Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting information

Total Synthesis and Structure Revision of Chrysamide B

Jinhong Chen, ‡[a] Junfang Li, ‡[a] Longqing Zhu, [a] Xue Peng, [a] Yiyue Feng, [a] Yingmei Lu, [a] Xiaoling Hu, [a] Jianpin Liang, [b] Quanyi Zhao, [a] Zhen Wang *[a]

a. School of Pharmacy, Lanzhou University, West Donggang Road. No. 199, Lanzhou 730000, China. E-mail: zhenw@lzu.edu.cn.

b. Key Laboratory of New Animal Drug Project of Gansu Province, Key Laboratory of Veterinary Pharmaceutical Development, Ministry of Agriculture, Lanzhou, Institute of Husbandry and Pharmaceutical Sciences of CAAS, Lanzhou.

‡. *These authors contributed equally to this work.*

Email: Zhen Wang* zhenw@lzu.edu.cn

Experimental details, characterization data of all products, copies of NMR spectra and crystal data.

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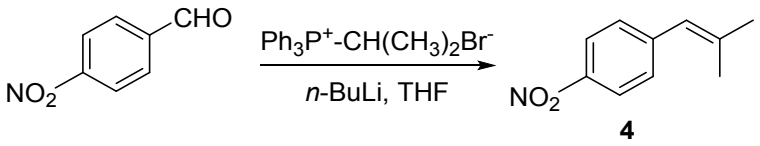
General Information:

All reactions were carried out in a dry solvent under dry oxygen or dry air atmosphere unless otherwise noted. NMR spectra were recorded on Bruker 400 MHz (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers. Proton chemical shifts are reported relative to a residual solvent peak ($CDCl_3$ at 7.26 ppm, d_6-DMSO at 2.50 ppm). Carbon chemical shifts are reported relative to a residual solvent peak ($CDCl_3$ at 77.3 ppm, d_6-DMSO at 39.5 ppm). The following abbreviations were used to designate multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were measured on a Brucker Daltonics Apex Ⅱ 47e Specification (for HRMS). Substrates were purchased from commercial sources and used as received.

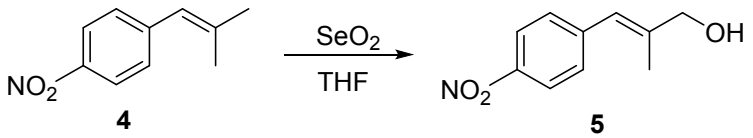
1-(2-methylprop-1-enyl)-4-nitrobenzene 4



4

Experimental: To a solution of Ph_3P^+-$CH(CH_3)_2Br^-$ [1](2.44 g, 6.36 mmol.) in tetrahydrofuran (25 mL), *n*-BuLi (3.64 mL, 1.6 M in hexanes, 5.83 mmol.) was added dropwise at -35 °C. After stirring for 1 h, the reaction mixture was added dropwise to a solution of 4-nitrobenzaldehyde (800 mg, 5.30 mmol.) in tetrahydrofuran (10 mL) at 0 °C. The mixture was stirred at room temperature for 4 h and then the resulting mixture was concentrated in vacuo. The residue was partitioned between EtOAc (2 × 50 mL) and satd NH_4Cl (50 mL), and the EtOAc layer was washed with satd NaCl, then dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give the olefin **4** as a yellow oil (807 mg, 86%). R_f = 0.6 (*n*-hexane/EtOAc, 40:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.7 Hz, 2H), 6.31 (s, 1H), 1.96 (s, 3H), 1.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.60, 145.45, 140.16, 129.16, 123.65, 123.40, 27.17, 19.65. IR (KBr): 3074, 2973, 2935, 1597, 1515, 1342, 1109, 862, 746 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{10}H_{11}NNaO_2$ $[M+Na]^+$ 200.0682, found 200.0683.

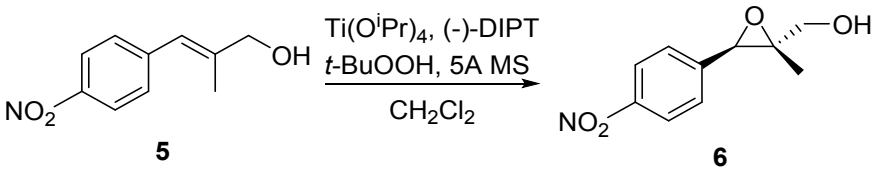
(Z)-2-methyl-3-(4-nitrophenyl)prop-2-enol 5



4 **5**

Experimental: To a solution of olefin **4** (600 mg, 3.39 mmol.) in dry tetrahydrofuran (17 mL), SeO_2 (941 mg, 8.48 mmol) was added and the mixture was stirred at 45 °C for 48 h. Then the reaction was cooled to room temperature and filtered over a pad of celite. The filtrate was concentrated, and the crude residue was purified by column chromatography on silica gel to yield allylic alcohol **5** as a colorless oil (392 mg, 60%). R_f = 0.6 (*n*-hexane/EtOAc, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.7 Hz, 2H), 7.41 (d, *J* = 8.7 Hz, 2H), 6.61 (s, 1H), 4.24 (d, *J* = 3.6 Hz, 2H), 1.91 (s, 3H), 1.72 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.03, 144.49, 141.81, 129.41, 123.49, 122.49, 68.08, 15.44. IR (KBr): 3062, 2988, 2920, 2853, 1597, 1517, 1344, 1247, 1046, 740 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{10}H_{11}NNaO_3$ $[M+Na]^+$ 216.0631, found 216.0636.

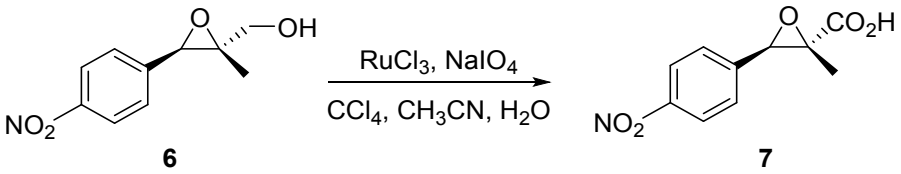
((2*R*,3*R*)-2-methyl-3-(4-nitrophenyl)oxiran-2-yl)methanol 6



5 **6**

Experimental: To a cooled (-35 °C) mixture of 5 Å molecular sieves (powdered, 907 mg), (-)-diisopropyl D-tartrate (64 mg, 0.27 mmol, 0.13 equiv), and titanium (IV) isopropoxide (77 mg, 0.27 mmol, 0.13 equiv) in dichloromethane (12 mL) was added *tert*-butyl hydroperoxide (2.41 mL, 1.5 M in decane, 3.62 mmol, 2.0 equiv) by over 10 min. The resulting mixture was stirred for 30 min at –35 °C. A solution of allylic alcohol **5** (350 mg, 1.81 mmol, 1 equiv) in dichloromethane (6 mL) was pre-dried over 500 mg 5Å molecular sieves for 110 min then added dropwise. The resulting mixture was stirred for 1 h at –35 °C then warmed to 0 °C and poured into a cooled (0 °C) suspension of Iron (II) sulfate (706 mg) and tartaric acid (204 mg) in water (5 mL). The resulting mixture was stirred for 15 min, and the aqueous phase was saturated with solid sodium chloride and extracted with dichloromethane. Emulsions were formed in some of the extractions, and were dispersed by filtration through celite. The organic phases were dried over Na_2SO_4, filtered through celite, and concentrated under reduced pressure. and the crude residue was purified by column chromatography on silica gel to give the epoxy alcohol **6** (354 mg, 94%). R_f = 0.4 (*n*-hexane/EtOAc, 1:1). Analysis by HPLC showed 95.66 % *ee* (Chiralcel AD-H, hexanes/*i*-PrOH 95/5, 0.6 mL/min flow rate, t_{major} = 51.637 min, t_{minor} = 60.100 min). $[\alpha]_D^{23}$ +17.0 (c 1.0, methanol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.8 Hz, 2H), 7.48 (d, *J* = 8.6 Hz, 2H), 4.30 (s, 1H), 3.88 (d, *J* = 12.7 Hz, 1H), 3.79 (d, *J* = 12.7 Hz, 1H), 2.04 (s, 1H), 1.07 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.42, 143.30, 127.27, 123.40, 99.92, 64.37, 59.12, 13.39. IR (KBr): 3120, 2958, 2924, 2853, 1603, 1519, 1346, 1071 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{10}H_{11}NNaO_4$ $[M+Na]^+$ 232.0580, found 232.0581.

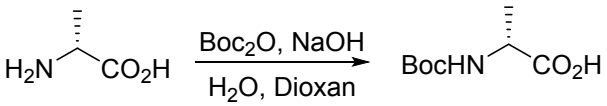
(2*S*,3*R*)-2-methyl-3-(4-nitrophenyl)oxirane-2-carboxylic acid 7



6 **7**

Experimental: To a solution of epoxy alcohol **6** (300 mg, 1.43 mmol) in 14 mL of $CCl_4/CH_3CN/H_2O$ (v/v/v, 2/2/1) was added $NaIO_4$ (768 mg, 3.59 mmol) and $RuCl_3•3H_2O$ (37 mg, 0.14 mmol) and the reaction mixture was stirred at room temperature for 24 h. The mixture was extracted with dichloromethane (3 × 15 mL). The combined organic extract was dried over Na_2SO_4, filtered and concentrated in vacuum to afford the epoxy-acid **7** (304 mg, 95%) as a colorless solid. m.p.: 127 °C-129 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.17 (s, 1H), 8.27 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.6 Hz, 2H), 4.47 (s, 1H), 1.34 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.51, 148.03, 140.46, 127.66, 123.66, 61.69, 59.81, 12.20. IR (KBr): 3150, 2869, 1720, 1523, 1347, 1180, 1083 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{10}H_9NNaO_4$ $[M+Na]^+$ 246.0373, found 246.0378.

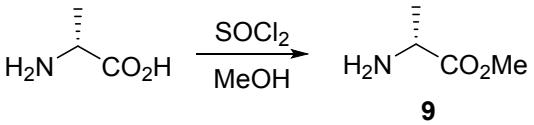
***N*-tert-Butoxycarbonyl-D-alanine 8**



8

Experimental: To a solution of D-alanine (2.50 g, 27.8 mmol) in H_2O (50 mL) and 1 M NaOH aq (28 mL) at 0 °C was added $(Boc)_2O$ (9.10 g, 41.7 mmol) in dioxane (50 mL). After being stirred at room temperature for 8 h, the reaction mixture was cooled to 0 °C, acidified with 3M HCl solution to pH 2-3, and diluted with EtOAc (50 mL). The mixture was extracted with EtOAc (3×70 mL), washed with brine, and dried over Na_2SO_4. Concentration gave the *N*-tert-Butoxycarbonyl-D-alanine in 96% yield as a white solid (5.07 g, 26.6 mmol). HRMS (ESI) m/z: calculated for $C_8H_{15}NNaO_4$ $[M+Na]^+$ 212.0893, found 212.0891.

D-alanine methyl ester hydrochloride 9

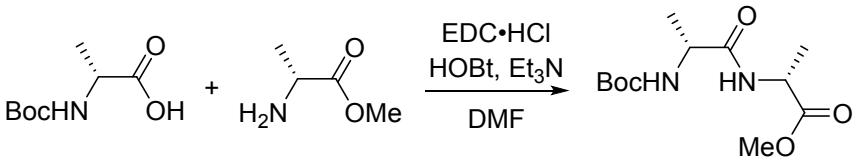


9

Experimental: Thionyl chloride (4 mL) was added dropwise to D-alanine (2.50 g, 27.8

mmol) in methanol (28 mL) at 0 °C and the solution was stirred at 45 °C for 6 h. Then the solvent was evaporated in vacuo and the resulting residue was diluted with diethyl ether and suspended in diethyl ether. The suspension was filtered to give D-alanine methyl ester hydrochloride as a white solid (3.46 g, 89%)[2].

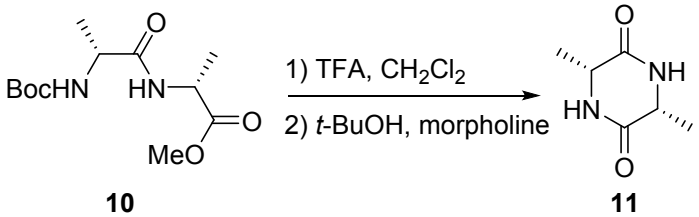
Methyl (tert-butoxycarbonyl)-D-alanyl-D-alaninate 10



8 **9** **10**

Experimental: To a solution of *N*-Boc-D-alanine **8** (2.50 g, 13.2 mmol.) and amino acid methyl ester hydrochloride **9** (1.84 g, 13.2 mmol.) in DMF (52ml), HOBt (2.14 g, 15.8 mmol.), EDC•HCl (3.04 g, 15.8 mmol.) and triethylamine (5.7 ml, 43.6 mmol.) were added. The reaction mixture was stirred overnight at room temperature. Then the mixture was diluted with EtOAc (50 mL) and organic layers were washed with water (3 × 50 ml), dried over Na_2SO_4, filtered and the solvent removed in vacuo. The resulting residue was purified using silica gel chromatography to give **10** as a white solid (2.68 g, 74%). R_f = 0.5 (*n*-hexane/EtOAc, 1:1). $[\alpha]_D^{23}$ +70.0 (c 1.0, methanol). m.p.: 108 °C-110 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (s, 1H), 5.02 (s, 1H), 4.56 (p, *J* = 7.2 Hz, 1H), 4.17 (s, 1H), 3.74 (s, 1H), 1.44 (s, 2H), 1.40 (d, *J* = 7.2 Hz, 1H), 1.35 (d, *J* = 7.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.14, 172.11, 155.39, 80.12, 52.41, 49.99, 47.97, 28.26, 18.33, 18.21. IR (KBr): 3312, 2984, 2939, 1748, 1713, 1694, 1668, 1538, 1456, 1392, 1368, 1167, 1072 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{12}H_{22}N_2NaO_5$ $[M+Na]^+$ 297.1421, found 297.1410.

(3*R*,6*R*)-3,6-dimethylpiperazine-2,5-dione 11

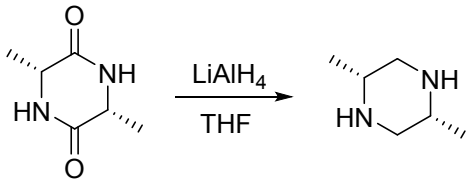


10 **11**

Experimental: Trifluoroacetic acid (1.2 mL) was added via syringe to a solution of the dipeptide **10** (1g, 3.65 mmol.) in dichloromethane (14 mL). The reaction mixture was stirred at 30 °C for 6 h, then the brown solution was concentrated under reduced pressure to afford a brown residue, which was dissolved in *t*-butanol (18 mL). The reaction mixture was stirred vigorously as morpholine (4 mL) was introduced slowly at room temperature. After 5 h, the diketopiperazine **11** precipitated as a white solid. The orange solution was removed under reduced pressure, and the resulting mixture was

diluted with dichloromethane and the product was collected by filtration and washed sequentially with dichloromethane to provide diketopiperazine **11** (450 mg, 87%) as a fluffy white solid. R_f = 0.7 (CH_2Cl_2/MeOH, 5:1). $[\alpha]_D^{23}$ +50.0 (c 1.0, methanol). m.p.: 256 °C-259 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.07 (s, 1H), 3.90 (q, *J* = 6.9 Hz, 1H), 1.25 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 169.02, 49.76, 18.47. IR (KBr): 3211, 3103, 2932, 1687, 1655, 1459, 1120, 1154 cm^{-1}. HRMS (ESI) m/z: calculated for $C_6H_{10}N_2NaO_2$ $[M+Na]^+$ 165.0634, found 165.0636.

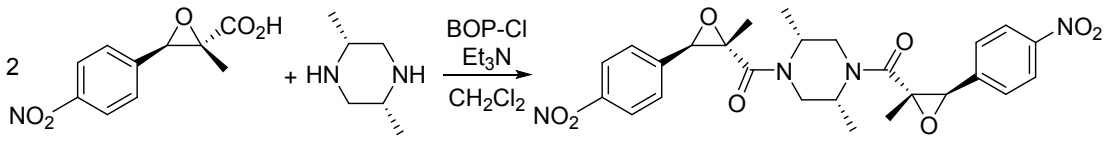
(2*R*,5*R*)-2,5-dimethylpiperazine 12



11 **12**

Experimental: To a solution of the diketopiperazine **11** (100 mg, 0.70 mmol) in tetrahydrofuran (7 mL) was added $LiAlH_4$ (214 mg, 5.62 mmol) over a period of 10 min with stirring at 0 °C. After the addition, the reaction mixture was refluxed for 8 h, then cooled to 0 °C, and carefully quenched by the addition of water. The mixture was filtered, and the filter cake was washed with dichloromethane. The combined filtrate was dried over Na_2SO_4, filtered through celite and evaporated under reduced pressure to give the piperazine **12**[3] (66 mg 83%) as a colorless oil. The crude product was used directly in the next reaction.

((2*R*,5*R*)-2,5-dimethylpiperazine-1,4-diyl)bis(((2S,3R)-2-methyl-3-(4-nitrophenyl)oxiran-2-yl)methanone) 2

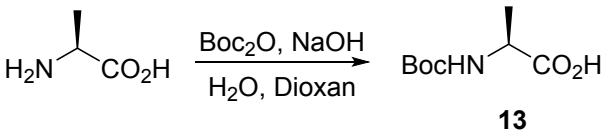


7 **12** **2**

Experimental: To a solution of piperazine **12** (20 mg, 0.175 mmol) in dichloromethane (2 mL) were added epoxy-acid **7** (86 mg, 0.386 mmol, 2.2 equiv) in dichloromethane (3 mL) and the solution was cooled to 0 °C in an ice-water bath. Then, BOP-Cl (117 mg, 0.456 mmol, 2.6 equiv) and triethylamine (69 µL, 0.525 mmol.) were added. The ice-water bath was removed and the reaction mixture was stirred overnight at room temperature. Then the mixture was diluted with dichloromethane (10 mL). The organic layers were washed with water (3 × 10 ml), dried over Na_2SO_4, filtered and the solvent removed in vacuo. The resulting residue was purified using silica gel chromatography (eluent: *n*-hexane/ EtOAc 1:1) to give a white solid compound **2** (68.7 mg, 74%). R_f =

0.4 (*n*-hexane/EtOAc, 1:2). $[\alpha]_D^{22}$ -128.0 (c 2.5, acetone). m.p.: 136 °C-138 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.5 Hz, 4H), 7.51 (d, *J* = 8.5 Hz, 4H), 4.44 (brs, 2H), 4.33 – 4.01 (brd, 4H), 3.05 (brs, 2H), 1.34 – 1.19 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.46, 147.80, 141.34, 127.46, 123.51, 63.70, 61.56, 50.18, 43.66, 15.25, 14.96. IR (KBr): 2973, 2932, 2879, 1644, 1525, 1480, 1433, 1347, 1180. 1110 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{26}H_{28}N4NaO_8$ $[M+Na]^+$ 547.1799, found 547.1795.

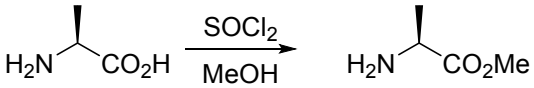
_N_-tert-Butoxycarbonyl-L-alanine 13



13

Experimental: To a solution of L-alanine (2.50 g, 27.8 mmol) in H_2O (50 mL) and 1 M NaOH aq (28 mL) at 0 °C was added $(Boc)_2O$ (9.10 g, 41.7 mmol) in dioxane (50 mL). After being stirred at room temperature for 8 h, the reaction mixture was cooled to 0 °C, acidified with 3M HCl solution to pH 2-3, and diluted with EtOAc (50 mL). The reaction mixture was extracted with EtOAc (3×70 mL), washed with brine, and dried over Na_2SO_4. Concentration gave the *N*-tert-Butoxycarbonyl-L-alanine in 94% yield as a white solid (4.96 g, 26.0 mmol). HRMS (ESI) m/z: calculated for $C_8H_{15}NNaO_4$ $[M+Na]^+$ 212.0893, found 212.0889.

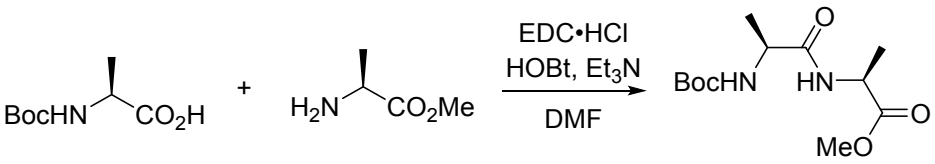
L-alanine methyl ester hydrochloride 14



14

Experimental: Thionyl chloride (4 mL) was added dropwise to L-alanine (2.50 g, 27.8 mmol) in methanol (28 mL) at 0 °C and the solution was stirred at 45 °C for 6 h. Then the solvent was evaporated in vacuo and the resulting residue was diluted with diethyl ether and suspended in diethyl ether. The suspension was filtered to give L-alanine methyl ester hydrochloride as a white solid (3.54 g, 91%).

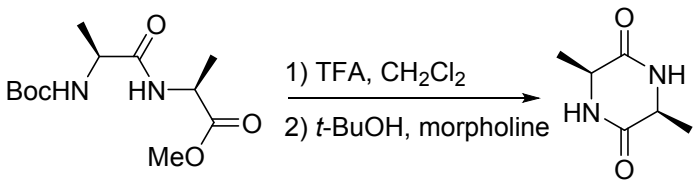
Methyl (tert-butoxycarbonyl)-L-alanyl-L-alaninate 15



13 **14** **15**

Experimental: To a solution of *N*-Boc-L-alanine **13** (2.50 g, 13.2 mmol.) and amino acid methyl ester hydrochloride **14** (1.84 g, 13.2 mmol.) in DMF (52ml), HOBt (2.14 g, 15.8 mmol.), EDC•HCl (3.04 g, 15.8 mmol.) and triethylamine (5.7 ml, 43.6 mmol.) were added. The the reaction mixture was stirred overnight at room temperature. Then the mixture was diluted with EtOAc (50 mL). The organic layers were washed with water (3 × 50 ml), dried over Na_2SO_4, filtered and the solvent removed in vacuo. The resulting residue was purified using silica gel chromatographyto give **15** as a white solid (2.64 g, 73%). R_f = 0.5 (*n*-hexane/EtOAc, 1:1). m.p.: 106 ^oC-109 ^oC. $[\alpha]_D^{23}$ -70.0 (c 1.0, methanol). ^1H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, 1H), 5.06 (s, 1H), 4.56 (p, J = 7.2 Hz, 1H), 4.17 (s, 1H), 3.73 (s, 3H), 1.43 (s, 6H), 1.39 (d, *J* = 7.2 Hz, 3H), 1.35 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.13, 172.16, 155.37, 80.07, 52.39, 49.98, 47.95, 28.24, 18.28. IR (KBr): 3317, 2984, 2939, 1746, 1694, 1666, 1530, 1453, 1392, 1368, 1169, 1072 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{12}H_{22}N_2NaO_5$ $[M+Na]^+$ 297.1421, found 297.1412.

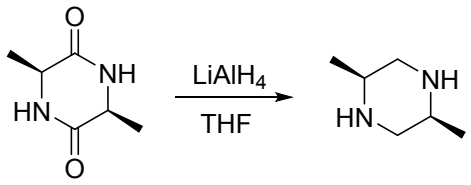
(3*S*,6*S*)-3,6-dimethylpiperazine-2,5-dione 16



15 **16**

Experimental: Trifluoroacetic acid (1.2 mL) was added via syringe to a solution of the dipeptide **15** (1g, 3.65 mmol.) in dichloromethane (14 mL). The reaction mixture was stirred at 30 ^oC for 6 h, then the brown solution was concentrated under reduced pressure to afford a brown residue, which was dissolved in *t*-butanol (18 mL). The reaction mixture was stirred vigorously as morpholine (4 mL) was introduced slowly at room temperature. After 5 h, the diketopiperazine **16** precipitated as a white solid. The orange solution was removed under reduced pressure, and the resulting mixture was diluted with dichloromethane and the product was collected by filtration and washed sequentially with dichloromethane to provide diketopiperazine **16** (460 mg, 89%) as a fluffy white solid. R_f = 0.7 (CH_2Cl_2/MeOH, 5:1). m.p.: 261 ^oC-263 ^oC. $[\alpha]_D^{23}$ -50.0 (c 1.0, methanol). ^1H NMR (400 MHz, DMSO) δ 8.08 (s, 2H), 3.90 (q, *J* = 6.9 Hz, 2H), 1.24 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (100 MHz, DMSO) δ 169.07, 49.78, 18.49. IR (KBr): 3185, 2984, 1687, 1657, 1459, 1439 1321, 1154, 1118 cm^{-1}. HRMS (ESI) m/z: calculated for $C_6H_{10}N_2NaO_2$ $[M+Na]^+$ 165.0634, found 165.0636.

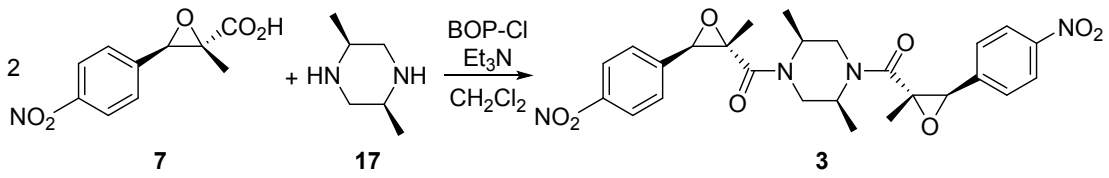
(2*S*,5*S*)-2,5-dimethylpiperazine 17



16 **17**

Experimental: To a solution of the diketopiperazine **16** (100 mg, 0.70 mmol) in tetrahydrofuran (7 mL) was added $LiAlH_4$ (214 mg, 5.62 mmol) over a period of 10 min with stirring at 0 °C. After the addition, the reaction mixture was refluxed for 8 h, then cooled to 0 °C, and carefully quenched by the addition of water. The mixture was filtered, and the filter cake was washed with dichloromethane. The combined filtrate was dried over Na_2SO_4, filtered through celite and evaporated under reduced pressure to give the piperazine **17** (64 mg 80%) as a colorless oil. The crude product was used directly in the next reaction.

((2*S*,5*S*)-2,5-dimethylpiperazine-1,4-diyl)bis(((2*S*,3*R*)-2-methyl-3-(4-nitrophenyl)oxiran-2-yl)methanone) 3



7 **17** **3**

Experimental: To a solution of piperazine **17** (20 mg, 0.175 mmol) in dichloromethane (2 mL) were added epoxy-acid **7** (86 mg, 0.386 mmol, 2.2 equiv) in dichloromethane (3 mL) and the solution was cooled to 0 °C in an ice-water bath. Then, BOP-Cl (117 mg, 0.456 mmol, 2.6 equiv) and triethylamine (69 μL, 0.525 mmol.) were added. The ice-water bath was removed and the reaction mixture was stirred overnight at room temperature. Then the mixture was diluted with dichloromethane (10 mL). The organic layers were washed with water (3 × 10 ml), dried over Na_2SO_4, filtered and the solvent removed in vacuo. The resulting residue was purified using silica gel chromatography (eluent: *n*-hexane/ EtOAc 1:1) to give a white solid compound **3** (70.6 mg, 76%). R_f = 0.5 (*n*-hexane/EtOAc, 1:2). m.p.: 139 °C-141 °C. $[\alpha]_D^{22}$ 148.0 (c 2.5, acetone), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 4.45 (brs, 1H), 4.27 (overlap, 2H), 3.34 – 3.08 (m, 1H), 1.31 (s, 3H), 1.24 (d, *J* = 4.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.67, 147.90, 141.13, 127.45, 123.61, 63.64, 61.87, 49.76, 43.80, 15.86, 14.89. IR (KBr): 2998, 2928, 1681, 1649, 1523, 1344, 1070 cm^{-1}. HRMS (ESI) m/z: calculated for $C_{26}H_{28}N_4NaO_8$ $[M+Na]^+$ 547.1799, found 547.1795.

Antibacterial assay

Experimental: The antibacterial activity was determined by broth microdilution against *S. aureus*(ATCC 25923), *S. albus*(ATCC 12228), *B. subtilis*(ATCC 5230), *E. coli*(ATCC 25922), *P. aeruginosa*(ATCC 10145), *Pasteurella multocida*(CVCC 408) and *Salmonella typhimurium*(ATCC 14028). Stock solutions of compounds were prepared in DMSO. The compound **2** and **3** were added to the test tube and serially diluted in Mueller-Hinton broth (the final concentration is 0.0625 μg/mL)[4]. Three Gram-positive bacteria, including *S. aureus*, *S. albus*, *P. aeruginosa*, and Gram-negative bacteria, *E. coli*, *P. aeruginosa*, *Pasteurella multocida*, *Salmonella typhimurium*, were cultivated and added to the tube. The initial concentration of bacteria cannot be lower than 10^5 CFU/mL. The broth was incubated at 36.7 ^oC for 18–24 h. The minimum inhibitory concentrations (MICs) were read when the change of clarity in the broth was observed in the control test tube. The ciprofloxacin was used as positive control.

1. G. G. Bianco, H. M. C. Ferraz, A. M. Costa, L. V. Costa-Lotufo, C. Pessoa, M. O. de Moraes, M. G. Schrems, A. Pfaltz, L. F. Silva, *J. Org. Chem*., 2009, **74**, 2561-2566.
2. R. Hata, H. Nonaka, Y. Takakusagi, K. Ichikawa, S. Sando, *Angew. Chem. Int. Ed*., 2016, **55**, 1765-1768.
3. R. F. Nystrom, W. G. Brown, *J. Am. Chem. Soc*., 1948, **70**, 3738-3740.
4. F. R. Cockerill, Clinical, I. Laboratory Standards, *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* : *Approved standard*, Clinical and Laboratory Standards Institute, 9th ed edn., 2012.

X-ray Crystallographic Study of Compound 3

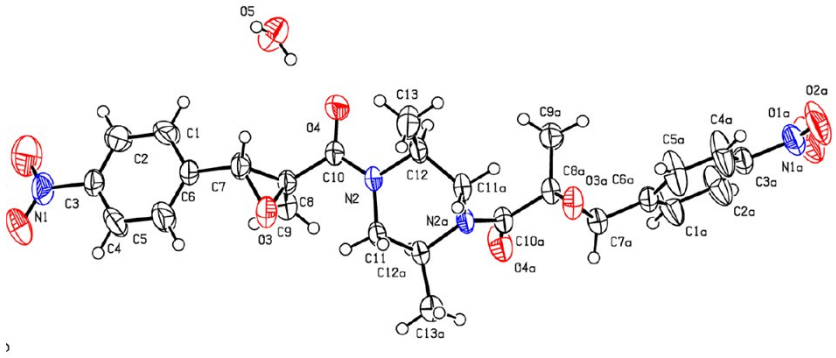


Figure S1. Single crystal X-ray structure of **3.**

Experimental:

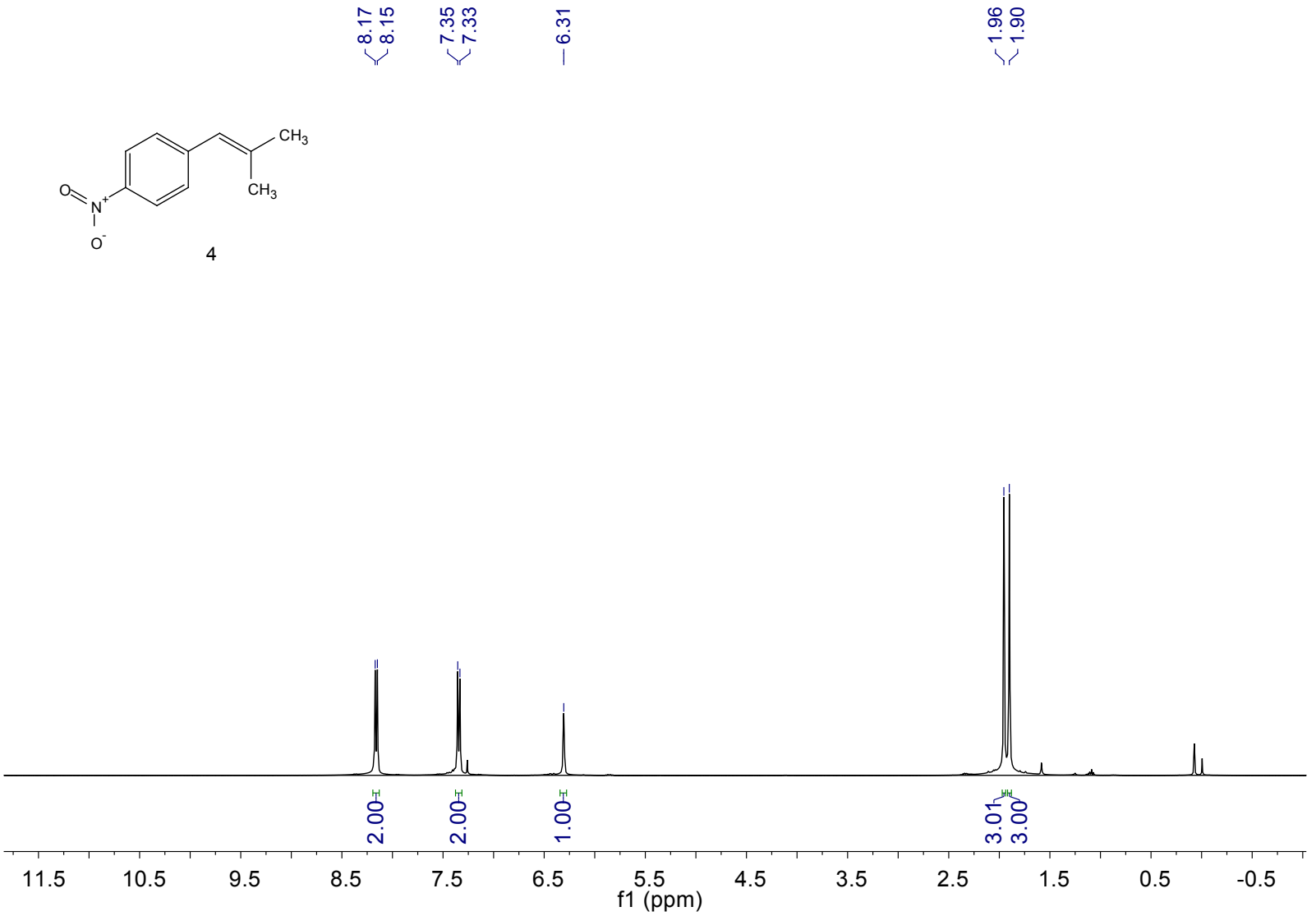
Single crystals of $C_{26}H_{28}N_4O_8$ were obtained by recrystallization from mixed solvents of dichloromethane and hexane. A suitable crystal was selected and carried out on a SuperNova, Dual, Cu at zero, Eos diffractometer. The crystal was kept at 292.9(8) K during data collection. Using Olex2 [1], the structure was solved with the Superflip [2] structure solution program using Charge Flipping and refined with the ShelXL [3] refinement package using Least Squares minimisation.

1. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.
2. Palatinus, L. & Chapuis, G. (2007). J. Appl. Cryst., 40, 786-790; Palatinus, L. & van der Lee, A. (2008). J. Appl. Cryst. 41, 975-984; Palatinus, L., Prathapa, S. J. & van Smaalen, S. (2012). J. Appl. Cryst. 45, 575-580.
3. Sheldrick, G.M. (2015). Acta Cryst. C71, 3-8.

Crystal Data for $C_{26}H_{30}N_4O_9$ (M =542.54 g/mol): monoclinic, space group I2 (no. 5), a = 9.9349(9) Å, b = 5.4755(5) Å, c = 25.351(3) Å, β = 101.162(10)°, V = 1352.9(2) Å^3, Z = 2, T = 292.9(8) K, μ(MoKα) = 0.102 mm^{-1}, $Dcalc$ = 1.332 g/cm^3, 3928 reflections measured (7.04° ≤ 2Θ ≤ 52.04°), 2177 unique (R_{int} = 0.0238, R_{sigma} = 0.0388) which were used in all calculations. The final R_1 was 0.0439 (>2sigma(I)) and wR_2 was 0.1064 (all data). CCDC 1834200.

Table 1 Crystal data and structure refinement for **3**.

Empirical formula	$C_{26}H_{30}N_4O_9$
Formula weight	542.54
Temperature/K	292.9(8)
Crystal system	monoclinic
Space group	I2
a/Å	9.9349(9)
b/Å	5.4755(5)
c/Å	25.351(3)
α/°	90.00
β/°	101.162(10)
γ/°	90.00
Volume/Å^3	1352.9(2)
Z	2
ρ_{calc}g/cm^3	1.332
μ/mm^{-1}	0.102
F(000)	572.0
Crystal size/mm^3	0.21 × 0.15 × 0.14
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	7.04 to 52.04
Index ranges	$-12 \leq h \leq 12$, $-6 \leq k \leq 6$, $-29 \leq l \leq 31$
Reflections collected	3928
Independent reflections	2177 [R_{int} = 0.0238, R_{sigma} = 0.0388]
Data/restraints/parameters	2177/1/183
Goodness-of-fit on F^2	1.052
Final R indexes [I>=2σ (I)]	R_1 = 0.0439, wR_2 = 0.0971
Final R indexes [all data]	R_1 = 0.0558, wR_2 = 0.1064
Largest diff. peak/hole / e Å^{-3}	0.13/-0.15
Flack parameter	- 1.0(18)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7.33
6.31
1.96
1.90
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CH3
O
N+
O-
4
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1.00
3.01
3.00
11.5
10.5
9.5
8.5
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6.5
5.5
4.5
3.5
2.5
1.5
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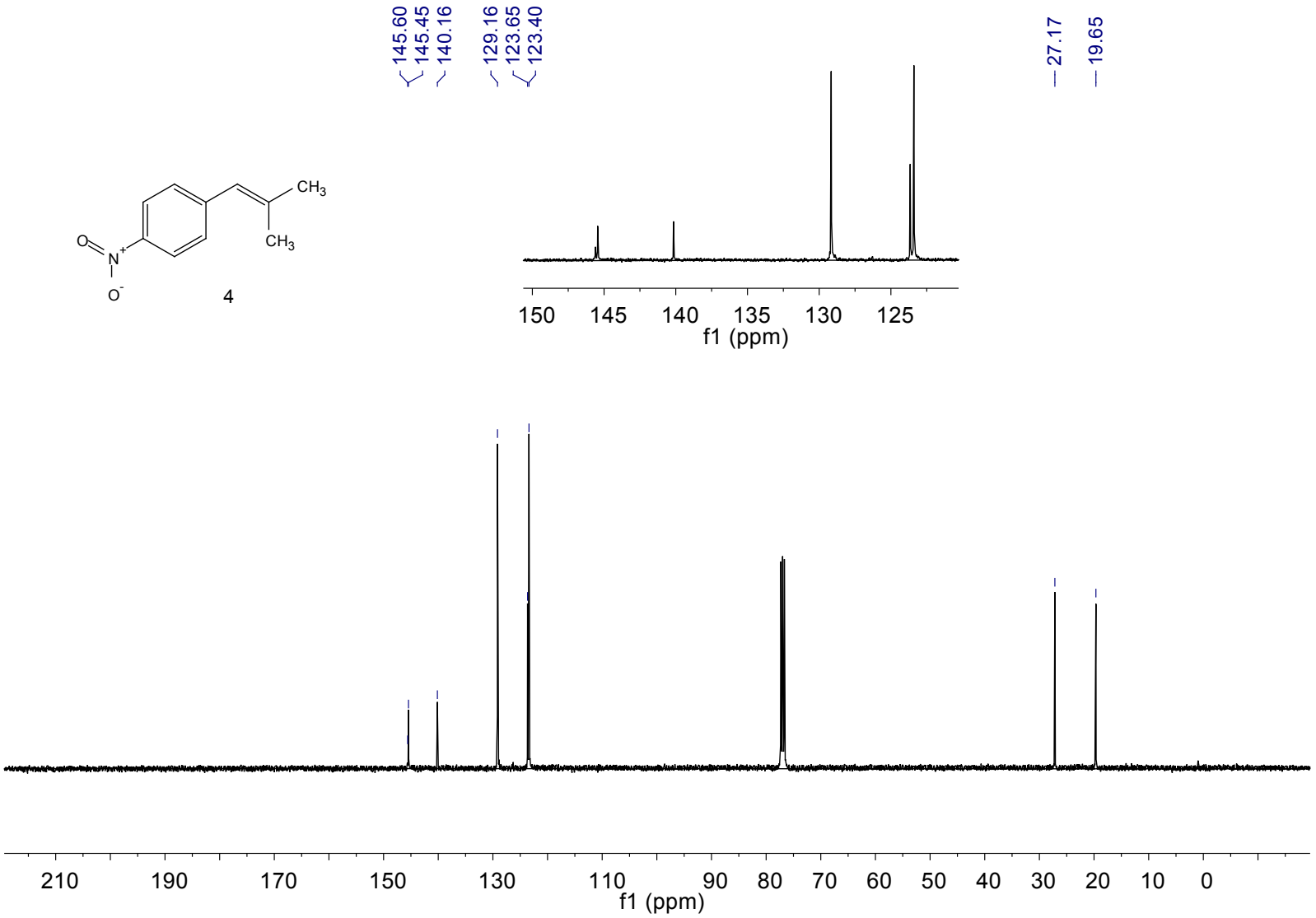
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135
130
125
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20
10
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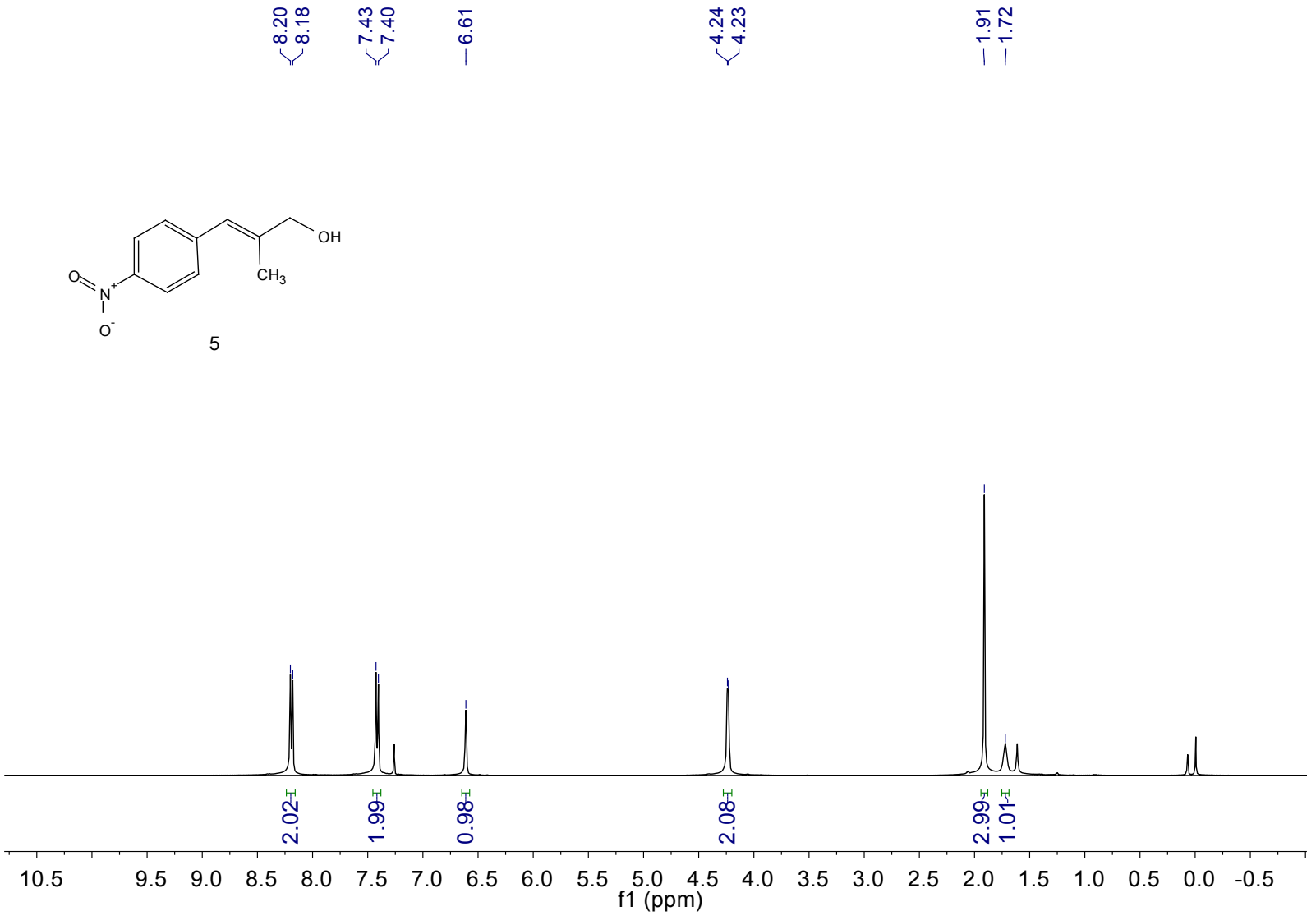
f1 (ppm)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O-
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1.99
0.98
2.08
2.99
1.01
10.5
9.5
9.0
8.5
8.0
7.5
7.0
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6.0
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5.0
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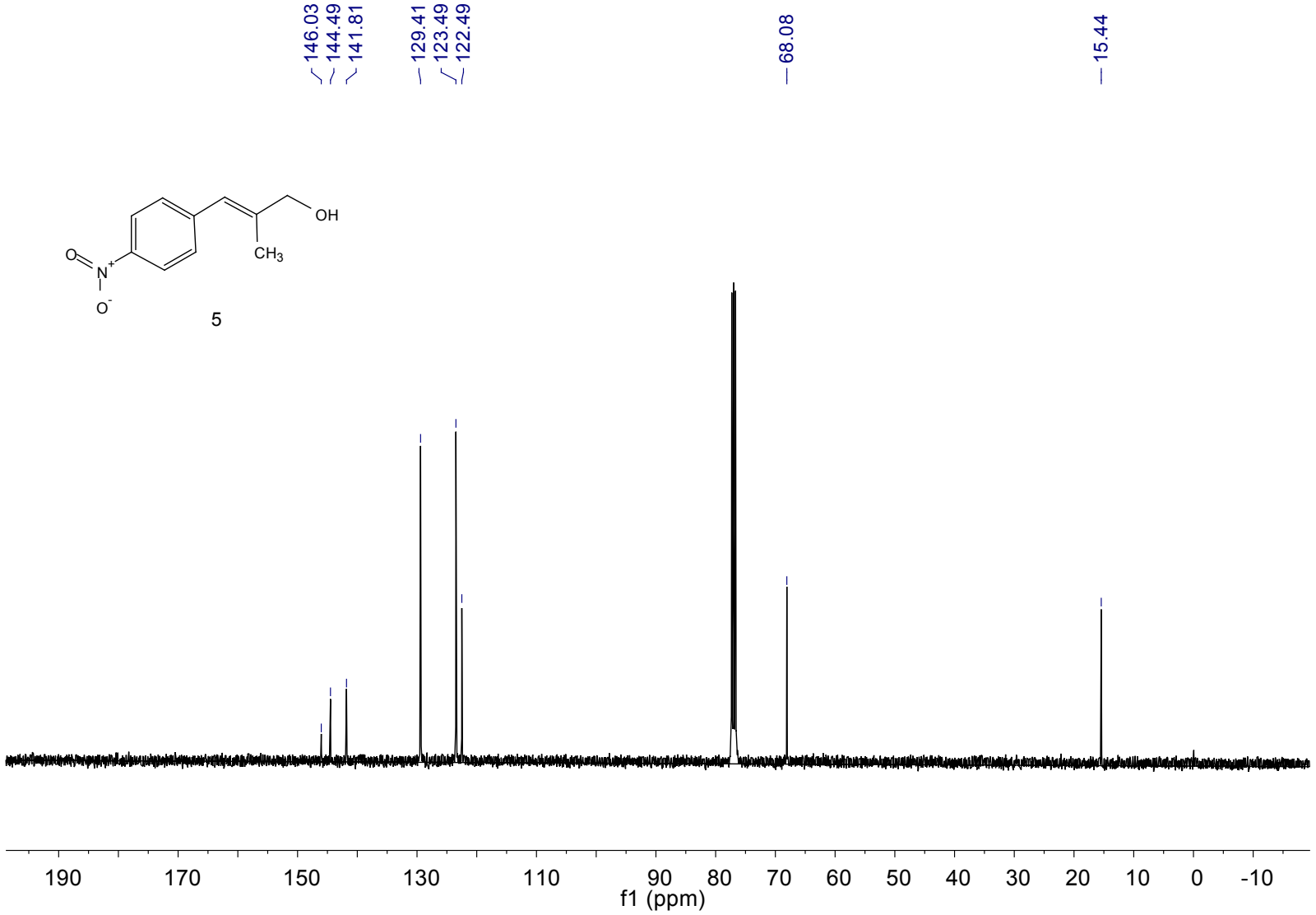
f1 (ppm)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N+
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130
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90
80
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30
20
10
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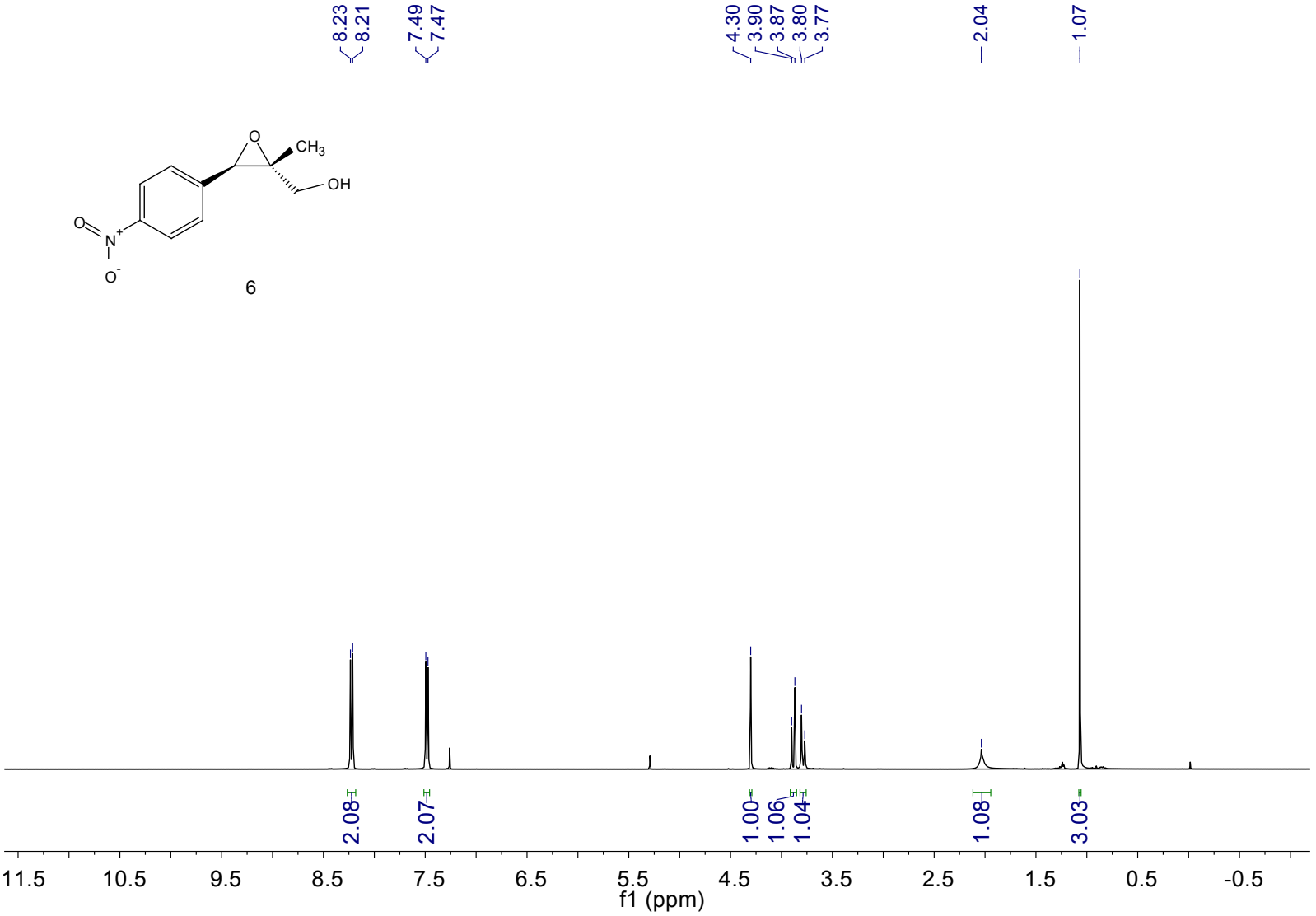
-10
f1 (ppm)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O
N+
O-
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2.08
2.07
1.00
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1.04
1.08
3.03
11.5
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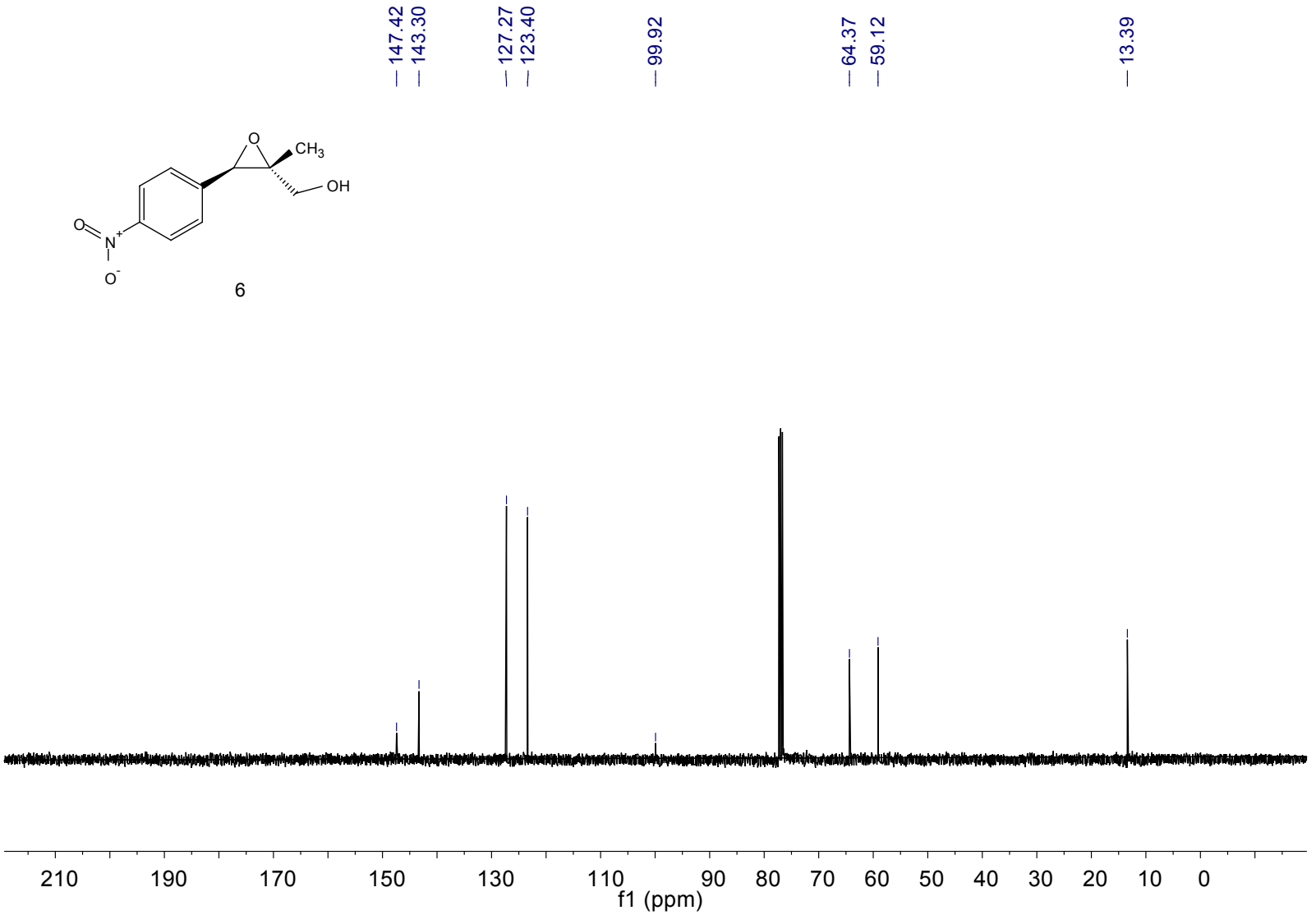
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30
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10
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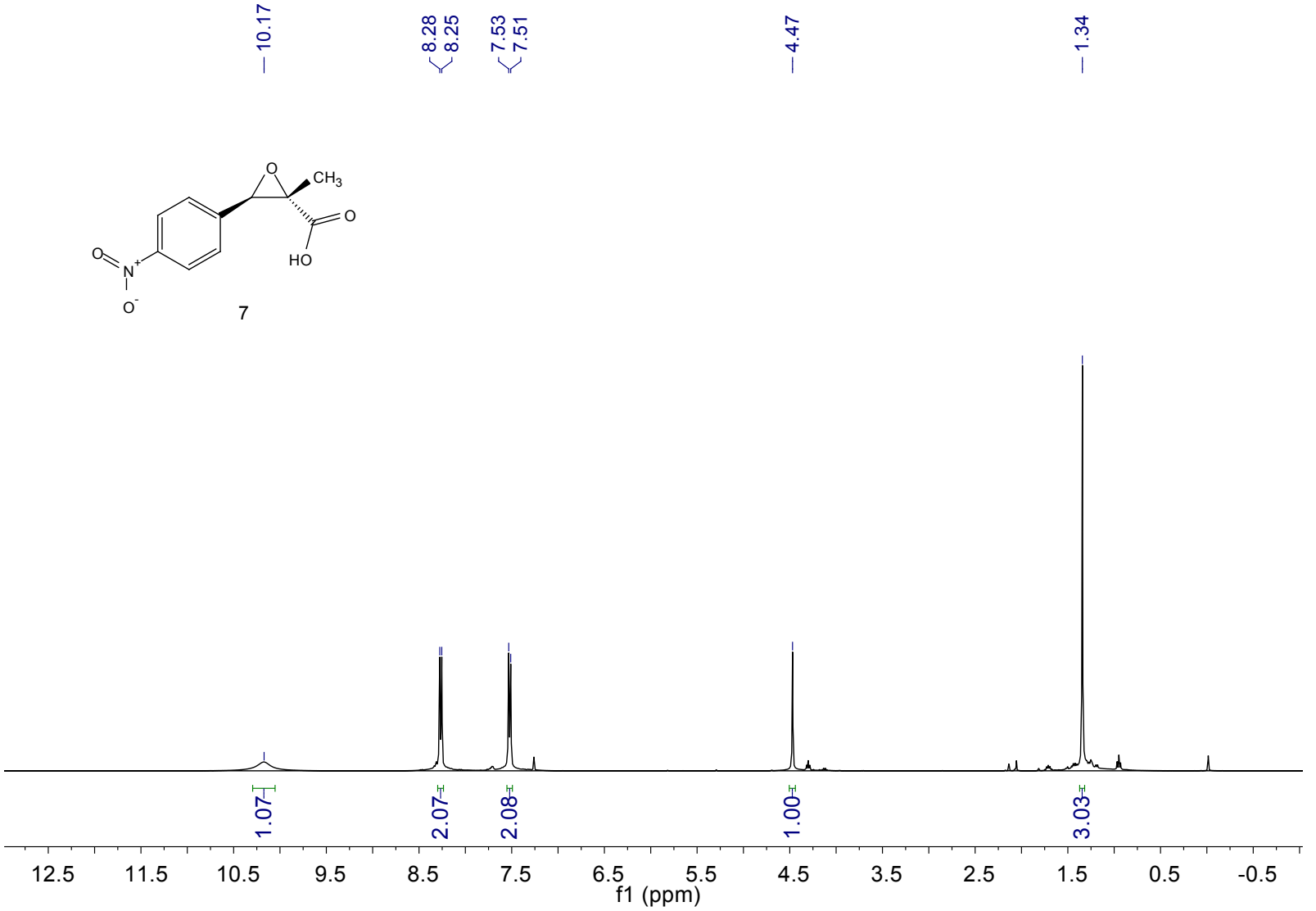
f1 (ppm)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HO
O
N+
O-
7
1.07
2.07
2.08
1.00
3.03
12.5
11.5
10.5
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5.5
4.5
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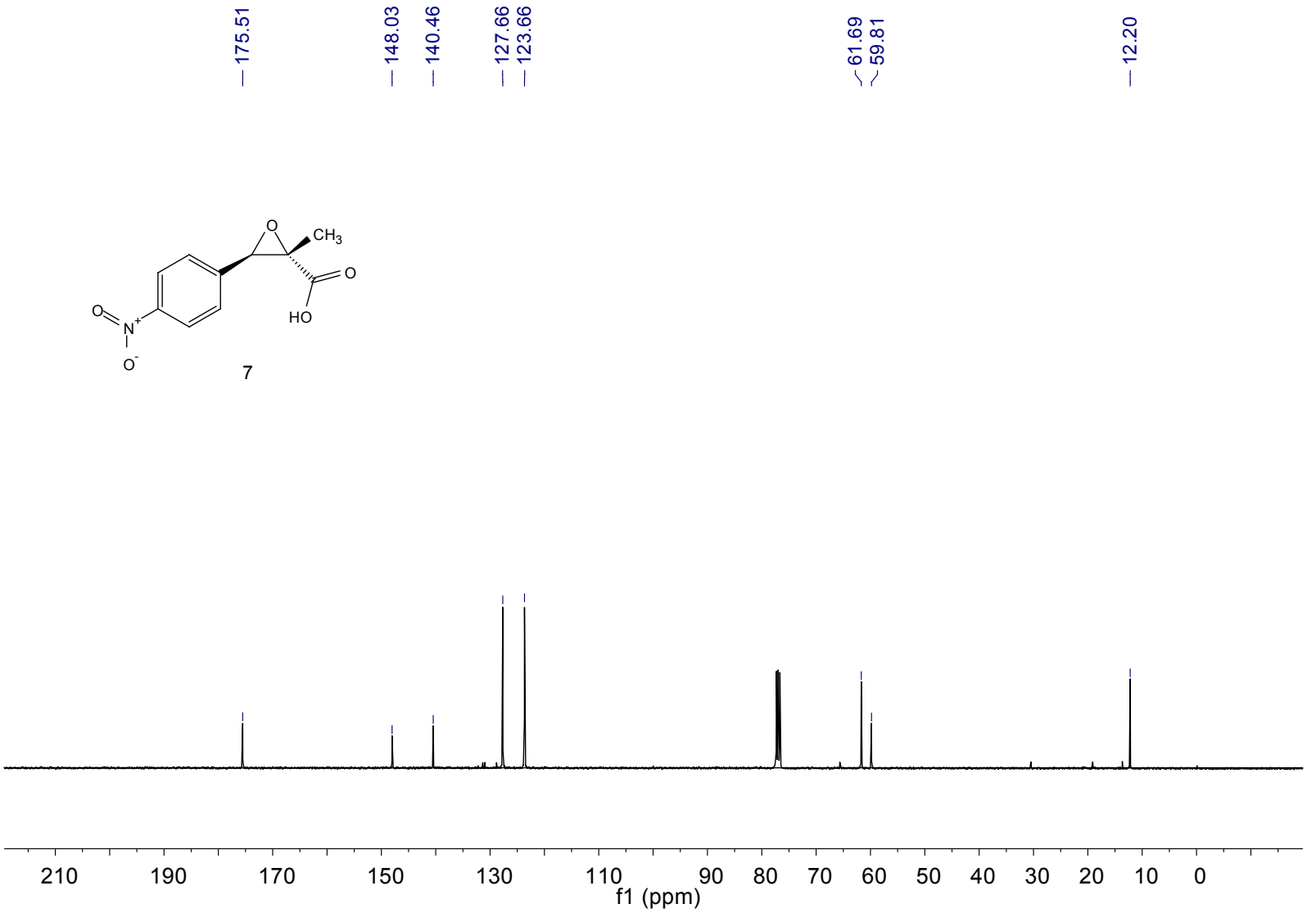
f1 (ppm)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N+
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90
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50
40
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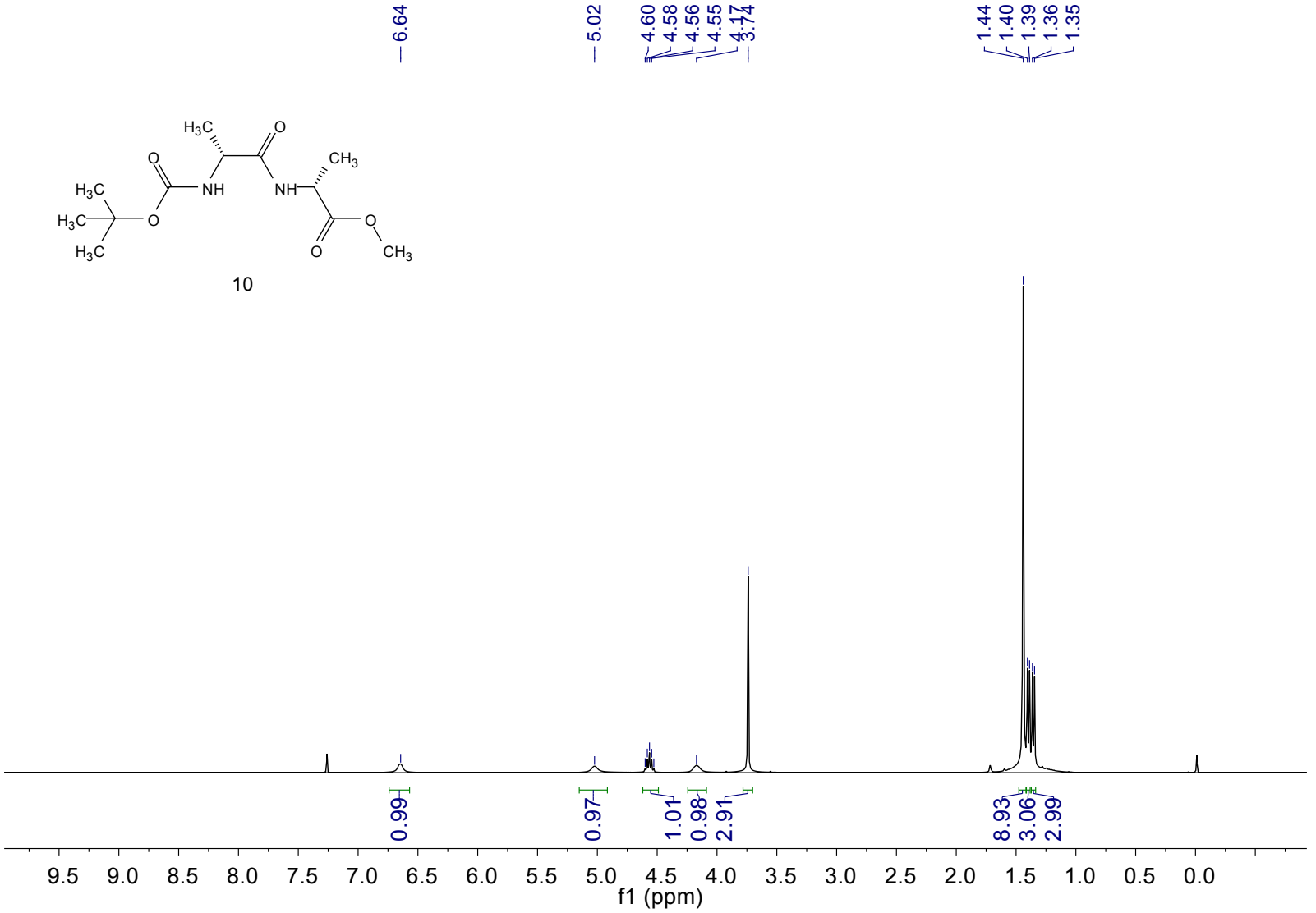
10
0
f1 (ppm)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0.97
1.01
0.98
2.91
8.93
3.06
2.99
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

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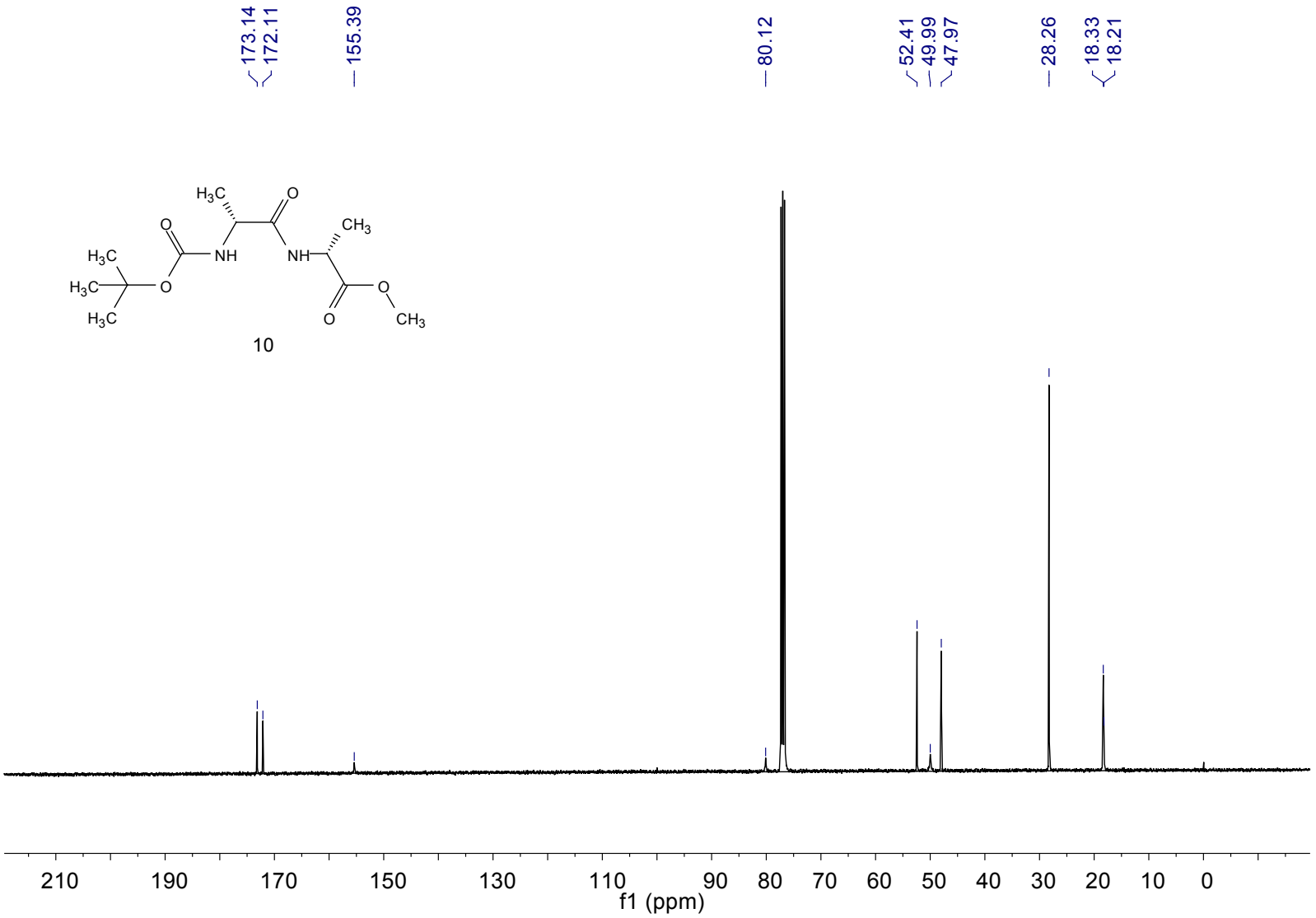
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O
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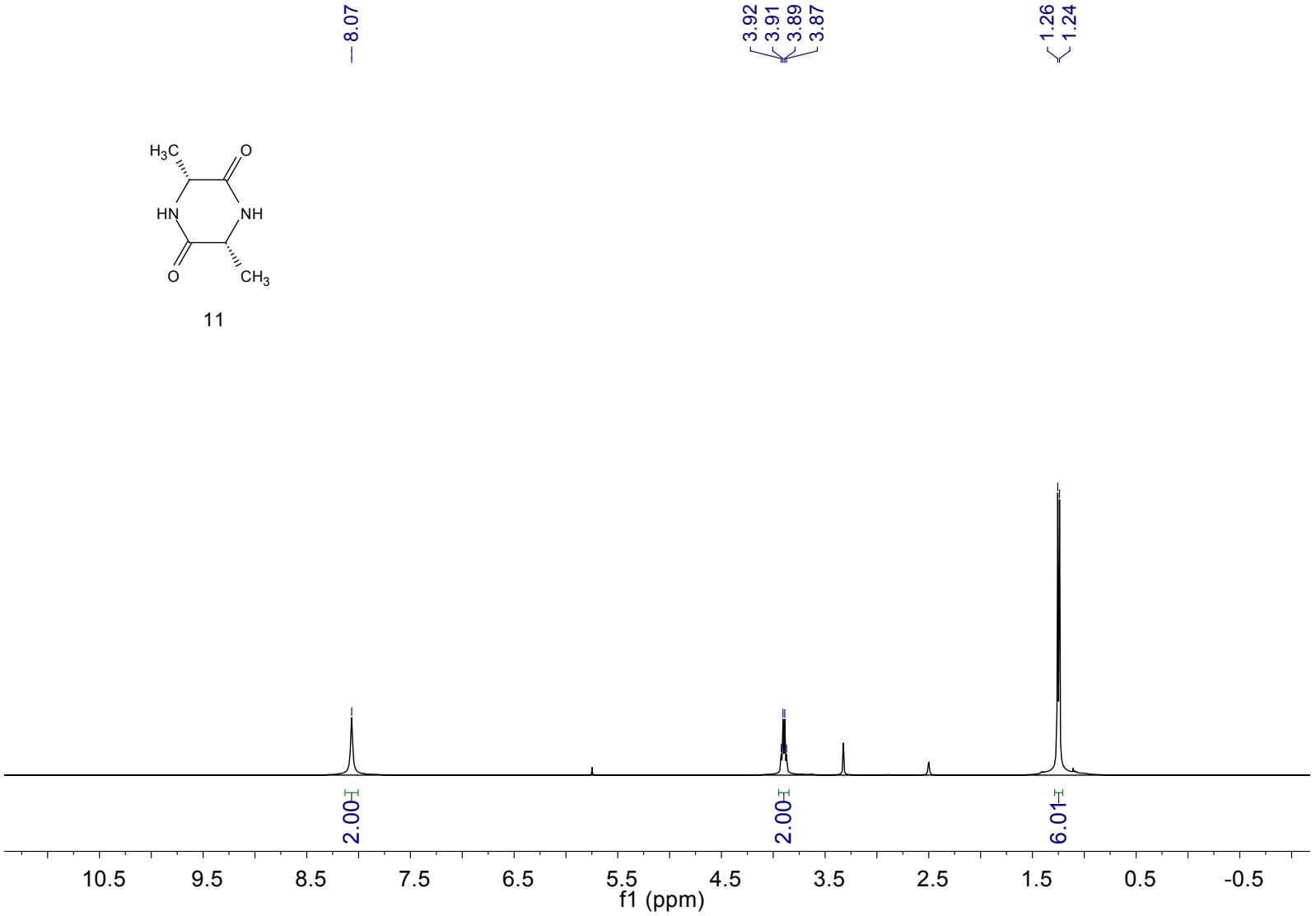
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4.5
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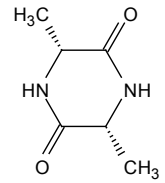
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11

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50
40
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20
10
0
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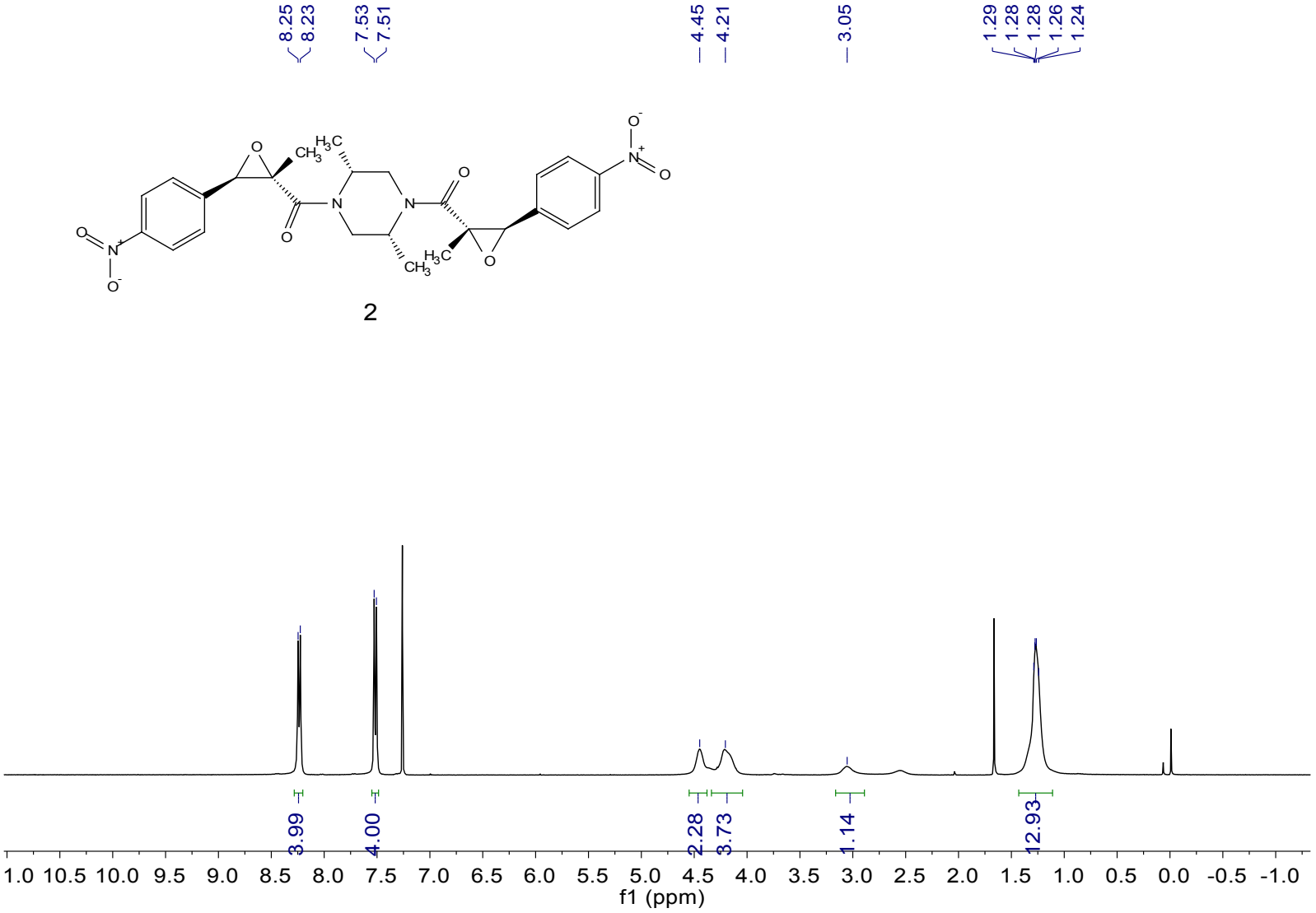
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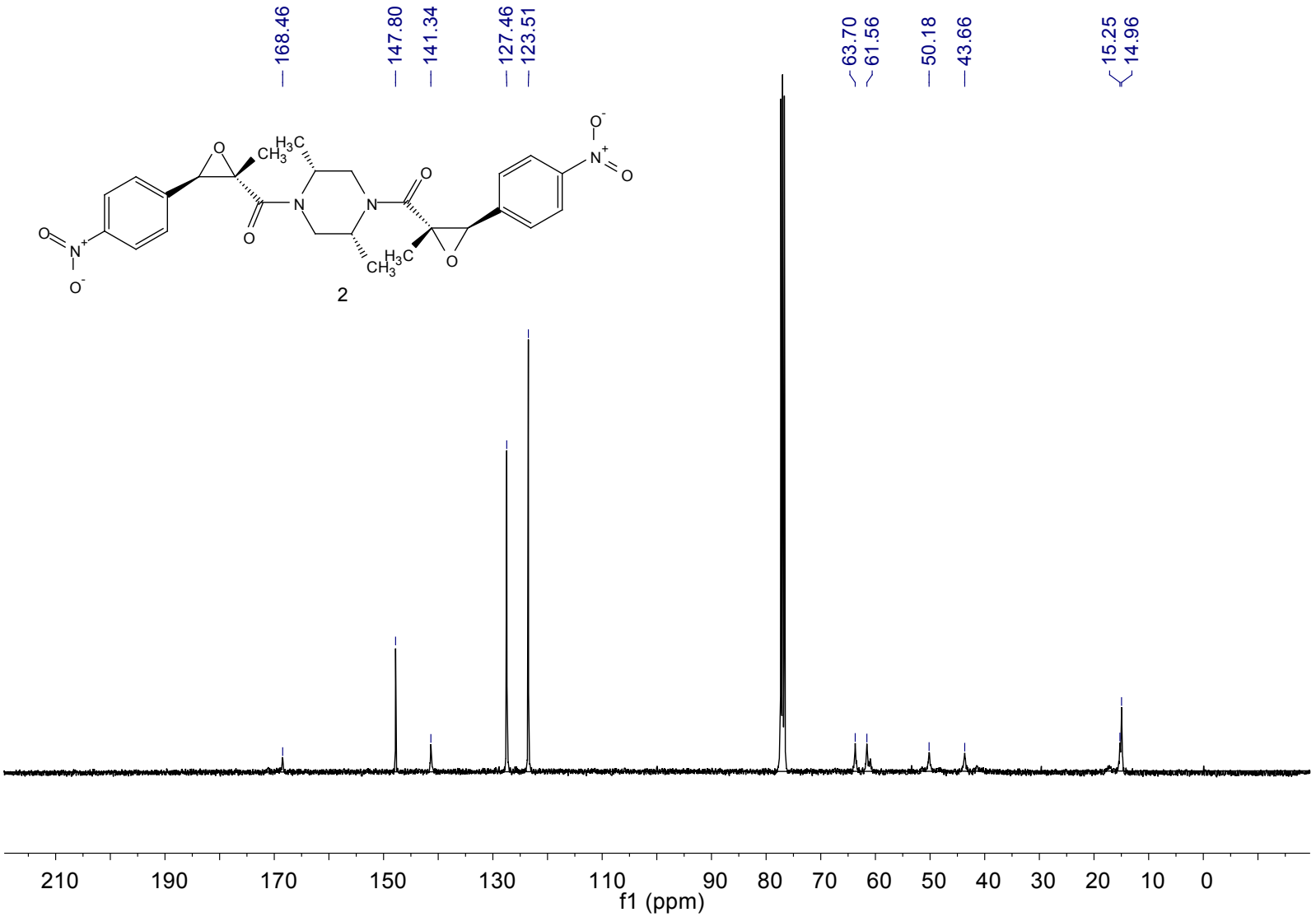
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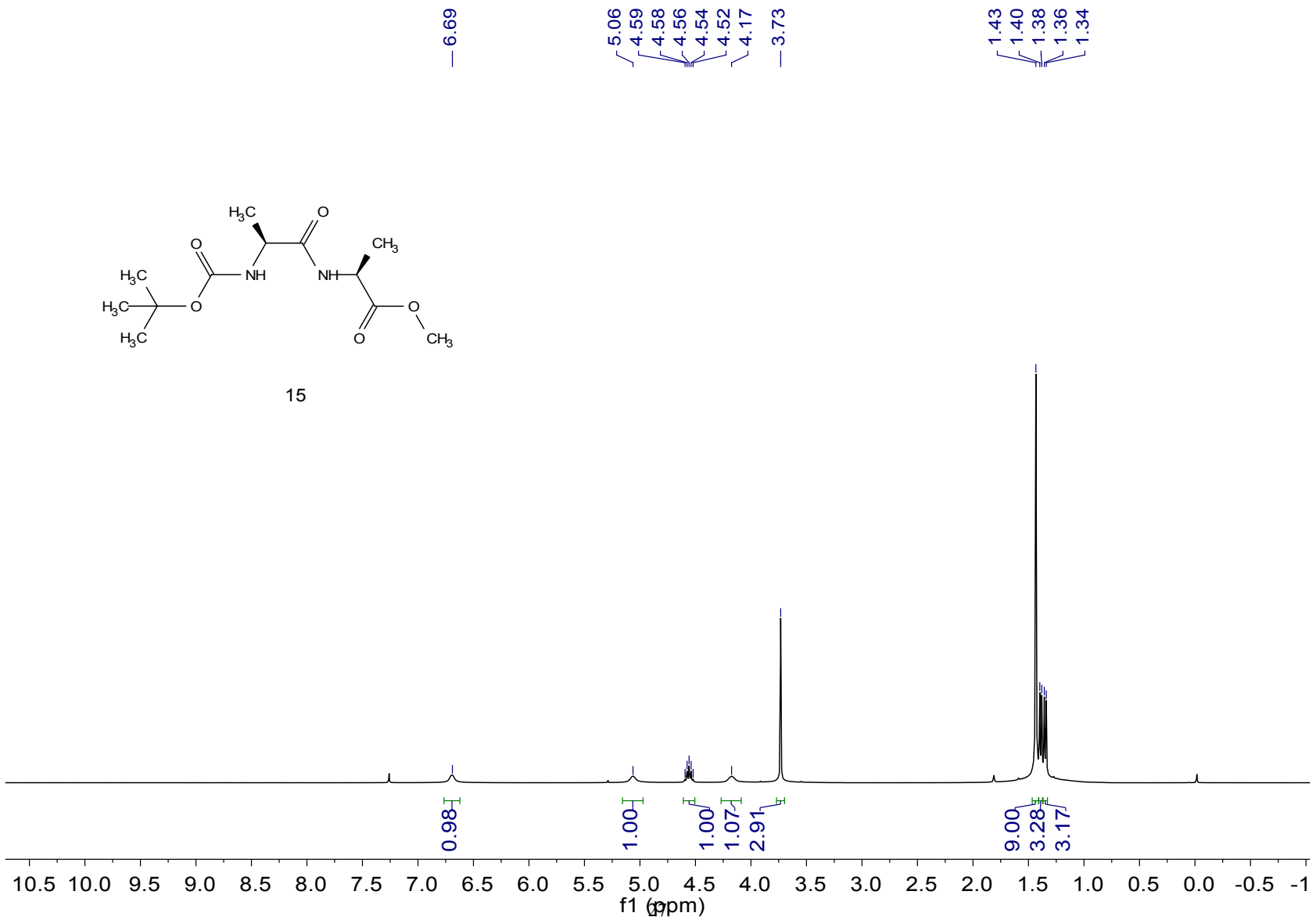
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9.00
3.28
3.17
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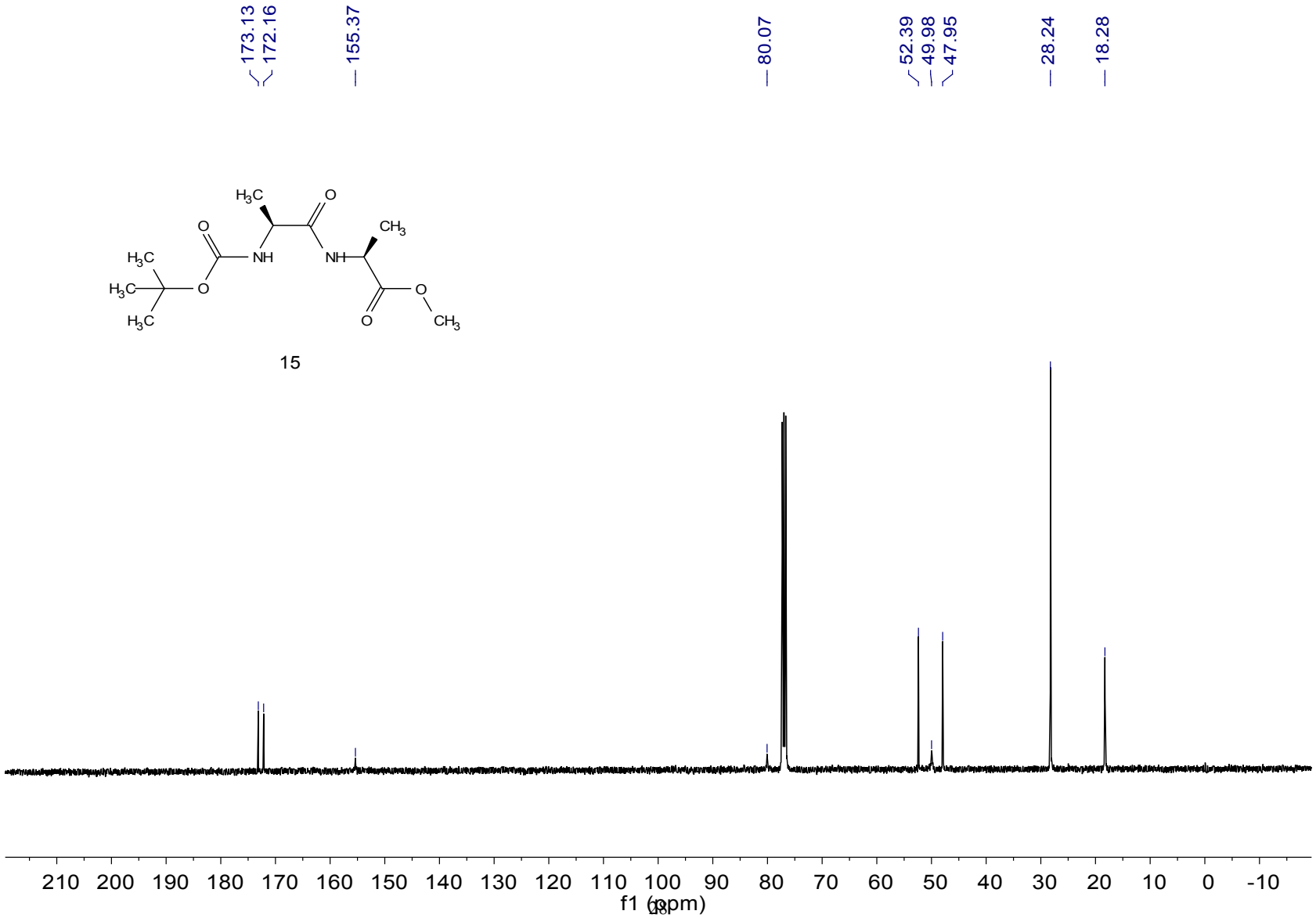
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NH
H3C
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CH3
15
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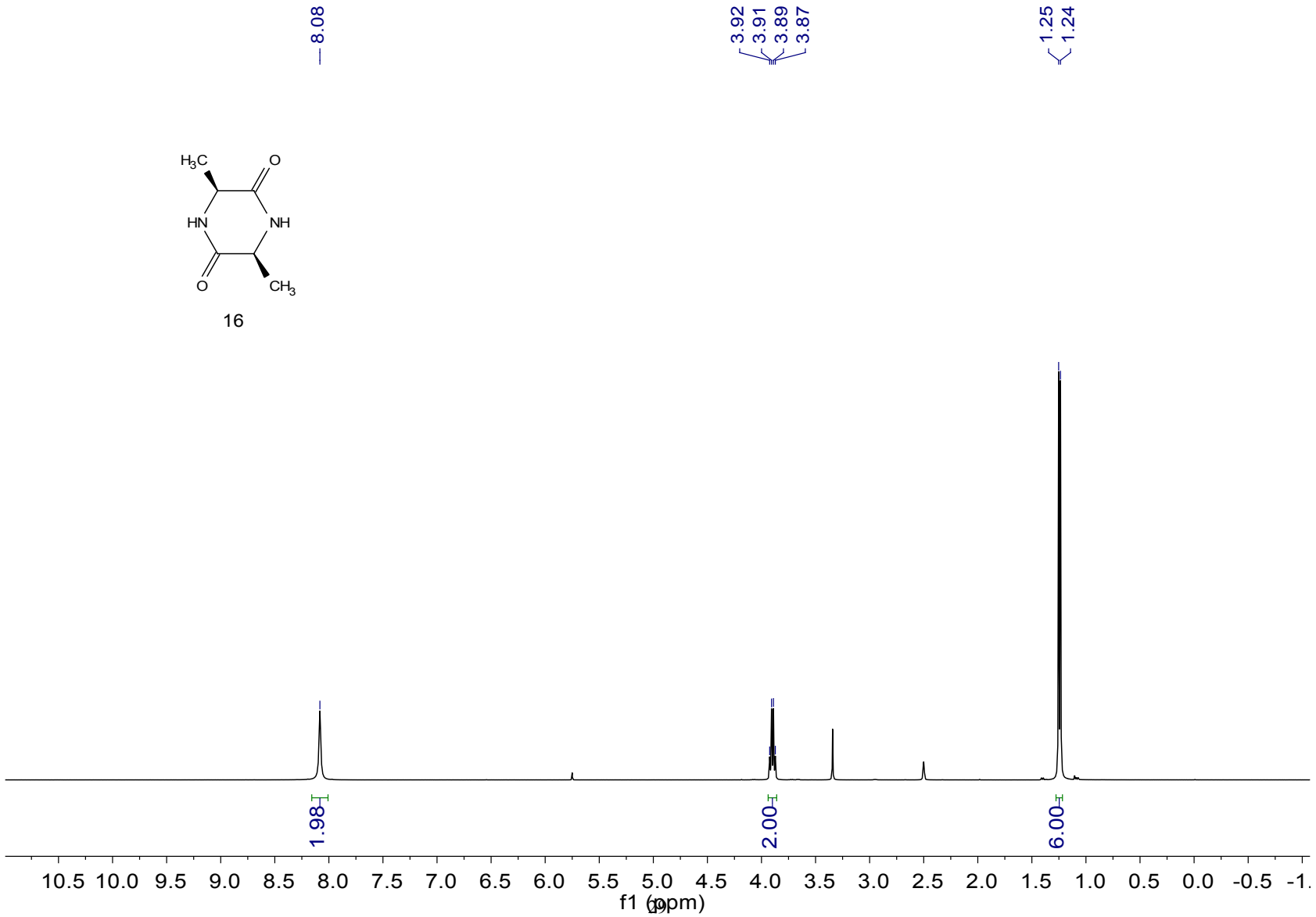
f1 (ppm)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6.00
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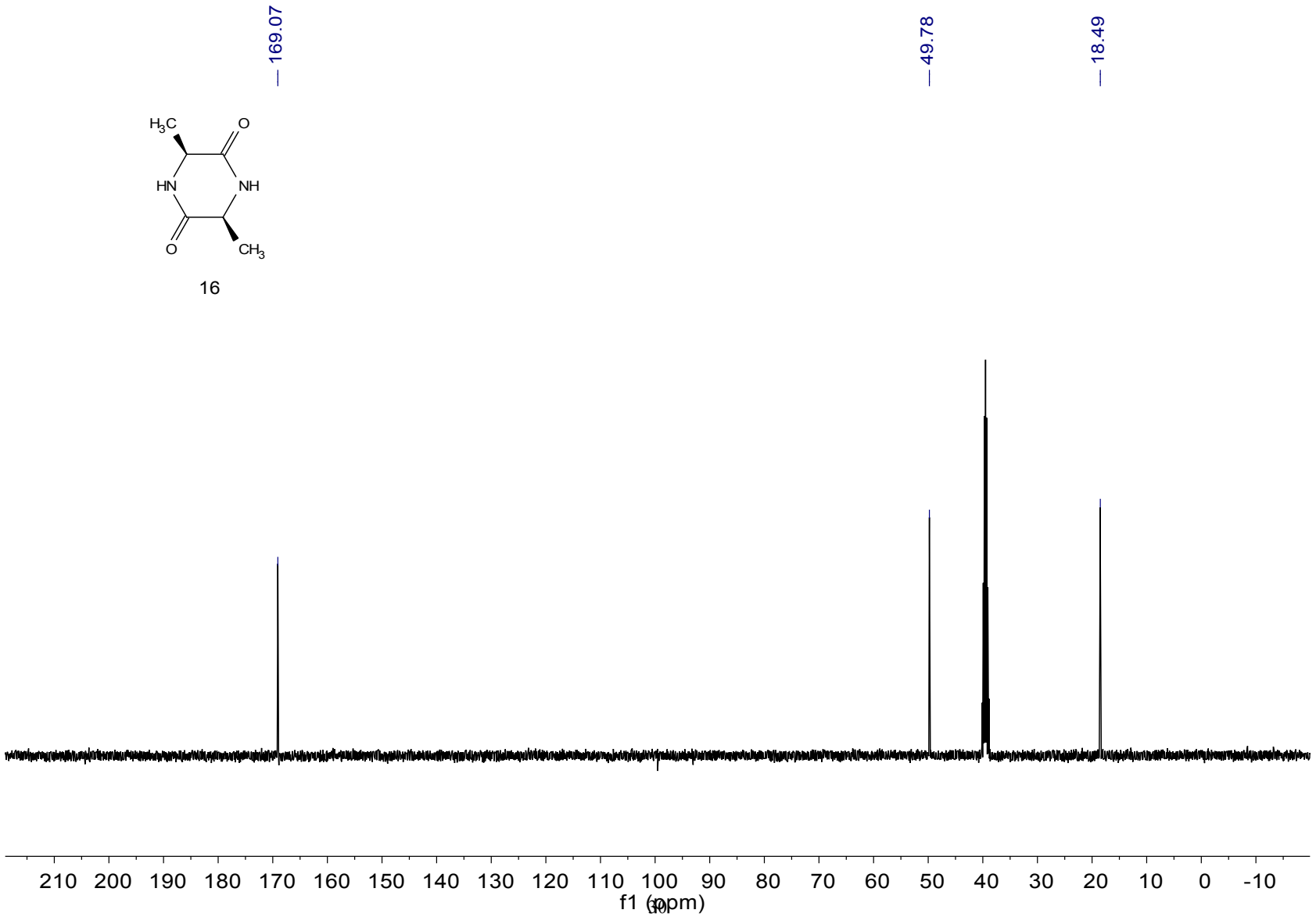
f1 (ppm)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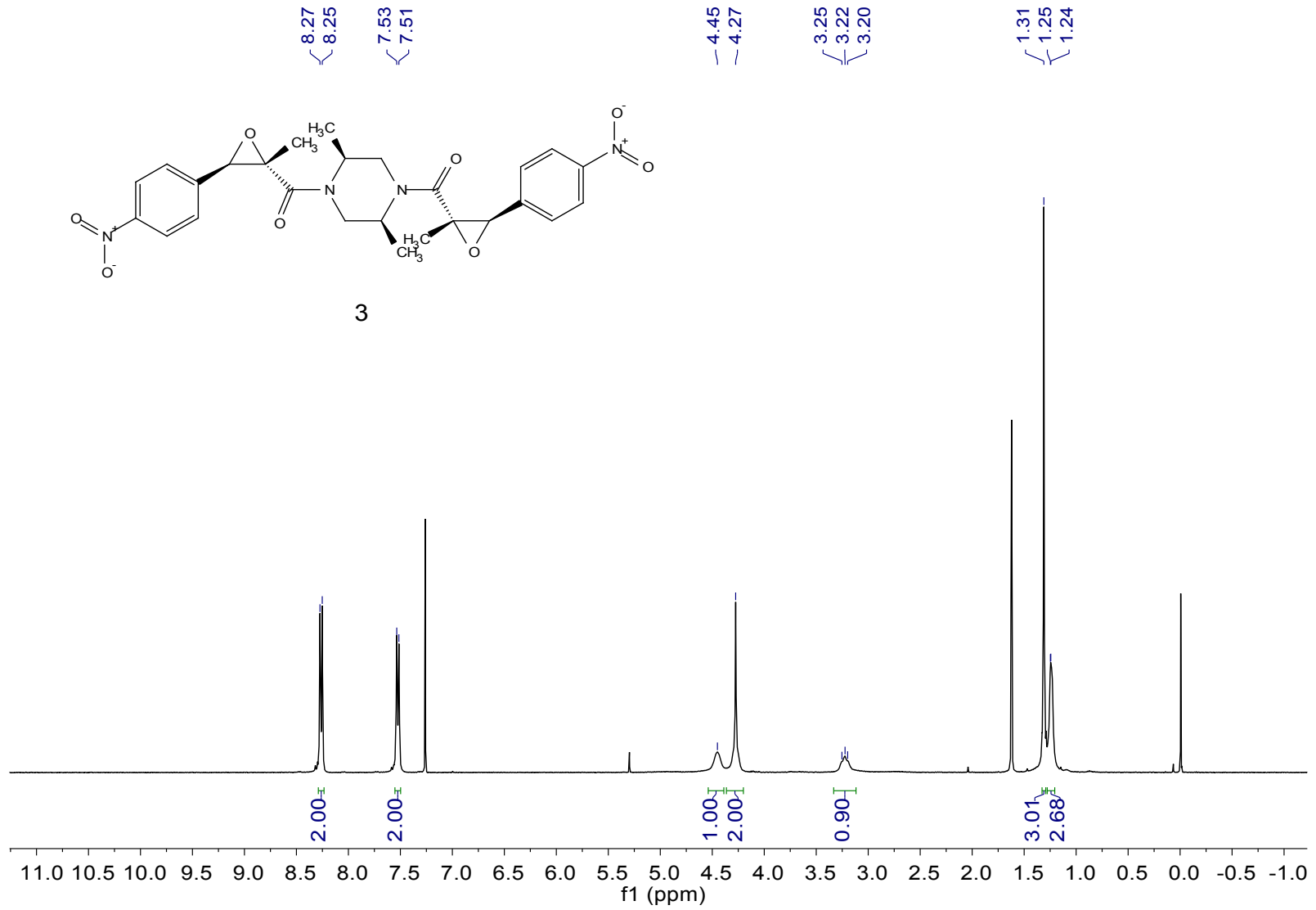
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1.25
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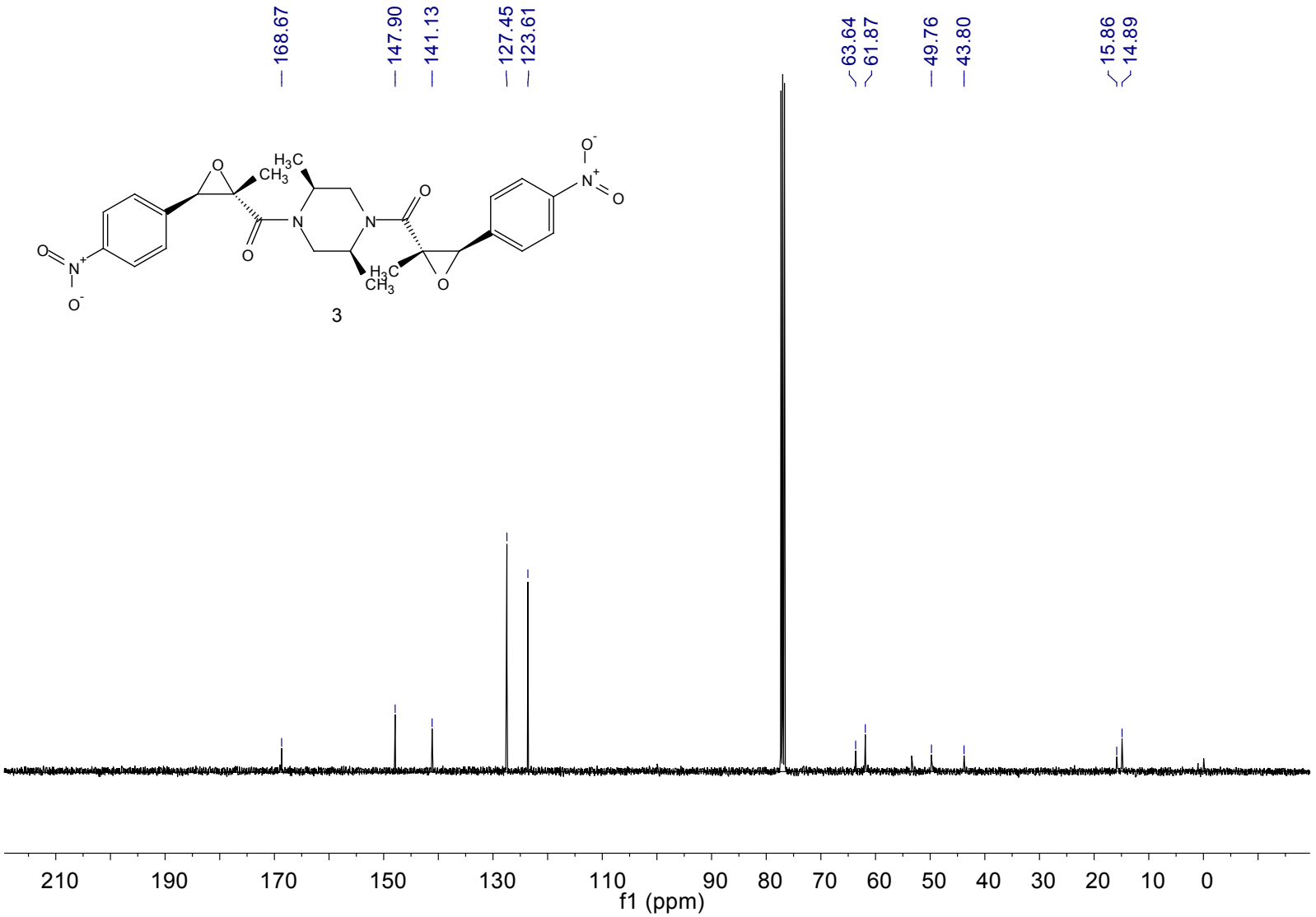
2.00
1.00
2.00
0.90
3.01
2.68
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)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130
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90
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70
60
50
40
30
20
10
0
f1 (ppm)