Folate Receptor-mediated Enhanced and Specific Delivery of Far-red Light-activatable Prodrugs of Combretastatin A-4 to FR-positive Tumor

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Supplementary Information (SI)

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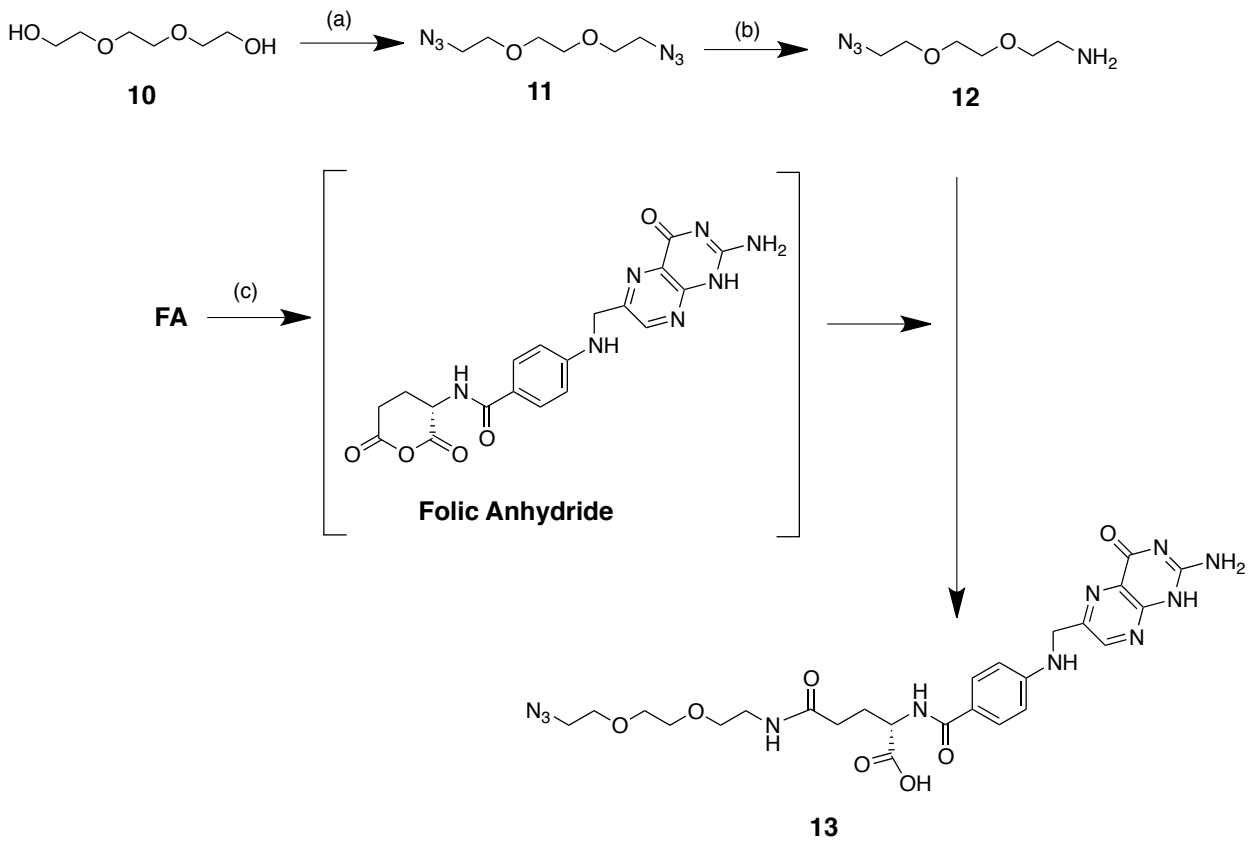
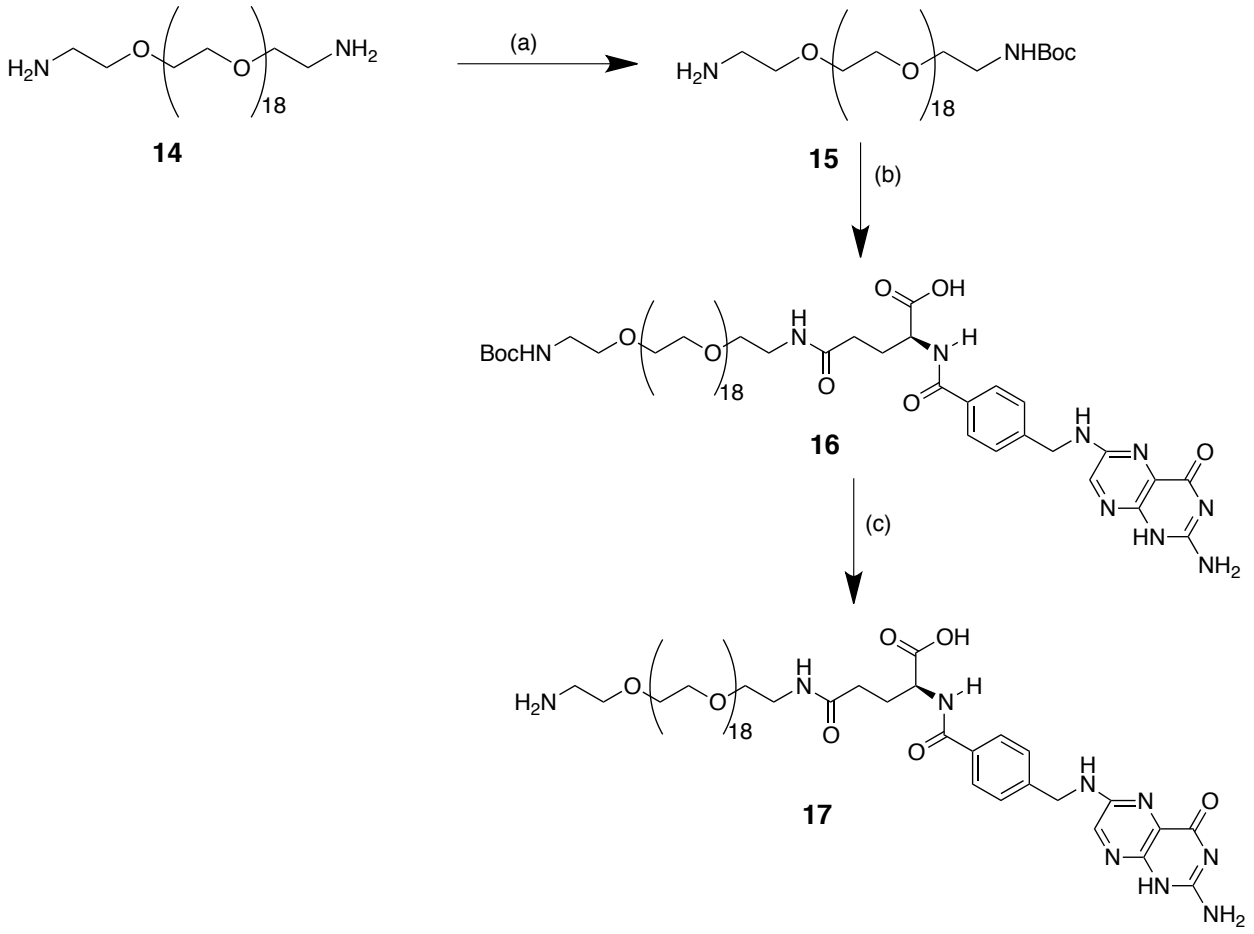


Scheme S1. Preparation of compound **13. (**a) Triethylene glycol (1 eq.), methanesulfonyl chloride (2 eq.), TEA (2 eq.) in 10 mL THF reflux at 120°C, 80% yield. (b) Diazide **11** (1 eq.), 0.65M H_3PO_4 (40 mL), PPh_3 (1 eq., 30 mL), 70% yield. (c) i) **FA** (1 eq.) DCC (6 eq.) DMF/pyridine (5:1 v/v), sonicate, 30 min, ii) add **12** (1 eq.) at rt, 24 h, iii) precipitate in cold Et_2O/Acetone (4:1 v/v), iv) Dialysis, 48 h, 65% yield.



Scheme S2. Synthesis for compounds **15** and **17.** (a) Diamine **14** (1 eq.), Boc_2O (1 eq.) TEA (3 eq.), anhydrous MeOH (9.31 mL), reflux, 24 h, 72% yield. (b) sonicated **FA** (1 eq) in DCC (6.7 eq), 30 min, DMF/Pyridine (5:1 v/v), **15** (1 eq.), rt, 36 h precipitate in cold Et_2O/Acetone (3:1 v/v), 73% yield. C) TFA, rt, 4 h.

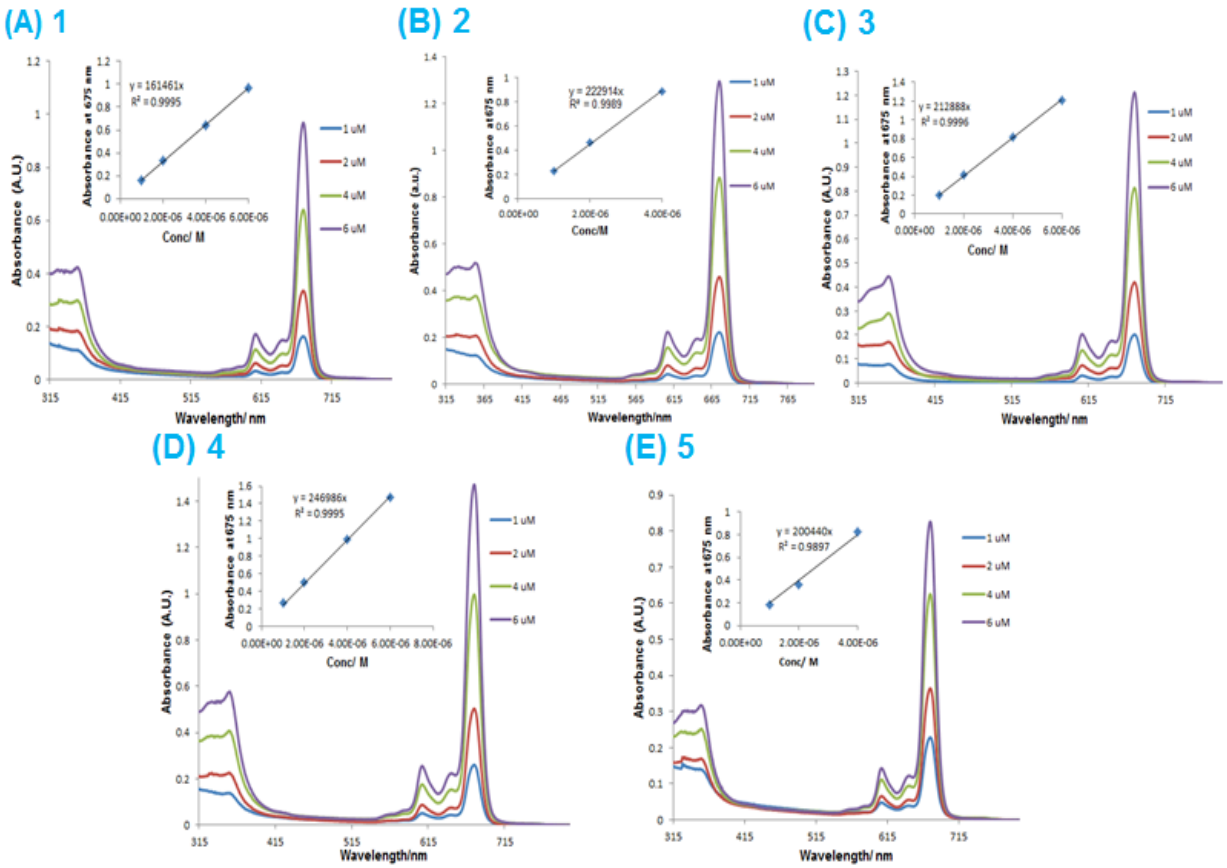


Figure S1. Absorption spectra of prodrugs (A) prodrug **1**, (B) prodrug **2**, (C) prodrug **3**, (D) prodrug **4,** and (E) prodrug **5** in DMF at different concentrations. The inset of each spectrum plots the Q-band absorbance vs. concentration of the conjugate.

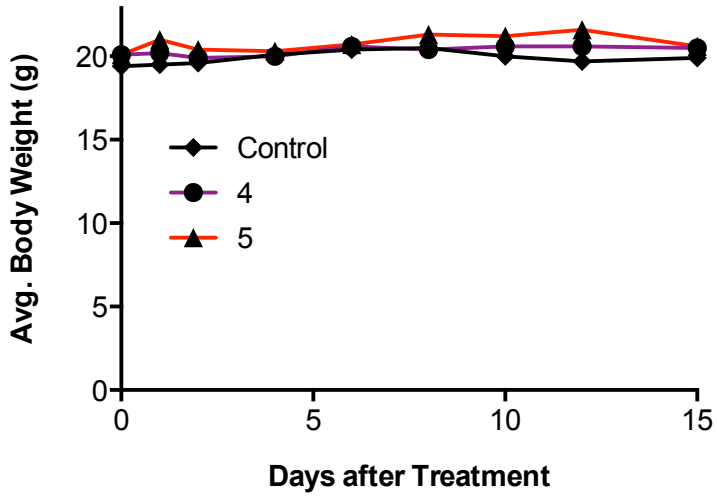


Figure S2. Body weight changes during the antitumor efficacy study. Changes are presented as an average of all three mice in each group. SD values were not added, for clarity.

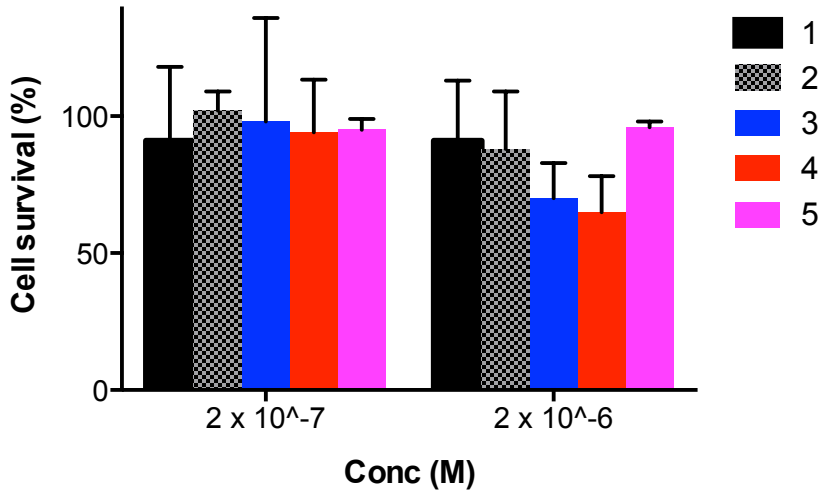


Figure S3. Dark toxicity of prodrugs **1**-**5**.

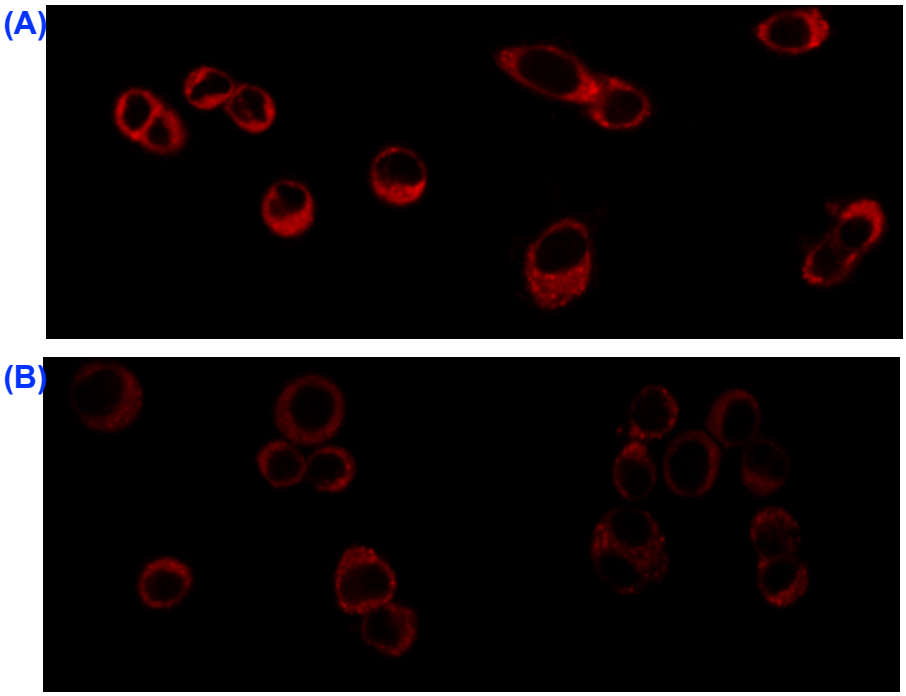


Figure S4. Confocal microscopy images of colon 26 cells incubated for 1 h 15 min with (A) prodrug 4 and (B) prodrug 5 at the concentration of 20 μM (excitation at 633 nm and emission at 640 – 700 nm).

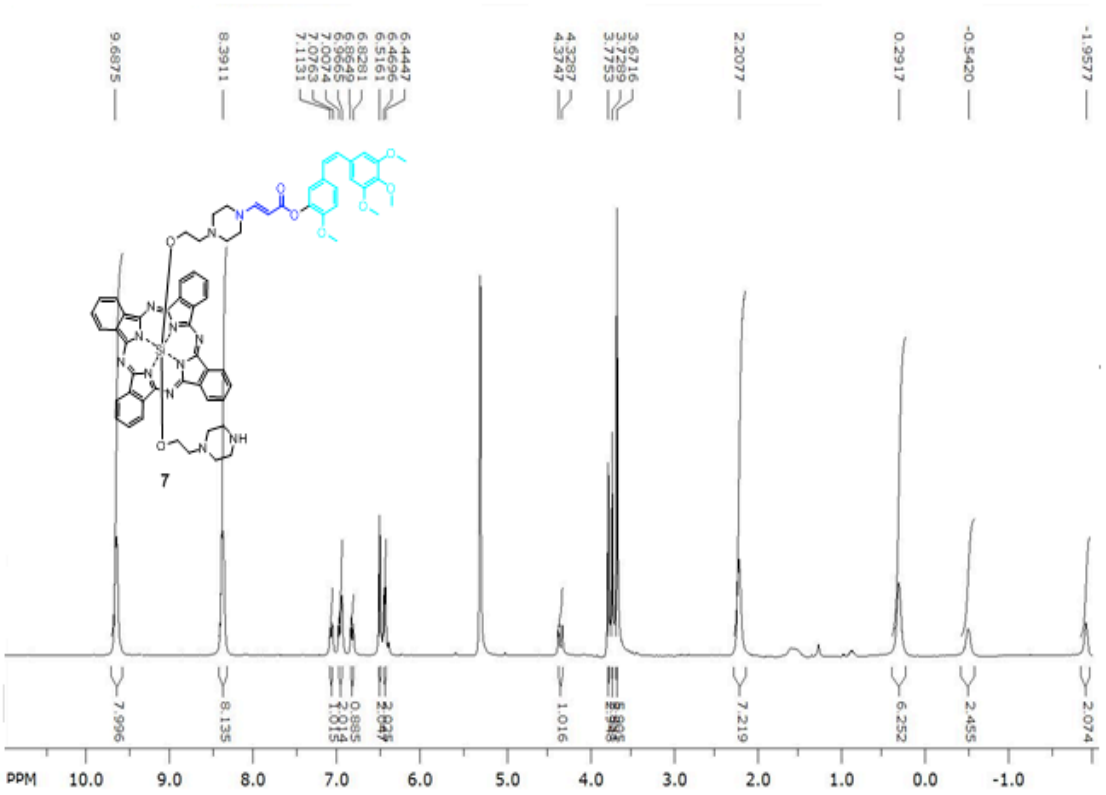


Figure S5: ^{1}H-NMR of compound **7**.

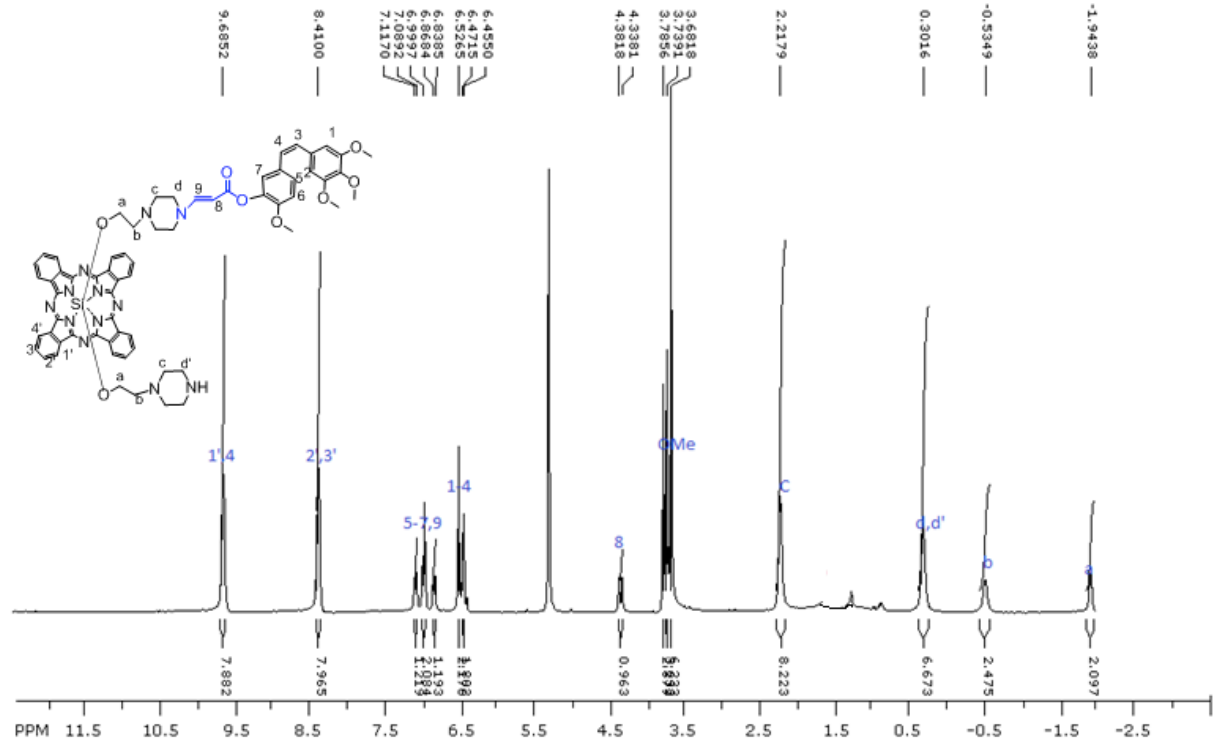


Figure S6: Peak Assignment on ^{1}H-NMR of compound **7**.

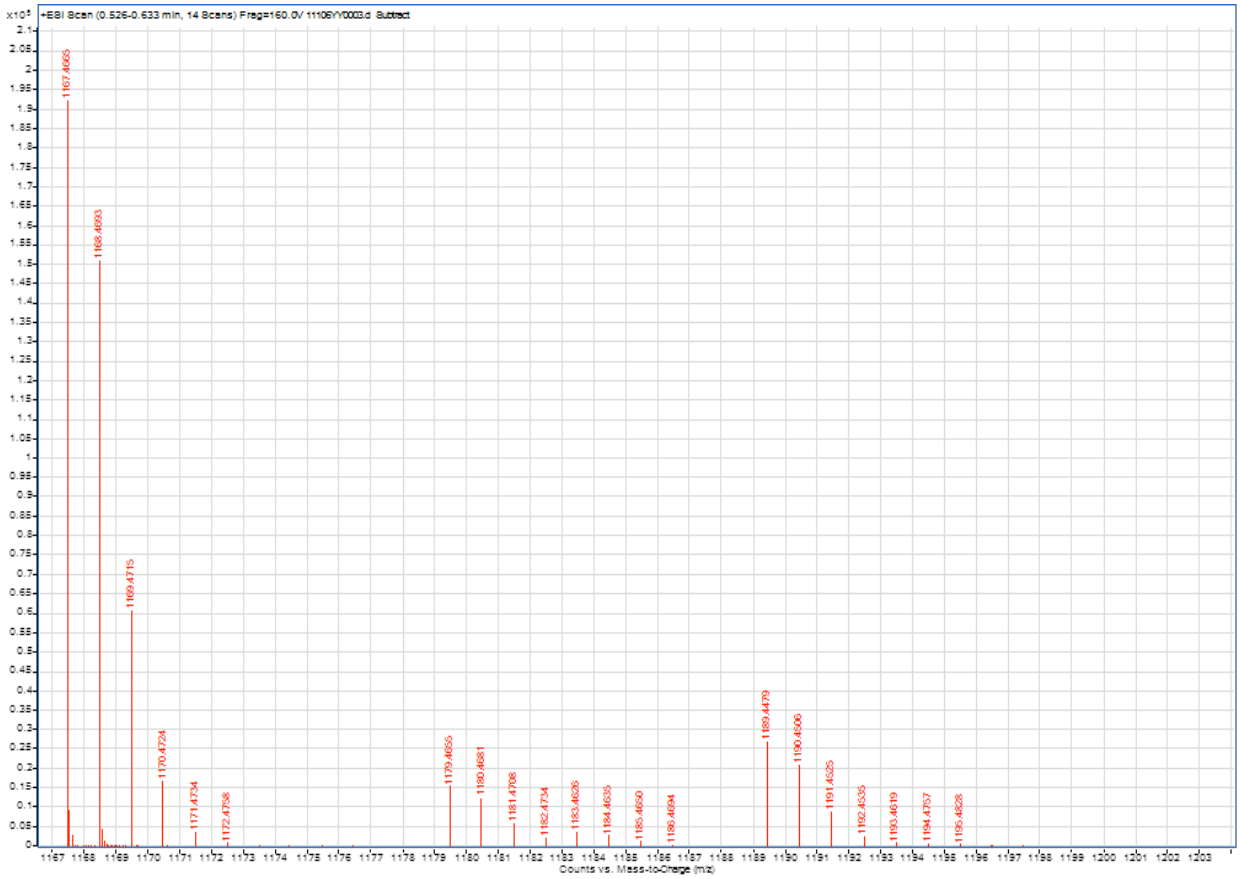


Figure S7. HRMS (ESI) of compound **7**. Calculated for $C_{65}H_{63}N_{13}O_8Si$ $[M + H]^+$: 1167.2661 and $C_{65}H_{61}N_{12}O_8SiNa$ $[M + Na]^+$: 1189.4481, found: m/z 1167.4665 and 1189.4479.

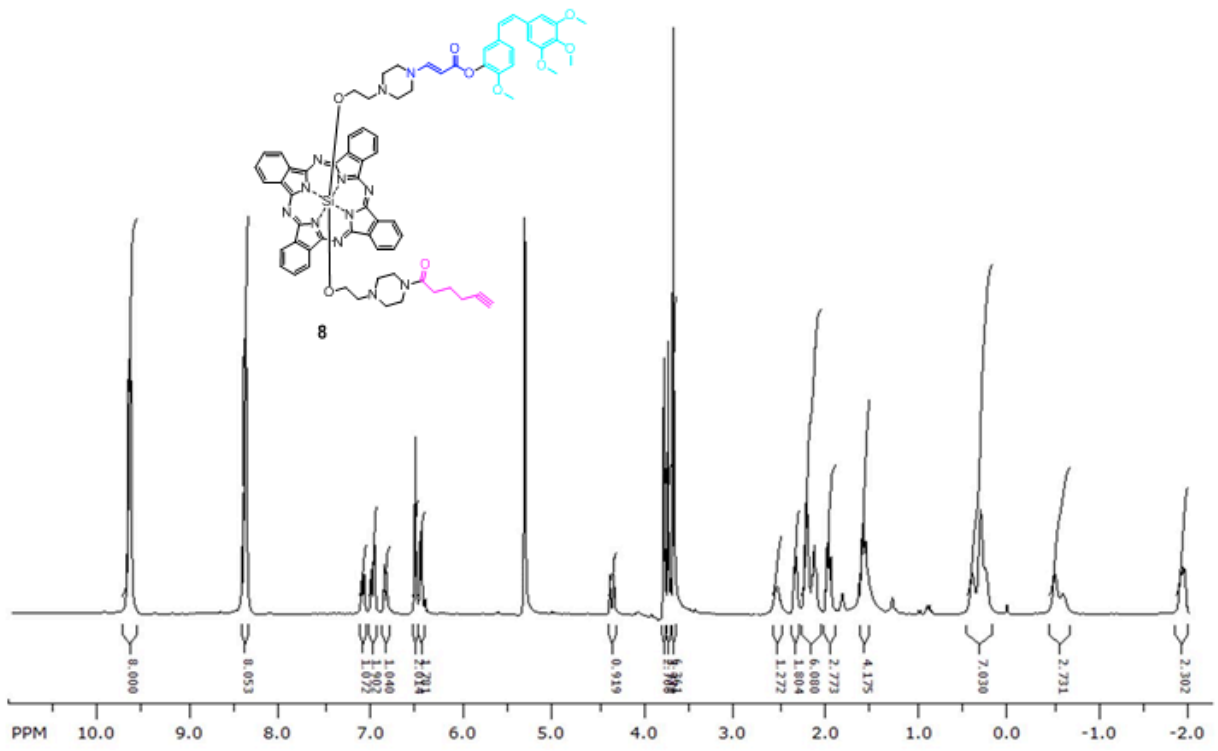


Figure S8. ^{1}H-NMR of compound **8**.

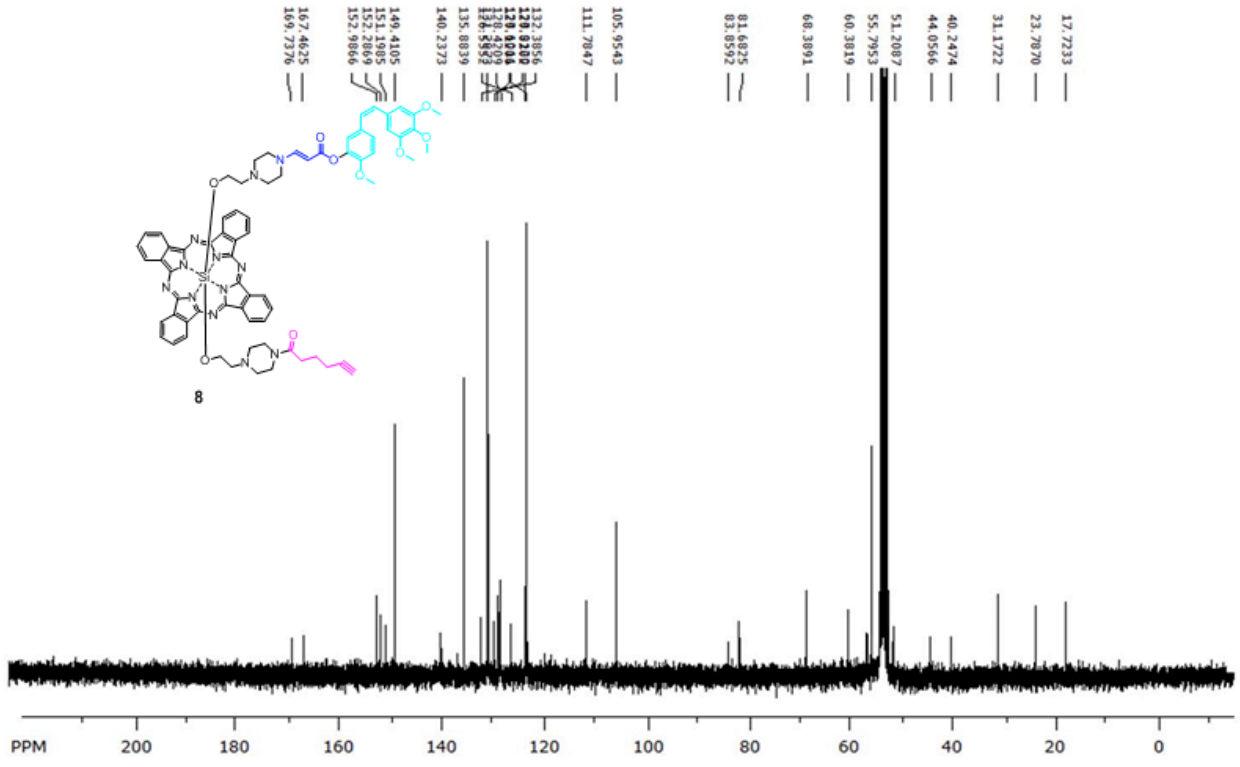


Figure S9. ^{13}C-NMR of compound **8**.

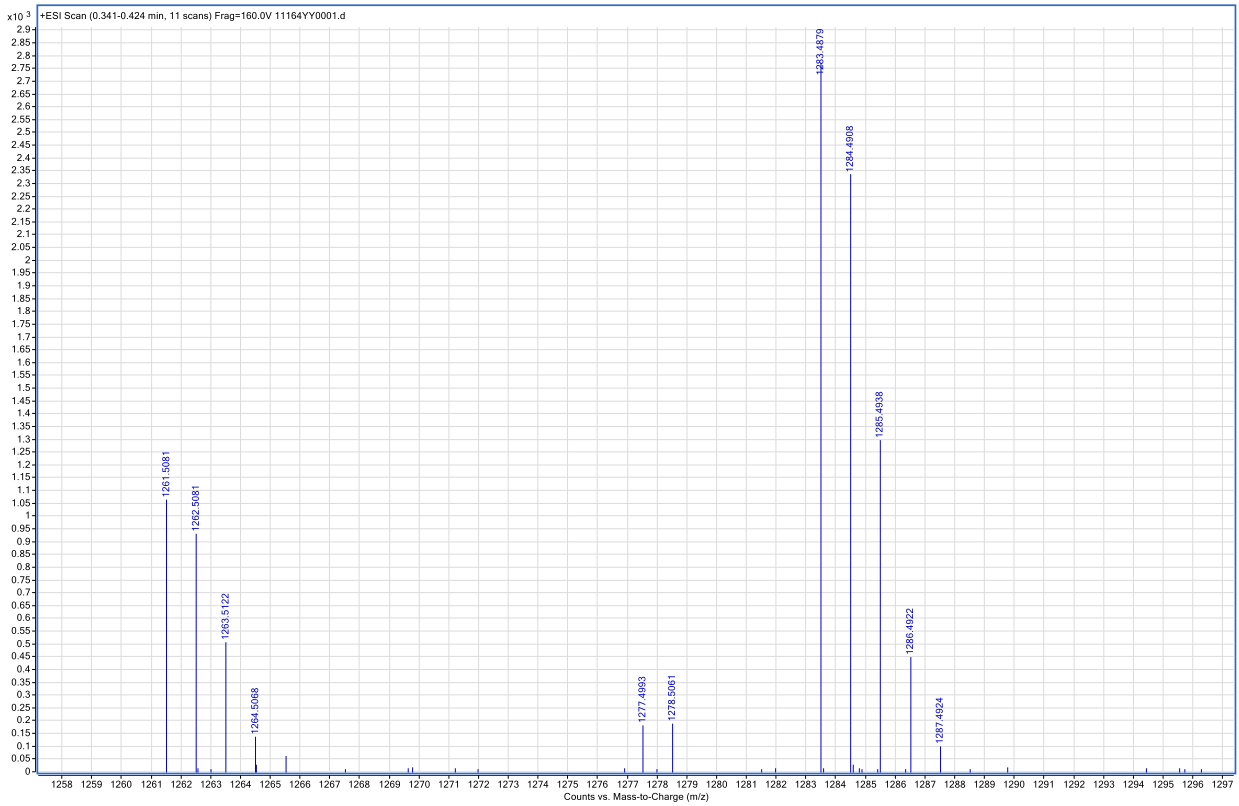


Figure S10. HRMS-ESI of compound **8.** Calculated for $C_{71}H_{69}N_{12}O_9Si$ $[M+H]^+$: 1261.5090 and $C_{71}H_{68}N_{12}O_9SiNa$ $[M+Na]^+$: 1283.4899, found: 1261.5077 and 1283.4891.

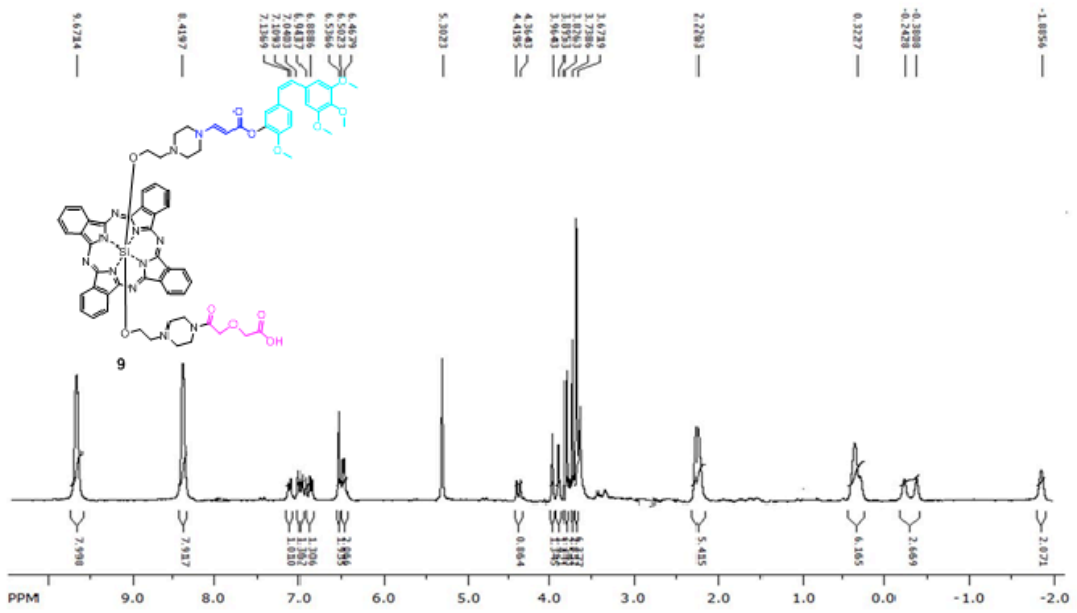


Figure S11. ^{1}H-NMR of compound **9**.

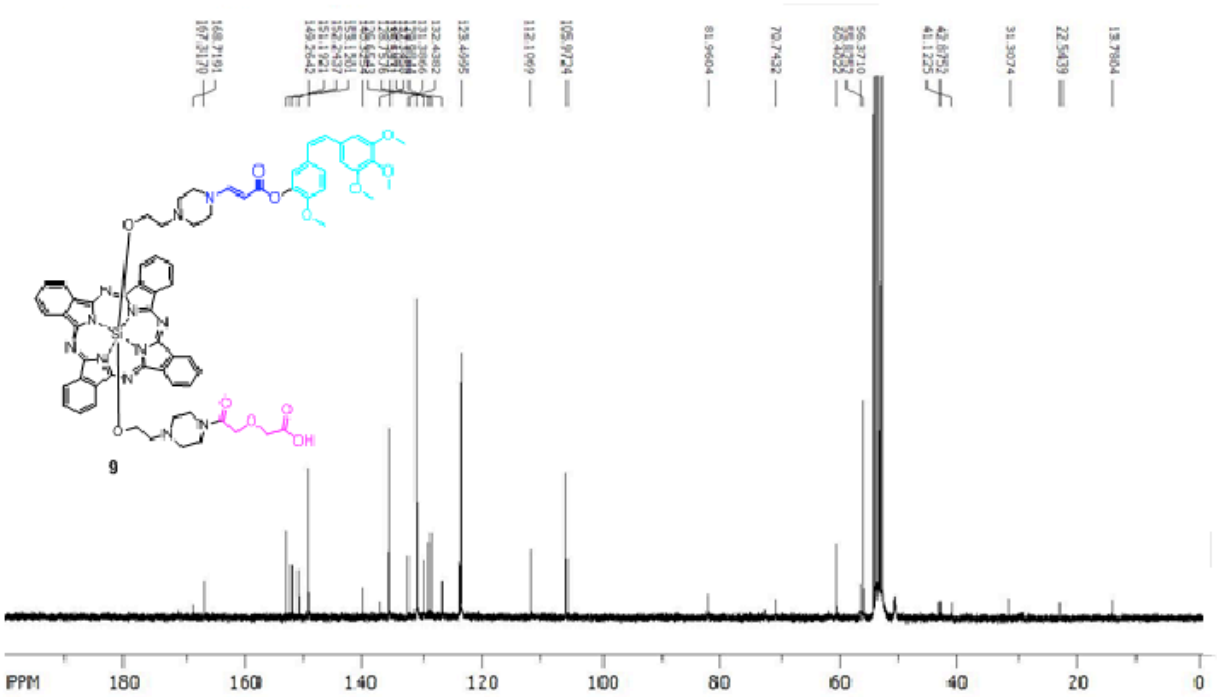


Figure S12. ^{13}C-NMR of compound **9**.

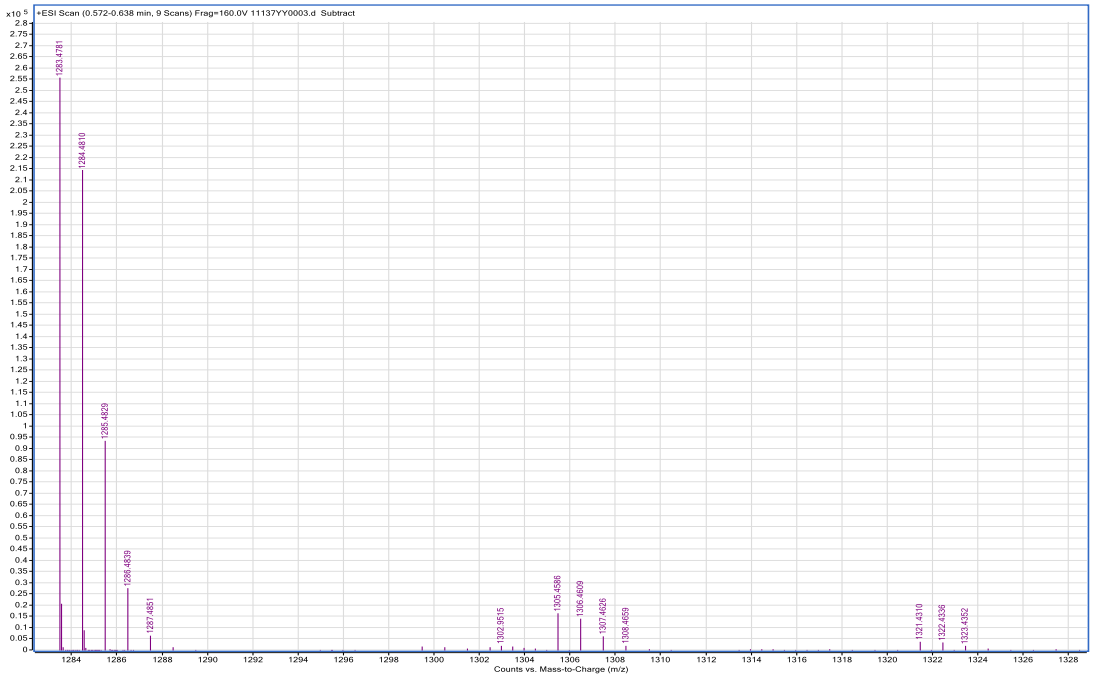


Figure S13. HRMS-ESI of compound **9**. Calculated for $C_{69}H_{66}N_{12}O_{12}Si$: $[M + H]^+$: 1283.4771 found m/z 1283.4781.

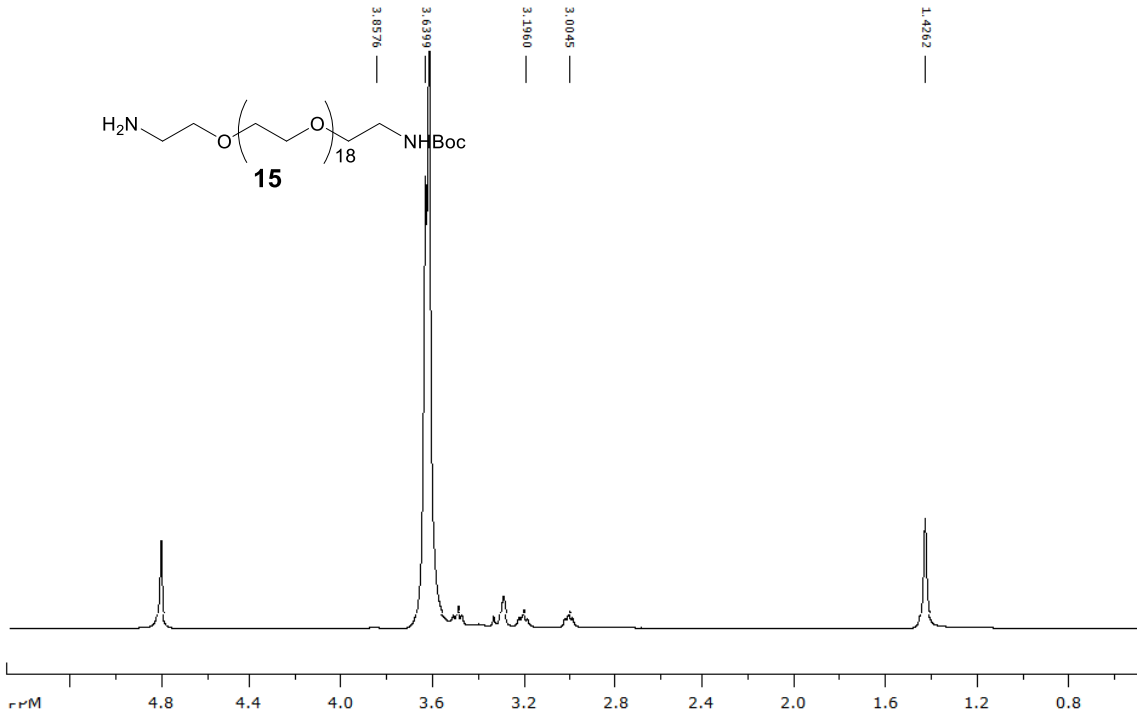


Figure S14. ^{1}H-NMR of compound **15**.

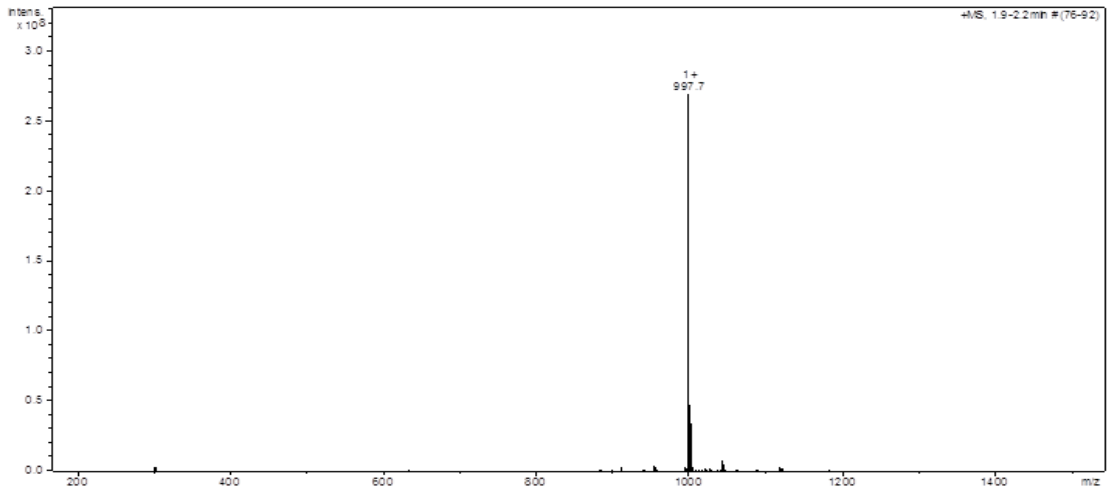


Figure S15. LRMS (ESI) of compound **15**. Calculated for $C_{45}H_{93}N_2O_{21}$ $[M + H]^+$:997.7, found: m/z 997.7.

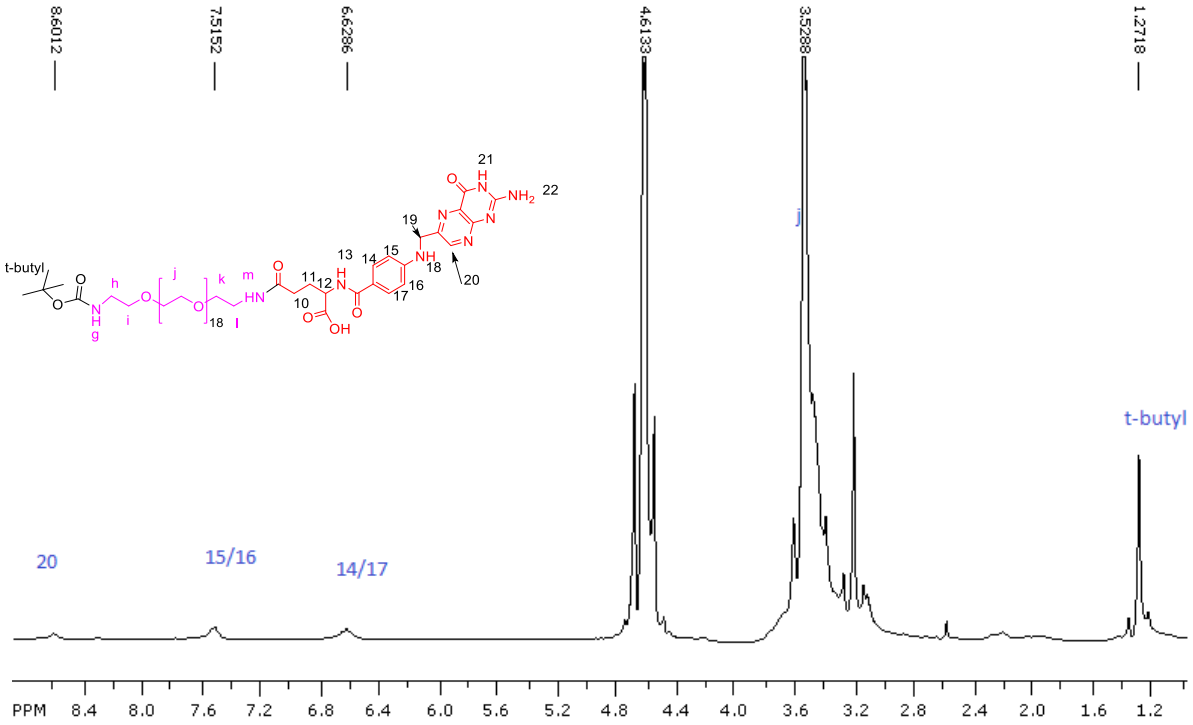


Figure S16. ^{1}H-NMR of compound **17**.

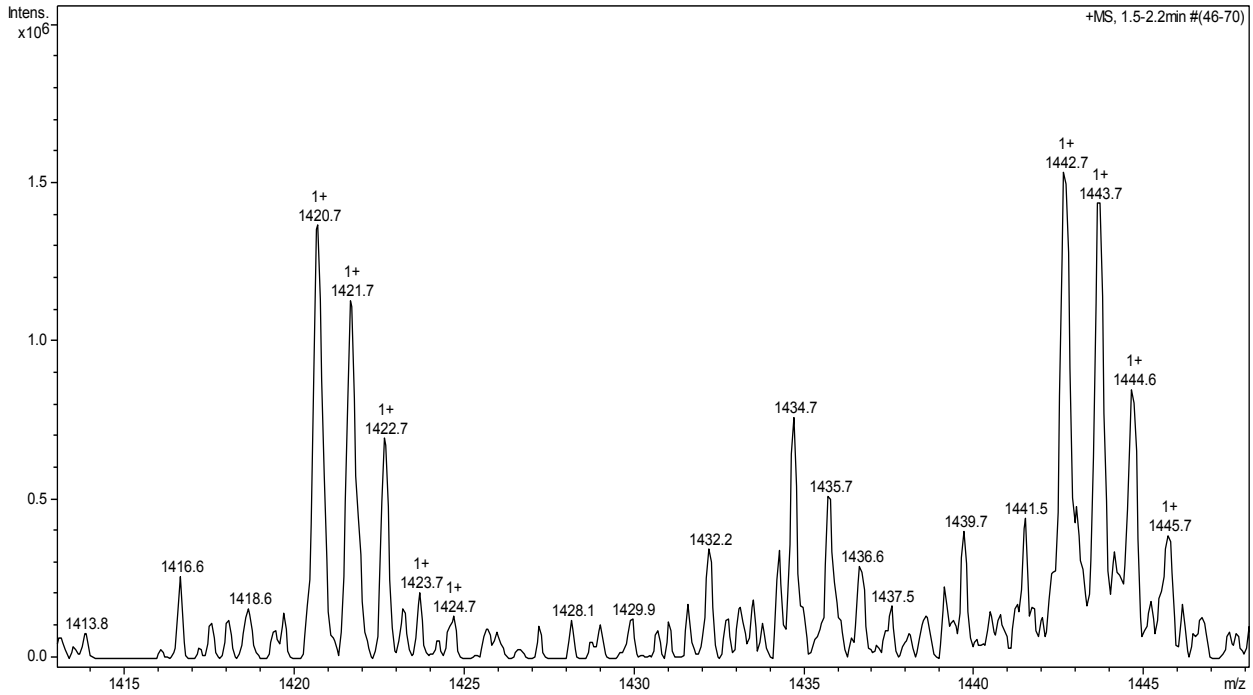


Figure S17. LRMS (ion trap) of **17**. Calculated $C_{64}H_{110}N_9O_{26}$ $[M+H]^+$: 1420.7, found: M/z 1420.7.

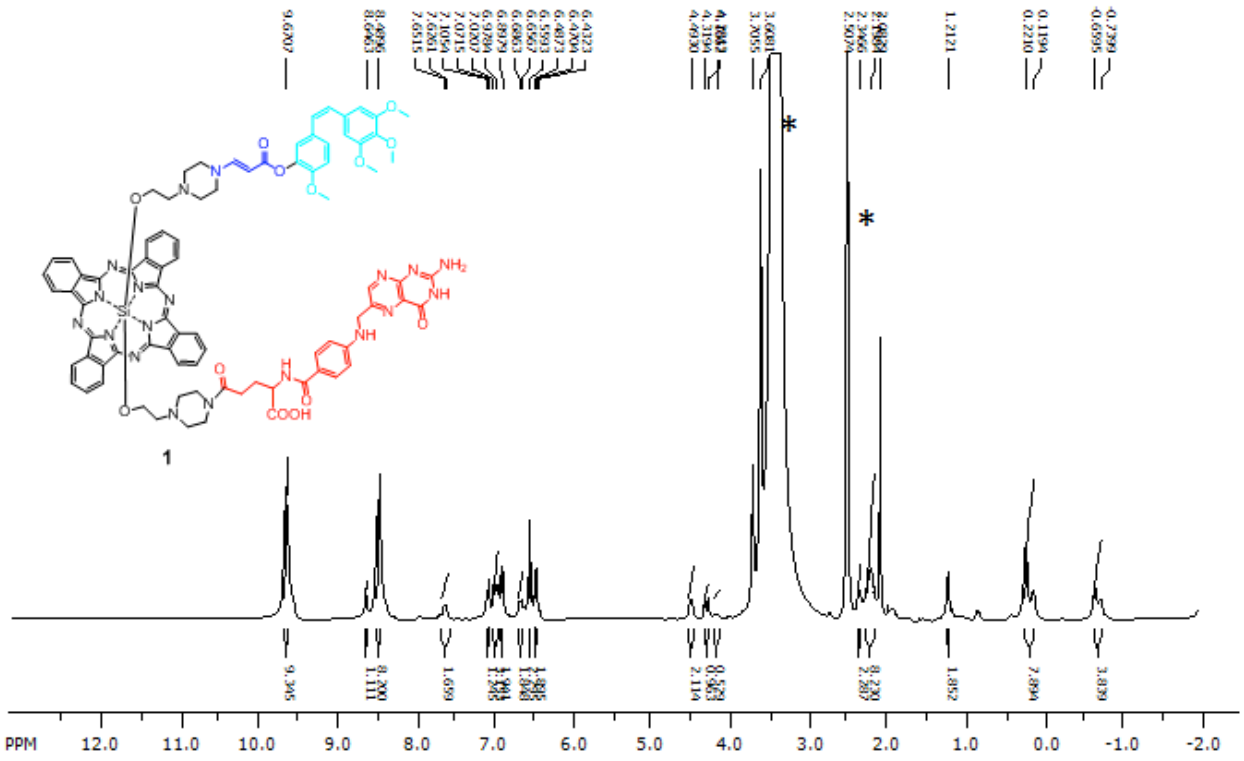


Figure S18. [1]H-NMR of prodrug **1**. *Peaks at 2.50, 3.60 were from solvents;(DMSO and H_2O, respectively).

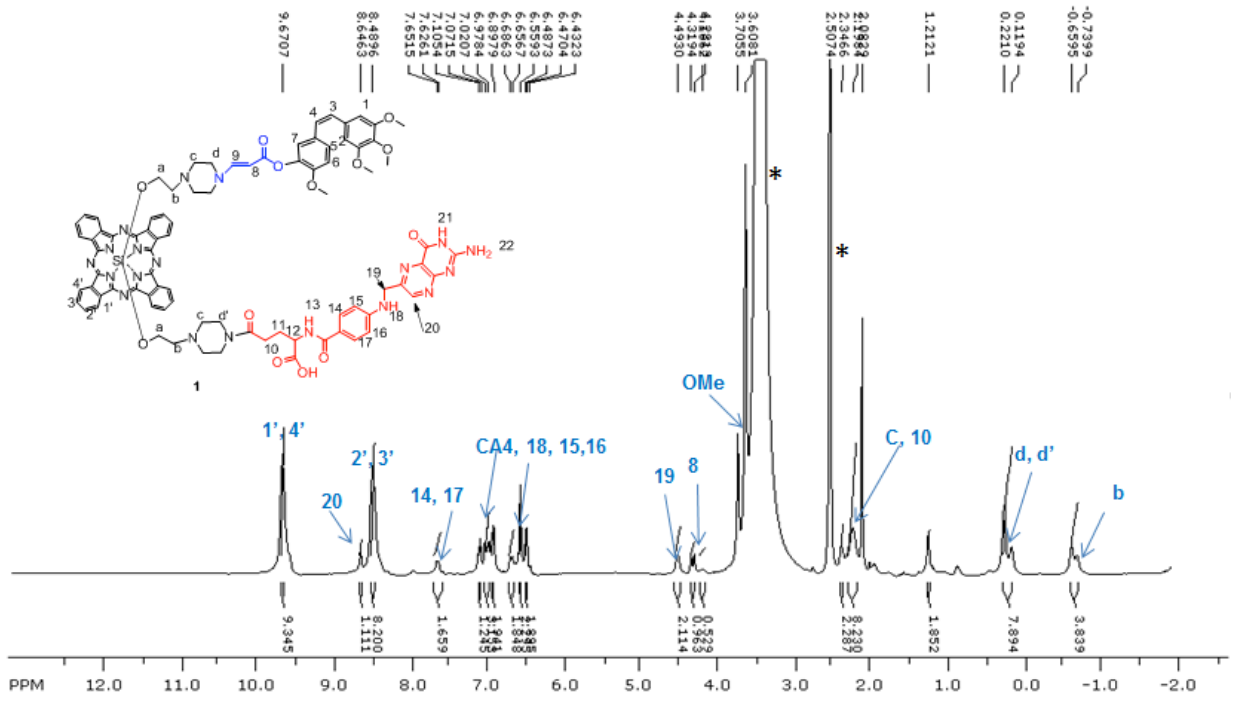


Figure S19. Assignment of peaks to the ^{1}H-NMR of prodrug **1**.

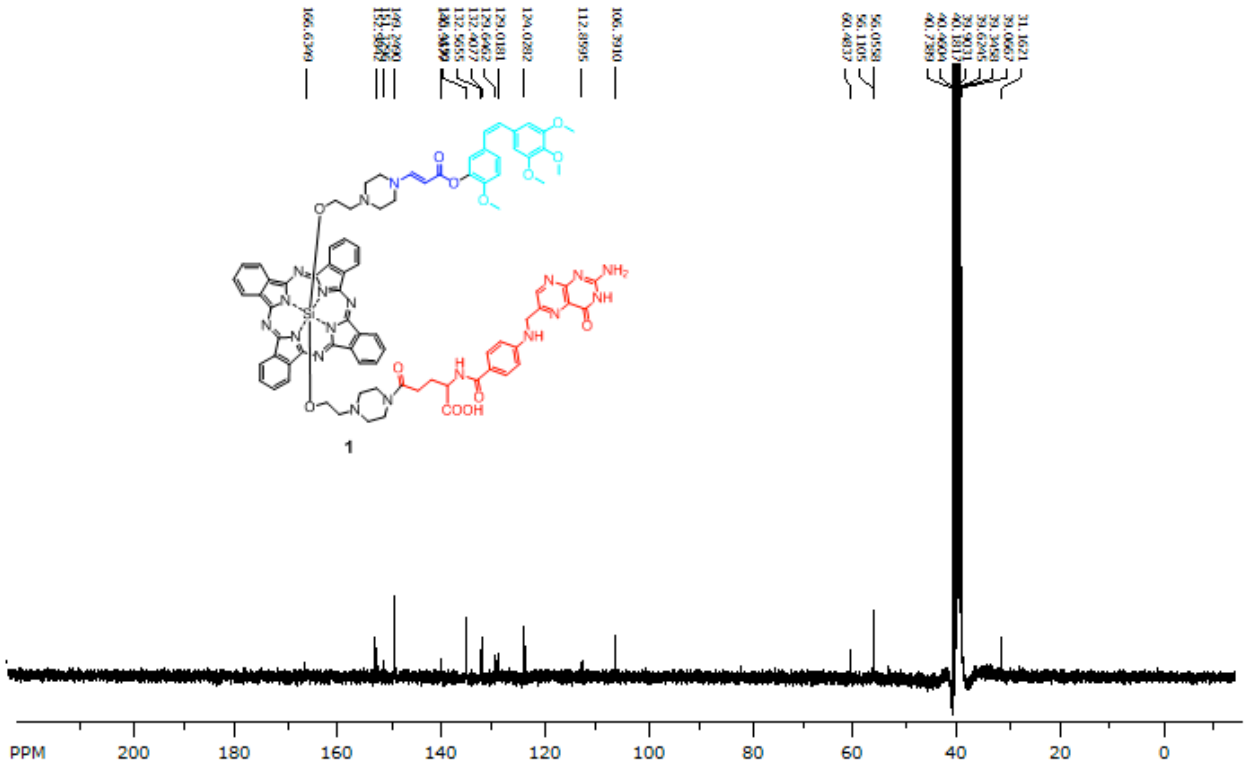


Figure S20. ^{13}C-NMR of prodrug **1**.

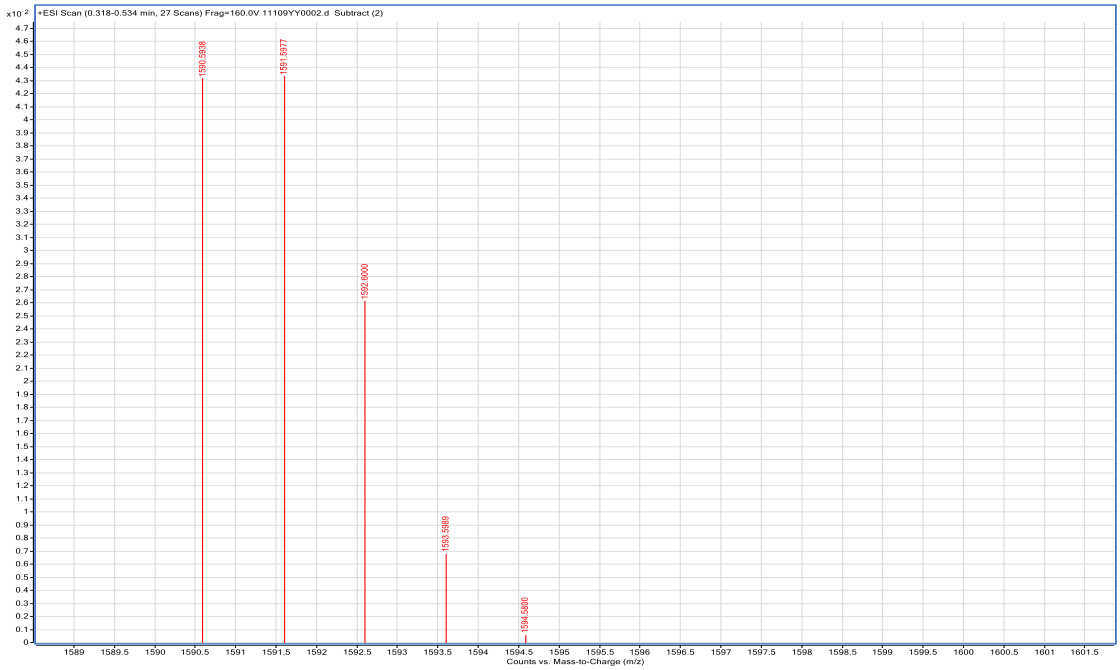
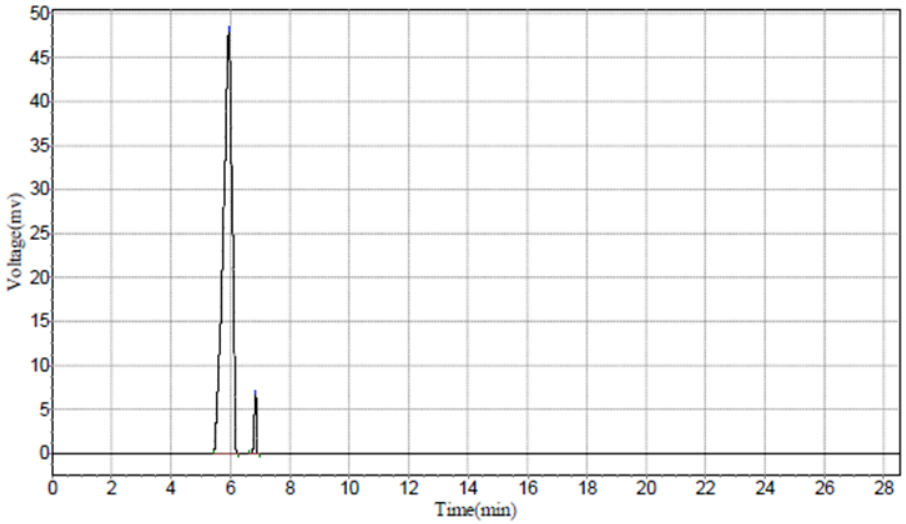


Figure S21. HRMS (ESI) of prodrug **1**. Calculated for $C_{84}H_{59}N_{20}O_{13}Si$ $[M + H]^+$: 1590.5952, found: 1590.5938.



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		5.940	47965.398	1001771.625	96.2589
2		6.848	6831.381	38933.301	3.7411
Total			54796.779	1040704.926	100.0000

Figure S22. HPLC chromatogram of prodrug **1**. Retention time 5.9 min. Method: Isocratic, solvent system: 50% ACN 50% H_2O, flow rate 0.5 mL/min, Detection at 254 nm, and purity: 96%.

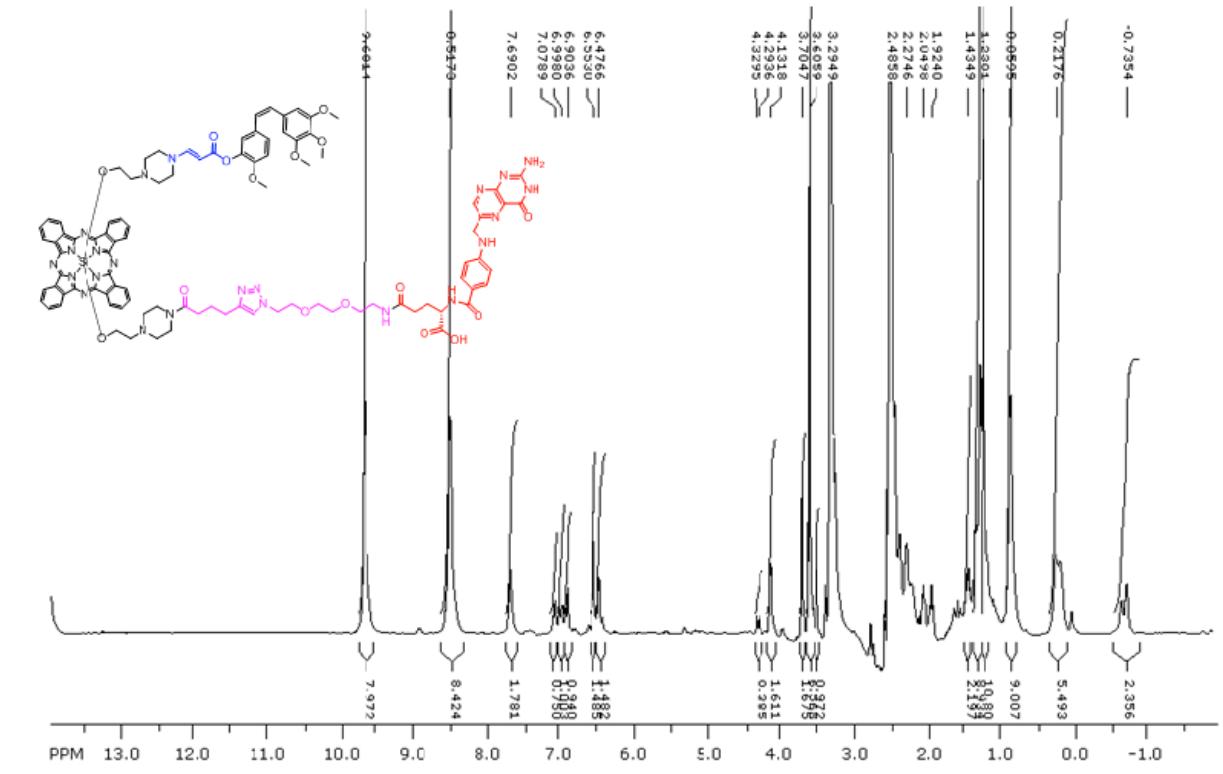


Figure S23. ^{1}H-NMR of prodrug **2**.

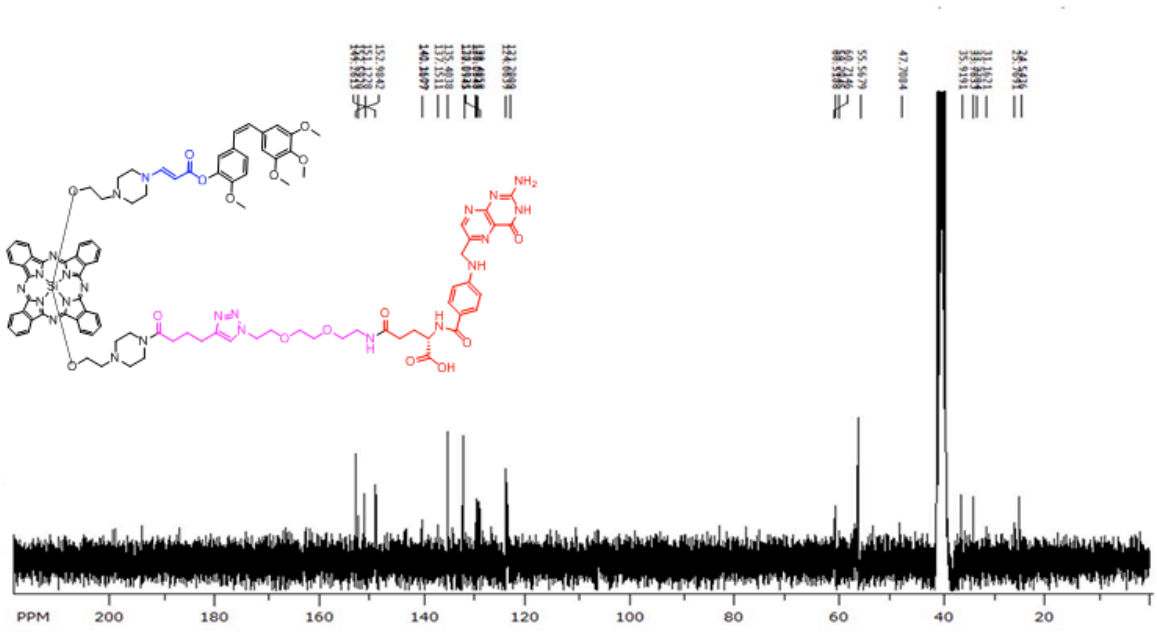


Figure S24. ^{13}C-NMR of prodrug **2**.

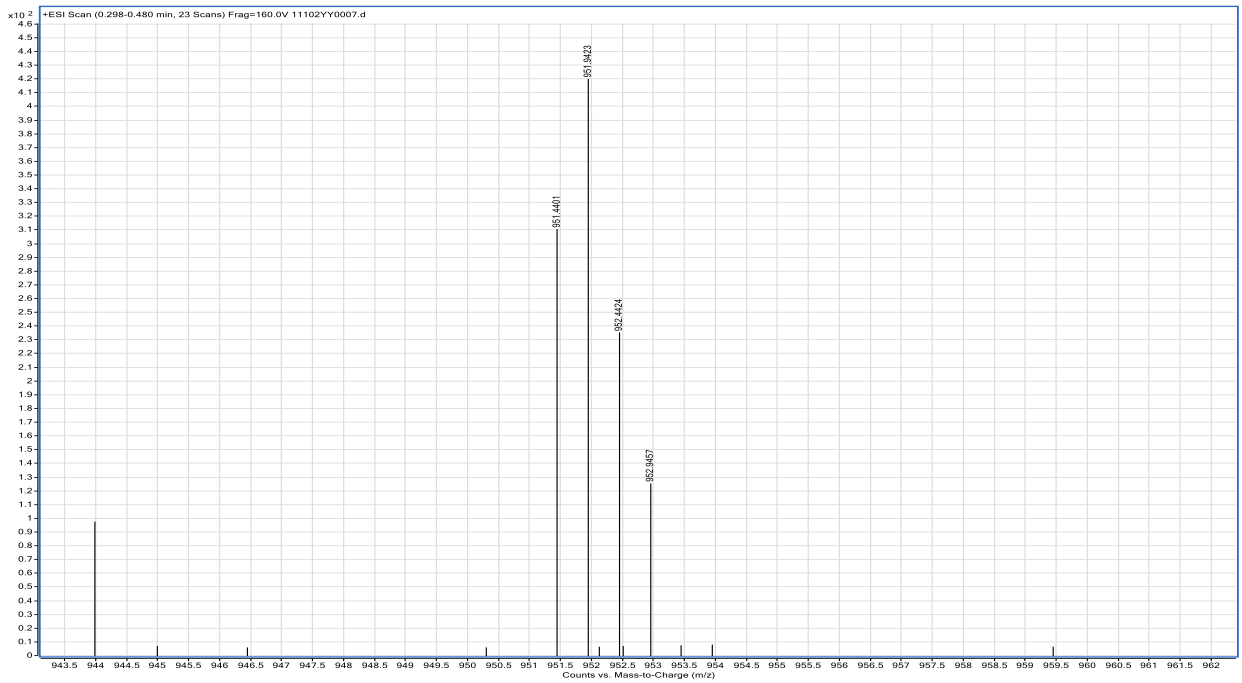
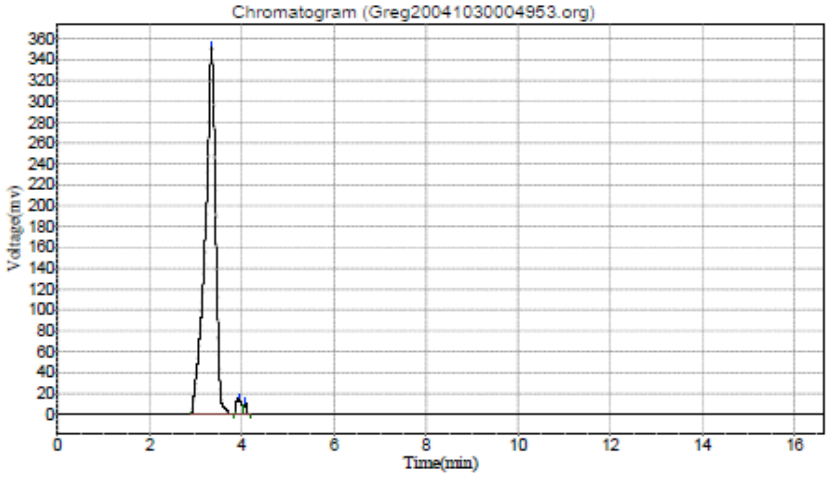


Figure S25. HRMS-ESI of prodrug **2.** Calculated for $[C_{96}H_{99}N_{23}O_{16}SiNa_2]^{+2}$ $[M+2Na]^{+2}$: 951.8602 , found: 951.9423.



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		3.348	355075.969	5549544.500	97.0861
2		3.932	16456.631	129944.125	2.2733
3		4.073	11506.306	36620.211	0.6406
Total			383038.905	5716108.836	100.0000

Figure S26. HPLC chromatogram of prodrug **2**. Retention time 3.4 min. Method: Isocratic, solvent system: 50% ACN 50% H_2O, flow rate 0.5 mL/min, Detection at 254 nm, and purity: 97%.

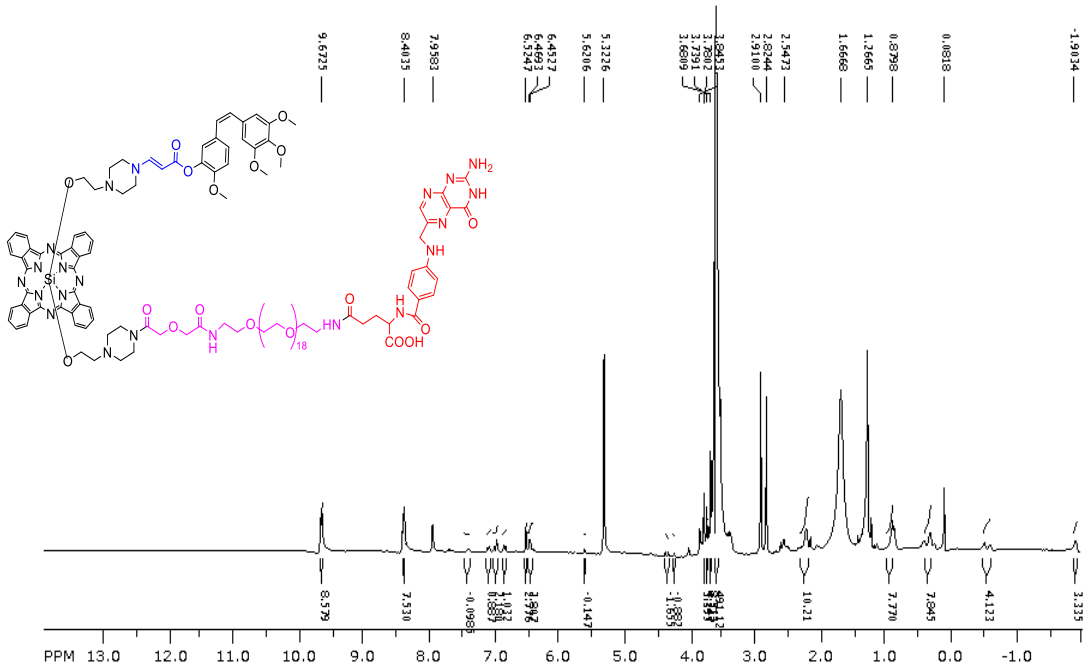


Figure S27. ^{1}H-NMR of prodrug **3**.

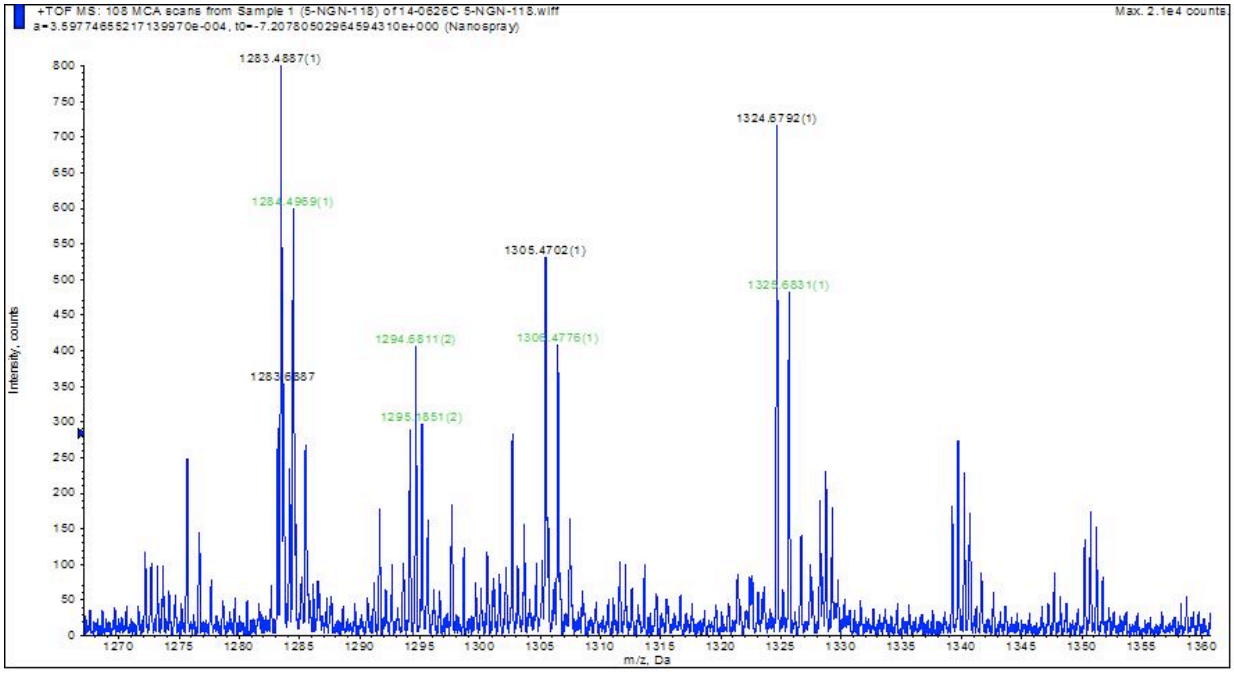
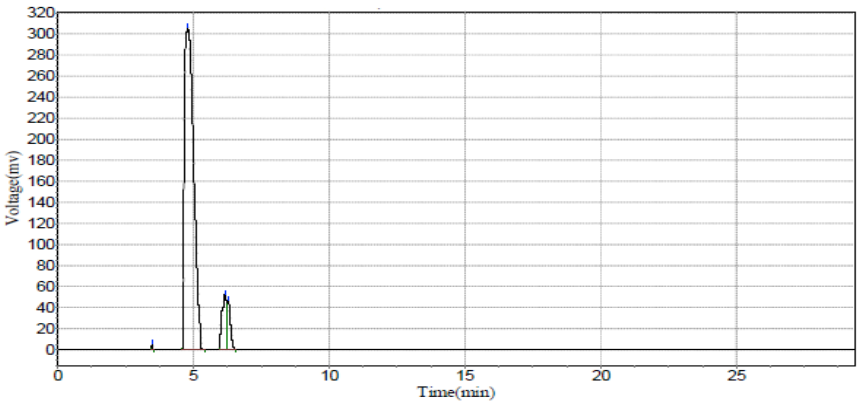


Figure S28. HRMS (ESI) of prodrug **3**: m/z 2584.1546 calcd for $[C_{128}H_{169}N_{21}O_{35}Si]^{+2}$ $[M+4H]^{+2}$: 1294.5947, found: 1294.6811.



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		3.465	5709.000	14762.400	0.1774
2		4.782	306122.813	7286394.000	87.5451
3		6.157	52182.656	622986.875	7.4851
4		6.273	47067.473	398874.313	4.7924
Total			411081.941	8323017.588	100.0000

Figure S29. HPLC chromatogram of prodrug **3**. Retention time 4.74 min. Method: Isocratic, solvent system: 60% ACN 40% H_2O, flow rate 0.5 mL/min, Detection at 254 nm, and purity: 87%.

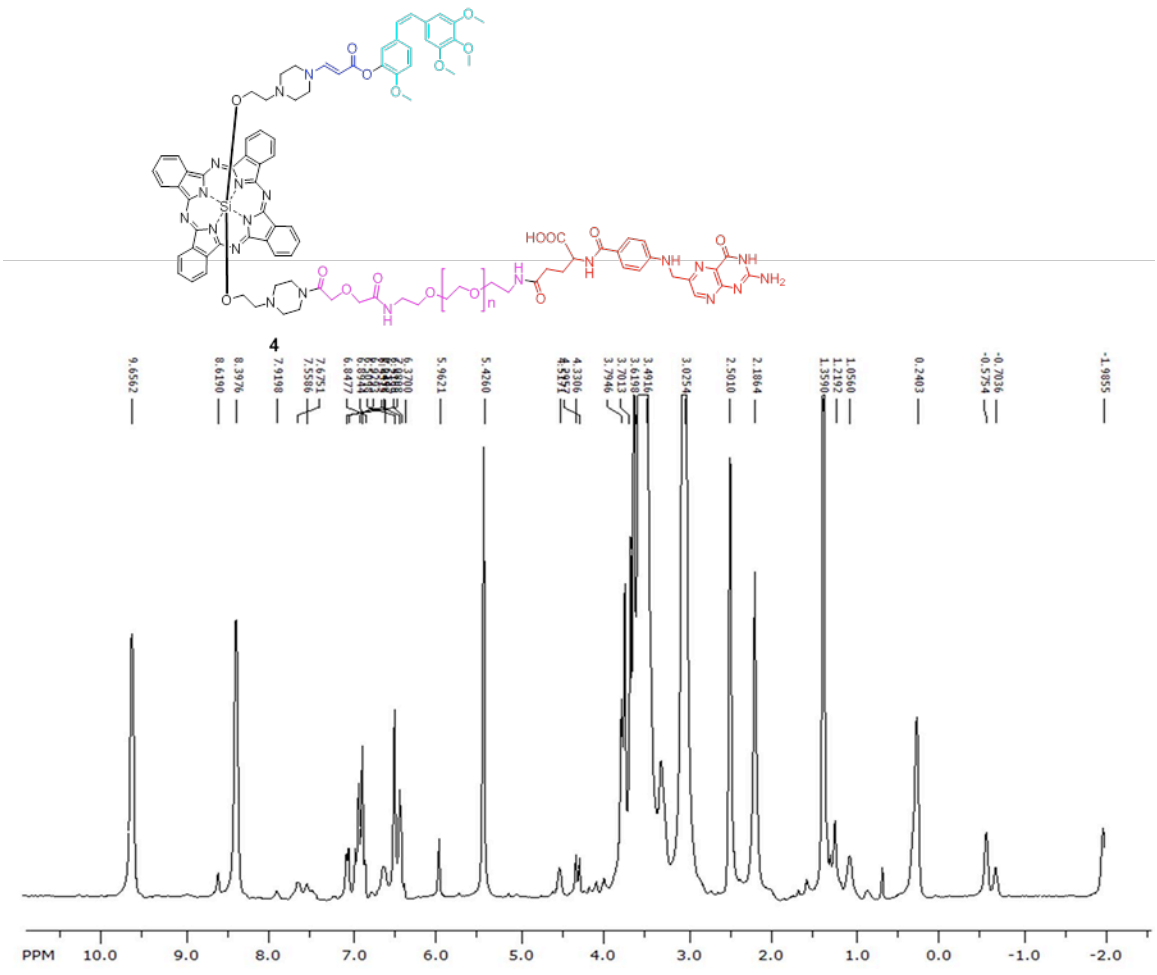


Figure S30: ^{1}H-NMR of prodrug **4**.

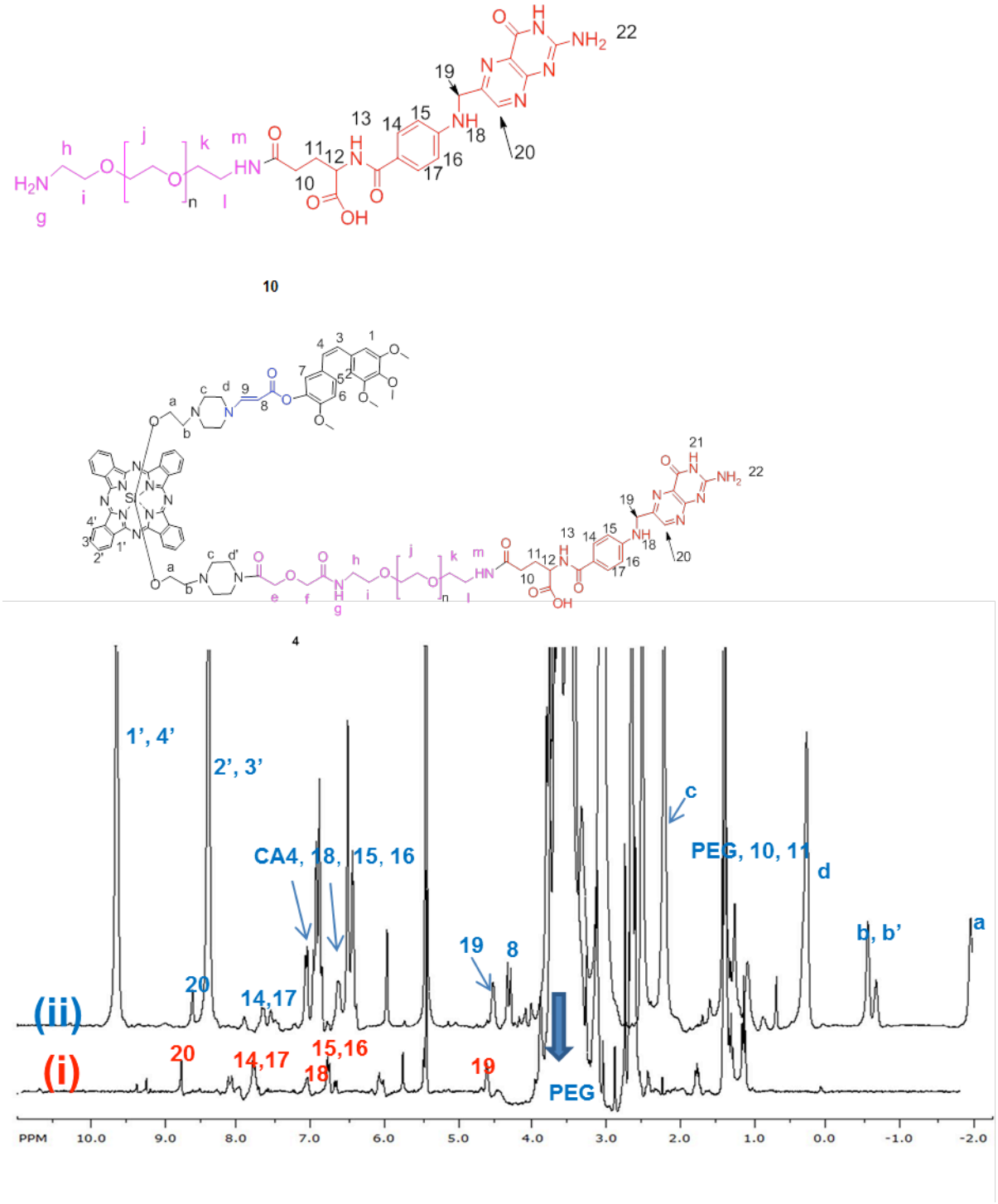


Figure S31. Assignment of peaks on the ^{1}H-NMR of (i) NH_2-PEG$_{\sim 45}$-FA and (ii) prodrug **4**. The peaks at 2.5, 3.05, and 5.39 ppm were from the following solvents: DMSO, H_2O, and DCM, respectively.

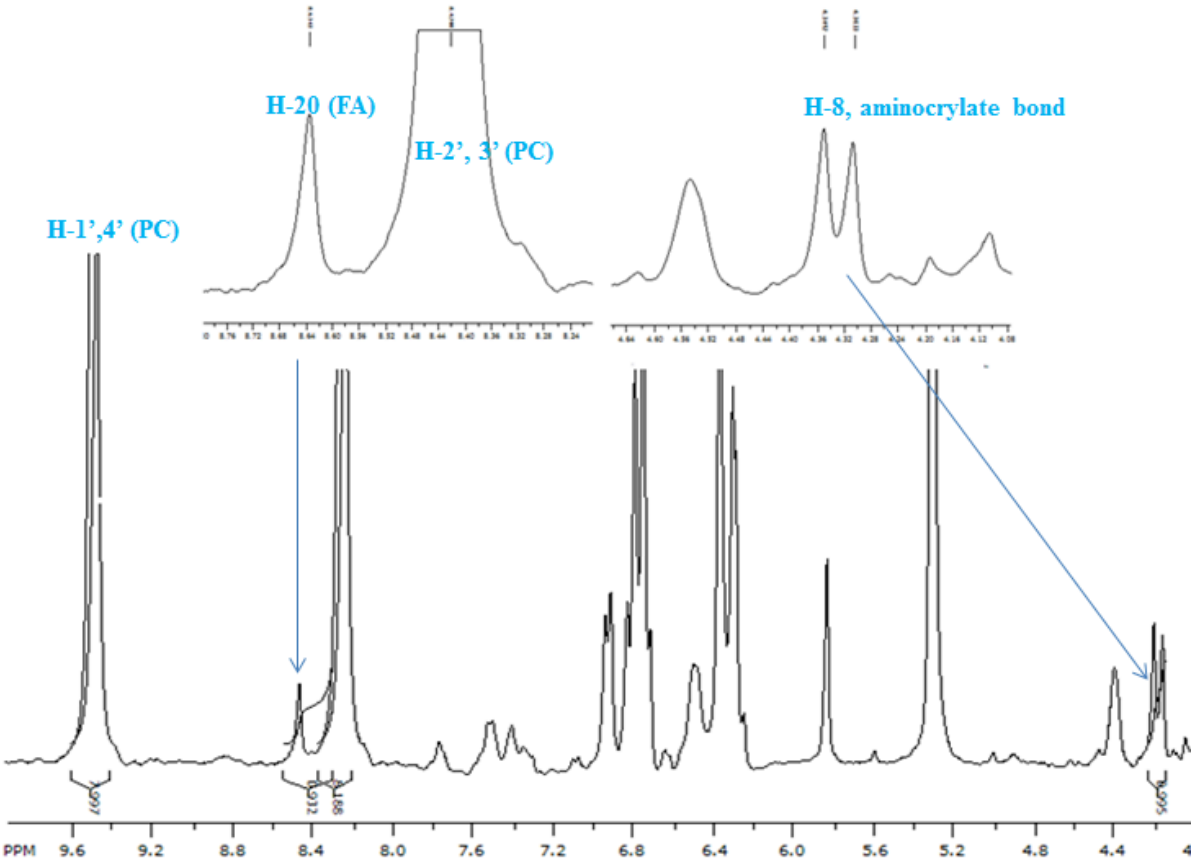


Figure S32: The integration of the ^{1}H-NMR peaks at 8.63 ppm (H-20) and 4.33 (H-8) ppm of FA and aminoacrylate bond respectively with respect to the peaks at 9.63 ppm (H-1', 4', 8H) and 8.39 ppm (H-2', 3', 8H) of the phthalocyanine (Pc) core. Based peaks indicate an almost 1:1 ratio (0.913 vs. 0.995) of the conjugated FA and CA4, thereby demonstrating a complete reaction between compounds **9** and **10** to produce the prodrug **4**. NB. These are the only two isolated peaks from compounds **9** and **10** that could be integrated with some degree of certainty. The rest of the peaks overlap with each other.

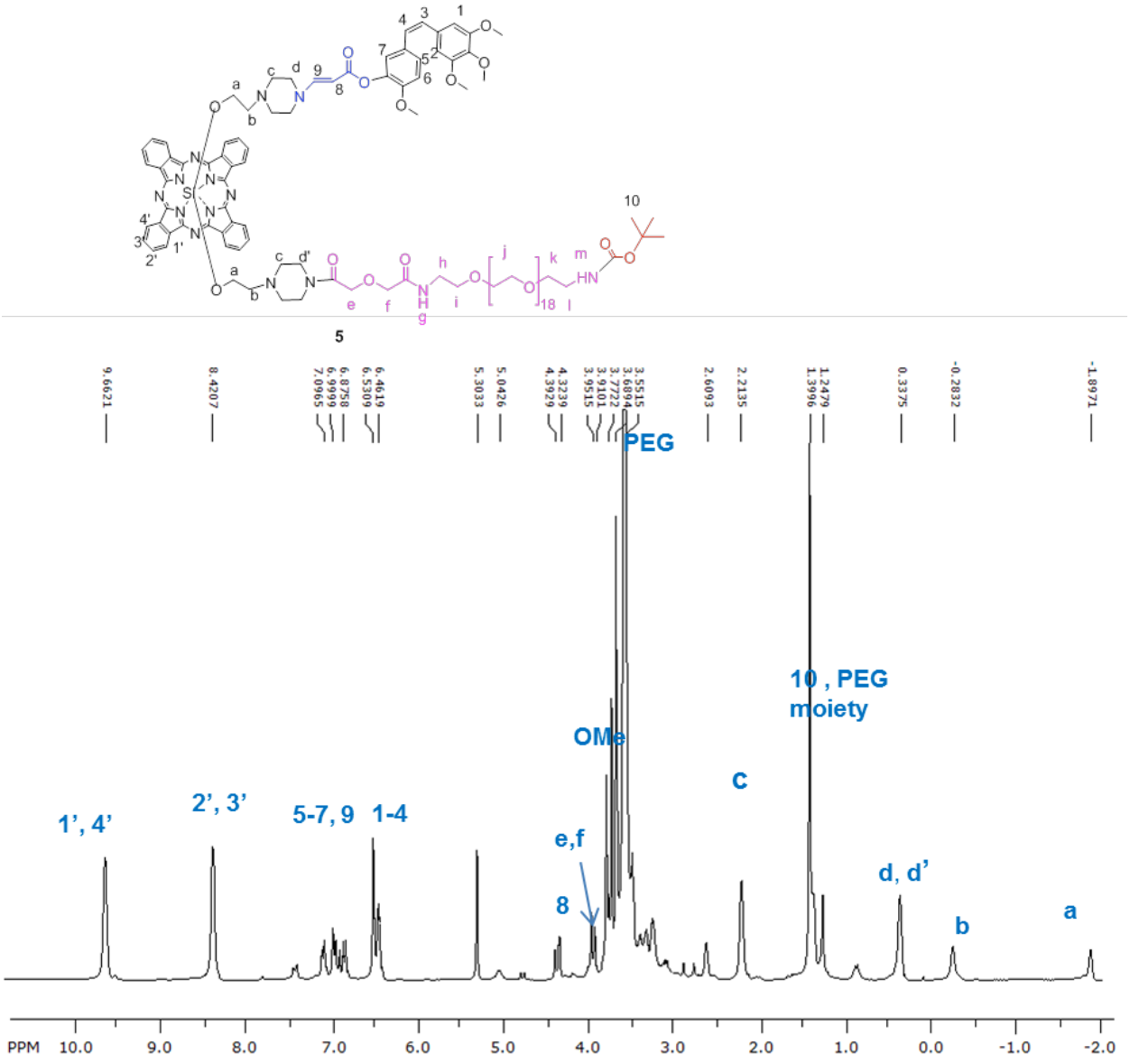


Figure S33. [1]H-NMR of Prodrug **5**. The peak at 5.30 ppm is the DCM peak.

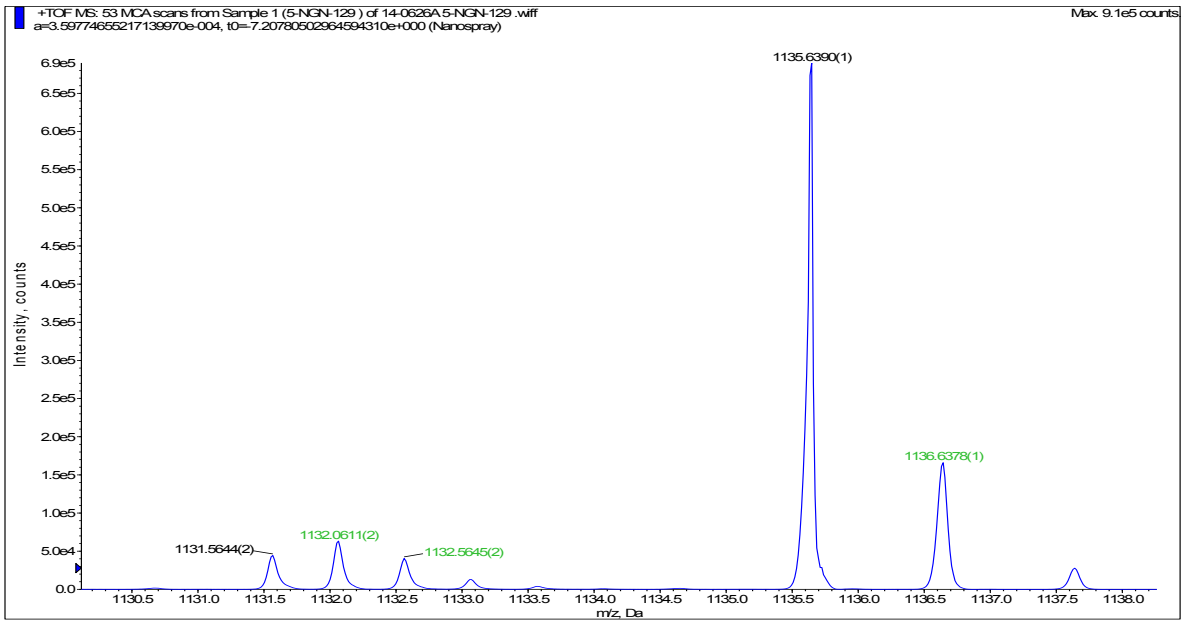
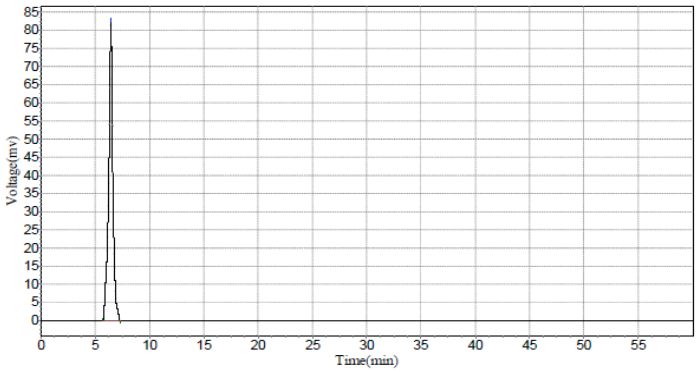


Figure S34. HRMS (TOF) of prodrug **5**. HRMS (ESI): m/z= 2261.0779 calcd for $[C_{114}H_{158}N_{14}O_{32}Si]^{+2}$ $[M+2H]^{+2}$: 1131.5468 (81.1%) and 1132.0462 (100%), found: 1131.5644 (81.1%) and 1132.0611(100%).



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		6.440	82370.523	2283782.500	100.0000
Total			82370.523	2283782.500	100.0000

Figure S35. HPLC chromatogram of prodrug **5**. Retention time 6.44 min. Method: Isocratic, solvent system: 60% ACN 40% H_2O, flow rate 0.5 mL/min.

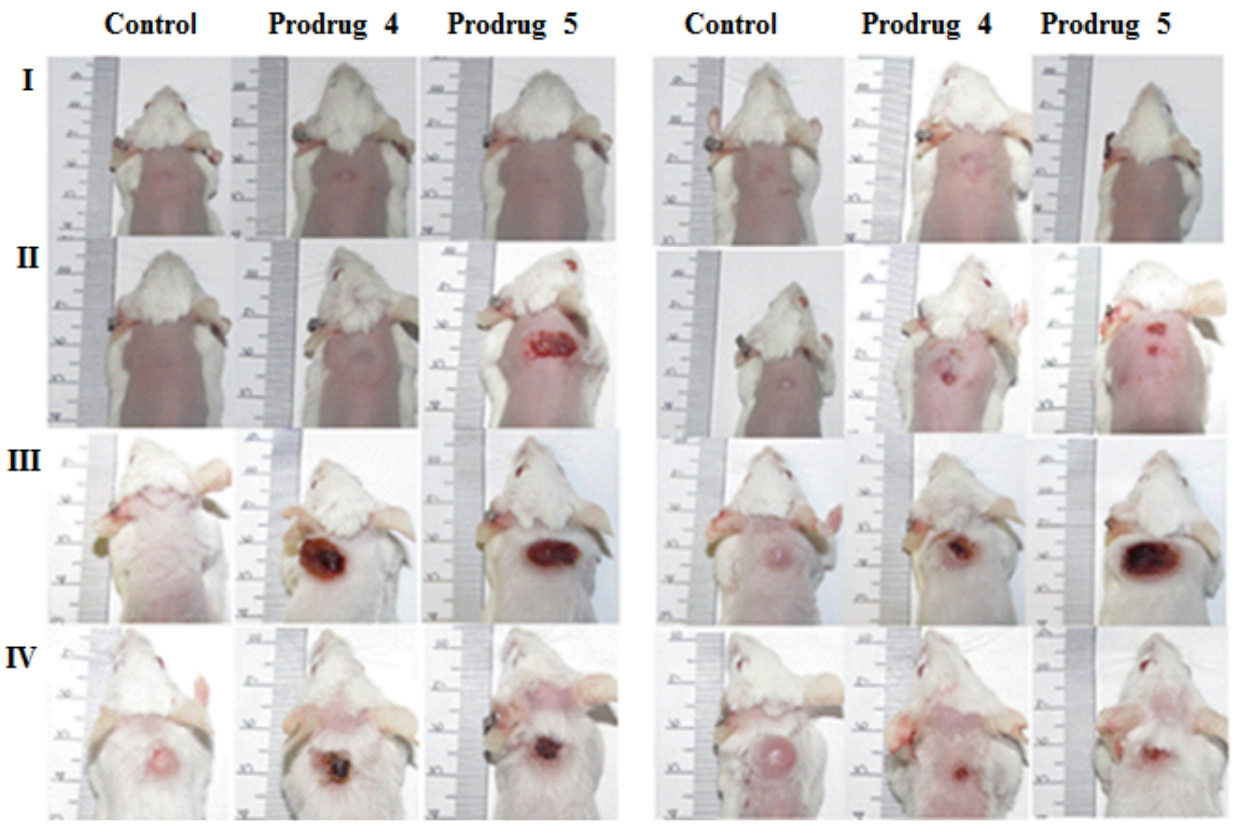


Figure S36. Photographic images of the other two groups of mice (3 mice per group) treated with prodrug (none, **4**, or **5** at 2 μmol/kg) with illumination (690 nm laser, 12 mm diameter circular beam, 100 mW/cm^2, 30 min,) 7 h post IV administration of the prodrug: (i) day 0 before illumination, (ii) day 1, (iii) day 6, and (iv) day 15 post-illumination.

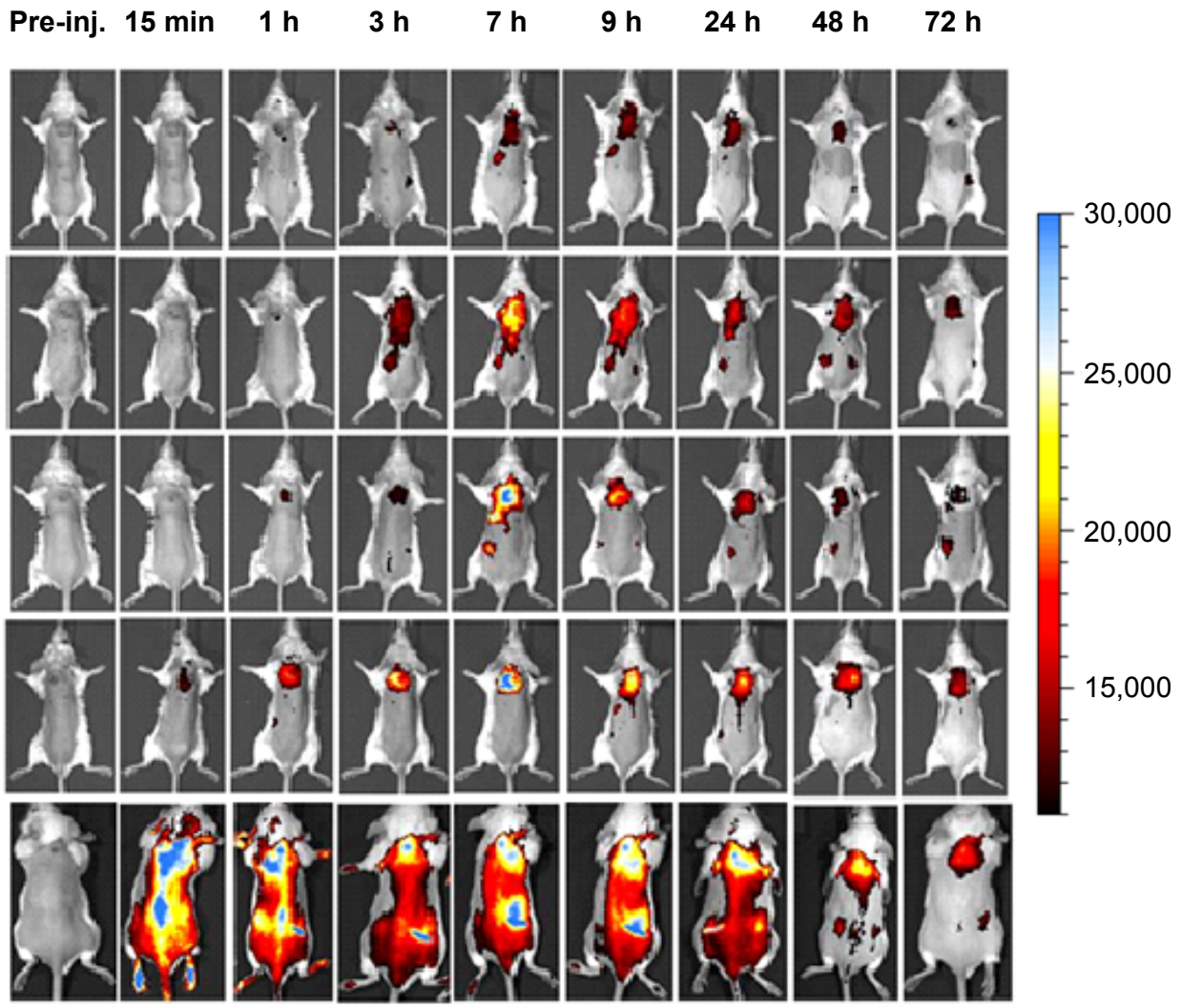


Figure S37. Additional data set of time-dependent preclinical fluorescence images of prodrugs **1**-**5** (Fig. 5A). Images of Balb/c mice bearing SC colon 26 tumors were taken before and at 0.25, 1, 3, 7, 9, 24, 48, and 72 h post IV injection of the prodrugs (2 μmol/kg).