Supporting Information:

Photocontrollable fluorogenic probes for visualizing near-membrane copper (II) in live cells

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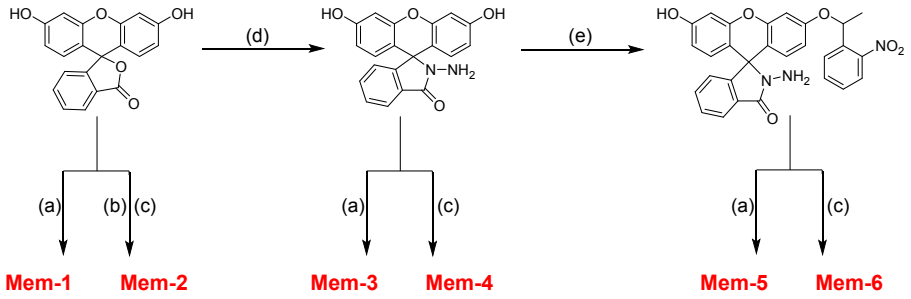
Synthesis and characterization:



Scheme S1. The synthesis route of fluorogenic probes and photocontrollable fluorogenic probes to visualize membrane copper (II) in live cells. (a) Triethylamine, Cholesteryl chloroformate, DMF, 0 °C, 49-60%; (b) Ethanol, H_2SO_4, reflux, quantitative; (c) Bromododecane, Cs_2CO_3, DMF, 60 °C, 50-62%; (d) Hydrazine hydrate, MeOH, reflux, 92%; (e) 1-(1-Bromoethyl)-2-nitrobenzene, Cs_2CO_3, DMF, 80 °C, 54%.

2-(3-oxo-6-(((((8R,9S,10R,13S,14S,17R)-10,13,17-trimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)carbonyl)oxy)-3H-xanthen-9-yl)benzoicacid (Mem-1).

Triethylamine (0.042 mL) was added to a solution of fluorescein (100 mg, 0.30 mmol) in DMF (4 mL), and the mixture was stirred under a dry nitrogen atmosphere for 5 mins. Cholesteryl chloroformate (135.15 mg, 0.30 mmol) in chloroform (1.5 mL) was

added dropwise to the solution. The reaction was stirred in an ice bath for 8 hrs. The mixture was poured into saturated ammonium chloride aqueous solution (10 mL), and then extracted with ethyl acetate (30 mL). The organic solution was washed three times with brine, dried over anhydrous Na_2SO_4 and filtered. Solvent of the organic solution was removed under reduced pressure and purified by flash column chromatography (silica gel, EA) affording **Mem-1** as a pale yellow liquid (yield 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 3 Hz, 1H), 7.63 (m, 2H), 7.16 (m, 2H), 6.67 (m, 5H), 5.43 (s, 1H), 4.62 (dd, J_1 = 4.5Hz, J_2 = 3 Hz,1H), 2.51 (d, *J* = 3 Hz, 2H), 2.23 (m, 1H), 1.84 (m, 6H), 1.51 (m, 6H), 0.94 (m, 25H), 0.69 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.15, 158.51, 153.15, 152.79, 152.36, 152.27, 152.03, 139.11, 135.44, 130.05, 129.23, 126.51, 125.26, 124.18, 123.51, 116.95, 11.6.85, 112.91, 110.48, 110.06, 103.29, 79.66, 56.83, 56.31, 50.15, 42.47, 39.86, 39.66, 38.02, 36.95, 39.69, 36.34, 35.92, 32.05, 31.99, 31.64, 29.82, 28.35, 28.14, 27.75, 24.42, 23.98, 22.94, 22.69, 21.20, 19.40, 18.86, 12.00.

Ethyl 2-(6-(dodecyloxy)-3-oxo-3H-xanthen-9-yl)benzoate (Mem-2). A slurry of fluorescein (500 mg, 1.5 mmol) in ethanol (75 mL) was treated with sulfuric acid (3.5 mL) and heated to reflux for 20 hrs. Then, ethanol was removed under reduced pressure. The residual yellow oil, Cs_2CO_3 (690 mg, 2.12 mmol) and bromododecane (347.38 mg, 1.39 mmol) were dissolved in anhydrous DMF (5.0 mL) under a dry nitrogen atmosphere. The reaction was stirred for 8 hrs at 60 °C followed by adding saturated aqueous ammonium chloride (10 mL). The mixture was extracted with ethyl acetate (20 mL). The organic solution was washed three times with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure and purified by flash column chromatography (silica gel, PE/EA=1:2 affording **Mem-2** as a hyacinth solid (yield 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.23 (d, *J* = 4.5 Hz, 1H), 7.67 (m, 2H), 7.28 (s, 1H), 6.84 (dd, J_1 = 9 Hz, J_2 = 4.5 Hz, 2H), 6.71 (d, *J* = 4.5 Hz, 1H), 6.52 (d, *J* = 6 Hz, 1H) 6.41 (s, 1 H), 4.02 (m, 4H), 1.79 (t, *J* = 6 Hz, 2H), 1.23 (t, *J* = 30 Hz, 18H), 0.92 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 158.72, 165.44, 163.81, 159.02, 154.38, 150.32, 134.37, 132.57, 131.27, 130.89, 130.49, 130.33, 129.92, 129.68, 129.00, 117.64, 114.84, 113.85, 105.78, 100.81, 69.04, 61.38, 31.97, 29.70, 29.63, 29.59, 29.39, 29.38, 29.00, 26.00, 22.74, 14.17, 13.65, 1.04.

Fluorescein hydrazide was synthesized according to modified literature procedures.[1] A solution of fluorescein (1.0 g, 3.0 mmol) in methanol (50 mL) was stirred in a dry 250 mL flask under a dry nitrogen atmosphere. Hydrazine hydrate (4.0 mL, 80%) was subsequently added dropwise by syringe over 10 mins with vigorous stirring. The reaction was then heated to 80 °C and continued at this temperature until fluorescein was consumed completely. The mixture solution was cooled to room temperature, and the solvent was removed in vacuo. Purification by flash column chromatography (silica gel, DCM/MeOH = 10:1) was afforded compound as a off-white crystal (yield 92%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.77 (t, *J* = 4.4 Hz, 1H), 7.49 (t, *J* = 3.5 Hz, 2H), 6.99 (t, *J* = 3.9 Hz 1H), 6.59 (d, *J* = 2.1 Hz, 2H), 6.38-6.47 (m, 4H).

2-Amino-3'-hydroxy-3-oxospiro[isoindoline-1,9'-xanthen]-6'-yl ((3S,8S,9S,10R,13R,14S)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-

2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl) carbonate (Mem-3) was synthesized by using the similar procedure described above in **Mem-1** by using fluorescein hydrazide (yield 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95 (t, *J* = 3 Hz, 1H), 7.81 (s, 1H), 7.48 (t, *J* = 3 Hz, 2H), 7.11 (m, 1H), 6.82 (dd, J_1 = 4.5 Hz, J_2 = 1.5 Hz, 1H), 6.70 (dd, J_1 = 4.5 Hz, J_2 = 0.9 Hz, 2H), 6.50 (m, 2H), 5.42 (d, *J* = 1.5 Hz, 1H), 4.60 (dd, J_1 = 6 Hz, J_2 =3 Hz, 1H), 3.69 (bs, NH_2), 2.49 (dd, J_1 = 10.5 Hz, J_2 = 4.5 Hz, 2H), 1.97 (m, 7H), 1.45 (m, 11H), 0.98 (m, 20H), 0.69 (s, 3H). ^{13}CNMR (75 MHz, $CDCl_3$): δ = 166.94, 158.22, 153.03, 152.89, 152.81, 151.80, 150.89, 139.13, 133.33, 129.33, 129.07, 128.44, 128.26, 124.04, 123.54, 123.48, 116.96, 116.28, 112.93, 110.20, 109.30, 103.63, 79.55, 65.75, 56.83, 56.32, 50.14, 42.47, 39.87, 39.66, 38.02, 36.95, 36.69, 36.33, 35.92, 32.05, 31.99, 28.35, 28.13, 27.74, 24.42, 23.98, 22.93, 22.69, 21.02, 19.40, 18.86, 12.00.

2-Amino-3'-(dodecyloxy)-6'-hydroxyspiro[isoindoline-1,9'-xanthen]-3-one (Mem-4) was synthesized by using the similar procedure described above in **Mem-2** by using fluorescein hydrazide (yield 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.94 (dd, J_1 = 3 Hz, J_2 = 1.5 Hz, 1H), 7.67 (t, *J* = 4.5 Hz, 2H), 7.05 (dd, J_1 = 3 Hz, J_2 = 1.5 Hz,1H), 6.77 (d, *J* = 1.2 Hz, 1H), 6.70 (s, 1H), 6.52 (m, 4H) 3.93 (t, *J* = 7.5 Hz, 2H), 3.68 (bs, $NH_{2)}$, 1.76 (t, *J* = 7.5 Hz, 2H), 1.27 (t, *J* = 24 Hz, 18H), 0.88 (t, *J* = 7.5Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.97, 160.48, 158.30, 153.38, 151.23, 133.26, 129.35, 128.85, 128.20, 128.13, 123.96, 123.43, 112.66, 112.17, 109.66, 109.19, 103.68, 101.81, 68.44, 66.23, 32.00, 29.74, 29.71, 29.69, 29.66, 29.46, 29.42, 29.22, 26.11, 22.76, 14.19.

2-Amino-3'-hydroxy-6'-(1-(2-nitrophenyl)ethoxy)spiro[isoindoline-1,9'-xanthen]-3-one. Compound was synthesized according to modified literature procedures.[1] 1-(1-Bromoethyl)-2-nitrobenzene (57 mg, 0.31 mmol) and fluorescein hydrazide (108 mg, 0.25 mmol) were added to a dried 25 mL flask. Anhydrous DMF (1 mL) was added, and the mixture was stirred under a dry nitrogen atmosphere for 15 mins. Cs_2CO_3 (196 mg, 0.6 mmol) was speedy added to the solution. The reaction was stirred for another 6 hrs at room temperature. The mixture was poured into saturated ammonium chloride aqueous solution (10 mL), and then the aqueous layer was further extracted with ethyl acetate (30 mL). The organic solution was washed three times with brine, dried over anhydrous Na_2SO_4 and filtered. Solvent of the organic solution was removed under reduced pressure and purified by flash column chromatography (silica gel, DCM/EA = 4:1) affording compound as a turquoise liquid (yield 54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04 (d, *J* = 8.4 Hz, 1H), 7.91 (m, 1H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.46 (m, 3H), 7.03 (dd, J_1 = 8.7, J_2 = 3.6 Hz, 1H), 6.69 (s, 1H), 6.61 (d, *J* = 9.3 Hz, 1H), 6.48 (m, 4H), 6.04 (m, 1H), 1.70 (d, *J* = 6.3 Hz, 3H).

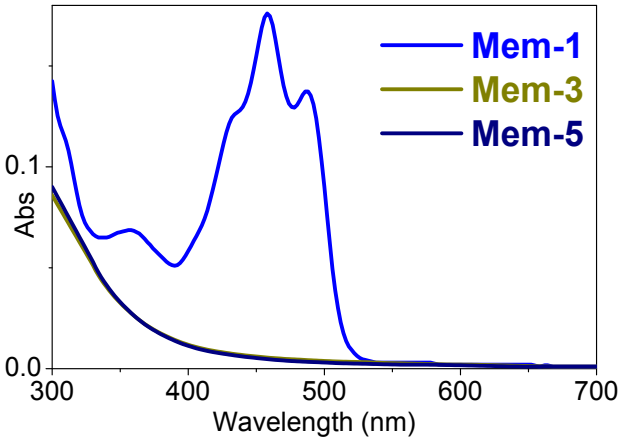


Fig. S1 Absorption spectra of **Mem-1/3/5** (5.0 μM) in ethanol. Abs = absorbance.

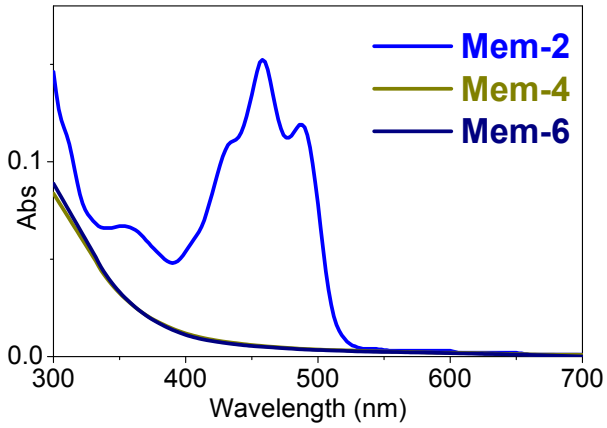


Fig. S2 Absorption spectra of **Mem-2/4/6** (5.0 μM) in ethanol. Abs = absorbance.

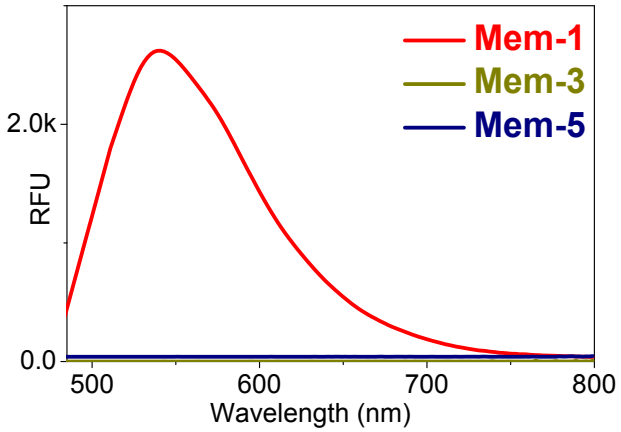


Fig. S3 Fluorescence emission spectra of **Mem-1/3/5** (5.0 μM) in ethanol. RFU = relative fluorescence units.

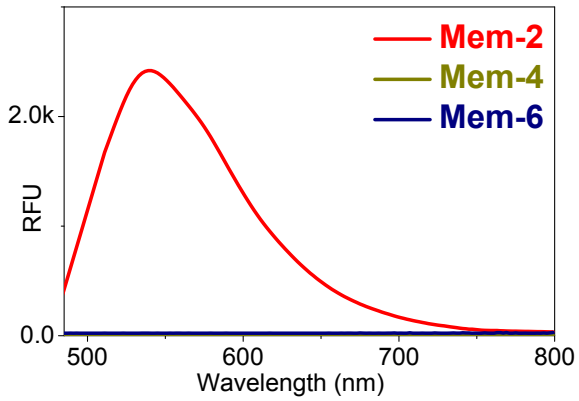


Fig. S4 Fluorescence emission spectra of **Mem-2/4/6** (5.0 μM) in ethanol. RFU = relative fluorescence units.

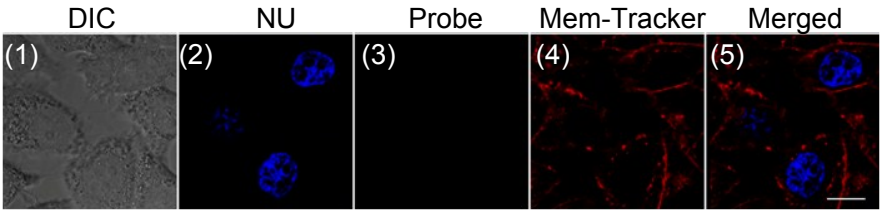


Fig. S5 One-photon excited fluorescence images of HepG2 cells upon treatment with DMSO as background control. Scale bar = 15 μm.

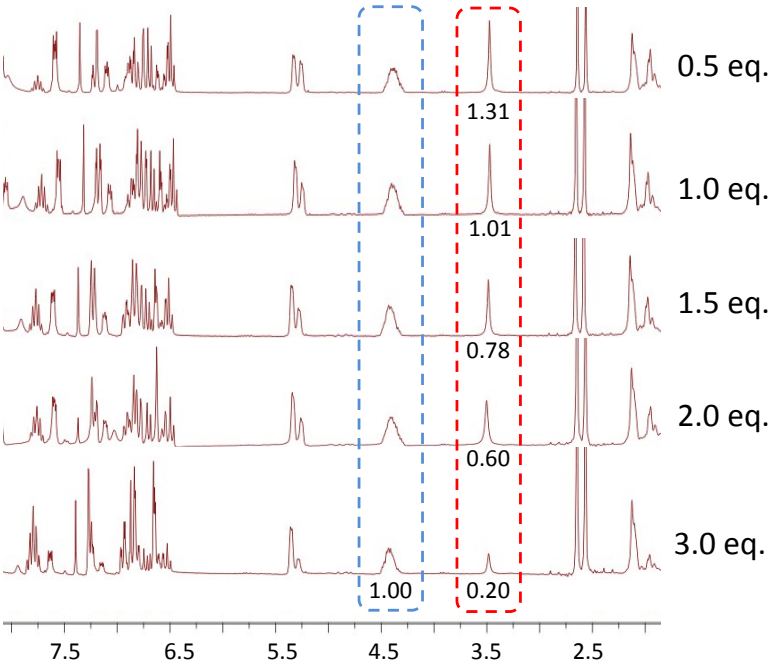


Fig. S6 ^{1}H-NMR titration for **Mem-3** against different equivalent copper (II) (0.5 - 3.0 eq.) in $CDCl_3$ for 60 mins incubation at room temperature.

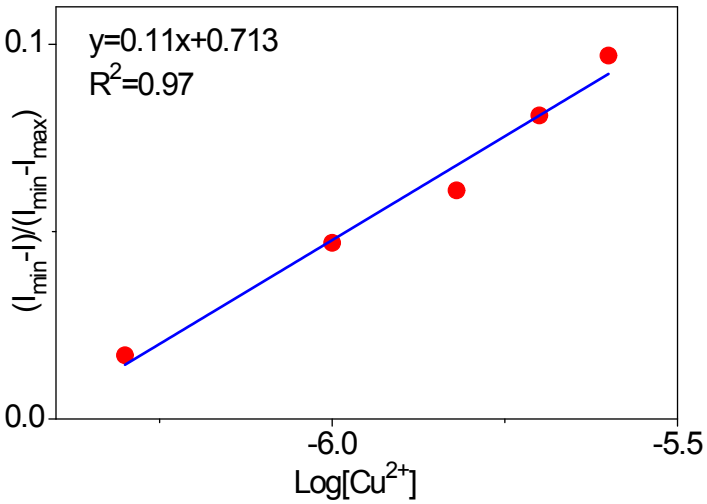


Fig. S7 Normalized of the fluorescence signal of uncaged **Mem-5** against various Cu^{2+} concentrations. The detection limit was determined as a reported method.[2]

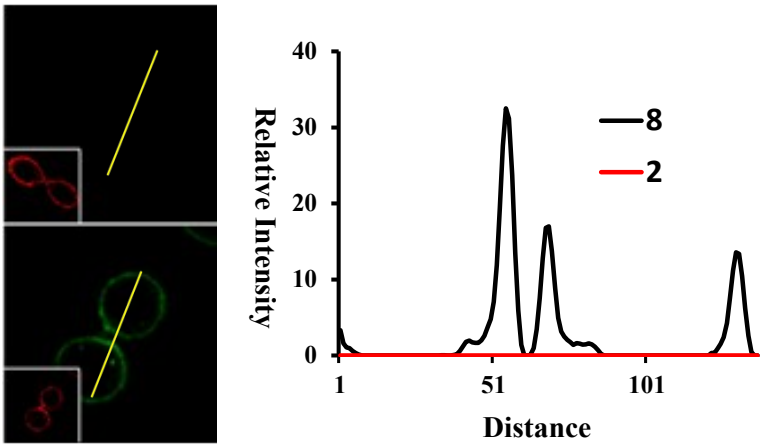


Fig. S8 Quantification analysis of images (2) and (8) in Fig. 6(e) in the maintext.

References

[1] (a) L. Yuan, W. Y. Lin, Z. M. Cao, L. L. Long and J. Z. Song, *Chem. Eur. J.*, **2011**, *17,* 689-696. (b) V. Dujols, F. Ford and A. W. Czarnik, *J. Am. Chem. Soc.*, **1997**, *119,* 7386-7387.

[2] (a) M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, *Anal. Chem*. **1996**, *68*, 1414-1418. (b) W. Y. Lin, L. Yuan, L. Long, C. Guo, J. Feng, *Adv. Funct. Mater*., **2008**, *18*, 2366-2372.

^{1}H and ^{13}C NMR spectra.

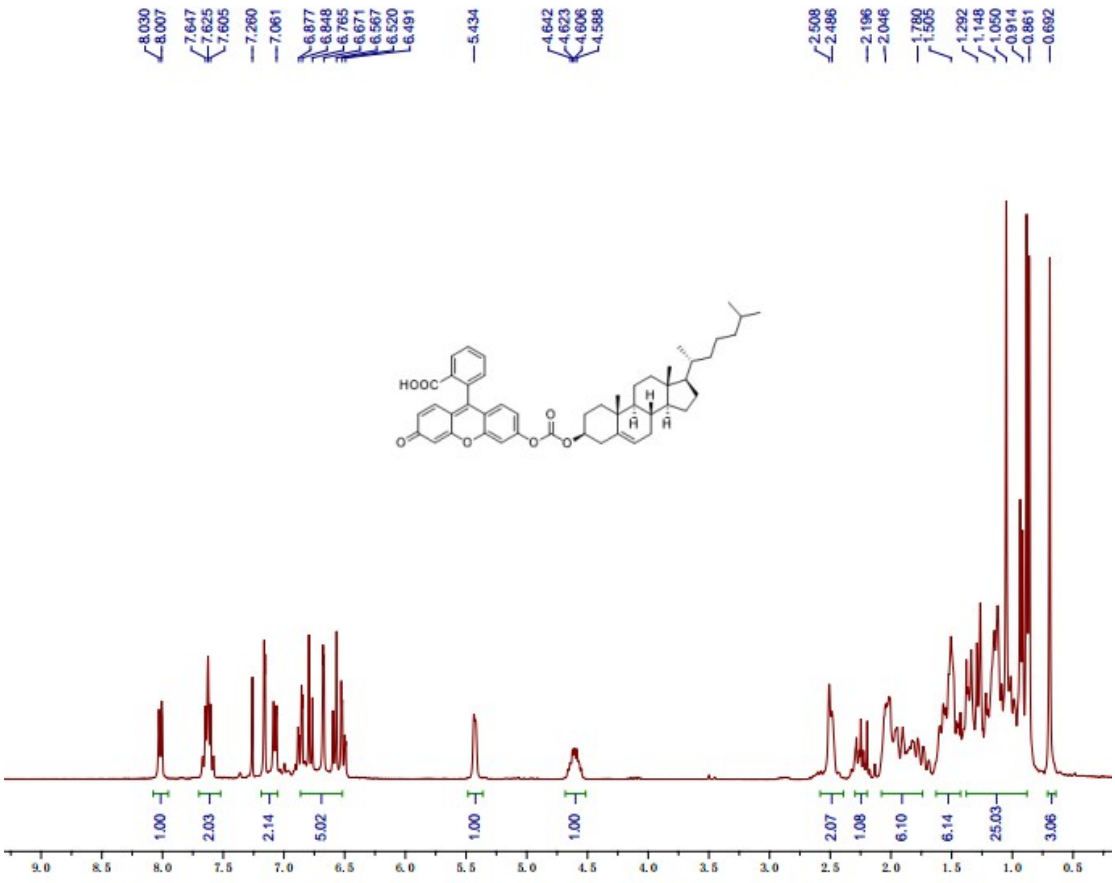


Fig. S9 ^{1}H NMR spectrum of **Mem-1**.

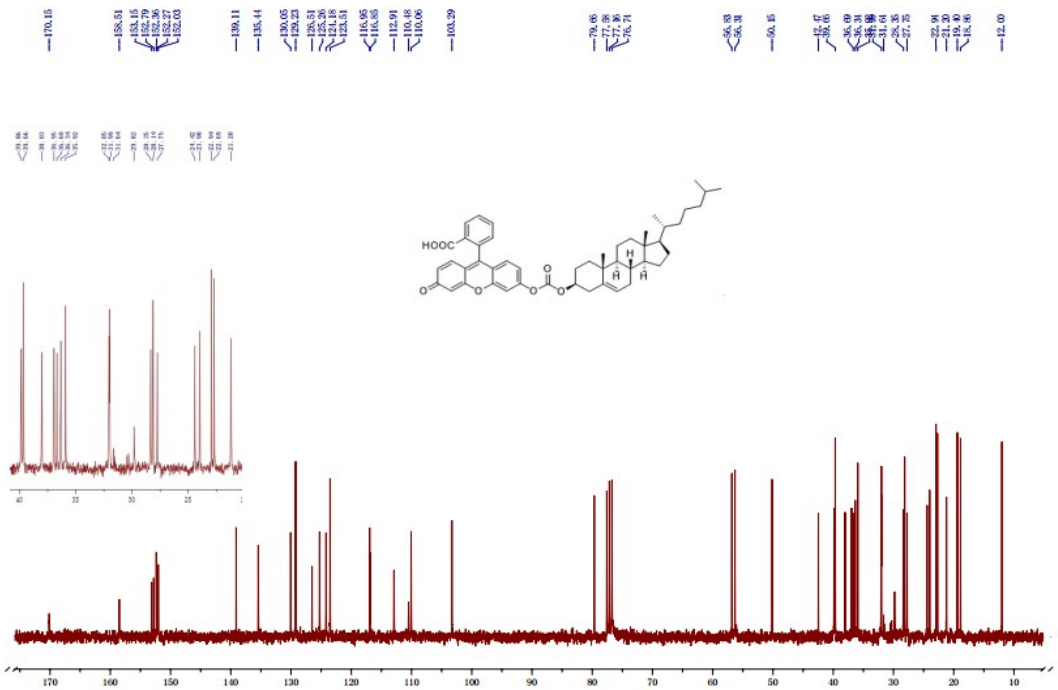


Fig. S10 ^{13}C NMR spectrum of **Mem-1**.

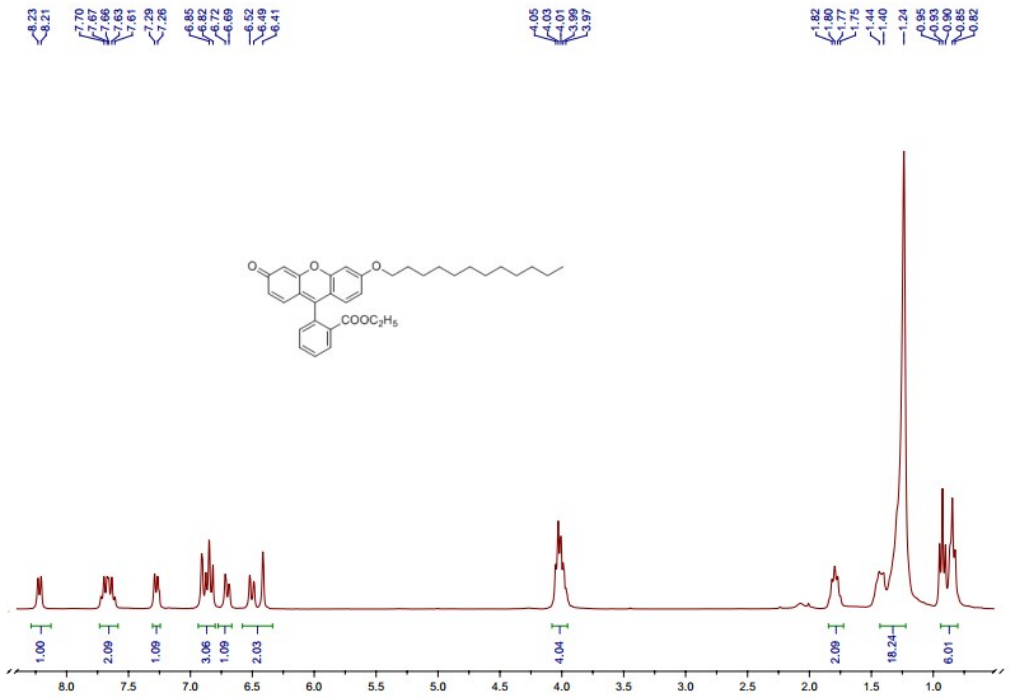


Fig. S11 ^{1}H NMR spectrum of **Mem-2**.

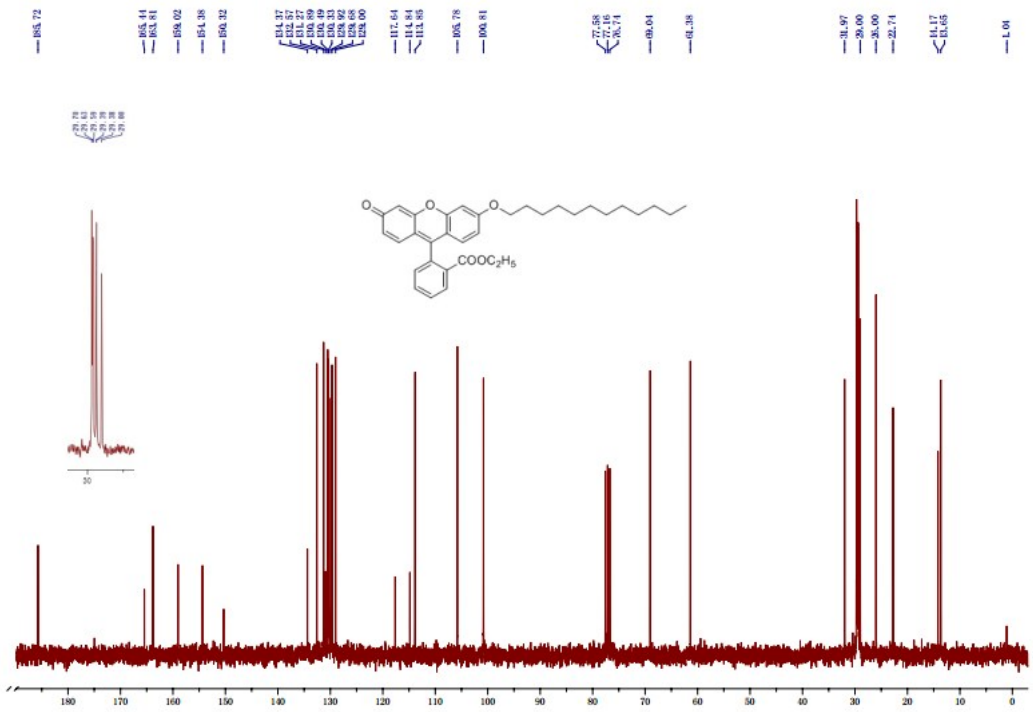


Fig. S12 ^{13}C NMR spectrum of **Mem-2**.

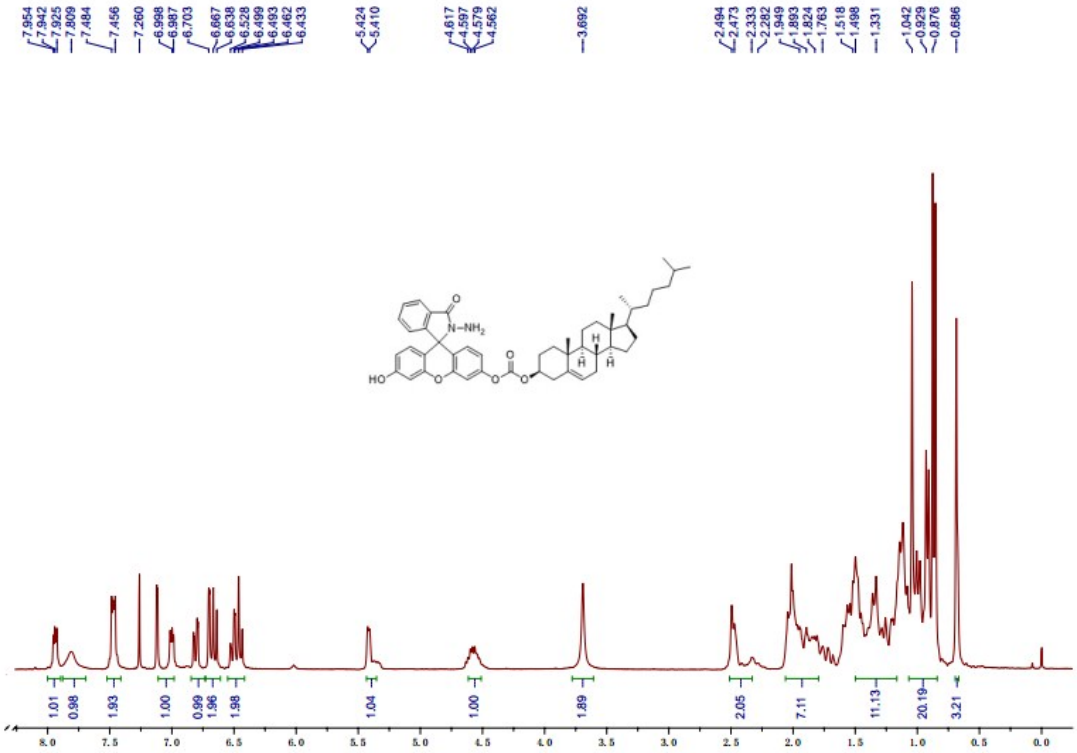


Fig. S13 ^{1}H NMR spectrum of **Mem-3**.

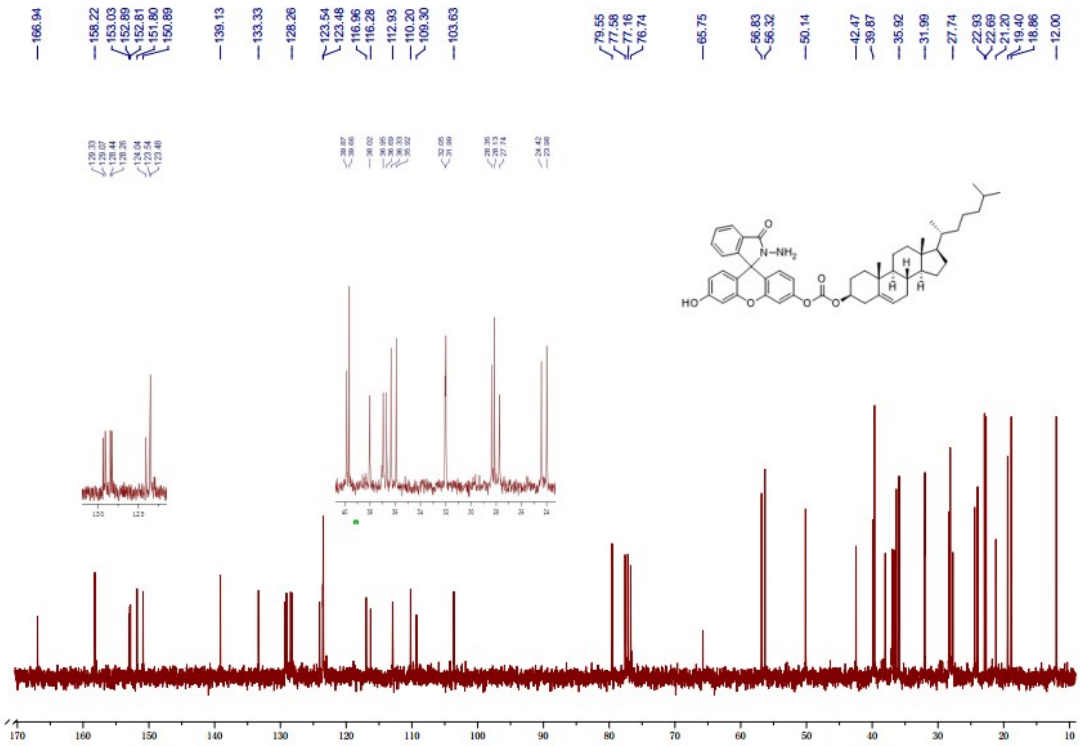


Fig. S14 ^{13}C NMR spectrum of **Mem-3**.

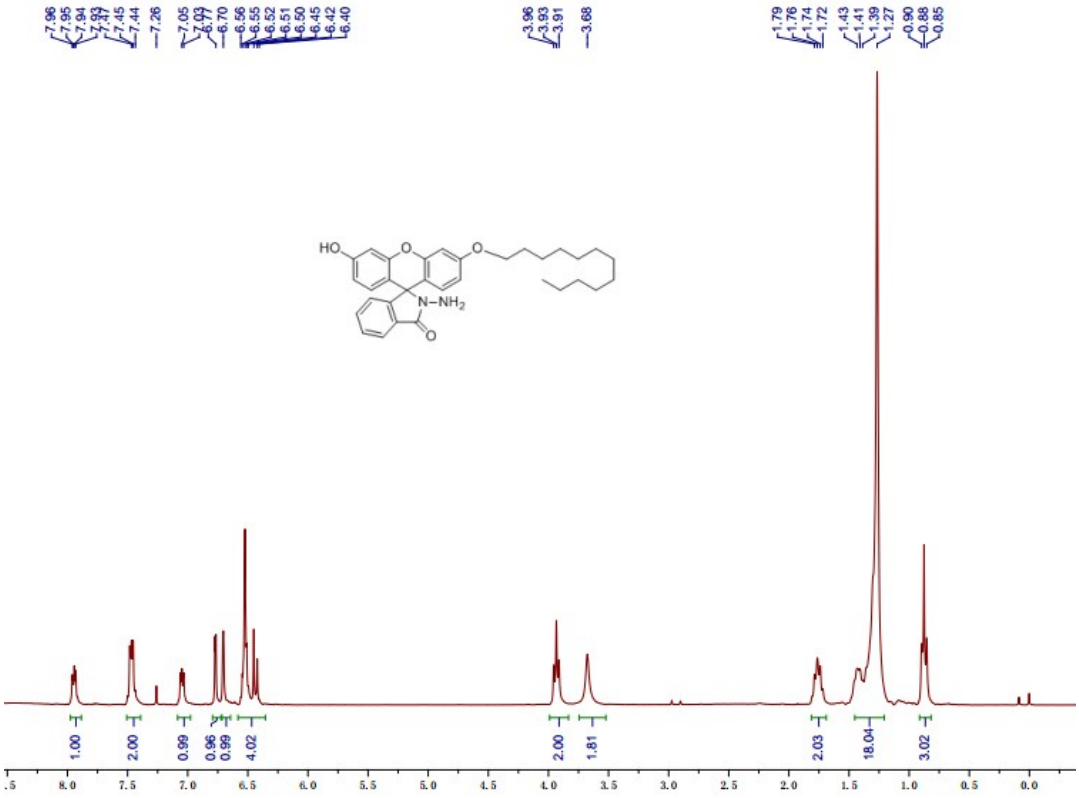


Fig. S15 ^{1}H NMR spectrum of **Mem-4**.

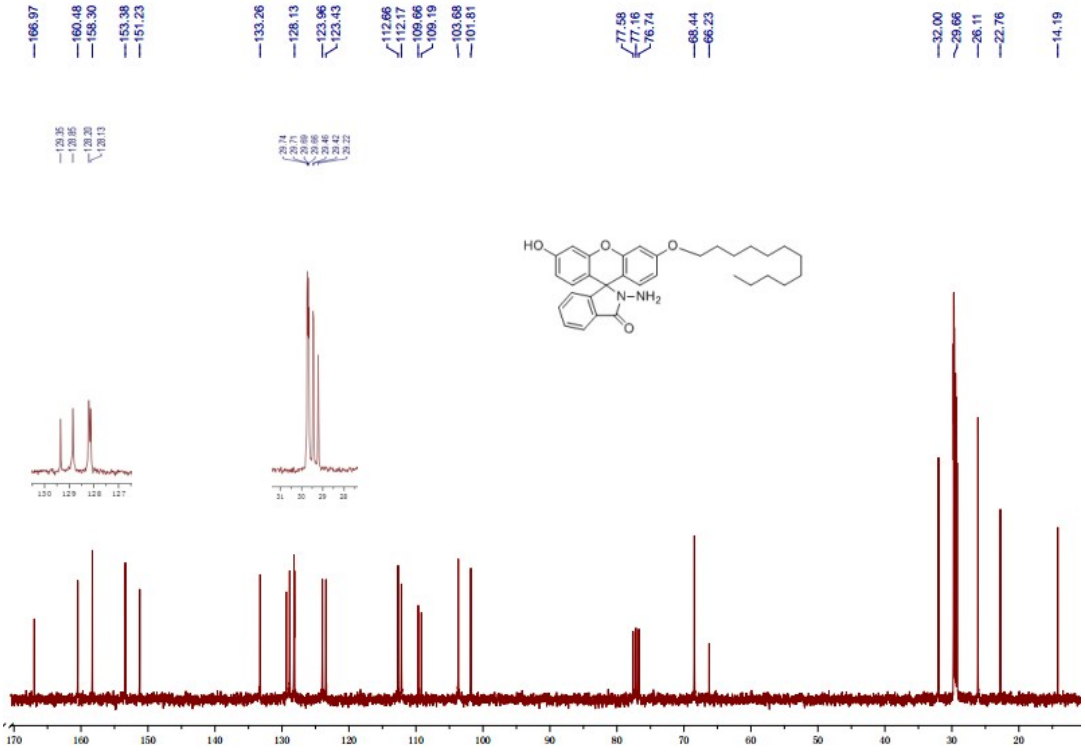


Fig. S16 ^{13}C NMR spectrum of **Mem-4**.

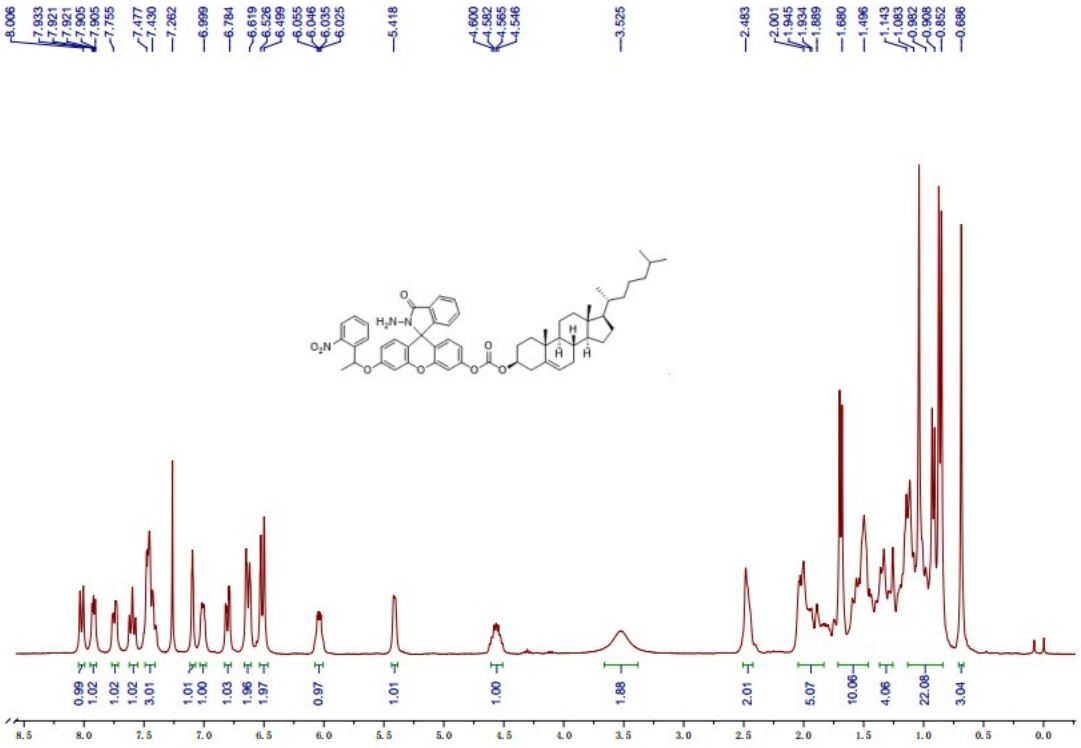


Fig. S17 ^{1}H NMR spectrum of **Mem-5**.

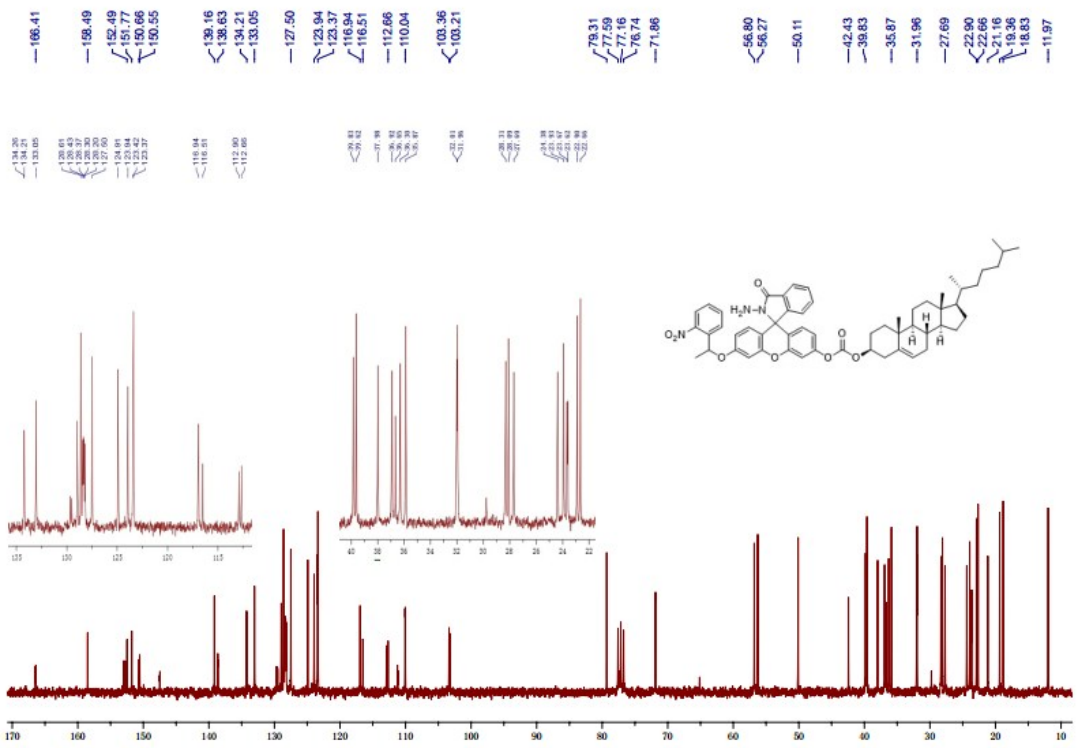


Fig. S18 ^{13}C NMR spectrum of **Mem-5**.

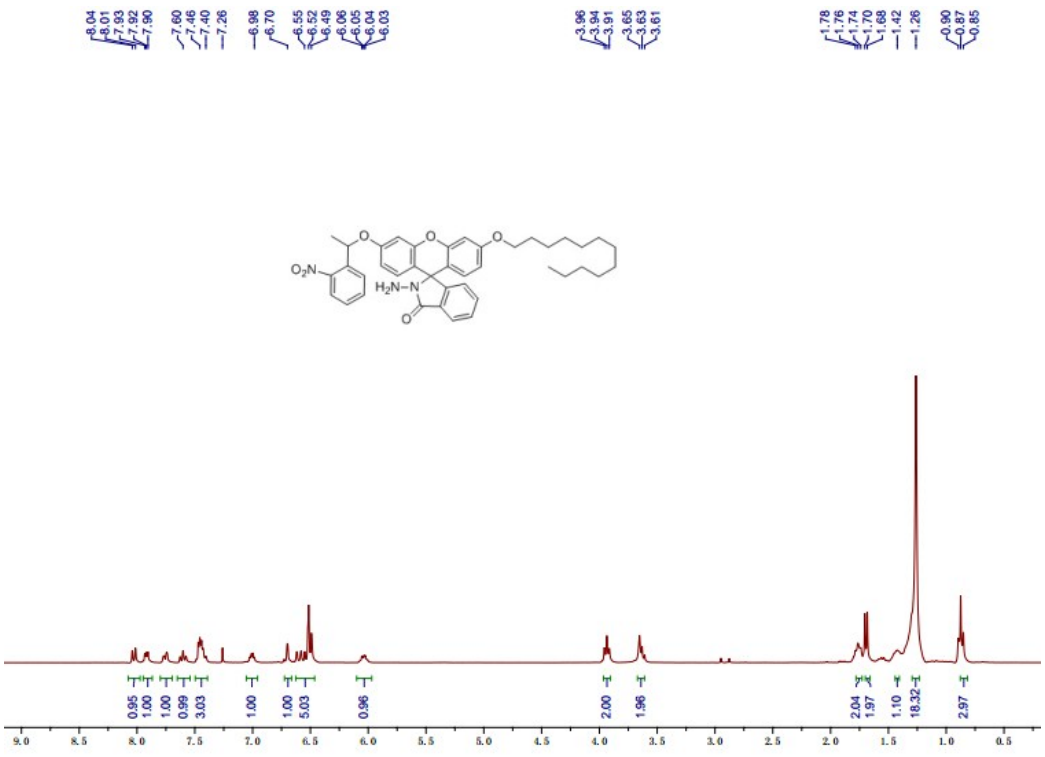


Fig. S19 ^{1}H NMR spectrum of **Mem-6**.

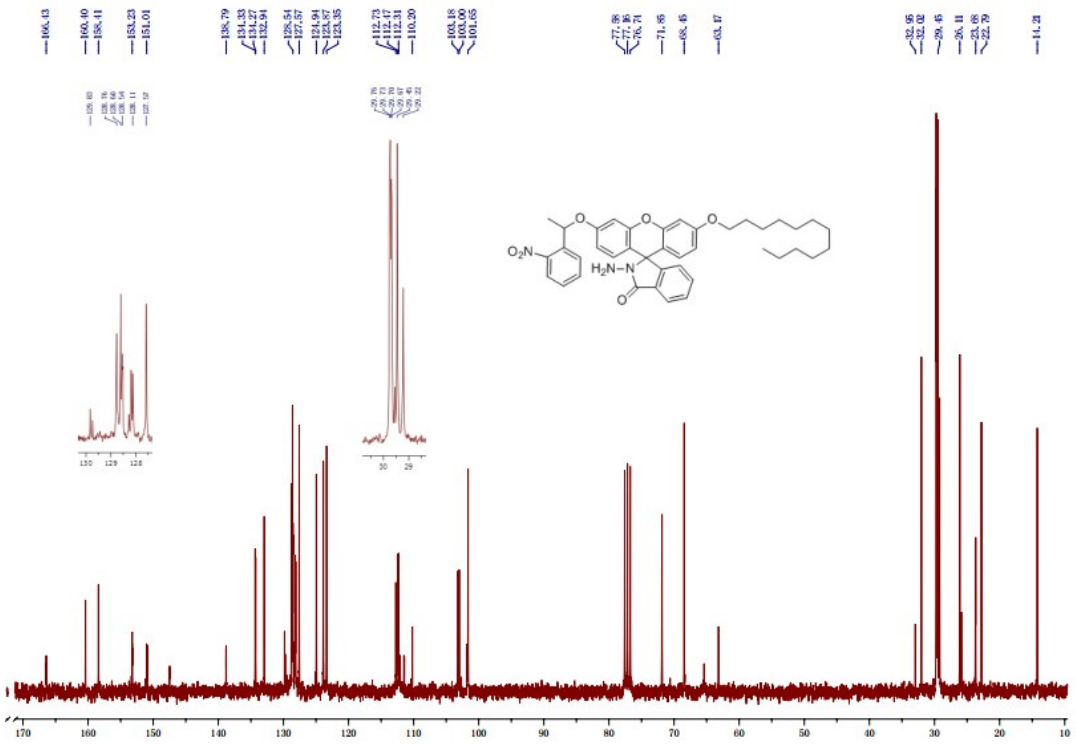


Fig. S20 ^{13}C NMR spectrum of **Mem-6**.