Supporting Information

Non-covalent Functionalized SWNTs as Potential Delivery Agents for Novel Bodipy-based PDT Sensitizers

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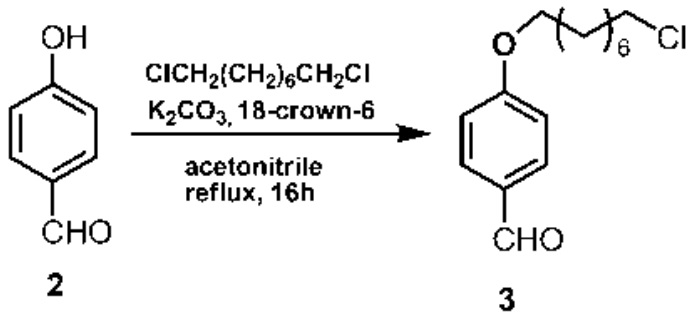
EXPERIMENTAL PROCEDURES

General:

All chemicals and solvents purchased from Aldrich were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker DPX-400 in $CDCl_3$ with TMS as internal reference. Absorption spectrometry was performed using a Varian spectrophotometer. Steady state fluorescence measurements were conducted using a Varian Eclipse spectrofluorometer. Column chromatography of all products was performed using Merck Silica Gel 60 (particle size: 0.040–0.063 mm, 230–400 mesh ASTM). Reactions were monitored by thin layer chromatography using fluorescent coated aluminum sheets. Solvents used for spectroscopy experiments were spectrophotometric grade. Mass spectrometry measurements were done at the Ohio State University Mass Spectrometry and Proteomics Facility, Ohio, U.S.A. AFM images were acquired in UNAM, Ankara, using PSIA XE-100E AFM and Multi75AI AFM tip. TEM images were aquired in UNAM, Ankara, using FEI Technai G2 F30 high resolution transmission electron microscope and carbon grid. 1-2 nm X 500 nm SWNT was purchased from Aldrich (catalog no: 652512).

Synthesis:

4-(8-chlorooctyloxy)benzaldehyde (3).

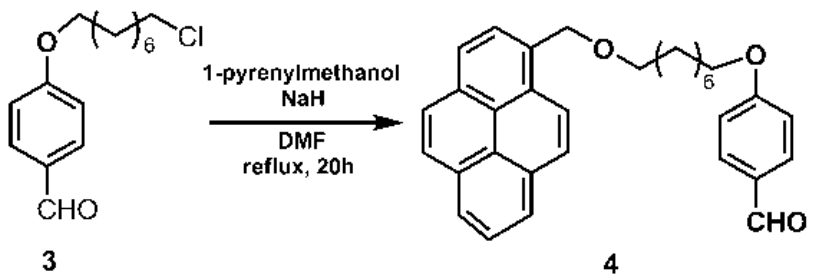


1,4-hydroxybenzaldehyde (1.25 g, 10 mmol) and 1,8 dichlorooctane (2.75 g, 15 mmol) were dissolved in acetonitrile (100 ml). K_2CO_3 (2.76 g, 20 mmol) and a few crystals of 18-crown-6 were added. The reaction mixture was refluxed for 16h. Then, acetonitrile was evaporated in vacuo and extracted with water and chloroform. Organic layer as dried with Na_2SO_4 and evaporated in vacuo.

The product was purified by silica gel column chromatography using $CHCl_3$/Hexane (80:20, v/v). Fraction containing compound **3** was collected then the solvent was removed under reduced pressure (3 mmol, 806 mg, 30%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 1.30-1.52 (m, 8H), 1.72-1.89 (m, 4H), 3.54 (t, *J*= 6.7 Hz, 2H), 4.05 (t, *J*= 6.5 Hz, 2H), 6. 98 (d, *J*= 8.76 Hz, 2H), 7.83 (d, *J*= 8.8 Hz, 2H), 9.89 (s, 1H) ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 22.85, 26.76, 28.76, 29.00, 29.13, 32.56, 45.08, 68.33, 114.74, 131.97, 164.22, 190.78. HRMS-ESI: calculated for [M+H] 269.1308, found 269.1303, Δ= 1.9 ppm

4-(8-(pyren-1-ylmethoxy)octyloxy)benzaldehyde (**4**).

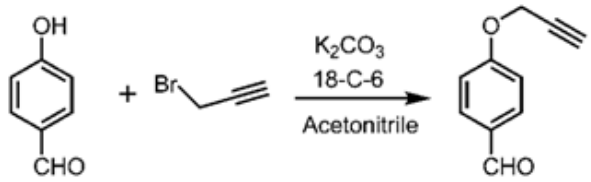


3 **4**

Pyrenylmethanol (696.84 mg, 3 mmol) was dissolved in DMF (5 ml). 120 mg 60% NaH was added slowly and the reaction mixture was stirred at room temperature for 20 min. 1,8-dichlorooctane **3** dissolved in DMF (5 ml) and added into the previous solution. After 20h of reflux, it was extracted with saturated $NaHCO_3$ aqueous solution and $CHCl_3$, organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$/Hexane (75:25, v/v). Fraction containing compound **4** was collected then the solvent was removed under reduced pressure (279 mg, 0.6 mmol, 20%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 1.22-1.42 (m, 8H), 1.62-1.79 (m, 4H), 3.62 (t, *J*= 6.5 Hz, 2H), 3.96 (t, *J*= 6.6 Hz, 2H), 5.23 (s, 2H), 6.96 (d, *J*=8.7 Hz, 2H), 7.82 (d, *J*=8.8 Hz, 2H), 8.01-8.13 (m, 4H), 8.14-8.21 (m, 4H), 8.40 (d, *J*=9.2 Hz, 1H), 9.88 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 25.8, 26.1, 29.0, 29.2, 29.8, 68.3, 70.4, 71.5, 114.7, 123.6, 124.5, 125.0, 125.2, 125.9, 126.9, 127.3, 127.4, 127.6, 129.4, 129.7, 130.8, 131.2, 131.3, 131.8, 132.0, 164.2, 190.8. HRMS-ESI: calculated for M+Na 487.2249, found 487.2217, Δ= 6.6 ppm

4-(prop-2-ynyloxy)benzaldehyde (5).

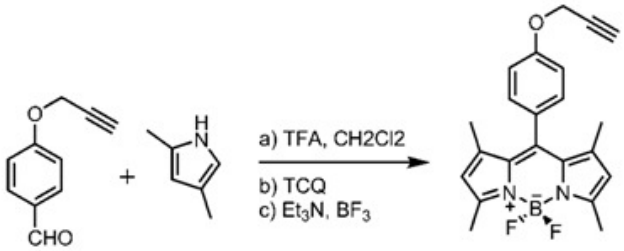


2 **5**

4-hydroxybenzaldehyde (2.45 g, 20 mmol) and propargyl bromide (2.5 g, 30 mmol) were dissolved in 100 ml acetonitrile. K_2CO_3 (5.5 g, mmol) and a few crystals of 18-crown-6 were added. The reaction was refluxed until all 4-hydroxybenzaldehyde consumed. The solvent was evaporated in vacuo, extracted with water and $CHCl_3$. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$/Hexane (50:50, v/v). Fraction containing compound **5** was collected then the solvent was removed under reduced pressure (2.85 g, 17.8 mmol, 89%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.58 (t, *J*=2.5 Hz, 1H) 4.79 (dd, J=1.48, 0.9 Hz), 7.11 (d, *J*=7.1 Hz, 2H), 7.87 (d, *J*=7.0 Hz, 2H), 9.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 55.9, 76.4, 77.5, 115.2, 131.9, 162.4, 190.8, 207.7. HRMS-ESI: calculated for M+Na 183.0422, found 183.0422, Δ= 0 ppm

1,3,5,7-Tetramethyl-8-(4-Propargyloxyphenyl)-4,4-difloroboradiaza-*s*-indacene (6).



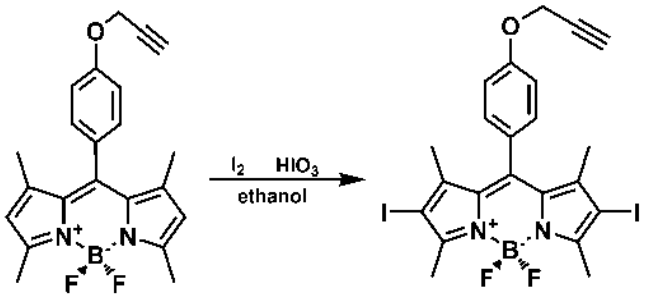
5 **6**

CH_2Cl_2 (300 ml) was purged with Ar for 30 min. 4-(prop-2-ynyloxy)benzaldehyde **5** (1 g, 6.2 mmol) and 2,4-dimethyl pyrrole (1.2 g, 12.6 mmol) were added. The color of the solution turned into red after the addition of 3 drops of trifluoroacetic acid. The reaction mixture was stirred at room

temperature for 12h. Then, tetrachloro-1,4-benzoquinone (1.52 g, 6.2 mmol) was added and the reaction mixture was stirred at room temperature for 45 min. Then triethyl amine (6 ml) and boron trifluoride diethyl etherate (6 ml) were added sequencially. After stirring at room temperature for 30 min, it was extracted with water. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$. Fraction containing compound **6** was collected then the solvent was removed under reduced pressure (260 mg, 0.68 mmol, 11%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 1.43 (s, 6H), 2.56-2.58 (m, 6H+1H), 4.77 (d, *J*=2.4 Hz, 2H), 5.99 (s, 2H), 7.10 (d, *J*=8.7 Hz), 7.21 (d, *J*=8.7 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 14.5, 14.6, 56.0, 75.9, 78.0, 115.6, 121.2, 128.0, 129.2, 131.8, 141.5, 143.1, 155.4, 158.1. HRMS-ESI: calculated for M+Na 401.1617, found 401.1644, Δ= 6.7 ppm

2,6-Diiodo-1,3,5,7-tetramethyl-8-(4-Propargyloxyphenyl)-4,4-difloroboradiaza-*s*-indacene (7).



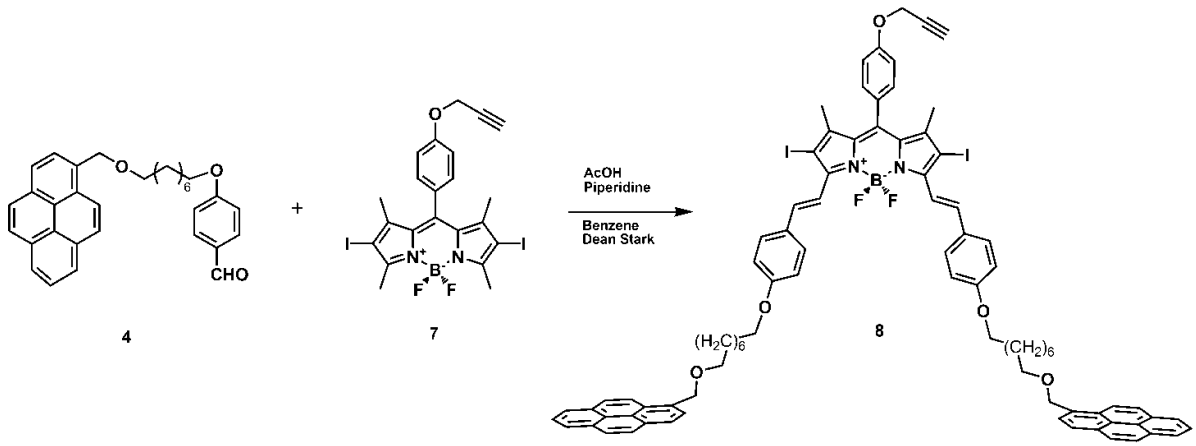
6 7

1,3,5,7-Tetramethyl-8-(4-Propargyloxyphenyl)-4,4-difloroboradiaza-*s*-indacene **6** (50 mg, 0.13 mmol) and I_2 (83 mg, 0.33 mmol) were dissolved in ethanol (100 ml). Iodic acid, HIO_3 (45.74 mg, 0.26 mmol) was dissolved in a few drops of water and added into previous solution. The reaction mixture was stirred at 60^{o}C for a few hours untill all reeactant was consumed. Then, saturated sodium thiosulfate solution was added (50 ml) and it was stirred at rrom temparature for additional 30 min. Then, it was extracted with $CHCl_3$ and water. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$/Hexane (50/50, v/v). Fraction containing compound **7** was collected then the solvent was removed under reduced pressure (59 mg, 0.09 mmol, 72%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 1.36 (s, 6H), 2.49 (s, 1H), 2.56 (s, 6H), 4.70 (s, 2H), 7.04 (d, *J*=8.8 Hz, 2H), 7.09 (d, *J*=8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 16.0, 17.1, 56.1, 76.1,

85.8, 100.1, 116.0, 127.8, 129.1, 131.6, 141.4, 146.0, 156.7, 158.6. HRMS-ESI: calculated for M+Na 652.9550, found 652.9515, Δ= 5.4 ppm

Double Knoevenagel condensation reaction (8).



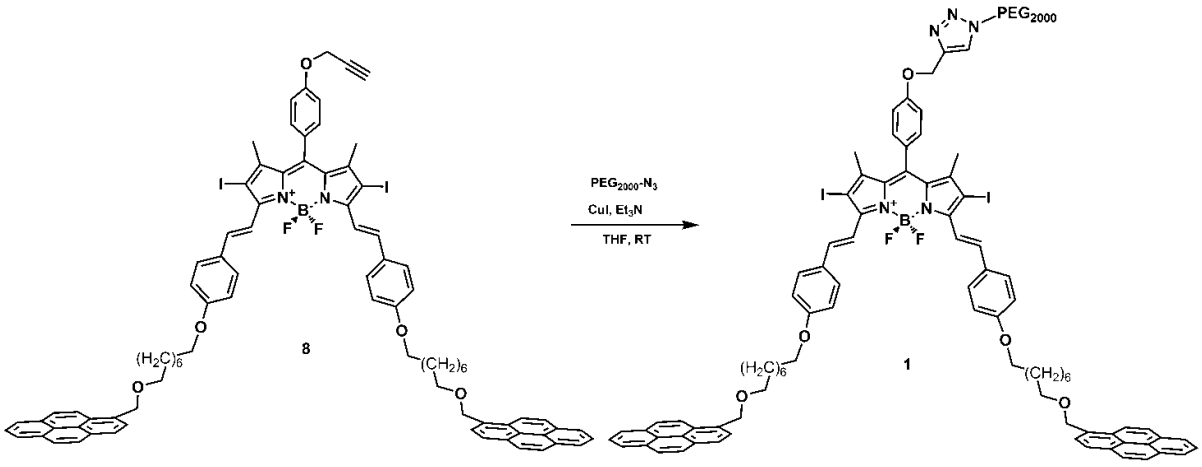
2,6-Diiodo-1,3,5,7-tetramethyl-8-(4-Propargyloxyphenyl)-4,4-difloroboradiaza-*s*-indacene **7** (38 mg, 0.06 mmol) and 1-(8-(4-formyl)-phenyloxyhexyloxymethyl)-pyrene **4** (70 mg, 0.15 mmol) dissolved in benzene (45 ml). Piperidine (0.2 ml) and glacial acetic acid (0.2 ml) were added. The solution was refluxed using Dean-Stark apparatus. When the solution was concentrated, reaction was followed by TLC until green-colored product became the major product. Then, benzene was evaporated in vacuo. It was extracted with $CHCl_3$ and water. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$. Fraction containing compound **8** was collected then the solvent was removed under reduced pressure (40 mg, 0.026 mmol, 44%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 1.15-1.78 (m, 30H), 2.61 (s, 1H), 3.61 (t, *J*= 6.6 Hz, 4H), 3.89 (t, *J*= 6.4 Hz, 4H), 4.81 (d, *J*=2.2 Hz, 2H), 5.21 (s, 4H), 6.85 (d, *J*=8.7 Hz, 4H), 7.11 (d, *J*=8.8 Hz, 2H), 7.19 (d, *J*=8.8 Hz, 2H), 7.55-7.64 (m, 4H+2H), 7.99-8.09 (m, 8H), 8.13-8.21 (m, 8H+2H), 8.39 (d, *J*=9.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 15.1, 18.2, 22.3, 25.9, 26.1, 29.1, 29.2, 29.3, 29.7, 29.8, 58.4, 68.0, 70.4, 71.5, 114.8, 123.6, 124.5, 125.1, 125.9, 126.9, 127.3, 127.4, 127.6, 129.2, 129.4, 131.2, 131.3, 131.8, 132.0, 160.3. MALDI: calculated for **8,** 1522.414, found 1522.319, Δ= 6.2 ppm.

Synthesis of Azido-PEG$_{2000}$:

PEG-N_3 was synthesized according to literature.[S1]

Click Reaction (1).



Double Knoevenagel condensation product, compound **8** (40 mg, 0.027 mmol) was dissolved in THF (2 ml). 2 molar equavalent CuI (10.3 mg, 0.054mmol), 50 molar equavalent triethyl amine (135.6 mg, 1.34 mmol) and 2 molar equavalent 1-azido polyethylene glycol (MW: 2000) (108 mg, 0.054 mmol) were added.[S2] The reaction mixture was stirred at room temperature for 12h, THF and triethyl amine were evaporated in vacuo. Organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The product was purified by silica gel column chromatography using $CHCl_3$/methanol (95/5, v/v). Fraction containing compound **1** was collected then the solvent was removed under reduced pressure (91 mg, 0.026 mmol, 95%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 1.19-1.42 (m, 16H), 1.47 (s, 6H), 1.60-1.71 (m, 8H), 3.37 (s, 3H), 3.51-3.71 (m, "PEG –OCH_2's" 210H), 3.78-3.90 (m, 8H), 5.17 (s, 4H), 5.25 (s, 2H), 6.84 (d, *J*=8.8 Hz, 4H), 7.11-7.17 (m, 4H), 7.53-7.62 (m, 4H+2H), 7.91 (s, 1H), 7.95-8.05 (m, 8H), 8.07-8.20 (m, 8H+2H), 8.45 (d, *J*=9.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 17.6, 25.8, 26.0, 29.0, 29.2, 29.6, 29.7, 46.9, 50.6, 59.0, 62.0, 68.0, 70.0, 70.6, 70.7, 71.9, 94.2, 106.0, 114.8, 123.5, 124.4, 125.1, 126.9, 127.3, 127.4, 127.5, 129.2, 129.3, 130.8, 131.1, 131.8, 139.1, 143.2, 150.4, 159.3, 160.3. MALDI: Distribution around 3298.384 with separation of 44.287 corresponding to etylene glycole unit.

Non-covalent Functionalization of SWNT with Photosensitizer:

Compound **1** was adsorbed on to the SWNT by means of sonication and unadsorbed molecules were eliminated through filtration.[S3] PBS buffer was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 1L water. 1 mM solution of photosensitizer was prepared by dissolving 70 mg compound **1** in 20 ml ethanol-PBS mixture (50/50, v/v). 3 mg SWNT was put into a veil containing 5 ml 1:1 ethanol-PBS mixture and sonicated for 15 min. Then, 5 ml of previous solution was added into the viel. The resultant mixture was sonicated for 1h while tempeature of the sonicator was maintained at around room temperature. The sample was centrifuged at 11000g for 2 hours to get rid of unsoluble SWNT via precipitation. Following this, the sample was centrifuged using Millipore 30kDa filter at 13000 g for 15 min to get rid of unadsorbed compound **1**. The filtrate was discarded and one more centrifugation was done. All green colored sample remained on the filter which was collected into a tube by centrifugation of the filter upside down at 1000 g for 3 min.

Singlet Oxygen Genaration Experiment:

Singlet oxygen generating capability of SWNT-adsorbed compound **1** was done using singlet oxygen trap molecule 1,3-diphenylisobenzofuran (DPBF). For light source 660 nm emitting 3000 mCd lead source was used. A solution containing DPBF (50 μM) and SWNT-adsorbed compound 1 (62 nM) was prepared in isopropanol. Concentration of the SWNT-adsorbed compound **1** was determined by comparing its UV-absorbance to the absorbance of compound **1** solution with known concentration. Another control solution was prepared with DPBF (50 μM) alone. Both solutions were aerated for 5 minutes. Then, absorbance spectrum of each solution was taken in 5 minutes intervals while the solutions were kept in dark. Then 660 nm light was exposed from 3-4 cm cell distance for 40 minutes and absorbance was taken in 5 minutes intervals for each solution. No decrease in absorbance was observed in dark (see Figure S4). When light was exposed, absorbance of DPBF decreased but only in the presence of compound **1** (see Figure S4).

References:

S1. *Pat.,* 528 398. 2007. *US Pat*., 7 301 003, 2025.

S2. W. T. Christian, C. Christensen, M. Meldal, *J. Org. Chem.,* 2002, **67**, 3057-3064.

S3. Z. Liu, W. Cai, L. He, N. Nakayama, K. Chen, X. Sun, *Nature Nanotechnology*, 2007, **2**,47-52

Results:

Quenching of pyrene emission when compound **1** is stacked on to the SWNT is shown in Figure S2 compared with the non-stacked compound **1** with equal absorbance (see Figure S1). Singlet oxygen generation results were shown in Figure S4.

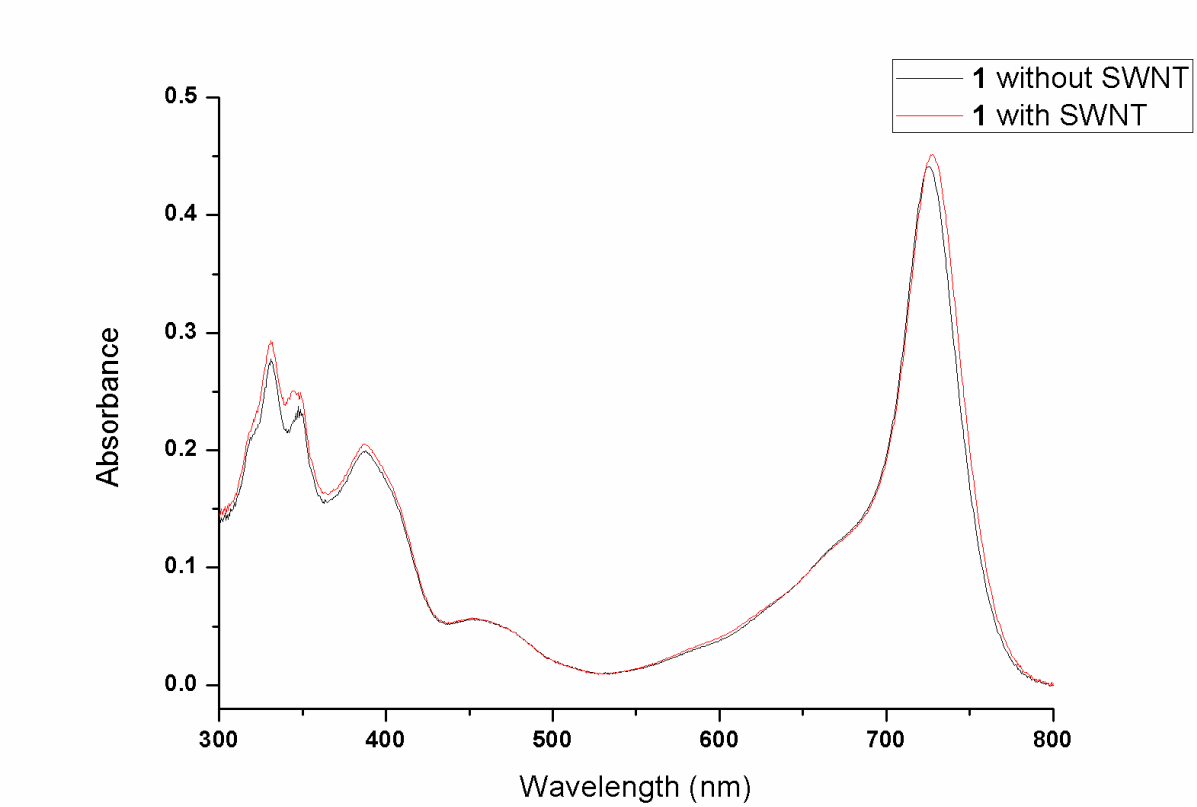


Figure S1. Absorbance of compound **1** in ethanol-PBS mixture (50/50, v/v). Red line: compound **1** before adsorbtion on to SWNT; dashed line: compound 13 after adsorbtion on to SWNT.

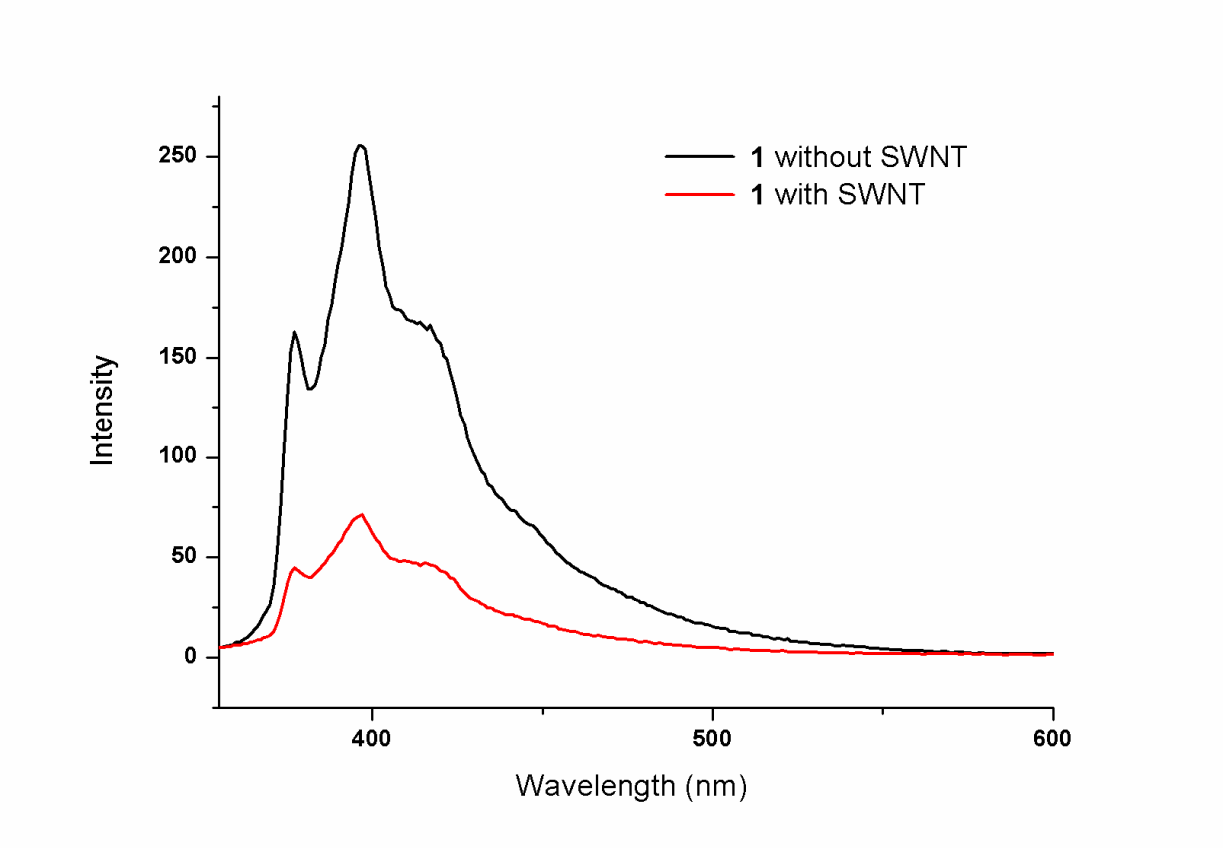


Figure S2. Fluorescence spectrum of compound **1** in ethanol-PBS mixture (50/50, v/v) excited at 343 nm. Red line: compound **1** before adsorbtion on SWNT; blue line: compound **1** after adsorbtion on SWNT. With equal absorbance (see Figure S1), emission of pyrene moity of adsorbed photosensitizer was quenched three folds compared to unadsorbed one.

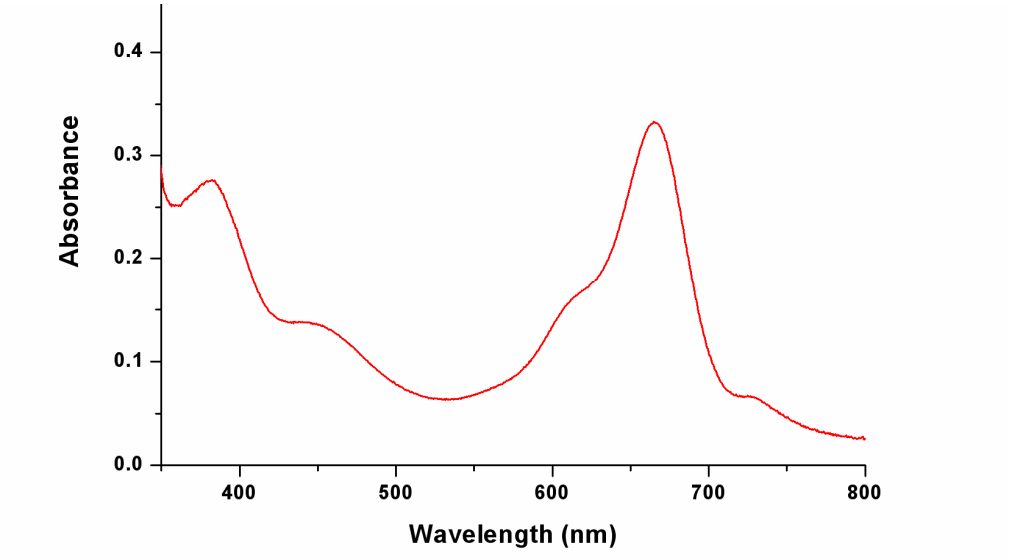


Figure S3. Absorbance spectrum of SWNT-adsorbed compound **1** in isopropanol. Absorption maxima in this solvent is at 666 nm.

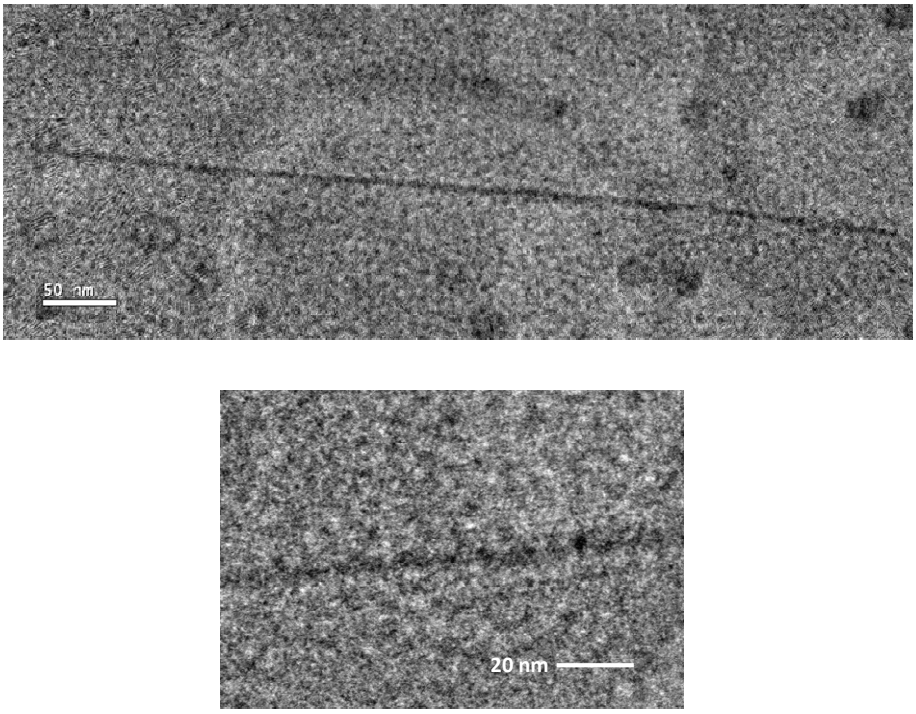


Figure S4. TEM image of SWNT functionalized with compound **1.**

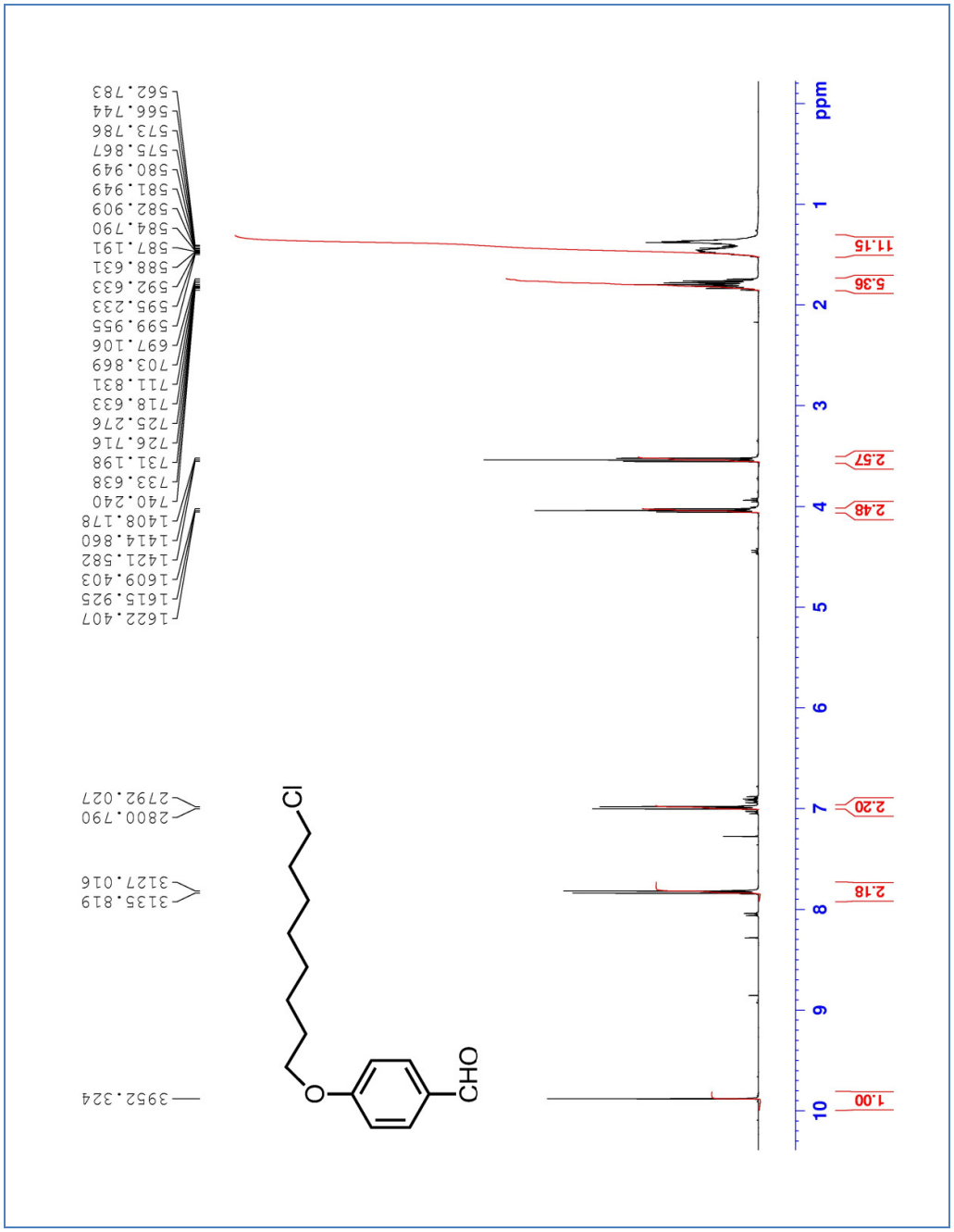


Figure S5. ^{1}H NMR spectrum of compound **3.**

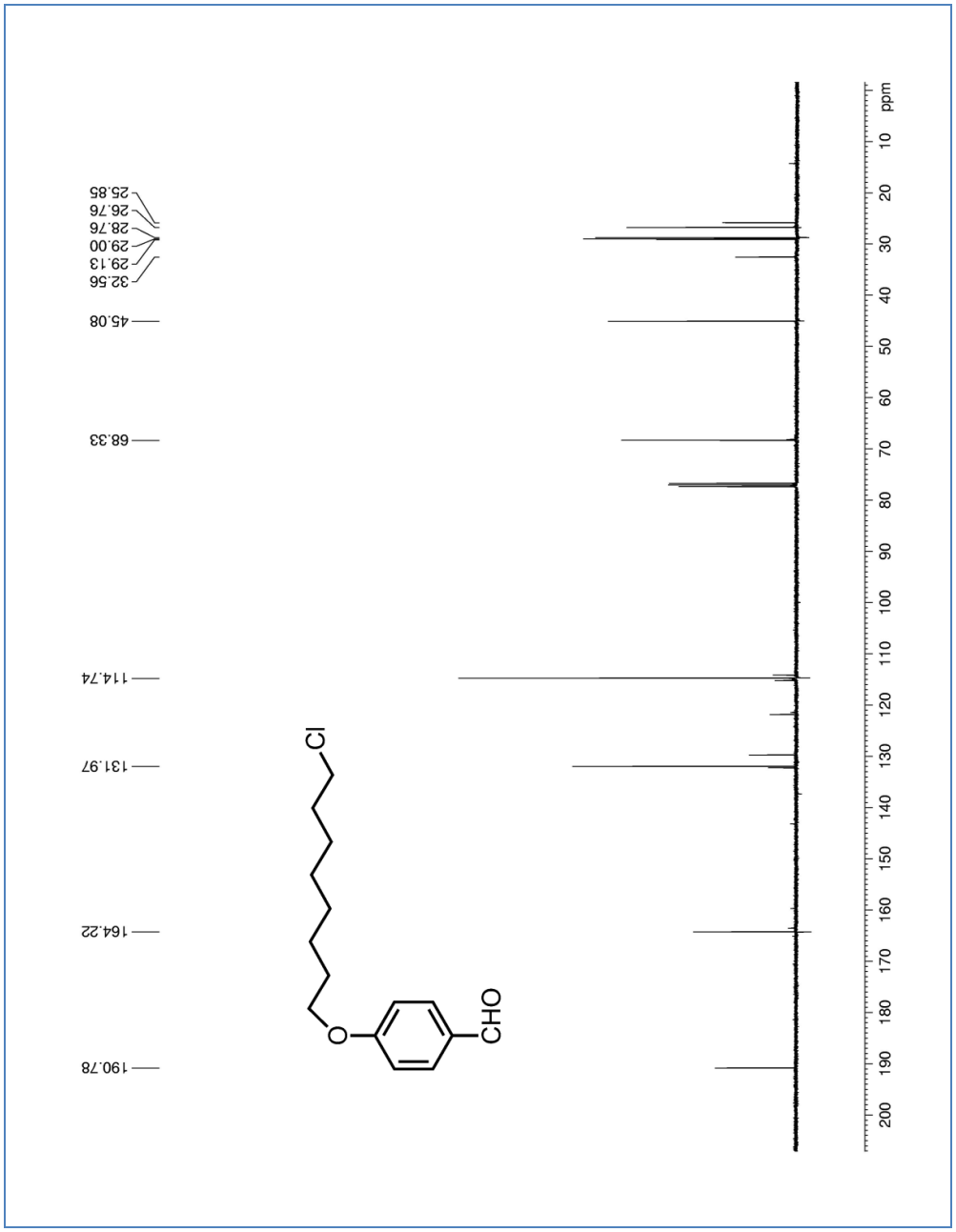


Figure S6. ^{13}C NMR spectrum of compound **3.**

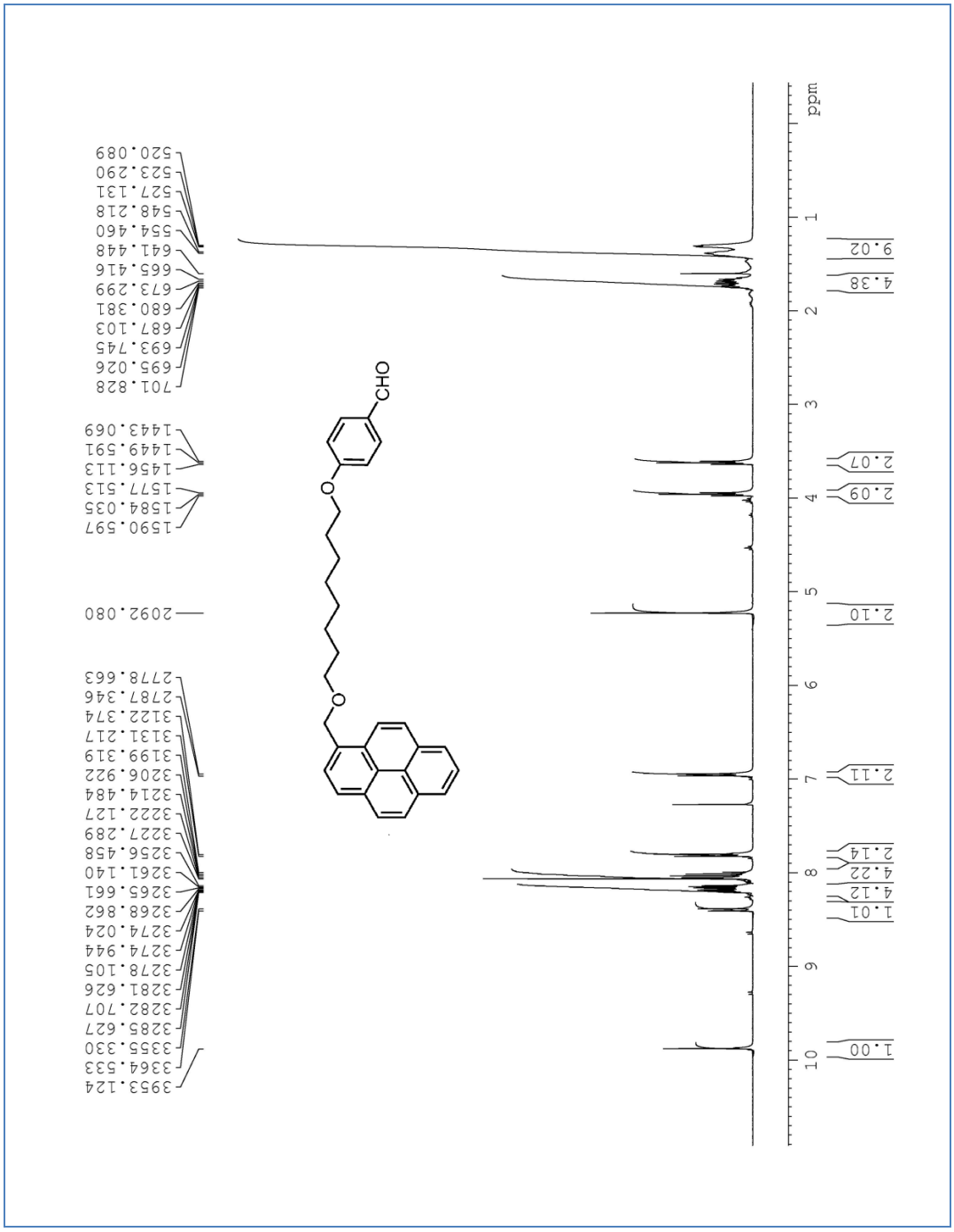


Figure S7. ^{1}H NMR spectrum of compound **4.**

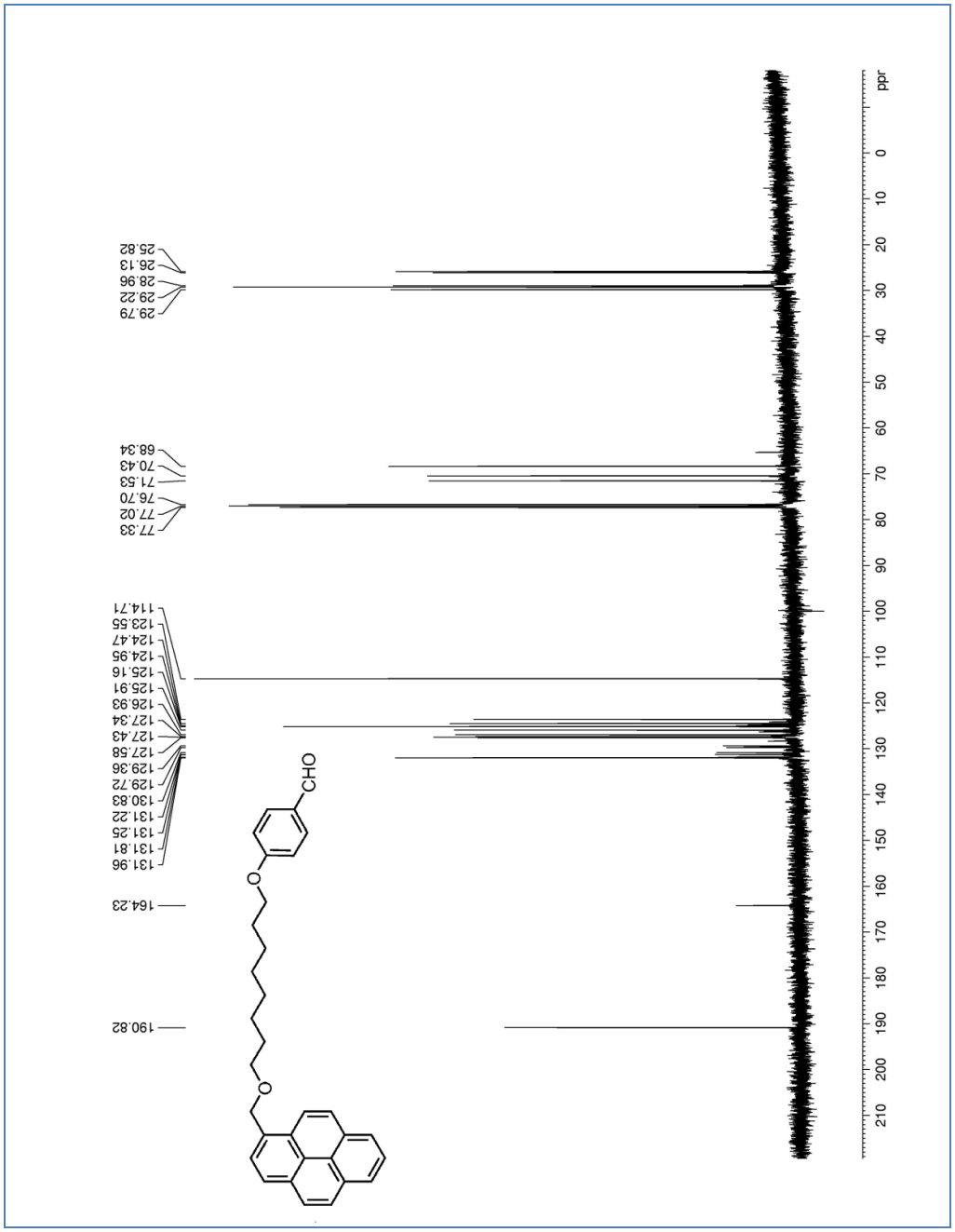


Figure S8. ^{13}C NMR spectrum of compound **4.**

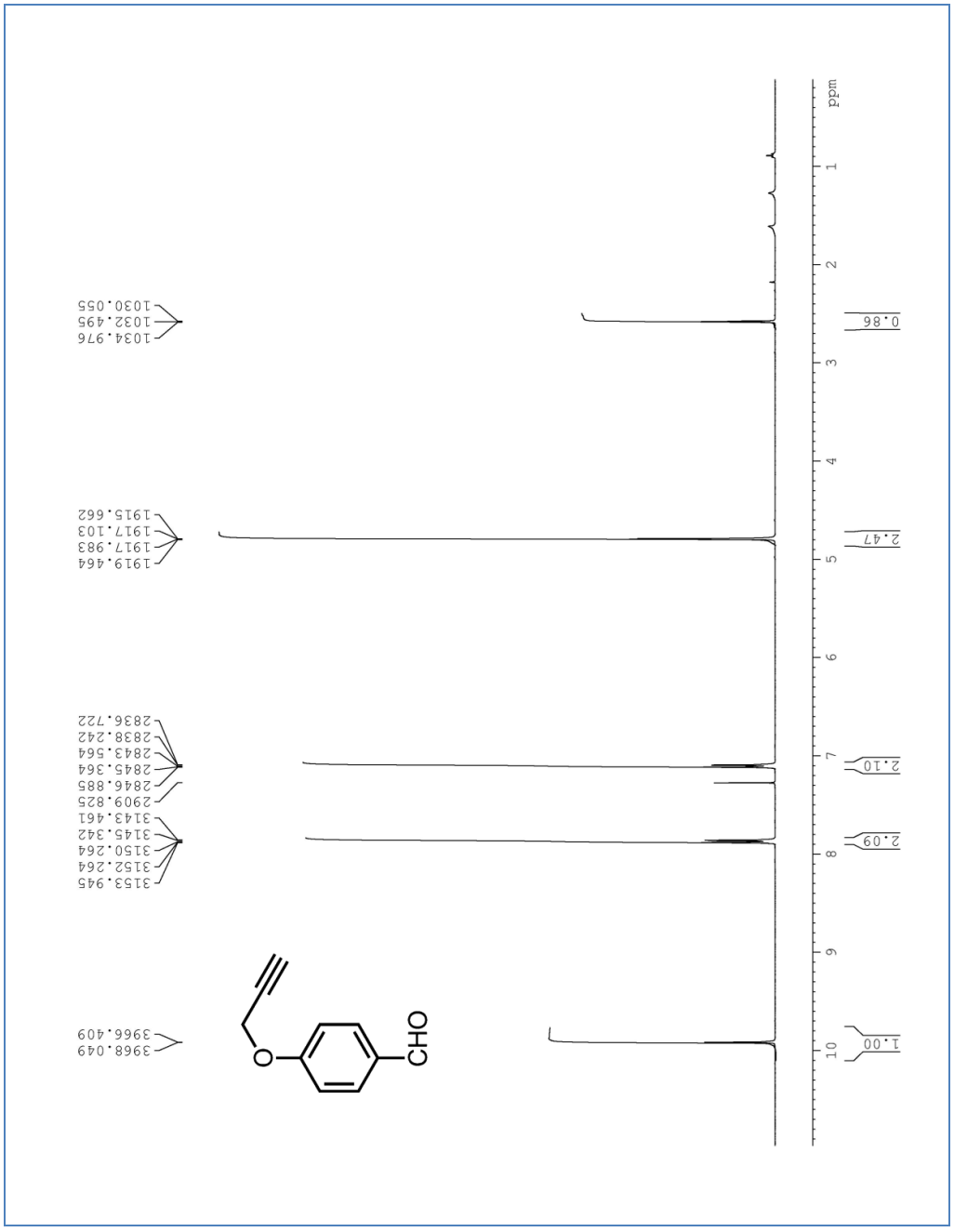


Figure S9. ^{1}H NMR spectrum of compound **5.**

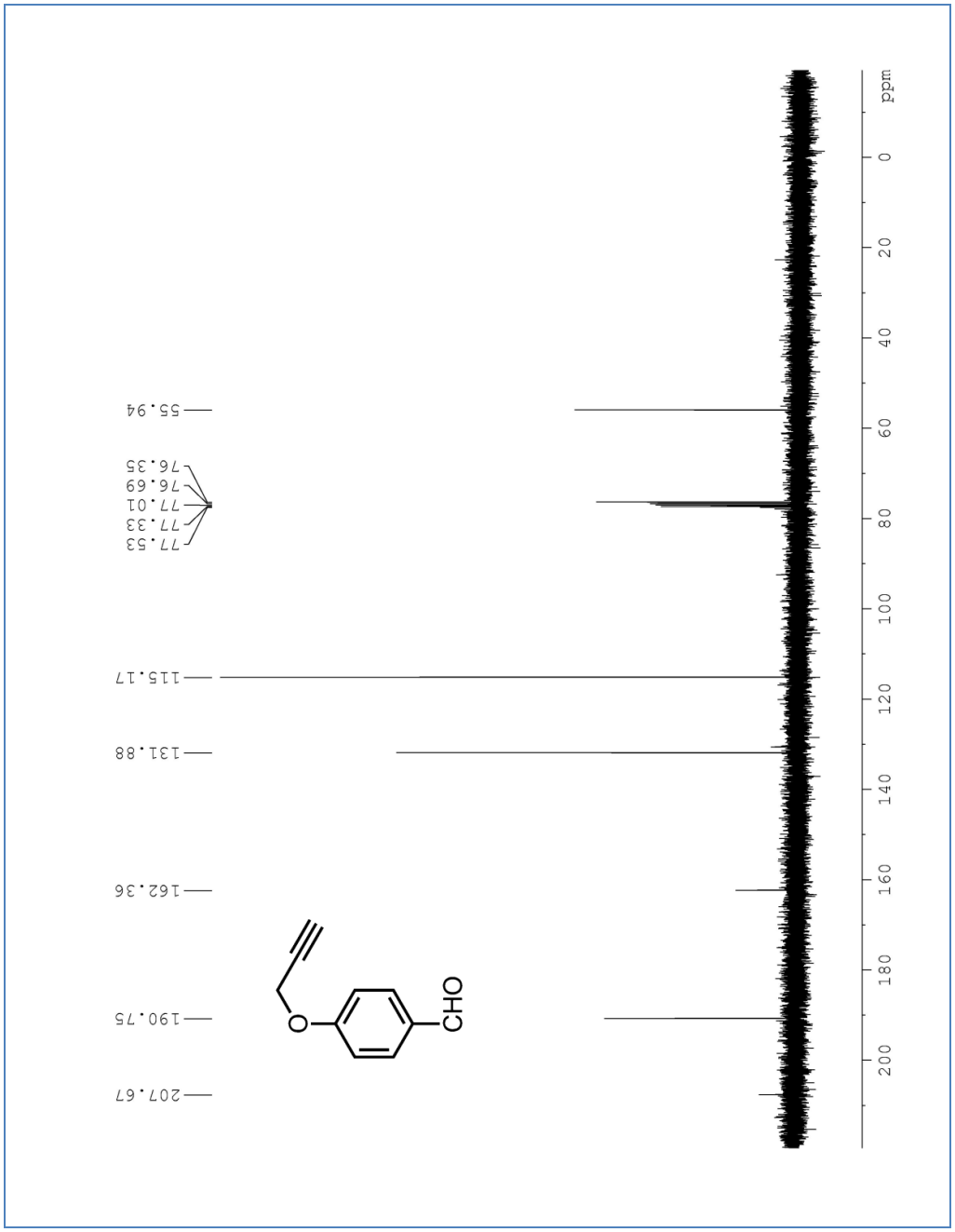


Figure S10. ^{13}C NMR spectrum of compound **5.**

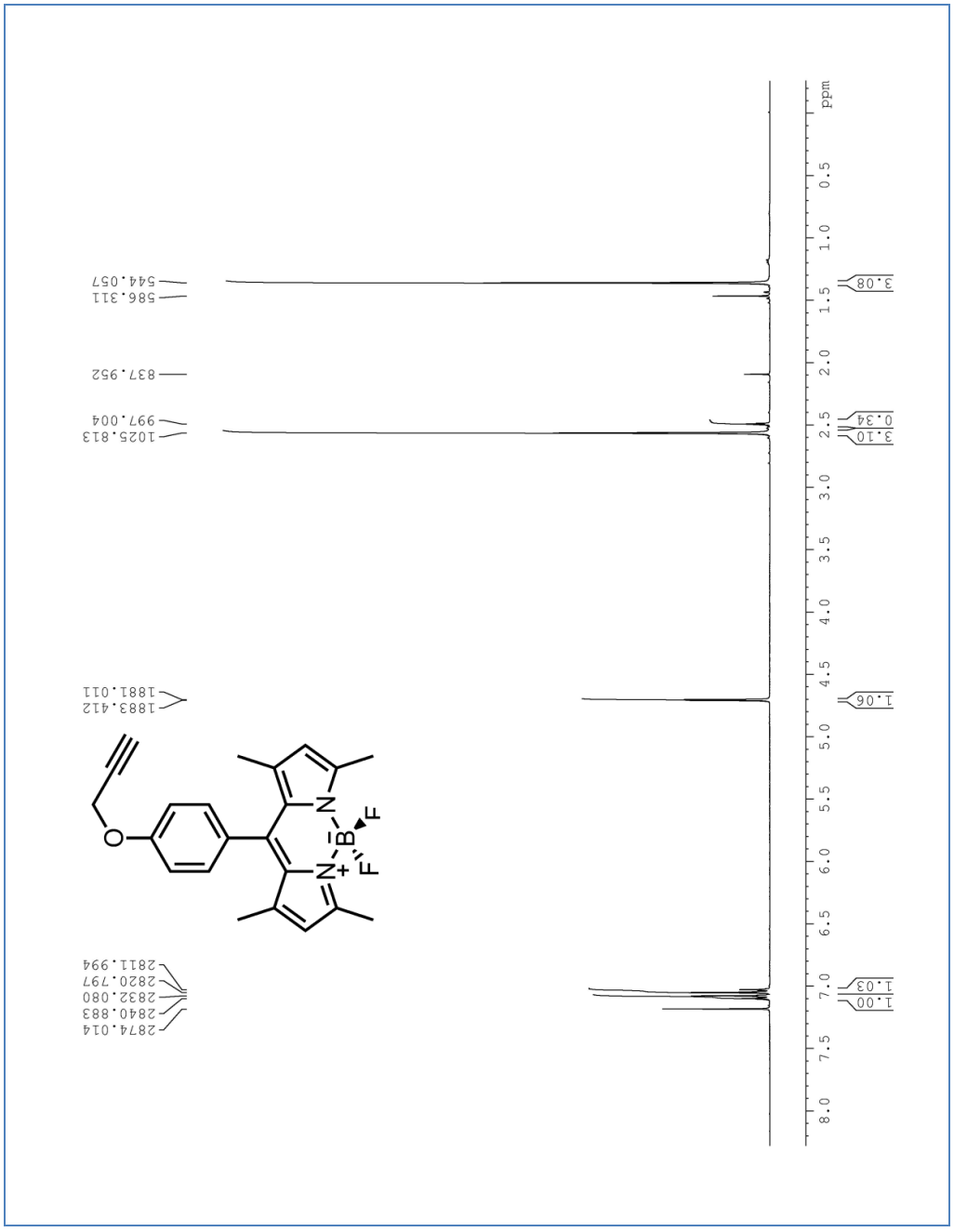


Figure S11. ^{1}H NMR spectrum of compound **6.**

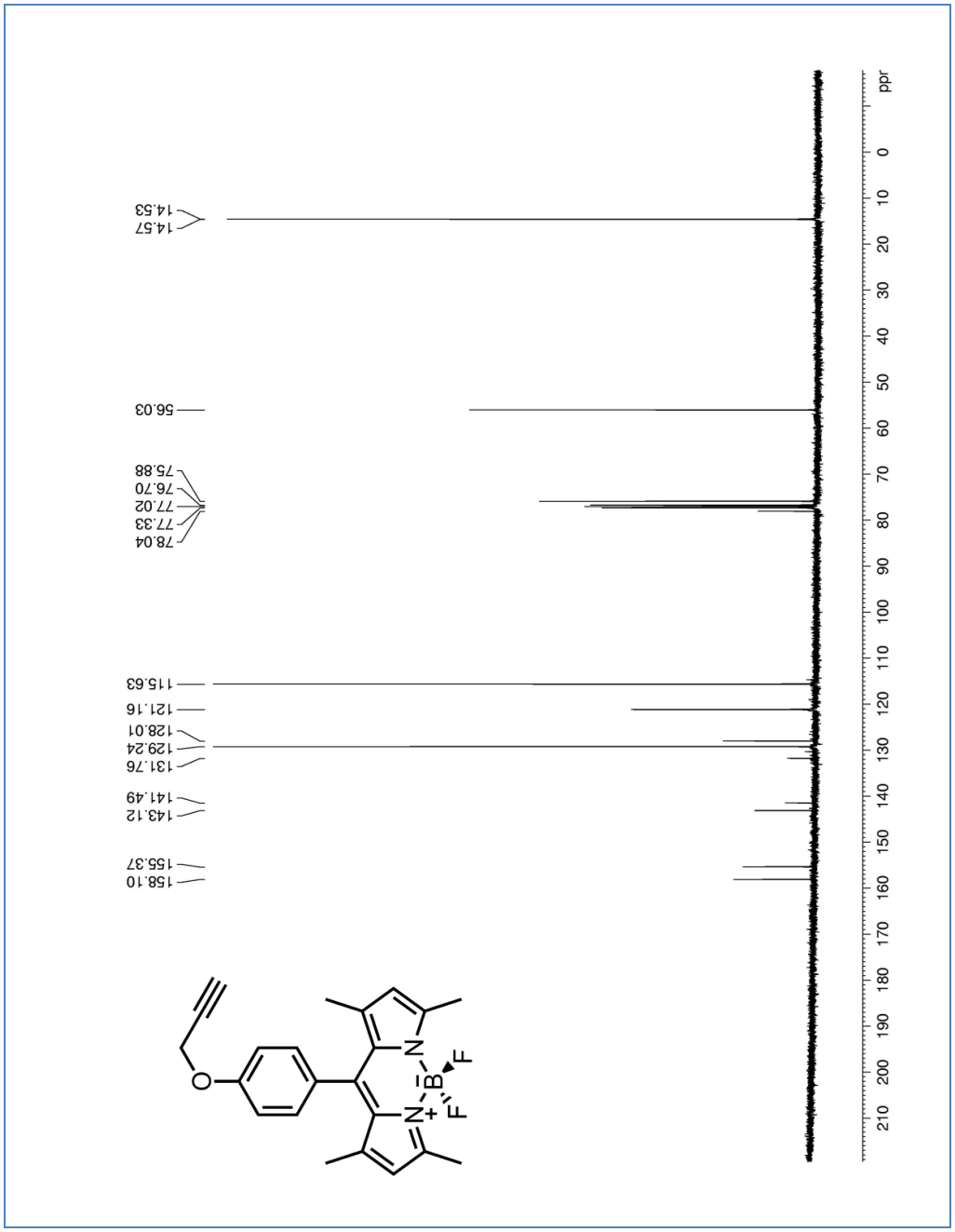


Figure S12. ^{13}C NMR spectrum of compound **6.**

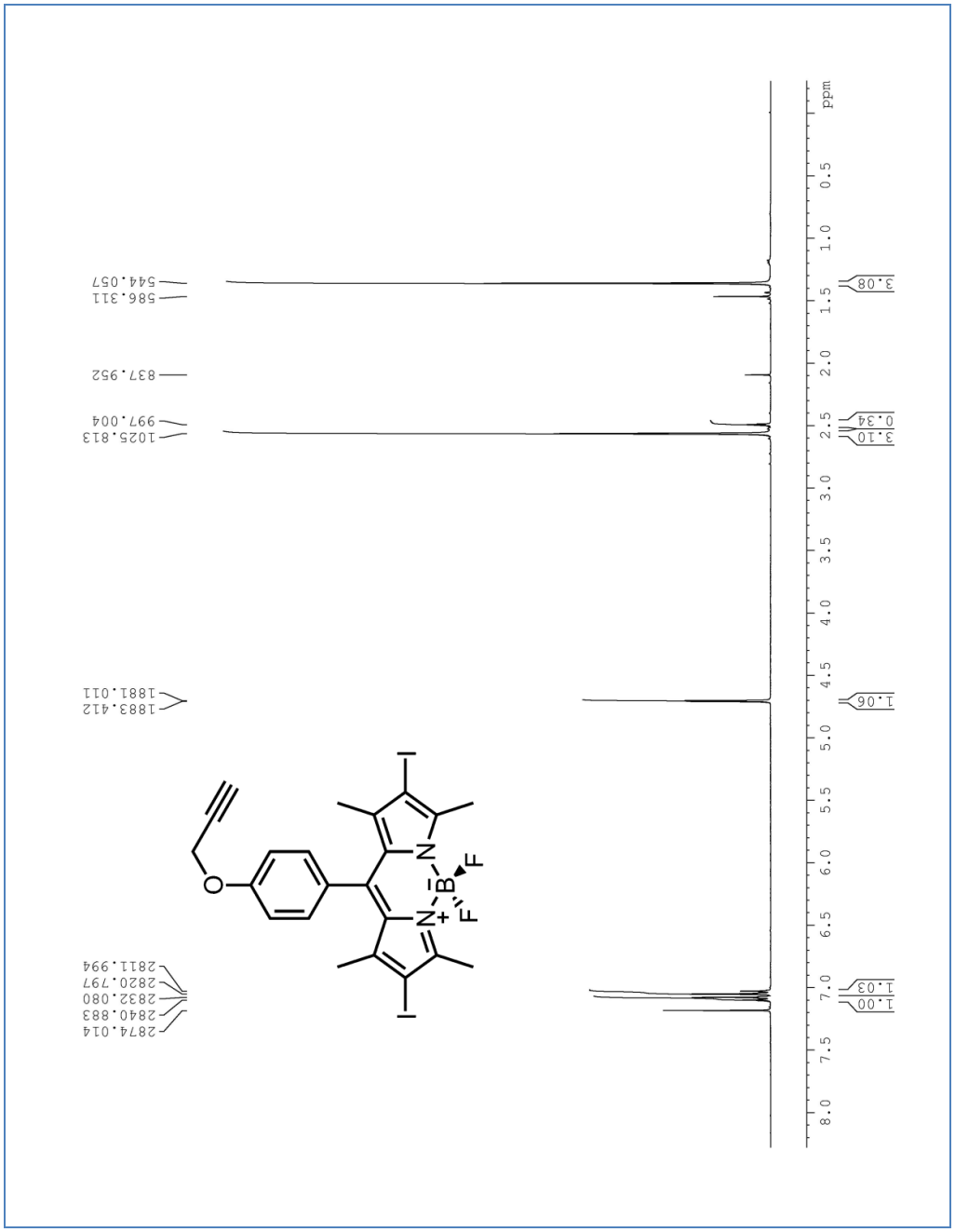


Figure S13. ^{1}H NMR spectrum of compound **7.**

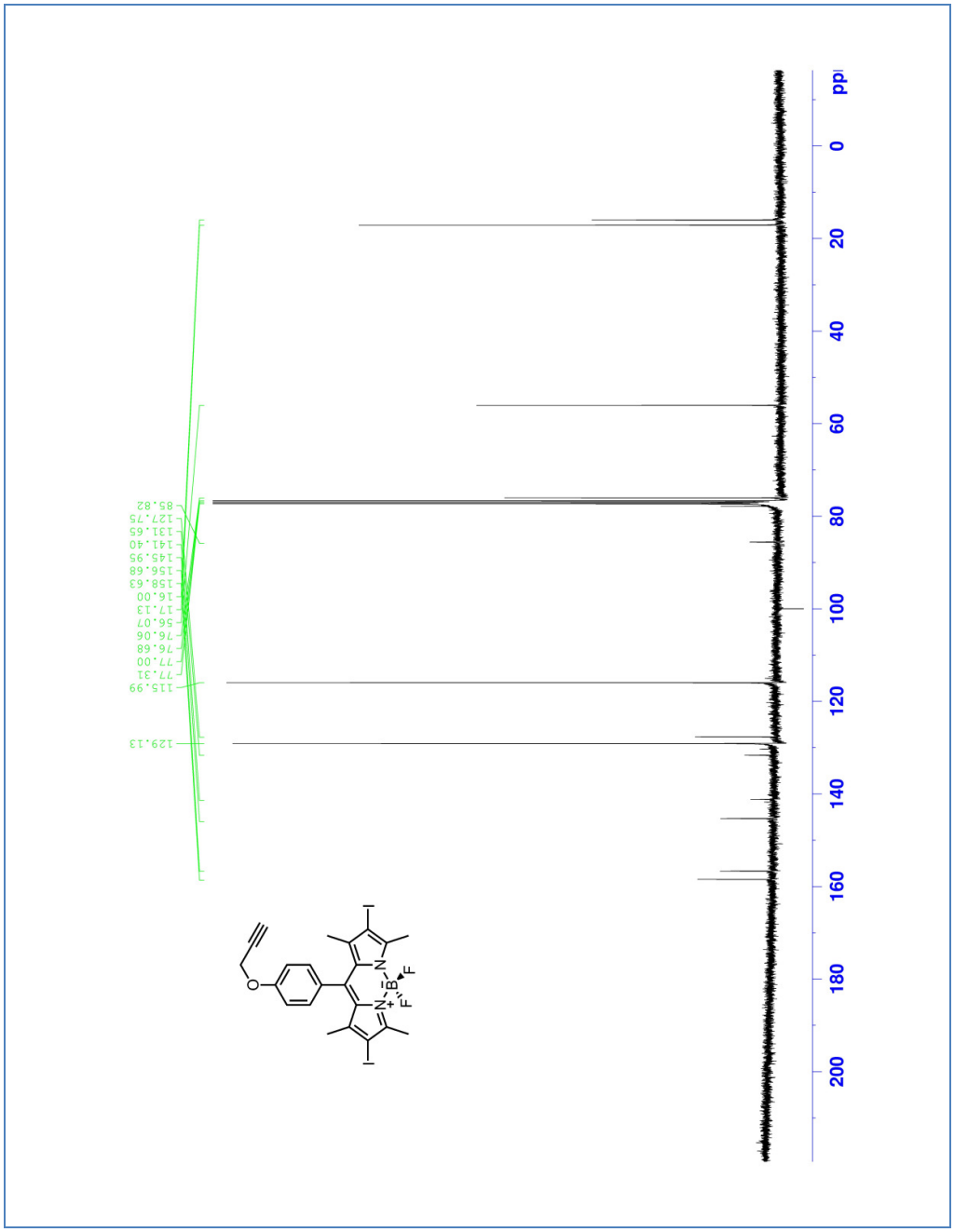


Figure S14 ^{13}C NMR spectrum of compound **7.**

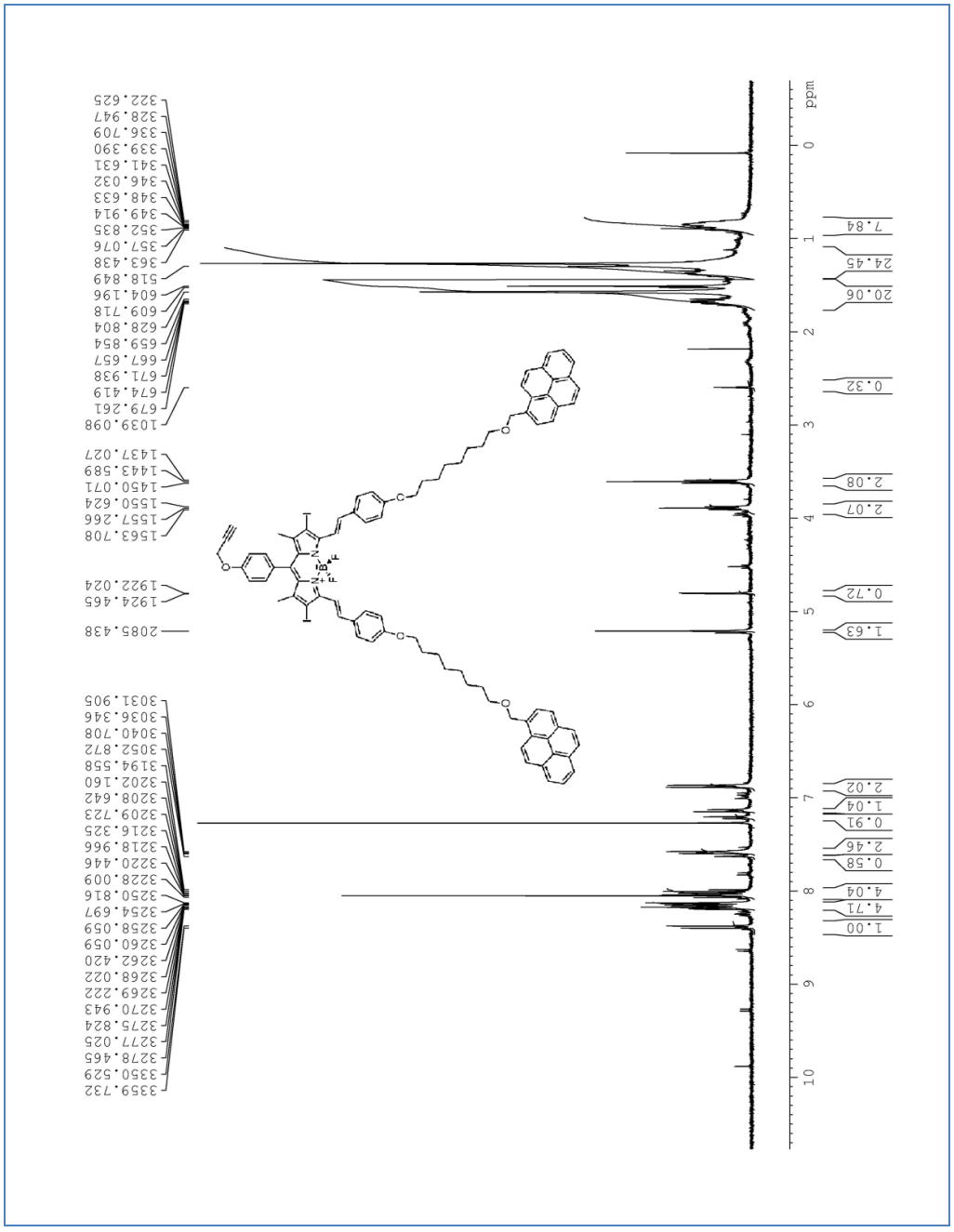


Figure S15. ^{1}H NMR spectrum of compound **8.**

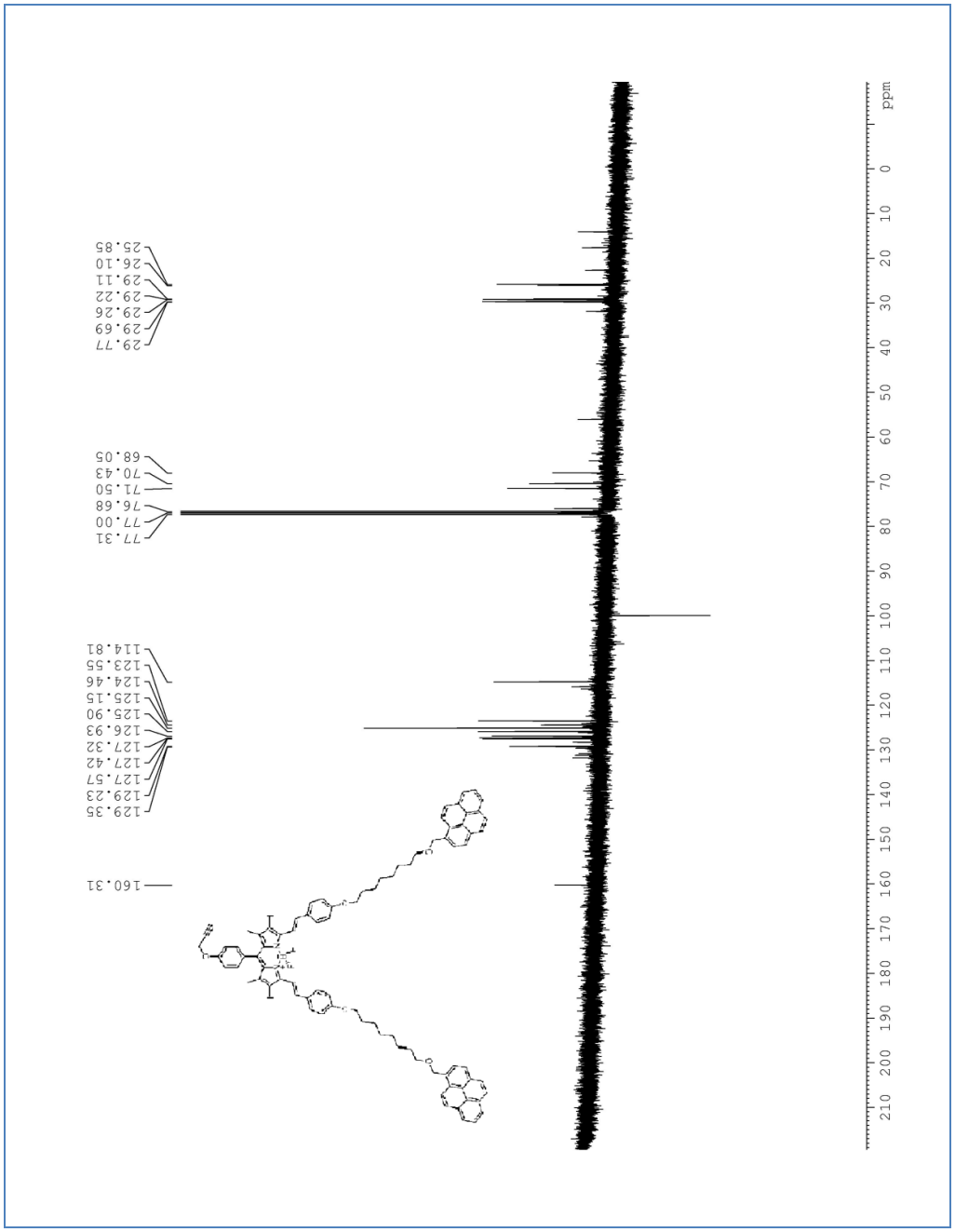


Figure S16. ^{13}C NMR spectrum of compound **8.**

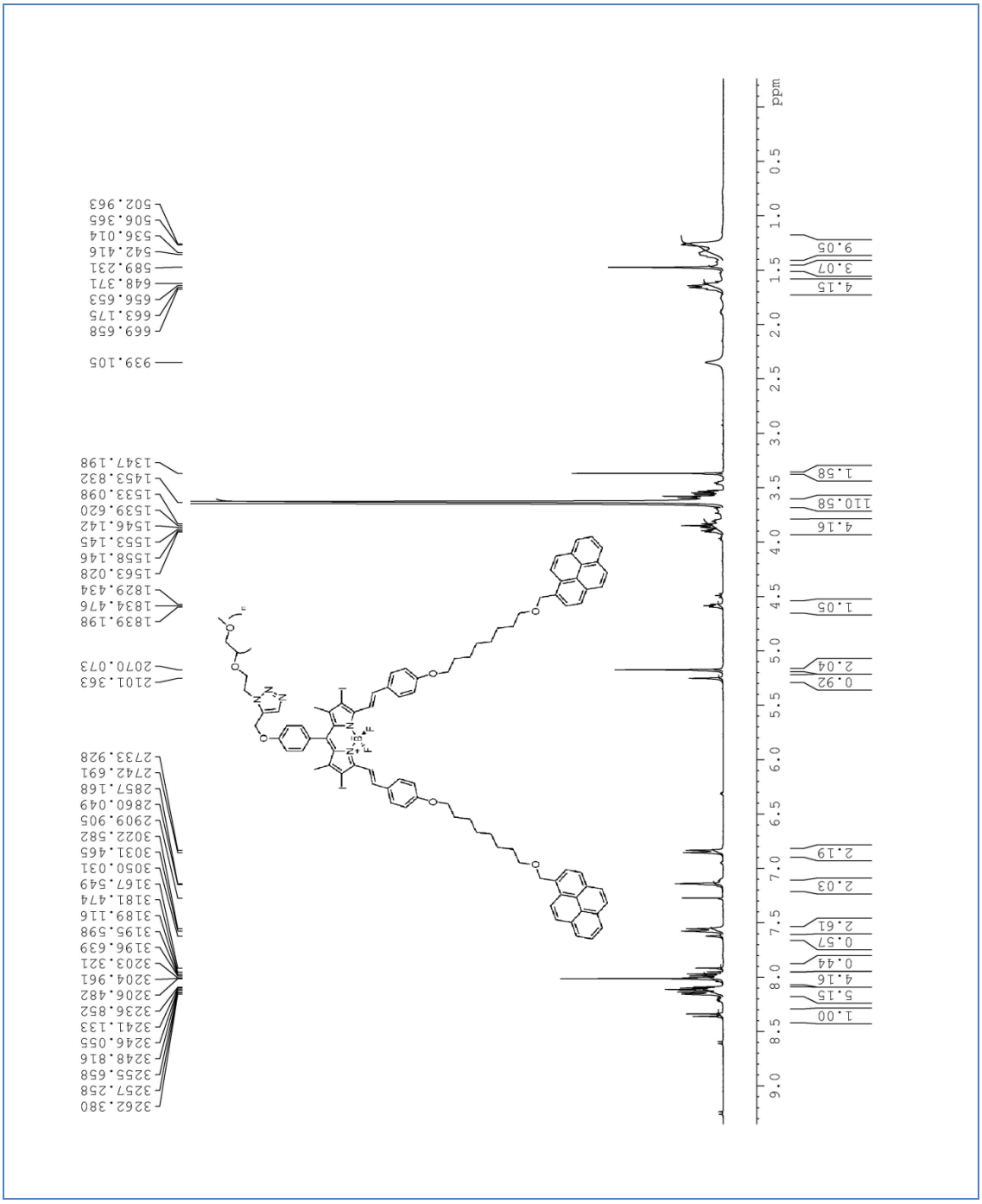


Figure S17. ^{1}H NMR spectrum of compound **1.**

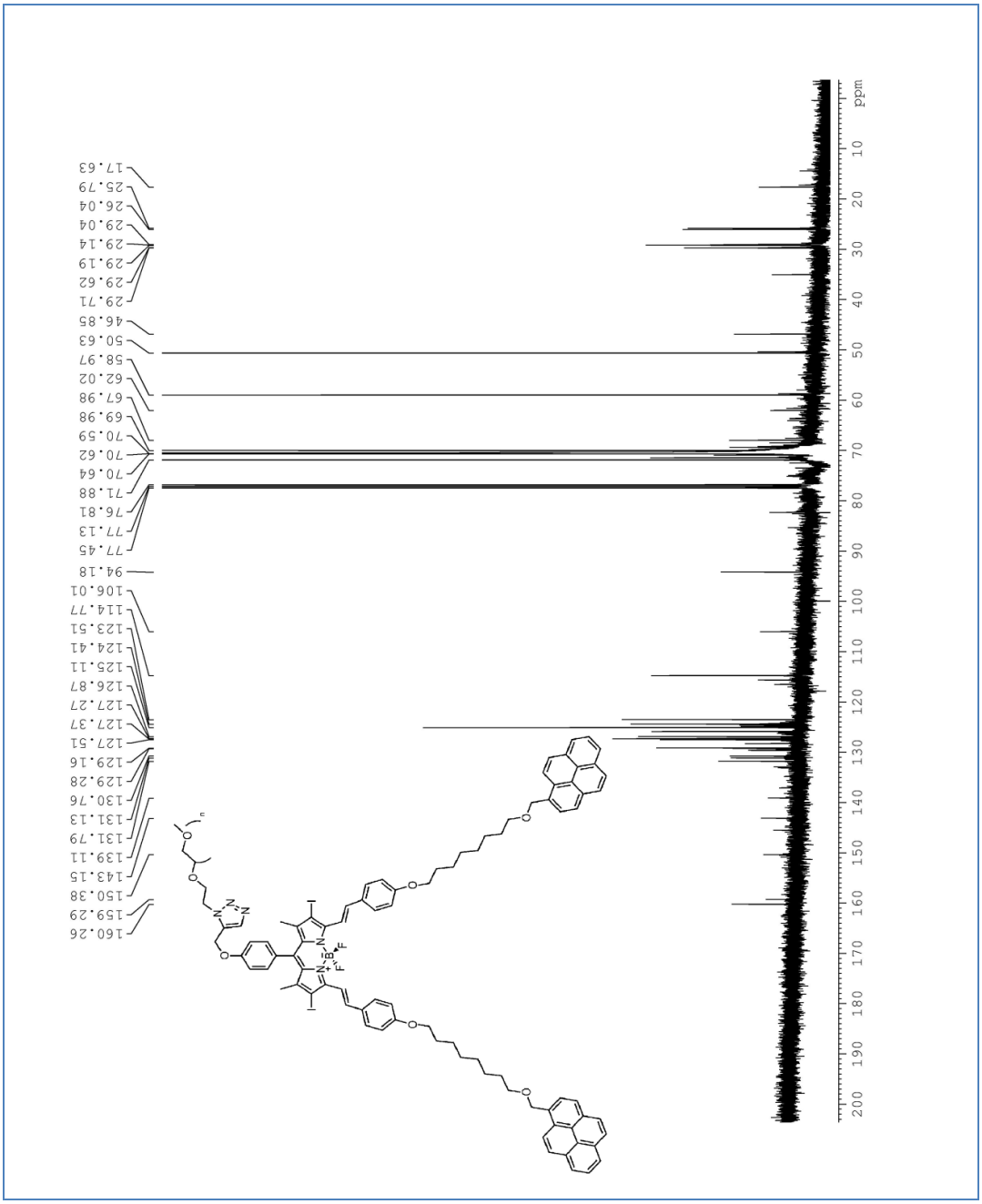


Figure S18. ^{13}C NMR spectrum of compound **1.**

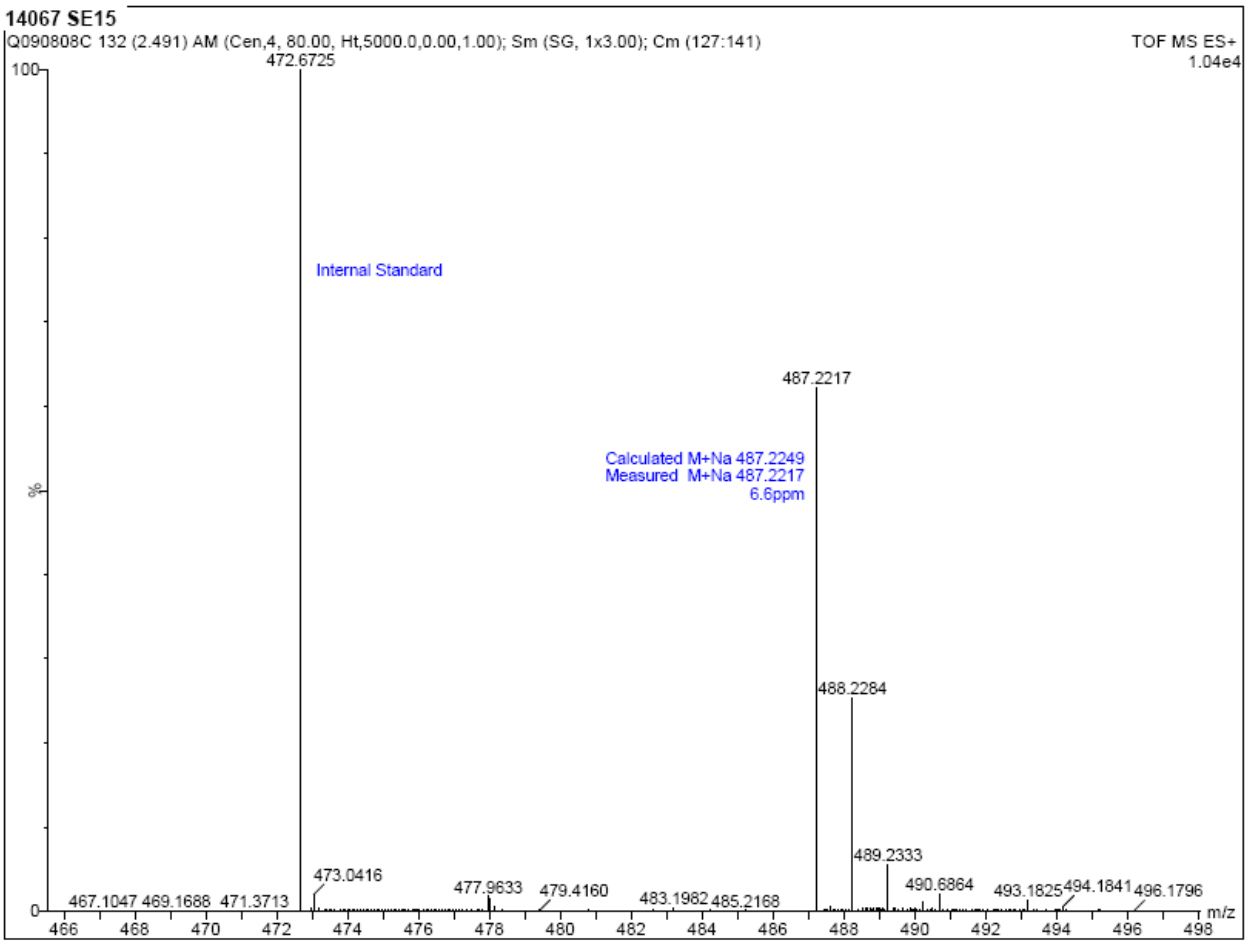


Figure S19. ESI-HRMS of compound **4**.

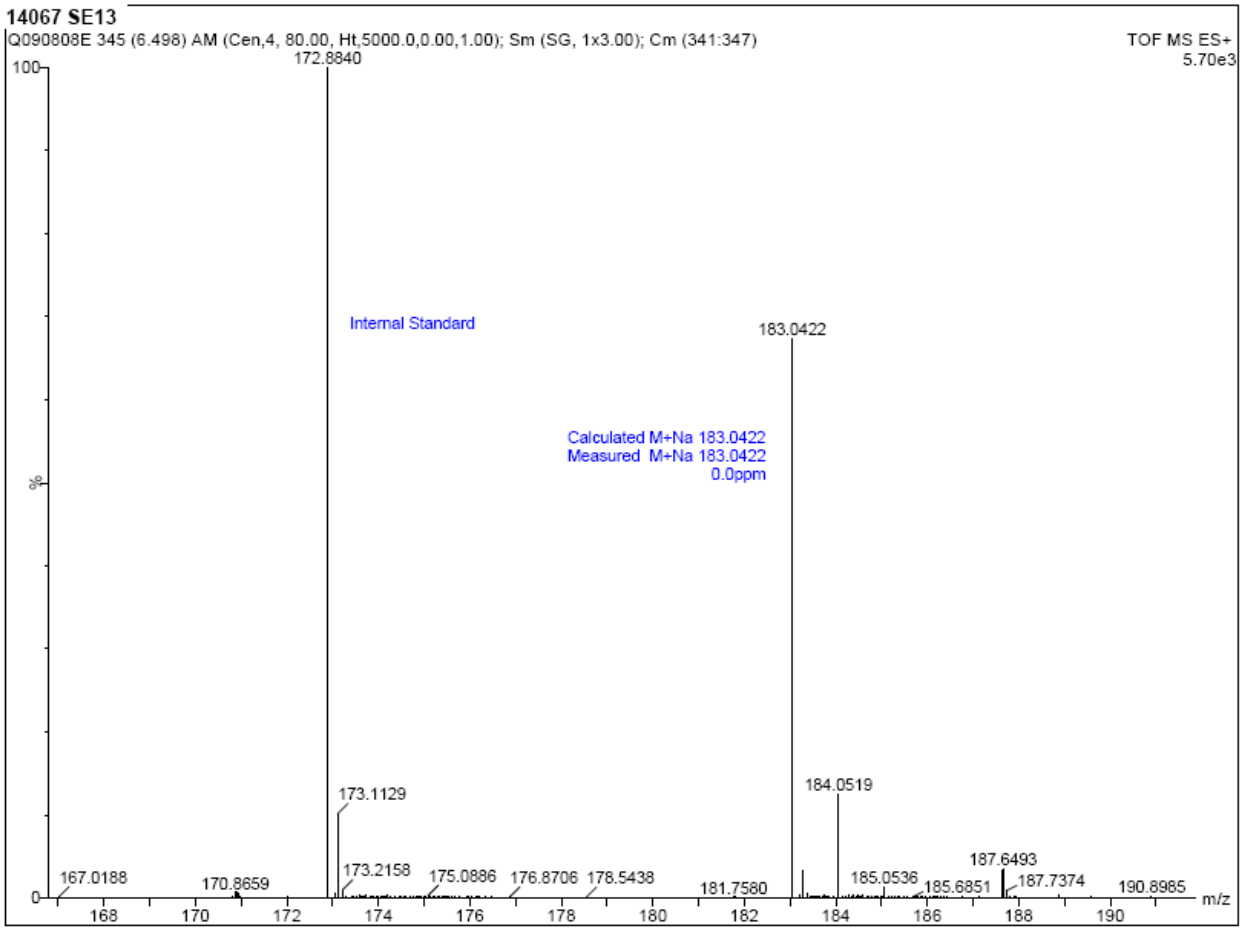


Figure S20. ESI-HRMS of compound **5**.

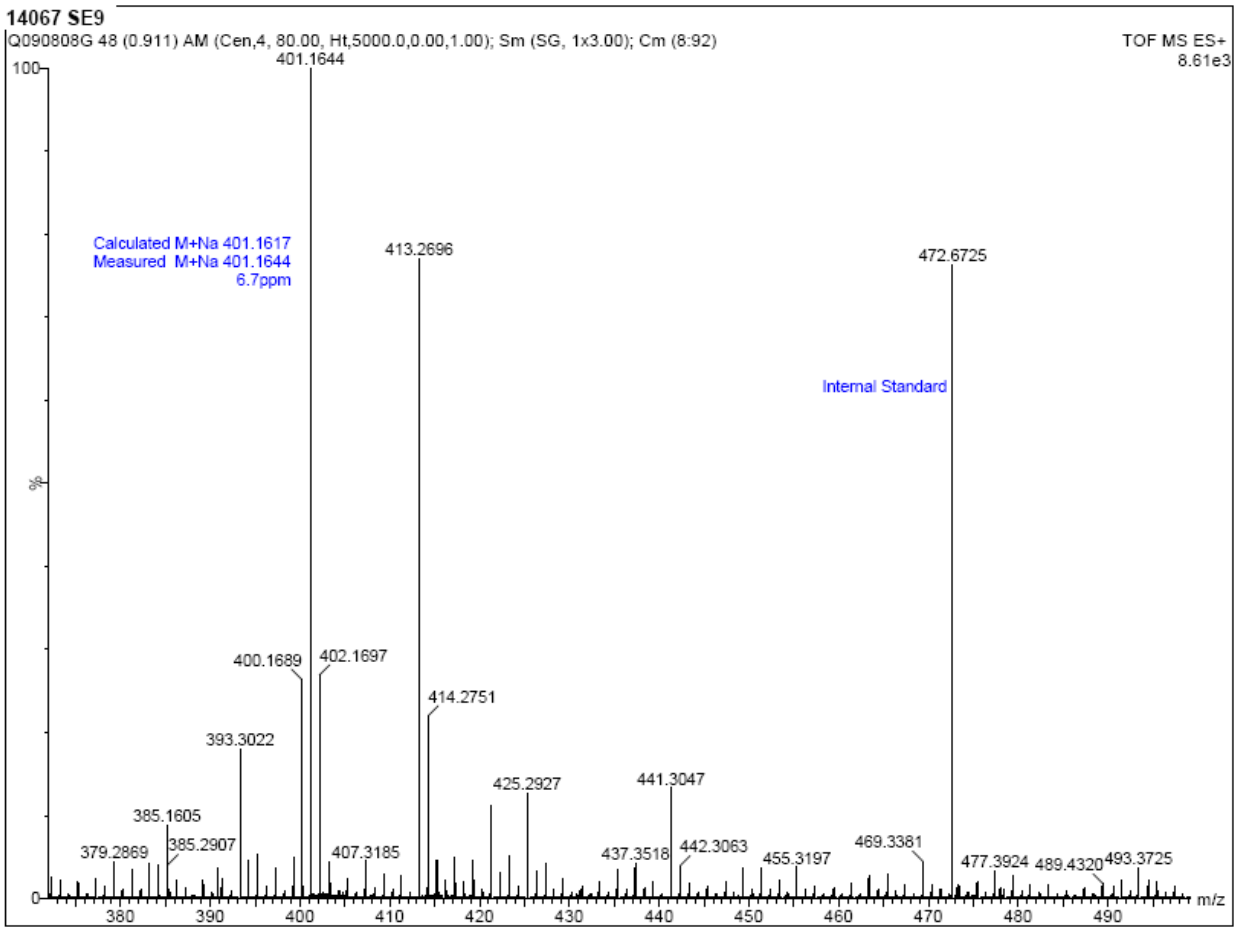


Figure S21. ESI-HRMS of compound **6**.

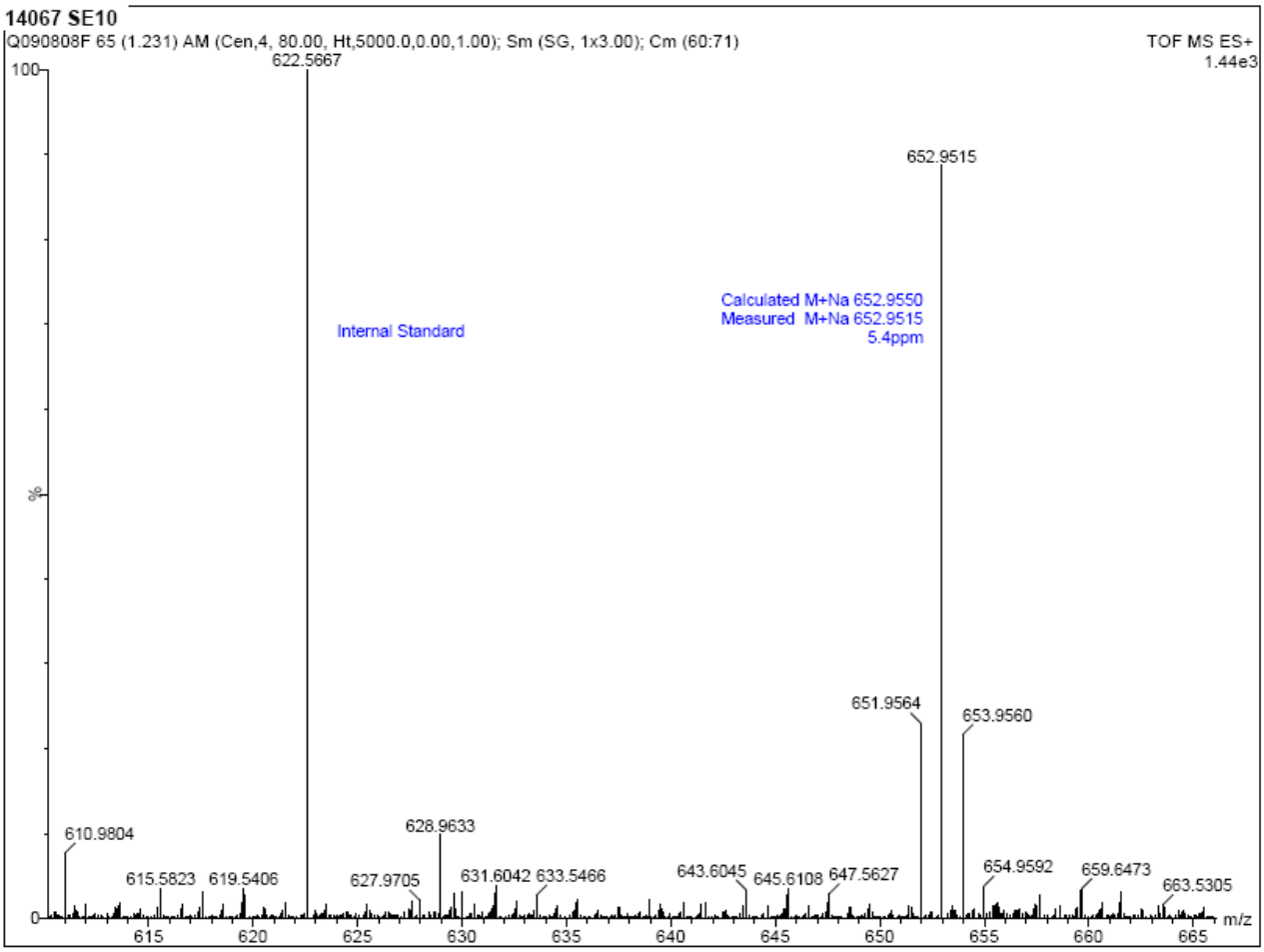
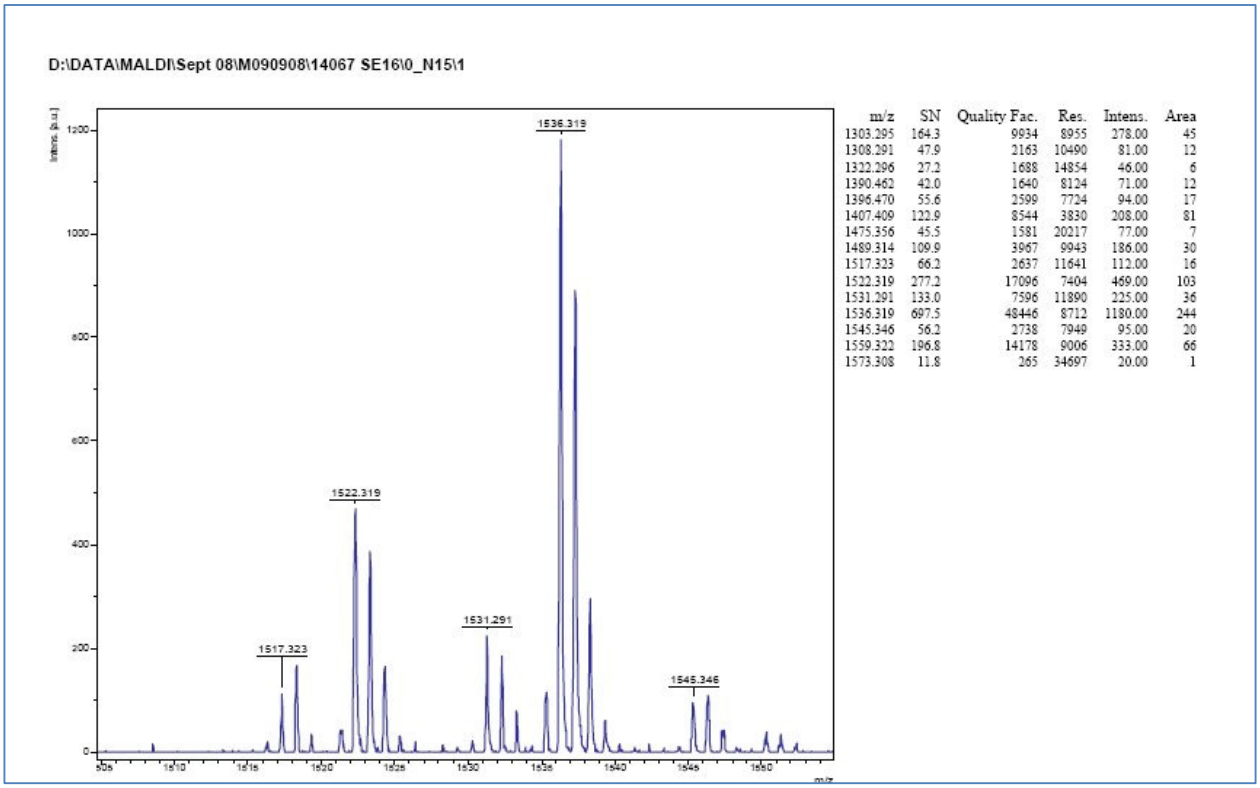
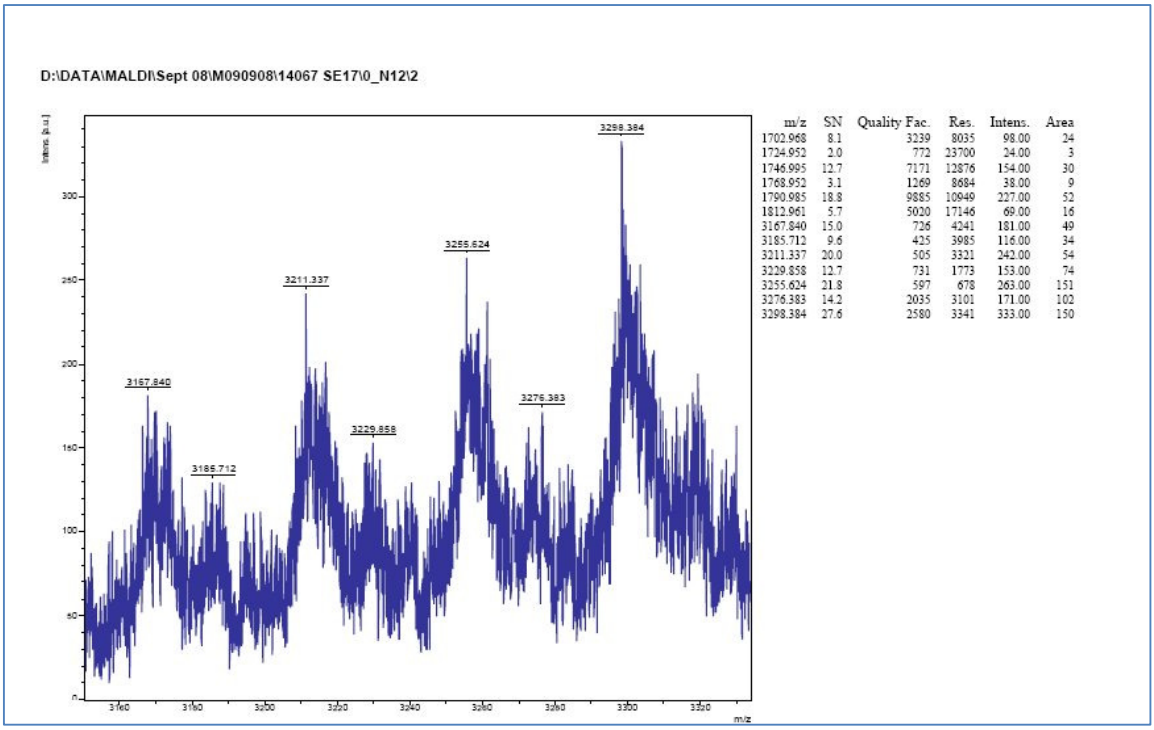


Figure S22. ESI-HRMS of compound **7**.



m/z	SN	Quality Fac.	Res.	Intens.	Area
1303.295	164.3	9934	8955	278.00	45
1308.291	47.9	2163	10490	81.00	12
1322.296	27.2	1688	14854	46.00	6
1390.462	42.0	1640	8124	71.00	12
1396.470	55.6	2599	7724	94.00	17
1407.409	122.9	8544	3830	208.00	81
1475.356	45.5	1581	20217	77.00	7
1489.314	109.9	3967	9943	186.00	30
1517.323	66.2	2637	11641	112.00	16
1522.319	277.2	17096	7404	469.00	103
1531.291	133.0	7596	11890	225.00	36
1536.319	697.5	48446	8712	1180.00	244
1545.346	56.2	2738	7949	95.00	20
1559.322	196.8	14178	9006	333.00	66
1573.308	11.8	265	34697	20.00	1

Figure S23. MALDI-MS of compound **8**.



m/z	SN	Quality Fac.	Res.	Intens.	Area
1702.968	8.1	3239	8035	98.00	24
1724.952	2.0	772	23700	24.00	3
1746.995	12.7	7171	12876	154.00	30
1768.952	3.1	1269	8684	38.00	9
1790.985	18.8	9885	10949	227.00	52
1812.961	5.7	5020	17146	69.00	16
3167.840	15.0	726	4241	181.00	49
3185.712	9.6	425	3985	116.00	34
3211.337	20.0	505	3321	242.00	54
3229.858	12.7	731	1773	153.00	74
3255.624	21.8	597	678	263.00	151
3276.383	14.2	2035	3101	171.00	102
3298.384	27.6	2580	3341	333.00	150

Figure S24. MALDI-MS of compound **1**.