Supporting Information for

A Mitochondrial-Targeted Nitroxide is a Potent Inhibitor of Ferroptosis

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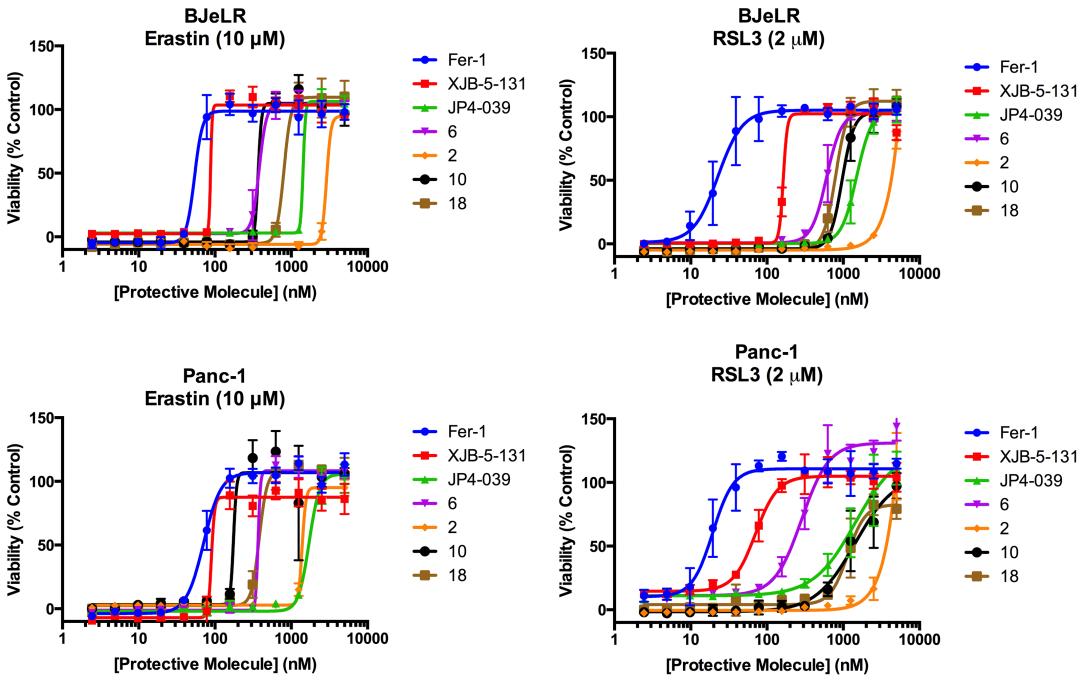
Supplementary Figures.



	EC_{50} (nM)			
	BJELR		PANC-1	
	Erastin	RSL3	Erastin	RSL3
Fer-1	76	21	70	27
XJB-5-131	86	165	89	70
JP4-039	1388	1422	1712	1440
2	2894	4064	1391	2727
6	376	590	367	285
10	368	964	174	1438
18	801	796	380	1146

Figure S1. Nitroxides are protective against ferroptosis in multiple cell lines.

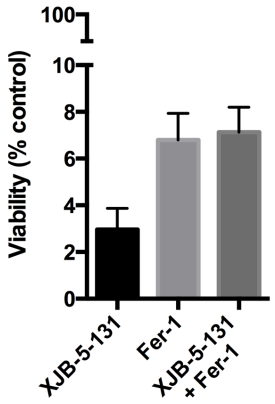
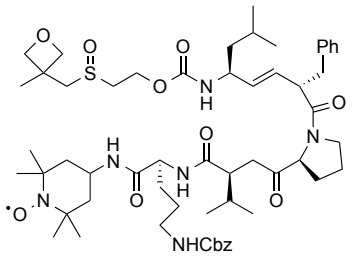
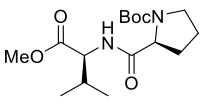


Figure S2. Co-treatment of HT-1080 cells with XJB-5-131 (100 nM) and ferrostatin-1 (20 nM) in the presence of erastin (10 μM).

General Experimental Part. All moisture- and air-sensitive reactions were performed in oven dried glassware under a positive pressure of argon. All reagents and solvents were used as received unless otherwise specified. THF and Et_2O were distilled over sodium/benzophenone ketyl; CH_2Cl_2 was distilled over CaH_2, MeCN and DMF were dried over molecular sieves. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F_{254} plates, 250 μm layer thickness) and visualization was accomplished with a 254/280 nm UV light and/or by staining with $KMnO_4$ solution (1.5 g $KMnO_4$ and 1.5 g K_2CO_3 in 100 mL of a 0.1% NaOH solution), a ninhydrin solution (2 g ninhydrin in 100 mL EtOH), a PMA solution (5 g phosphomolybdic acid in 100 mL EtOH), or a *p*-anisaldehyde solution (2.5 mL *p*-anisaldehyde, 2 mL AcOH and 3.5 mL conc. H_2SO_4 in 100 mL EtOH). Flash chromatography was performed on silica gel (40-63 μm). Melting points were determined on a Mel-Temp II capillary melting point apparatus fitted with a Fluke 51 II digital thermometer. Infrared spectra were recorded on an ATR spectrometer. NMR spectra were recorded on 300, 400 or 500 MHz instruments. Chemical shifts were reported in parts per million (ppm) and referenced to residual solvent. ^{1}H NMR spectra are tabulated as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s), number of protons. ^{13}C NMR spectra were obtained using a proton-decoupled pulse sequence and are tabulated by observed peak. LC-MS analyses were performed on a Shimadzu UFLC instrument equipped with an Applied Biosystem MDS SCIEX API 2000 mass spectrometer (ESI), under the following conditions: column: Varian Polaris C18-A (100 x 4.6 mm, 5μm) equilibrated at 40 °C; buffer A: 0.1% aqueous AcOH, buffer B: 0.1% AcOH in MeCN; 30 min gradient: 5% buffer B in buffer A for 1 min, then 5 to 95% buffer B in buffer A over 13 min, then 95% buffer B in buffer A for 4 min, then 95-5% buffer B in buffer A over 7 min, then 5% buffer B in buffer A for 5 min; flow rate: 0.2 mL/min; detection: TIC and/or UV λ = 254/280 nm. Mass spectra were obtained on a Waters Autospec double focusing mass spectrometer.

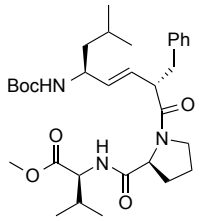


Benzyl ((4*S*)-4-((2*S*)-2-((2*S*)-1-((2*S*,5*S*,*E*)-2-Benzyl-7-methyl-5-(((2-(((3-methyloxetan-3-yl)methyl)sulfinyl)ethoxy)carbonyl)amino)oct-3-enoyl)pyrrolidine-2-carboxamido)-3-methylbutanamido)-5-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)amino)-5-oxopentyl)carbamate (2). To a solution of XJB-5-131[1] (50 mg, 0.050 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (40 µL, 0.54 mmol). The mixture was stirred for 4 h, and the solvent was evaporated. After drying on high vacuum for ~1 h, CH_2Cl_2 (1 mL), DIPEA (110 µL, 0.63 mmol) and activated carbonate **1**[2] (21 mg, 0.057 mmol) were added, and the resulting solution was stirred for 16 h. The solvent was evaporated, and the residue was purified by chromatography on SiO_2 (0-7.5% MeOH/CH_2Cl_2, eluted ~6%) to provide nitroxide **2** (28.6 mg, 52%) as a pink-orange solid: IR (ATR, neat) 3387, 3299, 2982, 2881, 2709, 1689, 1674, 1535, 1454, 1405, 1201, 1177, 1129, 831, 800, 719 cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{56}H_{85}O_{11}N_7S$ 1063.6022 (M+H), found 1063.6002.



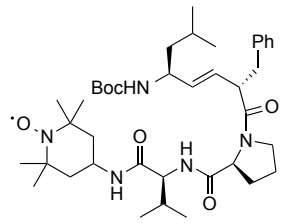
***tert*-Butyl (*S*)-2-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (3).** To *N*-Boc-L-proline (5.00 g, 23.2 mmol) and L-valine methyl ester hydrochloride (4.00 g, 23.6 mmol) in CH_2Cl_2 (115 mL) at 0 °C was added slowly *N,N*-diisopropylethylamine (9.80 mL, 58.1 mmol). The reaction mixture was stirred for 5 min, then T_3P (50 wt% in EtOAc, 15.20 mL, 25.53 mmol) was added slowly. The resulting mixture was allowed to warm to rt and stirred for 12 h. The solution was poured into saturated aqueous $NaHCO_3$, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layerss were dried ($MgSO_4$) and evaporated to give Boc-Pro-Val-OMe (7.62 g, 100%) as a white semi-solid. Spectral data are in accordance with literature values:[3] ^1H NMR (400 MHz, $CDCl_3$) δ 7.49 (br s, 1 H), 4.36-4.20 (m, 1 H), 3.72 (s, 3 H), 3.53-3.22 (m, 2 H),

2.37 (br s, 1 H), 2.17-2.14 (m, 2 H), 2.02-1.84 (m, 3 H), 1.46 (s, 9 H), 0.89 (dd, *J* = 8.5, 5.0 Hz, 6 H)



Methyl ((2*S*,5*S*,*E*)-2-benzyl-5-((*tert*-butoxycarbonyl)amino)-7-methyloct-3-enoyl)-L-prolyl-L-valinate (5). A solution of Boc-Pro-Val-OMe (**3**) (250 mg, 0.761 mmol) in a 4.0 N solution of HCl in 1,4-dioxane (3.5 mL, 14.0 mmol) was stirred at 0 °C for 30 min and at rt for an additional 1 h. Dioxane was removed in vacuo and the white foamy residue was dissolved in CH_2Cl_2, washed with 5% Na_2CO_3 solution, water and brine. The organic layer was dried ($MgSO_4$), and the solvent was evaporated to afford the crude amine as a pale yellow foamy gum, which was used for the next step without further purification.

To acid **4**[4] (200 mg, 0.553 mmol) at 0 °C was added a solution of the above crude amine in dry CH_2Cl_2 (16 mL), followed by HOBt (90 mg, 0.67 mmol), EDCI (125 mg, 0.652 mmol), and DMAP (7 mg, 0.06 mmol). The reaction mixture was stirred at rt for 42 h, then quenched with saturated aqueous NH_4Cl. The aqueous phase was extracted with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$) and purified by chromatography on SiO_2 (1% to 7.5% MeOH/CH_2Cl_2) to provide **5** (271 mg, 86% for 2 steps) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.2 Hz, 1 H), 7.27–7.14 (m, 5 H), 5.68 (dd, *J* = 15.2, 8.8 Hz, 1 H), 5.50 (d, *J* = 11.6 Hz, 1 H), 4.63 (br t, *J* = 7.2 Hz, 1 H), 4.57 (d, *J* = 8.0 Hz, 1 H), 4.43 (dd, *J* = 7.8, 5.0 Hz, 1 H), 4.16 (br s, 1 H), 3.75 (s, 3 H), 3.46 (t, *J* = 8.2 Hz, 1 H), 3.39 (q, *J* = 7.9 Hz, 1 H), 3.14 (dd, *J* = 13.2, 8.8 Hz, 1 H), 2.99 (q, *J* = 8.8 Hz, 1 H), 2.78 (dd, *J* = 13.4, 6.2 Hz, 1 H), 2.27 (br dd, *J* = 12.0, 6.8 Hz, 1 H), 2.16-2.04 (m, 1 H), 1.98-1.86 (m, 1 H), 1.78-1.71 (m, 1 H), 1.65-1.54 (m, 2 H), 1.44 (s, 9 H), 1.32-1.23 (m, 2 H), 0.90-0.86 (m, 12 H); IR (ATR, neat) 3316, 2959, 2876, 1742, 1707, 1685, 1623, 1525, 1436, 1366, 1247, 1204, 1174 cm^{-1}; HRMS (ESI$^+$) *m/z* calcd for $C_{32}H_{50}O_6N_3$ 572.3694 (M+H), found 572.3687.

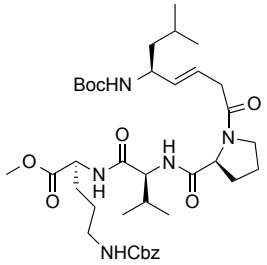


***tert*-Butyl ((4*S*,7*S*,*E*)-7-Benzyl-8-((*S*)-2-(((*S*)-1-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidin-1-yl)-2-methyl-8-oxooct-5-en-4-yl)carbamate (6).** A solution of **5** (250 mg, 0.437 mmol) in MeOH (12 mL) was treated at 0 °C with 1 N NaOH (4.3 mL, 4.3 mmol). The reaction mixture was stirred at rt for 3 h, then adjusted with 1 N HCl to pH ~4. The solution was extracted with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$) and evaporated to afford the corresponding acid as a white solid, which was immediately carried on for the next step.

To the above acid were added 4-amino-TEMPO (97.5 mg, 0.569 mmol), EDCI (105 mg, 0.548 mmol), HOBt (75 mg, 0.56 mmol), DMAP (55 mg, 0.45 mmol) and CH_2Cl_2 (8 mL). The resulting mixture was stirred at rt for 40 h, then poured into saturated aqueous NH_4Cl. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and the residue was purified by chromatography on SiO_2 to provide **6** (257 mg, 83% for 2 steps) as an orange foamy solid: IR (ATR, neat) 3303, 2963, 2932, 2871, 1704, 1681, 1651, 1505, 1364, 1244, 1167, 972, 749, 700 cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{40}H_{65}O_6N_5$ 711.4929 (M+H), found 711.4926.

A small sample of nitroxide **6** was reduced to the corresponding hydroxylamine for NMR analysis: To a solution of **6** (10 mg, 0.014 mmol) in MeOH (0.5 mL) was added L-(+)-ascorbic acid (5.3 mg, 0.030 mmol) at rt. The mixture was stirred for 15 min, and then evaporated. The residue was extracted with CH_2Cl_2 (1 mL) and water (1mL). The aqueous layer was washed with CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$), filtered and concentrated to provide the crude hydroxylamine. The material was dissolved in $CDCl_3$ and layered with 10% ascorbic acid in D_2O: ^1H NMR (500 MHz, $CDCl_3$, signals broadened due to rotamers) δ 7.24 (t, *J* = 7.5 Hz, 2 H), 7.17 (t, *J* = 8.0 Hz, 1 H), 7.13 (d, *J* = 8.0 Hz, 2 H), 5.59 (dd, *J* = 15.0, 8.5 Hz, 1 H), 5.46 (dd, *J* = 15.0, 5.0 Hz, 1 H), 4.48 (d, *J* = 6.0 Hz, 1 H), 4.20 (t, *J* = 5.5 Hz, 2 H), 4.07 (br s, 1 H), 3.50 (*t*, *J* = 7.5 Hz, 1 H), 3.38 (q, *J* = 7.8 Hz, 1 H), 3.11 (dd, *J* = 13.5, 8.5 Hz, 1 H), 3.06 (br s, 1 H), 2.77 (dd, *J* = 13.5, 6.5 Hz, 1 H), 2.23 (br s, 1 H), 2.13 (br s, 1 H), 1.93-1.83 (m, 3 H), 1.76 (br s, 2 H), 1.64 (br s, 2 H), 1.52 (br s, 1 H), 1.41 (s, 9 H), 1.31–1.20 (m, 16 H), 0.85 (dd, *J* = 8.5, 5.0 Hz, 12 H); ^{13}C NMR (125 MHz,

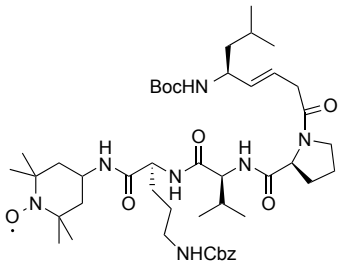
$CDCl_3$) δ 173.7, 171.6, 171.5, 170.3, 155.3, 139.1, 135.3, 129.2, 128.4, 127.4, 126.5, 79.3, 60.6, 58.3 53.5, 50.3, 47.3, 44.2, 40.4, 39.9, 30.4, 18.5, 18.0, 24.8, 24.7, 22.8, 22.4, 20.1, 19.5, 17.5.



Methyl (*S*)-5-(((benzyloxy)carbonyl)amino)-2-((*S*)-1-((*S,E*)-5-((*tert*-butoxycarbonyl)amino)-7-methyloct-3-enoyl)pyrrolidine-2-carboxamido)-3-methylbutanamido)pentanoate (9). A solution of Boc-Pro-Val-Orn(Z)-OMe (**7**)[5] (2.35 g, 4.08 mmol) in a 4.0 N HCl in 1,4-dioxane (20 mL, 80.00 mmol) was stirred at 0 °C for 30 min and at rt for an additional 1 h. Dioxane was removed in vacuo and the white foamy residue was dissolved in CH_2Cl_2, washed with 5% Na_2CO_3 solution, water and brine. The organic layer was dried ($MgSO_4$), and the solvent was evaporated. The crude amine was used without further purification.

To a solution of this amine in CH_2Cl_2 (15 mL) were added EDCI (700 mg, 3.65 mmol), HOBt (540 mg, 4.00 mmol), DMAP (41 mg, 0.33 mmol), and a solution of acid **8**[6] (900 mg, 3.32 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred at rt for 40 h, then poured into saturated NH_4Cl and water. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography on SiO_2 (0-10% MeOH/CH_2Cl_2) to give **9** (2.42 g, 67%) as a white foamy solid: ^{1}H NMR (500 MHz, $CDCl_3$, signals broadened due to rotamers) δ 7.45 (br s, 1 H), 7.23-7.18 (m, 5 H), 6.78 (br s, 1 H), 6.14 (br s, 1 H), 5.57–5.32 (m, 2 H), 4.97 (dd, *J* = 16.0, 12.5 Hz, 2 H), 4.55-4.49 (m, 1 H), 4.46-4.40 (m, 1 H), 4.32-4.23 (m, 1 H), 4.16-4.08 (m, 1 H), 3.61 (s, 3 H), 3.52 (br s, 1 H), 3.33 (q, *J* = 8 Hz, 1 H), 3.14–2.87 (m, 4 H), 2.07–1.72 (m, 6 H), 1.61-1.50 (m, 2 H), 1.49-1.38 (m, 2 H), 1.35 (s, 9 H), 1.30-1.23 (m, 1 H), 1.21-1.15 (m, 2 H), 0.81 (dd, *J* = 9.8, 6.8 Hz, 12 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 171.5, 171.3, 170.9, 156.6, 155.4, 136.8, 135.8, 128.2, 127.9, 127.7, 121.2, 78.6, 66.1, 60.2, 58.0, 52.1, 51.6, 49.7, 47.3, 44.5, 40.3, 38.8, 21.2, 29.1, 28.9, 28.8, 28.3, 25.8, 24.7, 24.6, 22.8, 22.6, 22.1, 19.0, 17.9; IR (ATR, neat) 3295, 2956, 2876, 1737, 1687,

1653, 1521, 1439, 1366, 1245, 1212, 1165 cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{38}H_{59}O_9N_5Na$ 752.4205 (M+H), found 752.4185.

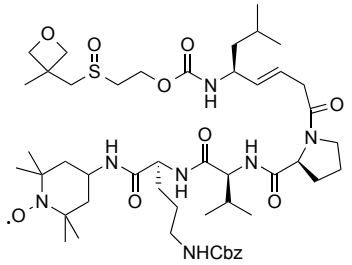


Benzyl ((*S*)-4-((*S*)-2-((*S*)-1-((*S*,*E*)-5-((*tert*-Butoxycarbonyl)amino)-7-methyloct-3-enoyl)pyrrolidine-2-carboxamido)-3-methylbutanamido)-5-((1-oxyl-2,2,6,6-tetramethylpiperidin-4-yl)amino)-5-oxopentyl)carbamate (10). A solution of **9** (1.50 g, 2.06 mmol) in MeOH (60 mL) was treated at 0 °C with 1 M NaOH (20.0 mL, 20.0 mmol). The reaction mixture was stirred at rt for 3 h, then treated with 1 M HCl (20.0 mL, 20.0 mmol). The solution was extracted with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$) and evaporated to afford the acid as a white solid, which was immediately carried on.

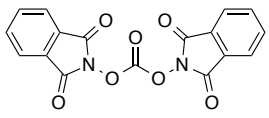
To a solution of the acid in CH_2Cl_2 (20 mL) were added *N*,*N*-diisopropylethylamine (520 µL, 3.08 mmol), 4-amino-TEMPO (460 mg, 2.69 mmol), EDCI (470 mg, 2.45 mmol), and HOBt (330 mg, 2.44 mmol). The reaction mixture was stirred at rt for 20 h, then quenched with saturated aqueous NH_4Cl. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and evaporated, and the residue was purified by chromatography on SiO_2 (1-10% MeOH/CH_2Cl_2) to provide **10** (1.15 g, 64%) as a pale orange-pink solid: HRMS (ESI^+) *m/z* calcd for $C_{46}H_{75}O_9N_7$ 869.5621 (M+H), found 869.5709; IR (ATR, neat) 3299, 3064, 2964, 2930, 2869, 1683, 1644, 1519, 1448, 1390, 1364, 1241, 1167 cm^{-1}.

A small sample of nitroxide **10** was reduced to the corresponding hydroxylamine for NMR analysis: To a solution of **10** (10 mg, 0.012 mmol) in MeOH (0.5 mL) was added L-(+)-ascorbic acid (6.1 mg, 0.035 mmol). The mixture was stirred for 15 min, and then evaporated. The residue was extracted with CH_2Cl_2 (1 mL) and water (1mL). The aqueous layer was washed with CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$), filtered and concentrated to provide the crude hydroxylamine. The material was dissolved in $CDCl_3$ and layered with 10% ascorbic acid in D_2O: ^1H NMR (500 MHz, $CDCl_3$, signals broadened due to rotamers) δ 7.32-7.27 (m, 5 H), 5.70-5.54 (m, 1 H), 5.51-5.39 (m, 1 H), 5.04 (dd, *J* = 15.5,

12.5 Hz, 2 H), 4.57–4.06 (m, 5 H), 3.69-3.52 (m, 1 H), 3.44 (br q, J = 7.5 Hz, 1 H), 3.18–2.90 (m, 4 H), 2.25–1.42 (m, 17 H), 1.39 (s, 9 H), 1.36–1.20 (m, 4 H), 1.20–1.07 (m, 12 H), 0.91–0.77 (m, 12 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 171.8, 171.2, 170.7, 156.8, 155.3, 136.7, 136.0, 128.4, 128.0, 121.7, 79.2, 66.5, 60.7, 59.4, 5.9, 50.3, 47.7, 44.7, 44.3, 40.8, 40.2, 38.9, 31.9, 30.9, 30.0, 29.7, 29.0, 28.5, 26.0, 25.0, 24.7, 23.2, 22.8, 22.3, 22.0, 19.9, 19.5, 17.9.

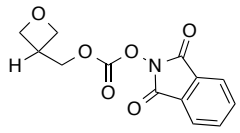


Benzyl ((4*S*)-5-((1-Oxyl-2,2,6,6-tetramethylpiperidin-4-yl)amino)-4-((2*S*)-3-methyl-2-((2*S*)-1-((5*S*,*E*)-7-methyl-5-(((2-(((3-methyloxetan-3-yl) methyl)sulfinyl)ethoxy)carbonyl)amino)oct-3-enoyl)pyrrolidine-2-carboxamido)butanamido)-5-oxopentyl)carbamate (11). To a solution of Boc-protected nitroxide **10** (175 mg, 0.201 mmol) in CH_2Cl_2 (4 mL) was added trifluoroacetic acid (150 µL, 2.02 mmol). The mixture was stirred for 4 h, and the solvent was evaporated. The residue was dried on high vacuum for ~1 h and treated with a solution of activated carbonate **1**[2] (90 mg, 0.25 mmol) in CH_2Cl_2 (2 mL), followed by DIPEA (375 µL, 2.15 mmol) upon which there was an immediate color change from yellow to dark red. The resulting solution was stirred for 16 h, the solvent was evaporated, and the residue was purified by chromatography on SiO_2 (1-7.5% MeOH/CH_2Cl_2, eluted ~6%) to provide carbamate **11** (162 mg, 83%) as a pale orange solid: IR (ATR, neat) 3308, 2989, 2709, 1671, 1534, 1451, 1405, 1199, 1173, 1126, 1071, 1025, 829, 800, 719 cm^{-1}; HRMS (ESI$^+$) *m/z* calcd for $C_{49}H_{79}O_{11}N_7S$ 973.5553 (M+H), found 973.5536.

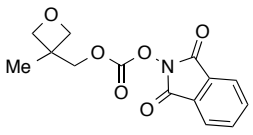


Bis(1,3-dioxoisoindolin-2-yl) carbonate (19). To a suspension of *N*-hydroxyphthalimide (3.90 g, 23.2 mmol) in CH_2Cl_2 (110 mL) was added triethylamine (3.30 mL, 23.3 mmol). The reaction mixture turned dark orange-brown while the hydroxyphthalimide went into solution. The mixture was cooled to 0 °C, and trichloromethyl chloroformate (670 µL, 5.50 mmol) was added slowly, during which time the reaction mixture became colorless and cloudy. The

solution was stirred at 0 °C for 30 min, then at room temperature for 1 h, and then poured into a 0.1 M HCl solution. The the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with 0.1 M HCl and water (2x), dried ($MgSO_4$), filtered and concentrated to provide bis(1,3-dioxoisoindolin-2-yl) carbonate **19** (2.85 g, 73%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35-8.32 (m, 1 H), 7.96-7.92 (m, 2 H), 7.89-7.82 (m, 5 H).

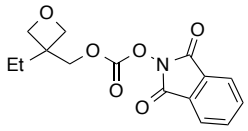


1,3-Dioxoisoindolin-2-yl (oxetan-3-ylmethyl) carbonate (12a). To a suspension of diphthalimidyl carbonate **19** (433 mg, 1.23 mmol) and 3-oxetanemethanol (0.10 mL, 1.2 mmol) in THF (12 mL) was added triethylamine (170 µL, 1.21 mmol). Upon addition of base, the suspension turned yellow, eventually progressing to a clear orange solution after 30 min. The reaction mixture was stirred for 4 h, and the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and washed with saturated aqueous $NaHCO_3$ (5 x 3 mL) until the organic layer became clear. The combined aqueous washings were extracted with EtOAc (10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to provide 1,3-dioxoisoindolin-2-yl (oxetan-3-ylmethyl) carbonate (**12a**, 0.325 g, 96%) as a white solid: Mp 88–93 °C; IR (ATR, CH_2Cl_2) 2975, 2888, 1819, 1787, 1732, 1365, 1230, 1183, 1128, 977, 874, 693 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93-7.89 (m, 2 H), 7.84-7.79 (m, 2 H), 4.86 (dd, J = 7.8, 6.6 Hz, 2 H), 4.60 (d, J = 7.2 Hz, 2 H), 4.51 (t, J = 6.2, 2 H), 3.49-3.39 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 152.5, 135.0, 128.6, 124.2, 73.4, 71.7, 33.8; HRMS (ESI$^+$) *m/z* calcd for $C_{13}H_{12}O_6N$ 278.0659 (M+H), found 278.0656.

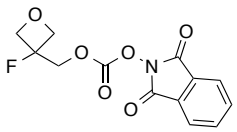


1,3-Dioxoisoindolin-2-yl ((3-methyloxetan-3-yl)methyl) carbonate (12b). To a suspension of diphthalimidyl carbonate **19** (0.354 g, 1.00 mmol) and 3-methyl-3-oxetanemethanol (0.10 mL, 0.99 mmol) in THF (12 mL) was added triethylamine (140 µL, 1.00 mmol). Upon addition of base, the suspension turned yellow, eventually progressing to a clear orange solution after 30 min. The reaction mixture was stirred for 4 h, and the solvent was

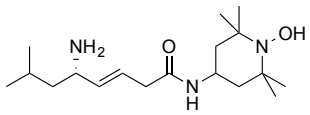
evaporated. The residue was dissolved in EtOAc (25 mL) and washed with saturated aqueous $NaHCO_3$ (5 x 3 mL) until the organic layer became clear. The combined aqueous washings were extracted with EtOAc (10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to provide 1,3-dioxoisoindolin-2-yl ((3-methyloxetan-3-yl)methyl) carbonate (**12b**, 0.287 g, 99%) as a waxy solid: IR (ATR, CH_2Cl_2) 3063, 2966, 2951, 2934, 1819, 1789, 1744, 1372, 1264, 1234, 1186, 954, 874, 730, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93-7.90 (m, 2 H), 7.84-7.80 (m, 2 H), 4.56 (d, J = 6.4 Hz, 2 H), 4.48 (s, 2 H), 4.44 (d, J = 6.4, 2 H), 1.42 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 152.6, 135.0, 128.6, 124.1, 79.0, 75.2, 39.3, 20.7; HRMS (ESI^+) *m/z* calcd for $C_{14}H_{14}O_6N$ 292.0816 (M+H), found 292.0813.



1,3-Dioxoisoindolin-2-yl ((3-ethyloxetan-3-yl)methyl) carbonate (12c). To a suspension of diphthalimidyl carbonate **19** (0.360 g, 1.02 mmol) and 3-ethyl-3-oxetanemethanol (0.120 mL, 1.01 mmol) in THF (10 mL) was added triethylamine (142 µL, 1.01 mmol). Upon addition of base, the suspension turned yellow, eventually progressing to a clear orange solution after 30 min. The reaction mixture was stirred for 4 h, and the solvent was evaporated. The residue was dissolved in EtOAc (25 mL) and washed with saturated aqueous $NaHCO_3$ (5 x 3 mL) until the organic layer became clear. The combined aqueous washings were extracted with EtOAc (10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to provide 1,3-dioxoisoindolin-2-yl ((3-ethyloxetan-3-yl)methyl) carbonate (**12c**, 0.283 g, 92%) as a colorless oil: IR (ATR, CH_2Cl_2) 2962, 2876, 1810, 1786, 1739, 1620, 1465, 1223, 1184, 1128, 979, 954, 874, 764, 695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.94-7.89 (m, 2 H), 7.85-7.81 (m, 2 H), 4.54 (s, 2 H), 4.51 (d, J = 6.4 Hz, 2 H), 4.47 (d, J = 6.0, 2 H), 1.84 (q, J = 7.5 Hz, 2 H), 0.96 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 152.6, 135.0, 128.6, 124.1, 73.0, 42.9, 26.3, 8.1; HRMS (ESI^+) *m/z* calcd for $C_{15}H_{16}O_6N$ 306.0972 (M+H), found 306.0970.



1,3-Dioxoisoindolin-2-yl ((3-fluorooxetan-3-yl)methyl) carbonate (12d). To a suspension of diphthalimidyl carbonate **19** (0.194 g, 0.551 mmol) and (3-fluorooxetan-3-yl)methanol[7] (0.059 g, 0.55 mmol) in THF (6 mL) was added triethylamine (77 μL, 0.55 mmol). Upon addition of base, the suspension turned yellow, eventually progressing to a clear orange solution after 30 min. The reaction mixture was stirred for 4 h, and the solvent was evaporated. The residue was dissolved in EtOAc (15 mL) and washed with saturated aqueous $NaHCO_3$ (5 x 3 mL) until the organic layer became clear. The combined aqueous washings were extracted with EtOAc (10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to provide 1,3-dioxoisoindolin-2-yl ((3-fluorooxetan-3-yl)methyl) carbonate (**12d**, 0.155 g, 95%) as a white solid: Mp 145–150 °C; IR (ATR, CH_2Cl_2) 3063, 2970, 2888, 1814, 1790, 1741, 1614, 1467, 1448, 1355, 1273, 1266, 1225, 1184, 1128, 1079, 958, 874, 762, 733, 695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.94-7.90 (m, 2 H), 7.85-7.81 (m, 2 H), 4.87 (dd, J = 18.0, 8.8 Hz, 2 H), 4.74 (d, J = 20.8 Hz, 2 H), 4.68-4.62 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 152.4, 135.0, 128.5, 124.2, 91.3 (d, J_{CF} = 214.0 Hz), 77.6 (d, J_{CF} = 24.0 Hz), 70.1 (d, J_{CF} = 26.0 Hz); HRMS (ESI^+) *m/z* calcd for $C_{13}H_{11}O_6NF$ 296.0565 (M+H), found 296.0563.



***tert*-Butyl (*S*,*E*)-(8-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate (14).** To a solution of JP4-039 (0.30 g, 0.71 mmol) in MeOH (6 mL) was added L-(+)-ascorbic acid (0.15 g, 0.85 mmol). The orange solution became colorless in less than 1 min. The mixture was stirred for 20 min then evaporated. The residue was extracted with CH_2Cl_2 (15 mL) and water (15 mL). The aqueous layer was washed with CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$), filtered and concentrated to provide the crude hydroxylamine that was used in the subsequent deprotection step without further purification.

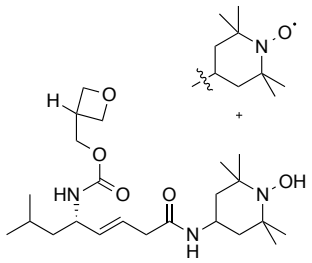
To a solution of the crude hydroxylamine in CH_2Cl_2 (6 mL) was added trifluoroacetic acid (1.05 mL, 14.1 mmol) and the mixture was stirred for 3 h. The reaction was concentrated, and the residue was dissolved in CH_2Cl_2 (30 mL). The organic layer was washed with 5% Na_2CO_3 (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated to provide *tert*-butyl (*S*,*E*)-(8-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-

5-en-4-yl)carbamate (**14**, 0.170 g, 74%) as a light yellow solid that was used without further purification.

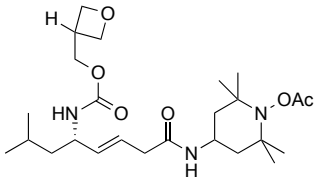
General procedure for the oxetane-containing JP4 analogs 13.[2]

To a solution of *tert*-butyl (*S*,*E*)-(8-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate (**14**, 0.20 mmol) in CH_2Cl_2 (2 mL) was added carbonate **12** (**12a**–**12d**, 0.22 mmol) followed by *N*,*N*-diisopropylethylamine (0.40 mmol) upon which there was an immediate color change to dark orange. The solution was stirred at room temperature for 24 h, and saturated aqueous $NaHCO_3$ was added. The layers were separated, and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were dried ($MgSO_4$), filtered, concentrated, and purified by chromatography on SiO_2 (CH_2Cl_2/MeOH, 15:1) to provide the corresponding pure products **13a**–**13d**.

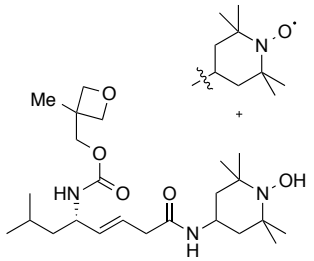
A small sample was reduced and acetylated for NMR analysis according to the following procedure: To a solution of **13** (**13a**–**13d**, nitroxide form, 0.02 mmol) in MeOH (0.5 mL) was added L-(+)-ascorbic acid (0.04 mmol). The orange solution became colorless in less than 1 min. The mixture was stirred for 30 min then acetic anhydride (0.08 mmol) and DMAP (0.01 mmol) were added and stirred for additional 2–12 h at room temperature. The solvent was evaporated and the crude mixture was re-dissolved in CH_2Cl_2 and extracted with water. The organic layers were dried ($MgSO_4$), filtered and concentrated to afford the corresponding acetylated product.



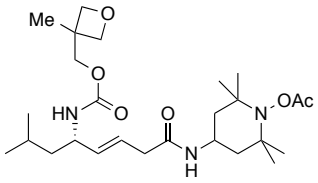
Oxetan-3-ylmethyl (*S*,*E*)-(8-((1-oxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate and/or oxetan-3-ylmethyl (*S*,*E*)-(8-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate (13a). Pale orange foam (nitroxide, 49 mg, 55%): IR (ATR, CH_2Cl_2) 3303, 3059, 2934, 2869, 1705, 1655, 1536, 1450, 1232, 1193, 1128, 1094, 1029, 982, 734 cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{23}H_{41}O_5N_3$ 439.3041 (M+H), found 439.3036.



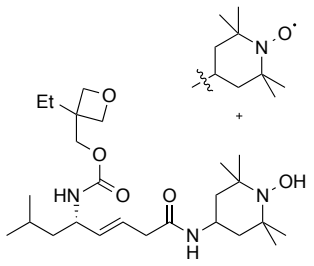
(*S*,*E*)-2,2,6,6-Tetramethyl-4-(7-methyl-5-(((oxetan-3-ylmethoxy)carbonyl)amino)oct-3-enamido)piperidin-1-yl acetate. White foam (7.3 mg, 95%): IR (ATR, CH_2Cl_2) 3515, 3308, 3058, 2933, 2889, 1764, 1701, 1648, 1536, 1465, 1454, 1359, 1255, 1215, 1193, 1077, 1028, 982, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.05-6.03 (m, 1 H), 5.70 (dt, *J* = 15.2, 7.5 Hz, 1 H), 5.41 (dd, *J* = 15.2, 7.2 Hz, 1 H), 4.80 (ddd, *J* = 8.1, 5.9, 2.3 Hz, 2 H), 4.73-4.72 (m, 1 H), 4.49 (td, *J* = 6.1, 2.5 Hz, 2 H), 4.34 (dd, *J* = 11.0, 6.2 Hz, 1 H), 4.27-4.16 (m, 2 H), 4.09-4.03 (m, 1 H), 3.31-3.24 (m, 1 H), 2.95 (d, *J* = 7.2 Hz, 2 H), 2.08 (s, 3 H), 1.85-1.79 (m, 2 H), 1.69-1.52 (m, 3 H), 1.39-1.34 (m, 2 H), 1.23 (s, 6 H), 1.05 (d, *J* = 6.8 Hz, 6 H), 0.92 (dd, *J* = 6.8, 3.2 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 155.9, 136.5, 124.6, 74.0, 65.5, 60.07, 60.02, 52.1, 44.8, 44.5, 43.6, 40.8, 39.9, 34.2, 31.9, 29.7, 24.6, 22.51, 22.43, 21.0, 19.1; HRMS (ESI^+) *m/z* calcd for $C_{25}H_{44}O_6N_3$ 482.3225 (M+H), found 482.3224.



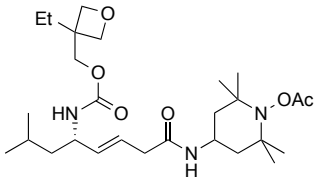
(3-Methyloxetan-3-yl)methyl (*S*,*E*)-(8-((1-oxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate and/or (3-methyloxetan-3-yl)methyl (*S*,*E*)-(8-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate (13b). Orange foam (nitroxide, 27 mg, 29%): IR (ATR, CH_2Cl_2) 3484, 3307, 3095, 2955, 2932, 2869, 1700, 1646, 1532, 1460, 1437, 1376, 1297, 1242, 1178, 1085, 1044, 1023, 973, 835, 764 cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{24}H_{43}O_5N_3$ 453.3197 (M+H), found 453.3194. White foam (hydroxylamine, 29 mg, 31%): HRMS (ESI^+) *m/z* calcd for $C_{24}H_{44}O_5N_3$ 454.3275 (M+H), found 454.3271.



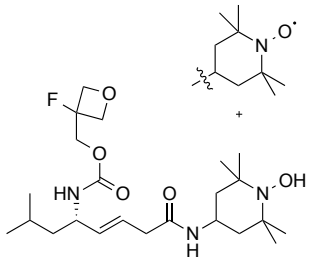
(*S*,*E*)-2,2,6,6-Tetramethyl-4-(7-methyl-5-((((3-methyloxetan-3-yl)methoxy)carbonyl)amino)oct-3-enamido)piperidin-1-yl acetate. White foam (6.3 mg, 79%): IR (ATR, CH_2Cl_2) 3516, 3303, 3059, 2933, 2876, 1761, 1700, 1646, 1532, 1465, 1450, 1420, 1365, 1245, 1215, 1193, 1127, 1094, 999, 982, 917, 797, 690 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.06-6.05 (m, 1 H), 5.70 (dt, *J* = 15.2, 7.5 Hz, 1 H), 5.42 (dd, *J* = 15.3, 7.8 Hz, 1 H), 4.79-4.78 (m, 1 H), 4.55-4.54 (m, 2 H), 4.38 (d, *J* = 6.0 Hz, 2 H), 4.23-4.18 (m, 2 H), 4.08-4.04 (m, 2 H), 2.95 (d, *J* = 7.0 Hz, 2 H), 2.08 (s, 3 H), 1.85-1.79 (m, 2 H), 1.68-1.63 (m, 2 H), 1.45-1.35 (m, 3 H), 1.33 (s, 3 H), 1.25-1.24 (m, 6 H), 1.06 (d, *J* = 8.5 Hz, 6 H), 0.93 (dd, *J* = 6.5, 4.0 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 156.0, 136.4, 124.5, 79.5, 68.9, 60.1, 52.1, 44.8, 44.6, 43.6, 40.8, 39.9, 39.3, 31.9, 29.7, 24.6, 22.7, 22.5, 22.4, 21.2, 21.0, 19.1; HRMS (ESI^+) *m/z* calcd for $C_{26}H_{46}O_6N_3$ 496.3381 (M+H), found 496.3381.



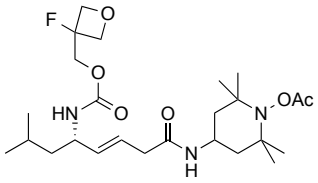
(3-Ethyloxetan-3-yl)methyl (*S*,*E*)-(8-((1-oxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate and/or (3-Ethyloxetan-3-yl)methyl (*S*,*E*)-(8-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate (13c). Pale orange oil (nitroxide, 40 mg, 30%): IR (ATR, CH_2Cl_2) 3328, 3065, 2955, 2949, 2869, 1704, 1648, 1529, 1460, 1264, 1240, 1117, 1087, 1051, 973, 731, 701 cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{25}H_{45}O_5N_3$ 467.3354 (M+H), found 467.3350. White solid (hydroxylamine, 40 mg, 30%): HRMS (ESI^+) *m/z* calcd for $C_{25}H_{46}O_5N_3$ 468.3432 (M+H), found 468.3430.



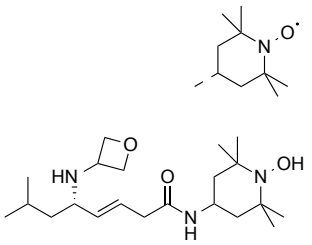
(*S*,*E*)-4-(5-((((3-Ethyloxetan-3-yl)methoxy)carbonyl)amino)-7-methyloct-3-enamido)-2,2,6,6-tetramethylpiperidin-1-yl acetate. White foam (11 mg, 96%): IR (ATR, CH_2Cl_2) 3514, 3298, 3059, 2934, 2882, 1764, 1701, 1670, 1654, 1646, 1629, 1534, 1465, 1450, 1359, 1232, 1215, 1192, 1094, 1029, 982 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.03-6.01 (m, 1 H), 5.70 (dt, *J* = 15.1, 7.5 Hz, 1 H), 5.43 (dd, *J* = 15.2 7.2 Hz, 1 H), 4.79-4.77 (m, 1 H), 4.50-4.48 (m, 2 H), 4.40 (d, *J* = 6.0 Hz, 2 H), 4.26-4.15 (m, 2 H), 4.12-4.03 (m, 2 H), 2.95 (d, *J* = 7.6 Hz, 2 H), 2.08 (s, 3 H), 1.87-1.55 (m, 7 H), 1.41-1.33 (m, 2 H), 1.25-1.23 (m, 6 H), 1.06 (d, *J* = 6.0 Hz, 6 H), 0.94-0.90 (m, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 156.0, 136.4, 124.3, 77.7, 66.8, 60.0, 52.0, 44.7, 44.6, 43.7, 42.7, 40.8, 39.9, 31.9, 29.7, 26.9, 24.6, 22.5, 22.4, 21.0, 19.1, 8.2; HRMS (ESI^+) *m/z* calcd for $C_{27}H_{48}O_6N_3$ 510.3538 (M+H), found 510.3538.



(3-Fluorooxetan-3-yl)methyl (*S*,*E*)-(8-((1-oxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate and/or (3-fluorooxetan-3-yl)methyl (*S*,*E*)-(8-((1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate (13d). Orange foam (nitroxide, 29 mg, 40%): IR (ATR, CH_2Cl_2) 3503, 3311, 2958, 1708, 1655, 1536, 1458, 1363, 1286, 1242, 1193, 1127, 1085, 973, 842, 735 cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{23}H_{40}O_5N_3F$ 457.2947 (M+H), found 457.2945. White foam (hydroxylamine, 14 mg, 19%): HRMS (ESI^+) *m/z* calcd for $C_{23}H_{41}O_5N_3F$ 458.3025 (M+H), found 458.3022.

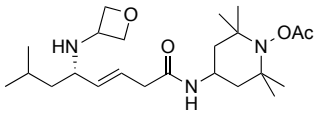


(*S*,*E*)-4-(5-((((3-Fluorooxetan-3-yl)methoxy)carbonyl)amino)-7-methyloct-3-enamido)-2,2,6,6-tetramethylpiperidin-1-yl acetate. White foam (8.6 mg, 84%): IR (ATR, CH_2Cl_2) 3339, 2955, 2945, 2882, 1760, 1713, 1647, 1532, 1454, 1355, 1264, 1212, 1182, 1119, 1085, 992, 971, 733 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.04-6.02 (m, 1 H), 5.71 (dt, *J* = 15.2, 7.5 Hz, 1 H), 5.41 (dd, *J* = 15.4, 7.4 Hz, 1 H), 4.80 (ddd, *J* = 18.9, 7.9, 3.3 Hz, 3 H), 4.66-4.40 (m, 4 H), 4.20 (tdt, *J* = 12.2, 8.1, 4.1 Hz, 1 H), 4.06 (quintet, *J* = 7.2 Hz, 1 H), 2.95 (d, *J* = 7.6 Hz, 2 H), 2.08 (s, 3 H), 1.85–1.79 (m, 2 H), 1.71-1.51 (m, 3 H), 1.37 (td, *J* = 7.3, 2.3 Hz, 2 H), 1.25-1.23 (m, 6 H), 1.05 (d, *J* = 8.8 Hz, 6 H), 0.92 (dd, *J* = 6.4, 4.0 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 155.2, 136.2, 124.8, 92.4 (d, J_{CF} = 211.1 Hz), 78.2 (dd, J_{CF} = 23.6, 6.1 Hz), 64.9 (d, J_{CF} = 25.5 Hz), 60.0, 52.3, 44.9, 44.6, 43.6, 40.8, 39.8, 31.91, 31.85, 29.7, 24.6, 22.5, 22.4, 20.99, 20.97, 19.1; HRMS (ESI^+) *m/z* calcd for $C_{25}H_{43}O_6N_3F$ 500.3130 (M+H), found 500.3128.

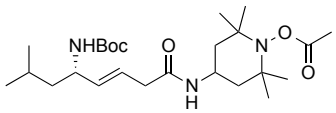


(*S*,*E*)-*N*-(1-Oxy-2,2,6,6-tetramethylpiperidin-4-yl)-7-methyl-5-(oxetan-3-ylamino)oct-3-enamide and/or (*S*,*E*)-*N*-(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)-7-methyl-5-(oxetan-3-ylamino)oct-3-enamide (16). To a solution of **14** (82 mg, 0.25 mmol) in DCE (4 mL) were added AcOH (14 μL, 0.25 mmol) and 3-oxetanone (17 μL, 0.27 mmol) at room temperature and stirred for 30 min. The reaction was cooled to 0 °C and sodium triacetoxyborohydride (75 mg, 0.35 mmol) was added and the reaction stirred at room temperature for 35 h. The reaction was quenched with saturated $NaHCO_3$ solution (10.0 mL) and extracted with CH_2Cl_2 (15.0 mL). The aqueous layer was back-extracted with CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$), filtered, concentrated, and purified by chromatography on SiO_2 (CH_2Cl_2/MeOH, 15:1) to provide **16** as a mixture of nitroxide and hydroxylamine. White foam (nitroxide, 15 mg, 16%): IR (ATR, CH_2Cl_2) 3296, 3059, 2934, 2869, 1629, 1544, 1450, 1420, 1359, 1340, 1232, 1215, 1178, 1094, 1029, 982, 956, 838, 734

cm^{-1}; HRMS (ESI^+) *m/z* calcd for $C_{21}H_{39}O_3N_3$ 381.2986 (M+H), found 381.2983. White foam (hydroxylamine, 25 mg, 25%): HRMS (ESI^+) *m/z* calcd for $C_{21}H_{40}O_5N_3$ 382.3064 (M+H), found 382.3060.

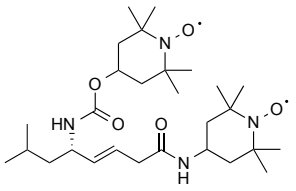


(*S*,*E*)-2,2,6,6-Tetramethyl-4-(7-methyl-5-(oxetan-3-ylamino)oct-3-enamido)piperidin-1-yl acetate. To a solution of **16** (a mixture of nitroxide and hydroxylamine, 8 mg, ca. 0.02 mmol) in MeOH (0.5 mL) was added L-(+)-ascorbic acid (7 mg, 0.04 mmol). Complete discoloration of the solution occurred within a few seconds. After stirring for 30 min at room temperature, acetic anhydride (8.4 μL, 0.082 mmol) and DMAP (1.3 mg, 0.010 mmol) were added and stirred at room temperature for 2 h. The solvent was evaporated, the mixture was re-dissolved in CH_2Cl_2 and extracted with water. The organic layers were dried ($MgSO_4$), filtered and concentrated to provide (*S*,*E*)-2,2,6,6-tetramethyl-4-(7-methyl-5-(oxetan-3-ylamino)oct-3-enamido)piperidin-1-yl acetate (3.5 mg, 40%) as a colorless oil: IR (ATR, CH_2Cl_2) 3492, 3301, 3279, 1762, 1646, 1542, 1465, 1359, 1215, 1193, 982, 956, 870, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.60 (dt, *J* = 14.9, 7.3 Hz, 1 H), 5.31-5.25 (m, 2 H), 4.77 (dt, *J* = 12.8, 6.5 Hz, 2 H), 4.37 (dt, *J* = 20.3, 6.3 Hz, 2 H), 4.21 (tdt, *J* = 12.2, 8.1, 4.1 Hz, 1 H), 3.96 (quintet, *J* = 6.7 Hz, 1 H), 3.04 (q, *J* = 7.7 Hz, 1 H), 2.91 (dd, *J* = 7.0, 1.0 Hz, 2 H), 2.10 (s, 3 H), 1.87-1.83 (m, 2 H), 1.60-1.50 (m, 4 H), 1.30 (td, *J* = 7.1, 1.6 Hz, 2 H), 1.25 (s, 6 H), 1.07 (s, 6 H), 0.89 (dd, *J* = 8.2, 6.6 Hz, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 138.0, 124.5, 80.6, 80.1, 60.1, 57.5, 51.6, 45.1, 44.8, 40.9, 40.2, 31.8, 24.7, 23.0, 22.3, 21.0, 19.1; HRMS (ESI^+) *m/z* calcd for $C_{23}H_{42}O_4N_3$ 424.3170 (M+H), found 424.3167.



(*S*,*E*)-4-(5-((*tert*-butoxycarbonyl)amino)-7-methyloct-3-enamido)-2,2,6,6-tetramethylpiperidin-1-yl acetate. To a solution of JP4-039 (40 mg, 0.094 mmol) in MeOH (0.2 mL) was added L-(+)-ascorbic acid (33 mg, 0.19 mmol). Complete discoloration of the solution occurred within a few seconds. After stirring at rt for 10-15 min, acetic anhydride

(35 μL, 0.38 mmol) and DMAP (5.7 mg, 0.047 mmol) were added and left to stir at rt for one hour. The solvent was evaporated, the crude re-dissolved in CH_2Cl_2 (0.2 mL) and extracted with water (2 x 0.5 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated to dryness to give the desired product as a pale yellow foam (37 mg, 84%): Mp 57.4–60.6 °C; IR (ATR, neat) 3307, 2958, 1761, 1685, 1651, 1646, 1527, 1363, 1245, 1200, 1172, 999, 869, 938 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.03 (br s, 1 H), 5.66 (dt, *J* = 6.8, 15.4 Hz, 1 H), 5.46 (dd, *J* = 6.8, 15.4 Hz, 1 H), 4.48 (br s, 1 H), 4.25–4.13 (m, 1 H), 4.00 (quintet, *J* = 7.1 Hz, 1 H), 2.94 (d, *J* = 7.3 Hz, 2 H), 2.09 (br s, 2 H), 2.07 (br s, 1 H), 1.86–1.78 (m, 2 H), 1.70–1.52 (m, 3 H), 1.44 (s, 9 H), 1.35–1.27 (m, 2 H), 1.22 (br s, 6 H), 1.04 (d, *J* = 8.7 Hz, 6 H), 0.91 (dd, *J* = 2.1, 6.6 Hz, 6 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.7, 170.5, 155.4, 137.2, 123.3, 79.5, 60.0, 51.4, 44.7, 44.5, 43.9, 40.9, 39.9, 31.8, 28.5, 24.6, 22.5, 20.9, 19.1; HRMS (ESI) *m/z* calcd for $C_{25}H_{46}N_3O_5$ 468.3432, found 468.3433.



1-Oxy-2,2,6,6-tetramethylpiperidin-4-yl (*S*,*E*)-(8-((1-oxy-2,2,6,6-tetramethylpiperidin-4-yl)amino)-2-methyl-8-oxooct-5-en-4-yl)carbamate (18). To a solution of 4-hydroxy-TEMPO (67 mg, 0.38 mmol) in DMF (700 μL) was added disuccinimidyl carbonate (115 mg, 0.449 mmol) and pyridine (62 μL, 0.075 mmol). The mixture was heated at 40 °C for 14 h to give **17**. Separately, trifluoroacetic acid (565 μL, 7.53 mmol) was added to a solution of JP4-039 (160 mg, 0.377 mmol) in CH_2Cl_2 (3.5 mL) at 0 °C. The reaction was stirred 3 h until the starting carbamate was consumed, and the solvent and excess acid were evaporated. The material was dissolved in EtOAc and washed 3x with 10% aqueous Na_2CO_3. The combined aqueous layers were extracted with EtOAc (2x), and the combined organic layers were dried (Na_2CO_3) and evaporated.

The TEMPO-mixed carbonate solution (**17**) was cooled to rt and transferred to a solution of the above amine and K_3PO_4 (83 mg, 0.38 mmol) in DMF (2.1 mL) and water (700 μL). The mixture was stirred 16 h, then diluted with water (20 mL) and EtOAc (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with 5% aqueous LiCl, dried ($MgSO_4$), and evaporated. The residue was

purified by chromatography on SiO_2 (40–80% EtOAc/hexanes) to provide bis-nitroxide **18** (110 mg, 56%) as a pale orange solid: Mp 89-92 °C; HRMS (ESI) *m/z* calcd for $C_{28}H_{51}N_4O_5$ 523.3854 (M+H), found 523.3846; IR (ATR, neat) 3310, 2972, 2934, 2871, 1713, 1649, 1528, 1465, 1363, 1316, 1239, 1178, 1116, 1085, 1049, 971, 776, 741, 685 cm^{-1}.

Cell Lines and Media. HT-1080 cells were obtained from ATCC and grown in DMEM with glutamine and sodium pyruvate (Corning 10-013) supplemented with 10% Heat-Inactivated FBS, 1% non-essential amino acids (Invitorgen), and 1% penicillin-streptomycin mix (Invitrogen). Panc-1 cells were obtained from ATCC and grown in DMEM with glutamine and sodium pyruvate (Corning 10-013) supplemented with 10% Heat-Inactivated FBS, and 1% penicillin-streptomycin mix (Invitrogen). BJeLR cells were grown in DMEM with glutamine and sodium pyruvate (Corning 10-013) supplemented with 20% Medium-199 (Sigma), 15% Heat-Inactivated FBS, and 1% penicillin-streptomycin mix (Invitrogen). All cells were maintained in a humidified environment at 37 ºC and 5% CO_2 in a tissue incubator.

Cell Viability Assay. 3,000 cells were seeded per well in black, clear bottom 384-well plates (Corning) and allowed to adhere overnight. The next day, the medium was replaced with 50 μL of growth medium and 5 μL medium containing erastin (10 μM) or (1*S*, 3*R*)-RSL3 (2 μM) and a dilution series of ferrostatin-1 or a nitroxide-containing molecule. 24 hours later, 6.1 μL of Presto Blue (Thermo-Fisher) were added. Cells were incubated for an additional 5 hours and the Presto Blue fluorescence intensity was measured using a Victor X5 plate reader (PerkinElmer)(ex/em 530/590). Background (no cells) fluorescence was subtracted and the resulting fluorescence intensities were averaged between biological replicates. From these data, dose-response curves and EC_{50} values were computed using Prism 6.0 (GraphPad).

References

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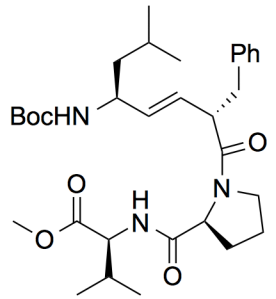

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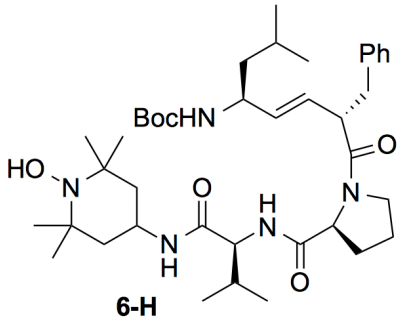
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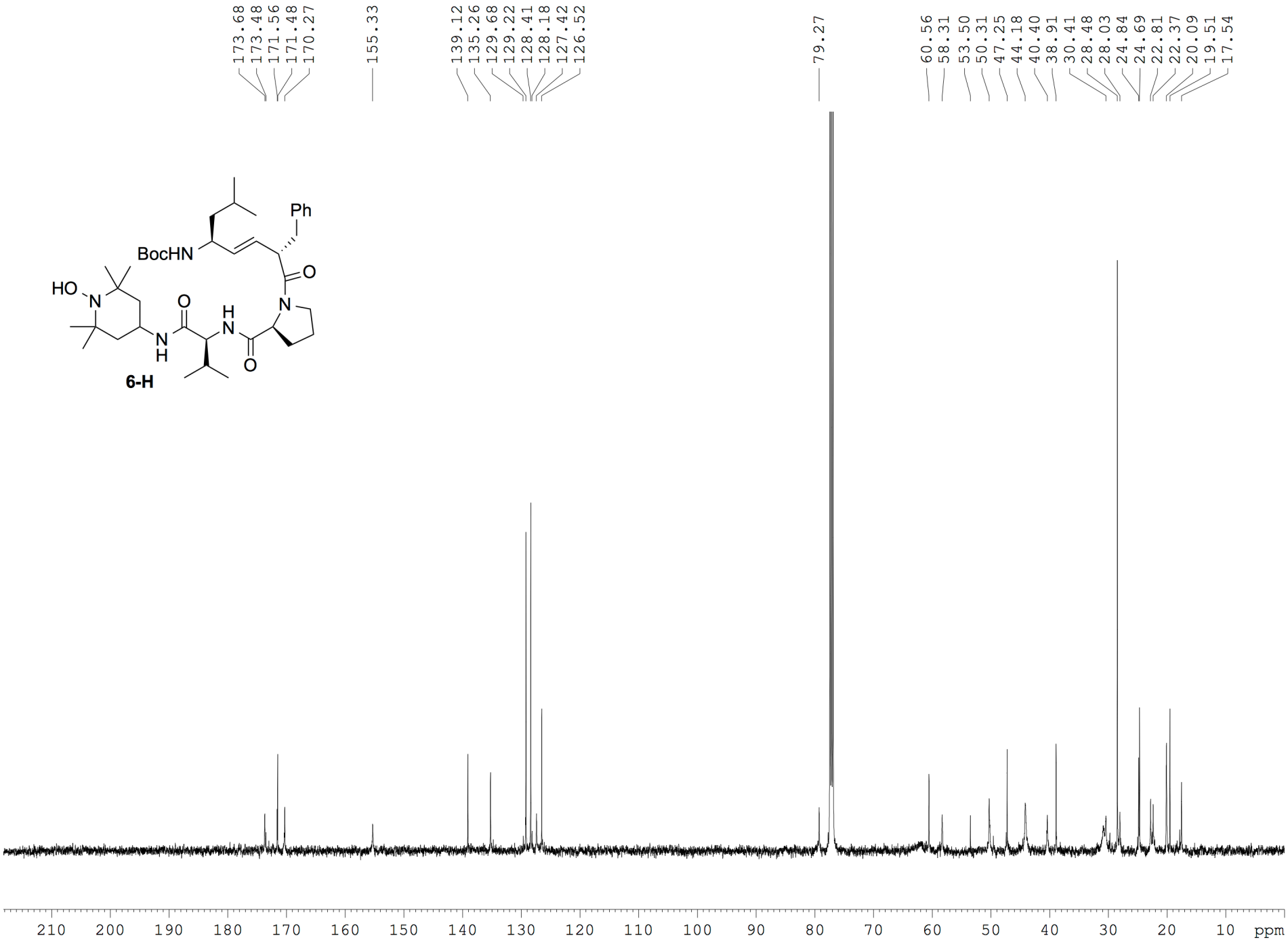
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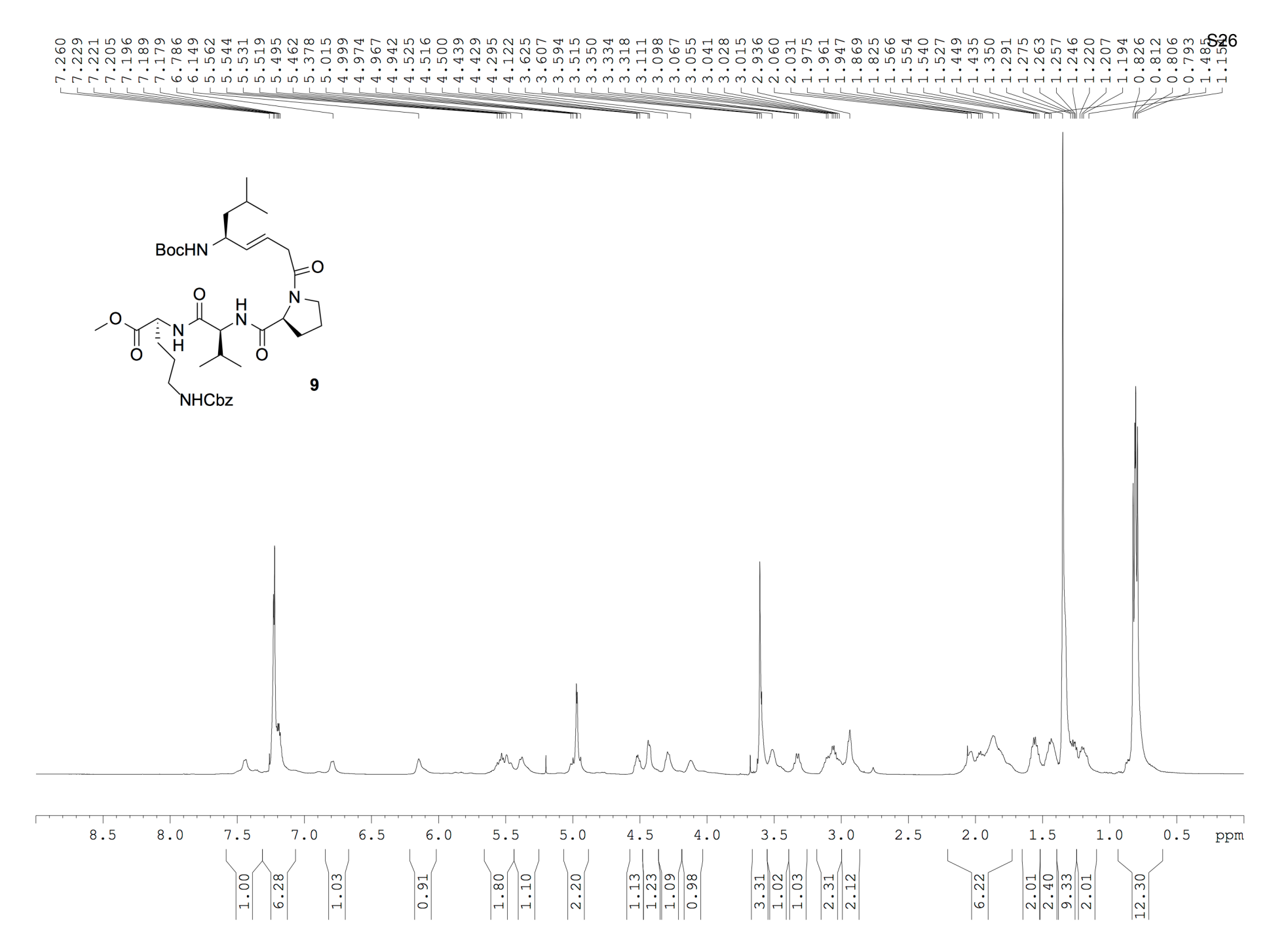
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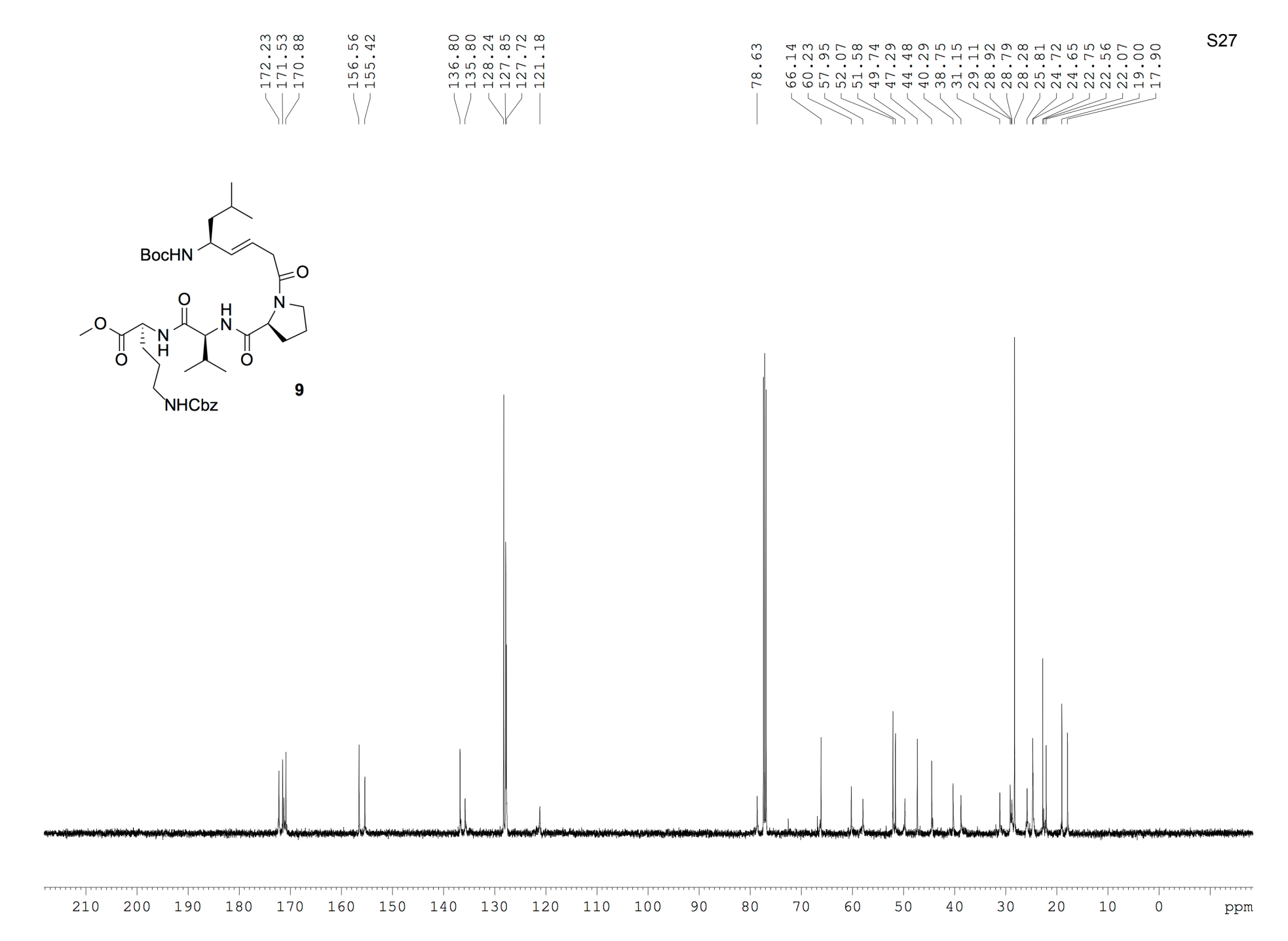
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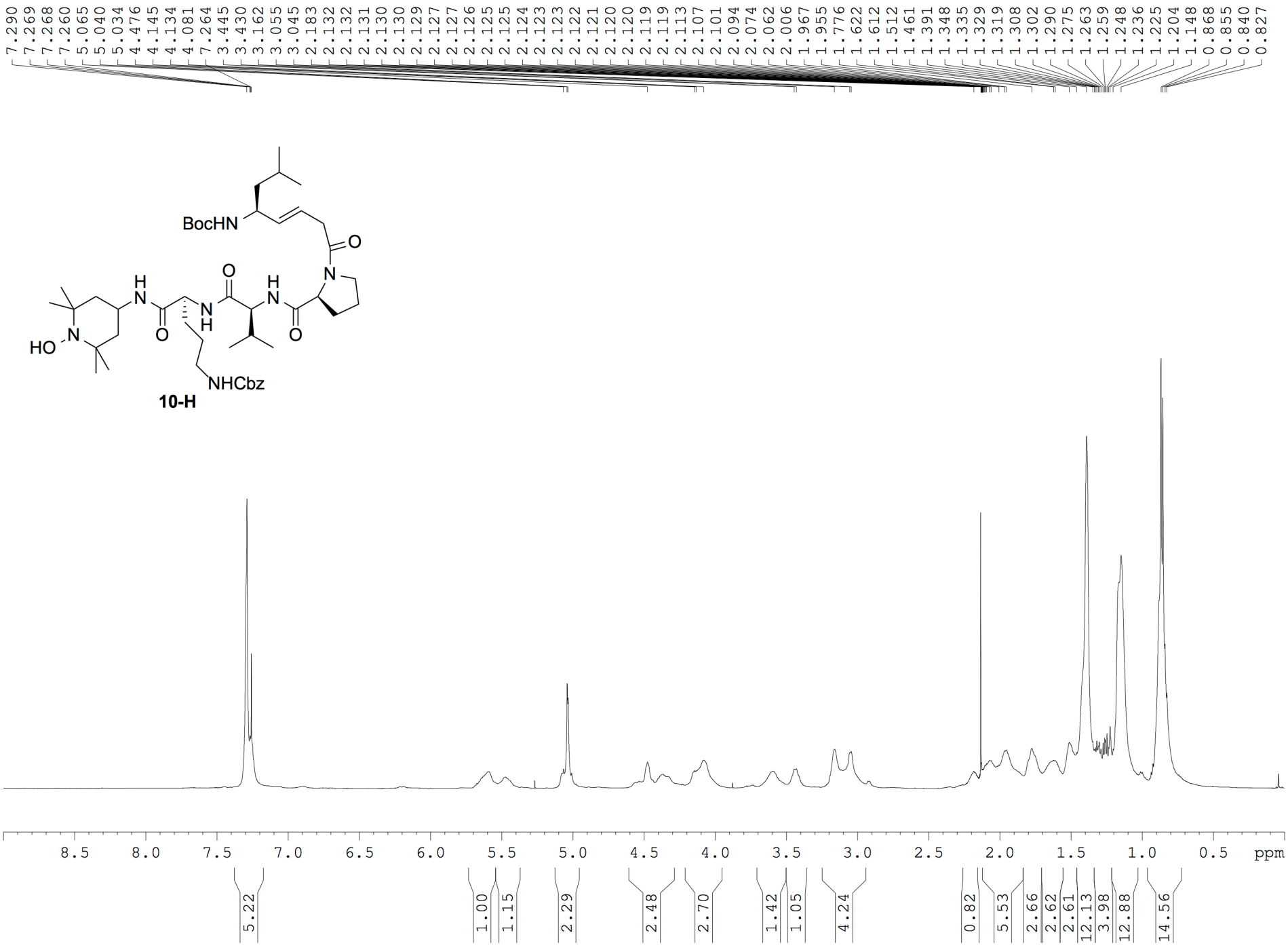
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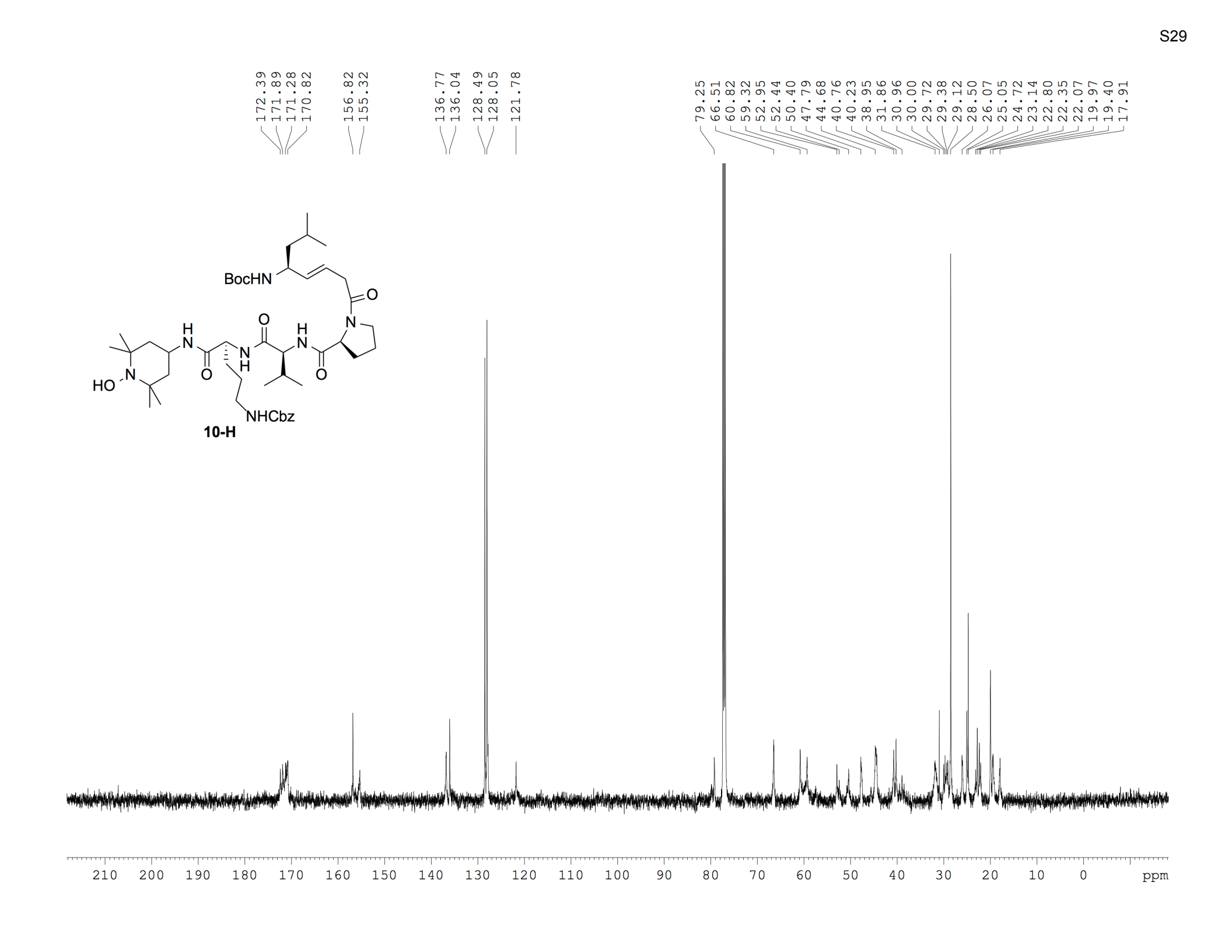
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128.05
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79.25
66.51
60.82
59.32
52.95
52.44
50.40
47.79
44.68
40.76
40.23
38.95
31.86
30.96
30.00
29.72
29.38
29.12
28.50
26.07
25.05
24.72
23.14
22.80
22.35
22.07
19.97
19.40
17.91
BocHN
HO
NHCbz
10-H
210
200
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100
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60
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30
20
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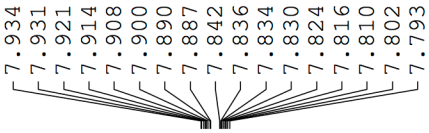
ppm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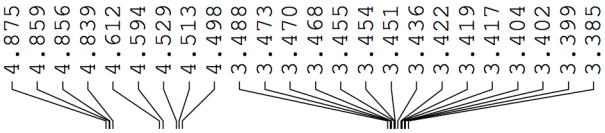
7.834
7.830
7.824
7.816
7.810
7.802
7.793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4.856
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4.513
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3.488
3.473
3.470
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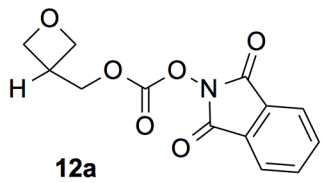
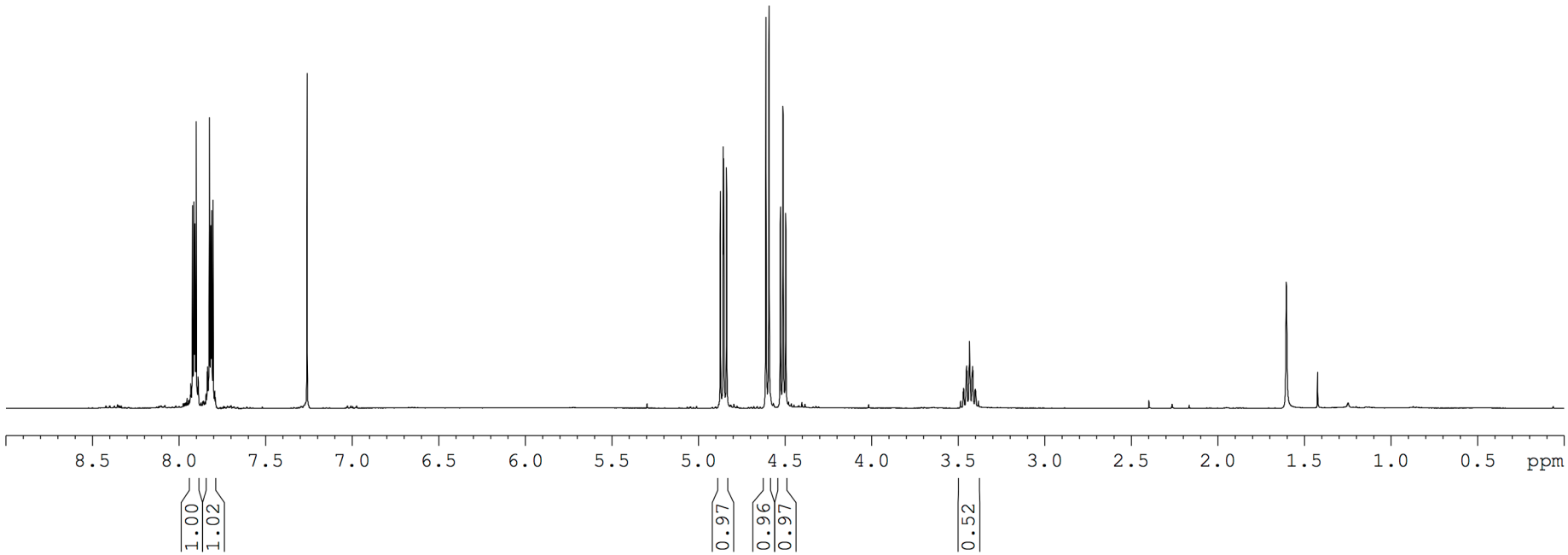
3.455
3.454
3.451
3.436
3.422
3.419
3.417
3.404
3.402
3.399
3.385


O
H
O
O
O
N
O
O

12a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5.5
5.0
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4.0
3.5
3.0
2.5
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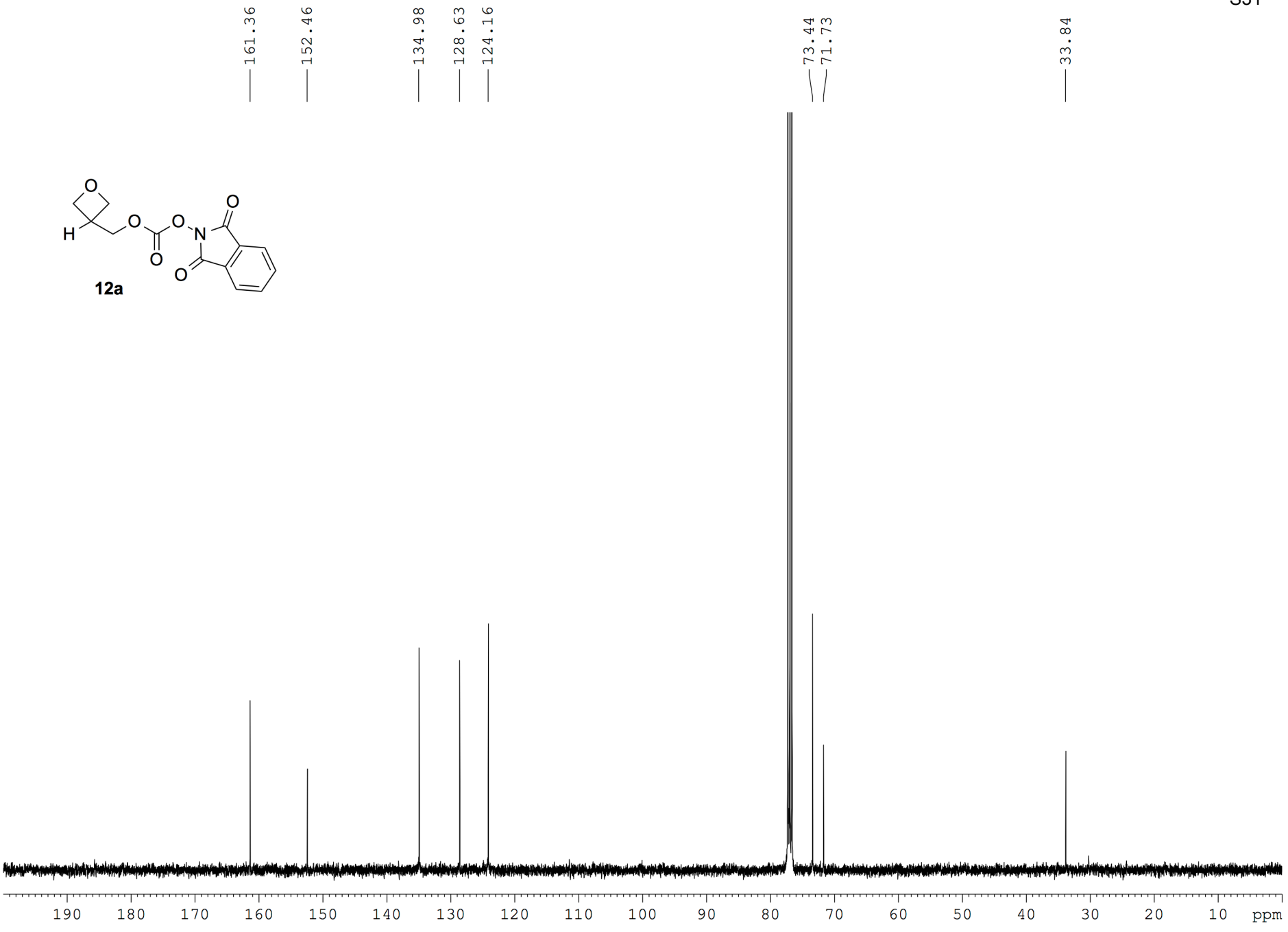
1.5
1.0
0.5
ppm
1.00
1.02
0.97
0.96
0.97
0.52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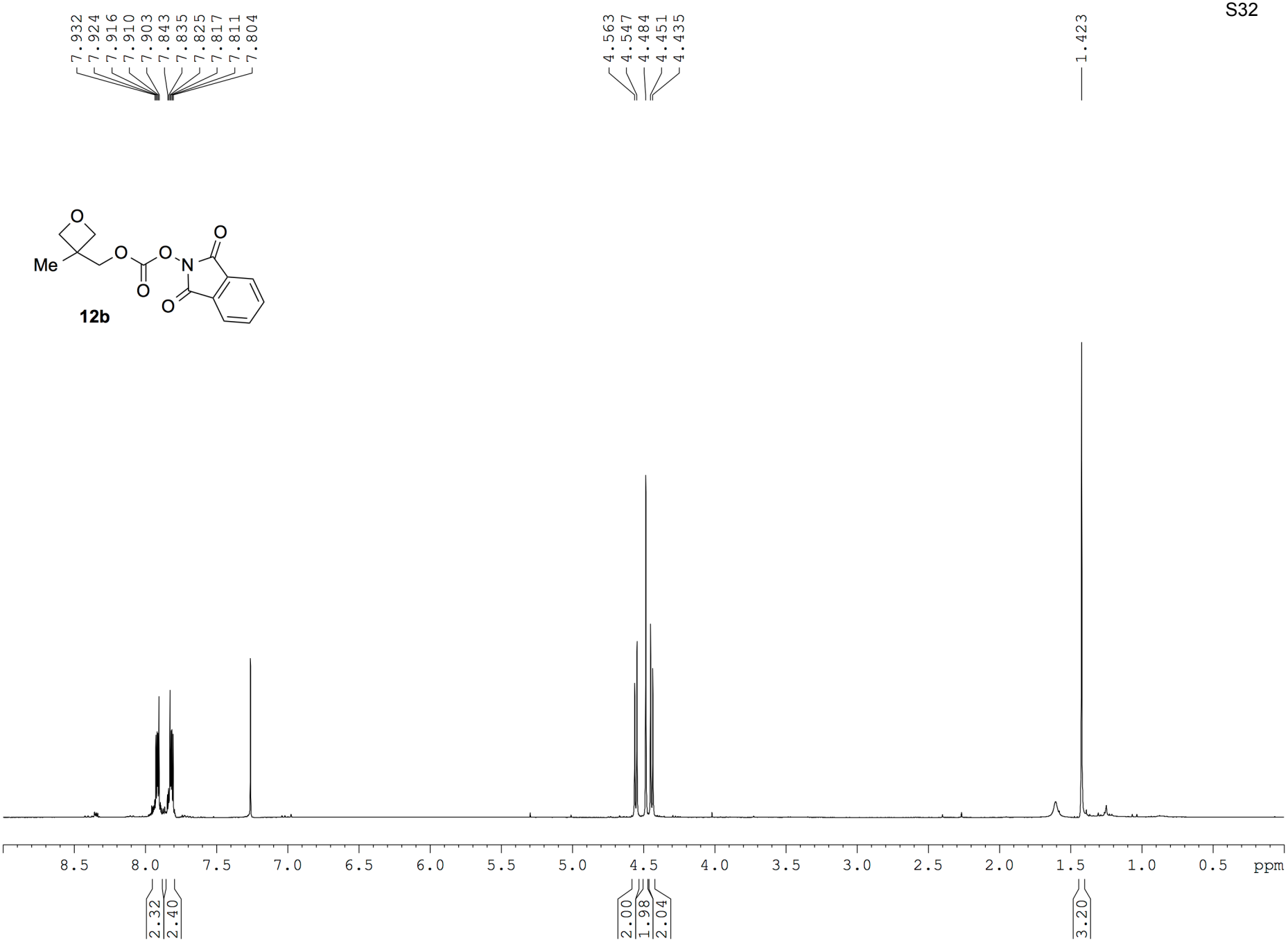
152.46
134.98
128.63
124.16
73.44
71.73
33.84
12a
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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7.916
7.910
7.903
7.843
7.835
7.825
7.817
7.811
7.804
4.563
4.547
4.484
4.451
4.435
1.423
Me
O
O
O
O
N
O
O
12b
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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1.5
1.0
0.5
ppm
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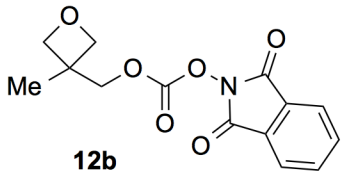
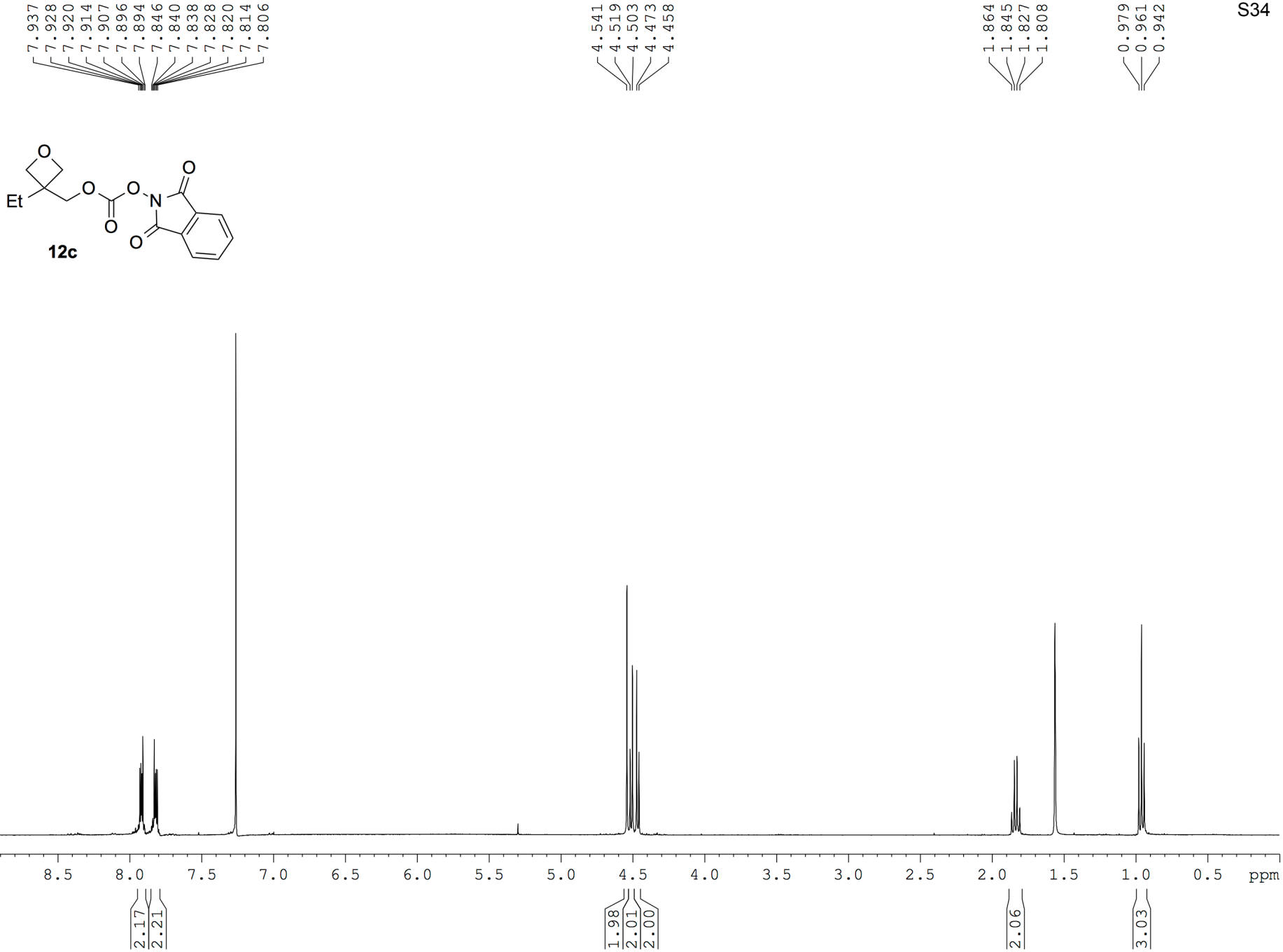
2.40
2.00
1.98
2.04
3.20


O
Me
O
O
O
N
O
O

12b

161.36
152.59
134.97
128.60
124.13
79.01
75.21
39.29
20.67
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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7.914
7.907
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7.894
7.846
7.840
7.838
7.828
7.820
7.814
7.806
4.541
4.519
4.503
4.473
4.458
1.864
1.845
1.827
1.808
0.979
0.961
0.942
Et
O
O
O
O
O
N
O
12c
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
ppm
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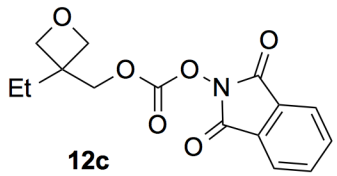
2.21
1.98
2.01
2.00
2.06
3.03

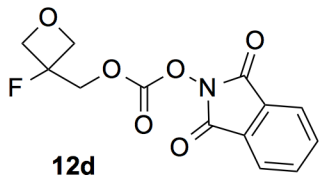

O
Et
O
O
O
N
O
O

12c

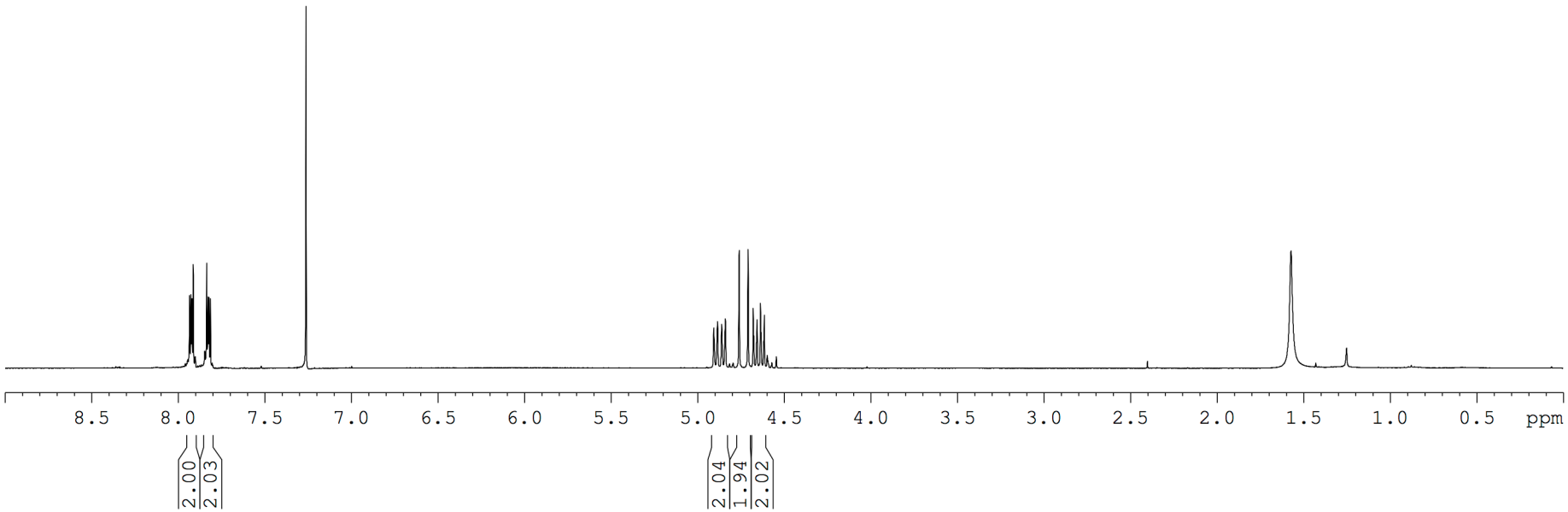
161.37
152.59
134.97
128.64
124.14
72.95
42.86
26.26
8.06
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

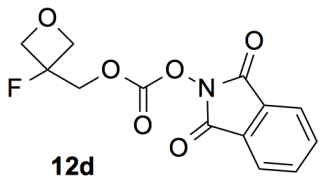
7.942
7.932
7.924
7.919
7.911
7.901
7.898
7.845
7.833
7.826
7.820
7.812

4.907
4.885
4.862
4.840
4.761
4.709
4.679
4.657
4.637
4.615



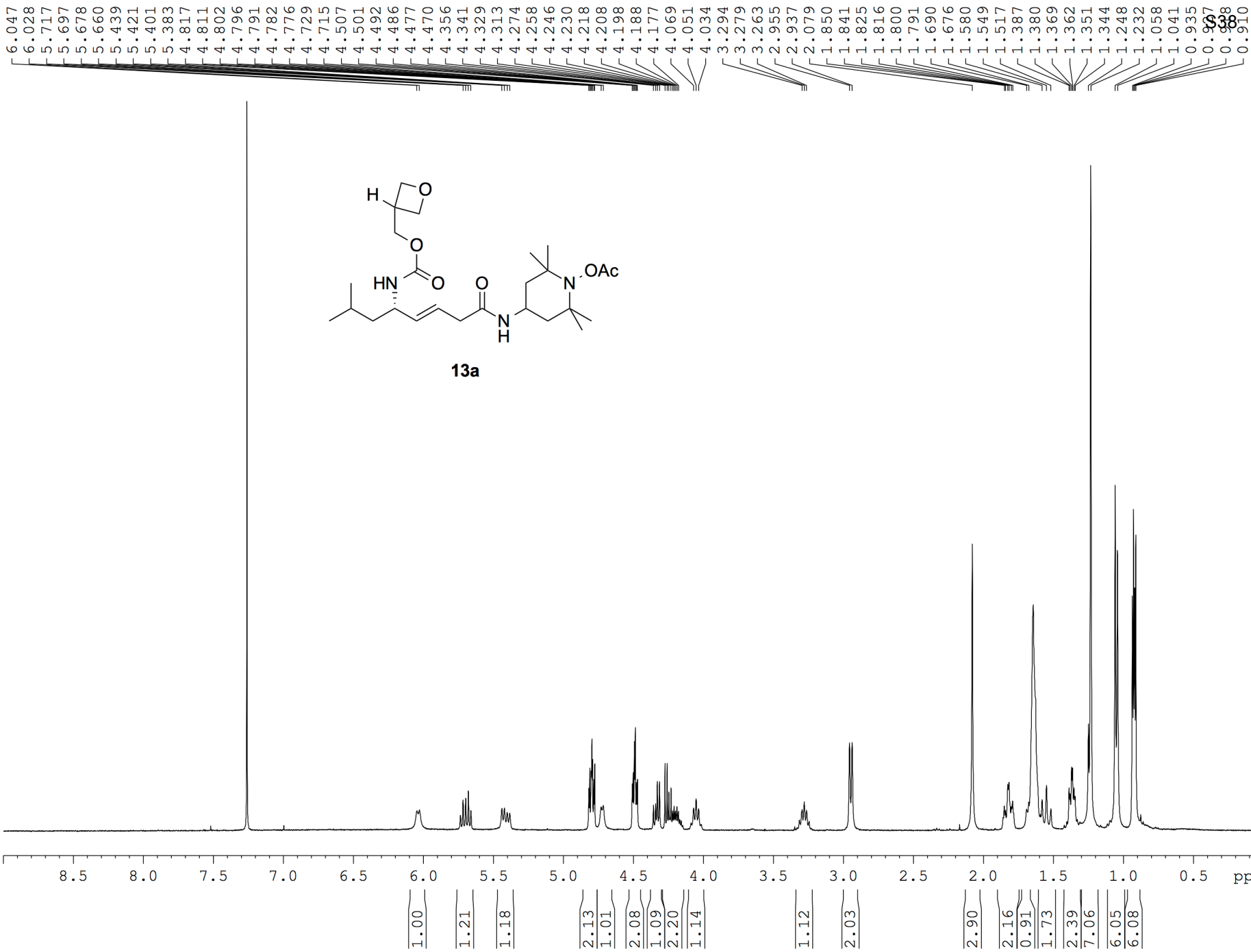
12d




O
F
O
O
O
N
O
O

12d

161.15
152.39
135.02
128.52
124.17
92.32
90.18
77.70
77.46
70.29
70.03
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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5.421
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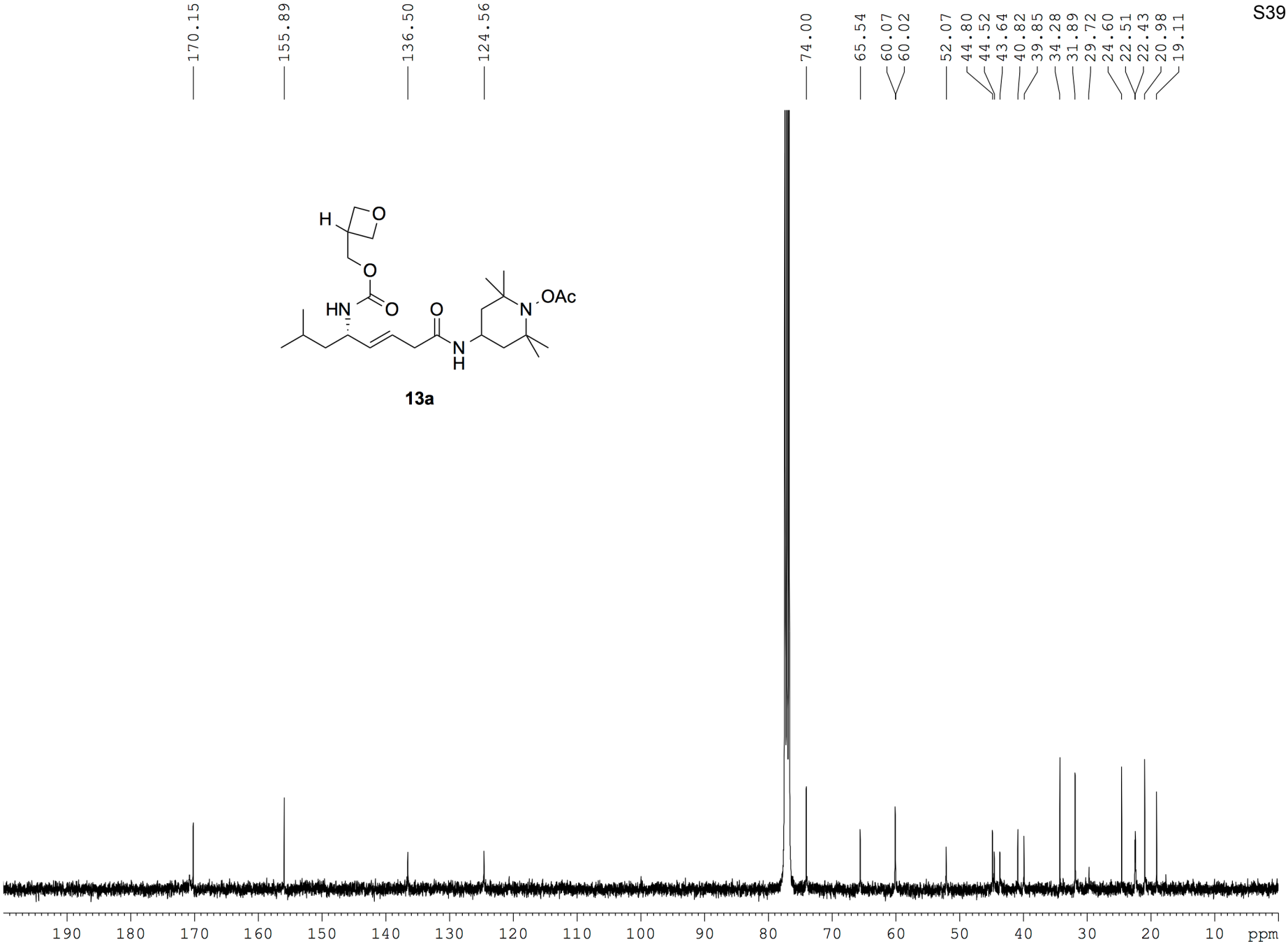
4.817
4.811
4.802
4.796
4.791
4.782
4.776
4.729
4.715
4.507
4.501
4.492
4.486
4.477
4.470
4.356
4.341
4.329
4.313
4.274
4.258
4.246
4.230
4.218
4.208
4.198
4.188
4.177
4.069
4.051
4.034
3.294
3.279
3.263
2.955
2.937
2.079
1.850
1.841
1.825
1.816
1.800
1.791
1.690
1.676
1.580
1.549
1.517
1.387
1.380
1.369
1.362
1.351
1.344
1.248
1.232
1.058
1.041
0.935
0.927
0.910
H
O
O
HN
O
O
N
OAc
N
H
13a
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
ppm
1.00
1.21
1.18
2.13
1.01
2.08
1.09
2.20
1.14
1.12
2.03
2.90
2.16
0.91
1.73
2.39
7.06
6.05
6.08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155.89
136.50
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74.00
65.54
60.07
60.02
52.07
44.80
44.52
43.64
40.82
39.85
34.28
31.89
29.72
24.60
22.51
22.43
20.98
19.11
H
O
O
HN
O
O
N
H
N
OAc
190
180
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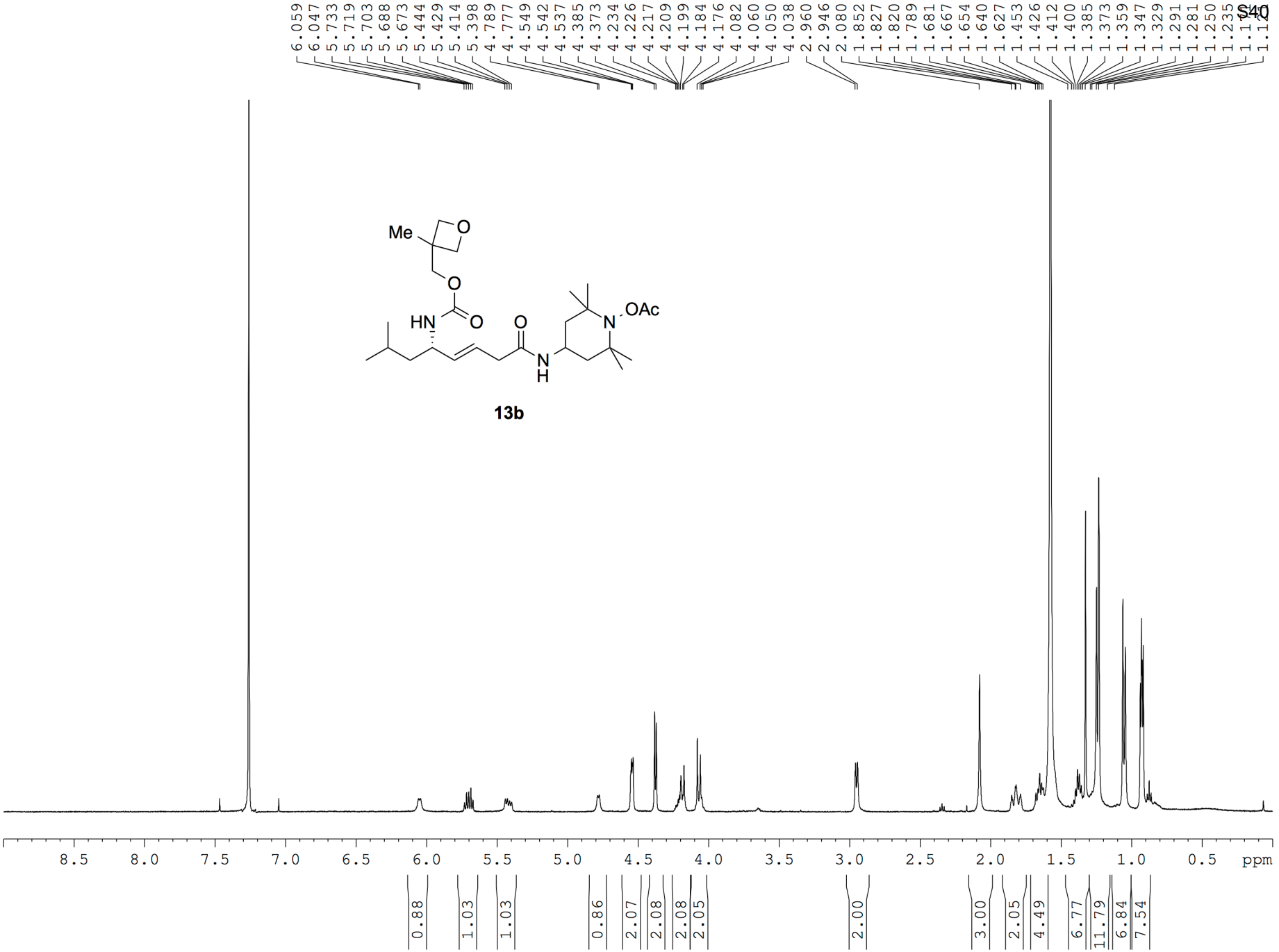
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

13a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5.414
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4.777
4.549
4.542
4.537
4.385
4.373
4.234
4.226
4.217
4.209
4.199
4.184
4.176
4.082
4.060
4.050
4.038
2.960
2.946
2.080
1.852
1.827
1.820
1.789
1.681
1.667
1.654
1.640
1.627
1.453
1.426
1.412
1.400
1.385
1.373
1.359
1.347
1.329
1.291
1.281
1.250
1.235
1.112
Me
O
O
HN
O
O
N
OAc
N
H
13b
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
ppm
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1.03
0.86
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2.08
2.05
2.00
3.00
2.05
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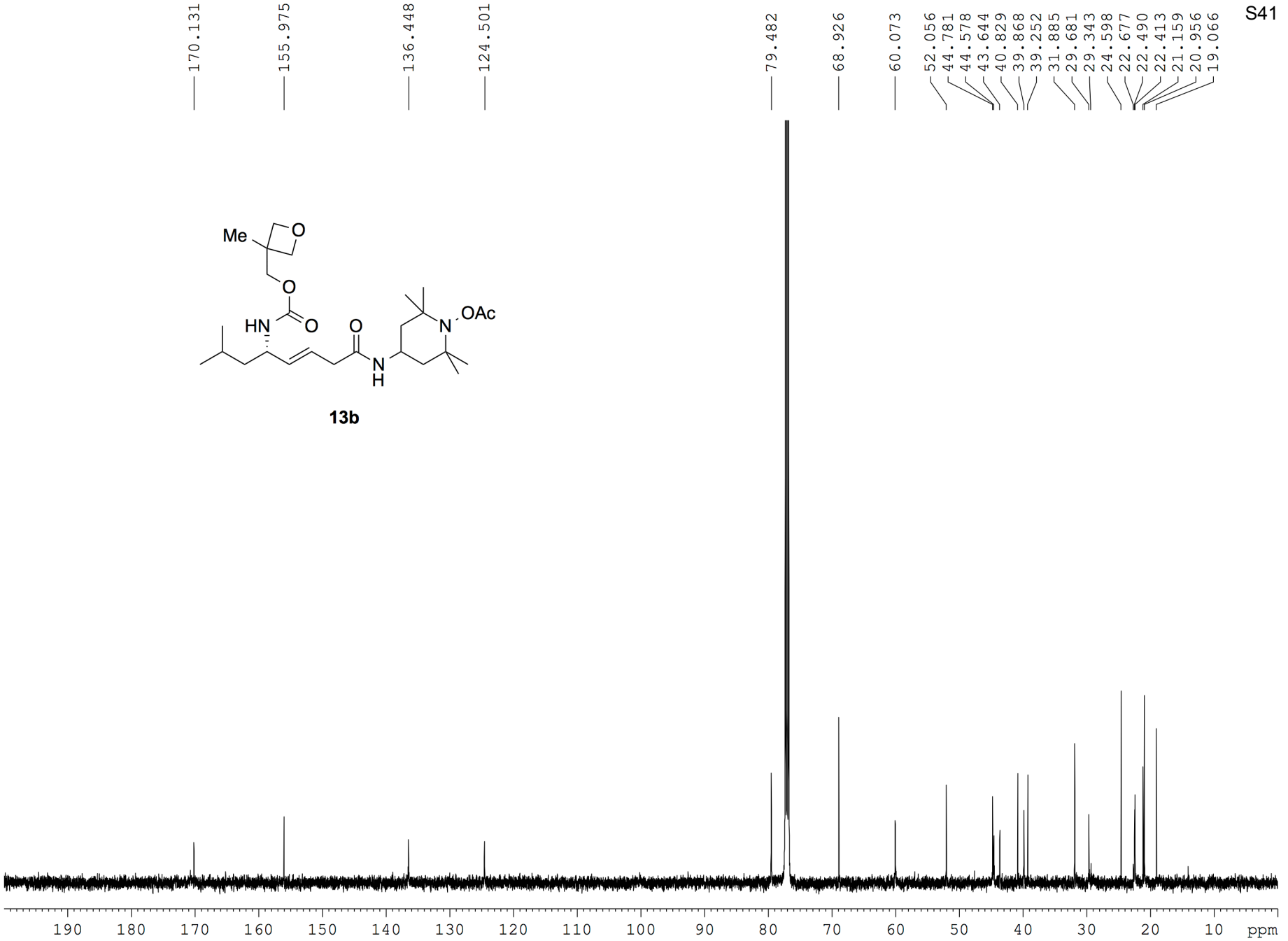
7.54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44.578
43.644
40.829
39.868
39.252
31.885
29.681
29.343
24.598
22.677
22.490
22.413
21.159
20.956
19.066
Me
O
O
HN
O
O
N
OAc
N
H
190
180
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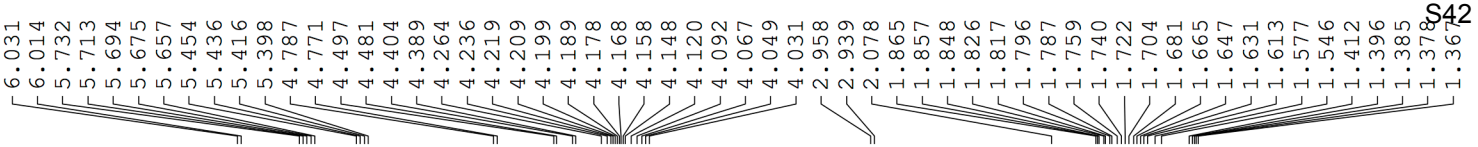
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

13b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5.454
5.436
5.416
5.398
4.787
4.771
4.497
4.481
4.404
4.389
4.264
4.236
4.219
4.209
4.199
4.189
4.178
4.168
4.158
4.148
4.120
4.092
4.067
4.049
4.031
2.958
2.939
2.078
1.865
1.857
1.848
1.826
1.817
1.796
1.787
1.759
1.740
1.722
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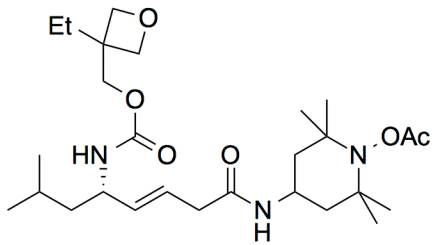
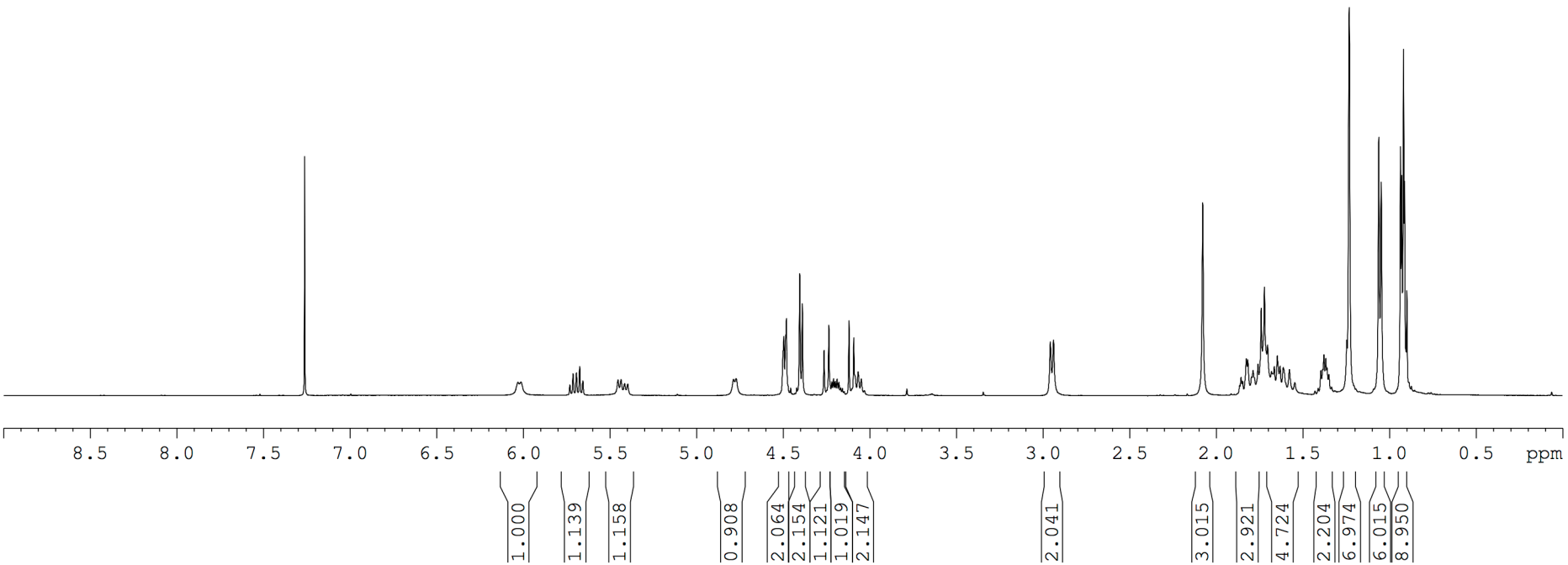
1.367


Et
O
O
HN
O
O
N
OAc
N
H

13c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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
ppm
1.000
1.139
1.158
0.908
2.064
2.154
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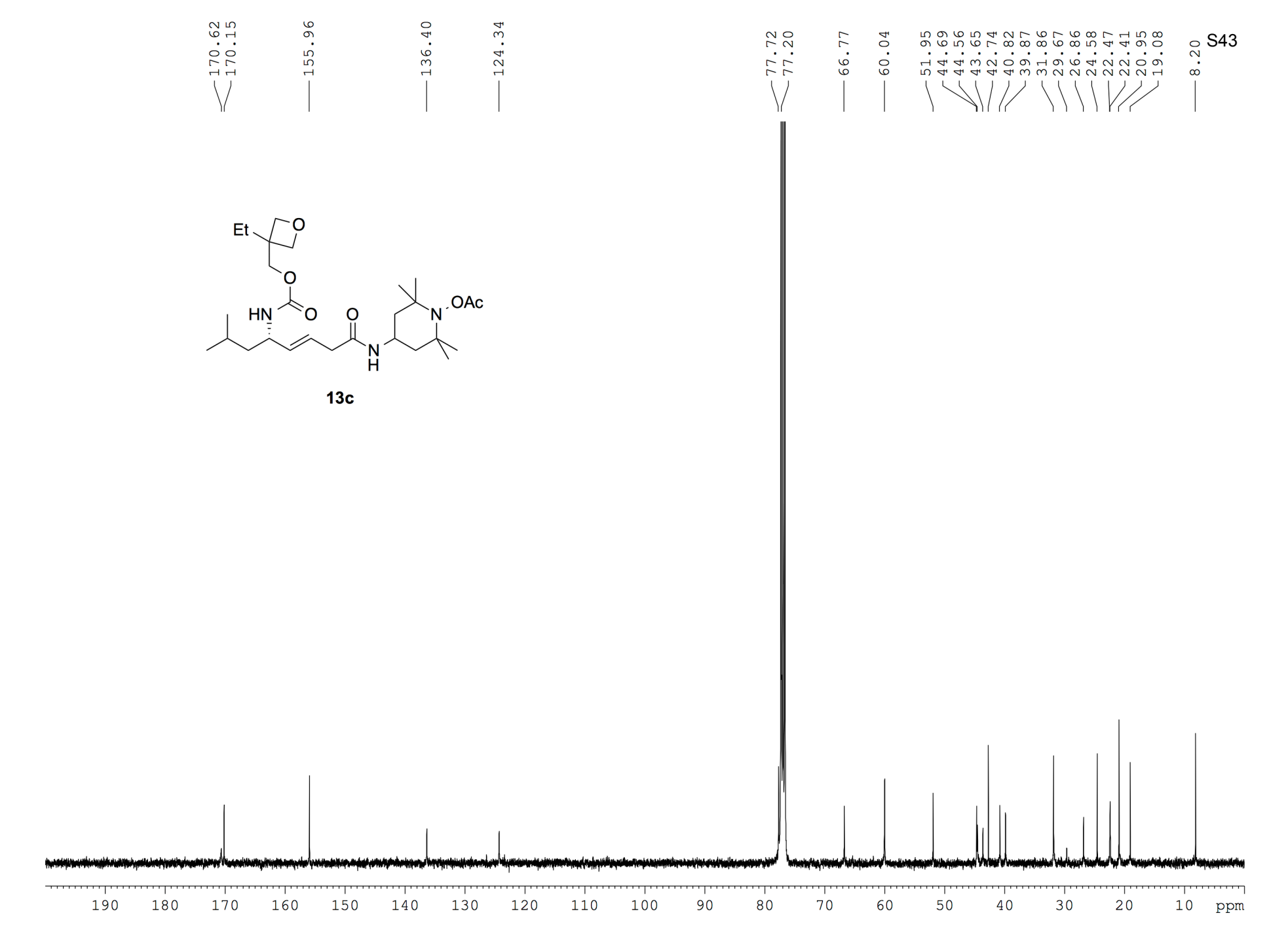
2.147
2.041
3.015
2.921
4.724
2.204
6.974
6.015
8.950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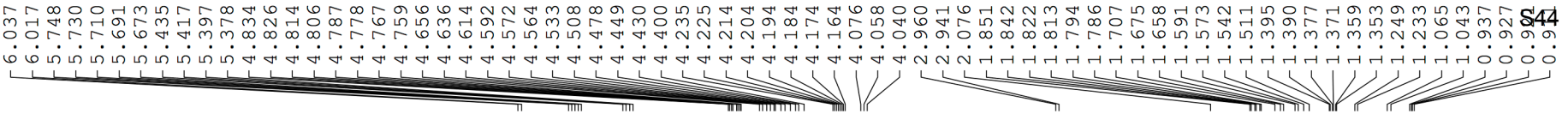
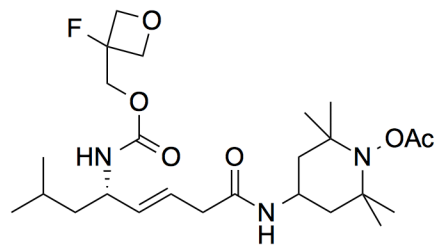
77.72
77.20
66.77
60.04
51.95
44.69
44.56
43.65
42.74
40.82
39.87
31.86
29.67
26.86
24.58
22.47
22.41
20.95
19.08
8.20
Et
O
O
HN
O
O
N
OAc
N
H
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

13c





13d

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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
ppm
0.93
1.04
1.00
2.78
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1.84
2.71
1.84
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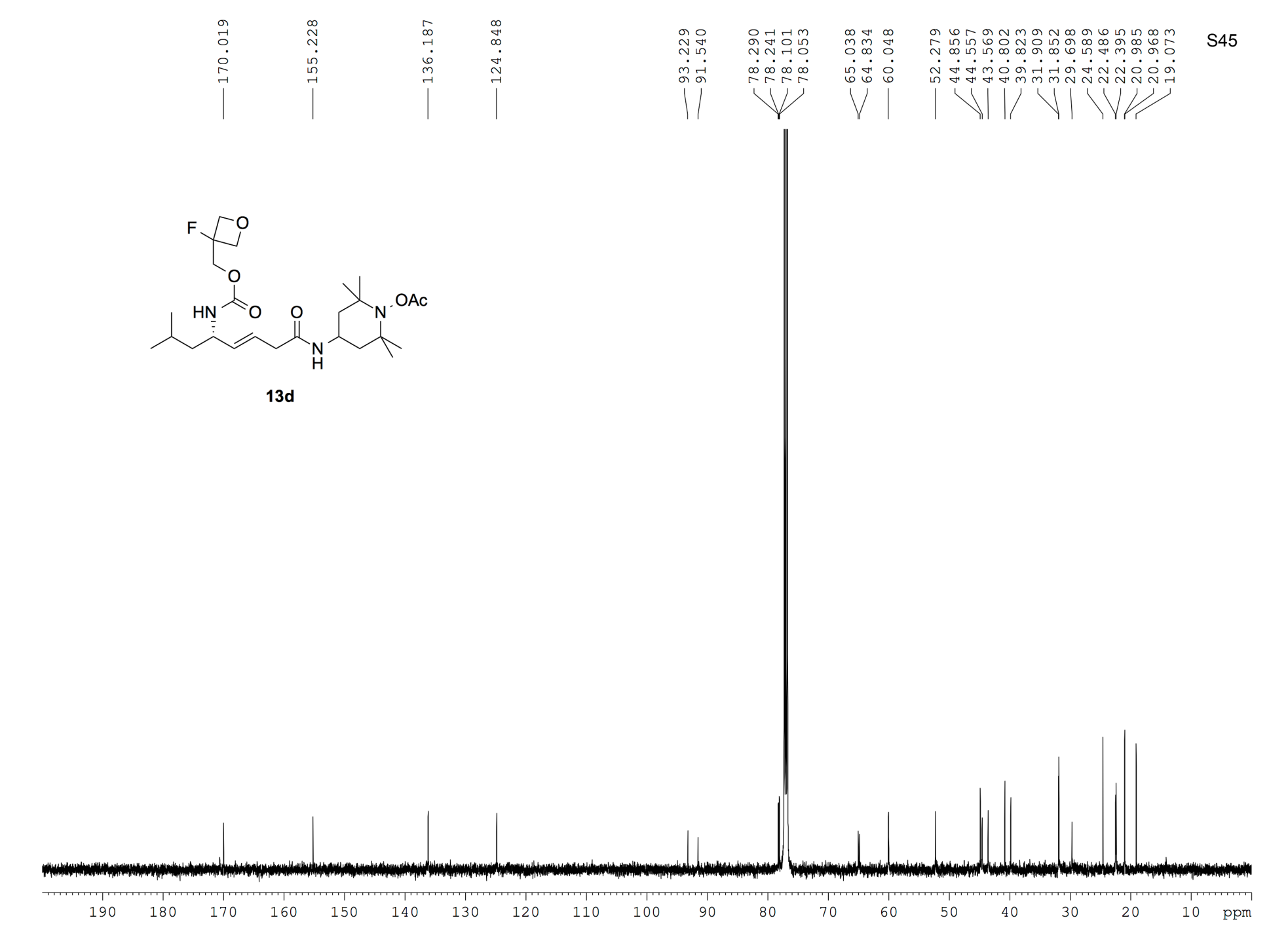
1.37
2.02
7.12
5.79
6.23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78.241
78.101
78.053
65.038
64.834
60.048
52.279
44.856
44.557
43.569
40.802
39.823
31.909
31.852
29.698
24.589
22.486
22.395
20.985
20.968
19.073
F
O
O
HN
O
O
N
OAc
N
H
190
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130
120
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100
90
80
70
60
50
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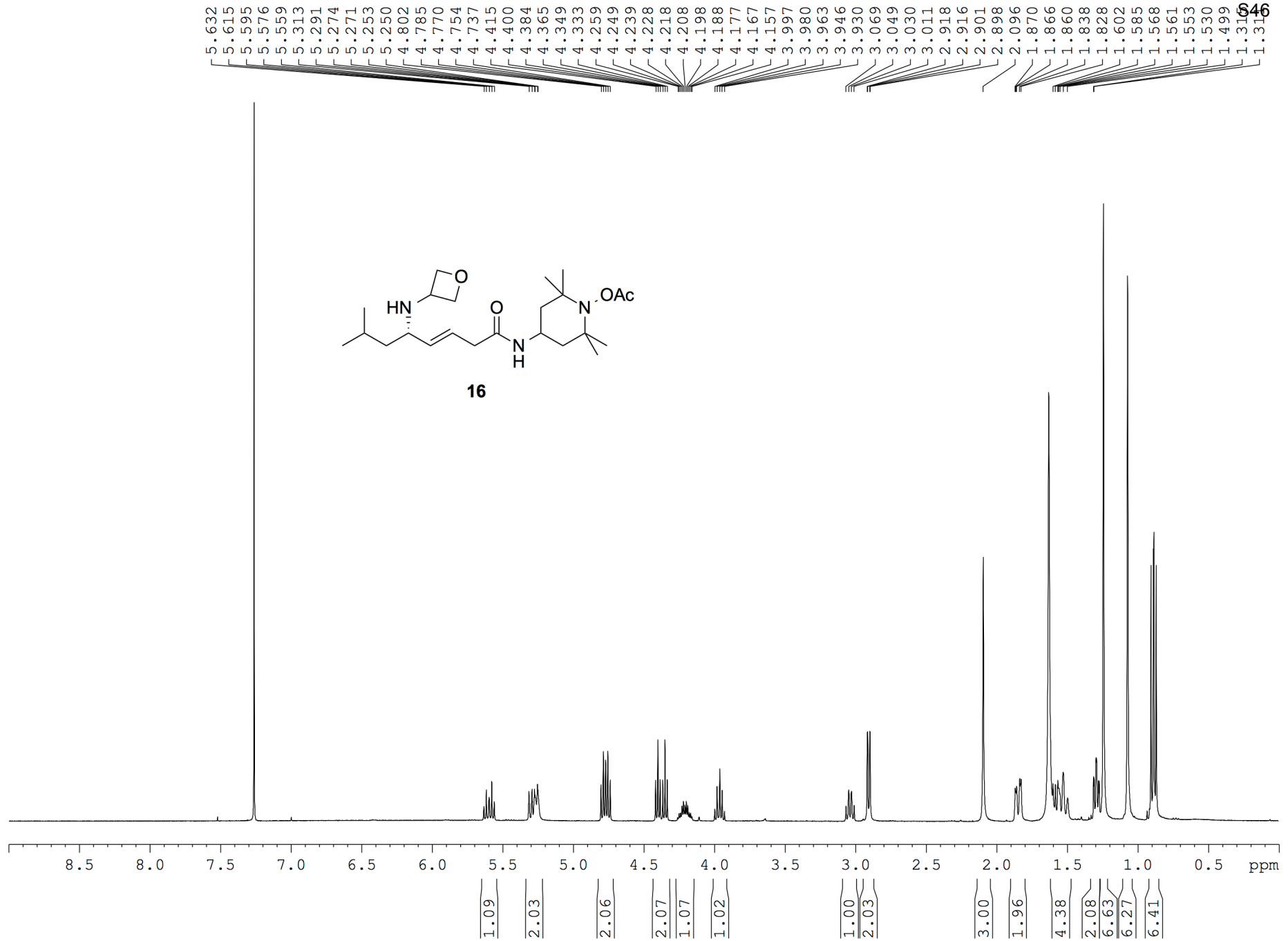
30
20
10
ppm

13d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5.559
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5.271
5.253
5.250
4.802
4.785
4.770
4.754
4.737
4.415
4.400
4.384
4.365
4.349
4.333
4.259
4.249
4.239
4.228
4.218
4.208
4.198
4.188
4.177
4.167
4.157
3.997
3.980
3.963
3.946
3.930
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3.049
3.030
3.011
2.918
2.916
2.901
2.898
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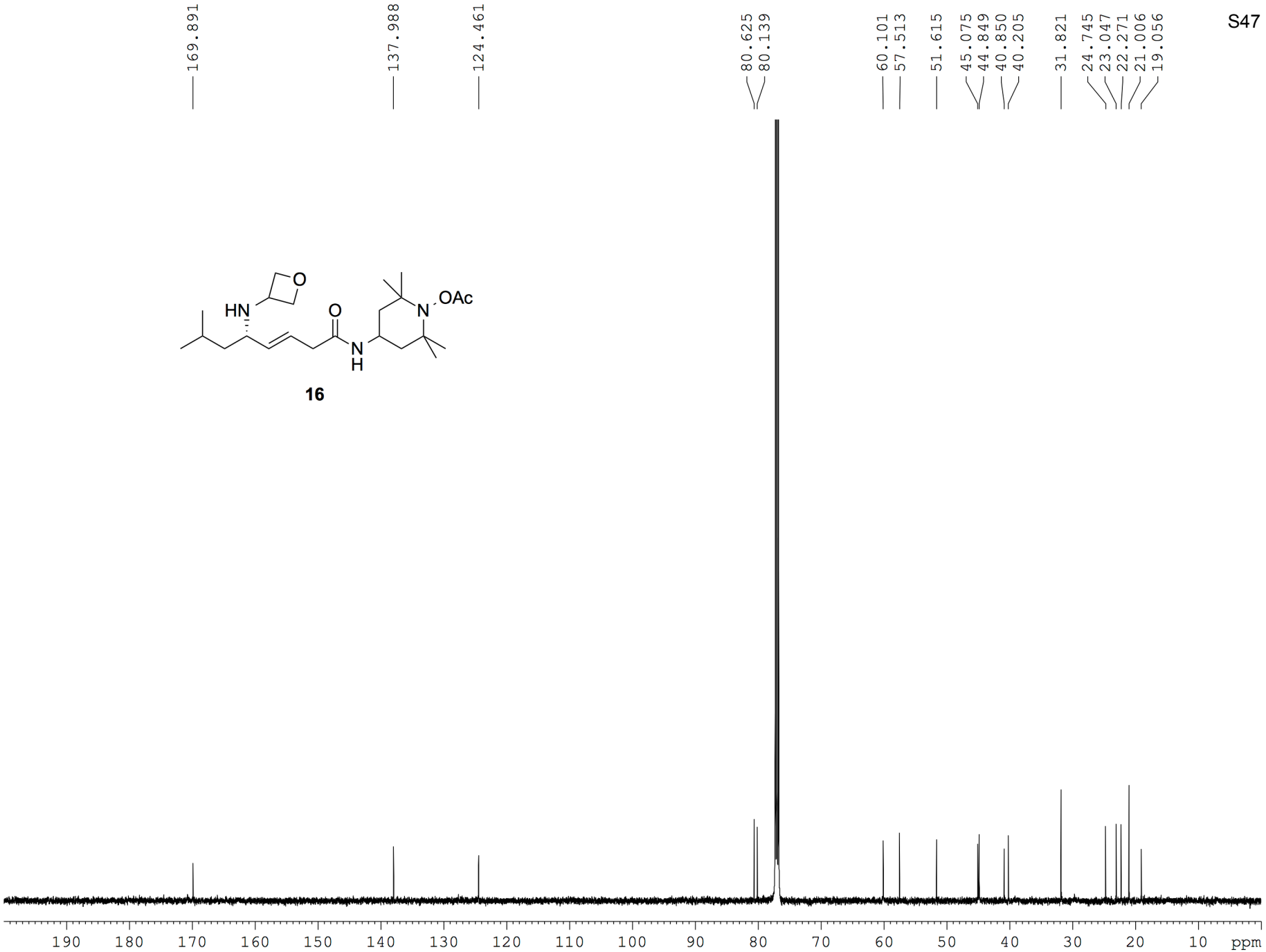
1.838
1.828
1.602
1.585
1.568
1.561
1.553
1.530
1.499
HN
O
O
N
H
N
OAc
16
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.09
2.03
2.06
2.07
1.07
1.02
1.00
2.03
3.00
1.96
4.38
2.08
6.63
6.27
6.41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137.988
124.461
80.625
80.139
60.101
57.513
51.615
45.075
44.849
40.850
40.205
31.821
24.745
23.047
22.271
21.006
19.056
HN
O
O
N
H
N
OAc
16
190
180
170
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150
140
130
120
110
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90
80
70
60
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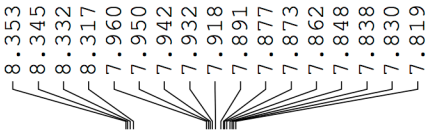
30
20
10
ppm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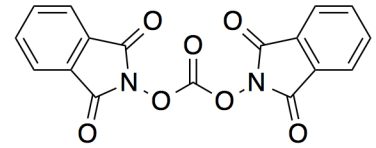
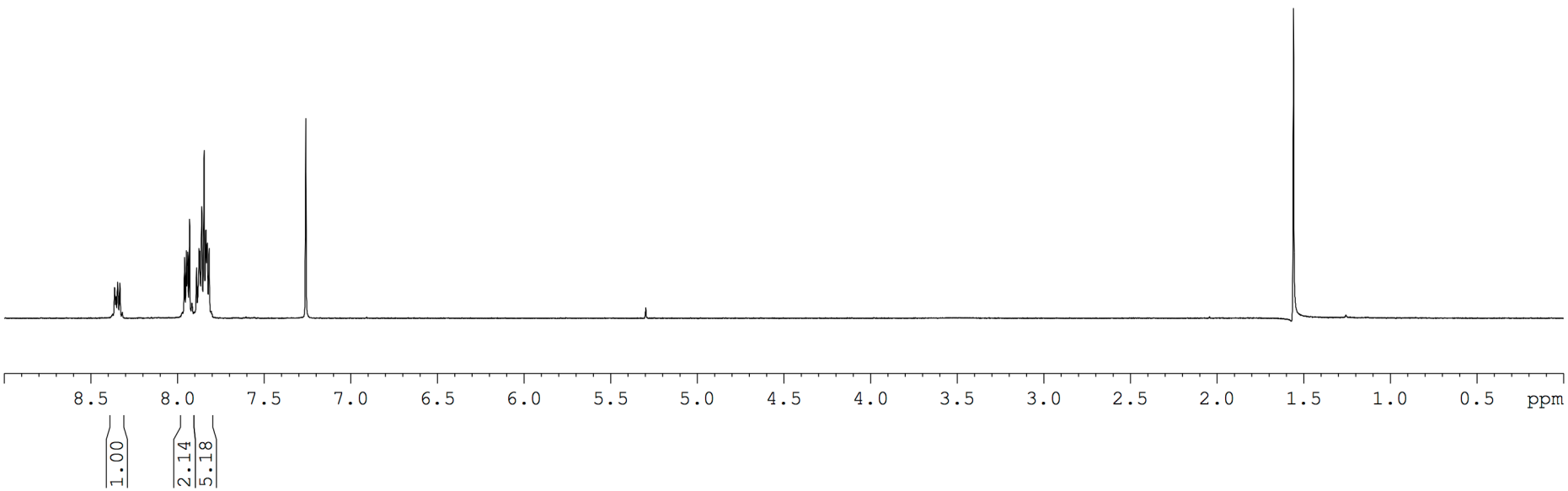
8.345
8.332
8.317
7.960
7.950
7.942
7.932
7.918
7.891
7.877
7.873
7.862
7.848
7.838
7.830
7.819



19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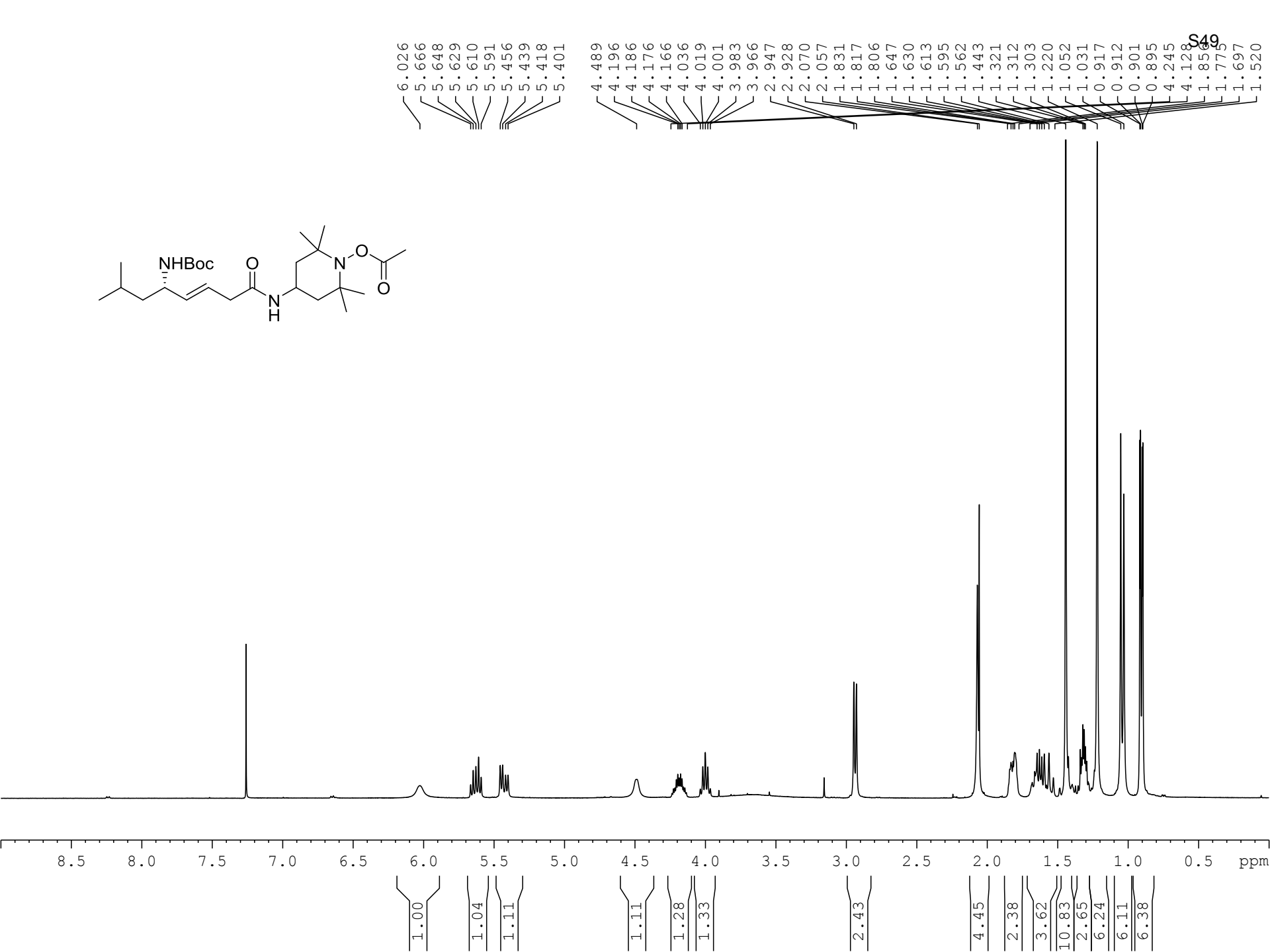
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
2.14
5.18


6.026
5.666
5.648
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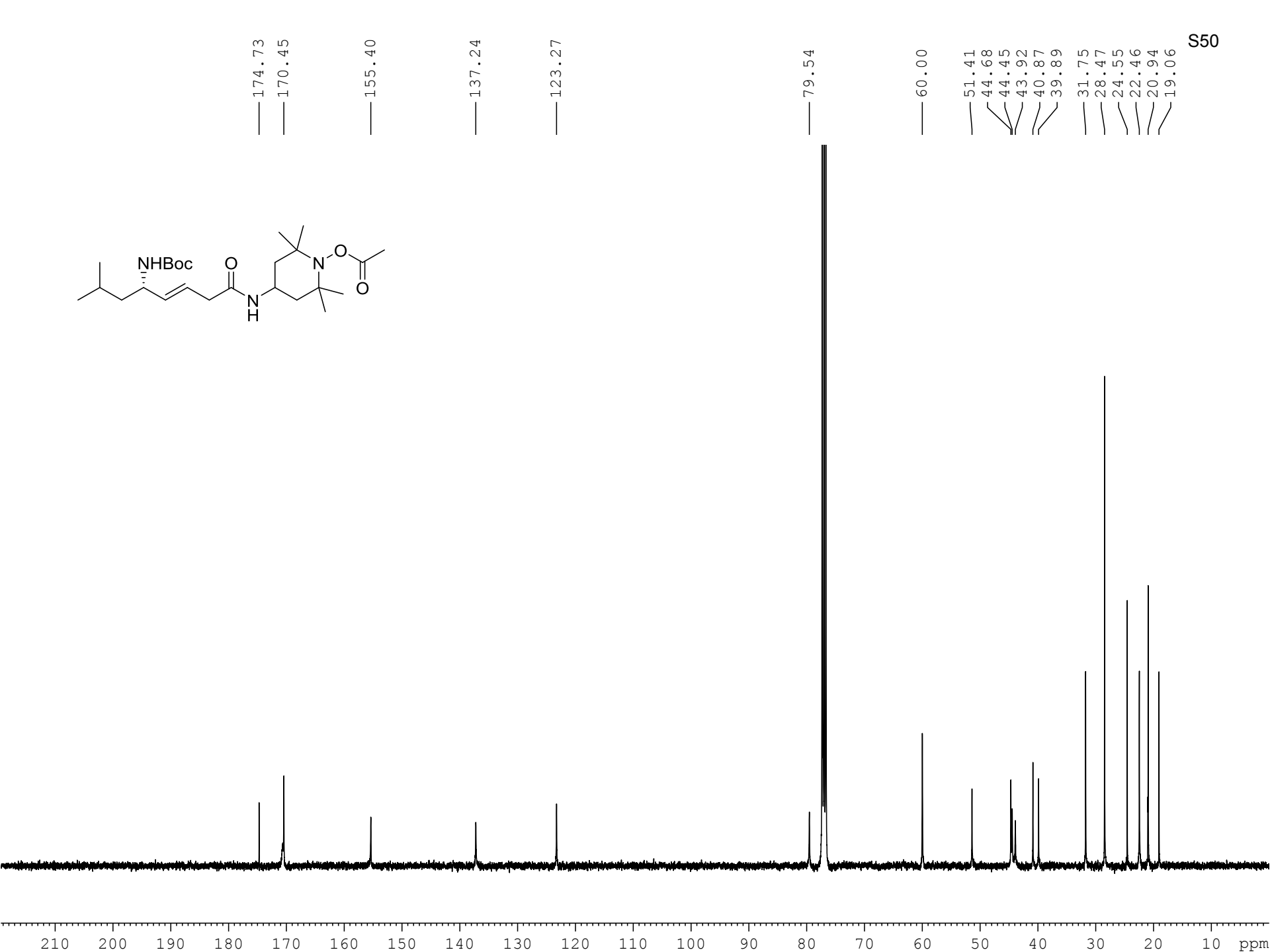
5.610
5.591
5.456
5.439
5.418
5.401
4.489
4.196
4.186
4.176
4.166
4.036
4.019
4.001
3.983
3.966
3.947
3.928
2.070
2.057
1.831
1.817
1.806
1.647
1.630
1.613
1.595
1.562
1.443
1.321
1.312
1.303
1.220
1.052
1.031
0.917
0.912
0.901
0.895
4.245
4.128
1.856
1.775
1.697
1.520
NHBoc
O
N
H
N
O
O
8.5
8.0
7.5
7.0
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
ppm
1.00
1.04
1.11
1.11
1.28
1.33
2.43
4.45
2.38
3.62
10.83
2.65
6.24
6.11
6.38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155.40
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123.27
79.54
60.00
51.41
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44.45
43.92
40.87
39.89
31.75
28.47
24.55
22.46
20.94
19.06
NHBoc
O
N
H
N
O
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm