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Supporting Information

Synthesis and Biological Evaluation of the Antimicrobial Natural Product Lipoxazolidinone A

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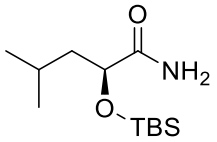
General Information

General information: THF and dichloromethane were purified using an alumina filtration system. Starting materials were purchased from a commercial chemical company and used as received. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F254 plates, 250 mm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a KMnO4 solution (1.5 g of $KMnO_4$, 10 g of K_2CO_3, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Reactions were also monitored by LC-MS (2.6 mm C18 50 x 2.10 mm column). Flash chromatography on SiO_2 was used to purify the crude reaction mixtures and performed on a flash system utilizing pre-packed cartridges and linear gradients.

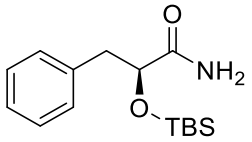
Melting points were determined using a capillary melting point apparatus. Infrared spectra were determined on a FT/IR spectrometer. ^1H, ^{13}C and NMR spectra were obtained on a 400, 500 or 700 MHz instrument in $CDCl_3$ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard ($CDCl_3$ = 7.26 ppm for ^1H and 77.16 ppm for ^{13}C). ^1H NMR spectra were run at 400 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, bs = broad singlet, dt = doublet of triplet, tt = triplet of triplet), number of protons, and coupling constant(s). ^{13}C NMR spectra were run at 100 MHz, 125 MHz or 175 MHz using a proton-decoupled pulse sequence with a d1 of 1 second unless otherwise noted, and are tabulated by observed peak. High-resolution mass spectra were obtained on an ion trap mass spectrometer using heated electrospray ionization (HESI).

General procedure for the preparation of O-TBS-α-hydroxy amides from α-amino acids

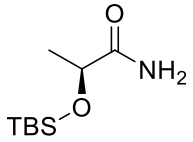
The α-hydroxy acid derivatives were synthesized following a known literature procedure.[1] In brief, the amino acid (10 mmol) was dissolved in 1 M H_2SO_4 (10 mmol) and cooled to 0 ˚C. Then 2 M $NaNO_2$ (20 mmol) was added dropwise and the reaction was allowed to slowly warm to room temperature and stirred overnight followed by addition of another aliquot of 2 M $NaNO_2$ (10 mmol) and the reaction was stirred for a further 24 h at room temperature. The reaction mixture was extracted with EtOAc (7 x 10 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$), filtered and concentrated. The resulting crude solid was dissolved in DMF (resulting in a 2 M solution) and imidazole (42 mmol) and TBSCl (21 mmol) were added and the reaction was stirred for 24 h at room temperature. The reaction was then diluted with EtOAc and washed with 10% citric acid (15 mL), sat. aq. $NaHCO_3$ (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and purified by flash column chromatography on SiO_2 (20% EtOAc:hex). To the resulting TBS protected hydroxy acid (5 mmol) was added *p*-toluenesulfonyl chloride (5 mmol), silica supported ammonium chloride (10 mmol)[2,3] and Et_3N (25 mmol) and the reaction was stirred for 15 minutes at room temperature and then diluted with EtOAc, filtered and concentrated. The residue was purified by flash column chromatography on SiO_2 (20% EtOAc:hex).



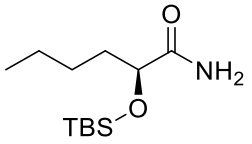
(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)-4-methylpentanamide (S1).[4] Yield: 187 mg (36%) of **S1** as a white solid. R_f = 0.18 (hexanes:EtOAc, 4:1); $[\alpha]_D$ = -39˚ (*c* = 2.4, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05 (s, 1 H), 6.46 (s, 1 H), 4.04 (dd, *J* = 7.3, 4.4 Hz, 1 H), 1.80-1.70 (m, 1 H), 1.60-1.44 (m, 2 H), 0.90-0.85 (m, 15 H), 0.06 (s, 3 H), 0.04 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.4, 72.5, 44.9, 25.9, 24.2, 23.6, 22.5, 18.2, -4.7, -5.0.



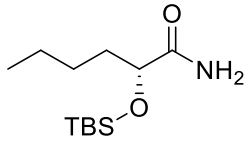
(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-phenylpropanamide (S2). Yield: 190 mg (32%) of **S2** as a white solid. R_f = 0.20 (hexanes:EtOAc, 1:1); $[\alpha]_D$ = -32˚ (*c* = 5.1, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30-7.16 (m, 5 H), 6.65 (bs, 1 H), 6.45 (bs, 1 H), 4.25 (dd, *J* = 7.7, 2.6 Hz, 1 H), 3.10 (dd, *J* = 13.6, 2.9 Hz, 1 H), 2.84 (dd, *J* = 13.6, 8.1 Hz, 1 H), 0.90 (s, 9 H), -0.08 (s, 3 H), -0.28 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.2, 137.5, 130.3,128.4, 126.9, 75.0, 42.0, 26.0, 18.2, -5.3, -5.4; IR (film) 3480, 3283, 3261, 2942, 2889, 1682, 1162 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{15}H_{26}NO_2Si$ $[M+H]^+$: 280.17278, found: 280.17278.



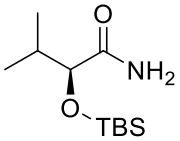
(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)propanamide (12). (*S*)-lactamide (1.0 g, 11 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0 ˚C and triethyl amine (2.3 mL, 17 mmol) and DMAP (130 mg, 1.1 mmol) were added followed by TBSCl (1.9 g, 12 mmol) as a solution in dichloromethane (6 mL). The reaction was slowly warmed to room temperature and stirred for 24 h, diluted with water (10 mL) and extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed with 10% citric acid (10 mL), saturated $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by chromatography on SiO_2 (3:1 hexanes:EtOAc) to yield 2.0 g (89%) of **12** as an oil: R_f = 0.25 (hexanes:EtOAc, 3:1); $[\alpha]_D$ = -7.5˚ (*c* = 10, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 6.60 (bs, 1 H), 5.64 (bs, 1 H), 4.21 (q, *J* = 7.0 Hz, 1 H), 1.38 (d, *J* = 6.8 Hz, 3 H), 0.92 (s, 9 H), 0.11 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$); δ = 179.7, 70.4, 33.9, 27.0, 22.5, 14.0; IR (film) 3480, 3282, 3195, 2955, 2931, 2860, 1693, 1121 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_9H_{22}NO_2Si$ $[M+H]^+$: 204.1414, found: 204.1411.



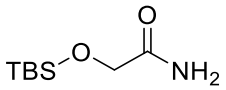
(*S*)-2-((tert-Butyldimethylsilyl)oxy)hexanamide (17). Yield: 240 mg (27%) of **17** as a colorless oil that solidified upon standing: R_f = 0.15 (hexanes:EtOAc, 4:1); $[\alpha]_D$ = -24˚ (*c* = 7.9, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 7.04 (bs, 1 H), 6.47 (bs, 1 H), 4.05 (t, *J* = 7.5 Hz, 1 H), 1.68-1.59 (m, 2 H), 1.32-1.23 (m, 4 H), 0.85-0.80 (m, 12 H), 0.02 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.2, 73.6, 35.1, 26.6, 26.0, 22.9, 18.3, 14.3, -4.6, -5.0. IR (film) 3483, 3287, 3197, 2954, 2860, 1688, 1469 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{12}H_{28}NO_2Si$ $[M+H]^+$: 246.1884, found: 246.1878.



(*R*)-2-((tert-Butyldimethylsilyl)oxy)hexanamide (S3). Yield: 90 mg (26%) of **S3** as a colorless oil that solidified upon standing: R_f = 0.15 (hexanes:EtOAc, 4:1); $[\alpha]_D$ = +23.5˚ (*c* = 2.1, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 6.52 (bs, 1 H), 6.30 (bs, 1 H), 4.11 (t, *J* = 7.5 Hz, 1 H), 1.75-1.65 (m, 2 H), 1.38-1.26 (m, 4 H), 0.91-0.85 (m, 12 H), 0.07 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$); δ = 177.8, 73.8, 35.3, 26.7, 26.2, 23.0, 18.4, 14.4, -4.4, -4.8; IR (film) 3483, 3287, 3197, 2954, 2860, 1688, 1469 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{12}H_{28}NO_2Si$ $[M+H]^+$: 246.1884, found: 246.1877.

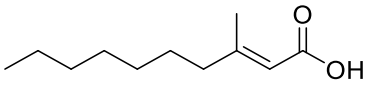


(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)-3-methylbutanamide (S4). Yield: 280 mg (30%) of **S4** as a white solid. R_f = 0.18 (hexanes:EtOAc, 4:1); $[\alpha]_D$ = -15˚ (*c* = 0.1, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.15 (s, 1 H), 6.30 (s, 1 H), 3.84 (d, *J* = 3.3 Hz, 1 H), 2.03-1.95 (m, 1 H), 0.89 (d, *J* = 7.0 Hz, 3 H), 0.85 (s, 9 H), 0.81 (d, *J* = 7.0 Hz, 3 H), 0.03 (s, 3 H), 0.01 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.5, 77.9, 32.7, 25.9, 19.4, 18.2, 16.4, -4.9, -5.0; IR (film) 3471, 3205, 3142, 2954, 2926, 1658, 1461 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{11}H_{26}NO_2Si$ $[M+H]^+$: 232.17273, found: 232.17273.

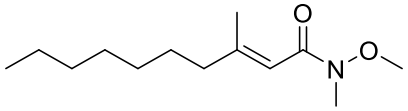


2-((*tert*-Butyldimethylsilyl)oxy)acetamide (S5). 2-hydroxy acetamide (250 mg, 3.3 mmol) was dissolved in DMF (2.5 mL) and imidazole (340 mg, 4.9 mmol) and TBSCl (560 mg, 3.6 mmol)

were added at room temperature. The resulting mixture was stirred for 24 h. The reaction was diluted with ethyl acetate (20 mL) and washed with 10% citric acid (15 mL), sat. aq. $NaHCO_3$ (15 mL), water (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by chromatography on SiO_2 (3:1 hexanes:EtOAc) to yield 530 mg (85%) of **S5** as an oily solid: R_f = 0.25 (hexanes:EtOAc, 3:1); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 6.83 (bs, 1 H), 6.59 (bs, 1 H), 4.03 (s, 2 H), 0.88 (s, 9 H), 0.07 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.3, 63.3, 26.1, 18.5, -5.3; IR (film) 3471, 3275, 3157, 2950, 2856, 1688, 1113 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_8H_{20}NO_2Si$ $[M+H]^+$: 190.1258, found: 190.1255.

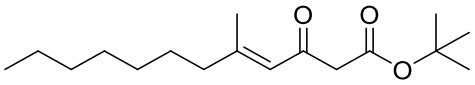


(*E*)-3-Methyldec-2-enoic acid (S6).[5,6] 1-iodoheptane (4.5 mL, 27 mmol) was dissolved in pentane (81 mL) and diethyl ether (54 mL) and cooled to -78 ˚C followed by addition of *t*BuLi (1.7 M solution in pentane, 33 mL, 56 mmol) and the reaction was allowed to stir at -78 ˚C for 5 minutes, the dry ice/acetone bath was removed and the reaction was warmed to room temperature and stirred for 1.5 h. The resulting heptyl Li solution was cooled to 0 ˚C and was added to a solution of CuI (2.55 g, 13.4 mmol) in THF (13.5 mL) at 0 ˚C. The reaction was stirred for 10 minutes and then cooled to -78 ˚C at which point a solution of 2-butynoic acid (0.50 g, 5.8 mmol) in THF (12 mL) was added and the reaction was stirred at -78 ˚C for 10 minutes and then warmed to -5 ˚C and stirred for 45 minutes. The reaction was poured into cooled (0 ˚C) 1 M HCl (100 mL) and extracted with EtOAc (3 x 30 mL), the combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered, concentrated and purified by chromatography on SiO_2 (10% EtOAc:hexanes) to yield 860 mg (81%) of **S6** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$); δ = 11.49 (bs, 1 H), 5.68 (s, 1 H), 2.17-2.13 (m, 5 H), 1.51-1.27 (m, 10 H), 0.88 (t, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$); δ = 172.9, 163.8, 115.3, 41.5, 32.0, 29.4, 29.3, 27.6, 22.9, 19.3, 14.3.

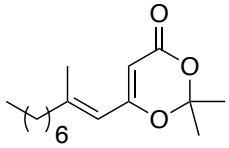


(*E*)-*N*-Methoxy-*N*,3-dimethyldec-2-enamide (S7). (*E*)-3-methyldec-2-enoic acid (**S6**) (120 mg, 0.66 mmol) was dissolved in THF (2.1 mL) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (140 mg, 0.79 mmol) was added followed by the addition of *N*-methylmorpholine (0.22 mL, 2.0 mmol). The reaction was stirred at room temperature for 1 h and *N,O*-dimethylhydroxylamine hydrochloride (66 mg, 0.66 mmol) was added and the reaction was stirred at room temperature overnight. Water (10 mL) was added and the reaction was extracted with Et_2O (2 x 10 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (5 mL), 1 M HCl (5 mL), brine (5 mL), dried ($MgSO_4$), filtered and concentrated to yield 150 mg (99%) of **S7** as an oil (90% purity): R_f = 0.33 (hexanes:EtOAc, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.99 (s, 1 H), 3.55 (s, 3 H), 3.07 (s, 3 H), 2.04-1.99 (m, 5 H), 1.38-1.34 (m, 2 H), 1.22-1.11 (m, 8 H), 0.76 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$); δ = 173.9, 156.7, 113.6, 61.2, 55.7, 40.1, 31.7, 29.0,

28.9, 27.4, 22.5, 18.4, 13.9; IR (film) 2955, 2931, 2856, 1657, 1638 1461, 1366 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{13}H_{26}NO_2$ $[M+H]^+$: 228.19581, found: 228.19579.



***tert*-Butyl (*E*)-5-methyl-3-oxododec-4-enoate (15).** *Tert*-butyl acetate (0.23 mL, 1.7 mmol) was added to freshly prepared solution of LDA (0.17 mL, 1.2 mmol diisopropyl amine; 0.71 mL, 1.2 mmol, 1.7 M *n*BuLi; 0.6 mL THF) at -78 ˚C and stirred for 15 minutes. Then (*E*)-*N*-methoxy-*N*,3-dimethyldec-2-enamide (**S7**) (130 mg, 0.57 mmol) was added as a solution in THF (0.6 mL). The reaction was stirred at -78 ˚C for 2 h. It was then removed from the dry ice/acetone bath and after 5 minutes at room temperature sat. aq. $NaHCO_3$ (5 mL) was added. Then the reaction was extracted with EtOAc (2 x 10 mL) and the combined organic layers were washed with saturated NH_4Cl (10 mL), dried ($MgSO_4$), filtered, concentrated *in vacuo* and purified by chromatography on SiO_2 (10% EtOAc:hexanes) to yield 89 mg (55%) of **15** as an oil: R_f = 0.15 (hexanes:EtOAc, 9:1); ^1H NMR (400 MHz, $CDCl_3$); δ = 6.08 (s, 1 H), 3.34 (s, 2 H), 2.13 (m, 5 H), 1.45 (s, 9 H), 1.26 (m, 10 H), 0.87 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 192.7, 167.2, 161.9, 122.1, 81.6, 52.3, 41.5, 31.9, 29.3, 29.2, 28.1, 27.6, 22.8, 19.8, 14.2; IR (film) 3007, 2957, 2927, 2855, 1734, 1685, 1620, 1249, 1144 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{17}H_{30}O_3Na$ $[M+Na]^+$: 305.20872, found: 305.20827.



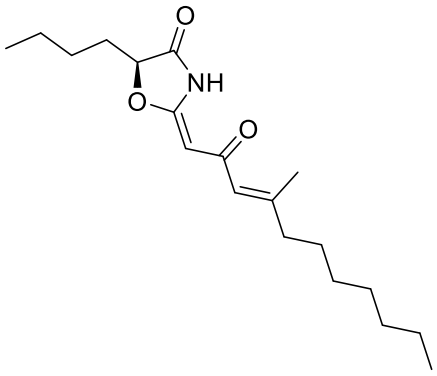
(*E*)-2,2-Diethyl-6-(2-methylnon-1-en-1-yl)-4*H*-1,3-dioxin-4-one (16). To a solution of **15** (0.3 mmol) in acetone (0.12 M) at -78 C was added sequentially trifluoroacetic anhydride (0.6 M), followed by trifluoroacetic acid (0.18 M), then acetic anhydride (0.74 M) dropwise. The resulting solution was slowly warmed to room temperature and stirred overnight. The reaction was then poured into saturated sodium bicarbonate and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, concentrated *in vacuo*, and purified by chromatography on SiO_2 (20% EtOAc:hexanes) to yield 61.2 mg (73%) of **16** as a colorless oil: R_f = 0.55 (20% EtOAc:hexanes); ^1H NMR (400 MHz, $CDCl_3$) ; δ = 6.79 (s, 1 H), 5.21 (s, 1 H), (2.17, m, 2 H), 2.14 (s, 3 H), 2.00 (s, 6 H), 0.90 (m, 10 H), 0.88 (m, 3 H).

General procedure for synthesis of 4-oxazolidinones:

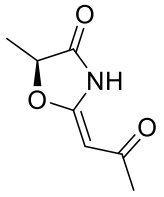
Acylated Meldrum's acid derivatives were synthesized following a previously reported procedure.[7] In brief, the carboxylic acid (1 mmol) was dissolved in dichloromethane (0.5 M) and cooled to 0 ˚C. Then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.1 mmol) and 4-dimethylaminopyridine (0.1 mmol) were added. Then Meldrum's acid (1.01 mmol) was added and the reaction was stirred at room temperature overnight. The insoluble urea was filtered, and

the solvent was removed *in vacuo,* redissolved in EtOAc and washed with HCl (15 mL, 1 M) and brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*.

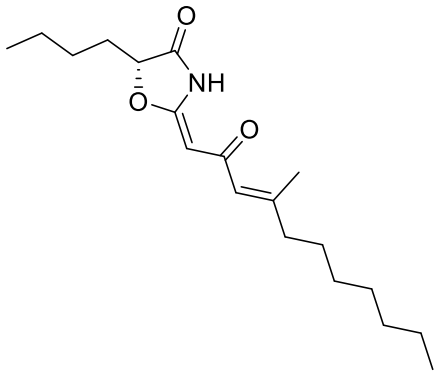
The crude acylated Meldrum's acid (or dioxinone derivative) and *O*-TBS-α-hydroxy amide (0.5 mmol) were dissolved in toluene (2 mL) and stirred at reflux for 1 h. The reaction was cooled to room temperature, concentrated *in vacuo*. The resulting crude imide (0.25 mmol) was dissolved in dichloromethane (2.5 mL) and trifluoroacetic acid (2.5 mL) was added at room temperature. The reaction was allowed to stir for 24 h and then concentrated *in vacuo* and purified by flash column chromatography on SiO_2 (20% EtOAc:hexanes). Yields are reported over the 3-step sequence.



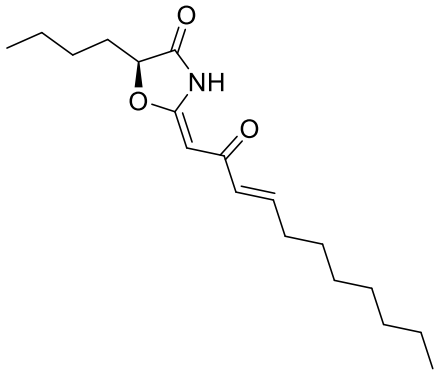
(*S*)-Lipoxazolidinone A (1). Prepared from dioxinone **16**. Yield 8.1 mg (52%) of **1** as a colorless oil: $[\alpha]_D$ = -34˚ (*c* = 0.78, MeOH) [Lit: $[\alpha]_D$ = -31˚ (*c* = 0.02, MeOH][9]; ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.89 (s, 1 H), 5.20 (s, 1 H), 4.62 (m, 1 H), 2.17 (s, 3 H), 2.12 (m, 2 H), 1.96 (m, 1 H), 1.81 (m, 1 H), 1.48-1.25 (m, 14 H), 0.93-0.86 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 189.9, 167.0, 158.3, 124.8, 94.9, 84.5, 79.1, 41.9, 32.2, 31.2, 29.7, 29.6, 28.1, 26.6, 23.1, 22.7, 19.6, 14.6, 14.2; IR (film) 2959, 2918, 2884, 1762, 1631, 1557, 1442 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{19}H_{32}NO_3$ $[M+H]^+$: 322.23767, found: 322.23754.



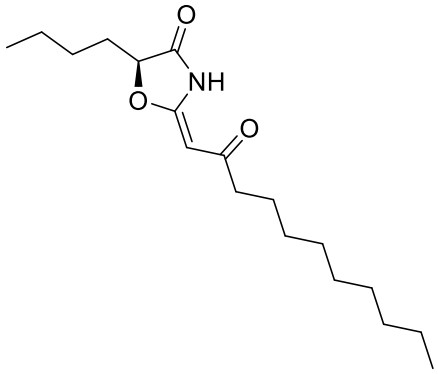
(*S,E*)-5-Methyl-2-(2-oxopropylidene)oxazolidin-4-one (14). Used commercially available dioxanone (2,2,6-trimethyl-1,3-dioxin-4-one). Yield: 87 mg (62%) of **14** as a white solid. R_f = 0.27 (hexanes:EtOAc, 1:1); $[\alpha]_D$ = -28 (*c* = 2.5, DCM); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.22 (s, 1 H), 4.70 (q, *J* = 7.0 Hz, 1 H), 2.13 (s, 3 H), 1.55 (d, *J* = 7.1 Hz, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$): δ = 197.6, 173.7, 165.2, 82.5, 75.3, 30.2, 16.9; IR (film) 3186, 2989, 2935, 1761, 1666, 1568 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_7H_{10}NO_3$ $[M+H]^+$: 156.06552, found: 156.06558.



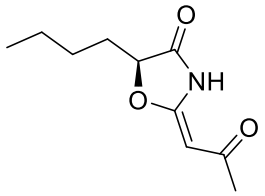
***Ent-(R)*-lipoxazolidinone A (18).** Yield 2.7 mg (49%) of **18** as a colorless oil: $[\alpha]_D$ = +30˚ (*c* = 0.09, dichloromethane); ^{1}H NMR (700 MHz, $CDCl_3$); δ = 5.89 (s, 1 H), 5.20 (s, 1 H), 4.62 (m, 1 H), 2.17 (s, 3 H), 2.13 (m, 2 H), 1.98 (m, 1 H), 1.82 (m, 1 H), 1.47-1.36 (m, 6 H), 1.28 (m, 8 H), 0.93-0.87 (m, 6 H); ^{13}C NMR (175 MHz, $CDCl_3$): δ = 189.8, 174.0, 166.9, 158.2, 124.6, 84.4, 78.9, 41.8, 32.1, 31.1, 29.6, 29.5, 27.9, 26.5, 23.0, 22.5, 19.5, 14.4, 14.1; IR (film) 2959, 2918, 2884, 1762, 1631, 1557, 1442 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{19}H_{32}NO_3$ $[M+H]^+$: 322.23767, found: 322.23806.



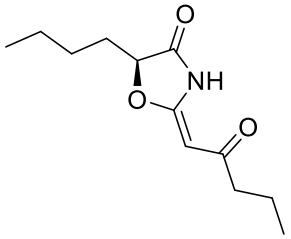
(*S*,*E*)-5-Butyl-2-((*E*)-2-oxoundec-3-en-1-ylidene)oxazolidin-4-one (19). Yield 6.2 mg (38%) of **19** as a white solid: $[\alpha]_D$ = -35˚ (*c* = 0.49, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 6.83 (dt, *J* = 15.8, 6.9 Hz, 1 H), 6.09 (d, *J* = 15.1 Hz, 1 H), 5.32 (s, 1 H), 4.65 (dd, *J* = 6.8, 4.1 Hz, 1 H), 2.22 (m, 2 H), 1.95 (m, 1 H), 1.83 (m, 1 H), 1.46-1.34 (m, 6 H), 1.27 (m, 8 H), 0.94-0.86 (m, 6 H); ^{13}C NMR (175 MHz, $CDCl_3$): δ = 188.1, 174.3, 168.0, 146.1, 130.3, 81.7, 79.2, 32.8, 32.1, 31.1, 29.5, 29.4, 28.6, 26.5, 23.0, 22.5, 14.4, 14.1; IR (film) 2958, 2920, 2886, 1768, 1628, 1559, 1441 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{18}H_{30}NO_3$ $[M+H]^+$: 308.22202, found: 308.22205.



(*S,E*)-5-Butyl-2-(2-oxoundecylidene)oxazolidin-4-one (20). Yield 11 mg (44%) of **20** as a white solid: $[\alpha]_D$ = -29˚ (*c* = 0.25, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.24 (s, 1 H), 4.63 (dd, *J* = 7.4, 4.2 Hz, 1 H), 2.35 (t, *J* = 7.1 Hz, 4 H), 1.97 (m, 1 H), 1.83 (m, 1 H), 1.61 (m, 4 H), 1.43-1.26 (m, 12 H), 0.94-0.86 (m, 6 H); ^{13}C NMR (175 MHz, $CDCl_3$): δ = 200.6, 173.1, 165.4, 81.6, 78.8, 43.1, 32.0, 30.9, 30.8, 29.6, 29.5, 29.4, 26.3, 25.4, 22.8, 22.3, 14.2, 13.9; IR (film) 2958, 2924, 2862, 1708, 1670, 1570, 1461 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{18}H_{32}NO_3$ $[M+H]^+$: 310.23767, found: 310.23771.

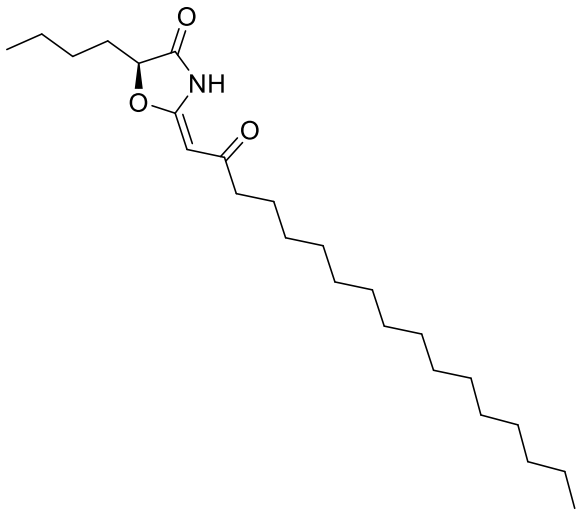


(*S,E*)-5-Butyl-2-(2-oxopropylidene)oxazolidin-4-one (21). Used commercially available dioxanone (2,2,6-trimethyl-1,3-dioxin-4-one). Yield 63 mg (72%) of **21** as a white solid: $[\alpha]_D$ = -37˚ (*c* = 0.7, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.24 (s, 1 H), 4.63 (dd, *J* = 7.4, 4.3 Hz, 1 H), 2.15 (s, 3 H), 1.96 (m, 1 H), 1.82 (m, 1 H), 1.38 (m, 4 H), 0.92 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$): δ = 197.7, 173.2, 165.5, 82.3, 79.0, 31.0, 30.2, 26.4, 22.5, 14.1; IR (film) 3184, 2957, 2935, 2863, 1767, 1665, 1574, 1431 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{10}H_{16}NO_3$ $[M+H]^+$: 198.11247, found: 198.11268.

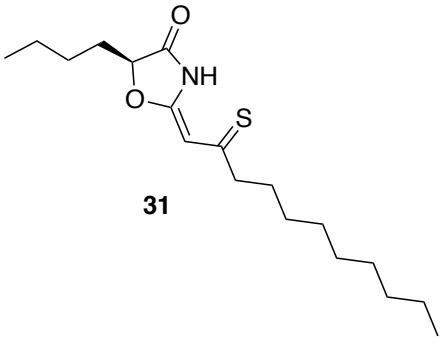


(*S,E*)-5-Butyl-2-(2-oxopentylidene)oxazolidin-4-one (22). Yield: 7 mg (39%) of **22** as a white solid: $[\alpha]_D$ = -33˚ (*c* = 0.09, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.24 (s, 1 H), 4.63 (dd, *J* = 7.5, 4.1 Hz, 1 H), 2.36 (t, *J* = 7.1 Hz, 2 H), 1.99 (m, 1 H), 1.82 (m, 1 H), 1.65 (m, 2 H), 1.39 (m, 4 H), 0.97-0.90 (m, 6 H); ^{13}C NMR (175 MHz, $CDCl_3$): δ = 200.4, 173.1, 165.5,

81.6, 78.8, 45.0, 30.8, 29.8, 26.3, 22.3, 18.8, 13.9; IR (film) 3182, 2952, 2935, 2861, 1765, 1664, 1568, 1442 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{12}H_{20}NO_3$ $[M+H]^+$: 226.14377, found: 226.14383.



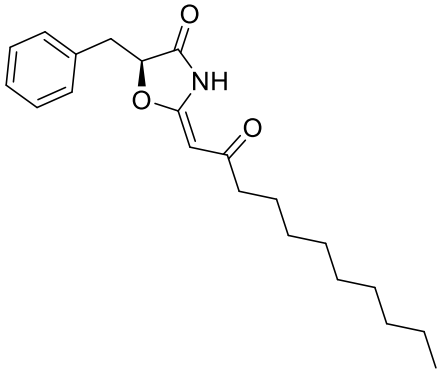
(*S,E*)-5-Butyl-2-(2-oxoheptadecylidene)oxazolidin-4-one (23). Yield: 14 mg (45%) of **23** as a white solid: R_f = 0.55 (hexanes:EtOAc, 4:1); $[\alpha]_D$ = -24˚ (*c* = 7.9, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.24 (s, 1 H), 4.63 (dd, *J* = 7.5, 4.2 Hz, 1 H), 2.34 (m, 4 H), 1.95 (m, 1 H), 1.85 (m, 1 H), 1.63 (m, 4 H), 1.45-1.25 (m, 24 H), 0.94-0.86 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.8, 179.9, 173.2, 81.6, 78.8, 43.1, 34.2, 32.1, 30.9, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 26.3, 25.5, 24.8, 22.9, 22.3, 14.3, 13.9; IR (film) 2955, 2915, 2848, 1756, 1670, 1468 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{24}H_{44}NO_3$ $[M+H]^+$: 394.33157, found: 394.33172.



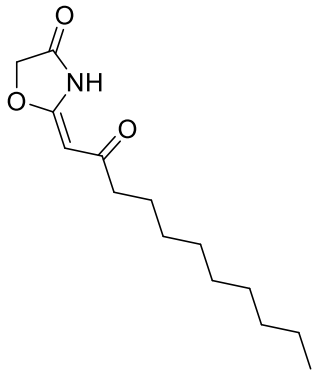
31

(S,E)-5-butyl-2-(2-thioxoundecylidene)oxazolidin-4-one (25). 4-oxazolidinone **19** (12 mg, 0.040 mmol) was dissolved in DCM (0.2 mL) and Lawesson's reagent (8.1 mg, 0.020 mmol) was added. The reaction was stirred for 2 h at room temperature and then concentrated *in vacuo* and purified by flash column chromatography on SiO_2 (10% EtOAc:hexanes) to yield: 9.1 mg (71%) of **25** as a yellow oil: $[\alpha]_D$ = -14˚ (*c* = 0.5, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 6.19 (s, 1 H), 4.67 (dd, *J* = 7.4, 4.5 Hz, 1 H), 2.81 (m, 2 H), 1.95 (m, 1 H), 1.71 (m, 1 H),

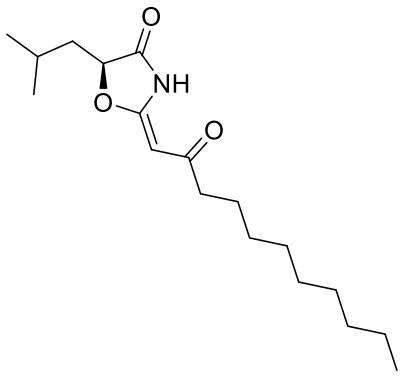
1.55 (m, 2 H), 1.48-1.25 (m, 16 H), 0.95-0.86 (m, 6 H).; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 209.6, 174.3, 169.6, 98.1, 79.1, 53.2, 43.9, 32.0, 30.0, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 24.0, 22.8, 14.2; IR (film) 3332, 2956, 2854, 1744, 1464, 1164 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{18}H_{32}NO_2S$ $[M+H]^+$: 326.21483, found: 326.21484.



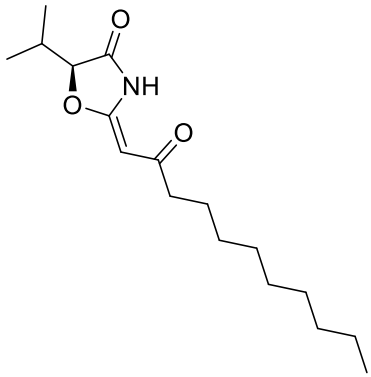
(*S,E*)-5-Benzyl-2-(2-oxoundecylidene)oxazolidin-4-one (26). Yield: 9.5 mg (47%) of **26** as a white solid: $[\alpha]_D$ = -31˚ (*c* = 0.05, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.20 (s, 1 H), 4.87 (dd, *J* = 6.8, 3.9 Hz, 1 H), 3.32 (dd, *J* = 14.8, 4.0 Hz, 1 H), 3.09 (dd, *J* = 14.8, 6.8 Hz, 1 H), 2.35 (t, *J* = 7.7 Hz, 2 H), 1.63 (m, 4 H), 1.26 (m, 10 H), 0.88 (t, *J* = 6.6 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.6, 172.1, 165.1, 134.0, 129.7, 128.8, 127.7, 81.7, 79.0, 43.1, 37.1, 32.0, 31.9, 29.6, 29.5, 29.4, 25.4, 22.8, 14.3; IR (film) 3184, 2955, 2924, 2865, 1742, 1676, 1570, 1015 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{21}H_{30}NO_3$ $[M+H]^+$: 344.22202, found: 344.22153.



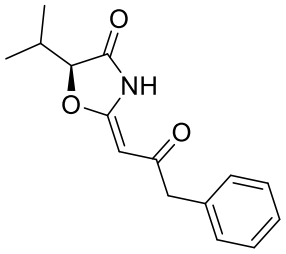
(*E*)-2-(2-Oxoundecylidene)oxazolidin-4-one (28). Yield 19 mg (51%) of **28** as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.30 (s, 1 H), 4.59 (s, 2 H), 2.36 (t, *J* = 7.2 Hz, 2 H), 1.62 (m, 2 H), 1.26 (m, 12 H), 0.87 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$): δ = 200.5, 171.2, 156.9, 82.3, 67.3, 43.2, 34.2, 32.2, 29.8, 29.6, 25.6, 25.0, 23.0, 14.4; IR (film) 2953, 2924, 2868, 1752, 1658, 1442 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{14}H_{24}NO_3$ $[M+H]^+$: 254.17507, found: 254.17513.



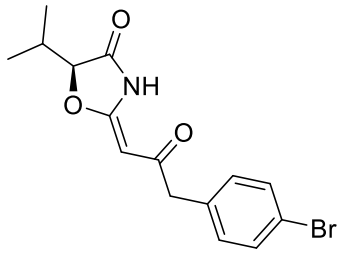
(*S,E*)-5-Isobutyl-2-(2-oxoundecylidene)oxazolidin-4-one (27). Yield: 19 mg (63%) of **27** as a white solid: $[\alpha]_D$ = -36˚ (*c* = 1.5, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.25 (s, 1 H), 4.67 (dd, *J* = 9.7, 3.6 Hz, 1 H), 2.37 (t, *J* = 7.7 Hz, 2 H), 1.90 (m, 1 H), 1.81 (m, 1 H), 1.70-1.57 (m, 3 H), 1.25 (m, 12 H), 0.99 (m, 6 H), 0.86 (t, *J* = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.4, 173.5, 165.8, 81.9, 77.8, 42.9, 39.9, 32.0, 29.5, 29.4, 29.4, 25.7, 25.0, 23.0, 22.8, 21.8, 14.2; IR (film) 3217, 2957, 2920, 2871, 1759, 1670, 1570, 1161 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{18}H_{32}NO_3$ $[M+H]^+$: 310.23767, found: 310.23705.



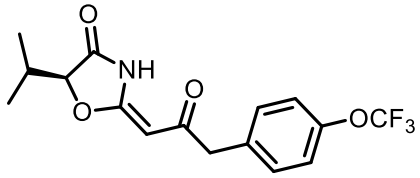
(*S,E*)-5-Isopropyl-2-(2-oxoundecylidene)oxazolidin-4-one (29). Yield: 33 mg (49%) of **29** as a white solid: $[\alpha]_D$ = -36˚ (*c* = 0.2, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 5.25 (s, 1 H), 4.47 (d, *J* = 3.8 Hz, 1 H), 2.37 (m, 2 H), 2.29 (m, 1 H), 1.59 (m, 2 H), 1.28 (m, 12 H), 1.11 (d, *J* = 7.0 Hz, 3 H), 0.97 (d, *J* = 6.9 Hz, 3 H) 0.85 (m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.0, 172.5, 165.7, 82.8, 81.3, 43.1, 32.0, 30.7, 29.6, 29.5, 29.4, 25.5, 22.8, 18.1, 15.7, 14.2; IR (film) 3238, 2958, 2925, 2854, 1769, 1667, 1580, 1014 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{17}H_{30}NO_3$ $[M+H]^+$: 296.22202, found: 296.22188.



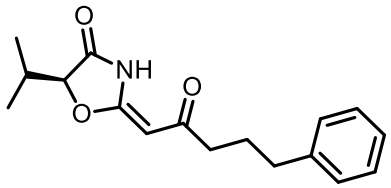
(*S,E*)-5-Isopropyl-2-(2-oxo-3-phenylpropylidene)oxazolidin-4-one (30). Yield: 11 mg (56%) of **30** as an oil: $[\alpha]_D$ = -37˚ (*c* = 1.1, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 7.34-7.23 (m, 5 H), 5.22 (s, 1 H), 4.45 (d, *J* = 3.8 Hz, 1 H), 3.67 (s, 2 H), 2.22 (m, 1 H), 1.09 (d, *J* = 7.0 Hz, 3 H), 0.95 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.9, 172.2, 166.1, 135.5, 129.6, 128.9, 127.1, 82.9, 81.1, 50.0, 30.7, 18.1, 15.8; IR (film) 3184, 2957, 2935, 2863, 1765, 1661, 1571, 1014 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{15}H_{18}NO_3$ $[M+H]^+$: 260.12812, found: 260.12806.



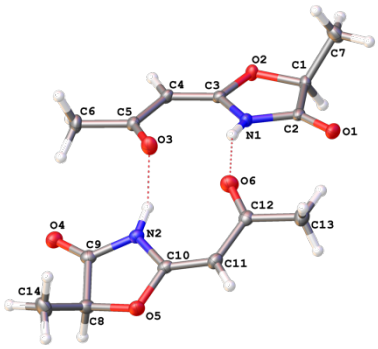
(*S,E*)-2-(3-(4-Bromophenyl)-2-oxopropylidene)-5-isopropyloxazolidin-4-one (31). Yield: 7.4 mg (51%) of **31** as a white solid: $[\alpha]_D$ = -26˚ (*c* = 0.6, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 7.46 (m, 2 H), 7.12 (m, 2 H), 5.19 (s, 1 H), 4.47 (d, *J* = 3.9 Hz, 1 H), 3.61 (s, 2 H), 2.28 (m, 1 H), 1.09 (d, *J* = 7.0 Hz, 3 H), 0.95 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 196.9, 172.1, 166.3, 134.5, 132.0, 131.4, 121.2, 83.1, 81.0, 49.2, 30.8, 18.1, 15.9; IR (film) 3211, 2957, 2935, 2863, 1765, 1662, 1578, 1011 cm^{-1}; HRMS (ESI) *m/z* calculated for $C_{15}H_{17}NO_3Br$ $[M+H]^+$: 338.03863, found: 338.03866.



(S,E)-5-isopropyl-2-(2-oxo-3-(4-(trifluoromethoxy)phenyl)propylidene)oxazolidin-4-one (32). Yield: 9.3 mg (25%) of **32** as an oil: $[\alpha]_D$ = -26˚ (*c* = 0.4, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 7.33 (m, 2 H), 7.18 (m, 2 H), 5.22 (s, 1 H), 4.47 (d, *J* = 3.8 Hz, 1 H), 3.67 (s, 2 H), 2.28 (m, 1 H), 1.10 (d, *J* = 7.0 Hz, 3 H), 0.97 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 200.3, 172.7, 167.4, 137.2, 131.9, 129.9, 129.7, 83.5, 81.8, 49.1, 30.8, 18.1, 15.9; HRMS (ESI) *m/z* calculated for $C_{16}H_{17}F_3NO_4$ $[M+H]^+$: 344.11042, found: 344.11017.



(*S,E*)-5-Isopropyl-2-(2-oxo-5-phenylpentylidene)oxazolidin-4-one (33). Yield: 25.8 mg (54%) of **33** as a white solid: $[\alpha]_D$ = -27˚ (*c* = 0.4, dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$); δ = 7.22 (m, 5 H), 5.36 (s, 1 H), 4.47 (d, *J* = 3.9 Hz, 1 H), 2.66 (t, *J* = *7.2 H*), 2.39 (m, 1H), 1.94 (t, *J* = 7.0 Hz, 3 H), 1.11 (t, *J* = 7.0 Hz, 3 H), 0.97 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 200.3, 172.7, 165.9, 142.1, 128.9, 126.4, 83.1, 81.6, 42.6, 35.8, 31.0, 27.2, 18.4, 16.1; HRMS (ESI) *m/z* calculated for $C_{17}H_{22}NO_3$ $[M+H]^+$: 288.15942, found: 288.15937.



Crystal data	
Compound	
Identification code	Rds308 (**14**)
chemical formula	$C_7H_9NO_3$
formula weight g/mol	155.15
temperature /K	100(2)
crystal system	monoclinic
space group	*P*2$_1$
***a* /Å**	4.3927(3)
***b* /Å**	18.3193(15)
***c* /Å**	9.2635(7)
α /°	90
β /°	93.409(4)
γ /°	90
***V* Å^3**	744.13(10)
Z	4
ρ_{calc} g/cm^3	1.385
***F*(000)**	328
θ range /°	2.20 to 31.07
reflections collected	20916
data / restr. / parms.	4784 / 1 / 209

R_{int}	0.0176
Goodness-of-fit	1.044
R_1[a] (I>2σ(I))	0.0278
wR_2[b] (I>2σ(I))	0.0754

[a] $R_1 = \Sigma(||F_o| - |F_c||) / \Sigma|F_o|$, [b] $wR_2 = [\Sigma (w(F_o^2 - F_c^2)^2) / \Sigma (w(F_o^2)^2]^{½}$; $w=1/[\sigma^2(F_o^2)+(aP)^2+bP]$, GOF= $\{\Sigma[w(F_o^2 - F_c^2)^2] / (\#reflns - \#parms)\}^{½}$

Single crystals suitable for structure analysis were selected under a microscope from the bulk and mounted on a MiTeGen mount with a minimum of paratone-N oil. Data were collected using a Bruker-Nonius X8 Kappa ApexII diffractometer by α and β scans using MoK α radiation(α = 0.71073 Å). Corrections for Lorentz and polarization effects, and absorption were made using SADABS[8]. The structure was solved with direct methods and refined using full-matrix least squares (on F^2) using the SHELX[9] software package. All non-hydrogen atoms were refined anisotropically. H atoms were added at calculated positions, with coordinates and U_{iso} values allowed to ride on the parent atom. H-bond donor atom positions were located from the difference map, and refined independently while maintaining typical riding values for U_{iso}. Final Data are deposited with Cambridge Structural Database, CCDC Deposition#: **CCDC 1453529**.

Biological data, bacterial strains and assay protocols

General information

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were obtained from the ATCC (29213, 33591) or provided by the Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) for distribution by BEI Resources, NIAID, NIH (NR45924, NR45926, NR45930, NR46062, NR45906, NR45898) and colonies were grown on solid media as instructed. The *A. baumannii* type strain ATCC 19606 was obtained from the American Type Culture Collection. The *lpxA* mutant strain 19606R was an LPS-deficient, colistin-resistant derivative of ATCC 19606 described previously.[10] *A. baumannii* cultures were grown on Mueller-Hinton (MH) agar or in cation-adjusted MH broth at 37 °C. Colistin sulfate (10 μg/mL) was added to overnight cultures where appropriate.

Mueller-Hinton broth (MHB, 211443-BD) and tryptic soy broth (TSB, Remel: R455052) were purchased from Fisher Scientific. Tryptic soy agar (TSA, cat. # 22091) and Linezolid (cat. # P70014) were purchased from Sigma-Aldrich. All assays were run in triplicate and repeated at least two separate times for MIC assays. All compounds were dissolved in molecular biology grade DMSO as 10 mM stock solutions. Optical densities were measured using a Thermo Scientific Genesys 20 spectrophotometer. All graphs were generated and analyzed using GraphPad Prism 7 or Excel (Office 2016).

Broth microdilution method for determination of minimum inhibitory concentration (MIC)[11]

MIC was determined by broth micro-dilution according to CLSI guidelines.[11] The test medium for most species was Mueller-Hinton broth (MHB). MSSA, MRSA and *S. epidermis* was grown in MHB for 6-8 h; this culture was used to inoculate fresh MHB (5 x 10^5 CFU/mL). The resulting bacterial suspension was aliquoted (1 mL) into 1.5 mL tubes and compound was added from a 10 mM DMSO stock to achieve the desired initial starting concentration (typically 128 μg/mL). Linezolid (from a 10 mM DMSO stock) was used as a positive control. Inoculated media not treated with compound served as the negative control. Rows 2-12 of a 96-well microtiter plate were filled at 100 μL/well from the remaining inoculated media. The samples containing test compounds and linezolid were then aliquoted (200μL) into the corresponding first row wells of the microtiter plate (two wells for each compound and two negative controls). Row 1 wells were mixed 6 to 8 times, then 100 μL was transferred to row 2. Row 2 wells were mixed 6 to 8 times, followed by a 100 μL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate. The plate was then covered and sealed with GLAD Press'n Seal® and incubated under stationary conditions at 37 °C. After 16 h, minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of compound at which no visible growth of bacteria was observed.

Hemolysis assay[12]

Hemolysis assays were performed on mechanically defibrinated sheep blood (Hemostat Labs: DSB50). Defibrinated blood (1.5 mL) was placed into a microcentrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was then removed and then the cells were resuspended in 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged, the supernatant was removed and cells were resuspended two additional times. The final cell suspension was then diluted 10-fold. Test compound solutions were made in PBS in small culture tubes and then added to aliquots of the 10-fold suspension dilution of blood. PBS was used as a negative control and a zero-hemolysis marker. Triton X (a 1% sample) was used as a positive control serving as the 100% lysis marker. Samples were then placed in an incubator at 37 °C while being shaken at 200 rpm for one hour. After one hour, the samples were transferred to microcentrifuge tubes and centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was then measured with a UV spectrometer at a 540 nm wavelength.

Bacterial membrane permeablization assay[13]

The backlight assay (Invitrogen) was used with *S. aureus* (ATCC 29213) to assess the membrane permeability of **29**. An overnight culture of S aureus in MHB was diluted 1:40 and grown to an optical density (OD600) of ~ 1.0. The cultures were centrifuged at 10,000 rpm for 15 minutes. The cell pellet was washed once with sterile water and resuspended in 1/10 the original volume and then diluted 1:20 in water and water containing various concentrations of **29**. Samples were incubated at 37 ˚C for 1 hour with shaking and then centrifuged at 10,000 rpm for 10 minutes. The cell pellet was washed once with water and then resuspended in the same amount of water. A 1:1 mixture of SYTO-9 and propidium iodide were added to the samples (3 μL/mL) and mixed well. The samples were then added to the wells of a 96-well plate and incubated in the dark for 15 minutes at room temperature. Green fluorescence (SYTO-9) was read at 530 nm and red

fluorescence (propidium iodide) was read at 645 nm (excitation wavelength = 485 nm). The ratio of green to red fluorescence was determined and expressed as a percentage of the control.

Single Step Resistance Studies[14]

For single step resistance, *S. aureus* (ATCC 29213) cells were grown to exponential phase and suspended in PBS at a concentration of approximately 10^8 cfu. 50 µL was plated onto triptic soy agar containing 2x, 4x, and 10x MIC of **29**. After 48 hours of incubation at 37 °C, no resistant colonies were detected, giving a calculated frequency of resistance of $<10^{-8}$.

Generation of a Resistant Mutant via Serial Passage[15]

To increase the chance of obtaining a resistant mutant a serial passage experiment was performed with incremental amounts of **29**. *S. aureus* (ATCC 29213) cells at exponential phase were diluted to an OD600 of 0.2 and cells were added to 0.25x MIC, 0.5x MIC, 1x MIC, 2x MIC, and 4x MIC of either **29** or ofloxacin. Every 24 hours, cultures were checked for growth by measuring OD600. Cultures with OD600 closest to 0.2 were diluted 1:100 in fresh MHB containing 0.25x MIC, 0.5x MIC, 1x MIC, 2x MIC, and 4x MIC of either **29** or ofloxacin. Serial passages continued for 25 days for ofloxacin and 55 days for **29**. Cultures with growth at 2x MIC of 4x MIC of antibiotic were plated on drug free agar plates and the MIC was determined.

Macromolecular Synthesis Assays

Bacteria and growth conditions: Macromolecular synthesis inhibition of the test agent was investigated using *S. aureus* MMX 100 (ATCC 29213). Cells were grown at 35°C overnight on Trypticase Soy Agar plus 5% sheep blood, and isolated colonies were used to inoculate 30 mL of MHB II. The culture was grown to early exponential growth phase (OD600 = 0.2 to 0.3) while incubating in a shaker at 35°C and 150 rpm.
DNA and RNA synthesis: When cells reached early exponential phase, 100 µl of culture was added to triplicate wells containing various concentrations of **29** or control antibiotics (2.5 µl) at 40X the required final concentration. A "no drug" control was included for all experiments. Following a 30 min pre-incubation at room temperature to allow for drug inhibition of a pathway, either [^{3}H] thymidine, at 2 µCi per well (DNA synthesis) or [^{3}H] uridine, at 0.5µCi per well (RNA synthesis) was added. Reactions were allowed to proceed at room temperature for 30 min and then stopped by adding 6 µl of cold 100% trichloroacetic acid (TCA). Reactions were incubated on ice for 30 min and the TCA-precipitated material was collected on a 25 mm GF/1.2 µm PES 96-well filter plate (Corning). After washing five times with 200 µl per well of cold 5% TCA, the filters were allowed to dry, and then counted using a Packard Top Count microplate scintillation counter.
Protein synthesis: When cells reached early exponential phase in MHB II, they were resuspended in M9 minimal medium and 100 µl of culture was added to triplicate wells containing various concentrations of **29** or control antibiotics (2.5 µl) at 40X the required final concentration. Following a 30 min pre-incubation at room temperature to allow for drug inhibition of protein synthesis, [^{3}H] leucine was added at 2.0 µCi per well. Reactions were allowed to proceed at room temperature for 40 min and then stopped by adding 12 µl of cold 50% trichloroacetic acid (TCA)/20% casamino acids. Reactions were incubated on ice for 30

min and the TCA-precipitated material was collected on a 25 mm GF/1.2 µm PES 96-well filter plate (Corning). After washing five times with 200 µl per well of cold 5% TCA, the filters were allowed to dry, and then counted using a Packard Top Count microplate scintillation counter.
Cell wall synthesis: When cells reached early exponential phase in MHB II, they were transferred to M9 minimal medium and added to 1.5 ml microfuge tubes (100 µl/tube in triplicate) containing various concentrations of **29** or control antibiotics (2.5 µl) at 40X the required final concentration. Following a 30 min pre-incubation at 37°C to allow for drug inhibition of cell wall synthesis, [^{14}C] N-acetylglucosamine (0.4 µCi/reaction) was added to each tube and incubated for 90 min in a 37°C heating block. Reactions were stopped through the addition of 100 µl of 8% SDS to each tube. Reactions were then heated at 95°C for 30 min in a heating block, cooled, briefly centrifuged, and spotted onto pre-wet nitrocellulose membrane filters (0.8 micron). After washing three times with 5 ml of 0.1% SDS, the filters were rinsed two times with 5 ml of deionized water, allowed to dry, and then counted using a Perkin Elmer Tri-Carb 4810TR Liquid Scintillation Analyzer.
Lipid synthesis: Bacterial cells were grown to early exponential growth phase in MHB II and 100 µl was added to 1.5 ml microfuge tubes (in triplicate) containing various concentrations of **29** or control antibiotics as described above. Following a 30 min pre-incubation at room temperature to allow for inhibition of lipid synthesis, [^{3}H] glycerol was added at 0.5 µCi per reaction. Reactions were allowed to proceed at room temperature for 40 min and then stopped through the addition of 375 µl chloroform/methanol (1:2), followed by vortexing for 20 seconds after each addition. Chloroform (125 µL) was then added to each reaction and vortexed, followed by the addition of 125 µl dH2O and vortexing. Reactions were centrifuged at 13,000 rpm in a microfuge for 10 min, and then 150 µl of the organic phase was transferred to a scintillation vial and allowed to dry in a fume hood for at least 1 hr. Samples were then counted using a Perkin Elmer Tri-Carb 4810TR Liquid Scintillation Analyzer.

In vitro Transcription/Translation Assay

Inhibition of cell-free transcription/translation was assessed using an *in vitro E. coli* S30 Extract System for Circular DNA assay with a firefly luciferase readout (Promega, Madison, WI). Each reaction mixture contained 10 µL of S30 premix, 7.5 µL of S30 extract, 2.5 µL of complete amino acid mix, 2.2 µL of nuclease free water, and 7% of antibiotic in DMSO. Antibiotics were dissolved in DMSO to give 10 mM stock solutions, and these were serially dilute to yield 0, 0.5, 1, and 2 times the MIC of the antibiotics. Control reaction mixtures received 7% DMSO and no antibiotic. The reaction mixtures were incubated with shaking for 30 minutes at 37 °C to allow the antibiotics time to adhere to transcription/translation machinery before the addition of plasmid DNA. After 30 minutes, the reaction mixtures were removed from the incubator, and the reactions initiated by the addition of 1 µg (1 µL) of plasmid pBEST*luc*™. The reaction mixtures were incubated with shaking for 30 minutes at 37 °C. Placement on ice for at least 5 minutes stopped the reactions. A 1:1 dilution of the reaction mixture in dilution buffer was prepared, and 15 µL was added to 50 µL of luciferase reagent for reading of luminescence at 560 nm on a Synergy™ 4 Multi-detection Microplate Reader (BioTek, Winooski, VT). All reaction mixtures were prepared in triplicate. Linezolid was used as the positive control.

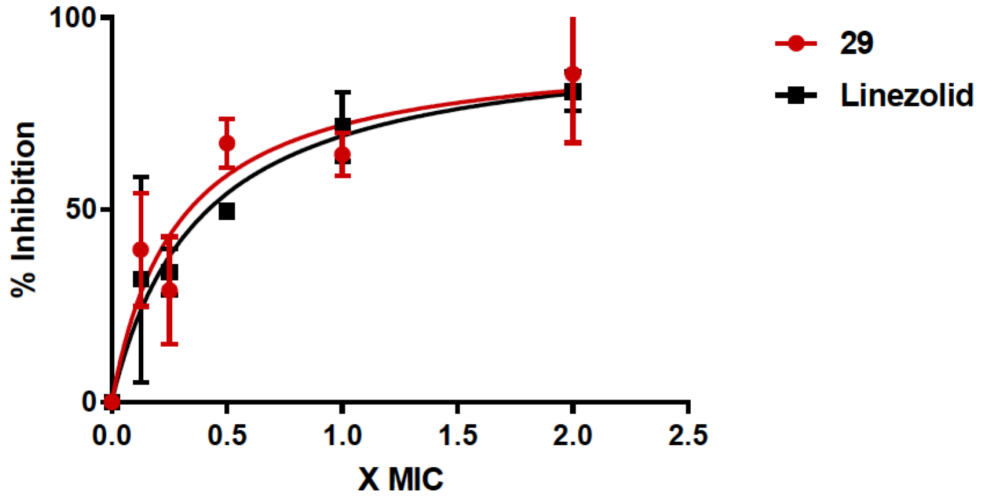


Figure S1. Protein synthesis inhibition by **29** and Linezolid.

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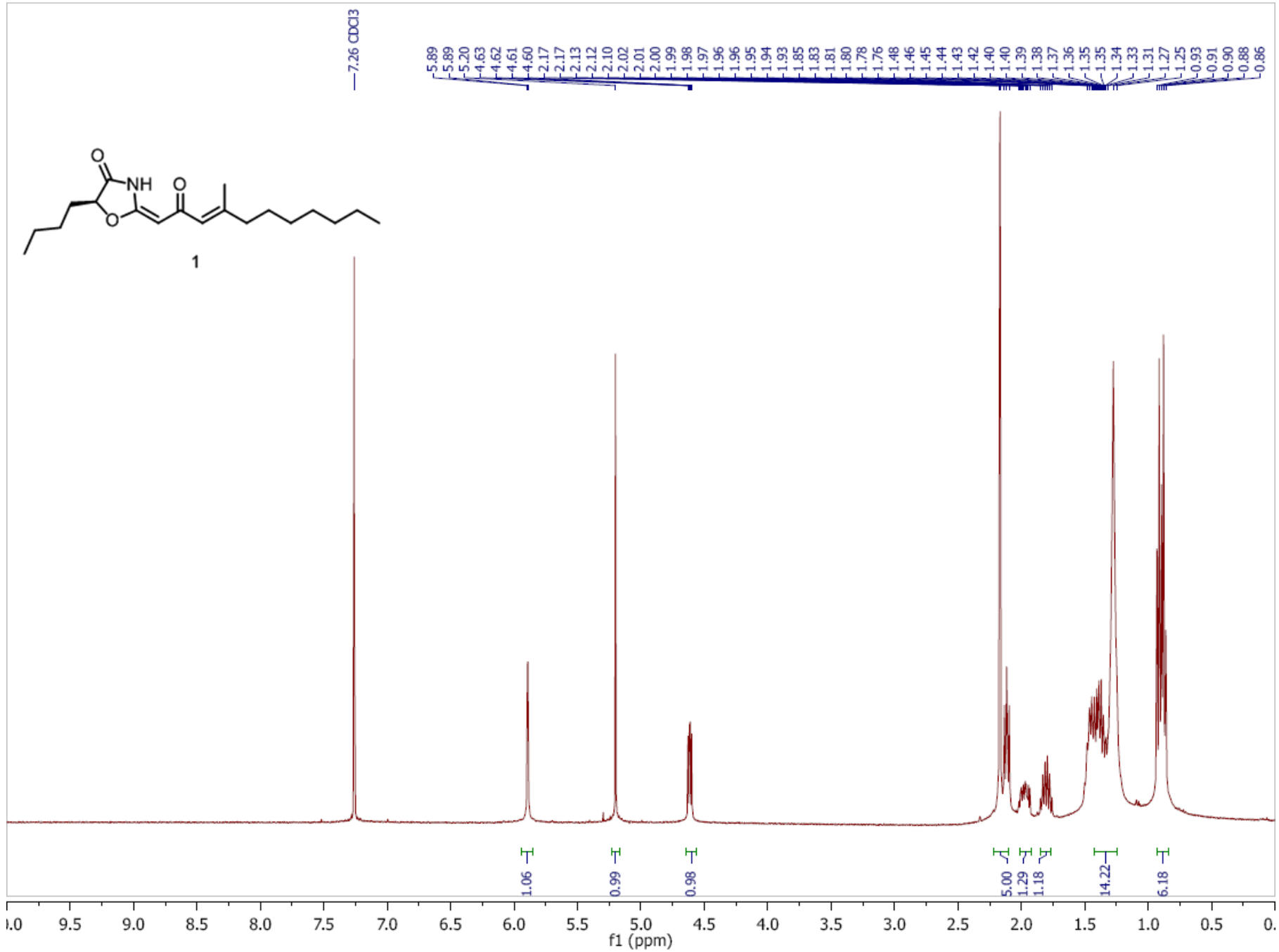

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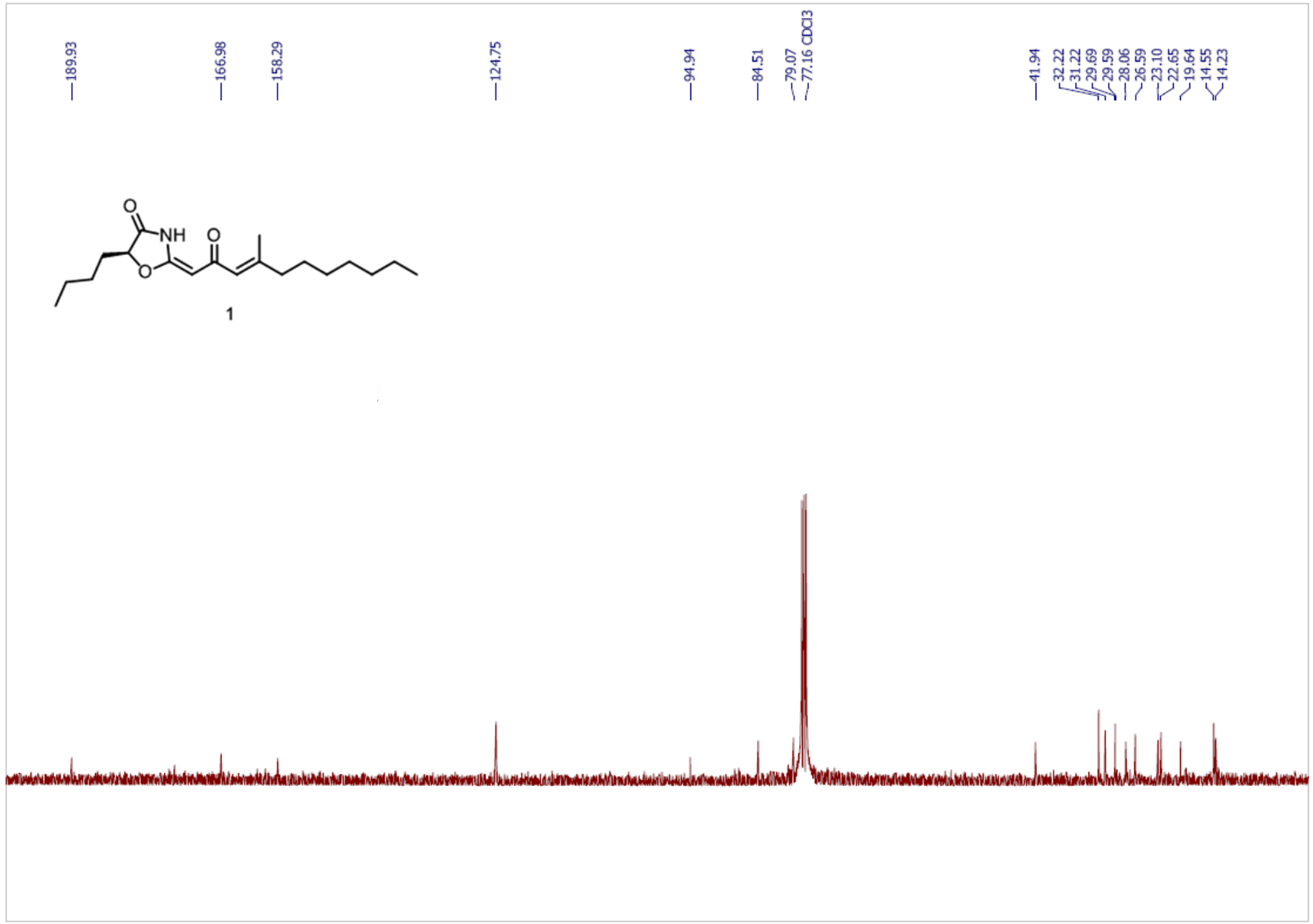

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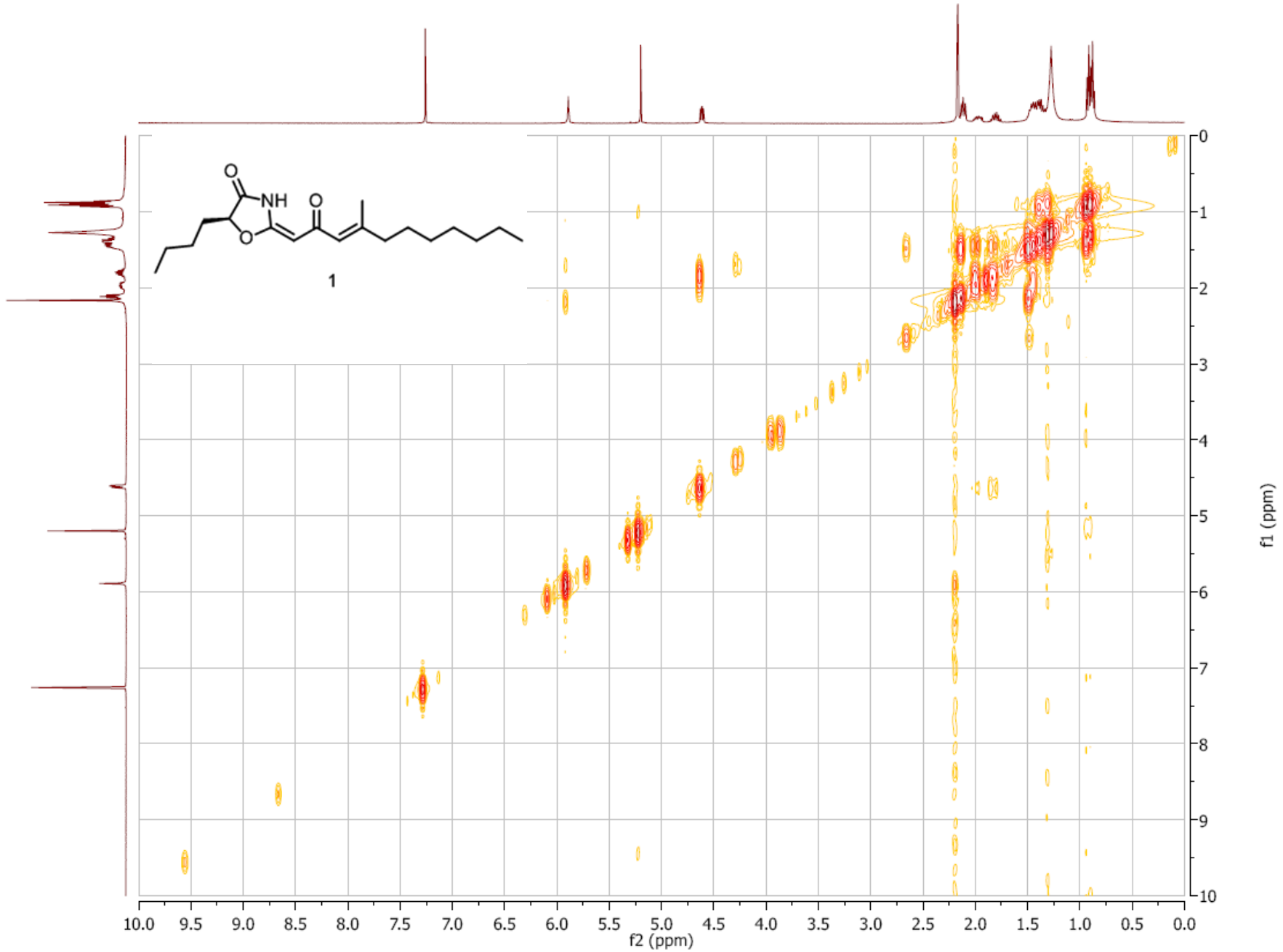

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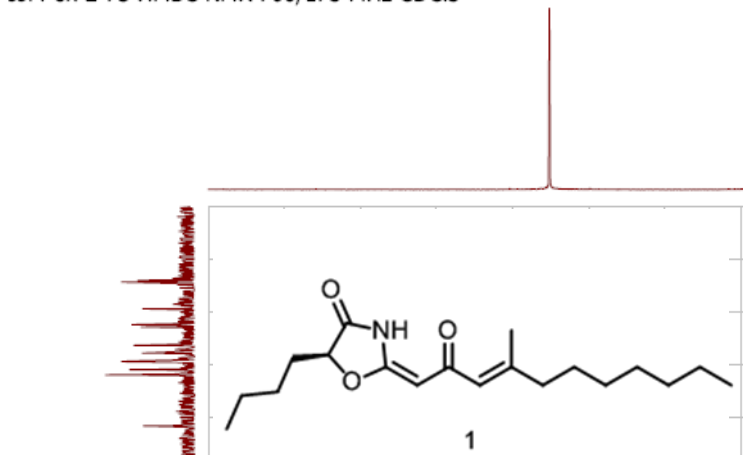

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f2 (ppm)

f1 (ppm)

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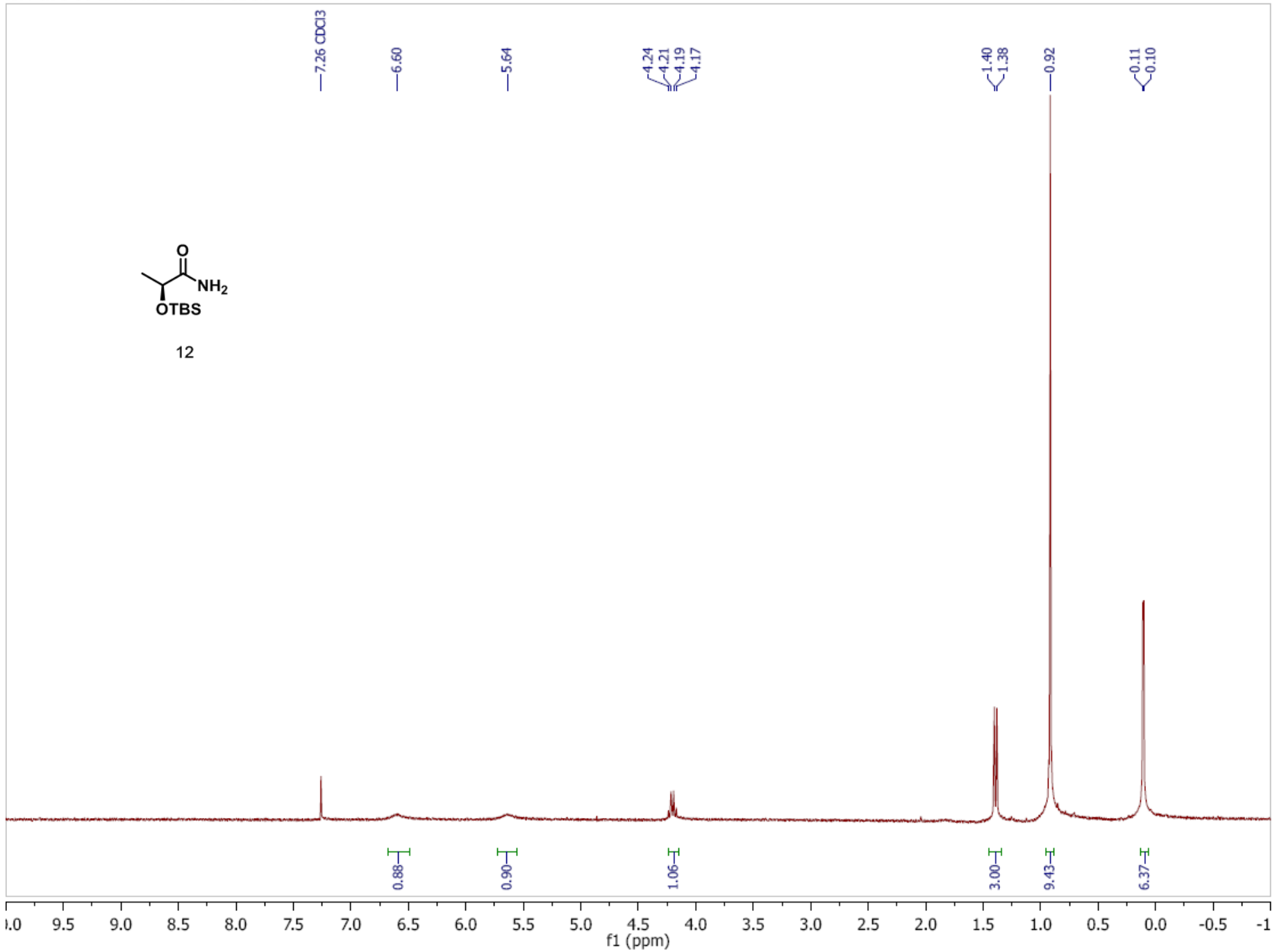

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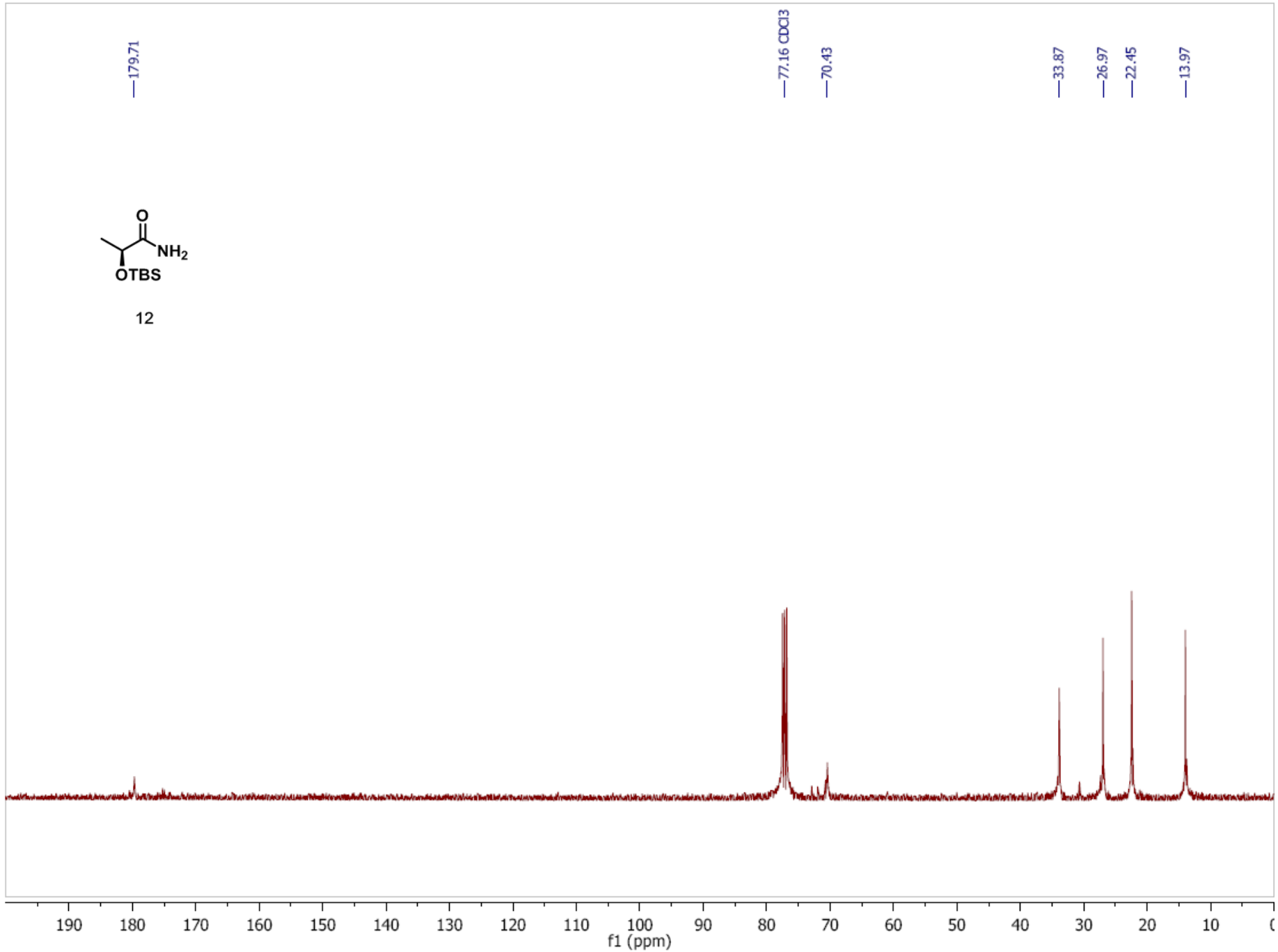

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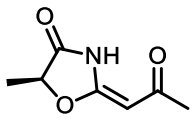

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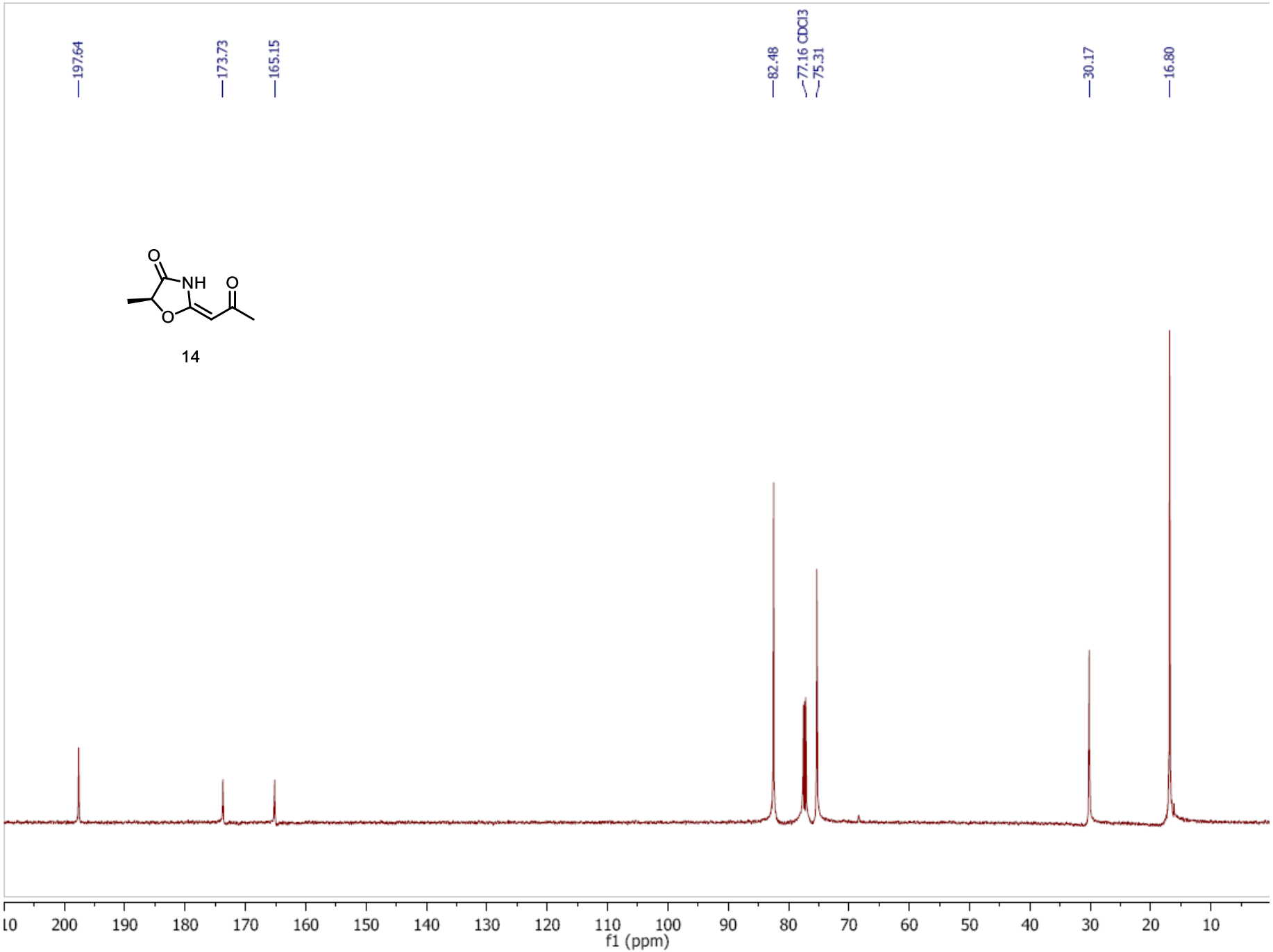

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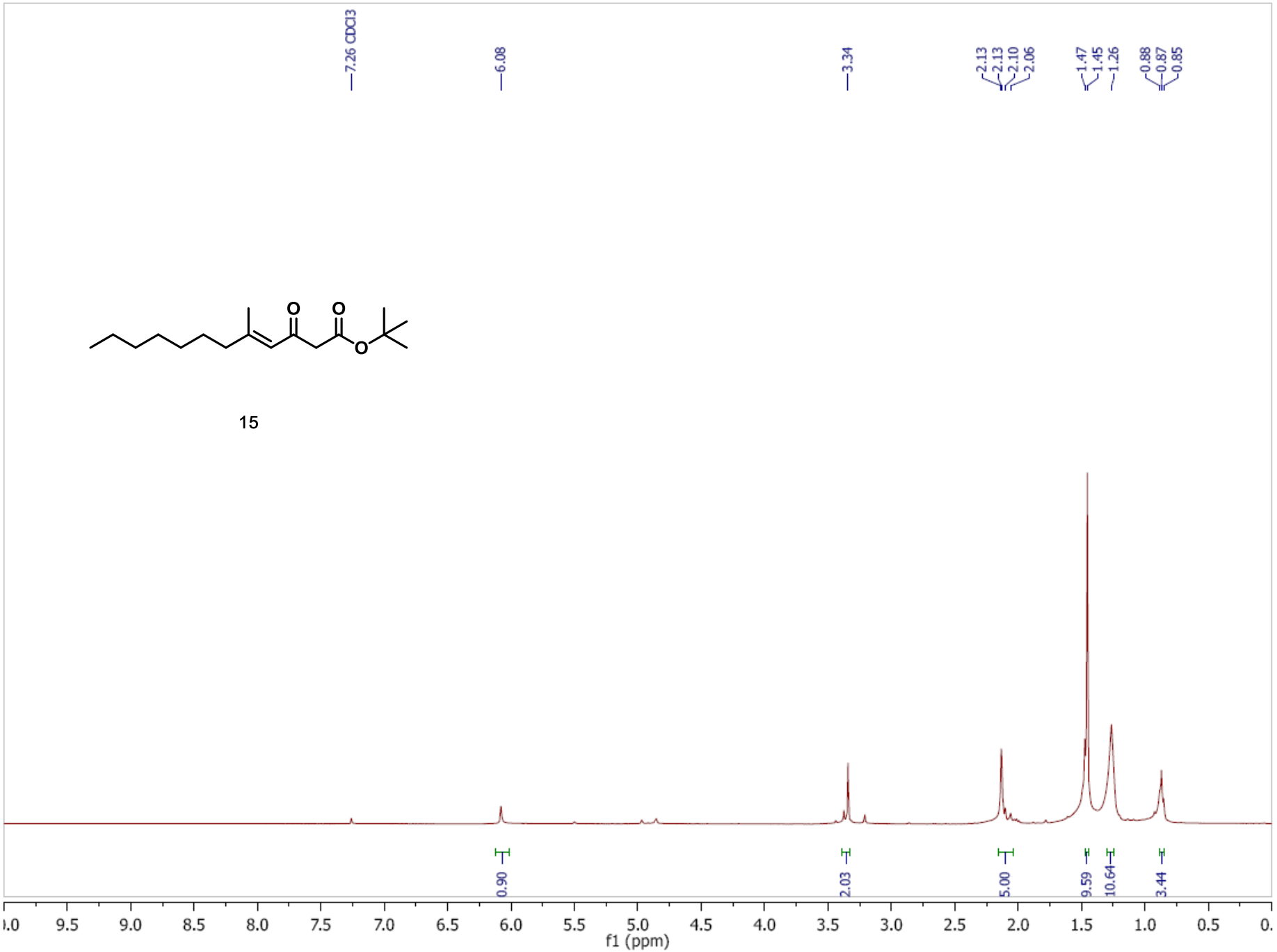

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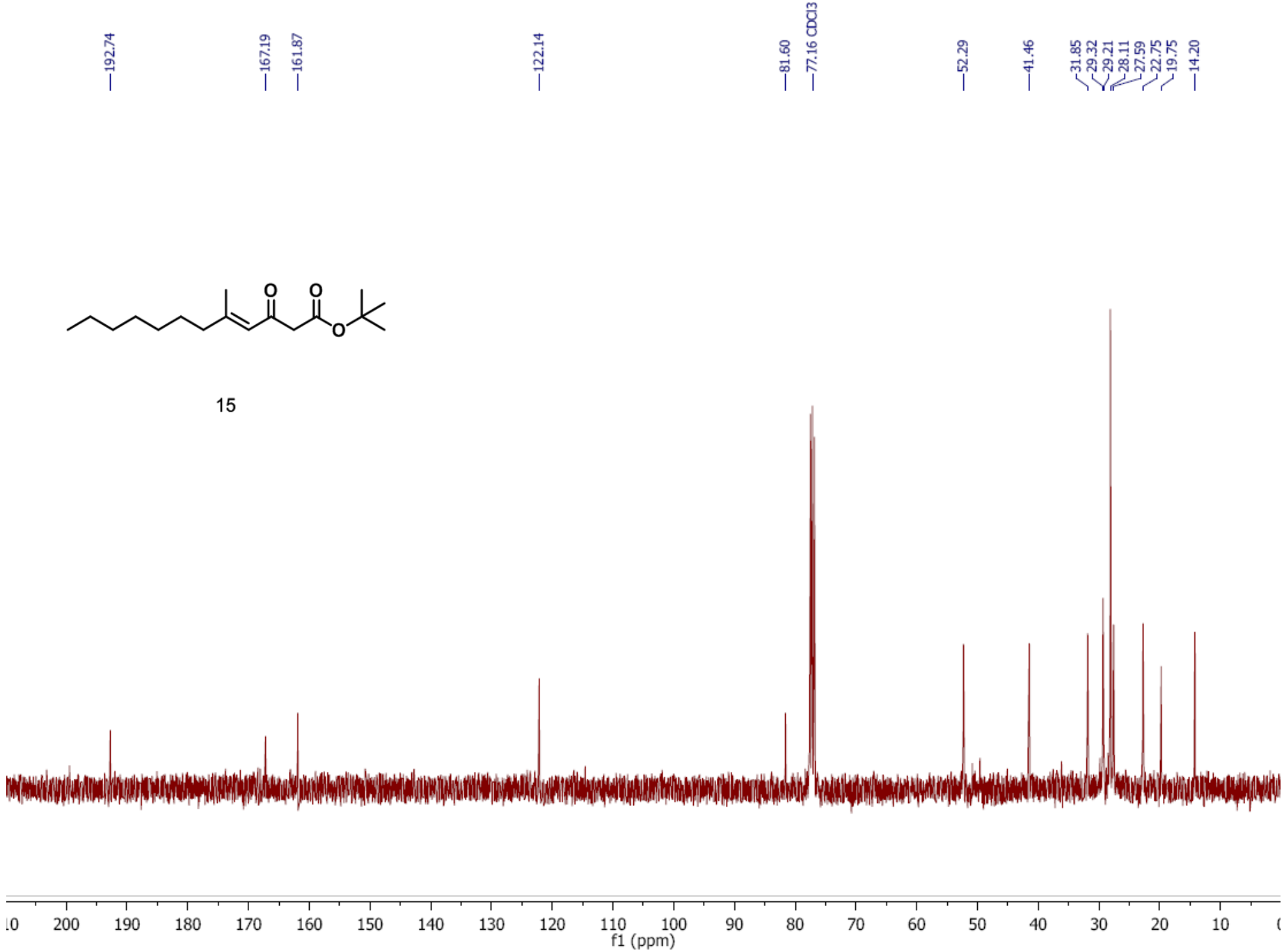

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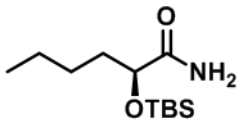

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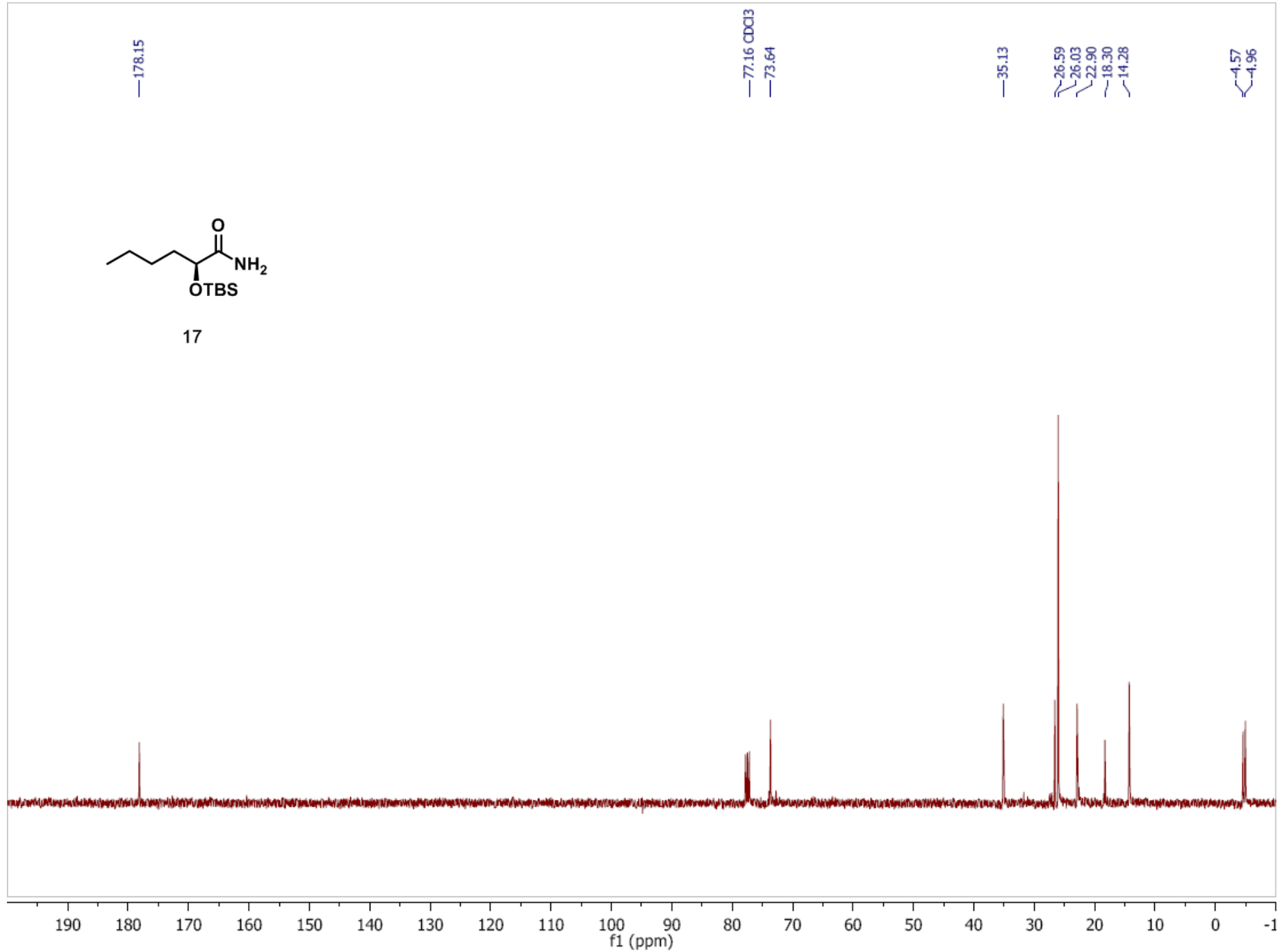

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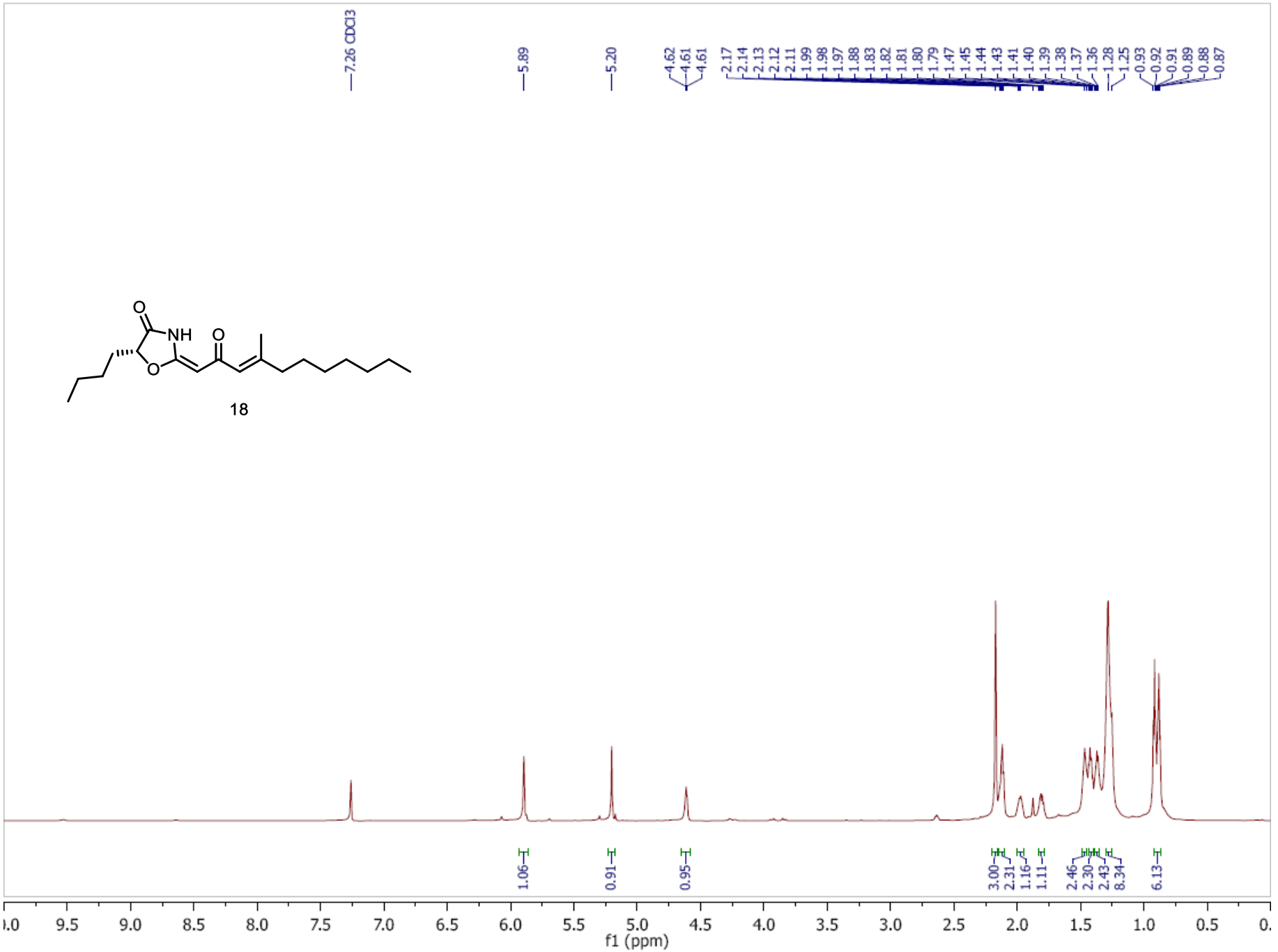

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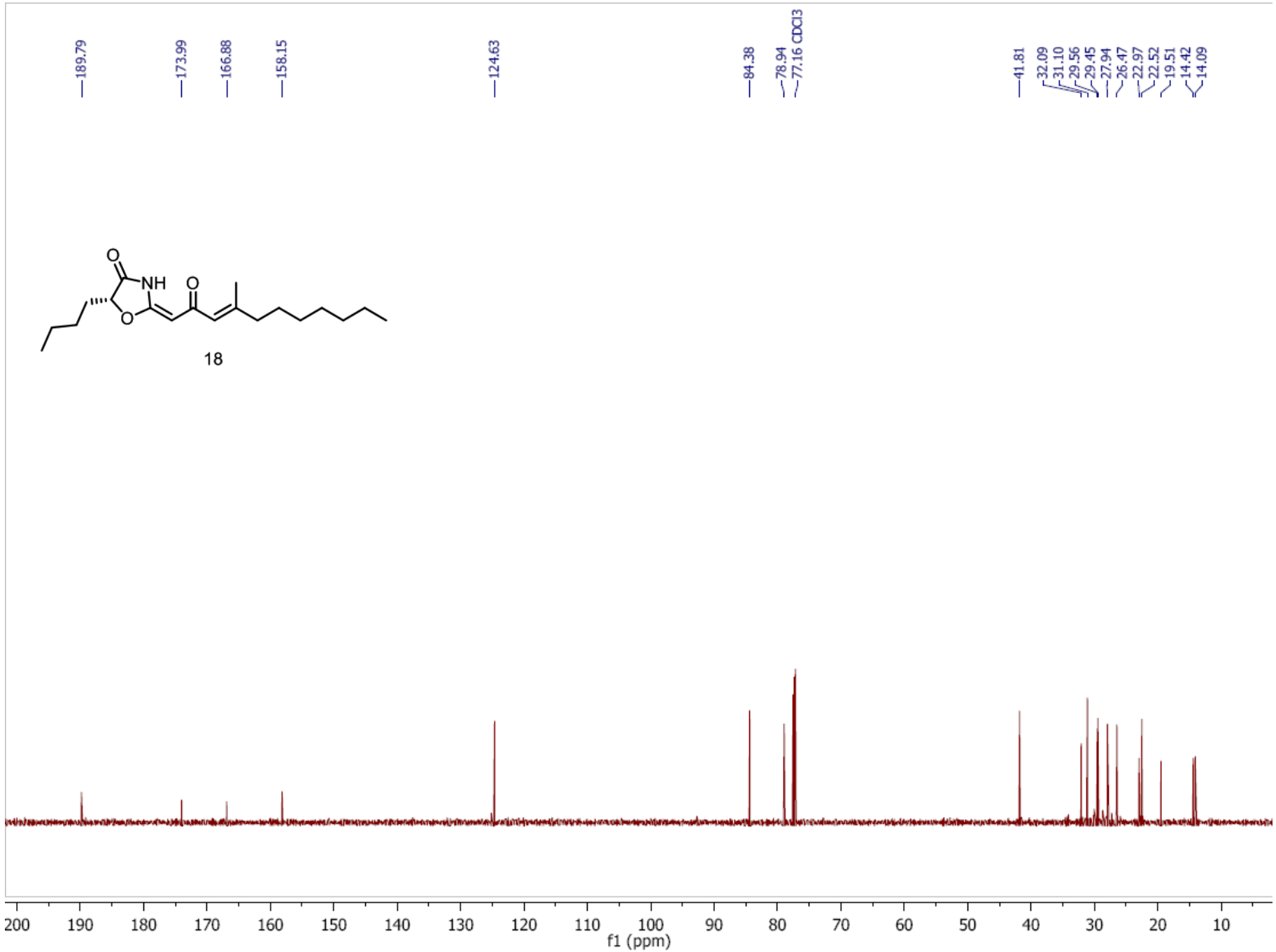

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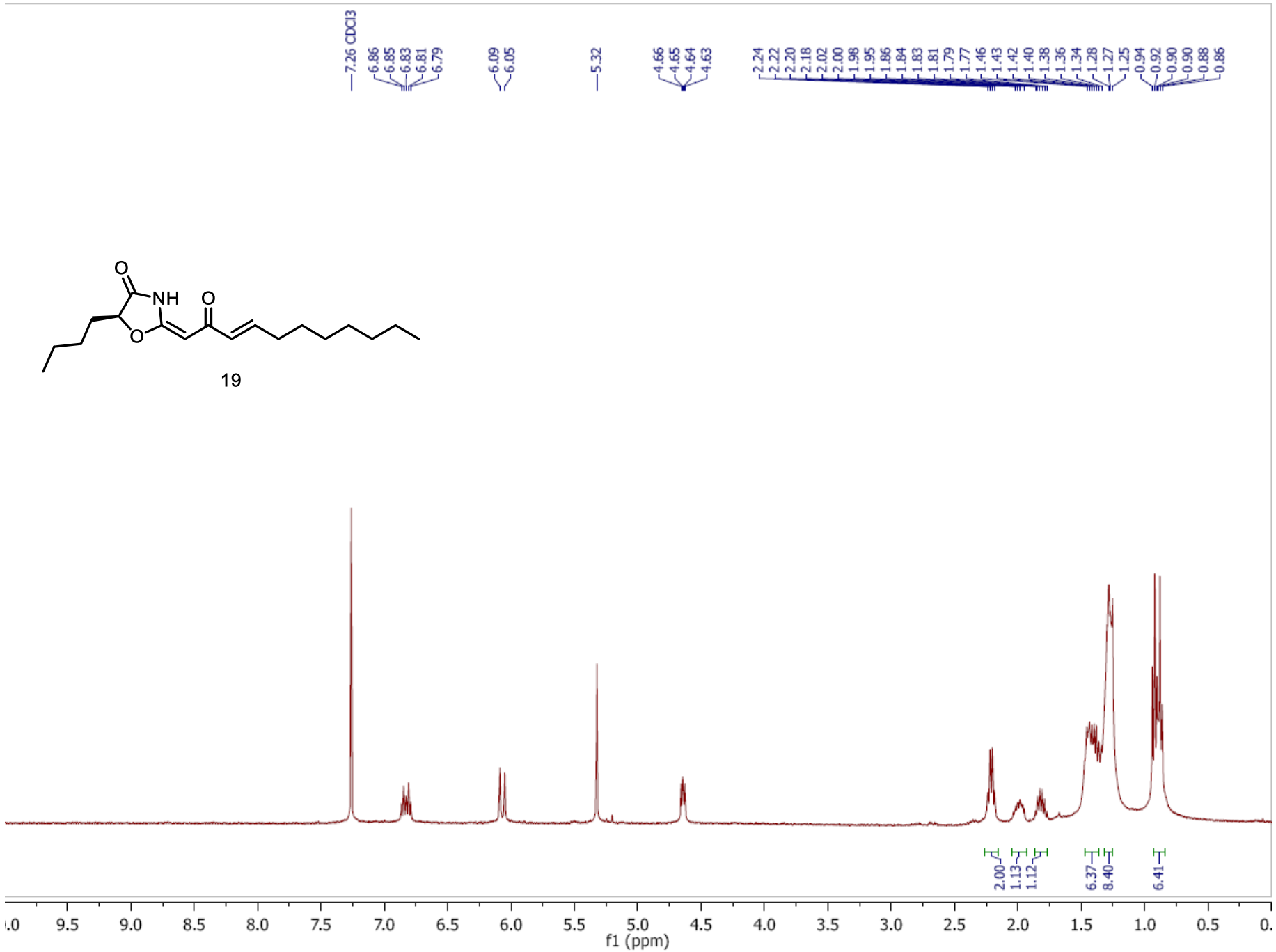

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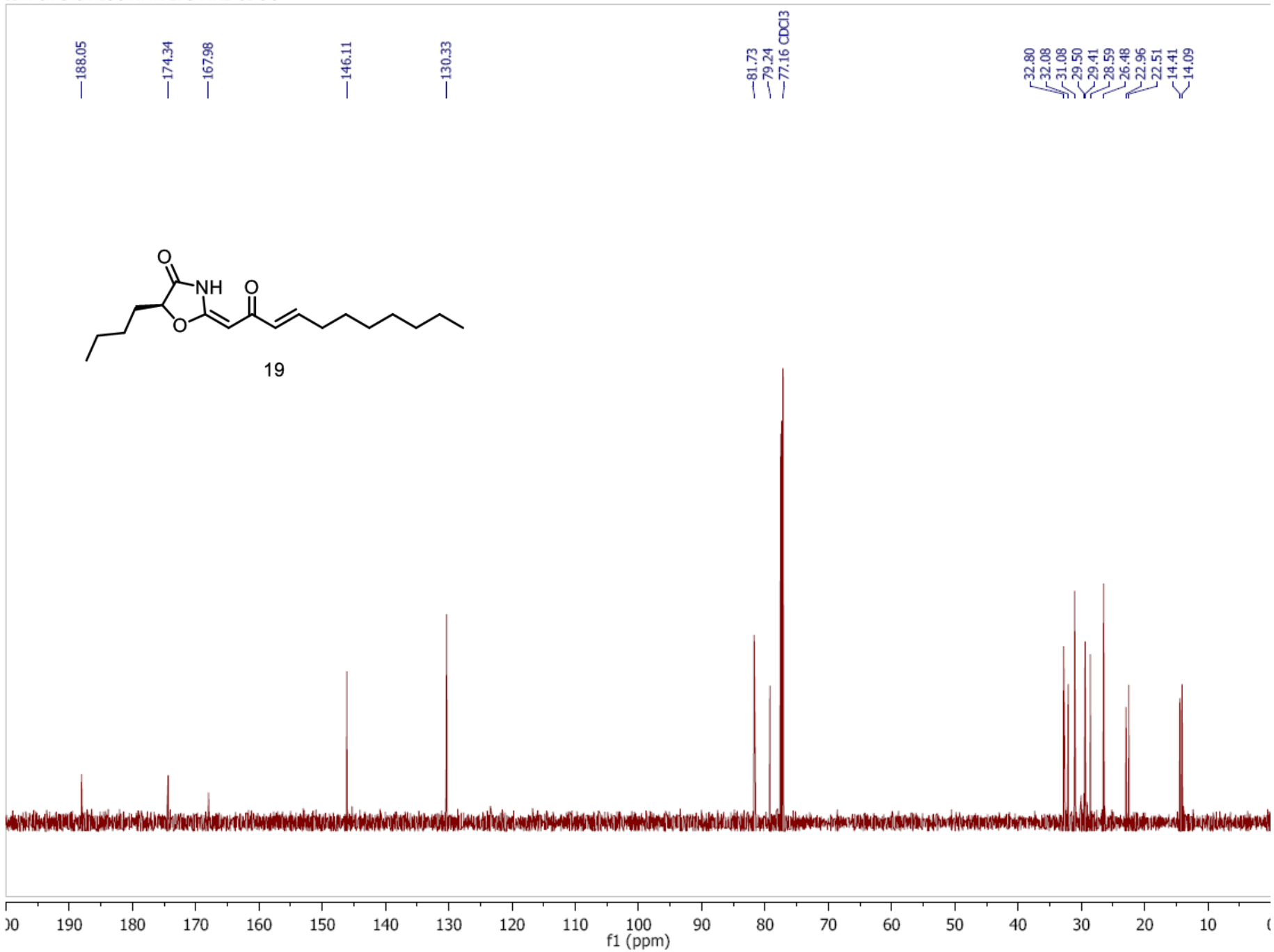

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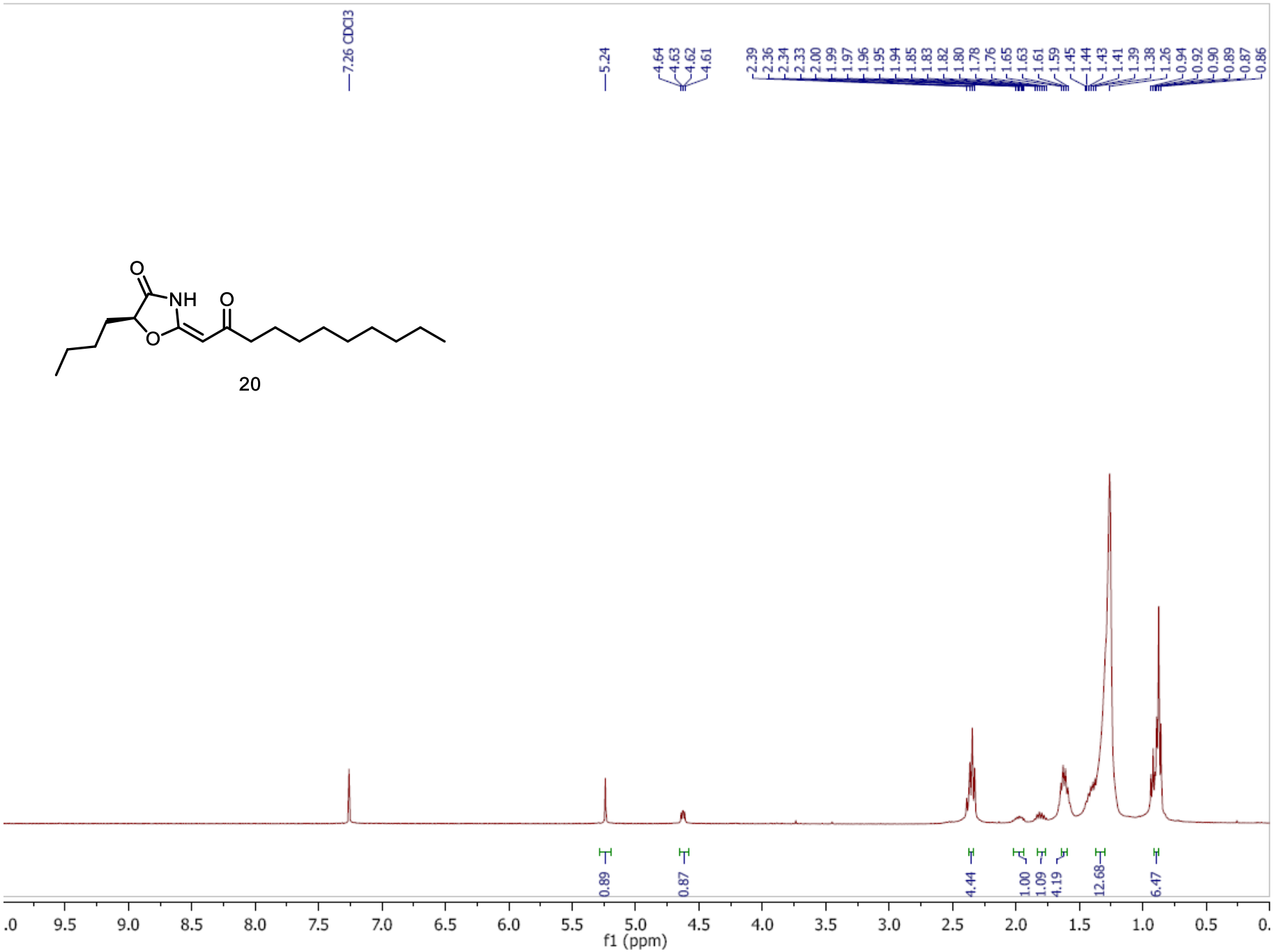

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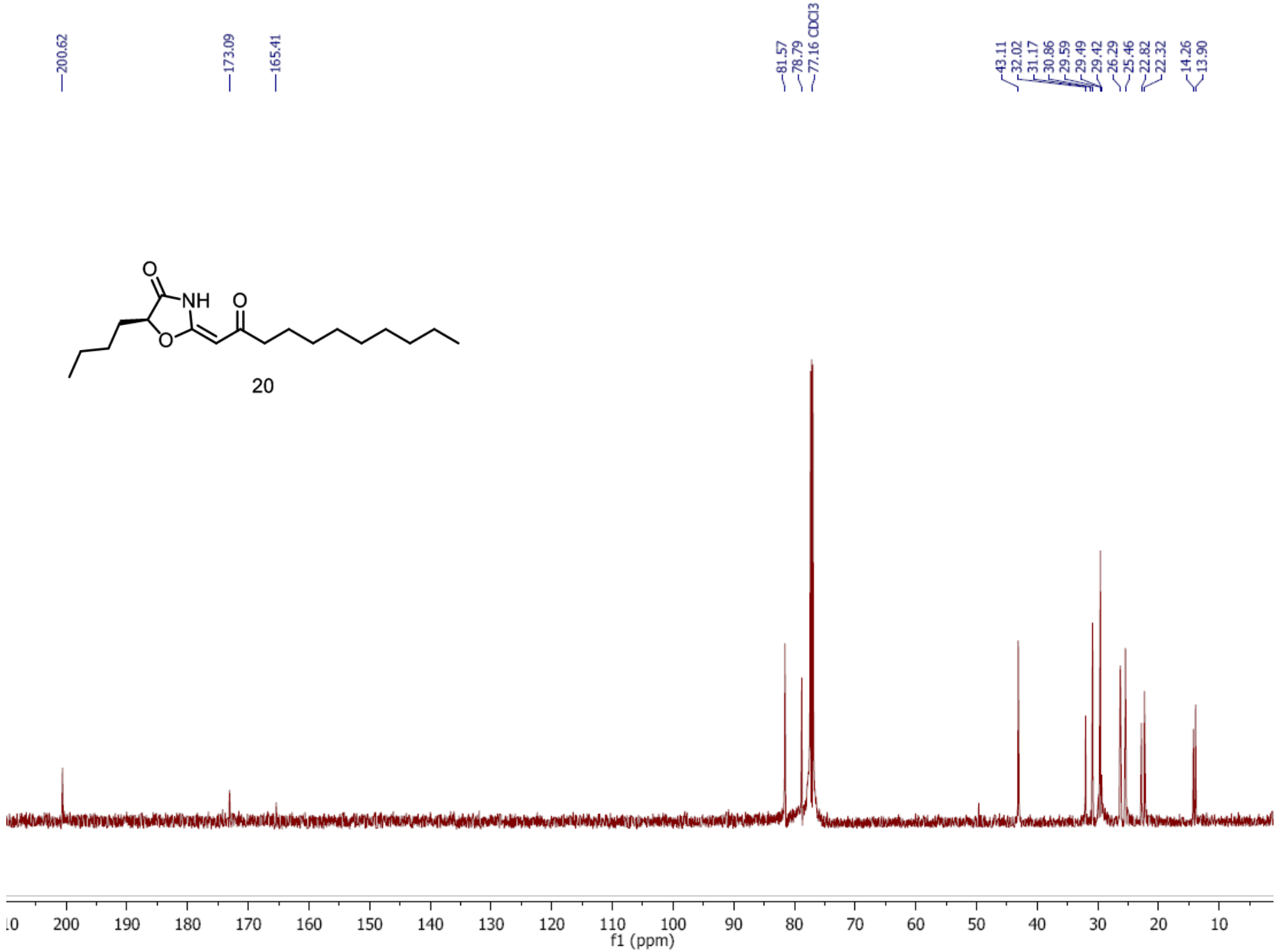

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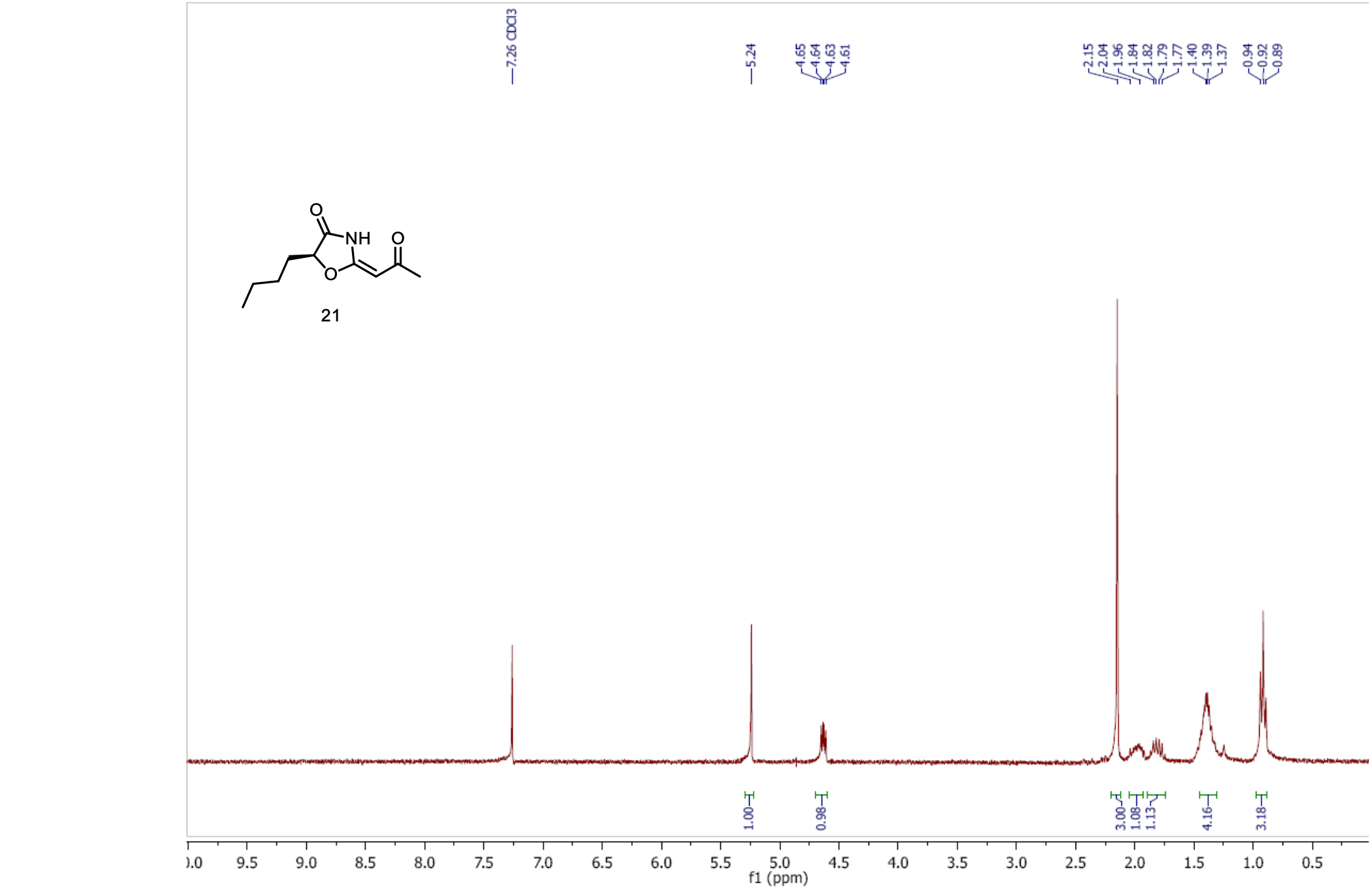

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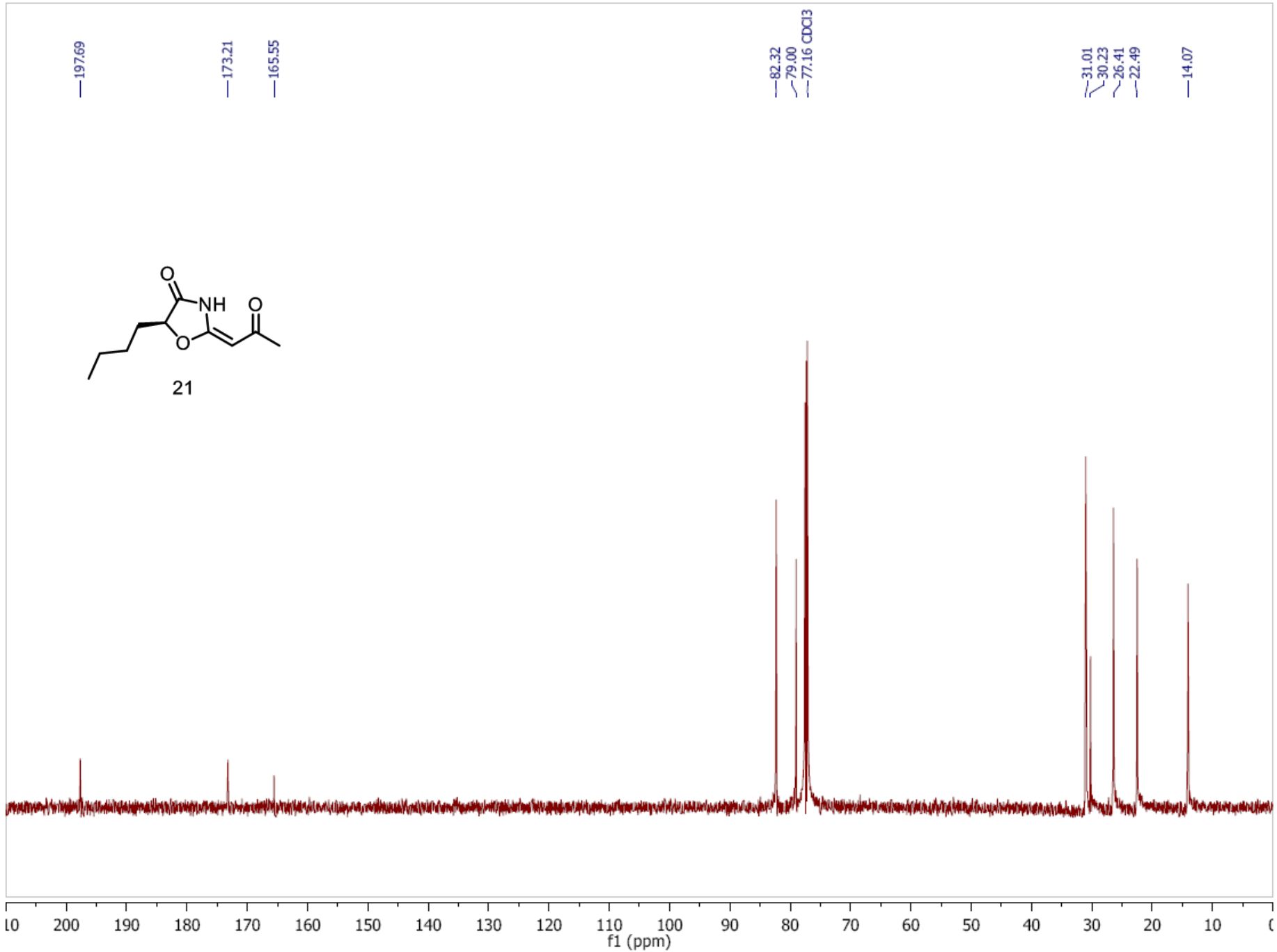

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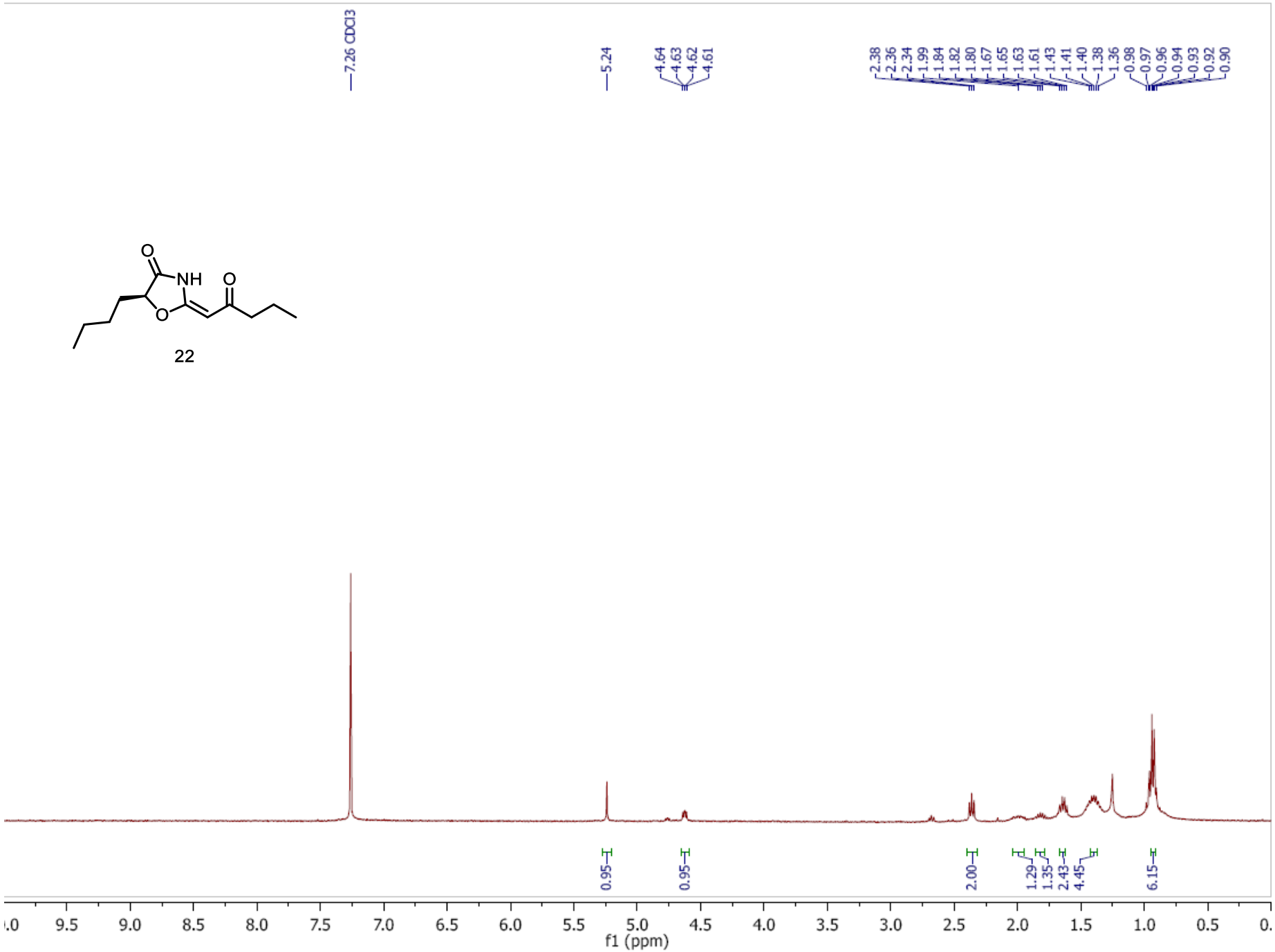

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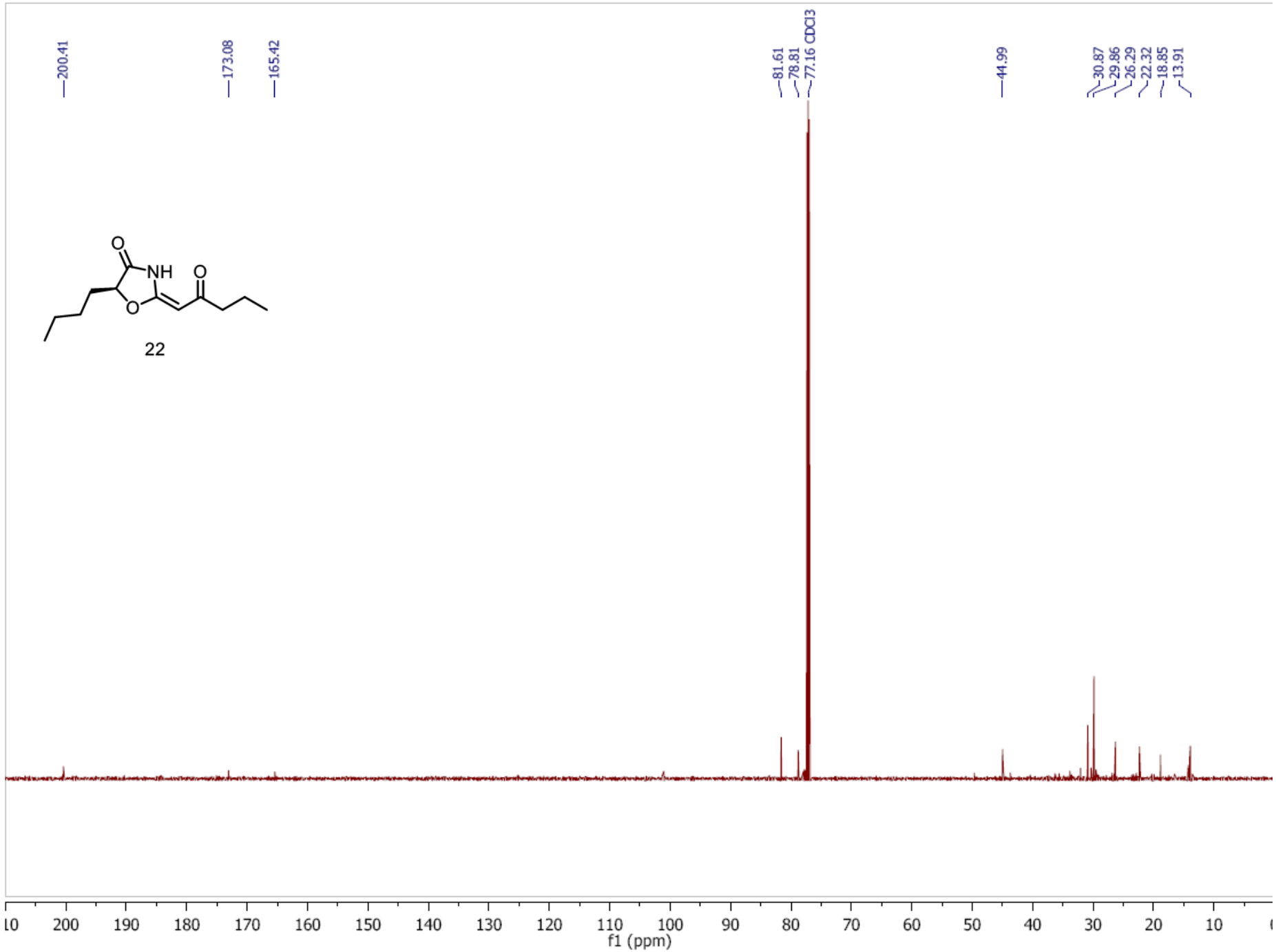

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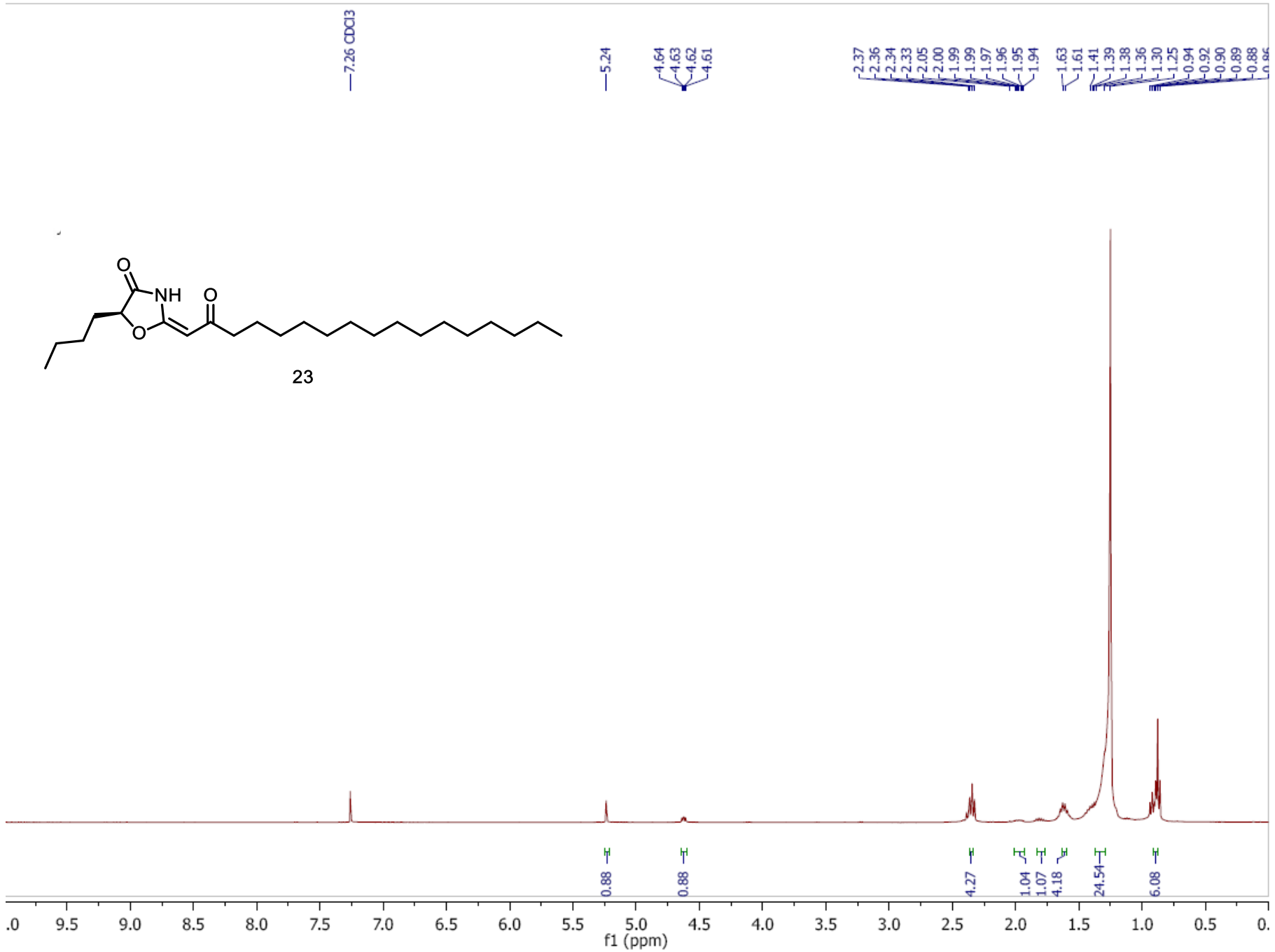

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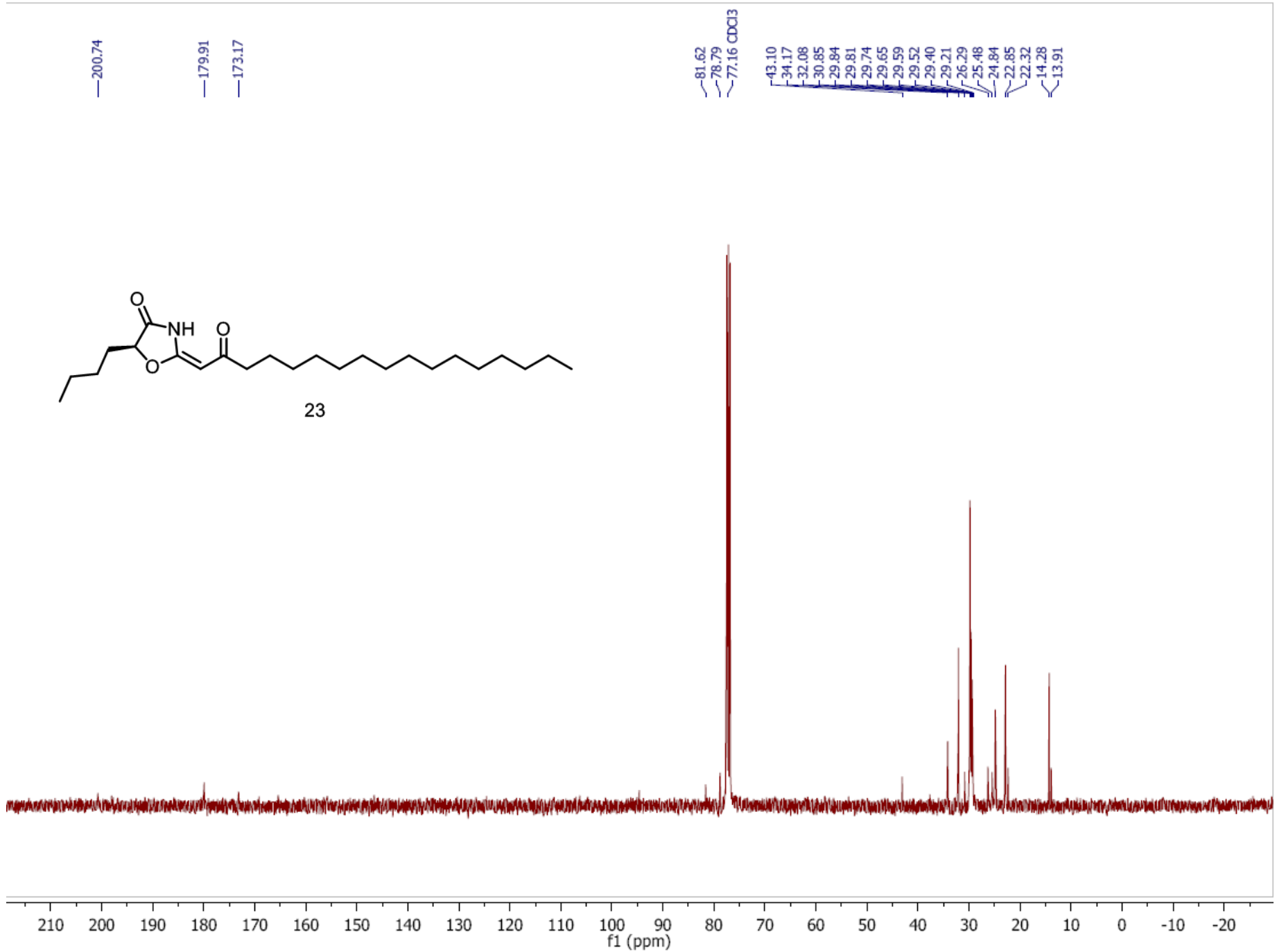

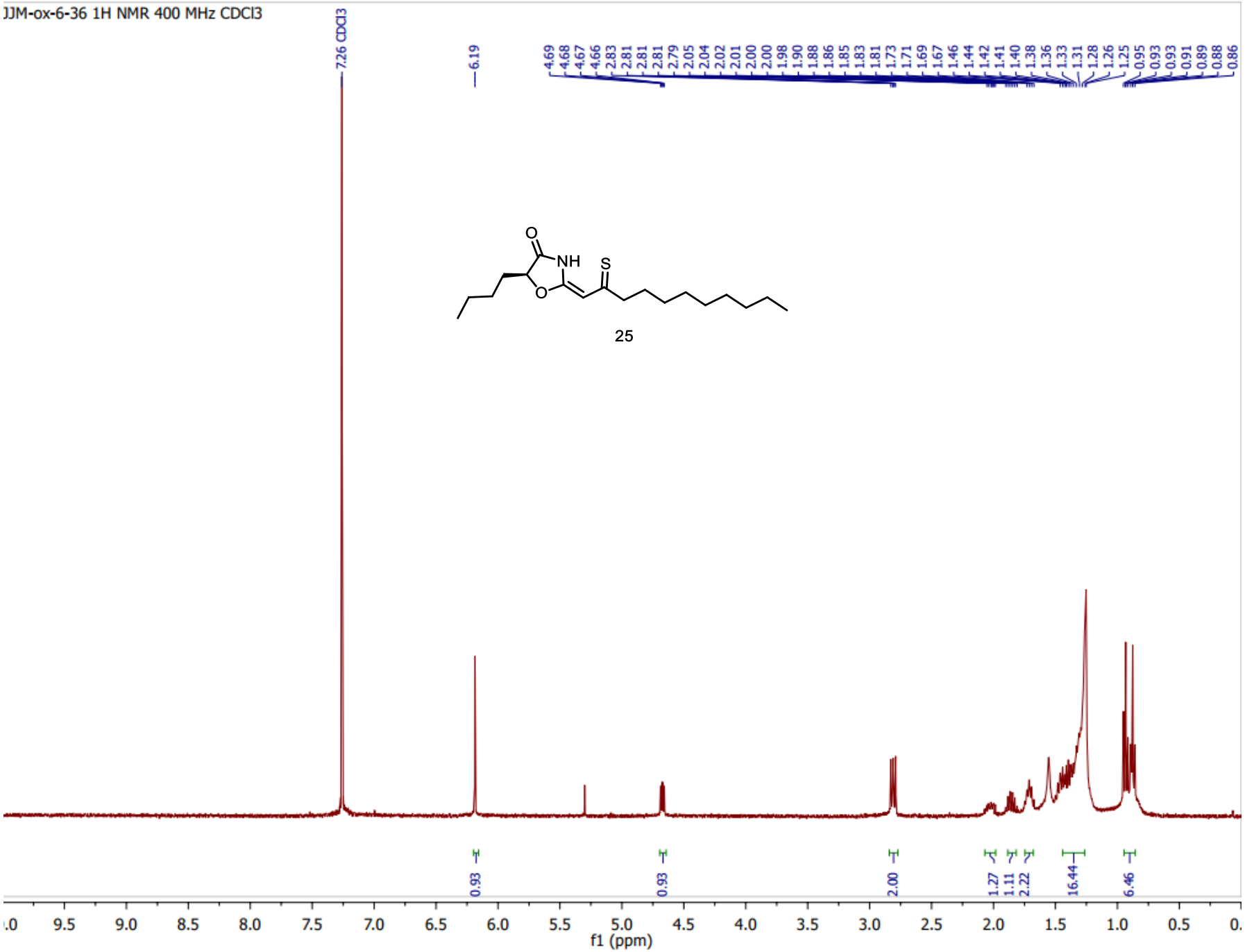
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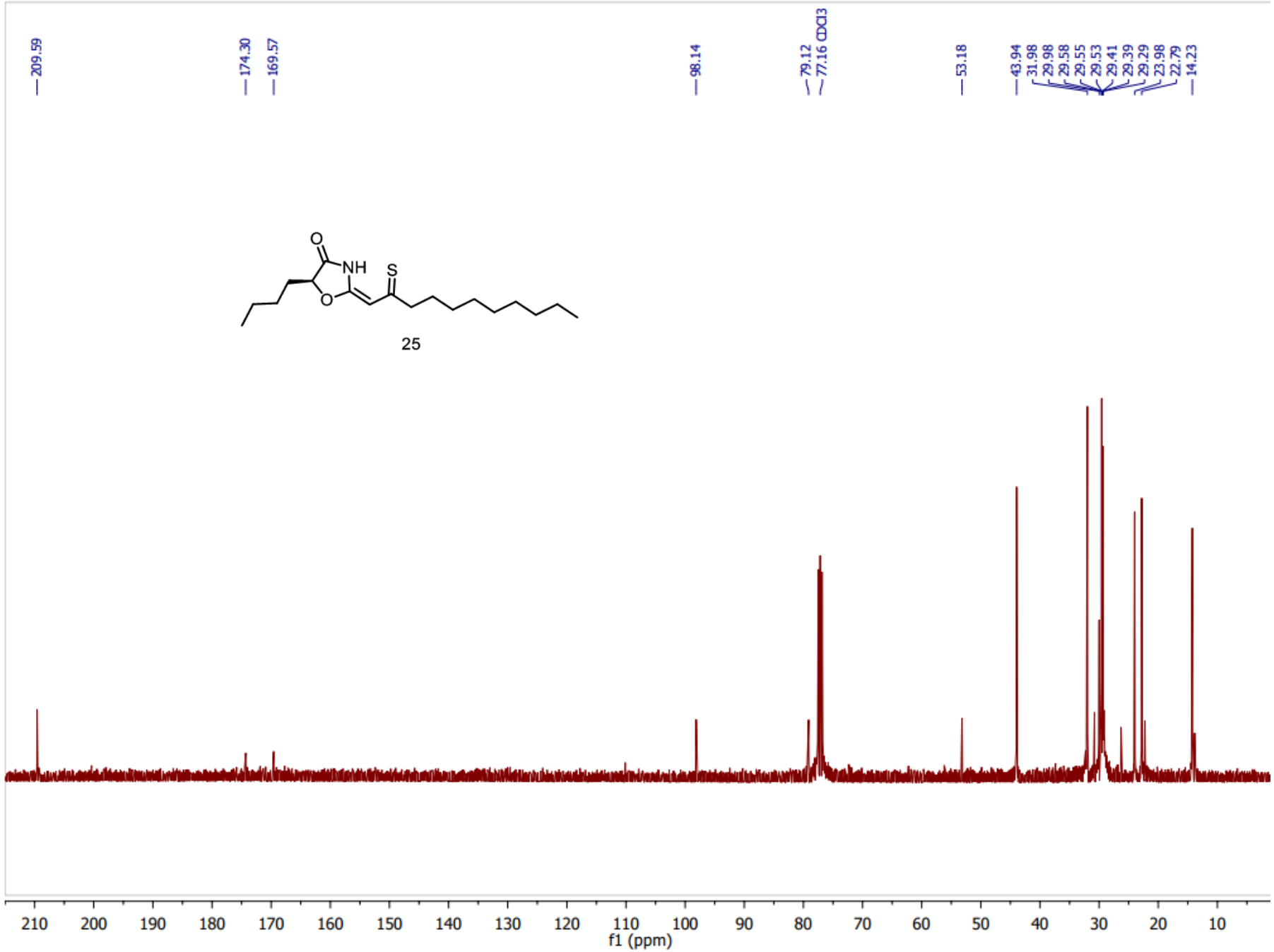
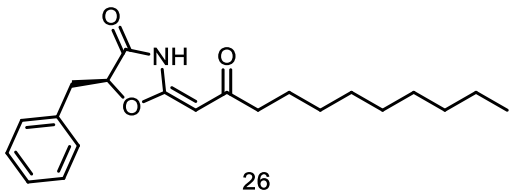
7.26 CDCl3
6.19
4.69 4.68 4.67 4.66 2.83 2.81 2.81 2.81 2.79 2.05 2.04 2.02 2.01 2.00 2.00 1.98 1.90 1.88 1.86 1.85 1.83 1.81 1.73 1.71 1.69 1.67 1.46 1.44 1.42 1.41 1.40 1.38 1.36 1.33 1.31 1.28 1.26 1.25 0.95 0.93 0.93 0.91 0.89 0.88 0.86
O
NH
S
O
25
0.93
0.93
2.00
1.27
1.11
2.22
16.44
6.46
9.5 9.0 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
f1 (ppm)

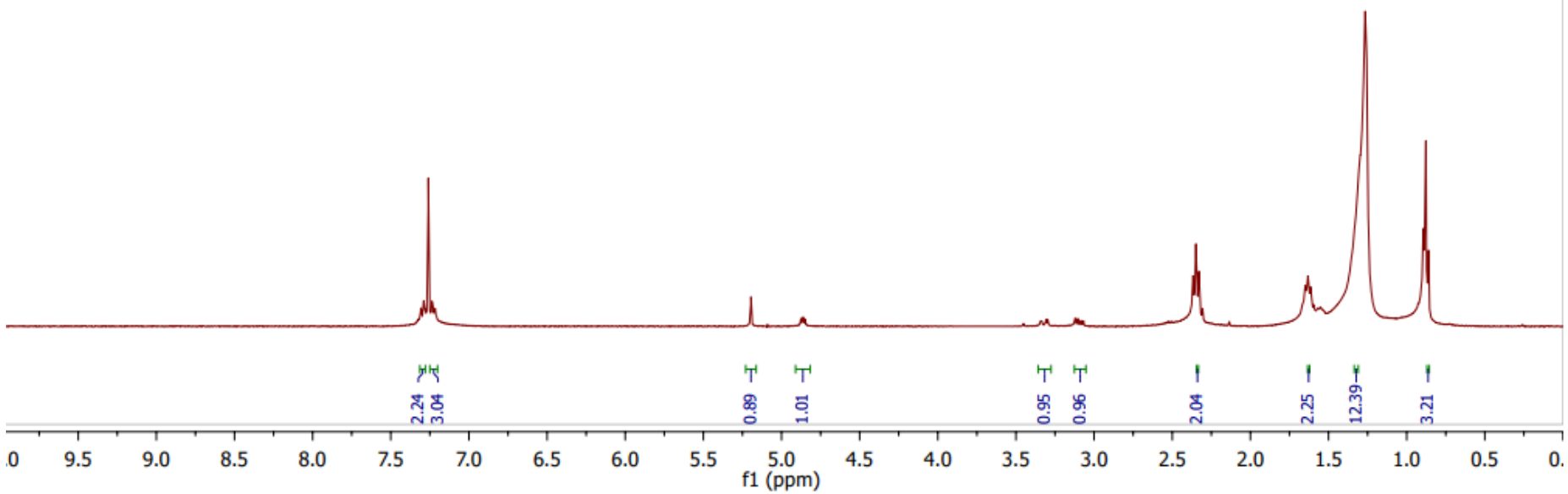
JJM-ox-6-36 13C NMR 100 MHz CDCl3



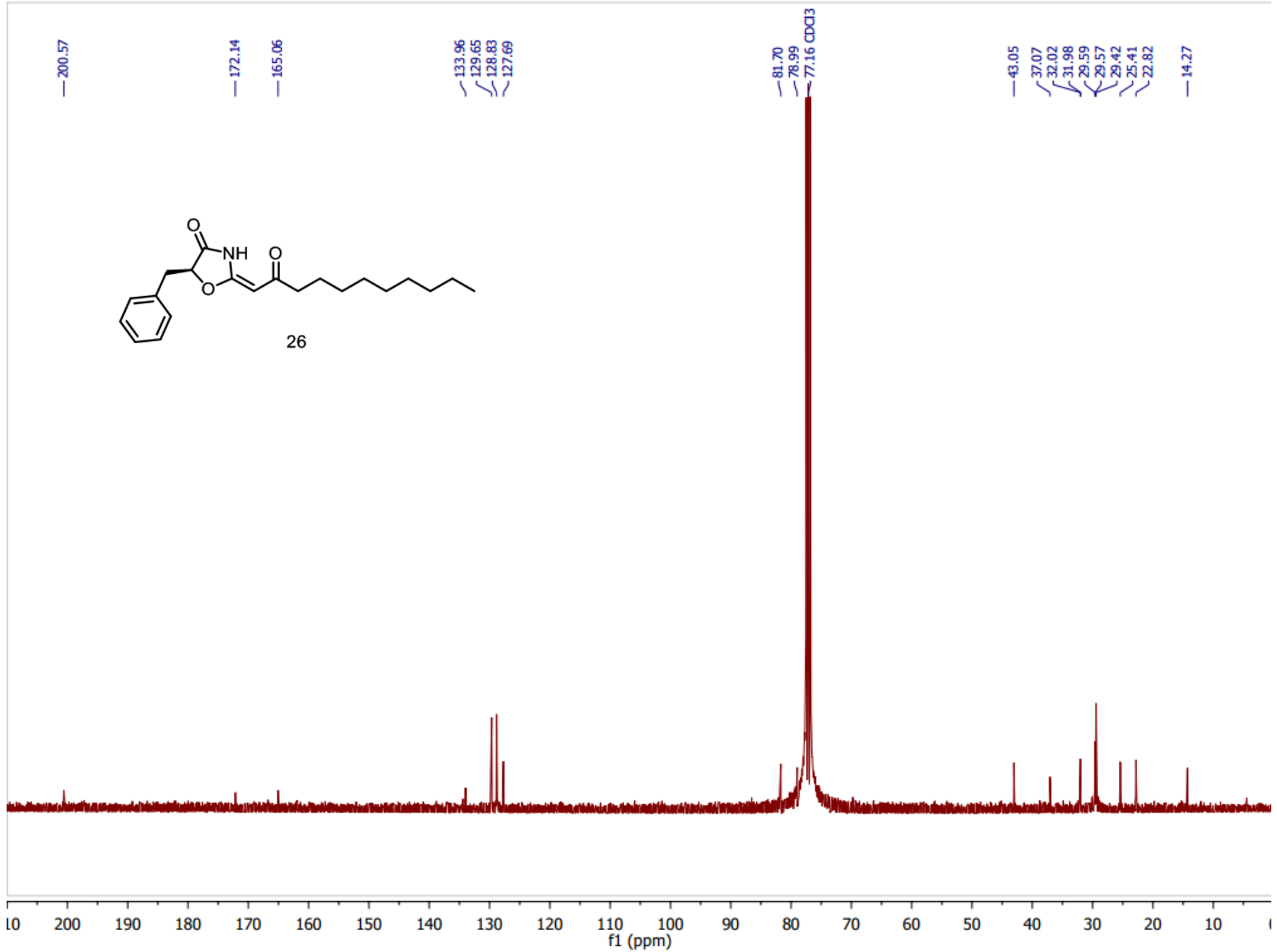
UM-ox-5-2 1H 400 MHz CDCl3

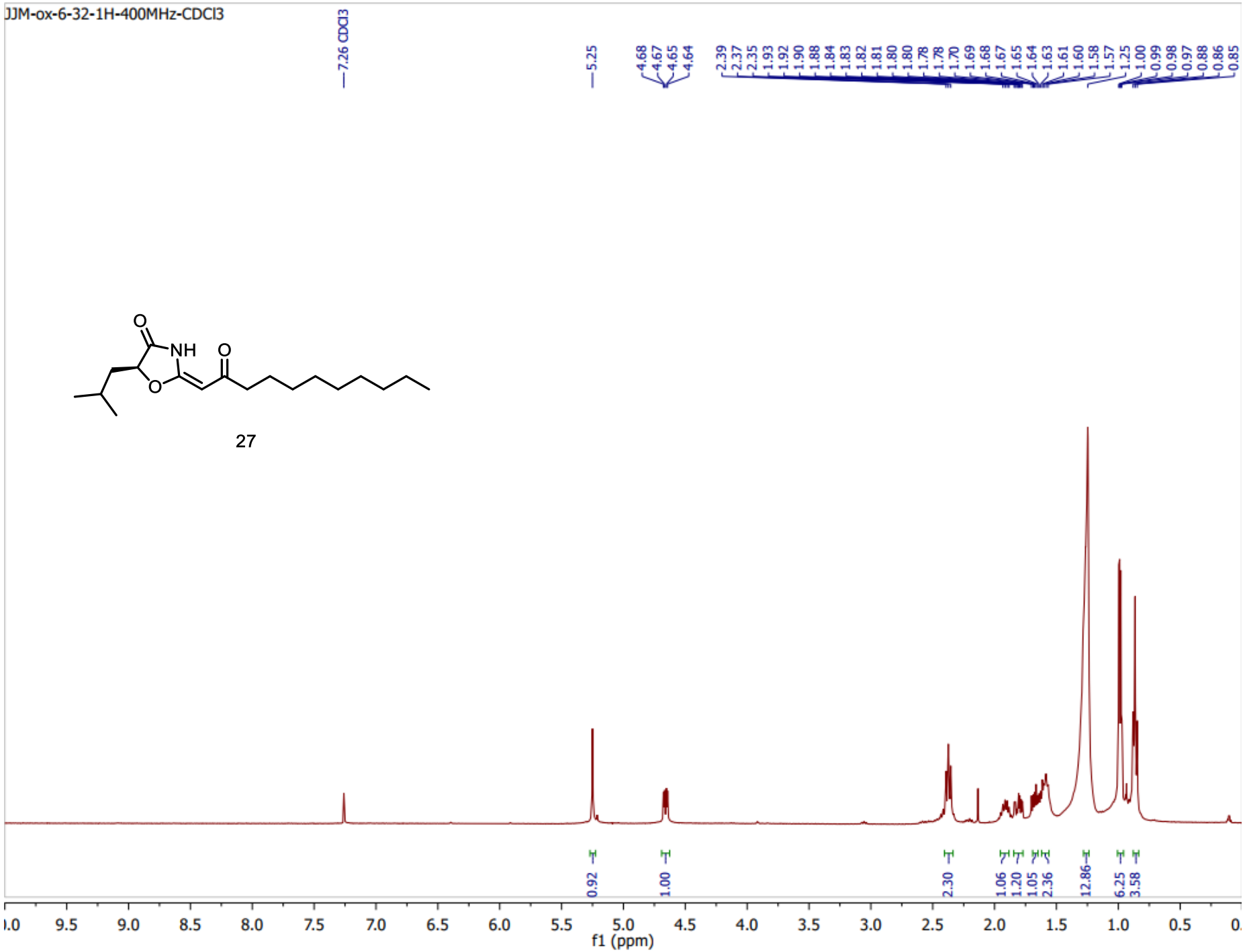


26

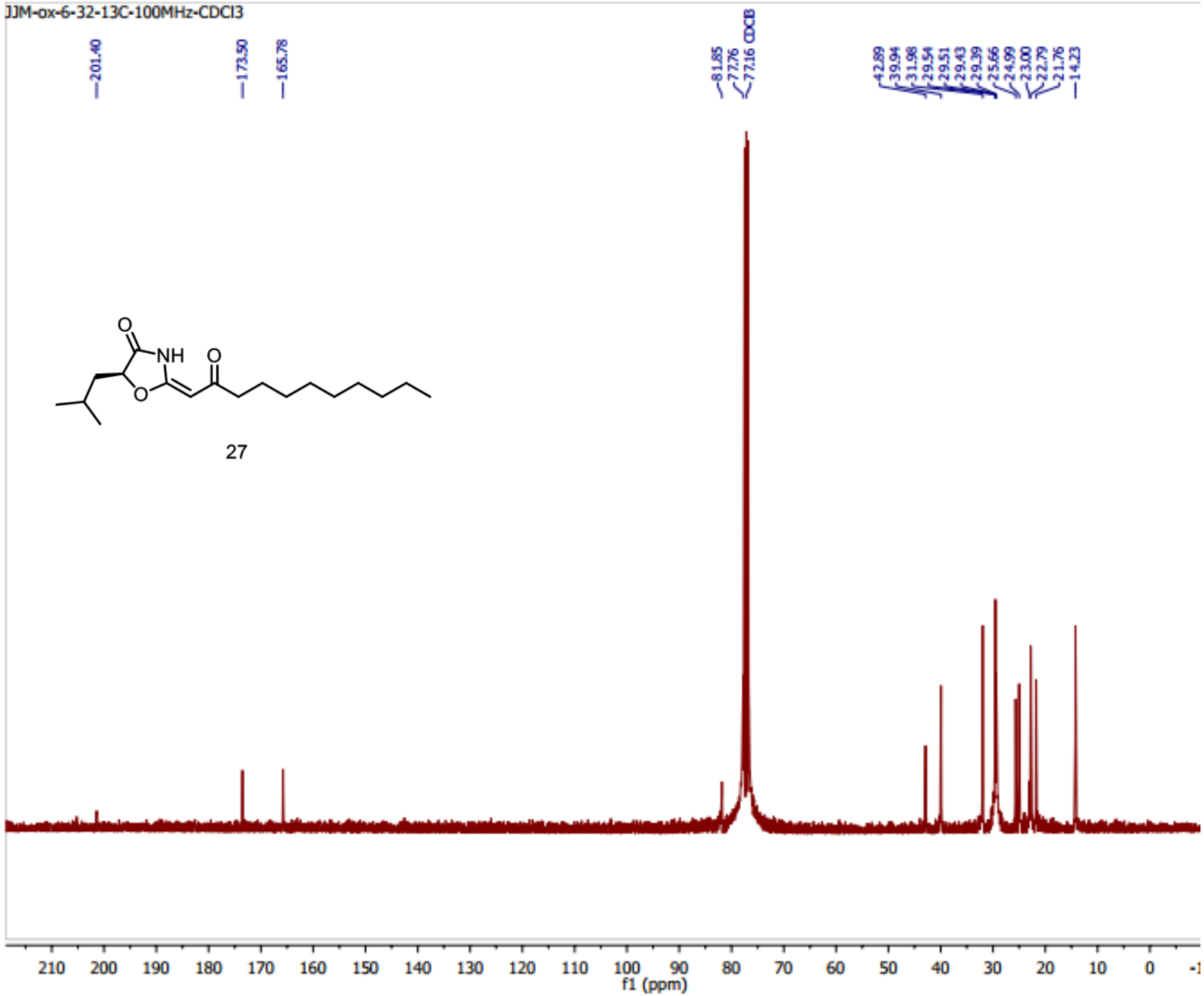


JJM-ox-5-2 13C NMR 100 MHz CDCl3

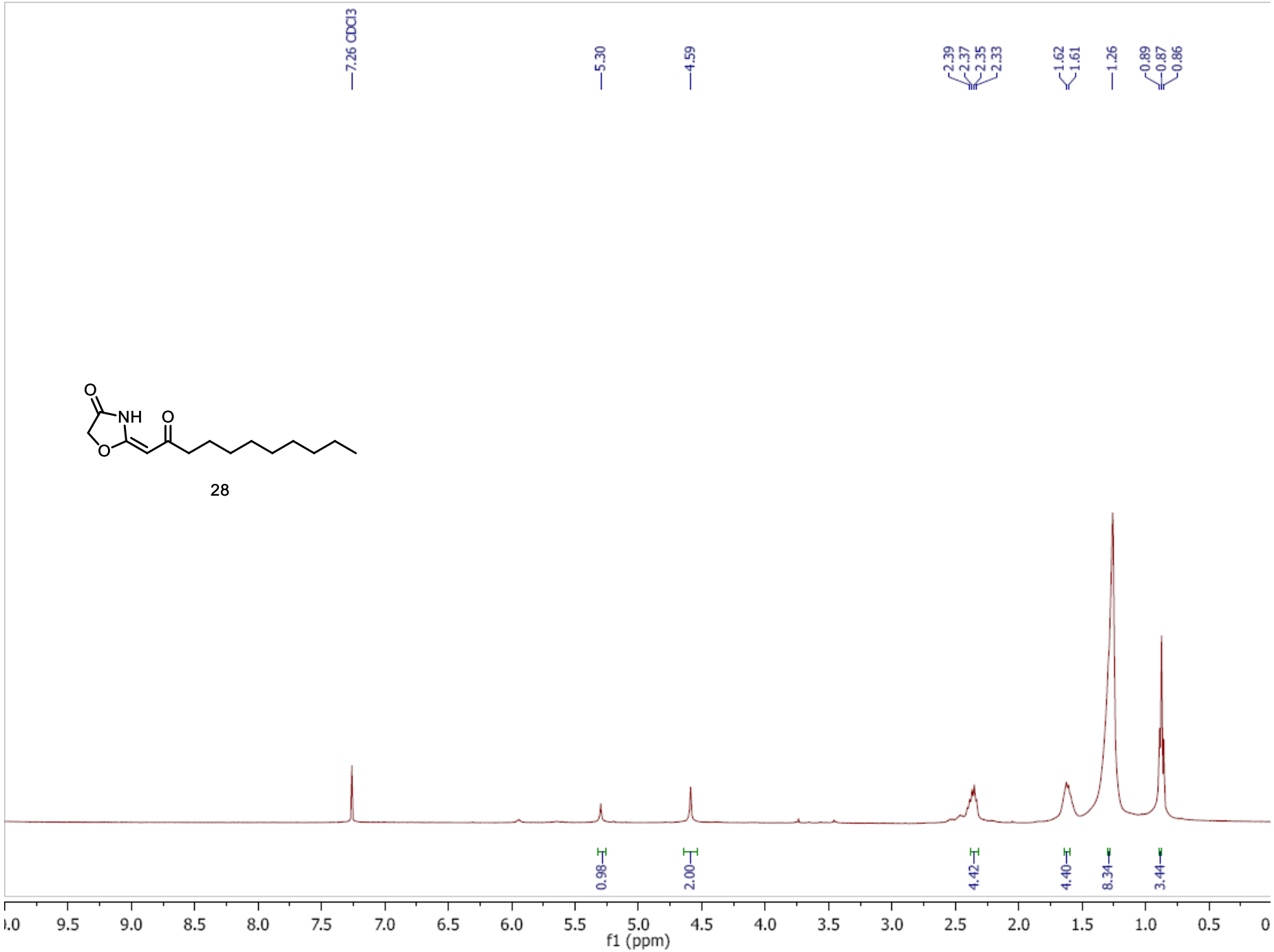



JJM-ox-6-32-1H-400MHz-CDCl3
7.26 CDCl3
5.25
4.68
4.67
4.65
4.64
2.39
2.37
2.35
1.93
1.92
1.90
1.88
1.84
1.83
1.82
1.81
1.80
1.80
1.78
1.78
1.70
1.69
1.68
1.67
1.65
1.64
1.63
1.61
1.60
1.58
1.57
1.25
1.00
0.99
0.98
0.97
0.88
0.86
0.85
O
NH
O
O
0.92
1.00
2.30
1.06
1.20
1.05
2.36
12.86
6.25
3.58
f1 (ppm)

27

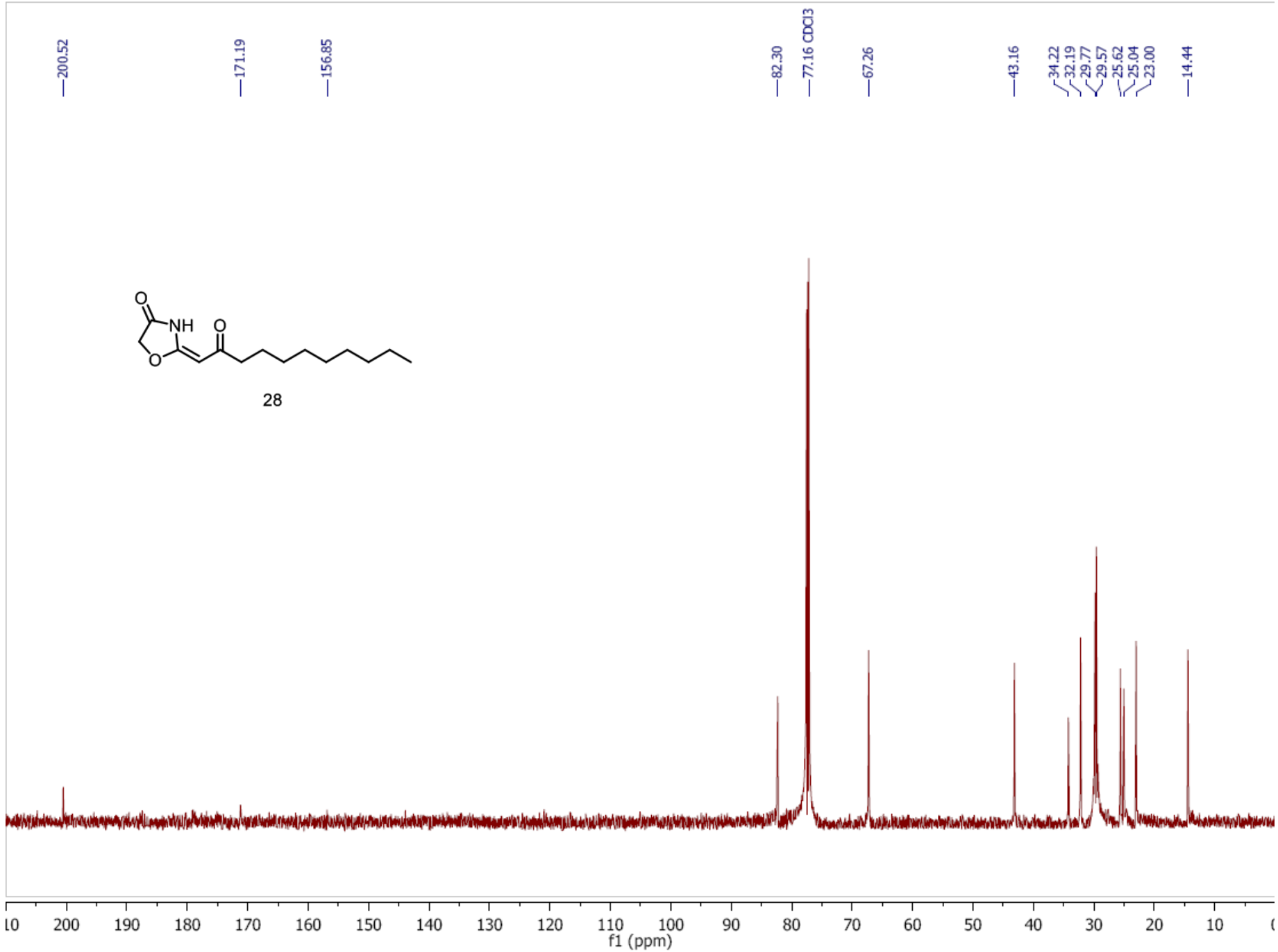

JJM-ox-6-32-13C-100MHz-CDCl3
201.40
173.50
165.78
81.85
77.76
77.16 CDCl3
42.89
39.94
31.98
29.54
29.51
29.43
29.39
25.66
24.99
23.00
22.79
21.76
14.23
O
NH
O
O
27
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

JJM-ox-5-3 1H NMR 400 MHz CDCl3

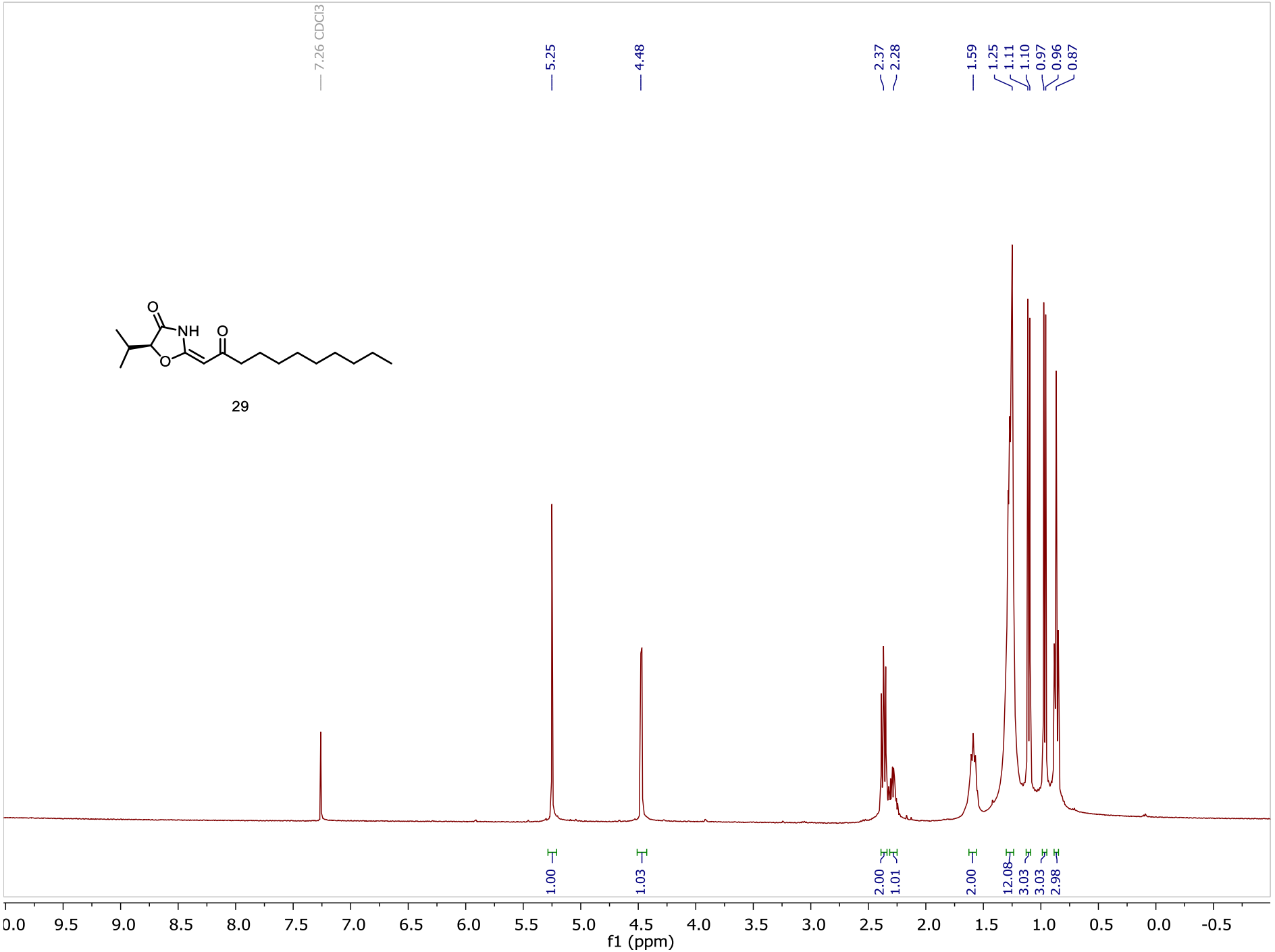


28

JJM-ox-5-3 13C NMR 175 MHz CDCl3

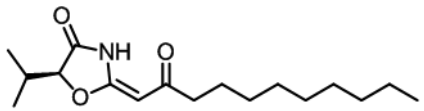


JJM-ox-6-33-1H-400MHz-CDCl3

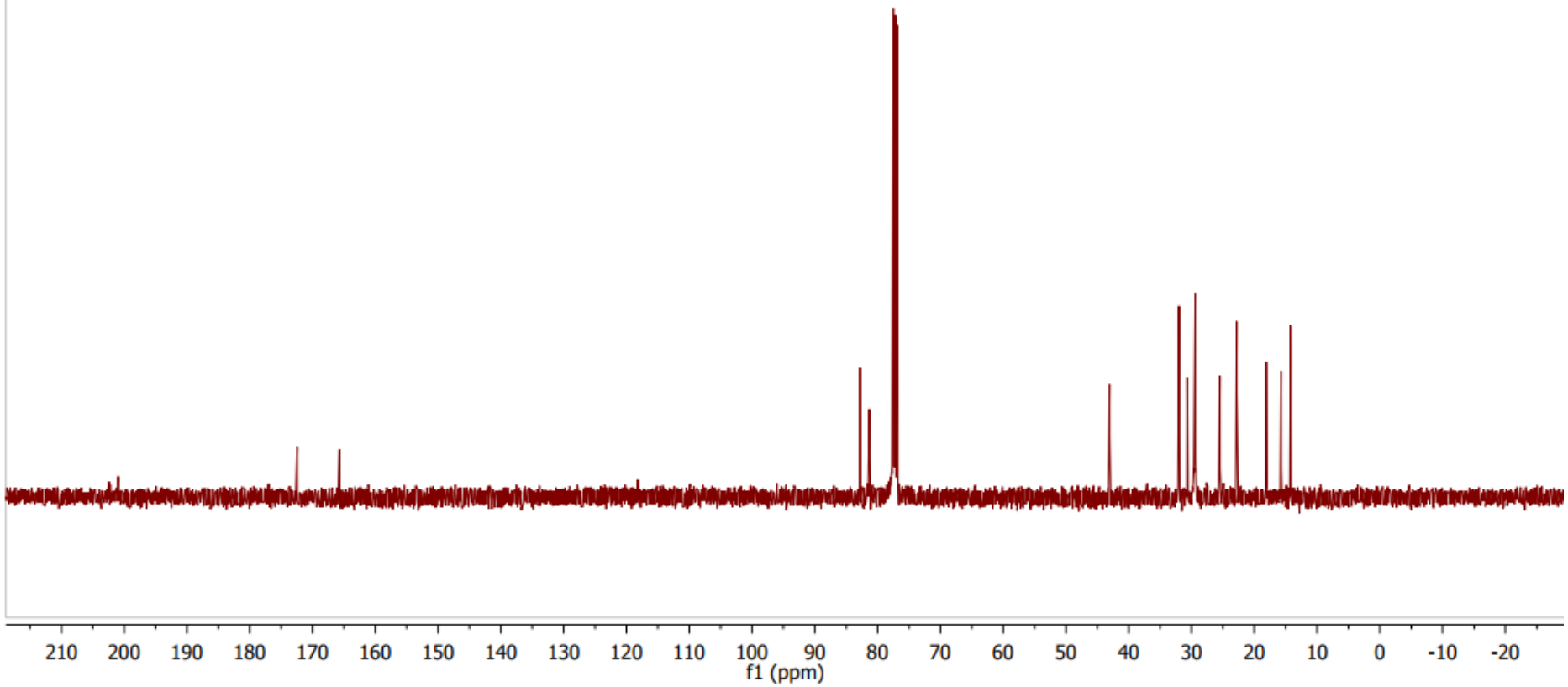


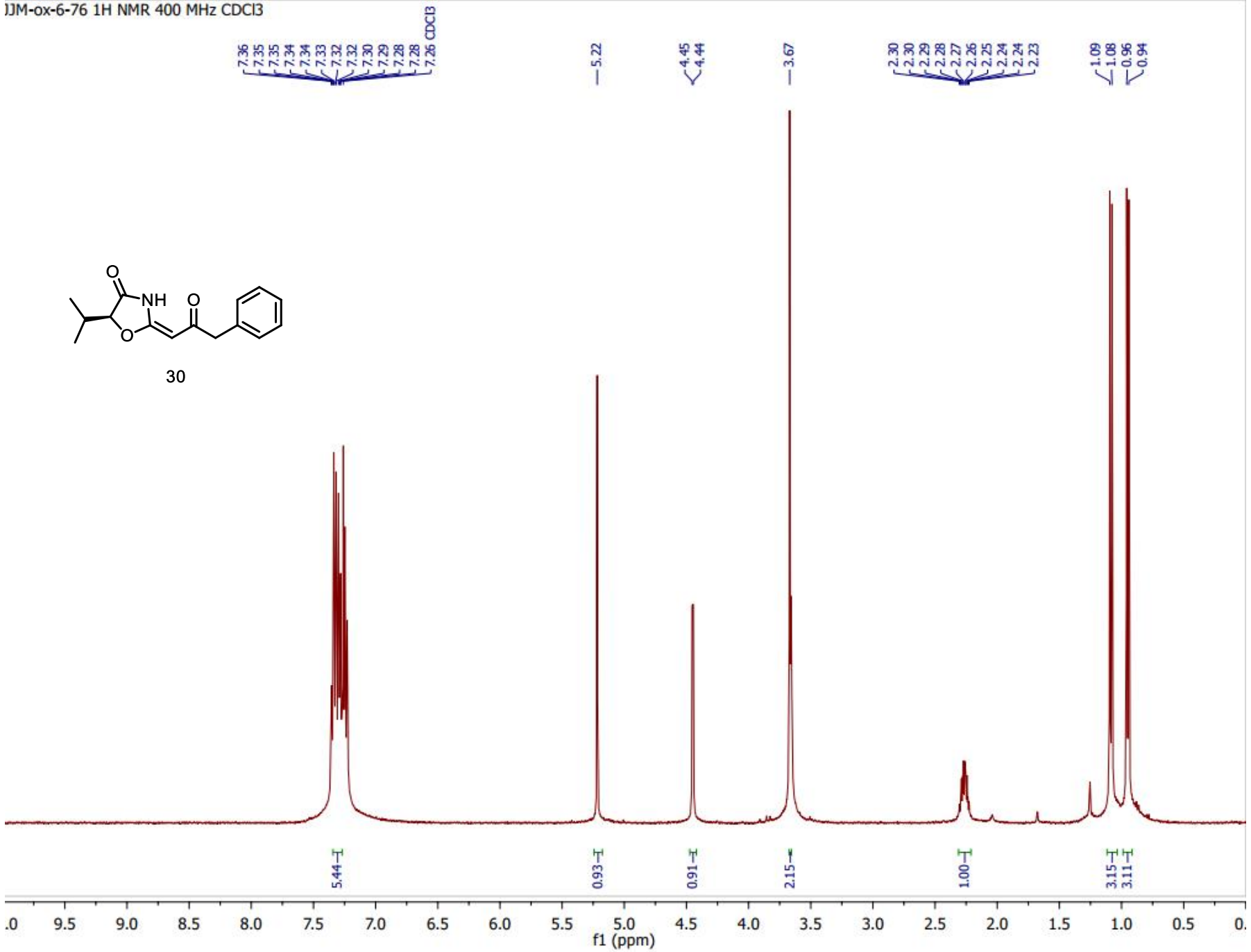
JJM-ox-6-33-13C-100MHz-CDCl3

200.95
172.45
165.69
82.79
81.31
77.16 CDCl3
43.06
31.99
30.68
29.56
29.55
29.47
29.40
25.49
22.80
18.10
15.73
14.24

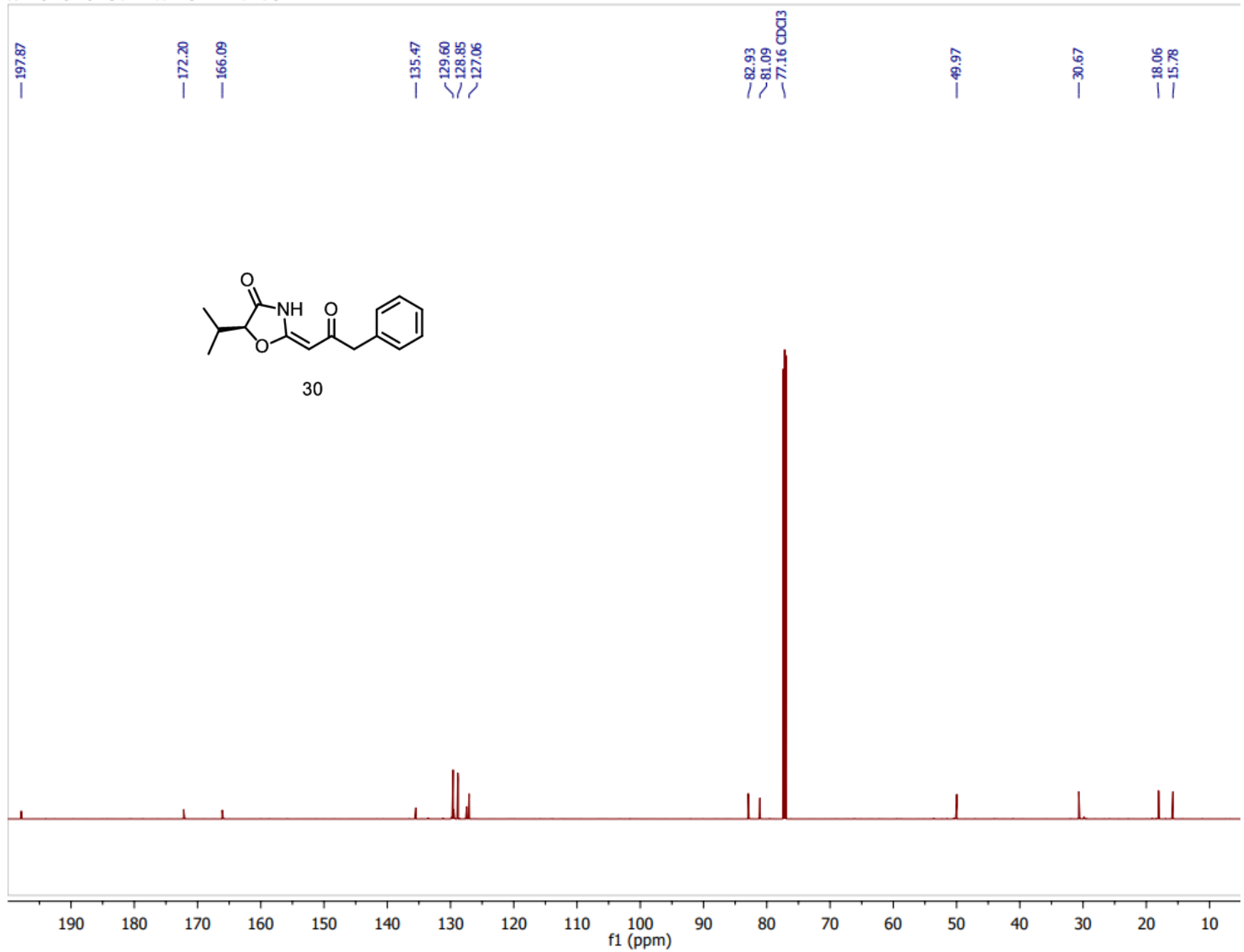


29

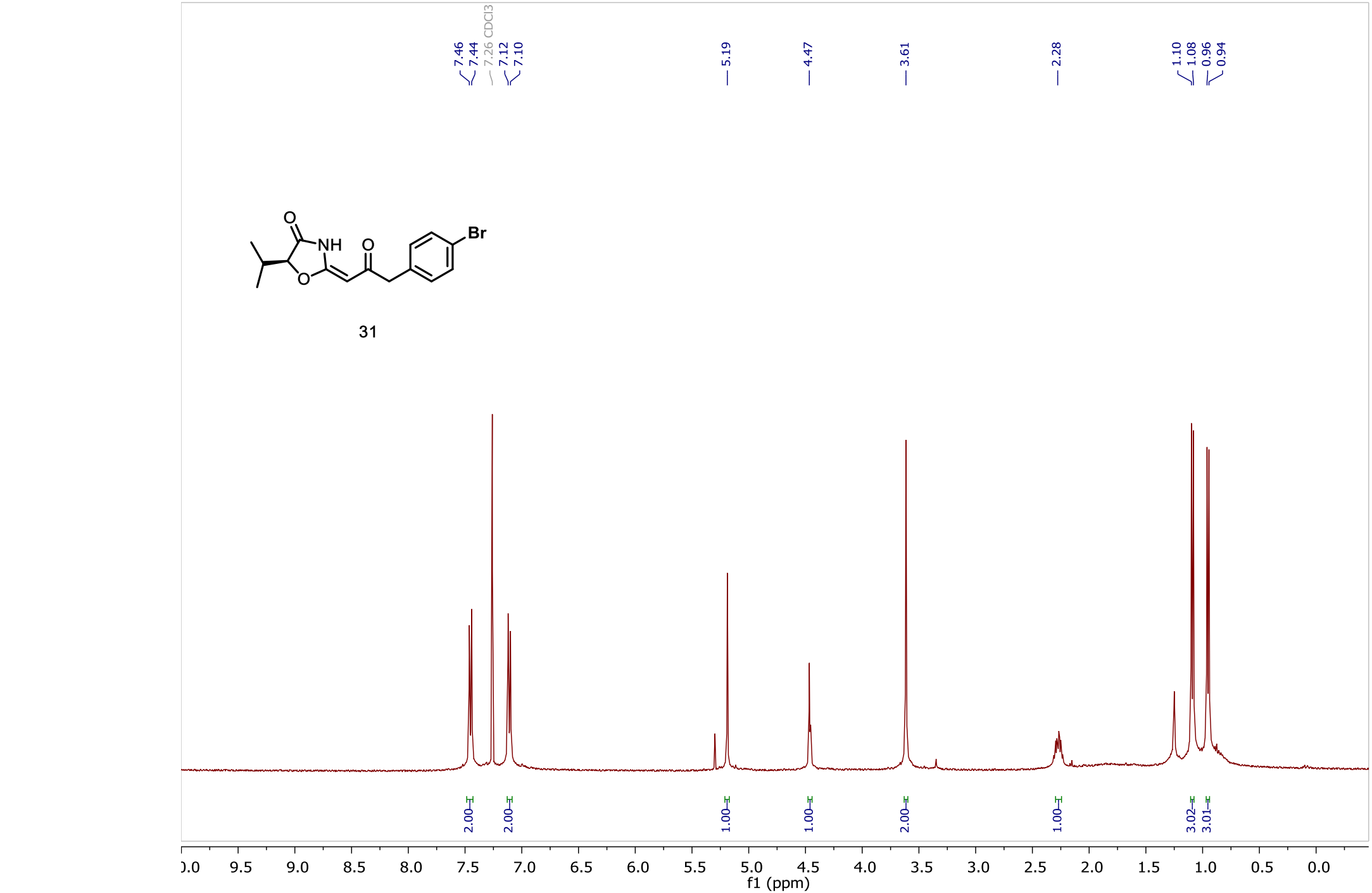



JJM-ox-6-76 1H NMR 400 MHz CDCl3
7.36
7.35
7.35
7.34
7.34
7.33
7.32
7.32
7.30
7.29
7.28
7.28
7.26 CDCl3
5.22
4.45
4.44
3.67
2.30
2.30
2.29
2.28
2.27
2.26
2.25
2.24
2.24
2.23
1.09
1.08
0.96
0.94
O
NH
O
O
30
5.44
0.93
0.91
2.15
1.00
3.15
3.11
f1 (ppm)

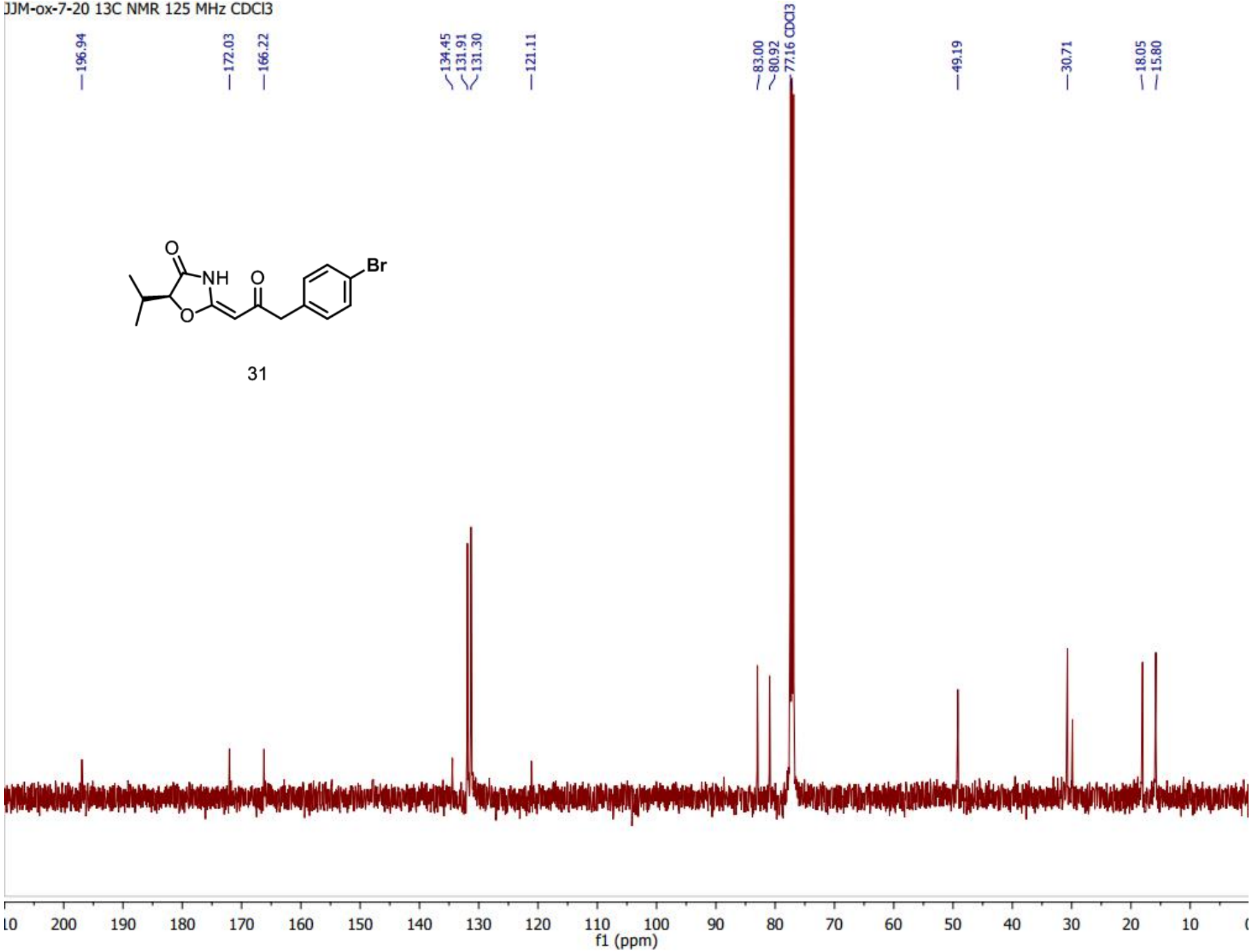
JJM-ox-6-76 13C NMR 125 MHz CDCl3



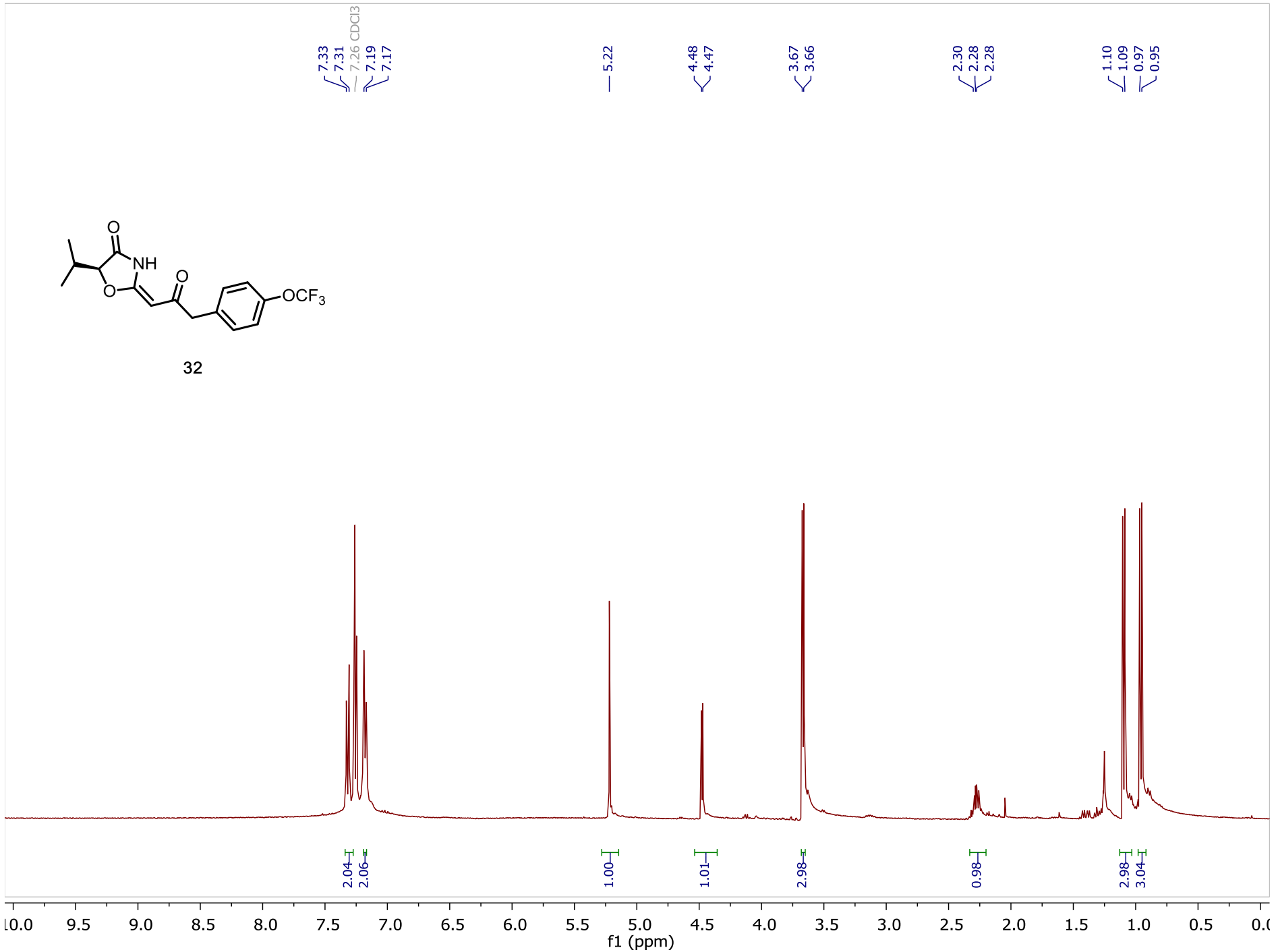
JJM-ox-7-20-1H-400MHz-CDCl3



31

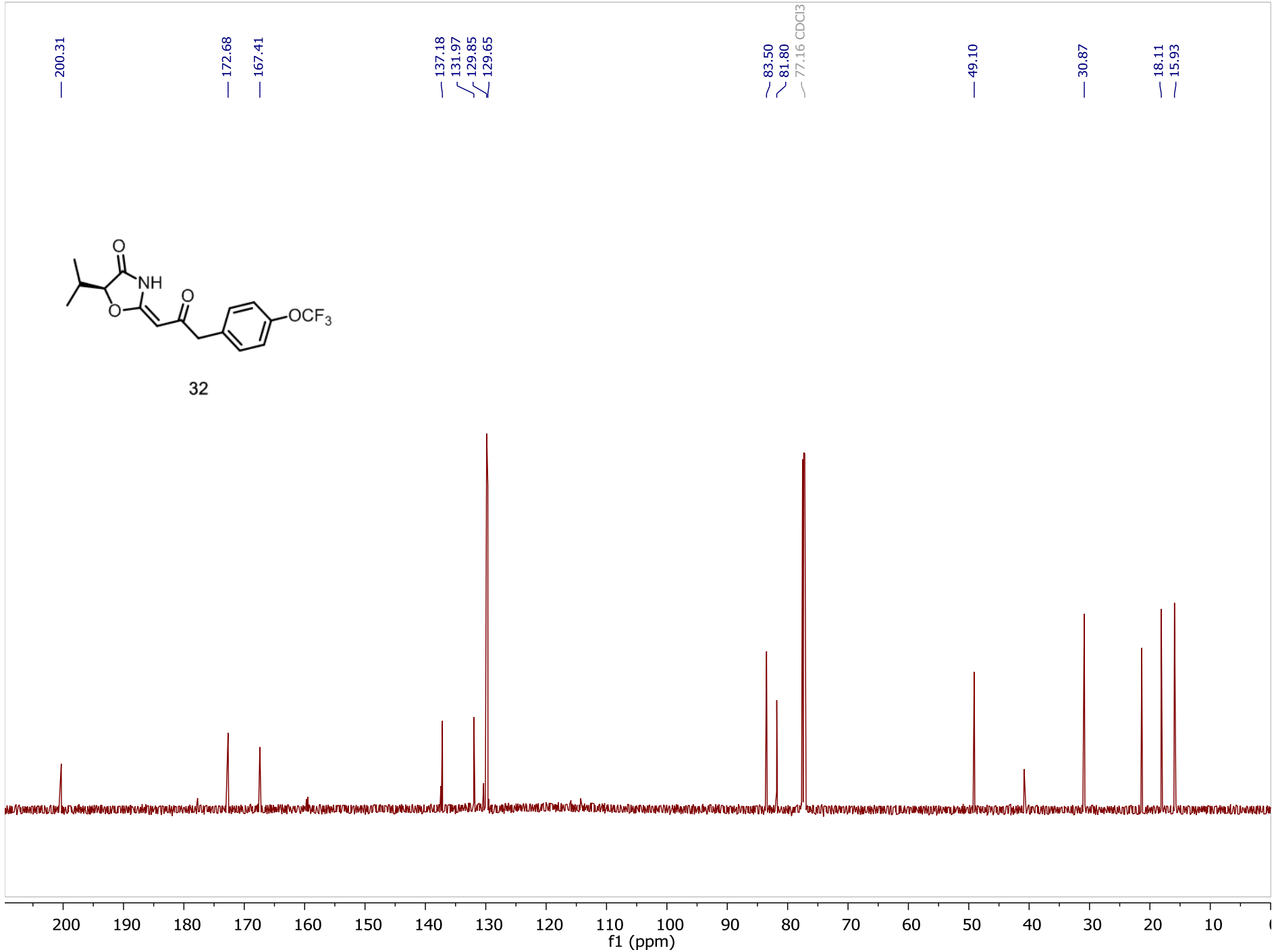

JJM-ox-7-20 13C NMR 125 MHz CDCl3
196.94
172.03
166.22
134.45
131.91
131.30
121.11
83.00
80.92
77.16 CDCl3
49.19
30.71
18.05
15.80
O
NH
O
O
Br
31
f1 (ppm)

KR0615AA_1H_CDCl3_400MHz_11_21_17

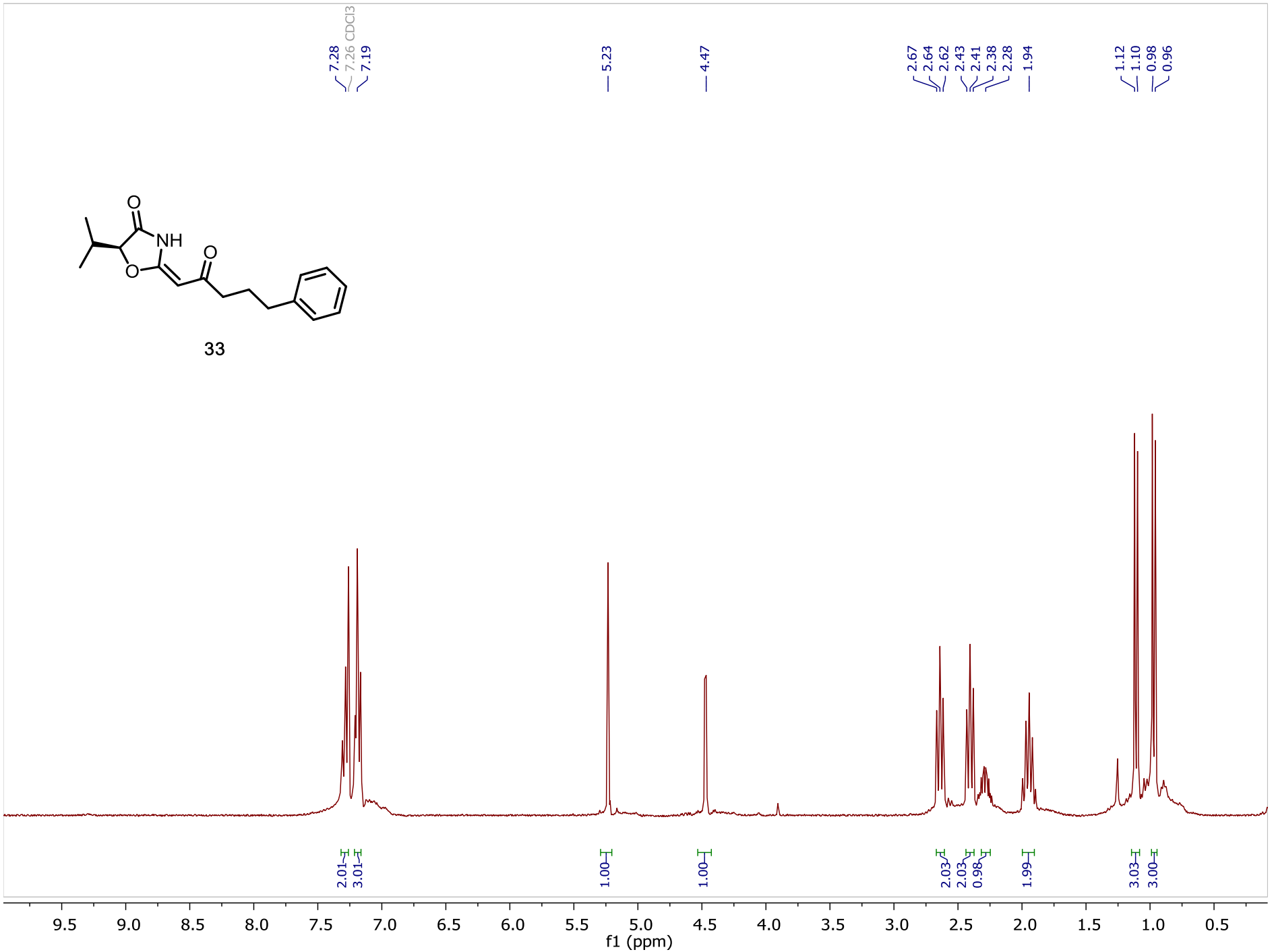


32

KR0620A.2.fid

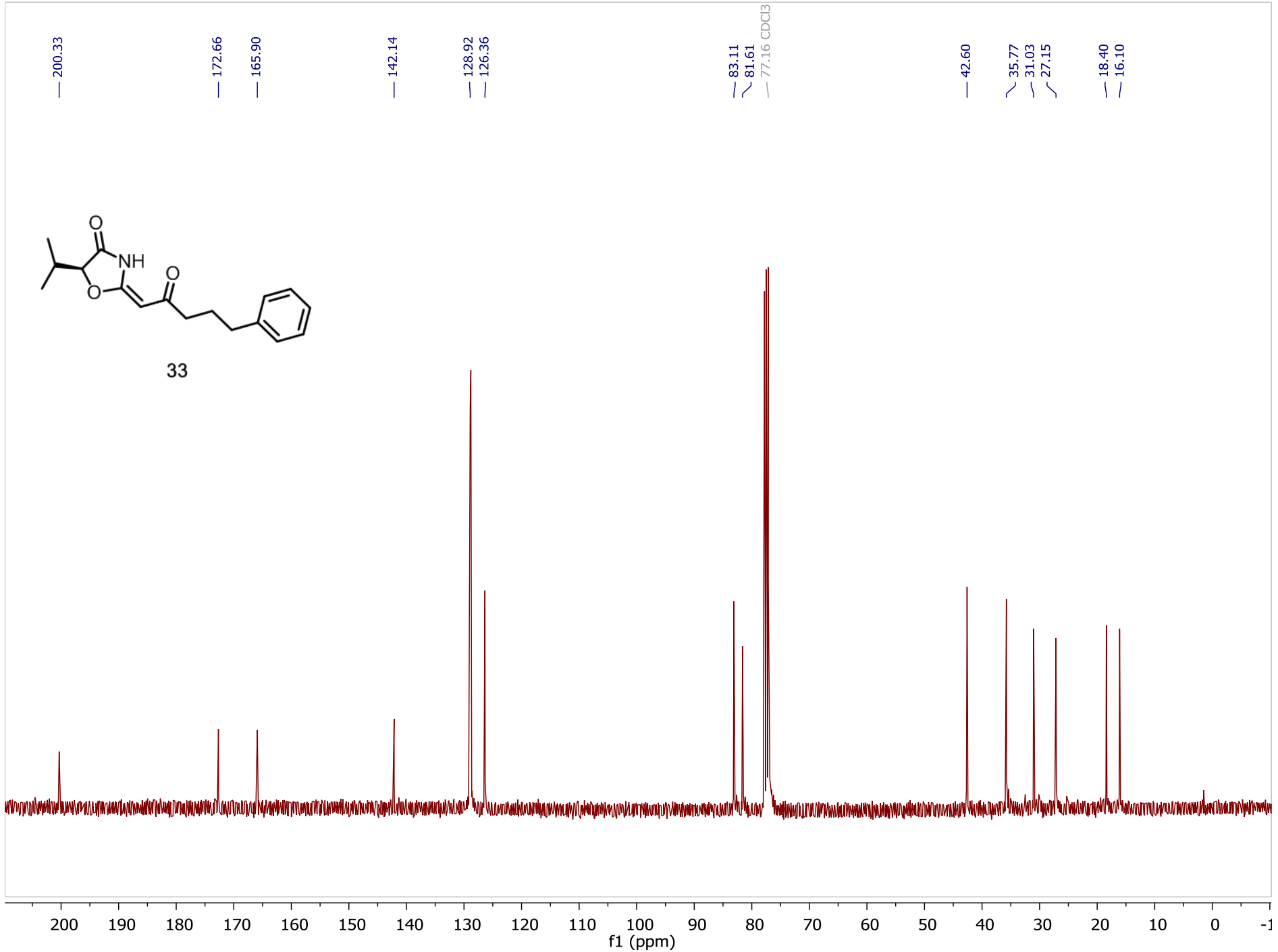


KR0537A_1H_CDCl3_300MHz_2_12_18

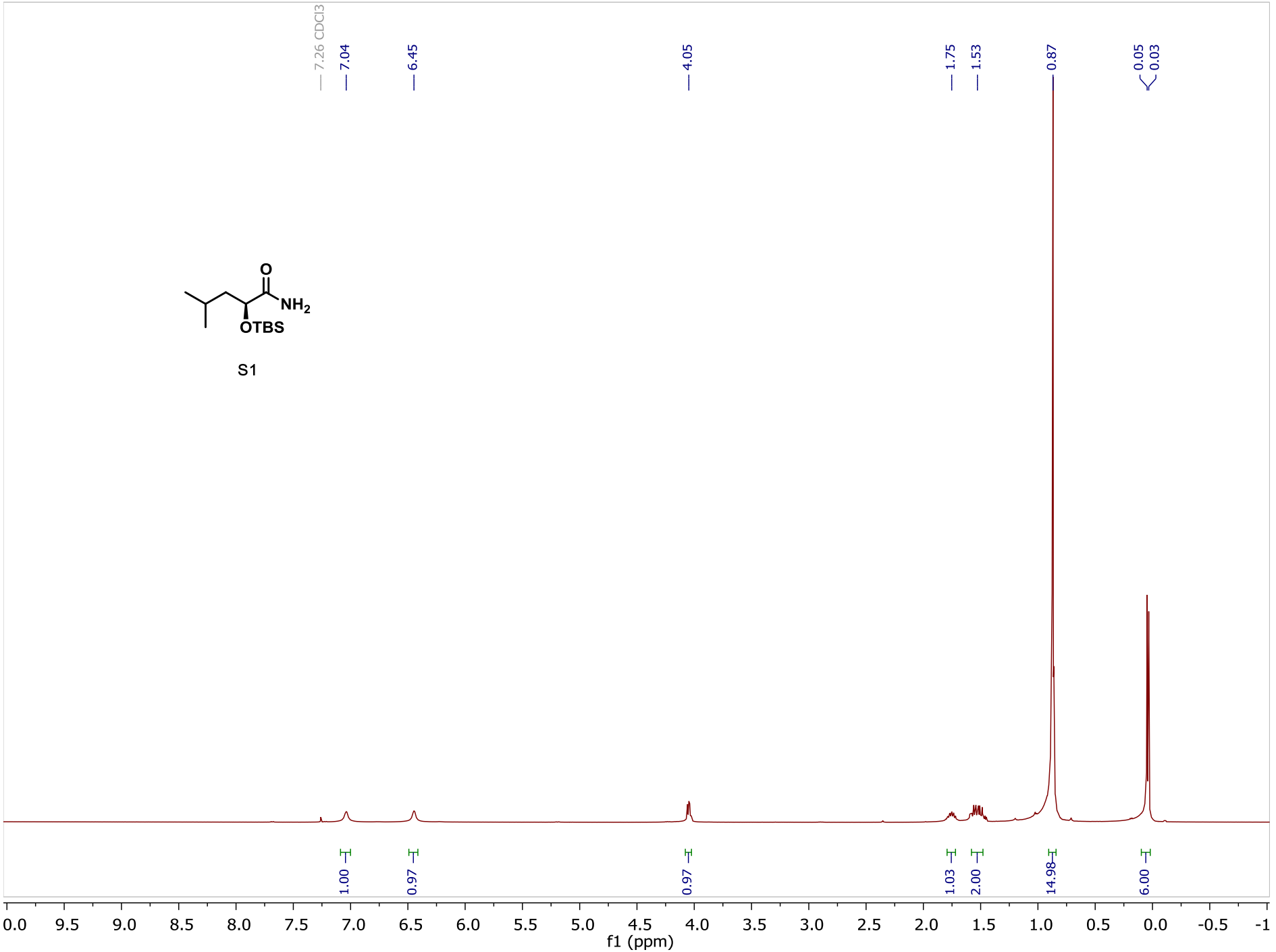


33

KR0537AA_13C_CDCl3_400MHz_10_14_17

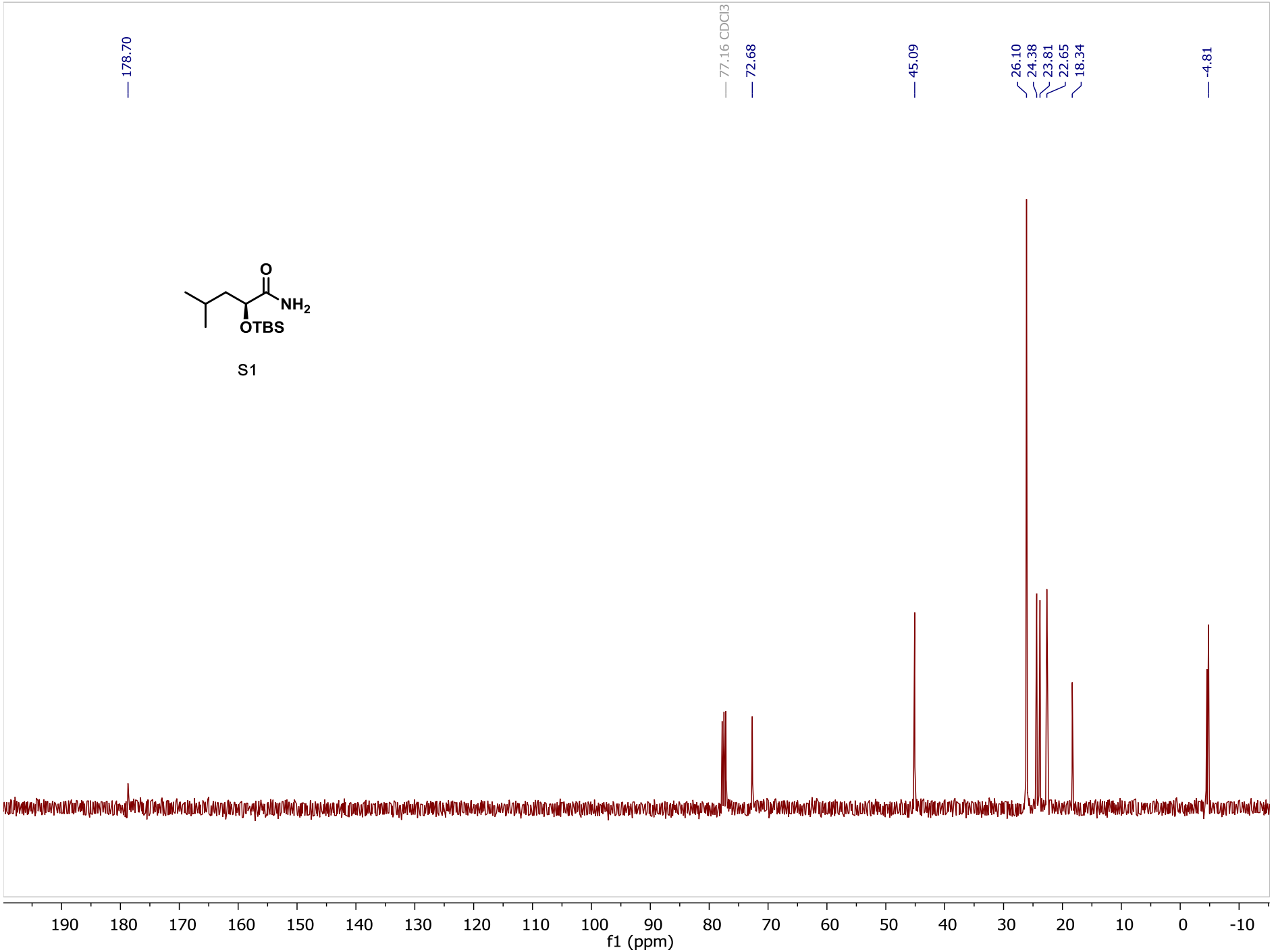


TEZ-02-19-1H-NMR-400MHz-CDCl3-03-02-2016

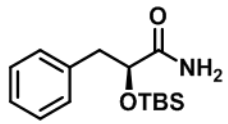


S1

TEZ-02-19-13C-NMR-100MHz-CDCl3-3-2-2016

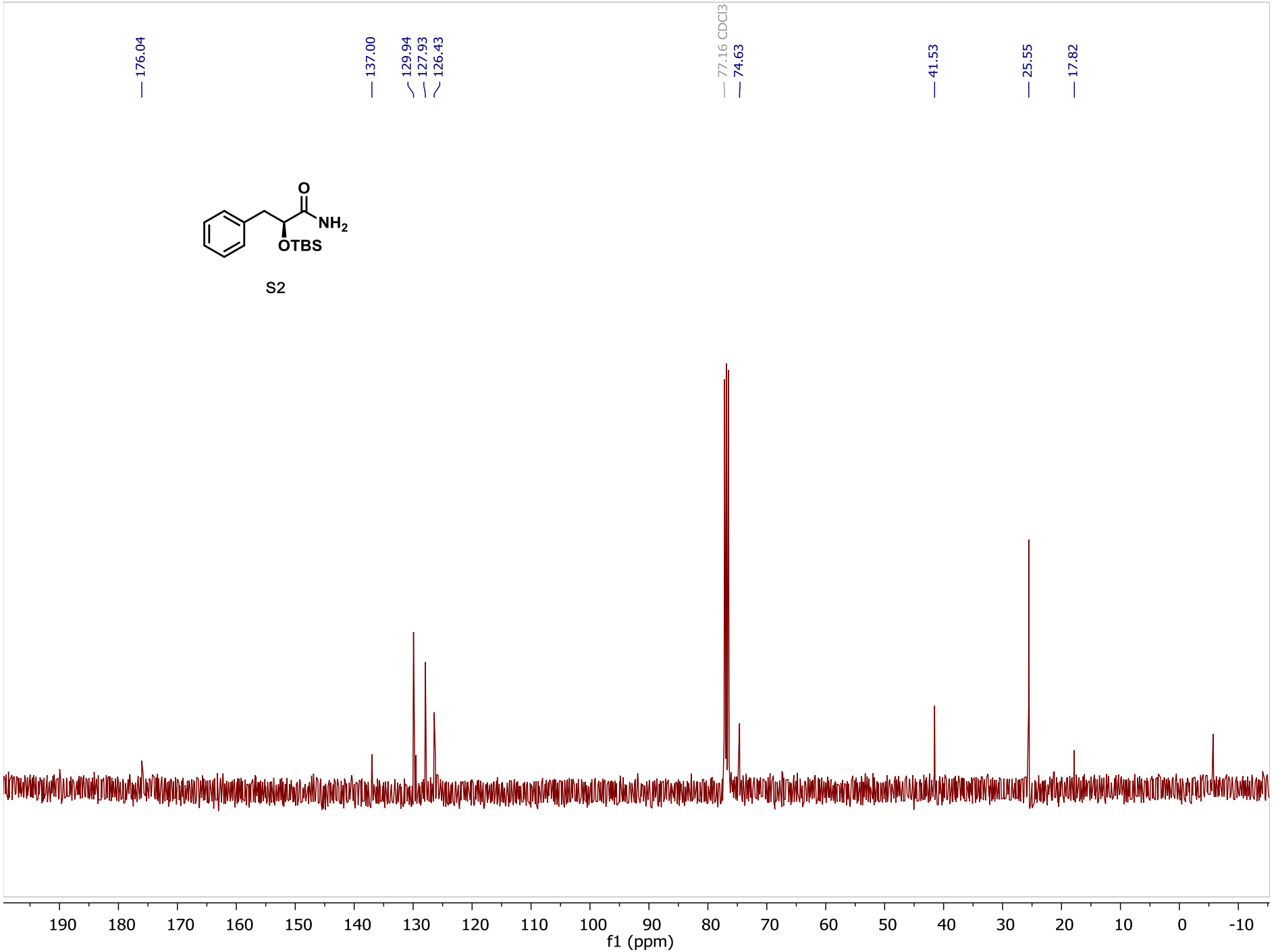


TEZ-01-69-1H

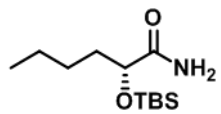


S2

TEZ-01-69-13C

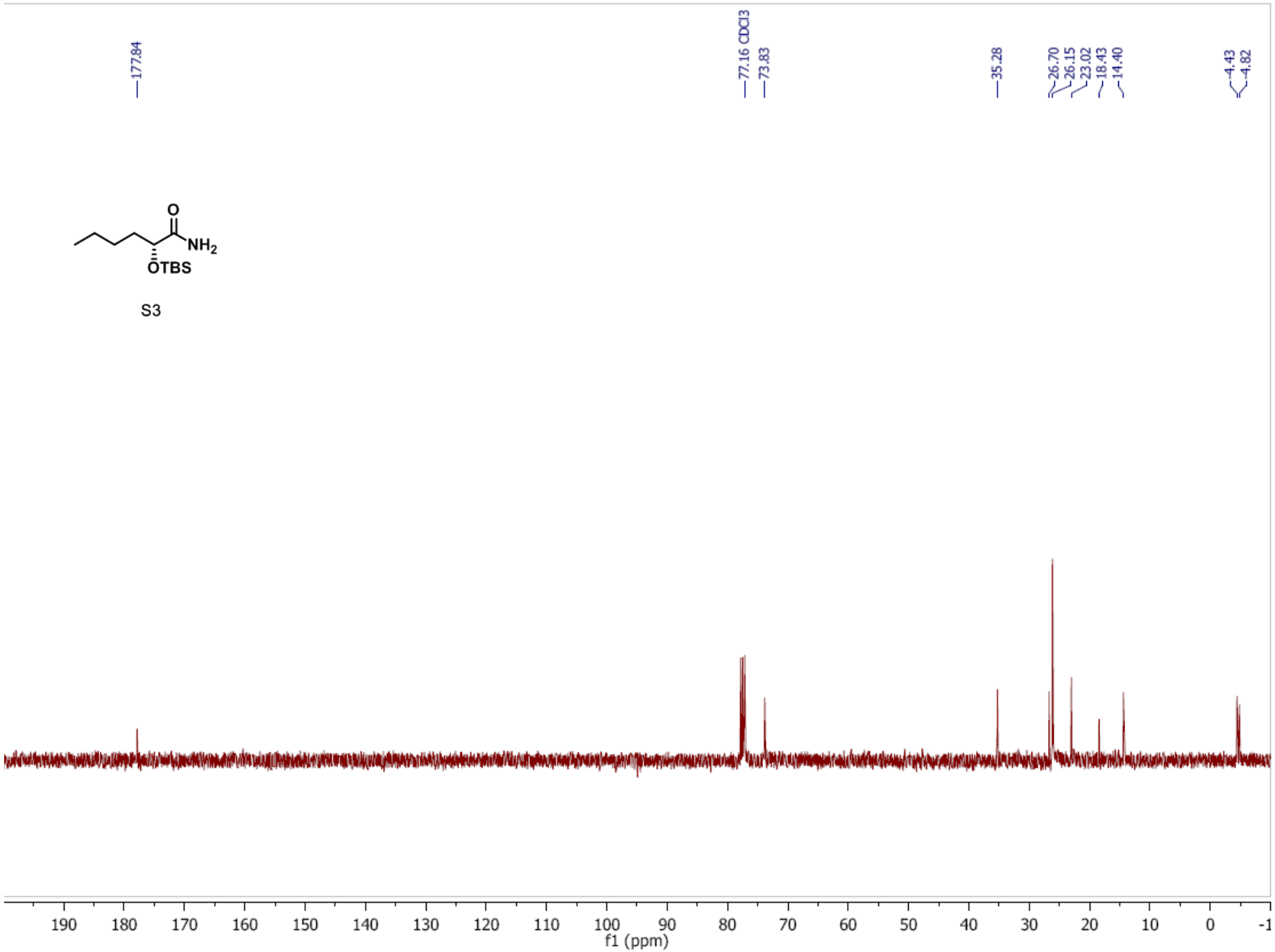

176.04
137.00
129.94
127.93
126.43
77.16 CDCl3
74.63
41.53
25.55
17.82
O
NH2
OTBS
S2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

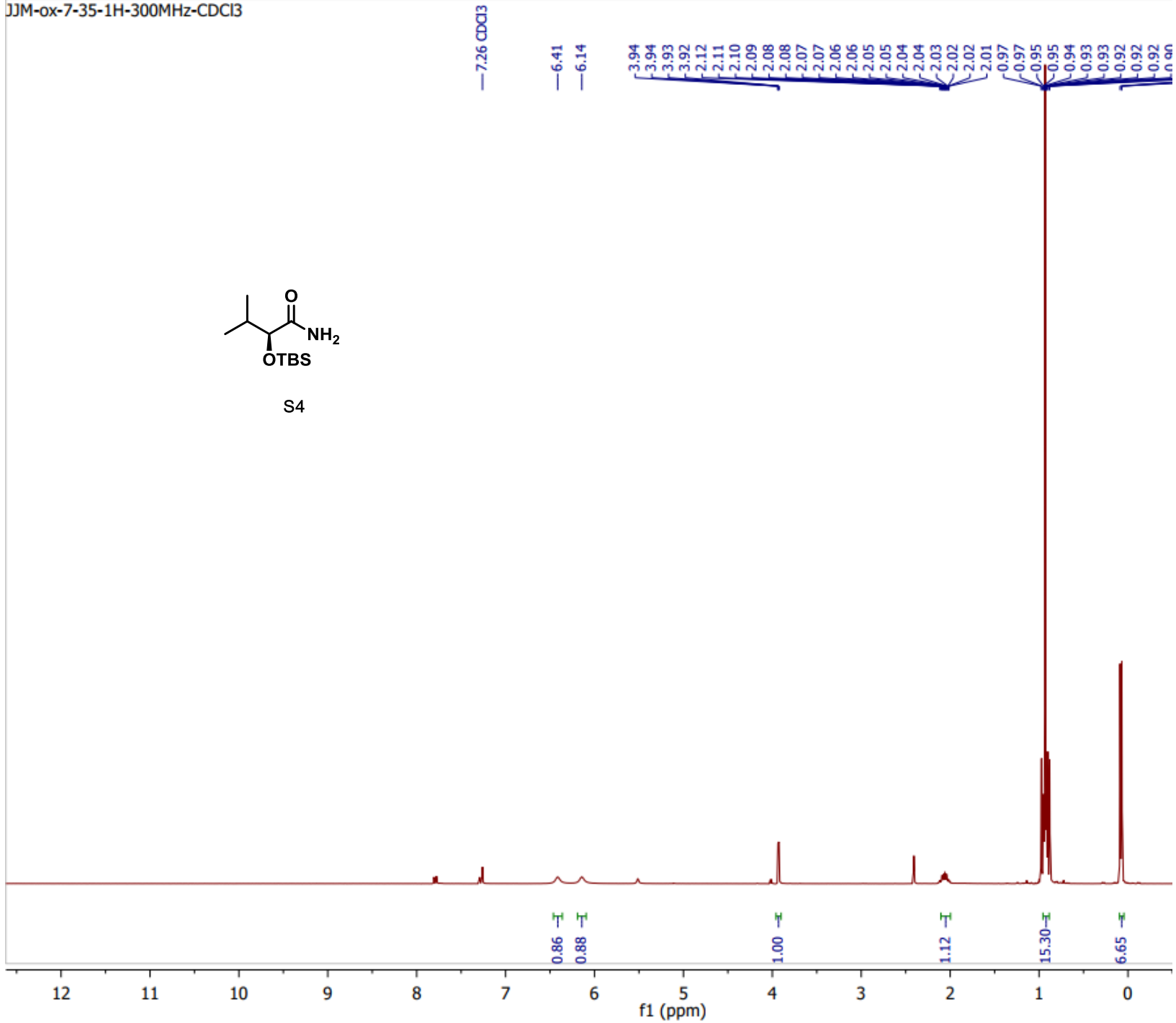
JJM-ox-3-43 1H NMR 400 MHz CDCl3



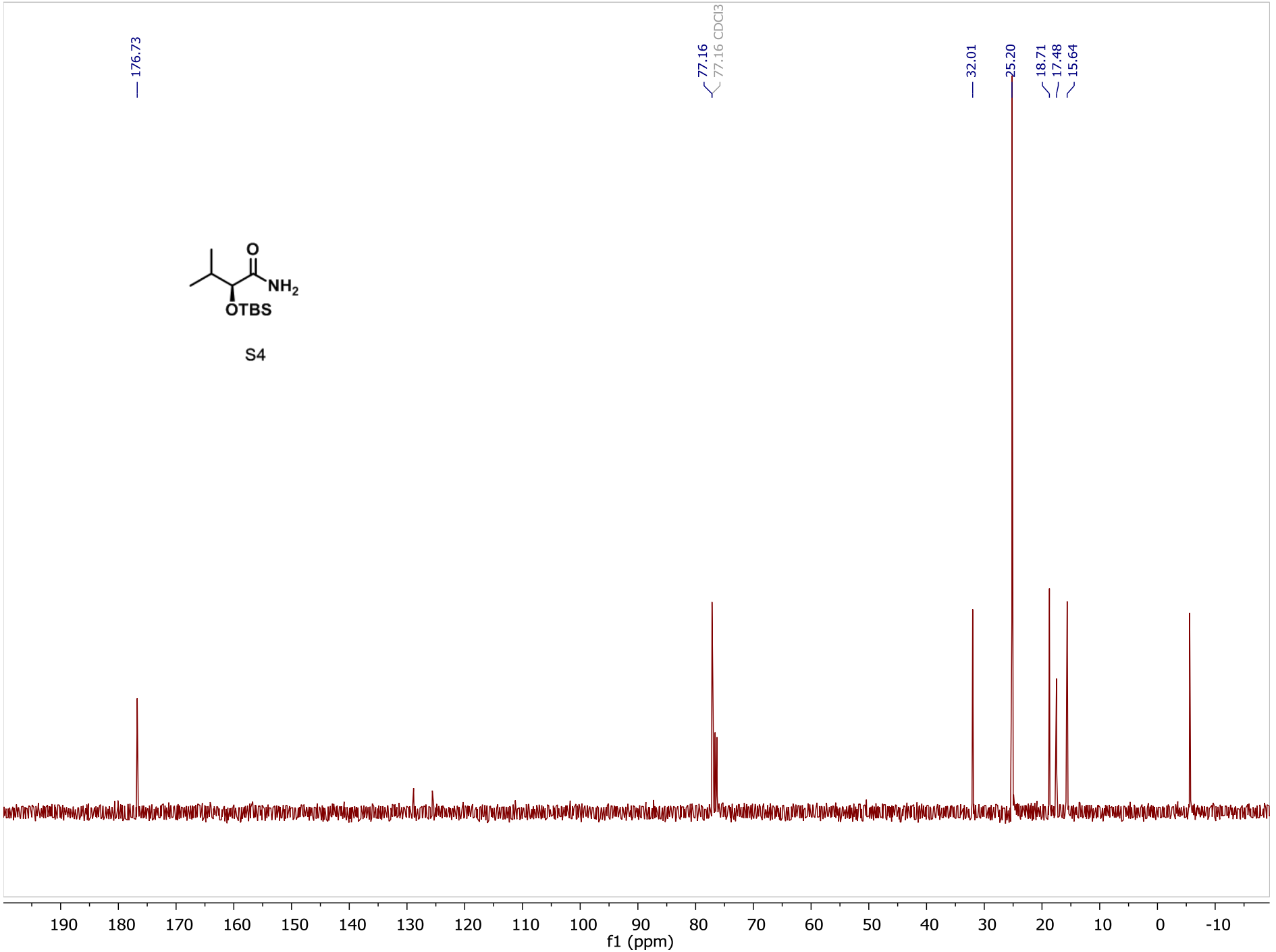
S3

JJM-ox-3-43 13C NMR 100 MHz CDCl3

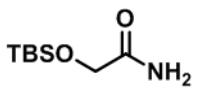



JJM-ox-7-35-1H-300MHz-CDCl3
7.26 CDCl3
6.41
6.14
3.94
3.94
3.93
3.92
2.12
2.11
2.10
2.09
2.08
2.08
2.07
2.07
2.06
2.06
2.05
2.05
2.04
2.04
2.03
2.02
2.02
2.01
0.97
0.97
0.95
0.95
0.94
0.93
0.93
0.92
0.92
0.92
O
NH2
OTBS
S4
0.86
0.88
1.00
1.12
15.30
6.65
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

TEZ-02-20-13C-NMR-100MHz-CDCl3-03-02-2016

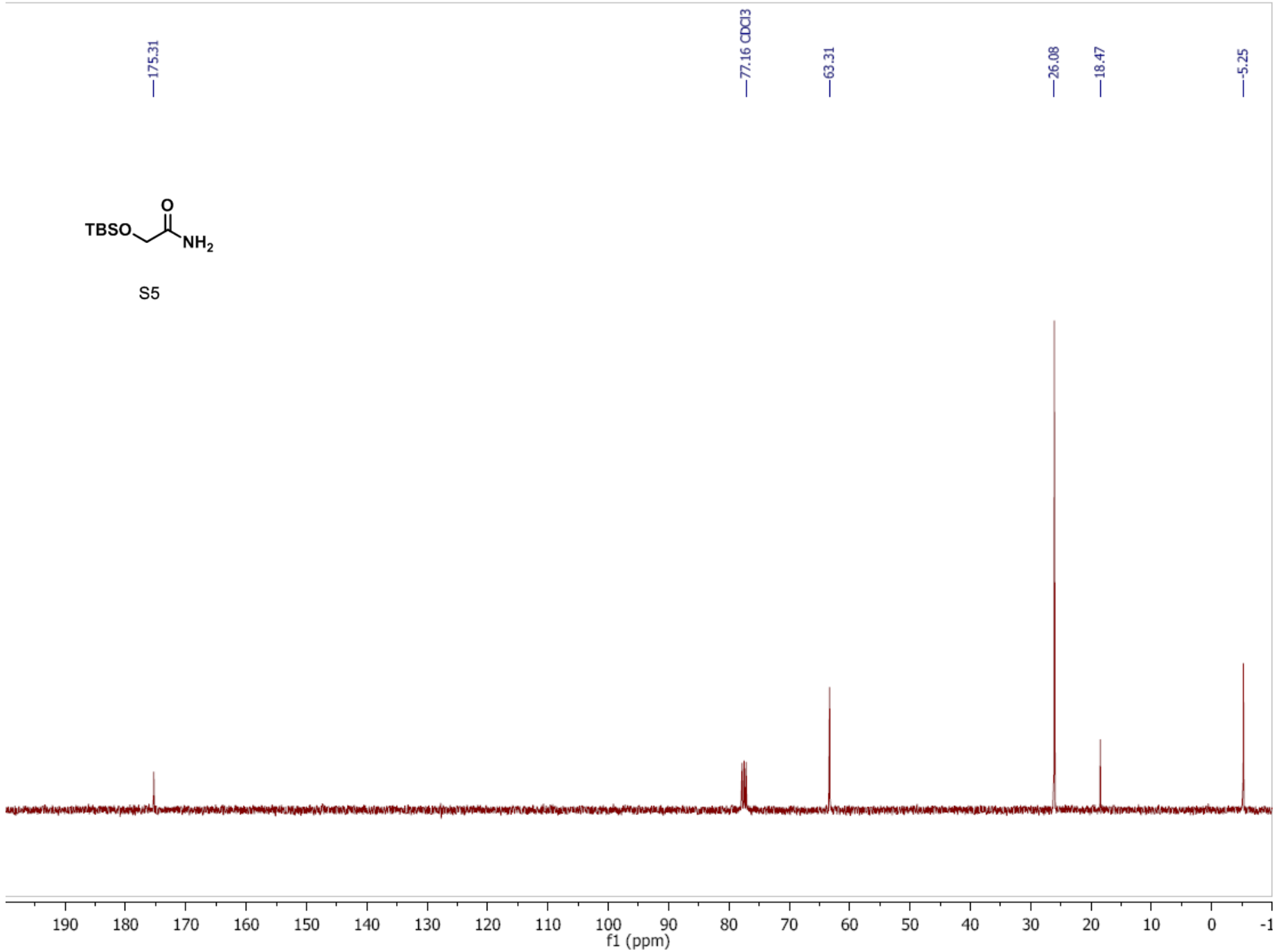


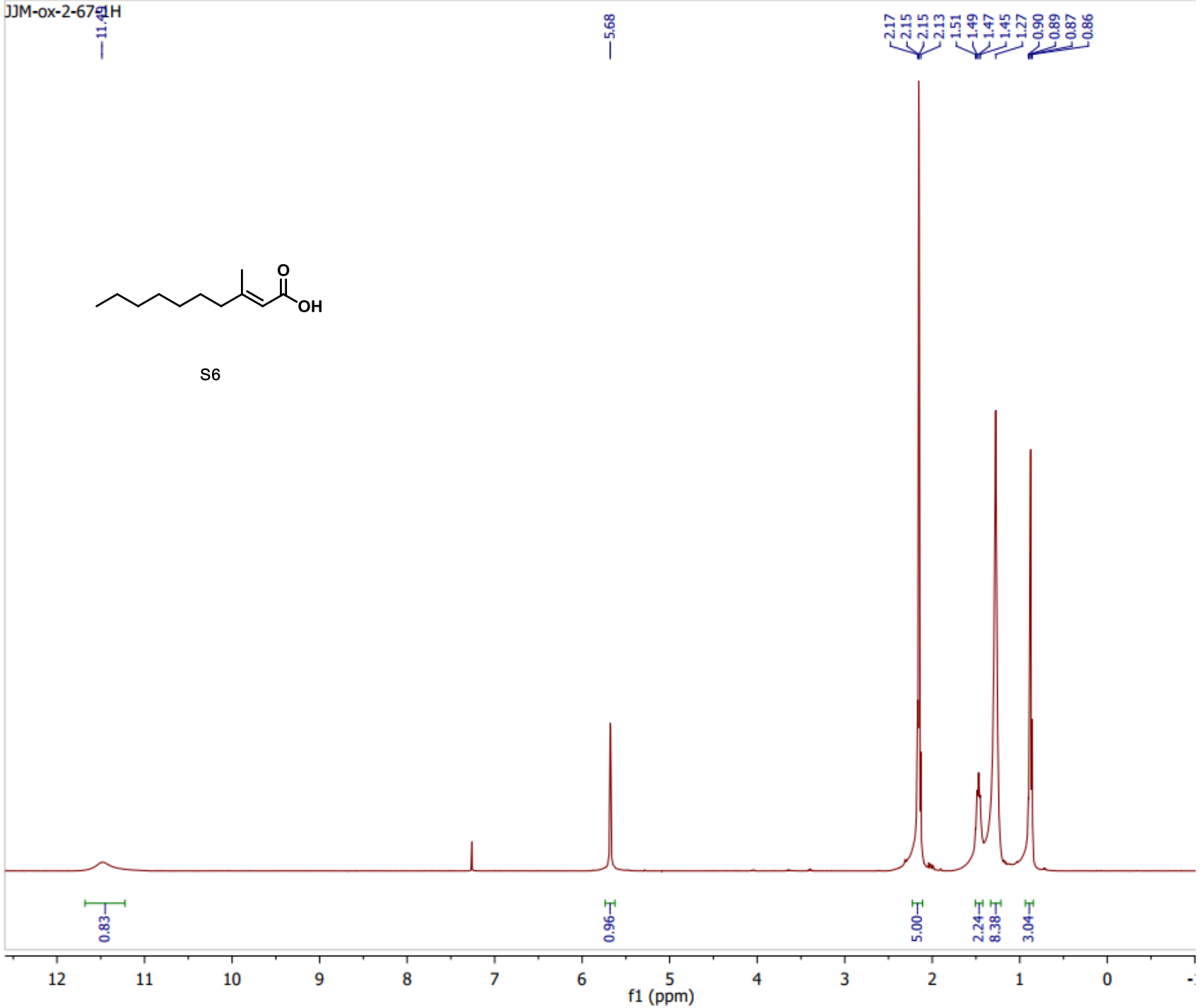
JJM-ox-01-73 1H 400MHz CDCl3



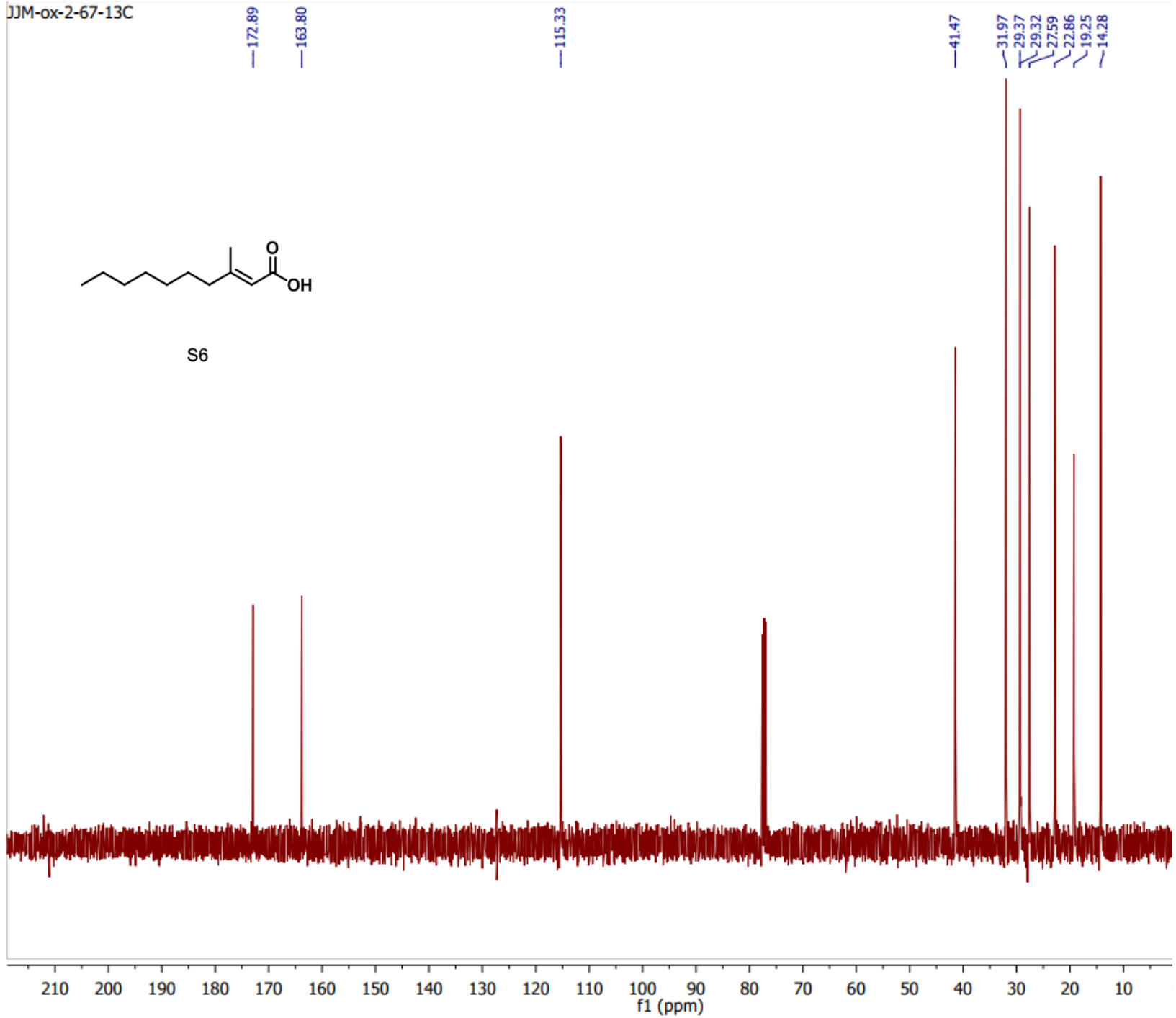
S5

JJM-ox-01-73 13C NMR 100 MHz CDCl3

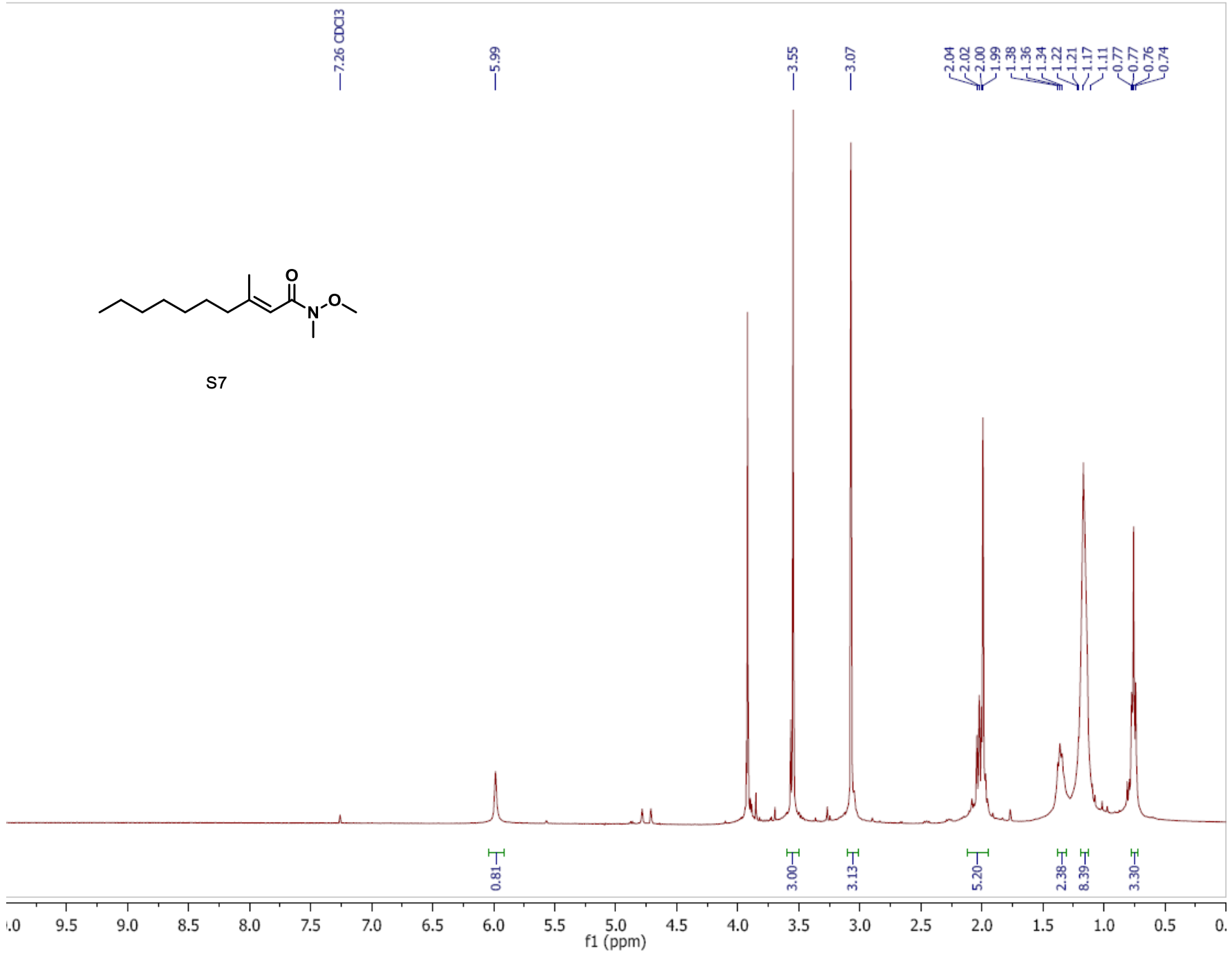



JJM-ox-2-67-1H
11.41
5.68
2.17
2.15
2.15
2.13
1.51
1.49
1.47
1.45
1.27
0.90
0.89
0.87
0.86
O
OH
0.83
0.96
5.00
2.24
8.38
3.04
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

S6

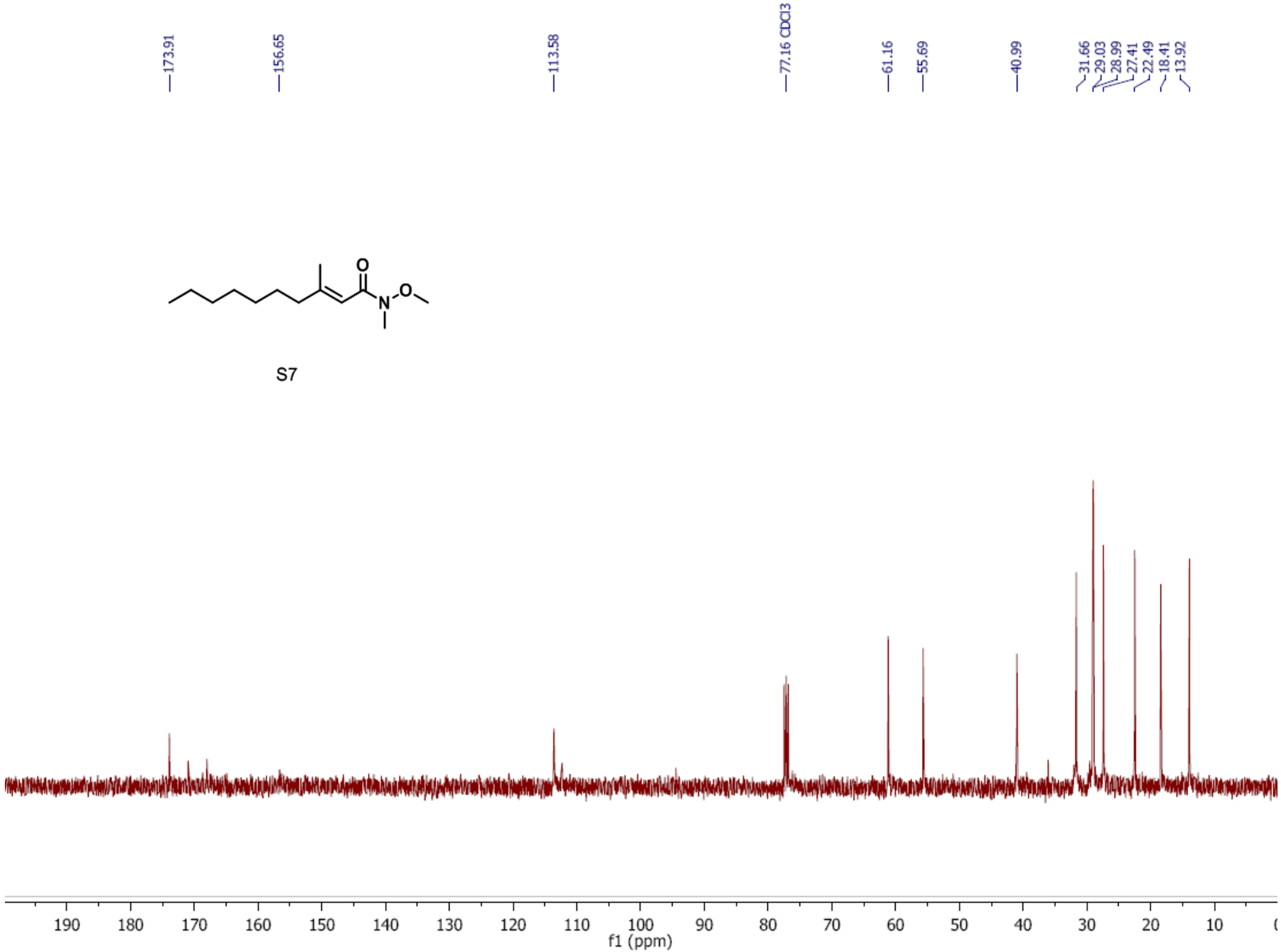

JJM-ox-2-67-13C
172.89
163.80
115.33
41.47
31.97
29.37
29.32
27.59
22.86
19.25
14.28
O
OH
S6
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

JJM-ox-5-52 1H NMR 400MHz CDCl3



S7

JJM-ox-5-52 13C NMR 100MHz CDCl3



S7