

Supporting Information

A Therapeutic Keypad Lock Decoded in Drug Resistant Cancer Cells

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1. General Methods

All reagents and solvents were purchased from commercial sources and used without further purification. Column chromatography was carried out using silica stationary phase (230–400 mesh, SiliCycle Inc., Canada). Analytical thin layer chromatography was performed on 0.25 mm thick precoated silica gel plates (60F254, Merck, Germany). Esterase enzyme is obtained from Sigma-Aldrich (Porcine Liver Esterase, Enzyme Commission Number 3.1.1.1). Compounds were visualized under UV light. All ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Inova instrument (400 MHz) at Selcuk University and Ataturk University respectively. ^1H NMR spectrum of PS3 is recorded on a 600 MHz Varian NMR Spectrometry at TÜBİTAK. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (J) are reported in hertz (Hz). Standard abbreviations indicating multiplicities are given: br = broad, d = doublet, m = multiplet, s = singlet, t = triplet. High-resolution mass spectrometry was carried out using Agilent 6530 Accurate-Mass Q-TOF LC/MS of the Eastern Anatolia Advanced Technology Research and Application Centre (DAYTAM, Erzurum, Turkey). LC-MS data were recorded using Shimadzu LCMS-2020 sSingle Quadrupole Liquid Chromatography Mass Spectrometer. For PDT, LED from Bright LED Electronics Corp. and model BL-BG43V4V with peak absorption value at 505 was used as a light source. For cell culture experiments MCF7 human breast adenocarcinoma cell line (ATCC) were used. Cells were visualised with Zeiss Fluorescence Microscopy.

2. Additional Figures

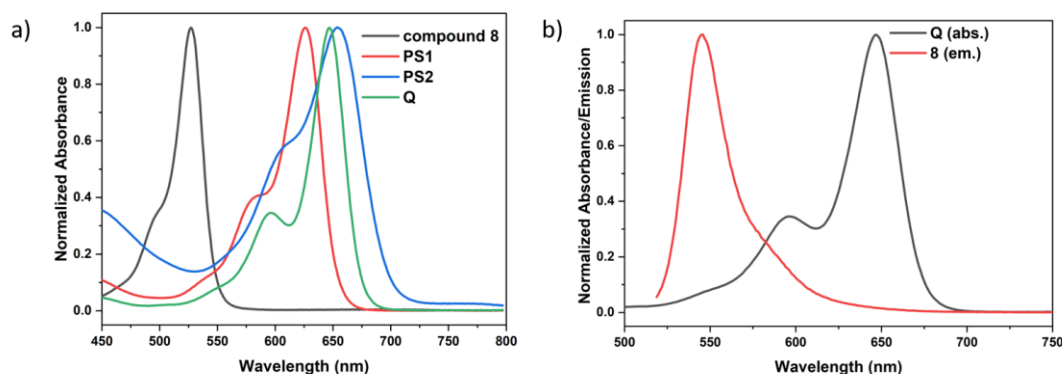


Figure S1. Normalized UV-Vis absorbance spectra of compounds 8, PS1, PS2 AND Q in DMSO (a), spectral overlap between emission spectrum of compound 8 and absorbance spectrum of Q. Emission spectra is recorded by exciting at 517 nm.

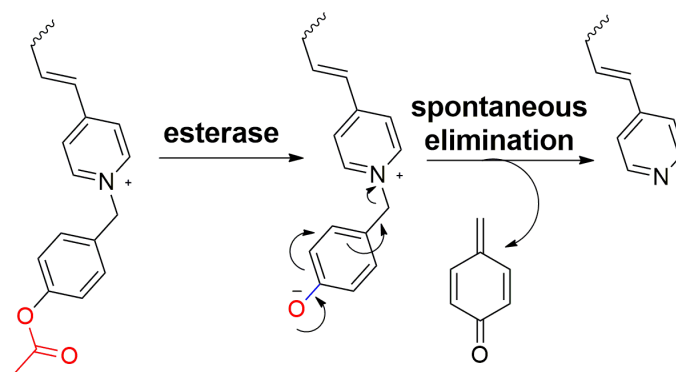


Figure S2. Mechanism of generation of pyridine bearing photosensitizer following hydrolysis of ester bond by esterase enzyme.

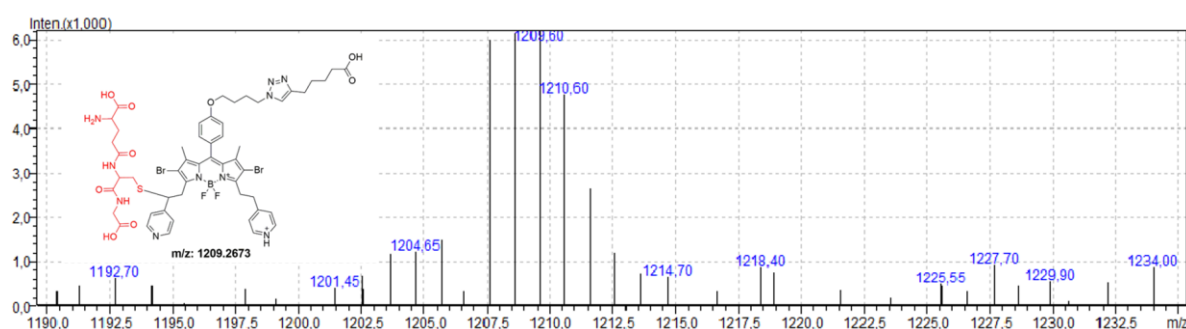


Figure S3. LC-MS spectrum of **PS3** incubated with 0.5 mM GSH for 90 min in 2% water in acetonitrile. Generation of GSH adduct with broken conjugation is observed with given m/z values.

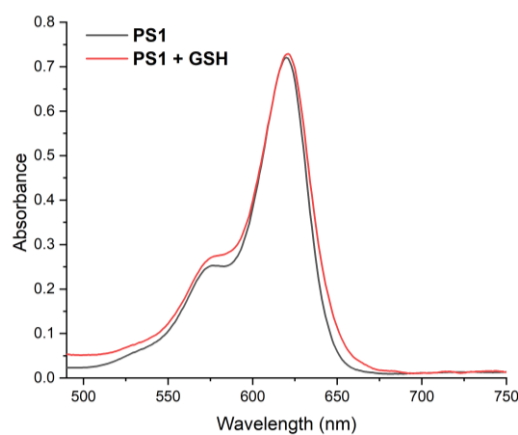


Figure S4. Change in the UV-Vis absorption spectra of **PS1** after incubation at 37°C alone (black) or with 0.5 mM GSH (red) for 90 min in 2% water in acetonitrile.

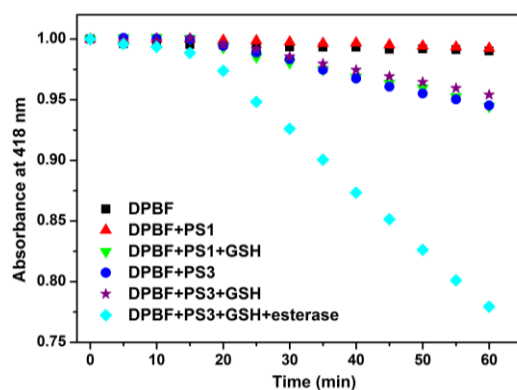


Figure S5. Comparison of the rate of decay of DPBF (50 μ M) at 418 nm using compound **PS1** (0.1 μ M) or **PS3** (0.1 μ M) I DMSO with 505 nm LED light from a distance of 30 cm. GSH bearing samples were incubated with 0.5 mM GSH for 90 min at 37°C prior to experiment. Likewise, esterase bearing samples were additionally incubated with 10U esterase for 60 min at 37°C following GSH incubation and then singlet oxygen generation experiment was performed. Samples were kept in dark during the first 15 min of the experiment.



Figure S6. 10 μ M **PS2** (left) and **PS2** after incubation with 0.5 mM GSH for 90 min at 37°C (right) in 2% PBS in acetonitrile. Colour change indicates broken conjugation. Product precipitates in time.

User Spectra

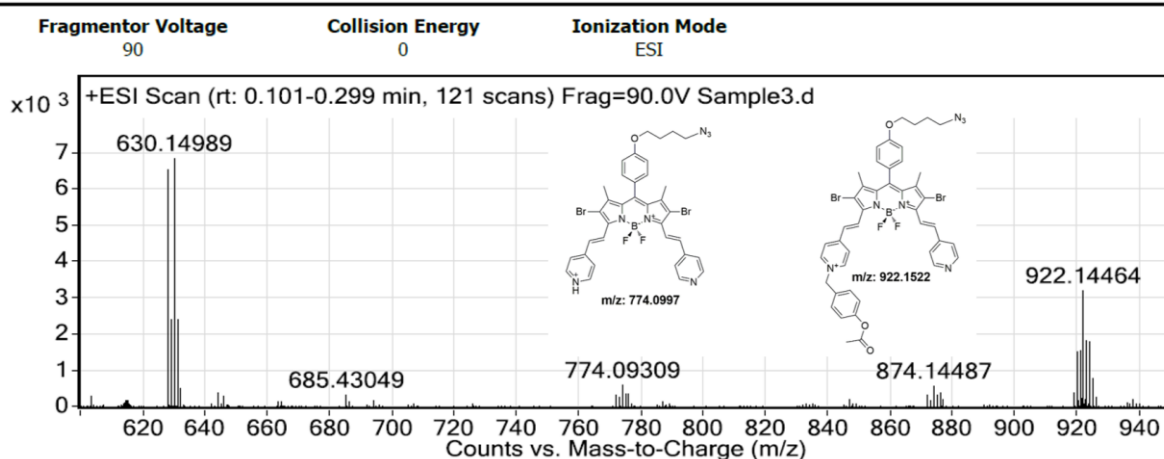


Figure S7. High Reselution Mass Spectrometry (HRMS) analysis of **PS2** incubated with 10U esterase for 1h in 2% water in THF. Generation of proposed products are observed with given m/z values.

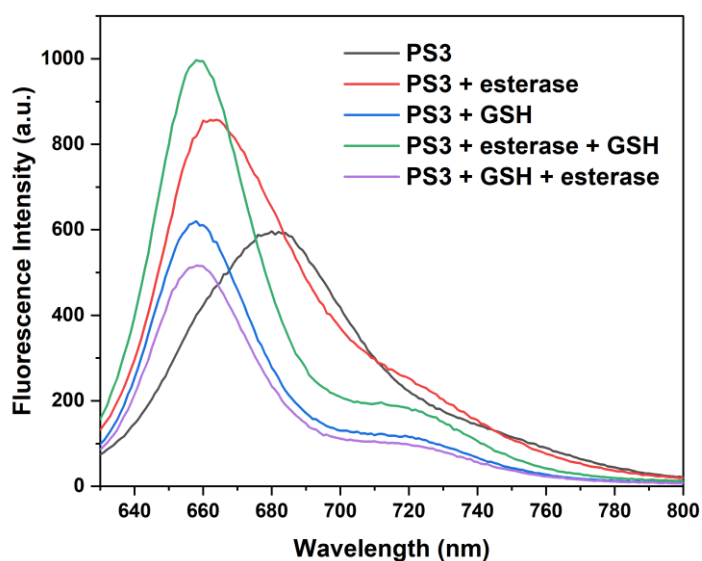
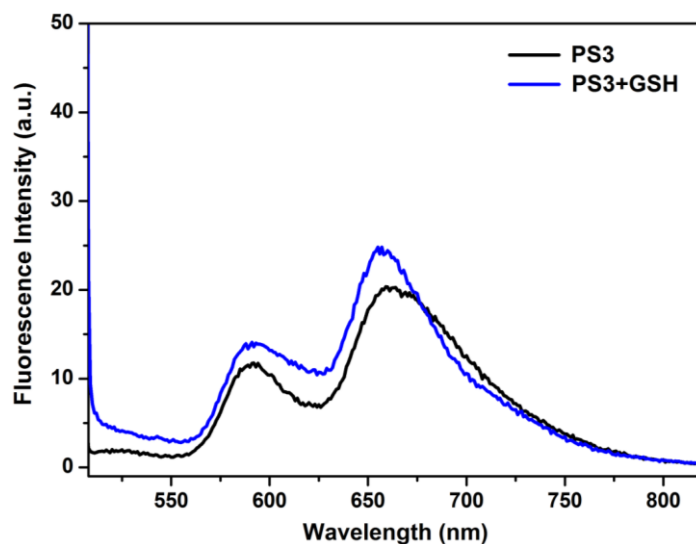


Figure S8. Change in emission spectra of **PS3** (10 μ M) in the absence and presence of combinations of esterase (10 U), GSH (0.5 mM) in THF:2% water. Esterase and GSH incubations were done for 60 min and 90 min respectively, at 37 $^{\circ}$ C. Spectra are recorded with excitation at 620 nm. Hypsochromic shift of emission wavelength is attributed to formation of pyridine bearing derivatives (in the case of esterase) or formation of GSH adduct/mono reduction of one of the styryl bonds (in the case of GSH). Esterase bearing samples display significant enhancement of fluorescence which would result from separation of emissive quencher module from the non-emissive photosensitizer (so that potential of energy transfer to PS module is decreased).



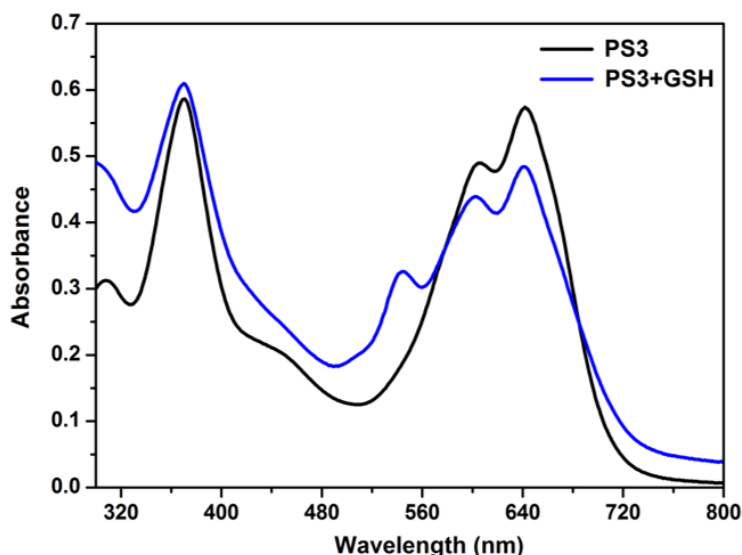


Figure S9. Change in emission (top) or absorbance (bottom) spectra of **PS3** (10 μ M) in the absence and presence of GSH (0.5 mM) in ACN:2%PBS buffer after incubation for 90 min at 37 $^{\circ}$ C, excitation at 500 nm.

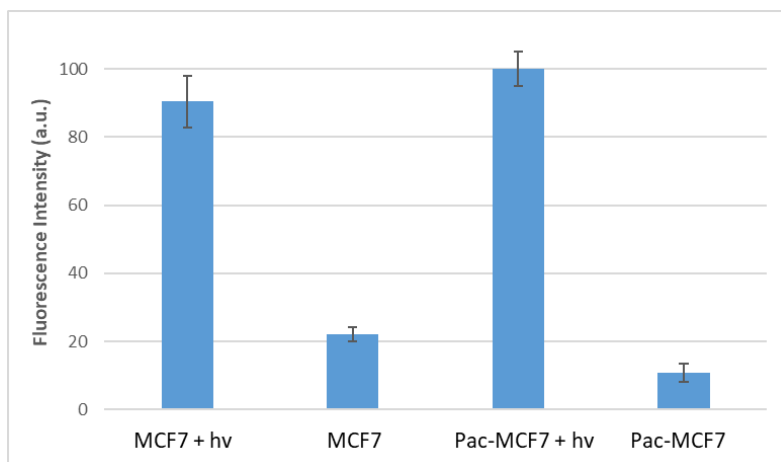


Figure S10. Relative fluorescence of cells stained with Annexin V. Fluorescence quantification is done using ImageJ software.

3. Synthesis and Other Experimental Procedures

3.1. Singlet Oxygen Generation Experiments

1,3-Diphenylisobenzofuran (DPBF) was used as a singlet oxygen trap. DPBF (50 mM) is dissolved in DMSO and solution was saturated with air for 10 minutes purging. Compounds **PS1** and **PS3** (0.1 μ M) were used for singlet oxygen generation experiments. Prior to experiment, **PS3** (10 μ M) is incubated with combination of 0.5 mM GSH and/or 10U esterase at 37 $^{\circ}$ C for 1h and 1.5h respectively. After incubation, samples are diluted to 0.1 μ M final photosensitizer concentration and singlet oxygen generation is analysed with DPBF. LED lamp with 505 nm peak value is used to irradiate samples. In the first 15 minutes of the measurement, samples were kept in dark and later they were irradiated at 5 min interval from a 30 cm distance. Change in

absorbance of DPBF at 418 nm were followed as a measure of singlet oxygen generation ability.

3.2. *Esterase and GSH Activity*

All compounds were dissolved in CH₃CN (ACN) to obtain a stock solution and, then the compounds were added to obtain a final concentration of 10 μ M. Esterase was dissolved in pure water and added to the sample solution (10U). In a 10 \times 10 mm quartz cuvette, the esterase solution (10 U, 40 μ L, dissolved in water) was mixed with **PS3** (60 μ L, dissolved in ACN) in THF (total volume of 2 mL). The mixture of the **PS3** and esterase was incubated at 37 $^{\circ}$ C for 60 min, then absorption and fluorescence spectrum were recorded. Also, glutathione (GSH) was dissolved in PBS (10 mm, pH 7.4). The GSH solution (40 μ L in PBS) was mixed with **PS3** (60 μ L, dissolved in ACN) in ACN (total volume of 2 mL) to obtain 0.5 mM final GSH concentration. Then the mixture of the **PS3** and GSH was incubated at 37 $^{\circ}$ C for 90 min. After absorption and fluorescence spectra measurements of mixtures were recorded. The same experiment was performed with 2% water in THF. For dual marker responsiveness, first GSH (0.5 mM) was added **PS3** (10 μ M) in THF:2% water, then the mixture incubated at 37 $^{\circ}$ C for 90 min, absorption and emission spectra of this solution was recorded. After same mixture added esterase (10 U) and incubated at 37 $^{\circ}$ C for 60 min. The activity of the esterase enzyme in the presence of GSH of compound **PS3** was followed by absorption and emission spectra.

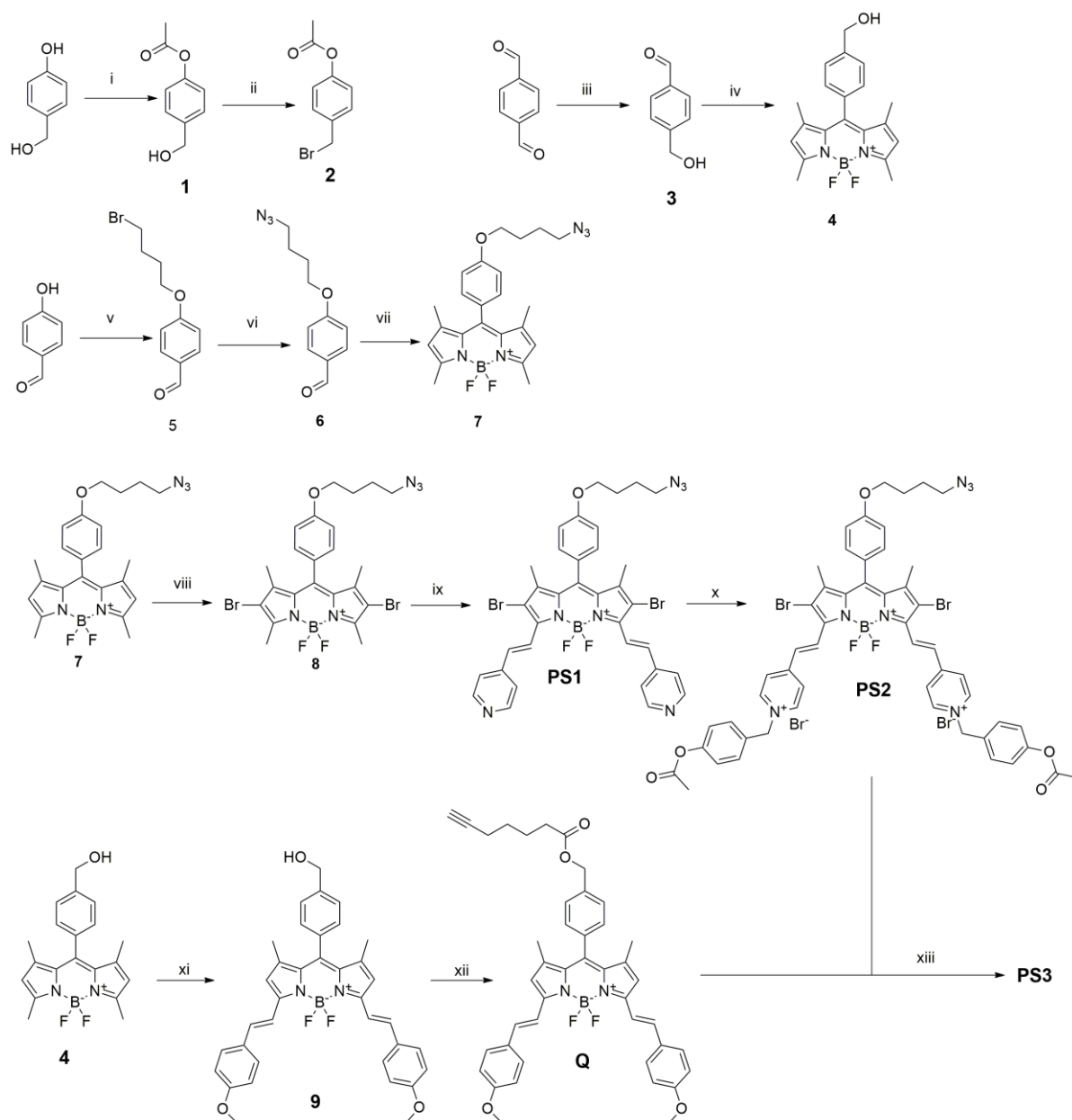
3.3. *Cell Culture Experiments*

MCF-7 human mammary carcinoma cells were used in the experiment. Cells were cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS), %1 gentamicin and incubated at 37 $^{\circ}$ C in 5% CO₂ with saturated humidity. Paclitaxel resistant cells (MCF7-Pac) were prepared by applying increasing dose of drug until cells are adopted to 400 nM of paclitaxel concentration, as previously described.³

Apoptotic cells were quantified using annexin V-FITC assay (BD Pharmingen) and analyzed by fluorescence microscope. Concentration of cells prior to staining is 4-5 \times 10⁵ cells per 3 mL volume. Cells were treated with **PS3** (25 μ M), irradiated with LED light (peak value 505 nm) for 4 hours then incubated in dark for 20 hours. After incubation period, cells were washed with 2 mL 1 x phosphate buffered saline (PBS)-/- (no calcium, no magnesium). Cells were fixed by formaldehyde. After gentle mixing, sample is centrifuged at 335 x g for 5 minutes. Cells were washed twice in PBS and Annexin V was added according to the manufacturer's recommendations [Annexin V Alexa Fluor 488 (Molecular Probes, A13201)]. Cells are incubated for 15 minutes at 37 $^{\circ}$ C in dark. Cells were washed with PBS. 4 μ L of PI was added (Sigma, Cat# P-4864-10ML) after dilution in 1 x Annexin V binding buffer (final PI concentration of 2 μ g/mL in each sample). Cells are incubated in the dark for 15 more minutes at 37 $^{\circ}$ C. 1 mL 1 x PBS-/- was added to each sample and mixed gently. Then apoptotic cells images were captured by using fluorescence microscope.

3.4. *Synthesis*

Compounds are synthesis as shown in Scheme S1.



Scheme S1. Synthesis of compounds **PS1-PS3** and **Q**. Reagents and conditions: (i) acetyl chloride, NEt_3 , DCM, rt, 24 h; (ii) NBS, PPh_3 , DCM, 0 °C, 2 h; (iii) NaBH_4 , EtOH, THF, rt, 2 h; (iv) a. 2,4-dimethyl pyrrole, TFA, DCM, N_2 ; b. *p*-chloranil, 3h; c. NEt_3 , $\text{BF}_3 \cdot \text{OEt}_2$; (v) 1,4-dibromobutane, K_2CO_3 , DMF, rt; (vi) NaN_3 , DMSO, 60 °C, 3h; (vii) a. 2,4-dimethyl pyrrole, TFA, DCM, N_2 ; b. *p*-chloranil, 3h; c. NEt_3 , $\text{BF}_3 \cdot \text{OEt}_2$; (viii) NBS, DCM, 0 °C to rt, 5h; (ix) 4-pyridinecarboxaldehyde, piperidine, acetic acid, benzene, 90 °C, 2h; (x) 4-(bromomethyl)phenyl acetate, dry ACN, 80 °C, 48h; (xi) 4-methoxybenzaldehyde, piperidine, acetic acid, benzen, 90 °C, 2h; (xii) 6-heptynoic acid, DCC, DMAP, rt, 24 h, DCM; (xiii) **PS2**, **Q**, $\text{Cu}(\text{MeCN})_4\text{PF}_6$, DCM, rt, 48h.

Synthesis of Compound 1

To a solution of 4-hydroxybenzyl alcohol (3.0 g, 24.1 mmol) in CH_2Cl_2 was added NEt_3 (3.36 mL, 24.1 mmol). This mixture added slowly acetyl chloride (1.72 mL, 24.1 mmol) at room temperature. After stirring 24 h, removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using progressively hexane:ethyl acetate (1:1) as the mobile phase to afford compound **1** as a colourless oil (2.23 g, 56%).

^1H NMR (400 MHz, Chloroform-*d*) δ 7.38 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.6 Hz, 2H), 4.68 (s, 2H), 2.30 (s, 3H).

^{13}C NMR (400 MHz, CDCl_3) δ 214.80, 169.82, 150.30, 138.72, 128.31, 121.91, 64.98, 21.34.

Synthesis of Compound 2

To a solution of compound **1** (1.39 g, 8.19 mmol) in anhydrous CH_2Cl_2 (20 mL) was cooled to 0 °C, and NBS (2.18 mg, 12.2 mmol) was added, then PPh_3 (3.43 g, 13.07 mmol) added gradually in small amounts. The reaction mixture was stirred for 2 h at 0 °C. After the solvent was removed, products were isolated by column chromatography using an *n*-hexane:EtOAc (4:1) mixture as an eluent. The compound **2** was obtained as white solid in the yield of 1.63 g (87%).

^1H NMR (400 MHz, Chloroform-*d*) δ 7.41 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H), 4.49 (s, 2H), 2.30 (s, 3H).

^{13}C NMR (400 MHz, Chloroform-*d*) δ 169.31, 150.58, 135.36, 130.28, 121.99, 32.65, 21.13.

Synthesis of Compound 3

Terephthalaldehyde (10 g, 75 mmol) was dissolved in 125 ml ethanol and 175 ml tetrahydrofuran. The reaction mixture is cooled to 5°C. Then, NaBH_4 (0.85 g, 22.5 mmol) was added to reaction mixture in small amounts over 20 minutes. After stirring 3 h at room temperature, the reaction mixture was acidified with dilute HCl (2 M), and extracted with DCM. The organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was used in the next step without purification. The compound **3** was afforded as off-white solid with %80 (8.0 gr) yield.

^1H NMR (400 MHz, Chloroform-*d*) δ 9.92 (s, 1H), 7.80 (d, J = 8.1 Hz, 2H), 7.46 (d, J = 7.9 Hz, 2H), 4.72 (d, J = 3.4 Hz, 2H), 4.61 (s, 1H).

^{13}C NMR (400 MHz, Chloroform-*d*) δ 192.60, 148.09, 136.13, 130.39, 127.01, 65.09.

HRMS: Theoretical m/z for $(\text{M}+\text{H})^+$: 137.0603, Experimental m/z for $(\text{M}+\text{H})^+$: 137.05858, Δ =13 ppm

Synthesis of Compound 4

To a solution of compound **3** (1.5 g, 11.0 mmol) in anhydrous CH_2Cl_2 (150 mL) was purged with N_2 for 30 minutes. Then, 2,4-dimethylpyrrole (2.30 g, 24.23 mmol) was added under N_2 . 5 drops of trifluoroacetic acid (TFA) were added to the reaction mixture, and the resulting mixture was stirred for 18 h under N_2 atmosphere at room temperature. After the complete consumption of aldehyde, *p*-chloranil (2.76 g, 11.0 mmol) was added to the reaction mixture. When the mixture was stirred for 3h, NEt_3 (9.21 mL) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (10.9 mL) were added to the mixture, respectively. After the mixture was additional stirred for 3h, it was washed twice with water, organic phase dried over Na_2SO_4 , and concentrated under reduced pressure. The crude product was purified by column chromatography using using *n*-hexane:ethyl acetate (1:1) as eluent. . The compound **4** was obtained as 246 mg orange solid (11%).

^1H NMR (400 MHz, CDCl_3): δ ppm; 7.49 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 5.97 (s, 2H), 4.80 (s, 2H), 2.55 (s, 6H), 1.37 (s, 6H).

^{13}C NMR (400 MHz, CDCl_3) δ 155.71, 143.32, 142.07, 141.76, 134.42, 129.55, 128.39, 127.61, 121.45, 65.02, 14.82, 14.72.

HRMS: Theoretical m/z for $(M+H)^+$: 355.1793, Experimental m/z for $(M+H)^+$: 355.17997, $\Delta=1.9$ ppm

Synthesis of Compound 5²

To a solution of 4-hydroxybenzaldehyde (5 g, 41 mmol) in anhydrous dimethyl formamide (DMF) was added K_2CO_3 (16.95 g, 123 mmol), then reaction mixture stirred 16h at room temperature. Then 1,4-dibromobutane (17.70 g, 82 mmol) was added dropwise with stirring, and the reaction mixture was stirred for 12 h at 100 °C. The solvent was removed, water (100 mL) was added to the residue, and product was extracted with dichloromethane (DCM). Organic phase was collected, dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified using silica column chromatography using *n*-hexane:ethyl acetate (4:1) mixture as mobile phase. The compound **5** was obtained as colourless oil in the yield of 8.13 g (77%).

1H NMR (400 MHz, $CDCl_3$): δ 9.86 (s, 1H), 7.81 (d, $J = 8.8$ Hz, 2H), 6.98 (d, $J = 8.8$ Hz, 2H), 4.07 (t, $J = 5.8$ Hz, 2H), 3.48 (t, $J = 6.6$ Hz, 2H), 2.07-1.99 (m, 4H) ppm.

^{13}C NMR (400 MHz, $CDCl_3$): δ ppm; 191.11, 164.15, 132.21, 130.27, 114.99, 67.59, 33.46, 29.53, 27.92.

HRMS: Theoretical m/z for $(M+H)^+$: 257.0177, Experimental m/z for $(M+H)^+$: 257.0172, $\Delta=1.95$ ppm

Synthesis of Compound 6³

Compound **5** (4.18 g, 16 mmol) were dissolved in DMSO (20 mL), and was added NaN_3 (1.6 g, 24 mmol). The reaction mixture was stirred 60 °C for 3 h, then was controlled by TLC. After the reaction was completed, it was cooled to room temperature; water (50 mL) was added to the mixture and was extracted with diethyl ether. The organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was used in the next step without purification. The compound **6** was acquired as colorless oil in quantitative yield.

1H NMR (400 MHz, $CDCl_3$): δ ppm; 9.84 (s, 1H), 7.79 (d, 2H, $J=8.8$ Hz), 6.96 (d, 2H, $J=8.7$ Hz), 4.04 (t, 2H, $J=6.1$ Hz), 3.42 (t, 2H, $J=6.7$ Hz), 1.87 (m, 2H), 1.79 (m, 2H).

^{13}C NMR (400 MHz, $CDCl_3$): δ ppm; 191.109, 164.18, 132.18, 130.39, 114.84, 67.81, 51.30, 26.54, 25.85.

HRMS: Theoretical m/z for $(M+H)^+$: 220.1086, Experimental m/z for $(M+H)^+$: 220.10808, $\Delta=2.36$ ppm

Synthesis of compound 7

To a solution of compound **6** (1.65 g, 7.5 mmol) in anhydrous CH_2Cl_2 (150 mL) was purged with N_2 for 30 minutes. Then, 2,4-dimethylpyrrole (1.94 mL, 18.7 mmol) was added under N_2 . 5-6 drops of trifluoroacetic acid (TFA) were added to the reaction mixture, and the resulting mixture was stirred for 18 h under N_2 atmosphere at room temperature. After the complete consumption of aldehyde, *p*-chloranil (2.40 g, 9.0 mmol) was added to the reaction mixture. When the mixture was stirred for 3h, NEt_3 (7.4 mL) and $BF_3 \cdot Et_2O$ (11.4 mL) were added to the mixture, respectively. After the mixture was additionally stirred for 3h, it was washed twice with water, organic phase dried over anhydrous Na_2SO_4 , and concentrated

under reduced pressure. The crude product was purified by column chromatography using hexane and ethyl acetate mixture (4:1 to 2:1) as eluent to obtain greenish orange solid with a yield of 28%.

^1H NMR (400 MHz, Chloroform-*d*) δ 7.04 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 5.96 (s, 2H), 4.02 (t, J = 5.9 Hz, 2H), 3.37 (t, J = 6.6 Hz, 2H), 2.53 (s, 6H), 1.97 – 1.70 (m, 4H), 1.42 (s, 2H).

^{13}C NMR (400 MHz, Chloroform-*d*) δ 159.66, 155.49, 143.38, 142.07, 132.07, 129.52, 127.34, 121.42, 115.35, 67.52, 51.43, 26.74, 25.99, 14.87, 14.78.

HRMS: Theoretical m/z for $(\text{M}+\text{H})^+$: 438.2277, Experimental m/z for $(\text{M}+\text{H})^+$: 438.23178, Δ =9.3 ppm

Synthesis of compound 8

A solution of compound **7** (300 mg, 0.68 mmol) in 40 mL of DCM was cooled to 0 °C in an ice bath. NBS (366 mg, 2 mmol) was added to this solution and the reaction was stirred for approximately 3 hours in the dark. After controlling with TLC, the reaction mixture was poured into water and extracted with DCM. The collected organic phases were dried over Na_2SO_4 and the solvent was removed. The crude product was purified by column chromatography using solvent mixture *n*-hexane:DCM (3:1) as eluent, giving a red solid in 72% yield.

^1H NMR (400 MHz, Chloroform-*d*) δ 7.13 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H), 4.06 (t, J = 5.9 Hz, 2H), 3.41 (t, J = 6.4 Hz, 2H), 2.60 (s, 6H), 1.98-1.80 (m, 4H), 1.43 (s, 6H).

^{13}C NMR (400 MHz, Chloroform-*d*) δ 160.09, 153.99, 142.52, 140.98, 131.24, 129.49, 126.64, 115.50, 112.03, 67.73, 51.42, 29.92, 26.72, 25.97, 14.15.

Synthesis of compound PS1

In a round bottom flask, compound **8** (870 mg, 1.46 mmol) were dissolved in benzene (10 mL). 4-Pyridinecarboxaldehyde (413 μL , 4.37 mmol), piperidine (400 μL) and acetic acid (400 μL) were added to reaction mixture. The solution was refluxed for 2-3 hours. Any water formed during the reaction was removed using a Dean-Stark apparatus. After controlling with TLC, it was cooled to room temperature; the reaction was quenched by water and extracted with DCM. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography with silica gel by using ethyl acetate:MeOH (95:5) as eluent. The pure product was obtained as a bright crimson solid in yield of 13% (287 mg).

^1H NMR (400 MHz, Chloroform-*d*) δ 8.67 (d, J = 5.7 Hz, 4H), 8.02 (d, J = 16.7 Hz, 2H), 7.85 (d, J = 16.7 Hz, 2H), 7.49 (d, J = 5.1 Hz, 4H), 7.18 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.5 Hz, 2H), 4.09 (t, J = 6.0 Hz, 2H), 3.42 (t, J = 6.6 Hz, 2H), 1.97-1.84 (m, 4H), 1.50 (s, 6H).

^{13}C NMR (400 MHz, Chloroform-*d*) δ 160.40, 150.61, 147.92, 144.01, 142.60, 136.52, 133.41, 129.54, 126.41, 122.18, 121.71, 116.28, 115.80, 110.82, 67.53, 51.63, 26.88, 26.03, 14.46.

HRMS: Theoretical m/z for $(\text{M}+\text{H})^+$: 774.0997, Experimental m/z for $(\text{M}+\text{H})^+$: 774.09845, Δ =1.61 ppm

Synthesis of compound PS2

A solution of compound **PS1** (134 mg, 0.17 mmol) in 10 mL of dry acetonitrile was added 4-(bromomethyl) phenyl acetate **2** (397 mg, 1.73 mmol), then the mixture was stirred at 80 °C for 48 h. After controlling with TLC, it was cooled to room temperature and removal of the solvent under vacuum. The residue dissolved in DCM, and it was precipitated in toluene twice, the compound **PS2** was collected by filtration, then the impurities washed off with the *n*-hexane solvent. The pure product was obtained as a dark green solid in yield of 87%. Due to low solubility and low stability in DMSO, ¹³C NMR spectrum cannot be recorded.

¹H NMR (400 MHz, DMSO-*d*₆) δ 9.19 (d, *J* = 6.3 Hz, 4H), 8.39 (d, *J* = 6.3 Hz, 4H), 8.15 (d, *J* = 16.6 Hz, 2H), 7.92 (d, *J* = 16.6 Hz, 2H), 7.60 (d, *J* = 8.6 Hz, 4H), 7.42 (d, *J* = 7.9 Hz, 2H), 7.23 (m, 6H), 5.86 (s, 4H), 4.12 (t, *J* = 6.4 Hz, 2H), 3.44 (t, *J* = 6.8 Hz, 2H), 2.27 (s, 6H), 1.82 (q, *J* = 7.1 Hz, 2H), 1.74 (q, *J* = 6.9 Hz, 2H), 1.51 (s, 6H).

HRMS: Theoretical *m/z* for (M)⁺₂: 535.6062, Experimental *m/z* for (M)⁺₂: 535.60385, Δ=4.39 ppm.

Synthesis of compound 9

In a round bottom flask, compound **4** (100 mg, 0.28 mmol) were dissolved in benzene (10 mL). *p*-anisaldehyde (115 mg, 0.85 mmol), piperidine (300 μL) and acetic acid (300 μL) were added to reaction mixture. The solution was refluxed for 2-3 hours. Water formed during the reaction was removed using a Dean-Stark apparatus. After controlling with TLC, it was cooled to room temperature; the reaction was quenched with water and extracted with DCM. The organic phase was dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography with silica gel by using acetone:hexane (50:50) as eluent. The pure product was obtained as green solid in yield of 58% (96 mg).

¹H NMR (400 MHz, CDCl₃) δ 7.64-7.55 (m, 6H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 16.3 Hz, 2H), 6.93 (d, *J* = 8.8 Hz, 4H), 6.60 (s, 2H), 4.80 (s, 2H), 3.84 (s, 6H), 1.42 (s, 6H).

¹³C NMR (400 MHz, CDCl₃) δ 160.63, 152.95, 142.01, 141.93, 140.90, 136.00, 134.74, 133.42, 129.79, 129.27, 128.98, 127.52, 117.73, 117.48, 114.50, 65.08, 55.61, 14.99.

HRMS: Theoretical *m/z* for (M+H)⁺: 591.2631, Experimental *m/z* for (M+H)⁺: 591.2659, Δ=4.7 ppm

Synthesis of compound Q

In a round bottom flask, 6-heptynoic acid (21 μL, 0.17 mmol) were dissolved in dry DCM (10 mL), DCC (171 mg, 0.85 mmol) and DMAP (21 mg, 20% mmol) were added into the solution and the reaction mixture was stirred for 1 hour at room temperature. Then, compound **5** (40 mg, 0.067 mmol) dissolved in dry DCM (5 mL) was then added to the mixture. Reaction was stirred at room temperature overnight. After controlling with TLC, solvent was evaporated under reduced pressure and the resulting crudes were purified by column chromatography with silica gel by using *n*-hexane:DCM (1:1) as eluent. The pure product was obtained as a dark blue-red solid in yield of 64% (30 mg).

¹H NMR (400 MHz, Chloroform-*d*) δ 7.68-7.55 (d + d, 2H + 4H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.22 (d, *J* = 16.2 Hz, 2H), 6.93 (d, *J* = 8.8 Hz, 4H), 6.61 (s, 2H), 5.22

(s, 2H), 3.85 (s, 6H), 2.44 (t, $J = 7.4$ Hz, 2H), 2.23 (td, $J = 7.0, 2.6$ Hz, 2H), 1.97 (t, $J = 2.6$ Hz, 1H), 1.80 (m, 2H), 1.60 (m, 2H), 1.42 (s, 6H).

^{13}C NMR (400 MHz, CDCl_3) δ 173.36, 160.43, 152.80, 151.93, 141.68, 137.52, 137.07, 135.86, 133.11, 129.56, 129.07, 128.45, 124.77, 117.58, 117.23, 114.28, 77.34, 68.70, 65.59, 55.40, 33.76, 29.71, 27.79, 24.03, 18.15, 14.75.

HRMS: Theoretical m/z for $(\text{M}+\text{H})^+$: 698.3127, Experimental m/z for $(\text{M}+\text{H})^+$: 698.3250, $\Delta=17.6$ ppm.

Synthesis of compound PS3

A solution of compound **PS2** (49 mg, 0.039 mmol) in 10 mL of dry DCM was added compound **Q** (45 mg, 0.063 mmol). Then $\text{Cu}(\text{MeCN})_4\text{PF}_6$ (75 mg, 0.2 mmol) were added into the solution and the reaction mixture was stirred for 48 hour at room temperature. After controlling with TLC, the resulting crude were diluted in DCM and washed with EDTA. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue dissolved in DCM, and it was precipitated in toluene twice, the compound **PS3** was collected by filtration, then the impurities washed off with the *n*-hexane solvent. The pure product was obtained as a dark blue solid in yield of 50% (35 mg). Due to low solubility and low stability in DMSO, ^{13}C NMR spectrum cannot be recorded.

^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 9.10 (m, 4H), 8.34 (d, $J = 18.4$ Hz, 2H), 8.12 (d, $J = 20.4$ Hz, 2H), 7.90 (d, $J = 15.8$ Hz, 2H), 7.85 (s, 1H), 7.55 (d, $J = 8.4$ Hz, 4H), 7.53 – 7.45 (m, 6H), 7.40 – 7.35 (m, 4H), 7.21 (d, $J = 8.4$ Hz, 6H), 7.00 (d, $J = 8.4$ Hz, 2H), 6.97 (d, $J = 8.7$ Hz, 2H), 6.88 (s, 2H), 5.80 (s, 4H), 5.19 (s, 2H), 4.36 (t, $J = 7.0$ Hz, 2H), 4.09 (t, $J = 6.8$ Hz, 2H), 3.75 (s, 6H), 3.41 (t, $J = 6.8$ Hz, 2H), 3.09 (s, 6H), 2.64 – 2.51 (m, 2H), 2.25 (s, 6H), 1.95 (m, 2H), 1.80 (m, 2H), 1.76 – 1.64 (m, 4H), 1.50 (s, 3H), 1.43 (s, 3H), 1.34 (s, 6H).

HRMS: Theoretical m/z for $(\text{M})^{+2}$: 884.76516, Experimental m/z for $(\text{M})^{+2}$: 884.76516, $\Delta=2.89$ ppm.

4. NMR and HRMS Spectra

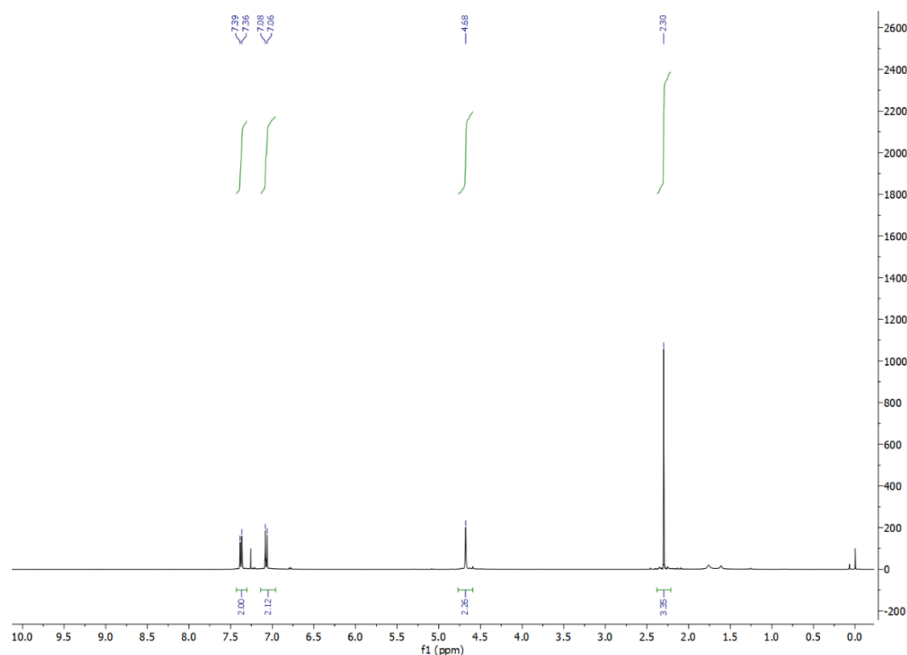


Figure S10. ^1H NMR spectrum of compound **1** (400 MHz, CDCl_3)

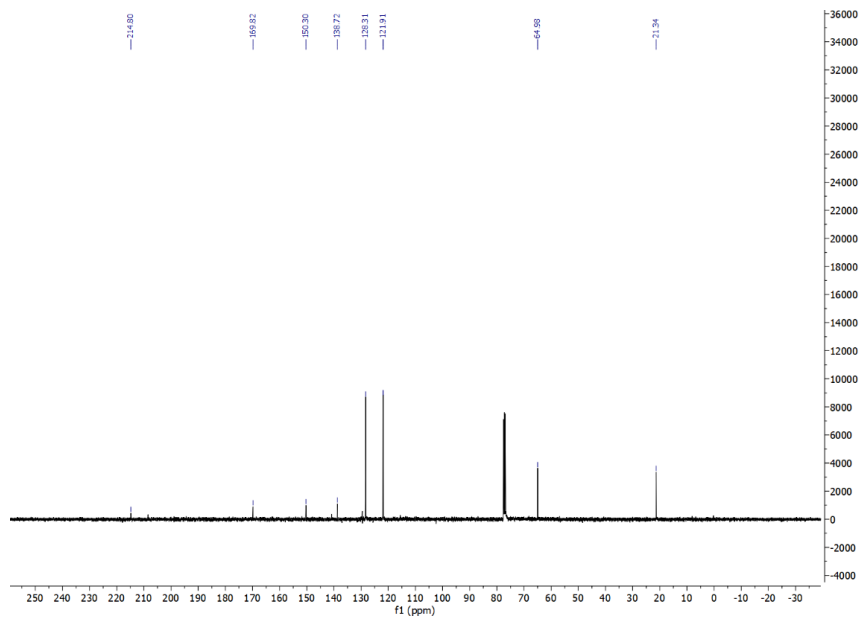


Figure S11. ¹³C NMR spectrum of compound **1** (400 MHz, CDCl₃)

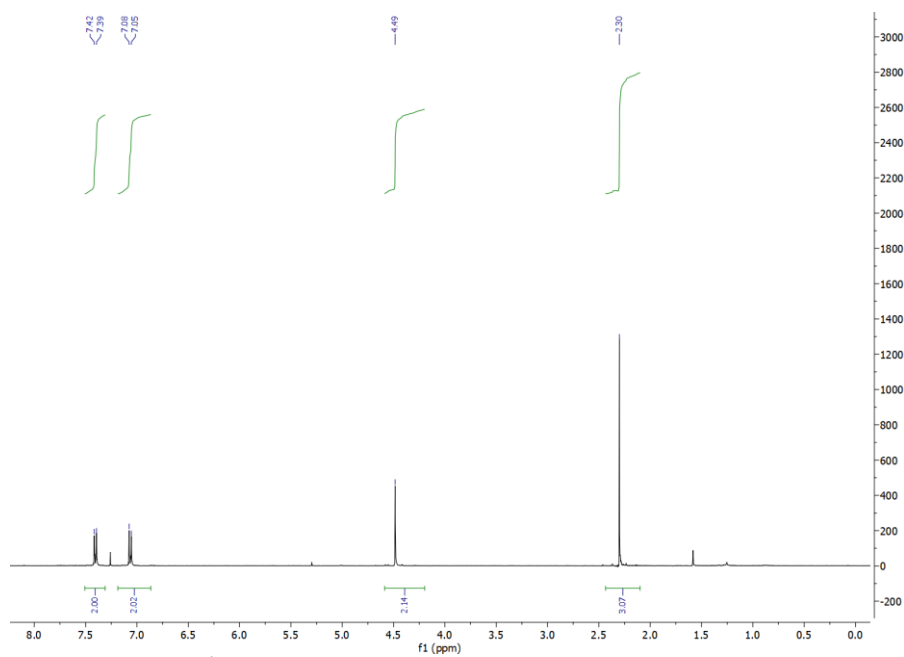


Figure S12. ¹H NMR spectrum of compound **2** (400 MHz, CDCl₃)

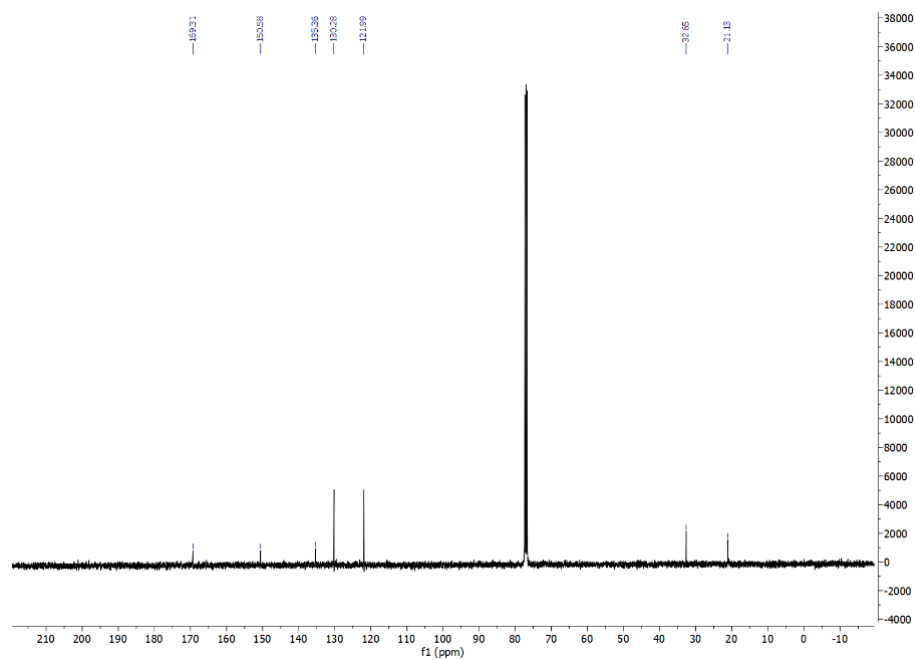


Figure S13. ¹³C NMR spectrum of compound **2** (400 MHz, CDCl₃)

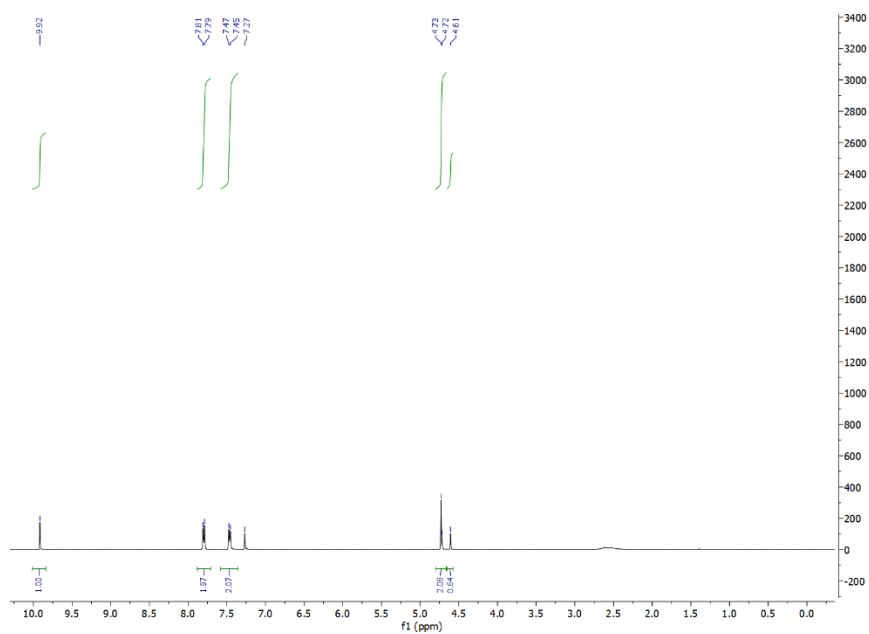


Figure S14. ¹H NMR spectrum of compound **3** (400 MHz, CDCl₃)

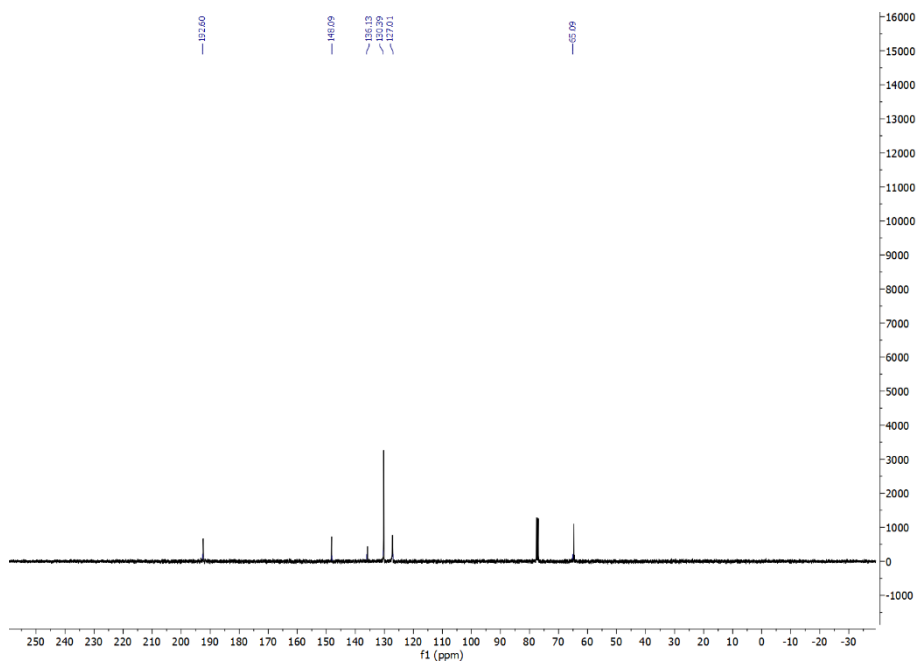


Figure S15. ¹³C NMR spectrum of compound **3** (400 MHz, CDCl₃)

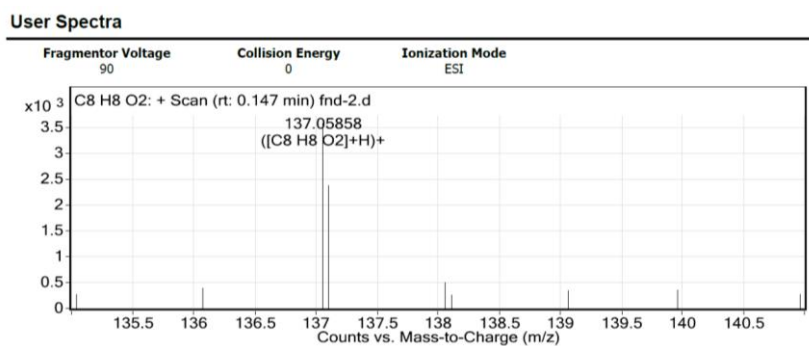


Figure S16. High Resolution Mass Spectrum of compound **3**.

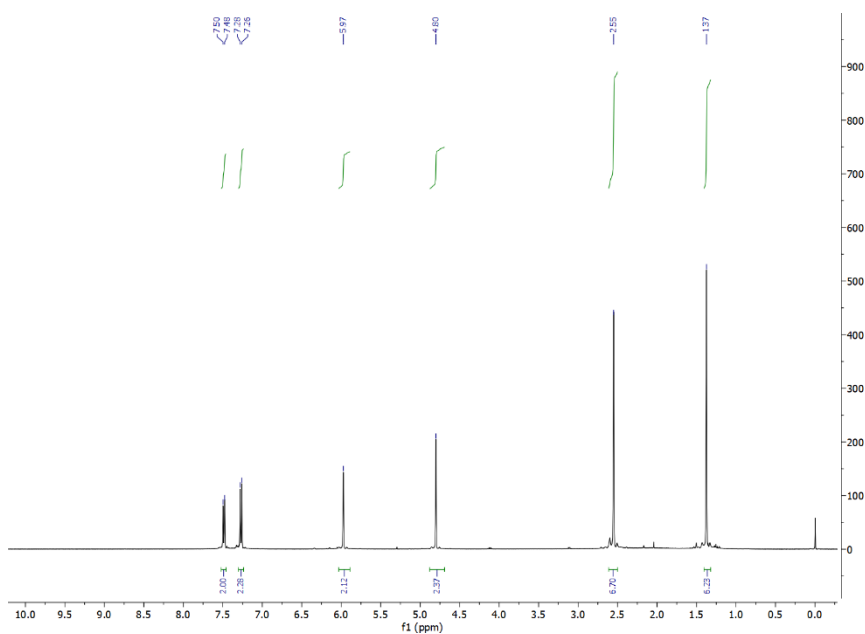


Figure S17. ¹H NMR spectrum of compound **4** (400 MHz, CDCl₃)

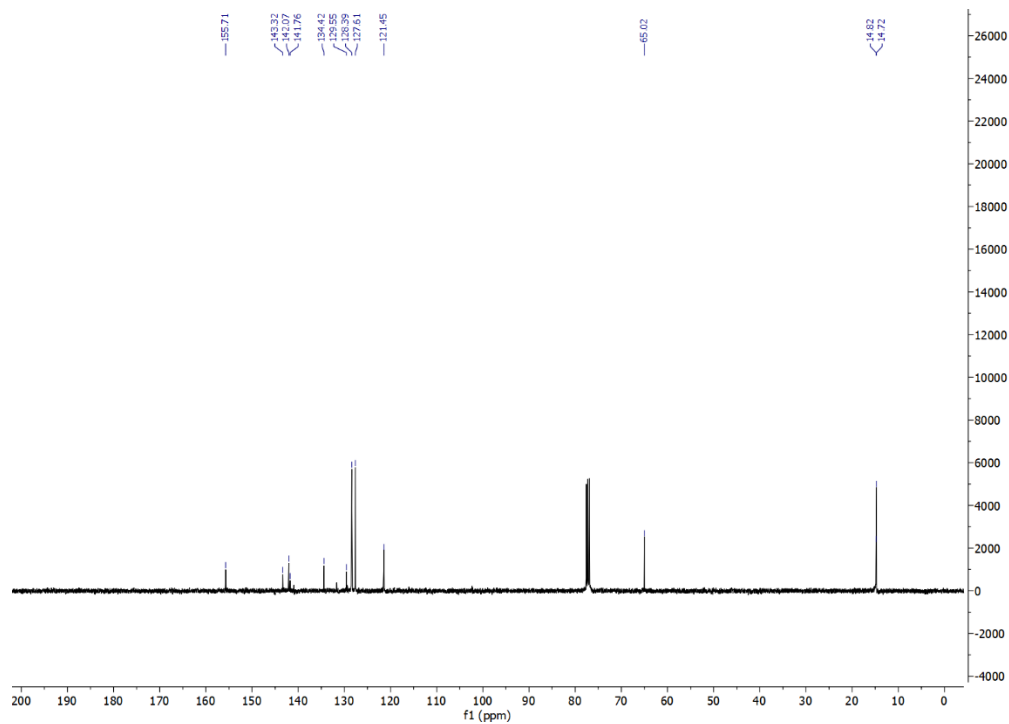


Figure S18. ¹³C NMR spectrum of compound **4** (400 MHz, CDCl₃)

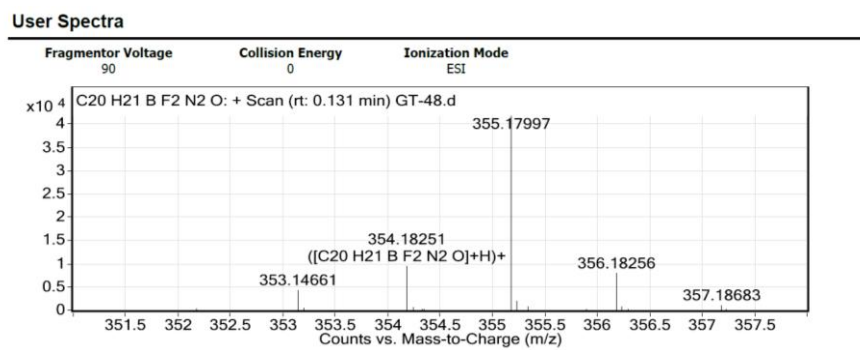


Figure S19. High Resolution Mass Spectrum of compound **4**.

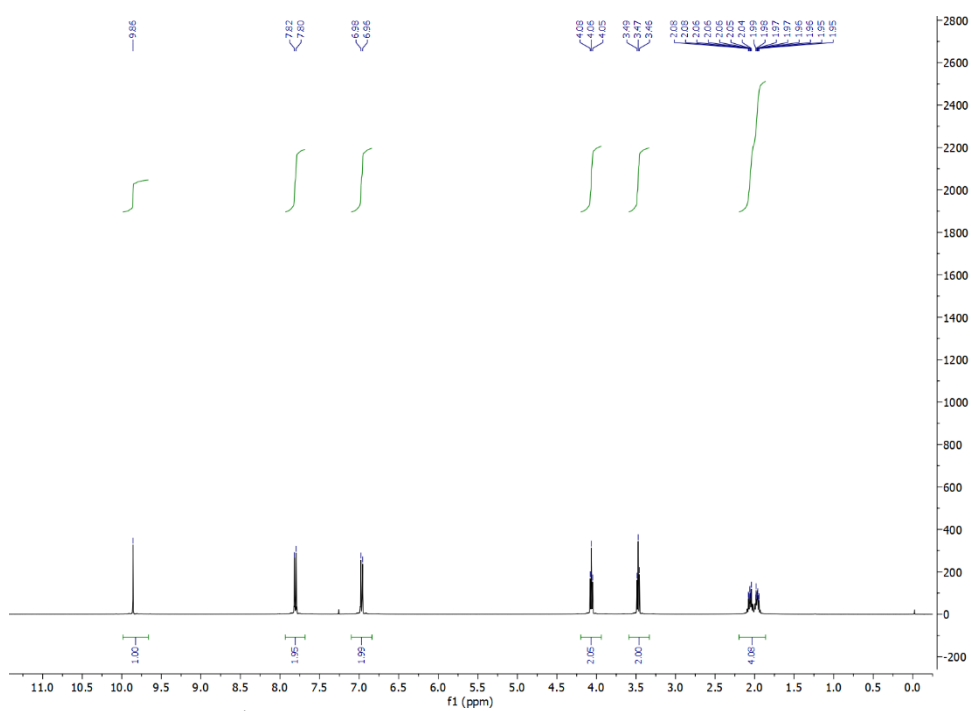


Figure S20. ¹H NMR spectrum of compound **5** (400 MHz, CDCl₃)

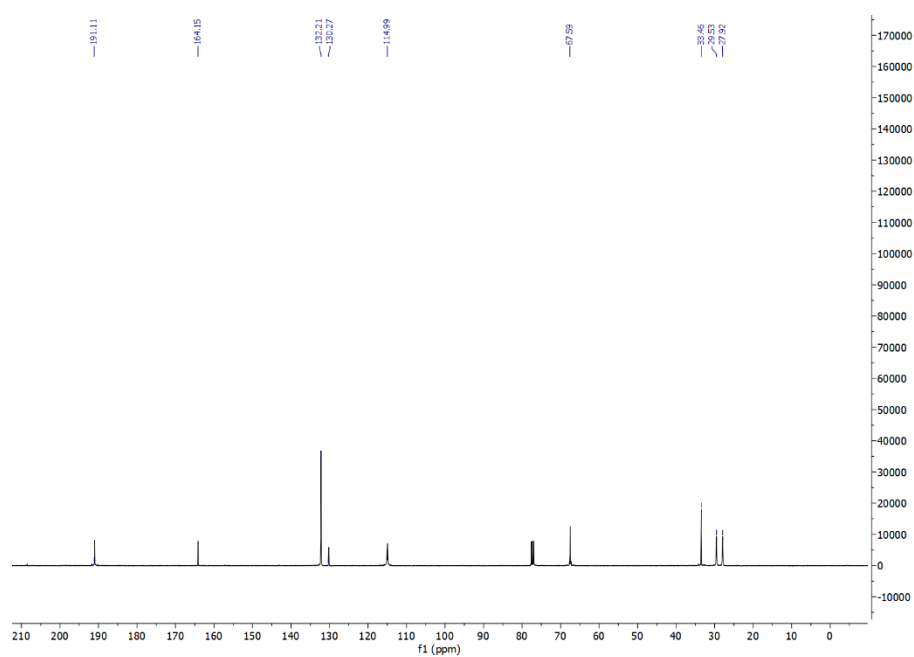


Figure S21. ¹³C NMR spectrum of compound **5** (400 MHz, CDCl₃)

User Spectra

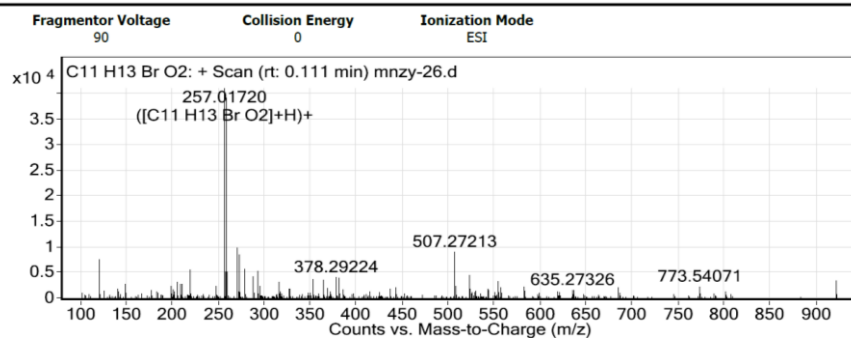


Figure S22. High Resolution Mass Spectrum of compound **5**

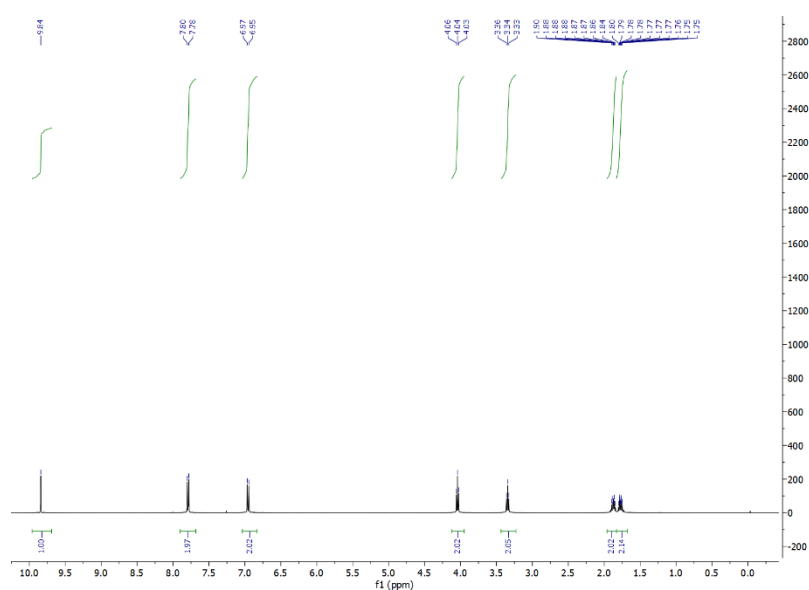


Figure S23. ^1H NMR spectrum of compound **6** (400 MHz, CDCl_3)

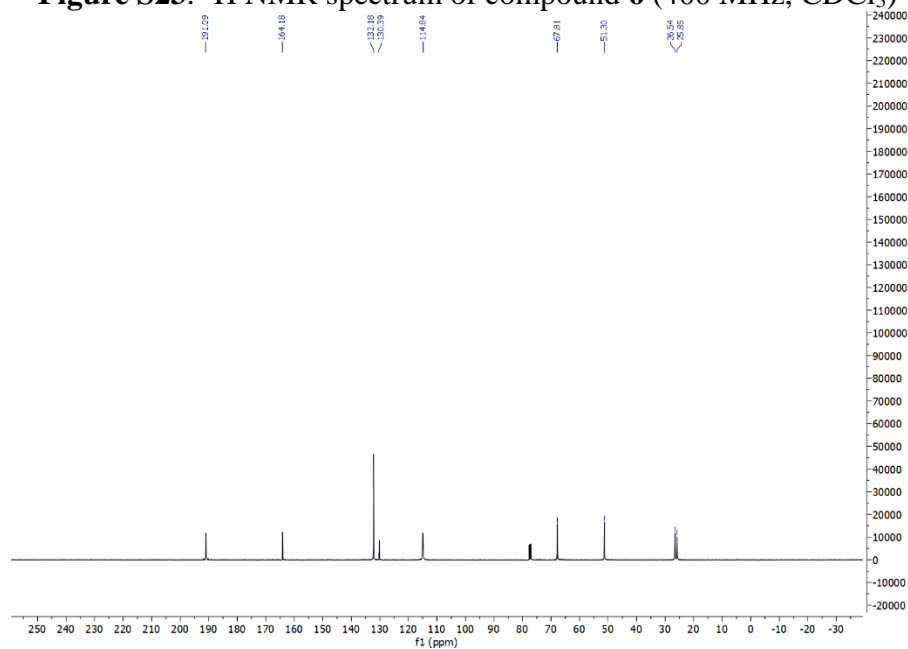


Figure S24. ^{13}C NMR spectrum of compound **6** (400 MHz, CDCl_3)

Fragmentor Voltage 90 Collision Energy 0 Ionization Mode ESI

x10⁴ C11 H13 N3 O2: + Scan (rt: 0.112 min) mnzy-78.d

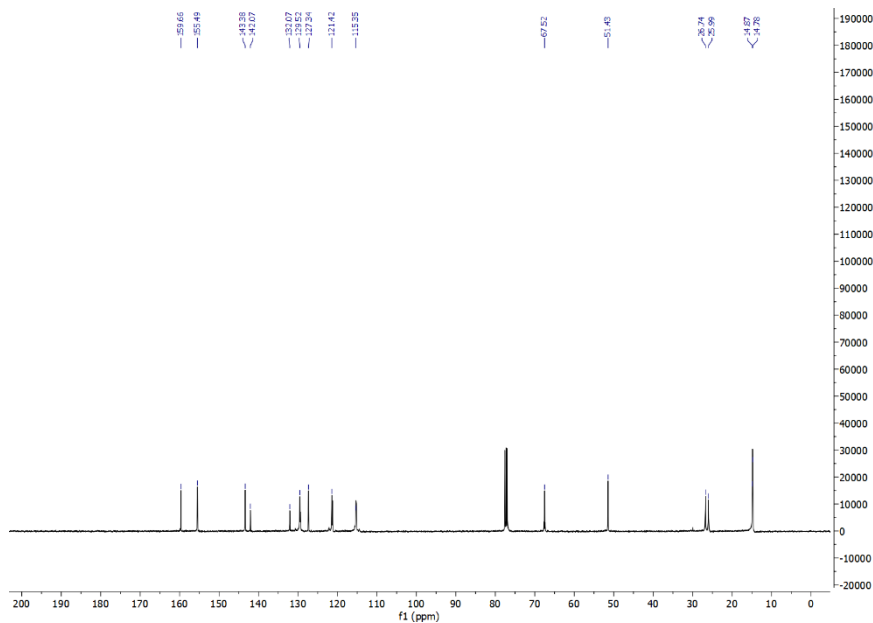
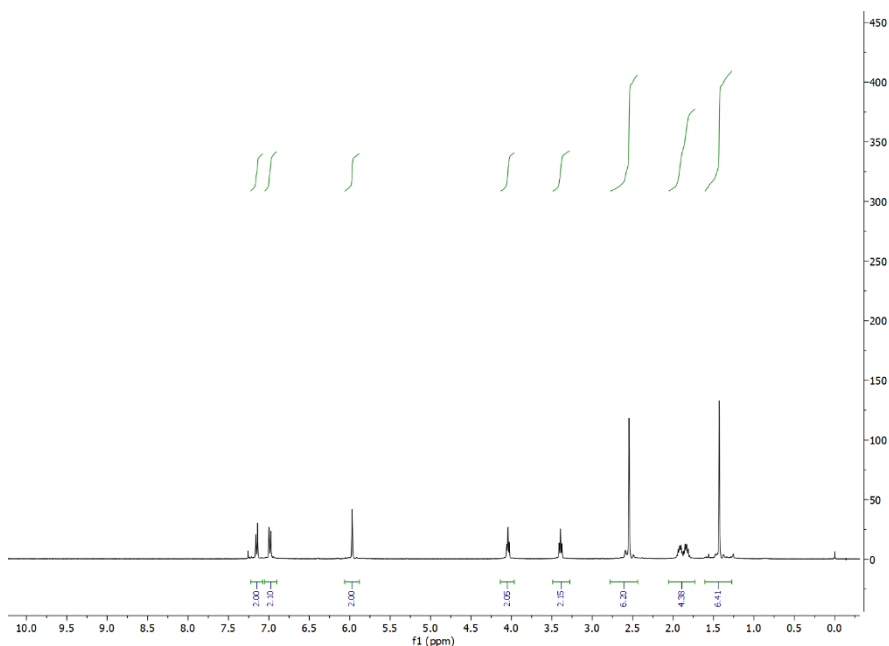
220.10808
([C11 H13 N3 O2]+H)+

475.17174

677.34491

922.00980

Counts vs. Mass-to-Charge (m/z)



User Spectra

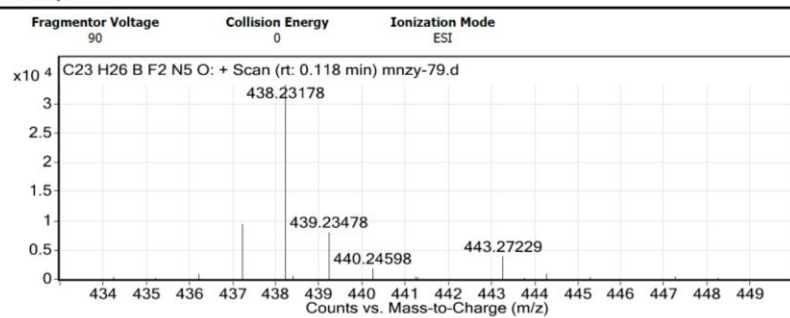


Figure S28. High Resolution Mass Spectrum of compound **7**.

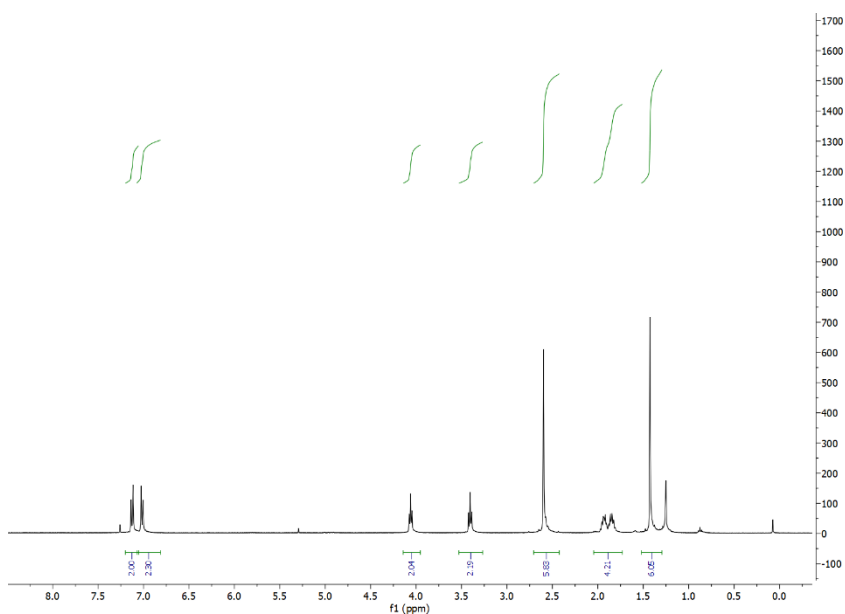


Figure S29. ¹H NMR spectrum of compound **8** (400 MHz, CDCl₃)

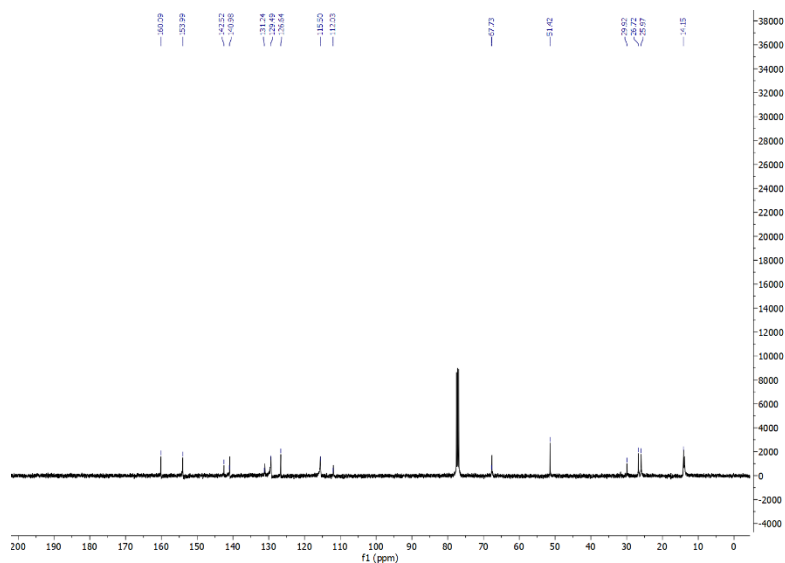


Figure S30. ¹³C NMR spectrum of compound **8** (400 MHz, CDCl₃)

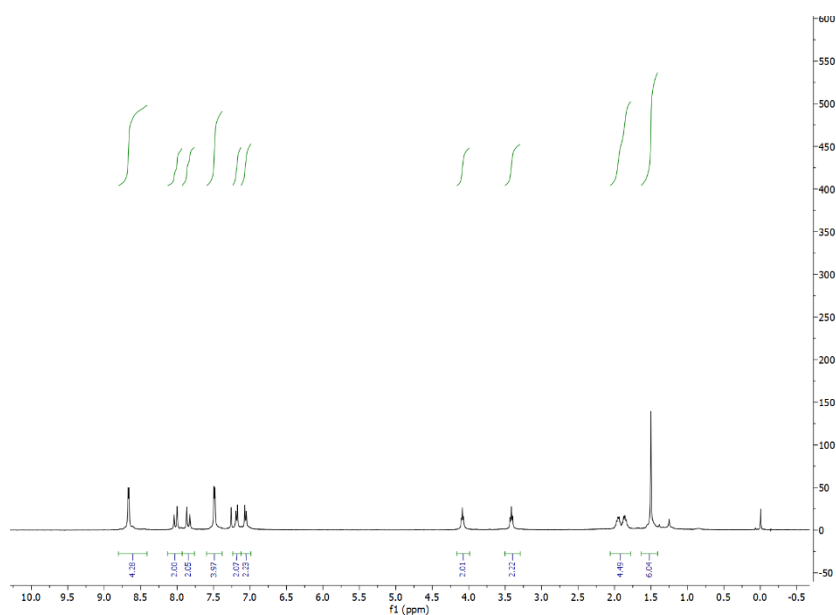


Figure S31. ^1H NMR spectrum of compound **PS1** (400 MHz, CDCl_3)

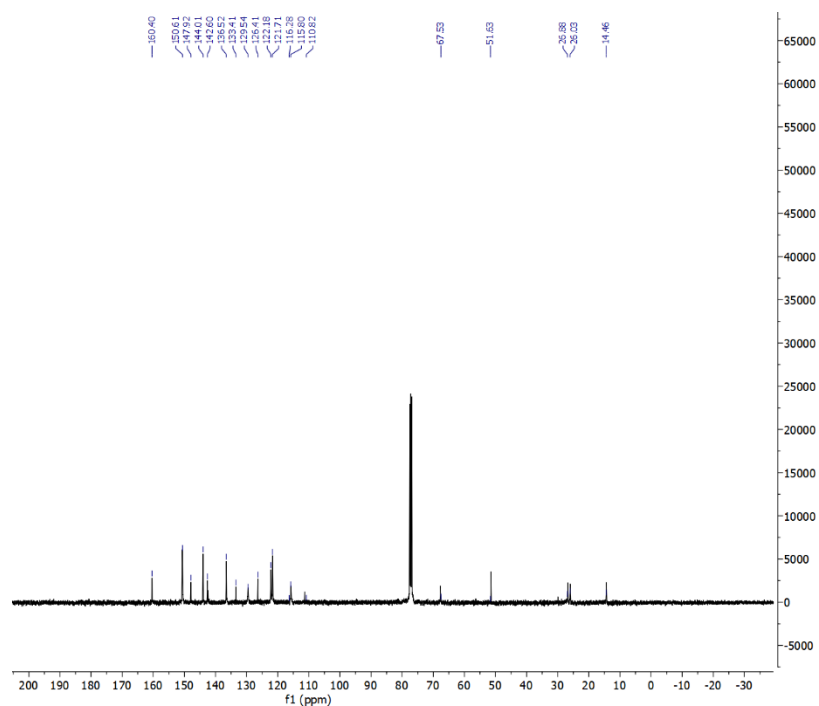


Figure S32. ^{13}C NMR spectrum of compound **PS1** (400 MHz, CDCl_3)

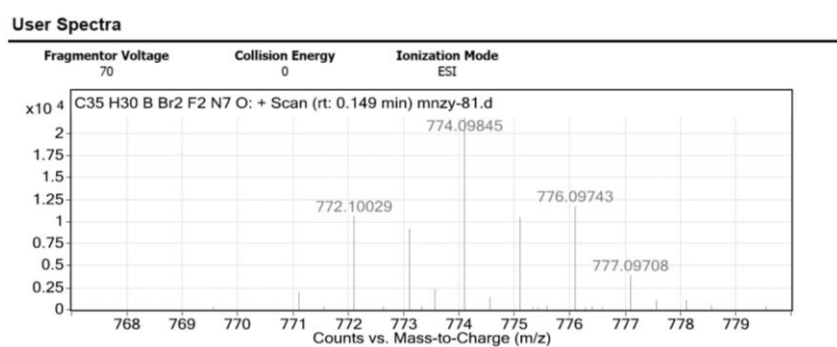


Figure S33. High Resolution Mass Spectrum of compound **PS1**.

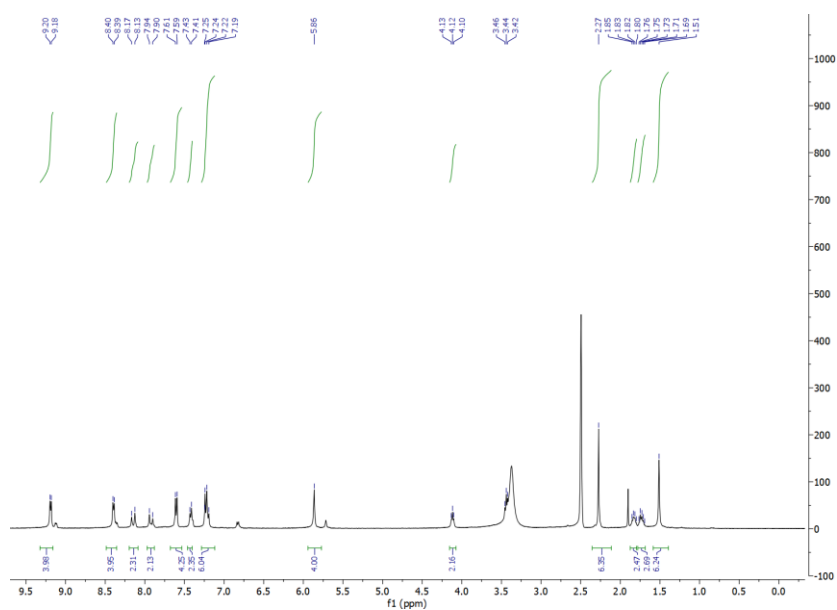


Figure S34. ^1H NMR spectrum of compound **PS2** (400 MHz, d-DMSO)

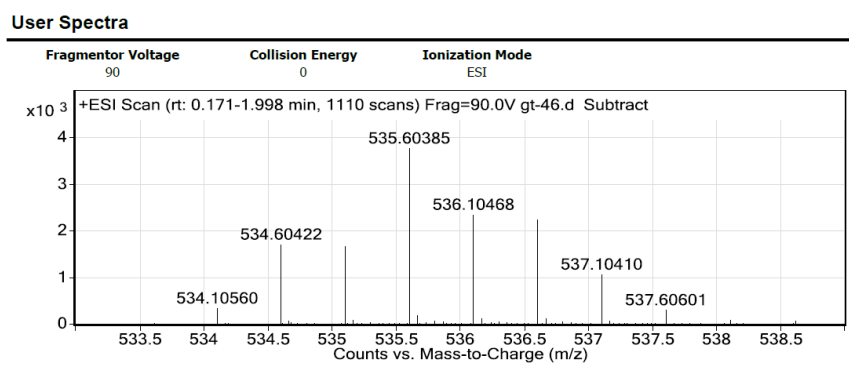


Figure S35. High Resolution Mass Spectrum of compound **PS2**.

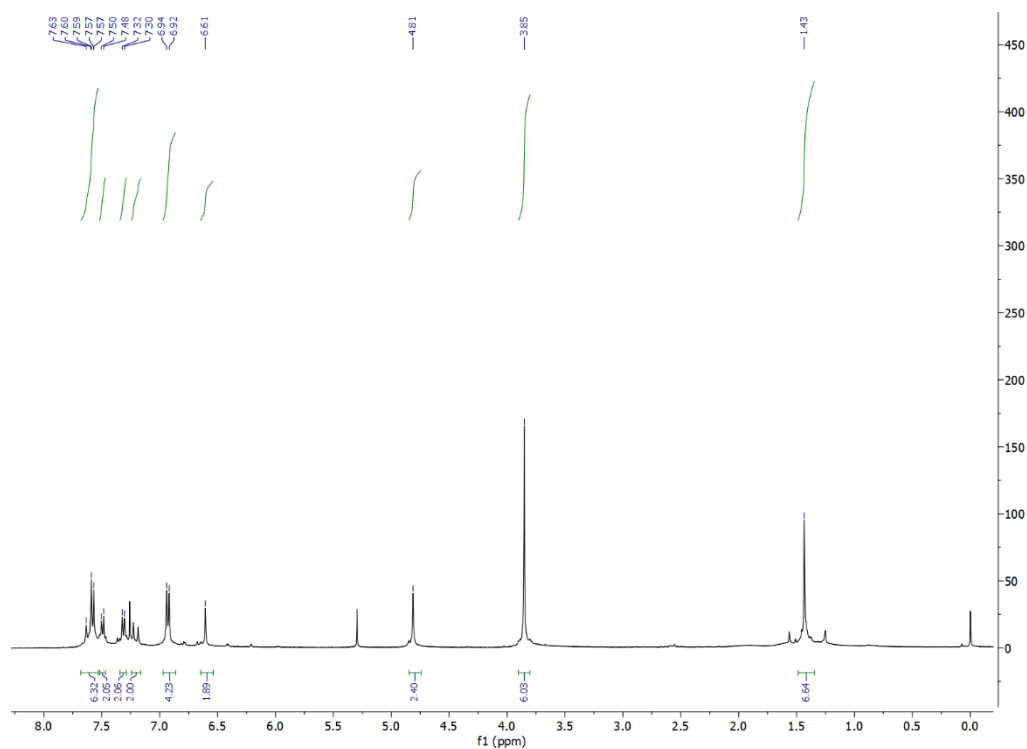


Figure S36. ¹H NMR spectrum of compound **9** (400 MHz, d-DMSO)

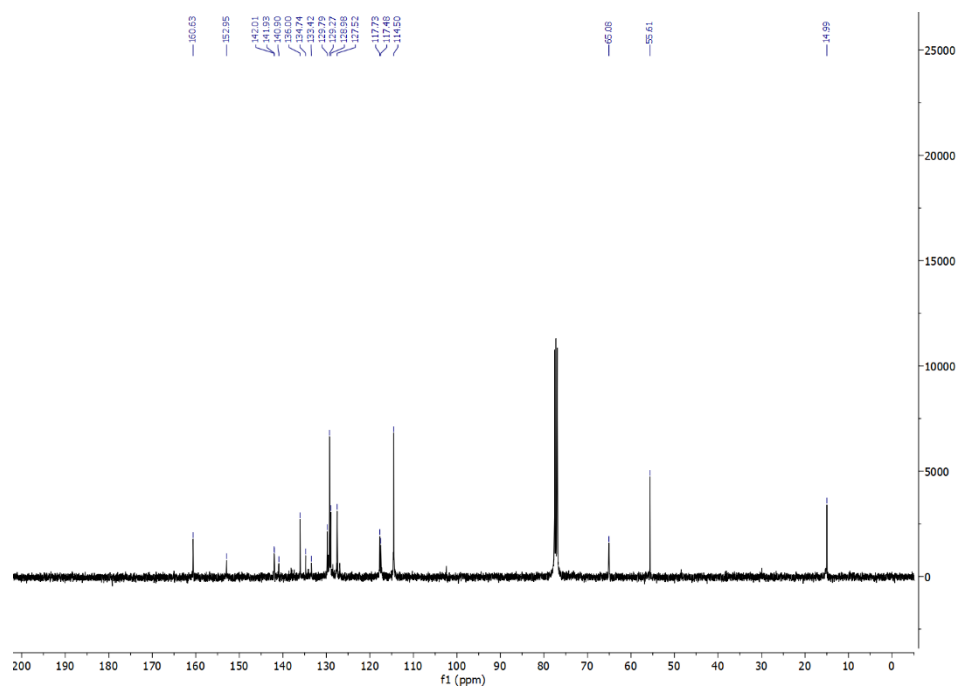
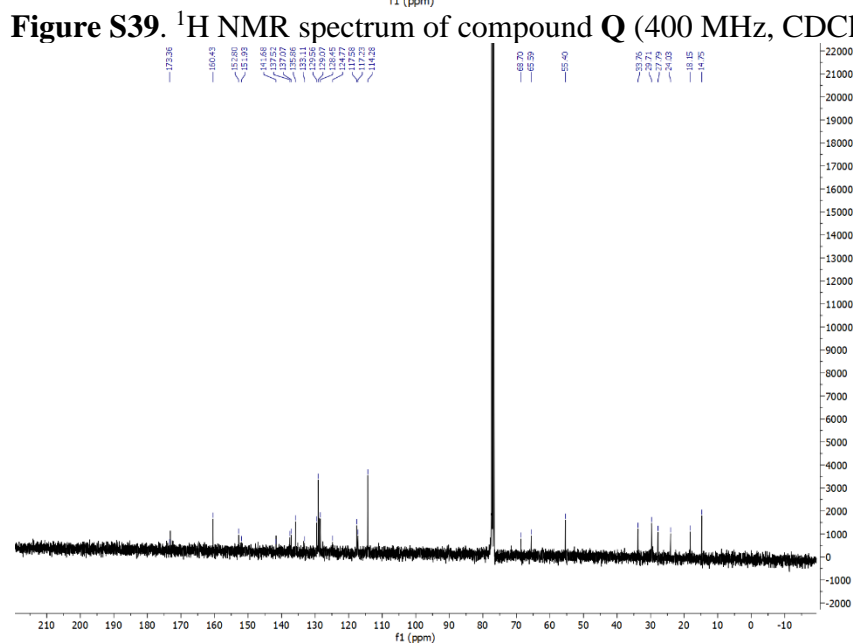
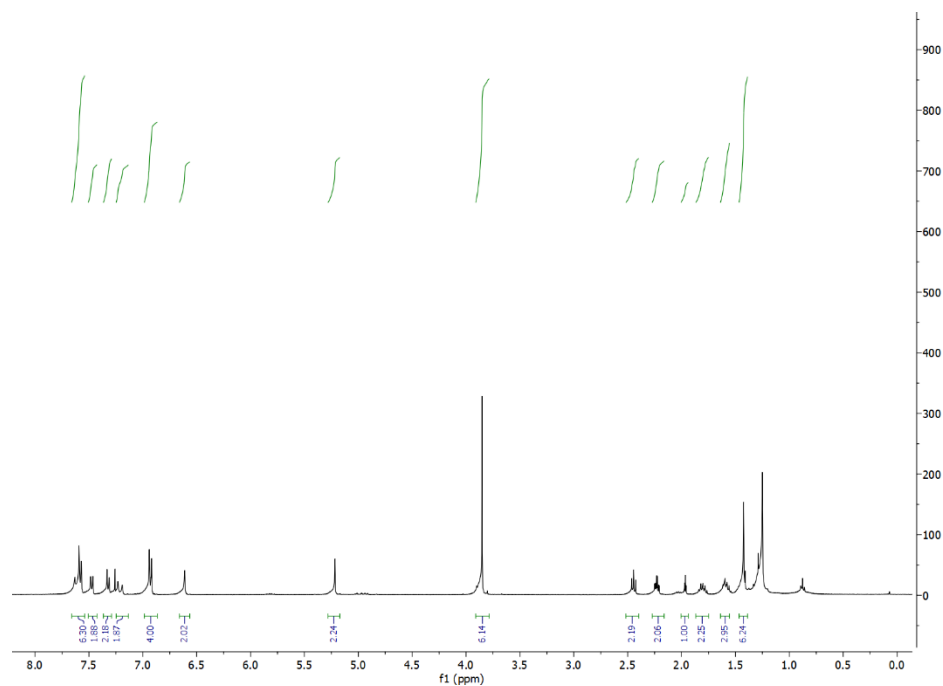
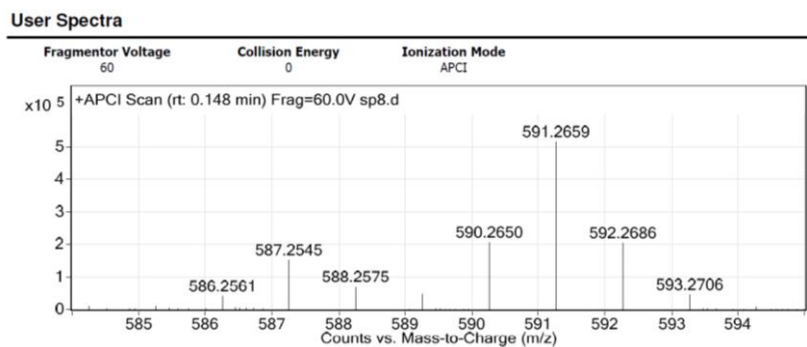


Figure S37. ¹³C NMR spectrum of compound **9** (400 MHz, d-DMSO)



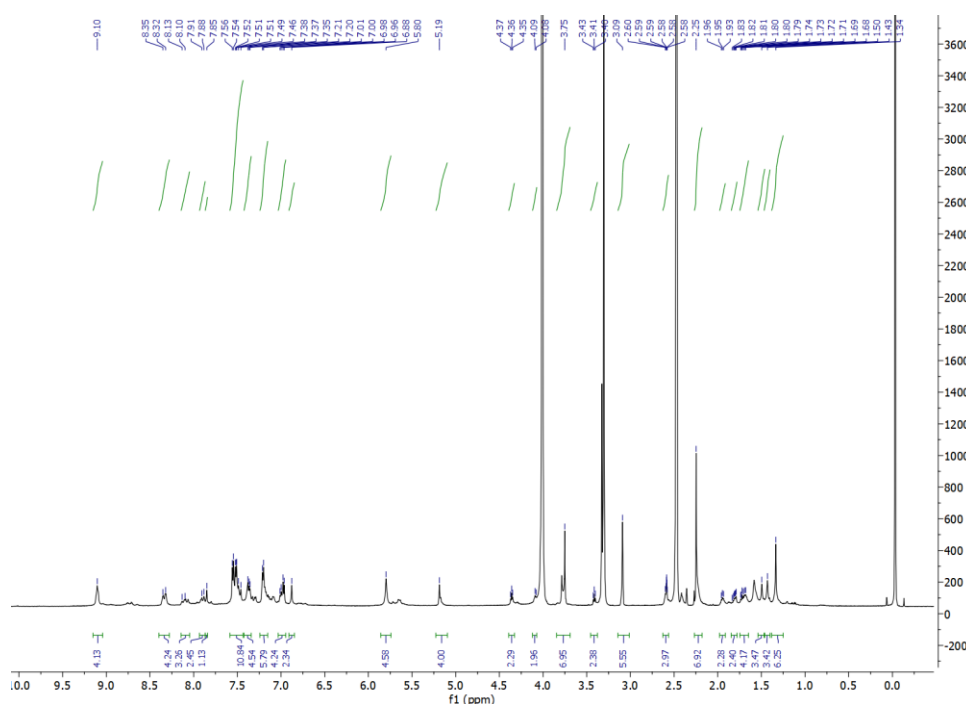
Fragmentor Voltage 90 Collision Energy 0 Ionization Mode ESI

x10³ C43 H41 B F2 N2 O4: + Scan (rt: 0.171 min) SEC17.d

698.32500
([C43 H41 B F2 N2 O4]+H)+

697.24744 700.29391

Counts vs. Mass-to-Charge (m/z)



Fragmentor Voltage 90 Collision Energy 0 Ionization Mode ESI

+ESI Scan (rt: 0.137 min) Frag=90.0V 60.d

Counts vs. Mass-to-Charge (m/z)

m/z	Relative Intensity (x10 ³)
883.26741	~1.0
884.26490	~3.2
884.76516	~6.0
885.76446	~4.8
886.26417	~3.0
886.76109	~2.0

5. References

- ¹ M.D. Kars, O.D. Iseri, U. Gunduz, J. Molnar, *Chemotherapy*, **2008**, 54, 194-200.
- ² P. Zhang, F. Deng, Y. Peng, H. Chen, Y. Gao, H. Li, *RSC Adv.* **2014**, 5, 47361-47367.
- ³ E.T. Ecik, E. Senkuytu, Z. Cebesoy, G.Y. Ciftci, *RSC Adv.* **2016**, 6, 47600-47606.