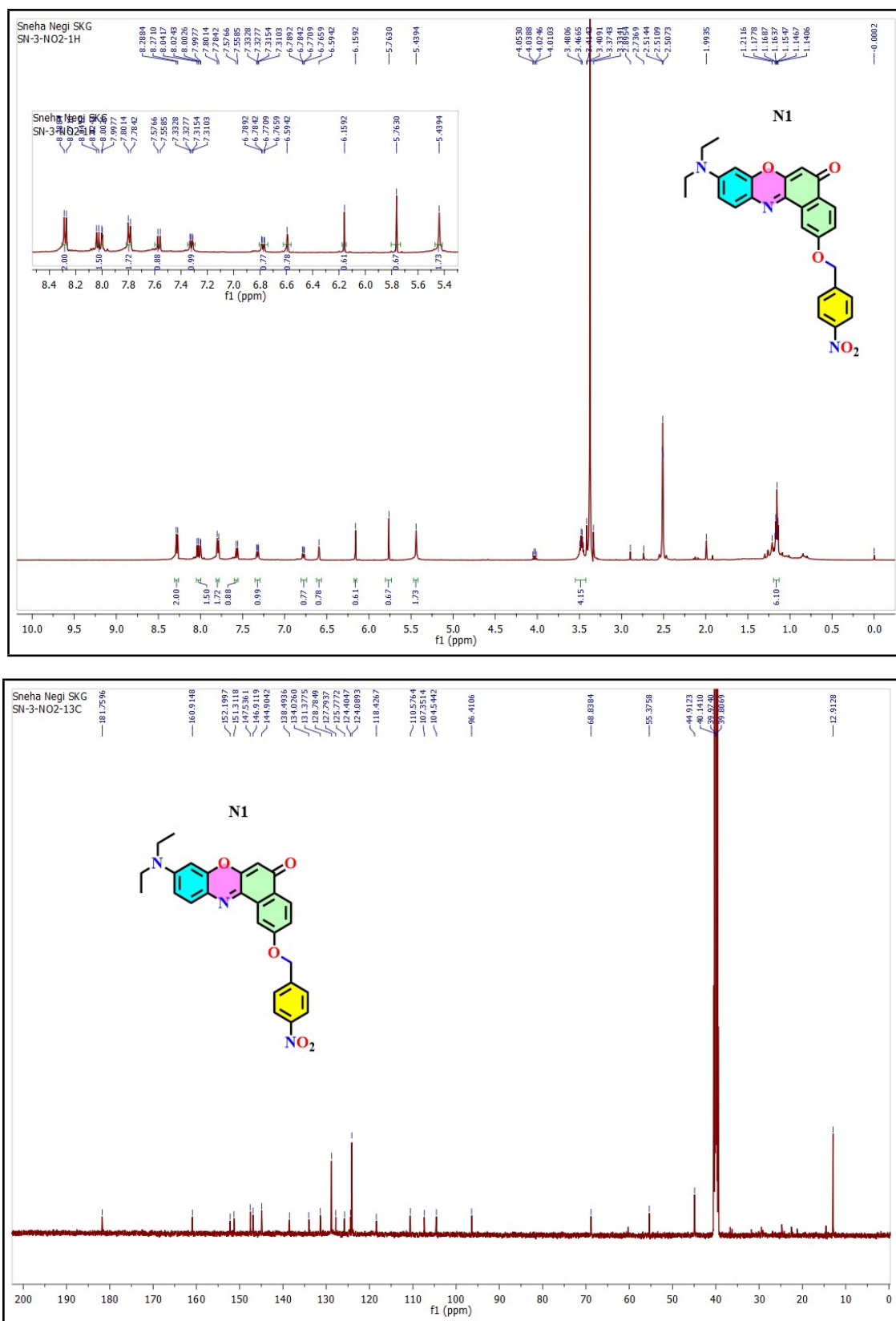
Video S2: Photoirradiation studies of **N1** in HeLa cells (Video attached as additional information)

Video S3: Cellular imaging of **N2** with continuous photoirradiation for 30 min in COS 7 cells

Video S4 Cellular imaging of **N3** with continuous photoirradiation for 30 min in COS 7 cells

## Spectral characterization

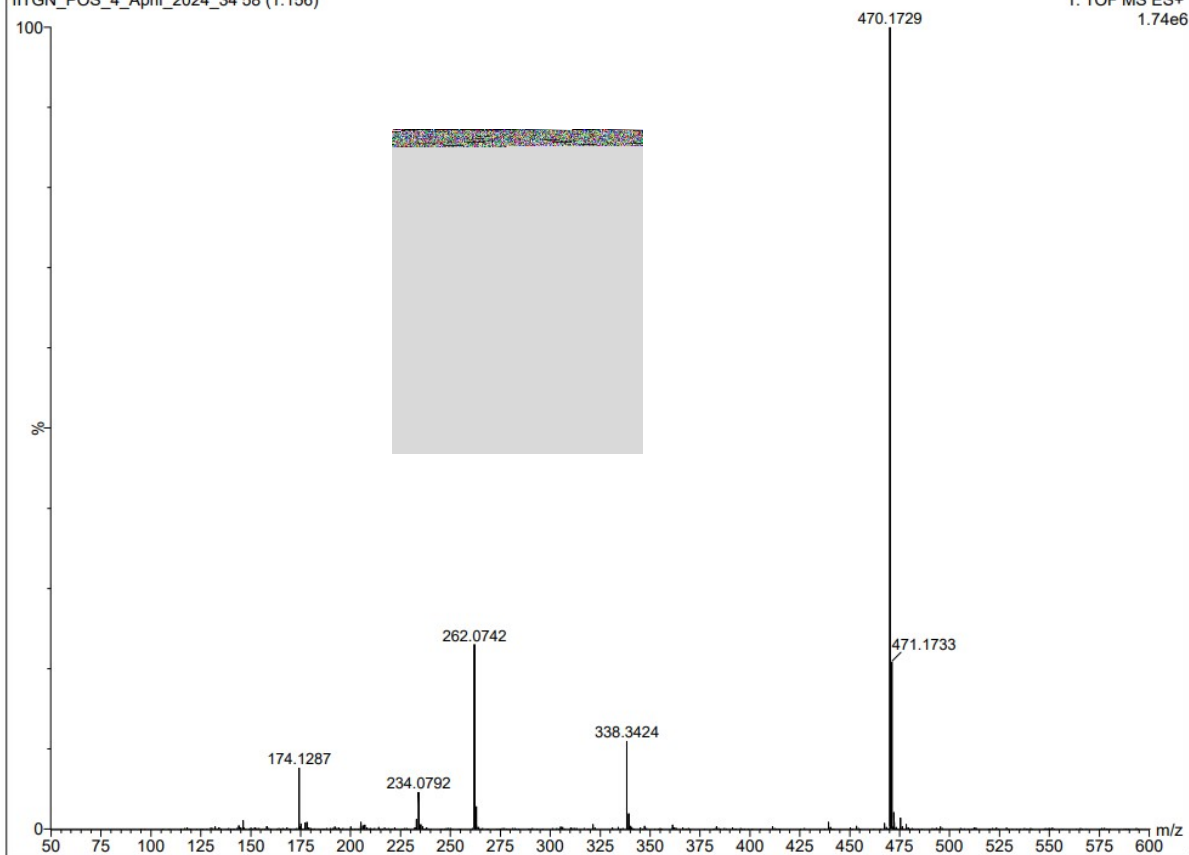
Fig S8:  $^1\text{H}$  and  $^{13}\text{C}$  NMR & Mass of N1



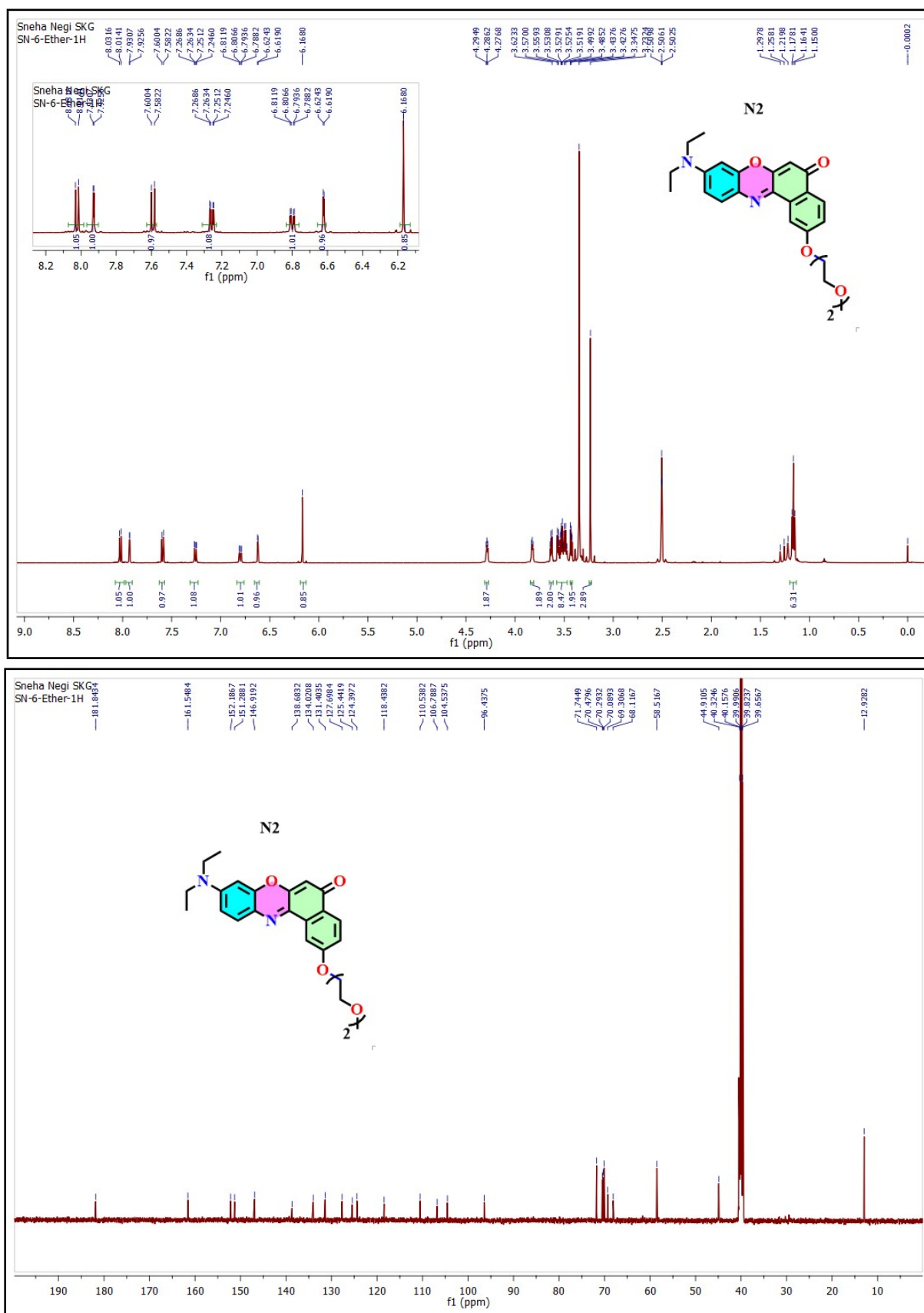
SKG\_SN\_NITRO

IITGN\_POS\_4\_April\_2024\_34 58 (1.156)

1: TOF MS ES+  
1.74e6



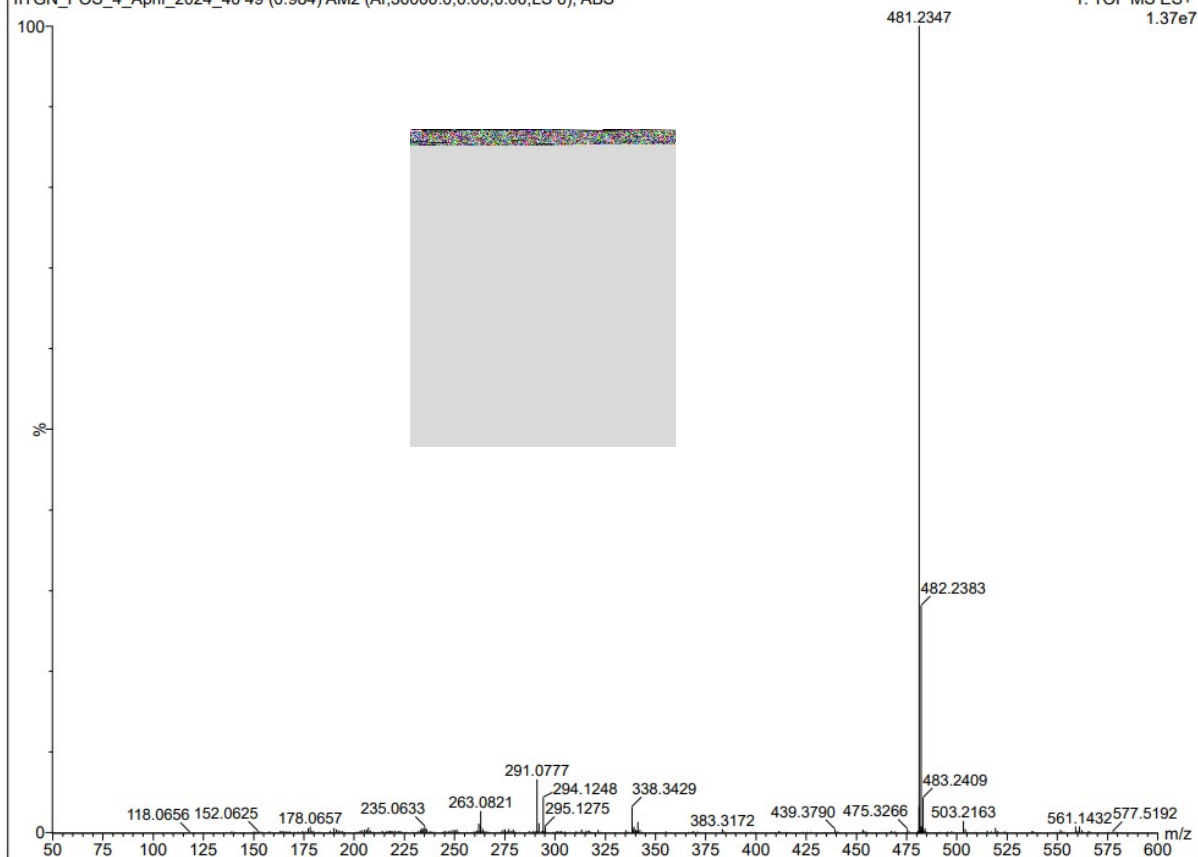
**Fig S9:  $^1\text{H}$  and  $^{13}\text{C}$  NMR & Mass of N2:**



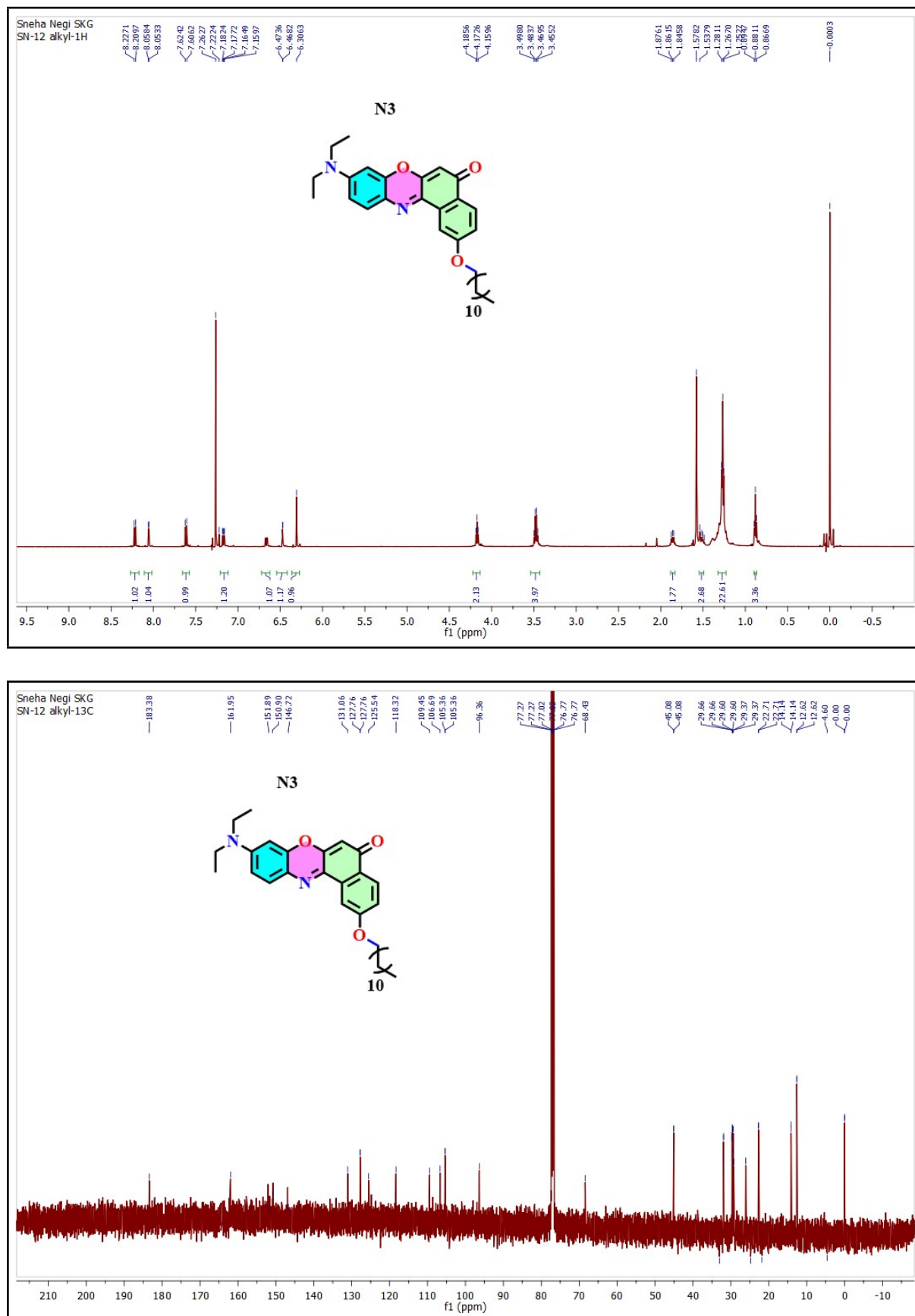
SKG\_SN\_ETHER

IIITGN\_POS\_4\_April\_2024\_40 49 (0.984) AM2 (Ar,30000.0,0.00,0.00,LS 6); ABS

1: TOF MS ES+  
1.37e7



**Fig S10:  $^1\text{H}$  and  $^{13}\text{C}$  NMR and Mass data of N3:**



SKG\_SN\_ALKYL

IITGN\_POS\_4\_April\_2024\_36 72 (1.412)

1: TOF MS ES+  
5.42e6

