

Supporting Information:

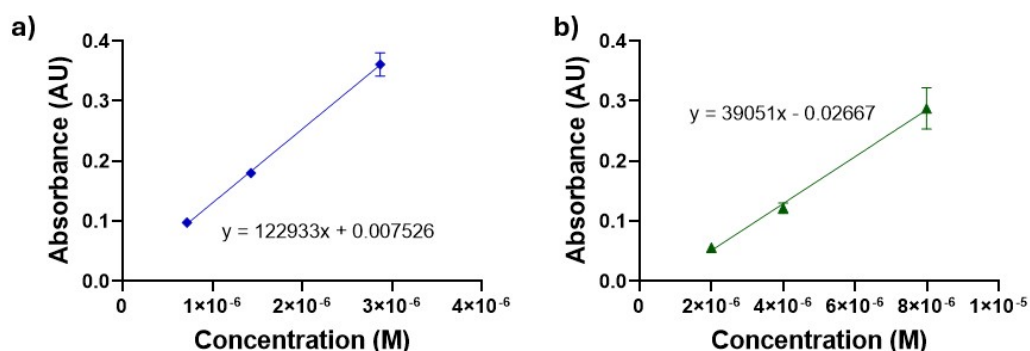


Figure S1: Extinction coefficients determined by qNMR. (a) **DB1** (b) **DB2**. Experiments were conducted in triplicate.

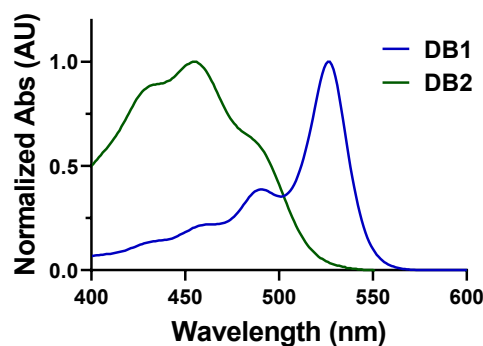


Figure S2: Normalized absorbance spectra of **DB1** and **DB2** in DMSO.

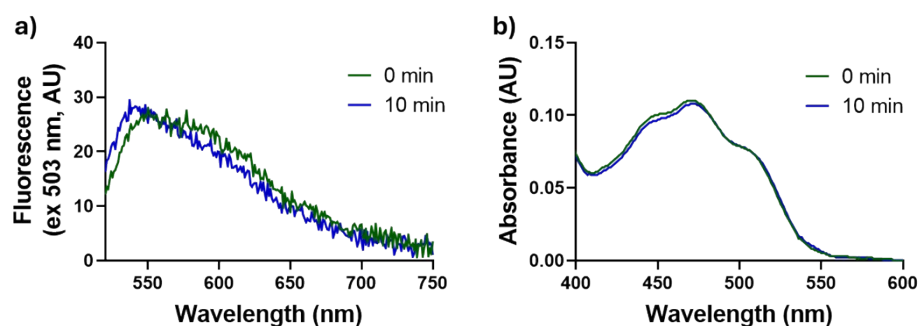


Figure S3: Incubation of **DB2** in 2% DMSO and PBS with NADH (No NTR). (a) Fluorescence spectra of **DB2** before and after 10-minute incubation with NADH. (b) Absorbance spectra of **DB2** before and after 10-minute incubation with NADH.

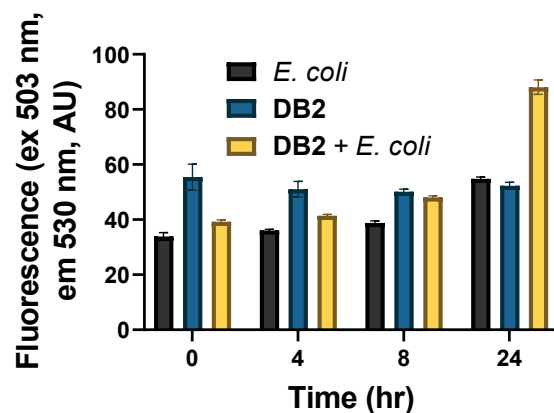


Figure S4: Activation of DB2 in *E. coli* monitored by fluorescence. While we observe an increase in fluorescence after 24 hrs, a similar increase in *B. subtilis* was observed at only 4 hrs with an almost 10-fold greater signal observed at 24 hrs. Experiments were conducted in triplicate.

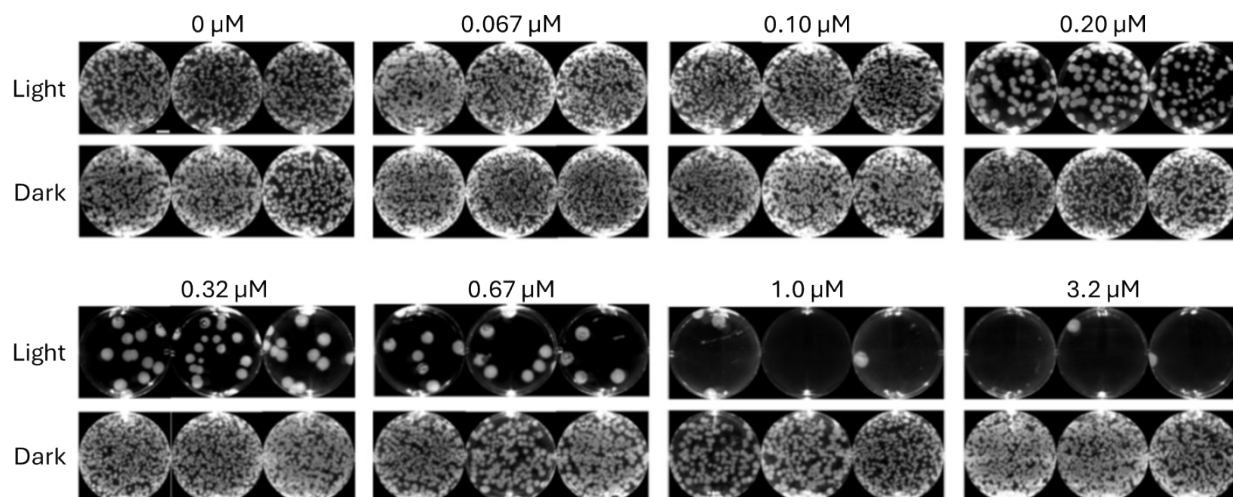
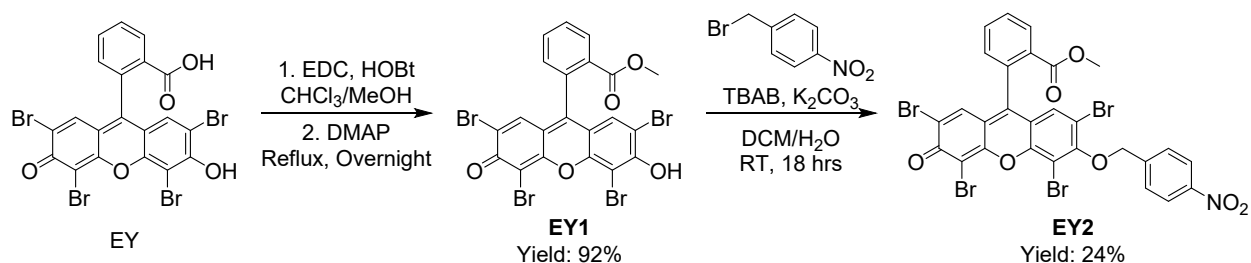


Figure S5: Cell viability assay using CFU counting. Images of colonies grown on agar pad are taken by Invitrogen iBright FL1500 imaging system and preprocessed with μ Manger or Fiji/ImageJ. Triplicates were used for statistical analysis.



Scheme S1: Synthesis of **EY2**. The carboxylic acid on EY is converted to a methyl ester in the first step to direct nucleophilic substitution of the 4-nitrobenzyl group towards the hydroxyl group in the subsequent step.

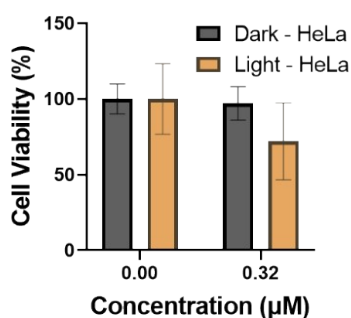


Figure S6: Cell viability of **DB2** in HeLa cells under dark or light conditions (10 minutes, 530 nm, 41.8 mW/cm²). Experiments were conducted in triplicate.

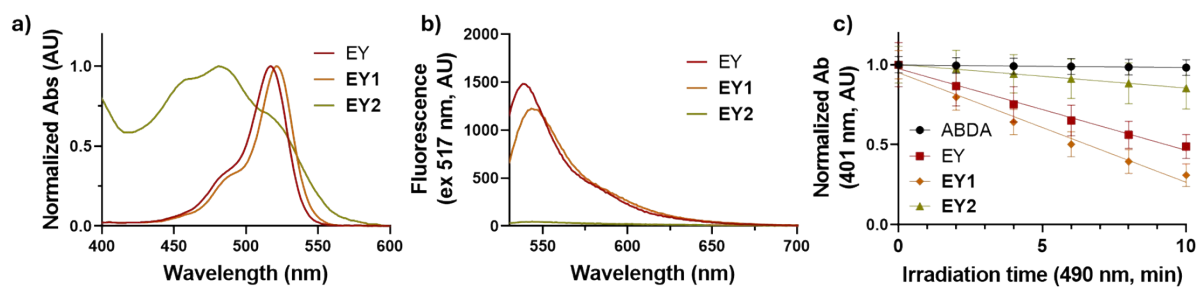


Figure S7: Photophysical characterizations of **EY2** in 2% DMSO and PBS. (a) Normalized absorbance spectra of compounds. (b) Fluorescence spectra of compounds. (c) ¹O₂ generation of compounds detected by ABDA under 490 nm irradiation (28.0 mW/cm²). EY was chosen as the standard and ABDA under irradiation serves as a control. Experiments were conducted in triplicate.

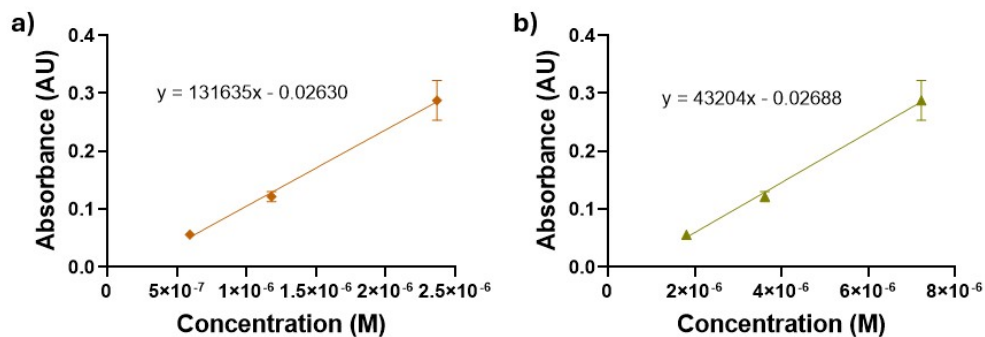


Figure S8: Extinction coefficients determined by qNMR. (a) **EY1** (b) **EY2**. Experiments were conducted in triplicate.

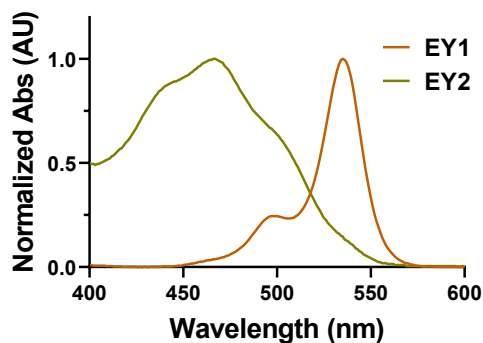


Figure S9: Normalized absorbance spectra of **EY1** and **EY2** in DMSO.

Table S1: Summary of Photophysical Data for **EY1** and **EY2**

	EY1	EY2
λ_{max} (abs, PBS)	521	481
λ_{max} (em, PBS)	544	543
λ_{max} (abs, DMSO)	535	466
ϵ ($\text{M}^{-1}\text{cm}^{-1}$, DMSO)*	132,000	43,000
Φ_f (DMSO)	0.61 ± 0.05	0.015 ± 0.002
Φ_{Δ} (PBS)	0.67 ± 0.11	0.13 ± 0.01

* Extinction coefficients were determined at the λ_{abs} in DMSO

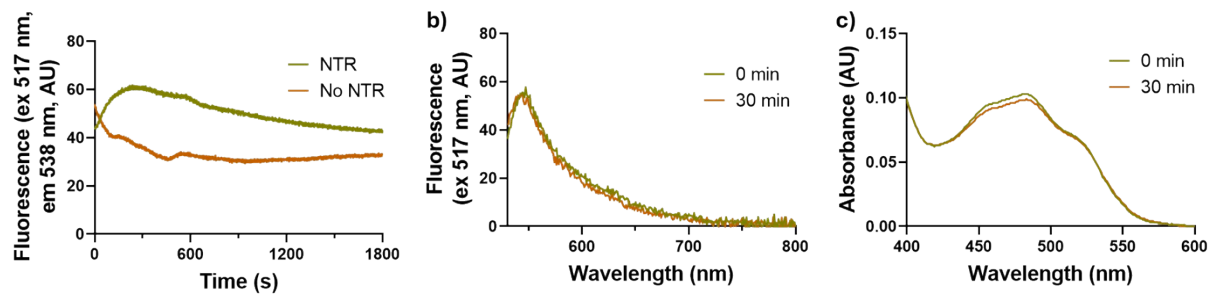


Figure S10: Nitroreductase reaction with **EY2** in 2% DMSO and PBS. (a) Fluorescence time-course of **DB2** with NTR and NADH. (b) Fluorescence spectra of **EY2** before and after 10-minute incubation with NTR and NADH. (c) Absorbance spectra of **EY2** before and after 10-minute incubation with NTR and NADH.

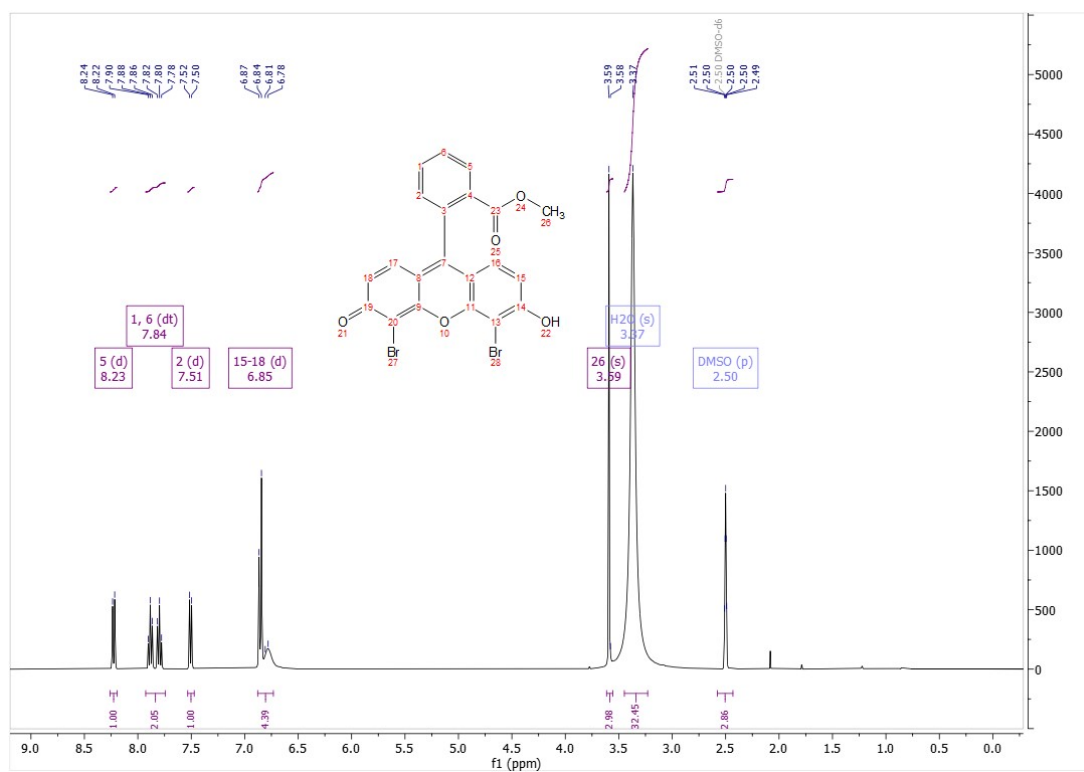


Figure S11: ¹H NMR of **DB1**

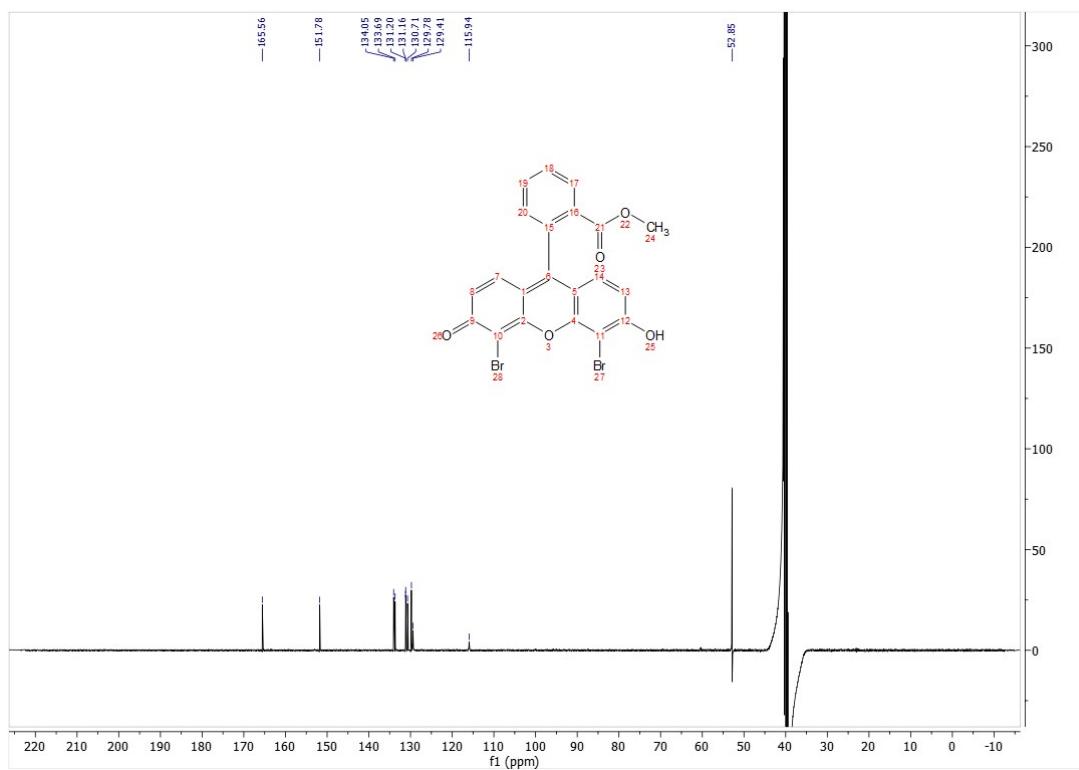


Figure S12: ^{13}C NMR of DB1

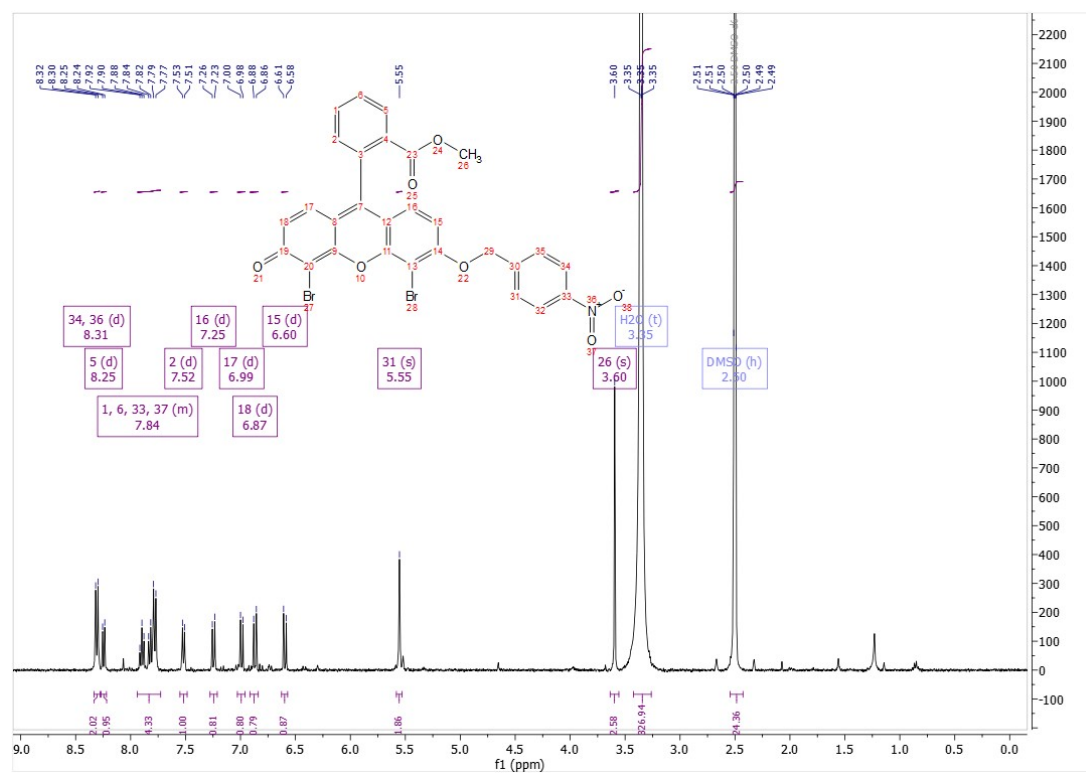


Figure S13: ^1H NMR of DB2

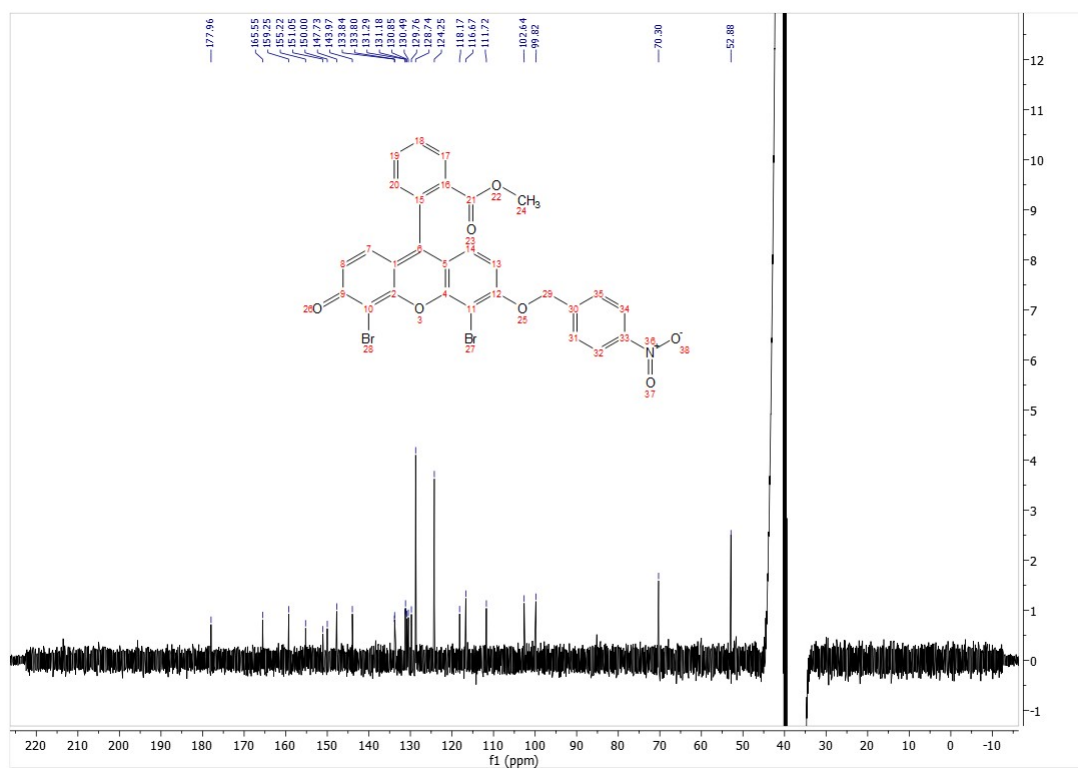


Figure S14: ^{13}C NMR of DB2

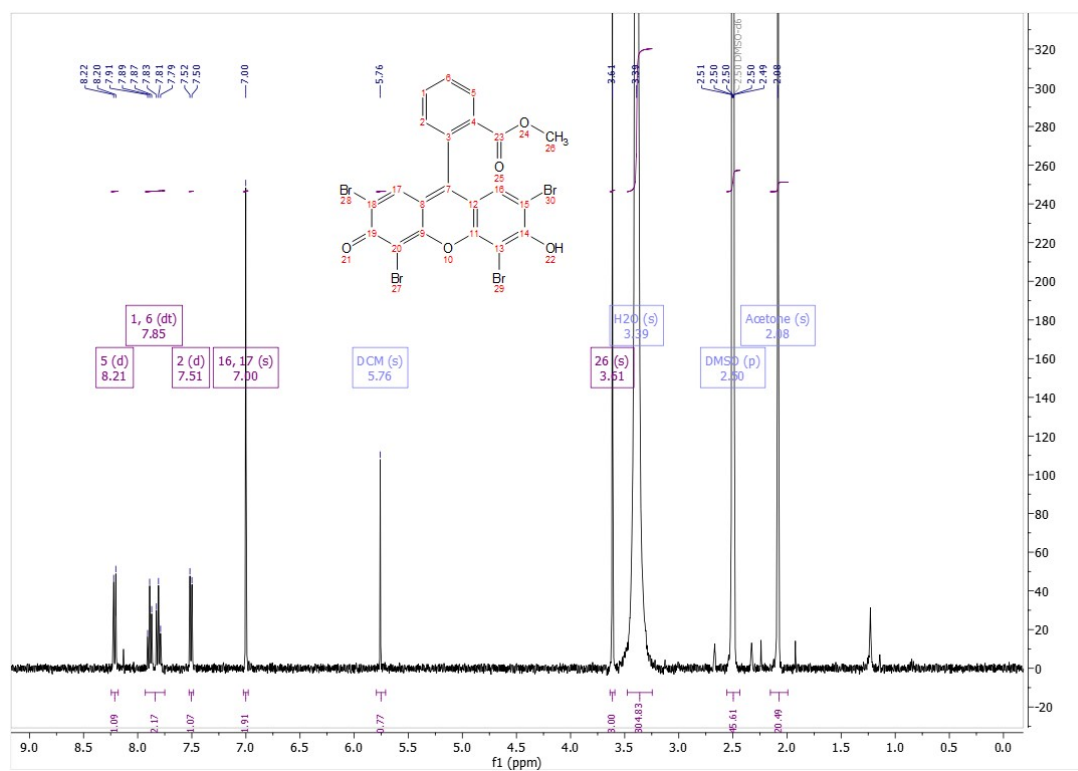
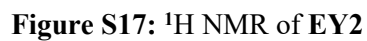
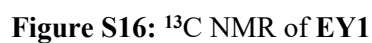


Figure S15: ^1H NMR of EY1



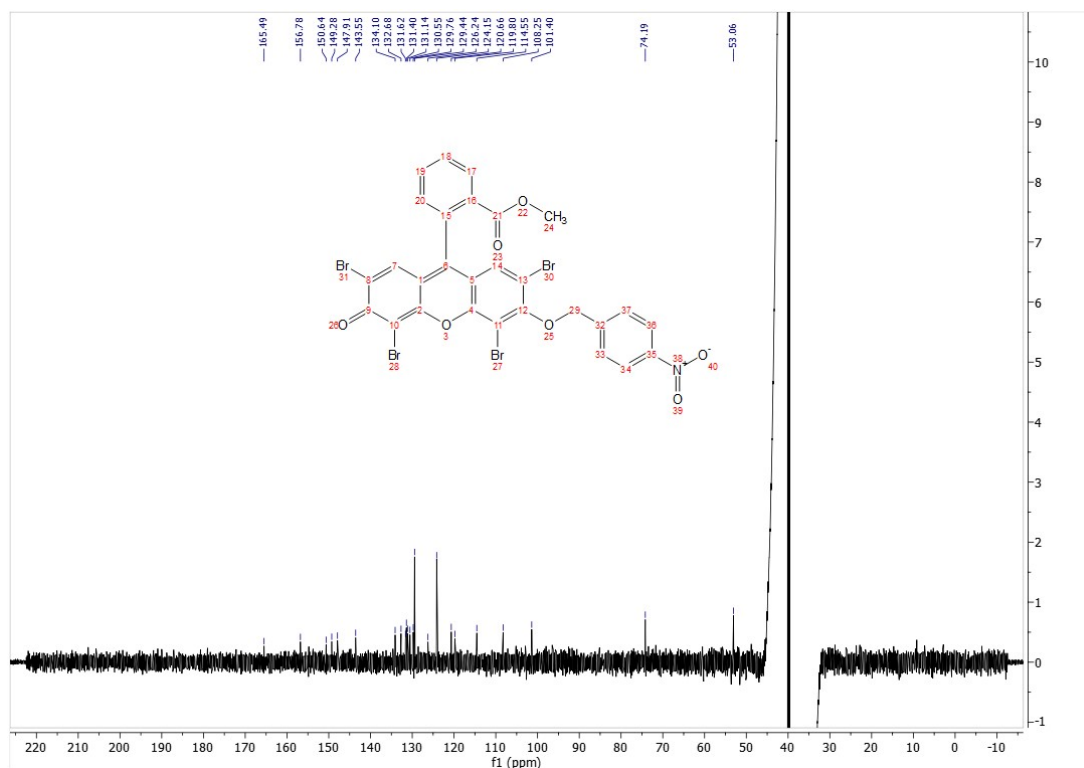


Figure S18: ^{13}C NMR of EY2

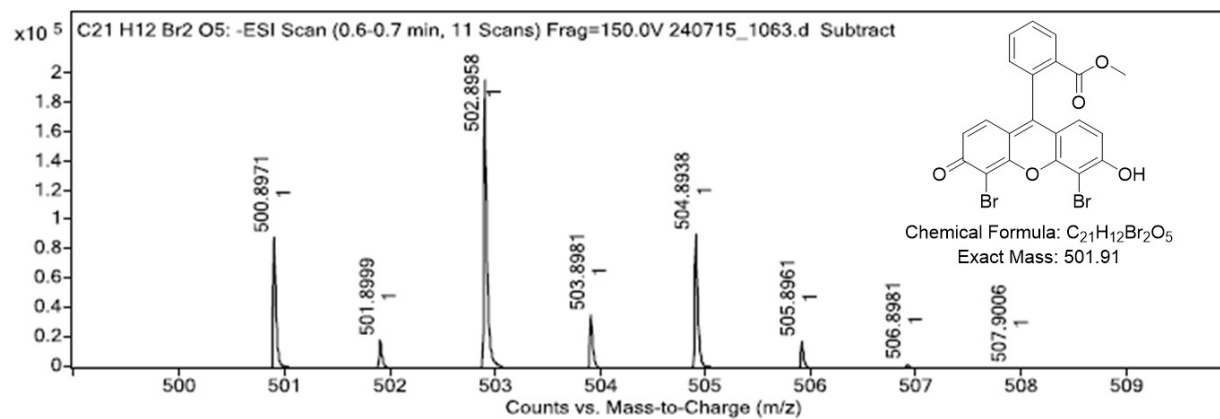


Figure S19: MS of DB1

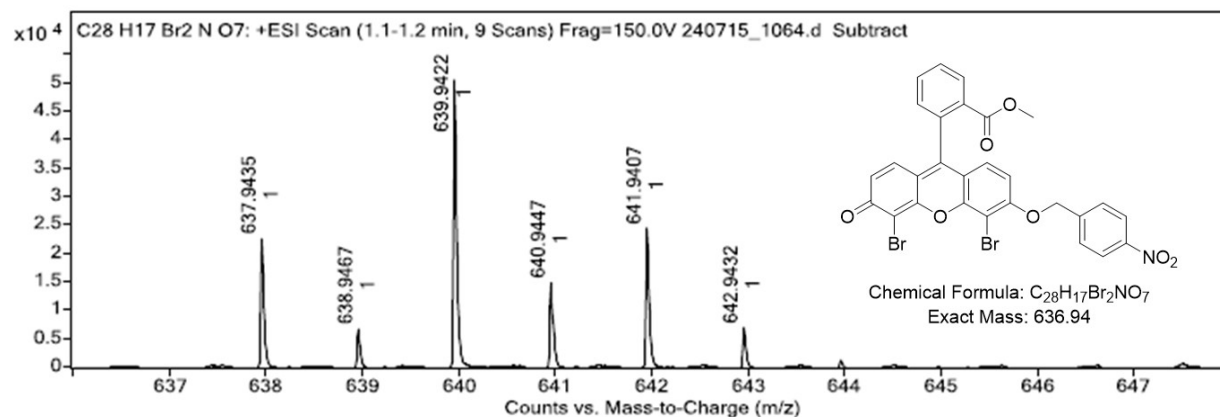


Figure S20: MS of DB2

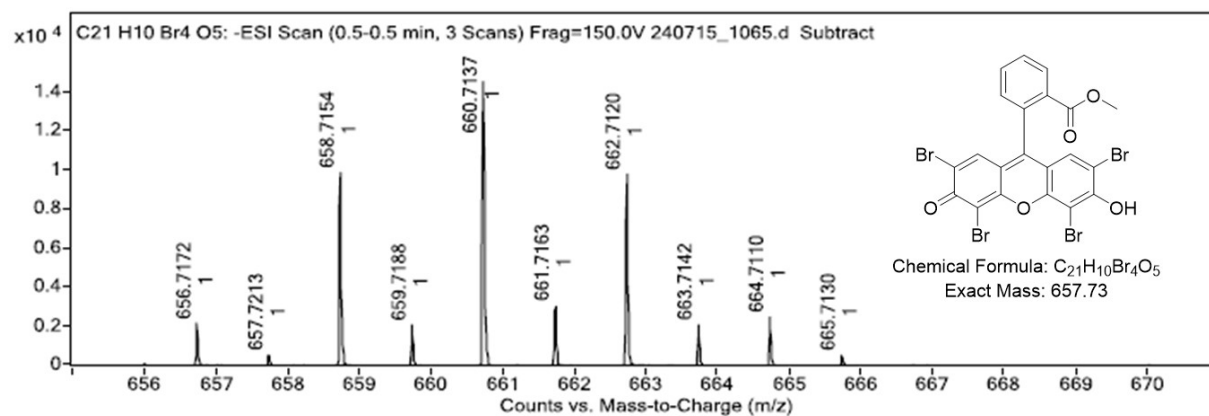


Figure S21: MS of EY1

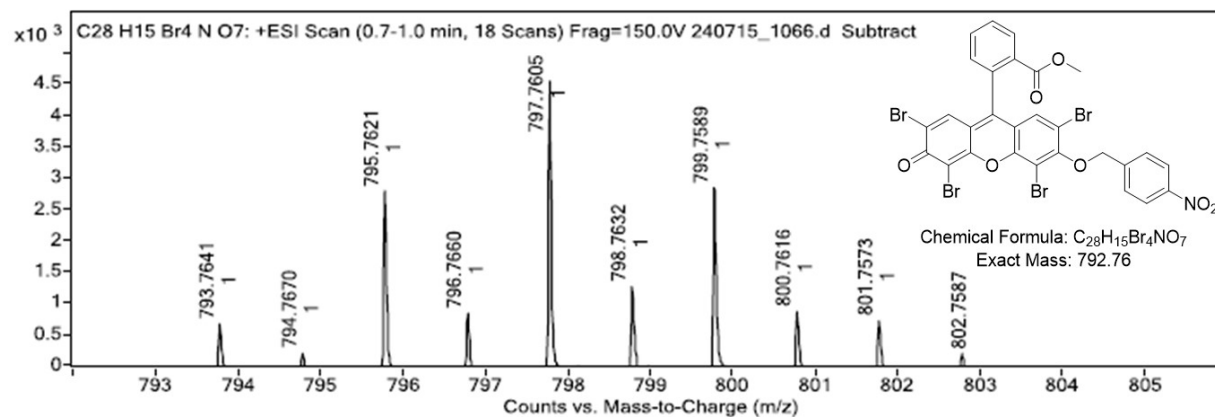


Figure S22: MS of EY2