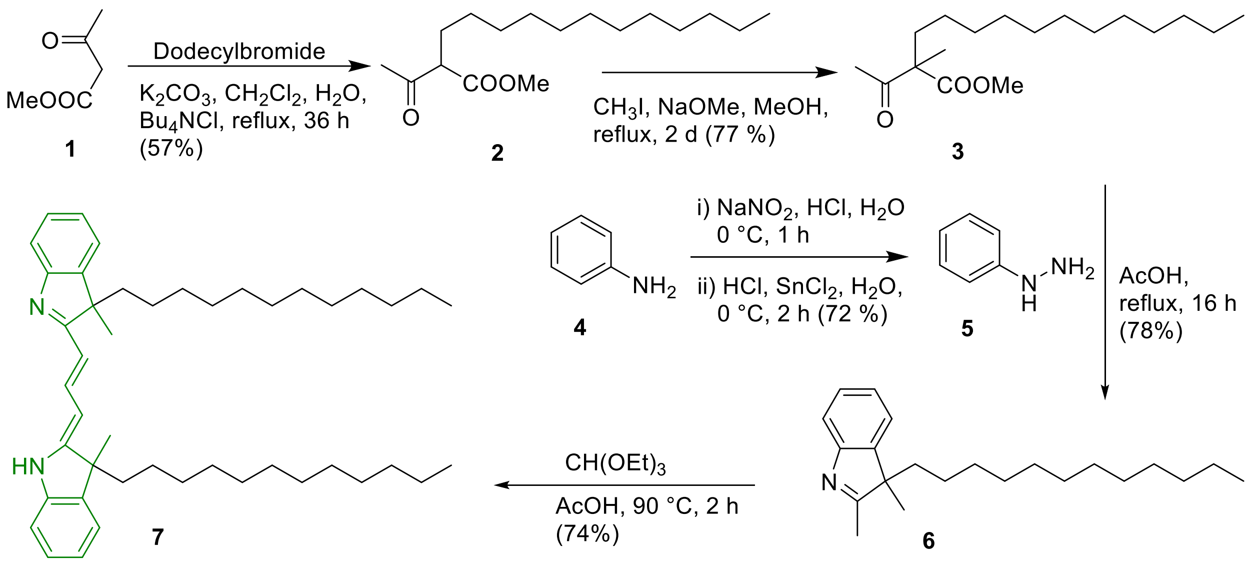
Supporting Information

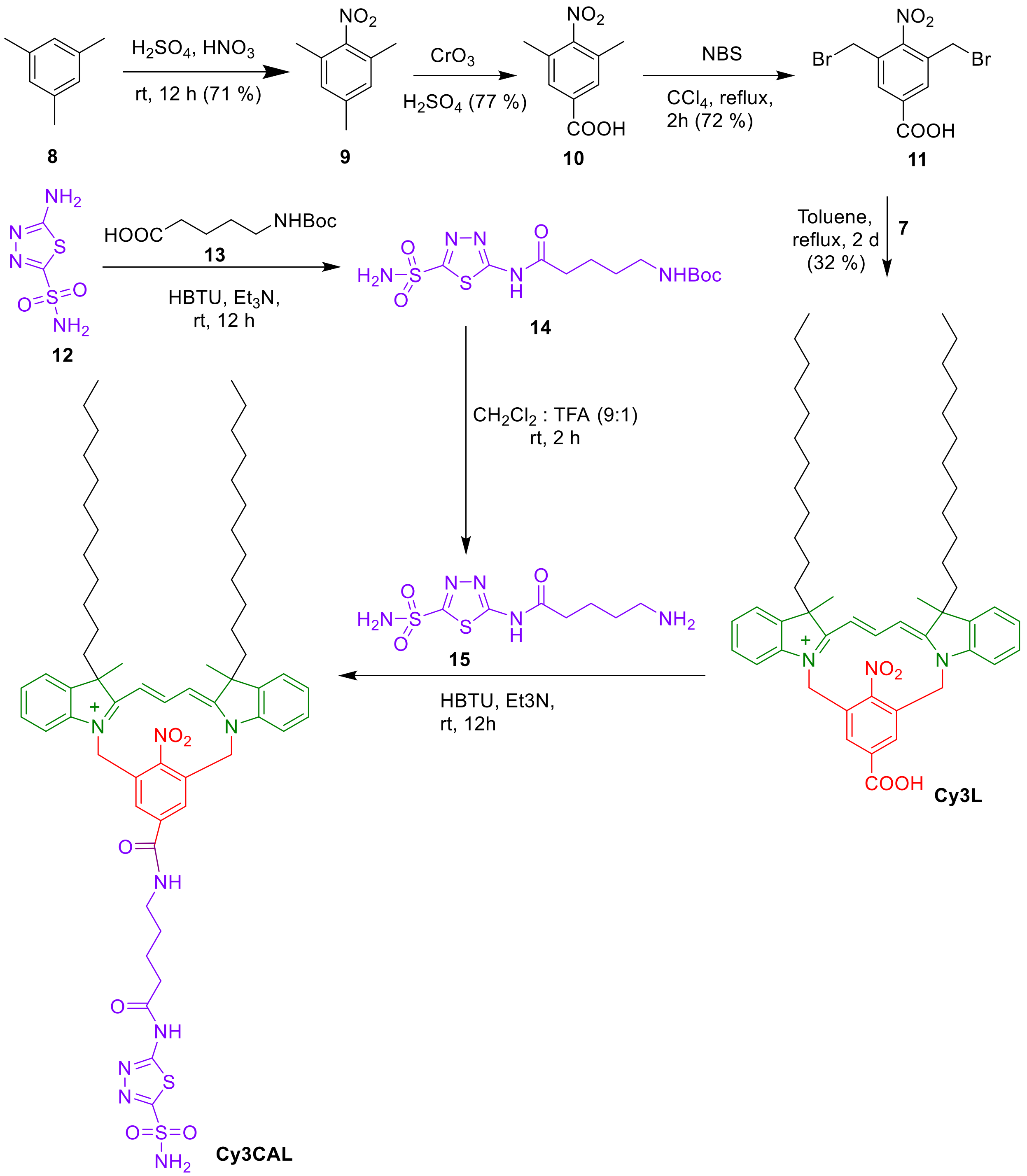
Photo-responsive Fluorescent Amphiphile for Target-specific and Image-guided Drug Delivery Applications

Subhasis Dey,[a†] Plaboni Sen,[b†] Anjali Patel,[c] BiswaMohan Prusty,[a] Siddhartha Sankar Ghosh,*[b] and Debasis Manna*[a]

[a]Department of Chemistry, Indian Institute of Technology Guwahati, Assam 781039, India
[b]Department of Bioscience and Bioengineering, Indian Institute of Technology Guwahati, Assam 781039, India
[c]Centre for the Environment, Indian Institute of Technology Guwahati, Assam 781039, India



Scheme S1: Synthetic routes to Cy3 derivative**.**



Scheme S2: Synthetic routes to macrocyclic photoresponsive fluorescent amphiphiles **Cy3CAL**.

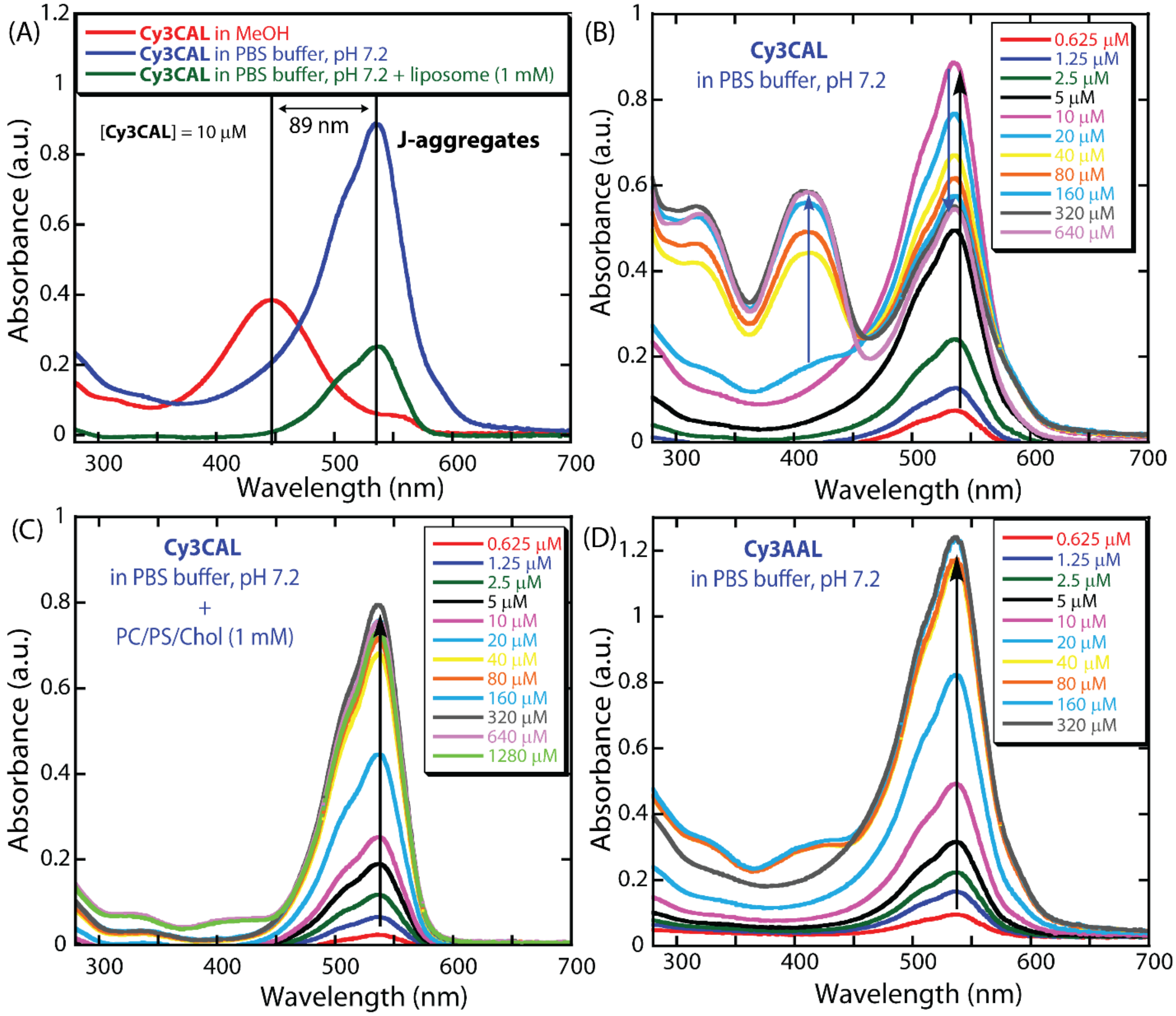


Fig. S1. UV-Vis spectra of **Cy3CAL** (10 µM) in MeOH, PBS buffer, pH 7.2, and in the presence of liposomes of PC/PS/Chol (60:20:20) (A). Concentration-dependent UV-Vis spectra of **Cy3CAL** in PBS buffer, pH 7.2 (B). Concentration-dependent UV-Vis spectra of **Cy3CAL** in the presence of liposomes of PC/PS/Chol (60:20:20) (C). Concentration-dependent UV-Vis spectra of **Cy3AAL** in PBS buffer, pH 7.2 (D).

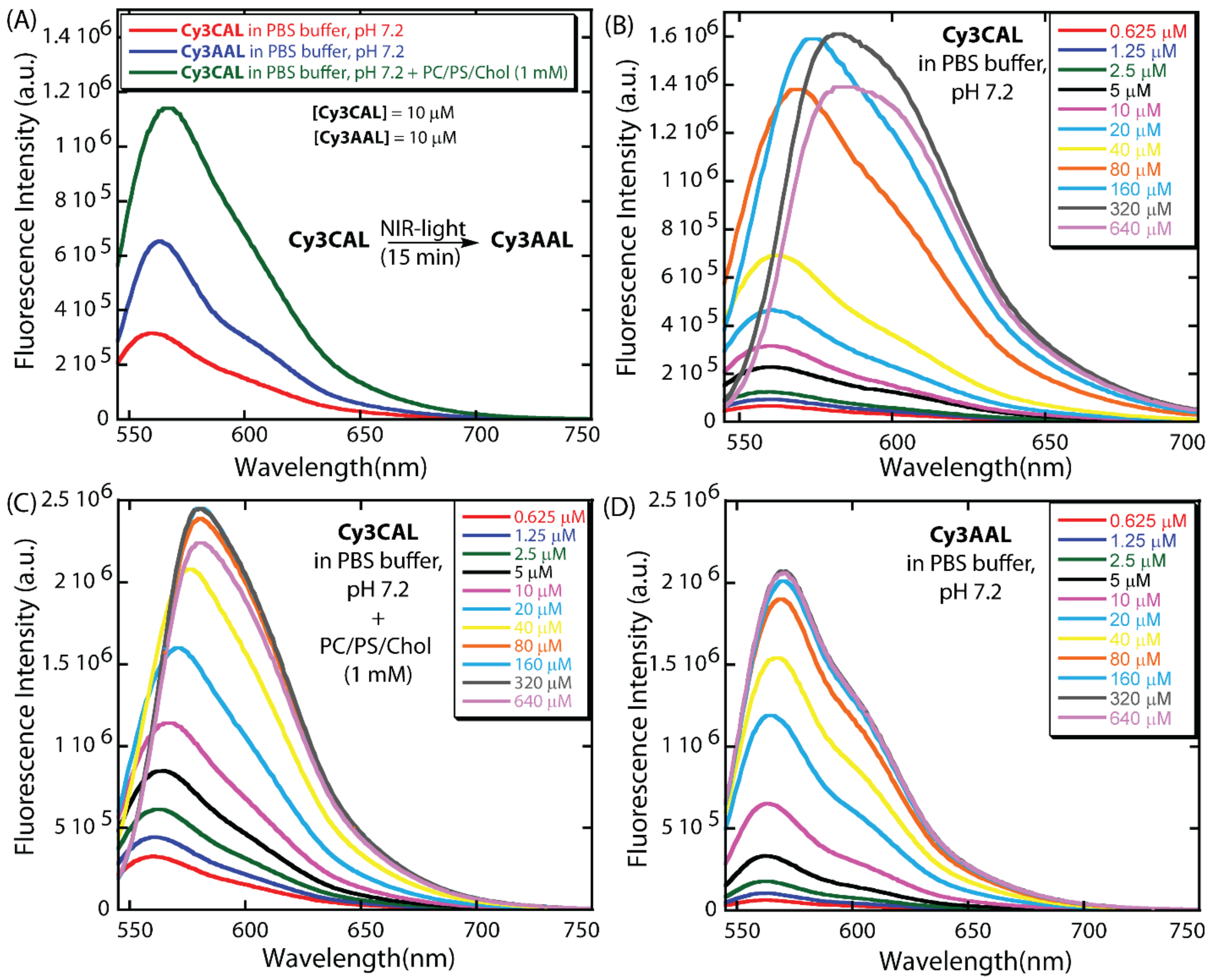


Fig. S2. Fluorescence spectra of **Cy3CAL** and **Cy3AAL** (10 μM) in PBS buffer, pH 7.2, and in the presence of liposomes of PC/PS/Chol (60:20:20) (A). Concentration-dependent fluorescence spectra of **Cy3CAL** in PBS buffer, pH 7.2 (B). Concentration-dependent fluorescence spectra of **Cy3CAL** in the presence of liposomes of PC/PS/Chol (60:20:20) (C). Concentration-dependent fluorescence spectra of **Cy3AAL** in PBS buffer, pH 7.2 (D). NIR laser light (808 nm, 1 W cm^{-2}) source was used to irradiate the samples for 15 minutes.

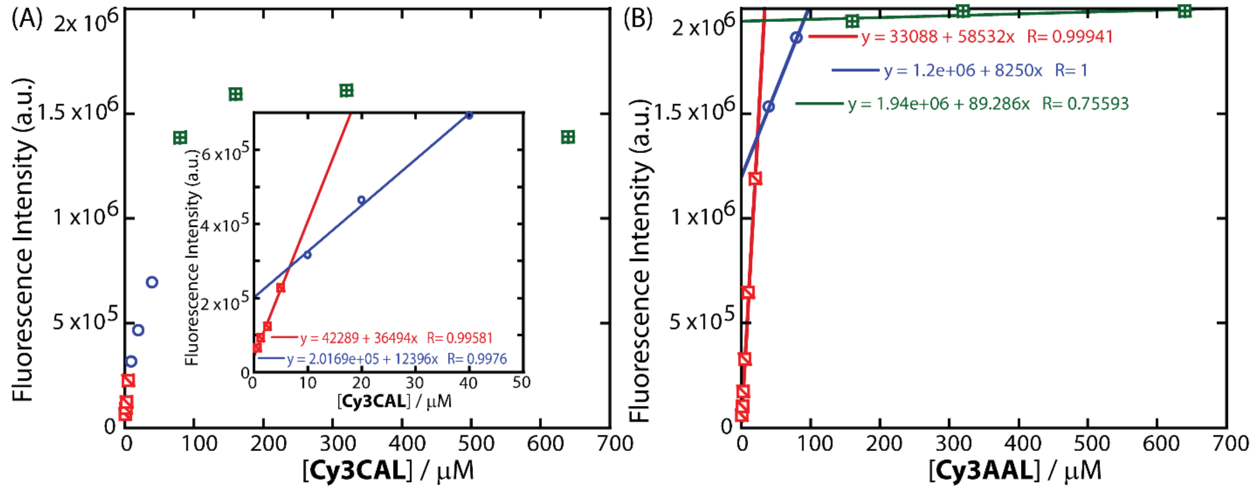


Fig. S3. Measurement of the critical aggregation constant of **Cy3CAL** and **Cy3AAL**.

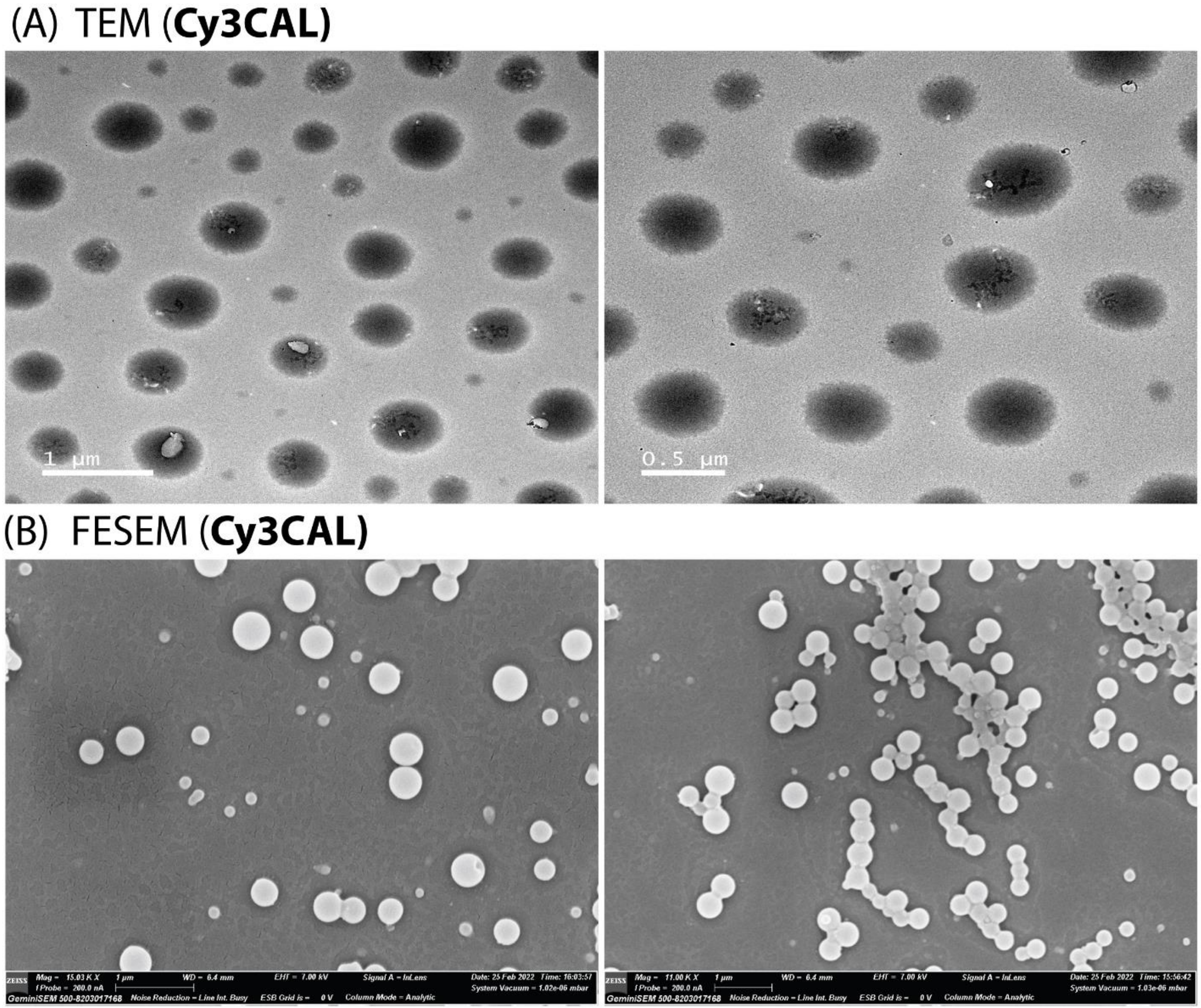


Fig. S4. Representative TEM images of **Cy3CAL** before light treatment (A). Representative FESEM images of **Cy3CAL** before light treatment (B).

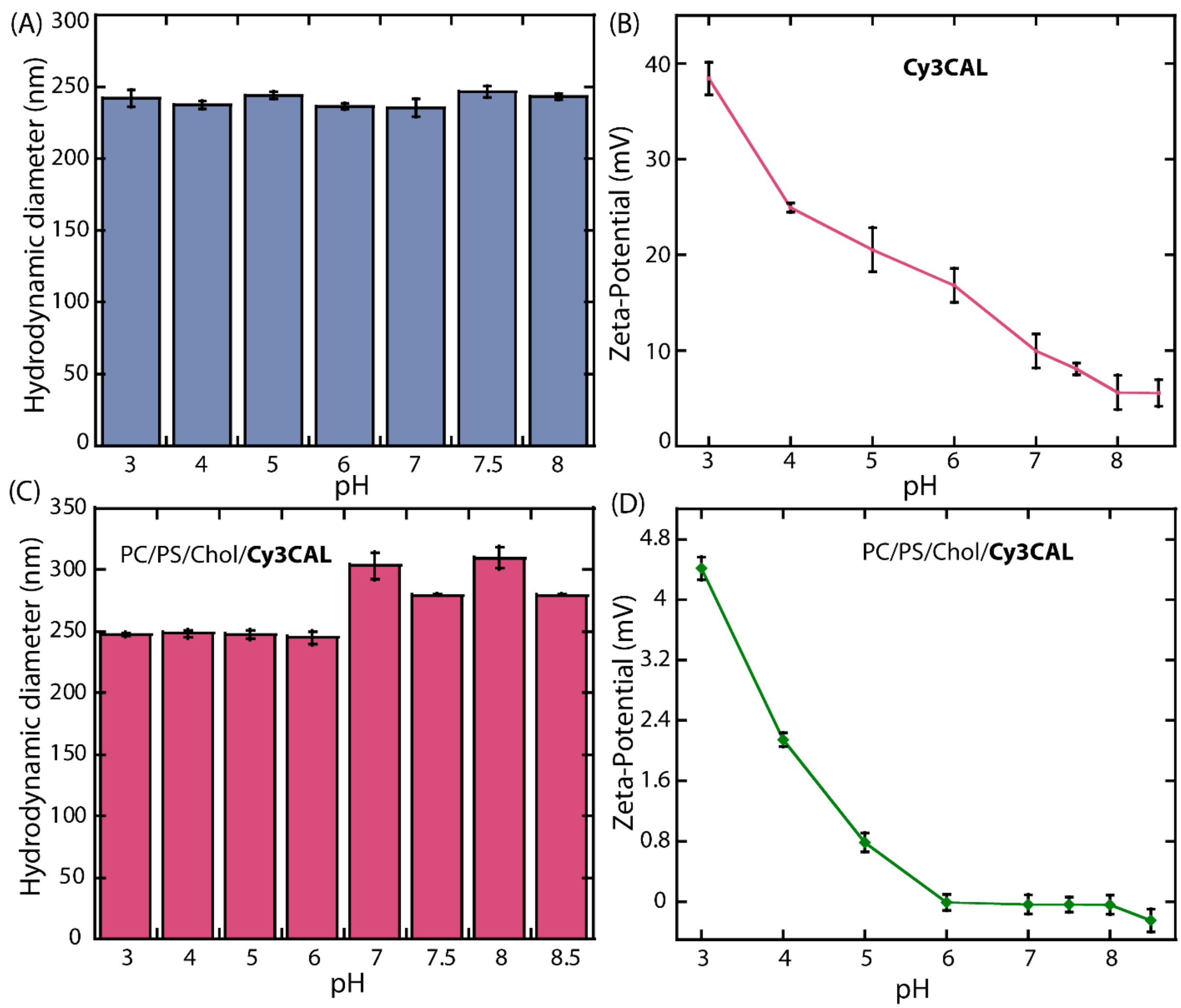


Fig. S5. Variation of hydrodynamic diameter (A) and zeta potential (B) of the soluble aggregates of **Cy3CAL** at various pH. Variation of hydrodynamic diameter (C) and zeta potential (D) of PC/PS/Chol/**Cy3CAL** (55/20/20/5) at various pH.

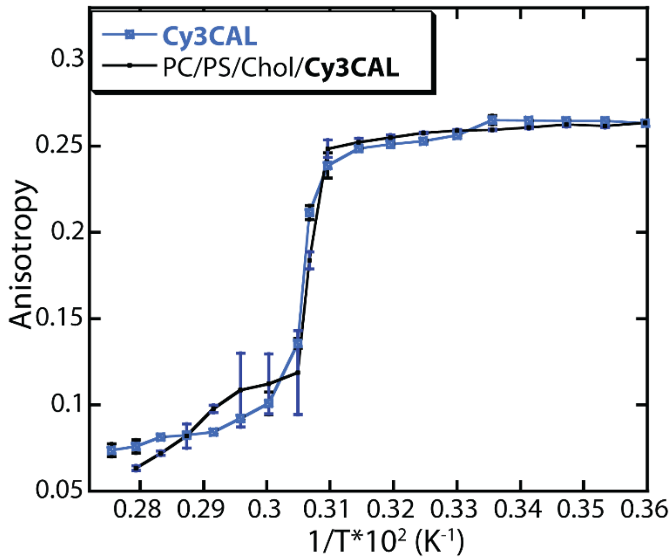


Fig. S6. The temperature-dependent fluorescence anisotropy measurement of environment-sensitive 1,6-diphenyl-1,3,5-hexatriene dye incorporated within the water-soluble aggregates.

NOTE: The photoreaction of the labile ortho-nitrobenzyl group is initiated by an intramolecular 1,5-H shift yielding aci-nitro protomers as a first intermediate. After that, cyclization to short-lived benzisoxazolidines is generated followed by ring opening yielding an o-nitrosoaldehyde group in the molecule.[1] However, the nitroso group may not be radically active in abstracting the hydrogen radicle from the other benzyl position.

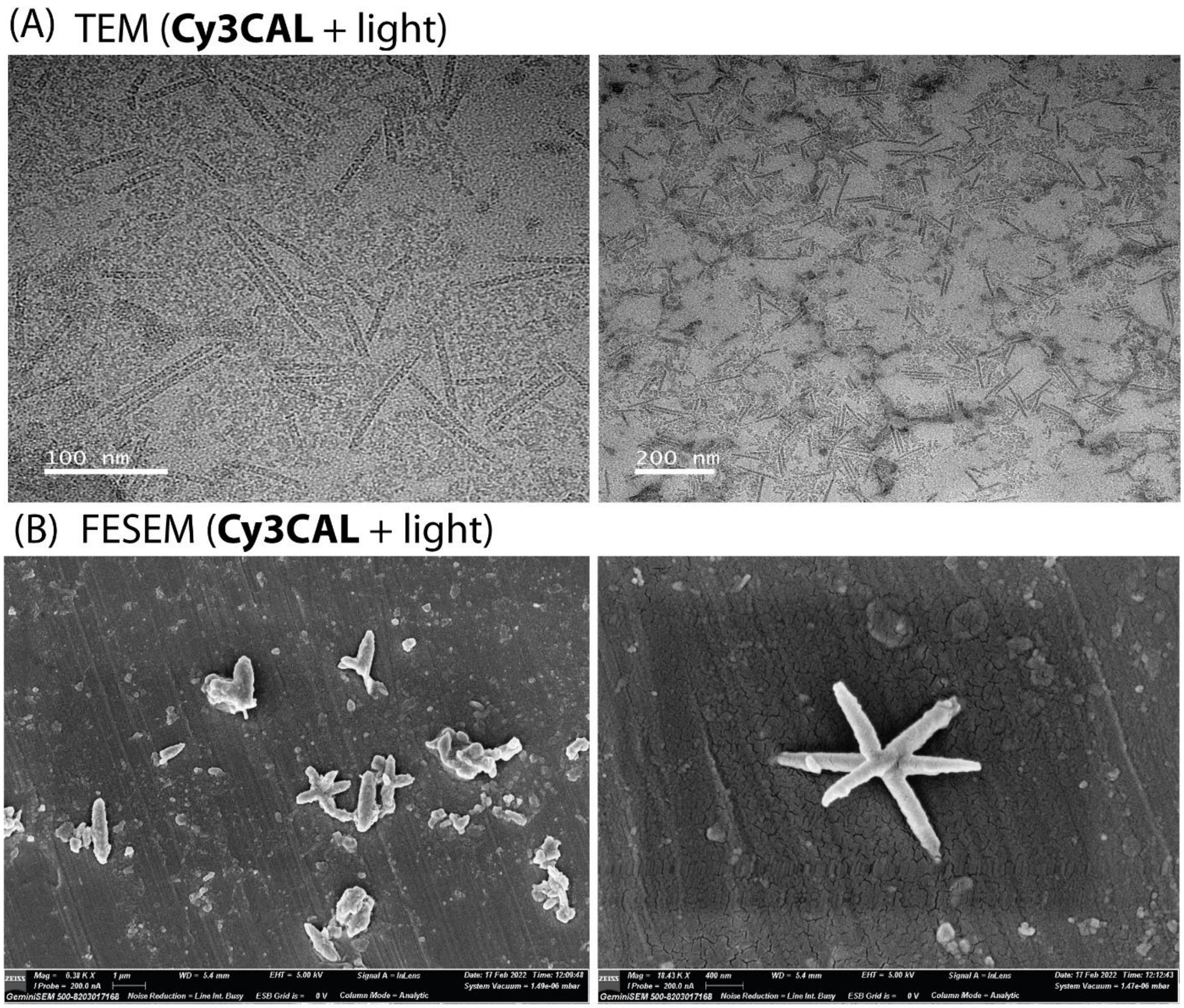


Fig. S7. Representative TEM images of **Cy3CAL** after light treatment (A). Representative FESEM images of **Cy3CAL** after light treatment (B).

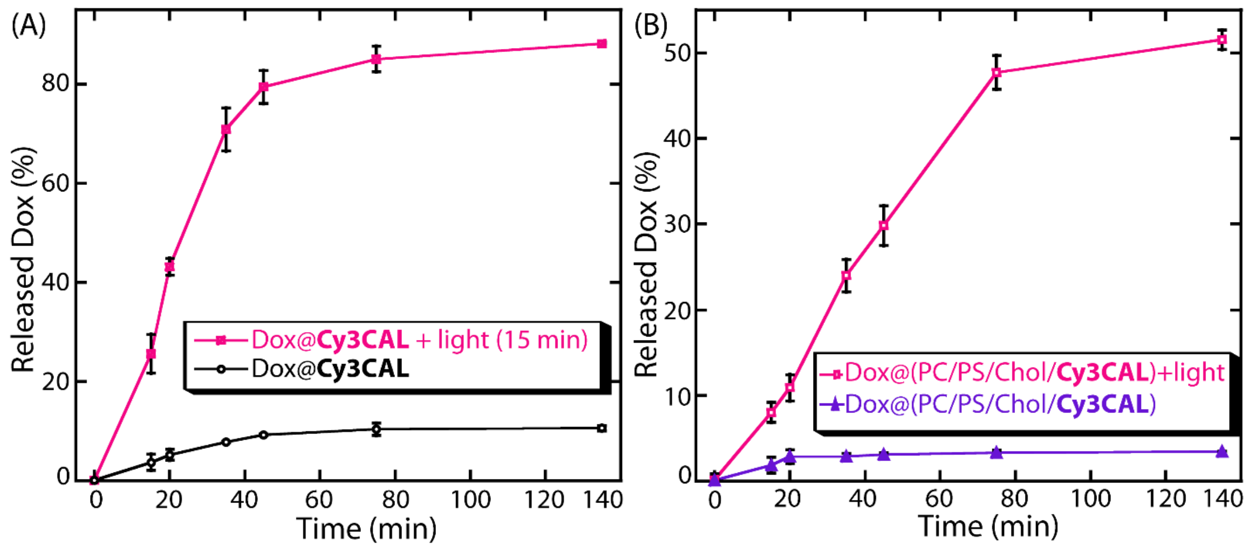


Fig. S8. The Dox release profiles of **Cy3CAL** without and with NIR-light treatment (15 min) (A). The Dox release profiles of lipid mixture of PC/PS/Chol/**Cy3CAL** without and with NIR-light treatment (15 min) (B). NIR laser light (808 nm, 1 W cm^{-2}) source was used to irradiate the samples for 15 minutes.

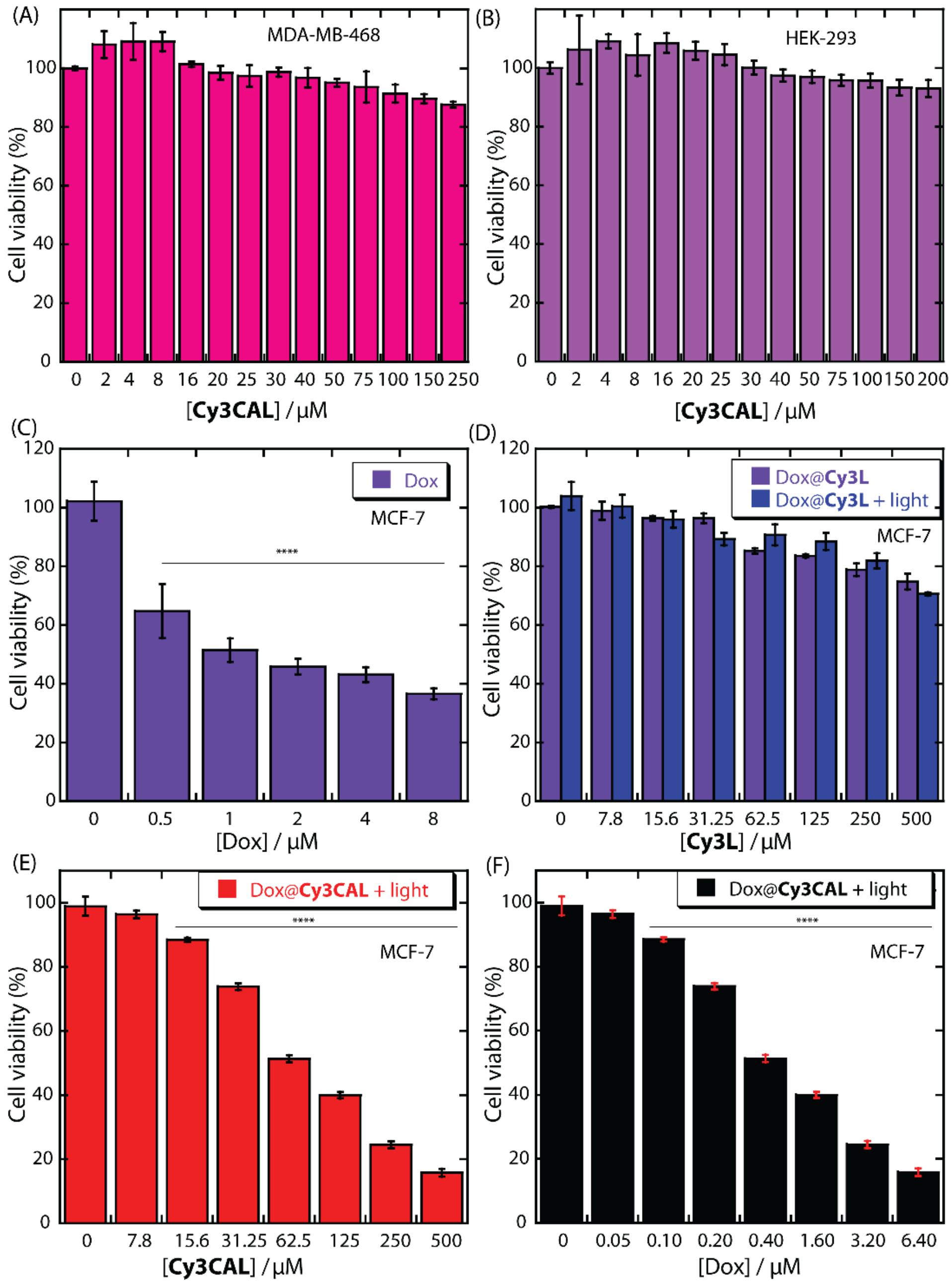


Fig. S9. Viabilities of MDA-MB-468 (A) and HEK-293 (B) cells in the presence of **Cy3CAL** at different concentrations. Viabilities of MCF-7 cells in the presence of **Dox** at different concentrations (C). Viabilities of MCF-7 cells in the presence of **Cy3CAL** (before and after light treatment for 15 min) at different concentrations (D). Viability of MCF-7 cells in the presence of Dox@**Cy3CAL** after light treatment with respect to the concentration of **Cy3CAL** (E) and DOX (F). NIR laser light (808 nm, 1 W cm^{-2}) source was used to irradiate the samples for 15 minutes.

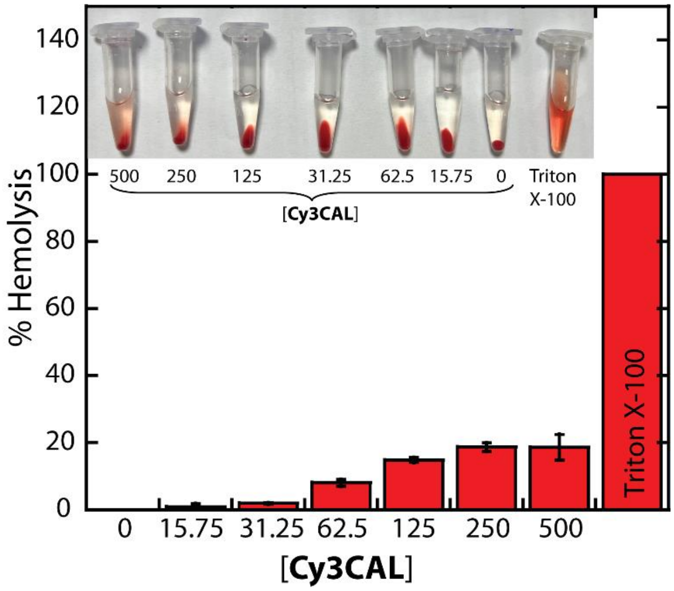


Fig. S10. Hemolytic activity of **Cy3CAL** at different concentrations.

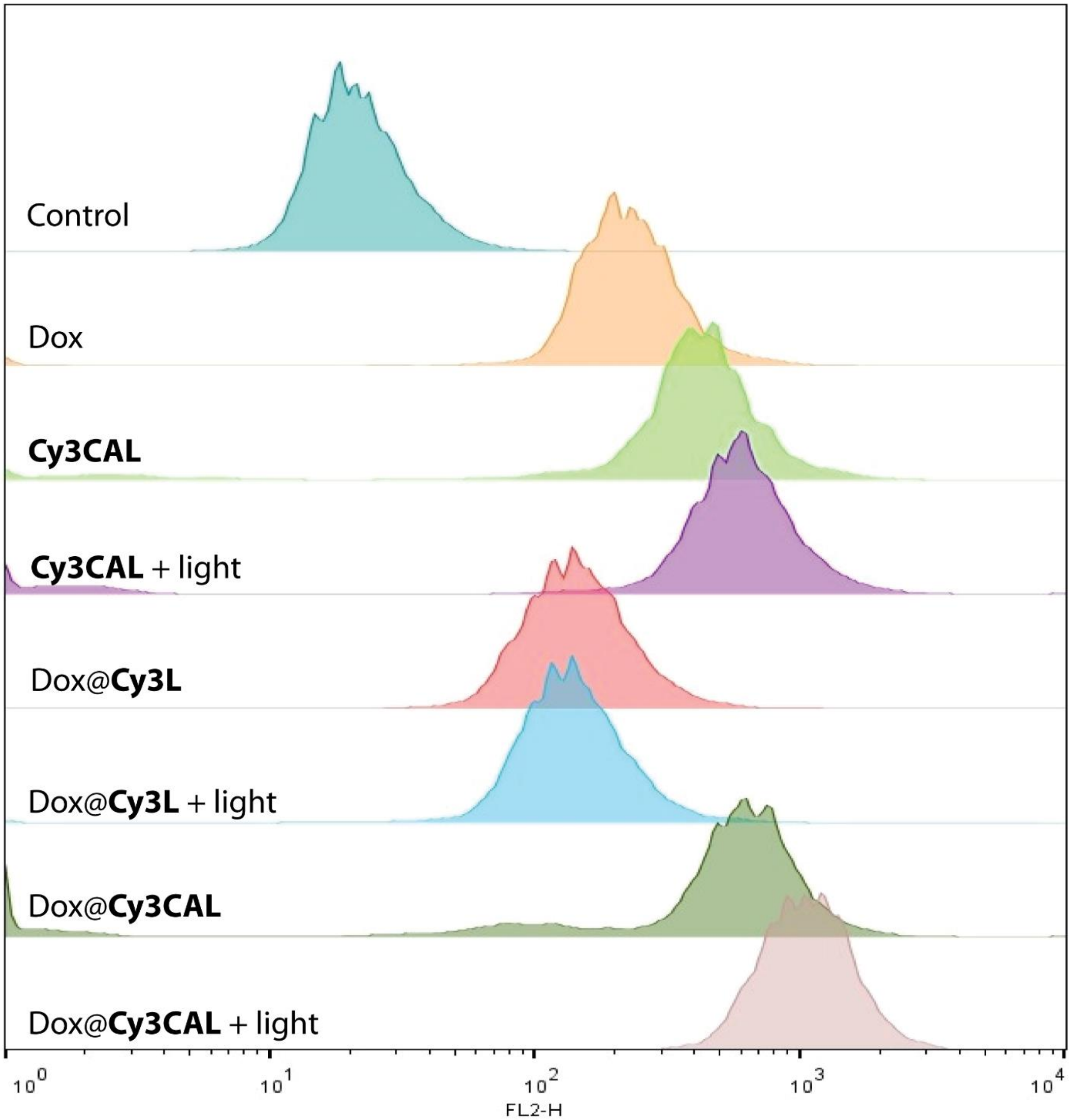


Fig. S11. Flow cytometry histograms after different treatments.

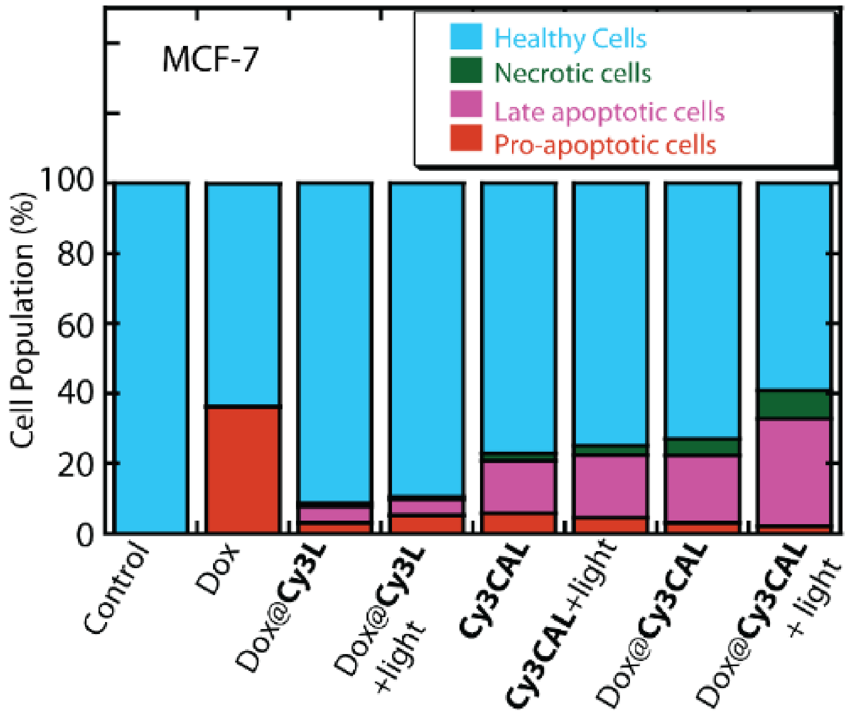


Fig. S12. The plot of the population of MCF-7 cells after various treatments (for 48 h).

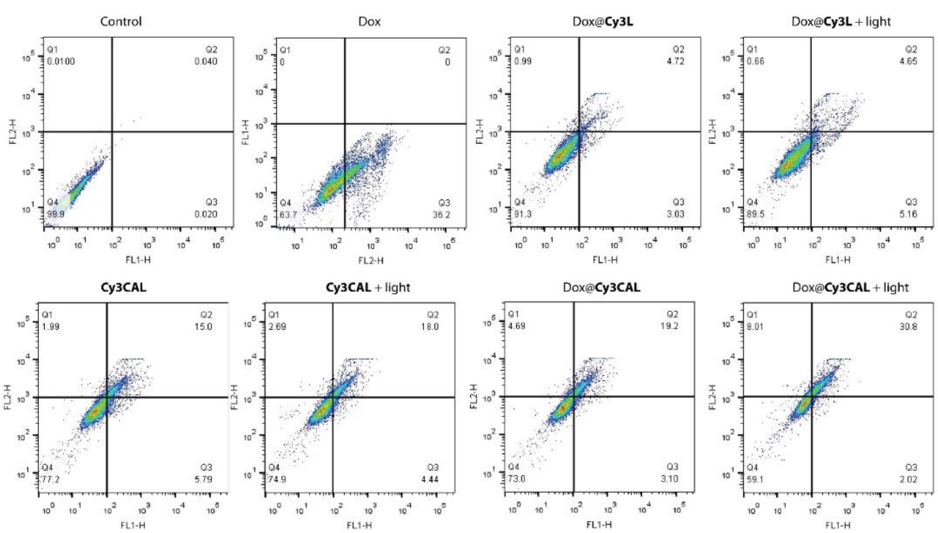
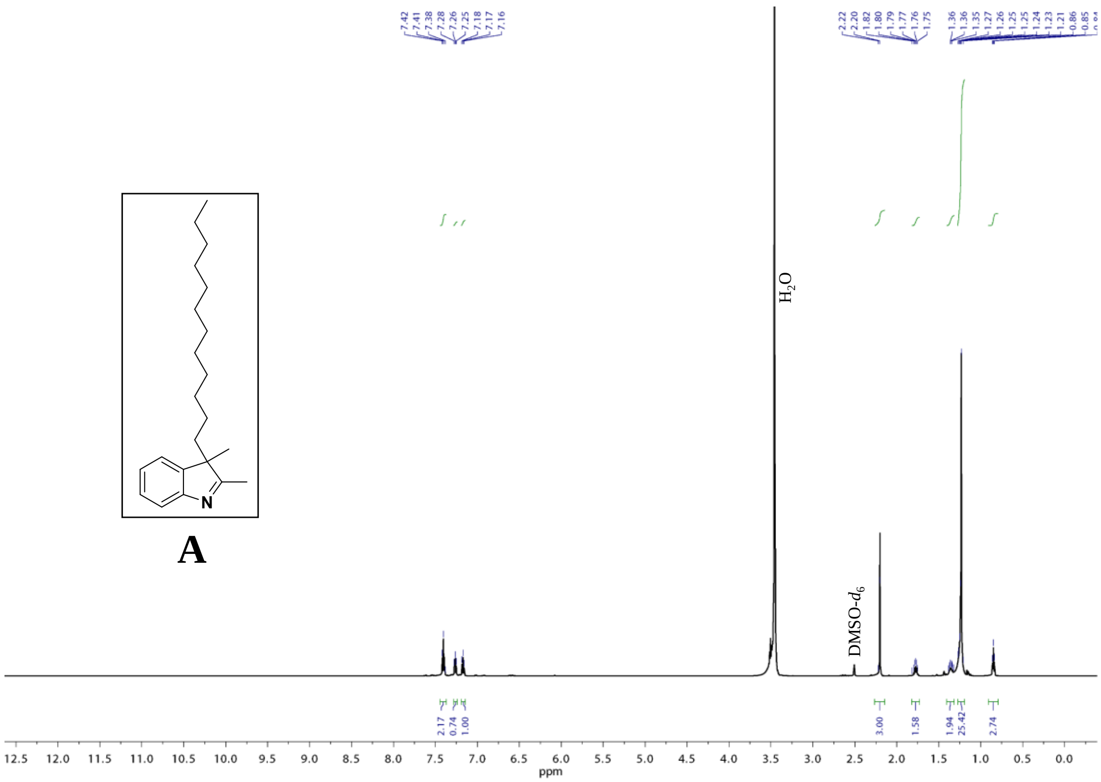


Fig. S13. Representative FACS data of MCF-7 cell populations after various treatments (for 48 h).

[1]H NMR and [13]C NMR spectra of synthesized compounds:



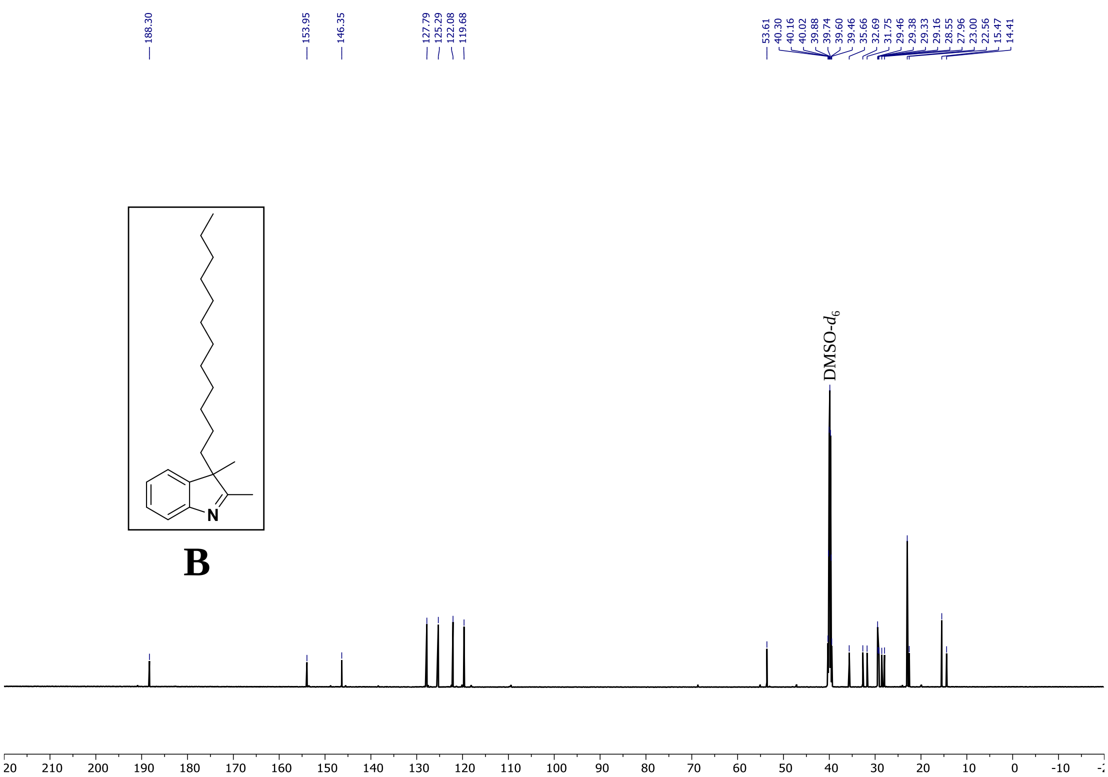
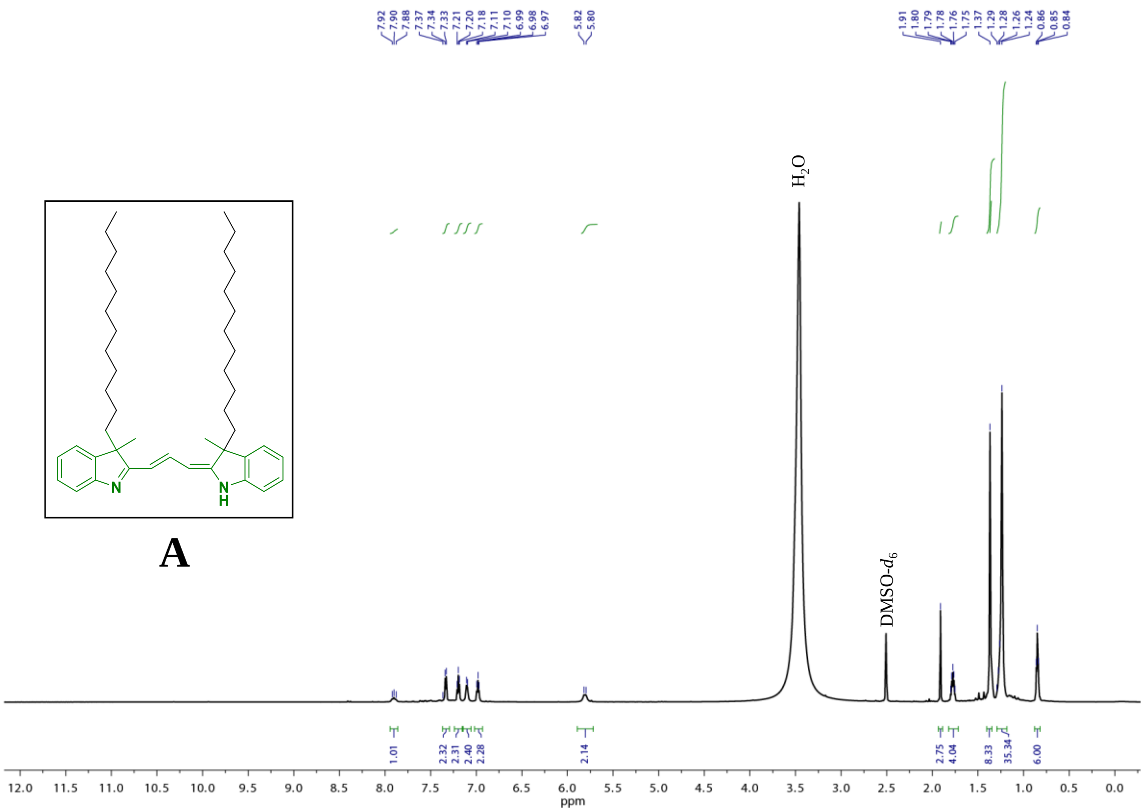


Fig. S14. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **6**.



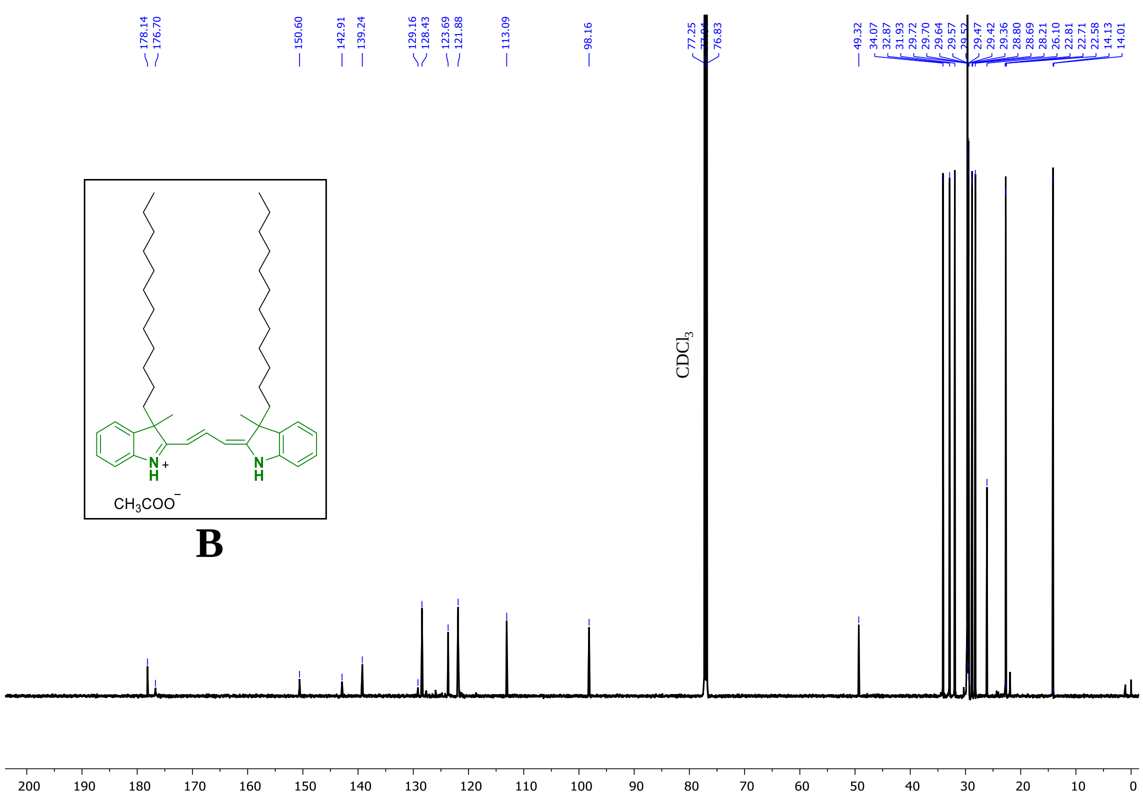
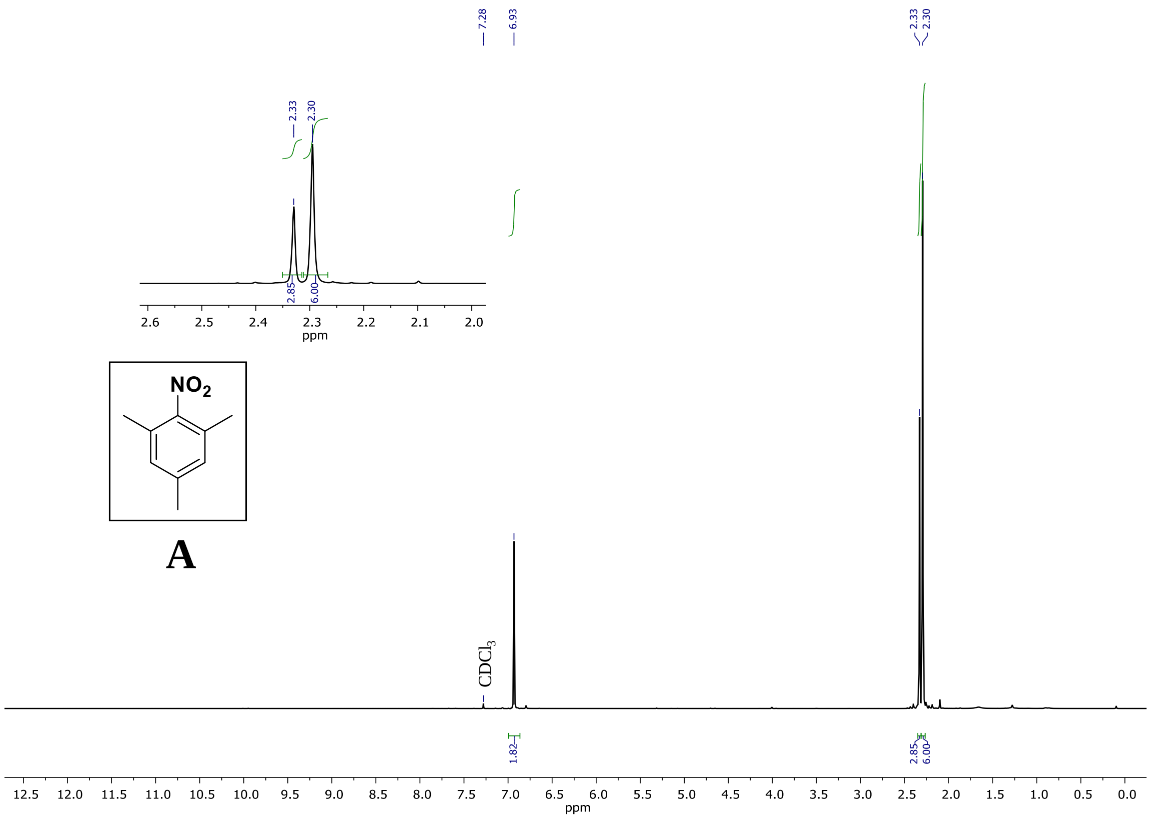


Fig. S15. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **7**.



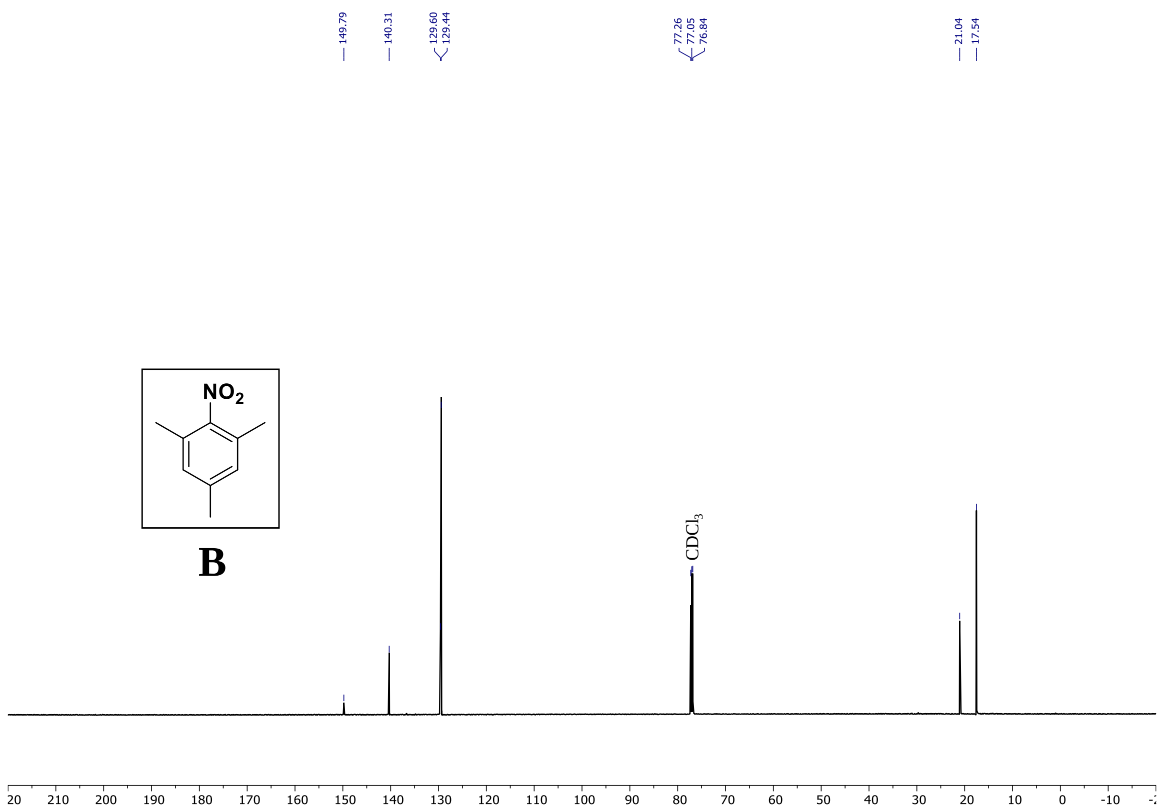


Fig. S16. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **9**.

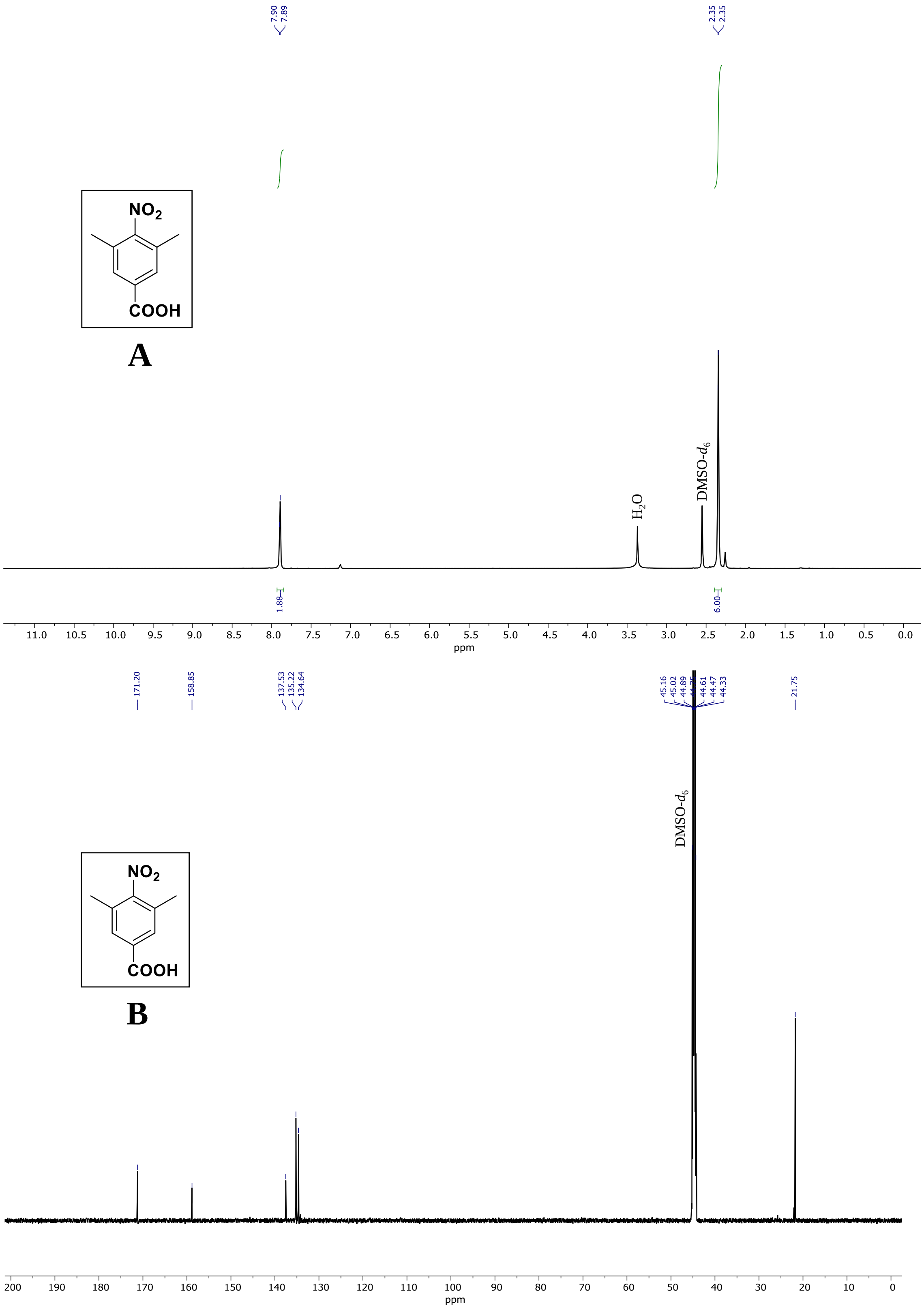
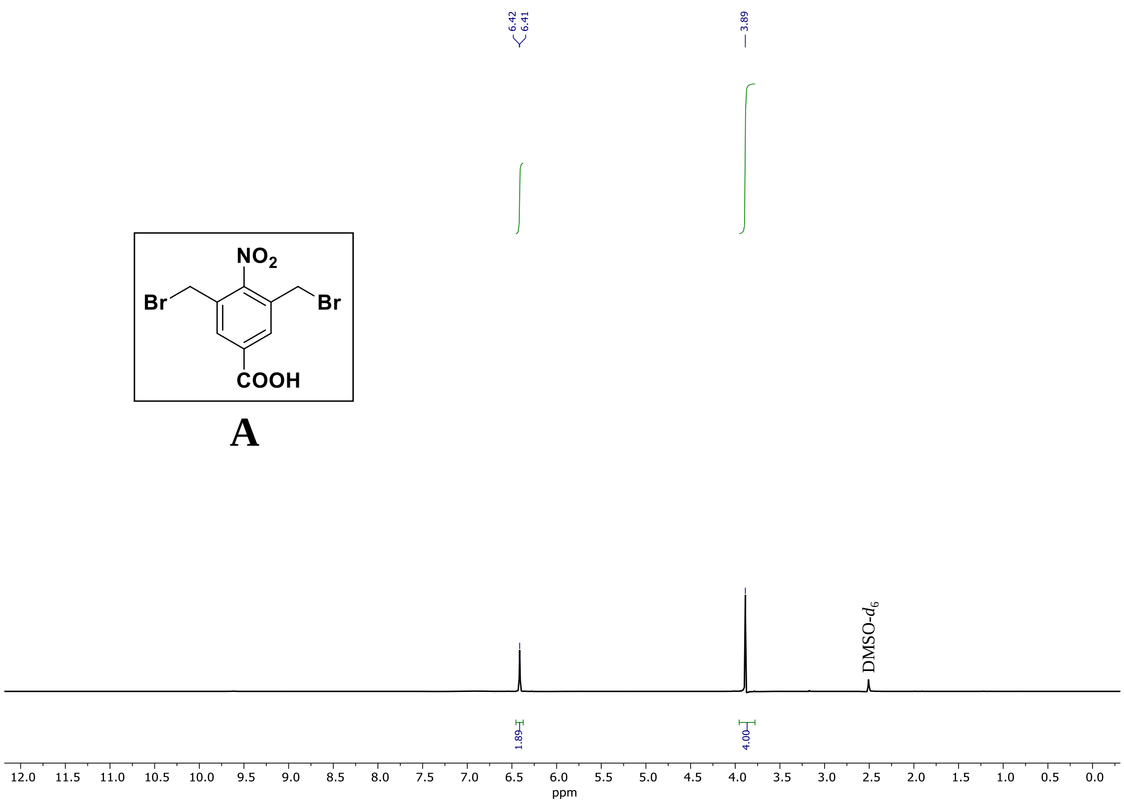


Fig. S17. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **10**.



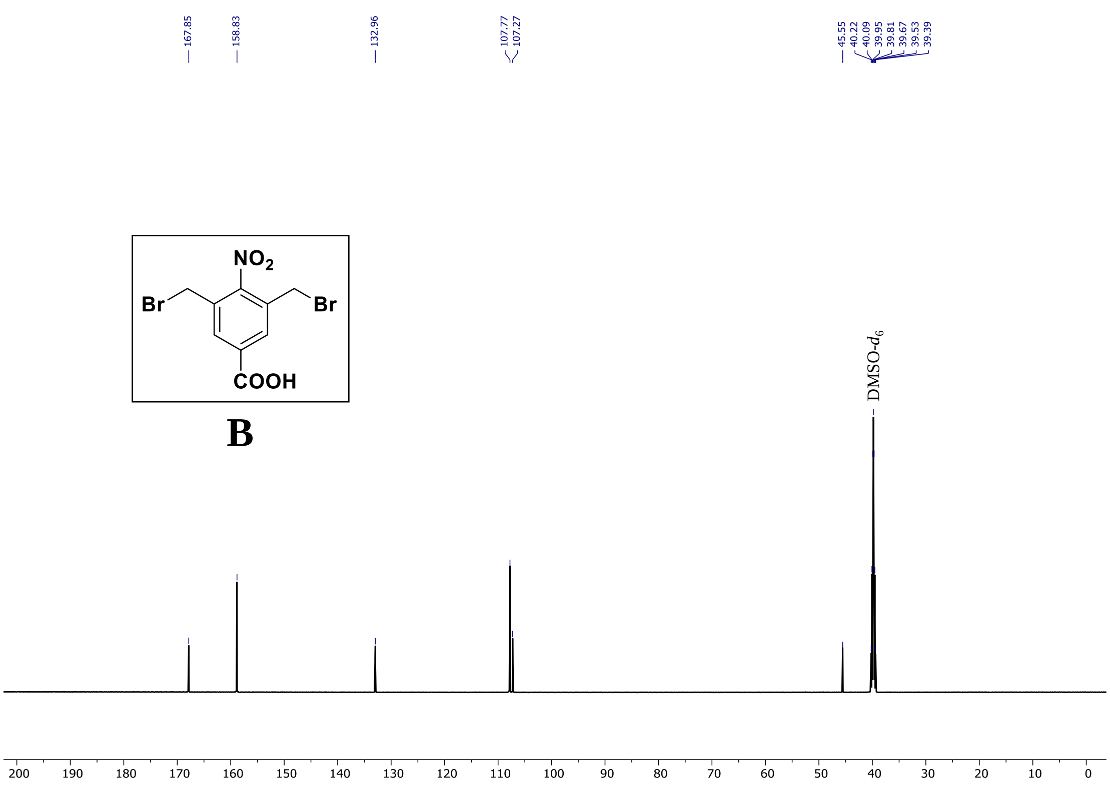


Fig. S18. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **11**.

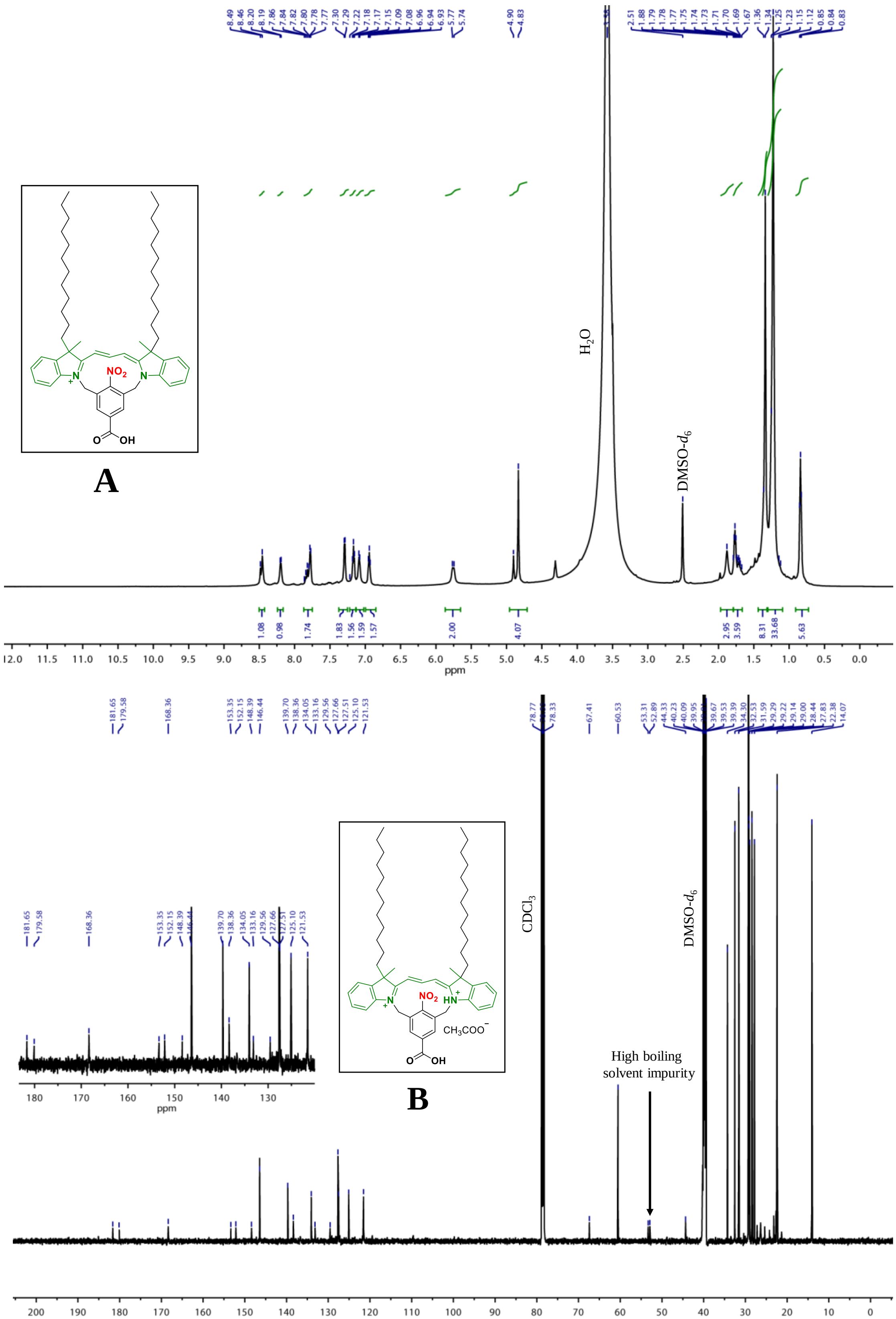


Fig. S19. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **Cy3L**.

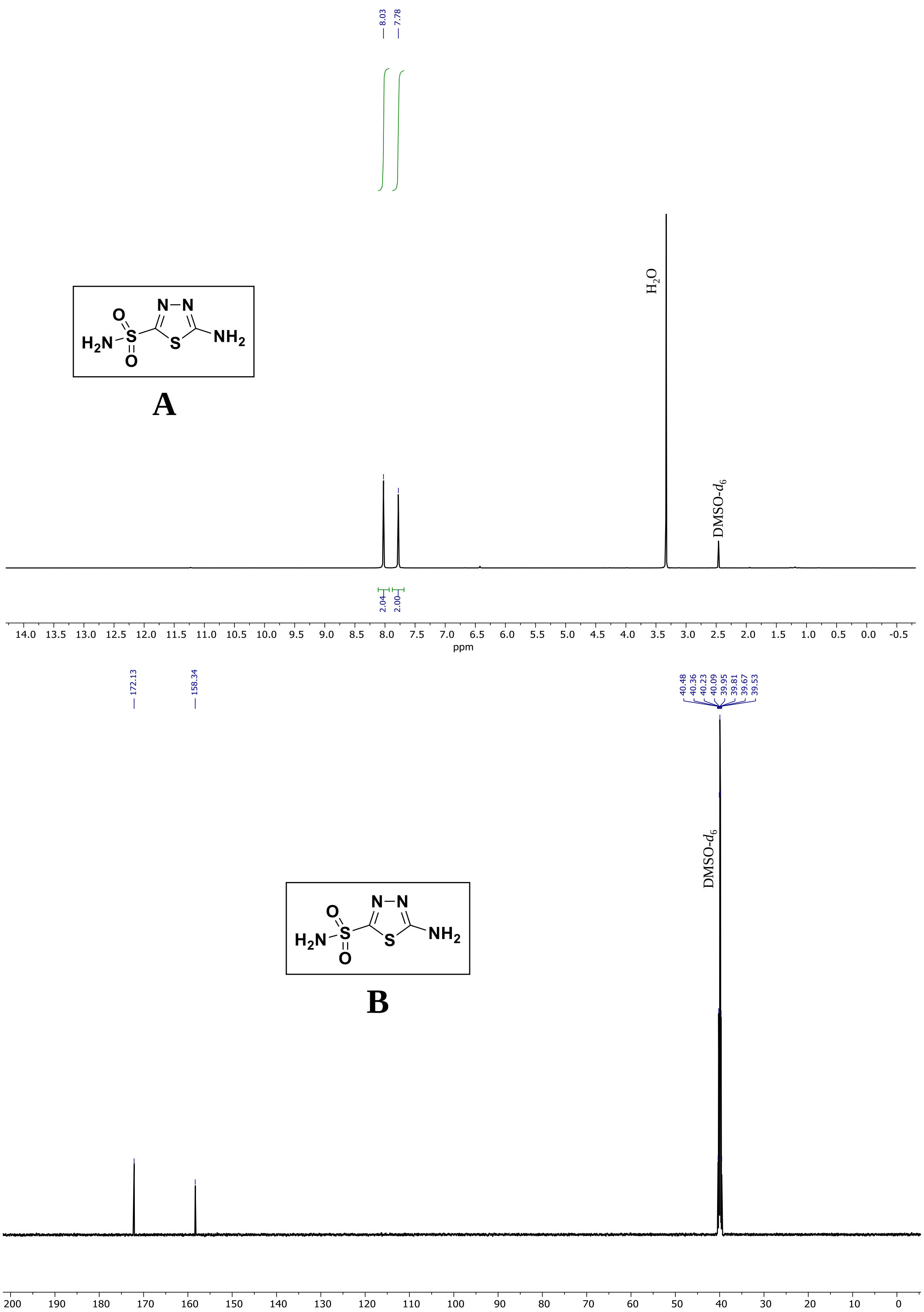


Fig. S20. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **12**.

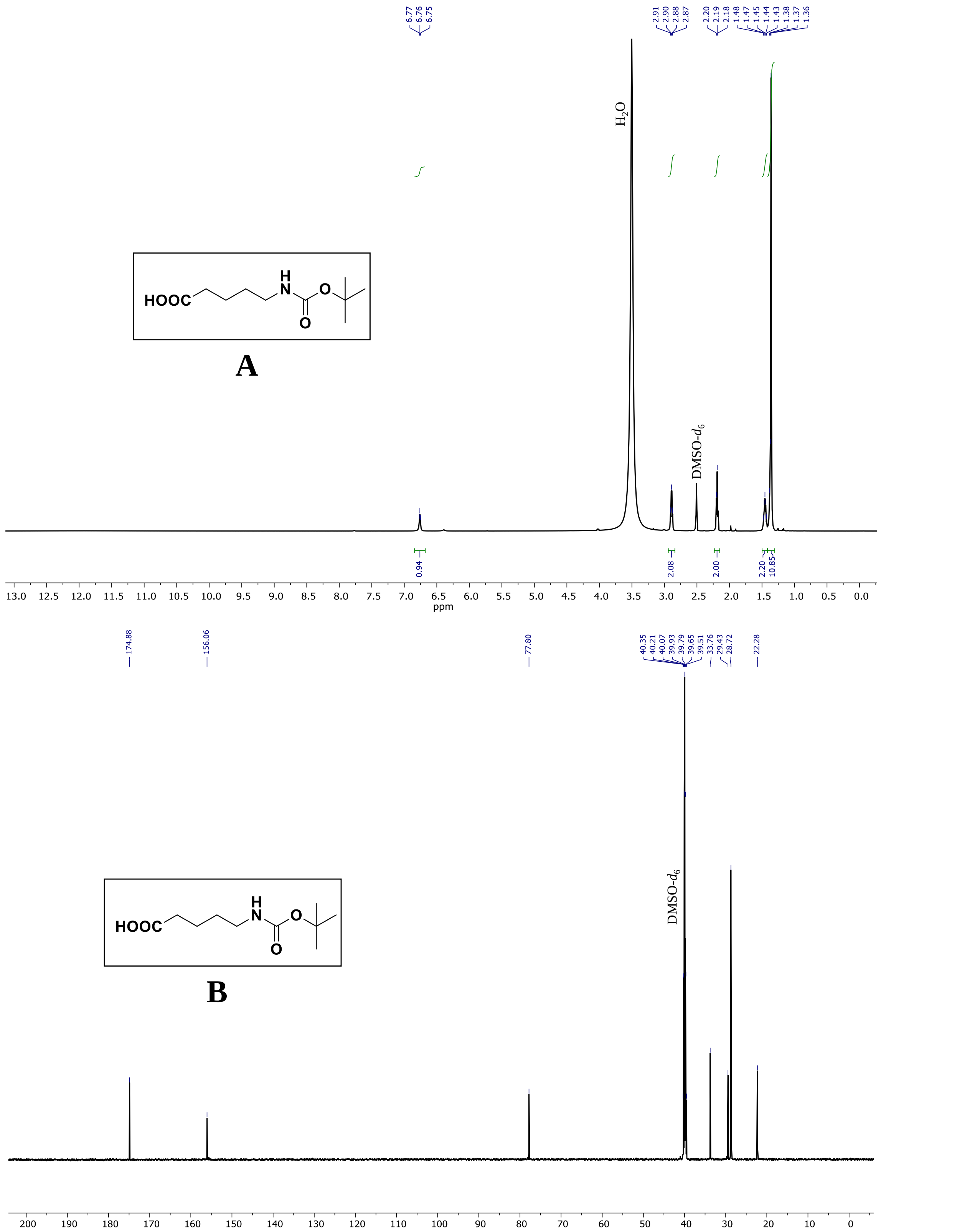
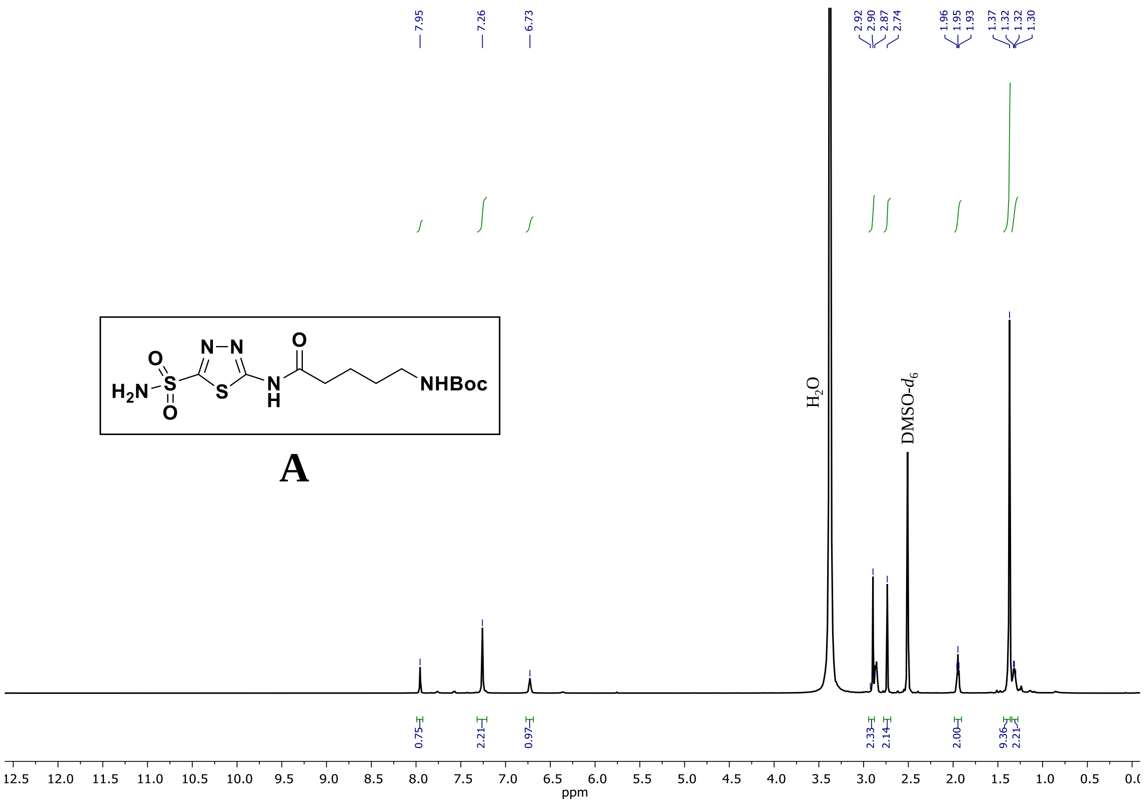


Fig. S21. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **13**.



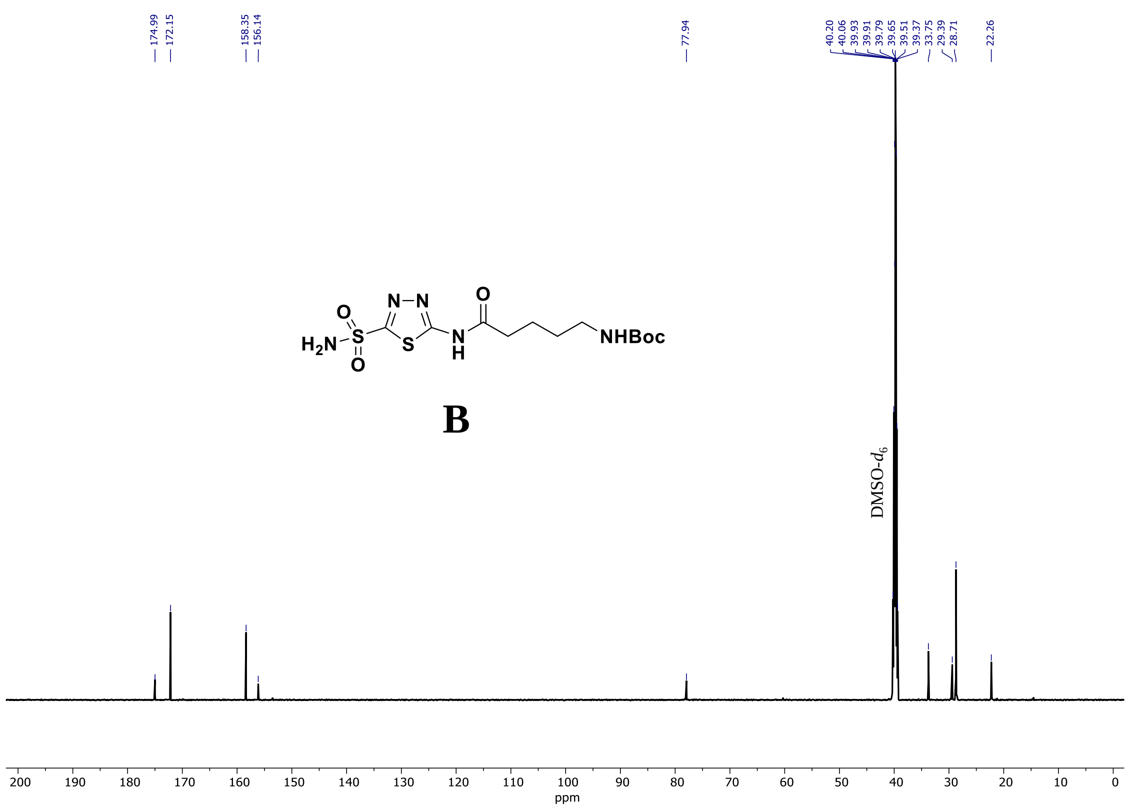


Fig. S22. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **14**.

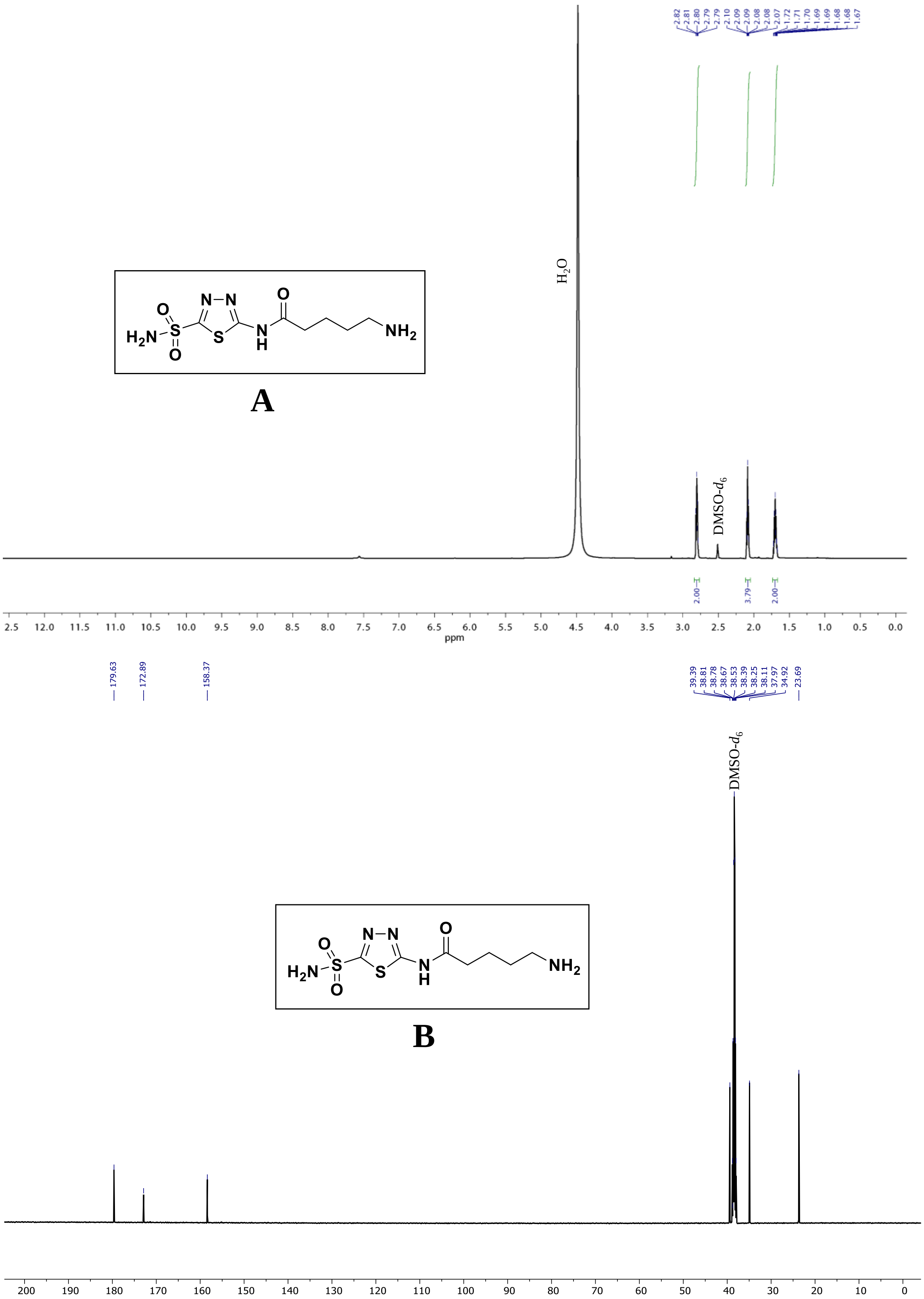


Fig. S23. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **15**.

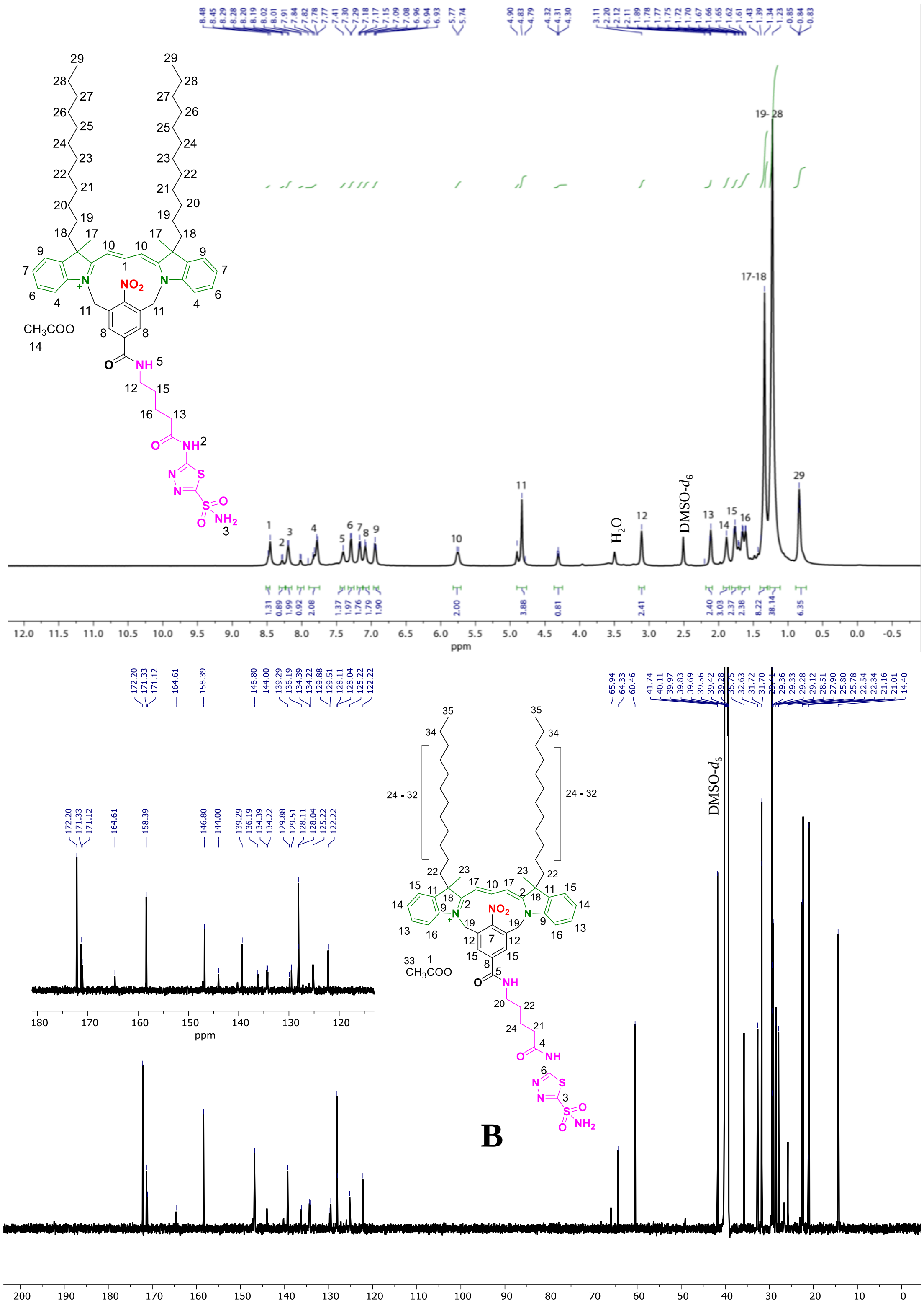


Fig. S24. [1]H NMR (A) and [13]C NMR (B) spectra of compound **Cy3CAL**.

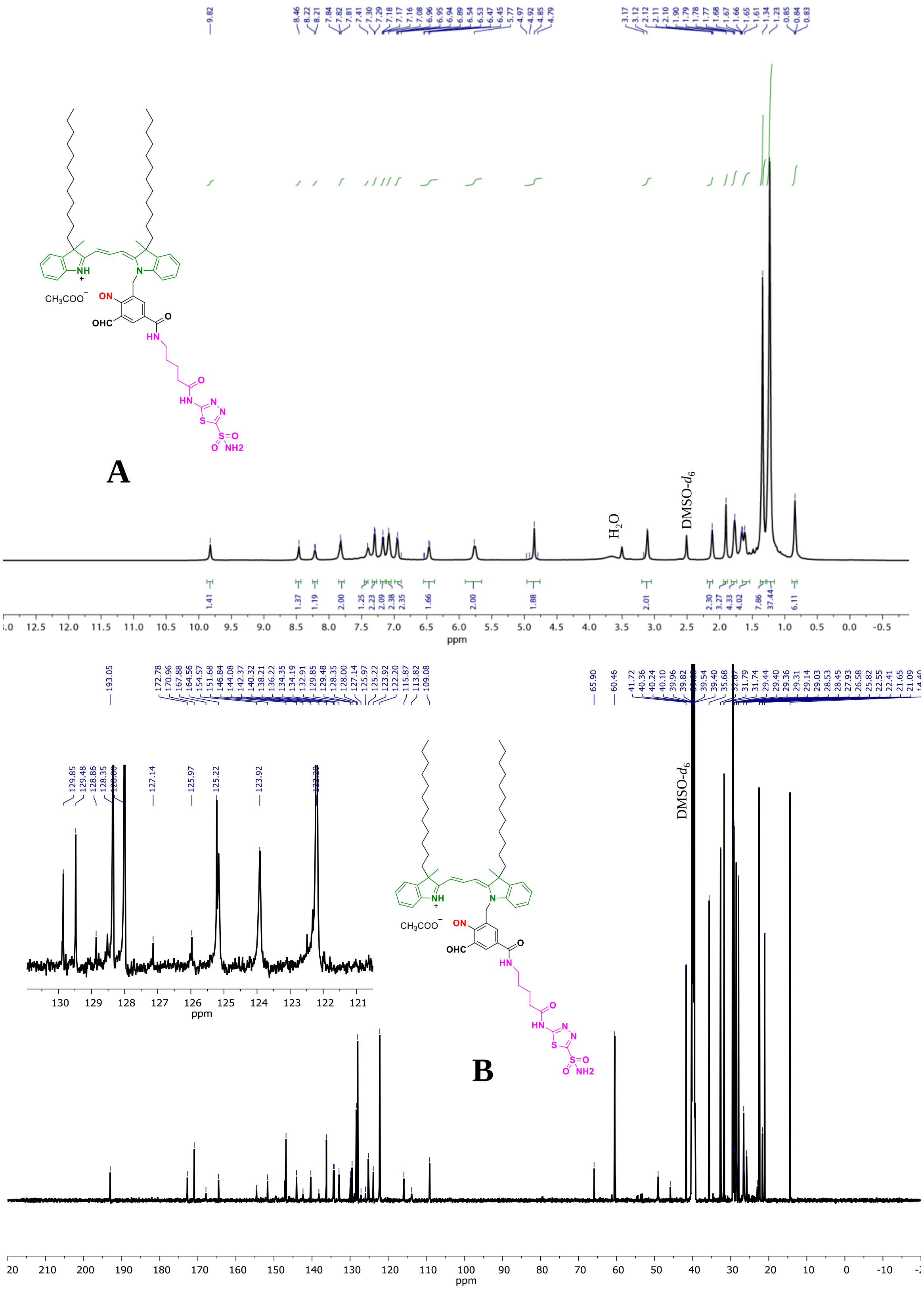


Fig. S25. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **Cy3AAL**.

Mass spectra

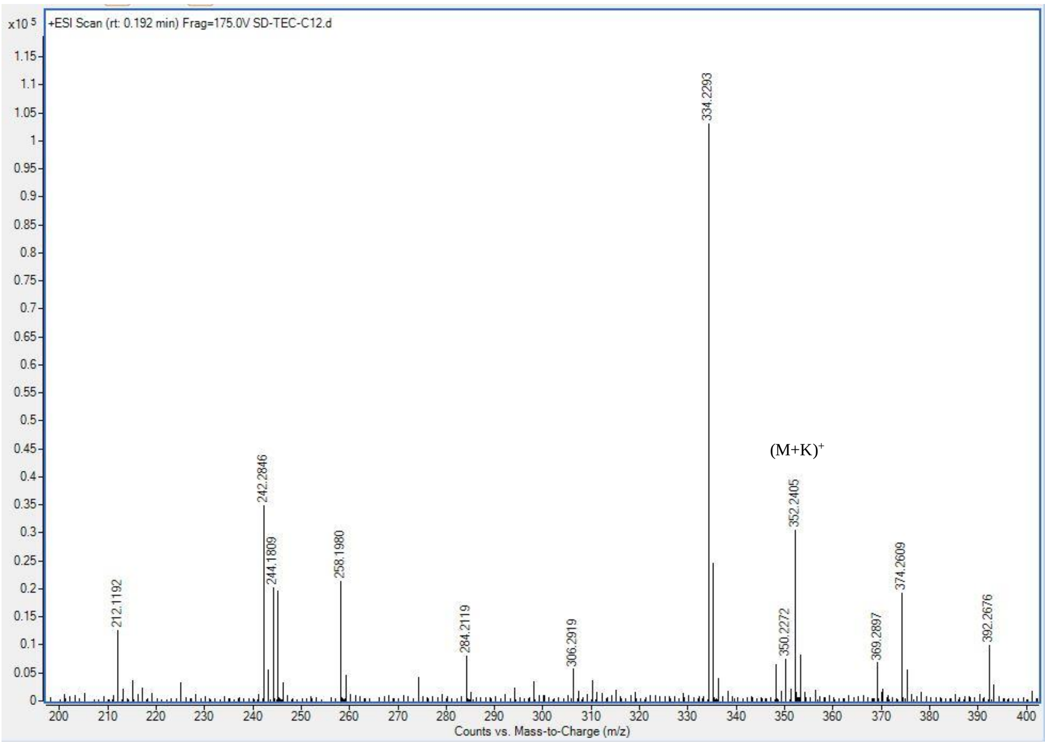


Fig. S26. Mass spectra of compound **6**.

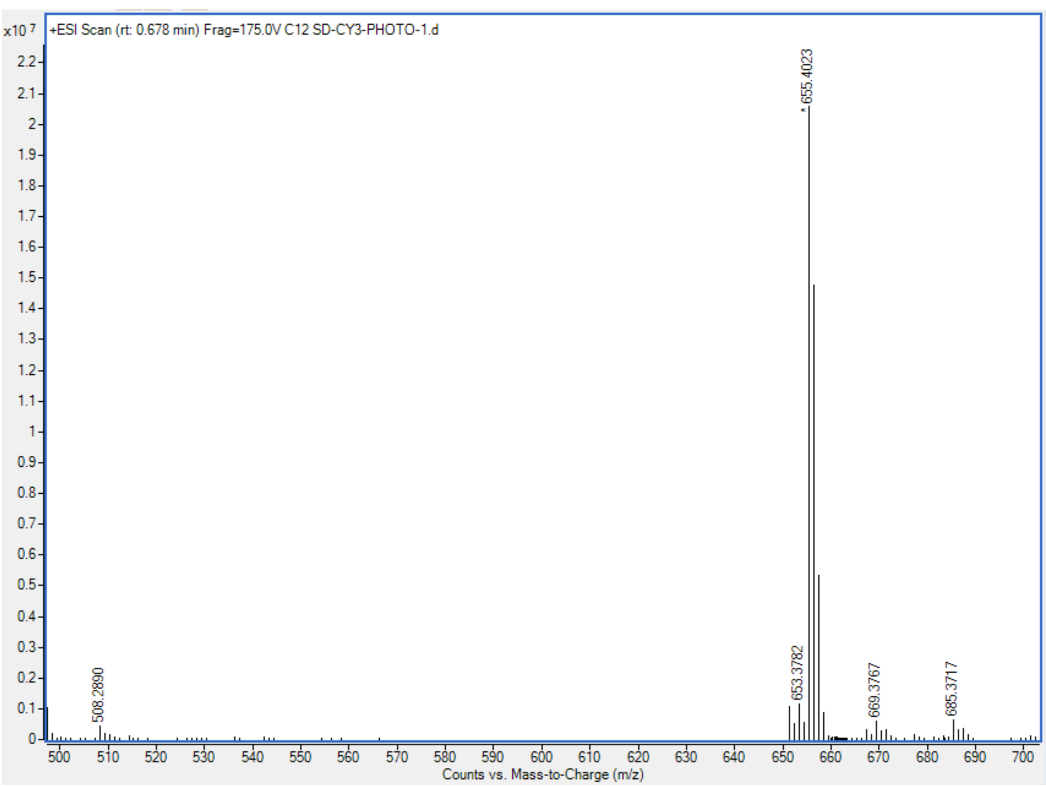


Fig. S27. Mass spectra of compound **7**.

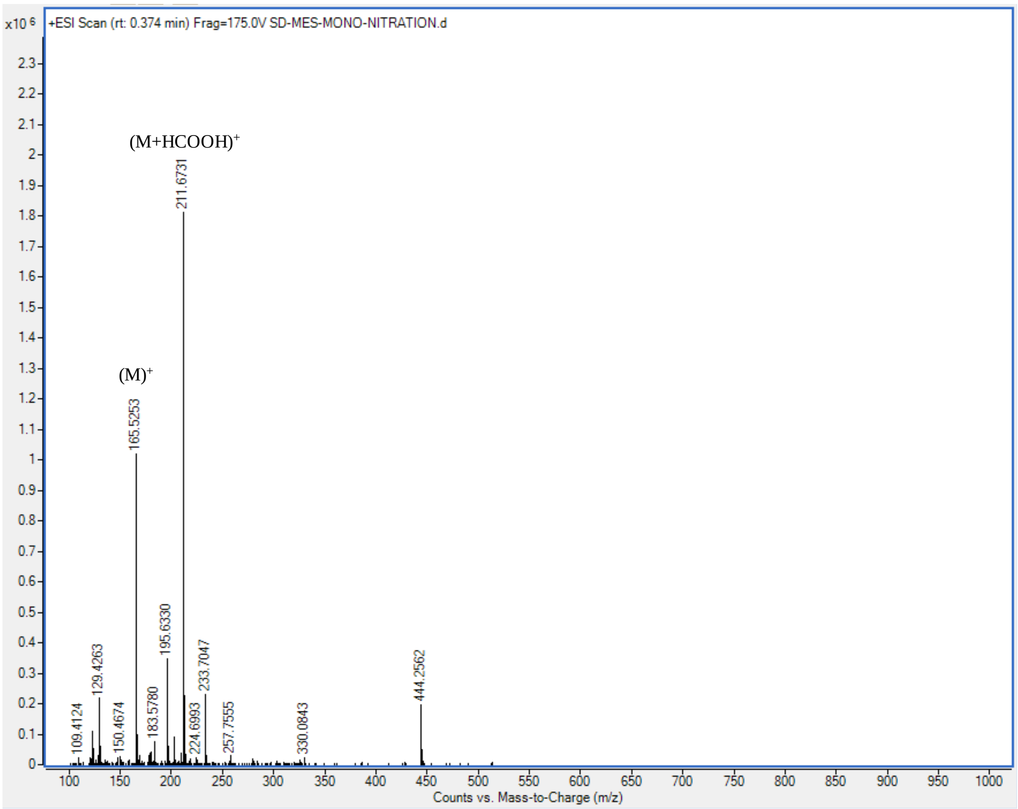


Fig. S28. Mass spectra of compound **9**.

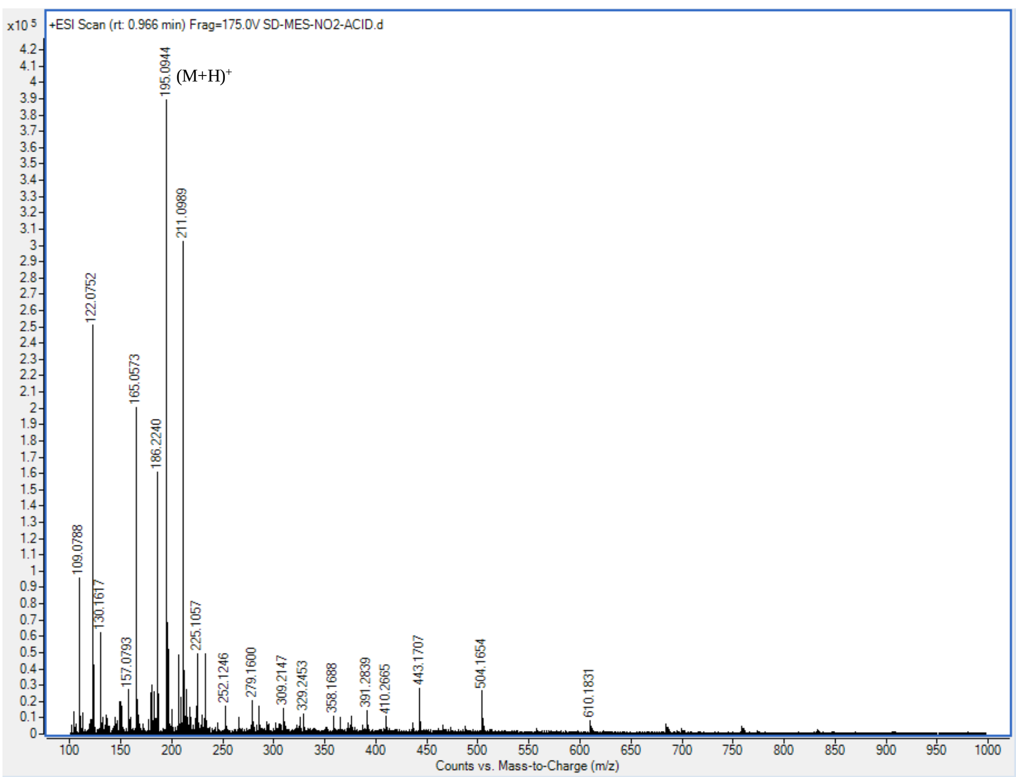


Fig. S29. Mass spectra of compound **10**.

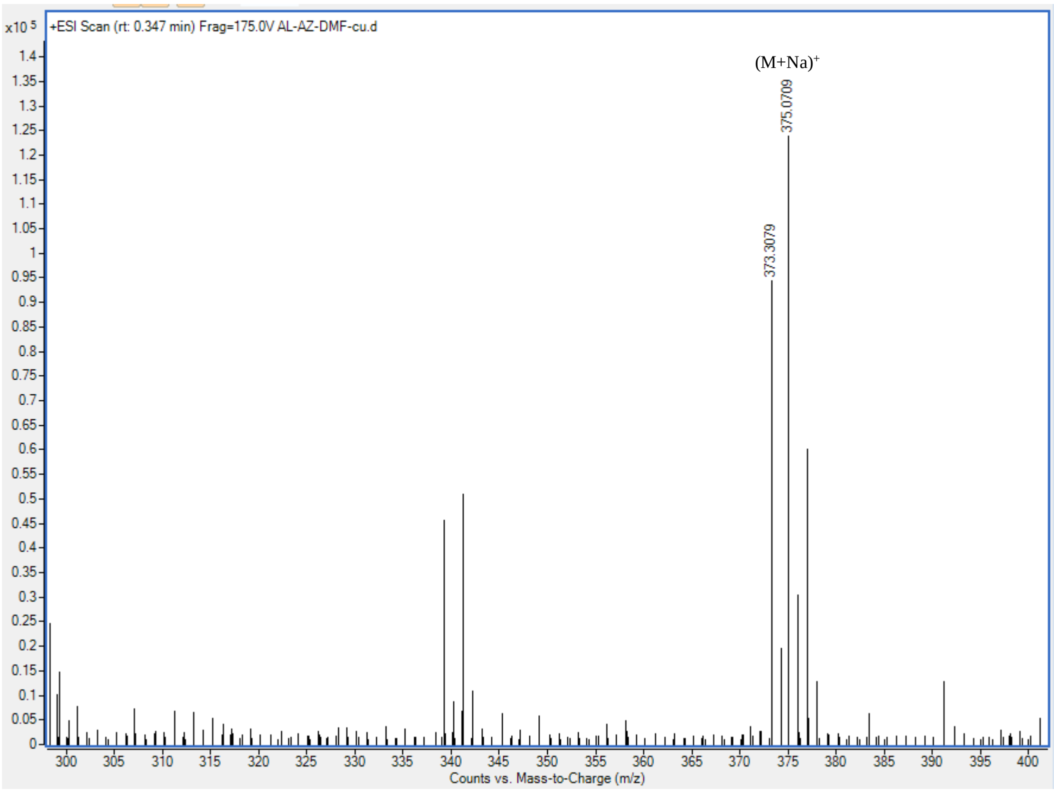


Fig. S30. Mass spectra of compound **11**.

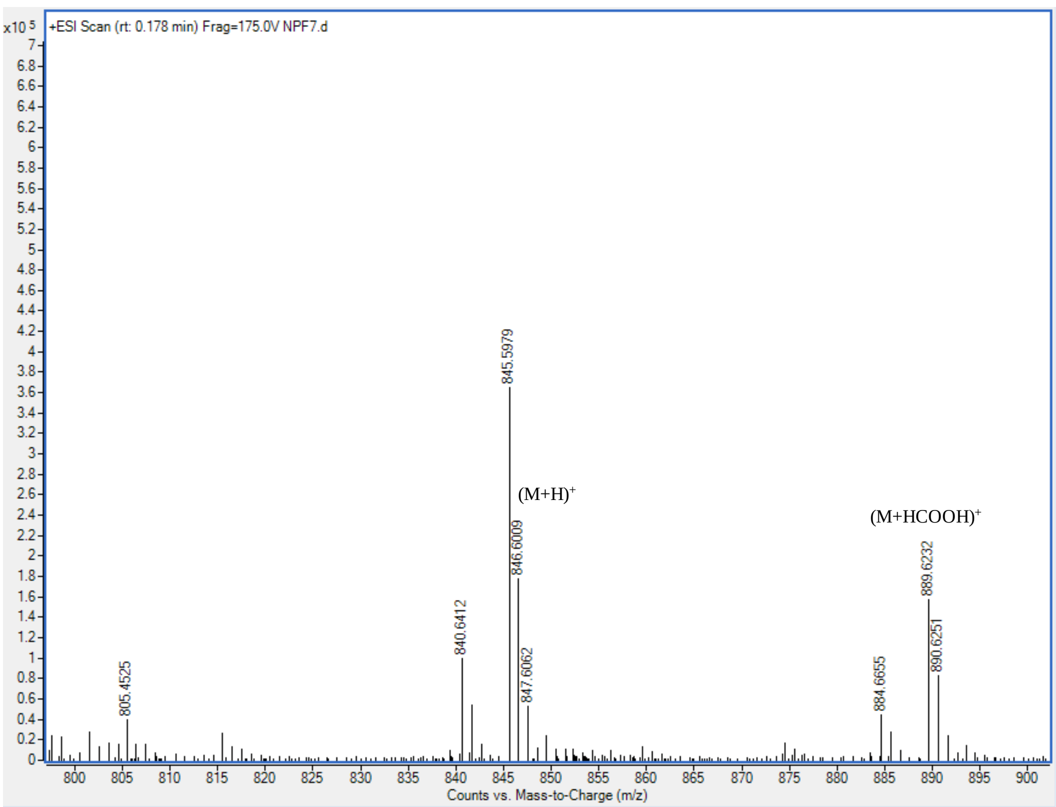


Fig. S31. Mass spectra of compound **Cy3L**.

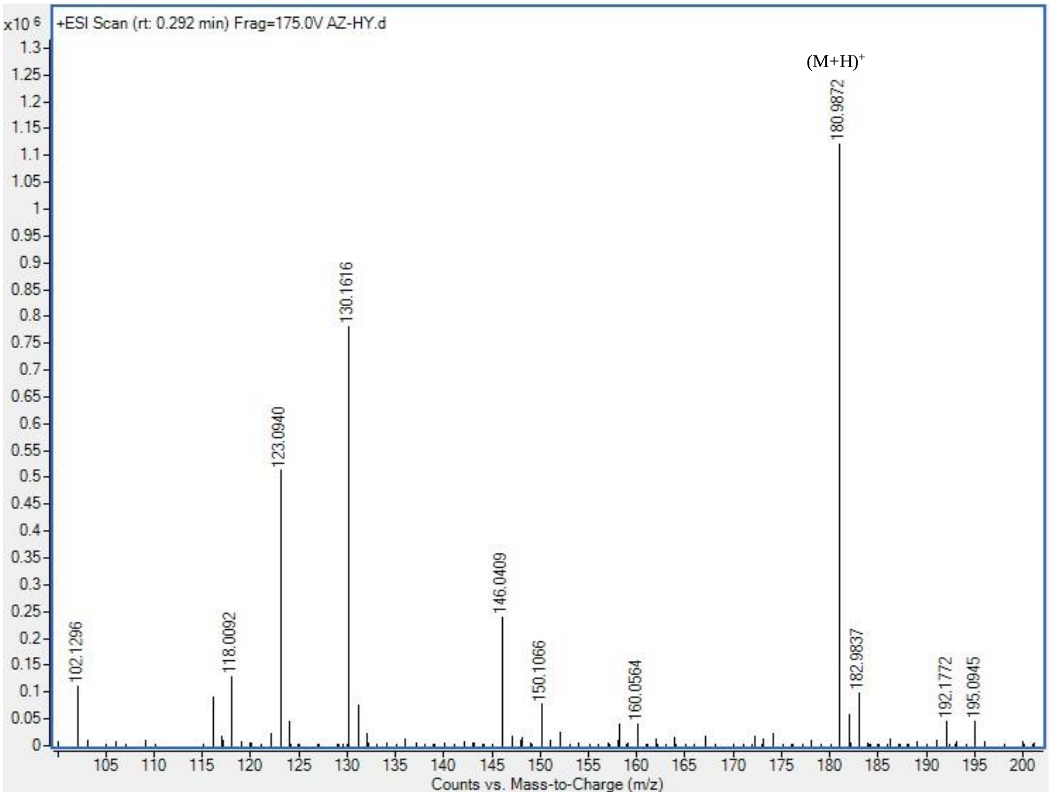


Fig. S32. Mass spectra of compound **12**.

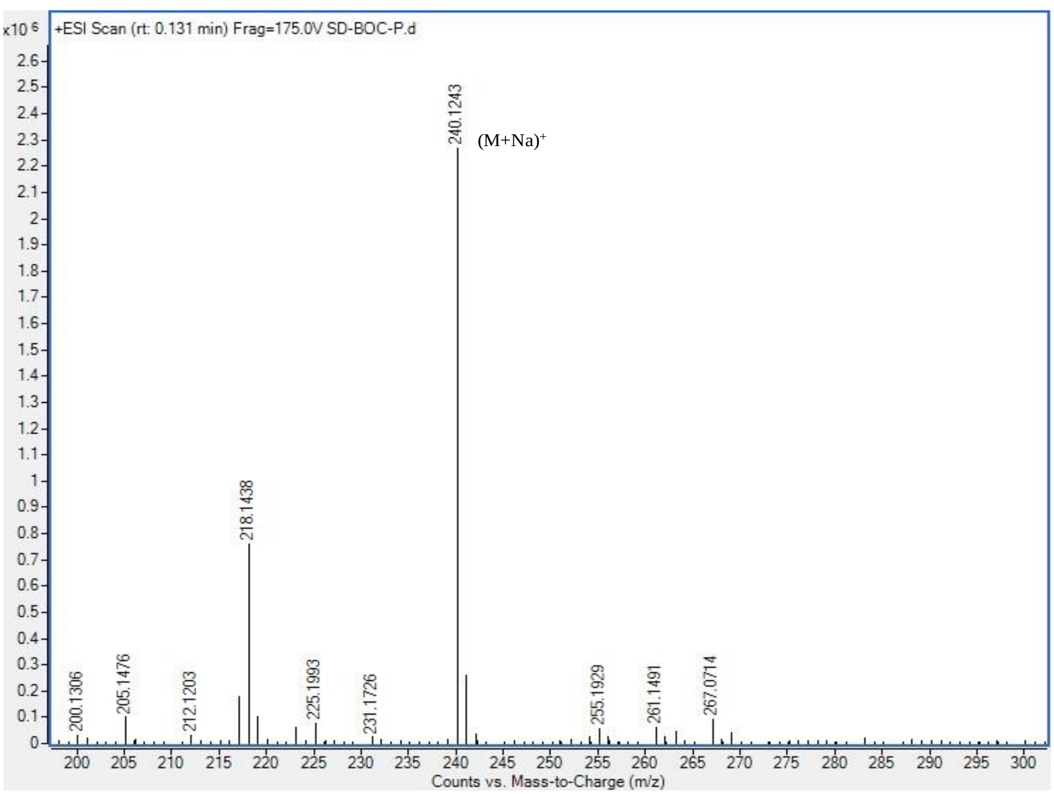


Fig. S33. Mass spectra of compound **13**.

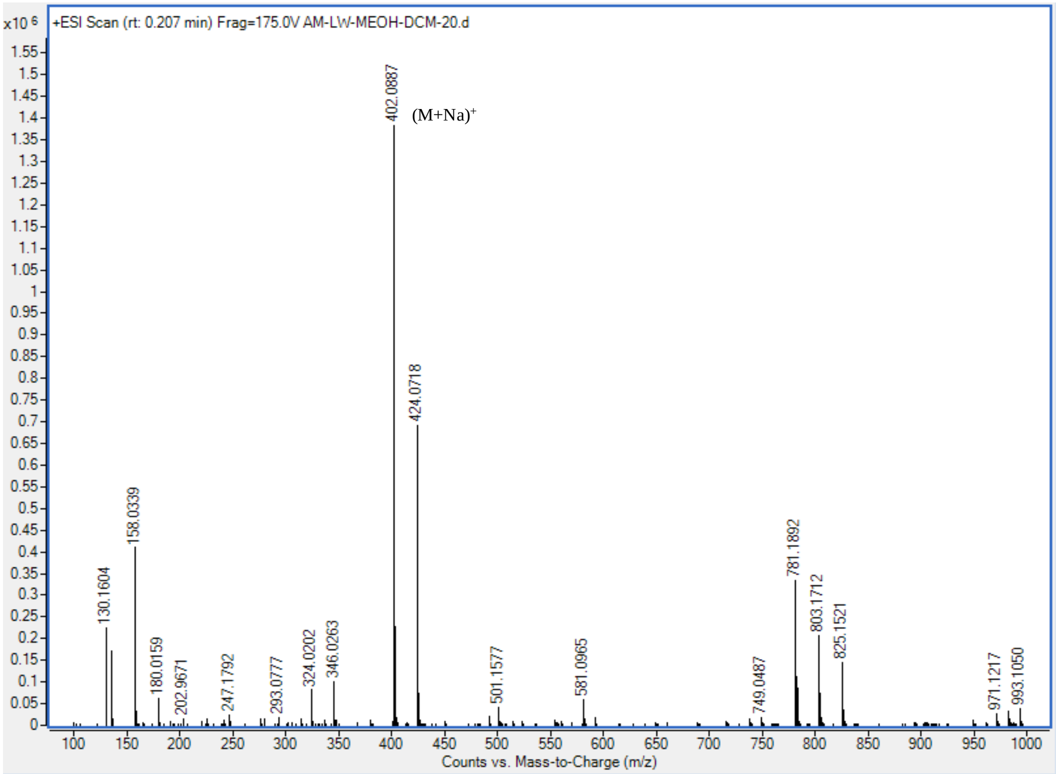


Fig. S34. Mass spectra of compound **14**.

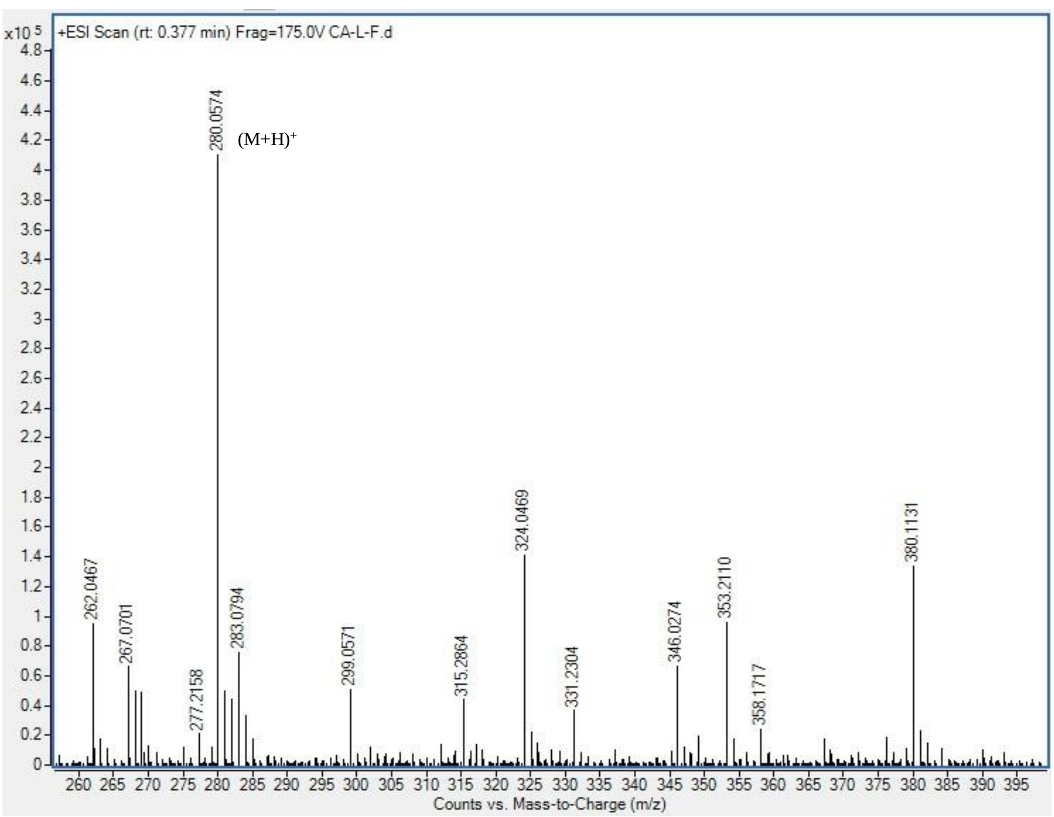


Fig. S35. Mass spectra of compound **15**.

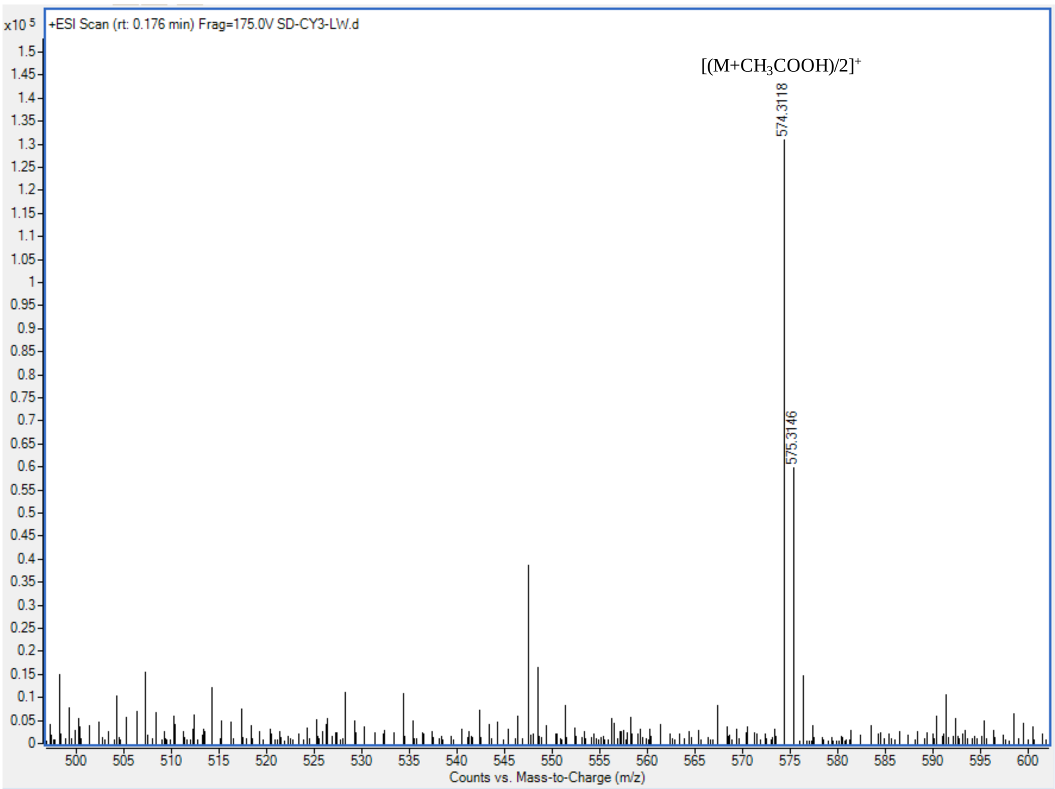
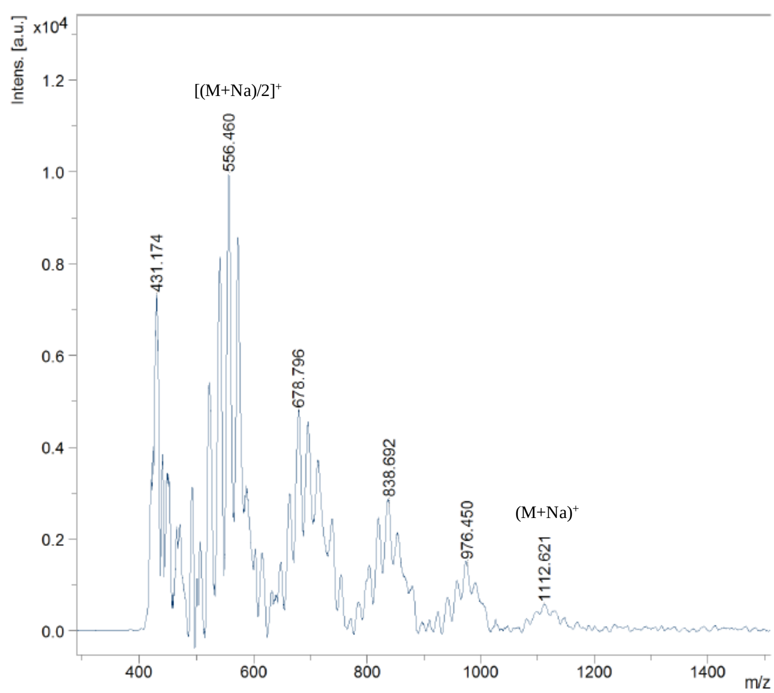


Fig. S36. Mass spectra of compound **Cy3CAL**.



m/z	S/N	QualityFac.	Res.	Intens.	Area
431.174	3		37.9	7332	89006
556.460	4		73.6	9956	92442
572.425	3		87.1	8576	89670
678.796				4767	
838.692				2698	
976.450				1404	
1112.621				576	

Fig. S37. MALDI-TOF spectra of compound **Cy3AAL**.

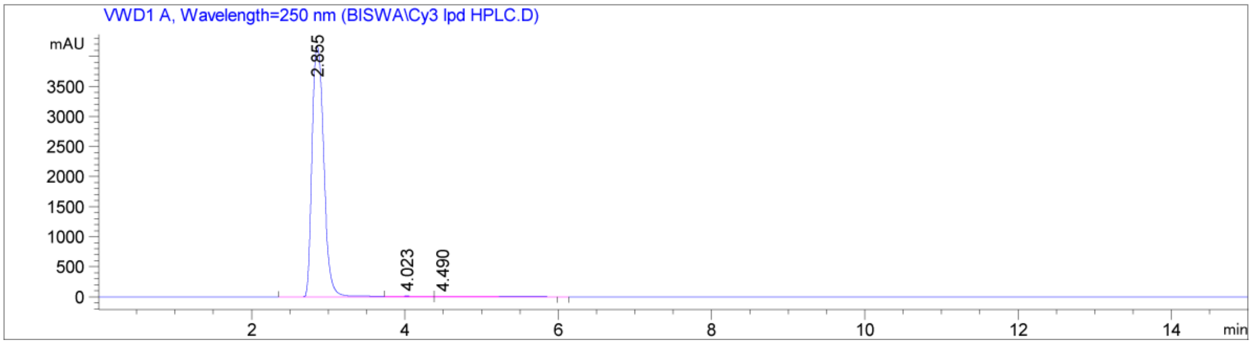


Fig. S38. HPLC trace of compound **Cy3CAL**.

Reference

1. Y. V. Il'ichev, M. A. Schworer and J. Wirz, *J. Am. Chem. Soc.*, 2004, **126**, 4581-4595.