

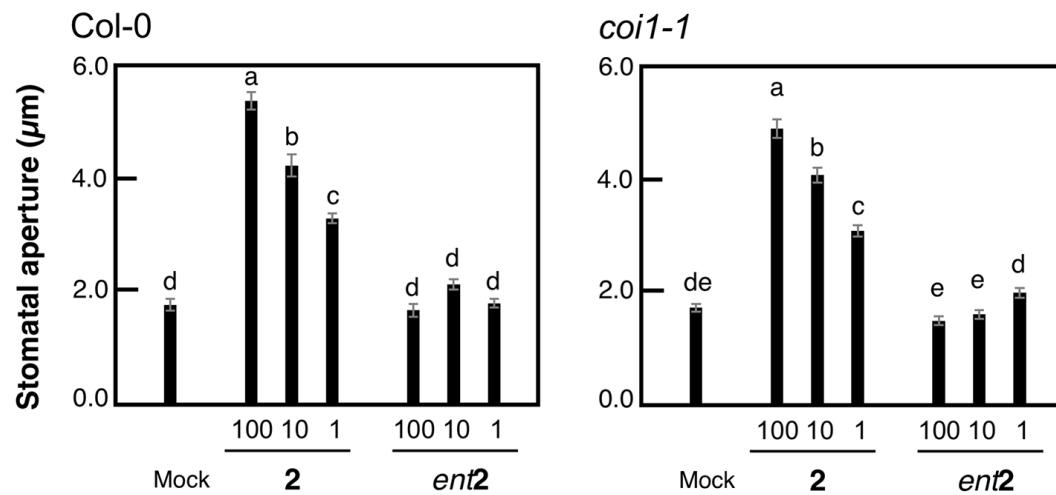
Supporting information for

The subcellular localization and binding protein of coronatine, a plant pathogen virulence factor, in the monocot plant *Commelina communis*, and the possible mode of action

Minoru Ueda, Kengo Hayashi, Syusuke Egoshi, Yasuhiro Ishimaru, Yousuke Takaoka, Hiroyuki Yamakoshi, Kosuke Dodo, and Mikiko Sodeoka

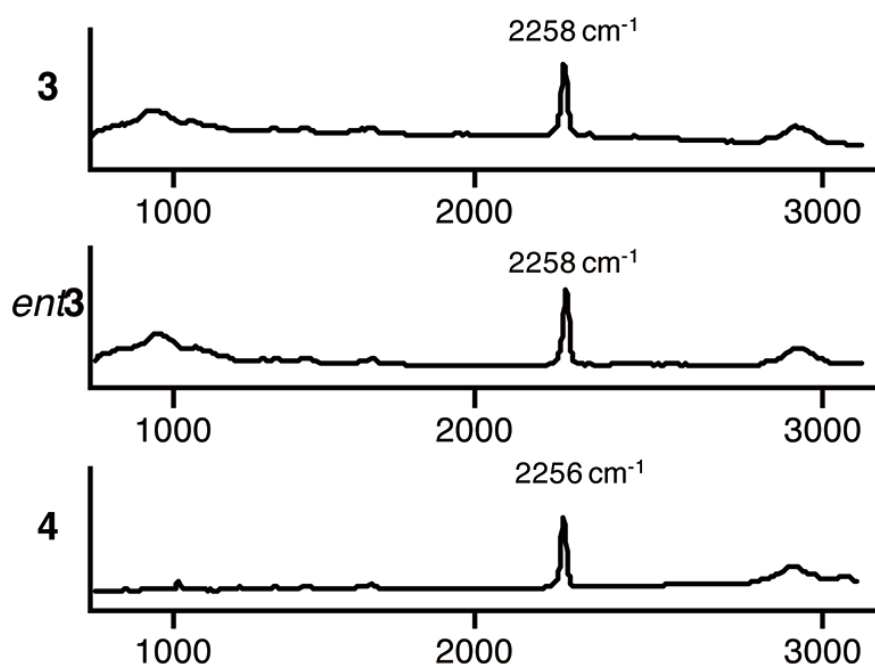
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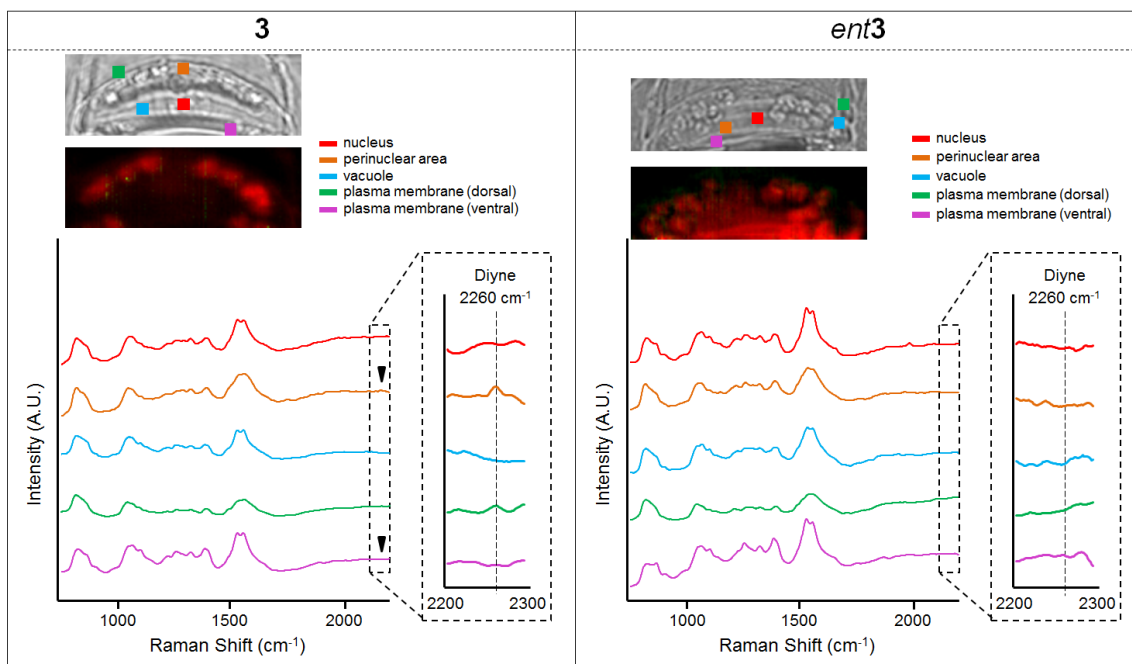
**Figure S1. Coronatine (2)-mediated stomatal aperture of dark-closed stomata of *Arabidopsis thaliana*.**

The dark-closed stomata of WT (Col-0) and *coi1-1* mutant of *A. thaliana* was treated by 1, 10, and 100 μM of **2** or *ent-2*, respectively. This data is an update of our old result in *ACS Cent. Sci.* **2017**, 3, 462. The stomatal aperture was re-measured according to a new method in ‘material and method’.



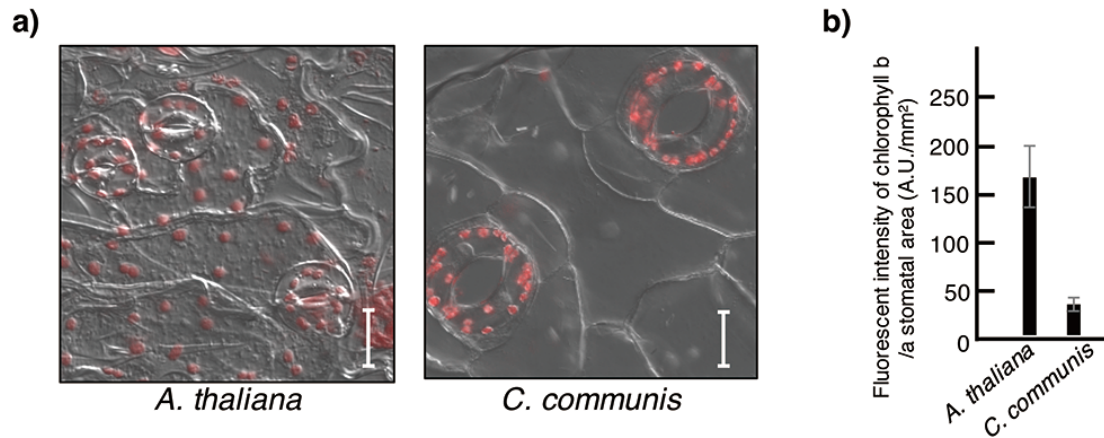
**Figure S2. Di-yne Raman signal of probes 3, *ent3*, and 4.**

Averaged Raman spectra of probes 3, *ent3*, and 4 as crystal forms. Di-yne Raman signal of probe 3/*ent3* was detected at 2258  $\text{cm}^{-1}$ , and di-yne Raman signal of probe 4 was detected at 2256  $\text{cm}^{-1}$ . The light intensity at the sample plane was calculated as 6.2  $\text{mW}/\mu\text{m}^2$  (3/*ent3*) or 6.0  $\text{mW}/\mu\text{m}^2$  (4) from the ratio of the measured laser power between the sample position and the area of the illumination line. The exposure time for each line was 10 s.



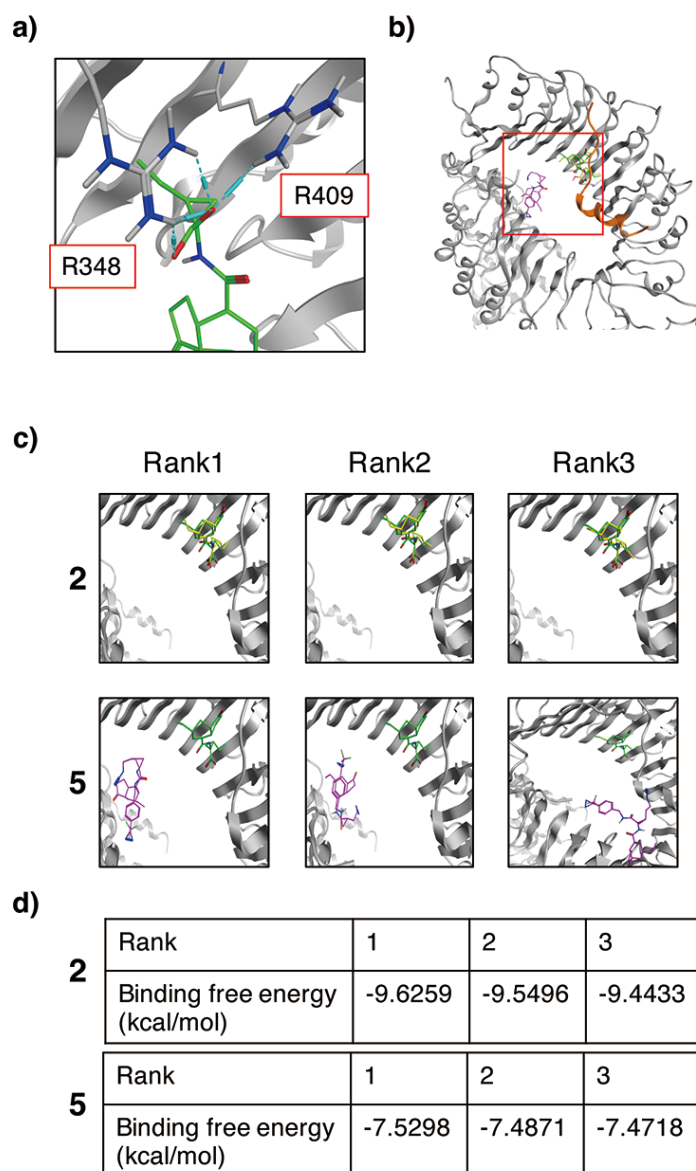
**Figure S3: Raman imaging with **3** and *ent3* using *Commelina communis*.**

Raman spectra and images of living guard cells of *Commelina communis* stained with 100  $\mu$ M of **3**/*ent3*. Raman spectra shows each subcellular area (average spectra of 6 pixel  $\times$  6 pixel: nuclear region in red, perinuclear region in orange, vacuole in cyan, dorsal plasma membrane in green, and ventral plasma membrane in purple) of the guard cell.



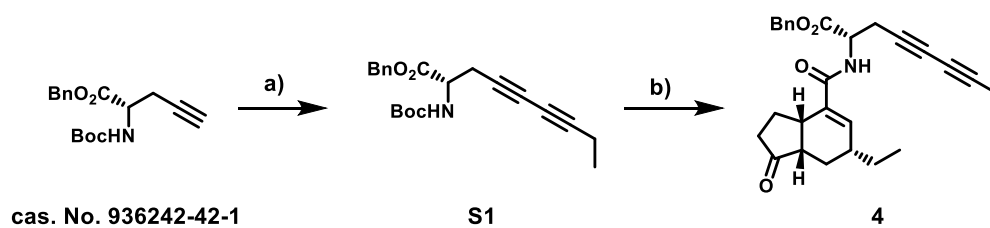
**Figure S4: Fluorescent imaging with *Arabidopsis* and *Commelina* plant**

a) Fluorescent imaging of peeled epidermis using a confocal microscope. The fluorescence of chlorophyll b is shown in red. Scale bars are 15  $\mu\text{m}$  in *A. thaliana* and 40  $\mu\text{m}$  in *C. communis*. b) Analysis of fluorescent intensity of chlorophyll b in a stomatal area. Bars represent the mean fluorescent intensity in a stomatal area with SE ( $n = 3$  stomata).



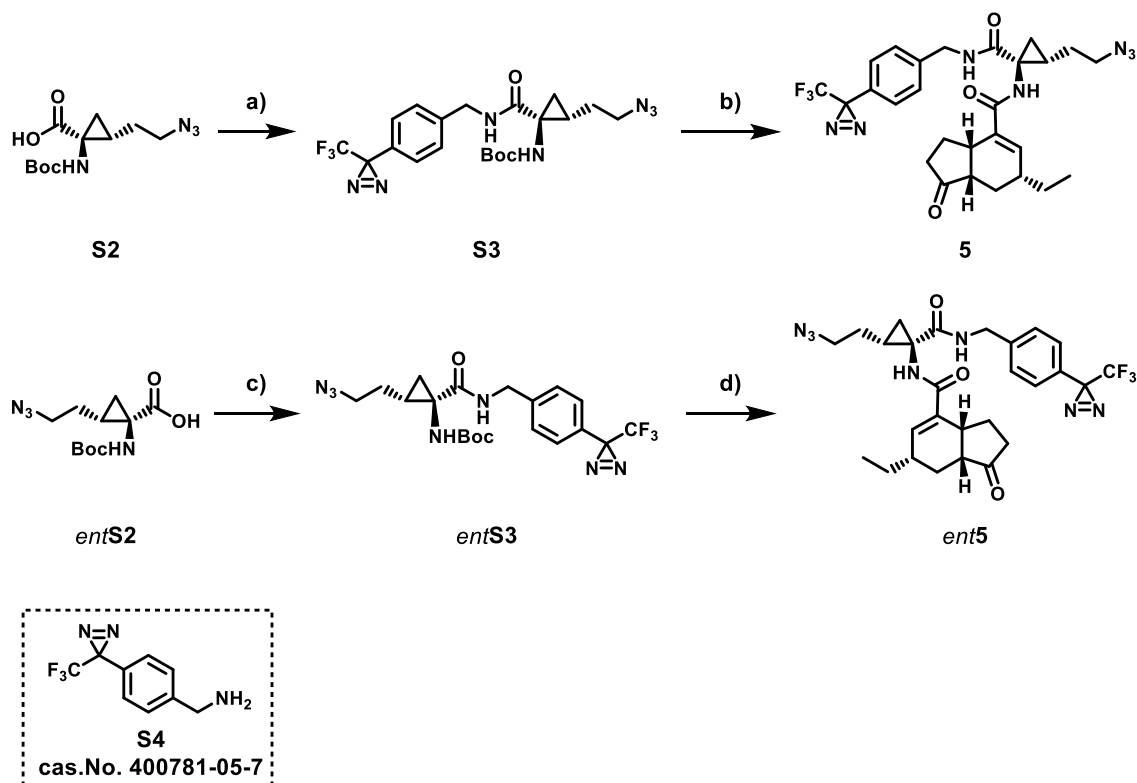
**Figure S5: The results of an *in silico* docking study using 2 and 5**

a) Ligand binding pocket in the crystal structure of the COI1/2/JAZ1 co-receptor complex (PDB ID: 3OGM). **2** is shown as green sticks, COI1 as gray ribbons and JAZ1 as orange ribbons. The hydrogen bond between the carboxylate group of **2** with R348 and R409 of COI1 is indicated by a cyan dotted line. b) Full structure of the *in silico* docking model between **2** or **5** and COI1-JAZ2 by MOE 2016.08 software shown in Figure 3b. **2** is shown as green sticks, COI1 as gray ribbons, JAZ2 as orange ribbons, calculated **2** as yellow sticks, and calculated **5** as magenta sticks, respectively. c-d) Top three high-scoring c) structures and d) binding free energies obtained by an *in silico* docking study between COI1/JAZ2 and **2** or **5**. **2** is shown as green sticks, COI1 as gray ribbons, JAZ2 as orange ribbons, calculated **2** as yellow sticks and calculated **5** as magenta sticks.



**Scheme S1.** Synthesis of compound **4**

a) 1-butyne, CuI, piperidine, 75%. b) 1) TFA, CH<sub>2</sub>Cl<sub>2</sub> 2) (+)-CFA, COMU, TEA, DMF, 10% over 2 steps.



**Scheme S2.** Synthesis of compounds **5** and *ent5*

a) **S4**, COMU, TEA, DMF, 7%. b) 1) TFA, CH<sub>2</sub>Cl<sub>2</sub> 2) (+)-CFA, COMU, TEA, DMF, 69% in 2 steps. c) **S4**, COMU, TEA, DMF, 7%. d) 1) TFA, CH<sub>2</sub>Cl<sub>2</sub> 2) (–)-CFA, COMU, TEA, DMF, 39% over 2 steps.



## Materials and Methods

### General materials and methods

All chemical reagents and solvents were obtained from commercial suppliers (Kanto Chemical Co. Ltd., Wako Pure Chemical Industries Co. Ltd., Nacalai Tesque Co., Ltd., Watanabe Chemical Industries Co. Ltd., GE Healthcare) and used without further purification. Ultraviolet (UV)-visible spectra were recorded on a UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). SDS-PAGE and western blotting were carried out using a Mini-Protean III electrophoresis apparatus (Bio-Rad, Hercules, CA). Chemiluminescence were observed on an LAS 4000 imaging system (Fujifilm, Tokyo, Japan). Reversed-phase high-performance liquid chromatography (HPLC) was carried out on a PU-4180 plus pump equipped with UV-4075 and MD-4010 detectors (JASCO, Tokyo, Japan) with detection at 220 nm. Both  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a JNM-ECS-400 spectrometer (JEOL, Tokyo, Japan) in deuterated chloroform. Fourier transform infrared (FT/IR) spectra were recorded on a FT/IR-4100 (JASCO, Tokyo, Japan). High-resolution (HR) electrospray ionization (ESI)-mass spectrometry (MS) analyses were conducted using a microTOF II (Bruker Daltonics Inc., Billerica, MA). Optical rotation was measured by a JASCO DIP-1000 polarimeter (JASCO, Tokyo, Japan). All anhydrous solvents were dried by standard techniques and freshly distilled before use or purchased in anhydrous form. Flash chromatography was carried out using dry-packed Chromatorex PSQ 100B silica gel (Fuji Silysia Chemical Ltd., Japan). All reactions were carried out under air unless stated otherwise.

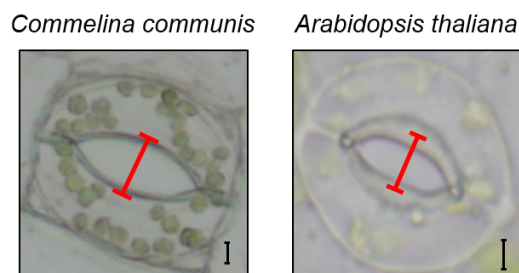
### Plant materials and growth conditions

*Commelina communis* was grown under a 16-h-light ( $75\ \mu\text{mol m}^{-2}\text{s}^{-1}$ ; cool-white fluorescent light) /8-h-dark cycle at 22 °C on soil in a Biotron LPH-240SP growth chamber (Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan). *Arabidopsis thaliana* (ecotype Col-0) was grown at the same conditions as *Commelina*. *Arabidopsis* wild-type plants, *etr1-1* and *ein2-1* mutants are in the genetic background of ecotype Columbia Col-0.

### Assessment of stomatal opening activity of the compounds

The abaxial leaf epidermis of 5-week-old *Commelina* plants was peeled and cut in a size of *ca.* 2 mm × 2 mm size. Before the treatment of test compounds, stomata were floated on buffer (10 mM MES–KOH 50 mM KCl, pH 6.2) and closed under dark conditions at 22 °C for 3 hours. Then, closed stomata were incubated for 3 hours with each compound in MES buffer at 22 °C for 3 hours in the dark. Micrographs were taken on an IX71

microscope (Olympus Corp., Japan) equipped with a DP72 CCD camera (Olympus Corp). The length of stomatal apertures was measured using ImageJ 1.45S software (<http://imagej.net/Welcome>). The stomatal aperture was measured as length between two guard cells as shown as red bar in the photographs below. Length of each scale bar indicates 5  $\mu\text{m}$  for *Commelina communis* are 2  $\mu\text{m}$  for *Arabidopsis thaliana*. The data were analyzed by one-way ANOVA followed by a Student-Newman-Keuls post hoc test among all means. Statistical analysis was conducted using CoStat version 6.400 software (CoHort Software, <http://www.cohort.com>).



#### **Pull-down assay of COI1-GST with MBP-JAZ2 and compounds**

The plasmids of GST-fused COI1 or ASK1 (pFB-GTE-COI1 and pFB-HTB-ASK1) were obtained from Addgene (<https://www.addgene.org/>). These proteins were co-expressed in Sf9 insect cells and purified by glutathione sepharose 4B (GE Healthcare, Ltd. UK).<sup>S1</sup> Recombinant MBP-fused JAZ2<sup>S2</sup> were expressed in *Escherichia coli* BL21 cells and purified in amylose resin (New England Biolabs) columns according to the previously reported protocols with slight change.<sup>S3,S4</sup> In the pull-down experiments, purified COI1-GST (5 nM) with ASK1-GST and probes (100 nM) in 500  $\mu\text{L}$  of incubation buffer (50 mM Tris-HCl, pH 7.8, containing 100 mM NaCl, 10% glycerol, 0.1% Tween20, 20 mM 2-mercaptoethanol, 100 nM inositol-1,2,4,5,6-pentakisphosphate (Ins(1,2,4,5,6)P<sub>5</sub>), 10 vol% complete<sup>®</sup> (protease inhibitor cocktail, according to the manufacture's (Roche) information) was added to amylose resin-bound MBP-JAZ2 (50  $\mu\text{L}$  suspension of amylose resin with 100 nM MBP-JAZ2). After 10 h incubation at 4 °C, the samples were washed with 500  $\mu\text{L}$  of fresh incubation buffer in triplicate. The washed amylose resin was resuspended in 50  $\mu\text{L}$  of SDS-PAGE loading buffer containing maltose (10 mM). After boiling for 10 min at 60 °C, the samples were subjected to SDS-PAGE analyses. The bound COI1-GST proteins were detected using anti-GST HRP conjugate (GE Healthcare). The MBP-JAZ2 proteins as input were detected using anti-MBP antibody (Wako Pure Chemical Industries Co Ltd.) and anti-rat IgG HRP conjugate (Santa Cruz Biotechnology, Inc.).

#### **Fluorescent imaging of peeled epidermis of *Commelina communis***

The abaxial leaf epidermis of 5-week-old *Commelina* or *Arabidopsis* Col-0 was peeled

off and cut in the size of *ca.* 2 mm × 2 mm size. Differential interference contrast (DIC) and fluorescent images (Ex. /Em. = 488 nm / 415–735 nm) were obtained on an LSM-710 confocal microscope (Carl Zeiss, Germany).

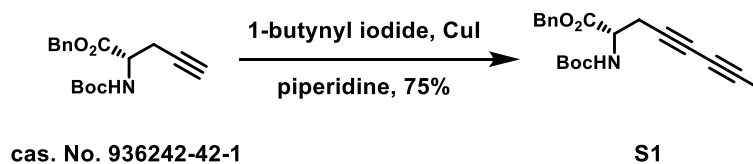
### ***In silico* docking study**

The initial structure of the COI1/2/ JAZ2 complex was obtained based on the previously reported crystal structure of COI1/2/JAZ1 (PDB ID: 3OGM) because the amino acid sequences of the JAZ1 degron peptide used in the X-ray crystallography were identical to that of JAZ2 (ELPIARRASLHRFLEKRKDR). The **2** in the COI1/2/JAZ2 complex was replaced **2** (as control) or **5** via docking simulations. Docking of these compounds were performed using MOE 2016.08 software (Chemical Computing Groups, Montreal, Canada). The ligand co-receptor binding free energy was calculated at Amber10 force field parameters<sup>S5</sup>. The London dG and GBVI/WSA dG algorithm was chosen to search for the best conformers<sup>S6</sup>. The obtained docked poses that showed the best score was indicated in Figure 2b.

### **Photoaffinity labeling experiments using peeled epidermis of *Commelina communis***

The abaxial leaf epidermis of 5-week-old *Commelina* plants were peeled off and the stomata was incubated and closed in MES buffer (10 mM MES-KOH 50 mM KCl, pH 6.2) at 22 °C under the dark condition for 3 hours. The epidermis with closed stomata were incubated for 3 hours under dark condition with **5** or *ent5* (10 μM) in MES buffer at 22 °C. The epidermis was irradiated with UV-light on ice for 20 min and then followed by washing with MES buffer. Washed epidermis was then transferred into fresh tubes and homogenized in HEPES buffer (100 mM HEPES-KOH, EDTA-free complete protease inhibitor cocktail (Roche), pH 7.4) on ice. The homogenate was centrifuged at 150,000 *g* for 30 min and the pellet was suspended in RIPA buffer (25 mM Tris-HCl, 150 mM NaCl, 1% NA-40, 1% sodium deoxycholate, 1% SDS, pH 7.6). The suspension was centrifuged at 15,000 *g* for 5 min and the supernatant was subjected to CuAAC reaction (5 mM aminoguanidine, 1 mM THPTA, 200 μM CuSO<sub>4</sub>, 5 mM sodium ascorbate, 100 μM Biotin-PEG4-alkyne (**6**, Aldrich)) for 1 h at rt and then denatured by heating at 60 °C for 10 min. Protein samples were subjected to SDS-PAGE and immunoblotting.

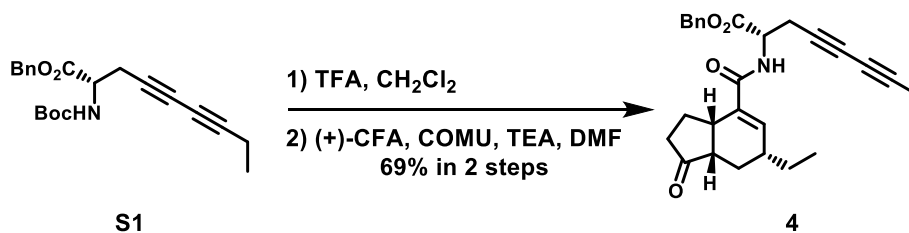
## Organic Synthetic Experimental Procedures and Product Physical Data



### (*S*)-benzyl 2-((*tert*-butoxycarbonyl)amino)nona-4,6-diynoate (**S1**)

1-Butynyl iodide (40  $\mu$ L, 405  $\mu$ mol) and copper (I) iodide (5 mg, 27  $\mu$ mol) were added to a solution of *N*-Boc-L-propargylglycine-OBn [CAS No.936242-42-1] (82.0 mg, 270  $\mu$ mol) in piperidine (0.54 mL) at 0  $^{\circ}$ C under an argon atmosphere. After 90 min of stirring, the reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  (30 mL). The reaction mixture was extracted with  $\text{CHCl}_3$  ( $2 \times 20$  mL), and then the organic layer was dried over  $\text{Na}_2\text{SO}_4$ . After evaporation, the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5/1) to give **S1** (72.4 mg, 204  $\mu$ mol, 75%) as a white solid.

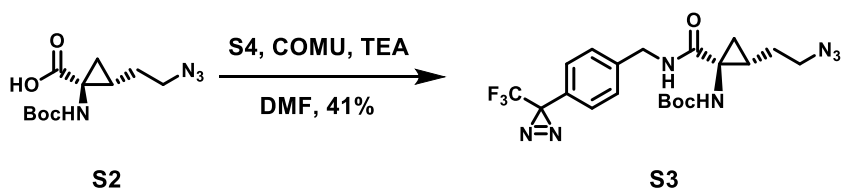
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 7.38-7.33 (m, 5H), 5.36 (d,  $J$  = 8.0 Hz, 1H), 5.23 (d,  $J$  = 12.6 Hz, 1H), 5.18 (d,  $J$  = 12.6 Hz, 1H), 4.50 (dt,  $J$  = 8.0, 4.4 Hz, 1H), 2.86 (dd,  $J$  = 16.8, 4.4 Hz, 1H), 2.79 (dd,  $J$  = 16.8, 4.4 Hz, 1H), 2.26 (q,  $J$  = 7.6 Hz, 2H), 1.45 (s, 9H), 1.17 (t,  $J$  = 7.6 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 170.4, 155.0, 135.1, 128.6 (2C), 128.4, 128.3 (2C), 80.3, 79.9, 71.0, 68.4, 67.5, 64.3, 52.1, 28.2 (3C), 23.8, 13.2, 12.9; IR (film)  $\text{cm}^{-1}$ : 3450, 2977, 2933, 2854, 2256, 1743, 1715, 1498, 1456, 1368, 1342, 1249, 1163, 1061, 1023, 753; HRMS (ESI, positive)  $m/z$   $[\text{M}+\text{Na}]^+$  calcd. for  $\text{C}_{21}\text{H}_{25}\text{NNaO}_4$  : 378.1676, Found : 378.1681;  $[\alpha]_{\text{D}}^{20} +3.1^{\circ}$  ( $c$  0.23,  $\text{CHCl}_3$ ).



(*S*)-benzyl 2-((3*aS*,6*R*,7*aS*)-6-ethyl-1-oxo-2,3,3*a*,6,7,7*a*-hexahydro-1*H*-indene-4-carboxamido)nona-4,6-diynoate (**4**)

TFA (0.5 mL) was added to a solution of **S1** (4.5 mg, 16.1  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) at rt. After stirring for 30 min, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in DMF (0.5 mL); then, (+)-CFA (2.1 mg, 10.0  $\mu\text{mol}$ ), COMU (6.9 mg, 16.0  $\mu\text{mol}$ ) and TEA (9.5  $\mu\text{L}$ , 40.0  $\mu\text{mol}$ ) were added at rt. After overnight stirring at 50  $^\circ\text{C}$ , the reaction mixture was quenched with 1N aqueous  $\text{KHSO}_4$  solution. The mixture was extracted with EtOAc ( $3 \times 10 \text{ mL}$ ) and then washed with brine ( $1 \times 10 \text{ mL}$ ). The organic layer was dried over  $\text{Na}_2\text{SO}_4$ . After evaporation, the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 8/1-3/1). Each solution was concentrated by evaporation to give **4** (3.1 mg, 7.0  $\mu\text{mol}$ , 69%) as a colorless crystalline solid.

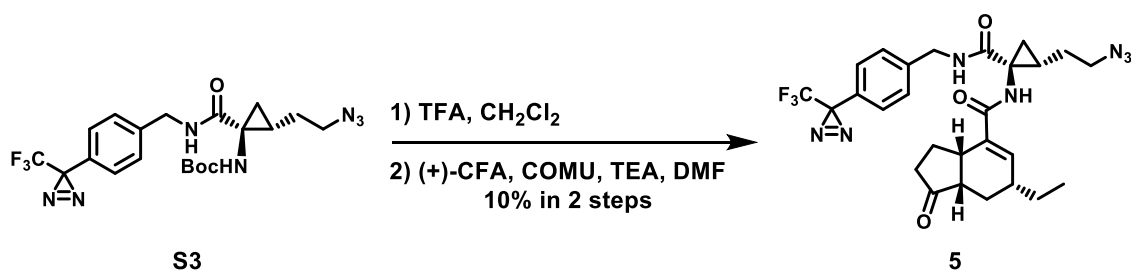
$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$ : 7.41-7.34 (m, 5H), 6.61 (d,  $J = 7.6 \text{ Hz}$ , 1H), 6.48 (s, 1H), 5.27 (d,  $J = 12.4 \text{ Hz}$ , 1H), 5.22 (d,  $J = 12.4 \text{ Hz}$ , 1H), 4.83 (dt,  $J = 7.6, 4.4 \text{ Hz}$ , 1H), 3.16 (dt,  $J = 11.2, 7.2 \text{ Hz}$ , 1H), 2.95 (dd,  $J = 16.8, 4.4 \text{ Hz}$ , 1H), 2.88 (dd,  $J = 16.8, 4.4 \text{ Hz}$ , 1H), 2.50-2.31 (m, 4H), 2.27 (q,  $J = 7.6 \text{ Hz}$ , 2H), 2.18 (brs, 1H), 1.90 (dt,  $J = 12.8, 4.8 \text{ Hz}$ , 1H), 1.66-1.37 (m, 3H), 1.15 (t,  $J = 7.6 \text{ Hz}$ , 3H), 1.08 (dt,  $J = 10.8, 2.8 \text{ Hz}$ , 1H), 1.01 (t,  $J = 7.6 \text{ Hz}$ , 3H);  $^{13}\text{C-NMR}$  (100MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 220.3, 170.4, 167.6, 138.0, 135.1, 135.0, 128.7 (2C), 128.6, 128.4 (2C), 80.0, 70.9, 69.2, 68.7, 67.8, 64.1, 50.9, 46.5, 38.2, 37.4, 36.1, 28.0, 27.8, 26.0, 23.3, 13.2, 12.9, 11.3; IR (film)  $\text{cm}^{-1}$ : 3330, 2966, 2938, 2877, 2860, 2258, 1741, 1661, 1627, 1523, 1457, 1382, 1343, 1316, 1275, 1213, 1189, 1051, 988; HRMS (ESI, positive)  $m/z$   $[\text{M}+\text{Na}]^+$  calcd. for  $\text{C}_{28}\text{H}_{31}\text{NNaO}_4$ : 468.2145, found: 468.2117;  $[\alpha]_{\text{D}}^{23} +99^\circ$  (*c* 0.17,  $\text{CHCl}_3$ ).



*tert*-butyl((1*S*,2*S*)-2-ethyl-1-((4-3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)carbamoyl)cyclopropylcarbamate (**S3**)

COMU (51.3 mg, 119  $\mu\text{mol}$ ) and TEA (10  $\mu\text{L}$ , 72  $\mu\text{mol}$ ) were added to a solution of **S2**<sup>S7</sup> (16.6 mg, 61.4  $\mu\text{mol}$ ) and 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzenemethanamine (**S4**; 11.0 mg, 51.1  $\mu\text{mol}$ ) in DMF (0.5 mL) at 0 °C under an argon atmosphere. The mixture was stirred for 13 h at rt. Then, the reaction mixture was diluted with EtOAc, successively washed with 1M HCl, saturated aqueous NaHCO<sub>3</sub>, and then with brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (*n*-Hexane/EtOAc = 15/1-3/1) to give **S3** (9.7 mg, 21  $\mu\text{mol}$ , 41%).

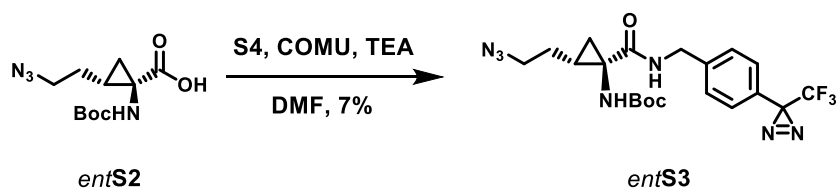
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$ : 7.38 (t,  $J$  = 5.6 Hz, 1H), 7.31 (d,  $J$  = 8.2 Hz, 2H), 7.14 (d,  $J$  = 8.2 Hz, 2H), 5.10 (s, 1H), 4.53 (d,  $J$  = 16.0 Hz, 1H), 4.38 (d,  $J$  = 16.0 Hz, 1H), 3.3 (dt,  $J$  = 16.0, 6.2 Hz, 2H), 1.87 (ddd,  $J$  = 14.4, 6.2, 5.7 Hz, 1H), 1.62 (dd,  $J$  = 7.6, 5.7 Hz, 2H), 1.52-1.45 (m, 2H), 1.40 (s, 9H), 1.13 (dd,  $J$  = 9.2, 5.0 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$ : 170.3, 156.0, 140.3, 128.1, 127.7, 126.8, 124.7 (q,  $^1J_{\text{CF}}$  = 274.6 Hz, CF<sub>3</sub>), 81.0, 51.0, 43.0, 39.8, 28.7 (q,  $^2J_{\text{CF}}$  = 38.2 Hz), 28.5, 28.1, 26.7, 20.7; IR (film) cm<sup>-1</sup>: 3323, 2980, 2932, 2097, 1709, 1652, 1520, 1456, 1393, 1366, 1344, 1242, 1159, 1052, 1023, 939; HRMS (ESI, positive)  $m/z$  [M+Na]<sup>+</sup> calcd. for C<sub>20</sub>H<sub>24</sub>F<sub>3</sub>N<sub>7</sub>NaO<sub>3</sub> 490.1785, Found 490.1790; [ $\alpha$ ]<sub>D</sub><sup>19</sup> +32.3 (*c* 0.305, CHCl<sub>3</sub>).



(3a*S*,6*R*,7a*S*)-6-ethyl-*N*-((1*S*, 2*S*)-2-ethyl-1-((4-(3-trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)carbamoyl)cyclopropyl)-1-oxo-2,3,3a,6,7,7a-hexahydro-1*H*-indene-4-carboxamide (**5**)

To a solution of **S3** (16.6 mg, 61.4  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added TFA (1 mL) under argon atmosphere. After stirring for 10 min, the reaction mixture was evaporated. The residue was dissolved in DMF (1.5 mL), then, (+)-CFA (4.6 mg, 22  $\mu$ mol), COMU (26.7 mg, 62.3  $\mu$ mol), and TEA (90  $\mu$ L, 646  $\mu$ mol) were added at 0 °C under argon atmosphere. After stirring for 12 h at rt, the reaction mixture was diluted with EtOAc, washed with 1M HCl, with saturated aqueous NaHCO<sub>3</sub>, and then with brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. After evaporation, the residue was purified by HPLC (ODS-HG ( $\phi$  20  $\times$  250 mm), 70% MeOH, 5 mL/min, UV 220 nm). Each solution was concentrated by evaporation to give **5** (1.2 mg, 2.1  $\mu$ mol, 10% in 2 steps) as a colorless crystalline solid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ <sub>H</sub> 7.38 (t, *J* = 6.0 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.14 (d, *J* = 8.2 Hz, 2H), 6.36 (s, 1H), 6.29 (s, 1H), 4.47 (dd, *J* = 15.6, 6.0 Hz, 1H), 4.43 (dd, *J* = 15.6, 6.0 Hz, 1H), 3.43-3.31 (m, 2H), 3.14-3.07 (m, 1H), 2.32-2.24 (m, 4H), 2.18-2.15 (m, 1H), 1.88 (dt, *J* = 13.2, 4.4 Hz, 1H), 1.82 (q, *J* = 6.8 Hz, 2H), 1.70 (dt, *J* = 14.0, 7.6 Hz, 1H), 1.64 (dd, *J* = 10.4, 5.8 Hz, 1H), 1.54-1.34 (m, 4H), 1.42-1.35 (m, 1H), 1.12 (dd, *J* = 9.6, 5.8 Hz, 2H), 1.02 (td, *J* = 13.2, 10.8 Hz, 1H), 0.98 (t, *J* = 7.6 Hz, 3H);  $\delta$ <sub>C</sub>: 219.5, 169.7, 169.5, 140.3, 138.0, 135.1, 128.1, 127.8, 126.8, 125.0 (q, <sup>1</sup>*J*<sub>CF</sub> = 273.6 Hz, CF<sub>3</sub>), 76.7, 50.8, 46.2, 43.2, 40.0, 38.0, 37.4, 36.1, 28.7 (q, <sup>2</sup>*J*<sub>CF</sub> = 40.0 Hz), 28.0 (3C), 27.8, 27.3, 25.9, 19.5, 11.3; IR (film) cm<sup>-1</sup>: 3316, 2928, 2855, 2362, 2094, 1740, 1653, 1558, 1540, 1519, 1507, 1457, 1344, 1263, 1233, 1184, 1156, 755; HRMS (ESI, positive) *m/z* [M+Na]<sup>+</sup> Calcd. for C<sub>27</sub>H<sub>30</sub>F<sub>3</sub>N<sub>7</sub>NaO<sub>3</sub> 580.2254, Found 580.2256; [ $\alpha$ ]<sub>D</sub><sup>24</sup> +83.6 (*c* 0.65, CHCl<sub>3</sub>).

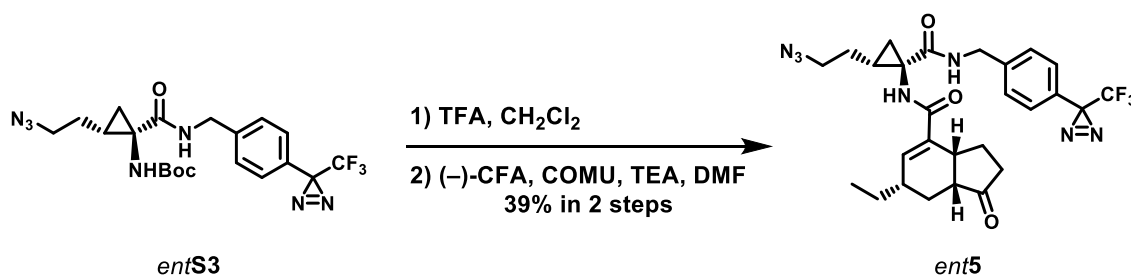


*tert*-butyl((1*R*,2*R*)-2-ethyl-1-((4-3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)carbamoyl)cyclopropyl)carbamate (*entS3*)

To a solution of *entS2*<sup>S7</sup> (10.3 mg, 38.1  $\mu\text{mol}$ ) and **S4** (9.4 mg, 43.7  $\mu\text{mol}$ ) in DMF (1 mL), COMU (25.3 mg, 59.1  $\mu\text{mol}$ ), TEA (30  $\mu\text{L}$ , 216  $\mu\text{mol}$ ) were added at 0 °C under argon atmosphere, and the mixture was stirred for 8 h at rt. Then, the reaction mixture was diluted with EtOAc and successively washed with 1M HCl, with saturated aqueous NaHCO<sub>3</sub>, and then with brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. After evaporation, the residue was purified by silica gel column chromatography (*n*-Hexane/EtOAc = 15/1-3/1). Each solution was concentrated by evaporation to provide *entS3* (1.3 mg, 2.78  $\mu\text{mol}$ , 7%) as a colorless crystalline solid.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$ : 7.31 (d,  $J$  = 8.2 Hz, 2H), 7.14 (d,  $J$  = 8.2 Hz, 2H), 5.10 (brs, 1H), 4.53 (d,  $J$  = 15.6 Hz, 1H), 4.38 (d,  $J$  = 15.6 Hz, 1H), 3.3 (dt,  $J$  = 16.0, 6.2 Hz, 2H), 1.86 (ddd,  $J$  = 14.4, 6.2, 5.3 Hz, 1H), 1.62 (dd,  $J$  = 7.6, 5.3 Hz, 2H), 1.52-1.44 (m, 2H), 1.40 (s, 9H), 1.13 (dd,  $J$  = 10.2, 5.3 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$  170.4, 156.3, 140.5, 128.3, 128.0, 127.0, 122.1 (q,  $^1J_{\text{CF}}$  = 273.6 Hz, CF<sub>3</sub>) 81.3, 51.2, 43.3, 39.9, 28.9 (q,  $^2J_{\text{CF}}$  = 40.2 Hz), 28.3, 28.0, 26.9, 20.7; IR (film) cm<sup>-1</sup> 3325, 2978, 2937, 2098, 1709, 1652, 1521, 1457, 1392, 1367, 1343, 1244, 1160, 1052, 1025, 939; HRMS (ESI, positive)  $m/z$  [M+Na]<sup>+</sup> Calcd. for C<sub>20</sub>H<sub>24</sub>F<sub>3</sub>N<sub>7</sub>NaO<sub>3</sub> 490.1785, Found 490.1784; [ $\alpha$ ]<sub>D</sub><sup>24</sup> -32.7 (*c* 0.230, CHCl<sub>3</sub>).





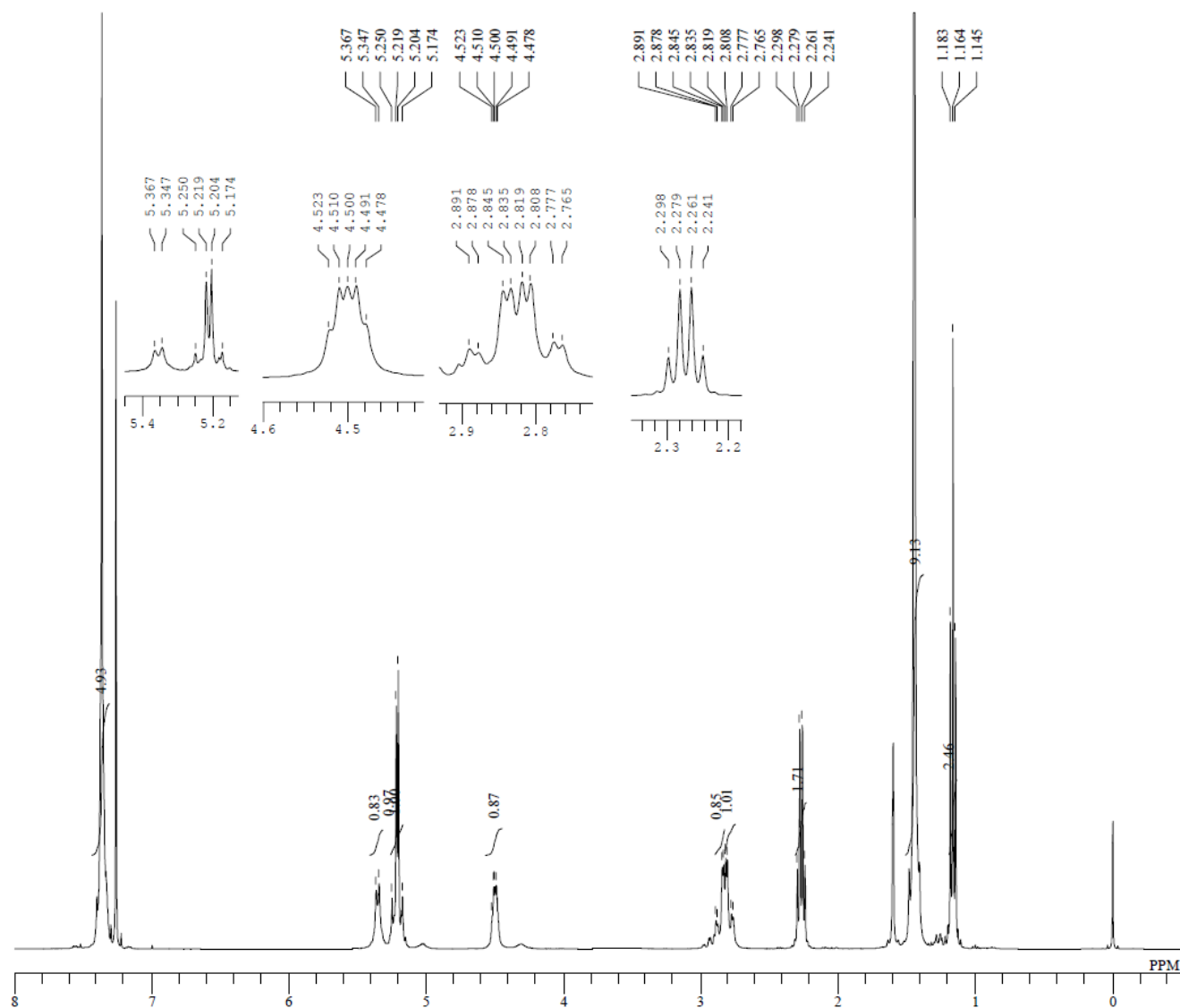
(3a*R*,6*S*,7a*R*)-6-ethyl-*N*-((1*R*, 2*R*)-2-ethyl-1-((4-(3-trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)carbamoyl)cyclopropyl)-1-oxo-2,3,3a,6,7,7a-hexahydro-1*H*-indene-4-carboxamide (*ent5*)

To a solution of *entS3* (1.3 mg, 2.8  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) was added TFA (0.5 mL) under argon atmosphere, and the mixture was stirred for 10 min. After stirring for 10 min, the reaction mixture was evaporated. The residue was dissolved in DMF (1.5 mL), then (–)-CFA (1.3 mg, 6.2  $\mu\text{mol}$ ), COMU (5.4 mg, 12.6  $\mu\text{mol}$ ), and TEA (10  $\mu\text{L}$ , 72  $\mu\text{mol}$ ) were added at 0 °C under argon atmosphere. After stirring for 12 h at rt, the reaction mixture was diluted with EtOAc and successively washed with 1M HCl, with saturated aqueous  $\text{NaHCO}_3$ , and with brine. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , and filtered. After evaporation, the residue was purified by HPLC (ODS-HG ( $\phi$  20  $\times$  250 mm), 70% MeOH, 5 mL/min, detection at 220 nm). Each solution was concentrated by evaporation to provide *ent5* (0.6 mg, 1.08  $\mu\text{mol}$ , 39% in 2steps) as a colorless crystalline solid.

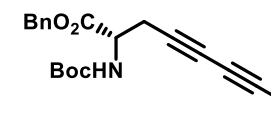
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  7.38 (t,  $J$  = 6.0 Hz, 1H), 7.31 (d,  $J$  = 8.2 Hz, 2H), 7.14 (d,  $J$  = 8.2 Hz, 2H), 6.36 (s, 1H), 6.29 (s, 1H), 4.45 (dd,  $J$  = 15.6, 6.0 Hz, 1H), 4.43 (dd,  $J$  = 15.6, 6.0 Hz, 1H), 3.43–3.31 (m, 2H), 3.14–3.07 (m, 1H), 2.42–2.24 (m, 4H), 2.17–2.14 (m, 1H), 1.88 (dt,  $J$  = 13.2, 4.4 Hz, 1H), 1.82 (q,  $J$  = 6.8 Hz, 2H), 1.70 (dt,  $J$  = 14.0, 7.6 Hz, 1H), 1.64 (dd,  $J$  = 10.4, 5.8 Hz, 1H), 1.54–1.34 (m, 4H), 1.42–1.35 (m, 1H), 1.12 (dd,  $J$  = 9.6, 5.8 Hz, 2H), 1.02 (td,  $J$  = 13.2, 10.8 Hz, 1H), 0.97 (t,  $J$  = 7.6 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$ : 219.5, 169.8, 169.6, 140.2, 138.1, 135.1, 128.2, 127.9, 126.8, 124.7 (q,  $^1J_{\text{CF}}$  = 272.7 Hz,  $\text{CF}_3$ ), 77.2, 50.8, 46.2, 43.2, 40.0, 38.0, 37.4, 36.1, 28.7 (q,  $^2J_{\text{CF}}$  = 50.5 Hz), 28.1, 28.0 (2C), 27.8, 27.3, 25.9, 19.5, 11.3; IR (film)  $\text{cm}^{-1}$  3317, 2930, 2857, 2361, 2096, 1738, 1653, 1558, 1540, 1519, 1507, 1457, 1344, 1262, 1231, 1185, 1154, 757; HRMS (ESI, positive)  $m/z$   $[\text{M}+\text{Na}]^+$  Calcd. for  $\text{C}_{27}\text{H}_{30}\text{F}_3\text{N}_7\text{NaO}_3$  580.2254, Found 580.2260;  $[\alpha]_{\text{D}}^{24}$  –80.6 ( $c$  0.70,  $\text{CHCl}_3$ ).

## References

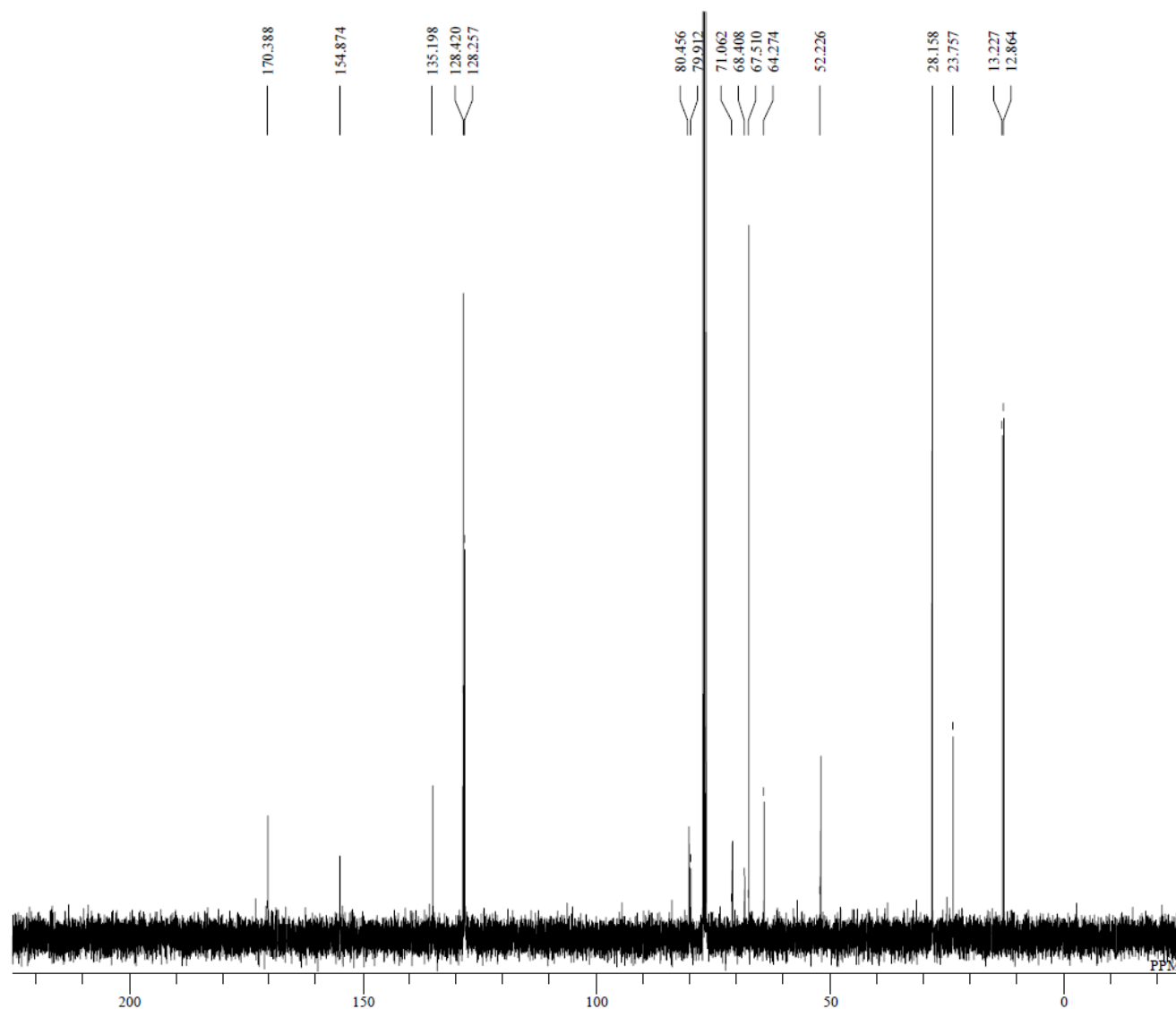
- S1) L. B. Sheard, X. Tan, H. Mao, J. Withers, G. Ben-Nissan, T. R. Hinds, Y. Kobayashi, F. F. Hsu, M. Sharon, J. Browse, S. Y. He, J. Rizo, G. A. Howe and N. Zheng, *Nature* 2010, **468**, 400-405.
- S2) S. Gimenez-Ibanez, M. Boter, A. Ortigosa, G. Garcia-Casado, A. Chini, M. G. Lewsey, J. R. Ecker, V. Ntoukakis and R. Solano, *New Phytol.*, 2017, **213**, 1378-1392
- S3) S. Fonseca, A. Chini, M. Hamberg, B. Adie, A. Porzel, R. Kramell, O. Miersch, C. Wasternack and R. Solano, *Nat. Chem. Biol.* 2009, **5**, 344-350.
- S4) S. Egoshi, Y. Takaoka, H. Saito, Y. Nukadzuka, K. Hayashi, Y. Ishimaru, H. Yamakoshi, K. Dodo, M. Sodeoka and M. Ueda, *RSC Adv.* 2016, **6**, 19404-19412.
- S5) H. J. Manen, Y. M Kraan, D. Roos and C. Otto, *J. Phys. Chem. B*, 2004, **108**, 18762-18771.
- S6) C. A. Lieber and A. M. Jansen, *Appl. Spectrosc.*, 2003, **57**, 1363–1367.
- S7) M. Okada, S. Egoshi and M. Ueda, *Biosci. Biotechnol. Biochem.*, 2010, **10**, 2092-2095.



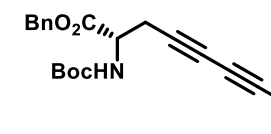
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 OBFIN 4.97 Hz  
 POINT 13107  
 FREQU 6024.10 Hz  
 SCANS 16  
 ACQTM 2.1758 sec  
 PD 5.0000 sec  
 PW1 4.84 usec  
 IRNUC 1H  
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 SLVNT CDCL3  
 EXREF 0.00 ppm  
 BF 1.20 Hz  
 RGAIN 38



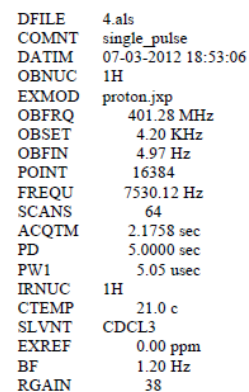
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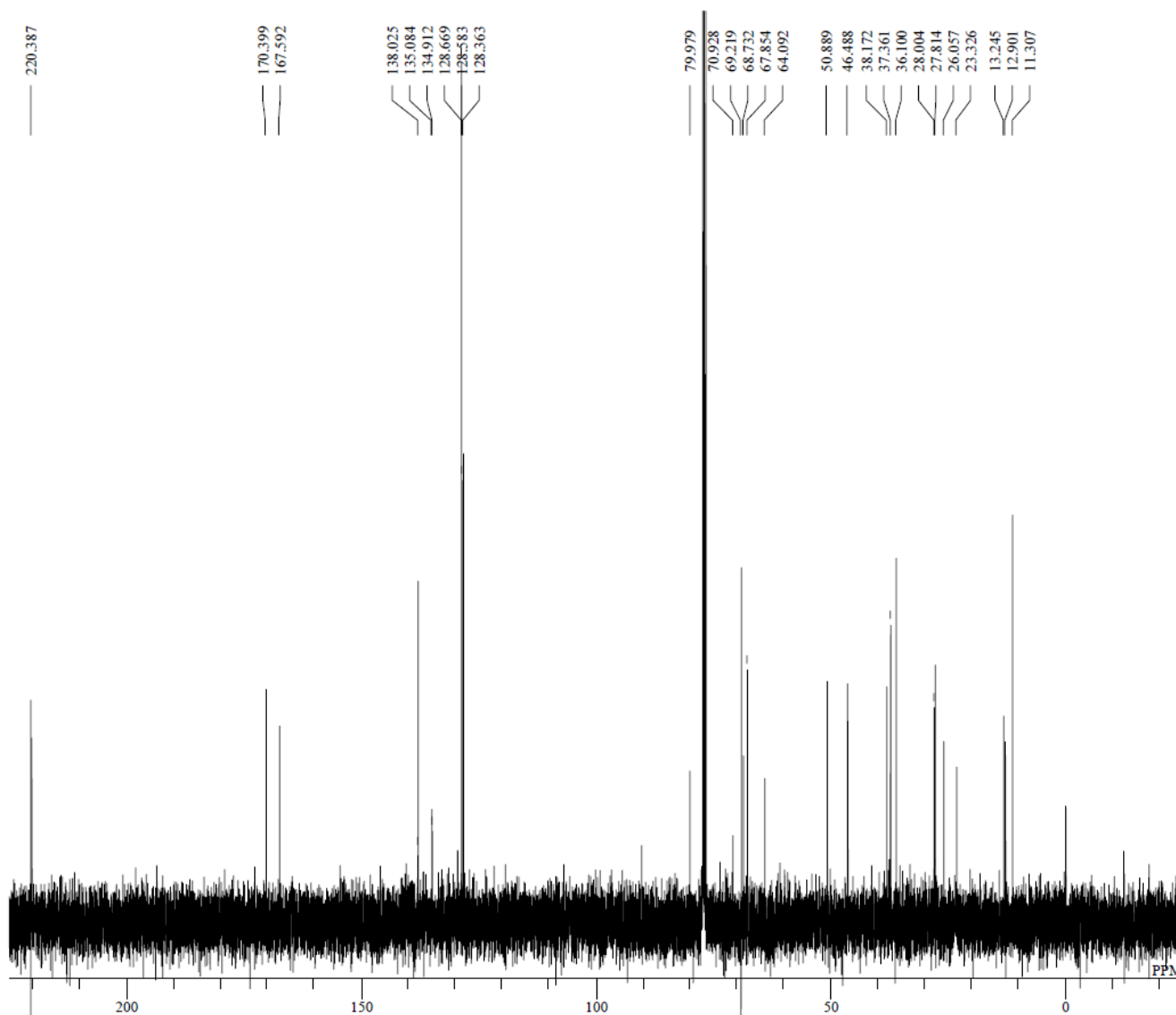


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 OBFIN 8.88 Hz  
 POINT 32767  
 FREQU 31565.66 Hz  
 SCANS 800  
 ACQTM 1.0381 sec  
 PD 1.0000 sec  
 PW1 2.88 usec  
 IRNUC <sup>1</sup>H  
 CTEMP 21.7 c  
 SLVNT CDCL<sub>3</sub>  
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 BF 0.18 Hz  
 RGAIN 60

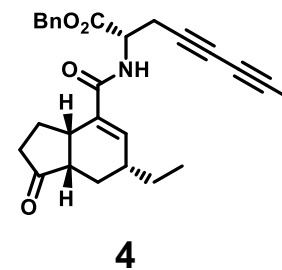


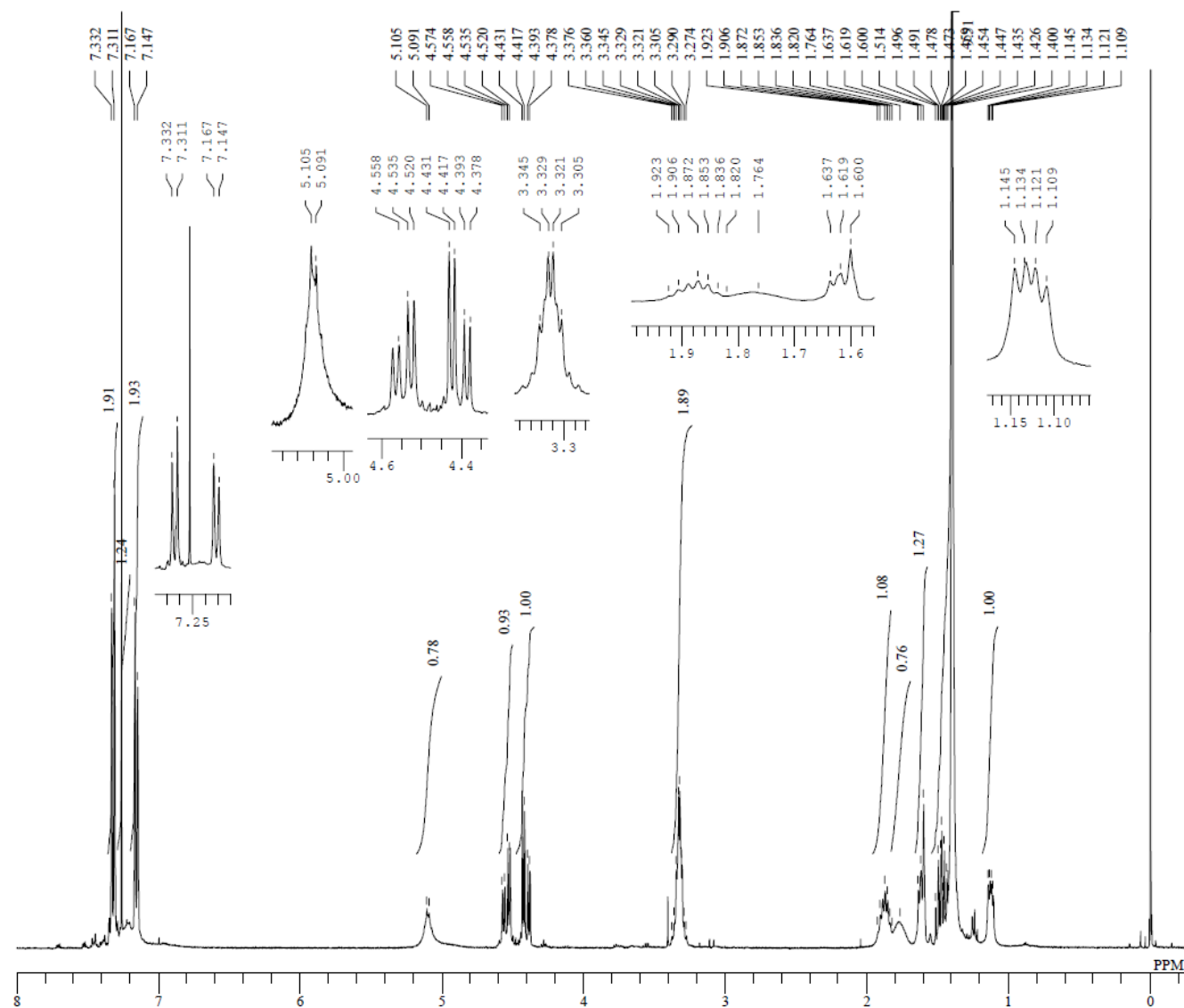
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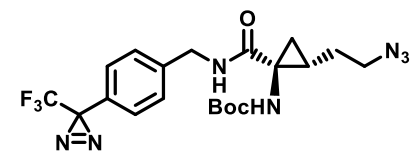


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 OBFIN 8.88 Hz  
 POINT 26214  
 FREQU 25252.53 Hz  
 SCANS 1024  
 ACQTM 1.0381 sec  
 PD 1.0000 sec  
 PW1 3.33 usec  
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 CTEMP 21.3 c  
 SLVNT CDCL<sub>3</sub>  
 EXREF 77.00 ppm  
 BF 0.18 Hz  
 RGAIN 60

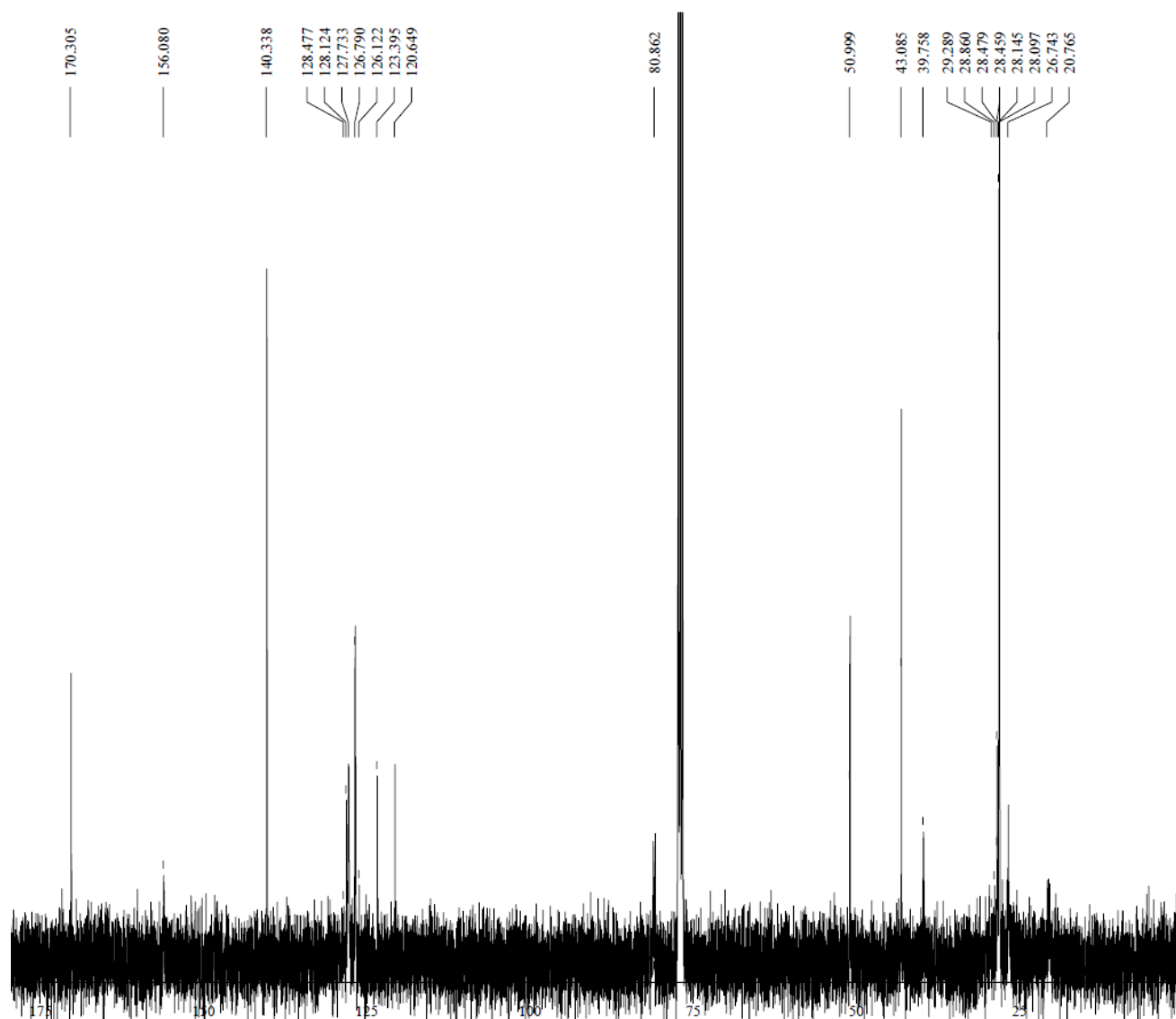




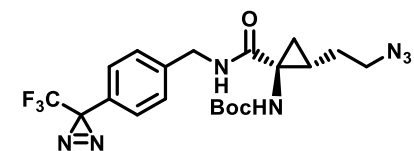
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 OBFIN 7.29 Hz  
 POINT 16384  
 FREQU 7503.00 Hz  
 SCANS 8  
 ACQTM 2.1837 sec  
 PD 5.0000 sec  
 PW1 2.95 usec  
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 CTEMP 21.8 c  
 SLVNT CDCL3  
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 RGAIN 62



**S3**

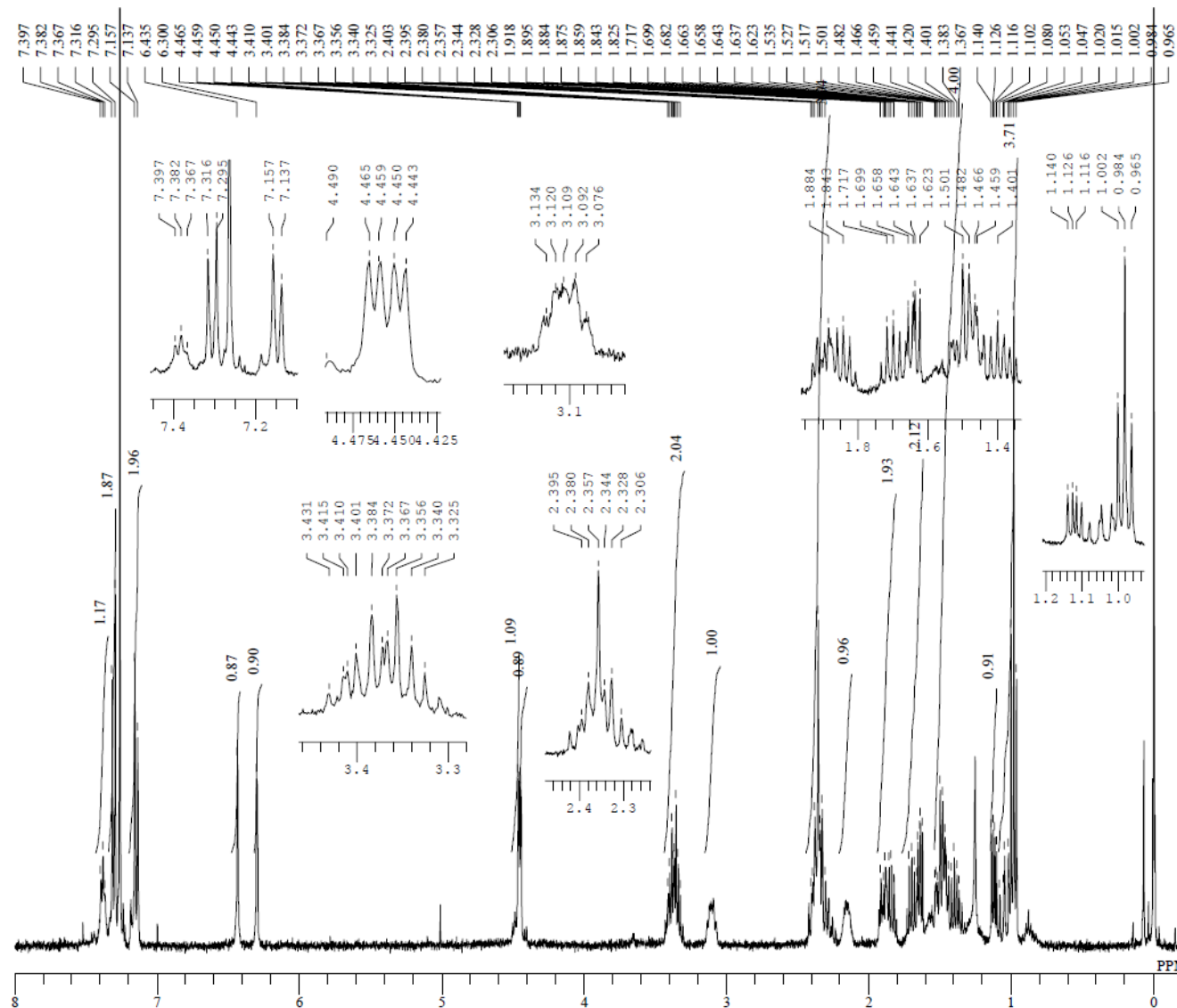


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 PD 2.0000 sec  
 PW1 3.27 usec  
 IRNUC <sup>1</sup>H  
 CTEMP 21.4 c  
 SLVNT CDCL<sub>3</sub>  
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 BF 0.18 Hz  
 RGAIN 50

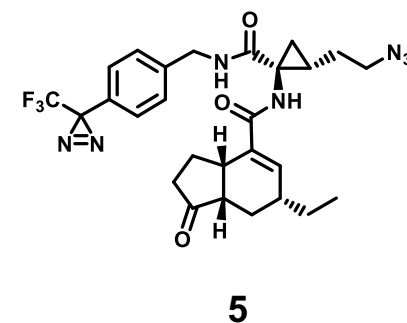


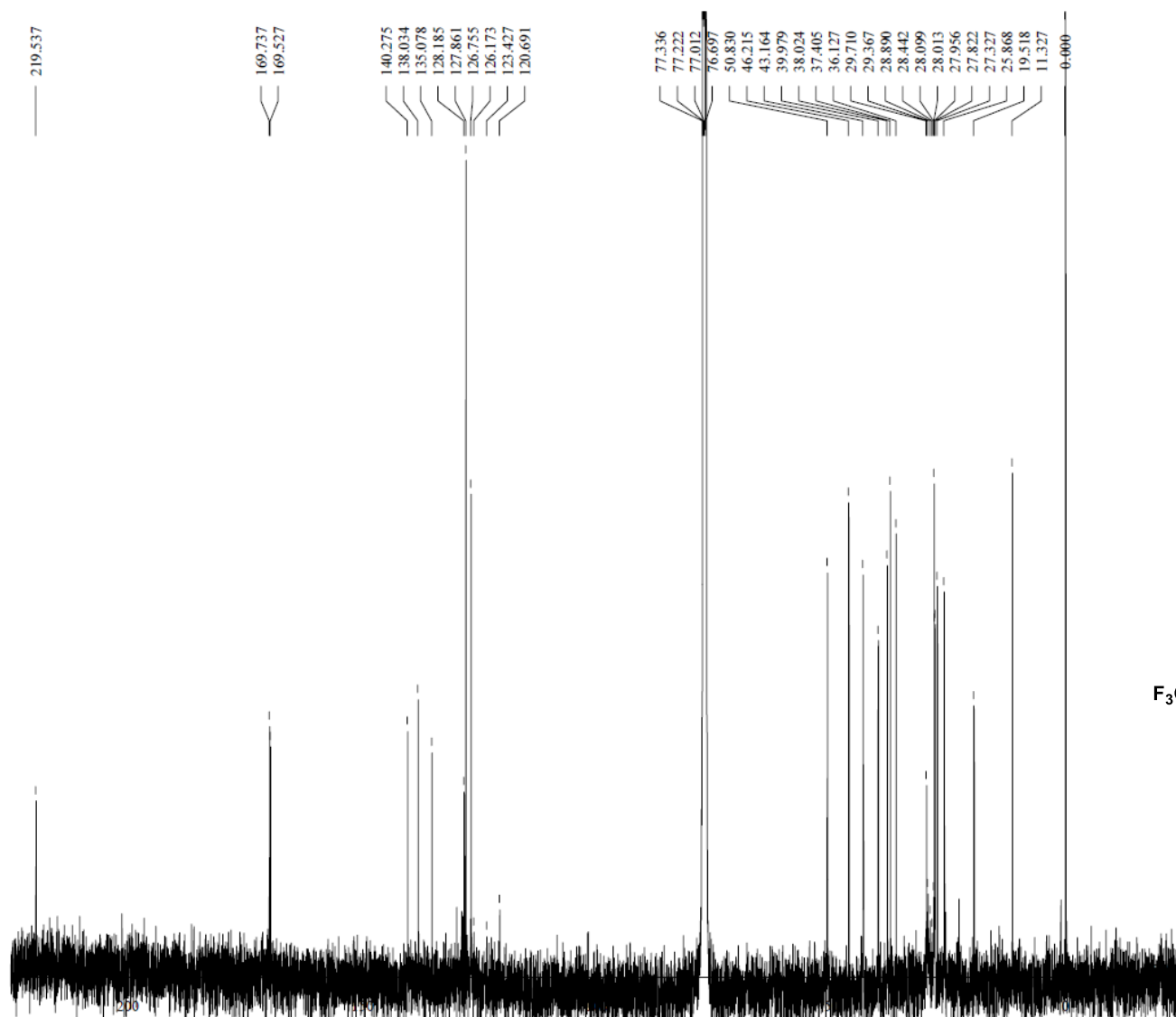
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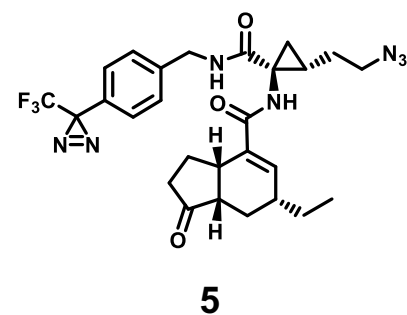


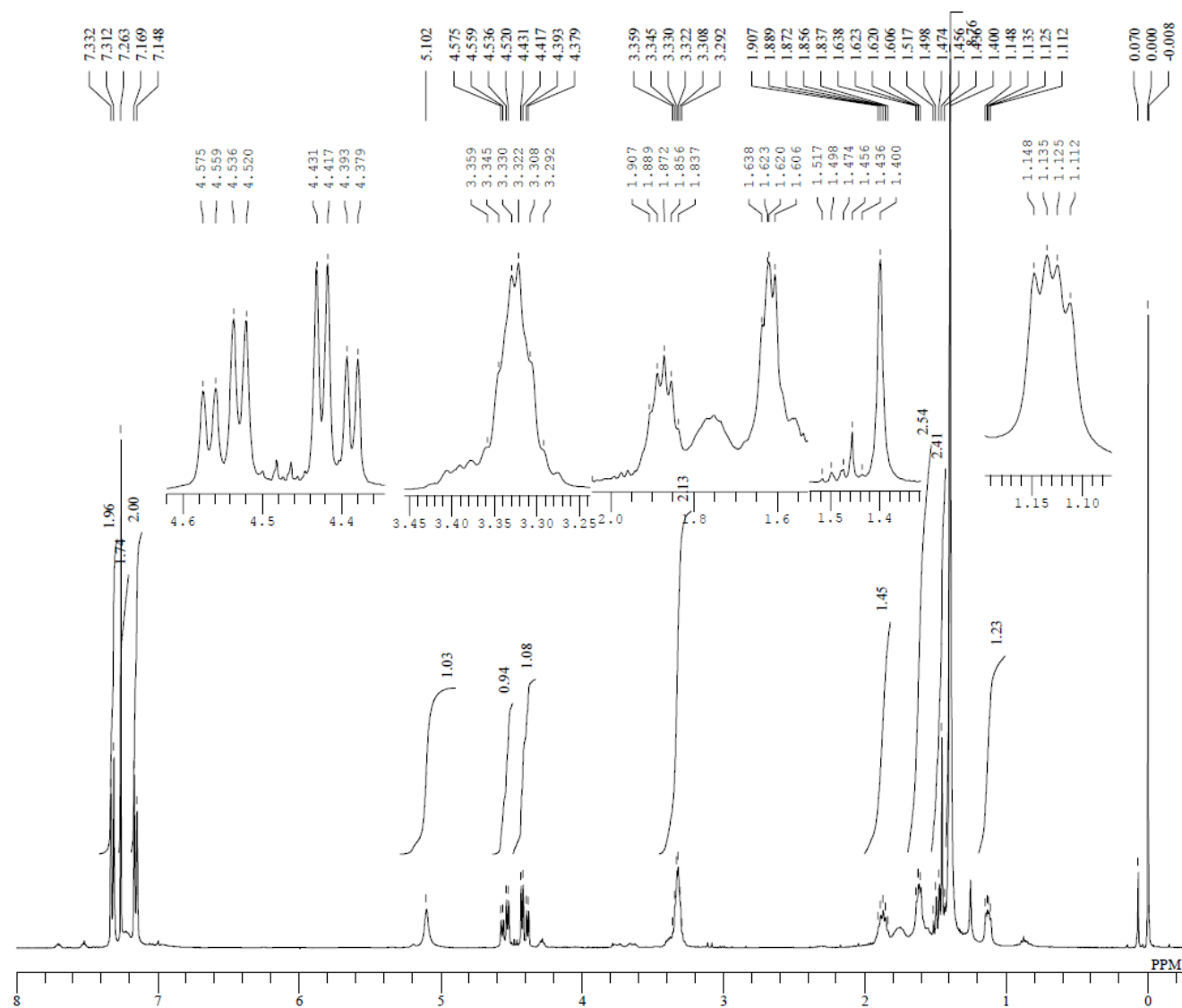
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 OBFIN 7.29 Hz  
 POINT 13107  
 FREQU 6002.40 Hz  
 SCANS 8  
 ACQTM 2.1837 sec  
 PD 5.0000 sec  
 PW1 2.95 usec  
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 CTEMP 21.6 c  
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 EXREF 0.00 ppm  
 BF 0.12 Hz  
 RGAIN 74



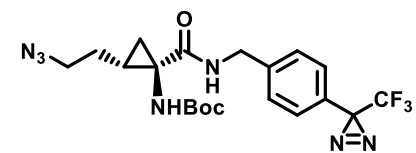


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 OBFIN 5.86 Hz  
 POINT 26214  
 FREQU 25125.63 Hz  
 SCANS 74130  
 ACQTM 1.0433 sec  
 PD 2.0000 sec  
 PW1 3.27 usec  
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 EXREF 0.00 ppm  
 BF 1.20 Hz  
 RGAIN 60

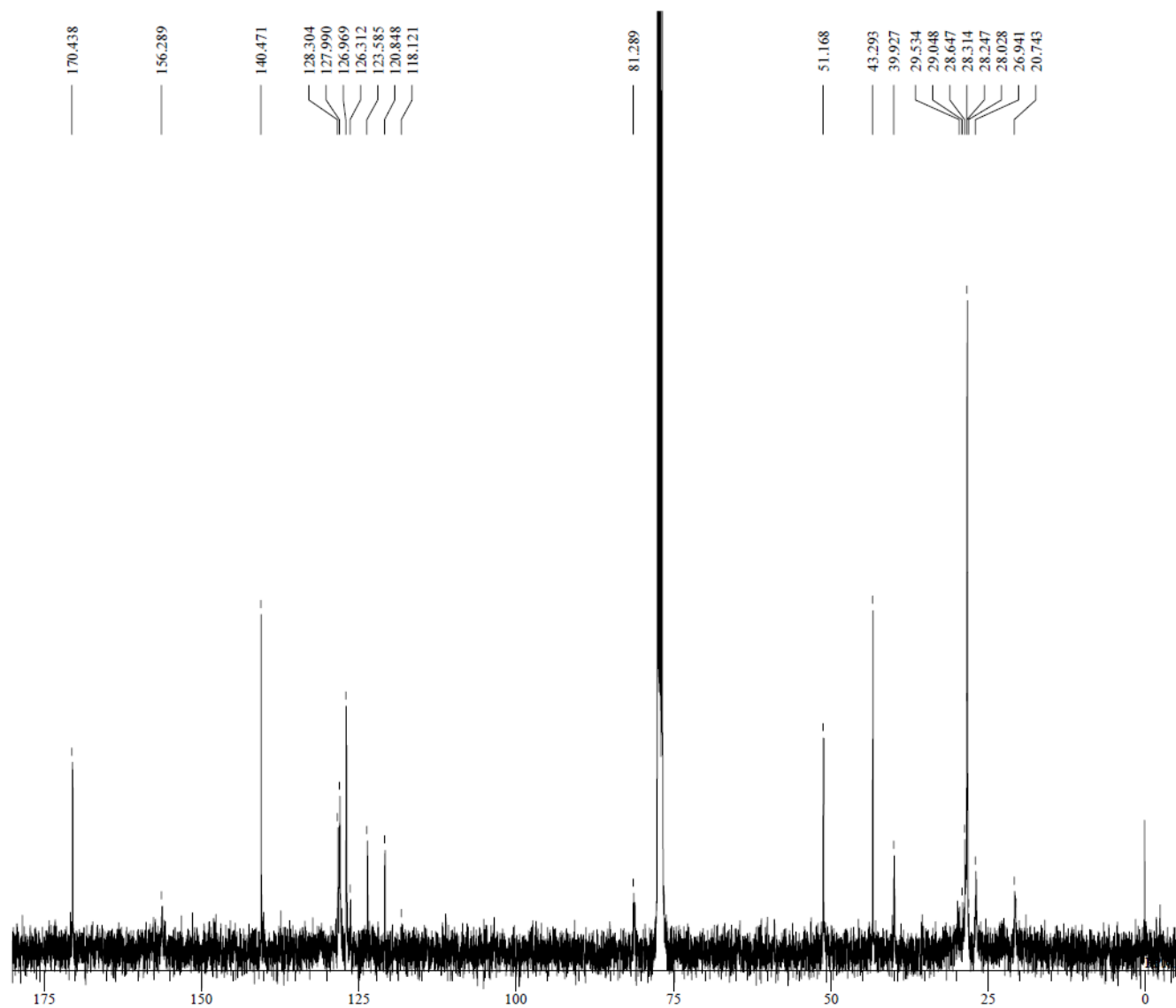




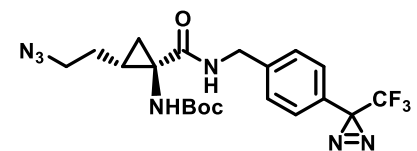
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 OBFIN 7.29 Hz  
 POINT 13107  
 FREQU 6002.40 Hz  
 SCANS 64  
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 PD 5.0000 sec  
 PW1 3.10 usec  
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 CTEMP 21.2 c  
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 EXREF 0.00 ppm  
 BF 0.12 Hz  
 RGAIN 48



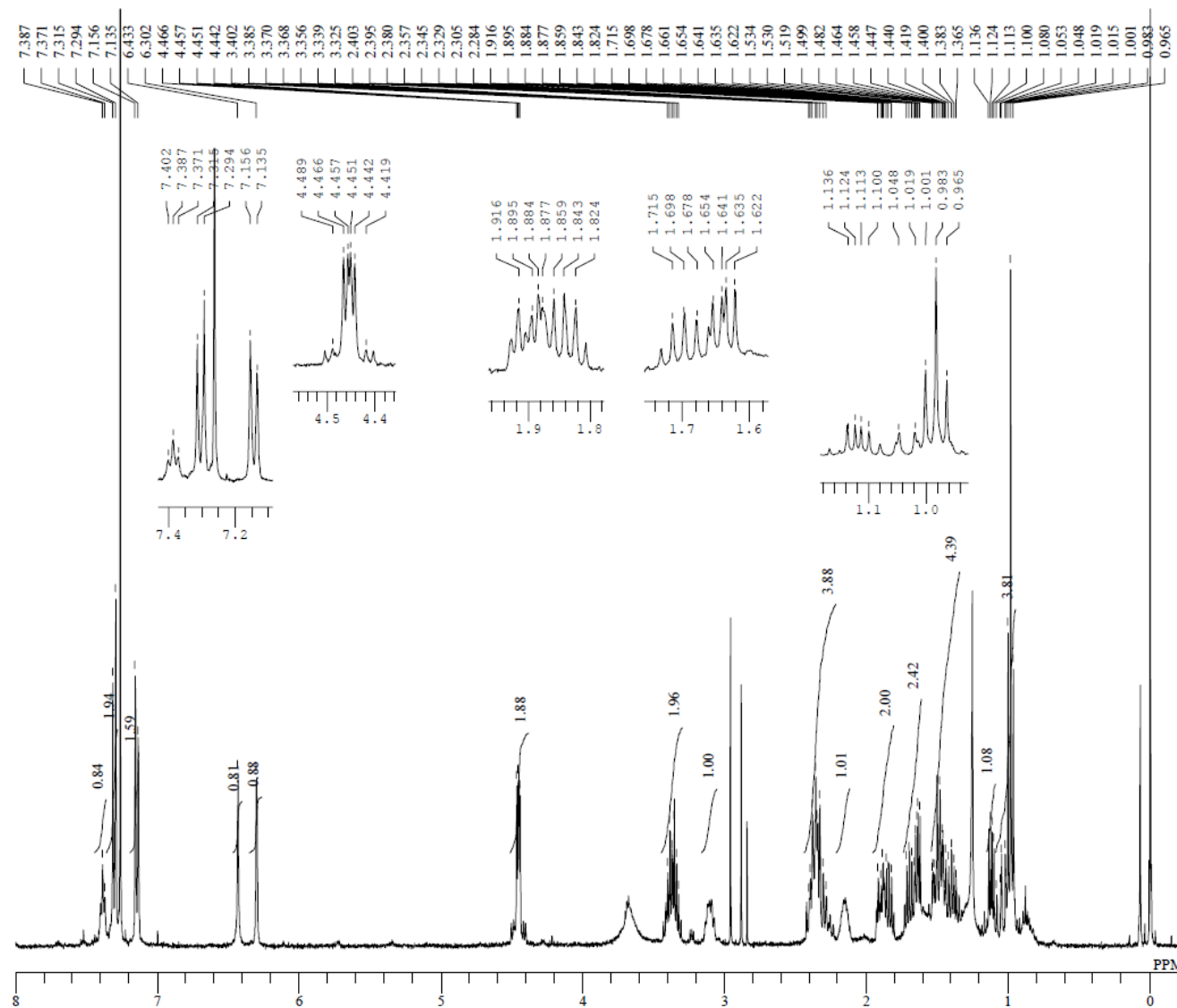
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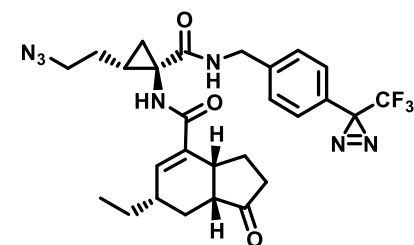
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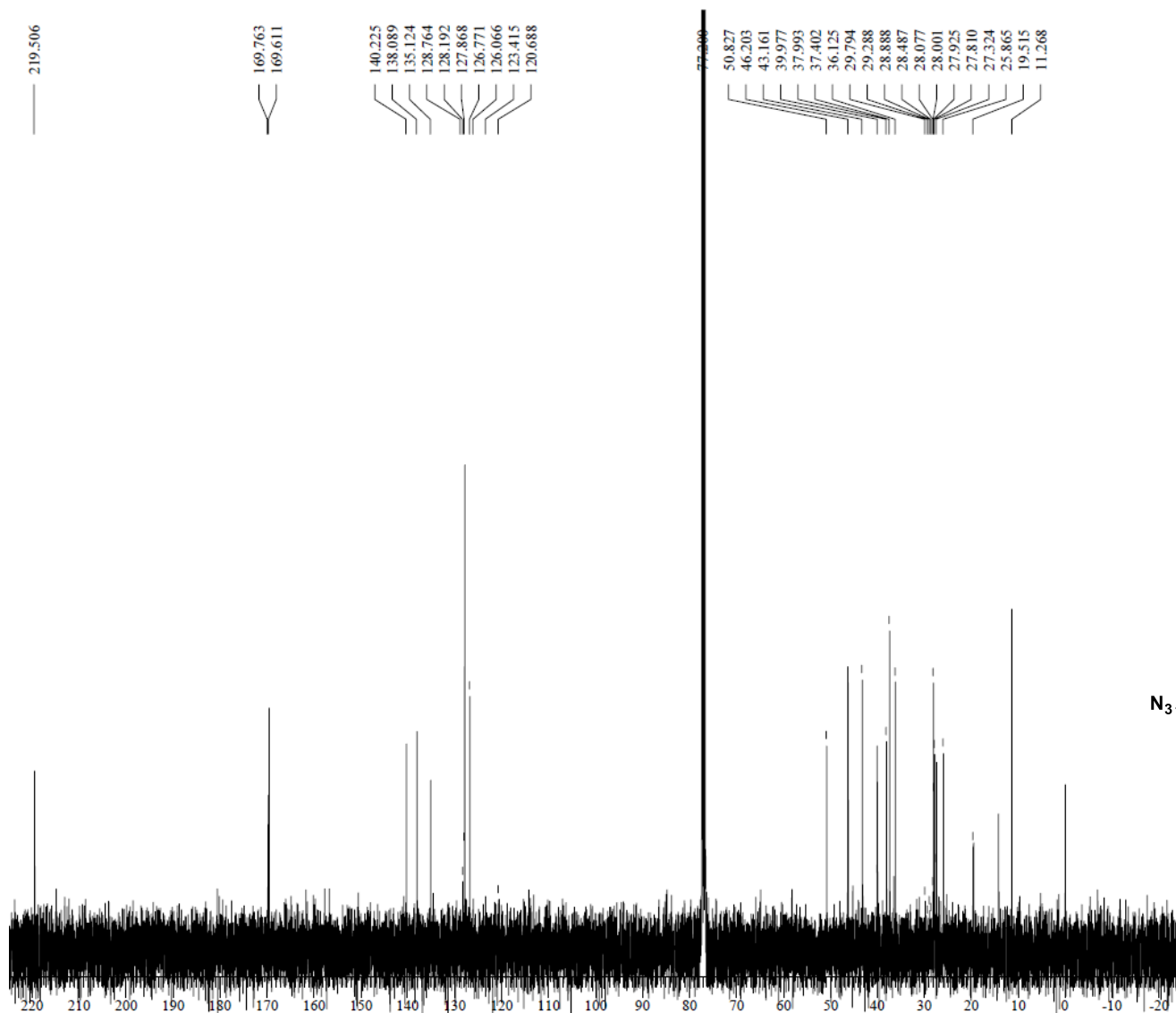
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 OBFIN 7.29 Hz  
 POINT 16384  
 FREQU 7503.00 Hz  
 SCANS 8  
 ACQTM 2.1837 sec  
 PD 5.0000 sec  
 PW1 2.95 usec  
 IRNUC 1H  
 CTEMP 21.6 c  
 SLVNT CDCL3  
 EXREF 0.00 ppm  
 BF 0.12 Hz  
 RGAIN 70



**ent5**



DFILE ent5.als  
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 DATIM 16-12-2017 14:32:10  
 OBNUC <sup>13</sup>C  
 EXMOD carbon.jxp  
 OBFRQ 100.53 MHz  
 OBSET 5.35 KHz  
 OBFIN 5.86 Hz  
 POINT 26214  
 FREQU 25125.63 Hz  
 SCANS 5007  
 ACQTM 1.0433 sec  
 PD 2.0000 sec  
 PW1 3.37 usec  
 IRNUC <sup>1</sup>H  
 CTEMP 21.9 c  
 SLVNT CDCL<sub>3</sub>  
 EXREF 77.00 ppm  
 BF 0.20 Hz  
 RGAIN 50

