

## Supporting Information

# PEGYLATED AND NANOPARTICLE-CONJUGATED SULFONIUM SALT PHOTO-TRIGGERS NECROTIC CELL DEATH

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**KEYWORDS:** *Oxygen-independent Photodynamic Therapy, Photoacid Generator, Silica Nanoparticles, Stimuli-responsive, Two-photon Microscopy*

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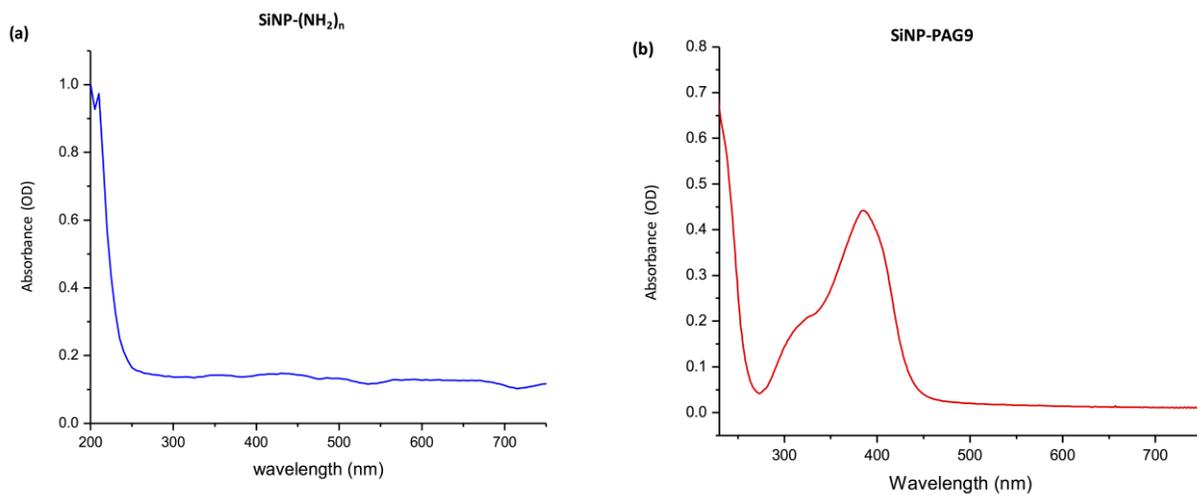


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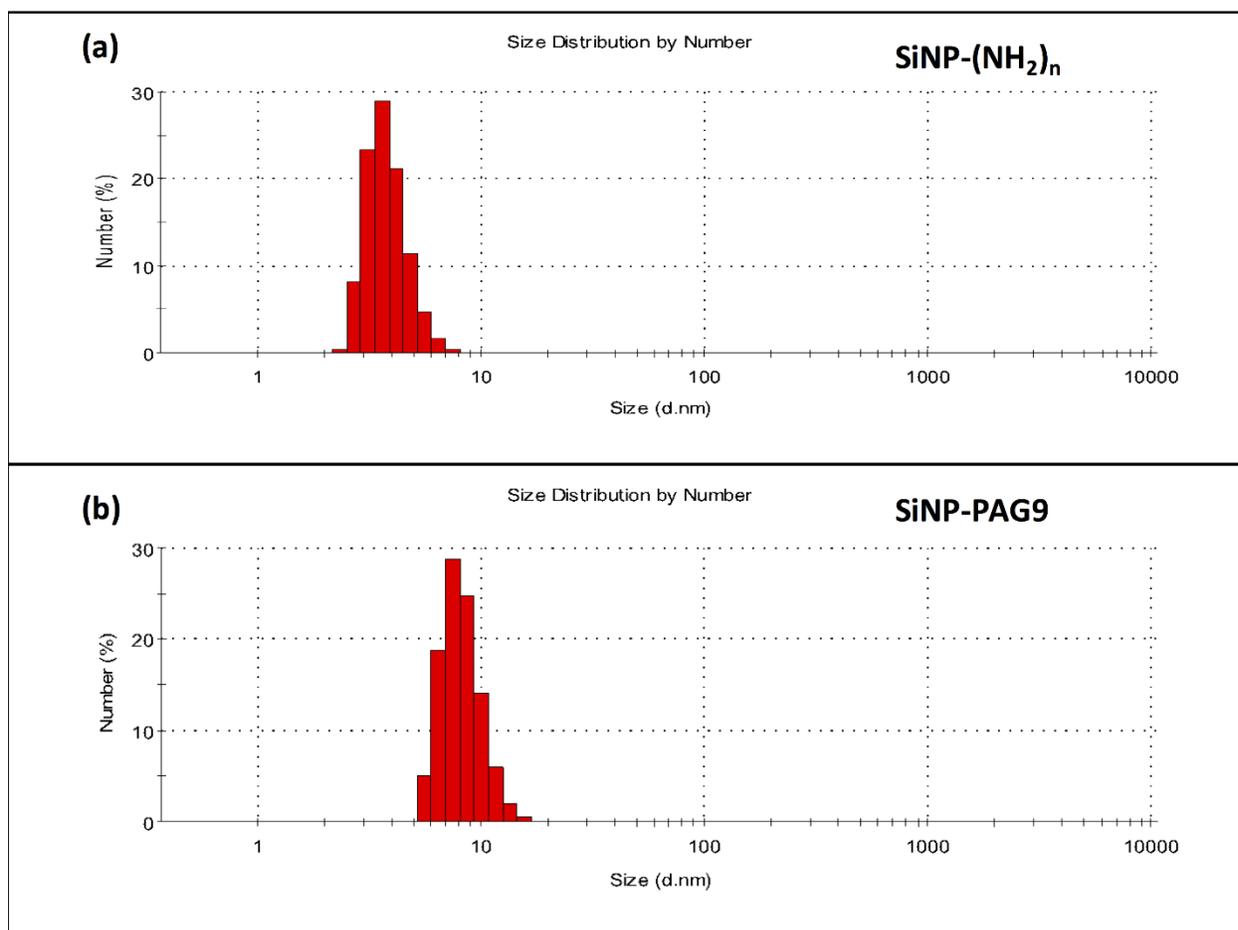


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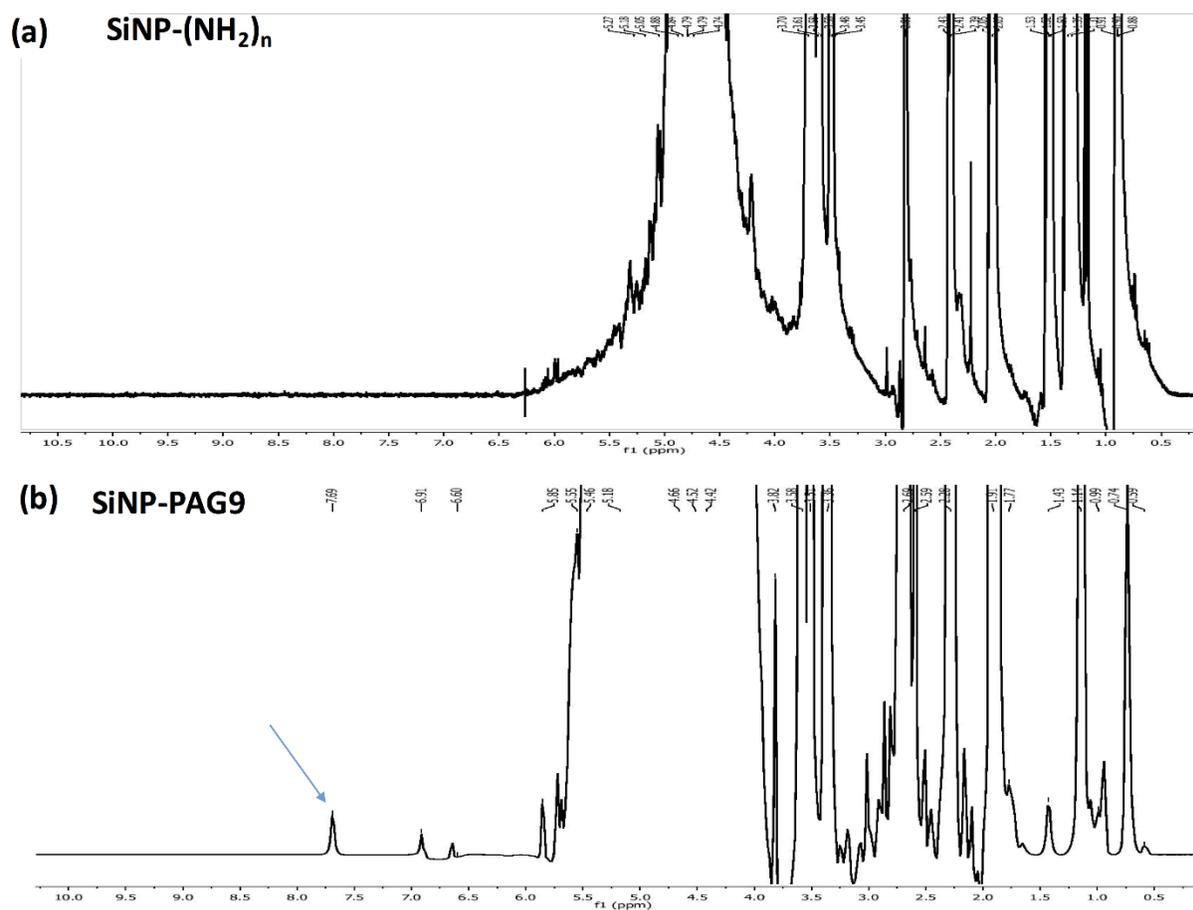


Figure S3. (a) <sup>1</sup>H NMR for Silica nanoparticles functionalized with amine (**SiNP-(NH<sub>2</sub>)<sub>n</sub>**). (b) <sup>1</sup>H NMR for Silica nanoparticles covalently bonded with compound 9 (**SiNP-PAG9**) in D<sub>2</sub>O.

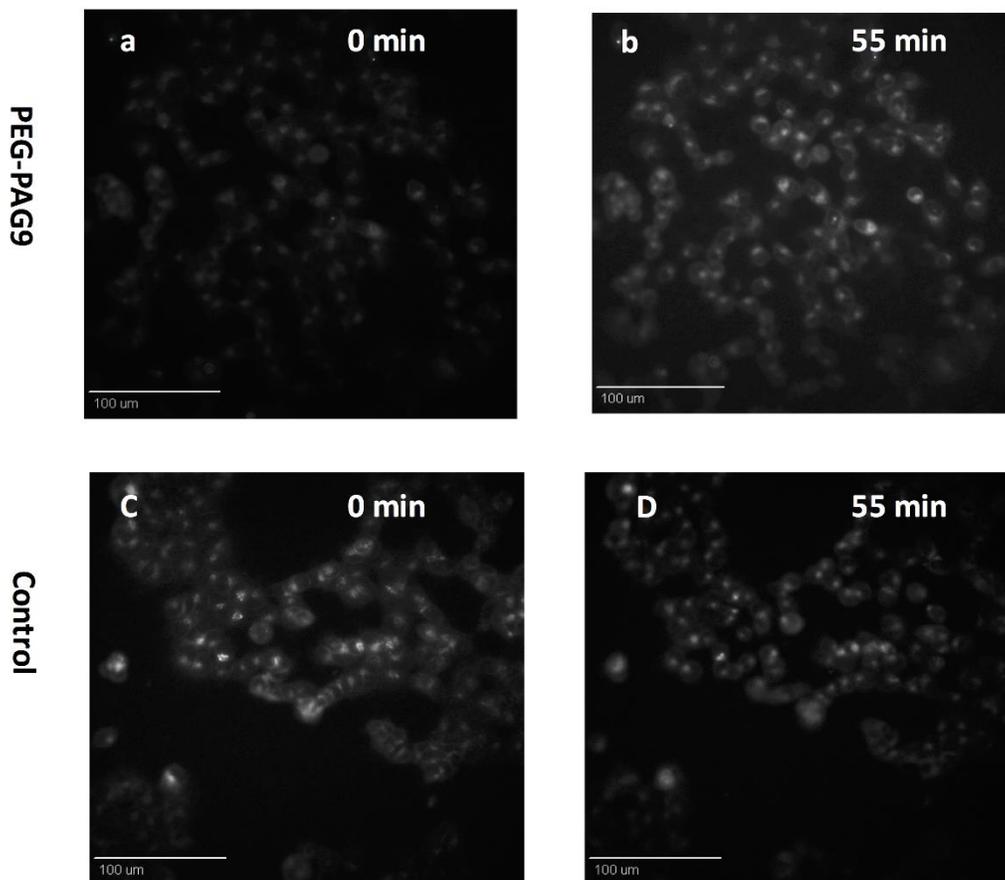


Figure S4. Acidic content increasing in the lysosomes as a function of exposure dose in HCT-116 cells incubated with **PEG-PAG9** (50  $\mu$ M) for 24 h and with 1  $\mu$ M of lysosensor Green 2 h. After 5 s irradiation using power light 5.4 mW/cm<sup>2</sup>. (a, b, c, d) cells were imaged with 1 min intervals time for 55 min with FITC channel. SlideBook was used to calculate the intensities at different time points.

## Synthesis

Phenyl(4-vinylphenyl) sulfane (**7**) was synthesized as previously reported [1]. All other chemicals and reagents were purchased from Aldrich or Acros Organics, and used as received unless otherwise noted.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic measurements were performed using Varian 400 or 500 NMR spectrometers at 400 or 400 MHz for  $^1\text{H}$  (referenced to TMS at  $\delta$ ) (0.0 ppm) and 125 or 75 MHz for  $^{13}\text{C}$  (referenced to  $\text{CDCl}_3$  at  $\delta$ ) 77.0 ppm). All glassware was flamed, dried, and cooled in a desiccator over calcium chloride. Most of reactions were carried out under  $\text{N}_2$  atmosphere. All sulfonium salt reactions and purifications were carried out under yellow light, red light, or in the dark

**Synthesis of 9-Ethyl-9H-fluorene (2):** Fluorine **1** (6.00 g, 36.01 mmol) was dissolved in dry THF (150 mL) under nitrogen gas and treated with n-BuLi (1.6 M in hexanes, 22.5 mL) at  $-78\text{ }^\circ\text{C}$  drop by drop over 30 min. After the addition complete, the temperature was raised to room temperature and the mixture was stirred for 1 h. The mixture was cooled to  $-78\text{ }^\circ\text{C}$  and a solution of ethylbromide (2.6 mL) was added. The temperature was allowed to rise to room temperature and mixture was left stirring for 24 h. The THF was evaporated from the orange solution in vacuum and then extracted with  $\text{CH}_2\text{Cl}_2$  yielding a yellow oil and purified by column chromatography using hexane to give 6.4 g (91% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.90 (d, 2H), 7.65 (d, 2H), 7.53 – 7.40 (m, 4H), 4.08 (t, 2H), 3.18 (d,  $J = 747.2$  Hz, 1H), 0.89 (t, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  147.31, 141.44, 126.98, 126.90, 124.41, 119.98, 119.87, 119.86, 77.48, 77.16, 76.85, 48.59, 25.83, 9.87.

**Synthesis of 3-(9-ethyl-9H-fluoren-9-yl)propanenitrile (3):** Compound **2** (6.00 g, 30.0 mmol) was dissolved in (28 mL) of dry *p*-dioxane at  $50\text{ }^\circ\text{C}$  temperature and degassed under vacuum and nitrogen. After that, 40% aqueous solution of Triton B (0.25 mL) was added, the mixture color was changed from light yellow to brown. This solution was then treated with acrylonitrile for 15 min. And the temperature was maintained around  $50\text{ }^\circ\text{C}$ . The resulting brown solution was stirred for 16 h. After which, it was neutralized with 10% HCl (aq) and extracted with  $\text{CH}_2\text{Cl}_2$ . The crude product was purified by column chromatography using 5:1 hexane/ethyl acetate to afford white solid (5.9 g mmol (77% yield) mp.  $99.2\text{--}100.0\text{ }^\circ\text{C}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.70 (d,  $J = 1.1$  Hz, 2H), 7.34 (dd,  $J = 3.6, 1.1$  Hz, 6H), 2.41 (m, 2H), 2.03(t, 2H), 1.49 (t,  $J = 4.0$  Hz, 2H), 0.31 (t, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  147.26, 141.43, 127.92, 127.81, 127.64, 122.83, 120.22, 119.88, 77.48, 77.16, 76.84, 54.87, 35.65, 32.85, 12.27, 8.30. HRMS (ESI-TOF)  $m/z$ :  $[\text{M} + \text{H}]^+$  Calculated for  $\text{C}_{18}\text{H}_{17}\text{N}$  248.1361; Found 248.1438.

**Synthesis of 3-(9-ethyl-9H-fluoren-9-yl)propanoic acid (4):** (4.00 g, 17.1 mmol) of compound **3** was dissolved in ethylene glycol (150 mL). (4 M, 50 mL) of KOH was added and heated to reflux for 16 h. After that, ethylene glycol was removed by vacuum distillation to afford yellow oil which was neutralized with 10% HCl (aq) and  $\text{CH}_2\text{Cl}_2$  was used for extraction. The crude product purified by column chromatography hexane/ethyl acetate (3:1) to produce (3.65 g) (85 % yield) mp.  $134.8\text{--}135\text{ }^\circ\text{C}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 6.7$  Hz, 2H), 7.44 – 7.17 (m, 6H), 2.56 – 2.24 (m, 2H), 2.06 (q,  $J = 7.3$  Hz, 2H), 1.68 – 1.49

(m, 2H), 0.34 (t,  $J = 7.3$  Hz, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  179.81, 148.45, 141.35, 127.35, 127.35. HRMS (ESI-TOF)  $m/z$ : [M-H]<sup>-</sup> Calculated for  $\text{C}_{18}\text{H}_{18}\text{O}_2$  265.1307; Found 265.1247

**Synthesis of compound 3-(9-ethyl-2-nitro-9H-fluoren-9-yl)propanoic acid (5):** (3.00 g, 11.9 mmol) of compound **4** was dissolved in glacial acetic acid (25 mL). The resulting solution was heated to 55 °C and (2 mL) of 70% Nitric acid was added drop wise. After the addition was complete, the mixture was stirred for 3 h and monitored by TLC. The reaction was stopped by adding 10 mL water and extracted with  $\text{CH}_2\text{Cl}_2$ . The afforded yellow oil was purified by column chromatography 3:1 hexane/ethyl acetate to give yellow solid (2.8 g,) (79% yield). mp. 134.8-136 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.50 – 8.17 (m, 2H), 7.92 – 7.76 (m, 2H), 7.59 – 7.40 (m, 3H), 2.64 – 2.33 (m, 2H), 2.26 – 2.03 (m, 2H), 1.79 – 1.45 (m, 2H), 0.34 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  179.39, 150.13, 149.79, 147.82, 147.25, 139.00, 129.63, 128.03, 123.81, 123.29, 121.46, 120.07, 118.50, 77.39, 77.07, 76.75, 55.35, 33.87, 32.72, 28.90, 8.25. HRMS (ESI-TOF)  $m/z$ : [M-H]<sup>-</sup> Calculated for  $\text{C}_{18}\text{H}_{17}\text{NO}_4$  310.1158; Found 310.1073.

**Synthesis of 3-(9-ethyl-2-iodo-7-nitro-9H-fluoren-9-yl)propanoic acid (6):** (2.00 g, 6.7 mmol) of compound **5** was dissolved in (30 mL) glacial acetic acid and placed into three neck flask fitted with condenser, nitrogen gas and thermometer. The mixture was degassed with nitrogen for 15 min. then  $\text{I}_2$  (850 mg, 3.3 mmol) was added and the resulting orange solution was stirred for 30 min. After that, (924 mg, 13.4 mmol) of  $\text{NaNO}_2$  followed by concentrated  $\text{H}_2\text{SO}_4$  (1.5 mL) was added and the reaction heated to 125 °C and monitored by TLC for 3 h. After the reaction had completed, the reaction was cooled down and extracted with ethyl acetate. The resulting yellow oil was purified by column chromatography using 3:1 hexane/ ethyl acetate to give a yellow solid (2.52 g) (90% yield), mp. 181-182 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.28 (d,  $J = 8.4, 2.1$  Hz, 1H), 8.20 (s,  $J = 3.2$  Hz, 1H), 7.86 – 7.73 (m, 3H), 7.54 (d,  $J = 8.6$  Hz, 1H), 2.60 – 2.33 (m, 2H), 2.24 – 1.97 (m, 2H), 1.74 – 1.47 (m, 2H), 0.33 (t,  $J = 7.3$  Hz, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  179.58, 152.56, 149.59, 147.98, 147.08, 138.95, 137.59, 133.01, 124.29, 123.26, 120.65, 118.89, 96.15, 77.73, 77.41, 77.09, 55.93, 34.06, 32.98, 29.27, 8.60. HRMS (ESI-TOF)  $m/z$ : [M-H]<sup>-</sup> Calculated for  $\text{C}_{18}\text{H}_{16}\text{INO}_4$  436.0124; Found 436.0046.

**Synthesis of 3-(9-ethyl-2-nitro-7-(4-(phenylthio)styryl)-9H-fluoren-9-yl)propanoic acid (8):** In 2 mL of dry DMF (500 mg, 1.18 mmol) of compound **6** and (200 mg, 0.94 mmol) of compound **7** (phenyl (4-vinylphenyl) sulfane) were dissolved with 0.01g, (10% molar) of Palladium acetate, and (1.7 mL) of triethylamine. The resulting brown solution was purged with nitrogen for 10 min. while stirred. The microwave was adjusted to closed vessel standard mode; maximum temperature 160 °C; maximum pressure 250 psi; maximum power 60 W, and high speed stirring. The run time (a time at which the reaction approaches maximum temperature or pressure) was adjusted for 2 min. and the hold time 30 min. the reaction was monitored by TLC. After starting material was gone, the mixture cooled down and celite plug was used to filter it. The filtrate was extracted with  $\text{CH}_2\text{Cl}_2$  and washed with water 4x, dried with anhydrous

magnesium sulfate and concentrated to afford brown oil which was purified by column chromatography using hexane/ethyl acetate (2:1) to give a yellow crystal (485mg) (99%). mp. 81-82 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.29 (d, *J* = 8.4, 2.1 Hz, 1H), 8.22 (s, *J* = 1.8 Hz, 1H), 7.79 (q, *J* = 8.1, 6.1 Hz, 2H), 7.42 (m, *J* = 14.4, 13.5, 9.2, 7.6, 3.5 Hz, 11H), 7.18 (s, 2H), 2.46 (m, *J* = 8.2 Hz, 2H), 2.24 – 2.10 (m, 2H), 1.62 (t, *J* = 13.2 Hz, 2H), 0.37 (t, *J* = 7.3 Hz, 3H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 178.16, 150.76, 149.98, 147.45, 147.12, 138.77, 138.59, 135.95, 135.68, 135.19, 131.49, 131.45, 130.80, 129.31, 129.06, 128.50, 127.38, 127.30, 126.78, 123.94, 121.73, 120.96, 119.97, 118.46, 77.33, 77.22, 77.01, 76.70, 55.33, 33.93, 32.82, 28.73, 8.30. HRMS (ESI-TOF) *m/z*: [M-H]<sup>-</sup> Calculated for C<sub>32</sub>H<sub>27</sub>NO<sub>4</sub>S 520.1661; Found 520.1592.

**Synthesis of 4-(2-(9-(2-carboxyethyl)-9-ethyl-7-nitro-9H-fluoren-2-yl)vinyl)phenyl) Diphenyl**

**sulfoniumhexafluorophosphate (9)**: (370 mg, 0.7101 mmol) of compound **8** was dissolved in (2 mL) of chlorobenzene in a (2 mL) glass reaction vessel, 302.8 mg, (0.711 mmol) of diphenyliodonium hexafluoro phosphate and 0.005 g (5% molar) of copper (II) benzoate were mixed in the dark while purged with nitrogen for 10 min. the microwave was adjusted to closed vessel standard mode; maximum temperature 125 °C, maximum pressure 40 psi; maximum power 100 W, high speed stirring. The run time (the time at which the reaction approaches max temperature or pressure) was set for 1 min. and the hold time 15 min. TLC was used to monitor the reaction every 2 min. upon the completion the solvent was evaporated to produce a brown crude product. The resulting crude was purified by column chromatography using DCM/Methanol 9:1 to afford a yellow solid crystal (470 mg) (87%) mp. 63-64 °C. <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.28 (s, *J* = 2.9 Hz, 1H), 8.25 (d, *J* = 2.0 Hz, 1H), 8.15 – 7.97 (m, 4H), 7.93 – 7.75 (m, 14H), 7.66 (d, *J* = 16.3 Hz, 1H), 7.57 (d, *J* = 16.4 Hz, 1H), 2.19 (m, *J* = 30.8, 14.6, 7.7 Hz, 4H), 1.49 – 1.08 (m, 2H), 0.23 (t, *J* = 7.3 Hz, 3H). <sup>13</sup>C NMR (101 MHz, DMSO) δ 175.81, 152.84, 152.07, 147.67, 143.97, 139.85, 138.24, 135.08, 134.23, 132.65, 132.15, 131.98, 129.64, 128.02, 127.73, 126.26, 124.24, 123.31, 122.85, 122.30, 121.48, 119.12, 116.14, 56.32, 36.17, 32.17, 9.11. HRMS (ESI-TOF) *m/z*: [M+H]<sup>+</sup> Calculated for C<sub>38</sub>H<sub>32</sub>NO<sub>4</sub>S<sup>+</sup> 598.2047; Found 598.2073.

**Synthesis of 4-(2-(9-ethyl-9-(3-((3-methoxypropyl)amino)-3-oxopropyl)-7-nitro-9H-fluoren-2-yl)vinyl)phenyl)diphenylsulfonium (PEG-PAG 9)**

Benzotriazole (64 mg, 0.005 mmol) was dissolved in (10 mL) dry DCM in 25 mL round bottom flask while it was purged with nitrogen and stirred at room temperature until the solution became clear. After that 0.1 mL of thionyl chloride was added. After the addition had completed, the solution was treated with (100 mg, 0.13 mmol) of compound **9** and the reaction was stirred for another 2 h. Then, 1 equivalent mmol of polyethyleneglycole (PEG) was added and stirred for 3 h. the DCM was evaporated to give crude dark yellow oil. The afford crude was purified by column chromatography 5:1 DCM/ Methanol to produce brown oil (165 mg), (70% yield). <sup>1</sup>H NMR (400 MHz, MeOD) δ 8.29 – 8.13 (m, 2H), 7.91 (dd, *J* = 18.5, 9.2 Hz, 4H), 7.80 – 7.61 (m, 14H), 7.51 (d, *J* = 10.1 Hz, 1H), 7.27 (d, *J* = 4.4 Hz, 1H), 3.76 – 3.15 (m, 96H), 2.51 – 2.33 (m, 2H), 2.20 – 2.00 (m, 2H), 1.39 (dd, *J* = 17.1, 9.1 Hz, 2H), 0.22 (t, *J* = 6.0 Hz, 3H). <sup>13</sup>C NMR (101 MHz, MeOD) δ 174.10, 151.58, 151.00, 147.80, 147.64,

144.50, 140.17, 138.15, 134.77, 134.02, 131.88, 131.71, 131.15, 129.33, 127.71, 126.96, 125.54, 123.77, 122.38, 122.10, 121.95, 120.56, 118.72, 71.89, 70.46, 70.41, 70.34, 70.27, 70.07, 69.24, 58.03, 55.97, 48.64, 48.57, 48.43, 48.36, 48.22, 48.15, 48.00, 47.93, 47.72, 47.51, 47.29, 39.20, 34.76, 32.69, 30.71, 7.58. HRMS (ESI-TOF) m/z: [M- PF<sub>6</sub>]- Calculated for C<sub>41</sub>H<sub>39</sub>N<sub>2</sub>O<sub>4</sub>S<sup>+</sup> 1623.8026; Found 1623.8461.

**Synthesis of amine-terminated silica nanoparticles SiNP(NH<sub>2</sub>)<sub>n</sub>:** Synthesis of (SiNP(NH<sub>2</sub>)<sub>n</sub>) was performed following the method described by P.N. Prasad et al. with some modifications [2]. The synthesized silica nanoparticles and their bioconjugates with compound **9** were schematically described by **Scheme 1**. Briefly, the nanoparticles were synthesized by adding polymeric organically modified silica solution into the Tween-80/ 1-butanol micelle solution dissolved in deionized water. First, 0.5 g of Triethoxyvinylsilane (VTES, 97%) was dissolved in 5 mL of N-methyl-2-pyrrolidone (NMP) and condensed in the presence of 200 µL of ammonium hydroxide (28–30%) at room temperature with 16 h stirring in order to get a clear solution of prepolymerized silica solution. After that, the prepolymerized silica solution was filtered using a membrane filter (0.2 µm pore size). Micelles were prepared by mixing 0.1 g of Tween-80 and 0.2 mL of 1-butanol in 5 mL of deionized water. 100 µL of silica solution was mixed with 0.2 mL of NMP and then added to the prepared micelle dispersions drop by drop to induce nanoprecipitation under vigorous stirring. After 10 min, 20 µL of (3-aminopropyl)triethoxysilane (APTES) was added in order to functionalize the surface of SiNPs. The mixture was stirred for further 24 h in order to be sure the sol-gel condensation within the co precipitation nanoparticles was complete. Purification of nanoparticles was performed by dialyzing the dispersion against deionized water in a 10 KDa cutoff cellulose membrane to remove Tween-80 and 1-butanol for 48 h. The solution was then filtered with a 0.2 µm cutoff membrane filter.

**Conjugation of amine-terminated silica nanoparticles SiNP-PAG9:** A 0.2 mL of 0.1 M EDC solution was added to compound **9** (3.5 mg) dissolved in 0.5 mL of NMP. The mixture was stirred for 25 min and then 5 mL of stock solution of amine-terminated silica nanoparticles was added. The reaction between amine groups with PAG was allowed to proceed for 3 h. After that, the reaction mixture was dialyzed against deionized water in the dark for 48 h in order to remove unreacted molecules. Finally, the sample was filtered with a 0.2 µm cutoff membrane filter and stored at 5 °C for later use.

#### **Cell Culture:**

Cells HCT-116 (ATCC, USA) were cultured in RPMI-1640 medium, supplemented with 10% FBS, 1% penicillin-streptomycin, at 37 °C in a 95% humidified atmosphere containing 5% CO<sub>2</sub>.

#### **Photocytotoxicity Assay:**

In 96-well black wall clear bottom plates (Corning, USA) HCT-116 cells were seeded at 8 × 10<sup>4</sup> cells/well and incubated for 48 hours. For dark cytotoxicity experiment, **PEG-PAG9** or **SiNP-PAG9** was diluted to 1.5 µM, 3.12 µM, 6.25 µM, 12.5 µM, 25 µM, 50 µM, and 100 µM from the stock solution. Cells

were then incubated with diluted PEG-PAG9 or SiNP-PAG9 for another 24 hours. Cell viability was determined with CellTiter 96 AQueos One Solution Reagent (Promega, USA). For photocytotoxicity experiment, 50  $\mu\text{M}$  of **PEG-PAG9** or 10  $\mu\text{M}$  **SiNP-PAG9** was added to the HCT-116 Cells. The 96-well black wall plate was placed on an inverted microscope (Olympus IX81) coupled with a 100 W mercury lamp. The distance between the bottom of the plate and objective was set at 1 cm in order to irradiate the whole well by the UV light. A customized filter cube was employed (Ex 377/50, DM 409, Em 460/50) to excite PAG. The final power reaching the plate was 5.4 mW/cm<sup>2</sup>. Different irradiation times were used (0 min, 1 min, 5 min, and 10 min). After irradiation, the plate was incubated for another 24 hours before the cell viability was measured.

#### ***Live Cell Imaging of PEG-PAG9 and SiNP-PAG9***

In 40 mm Poly-D-lysine functionalized coverslips, cells were cultured for 48 hours. After that, **PEG-PAG9** at 50 $\mu\text{M}$  or **SiNP-PAG9** 10 $\mu\text{M}$  concentration was added to the cells. 24 hours later, coverslip was washed with PBS twice and set onto a bioptics live cell imaging chamber. After irradiation with UV lamp for 2000 s (5.4 mW/cm<sup>2</sup>) cells were imaged with confocal microscope (Olympus IX-81) at 1 min interval for 4 hours by using DIC channel. Cells were kept in 37 °C RPMI-1640 whole culture medium with 5% CO<sub>2</sub>, while imaging.

#### ***Calculation of the increasing in acidity in lysosomes***

In order to calculate the increasing in the acidity of lysosomes, cells were incubated with **PEG-PAG9** at 50 $\mu\text{M}$  concentration for 24 hours. Then 2mM of LysoSensor Green (Invitrogen, USA) was added for another 2 hours. After 2000 ms irradiation, cells were imaged at 1 min interval for 60 min with FITC filter cube (Ex 477/50, DM 507, DM 536/40) to collect fluorescence of LysoSensor Green. The fluorescence intensities were calculated at different time points with SlideBook. Cells were kept in 37 °C RPMI-1640 whole culture medium with 5% CO<sub>2</sub> while imaging.

#### ***Estimation of Lysosomal pH Drop***

The pH drop in Lysosome was measured according to method reported by [3]. A solution of **PEG-PAG9** (50 $\mu\text{M}$ ) and Rh B Base (0.1mM) in DCM mixed in the dark and exposed to generate a dose dependent calibration curve by assuming the number of acid molecules generated the same as the number of Rh B Base molecules converted to Rh B+.

#### ***Photophysical characterizations:***

##### ***Absorption spectra:***

Absorption spectra were recorded by using Agilent 8453UV-vis spectrophotometer. Steady state fluorescence spectra were measured by a EDINBURGH INSTRUMENTS FLS980 spectrofluorimeter.

##### ***Fluorescence Quantum Yield Measurement:***

Relative to Rhodamin 6G (Rh6G) in ethanol as a standard, fluorescence quantum yield was determined. The measurement was made in the photon-counting regime of a PMT by using an L-format configuration using EDINBURGH INSTRUMENTS FLS980 spectrofluorimeter. The measurement was performed at room temperature in 1 cm quartz cuvettes with concentration  $1 \times 10^{-6}$  M.

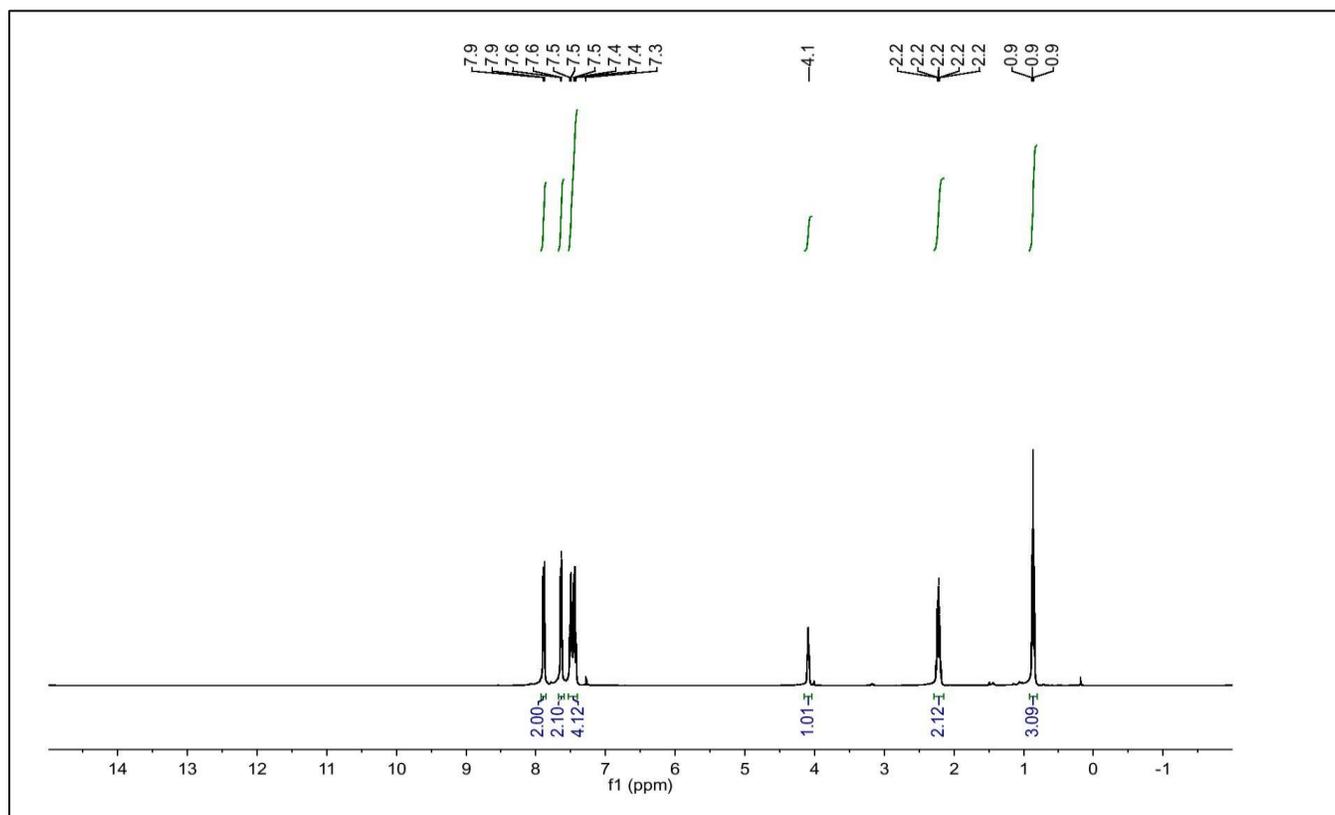
### **Photoacid Quantum yield Measurement**

Photoacid quantum yield was measured by using selective exciting PAG solution at the suitable wavelength using LOCTITE. The power of the incident light was measured by using Ophir Power Star power meter which equipped with a UV 1.44 cm<sup>2</sup> detector head. To calculate the quantum yield Rhodamine B base was used as a sensor for the photoacid generator, and observed the change on the optical density of sulfonium salt with not exceed 5% (17). The quantum yield for photoacid generator was calculated by using the following equation:

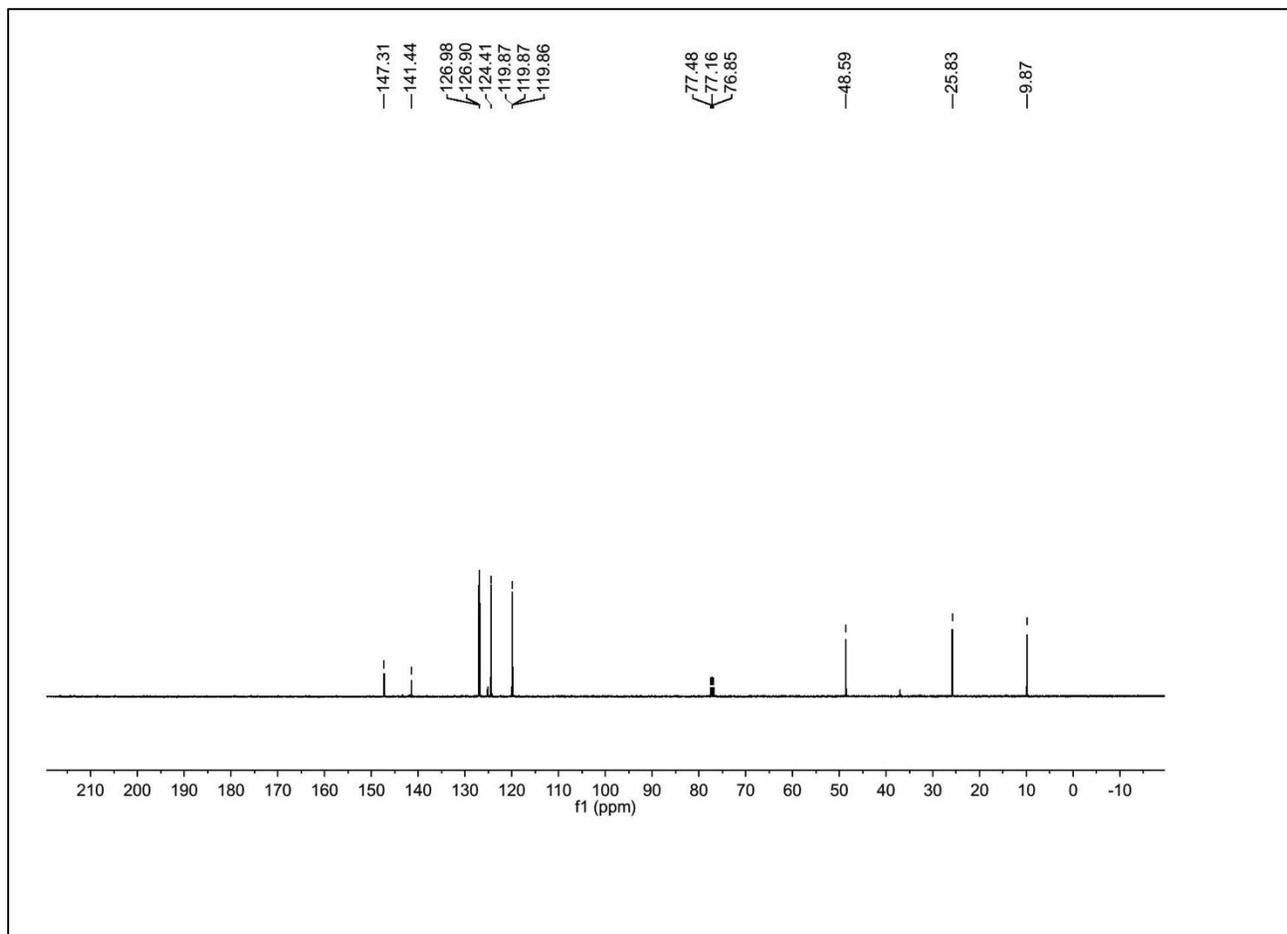
$$\phi_H = \frac{\Delta OD_{555} \cdot N_A}{10^3 \cdot \epsilon_{555}^{RhB} \cdot I_o \cdot [1 - 10^{-\bar{D}}] \cdot \Delta t}$$

where  $\Delta OD^{555}$  is the change in the optical density during the generation of Rhodamine B measured at 555nm,  $N_A$  is the Avogadro number,  $\epsilon_{555}^{RhB}$  is the extinction coefficient of rhodamine B at 555 nm,  $\bar{D}$  is the average absorption intensity,  $I_o$  is the Intensity of irradiation (photon/cm<sup>2</sup>.sec), and  $\Delta t$  is the time of irradiation in sec.

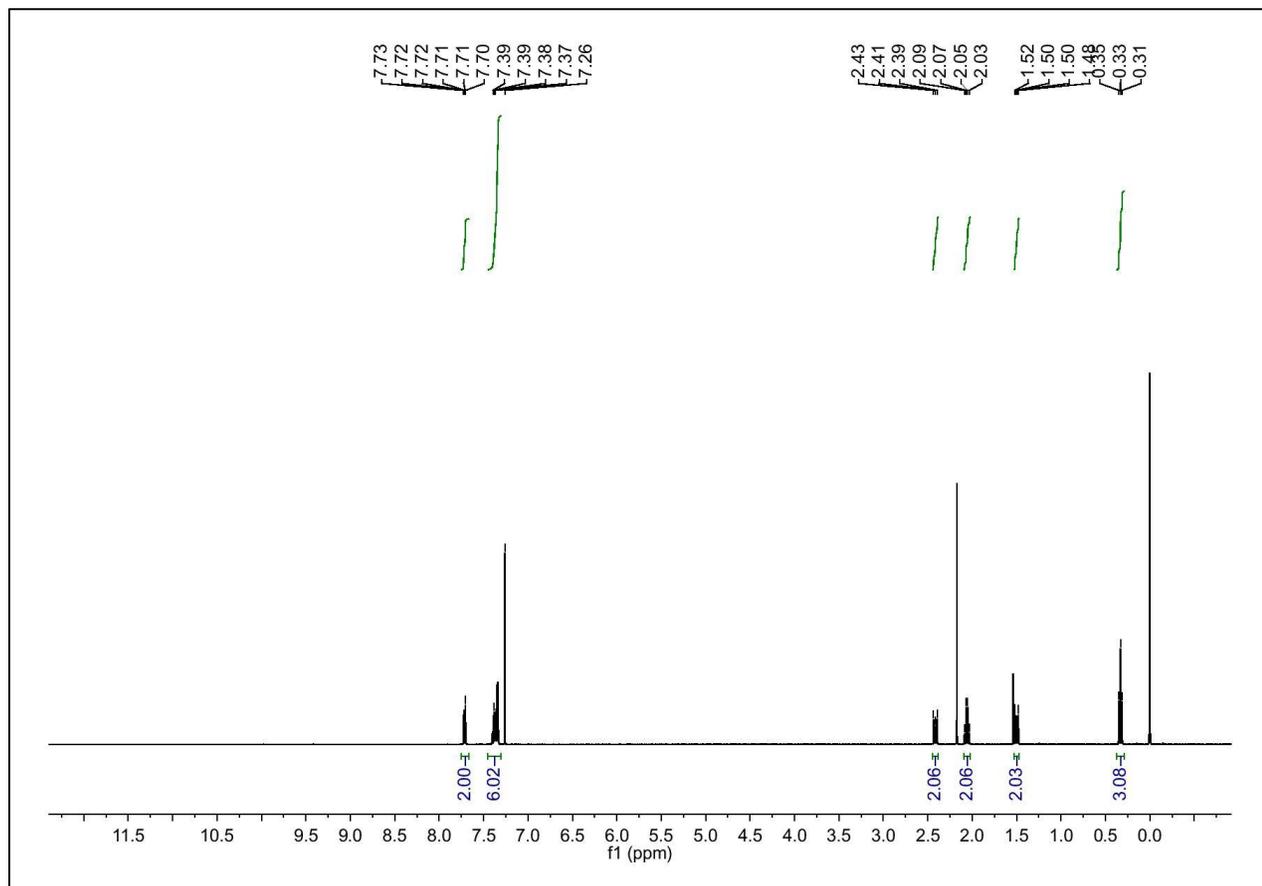
# <sup>1</sup>H NMR of 2



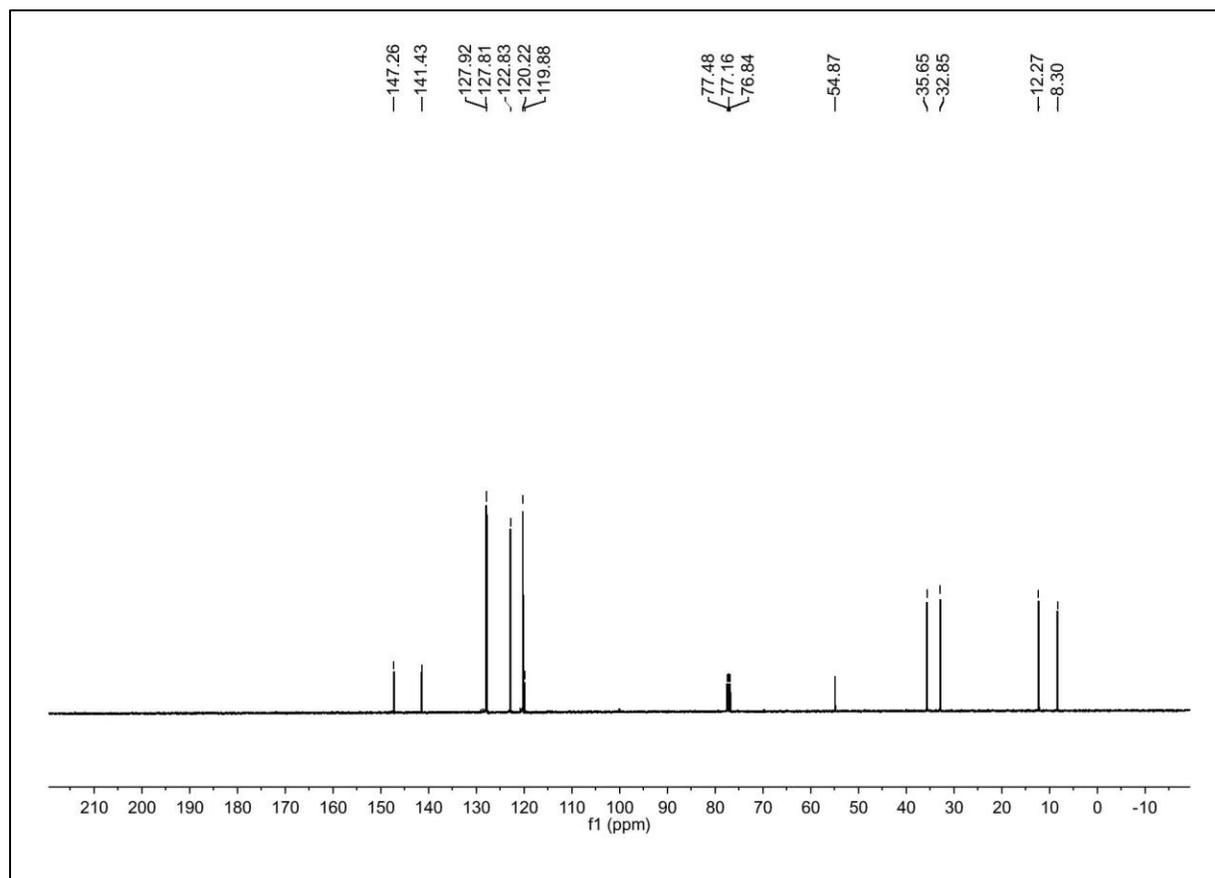
### $^{13}\text{C}$ NMR of 2



# <sup>1</sup>H NMR of 3

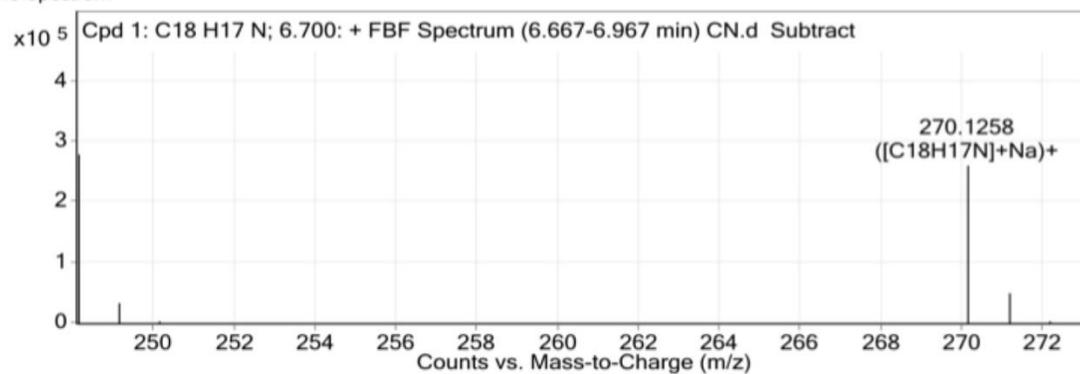


### $^{13}\text{C}$ NMR of 3

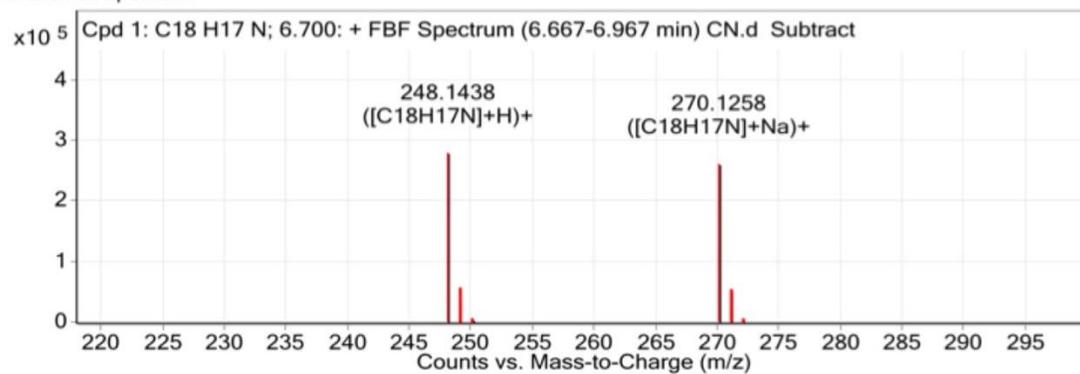


### LC-MASS OF 3

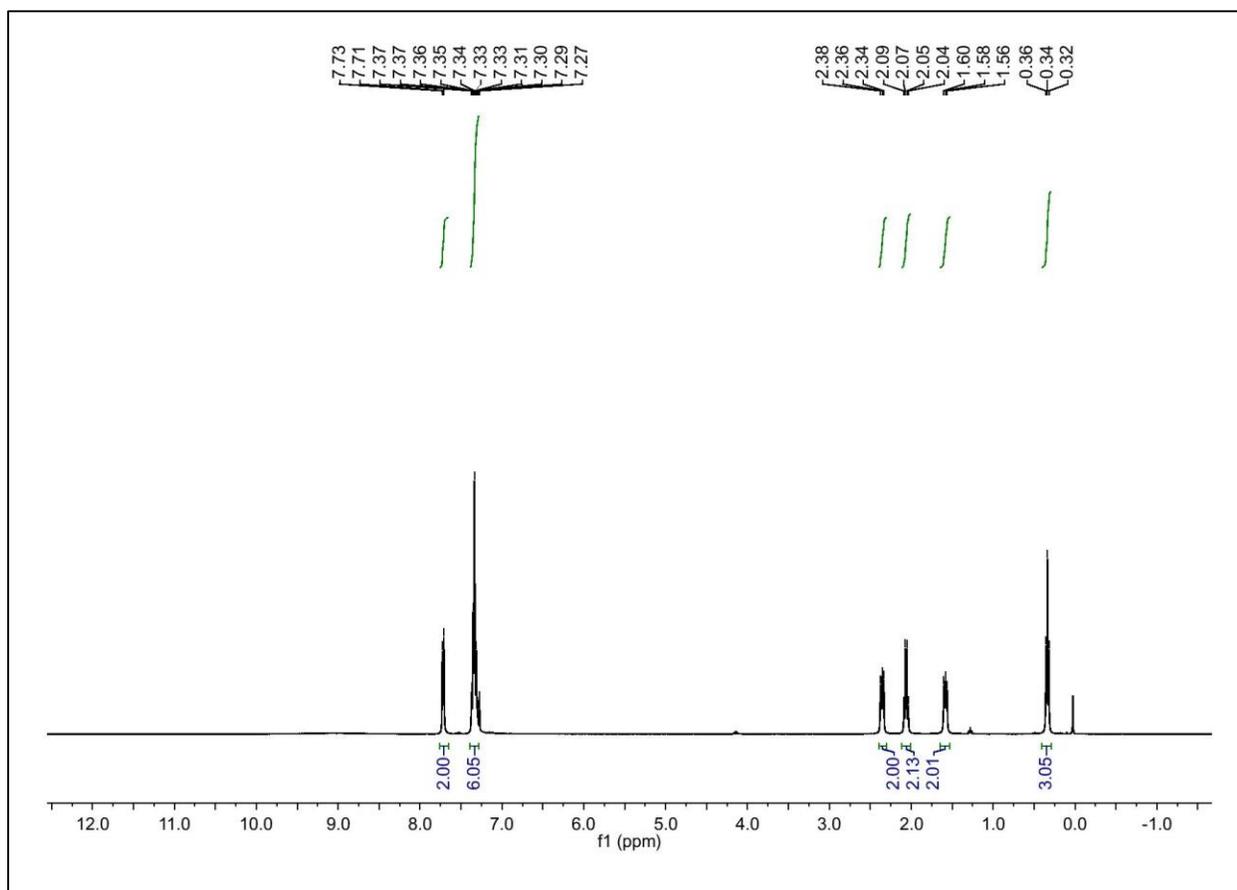
MS Spectrum



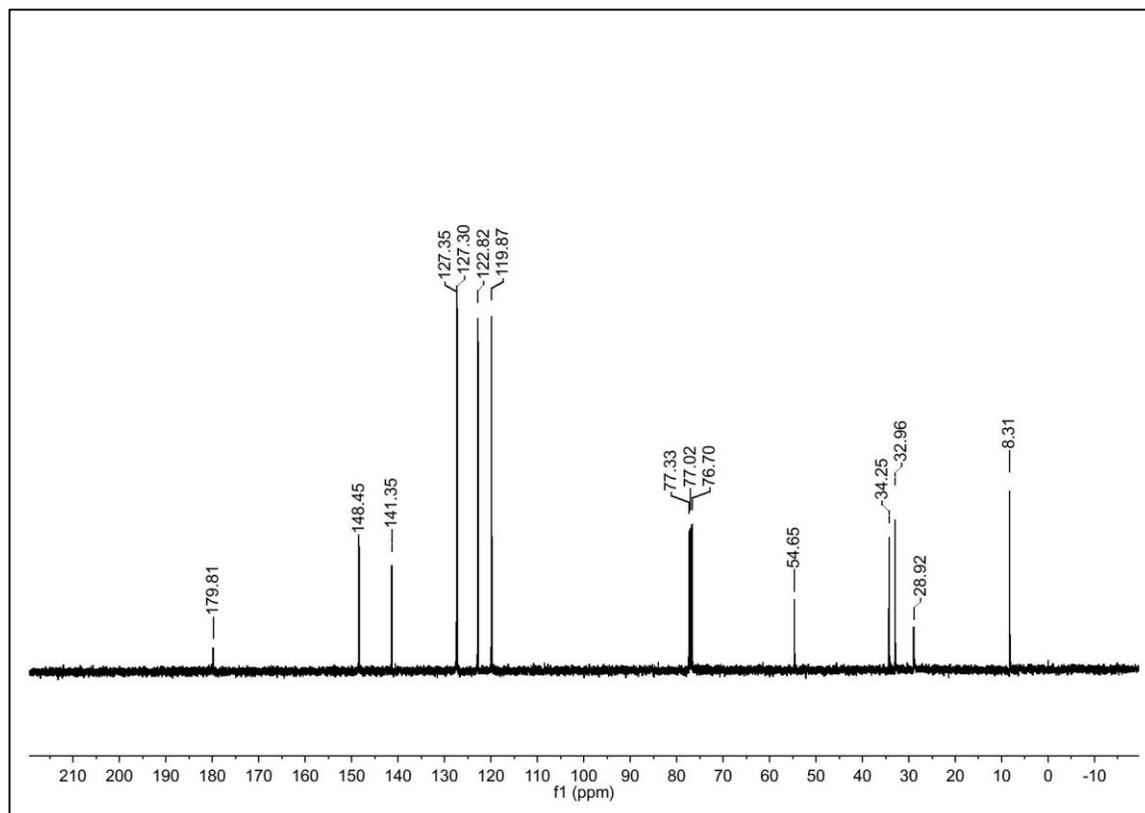
MS Zoomed Spectrum



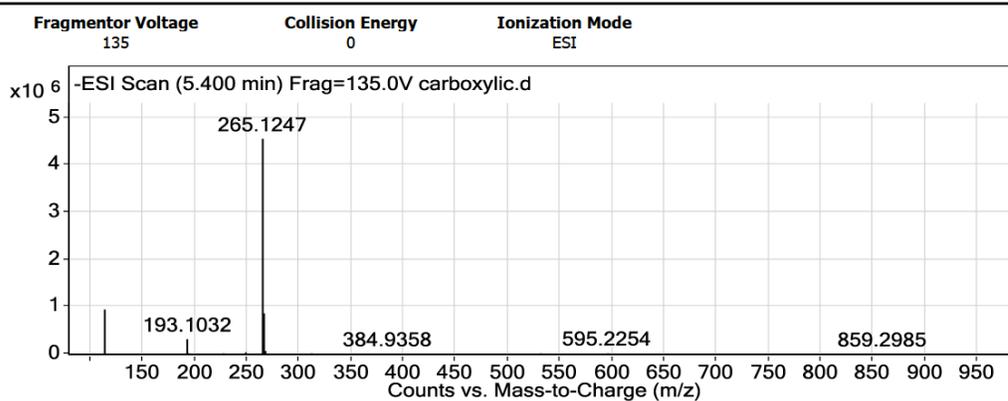
# <sup>1</sup>H NMR of 4



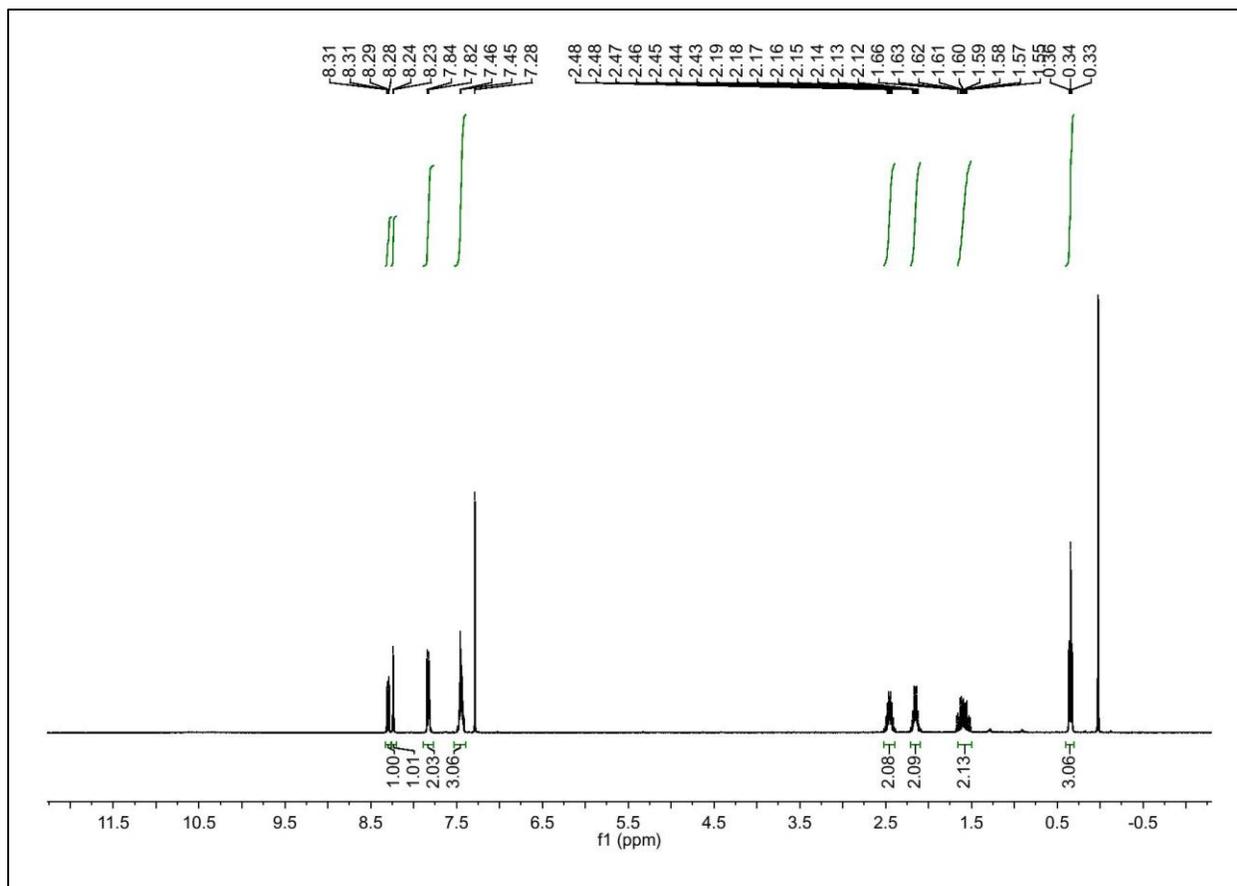
### <sup>13</sup>C NMR of 4



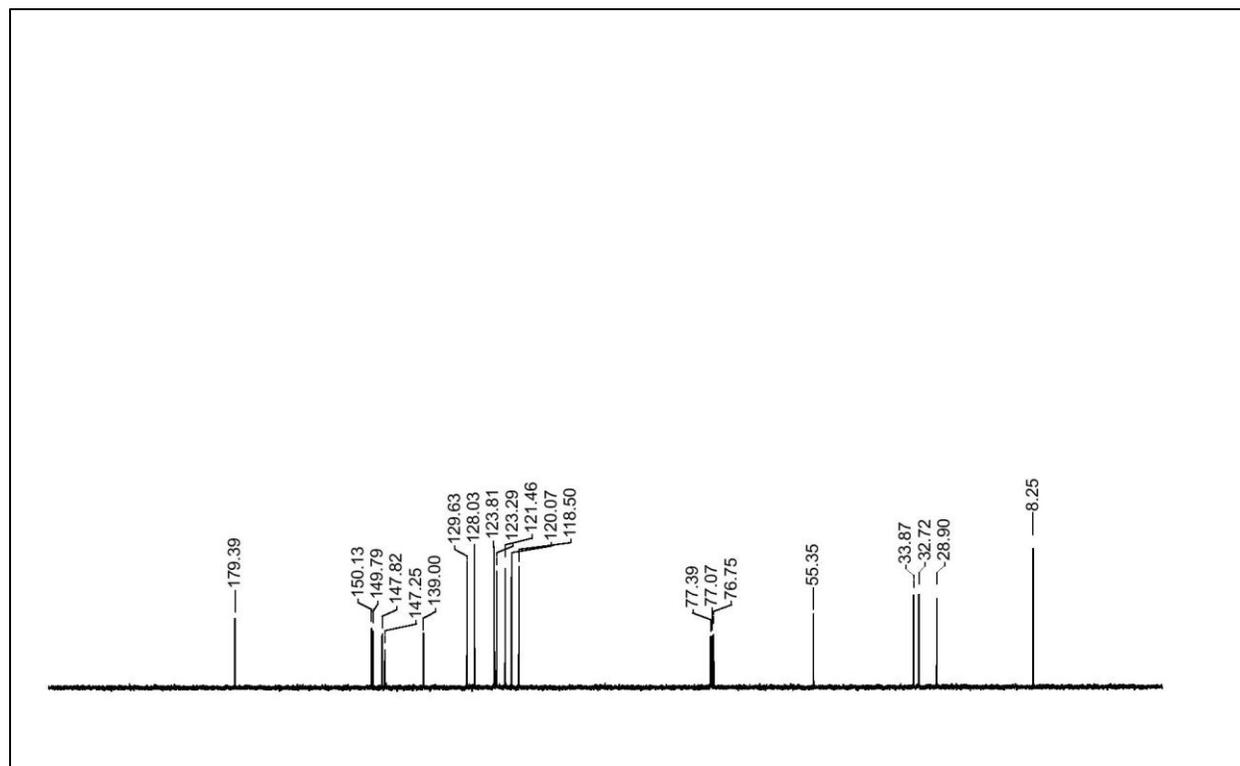
### LC-MASS of 4



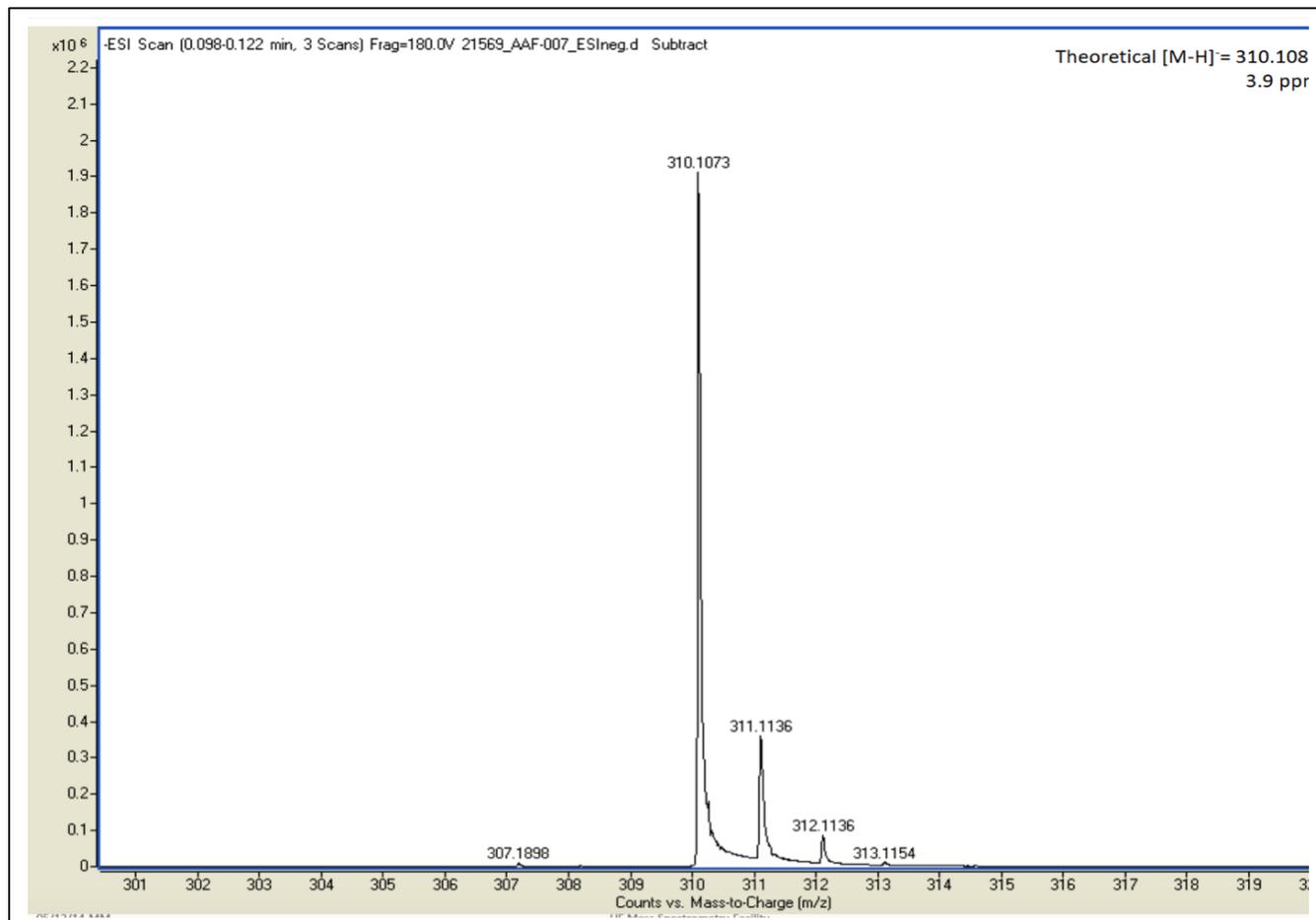
# <sup>1</sup>H NMR of 5



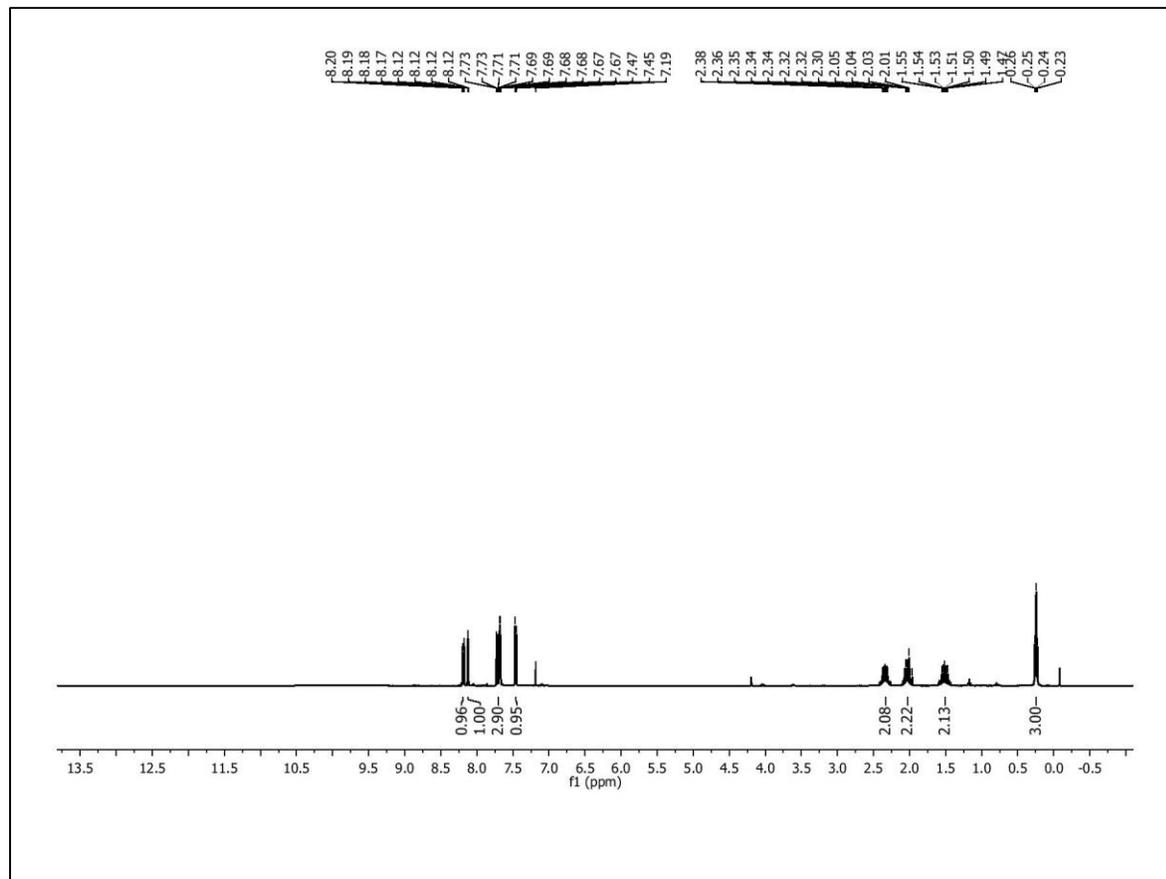
# $^{13}\text{C}$ NMR of 5



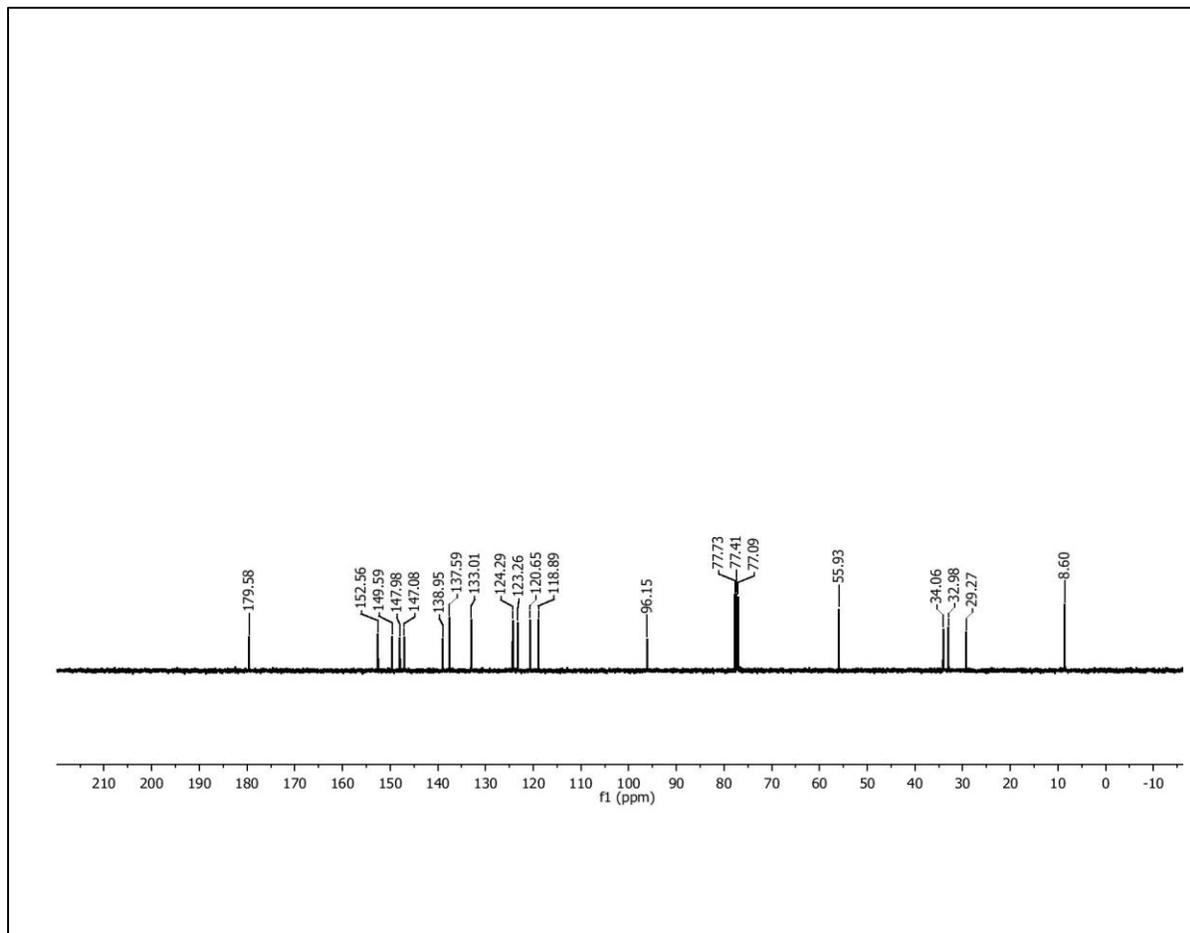
# HRMS of 5



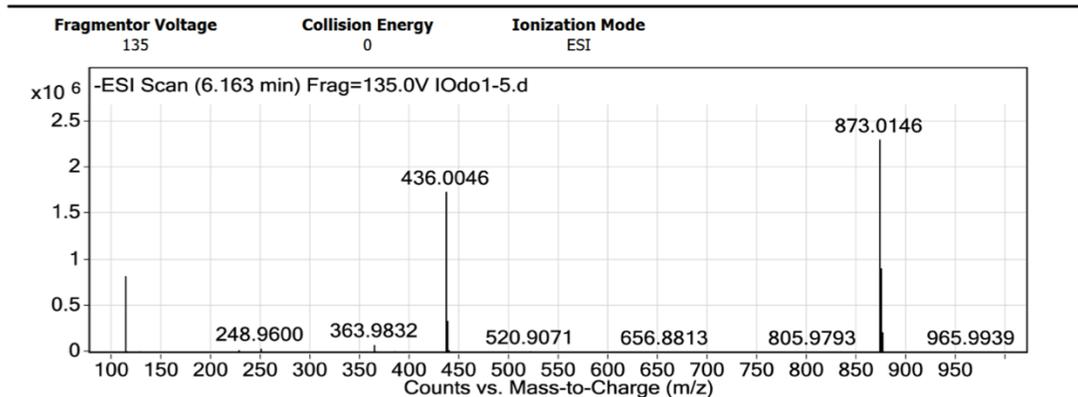
# <sup>1</sup>H NMR of 6



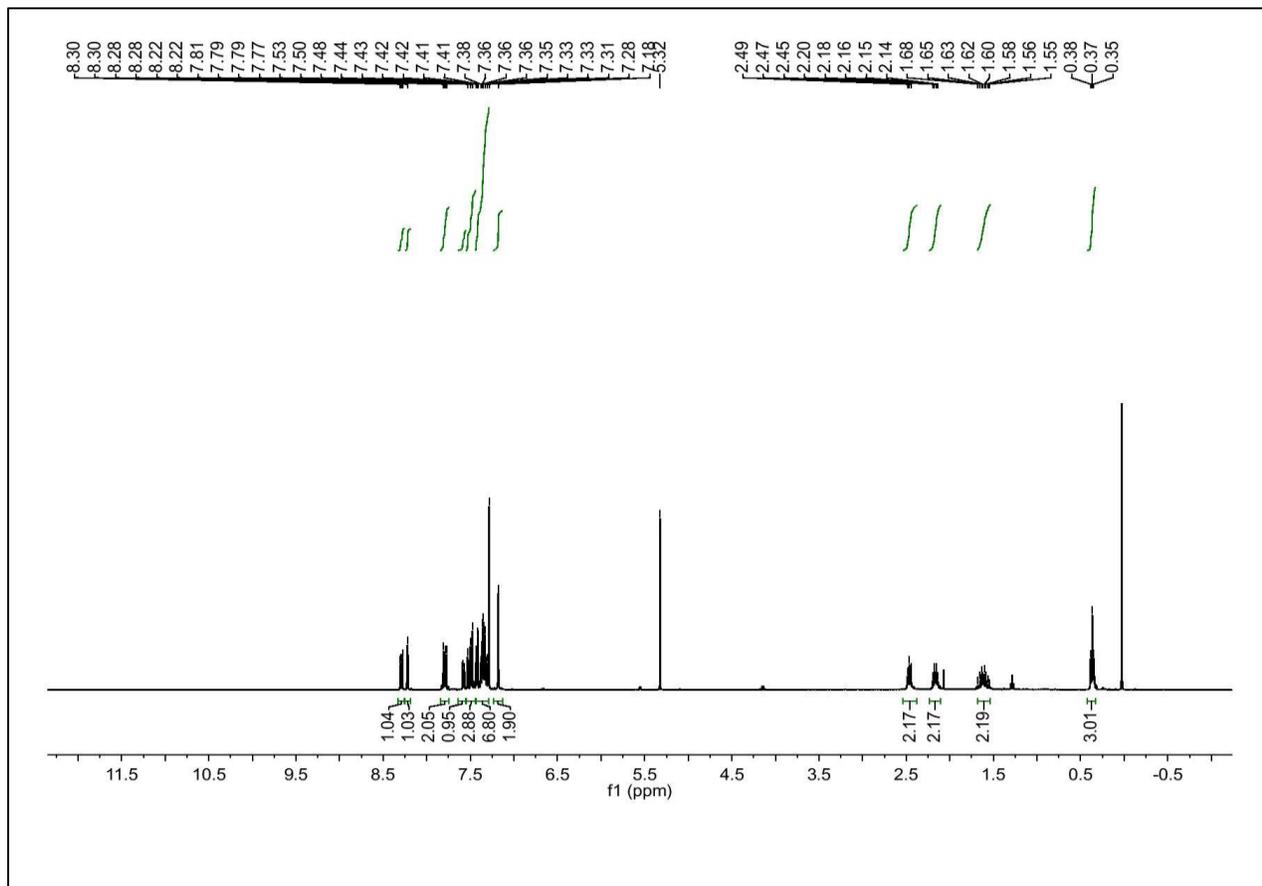
# $^{13}\text{C}$ NMR of 6



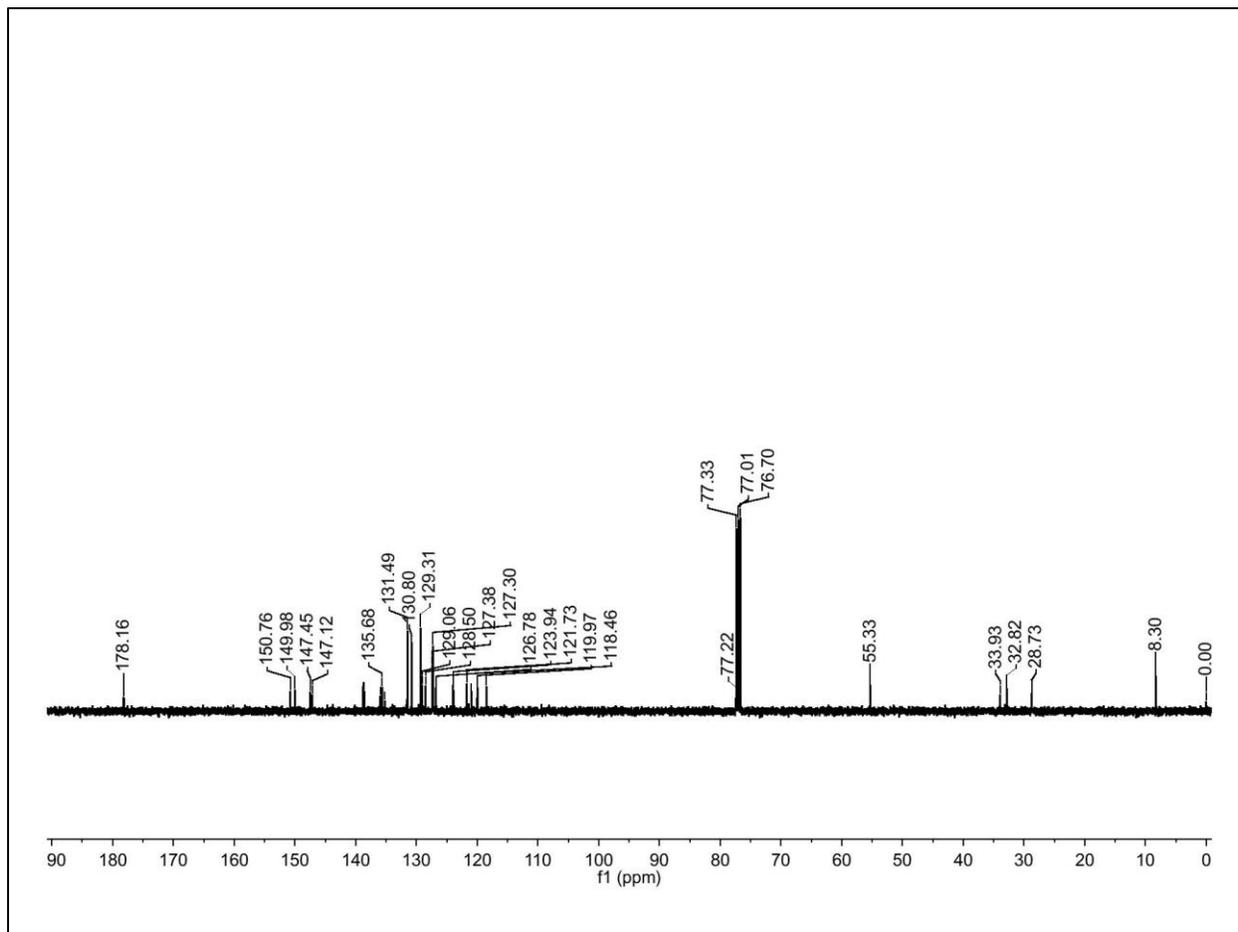
# LC-MASS of 6



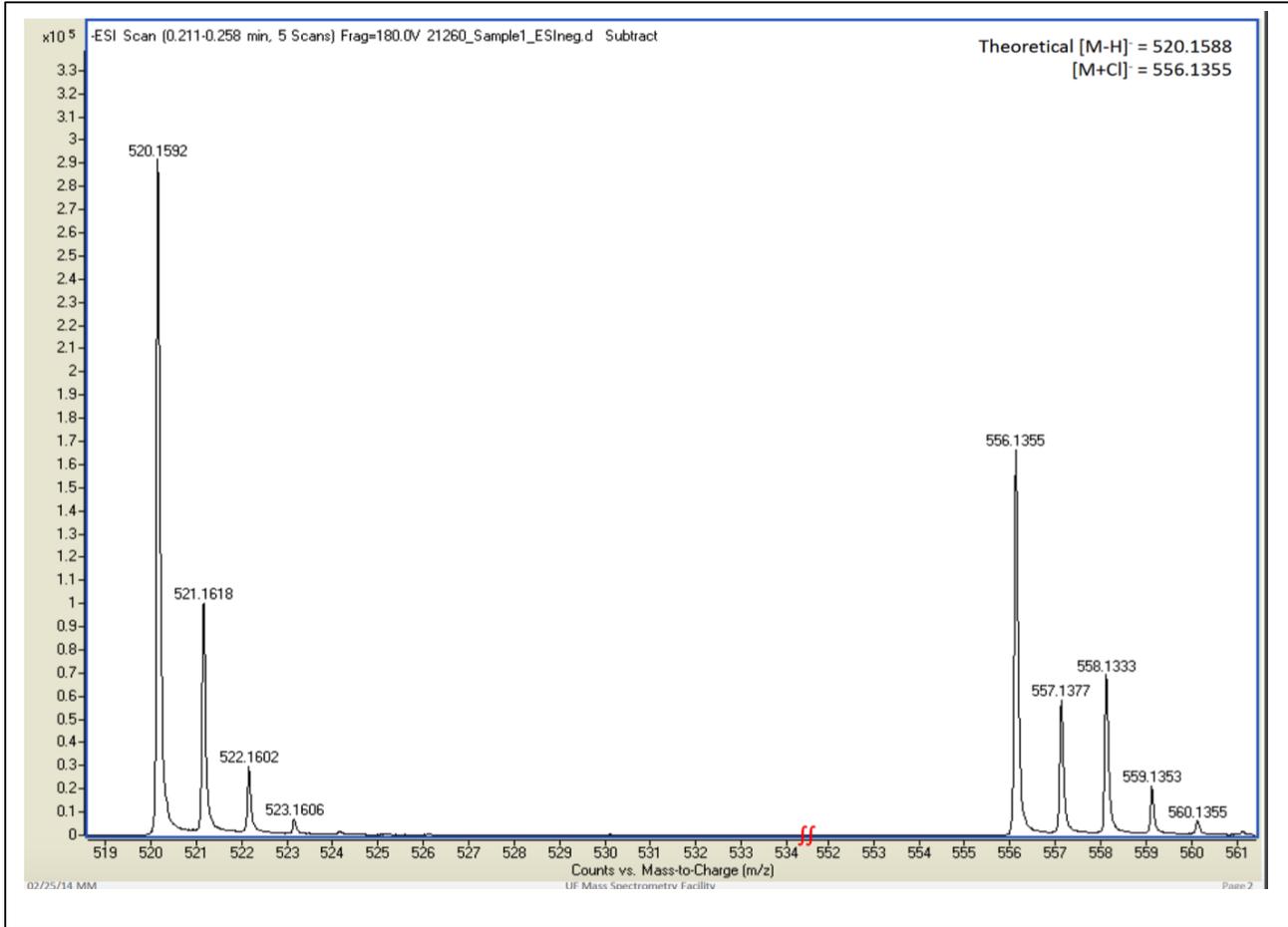
# <sup>1</sup>H NMR of 8



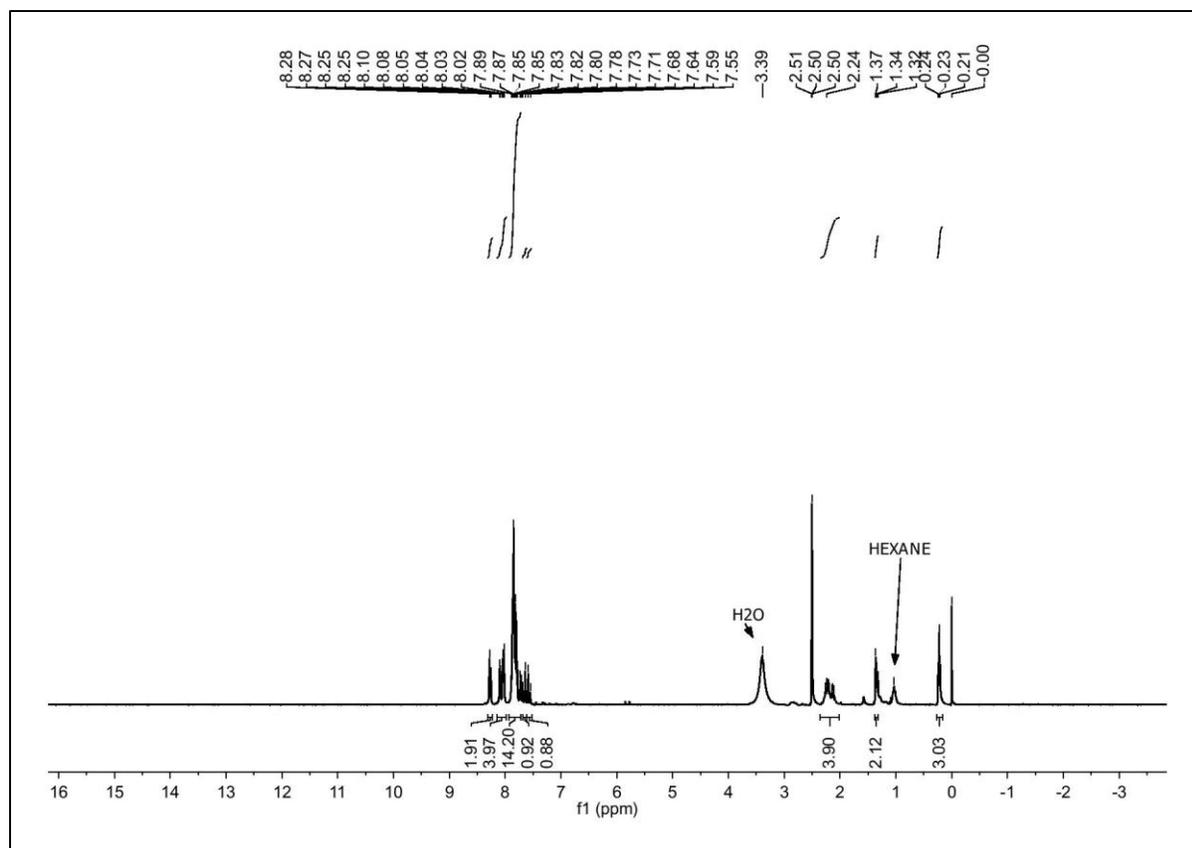
# $^1\text{H}$ NMR of 8



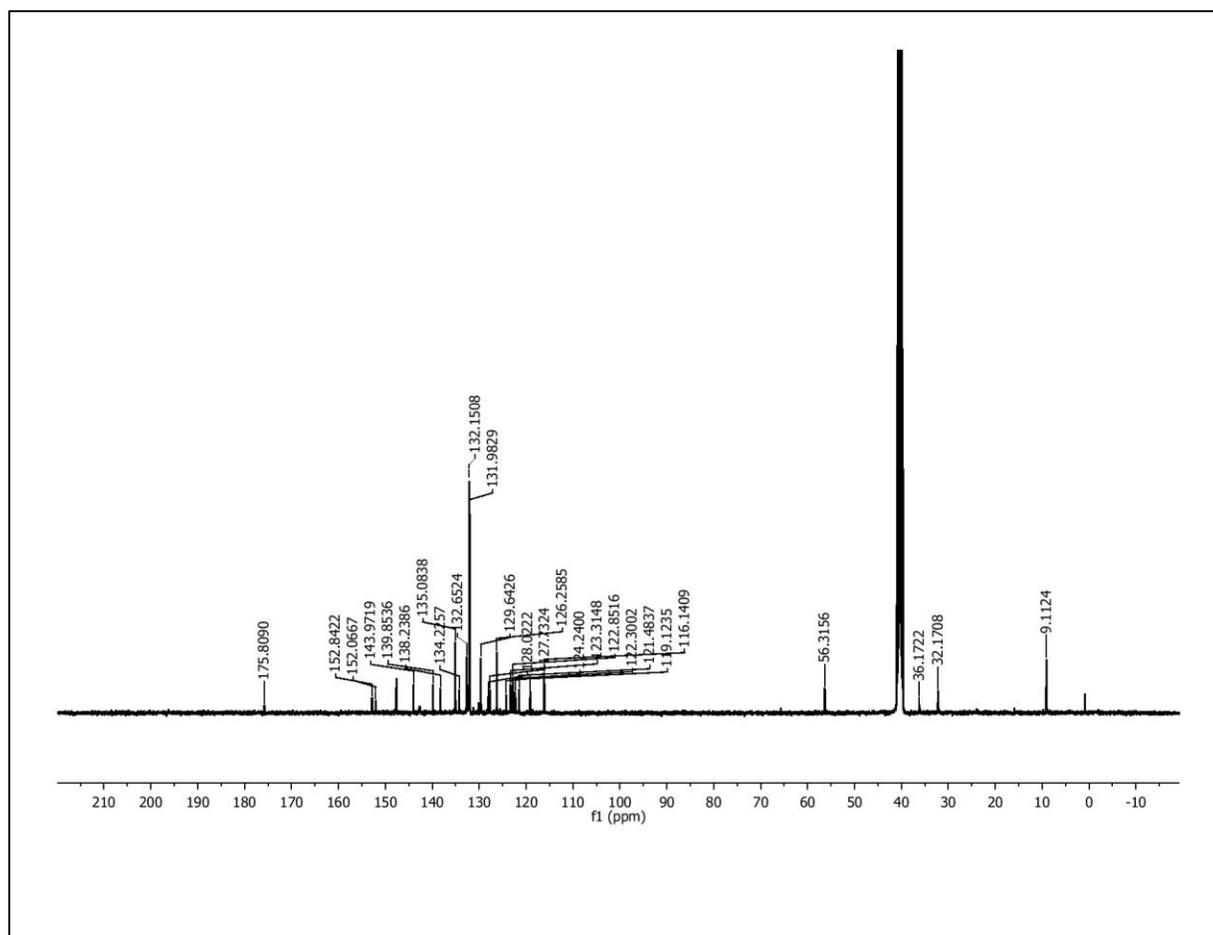
# HRMS of 8



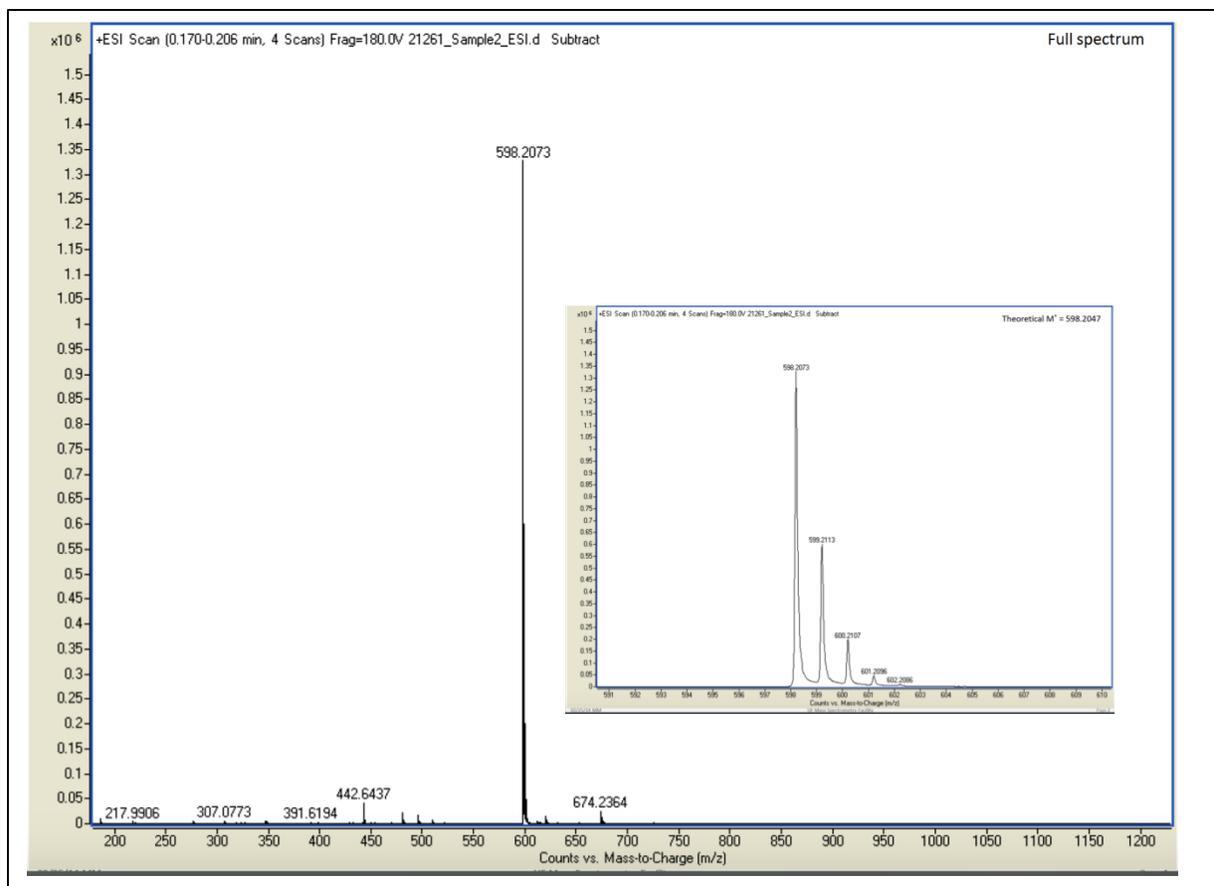
# <sup>1</sup>H NMR of 9



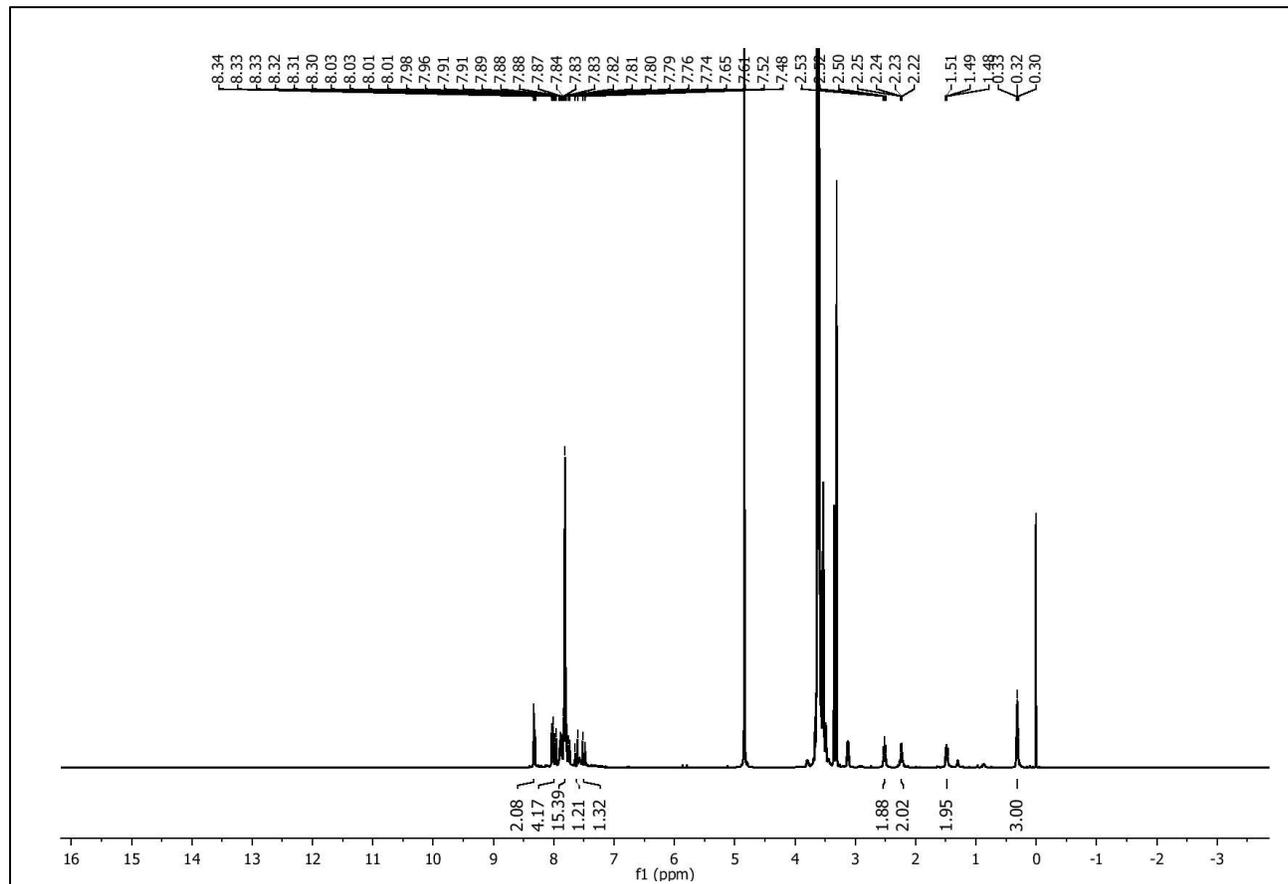
# $^{13}\text{C}$ NMR of 9



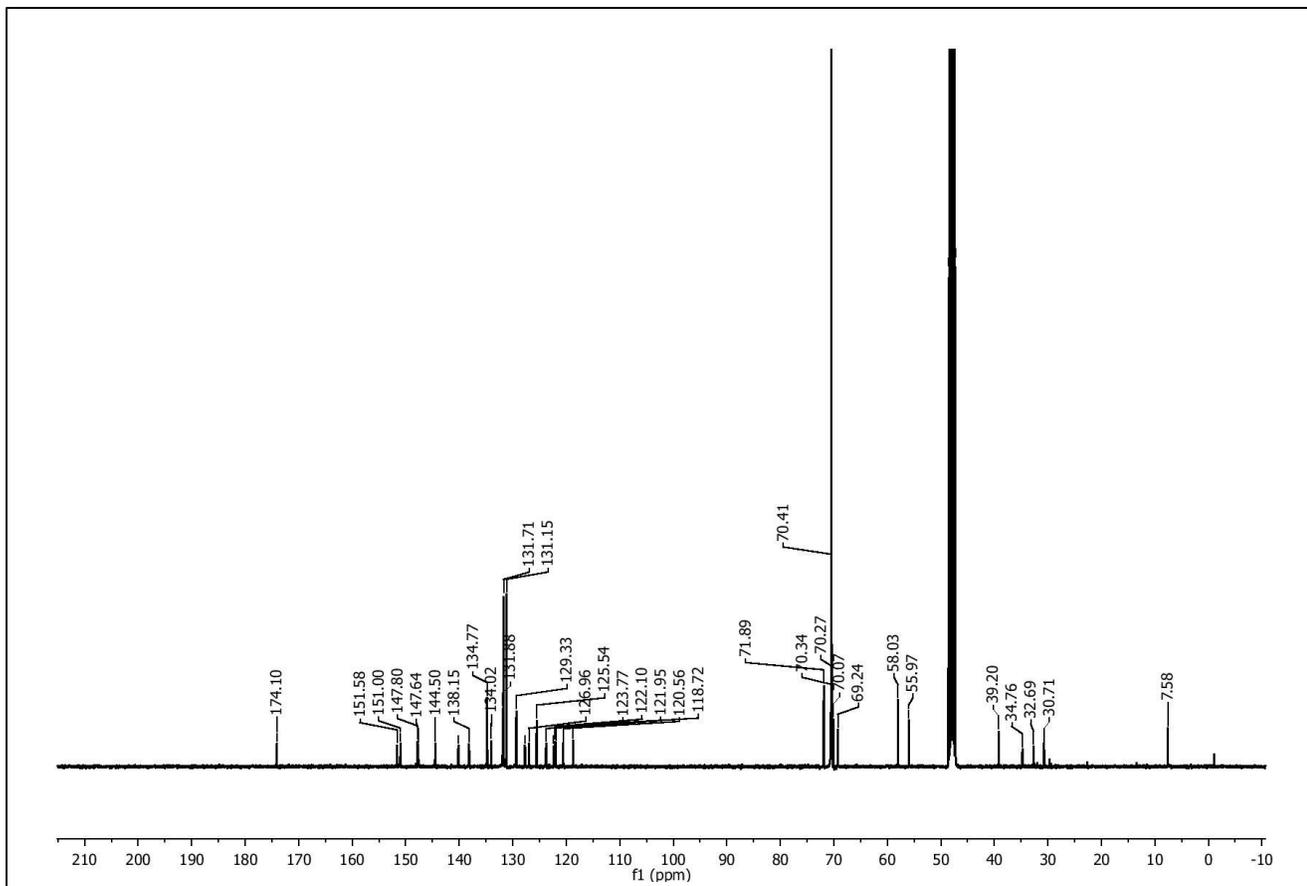
# HRMS of 9



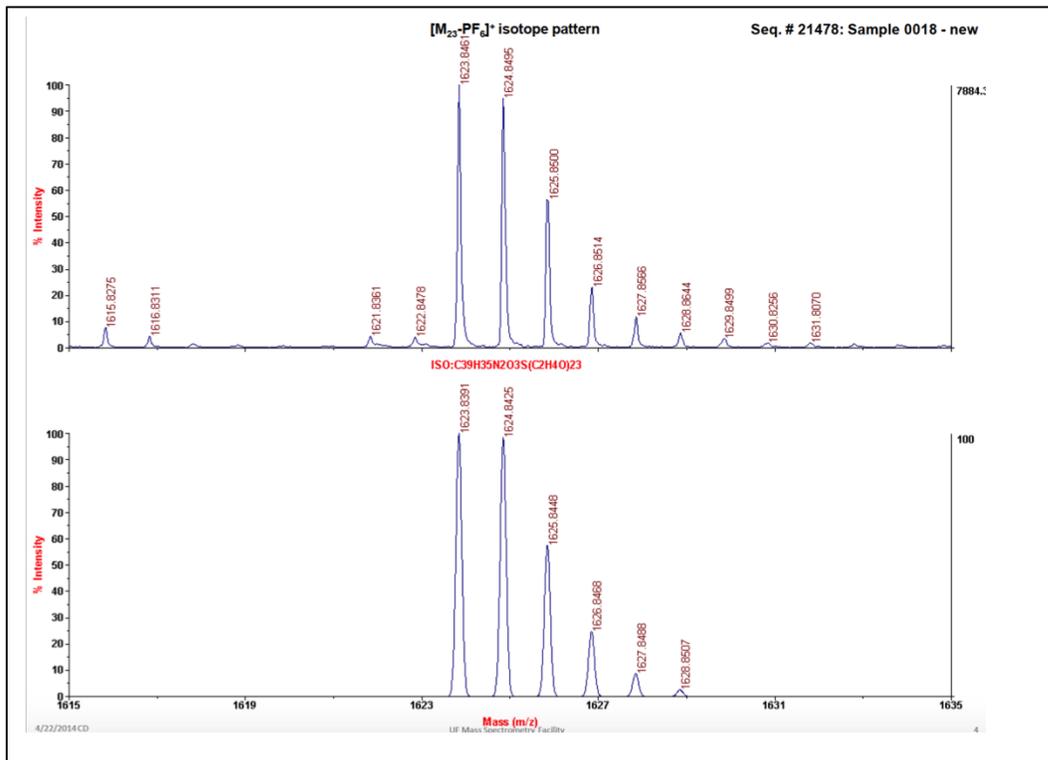
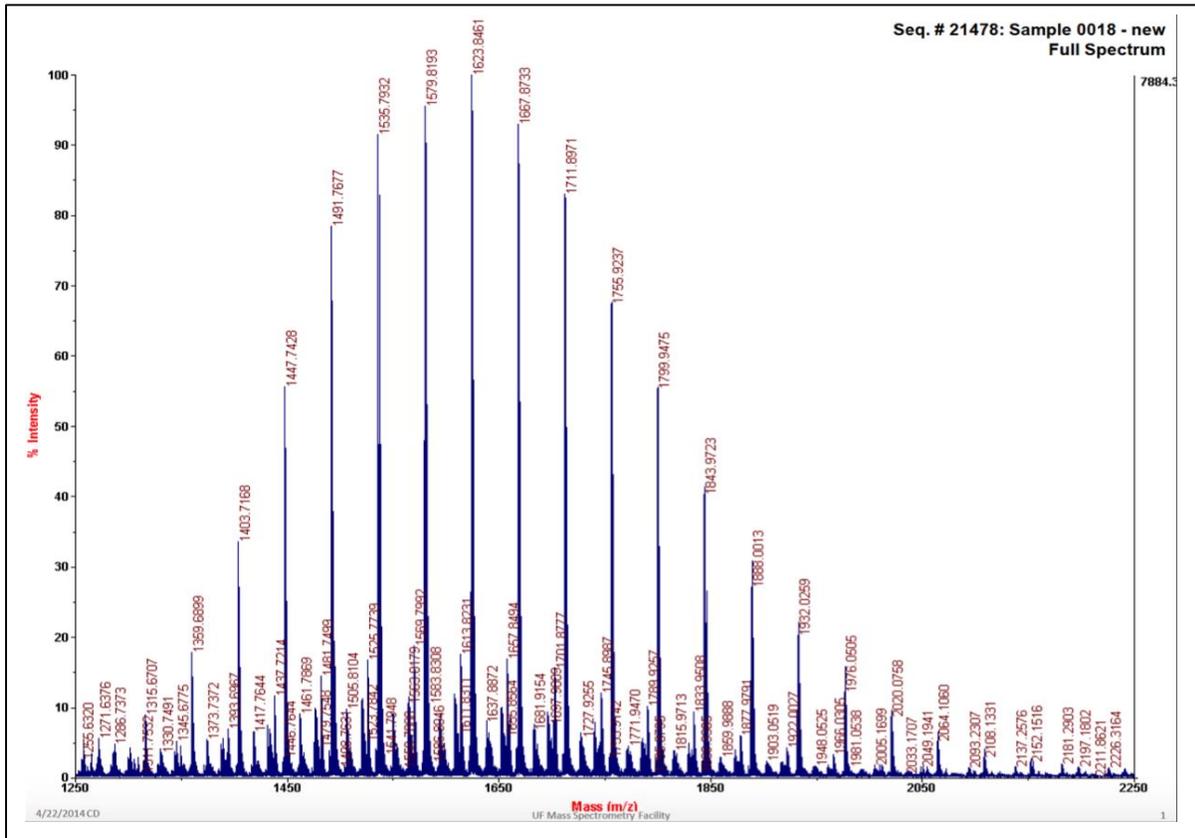
# <sup>1</sup>H NMR of PEG-PAG9



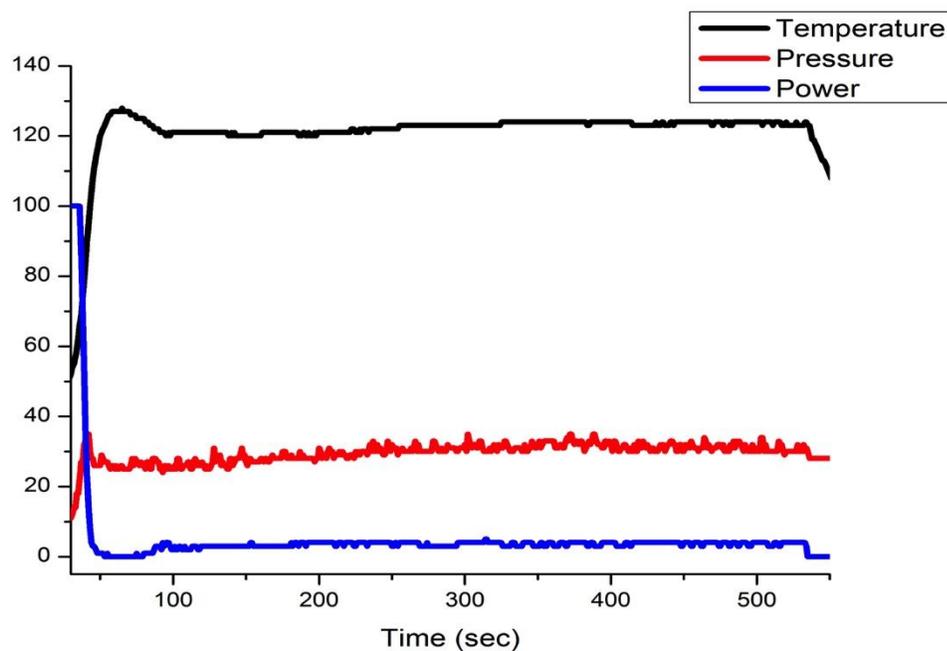
# <sup>13</sup>C NMR of PEG-PAG9



# MALDI-TOF-MS of PEG-PAG9



## Microwave Vessel Conditions in Synthesis of 9



### References

1. Yanez, C. O.; Andrade, C. D.; Belfield, K. D. *Chem. Commun.* 2009, 827.
2. R. Kumar, I. Roy, T. Y. Ohulchansky, L. N. Goswami, A. C. Bonoiu, E. J. Bergey, K. M. Trampusch, A. Maitra, and P. N. Prasad, *ACS Nano* 2(3), 449–456 (2008).
3. X. L. Yue, C. O. Yanez, S. Yao and K. D. Belfield, *J. Am. Chem. Soc.*, 2013, 135, 2112.