Electronic Supplementary Material (ESI) for ChemComm.

Trifunctional fatty acid derivatives: the impact of diazirine placement

Supporting Information

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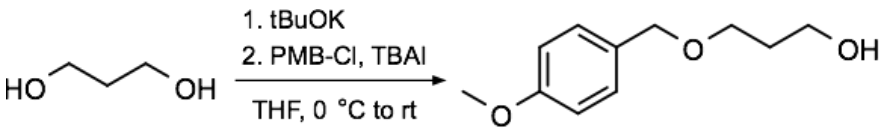
METHODS

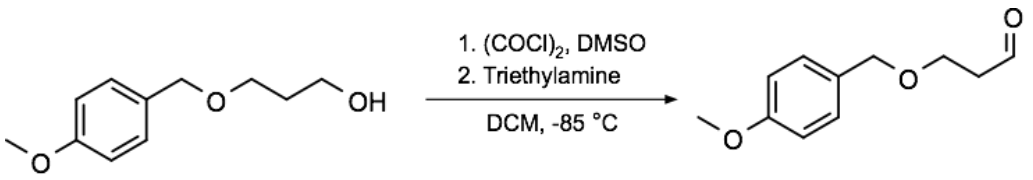
Synthesis

All chemicals were purchased from commercial suppliers and were used without further purification. Solvents were of ACS chemical grade (Fisher Scientific) and were used without further purification. Analytical thin-layer chromatography was performed on silica gel 60 F254 aluminum-backed plates (Millipore Sigma) and spots were visualized either by UV light (254 nm for PMB-containing compounds; 365 nm for coumarin-containing compounsd) or potassium permanganate staining (1.5 g $KMnO_4$, 10g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL of water). Flash column chromatography was performed with manually packed columns using Thermo Scientific Chemical Silica gel (0.035-0.070mm, 60 Å). High pressure liquid chromatography (HPLC) was performed on a Varian Prostar 210 (Agilent) using Polars 5 C18-A columns (Analytical: 150 x 4.6 mm, 3 μm; Preparative: 150 x 21.2 mm, 5 μm). Mass spectra were obtained on an Advion Expressions CMS mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker DPX spectrometer at 400 MHz. Chemical shifts are reported as parts per million (ppm) downfield from solvent references. Spectral characterization can be found in the Supporting Information.

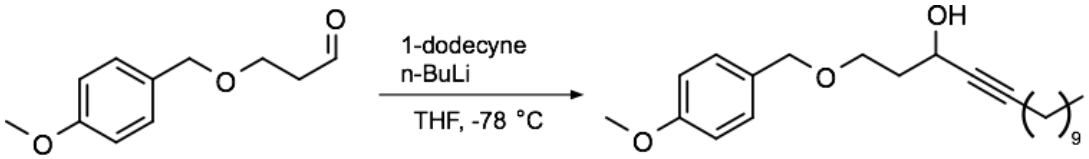
Synthesis of 1-10 FA (2)



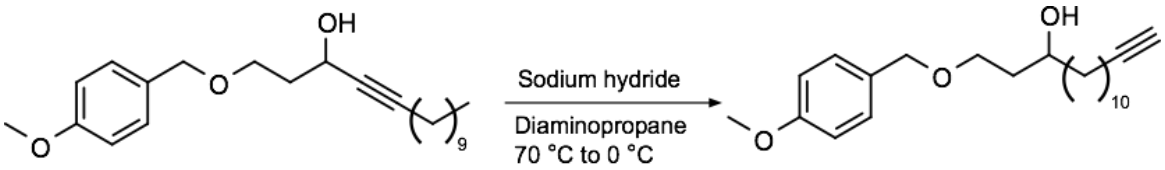
3-[4-Methoxyphenyl)methoxy]propan-1-ol (4). To a 0˚C solution of 1,3-propanediol (18.9 mL, 262.8 mmol, 1 equivalent) in anhydrous THF (150 mL) was added a suspension of potassium *tert*-butoxide (14.75 g, 131 mmol, 1 equivalent) in anhydrous THF (150 mL), dropwise. To the resulting viscous suspension of alkoxide was added tetrabutylammonium iodide (9.7083 g, 18.95 mmol, 0.075 equivalents) and para-methoxylbenzyl chloride (14.4 mL, 0.5 equivalents). Once the addition was complete, the reaction was allowed to warm to room temperature and stirred overnight. TLC (2:1 hexanes : ethyl acetate) showed the formation of the desired product (Rf = 0.2) as well as the bis-protected byproduct (Rf = 0.75). Water (100 mL) was added, and the aqueous layer was extracted 3 x 100 mL with ethyl acetate and the combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The orange oil was purified by flash chromatography, with a gradient of 2 : 1 hexanes : ethyl acetate to pure ethyl acetate, yielding **4**, 15.3 g, in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.255 (dt, J = 8.8, J = 2.8, 2H), 6.881(dt, J = 8.8, J = 2.8, 2H), 4.455(s, 2H), 3.806(s, 3H), 3.776(t, J = 5.6, 2H), 3.641(t, J = 5.6, 2H), 1.855(quint, J = 5.6, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 159.2, 130.2, 129.3, 113.8, 68.9, 61.7, 55.3, 32.1; MS (*m*/*z*): $[M - H_2O + H]^+$ calcd. for $C_{11}H_{16}O_3$, 179.107; found 179.2



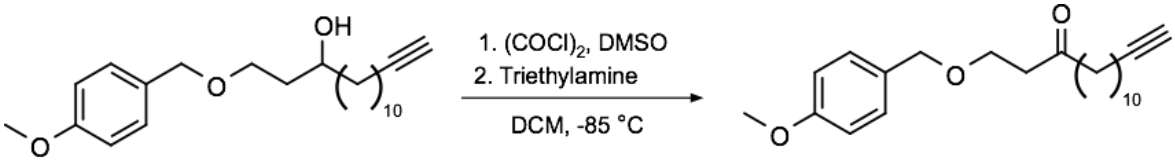
3-[4-Methoxyphenyl)methoxy]propanal (6). Dimethyl sulfoxide (10.8 mL, 152.6 mmol, 2.4 equivalents) was diluted in dichloromethane (10 mL) and added, dropwise, to a stirring solution of oxalyl chloride (6 mL, 69.95 mmol,, 1.1 equivalents) in dichloromethane (200 mL) at -85˚C. After the evolution of gas had ceased, **4** (12.48 g, 63.59 mmol, 1 equivalent), diluted in 10 mL dichloromethane, was added dropwise. This mixture was stirred at -85˚C for thirty min, when the appearance of the sulfonium intermediate by TLC (3:1 hexanes : ethyl acetate, Rf = 0), and then triethylamine (40 mL, 292.5 mmol, 4.6 equivalents) was added, dropwise, and product was observed by TLC (3:1 hexanes : ethyl acetate, Rf = 0.6). The reaction was allowed to warm to room temperature, and then 200 mL of water was added. The aqueous layer was extracted 2x100mL with dichloromethane. The combined organic layers were washed 2 x 200 mL with 10% citric acid, 1 x 100 mL brine, then dried over $MgSO_4$ and concentrated under reduced pressure, yielding 11.56 g of **6** (85 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.786(t, J = 1.8, 1H), 7.26(dt, J = 8.8, J = 2.8, 2H), 6.891(dt, J = 8.8, J = 8.8, 2H), 4.463(s, 2H), 3.805(s, 3H), 3.785(t, J = 6, 2H), 2.684(td, J = , J = 2, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 201.3, 159.4, 130.0, 129.5, 113.9, 73.0, 63.6, 55.4, 44.0



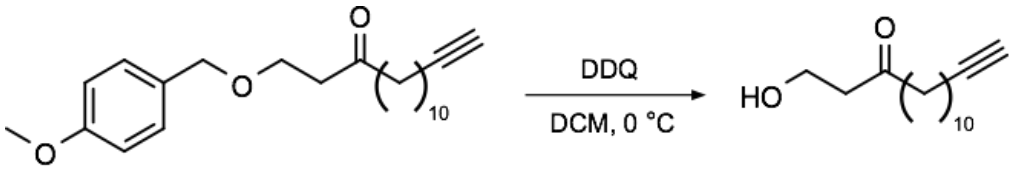
1-[(4-Methoxyphenyl)methoxy]pentadec-4-yn-3-ol (8). To a -78 ˚C solution of 1-dodecyne (11.5 mL, 54.01 mmol, 1 equivalent) in anhydrous tetrahydrofuran (200 mL) was added 2.5 M *n*-butyllithium (32.4 mL, 81.01 mmol, 1.5 equivalents), dropwise. This was stirred for thirty min, producing an off-white slurry, following which a 0 ˚C solution of **6** (10.49 g, 54.01 mmol, 1 equivalent) in anhydrous tetrahydrofuran (100 mL) was added, dropwise. The reaction was stirred for 1 h at -23 ˚C, then overnight at room temperature. Following the appearance of product by TLC (4:1 hexanes : ethyl acetate, Rf = 0.5), the reaction was quenched with 10 % citric acid and then acidified to pH 4 with the same. A further 200 mL water was added, and then the aqueous layer was extracted 2 x 150 mL with ethyl acetate, washed 1 x 200 mL with brine, dried over magnesium sulfate and concentrated under reduced pressure. The crude reside was purified by flash chromatography (4:1 hexanes : ethyl acetate), yielding 19.67 g of **8** as a yellow oil (50.6 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.267(dt, J = 8.8, J = 2.8, 2H), 6.908(dt, J = 8.8, J = 2.8, 2H), 4.596(m, 1H), 4.482(s, 2H), 3.823(m, 1H), 3.803(s, 3H), 3.662(m, 1H), 2.210(td, J = 7, J = 2, 2H), 2.066(m, 1H), 1.943(m, 1H), 1.577(br, 1H), 1.509(quint, J = 7, 4H), 1.380(m, 2H), 1.282(s, 12H), 0.916(t, J = 6.8, 2H); MS (*m*/*z*): $[M - H_2O + H]^+$ calcd. for $C_{23}H_{36}O_3$, 343.263; found 343.5



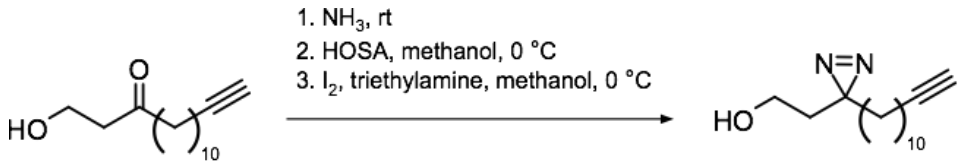
1 - [4 - Methoxyphenyl)methoxy]pentadec-14-yn-3-ol (10). 60% sodium hydride in mineral oil (3.003 g, 75.02 mmol, 5.5 equivalents) was suspended in diaminopropane (50 mL) under argon atmosphere. The suspension was heated to 70 ˚C and stirred for 1 h, resulting in a clear brown solution. This solution was cooled to 0 ˚C and **8** (4.917 g, 13.64 mmol, 1 equivalent) was added, dropwise, resulting in a dark, red-black solution. Once TLC showed consumption of starting material, after about 15 min (4:1 hexanes : ethyl acetate, product Rf = 0.45), the reaction was quenched by pouring slowly over a large excess of ice, extracted 3 x 50 mL with dichloromethane, and the combined organic layers were washed 2 x 50 mL with 10% ice-cold citric acid and 1 x 50 mL brine, dried over magnesium sulfate, and concentrated. The crude orange residue was purified by flash chromatography (4:1 hexanes : ethyl acetate), yielding an orange oil, **10**, 3.48 g (70.8 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.26(dt, J = 8.8, J = 2.8, 2H), 6.884(dt, J = 8.8, J = 2.8, 2H), 4.452(s, 2H), 3.803(s, 3H), 3.684(m, 1H), 3.626(m, 1H), 2.173(td, J = 7, J = 2.4, 2H), 1.835(t, J = 2.4, 1H), 1.714(m, 2H), 1.538(m, 6H), 1.397(m, 5H), 1.271(s, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 159.4, 130.1, 129.4, 113.9, 73.1, 71.7, 69.2, 68.1, 55.4, 37.6, 36.5, 29.8, 29.7, 29.6, 29.6, 29.2, 28.9, 28.6, 27.9, 25.7, 18.5; MS (*m*/*z*): $[M - H_2O + H]^+$ calcd. for $C_{23}H_{36}O_3$, 343.263; found 343.5



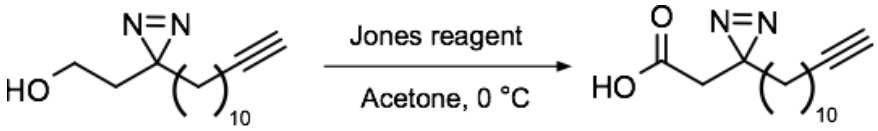
1-[4-Methoxyphenyl)methoxy]pentadec-14-yn-3-one (12). Dimethyl sulfoxide (4.4 mL, 62.34 mmol, 2.4 equivalents) was diluted in dichloromethane (5 mL) and added, dropwise, to a stirring solution of oxalyl chloride (2.5 mL, 28.573 mmol, 1.1 equivalents) in dichloromethane (200 mL) at -85˚C. After the evolution of gas had ceased, **10** (9.365 g, 25.975 mmol, 1 equivalent) was added dropwise. This mixture was stirred at -85˚C for thirty min, and then triethylamine (16.7 mL, 119.49 mmol, 4.6 equivalents) was added, dropwise. The reaction was allowed to warm to room temperature over 1 h, and after the appearance of product by TLC (4:1 hexanes : ethyl acetate, Rf = 0.6), 200 mL of water were added. The aqueous layer was extracted 2x100mL with dichloromethane. The combined organic layers were washed 2 x 200 mL with 10% citric acid, 1 x 100 mL brine, then dried over $MgSO_4$ and concentrated under reduced pressure, yielding 8.635 g of **12** (93% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.223(dt, J = 8.8, J = 2.8, 2H), 6.877(dt, J = 8.8, J = 2.8, 2H), 4.430(s, 2H), 3.797(s, 3H), 3.702(t, J = 6.4, 2H), 2.668(t, J = 6.4, 2H), 2.418(t, J = 7.6, 2H), 2.177(td, J = 7, J = 2.4, 2H), 1.933(t, J = 2.4, 1H), 1.496(m, 4H), 1.375(m, 2H), 1.258(s, 12H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 209.7, 159.3, 130.3, 129.4, 113.9, 84.9, 73.0, 65.5, 60.5, 55.4, 43.5, 43.2, 42.9, 29.5, 29.5, 29.3, 29.2, 28.8, 28.6, 25.8, 23.7, 2.4, 21.1, 18.5, 14.3, 13.9; MS (*m*/*z*): $[M + H]^+$ calcd. for $C_{23}H_{34}O_3$, 359.258; found 359.3



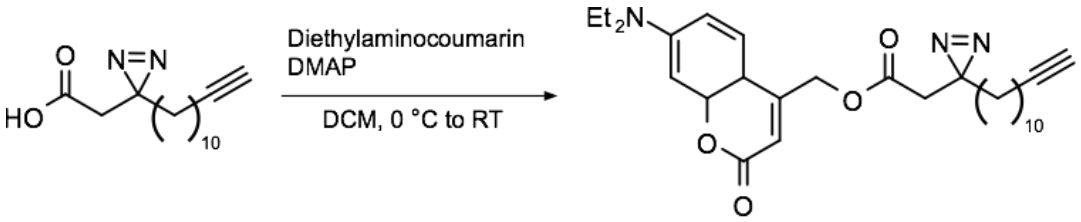
1-Hydroxypentadec-14-yn-3-one (14). To a 0 ˚C solution of **12** (8.635 g, 24.08 mmol, 1 equivalent) in DCM (500 mL), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone was added as a solid, followed by water (50 mL). The reaction was stirred for 30 min at 0 ˚C and then for 1 h at room temperature. Once the reaction had gone to completion, by TLC (4:1 hexanes : ethyl acetate, Rf = 0.1, $KMnO_4$ staining), the reaction was poured over 500 mL saturated sodium bicarbonate, extracted 2 x 200 mL DCM, then washed 2 x 300 mL with 10% sodium bicarbonate and 2 x 300 mL brine. The crude orange residue was purified by flash chromatography, 3:1 hexanes:ethyl acetate, yielding **14** (4.3 g, 17.17 mmol) as a pale orange semi-crystalline solid in 70.8 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.837(m, 2H), 2.666(t, J = 5.4, 2H), 2.431(t, J = 7.4, 2H), 2.175(td, J = 7, J = 2.4, 2H), 1.937(t, J = 2.4, 1H), 1.499(m, 4H), 1.378(m, 2H), 1.275(s, 10H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 68.1, 57.7, 35.7, 33.3, 29.5, 29.4, 29.2, 29.1, 29.0, 28.8, 28.5, 23.9, 18.5



2-[3-(Dodec-11-yn-1-yl)-3H-diazirin-3-yl]ethan-1-ol (16). 14 (3.5 g, 1 equivalent) was placed in an oven-dried bomb flask and cooled to -85 ˚C. Ammonia (25 mL) was condensed on top of the compound and the bomb flask was capped. The mixture was allowed to warm to room temperature, stirring, whereupon **14** dissolved in the ammonia. The reaction stirred for 24hr at room temperature, and then the ammonia was evaporated overnight, into an aqueous 10% citric acid bath. Hydroxylaminesulfonic acid (3.65 g, 2.2 equivalents) was dissolved in 15 mL anhydrous methanol and added to the residue in the bomb flask at 0 ˚C. This was stirred for 1 h at rt, and then the reaction mixture was filtered to remove white solids and the methanol was evaporated. The residue was dissolved in 50 mL ethyl acetate and washed 1x 25 mL 10% citric acid and 1x25 mL brine, dried over MgSO4, and concentrated. The crude residue was oxidized without further purification, by dissolving in 50 mL methanol, cooling to 0 ˚C, adding TEA (4.1 mL, 2 equivalents), and I_2 (3.73 g, 1 equivalent) portion wise as a solid until the brown iodine color persisted. Methanol was once again evaporated, the residue was dissolved in 50 mL ethyl acetate and washed 4x 50mL with sodium thiosulfate and 1x 50mL brine. TLC (4:1 hexanes : ethyl acetate) indicated consumption of starting material and a complex mixture of products; isolation of the spot with Rf = 0.65 resulted in the correct product. The crude reside was purified by flash chromatography (4:1 hexanes : ethyl acetate) yielding **16** (0.939 g) in 25.6 % yield as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.454(m, 2H), 2.171(td, J = 7, J = 2.4, 2H), 1.937(t, J = 2.4, 1H), 1.679(t, J = 6.4, 2H), 1.517(m, 2H), 1.413(m, 4H), 1.253(m, 15H), 1.090(m, 3H); MS (*m*/*z*): [M + NH4]$^+$ calcd. for $C_{15}H_{26}N_2O$, 268.238; found 268.1

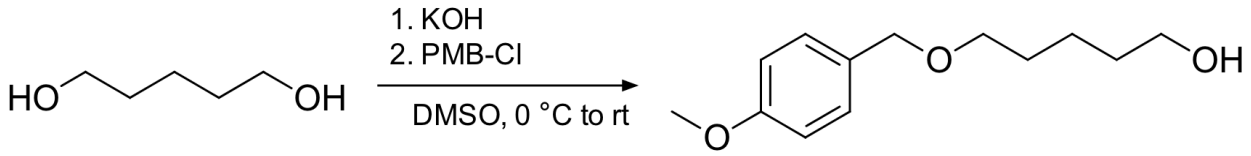


2-[3-(Dodec-11-yn-1-yl)-3H-diazirin-3-yl]acetic acid (18). **16** (50 mg, 1 equivalent) was dissolved in acetone (20 mL) and cooled to 0 ˚C. Jones reagent (2 M, 100μL, 1 equivalent) was added, dropwise, until the pink color of the reagent persisted. The reaction was stirred for 15 min at 0˚C, and after the appearance of product by TLC (8:1 hexanes : ethyl acetate, Rf = 0.05, $KMnO_4$ staining) was quenched with isopropanol, producing a bright, blue-green precipitate. The precipitate was filtered and then the acetone was removed under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and washed twice with saturated sodium bicarbonate and once with brine, dried over magnesium sulfate, and concentrated, yielding pure **18** (41.9 mg, 79.3 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.301(s, 2H), 2.175(td, J = 7, J = 2.4, 2H), 1.936(t, J = 2.4, 1H), 1.536(m, 5H), 1.379(m, 3H), 1.259(m, 12H), 1.086(m, 3H); MS (*m*/*z*): $[M + 2H]^+$, calcd. for 2($C_{15}H_{24}N_2O_2$), 530.382, found 530.3.

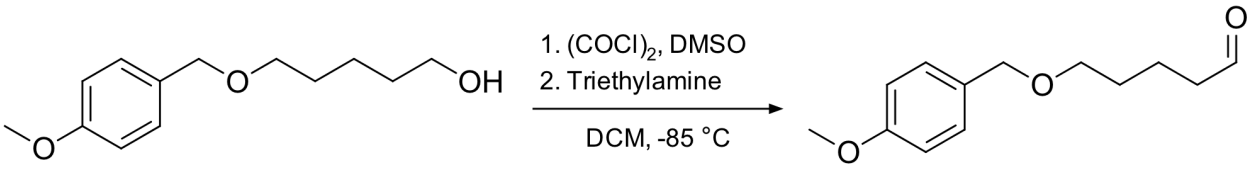


[7-(Diethlamino)-2-oxo-2H-chromen-4-yl]methyl 2-[3-(prop-2-yn-1-yl)-3H-diazirin-3-yl] acetate (1-10 FA). 8 (100 mg, 1 equivalent) was dissolved in 10 mL DCM. EDC (163 mg, 2.1 equivalents) and DMAP (10 mg, 0.2 equivalents) were added and stirred together for 15 min, as the mixture gradually turned orange. 7-(diethylamino) 4-hydroxymethylcoumarin was dissolved in 5 mL DCM. The coumarin and the activated compound **8** were both cooled to 0 ˚C and the coumarin added was added to compound **8**. The reaction was allowed to warm to room temperature overnight. Appearance of product was observed by TLC (2:1 hexanes : ethyl acetate, Rf = 0.2), and the reaction was poured over citric acid, extracted 2 x 15 mL with DCM, washed once with brine, and concentrated under reduced pressure. The crude residue was purified initially by flash chromatography (2:1 hexanes : ethyl acetate), and then by preparative LC-MS, with a gradient of 50-100% acetonitrile in water over 35 min; pure product appeared at 18.33 min as a pale yellow solid (130 mg, 64.9 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.329 (d, J = 8.8, 1H), 6.649 (dd, J = 8.6, J = 1.6, 1H), 6.571 (d, J = 2.8, 1H), 6.192 (t, J = 1.2, 1H), 5.270 (d, J = 1.6, 2H), 3.439 (q, J = 7.2, 4H), 2.400 (s, 2H), 2.191 (td, J = 7.2, J = 2.4, 2H), 1.942 (t, J = 2.4, 2H), 1.536 (m, 6H), 1.382 (m, 2H), 1.243 (m, 22H), 1.083 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 168.8, 161.8, 156.4, 150.5, 148.8, 124.6, 109.3, 107.3, 98.5, 84.9, 68.2, 62.1, 45.2, 39.8, 32.7, 29.9, 29.5, 29.5, 29.4, 29.2, 29.2, 28.9, 28.6, 26.2, 23.9, 18.5, 12.5 (m/z): [M + H]+, calcd. For $C_{29}H_{39}N_3O_4$, 494.2948, found 494.3

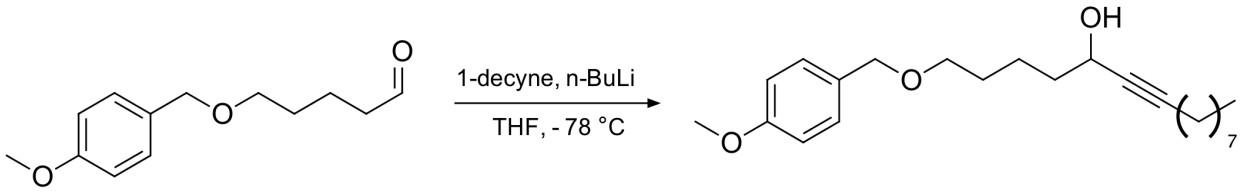
Synthesis of 3-8 FA (3)



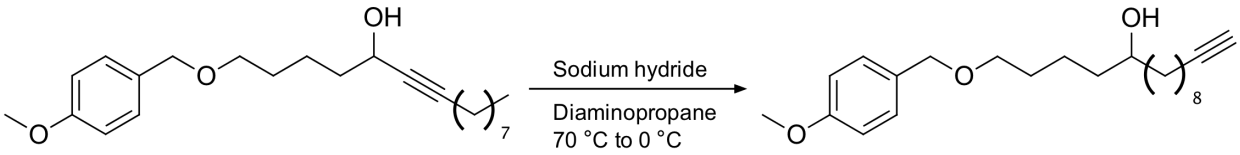
5-[(4-Methoxyphenyl)methoxy]pentan-1-ol (5) To a 0°C solution of 1,5-pentanediol (9.5 g, 91.21 mmol, 1 equivalent) in dimethyl sulfoxide (40 mL) was added potassium hydroxide (8 g, 91.21 mmol, 1 equivalent). The reaction was stirred at room temperature for 30 min, and then cooled to 0 °C for the addition of para-methoxybenzyl chloride (7.142 g, 45.60 mmol, 0.5 equivalents), and then returned to room temperature and stirred for 1.5 h. TLC (2:1 hexanes : ethyl acetate, UV visualization) showed the formation of the desired product (Rf = 0.15) as well as the bis-protected by-product (Rf = 0.6). Water (100 mL) was added, and the aqueous layer was extracted 3 x 100 mL with ether and the combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The orange oil was purified by flash chromatography, with a gradient of 20% ethyl acetate in hexanes to 50% ethyl acetate in hexanes, yielding **5**, 15.0 g, in 73.3% yield. 1H NMR (400 MHz, CDCl3) δ = 7.25 (d, J = 8.8, 2H), 6.86 (d, J = 8.8, 2H), 4.24 (s, 2H), 3.79 (s, 3H), 3.62 (t, J = 6.4, 2H), 3.44 (t, J = 6.4, 2H), 1.66-1.41 (m, 6H); 13C NMR (400 MHz, CDCl3) δ = 1591.2, 130.7, 129.3, 113.8, 72.6, 70.0, 62.8, 55.3, 32.5, 29.5, 22.5



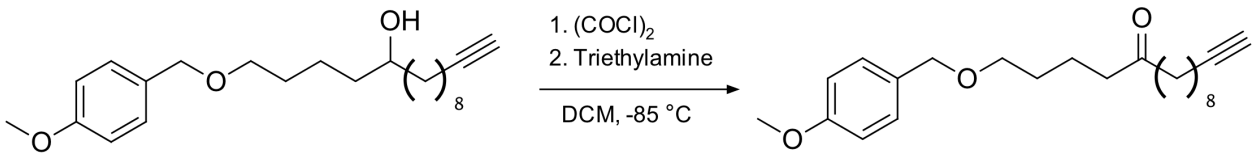
5-[(4-Methoxyphenyl)methoxy]pentanal (7) Dimethyl sulfoxide (12.18 mL, 171.32 mmol, 2.4 equivalents) was diluted in dichloromethane (10 mL) and added, dropwise, to a stirring solution of oxalyl chloride (9,97 g, 78.82 mmol,, 1.1 equivalents) in dichloromethane (50 mL) at -85°C. After the evolution of gas had ceased, **5** (16.0 g, 71.38 mmol, 1 equivalent), diluted in 10 mL dichloromethane, was added dropwise. This mixture was stirred at -85°C for thirty min, with the appearance of the sulfonium intermediate by TLC (2:1 hexanes : ethyl acetate, Rf = 0). Triethylamine 45.46 mL, 328.36 mmol, 4.6 equivalents) was then added, dropwise, and product was observed by TLC (2:1 hexanes : ethyl acetate, Rf = 0.4, UV visualization). The reaction was allowed to warm to room temperature, and then 200 mL of water was added. The aqueous layer was extracted 2x100mL with dichloromethane. The combined organic layers were washed 2 x 200 mL with 10% citric acid, 1 x 100 mL brine, then dried over $MgSO_4$ and concentrated under reduced pressure, yielding 14.96 g of **7** (67 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.75 (t, J = 1.6, 1H), 7.25 (d, J = 8.8, 2H), 6.87 (d, J = 8.8, 2H), 4.42 (s, 2H), 3.8- (s, 3H), 3.46 (t, J = 6, 2H), 2.45 (td, J = 7.2, J = 1.6, 2H), 1.77-1.6 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 202.6, 159.2, 130.5, 129.3, 113.8, 72.6, 69.5, 55.3, 43.6, 29.1, 18.9



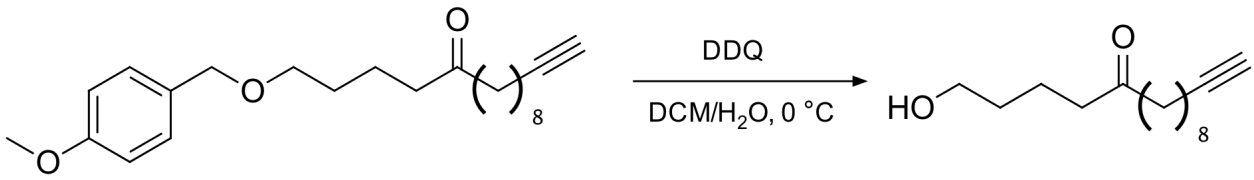
1-[(4-Methoxyphenyl)methoxy]pentadec-6-yn-5-ol (9) To a -78 ˚C solution of 1-decyne (14.04 mL, 94.34 mmol, 1.5 equivalent) in anhydrous tetrahydrofuran (200 mL) was added 2.5 M n-butyllithium (37 mL, 94.54 mmol, 1.5 equivalents), dropwise. This was allowed to warm to 0 ˚C and stirred for 1h, following which a 0 ˚C solution of **7** (14.0 g, 63.024 mmol, 1 equivalent) in anhydrous tetrahydrofuran (10 mL) was added, dropwise. The reaction was stirred for 1 h at 0 ˚C, then overnight at room temperature. Following the appearance of product by TLC (2:1 hexanes : ethyl acetate, Rf = 0.4, UV visualization), the reaction was quenched with 10 % citric acid and then acidified to pH 4 with the same. A further 200 mL water was added, and then the aqueous layer was extracted 2 x 150 mL with ethyl acetate, washed 1 x 200 mL with brine, dried over magnesium sulfate and concentrated under reduced pressure. The crude reside was purified by flash chromatography (gradient of 10 % ethyl acetate in hexanes to 20 % ethyl acetate in hexanes), yielding 17.007 g of **9** as a yellow oil (50.6 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.26 (d, J = 8.8, 2H), 6.87 (d, J = 8.8, 2H), 4.43 (s, 2H), 4.36 (t, J = 7.2, 1H), 3.8 (s, 3H), 3.44 (t, J = 6, 2H), 2.19 (td, J = 7.2, J = 1.6, 2H), 1.72-1.61 (m, 4H), 1.56-1.46 (m, 4H), 1.27 (m, 10H), 0.88 (t, U = 7.2, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 159.1, 130.7, 129.2, 113.8, 85.7, 81.2, 72.6, 70.0, 62.7, 55.3, 38.9, 31.8, 29.4, 29.2, 29.1, 28.9, 28.7, 22.7, 22.0, 18.7, 14.1



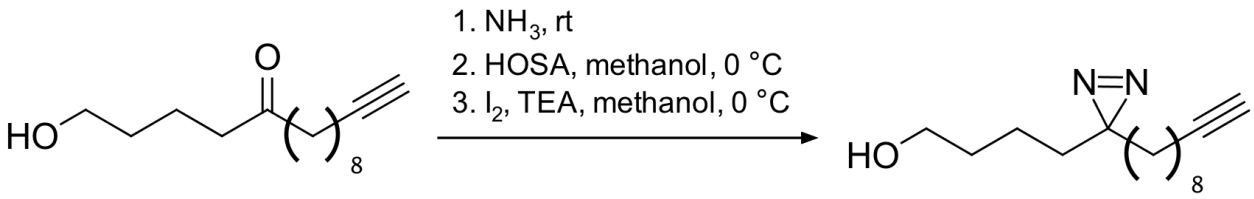
1-[(4-Methoxyphenyl)methoxy]pentadec-14-yn-5-ol (11) 60% sodium hydride in mineral oil (1.831 g, 76.33 mmol, 5.5 equivalents) was suspended in diaminopropane (50 mL) under argon atmosphere. The suspension was heated to 70 ˚C and stirred for 1 h, resulting in a clear brown solution. This solution was cooled to 0 ˚C and **9** (5 g, 13.82 mmol, 1 equivalent), dissolved in 5 mL diaminopropane was added, dropwise, resulting in a dark, red-black solution. Once TLC showed consumption of starting material, after about 2 h (2:1 hexanes : ethyl acetate, product Rf = 0.3, UV visualization), the reaction was quenched by diluting in 50 mL DCM and slowly adding 100 mL ice-cold water, extracted 3 x 50 mL with dichloromethane, and the combined organic layers were washed 2 x 50 mL with 10% ice-cold citric acid and 1 x 50 mL brine, dried over magnesium sulfate, and concentrated. The crude orange residue was purified by flash chromatography (20 % ethyl acetate in hexanes), yielding an orange oil, **11**, 2.31 g (46.4 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 (d, J = 8.8, 2H), 6.80 (d, J = 8.8, 2H), 4.36 (s, 2H), 3.74 (s, 3H), 3.51 (m, 1H), 3.38 (t J = 6.4, 2H), 2.11 (td(J = 7.2, J = 2.8, 2H), 1.87 (t, J = 2.8, 1H), 1.53 (m, 4H), 1.47-1.19 (m, 16H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 1591., 130.7, 129.3, 113.8, 84.8, 72.6, 71.8, 70.0, 68.1, 55.3, 37.5, 37.2, 29.7, 29.6, 29.5, 29.1, 28.7, 28.5, 25.6, 22.4, 18.4



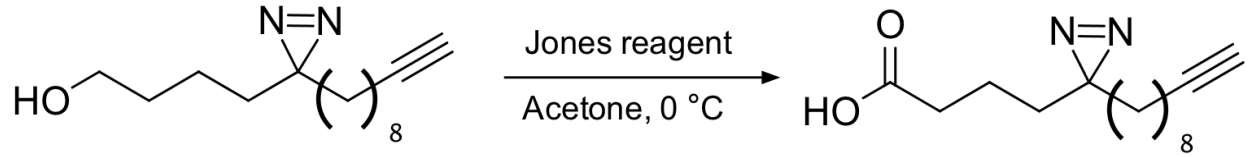
1-[(4-Methoxyphenyl)methoxy]pentadec-14-yn-5-one (13) Dimethyl sulfoxide (0.85 mL, 4.99 mmol, 2.4 equivalents) was diluted in dichloromethane (5 mL) and added, dropwise, to a stirring solution of oxalyl chloride (0.47 mL, 5.49 mmol, 1.1 equivalents) in dichloromethane (50 mL) at -85˚C. After the evolution of gas had ceased, **11** (1.8 g, 4.99 mmol, 1 equivalent) was added dropwise. This mixture was stirred at -85˚C for thirty min, and then triethylamine (3.2 mL, 22.98 mmol, 4.6 equivalents) was added, dropwise. The reaction was allowed to warm to room temperature over 1 h, and 200 mL of water were added. The aqueous layer was extracted 2x100 mL with dichloromethane. The combined organic layers were washed 2 x 200 mL with 10% citric acid, 1 x 100 mL brine, then dried over $MgSO_4$ and concentrated under reduced pressure, yielding 1.7 g of **13** (93 % yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.12 (d, J = 8.8, 2H), 6.81 (d, J = 8.8, 2H), 4.35 (s, 2H), 3.74 (s, 3H), 3.37 (t = J = 6.4, 2H), 2.35 - 2.28 (m, 4H), 2.11 (td, J = 7.2, J = 2.8, 2H), 1.87 (t, J = 2.8, 1H), 1.58-1.41 (m, 8H), 1.32-1.29 (m, 2H), 1.47-1.19 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 159.1, 130.7, 129.3, 113.8, 83.4, 76.0, 72.6, 70.1, 70.0, 55.3, 36.0, 31.8, 29.7, 29.0, 28.9, 28.8, 27.8, 22.6, 22.4, 18.8, 14.1



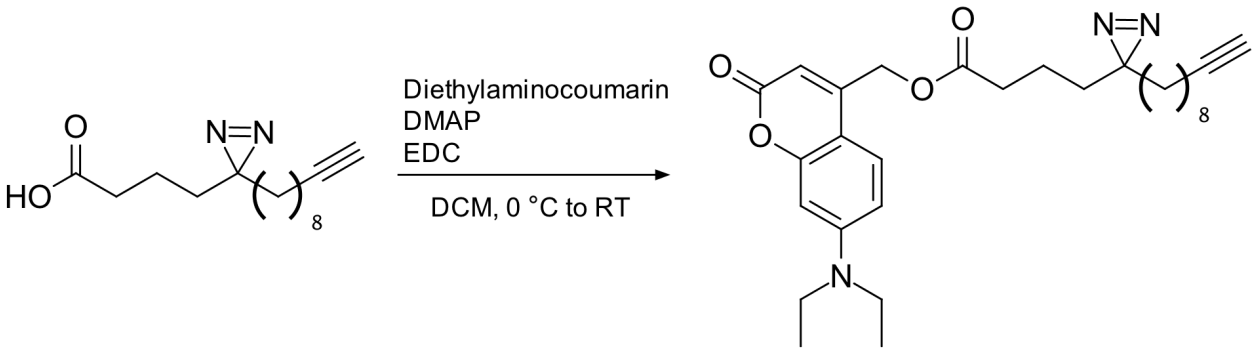
1-Hydroxypentadec-14-yn-5-one (15) To a 0 ˚C solution of **13** (4.8 g, 13.4 mmol, 1 equivalent) in DCM (120 mL), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (6.082 g, 26.8 mol, 2 equivalents) was added as a solid, followed by water (15 mL). The reaction was stirred for 30 min at 0 ˚C and then for 1 h at room temperature. Once the reaction had gone to completion, by TLC (3:1 hexanes : ethyl acetate, Rf = 0.2, $KMnO_4$ staining) The reaction was then poured over 500 mL saturated sodium bicarbonate, extracted 2 x 200 mL DCM, then washed 2 x 300 mL with 10% sodium bicarbonate and 2 x 300 mL brine. The crude orange residue was purified by flash chromatography (gradient of 30 % ethyl acetate to 50 % ethyl acetate in hexanes) yielding **15** (2.6 g, 17.17 mmol) as a pale orange semi-crystalline solid in 59 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.62 (t, J = 6.4, 2H), 2.47 (t, J = 7.2, 2H), 2.40 (t, J = 7.2, 2H), 2.17 (td, J = 7.2, J = 2.8, 2H), 1.94 (t, J = 2.8, 1H), 1.68-1.64 (m, 2H), 1.57-1.41 (m, 6H), 1.42-1.37 (m, 2H), 1.28-1.15 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 2.11.6, 84.8, 68.1, 62.3, 42.8, 42.3, 32.2, 29.3, 28.9, 28.7, 28.4, 23.9, 19.7, 18.4



4-[3-(Dec-9-yn-1-yl)-3H-diazirin-3-yl]butan-1-ol (17) 26 (440 mg, 1 equivalent) was placed in an oven-dried bomb flask and cooled to -85 ˚C. Ammonia (25 mL) was condensed on top of the compound and the bomb flask was capped. The mixture was allowed to warm to room temperature, stirring, whereupon 17 dissolved in the ammonia. The reaction stirred for 24hr at room temperature, and then the ammonia was evaporated overnight, into an aqueous 10% citric acid bath. Hydroxylaminesulfonic acid (459 mg, 2.2 equivalents) was dissolved in 15 mL anhydrous methanol and added to the residue in the bomb flask at 0 ˚C. This was stirred for 1 hr at rt, and then the reaction mixture was filtered to remove white solids and the methanol was evaporated. The residue was dissolved in 50 mL ethyl acetate and washed 1x 25 mL 10% citric acid and 1x25 mL brine, dried over MgSO4, and concentrated. The crude residue was oxidized without further purification, by dissolving in 50 mL methanol, cooling to 0 ˚C, adding TEA (4.1 mL, 2 equivalents), and I_2 (468 g, 1 equivalent) portion wise as a solid until the brown iodine color persisted. Methanol was once again evaporated, the residue was dissolved in 50 mL ethyl acetate and washed 4x 50mL with sodium thiosulfate and 1x 50mL brine. TLC (2:1 hexanes: ethyl acetate, potassium permanganate staining) indicated a complex mixture of products; isolation of the spot with Rf = 0.45 resulted in the correct product. The crude reside was purified by flash chromatography (9:1 hexanes : ethyl acetate) yielding **17** (97.5 mg) in 21 % yield as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.54 (t, J = 6.4, 2H), 2.11 (td, J = 7.2, J= 2.8, 2H), 1.87 (t, J = 2.8, 1H), 1.68-1.64 (m, 2H), 1.33-1.27 (m, 6H), 1.21-1.15 (m, 6H), 1.14-1.08 (m, 2H), 1.03-0.97 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 84.8, 77.2, 68.1, 62.6, 32.8, 32.7, 32.2, 29.2, 29.1, 28.9, 28.7, 28.4, 23.8, 20.2, 18.4



4-[3-(Dec-9-yn-1-yl)-3H-diazirin-3-yl] butanoic acid (19) 17 (94 mg, 1 equivalent) was dissolved in acetone (20 mL) and cooled to 0 ˚C. Jones reagent (2 M, 200μL, 1 equivalent) was added, dropwise, until the pink color of the reagent persisted. The reaction was stirred for 15 min at 0˚C, and after the appearance of product by TLC (2:1 hexanes : ethyl acetate, Rf = 0.3, $KMnO_4$ staining) then quenched with isopropanol, producing a bright, blue-green precipitate. The precipitate was filtered and then the acetone was removed under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and washed 2x with saturated sodium bicarbonate and 1x brine, dried over magnesium sulfate, and concentrated, yielding pure **19** (55.9 mg, 56 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.25 (t, J = 7.2, 2H), 2.11 (td, J = 7.2, J = 2.8, 2H), 1.87 (t, 2.8, 1H), 1.46-1.14 (m, 16H), 1.14-0.99 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 178.3, 84.8, 68.1, 33.1, 32.7, 32.2, 29.7, 29.2, 29.1, 28.9, 28.7, 28.4, 23.8, 19.0, 18.4



[7-(Diethylamino)-2-oxo-2H-chromen-4-yl] methyl 4-[3-(dec-9-yn-1-yl)-3H-diazirin-yl] butanoate (3-8 Fatty Acid, 3) 19 (54 mg, 1 equivalent) was dissolved in 10 mL DCM. EDC (78.3 mg, 2 equivalents) and DMAP (4.99 mg, 0.2 equivalents) were added and stirred together for 15 min, as the mixture gradually turned orange. 7-(Diethylamino) 4-hydroxymethylcoumarin (55.6 mg, 1.1 equivalents) was dissolved in 5 mL DCM. The coumarin and the activated compound **19** were both cooled to 0 ˚C and the coumarin added was added to compound **19**. The reaction was allowed to warm to room temperature overnight. Appearance of product was observed by TLC (3:1 hexanes : ethyl acetate, Rf = 0.1, potassium permanganate and UV visualization), and the reaction was poured over citric acid, extracted 2 x 15 mL with DCM, washed once with brine, and concentrated under reduced pressure. The crude residue was purified initially by flash chromatography (gradient of 5 % to 10 % ethyl acetate in hexanes) to produce 7 as a pale yellow solid (130 mg, 64.9 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.21 (d, J = 8.8, 1H), 6.51 (dd, J = 8.8, J = 2.8, 1H), 6.45 (d, J = 2.8, 1H), 6.04 (s, 1H), 5.15 (s, 2H), 3.35 (q, J = 7.2, 4H), 2.34 (t, J = 7.2, 2H), 2.1 (td, J = 7.2, J = 2.8, 2H), 1.87 (t, J = 2.8, 1H), 1.48-1.14 (m, 4H), 1.37-1.16 (m, 12H), 1.14 (t, J = 7.2, 6H), 1.03-0.98 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 172.3, 161.9, 156.3, 150.7, 149.3, 124.4, 108.7, 106.5, 106.0, 97.9, 84.7, 68.1, 61.4, 44.8, 33.3, 32.7, 32.3, 29.2, 29.1, 28.9, 28.7, 28.4, 28.2, 23.8, 19.2, 18.4, 12.4

Cell culture Huh7 cells were maintained in standard tissue-culture treated vessels in DMEM supplemented with 10% FBS, 1% non-essential amino acids and 1% penicillin-streptomycin at 37 ˚C and 5% CO_2.

Antibodies and chemicals

- Antibodies
    - anti-Giantin (mouse, abcam ab37266)
    - anti-PDI (rabbit, Cell Signaling 3501S)
    - Goat anti-rabbit IgG (H+L) Alexa Fluor Plus 488 (Invitrogen A32731TR)
    - Goat anti-Mouse IgG (H+L) Alexa Fluor 555 (Invitrogen A-21422)
    - anti-PITPNB (rabbit, Proteintech, 13110)
    - Anti-rabbit IgG, HRP-linked Antibody (Cell Signaling, #7074)
- Chemicals
    - A647 picolyl azide (Vector laboratories CCT-1300)
    - Picolyl azide-agarose beads (Vector laboratories CCT-1408)
    - Biotin azide (Vectory laboratories CCT-1265)

- Monomeric avidin agarose resin (Thermo Scientific 20228)

Uncaging and photocrosslinking For uncaging and photocrosslinking, two lamps were primarily used: a UV type 1 LED lamp (Nailstar model NS-02, https://www.amazon.de/gp/product/B01286DTFQ/ref=ppx_yo_dt_b_asin_title_o02_s00?ie=UTF8&psc=1) for uncaging — "Uncaging Lamp", and a 36W/365 nm lamp (MelodySusie, Pro04 model, https://www.amazon.com/MelodySusie-Professional-Setting-Manicure-Pedicure/dp/B012MEZP2E?ref_=ast_sto_dp&th=1&psc=1) for photocrosslinking — "Photocrosslinking Lamp". Unless stated otherwise below, each lamp was used for 5 minutes. For the lamp comparison experiment (see below), an additional lamp was used, a 1,000 W high-power Mercury-Xenon lamp (Newport), fitted with either a 400 nm high-pass filter (for uncaging) or a 345 nm high-pass filter (for photocrossinking).

Lamp comparison Huh7 cells were seeded in 24-well plates containing glass coverslips and grown to 75% confluence. 8-3 FA was diluted to 50 μM in complete media, 200 μL probe solution was added to each well, and was allowed to sit on cells for 30 min at 37 ˚C prior to uncaging. Cells were uncaged either with the Uncaging Lamp or with the Newport lamp fitted with a 400 nm high-pass filter, for either 2 min or 5 min, and then photocrosslinked either with the Photocrosslinking Lamp or with the Newport lam fitted with a 345 nm high-pass filter. A "no light" background control was also included, which had been treated with 8-3 FA in the same manner as above but kept in the dark prior to fixation. Cells were fixed in methanol for 20 min. Cells were washed three times with PBS to remove organic solvent, and then 200 μL (for 24-well plates) of a click mix were added (1 mM copper sulfate, 1 mM sodium ascorbate, 100 μM TBTA, 2 μM A647 picolyl azide, in PBS). The reaction was allowed to proceed for 1 h in the dark. Cells were stained with DAPI (1:1000 in PBS) for 10 minutes. Coverslips were mounted on slides and imaged on a Zeiss LSM 980 Laser-Scanning 4-channel confocal microscope.

Analysis of trifunctional lipids by thin-layer chromatography *Probe labeling* Huh7 cells were seeded in 6-well plates and grown to 75% confluence. Dilutions of trifunctional probe were made in complete media, for a final concentration of 50 μM for each fatty acid, with a corresponding DMSO control. 1 mL probe dilution was added to each plate and allowed to sit on cells for 30 min at 37 ˚C prior to uncaging. Dishes were exposed to 400 nm light via the Uncaging Lamp for five min to uncage the probe. Probe solutions were removed and replaced with DMEM, and then cells were returned to the incubator for 1 or 24 h to allow for metabolism. *Lipid extraction* Lipids were extracted with a modified Bligh-Dyer extraction; briefly, dishes were rinsed 4x with PBS, and 1 mL of a 2 : 0.8 methanol:water mixture was added, and cells were scraped into this mixture and moved to a glass tube, to which 1 mL of chloroform was added. Tubes were vortexed to mix, and the layers were allowed to separate at -20 ˚C for 1 h to overnight. Tubes were centrifuged (1,000 x g for 10 min) to ensure complete separation of layers, and then the chloroform layer was removed to a fresh tube and then dried under a stream of nitrogen. *Click labeling* Extracted lipids were re-dissolved in 10 μL chloroform, and 40 μL of a click mix (5 μL each of 1 mM TBTA, 10 mM copper sulfate, 10 mM sodium ascorbate, and 10 mM 3-azido-7-diethylaminocoumarin, and 20 μL ethanol) was added. The reaction was allowed to proceed for 3 h in the dark, and then extracts were once more dried under a stream of nitrogen. *Plating and running TLC* Extracted lipids were re-dissolved in 10 μL chloroform and plated on 10x10 cm HPTLC silica 60 Å glass plates without F254 fluorophore. Lipids were resolved by

a 2-step system: first using chloroform/methanol/saturated aqueous ammonium hydroxide 65:25:4 for 6cm, then drying, and then using hexanes/ethyl acetate 1:1 for 9cm. Fluorescently labeled lipids were visualized using a Sapphire molecular imager in the Cy2 channel. Images were processed in Fiji software to subtract background via the rolling-ball method with radius = 40 px.

Subcellular visualization of lipids by confocal microscopy *Probe labeling* Huh7 cells were seeded on coverslips in 24-well plates, and grown to about 70% confluence. Dilutions of trifunctional probe were made in complete media to a final concentration of 50 μM for each fatty acid. 200 μL of probe dilution was added to each plate and allowed to sit on cells for 30 min at 37 ˚C prior to uncaging. Dishes were exposed to 400 nm light for fives minutes to uncage the probe, using the Uncaging Lamp and returned to the incubator for 5, 30, or 60 min to allow for metabolism, then exposed to 350 nm light for five minutes to photo-crosslink the probe, using the Photocrosslinking Lamp and immediately fixed. Cells were fixed by washing twice with PBS, then left in methanol for 20 min. *Click labeling* Cells were washed three times with PBS to remove organic solvent, and then 200 μL (for 24-well plates) or 70 μL (for 96-well plates) of a click mix were added (1 mM copper sulfate, 1 mM sodium ascorbate, 100 μM TBTA, 2 μM A647 picolyl azide, in PBS). The reaction was allowed to proceed for 1 h in the dark. *Antibody staining* Click mix was removed, cells were washed twice with PBS, and blocking buffer (2% BSA, 0.1% Triton-X-100 in PBS) was added. Cells were blocked for 1 h before the addition of primary antibodies. Primary antibodies (anti-Giantin, anti-PDI, catalogue numbers listed above) were diluted 1:250 in blocking buffer and left on cells, with rocking, overnight at 4 ˚C. The next day, primary antibodies were removed, cells were washed three times with PBS, and fluorescent secondary antibodies were added, either A488 anti-rabbit or A555 anti-mouse, 1:500 dilution in blocking buffer, for 1 h at room temperature, with rocking. Secondary antibodies were removed, cells were washed three times with PBS, and DAPI (1:1000) was added for ten min. Cells were imaged within a week after staining, on a Zeiss LSM 980 Laser-Scanning 4-channel confocal microscope with Airyscan.2[1]. *Image analysis* Pearson's correlation coefficients between the lipid signal and the signal for each organelle marker were calculated using a CellProfiler pipeline[2]. Individual cells were selected based off of regions of intensity of the lipid signal and coefficients were calculated within each cell.

Isolation and identification of protein-lipid complexes by LC-MS/MS *Probe labeling* Huh7 cells were seeded in 10cm dishes and grown to 90% confluence. Dilutions of trifunctional probe were made in complete media to a final concentration of 50 μM for each fatty acid. 3 mL of probe dilution was added to each plate and allowed to sit on cells for 30 min at 37 ˚C prior to uncaging for 5 minutes with the Uncaging Lamp. Cells treated with fatty acids were subjected to photocrosslinking for five minutes with the Photocrosslinking Lamp 1 h after uncaging. After photocrosslinking, cells were washed three times with PBS and scraped into 2 mL of ice-cold PBS. Cells were pelleted by centrifugation (1,000 x g for 5 min), supernatant was decanted, and cells were resuspended in 500 μL PBS. *Sample preparation for proteomics* Cells were lysed by probe sonication, on ice, in three 15-second bursts. Lysates were subjected to a click reaction with picolyl azide agarose beads: 200 μL azide beads were washed once in DI water, then added to the cell lysate, along with copper sulfate (1mM, final concentration), sodium ascorbate (1 mM, final concentration), and TBTA (100 μM, final concentration). Samples were rotated at room temperature for 1 h. Beads were spun down (1,000 x g for 2 min), transferred to 2 mL centrifuge columns, and washed extensively: 3x with PBS, 5x with bead wash buffer 1 (100 mM Tris-HCl, pH = 8.0, 250 mM NaCl, 5 mM EDTA, 1% SDS), 10x with bead wash buffer 2 (100 mM Tris-HCl, pH

= 8.0, 8M urea). Beads were transferred from the column in PBS to a clean eppendorf tube and spun down. Isolated proteins were reduced (by resuspending them in 1 mM digestion buffer [100 mM Tris-HCl, pH = 8.0, 2 mM $CaCl_2$, 10% ACN], adding DTT to 10 mM, and incubating at 42 ˚C for 30 min), alkylated (by spinning down beads and resuspending them in 1 mL 40 mM aqueous iodoacetamide and incubating them at room temperature in the dark for 30 min). Bead-bound proteins were then digested by spinning them down, adding 50 μL of digestion buffer and 1 μL of LC-MS grade trypsin, and shaken at 37 ˚C overnight. Peptides were then desalted on C18 columns and the eluent was frozen at -80 ˚C. *Identification of isolated proteins by LC-MS/MS.* Dried peptides were shipped to the EMBL proteomics core facility where they were TMT-labeled using the TMT-16-plex system and analyzed by LC-MS/MS on an Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific). Peptides were separated using an Ultimate 3000 nano RSLC system (Dionex) equipped with a precolumn (C18 PepMam100, 5mm, 300 μm i.d., 5 μm, 100 Å) and an analytical column (Acclaim PepMap 100. 75 x 50 cm C18, 3 mm, 100 Å) connected to a nanospray-Flex ion source. The peptides were loaded onto the precolumn in solvent A (0.1% formic acid) and eluted in a gradient of solvent B (0.1% formic acid in acetonitrile) from 2 to 85% over 120 min at 0.3 μL per minute. Data were collected in positive ion mode with a spray voltage of 2.4 kV and capillary temperature of 275 ˚C. Full scan MS spectra with a mass range of 300–1500 m/z were acquired in profile mode using a resolution of 120,000. AGC target was set to 50% and a max injection time of 250 ms. Precursors were isolated using the quadrupole with a window of 1.4 m/z and fragmentation was triggered by HCD in fixed collision energy mode with fixed collision energy of 30%. MS2 spectra were acquired with the Orbitrap with a resolution of 15.000. Normalized AGC target was set to 200% and a max injection time of 32 ms. *Analysis of proteomics data.* Acquired data were analyzed using IsobarQuant[3] and Mascot V.24 (Matrix Science) using a reverse UniProt FASTA database (UP000005640) including common contaminants. Only proteins that were quantified with two unique peptide matches in both replicates were kept for the analysis. A variance stabilization normalization was performed on the log2 raw data[4], and enrichments of proteins in the (+) UV condition over the (-) UV condition was calculated using LIMMA analysis[5]. A protein is considered a "hit" in a certain condition if the false discovery rate is smaller than 0.05 and the fold change is at least 2; a protein is considered a "candidate" if a the false discovery rate is smaller than 0.2 and the fold change is at least 1.5.

In gel fluorescence of protein-lipid complexes *Probe labeling* Huh7 cells were seeded in 10cm dishes and grown to 90% confluence, in triplicate for each condition. Dilutions of trifunctional probe were made in complete media to a final concentration of 50 μM for each fatty acid. 3 mL of probe dilution was added to each plate and allowed to sit on cells for 30 min at 37 ˚C prior to uncaging for 5 minutes with the Uncaging Lamp. Cells treated with fatty acids were subjected to photocrosslinking for five minutes with the Photocrosslinking Lamp 1 hour after uncaging. (-) UV controls for each probe were prepared by keeping probe-treated cells in the dark prior to harvest. After photocrosslinking, cells were washed three times with PBS and scraped into 2 mL of ice-cold PBS. Cells were pelleted by centrifugation (1,000 x g for 5 min), supernatant was decanted, and cells were resuspended in 250 μL PBS. *Lysis, click reaction, and gel running* Cells were lysed by probe sonication, on ice, in three 15-second bursts. Lysates were subjected to a BCA assay to determine protein concentration, and amount of protein in each condition were normalized. 25 μL of lysate, diluted in PBS to produce a uniform 32.5 μg of protein per condition, was subjected to a click reaction with A647 picolyl azide (by adding 2 μL of a master mix containing the azide, copper sulfate, sodium ascorbate, and TBTA, for a final con-

centration of 20 μM azide, 100 μM TBTA, 1 mM copper sulfate, and 1 mM sodium ascorbate), for 1 hour at room temperature, in the dark. Proteins were gently solubilized for the gel by incubating with Laemmli buffer for 30 minutes at 60 ˚C, and then run on a 12.5% SDS-PAGE gel. The far-red fluorescence was visualized on an Azure Sapphire Biomolecular Imager. Following fluorescent visualization, the gel was stained with Blazin' Blue Protein Gel Stain to verify the uniformity of protein amount between samples.

Biotin pulldown and western blotting of protein-lipid complexes *Pulldown* The same lysates prepared in the "in gel fluorescence" section above were used for pulldown experiments. Protein amounts were normalized by dilution with PBS based on the BCA assay, with a total of 260 μg of protein per sample, in 500 μL total. Lysates were subjected to a click reaction with biotin azide (by adding 5.15 μL of a master mix containing the azide, copper sulfate, sodium ascorbate, and TBTA, for a final concentration of 5 μM azide, 100 μM TBTA, 1 mM copper sulfate, and 1 mM sodium ascorbate), for 2.5 hours at room temperature, rocking. Excess biotin azide was removed on three sequential Zeba desalting columns (7K MWCO): columns were equilibrated three times with PBS, and the click reaction was loaded onto the column and spun through following manufacturer's instructions. Flow-through from the first column was loaded onto the second column, and so on. Flow-through from the third column was incubated overnight at 4 ˚C with 200 μL monomeric avidin agarose resin. In the morning, beads were washed four times with 1 mL of a wash buffer (0.05% Triton-X-100 in PBS), spinning at 2,500 x g for 2 minutes after each wash. Proteins were eluted off beads by adding 60 μL of 1x Laemmli buffer and incubating at 60 ˚C for 30 minutes. *Western blotting* Eluent from the agarose beads, as well as the input whole-cell lysate, was run on a 12.5% SDS-PAGE gel and then transferred to a PVDF membrane. After blocking in 3% BSA in TBST, the membrane was treated with anti-PITPNB primary antibody (1:1000) overnight at 4 ˚C, and then an anti-rabbit HRP secondary antibody (1:5000) for 1 hour at room temperature, before treatment with a chemiluminescent substrate and visualization on an ImageQuant 4000 imager.

References

1. Huff, J., *Nature Methods* **2015,** *12* (12), i-ii.
2. McQuin, C.; Goodman, A.; Chernyshev, V.; Kamentsky, L.; Cimini, B. A.; Karhohs, K. W.; Doan, M.; Ding, L.; Rafelski, S. M.; Thirstrup, D.; Wiegraebe, W.; Singh, S.; Becker, T.; Caicedo, J. C.; Carpenter, A. E., *PLoS Biol* **2018,** *16* (7).
3. Franken, H.; Mathieson, T.; Childs, D.; Sweetman, G. M. A.; Werner, T.; Tögel, I.; Doce, C.; Gade, S.; Bantscheff, M.; Drewes, G.; Reinhard, F. B. M.; Huber, W.; Savitski, M. M., *Nature Protocols* **2015,** *10* (10), 1567-1593.
4. Huber, W.; von Heydebreck, A.; Sueltmann, H.; Poustka, A.; Vingron, M., *Bioinformatics* **2002,** *18 Suppl. 1*, S96-S104.
5. Ritchie, M. E.; Phipson, B.; Wu, D.; Hu, Y.; Law, C. W.; Shi, W.; Smyth, G. K., *Nucleic Acids Res* **2015,** *43* (7).

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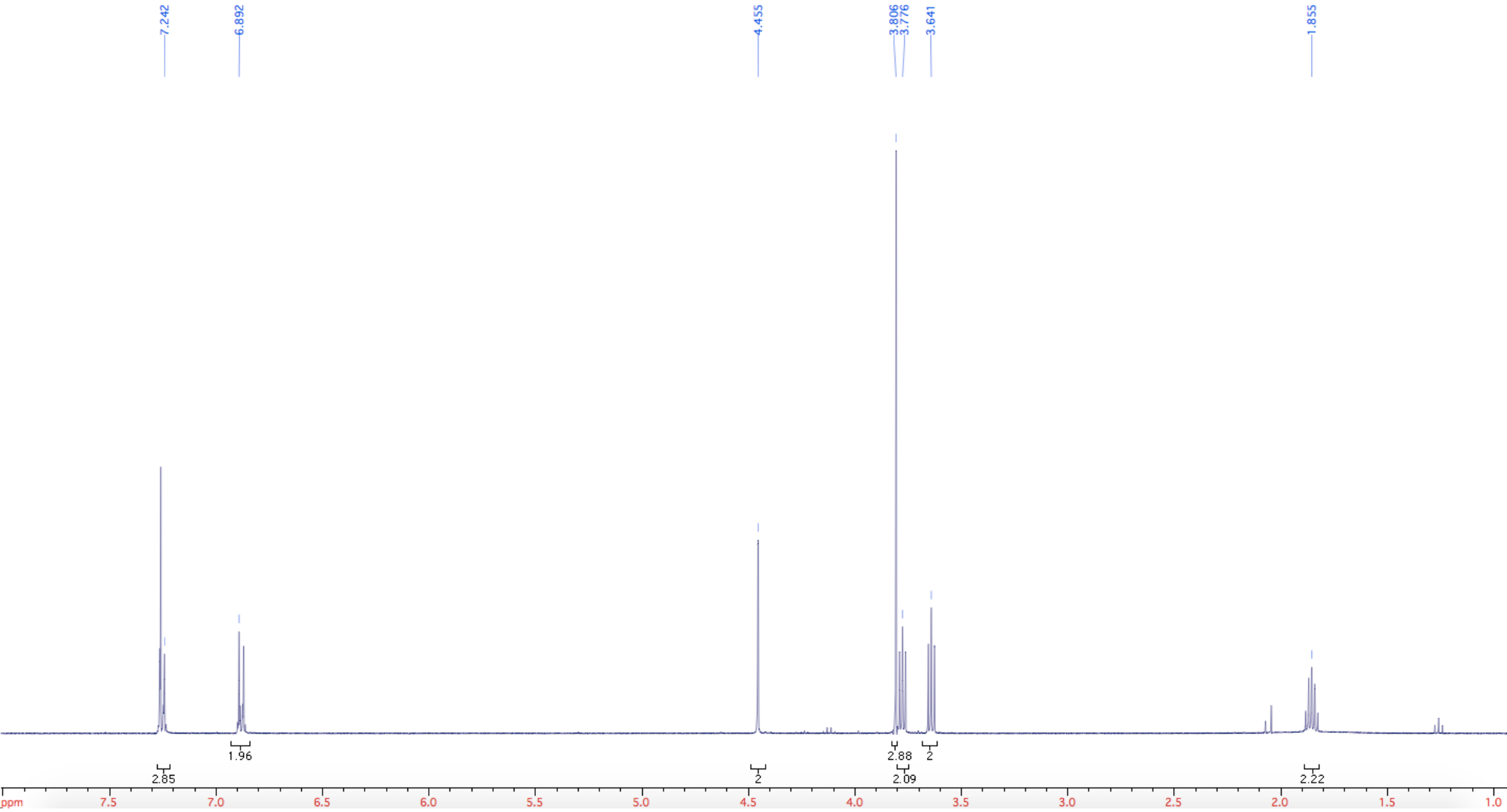
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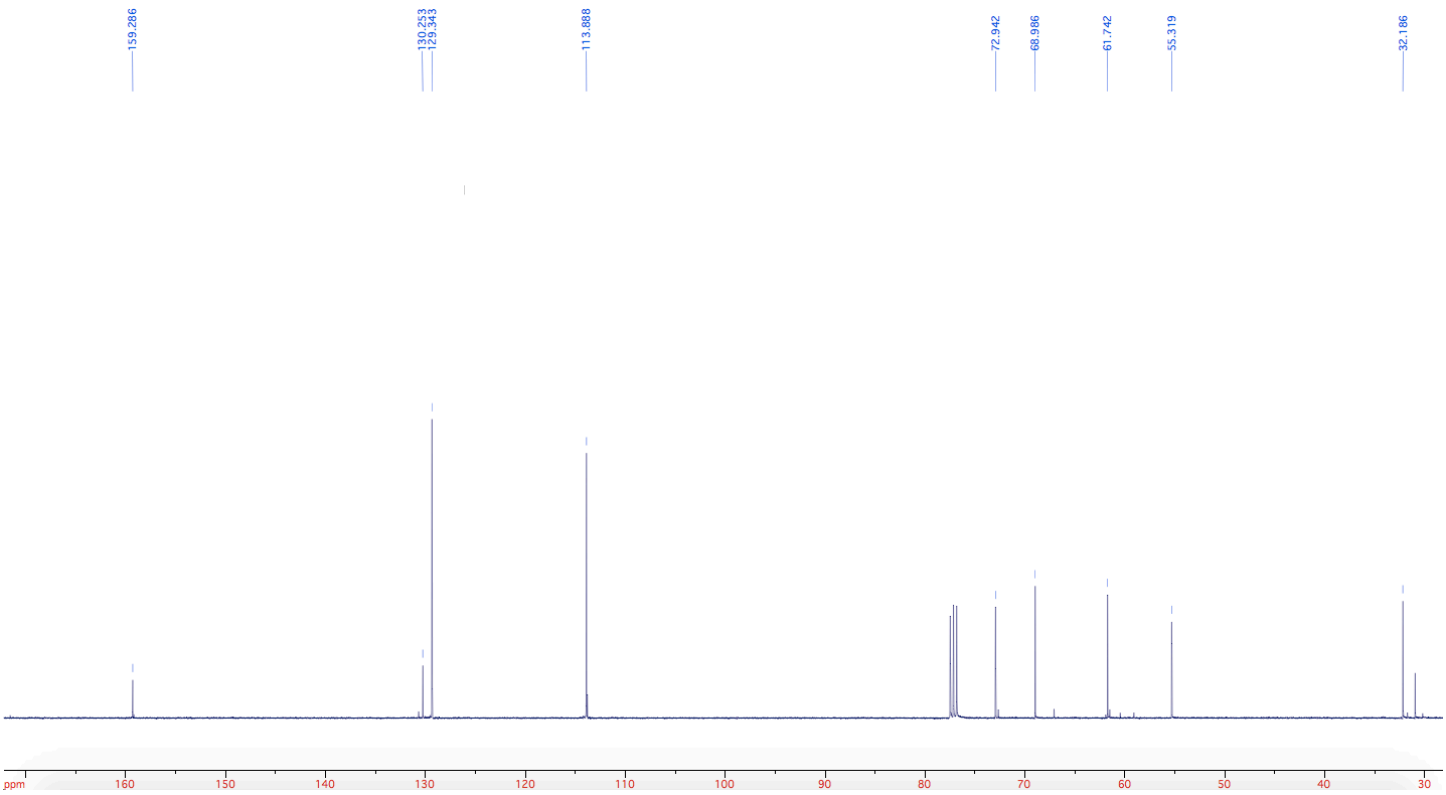
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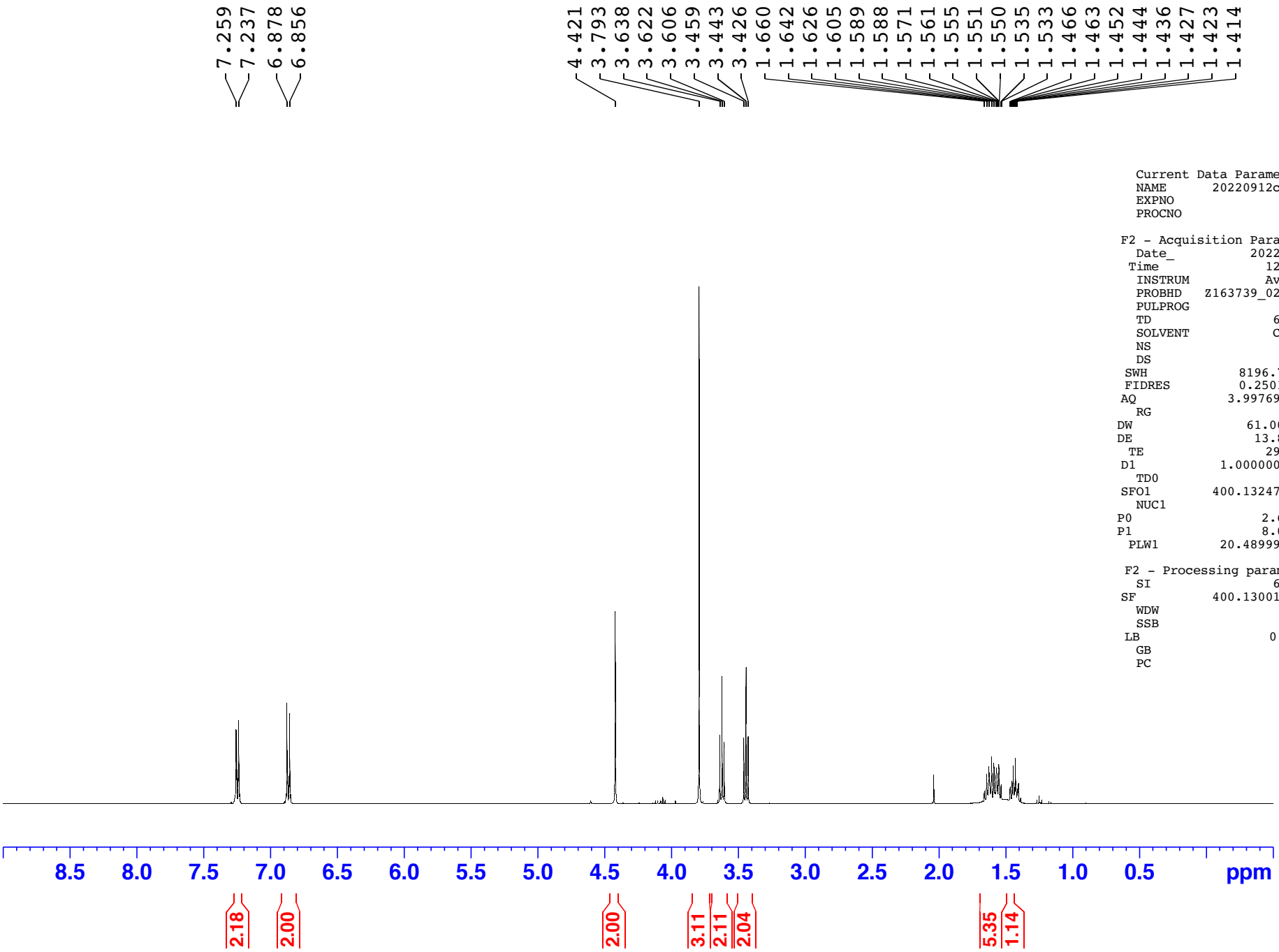
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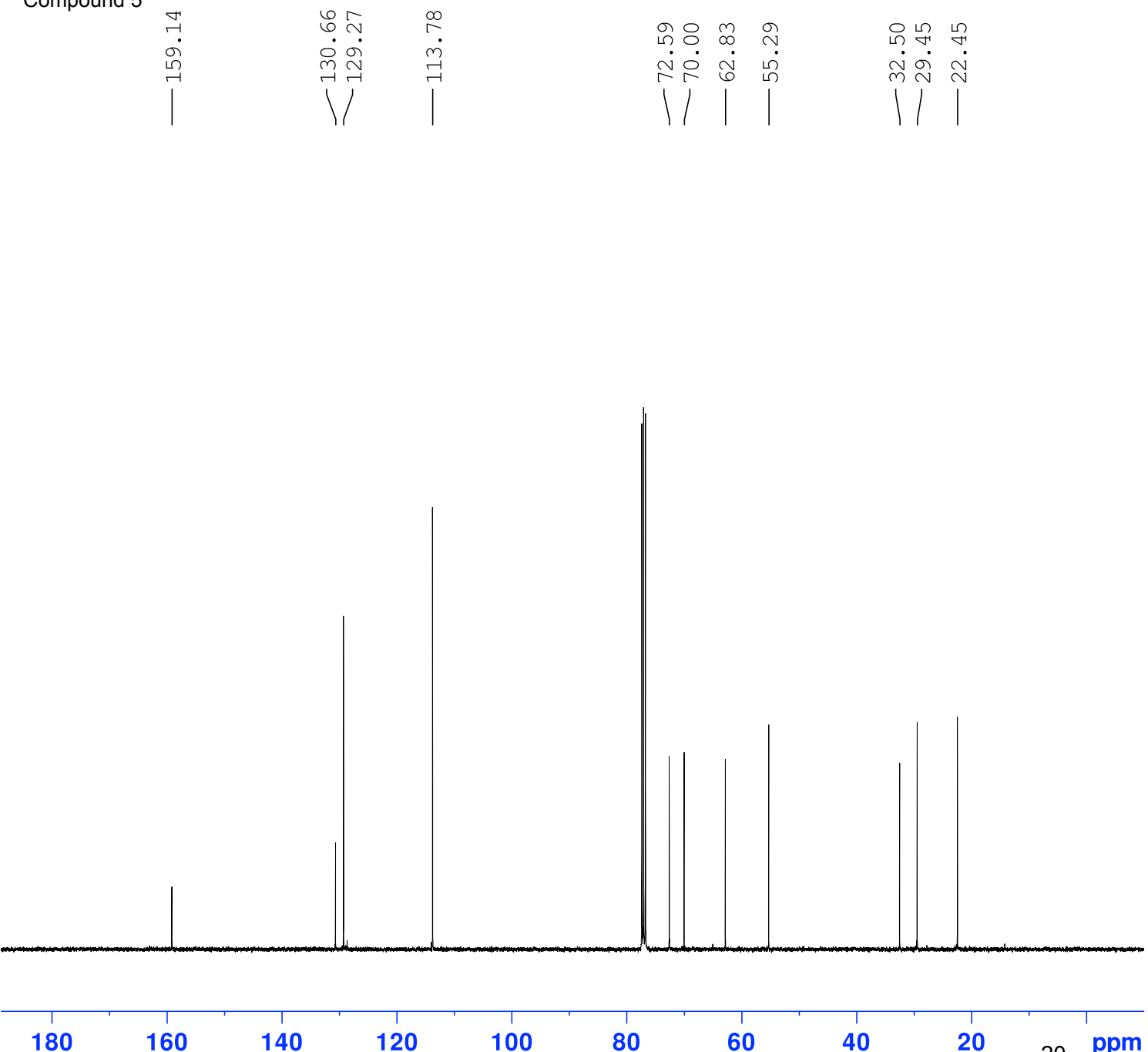
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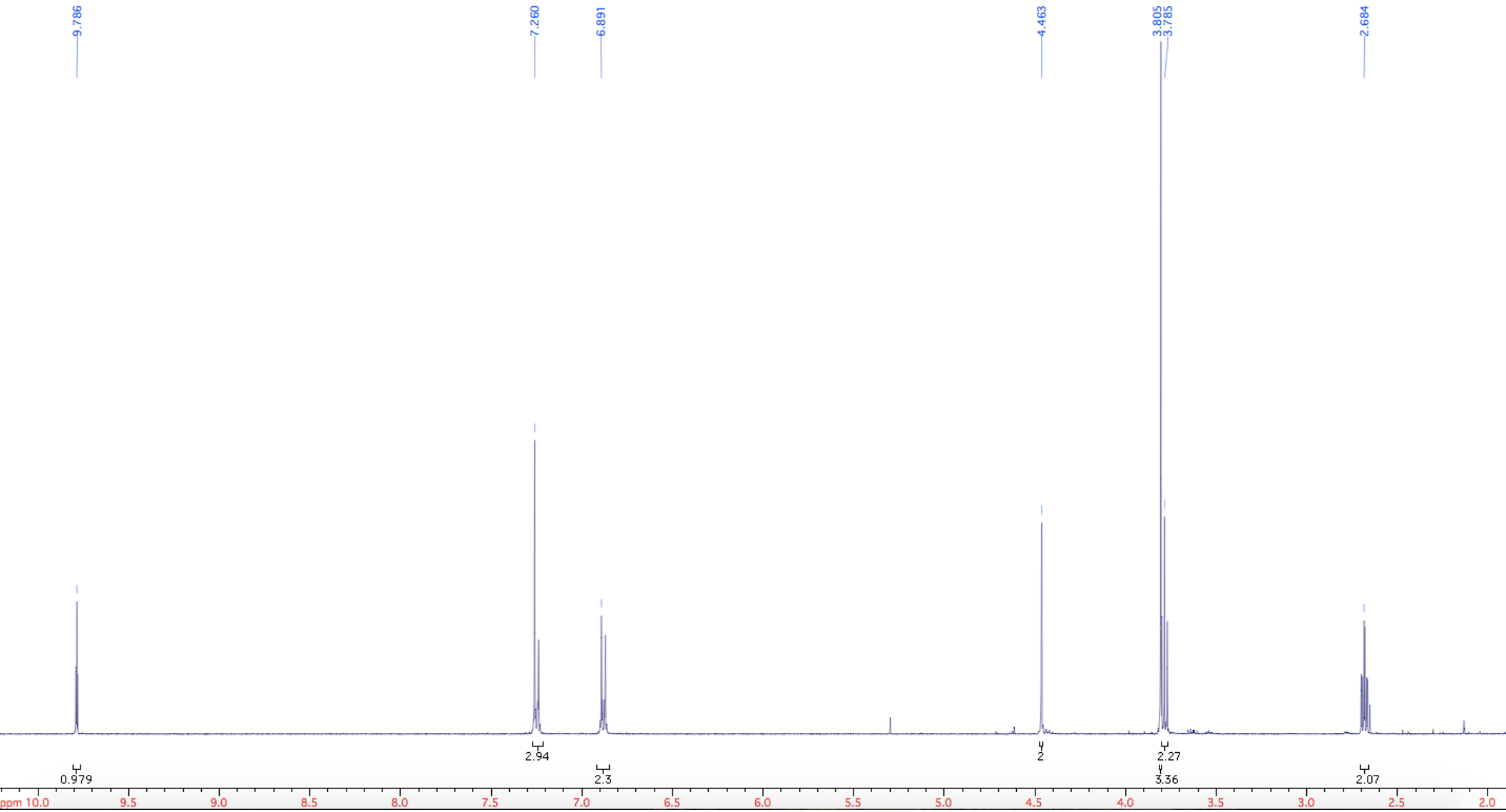
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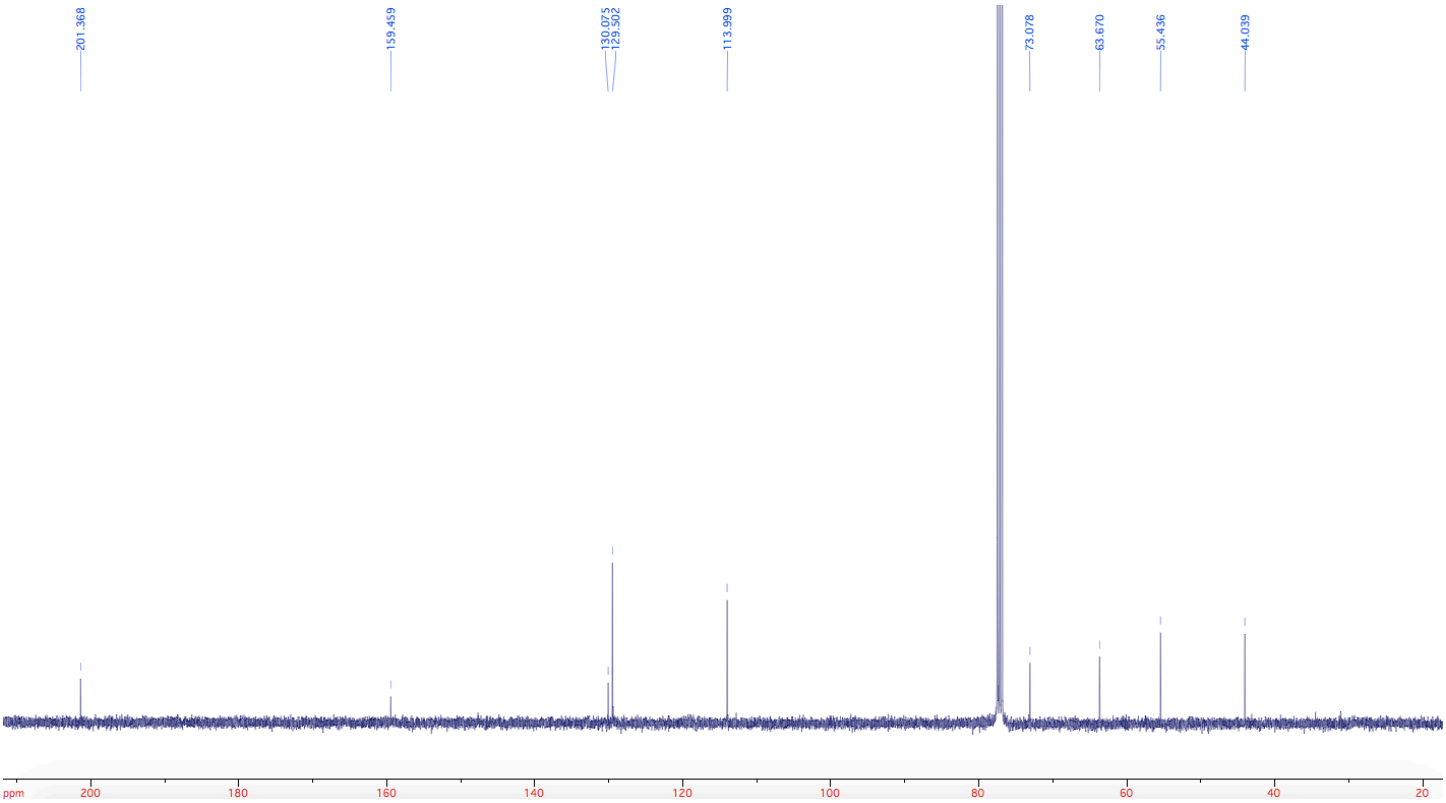
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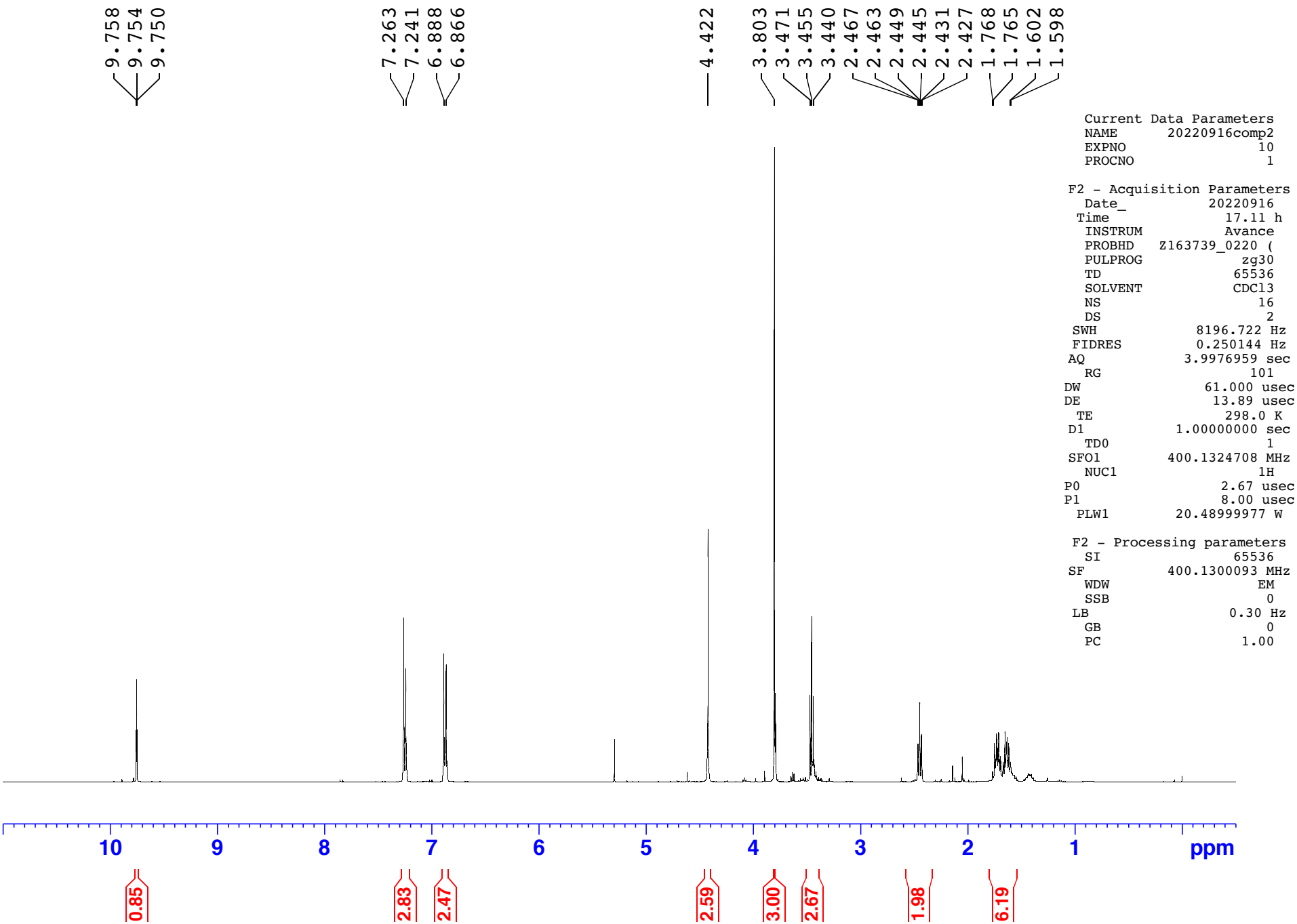
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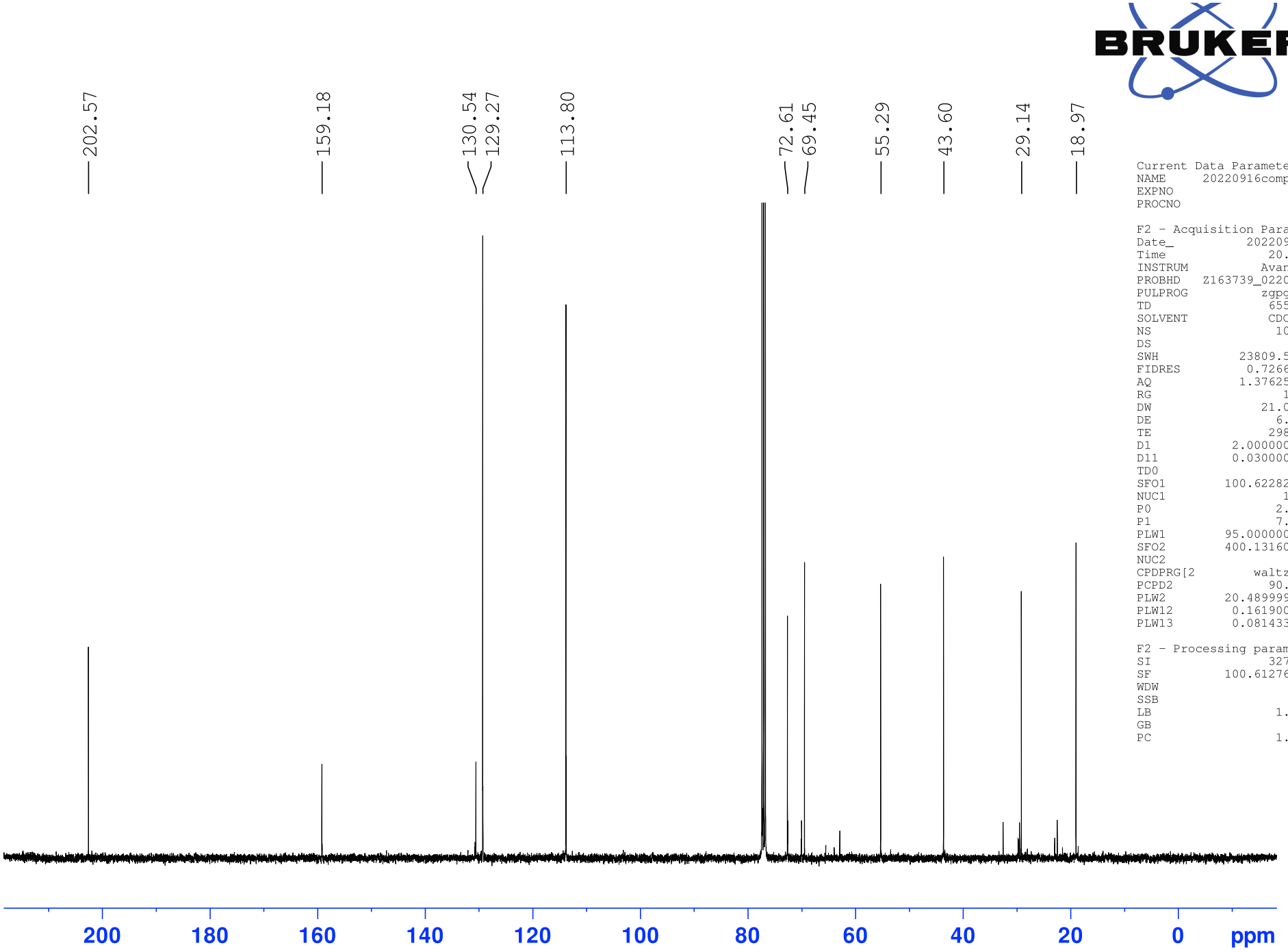


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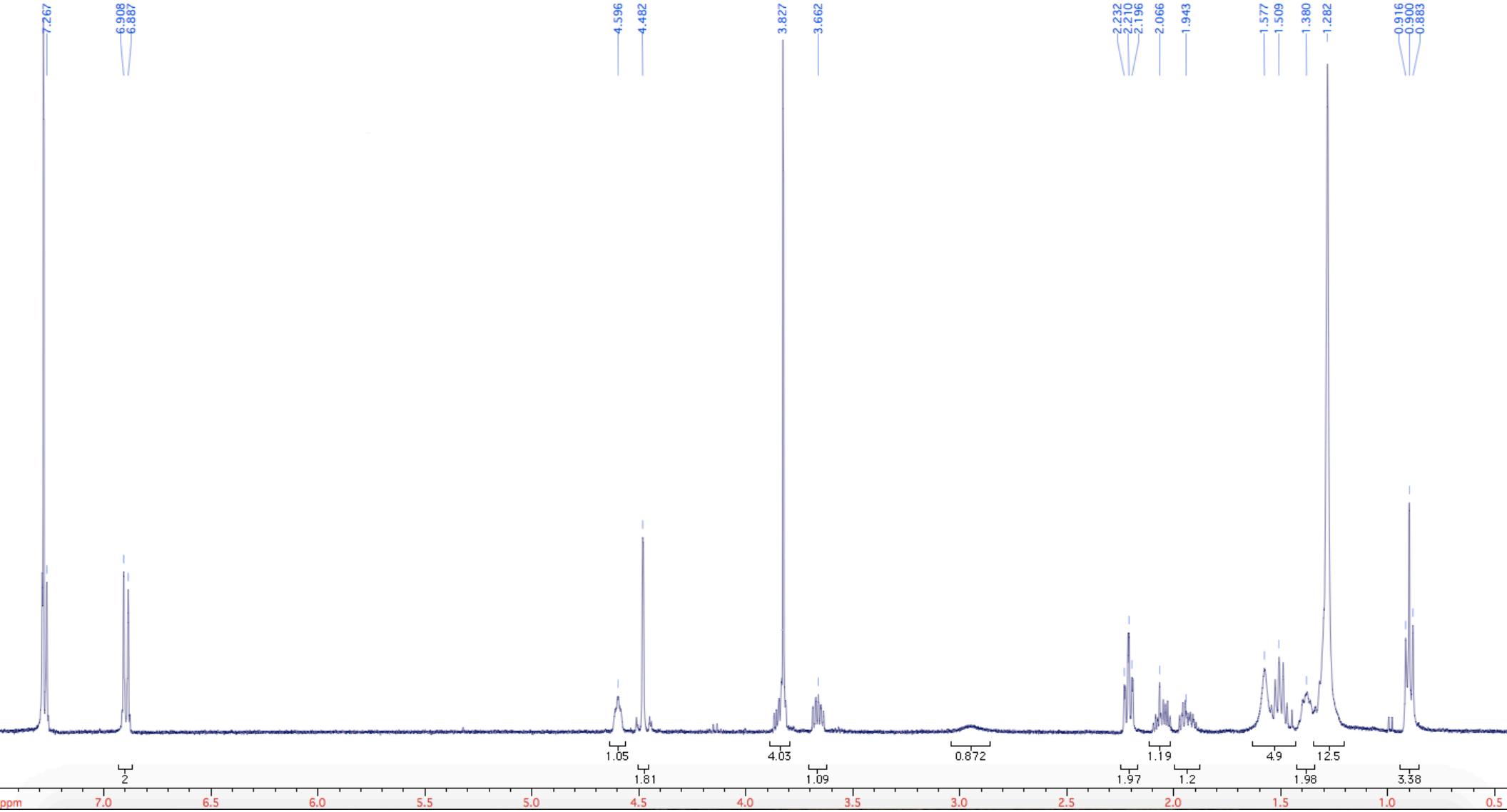
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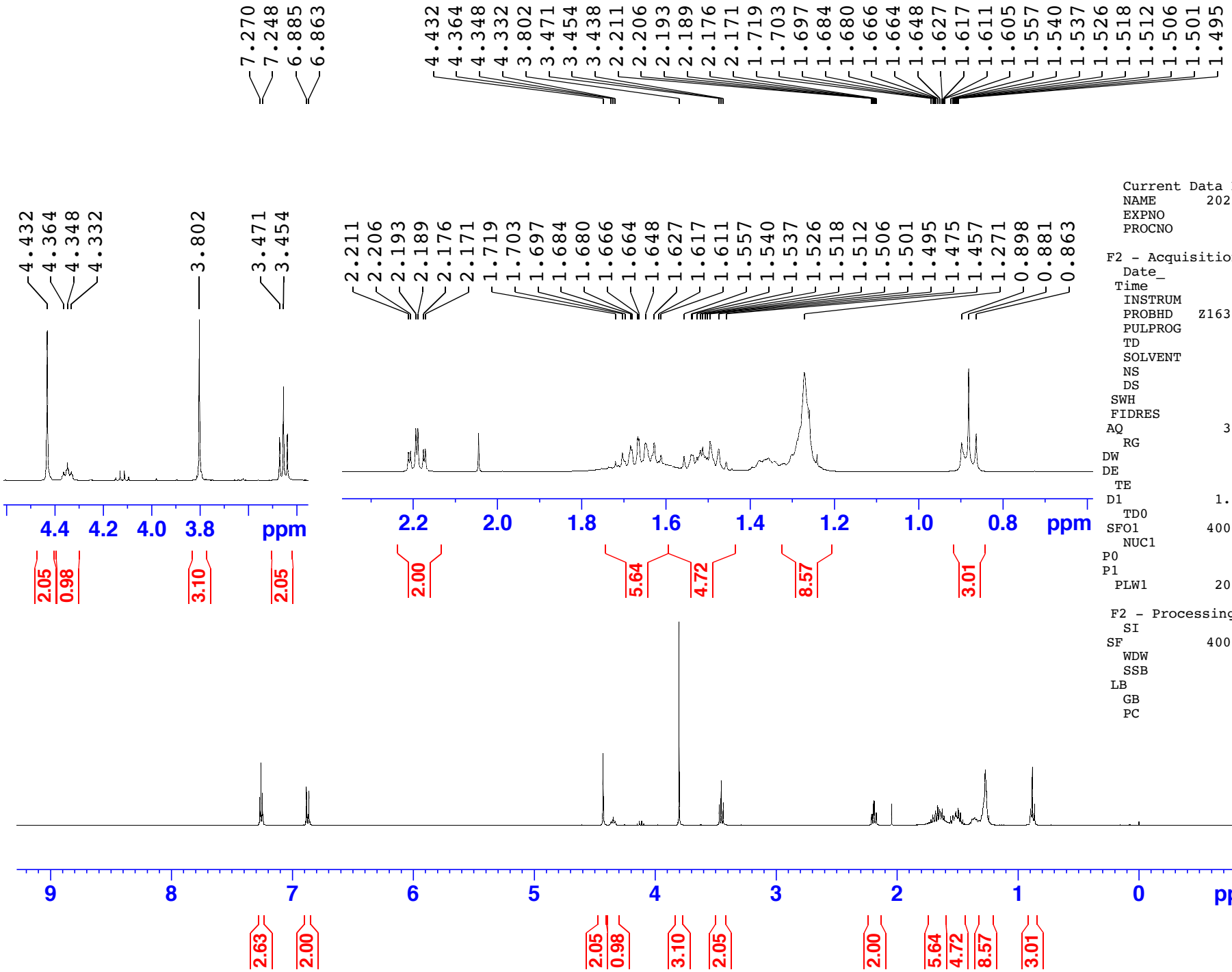


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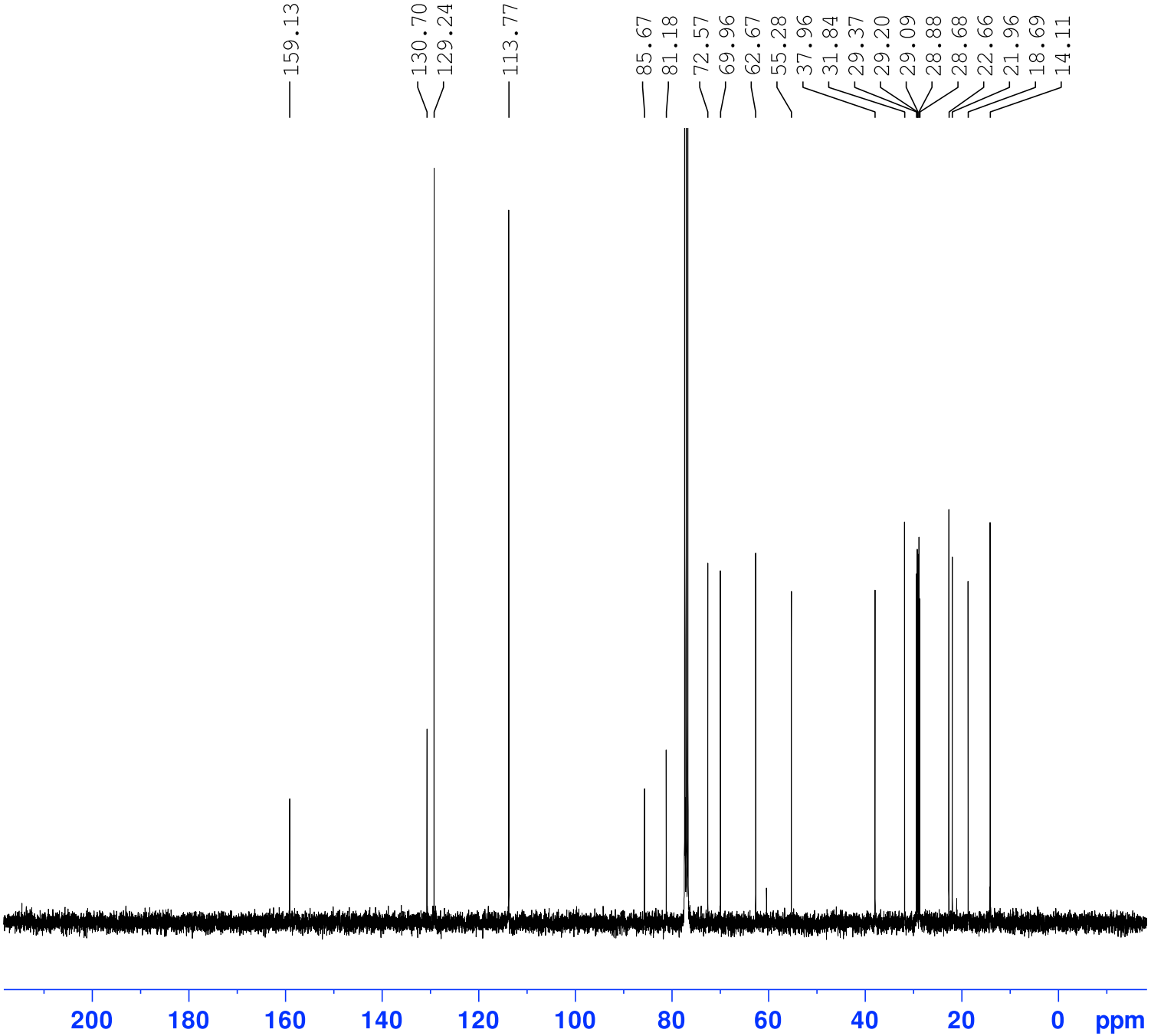

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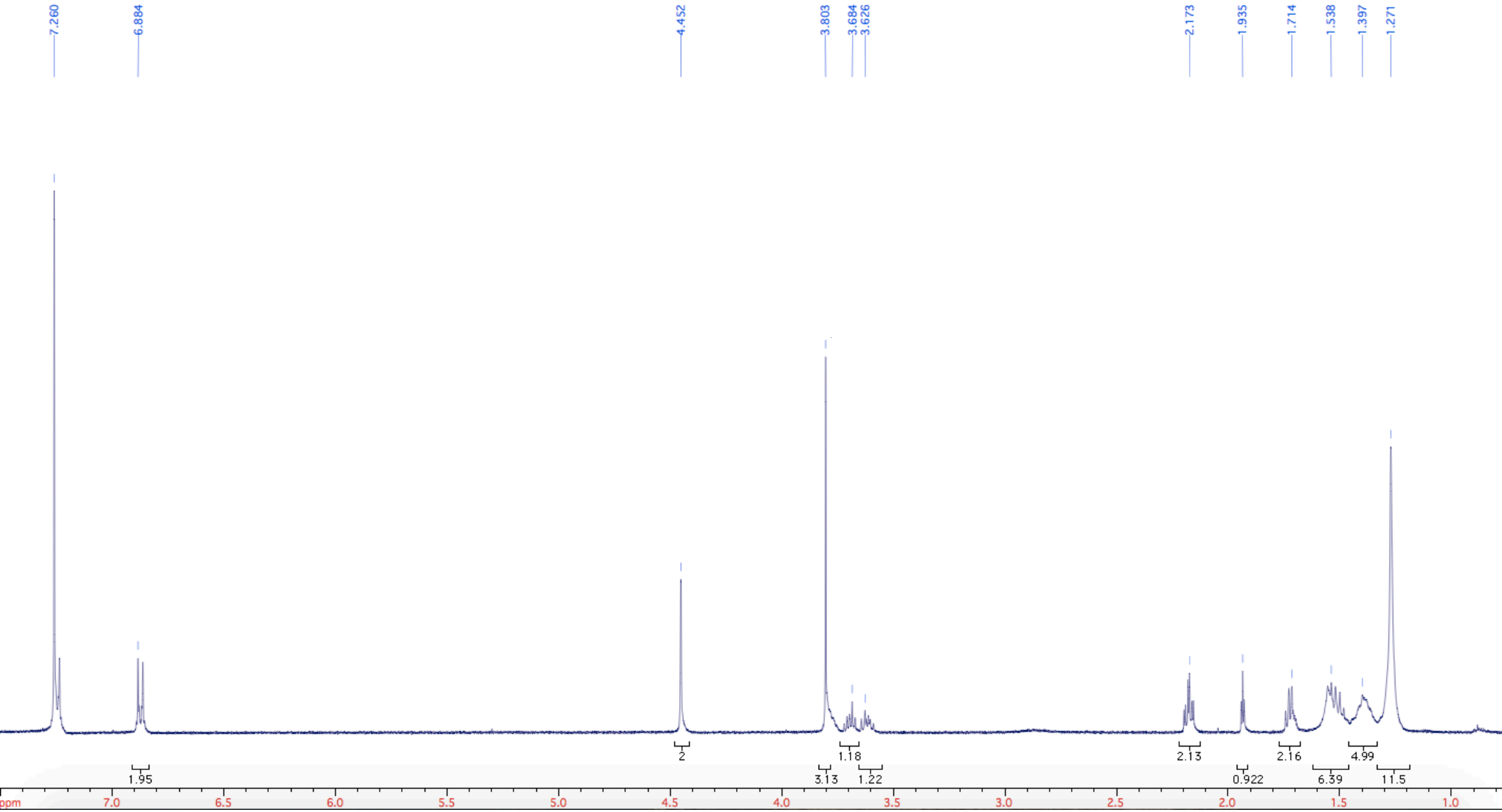
Compound 10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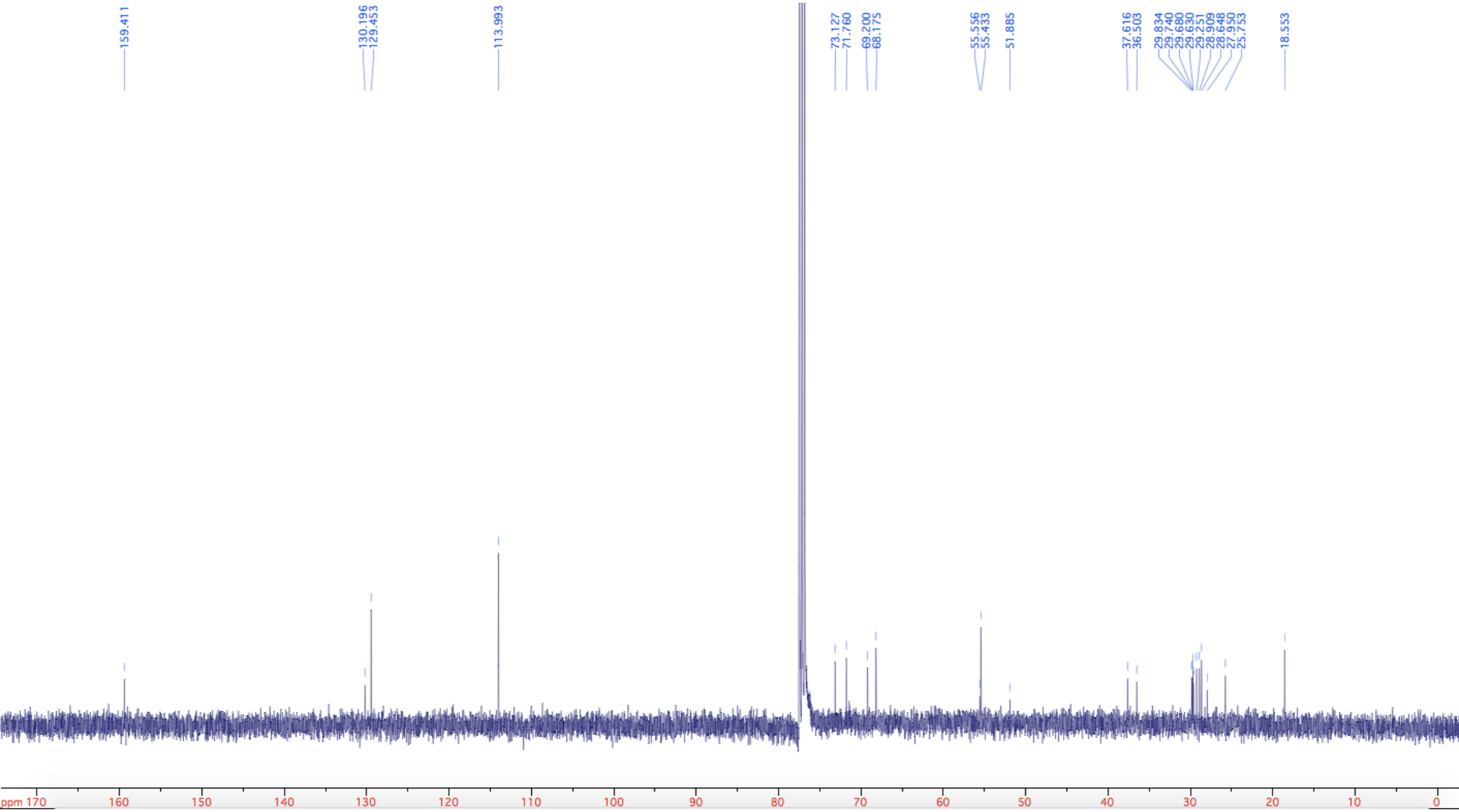
Compound 10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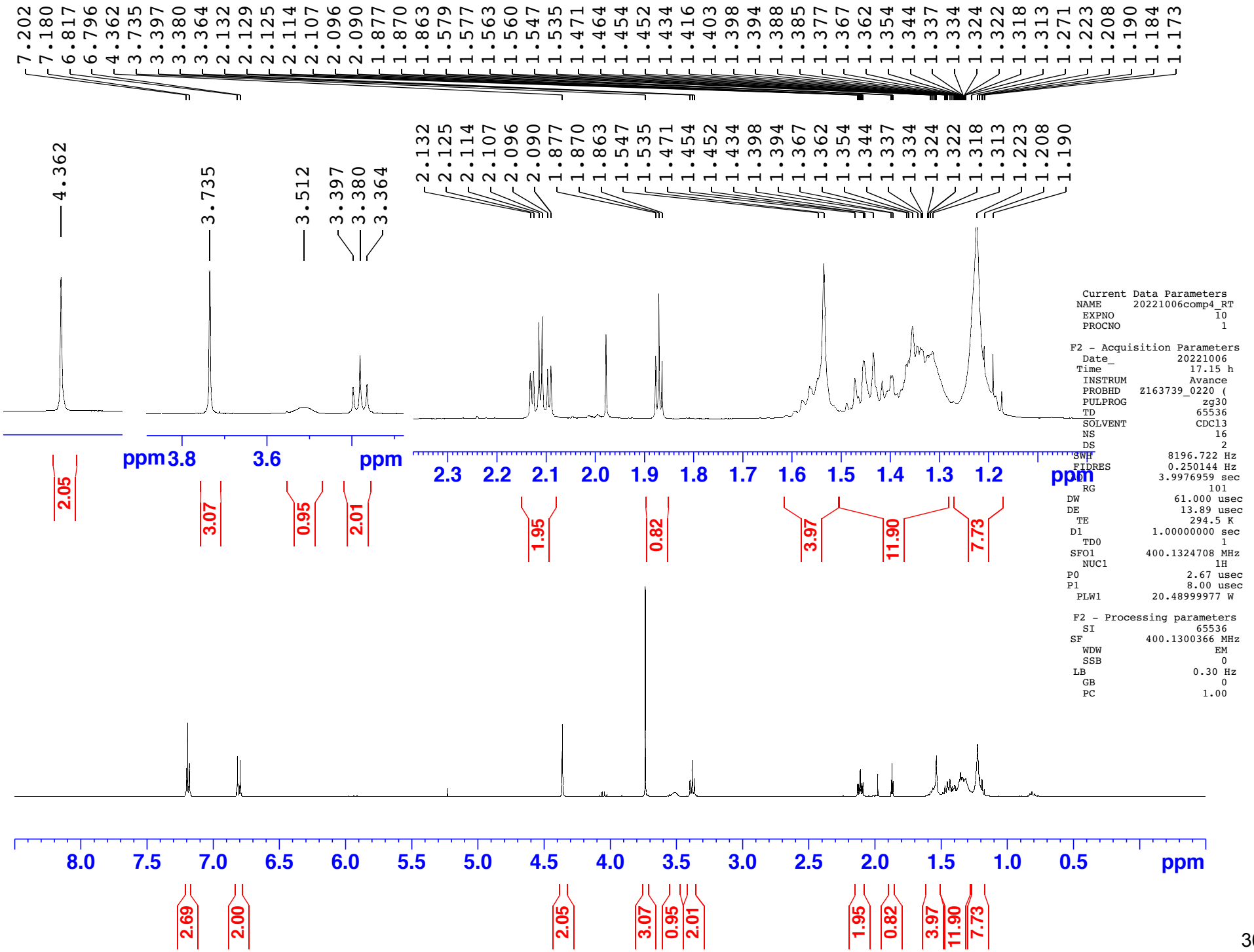
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TE 294.5 K
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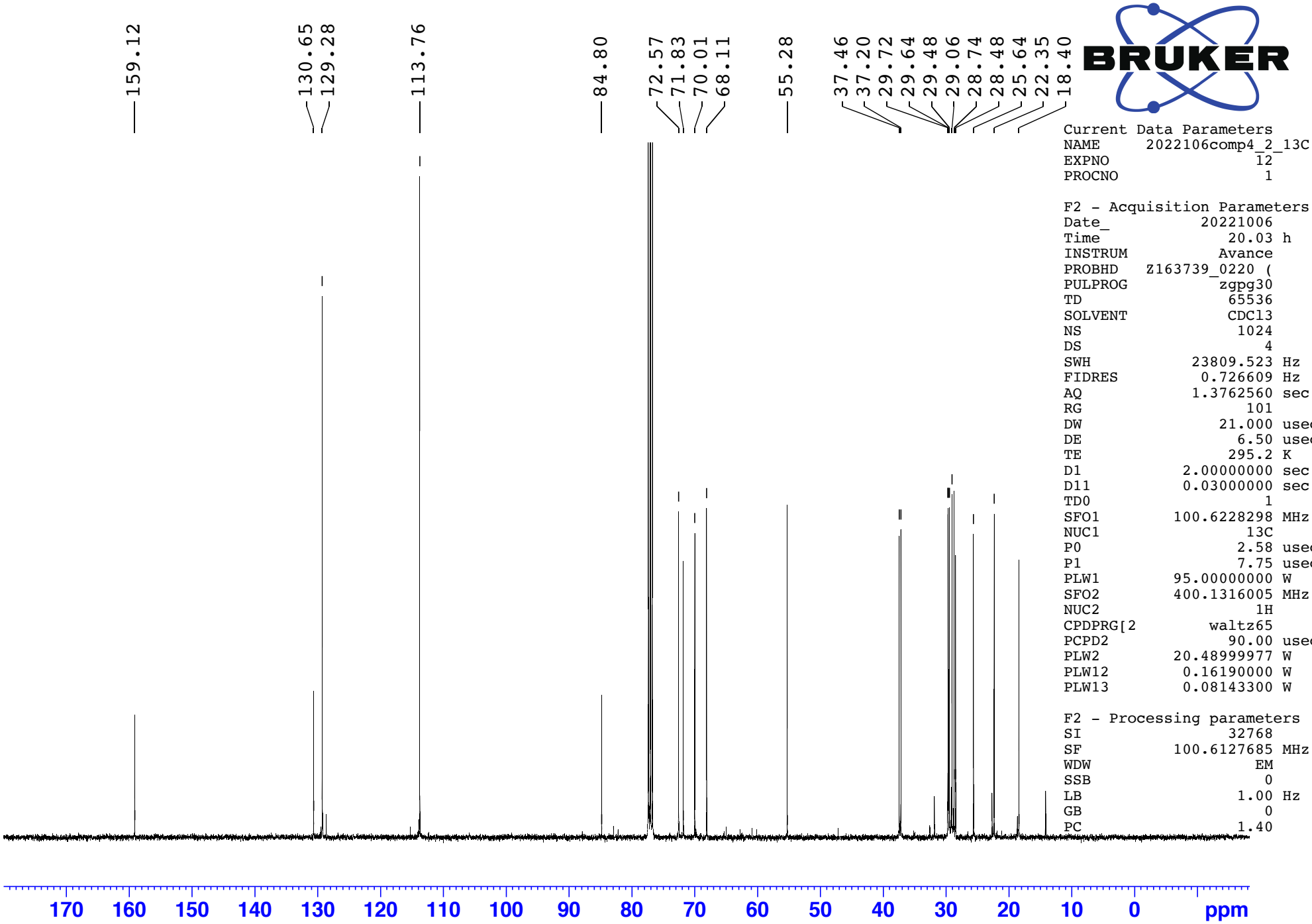
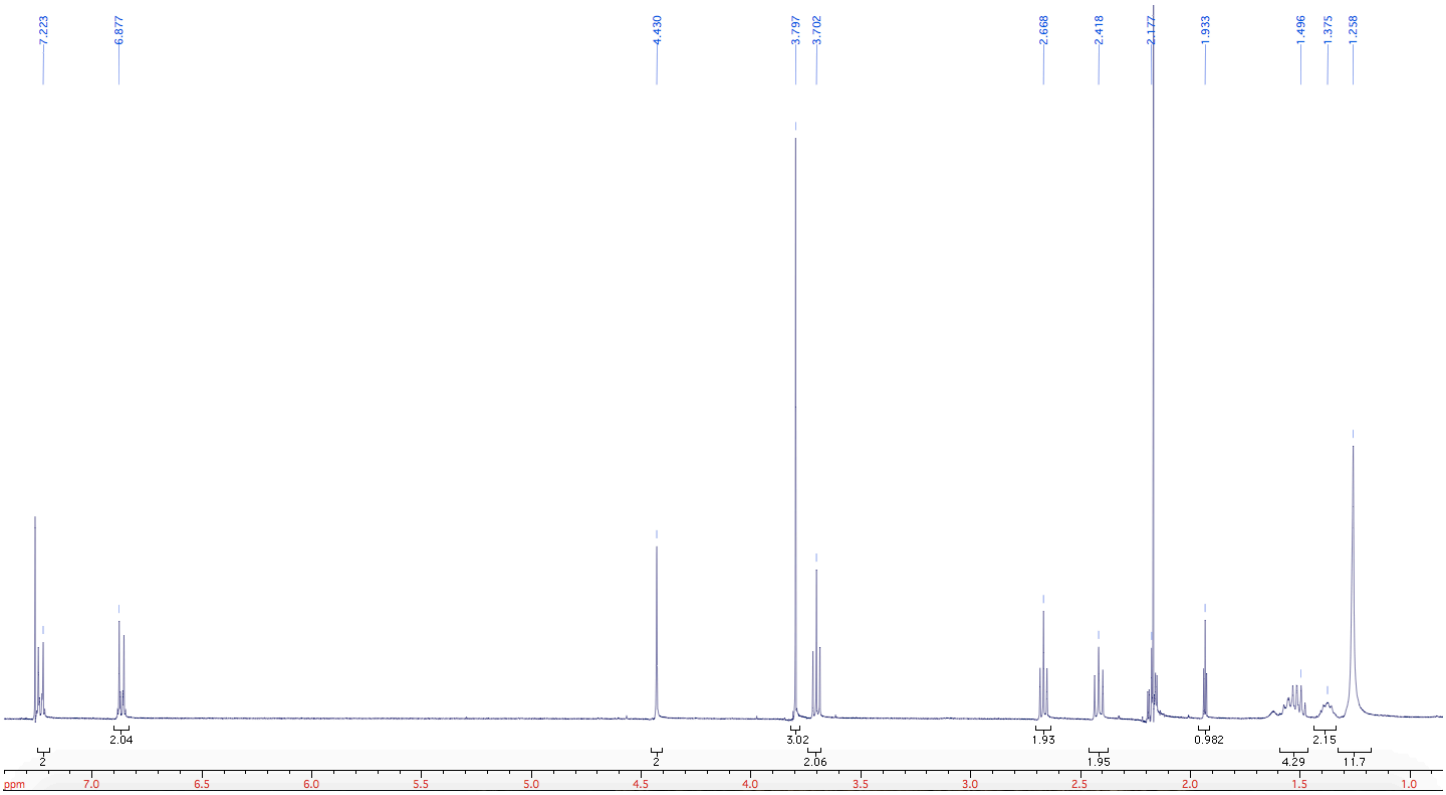
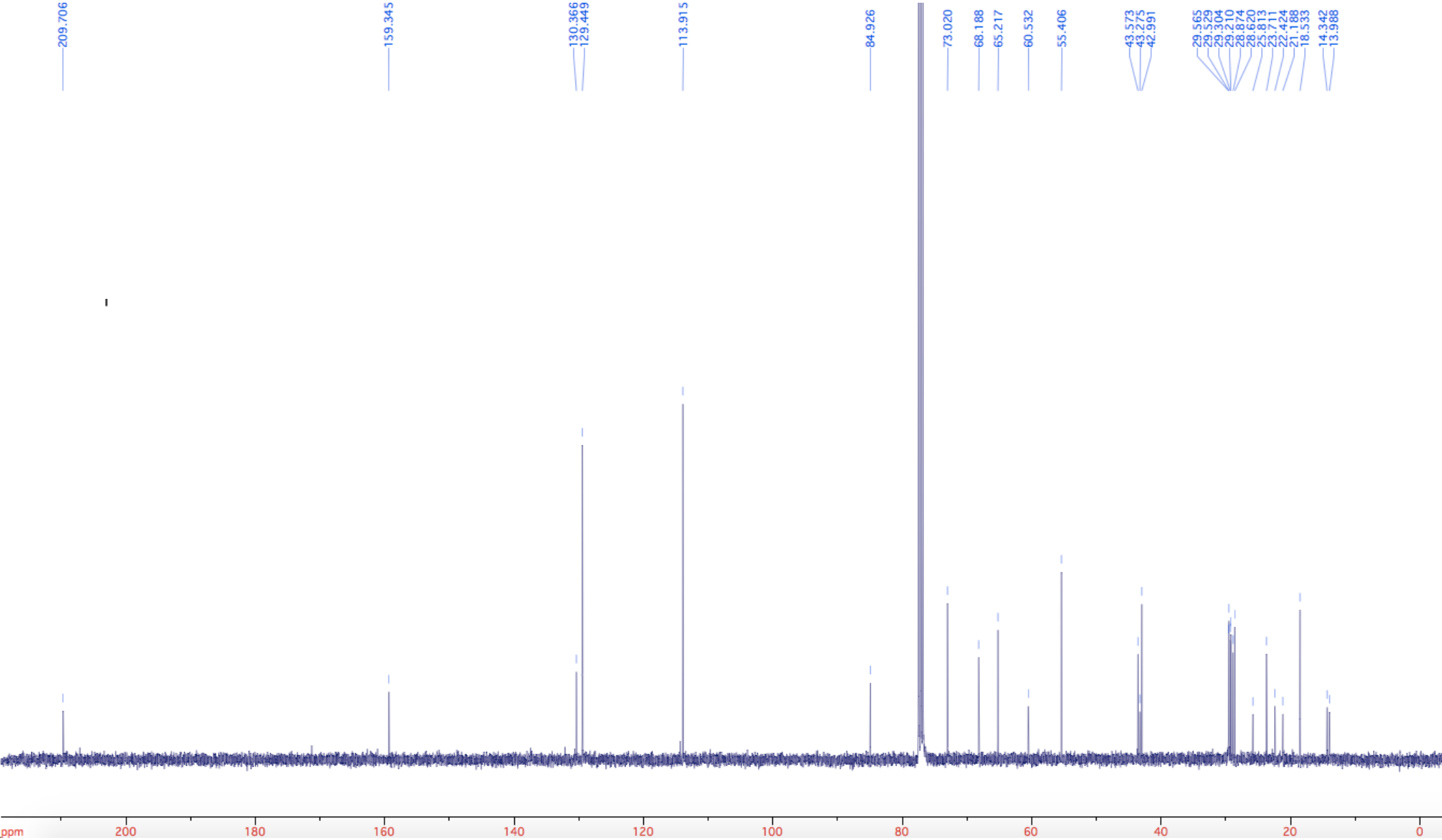
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.69 2.00 2.05 3.07 0.95 2.01 1.95 0.82 3.97 11.90 7.73


159.12
130.65
129.28
113.76
84.80
72.57
71.83
70.01
68.11
55.28
37.46
37.20
29.72
29.64
29.48
29.06
28.74
28.48
25.64
22.35
18.40
BRUKER
Current Data Parameters
NAME 2022106comp4_2_13C
EXPNO 12
PROCNO 1
F2 - Acquisition Parameters
Date_ 20221006
Time 20.03 h
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 23809.523 Hz
FIDRES 0.726609 Hz
AQ 1.3762560 sec
RG 101
DW 21.000 usec
DE 6.50 usec
TE 295.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6228298 MHz
NUC1 13C
P0 2.58 usec
P1 7.75 usec
PLW1 95.00000000 W
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 90.00 usec
PLW2 20.48999977 W
PLW12 0.16190000 W
PLW13 0.08143300 W
F2 - Processing parameters
SI 32768
SF 100.6127685 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

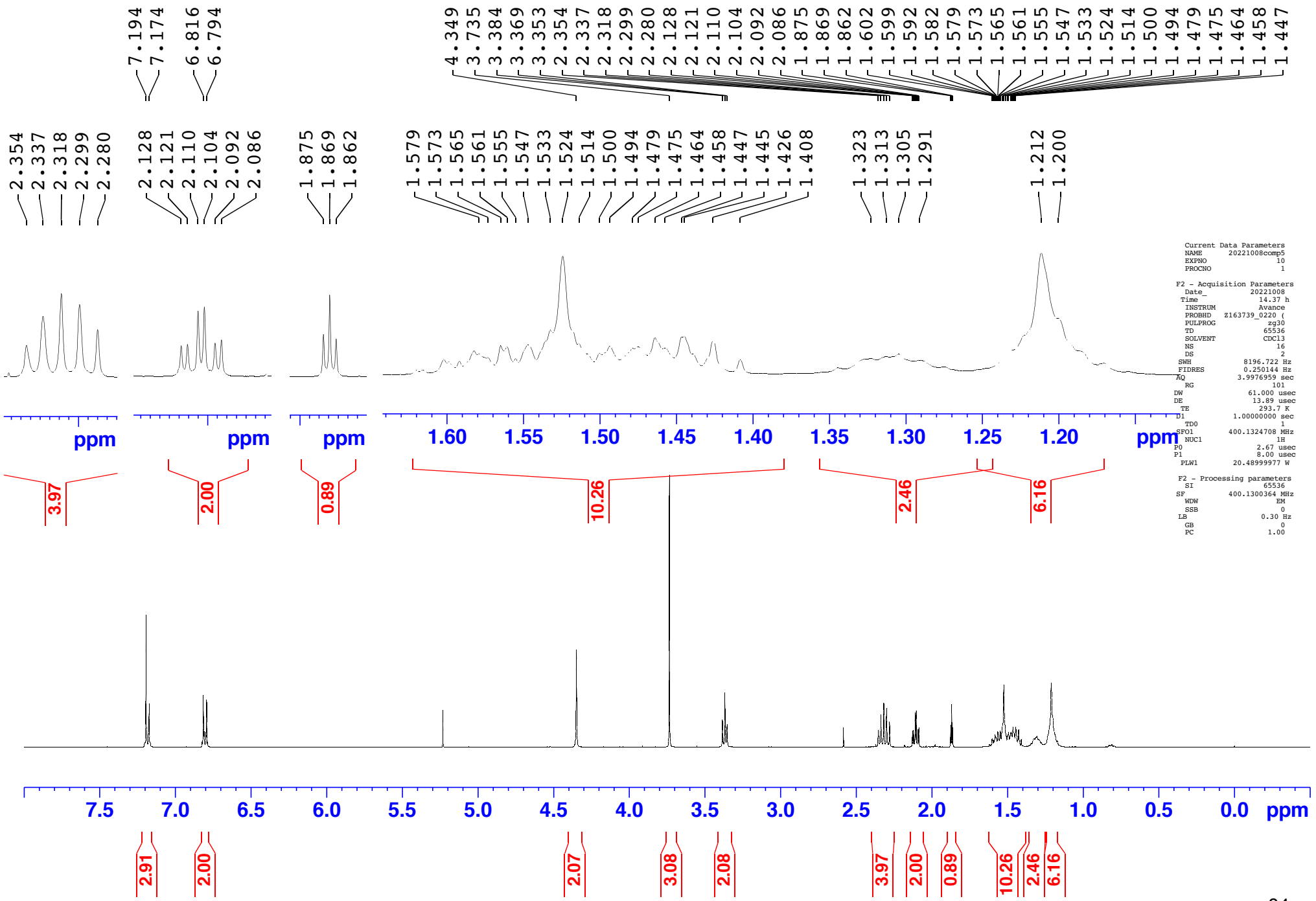
Compound 12


7.223
6.877
4.430
3.797
3.702
2.668
2.418
2.177
1.933
1.496
1.375
1.258
2
2.04
2
3.02
2.06
1.93
1.95
0.982
4.29
2.15
11.7
ppm
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

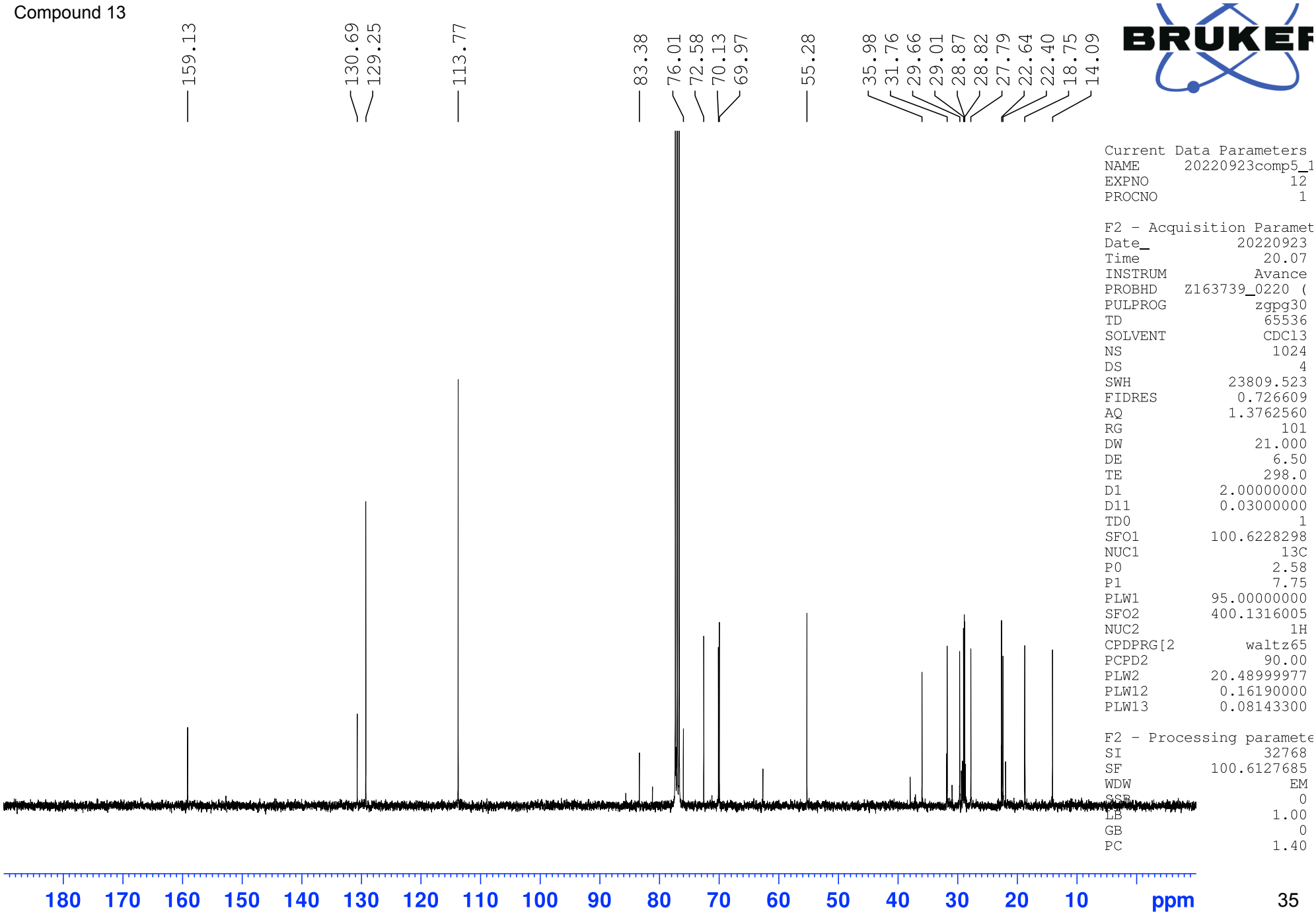
Compound 12


209.706
159.345
130.366
129.449
113.915
84.926
73.020
68.188
65.217
60.532
55.406
43.573
43.275
42.991
29.565
29.529
29.304
29.210
28.874
28.620
25.813
23.711
22.424
21.188
18.533
14.342
13.988
ppm
200
180
160
140
120
100
80
60
40
20
0

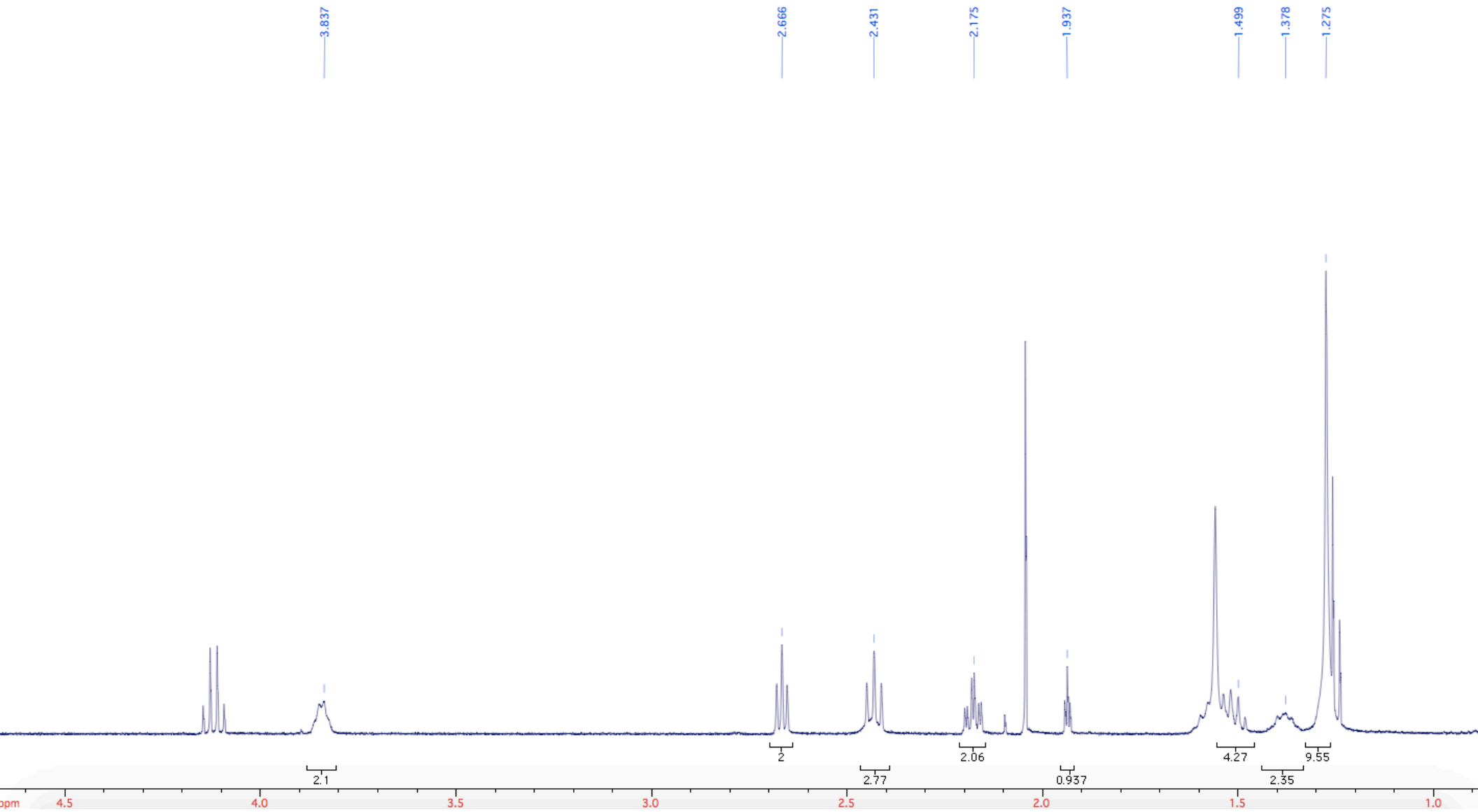
Compound 13

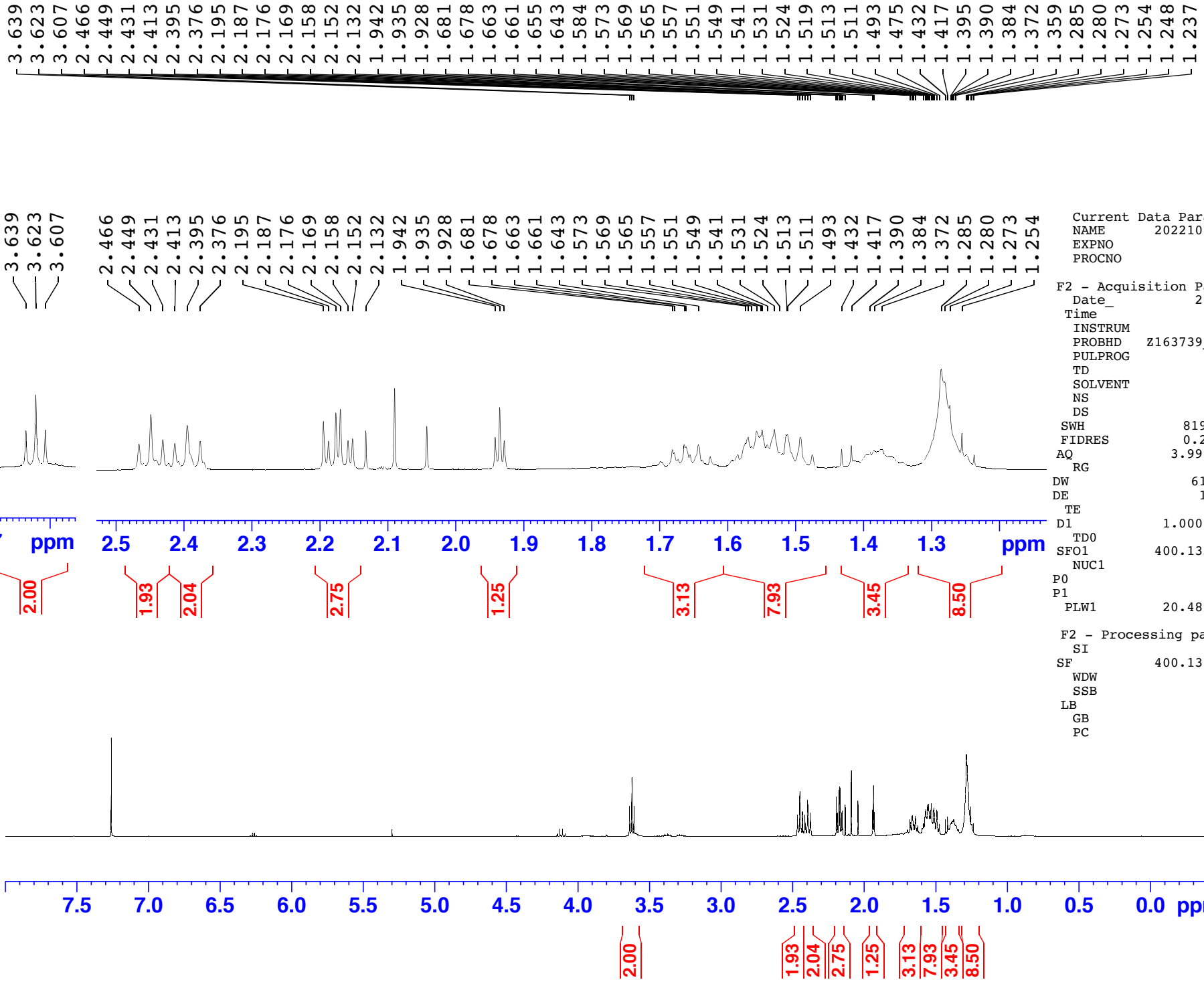

7.194
7.174
6.816
6.794
4.349
3.735
3.384
3.369
3.353
2.354
2.337
2.318
2.299
2.280
2.128
2.121
2.110
2.104
2.092
2.086
1.875
1.869
1.862
1.579
1.573
1.565
1.561
1.555
1.547
1.533
1.524
1.514
1.500
1.494
1.479
1.475
1.464
1.458
1.447
1.445
1.426
1.408
1.323
1.313
1.305
1.291
1.212
1.200
ppm
3.97
2.00
0.89
10.26
2.46
6.16
Current Data Parameters
NAME 20221008comp5
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20221008
Time 14.37 h
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8196.722 Hz
FIDRES 0.250144 Hz
AQ 3.9976959 sec
RG 101
DW 61.000 usec
DE 13.89 usec
TE 293.7 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324708 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 20.48999977 W
F2 - Processing parameters
SI 65536
SF 400.1300364 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
2.91
2.00
2.07
3.08
2.08
3.97
2.00
0.89
10.26
2.46
6.16

Compound 13

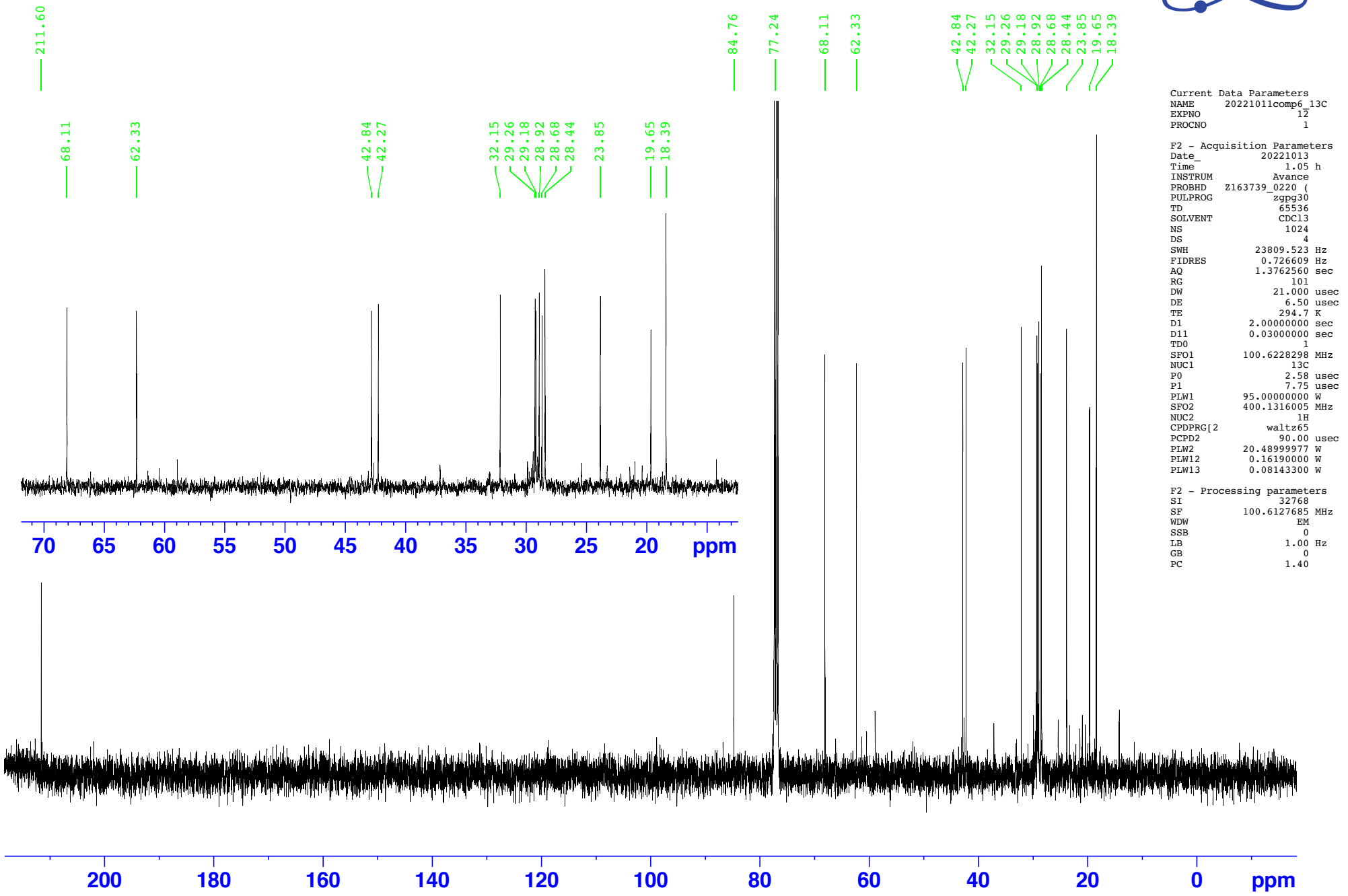

159.13
130.69
129.25
113.77
83.38
76.01
72.58
70.13
69.97
55.28
35.98
31.76
29.66
29.01
28.87
28.82
27.79
22.64
22.40
18.75
14.09
Current Data Parameters
NAME 20220923comp5_1
EXPNO 12
PROCNO 1
F2 - Acquisition Paramet
Date_ 20220923
Time 20.07
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 23809.523
FIDRES 0.726609
AQ 1.3762560
RG 101
DW 21.000
DE 6.50
TE 298.0
D1 2.00000000
D11 0.03000000
TD0 1
SFO1 100.6228298
NUC1 13C
P0 2.58
P1 7.75
PLW1 95.00000000
SFO2 400.1316005
NUC2 1H
CPDPRG[2 waltz65
PCPD2 90.00
PLW2 20.48999977
PLW12 0.16190000
PLW13 0.08143300
F2 - Processing paramete
SI 32768
SF 100.6127685
WDW EM
SSB 0
LB 1.00
GB 0
PC 1.40
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Compound 14


3.837
2.666
2.431
2.175
1.937
1.499
1.378
1.275
2.1
2
2.77
2.06
0.937
4.27
2.35
9.55
ppm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0




```
Current Data Parameters
NAME      20221011comp6
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20221011
Time             16.47 h
INSTRUM          Avance
PROBHD   Z163739_0220 (
PULPROG          zg30
TD              65536
SOLVENT         CDCl3
NS                 16
DS                  2
SWH          8196.722 Hz
FIDRES       0.250144 Hz
AQ           3.9976959 sec
RG                101
DW             61.000 usec
DE             13.89 usec
TE             293.9 K
D1          1.00000000 sec
TD0                1
SFO1       400.1324708 MHz
NUC1               1H
P0             2.67 usec
P1             8.00 usec
PLW1       20.48999977 W

F2 - Processing parameters
SI              65536
SF         400.1300098 MHz
WDW               EM
SSB                0
LB              0.30 Hz
GB                 0
PC              1.00
```



Current Data Parameters

NAME	20221011comp6_13C
EXPNO	12
PROCNO	1

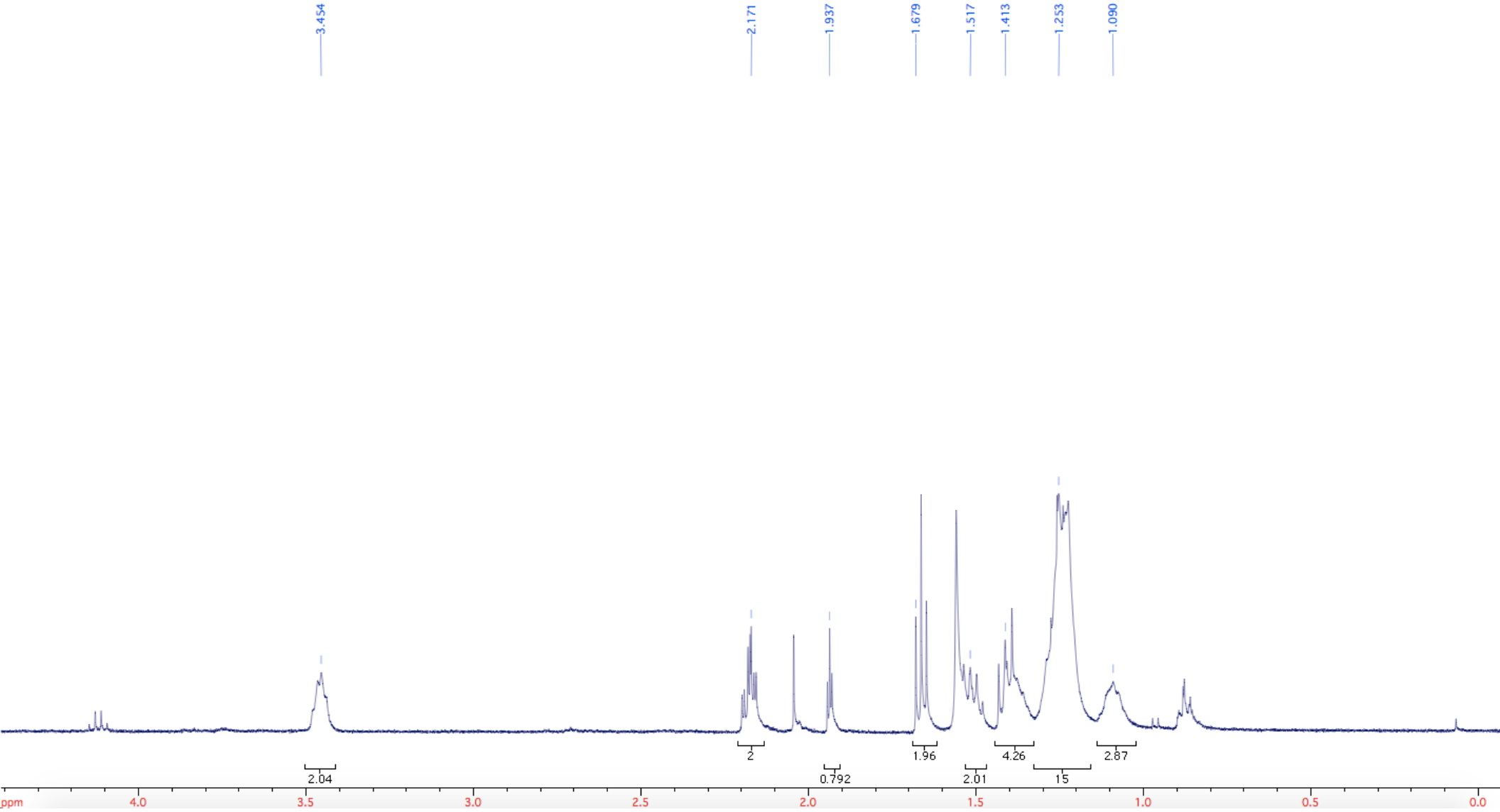
F2 - Acquisition Parameters

Date_	20221013
Time	1.05 h
INSTRUM	Avance
PROBHD	Z163739_0220 (
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	1024
DS	4
SWH	23809.523 Hz
FIDRES	0.726609 Hz
AQ	1.3762560 sec
RG	101
DW	21.000 usec
DE	6.50 usec
TE	294.7 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
SFO1	100.6228298 MHz
NUC1	13C
P0	2.58 usec
P1	7.75 usec
PLW1	95.00000000 W
SFO2	400.1316005 MHz
NUC2	1H
CPDPRG[2	waltz65
PCPD2	90.00 usec
PLW2	20.48999977 W
PLW12	0.16190000 W
PLW13	0.08143300 W

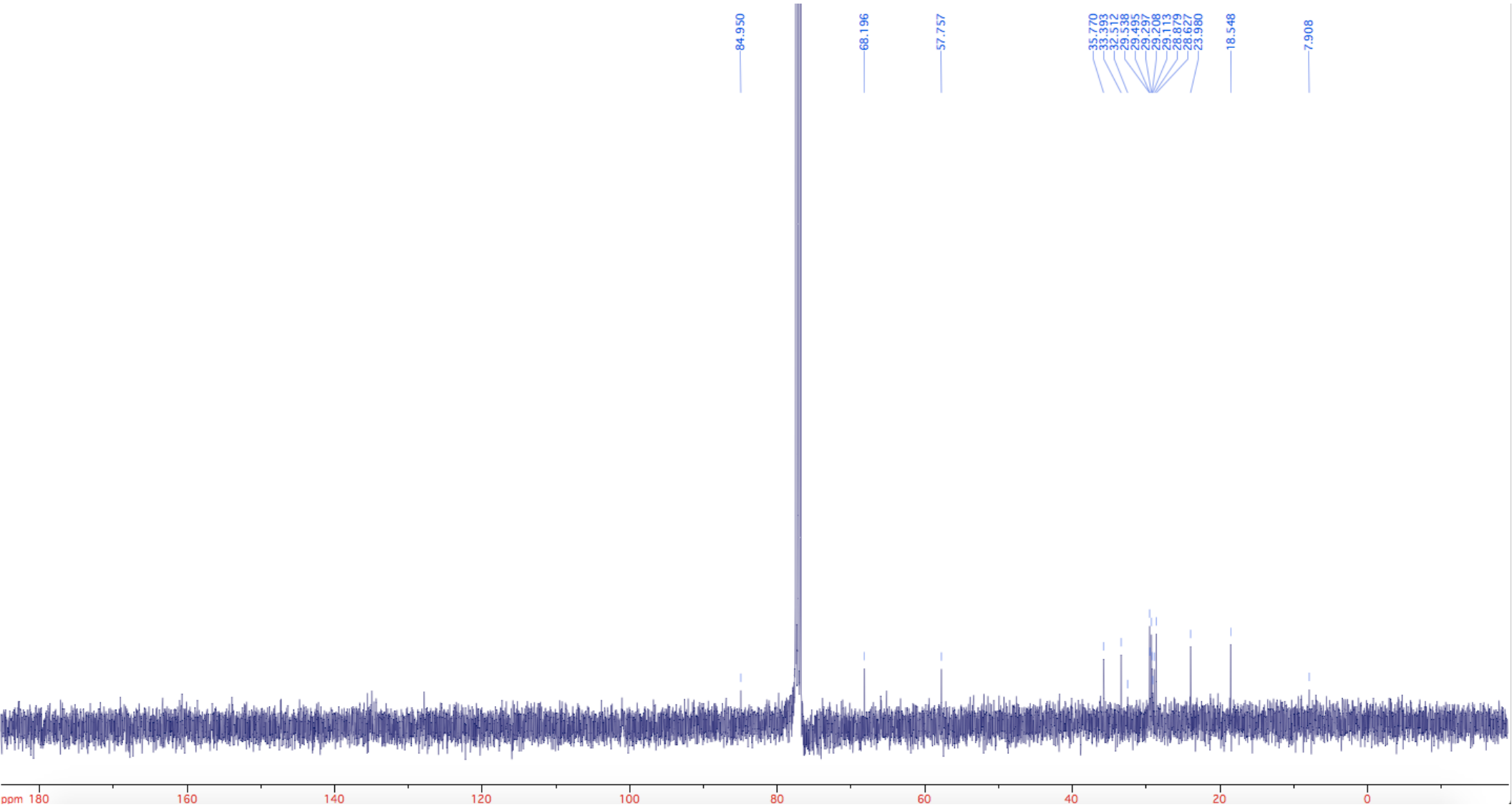
F2 - Processing parameters

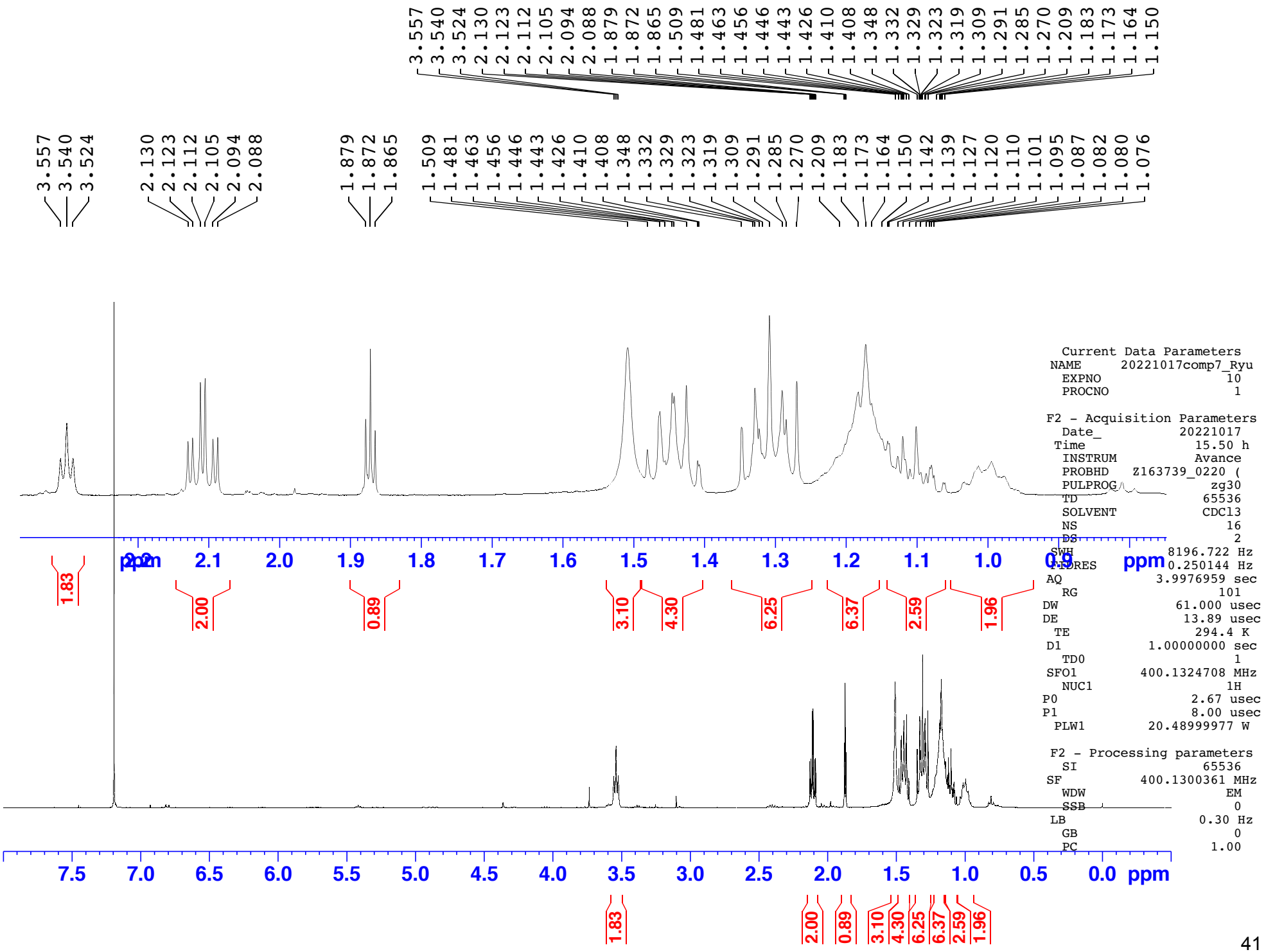
SI	32768
SF	100.6127685 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

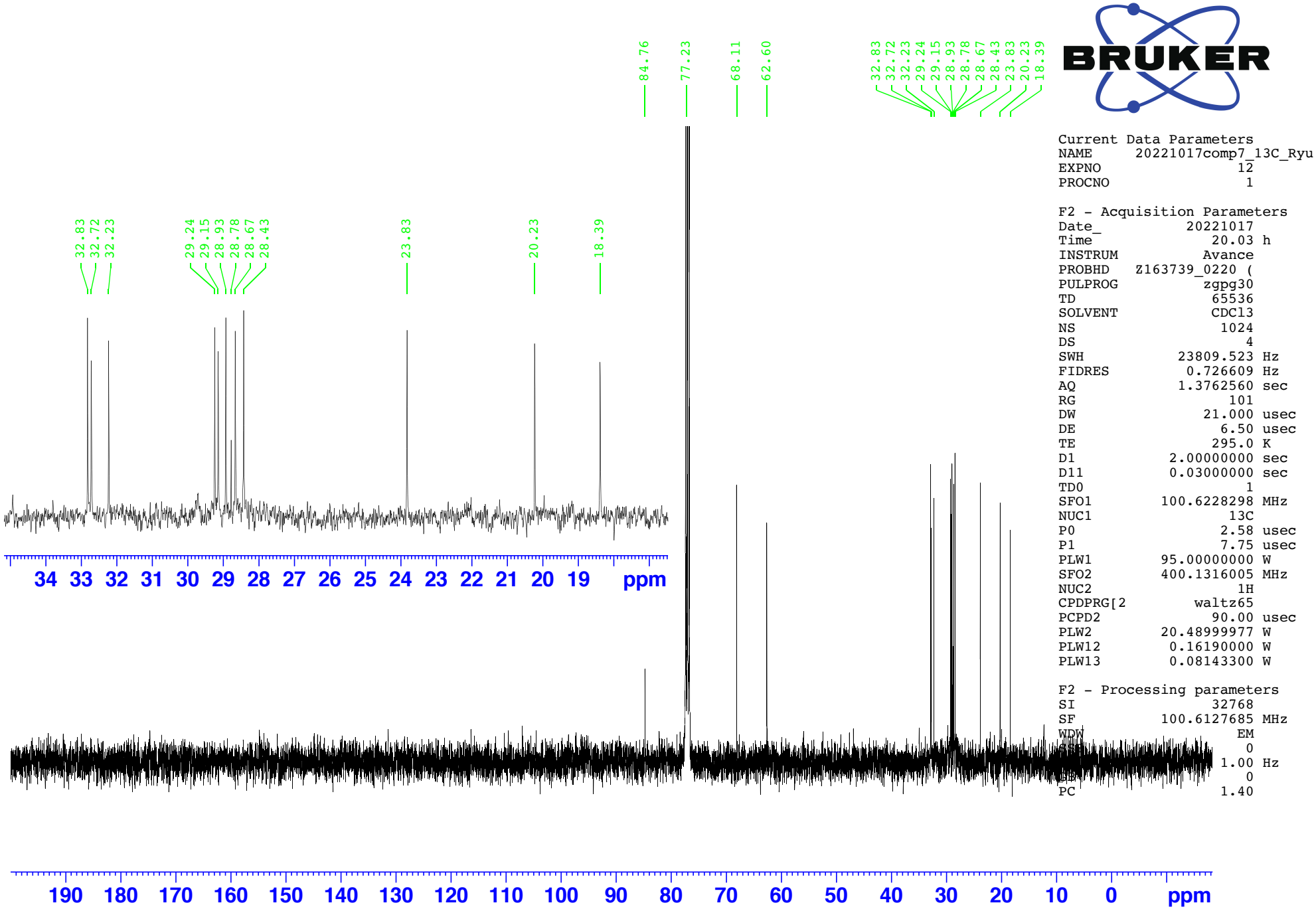
Compound 16


3.454
2.171
1.937
1.679
1.517
1.413
1.253
1.090
2.04
2
0.792
1.96
2.01
4.26
15
2.87
ppm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Compound 16


84.950
68.196
57.757
35.770
33.393
32.512
29.538
29.495
29.297
29.208
29.113
28.879
28.627
23.980
18.548
7.908
ppm 180
160
140
120
100
80
60
40
20
0

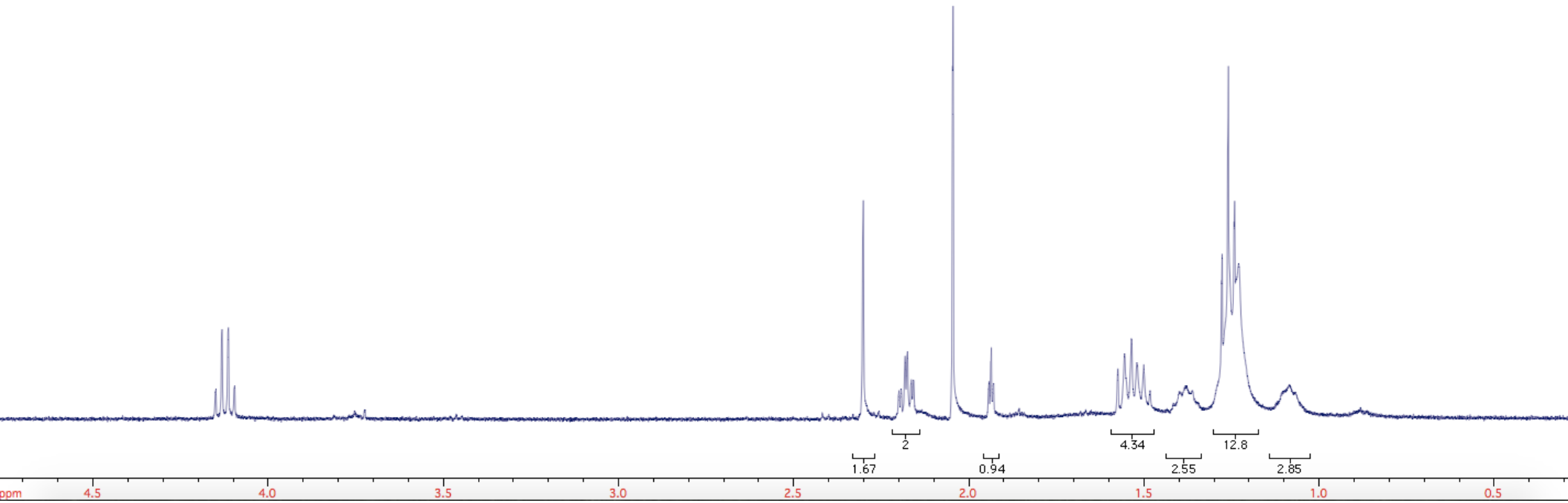

3.557 3.540 3.524 2.130 2.123 2.112 2.105 2.094 2.088 1.879 1.872 1.865 1.509 1.481 1.463 1.456 1.446 1.443 1.426 1.410 1.408 1.348 1.332 1.329 1.323 1.319 1.309 1.291 1.285 1.270 1.209 1.183 1.173 1.164 1.150
1.83 2.00 0.89 3.10 4.30 6.25 6.37 2.59 1.96
ppm
Current Data Parameters
NAME 20221017comp7_Ryu
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20221017
Time 15.50 h
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8196.722 Hz
FIDRES 0.250144 Hz
AQ 3.9976959 sec
RG 101
DW 61.000 usec
DE 13.89 usec
TE 294.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324708 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 20.48999977 W
F2 - Processing parameters
SI 65536
SF 400.1300361 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

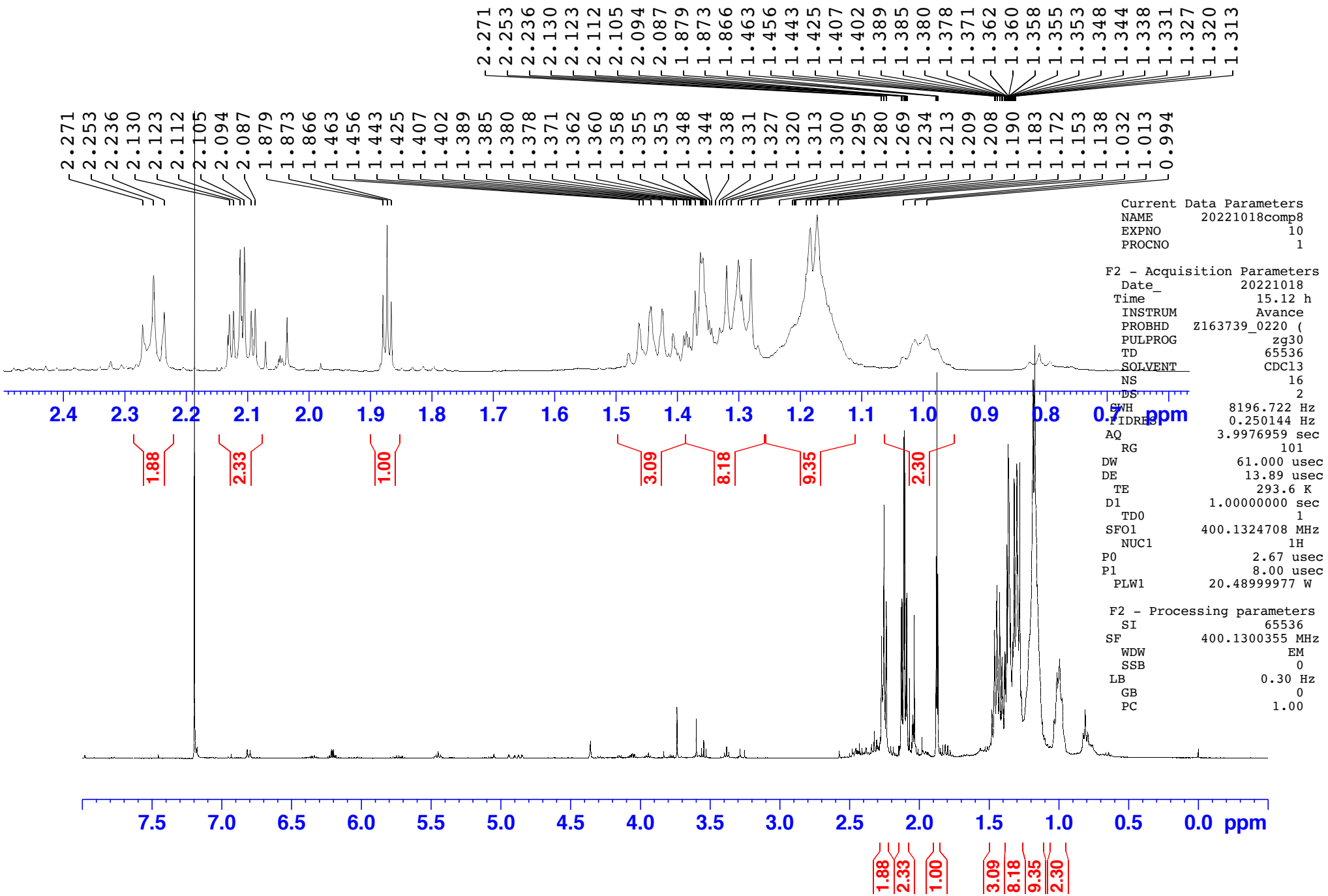


BRUKER

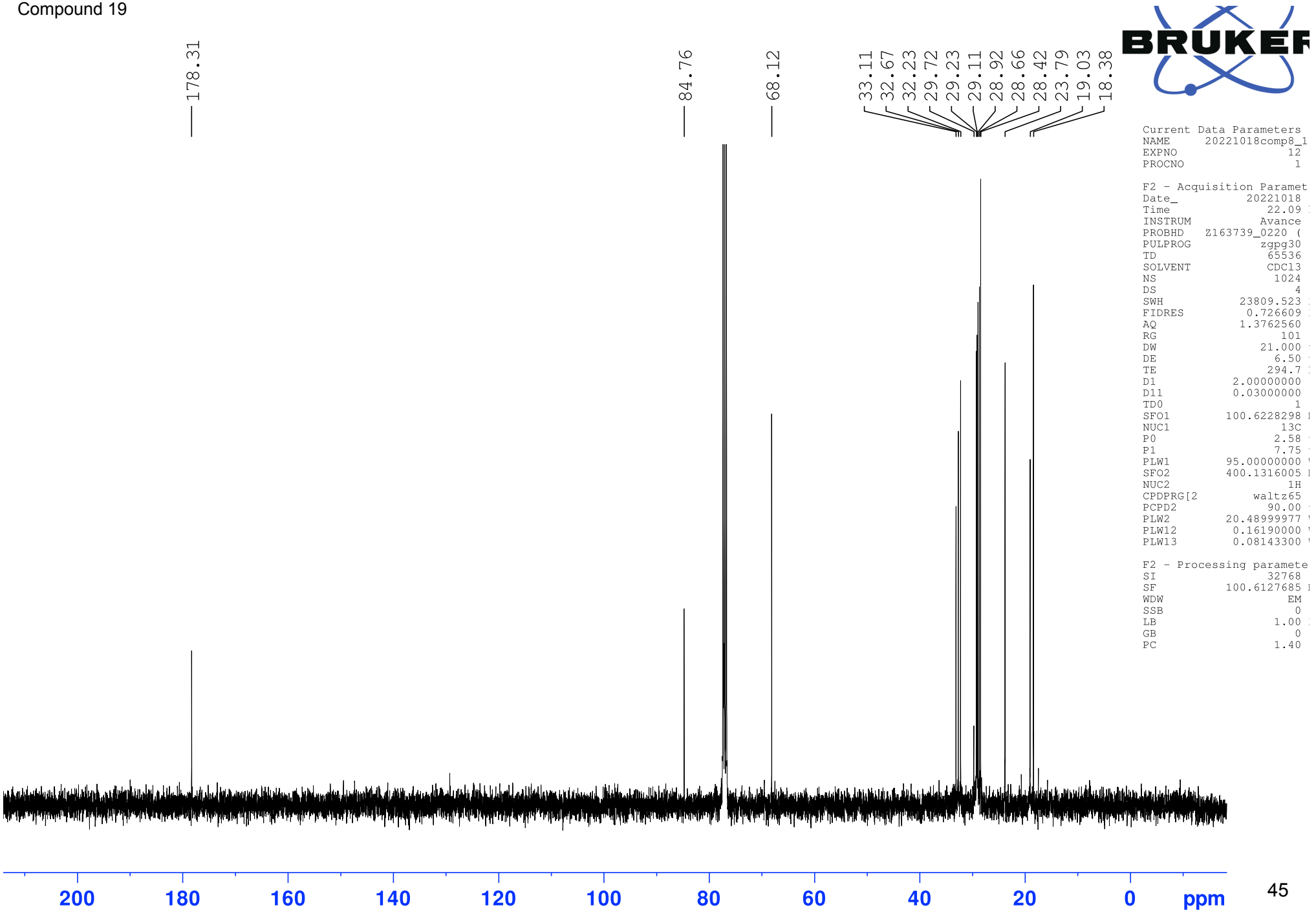
```
Current Data Parameters
NAME      20221017comp7_13C_Ryu
EXPNO                        12
PROCNO                        1

F2 - Acquisition Parameters
Date_              20221017
Time                  20.03 h
INSTRUM              Avance
PROBHD     Z163739_0220 (
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                     1024
DS                        4
SWH              23809.523 Hz
FIDRES            0.726609 Hz
AQ                1.3762560 sec
RG                      101
DW                 21.000 usec
DE                  6.50 usec
TE                 295.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                       1
SFO1           100.6228298 MHz
NUC1                    13C
P0                   2.58 usec
P1                   7.75 usec
PLW1           95.00000000 W
SFO2           400.1316005 MHz
NUC2                    1H
CPDPRG[2            waltz65
PCPD2               90.00 usec
PLW2           20.48999977 W
PLW12           0.16190000 W
PLW13           0.08143300 W

F2 - Processing parameters
SI                    32768
SF             100.6127685 MHz
WDW                      EM
                          0
                   1.00 Hz
                          0
PC                    1.40
```

Compound 18

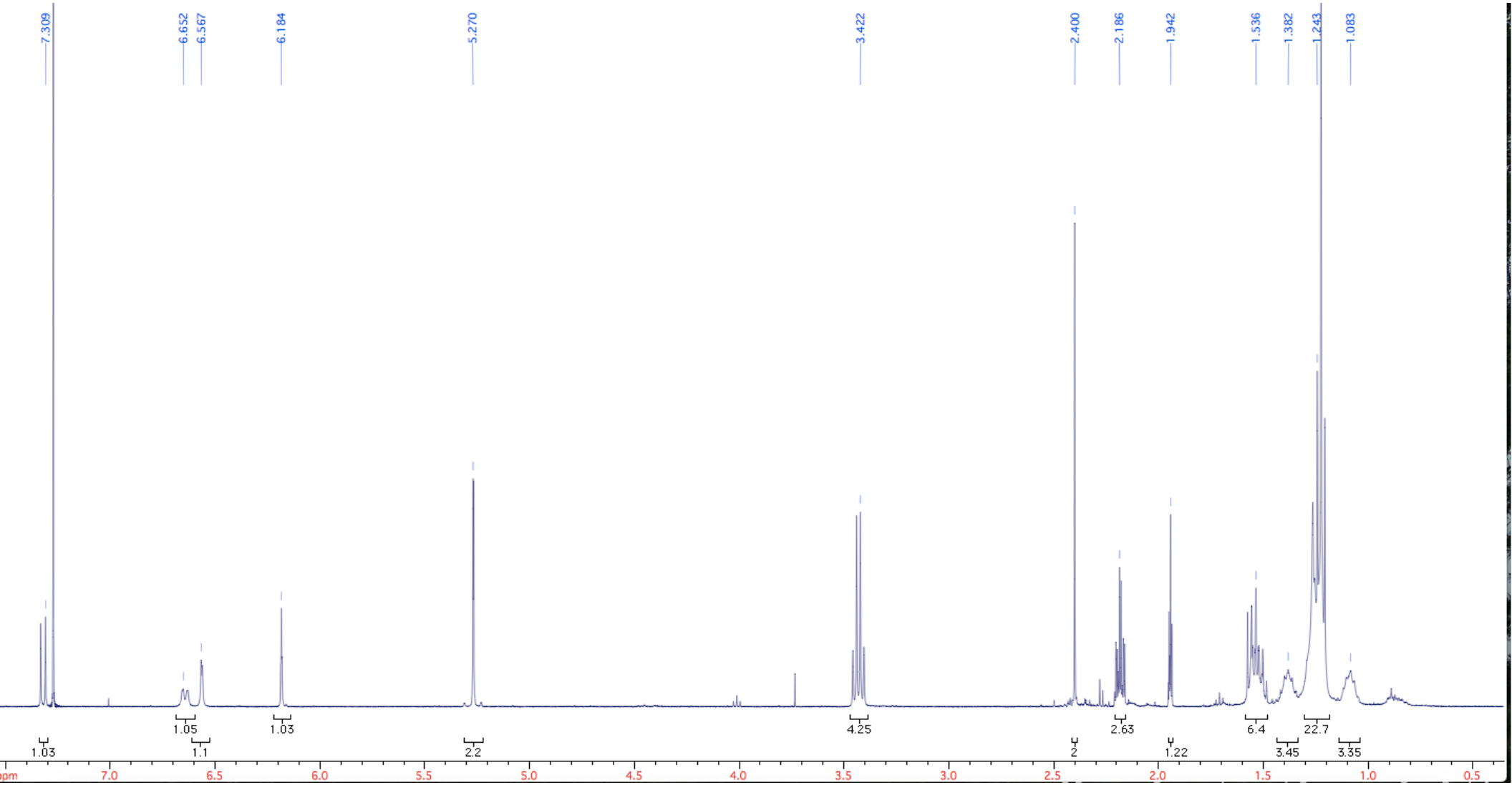



2.271 2.253 2.236 2.130 2.123 2.112 2.105 2.094 2.087 1.879 1.873 1.866 1.463 1.456 1.443 1.425 1.407 1.402 1.389 1.385 1.380 1.378 1.371 1.362 1.360 1.358 1.355 1.353 1.348 1.344 1.338 1.331 1.327 1.320 1.313
2.271 2.253 2.236 2.130 2.123 2.112 2.105 2.094 2.087 1.879 1.873 1.866 1.463 1.456 1.443 1.425 1.407 1.402 1.389 1.385 1.380 1.378 1.371 1.362 1.360 1.358 1.355 1.353 1.348 1.344 1.338 1.331 1.327 1.320 1.313 1.300 1.295 1.280 1.269 1.234 1.213 1.209 1.208 1.190 1.183 1.172 1.153 1.138 1.032 1.013 0.994
2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7 ppm
1.88 2.33 1.00 3.09 8.18 9.35 2.30
Current Data Parameters
NAME 20221018comp8
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20221018
Time 15.12 h
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8196.722 Hz
FIDRES 0.250144 Hz
AQ 3.9976959 sec
RG 101
DW 61.000 usec
DE 13.89 usec
TE 293.6 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324708 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 20.48999977 W
F2 - Processing parameters
SI 65536
SF 400.1300355 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
1.88 2.33 1.00 3.09 8.18 9.35 2.30

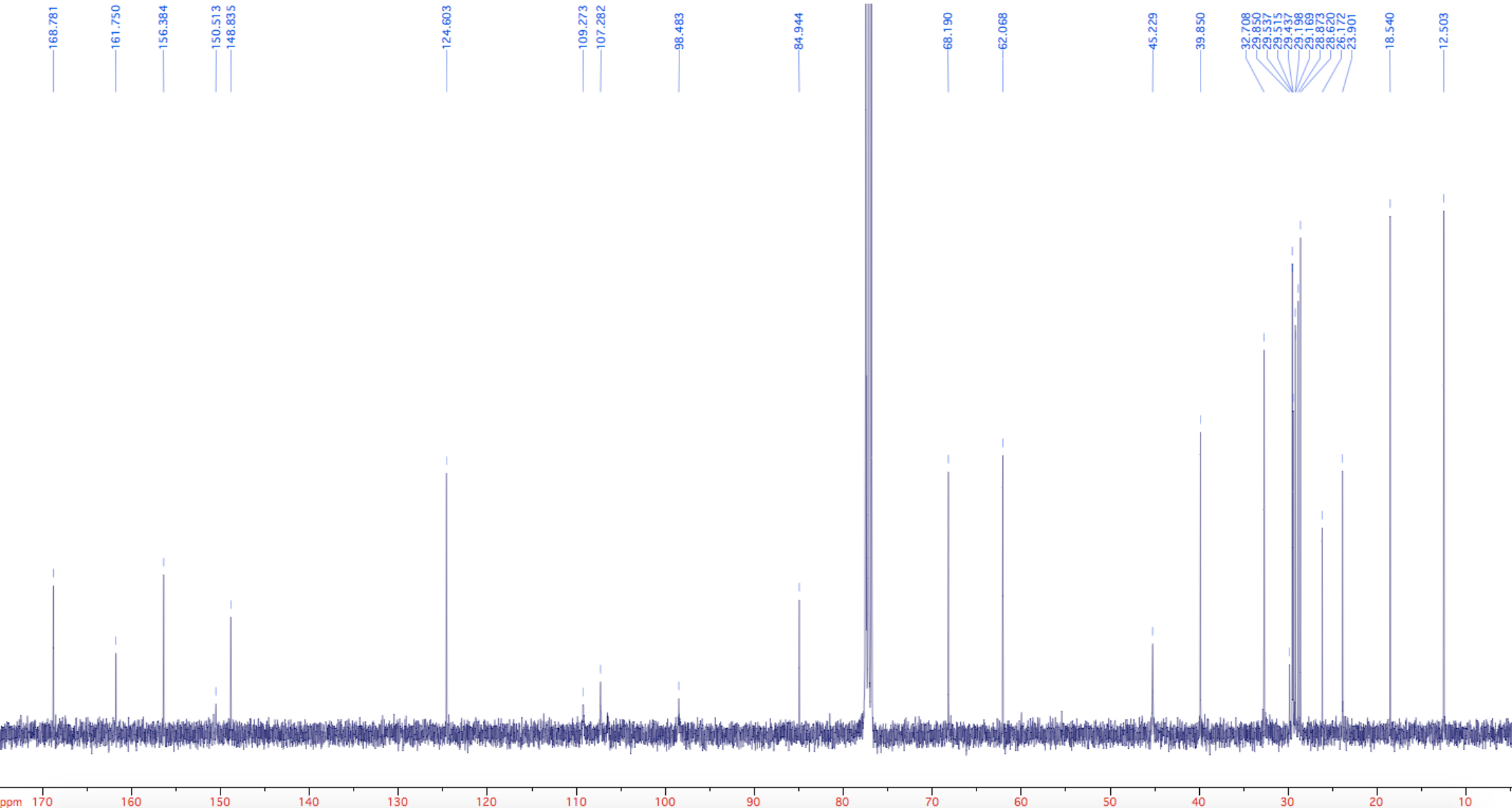
Compound 19

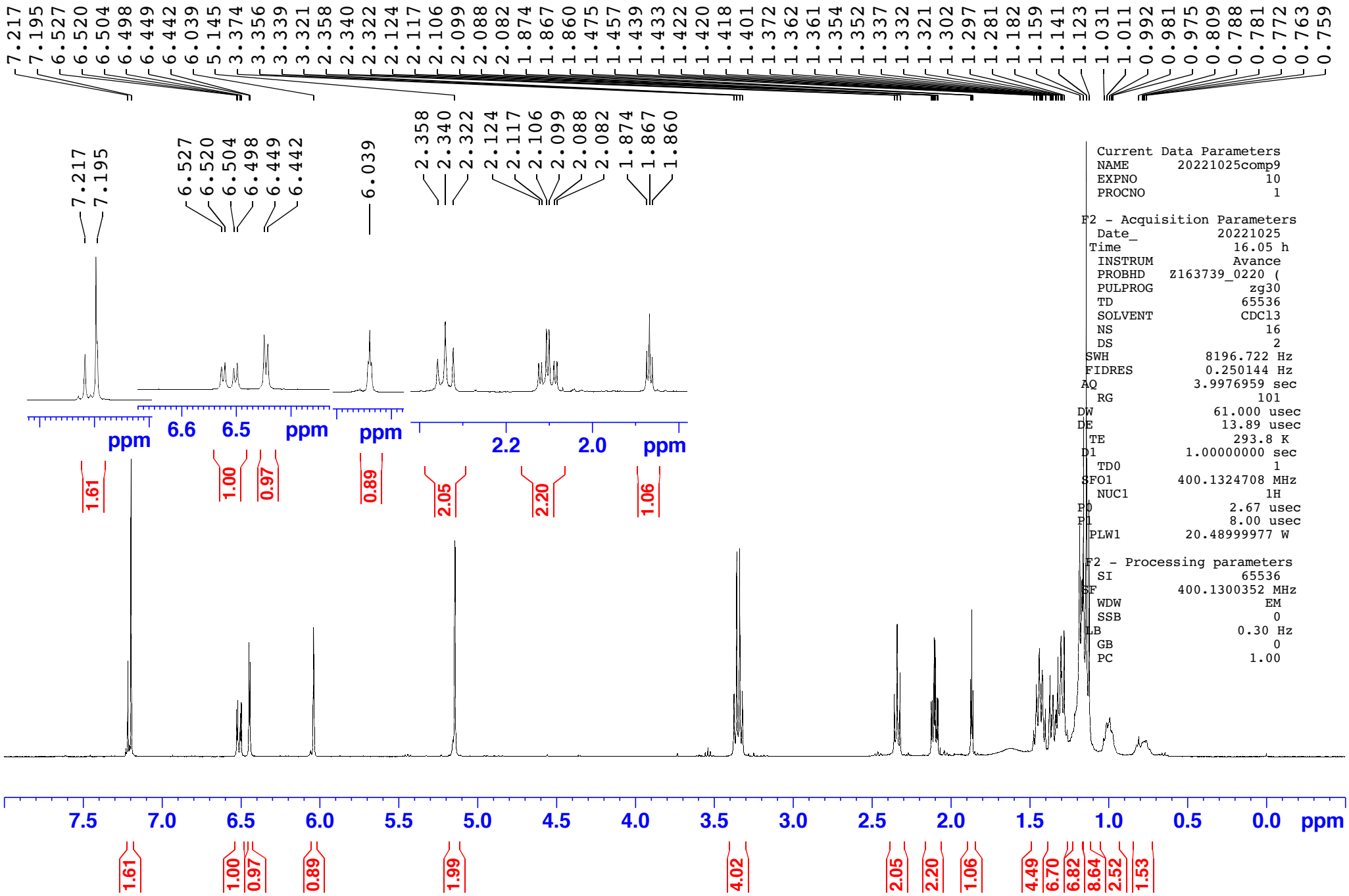

178.31
84.76
68.12
33.11
32.67
32.23
29.72
29.23
29.11
28.92
28.66
28.42
23.79
19.03
18.38
Current Data Parameters
NAME 20221018comp8_1
EXPNO 12
PROCNO 1
F2 - Acquisition Paramet
Date_ 20221018
Time 22.09
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 23809.523
FIDRES 0.726609
AQ 1.3762560
RG 101
DW 21.000
DE 6.50
TE 294.7
D1 2.00000000
D11 0.03000000
TD0 1
SFO1 100.6228298
NUC1 13C
P0 2.58
P1 7.75
PLW1 95.00000000
SFO2 400.1316005
NUC2 1H
CPDPRG[2 waltz65
PCPD2 90.00
PLW2 20.48999977
PLW12 0.16190000
PLW13 0.08143300
F2 - Processing paramete
SI 32768
SF 100.6127685
WDW EM
SSB 0
LB 1.00
GB 0
PC 1.40
200
180
160
140
120
100
80
60
40
20
0
ppm

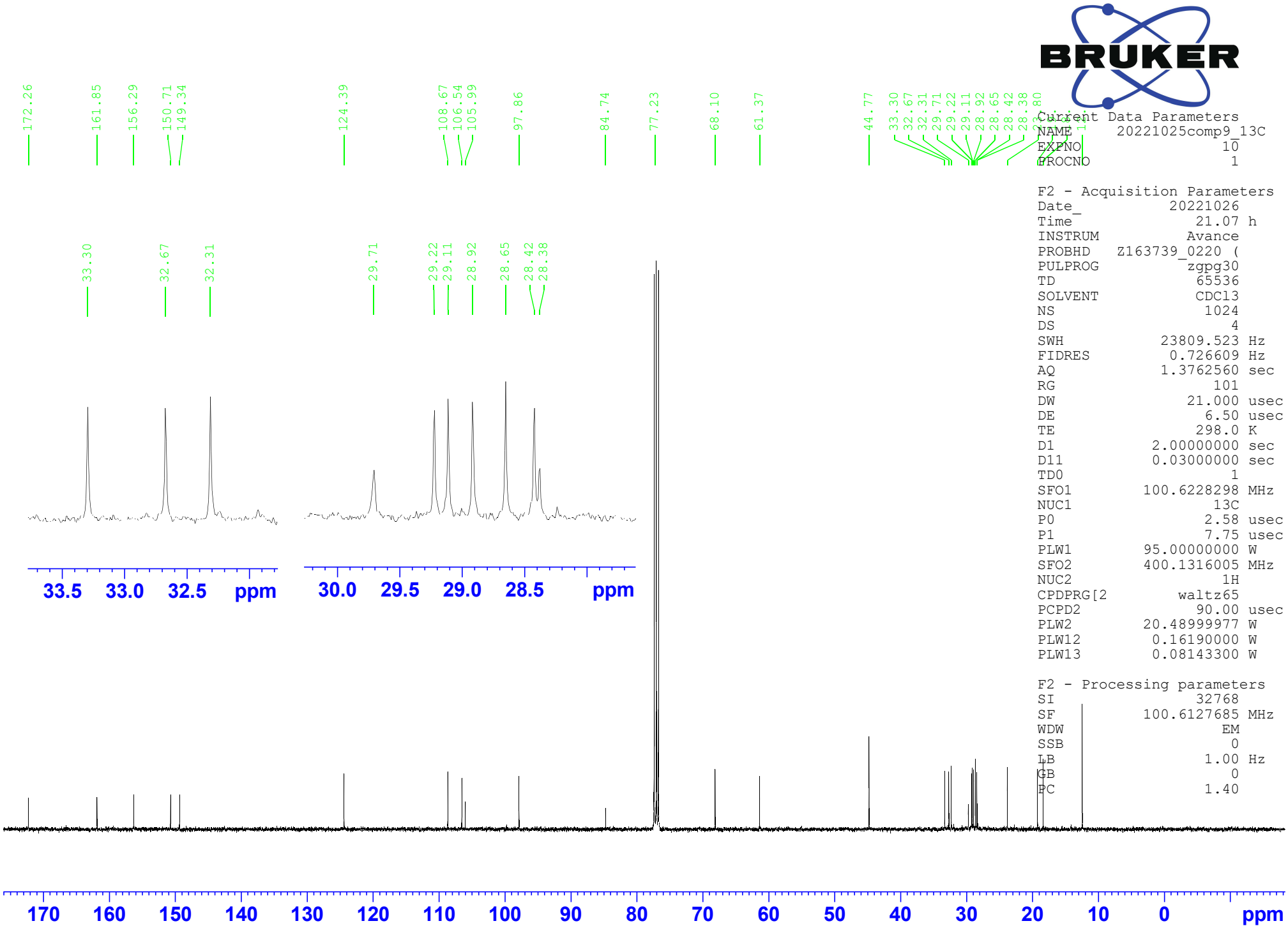
Compound 2, 1-10 FA



Compound 2, 1-10 FA




7.217 7.195 6.527 6.520 6.504 6.498 6.449 6.442 6.039 5.145 3.374 3.356 3.339 3.321 2.358 2.340 2.322 2.124 2.117 2.106 2.099 2.088 2.082 1.874 1.867 1.860 1.475 1.457 1.439 1.433 1.422 1.420 1.418 1.401 1.372 1.362 1.361 1.354 1.352 1.337 1.332 1.321 1.302 1.297 1.281 1.182 1.159 1.141 1.123 1.031 1.011 0.992 0.981 0.975 0.809 0.788 0.781 0.772 0.763 0.759
ppm
1.61 1.00 0.97 0.89 2.05 2.20 1.06
1.61 1.00 0.97 0.89 1.99 4.02 2.05 2.20 1.06 4.49 6.70 6.82 8.64 2.52 1.53
Current Data Parameters
NAME 20221025comp9
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20221025
Time 16.05 h
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8196.722 Hz
FIDRES 0.250144 Hz
AQ 3.9976959 sec
RG 101
DW 61.000 usec
DE 13.89 usec
TE 293.8 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324708 MHz
NUC1 1H
P0 2.67 usec
P1 8.00 usec
PLW1 20.48999977 W
F2 - Processing parameters
SI 65536
SF 400.1300352 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



Current Data Parameters
NAME 20221025comp9_13C
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20221026
Time 21.07 h
INSTRUM Avance
PROBHD Z163739_0220 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 23809.523 Hz
FIDRES 0.726609 Hz
AQ 1.3762560 sec
RG 101
DW 21.000 usec
DE 6.50 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6228298 MHz
NUC1 13C
P0 2.58 usec
P1 7.75 usec
PLW1 95.00000000 W
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz65
PCPD2 90.00 usec
PLW2 20.48999977 W
PLW12 0.16190000 W
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F2 - Processing parameters
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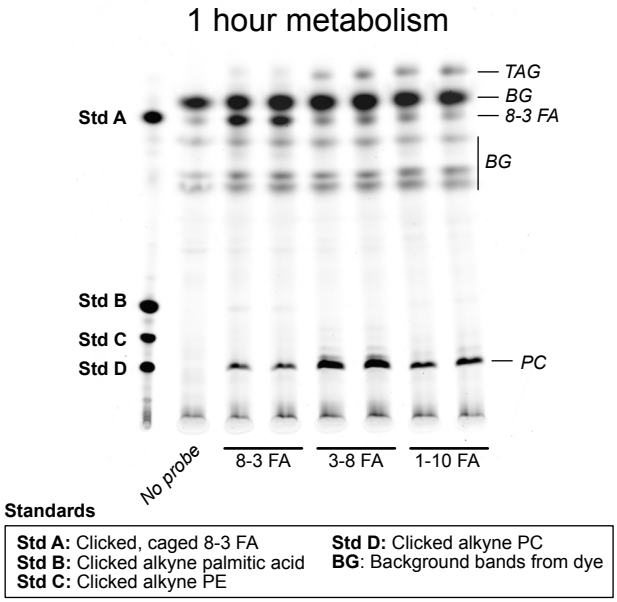
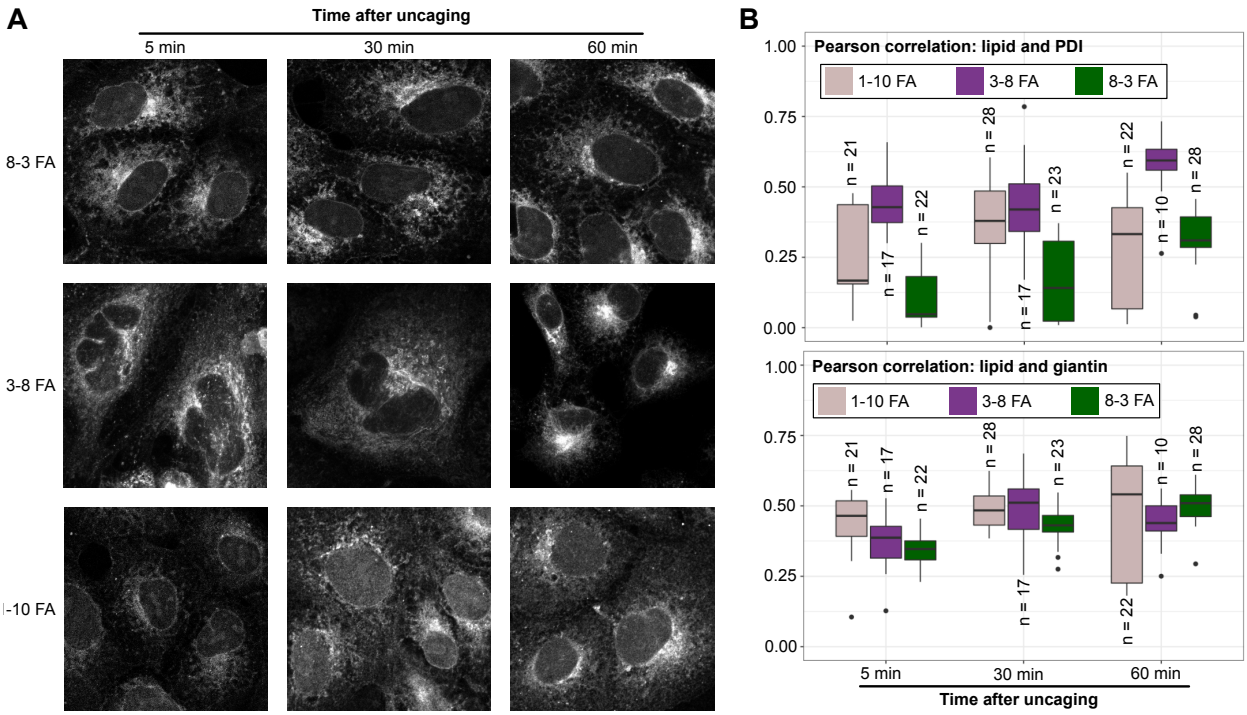
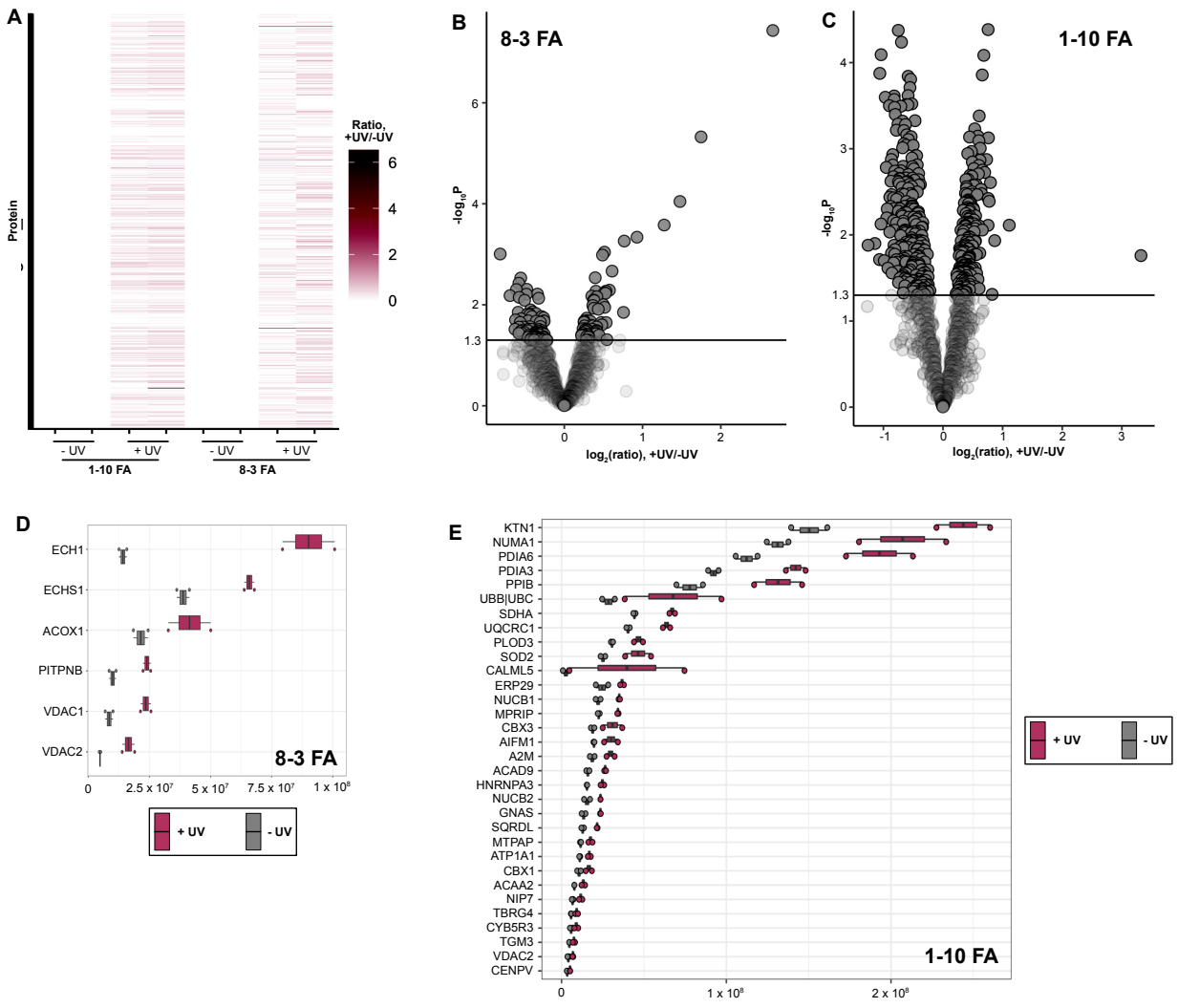
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



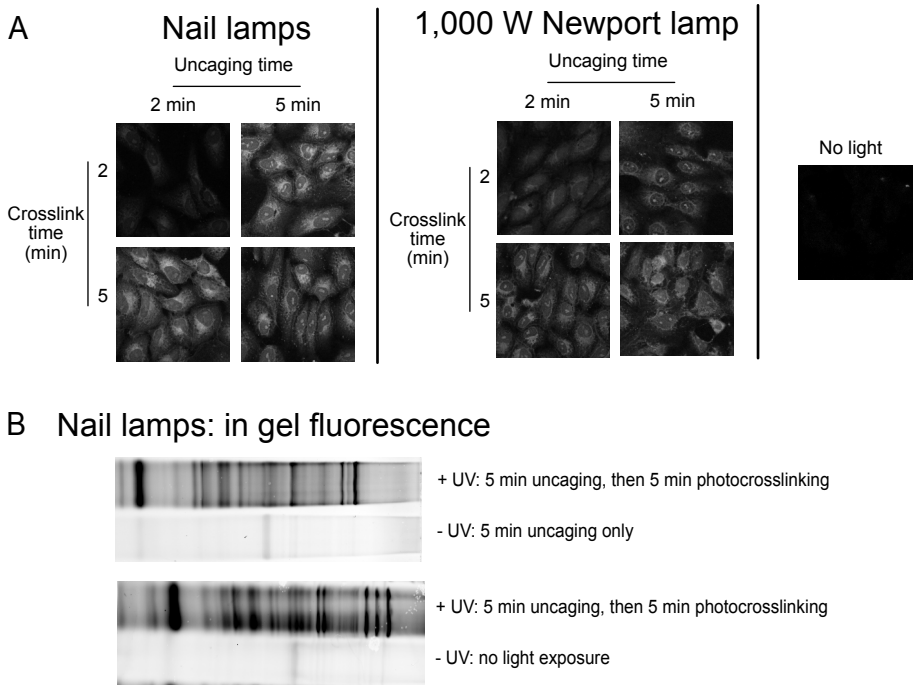
Supplementary Figure 1: Metabolism of trifunctional fatty acids TLC of lipid extracts from Huh7 cells treated with 8-3 FA, 3-8 and 1-10 FA and harvested 1 hour after uncaging. TAG = triacylglyerol; BG = background; PE = phosphatidylethanolamine; PC = phosphatidylcholine.



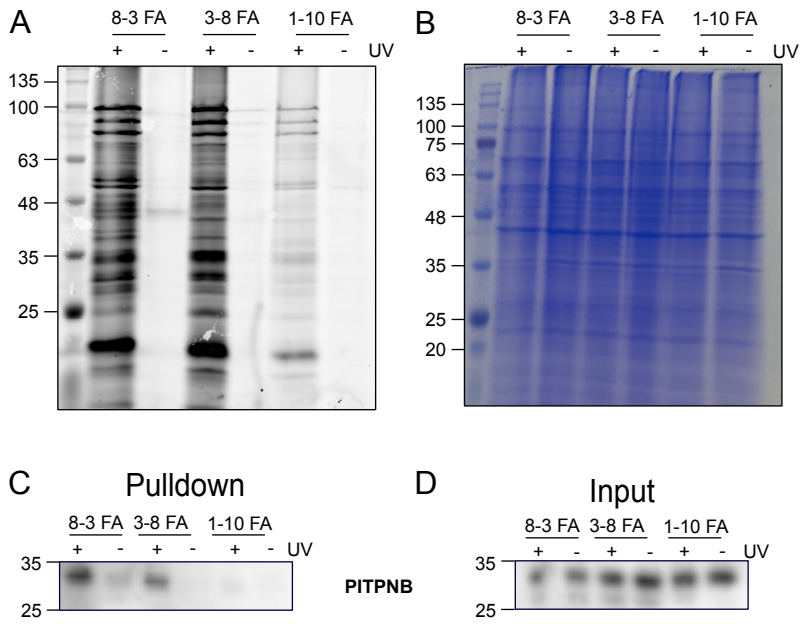
Supplementary Figure 2: Subcellular localization of trifunctional fatty acids. (**A**) Representative images of Huh7 cells treated with 8-3 FA, 3-8 FA, or 1-10 FA, exposed to 400nm light to uncage the probe, and crosslinked with 350nm light 5, 30, or 60 minutes after uncaging, and subjected to click reactions with A647 picolyl azide. Representative images of the far red signal at each timepoint are shown; images are representative of four biological replicates over two independent experiments (**B**) Colocalization of each fatty acid with markers for the ER (PDI) or Golgi (Giantin). Pearson coefficients were calculated for individual cells using a Cellprofiler pipeline; the number of cells used for each condition are indicated by their respective boxplots.



Supplementary Figure 3 Overview of proteomic analysis. Huh7 cells were treated with 8-3 FA or 1-10 FA, uncaged, and photocrosslink 1 hour after uncaging (+ UV), or uncaged without subsequent photocrosslinking (- UV). Cell extracts were lysed by probe sonication and subjected to a click reaction with azide agarose prior to washing to removing nonbound proteins and trypsin digestion. Tryptic digests were desalted and analyzed by LC-MS/MS. (**A**) Heat map of the log-transformed ratio of the intensity of each protein in the +UV sample versus the intensity for that protein in the - UV sample (**B**) Volcano plot of negative log-transformed p-values and fold changes (+UV over -UV) from the Limma analysis of 8-3 FA (**C**) Volcano plot of negative log-transformed p-values and fold changes (+UV over -UV) from the Limma analysis of 1-10 FA (**D)** Normalized intensities for the "hit" and "candidate" proteins in the 8-3 FA-treated samples (**E**) Normalized intensities for the "hit" and "candidate" proteins in the 1-10 FA-treated samples



Supplementary Figure 4 Lamp comparison (**A**) Confocal microscopy of cells treated with 8-3 FA and then uncaged and photocrosslinked for the indicated times with either the nail lamps described in the Supplementary Methods or a 1,000W Mercury-Xenon light source fitted with 400 nm (for uncaging) or 345 nm (for photocrosslinking) high-pass filters. (**B**) In-gel fluorescence of lysates from cells treated with 8-3 FA and then uncaged and photocrosslinked for 5 minutes with the nail lamps; negative controls are either 1) uncaging for five minutes only (no photocrosslinking) or 2) no light exposure, maintained in the dark until cells were scraped for lysis



Supplementary Figure 5 In-gel fluorescence and biotin pulldowns (**A**) In-gel fluorescence of lysates from Huh7 cells treated with the indicated probe, in the presence or absence of UV irradiation (from "Photocrosslinking Lamp"). Shown is fluorescence from an A647 picolyl azide clicked to the lysate. (**B**) Blazin' Blue staining of total protein from the gel in A. (**C**) PITPNB staining of protein-lipid complexes subjected to a click reaction with biotin azide, pulled down with monomeric avidin agarose, and eluted with Laemmli buffer. Image is representative of three independent experiments. (**D**) PITPNB staining of the input lysate that was then subjected to the pulldown in (C)