

Supporting Information

# **Fusarisetins: Structure-function studies on a novel class of cell migration inhibitors**

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## General Procedures

Unless indicated, all commercially available reagents and anhydrous solvents were purchased at the highest commercial quality and were used as received without further purification. All non-aqueous reactions were carried out under argon atmosphere using dry glassware that had been flame-dried under a stream of argon unless otherwise noted. Anhydrous tetrahydrofuran (THF) and dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh) using hexanes-EtOAc or CH<sub>2</sub>Cl<sub>2</sub>-MeOH mixtures of increasing polarity. The progress of all the reactions was monitored by thin-layer chromatography (TLC) using glass plates precoated with silica gel-60 F254 to a thickness of 0.5 mm (Merck), and compounds were visualized by irradiation with UV light and/or by treatment with a solution of ninhydrin stain or Ceric Ammonium Molybdate (CAM) stain followed by heating. <sup>13</sup>C NMR and <sup>1</sup>H NMR spectra were recorded on a 400 MHz, 500 MHz Varian instrument or a 500 MHz JEOL instrument. CDCl<sub>3</sub> was treated with flame dried K<sub>2</sub>CO<sub>3</sub>, chemical shifts (δ) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak reference (CDCl<sub>3</sub> or CD<sub>3</sub>OD), with the abbreviations s, br s, d, t, q, quint, m, td, dt and qd denoting singlet, broad singlet, doublet, triplet, quartet, quintet, multiplet, quartet of doublets, triplet of doublets, doublet of triplets and quartet of doublets, respectively. *J* = coupling constants given in Hertz (Hz). High resolution Mass spectra (HRMS) were recorded on a trisector WG AutoSpecQ spectrometer. Optical rotation data were collected on a Jasco P-1010 polarimeter using HPLC grade anhydrous CHCl<sub>3</sub> or anhydrous MeOH. Microwave experiments were carried out in Biotage (model:Initiator) microwave reactor using high pressure vessels. Cell cultures were incubated in NABCO CO<sub>2</sub> 6000 incubator and biological assays were performed in 24-well Falcon Multiwell (3047) cell dishes. Micrographs were processed with ImageJ software.

Fusarisetin A (**1**) and B (**2**) were synthesized as previously described.<sup>1</sup> The compounds were isolated after column chromatography as single isomers in greater than 95% purity (defined by <sup>1</sup>H NMR). **1** and **2** were spectroscopically identical (<sup>1</sup>H and <sup>13</sup>C NMR) to the ones reported in the initial isolation manuscript.<sup>2</sup> The synthesis was enantiopure since it departed from commercially available R-(+)-citronellal (greater than 95% ee). Fusarisetin A (**1**): [α]<sub>D</sub><sup>25</sup> = +85.3, *c* = 0.2, MeOH; natural:[α]<sub>D</sub><sup>25</sup> = +84.6, *c* = 0.2, MeOH. Fusarisetin B (**2**): [α]<sub>D</sub><sup>25</sup> = +51.2, *c* = 0.15, CHCl<sub>3</sub>; natural:[α]<sub>D</sub><sup>25</sup> = +84.9, *c* = 0.2, MeOH.

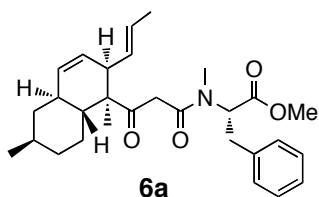
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<sup>1</sup> J. Xu, E. J. E. Caro-Diaz, M. H. Lacoske, C.-I. Hung, C. Jamora, and E. A. Theodorakis, *Chem. Sci.*, 2012, **3**, 3378–3386.

<sup>2</sup> J. H. Jang, Y. Asami, J.-P. Jang, S. O. Kim, D. O. Moon, K. S. Shin, D. Hashizume, M. Muroi, T. Saito, H. Oh, B. Y. Kim, H. Osada and J. S. Ahn, *J. Am. Chem. Soc.*, 2011, **133**, 6865–6867

## SYNTHETIC PROCEDURES

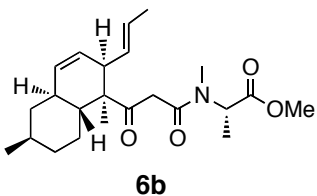
**Amino esters 5a-5d:** All amine coupling partners are commercially available from Sigma Aldrich (CDSO15644, PH006931) and FCH Group (FCH1120224, FCH1241743). They also can be readily prepared using standard reductive amination strategies.<sup>3</sup>



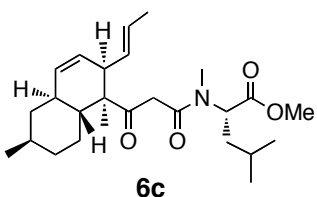
**C<sub>3</sub>-benzyl  $\beta$ -ketoamide 6a:** To a solution  $\beta$ -ketoacid **4**<sup>4</sup> (42 mg, 0.144 mmol) was transferred to a round bottom flask which contained *N*-methyl phenyl alanine methyl ester (33 mg, 0.173 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.6 mL). To this solution was added, DMF (0.1 mL), *O*-(7-azabenzotriazol-1-yl)-*N,N,N,N*-tetramethyluronium hexafluorophosphate (HATU, 54 mg, 0.144 mmol) and cooled to 0 °C, followed by adding in the diisopropylethylamine (DIPEA, 0.16 ml, 0.36 mmol) dropwise. The reaction was stirred at rt for 2 hrs before it was acidified with 2M HCl solution to pH = 2. The mixture was then diluted with EtOAc, sequentially washed with 2M HCl solution, NaHCO<sub>3</sub> and brine. The organic layer was dried over MgSO<sub>4</sub> and concentrated in *vacuo* to afford the desired ketoamide **6a** as a yellow oil (95%, 64 mg). This ketoamide was used directly to the next step without further purification. The <sup>1</sup>H NMR and <sup>13</sup>C NMR can be complicated due to enol-keto tautomers and amide rotamers. [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -80.4 (c = 1.0, CHCl<sub>3</sub>), <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, complicated by enol-keto tautomer and amide rotamers)  $\delta$ : 7.24 (m, 5H), 5.41-5.30 (m, 2H), 5.19 (m, 2H), 3.71 (m, 3H), 3.56 (d, *J* = 15.6 Hz, 1H), 3.35 (dd, *J* = 14.1, 5.5 Hz, 1H), 3.22 (d, *J* = 16.0 Hz, 1H), 3.04 (m, 1H), 2.79 (m, 3H), 2.47 (br m, 1H), 1.80-1.65 (m, 6H), 1.56 (d, *J* = 6 Hz, 3H), 1.57- 1.40 (m, 1H), 1.08 (s, 3H), 1.05 (m, 1H), 0.90 (m, 1H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.84 (m, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$ : 206.5, 171.2, 168.3, 137.3, 131.4, 130.7, 129.1, 128.6, 126.8, 126.5, 126.3, 58.7, 53.5, 52.4, 49.5, 45.6, 42.0, 39.8, 38.5, 35.6, 34.9, 33.8, 33.5, 29.8, 27.2, 22.6, 17.9, 17.1. HRMS (ESI) *m/e* 466.2952 [M+H<sup>+</sup>] calcd for C<sub>29</sub>H<sub>40</sub>NO<sub>4</sub><sup>+</sup>: 466.2950.

<sup>3</sup> White, K. N.; Konopelski, J. P. *Org. Lett.* **2005**, *7*, 4111–4112

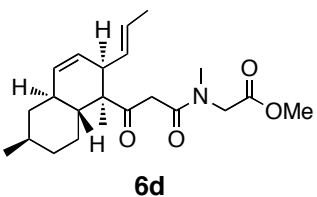
<sup>4</sup> J. Xu, E. J. E. Caro-Diaz, M. H. Lacoske, C.-I. Hung, C. Jamora, and E. A. Theodorakis, *Chem. Sci.*, 2012, **3**, 3378–3386



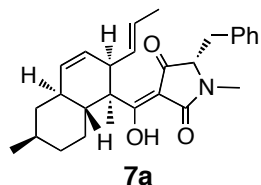
**C<sub>3</sub>-methyl β-ketoamide 6b:** Same procedure as **6a** yielded **6b** (35 mg, 92%) as pale yellow oil.  $[\alpha]_D^{25} = -104.9$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ),  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , complicated by enol-keto tautomer and amide rotamers)  $\delta$ : 5.40 (m, 2H), 5.16 (m, 2H), 3.71 (m, 3H), 3.44 (m, 2H), 2.86 (m, 3H), 2.54 (br m, 1H), 1.80-1.62 (m, 6H), 1.59 (d,  $J = 8.0$  Hz, 3H), 1.52- 1.38 (m, 4H), 1.24 (d,  $J = 5.2$  Hz, 3H), 1.22 (s, 3H), 1.20 – 0.96 (m, 1H), 0.90 (m, 1H), 0.89 (d,  $J = 6.9$  Hz, 3H), 0.84 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 207.1, 172.4, 168.1, 130.6, 129.4, 126.6, 126.0, 68.4, 52.6, 52.2, 42.0, 40.0, 38.5, 35.6, 33.5, 31.1, 29.8, 28.9, 27.3, 22.7, 17.9, 17.3, 14.6. HRMS (ESI)  $m/e$  390.2639  $[\text{M}+\text{H}^+]$  calcd for  $\text{C}_{23}\text{H}_{36}\text{NO}_4^+$ : 390.2637.



**C<sub>3</sub>-isobutyl β-ketoamide 6c:** Same procedure as **6a** yielded **6c** (31 mg, 87%) as pale yellow oil  $[\alpha]_D^{25} = -134.3$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ),  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , complicated by enol-keto tautomer and amide rotamers)  $\delta$ : 5.40 (m, 2H), 5.17 (m, 2H), 3.69 (s, 3H), 3.48 – 3.30 (m, 2H), 2.93 (m, 1H), 2.85 (s, 3H), 2.56 (br m, 1H), 1.80-1.62 (m, 6H), 1.59 (d,  $J = 6.0$  Hz, 3H), 1.52- 1.38 (m, 4H), 1.25 (s, 3H), 1.21 (s, 3H), 1.20 – 0.96 (m, 1H), 0.98 – 0.84 (m, 9H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 206.9, 171.9, 168.7, 131.1, 130.7, 126.9, 126.6, 59.9, 53.6, 51.9, 49.7, 46.4, 42.0, 39.9, 35.6, 33.5, 31.9, 29.8, 27.3, 24.8, 22.6, 18.0, 17.3, 15.7. HRMS (ESI)  $m/e$  432.3108  $[\text{M}+\text{H}^+]$  calcd for  $\text{C}_{26}\text{H}_{42}\text{NO}_4^+$ : 432.3110

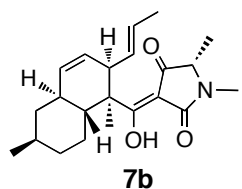


**C<sub>3</sub>-dihydro β-ketoamide 6d:** Same procedure as **6a** yielded **6d** (52 mg, 90%) as pale yellow oil.  $[\alpha]_D^{25} = -127.0$  ( $c = 4.6$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , complicated by enol-keto tautomer and amide rotamers)  $\delta$ : 5.36-5.31 (m, 2H), 5.22-5.12 (m, 2H), 4.20-4.06 (m, 2H), 3.76-3.70 (s, 3H), 3.46-3.33 (m, 1H), 3.03-2.95 (s, 3H), 2.55-2.38 (m, 1H), 1.78-1.56 (m, 9H), 1.25-1.21 (s, 3H), 1.12-0.92 (m, 3H), 0.85 (d,  $J = 6.4$  Hz, 3H), 0.83 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  206.8, 169.7, 168.2, 131.1, 130.5, 126.6, 126.5, 53.5, 52.2, 49.6, 49.3, 45.7, 42.0, 40.0, 38.4, 37.3, 35.6, 33.4, 27.2, 22.5, 17.9, 17.3; HRMS (ESI)  $m/e$  398.2303  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{22}\text{H}_{33}\text{NO}_4\text{Na}$ : 398.2302.



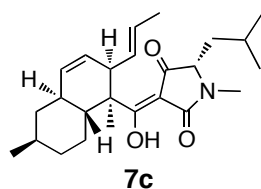
**C<sub>3</sub>-phenyl tetramic acid 7a:**  $\beta$ -ketoamide **6a** (45 mg, 0.097 mmol) was dissolved in MeOH (5.3 ml) and then NaOMe solution (0.97 mL, 0.5 M in MeOH) was added dropwise. The reaction was stirred at rt for 30 mins before it was quenched with 1M HCl. The mixture was diluted with water

and CH<sub>2</sub>Cl<sub>2</sub> and separated, the aqueous layer was further extracted with CH<sub>2</sub>Cl<sub>2</sub> (5x). The combined organic phase was dried over MgSO<sub>4</sub> and concentrated to yield tetramic acid **5a** (40 mg, 95%) as a red-brown oil.  $[\alpha]_D^{25} = -124.1$  ( $c = 1.0$ , CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  : 7.22 (m, 3H), 7.12 (m, 2H), 5.39 (br s, 2H), 5.24 (m, 1H), 5.16 (m, 1H), 3.83 (t,  $J = 4.5$  Hz, 1H), 3.74 (m, 1H), 3.36 (br s, 1H), 2.91 (br s, 4H), 1.84 -1.58 (m, 4H), 1.50-1.40 (m, 2H), 1.53 (d,  $J = 4.2$  Hz, 3H), 1.33 (s, 3H), 1.15-0.95 (m, 3H), 0.90 (d,  $J = 6.6$  Hz, 3H), 0.87 (m, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  : 198.1, 191.1, 176.7, 135.4, 131.1, 130.0, 129.5, 128.6, 127.1, 126.8, 124.4, 100.4, 67.0, 53.6, 48.7, 45.2, 42.4, 39.9, 38.7, 35.8, 33.6, 29.8, 28.9, 22.6, 18.1, 13.8; HRMS (ESI)  $m/e$  456.2509 [M+Na<sup>+</sup>] calcd for C<sub>28</sub>H<sub>35</sub>NO<sub>3</sub>Na<sup>+</sup>: 456.2510



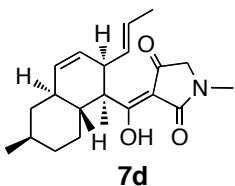
**C<sub>3</sub>-methyl tetramic acid 7b:** Same procedure as **7a** afforded **7b** (32 mg, 99%) as red-brown oil.  $[\alpha]_D^{25} = -148.9$  ( $c = 1.0$ , CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  : 5.39 (br s, 2H), 5.27 (m, 1H), 5.19 (m, 1H), 3.70 (m, 1H), 3.42 (br s, 1H), 2.98 (s, 3H), 1.97 (m, 1H), 1.86-1.58 (m, 4H), 1.56 (m, 3H),

1.46 (d,  $J = 5.0$  Hz, 3H), 1.40 (m, 3H), 1.19-1.05 (m, 3H), 0.91 (d,  $J = 6.3$  Hz, 3H), 0.86 (m, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) : 198.7, 192.3, 176.0, 131.2, 130.5, 130.0, 126.9, 100.3, 57.4, 48.6, 45.2, 42.4, 40.1, 38.5, 35.8, 33.7, 29.7, 28.4, 26.9, 22.5, 18.0, 15.2; HRMS (ESI)  $m/e$  380.2196 [M+Na<sup>+</sup>] calcd for C<sub>22</sub>H<sub>31</sub>O<sub>4</sub>NNa<sup>+</sup>: 380.2194

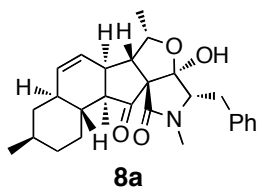


**C<sub>3</sub>-isobutyl tetramic acid 7c:** Same procedure as **7a** afforded **7c** (28 mg, 99%) as red-brown oil.  $[\alpha]_D^{25} = -178.3$  ( $c = 1.0$ , CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  : 5.40 (br s, 2H), 5.24 (m, 2H), 3.59 (m, 1H), 3.36 (br s, 1H), 2.98 (s, 3H), 1.97 (m, 1H), 1.81 (m, 3H), 1.68 (m, 1H), 1.46 (d,  $J =$

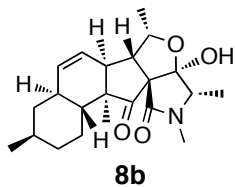
5.0 Hz, 3H), 1.34 (d,  $J = 6.9$  Hz, 3H), 1.13-1.04 (m, 3H), 0.91 (d,  $J = 6.3$  Hz, 3H), 0.84 (m, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) : 204.5, 191.8, 176.6, 131.5, 131.1, 130.0, 127.1, 101.1, 65.0, 48.5, 44.9, 42.4, 40.0, 38.7, 35.9, 33.7, 29.8, 28.5, 27.2, 24.9, 22.6, 18.1, 17.8, 14.3, 12.4; HRMS (ESI)  $m/e$  422.2666 [M+Na<sup>+</sup>] calcd for C<sub>25</sub>H<sub>37</sub>O<sub>3</sub>NNa<sup>+</sup>: 422.2663



**C<sub>3</sub>-dihydro tetramic acid 7d:** Same procedure as **7a** afforded **7d** (47 mg, 99%) as red-brown oil.  $[\alpha]_D^{25} = -189.6$  ( $c = 2.2$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  : 5.39 (br s, 2H), 5.10 (m, 2H), 3.62 (m, 2H), 3.32 (br s, 1H), 3.02 (s, 3H), 1.95 (m, 1H), 1.86-1.58 (m, 4H), 1.53 (d,  $J = 5.2$  Hz, 3H), 1.50-1.40 (m, 3H), 1.15-0.95 (m, 3H), 0.90 (d,  $J = 6.6$  Hz, 3H), 0.87 (m, 1H);  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  : 198.1, 188.7, 176.8, 131.0, 129.9, 127.1, 126.8, 100.6, 57.0, 48.6, 45.1, 42.3, 40.1, 38.7, 35.8, 33.6, 28.9, 28.4, 22.6, 18.0, 14.2; HRMS (ESI)  $m/e$  366.2039  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{21}\text{H}_{29}\text{NO}_3\text{Na}$ : 366.2040.

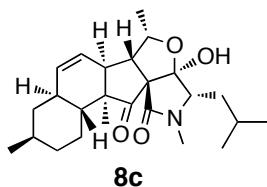


**C<sub>3</sub>-phenyl fusarisetin 8a:** A solution of tetramic acid (**7a**, 12 mg, 0.027 mmol) in acetic acid (0.1 ml) was added ceric ammonium nitrate (CAN, 16 mg, 0.027 mmol). The mixture was stirred at rt under oxygen atmosphere (1 atm, balloon) for 30 mins. The reaction was diluted with  $\text{CH}_2\text{Cl}_2$ , passed through a short silica pad, washed with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (20:1) and concentrated in *vacuo*. The residue obtained above was then dissolved in anhydrous MeOH (0.5 ml), followed by the addition of thiourea (22 mg, 0.27 mmol). This reaction was heated in a sealed microwave vial at  $70^\circ\text{C}$  for 1 hr. The reaction was allowed to cool to rt and was concentrated in *vacuo*. The crude product was purified *via* preparative TLC using  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (95/5) to afford **6a** (5.2 mg, 42%) as a semisolid.  $[\alpha]_D^{25} = +58.5$  ( $c = 0.53$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  : 7.29 (m, 4H), 7.22 (m, 1H), 5.72 (m, 1H), 5.52 (d,  $J = 9.7$  Hz, 1H), 4.45 (m, 1H), 3.81 (m, 1H), 3.15 (m, 1H), 3.08 (m, 1H), 3.02 (dd,  $J = 10.8, 6.3$  Hz, 1H), 2.73 (s, 3H), 2.60 (dd,  $J = 10.9, 4.6$  Hz, 1H), 2.49 (s, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.42 (d,  $J = 6.3$  Hz, 3H), 1.05 (m, 1H), 1.00 (s, 3H), 0.89 (d,  $J = 6.9$  Hz, 3H), 0.85 (m, 1H);  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta$  : 211.7, 169.3, 138.3, 133.0, 129.5, 129.2, 126.9, 125.4, 109.1, 79.8, 75.1, 70.4, 54.5, 54.2, 43.5, 41.9, 37.5, 36.9, 36.8, 35.2, 33.2, 30.0, 29.9, 25.5, 22.6, 17.9, 14.3. HRMS (ESI)  $m/e$  472.2458  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{28}\text{H}_{35}\text{O}_4\text{NNa}^+$ : 472.2459.

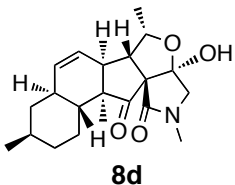


**C<sub>3</sub>-methyl tetramic acid 8b:** Same procedure as **8a** afforded **8b** (6.2 mg, 25%) as a semisolid.  $[\alpha]_D^{25} = +46.1$  ( $c = 0.62$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  : 5.72 (m, 1H), 5.52 (d,  $J = 10.4$  Hz, 1H), 4.56 (qd,  $J = 6.3, 3.5$  Hz,

1H), 3.97 (m, 2H), 3.52 (dd,  $J = 6.9, 2.9$  Hz, 1H), 2.92 (s, 3H), 2.73 (dd,  $J = 10.3, 4.0$  Hz, 1H), 2.37 (dd,  $J = 9.8, 4.6$  Hz, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.26 (d,  $J = 6.4$  Hz, 3H), 1.05 (m, 2H), 0.96 (s, 3H), 0.89 (d,  $J = 6.3$  Hz, 3H), 0.85 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 213.1, 169.9, 132.5, 124.6, 109.3, 83.7, 74.8, 67.4, 59.2, 57.7, 55.4, 50.3, 41.7, 36.8, 36.5, 35.2, 33.0, 29.1, 25.4, 22.5, 22.4, 14.2; HRMS (ESI)  $m/e$  396.2145  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{25}\text{H}_{37}\text{NO}_4\text{Na}$ : 396.2143.

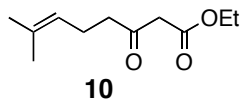


**8c:** Same procedure as **8a** afforded **8c** (4.0 mg, 21%) as a semisolid.  $[\alpha]_D^{25} = +34.7$  ( $c = 0.4$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.71 (ddd,  $J = 10.3, 4.9, 2.5$  Hz, 1H), 5.53 (d,  $J = 9.8$  Hz, 1H), 4.42 (quint,  $J = 6.4$ , 1H), 3.97 (m, 2H), 3.52 (dd,  $J = 6.9, 2.9$  Hz, 1H), 2.92 (s, 3H), 2.73 (dd,  $J = 10.3, 4.0$  Hz, 1H), 2.37 (dd,  $J = 9.8, 4.6$  Hz, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.26 (d,  $J = 6.4$  Hz, 3H), 1.05 (m, 2H), 0.96 (s, 3H), 0.89 (d,  $J = 6.3$  Hz, 3H), 0.85 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 213.1, 169.9, 132.5, 124.6, 109.3, 83.7, 74.8, 67.4, 59.2, 57.7, 55.4, 50.3, 41.7, 36.8, 36.5, 35.2, 33.0, 29.1, 25.4, 22.5, 22.4, 14.2; HRMS (ESI)  $m/e$  438.2615  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{25}\text{H}_{37}\text{NO}_4\text{Na}$ : 438.2617.

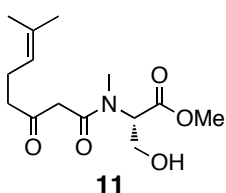


**8d:** Same procedure as **8a** afforded **8d** (7.1 mg, 32%) as a semisolid.  $[\alpha]_D^{25} = +44.6$  ( $c = 0.6$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.71 (ddd  $J = 10.3, 5.2, 2.9$  Hz, 1H), 5.53 (d,  $J = 10.3$  Hz, 1H), 4.31 (qd,  $J = 6.3, 6.3$  Hz, 1H), 3.62 (d,  $J = 10.3$  Hz, 1H), 3.52 (d,  $J = 10.3$  Hz, 1H), 3.04 (br s, 1H), 2.92 (dd,  $J = 10.9, 5.7$  Hz, 1H), 2.90 (s, 3H), 2.48 (dd,  $J = 10.3, 4.6$  Hz, 1H), 1.82 (m, 2H), 1.73 (m, 1H), 1.63 (m, 1H), 1.54 (m, 2H), 1.45 (d,  $J = 6.9$  Hz, 3H), 1.09 (qd,  $J = 13.2, 3.5$  Hz, 1H), 1.01 (qd,  $J = 11.5, 3.4$  Hz, 1H), 0.97 (s, 3H), 0.89 (d,  $J = 6.9$  Hz, 3H), 0.85 (q,  $J = 12.6$  Hz, 1H);  $^{13}\text{C}$  NMR (200 MHz,  $\text{CDCl}_3$ ):  $\delta$  213.0, 169.4, 132.8, 125.2, 107.2, 77.9, 74.3, 61.1, 54.5, 54.4, 43.4, 41.7, 37.2, 36.5, 35.2, 33.0, 29.8, 25.5, 22.4, 16.8, 14.0; HRMS (ESI)  $m/e$  382.1990  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{21}\text{H}_{29}\text{NO}_4\text{Na}$ : 382.1989.

## Synthesis of CDE ring core analog



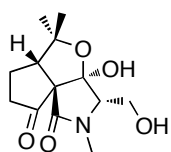
**$\beta$ -keto ester 10:** To a solution of ethyl acetoacetate (1.0 g, 0.98 mL, 7.68 mmol) in THF at 0 °C was added all at once NaH (60% in mineral oil, 340 mg, 8.45 mmol). The reaction was allowed to stir for 30 mins and then *n*-BuLi (1.6 M in Hexanes, 5.8 mL, 8.07 mmol) was added drop wise to form a bright blood-orange solution. The mixture was stirred at this temperature for 30 mins, followed by rapid addition of 3,3-dimethylallyl bromide. The reaction was stirred at 0 °C for 3 hours, then allowed to reach room temperature and stirred for an additional 4 hours when TLC showed consumption of starting material. The reaction mixture was then quenched with saturated NH<sub>4</sub>Cl solution and extracted with diethyl ether (3x). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by silica flash column chromatography to yield  $\beta$ -keto ester **8** (1.1 g, 73 %) as a clear oil. *R*<sub>f</sub>: 0.3 (10:1, Hex:EtOAc); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 5.05 (t, *J* = 8.8 Hz, 1H), 4.19 (q, *J* = 8.8 Hz, 1H), 3.43 (s, 2H), 2.56 (t, *J* = 9.4 Hz, 2H), 2.27 (q, *J* = 8.8 Hz, 1H), 1.67 (s, 3H), 1.61 (s, 3H), 1.28 (t, *J* = 8.8 Hz, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  : 203.1, 169.6, 134.7, 119.7, 61.2, 59.7, 29.0, 26.9, 25.7, 17.7, 14.0; HRMS (ESI) *m/e* 198.1256 [M+Na]<sup>+</sup> calcd for C<sub>11</sub>H<sub>18</sub>O<sub>3</sub>Na<sup>+</sup>: 198.1257.



**$\beta$ -ketoamide 11:** To a solution of  $\beta$ -keto ester **10** (110 mg, 0.56 mmol) in EtOH (2 mL) was added KOH (1M in H<sub>2</sub>O, 1 mL) at room temperature. The reaction mixture was stirred for 10 hours at which time the reaction was diluted with CH<sub>2</sub>Cl<sub>2</sub> and was quenched with 1M HCl. The layers were separated and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude residue was used without further purification. To a solution of crude  $\beta$ -keto acid (40 mg, 0.24 mmol) and freshly prepared *N*-methyl serine methyl ester (45 mg, 0.34 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.8 mL) was added consecutively HOBt (70.4 mg, 0.46 mmol) and EDC (71.4 mg, 0.45 mmol) at room temperature. The reaction was stirred overnight at this temperature and was then diluted with EtOAc and quenched with 1M HCl. The aqueous layers was extracted with EtOAc (3x), washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude residue was used was purified via silica flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH, 200:1 to 20:1) to yield **9** (65 mg, 96%) as a pale



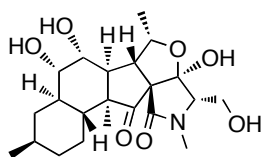
yellow oil.  $R_f$ : 0.44 (15:1,  $\text{CH}_2\text{Cl}_2$ :MeOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ , complicated by enol-keto tautomer and amide rotamers)  $\delta$ : 5.11 - 5.05 (m, 1H), 4.83 (dd,  $J = 7.3, 5.4$  Hz, 1H), 4.54 (dd, minor), 4.06 (dd,  $J = 11.6, 4.9$  Hz, 1H), 4.00 (dd,  $J = 11.8, 7.4$  Hz, 1H), 3.78 (s, minor), 3.76 (s, 3H), 3.67 (s, minor), 3.62 (s, 2H), 3.04 (s, minor), 3.02 (s, 3H), 2.59 (t,  $J = 7.4$  Hz, 2H), 2.27 (q,  $J = 7.5$  Hz, 2H), 1.66 (s, 3H), 1.61 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 204.5, 169.9, 168.1, 133.4, 122.2, 61.0, 60.4, 52.6, 49.7, 43.1, 35.6, 25.8, 22.4, 17.9; HRMS (ESI)  $m/e$  308.1468  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{14}\text{H}_{23}\text{O}_5\text{NNa}^+$ : 308.1470.



**12**

**CDE ring analog 10:**  $\beta$ -ketoamide **11** (45 mg, 0.16 mmol) was dissolved in MeOH (5.3 ml) and then NaOMe solution (1.6 ml, 0.5 M in MeOH) was added dropwise. The reaction was stirred at rt for 30 mins before it was quenched with 1M HCl. The mixture was diluted with water and  $\text{CH}_2\text{Cl}_2$ , separated, and the aqueous layer was further extracted with  $\text{CH}_2\text{Cl}_2$  (5x). The combined organic phase was dried over  $\text{MgSO}_4$  and concentrated to yield the corresponding tetramic acid (40 mg, 98%) as a dark red oil. This tetramic acid was dissolved in acetic acid (0.8 mL) and ceric ammonium nitrate (CAN, 88 mg, 0.16 mmol) was added. The reaction was stirred under oxygen atmosphere (1 atm) for 30 mins and then diluted with  $\text{CH}_2\text{Cl}_2$  (5 ml), passed through a short silica pad, washed with  $\text{CH}_2\text{Cl}_2$ /MeOH (20:1, 20 ml) and concentrated in *vacuo*. The crude residue above was dissolved in anhydrous MeCN (0.8 ml), followed by the addition of CuCl (160 mg, 1.6 mmol). This reaction was stirred at rt for 2 hr, and was then concentrated in *vacuo* and purified via preparative TLC ( $\text{CH}_2\text{Cl}_2$ :MeOH 25:1, 4 times) to afford tricyclic core analog **10** (6.2 mg, 14 %) as a clear oil.  $R_f$ : 0.22 (15:1,  $\text{CH}_2\text{Cl}_2$ :MeOH);  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 3.92 (m, 2H), 3.53 (t,  $J = 4.0$  Hz, 1H), 3.1 (t,  $J = 9.2$  Hz, 1H), 2.94 (s, 3H), 2.49 (m, 2H), 2.14 (m, 2H), 1.45 (s, 3H), 1.18 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 211.9, 170.7, 108.9, 87.2, 68.8, 59.6, 55.1, 41.2, 30.6, 29.9, 29.2, 25.2, 22.7; HRMS (ESI)  $m/e$  269.1263  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{13}\text{H}_{19}\text{O}_5\text{NNa}^+$ : 269.1261

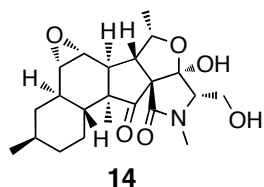
### Synthesis of Analogs 13-16



**13**

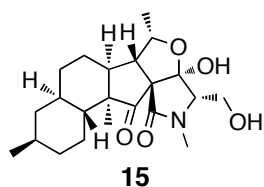
**Dihydroxy fusarisetin 13 :** To a solution of **1** (4 mg, 0.01 mmol) in acetone: $\text{H}_2\text{O}$  (9:1, 0.5 mL) was added sequentially NMO (1.4 mg, 0.01 mmol) and  $\text{OsO}_4$  (4% w/w in  $\text{H}_2\text{O}$ , 13  $\mu\text{L}$ ) at room temperature. The

reaction mixture was stirred for 16 hours was then diluted with EtOAc and water, extracted with EtOAc (3x), washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by prep TLC (20:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH 3x) to yield **13** (2.6 mg, 62%) as a white solid.  $[\alpha]_D^{25} = +65.6$  ( $c = 0.26$ , CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 4.39 (quint,  $J = 6.9$  Hz, 1H), 4.00-3.90 (m, 3H), 3.56 (dd,  $J = 4.6, 2.3$  Hz, 1H), 3.25 (dd,  $J = 12.1, 6.3$  Hz, 1H), 2.94 (s, 3H), 2.25 (m, 1H), 1.76-1.63 (m, 4H), 1.55-1.45 (m, 2H), 1.39 (d,  $J = 6.4$  Hz, 3H), 1.04 (s, 3H), 0.98-0.91 (m, 2H), 0.84 (d,  $J = 6.3$  Hz, 3H), 0.80 (m, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$ : 211.4, 169.4, 108.6, 83.8, 78.7, 74.7, 73.3, 69.5, 69.1, 67.2, 59.0, 54.2, 47.6, 47.3, 38.3, 37.0, 36.5, 34.5, 32.0, 29.0, 25.4, 22.6, 17.1; HRMS (ESI)  $m/e$  446.2149 [M+Na]<sup>+</sup> calcd for C<sub>22</sub>H<sub>33</sub>O<sub>7</sub>NNa<sup>+</sup>: 446.2150.



**Epoxy fusarisetin 14** : To a solution of **1** (3 mg, 0.0077 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.13 mL) at 0°C was added *m*-CPBA (70% w/w, 2.3 mg). The reaction was allowed to reach room temperature and stirred for 3 hours.

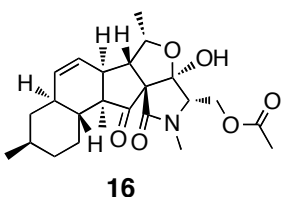
The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, quenched with saturated aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and saturated aq. NaHCO<sub>3</sub> (1:1) and stirred for 15 mins. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x). The combined organic layers were washed with saturated aq. NaHCO<sub>3</sub>, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude residue was purified by prep TLC (30:1, CH<sub>2</sub>Cl<sub>2</sub>:MeOH, 4x) to yield **14** (2.4 mg, 77%) as a white powder.  $[\alpha]_D^{25} = +72.8$  ( $c = 0.24$ , CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 5.72 (m, 1H), 5.52 (d,  $J = 10.4$  Hz, 1H), 4.56 (qd,  $J = 6.3, 3.5$  Hz, 1H), 3.97 (m, 2H), 3.52 (dd,  $J = 6.9, 2.9$  Hz, 1H), 2.92 (s, 3H), 2.73 (dd,  $J = 10.3, 4.0$  Hz, 1H), 2.37 (dd,  $J = 9.8, 4.6$  Hz, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.26 (d,  $J = 6.4$  Hz, 3H), 1.05 (m, 2H), 0.96 (s, 3H), 0.89 (d,  $J = 6.3$  Hz, 3H), 0.85 (m, 1H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$ : 213.2, 169.3, 109.0, 78.8, 73.8, 68.9, 59.2, 57.6, 51.1, 48.8, 41.0, 35.6, 34.7, 33.2, 33.0, 32.1, 28.7, 25.2, 22.9, 14.3; HRMS (ESI)  $m/e$  428.2044 [M+Na]<sup>+</sup> calcd for C<sub>22</sub>H<sub>31</sub>O<sub>6</sub>NNa<sup>+</sup>: 428.2041.



**Saturated decalin fusarisetin 15**: To a solution of **1** (3 mg, 0.0077 mmol) in EtOAc (0.1 mL) was added Pd/C (0.5 mg). The mixture was stirred under an atmosphere of H<sub>2</sub> (5 atm) for 1 hour, at which time the mixture was filtered through a plug of celite, rinsed with EtOAc and

concentrated to yield analog **15** (2.5 mg, 95%) as a clear oil.  $[\alpha]_D^{25} = +79.4$  ( $c = 0.25$ , CHCl<sub>3</sub>);

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 4.56 (quint,  $J = 6.4$  Hz, 1H), 4.05-3.91 (m, 3H), 3.55 (dd,  $J = 4.9$ , 2.5 Hz, 1H), 3.25 (dd,  $J = 11.7$ , 5.9 Hz, 1H), 2.94 (s, 3H), 2.23 (m, 1H), 1.73-1.59 (m, 4H), 1.57-1.42 (m, 3H), 1.39 (d,  $J = 6.9$  Hz, 3H), 1.31 (m, 2H), 1.04 (s, 3H), 0.98-0.91 (m, 2H), 0.84 (d,  $J = 6.9$  Hz, 3H), 0.78 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 212.2, 169.9, 132.5, 124.6, 109.3, 83.7, 74.8, 67.4, 59.2, 57.7, 55.4, 50.3, 41.7, 36.8, 36.5, 35.2, 33.0, 29.1, 25.4, 22.5, 22.4, 14.2; HRMS (ESI)  $m/e$  414.2251  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{22}\text{H}_{33}\text{O}_5\text{NNa}^+$ : 414.2254.



**Aceto-fusarisetin 16:** To a solution of **1** (4 mg, 0.01 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.3 mL) at  $0^\circ\text{C}$  was added sequentially 4-DMAP (1.3 mg, 0.01) and  $\text{Ac}_2\text{O}$  (0.1 mL, 0.1 M in  $\text{CH}_2\text{Cl}_2$ ). The reaction mixture was raised to r.t. temperature a stirred for 30 mins diluted with  $\text{CH}_2\text{Cl}_2$  then quenched with saturated  $\text{NH}_4\text{Cl}$ . The mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3x), the combined organic layers washed with saturated  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The residue was purified by prep TLC (hexanes:EtOAc, 20:1, 3x) to yield **16** (3.4 mg, 78%) as a pale yellow oil.  $[\alpha]_D^{25} = +52.1$  ( $c = 0.34$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.72 (ddd,  $J = 10.3$ , 5.2, 2.9 Hz, 1H), 5.53 (d,  $J = 9.7$  Hz, 1H), 4.46 (dd,  $J = 12.0$ , 6.3 Hz, 1H), 4.39 (m, 1H), 3.63 (dd,  $J = 6.9$ , 4.0 Hz, 1H), 2.97 (dd,  $J = 11.5$ , 5.7 Hz, 1H), 2.94 (s, 3H), 2.58 (dd,  $J = 9.5$ , 4.6 Hz, 1H), 2.14 (s, 3H), 1.87-1.78 (m, 3H), 1.75-1.67 (m, 2H), 1.60-1.51 (m, 2H), 1.46 (d,  $J = 6.3$  Hz, 3H), 1.11-1.05 (m, 2H), 0.98 (s, 3H), 0.89 (d,  $J = 6.9$  Hz, 3H), 0.83 (m, 1H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$ : 211.8, 170.7, 169.4, 133.1, 125.3, 107.9, 78.9, 74.6, 67.9, 61.6, 54.5, 43.5, 41.8, 37.5, 36.7, 33.1, 29.2, 25.5, 22.9, 22.5, 21.0, 17.4, 14.3; HRMS (ESI)  $m/e$  454.2200  $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{24}\text{H}_{33}\text{O}_6\text{NNa}^+$ : 454.2201.

## BIOLOGICAL ASSAYS AND PROCEDURES

### Immunofluorescence Assay:

*Cell culturing:* MDA-MB-231 cells ( $1 \times 10^4$  cells/24-well plate) were plated in 12-well cell culture dishes (with circular cover slips installed inside the wells) and incubated for 24 hrs at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS). The wells were then aspirated and given DMEM (10% FBS) containing 10  $\mu\text{g}/\text{mL}$  of Cytochalasin D (**2**) (Sigma Aldrich, C8273), 10  $\mu\text{g}/\text{mL}$  of Fusarisetin A (isolated as described

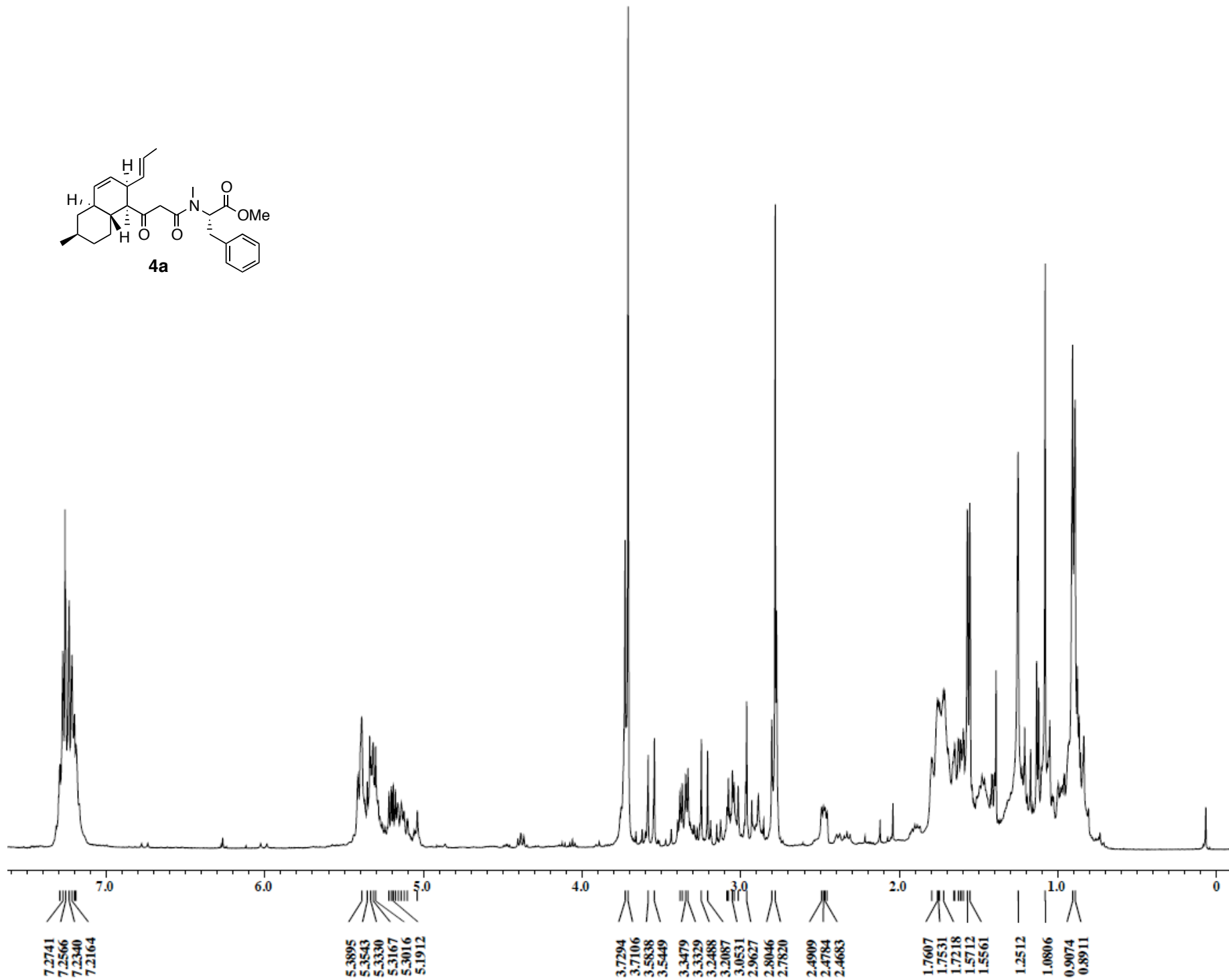
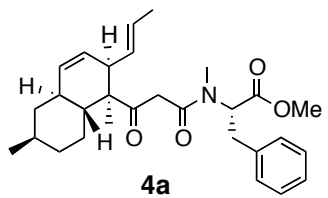
in General Procedures, purity greater than 95%) or appropriate amount of DMSO (vehicle control) then incubated for 4 hrs (37 °C, 5% CO<sub>2</sub>). At t = 4hrs, select coverslips were removed from wells and washed for fixing. Media was then removed from remaining wells containing Cytochalasin D by aspiration and wells were washed with 10% Phosphate-buffered Saline (PBS, 3x), given DMEM (10% FBS) containing 10 mg/mL of Fusarisetin A, or appropriate amount of DMSO (vehicle control) and incubated for 4 hrs (37 °C, 5% CO<sub>2</sub>). At t = 8hrs, coverslips were removed and washed for fixing. The well containing Fusarisetin A (since t = 0) was allowed to incubate for an additional 16 hrs (37 °C, 5% CO<sub>2</sub>) at which time the coverslip was removed and washed (PBS 3x) for fixing.

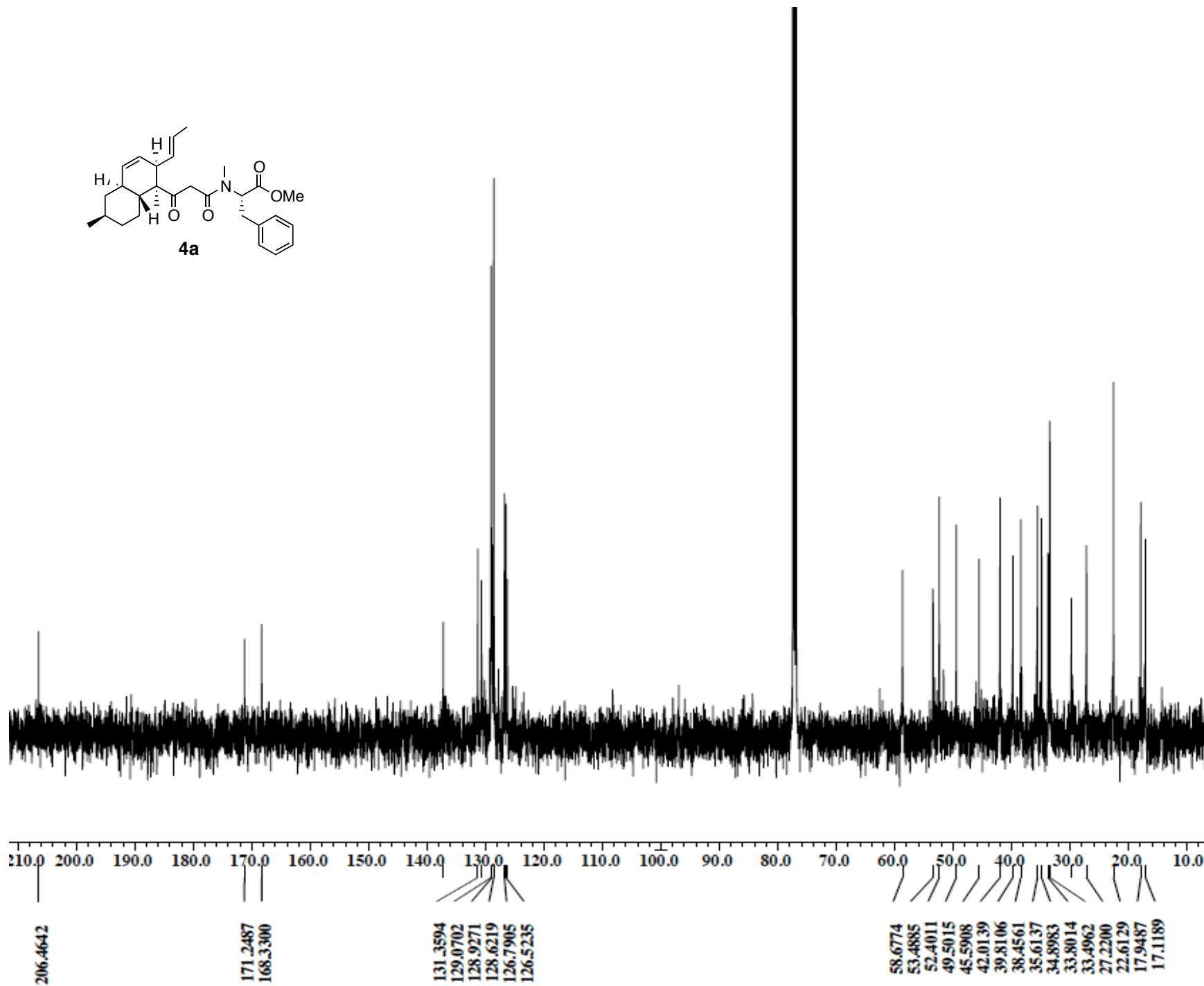
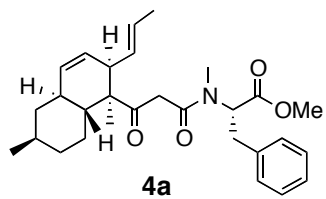
*Fixing and Staining Protocol:* All removed cover slips were washed with PBS (3x) then treated with paraformaldehyde (4% in PBS) for 10 mins. The formaldehyde solution was aspirated and the slips were washed with PBS (3x). At this time, they were treated with blocking buffer [0.1% Triton X, 3% bovine serum albumin (BSA) both in PBS] for 30 mins. Then the slips were treated with FITC-conjugated Phalloidin (Sigma Aldrich, P5282) and Rhodamine-conjugated anti-tubulin antibody (Cytoskeleton Inc., TL590M) both at 1:100 dilution in blocking buffer for 1hr, then washed with PBS (3x). The coverslips were finally mounted onto microscope slides with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Life Technologies, D1306) solution. Fluorescent images were taken at 40x magnification.

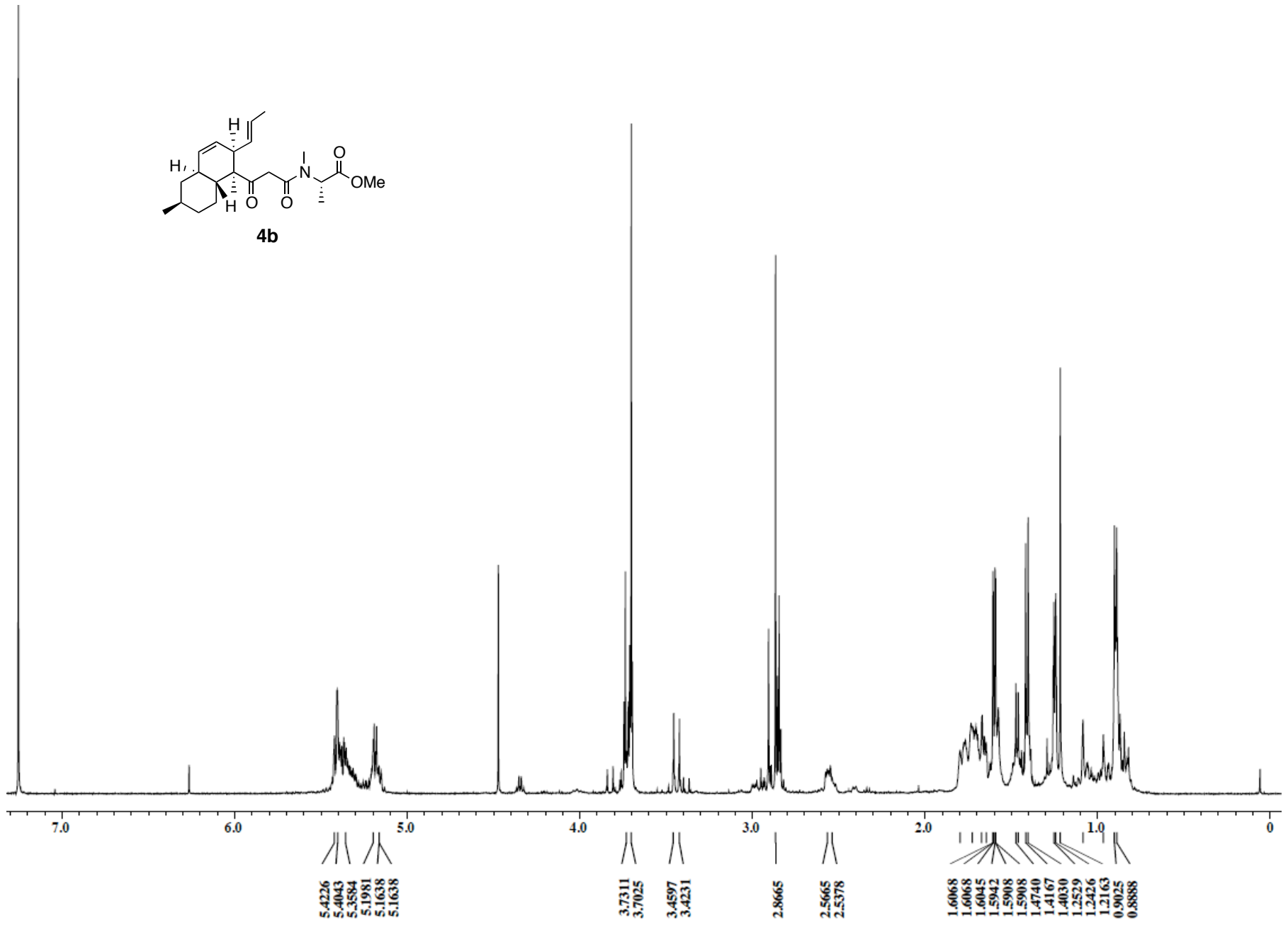
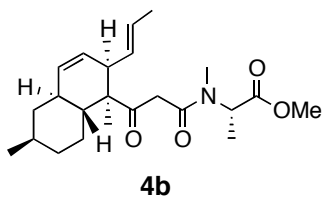
**Scratch wound assay:** This assay was used for general screening of compounds. MDA-MB-231 cells (5 x10<sup>5</sup> cells/24-well plate) were plated in cell culture dishes, and after 24 h of incubation at 37 °C in 5% CO<sub>2</sub> in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), the confluent monolayer of cells was scratched with a pipette tip to create a cell-free zone in each well. The medium was aspirated and each well washed with 10% Phosphate-buffered Saline (PBS) solution to remove any detached cells. The PBS was aspirated and replaced with fresh DMEM medium (500 µL) containing fusarisetins (1, 3, 10, 50, 100 and 200 µg/mL) and corresponding amount of DMSO (vehicle control, < 5%). The scratch-wounds (cell-free zones) were photographed under a microscope (10x magnification) at time = 0 , then after 48 to determine migration of the cells into the cell free-zone, the cells were photographed again under microscope (10x magnification). Relative migration values were determined (means ± SD for triplicate, n = 3) by the ratio *original wound area/area occupied by migrated cells* (as

measured by ImageJ software) then standardized to DMSO controls. *Note:* All fusarisetins were synthesized as described above and purified using column chromatography or preparative TLC; their purity was greater than 85% as determined by  $^1\text{H}$  NMR. With the exception of compound **11** that was synthesized as a racemic mixture, all other fusarisetins were synthesized in enantiomerically pure form.

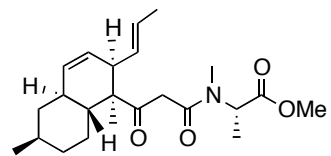
**Boyden-Chamber Transwell assay:** Transwell cell migration assays were carried out using Transwell membrane filter inserts (BioExpress Transwell PC well insert, 6.5 mm diameter) in a 24-well tissue-culture plate. The Transwell filter has 8  $\mu\text{m}$  pore-size membranes. MDA-MB-231 cells ( $5 \times 10^5$  cells/well) suspended in serum free DMEM medium with diverse concentrations of fusarisetin A were added to the upper chambers, and DMEM medium containing 10% FBS was placed in the lower well, then incubated for 24 h at 37 °C in 5%  $\text{CO}_2$ . Non-invading cells on the upper surface of the membrane were removed by wiping them out with a cotton swab, and migrated cells on the lower surface were fixed with 4% formaldehyde solution and stained with Crystal Violet staining solution. The number of invaded cells per membrane was counted under a light microscope at 10x magnification. Values were determined counting cells that had migrated across the membrane insert and calculating the ratio of migrated cells with fusarisetins (**13** and **16**, at variable concentrations 1-127  $\mu\text{M}$ ) to migrated cells with the corresponding concentration of DMSO. The values are presented as the means  $\pm$  SD for triplicate samples ( $n = 3$ ).



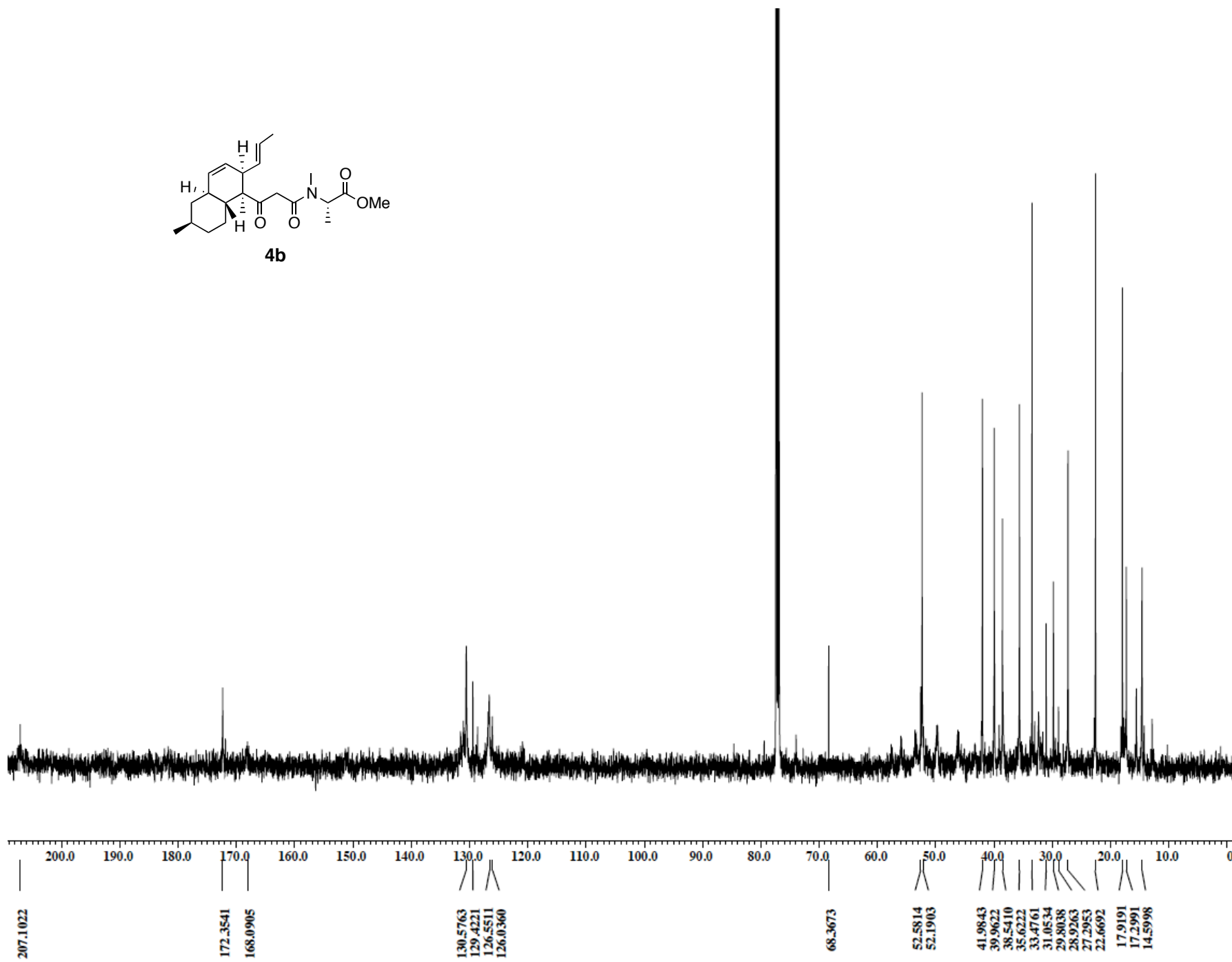


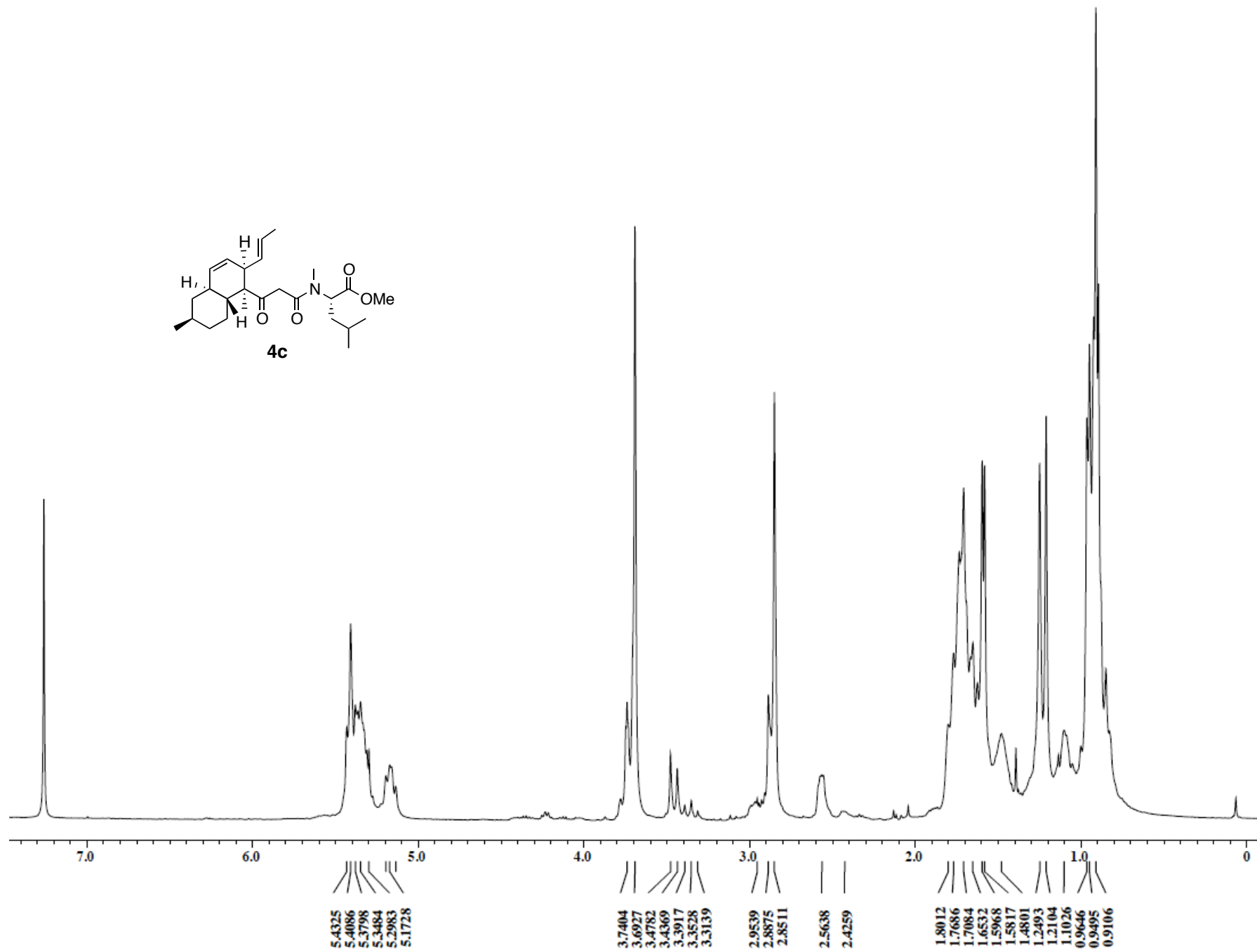
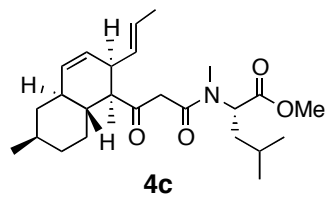


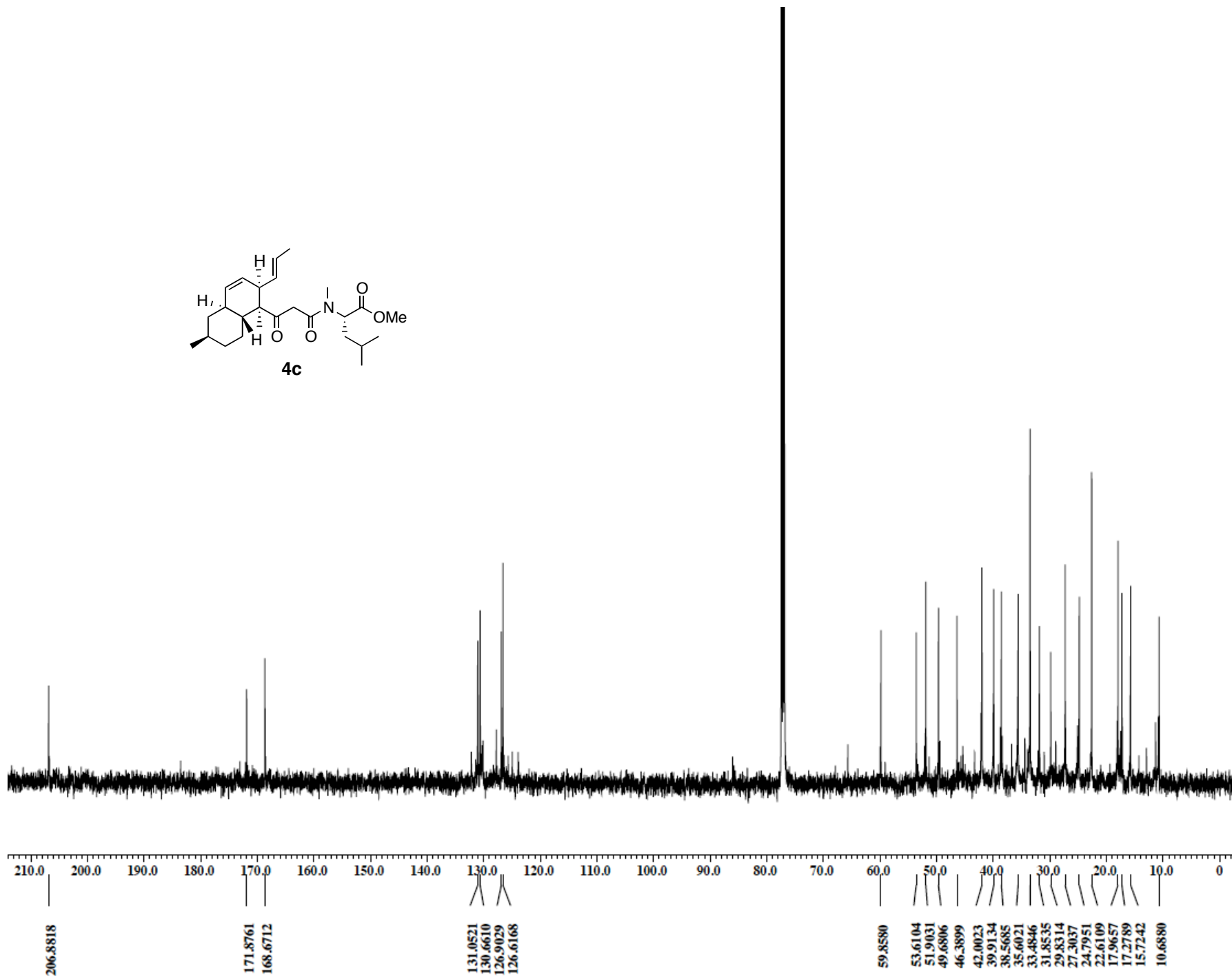
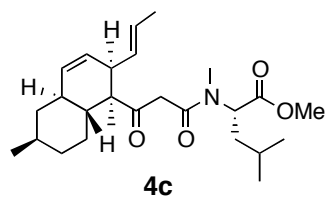


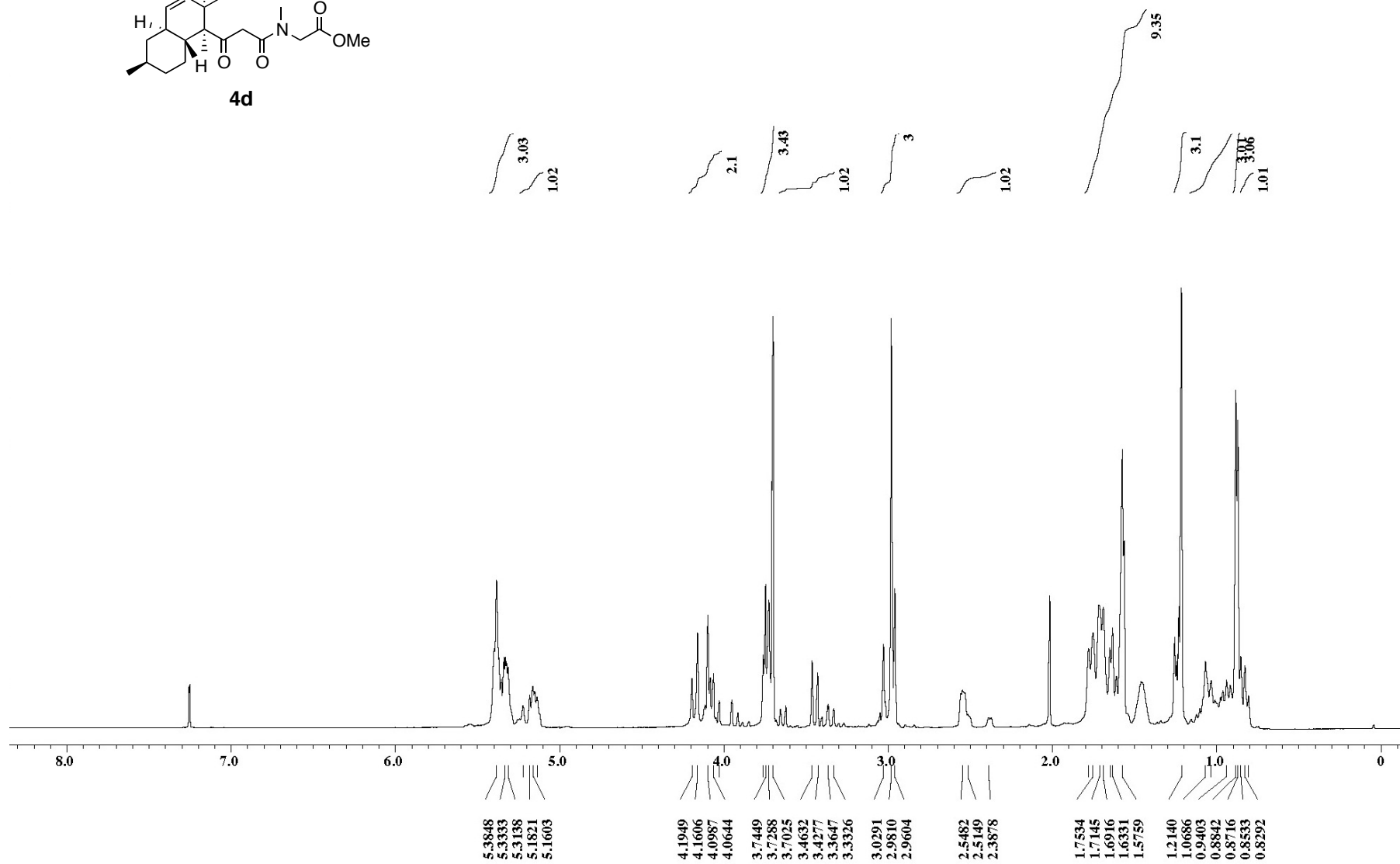
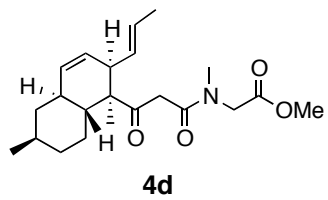


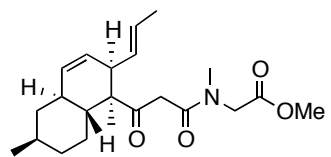
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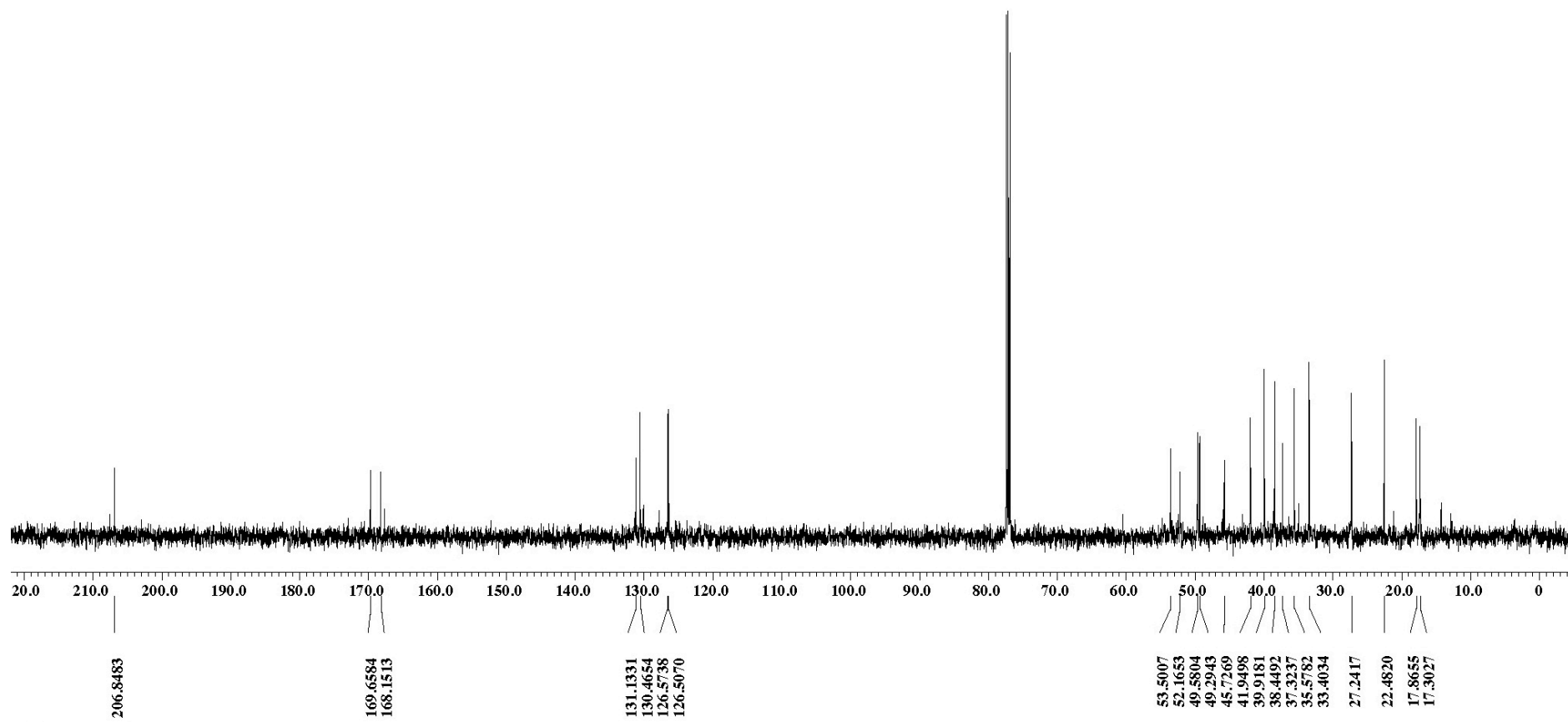


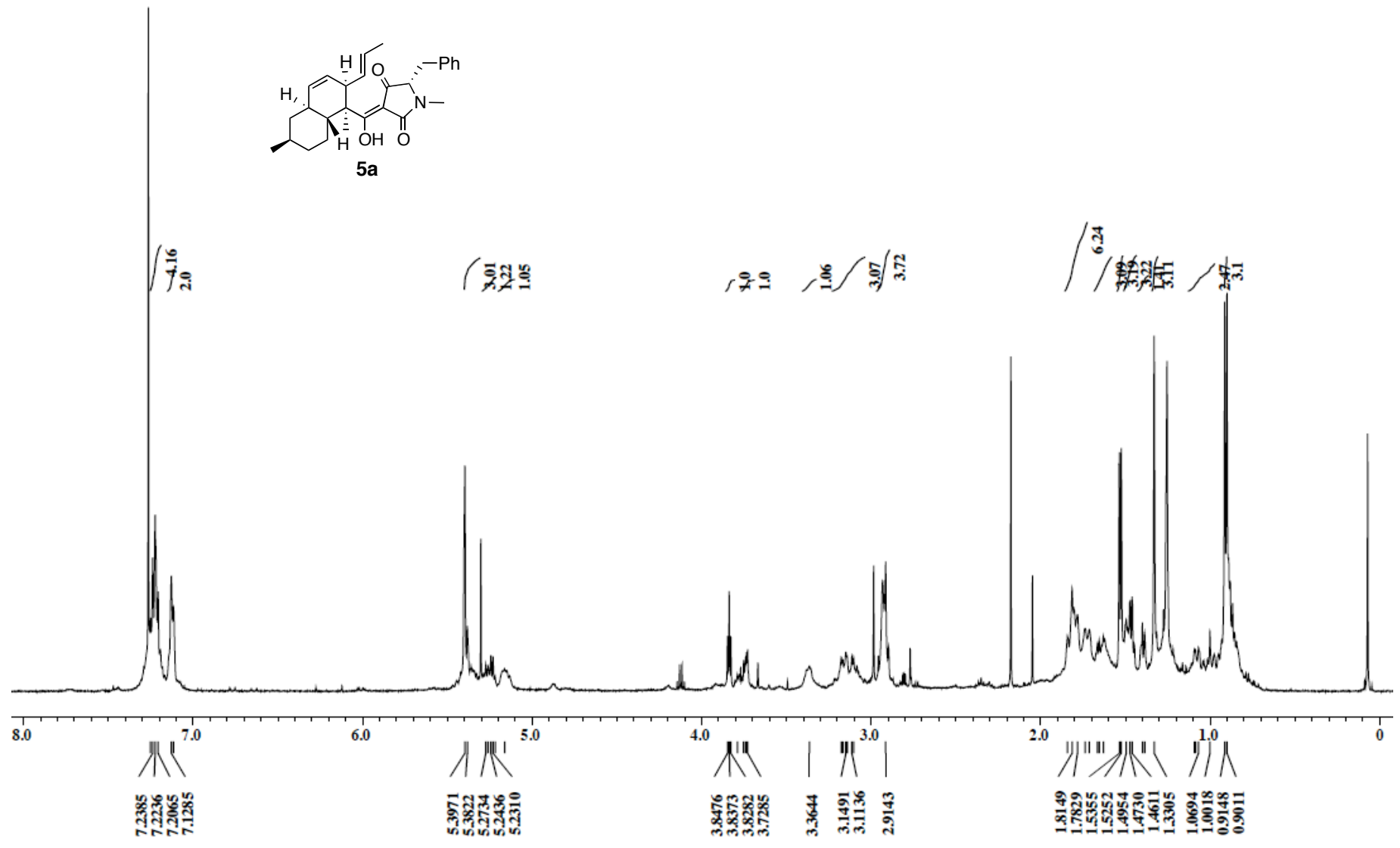
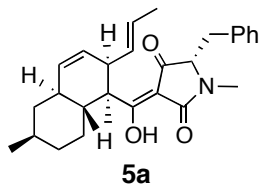


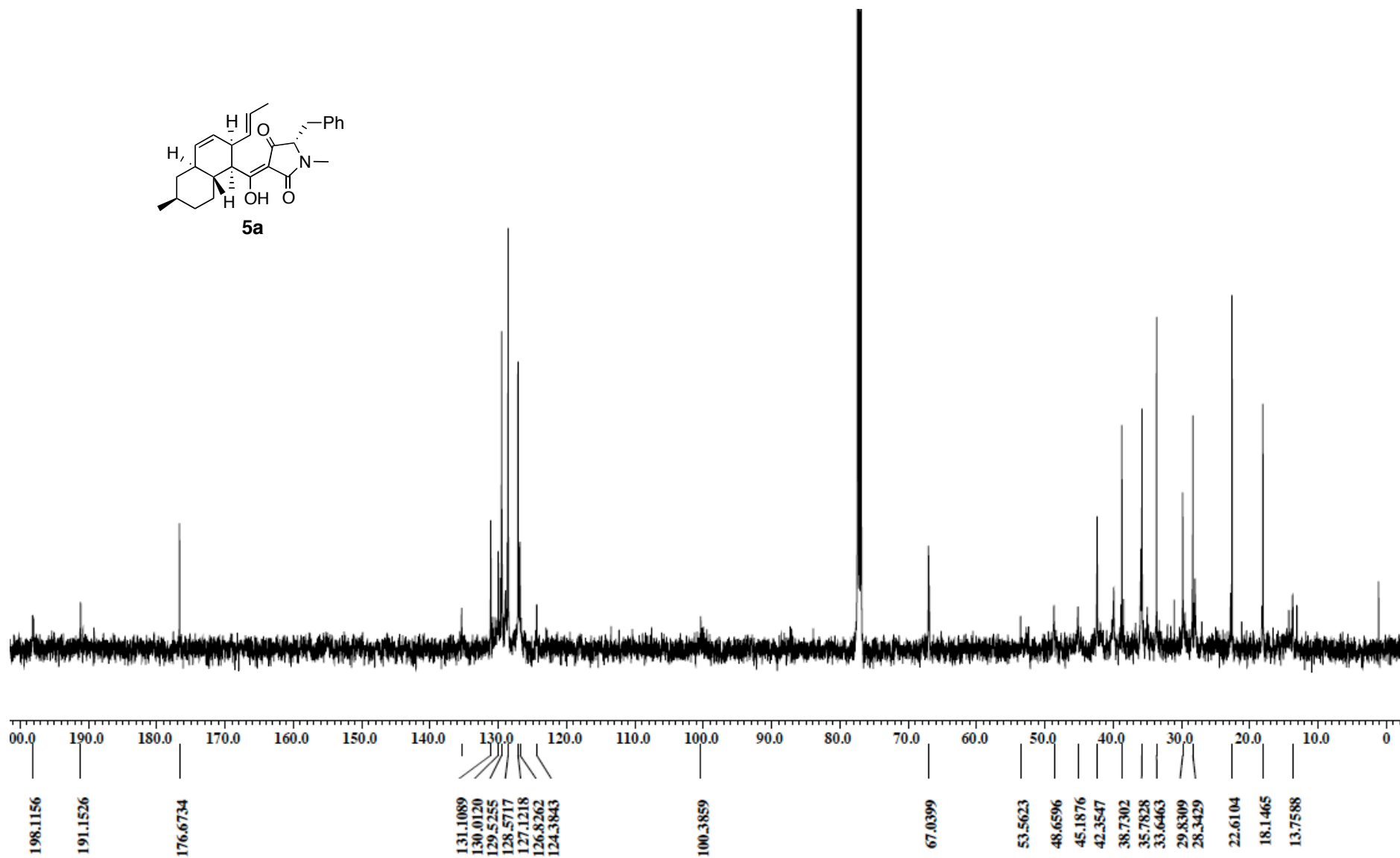
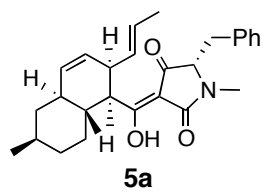


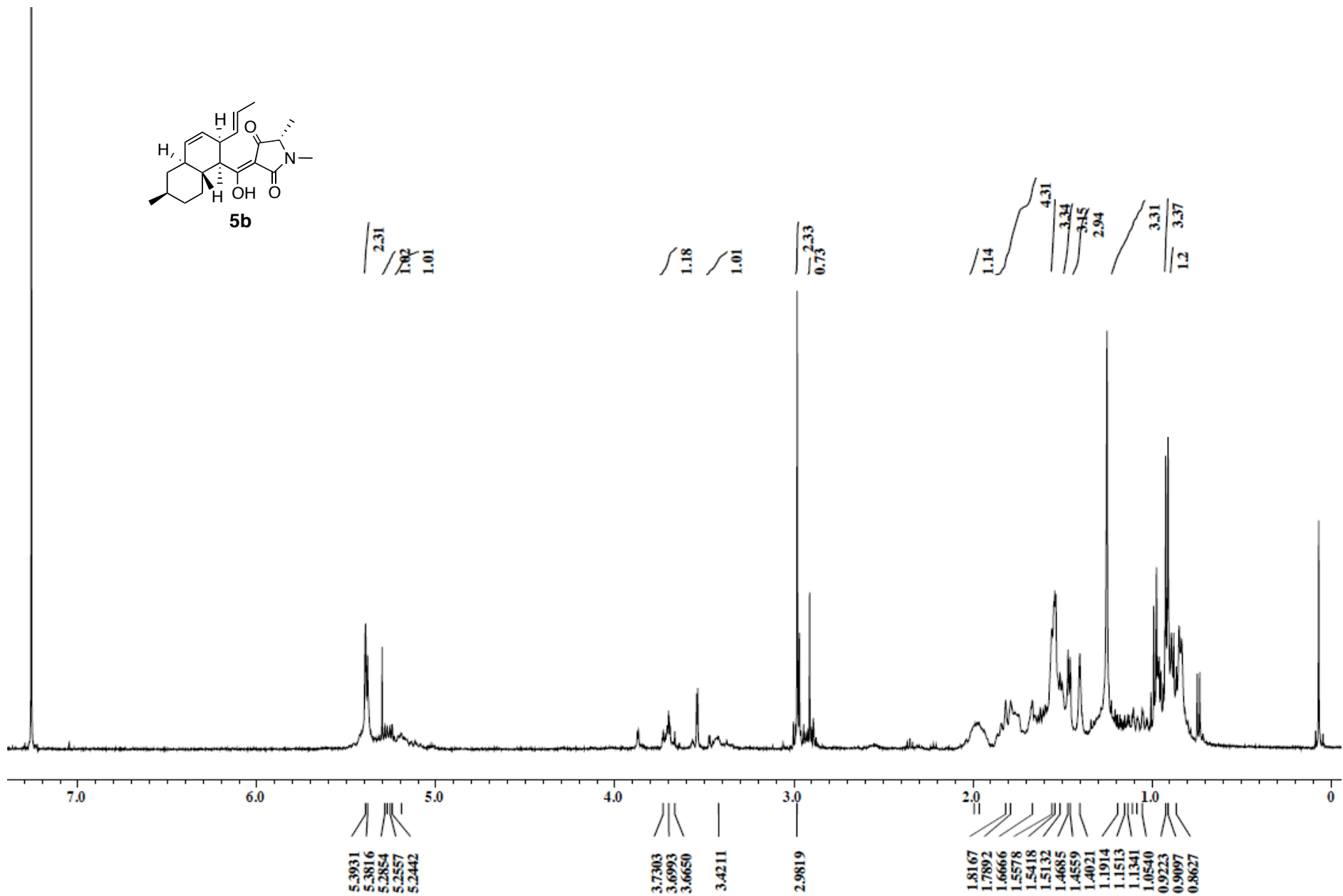
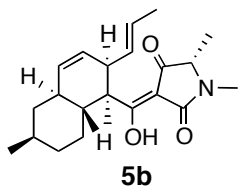


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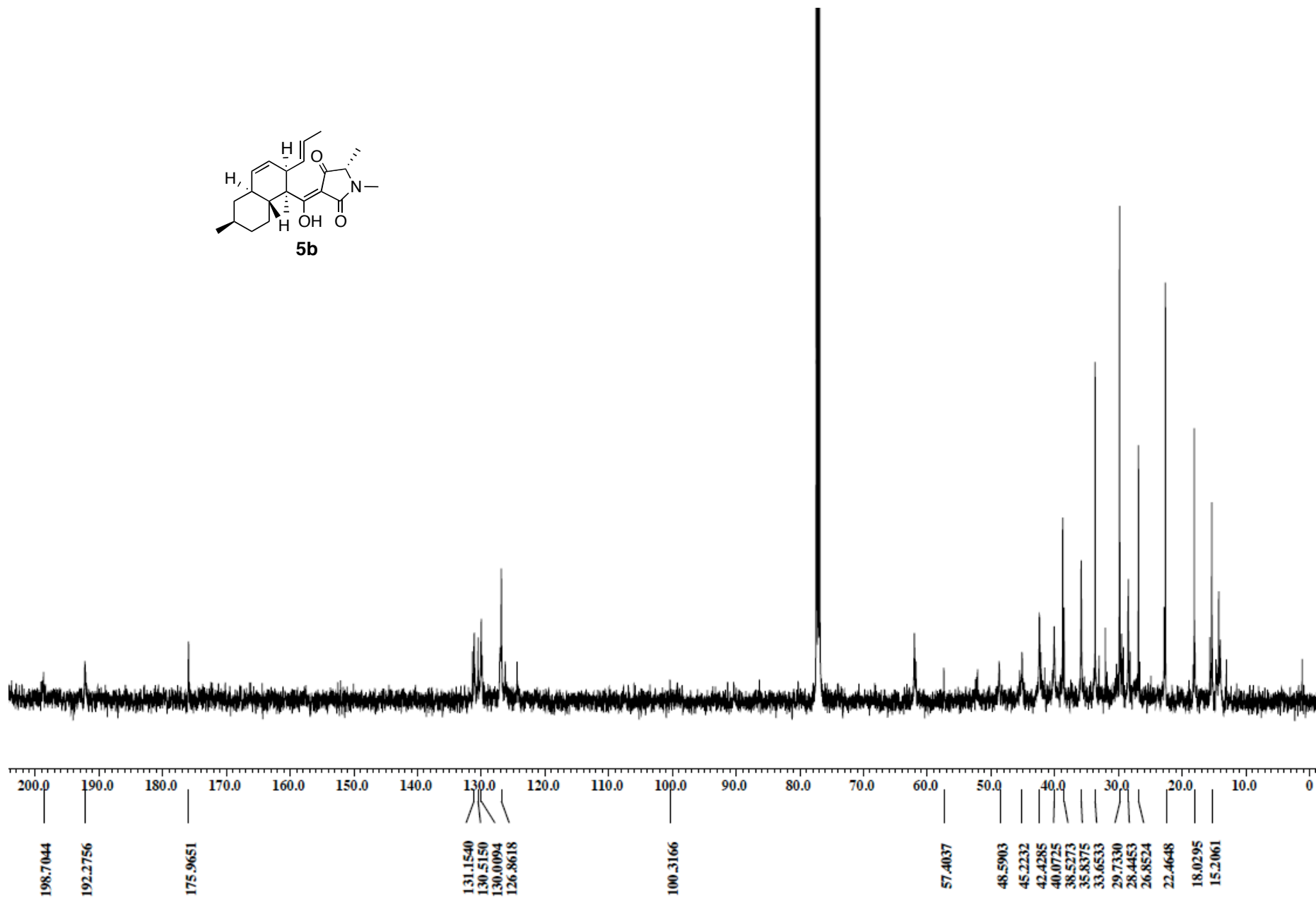
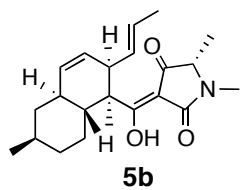


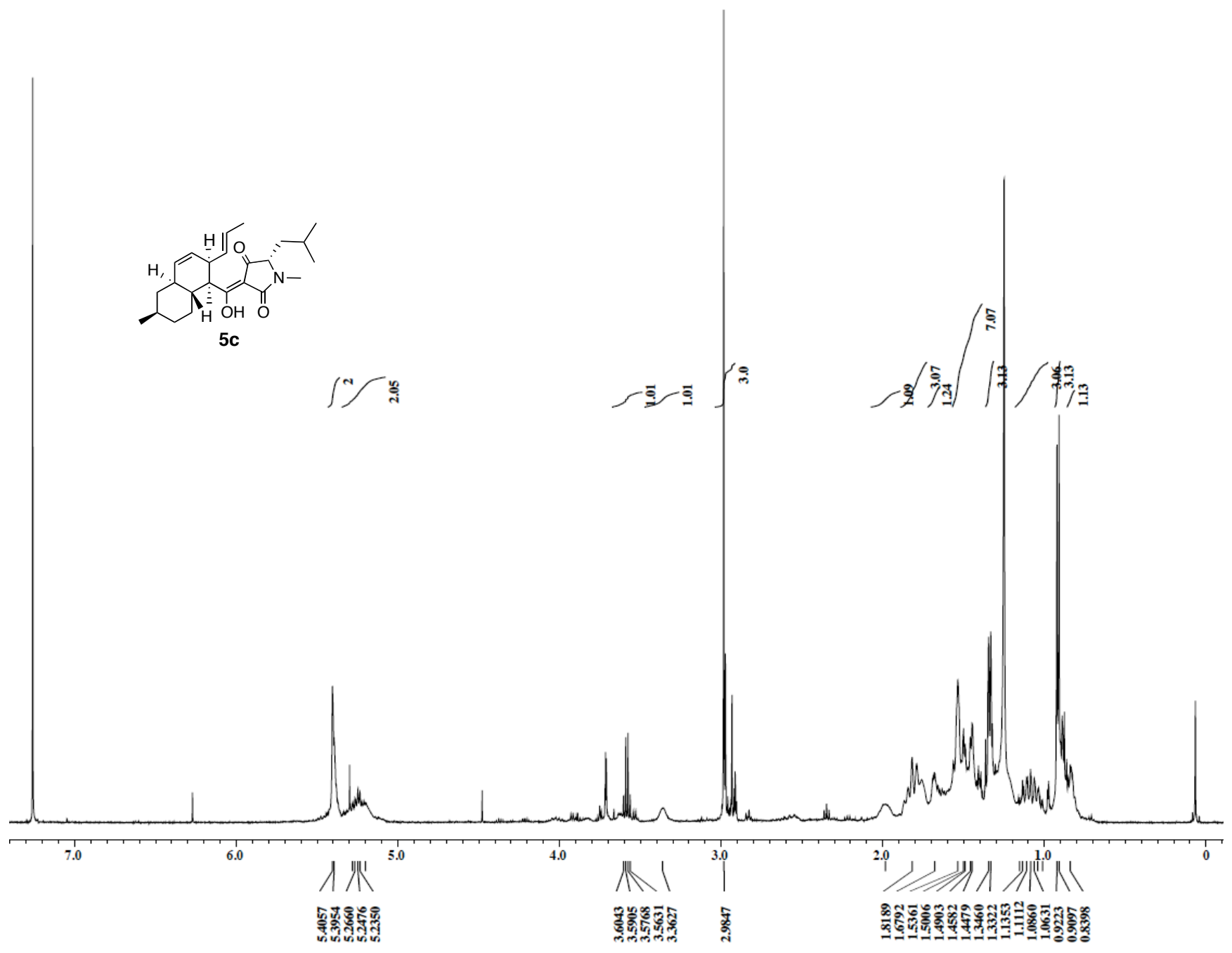
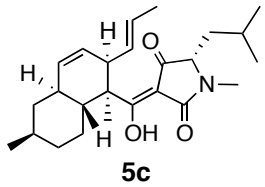


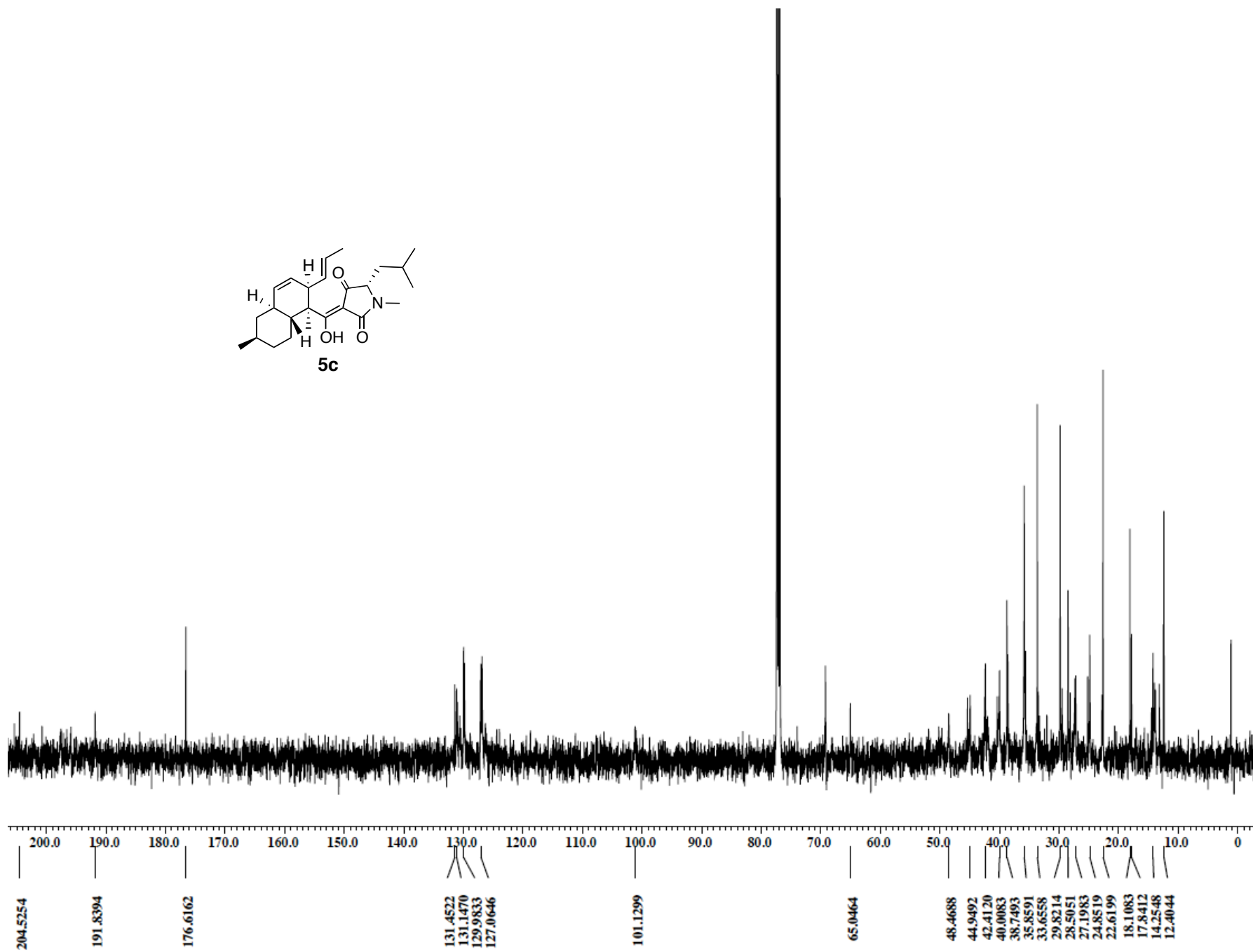
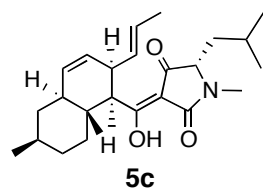


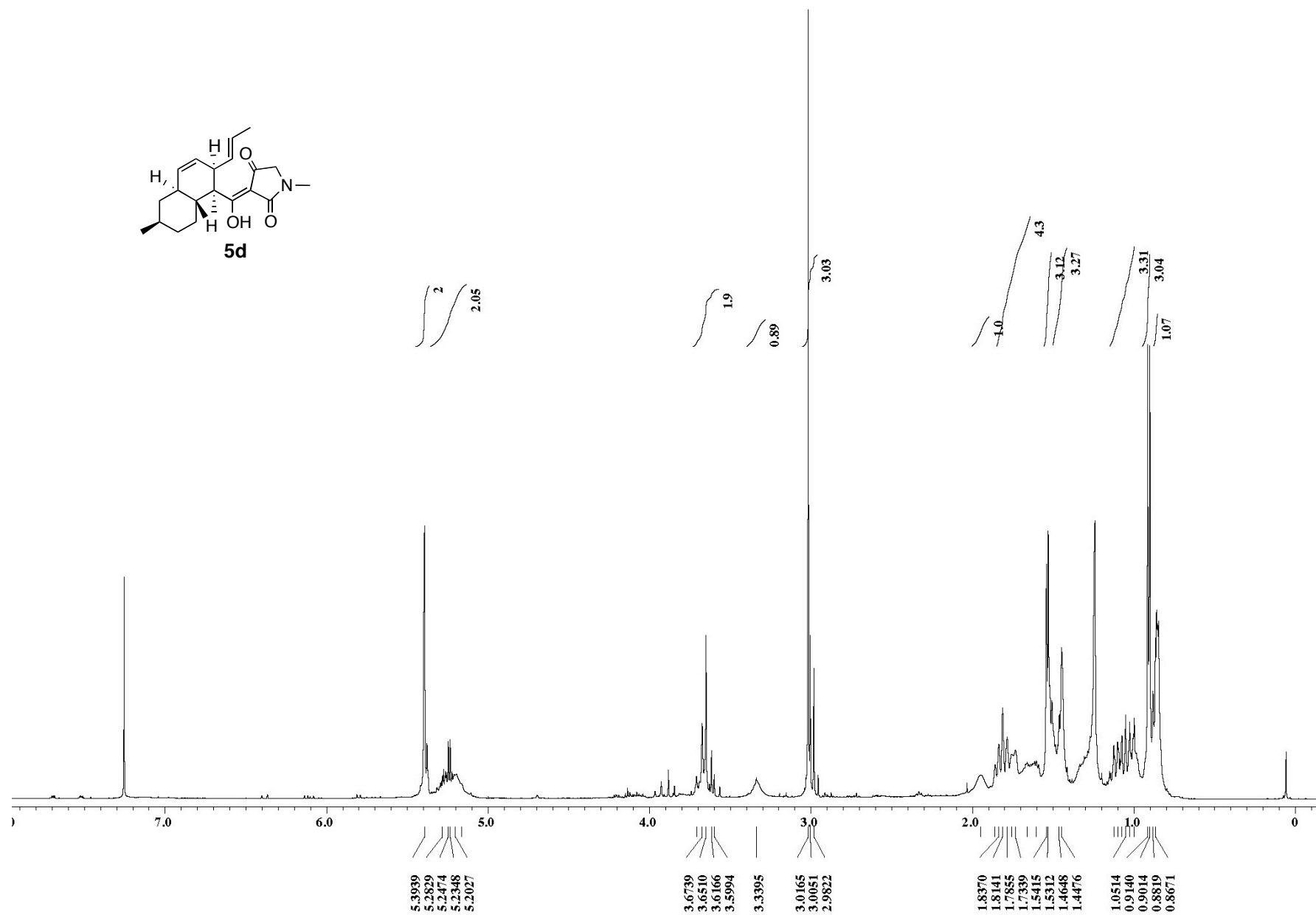
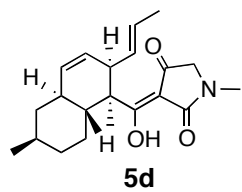


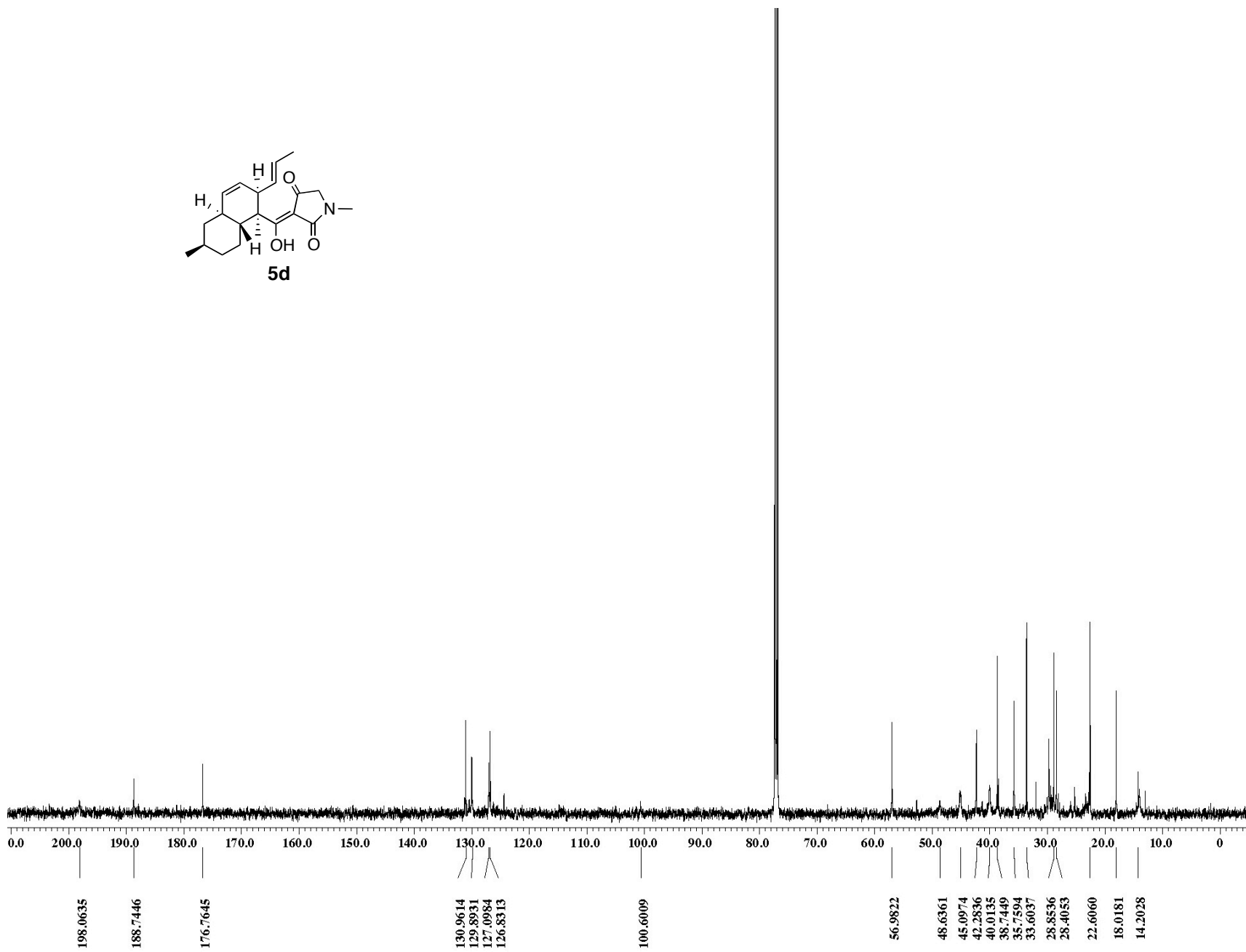
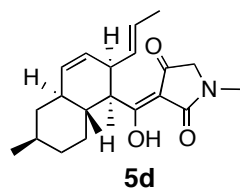


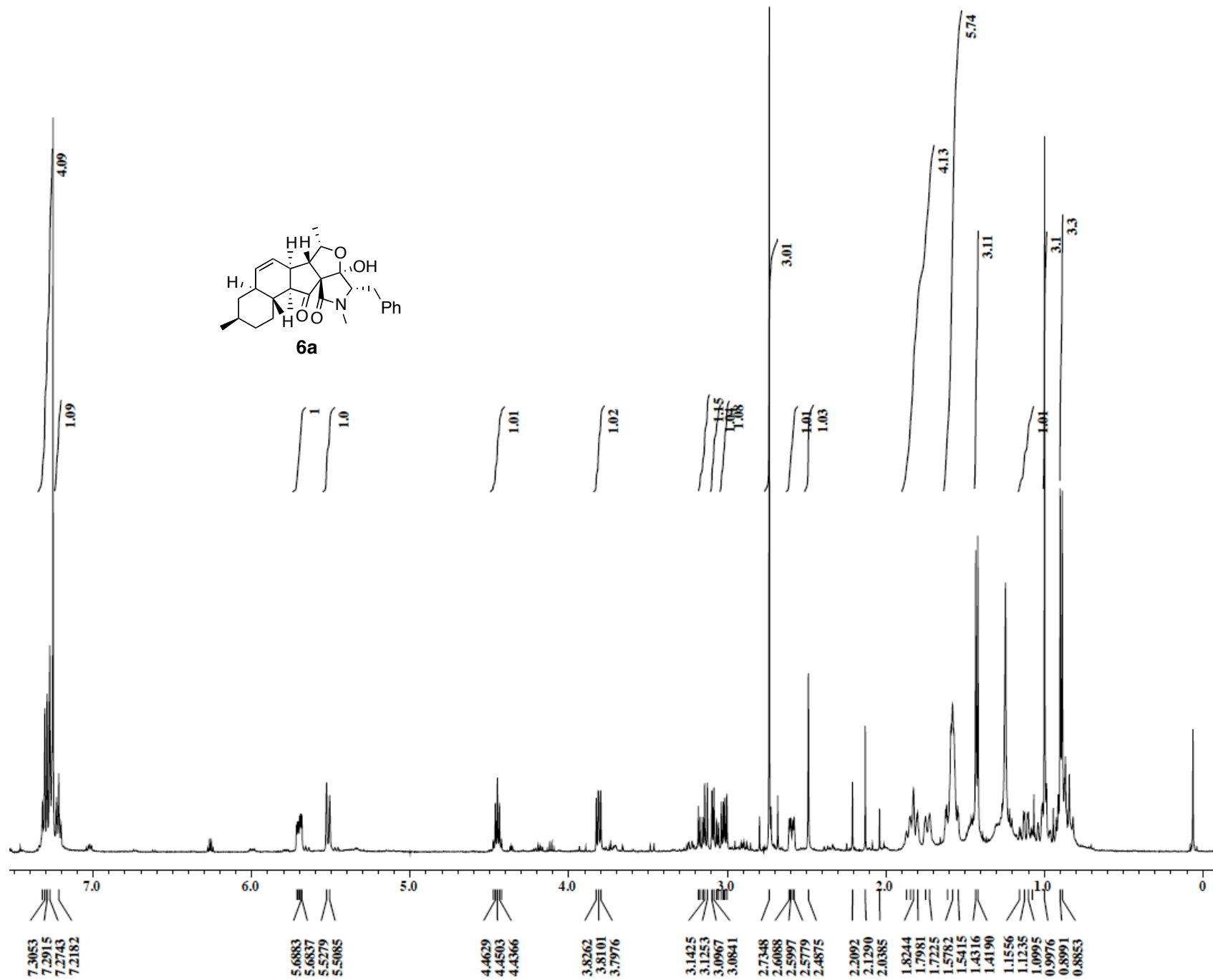
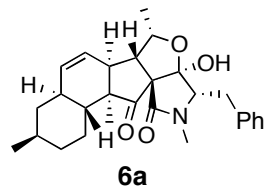


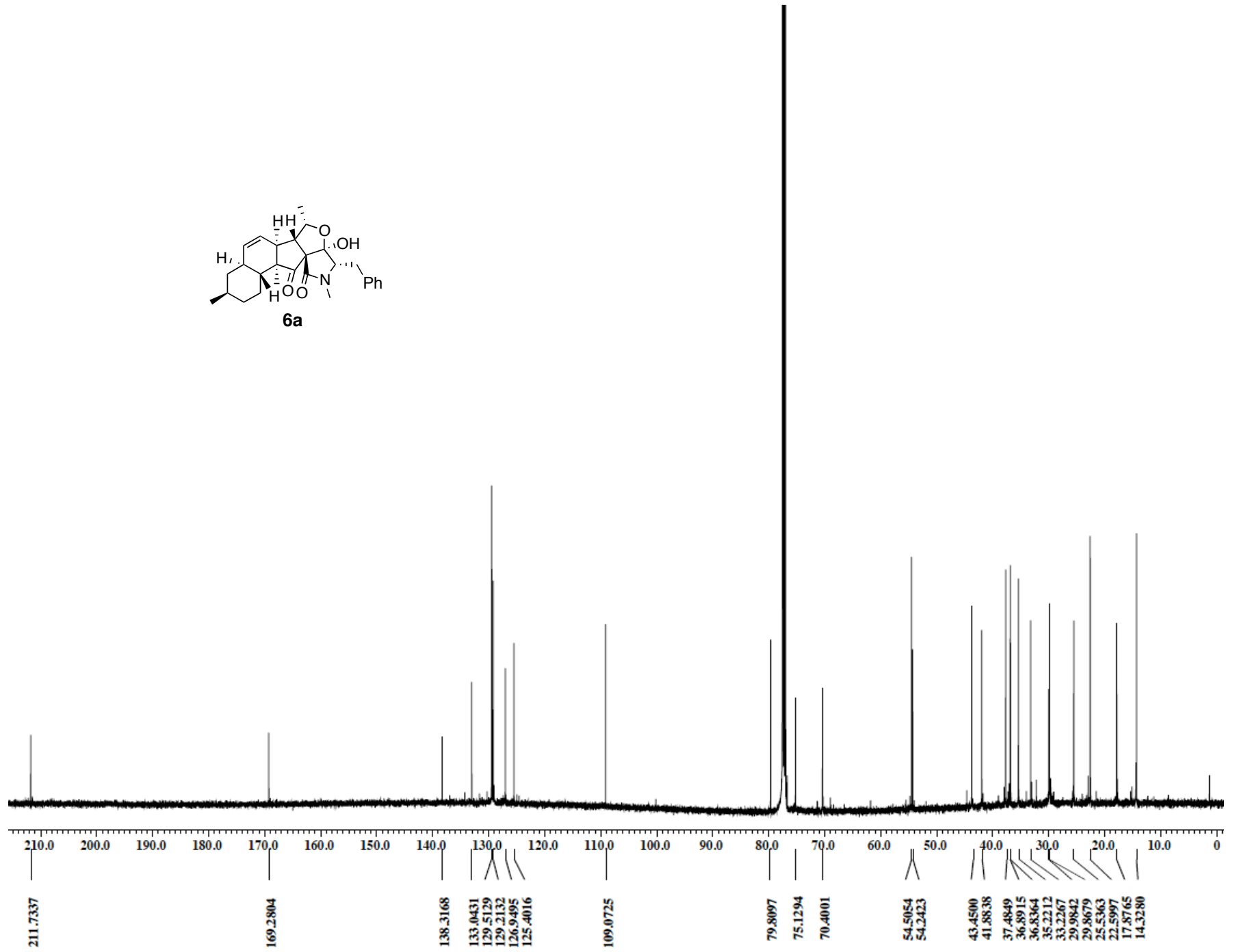
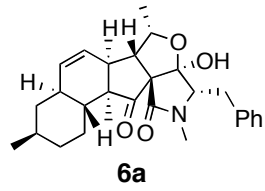


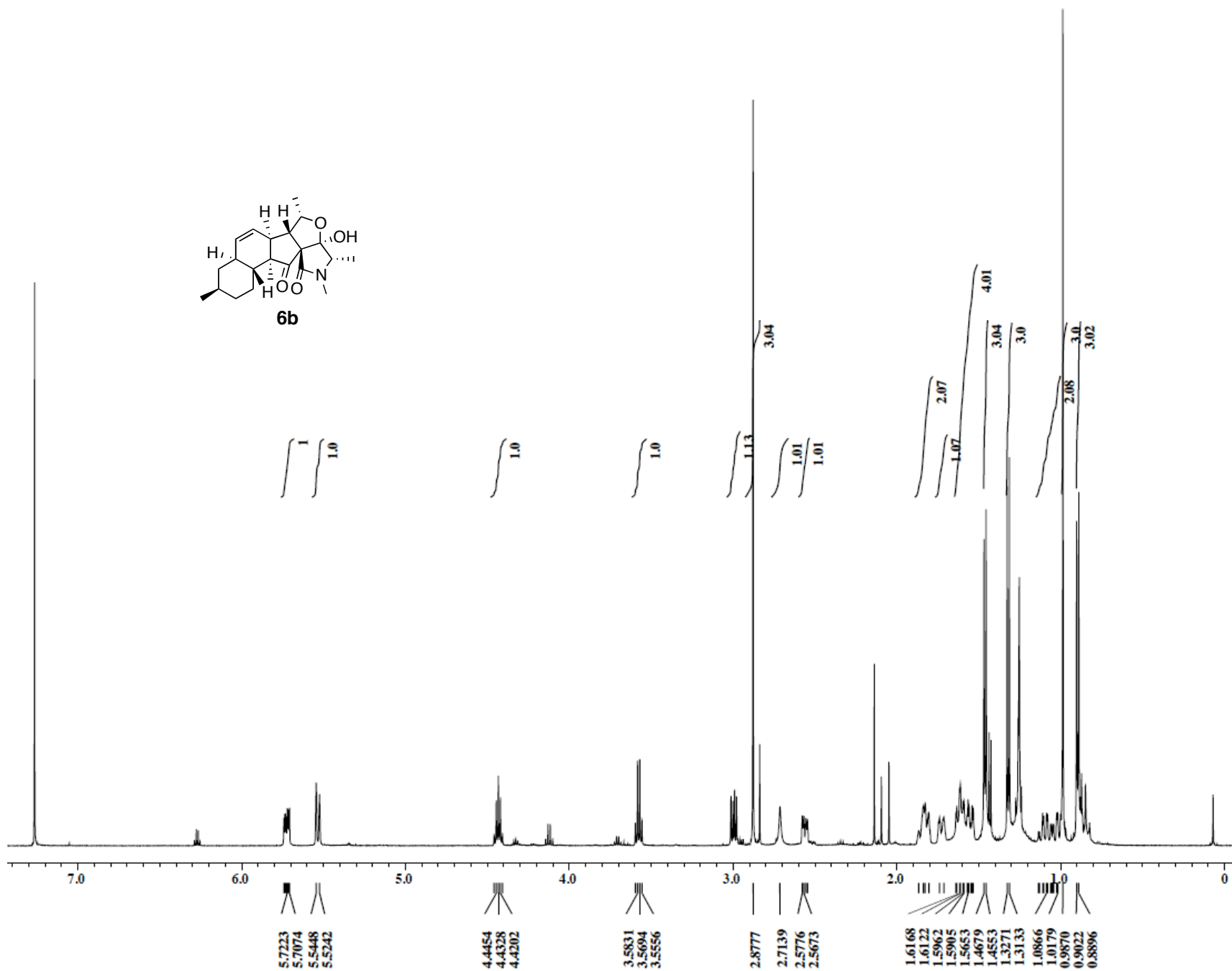
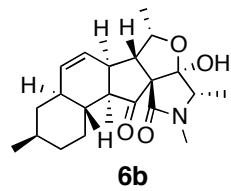




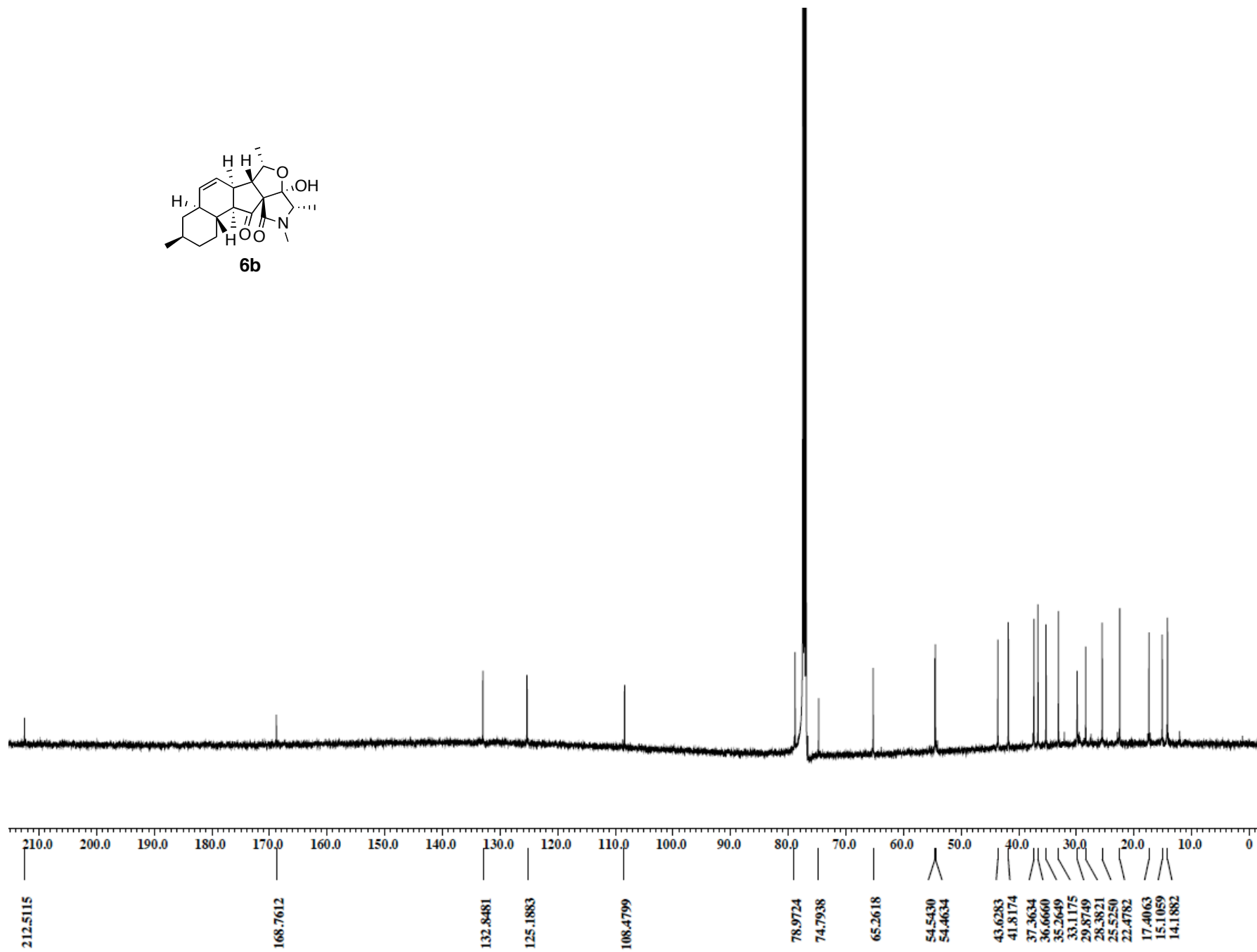
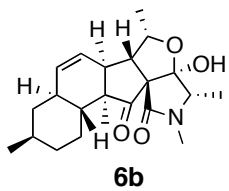


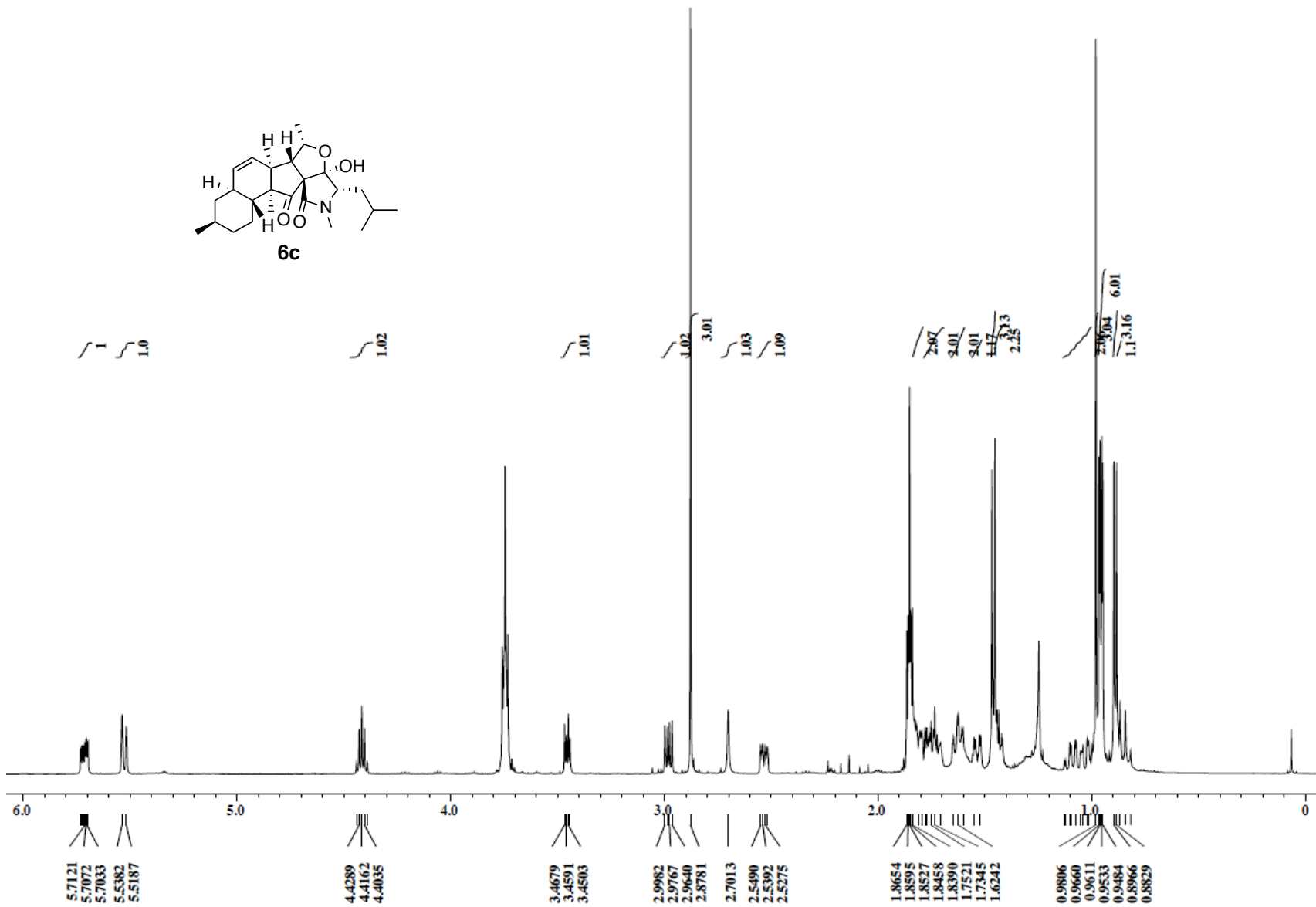
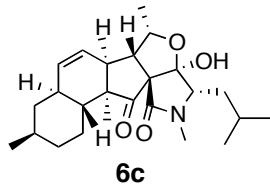


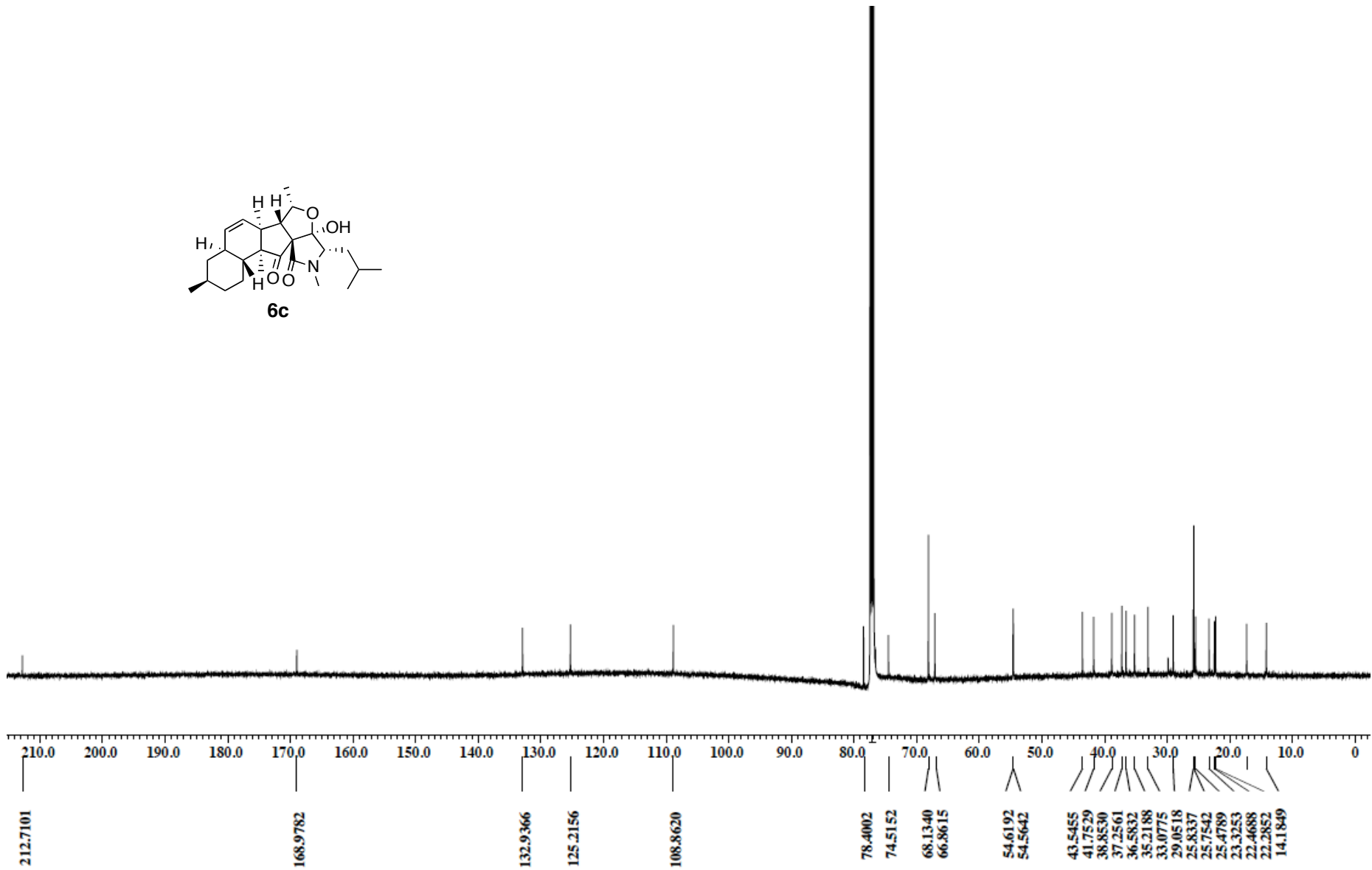
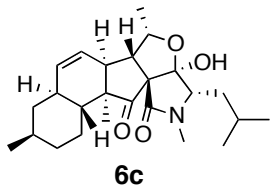


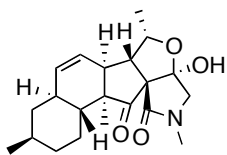




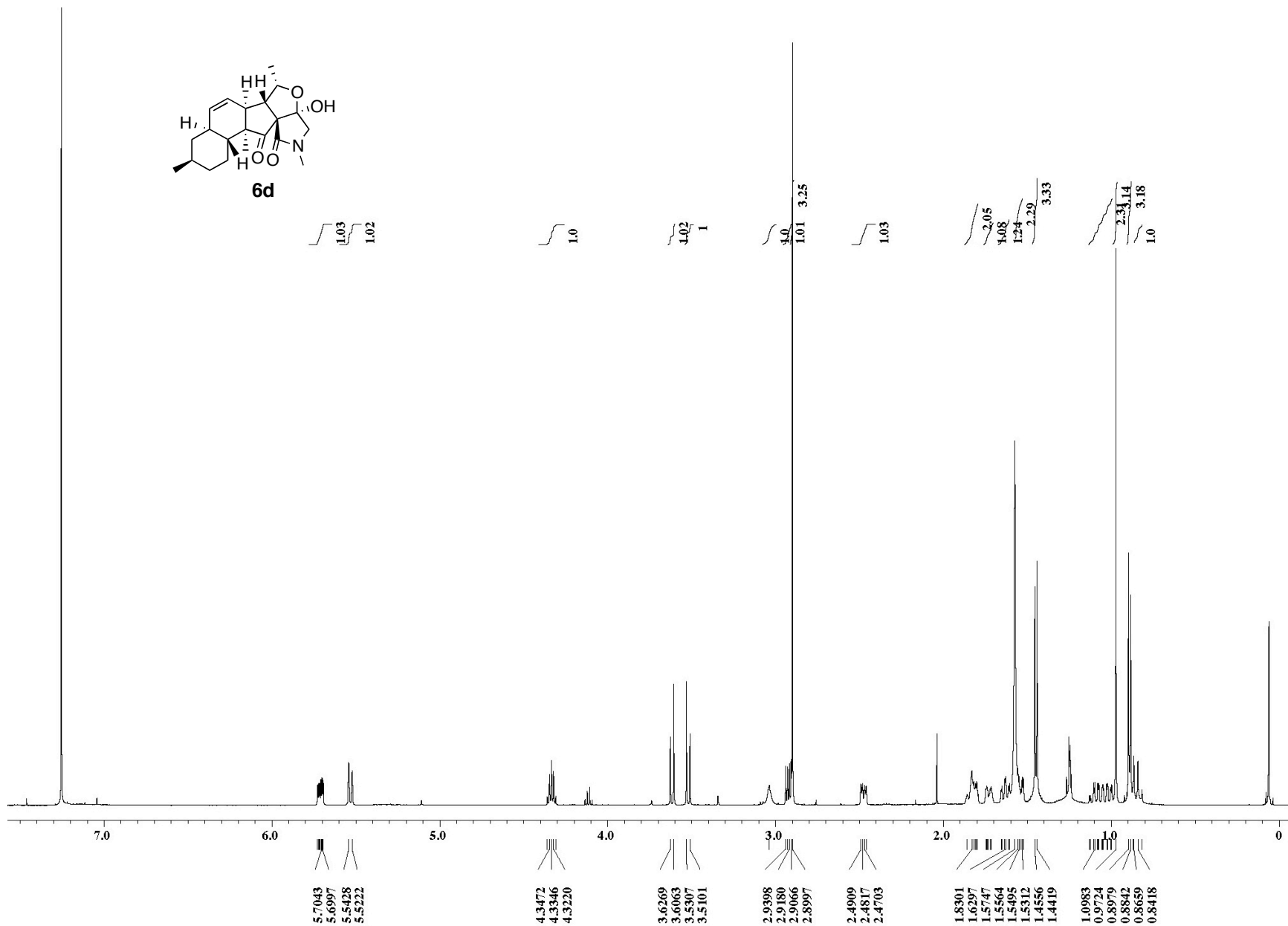


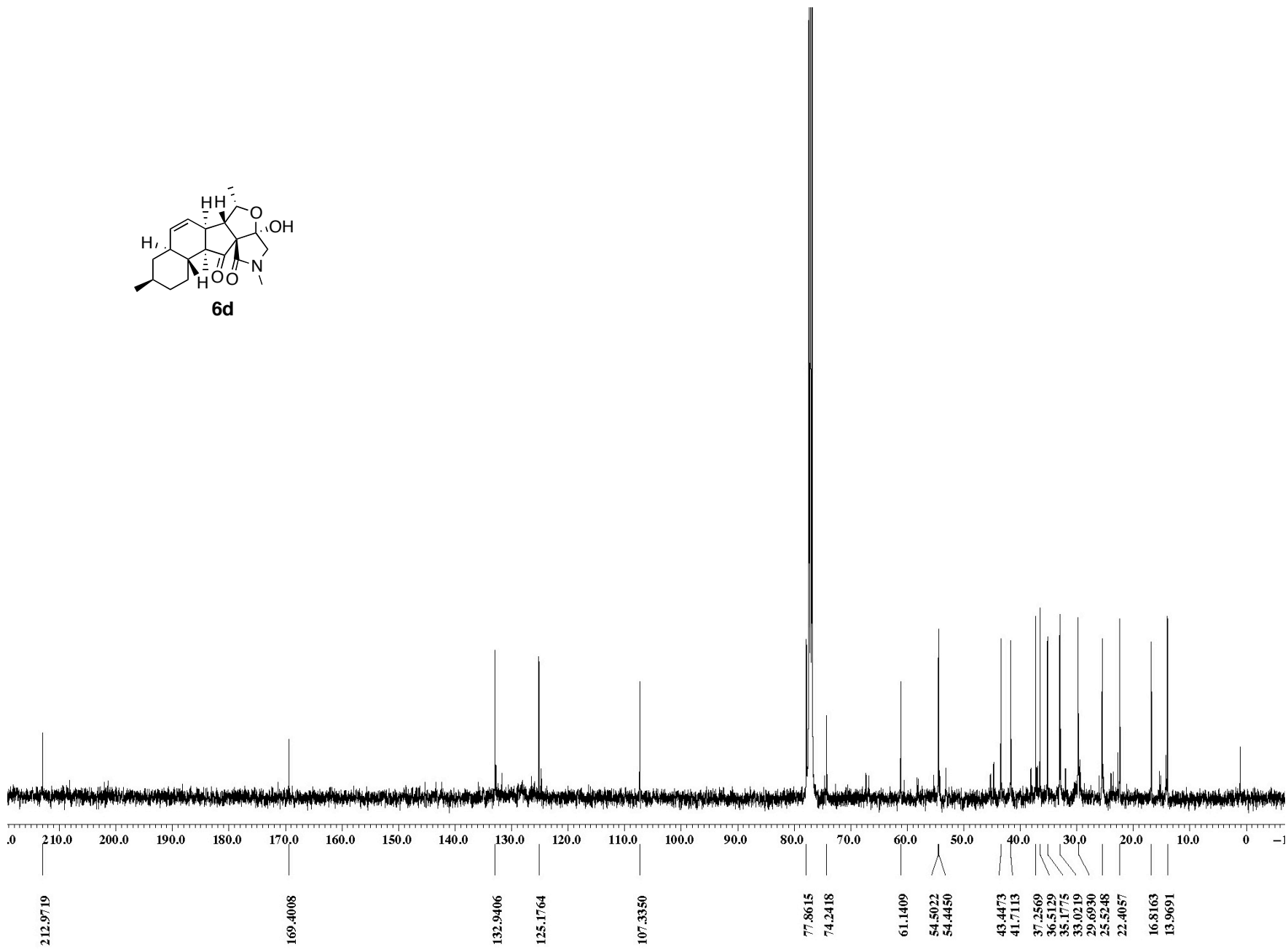
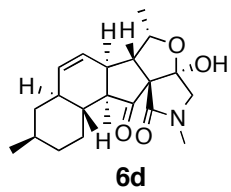


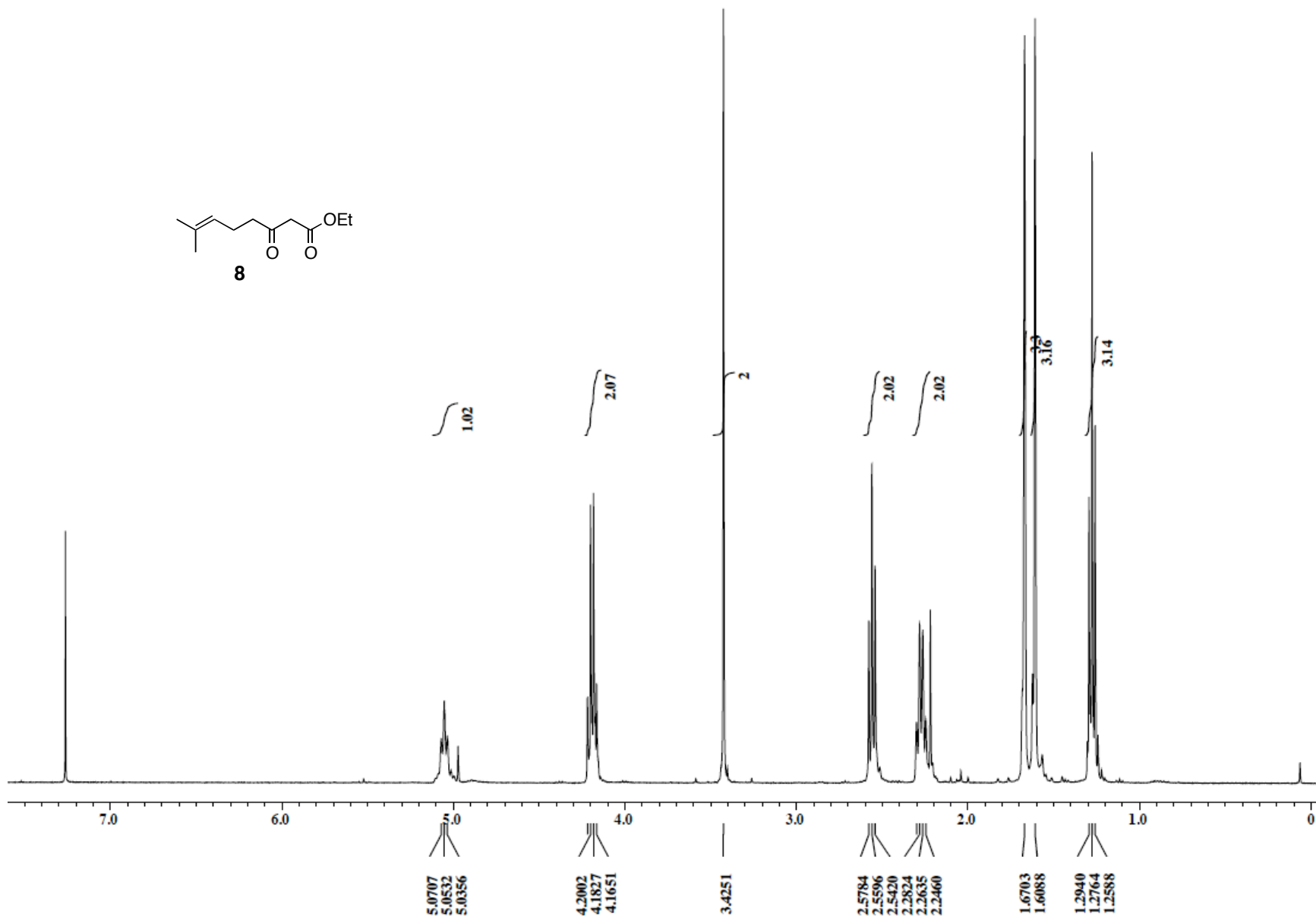
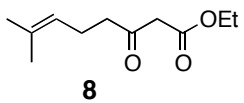


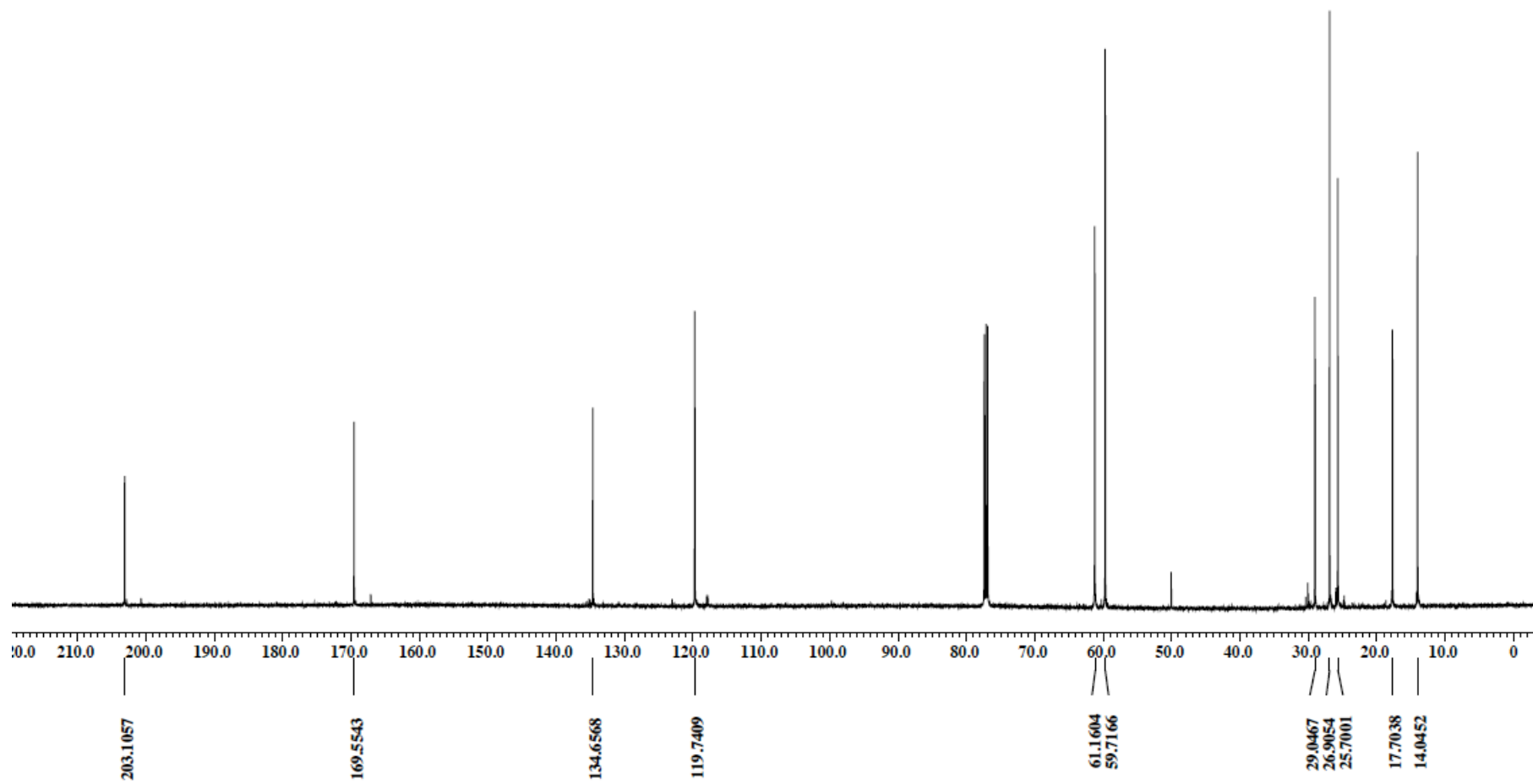
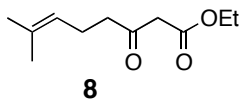


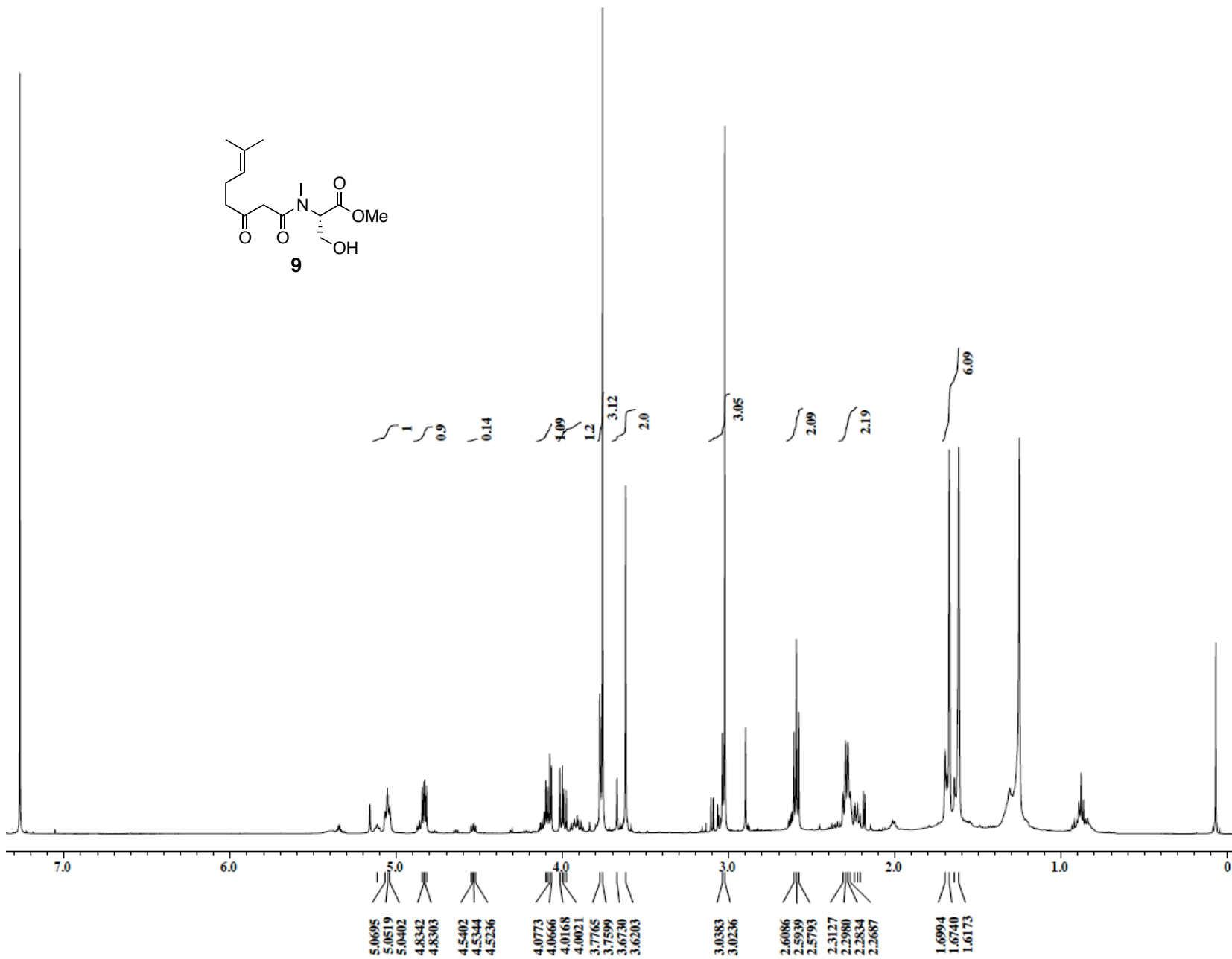
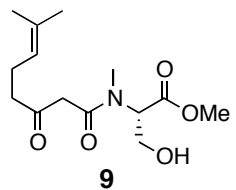
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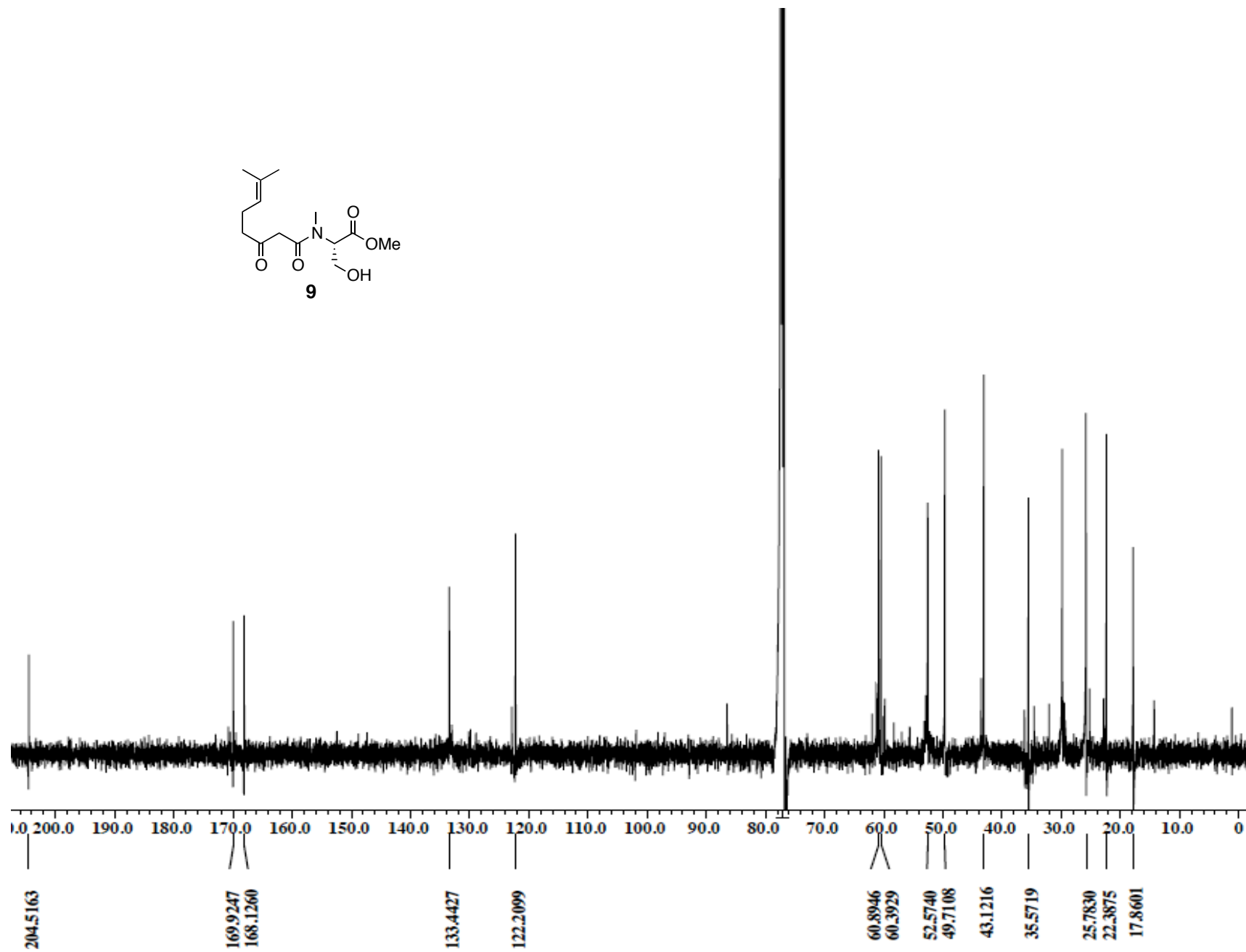
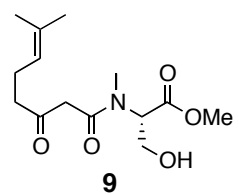


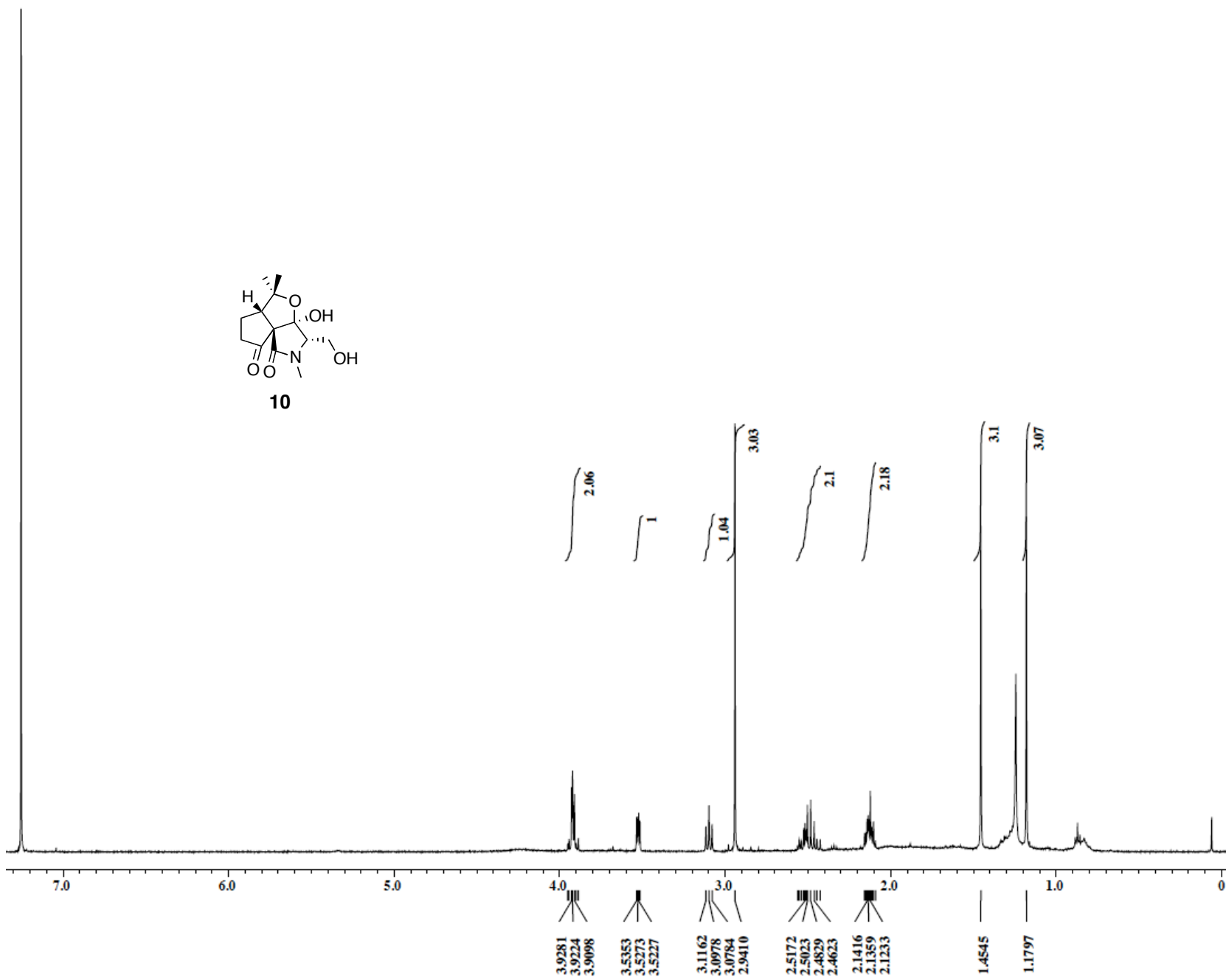
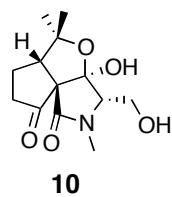


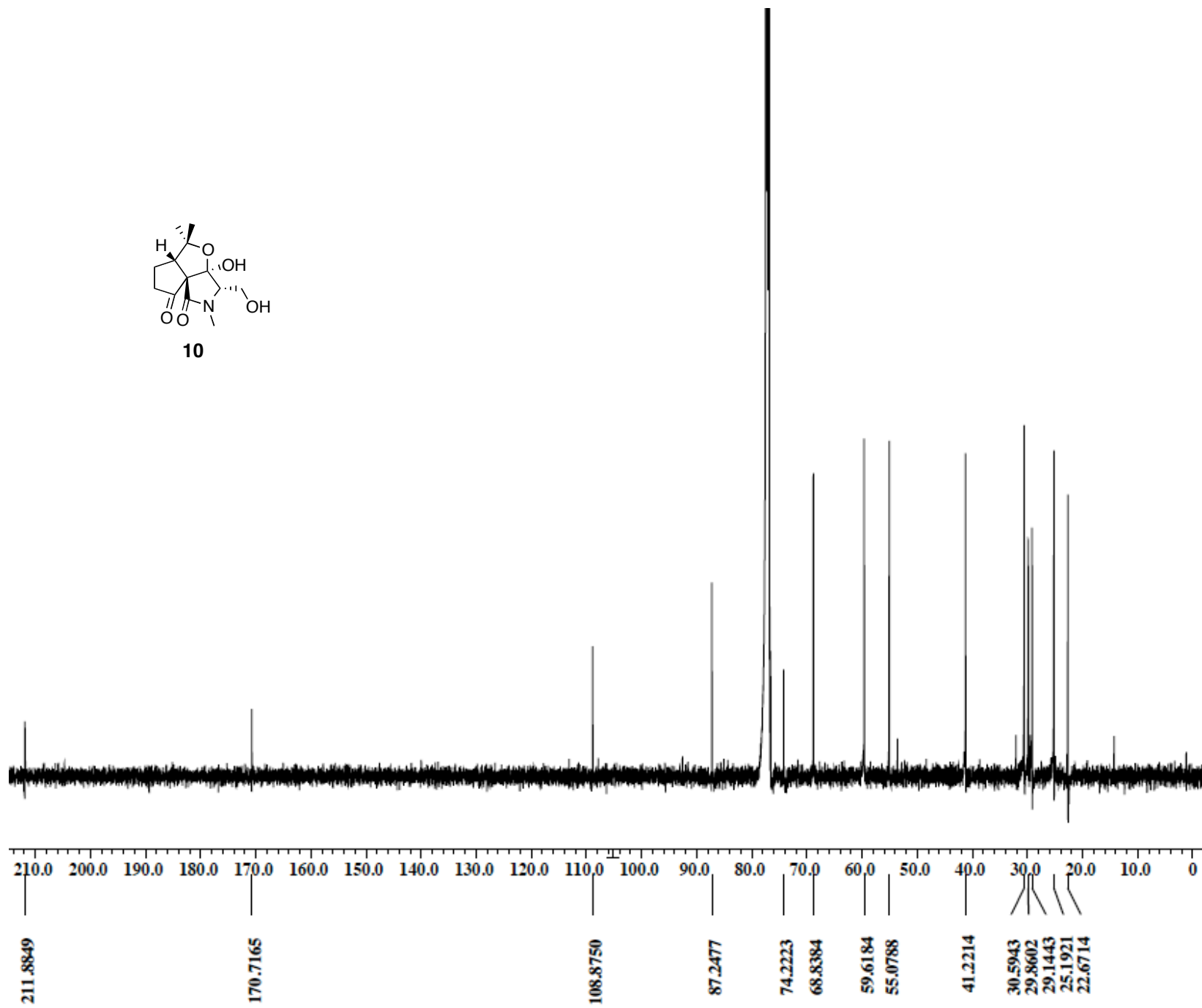
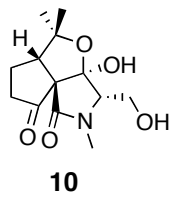


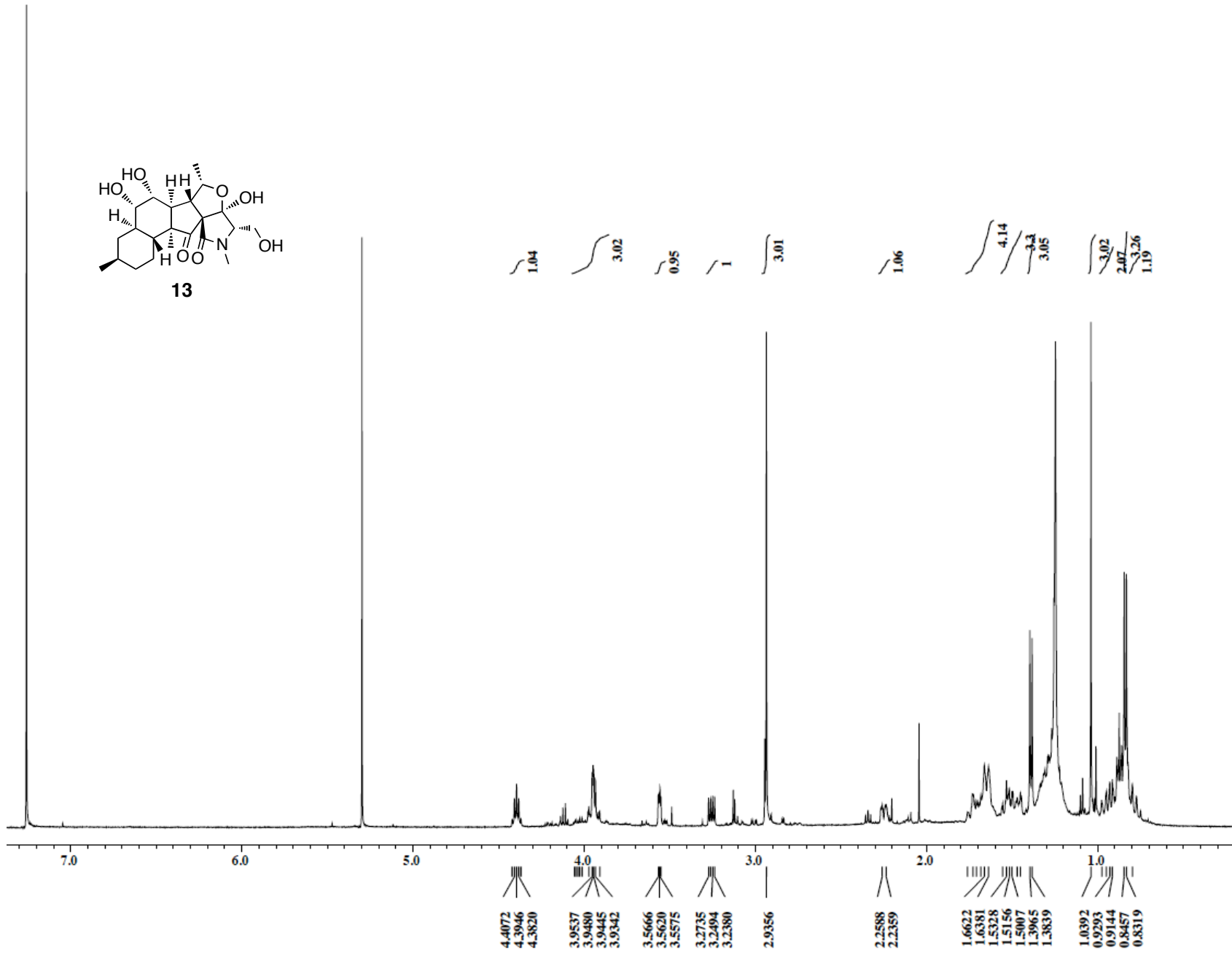
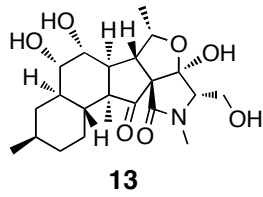


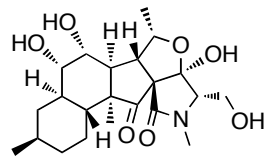




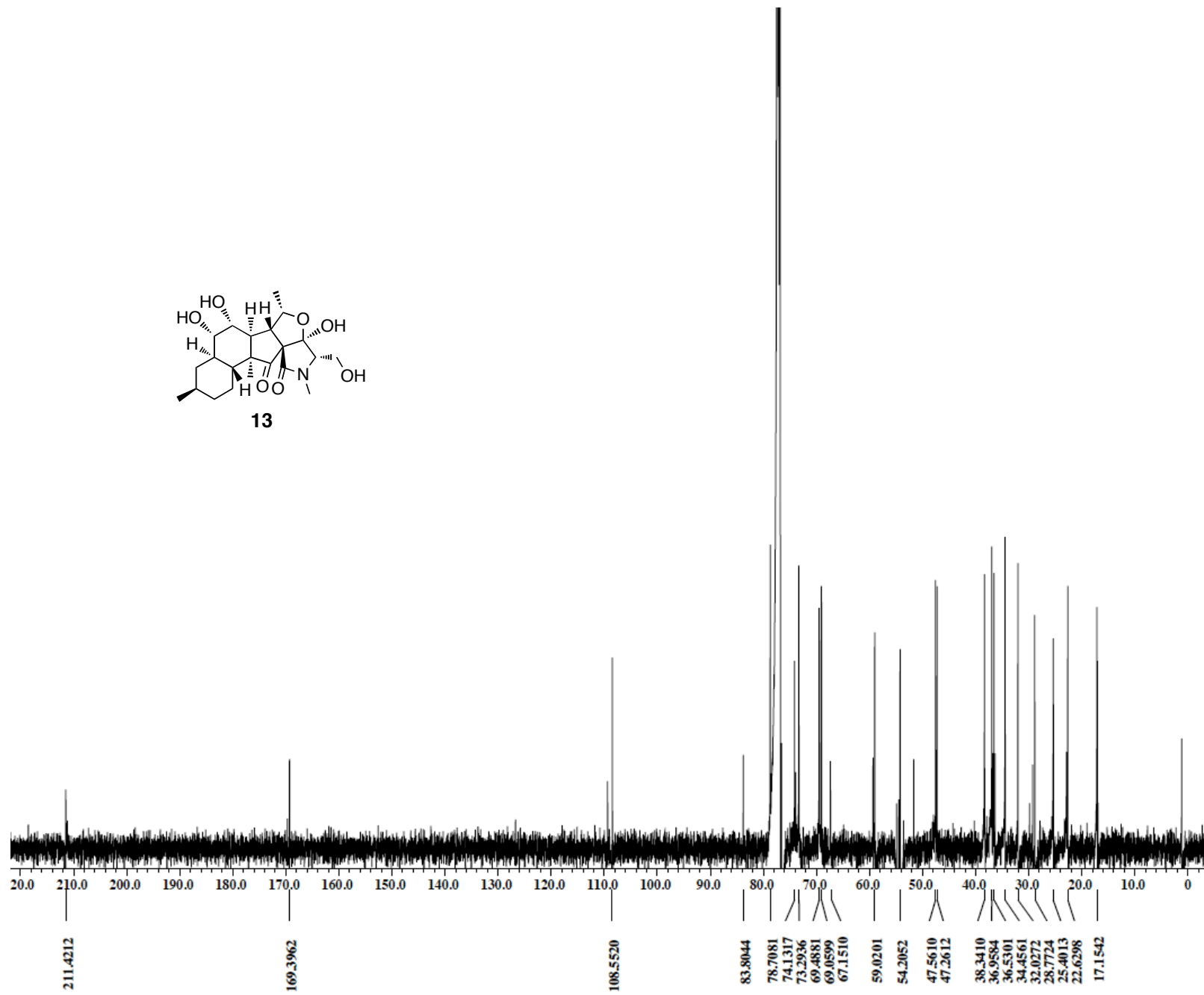


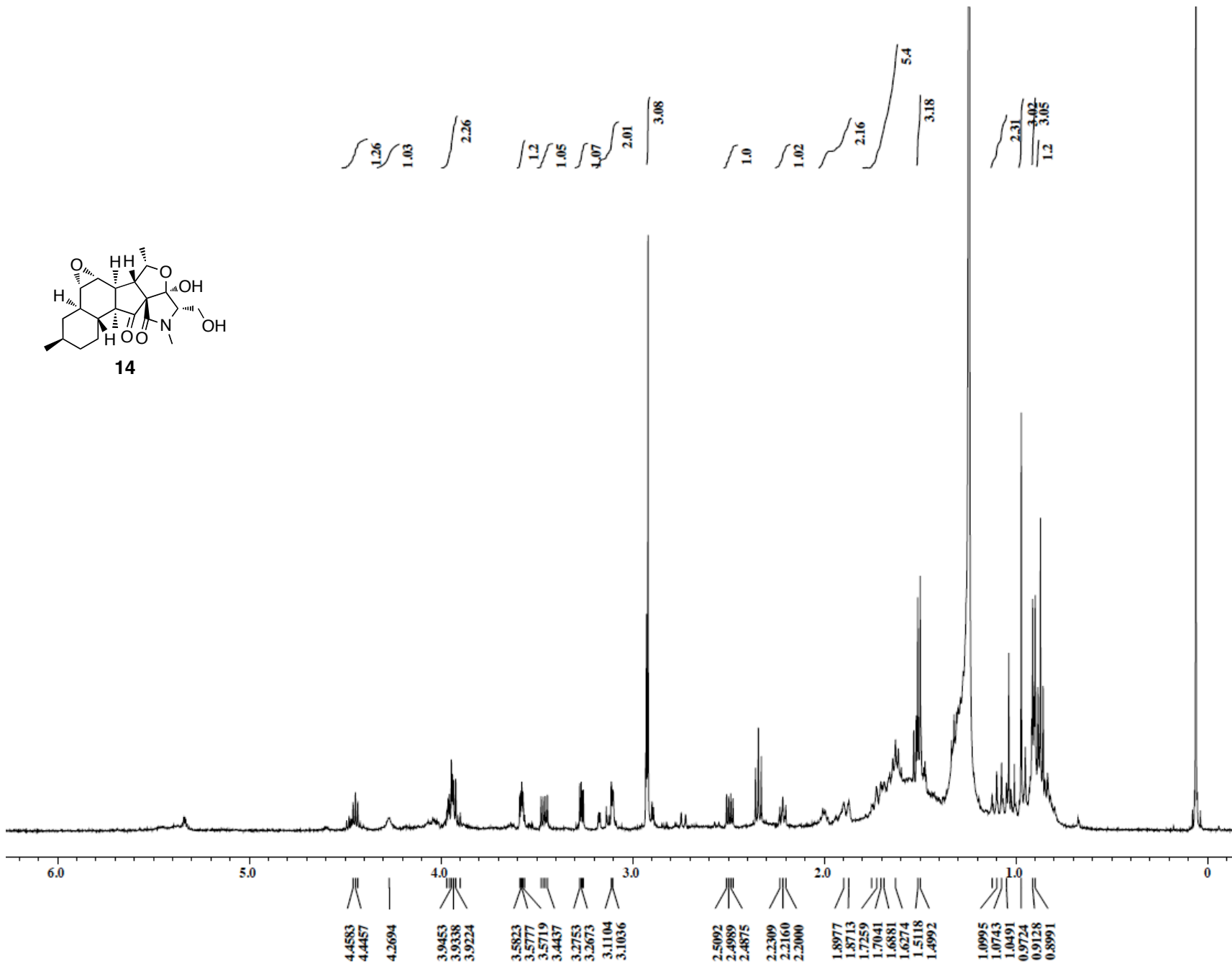
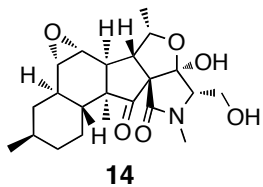


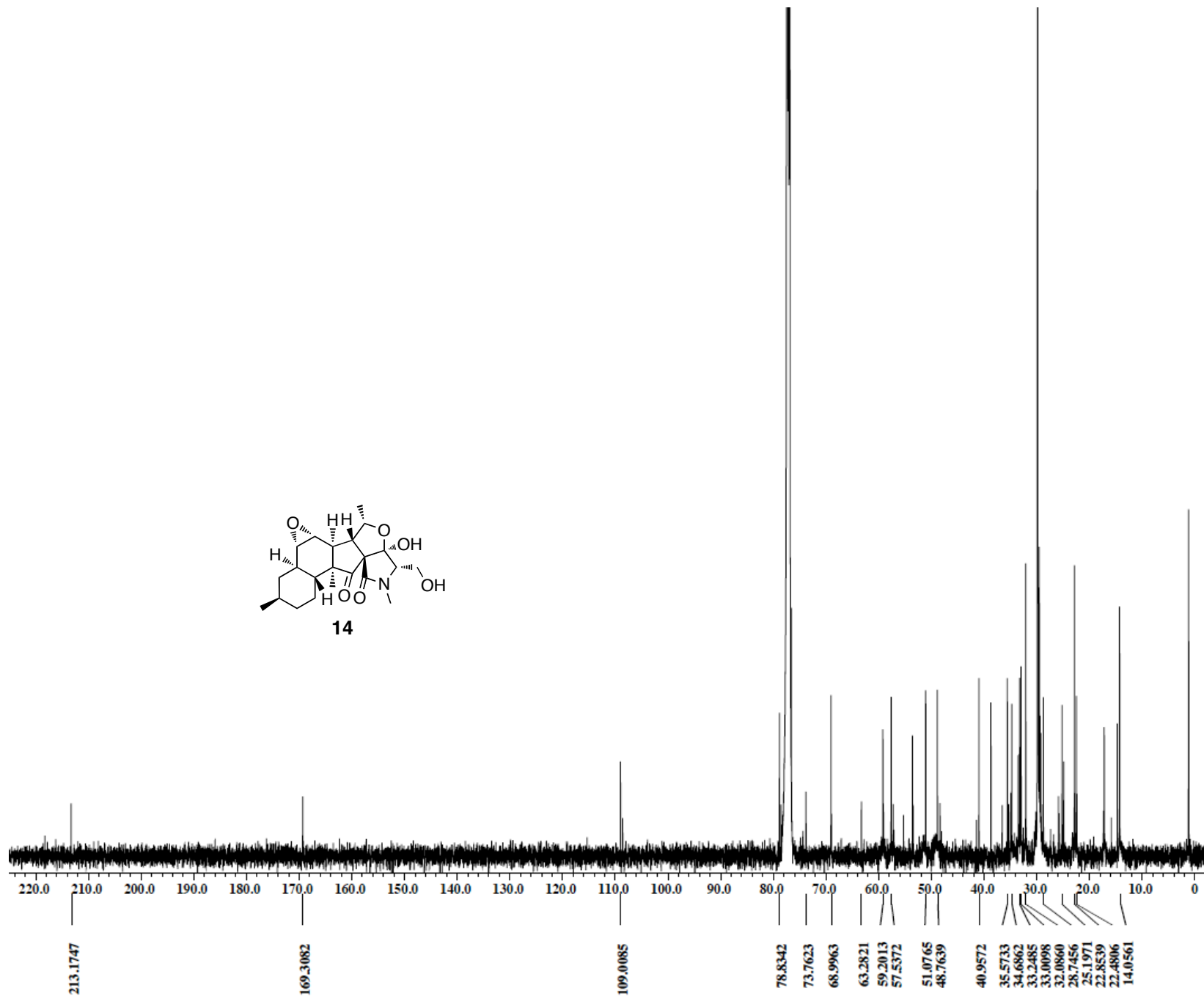
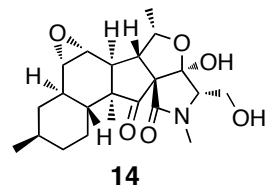


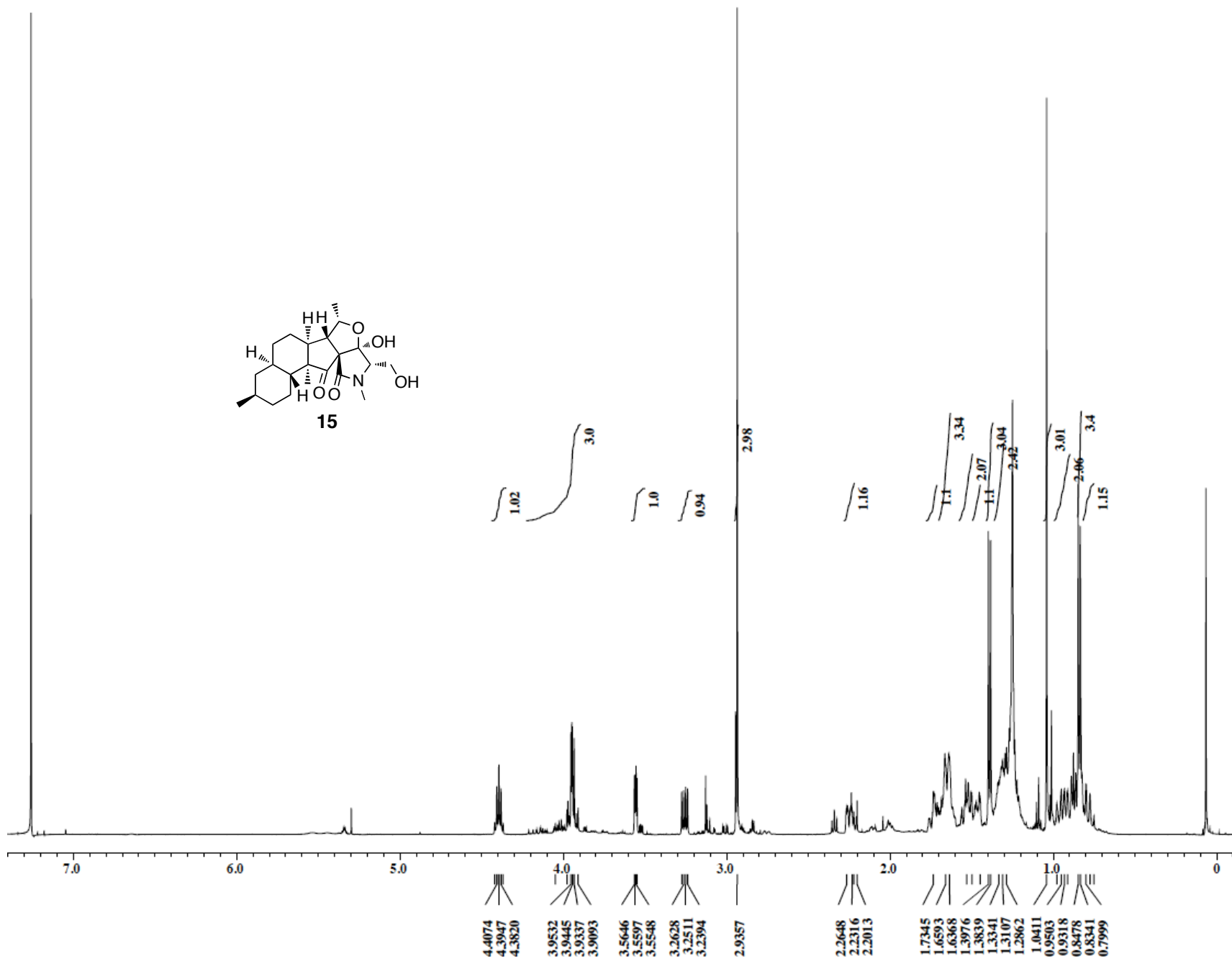
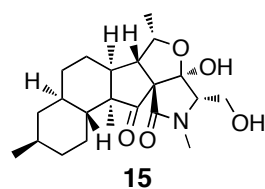


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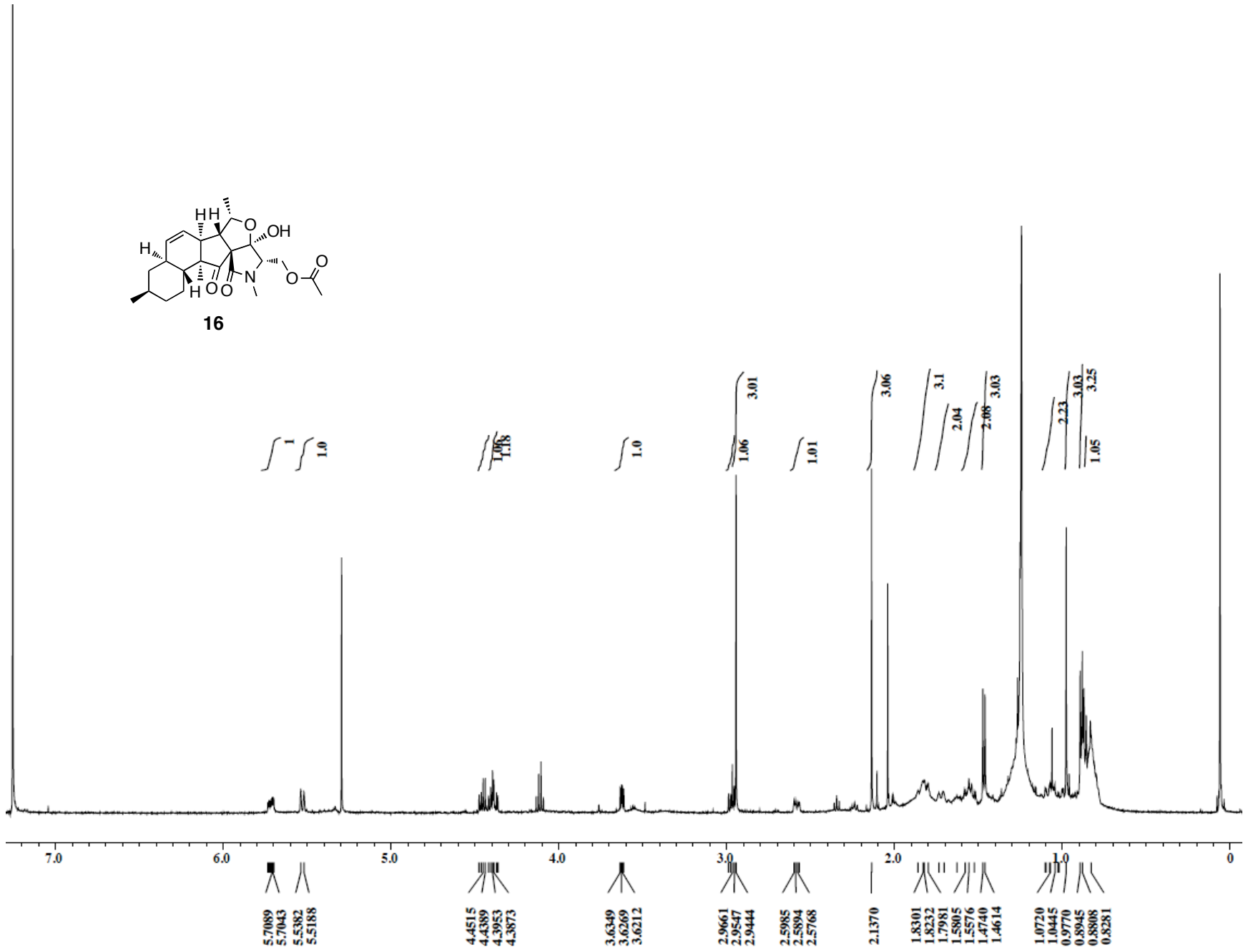
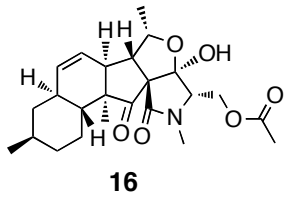


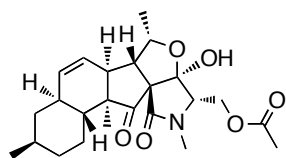












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