

# Structure optimization of gatastatin for the development of $\gamma$ -tubulin-specific inhibitor

Kana Shintani, Haruna Ebisu, Minagi Mukaiyama, Taisei Hatanaka, Takumi Chinen, Daisuke Takao, Yoko Nagumo, Akira Sakakura, Ichiro Hayakawa, and Takeo Usui

## Contents of SI

### Biological assays

Calculation of IC <sub>50</sub> value.....	S2
In vitro microtubule polymerization assay.....	S2
Immunofluorescence microscopy.....	S2
Nucleation assay.....	S2
GTP binding assay.....	S3

### Supporting Data

Supporting Figure 1.....	S4
Supporting Figure 2.....	S4
Supporting Figure 3.....	S5
Supporting Figure 4.....	S5

### Chemistry

General method.....	S6
Synthesis of gatastatin ( <b>1</b> ) and B-ring modified gatastatin derivatives ( <b>3–5</b> ).....	S7
Synthesis of <i>O</i> <sup>6</sup> -modified gatastatin derivatives ( <b>6–10</b> ) and <i>O</i> <sup>6</sup> -modified 3',4'-acetonide gatastatin derivatives ( <b>11–15</b> ) .....	S10
<sup>1</sup> H and <sup>13</sup> C NMR spectra of synthesized compounds.....	S17

### Calculation of IC<sub>50</sub> value

HeLa cells were grown in DMEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere containing 5 % CO<sub>2</sub>. Various concentrations of each compound were then added and incubated for 48 h. Cell growth was determined using WST-8 counting kit (Dojindo). IC<sub>50</sub> value was expressed as mean ± SD from three independent experiments.

### In vitro microtubule (MT) polymerization assay

Tubulin was prepared from porcine brain using high-molarity buffer (*Protein Expr. Purif.*, **2003**, 32, 83). Tubulin was dissolved in MES buffer (100 mM morpholine ethanesulfonic acid, 0.5 mM MgCl<sub>2</sub>, and 1 mM ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid, pH 6.8), and stored at –80 °C. For polymerization assay, porcine brain tubulin (1 mg/ml) was incubated with 1 mM GTP and 30 µM gatastatin derivatives in the presence of 0.8 M or 0.2 M glutamate for 30 min at 37°C. In control condition, 0.8 M glutamate stimulates MT polymerization but not 0.2 M glutamate. After 30 min incubation, polymerized and unpolymerized tubulin were separated by ultracentrifugation (200,000 × g, 10 min, 25°C). After electrophoresis on 10% SDS-PAGE, amount of tubulin was quantified by Image J. Values are mean ± SD from three independent experiments.

### Immunofluorescence microscopy

HeLa cells were seeded in 8 well chamber (IWAKI, 5232-008) at the density of 6.0 × 10<sup>3</sup> cells/200 µl/well and treated with each compound (30 µM) for 24 h. Cells were fixed with MeOH at –20°C for 5 min. After washing with PBS for 10 min, fixed-cells were incubated with anti α-tubulin antibody (1:1000 dilution DM1A, Abcam, ab32293) and pericentrin (1:1000 dilution Abcam, ab4448) antibodies at 37°C for 1 h. After washing with PBS once, the cell were incubated with Alexa<sup>488</sup>-conjugated anti-mouse IgG, Alexa<sup>568</sup>-conjugated anti-rabbit IgG respectively (1:2000 dilution) and 1 µg/ml Hoechst 33258 at 37°C for 40 min. After washing the cells with PBS four times, cover glasses were mounted with Dako fluorescent medium(Dako North America, S3023). About 300 mitotic cells/each sample were observed under a fluorescent microscope (Leica LAS AF 6000, Leica Microsystems, Wetzlar, Germany) and the morphology of cells were classified.

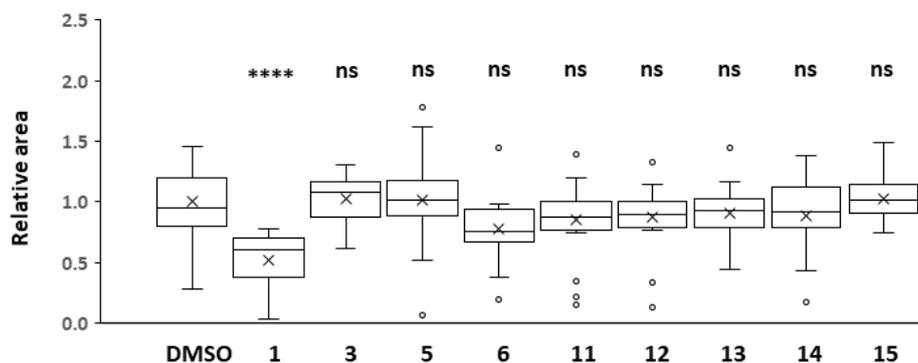
### Nucleation Assay

HeLa cells were seeded in 8 well chamber (IWAKI, 5232-008) at the density of 6.0 × 10<sup>3</sup> cells/200 µl/well. After 1 day, the cells were treated with 20 µM STLC for 6 h. Then, the cells were washed by cold (4°C) medium and incubated on ice to depolymerize MT. After 1 h, the cells were treated with 1 % DMSO, 30 µM gatastatin, 10 µM colchicine or gatastatin derivatives for 15 min on ice. During the process of drug treatment on ice, control cells were fixed with MeOH (DMSO 0 min). After changing the medium to warm (30°C) medium containing drug or PBS (DMSO 0 min sample), cells were incubated at 30°C for 3 min.

**GTP binding Assay**

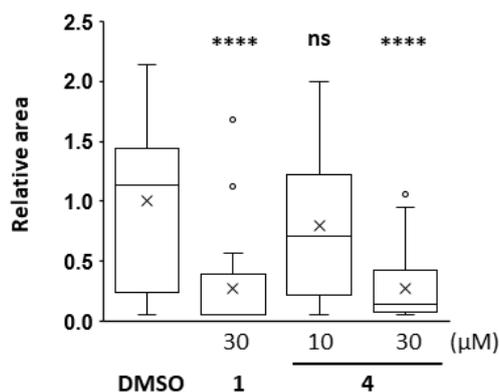
For analysis of the effect of gatastatin and derivative **9** on GTP binding of  $\gamma$ -tubulin, 70 nM of purified human  $\gamma$ -tubulin in GTP binding buffer (50 mM MES, 5 mM MgCl<sub>2</sub>, 1 mM EGTA, pH 6.6) was incubated with or without compounds for 15 min at 20°C. A final concentration of 100 nM of  $\alpha$ -[<sup>32</sup>P]-GTP was added and incubated at 20°C for 30 min. After incubation, bound  $\alpha$ -[<sup>32</sup>P]-GTP was cross-linked to  $\gamma$ -tubulin for 60 sec by illumination with a UV lamp using CL-1000 Ultraviolet Crosslinker. Protein with the cross-linked GTP was separated from unbound nucleotide by TCA precipitation and SDS-PAGE, and <sup>32</sup>P labeling was measured using a Typhoon FLA-9000 (General Electronic Company). Band intensities were quantified with ImageJ software.

## Supporting Information



### Supporting Figure 1. Effects of gatastatin derivatives 3, 5, 6, and 11-15 on MT nucleation.

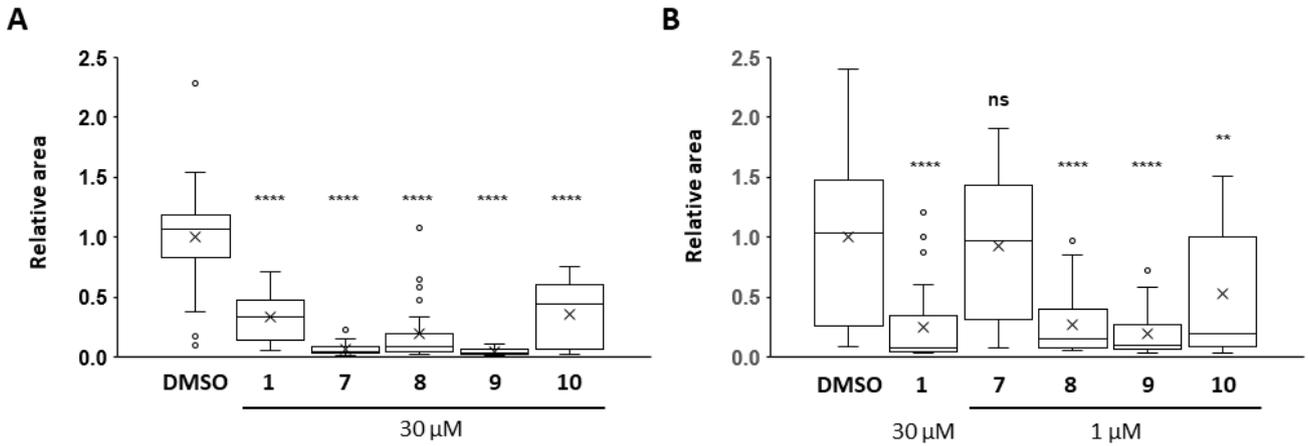
MT area nucleated from centrosomes ( $n = 20\sim 25$ ) were measured. Representative data of two independent experiments is shown. Gatastatin derivatives 3, 5, 6, and 11-15 showed no inhibitory activity against MT nucleation at 30  $\mu\text{M}$ . We used GraphPad Prism 6 to perform ANOVA. \*\*\*\*,  $p < 0.0001$ ; ns, no significance.



### Supporting Figure 2. Effects of gatastatin derivatives 4 on MT nucleation.

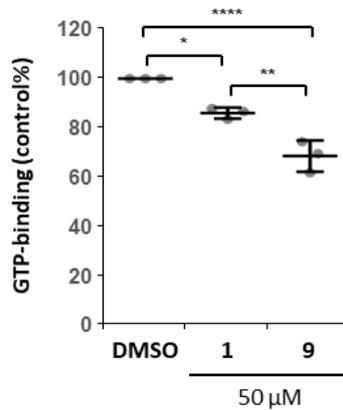
MT area nucleated from centrosomes ( $n = 20\sim 25$ ) were measured. Representative data of two independent experiments is shown. Gatastatin derivatives 4 inhibited MT nucleation at 30, but not 10  $\mu\text{M}$ . We used GraphPad Prism 6 to perform ANOVA. \*\*\*\*,  $p < 0.0001$ ; ns, no significance.

Supporting Information



**Supporting Figure 3. Effects of gatastatin derivatives 7-10 on MT nucleation.**

MT area nucleated from centrosomes ( $n = 20\sim 22$ ) were measured. Representative data of two independent experiments is shown. **A.** Gatastatin derivatives **7-10** inhibited MT nucleation at  $30\ \mu\text{M}$ . **B.** Gatastatin derivatives **8** and **9** inhibited MT nucleation even at  $1\ \mu\text{M}$ . We used GraphPad Prizm 6 to perform ANOVA. \*\*\*\*,  $p < 0.0001$ ; \*\*,  $p < 0.01$ ; ns, no significance.



**Supporting Figure 4. Effects of gatastatin derivative 9 on GTP-binding of  $\gamma$ -tubulin.**

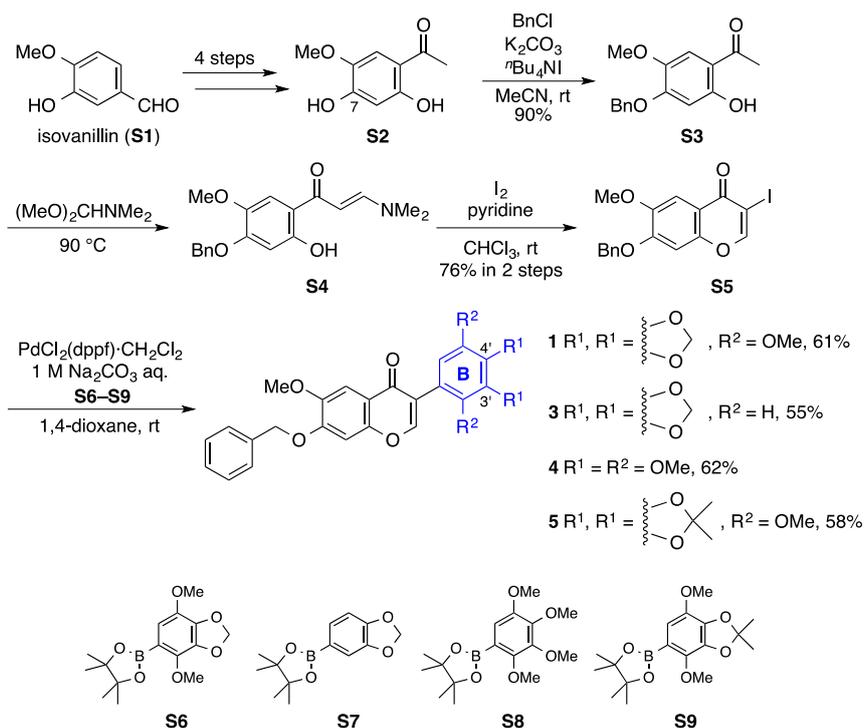
The effect of derivative **9** on GTP binding of  $\gamma$ -tubulin were measured. Three independent experiments ( $n = 3$ ) is shown. Gatastatin derivative **9** inhibited GTP binding of  $\gamma$ -tubulin at  $50\ \mu\text{M}$ . We used GraphPad Prizm 7 to perform ANOVA. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*\*,  $p < 0.001$ .

**Chemistry****General method**

All chemicals were used as obtained from commercial supplies unless otherwise noted. Anhydrous acetone,  $\text{CHCl}_3$ , MeCN, 1,4-dioxane,  $\text{CH}_2\text{Cl}_2$ , MeOH were purchased from Wako Pure Chemical Industries Ltd or KANTO Chemical Co., Inc, and used without further drying. TLC analyses were conducted on E. Merck precoated silica gel 60 F<sub>254</sub> (0.25 mm layer thickness). Kanto silica gel 60 N or E. Merck silica gel 60 were used for column chromatography. Melting point was measured on a J-SCIENCE RFS-10 micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a HORIBA FT-720 instrument and only selected peaks are reported in wavenumbers ( $\text{cm}^{-1}$ ).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Varian 400-MR spectrometer. The  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts were referenced to the solvent peaks,  $\delta_{\text{H}}$  7.26 (residual  $\text{CHCl}_3$ ) and  $\delta_{\text{C}}$  77.0 ppm ( $\text{CDCl}_3$ ). *J* values are given in Hz. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. High resolution ESI (electrospray ionization)/TOF (time-of-flight) mass spectra were recorded on an Agilent Technologies G6520 spectrometer.

**Synthesis of gatastatin (1) and B-ring modified gatastatin derivatives (3–5)**

Preparation of gatastatin (**1**) and B-ring modified gatastatin derivatives (**3–5**) is shown in Scheme S1. Isovanillin (**S1**) was converted to acetophenone **S2** using our previously reported procedure.<sup>S1</sup> Benzoylation at the *O*<sup>7</sup>-hydroxy group in **S2** and condensation of the resulting **S3** with *N,N*-dimethylformamide produced enamine **S4**, which was transformed into iodochromone **S5**. Suzuki–Miyaura coupling<sup>S2</sup> between iodochromone **S5** and the pinacol boronates **S6–S9**<sup>1</sup> produced gatastatin (**1**) and B-ring modified gatastatin derivatives (**3–5**).



**Scheme S1.** Synthesis of gatastatin (**1**) and B-ring modified gatastatin derivatives (**3–5**)

**1-(4-(Benzyloxy)-2-hydroxy-5-methoxyphenyl)ethan-1-one (S3).** To a stirred solution of the acetophenone **S2** (12.3 g, 67.3 mmol) in MeCN (300 mL) were added  $\text{K}_2\text{CO}_3$  (18.6 g, 135 mmol), BnCl (7.80 mL, 67.8 mmol), and  $n\text{Bu}_4\text{NI}$  (30.0 g, 81.2 mmol) at room temperature. After being stirred at room temperature for 22 h, the reaction mixture was diluted with saturated aqueous  $\text{NaHCO}_3$  (200 mL) and extracted with EtOAc ( $3 \times 400$  mL). The combined extracts were washed with brine (600 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (300 g, hexane–EtOAc 15 : 1  $\rightarrow$  6 : 1  $\rightarrow$  5 : 1  $\rightarrow$  3 : 1) to give benzyl ether **S3** (16.4 g, 90%) as a white solid: mp 124–126 °C; IR (neat) 3020, 1631, 1506, 1265, 1215, 1063  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  12.60 (s, 1H), 7.49–7.28 (m, 5H), 7.09 (s, 1H), 6.48 (s, 1H), 5.17 (s, 2H), 3.86 (s, 3H), 2.55 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  202.1, 159.8, 155.9, 142.1, 135.6, 128.7 (2C), 128.2, 127.3 (2C), 112.4, 111.9, 101.9, 70.7, 56.9, 26.3; HRMS (ESI)  $m/z$  273.1125, calcd for  $\text{C}_{16}\text{H}_{17}\text{O}_4$   $[\text{M}+\text{H}]^+$  273.1127.

## Supporting Information

**7-(Benzyloxy)-3-iodo-6-methoxy-4H-chromen-4-one (S5).** Benzyl ether **S3** (3.46 g, 12.7 mmol) was dissolved in *N,N*-dimethylformamide dimethyl acetal (15.0 mL, 0.11 mmol) and stirred at 90 °C for 5 h. The reaction mixture was concentrated *in vacuo* to give crude enamine **S4** (4.53 g), which was used for the next reaction without further purification.

To a stirred solution of crude enamine **S4** (4.53 g) in CHCl<sub>3</sub> (90 mL) were added pyridine (5.60 mL, 69.5 mmol) and I<sub>2</sub> (3.49 g, 27.7 mmol) at 0 °C. After being stirred in dark at room temperature for 20 h, the mixture was diluted with saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (100 mL) at room temperature and extracted with CHCl<sub>3</sub> (3 × 110 mL). The combined extracts were washed with brine (200 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (60 g, hexane–EtOAc 20 : 1 → 10 : 1 → 8 : 1 → 4 : 1) to give iodochromone **S5** (4.32 g, 76% in two steps) as a yellow solid: mp 191–193 °C; IR (neat) 1618, 1506, 1431, 1288, 1215, 1063 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.18 (s, 1H), 7.54 (s, 1H), 7.46–7.34 (m, 5H), 6.86 (s, 1H), 5.23 (s, 2H), 3.96 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.4, 156.8, 153.7, 152.0, 148.5, 135.3, 128.8 (2C), 128.4, 127.3 (2C), 115.3, 105.2, 101.0, 86.5, 71.2, 56.4; HRMS (ESI) *m/z* 408.9929, calcd for C<sub>17</sub>H<sub>14</sub>IO<sub>4</sub> [M+H]<sup>+</sup> 408.9937.

### Typical procedure for the preparation of B-ring modified gatastatin derivatives

**3-(Benzo[d][1,3]dioxol-5-yl)-7-(benzyloxy)-6-methoxy-4H-chromen-4-one (3).** All solvents were degassed by freeze–thawing. To a stirred solution of pinacol boronate **S7** (61.2 mg, 247 μmol) in 1,4-dioxane (600 μL) was added PdCl<sub>2</sub>(dppf)·CH<sub>2</sub>Cl<sub>2</sub> (18.2 mg, 22.3 μmol) in a glove box, and were added solution of iodochromone **S5** (81.6 mg, 200 μmol) in 1,4-dioxane (1.35 mL) and aqueous 1 M Na<sub>2</sub>CO<sub>3</sub> (800 μL, 800 μmol) at room temperature. The mixture was stirred at room temperature under a stream of Ar for 15 h and diluted with H<sub>2</sub>O (6.0 mL) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at room temperature. The layers were separated, and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 10 mL). The organic layer and extracts were combined, washed with brine (20 mL × 2), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered with Florisil (2.0 g), and concentrated. The crude product was purified by column chromatography on silica gel (60 g, hexane–EtOAc 8 : 1 → 6 : 1 → 4 : 1) to give a yellow solid (containing Pd-metal; 46.7 mg). The resultant yellow solid was dissolved in CHCl<sub>3</sub> (3 mL), and SiliaBond (SILICYCLE, SiliaMetS<sup>®</sup> Thiourea, 250 mg) was added. The resulting mixture was stirred at room temperature for 1 h, filtered, and concentrated to give **3** (44.5 mg, 55%) as a white solid: mp 148–150 °C; IR (neat) 3020, 1635, 1604, 1506, 1433, 1272, 1214, 1039 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.88 (s, 1H), 7.63 (s, 1H), 7.48–7.34 (m, 5H), 7.09 (d, *J* = 1.6 Hz, 1H), 6.96 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.89 (s, 1H), 6.86 (s, *J* = 8.0 Hz, 1H), 5.98 (s, 2H), 5.25 (s, 2H), 3.99 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 175.3, 153.3, 152.0 (2C), 148.1, 147.6, 147.5, 135.5, 128.8 (2C), 128.3, 127.3 (2C), 125.9, 124.4, 122.3, 118.0, 109.7, 108.3, 105.1, 101.2, 101.1, 71.1, 56.4; HRMS (ESI) *m/z* 403.1176, calcd for C<sub>24</sub>H<sub>19</sub>O<sub>6</sub> [M+H]<sup>+</sup> 403.1182.

**7-(Benzyloxy)-6-methoxy-3-(2,3,4,5-tetramethoxyphenyl)-4H-chromen-4-one (4).** White solid; mp 148–150 °C; IR (neat) 3020, 1635, 1604, 1506, 1466, 1433, 1269, 1215, 1115 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.96 (s, 1H), 7.64 (s, 1H), 7.49–7.35 (m, 5H), 6.92 (s, 1H), 6.70 (s, 1H), 5.27 (s, 2H), 3.99

### Supporting Information

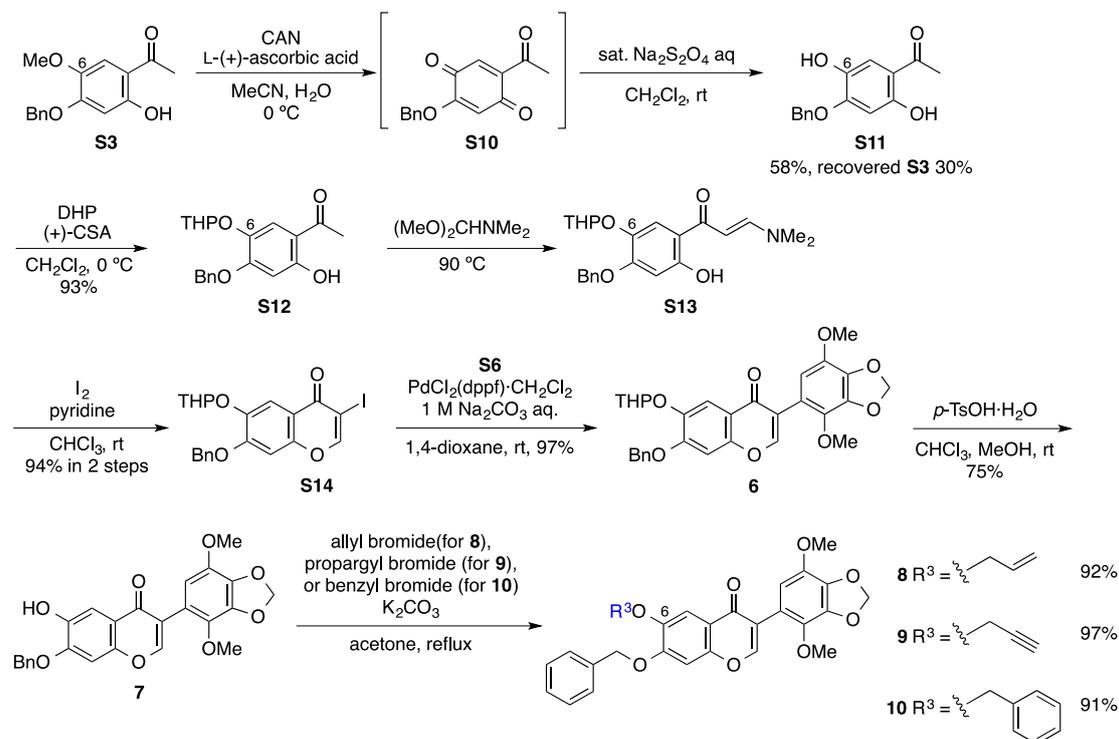
(s, 3H), 3.96 (s, 3H), 3.93 (s, 3H), 3.84 (s, 3H), 3.71 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.5, 153.9, 153.3, 152.0, 149.2, 148.1, 147.2, 145.7, 143.0, 135.5, 128.8 (2C), 128.3, 127.3 (2C), 121.1, 120.2, 118.1, 109.2, 105.1, 101.3, 71.1, 61.19, 61.15 (2C), 56.4, 56.1; HRMS (ESI)  $m/z$  479.1710, calcd for  $\text{C}_{27}\text{H}_{27}\text{O}_8$   $[\text{M}+\text{H}]^+$  479.1706.

**7-(Benzyloxy)-3-(4,7-dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-6-methoxy-4H-chromen-4-one (5).** White solid; mp 164–167 °C; IR (neat) 3020, 1635, 1606, 1506, 1466, 1431, 1267, 1215, 1141  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.88 (s, 1H), 7.63 (s, 1H), 7.47–7.34 (m, 5H), 6.89 (s, 1H), 6.48 (s, 1H), 5.26 (s, 2H), 3.98 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 1.75 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.5, 153.5, 153.2, 152.0, 148.0, 139.1, 139.0, 136.7, 136.6, 135.5, 128.8 (2C), 128.3, 127.2 (2C), 121.6, 119.6, 118.0, 117.2, 108.6, 105.1, 101.3, 71.1, 60.2, 56.5, 56.4, 25.9 (2C); HRMS (ESI)  $m/z$  491.1709, calcd for  $\text{C}_{28}\text{H}_{27}\text{O}_8$   $[\text{M}+\text{H}]^+$  491.1706.

**Gatastatin (1).** White solid; mp 152–155 °C; IR (neat) 3018, 1647, 1508, 1456, 1296, 1217, 1051  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.86 (s, 1H), 7.62 (s, 1H), 7.48–7.34 (m, 5H), 6.89 (s, 1H), 6.51 (s, 1H), 6.01 (s, 2H), 5.26 (s, 2H), 3.98 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.4, 153.4, 153.2, 152.0, 148.0, 139.1, 138.9, 137.0, 136.7, 135.5, 128.8 (2C), 128.3, 127.2 (2C), 121.6, 118.0, 117.9, 110.0, 105.1, 101.8, 101.3, 71.1, 60.1, 56.8, 56.3; HRMS (ESI)  $m/z$  463.1388, calcd for  $\text{C}_{26}\text{H}_{23}\text{O}_8$   $[\text{M}+\text{H}]^+$  463.1393.

### Synthesis of *O*<sup>6</sup>-modified gatastatin derivatives (6–10) and *O*<sup>6</sup>-modified 3',4'-acetonide gatastatin derivatives (11–15)

We prepared *O*<sup>6</sup>-modified gatastatin derivatives (6–10) from acetophenone **S3** (Scheme S2). Oxidative cleavage of the methyl group at the *O*<sup>6</sup>-position of acetophenone **S3** produced hydroquinone **S11**. In this reaction, **S3** is treated with CAN in the presence of L-(+)-ascorbic acid as a radical scavenger to generate benzoquinone **S10**, which is reduced using sodium thiosulfate to provide hydroquinone **S11**. Selective introduction of a THP group at the *O*<sup>6</sup>-position in **S11** produced acetophenone **S12**. Condensation of **S12** with *N,N*-dimethylformamide acetal and subsequent iodination of enamine **S13** generated iodochromone **S14**. Joining of iodochromone **S14** and pinacol boronate **S6** using Suzuki–Miyaura coupling<sup>S2</sup> gave **6**, which was transformed into *O*<sup>6</sup>-hydroxy gatastatin (**7**). Alkylation of the *O*<sup>6</sup>-hydroxy group of **7** with allyl bromide, propargyl bromide, and benzyl bromide produced *O*<sup>6</sup>-alkylated gatastatin derivatives (**8–10**), respectively. Also, *O*<sup>6</sup>-modified 3',4'-acetonide gatastatin derivatives (**11–15**) were prepared from **S14** in the same manner using pinacol boronate **S9** (Scheme S3).



**Scheme S2.** Synthesis of *O*<sup>6</sup>-modified gatastatin derivatives (6–10)

**1-(4-(Benzyloxy)-2,5-dihydroxyphenyl)ethan-1-one (S11).** To a stirred solution of acetophenone **S3** (204 mg, 749 μmol) and L-(+)-ascorbic acid (158 mg, 899 μmol) in MeCN (6.0 mL) was added CAN (1 M solution in H<sub>2</sub>O, 1.50 mL, 1.50 mmol) at 0 °C. After being stirred at 0 °C for 1 h, the reaction mixture were added L-(+)-ascorbic acid (199 mg, 1.13 mmol), CHCl<sub>3</sub> (25 mL), and H<sub>2</sub>O (25 mL) at 0 °C. The layers were separated, and the aqueous layer was extracted with CHCl<sub>3</sub> (2 × 30 mL). The organic layer and extracts were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The resultant mixture was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and added with saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> (5 mL) at room temperature.

## Supporting Information

After being stirred at room temperature for 1 h, the resultant mixture was separated, and the aqueous layer was extracted with CHCl<sub>3</sub> (2 × 6 mL). The organic layer and extracts were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (8.0 g, hexane–acetone 30 : 1 → 10 : 1 → 5 : 1) to give hydroquinone **S11** (118 mg, 58%) as a yellow solid and acetophenone **S3** (57.3 mg, 30%): mp 159–161 °C; IR (neat) 3566, 3020, 2974, 1699, 1635, 1506, 1458, 1219 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 12.46 (s, 1H), 7.43–7.40 (m, 5H), 7.24 (s, 1H), 6.53 (s, 1H), 5.14 (s, 2H), 2.54 (s, 3H). A signal due to one proton (OH) was not observed; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 202.7, 158.6, 152.7, 138.0, 134.9, 128.9 (2C), 128.8, 128.0 (2C), 114.1, 112.6, 100.8, 71.2, 26.5; HRMS (ESI) *m/z* [M+H]<sup>+</sup> 259.0967, calcd for C<sub>15</sub>H<sub>15</sub>O<sub>4</sub> [M+H]<sup>+</sup> 259.0970.

**1-(4-(Benzyloxy)-2-hydroxy-5-((tetrahydro-2H-pyran-2-yl)oxy)phenyl)ethan-1-one (S12).** To a stirred solution of hydroquinone **S11** (1.40 g, 5.43 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (85.0 mL) were added DHP (2.00 mL, 22.1 mmol) and (+)-CSA (64.0 mg, 273 μmol) at 0 °C. After being stirred at 0 °C for 2 h, the reaction mixture was added Et<sub>3</sub>N (150 μL, 1.08 mmol) and stirred at 0 °C for 10 min. The resultant mixture was diluted with saturated aqueous NaHCO<sub>3</sub> (100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 120 mL). The organic layer and extracts were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (33 g, hexane–EtOAc 30 : 1 → 20 : 1 → 15 : 1 → 8 : 1) to give THP ether **S12** (1.74 g, 93%) as a white solid: mp 102–105 °C; IR (neat) 3566, 3020, 2976, 1635, 1508, 1373, 1329, 1254, 1217 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 12.60 (s, 1H), 7.43–7.32 (m, 6H), 6.50 (s, 1H), 5.26 (t, *J* = 3.2 Hz, 1H), 5.11 (s, 2H), 4.05 (m, 1H), 3.61 (m, 1H), 2.52 (s, 3H), 2.00–1.81 (m, 3H), 1.69–1.60 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 202.4, 160.7, 157.3, 138.6, 135.8, 128.5 (2C), 128.0, 127.0 (2C), 120.8, 112.3, 101.8, 99.0, 70.4, 62.2, 30.2, 26.2, 25.2, 18.7; HRMS (ESI) *m/z* 343.1546, calcd for C<sub>20</sub>H<sub>23</sub>O<sub>5</sub> [M+H]<sup>+</sup> 343.1546.

**7-(Benzyloxy)-3-iodo-6-((tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (S14).** THP ether **S12** (1.00 g, 2.93 mmol) was dissolved in *N,N*-dimethylformamide dimethyl acetal (10.0 mL, 75.3 mmol) and stirred at 90 °C for 5 h. The reaction mixture was concentrated *in vacuo* to give crude enamine **S13** (1.21 g), which was used for the next reaction without further purification.

To a stirred solution of crude enamine **S13** (205 mg) in CHCl<sub>3</sub> (12.0 mL) were added pyridine (400 μL, 4.97 mmol) and I<sub>2</sub> (261 mg, 2.07 mmol) at 0 °C. After being stirred in dark at room temperature for 23 h, the mixture was diluted with saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (15 mL) at room temperature and extracted with CHCl<sub>3</sub> (3 × 20 mL). The combined extracts were washed with brine (50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (10 g, hexane–EtOAc 12 : 1 → 10 : 1 → 8 : 1 → 4 : 1) to give iodochromone **S14** (223 mg, 94% in two steps) as a yellow solid: mp 166–170 °C; IR (neat) 3020, 2976, 1647, 1616, 1496, 1456, 1367, 1215 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.18 (s, 1H), 7.84 (s, 1H), 7.46–7.34 (m, 5H), 6.90 (s,

## Supporting Information

1H), 5.54 (t,  $J = 3.0$  Hz, 1H), 5.21 (s, 2H), 3.96 (m, 1H), 3.62 (m, 1H), 2.01–1.88 (m, 3H), 1.72–1.62 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.3, 156.8, 155.0, 152.8, 145.4, 135.6, 128.7 (2C), 128.2, 127.0 (2C), 115.5, 112.5, 101.4, 97.7, 86.6, 70.9, 62.2, 30.1, 25.1, 18.6; HRMS (ESI)  $m/z$  479.0351, calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_5$   $[\text{M}+\text{H}]^+$  479.0355.

**7-(Benzyloxy)-3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one (6).** All solvents were degassed by freeze–thawing. The mixture of pinacol boronate **S6** (100 mg, 325  $\mu\text{mol}$ ) and iodochromone **S14** (129 mg, 270  $\mu\text{mol}$ ) was added  $\text{PdCl}_2(\text{dppf})\cdot\text{CH}_2\text{Cl}_2$  (11.5 mg, 14.1  $\mu\text{mol}$ ) in a glove box, and were added 1,4-dioxane (6.0 mL) and aqueous 1 M  $\text{Na}_2\text{CO}_3$  (1.90 mL, 1.90 mmol) at room temperature. The mixture was stirred at room temperature under a stream of Ar for 15.5 h and diluted with  $\text{H}_2\text{O}$  (10.0 mL). The mixture was extracted with EtOAc (3  $\times$  12 mL). The combined extracts were washed with brine (30 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3.5 g, hexane–EtOAc 8 : 1  $\rightarrow$  6 : 1  $\rightarrow$  3 : 1) to give a yellow solid (containing Pd-metal; 142 mg). The resultant yellow solid was dissolved in EtOAc (10 mL), and SiliaBond (SILICYCLE, SiliaMetS<sup>®</sup> Thiourea, 714 mg) was added. The resulting mixture was stirred at room temperature for 1 h, filtered, and concentrated to give **6** (141 mg, 97%) as a white solid: mp 88–92  $^\circ\text{C}$ ; IR (neat) 3020, 2978, 1646, 1607, 1522, 1468, 1423, 1352, 1295  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.91 (s, 1H), 7.85 (s, 1H), 7.47–7.32 (m, 5H), 6.93 (s, 1H), 6.50 (s, 1H), 6.00 (s, 2H), 5.57 (t,  $J = 3.0$  Hz, 1H), 5.22 (s, 2H), 4.01 (m, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.62 (m, 1H), 2.05–1.88 (m, 3H), 1.71–1.58 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.3, 154.6, 153.4, 152.8, 144.8, 139.0, 138.9, 136.9, 136.7, 135.8, 128.6 (2C), 128.1, 126.9 (2C), 121.5, 118.1, 118.0, 112.7, 110.0, 101.7, 101.7, 97.7, 70.8, 62.1, 60.1, 56.8, 30.1, 25.1, 18.6; HRMS (ESI)  $m/z$  533.1806, calcd for  $\text{C}_{30}\text{H}_{29}\text{O}_9$   $[\text{M}+\text{H}]^+$  533.1812.

**7-(Benzyloxy)-3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-hydroxy-4*H*-chromen-4-one (7).** To a stirred solution of **6** (17.1 mg, 32.1  $\mu\text{mol}$ ) in  $\text{CHCl}_3$ –MeOH (2 : 1, 10.5 mL) was added *p*-TsOH $\cdot\text{H}_2\text{O}$  (1.20 mg, 6.31  $\mu\text{mol}$ ) at room temperature. After the mixture was stirred at room temperature for 1.5 h,  $\text{Et}_3\text{N}$  (20.0  $\mu\text{L}$ , 143  $\mu\text{mol}$ ) and  $\text{H}_2\text{O}$  (3 mL) were added. The layers were separated, and the aqueous layer was extracted with  $\text{CHCl}_3$  (2  $\times$  5 mL). The organic layer and extracts were combined, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (6.0 g, hexane–EtOAc 3 : 1  $\rightarrow$  1 : 1) to give **7** (10.8 mg, 75%) as a white solid: mp 224–226  $^\circ\text{C}$ ; IR (neat) 3608, 3020, 2976, 1647, 1558, 1541, 1219  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.88 (s, 1H), 7.74 (s, 1H), 7.46–7.45 (m, 5H), 6.96 (s, 1H), 6.52 (s, 1H), 6.02 (s, 2H), 5.23 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H). A signal due to one proton (OH) was not observed;  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.5, 153.6, 151.5, 150.8, 144.1, 139.1, 139.0, 137.0, 136.7, 134.8, 128.94 (2C), 128.90, 127.9 (2C), 121.5, 118.9, 118.0, 110.0, 109.0, 101.8, 100.3, 71.6, 60.2, 56.9; HRMS (ESI)  $m/z$  449.1233, calcd for  $\text{C}_{25}\text{H}_{21}\text{O}_8$   $[\text{M}+\text{H}]^+$  449.1236.

### Typical procedure for the preparation of *O*<sup>6</sup>-alkylated gatastatin derivatives

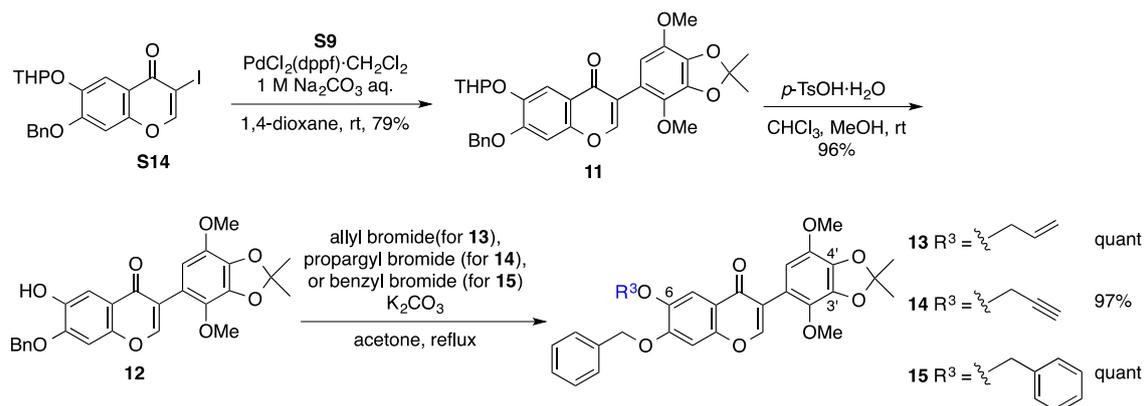
**6-(Allyloxy)-7-(benzyloxy)-3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-4*H*-chromen-4-one (8).** To a

### Supporting Information

stirred solution of **7** (15.6 mg, 34.8  $\mu\text{mol}$ ) in acetone (6.0 mL) were added  $\text{K}_2\text{CO}_3$  (9.80 mg, 70.9  $\mu\text{mol}$ ) and allyl bromide (4.40  $\mu\text{L}$ , 52.0  $\mu\text{mol}$ ) at room temperature. After being stirred at reflux for 8.5 h, the mixture was diluted with saturated aqueous  $\text{NaHCO}_3$  (10 mL) and extracted with  $\text{CHCl}_3$  ( $3 \times 12$  mL). The combined extracts were washed with brine (30 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (2.0 g, hexane–EtOAc 7 : 1  $\rightarrow$  5 : 1  $\rightarrow$  3 : 1) to give allyl ether **8** (15.7 mg, 92%) as a white solid: mp 146–148  $^\circ\text{C}$ ; IR (neat) 3020, 2933, 1635, 1608, 1506, 1456, 1268, 1097, 1038  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.86 (s, 1H), 7.64 (s, 1H), 7.48–7.34 (m, 5H), 6.91 (s, 1H), 6.51 (s, 1H), 6.11 (ddt,  $J = 17.2, 10.4, 5.2$  Hz, 1H), 6.01 (s, 2H), 5.48 (ddt,  $J = 17.2, 1.6, 1.6$  Hz, 1H), 5.32 (ddt,  $J = 10.4, 1.6, 1.6$  Hz, 1H), 5.26 (s, 2H), 4.71 (ddd,  $J = 5.2, 1.6, 1.6$  Hz, 2H), 3.86 (s, 3H), 3.84 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.4, 153.6, 153.4, 152.1, 146.9, 139.1, 138.9, 137.0, 136.7, 135.7, 132.6, 128.7 (2C), 128.2, 127.1 (2C), 121.6, 118.1, 118.0, 117.9, 110.0, 106.9, 101.8, 101.6, 71.0, 69.9, 60.2, 56.8; HRMS (ESI)  $m/z$  489.1546, calcd for  $\text{C}_{28}\text{H}_{25}\text{O}_8$   $[\text{M}+\text{H}]^+$  489.1549.

**7-(Benzyloxy)-3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-(prop-2-yn-1-yloxy)-4H-chromen-4-one (9)**. White solid; mp 174–176  $^\circ\text{C}$ ; IR (neat) 3307, 3020, 2976, 1635, 1608, 1506, 1456, 1419, 1295, 1261, 1215, 1097  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.87 (s, 1H), 7.78 (s, 1H), 7.48–7.33 (m, 5H), 6.92 (s, 1H), 6.51 (s, 1H), 6.02 (s, 2H), 5.26 (s, 2H), 4.87 (d,  $J = 2.2$  Hz, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 2.54 (t,  $J = 2.2$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.3, 153.7, 153.5, 152.6, 145.7, 139.1, 138.9, 137.0, 136.7, 135.5, 128.8 (2C), 128.3, 127.2 (2C), 121.7, 117.88, 117.86, 110.0, 108.1, 101.8, 101.7, 77.8, 76.3, 71.1, 60.2, 57.0, 56.8; HRMS (ESI)  $m/z$  487.1385, calcd for  $\text{C}_{28}\text{H}_{23}\text{O}_8$   $[\text{M}+\text{H}]^+$  487.1393.

**6,7-Bis(benzyloxy)-3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-4H-chromen-4-one (10)**. White solid; mp 154–158  $^\circ\text{C}$ ; IR (neat) 3020, 2976, 1608, 1456, 1296, 1245, 1153, 1037  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.87 (s, 1H), 7.73 (s, 1H), 7.51–7.32 (m, 10H), 6.92 (s, 1H), 6.51 (s, 1H), 6.01 (s, 2H), 5.27 (s, 2H), 5.25 (s, 2H), 3.86 (s, 3H), 3.84 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.4, 153.8, 153.5, 152.2, 147.1, 139.1, 138.9, 137.0, 136.7, 136.4, 135.8, 128.7 (2C), 128.5 (2C), 128.2, 128.0, 127.4 (2C), 127.0 (2C), 121.6, 117.99, 117.97, 110.0, 107.5, 101.8, 101.7, 71.04, 70.96, 60.2, 56.8; HRMS (ESI)  $m/z$  539.1709, calcd for  $\text{C}_{32}\text{H}_{27}\text{O}_8$   $[\text{M}+\text{H}]^+$  539.1706.

Synthesis of  $O^6$ -modified 3',4'-acetonide gatastatin derivatives (**11–15**)Scheme S3. Synthesis of  $O^6$ -modified 3',4'-acetonide gatastatin derivatives (**11–15**)

**7-(Benzyloxy)-3-(4,7-dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-6-((tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (**11**).** All solvents were degassed by freeze–thawing. The mixture of pinacol boronate **S9** (29.5 mg, 91.9  $\mu\text{mol}$ ) and iodochromone **S14** (36.6 mg, 76.5  $\mu\text{mol}$ ) was added  $\text{PdCl}_2(\text{dppf}) \cdot \text{CH}_2\text{Cl}_2$  (3.10 mg, 3.80  $\mu\text{mol}$ ) in a glove box, and were added 1,4-dioxane (2.0 mL) and aqueous 1 M  $\text{Na}_2\text{CO}_3$  (540  $\mu\text{L}$ , 540  $\mu\text{mol}$ ) at room temperature. The mixture was stirred at room temperature under a stream of Ar for 15 h and diluted with  $\text{H}_2\text{O}$  (5.0 mL). The mixture was extracted with EtOAc (3  $\times$  6 mL). The combined extracts were washed with brine (15 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (1.5 g, hexane–EtOAc 10 : 1  $\rightarrow$  6 : 1  $\rightarrow$  4 : 1) to give a yellow solid (containing Pd-metal; 34.2 mg). The resultant yellow solid was dissolved in EtOAc (3.0 mL), and SiliaBond (SILICYCLE, SiliaMetS<sup>®</sup> Thiourea, 102 mg) was added. The resulting mixture was stirred at room temperature for 1 h, filtered, and concentrated to give **11** (34.0 mg, 79%) as a white solid: mp 68–72  $^\circ\text{C}$ ; IR (neat) 3020, 2962, 1647, 1522, 1458, 1419, 1375, 1261  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.91 (s, 1H), 7.84 (s, 1H), 7.48–7.34 (m, 5H), 6.91 (s, 1H), 6.46 (s, 1H), 5.55 (t,  $J$  = 3.0 Hz, 1H), 5.20 (s, 2H), 4.01 (m, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.63 (m, 1H), 2.04–1.91 (m, 3H), 1.73 (s, 6H), 1.70–1.59 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.3, 154.5, 153.4, 152.7, 144.7, 139.0, 138.8, 136.6, 136.5, 135.7, 128.5 (2C), 128.0, 126.8 (2C), 121.5, 119.4, 118.1, 117.1, 112.6, 108.6, 101.6, 97.5, 70.7, 62.0, 60.0, 56.4, 30.0, 25.7 (2C), 25.0, 18.5; HRMS (ESI)  $m/z$  561.2126, calcd for  $\text{C}_{32}\text{H}_{33}\text{O}_9$   $[\text{M}+\text{H}]^+$  561.2125.

**7-(Benzyloxy)-3-(4,7-dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-6-hydroxy-4H-chromen-4-one (**12**).** To a stirred solution of **11** (160 mg, 285  $\mu\text{mol}$ ) in  $\text{CHCl}_3$ –MeOH (2 : 1, 18.0 mL) was added  $p\text{-TsOH} \cdot \text{H}_2\text{O}$  (9.20 mg, 48.4  $\mu\text{mol}$ ) at 0  $^\circ\text{C}$ . After the mixture was stirred at room temperature for 5 h,  $\text{Et}_3\text{N}$  (20.0  $\mu\text{L}$ , 143  $\mu\text{mol}$ ) and  $\text{H}_2\text{O}$  (10 mL) were added. The layers were separated, and the aqueous layer was extracted with  $\text{CHCl}_3$  (3  $\times$  12 mL). The organic layer and extracts were combined, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3.0 g, hexane–EtOAc 3 : 1  $\rightarrow$  1 : 1) to give **12** (123 mg, 96%) as a white solid: mp 200–203  $^\circ\text{C}$ ; IR (neat) 3608, 3020, 2960, 1635, 1508, 1457, 1271, 1217  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.88 (s, 1H), 7.75 (s, 1H), 7.45–7.40 (m, 5H), 6.95 (s, 1H), 6.48 (s, 1H), 5.83 (s, 1H), 5.23 (s, 2H), 3.85

## Supporting Information

(s, 3H), 3.80 (s, 3H), 1.75 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.6, 153.6, 151.5, 150.7, 144.1, 139.2, 139.0, 136.8, 136.6, 134.8, 128.94 (2C), 128.89, 127.9 (2C), 121.5, 119.6, 118.9, 117.2, 109.0, 108.7, 100.3, 71.6, 60.2, 56.6, 25.9 (2C); HRMS (ESI)  $m/z$  477.1551, calcd for  $\text{C}_{27}\text{H}_{25}\text{O}_8$   $[\text{M}+\text{H}]^+$  477.1549.

### Typical procedure for the preparation of $\text{O}^6$ -alkylated 3',4'-acetonide gatastatin derivatives

**6-(Allyloxy)-7-(benzyloxy)-3-(4,7-dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-4H-chromen-4-one (13).** To a stirred solution of **12** (15.2 mg, 31.9  $\mu\text{mol}$ ) in acetone (3.0 mL) were added  $\text{K}_2\text{CO}_3$  (8.80 mg, 63.7  $\mu\text{mol}$ ) and allyl bromide (4.10  $\mu\text{L}$ , 48.5  $\mu\text{mol}$ ) at room temperature. After being stirred at reflux for 10 h, the mixture was diluted with saturated aqueous  $\text{NaHCO}_3$  (5.0 mL) and extracted with  $\text{CHCl}_3$  (3  $\times$  6 mL). The combined extracts were washed with brine (15 mL), dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated. The crude product was purified by column chromatography on silica gel (2.0 g, hexane–EtOAc 7 : 1  $\rightarrow$  3 : 1  $\rightarrow$  1 : 1) to give allyl ether **13** (16.4 mg, quant) as a white solid: mp 125–129  $^\circ\text{C}$ ; IR (neat) 3018, 2937, 1639, 1606, 1502, 1454, 1267, 1141, 1054  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.88 (s, 1H), 7.64 (s, 1H), 7.48–7.34 (m, 5H), 6.90 (s, 1H), 6.47 (s, 1H), 6.11 (ddt,  $J$  = 17.2, 10.4, 5.2 Hz, 1H), 5.48 (ddt,  $J$  = 17.2, 1.6, 1.6 Hz, 1H), 5.32 (ddt,  $J$  = 10.4, 1.6, 1.6 Hz, 1H), 5.26 (s, 2H), 4.71 (ddd,  $J$  = 5.2, 1.6, 1.6 Hz, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 1.75 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.5, 153.59, 153.56, 152.1, 146.9, 139.1, 139.0, 136.8, 136.6, 135.7, 132.6, 128.7 (2C), 128.2, 127.1 (2C), 121.6, 119.6, 118.1, 118.0, 117.2, 108.7, 107.0, 101.6, 71.0, 69.9, 60.2, 56.6, 25.9 (2C); HRMS (ESI)  $m/z$  517.1868, calcd for  $\text{C}_{30}\text{H}_{29}\text{O}_8$   $[\text{M}+\text{H}]^+$  517.1862.

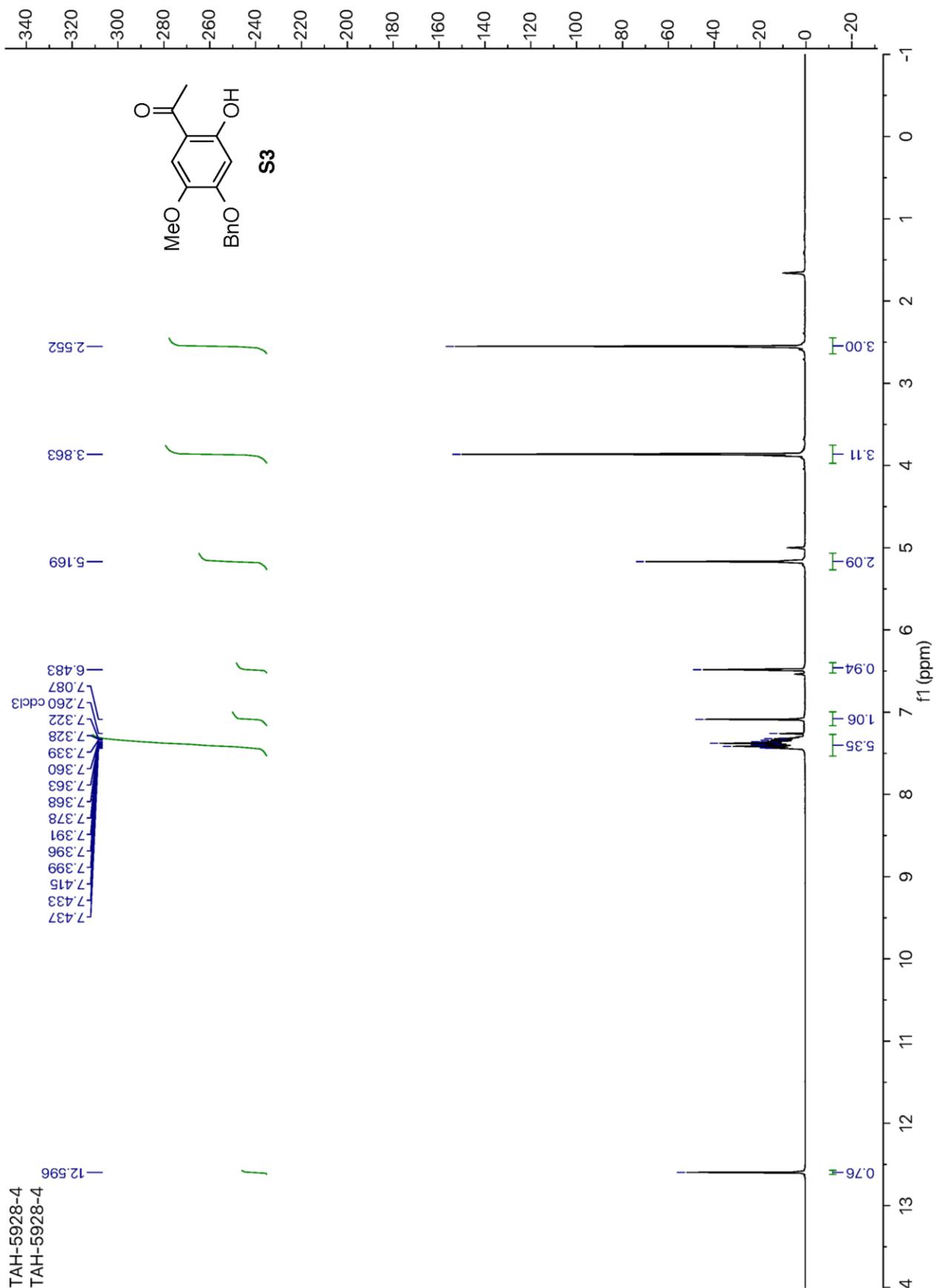
**7-(Benzyloxy)-3-(4,7-dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-6-(prop-2-yn-1-yloxy)-4H-chromen-4-one (14).** White solid; mp 143–147  $^\circ\text{C}$ ; IR (neat) 3307, 3018, 2933, 1641, 1606, 1502, 1460, 1377, 1269, 1214, 1089  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.88 (s, 1H), 7.79 (s, 1H), 7.50–7.32 (m, 5H), 6.92 (s, 1H), 6.48 (s, 1H), 5.26 (s, 2H), 4.87 (d,  $J$  = 2.4 Hz, 2H), 3.85 (s, 3H), 3.80 (s, 3H), 2.54 (t,  $J$  = 2.4 Hz, 1H), 1.75 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.4, 153.7, 153.6, 152.6, 145.7, 139.1, 139.0, 136.8, 136.6, 135.5, 128.8 (2C), 128.3, 127.2 (2C), 121.7, 119.6, 117.9, 117.1, 108.7, 108.2, 101.7, 77.8, 76.3, 71.1, 60.2, 57.0, 56.6, 25.9 (2C); HRMS (ESI)  $m/z$  515.1700, calcd for  $\text{C}_{30}\text{H}_{27}\text{O}_8$   $[\text{M}+\text{H}]^+$  515.1706.

**6,7-Bis(benzyloxy)-3-(4,7-dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-4H-chromen-4-one (15).** White solid; mp 64–69  $^\circ\text{C}$ ; IR (neat) 3020, 2979, 1602, 1516, 1456, 1419, 1215  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.88 (s, 1H), 7.74 (s, 1H), 7.51–7.32 (m, 10H), 6.92 (s, 1H), 6.47 (s, 1H), 5.27 (s, 2H), 5.25 (s, 2H), 3.85 (s, 3H), 3.80 (s, 3H), 1.75 (s, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  175.5, 153.8, 153.5, 152.2, 147.1, 139.1, 139.0, 136.8, 136.6, 136.5, 135.8, 128.7 (2C), 128.5 (2C), 128.2, 128.0, 127.4 (2C), 127.0 (2C), 121.6, 119.6, 118.0, 117.1, 108.6, 107.6, 101.7, 71.05, 70.95, 60.2, 56.6, 25.9 (2C); HRMS (ESI)  $m/z$  567.2015, calcd for  $\text{C}_{34}\text{H}_{31}\text{O}_8$   $[\text{M}+\text{H}]^+$  567.2019.

**References**

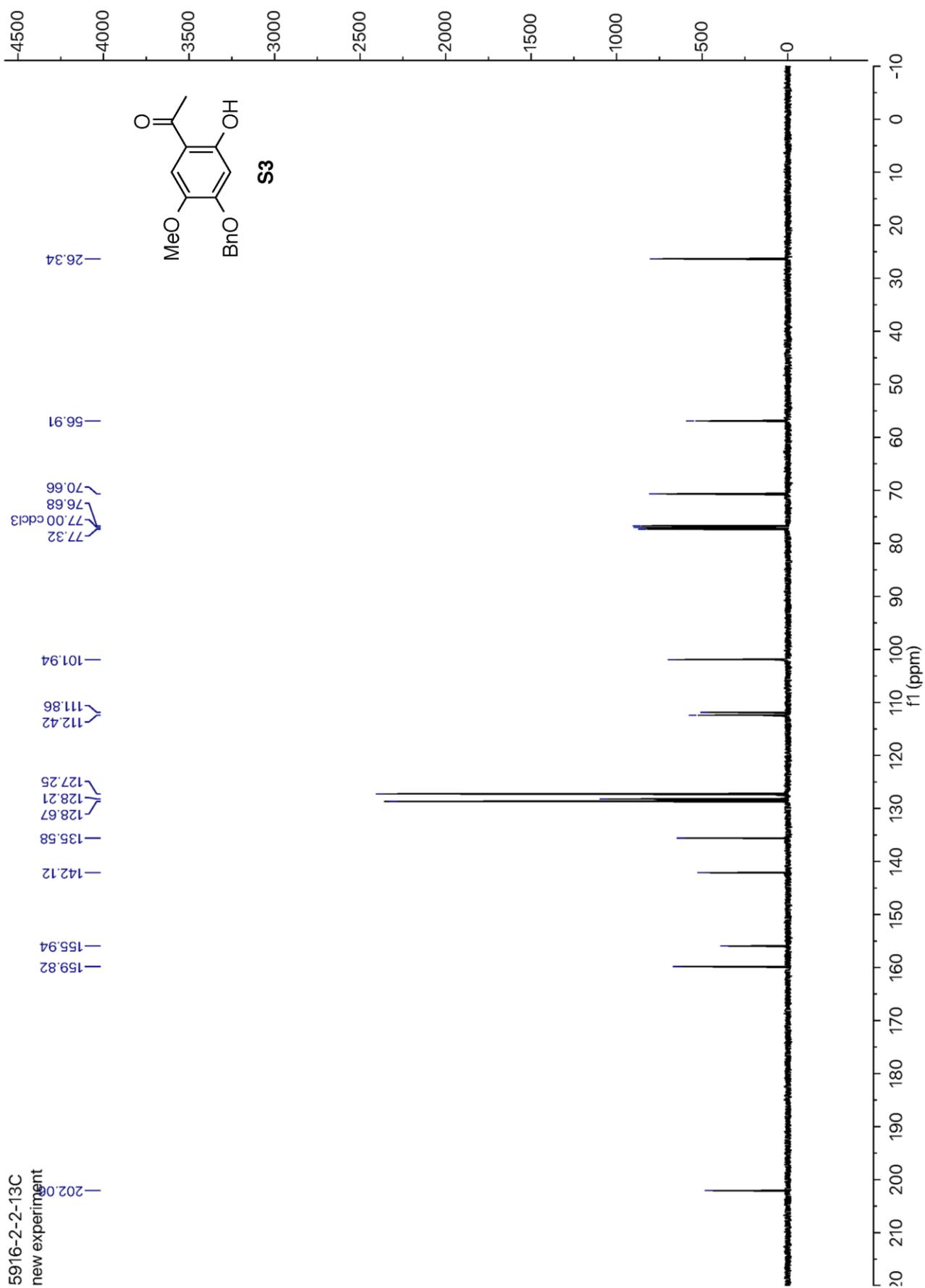
- S1) Hayakawa, I.; Shioda, S.; Ikedo, A.; Kigoshi, H. *Bull. Chem. Soc. Jpn.* **2014**, *87*, 544–549.  
S2) Hoshino, Y.; Miyaura, N.; Suzuki, A. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3008–3010.

Supporting Information



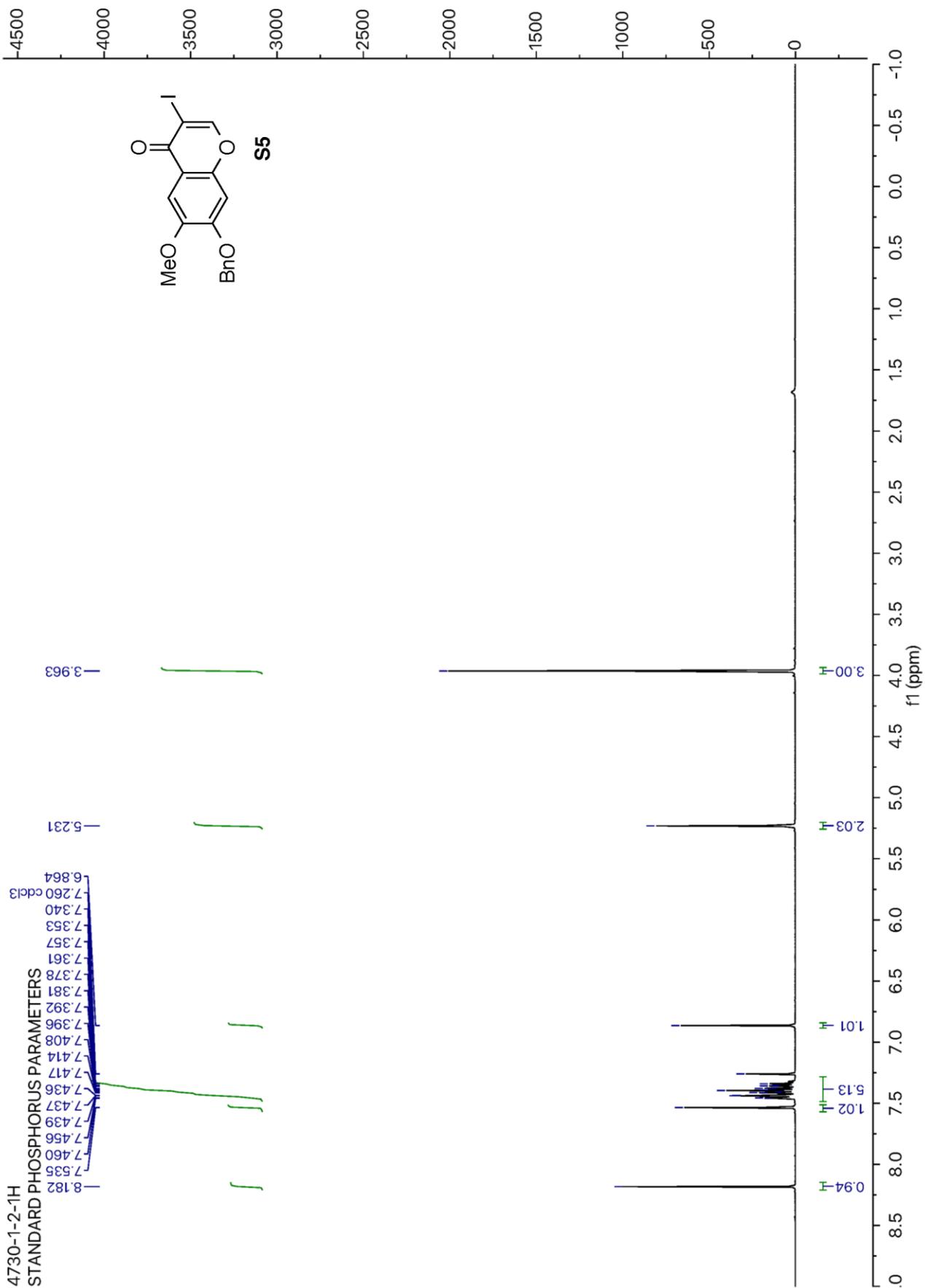
<sup>1</sup>H NMR spectra of **S3** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



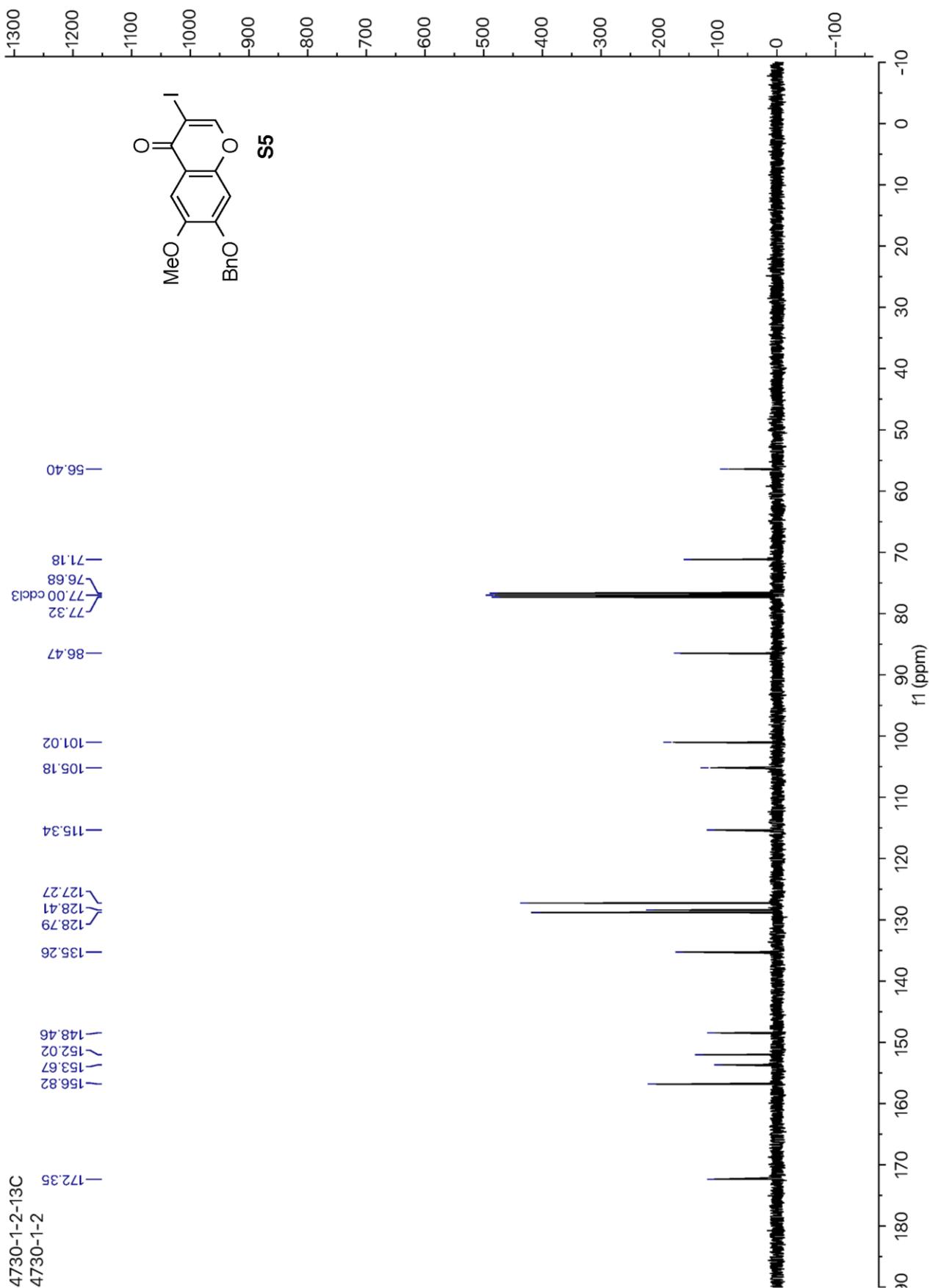
<sup>13</sup>C NMR spectra of **S3** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



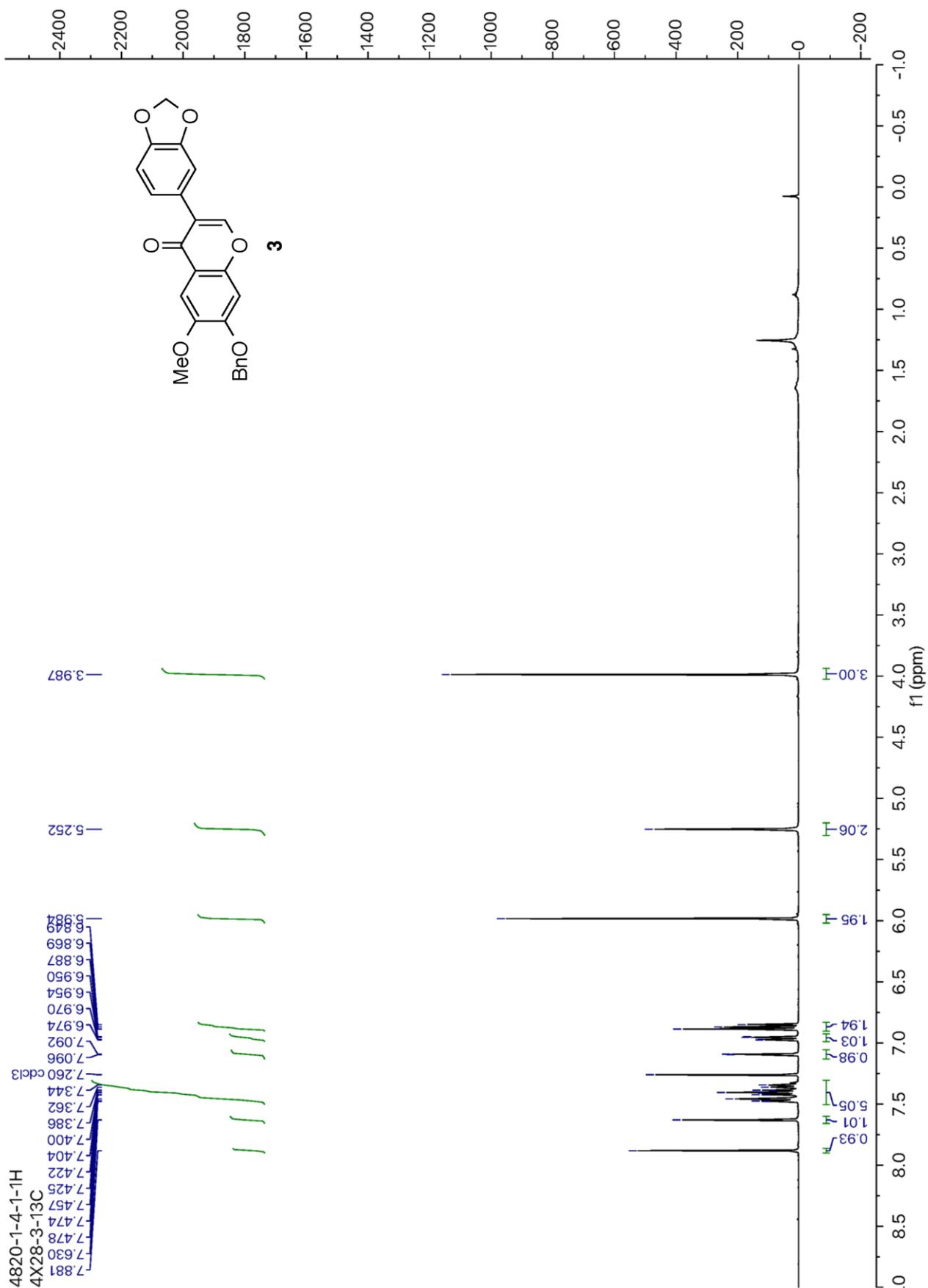
<sup>1</sup>H NMR spectra of **S5** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



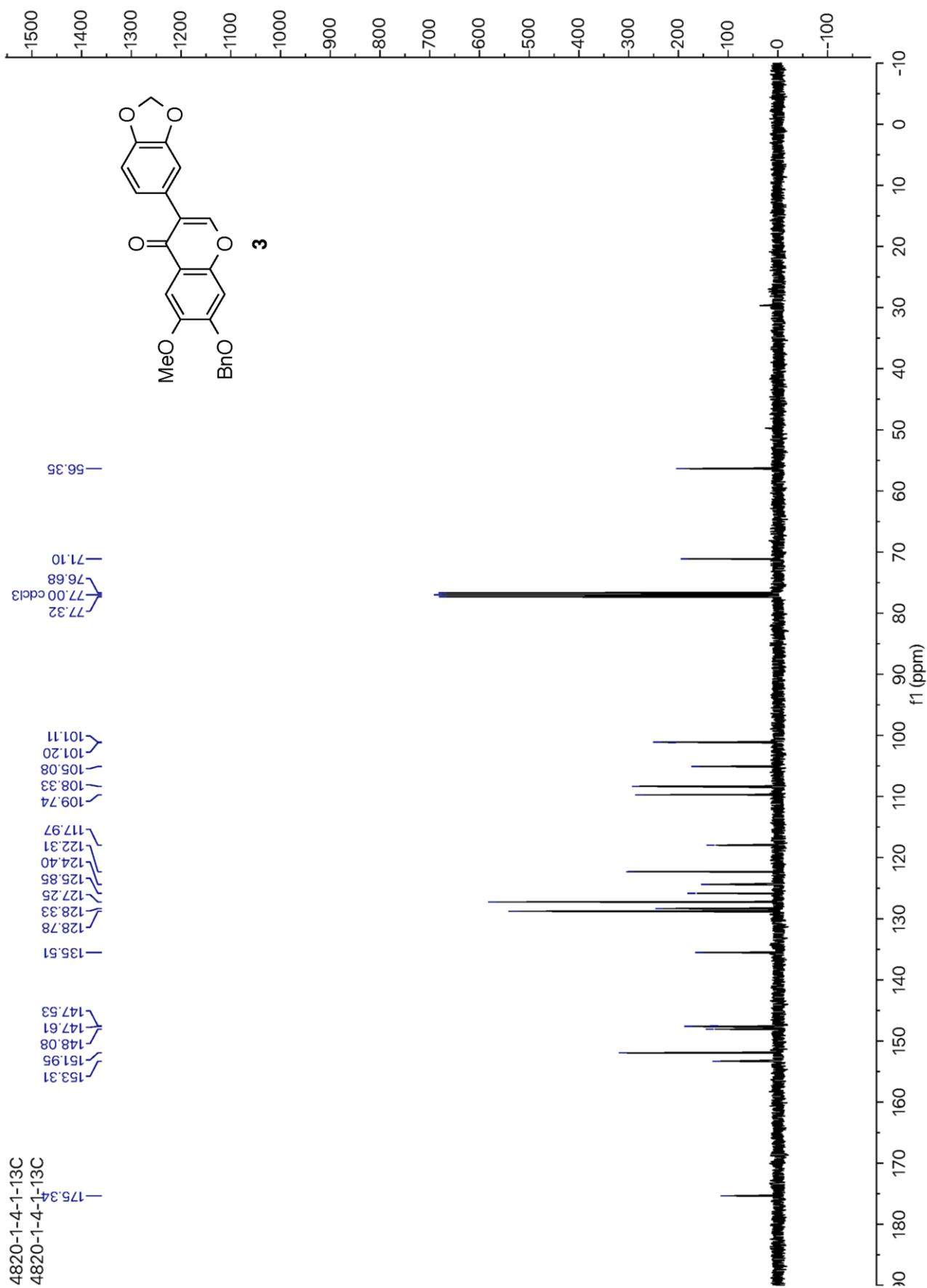
$^{13}\text{C}$  NMR spectra of **S5** (100 MHz,  $\text{CDCl}_3$ )

Supporting Information



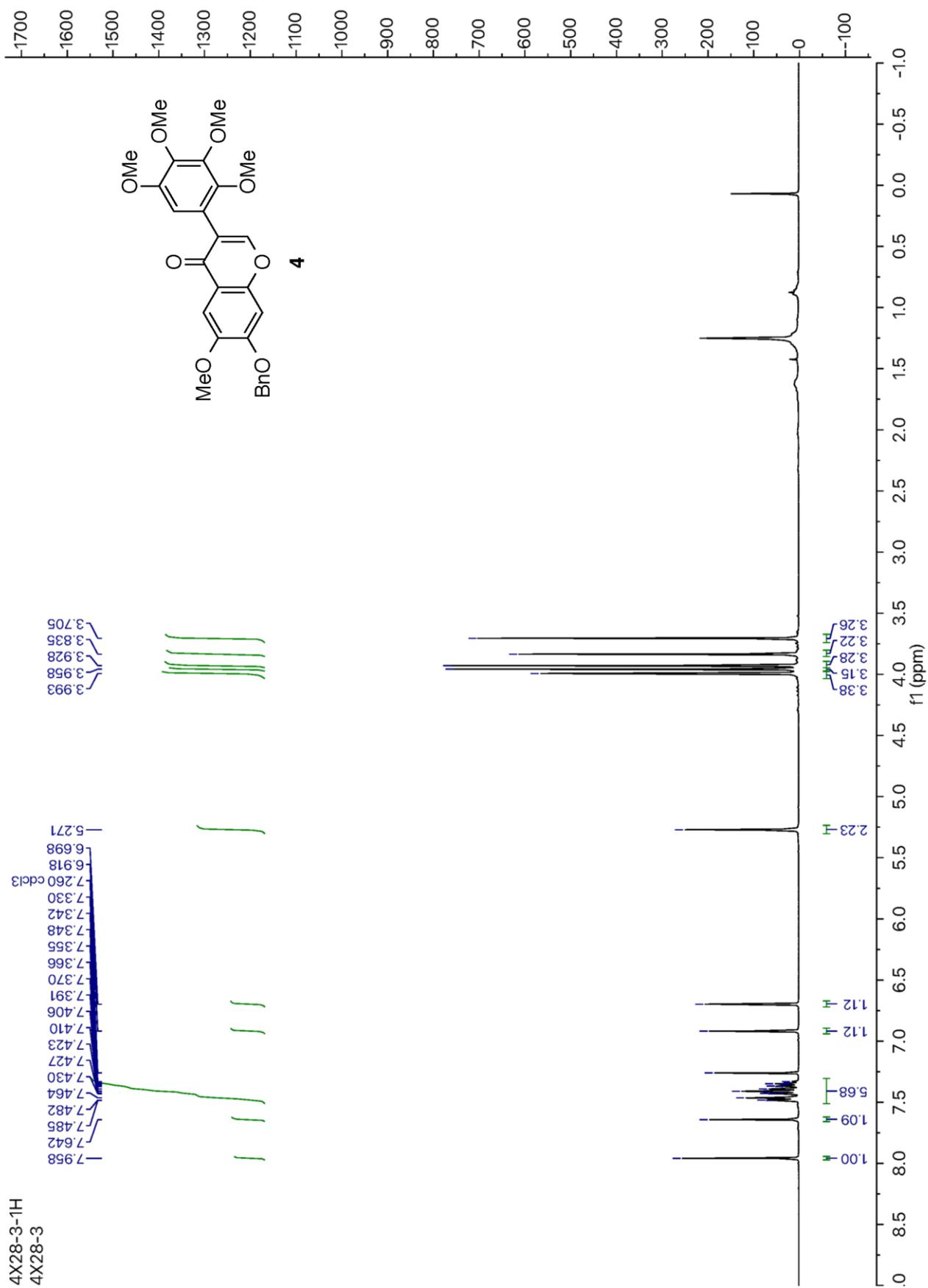
<sup>1</sup>H NMR spectra of **3** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



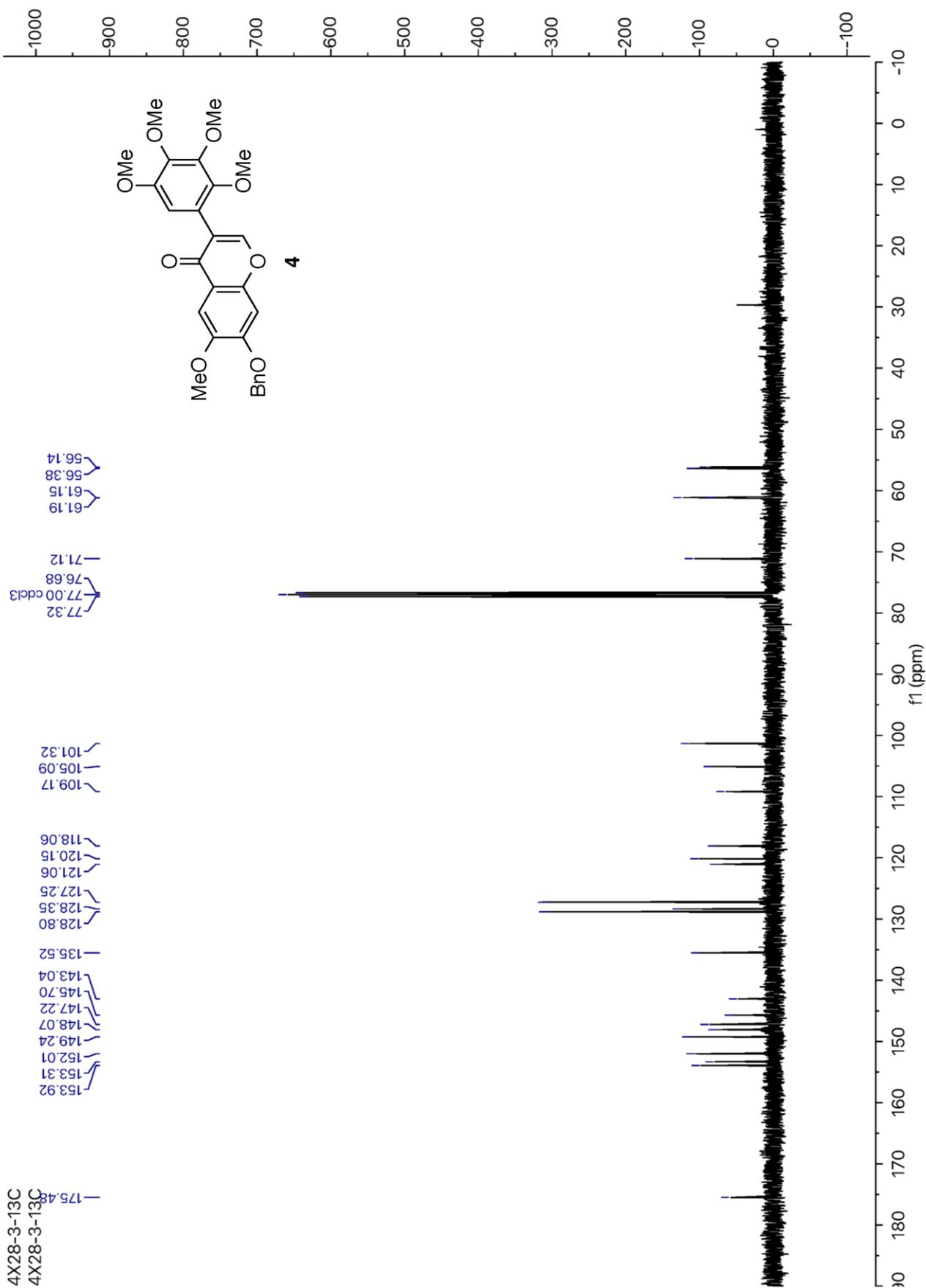
<sup>13</sup>C NMR spectra of **3** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



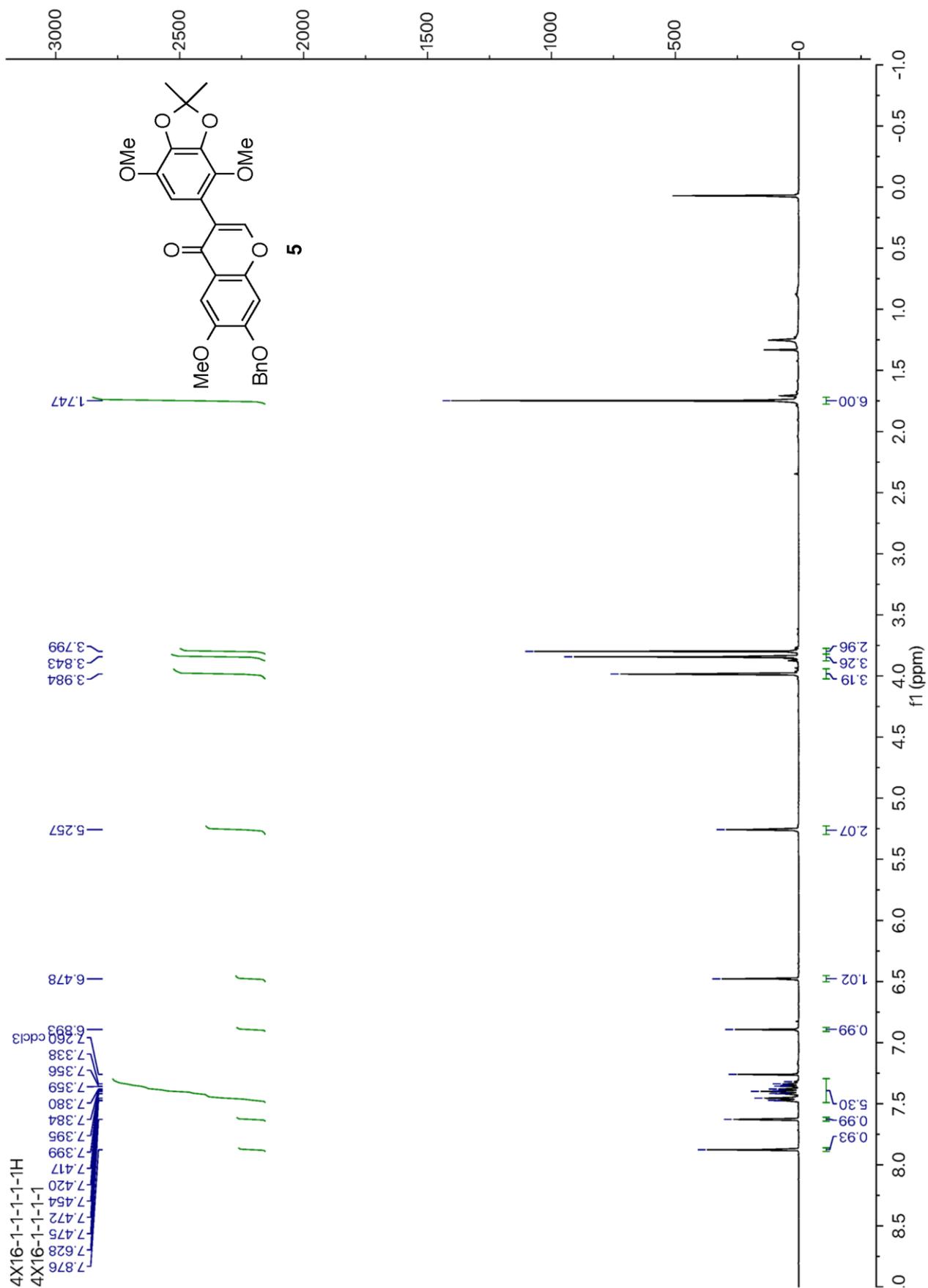
<sup>1</sup>H NMR spectra of **4** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



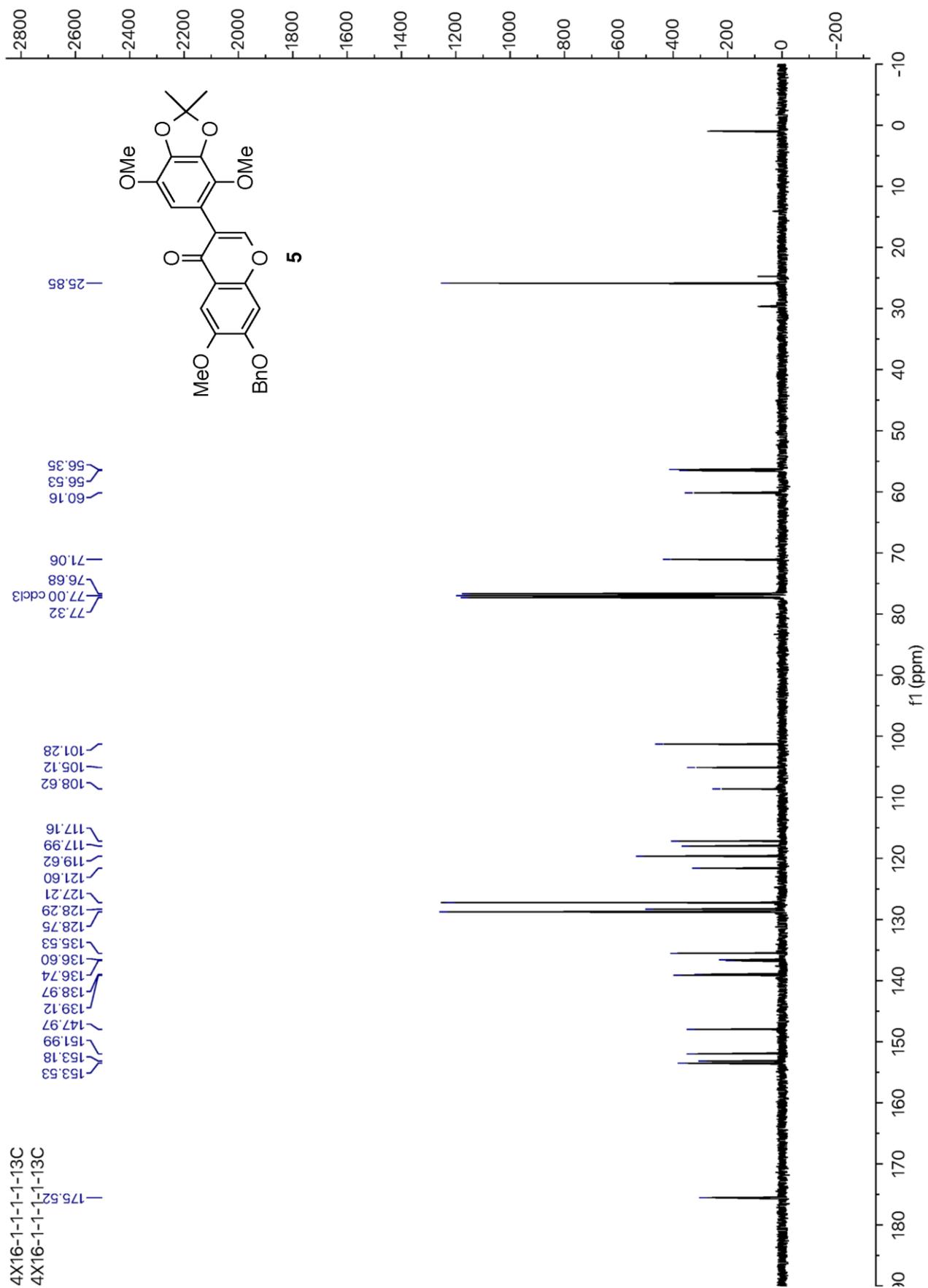
<sup>13</sup>C NMR spectra of **4** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



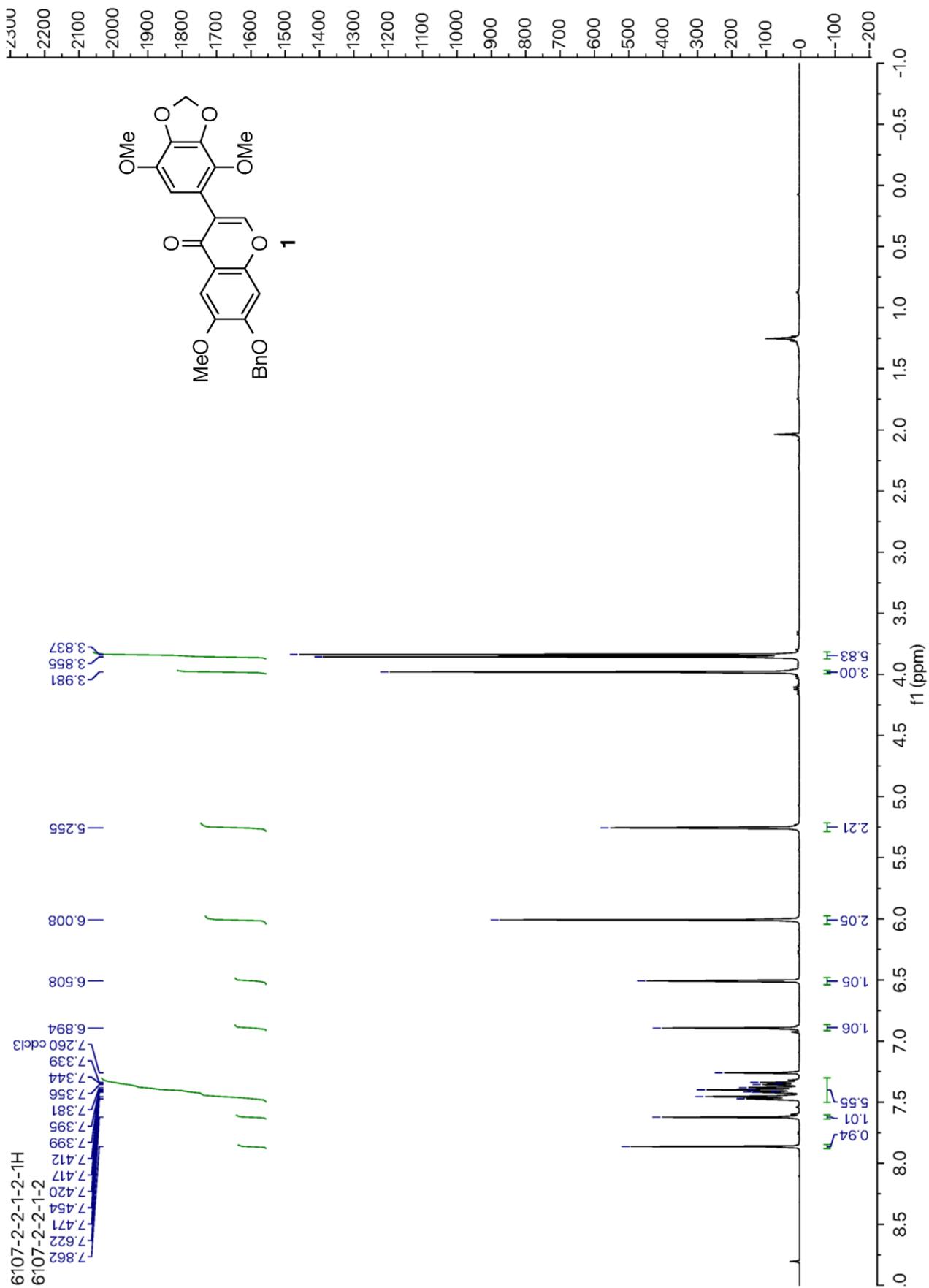
$^1\text{H}$  NMR spectra of **5** (400 MHz,  $\text{CDCl}_3$ )

Supporting Information



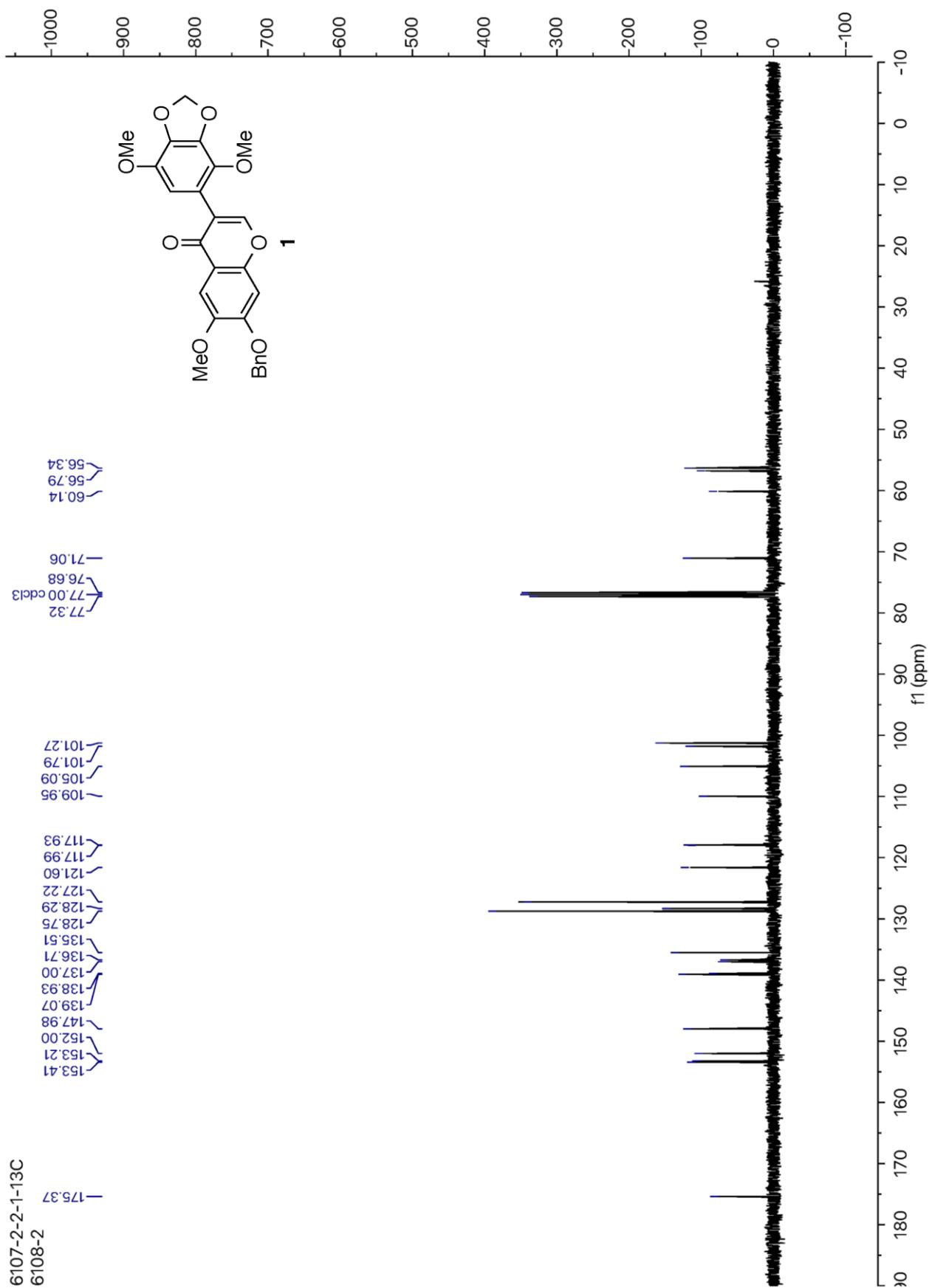
<sup>13</sup>C NMR spectra of **5** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



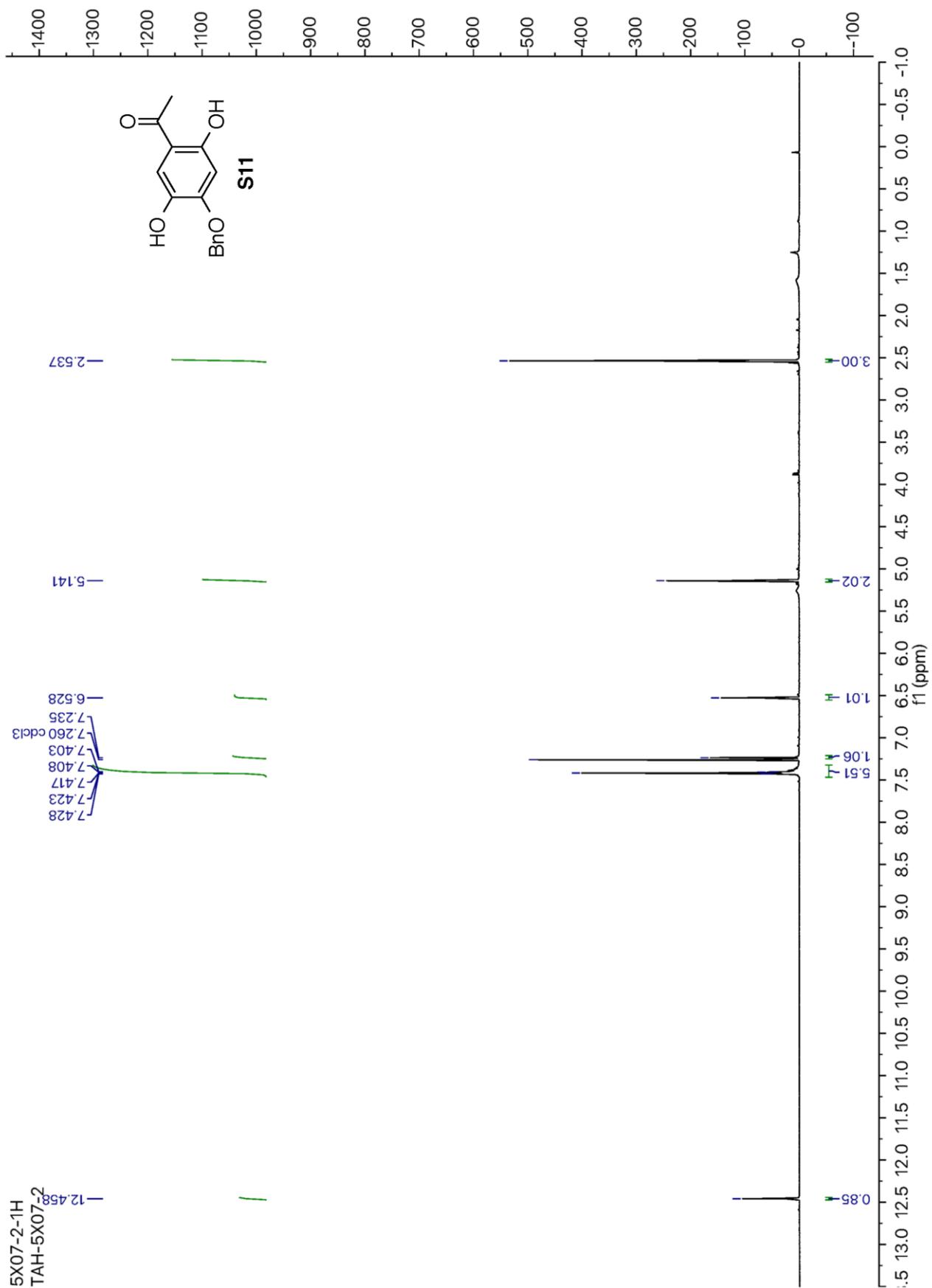
<sup>1</sup>H NMR spectra of **1** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



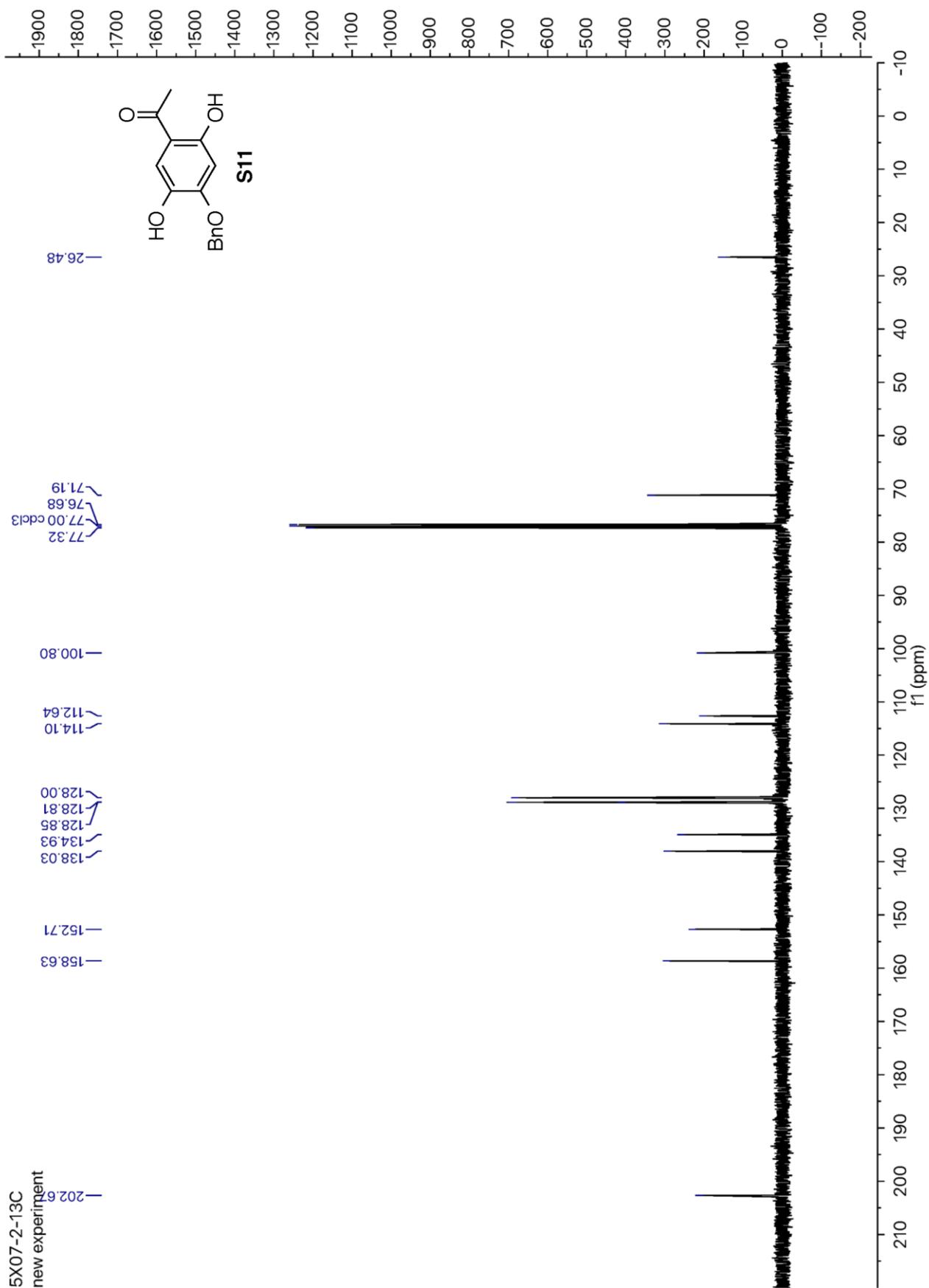
<sup>13</sup>C NMR spectra of **1** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



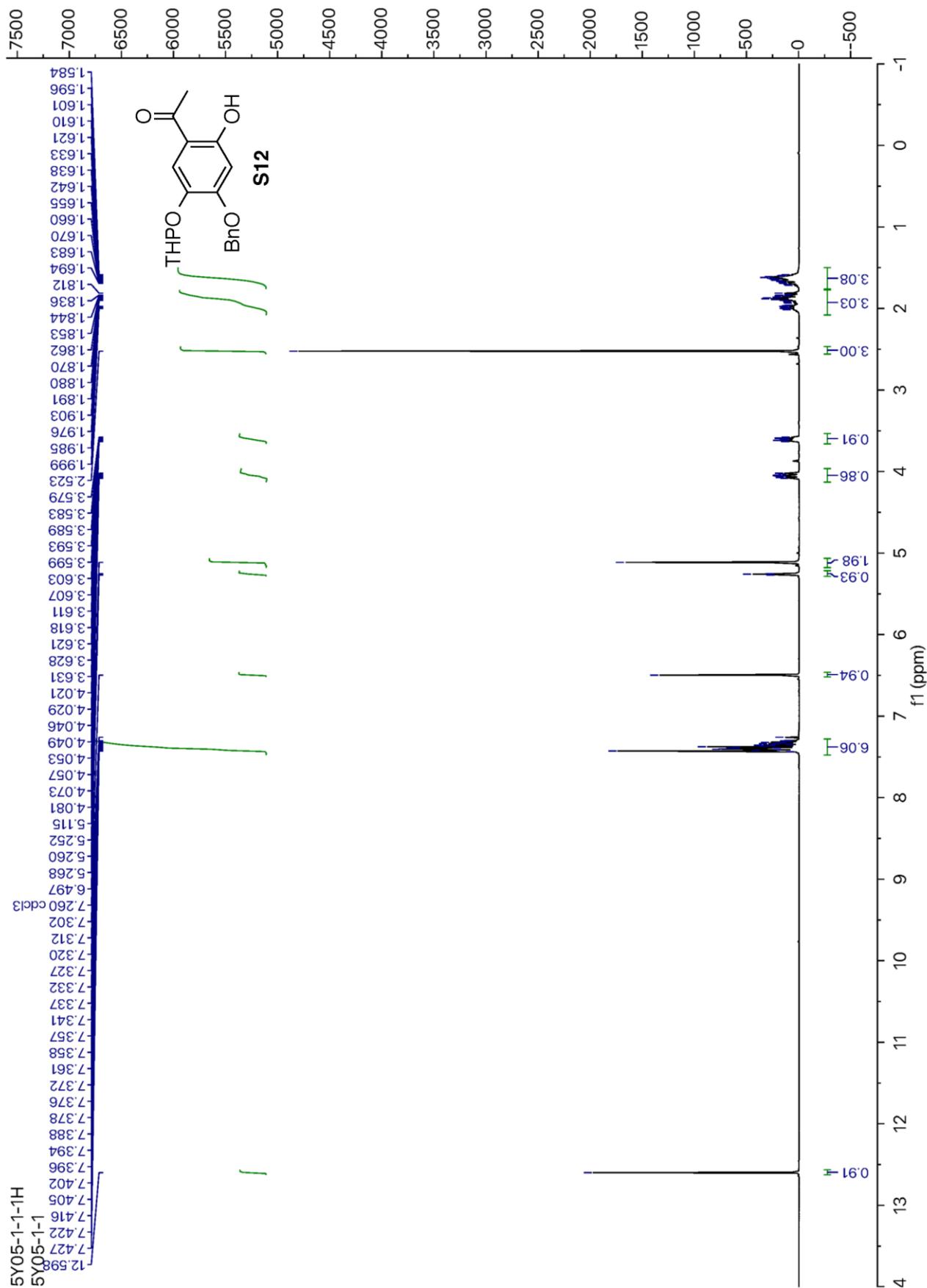
<sup>1</sup>H NMR spectra of **S11** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



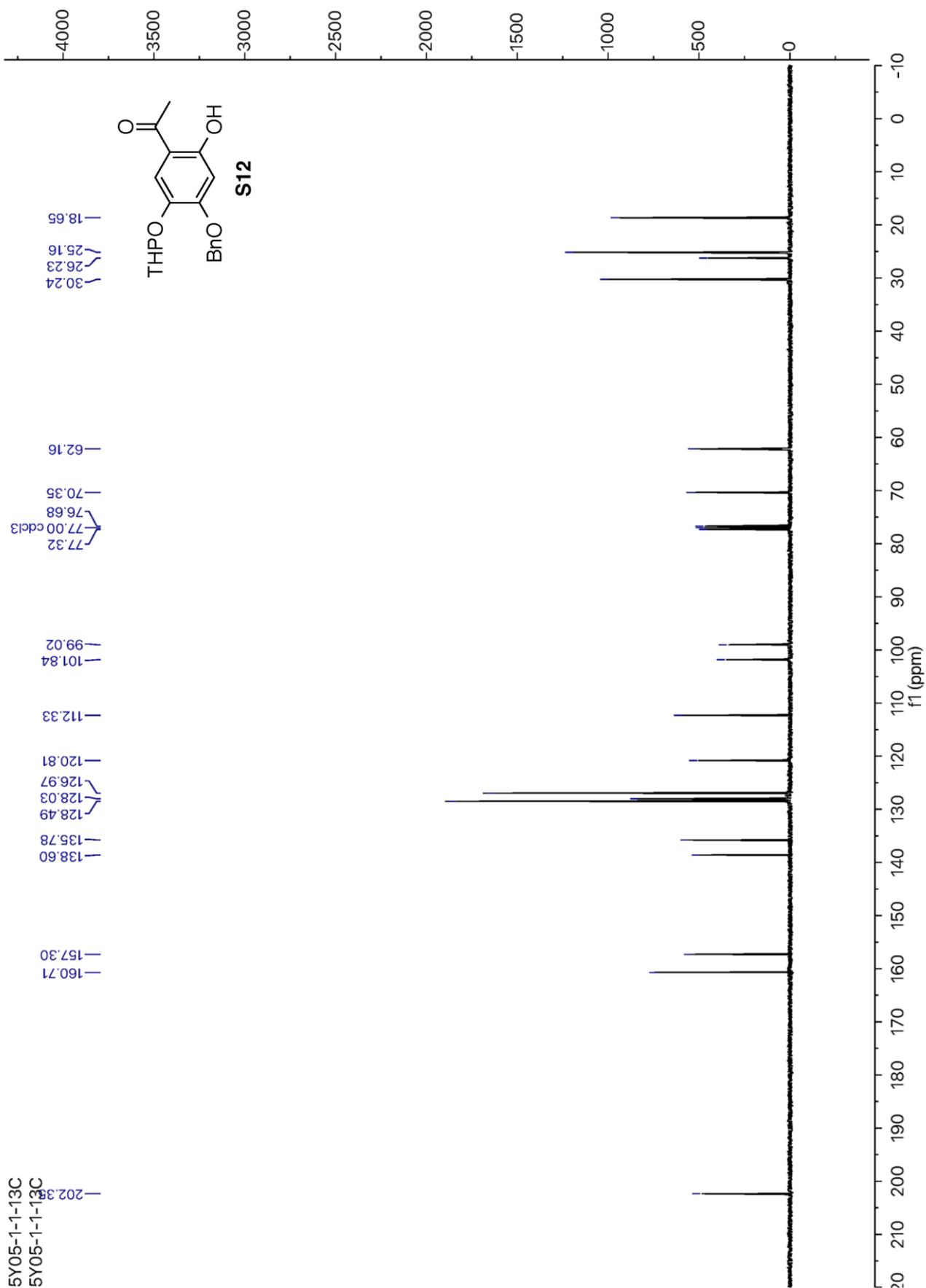
<sup>13</sup>C NMR spectra of **S11** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



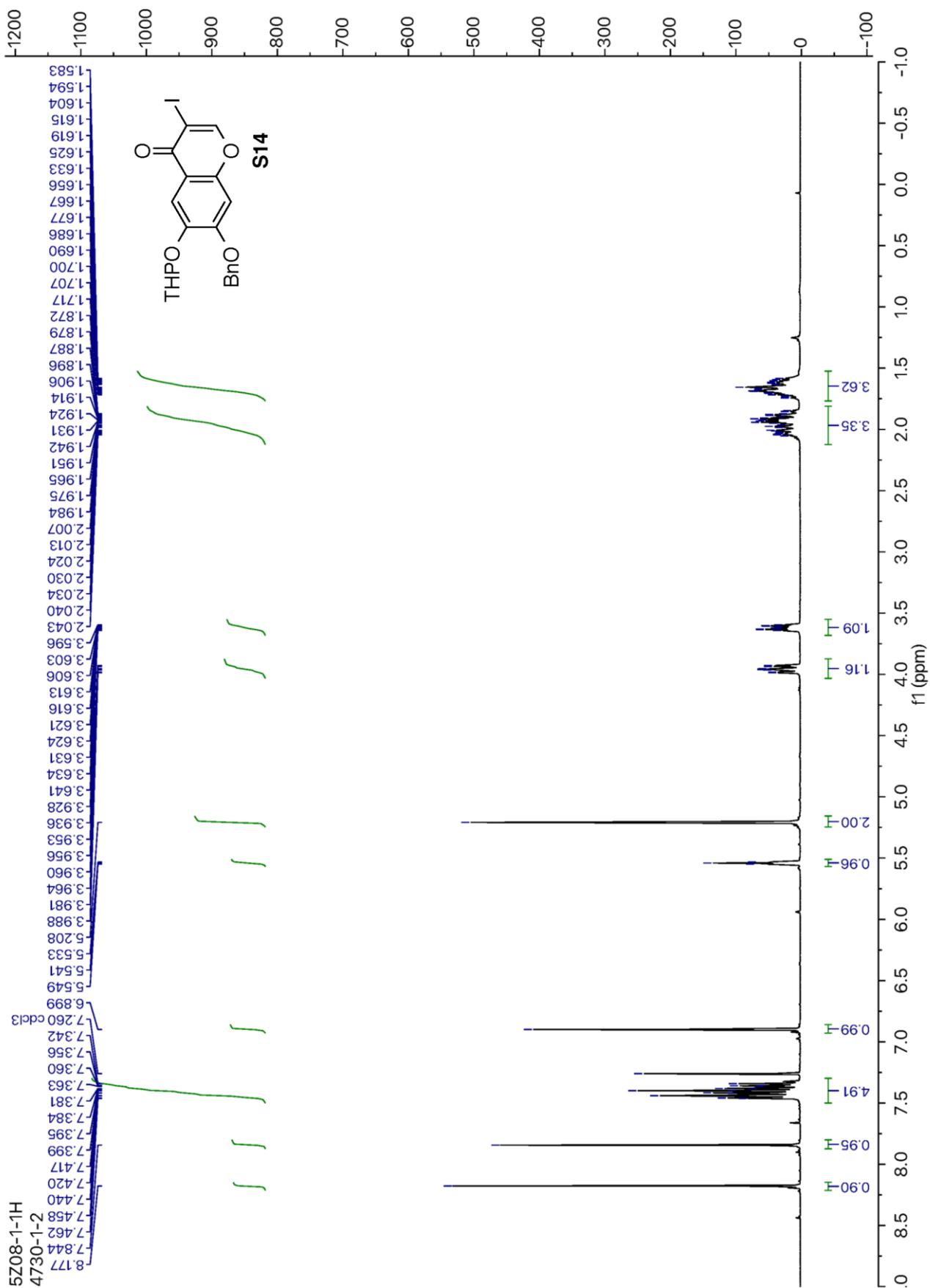
<sup>1</sup>H NMR spectra of **S12** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



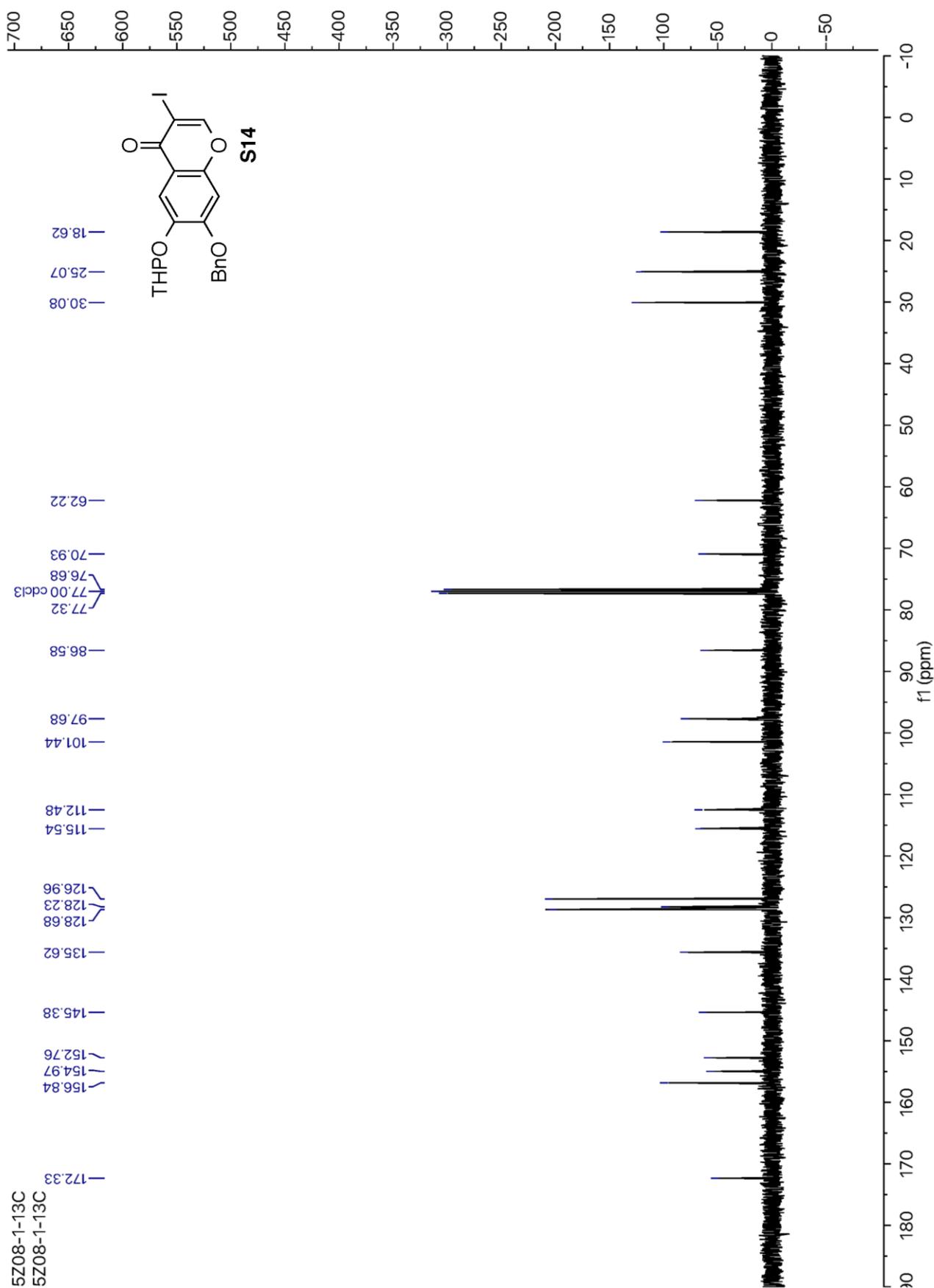
<sup>13</sup>C NMR spectra of **S12** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



<sup>1</sup>H NMR spectra of **S14** (400 MHz, CDCl<sub>3</sub>)

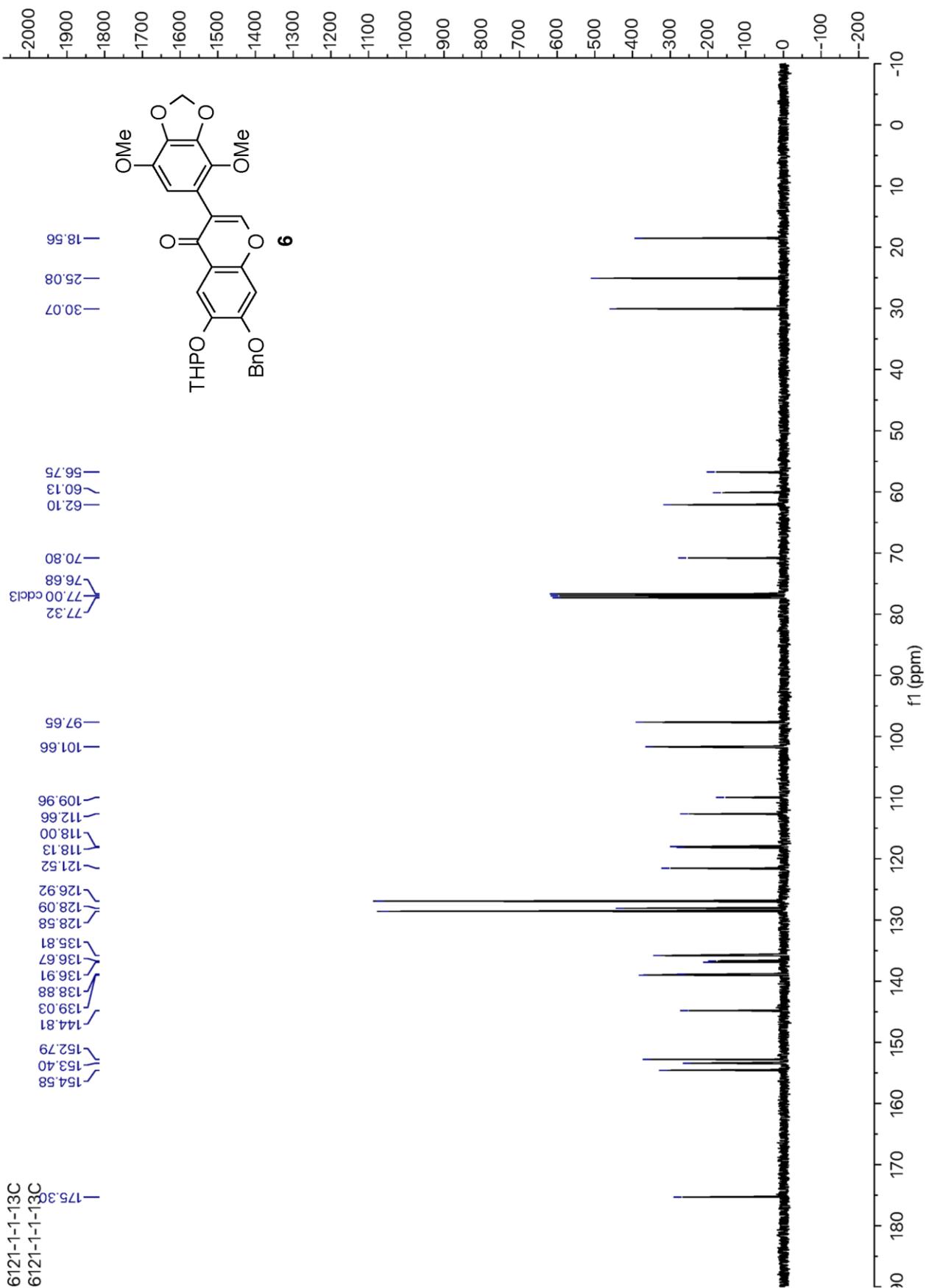
Supporting Information



$^{13}\text{C}$  NMR spectra of **S14** (100 MHz,  $\text{CDCl}_3$ )

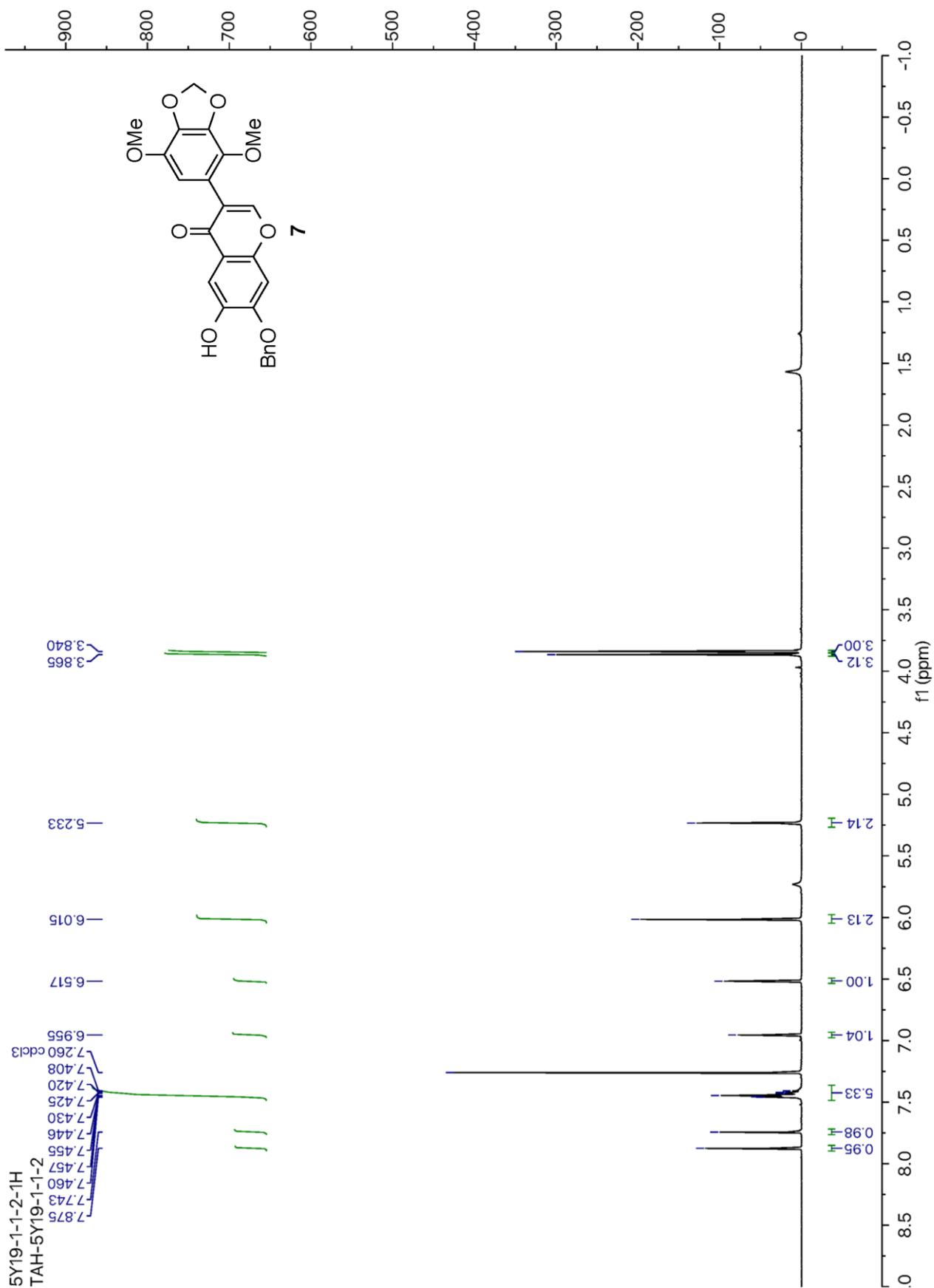


Supporting Information



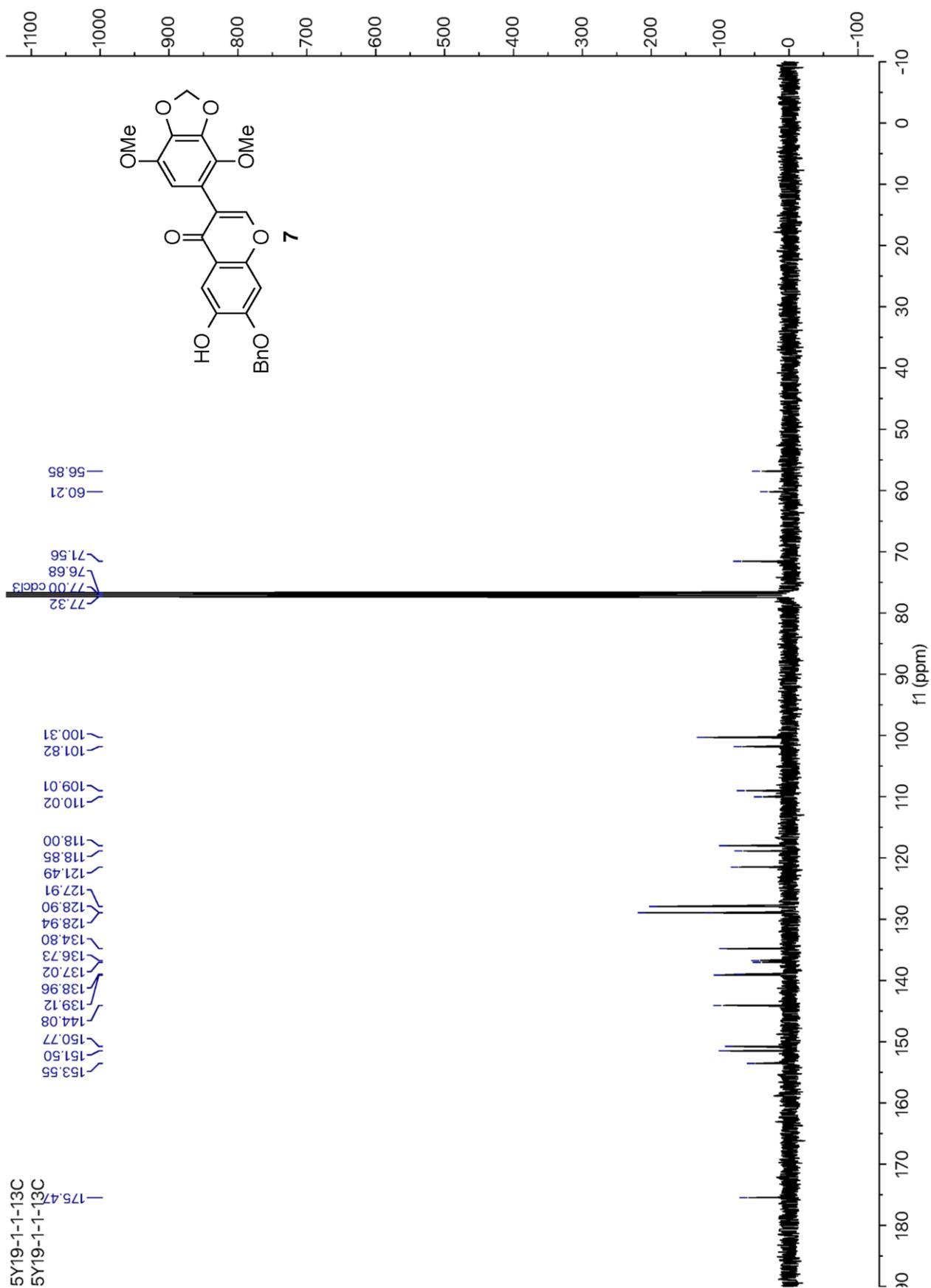
<sup>13</sup>C NMR spectra of **6** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



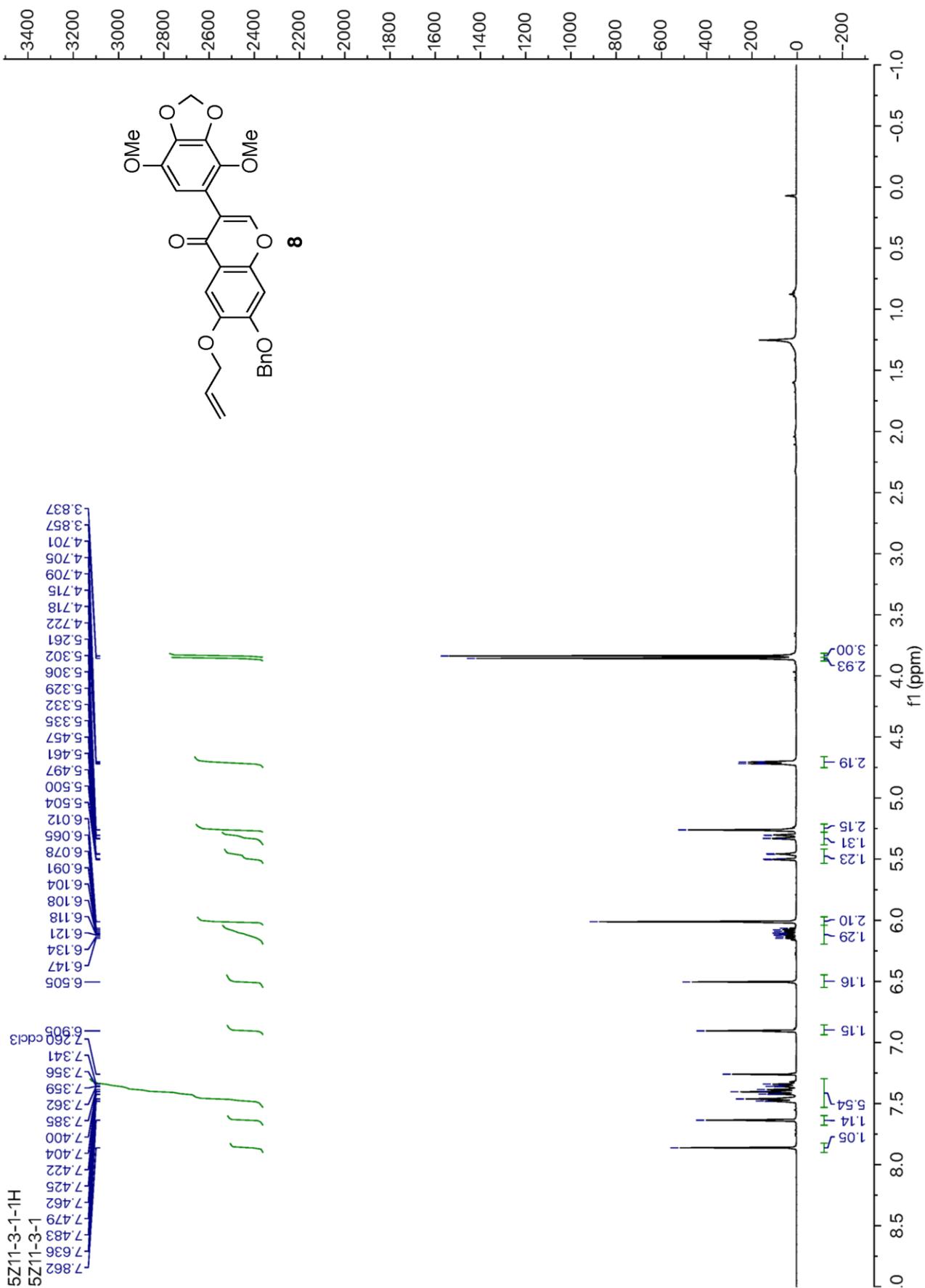
<sup>1</sup>H NMR spectra of **7** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



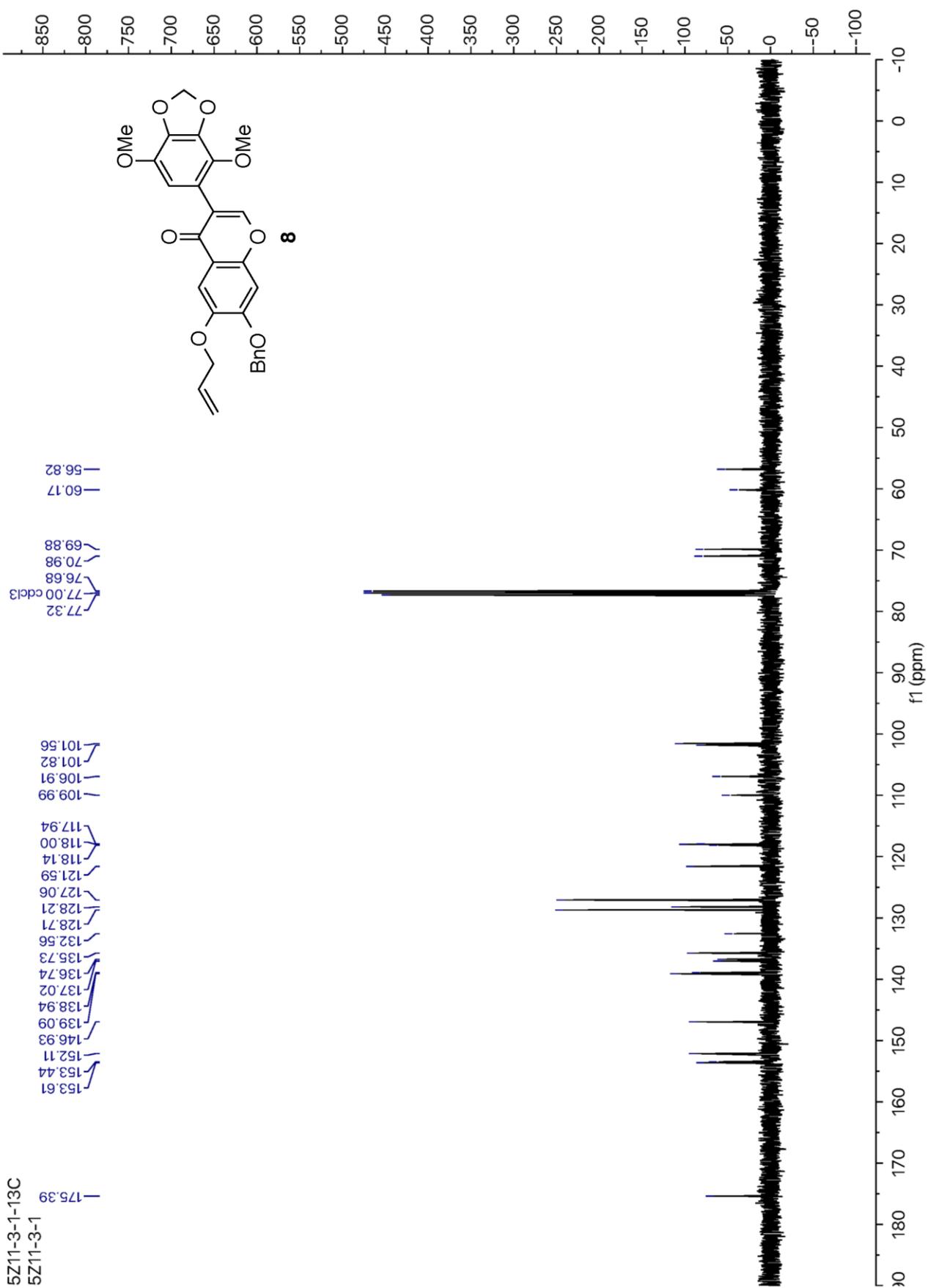
<sup>13</sup>C NMR spectra of **7** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



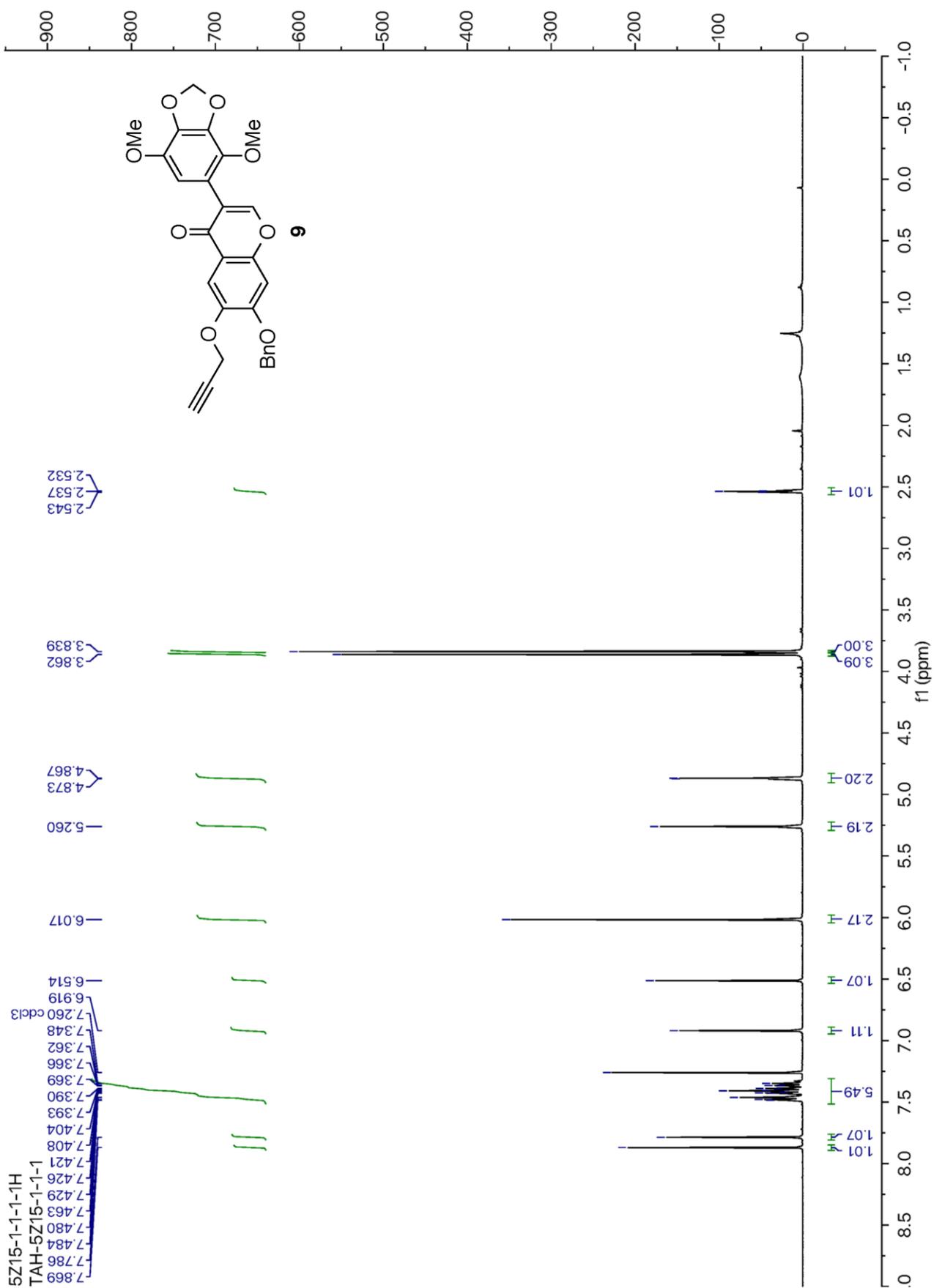
<sup>1</sup>H NMR spectra of **8** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



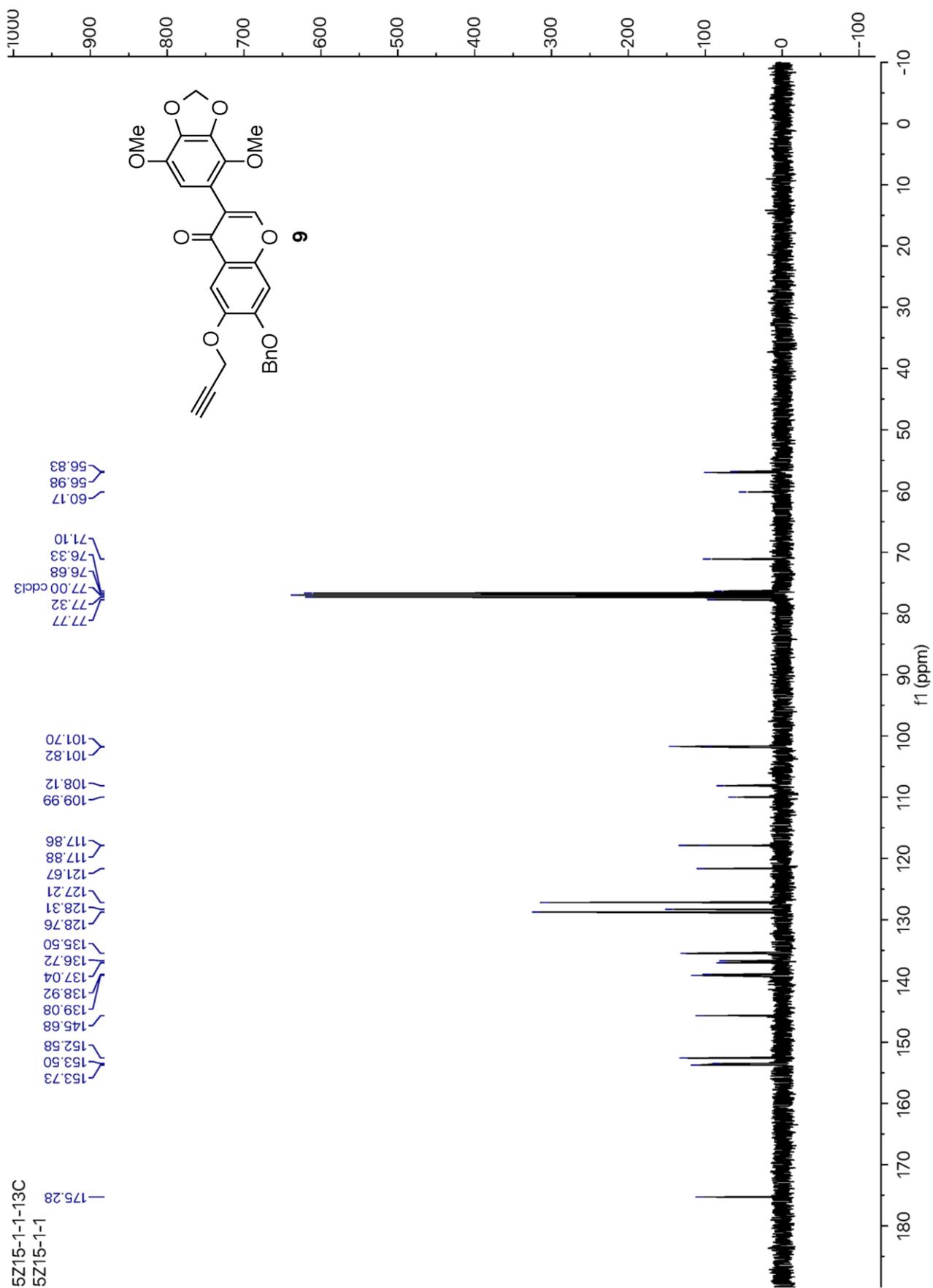
<sup>13</sup>C NMR spectra of **8** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



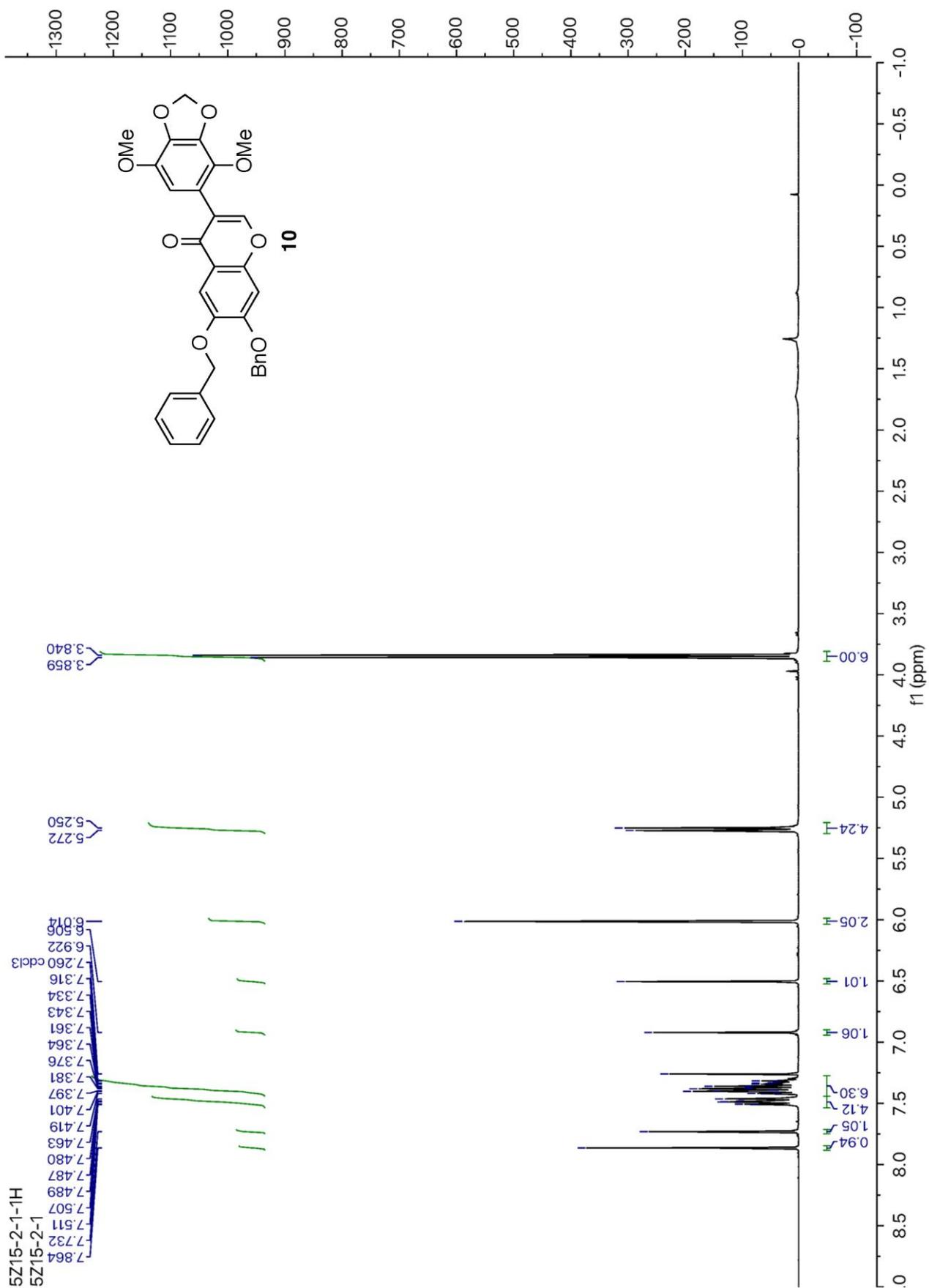
<sup>1</sup>H NMR spectra of **9** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



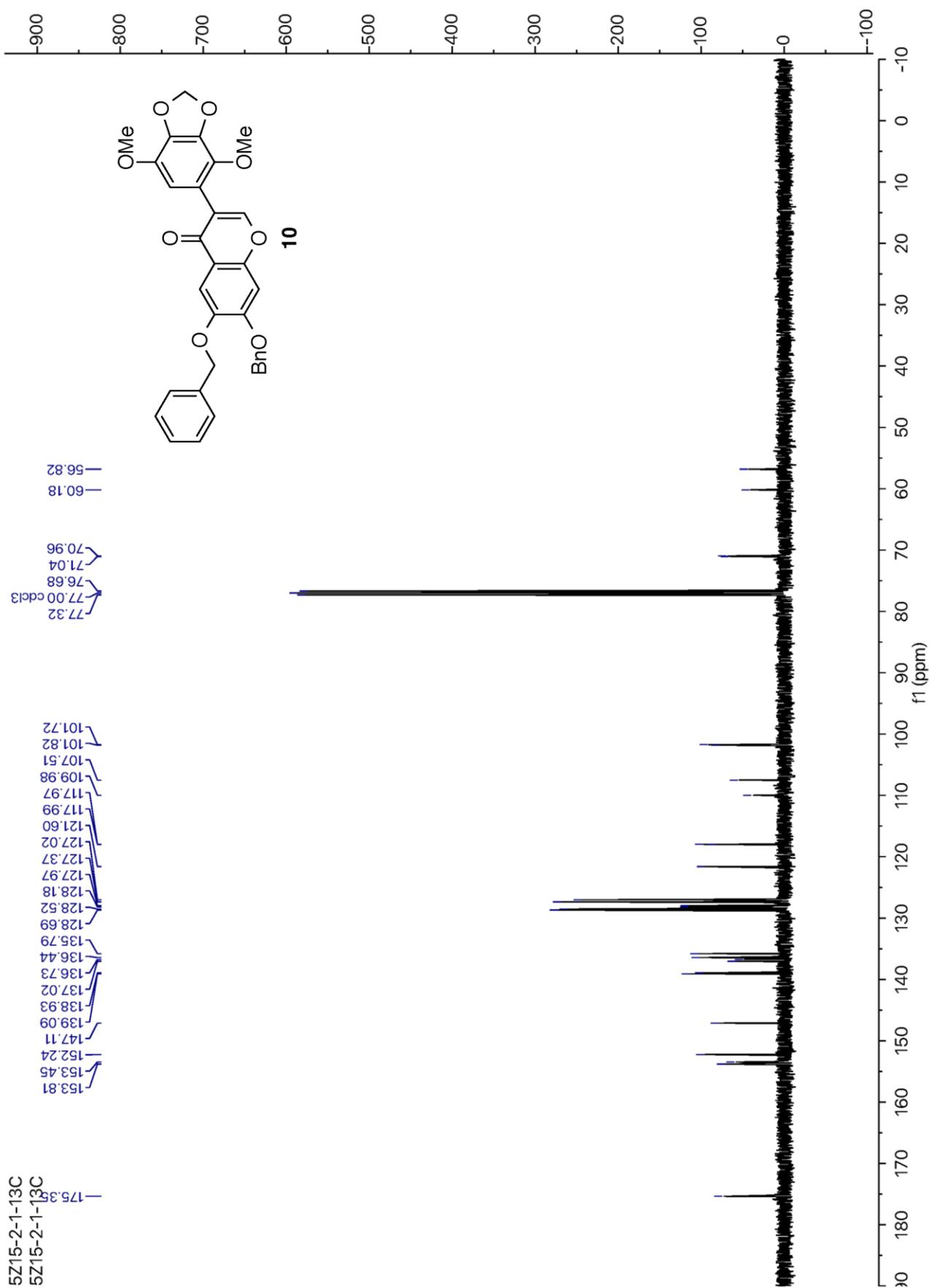
<sup>13</sup>C NMR spectra of **9** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



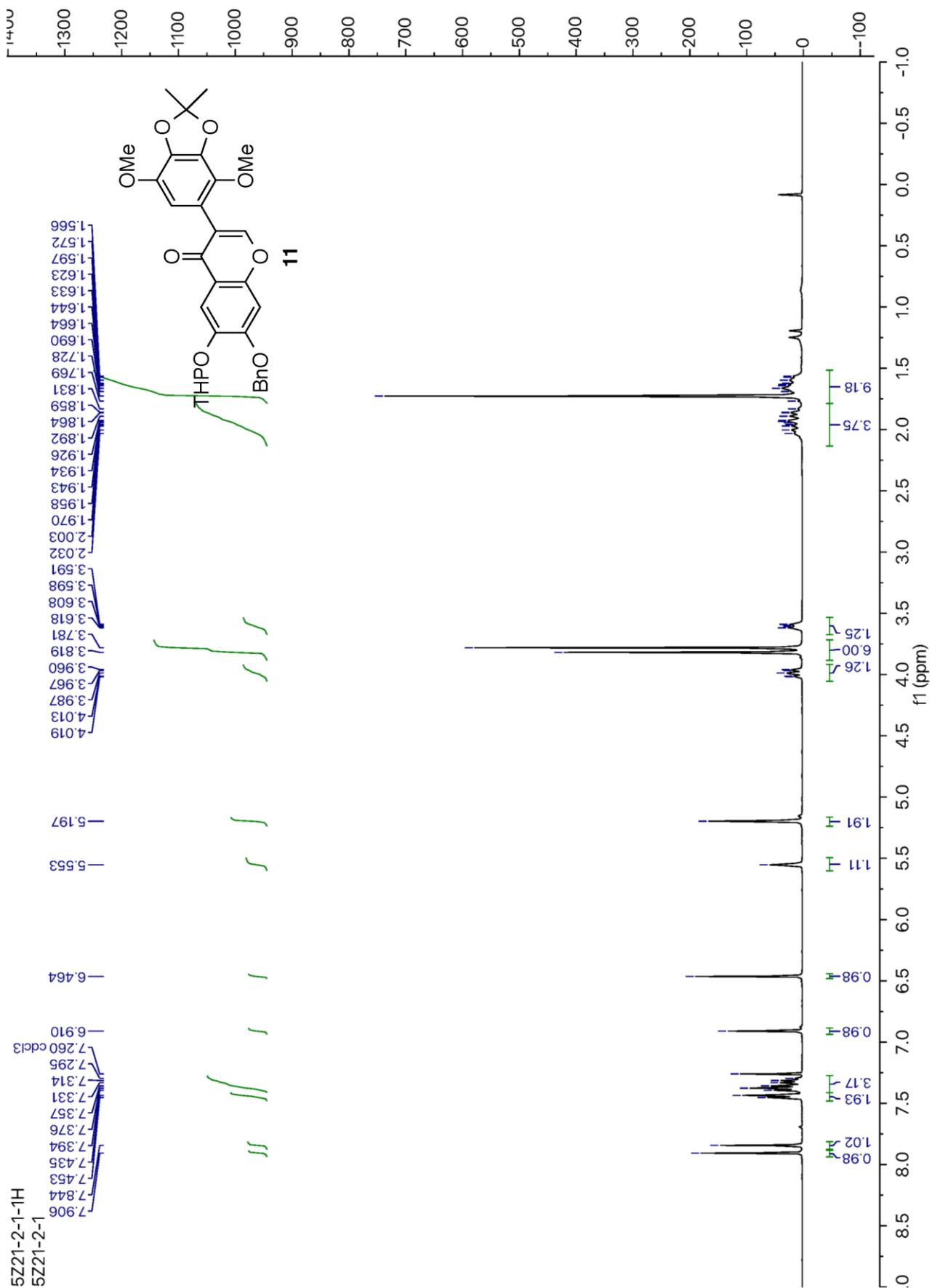
<sup>1</sup>H NMR spectra of **10** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



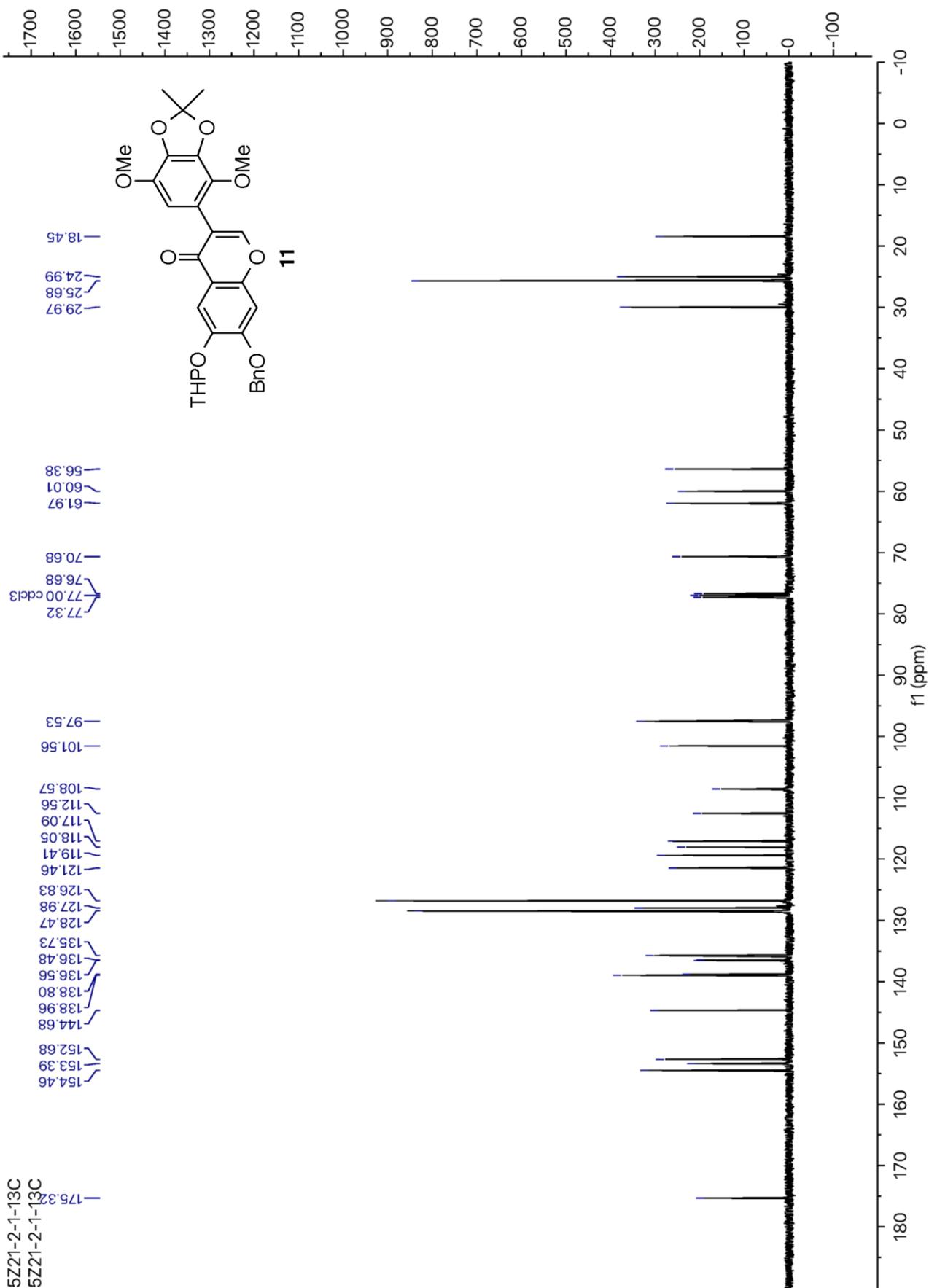
<sup>13</sup>C NMR spectra of **10** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



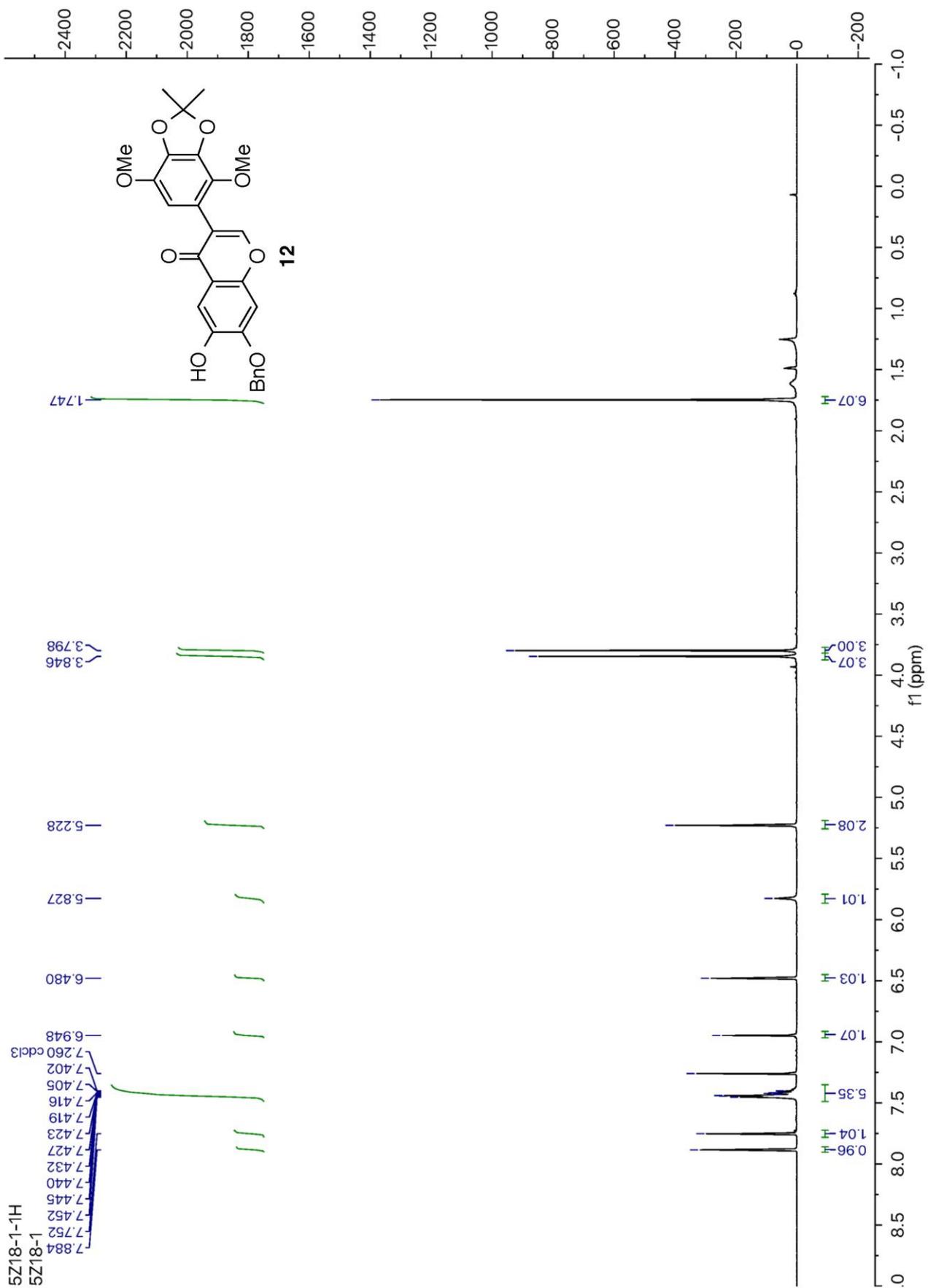
<sup>1</sup>H NMR spectra of **11** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



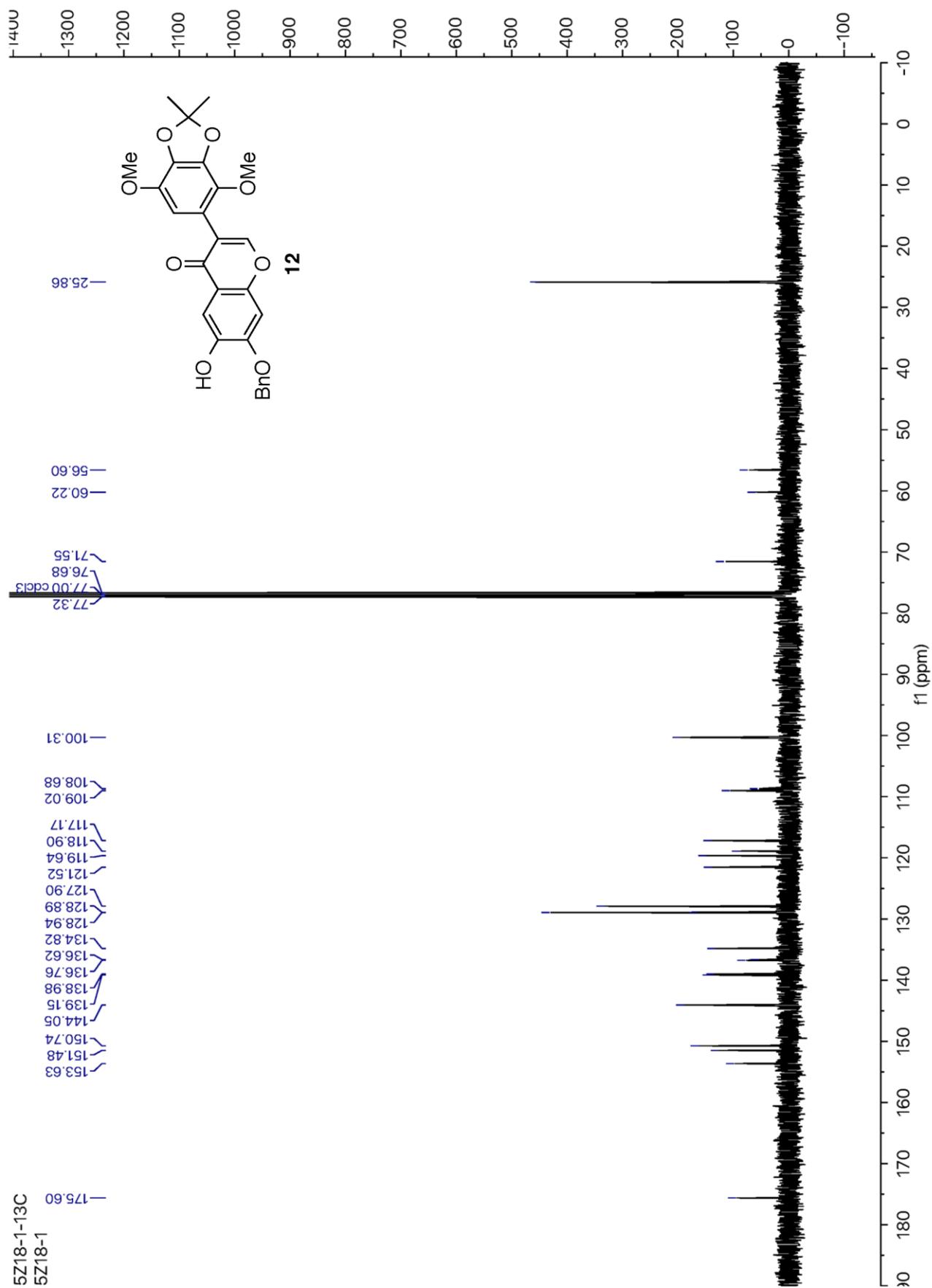
<sup>13</sup>C NMR spectra of **11** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



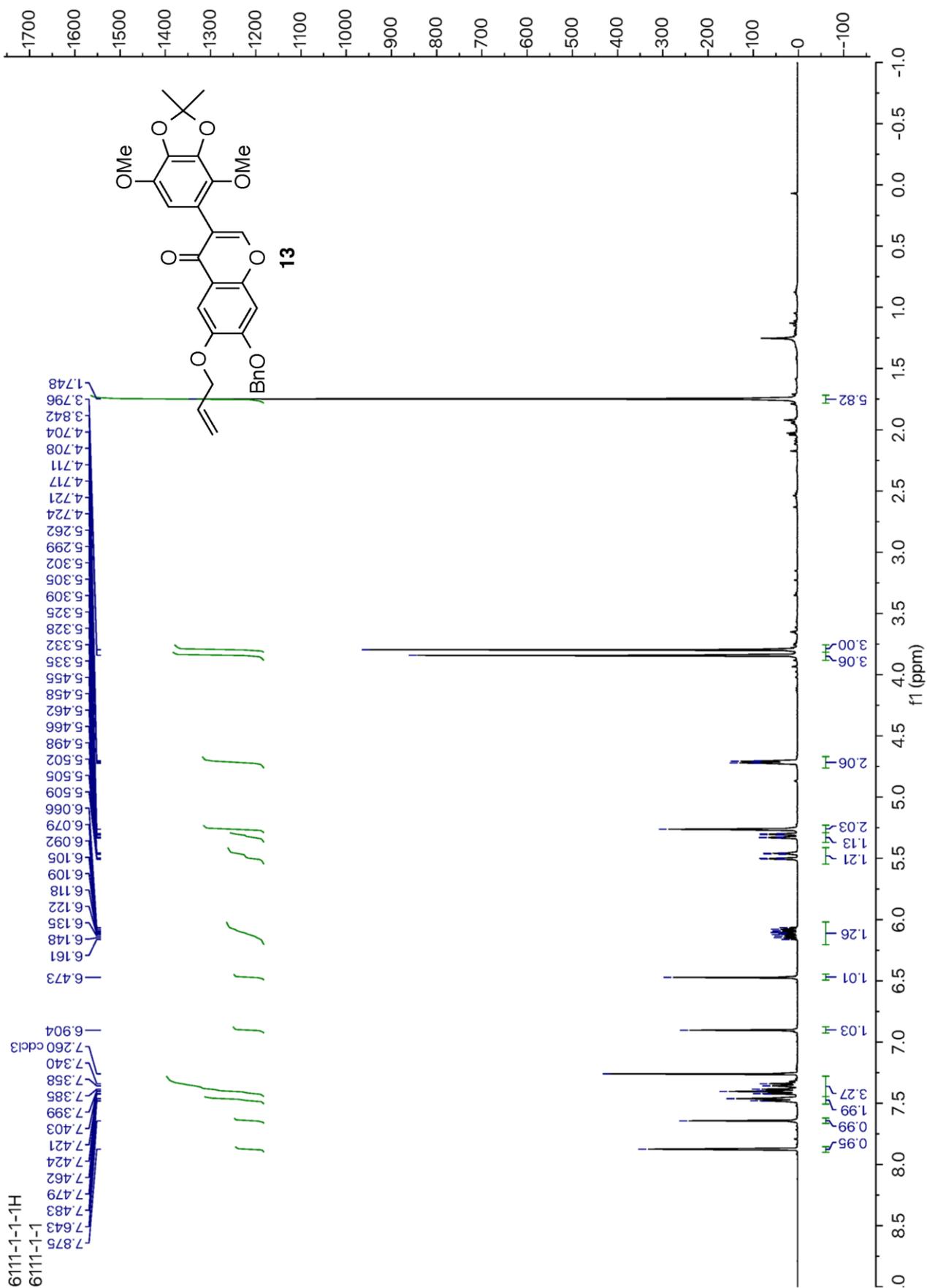
<sup>1</sup>H NMR spectra of **12** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



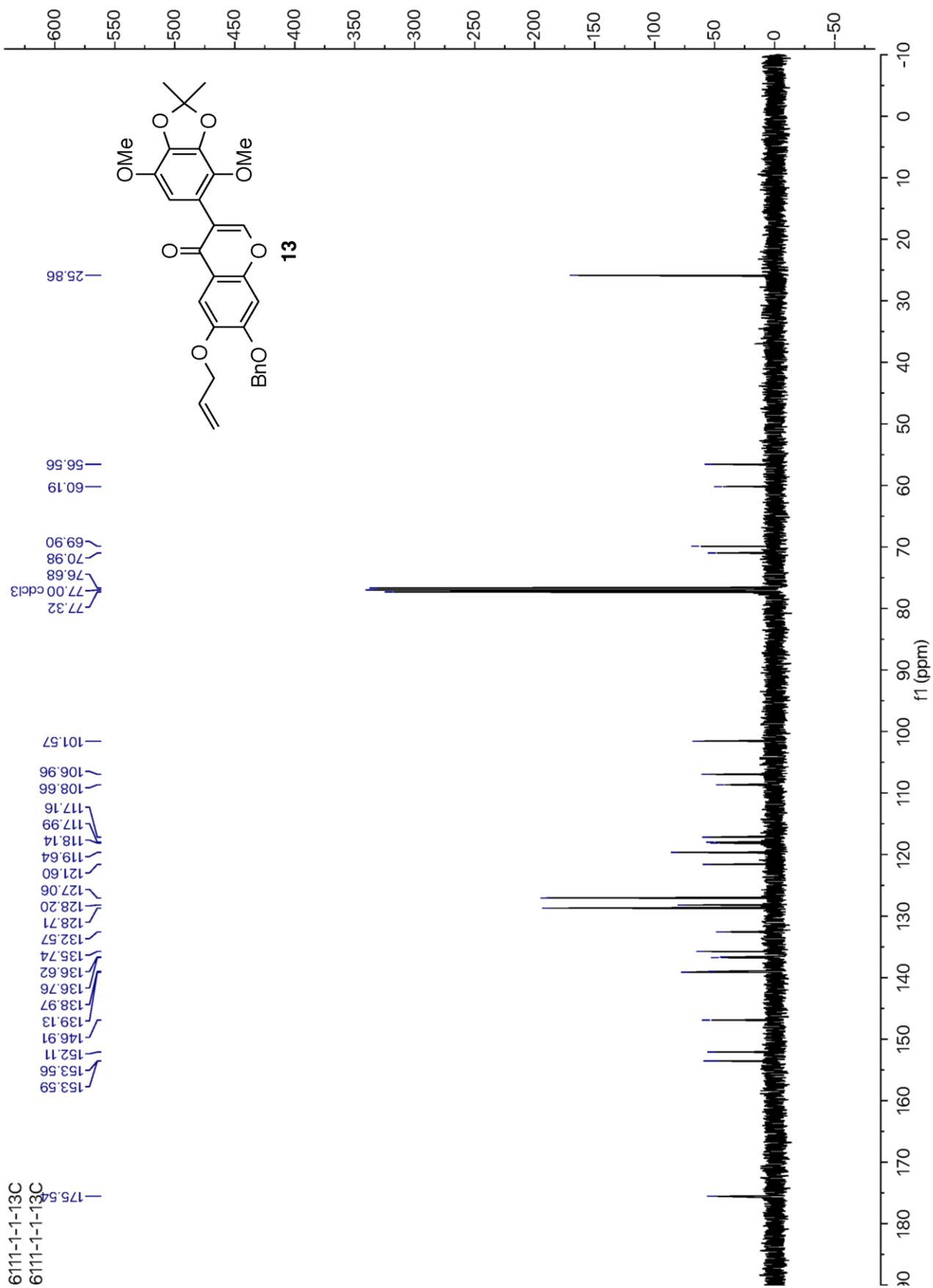
<sup>13</sup>C NMR spectra of **12** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



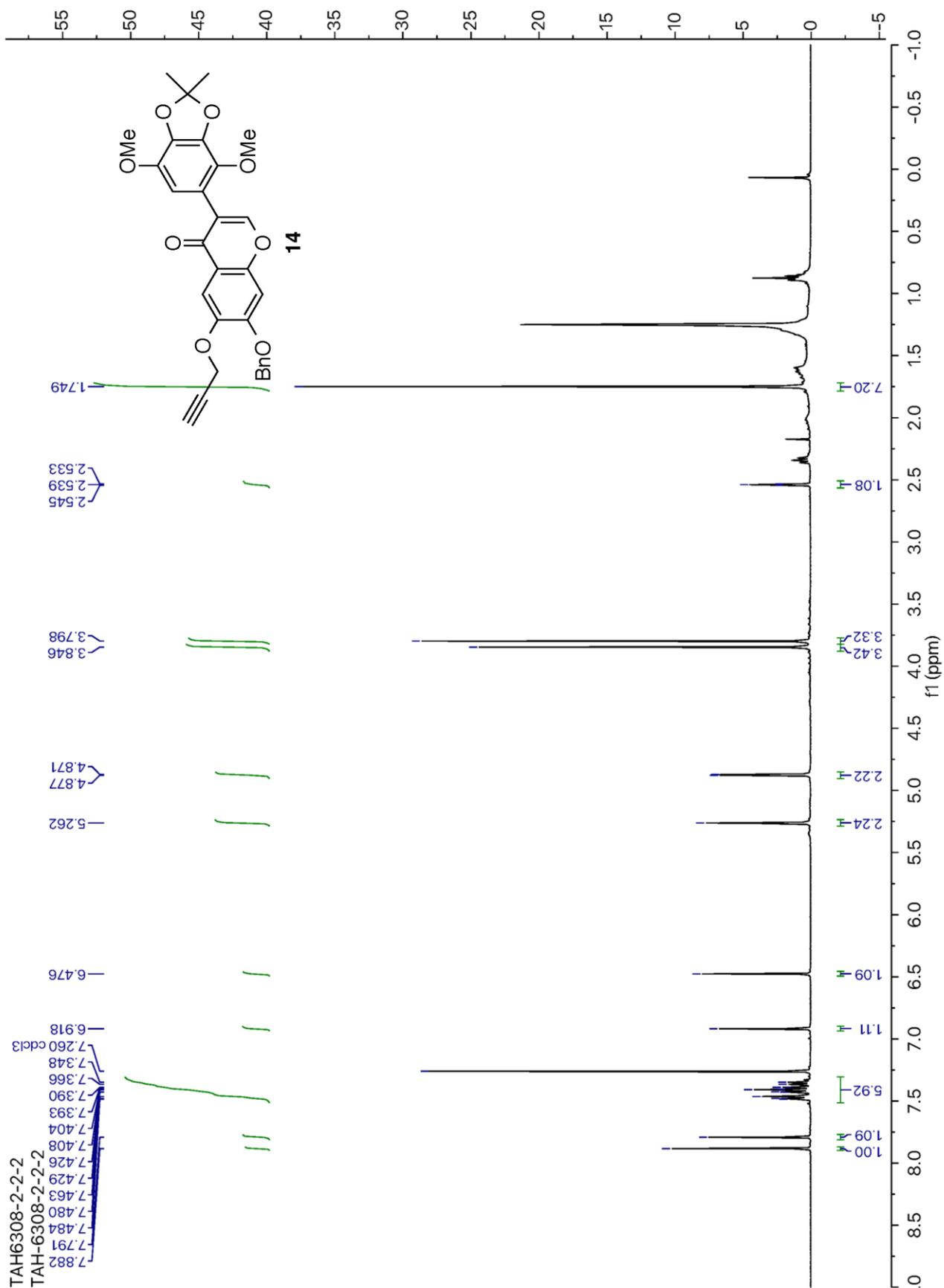
<sup>1</sup>H NMR spectra of **13** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



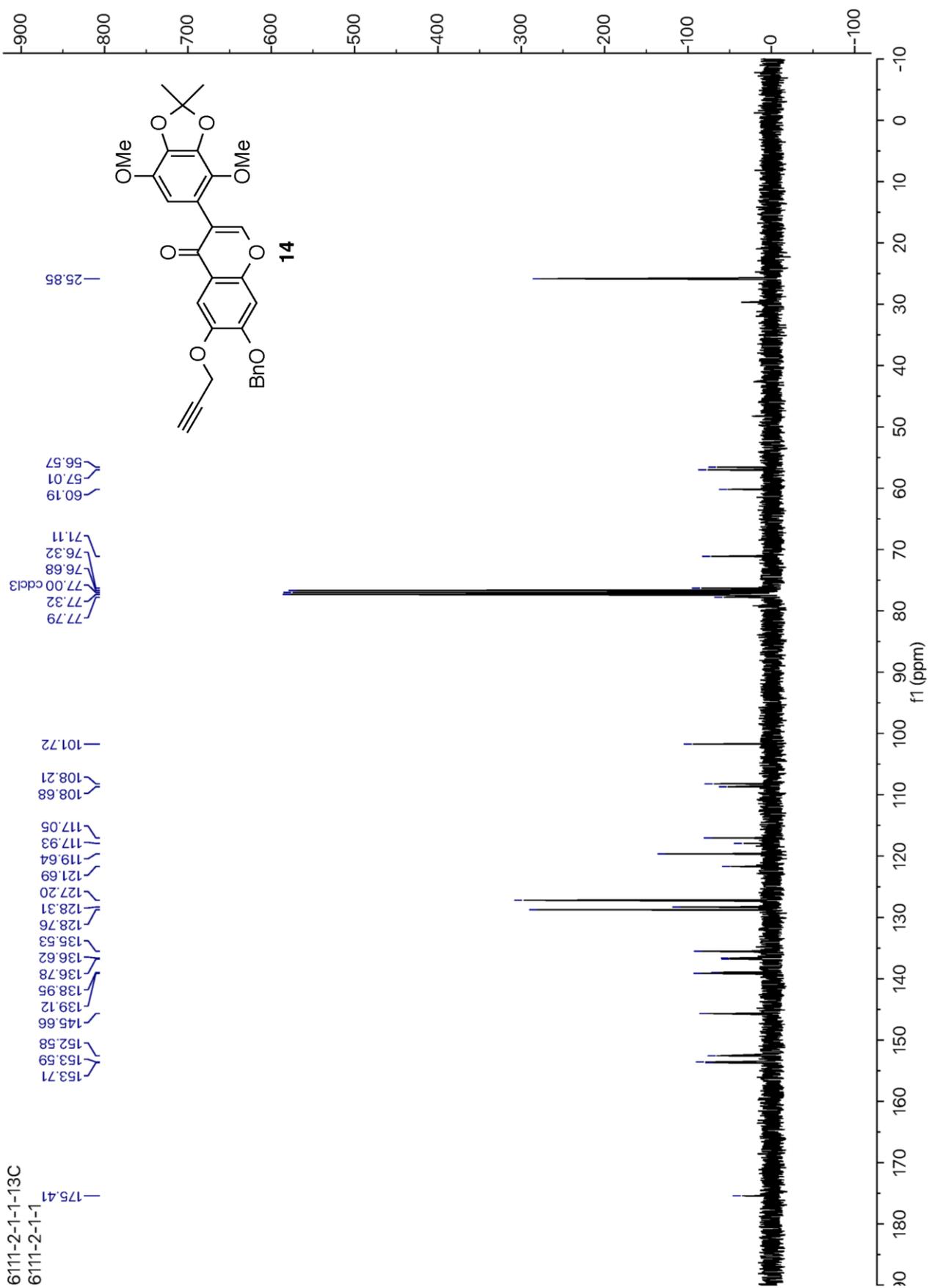
<sup>13</sup>C NMR spectra of **13** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



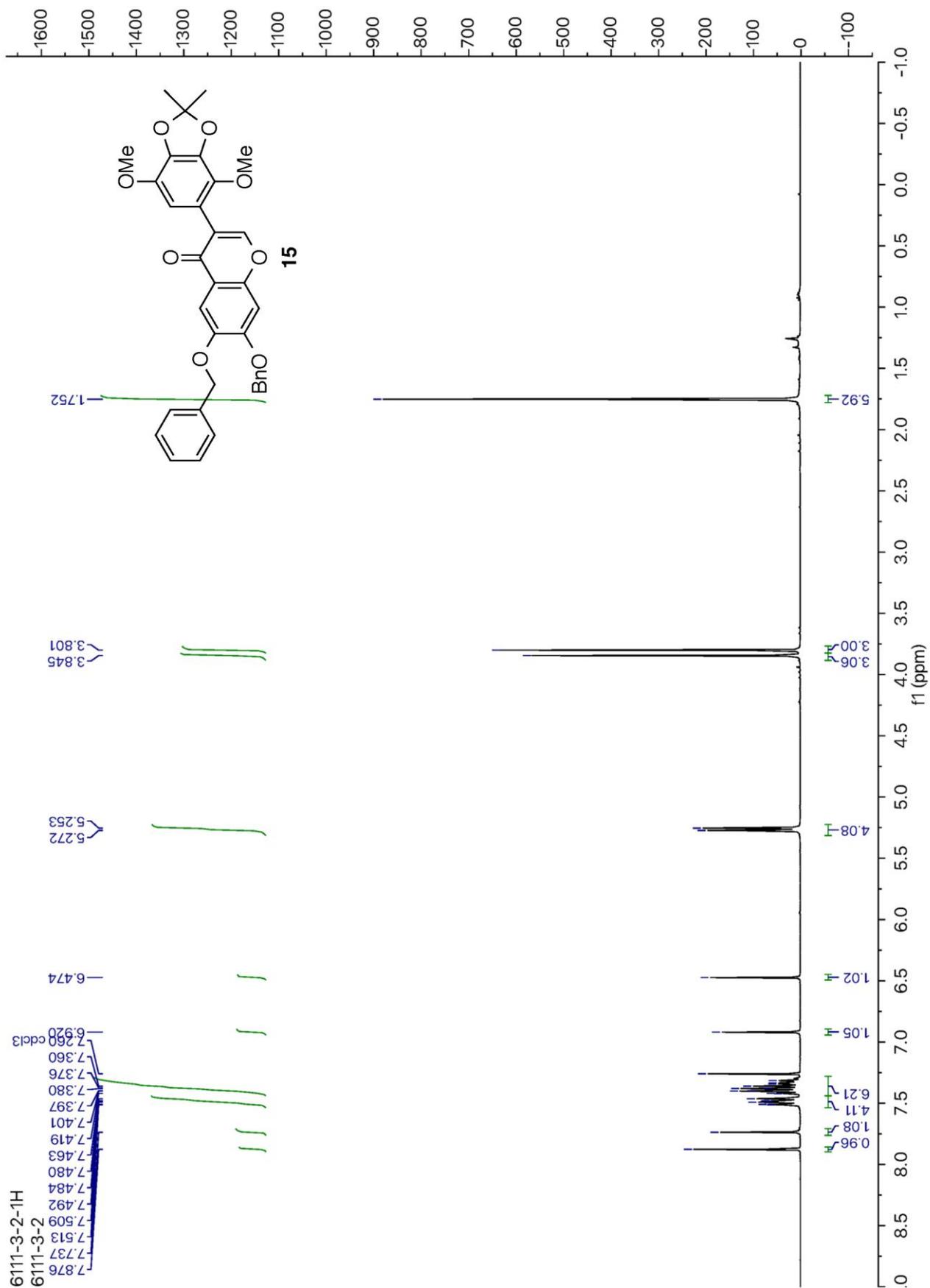
<sup>1</sup>H NMR spectra of **14** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



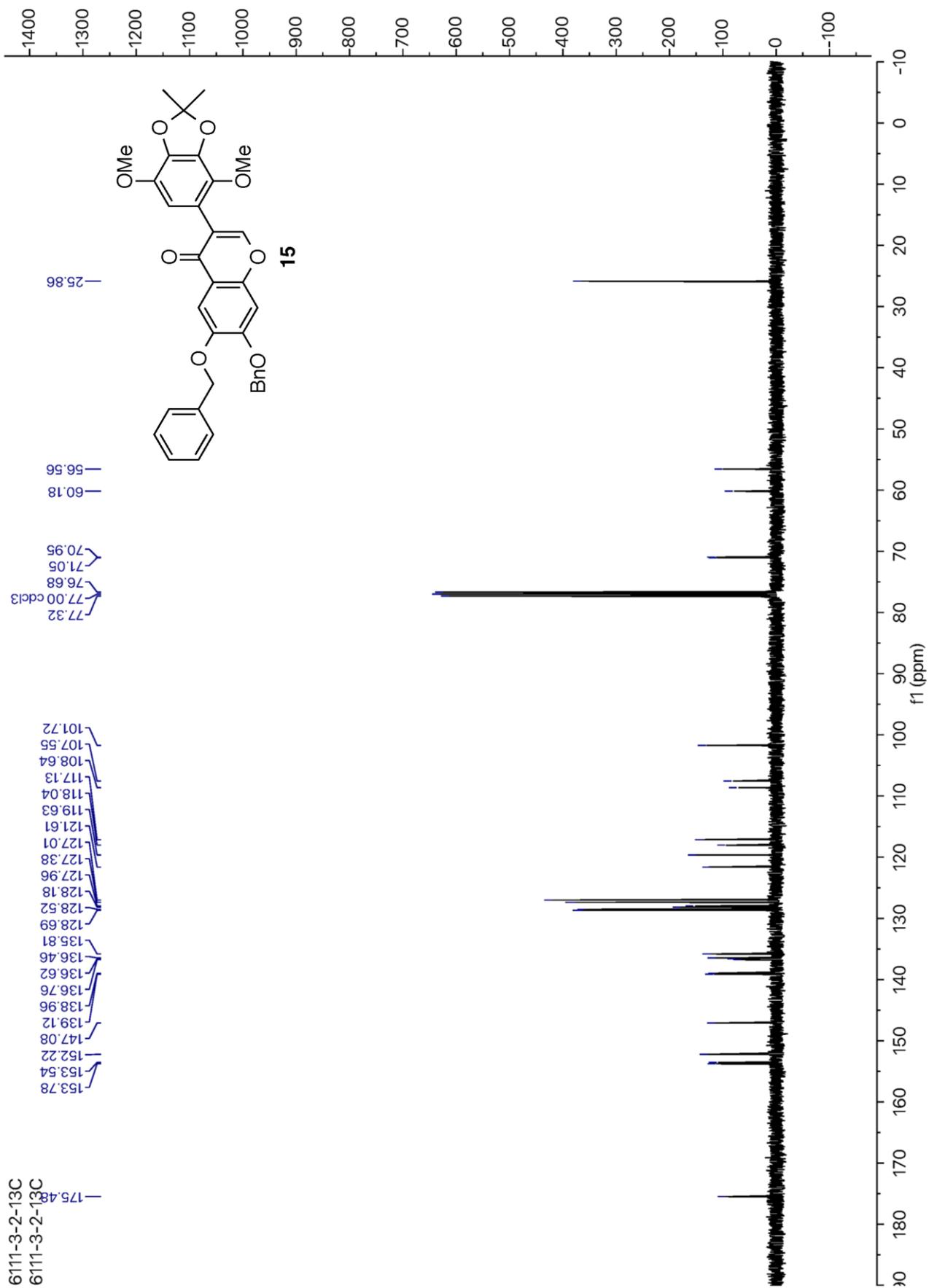
<sup>13</sup>C NMR spectra of **14** (100 MHz, CDCl<sub>3</sub>)

Supporting Information



<sup>1</sup>H NMR spectra of **15** (400 MHz, CDCl<sub>3</sub>)

Supporting Information



<sup>13</sup>C NMR spectra of **15** (100 MHz, CDCl<sub>3</sub>)