Sulfur-Tetrazine as Highly Efficient Visible-Light Activatable Photo-Trigger for Designing Photoactivatable Fluorescence Molecules

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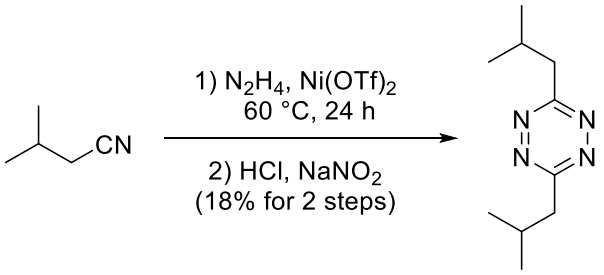
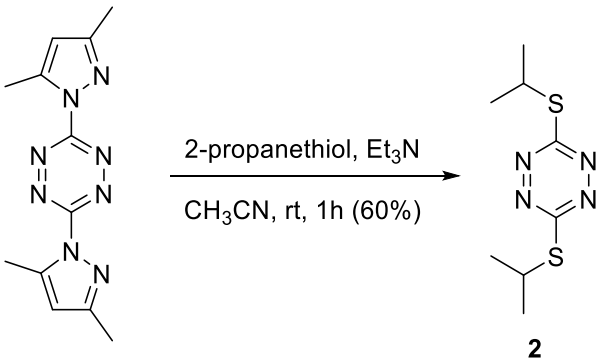
Supporting Information

1. Synthesis and Characterization



1

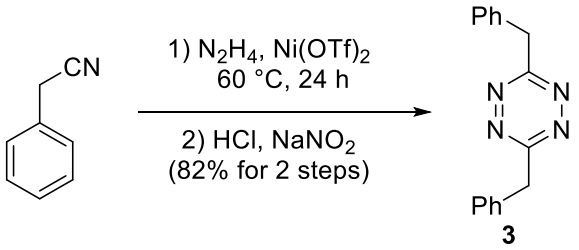
Synthesis of compound 1. Isovaleronitrile (200 μL, 1.91 mmol), Nickel trifluoromethanesulfonate (340 mg, 0.95 mmol) were loaded into a Schlenk tube under N_2 flow, followed by the addition of hydrazine (3 mL, 95.5 mmol). The reaction was stirred for 24 h at 60 °C, then, cooled to 0 °C, sodium nitrite (1.31 g, 19.1 mmol) in 10 mL of water was added, and the pH was adjusted to 3.0 with 1M HCl, the organic phase was extracted with dichloromethane, dried with anhydrous sodium sulfate, and filtered, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 5% ethyl acetate in hexane). Compound **1** (34 mg, 18% yield) was obtained ad a dark red oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.16 (d, *J* = 7.2 Hz, 2H), 2.37 (dh, *J* = 13.6, 6.8 Hz, 1H), 1.01 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.59, 43.59, 28.64, 22.44. ESIMS $[M+H]^+$ calcd. for $C_{10}H_{18}N_4$ 195.15, found 195.15.



2

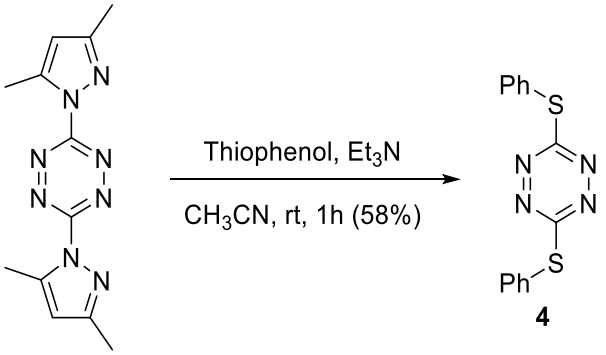
Synthesis of compound 2. To a solution of pyrazole tetrazine (27 mg, 0.1 mmol) and triethylamine (7 μL, 0.05 mmol) in acetonitrile (10 mL), 2-propanethiol (18.5 μL, 0.2 mmol) was added dropwise. The resulting solution was stirred at room temperature for 1 hour. The solution was extracted three times with ethyl acetate, dried with sodium sulfate, and the solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, 10% ethyl acetate in hexanes) to afford compound **2** as a red solid (14 mg, 60% yield). ^{1}H NMR (600 MHz,

$CDCl_3$) δ 4.09 (hept, *J* = 6.8 Hz, 2H), 1.50 (d, *J* = 6.8 Hz, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.67, 36.21, 22.80. ESIMS $[M+H]^+$ calcd. for $C_8H_{14}N_4S_2$ 231.06, found 231.07.



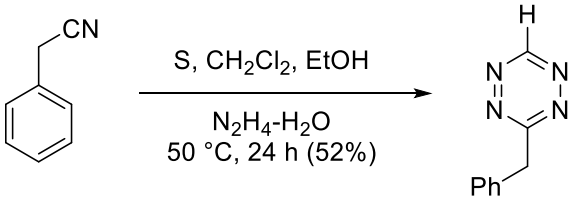
3

Synthesis of compound 3. Phenylacetoitrile (250 mg, 2.13 mmol), nickel trifluoromethanesulfonate (390 mg, 1.06 mmol) were loaded into a Schlenk tube under N_2 flow, followed by the addition of hydrazine (3.4 mL, 106 mmol). The reaction was stirred for 24 h at 60 °C, then, cooled to 0 °C, sodium nitrite (1.46 g, 21.3 mmol) in 10 mL of water was added, and the pH was adjusted to 3.0 with 1M HCl, the organic phase was extracted with dichloromethane, dried with anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 10% ethyl acetate in hexane). Compound **3** (228 mg, 82% yield) was obtained as a dark red solid. ^1H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.5 Hz, 4H), 7.31 (t, *J* = 7.6 Hz, 4H), 7.24 (d, *J* = 7.3 Hz, 2H), 4.60 (s, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.34, 135.95, 129.42, 129.04, 127.51, 41.39. ESIMS $[M+H]^+$ calcd. for $C_{16}H_{14}N_4$ 263.12, found 263.12.



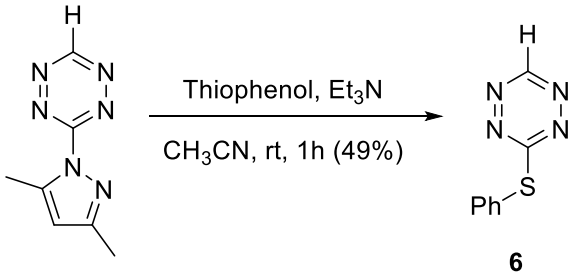
4

Synthesis of compound 4. To a solution of pyrazole tetrazine (27 mg, 0.1 mmol) and triethylamine (7 μL, 0.05 mmol) in acetonitrile (10 mL), thiophenol (22 mg, 0.2 mmol) was added dropwise. The resulting solution was stirred at room temperature for 1 hour. The solution was extracted three times with ethyl acetate, dried with sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 10% ethyl acetate in hexanes) to afford compound **4** as a red solid (17 mg, 58% yield). ^1H NMR (600 MHz, $CDCl_3$) δ 7.65 – 7.60 (m, 4H), 7.47 – 7.41 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.11, 135.74, 130.52, 129.95, 125.86. ESIMS $[M+H]^+$ calcd. for $C_{14}H_{10}N_4S_2$ 299.03, found 299.04.



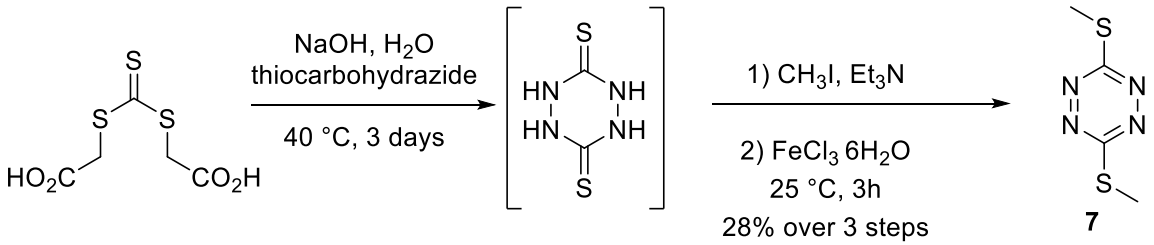
5

Synthesis of compound 5. Phenylacetoitrile (235 mg, 2 mmol), Sulfur (128 mg, 4 mmol) and dichloromethane (127 μL, mmol) were loaded into a Schlenk tube under N_2 flow, followed by the addition of hydrazine hydrate (0.8 mL, 16 mmol). The reaction was stirred for 24 h at 50 °C, then, cooled to 0 °C, sodium nitrite (1.4 g, 20 mmol) in 10 mL of water was added, and the pH was adjusted to 3.0 with 1M HCl, the organic phase was extracted with dichloromethane, dried with anhydrous sodium sulfate and filtered, the solvent was removed under reduced pressure and then purified by column chromatography (silica gel, 5% ethyl acetate in hexane). Compound **5** (180 mg, 52% yield) was obtained as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.19 (s, 1H), 7.42 (d, *J* = 7.2 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 3H), 4.66 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.03, 158.14, 135.65, 129.38, 129.11, 128.05, 127.63, 41.91. ESIMS $[M+H]^+$ calcd. for $C_9H_4N_4$ 173.07, found 173.06.



6

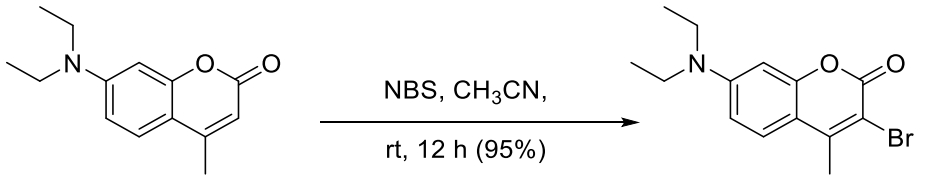
Synthesis of compound 6. To a solution of 3H-pyrazole tetrazine (6 mg, 0.03 mmol) and triethylamine (7 μL, 0.05 mmol) in acetonitrile (5 mL), thiophenol (3.8 mg, 0.034 mmol) was added dropwise. The resulting solution was stirred at room temperature for 1 hour. The solution was extracted three times with ethyl acetate, dried with sodium sulfate, and the solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, 10% ethyl acetate in hexanes) to afford compound **6** as a red solid (3.1 mg, 49% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.97 (s, 1H), 7.68 (dd, *J* = 7.9, 1.5 Hz, 2H), 7.53 – 7.48 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.57, 156.25, 144.30, 135.77, 130.80, 130.12. ESIMS $[M+H]^+$ calcd. for $C_8H_6N_4S$ 191.03, found 191.05.



7

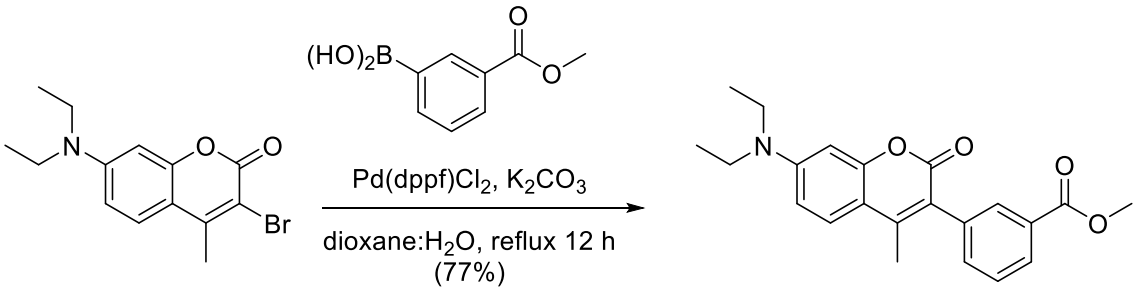
Synthesis of compound 7. A suspension of bis(carboxymethyl)trithiocarbonate (4.62 g, 20.4 mmol) in H_2O (60 mL) was treated with NaOH (1.63 g, 40.8 mmol). The resulting mixture was

added to a suspension of thiocarbohydrazide (2.21 g, 20.4 mmol) in H_2O (40 mL) under a N_2 and stirred at 40 °C for 3 days. The white precipitate was then collected by filtration, washed with H_2O (30 mL × 2), and dried to afford 3,6- bis(dithiotetrahydro)-1,2,4,5-tetrazine (1.36 g, 45%). A suspension of 3,6-bis(dithiotetrahydro)-1,2,4,5-tetrazine (1.36 g, 9.1 mmol) in EtOH (15 mL) was treated with Et_3N (2.58 mL, 18.3 mmol) and MeI (1.2 mL, 18.3 mmol) under N2 atmosphere at 0°C. The mixture was warmed to room temperature and stirred for 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in EtOH (15 mL) and treated with $FeCl_3 \cdot 6H_2O$ (6.1 g, 22.8 mmol). The mixture was stirred for additional 3 h at room temperature, then diluted with H_2O (30 mL) and extracted with EtOAc (30 mL × 3). The combined organic phase was washed with saturated aqueous NaCl, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, 10% ethyl acetate in hexanes) to afford compound **7** (880 mg, 28% over 3 steps) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.70 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.93, 13.57. ESIMS $[M+H]^+$ calcd. for $C_4H_6N_4S_2$ 175.00, found 175.01.



8

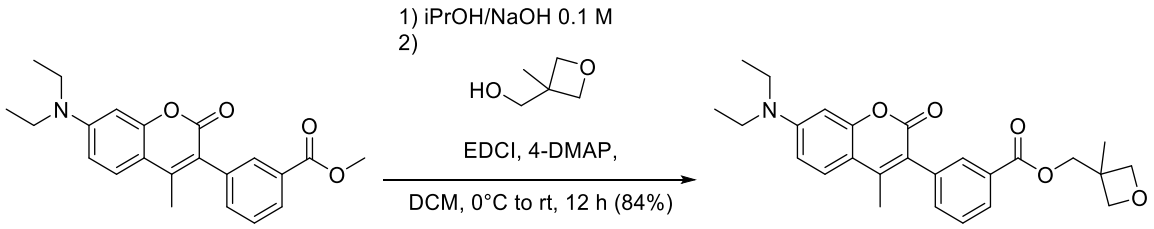
Synthesis of compound 8. 7-Diethylamino-4-methyl coumarin (2.31g, 10 mmol) was dissolved in acetonitrile (50 mL), under N_2 atmosphere. N-bromosuccinimide (1.95 g, 11 mmol) was added slowly and the resulting solution was stirred for 12 h. The reaction mixture was washed with water, the organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 20% ethyl acetate in hexanes) to afford compound **8** as a yellow solid (3.26 g, 95% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, *J* = 9.1 Hz, 1H), 6.60 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.48 (d, *J* = 2.6 Hz, 1H), 3.41 (q, *J* = 7.2 Hz, 4H), 2.52 (s, 3H), 1.20 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 158.34, 154.58, 151.66, 150.73, 126.20, 109.09, 105.75, 97.37, 44.92, 19.27, 12.55. ESIMS $[M+H]^+$ calcd. for $C_{14}H_{16}BrNO_2$ 310.03, 312,03, found 310.03, 312,03.



8 **9**

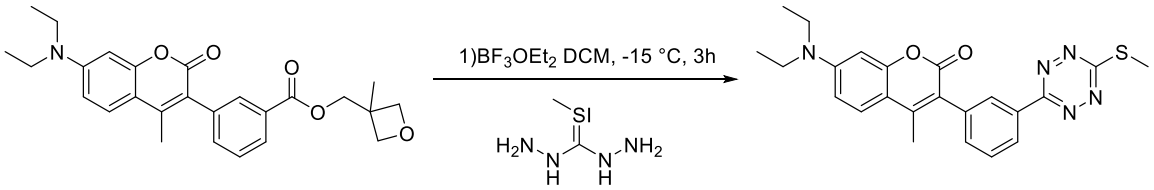
Synthesis of compound 9. Compound **8** (621 mg, 2 mmol), 3-(Methoxycarbonyl)phenylboronic acid (540 mg, 3 mmol), K_2CO_3 (829 mg, 6 mmol), and [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride ($Pd(dppf)Cl_2$) (73 mg, 0.1 mmol) were

charged into a pressure tube under N_2 atmosphere. Dioxane and water (3:1, 15 mL) were added and the resulting solution was refluxed for 12 h. The reaction mixture was cooled to room temperature and extracted three times with ethyl acetate. The organic layers were combined, dried over sodium sulfate, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 20% ethyl acetate in hexanes) to afford compound **9** as a yellow solid (587 mg, 77% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (ddd, *J* = 6.1, 2.8, 1.7 Hz, 1H), 7.98 (q, *J* = 1.1 Hz, 1H), 7.52 – 7.49 (m, 2H), 7.46 (d, *J* = 9.0 Hz, 1H), 6.62 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.55 (d, *J* = 2.7 Hz, 1H), 3.91 (s, 3H), 3.43 (q, *J* = 7.2 Hz, 4H), 2.22 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.30, 167.11, 162.02, 155.32, 150.56, 148.97, 135.83, 135.36, 131.90, 130.37, 128.97, 128.55, 126.32, 120.13, 109.43, 108.80, 97.60, 60.53, 52.27, 44.91, 16.48, 12.60. ESIMS $[M+H]^+$ calcd. for $C_{22}H_{23}NO_4$ 366.16, found 366.16.



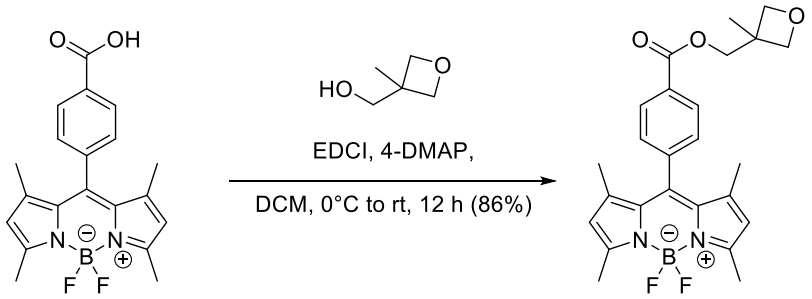
9 **10**

Synthesis of compound 10. Compound **9** (365 mg, 1 mmol) was dissolved in a 1:1 mixture of isopropyl alcohol/NaOH 0.1M (15 mL). The solution was stirred for 4 h until IPA was evaporated under reduced pressure. The solution was acidified with aq. HCl 0.5 M until pH = 5. The solution was extracted three times with dichloromethane. The organic layers were collected, dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The intermediate Coumarin-CO_2H was used directly into the next step without further purification. Coumarin-CO_2H was added to a solution of 3-methyl-2-oxetanemethanol (112 mg, 1.1 mmol), EDCI (191 mg, 1 mmol) and 4-DMAP (12 mg, 0.1 mmol) in dichloromethane (15 mL) at 0 °C under N_2. The solution was allowed to warm slowly to room temperature and stirred for a total of 12 h. The solution was diluted with DCM, washed with sodium bicarbonate$_{(aq.)}$, the organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel 35% ethyl acetate in hexanes to afford compound **10** as a yellow solid (365 mg, 84% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 – 8.03 (m, 1H), 7.97 (td, *J* = 1.7, 0.7 Hz, 1H), 7.56 – 7.51 (m, 2H), 7.47 (d, *J* = 9.0 Hz, 1H), 6.63 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.55 (d, *J* = 2.5 Hz, 1H), 4.64 (d, *J* = 6.0 Hz, 2H), 4.45 (d, *J* = 6.0 Hz, 2H), 4.41 (s, 2H), 3.44 (q, *J* = 7.1 Hz, 4H), 2.23 (s, 3H), 1.43 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.41, 161.92, 155.19, 150.42, 148.91, 135.83, 135.52, 131.77, 129.98, 128.89, 128.54, 126.22, 119.88, 109.25, 108.69, 97.44, 79.68, 69.16, 44.80, 39.31, 21.32, 16.37, 12.46. ESIMS $[M+H]^+$ calcd. for $C_{26}H_{29}NO_5$ 436.20, found 435.19.



10 **11**

Synthesis of compound 11. (3-Methyloxetan-3-yl)methyl carboxylic ester Compound **24** (86 mg, 0.2 mmol) was dissolved in dichloromethane (0.2 mL) in a pressure tube under N_2 and the solution was cooled to -15 °C using an ice-salt bath. Boron trifluoride diethyl etherate (25 μL, 0.2 mmol) was added to the solution and stirred at -15 °C for 3 h. Pyridine (50 μL, 0.62 mmol), DMF (200 μL) and methyl thiocarbohydrazide (50 mg, 0.2 mmol) were added. The resulting solution was heated to 80 °C and stirred for an additional 1 h. The solution was cooled to room temperature, PIDA (66 mg, 0.2 mmol) was added slowly, and the mixture was stirred for 1 h at room temperature. The solution was washed with aq. sodium bicarbonate and extracted three times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered,5 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 20% ethyl acetate in hexanes) to afford SMeTz-Coumarin **11** as a red solid (32 mg, 38 % yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 (dt, *J* = 7.9, 1.5 Hz, 1H), 8.47 (t, *J* = 1.8 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.58 (dt, *J* = 7.6, 1.5 Hz, 1H), 7.48 (d, *J* = 9.1 Hz, 1H), 6.64 (dd, *J* = 9.1, 2.7 Hz, 1H), 6.57 (d, *J* = 2.7 Hz, 1H), 3.44 (q, *J* = 7.1 Hz, 4H), 2.80 (s, 3H), 2.30 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.45, 162.40, 162.05, 155.37, 150.60, 149.02, 136.75, 134.82, 131.83, 129.86, 129.45, 126.86, 126.37, 120.25, 109.49, 108.85, 97.66, 44.95, 16.57, 13.58, 12.63. HRMS $[M+H]^+$ calcd. for $C_{23}H_{23}N_5O_2S$ 434.1651, found 434.1649.

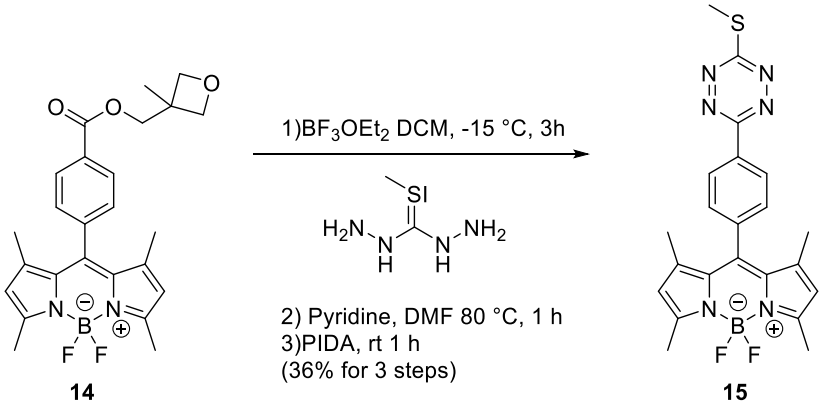


13 **14**

Compound **13** was obtained following the procedure reported in 2020[1].

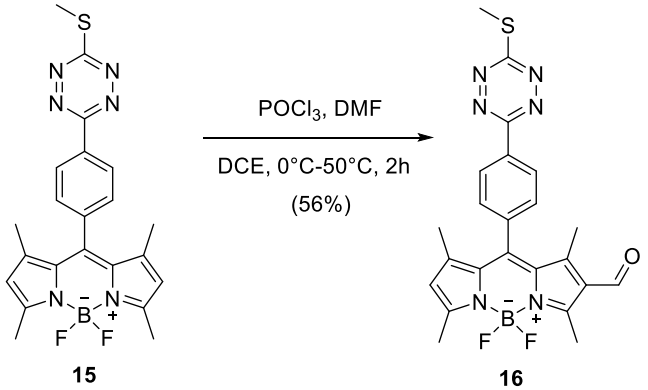
Synthesis of compound 14. BODIPYpPhCO_2H **13** (368 mg, mmol) was added to a solution of 3-methyl-2-oxetanemethanol (112 mg, 1.1 mmol), EDCI (191 mg, 1 mmol) and 4-DMAP (12 mg, 0.1 mmol) in dichloromethane (15 mL) at 0 °C under N_2. The solution was allowed to warm slowly to room temperature and stirred for a total of 12 h. The solution was diluted with DCM, washed with aq. sodium bicarbonate, the organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 30% ethyl acetate in hexanes to afford compound **14** as a red solid (388mg, 86% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.22 – 8.17 (m, 2H), 7.44 – 7.40 (m, 2H), 5.99 (s, 2H), 4.68 (d, *J* = 6.1 Hz, 2H),

4.50 (d, *J* = 6.1 Hz, 2H), 4.44 (s, 2H), 2.56 (s, 6H), 1.47 (s, 3H), 1.36 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.07, 156.20, 142.99, 140.31, 140.19, 131.02, 130.60, 128.67, 121.67, 79.74, 69.55, 39.41, 21.48, 14.79. ESIMS $[M+H]^+$ calcd. for $C_{25}H_{27}BF_2N_2O_3$ 453.20, found 453.20.



14 15

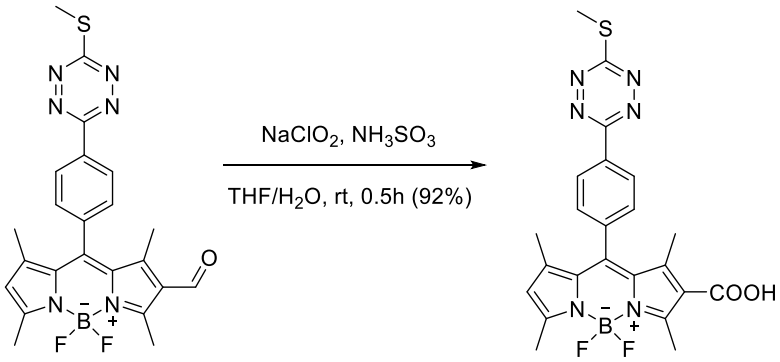
Synthesis of compound 15. BODIPYpPh(3-methyloxetan-3-yl)methyl carboxylic ester **14** (90 mg, 0.2 mmol) was dissolved in dichloromethane (0.2 mL) in a pressure tube under N_2 and the solution was cooled to -15 °C using an ice-salt bath. Boron trifluoride diethyl etherate (25 μL, 0.2 mmol) was added to the solution and stirred at -15 °C for 3 h. Pyridine (50 μL, 0.62 mmol), DMF (200 μL) and methyl thiocarbohydrazide (50 mg, 0.2 mmol) were added. The resulting solution was heated to 80 °C and stirred for an additional 1 h. The solution was cooled to room temperature, PIDA (66 mg, 0.2 mmol) was added slowly, and the mixture was stirred for 1 h at room temperature. The solution was washed with sodium bicarbonate$_{(aq.)}$ and extracted three times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 10% ethyl acetate in hexanes) to afford SMepTz-BODIPY **15** as a red solid (32 mg, 36 % yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.68 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 6.01 (s, 2H), 2.82 (s, 3H), 2.58 (s, 6H), 1.45 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.80, 161.79, 156.04, 142.90, 140.24, 139.21, 132.43, 131.04, 129.25, 128.12, 121.53, 14.65, 13.49. HRMS $[M-H]^+$ calcd. for $C_{22}H_{21}BF_2N_6S$ 451.1688, found 451.1691.



15 16

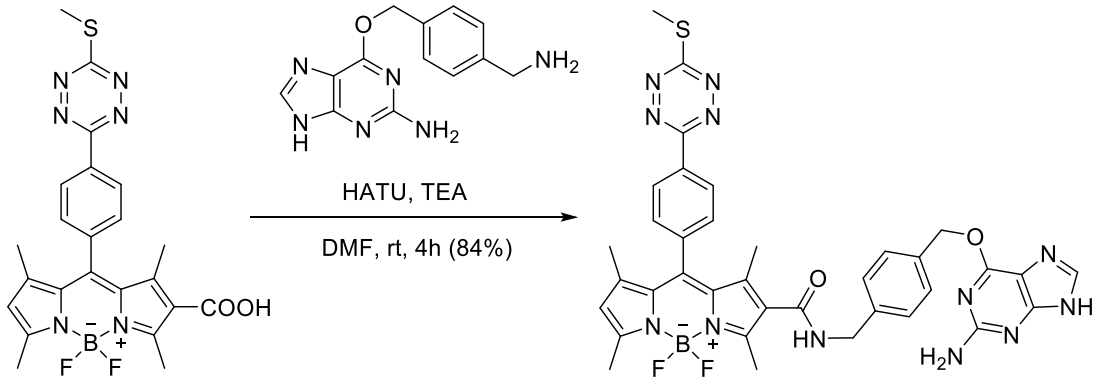
Synthesis of compound 16. Phosphoryl chloride (2 ml) and dimethylformamide (DMF) (2 ml) were mixed in a pressure tube under N_2 and the solution was cooled to 0 °C using an ice bath for 30 minutes. SMepTz-BODIPY (38 mg, 0.08 mmol) was dissolved in dichloromethane (10 mL) and then

added into the reaction tube. The resulting solution was heated to 50 °C for 2 h. The solution was cooled to room temperature, reaction was quenched by sodium bicarbonate$_{(aq.)}$ and extracted three times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 10% ethyl acetate in hexanes) to afford **16** as red solid (17 mg, 56% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.71 (d, J = 8.3 Hz, 2H), 7.53 (d, J = 8.3 Hz, 2H), 6.17 (s, 1H), 2.82 (s, 3H), 2.81 (s, 3H), 2.62 (s, 3H), 1.72 (s, 3H), 1.48 (s, 3H). ESIMS $[M+H]^+$ calcd. for $C_{23}H_{21}BF_2N_6OS$ 479.16, found 479.16. ^{13}C NMR (151 MHz, CDCl3) δ 186.28, 176.42, 162.72, 162.01, 157.40, 147.38, 143.11, 142.55, 138.62, 134.19, 133.47, 129.82, 129.42, 128.81, 126.88, 124.79, 30.12, 15.56, 13.92, 12.33.



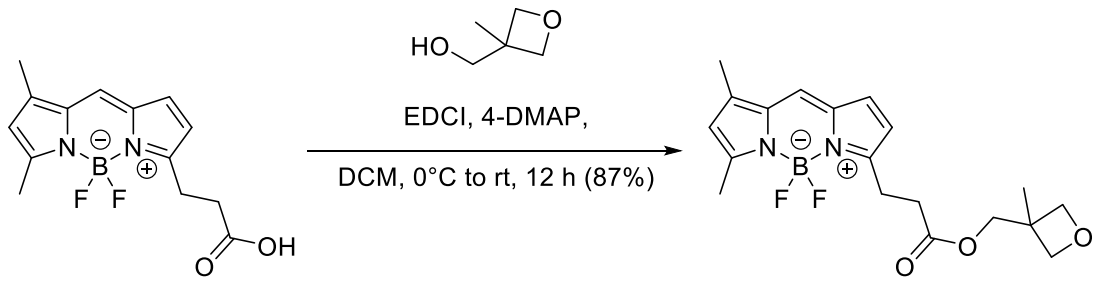
16 **17**

Synthesis of compound 17. Compound 16 (17 mg, 0.036 mmol) was dissolved in THF (6 mL) and H2O (2 mL) in a 50 mL round bottom flask, sulfamic acid (6.9 mg, 0.071 mmol, 2 eq.) and sodium chlorite (6.4 mg, 0.071 mmol) were added and stirred at room temperature for 30 minutes. The reaction was diluted with ethyl acetate and poured into a solution of sodium thiosulfate. The organic layer was extracted three times with ethyl acetate, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by column chromatography (3% methanol in dichloromethane) to afford SMepTz-BODIPY-COOH **17** (16.4 mg, 92% yield) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.76 (d, J = 8.5 Hz, 2H), 7.58 (d, J = 8.5 Hz, 2H), 6.19 (s, 1H), 2.90 (s, 3H), 2.86 (s, 3H), 2.67 (s, 3H), 1.78 (s, 3H), 1.52 (s, 3H). ESIMS $[M+H]^+$ calcd. for $C_{23}H_{21}BF_2N_6O_2S$ 495.16, found 495.17. ^{13}C NMR (151 MHz, DMSO) δ 175.06, 165.32, 161.16, 160.14, 155.07, 145.85, 142.84, 142.27, 137.41, 132.79, 132.21, 129.13, 128.66, 127.86, 123.85, 120.96, 54.62, 14.36, 13.09.



17 18

Synthesis of compound 18. Compound **17** (4 mg, 0.008 mmol), Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium (HATU, 3.34 mg, 0.0088 mmol, 1.1 eq) and 6-[[4-(Aminomethyl)phenyl]methoxy]-9H-purin-2-amine (4.32 mg, 0.016 mmol, 2 eq) were mixed in anhydrous DMF in a 20 ml vail. Triethylamine (3.3 µl, 0.024 mmol, 3 eq) was added and stirred at room temperature for 3.5 hours. The reaction was poured into DI H_2O and extracted with ethyl acetate for three times, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The product was purified by TLC to afford **SMepTz-BODIPY-SNAP (18)** (5.1 mg, 84% yield) as red solid. ^{1}H NMR (600 MHz, CDCl3_3mm_tubes) δ 8.58 (d, J = 5.8 Hz, 2H), 7.37 (s, 5H), 7.30 (d, J = 6.4 Hz, 2H), 6.05 (s, 1H), 5.32 (s, 2H), 5.05 (s, 2H), 4.57 (s, 2H), 2.78 (s, 3H), 2.70 (s, 3H), 2.57 (s, 3H), 1.43 (d, J = 9.7 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 174.91, 163.74, 161.06, 159.72, 159.51, 158.03, 155.05, 151.99, 144.42, 141.66, 139.07, 138.79, 137.65, 137.34, 135.16, 132.67, 131.39, 129.04, 128.79, 128.65, 128.40, 127.83, 127.17, 122.72, 113.35, 66.35, 54.78, 42.08, 14.33, 13.10, 12.91, 12.56. HRMS $[M+H]^+$ calcd. for $C_{36}H_{33}BF_2N_{12}O_2S$ 747.2710, found 747.2715.

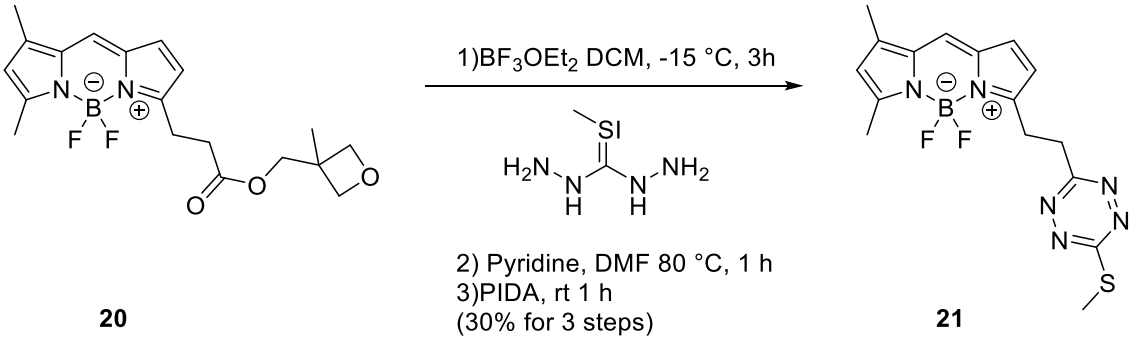


19 20

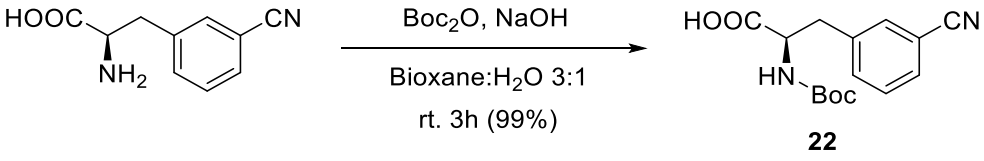
Compound **19** was obtained following the procedure reported in 2020[1].

Synthesis of compound 20. BODIPY propionic acid **19** (200 mg, 0.68 mmol) was added to a solution of 3-Methyl-2-oxetanemethanol (76 mg, 0.75 mmol), EDCI (130 mg, 0.68 mmol) and 4-DMAP (8 mg, 0.07 mmol) in dichloromethane (15 mL) at 0 °C under N_2. The solution was allowed to warm slowly to room temperature and stirred for a total of 12 h. The solution was diluted with DCM, washed with aq. sodium bicarbonate, the organic layer was dried over sodium sulfate, and

the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 25% ethyl acetate in hexanes to afford compound **20** as a thick red oil (222 mg, 87% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08 (s, 1H), 6.88 (d, *J* = 4.1 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 4.48 (d, *J* = 6.0 Hz, 2H), 4.35 (d, *J* = 6.1 Hz, 2H), 4.19 (s, 2H), 3.31 (t, *J* = 7.5 Hz, 2H), 2.82 (t, *J* = 7.5 Hz, 2H), 2.56 (s, 3H), 2.25 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.73, 160.78, 156.93, 144.12, 135.43, 133.41, 128.11, 123.98, 120.68, 116.66, 79.78, 69.12, 39.21, 33.41, 24.06, 21.29, 15.11, 11.46. ESIMS $[M+H]^+$ calcd. for $C_{19}H_{23}BF_2N_2O_3$ 337.17, found 377.16.

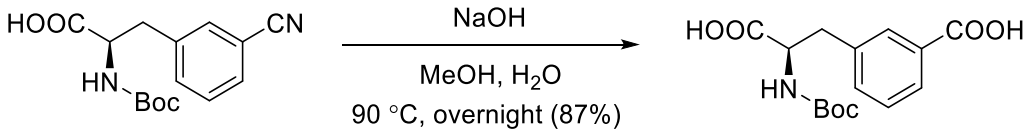


Synthesis of compound 21. (3-methyloxetan-3-yl)methyl carboxylic ester BODIPY **20** (75 mg, 0,2 mmol) was dissolved in dichloromethane (0.2 mL) in a pressure tube under N_2 and the solution was cooled to -15 °C using an ice-salt bath. Boron trifluoride diethyl etherate (25 μL, 0.2 mmol) was added to the solution and stirred at -15 °C for 3 h. Pyridine (50 μL, 0.62 mmol), DMF (200 μL) and methyl thiocarbohydrazide (50 mg, 0.2 mmol) were added. The resulting solution was heated to 80 °C and stirred for an additional 1 h. The solution was cooled to room temperature, PIDA (66 mg, 0.2 mmol) was added slowly, and the mixture was stirred for 1 h at room temperature. The solution was washed with aq. sodium bicarbonate and extracted three times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 15% ethyl acetate in hexanes) to afford SMeFretTz-BODIPY **21** as a red solid (22 mg, 30% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.09 (s, 1H), 6.87 (d, *J* = 4.1 Hz, 1H), 6.25 (d, *J* = 4.2 Hz, 1H), 6.12 (s, 1H), 3.71 (t, *J* = 8.0 Hz, 2H), 3.61 (t, *J* = 7.7 Hz, 2H), 2.73 (s, 3H), 2.56 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.71, 166.98, 161.17, 156.06, 144.31, 135.61, 133.41, 127.99, 124.05, 120.80, 116.78, 33.62, 26.85, 15.15, 13.49, 11.48. HRMS $[M+Na]^+$ calcd. for $C_{16}H_{17}BF_2N_6S$ 397.1194, found 397.1195.



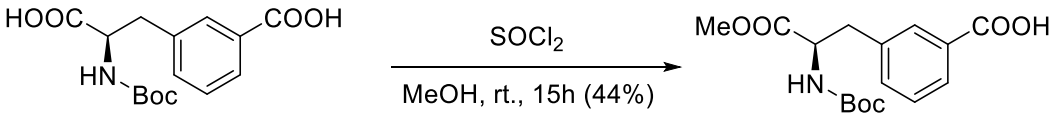
Synthesis of compound 22. 3-Cyano-L-phenylalanine (2 g, 10.55 mmol) in the mixture of dioxane 30 ml in a 200 ml round-bottom flask. Sodium hydroxide (840 mg, 21 mmol, 2 eq) was dissolved in DI water 10 ml, added into the flask slowly. Di-tert-butyl decarbonate (2.9 ml, 12.6 mmol, 1.2 eq) was added dropwise into the flask. Stir the reaction at room temperature for three hours before neutralizing with 1 N HCl and extracted with ethyl acetate for three times. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced

pressure to afford white solid **22** (3.0 g, 99% yield) without purification. ^{1}H NMR (600 MHz, MeOD) δ 7.66 – 7.56 (m, 3H), 7.47 (dd, J = 18.0, 10.4 Hz, 1H), 4.36 (dt, J = 28.6, 14.3 Hz, 1H), 3.28 – 3.18 (m, 1H), 3.01 – 2.90 (m, 1H), 1.36 (d, J = 20.6 Hz, 9H). ESIMS $[M+H]^+$ calcd. for $C_{15}H_{18}N_2O_4$ 291.13, found 291.13.



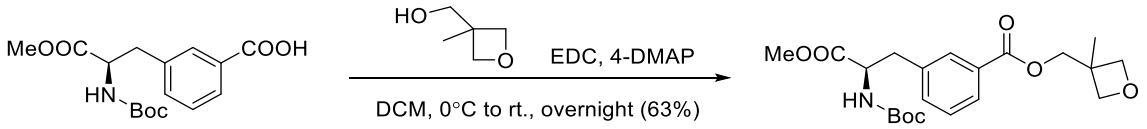
22 **23**

Synthesis of compound 23. To Compound **22** (2 g, 6.89 mmol) dissolved in methanol (17 ml) was added NaOH (1.38 g, 34.46 mmol, 5 eq) dissolved in DI water (17 ml). The reaction was heated at 90 ^{o}C overnight. The reaction was cooled down to room temperature, neutralized by 1 N HCl and extracted with ethyl acetate three times. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure to afford while solid **23** (1.8 g, 87% yield) without purification. ^{1}H NMR (600 MHz, MeOD) δ 7.91 (d, J = 8.8 Hz, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 4.40 – 4.31 (m, 1H), 3.24 (dd, J = 13.8, 4.9 Hz, 1H), 2.97 (dt, J = 13.9, 9.1 Hz, 1H), 1.35 (d, J = 18.2 Hz, 9H). ^{13}C NMR (151 MHz, MeOD) δ 175.34, 170.10, 158.03, 139.53, 135.31, 132.28, 132.07, 129.78, 129.41, 80.84, 56.42, 38.79, 28.92. ESIMS $[M+H]^+$ calcd. for $C_{15}H_{19}NO_6$ 309.13, found 309.09.



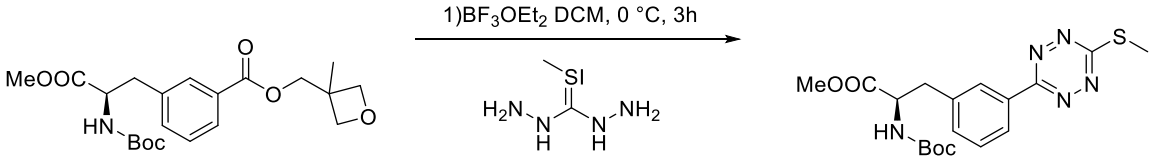
23 **24**

Synthesis of compound 24. To Compound **23** (600 mg, 1.94 mmol) dissolved in methanol (10 ml) was added $SOCl_2$ (7 µl, 0.097 mmol, 5 mol%). Stir the reaction at room temperature for 15 hours. Pour the reaction into sat. $NaHCO_3$, followed by neutralization with 1 N HCl and extraction with ethyl acetate for three times. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 10% methanol in DCM) to afford white solid **24** (274 mg, 44% yield). ^{1}H NMR (600 MHz, MeOD) δ 7.89 (d, J = 6.1 Hz, 2H), 7.44 (t, J = 10.9 Hz, 1H), 7.40 (t, J = 7.7 Hz, 1H), 4.38 (dd, J = 8.6, 5.6 Hz, 1H), 3.70 (s, 3H), 3.18 (dd, J = 13.8, 5.3 Hz, 1H), 2.97 (dd, J = 13.7, 9.2 Hz, 1H), 1.35 (s, J = 16.6 Hz, 9H). ^{13}C NMR (151 MHz, MeOD) δ 174.33, 170.14, 158.13, 139.41, 135.37, 132.10, 129.99, 129.62, 81.07, 56.76, 53.11, 38.87, 29.04. ESIMS $[M+H]^+$ calcd. for $C_{16}H_{21}NO_6$ 323.14, found 323.14.



24 **25**

Synthesis of compound 25 To the mixture of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 195 mg, 1.02 mmol, 1.2 eq) and 4-Dimethylaminopyridine (DMAP, 10 mg, 0.085 mmol, 0.1 eq) in dichloromethane 5 ml stirring in ice bath were added (3-methyloxetan-3-yl)methanol (93 µl, 0.94 mmol, 1.1 eq) and compound **24**. The reaction was stirred in ice bath for 15 minutes and then in room temperature overnight. The reaction was added into excess DMC and washed with sat. $NaHCO_3$ and brine. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 40% ethyl acetate in hexanes) to afford white solid **25** (217 mg, 63% yield). ^{1}H NMR (600 MHz, CDCl3) δ 7.89 (d, J = 7.0 Hz, 1H), 7.78 (s, 1H), 7.36 – 7.30 (m, 2H), 5.13 (d, J = 7.9 Hz, 1H), 4.58 (dd, J = 6.0, 1.0 Hz, 2H), 4.39 (dd, J = 13.9, 3.8 Hz, 2H), 4.35 (d, J = 2.3 Hz, 2H), 3.67 (s, 3H), 3.16 (dd, J = 13.8, 5.4 Hz, 1H), 3.04 (dd, J = 13.7, 6.4 Hz, 1H), 1.38 (s, 3H), 1.35 (s, 9H). ^{13}C NMR (151 MHz, CDCl3) δ 172.10, 166.37, 155.10, 136.86, 134.20, 130.49, 130.15, 128.70, 128.33, 79.58, 69.15, 54.39, 52.31, 39.34, 38.15, 28.29, 21.30. ESIMS $[M+H]^+$ calcd. for $C_{21}H_{29}NO_7$ 408.20, found 410.20.



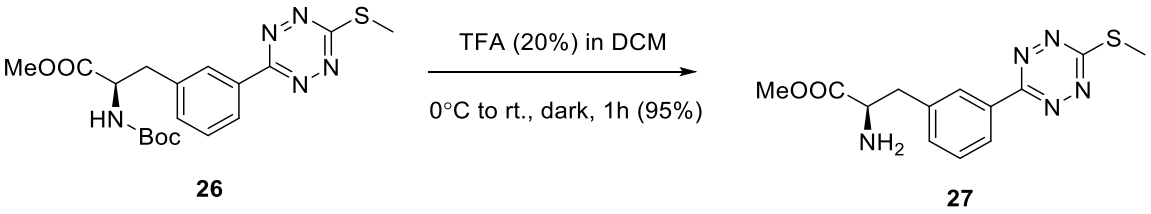
25

2) Pyridine, DMF 80 °C, 1 h
3) PIDA, rt 1 h
(30% for 3 steps)

26

Synthesis of compound 26. Compound **25** (150 mg, 0.61 mmol) was dissolved in dichloromethane (0.75 mL, 1 M) in a pressure tube under N_2 and the solution was cooled to 0 °C using an ice-salt bath. Boron trifluoride diethyl etherate (91 µL, 0.74 mmol) was added to the solution and stirred at 0 °C for 3 h. Pyridine (148 µL, 1.83 mmol), DMF (600 µl, 1 M) and methyl thiocarbohydrazide (106 mg, 0.43mmol, 0.7 eq) were added. The resulting solution was heated to 80 °C and stirred for an additional 1 h. The solution was cooled to room temperature, PIDA (138 mg, 0.43 mmol, 0.7 eq) was added slowly, and the mixture was stirred for 1 h at room temperature. The solution was washed with aq. sodium bicarbonate and extracted three times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, 15% ethyl acetate in hexanes) to afford compound **26** as a red solid (75 mg, 30% yield) ^{1}H NMR (600 MHz, CDCl3) δ 8.41 (d, J = 7.8 Hz, 1H), 8.31 (s, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 5.05 (d, J = 7.6 Hz, 1H), 4.66 (d, J = 6.9 Hz, 1H), 3.75 (s, 3H), 3.27 (dd, J = 13.7, 5.3 Hz, 1H), 3.16 (dd, J = 13.7, 6.1 Hz, 1H), 2.80 (s, 3H), 1.41 (s, 9H). ^{13}C NMR (151 MHz, CDCl3) δ 175.60, 172.30, 162.37,

155.24, 137.66, 133.51, 132.08, 129.69, 128.54, 126.43, 80.30, 54.64, 52.63, 38.64, 28.49, 13.64. HRMS $[M+Na]^+$ calcd. for $C_{18}H_{23}N_5O_4S$ 428.1368, found 428.1370.



26 **27**

Synthesis of compound 27. Compound **26** (5 mg, 0.012 mmol) was dissolved in 1 ml dichloromethane, TFA 200 µl was added dropwise in ice bath, then stir at room temperature for 1 hour. The solution was washed with aq. sodium bicarbonate and extracted three times with ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure to afford ***m*-Stet-Phe (27)** (3.6 mg, 98% yield) without purification. ^{1}H NMR (600 MHz, CDCl3) δ 8.39 (d, J = 7.8 Hz, 1H), 8.37 (s, 1H), 7.51 (t, J = 7.7 Hz, 1H), 7.44 (d, J = 7.5 Hz, 1H), 3.81 (dd, J = 8.5, 5.8 Hz, 1H), 3.73 (s, 3H), 3.20 (dd, J = 13.6, 5.1 Hz, 1H), 2.97 (dd, J = 13.6, 8.0 Hz, 1H), 2.78 (s, 3H). ^{13}C NMR (151 MHz, CDCl3) δ 175.54, 175.47, 162.37, 138.83, 133.50, 132.07, 129.67, 128.37, 126.23, 55.93, 52.32, 41.20, 13.61. HRMS $[M+H]^+$ calcd. for $C_{13}H_{15}N_5O_2S$ 306.1025, found 306.1024.

2. Tables

Table S1. Oligonucleotide primers

Oligonucleotide	Sequence (5'-3')
mz165	TATAATTACCTGCGTAAACTGGATCGCA
mz166	CGCAGGTAATTATATCCCGTCGGTGCCAGCATC
mz167	GGATTCTCCCAAATGGGCAGCGGTTGTAC
mz168	CATTTGGGAGAATCCAACCATGGTAAATTCTTCCAGGTGTTC
mz324	CTACCGGTCGCCACCATGGTGAGCAAGGGCGAGG
mz325	AGATCTGAGTCCGGACTTGTACAGCTCGTCCATGCCG
mz326	TCCGGACTCAGATCTCGAGCTC
mz327	GGTGGCGACCGGTAGCG
mz163	TAGGTCTATATCACCGCCGACAAGC
mz164	GGTGATATAGACCTAGTGGCTGTTGAAGTTGTACTCCA

Table S2. DNA sequence of Tet3.0-R2-84

Gene Name	Sequence (5'-3')
Tet3.0-R2-84	ATGGATAAAAAACCGCTGGACGTTCTGATCTCCGCTACGGGTCTGTGGATGAGCCGCACGGGT ACGCTGCATAAAATTAAACACCACGAAGTGTCACGTTCGAAAATCTATATCGAAATGGCGTGCG GTGATCATCTGGTGGTTAACAATAGCCGTTCTTGTCGCACCGCGCGTGCCTTTCGCCATCACAA ATACCGCAAAACGTGCAAACGTTGTCGCGTGTCAGATGAAGACATTAACAATTTCCTGACCCGT AGTACGGAATCCAAAAACTCAGTGAAAGTTCGCGTCGTGAGTGCTCCGAAAGTTAAAAAAGCGA TGCCGAAAAGTGTCTCCCGTGCCCCGAAACCGCTGGAAAACTCAGTGTCGGCAAAAGCTTCCA CCAATACGAGCCGCTCTGTTCCGTCGCCGGCAAAAAGCACCCCGAACAGCTCTGTCCCGGCAA GCGCACCGGCACCGTCTCTGACGCGTAGTCAGCTGGATCGCGTGGAAGCCCTGCTGTCCCCG GAAGACAAAATCTCACTGAATATGGCAAAACCGTTTCGTGAACTGGAACCGGAACTGGTTACCC GTCGCAAAAACGATTTCCAACGTCTGTATACGAATGATCGCGAAGACTACCTGGGTAAACTGGA ACGTGATATCACCAAATTTTTCGTGGACCGCGGCTTTCTGGAAATCAAATCTCCGATTCTGATCC CGGCTGAATATGTTGAACGCATGGGTATTAACAATGATACCGAACTGAGTAAACAGATTTTTCGT GTGGATAAAAACCTGTGCCTGCGGCCGATGCTGGCACCGACGGGATATAATTACCTGCGTAAA CTGGATCGCATTCTGCCGGGTCCGATTAAAATCTTTGAAGTGGGCCCGTGTTATCGTAAAGAAT CGGATGGCAAAGAACACCTGGAAGAATTTACCATGGTTGGATTCTCCCAAATGGGCAGCGGTT GTACGCGCGAAAATCTGGAAGCGCTGATCAAAGAATTCCTGGATTACCTGGAAATCGACTTCGA AATCGTCGGTGATTCTTGCATGGTGTTTGGCGATACCCTGGACATCATGCATGGTGACCTGGAA CTGAGTTCCGCTGTTGTCGGTCCGGTCAGCCTGGATCGTGAATGGGGCATTGACAAACCGTGG ATCGGCGCGGGTTTTGGCCTGGAACGCCTGCTGAAAGTTATGCACGGCTTCAAAAACATCAAAC GTGCGTCTCGCTCGGAATCGTATTACAACGGCATCTCAACCAATCTGTAA

Table S3. Turn on Photolysis kinetics of alkyl- and sulfur-tetrazine derivatives. The rate constants were calculated based on the characteristic absorption of each compound in 1 mM CH_3CN.

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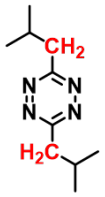
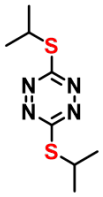
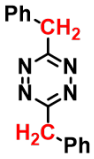
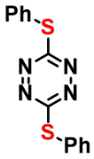
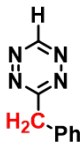
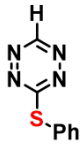
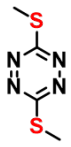
Structure							
K_{254} (nM/s)	9	1518	278	3208	254	2721	943
K_{365} (nM/s)	<1	19	<1	222	<1	174	17
K_{405} (nM/s)	N/A	7	N/A	148	N/A	83	6

Table S4. Turn on ratios of tetrazine-dye conjugated measured at the maximum of emission. 1mM concentration in CH_3CN.

Compound	I/I_0 254 nm	I/I_0 365 nm	I/I_0 405 nm	I/I_0 510 nm
MeTz-Coumarin	86	3	2	1
SMeTz-Coumarin	323	61	12	1
MepTz-BODIPY	178	15	3	1
SMepTz-BODIPY	431	187	134	84
SMeFretTz-BODIPY	110	30	27	12

Table S5. Photophysical data of fluorophore tetrazine conjugates and their nitrile analogues.

Compound name	l_{Abs} (nm)	$Ex10^4(M^{-1}\ cm^{-1})$	l_{Em} (nm)	$\Phi_{F\ MeCN}$
MeTz-Coumarin	347	1.8	416	<0.001[a]
SMeTz-Coumarin	382	2.8	465	<0.001[a]
CN-Coumarin	351	1.9	417	0.65[a]
MepTz-BODIPY	490	1.9	509	0.004[b]
SMepTz-BODIPY	498	4.2	509	<0.001[b]
CN-BODIPY	492	3.6	509	0.60[b]
SMeFretTz-BODIPY	501	4.7	510	0.005[b]

a) Quinine sulfate in 0.5 M H_2SO_4 was used as reference. b) Fluorescein in 0.1 M NaOH was used as reference. c) 254 nm (1400 mW cm^{-2}). d) 365 nm (1400 mW cm^{-2}).

3. Spectrums

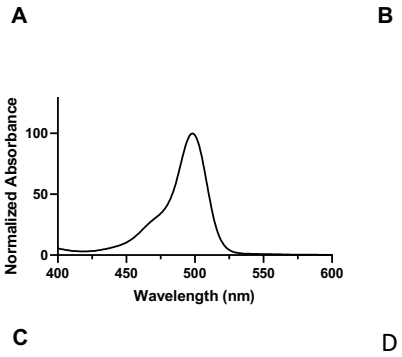
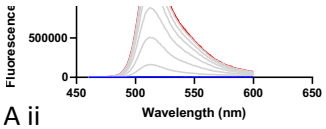


Figure S1. Absorbance spectra of (A) SMepTz-BODIPY, (B) SMeTz-Coumarin, (C) SMeFretTz-BODIPY and (D) *m*-Stet-Phe. Absorbance of pTz-BODIPY, Tz-Coumarin were reported in 2020[1].

A i

B i

C i



A ii

B ii

C ii

A iii

B iii

C iii

Figure S2. Emission spectra of (A) SMepTz-BODIPY, (B) SMeTz-Coumarin and (C) SMeFretTz-BODIPY before (blue) and after (red) 254 nm (i), 365 (i), 405 (iii) photoactivation. Fluorescence changing of pTz-BODIPY, Tz-Coumarin during irradiation were reported in 2020[1].

4. Photolysis Graphs

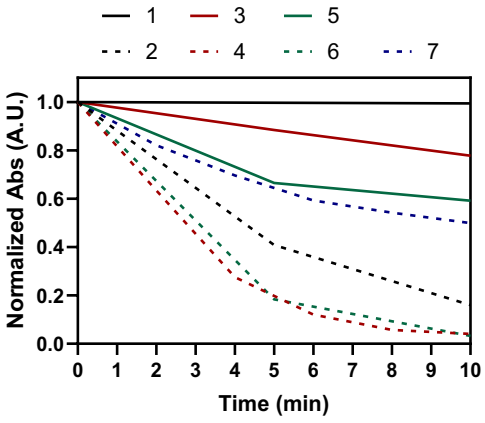


Figure S3. The absorption declinations of sulfur-tetrazine (dash) and alkyl tetrazine (solid) derivatives during photodissociation with UV 254. (Numbers are consistent with Table S3.)

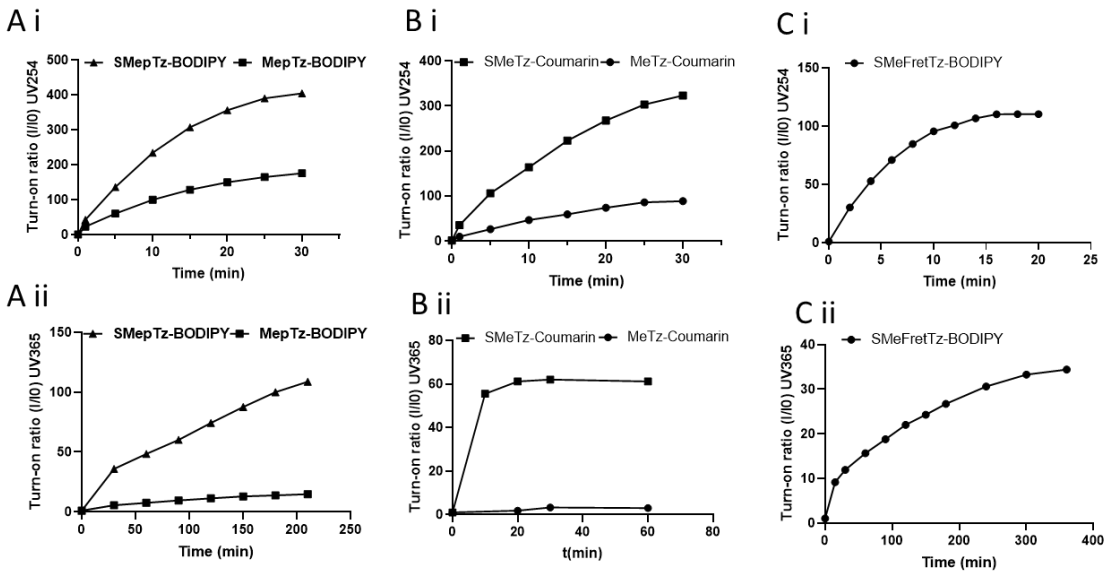


Figure S4. Photolysis rate of MepTz-BODIPY and SMepTz-BODIPY (A), MeTz-Coumarin and SMeTz-Coumarin (B) and SMeFretCoumarin (C) under UV 254 (i) and UV 365 (ii).

5. Protein ESI LC-MS

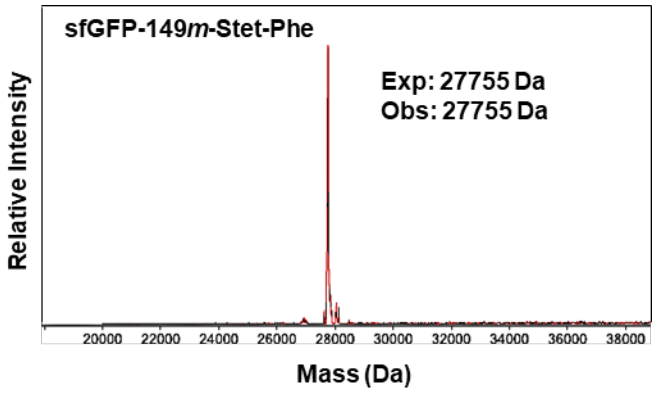


Figure S5. ESI-LC-MS analysis of sfGFP-149*m*-Stet-Phe extracted from *E. coli*. shows a single major peak at 27755 ± 1 Da consistent with the expected molecule weight.

6. Cell Imaging

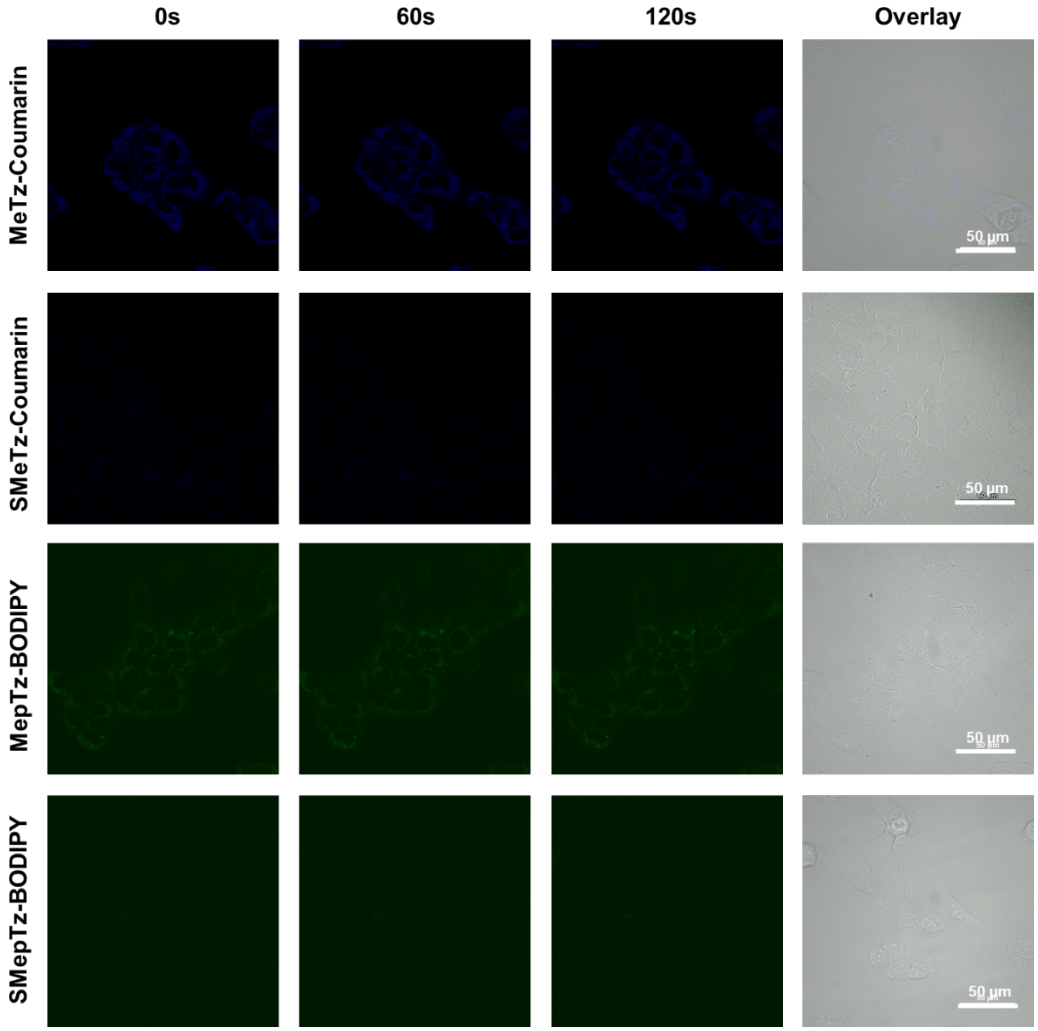


Figure S6. Incubation of MeTz-Coumarin, SMeTz-Coumarin, MepTz-BODIPY, and SMepTz-BODIPY with A431 cells keeping in darkness. The images were obtained by Nikon A1 confocal. Scale = 50 µm.

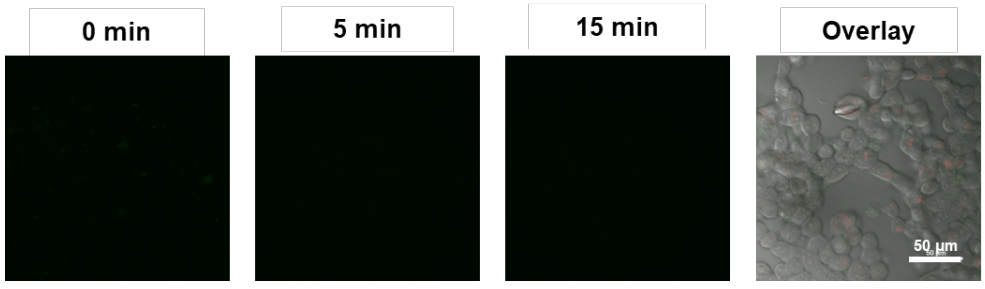


Figure S7. Confocal image of live HEK 293T cells expressing sfGFP-149m-Stet-Phe keeping in dark for different time points. Scale bar = 50 µm.

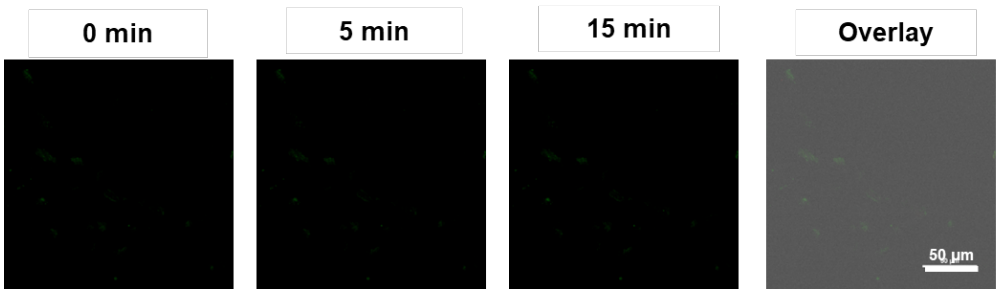


Figure S8. Confocal image of live U2OS cells expressing NUP96-SNAP tag keeping in dark for different times. Scale bar = 50 µm.

7. Cell Viability Test

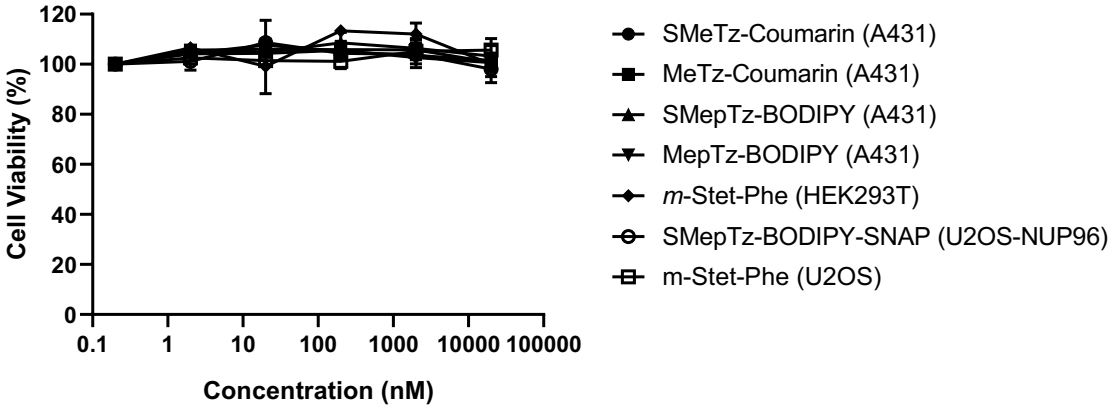


Figure S9. Cell viability of all the fluorescent probes and ncAA to the cell lines they incubated with in this research study. The cell viabilities of SMeTz-Coumarin, MeTz-Coumarin, SMepTz-BODIPY and MepTz-BODIPY were tested with A431. The cell viability ncAA m-Stet-Phe was tested with both HEK293T and U2OS. The cell viability of SMepTz-BODIPY-SNAP was tested with U2OS-NUP96.

8. Stability Test

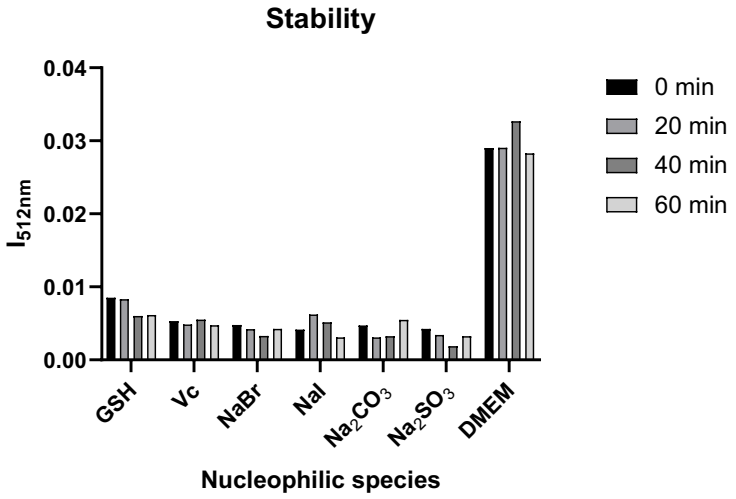


Figure S10. Stability of *m*-Stet-Phe with common nucleophiles of biological systems. The absorptions of *m*-Stet-Phe were collected in a 20 μM HBSS solution (pH 7.4) with 5 mM GSH, or 200 μM Vc, NaBr, NaI, Na_2CO_3, Na_2SO_3, or DMEM medium at different time points.

9. NMR

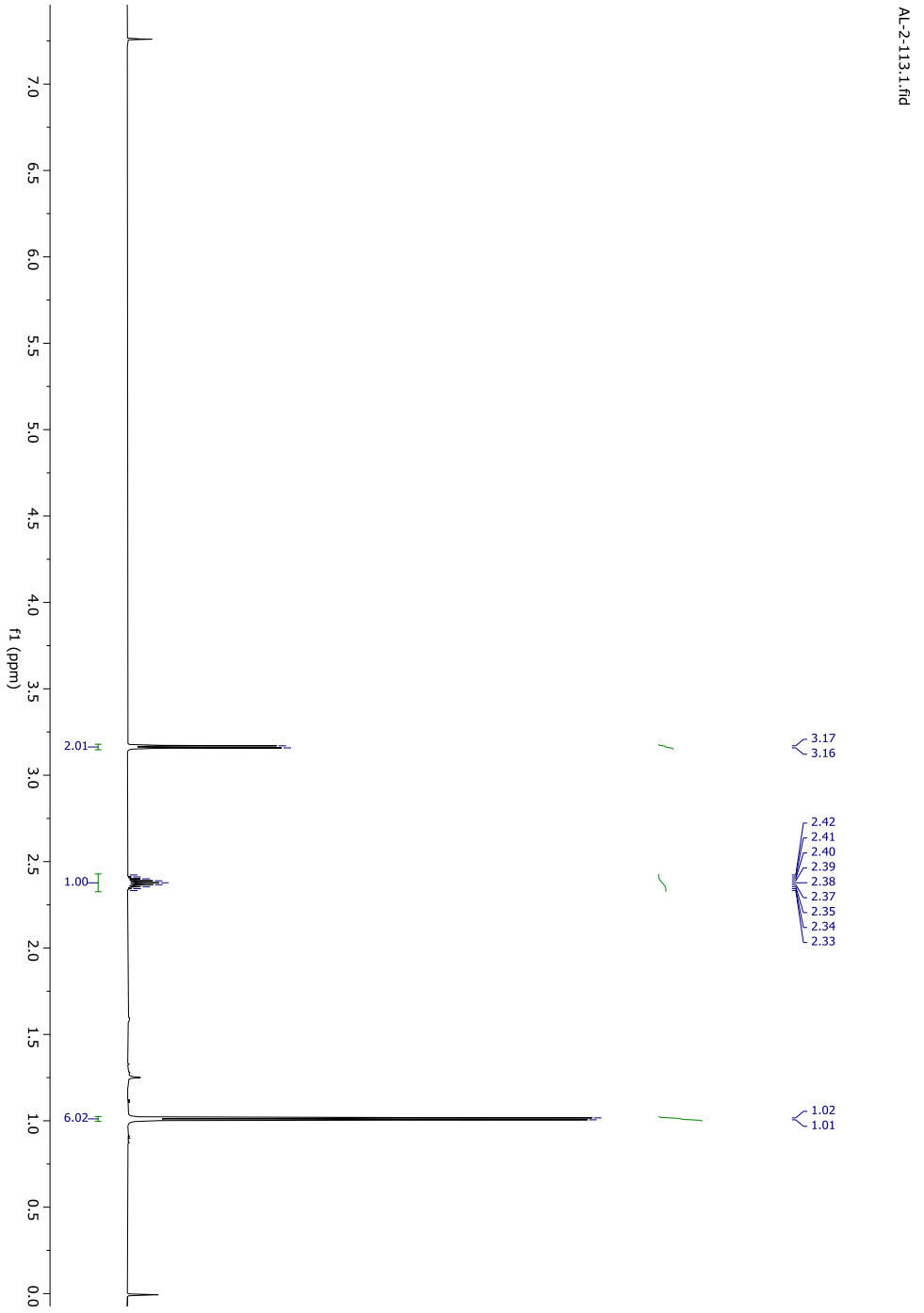


Figure S11. ^{1}H NMR spectrum of compound 1.

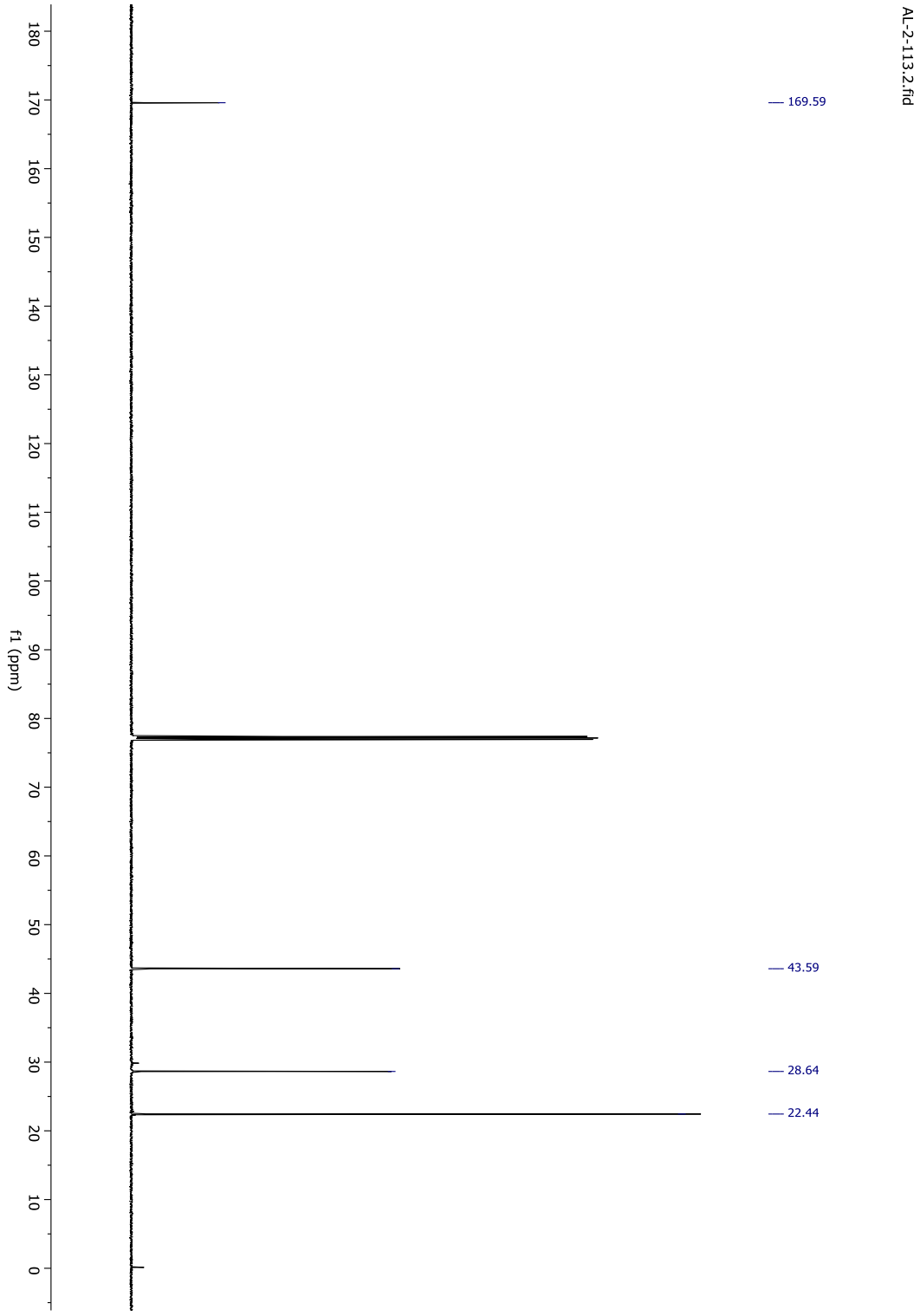


Figure S12. ^{13}C NMR spectrum of compound 1.

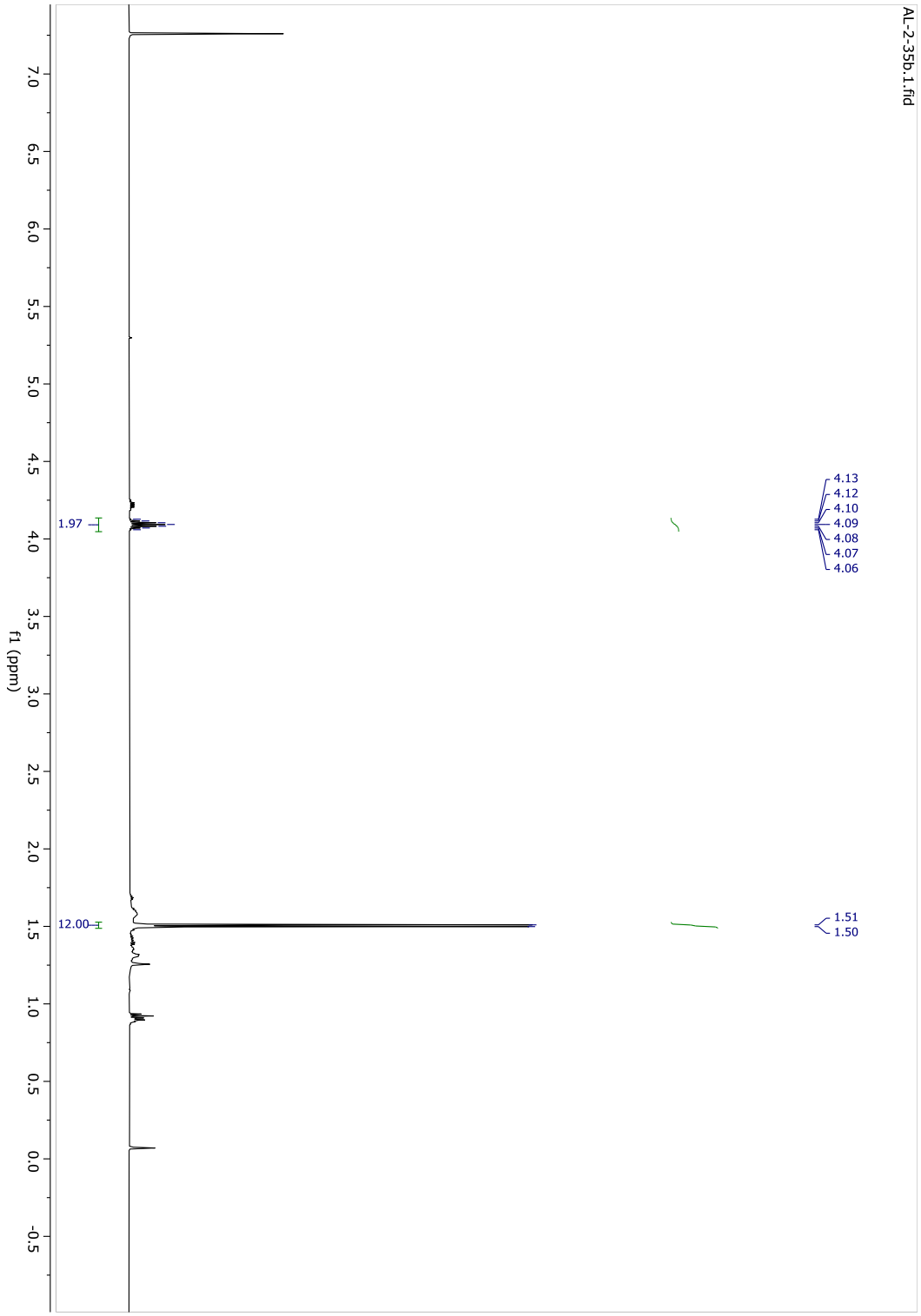


Figure S13. ^{1}H NMR spectrum of compound 2.

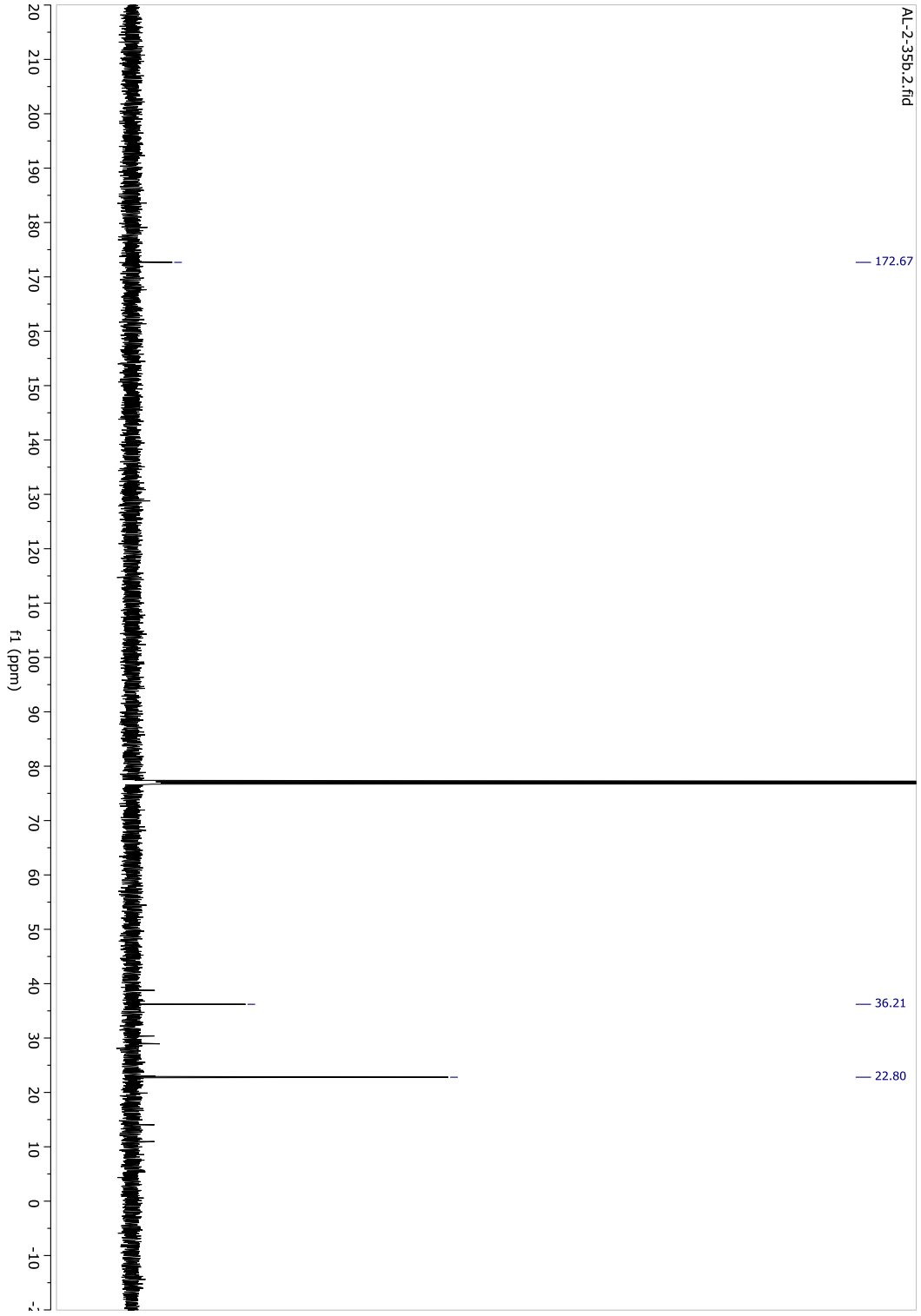


Figure S14. ^{13}C NMR spectrum of compound 2.

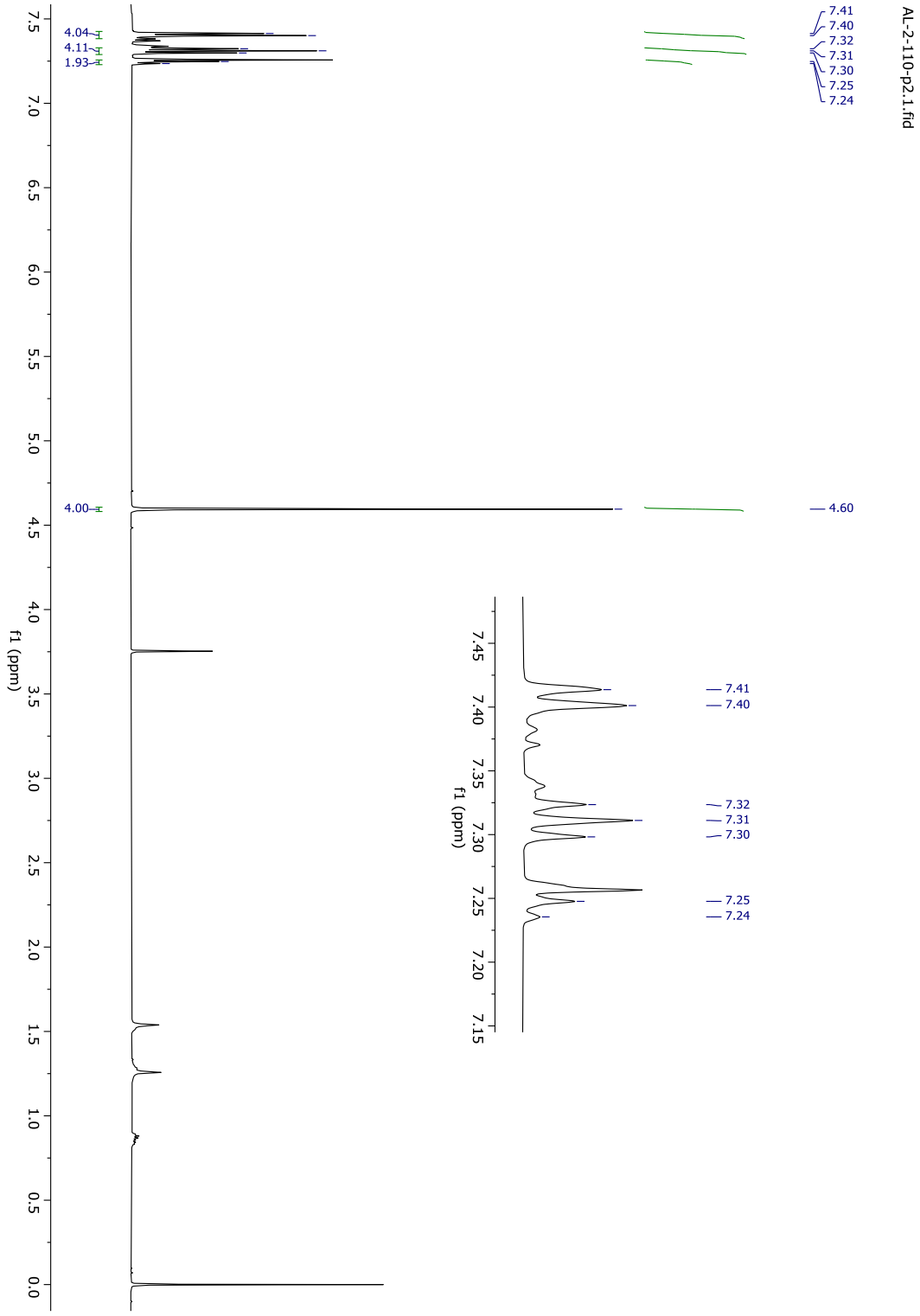


Figure S15. ^{1}H NMR spectrum of compound 3.

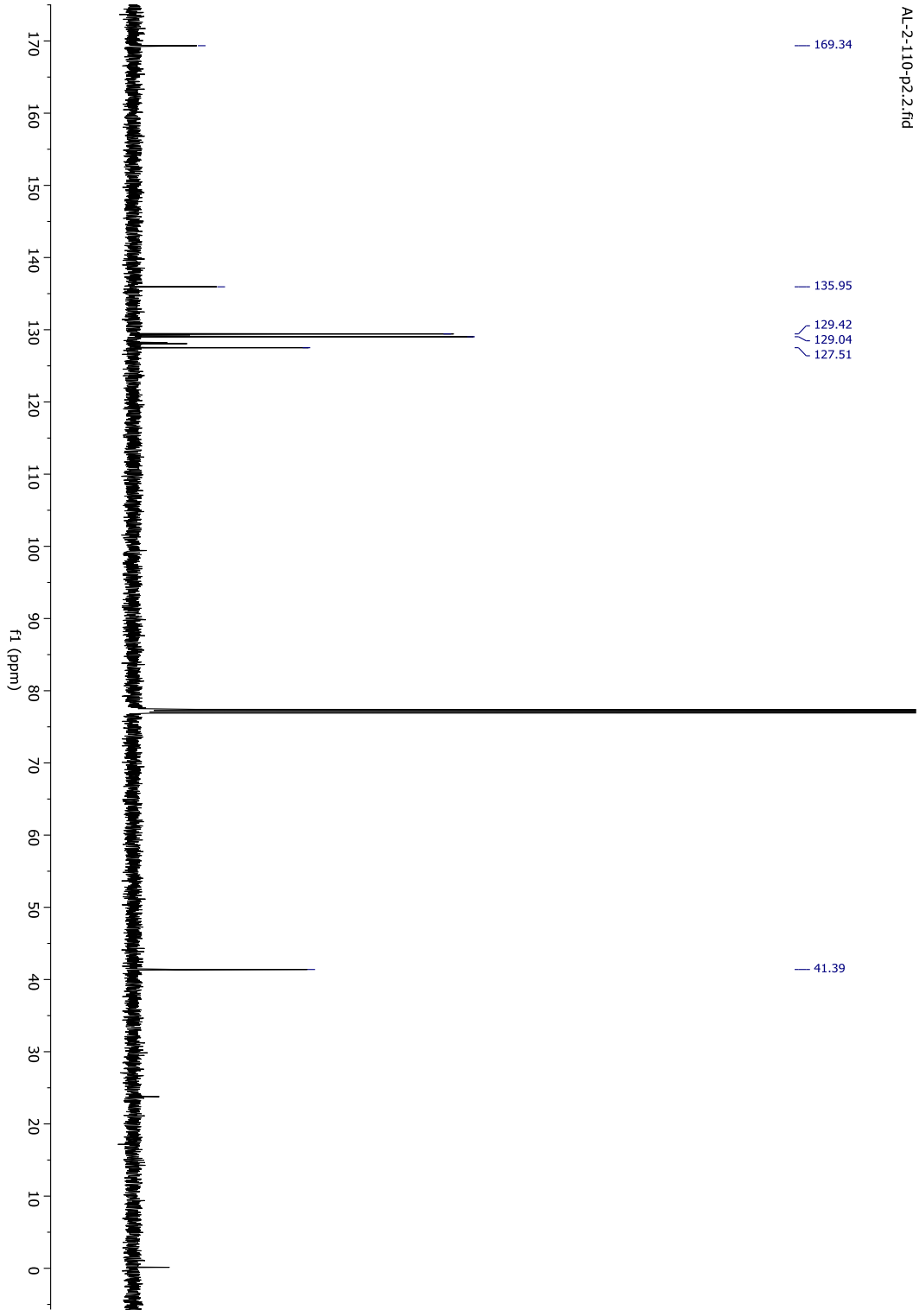


Figure S16. ^{13}C NMR spectrum of compound 3.

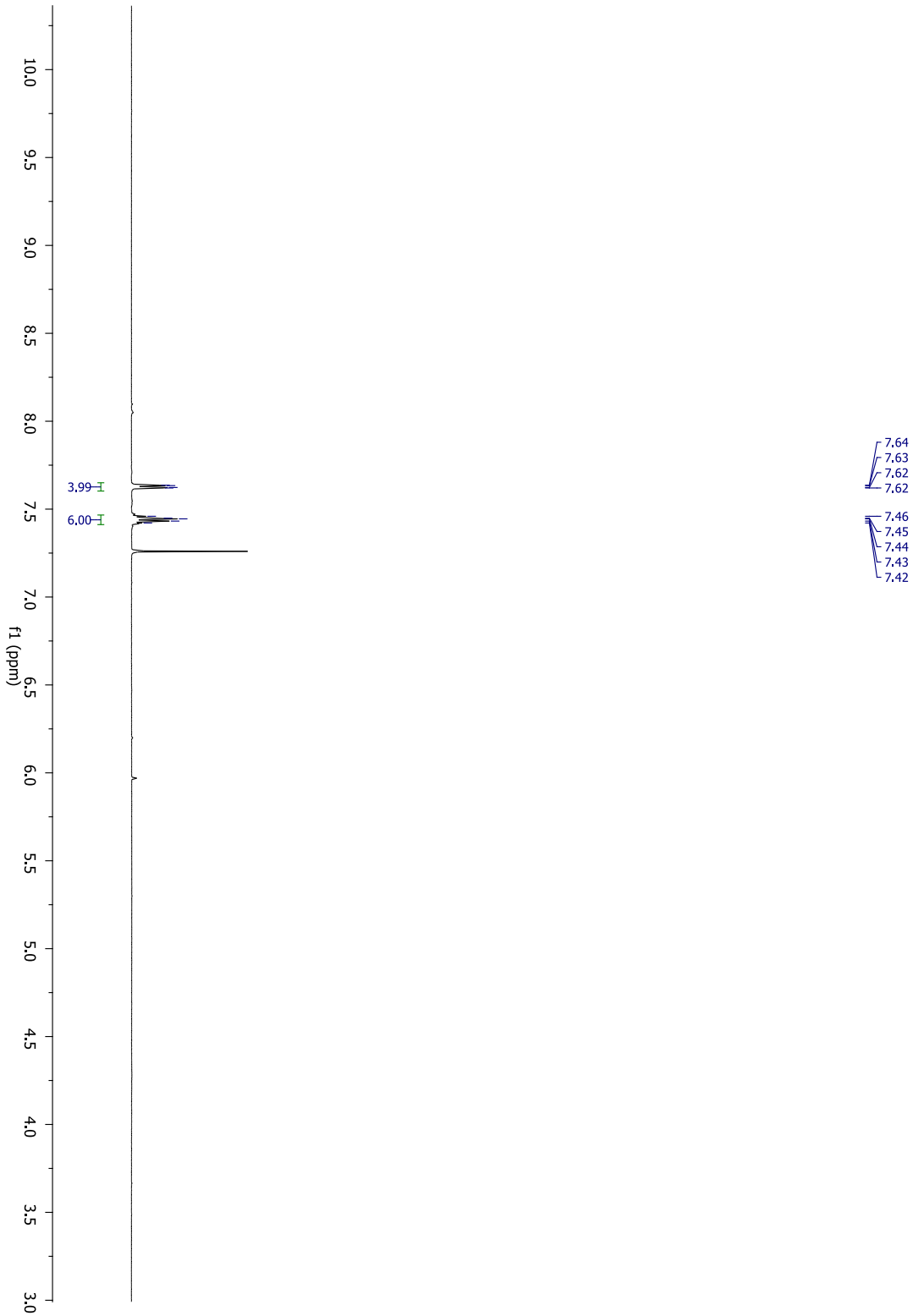


Figure S17. ^{1}H NMR spectrum of compound 4.

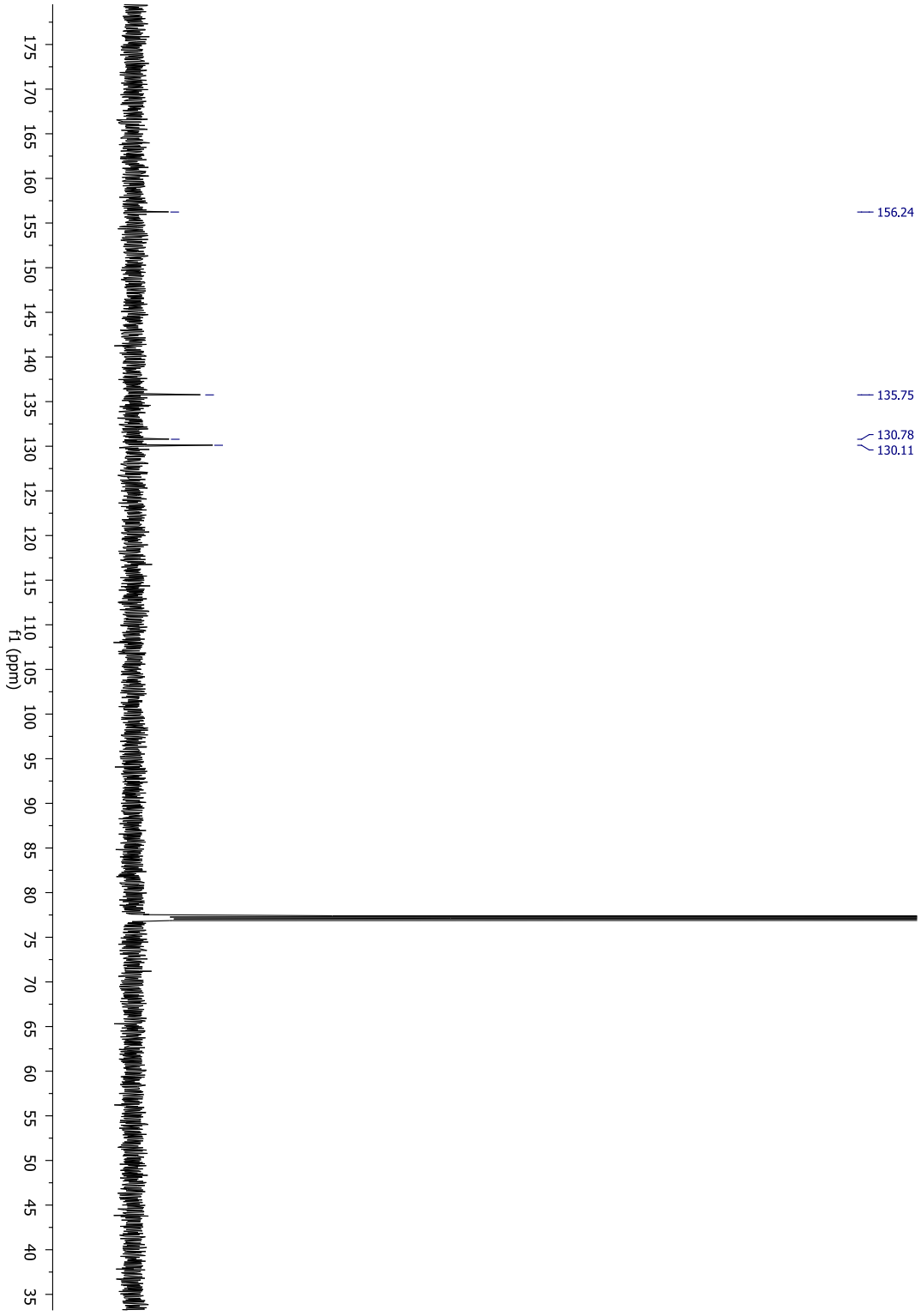


Figure S18. ^{13}C NMR spectrum of compound 4.

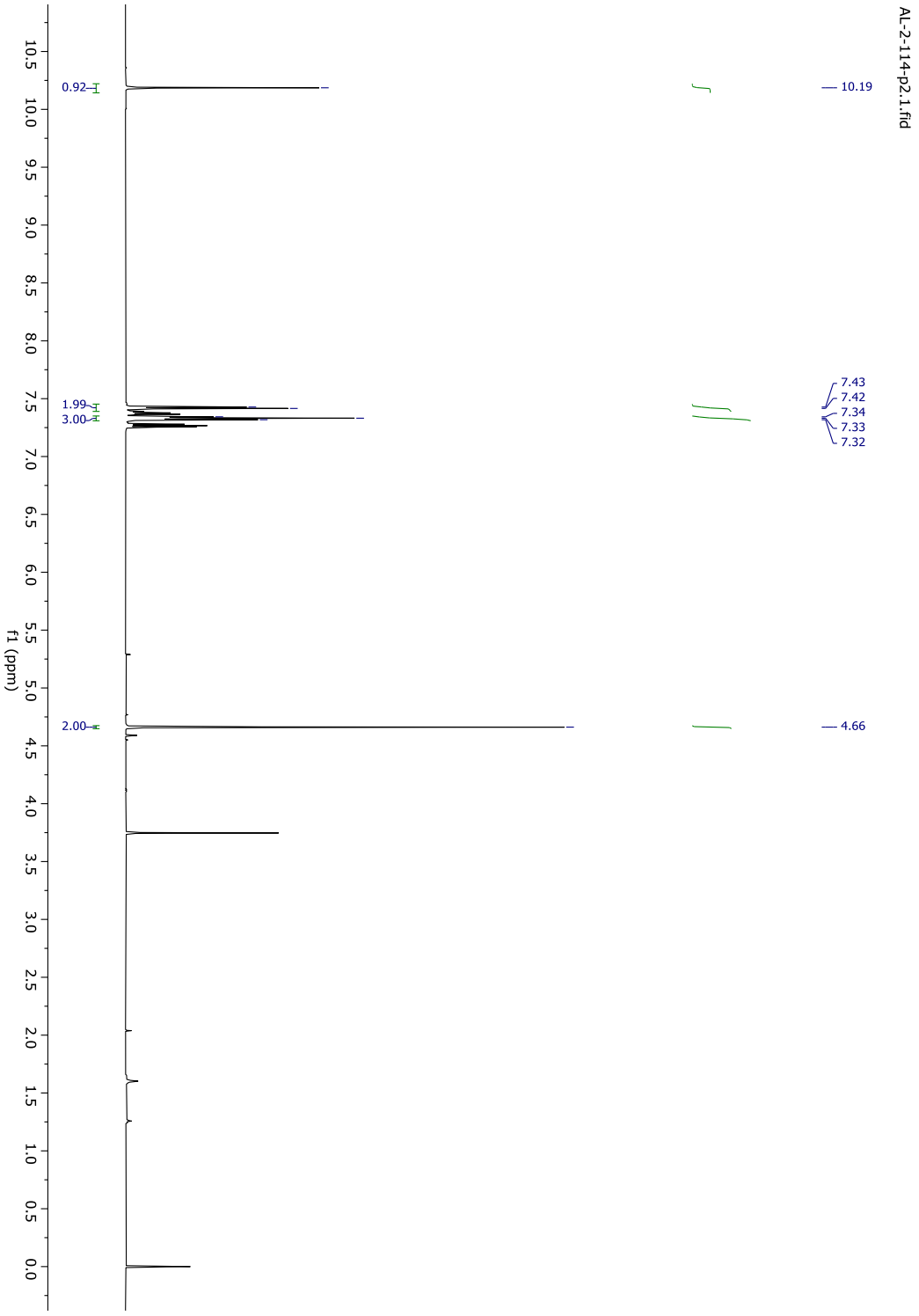


Figure S19. ^{1}H NMR spectrum of compound 5.

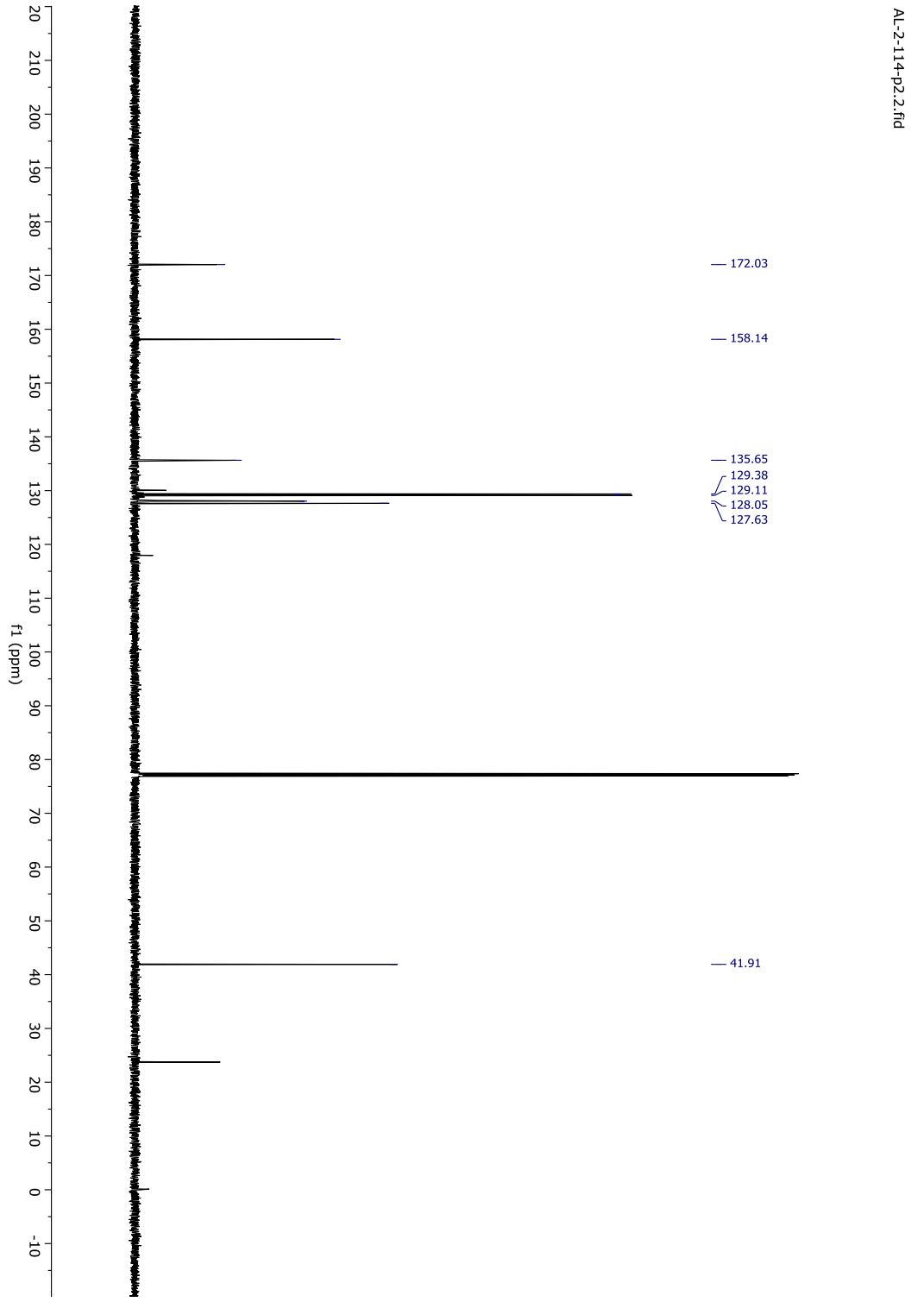


Figure S20. ^{13}C NMR spectrum of compound 5.

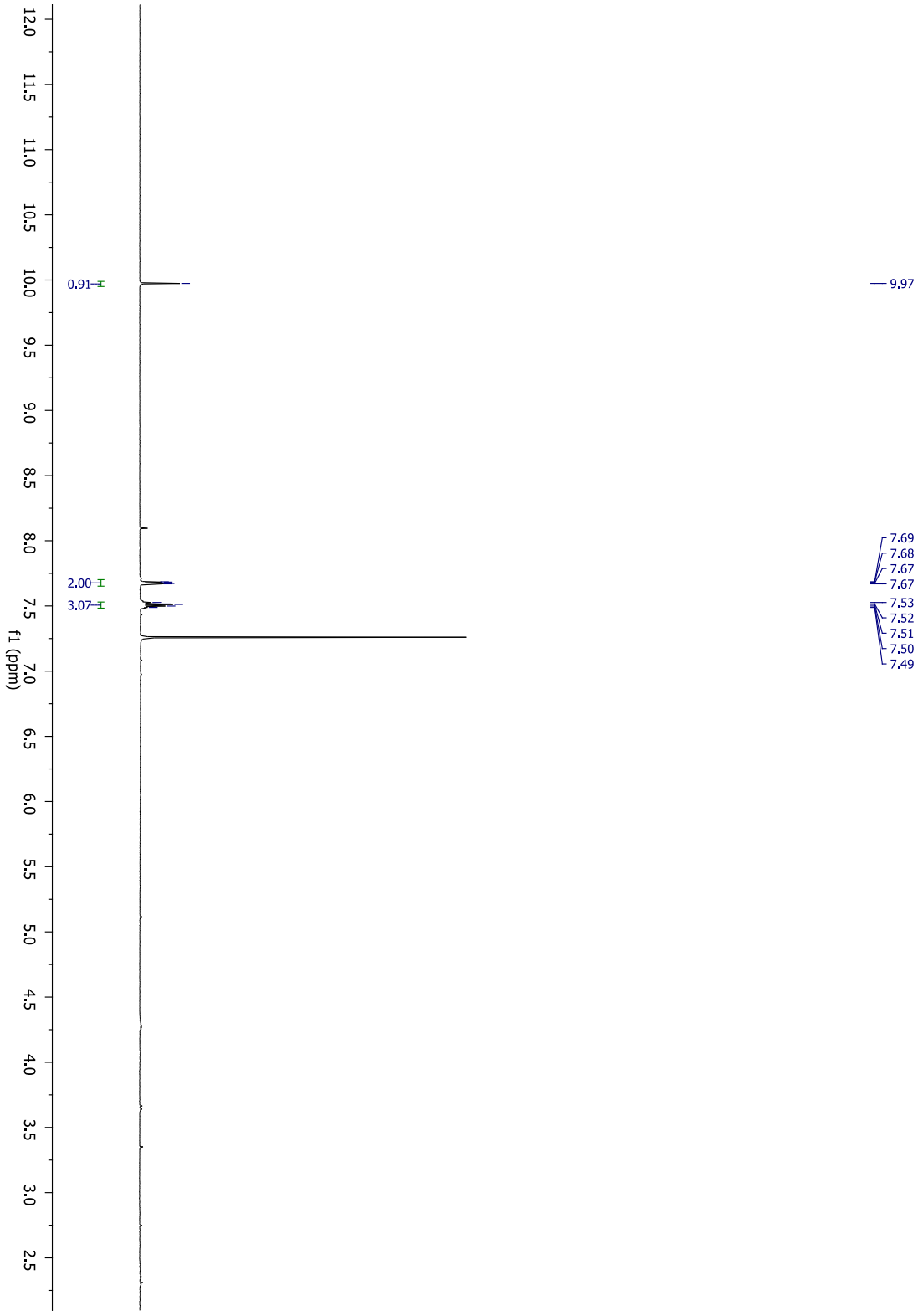


Figure S21. ^{1}H NMR spectrum of compound 6.

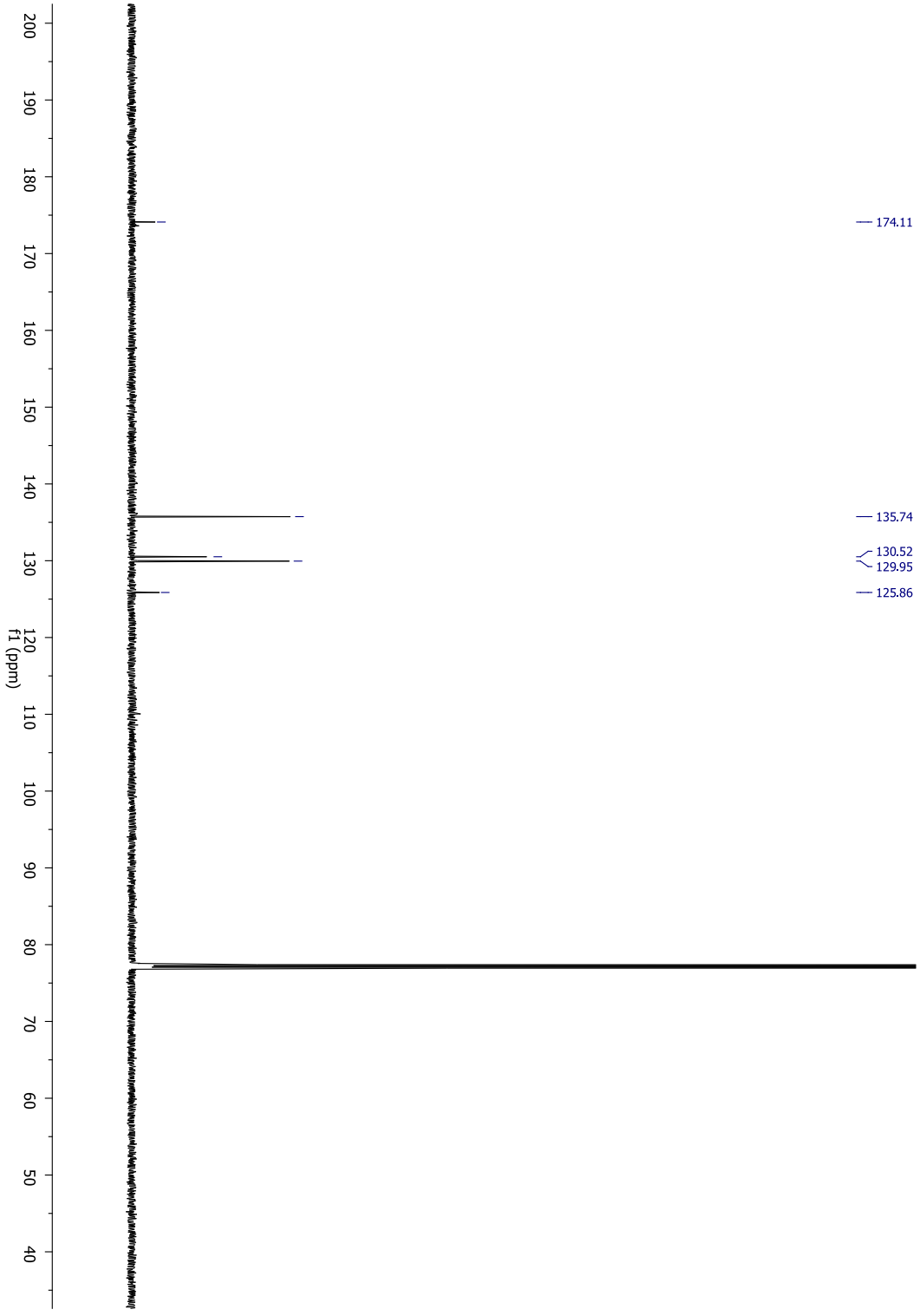


Figure S22. ^{13}C NMR spectrum of compound 6.

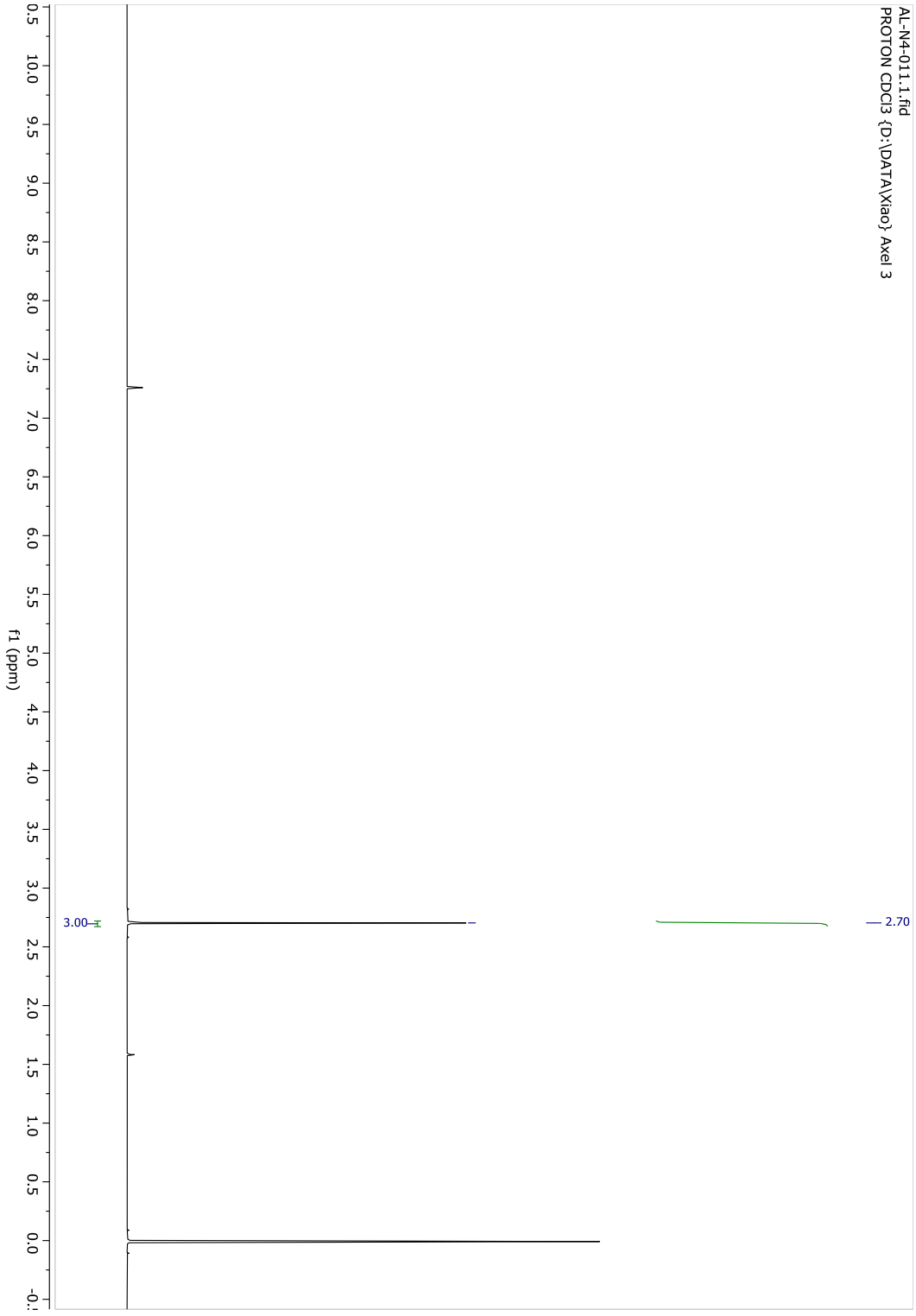


Figure S23. ^{1}H NMR spectrum of compound 7.

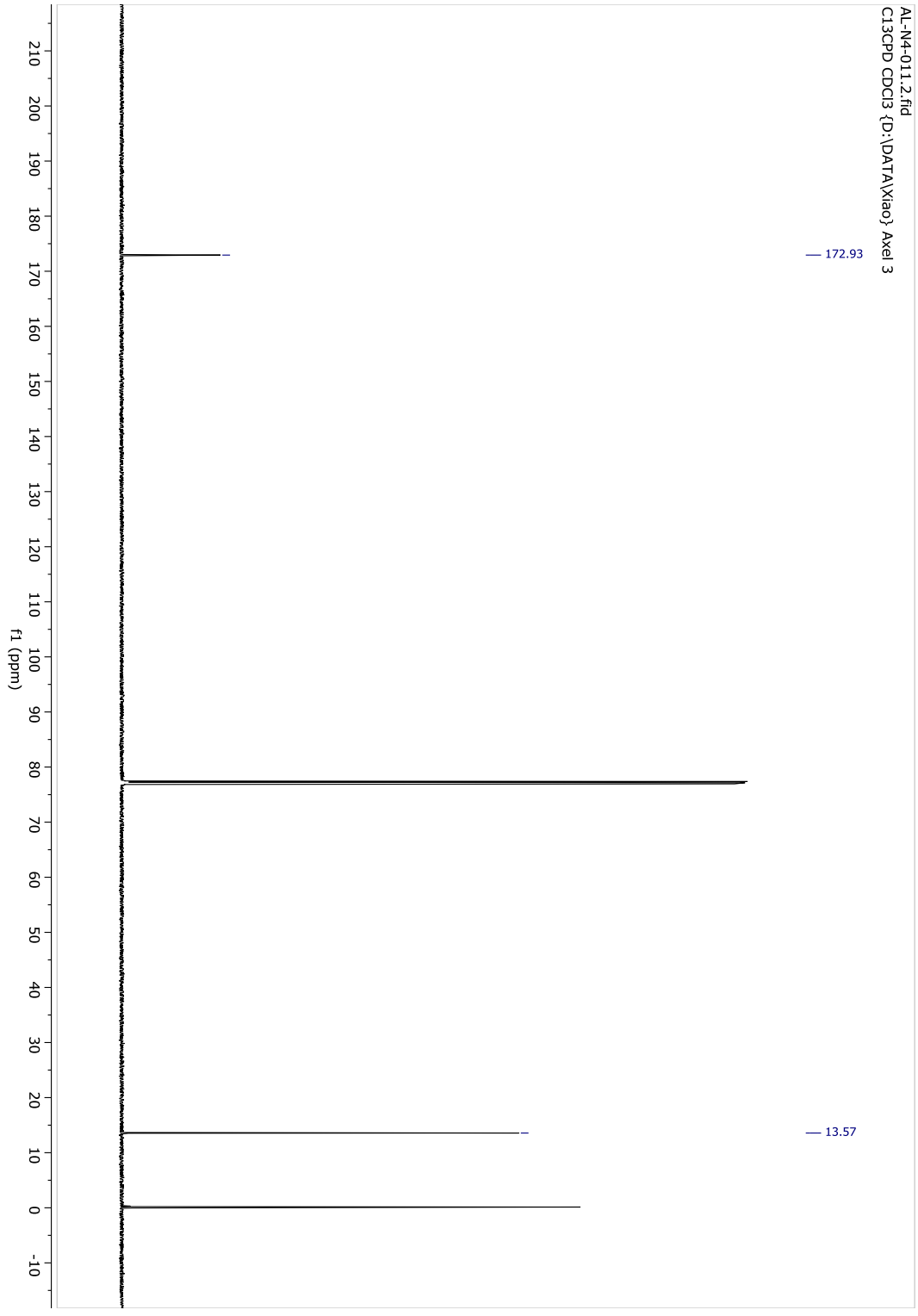


Figure S24. ^{13}C NMR spectrum of compound 7.

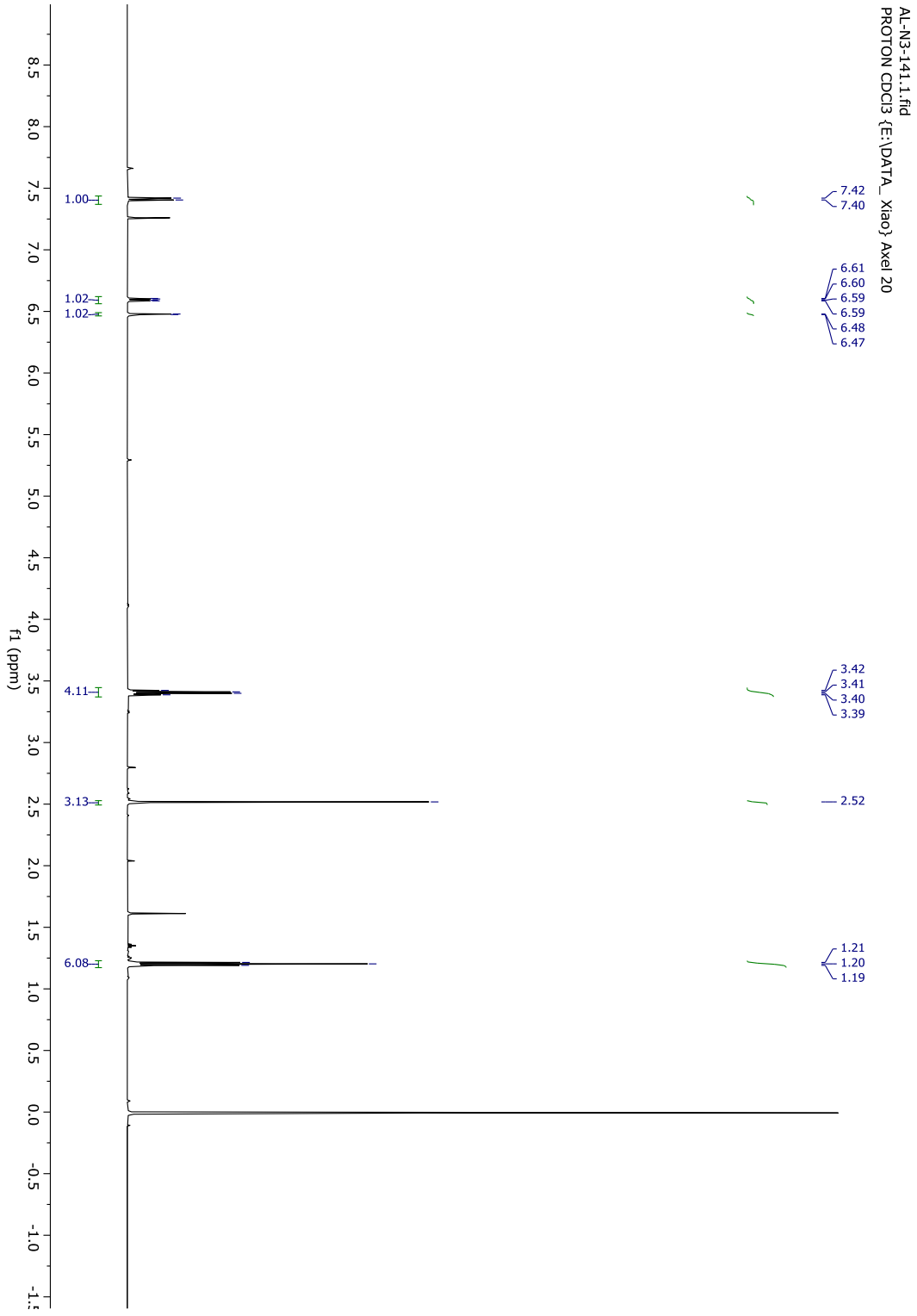


Figure S25. ^{1}H NMR spectrum of compound 8.

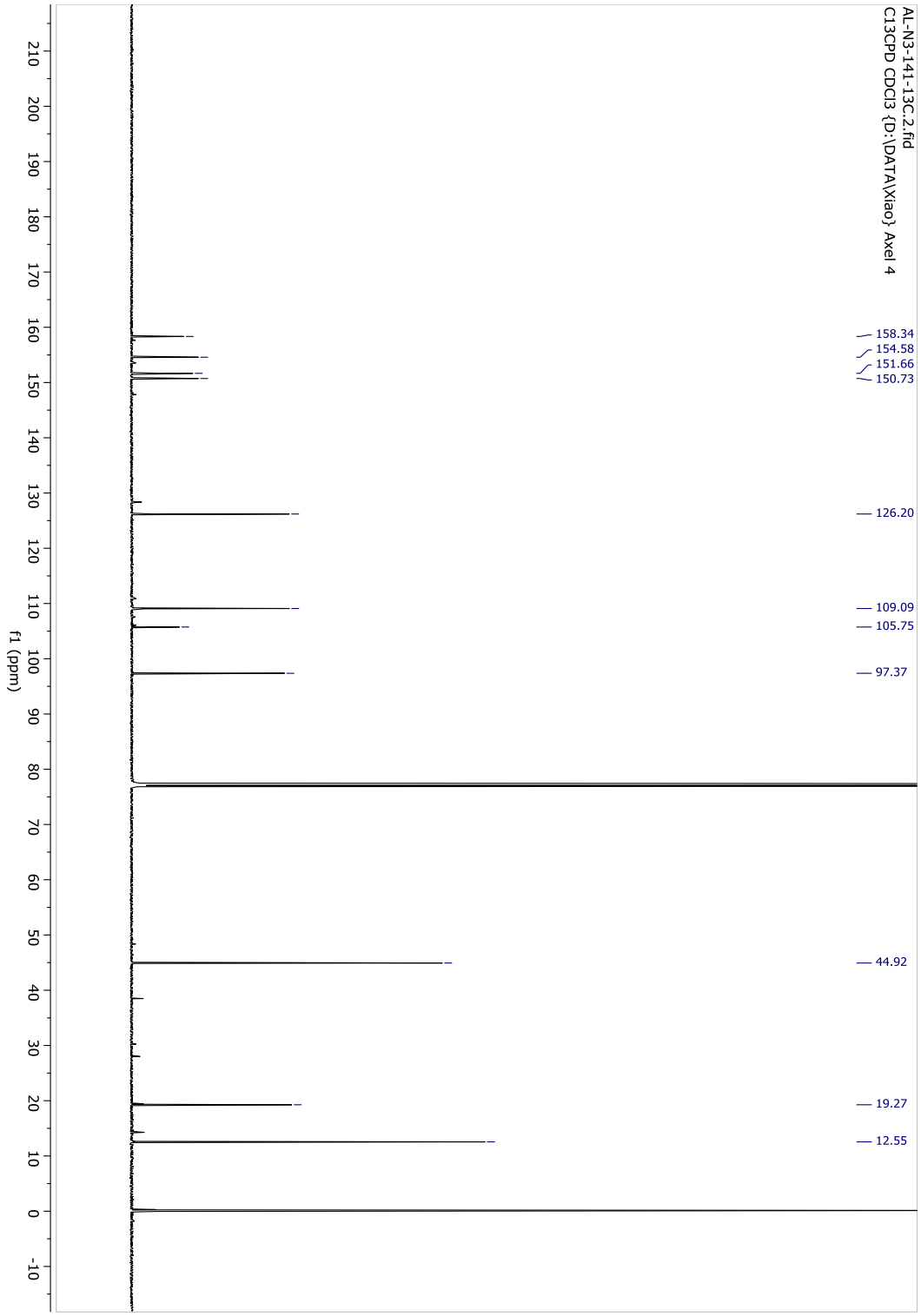


Figure S26. ^{13}C NMR spectrum of compound 8.

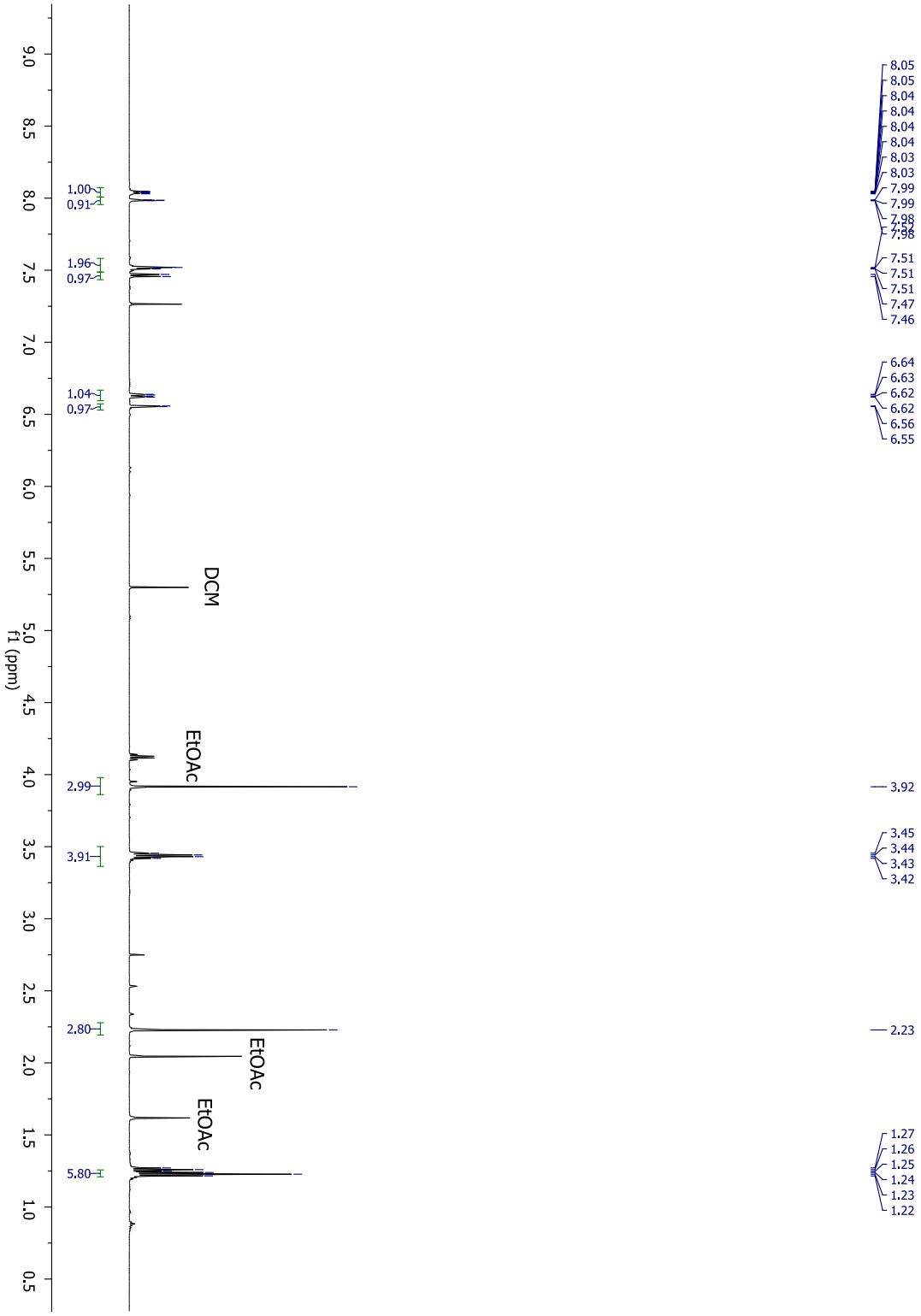


Figure S27. ^{1}H NMR spectrum of compound 9.

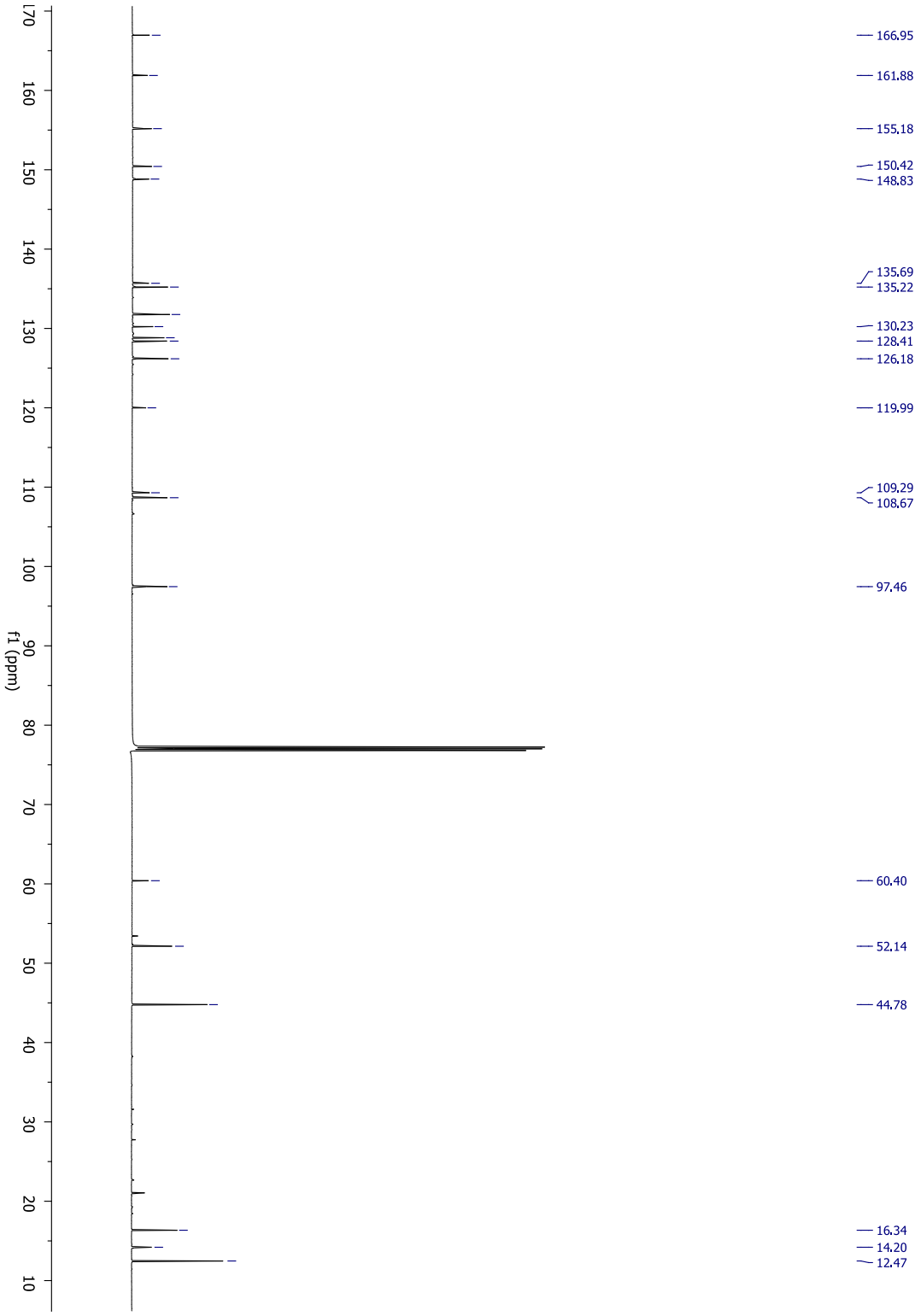


Figure S28. ^{13}C NMR spectrum of compound 9.

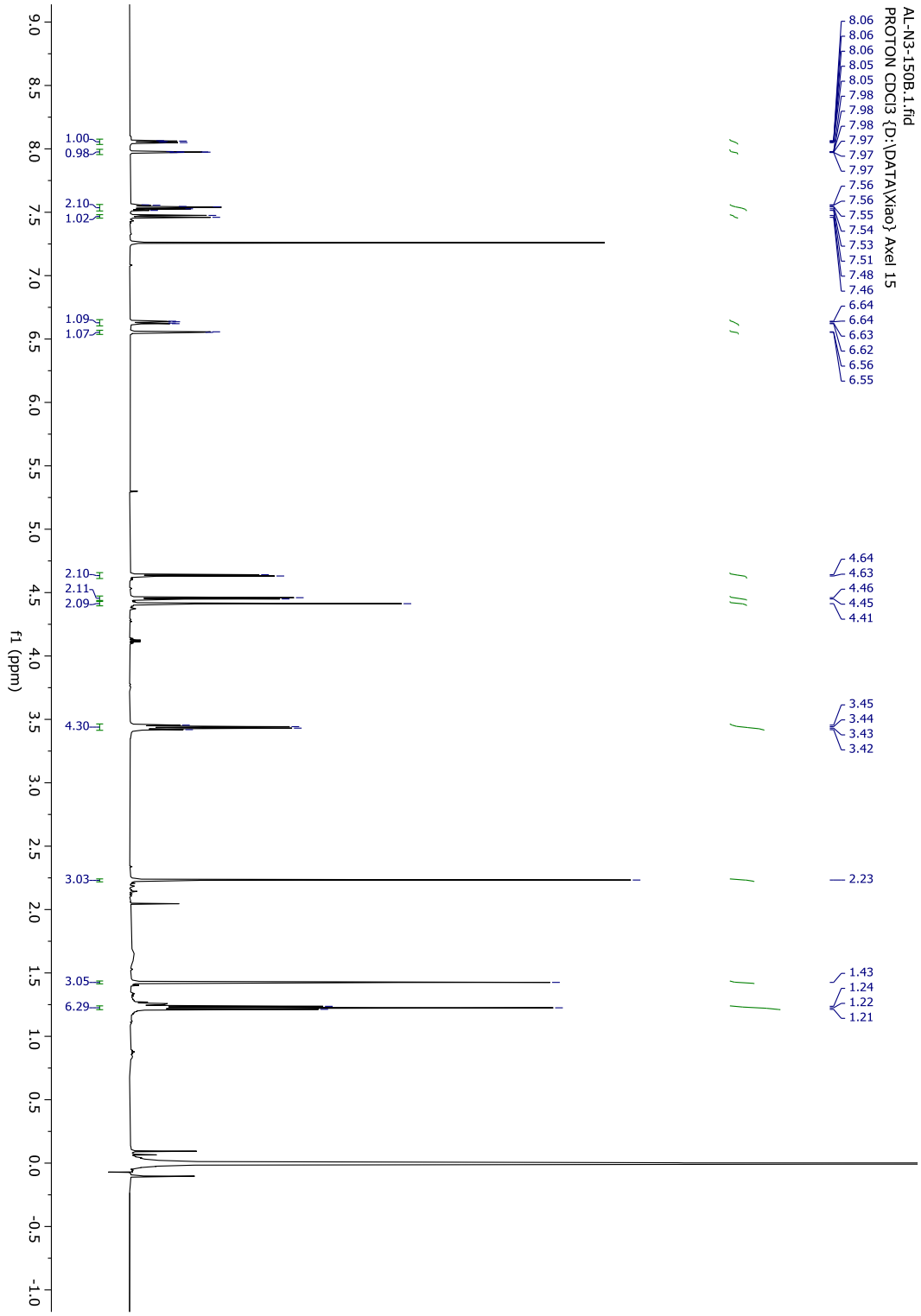


Figure S29. ^{1}H NMR spectrum of compound 10.

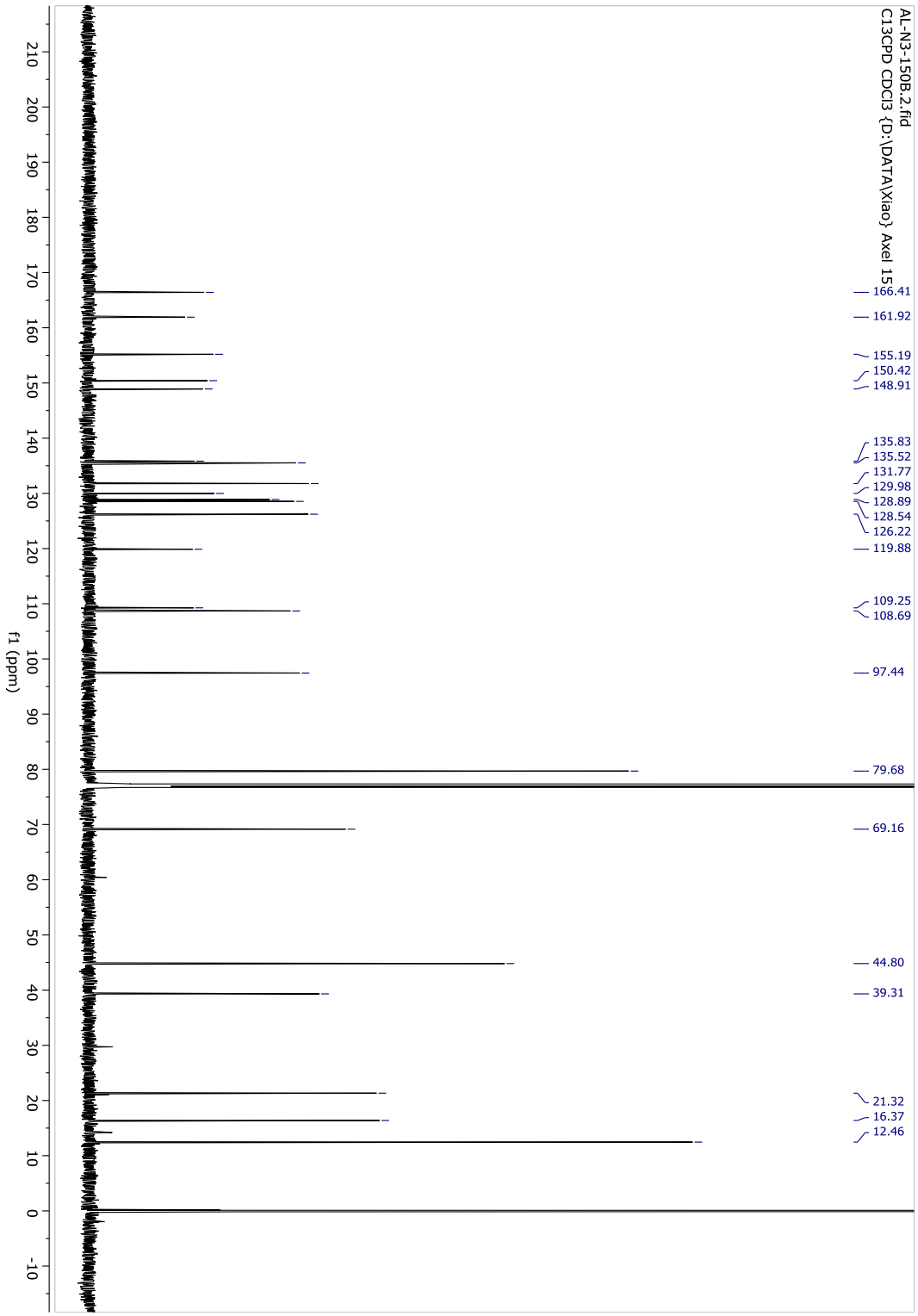


Figure S30. ^{13}C NMR spectrum of compound 10.

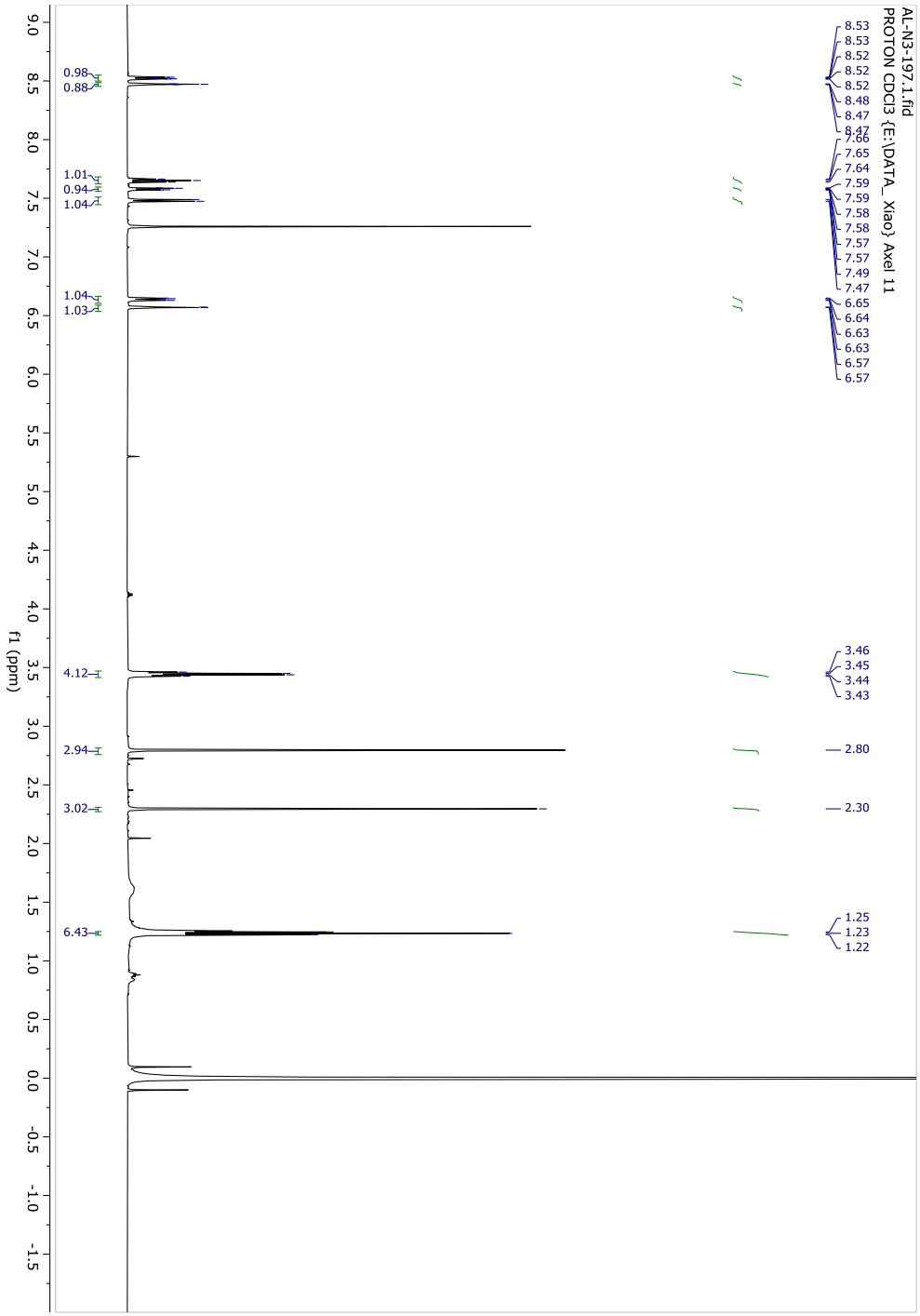


Figure S31. ^{1}H NMR spectrum of compound 11.

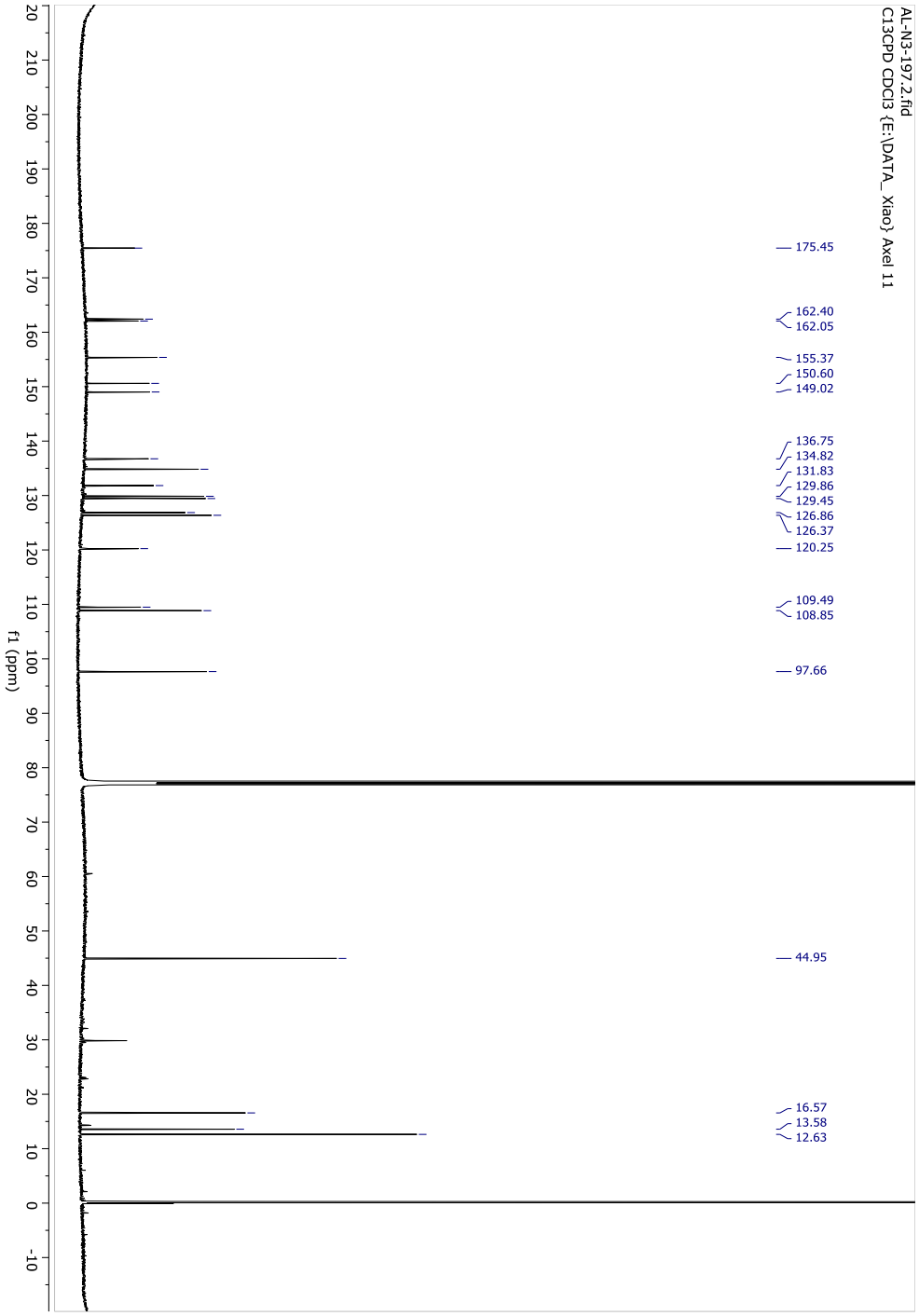


Figure S32. ^{13}C NMR spectrum of compound 11.

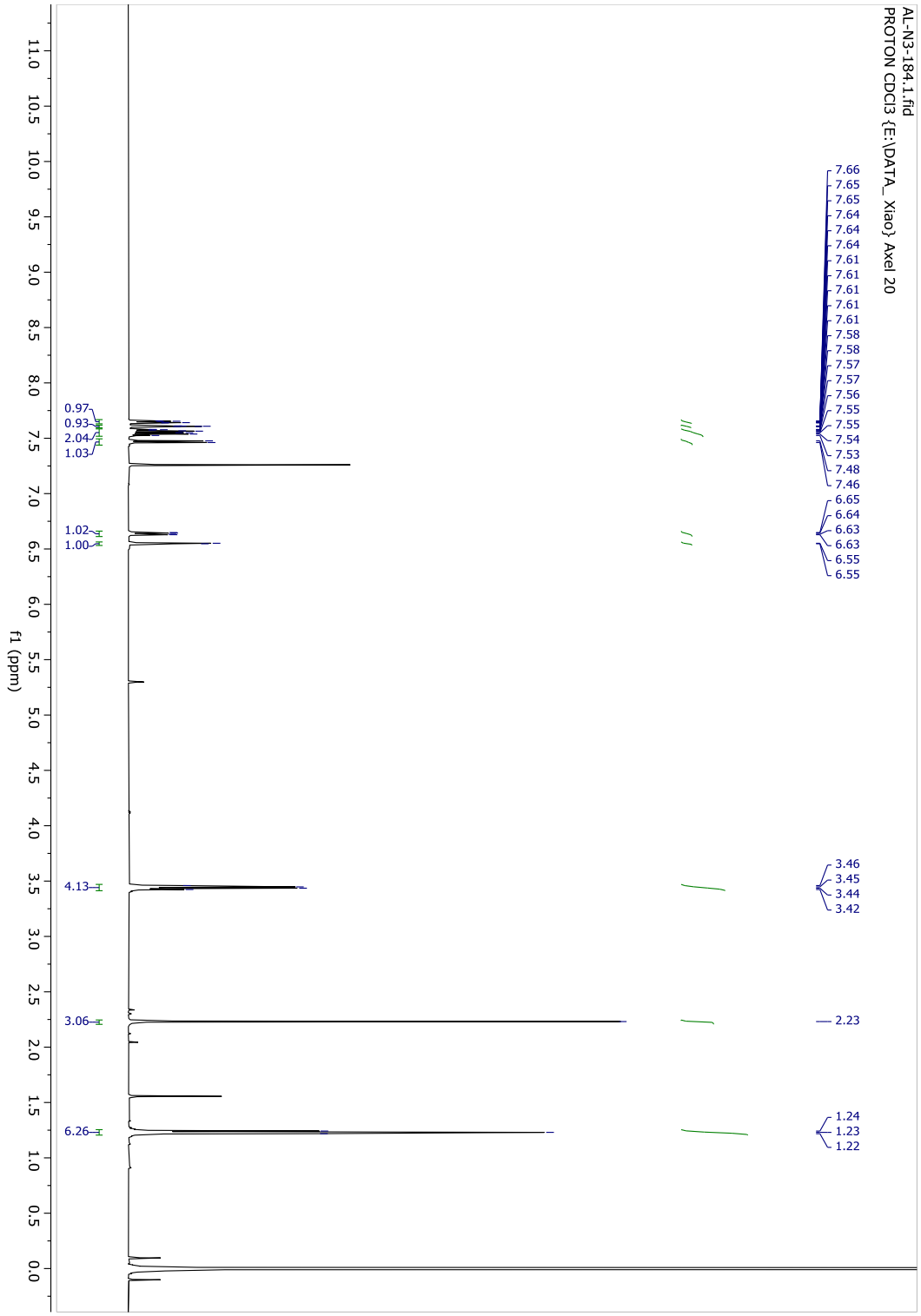


Figure S33. ^{1}H NMR spectrum of compound 12.

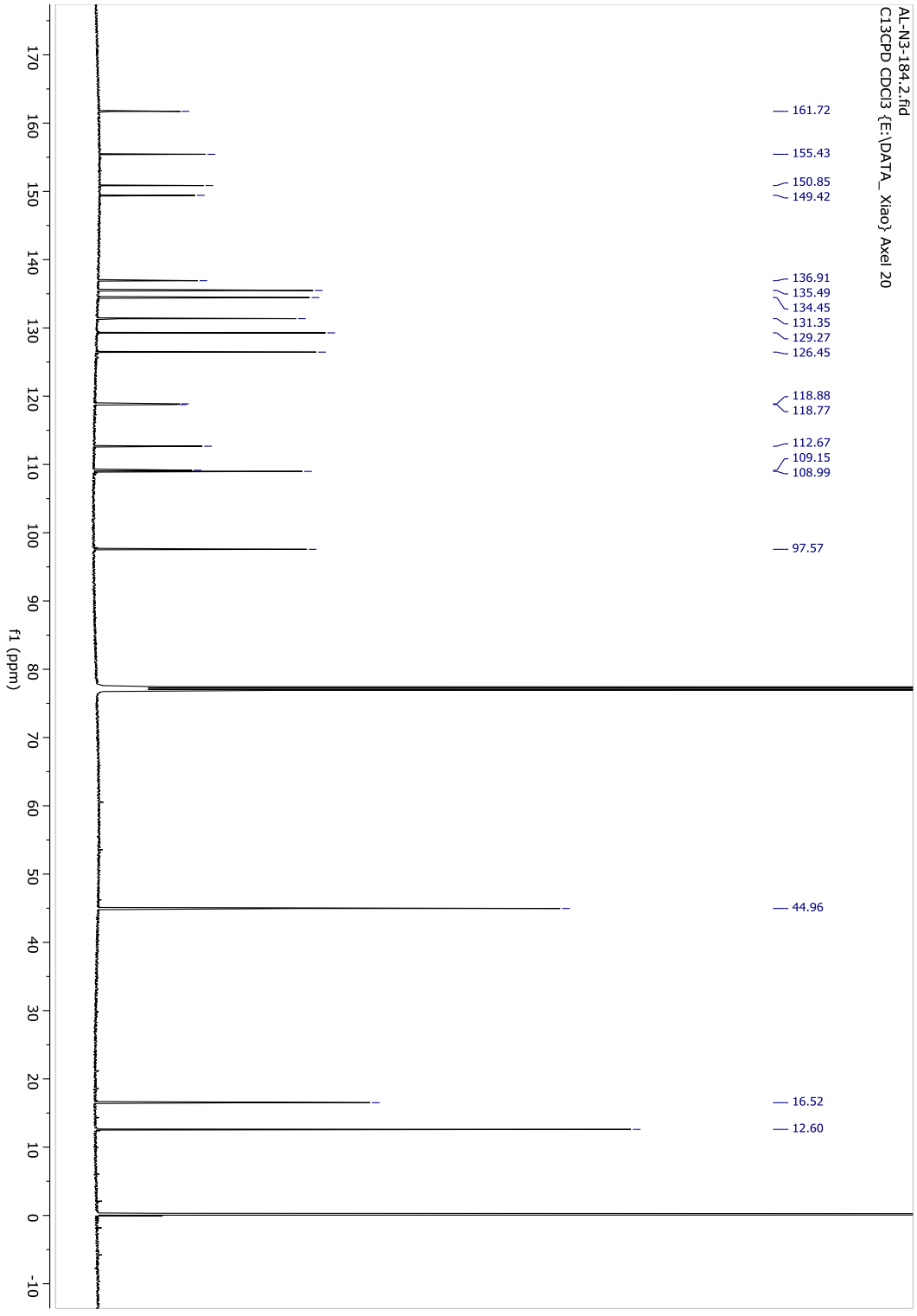


Figure S34. ^{13}C NMR spectrum of compound 12.

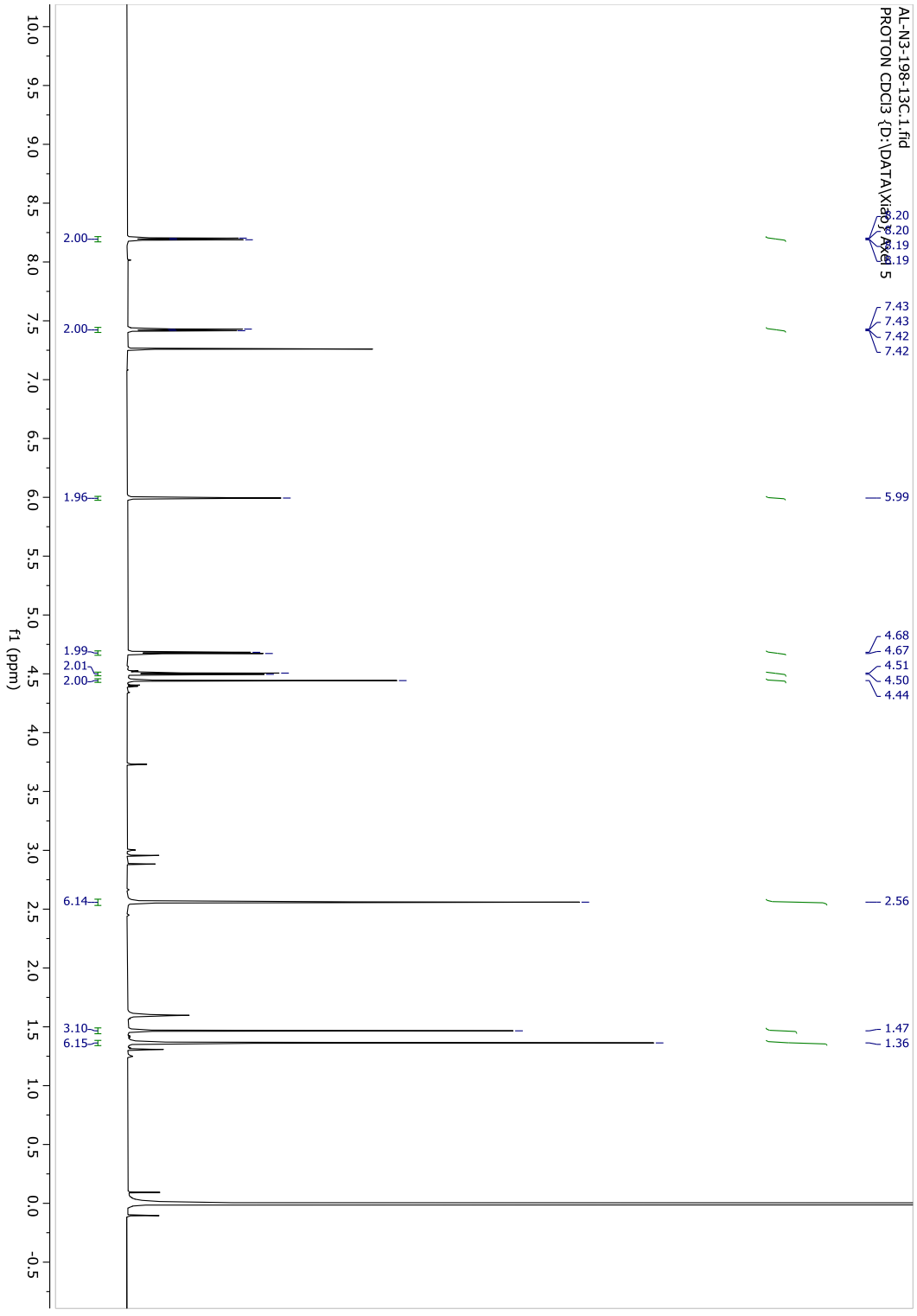


Figure S35. ^{1}H NMR spectrum of compound 14.

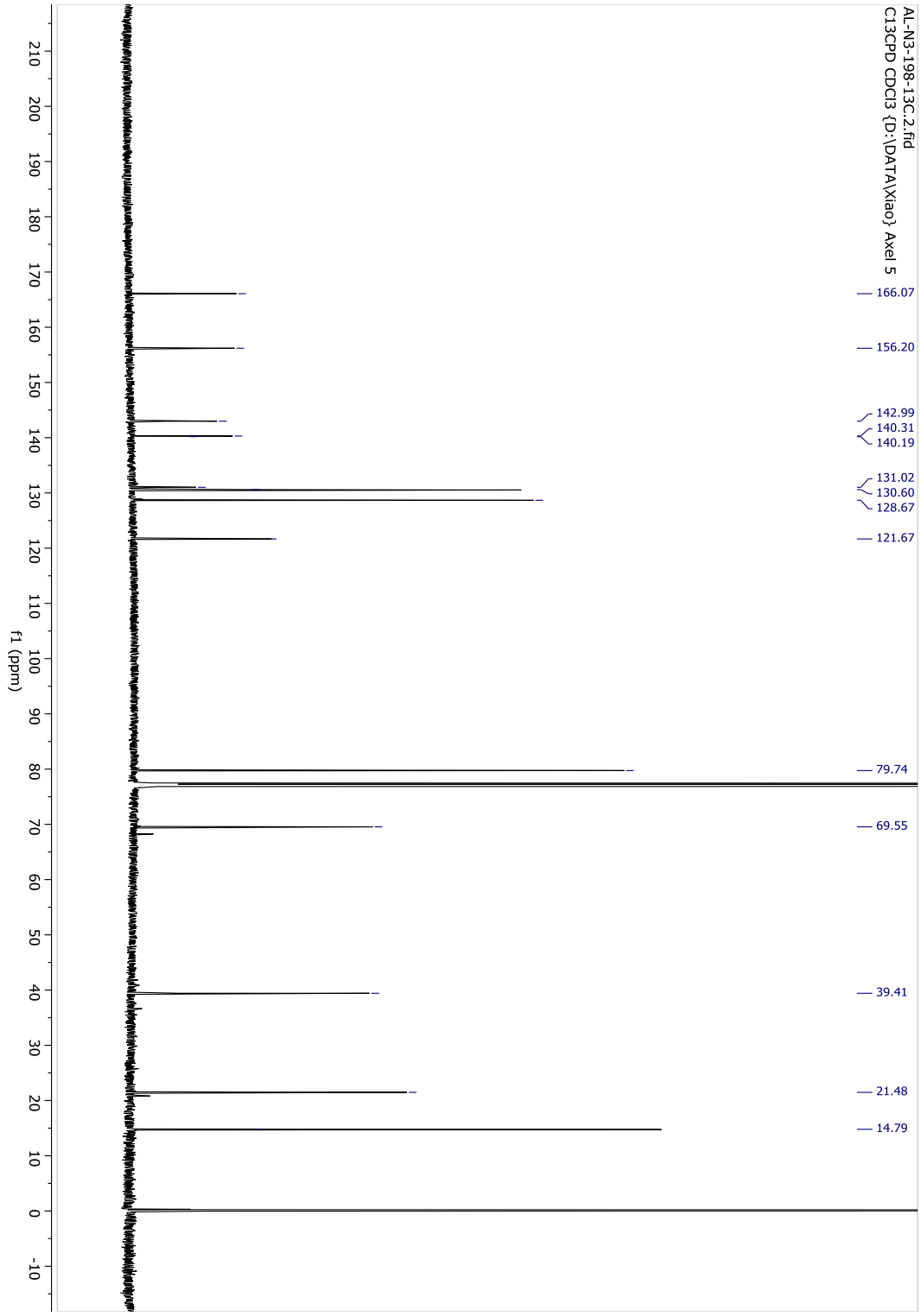


Figure S36. ^{13}C NMR spectrum of compound 14.

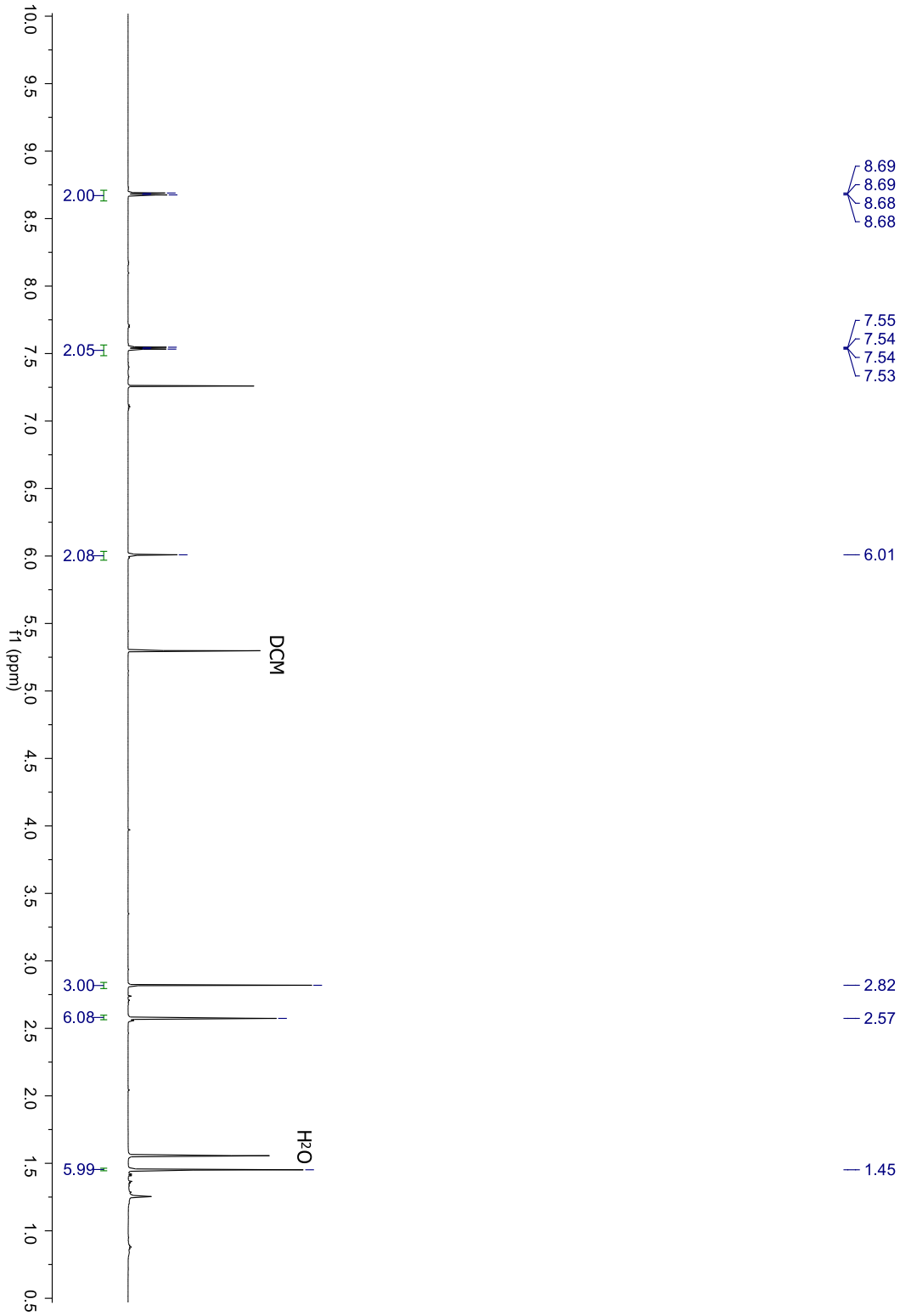


Figure S37. ^{1}H NMR spectrum of compound 15.

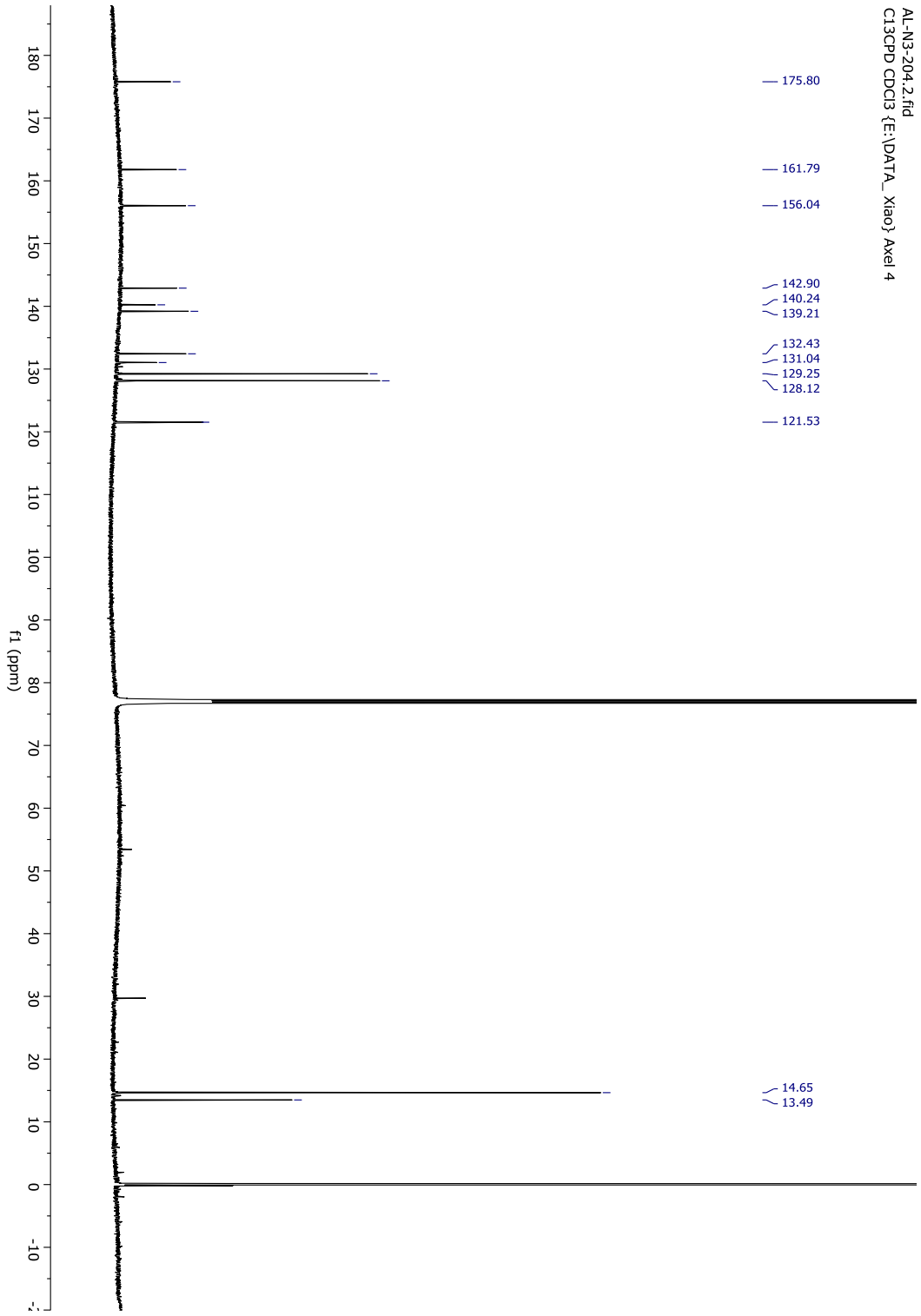


Figure S38. ^{13}C NMR spectrum of compound 15.

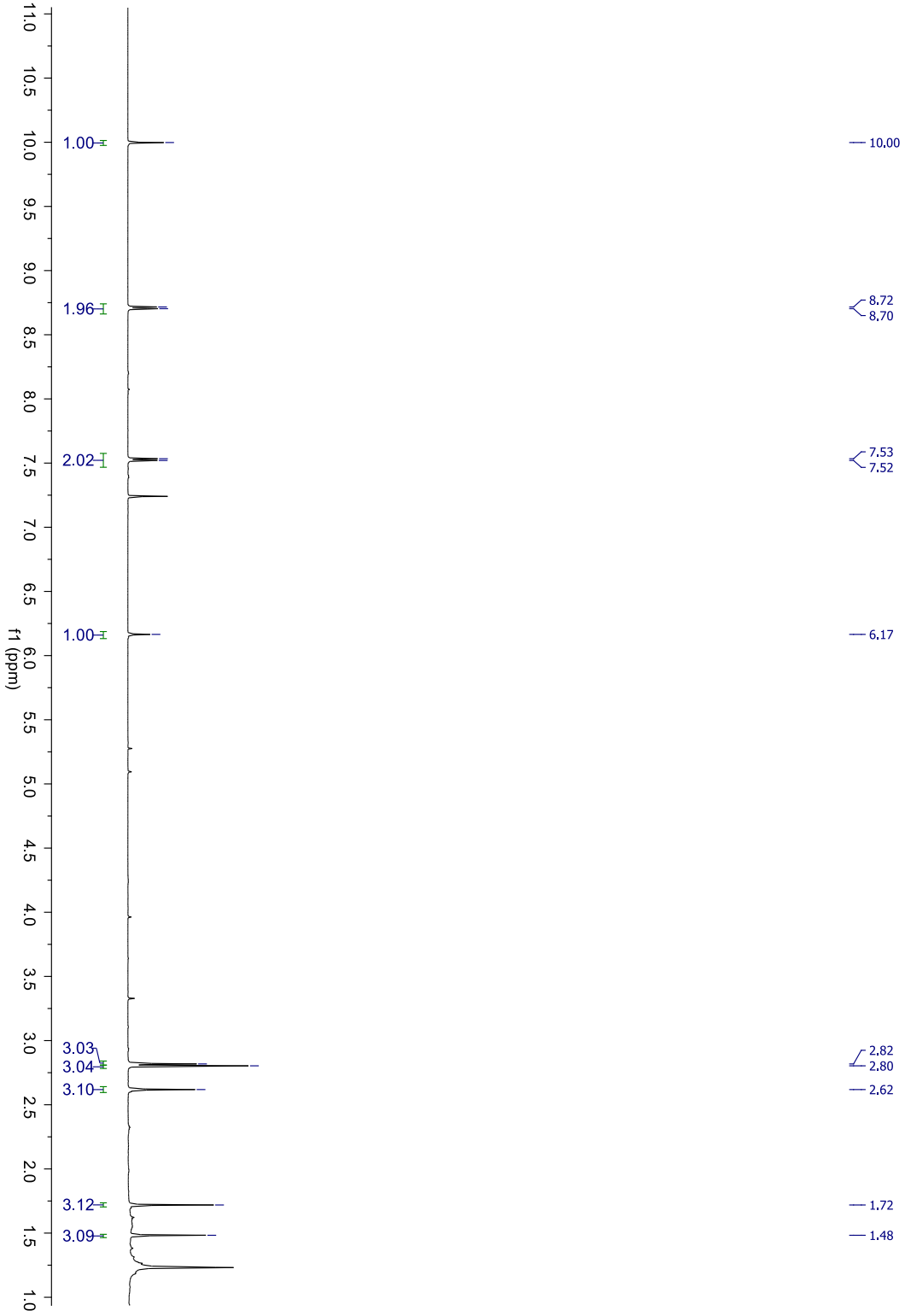


Figure S39. ^{1}H NMR spectrum of compound 16.

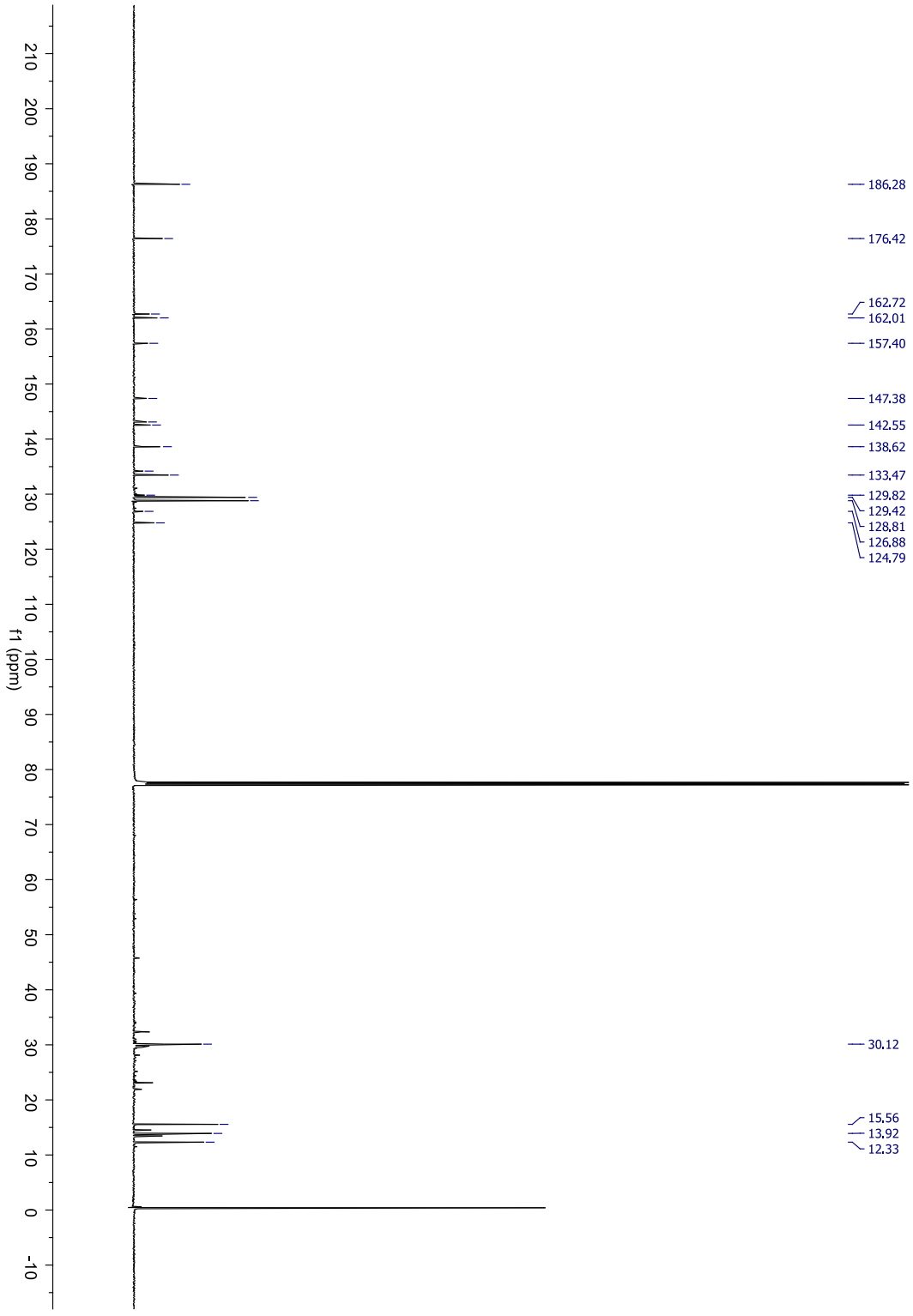


Figure S40. ^{13}C NMR spectrum of compound 16.

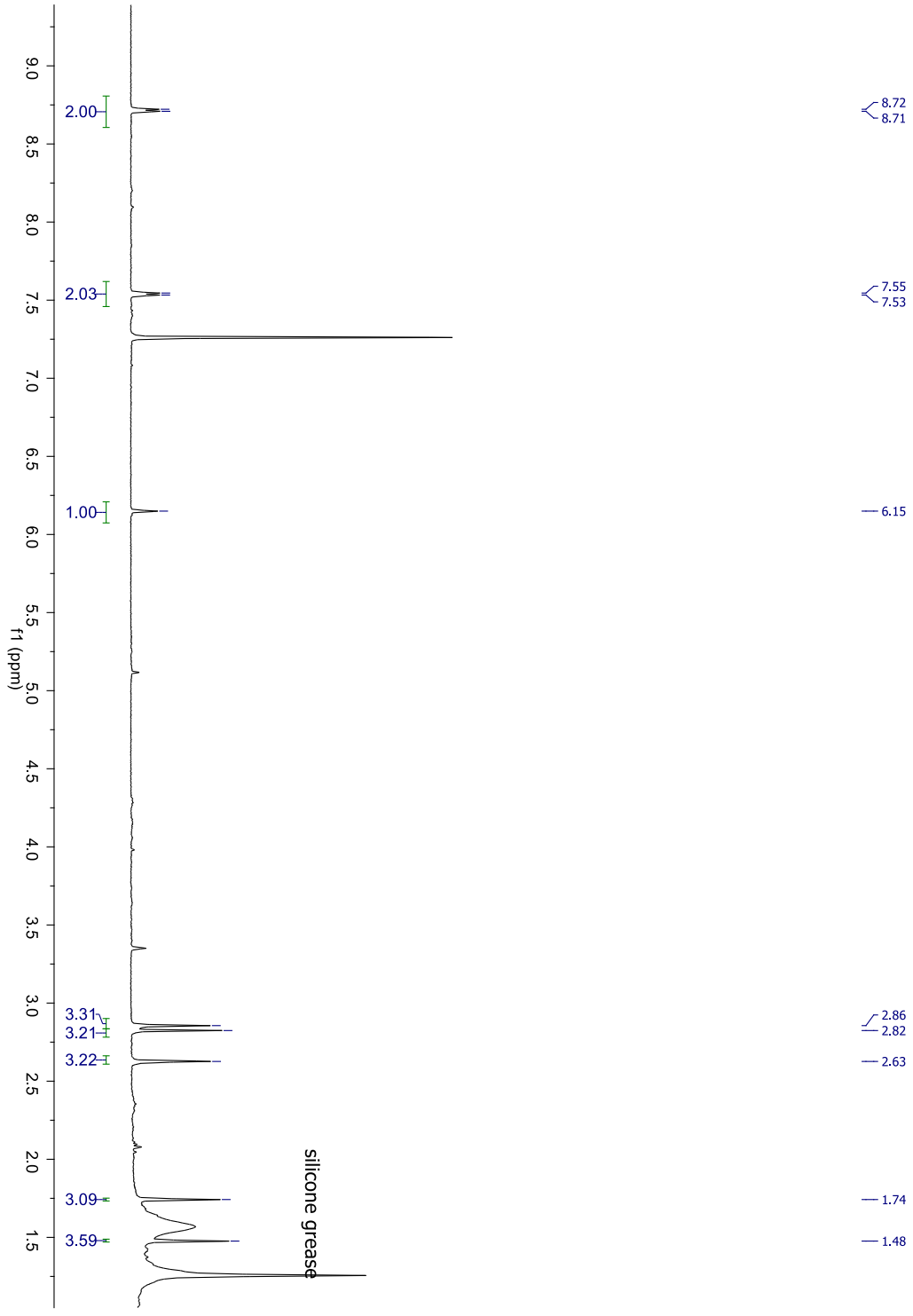


Figure S41. ^{1}H NMR spectrum of compound 17.

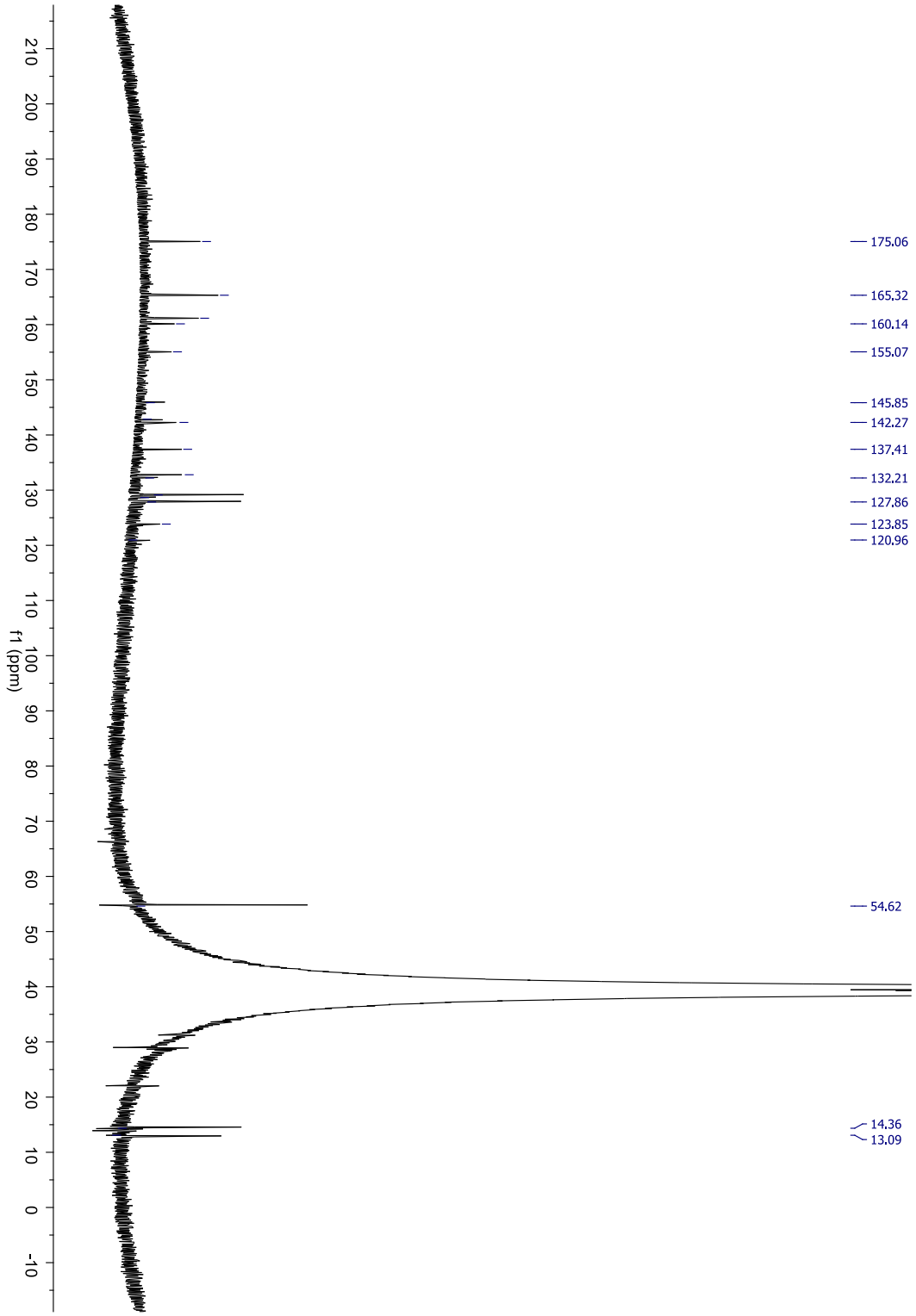


Figure S42. ^{13}C NMR spectrum of compound 17.

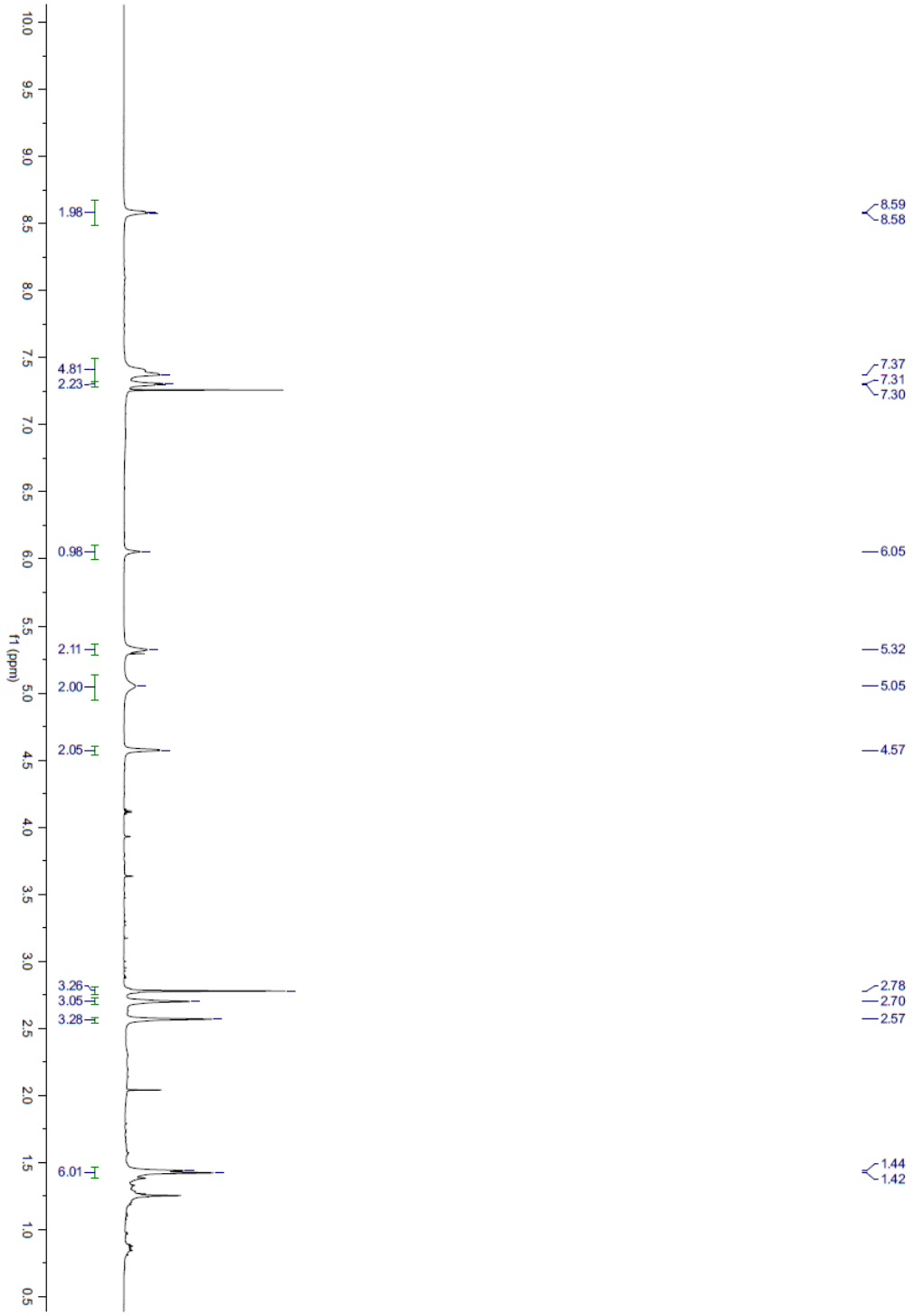


Figure S43. ^{1}H NMR spectrum of compound 18.

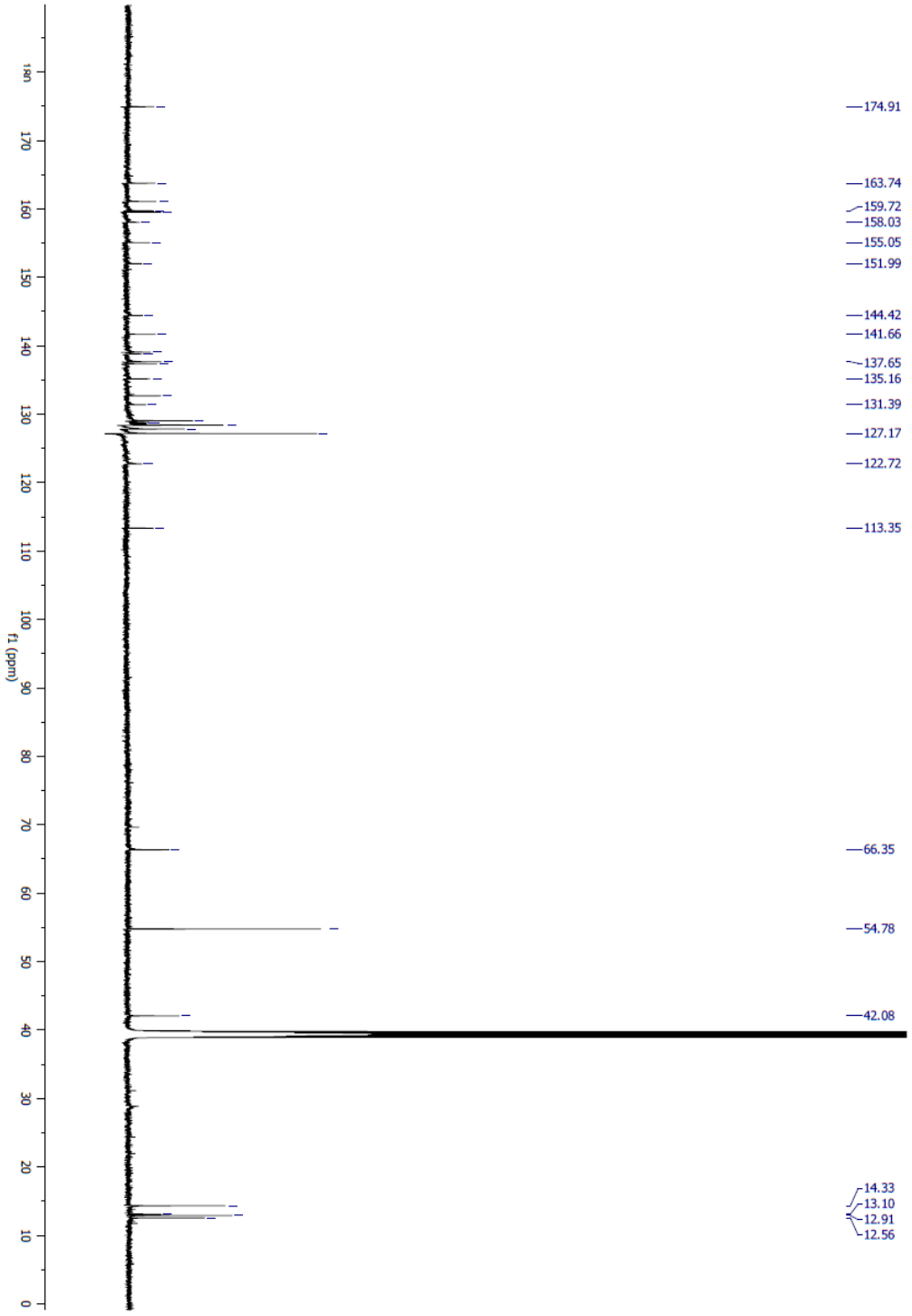


Figure S44. ^{13}C NMR spectrum of compound 18.

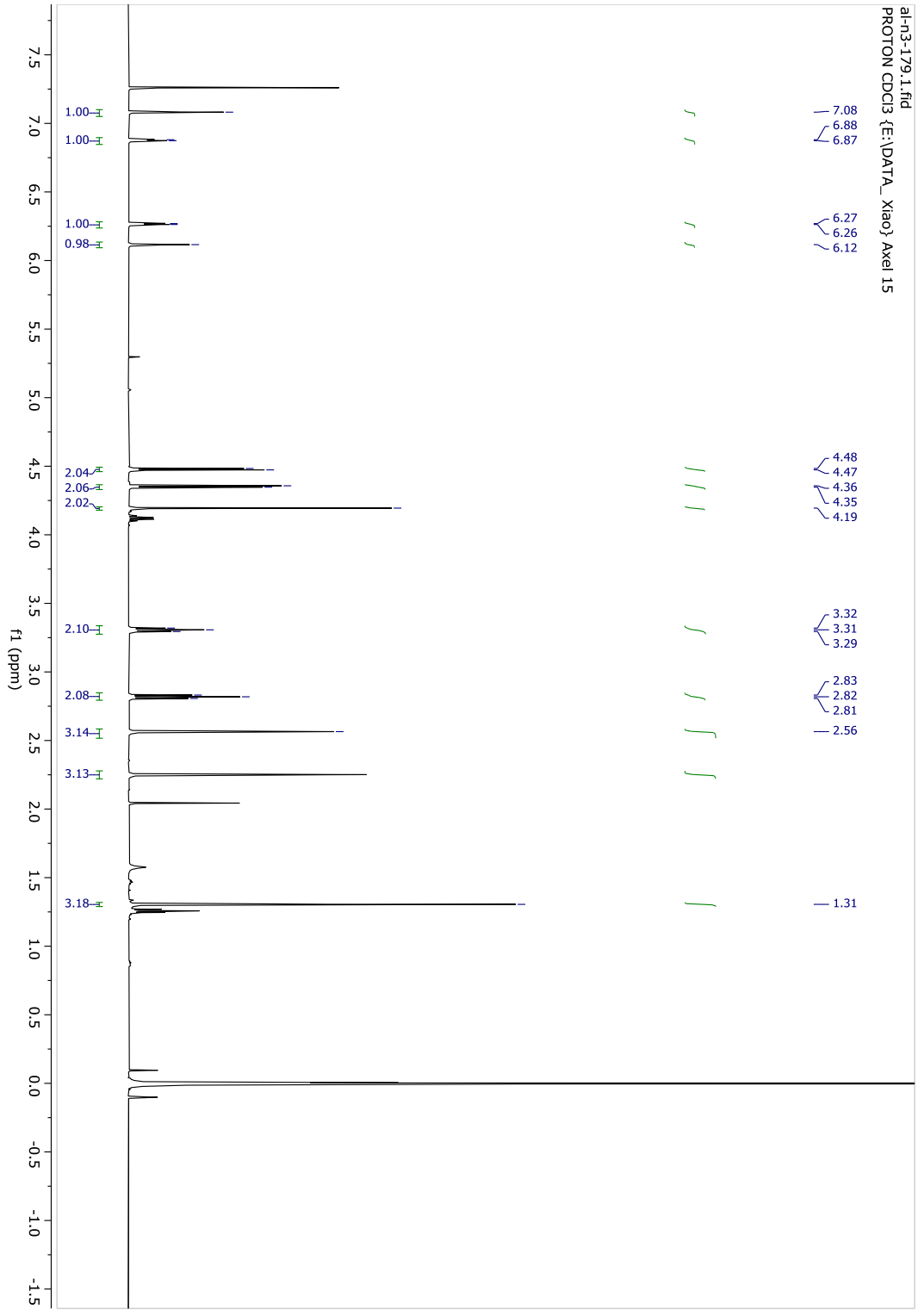


Figure S45. ^{1}H NMR spectrum of compound 20.

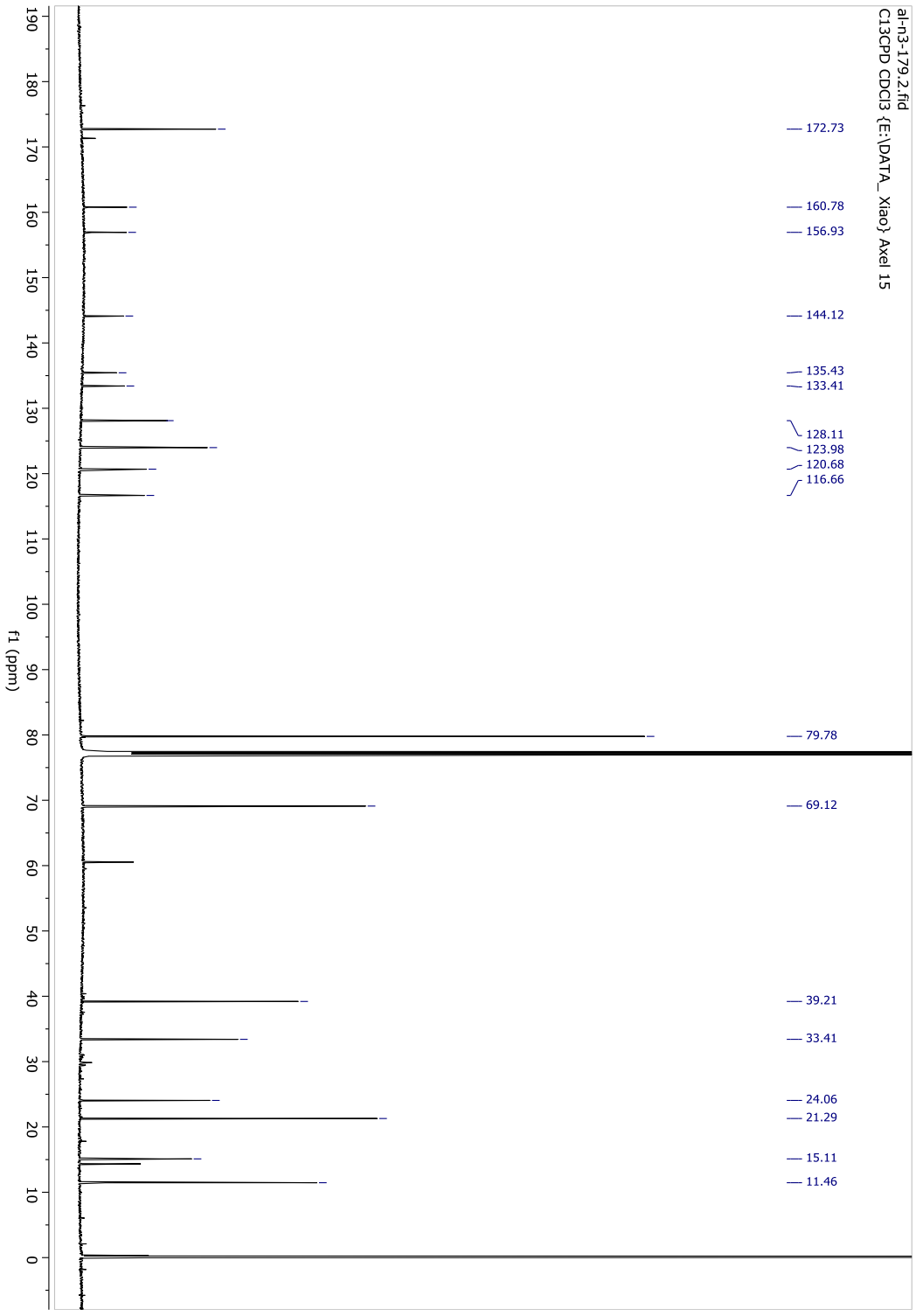


Figure S46. ^{13}C NMR spectrum of compound 20.

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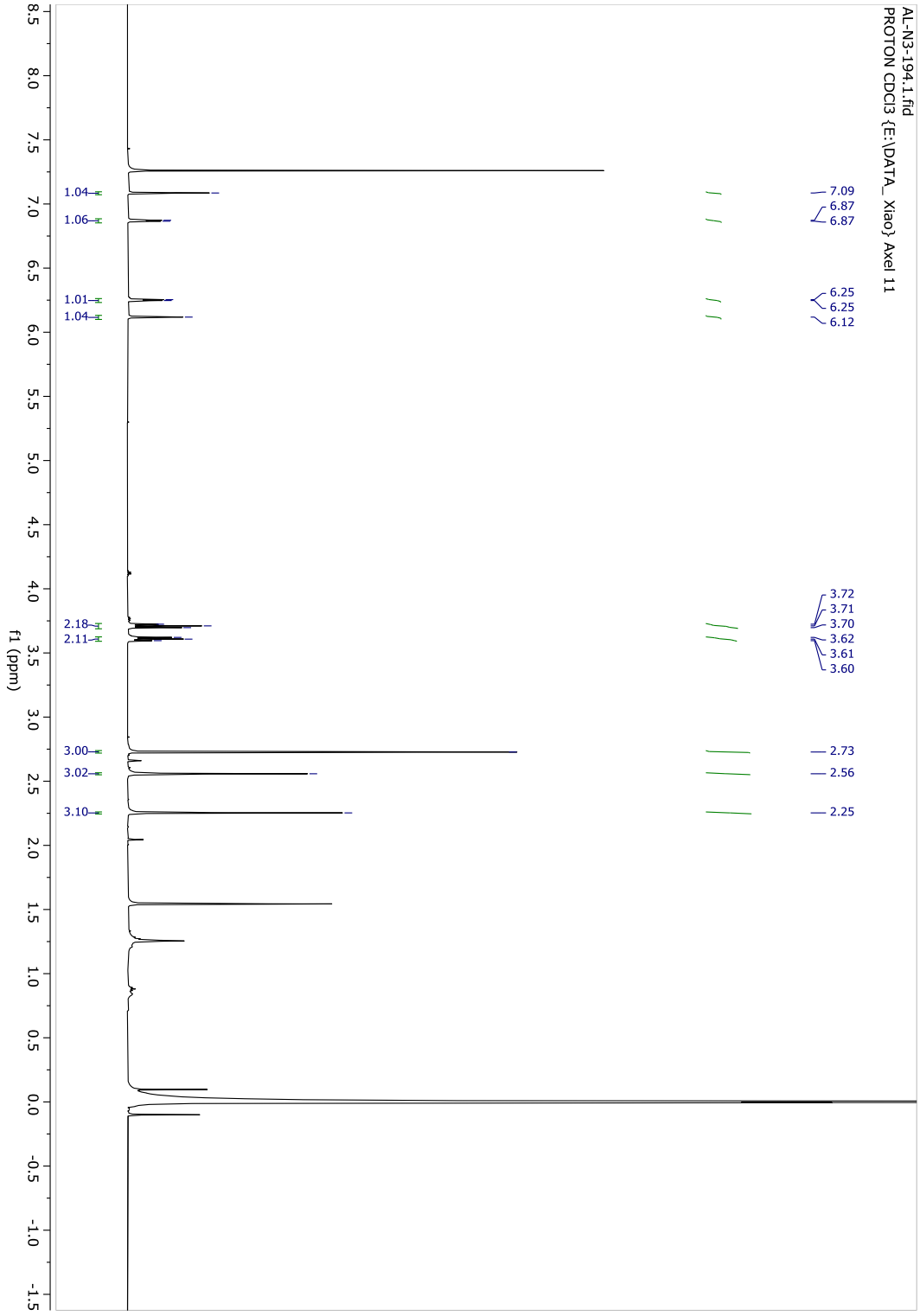

Figure S47. ^{1}H NMR spectrum of compound 21.

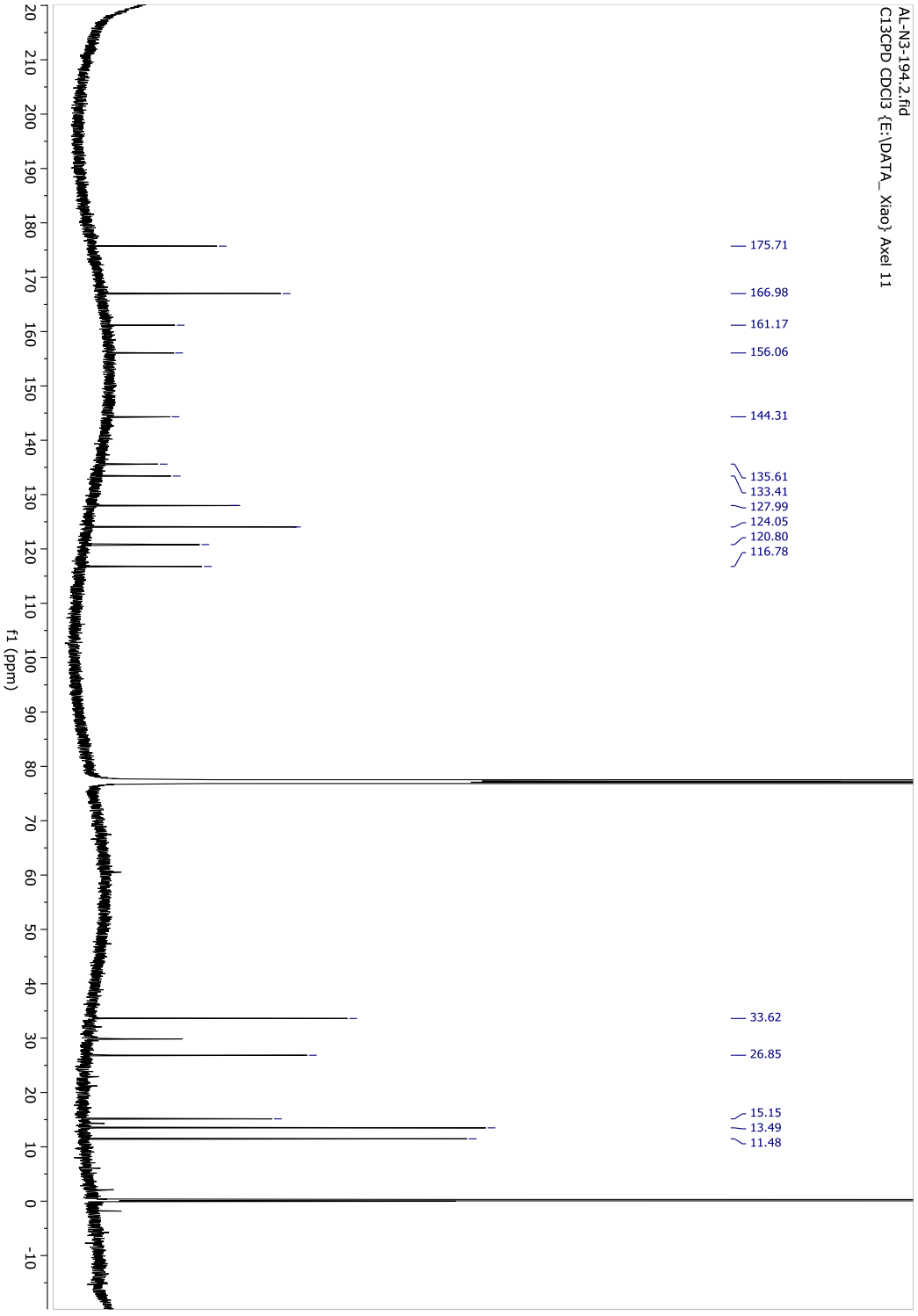

Figure S48. ^{13}C NMR spectrum of compound 21.

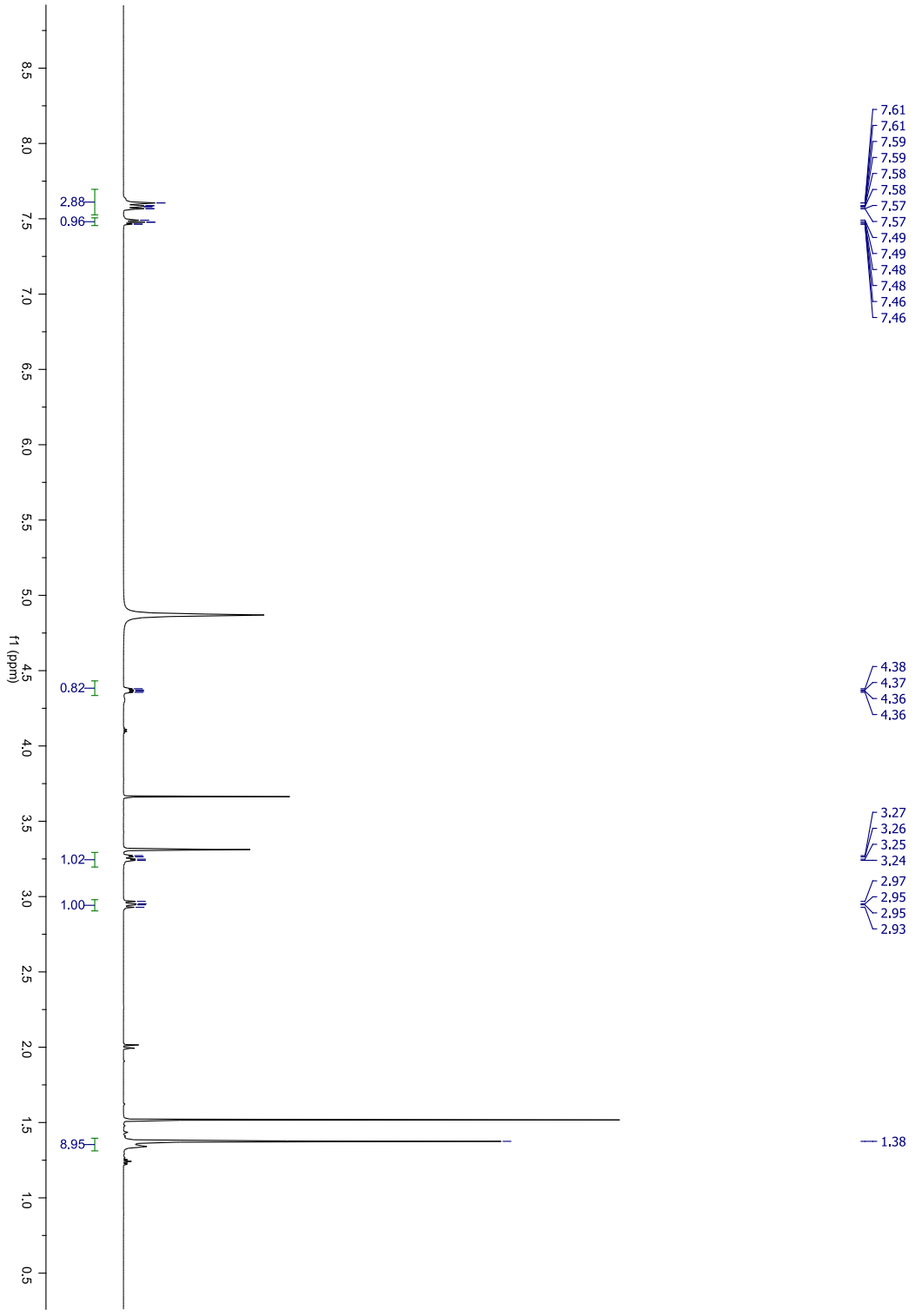

Figure S49. ^{1}H NMR spectrum of compound 22.

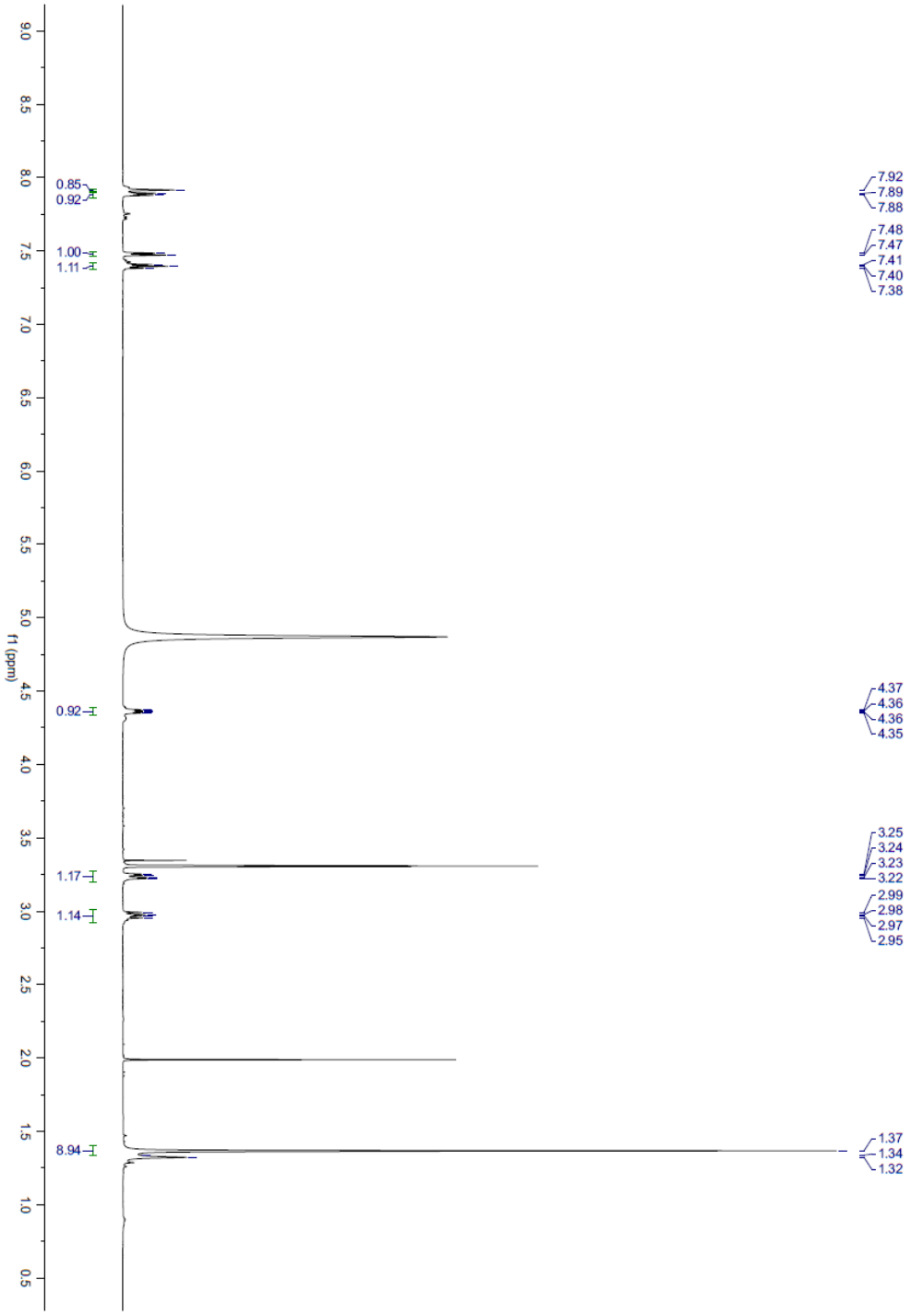

Figure S50. ^{1}H NMR spectrum of compound 23.

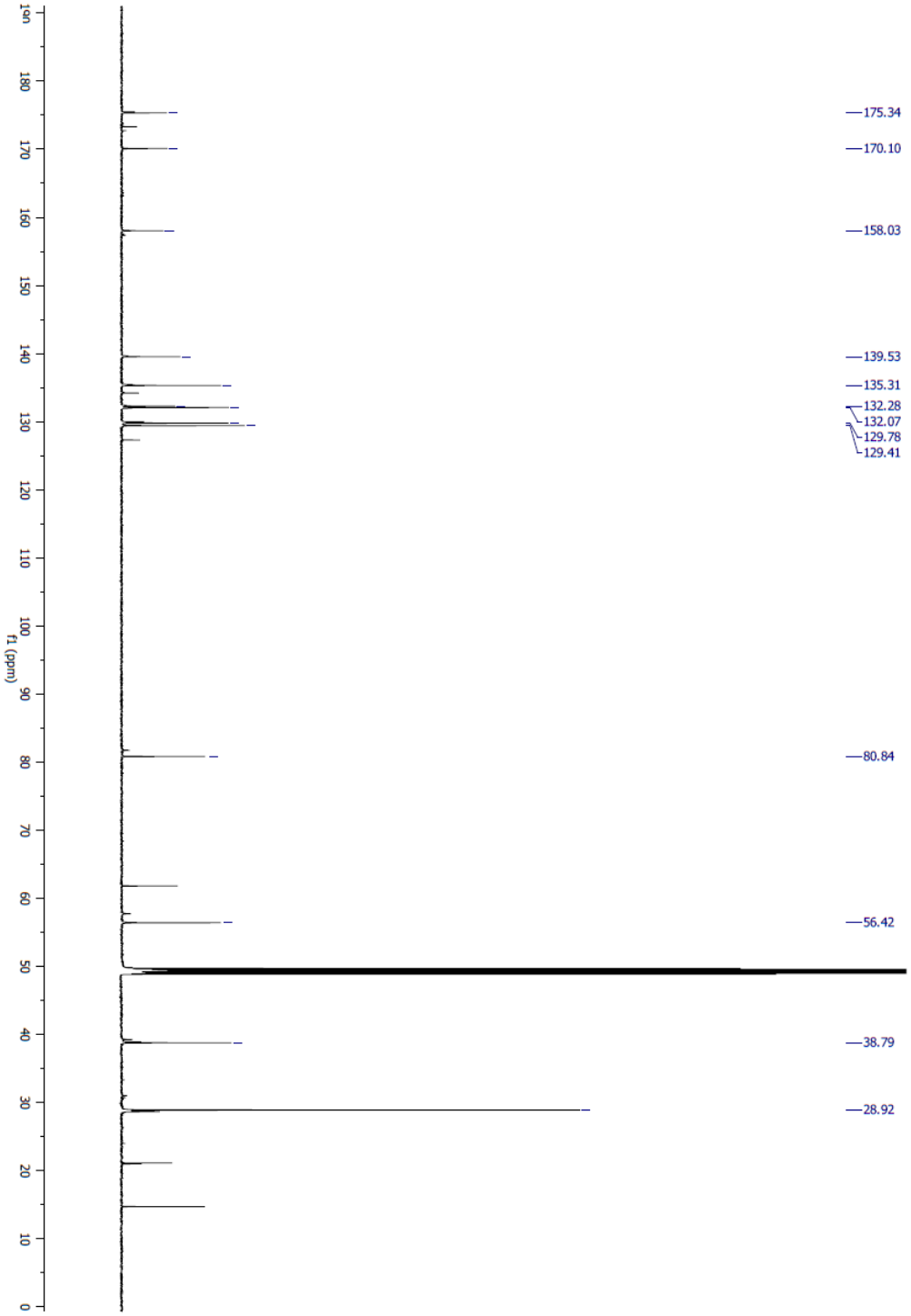

Figure S51. ^{13}C NMR spectrum of compound 23

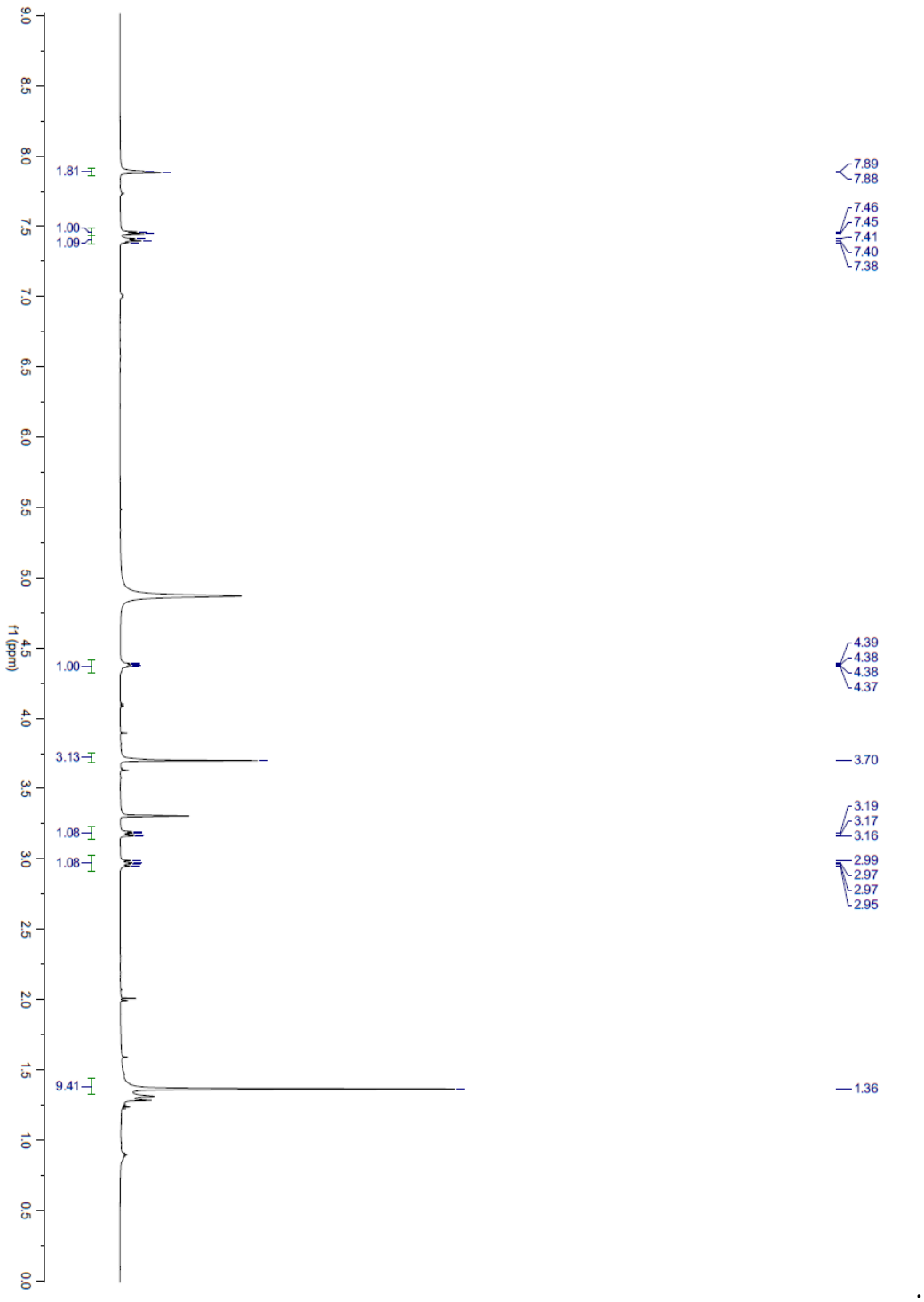

Figure S52. ^{1}H NMR spectrum of compound 24.

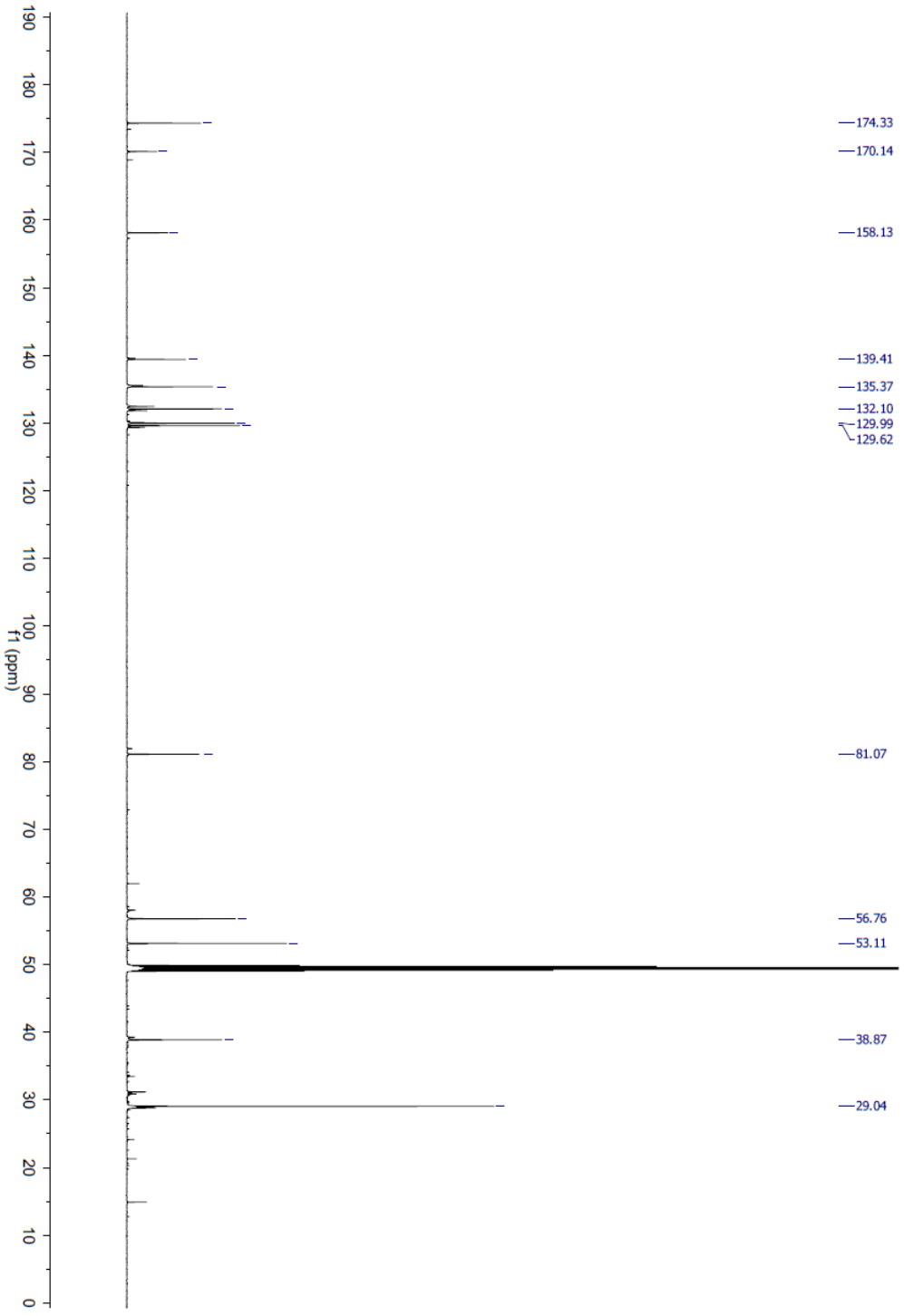

Figure S53. ^{13}C NMR spectrum of compound 24.

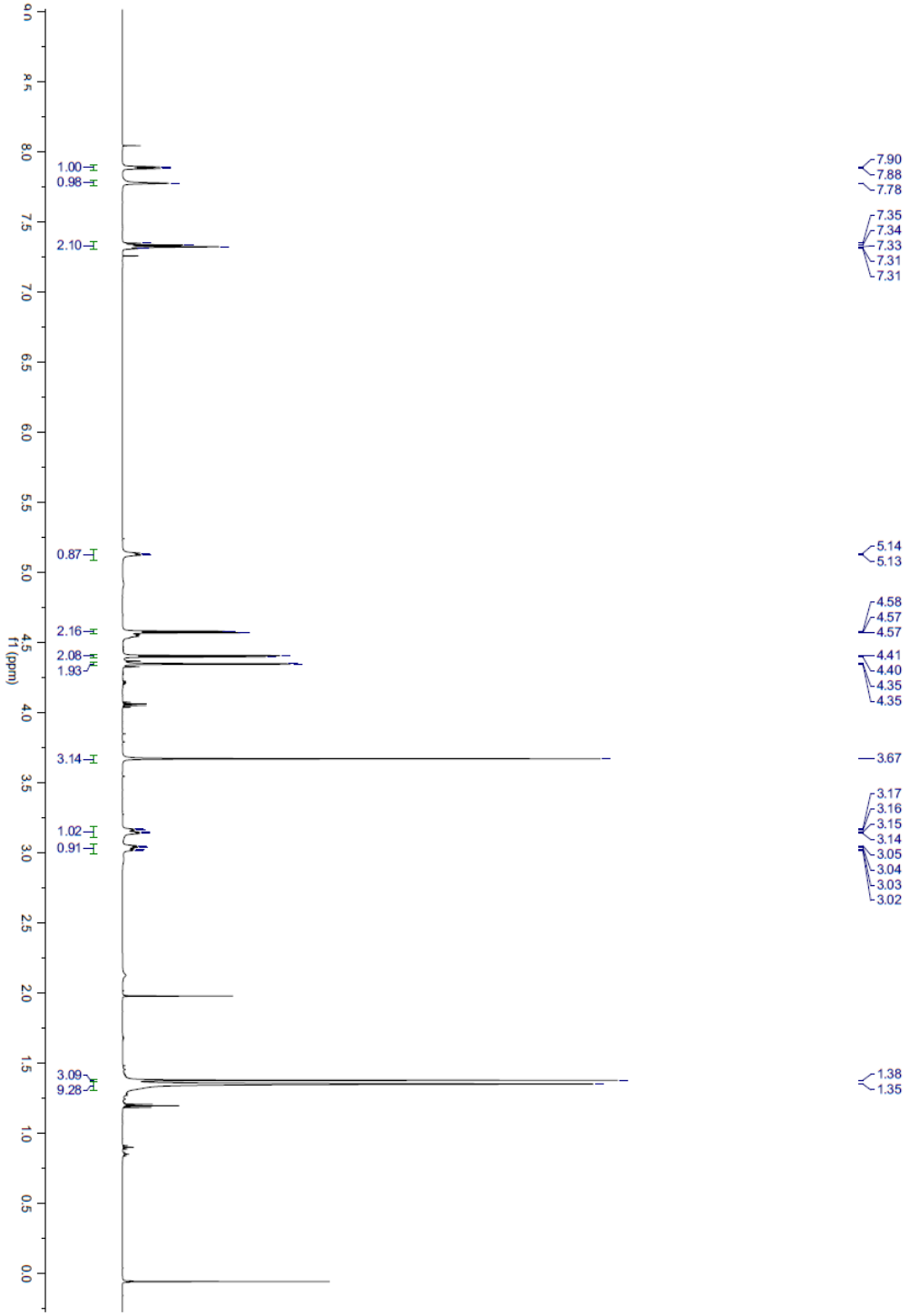

Figure S54. ^{1}H NMR spectrum of compound 25.

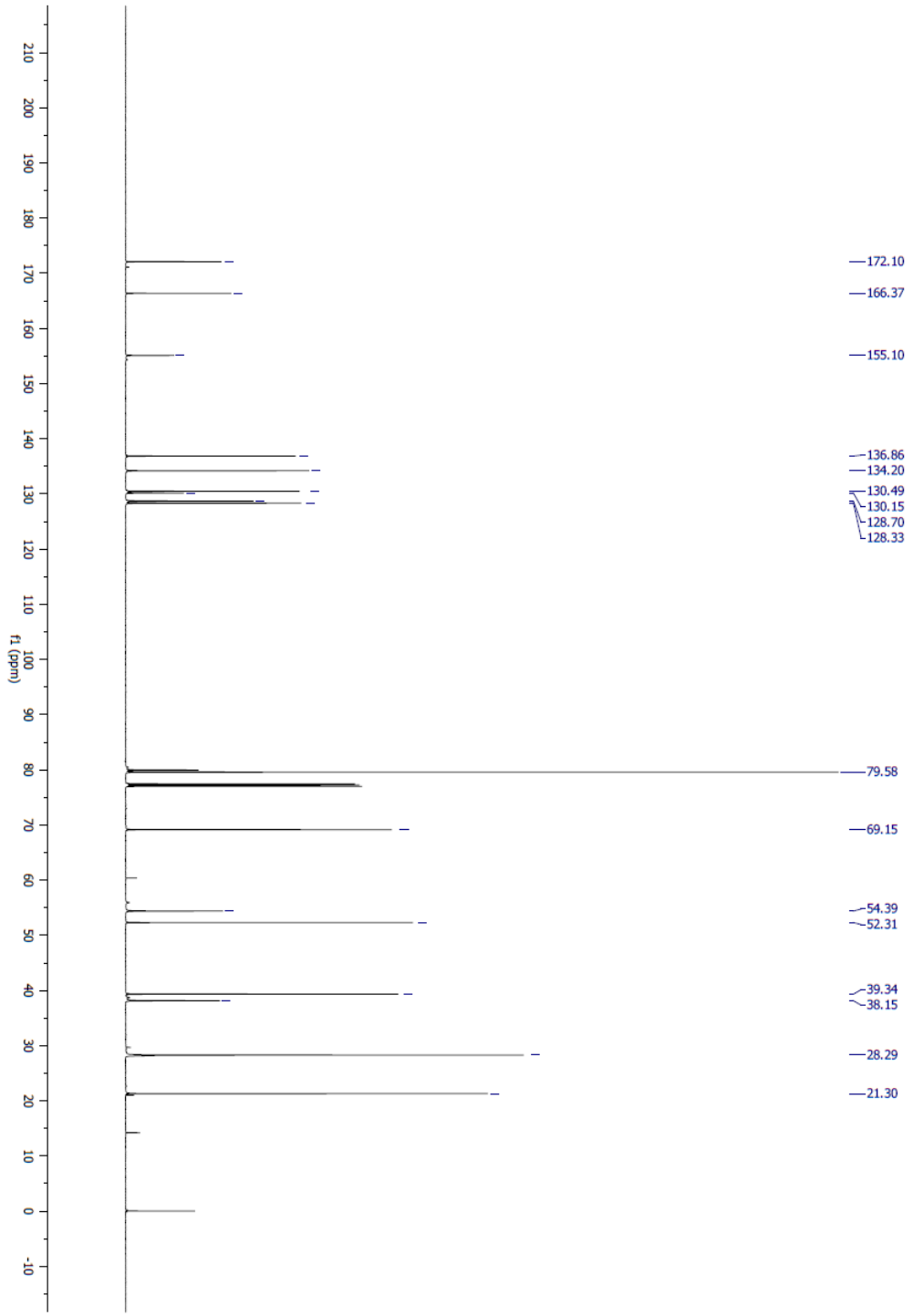

Figure S55. ^{13}C NMR spectrum of compound 25.

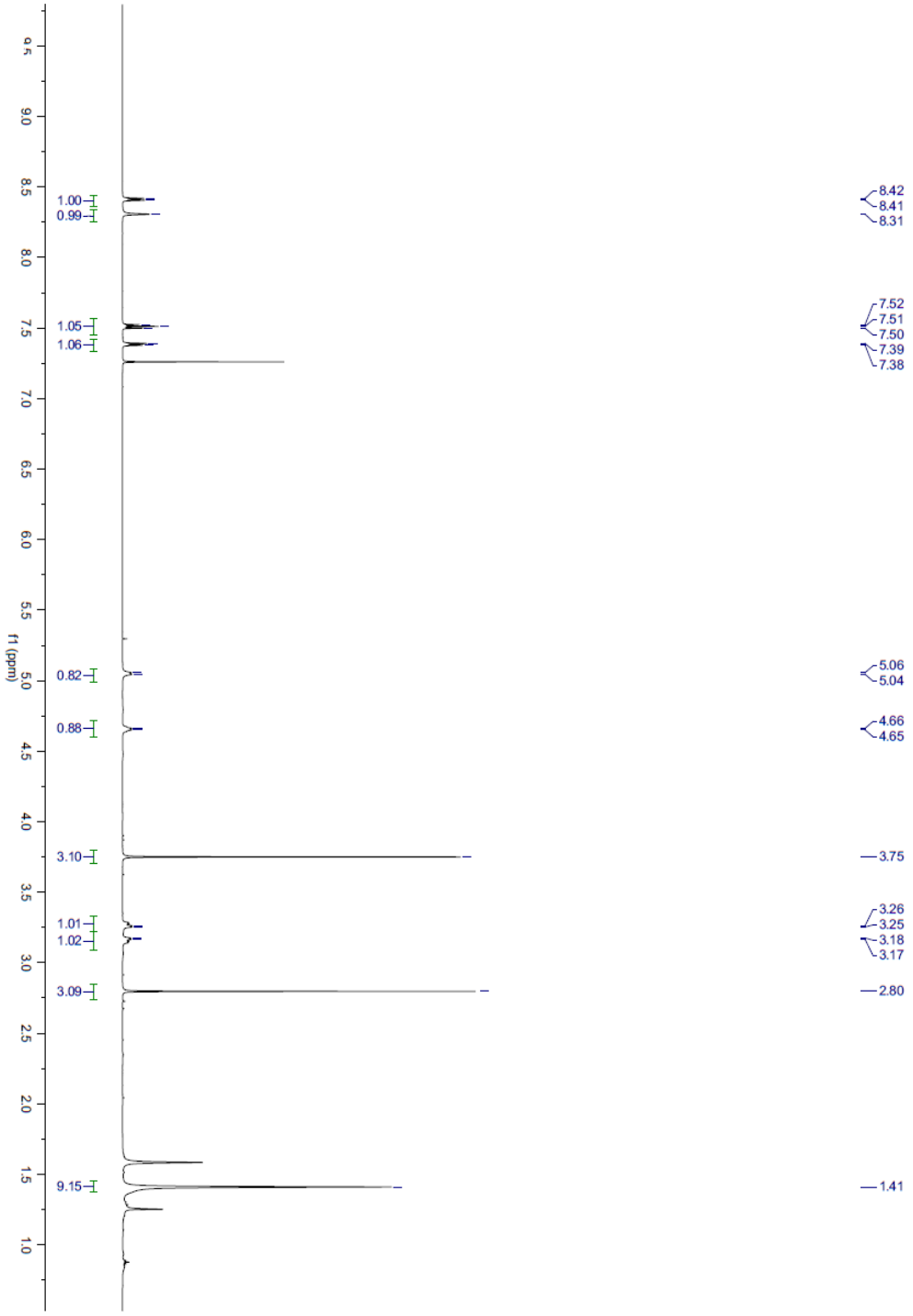

Figure S56. ^{1}H NMR spectrum of compound 26.

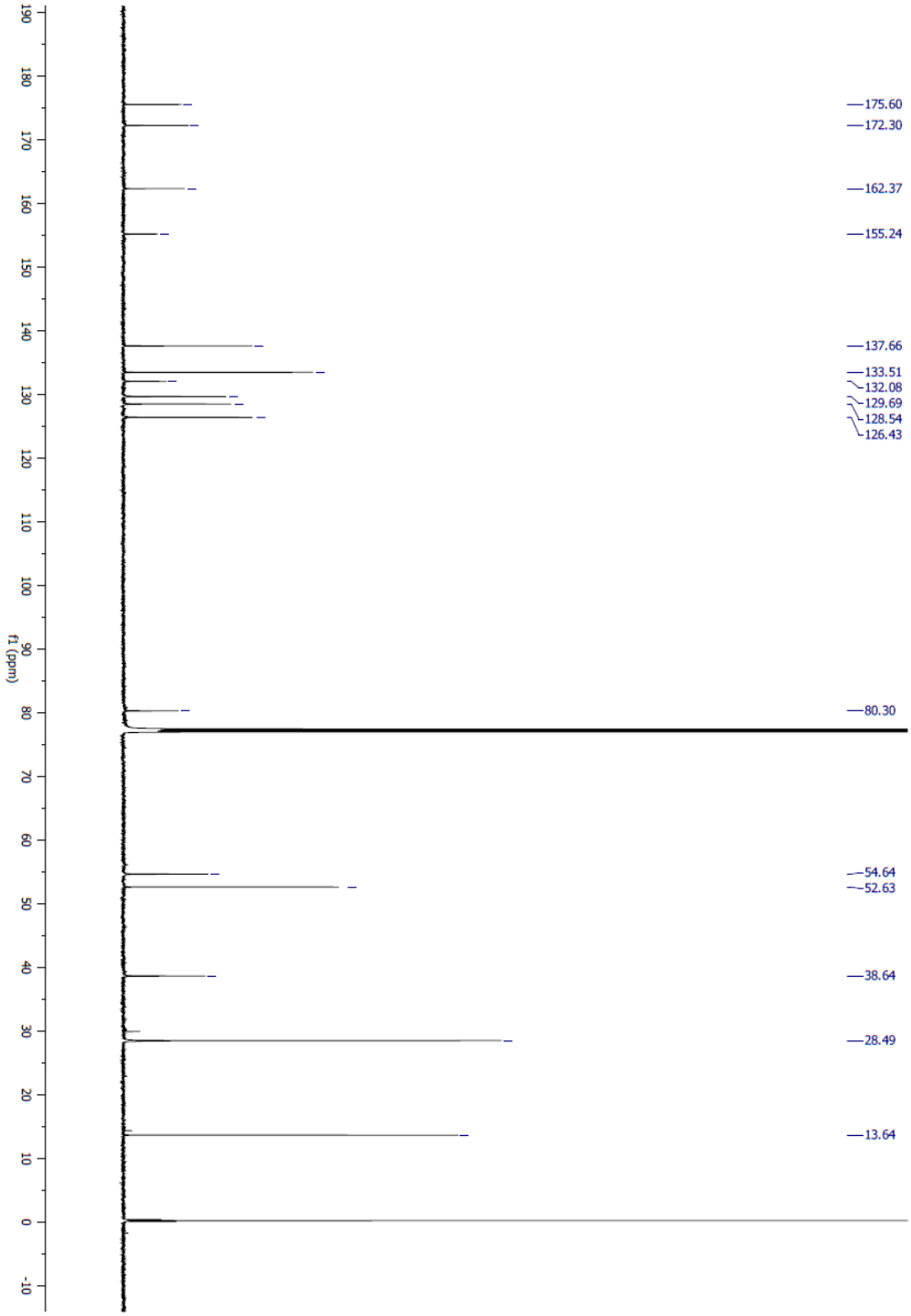

Figure S57. ^{13}C NMR spectrum of compound 26.

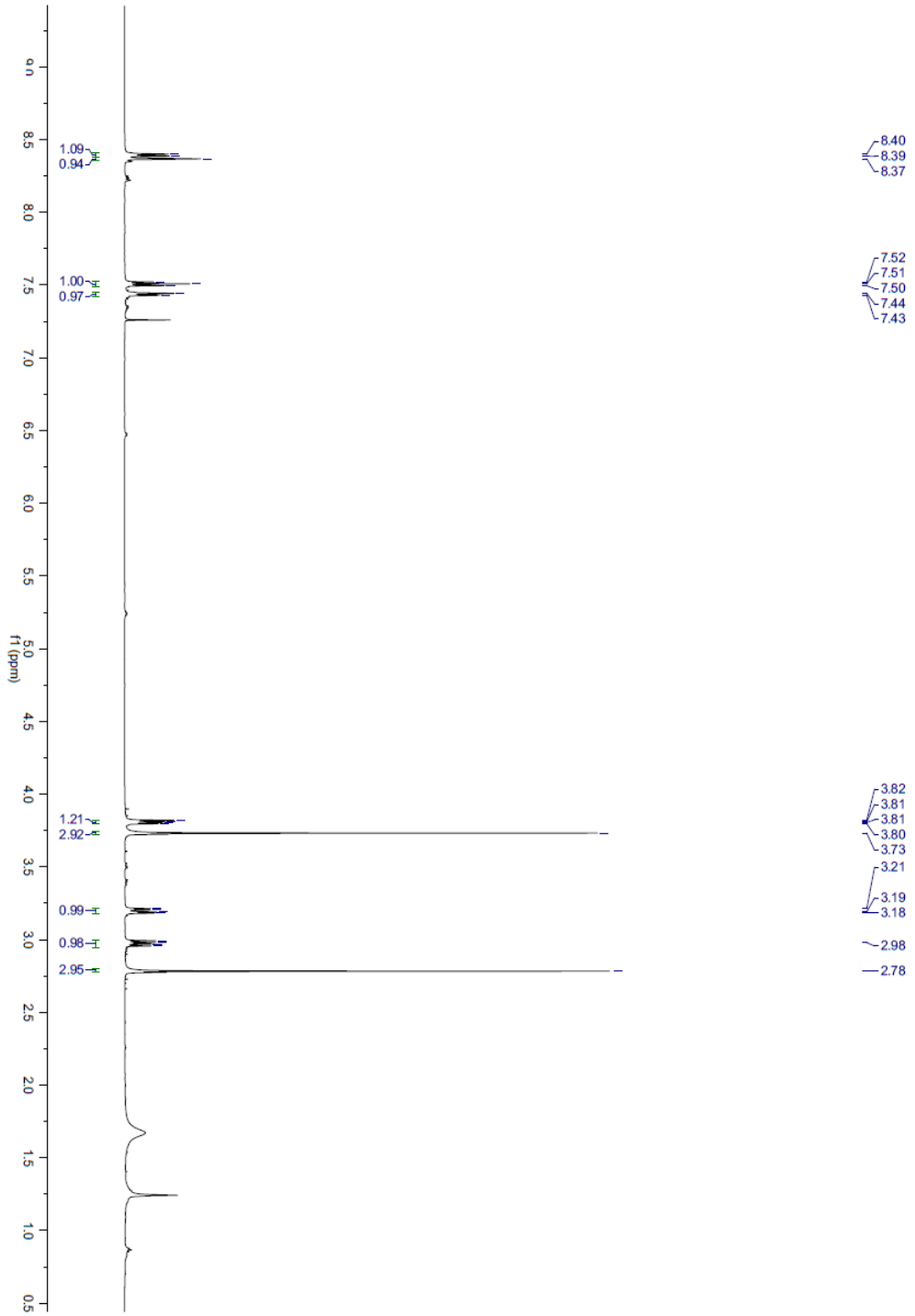

Figure S58. ^{1}H NMR spectrum of compound 27.

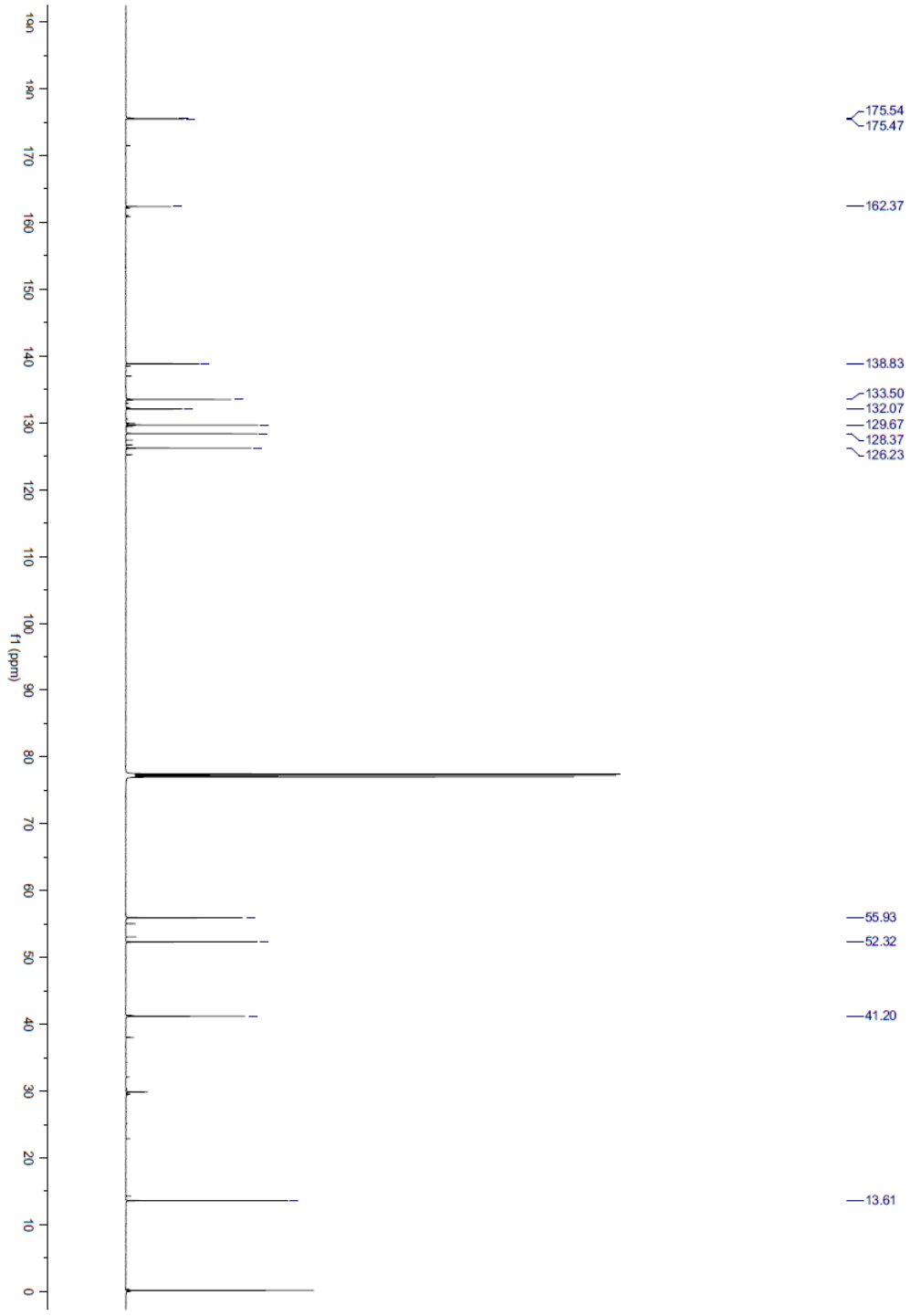

Figure S59. ^{13}C NMR spectrum of compound 27.

10. HPLC and HR-MS

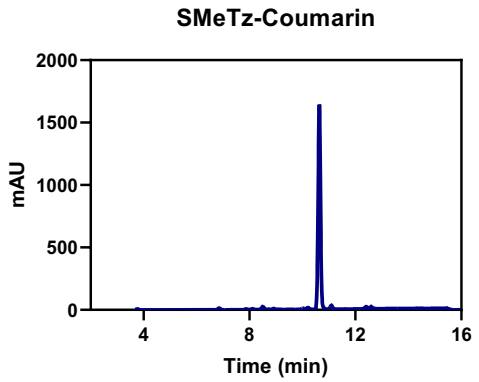

Figure S60. HPLC of compound 11.

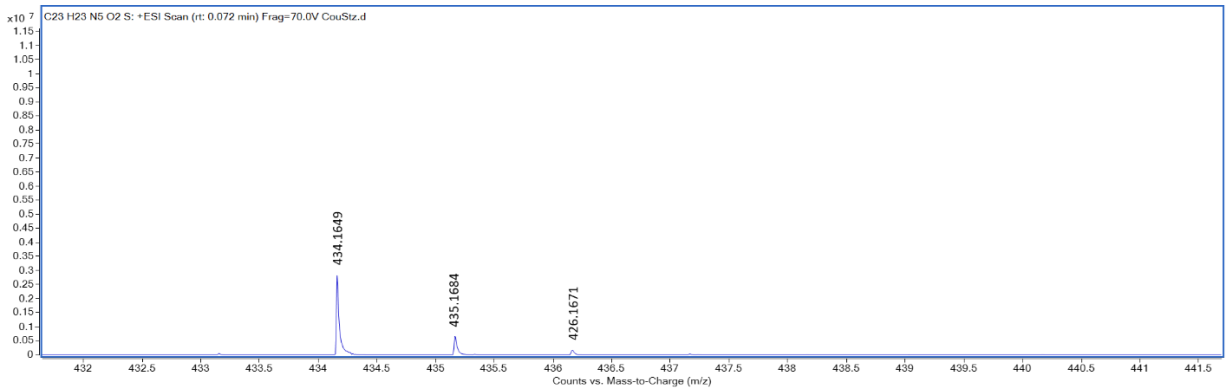

Figure S61. HR-MS of compound 11.

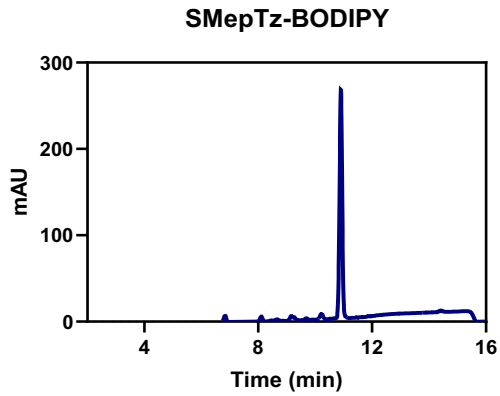

Figure S62. HPLC of compound 15.

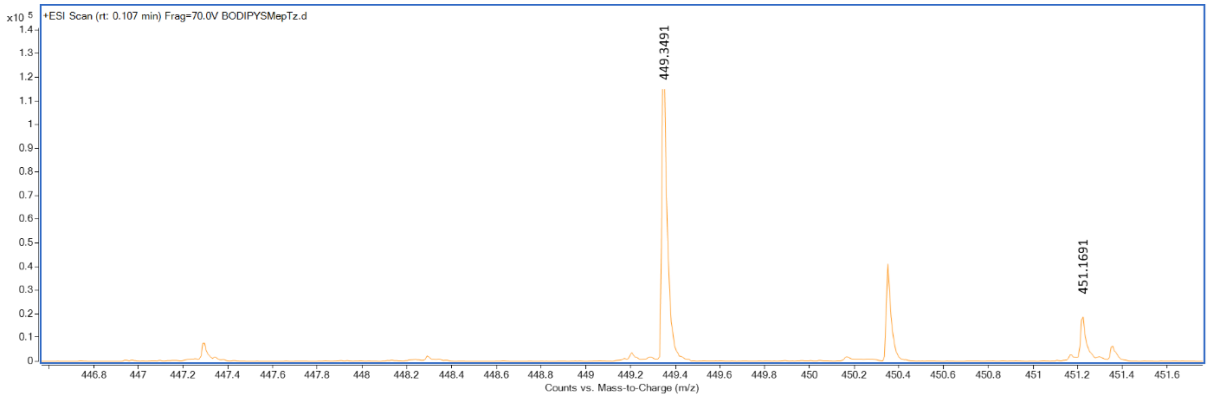

Figure S63. HR-MS of compound 15.

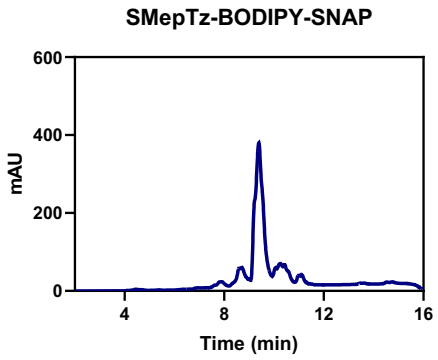

Figure S64. HPLC of compound 18.

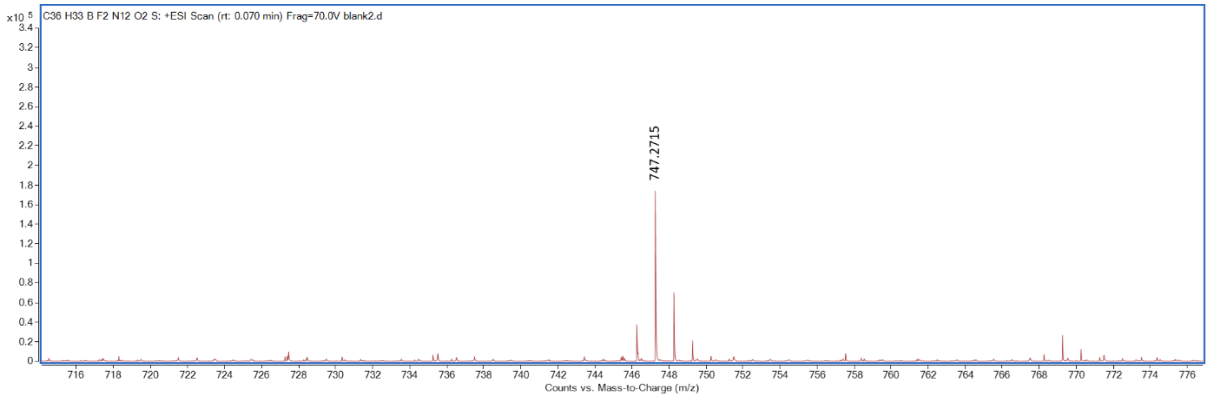

Figure S65. HR-MS of compound 18.

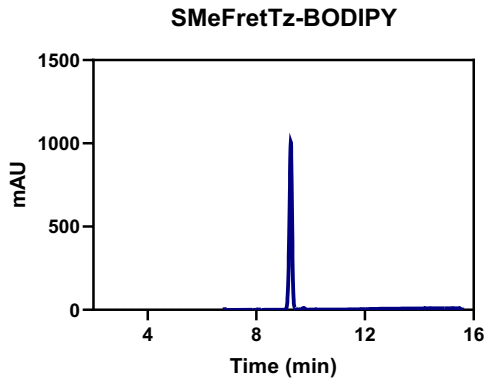

Figure S66. HPLC of compound 21.

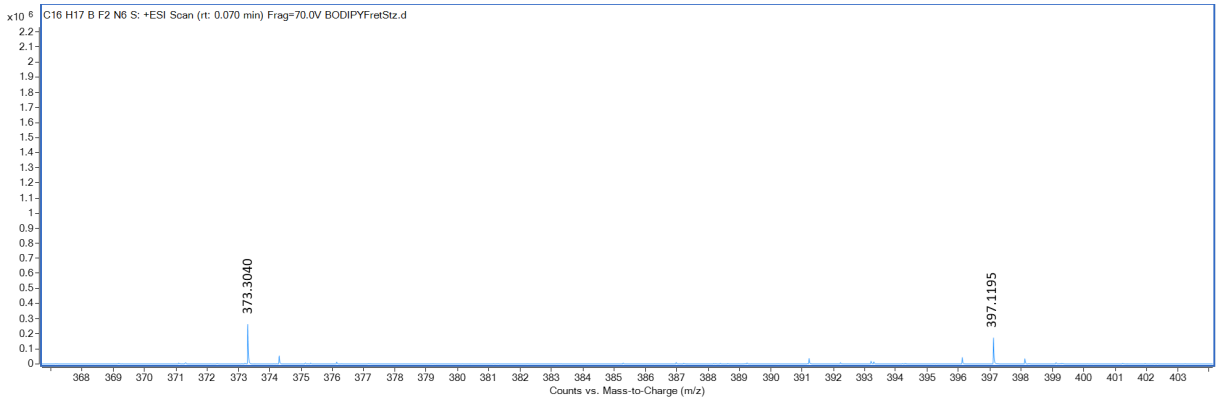

Figure S67. HR-MS of compound 21.

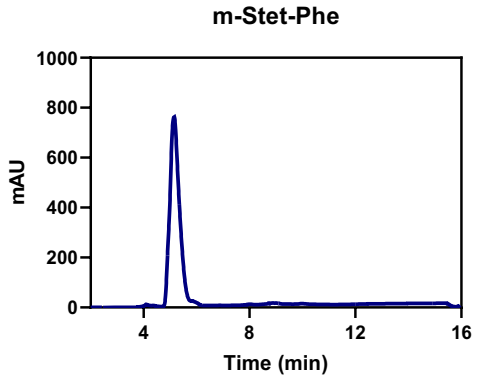

Figure S68. HPLC of compound 27.

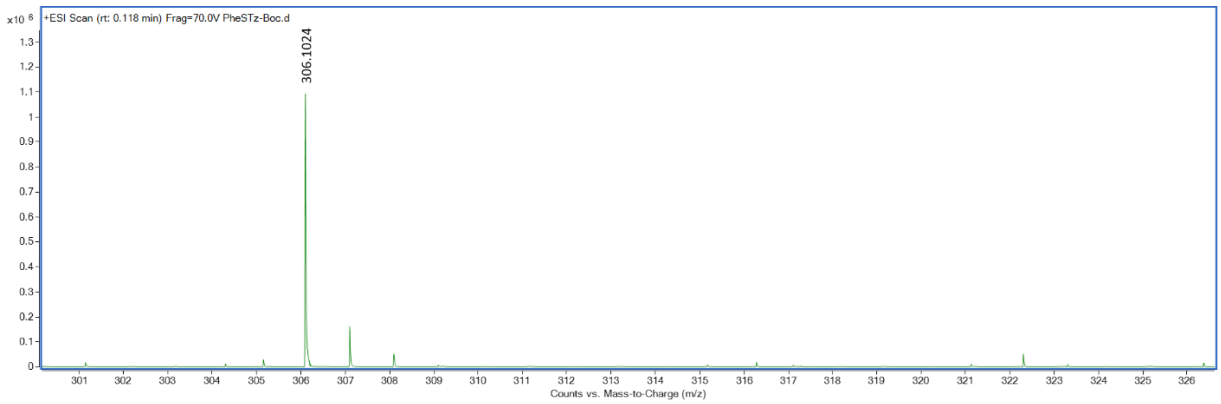

Figure S69. HR-MS of compound 27.

11. Reference

[1] Axel Loredo, Juan Tang, Lushun Wang, Kuan-Lin Wu, Zane Peng and Han Xiao, *Chem. Sci*., 2020, **11**, 4410-4415.