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Supporting Information

Pretargeted radiotherapy and synergistic treatment of metastatic, castration-resistant prostate cancer using cross-linked, PSMA-targeted lipoic acid nanoparticles

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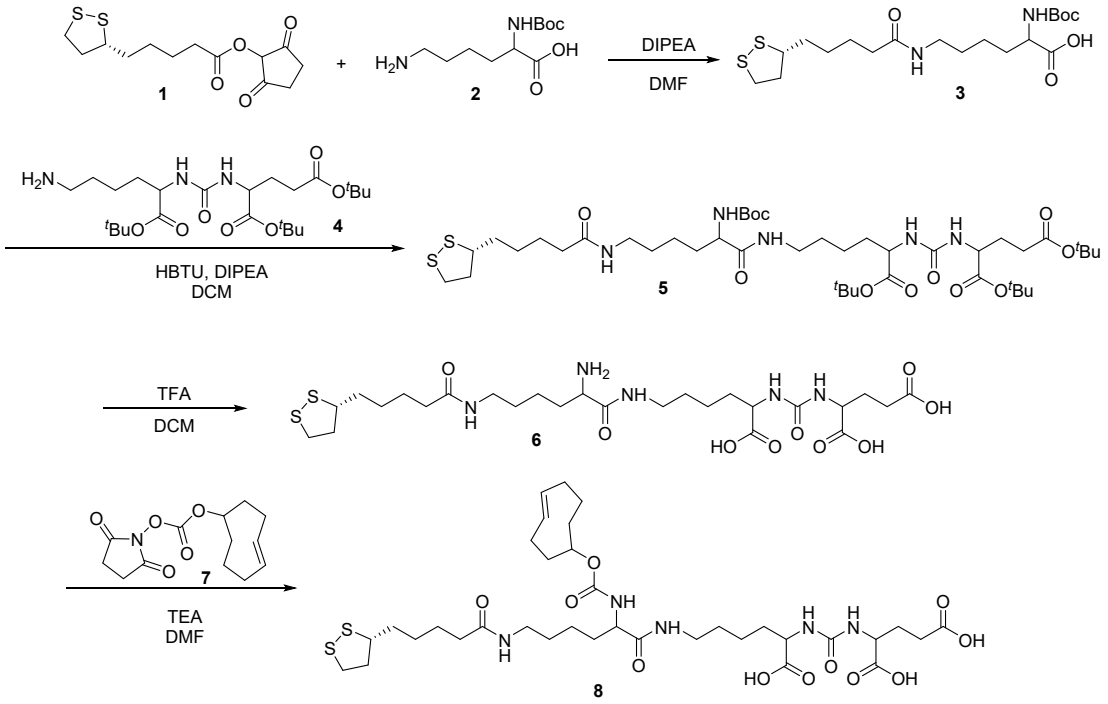
1 Reagents and Apparatus

All chemical reagents were purchased from J&K Scientific, TCI, Energy Chemical, Bide Pharm, and Xi'an Confluore Biological Technology. Na[^{131}I] was obtained from Syncor Pharmaceutical Co., Ltd. (Chengdu, China).

The ^{1}H and ^{13}C NMR spectra were taken on Bruker nuclear magnetic resonance spectrometer (^{1}H, 400 MHz; ^{13}C, 101 MHz). High-resolution mass spectra (HRMS) were recorded on a Bruker micro-TOF-QII time of flight mass spectrometer with electrospray ionization. Confocal fluorescence images were recorded on a LSM 880 system (ZEISS, Germany). Pre-targeted single photon emission computed tomography/computed tomography imaging was performed using an NM/CT 670 system (GE, USA).

2 Synthesis

2.1 Synthesis of PLAA (8)



Scheme S1. Synthesis of PLAA (8).

N^6-(5-(1,2-Dithiolan-3-yl)pentanoyl)-N^2-(*tert*-butoxycarbonyl)lysine (3)

Compounds **1**[1] (200.0 mg, 0.600 mmol, 1.0 equiv) and **2** (J&K Scientific, 198.2 mg, 0.800 mmol, 1.3 equiv) were dissolved in DMF (5.0 mL). *N*,*N*-Diisopropylethylamine (DIPEA) (195.0 mg, 1.500 mmol, 2.5 equiv) was then added, and the mixture was stirred at room temperature (rt) overnight. The reaction mixture was diluted with water and extracted with DCM. The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The desired product (**3**) was obtained in 90% yield (312.9 mg, 0.719 mmol) after purification by silica gel column chromatography (DCM:MeOH = 60:1 ~ 20:1).

^{1}H NMR (400 MHz, CD_3OD) δ 4.05 (dd, J = 9.0, 4.8 Hz, 1H), 3.57 (dq, J = 8.8, 6.1 Hz, 1H), 3.21–3.06 (m, 4H), 2.51–2.40 (m, 1H), 2.18 (t, J = 7.4 Hz, 2H), 1.93–1.76 (m, 2H), 1.76–1.58 (m, 5H), 1.44 (s, 15H). **^{13}C NMR** (101 MHz, CD_3OD) δ 176.24, 175.97, 158.15, 80.45, 57.53, 54.81, 41.30, 40.05, 39.35, 36.91, 35.74, 32.46, 29.98, 29.88, 28.75, 26.77, 24.31. **HRMS** (ESI) $[M + Na]^+$ *m/z* calcd. for $[C_{19}H_{34}N_2NaO_5S_2]^+$ 457.1801, found 457.1784.

Tri-*tert*-butyl 6-(4-(5-(1,2-dithiolan-3-yl)pentanamido)butyl)-2,2-dimethyl-4,7,15-trioxo-3-oxa-5,8,14,16-tetraazanonadecane-13,17,19-tricarboxylate (5)

Compounds **3** (150.0 mg, 0.350 mmol, 1.0 equiv), compound **4**[2] (247.7 mg, 0.520 mmol, 1.5 equiv), and

(2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 196.3 mg, 0.520 mmol, 1.5 equiv) were dissolved in DCM (5.0 mL). DIPEA (89.2 mg, 0.690 mmol, 2.0 equiv) was then added, and the mixture was stirred at rt overnight. The reaction solution was concentrated *in vacuo.* Purification by silica gel column chromatography (DCM:MeOH = 40:1) afforded compound **5** in 35% yield (109.0 mg, 0.120 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (q, *J* = 5.4 Hz, 1H), 6.27 (dd, *J* = 11.9, 8.4 Hz, 1H), 4.04 (dt, *J* = 8.6, 5.7 Hz, 1H), 3.94 (q, *J* = 5.7 Hz, 1H), 3.80 (s, 1H), 3.66–3.55 (m, 1H), 3.22–2.94 (m, 6H), 2.41 (dq, *J* = 12.5, 6.3 Hz, 1H), 2.30–2.14 (m, 2H), 2.03 (t, *J* = 7.3 Hz, 2H), 1.92–1.81 (m, 2H), 1.72–1.14 (m, 56H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.25, 171.89, 171.86, 171.69, 171.40, 157.09, 155.25, 80.57, 80.24, 79.71, 77.88, 56.10, 54.26, 53.01, 52.15, 39.90, 39.42, 38.21, 38.09, 35.24, 34.13, 31.81, 31.70, 30.86, 28.89, 28.72, 28.34, 28.18, 27.72, 27.64, 27.63, 25.07, 22.93, 22.43. **HRMS** (ESI) $[M + H]^+$ *m/z* calcd. for $[C_{43}H_{78}N_5O_{11}S_2]^+$ 904.5134, found 904.5133.

14-Amino-24-(1,2-dithiolan-3-yl)-5,13,20-trioxo-4,6,12,19-tetraazatetracosane-1,3,7-tricarboxylic acid (6)

Trifluoroacetic acid (TFA, 1.0 mL) was added upon stirring to a solution of compound **5** (101.0 mg, 0.110 mmol) in anhydrous DCM (1.0 mL). After stirring at rt for 6 h, the reaction solution was added dropwise into 20.0 mL of diethyl ether. The mixture was then cooled at 4 °C and filtered. Compound **6** was obtained as a white precipitate without further purification (70.5 mg, 99%, 0.110 mmol).

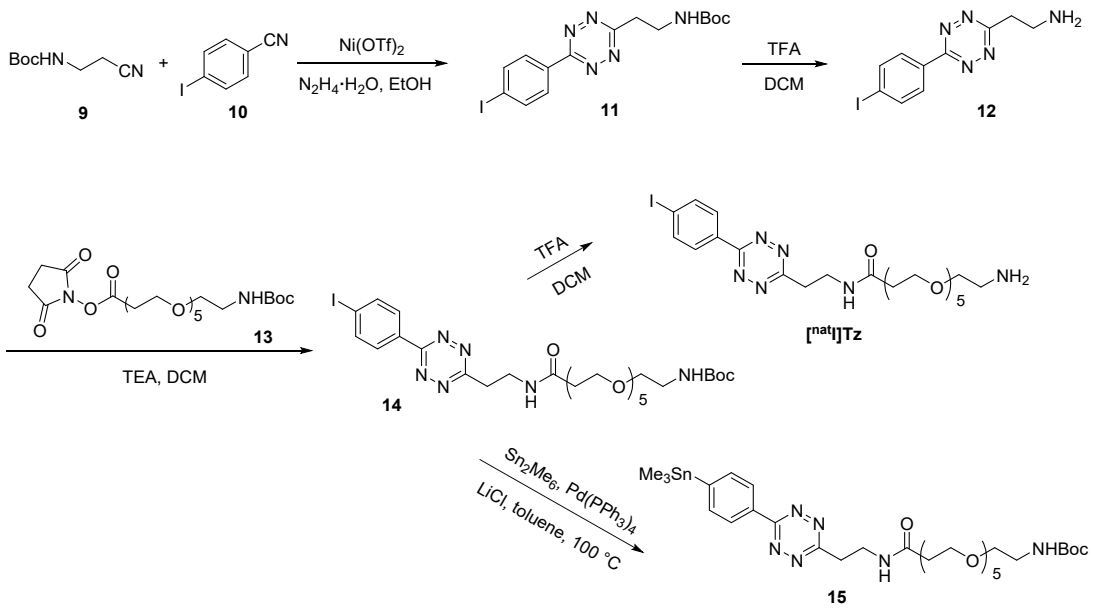
^{1}H NMR (400 MHz, CD_3OD) δ 4.30 (ddd, *J* = 21.1, 8.5, 5.0 Hz, 2H), 3.78 (t, *J* = 6.6 Hz, 1H), 3.59 (dq, *J* = 9.0, 6.4 Hz, 1H), 3.30–3.09 (m, 5H), 2.52–2.39 (m, 2H), 2.18 (dt, *J* = 21.3, 6.9 Hz, 3H), 1.96–1.77 (m, 5H), 1.76–1.52 (m, 9H), 1.51–1.40 (m, 6H), 1.33 (d, *J* = 4.6 Hz, 2H). **HRMS** (ESI) $[M + H]^+$ *m/z* calcd. for $[C_{26}H_{46}N_5O_9S_2]^+$ 636.2732, found 636.2742.

(*E*)-14-(((cyclooct-4-en-1-yloxy)carbonyl)amino)-24-(1,2-dithiolan-3-yl)-5,13,20-trioxo-4,6,12,19-tetraazatetracosane-1,3,7-tricarboxylic acid (8, PLAA)

Compounds **6** (9.7 mg, 0.015 mmol, 1.0 equiv) and **7** (Xi'an Confluore Biological Technology, 6.5 mg, 0.018 mmol, 1.2 equiv) were dissolved in DMF (1.0 mL). Triethylamine (TEA, 9.0 mg, 0.080 mmol, 5.3 equiv) was then added, and the mixture was stirred at rt for 6 h. After reaction completion, the reaction solution was added dropwise into 25.0 mL of diethyl ether. The mixture was then cooled at 4 °C, and the formed precipitate was filtered out, then purified by thin-layer chromatography (DCM:MeOH = 15:1) to afford PLAA as a white solid in 79% yield (9.1 mg, 0.011 mmol).

^{1}H NMR (400 MHz, CD_3OD) δ 5.61 (dt, *J* = 14.9, 7.4 Hz, 1H), 5.48 (ddd, *J* = 15.7, 10.6, 3.5 Hz, 1H), 4.37–4.22 (m, 3H), 4.06–3.95 (m, 1H), 3.59 (dq, *J* = 8.8, 6.3 Hz, 1H), 3.24–3.07 (m, 5H), 2.48–2.28 (m, 5H), 2.17 (dt, *J* = 18.9, 6.4 Hz, 3H), 2.05–1.82 (m, 7H), 1.74–1.60 (m, 8H), 1.56–1.38 (m, 10H), 1.33 (d, *J* = 4.2 Hz, 4H). **^{13}C NMR** (101 MHz, CD_3OD) δ 176.49, 176.12, 176.01, 175.09, 174.39, 160.12, 158.25, 136.04, 133.79, 82.24, 57.56, 56.49, 54.00, 53.65, 42.23, 41.33, 40.01, 39.77, 39.56, 39.37, 39.14, 36.94, 35.76, 35.17, 33.49, 33.00, 32.17, 31.51, 31.19, 30.01, 29.91, 29.06, 26.79, 24.29, 23.63. **HRMS** (ESI) $[M + H]^+$ *m/z* calcd. for $[C_{35}H_{58}N_5O_{11}S_2]^+$ 788.3569, found 788.3572.

2.2 Synthesis of [^{nat}I]Tz and compound 15



Scheme S2. Synthesis of [^{nat}I]Tz and compound 15.

***tert*-Butyl (2-(6-(4-iodophenyl)-1,2,4,5-tetrazin-3-yl)ethyl)carbamate (11)**

Compound **9** (3.3 g, 14.410 mmol, 1.0 equiv), compound **10** (1.00 g, 5.880 mmol, 0.4 equiv), and $Ni(OTf)_2$ (632.0 mg, 1.930 mmol, 0.1) were dissolved in dioxane (2.0 mL). Hydrazine hydrate (98%, 4.0 mL, 82.301 mmol, 5.7 equiv) was then added at 0 °C, and the mixture was stirred at 60 °C overnight. After reaction completion, the reaction solution was cooled in an ice bath, and a cold aqueous $NaNO_2$ solution (4.46 g, 100.0 mL, 64.637 mmol, 4.5 equiv) was added, followed by slow addition of 1 M HCl with vigorous stirring. The reaction mixture turned bright red, and gas production was observed. The addition of HCl was continued until gas production stopped, and the pH reached 3.0–4.0. Then, the mixture was extracted with DCM (50.0 mL × 3), and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The obtained residue was purified by silica gel column chromatography (DCM:MeOH = 50:1 ~ 30:1) to afford a pink solid in 24% yield (600.0 mg, 1.40 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30–8.20 (m, 2H), 8.10–8.01 (m, 2H), 3.48 (q, J = 6.2 Hz, 2H), 3.38 (dd, J = 6.9, 5.3 Hz, 2H), 1.26 (s, 9H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 168.53, 163.52, 156.03, 138.90, 131.83, 129.51, 101.15, 78.23, 35.98, 28.58.

2-(6-(4-Iodophenyl)-1,2,4,5-tetrazin-3-yl)ethan-1-amine (12)

TFA (1.0 mL) was added dropwise upon stirring to a solution of compound **11** (600.0 mg, 1.410 mmol) in anhydrous DCM (4.0 mL) in an ice bath. After stirring at rt for 1 h, the reaction solution was concentrated *in vacuo,* and the obtained residue was purified by silica gel column chromatography (DCM:MeOH = 10:1) to afford a pink solid in 93% yield (428.0 mg, 1.30 mmol).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, J = 8.5 Hz, 2H), 8.09 (d, J = 8.5 Hz, 2H), 7.99 (s, 2H), 3.65

(t, J = 6.9 Hz, 2H), 3.46 (t, J = 6.9 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 167.31, 163.87, 139.01, 131.70, 129.63, 101.45, 37.34, 32.70. **HRMS** (ESI) $[M + H]^+$ *m/z* calcd. for $[C_{10}H_{10}IN_5]^+$ 328.0054, found 328.0060.

***tert*-Butyl (21-(6-(4-iodophenyl)-1,2,4,5-tetrazin-3-yl)-18-oxo-3,6,9,12,15-pentaoxa-19-azahenicosyl)carbamate (14)**

Compounds **12** (150.0 mg, 0.460 mmol, 1 equiv) and **13** (Xi'an Confluore Biological Technology, 200.0 mg, 0.390 mmol, 0.8 equiv) were dissolved in DCM (10.0 mL). TEA (185.0 mg, 1.850 mmol, 4.0 equiv) was then added, and the mixture was stirred at rt for 3 h. After reaction completion, the mixture was concentrated *in vacuo,* and the obtained residue was purified by silica gel column chromatography (DCM:MeOH = 100:1 ~ 50:1) to afford a pink solid in 85% yield (198.0 mg, 0.365 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39–8.25 (m, 2H), 8.04–7.86 (m, 2H), 7.15 (s, 1H), 5.14 (s, 1H), 3.88 (q, J = 6.3 Hz, 2H), 3.74–3.49 (*m*, 23H), 3.31 (*q*, J = 5.4 Hz, 2H), 2.43 (*td*, J = 5.7, 2.3 Hz, 2H), 1.43 (*s*, 10H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 173.24, 170.82, 168.49, 163.63, 158.96, 158.75, 156.05, 138.93, 131.87, 129.57, 118.61, 116.63, 101.15, 78.03, 70.23, 70.08, 69.97, 69.94, 69.62, 67.13, 46.04, 37.55, 36.54, 35.29, 28.67, 25.67.

1-Amino-*N*-(2-(6-(4-iodophenyl)-1,2,4,5-tetrazin-3-yl)ethyl)-3,6,9,12,15-pentaoxaoctadecan-18-amide ([^{nat}I]Tz)

TFA (1.0 mL) was added dropwise upon stirring to a solution of compound **14** (194.0 mg, 0.230 mmol) in anhydrous DCM (4.0 mL) in an ice bath. After stirring at rt for 3 h, the reaction mixture was concentrated *in vacuo,* and the obtained residue was purified by silica gel column chromatography (DCM:MeOH = 10:1) to afford a pink solid in 93% yield (155.0 mg, 0.350 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.31 (d, J = 8.6 Hz, 2H), 7.93 (d, J = 8.6 Hz, 2H), 3.99–3.93 (m, 2H), 3.87 (q, J = 6.5 Hz, 2H), 3.81–3.77 (m, 2H), 3.76–3.68 (m, 6H), 3.67–3.55 (m, 12H), 3.15–3.07 (m, 2H), 2.62 (t, J = 6.0 Hz, 2H). **^{13}C NMR** (151 MHz, DMSO-d_6) δ 173.23, 170.82, 168.48, 163.63, 138.94, 131.85, 129.57, 101.19, 70.20, 70.13, 70.08, 69.93, 67.14, 67.12, 60.22, 39.08, 37.56, 36.51, 35.29, 25.69, 21.22, 14.54. **HRMS** (ESI) $[M + H]^+$ *m/z* calcd. for $[C_{23}H_{36}IN_6O_6]^+$ 619.1736, found 619.1618.

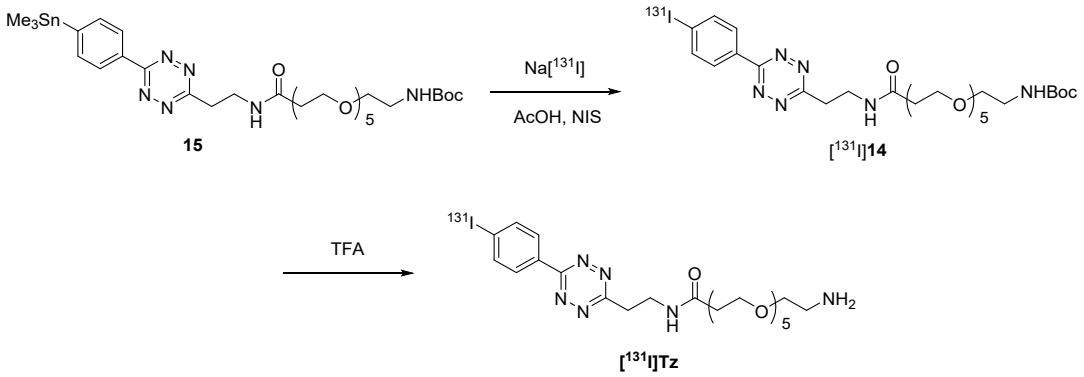
***tert*-Butyl(18-oxo-21-(6-(4-(trimethylstannyl)phenyl)-1,2,4,5-tetrazin-3-yl)-3,6,9,12,15-pentaoxa-19-azahenicosyl)carbamate (15)**

Compound **14** (142.0 mg, 0.190 mmol, 1 equiv), $Pd(PPh_3)_4$ (46.0 mg, 0.040 mmol, 0.2 equiv), and LiCl (50.0 mg, 1.179 mmol, 6.2 equiv) were dissolved in toluene (5.0 mL) under argon. Sn_2Me_6 (84.0 mg, 0.260 mmol, 1.4 equiv) was then added, and the reaction mixture was stirred at 100 °C for 3 h. Next, the reaction solution was filtered and washed twice with DCM. The combined organic layers were concentrated *in vacuo,* and the obtained residue was purified by silica gel column chromatography

(DCM:MeOH = 100:1 ~ 50:1) to afford a dark red solid in 30% yield (37.3 mg, 0.064 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57–8.44 (m, 2H), 7.82–7.62 (m, 2H), 7.14 (s, 1H), 5.17 (s, 1H), 3.90 (q, *J* = 6.3 Hz, 2H), 3.69 (t, *J* = 5.7 Hz, 2H), 3.65–3.55 (m, 19H), 3.53 (t, *J* = 5.1 Hz, 2H), 3.49 (s, 1H), 3.30 (d, *J* = 5.3 Hz, 2H), 1.43 (s, 10H), 0.36 (s, 9H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.12, 168.33, 164.80, 149.55, 136.82, 131.57, 127.06, 70.70, 70.66, 70.57, 70.39, 70.35, 70.28, 67.25, 40.51, 37.38, 36.99, 35.04, 28.57, -9.33. **HRMS** (ESI) $[M + Na]^+$ *m/z* calcd. for $[C_{31}H_{52}N_6NaO_8Sn]^+$ 779.2761, found 779.2781.

2.3 Preparation of [^{131}I]Tz



Scheme S3. Preparation of [^{131}I]Tz.

A solution of compound **15** (2.0 mg) in dichloromethane (200.0 μL) was added to a mixture of acetic acid (300.0 μL) and NIS methanol solution (0.4 mg/mL, 300.0 μL). Na[^{131}I] (40.0 μL, 11.5 mCi) was subsequently added, and the mixture was vortexed for several seconds, then incubated at room temperature for 45 min. The reaction was quenched with sodium citrate in deionized water (300.0 μL, 0.8 mg/mL), then the resulting solution was diluted in acetonitrile, extracted three times with dichloromethane (100.0 μL), and the isolated compound **14** was incubated with 200.0 μL of TFA at rt for 2 h to give the ^{131}I-labeled tetrazine derivative **[^{131}I]Tz** (8.5 mCi), which was purified by radio-HPLC.

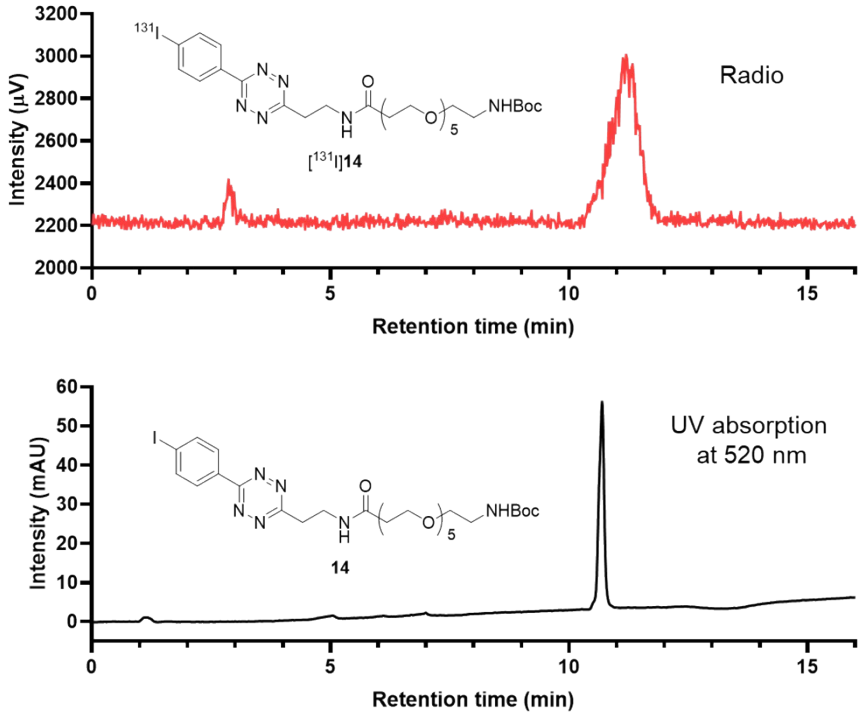


Figure S1. HPLC profile of [^{131}I]14 and compound 14.

3 Experimental procedures and supplementary figures

*3.1**Preparation and characterization of cPLANPs.***

A 1 mg/mL solution of PLAA in PBS (pH = 7.2) was left at 25 °C for 1–3 minutes, and nanoparticles formed spontaneously. The resulting pale blue emulsion was irradiated under UV light (365 nm) for 2 h to give cPLANPs through a ring-opening disulfide exchange polymerization reaction. The size and zeta potential of cPLANPs were determined by DLS (Brookhaven NanoBrook 90plus Zeta, NY, USA) and their morphology was observed after negative staining with 2% phosphotungstic acid solution by TEM (FEI Talos F200S, OR, USA).

*3.2 **Cellular uptake of cPLANPs.***

LNCaP and PC3 cells were seeded into 35-mm glass-bottom dishes, incubated for 48 h at a density of ~80%, and then treated with cPLANPs (10 ng/mL) for 2 h. Afterward, the culture medium was removed, and the cells were washed three times with PBS and incubated with **Tz-Cy5** (1 μM) for another 1 h. The cells were finally washed briefly with PBS and the cell nuclei were stained with Hoechst 33342 (2 μM) for 5 min, washed three times with PBS, and imaged with a confocal fluorescence microscope (Zeiss 880, Germany).

3.3 ***Synergistic killing effect of cPLANPs on LNCaP cells.***

LNCaP cells were incubated with different concentrations of cPLANPs (0, 50, 200, 500, 1000 μg/mL) at 37 °C under normoxic conditions (20% O_2/5% CO_2) and the harvested cells were analyzed by Western blotting. Briefly, the protein bands were incubated at 4 °C overnight with primary antibodies (1:500) against BCL-2 (Abcam, Cambridge, UK), caspase-3 (Abcam, Cambridge, UK), or *β*-actin (SAB, College Park, USA), then washed three times with Tris-buffered saline containing 0.1% Tween-20 (TBST), and incubated with secondary antibodies ((SAB, College Park, USA), diluted at 1:5000) at 25 °C for 2 h. The bands were washed again three times with TBST and visualized with ECL reagents (Millipore, Massachusetts, USA) and a ChemiDoc XR+UV illuminator (Bio-Rad, Hercules, CA, USA).

3.4 ***Simulation of cPLANPs-based, pre-targeted radiotherapy in LNCaP cells.***

LNCaP cells were seeded in six-well plates (3×10^6 cells/well) and incubated in culture medium overnight at 37 °C under normoxic conditions (20% O_2/5% CO_2). The culture medium was then removed, and the cells were incubated with cPLANPs (10 ng/mL) for 2 h. The culture medium was removed again and the cells were washed three times with PBS, then treated for 1 h with fresh culture medium containing **[^{131}I]Tz** of different radioactivity. Afterward, the cells were washed again with PBS, incubated for another 48 h, and stained with calcein-AM and PI for live-or-dead cell imaging or with Hoechst 33342 for nuclei imaging. Stained cells were visualized by confocal fluorescence microscopy (Zeiss 880, Germany). The same process was used to determine cell apoptosis by Annexin-V-FITC/PI staining and flow cytometry.

3.5 ***ϒ-H2AX immunofluorescence assay.***

LNCaP cells were seeded on 35-mm glass bottom dishes, incubated in culture medium at 37 °C under normoxic conditions (20% O_2/5% CO_2) for 48 h, then treated with cPLANPs (10 ng/mL) for 2 h. The culture medium was then removed, and the cells were washed three times with PBS and treated with fresh culture medium containing **[^{131}I]Tz** (100 μCi) for 1 h. The solution was decanted and the cells were incubated for another 48 h at 37 °C under normoxic conditions after washing several times with PBS. The ϒ-H2AX immunofluorescence assay was then performed following the manufacturer's instructions. Briefly, the cells were fixed with 4% paraformaldehyde at 25 °C for 10 min, washed with PBS, permeabilized by incubation with 0.3% (v/v) Triton X-100 at 4 °C for 30 min, blocked with 5% bovine serum albumin solution at 25 °C for 1 h, washed again three times with PBS, and incubated with anti-ϒ-H2AX antibody (Cell Signaling Technology, Boston, USA, diluted at 1:500) at 37 °C for 2 h. The antibody solution was then removed, and the cells were incubated with AlexaFluor 488-conjugated secondary antibody (Jackson Immuno Reseacher, Pennsylvania, USA, diluted at 1:1000) at 37 °C for 1 h. Nuclei were stained with Hoechst 33342. Finally, cells were observed under a confocal fluorescence microscope (Zeiss 880).

3.6 ***Tumor model.***

Male Balb/c nude mice (5 weeks old, 20 g) were obtained from Chengdu Dossy Experimental Animals

Co., Ltd. (Chengdu, China) and housed under specific pathogen-free conditions in the animal care facility at Sichuan University. All in vivo experiments were approved by the Animal Care and Use Committee and Ethics Committee of West China Hospital, Sichuan University (Approval No. 20220512004). LNCaP cells (~10^7 cells) suspended in 100 μL PBS/matrigel (3:1, v/v) were subcutaneously injected into the right axillary of each mouse. When the tumor volume reached 90–120 mm^3, the length and width of the tumors were measured using a vernier caliper, and the tumor volume was calculated as follows: tumor volume = 0.5 × $width^2$ × length.

3.7 ***In vivo anti-tumor activity.***

The in vivo anti-tumor efficacy of the developed strategy was evaluated in male Balb/c nude mice bearing LNCaP tumors. When the tumor volume reached 90–120 mm^3, the mice were randomly divided into five groups (n = 5). In three groups, cPLANPs were intravenously administered every three days at a dose of 5 mg/kg, for a total of five times. At 24 h after each injection, **[^{131}I]Tz** (200 μCi), **[^{nat}I]Tz**, or saline was intravenously injected via the tail vein. In another group, mice were intravenously administered with saline every three days for five times. At 24 h after each injection, **[^{131}I]Tz** (200 μCi) was intravenously injected via the tail vein. In the last group, mice were treated with saline alone; these animals served as the control.

During treatment, the length and width of tumors and the body weight of mice were recorded. At the end of the experiment, all animals were sacrificed, and their tumors and visceral organs were collected for additional experiments. The tumor tissues were snap-frozen in optimal cutting temperature and stored at −80 °C. The tumor tissues were sectioned into 5-μm slices, stained with TUNEL reagent, and observed on a microscope (VS2000, Olympus, Tokyo, Japan). In order to determine the expression of BCL-2 and caspase-3 in vivo, tumor tissues were cut into pieces and sonicated in RIPA buffer. Total protein levels were measured using the bicinchoninic acid protein assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions, then lysates were analyzed by Western blotting as described above. Brain, heart, liver, spleen, lung, and kidney samples were also fixed in 4% paraformaldehyde for 24 h, embedded into paraffin, sectioned into slices, and stained with hematoxylin–eosin to assess the safety profile of cPLANP-based, pre-targeted radiotherapy.

3.8 ***The critical aggregation concentration of PLAA***

A mixture of Nile Red (final concentration, 1.0 μM) and PLAA (final concentration, 0.03–40 mM) in PBS were shaken at rt overnight. The fluorescence emission intensity of Nile Red at 525 nm (λ_{ex} = 485 nm) was measured with a FluoroMax-4 fluorometer (Horiba, Kyoto, Japan). The critical aggregation concentration of PLAA in PBS was determined at 2.1 mM as the intersection of the tangents to the two linear segments of the graph.

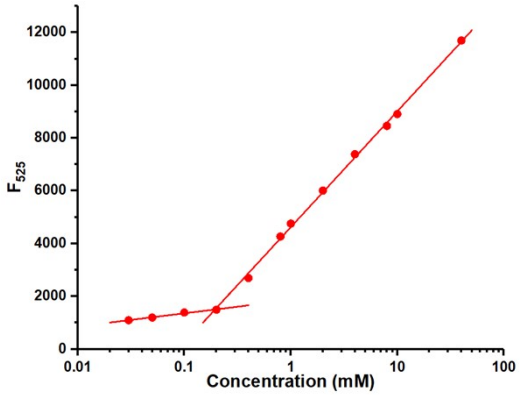


Figure S2. Fluorescence emission intensity of Nile red (1.0 μM) at 525 nm as a function of the concentration of PSMA-targeted lipoic acid amphiphile (PLAA).

3.9 ***In vitro stability of cPLANPs***

cPLANPs at different concentrations (10, 100, 200 μg/mL) were dissolved in RPMI-1640 by itself or supplemented with 10% (v/v) FBS, and their average size was monitored by DLS at 25 ºC for 72 h.

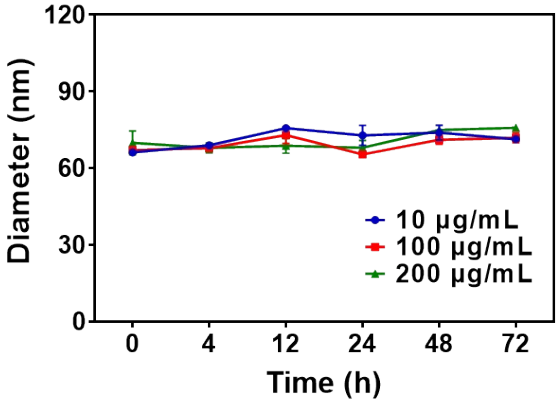


Figure S3. *In vitro* stability of cross-linked, PSMA-targeted lipoic acid-based nanoparticles upon incubation in serum-free RPMI-1640 at 25 ºC.

3.10 ***In vitro cytotoxicity of cPLANPs***

The cytotoxicity of cPLANPs against PC3 cells (PSMA-negative cells), LNCaP cells (PSMA-positive cells), and MSC cells was evaluated using the CCK-8 assay. LNCaP cells were cultured in RPMI-1640 supplemented with 10% FBS, while PC3 and MSC cells were cultured in Dulbecco's modified Eagle medium containing 10% FBS. All cells were seeded into 96-well plates at a density of 1×10^5 cells/well and incubated for another 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were then treated with various cPLANP concentrations (0, 1, 10, 50, 100, 200, 500 μg/mL) for another 24, 48,

and 72 h. Afterward, the culture medium was removed and replaced with 150 μL of fresh medium containing 10% (v/v) CCK8 solution, followed by incubation for another 2 h. Absorbance at 450 nm was measured using a microplate reader (Tecan Spark, Maennedorf, Switzerland).

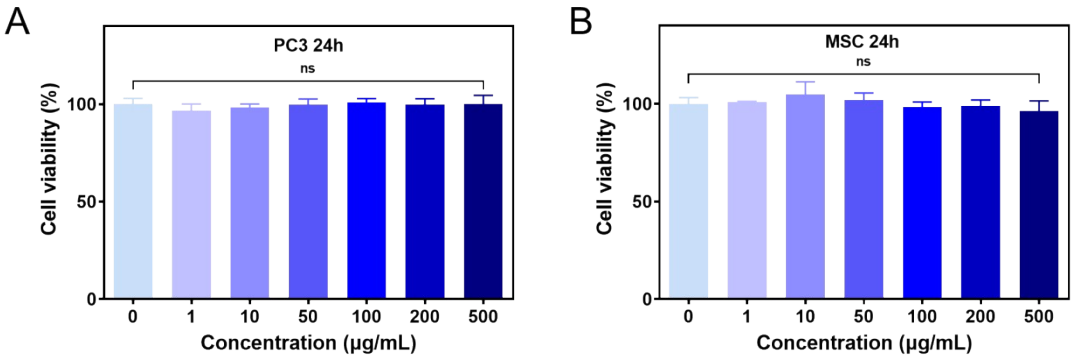


Figure S4. Viability of (**A**) PC3 cells (PSMA-negative) and (**B**) MSC cells (normal control) after 24-h treatment with the indicated concentrations of cPLANPs.

3.11 ***In vivo SPECT/CT imaging***

LNCaP tumor-bearing mice were randomly divided into three groups. In the first group, mice received 200 μL of cPLANPs (5 mg/kg) intravenously through the tail vein, followed 24 h later by **[^{131}I]Tz** (200 μCi). In the second group, mice were administered only with **[^{131}I]Tz** (200 μCi), while in the third group, mice were first injected with PLAA, followed 24 h later by **[^{131}I]Tz** (200 μCi).

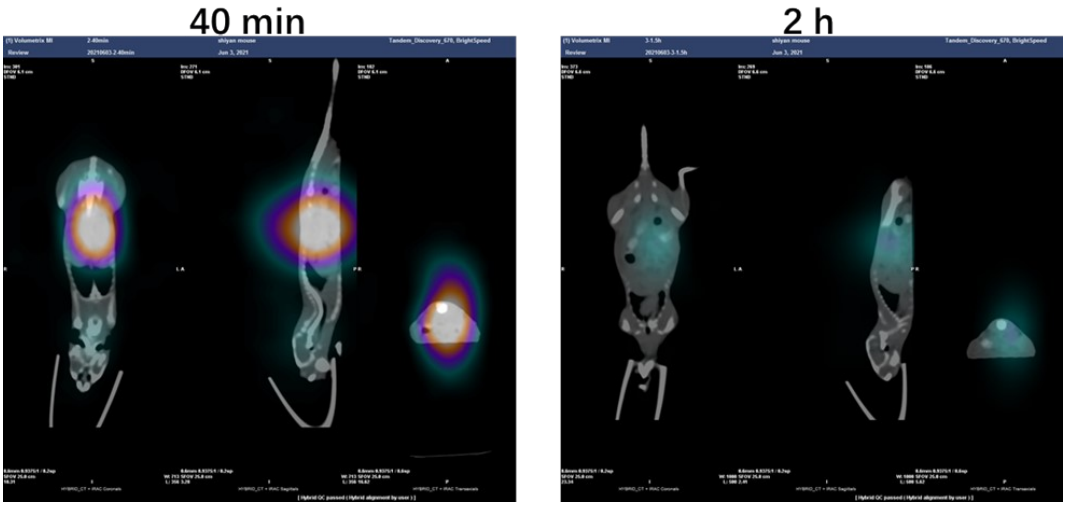


Figure S5. Pre-targeted single photon emission computed tomography/computed tomography imaging of mice bearing LNCaP tumors at different time points after intravenous injection of **[^{131}I]Tz** (200 μCi). Imaging was performed using an NM/CT 670 system (GE, USA)

3.12 supplementary figures S6

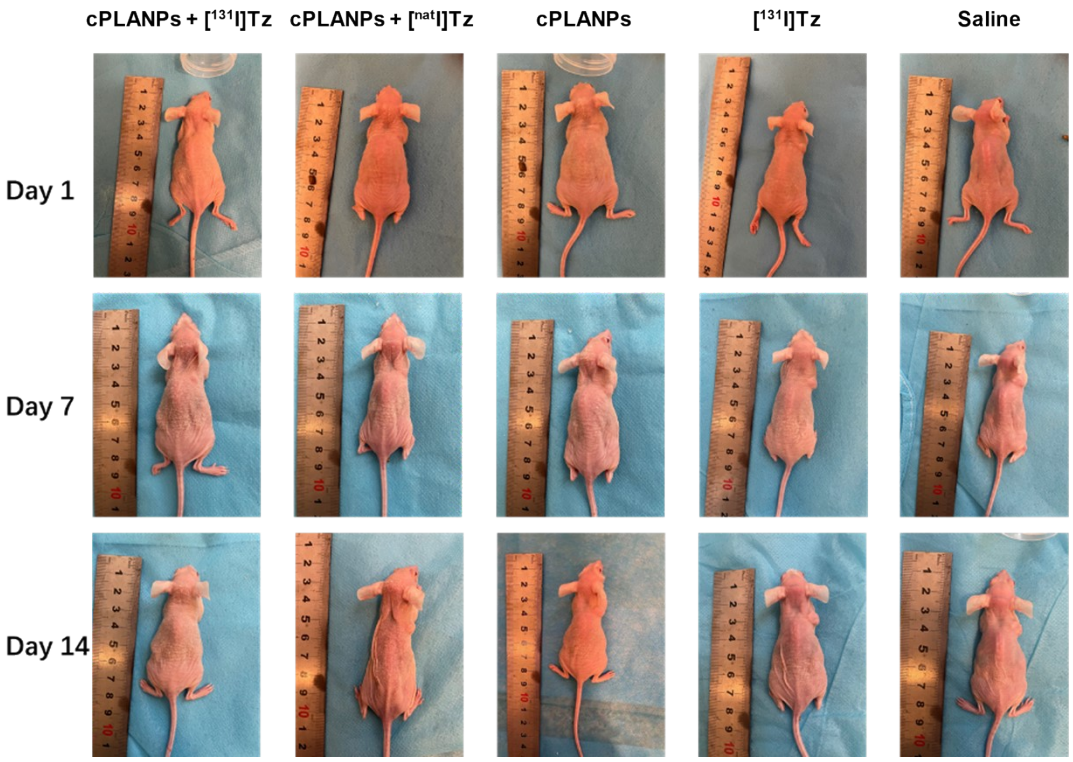


Figure S6. Appearance of mice bearing LNCaP tumors at different time points after the indicated treatments. cPLANPs, cross-linked, PSMA-targeted lipoic acid-based nanoparticles; **[^{131}I]Tz**, ^{131}I-labeled 1,2,4,5-tetrazine derivative.

3.13 Western Blot

The expression levels of BCL-2 and caspase-3 in cells were evaluated by Western Blot Assay. First, LNCaP cells treated with 0–1000 μg/mL cPLANPs for 48 h were collected and lysed with RIPA lysis buffer. The total protein concentration was measured using a BCA kit. The concentration of SDS-PAGE gel should be determined according to the size of the protein band, and the preparation method followed the instructions of the kit. After the SDS-PAGE gel was prepared, the same amount of protein was added into each well of SDS-PAGE gel, running at 90 V for 40 min, and then 120 V for another 1 h. After that, the gel was transferred to a PVDF membrane (MerckMillipore, IPVH00010), cleaned with TBST buffer 3 times, and sealed with skim milk for 1 h. Following cleaning with TBST buffer 3 times again, and incubated for 12 h at 4 °C with specific antibodies: rabbit polyclonal antibody to Bcl-2 (Affinity, AF6139, dilution 1/500) and rabbit monoclonal [EPR18297] to Caspase-3 (abcam, ab184787, dilution 1/500). On the second day, the PVDF membrane was added to the secondary antibody and incubated at room temperature for 1 h. Finally, the ECL substrate was added and the protein blots were visualized on the ChemiDoc Imaging System (ChemiScope 6100, China).

3.14 ***Statistical analysis.***

Statistical analysis was performed using GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA, USA). Differences between two groups were assessed for significance using Student's t test, while differences among multiple groups were assessed using analysis of variance. All data were expressed as mean ± standard deviation (SD). Differences associated with $P < 0.05$ were considered statistically significant.

3.15 ***Raw western bot data for Figure 2D.***

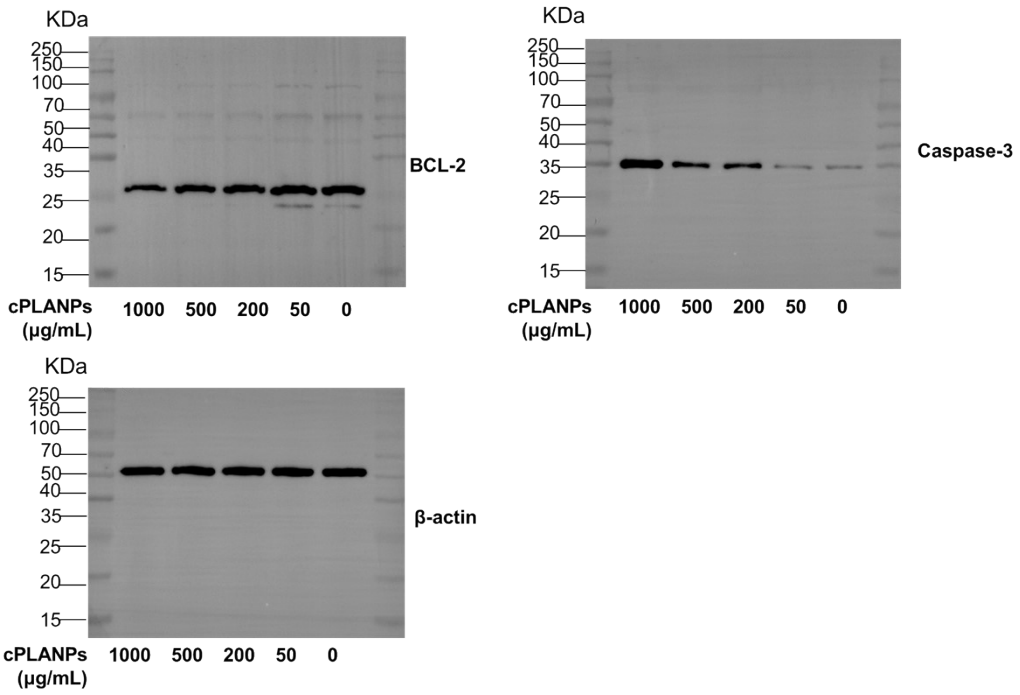


Figure S7. Raw western bot data for Figure 2D.

3.16 ***Raw western bot data for Figure 5C.***

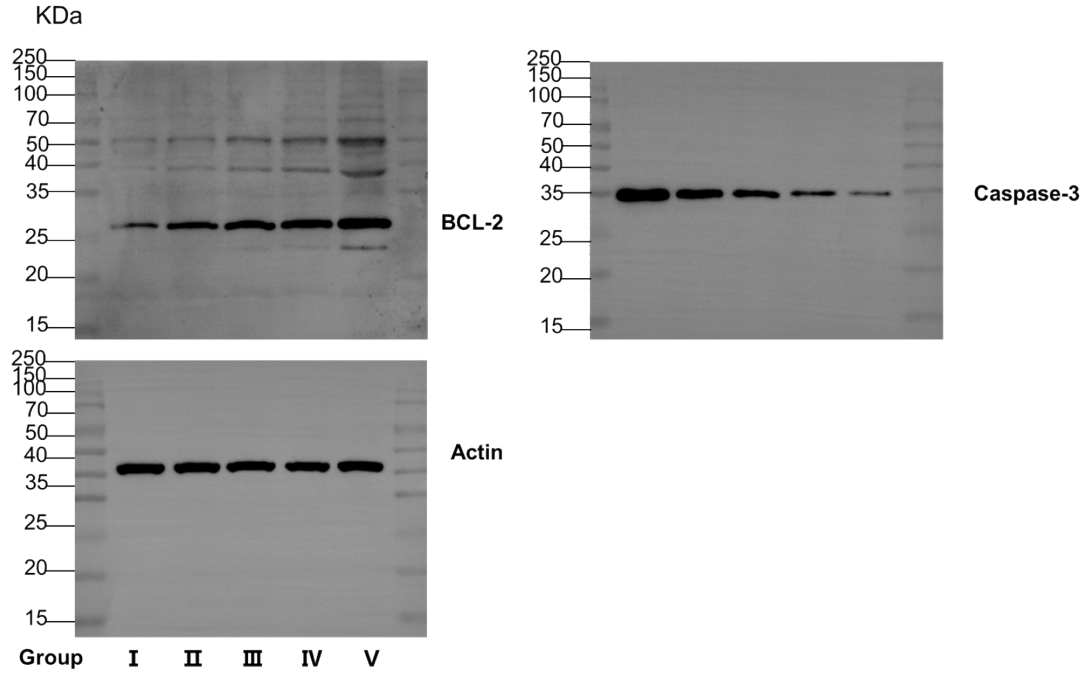


Figure S8. Raw western bot data for Figure 5C.

3.17 ***Raw western bot data for poly ADP-ribose polymerase (PARP) in LNCaP cells.***

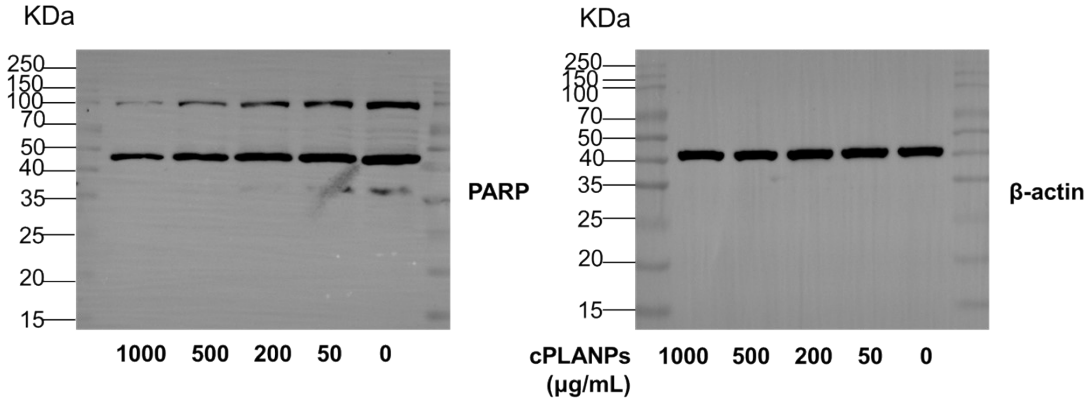


Figure S9. Representative Western blots against poly ADP-ribose polymerase (PARP) in LNCaP cells.

3.18 ***Relative expression of poly ADP-ribose polymerase (PARP) in LNCaP cells.***

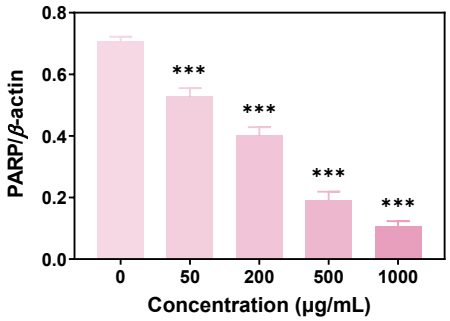


Figure S10. Relative expression of poly ADP-ribose polymerase (PARP) in LNCaP cells.

3.19 ***Raw western bot data for C4-2.***

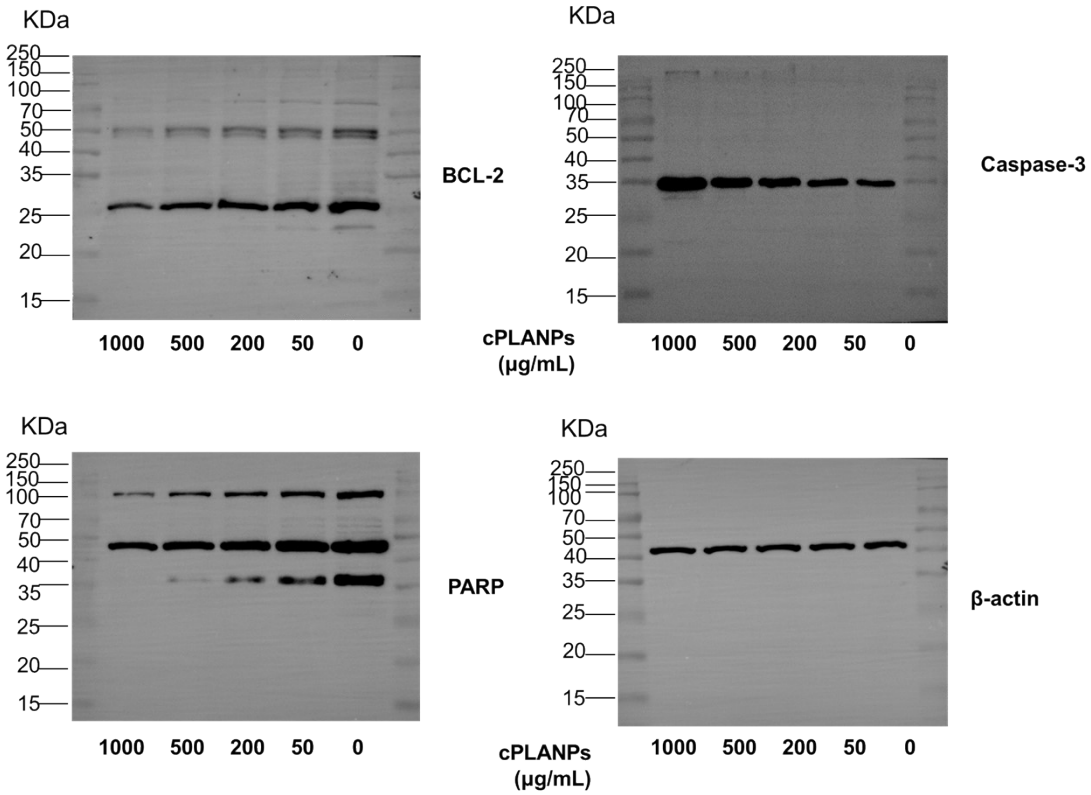


Figure S11. Representative Western blots against B-cell lymphoma-2 (BCL-2), caspase-3 and poly ADP-ribose polymerase (PARP) in C4-2 cells.

3.20 ***Relative expression of poly ADP-ribose polymerase (PARP) in C4-2 cells.***

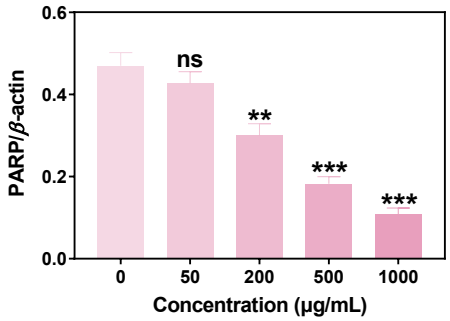


Figure S12. Relative expression of poly ADP-ribose polymerase (PARP) in C4-2 cells.

3.21 ***Pre-targeted fluorescence imaging in C4-2 cells***.

C4-2 cells were seeded into 35-mm glass-bottom dishes, incubated for 48 h at a density of ~80%, and then treated with cPLANPs (10 ng/mL) for 2 h. Afterward, the culture medium was removed, and the cells were washed three times with PBS and incubated with **Tz-Cy5** (1 μM) for another 1 h. The cells were finally washed briefly with PBS, and imaged with a confocal fluorescence microscope (Zeiss 880, Germany).

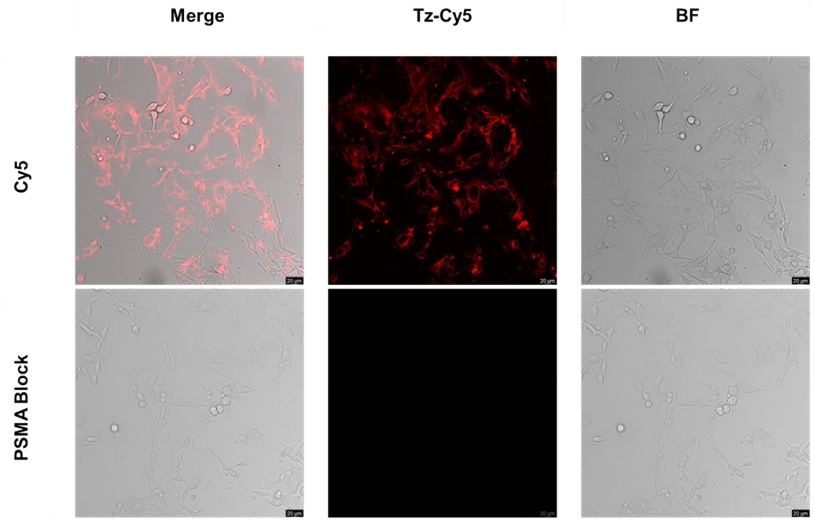


Figure S13. Pre-targeted fluorescence imaging of C4-2 cells treated with cPLANPs (10 ng/mL) and **Tz-Cy5** (1 μM).

4 References

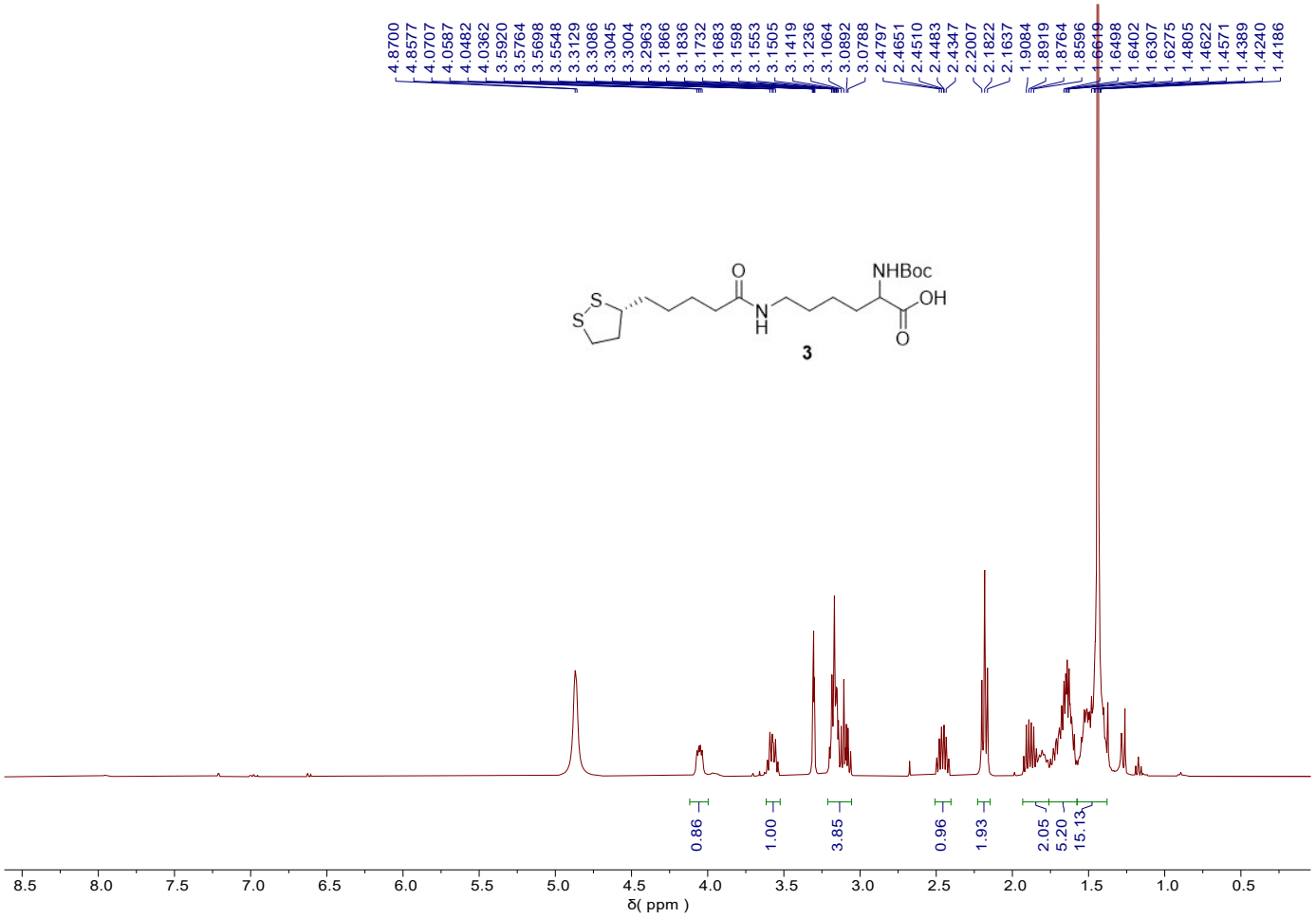
1. Fragai, M.; Comito, G.; Di Cesare Mannelli, L.; Gualdani, R.; Calderone, V.; Louka, A.; Richichi, B.; Francesconi, O.; Angeli, A.; Nocentini, A.; Gratteri, P.; Chiarugi, P.; Ghelardini, C.; Tadini-

Buoninsegni, F.; Supuran, C. T.; Nativi, C., Lipoyl-Homotaurine Derivative (ADM_12) Reverts Oxaliplatin-Induced Neuropathy and Reduces Cancer Cells Malignancy by Inhibiting Carbonic Anhydrase IX (CAIX). *J. Med. Chem.* **2017,** *60* (21), 9003-9011.

2. Maresca, K. P.; Hillier, S. M.; Femia, F. J.; Keith, D.; Barone, C.; Joyal, J. L.; Zimmerman, C. N.; Kozikowski, A. P.; Barrett, J. A.; Eckelman, W. C.; Babich, J. W., A Series of Halogenated Heterodimeric Inhibitors of Prostate Specific Membrane Antigen (PSMA) as Radiolabeled Probes for Targeting Prostate Cancer. *J. Med. Chem.* **2009,** *52* (2), 347-357.

5 NMR Spectra and High-resolution Mass Spectra

^{1}H and ^{13}C NMR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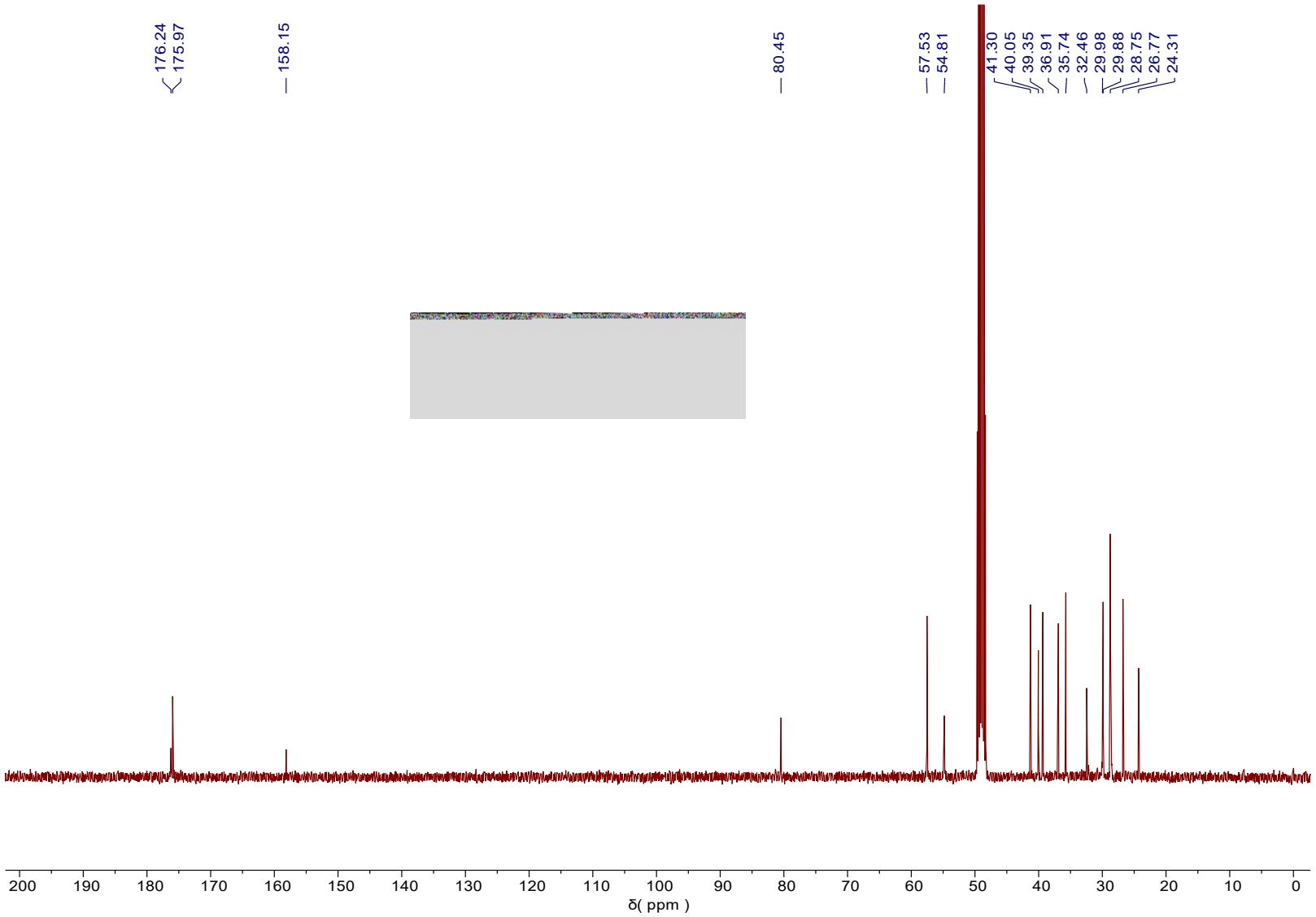
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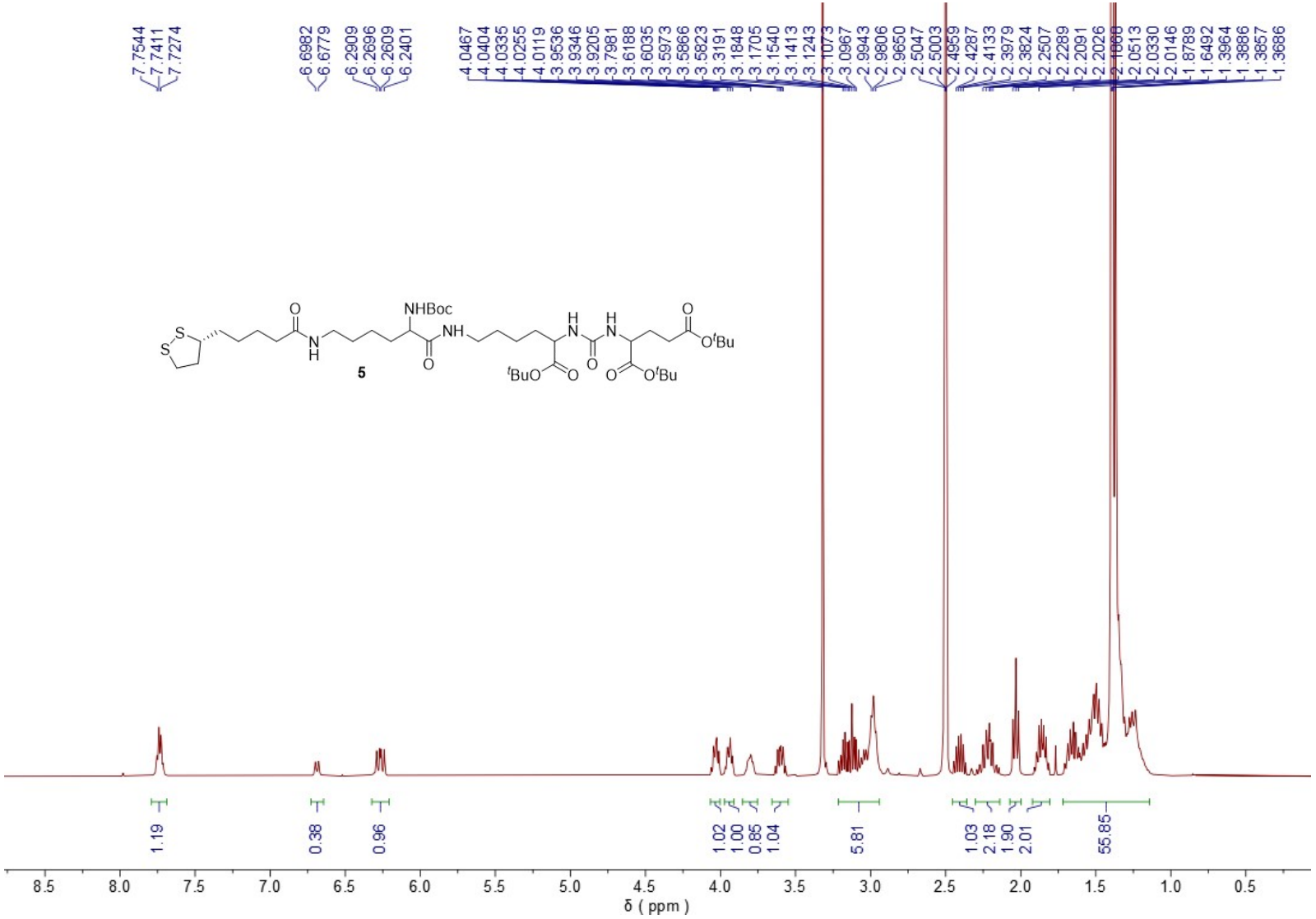
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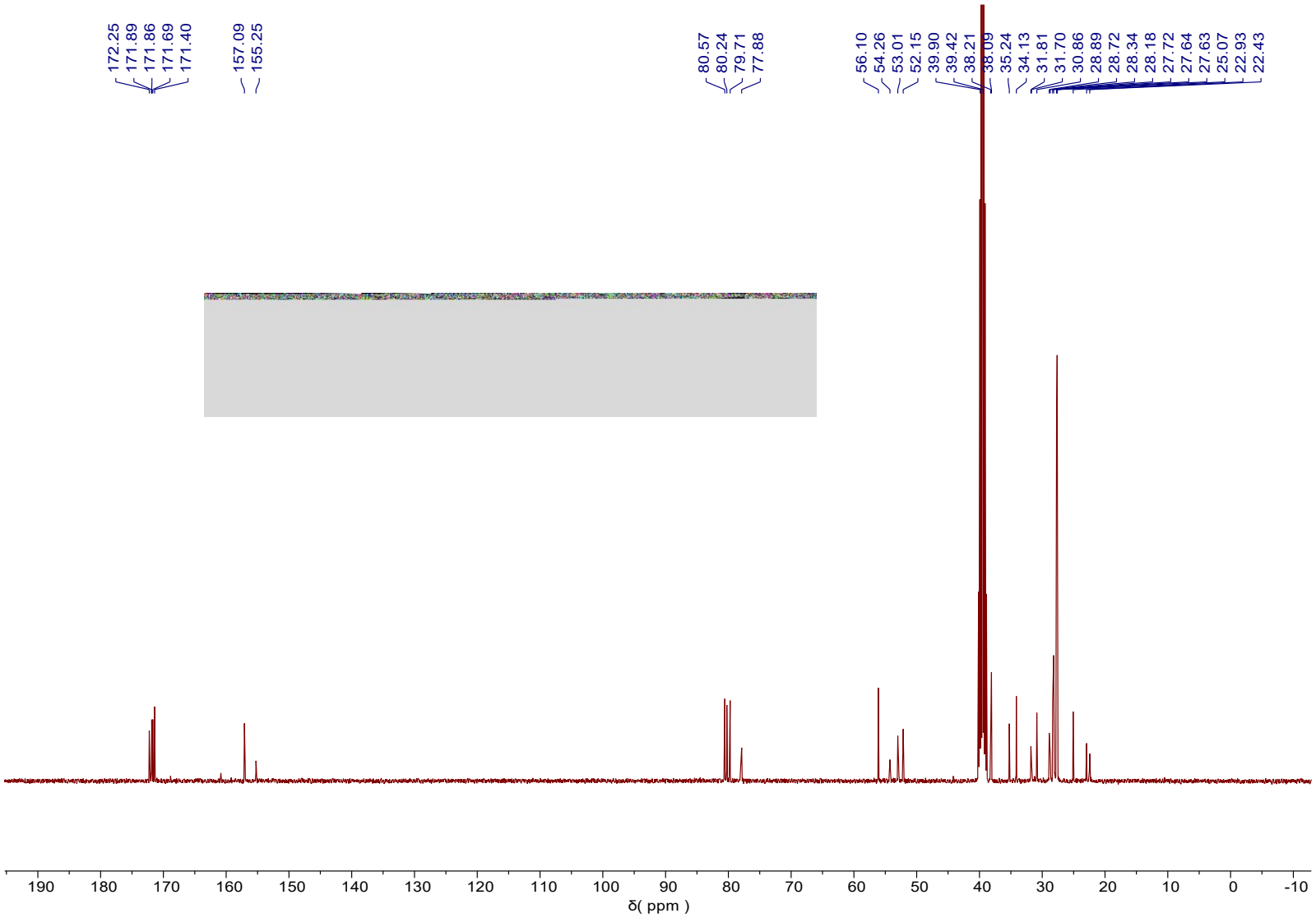
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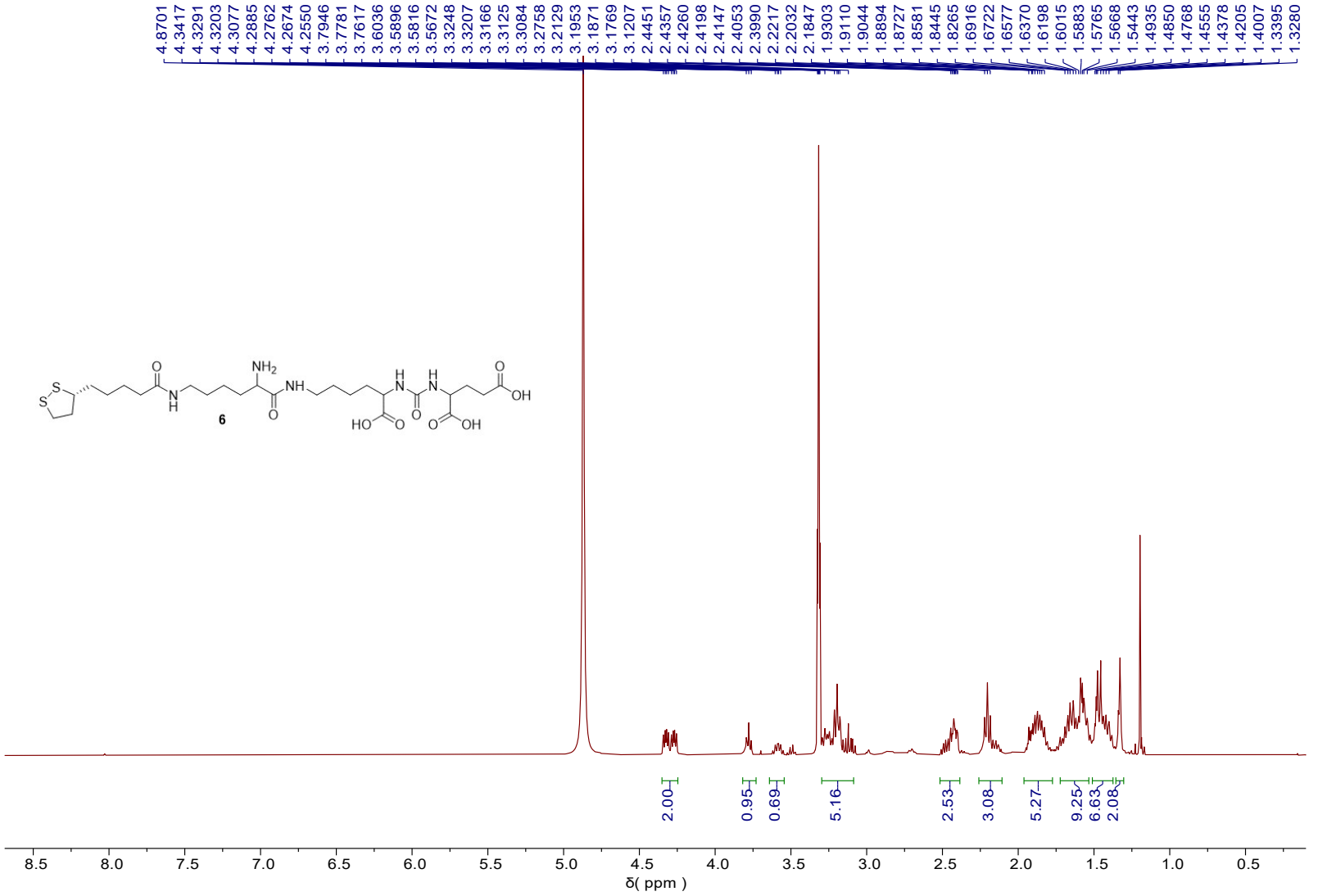
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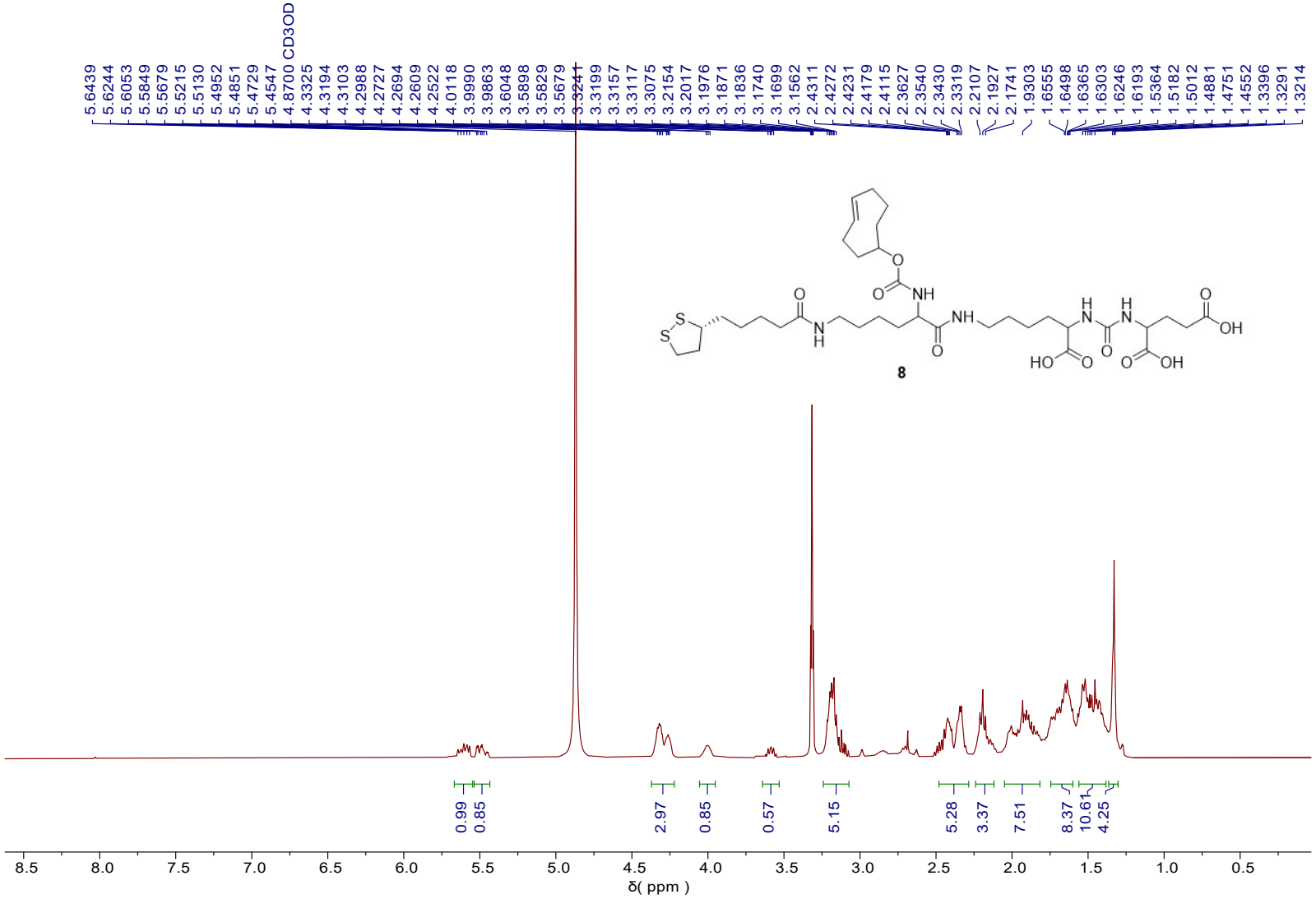
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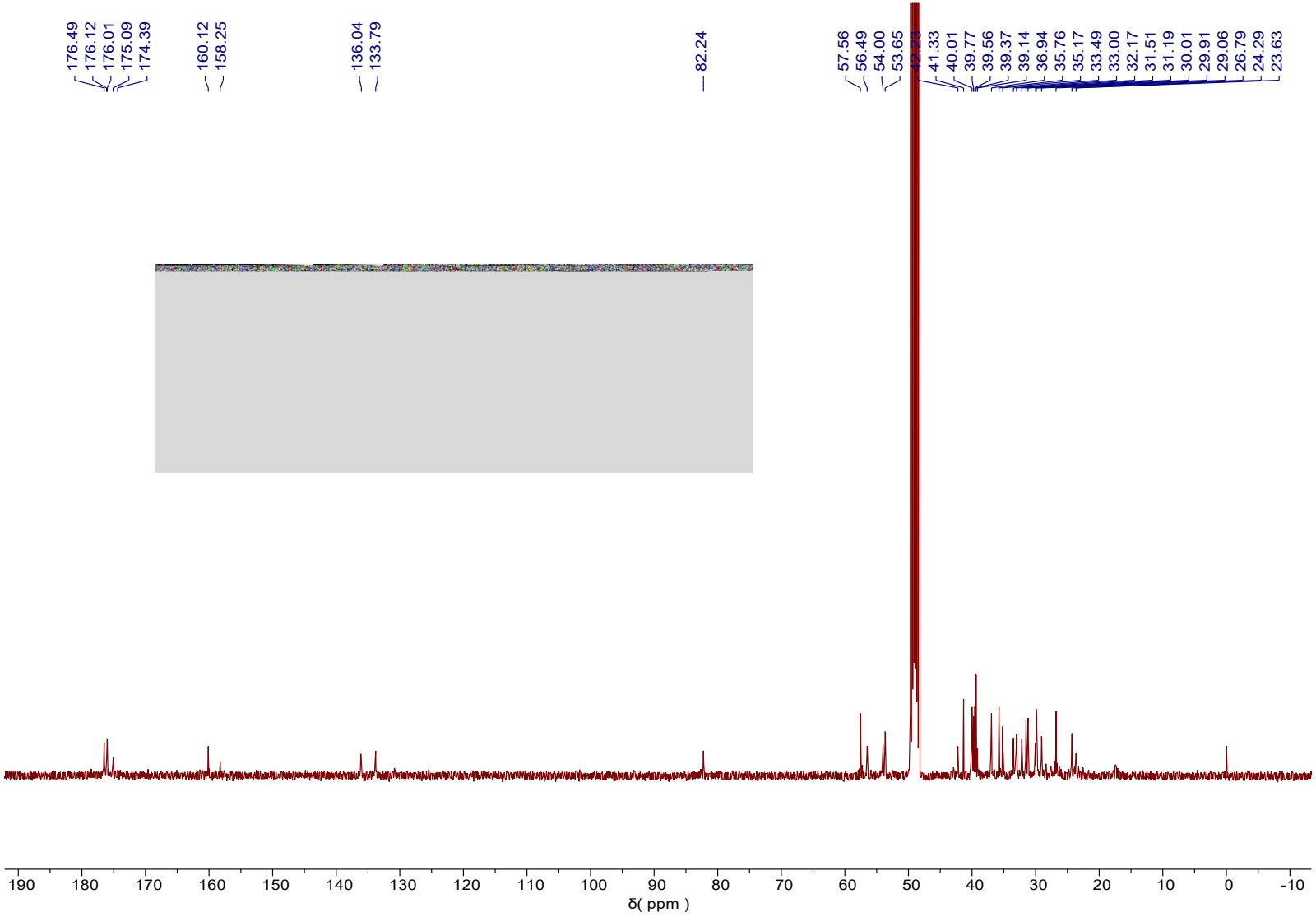
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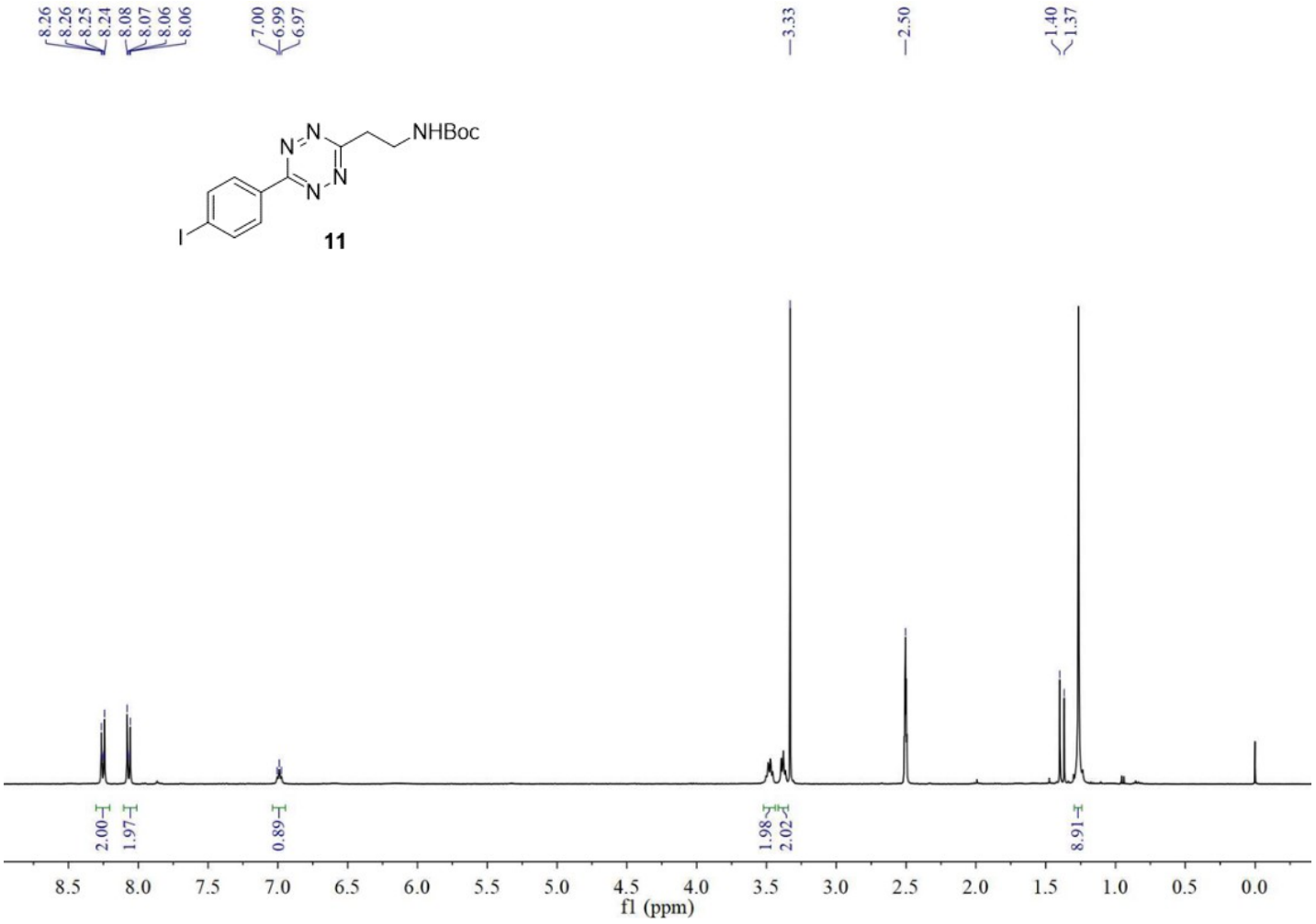
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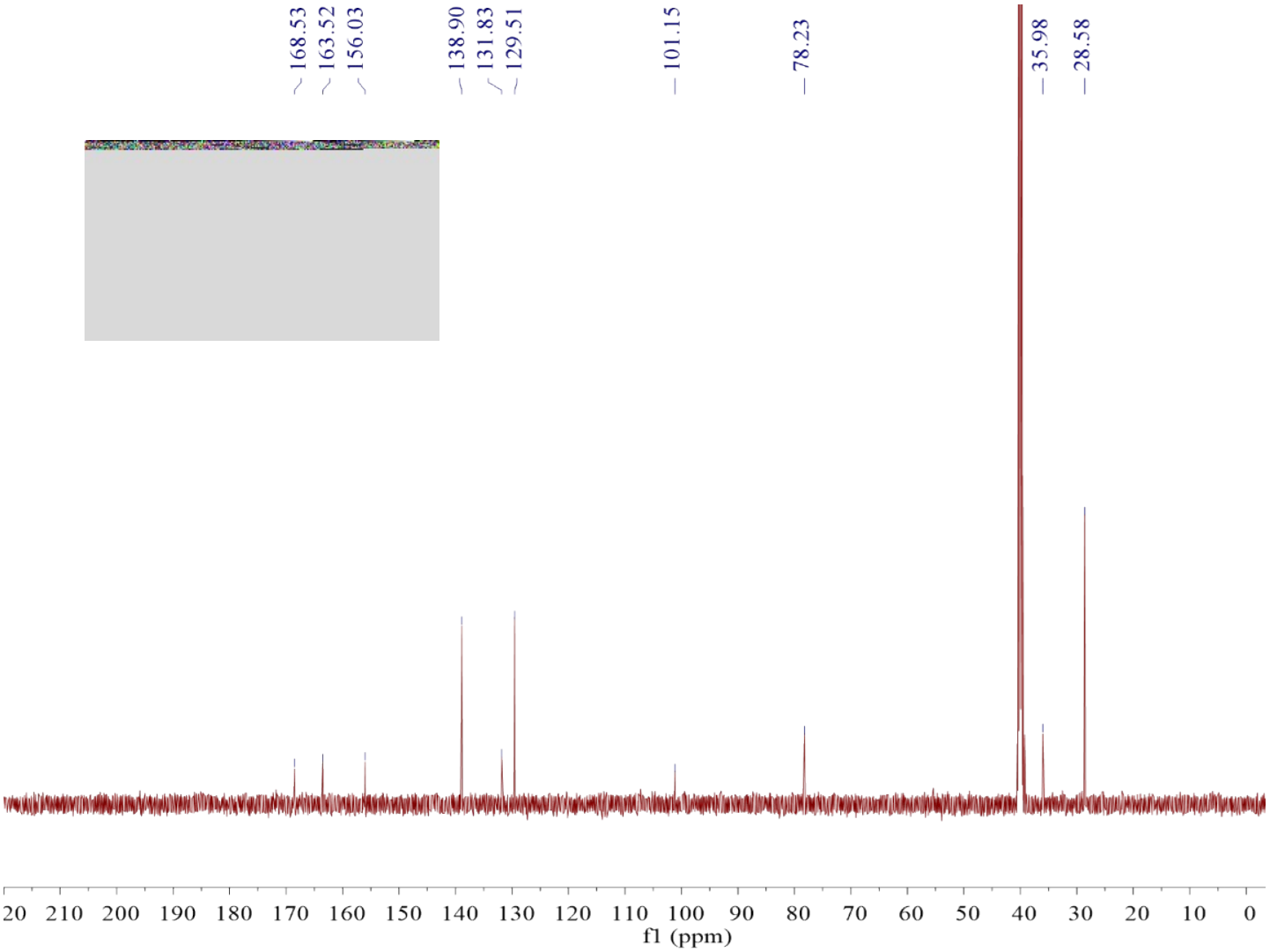
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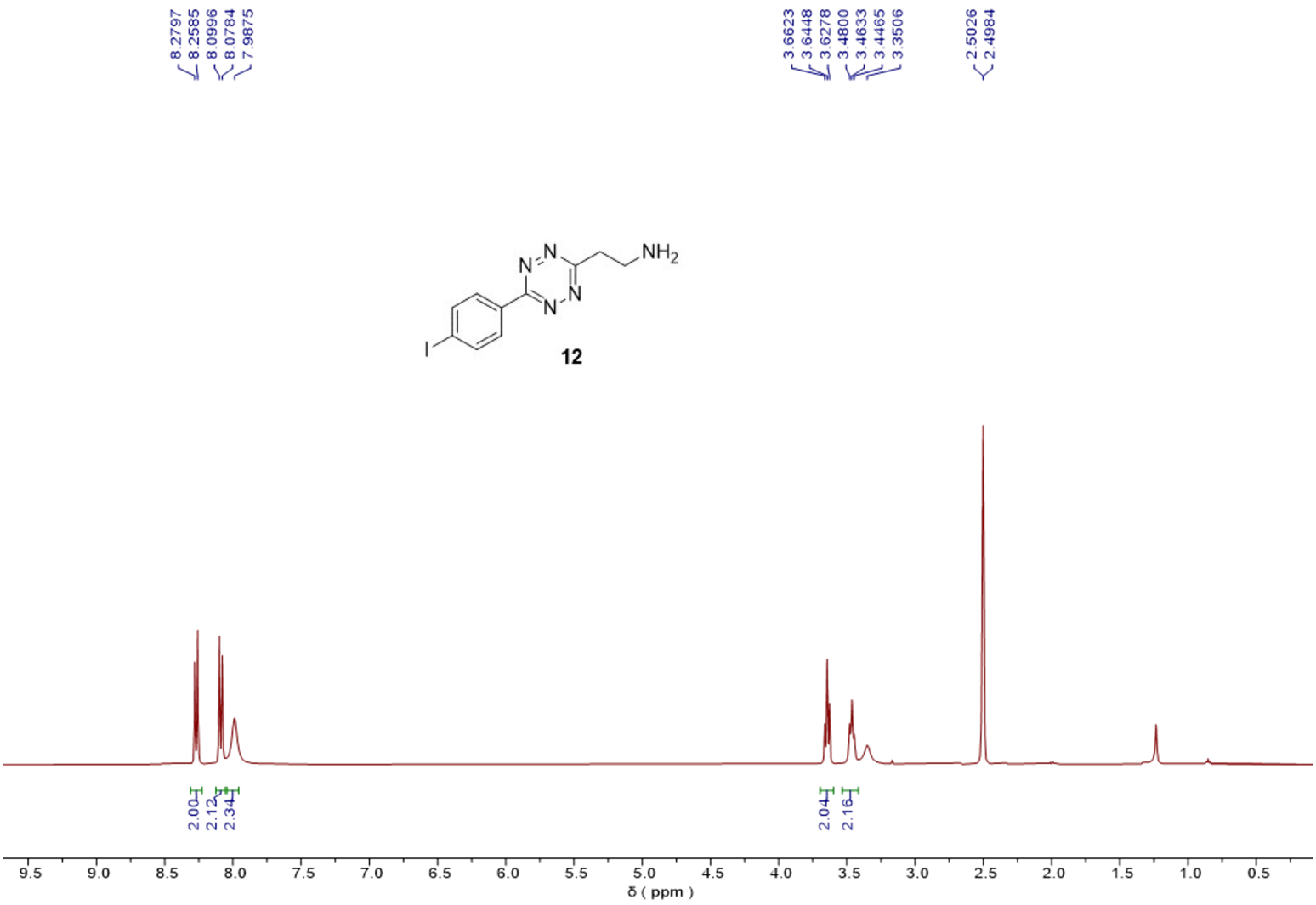
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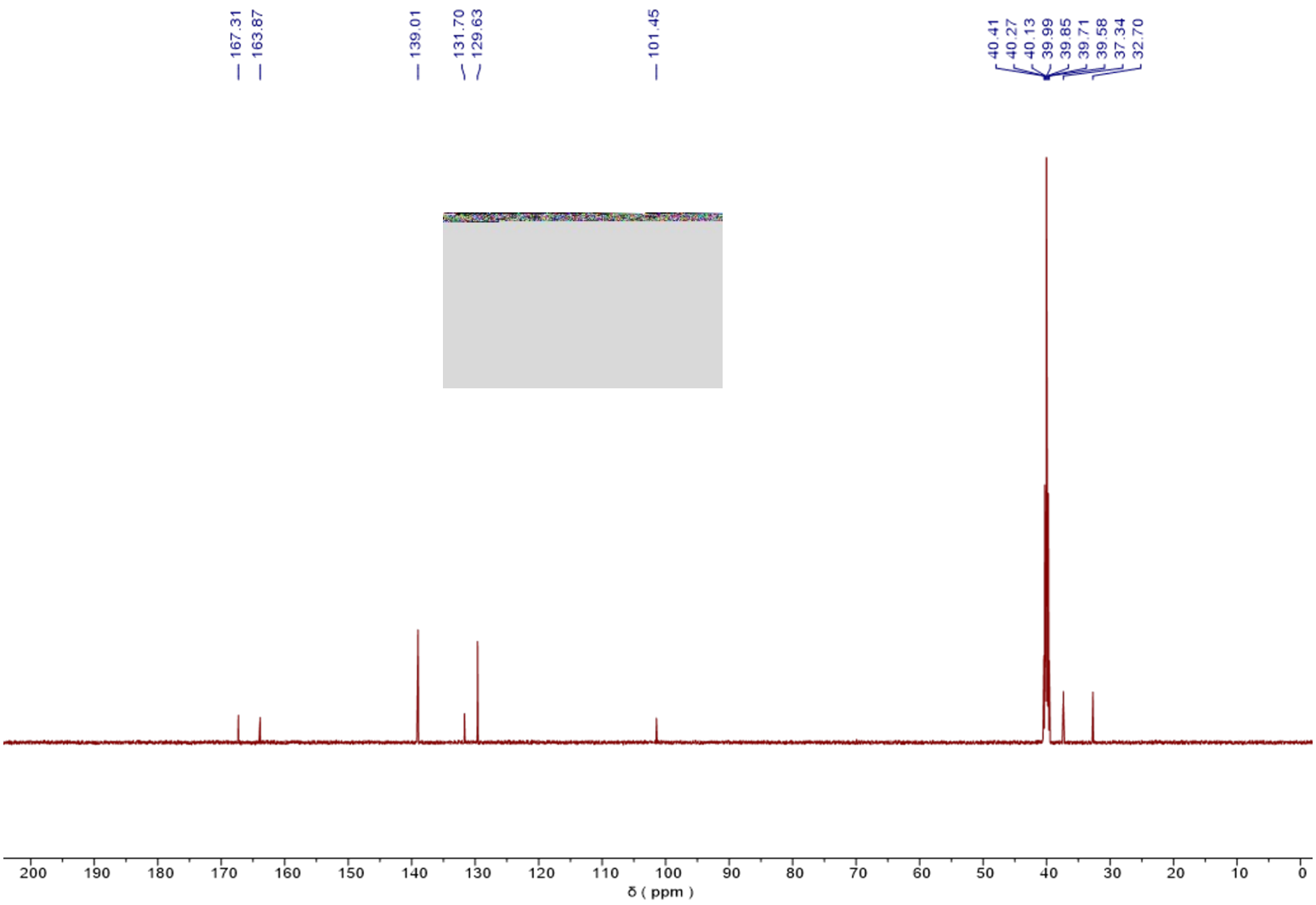
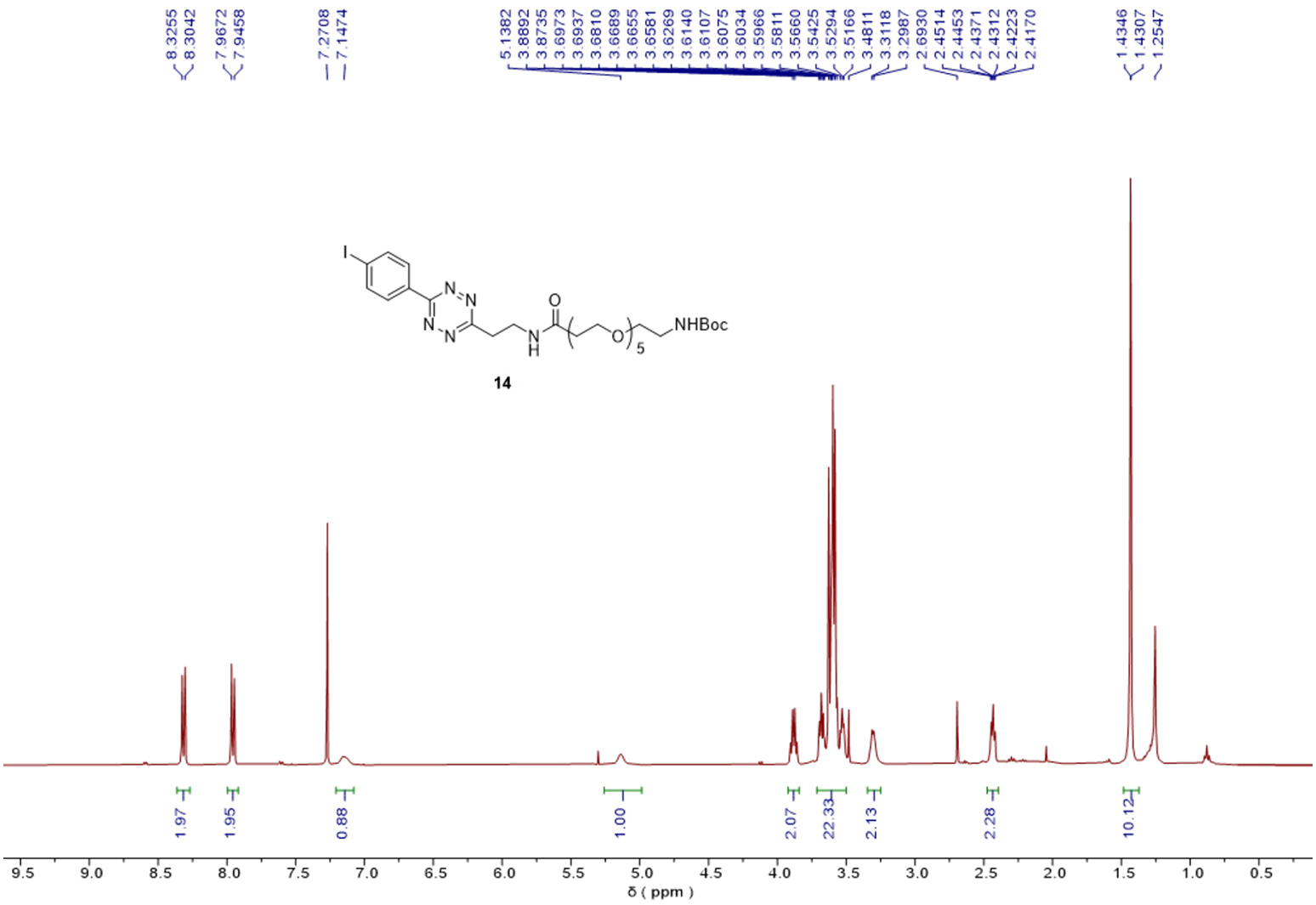
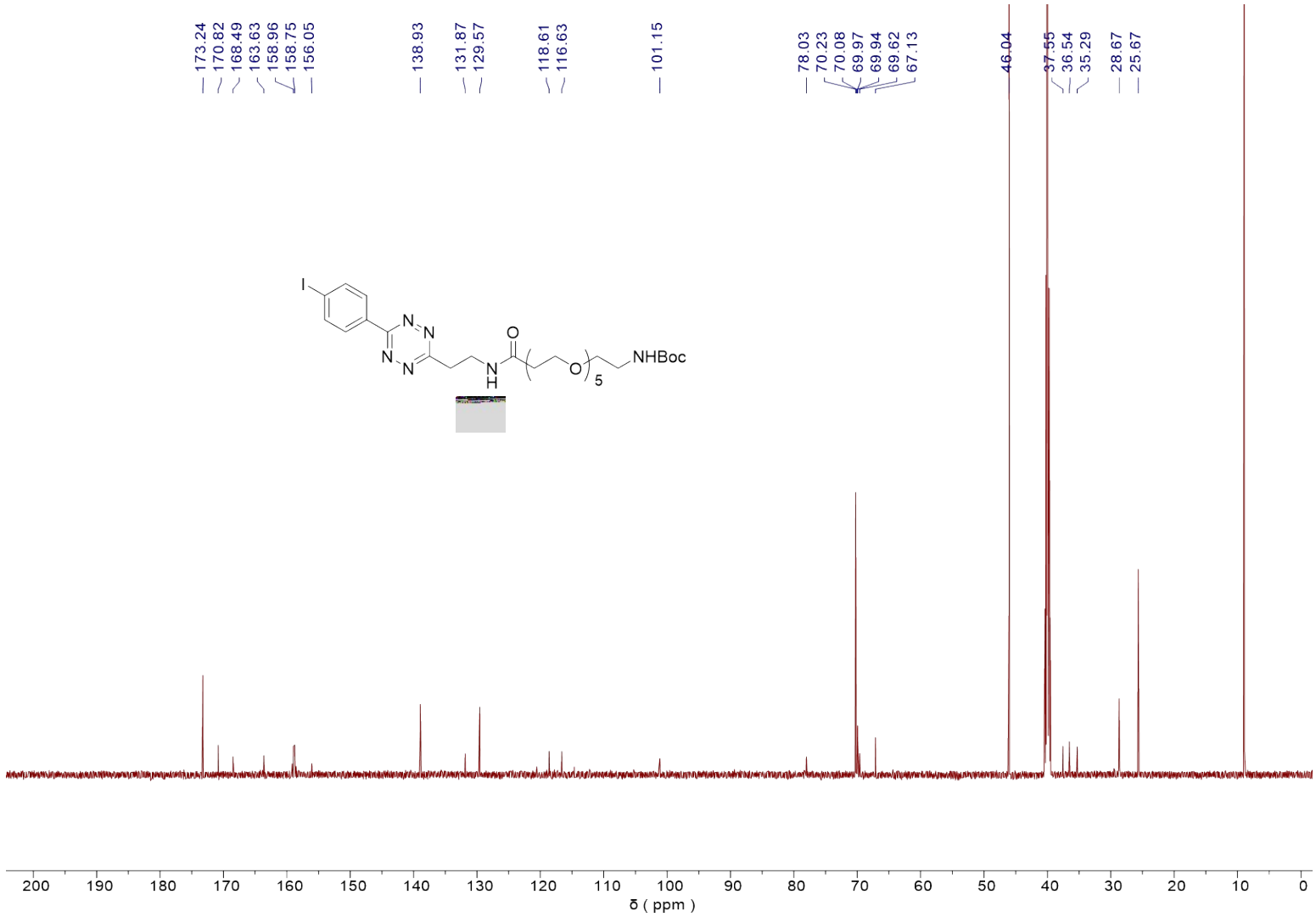
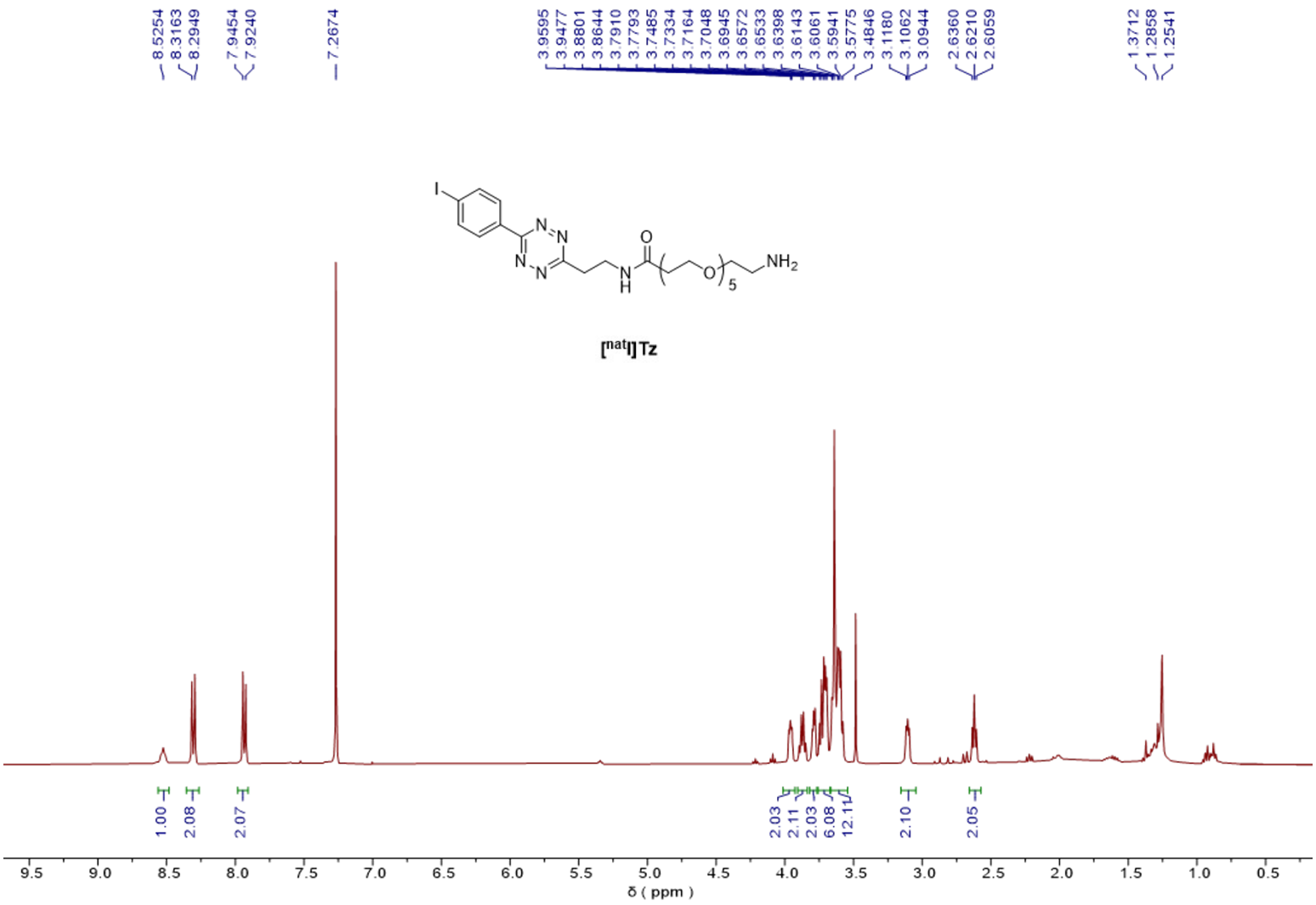
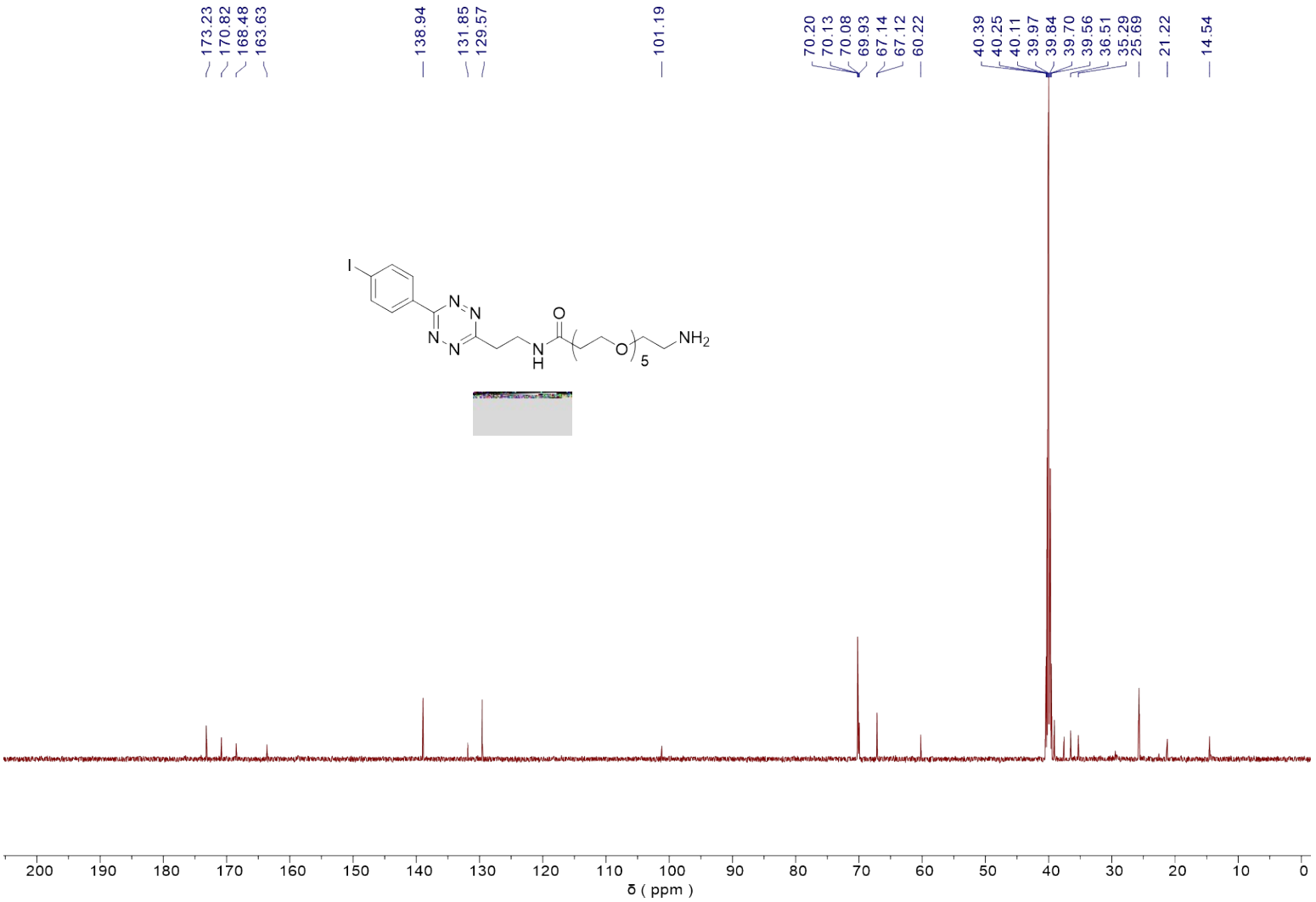
δ (ppm)

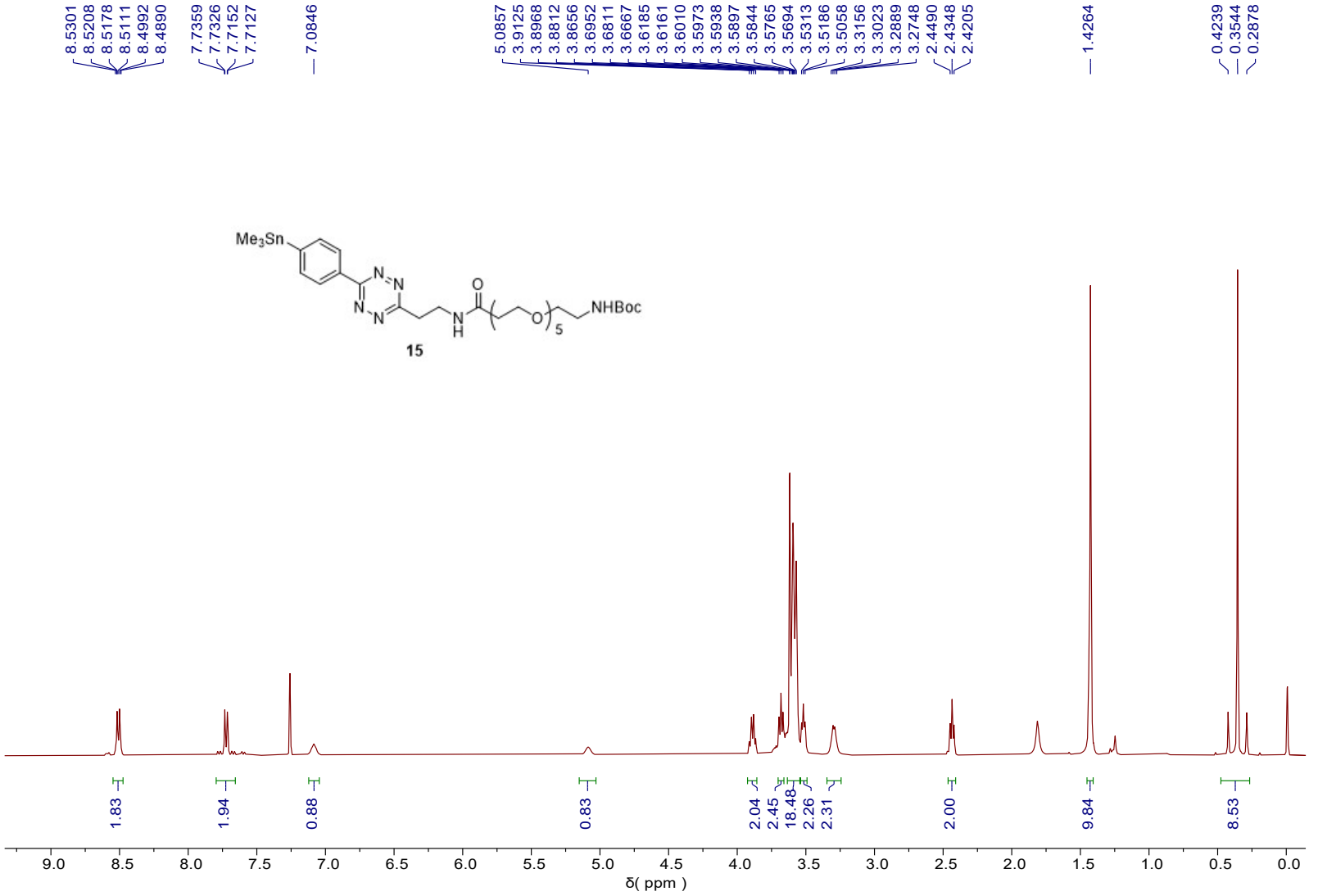

167.31
163.87
139.01
131.70
129.63
101.45
40.41
40.27
40.13
39.99
39.85
39.71
39.58
37.34
32.70
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
δ (ppm)

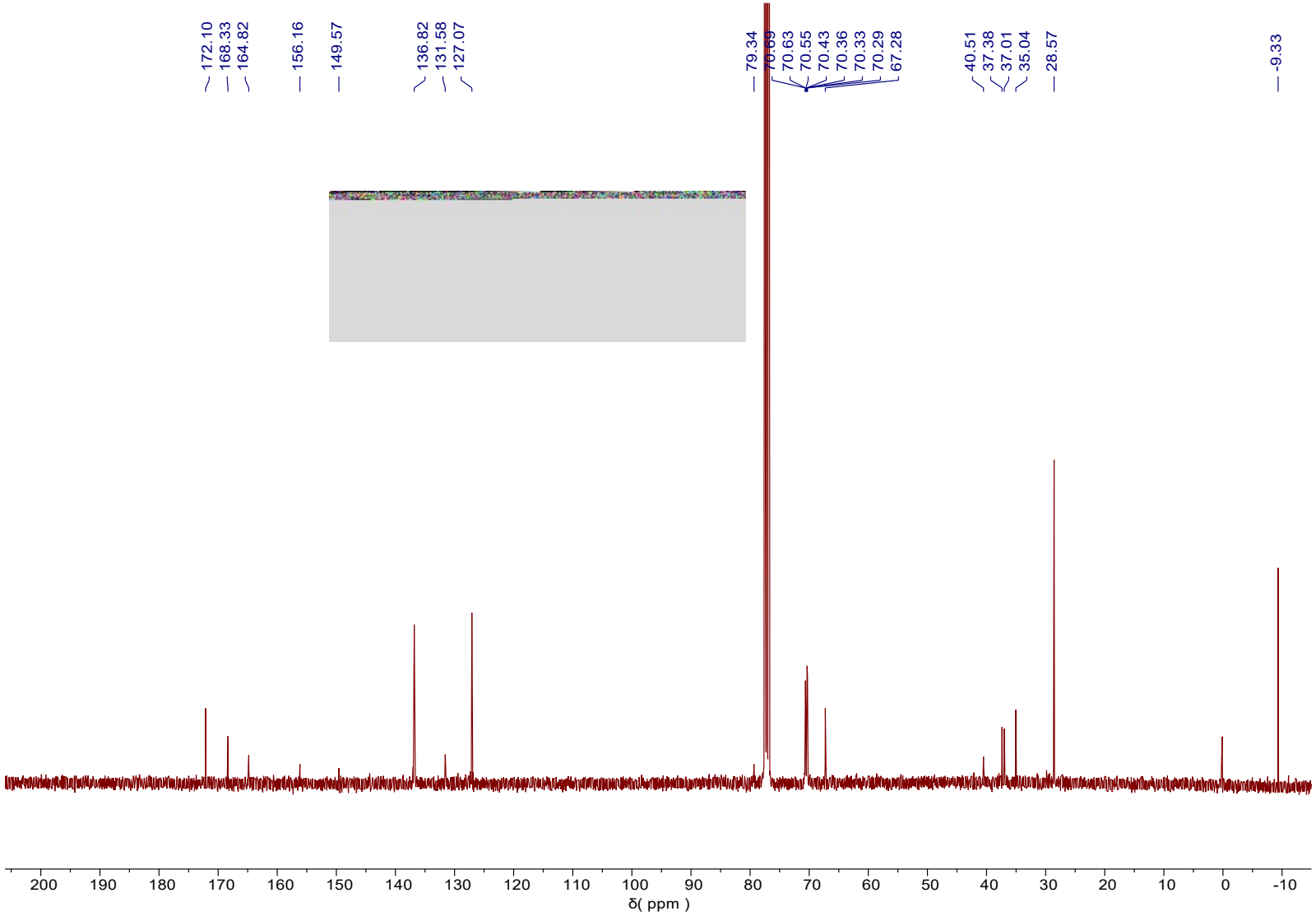

8.3255
8.3042
7.9672
7.9458
7.2708
7.1474
5.1382
3.8892
3.8735
3.6973
3.6937
3.6810
3.6689
3.6655
3.6581
3.6269
3.6140
3.6107
3.6075
3.6034
3.5966
3.5811
3.5660
3.5425
3.5294
3.5166
3.4811
3.3118
3.2987
2.6930
2.4514
2.4453
2.4371
2.4312
2.4223
2.4170
1.4346
1.4307
1.2547
I
N
N
N
N
H
O
O
5
NHBoc
14
1.97
1.95
0.88
1.00
2.07
22.33
2.13
2.28
10.12
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
δ (ppm)


173.24
170.82
168.49
163.63
158.96
158.75
156.05
138.93
131.87
129.57
118.61
116.63
101.15
78.03
70.23
70.08
69.97
69.94
69.62
67.13
46.04
37.55
36.54
35.29
28.67
25.67
I
N
N
N
N
H
O
O
5
NHBoc
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
δ (ppm)

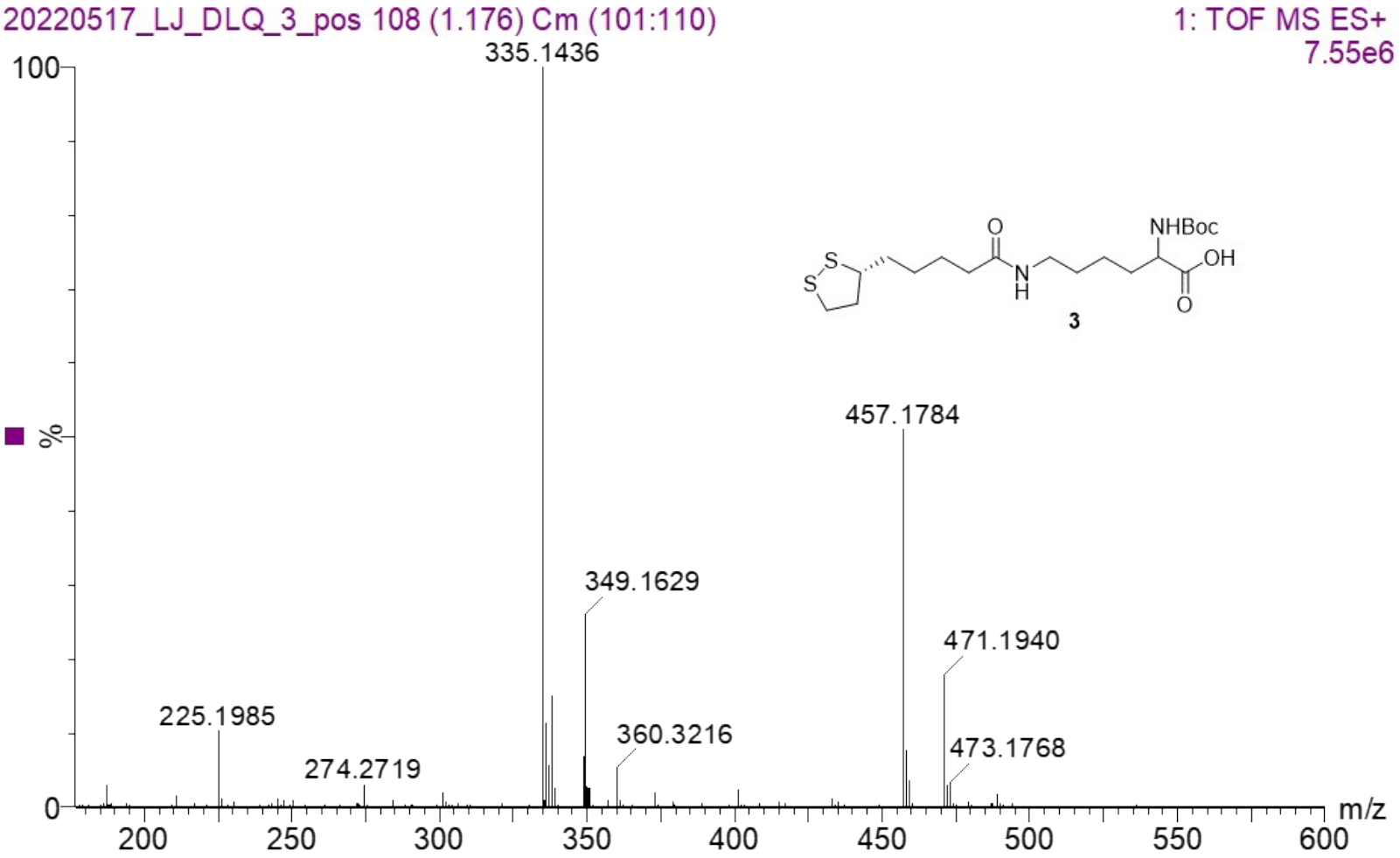

8.5254
8.3163
8.2949
7.9454
7.9240
7.2674
3.9595
3.9477
3.8801
3.8644
3.7910
3.7793
3.7485
3.7334
3.7164
3.7048
3.6945
3.6572
3.6533
3.6398
3.6143
3.6061
3.5941
3.5775
3.4846
3.1180
3.1062
3.0944
2.6360
2.6210
2.6059
1.3712
1.2858
1.2541
I
N
N
N
N
H
O
O
5
NH2
[matI]Tz
1.00
2.08
2.07
2.03
2.11
2.03
6.08
12.11
2.10
2.05
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
δ (ppm)

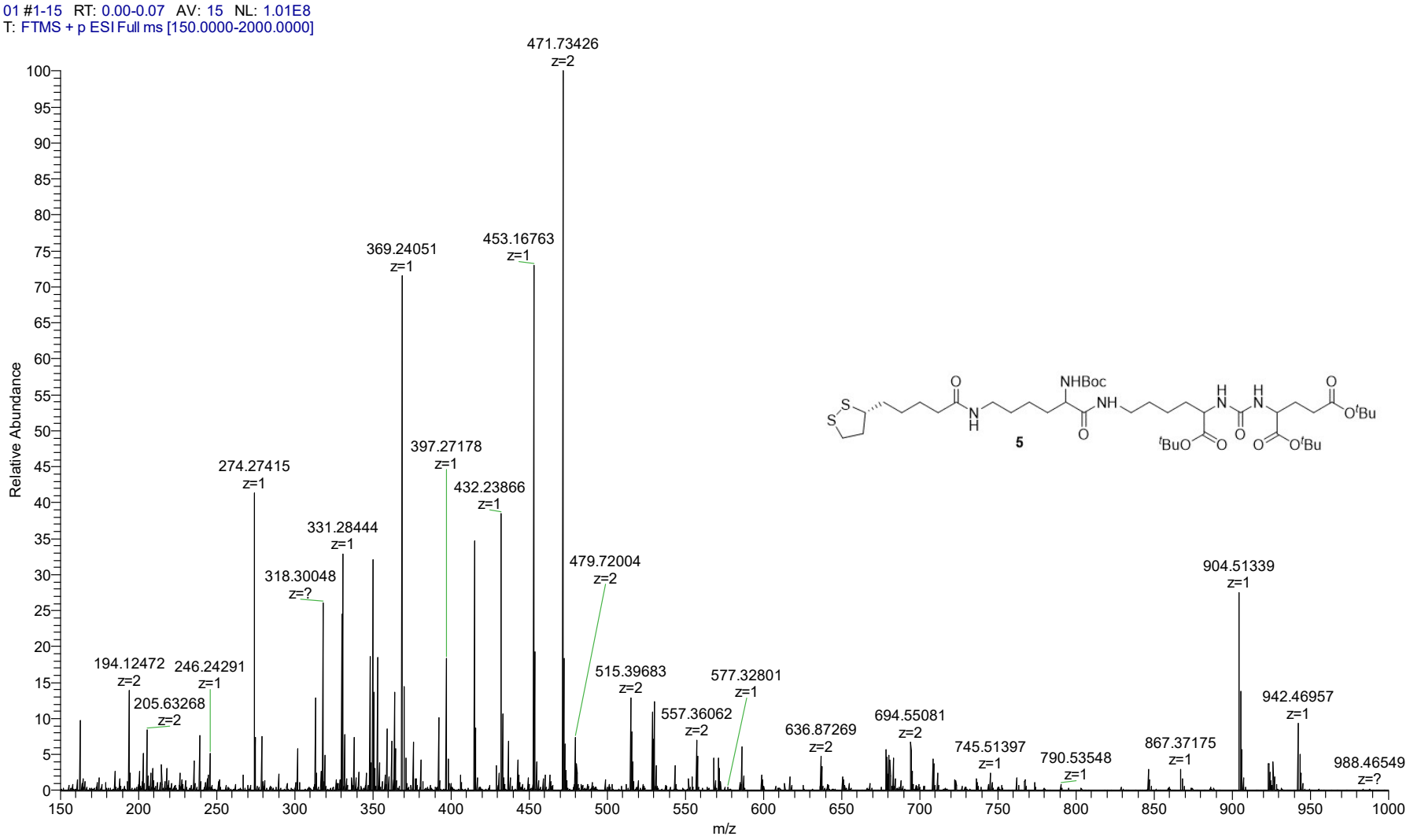

173.23
170.82
168.48
163.63
138.94
131.85
129.57
101.19
70.20
70.13
70.08
69.93
67.14
67.12
60.22
40.39
40.25
40.11
39.97
39.84
39.70
39.56
36.51
35.29
25.69
21.22
14.54
I
N
N
N
N
H
O
O
5
NH2
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
δ (ppm)

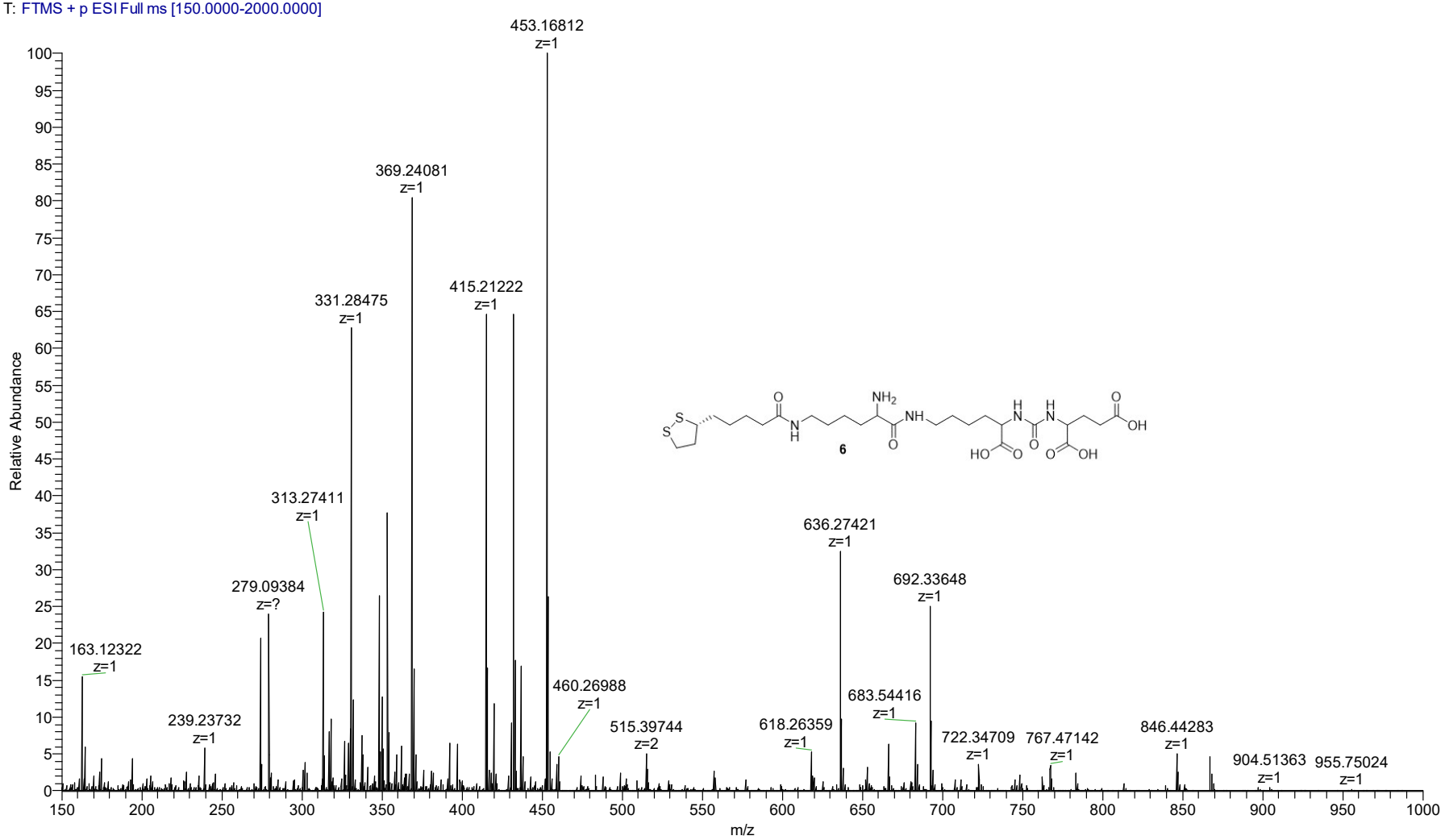

8.5301
8.5208
8.5178
8.5111
8.4992
8.4890
7.7359
7.7326
7.7152
7.7127
7.0846
5.0857
3.9125
3.8968
3.8812
3.8656
3.6952
3.6811
3.6667
3.6185
3.6161
3.6010
3.5973
3.5938
3.5897
3.5844
3.5765
3.5694
3.5313
3.5186
3.5058
3.3156
3.3023
3.2889
3.2748
2.4490
2.4348
2.4205
1.4264
0.4239
0.3544
0.2878
Me3Sn
N
N
N
N
H
O
O
5
NHBoc
15
1.83
1.94
0.88
0.83
2.04
2.45
18.48
2.26
2.31
2.00
9.84
8.53
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
δ(ppm)

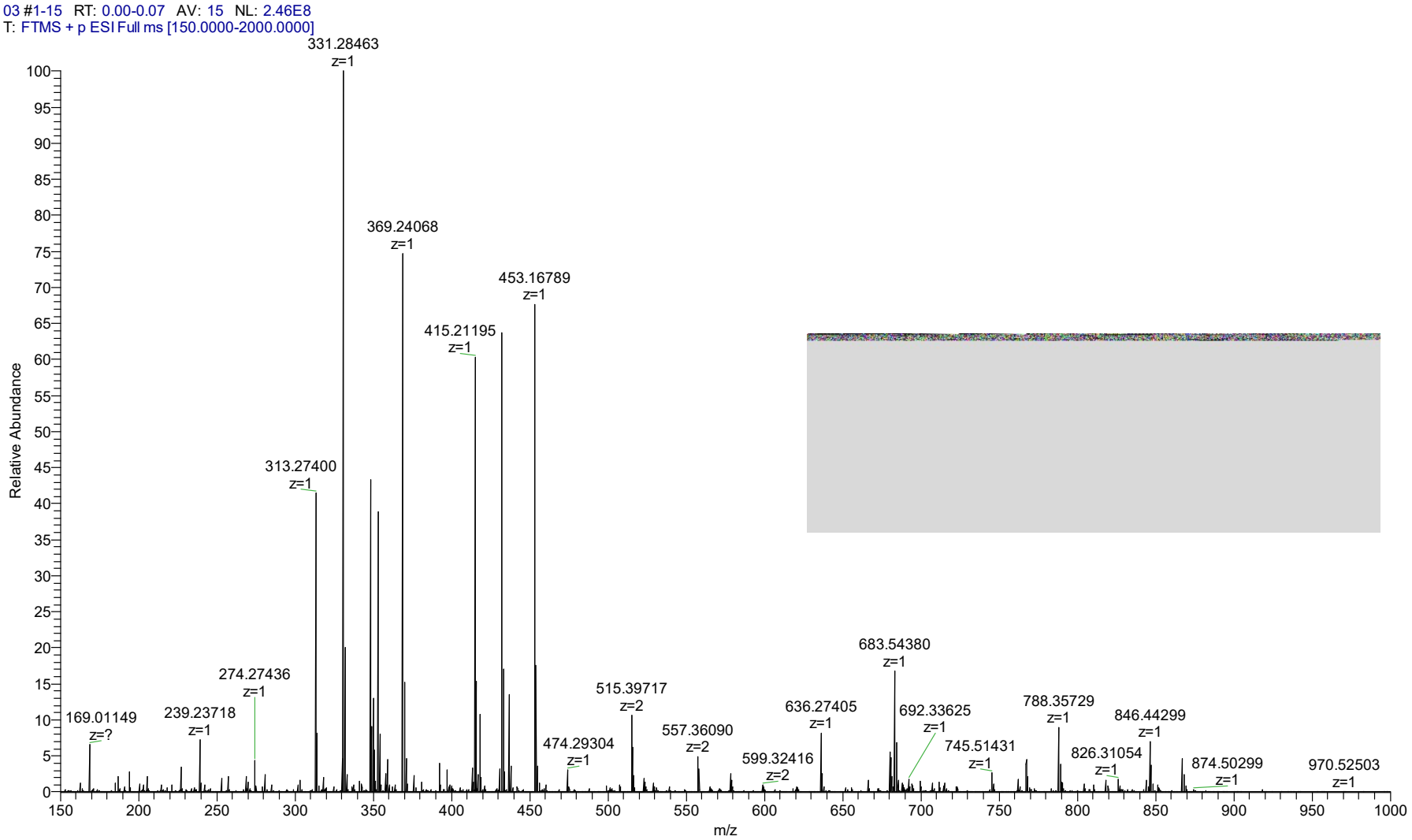

172.10
168.33
164.82
156.16
149.57
136.82
131.58
127.07
79.34
70.69
70.63
70.55
70.43
70.36
70.33
70.29
67.28
40.51
37.38
37.01
35.04
28.57
-9.33
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
δ(ppm)

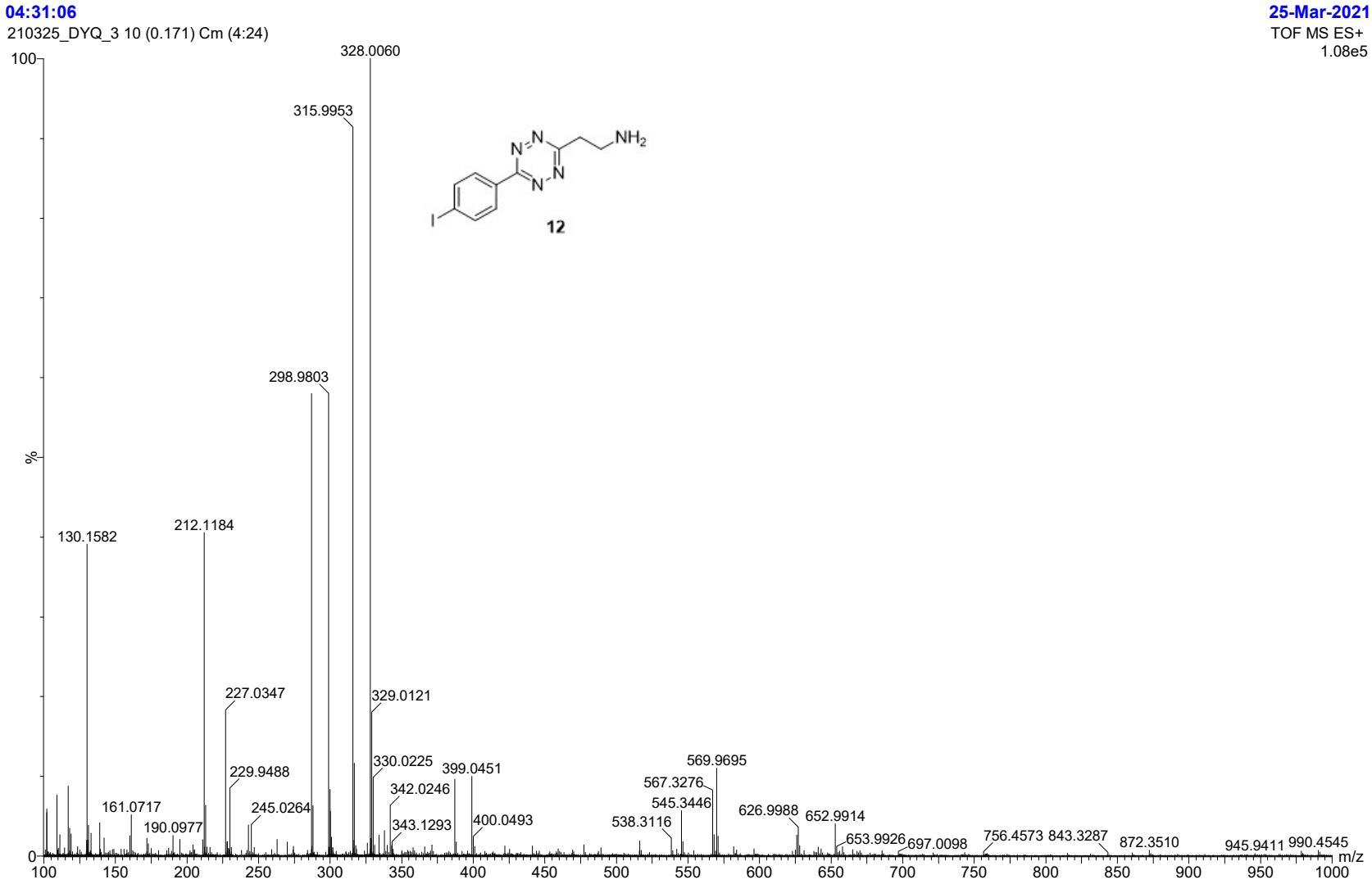
HRMS

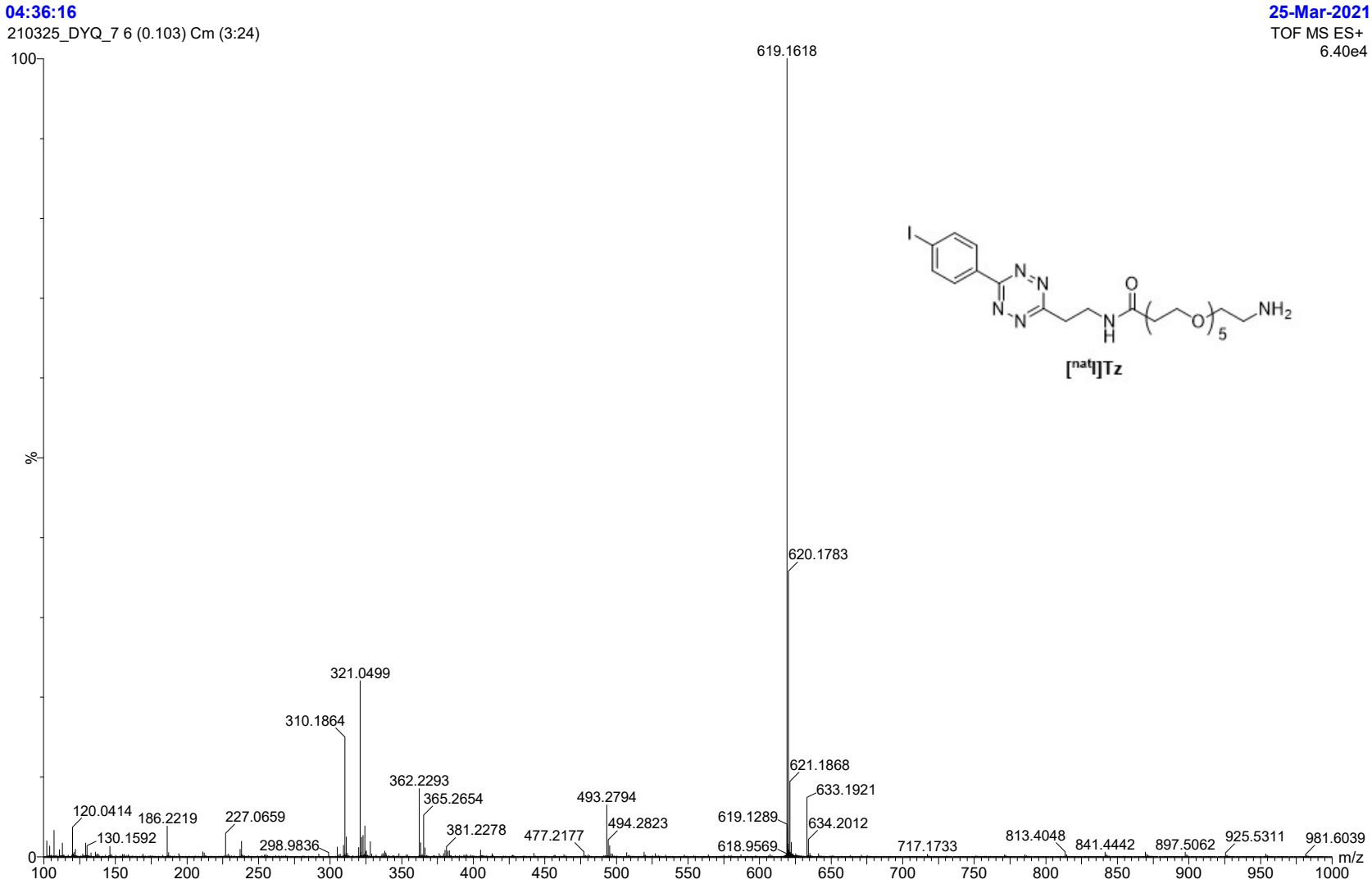

20220517_LJ_DLQ_3_pos 108 (1.176) Cm (101:110)
1: TOF MS ES+
7.55e6
100
%
0
335.1436
457.1784
349.1629
471.1940
225.1985
360.3216
473.1768
274.2719
m/z
200
250
300
350
400
450
500
550
600
NHBoc
OH
O
S
S
N
H
O
3


01 #1-15 RT: 0.00-0.07 AV: 15 NL: 1.01E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
Relative Abundance
m/z
471.73426 z=2
369.24051 z=1
453.16763 z=1
397.27178 z=1
274.27415 z=1
432.23866 z=1
331.28444 z=1
318.30048 z=?
479.72004 z=2
194.12472 z=2
246.24291 z=1
205.63268 z=2
515.39683 z=2
577.32801 z=1
557.36062 z=2
636.87269 z=2
694.55081 z=2
745.51397 z=1
790.53548 z=1
867.37175 z=1
904.51339 z=1
942.46957 z=1
988.46549 z=?
NHBoc
NH
5


02 #1-15 RT: 0.00-0.07 AV: 15 NL: 1.04E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
453.16812
z=1
369.24081
z=1
331.28475
z=1
415.21222
z=1
313.27411
z=1
279.09384
z=?
163.12322
z=1
239.23732
z=1
460.26988
z=1
515.39744
z=2
636.27421
z=1
692.33648
z=1
683.54416
z=1
618.26359
z=1
722.34709
z=1
767.47142
z=1
846.44283
z=1
904.51363
z=1
955.75024
z=1
6
Relative Abundance
m/z


03 #1-15 RT: 0.00-0.07 AV: 15 NL: 2.46E8
T: FTMS + p ESI Full ms [150.0000-2000.0000]
331.28463
z=1
369.24068
z=1
453.16789
z=1
415.21195
z=1
313.27400
z=1
274.27436
z=1
169.01149
z=?
239.23718
z=1
515.39717
z=2
474.29304
z=1
557.36090
z=2
599.32416
z=2
636.27405
z=1
683.54380
z=1
692.33625
z=1
745.51431
z=1
788.35729
z=1
826.31054
z=1
846.44299
z=1
874.50299
z=1
970.52503
z=1
Relative Abundance
m/z


04:31:06
25-Mar-2021
210325_DYQ_3 10 (0.171) Cm (4:24)
TOF MS ES+
1.08e5
328.0060
315.9953
298.9803
212.1184
130.1582
227.0347
329.0121
330.0225
229.9488
399.0451
342.0246
161.0717
245.0264
190.0977
343.1293
400.0493
569.9695
567.3276
545.3446
538.3116
626.9988
652.9914
653.9926
697.0098
756.4573
843.3287
872.3510
945.9411
990.4545
NH2
12
%
m/z


04:36:16
25-Mar-2021
210325_DYQ_7 6 (0.103) Cm (3:24)
TOF MS ES+
6.40e4
100
%
0
619.1618
620.1783
321.0499
310.1864
621.1868
362.2293
633.1921
365.2654
493.2794
120.0414
186.2219
227.0659
619.1289
634.2012
381.2278
494.2823
130.1592
298.9836
477.2177
618.9569
717.1733
813.4048
841.4442
897.5062
925.5311
981.6039
m/z
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
I
N
N
N
N
H
O
O
5
NH2
[natI]Tz