Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis of Acylguanidine Zanamivir Derivatives as Neuraminidase Inhibitors and Evaluation of Their Bio-activities

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Materials.

Dichloromethane (DCM, Merck), acetonitrile (CH_3CN, Merck), tetrahydrofuran (THF, Merck), ethanol (EtOH, Merck), trifluoroacetic acid (TFA, Acros), sodium hydride (NaH, Acros), triethyl amine (NEt_3, Merck), diisopropylamine (DiPEA, Merck), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbonate (EDC, Merck), di-tert-butyl-dicarbonate (Boc_2O, Acros), potassium carbonate (K_2CO_3, Merck), *N*-hydroxysuccinimide (NHS, Aldrich), and tetrabutylammonium thiocyanate ($(n\text{-}Bu)_4NSCN$, TCI) were used as received. All of the commercially available reagents were used without further purification. Anhydrous solvents were purchased from Merck and were used as received. Analytical thin-layer chromatography (TLC) and reverse-phase TLC were performed using pre-coated plates (Silica Gel 60 F254 and 60 RP-18F254S, respectively, Merck). Silica gel 60 and C-18 reverse-phase gel (Merck) were used for flash chromatography.

General Measurements.

^1H and ^{13}C NMR spectra were recorded using a Bruker AV-400 spectrometer or a Varian MR-400 MHz spectrometer. The proton chemical shifts are reported in parts per million (ppm) relative to the methyl quintuplet at 3.31 ppm for the residual CD_3OD in methanol-d^4. The carbon chemical shifts are reported in parts per million relative to the internal ^{13}C signals in CD_3OD-d^4 (49.00 ppm). The purity of the final products was determined using a Grace Vydac analytic HPLC column with solvent A (ddH_2O+0.1% TFA) and solvent B (MeCN) as the eluents. Mass spectra were obtained using an FAB JMS-700 double-focusing mass spectrometer (JEOL, Tokyo, Japan) and an ESI Finnigan LCQ mass spectrometer (Thermo Finnigan, San Jose, CA, USA) in negative or positive mode.

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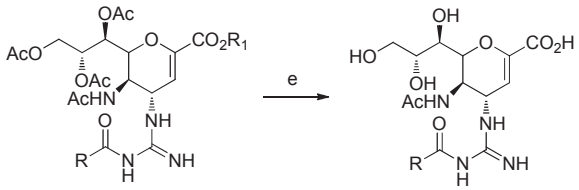


	R1	Condition	Temperature&Time	Yield
Entry 1	Me	1. NaOMe / MeOH 2. NaOH / H_2O	r.t.,	<40%
Entry 2	Me	LiOH / H_2O / MeOH	r.t., 2hr	20~50%
Entry 3	Me	LiOH / H_2O / MeOH	r.t., 12hr	30~60%
Entry 4	Me	NaOH / H_2O / MeOH	0°C, 20min	35~60%
Entry 5	Me	LiI / Pyr.	reflux, 12hr	N.R.[a]
Entry 6	Et	NaOH / H_2O / MeOH	r.t., 2hr	<60%
Entry 7	Et	LiOH / H_2O / MeOH	0°C, 20min	<70%
Entry 8	Et	K_2CO_3 / EtOH	r.t., 2hr	70~90%

[a] means that no reaction

General □r□e□ure □□r t□e s□nt□esis □□□□m□□un□s □a□□a□.

Compound □[1] was synthesized using a modification of the procedures reported in the literature. A solution of 1H-pyrazole-1-carboxamidine □ (2.0 g, 13.62 mmol) and Boc_2O (4.30 g, 27.24 mmol) in anhydrous DMF (30 ml) and DCM (30 ml) was added to Et_3N (3.77 ml, 13.62 mmol) at 4 °C.[2] The resulting solution was stirred at room temperature under N_2 for 8 h. The solvent was evaporated after the completion of the reaction, as monitored by TLC, and the product was puri□ed by column chromatography (hexane□AcOEt 4□1, R_f □ 0.25) to give 1H-pyrazole-N-Boc-1-carboxamidine (3.06 g, 91%). NaH (48 mg, 1.21 mmol) was added to a solution of the above pure product (200 mg, 0.81 mmol) in anhydrous THF (8 ml, 0.1 M) at 4 °C, and the resulting solution was stirred at 4 □C for 0.5 h, followed

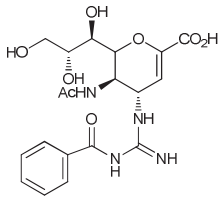
by the addition of the activated acid (0.97 mmol). The reaction was stirred at 4 °C for 4 h, quenched by adding MeOH and then concentrated. The desired isomers were purified by column chromatography (hexane–AcOEt 4–1, R_f = 0.10–0.25) to give isomer α (yield 40%–60%). Et_3N (0.035 mL, 0.25 mmol) was added to a stirred solution of isomer α (0.25 mmol) and compound β (100 mg, 0.23 mmol) in DCM (3 mL), and the resulting solution was stirred at room temperature for 6 h. Then, 10% hydrochloride solution was added, and the mixture was extracted with EA. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography using silica gel (hexane–AcOEt 1–1, R_f = 0.10–0.25) to give isomer γ (yield 75%–87%). A solution of TFA (1 mL) in 3 mL of DCM was added to isomer γ (0.2 mmol) at 0 °C. The reaction was stirred at room temperature for 2 h. After the removal of the solvent, the product was purified using silica gel chromatography (MeOH–DCM 1–9, R_f = 0.15–0.35) to give compound δ (yield 95%) as a white syrup. To a mixture of compound δ (0.1 mmol) in EtOH (0.01 M) at 0 °C was added dropwise an aqueous solution of potassium carbonate (0.04 M). The solution was stirred for 30 min in an ice bath. The mixture was then neutralized with IR120 and concentrated under reduced pressure. The residue was purified by column chromatography (H_2O (with 0.1% TFA)–MeCN (7–3), R_f 0.21–0.60) using C-18 reverse-phase gel to give compound ε (yield 70%–90%).

General procedure for the synthesis of mannosamine analogs.

Compounds ζ[3] and η[4] were synthesized using a modification of the reported procedures. To a solution of compound ζ (0.51 mmol) in DMF (5 mL) was added EDC (176 mg, 0.92 mmol), DIPEA (0.16 mL, 0.92 mmol), and amine compound θ (200 mg, 0.46 mmol). The mixture was stirred at room temperature overnight. After the DMF was removed, the mixture was purified by silica gel chromatography

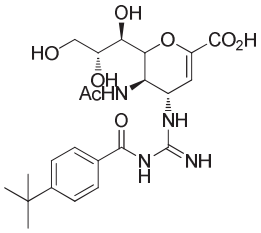
(hexane–AcOEt 4–1, R_f = 0.15–0.20) to give compound ‡‡ (yield 65%–83%) as a pale yellow syrup. The Pbf-protecting group in compound ‡‡ (0.2 mmol) was removed by treatment with 4 mL of TFA–DCM (1–3) for 2 h. After the removal of the solvent, the product was purified using silica gel chromatography (MeOH–DCM 1–9, R_f = 0.15–0.35) to give compound ‡‡ (yield 70%–83%) as a white syrup. An aqueous solution of potassium carbonate was added dropwise to a solution of compound ‡‡ (0.1 mmol) in EtOH (0.001 M) at 0 °C, and the resulting mixture was stirred for 30 min in an ice bath. The mixture was neutralized with IR120 and dried under vacuum. The residue was purified by column chromatography (H_2O (with 0.1% TFA)–MeCN 7–3, R_f = 0.49–0.65) using C-18 reverse-phase gel to give compound ‡‡ (yield 73%–88%).

Assignments for compound ‡‡



4-Guanidino-5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enonic acid (‡‡)

R_f = 0.48 (EA–MeOH–H_2O, 3–2–1). ^{1}H NMR (400 MHz, CD_3OD) δ 2.02 (s, 3H), 3.67-3.74 (m, 2H), 3.80 (dd, *J* = 3.2, 11.2 Hz, 1H), 3.83-3.88 (m, 1H), 4.39 (dd, *J* = 7.2, 8.8 Hz, 1H), 4.52 (dd, *J* = 3.6, 8.8 Hz, 1H), 4.74 (dd, *J* = 2.4, 7.6 Hz, 1H), 5.90 (d, *J* = 2.4 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 22.63, 30.67, 51.57, 64.69, 70.49, 71.62, 77.39, 104.09, 129.36, 130.05, 132.82, 135.16, 149.75, 156.24, 163.23, 169.97, 174.55. HRMS (ESI-TOF) calcd for $C_{19}H_{25}N_4O_8$ [M + H]$^+$ = 437.1672 , found = 437.1675.



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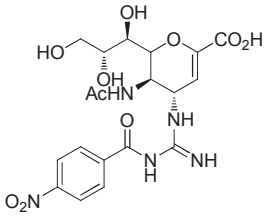
R_f □0.53 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.35 (s, 9H), 2.02 (s, 3H), 3.67-3.74 (m, 2H), 3.80-3.90 (m, 2H), 4.38 (dd, *J* □7.6, 8.0 Hz, 1H), 4.52 (dd, *J* □3.6, 8.4 Hz, 1H), 4.69-4.71 (br, 1H), δ 5.94 (d, *J* □2.8 Hz, 1H), 7.61 (d, *J* □8.4 Hz, 2H), 7.93 (d, *J* □8.4 Hz, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.63, 31.36, 36.10, 51.34, 64.68, 70.60, 71.53, 77.43, 105.50, 127.11, 129.32, 129.59, 148.31, 156.15, 159.43, 165.98, 169.58, 174.58□HRMS (ESI-TOF) calcd for $C_{30}H_{41}N_4O_{11}$ □M + H$^+$□□ 493.2298 , found □493.2312.



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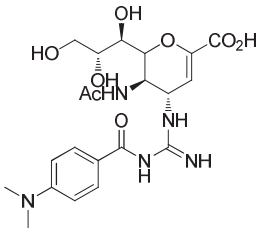
R_f □0.43 (EA□MeOH□H_2O 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 2.02 (s, 3H), 3.69 (dd, *J* □5.2, 11.6 Hz, 1H), 3.72-3.74 (m, 1H), 3.81 (dd, *J* □ 3.2, 11.6 Hz, 1H), 3.85-3.88 (m, 1H), 4.35-4.38 (br, 1H), 4.53-4.55 (m, 1H), 4.67-4.69 (br, 1H), 5.90 (d, *J* □3.2 Hz, 1H), 8.20 (d, *J* □8.0 Hz, 2H), 8.40 (d, *J* □8.0 Hz, 1H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.74, 30.70, 51.57, 64.77, 70.05, 71.62, 77.62, 107.79, 124.01, 131.13, 145.83, 150.90, 163.18, 168.69, 174.34, 176.13□HRMS (FAB) calcd for $C_{19}H_{24}N_5O_{10}$ □M + H$^+$□□482.1523 , found □482.1529.



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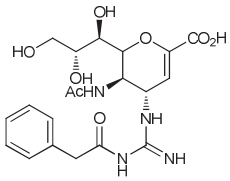
R_f □0.43 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.94 (s, 3H), 3.07 (s, 6H), 3.63-3.70 (m, 2H), 3.81 (dd, *J* □ 2.4, 11.6 Hz, 1H), 3.90-3.94 (m, 1H), 4.33-4.35 (m, 2H), 5.02 (br, 1H), 5.92 (d, *J* □2.0 Hz, 1H), 6.89 (d, *J* □ 8.8 Hz, 2H), 7.76 (d, *J* □ 8.8 Hz, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.62, 40.24, 62.83, 64.83, 67.30, 69.95, 71.23, 78.41, 111.83, 112.16, 121.56, 129.95, 146.09, 154.33, 165.52, 170.79, 174.60□HRMS (FAB) calcd for $C_{19}H_{24}N_5O_{10}$ □M + H$^+$□□482.1523, found□ 482.1529.



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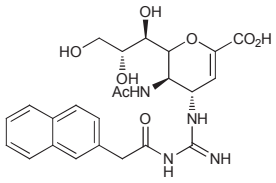
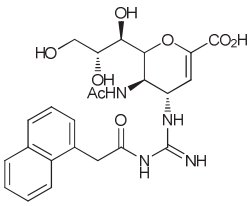
(□R_f □ 0.47 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.98 (s, 3H), 3.68-3.72 (m , 2H), 3.79 (s, 2H), 3.80 (dd, *J* □3.2, 11.2 Hz, 1H), 3.85-3.89 (m , 1H), 4.39 (dd, *J* □8.4, 8.8 Hz, 1H), 4.47 (dd, *J* □3.6, 8.8 Hz, 1H), 4.74 (dd, *J* □2.4, 8.4 Hz, 1H), 5.90 (d, *J* □2.4 Hz, 1H), 7.25-7.28 (m, 1H), 7.29-7.31 (m, 4H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.59, 44.34, 51.15, 64.48, 70.18, 71.53, 77.62, 106.66, 119.44, 128.49, 129.68, 130.55, 134.15, 147.29, 155.72, 165.36, 174.51, 175.27□HRMS (MALDI) calcd for $C_{20}H_{26}N_4O_8$ □M + H$^+$□□451.1751, found□451.1739.



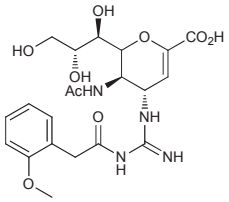
□□□ □etami□□□□□□an □□□r □□□□□*N*□□□□na□□t□alen□□□□l□a□et□l□□uani□in□□□□□□□□□tri□e □□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□

R_f □ 0.55 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.98 (s, 3H), 3.65-3.70 (m, 2H), 3.80 (dd, *J* □3.2, 11.2 Hz, 1H), 3.81-3.86 (m, 1H), 3.97 (s, 2H), 4.28 (dd, *J* □8.4, 8.8 Hz, 1H), 4.49 (dd, *J* □3.2, 8.8 Hz, 1H), 4.74 (dd, *J* □2.8, 8.0 Hz, 1H), 5.90 (d, *J* □2.8 Hz, 1H), 7.43 (dd, *J* □1.2, 8.4 Hz, 1H), 7.45-7.50 (m, 2H), 7.80 (s, 1H), 7.82-7.84 (m, 3H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.46, 44.61, 50.82, 64.70, 70.98, 71.35, 77.25, 106.03, 127.16, 127.38, 128.29, 128.65, 128.69, 129.42, 129.54, 131.52, 134.14, 134.92, 147.43, 155.50, 164.99, 174.47, 174.82□HRMS (MALDI) calcd for $C_{20}H_{26}N_4O_8$ □M + H$^+$□□451.1751, found□451.1739.



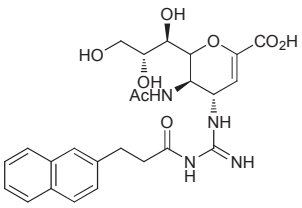
□□□ □etami□□□□□□an □□□r □□□□□*N*□□□□na□□t□alen□□□□l□a□et□l□□uani□in□□□□□□□□□tri□e □□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□

R_f □ 0.55 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.97 (s, 3H), 3.65-3.69 (m, 2H), 3.79 (dd, *J* □3.2, 11.2 Hz, 1H), 3.81-3.86 (m, 1H), 3.97 (s, 2H), 4.28 (dd, *J* □7.6, 8.0 Hz, 1H), 4.29 (s, 2H), 4.49 (dd, *J* □0.8, 7.6 Hz, 1H), 4.77 (dd, *J* □2.8, 6.8 Hz, 1H), 5.90 (d, *J* □2.8 Hz, 1H), 7.44-7.49 (m, 2H), 7.51-7.57 (m, 2H), 7.86 (dd, *J* □2.8, 6.8 Hz, 1H), 7.90 (d, *J* □7.6 Hz, 1H), 7.98 (d, *J* □8.0 Hz, 1H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.49, 42.18, 50.82, 64.71, 70.92, 71.35, 77.31, 106.20, 124.72, 126.57, 127.04, 127.65, 129.69, 129.71, 129.83, 130.29, 133.50, 135.40, 147.30, 155.46, 164.88, 174.47, 174.78□HRMS (MALDI) calcd for $C_{20}H_{26}N_4O_8$ □M + H$^+$□□451.1751, found□451.1743.



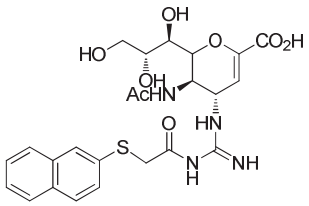
□□□ □etami□□□□□□an□□□r□□□□*N*□□□□□□met□□□□□□en□l□a□et□l□uani□in□□□□□□□□tri□e□□□□□ □□l□□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□□□

R_f □0.42 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 2.00 (s, 3H), 3.66-3.73 (m, 2H), 3.76 (s, 2H), 3.78-3.88 (m, 2H), 3.82 (s, 3H), 4.30 (dd, *J* □8.4, 8.8 Hz, 1H), 4.48 (dd, *J* □0.8, 8.4 Hz, 1H), 4.74 (dd, *J* □2.0, 7.2 Hz, 1H), 5.90 (d, *J* □2.8 Hz, 1H), 6.93 (dd, *J* □7.2, 7.6 Hz, 1H), 6.98 (d, *J* □8.4 Hz, 1H), 7.20 (d, *J* □7.6 Hz, 1H), 7.30 (dd, *J* □7.2, 8.4 Hz, 1H) □^{13}C NMR (100 MHz, CD_3OD) δ 22.61, 39.67, 51.36, 55.99, 61.54, 64.10, 69.64, 72.09, 77.52, 105.55, 111.68, 121.67, 122.75, 130.31, 132.42, 155.69, 158.95, 162.97, 173.12, 174.31, 175.2760 □HRMS (MALDI) calcd for $C_{21}H_{28}N_4O_9$ □M + H$^+$□ □480.1856 , found □480.1850



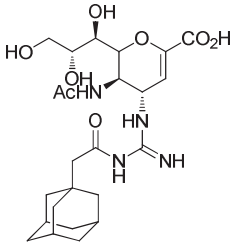
□□□ □etami□□□□□□an□□□r□□□□*N*□na□□t□alen□□□□l□□r□□an□□l□uani□in□□□□□□□□tri□e□□□□□ □□l□□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□i□

R_f □0.53 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.97 (s, 3H), 1.97 (br, 2H), 3.15 (t, *J* □7.6 Hz, 1H), 3.66-3.70 (m, 2H), 3.80 (dd, *J* □3.2, 11.2 Hz, 1H), 3.81-3.85 (m, 1H), 4.28 (br, 1H), 4.49 (dd, *J* □0.8, 8.0 Hz, 1H), 4.52 (br, 1H), 5.87 (d, *J* □3.2 Hz, 1H), 7.37 (m, 3H), 7.70 (s, 1H), 7.78 (m, 3H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.48, 31.26, 39.54, 39.65, 50.77, 64.72, 71.15, 71.34, 77.19, 106.02, 126.57, 127.15, 127.68, 127.92, 128.50, 128.61, 129.25, 133.75, 135.09, 138.79, 147.43, 155.28, 164.99, 174.48, 176.07 HRMS (ESI-TOF) calcd for $C_{25}H_{30}N_4O_8$ □M + H$^+$□ □514.2064 , found □514.2060.



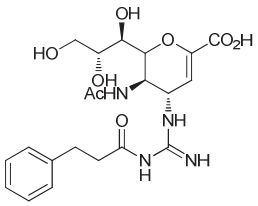
□□□ □etami□□□□□□an□□□r□□□□*N*□□□□na□□t□alen□□□□lt□i□a□et□l□uani□in□□□□□□□□□t ri□e□□□□□ □□l□□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□□□

R_f □ 0.51 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.92 (s, 3H), 3.65-3.69 (m, 2H), 3.80 (dd, *J* □ 2.8, 11.2 Hz, 1H), 3.80-3.85 (m, 1H), 3.96 (s, 2H), 4.28 (br, 1H), 4.47 (dd, *J* □ 0.8, 8.4 Hz, 1H), 4.57 (br, 1H), 5.87 (d, *J* □ 2.4 Hz, 1H), 7.45-7.51 (m, 3H), 7.79-7.83 (m, 3H), 7.92 (s, 1H) □^{13}C NMR (100 MHz, CD_3OD) δ 22.47, 39.70, 50.95, 64.71, 70.80, 71.33, 77.33, 106.23, 127.55, 127.90, 128.42, 128.75, 129.97, 132.38, 133.84, 135.12, 147.28, 155.34, 164.89, 172.78, 174.46 HRMS (ESI-TOF) calcd for $C_{24}H_{28}N_4O_8S$ □M + H□$^+$ □533.1706 , found □533.1713.



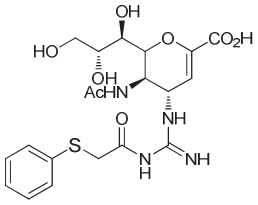
□□□ □etami□□□□□□an□□□r□□□□*N*□□□□a□amantan□□□□l□a□et□l□uani□in□□□□□□□□□tri□e □□□□□ □□l□□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□□□

R_f □ 0.57 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.68 (m, 10H), 1.75 (m, 3H), 1.97 (m, 2H), 2.00 (s, 3H), 2.19 (s, 2H), 3.68-3.72 (m, 2H), 3.80 (dd, *J* □ 3.2, 11.2 Hz, 1H), 3.85-3.89 (m, 1H), 4.34 (dd, *J* □ 8.4, 8.8 Hz, 1H), 4.49 (dd, *J* □ 3.6, 8.8 Hz, 1H), 4.62 (dd, *J* □ 2.4, 8.4 Hz, 1H), 5.92 (d, *J* □ 2.4 Hz, 1H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.56, 30.06, 34.68, 37.67, 43.27, 50.96, 52.11, 64.69, 70.65, 71.38, 77.47, 106.55, 147.26, 155.36, 165.03, 174.40, 175.13 □HRMS (ESI-TOF) calcd for $C_{20}H_{26}N_4O_8$ □M + H□$^+$ □509.2611 , found □509.2615



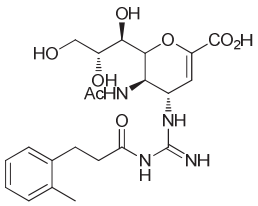
□□□ □etami□□□□□□□an□□□r□□□□□*N*□□□□□□□en□l□r□□an□□l□uani□in□□□□□□□□□tri□e□□□□□ □ □l□□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□l□

R_f □ 0.49 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.99 (s, 3H), 2.80 (t, *J* □ 8.0 Hz, 2H), 2.95 (t, *J* □ 8.0 Hz, 2H), 3.67-3.70 (m, 2H), 3.80 (dd, *J* □ 2.8 , 11.6 Hz, 1H), 3.83-3.88 (m, 1H), 4.29 (dd, *J* □ 7.2, 8.4 Hz, 1H), 4.45 (dd, *J* □ 1.2, 8.4 Hz, 1H), 4.56 (dd, *J* □ 2.8, 7.2 Hz, 1H), 5.90 (d, *J* □ 2.8 Hz, 1H), 7.16-7.29 (m, 5H) □^{13}C NMR (100 MHz, CD_3OD) δ 22.57, 31.23, 39.65, 50.91, 64.73, 70.92, 71.46, 77.39, 106.04, 127.48, 129.40, 129.60, 132.82, 141.30, 147.60, 155.38, 165.20, 174.49, 176.18 HRMS (MALDI) calcd for $C_{21}H_{28}N_4O_8$ □M + H□$^+$ □ □465.1907 , found □ 465.1925.



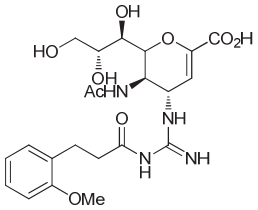
□□□ □etami□□□□□□□an□□□r□□□□□*N*□□□□□□□en□lt□i□a□et□l□□uani□in□□□□□□□□□tri□e□□□□□ □ □□l□□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□m□

R_f □ 0.45 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.98 (s, 3H), 3.67-3.70 (m, 2H), 3.81 (dd, *J* □ 2.8, 11.6 Hz, 1H), 3.83-3.88 (m, 3H), 4.28 (dd, *J* □ 7.2, 8.4 Hz, 1H), 4.49 (dd, *J* □ 0.8, 8.0 Hz, 1H), 4.56 (dd, *J* □ 2.4, 7.2 Hz, 1H), 5.89 (d, *J* □ 3.2 Hz, 1H), 7.28 (dd, *J* □ 1.2, 7.2 Hz, 1H), 7.34 (dd, *J* □ 1.2, 7.2 Hz, 2H), 7.45 (d, *J* □ 7.2 Hz, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.29, 39.71, 39.65, 50.66, 64.50, 70.77, 71.10, 77.06, 105.89, 128.50, 130.419, 131.34, 134.79, 147.13, 155.10, 164.66, 172.49, 174.26 HRMS (ESI-TOF) calcd for $C_{20}H_{26}N_4O_8S$ □M + H□$^+$ □483.1550 , found □483.1552.



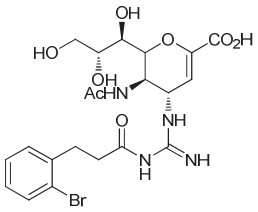
□□□ □etami□□□□□□□an □□□r□□□□□*N*□□□□□□□t□l□l□r□an□l□uani□in□□□□□□□□□tri□e□□□□ □□l□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□n□

R_f □0.52 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 2.00 (s, 3H), 2.33 (s, 3H), 2.80 (br, 2H), 2.98 (t, *J* □8.0 Hz, 2H), 3.67-3.70 (m, 2H), 3.80 (dd, *J* □3.2 , 11.2 Hz, 1H), 3.83-3.88 (m, 1H), 4.29 (br, 1H), 4.50 (dd, *J* □1.2, 8.0 Hz, 1H), 4.55 (dd, *J* □2.8, 7.2 Hz, 1H), 5.90 (d, *J* □3.2 Hz, 1H), 7.16-7.29 (m, 4H) □^{13}C NMR (100 MHz, CD_3OD) δ 19.28, 22.52, 28.41, 30.85, 38.36, 50.91, 64.68, 70.92, 71.37, 77.20, 105.75, 127.21, 127.67, 129.40, 129.69, 131.37, 137.09, 139.24, 147.75, 155.33, 165.38, 174.47, 176.23 HRMS (ESI-TOF) calcd for $C_{22}H_{30}N_4O_8$ □M + H$^+$□ □478.2064 , found □478.2055.



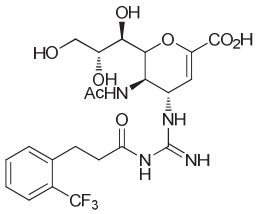
□□□ □etami□□□□□□□an □□□r□□□□□*N*□□□□□□□met□□□□□□en□l□□r□□an□□l□uani□in□□□□□□□□ □tri□e□□□□□ □□l□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□□□□

R_f □0.48 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) 1.99 (s, 3H), 2.77 (t, *J* □8.0 Hz, 2H), 2.95 (t, *J* □8.0 Hz, 2H), 3.67-3.70 (m, 2H), 3.77-3.83 (m, 6H), 4.25 (dd, *J* □7.2, 8.4 Hz, 1H), 4.40 (dd, *J* □1.2, 8.4 Hz, 1H), 4.52 (dd, *J* □2.8, 7.2 Hz, 1H), 5.75 (d, *J* □2.8 Hz, 1H), 6.80 (dd, *J* □7.6, 8.0 Hz, 1H), 6.90 (d, *J* □8.0 Hz, 1H), 7.10 (d, *J* □7.6 Hz, 1H), 7.18 (dd, *J* □7.6, 8.0 Hz, 1H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.63, 26.46, 38.07, 54.57, 55.74, 64.57, 70.40, 71.65, 77.32, 104.68, 111.47, 121.55, 129.05, 129.12, 131.05, 155.53, 158.86, 174.43, 176.81 HRMS (ESI-TOF) calcd for $C_{21}H_{28}N_4O_8$ □M + H$^+$□ □495.2091 , found □495.2094.



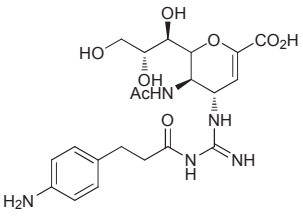
□□□ □etami□□□□□□an□□□r□□□□N□□□□□□□□r□m□□□en□l□□r□□an□□l□□uani□in□□□□□□□□t ri□e□□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□

R_f □0.45 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 2.00 (s, 3H), 2.81 (t, *J* □7.6 Hz, 2H), 3.11 (t, *J* □7.6 Hz, 2H), 3.65-3.70 (m, 2H), 3.80 (dd, *J* □2.8, 11.6 Hz, 1H), 3.83-3.88 (m, 1H), 4.28 (dd, *J* □7.2, 8.4 Hz, 1H), 4.50 (dd, *J* □1.2, 8.4 Hz, 1H), 4.60 (dd, *J* □2.8, 7.2 Hz, 1H), 5.90 (d, *J* □2.8 Hz, 1H), 7.13 (dt, *J* □2.0, 8.0 Hz, 1H), 7.28 (dt, *J* □2.0, 8.0 Hz, 1H), 7.35 (d, *J* □8.0 Hz, 1H), 7.55 (d, *J* □8.0 Hz, 1H)□ 13C NMR (100 MHz, CD3OD) δ 22.52, 31.41, 37.75, 50.78, 64.72, 71.06, 71.35, 77.23, 106.11, 125.11, 128.99, 129.60, 131.86, 134.00, 140.45, 147.41, 155.30, 164.98, 174.49, 175.64 HRMS (ESI-TOF) calcd for $C_{21}H_{28}N_4O_8$ □M + H□$^+$ □543.1091 , found □543.1082.



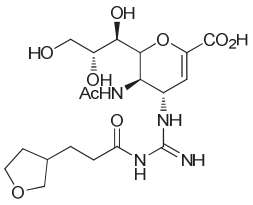
□□□ □etami□□□□□□an□□□r□□□□N□□□□□□□□tri□lu□r□met□□l□□□en□l□□r□□an□□l□□uani□i n□□□□□□□□□tri□e□□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□

R_f □0.39 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.99 (s, 3H), 2.80 (t, *J* □7.6 Hz, 2H), 3.14 (t, *J* □7.6 Hz, 2H), 3.65-3.70 (m, 2H), 3.80 (dd, *J* □2.0 , 3.2 Hz, 1H), 3.83-3.88 (m, 1H), 4.32 (dd, *J* □8.0, 8.4 Hz, 1H), 4.47 (dd, *J* □0.8, 8.8 Hz, 1H), 4.66 (dd, *J* □2.4, 7.6 Hz, 1H), 5.91 (d, *J* □3.2 Hz, 1H), 7.38 (t, *J* □7.6 Hz, 1H), 7.48 (d, *J* □7.6 Hz, 1H), 7.55 (t, *J* □7.6 Hz, 1H), 7.64 (d, *J* □7.6 Hz, 1H) □^{13}C NMR (100 MHz, CD_3OD) δ 22.54, 27.72, 39.28, 50.87, 64.75, 70.88, 71.39, 77.26, 106.11, 127.16, 128.10, 132.42, 133.61, 139.98, 147.49, 155.38, 165.06, 174.55, 176.55 HRMS (ESI-TOF) calcd for $C_{21}H_{28}N_4O_8$ □M + H□$^+$ □535.1859 , found □535.1855.



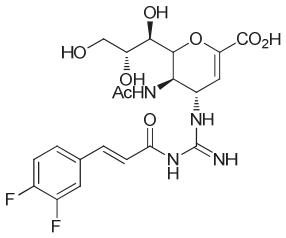
5-acetamido-4-guanidino-2,6-anhydro-3,4,5-trideoxy-*N*-(3-aminophenethyl)-D-glycero-D-galacto-non-2-enonic acid

R_f = 0.21 (EA:MeOH:H_2O, 3:2:1). ^{1}H NMR (400 MHz, CD_3OD) δ 1.99 (s, 3H), 2.84 (br, 2H), 3.03 (t, *J* = 7.6 Hz, 2H), 3.66-3.70 (m, 2H), 3.81 (dd, *J* = 2.8, 11.6 Hz, 1H), 3.84-3.86 (m, 1H), 4.30 (br, 1H), 4.50 (dd, *J* = 1.2, 7.6 Hz, 1H), 4.57 (dd, *J* = 2.8, 7.6 Hz, 1H), 5.89 (d, *J* = 2.8 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 22.37, 30.24, 31.14, 36.07, 38.98, 50.55, 64.56, 70.89, 71.22, 71.46, 77.19, 105.98, 123.67, 131.06, 131.13, 142.06, 147.30, 155.25, 164.87, 174.30, 175.79 HRMS (ESI-TOF) calcd for $C_{21}H_{29}N_5O_8$ [M + H]$^+$ 479.2016 , found 479.2007.



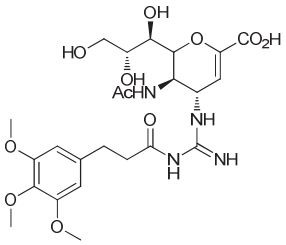
5-acetamido-4-guanidino-2,6-anhydro-3,4,5-trideoxy-*N*-(tetrahydrofuran-2-ylmethyl)-D-glycero-D-galacto-non-2-enonic acid

R_f = 0.51 (EA:MeOH:H_2O, 3:2:1). ^{1}H NMR (400 MHz, CD_3OD) δ 1.52-1.60 (m , 1H), 1.71-1.78 (m, 2H), 1.99 (s , 3H), 2.05-2.13 (m, 1H), 2.21-2.28 (m, 1H), 2.52 (br, 2H), 3.37 (dd, *J* = 6.8, 8.0 Hz, 2H), 3.66-3.90 (m, 7H), 4.30 (dd, *J* = 7.6, 8.0 Hz, 1H), 4.51 (dd, *J* = 1.2, 8.0 Hz, 1H), 4.58 (dd, *J* = 2.8, 7.6 Hz, 1H), 5.92 (d, *J* = 2.8 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 22.59, 28.60, 32.94, 36.66, 39.79, 51.11, 64.73, 68.81, 70.92, 71.48, 73.87, 77.39, 105.45, 148.30, 155.53, 165.20, 174.44, 176.79 HRMS (ESI-TOF) calcd for $C_{18}H_{28}N_4O_9$ [M + H]$^+$ 444.1856 , found 444.1848.



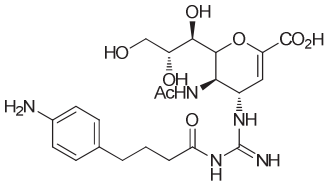
□□□ □etami□□□□□□an □□□r□□□□□*N*□□□***E***□□□□□□□□□i□lu□r□□□en□l□□□□□r□□en□l□□uani□in □□□□□□□□□tri□e□□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□t□

R_f □0.29 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 2.02 (s, 3H), 3.70 (dd, *J* □5.2, 11.2 Hz, 1H), 3.71 -3.73 (m, 1H), 3.82 (dd, *J* □ 3.2, 11.2 Hz, 1H), 3.86-3.90 (m, 1H), 4.37 (t, *J* □ 8.4 Hz, 1H), 4.50-4.51 (m, 1H), 4.72-4.75 (br, 1H), 5.96 (d, *J* □ 2.8 Hz, 1H), 6.72 (d, *J* □ 15.2 Hz, 1H), 7.33 (td, *J* □ 8.4, 18.4 Hz, 1H), 7.48-7.49 (br, 1H), 7.63 (t, *J* □ 8.4 Hz, 1H), 7.80 (t, *J* □ 15.2 Hz, 1H)□^{13}C NMR (100 MHz, CD_3OD) 22.57, 30.67, 51.08, 64.69, 70.68, 71.54, 77.58, 106.44, 117.98, 118.12, 119.03, 119.17, 120.53, 127.11, 132.89, 145.61, 147.44, 156.01, 165.14, 168.23, 174.55 □HRMS (FAB) calcd for $C_{21}H_{25}N_4O_8F_2$ □M + H$^+$□ □499.1640 , found □ 499.1630.



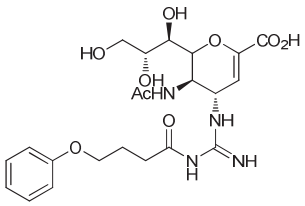
□□□ □etami□□□□□□an □□□r□□□□□*N*□□□□□□□□□□□□trimet□□□□□□en□l□□r□□an□□l□□uani□in□□ □□□□□□□□tri□e□□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□u□

R_f □0.37 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.99 (s, 3H), 2.79 (t, *J* □6.8 Hz, 2H), 2.93 (t, *J* □6.8 Hz, 2H), 3.66-3.70 (m, 2H), 3.72 (s, 3H), 3.79-3.86 (m, 2H), 3.81 (s, 6H), 4.26 (dd, *J* □7.2, 8.0 Hz, 1H), 4.51 (dd, *J* □0.8, 8.0 Hz, 1H), 4.56 (dd, *J* □3.2, 7.2 Hz, 1H), 5.90 (d, *J* □3.2 Hz, 1H), 6.55 (s, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.52, 31.48, 39.81, 56.60, 61.09, 64.72, 71.08, 71.34, 77.23, 106.20, 106.75, 137.61, 147.33, 150.58, 154.54, 155.30, 164.90, 174.51, 176.15 HRMS (ESI-TOF) calcd for $C_{25}H_{34}N_4O_{11}$ □M + H$^+$□ □568.2381 , found □568.2375.



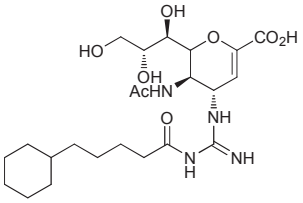
□□□ □etami□□□□□□an□□□r□□□□*N*□□□□□□amin□□□en□l□□utan□□l□uani□in□□□□□□□□□tri□e□□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□

R_f □0.33 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.94-1.97 (m, 2H), 2.00 (s , 3H), 2.52 (t, *J* □6.0 Hz, 2H), 2.74 (t, *J* □7.6 Hz, 2H), 3.66-3.71 (m, 2H), 3.81 (dd, *J* □3.2, 11.6 Hz, 1H), 3.84-3.87 (m, 1H), 4.31 (dd, *J* □7.6, 8.4 Hz, 1H), 4.45 (dd, *J* □1.2, 7.6 Hz, 1H), 4.56 (dd, *J* □2.8, 7.6 Hz, 1H), 5.92 (d, *J* □2.8 Hz, 1H), 7.32 (d, *J* □6.4 Hz, 2H), 7.40 (d, *J* □8.4 Hz, 2H) □^{13}C NMR (100 MHz, CD_3OD) δ 22.50, 26.75, 35.17, 36.99, 50.96, 64.68, 70.75, 71.33, 77.34, 106.33, 124.02, 129.94, 131.26, 144.15, 147.27, 155.42, 164.89, 174.45, 176.64 HRMS (ESI-TOF) calcd for $C_{22}H_{31}N_5O_8$ □M + H□$^+$ □493.2173 , found □493.2171



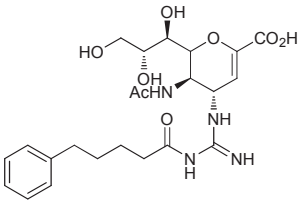
□□□ □etami□□□□□□an□□□r□□□□*N*□□□□□□en□□□□utan□□l□uani□in□□□□□□□□□tri□e□□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□□

R_f □ 0.47 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.99 (s, 3H), 2.14 (t, *J* □6.0 Hz, 2H), 1.99 (s, 3H), 2.68 (s, 3H), 3.69 (dd, *J* □5.2, 11.2 Hz, 1H), 3.70 (dd, *J* □1.2, 8.8 Hz, 1H), 3.80 (dd, *J* □3.2, 11.2 Hz, 1H), 3.86 (ddd, *J* □3.2, 5.2, 8.8 Hz, 1H), 4.04 (d, *J* □6.0 Hz, 1H), 4.30 (dd, *J* □7.6, 8.4 Hz, 1H), 4.49 (dd, *J* □1.2, 8.4 Hz, 1H), 4.62 (dd, *J* □2.8, 7.6 Hz, 1H), 5.90 (d, *J* □2.8 Hz, 1H), 6.88-6.91 (m, 3H), 7.24-7.26 (m, 2H) □^{13}C NMR (100 MHz, CD_3OD) δ 22.54, 25.18, 34.71, 50.91, 64.69, 67.59, 71.43, 77.41, 106.35, 115.45, 121.83, 130.45, 147.34, 155.47, 160.12, 164.98, 174.47 176.77 □HRMS (MALDI) calcd for $C_{22}H_{30}N_4O_8$ □M + H□$^+$ □495.2013 , found □495.2018.



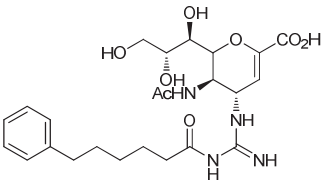
□□□ □etami□□□□□□an□□□r□□□□*N*□□□□□□□l□□e□□l□entan□□l□□uani□in□□□□□□□□□tri□e□□□
□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□□

R_f □0.59 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 0.87-0.92 (m, 2H), 1.17-1.27 (m, 6H), 1.37 (quintet, *J* □ 6.0 Hz, 2H), 1.63 (quintet, *J* □ 7.6 Hz, 2H), 1.67-1.73 (m, 2H), 2.00 (s, 3H), 2.46 (t, *J* □6.8 Hz, 2H), 3.62-3.71 (m, 2H), 3.81 (dd, *J* □3.2, 14.4 Hz, 1H), 3.82-3.87 (m, 1H), 4.31 (dd, *J* □7.6, 8.0 Hz, 1H), 4.50 (dd, *J* □ 1.2, 8.0 Hz, 1H), 4.61 (dd, *J* □2.8, 6.8 Hz, 1H), 5.92 (d, *J* □2.8 Hz, 1H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.51, 25.59, 27.21, 27.44, 27.74, 34.47, 37.86, 38.21, 38.75, 50.85, 64.73, 70.82, 71.35, 77.39, 106.35, 147.23, 155.47, 164.90, 174.49, 176.10 HRMS (ESI-TOF) calcd for $C_{23}H_{38}N_4O_8$ □M + H□$^+$ □498.2690 , found □498.2692.



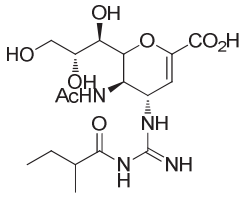
□□□ □etami□□□□□□an□□□r□□□□*N*□□□□□□□en□l□entan□□l□□uani□in□□□□□□□□□tri□e□□□□□□
□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□□

R_f □ 0.56 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.68 (quintet, *J* □ 3.6 Hz, 4H), 2.00 (s, 3H), 2.49 (br, 2H), 2.63 (t, *J* □6.8 Hz, 2H), 3.66-3.70 (m, 2H), 3.81 (dd, *J* □3.2, 8.4 Hz, 1H), 3.82-3.87 (m, 1H), 4.30 (dd, *J* □7.6, 8.0 Hz, 1H), 4.50 (dd, *J* □1.2, 8.0 Hz, 1H), 4.60 (dd, *J* □2.8, 7.2 Hz, 1H), 5.91 (d, *J* □3.2 Hz, 1H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.51, 24.85, 31.73, 36.43, 37.67, 50.85, 64.73, 70.88, 71.35, 77.35, 106.35, 126.83, 129.34, 129.39, 143.24, 147.29, 155.47, 164.93, 174.49, 176.90 HRMS (ESI-TOF) calcd for $C_{23}H_{32}N_4O_8$ □M + H□$^+$ □492.2220 , found □ 492.2227.



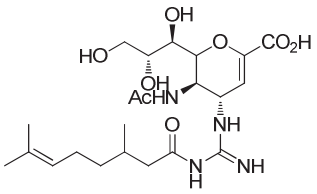
□□□ □etami□□□□□□□an□□□r□□□□□*N*□□□□□□en□l□e□an□l□□uani□in□□□□□□□□□□tri□e□□□□□ □□ l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□□

R_f □ 0.53 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.38 (m, 2H), δ 1.66 (m, 4H), 1.99 (s, 3H), 2.46 (t, *J* □ 6.0 Hz, 2H), 2.61 (t, *J* □ 7.6 Hz, 2H), 3.66-3.70 (m, 2H), 3.81 (dd, *J* □ 2.8, 11.6 Hz, 1H), 3.82-3.87 (m, 1H), 4.29 (dd, *J* □ 7.6, 8.4 Hz, 1H), 4.49 (dd, *J* □ 1.2, 8.4 Hz, 1H), 4.58 (dd, *J* □ 3.2, 7.2 Hz, 1H), 5.89 (d, *J* □ 3.2 Hz, 1H), 7.11-7.17 (m, 3H), 7.22-7.25 (m, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.52, 25.11, 29.45, 32.27, 36.59, 37.76, 50.91, 64.69, 70.85, 71.37, 77.28, 105.99, 126.71, 129.28, 129.38, 132.82, 143.61, 147.60, 155.43, 165.30, 174.49, 176.98 HRMS (ESI-TOF) calcd for $C_{24}H_{34}N_4O_8$ □M + H$^+$□ □506.2377 , found □506.2385.



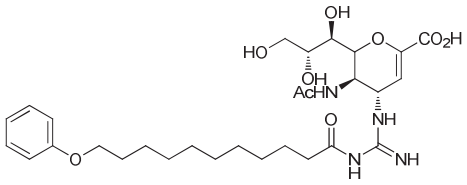
□□□ □etami□□□□□□□an□□□r□□□□□*N*□□□□met□□l□utan□□l□□uani□in□□□□□□□□□□tri□e□□□□□ □□ l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□aa□

R_f □ 0.49 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 0.91 (dd, *J* □ 7.2, 7.6 Hz, 1H), 1.15 (d, *J* □ 6.8 Hz, 1H), 1.47 (ddq, *J* □ 6.8, 7.2, 14.4 Hz, 1H), 1.47 (ddq, *J* □ 6.8, 7.6, 14.4 Hz, 1H), 1.97 (s, 3H), 2.46-2.48 (br, 1H), 3.68 (dd, *J* □5.2, 11.6 Hz, 1H), 3.69-3.71 (m, 1H), 3.82 (dd, *J* □ 3.2, 11.6 Hz, 1H), 3.85-3.88 (m, 1H), 4.32 (dd, *J* □ 8.4, 8.4 Hz, 1H), 4.48-4.50 (m, 1H), 4.64-4.67 (br, 1H), 5.90 (d, *J* □2.8 Hz, 1H) □ ^{13}C NMR (100 MHz, CD_3OD) δ 11.67, 16,63, 16.70, 22.59, 27.54, 44.44, 51.09, 64.69, 70.62, 71.47, 77.51, 106.22, 147.64, 155.75, 165.38, 174.46, 180.70 □HRMS (FAB) calcd for $C_{17}H_{29}N_4O_8$ □M + H$^+$□ □417.1985 , found □417.1980.



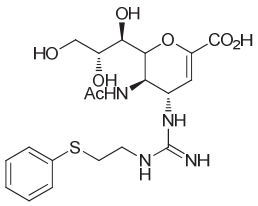
□□□ □etami□□□□□□an □□□r□□□□N□□□□□□imet□□l□□□□ten□□l□uani□in□□□□□□□□tri□e□□ □□□ □□l□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□a□□

R_f □0.52 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 0.99 (d, *J* □6.4 Hz, 3H), 1.17 (s, 6H), 1.23-1.26 (m, 1H), 1.33-1.44 (m, 5H), 2.00 (s, 3H), 2.28 (dd, *J* □ 6.0, 12.8 Hz), 2.48 (dd, *J* □ 4.0, 14.4 Hz, 1H), 3.68 (dd, *J* □5.2, 11.2 Hz, 1H), 3.69-3.72 (m, 1H), 3.81 (dd, *J* □3.2, 11.2 Hz, 1H), 3.83-3.87 (m, 1H), 4.30 (dd, *J* □ 7.6, 7.6 Hz, 1H), 4.51 (dd, *J* □3.6, 8.0 Hz, 1H), 4.60 (dd, *J* □3.2, 7.6 Hz, 1H), 5.92 (d, *J* □3.2 Hz, 1H) □^{13}C NMR (100 MHz, CD_3OD) δ 19.85, 22.56, 22.64, 29.16, 29.24, 31.25, 38.25, 44.74, 45.35, 51.02, 64.75, 70.96, 71.36, 71.47, 77.26, 105.14, 148.45, 155.43, 166.01, 174.41, 176.57 □HRMS (FAB) calcd for $C_{22}H_{37}N_4O_8$ □M + H□$^+$ □ 485.2611 , found □485.2601.



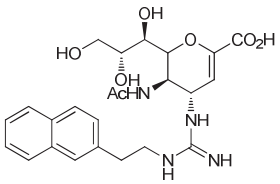
□□□ □etami□□□□□□an □□□r□□□□N□□□□□□□en□□□un□e□an□□l□uani□in□□□□□□□□tri□e□□ □□□ □□l□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□a□□

R_f □ 0.57 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.32 (m, 10H), 1.44 (quintet, *J* □6.8 Hz, 2H), 1.64 (quintet, *J* □6.8 Hz, 2H), 1.74 (quintet, *J* □6.8 Hz, 2H), 2.00 (s, 3H), 2.48 (t, *J* □7.2 Hz, 2H), 3.71-3.75 (m, 2H), 3.81 (dd, *J* □3.2, 11.6 Hz, 1H), 3.86-3.96 (m, 4H), 4.36 (dd, *J* □8.8, 9.2 Hz, 1H), 4.46 (dd, *J* □0.8, 9.2 Hz, 1H), 4.72 (dd, *J* □3.2, 6.8 Hz, 1H), 5.93 (d, *J* □3.2 Hz, 1H), 6.85-6.88 (m, 3H), 7.20 (dd, *J* □7.6, 8.0 Hz, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.33, 25.08, 26.92, 29.75, 30.11, 30.18, 30.22, 30.37, 37.63, 50.81, 64.44, 68.58, 70.51, 71.23, 77.03, 105.09, 115.24, 121.24, 130.15, 148.11, 155.25, 160.32, 165.86, 174.21, 176.87 HRMS (ESI-TOF) calcd for $C_{29}H_{44}N_4O_9$ □M + H□$^+$ □592.3108 , found □592.3102.



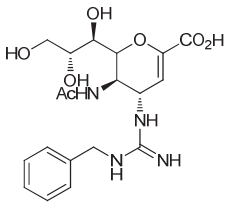
□□□ □etami□□□□□□□an □□□r□□□□□*N*□□□□□□□en□lt□i□et□□l□□uani□in□□□□□□□□□tri□e□□□□□ □
□l□□er□□□ □□ala□t□n□n□□□en□ni□a□i□ □□□a□□

R_f □0.49 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.99 (s, 3H), 3.16 (t, *J* □6.4 Hz, 2H), 3.43 (t, *J* □6.4 Hz, 2H), 3.64-3.69 (m, 2H), 3.81 (dd, *J* □3.2, 11.2 Hz, 1H), 3.82-3.88 (m, 1H), 4.22 (t, *J* □7.6 Hz, 1H), 4.38-4.41 (m, 2H), 5.86 (d, *J* □2.8 Hz, 1H), 7.24 (m, 1H), 7.33 (m, 2H), 7.42 (m, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.67, 33.81, 41.92, 51.70, 64.70, 70.42, 71.26, 77.66, 108.22, 127.95, 130.33, 131.21, 136.07, 146.39, 157.67, 165.26, 174.28 HRMS (ESI-TOF) calcd for $C_{20}H_{28}N_4O_7S$ □M + H$^+$□ □468.1679 , found □468.1671.



□□□ □etami□□□□□□□an □□□r□□□□□*N*□□□□□na□□t□alen□□□□l□et□□l□□uani□in□□□□□□□□□tri□e□
□□□□ □□l□□er□□□ □□ala□t□□n□n□□□en□ni□a□i□ □□□ae□

R_f □0.65 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz , CD_3OD) δ 1.96 (s, 3H), 3.06 (t, *J* □7.2 Hz, 2H), 3.59 (t, *J* □7.2 Hz, 2H), 3.64-3.69 (m, 2H), 3.81 (dd, *J* □2.8, 11.2 Hz, 1H), 3.83-3.88 (m, 1H), 4.20 (br, 1H), 4.37-4.39 (m, 2H), 5.78 (d, *J* □2.8 Hz, 1H), 7.40-7.48 (m, 3H), 7.73 (s, 1H), 7.83 (m, 3H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.63, 36.08, 43.86, 51.53, 64.72, 70.42, 71.26, 77.67, 108.50, 126.74, 127.27, 128.11, 128.47, 128.56, 128.67, 129.43, 133.91, 135.08, 136.76, 146.69, 157.60, 165.10, 174.48 HRMS (ESI-TOF) calcd for $C_{24}H_{30}N_4O_7$ □M + H$^+$□ □486.2114 , found □ 486.2117.



□□□ □etami□□□□□□□an□□□r□□□□□N□□en□□l□uani□in□□□□□□□□□tri□e□□□□□ □□l□□er□□□ □□al a□t□□n□n□□□en□ni□a□i□ □□□a□□

R_f □0.52 (EA□MeOH□H_2O, 3□2□1)□^{1}H NMR (400 MHz, CD_3OD) δ 1.97 (s, 3H), 3.66 (dd, *J* □1.2, 11.2 Hz, 1H), 3.80 (dd, *J* □3.2, 11.2 Hz, 1H), 3.68 (dd, *J* □5.2, 7.2 Hz, 1H), 3.85-3.89 (m, 1H), 4.26 (dd, *J* □8.4, 9.6 Hz, 1H), 4.41 (dd, *J* □1.2, 9.6 Hz, 1H), 4.42-4.45 (m, 2H), 4.47-4.52 (m, 1H), 5.87 (d, *J* □2.8 Hz, 1H), 7.32-7.34 (m, 3H), 7.37-7.41 (m, 2H)□^{13}C NMR (100 MHz, CD_3OD) δ 22.69, 46.18, 51.92, 64.71, 70.23, 71.26, 77.73, 108.31, 128.37, 129.10, 129.98, 137.53, 147.07, 157.71, 165.34, 174.28□ HRMS (ESI-TOF) calcd for $C_{19}H_{26}N_4O_7$ □M + H$^+$□ □422.1801 , found □422.1809.

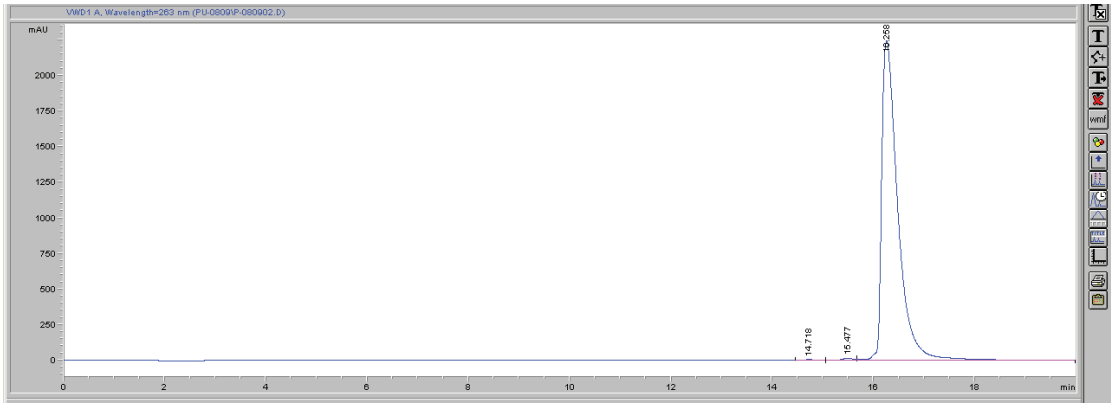
□ □□□ □□t□e □□m□□un□ □□

HPLC spectrum of compound □□(purity □99%)

Column□Vydac C18-column (Cat□218TP54, 4.6mm I.D. ∗250 mm (□ μM)

Eluent□H_2O (0.1% TFA)□Acetonitrile

	H_2O(0.1%TFA)	Acetonitrile	Flow rate (ml□min)
0 min	100%	0%	1
10 min	72%	28%	1
15 min	65%	35%	1
20 min	50%	50%	1



#	Time	Area	Height	Width	Area%	Symmetry
1	14.718	126.2	6.8	0.2616	0.250	0.491
2	15.477	301.4	17.3	0.2385	0.597	1.123
3	16.258	50089.2	2251	0.3295	99.153	0.43

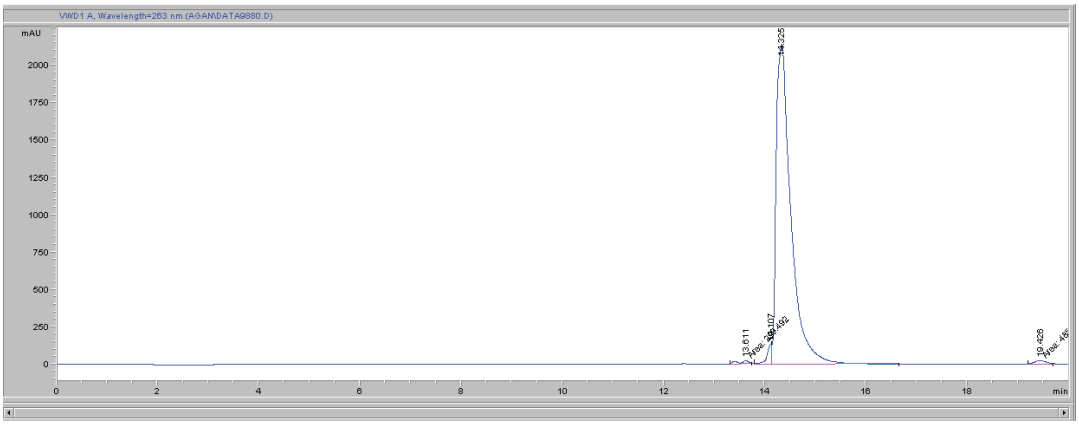
□□□□ □□t□e □□m□□un□ □□a□

HPLC spectrum of compound □□**a**□(purity □99%)

Column□Vydac C18-column (Cat□218TP54, 4.6mm I.D. ∗250 mm (□ μM)

Eluent□H_2O (0.1% TFA)□Acetonitrile

	H_2O(0.1%TFA)	Acetonitrile	Flow rate (ml□min)
0 min	100%	0%	1
10 min	72%	28%	1
15 min	65%	35%	1
20 min	50%	50%	1



#	Time	Area	Height	Width	Area%	Symmetry
1	13.611	299.5	19.2	0.26	0.662	2.889
2	14.107	853.7	132.9	0.0953	1.886	6.63
3	14.325	43620.2	2146.2	0.2939	96.379	0.535
4	19.426	485.7	26.7	0.3033	1.073	0.997

□euramini□ase in□i□iti□n assa□

Influenza virus A□□ SN□33 (H1N1) and influenza virus A□Taiwan□3446□2002(H3N2) were kindly provided by Dr. Shin-Ru Shih (The Clinical Virology Laboratory of Chang Gung Memorial Hospital (Linkou, Taoyuan)). As the source of viral neuramindase stock, large-scale influenza virus suspensions were prepared from MDCK cells infected with influenza virus (MOI 0.01) for 72 hrs. To inactivate viral infectivity, virus suspensions were treated with formaldehyde at a final concentration of 0.01% at 37 ˚C for 30 min, basically the same as our previous report.[5,6] Such preparations were safe for handling on the bench because the viral titer is under the detection limit but without decreasing the NA activity. Aliquots of the inactivated

virus supernatants were stored at −80 °C. The NA enzymatic activity was measured using the fluorogenic substrate MU-NANA, according to the method of Potier et al.[7] The viral stock was titrated in two-fold dilutions in 32.5 mM MES (pH 6.0)-4 mM $CaCl_2$, and the dilution of NA stock giving rise to approximately 5□1 to 10□1 signal to noise (s□n) ratio was employed in the NA inhibition assays. Fluorometric determinations were quantified with a Fluoroskan spectrofluorometer (Labsystems,Helsinki, Finland) based on the release of the fluorescent product 4-methyl-umbelliferone (4MU) using excitation and emission wavelengths of 360 and 460 nm, respectively. For NA inhibition assays, the appropriate viral NA dilution (10 µl) were preincubated with 10 µl zanamivir derivatives at variable concentrations for 30 min at 37 □C in 96-well, and the fluorogenic substrate was added at a final concentration of 100 µM in assay buffer (32.5mM 2-(N-morpholino)-ethanesulfonic acid, 4mM CaCl2 at pH 6.5) for 1 hour. The enzymatic reaction was terminated by the addition of 150 µL stop solution (25% ethanol, 0.1M glycine, pH 10.7). IC50 determination was performed with GraphPad Prism 4.

□e□eren□e□

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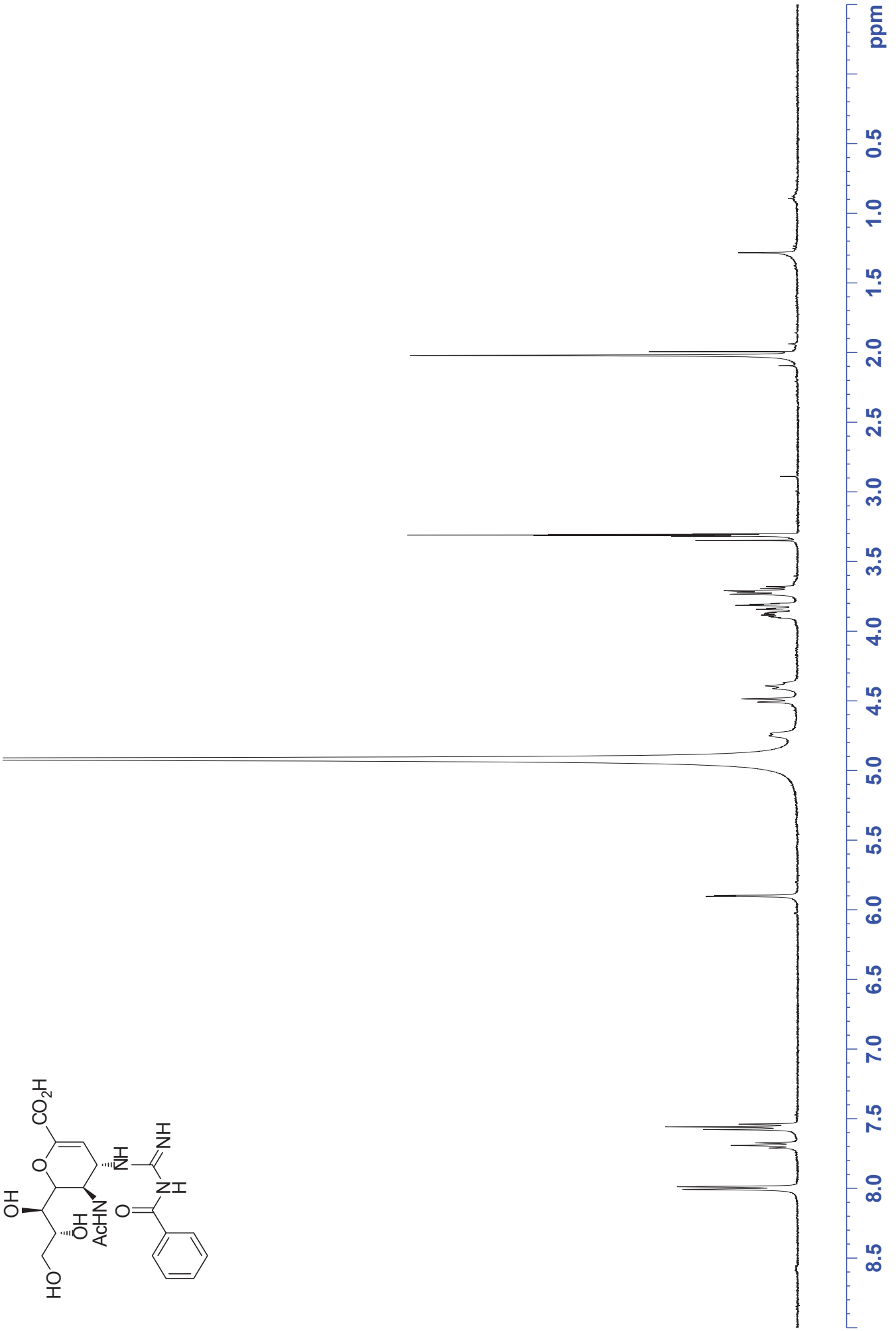

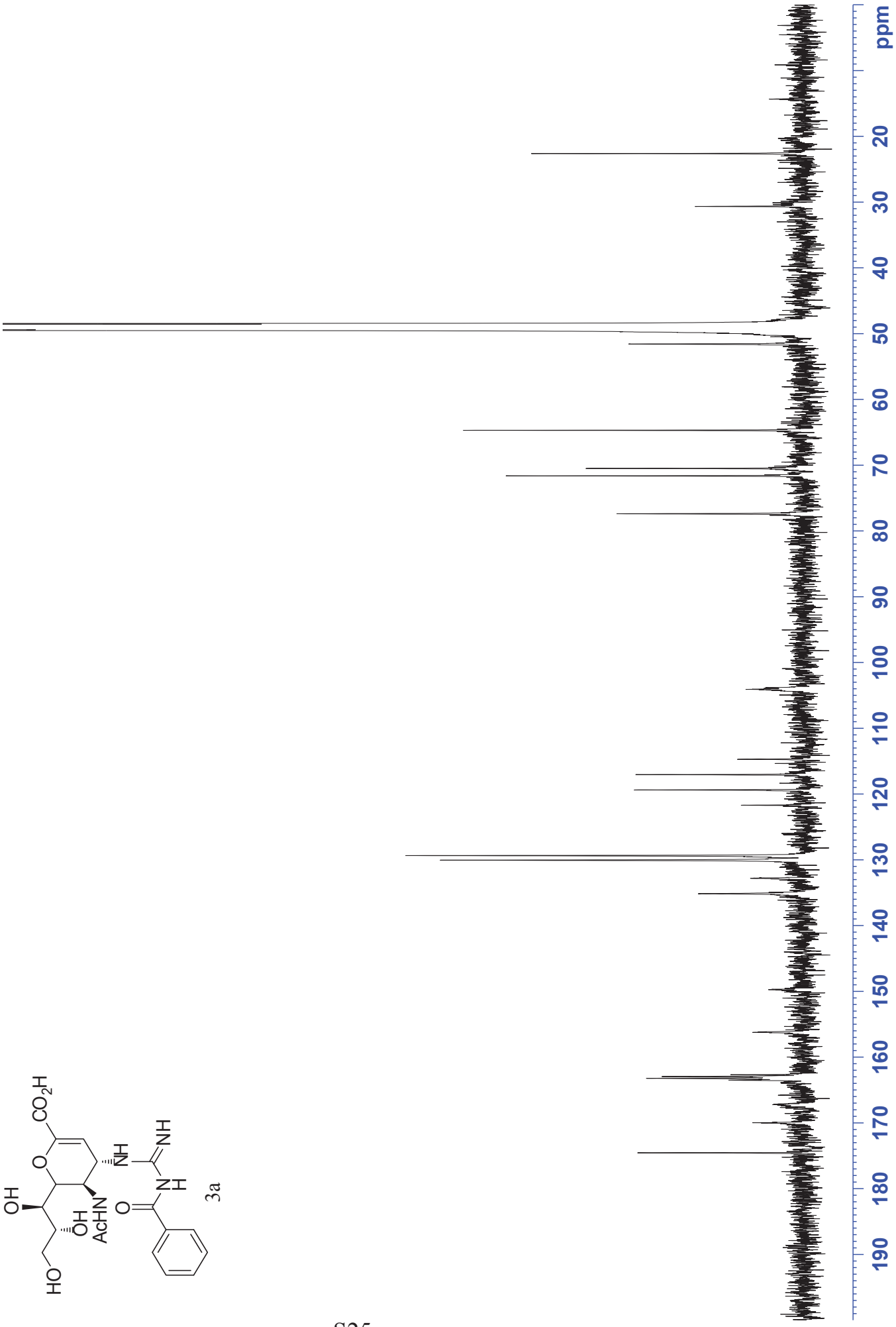
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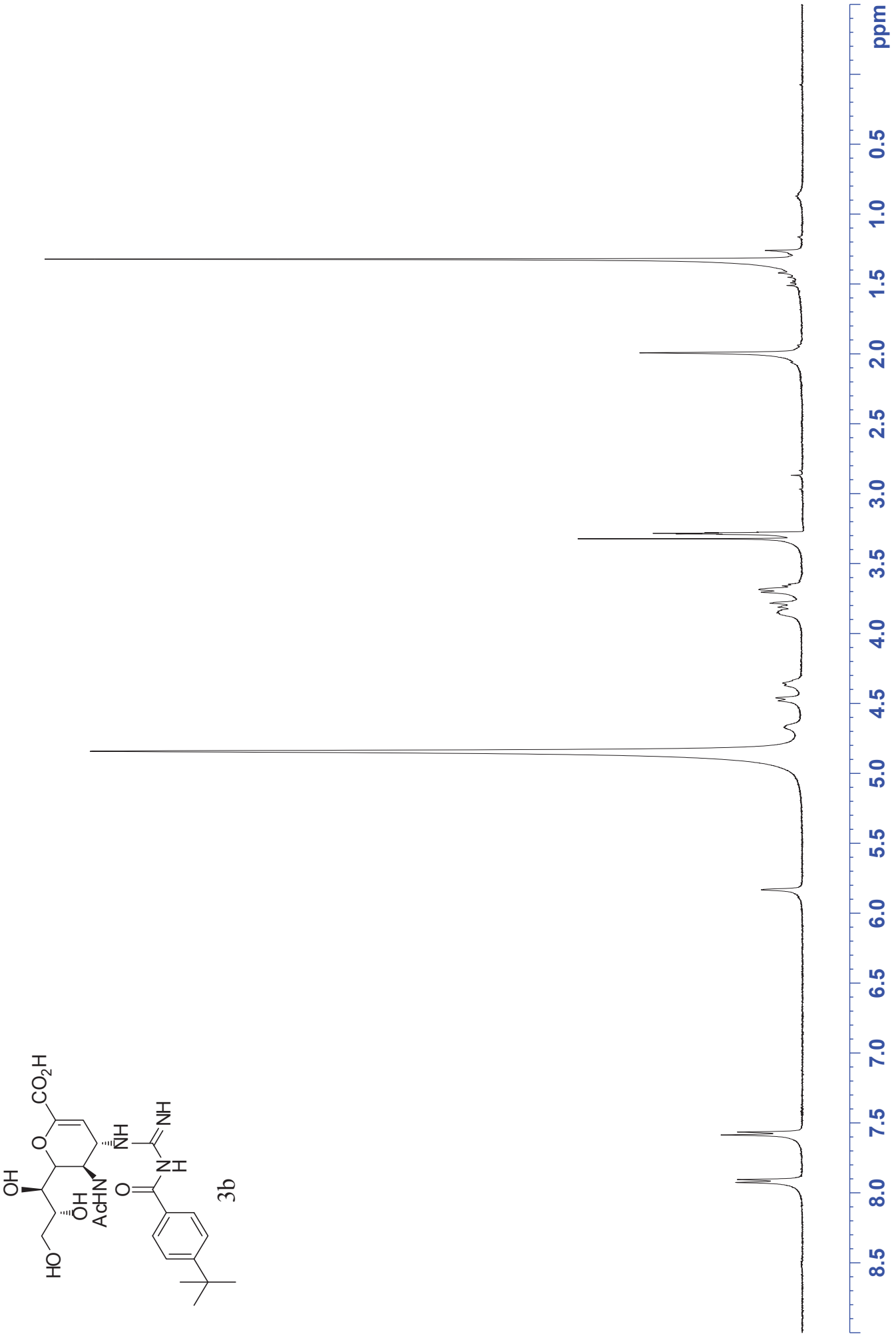
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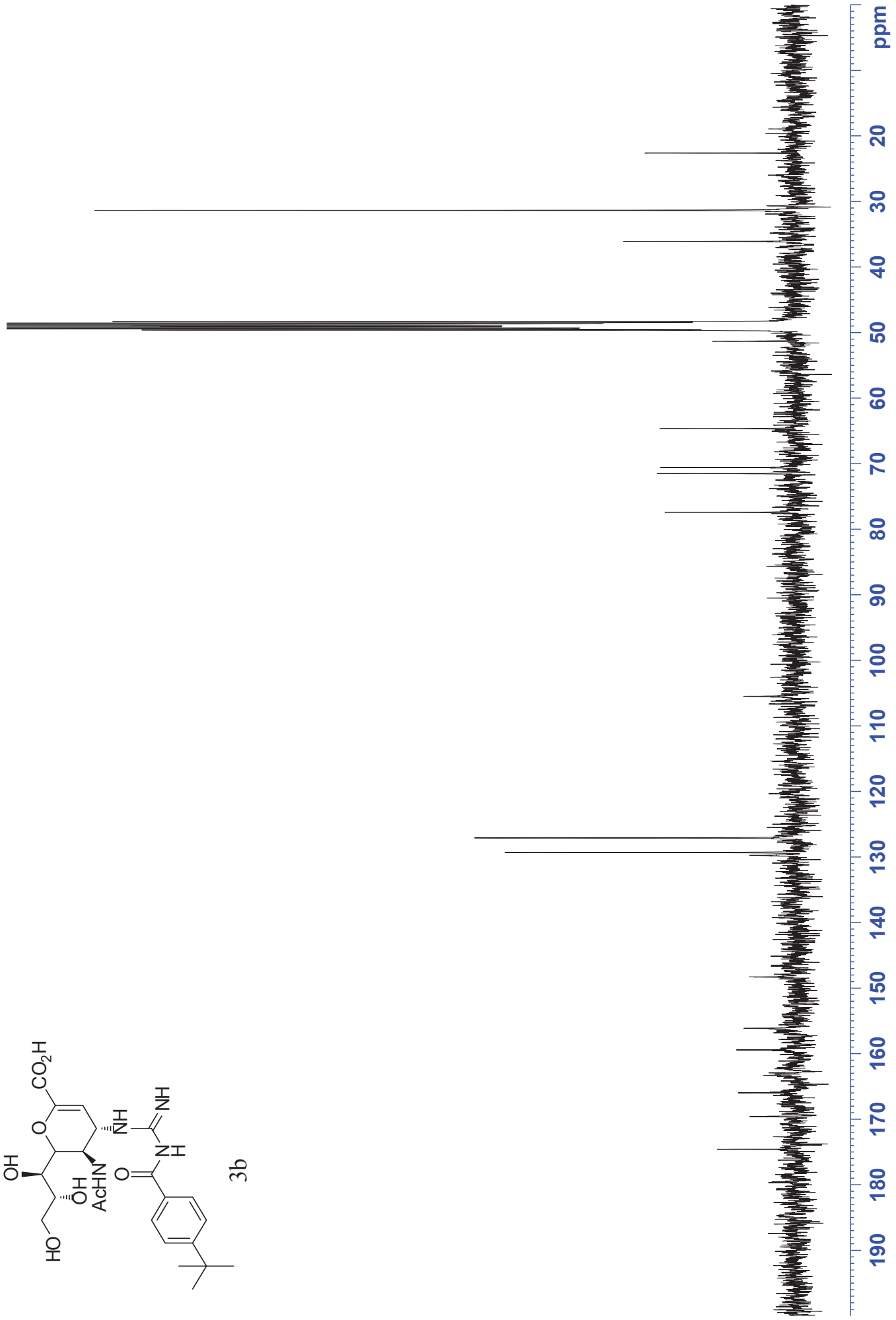

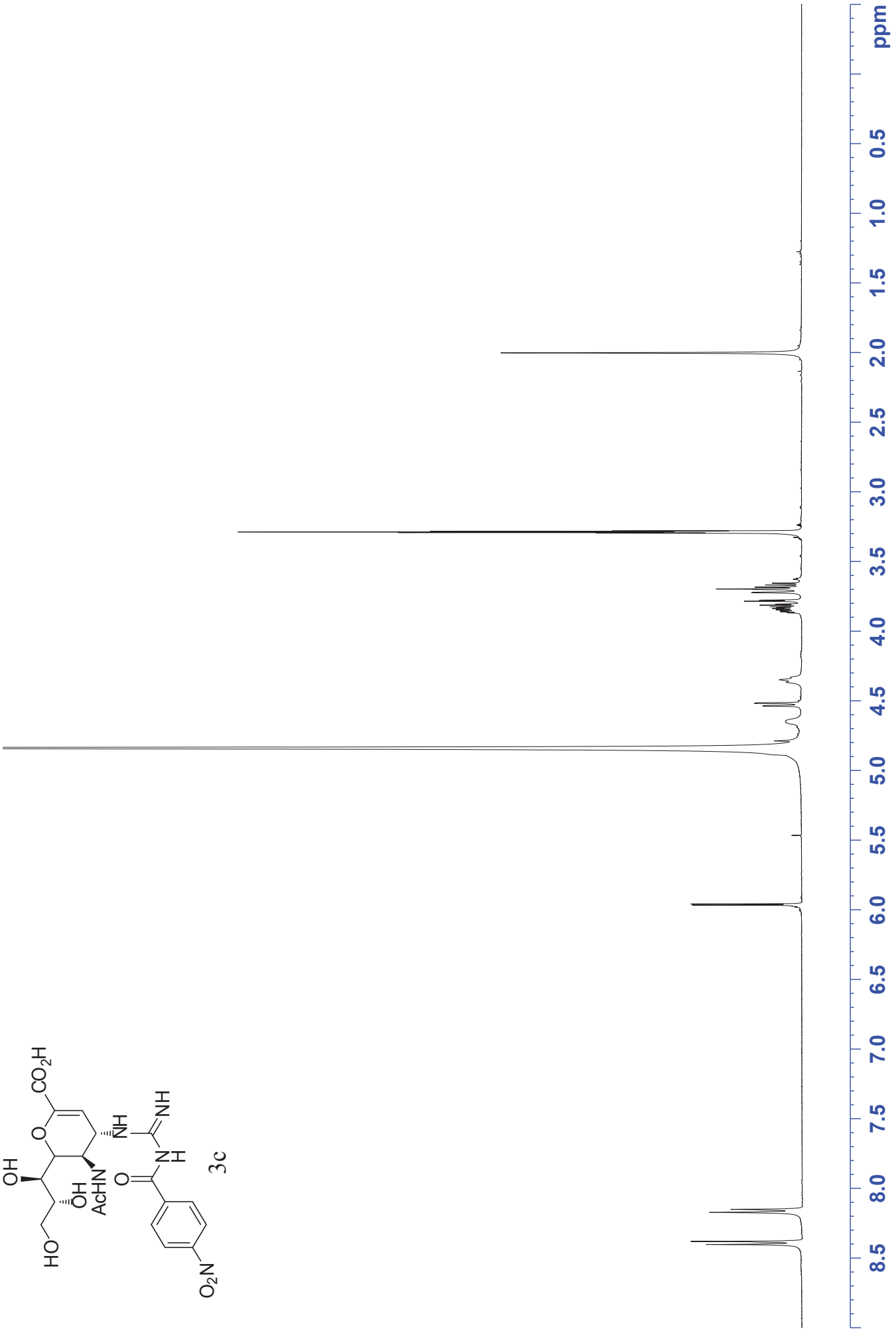

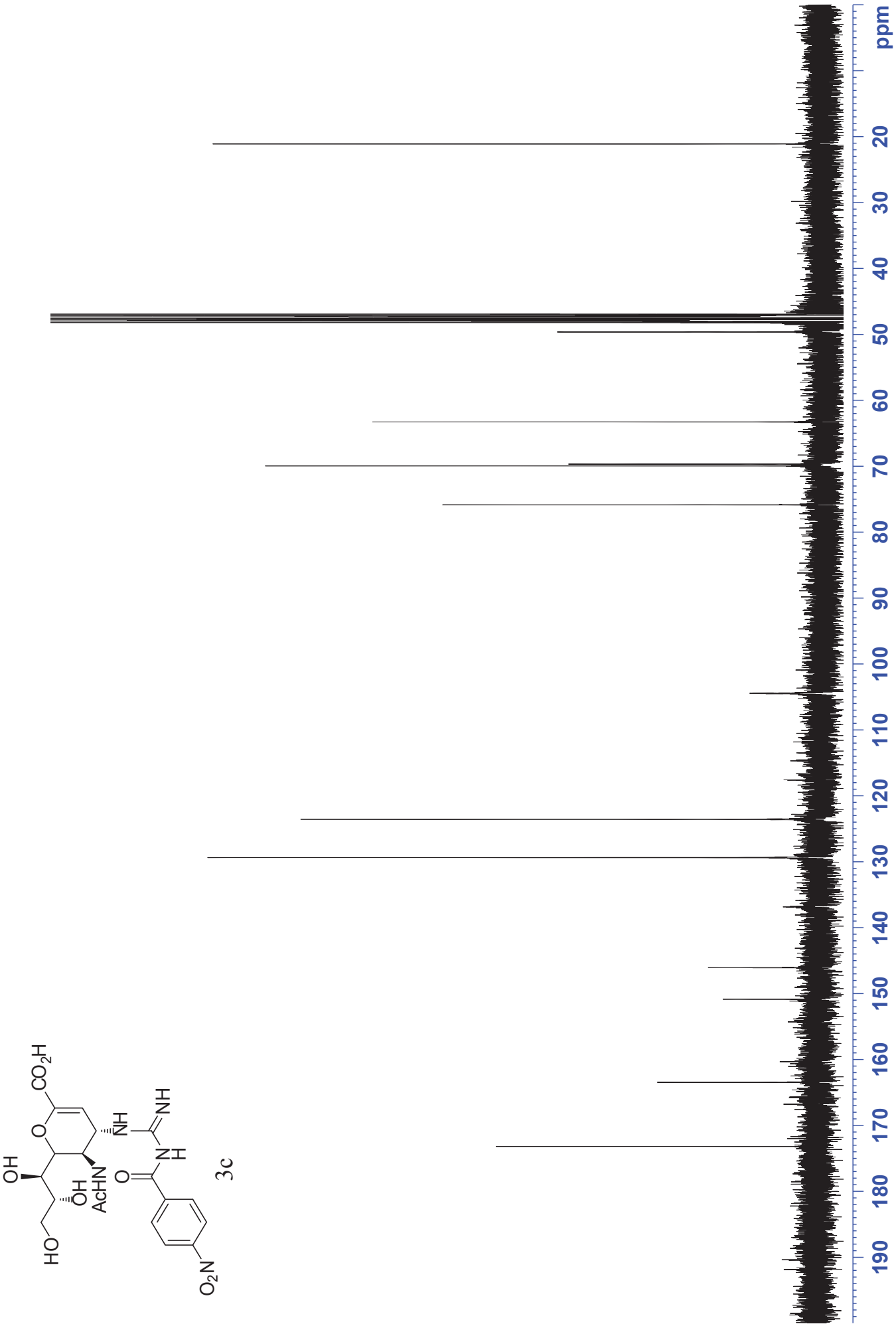

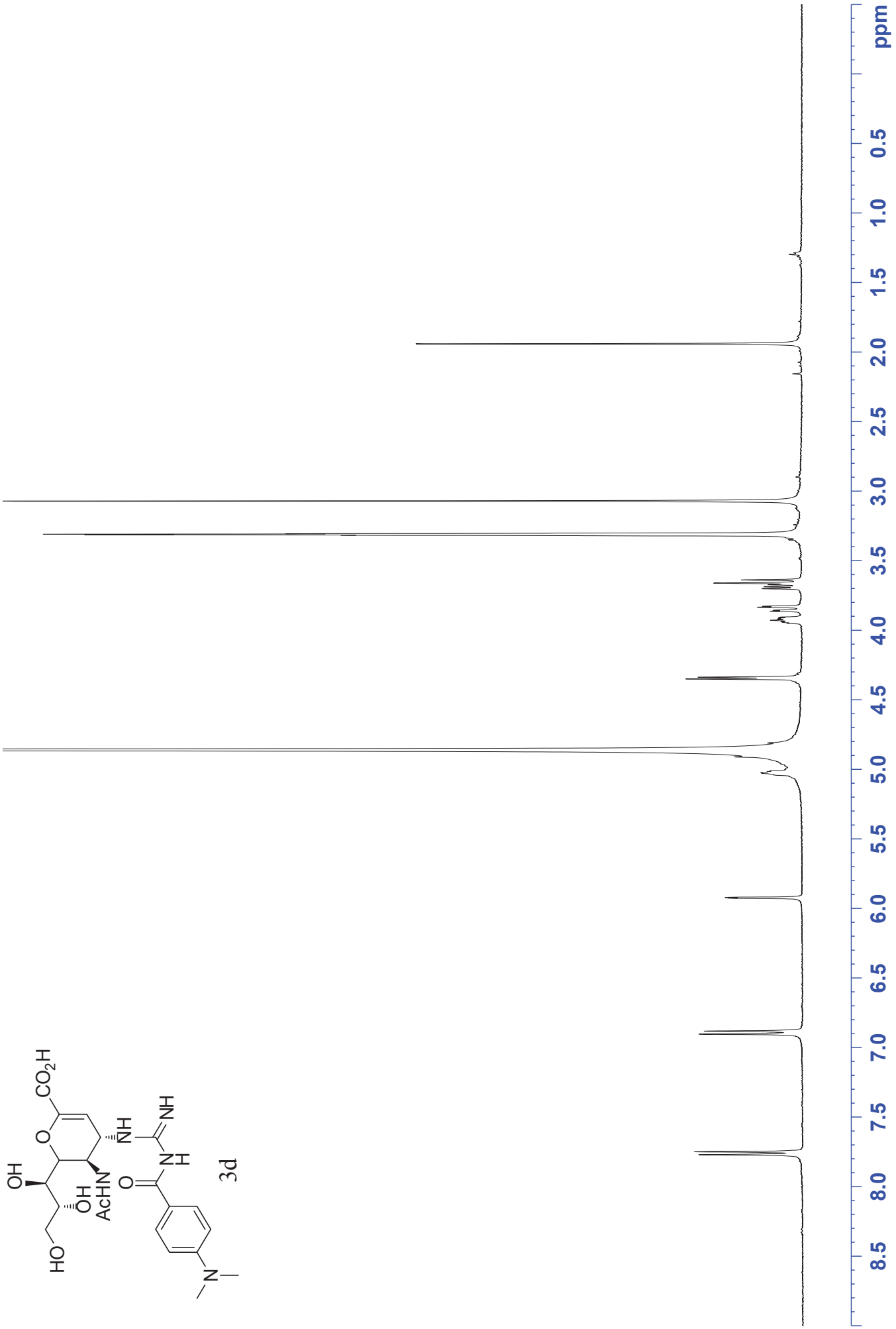

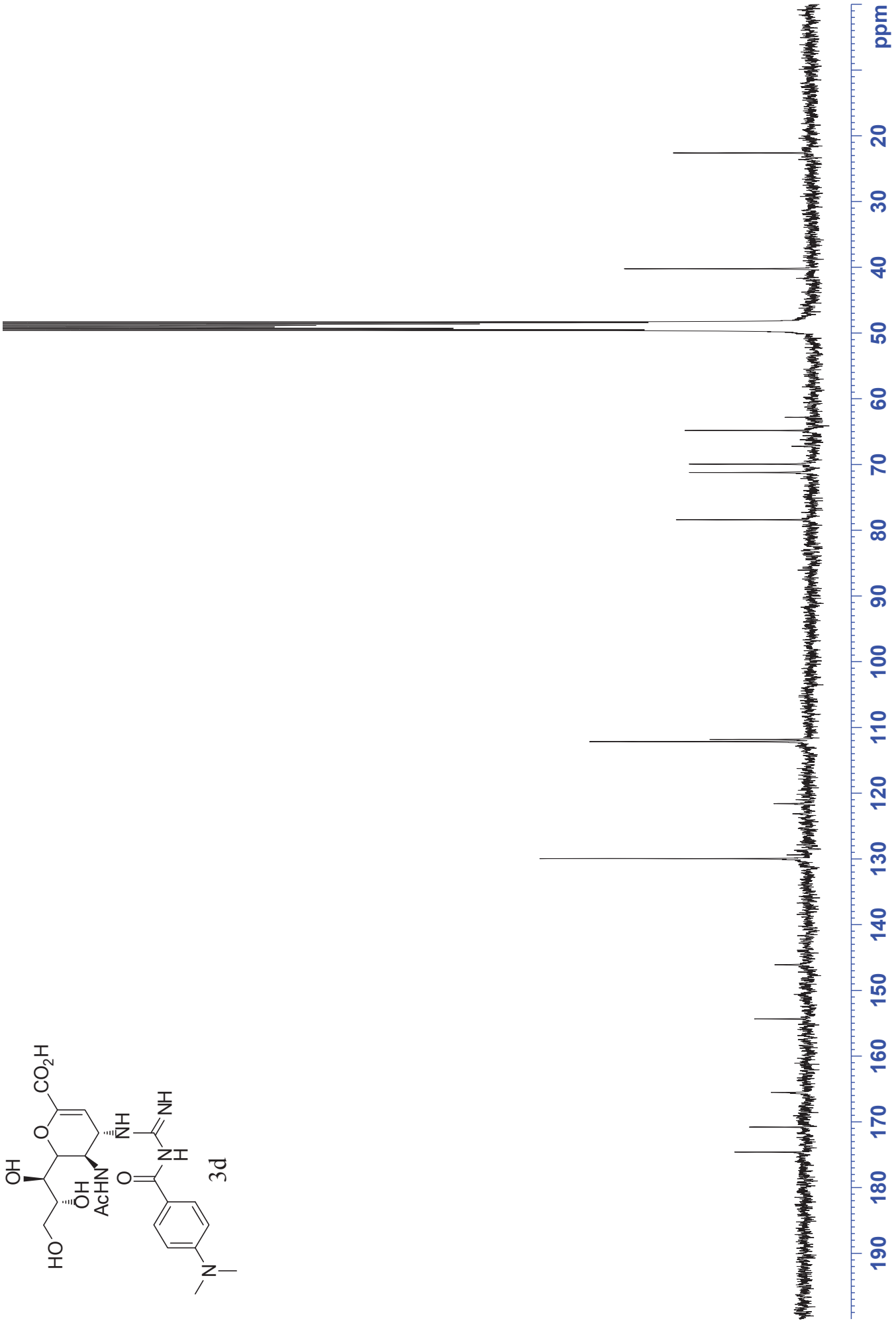

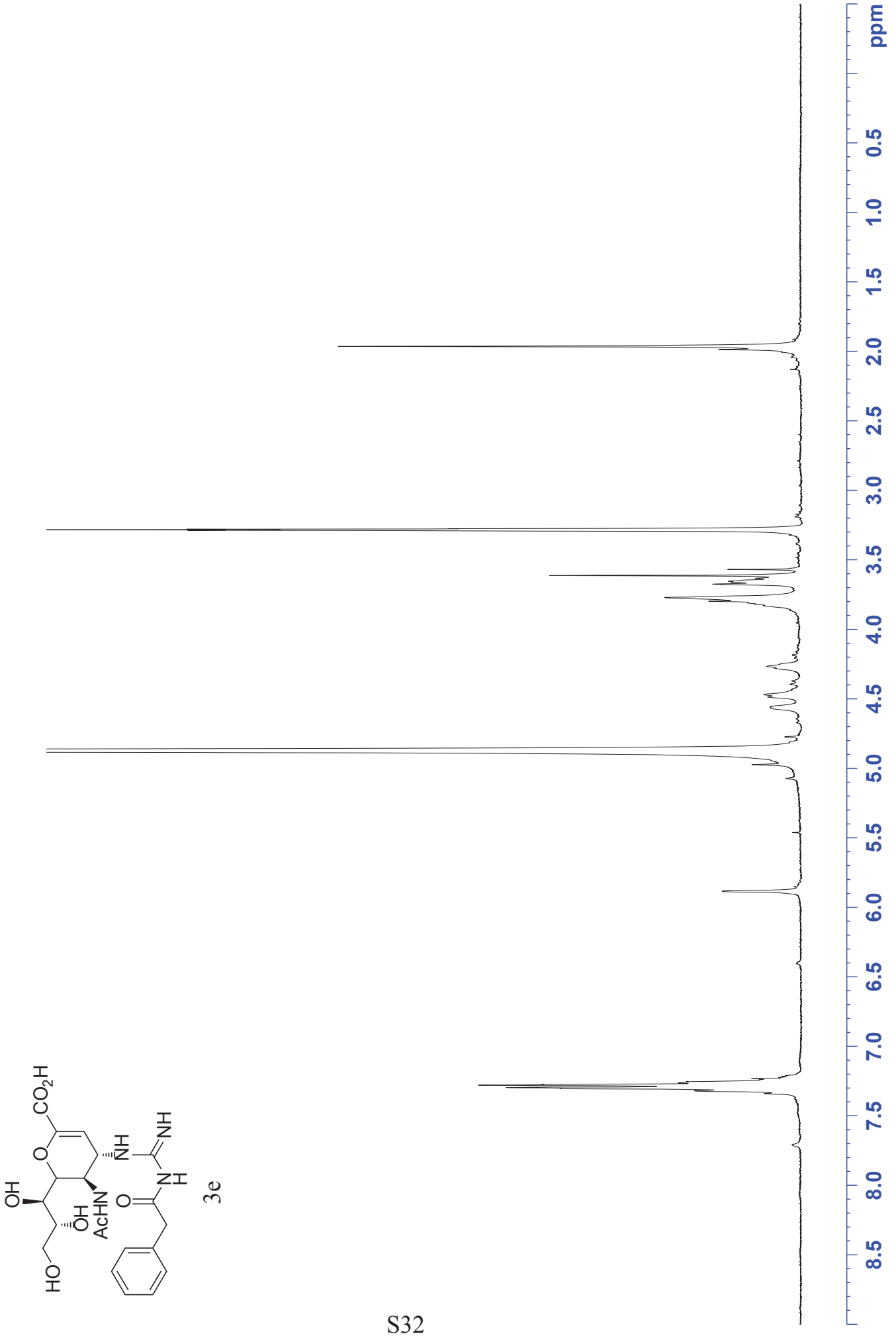

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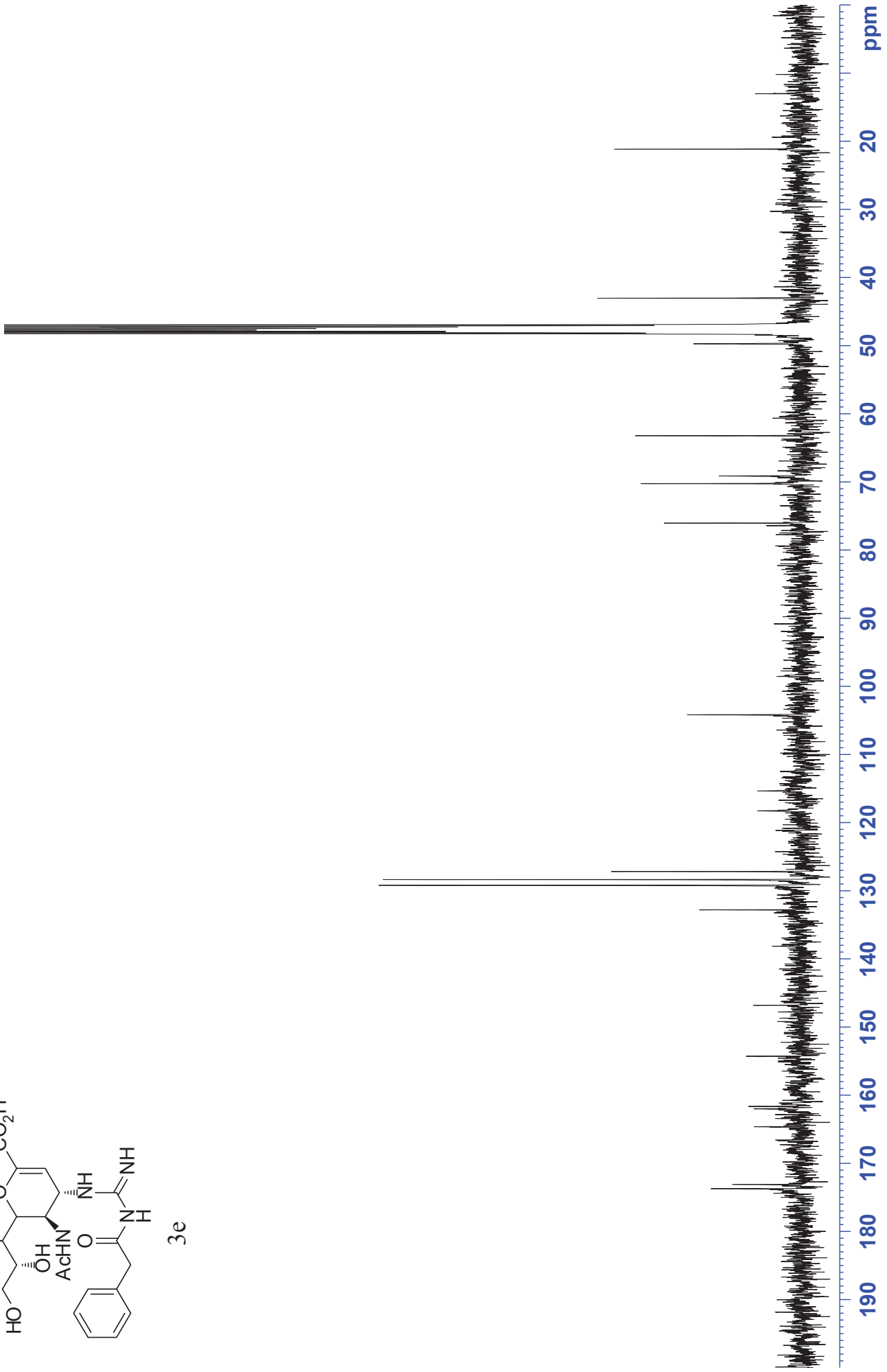

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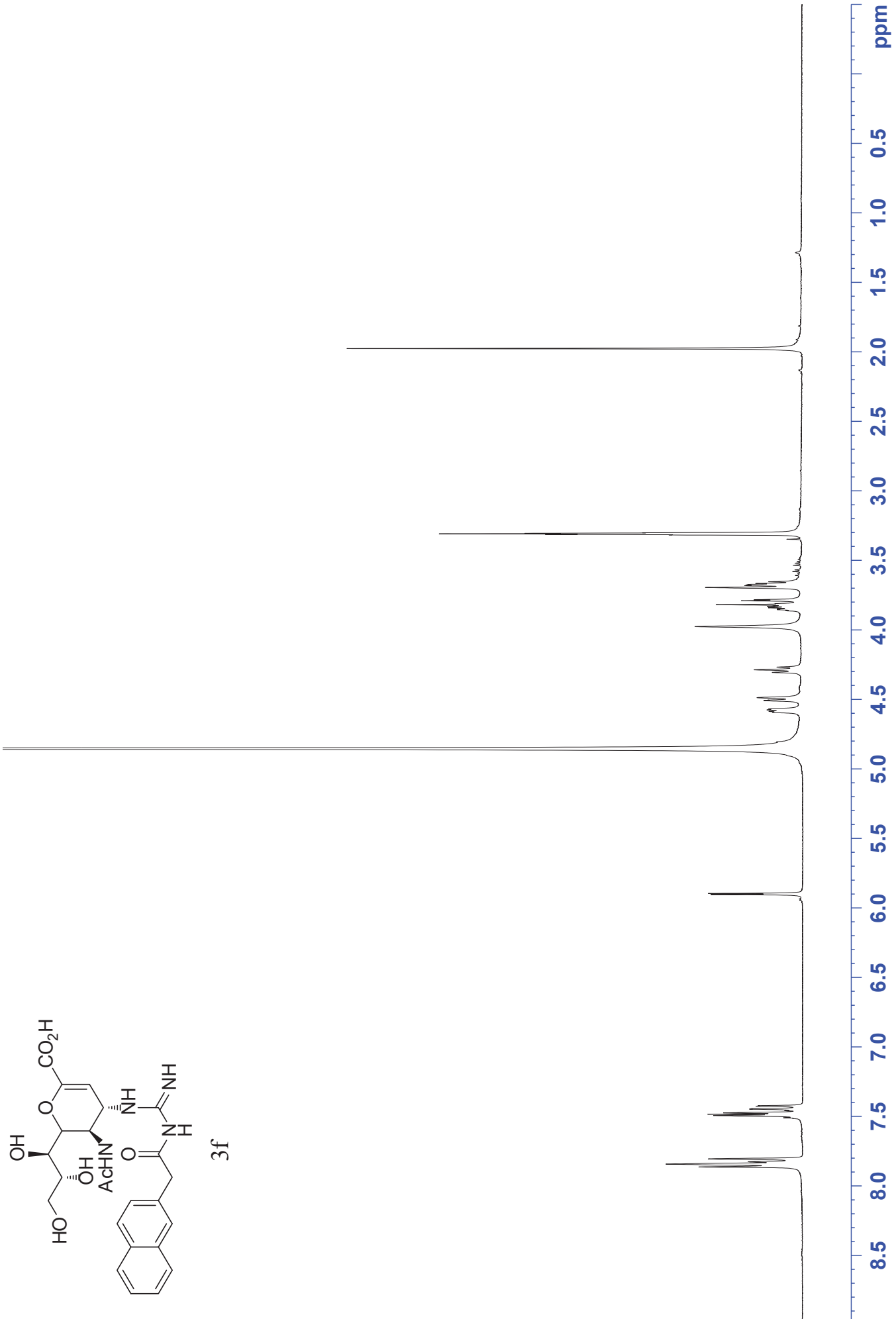

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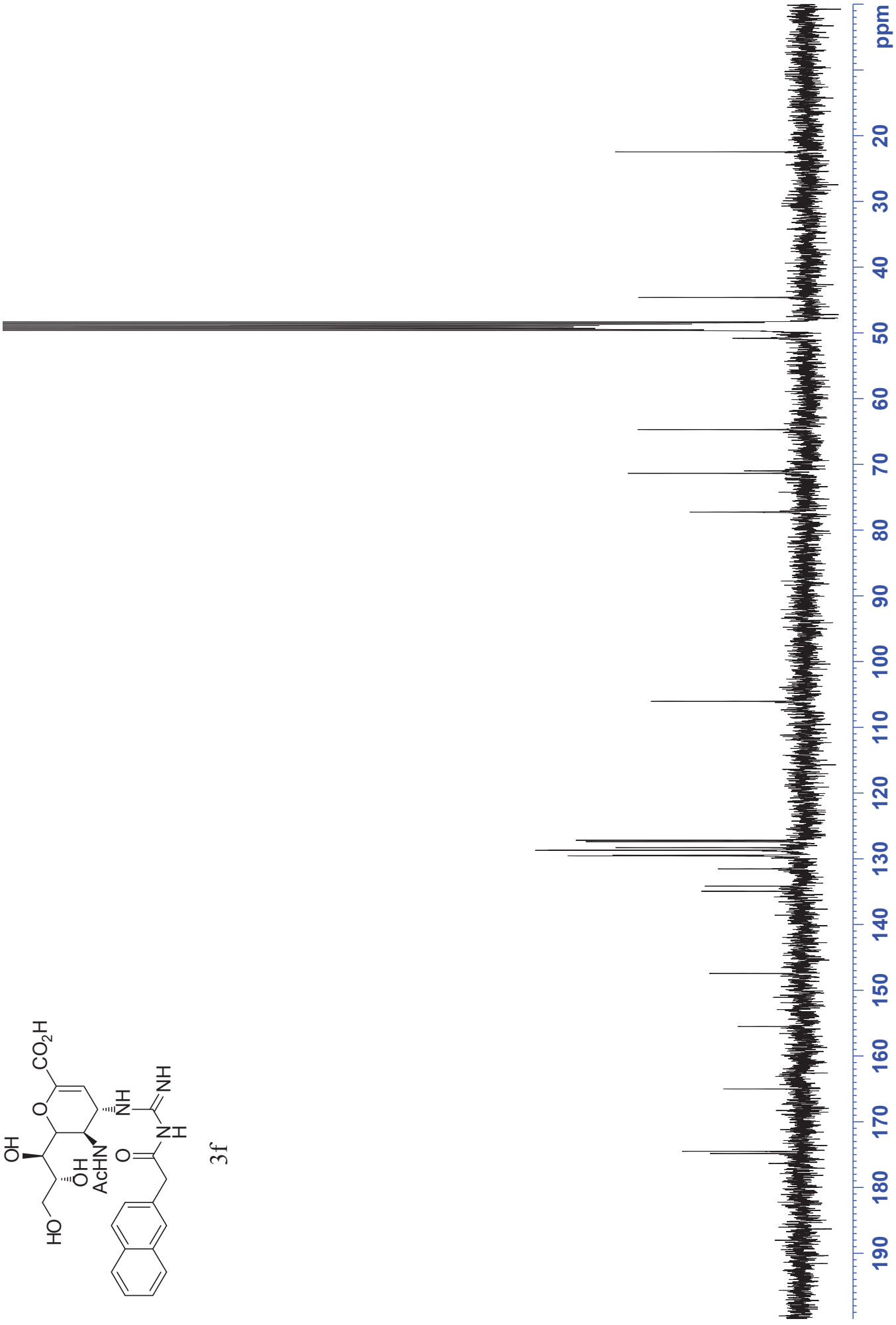

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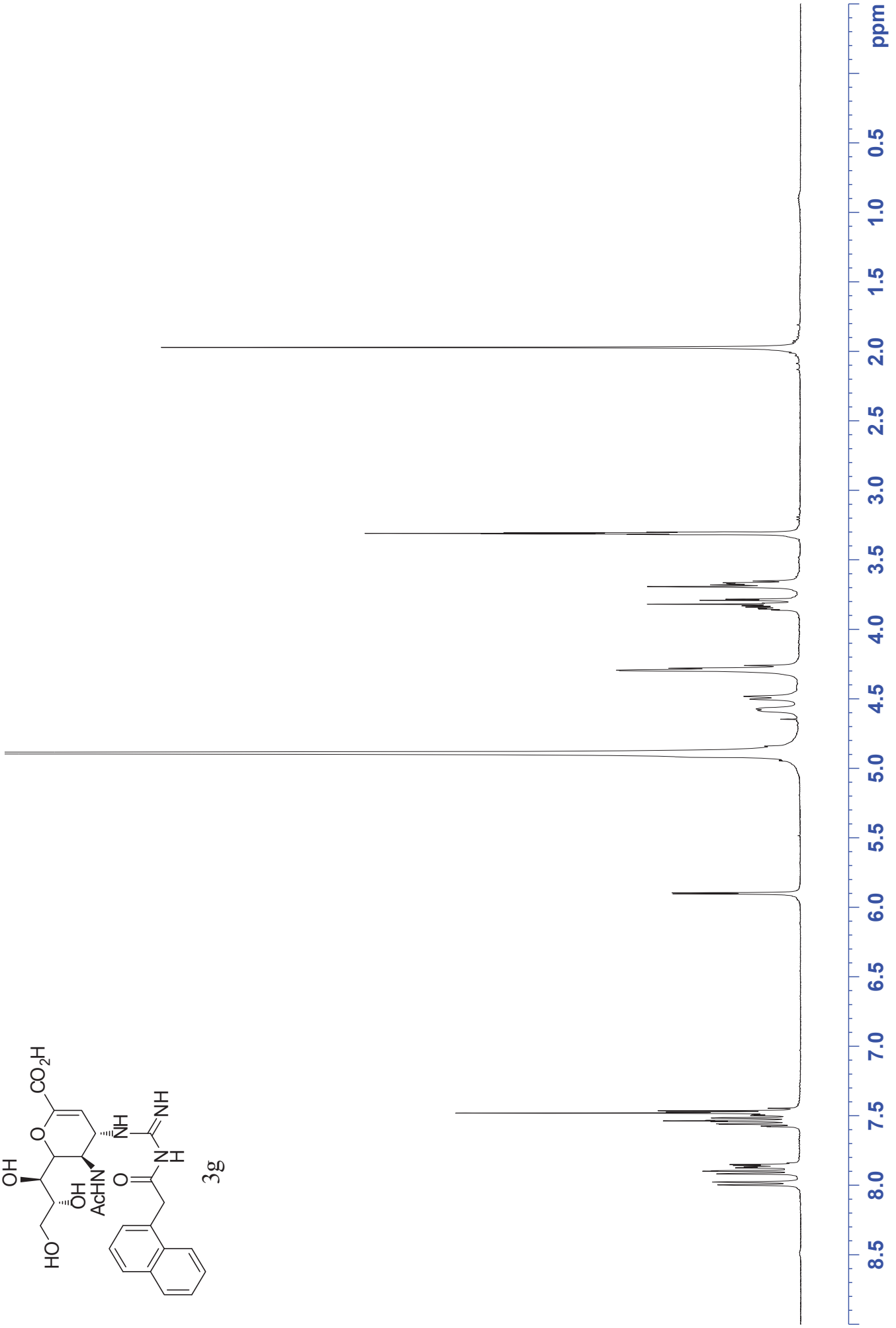

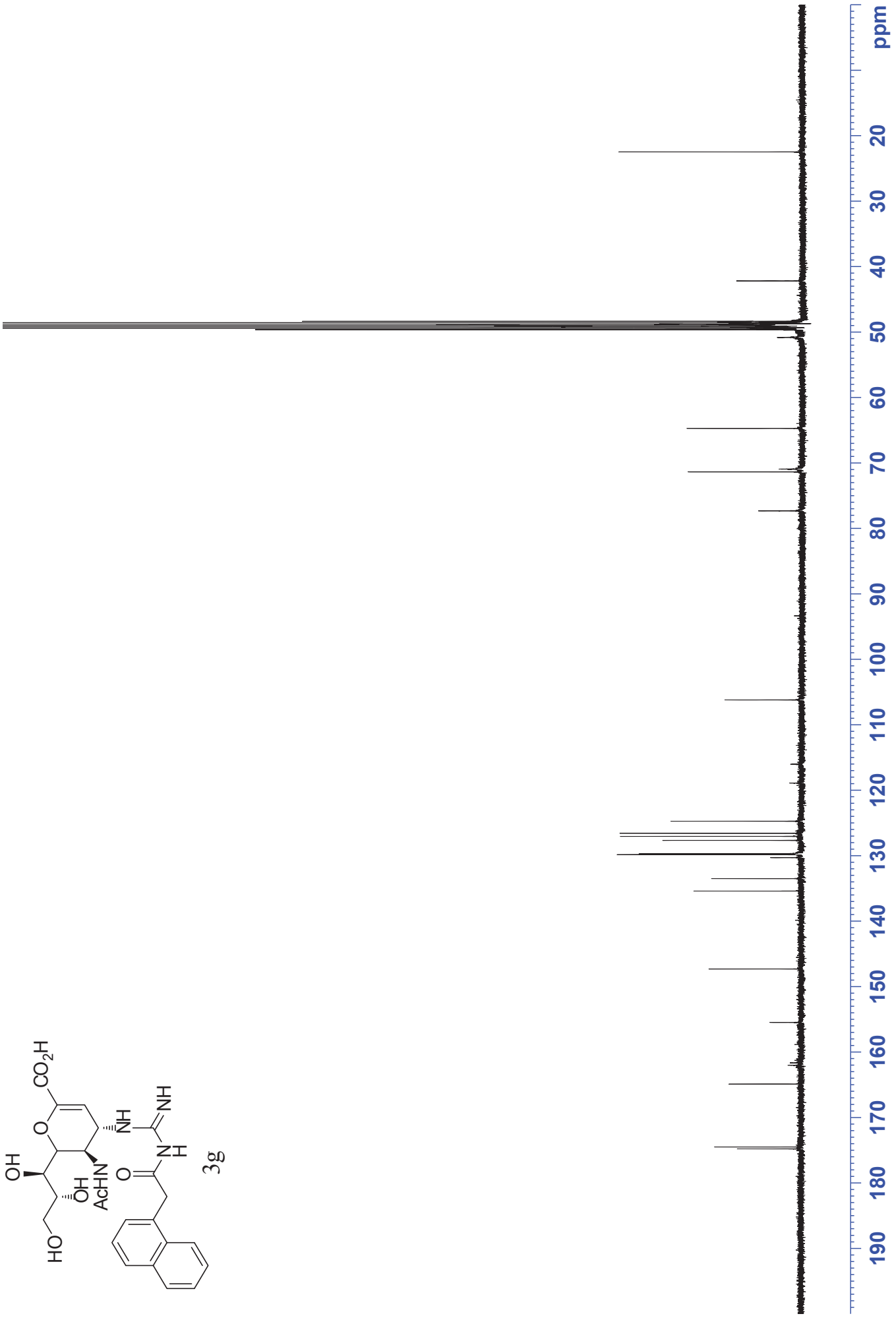

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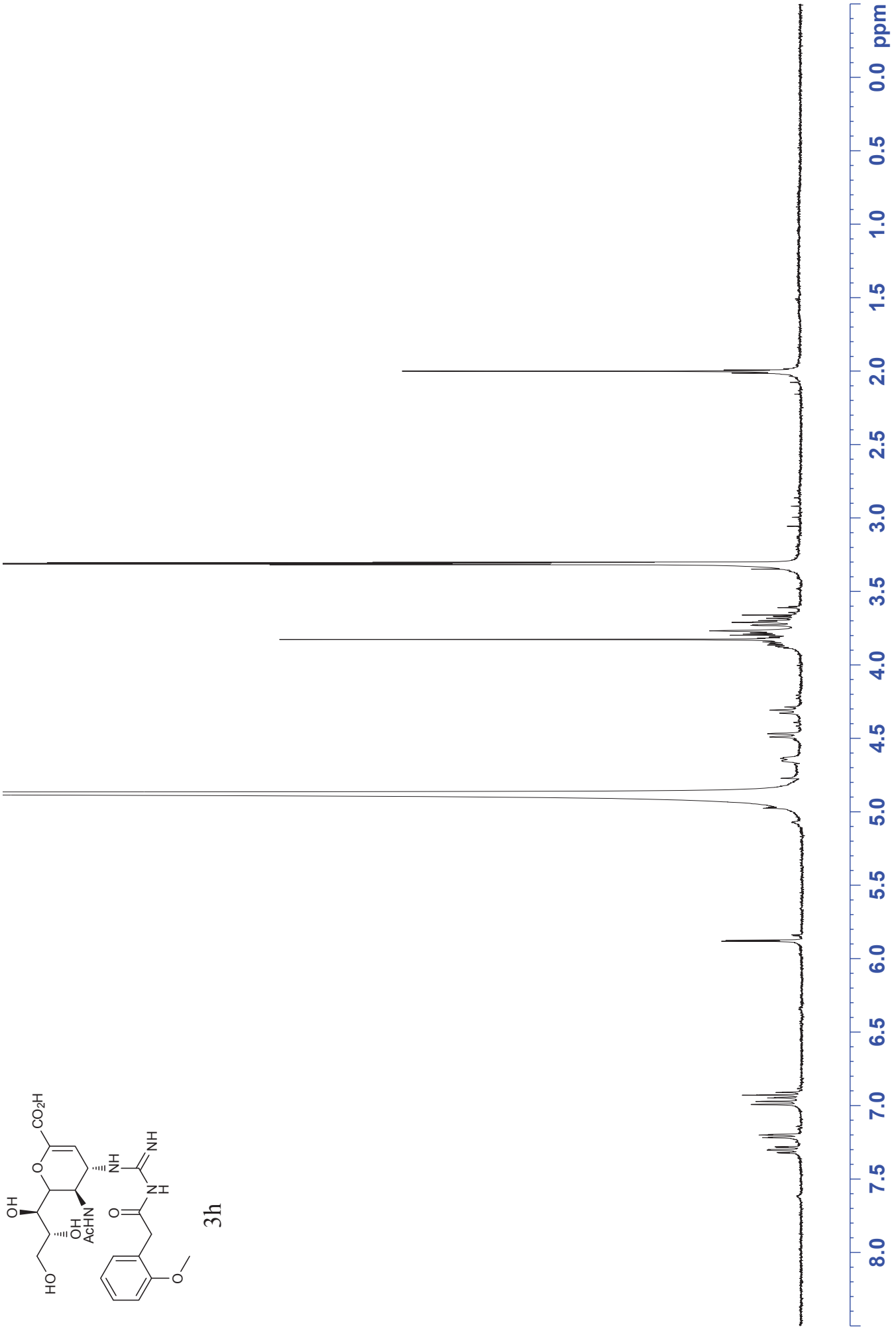

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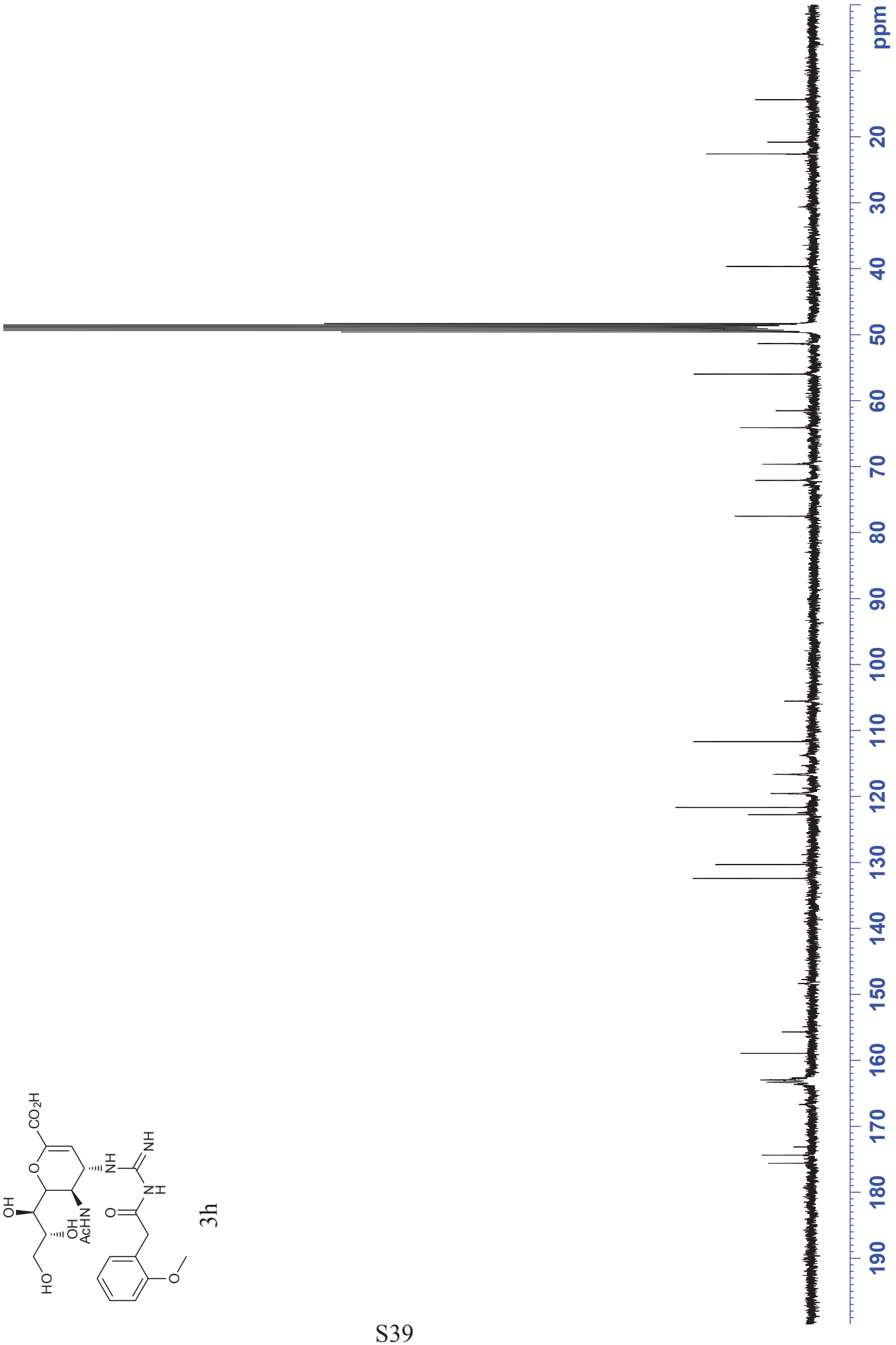

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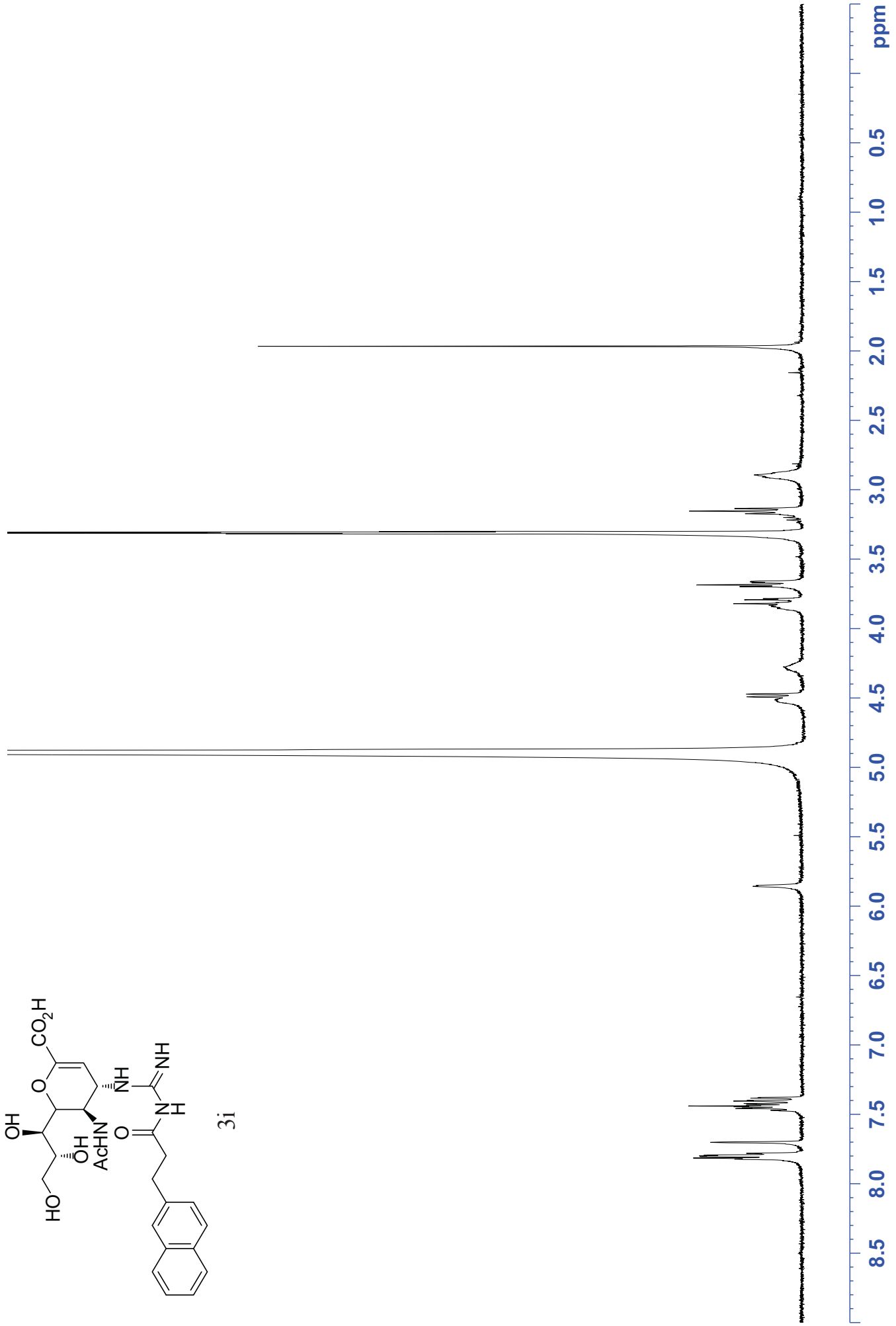

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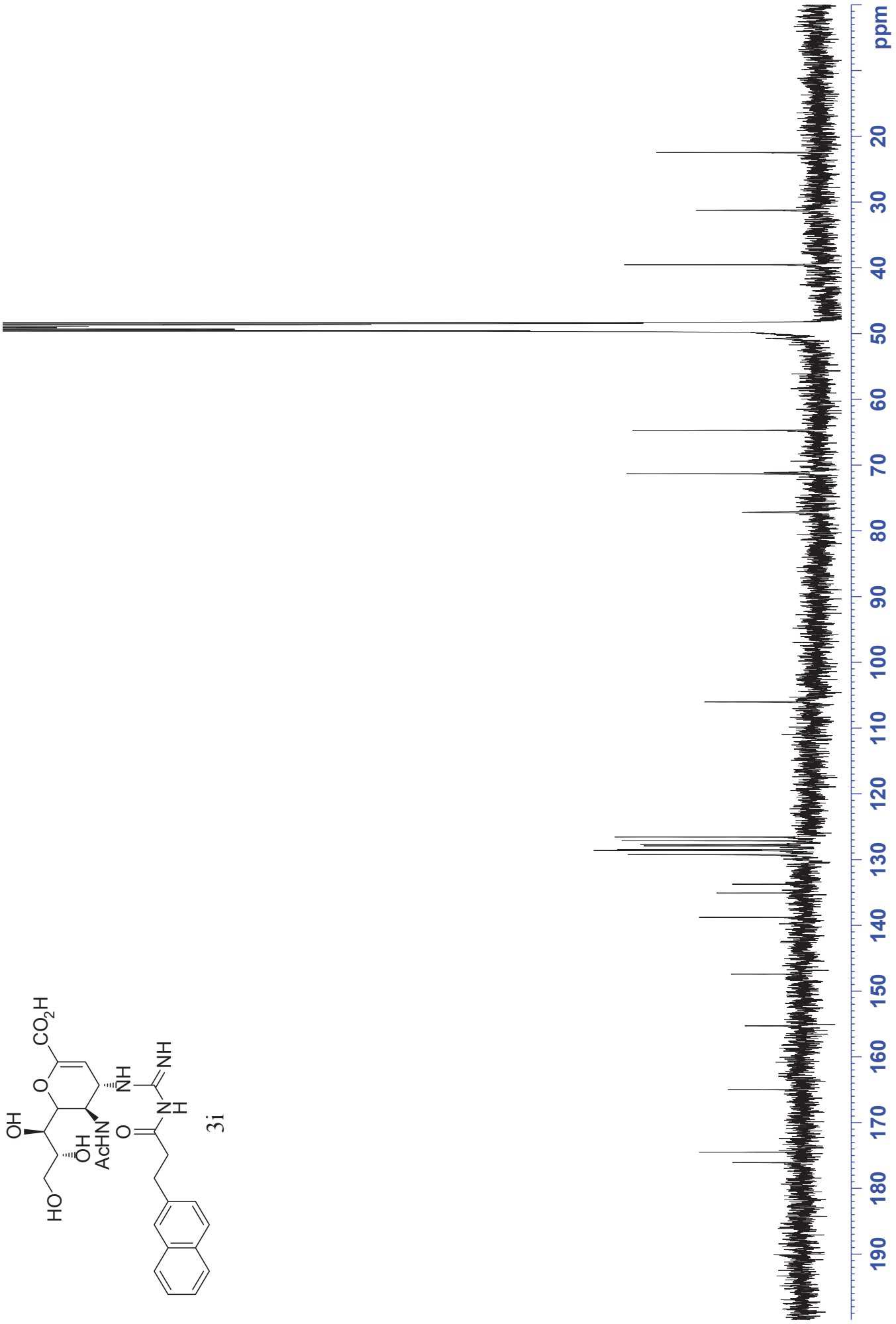

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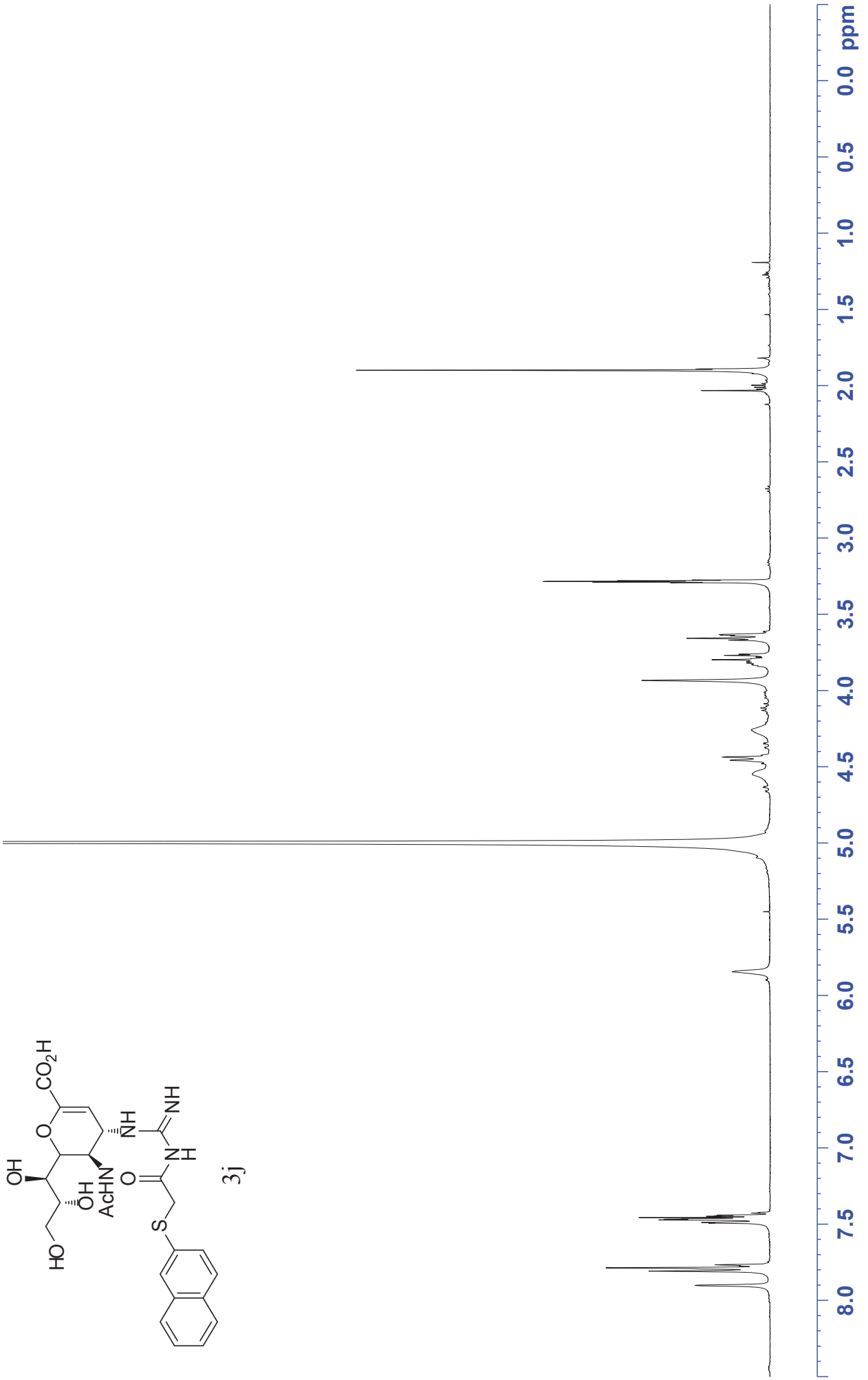

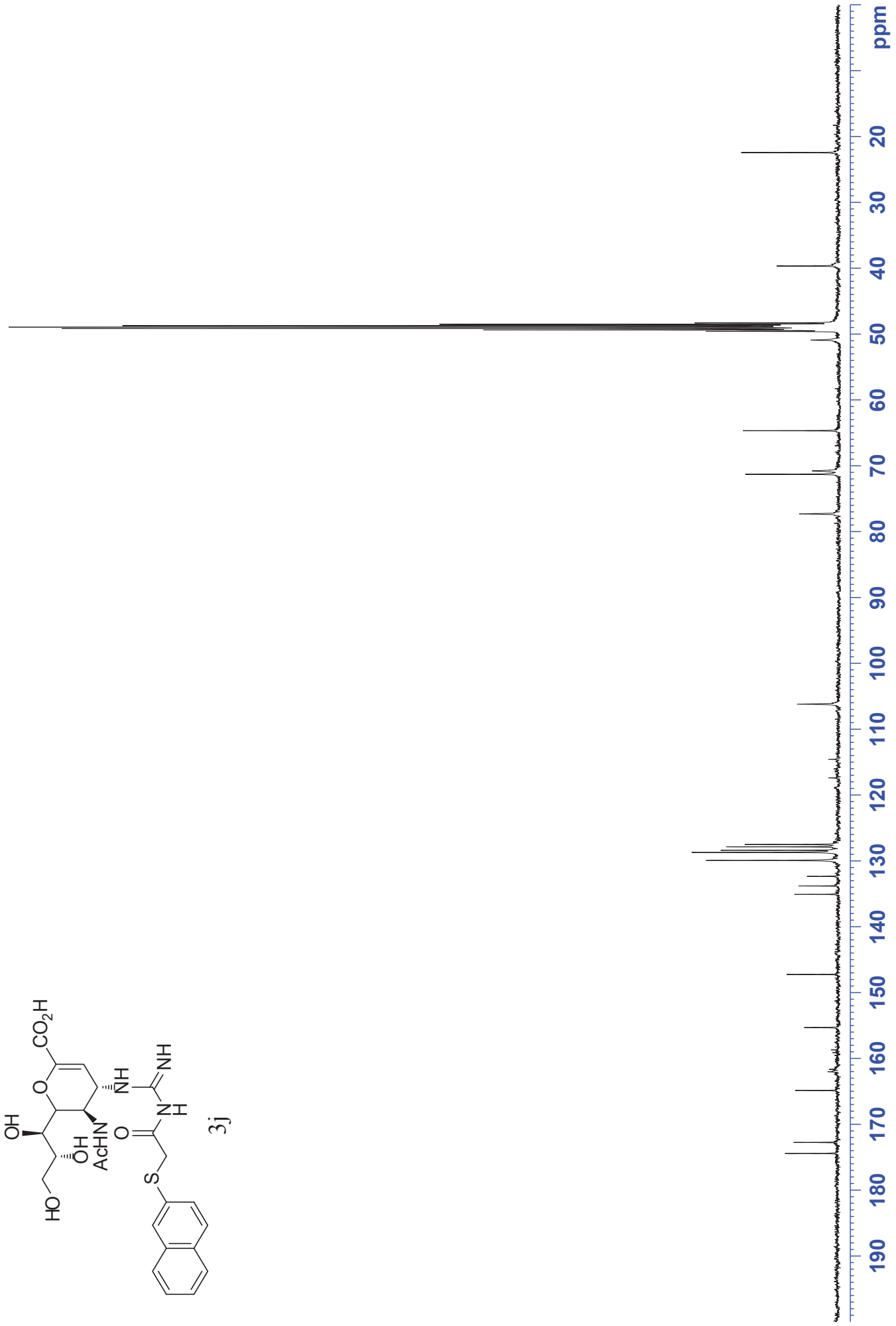

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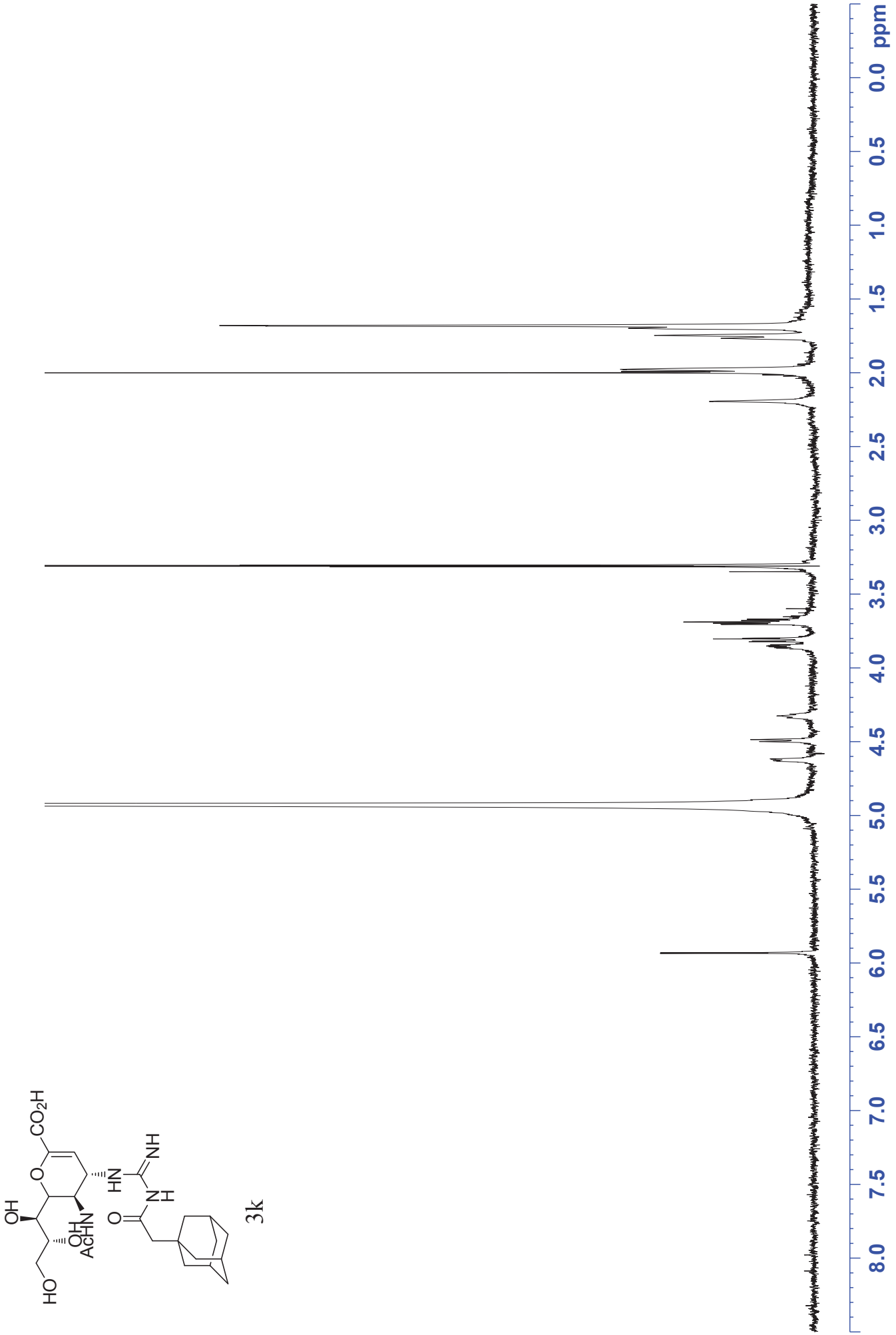

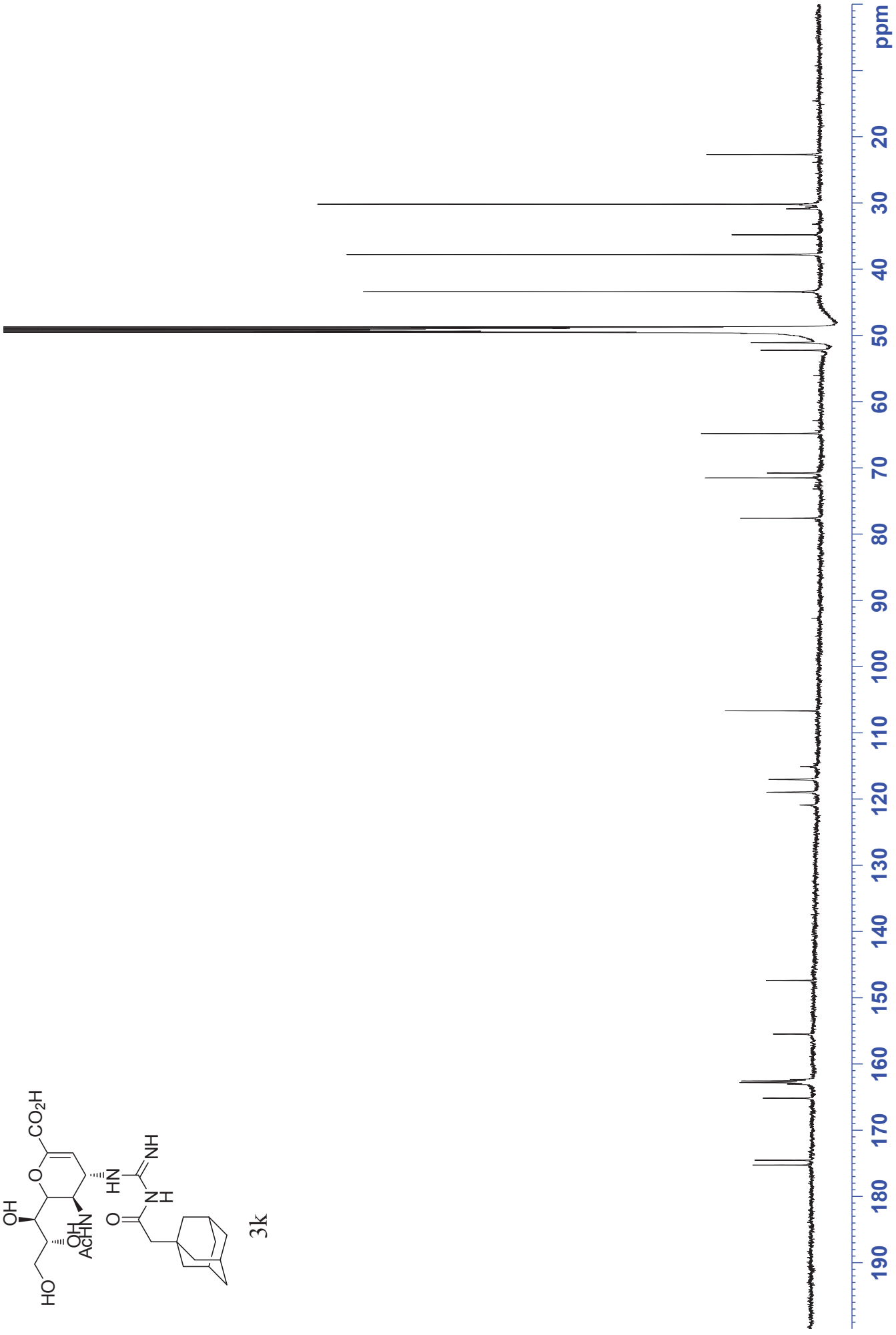

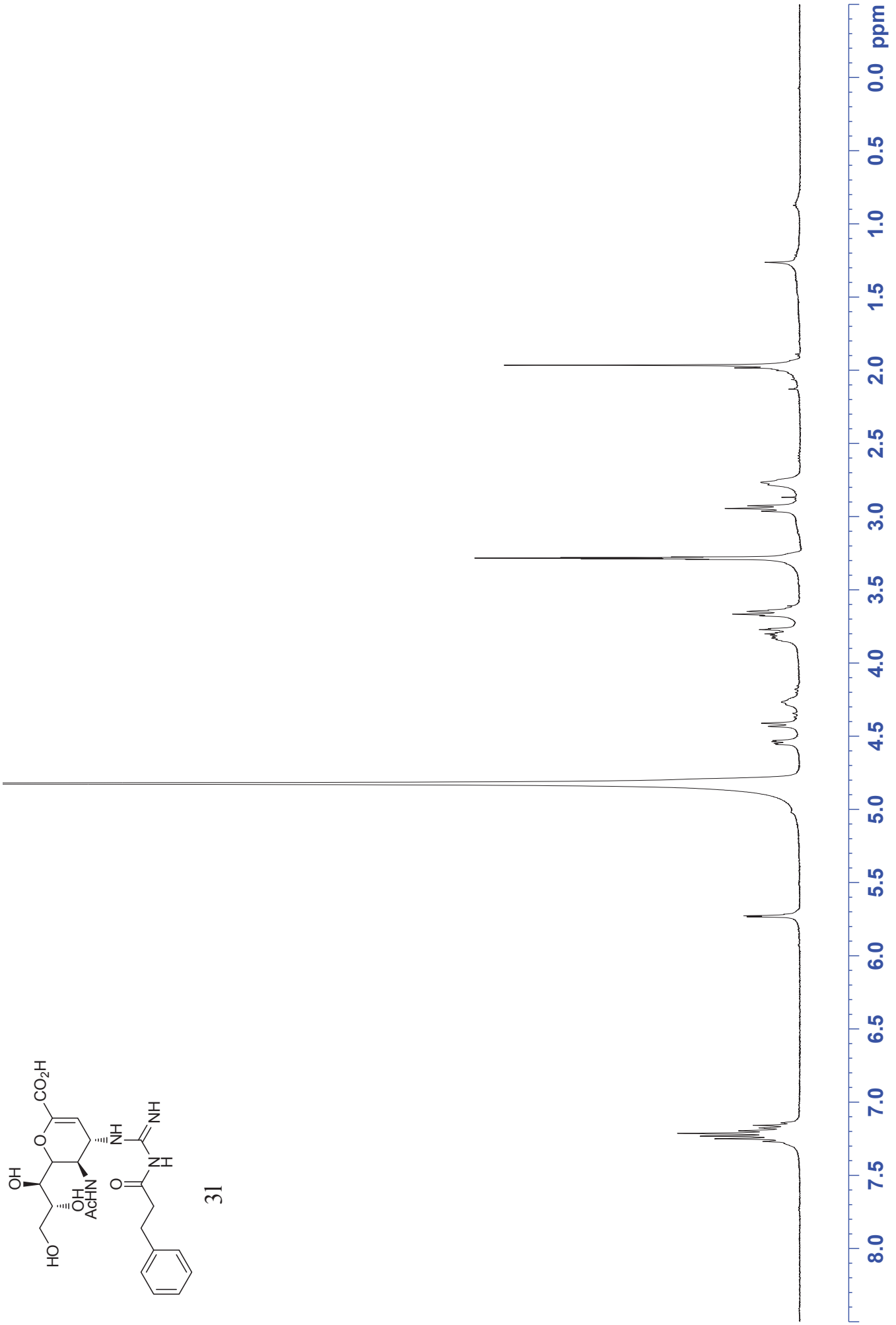

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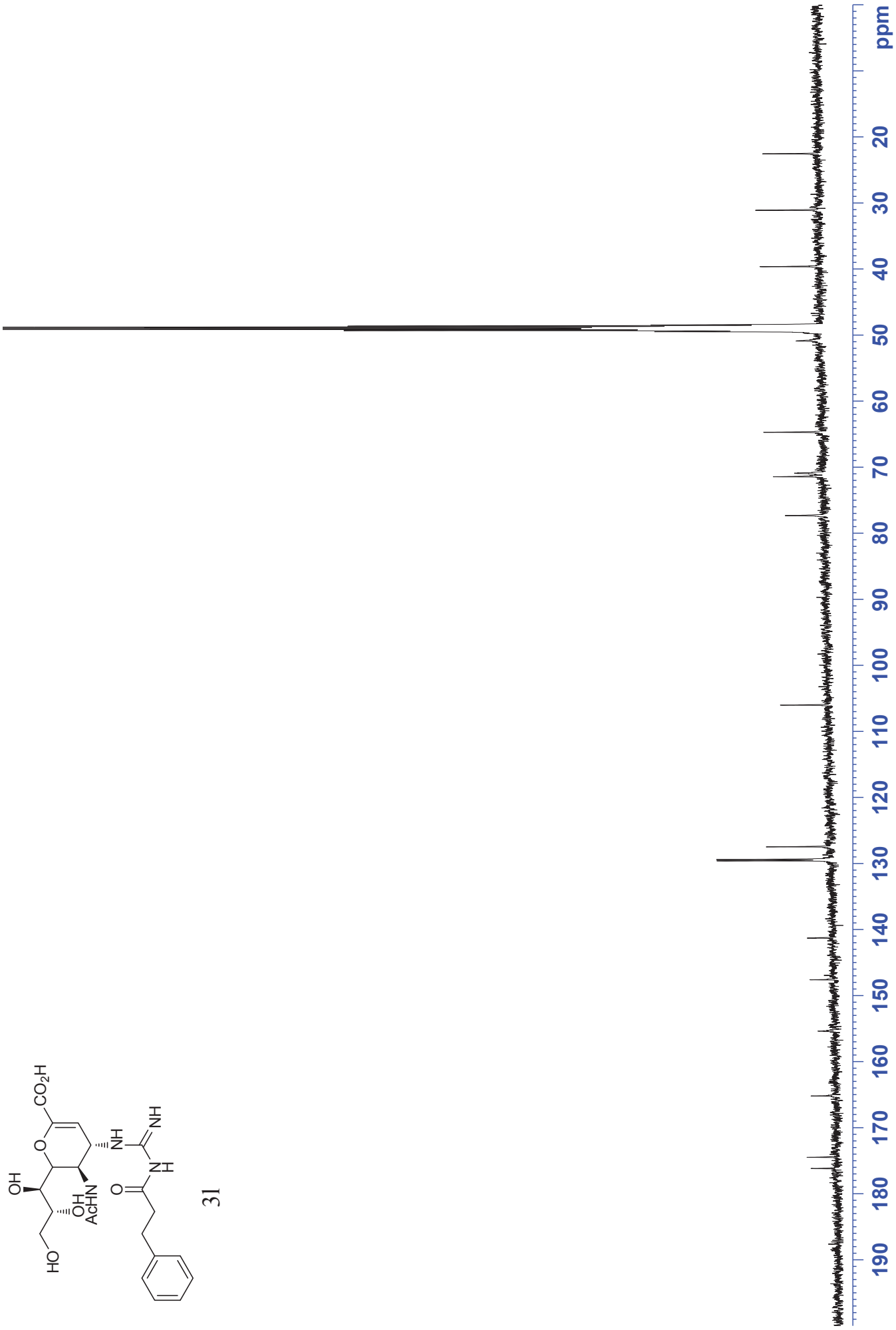

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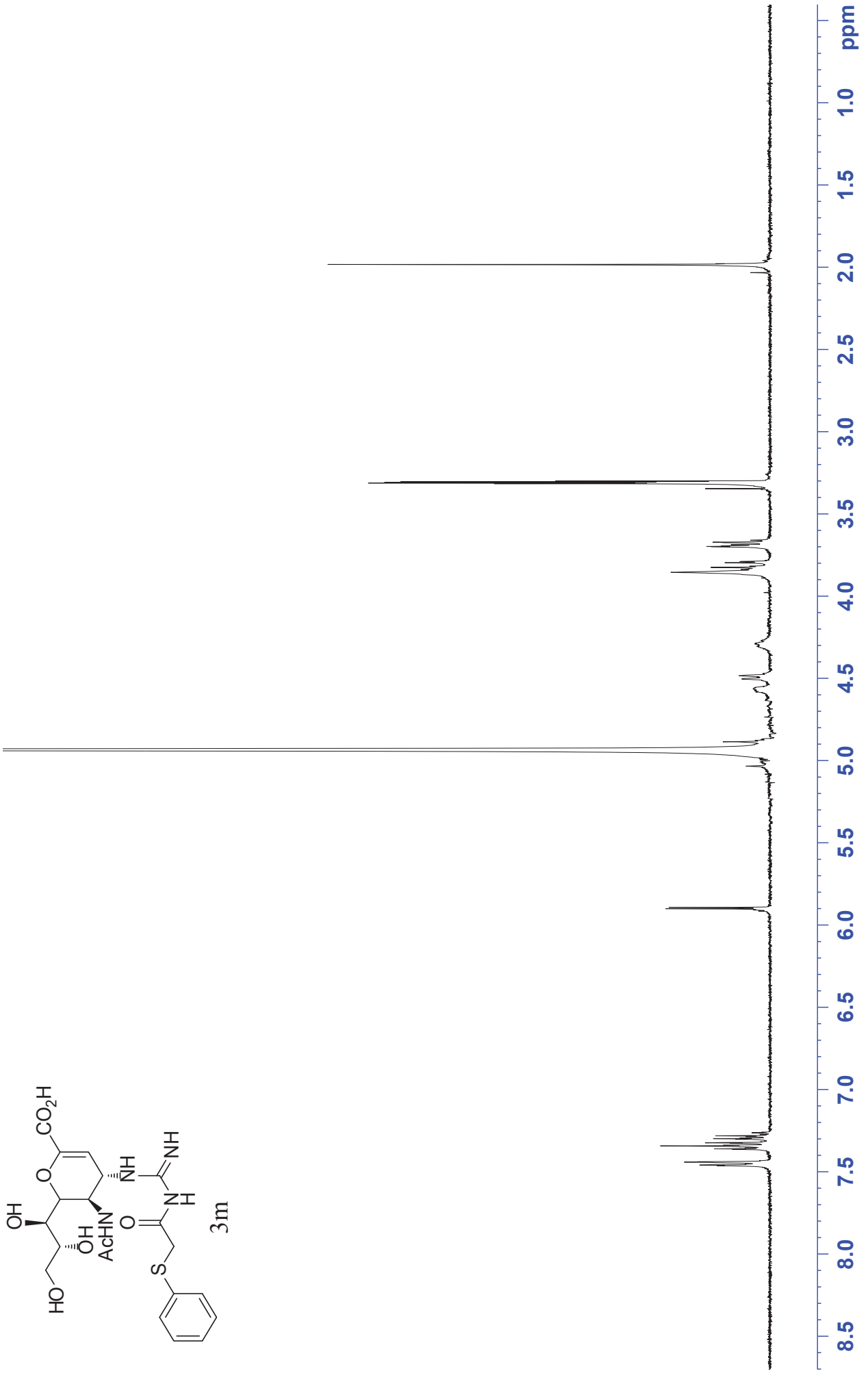

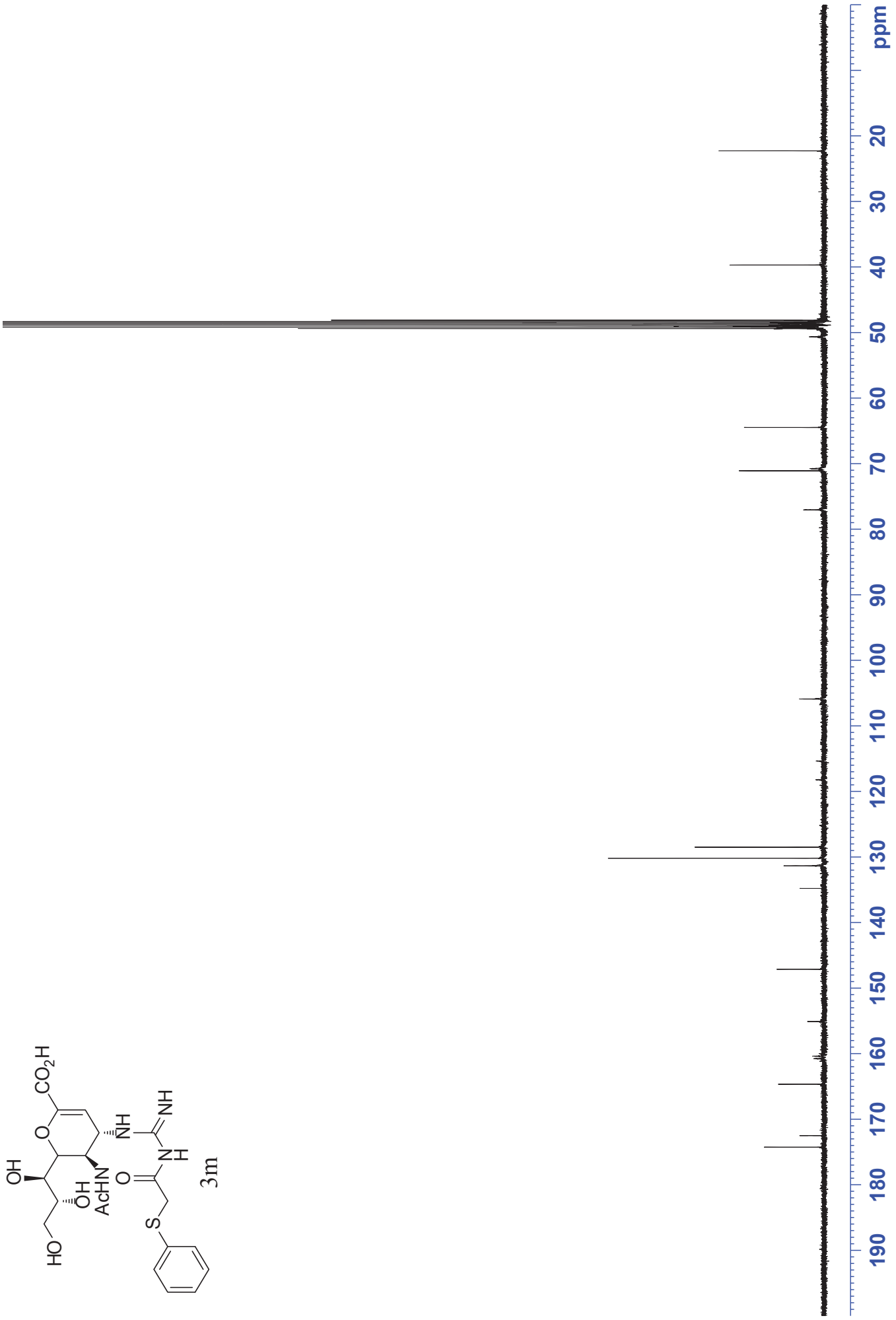
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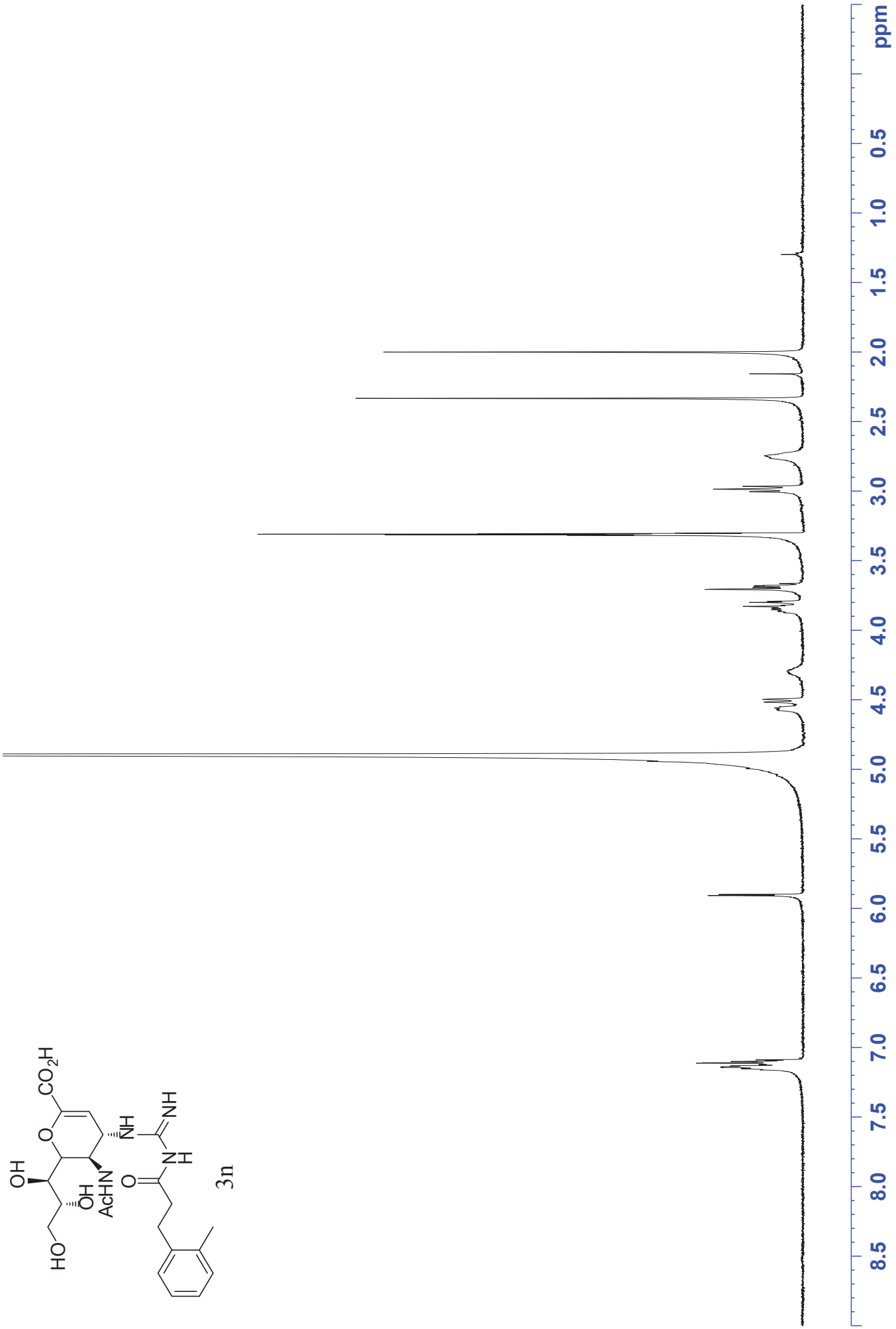

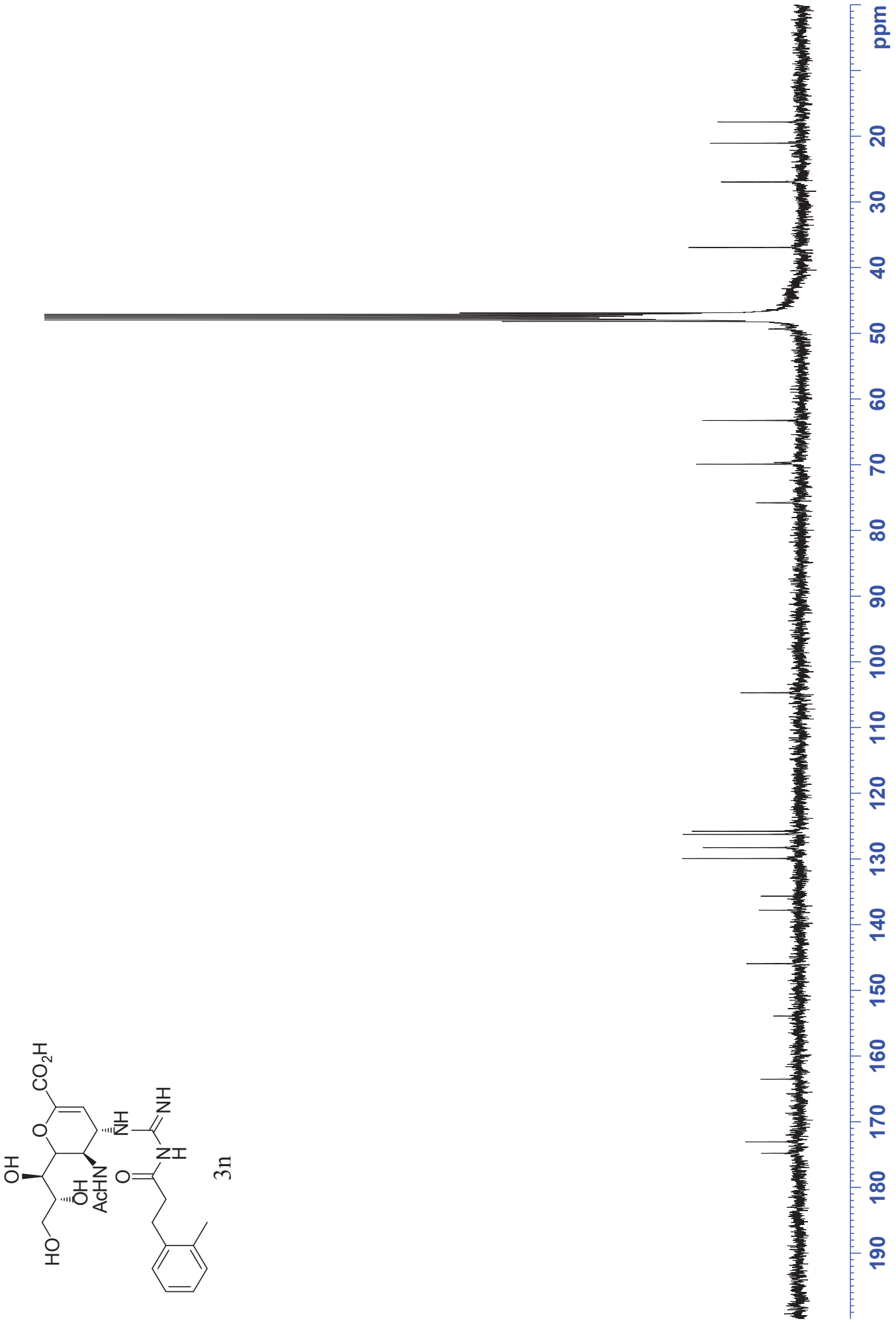
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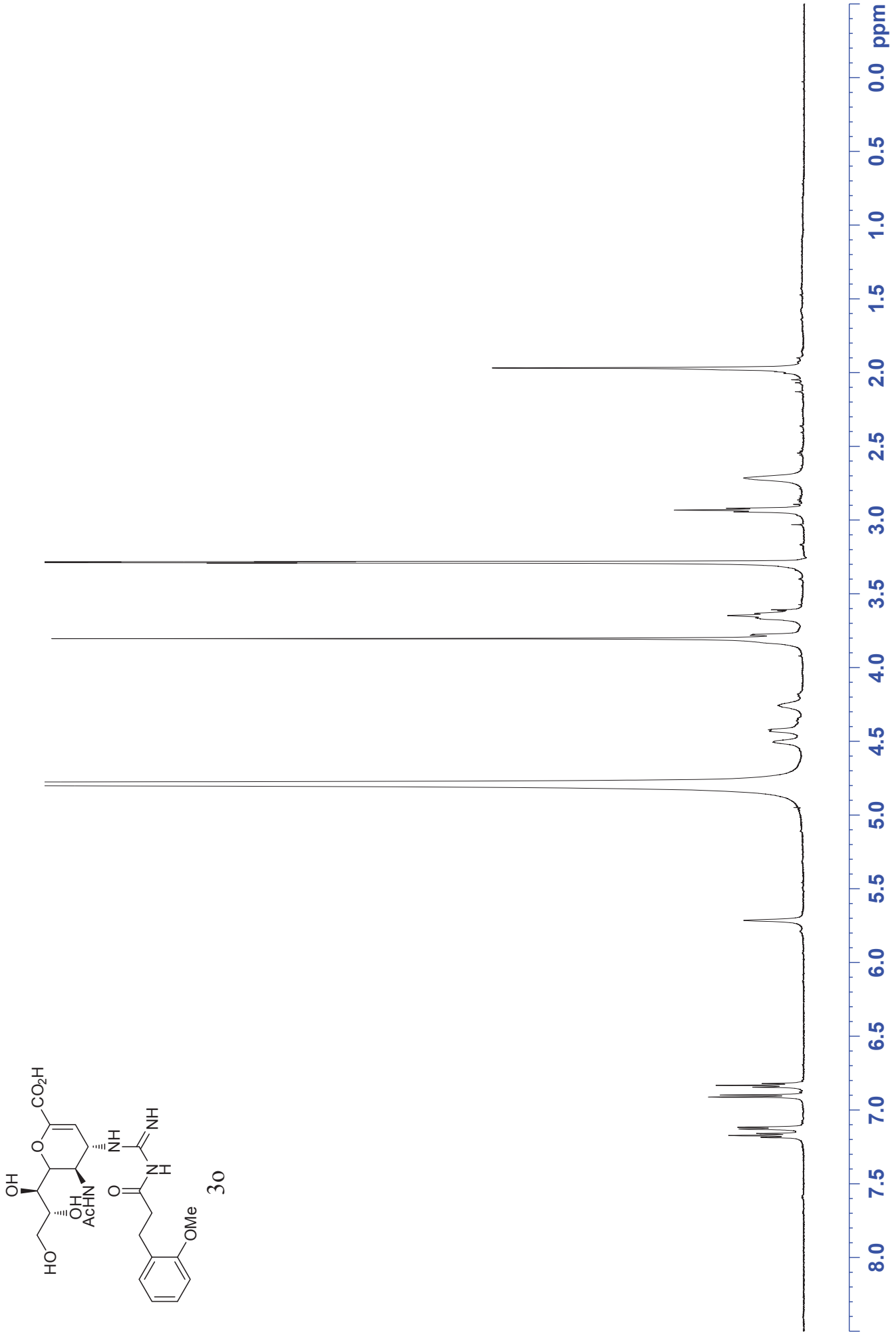

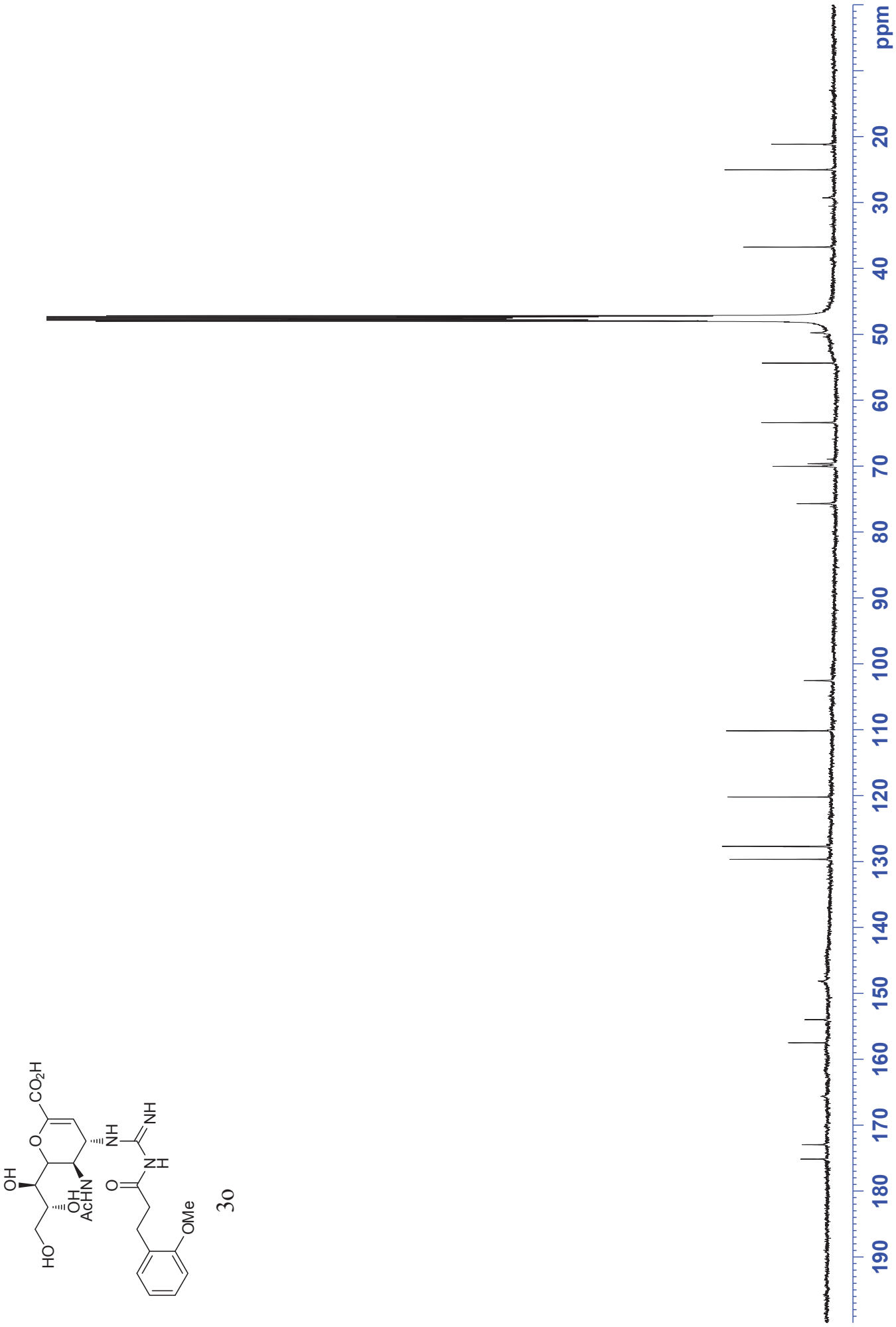
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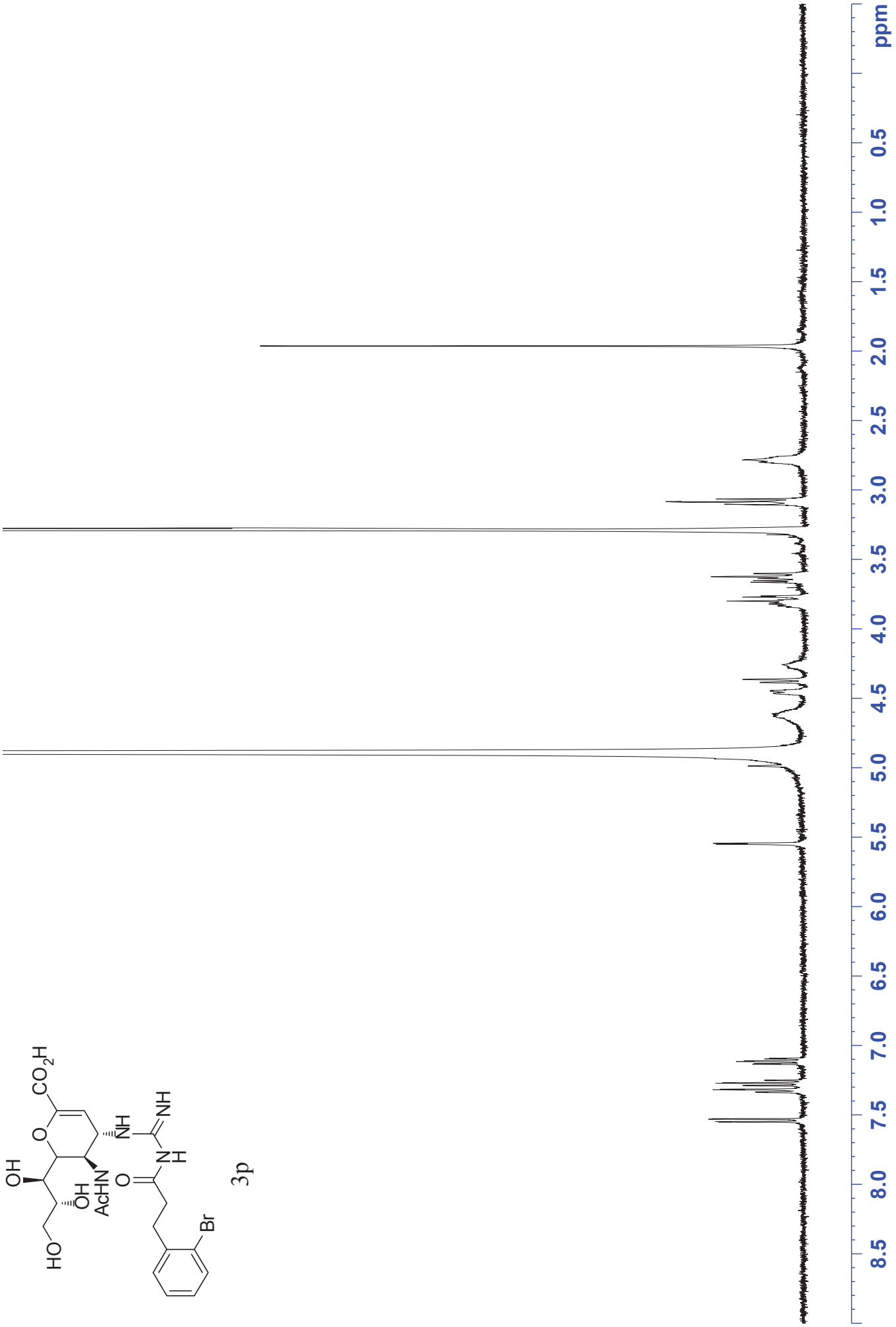

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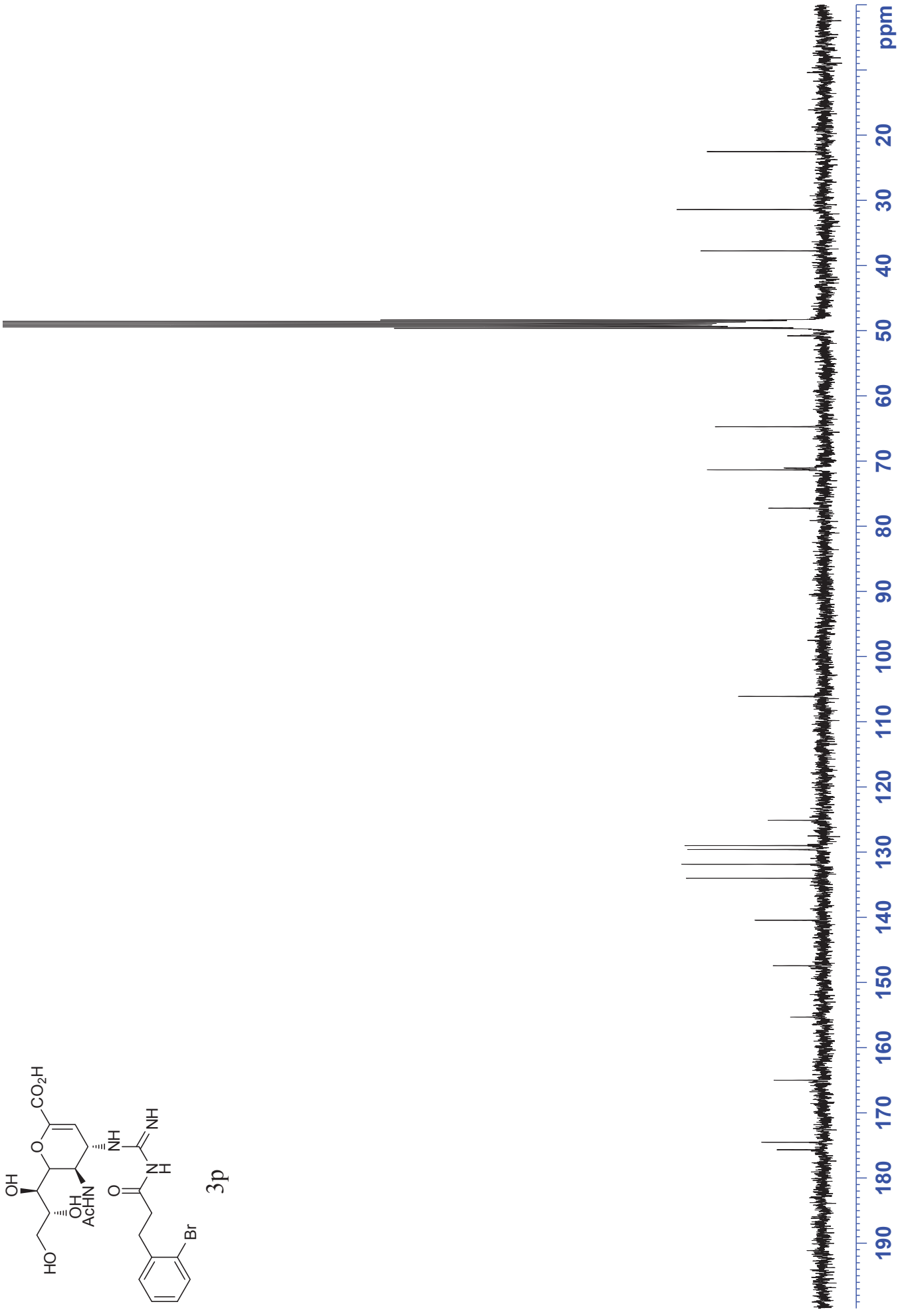

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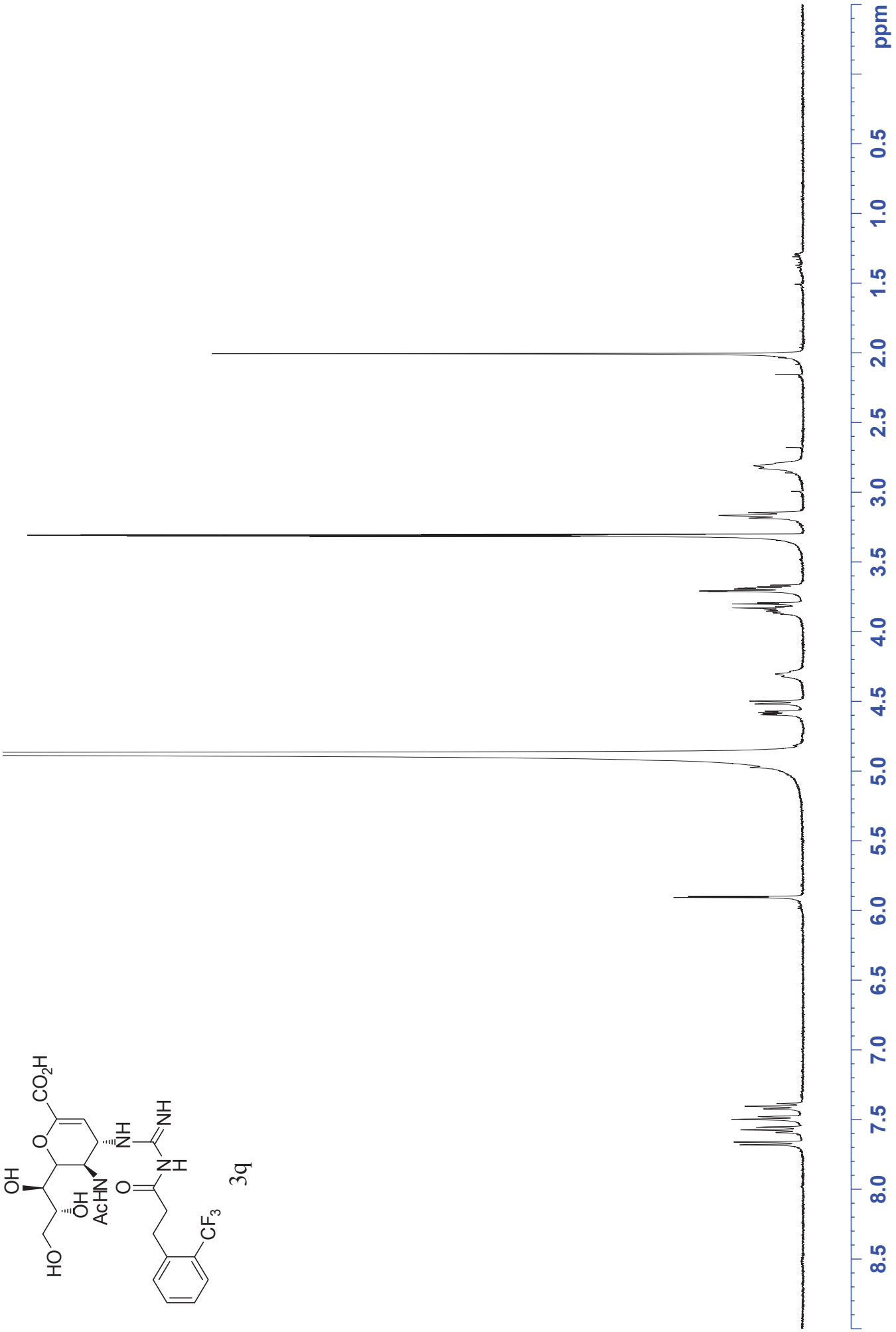

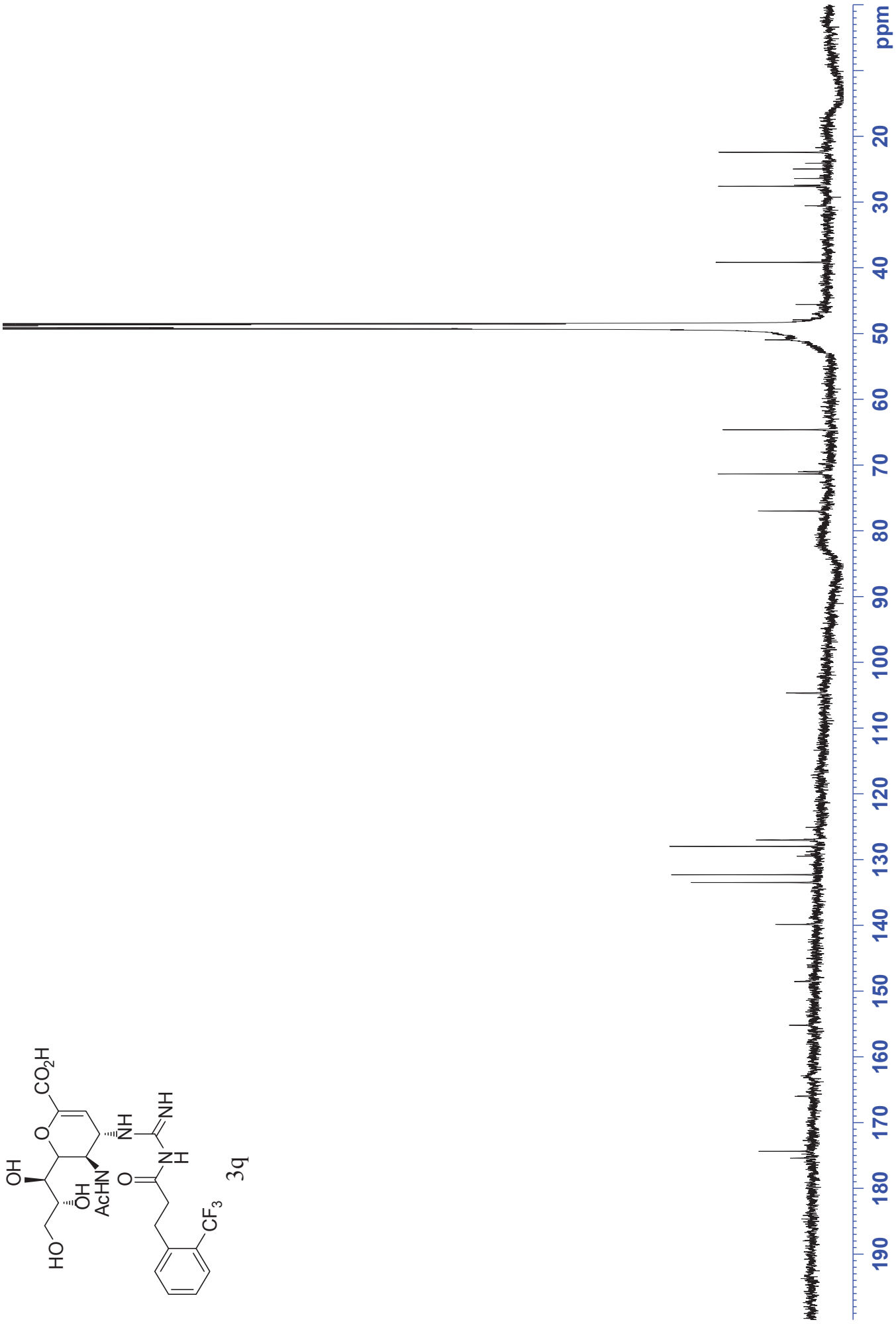

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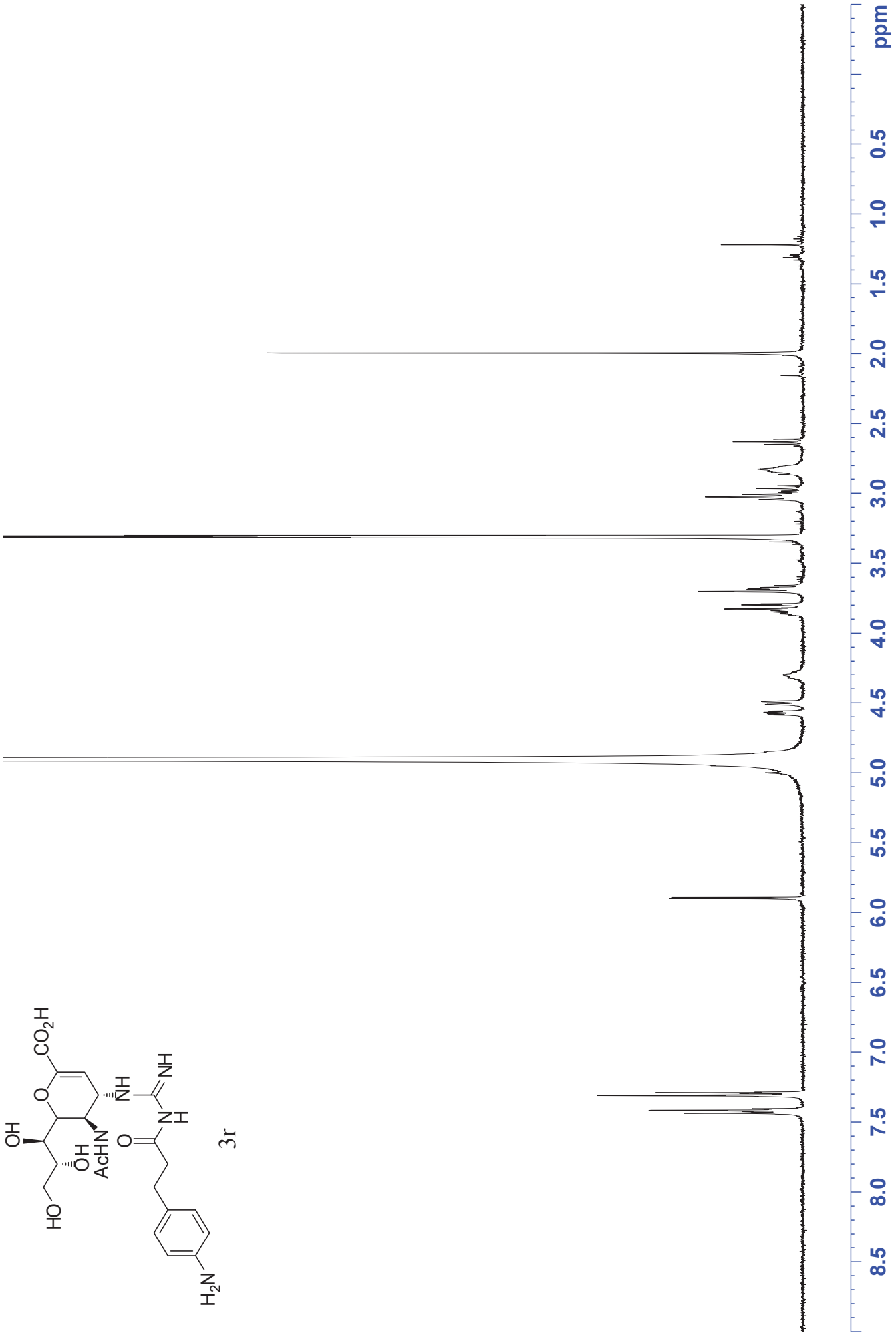

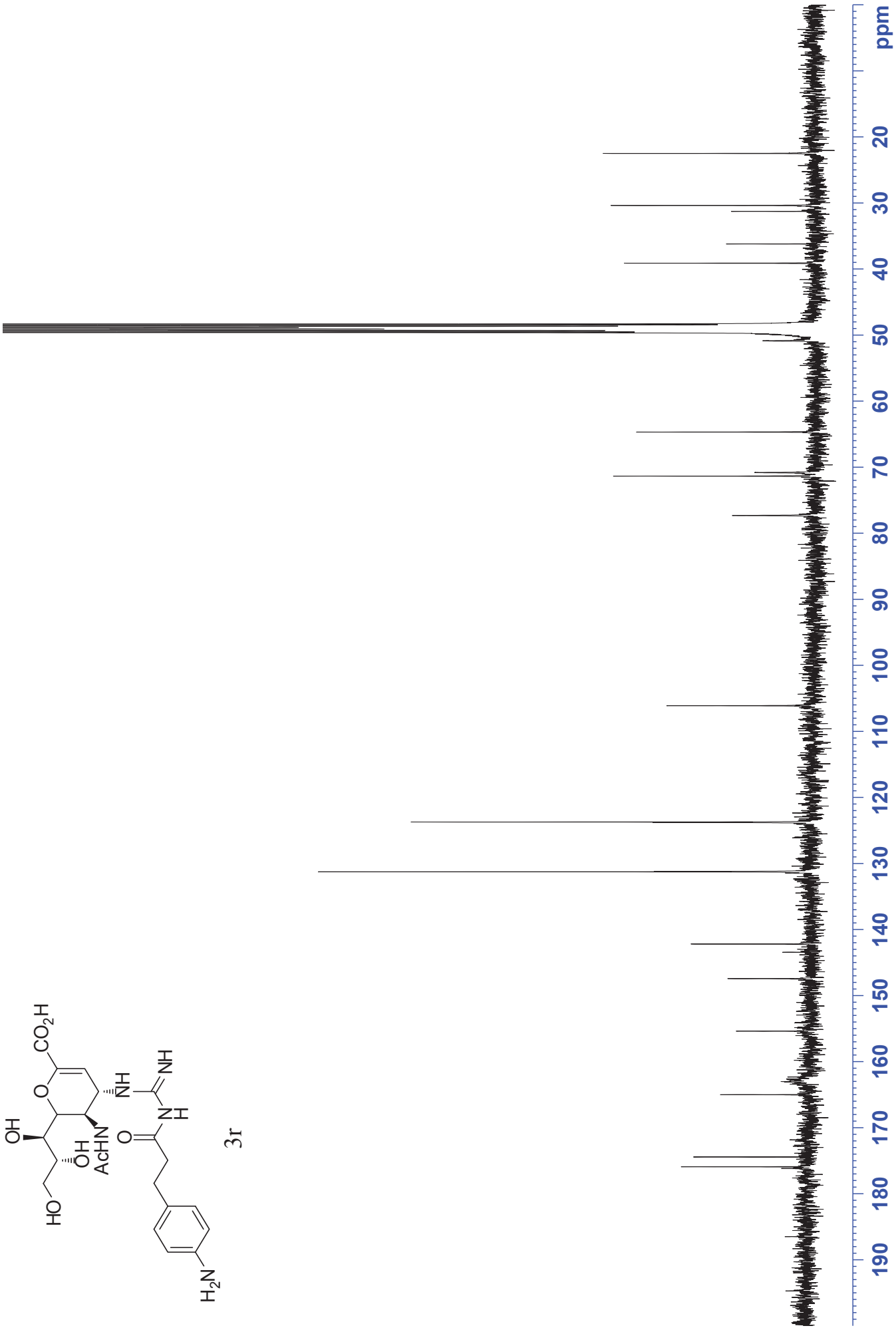

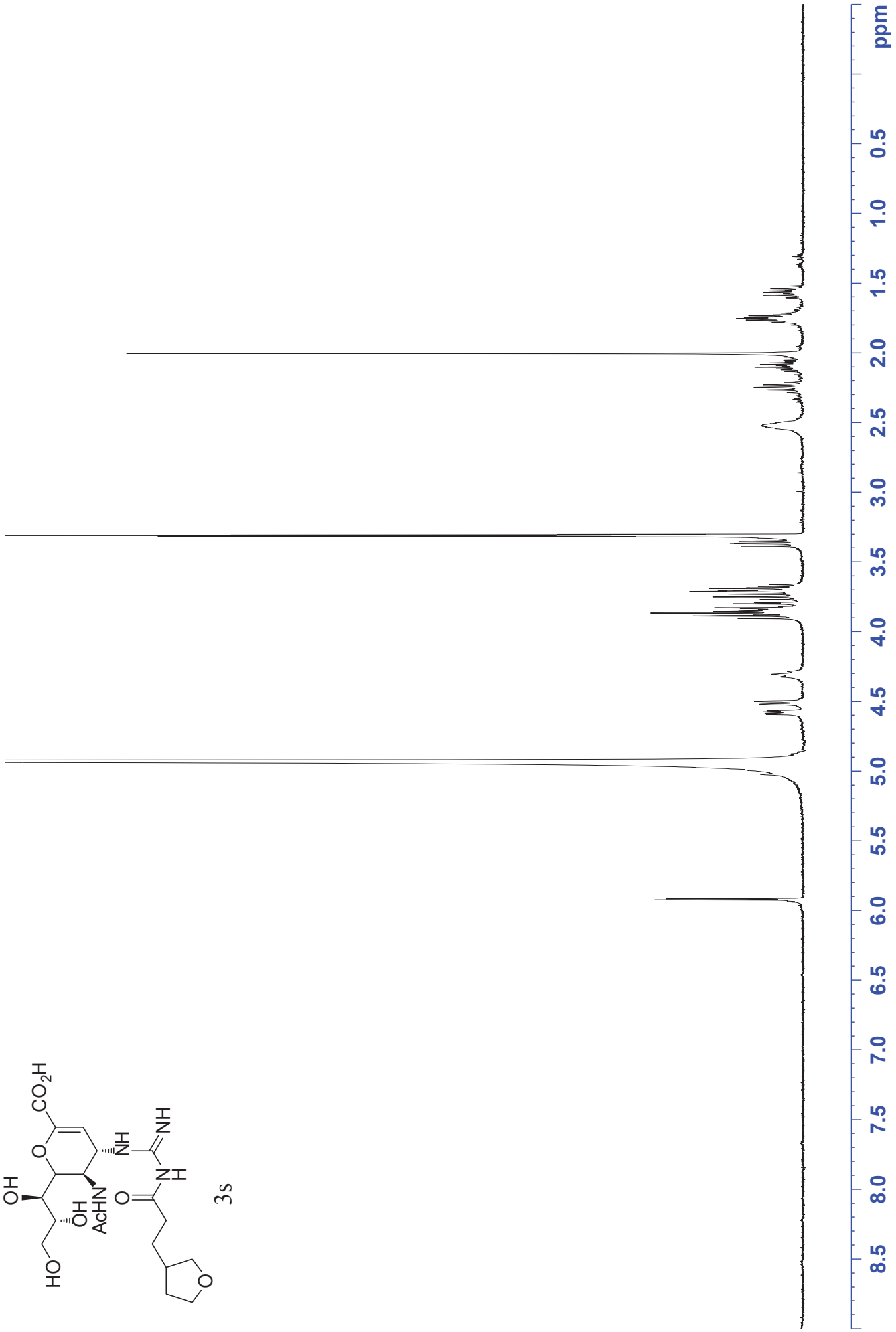
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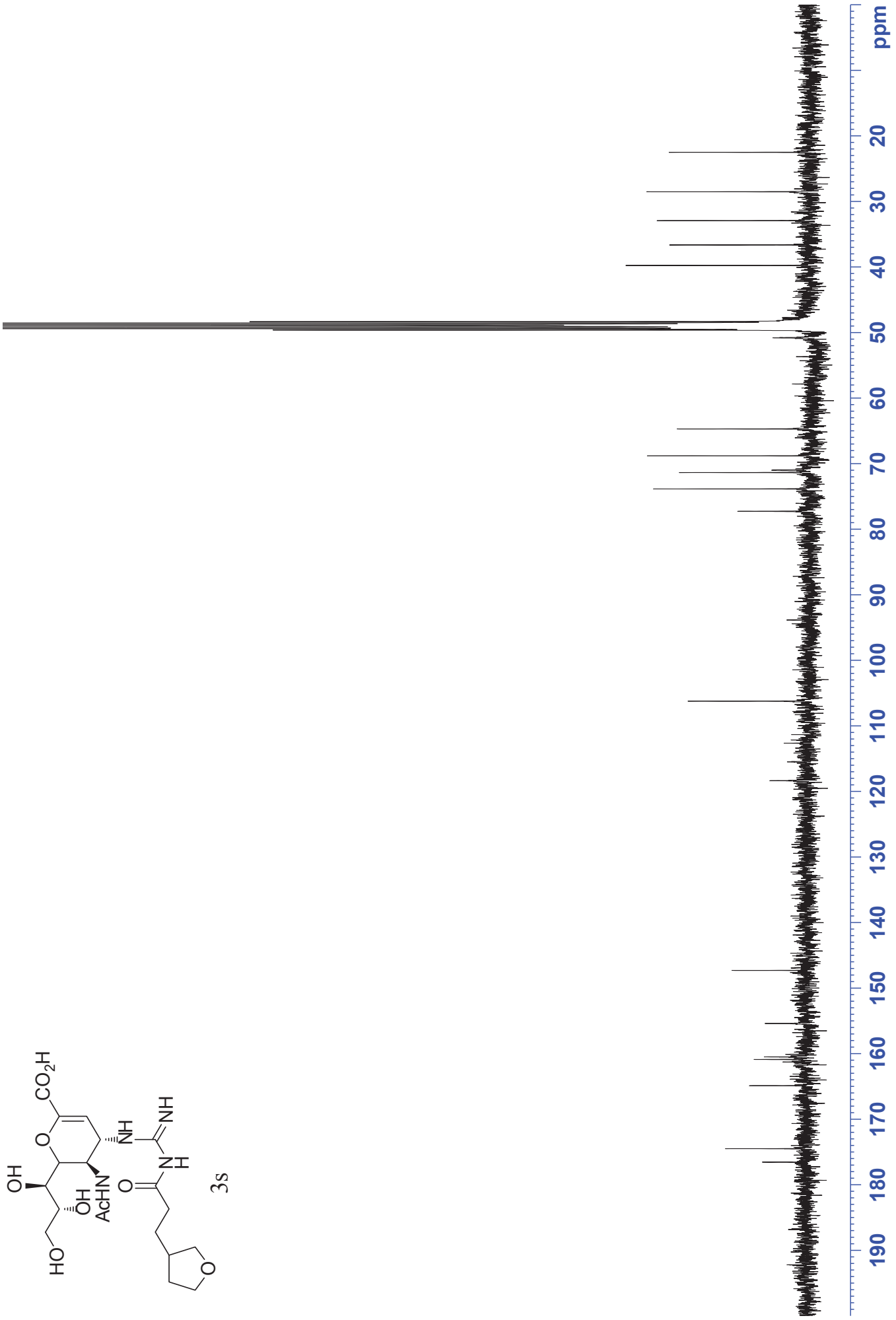

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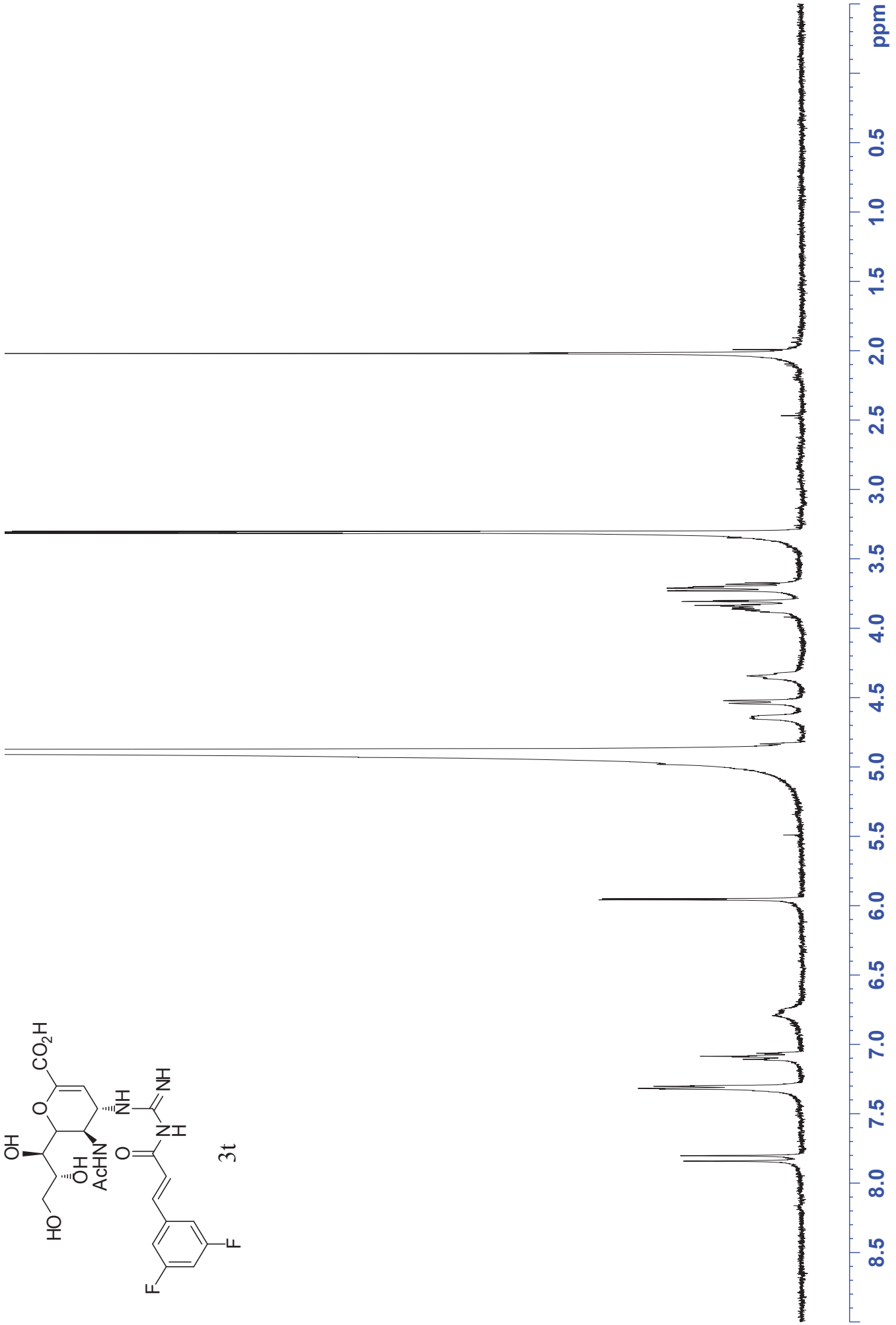

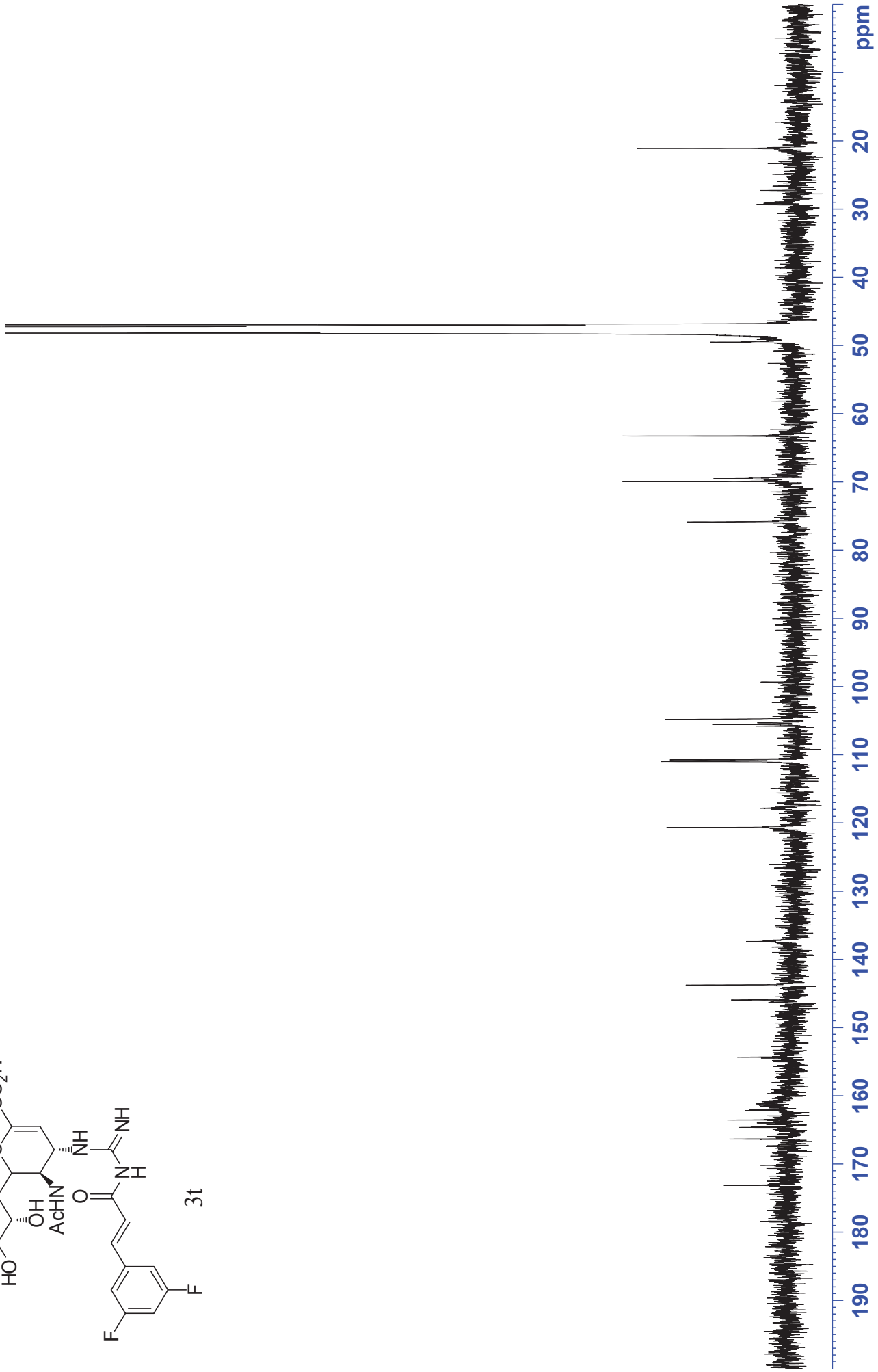

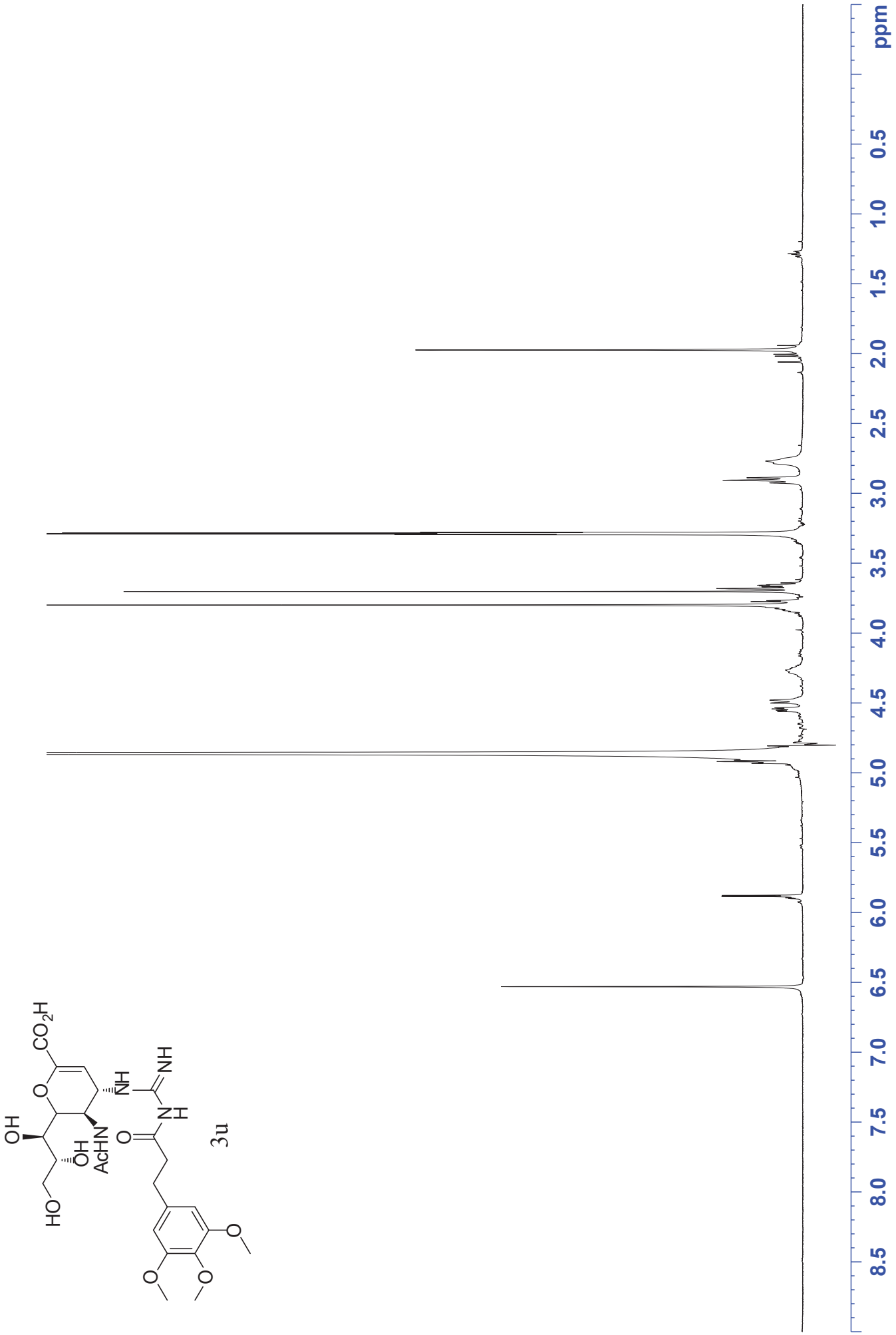

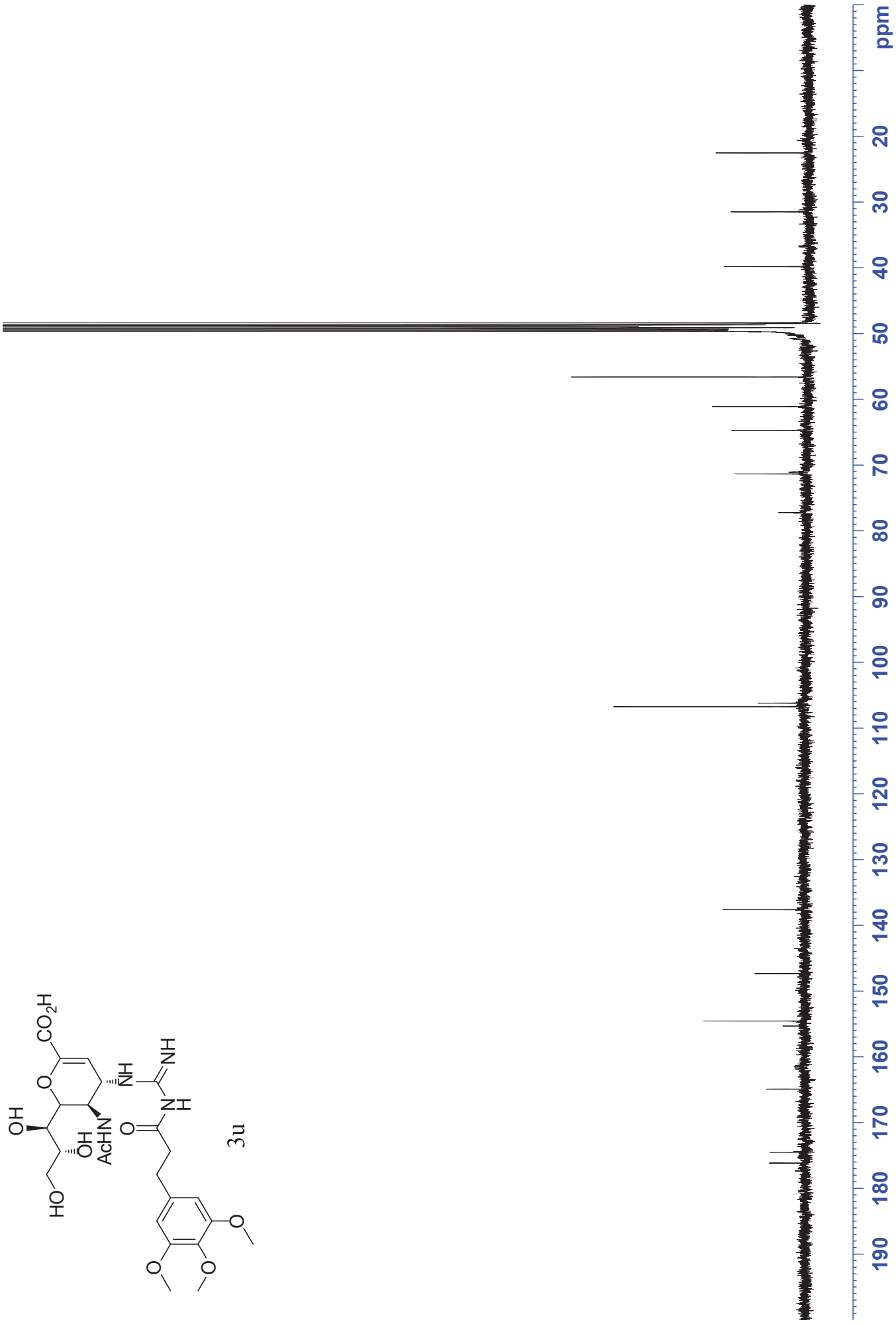

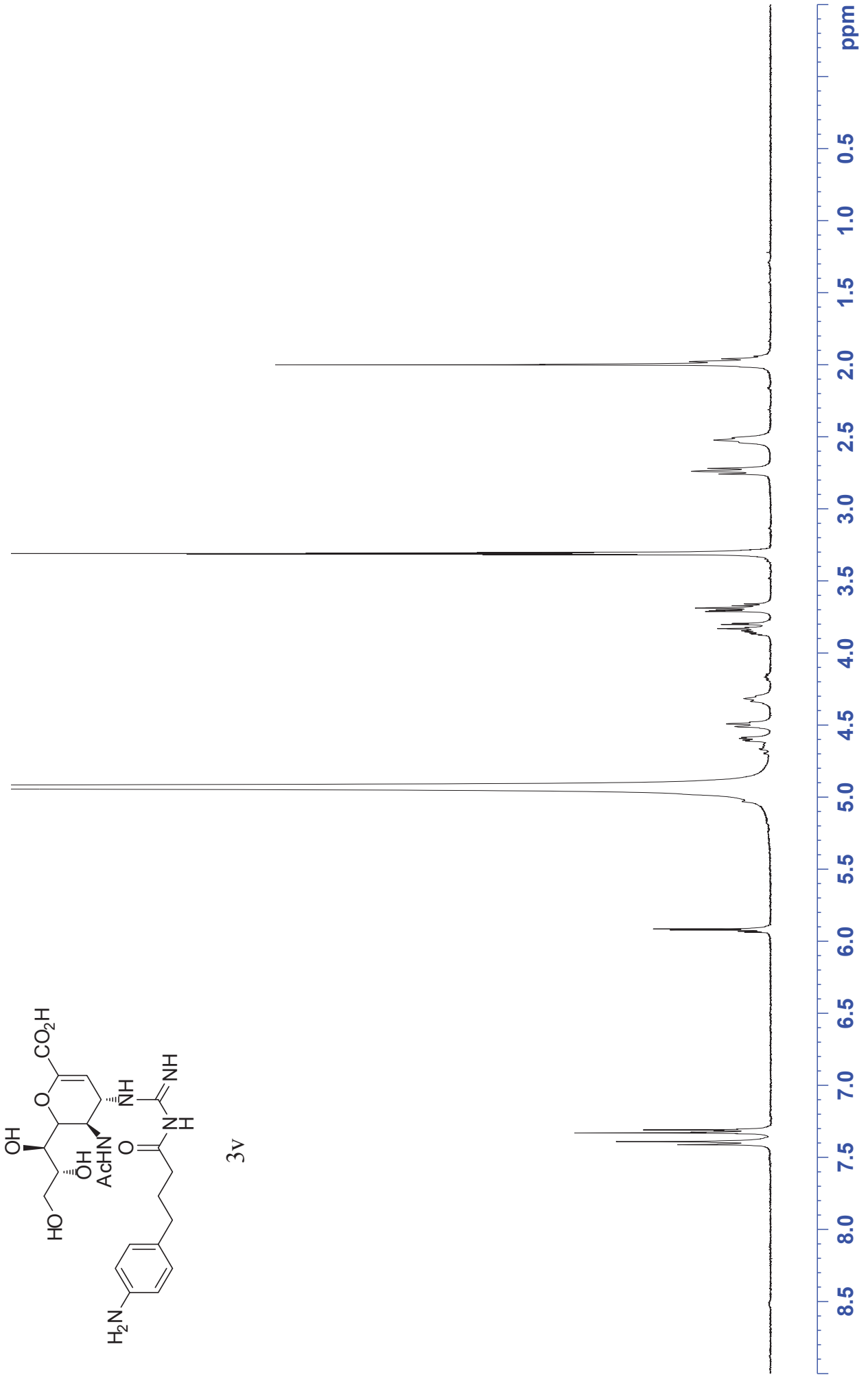

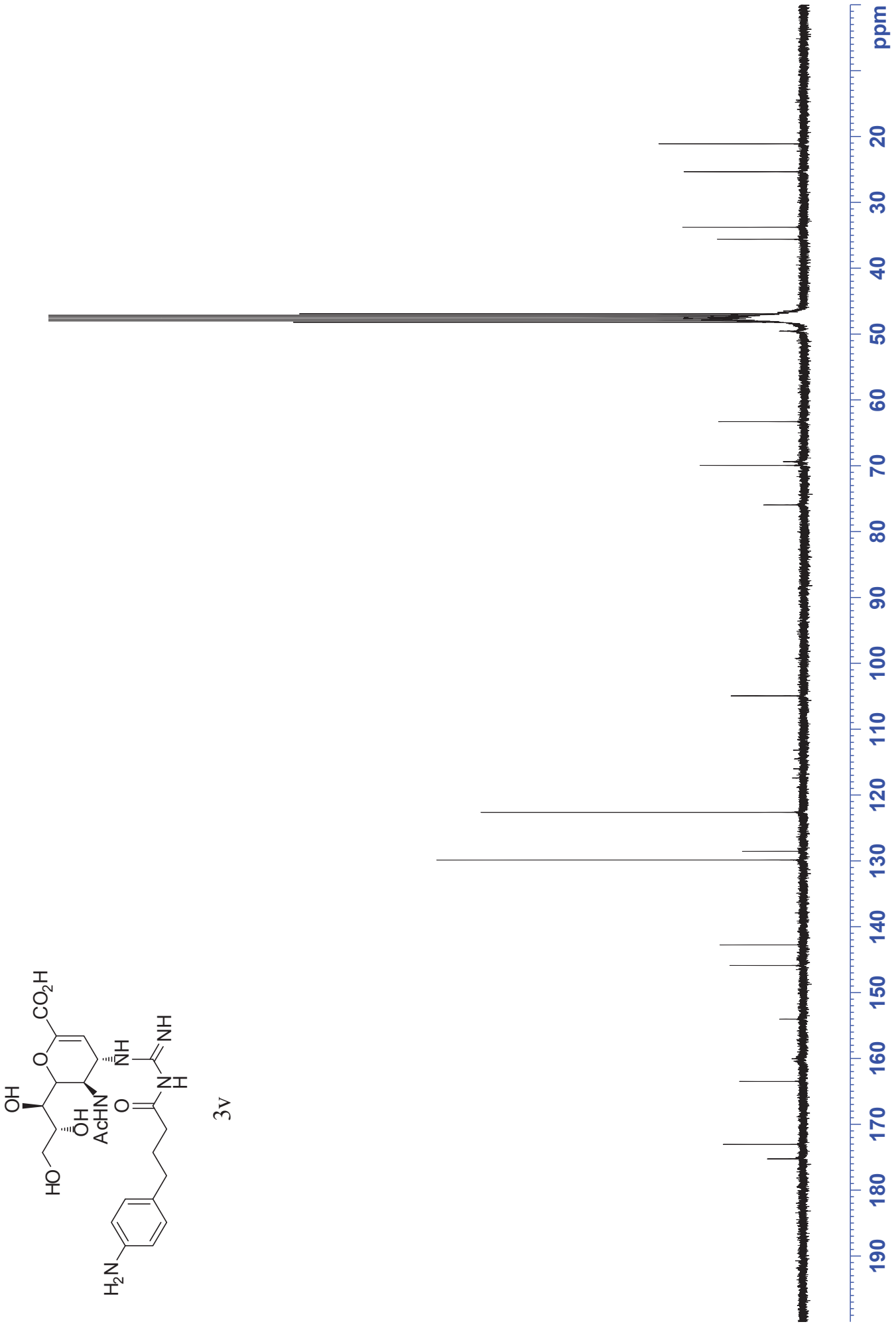

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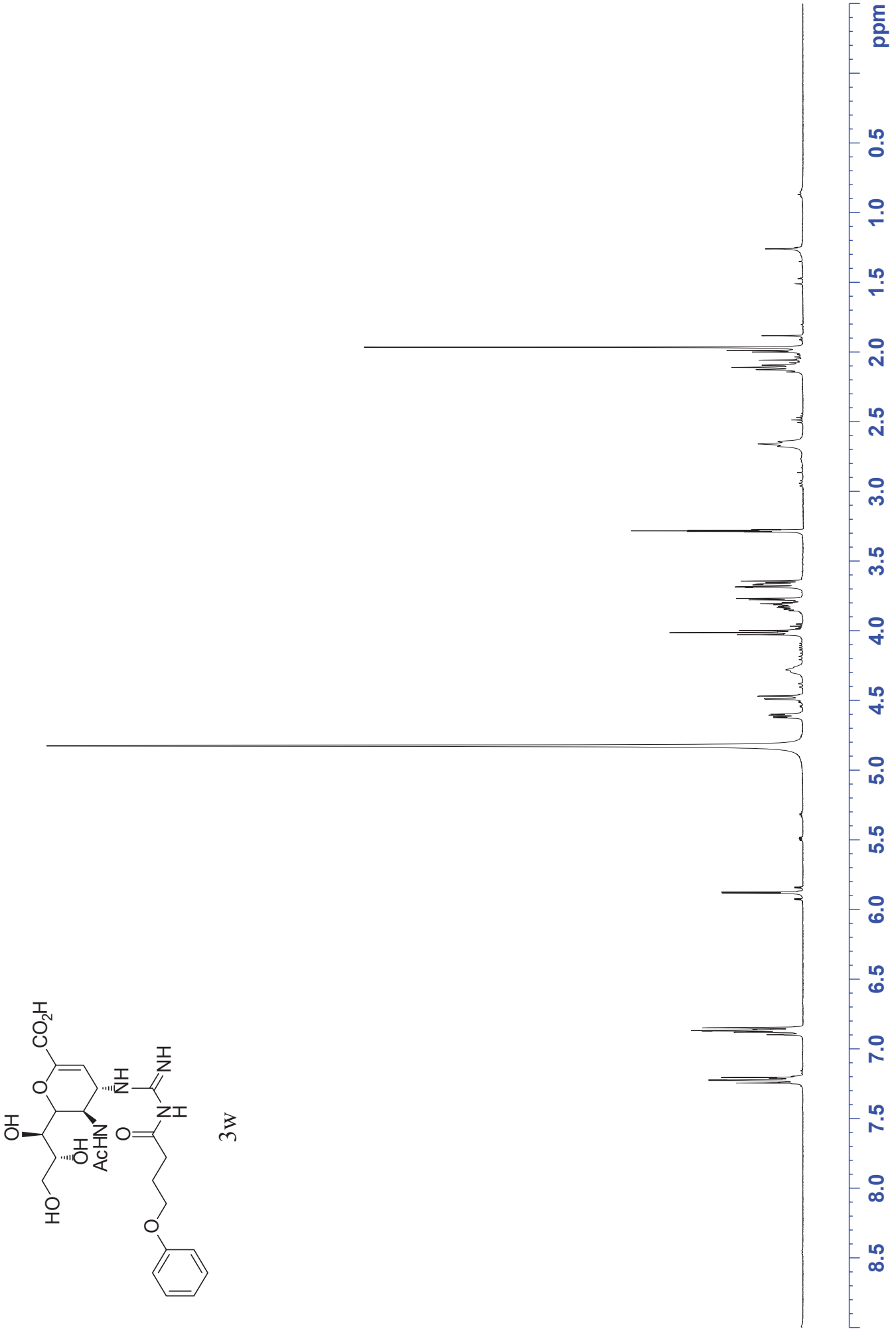
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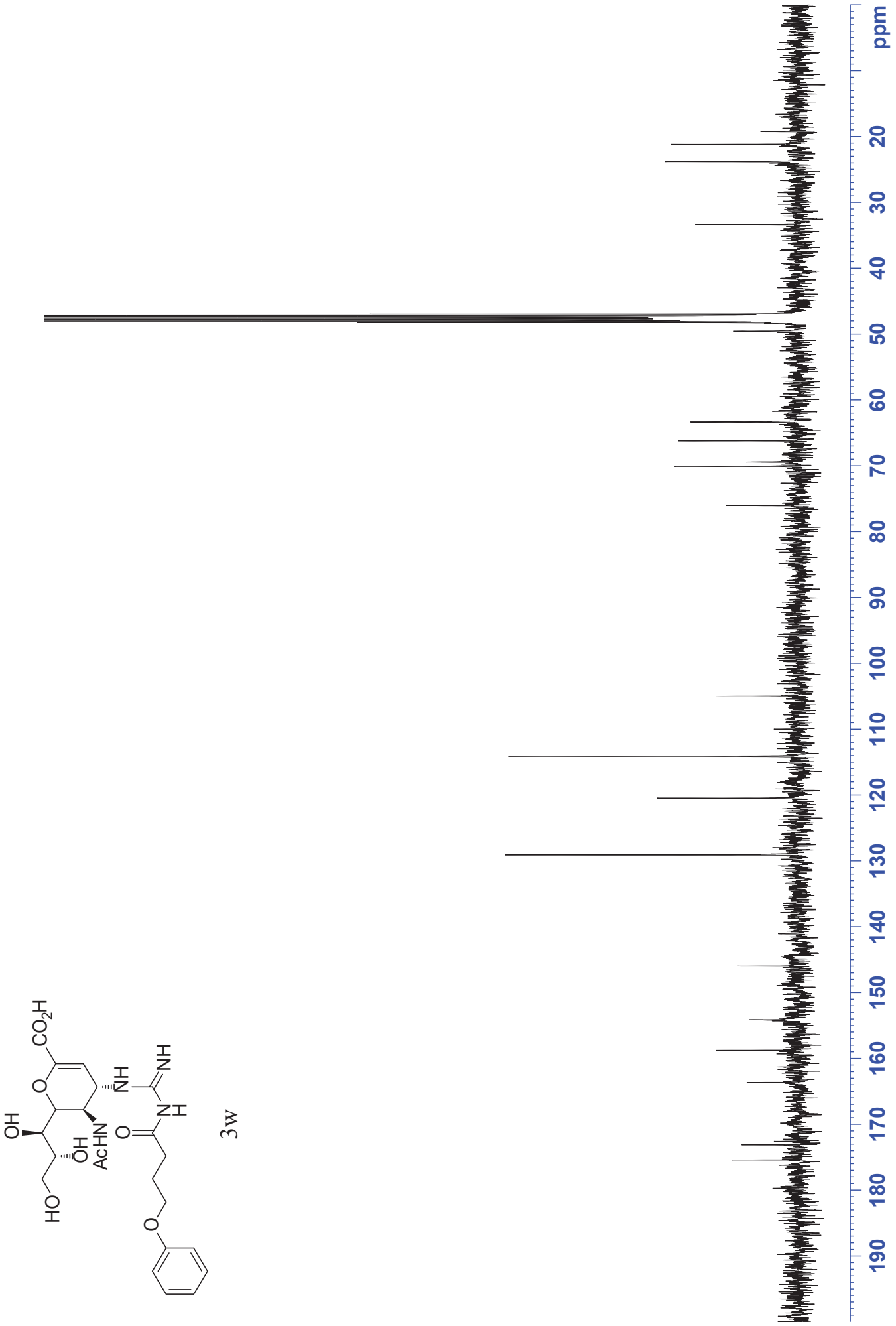

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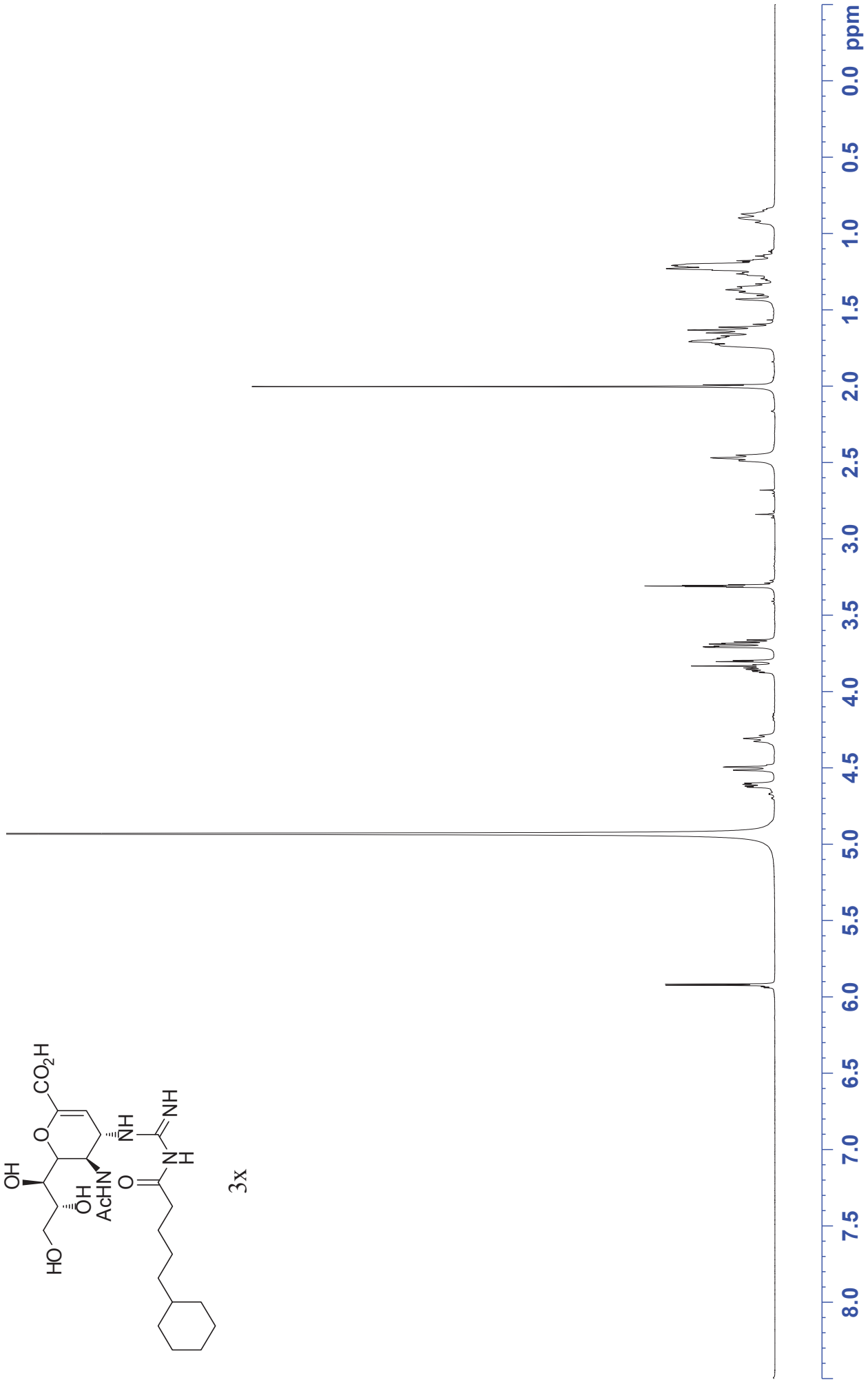

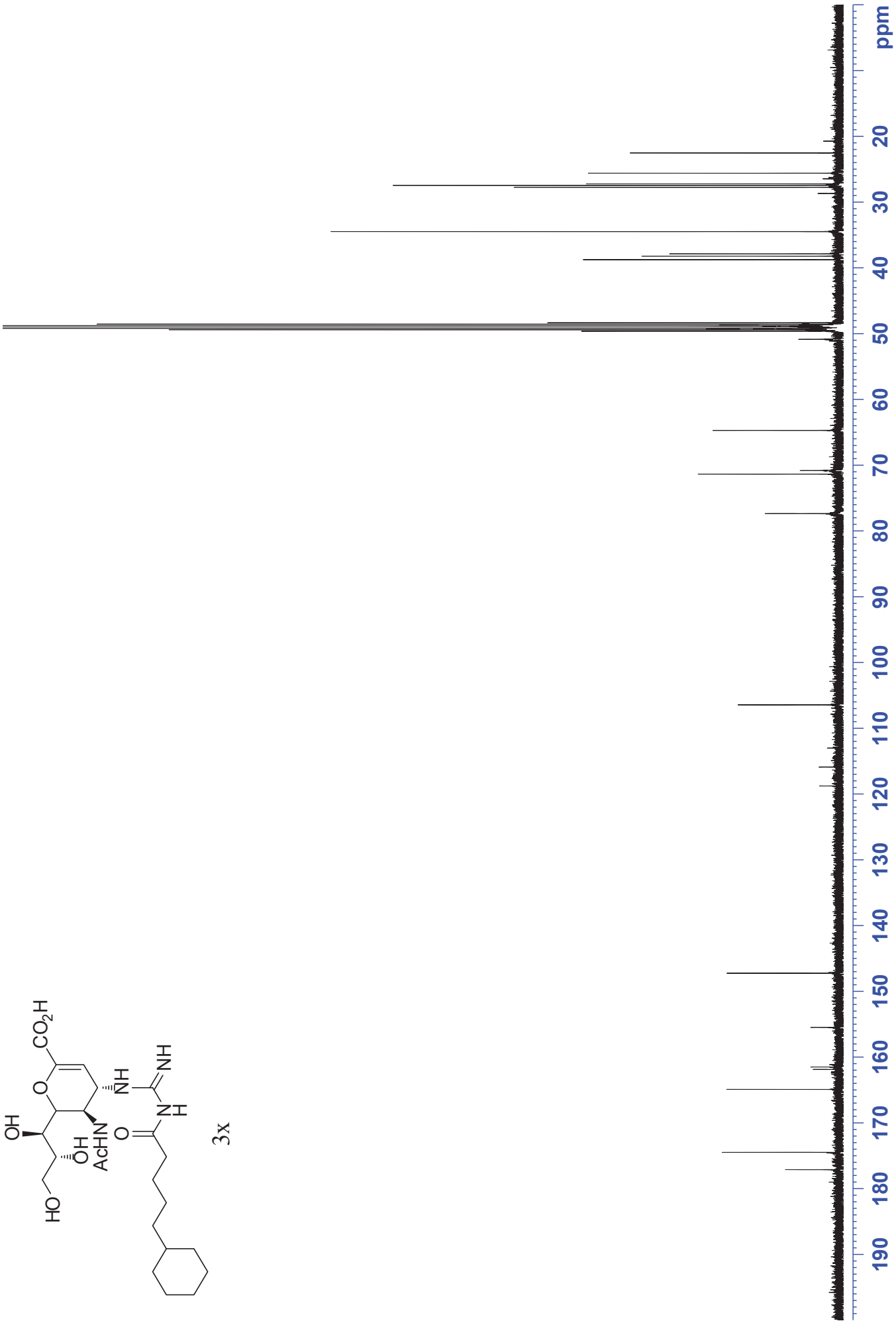

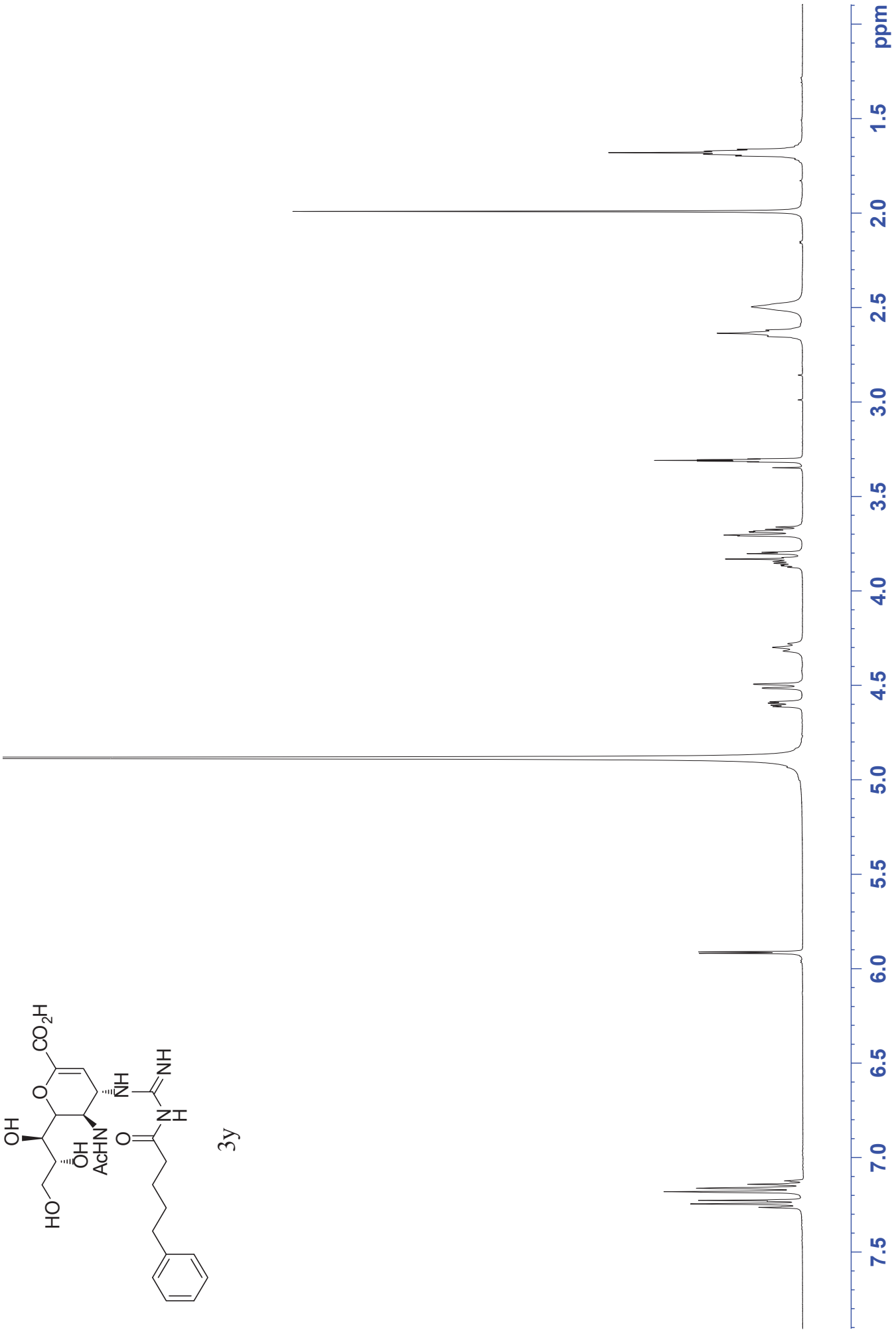

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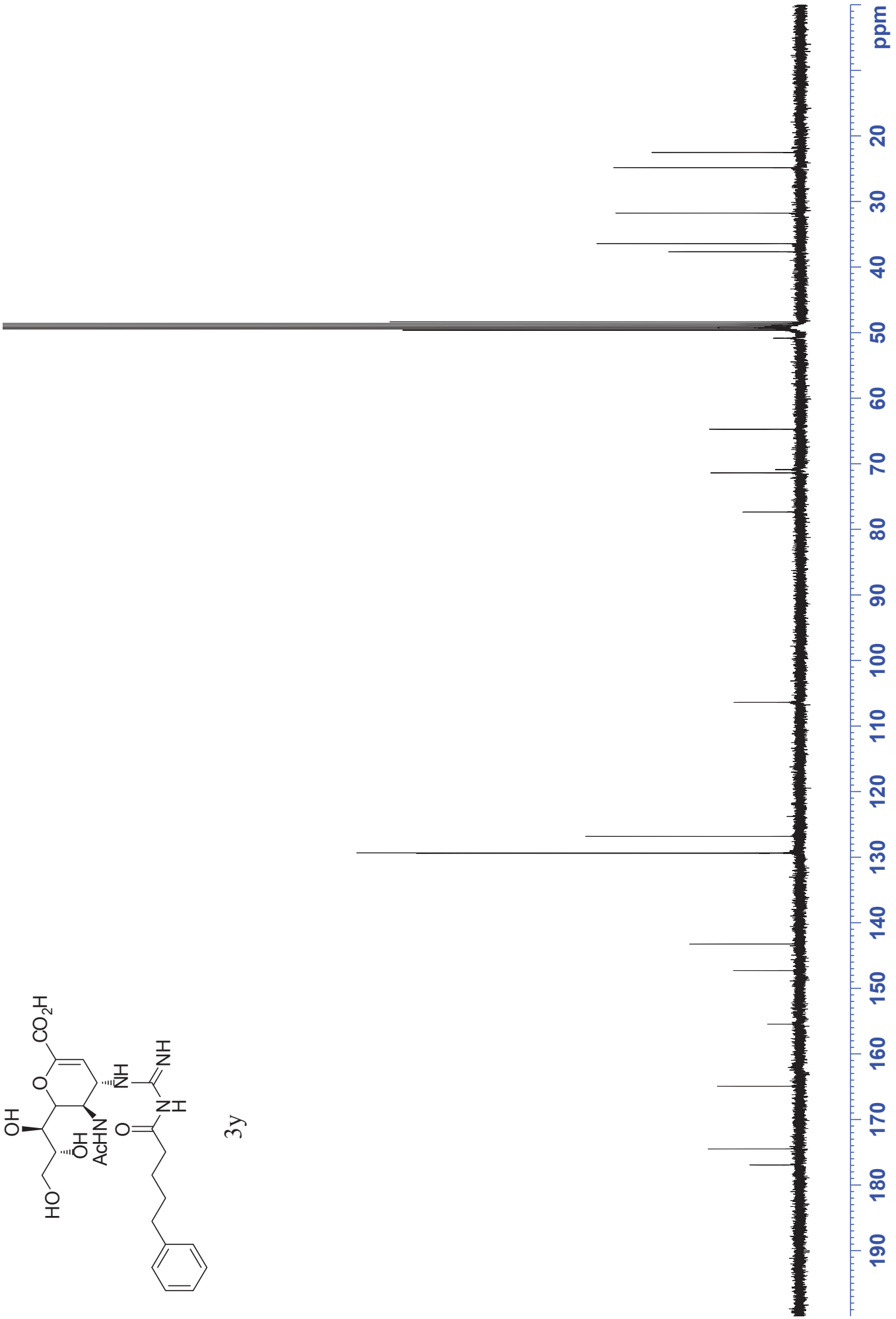

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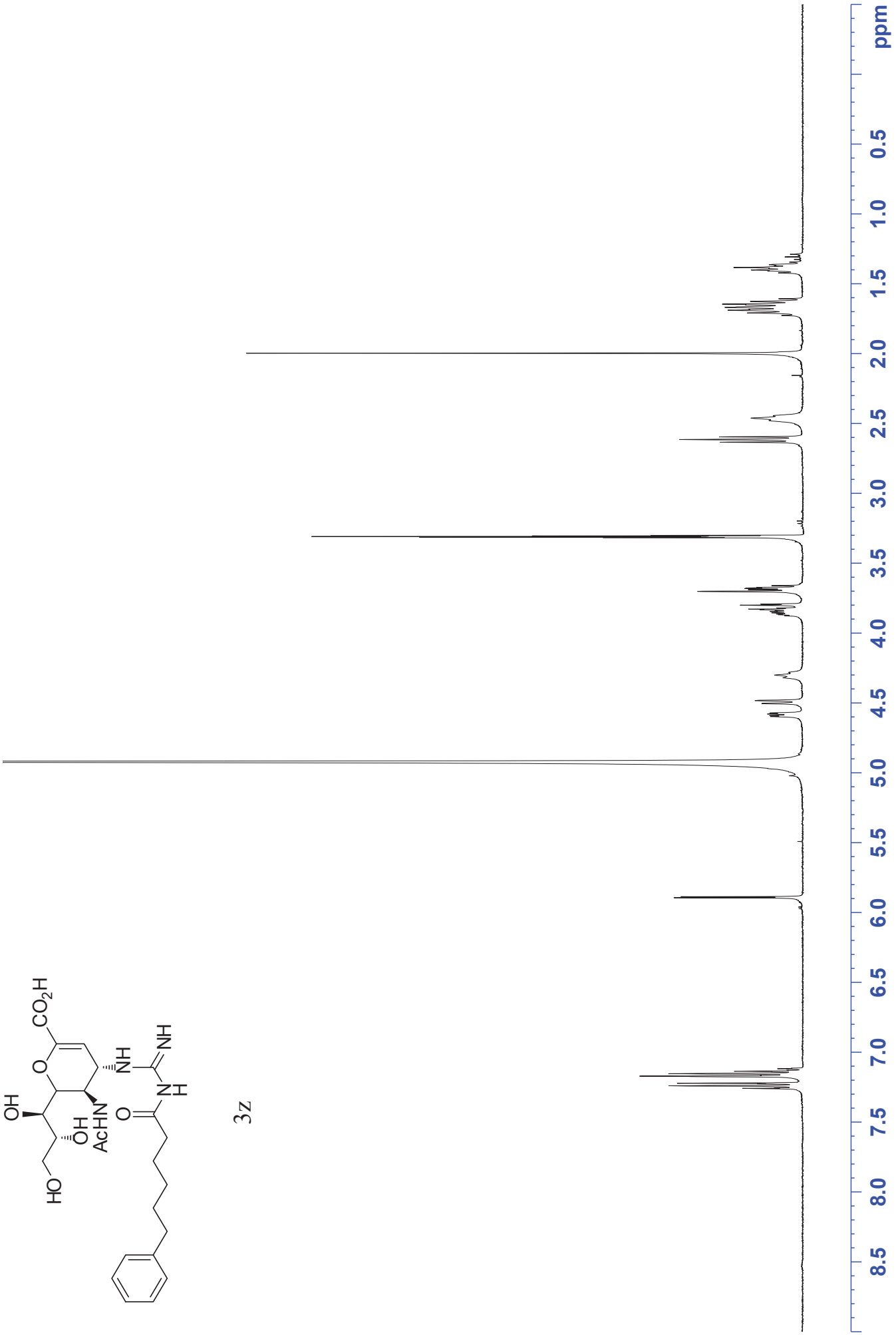

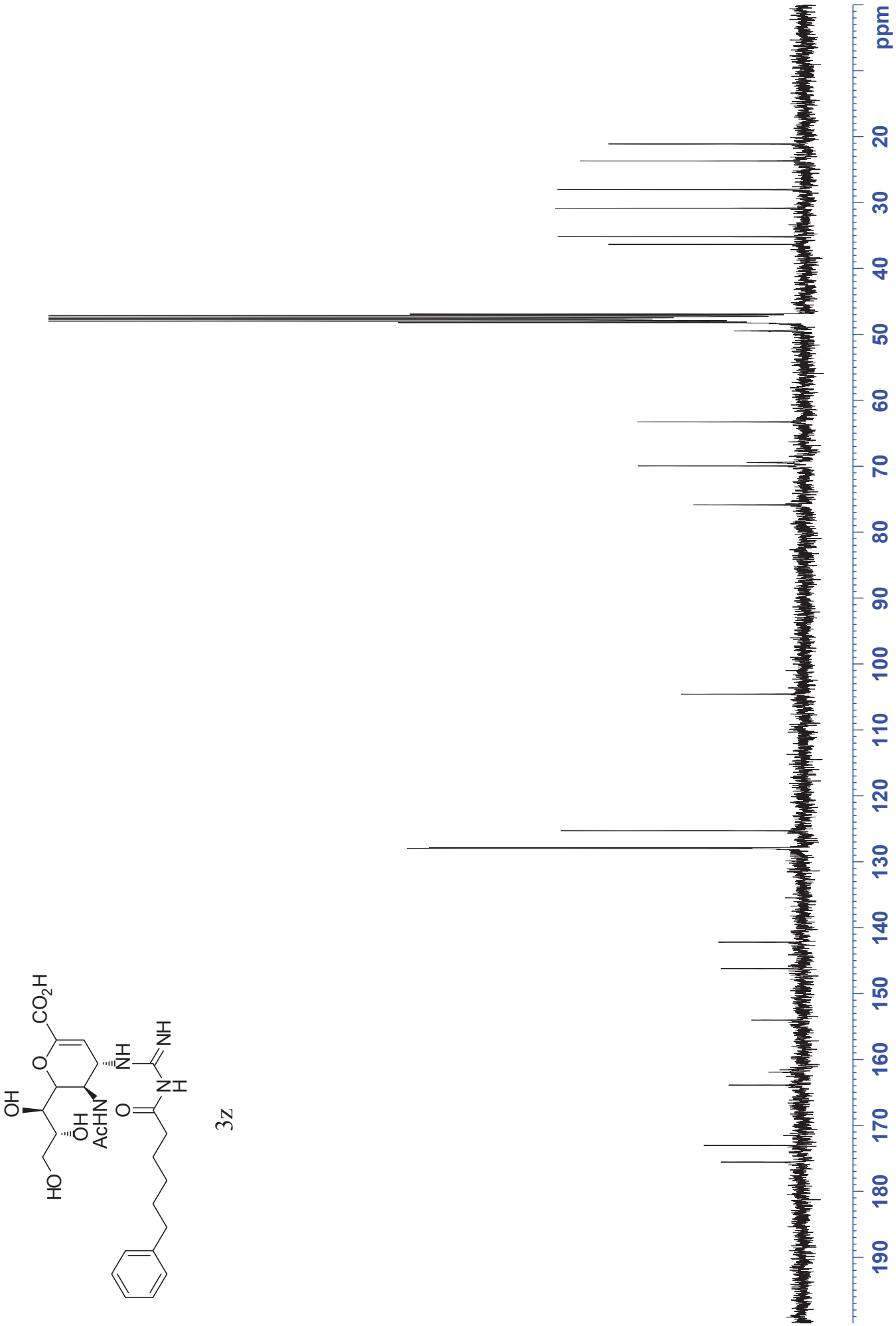

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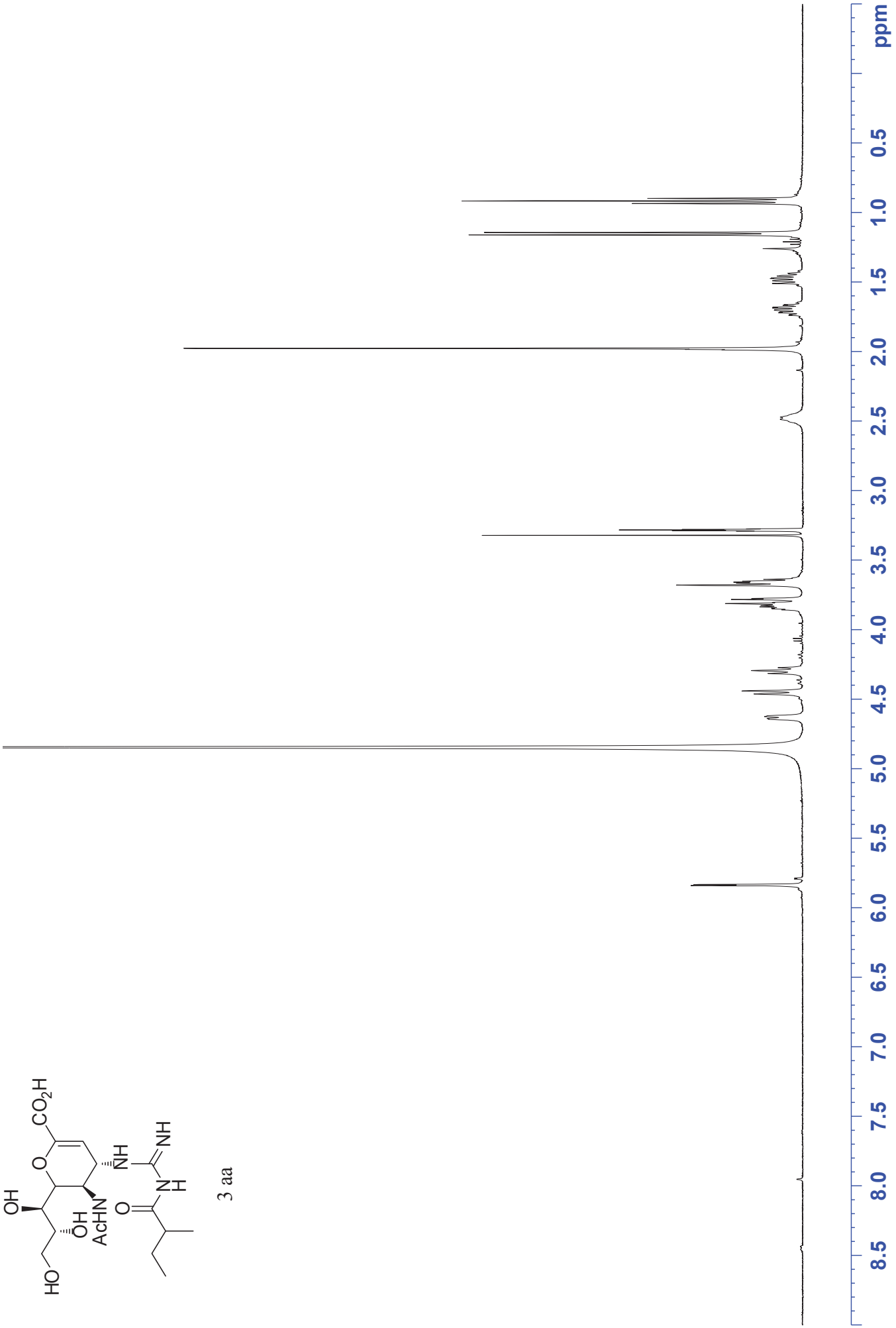

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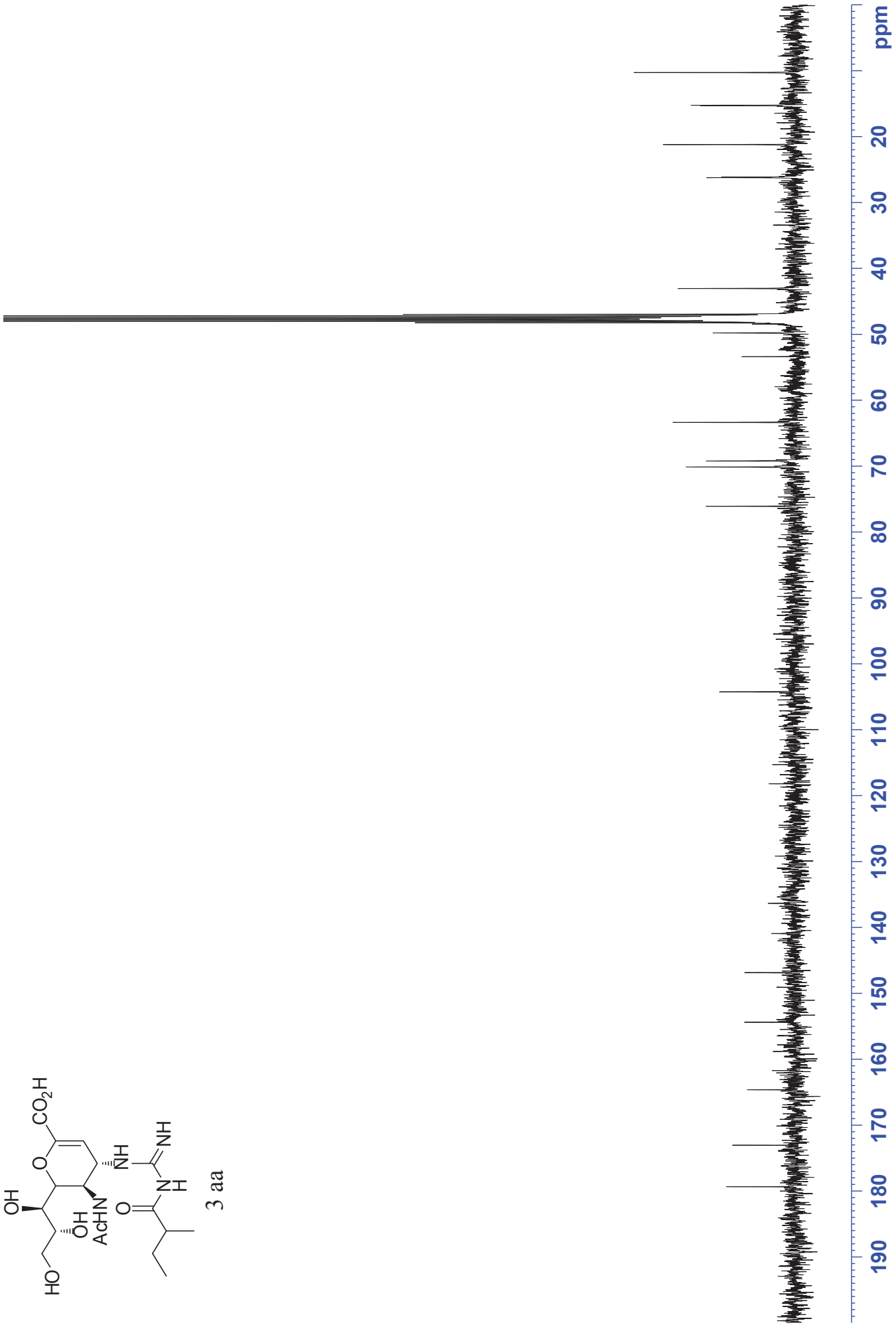
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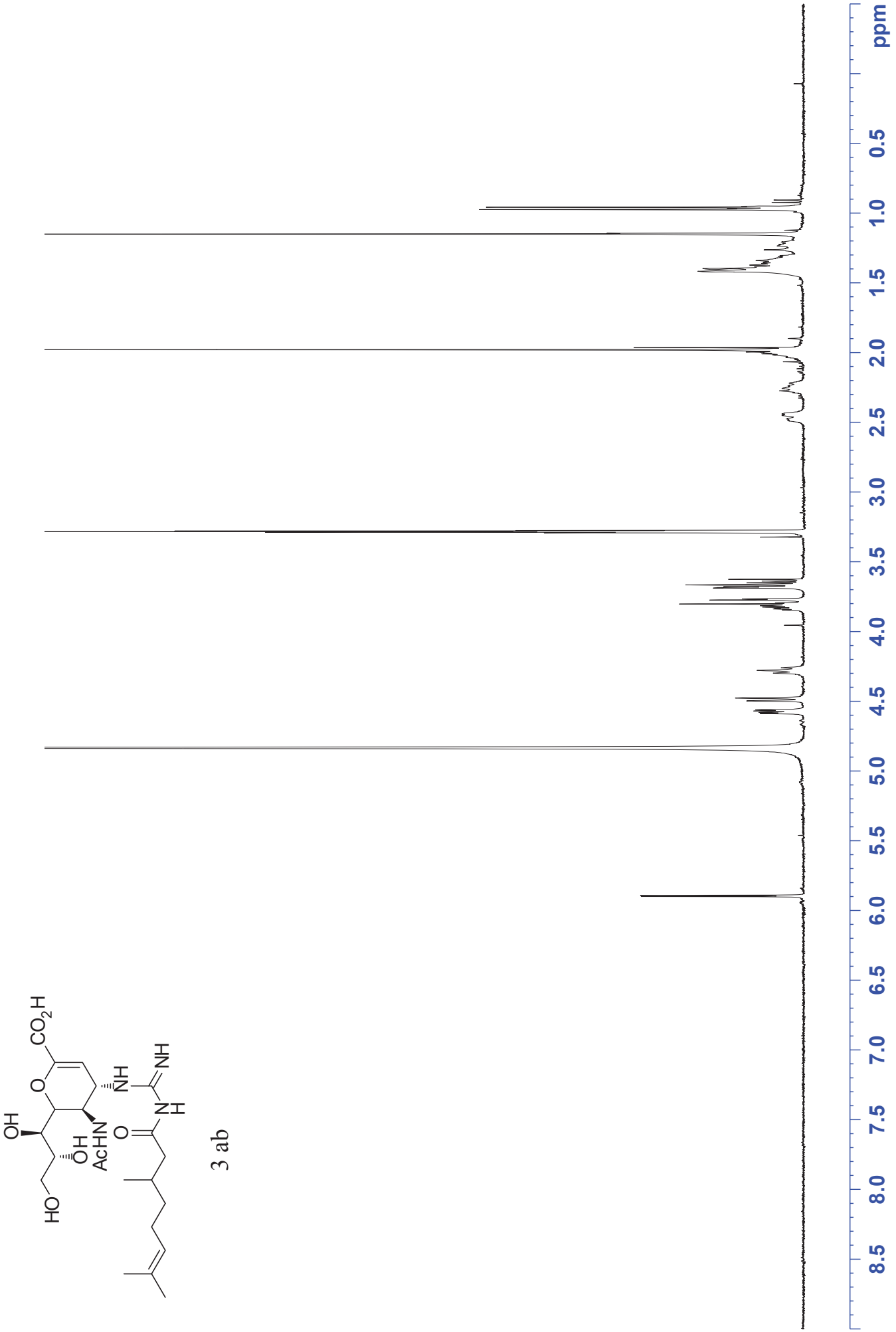

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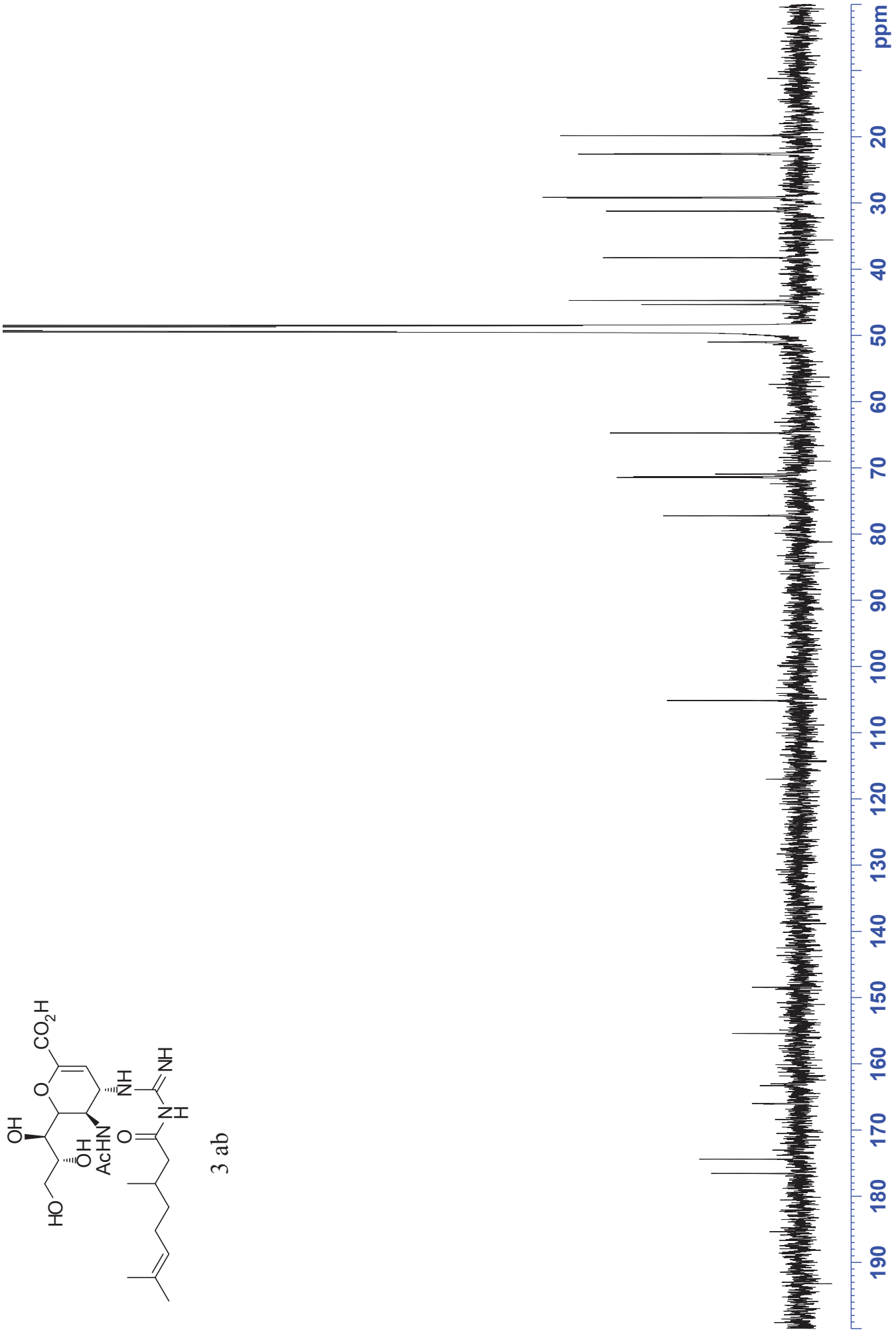

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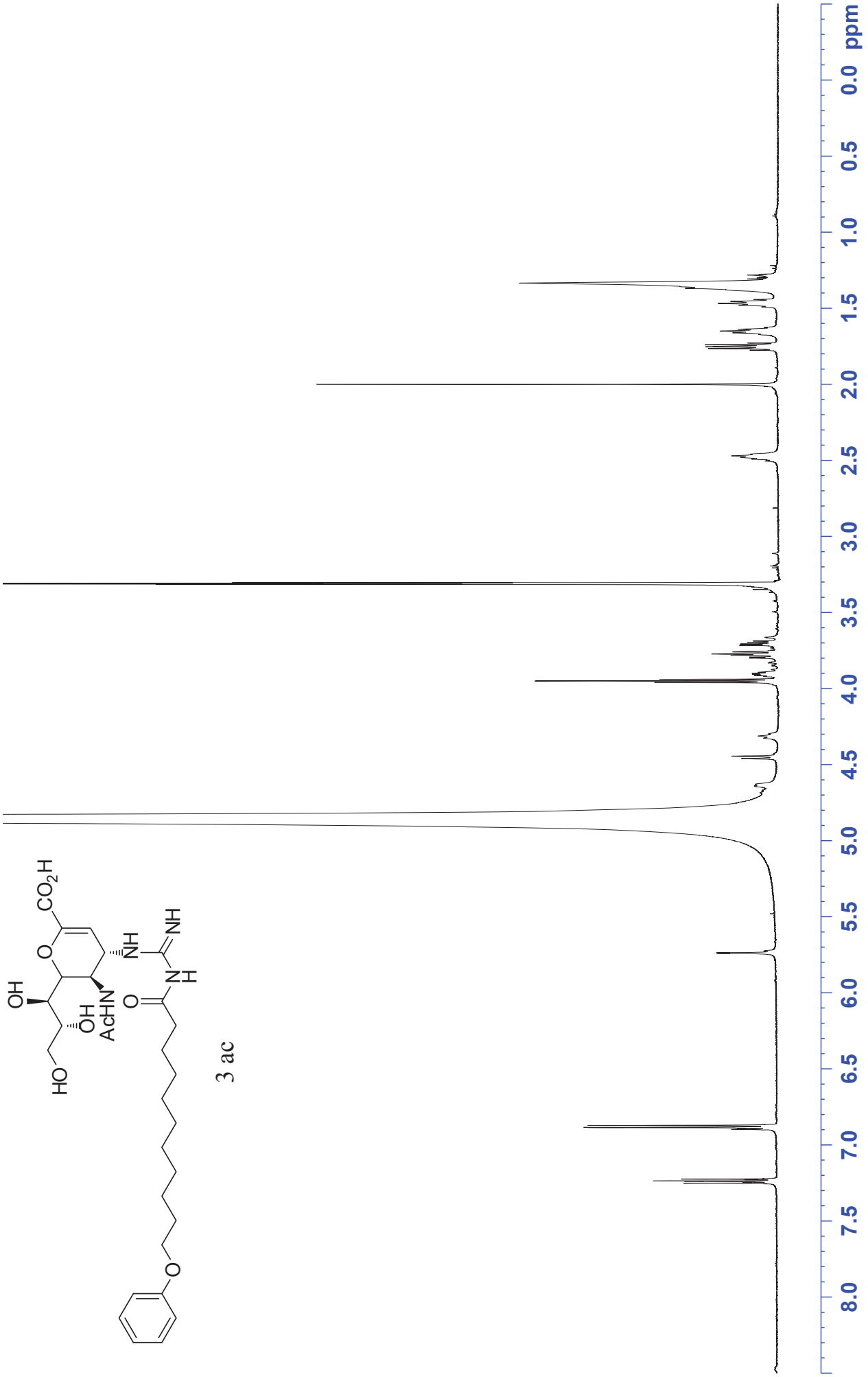

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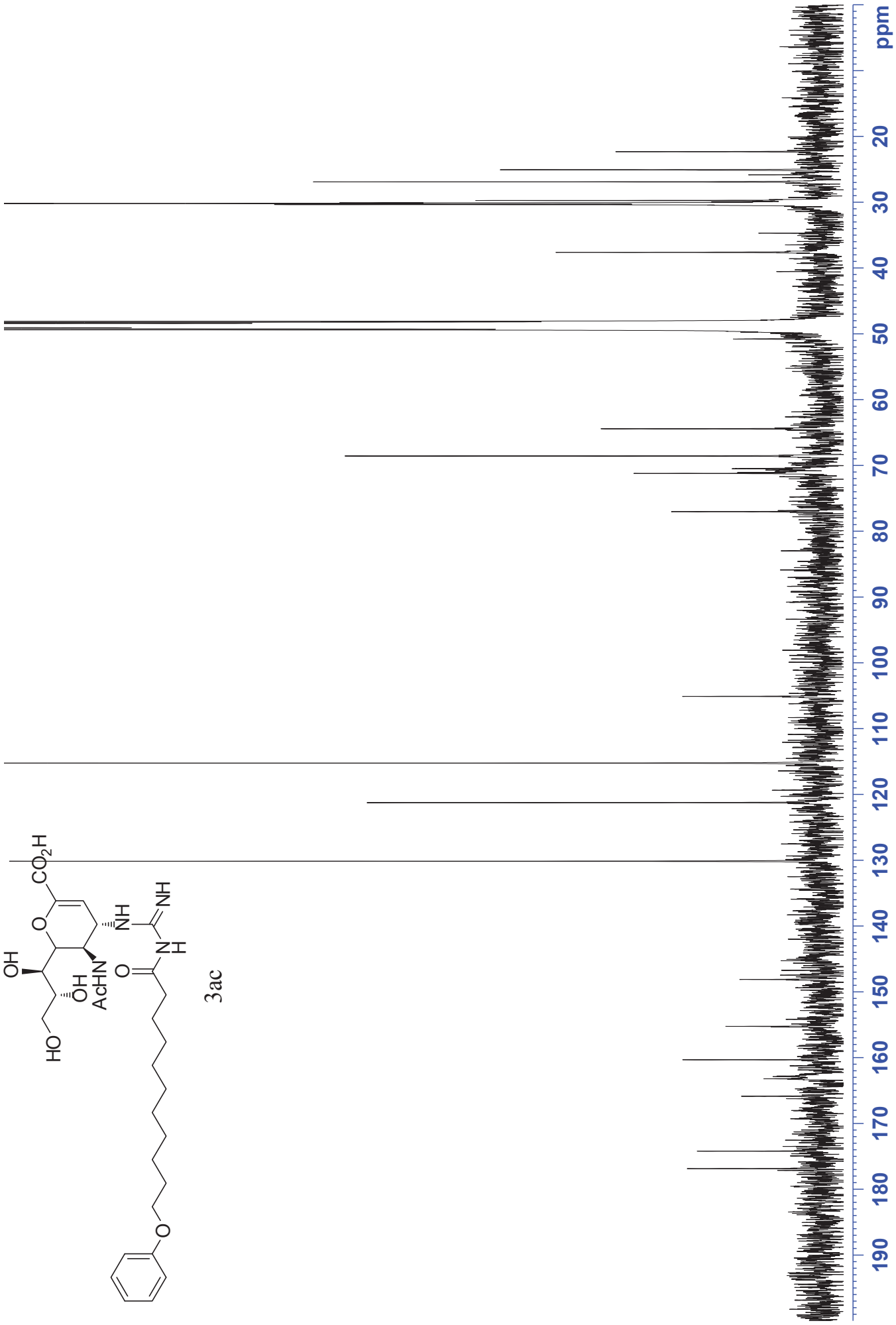

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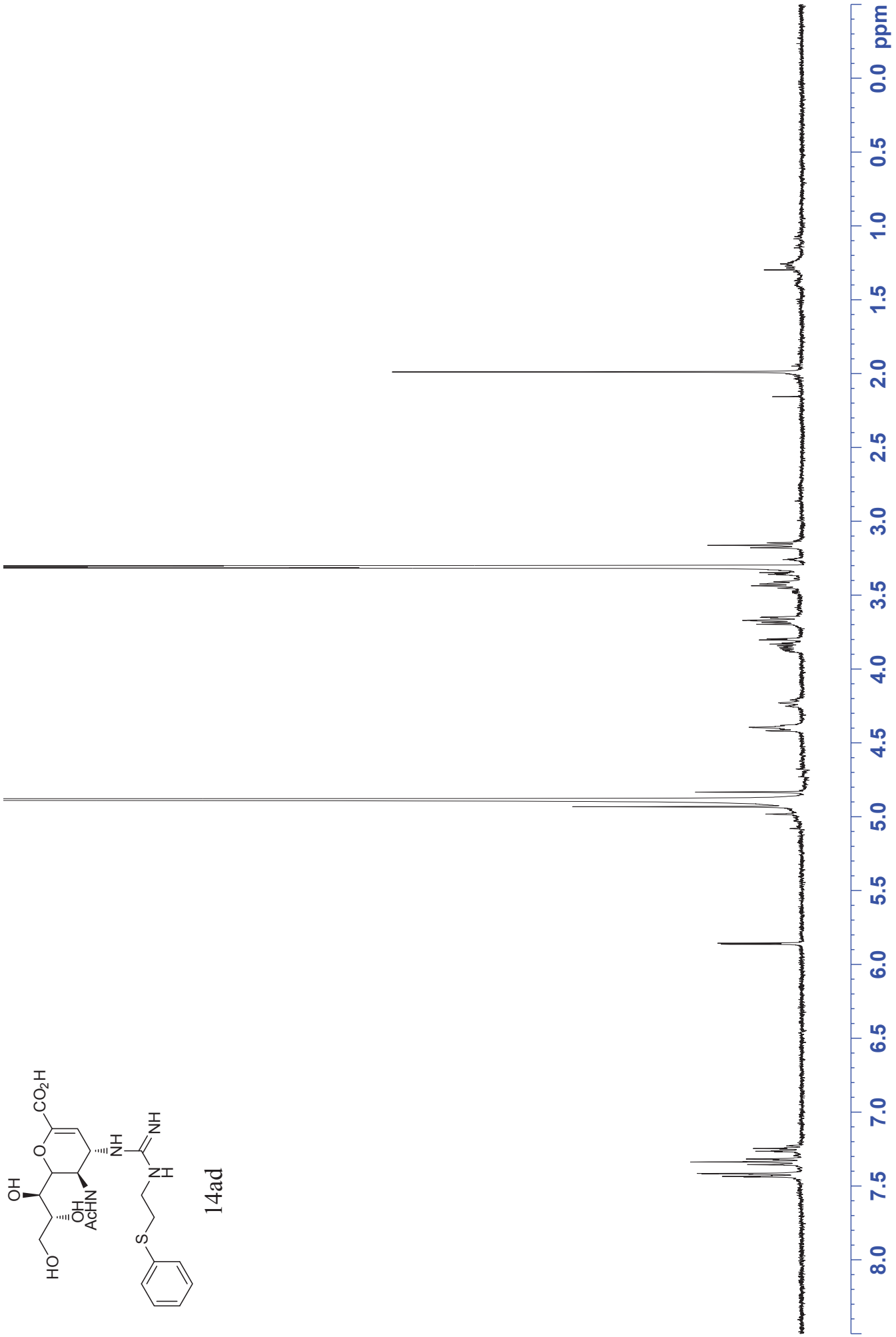
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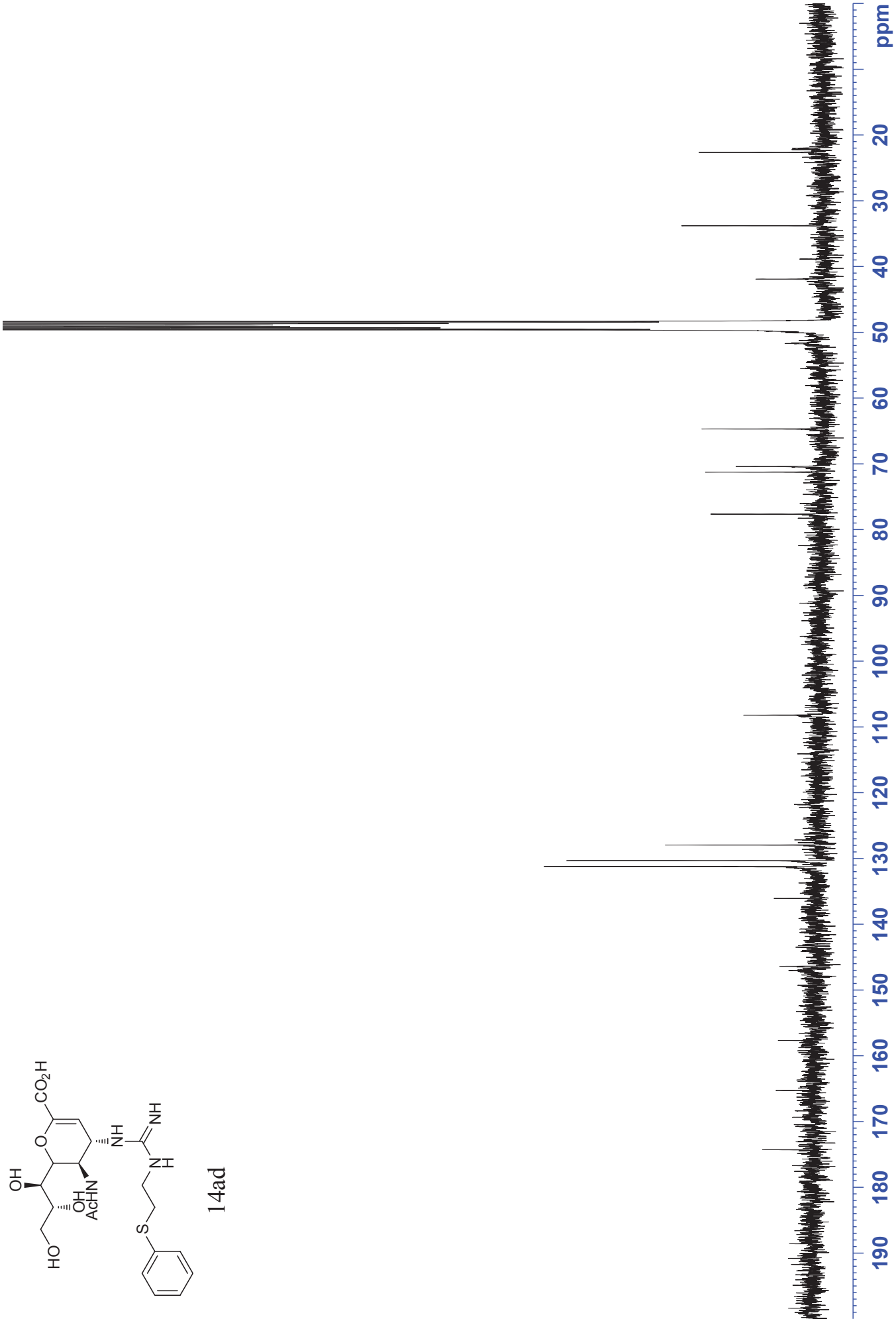
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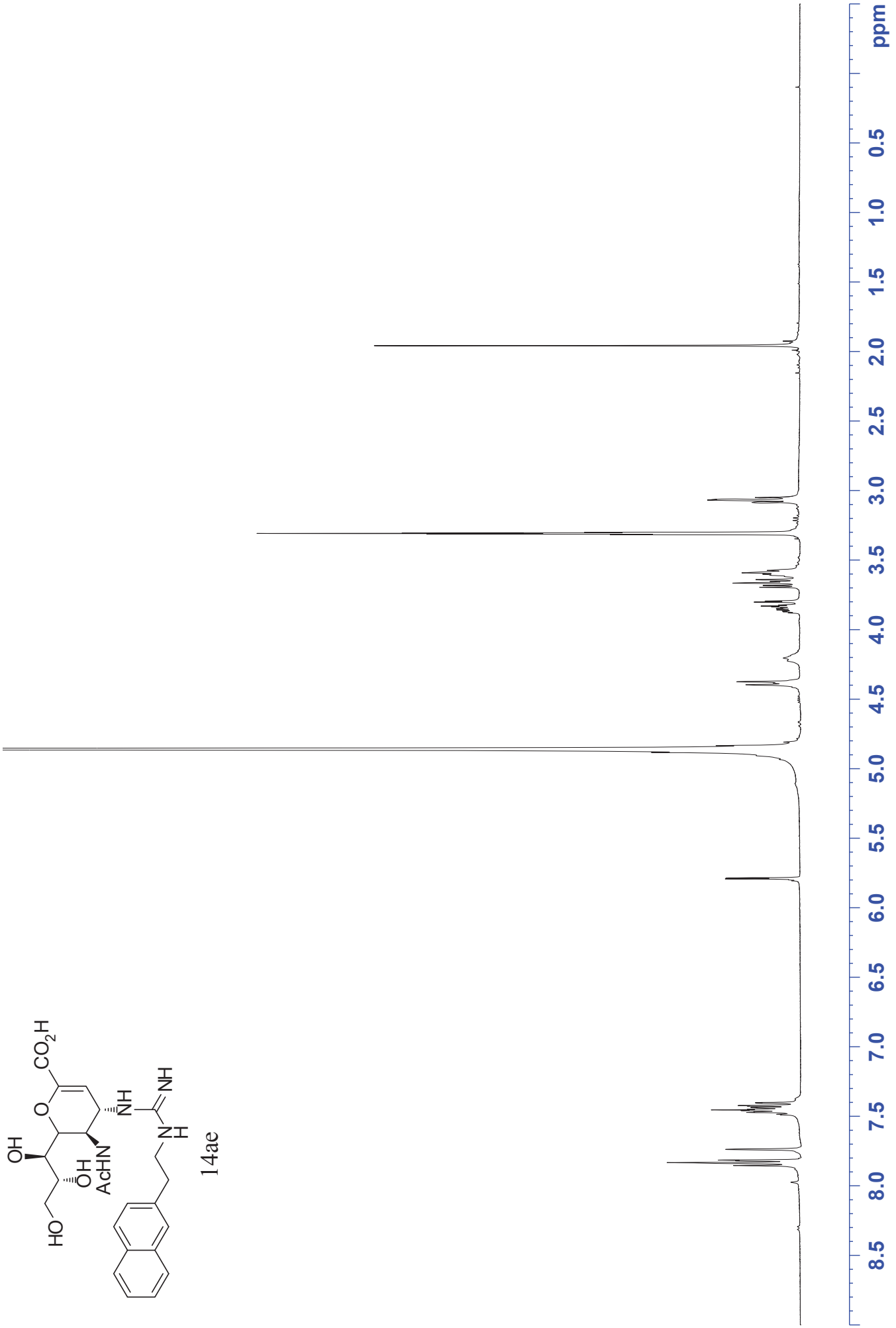
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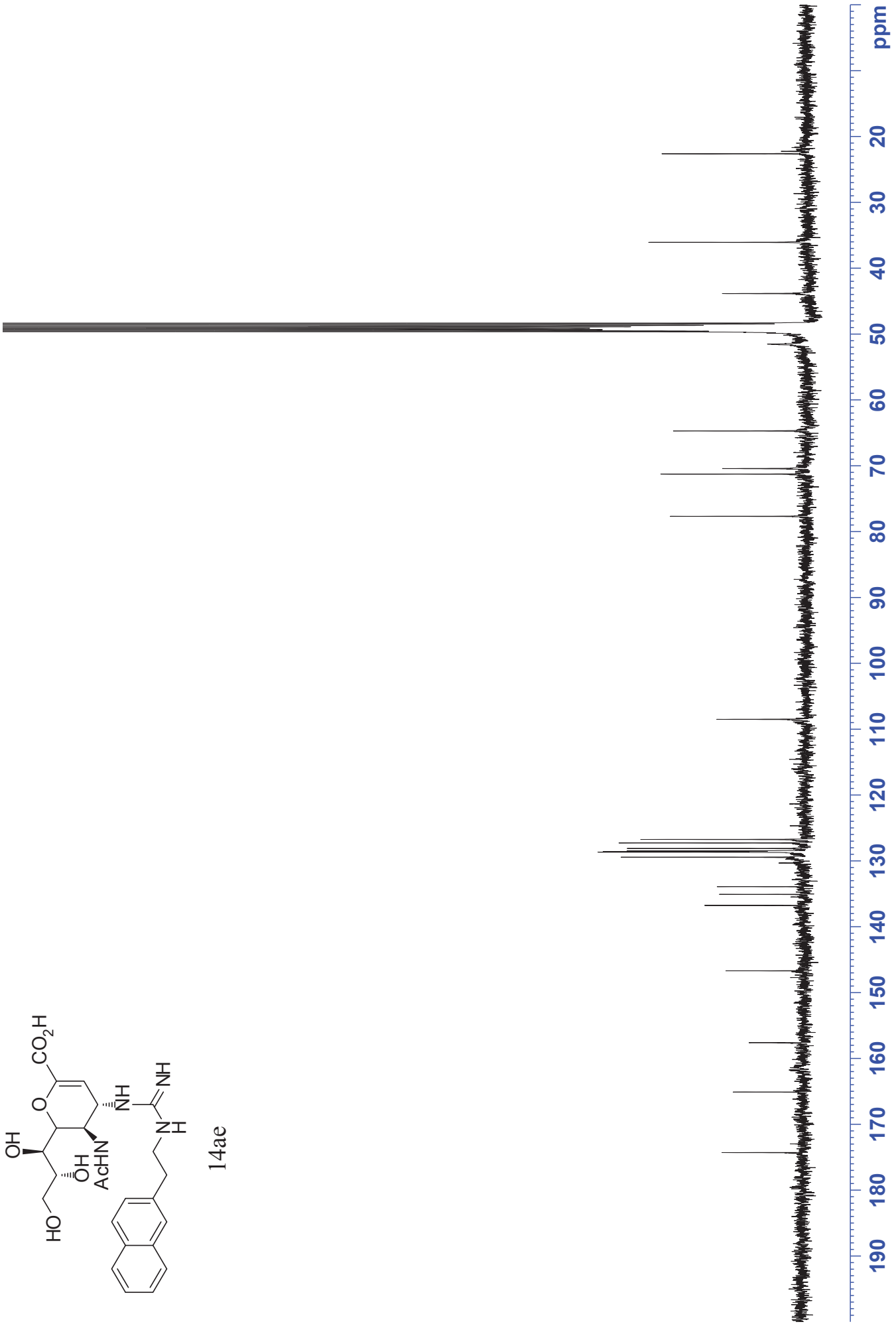

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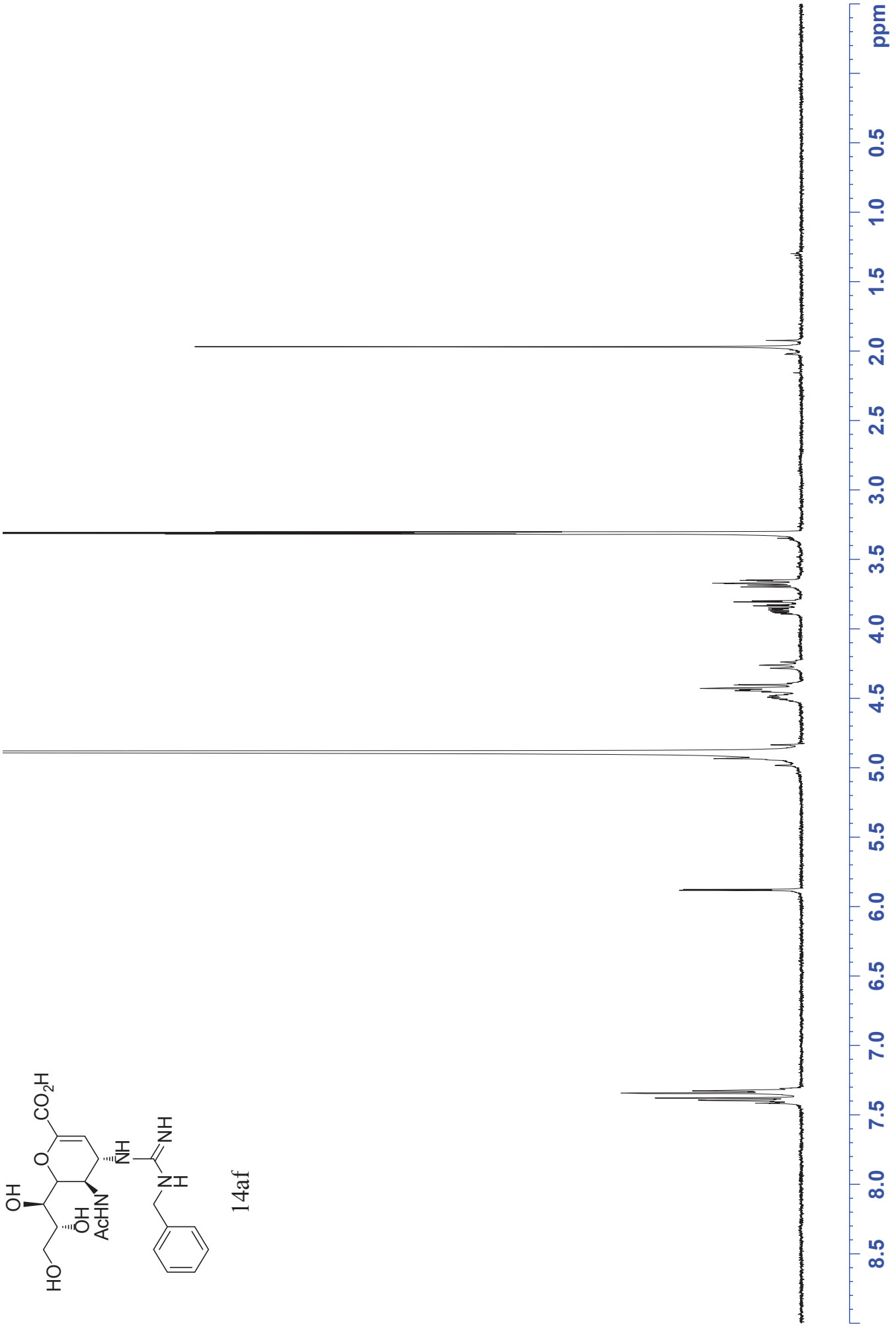
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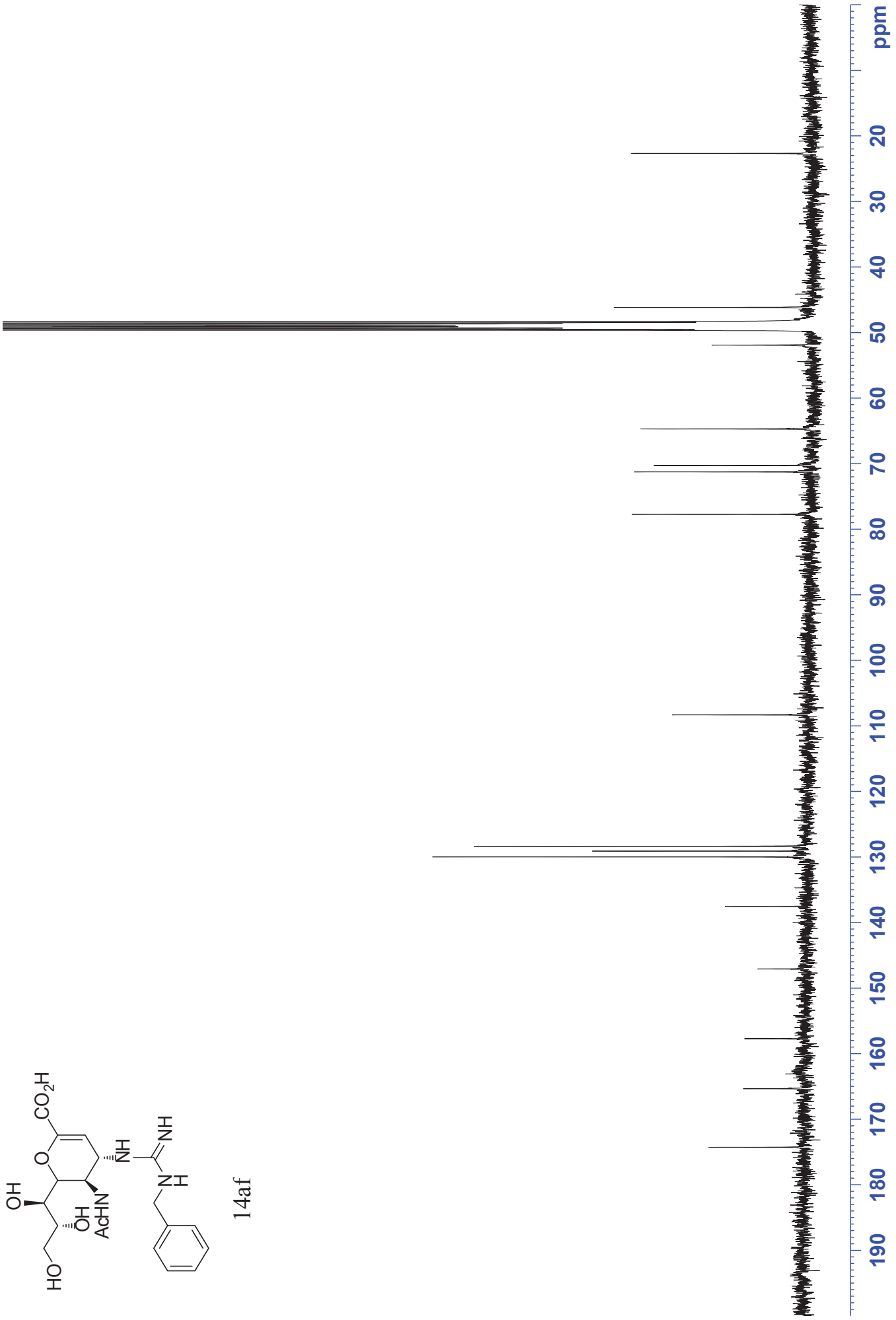

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