Supporting Information

for

DPAGT1 Inhibitors of Capuramycin Analogues and Their Antimigratory Activities of Solid Tumors

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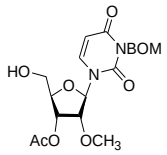
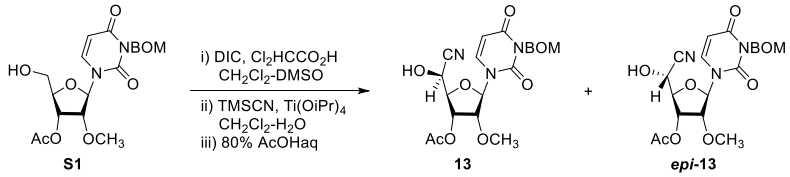
General

All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. THF, CH_2Cl_2, and DMF were purified via Innovative Technology's Pure-Solve System. All reactions were performed under an Argon atmosphere. All stirring was performed with an internal magnetic stirrer. Reactions were monitored by TLC using 0.25 mm coated commercial silica gel plates (EMD, Silica Gel $60F_{254}$). TLC spots were visualized by UV light at 254 nm, or developed with ceric ammonium molybdate or anisaldehyde or copper sulfate or ninhydrin solutions by heating on a hot plate. Reactions were also monitored by using SHIMADZU LCMS-2020 with solvents: A: 0.1% formic acid in water, B: acetonitrile. Flash chromatography was performed with SiliCycle silica gel (Purasil 60 Å, 230-400 Mesh). Proton magnetic resonance (^{1}H-NMR) spectral data were recorded on 400, and 500 MHz instruments. Carbon magnetic resonance (^{13}C-NMR) spectral data were recorded on 100 and 125 MHz instruments. For all NMR spectra, chemical shifts (δH, δC) were quoted in parts per million (ppm), and *J* values were quoted in Hz. ^{1}H and ^{13}C NMR spectra were calibrated with residual undeuterated solvent ($CDCl_3$: δH = 7.26 ppm, δC = 77.16 ppm; CD_3CN: δH = 1.94 ppm, δC = 1.32ppm; CD_3OD: δH =3.31 ppm, δC =49.00 ppm; DMSO-d_6: δH = 2.50 ppm, δC = 39.52 ppm; D_2O: δH = 4.79 ppm) as an internal reference. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, dd = double doublets, t = triplet, q = quartet, quin = quintet, hept = heptet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Perkin-Elmer FT1600 spectrometer. HPLC analyses were performed with a Shimadzu LC-20AD HPLC system. HR-MS data were obtained from a Waters Synapt G2-Si (ion mobility mass spectrometer with nanoelectrospray ionization).



S1

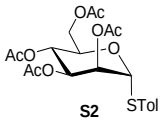
(2*R*,5*R*)-5-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl acetate (S1). The title compound was synthesized according to the reported procedure[1]: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.71 (d, *J* = 8.2 Hz, 1H), 7.40 – 7.26 (m, 5H), 5.77 (d, *J* = 6.4 Hz, 1H), 5.75 (d, *J* = 2.6 Hz, 1H), 5.48 (d, *J* = 2.2 Hz, 2H), 5.23 (t, *J* = 5.2 Hz, 1H), 4.71 (s, 2H), 4.23 – 4.17 (m, 2H), 3.99 (dd, *J* = 12.6, 2.0 Hz, 1H), 3.78 (dd, *J* = 12.6, 2.1 Hz, 1H), 3.48 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.59, 162.49, 151.01, 139.77, 137.77, 128.31 (2C), 127.70, 127.67 (2C), 102.21, 90.31, 82.65, 81.20, 72.31, 70.27, 70.01, 61.24, 58.95, 20.81; HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{25}N_2O_8$ [M + H] 421.1611, found: 421.1641.



S1 **13** ***epi*-13**

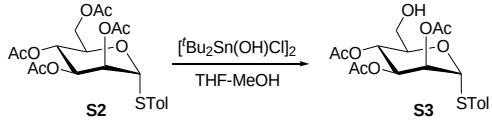
(2*R*,5*R*)-5-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-((*S*)-cyano(hydroxy)methyl)-4-methoxytetrahydrofuran-3-yl acetate (13). To a stirred solution of **S1** (1.72 g, 4.09 mmol) and dichloroacetic acid (0.51 mL, 6.14 mmol) in a 10:1 mixture of CH_2Cl_2 and DMSO (18.4 mL) was added DIC (1.28 mL, 8.18 mmol) at 0 °C. After being stirred for 2h, H_2O (0.16 mL), TMSCN (1.02 mL, 8.18 mmol) and Ti(O^{i}Pr)$_4$ (2.42 mL, 8.18 mmol) were added to the reaction solution. After being stirred for 8h at r.t., the solution was concentrated *in vacuo*. The crude mixture was suspended to a 4:1 mixture of AcOH and H_2O (50 mL). After being stirred for 13h at r.t., the solution was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 2/1 - 1/2) to afford 0.70 g (38%) of **13** and 0.66 g (36%) of ***epi*-13**. Data for **13**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.27 (m, 5H), 7.21 (d, *J* = 8.1 Hz, 1H), 5.82 (d, *J* = 8.1 Hz, 1H), 5.49 (s, 2H), 5.38 (d, *J* = 6.9 Hz, 1H), 5.35 (dd, *J* = 5.7, 2.4 Hz, 1H), 4.71 (s, 2H), 4.71 – 4.69 (m, 1H), 4.54 (dd, *J* = 6.9, 5.7 Hz, 1H), 4.36 (t, *J* = 2.2 Hz, 1H), 3.39 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.20, 161.85, 151.38, 141.95, 137.51, 128.42 (2C), 127.84, 127.66 (2C), 117.13, 103.23, 95.16, 84.08, 78.39, 72.52, 70.47, 70.31, 61.79, 59.23, 20.71; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{24}N_3O_8$ [M + H] 446.1563, found: 446.1568. Data for **(2*R*,5*R*)-5-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-((*R*)-cyano(hydroxy)methyl)-4-methoxytetrahydrofuran-3-yl acetate (*epi*-13)**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.53 (d, *J* = 8.1 Hz, 1H), 7.38 – 7.27 (m, 5H), 5.80 (d, *J* = 5.5 Hz, 1H), 5.78 (d, *J* = 8.2 Hz, 1H), 5.50 – 5.43 (m, 3H), 4.82 (d, *J* = 2.6 Hz, 1H), 4.70 (s, 2H), 4.37 (dd, *J* = 4.0, 2.6 Hz, 1H), 4.19 (t, *J* = 5.4 Hz, 1H), 3.44 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.02, 162.37, 151.07, 139.71, 137.50, 128.38 (2C),

127.85, 127.70 (2C), 116.60, 102.71, 90.45, 82.72, 80.66, 72.43, 70.43, 69.12, 60.90, 59.37, 20.73; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{24}N_3O_8$ [M + H] 446.1563, found: 446.1580.



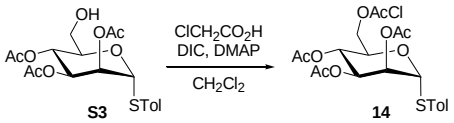
S2

(2*R*,3*R*,4*S*,5*S*,6*R*)-2-(Acetoxymethyl)-6-(*p*-tolylthio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S2). The title compound was synthesized according to the reported procedure[1]: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 (d, *J* = 8.2 Hz, 2H), 7.12 (d, *J* = 7.9 Hz, 2H), 5.50 – 5.48 (m, 1H), 5.41 (d, *J* = 1.5 Hz, 1H), 5.32 (dd, *J* = 5.6, 1.7 Hz, 2H), 4.56 (dp, *J* = 8.1, 2.5 Hz, 1H), 4.30 (dd, *J* = 12.2, 5.9 Hz, 1H), 4.10 (dd, *J* = 12.2, 2.4 Hz, 1H), 2.33 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.56, 169.92, 169.81, 169.74, 138.44, 132.58 (2C), 129.93 (2C), 128.72, 85.99, 70.82, 69.34, 69.32, 66.34, 62.46, 21.13, 20.89, 20.72, 20.70, 20.65; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{27}O_9S$ [M + H] 455.1376, found: 455.1395.



S2 **S3**

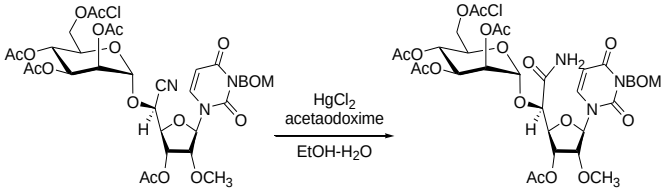
(2*R*,3*R*,4*S*,5*S*,6*R*)-2-(Hydroxymethyl)-6-(*p*-tolylthio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S3). To a stirred solution of **S2** (11.1 g, 24.3 mmol) in a 1:4 mixture of THF and MeOH (25 mL) was added [^{t}Bu$_2$Sn(OH)Cl]$_2$ (1.39 g, 2.43 mmol). After being stirred for 24h at r.t., the solution was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 2/1 - 1/1) to afford 8.01 g (80%) of **S3**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 5.51 (dd, *J* = 3.3, 1.6 Hz, 1H), 5.42 (d, *J* = 1.5 Hz, 1H), 5.38 (dd, *J* = 10.1, 3.3 Hz, 1H), 5.29 (t, *J* = 10.0 Hz, 1H), 4.30 (ddd, *J* = 9.7, 4.2, 2.3 Hz, 1H), 3.69 (dd, *J* = 12.7, 2.5 Hz, 1H), 3.63 (dd, *J* = 12.8, 4.2 Hz, 1H), 2.32 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.78, 169.96, 169.81, 138.43, 132.58 (2C), 129.99 (2C), 128.73, 86.04, 71.62, 70.88, 69.10, 66.51, 61.20, 21.11, 20.87, 20.75, 20.66; HRMS (ESI+) *m*/*z* calcd for $C_{19}H_{25}O_8S$ [M + H] 413.1270, found: 413.1277.



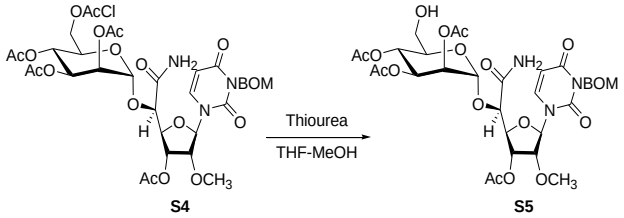
S3 **14**

(2*R*,3*R*,4*S*,5*S*,6*R*)-2-((2-Chloroacetoxy)methyl)-6-(*p*-tolylthio)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (14). To a stirred solution of **S3** (8.01 g, 19.4 mmol), chloroacetic acid (2.75 g, 29.1 mmol) and DMAP (0.24 g, 1.94 mmol) in CH_2Cl_2 (50 mL) was added

DIC (4.56 mL, 29.1 mmol). After being stirred for 4h at r.t., the reaction mixture was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 3/1 - 2/1) to afford 8.92 g (94%) of **14**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 2H), 5.49 (d, *J* = 2.2 Hz, 1H), 5.42 (d, *J* = 1.6 Hz, 1H), 5.32 (d, *J* = 6.4 Hz, 2H), 4.59 – 4.54 (m, 1H), 4.40 (dd, *J* = 12.2, 5.9 Hz, 1H), 4.21 (dd, *J* = 12.1, 2.2 Hz, 1H), 4.03 (d, *J* = 1.1 Hz, 2H), 2.33 (s, 3H), 2.14 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.88, 169.81, 169.78, 166.93, 138.51, 132.48 (2C), 129.98 (2C), 128.56, 85.84, 70.70, 69.19, 69.17, 66.19, 63.95, 40.58, 21.13, 20.87, 20.71, 20.64; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{26}ClO_9S$ [M + H] 489.0986, found: 489.1003.

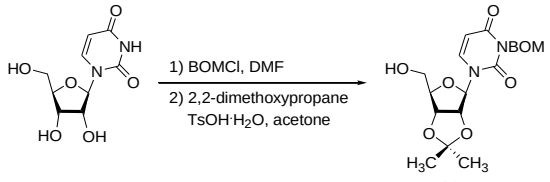


(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-1-((2*S*,5*R*)-3-Acetoxy-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-methoxytetrahydrofuran-2-yl)-2-amino-2-oxoethoxy)-6-((2-chloroacetoxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S4). To a stirred solution of **15** (0.24 g, 0.29 mmol) in a 9:1 mixture of EtOH and H_2O (2.9 mL) were added $HgCl_2$ (0.16 g, 0.59 mmol) and acetaldoxime (0.18 mL, 2.9 mmol). After being stirred for 13h at r.t., the reaction mixture was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexanes/EtOAc = 1/2 - $CHCl_3$/MeOH = 96/4) to afford **S4** (0.21 g, 87%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.53 (d, *J* = 8.2 Hz, 1H), 7.39 – 7.30 (m, 5H), 6.36 (brs, 1H), 6.06 (d, *J* = 8.0 Hz, 1H), 5.97 (d, *J* = 4.1 Hz, 1H), 5.76 (brs, 1H), 5.53 – 5.44 (m, 2H), 5.40 – 5.27 (m, 2H), 5.19 (dd, *J* = 10.0, 3.3 Hz, 1H), 5.11 (t, *J* = 5.8 Hz, 1H), 4.99 (d, *J* = 2.0 Hz, 1H), 4.70 (s, 2H), 4.68 (d, *J* = 6.5 Hz, 1H), 4.53 (dd, *J* = 6.1, 2.2 Hz, 1H), 4.42 (d, *J* = 2.2 Hz, 1H), 4.37 (dd, *J* = 12.4, 5.3 Hz, 1H), 4.21 (dd, *J* = 12.3, 2.4 Hz, 1H), 4.15 (d, *J* = 2.4 Hz, 2H), 4.01 (dd, *J* = 5.6, 4.2 Hz, 1H), 3.46 (s, 3H), 2.18 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.22, 170.18, 170.04, 169.67, 169.53, 167.06, 162.40, 150.91, 137.75, 137.58, 128.34, 128.32 (2C), 127.70 (2C), 103.35, 97.10, 88.44, 80.95, 76.39, 72.25, 70.51, 70.33, 69.76, 68.72, 68.68, 64.98, 63.43, 59.03, 40.65, 20.76, 20.67, 20.63 (2C); HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{43}ClN_3O_{18}$ [M + H] 828.2230, found: 828.2246.



S4 **S5**

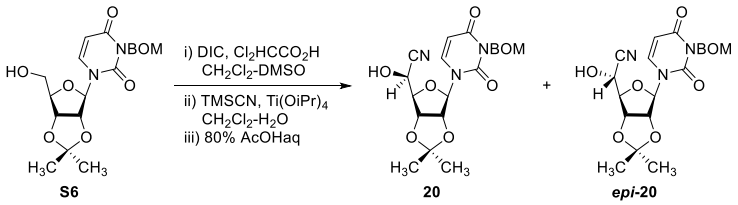
(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-1-((2*S*,5*R*)-3-Acetoxy-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-methoxytetrahydrofuran-2-yl)-2-amino-2-oxoethoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S5). To a solution of **S4** (0.21 g, 0.26 mmol) in a 1:1 mixture of THF and MeOH (2.6 mL) was added thiourea (0.059 g, 0.77 mmol). After being stirred for 11h at 50 °C , the reaction mixture was concentrated *in vacuo*. The residue was quenched with H_2O, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98/2 - 97/3 - 96/4) to afford **S5** (0.15 g, 75%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 8.2 Hz, 1H), 7.40 – 7.28 (m, 5H), 6.46 (brs, 1H), 6.10 (d, *J* = 8.2 Hz, 1H), 5.93 (d, *J* = 2.7 Hz, 1H), 5.88 (brs, 1H), 5.50 (d, *J* = 9.8 Hz, 1H), 5.46 (d, *J* = 9.5 Hz, 1H), 5.41 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.28 – 5.22 (m, 1H), 5.17 (dd, *J* = 10.1, 3.3 Hz, 1H), 5.01 (dd, *J* = 7.8, 5.5 Hz, 1H), 4.99 (d, *J* = 1.9 Hz, 1H), 4.70 (s, 2H), 4.58 (dd, *J* = 7.9, 2.2 Hz, 1H), 4.43 (d, *J* = 2.2 Hz, 1H), 4.03 (dd, *J* = 5.3, 2.6 Hz, 1H), 3.70 (ddd, *J* = 9.5, 4.7, 2.9 Hz, 1H), 3.60 (dd, *J* = 5.8, 3.9 Hz, 1H), 3.49 (s, 3H), 3.38 (d, *J* = 14.3 Hz, 1H), 2.16 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.42, 170.38, 170.18, 170.04, 162.54, 150.77, 137.75, 137.36, 128.32 (2C), 127.73 (2C), 127.70, 103.06, 97.25, 88.88, 81.14, 80.39, 75.76, 72.92, 72.26, 70.25, 69.52, 68.83, 68.75, 65.42, 61.25, 58.98, 20.78, 20.74, 20.66, 20.59; HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{42}N_3O_{17}$ [M + H] 752.2514, found: 752.2522.



S6

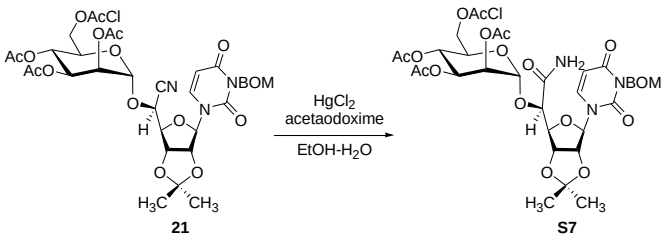
3-((Benzyloxy)methyl)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione (S6). To a stirred solution of uridine (14.7g, 60 mmol) in DMF (50 mL) were added BOMCl (6.87 mL, 50 mmol) and DBU (11.2 mL, 75 mmol). After being stirred for 6h at 0 °C, the reaction was quenched with 1M aq. HCl, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. To a stirred solution of the crude mixture and 2,2-dimethoxypropane (18.4 mL, 150 mmol) in acetone (50 mL) was added $TsOH·H_2O$ (0.95 g, 5.0 mmol). After being stirred for 8h at r.t., the solution was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 1/2)

to afford 15.8 g (78% for 2 steps) of **S6**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.26 (m, 6H), 5.73 (d, *J* = 8.1 Hz, 1H), 5.56 (d, *J* = 2.6 Hz, 1H), 5.48 (d, *J* = 9.7 Hz, 1H), 5.44 (d, *J* = 9.7 Hz, 1H), 4.97 (dd, *J* = 6.4, 2.7 Hz, 1H), 4.94 (dd, *J* = 9.5, 3.1 Hz, 1H), 4.69 (s, 2H), 4.29 (q, *J* = 3.0 Hz, 1H), 3.91 (dd, *J* = 12.0, 2.5 Hz, 1H), 3.79 (dd, *J* = 12.0, 3.5 Hz, 1H), 1.57 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.50, 151.00, 141.27, 137.70, 128.30 (2C), 127.70, 127.59 (2C), 114.25, 102.01, 96.49, 86.93, 83.83, 80.23, 72.36, 70.32, 62.63, 27.21, 25.22; HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{25}N_2O_7$ [M + H] 405.1662, found: 405.1681.

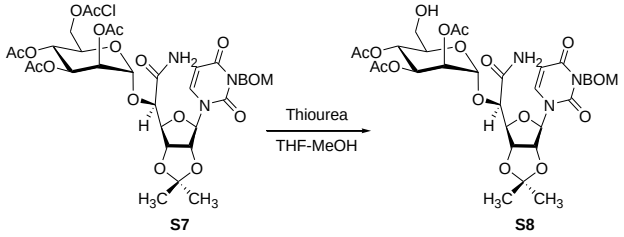


S6 20 *epi*-20

(*S*)-2-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-2-hydroxyacetonitrile (20). To a stirred solution of **S6** (2.89 g, 7.15 mmol) and dichloroacetic acid (0.88 mL, 10.7 mmol) in a 10:1 mixture of CH_2Cl_2 and DMSO (31.5 mL) was added DIC (2.24 mL, 14.3 mmol) at 0 °C. After being stirred for 2h, H_2O (0.29 mL), TMSCN (1.35 mL, 14.3 mmol) and Ti(O^{i}Pr)$_4$ (4.23 mL, 14.3 mmol) were added to the reaction solution. After being stirred for 6h at r.t., the solution was concentrated *in vacuo*. The crude mixture was suspended to a 4:1 mixture of AcOH and H_2O (50 mL). After being stirred for 9h at r.t., the solution was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 2/1 - 1/1) to afford 1.18 g (38%) of **20** and 1.03 g (34%) of ***epi*-20**. Data for **20**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.30 (m, 5H), 7.15 (d, *J* = 8.0 Hz, 1H), 5.80 (d, *J* = 8.1 Hz, 1H), 5.49 (d, *J* = 9.8 Hz, 1H), 5.44 (d, *J* = 9.8 Hz, 1H), 5.35 (d, *J* = 2.8 Hz, 1H), 5.14 (dd, *J* = 6.6, 2.8 Hz, 1H), 5.07 (dd, *J* = 6.6, 3.5 Hz, 1H), 4.72 – 4.68 (m, 3H), 4.40 (dd, *J* = 3.5, 2.3 Hz, 1H), 1.57 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.91, 151.68, 142.29, 137.52, 128.41 (2C), 127.87, 127.58 (2C), 117.55, 115.20, 102.97, 99.49, 86.78, 82.89, 79.50, 72.60, 70.48, 62.12, 27.09, 25.10; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{24}N_3O_7$ [M + H] 430.1614, found: 430.1630. Data for **(*R*)-2-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-2-hydroxyacetonitrile (*epi*-20)**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.27 (m, 5H), 7.18 (d, *J* = 8.1 Hz, 1H), 5.77 (d, *J* = 8.0 Hz, 1H), 5.49 – 5.41 (m, 3H), 5.10 (dd, *J* = 6.6, 2.9 Hz, 1H), 5.07 (dd, *J* = 6.5, 2.3 Hz, 1H), 4.76 (d, *J* = 5.3 Hz, 1H), 4.68 (s, 2H), 4.36 (dd, *J* = 5.3, 2.9 Hz, 1H), 1.58 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.20, 151.16, 141.86, 137.43, 128.40 (2C), 127.89, 127.67 (2C), 117.41, 114.95, 102.67, 98.31, 87.29, 83.40, 80.68, 72.55, 70.42, 61.78, 27.02, 25.06; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{24}N_3O_7$ [M + H] 430.1614, found: 430.1633.

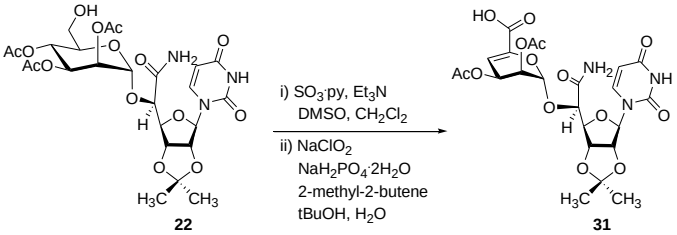


(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((*R*)-2-Amino-1-((3a*R*,4*S*,6*R*,6a*R*)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-2-oxoethoxy)-6-((2-chloroacetoxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S7). To a stirred solution of **21** (0.38 g, 0.49 mmol) in a 9:1 mixture of EtOH and H_2O (4.9 mL) were added $HgCl_2$ (0.27 g, 0.98 mmol) and acetaldoxime (0.30 mL, 4.9 mmol). After being stirred for 12h at r.t., the reaction mixture was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexanes/EtOAc = 1/2 - $CHCl_3$/MeOH = 97/3) to afford **S7** (0.36 g, 91%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.27 (m, 6H), 6.41 (brs, 1H), 5.86 (d, *J* = 8.1 Hz, 1H), 5.81 (brs, 1H), 5.70 (d, *J* = 2.1 Hz, 1H), 5.49 (d, *J* = 9.9 Hz, 1H), 5.46 (d, *J* = 9.8 Hz, 1H), 5.33 – 5.26 (m, 2H), 4.98 (dd, *J* = 6.3, 4.9 Hz, 1H), 4.93 – 4.91 (m, 1H), 4.89 (dd, *J* = 6.3, 2.0 Hz, 1H), 4.69 (s, 2H), 4.41 (d, *J* = 4.2 Hz, 1H), 4.33 (t, *J* = 4.6 Hz, 1H), 4.28 (dd, *J* = 12.1, 5.3 Hz, 1H), 4.18 (d, *J* = 2.5 Hz, 1H), 4.16 – 4.11 (m, 2H), 4.10 (s, 2H), 2.14 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.57 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.19, 170.09, 170.01, 169.63, 166.94, 162.40, 150.62, 140.32, 137.80, 128.29 (2C), 127.69, 127.65 (2C), 115.00, 102.60, 97.38, 93.65, 85.88, 84.08, 79.80, 77.63, 72.31, 70.33, 69.80, 69.05, 68.60, 65.36, 63.41, 40.56, 27.32, 25.55, 20.77, 20.67, 20.63; HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{43}ClN_3O_{17}$ [M + H] 812.2281, found: 812.2314.



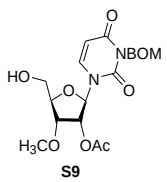
(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((*R*)-2-Amino-1-((3a*R*,4*S*,6*R*,6a*R*)-6-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-2-oxoethoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S8). To a solution of **S7** (0.36 g, 0.45 mmol) in a 1:1 mixture of THF and MeOH (4.5 mL) was added thiourea (0.10 g, 1.34 mmol). After being stirred for 11h at 50 °C, the reaction mixture was concentrated *in vacuo*. The residue was quenched with H_2O, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98/2 - 97/3 - 96/4) to afford **S8** (0.25 g, 76%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39

– 7.27 (m, 6H), 6.43 (brs, 1H), 5.83 (d, *J* = 8.1 Hz, 1H), 5.74 (brs, 1H), 5.68 (d, *J* = 2.1 Hz, 1H), 5.46 (s, 2H), 5.33 – 5.27 (m, 2H), 5.18 (t, *J* = 9.9 Hz, 1H), 5.08 (dd, *J* = 6.5, 4.5 Hz, 1H), 4.92 – 4.88 (m, 2H), 4.70 (s, 2H), 4.43 (d, *J* = 5.7 Hz, 1H), 4.33 (dd, *J* = 5.7, 4.5 Hz, 1H), 3.89 (dt, *J* = 10.0, 3.8 Hz, 1H), 3.50 (d, *J* = 3.9 Hz, 2H), 2.14 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.56 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.35, 170.16, 170.10, 170.09, 162.46, 150.72, 140.56, 137.69, 128.33 (2C), 127.74, 127.69 (2C), 114.84, 102.50, 97.03, 94.40, 86.72, 84.25, 80.13, 77.92, 72.35, 72.04, 70.23, 69.20, 68.59, 65.87, 61.25, 27.23, 25.41, 20.81, 20.73, 20.69; HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{42}N_3O_{16}$ [M + H] 736.2565, found: 736.2586.

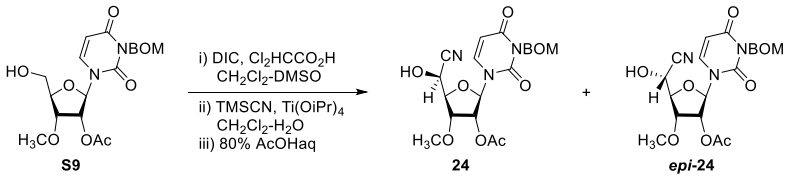


22 **31**

(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-((*R*)-2-amino-1-((3a*R*,4*S*,6*R*,6a*R*)-6-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-2-oxoethoxy)-3,4-dihydro-2*H*-pyran-6-carboxylic acid (31). To a stirred solution of **22** (0.17 g, 0.27 mmol) and DMSO (0.19 mL, 2.72 mmol) in a 5:1 mixture of CH_2Cl_2 and Et_3N (1.4 mL) was added SO_3·pyridine (0.43 g, 2.72 mmol). After being stirred for 3h at r.t., the reaction mixture was added H_2O (0.27 mL) and passed through a silica gel pad ($CHCl_3$/MeOH = 92/8). To a stirred solution of the crude mixture in ^{t}BuOH (1.0 mL) and 2-methyl-2-butene (0.5 mL) was added a solution of $NaClO_2$ (0.12 g, 1.36 mmol) and NaH_2PO_4·$2H_2O$ (0.21 g, 1.36 mmol) in H_2O (1.0 mL). After being stirred for 5h at r.t., the reaction extracted with $CHCl_3$/MeOH (9/1). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) to afford **31** (0.13 g, 81%): ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.88 (d, *J* = 8.1 Hz, 1H), 5.88 (d, *J* = 3.6 Hz, 1H), 5.84 (d, *J* = 8.1 Hz, 1H), 5.80 (dd, *J* = 2.7, 1.5 Hz, 1H), 5.53 (dd, *J* = 4.5, 2.5 Hz, 1H), 5.41 (ddd, *J* = 4.7, 3.4, 1.6 Hz, 1H), 5.28 (d, *J* = 3.3 Hz, 1H), 4.84 (d, *J* = 1.9 Hz, 1H), 4.77 (dd, *J* = 6.2, 2.1 Hz, 1H), 4.73 (d, *J* = 2.1 Hz, 1H), 4.62 (dd, *J* = 6.1, 3.7 Hz, 1H), 2.07 (s, 3H), 2.03 (s, 3H), 1.53 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (101 MHz, MeOD) δ 172.67, 171.86, 171.50, 168.09, 166.27, 152.17, 148.19, 142.21, 115.19, 104.36, 103.01, 98.08, 93.58, 87.18, 86.07, 82.82, 78.22, 65.27, 65.01, 27.45, 25.47, 20.69, 20.57; HRMS (ESI+) *m*/*z* calcd for $C_{23}H_{28}N_3O_{14}$ [M + H] 570.1571, found: 570.1585.

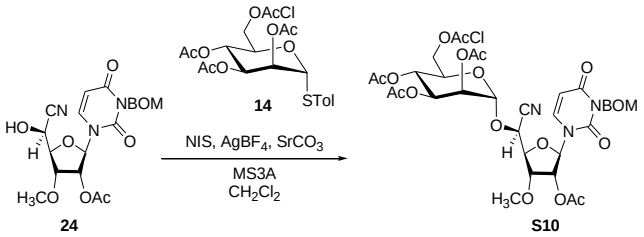

S9

(2*R*,5*R*)-2-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-5-(hydroxymethyl)-4-methoxytetrahydrofuran-3-yl acetate (S9). The title compound was synthesized according to the reported procedure[1]: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 8.2 Hz, 1H), 7.39 – 7.27 (m, 5H), 5.78 (d, *J* = 3.7 Hz, 1H), 5.75 (d, *J* = 8.1 Hz, 1H), 5.46 (d, *J* = 1.7 Hz, 2H), 5.43 (dd, *J* = 4.7, 3.7 Hz, 1H), 4.69 (s, 2H), 4.10 (s, 2H), 4.02 (dd, *J* = 12.2, 1.7 Hz, 1H), 3.80 (dd, *J* = 12.0, 1.8 Hz, 1H), 3.41 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.85, 162.51, 150.87, 139.88, 137.74, 128.31 (2C), 127.68, 127.66 (2C), 102.22, 91.10, 82.75, 73.84, 72.25, 70.30, 61.30, 20.71; HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{25}N_2O_8$ [M + H] 421.1611, found: 421.1630.

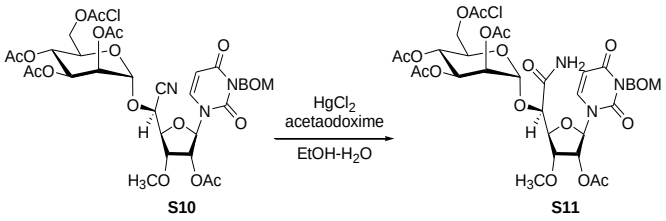


S9 24 *epi*-24

(2*R*,5*R*)-2-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-5-((*S*)-cyano(hydroxy)methyl)-4-methoxytetrahydrofuran-3-yl acetate (24). To a stirred solution of **S9** (4.14 g, 9.85mmol) and dichloroacetic acid (1.22 mL, 14.8 mmol) in a 10:1 mixture of CH_2Cl_2 and DMSO (42.9 mL) was added DIC (3.08 mL, 19.7 mmol) at 0 °C. After being stirred for 2h, H_2O (0.39 mL), TMSCN (2.46 mL, 19.7 mmol) and Ti(O^{i}Pr)$_4$ (5.83 mL, 19.7 mmol) were added to the reaction solution. After being stirred for 8h at r.t., the solution was concentrated *in vacuo*. The crude mixture was suspended to a 4:1 mixture of AcOH and H_2O (50 mL). After being stirred for 12h at r.t., the solution was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 2/1 - 1/2) to afford 1.84 g (42%) of **24** and 1.87 g (43%) of ***epi*-24**. Data for **24**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.26 (m, 6H), 5.79 (d, *J* = 8.1 Hz, 1H), 5.53 (d, *J* = 4.5 Hz, 1H), 5.49 (d, *J* = 5.4 Hz, 1H), 5.46 (d, *J* = 1.5 Hz, 2H), 4.70 (s, 2H), 4.64 (d, *J* = 2.1 Hz, 1H), 4.29 (t, *J* = 5.5 Hz, 1H), 4.24 (dd, *J* = 5.3, 1.9 Hz, 1H), 3.41 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.94, 162.18, 151.22, 141.38, 137.47, 128.41 (2C), 128.39, 127.86, 127.69 (2C), 117.53, 102.82, 94.65, 83.19, 73.05, 72.48, 70.45, 61.21, 59.28, 20.62; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{24}N_3O_8$ [M + H] 446.1563, found: 446.1576. Data for **(2*R*,5*R*)-2-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-5-((*R*)-cyano(hydroxy)methyl)-4-methoxytetrahydrofuran-3-yl acetate (*epi*-24)**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.27 (m, 6H), 5.82 (d, *J* = 5.9 Hz, 1H), 5.77 (d, *J* = 8.1 Hz, 1H), 5.45 (d, *J* = 3.1 Hz, 2H), 5.39 (t, *J* = 5.7 Hz, 1H), 4.78 (d, *J* = 2.8 Hz, 1H), 4.69 (s, 2H), 4.31 – 4.25 (m, 2H), 3.46 (s, 3H), 2.15 (s, 3H): ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.91, 162.39, 151.03, 140.34, 137.37, 128.41 (2C), 127.90, 127.77 (2C), 116.79, 102.81, 91.50, 83.44, 73.02, 72.41, 70.43, 61.44, 58.90, 20.60; HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{24}N_3O_8$ [M + H] 446.1563, found: 446.1569.

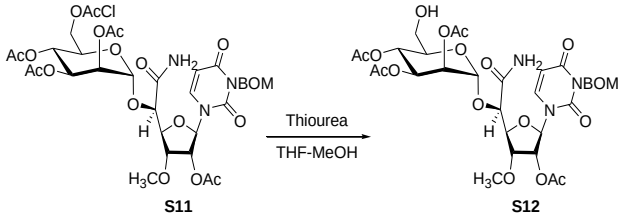


(2*S*,3*S*,4*S*,5*R*,6*R*)-2-((1*S*)-((2*R*,5*R*)-4-Acetoxy-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-methoxytetrahydrofuran-2-yl)(cyano)methoxy)-6-((2-chloroacetoxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S10). To a stirred suspension of **24** (0.72 g, 1.62 mmol), **14** (1.59 g, 3.24 mmol), MS3A (2.2 g) and $SrCO_3$ (1.20 g, 8.11 mmol) in CH_2Cl_2 (40.5 mL) were added $AgBF_4$ (0.16 g, 0.81 mmol) and NIS (1.09 g, 4.86 mmol) at 0 ºC. After 13h, the reaction mixture was added Et_3N (2.0 mL) and passed through a silica gel pad (hexanes/EtOAc = 1/4). The solution was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 2/1 - 1/2) to afford **S10** (1.05 g, 80%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.28 (m, 6H), 5.94 (d, *J* = 12.1 Hz, 1H), 5.94 (s, 1H), 5.49 (d, *J* = 9.8 Hz, 1H), 5.45 (d, *J* = 9.8 Hz, 1H), 5.38 – 5.28 (m, 3H), 5.19 (d, *J* = 3.4 Hz, 1H), 5.17 – 5.16 (m, 1H), 4.77 (d, *J* = 4.3 Hz, 1H), 4.69 (s, 2H), 4.39 – 4.32 (m, 1H), 4.28 (dd, *J* = 6.2, 4.2 Hz, 1H), 4.22 (dd, *J* = 12.0, 2.5 Hz, 1H), 4.16 – 4.13 (m, 1H), 4.10 (s, 2H), 3.89 (ddd, *J* = 10.2, 5.4, 2.4 Hz, 1H), 3.43 (s, 3H), 2.18 (s, 6H), 2.05 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.79, 169.73, 169.54, 169.51, 166.81, 162.19, 150.76, 138.57, 137.70, 128.32 (2C), 127.72, 127.70 (2C), 114.28, 103.37, 96.10, 90.33, 80.52, 77.61, 72.97, 72.29, 70.36, 70.00, 68.49, 68.01, 65.26, 64.29, 63.51, 59.37, 40.53, 20.70, 20.64, 20.62, 20.56; HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{41}ClN_3O_{17}$ [M + H] 810.2125, found: 810.2144.



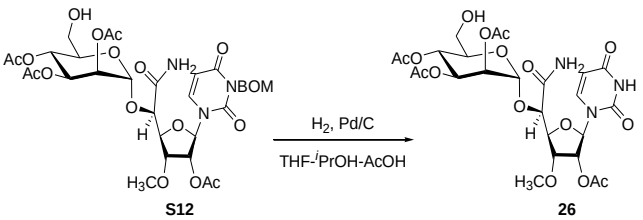
(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-1-((2*S*,5*R*)-4-Acetoxy-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-methoxytetrahydrofuran-2-yl)-2-amino-2-oxoethoxy)-6-((2-chloroacetoxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S11). To a stirred solution of **S10** (1.05 g, 1.30 mmol) in a 9:1 mixture of EtOH and H_2O (5.0 mL) were added $HgCl_2$ (0.70 g, 2.59 mmol) and acetaldoxime (0.79 mL, 13.0 mmol). After being stirred for 14h at r.t., the reaction mixture was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexanes/EtOAc = 1/2 - $CHCl_3$/MeOH = 96/4) to afford **S11** (0.87 g, 81%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 (d, *J* = 8.2 Hz, 1H), 7.38 – 7.30 (m, 5H), 6.03 (d, *J* = 8.2 Hz, 1H), 5.95 (d, *J* = 4.0 Hz, 1H), 5.77 – 5.71 (m, 1H),

5.48 (d, *J* = 9.5 Hz, 1H), 5.45 (d, *J* = 9.3 Hz, 1H), 5.39 – 5.37 (m, 1H), 5.35 (d, *J* = 10.1 Hz, 1H), 5.29 – 5.27 (m, 1H), 5.21 (dd, *J* = 10.1, 3.3 Hz, 1H), 4.99 – 4.98 (m, 1H), 4.69 (s, 2H), 4.42 (dd, *J* = 12.3, 5.2 Hz, 1H), 4.38 (s, 2H), 4.25 (dd, *J* = 12.2, 2.7 Hz, 1H), 4.13 (dd, *J* = 9.6, 2.3 Hz, 1H), 4.11 (d, *J* = 1.8 Hz, 2H), 4.04 – 4.01 (m, 1H), 3.40 (s, 1H), 3.39 (s, 3H), 2.17 (s, 3H), 2.16 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.16, 170.06, 169.89, 169.80, 169.61, 166.91, 162.35, 150.77, 138.11, 137.75, 128.36, 128.32 (2C), 127.69 (2C), 103.37, 97.07, 89.01, 81.18, 77.48, 73.01, 72.25, 70.32, 69.99, 68.81, 68.73, 65.20, 63.60, 58.88, 40.59, 21.06, 20.78, 20.67, 20.64; HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{43}ClN_3O_{18}$ [M + H] 828.2230, found: 828.2252.



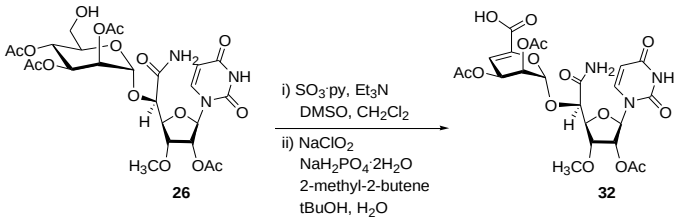
S11 **S12**

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-1-((2*S*,5*R*)-4-Acetoxy-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-methoxytetrahydrofuran-2-yl)-2-amino-2-oxoethoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S12). To a solution of **S11** (0.87 g, 1.05 mmol) in a 1:1 mixture of THF and MeOH (3.0 mL) was added thiourea (0.24 g, 3.16 mmol). After being stirred for 14h at 50 °C , the reaction mixture was concentrated *in vacuo*. The residue was quenched with H_2O, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98/2 - 97/3 - 96/4) to afford **S12** (0.61 g, 77%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44 (d, *J* = 8.2 Hz, 1H), 7.38 – 7.30 (m, 5H), 6.34 (brs, 1H), 6.02 (d, *J* = 8.2 Hz, 1H), 5.92 (d, *J* = 3.6 Hz, 1H), 5.72 (brs, 1H), 5.47 (d, *J* = 2.2 Hz, 2H), 5.39 – 5.36 (m, 1H), 5.30 – 5.25 (m, 2H), 4.98 (d, *J* = 1.8 Hz, 1H), 4.70 (s, 2H), 4.38 (dt, *J* = 9.4, 3.0 Hz, 2H), 4.07 – 4.02 (m, 1H), 3.93 – 3.88 (m, 1H), 3.68 – 3.64 (m, 2H), 3.41 (d, *J* = 5.4 Hz, 1H), 3.36 (s, 3H), 2.16 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.51, 170.23, 170.19, 170.02, 169.87, 162.39, 150.74, 138.15, 137.75, 128.34 (2C), 127.71, 127.69 (2C), 103.25, 97.28, 89.41, 81.39, 77.51, 73.21, 72.44, 72.28, 70.31, 68.96, 68.79, 65.59, 61.24, 58.92, 20.80, 20.74, 20.68, 20.67; HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{42}N_3O_{17}$ [M + H] 752.2514, found: 752.2535.



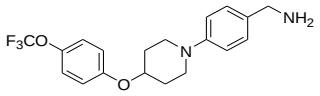
S12 **26**

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-1-((2*S*,5*R*)-4-Acetoxy-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-methoxytetrahydrofuran-2-yl)-2-amino-2-oxoethoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (26). To a stirred solution of **S12** (0.61 g, 0.81 mmol) and AcOH (0.12 mL) in a 1:1 mixture of THF and ^{i}PrOH (6.0 mL) was added Pd/C (0.49 g). H_2 gas was introduced and the reaction mixture was stirred under H_2 atmosphere. After being stirred for 5h at r.t., the solution was filtered through Celite and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 97/3 - 92/8) to afford **26** (0.39 g, 77%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.29 (brs, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 6.93 (brs, 1H), 6.00 (d, *J* = 4.5 Hz, 2H), 5.90 (d, *J* = 8.2 Hz, 1H), 5.54 (dd, *J* = 3.3, 1.7 Hz, 1H), 5.37 (t, *J* = 4.7 Hz, 1H), 5.12 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.97 (d, *J* = 1.6 Hz, 1H), 4.51 (dd, *J* = 12.1, 5.6 Hz, 1H), 4.46 (dd, *J* = 5.7, 1.8 Hz, 1H), 4.41 (d, *J* = 1.8 Hz, 1H), 4.38 (dd, *J* = 12.2, 2.2 Hz, 1H), 4.18 (t, *J* = 5.3 Hz, 1H), 4.02 (ddd, *J* = 7.8, 5.6, 2.0 Hz, 1H), 3.90 (t, *J* = 9.9 Hz, 1H), 3.40 (s, 3H), 2.15 (s, 6H), 2.13 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.25, 171.00, 170.66, 170.41, 163.33, 150.05, 139.56, 103.71, 96.81, 87.36, 81.28, 77.46, 75.50, 73.04, 72.36, 71.12, 68.99, 65.05, 63.24, 58.56, 20.82, 20.81, 20.74, 20.72; HRMS (ESI+) *m*/*z* calcd for $C_{25}H_{34}N_3O_{16}$ [M + H] 632.1939, found: 632.1958.



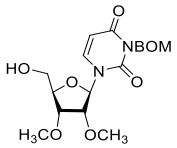
26 **32**

(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-((1*R*)-1-((2*S*,5*R*)-4-acetoxy-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-methoxytetrahydrofuran-2-yl)-2-amino-2-oxoethoxy)-3,4-dihydro-2*H*-pyran-6-carboxylic acid (32). To a stirred solution of **26** (0.39 g, 0.62 mmol) and DMSO (0.44 mL, 6.23 mmol) in a 5:1 mixture of CH_2Cl_2 and Et_3N (3.1 mL) was added SO_3·pyridine (0.99 g, 6.23 mmol). After being stirred for 1h at r.t., the reaction mixture was added H_2O (0.62 mL) and passed through a silica gel pad ($CHCl_3$/MeOH = 93/7). To a stirred solution of the crude mixture in ^{t}BuOH (2.0 mL) and 2-methyl-2-butene (1.0 mL) was added a solution of $NaClO_2$ (0.28 g, 3.11 mol) and NaH_2PO_4·$2H_2O$ (0.49 g, 3.11 mmol) in H_2O (2.0 mL). After being stirred for 2h at r.t., the reaction extracted with $CHCl_3$/MeOH (9/1). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) to afford **32** (0.30 g, 82%): ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.80 (d, *J* = 8.2 Hz, 1H), 5.95 (d, *J* = 4.9 Hz, 1H), 5.92 (d, *J* = 8.1 Hz, 1H), 5.80 – 5.78 (m, 1H), 5.70 (dd, *J* = 4.5, 2.5 Hz, 1H), 5.59 – 5.56 (m, 1H), 5.31 (d, *J* = 3.2 Hz, 1H), 5.29 (t, *J* = 5.1 Hz, 1H), 4.80 (d, *J* = 1.9 Hz, 1H), 4.47 (dd, *J* = 4.8, 1.8 Hz, 1H), 3.93 (t, *J* = 5.0 Hz, 1H), 3.40 (s, 3H), 2.08 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (101 MHz, MeOD) δ 173.04, 172.10, 171.63, 171.35, 168.19, 166.08, 152.15, 148.06, 141.34, 104.67, 103.84, 97.92, 88.21, 84.13, 79.65, 76.98, 75.16, 65.37, 65.05, 59.67, 20.77, 20.57, 20.44; HRMS (ESI+) *m*/*z* calcd for $C_{23}H_{28}N_3O_{15}$ [M + H] 586.1520, found: 586.1543.



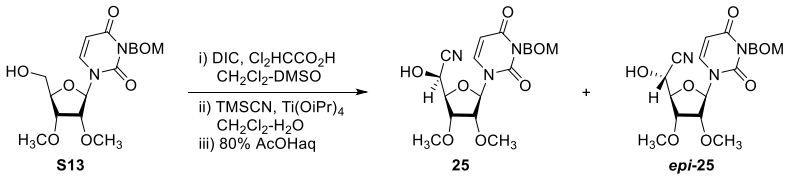
34

(4-(4-(4-(Trifluoromethoxy)phenoxy)piperidin-1-yl)phenyl)methanamine (34). The title compound was synthesized according to the reported procedure[2]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.2 Hz, 2H), 7.14 (d, *J* = 8.6 Hz, 2H), 6.97 – 6.87 (m, 4H), 4.43 (tt, *J* = 7.7, 3.8 Hz, 1H), 3.79 (s, 2H), 3.49 (ddd, *J* = 11.7, 7.2, 3.7 Hz, 2H), 3.09 (ddd, *J* = 12.2, 8.2, 3.6 Hz, 2H), 2.15 – 2.06 (m, 2H), 1.98 – 1.88 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.8, 150.2, 142.8, 134.6, 128.0 (2C), 122.5 (2C), 116.83 (2C), 116.76 (2C), 72.9, 46.9 (2C), 45.9, 30.4 (2C); HRMS (ESI+) *m*/*z* calcd for $C_{19}H_{22}F_3N_2O_2$ [M + H] 367.1633, found 367.1628.



S13

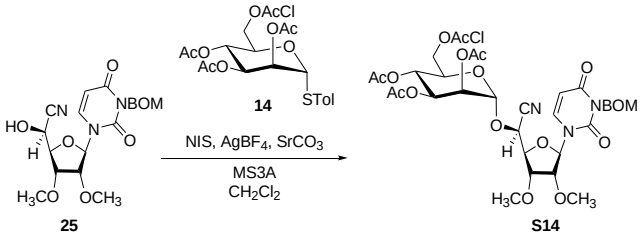
3-((Benzyloxy)methyl)-1-((2*R*,5*R*)-5-(hydroxymethyl)-3,4-dimethoxytetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (S13). The title compound was synthesized according to the reported procedure[1]: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.81 (d, *J* = 8.1 Hz, 1H), 7.39 – 7.23 (m, 5H), 5.74 (d, *J* = 4.6 Hz, 2H), 5.73 (d, *J* = 6.4 Hz, 2H), 5.47 (d, *J* = 1.4 Hz, 2H), 4.71 (s, 2H), 4.17 (dt, *J* = 6.9, 2.2 Hz, 1H), 4.08 – 4.03 (m, 2H), 3.91 (dd, *J* = 6.8, 4.9 Hz, 1H), 3.80 (dd, *J* = 12.3, 2.2 Hz, 1H), 3.59 (s, 3H), 3.45 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.72, 150.80, 139.99, 137.74, 128.29 (2C), 127.70, 127.63 (2C), 101.61, 90.64, 82.30, 81.06, 76.53, 72.30, 70.19, 60.93, 58.41, 58.15; HRMS (ESI+) *m*/*z* calcd for $C_{19}H_{25}N_2O_7$ [M + H] 393.1662, found 393.1675.



S13 25 *epi*-25

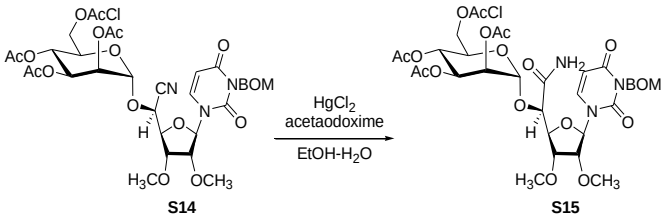
(2*S*)-2-((2*R*,5*R*)-5-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethoxytetrahydrofuran-2-yl)-2-hydroxyacetonitrile (25). To a stirred solution of **S13** (1.76 g, 4.48 mmol) and dichloroacetic acid (0.55 mL, 6.73 mmol) in a 10:1 mixture of CH_2Cl_2 and DMSO (19.7 mL) was added DIC (1.40 mL, 8.97 mmol) at 0 °C. After being stirred for 3h, H_2O (0.18 mL), TMSCN (0.85 mL, 8.97 mmol) and $Ti(O^iPr)_4$ (2.65

mL, 8.97 mmol) were added to the reaction solution. After being stirred for 6h at r.t., the solution was concentrated *in vacuo*. The crude mixture was suspended to a 4:1 mixture of AcOH and H_2O (25 mL). After being stirred for 10h at r.t., the solution was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 2/1 - 1/2) to afford 0.73 g (39%) of **25** and 0.73 g (39%) of ***epi*-25**. Data for **25**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.30 (m, 6H), 5.79 (d, *J* = 8.1 Hz, 1H), 5.48 (s, 2H), 5.47 – 5.44 (m, 1H), 4.71 (s, 2H), 4.63 (d, *J* = 2.1 Hz, 1H), 4.37 (t, *J* = 5.4 Hz, 1H), 4.34 (dd, *J* = 4.0, 2.0 Hz, 1H), 4.02 (dd, *J* = 5.4, 4.0 Hz, 1H), 3.50 (s, 3H), 3.48 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.14, 151.22, 141.68, 137.54, 128.40 (2C), 127.84, 127.67 (2C), 117.49, 102.76, 94.96, 82.95, 79.40, 77.56, 72.50, 70.39, 61.69, 58.60, 58.44; HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{24}N_3O_7$ [M + H] 418.1614, found 418.1633. Data for **(2*R*)-2-((2*R*,5*R*)-5-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethoxytetrahydrofuran-2-yl)-2-hydroxyacetonitrile (*epi*-25)**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.27 (m, 6H), 5.78 (d, *J* = 8.1 Hz, 1H), 5.57 (d, *J* = 6.3 Hz, 1H), 5.47 (d, *J* = 9.7 Hz, 1H), 5.44 (d, *J* = 9.7 Hz, 1H), 4.78 (d, *J* = 2.5 Hz, 1H), 4.70 (s, 2H), 4.34 – 4.30 (m, 2H), 4.12 (dd, *J* = 5.4, 2.9 Hz, 1H), 3.53 (s, 3H), 3.49 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.26, 151.07, 141.31, 137.41, 128.41, 127.90, 127.71, 116.84, 102.73, 93.60, 83.46, 79.51, 77.23, 72.49, 70.38, 61.91, 58.49, 58.11; HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{24}N_3O_7$ [M + H] 418.1614, found 418.1638.

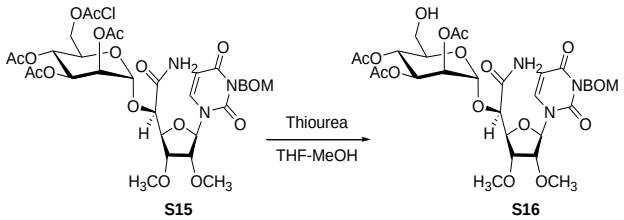


(2*S*,3*S*,4*S*,5*R*,6*R*)-2-((1*S*)-((2*R*,5*R*)-5-(3-((Benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethoxytetrahydrofuran-2-yl)(cyano)methoxy)-6-((2-chloroacetoxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S14). To a stirred suspension of **25** (0.20 g, 0.48 mmol), **14** (0.47 g, 0.95 mmol), MS3A (0.60 g) and $SrCO_3$ (0.35 g, 2.39 mmol) in CH_2Cl_2 (11.9 mL) were added $AgBF_4$ (0.047 g, 0.24 mmol) and NIS (0.21 g, 0.95 mmol) at 0 °C. After being stirred for 20h, the reaction mixture was added Et_3N (2.0 mL) and passed through a silica gel pad (hexanes/EtOAc = 1/4). The solution was concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc = 2/1 - 1/2) to afford **S14** (0.32 g, 87%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.46 (d, *J* = 8.3 Hz, 1H), 7.39 – 7.27 (m, 5H), 6.04 (d, *J* = 8.3 Hz, 1H), 5.96 (d, *J* = 2.1 Hz, 1H), 5.49 (d, *J* = 9.7 Hz, 1H), 5.46 (d, *J* = 9.7 Hz, 1H), 5.40 (dd, *J* = 3.4, 2.0 Hz, 1H), 5.29 (t, *J* = 10.0 Hz, 1H), 5.19 (d, *J* = 1.9 Hz, 1H), 5.12 (dd, *J* = 10.1, 3.4 Hz, 1H), 4.77 (d, *J* = 3.1 Hz, 1H), 4.71 (s, 2H), 4.39 (dd, *J* = 7.6, 3.1 Hz, 1H), 4.35 – 4.27 (m, 2H), 4.11 (s, 2H), 4.02 – 3.96 (m, 1H), 3.85 (dd, *J* = 7.7, 5.0 Hz, 1H), 3.77 (dd, *J* = 10.0, 4.6 Hz, 1H), 3.63 (s, 3H), 3.43 (s, 3H), 2.19 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.76, 169.50, 169.46, 166.84, 162.35, 150.71, 137.75, 137.25,

128.31 (2C), 127.71 (2C), 114.36, 103.02, 95.84, 89.16, 80.49, 80.45, 79.68, 72.28, 70.25, 70.21, 68.50, 67.79, 65.03, 63.44, 62.75, 58.57, 58.08, 40.50, 20.68, 20.63, 20.62, 20.53; HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{41}ClN_3O_{16}$ [M + H] 782.2175, found 782.2197.

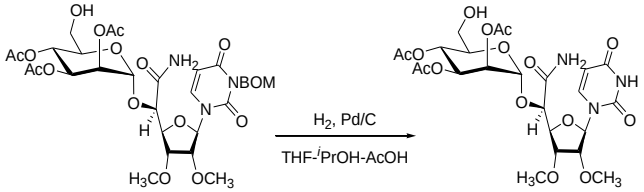


(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-2-Amino-1-((2*S*,5*R*)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethoxytetrahydrofuran-2-yl)-2-oxoethoxy)-6-((2-chloroacetoxy)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S15). To a stirred solution of **S14** (0.32 g, 0.42 mmol) in a 9:1 mixture of EtOH and H_2O (4.2 mL) were added $HgCl_2$ (0.23 g, 0.83 mmol) and acetaldoxime (0.25 mL, 4.15 mmol). After being stirred for 11h at r.t., the reaction mixture was concentrated *in vacuo*. The residue was quenched with aq. $NaHCO_3$, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexanes/EtOAc = 1/2 - $CHCl_3$/MeOH = 96/4) to afford **S15** (0.29 g, 88%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 8.2 Hz, 1H), 7.39 – 7.27 (m, 5H), 6.35 (brs, 1H), 6.08 (d, *J* = 8.1 Hz, 1H), 5.89 (d, *J* = 1.1 Hz, 1H), 5.83 (brs, 1H), 5.48 (d, *J* = 9.7 Hz, 1H), 5.45 (d, *J* = 9.7 Hz, 1H), 5.41 (dd, *J* = 3.4, 1.9 Hz, 1H), 5.30 (d, *J* = 10.0 Hz, 1H), 5.17 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.03 (d, *J* = 1.9 Hz, 1H), 4.71 (s, 2H), 4.48 (dd, *J* = 8.8, 2.6 Hz, 1H), 4.41 (d, *J* = 2.6 Hz, 1H), 4.34 (dd, *J* = 12.2, 5.5 Hz, 1H), 4.27 (dd, *J* = 12.2, 2.5 Hz, 1H), 4.11 (s, 2H), 3.91 (dd, *J* = 4.8, 1.1 Hz, 1H), 3.90 – 3.85 (m, 1H), 3.76 (dd, *J* = 8.7, 4.8 Hz, 1H), 3.63 (s, 3H), 3.38 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H); HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{43}ClN_3O_{17}$ [M + H] 800.2281, found 800.2314.



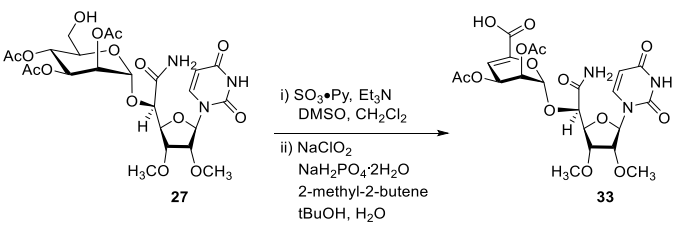
(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-2-Amino-1-((2*S*,5*R*)-5-(3-((benzyloxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethoxytetrahydrofuran-2-yl)-2-oxoethoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (S16). To a solution of **S15** (0.29 g, 0.37 mmol) in a 1:1 mixture of THF and MeOH (1.0 mL) was added thiourea (0.065 g, 0.86 mmol). After being stirred for 8h at 50 °C, the reaction mixture was concentrated *in vacuo*. The residue was quenched with H_2O, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude

product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98/2 - 97/3 - 96/4) to afford **S16** (0.21 g, 78%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.61 (d, *J* = 8.2 Hz, 1H), 7.40 – 7.28 (m, 5H), 6.43 (brs, 1H), 6.06 (d, *J* = 8.2 Hz, 1H), 5.87 (d, *J* = 1.2 Hz, 1H), 5.84 (brs, 1H), 5.47 (d, *J* = 1.5 Hz, 2H), 5.42 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.29 (t, *J* = 9.9 Hz, 1H), 5.20 (dd, *J* = 10.2, 3.4 Hz, 1H), 5.02 (d, *J* = 1.7 Hz, 1H), 4.71 (s, 2H), 4.48 – 4.43 (m, 2H), 3.91 (dd, *J* = 4.8, 1.3 Hz, 1H), 3.76 (dd, *J* = 8.5, 4.8 Hz, 1H), 3.74 – 3.69 (m, 1H), 3.67 – 3.63 (m, 2H), 3.63 (s, 3H), 3.38 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.33, 170.18, 170.15, 170.13, 162.57, 150.64, 137.77, 137.44, 128.32 (2C), 127.71 (2C), 102.65, 96.73, 89.00, 80.73, 80.69, 75.21, 72.29, 72.26, 70.16, 68.81, 68.76, 65.49, 61.03, 58.46, 57.73, 20.80, 20.74, 20.67; HRMS (ESI+) *m*/*z* calcd for $C_{32}H_{42}N_3O_{16}$ [M + H] 724.2565, found 724.2599.



S16 27

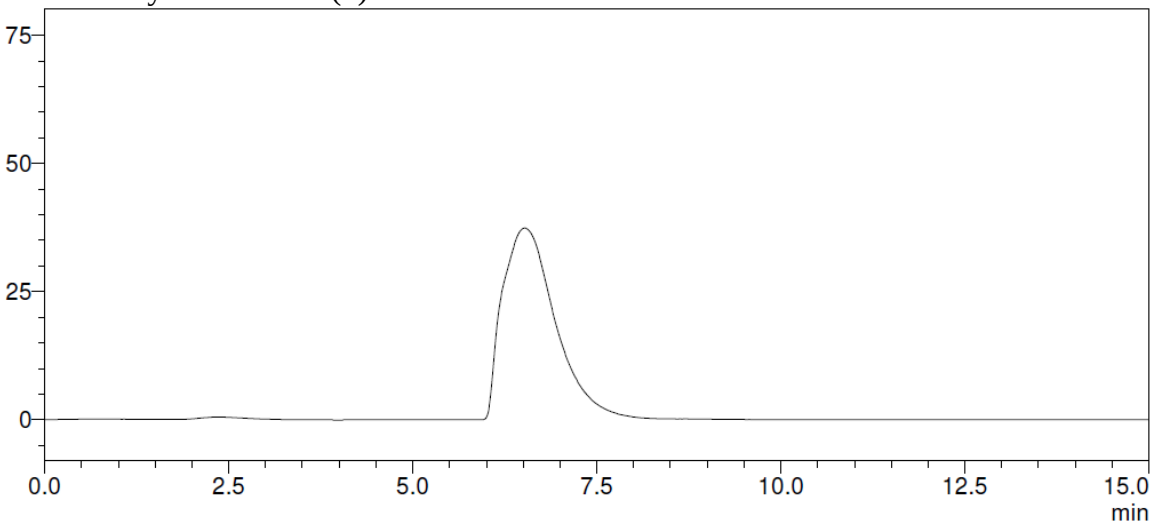
(2*R*,3*S*,4*S*,5*R*,6*R*)-2-((1*R*)-2-Amino-1-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethoxytetrahydrofuran-2-yl)-2-oxoethoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (27). To a stirred solution of **S16** (0.21 g, 0.29 mmol) and AcOH (0.04 mL) in a 1:1 mixture of THF and ^{i}PrOH (2.0 mL) was added Pd/C (0.16 g). H_2 gas was introduced and the reaction mixture was stirred under H_2 atmosphere. After being stirred for 4h at r.t., the solution was filtered through Celite and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 97/3 - 92/8) to afford **27** (0.14 g, 82%): ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.56 (brs, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.16 (brs, 1H), 6.05 (brs, 1H), 5.94 (s, 1H), 5.91 (d, *J* = 8.1 Hz, 1H), 5.59 – 5.56 (m, 1H), 5.03 (dd, *J* = 9.6, 3.3 Hz, 1H), 4.98 (s, 1H), 4.53 (dd, *J* = 12.1, 4.8 Hz, 1H), 4.49 – 4.44 (m, 2H), 4.34 (d, *J* = 12.0 Hz, 1H), 4.00 (dd, *J* = 8.6, 4.8 Hz, 1H), 3.90 (d, *J* = 5.5 Hz, 1H), 3.87 (d, *J* = 10.0 Hz, 1H), 3.83 – 3.77 (m, 1H), 3.59 (s, 3H), 3.43 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.66, 171.02, 170.90, 170.52, 163.75, 149.93, 138.98, 103.07, 96.65, 87.79, 81.38, 80.68, 76.11, 73.90, 72.11, 71.18, 68.76, 64.67, 62.78, 58.35, 57.46, 20.81 (2C), 20.76; HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{34}N_3O_{15}$ [M + H] 604.1990, found 604.2014.



27 33

(2*S*,3*S*,4*S*)-3,4-Diacetoxy-2-((1*R*)-2-amino-1-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethoxytetrahydrofuran-2-yl)-2-oxoethoxy)-3,4-dihydro-2*H*-pyran-6-carboxylic acid (33). To a stirred solution of **27** (0.14 g, 0.23 mmol) and DMSO (0.17 mL, 2.34 mmol) in a 5:1 mixture of CH_2Cl_2 and Et_3N (1.2 mL) was added SO_3·pyridine (0.39 g, 2.34 mmol). After being stirred for 3h at r.t., the reaction mixture was added H_2O (0.23 mL) and passed through a silica gel pad ($CHCl_3$/MeOH = 92/8). To a stirred solution of the crude mixture in ^{t}BuOH (1.0 mL) and 2-methyl-2-butene (0.5 mL) was added a solution of $NaClO_2$ (0.11 g, 1.17 mmol) and NaH_2PO_4·$2H_2O$ (0.18 g, 1.17 mmol) in H_2O (1.0 mL). After being stirred for 5h at r.t., the reaction extracted with $CHCl_3$/MeOH (9/1). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) to afford **33** (0.11 g, 85%): ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.78 (d, *J* = 8.2 Hz, 1H), 5.94 (s, 1H), 5.92 (d, *J* = 3.2 Hz, 1H), 5.82 (t, *J* = 2.1 Hz, 1H), 5.66 (dd, *J* = 4.6, 2.5 Hz, 1H), 5.57 (ddd, *J* = 4.7, 3.2, 1.6 Hz, 1H), 5.31 (d, *J* = 3.2 Hz, 1H), 4.78 (d, *J* = 1.9 Hz, 1H), 4.51 (dd, *J* = 4.4, 1.9 Hz, 1H), 3.87 (t, *J* = 4.7 Hz, 1H), 3.82 (t, *J* = 5.0 Hz, 1H), 3.45 (s, 3H), 3.44 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (101 MHz, MeOD) δ 173.20, 172.12, 171.62, 168.06, 166.08, 152.12, 148.47, 141.11, 104.32, 103.83, 98.17, 87.88, 83.90, 83.29, 78.97, 77.77, 65.15, 65.06, 58.88, 58.65, 20.76, 20.58; HRMS (ESI+) *m/z* calcd for $C_{22}H_{28}N_3O_{14}$ [M + H] 558.1571, found 558.1595.

HPLC analysis of I-CAP (**3**)

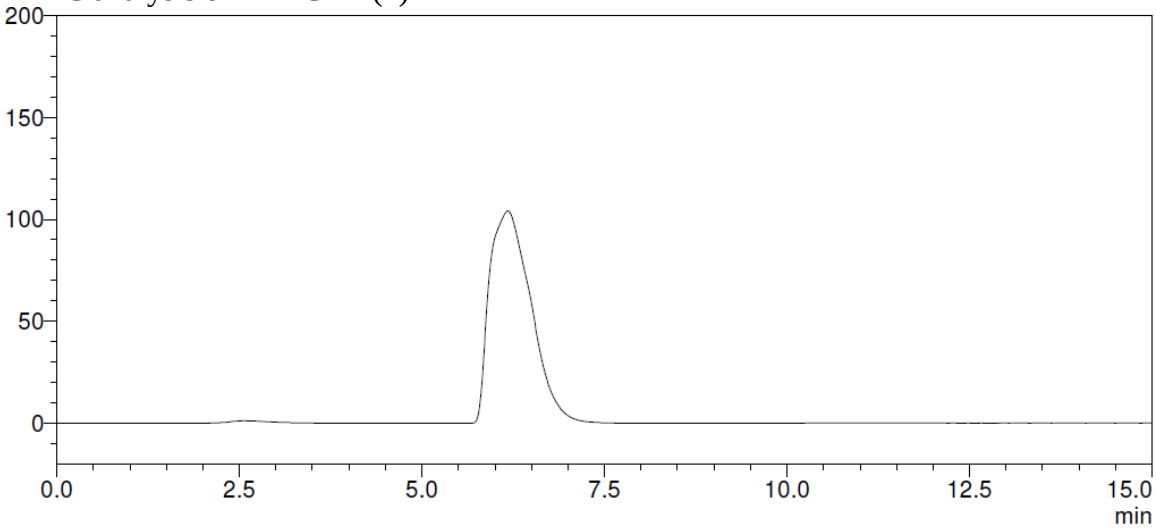


Area % purity: 97.7%

Conditions:

column: Kinetex® (C18, 5 μm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of DM-CAP (**4**)

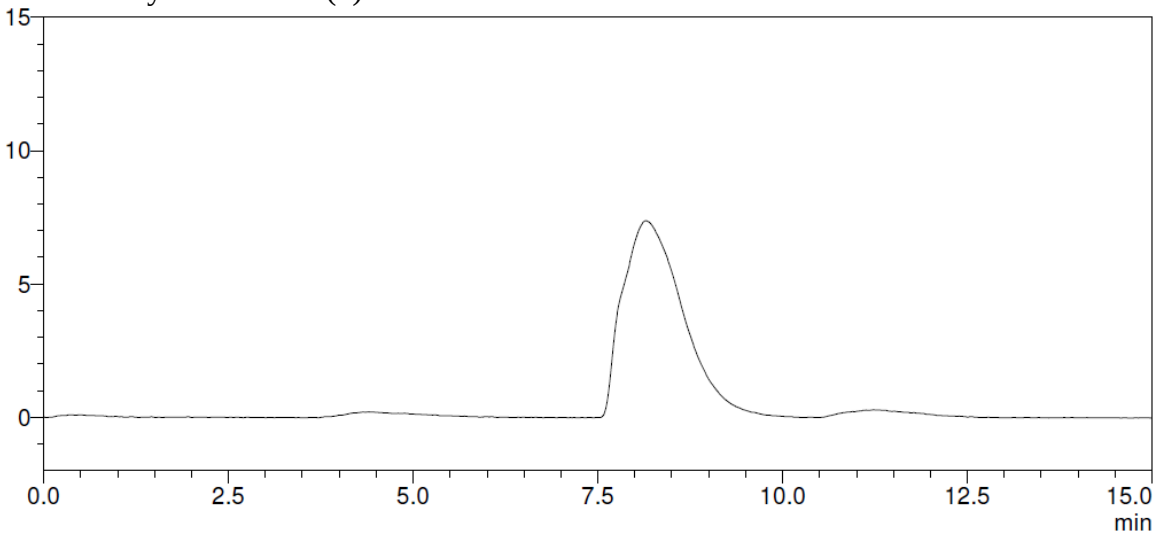


Area % purity: 97.8%

Conditions:

column: Kinetex® (C18, 5 µm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of CPPB (**5**)

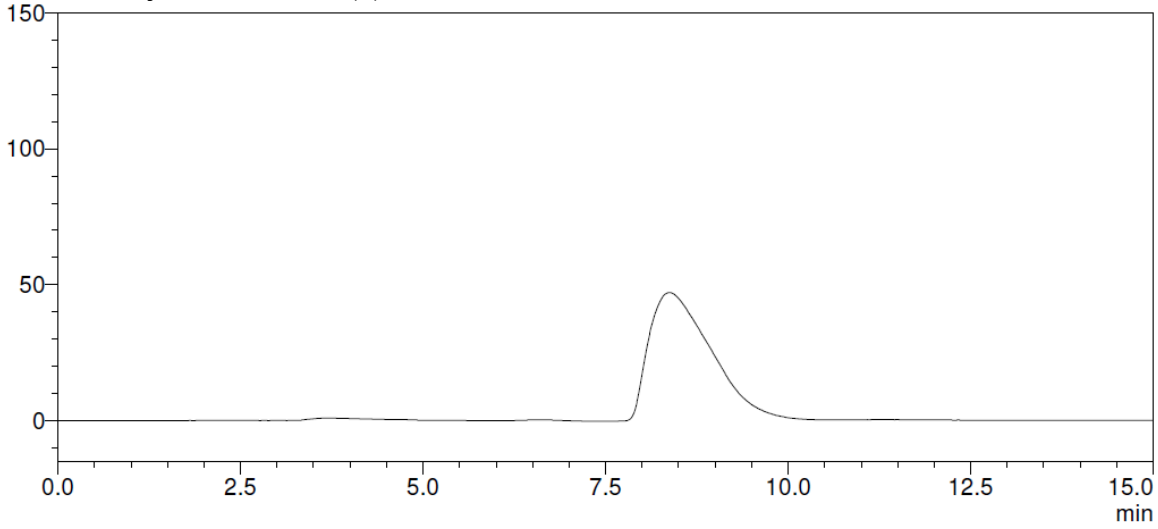


Area % purity: 95.2%

Conditions:

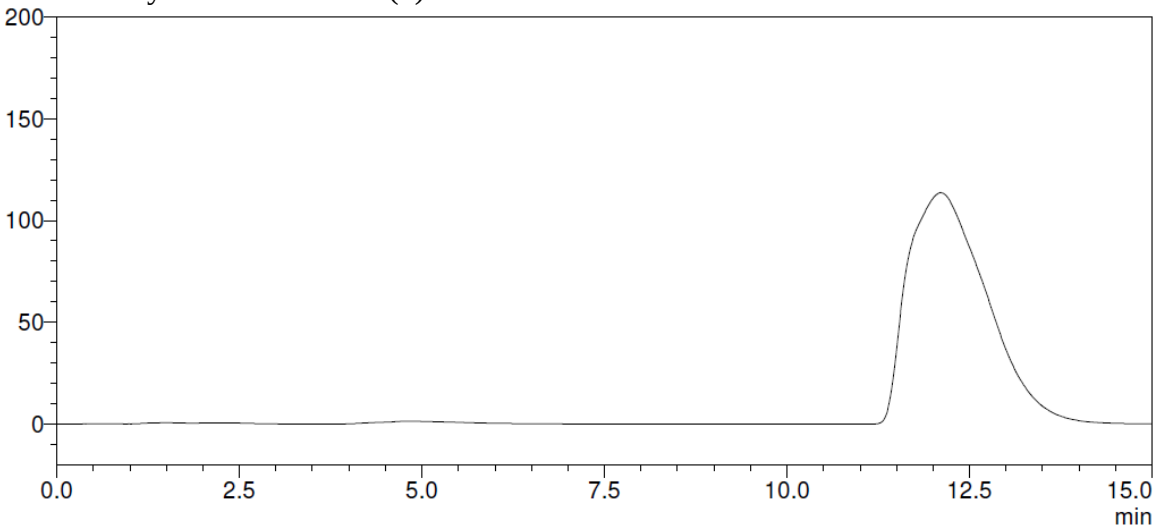
column: Kinetex® (C18, 5 µm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of I-CPPB (**6**)



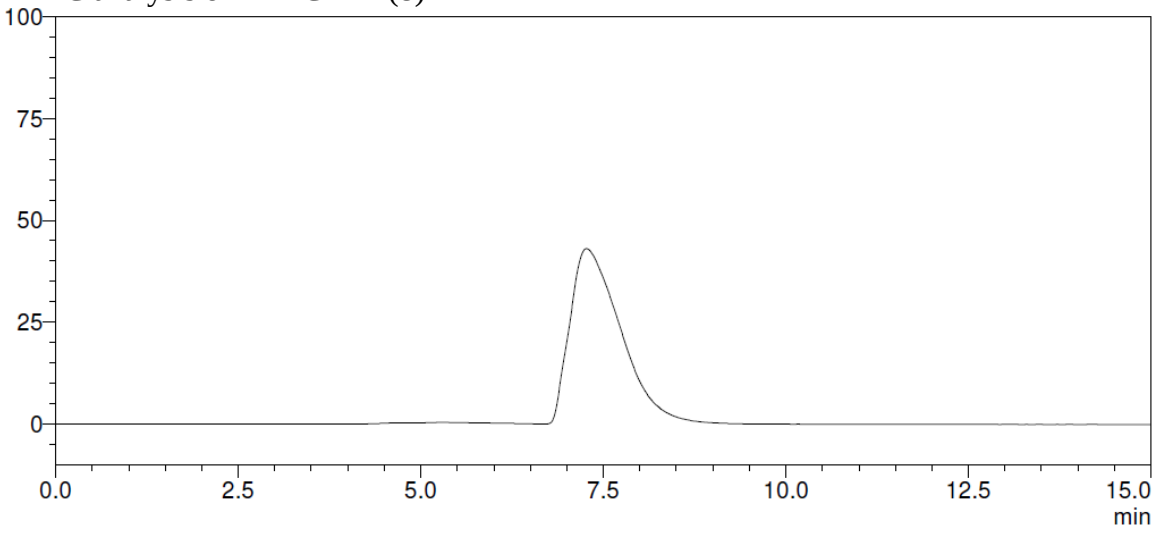
Area % purity: 98.9%
Conditions:
column: Kinetex® (C18, 5 μm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of OM-CPPB (**7**)



Area % purity: 97.1%
Conditions:
column: Kinetex® (C18, 5 μm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of DM-CPPB (**8**)

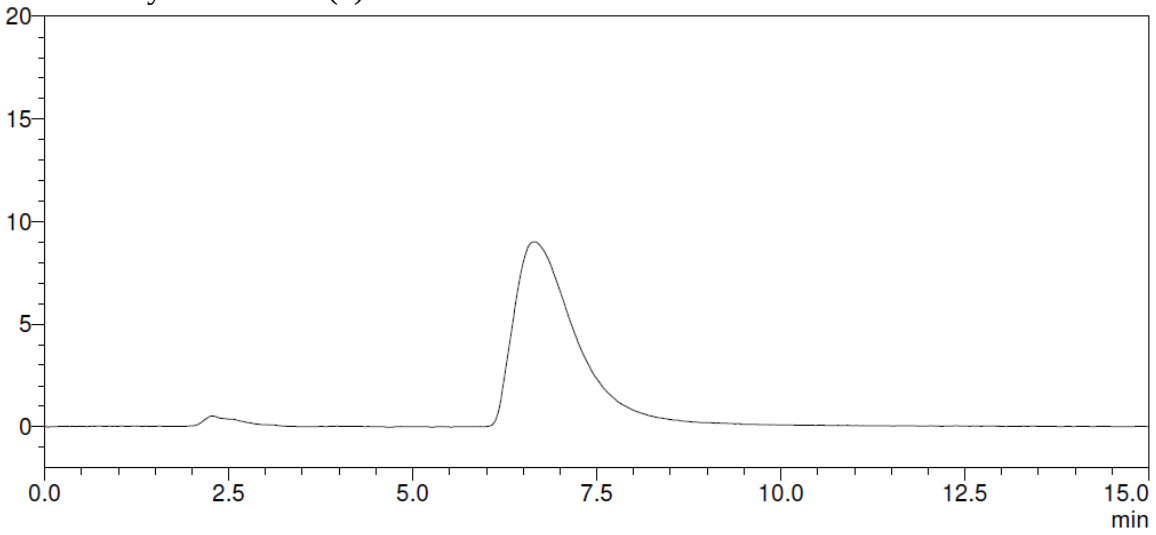


Area % purity: 97.6%

Conditions:

column: Kinetex® (C18, 5 μm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of CPPA (**9**)

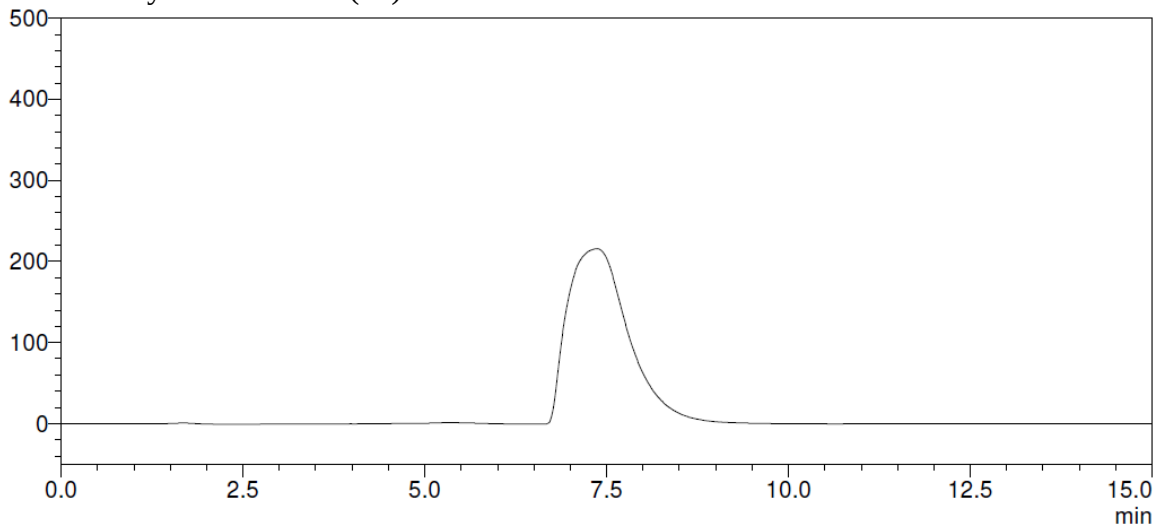


Area % purity: 96.0%

Conditions:

column: Kinetex® (C18, 5 μm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of I-CPPA (**10**)

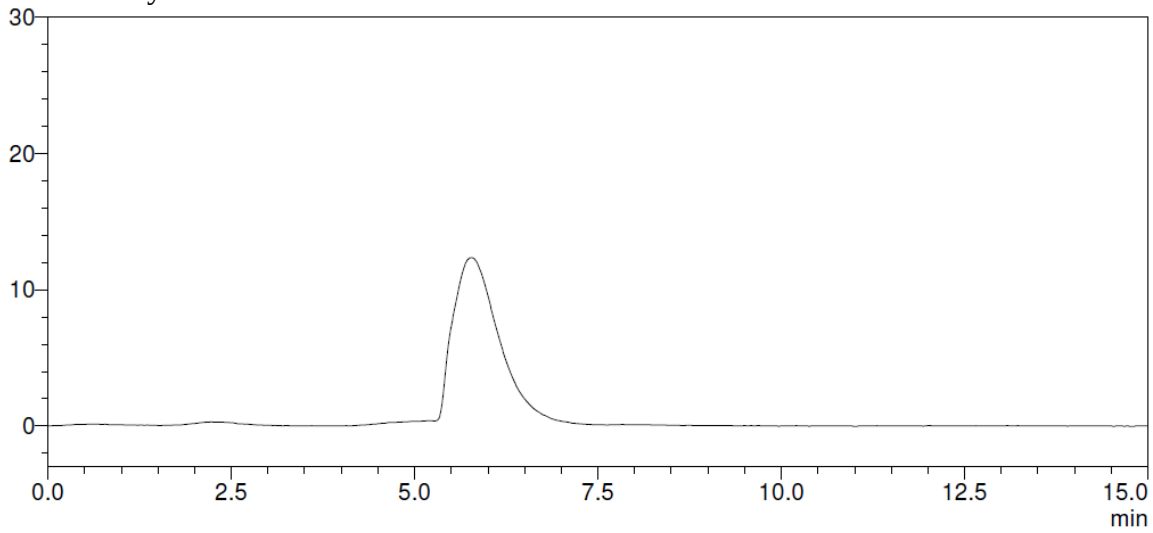


Area % purity: 97.9%

Conditions:

column: Kinetex® (C18, 5 µm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

HPLC analysis of **A500359F**



Area % purity: 95.5%

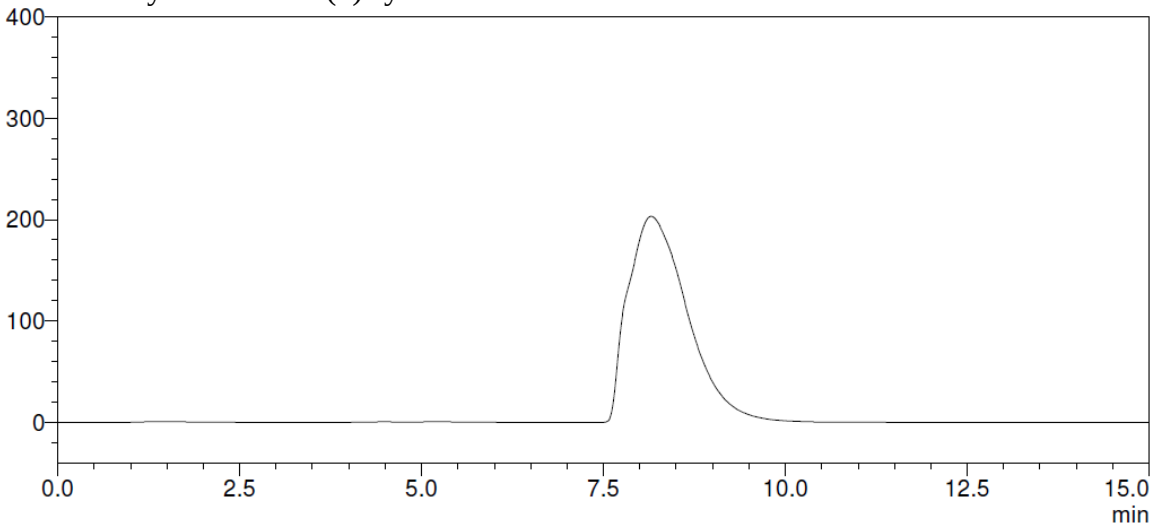
Conditions:

column: Kinetex® (C18, 5 µm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

Synthesis of CPPB (5) from A500359F

To a stirred solution of **A-500359F** (11 mg, 0.024 mmol), **34** (26 mg, 0.072 mmol), Glyceroacetonide-Oxyma (16 mg, 0.072 mmol) and NMM (26 mL, 0.24 mmol) in DMF (0.49 mL) was added EDCI (23 mg, 0.12 mmol). After being stirred for 5h at r.t., the reaction mixture was filtered, and diluted with water. The product was extracted with $CHCl_3$/MeOH (9/1). The combined extracts were derived over Na_2SO_4, and concentrated *in vacuo*. The crude mixture was purified by DOWEX 50Wx4 (MeOH : NH_4OH = 4 : 1) followed by reverse-phase HPLC [column: Luna (C_{18}, 10 mm, 100 Å, 250 x 10 mm), solvents: 65:35 MeOH : H_2O, flow rate: 3.0 mL/min, UV: 254 nm, retention time: 18 min] to afford **5** (17 mg, 92%): ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.86 (d, *J* = 8.1 Hz, 1H), 7.20 (dd, *J* = 10.9, 8.6 Hz, 4H), 7.02 (d, *J* = 9.1 Hz, 2H), 6.97 (d, *J* = 8.7 Hz, 2H), 5.98 (dd, *J* = 3.3, 1.0 Hz, 1H), 5.80 (d, *J* = 4.4 Hz, 1H), 5.76 (d, *J* = 8.2 Hz, 1H), 5.21 (d, *J* = 4.6 Hz, 1H), 4.68 (d, *J* = 2.0 Hz, 1H), 4.54 (dp, *J* = 7.3, 3.6 Hz, 1H), 4.48 (dd, *J* = 5.2, 2.0 Hz, 1H), 4.44 (d, *J* = 14.5 Hz, 1H), 4.39 (t, *J* = 3.9 Hz, 1H), 4.33 (d, *J* = 14.6 Hz, 1H), 4.20 (t, *J* = 4.7 Hz, 1H), 4.06 – 4.03 (m, 1H), 3.66 (t, *J* = 5.1 Hz, 1H), 3.51 – 3.45 (m, 3H), 3.19 (s, 3H), 3.08 (ddd, *J* = 12.3, 8.6, 3.4 Hz, 3H), 2.14 – 2.08 (m, 2H), 1.86 (dtd, *J* = 12.2, 8.3, 3.5 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ 173.68, 166.16, 163.31, 157.62, 152.18, 152.08, 144.15, 141.83, 130.91, 129.76 (2C), 123.58 (2C), 118.03 (4C), 109.58, 102.74, 100.64, 90.83, 83.32, 80.35, 77.51, 74.27, 74.01, 67.61, 63.48, 58.61, 48.20 (2C), 43.51, 31.47 (2C); HRMS (ESI+) *m/z* calcd for $C_{36}H_{41}F_3N_5O_{13}$ [M + H] 808.2653, found 808.2674.

HPLC analysis of CPPB (**5**) synthesized from **A500359F**



Area % purity: 98.3%

Conditions:

column: Kinetex® (C18, 5 μm, 100 Å, 250 x 4.60 mm), solvents: 30:70 MeOH:H_2O, flow rate: 1.0 mL/min, UV: 254 nm

Bacterial strains and growth of bacteria

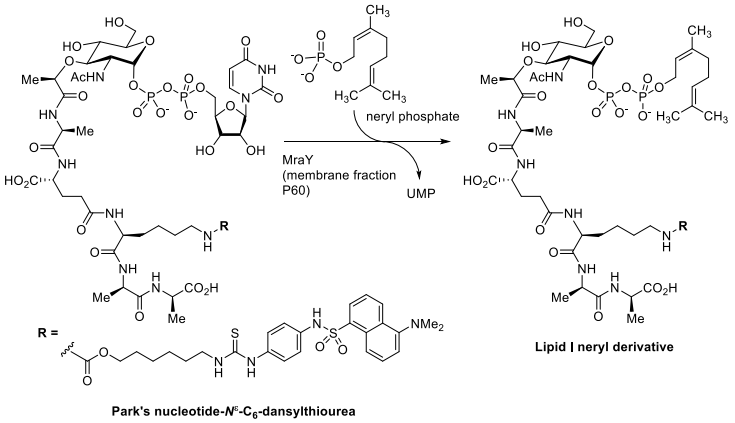
Mycobacterium smegmatis (ATCC 607) and *E. coli* (ATCC 10798) were obtained from American Type Culture Collection (ATCC). *Mycobacterium avium* 2285, *Myobacterium kansasii* 824, *Mycobacterium abscessus* DJO-44273, and *Mycobacterium tuberculosis* H37Rv were acquired from EBI. A single colony of *Mycobacterium spp.* was obtained on Difco Middlebrook 7H10 nutrient agar enriched with albumin, dextrose, and catalase (ADC). The others were obtained on the recommended agar media. A single colony of *E. coli* were grown on tryptic soy agar for 24h at 37ºC in a static incubator and cultured in tryptic soy broth until log phase to be an optical density (OD) of 0.2-0.5. The OD was monitored at 600 and 570 nm using a 96-well microplate reader.

MIC assays

Minimum inhibitory concentrations were determined by broth dilution microplate alamar blue assay or by OD measurement. All compounds were stored in DMSO or water (1 mg/100 μL concentration). This concentration was used as the stock solution for all MIC studies. Each compound from stock solution was placed in the first well of a sterile 96 well plate and a serial dilution was conducted with the culturing broth (total volume of 10 μL). The bacterial suspension at log phase (190 μL) was added to each well (total volume of 200 μL), and was incubated for 24h at 37 ˚C. 20 μL of resazurin (0.02%) was added to each well and incubated for 4h for *Mycobacterium spp.* (National Committee for Clinical Laboratory Standards (NCCLS) method (pink = growth, blue = no visible growth)). The OD measurements were performed for all experiments prior to colorimetric. The MIC values were determined according to the colorimetric assays using resazurin. The absorbance of each well was also measured at 570 and 600 nm via UV-Vis.

MraY assays

MraY assay substrates, Park's nucleotide-N^{ε}-C_6-dansylthiourea and neryl phosphate, were chemically synthesized according to the reported procedures.[3]

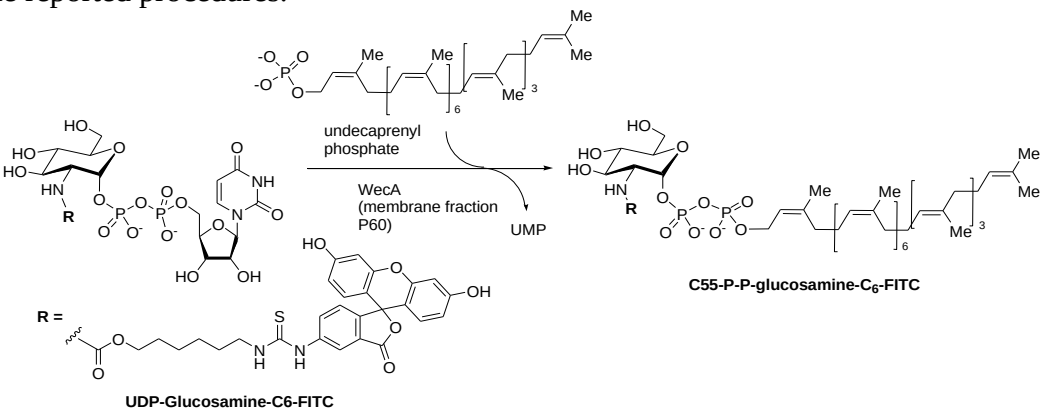


Park's nucleotide-N^{ε}-C_6-dansylthiourea (2 mM stock solution, 1.88 μL), $MgCl_2$ (0.5 M, 5 μL), KCl (2 M, 5 μL), Triton X100 (0.5%, 5.63 μL), Tris-HCl buffer (pH 8.0, 50 mM), neryl phosphate (0.1 M, 2.25 μL), and inhibitor molecule (0 - 50 μg/mL in Tris-HCl buffer)

were placed in a 1.5 mL Eppendorf tube. To a stirred reaction mixture, P-60 (10μL) was added (total volume of reaction mixture: 50 μL adjust with Tris-HCl buffer). The reaction mixture was incubated for 2h at room temperature (26 ºC) and quenched with $CHCl_3$ (100μL). Two phases were mixed via vortex and centrifuged at 25,000 xg for 10min. The upper aqueous phase was assayed via reverse-phase HPLC. The water phase (10 μL) was injected into HPLC (solvent: CH_3CN/0.05 M aq. NH_4HCO_3 = 25:75; UV: 350 nm; flow rate: 0.5 mL/min; column: Kinetex 5μm C8, 100 A, 150 x 4.60 mm), and the area of the peak for lipid I-neryl derivative was quantified to obtain the IC_{50} value. The IC_{50} values were calculated from plots of the percentage product inhibition versus the inhibitor.

WecA assays

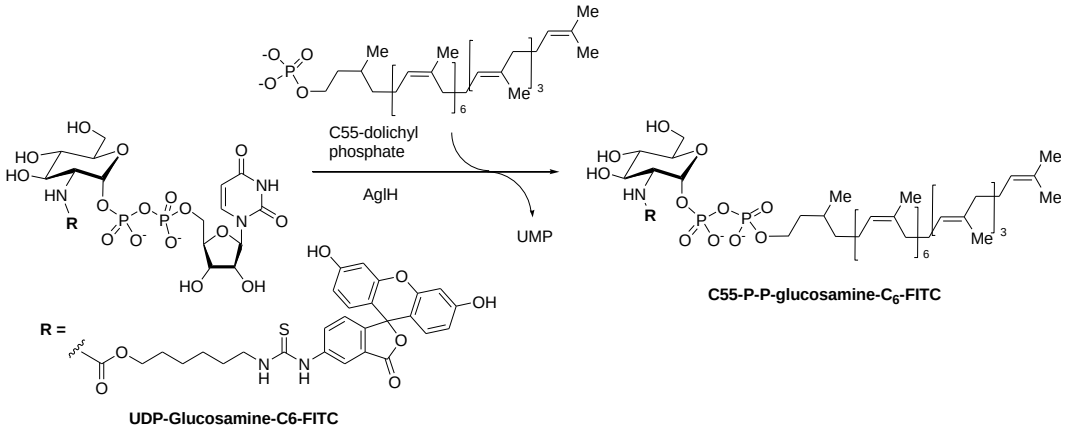
WecA assay substrate, UDP-Glucosamine-C6-FITC was chemically synthesized according to the reported procedures.[4]



UDP-Glucosamine-C6-FITC (2 mM stock solution, 0.56 μL), $MgCl_2$ (0.5 M, 4 μL), β-mercaptoethanol (50 mM, 5 μL), CHAPS (5%, 11.25 μL), Tris-HCl buffer (pH 8.0, 50 mM), undecaprenyl phosphate (4 mM, 1.4 μL), and inhibitor molecule (0 - 50 μg/mL in Tris-HCl buffer) were place in a 1.5 μL Eppendorf tube. To a stirred reaction mixture, P-60 (10 μL) was added (total volume of reaction mixture: 50 μL adjust with Tris-HCl buffer). The reaction mixture was incubated for 2h at 37 ºC and quenched with n-butanol (150 μL). Two phases were mixed via vortex and centrifuged at 10,000 xg for 3min. The upper organic phase was assayed via reverse-phase HPLC. The organic phase (30 μL) was injected into HPLC (solvent: gradient elution of 85:15 to 95:5 MeOH/0.05 M aq. NH_4HCO_3 over 20min; UV: 485 nm; flow rate: 0.5 ml/ min; column: Kinetex 5 μm C8, 100 Å, 150 x 4.60 mm), and the area of the peak for C55-P-P-glucosamine-C_6-FITC was quantified to obtain the IC_{50} value. The IC_{50} values were calculated from plots of the percentage product inhibition versus the inhibitor concentration.

AglH assays

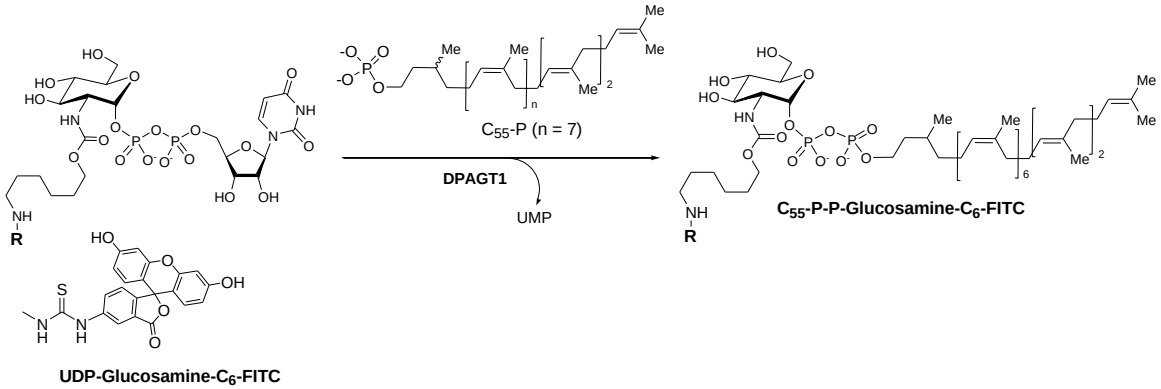
AglH assays were performed as the procedure described for WecA assays, but used *Mj*AglH and α-dihydroundecaprenyl phosphate (C_{55}-dolichyl phosphate) instead of WecA and undecaprenyl phosphate.[5]



UDP-Glucosamine-C6-FITC (2 mM stock solution, 0.56 μL), $MgCl_2$ (0.5 M, 4 μL), β-mercaptoethanol (50 mM, 5 μL), CHAPS (5%, 11.25 μL), Tris-HCl buffer (pH 8.0, 50 mM), C55-dolichyl phosphate (4 mM, 1.4 μL), and inhibitor molecule (0 - 50 μg/mL in Tris-HCl buffer) were place in a 1.5 mL Eppendorf tube. To a stirred reaction mixture, AglH solution (10 μL) was added (total volume of reaction mixture: 50 μL adjust with Tris-HCl buffer). The reaction mixture was incubated for 2h at 37 ºC and quenched with n-butanol (150 μL). Two phases were mixed via vortex and centrifuged at 10,000 xg for 3min. The upper organic phase was assayed via reverse-phase HPLC. The organic phase (30 μL) was injected into HPLC (solvent: gradient elution of 85:15 to 95:5 MeOH/0.05 M aq. NH_4HCO_3 over 20min; UV: 485 nm; flow rate: 0.5 ml/ min; column: Kinetex 5 μm C8, 100 Å, 150 x 4.60 mm), and the area of the peak for C55-P-P-glucosamine-C_6-FITC was quantified to obtain the IC_{50} value. The IC_{50} values were calculated from plots of the percentage product inhibition versus the inhibitor concentration.

DPAGT1 assays

DPAGT1 assays were performed as the procedure described for AglH assays, but used DPAGT1.[6]



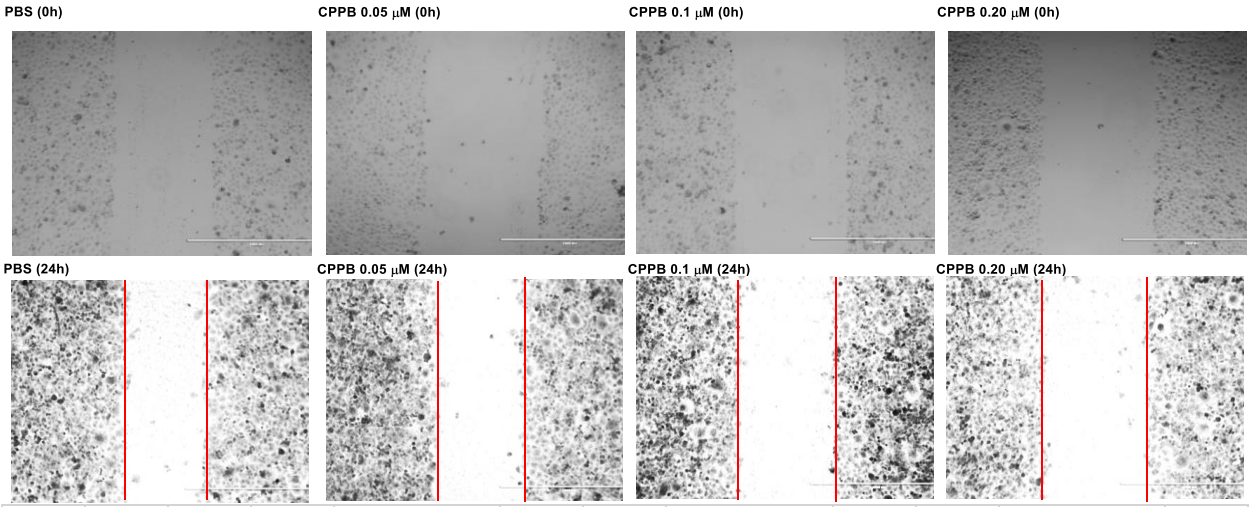
UDP-Glucosamine-C_6-FITC (2 mM stock solution, 0.56 μL), $MgCl_2$ (0.5 M, 4 μL), β-mercaptoethanol (50 mM, 5 μL), CHAPS (20%, 2.5 μL), Tris-HCl buffer (pH 8.0, 50 mM), C_{55}-dolichyl phosphate (4 mM, 1.68 μL), and inhibitor molecule (0 - 50 μg/mL in Tris-HCl buffer) were place in a 1.5 mL Eppendorf tube. To a stirred reaction mixture, DPAGT1 solution (10 μL) was added (total volume of reaction mixture: 50 μL adjust with Tris-HCl

buffer). The reaction mixture was incubated for 2h at 37 ^{o}C and quenched with n-butanol (150 μL). Two phases were mixed via vortex and centrifuged at 10,000 xg for 3min. The upper organic phase was assayed via reverse-phase HPLC. The organic phase (30 μL) was injected into HPLC (solvent: gradient elution of 85:15 to 95:5 MeOH/0.05 M aq. NH_4HCO_3 over 20min.; UV: 485 nm; flow rate: 0.5 mL/ min; column: Kinetex 5 μm C8, 100 Å, 150 x 4.60 mm), and the area of the peak for C_{55}-P-P-glucosamine-C_6-FITC was quantified to obtain the IC_{50} value. The IC_{50} values were calculated from plots of the percentage product inhibition versus the inhibitor concentration.

Scratch assay

A confluent monolayer was formed in 24-well plates. The monolayer was scratched by a sterile 200 μL pipette tip and washed with PBS to remove cell debris. Complete medium with CPPB (**5**) (0, 0.05, 0.1, 0.2 μM) were added and scratched areas were photographed with microscope. The scratched cells were incubated at 37 °C, 5% CO_2. After 24h, medium was removed and cells were stained with a 1:1 mixture of crystal violet and PBS for 5min, washed with PBS twice, and photographed with microscope. Wound areas were measured and recovered areas were calculated.

Figure S1A. Analysis of migration inhibition of PANC-1.



PBS				CPPB 0.05μM				CPPB 0.1μM				CPPB 0.2μM		
0h	24h	%closure		0h	24h	%closure		0h	24h	%closure		0h	24h	%closure
0.9224	0.7694	16.58716		1.1366	0.781	31.28629		0.9563	0.7837	18.04873		1.2142	0.9959	17.97892
0.9584	0.5755	39.952		1.1389	0.7427	34.78795		0.9745	0.8299	14.83838		1.2191	0.9451	22.4756
0.9153	0.5508	39.82301		1.1366	0.8392	26.16576		0.954	0.6747	29.27673		1.2142	0.843	30.57157
0.9638	0.526	45.42436		1.1707	0.8065	31.10959		0.9922	0.757	23.7049		1.2349	0.9349	24.29347
1.0177	0.5633	44.6497		1.1941	0.7025	41.16908		1.0457	0.7419	29.05231		1.2966	1.1444	11.73839

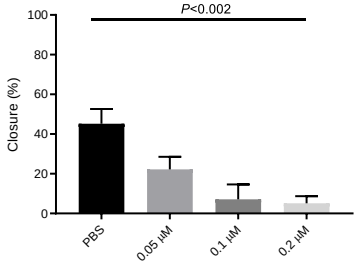
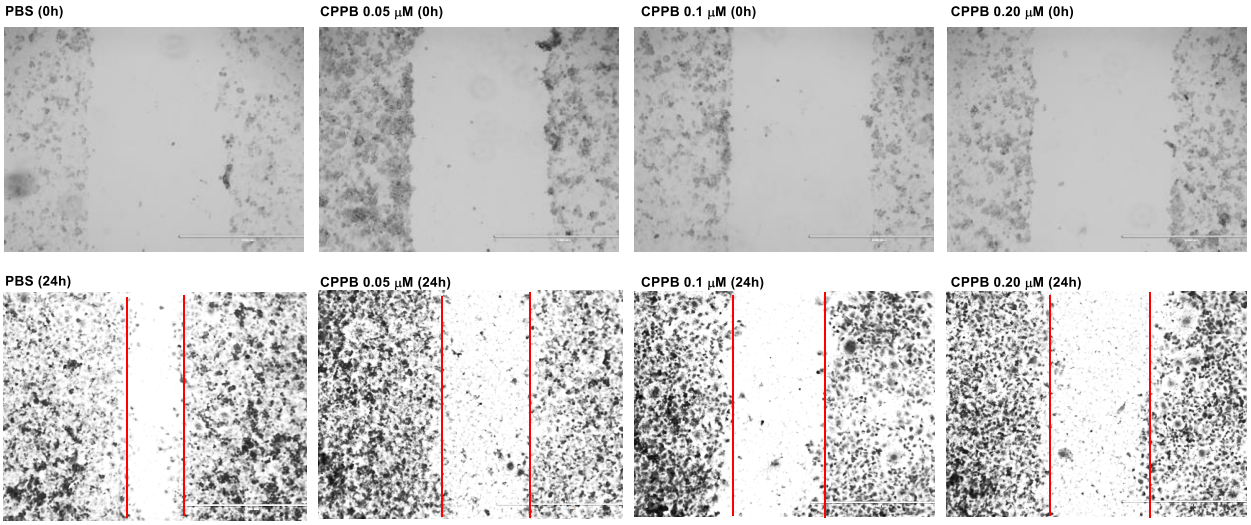


Figure S1B. Analysis of migration inhibition of PD002.



PBS				CPPB 0.05μM				CPPB 0.1μM				CPPB 0.2μM		
0h	24h	%closure		0h	24h	%closure		0h	24h	%closure		0h	24h	%closure
1.2905	0.5376	58.34173		1.1679	0.5325	54.40534		1.1459	0.8198	28.45798		0.9814	0.7852	19.99185
1.3473	0.4855	63.96497		1.1119	0.6251	43.78092		1.1814	0.701	40.66362		1.0246	0.7978	22.13547
1.3473	0.518	61.55274		1.2686	0.732	42.2986		1.2192	0.7472	38.71391		1.0021	0.8409	16.08622
1.3419	0.3756	72.00984		1.2839	0.6888	46.35096		1.2497	0.5462	56.29351		1.1403	0.925	18.881
1.3137	0.4698	64.23841		1.2864	0.7164	44.3097		1.2209	0.6014	50.74126		1.0993	0.9277	15.60993

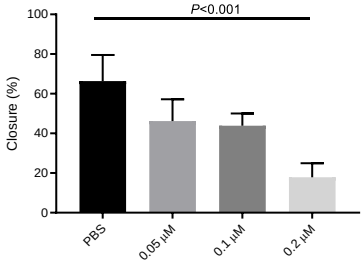
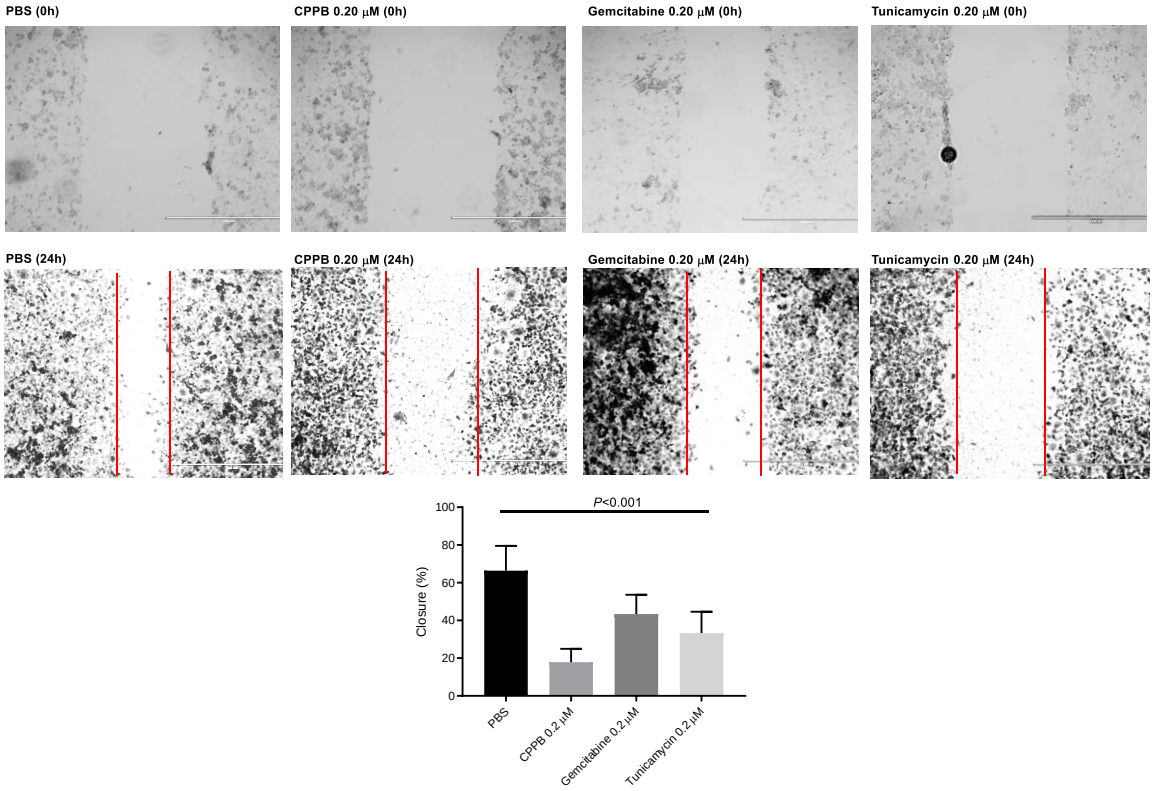
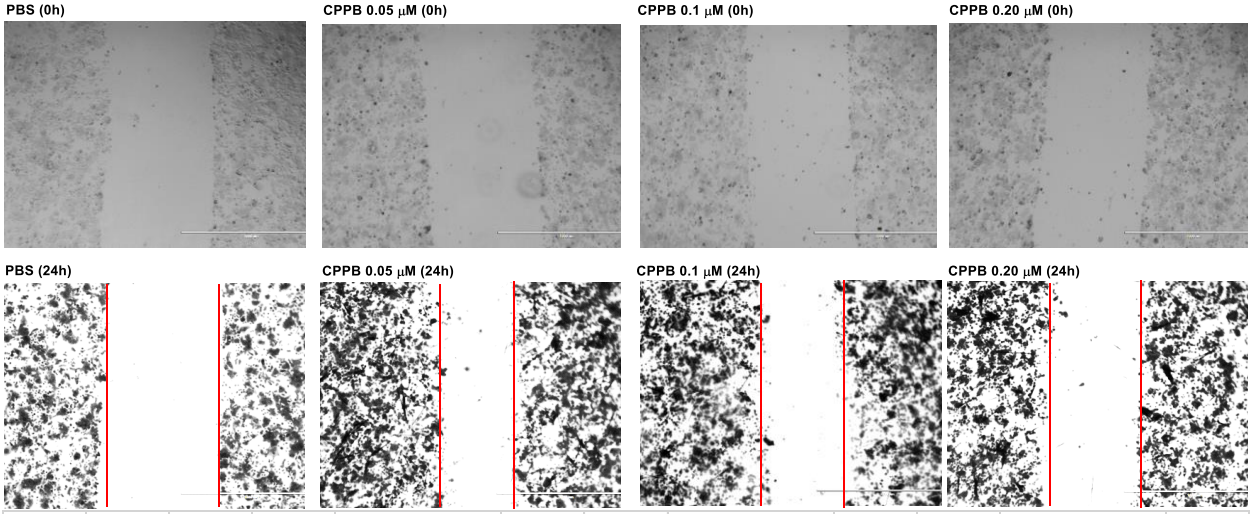


Figure S1C. Comparisons of migration inhibition of PD002 by treatment with CPPB, gemcitabine, and tunicamycin in wound healing (scratch) assays.[a]



[a]All images were acquired at 24h.

Figure S1D. Analysis of migration inhibition of AsPC-1.



PBS				CPPB 0.05μM				CPPB 0.1μM				CPPB 0.2μM		
0h	24h	%closure		0h	24h	%closure		0h	24h	%closure		0h	24h	%closure
1.1202	0.6497	42.00143		1.2056	0.9621	20.19741		1	0.829	17.1		0.888	0.8117	8.592342
1.1896	0.7482	37.10491		1.3113	1.0346	21.1012		1.0014	0.7941	20.70102		0.9326	0.8244	11.60197
1.0831	0.6704	38.10359		1.2664	0.9878	21.99937		0.9258	0.7222	21.99179		0.9316	0.862	7.471018
1.0242	0.6073	40.70494		1.2446	1.0044	19.29937		0.915	0.7192	21.39891		0.9041	0.7884	12.79726
1.0173	0.6653	34.6014		1.2625	1.0125	19.80198		0.927	0.7472	19.3959		0.9453	0.8555	9.49963
1.0616	0.5881	44.60249		1.3183	1.0091	23.45445		0.934	0.7341	21.40257		0.9924	0.8733	12.00121
1.0263	0.6209	39.50112		1.3182	1.069	18.90457		0.9441	0.8015	15.10433		1.0165	0.8904	12.40531
1.0524	0.5757	45.29647		1.3117	1.01	23.00069		1.0182	0.8634	15.2033		1.1768	1.0898	7.39293
1.0346	0.5628	45.60217		1.3507	1.0049	25.60154		1.0171	0.8615	15.2984		1.2718	1.1357	10.70137
1.0952	0.5958	45.59898		1.3037	1.0104	22.49751		1.0114	0.8708	13.90152		1.2231	1.1201	8.421225
1.124	0.607	45.99644		1.3529	1.0188	24.6951		1.1283	0.9263	17.90304		1.2291	1.0878	11.49622
1.101	0.643	41.59855		1.3416	1.0961	18.29905		0.8307	0.6837	17.69592		1.3053	1.1644	10.79445
1.1259	0.6463	42.59703		1.116	0.7946	28.79928		1.0105	0.8114	19.70312		1.3029	1.1719	10.05449
1.1103	0.6506	41.40322		1.0833	0.7789	28.09933		1.1237	0.9513	15.34217		1.3615	1.2159	10.69409
1.1471	0.6814	40.59803		1.0811	0.7794	27.90676		1.1739	1.0095	14.0046		1.3911	1.2812	7.900223
1.1523	0.6211	46.09911		1.071	0.8214	23.30532		1.1236	0.945	15.89534		1.4017	1.2909	7.904687
1.1546	0.6374	44.79473		1.1878	0.8754	26.30072		1.2464	1.0258	17.69897				
1.0963	0.6063	44.69579		1.1048	0.9291	15.90333		1.214	1.0222	15.79901				
1.2443	0.698	43.9042		1.2355	1.0057	18.59976		1.2645	1.0267	18.80585				
1.2544	0.7476	40.40179		1.229	0.9623	21.70057		1.2654	1.0693	15.49708				
1.2672	0.74	41.60354		1.2291	1.0091	17.89928		1.3561	1.1255	17.00465				
1.2899	0.8023	37.80138						1.3803	1.1332	17.90191				
1.3807	0.8008	42.00043						1.4222	1.1491	19.20264				
1.39	0.8762	36.96403												
1.1324	0.6896	39.10279												
1.0863	0.6083	44.00258												
1.0895	0.6886	36.7967												
1.1169	0.7271	34.90017												
1.1561	0.6694	42.09843												
1.1433	0.7317	36.00105												
1.1657	0.6609	43.30445												
1.1609	0.6594	43.19924												
1.2023	0.6865	42.90111												
1.1897	0.671	43.59923												
1.2856	0.72	43.99502												
1.203	0.7014	41.69576												
1.2197	0.744	39.00139												
1.1852	0.6756	42.99696												
1.2289	0.7623	37.96892												
1.4237	0.8699	38.89864												

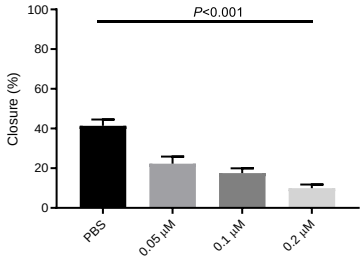
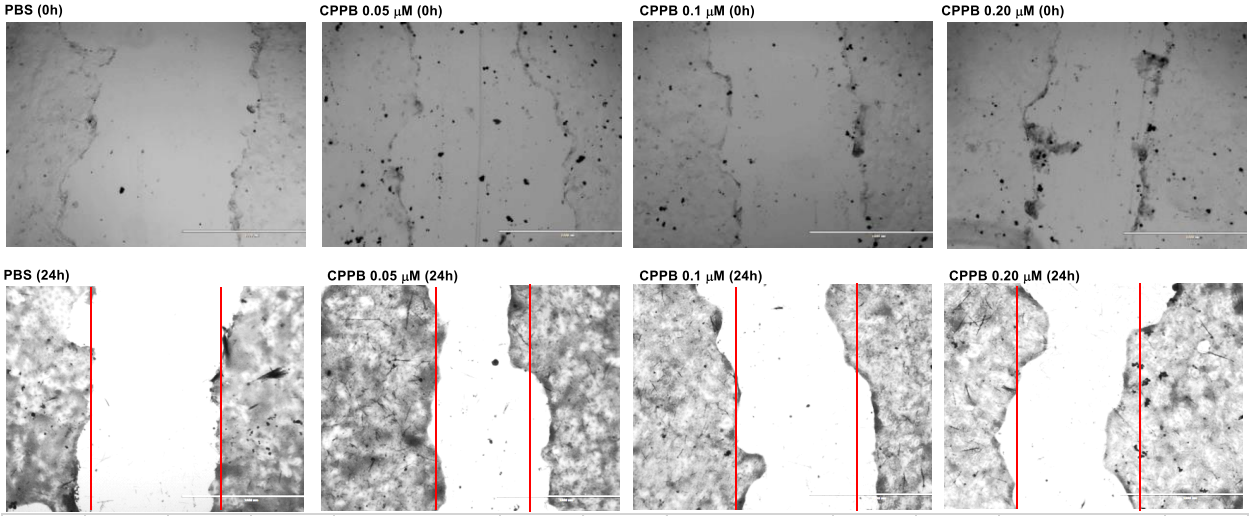


Figure S1E. Analysis of migration inhibition of Capan-1.



PBS				CPPB 0.05μM				CPPB 0.1μM				CPPB 0.2μM		
0h	24h	%closure		0h	24h	%closure		0h	24h	%closure		0h	24h	%closure
1.4625	1.1071	24.30085		1.5753	1.2823	18.59963		1.9965	1.5094	24.3977		1.3607	1.1076	18.60072
1.1401	0.6954	39.00535		1.1492	0.9745	15.20188		1.4587	1.1611	20.40173		1.1822	1.1231	4.999154
1.2529	0.9008	28.1028		1.377	1.1498	16.49964		1.1065	0.8974	18.89742		1.0793	0.9595	11.09979
1.1594	0.6922	40.29671		1.2096	0.9459	21.8006		1.3102	1.0521	19.69928		1.2914	1.121	13.19498
1.236	0.8801	28.7945		1.4973	1.2308	17.7987		1.3016	0.9788	24.80025		1.1568	1.0829	6.388313
1.2417	0.8431	32.10115		1.397	1.1749	15.89835		1.4161	1.0706	24.39799		1.2403	1.1981	3.402403
1.6172	1.1191	30.80015		1.0924	0.8302	24.0022		1.4164	1.2053	14.90398		1.3245	1.261	4.794262
1.2064	0.8891	26.30139		1.7816	1.4556	18.29816		1.3186	1.0945	16.9953		1.1854	1.1101	6.352286
1.1487	0.8179	28.79777		1.6739	1.2755	23.8007		1.2036	0.9665	19.69924		1.208	1.1611	3.88245
1.1336	0.7402	34.7036		1.3164	1.0518	20.10027		1.3646	1.0426	23.59666		1.1683	1.1025	5.632115
1.0483	0.7548	27.99771		2.1668	1.7248	20.39874		1.2981	1.0826	16.60119		1.2234	1.1868	2.991663
1.2839	0.9346	27.20617		1.0901	0.8339	23.50243		1.2879	1.0007	22.29987		1.1968	1.1636	2.774064
1.1493	0.87	24.30175		1.7889	1.195	33.19917		1.1809	0.8609	27.09798		1.2673	1.2214	3.621873
1.6869	1.2568	25.49647		1.5153	1.2077	20.29961		1.5736	1.2951	17.69827		1.2416	1.1976	3.543814
1.7306	1.3672	20.9985		1.3192	1.0686	18.99636		1.3702	1.1455	16.39907				
1.4137	1.0772	23.80279						1.3664	1.1587	15.20053				
1.5953	1.1135	30.20122												
1.3062	1.0319	20.99985												
1.4962	1.1267	24.6959												
1.7921	1.3781	23.10139												
1.504	1.1551	23.19814												
1.5016	1.0737	28.49627												

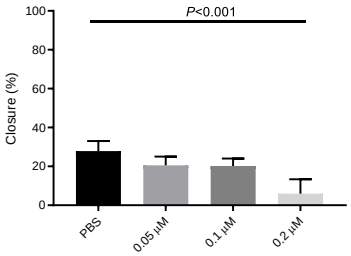
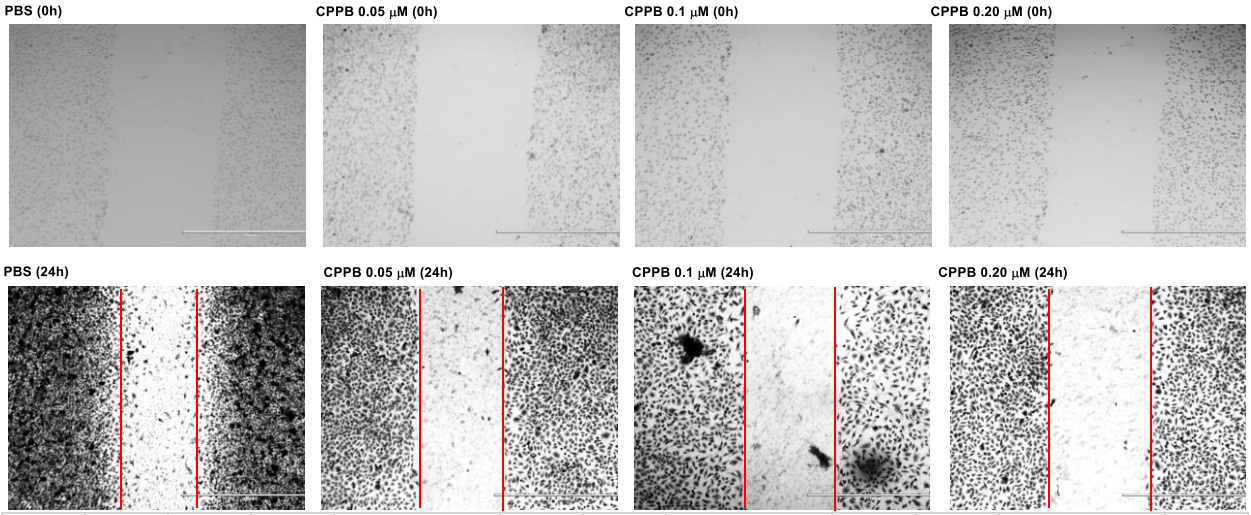


Figure S1F. Analysis of migration inhibition of SiHa.



PBS				CPPB 0.05μM				CPPB 0.1μM				CPPB 0.2μM		
0h	24h	%closure		0h	24h	%closure		0h	24h	%closure		0h	24h	%closure
1.131	0.5357	52.63484		1.0945	0.8928	18.42851		1.1284	1.0702	5.157745		1.1702	1.022	12.6645
1.1378	0.5703	49.87696		1.1537	0.8772	23.96637		1.1514	1.059	8.025013		1.1082	1.0271	7.318174
1.0545	0.5699	45.95543		1.0418	0.8544	17.9881		1.0314	0.9601	6.912934		1.0902	1.0167	6.741882
1.1266	0.7391	34.39553		1.1414	0.9128	20.02804		1.1026	1.0792	2.122256		1.1357	1.1045	2.747204
1.0655	0.6455	39.41811		1.0766	0.8946	16.90507		1.0691	0.9777	8.549247		1.0509	0.9995	4.891046
1.0253	0.5249	48.80523		1.0384	0.6963	32.94492		1.0201	0.8835	13.39084		1.0175	0.9995	1.769042

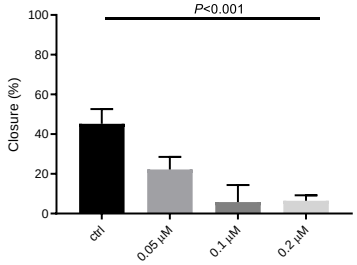
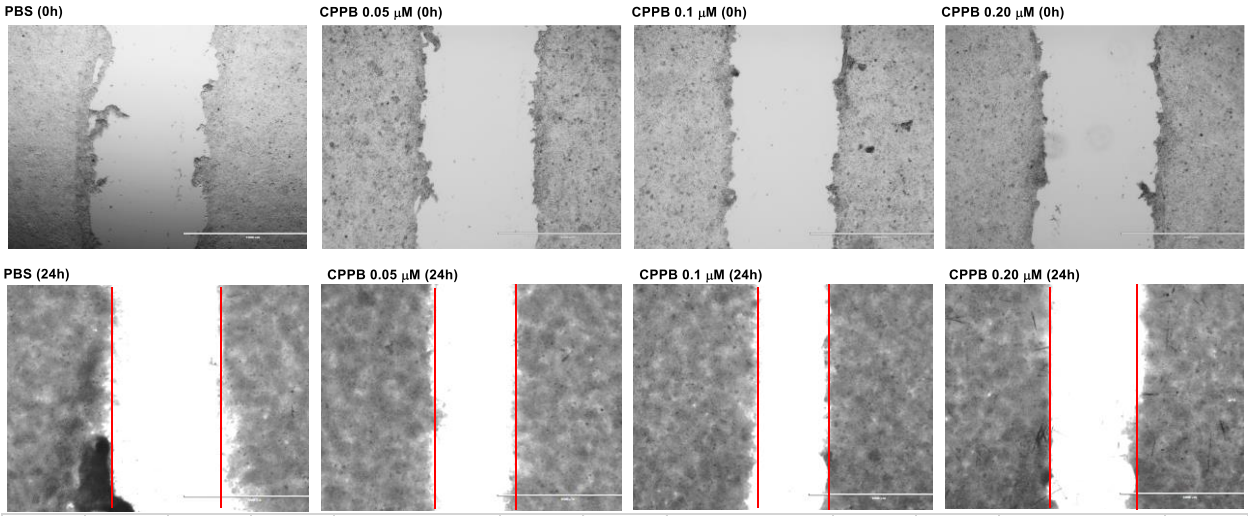
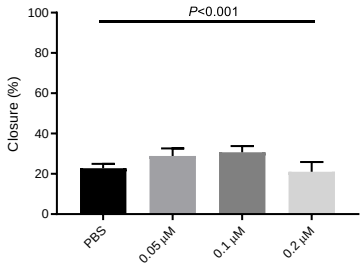


Figure S1G. Analysis of migration inhibition of HCT-116.



PBS				CPPB 0.05μM				CPPB 0.1μM				CPPB 0.2μM		
0h	24h	%closure		0h	24h	%closure		0h	24h	%closure		0h	24h	%closure
1.5053	1.0603	29.56221		1.4222	0.9657	32.09816		2.0629	1.4358	30.39895		1.6537	1.1708	29.20119
1.6171	1.174	27.4009		1.4494	1.0711	26.10046		1.23	0.8721	29.09756		1.9951	1.4644	26.60017
1.7035	1.1152	34.53478		1.6431	1.1436	30.39985		1.3805	0.9401	31.90148		1.8349	1.3266	27.70178
1.6962	1.1195	33.99953		1.5439	1.1903	22.90304		1.5635	1.0256	34.40358		1.8247	1.3794	24.40401
2.2438	1.611	28.20216		1.7176	1.235	28.09735		1.5077	1.0584	29.80036		1.5477	1.3094	15.39704
1.59	1.1607	27		1.935	1.4261	26.29974		1.6479	1.2326	25.20177		1.4744	1.2296	16.60336
1.3777	0.9093	33.99869		1.4917	1.1217	24.80391		1.7368	1.1133	35.89936		1.4891	1.1793	20.80451
1.2339	0.8736	29.2001		1.5398	1.0147	34.10183		1.5942	1.1	30.99987		1.3503	1.1221	16.89995
1.2266	0.8549	30.30328		1.445	1.0751	25.59862		1.584	1.1389	28.09975		1.1726	1.0049	14.30155
1.4337	0.9692	32.39869		1.5986	1.1302	29.30064		1.7505	1.2656	27.70066		1.1176	0.8784	21.40301
1.7329	1.2078	30.30181		1.5009	0.9876	34.19948		1.9446	1.3126	32.50026		1.0392	0.7555	27.29985
1.1772	0.8582	27.0982		1.5985	1.1605	27.40069		2.3824	1.6438	31.00235		1.0788	0.8695	19.40119
				1.8095	1.1327	37.4026		2.6203	1.9311	26.30233		1.1234	0.874	22.20046
				2.0571	1.4811	28.00058		0.9566	0.6868	28.20406		1.0235	0.8597	16.00391
				2.3298	1.7194	26.19967		0.9293	0.631	32.09943		0.9906	0.7836	20.89643
				2.3297	1.7869	23.29914		1.0234	0.7123	30.39867		0.9532	0.7883	17.29962
				1.9191	1.3856	27.79949		0.9431	0.6771	28.20486				
				1.8512	1.3347	27.90082		0.9551	0.6113	35.99623				
				1.8085	1.2822	29.10147		1.0398	0.6759	34.99711				
				1.8875	1.2986	31.2								
				1.0256	0.6584	35.80343								
				1.0867	0.7596	30.1003								
				1.0506	0.7564	28.00305								
				0.9818	0.7108	27.60236								
				1.1131	0.7302	34.39943								
				0.9354	0.6651	28.89673								
				1.0654	0.8129	23.70002								
				0.9039	0.6481	28.29959								

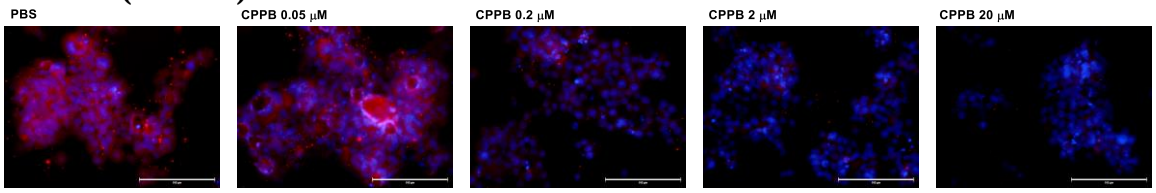


Immunofluorescent staining

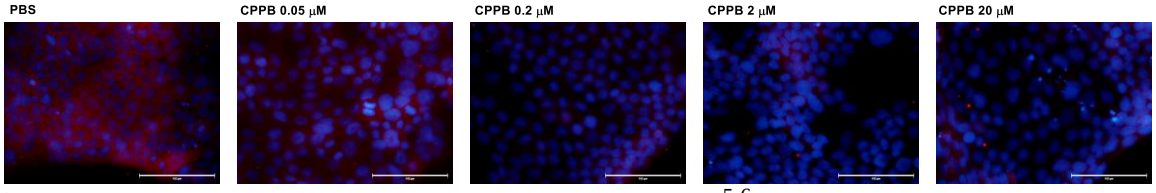
A confluent monolayer was formed in Nunc™ Lab-Tek™ II CC2™ chamber slide (8 well, Thermo Scientific, Cat. # 154941PK). Complete medium with CPPB (**5**, 0 - 20 μM) was added and incubated at 37 °C, 5% CO_2. After 24h (for snail and E-cadherin, 72h for DPAGT1), medium was removed and cells were washed with PBS (3 times), then fixed with 4% paraformaldehyde in PBS for 30min at 4 °C. After washing with 0.2% Tween-20/PBS for 3 times, permeabilized with 0.25% Triton X100 in PBS for 30min at 4 °C. After washing with 0.2% Tween-20/PBS for 3 times, treated with blocking buffer (0.2% Tween-20, 1% NGS, 1% BSA in PBS) for 2h at 4 °C. Then the cells were treated with primary antibody (snail: Snail (C15D3) rabbit mAb (Cell Signaling Technology, Cat. # 3879S), E-cadherin: E-Cadherin (4A2) mouse mAb (Cell Signaling Technology, Cat. # 14472S), DPAGT1: DPAGT1 polyclonal antibody (Invitrogen, Cat. # PA5-72704)) in blocking buffer (0.4% v/v) for overnight at 4 °C. The cells were washed with 0.2% Tween-20/PBS (3 times) and treated with secondary antibody (for snail and DPAGT1: goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor 568 (Invitrogen, Cat. # A11011), for E-cadherin: goat anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor Plus 647 (Invitrogen, Cat. # A32728)) in blocking buffer (0.2% v/v) for 2h at r.t. in the dark. After another 3-times washing with 0.2% Tween-20/PBS, the cells were treated with DAPI Fluoromount-G® (SouthernBiotech, Cat. # 0100-20) and covered with glass slide for fluorescence microscopy analysis.

Figure S2. Immunofluorescent staining: Effect of a DPAGT1 inhibitor, CPPB, on Snail in pancreatic cancer cells (AsPC-1 and Capan-1).[a]

A: Snail (AsPC-1)



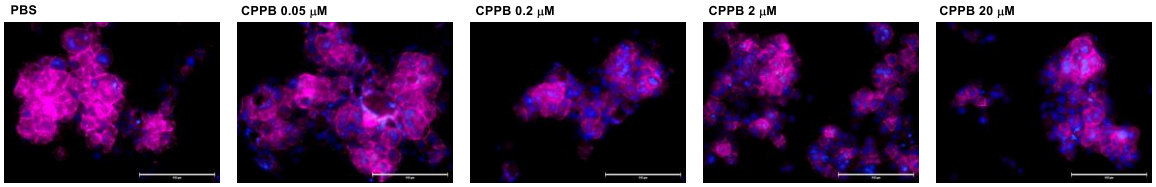
B: Snail (Capan-1)



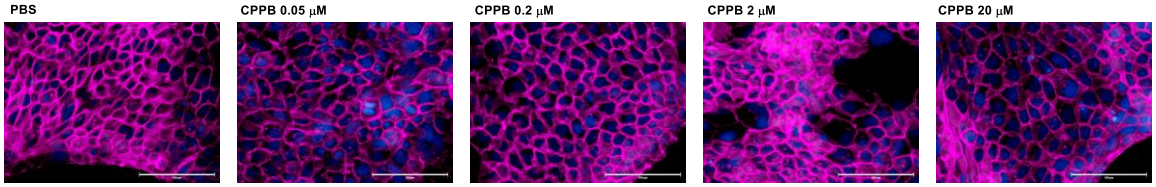
[a]Fluorescent microscopy images at 40x. The cells (1 x $10^{5\text{-}6}$) were treated with CPPB (0.05, 0.2, 2.0, and 20 μM) or PBS for 24h. The cells were treated with Snail (C15D3) rabbit mAb (Cell Signaling Technology), followed by goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor™ 568. DAPI (4',6-diamidino-2-phenylindole), a blue fluorescent DNA dye, was used to mark the nucleus.

Figure S3. Immunofluorescent staining: Effect of a DPAGT1 inhibitor, CPPB, on E-Cadherin in pancreatic cancer cells (AsPC-1 and Capan-1).[a]

A: E-Cadherin (AsPC-1)



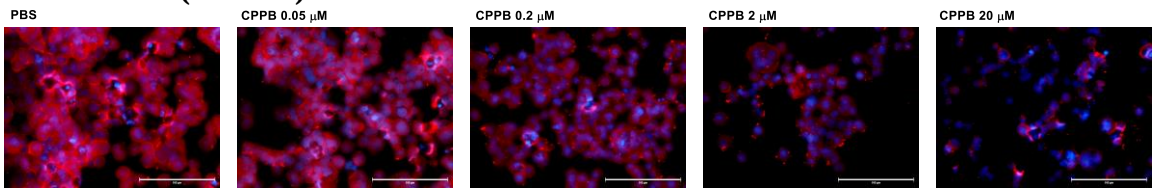
B: E-Cadherin (Capan-1)



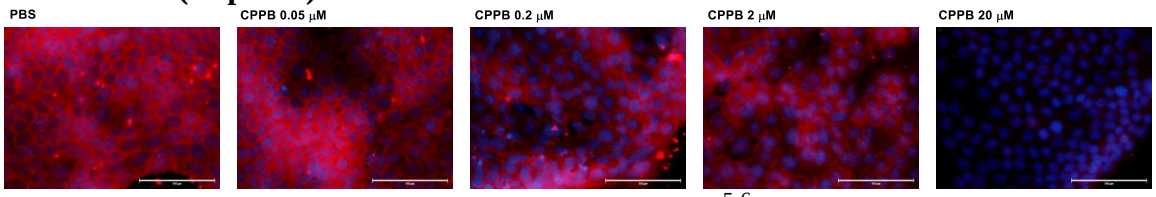
[a]Fluorescent microscopy images at 40x. The cells (1 x 10^{5-6}) were treated with CPPB (0.05, 0.2, 2.0, and 20 μM) or PBS for 24h. The cells were treated with E-Cadherin (4A2) mouse mAb (Cell Signaling Technology), followed by goat anti-mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor™ Plus 647. DAPI (4',6-diamidino-2-phenylindole), a blue fluorescent DNA dye, was used to mark the nucleus.

Figure S4. Immunofluorescent staining: The DPAGT1 expression level in the selected cancer cell lines (AsPC-1 and Capan-1) treated with CPPB.[a]

A: DPAGT1 (AsPC-1)



B: DPAGT1 (Capan-1)



[a]Fluorescent microscopy images at 40x. The cells (1 x 10^{5-6}) were treated with CPPB (0.05, 0.2, 2.0, and 20 μM) or PBS for 72h. The cells were treated with DPAGT1 polyclonal antibody (Invitrogen, PA5-72704), followed by goat anti-rabbit IgG (H+L) cross-adsorbed secondary antibody, Alexa Fluor™ 568. DAPI (4',6-diamidino-2-phenylindole), a blue fluorescent DNA dye, was used to mark the nucleus.

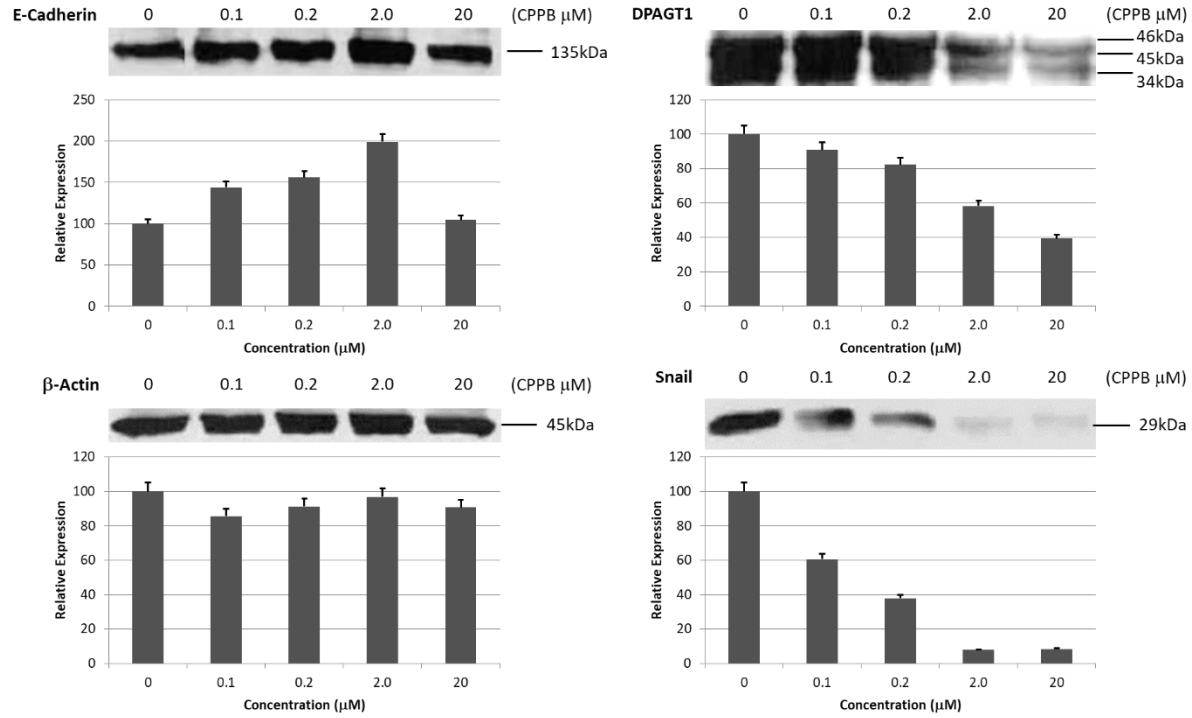
SDS-PAGE and Western blotting assay

The medium of the cells grown in 10 cm cell culture plate was removed, and the cells were washed once with PBS, and lysed with Pierce RIPA buffer (Thermo Scientific, Cat. #

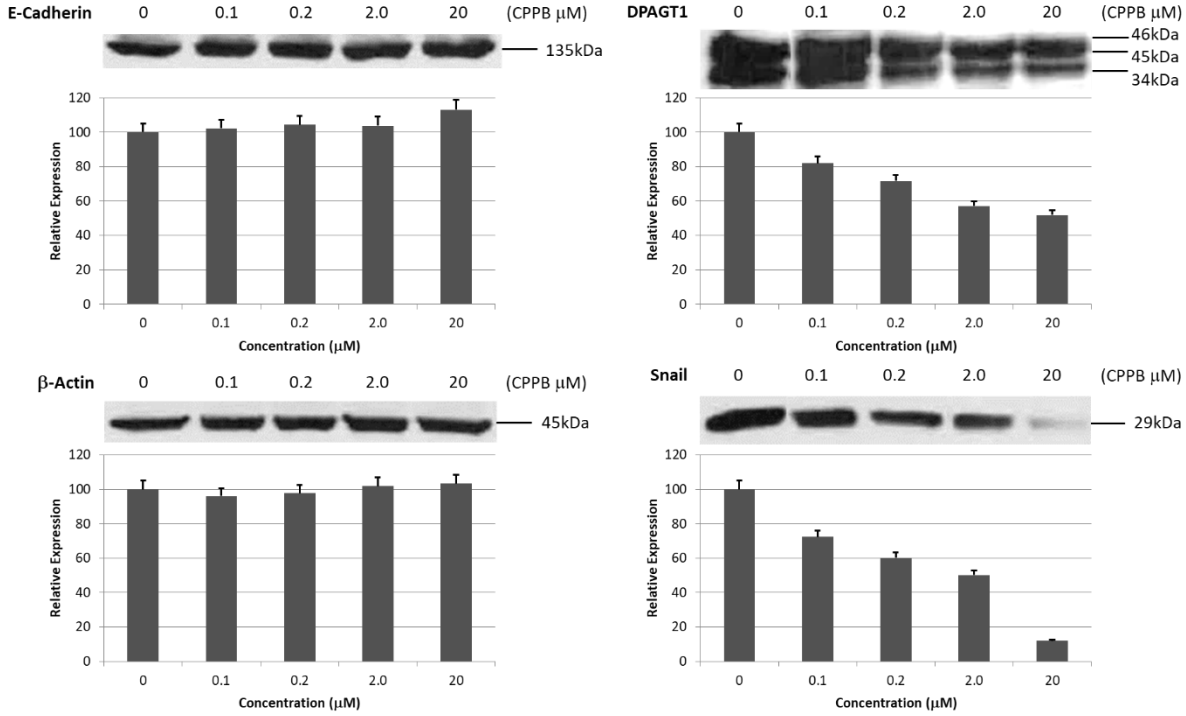
89901) containing 1x Pierce protease and phosphatase inhibitors (Thermo Scientific, Cat. # 88668). The cell lysate was pelleted down at 15,200 xg at 4 ^{o}C for 30min, the cell supernatant was transferred to a fresh Eppendorf tube, and 5 uL of sample was quantitated by using (Quick Start Bradford Dyed Reagent, Biorad, Cat. # 500-0205). 30 µL (1.5 mg total protein /mL) of each protein sample was analyzed by SDS-PAGE (10% gel) followed by Western blotting, and chemiluminescence. Precision Plus Dual Color (Biorad, Cat. # 161-0374) was used as protein standard marker. E-Cadherin (24E10) rabbit mAb (Cell Signaling Technology, Cat. #3195), monoclonal anti-DPAGT1 antibody produced in mouse clone 1G1 (Sigma Aldrich, Cat. #SAB1402754), β-actin (8H10D10) mouse mAb (Cell Signaling Technology, Cat. #3700) and Snail (C15D3) rabbit mAb (Cell Signaling Technology, Cat. #3879) were used as primary antibody. Anti-rabbit IgG, HRP-linked antibody (Cell Signaling Technology, Cat. #7074) or anti-mouse IgG, HRP-linked antibody (Cell Signaling Technology, Cat. #7076) were used as secondary antibody. Clarity Western ECL Substrate (Biorad, Cat. # 170-5060) was used to develop the probe signal, and Classic Blue BX film (MidSci, Ref. # 604 5983) was used for chemiluminescence.

Figure S5. Western blotting assay for selected cancer cell lines (PD002, PANC-1 and HCT-116) treated with CPPB.[a]

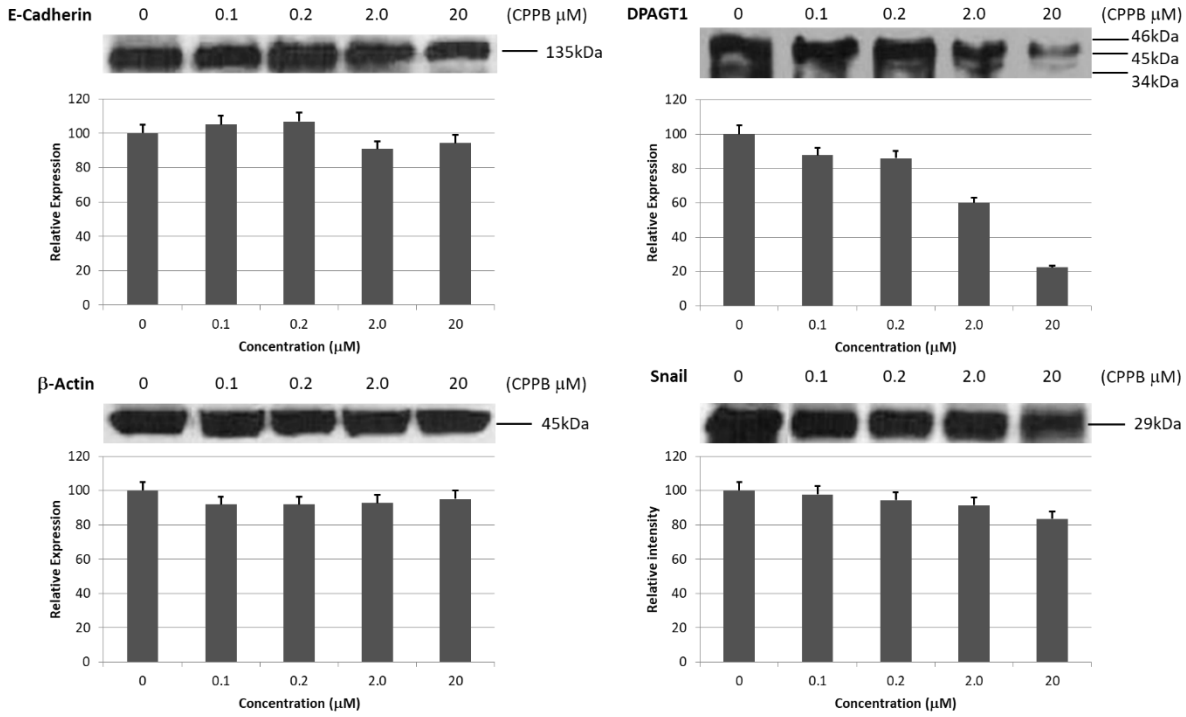
A: PD002[b]



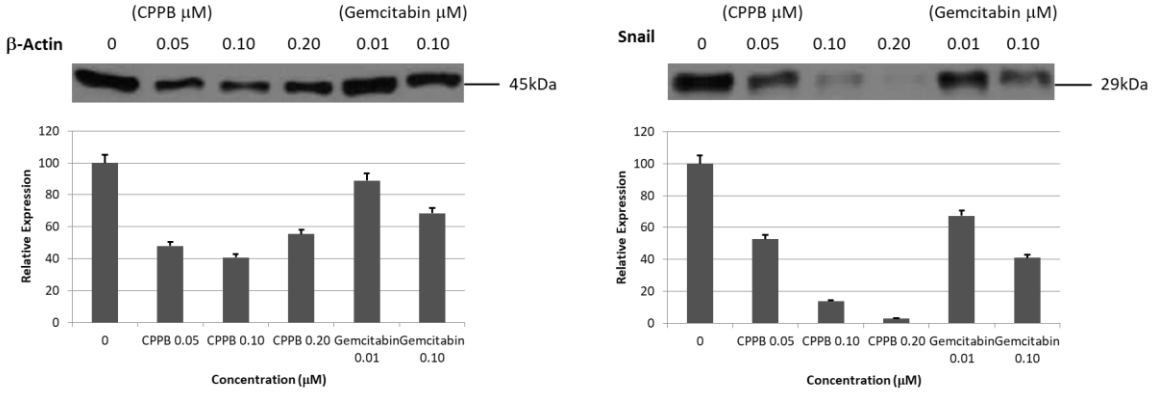
B: PANC-1[c]



C: HCT-116[d,f]



D: SiHa[e]



[a]The relative expression level was quantified by using Image Studio™ Lite quantification software. [b]Exposure time: E-Cadherin (5min), DPAGT1 (15min), β-actin (30sec), Snail (2min). [c]Exposure time: E-Cadherin (5min), DPAGT1 (15min), β-actin (2sec), Snail (2min). [d]Exposure time: E-Cadherin (5min), DPAGT1 (2min), β-actin (10sec), Snail (2min). [e]Exposure time: β-actin (5sec), Snail (2min). [e]The cell lysate for DPAGT1 was prepared by ultracentrifugation (130,000 xg for 1h at 4 °C) and 30 μL of the lysate was analyzed.

Synergistic effect of CPPB with paclitaxel

The synergistic or antagonistic activities of CPPB (**5**) with paclitaxel were assessed *in vitro* via micro dilution broth checkerboard technique. PD002 cells (180 μL, $1x10^4$/mL) were places in each well of a 96well plate. The cells were treated with a combination of CPPB (0-50 μM) and paclitaxel (5-0.024 μM), and cultured at 37 °C for 72h under 5% CO_2. Antiproliferation kinetic of each well were monitored by using an IncuCyte Live-Cell Imaging System (Essen BioScience, Ann Arbor, MI).

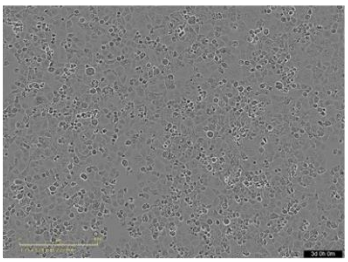
Figure S6. Fractional Inhibitory Concentration (FIC) of a combination of CPPB and paclitaxel against a patient-derived pancreatic adenocarcinoma PD002.[a]
ΣFIC[b] index for the wells at growth–no growth interface.

	1	2	3	4	5	6	7	8	9	10	11	12	C_A: CPPB (μM)
A	0.001953	0.003906	0.007813	0.01563	0.03125	0.06250	0.1250	0.2500	0.5000	1.0000	2.0000	4.0000	0
B	0.002239	0.004192	0.008098	0.01591	0.03154	0.06279	0.1253	0.2503	0.5003	1.0003	2.0003	4.0003	0.01
C	0.003382	0.005335	0.009241	0.01705	0.03268	0.06393	0.1264	0.2514	0.5014	1.0014	2.0014	4.0014	0.05
D	0.004810	0.006763	0.01067	0.01848	0.03411	0.06536	0.1279	0.2529	0.5029	1.0029	2.0029	4.0029	0.1
E	0.007667	0.009621	0.01353	0.02134	0.03696	0.06821	0.1307	0.2557	0.5057	1.006	2.0057	4.0057	0.2
F	0.05910	0.06105	0.06496	0.07277	0.08839	0.1196	0.1821	0.3071	0.5571	1.05714	2.0571	4.0571	2.0
G	0.5734	0.5753	0.5792	0.5871	0.6027	0.6339	0.6964	0.8214	1.0714	1.5714	2.5714	4.5714	20.0
H	1.4305	1.4325	1.4364	1.4442	1.4598	1.4911	1.5536	1.6786	1.9286	2.4286	3.4286	5.4286	50.0
	0.0024	**0.0049**	**0.0098**	**0.020**	**0.039**	**0.078**	**0.16**	**0.31**	**0.63**	**1.25**	**2.5**	**5**	C_B: Paclitaxel (μM)

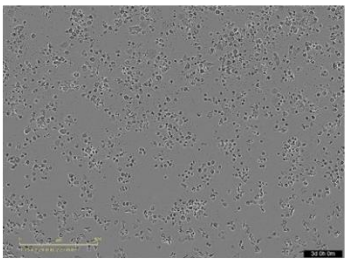
Highlighted in orange: growth, others: non-growth
[a]The IC_{50} values of CPPB and paclitaxel against PD002 are 35.0 and 1.25 μM, respectively. [b]ΣFIC is the sum of fractional inhibitory concentration calculated by the equation $\Sigma FIC = FIC_A + FIC_B = C_A/IC_{50A} + C_B/IC_{50B}$. MIC_A and MIC_B: MIC of drugs A and B, C_A and

C_B=concentrations of drugs A and B used in combination. In these interaction studies, ΣFIC of less than 0.5 represents synergistic activity.

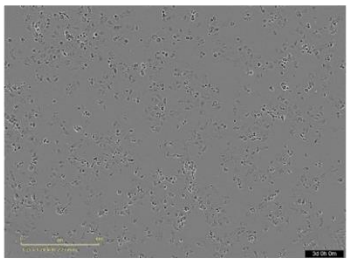
CPPB 0 μM, Paclitaxel 0 μM



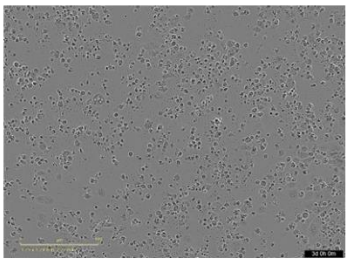
CPPB 0 μM, Paclitaxel 0.63 μM



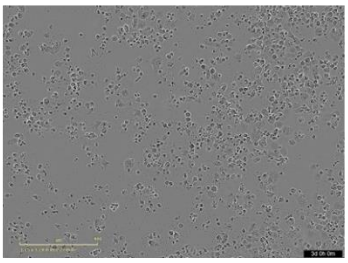
CPPB 0.1 μM, Paclitaxel 0.16 μM



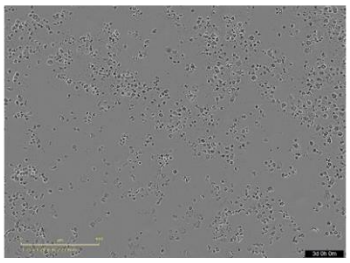
CPPB 0.2 μM, Paclitaxel 0.020 μM



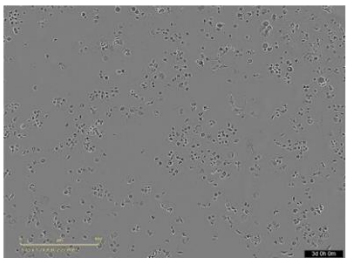
CPPB 2.0 μM, Paclitaxel 0.0024 μM



CPPB 20 μM, Paclitaxel 0.0024 μM



CPPB 50 μM, Paclitaxel 0.0024 μM



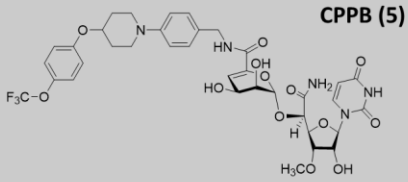
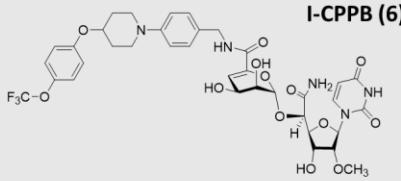
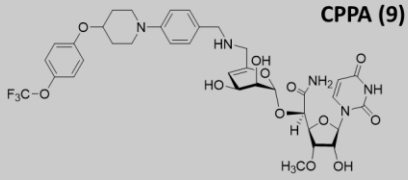
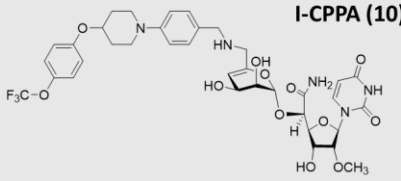
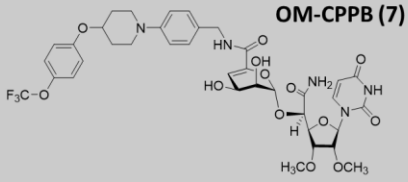
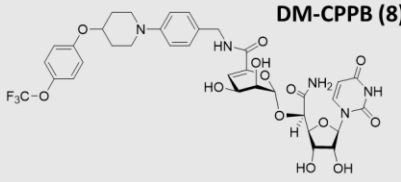
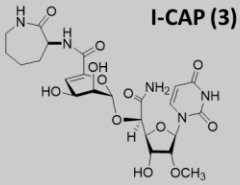
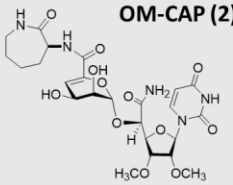
Computational methods – DPAGT1 inhibitor study

Protein preparation. All molecular modeling and docking studies were performed using the experimental structure of the human GPT (DPAGT1, H129 variant) with bound tunicamycin (PDB 6BW6).[7] The biological unit was downloaded and prepared using the Protein Preparation Wizard of the Maestro Small Molecule Drug Discovery Suite (Schrödinger, LLC).[8] Hydrogens were added, when applicable, and protonation and tautomeric states were assigned using the Epic program.[9] Lone waters were removed and the protein was refined by optimizing H-bond assignments and performing a restrained minimization using MacroModel.[10]

Docking site preparation. The docking receptor grid was prepared using Schrödinger's Glide program.[11, 12] The docking grid was defined as 25 Å region centroid of the bound tunicamycin compound. Van der Waals radius scaling was employed with a scaling factor of 1.0 and partial charge cutoff of 0.25 (default values). No docking constraints or excluded volumes were defined. Hydroxyl and thiol groups within close proximity to the bound tunicamycin compound (≤ 3Å) were defined as rotatable.

Inhibitor docking. Compounds were built and prepared for docking using the LigPrep program using default settings (Schrödinger, LLC).[11] The DPAGT1 inhibitors, non-inhibitors, and weak inhibitor reported herein were docked into the prepared protein using Schrödinger's Glide program using XP (extra precision) settings using the grid described above.[13]

Table S1. DPAGT1 inhibitors of capuramycin analogues possessing antimigratory activities of pancreatic cancer cell lines.

Compound	IC_{50} (DPAGT1)	Glide XP Score
CPPB (5) 	200 nM	-16.6
I-CPPB (6) 	600 nM	-12.4
CPPA (9) 	>50 μM	-14.5
I-CPPA (10) 	>50 μM	-14.6
OM-CPPB (7) 	20 μM	-13.6
DM-CPPB (8) 	>50 μM	-12.9
I-CAP (3) 	11.5 μM	-10.9
OM-CAP (2) 	4.5 μM	-12.9

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[10] Schrödinger Release 2019-4: MacroModel, Schrödinger, LLC, New York, NY, **2019**.

[11] Schrödinger Release 2019-4: LigPrep, Schrödinger, LLC, New York, NY, **2019**.

[12] Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: a new approach for rapid accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *25*, 1739-1749.

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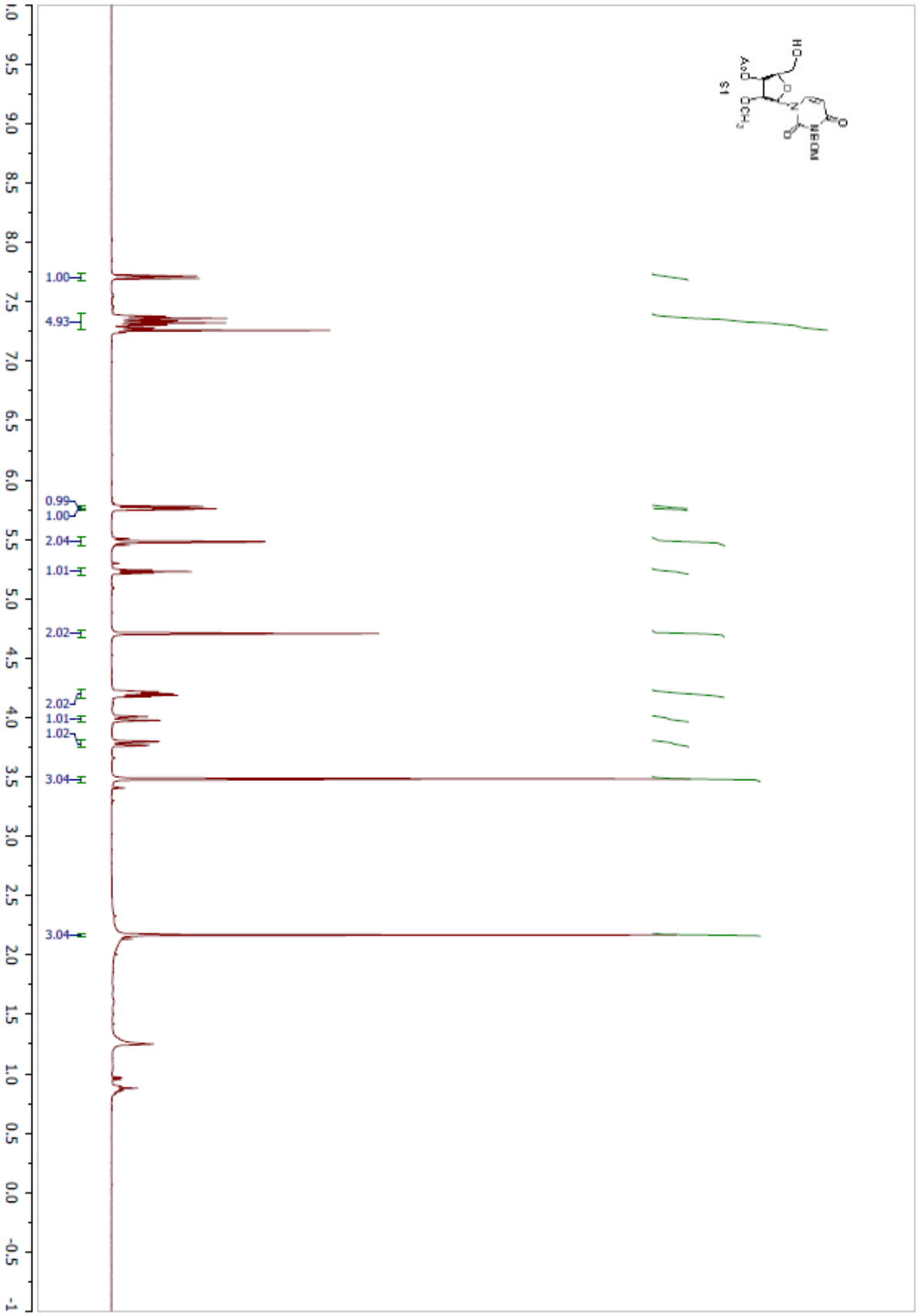
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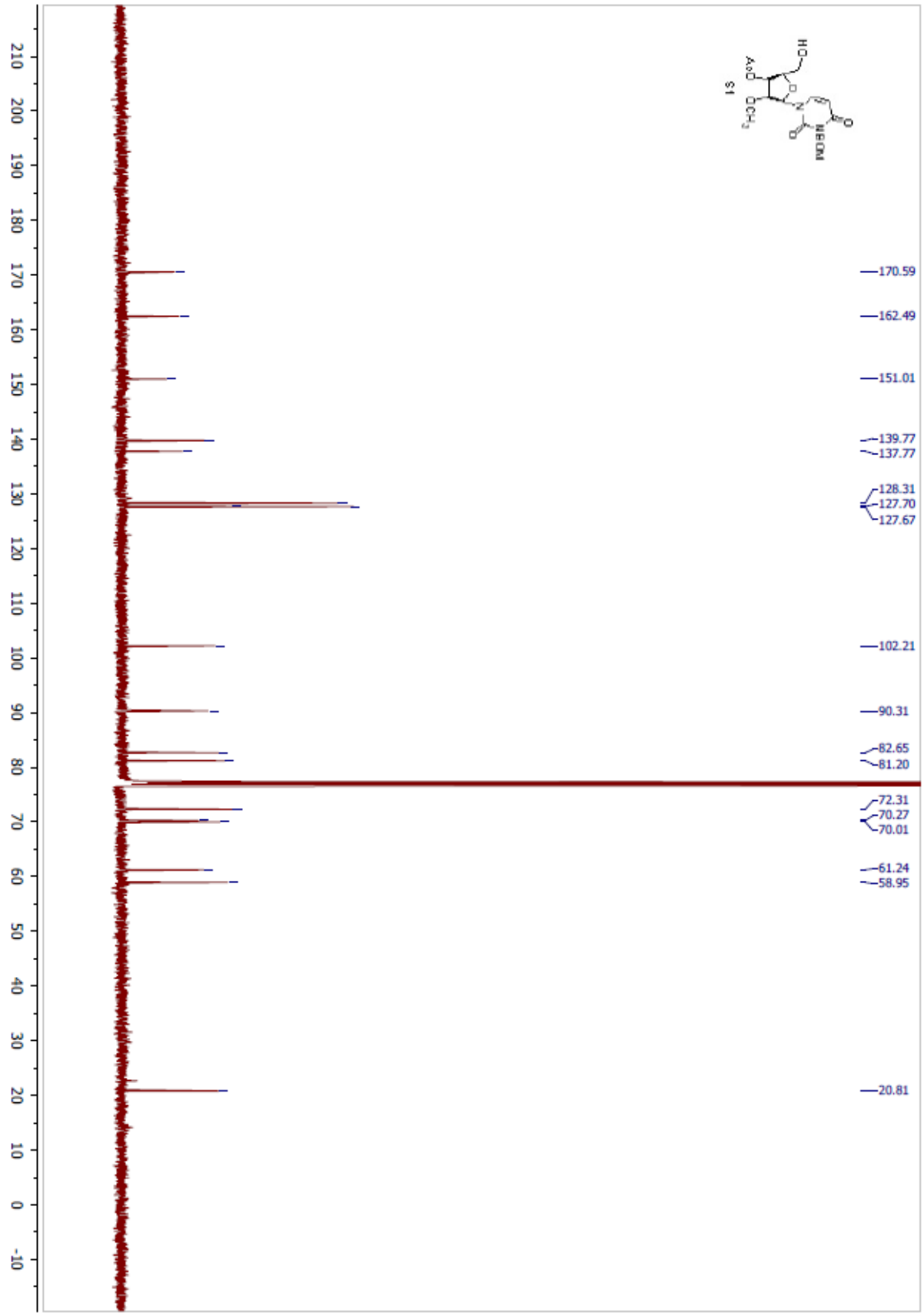
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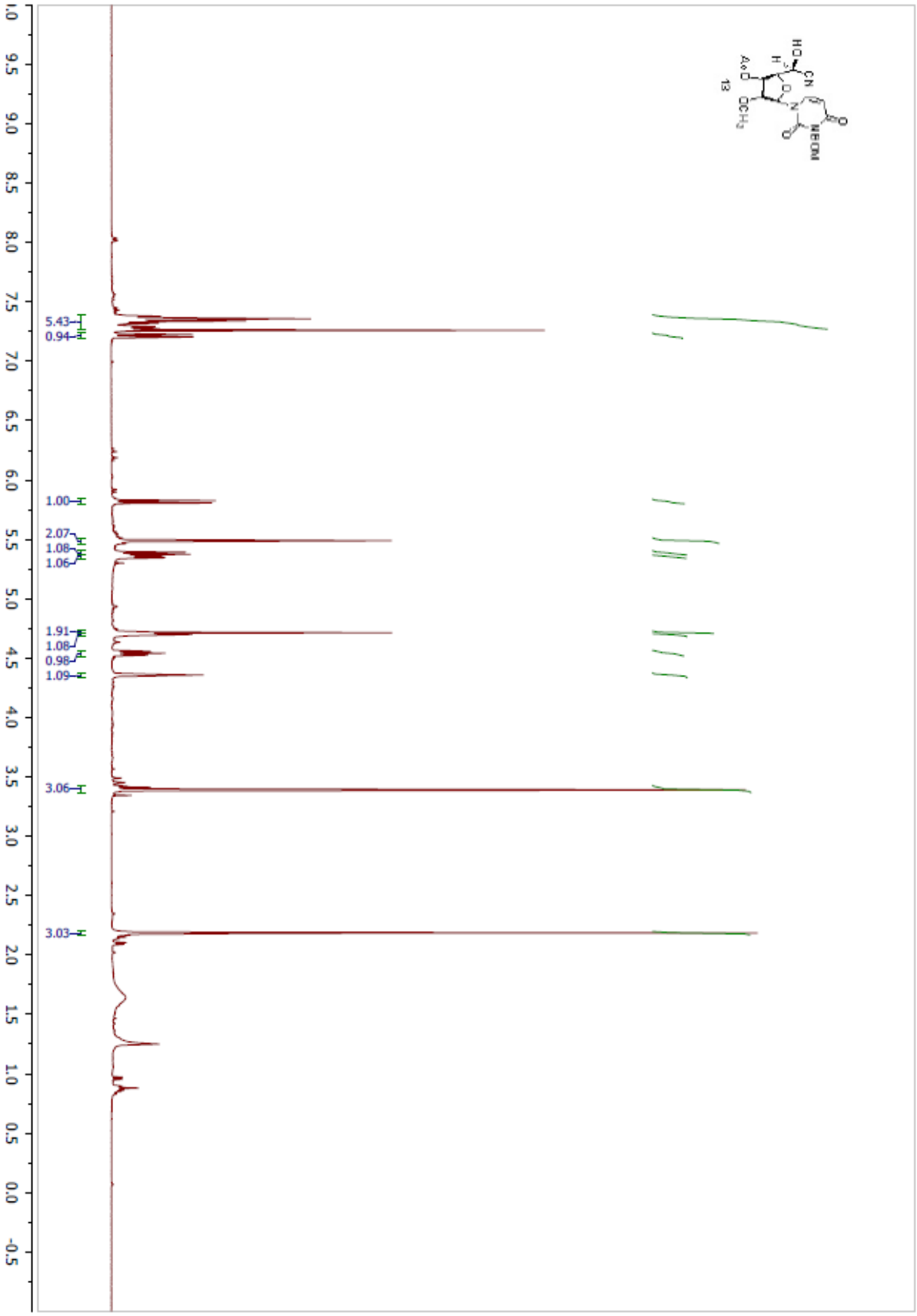
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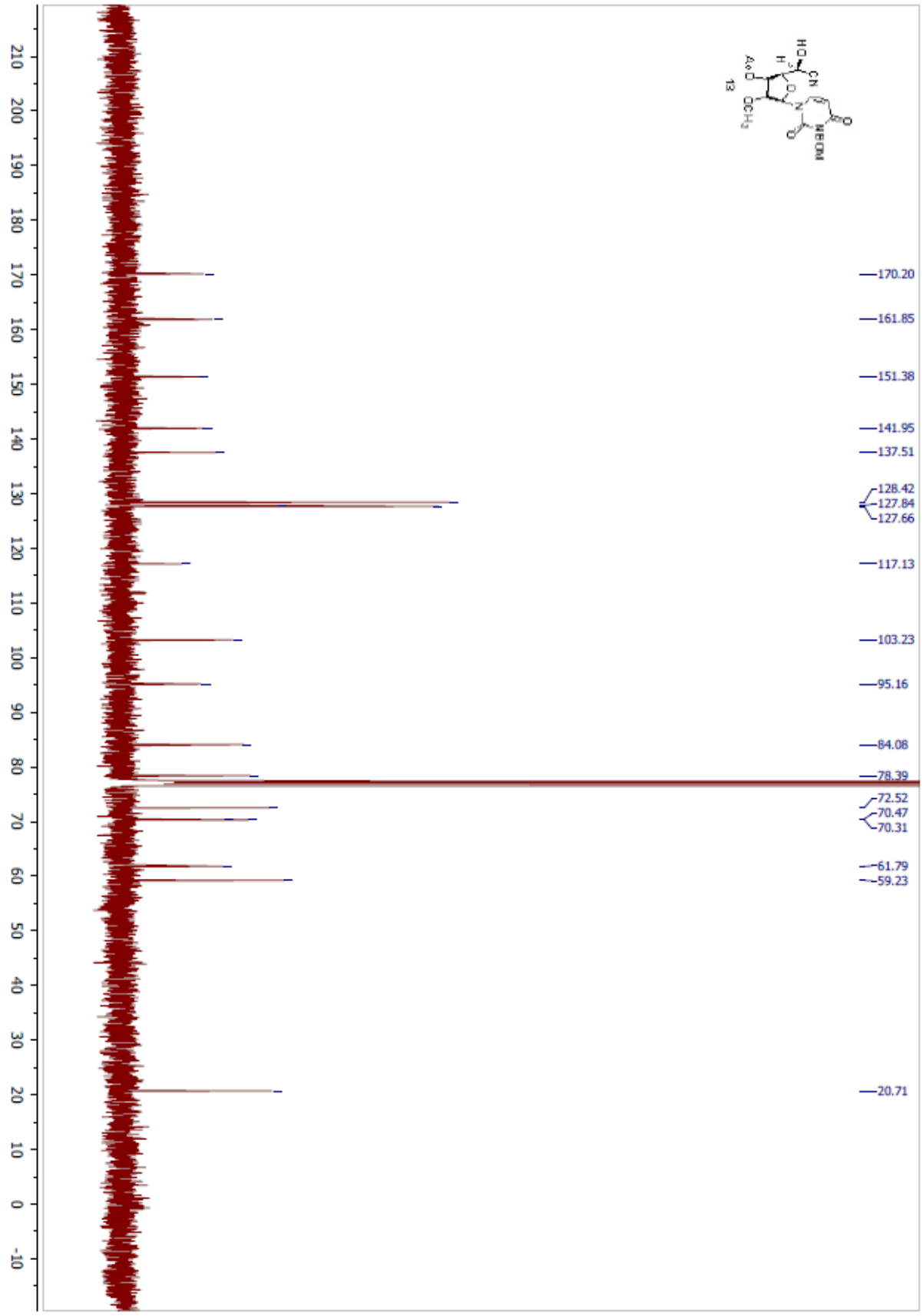
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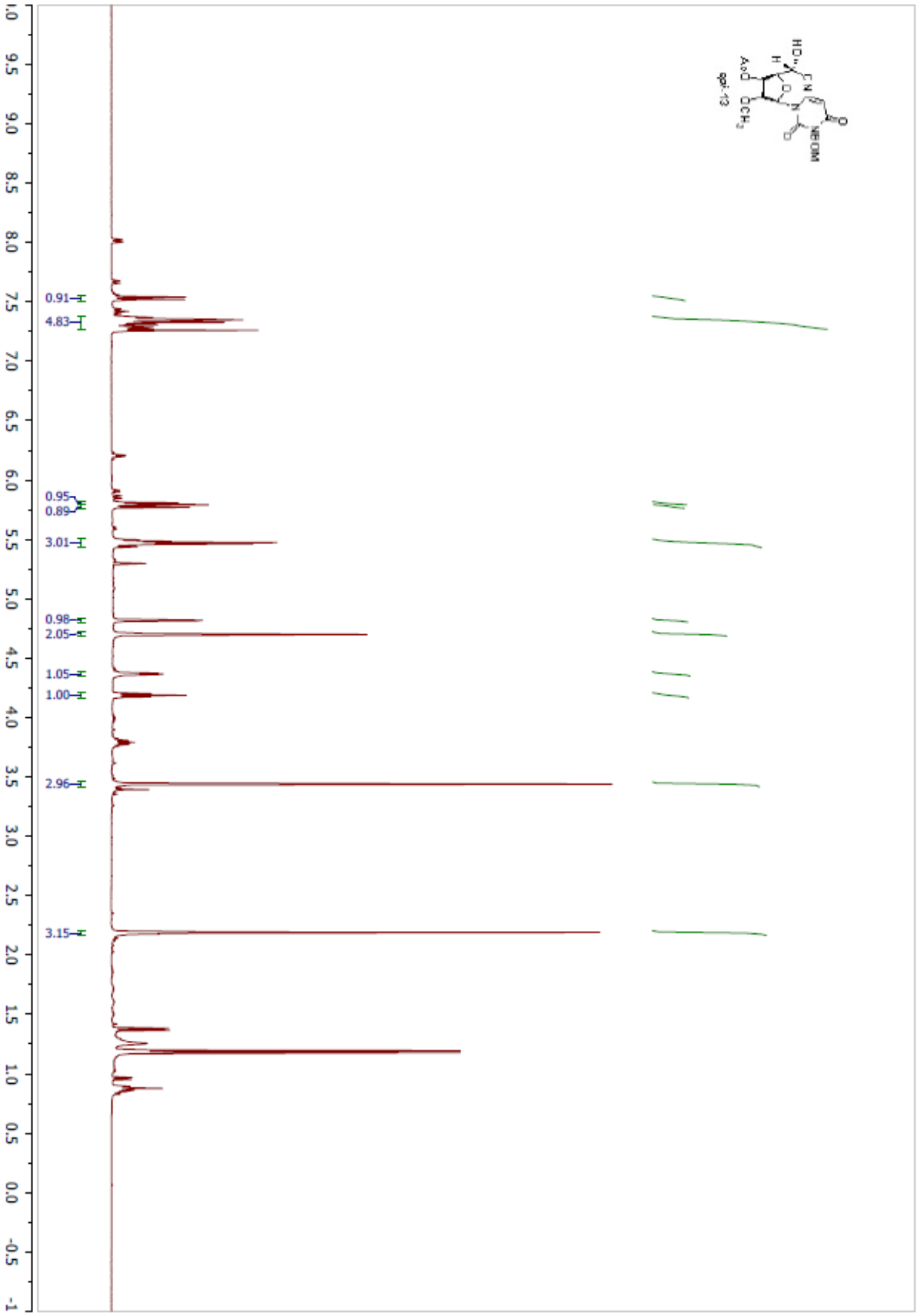
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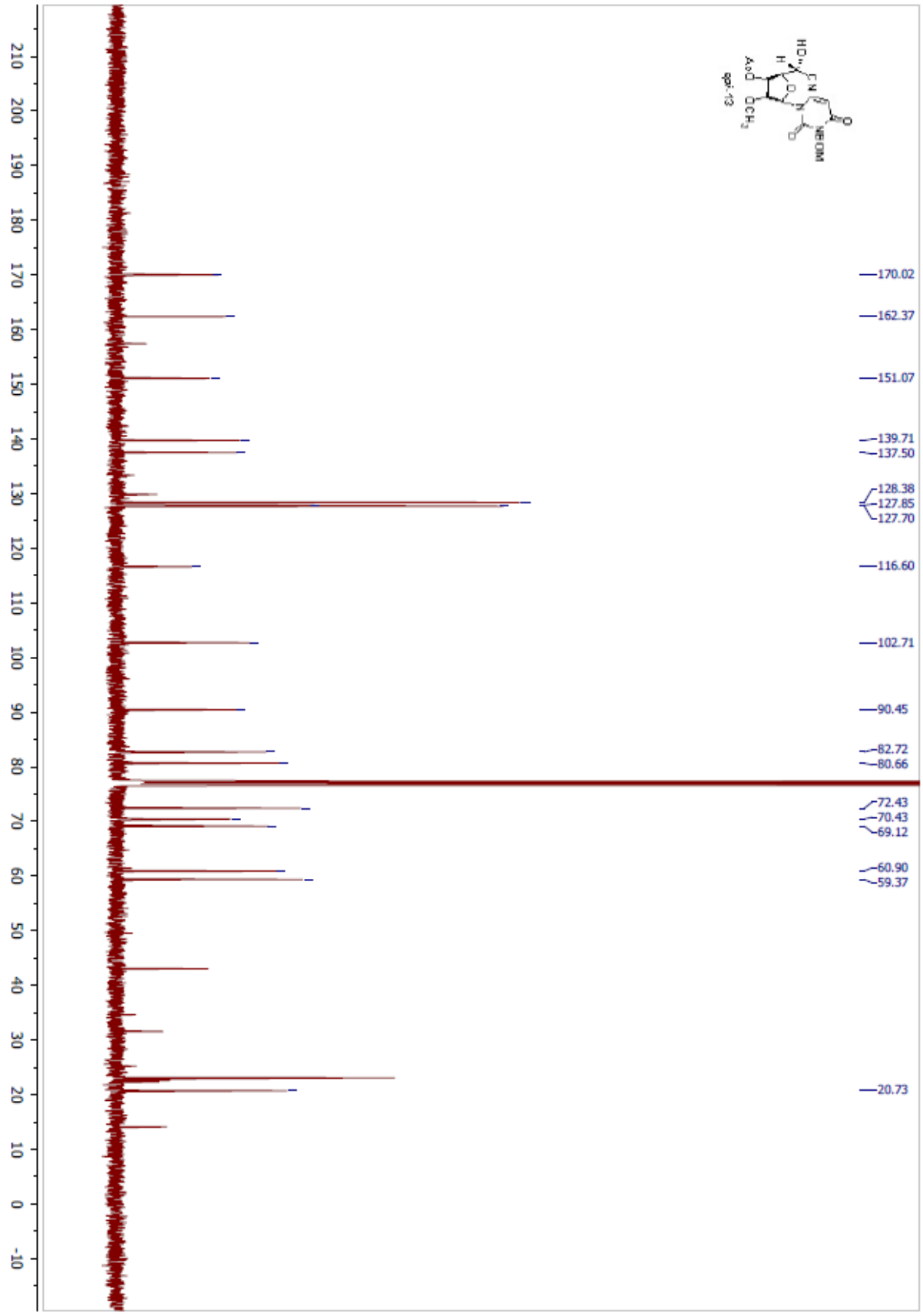
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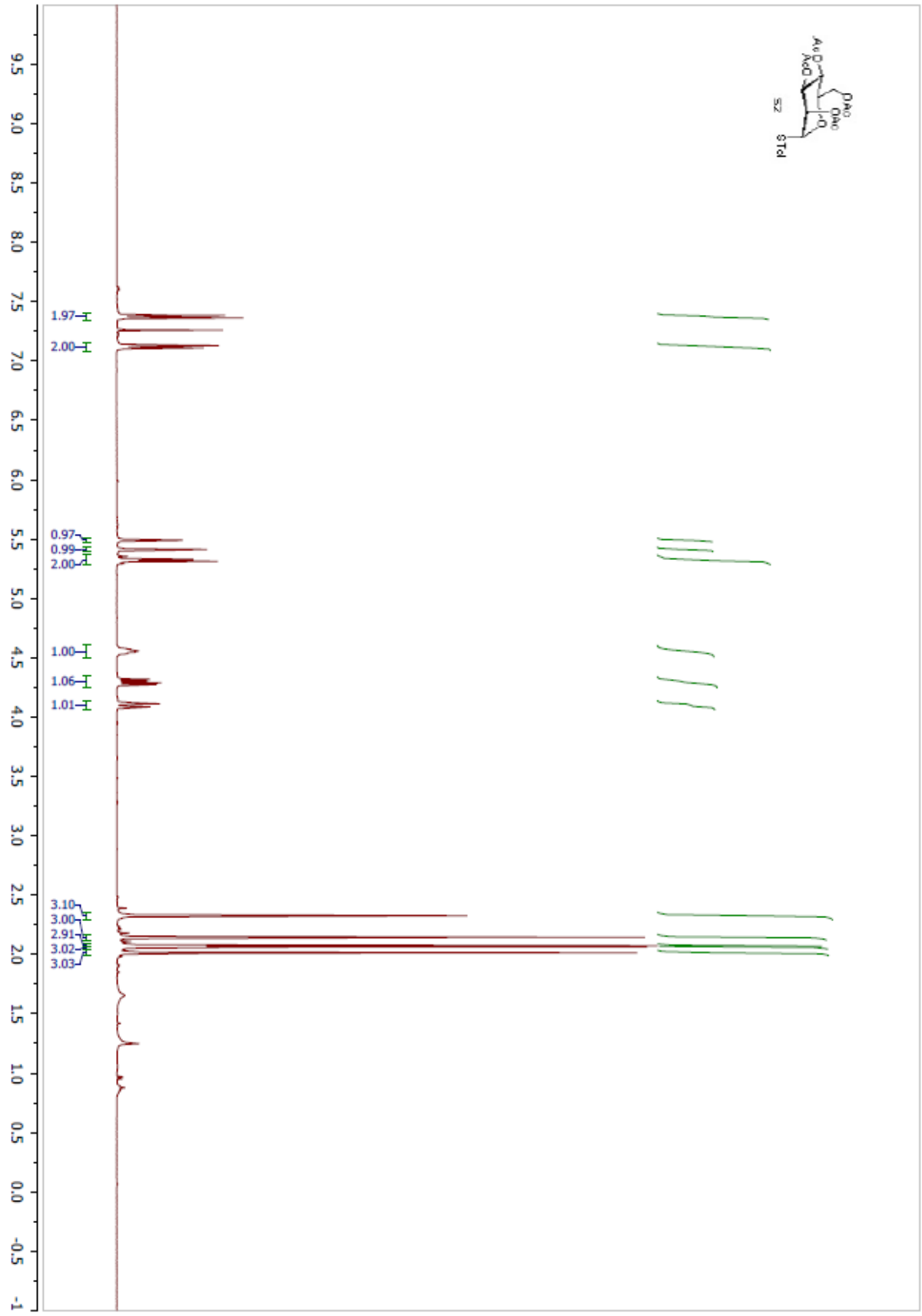
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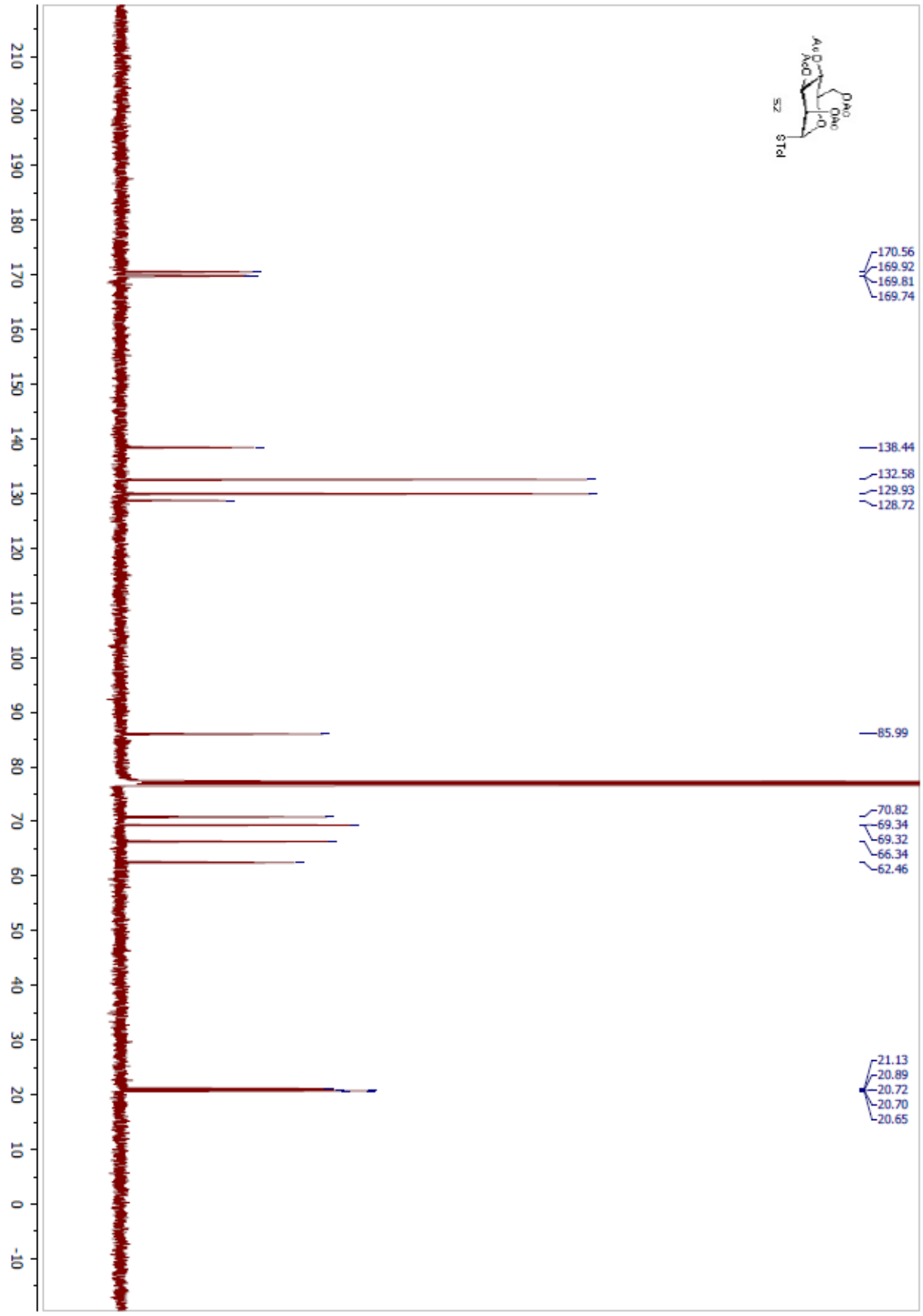
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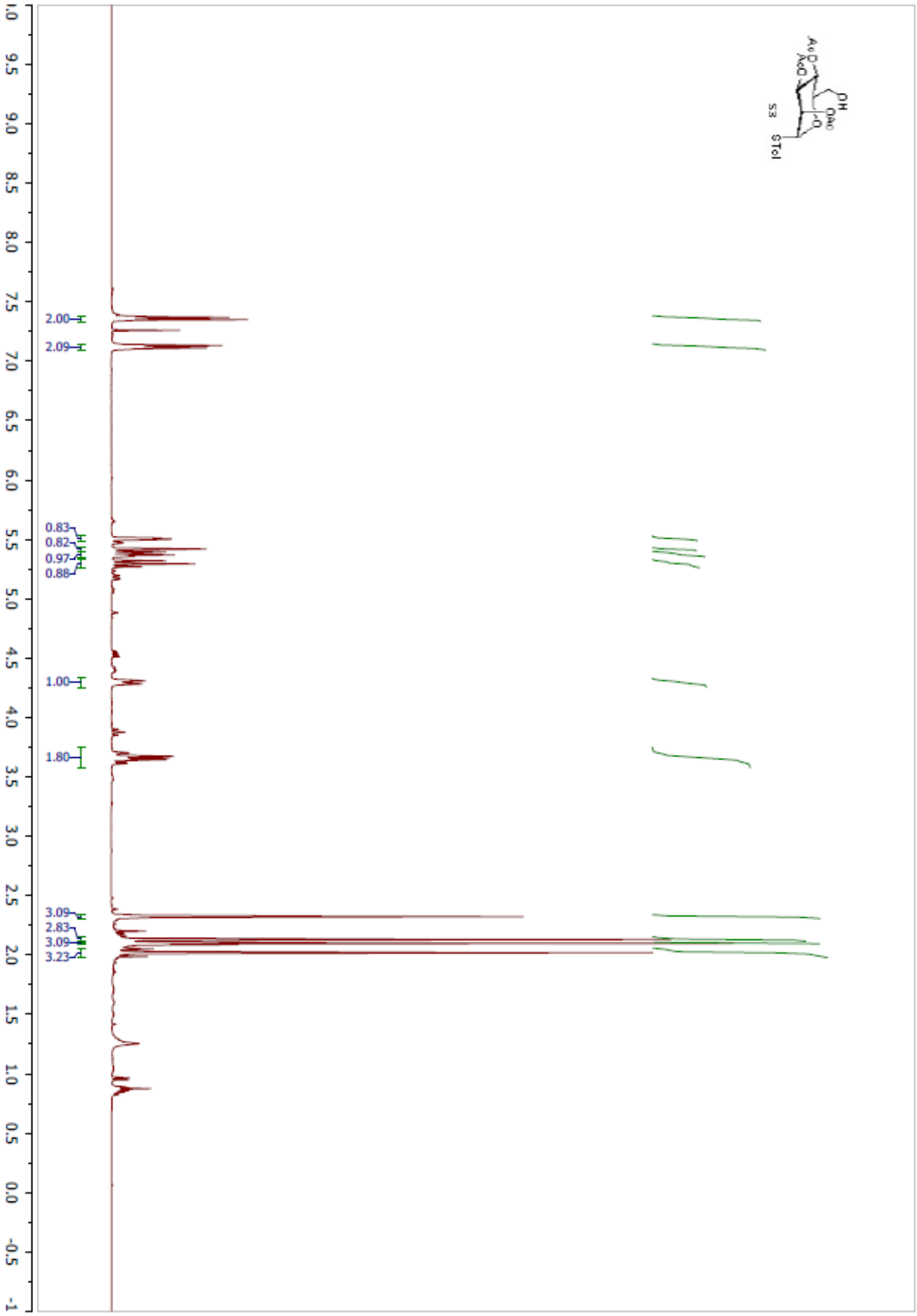
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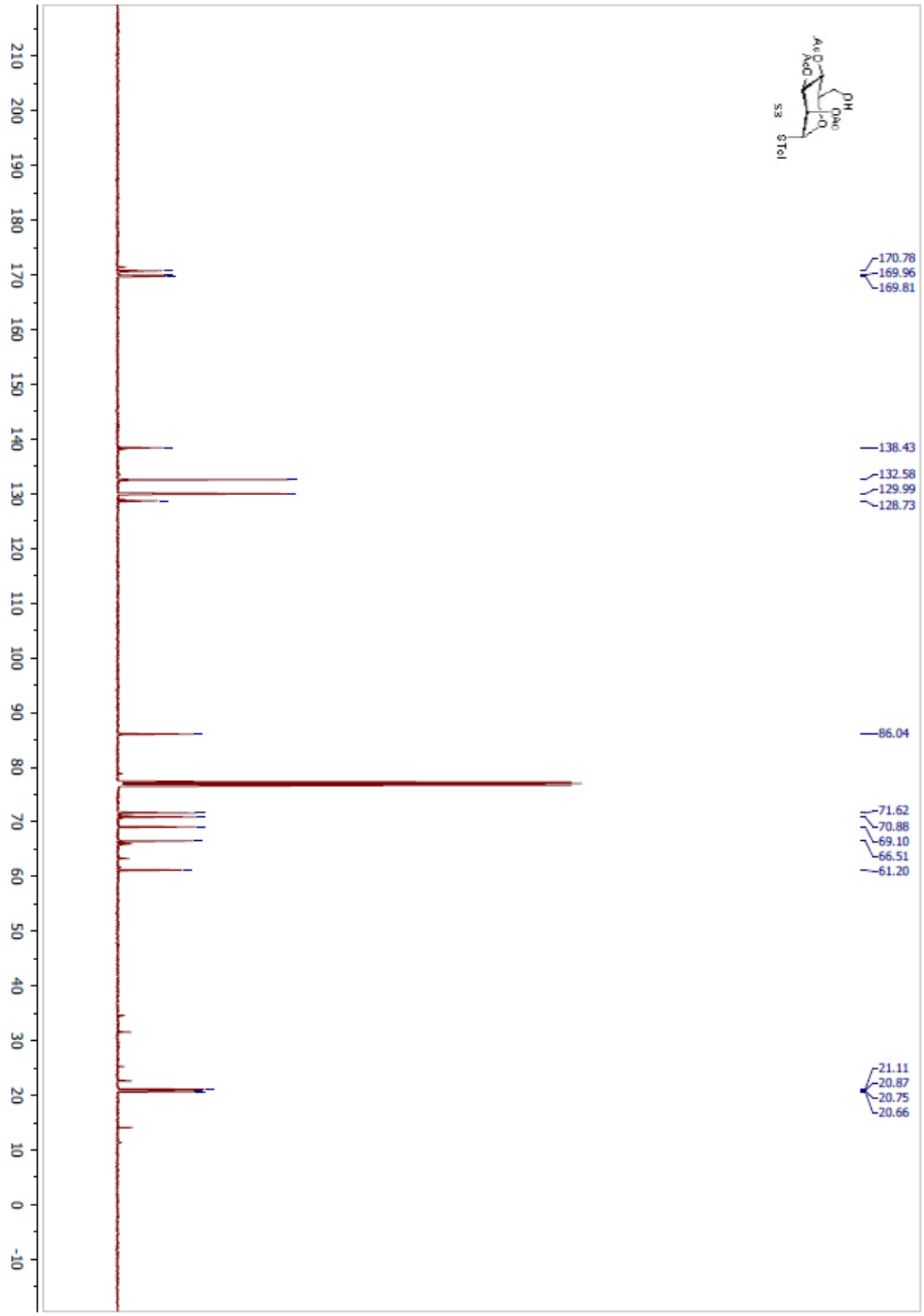
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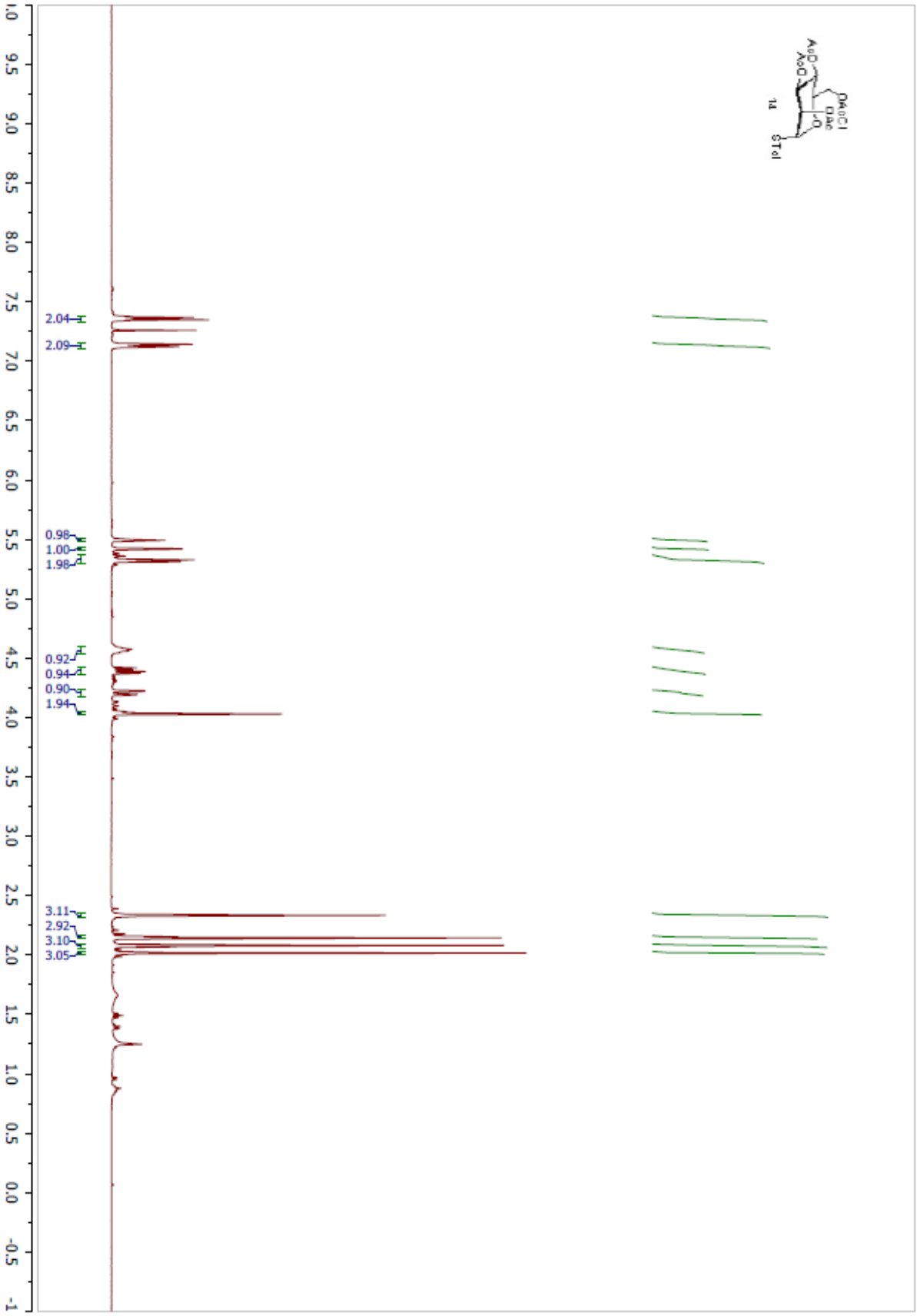
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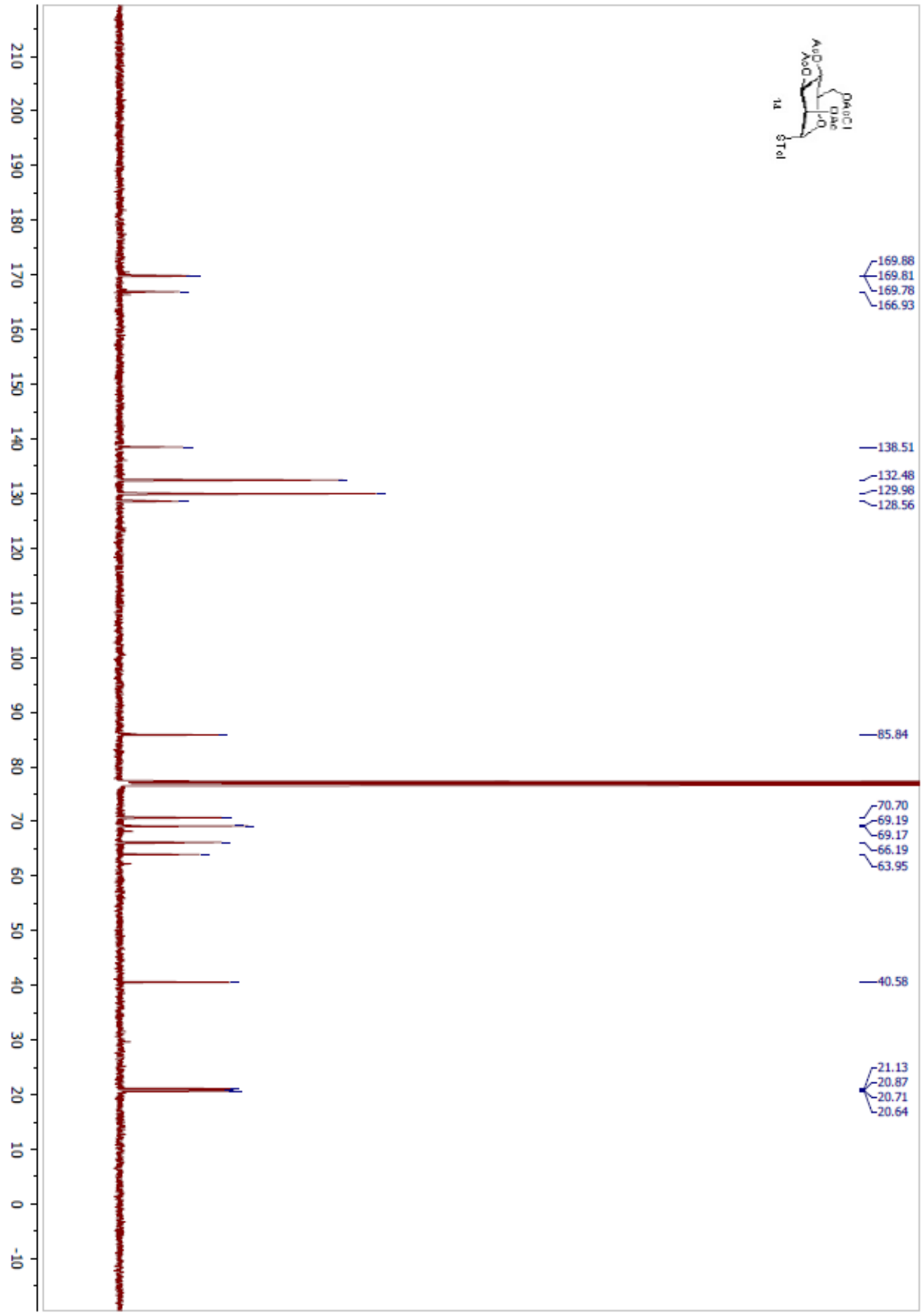
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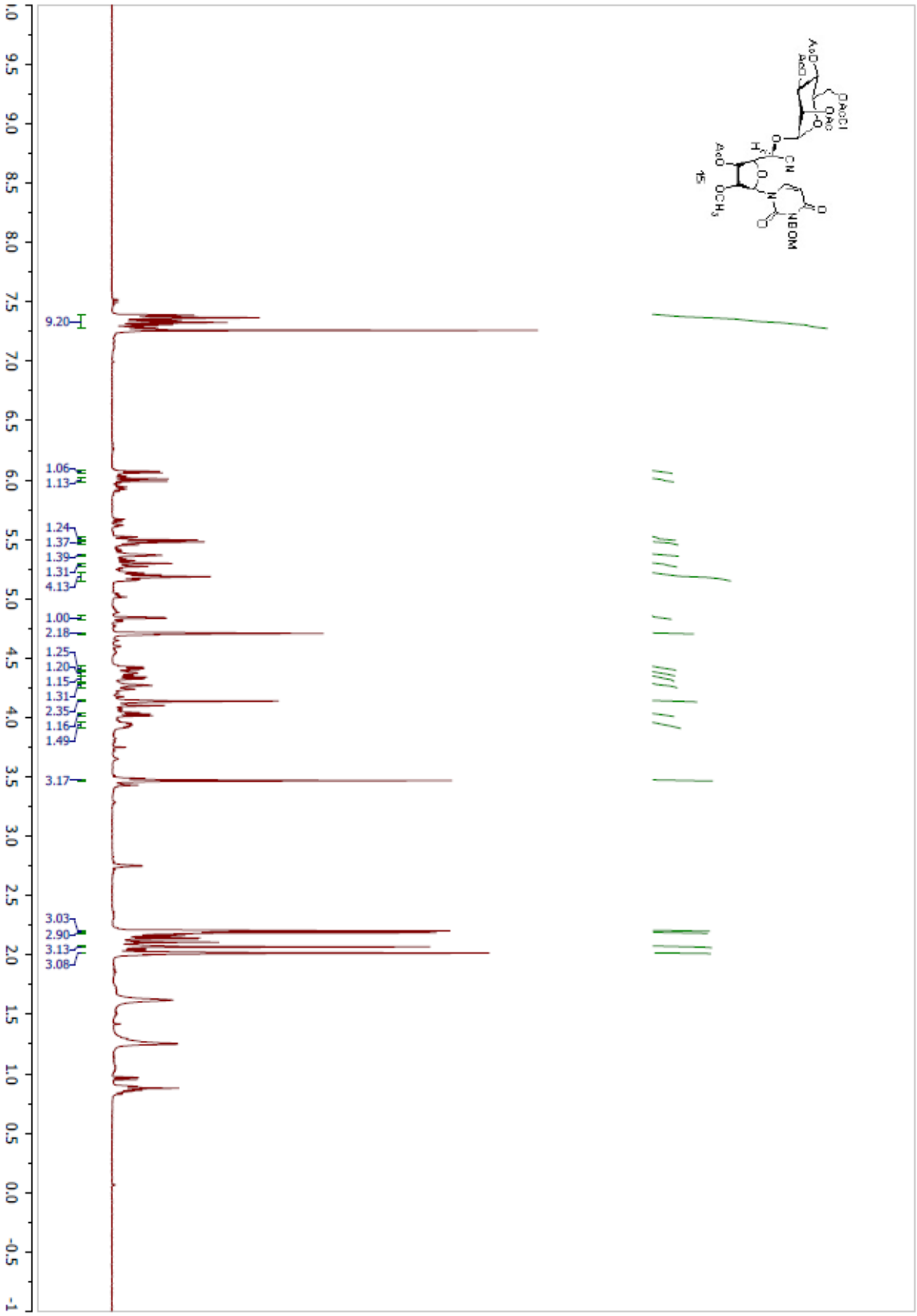
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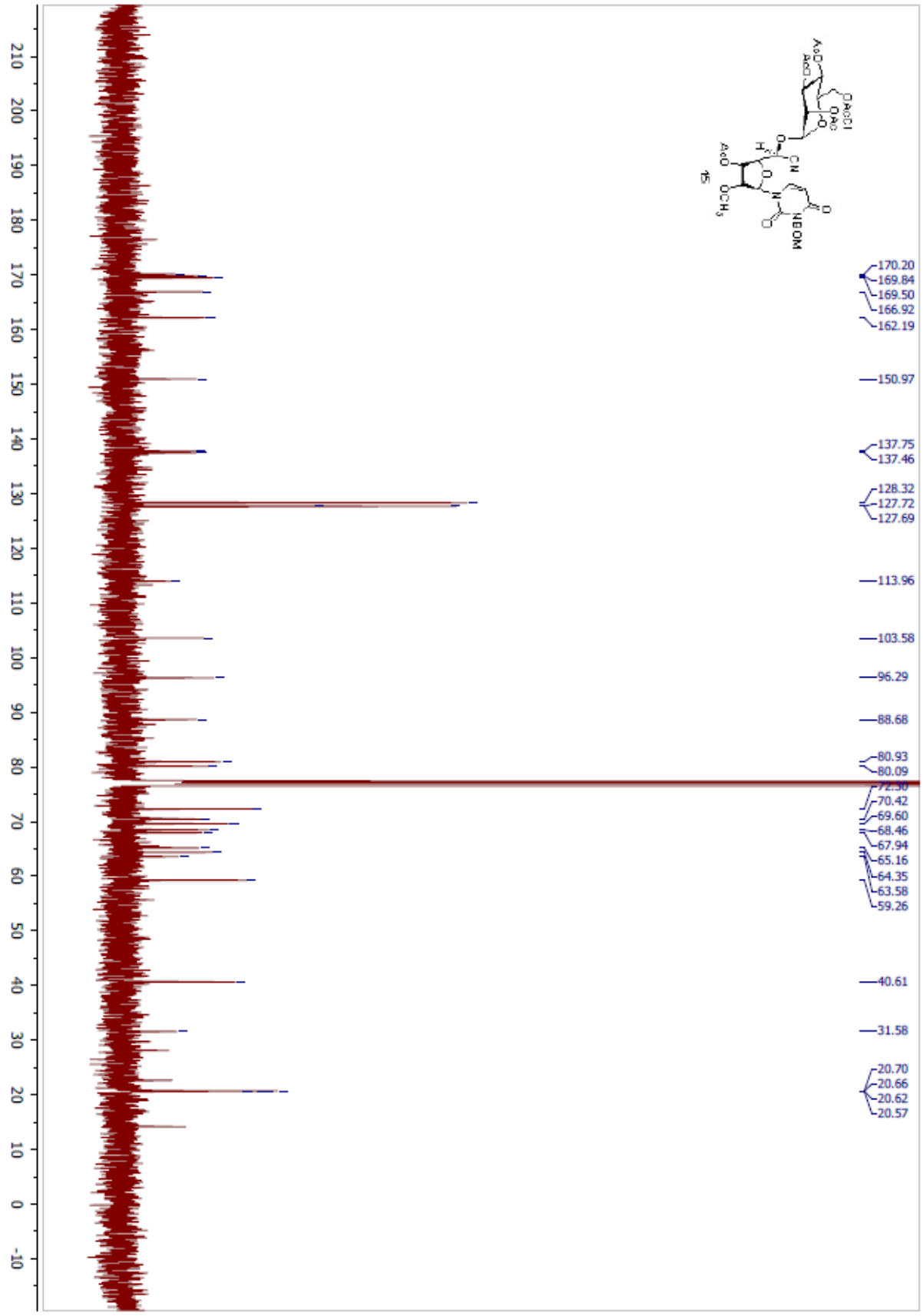
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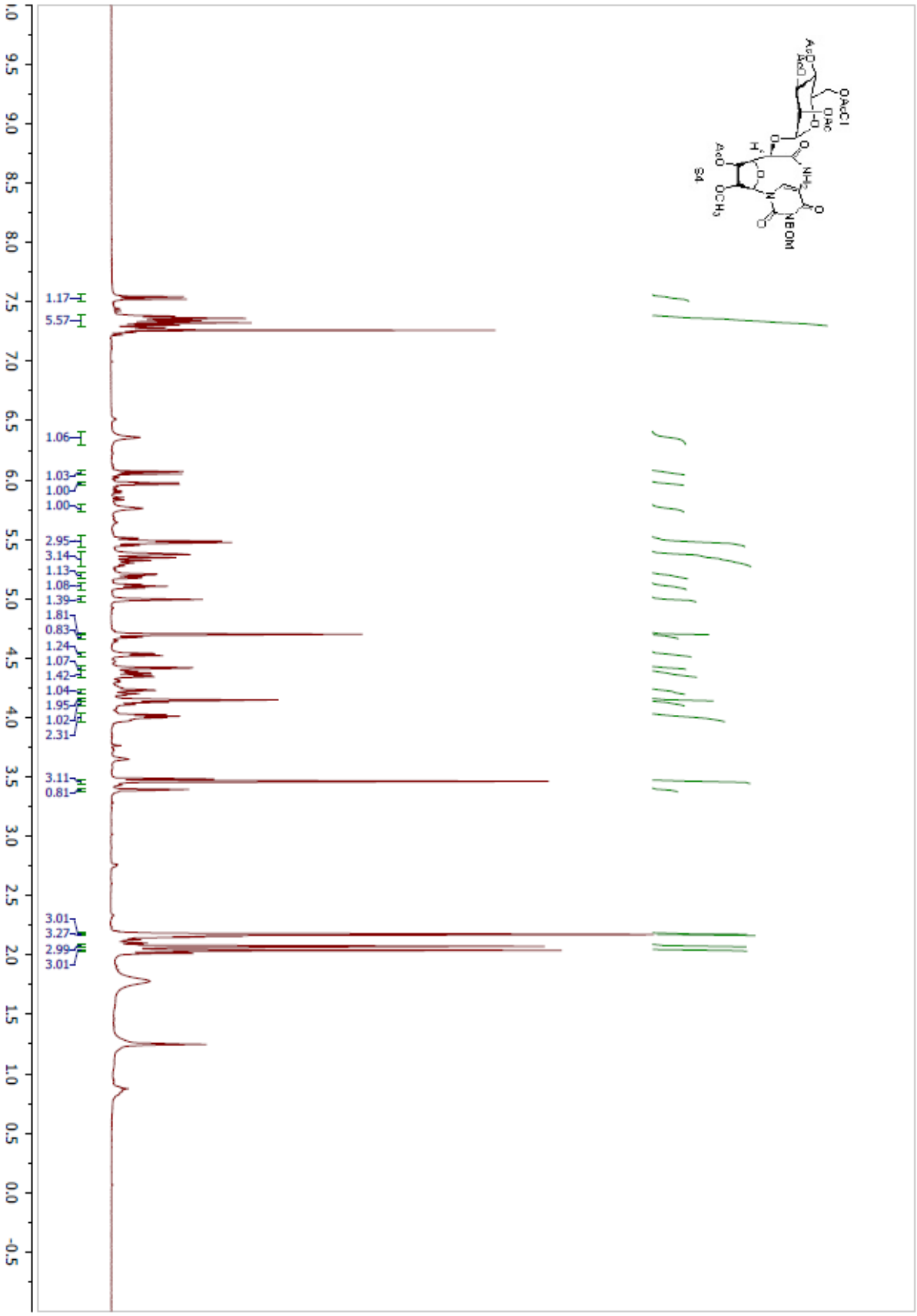
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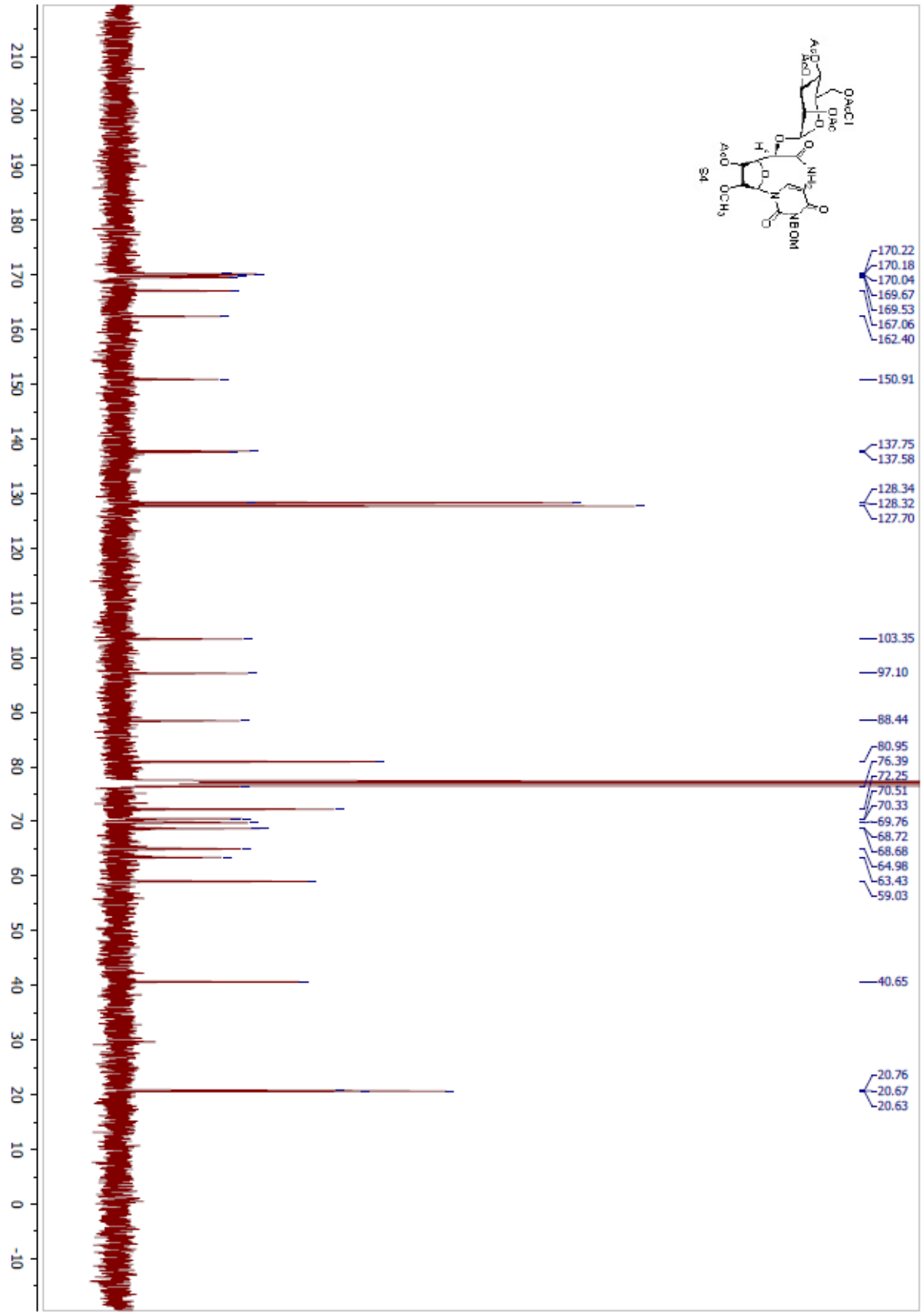
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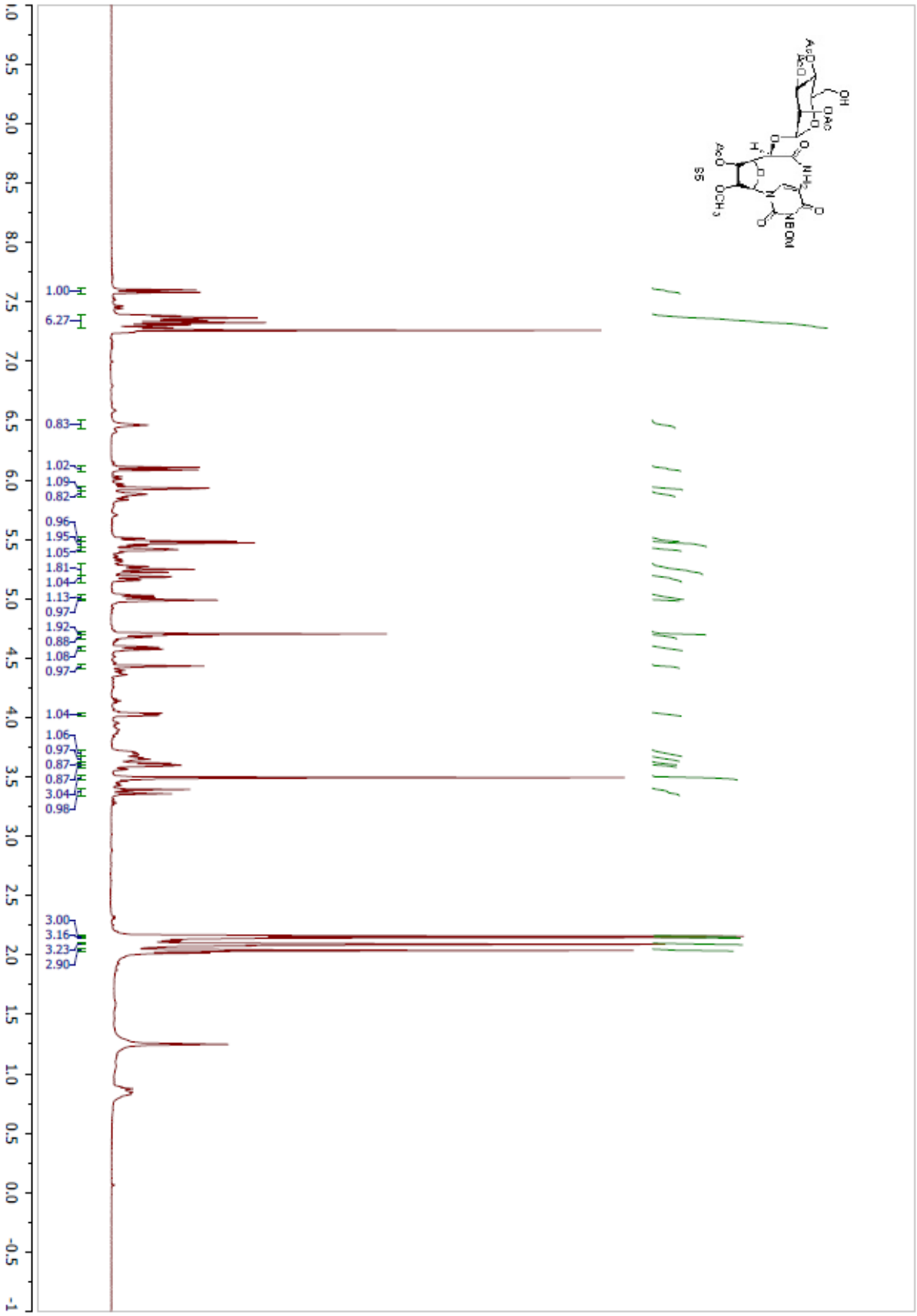
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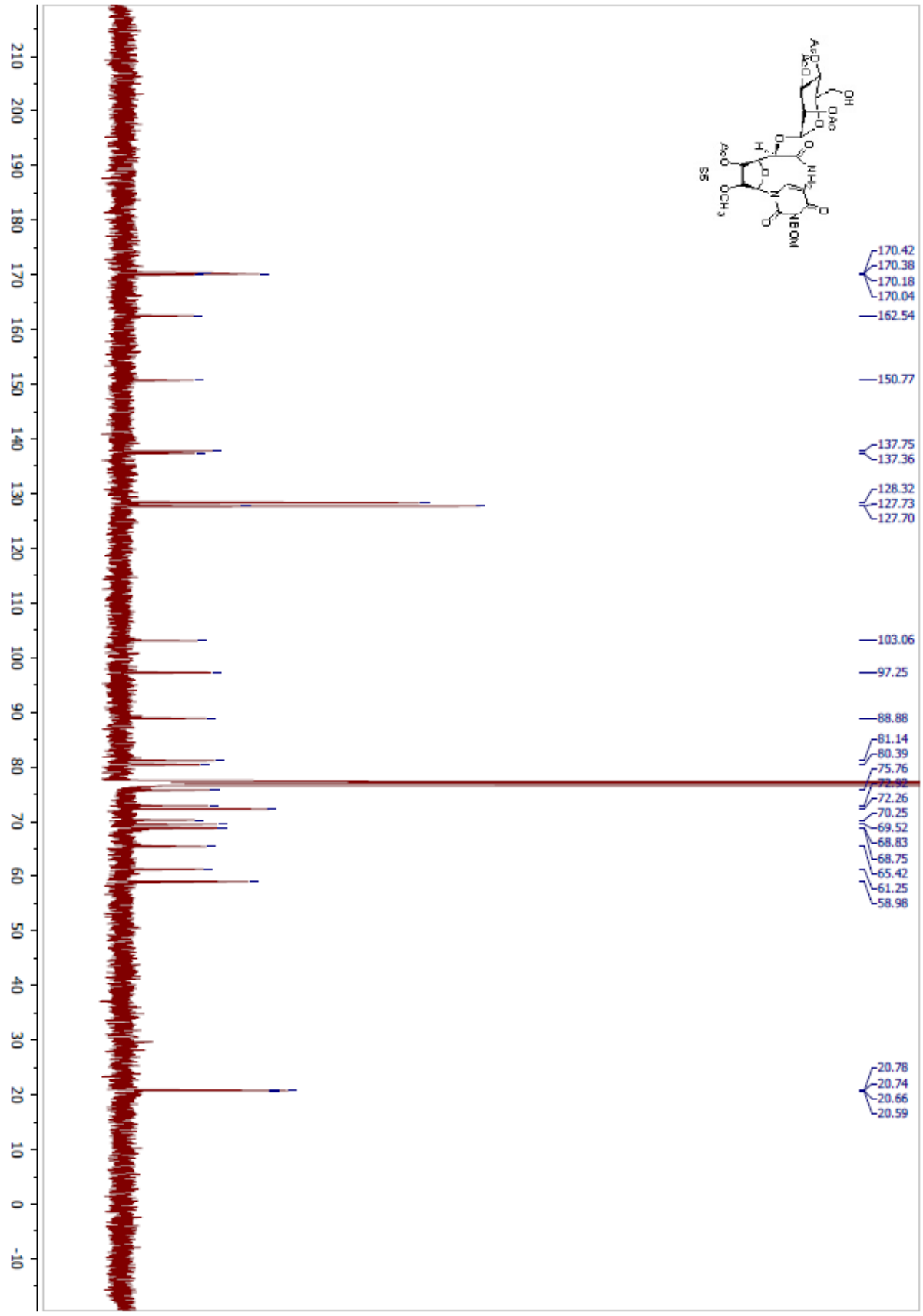
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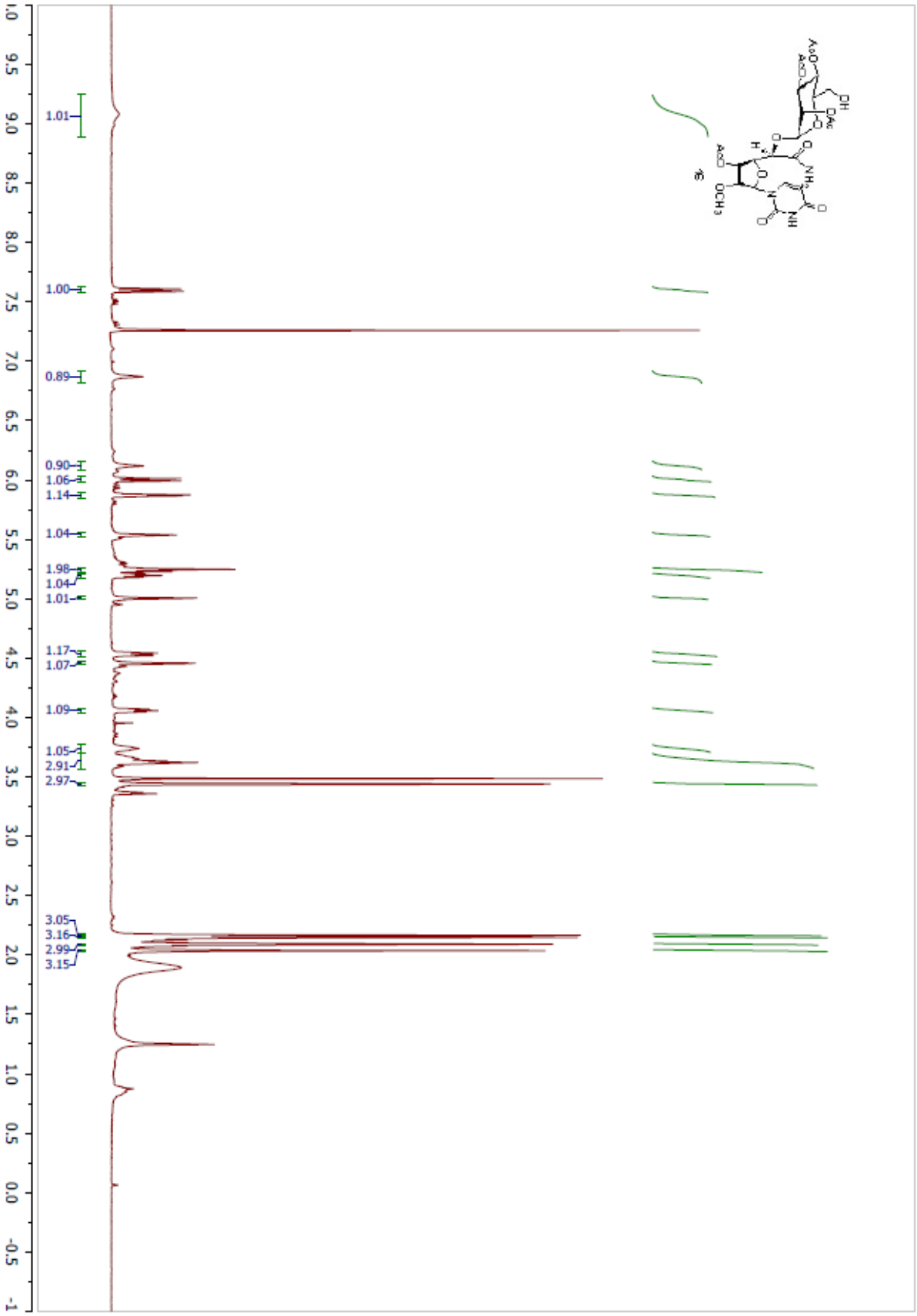
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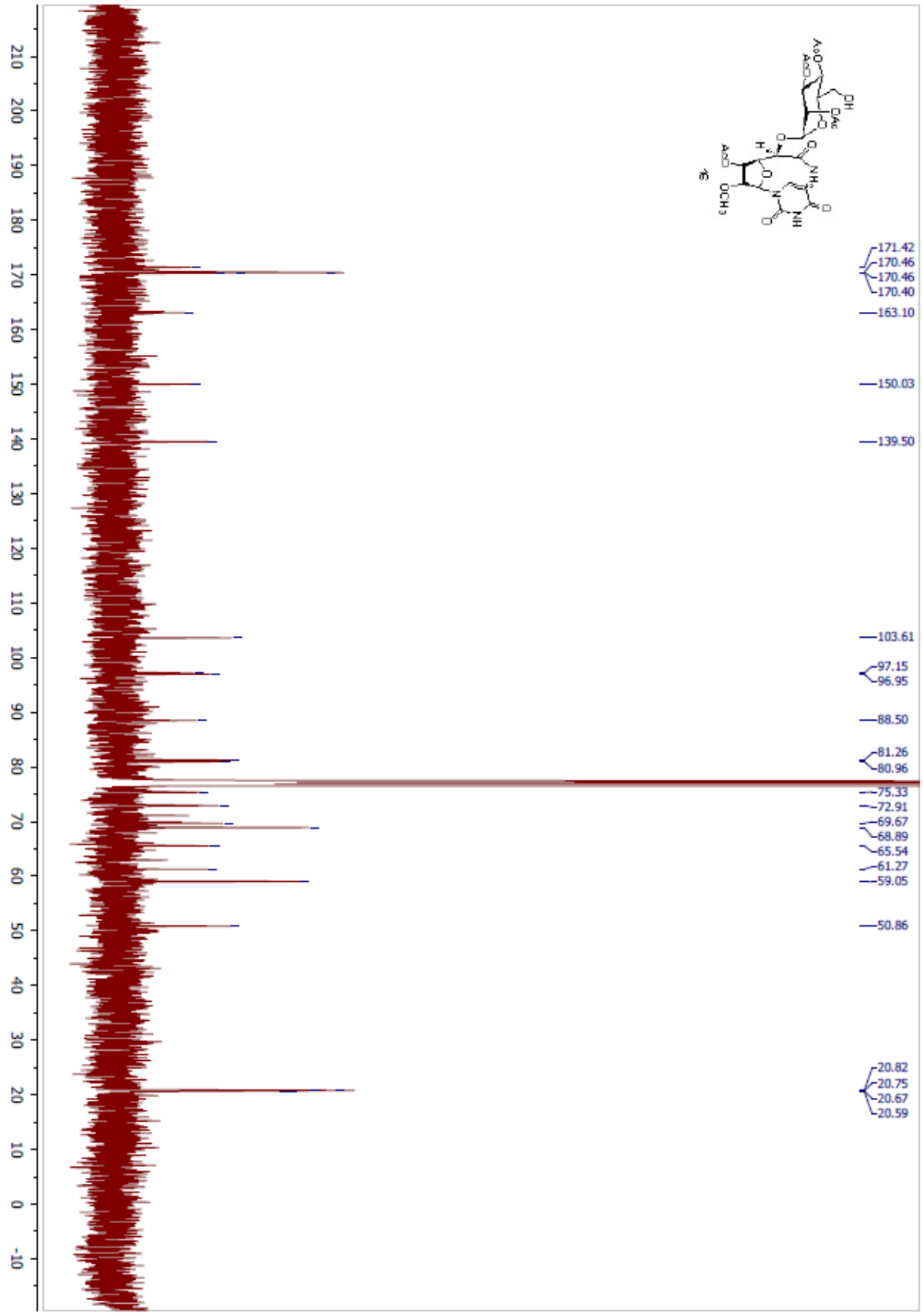
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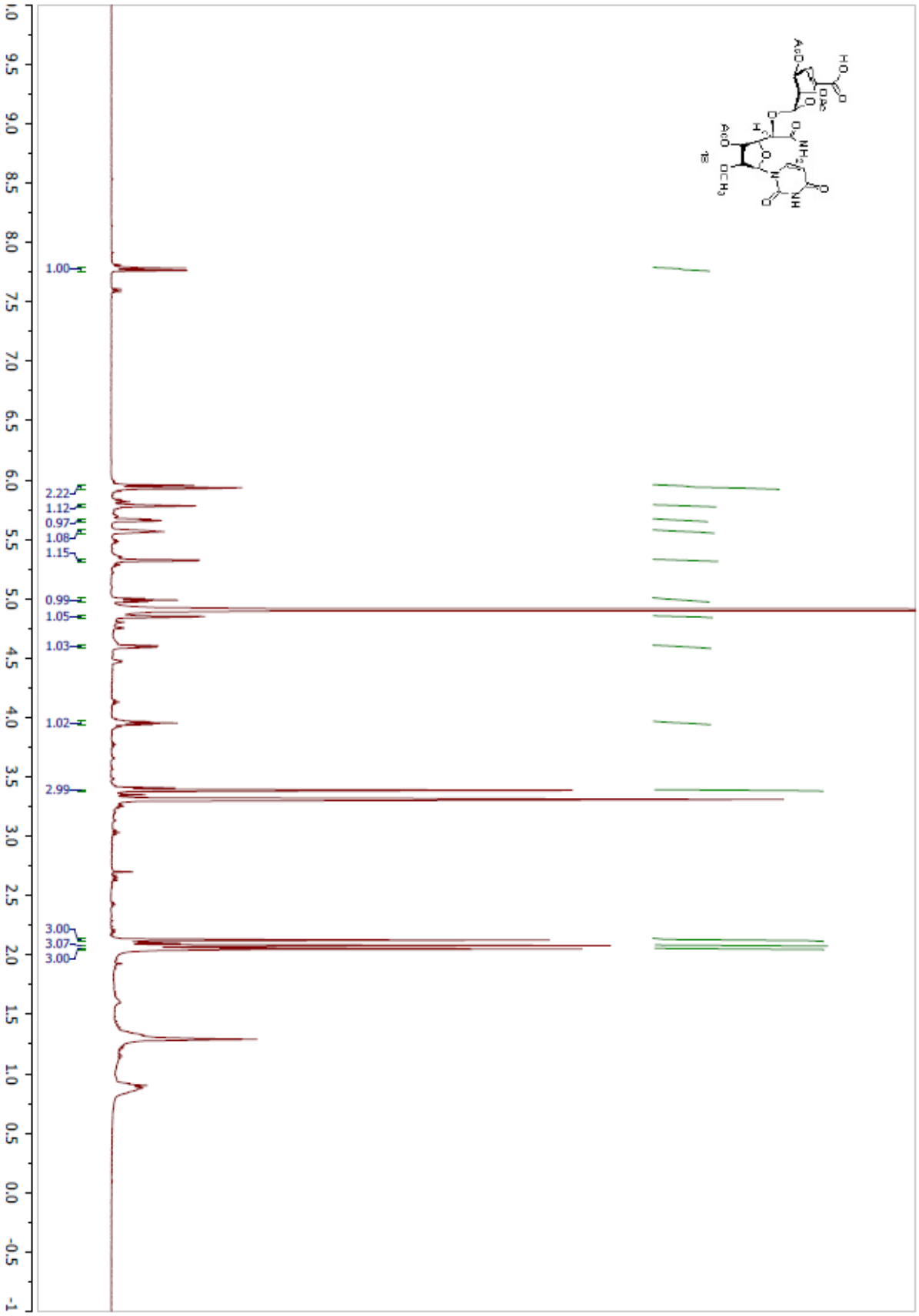
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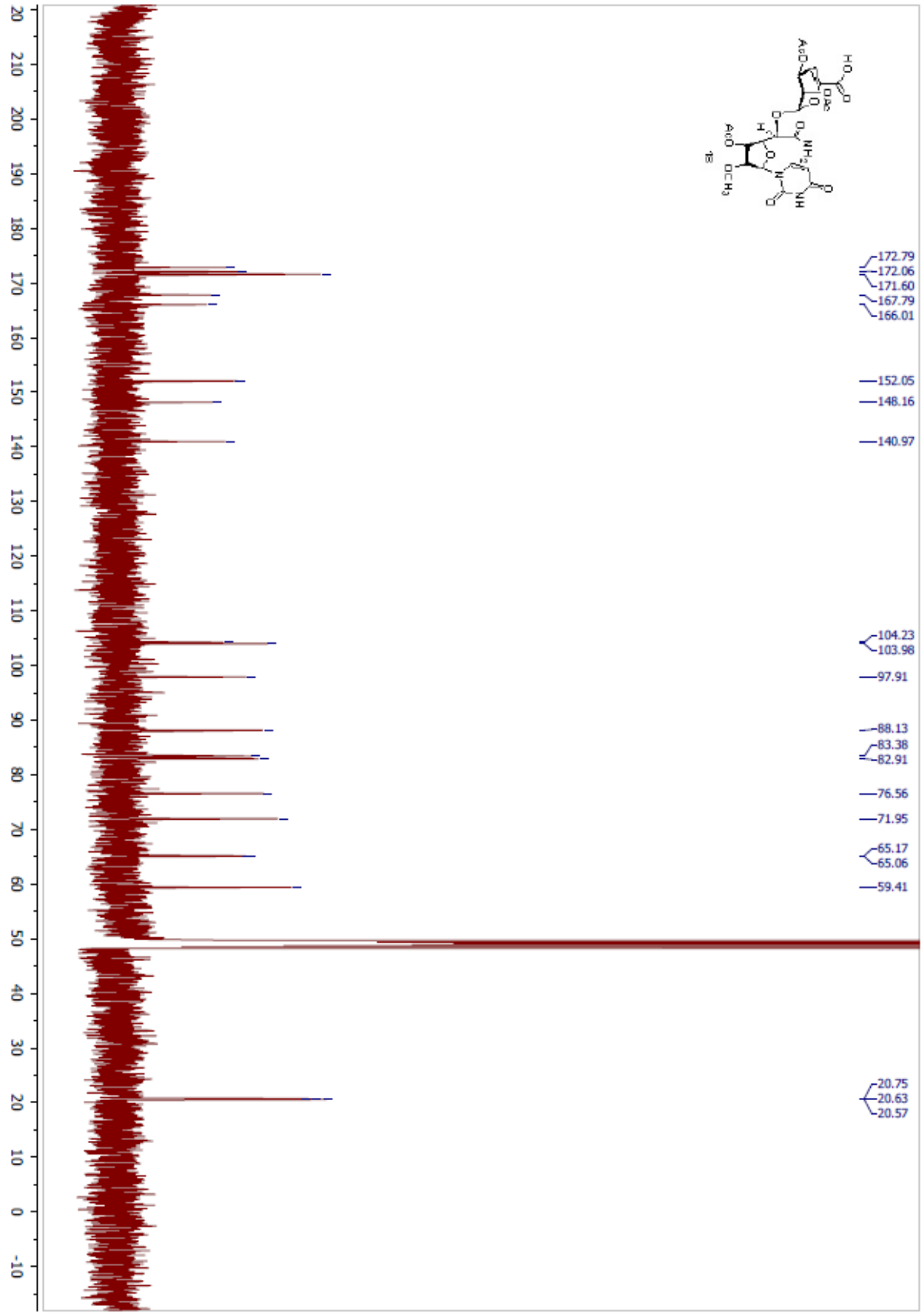
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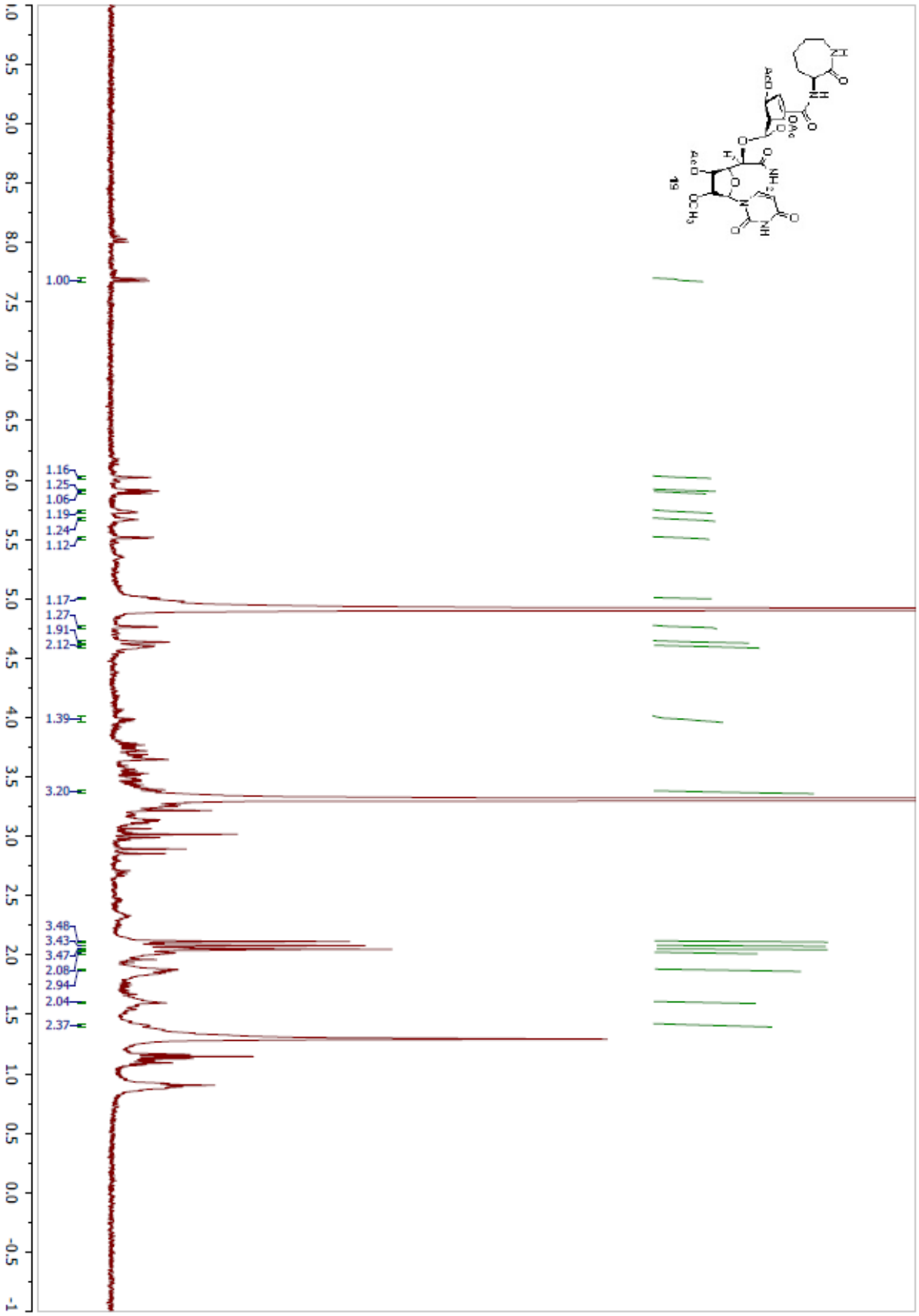
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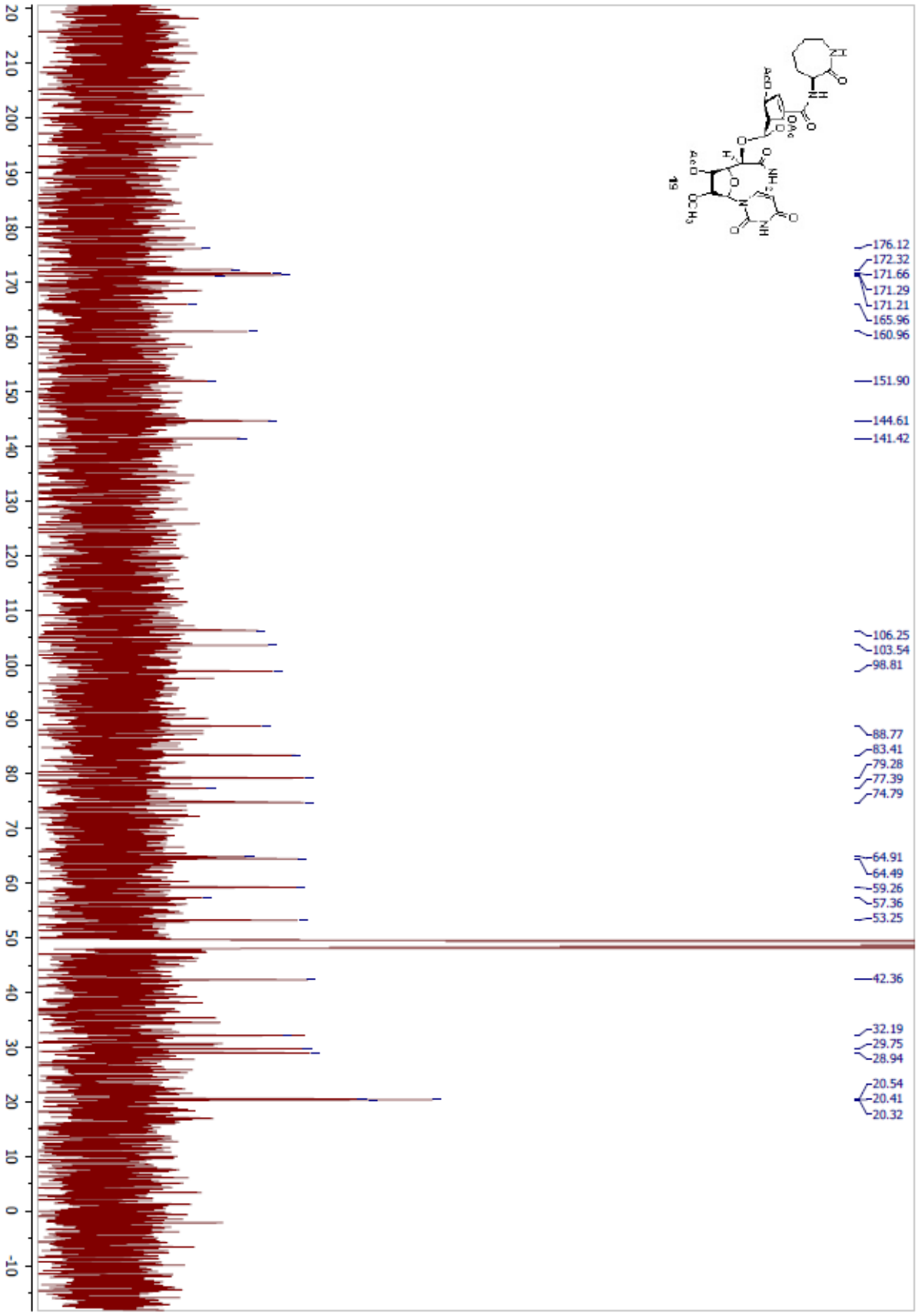
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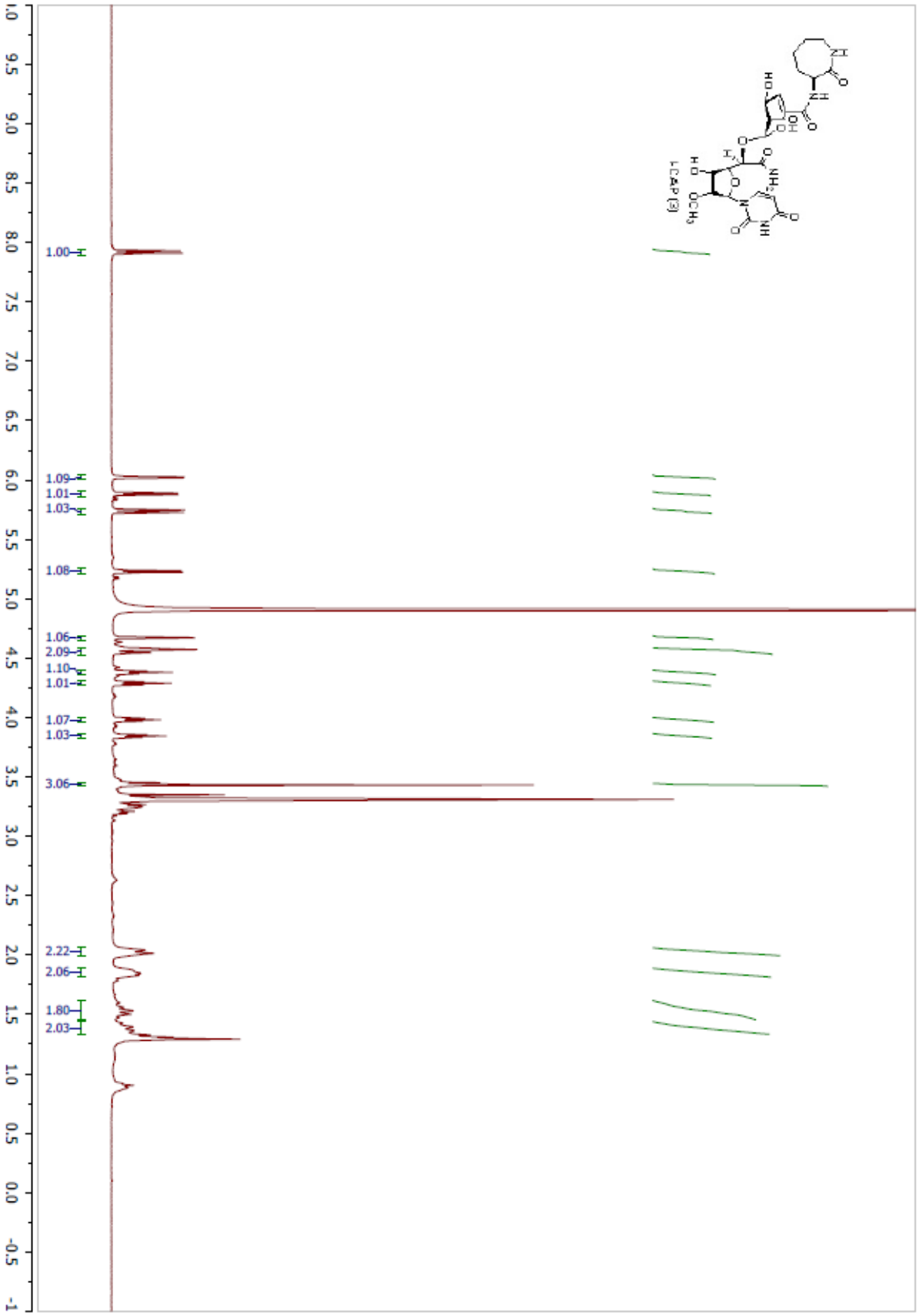
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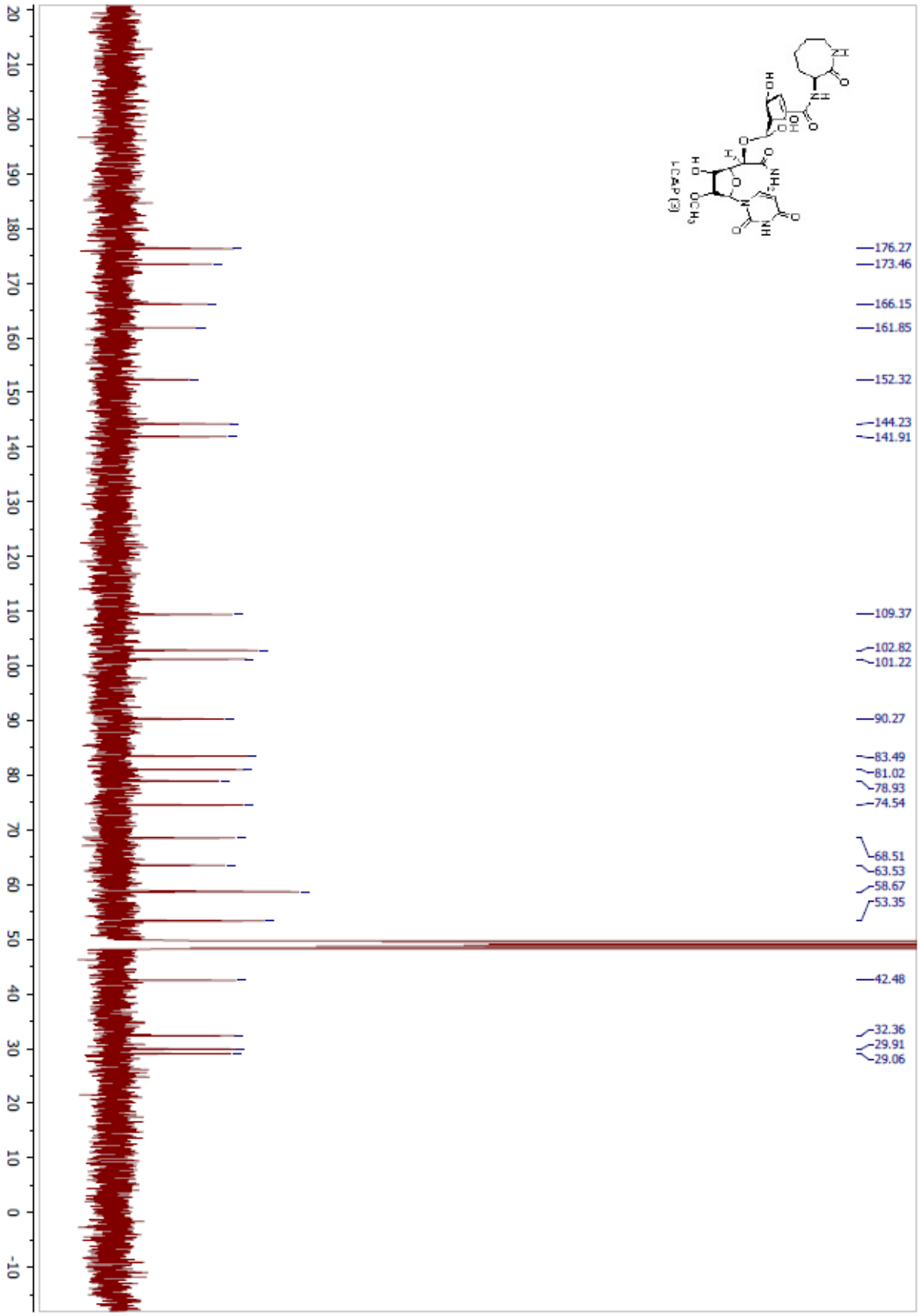
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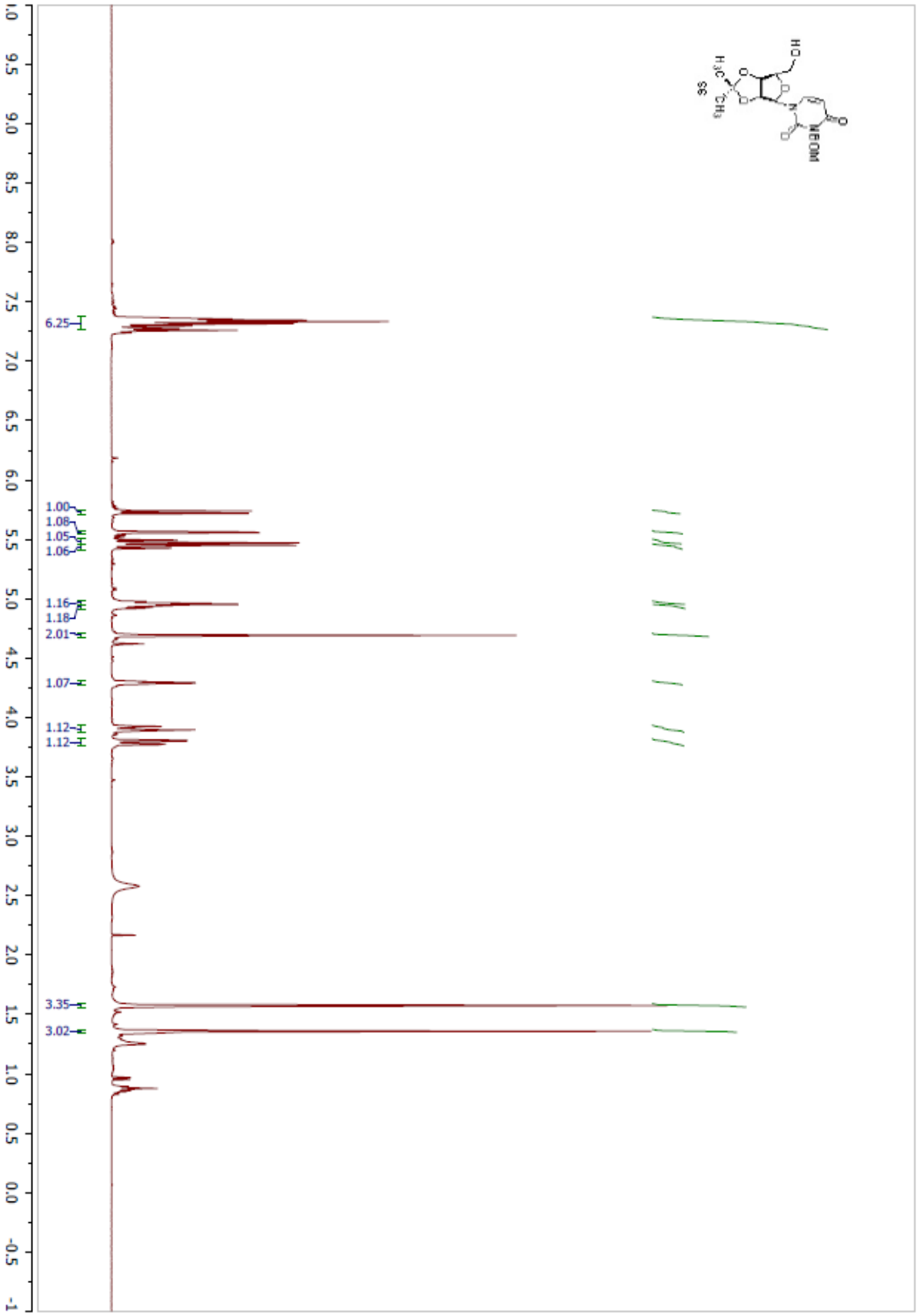
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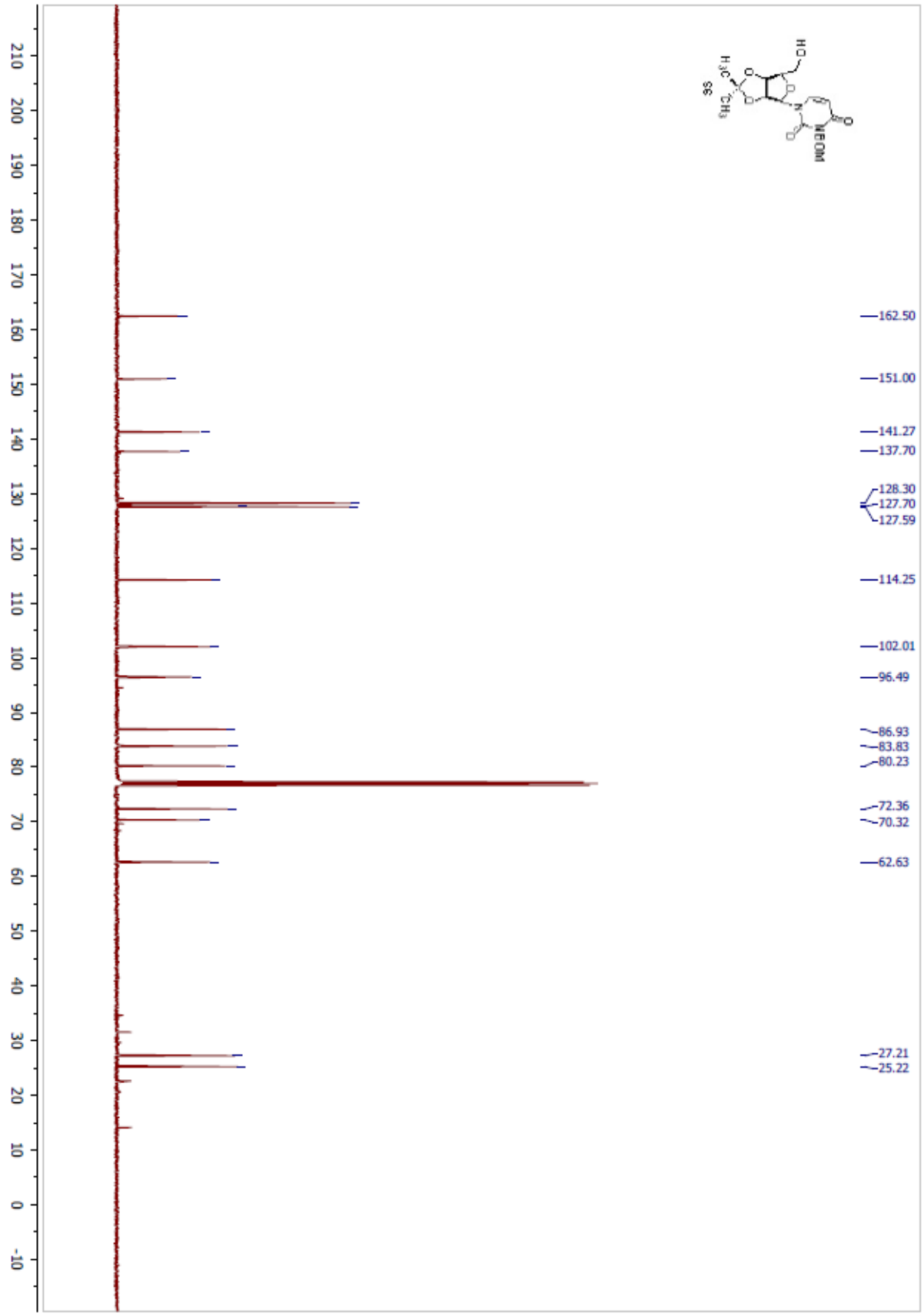
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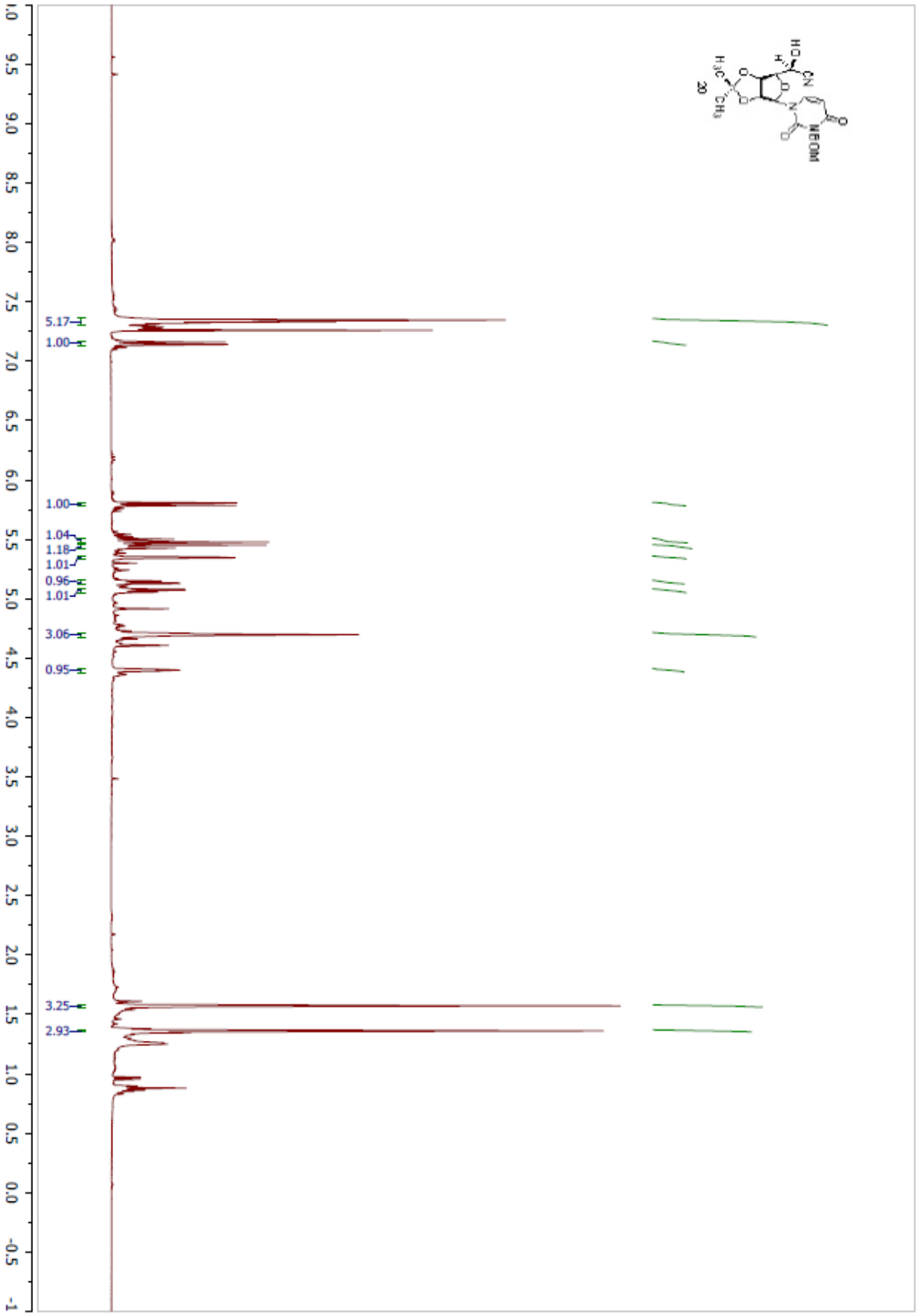
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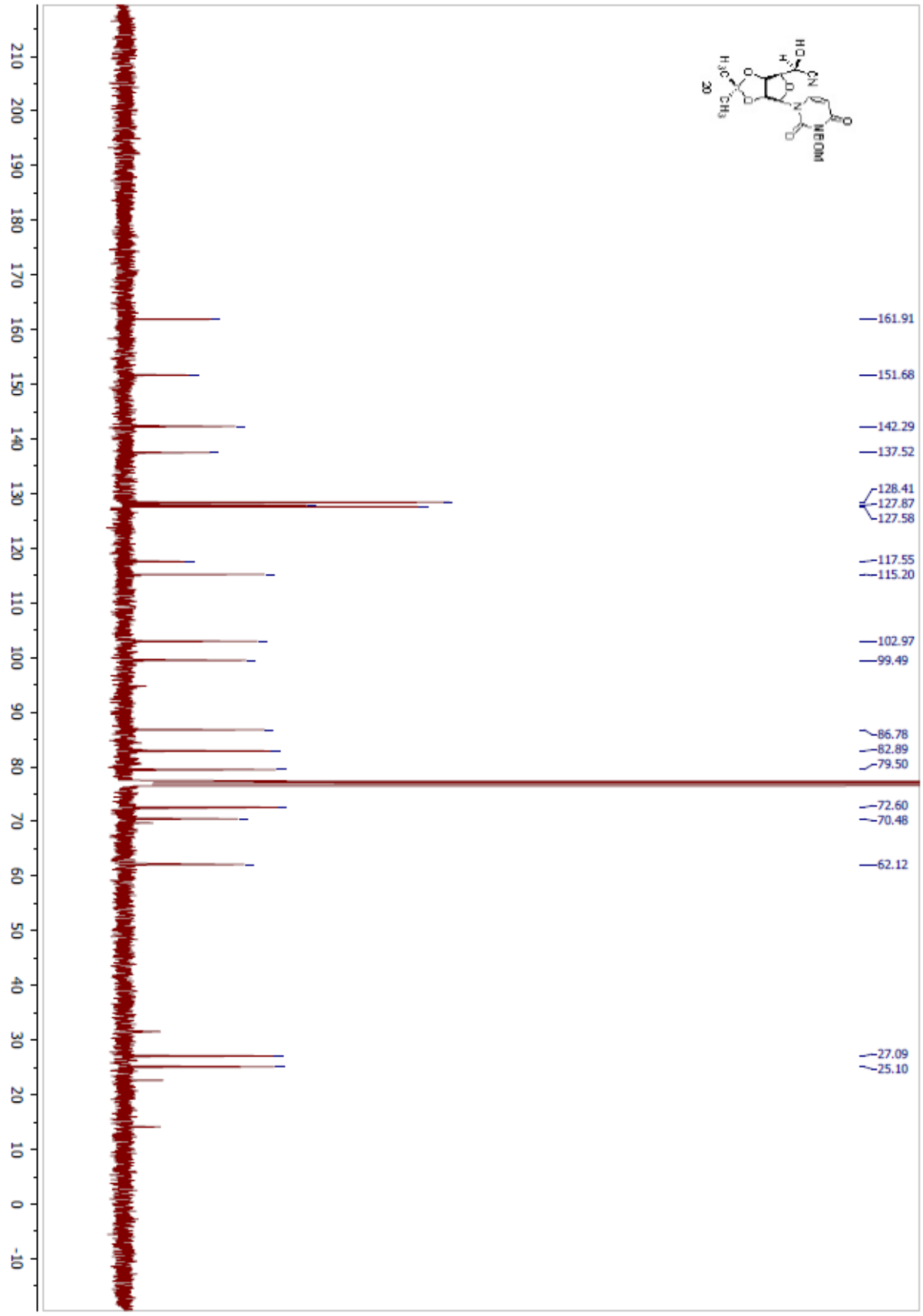
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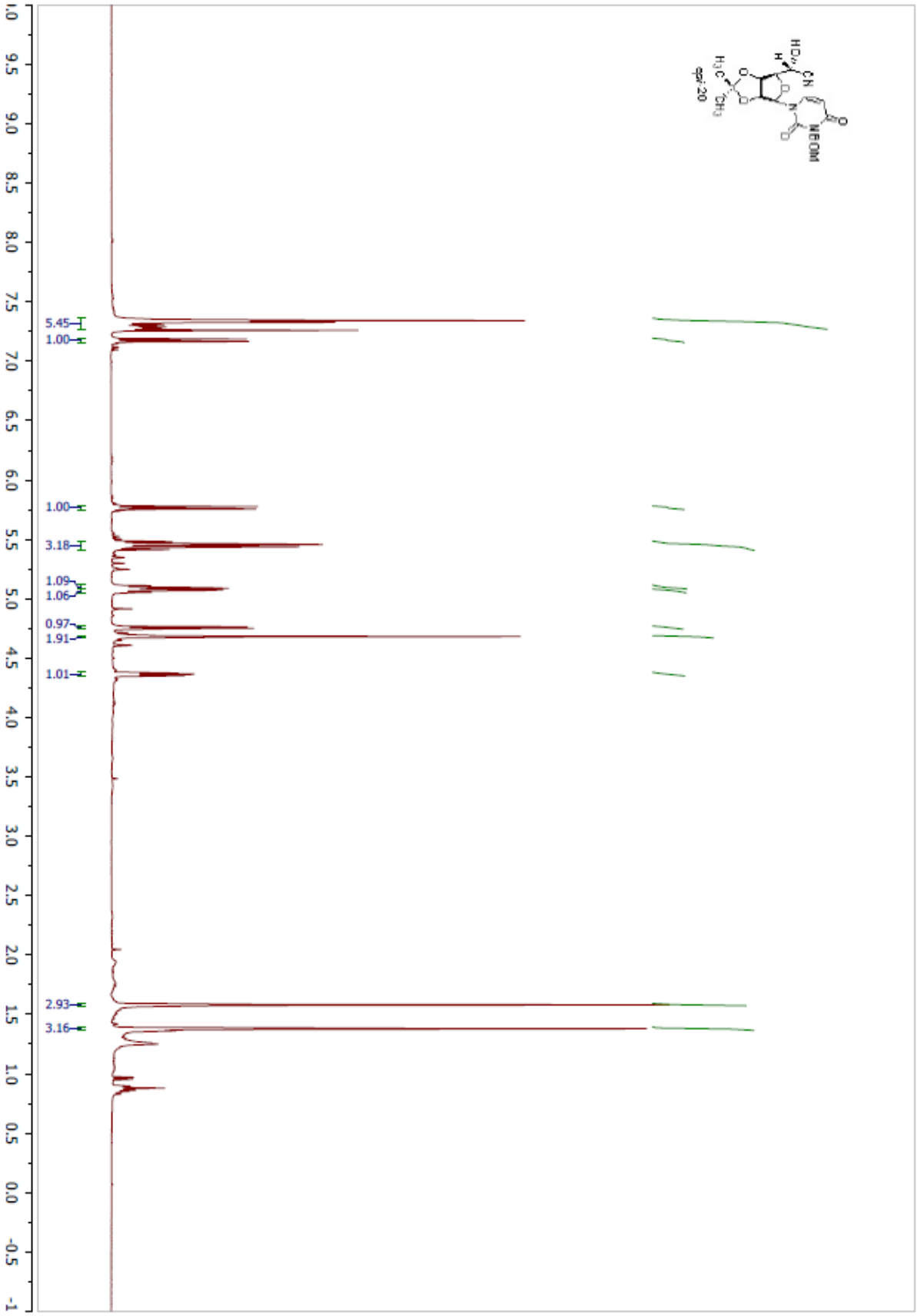
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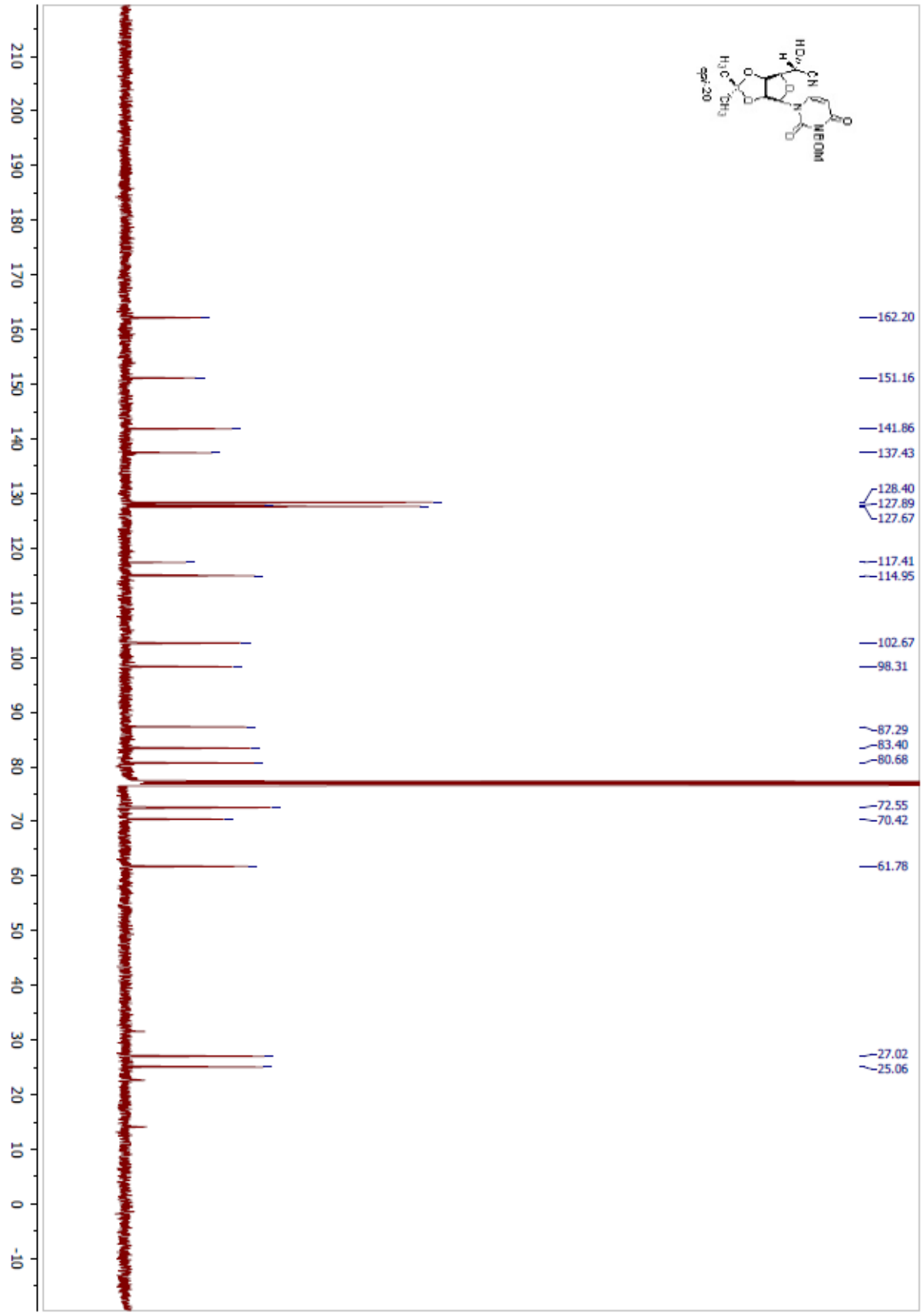
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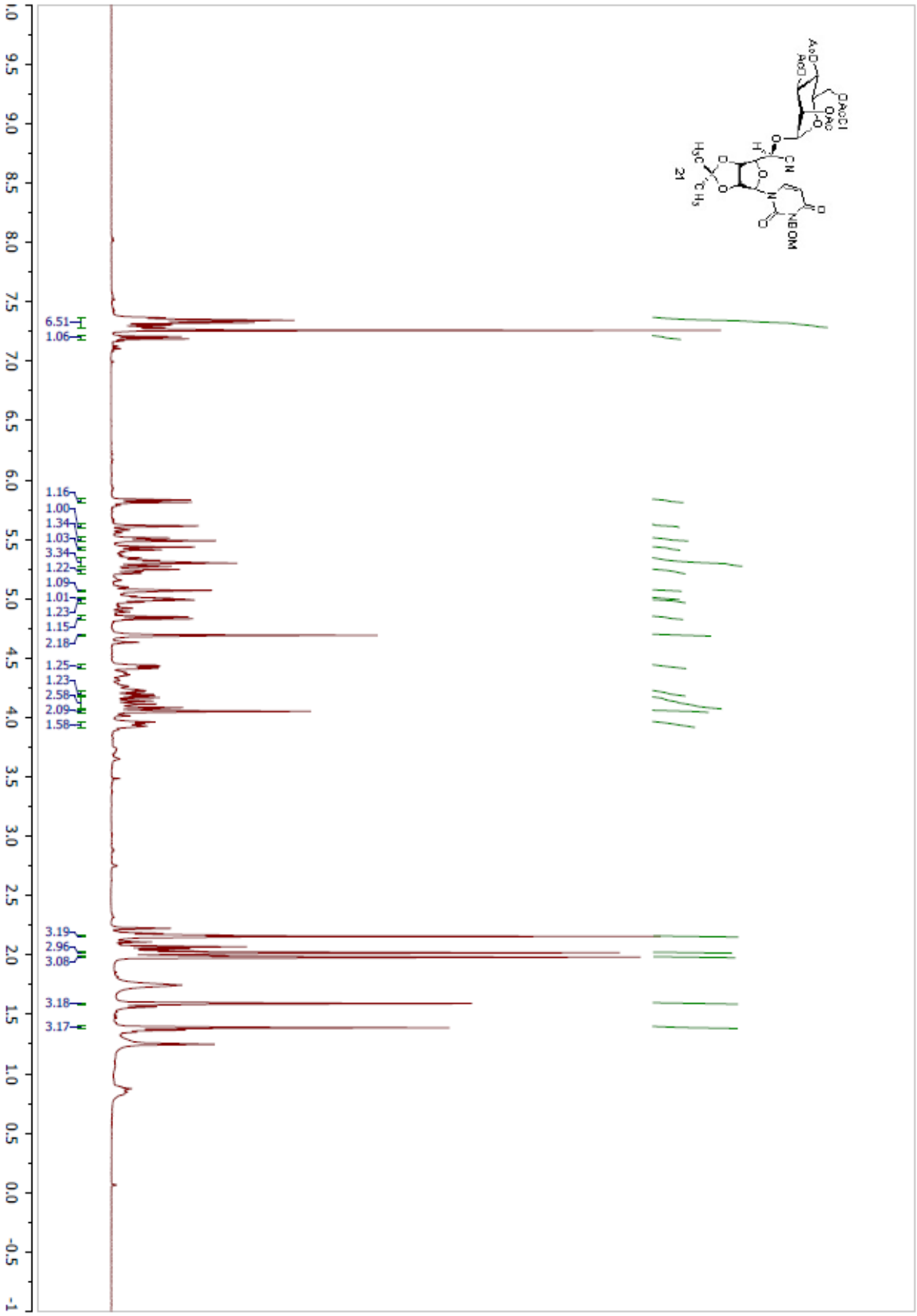
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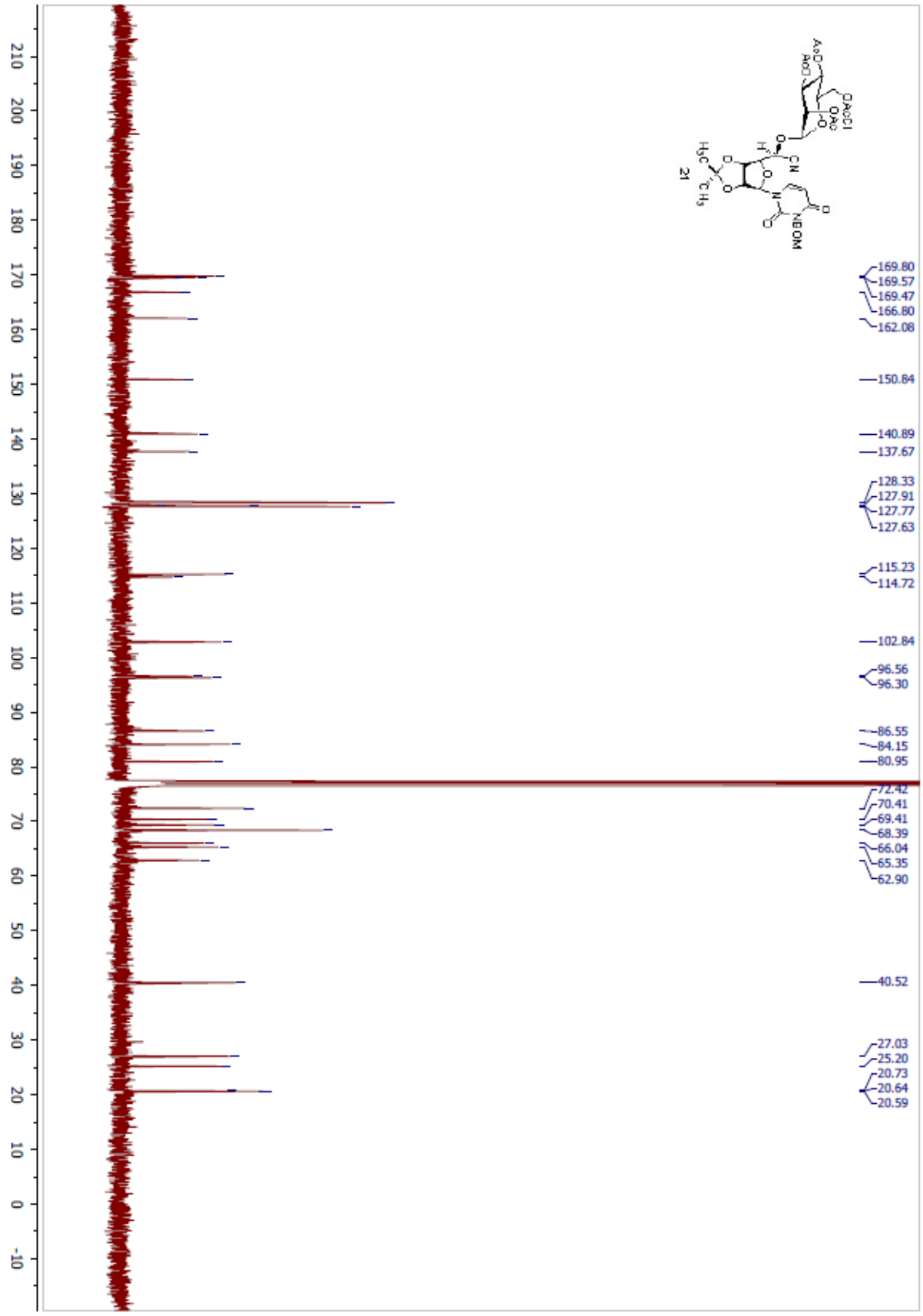
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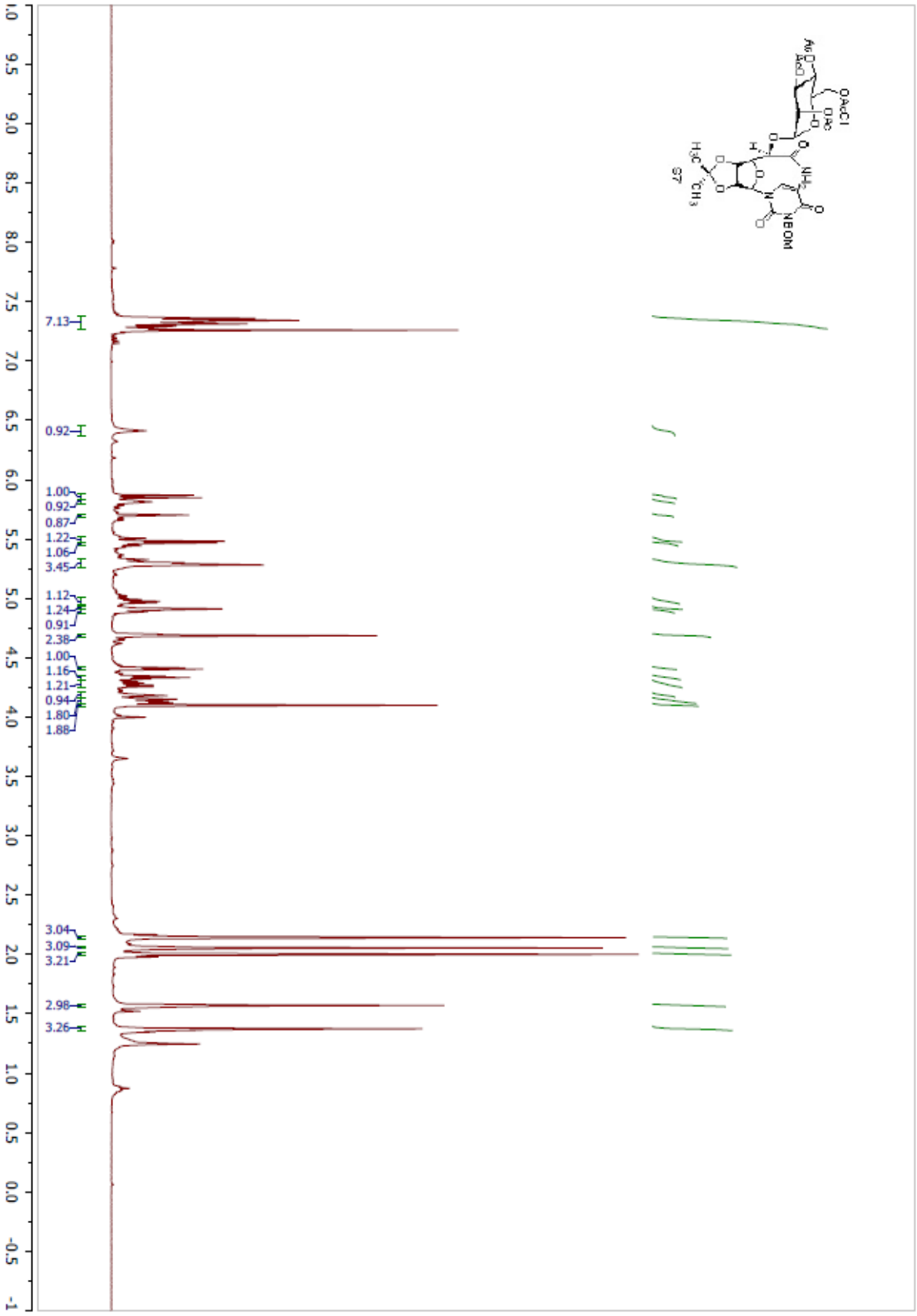
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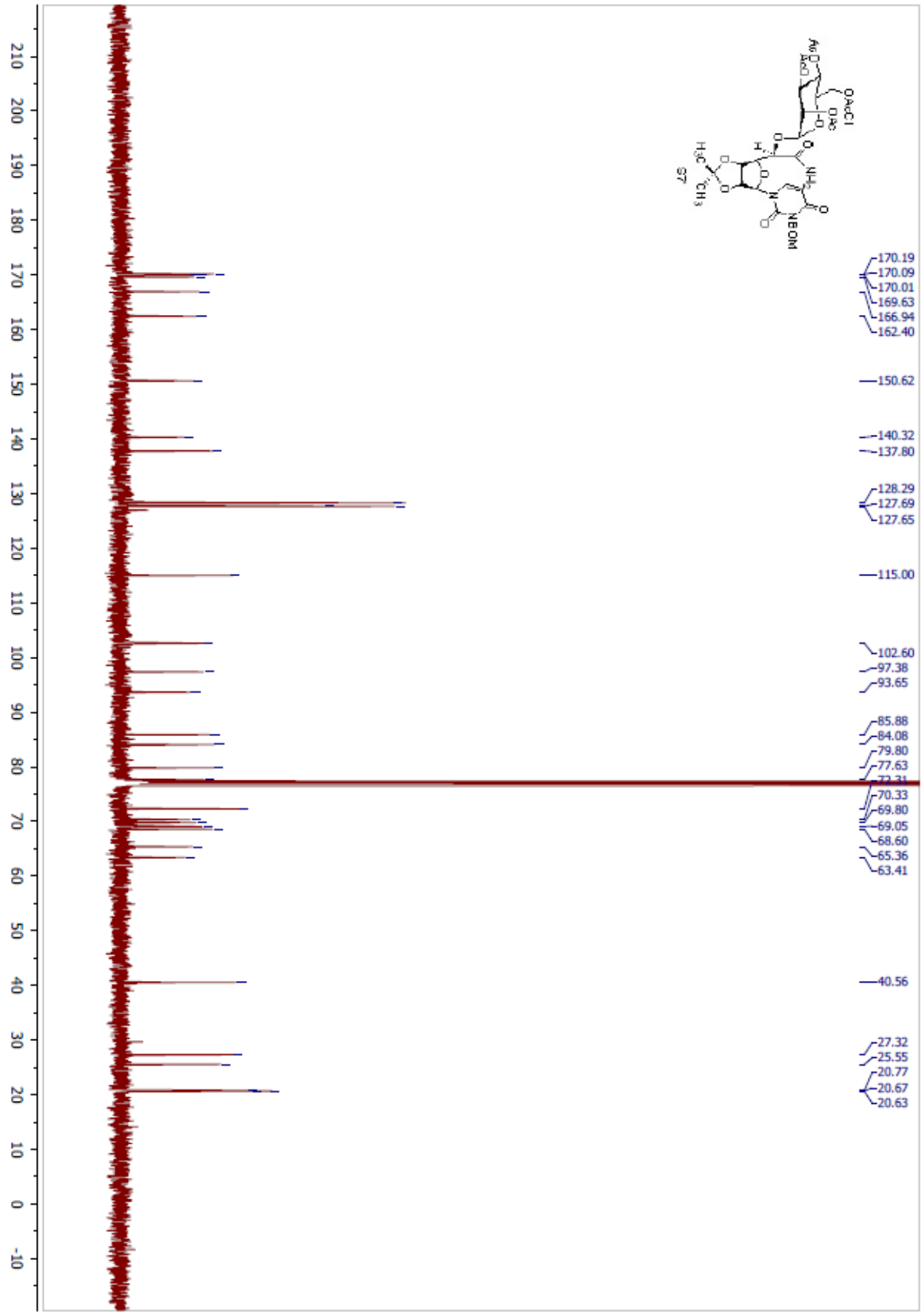
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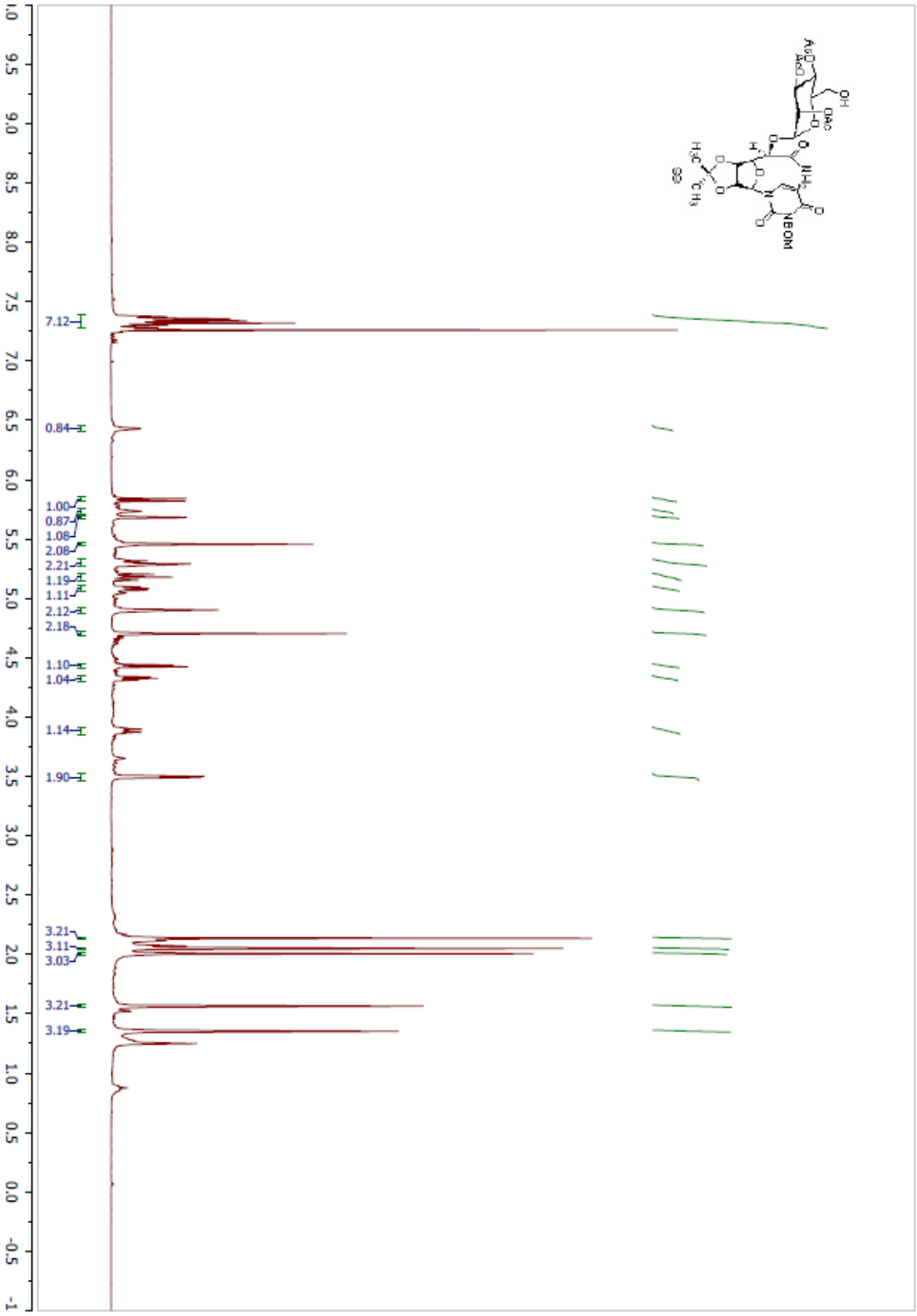
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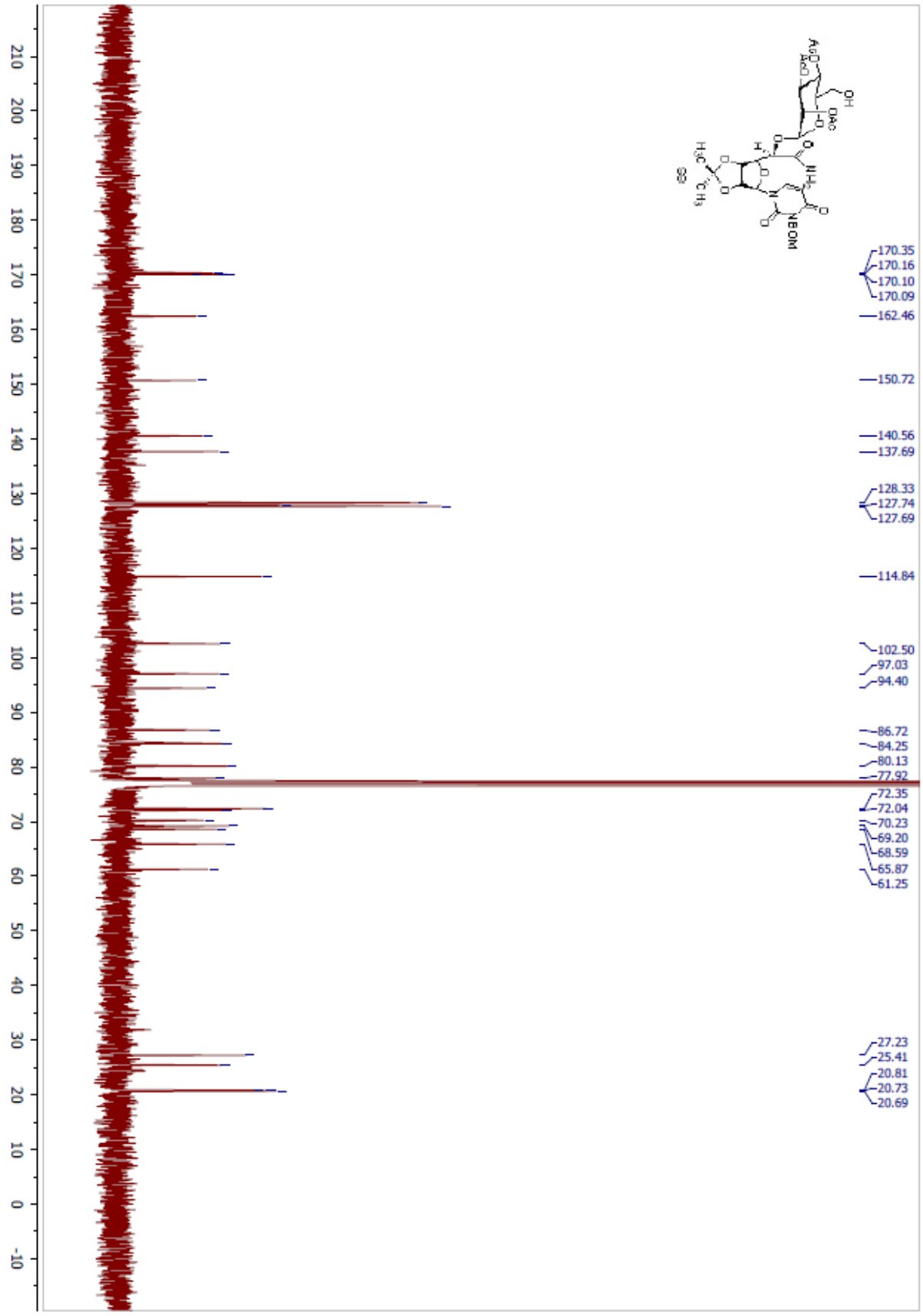
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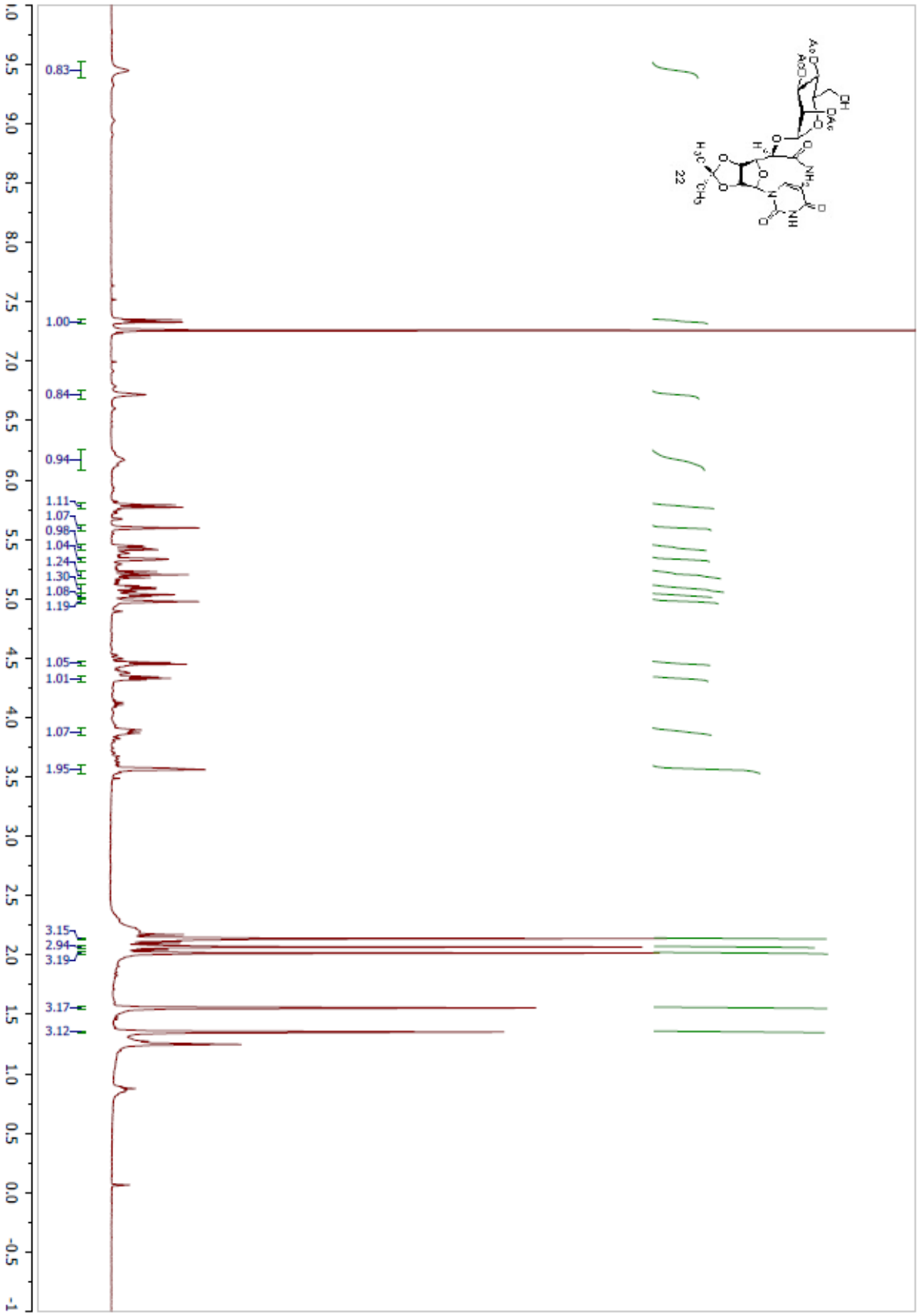
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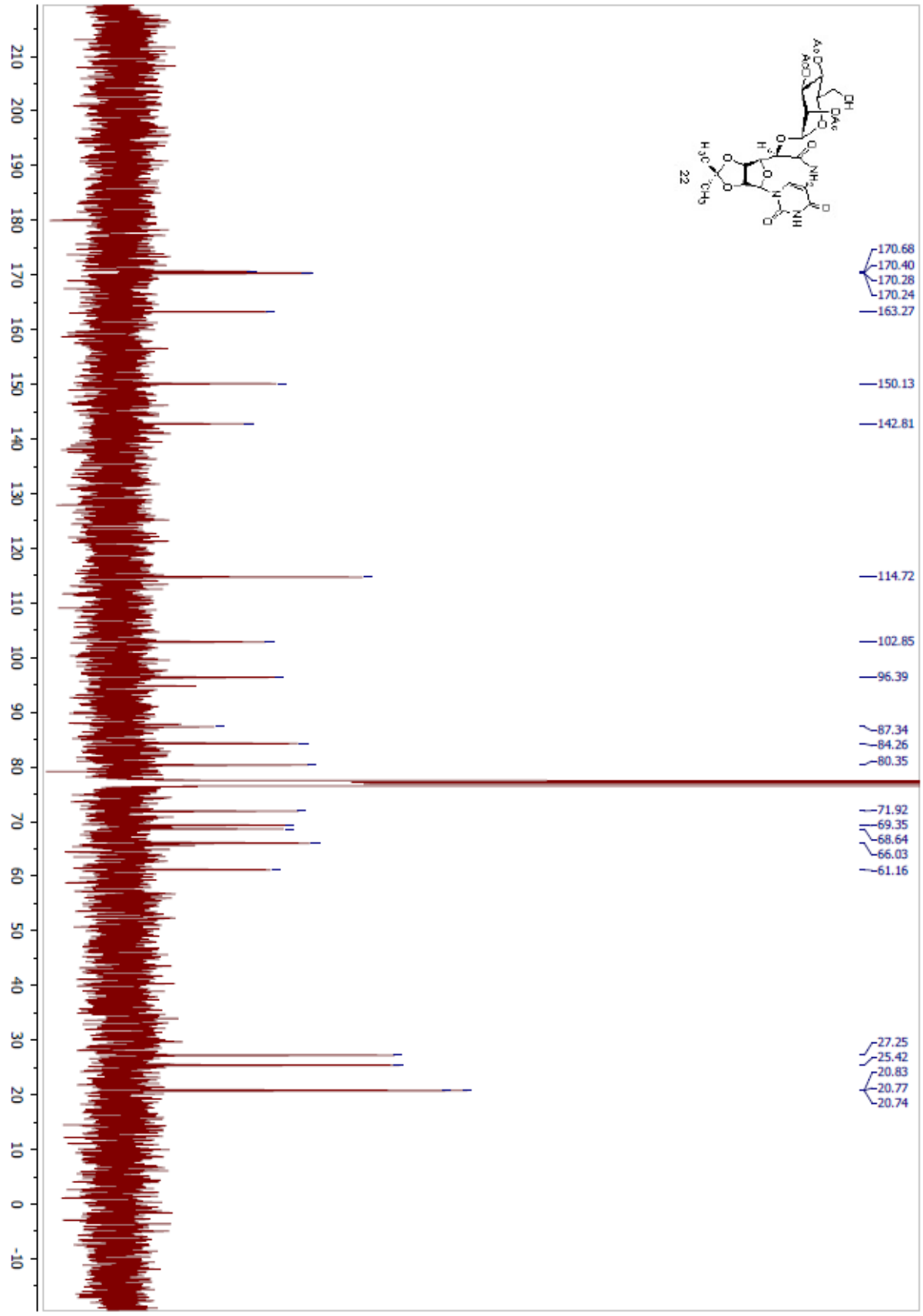
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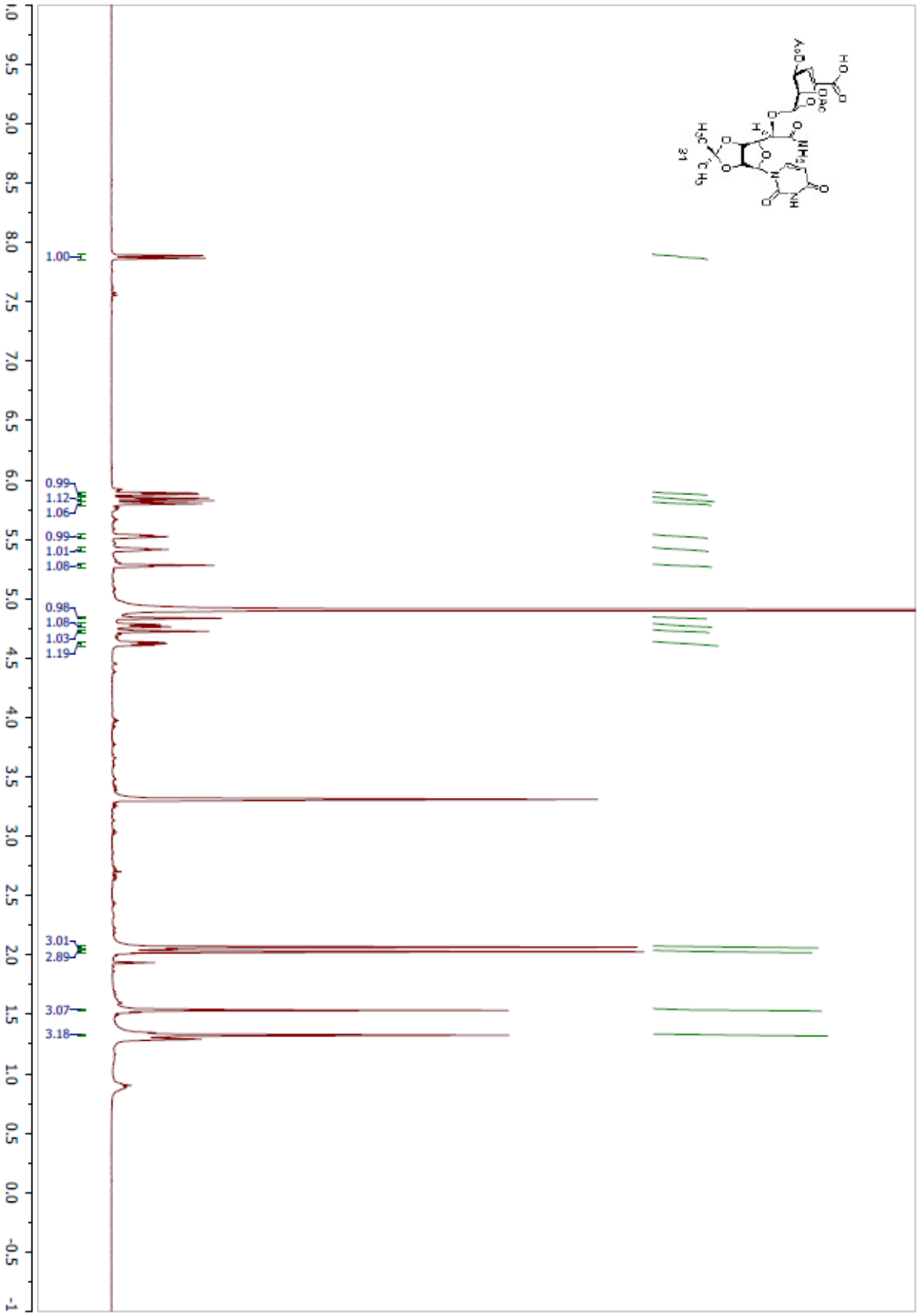
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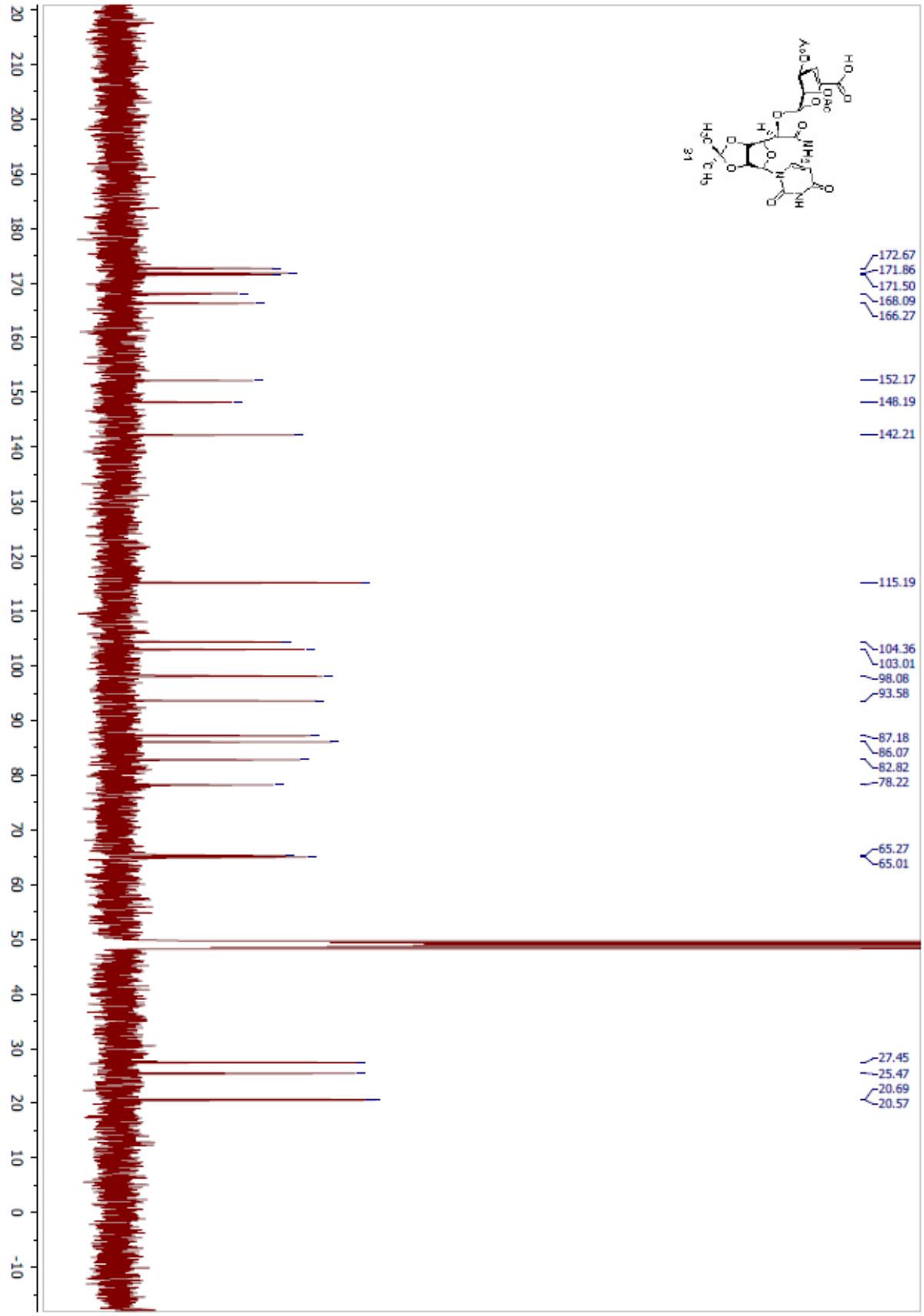
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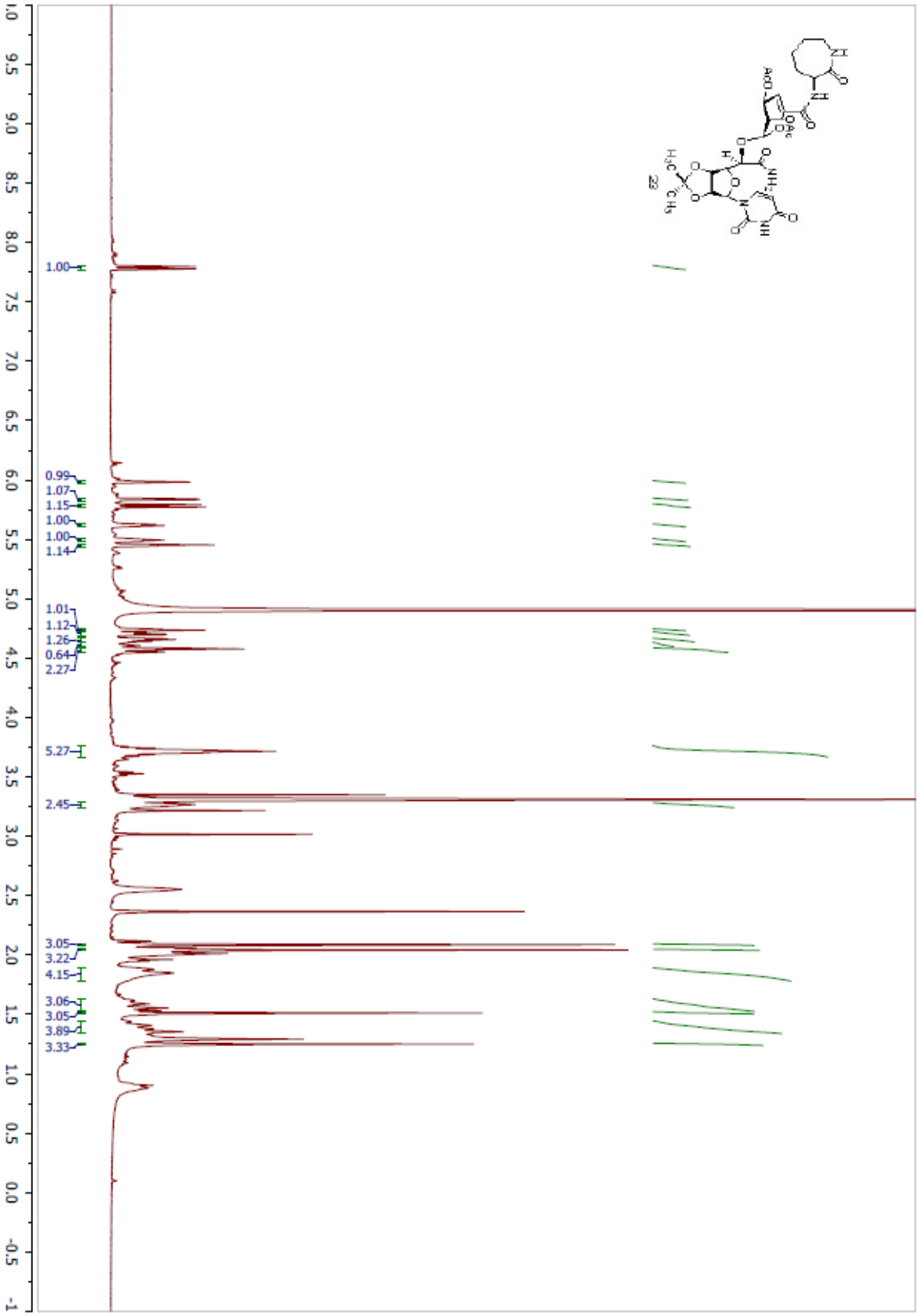
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4.15
3.06
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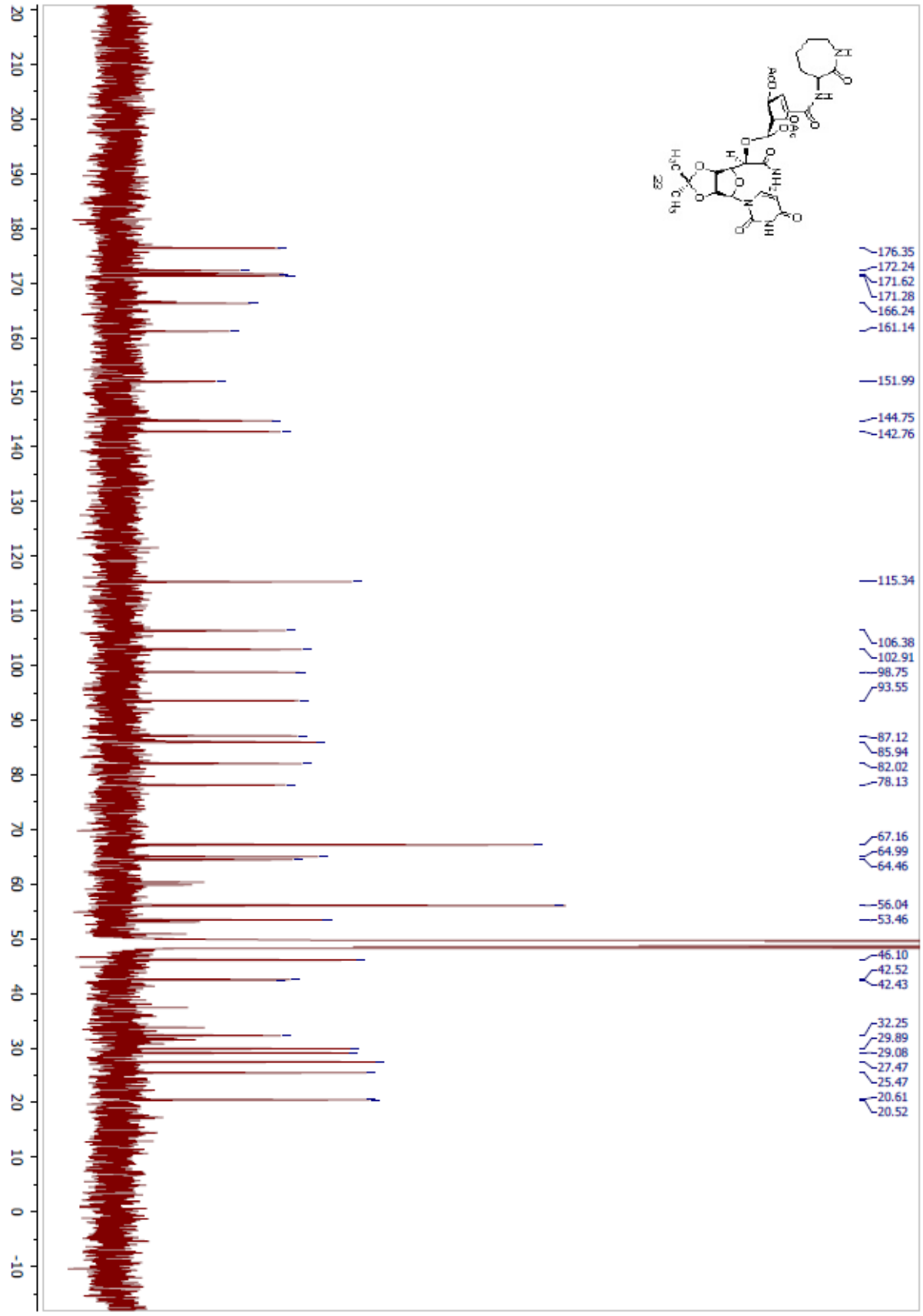
3.33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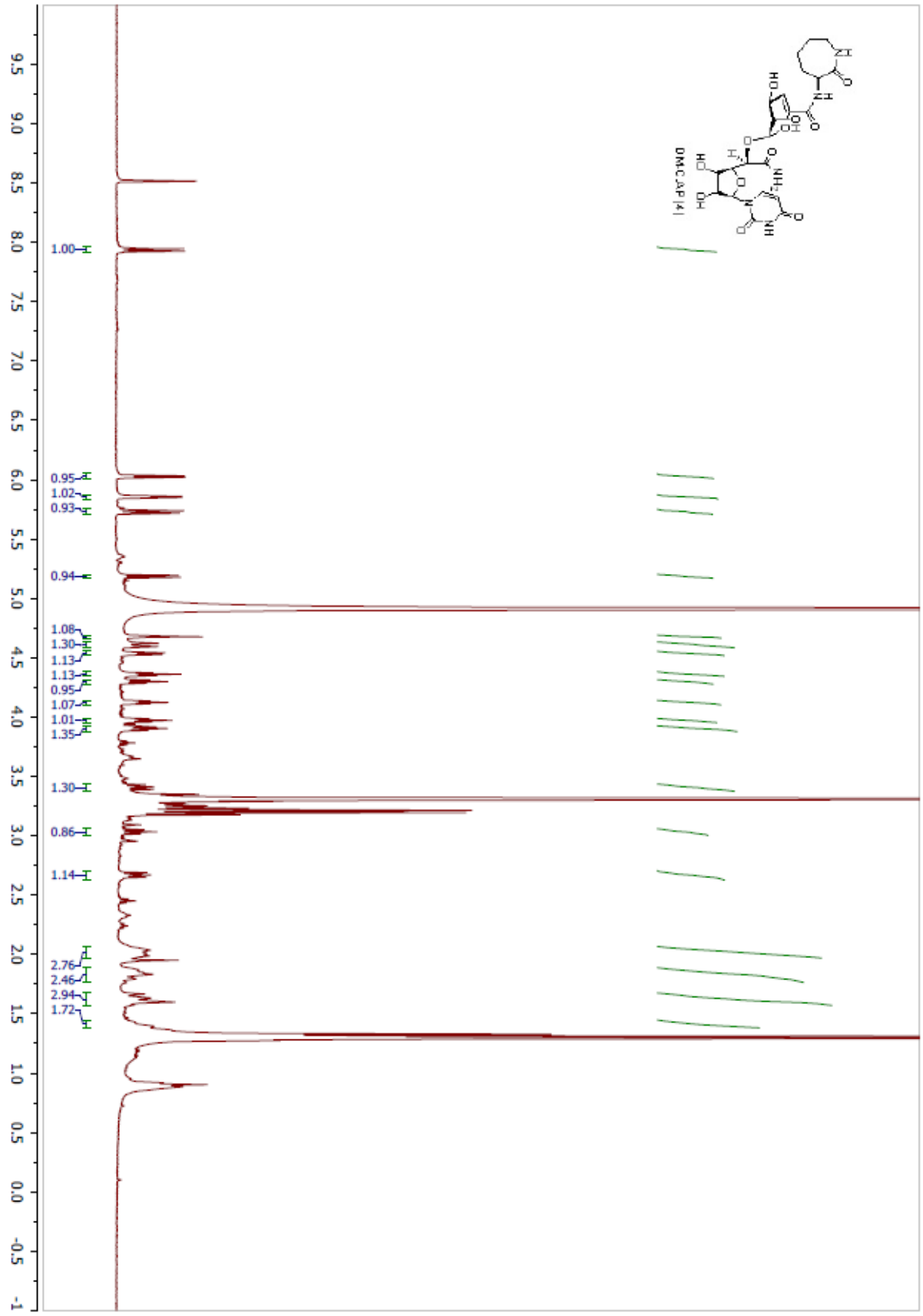
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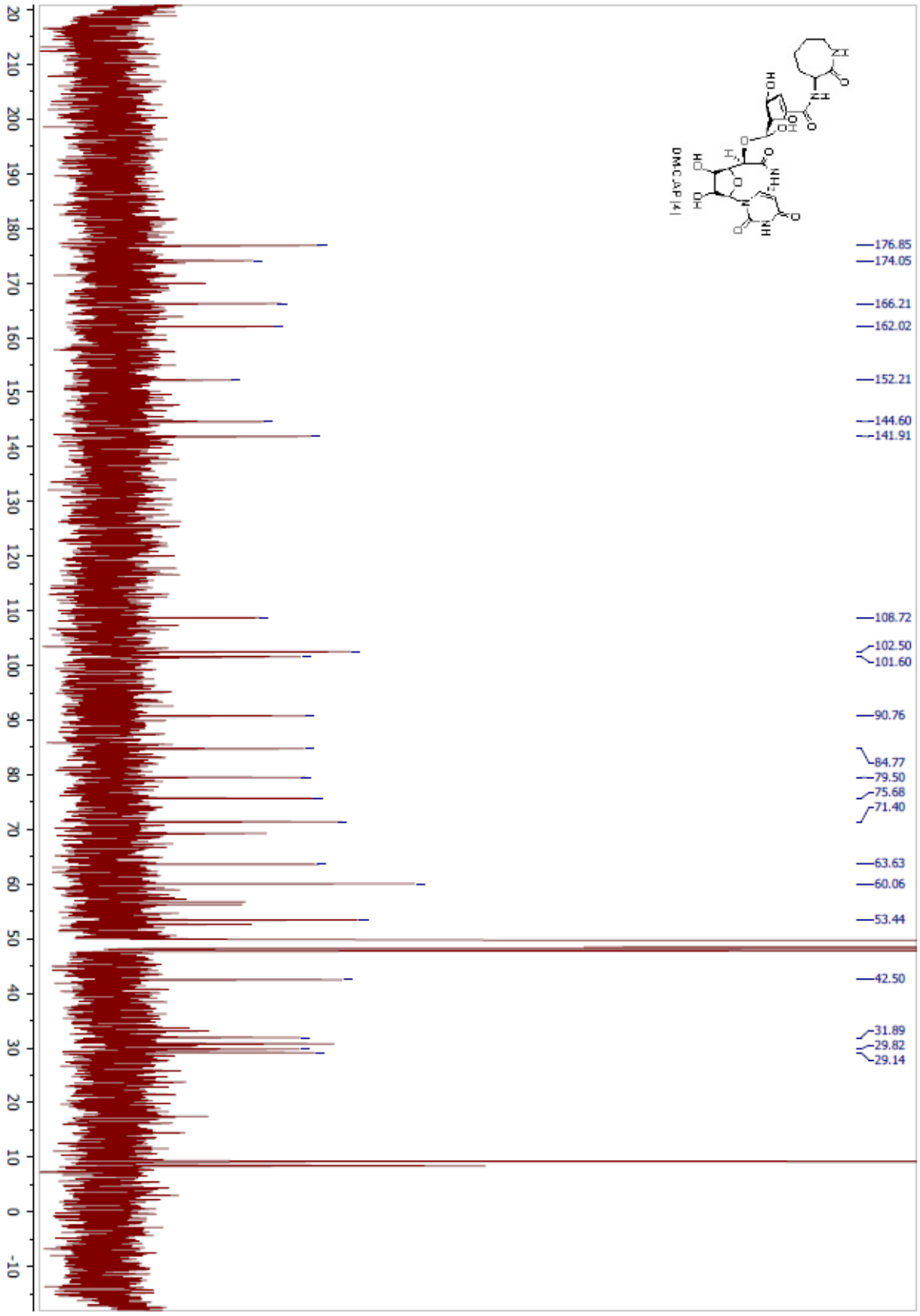
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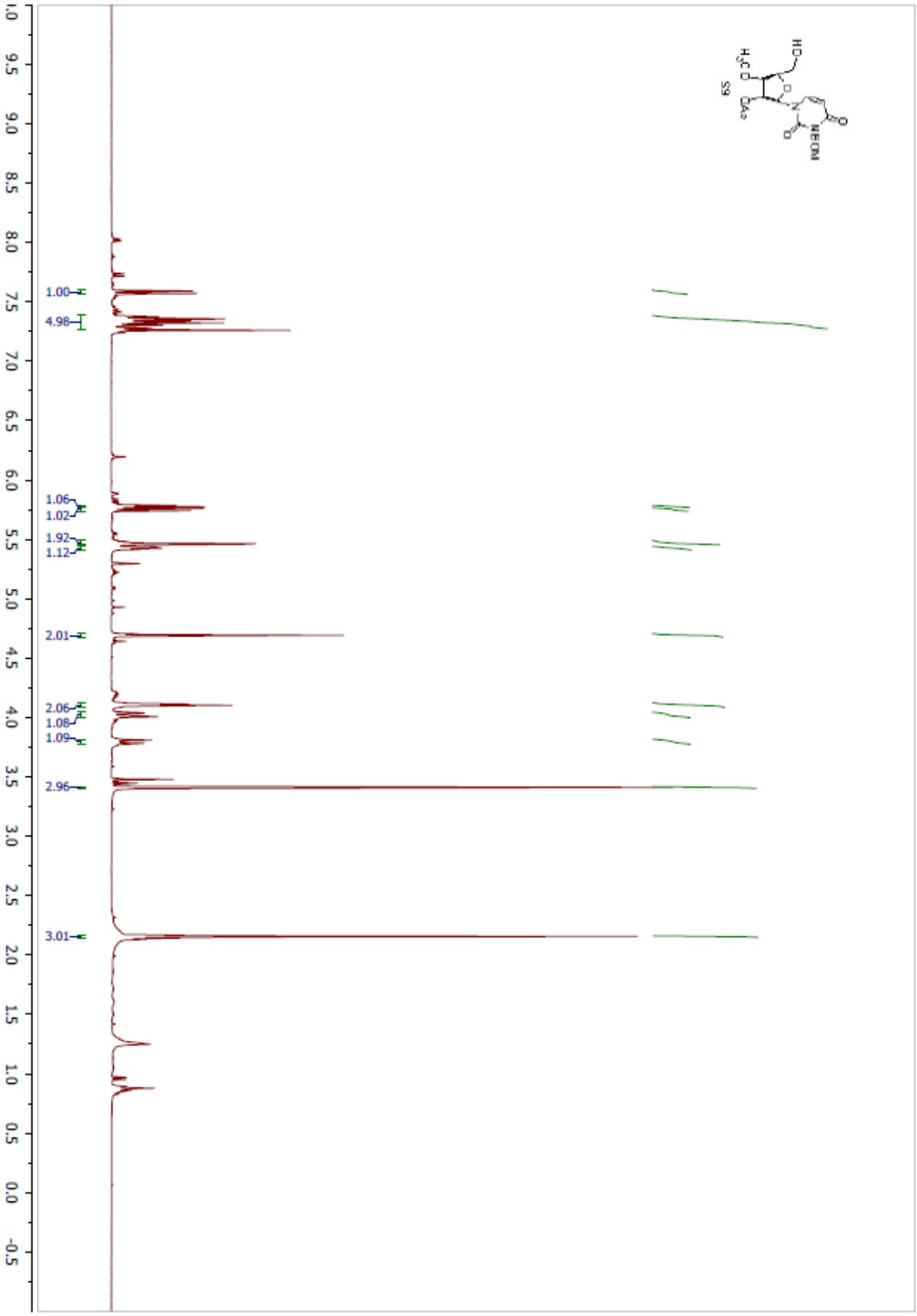
31.89
29.82
29.14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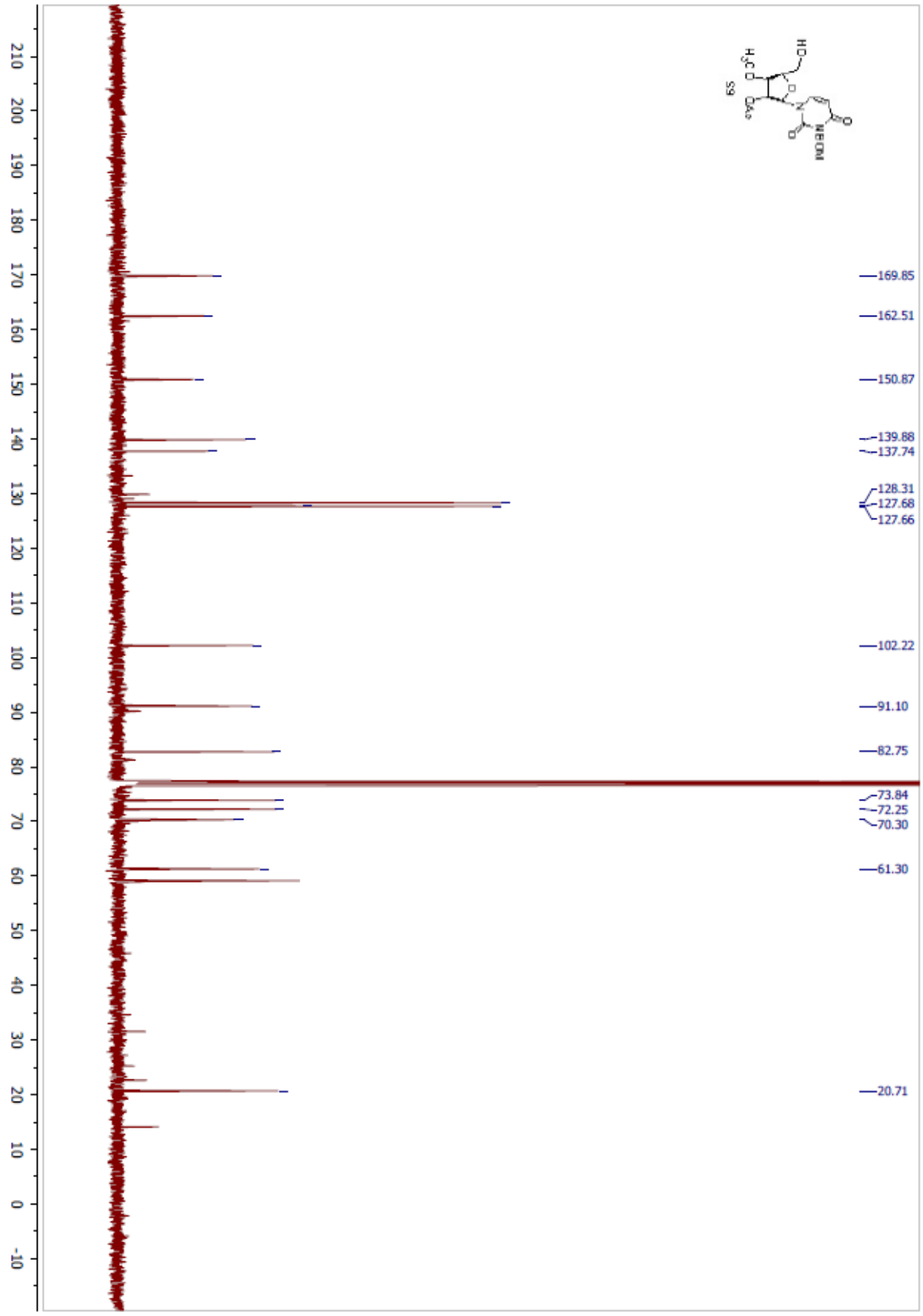
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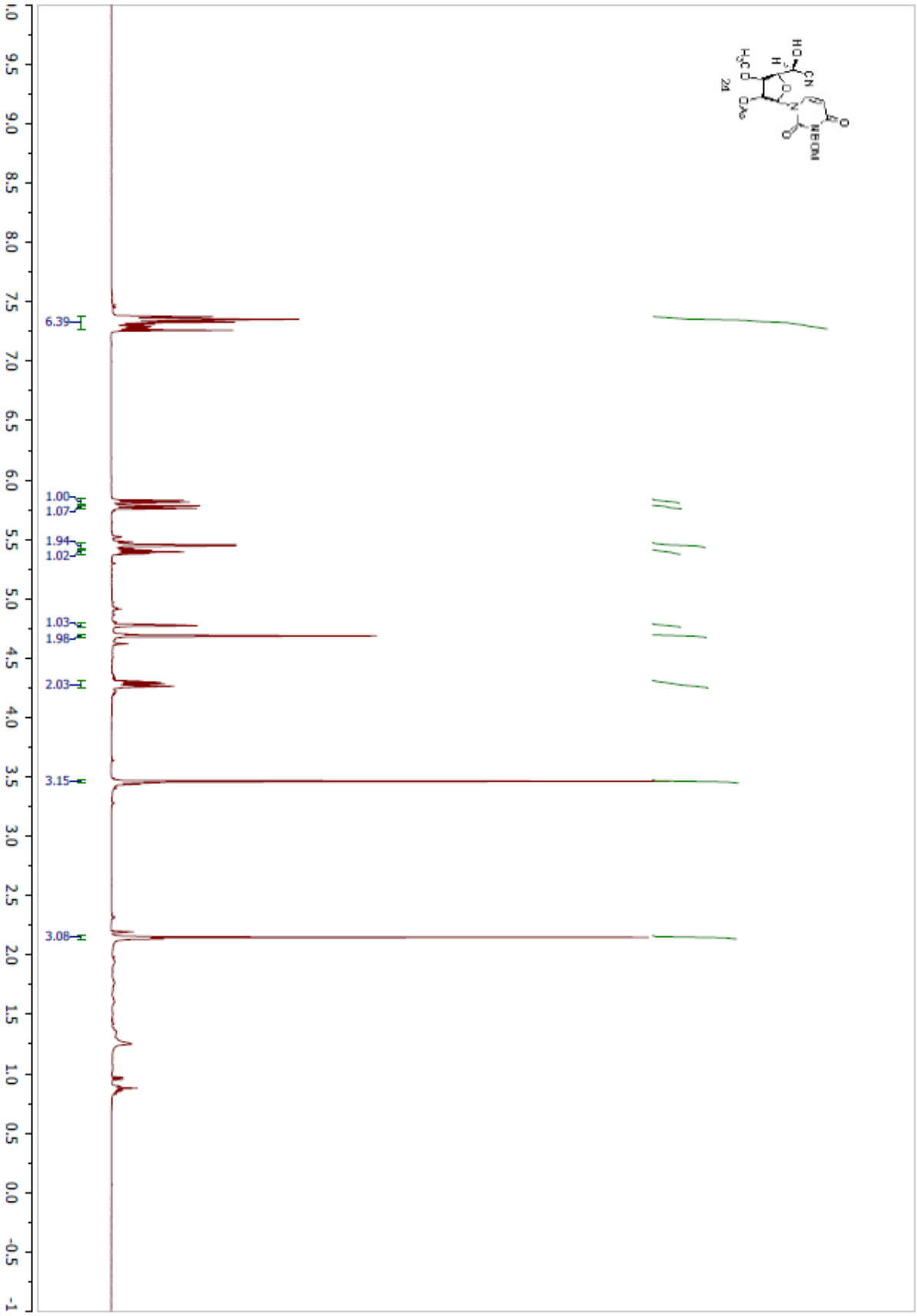
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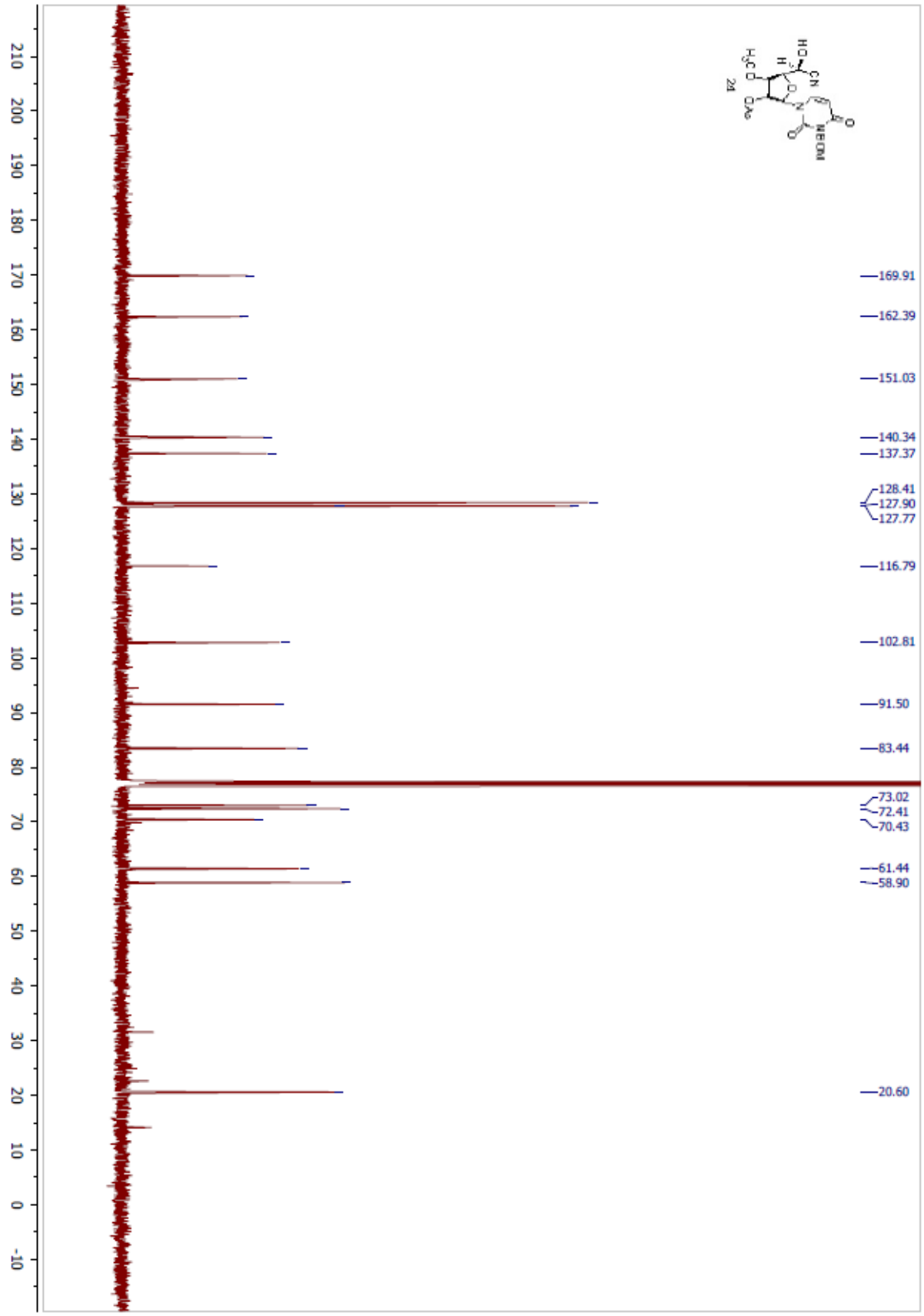
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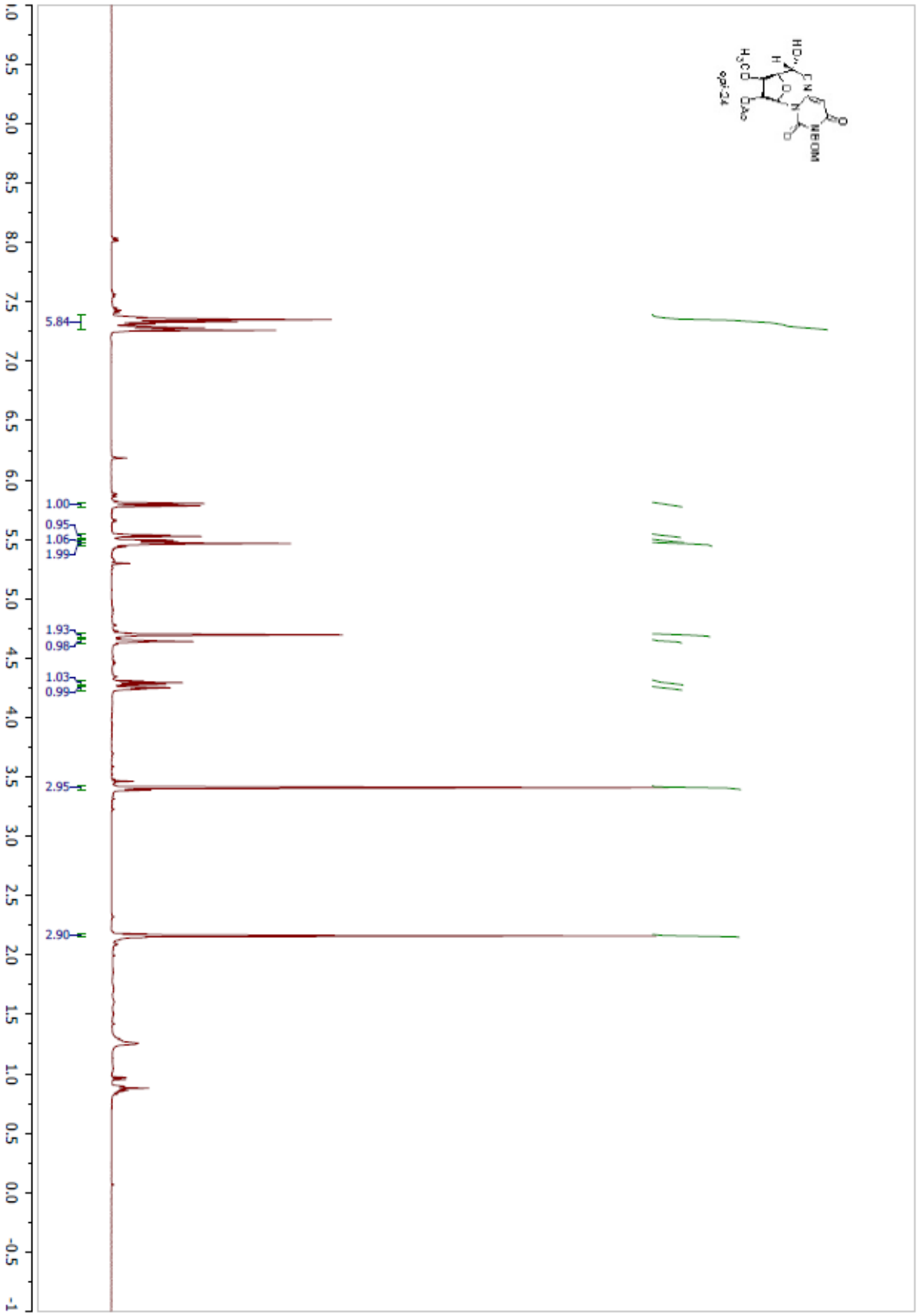
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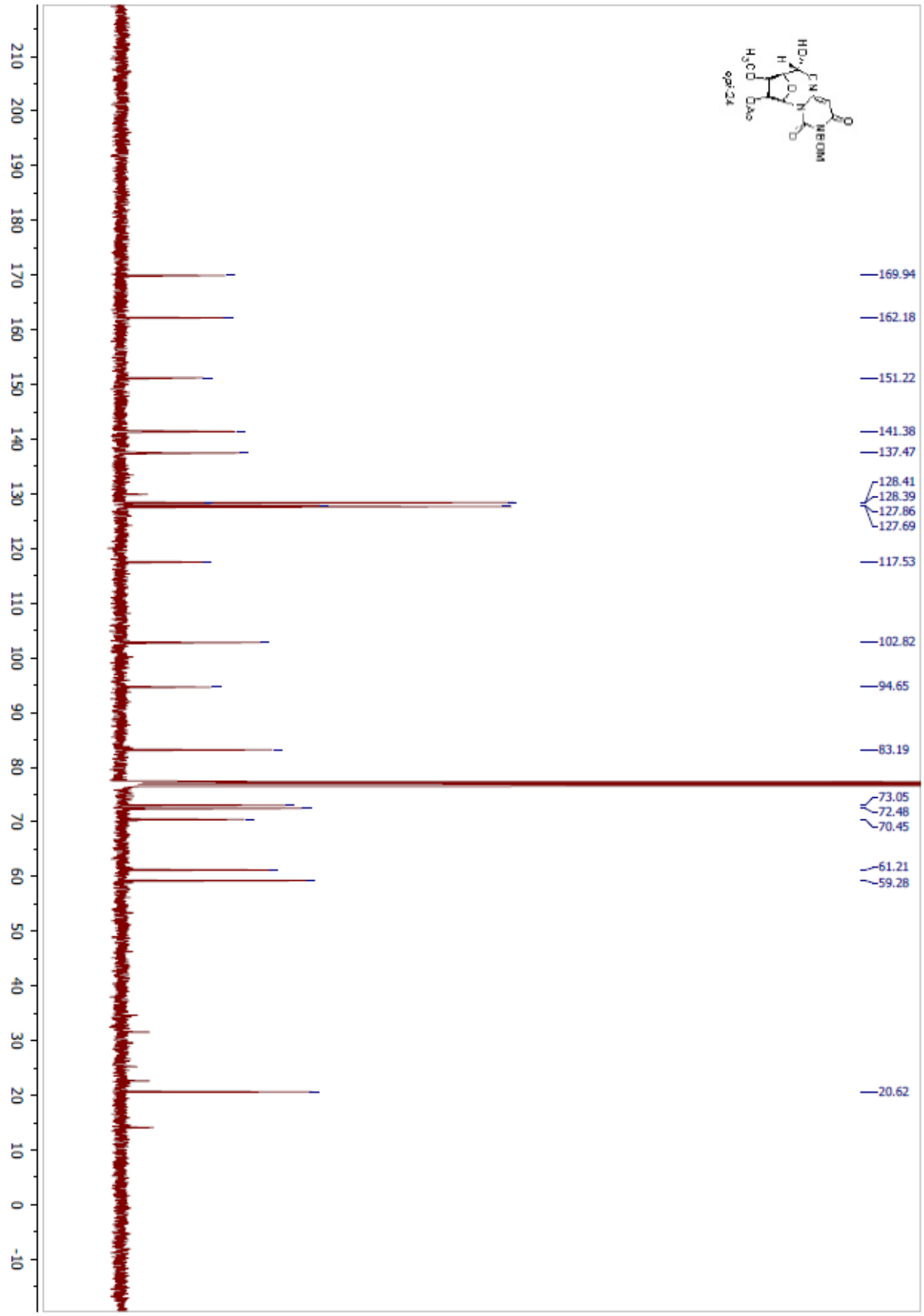
epi-24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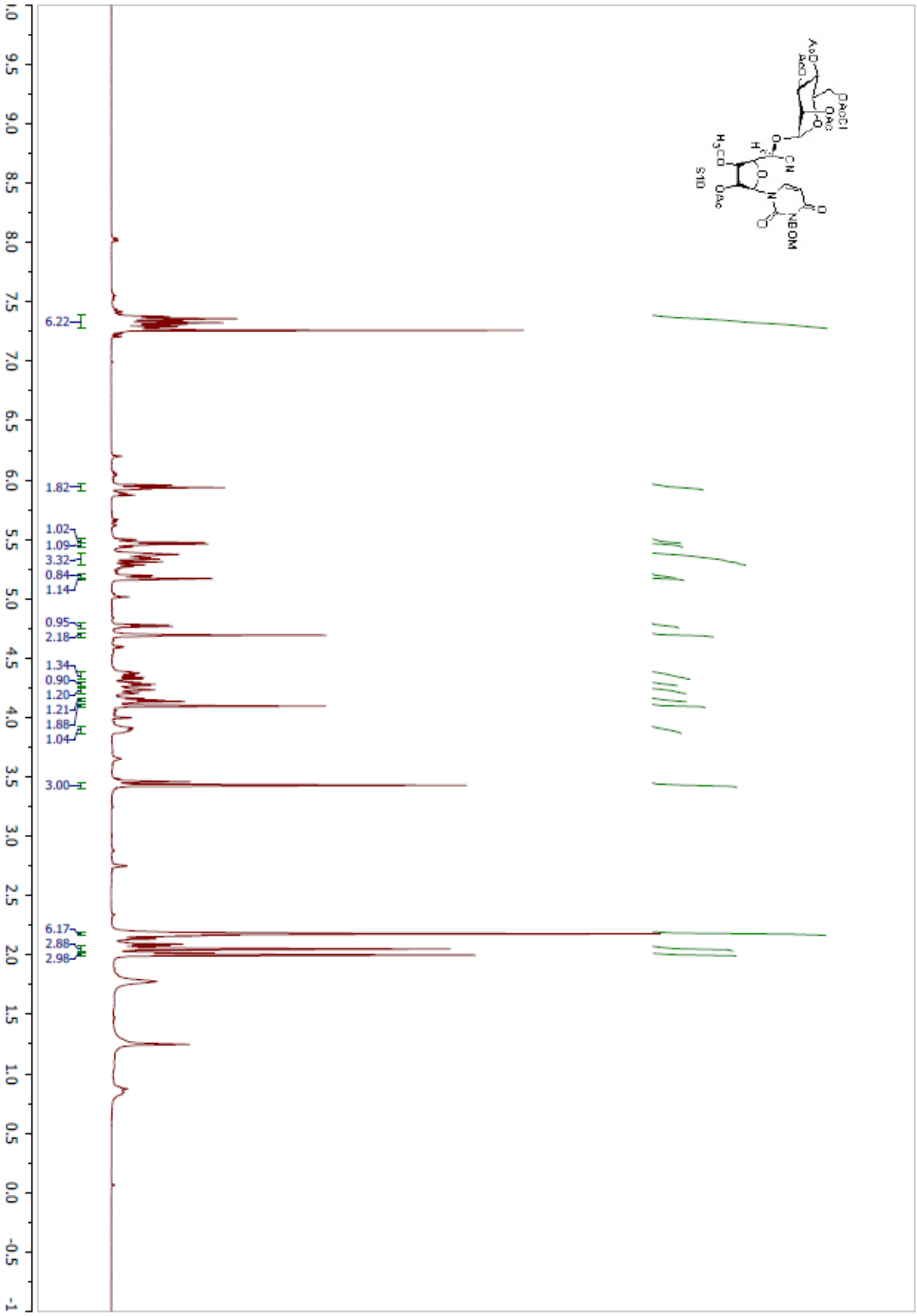
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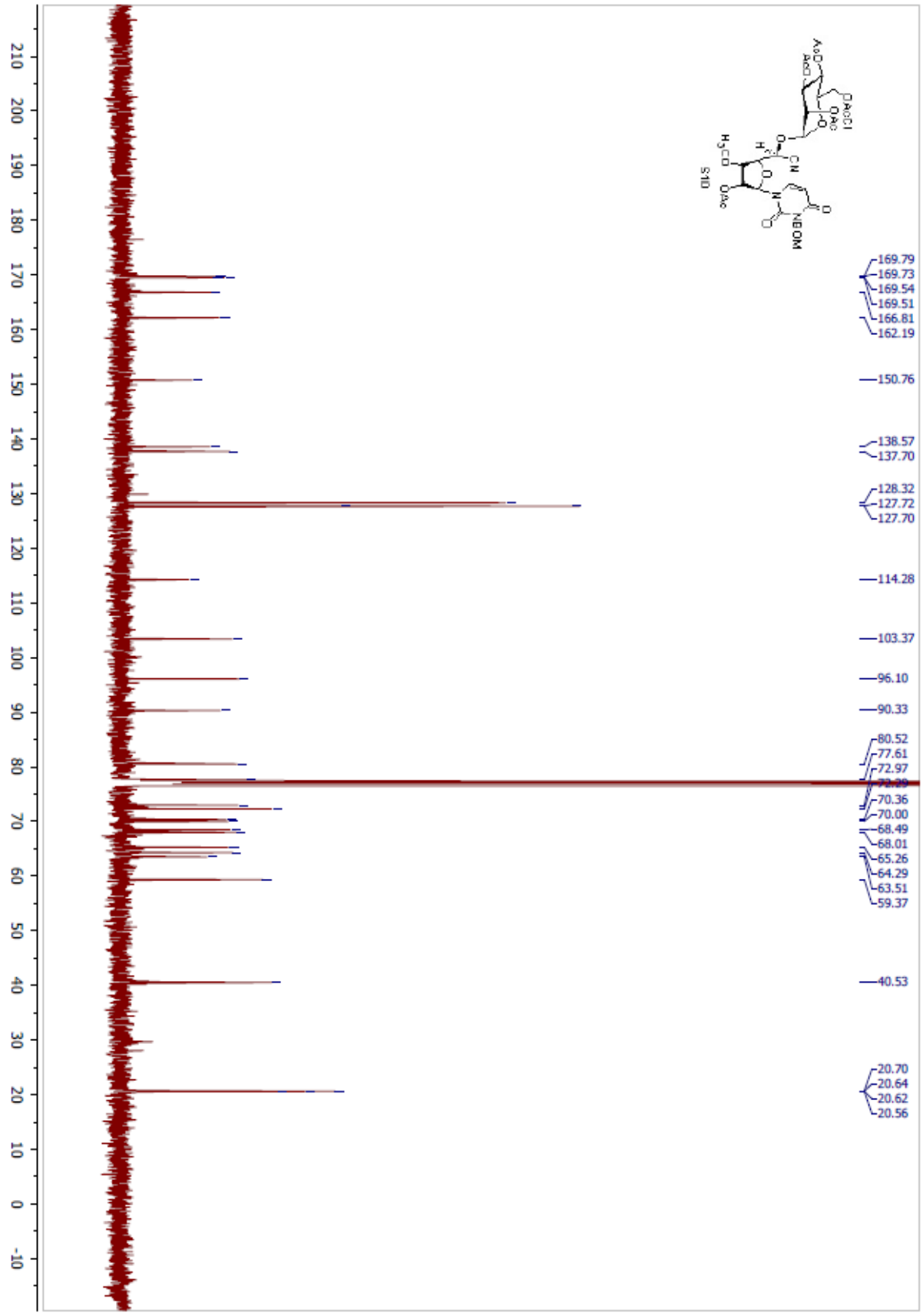
S1D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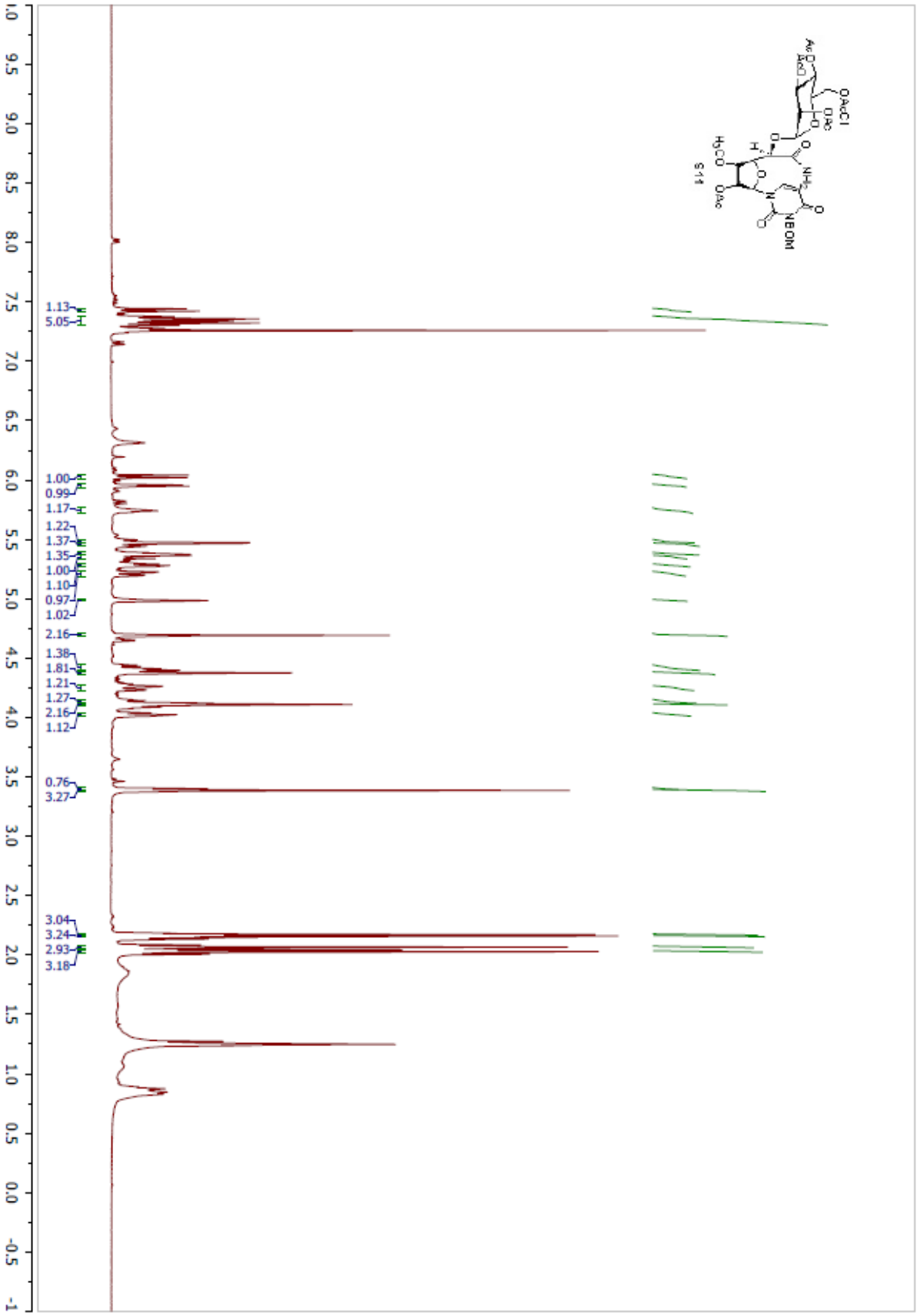
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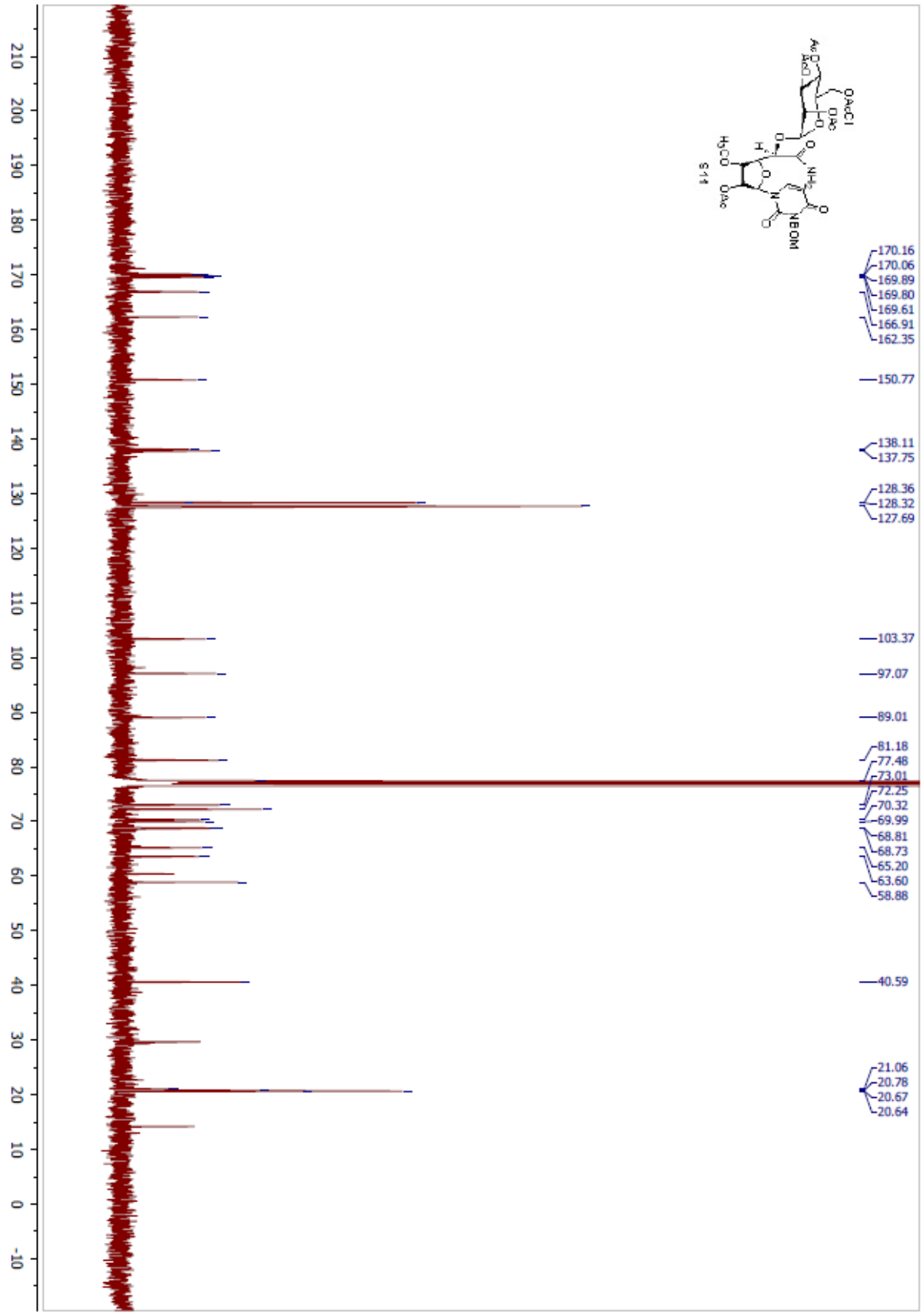
NBOM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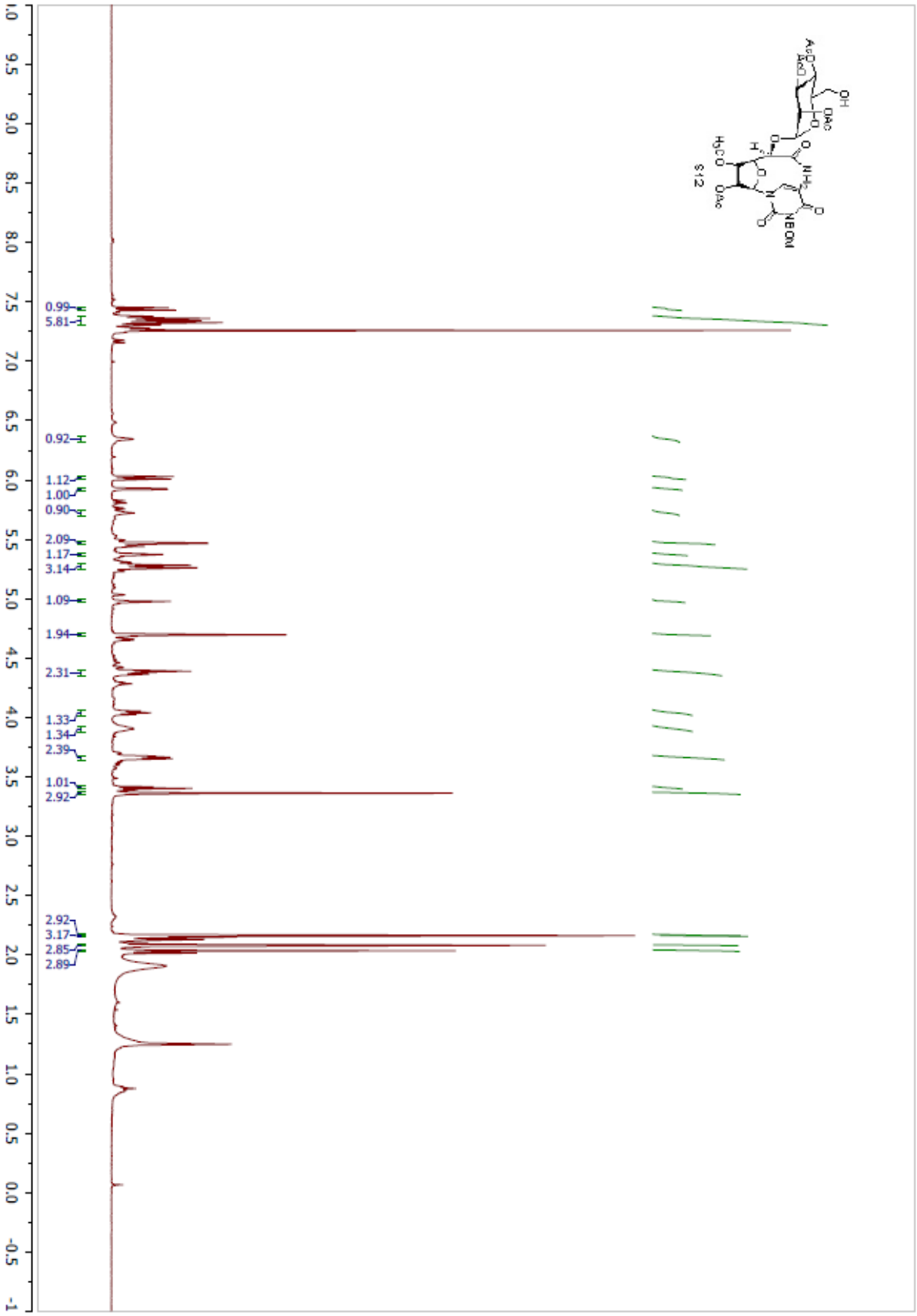
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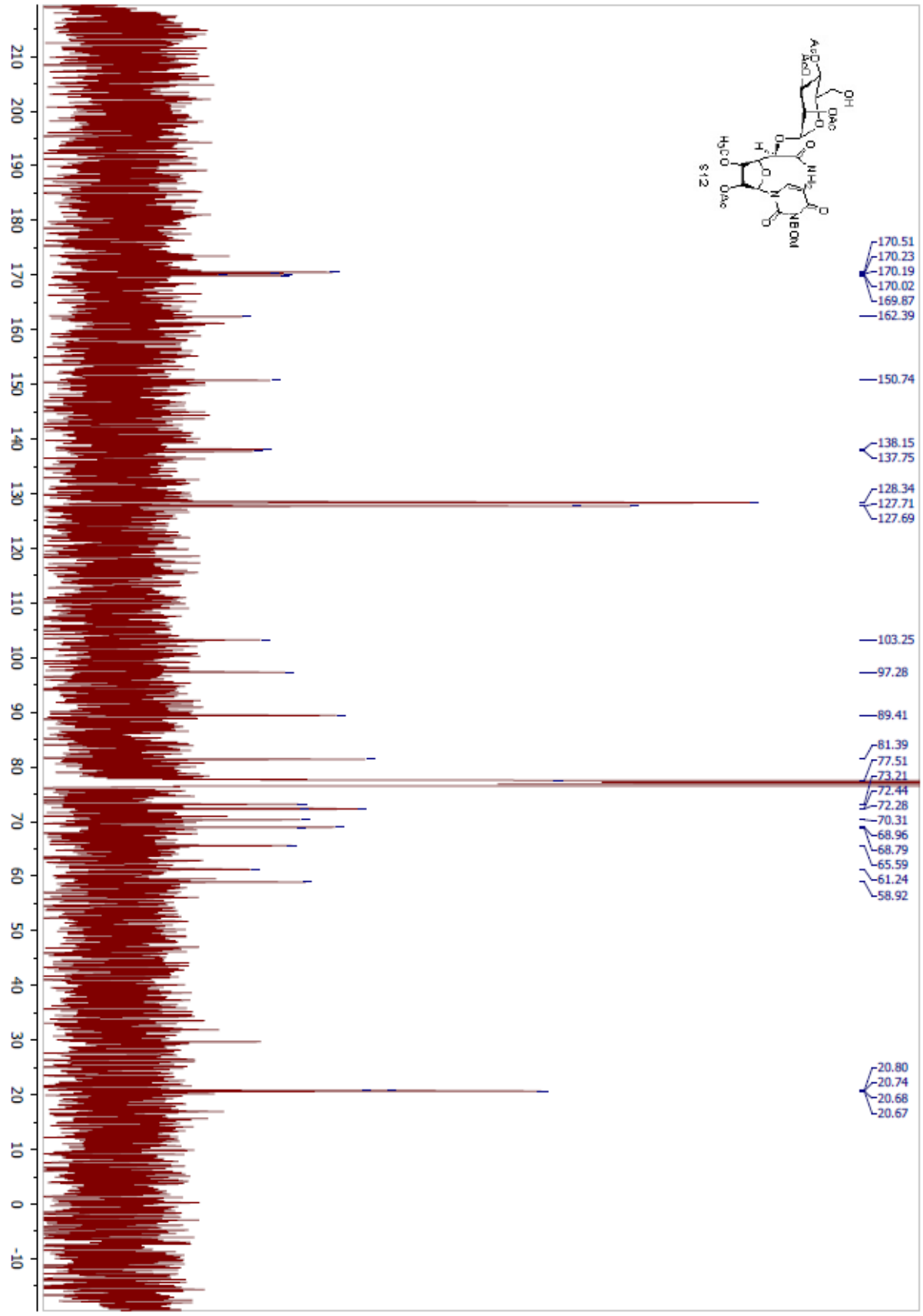
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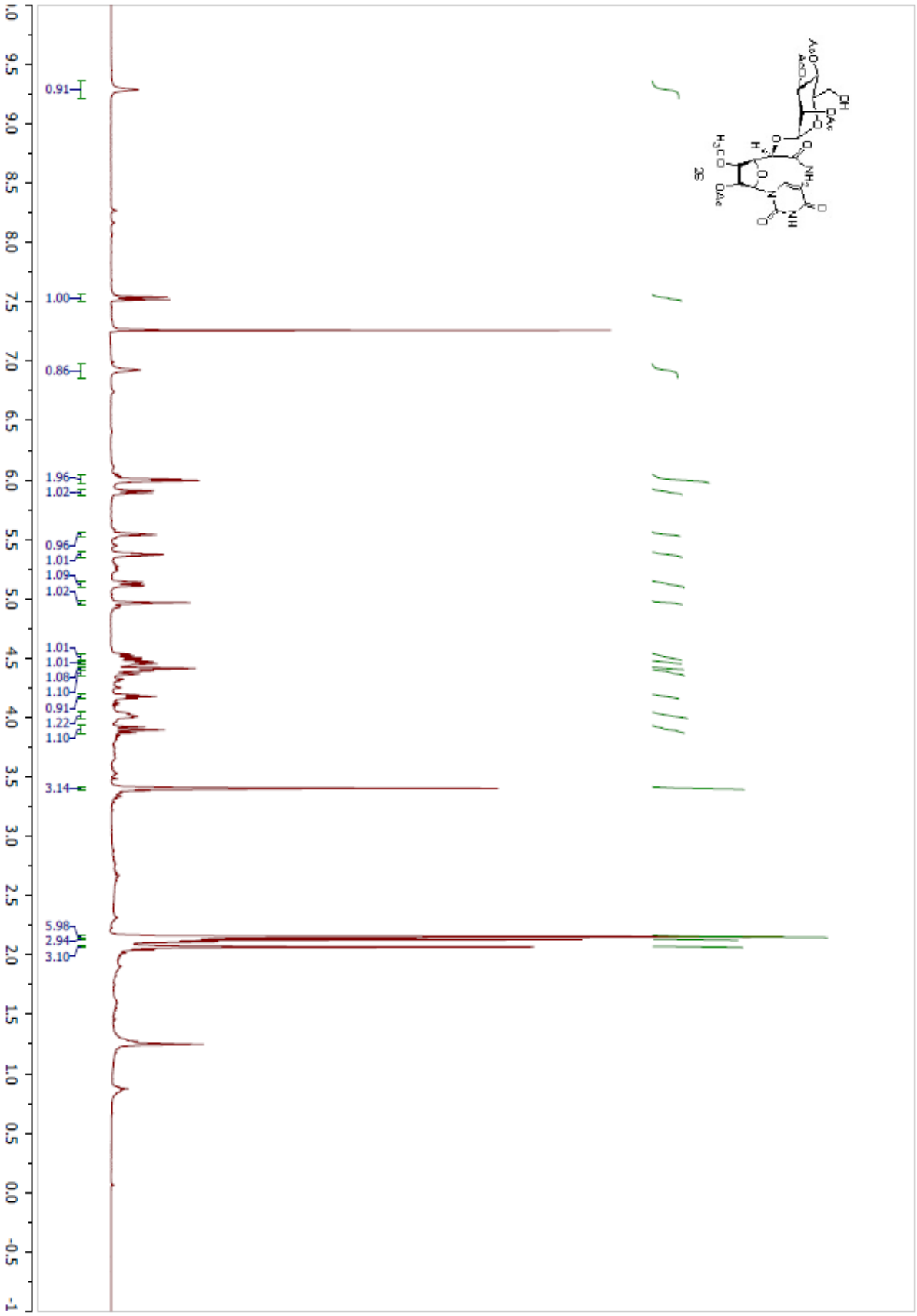
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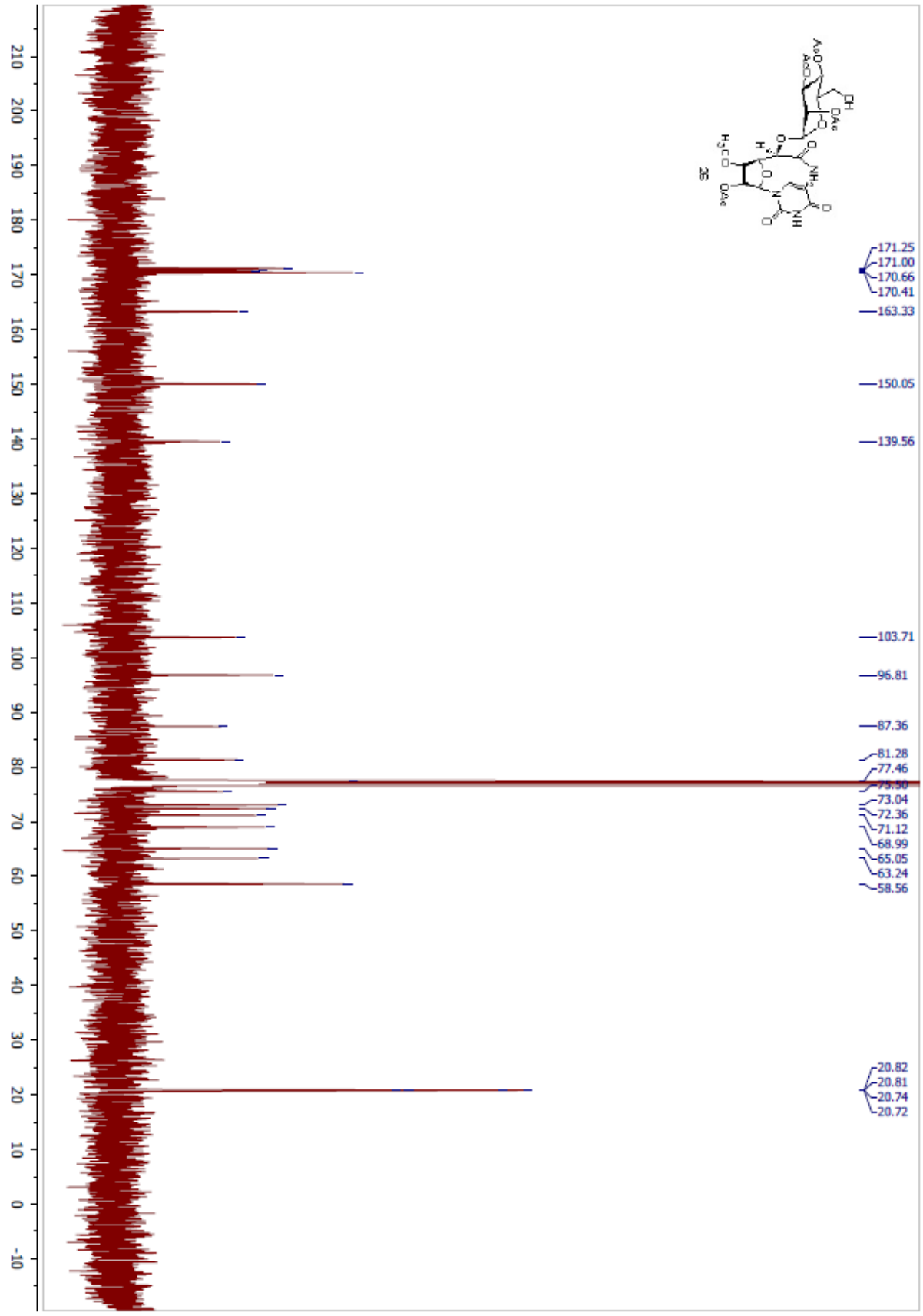
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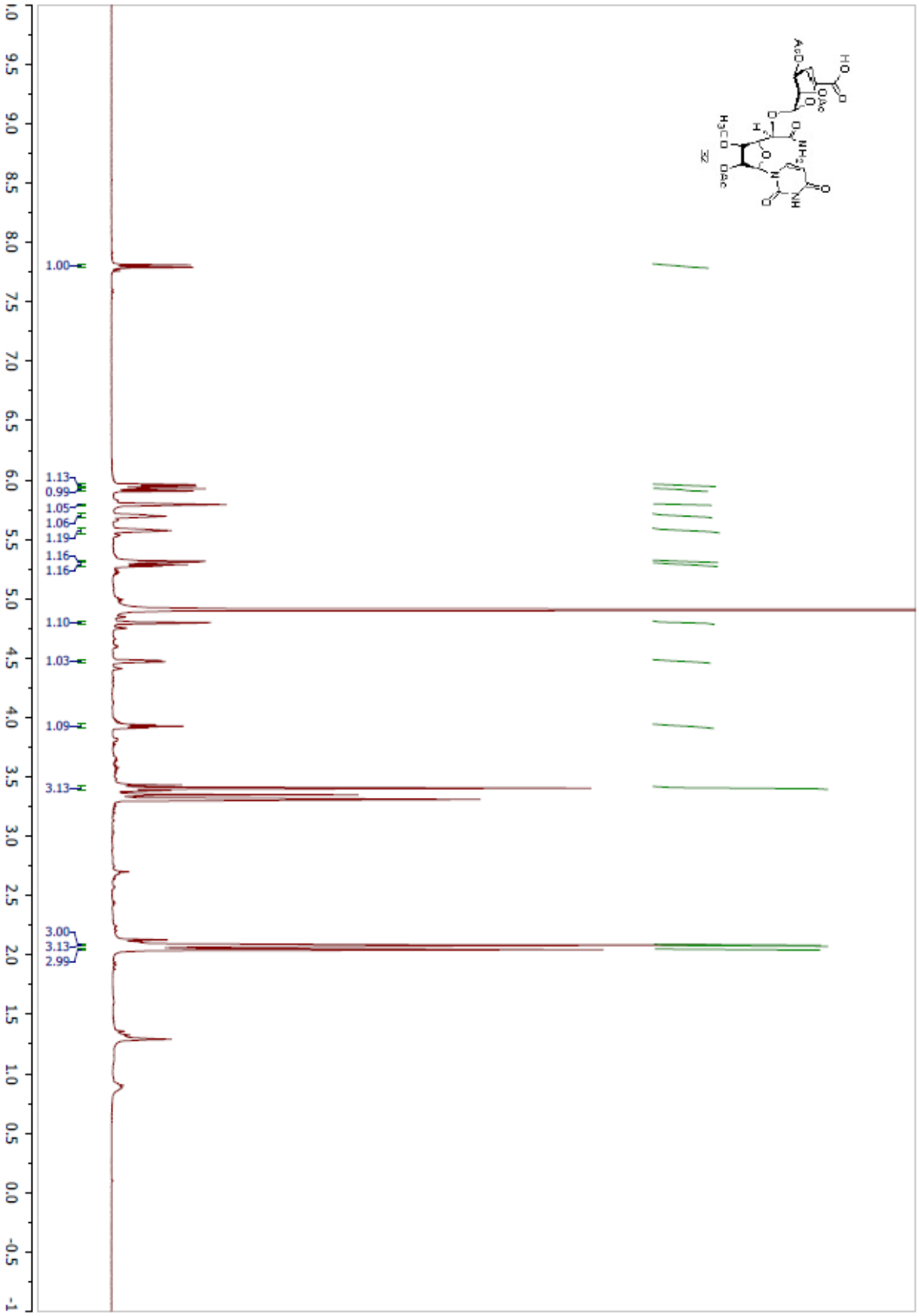
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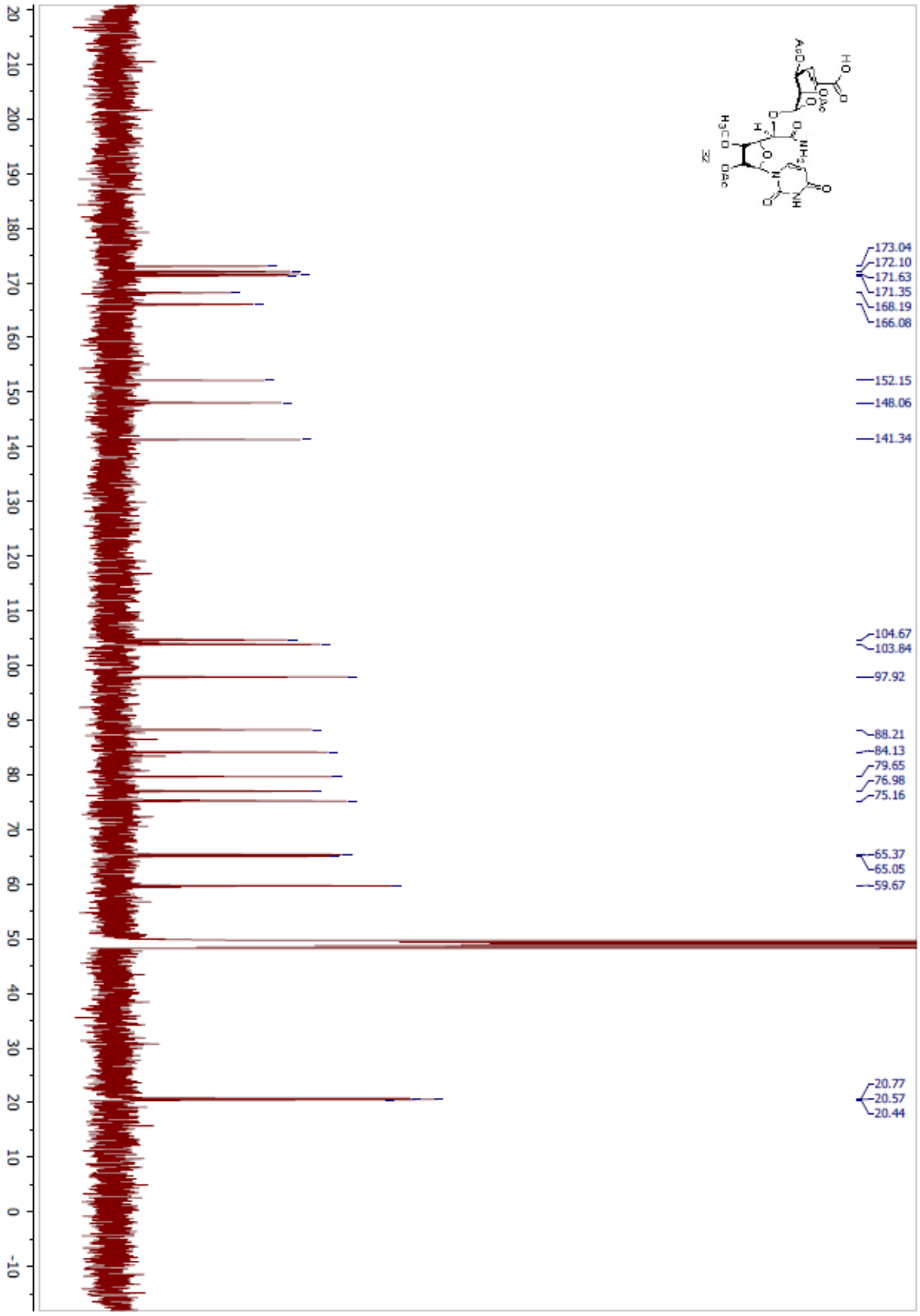
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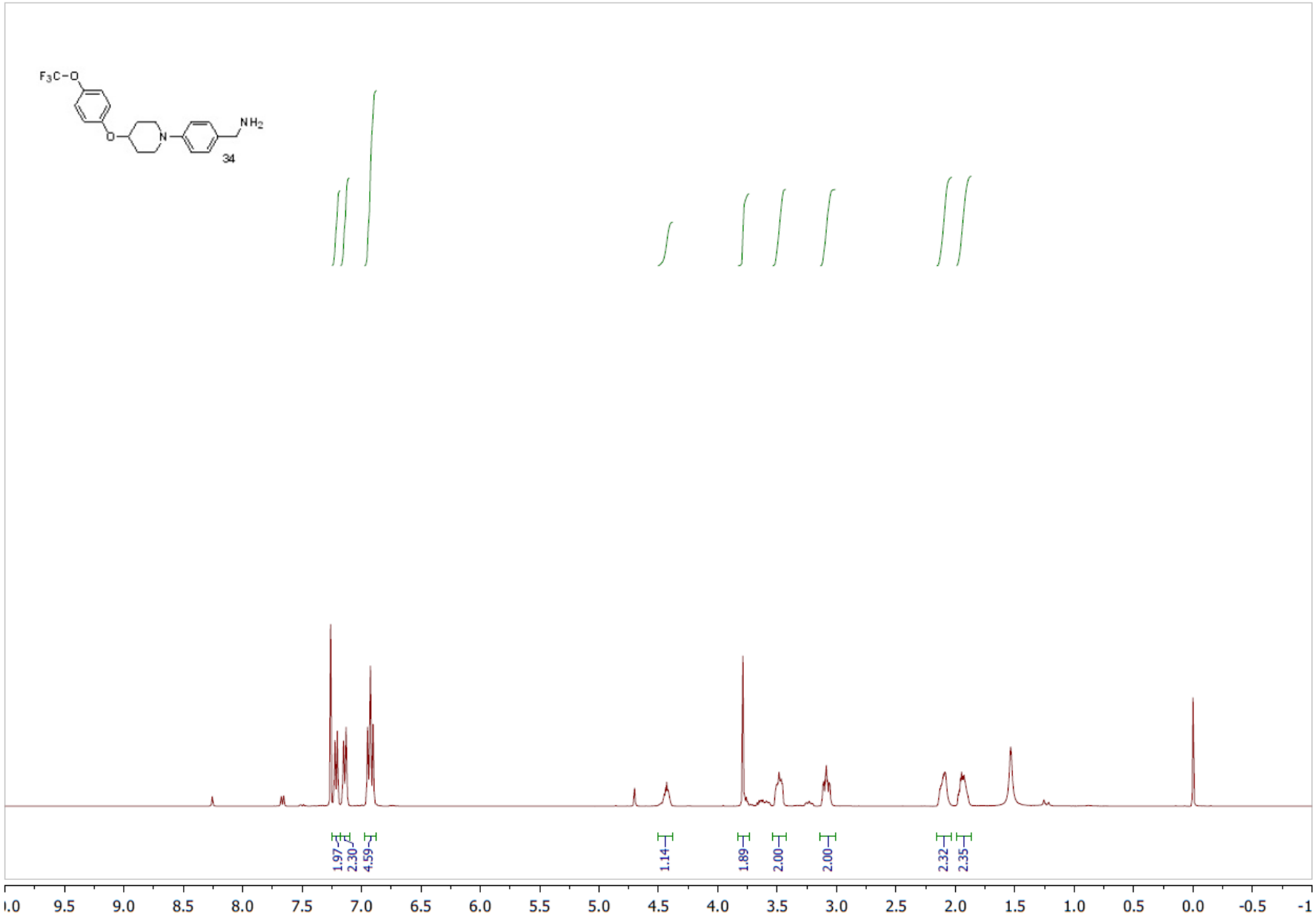
F3C-O
O
N
NH2
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2.00
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2.35
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9.0
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7.5
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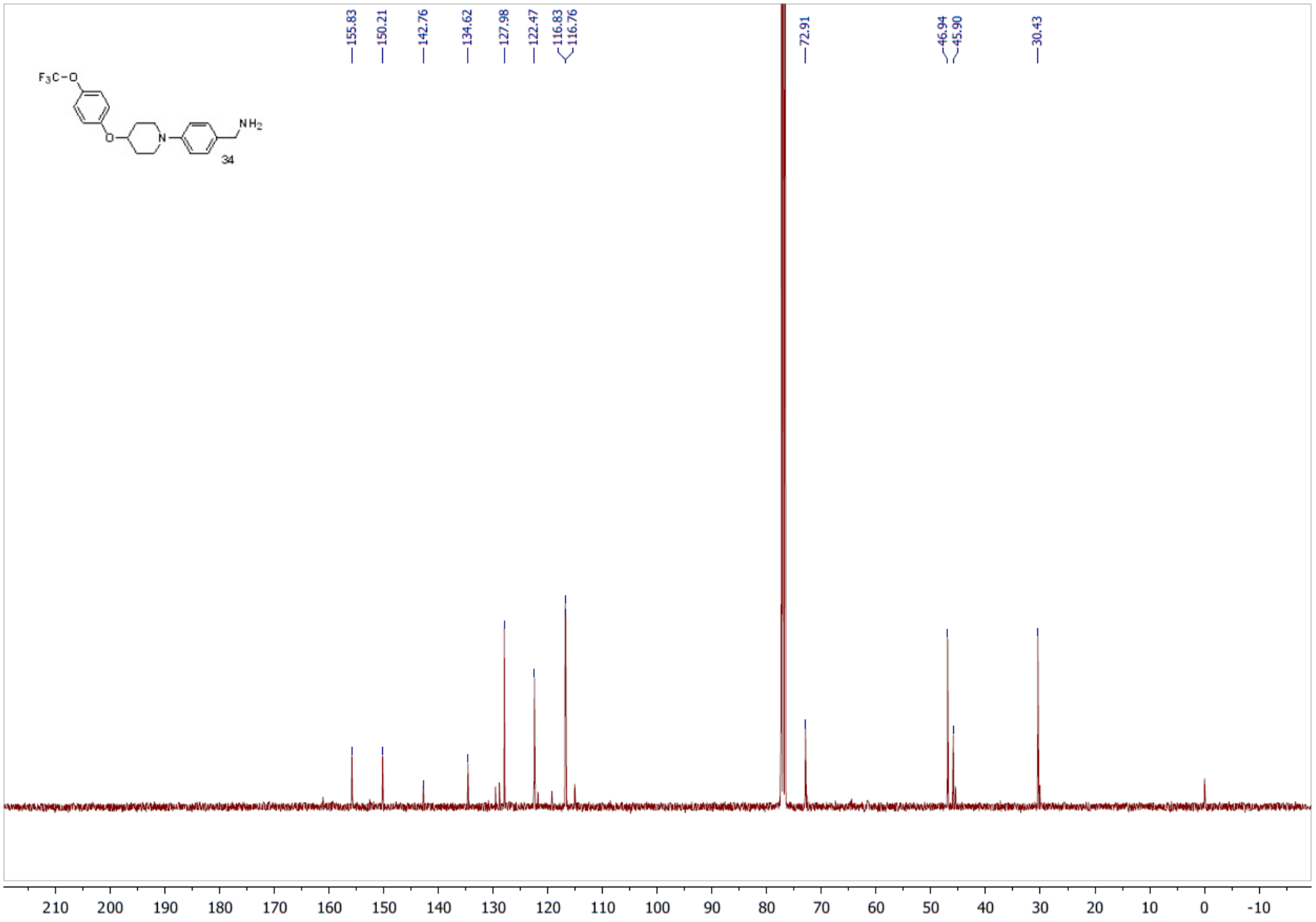
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O
N
NH2
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210
200
190
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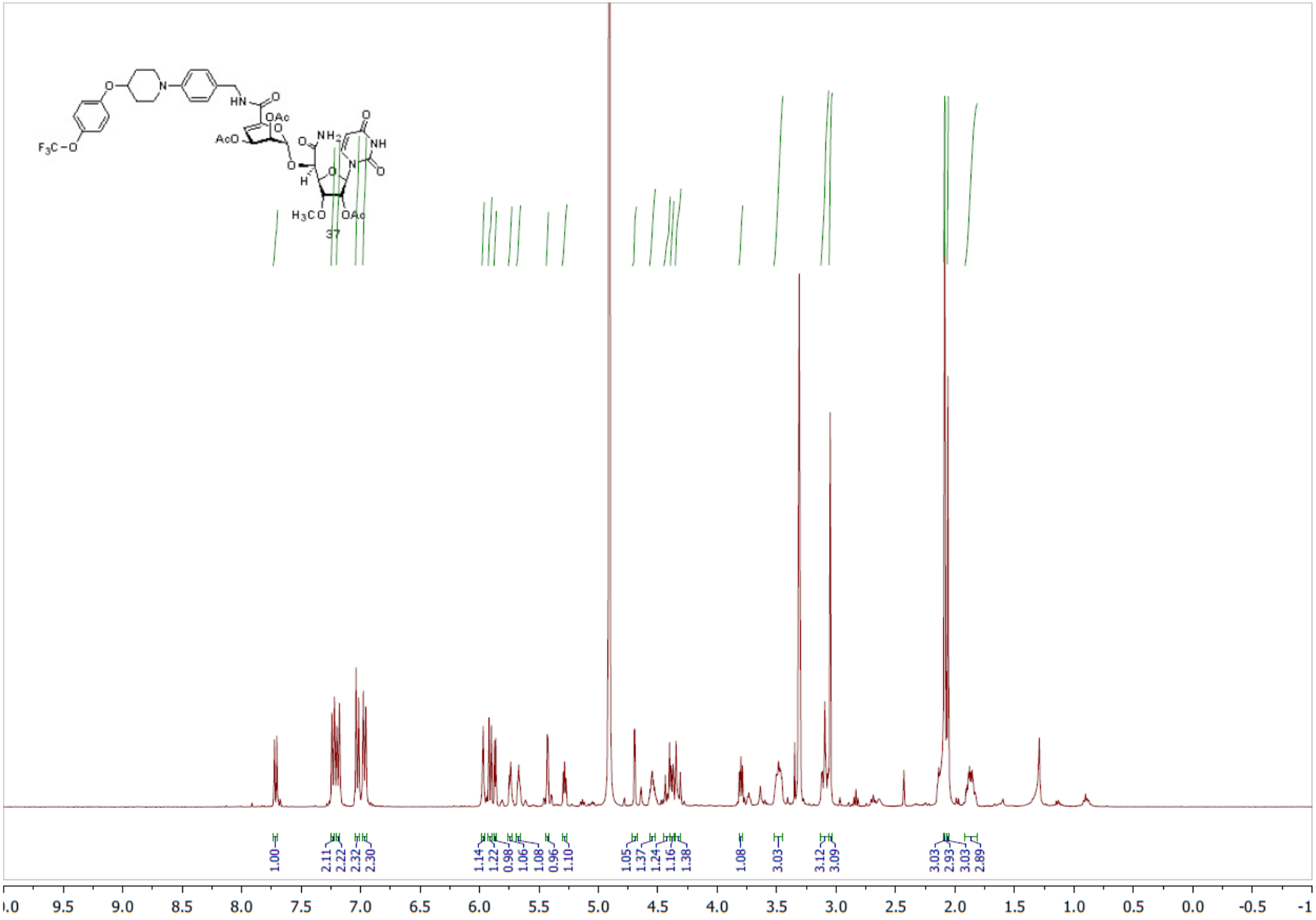
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AcO
NH2
NH
H3CO
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1.08
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1.38
1.08
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3.03
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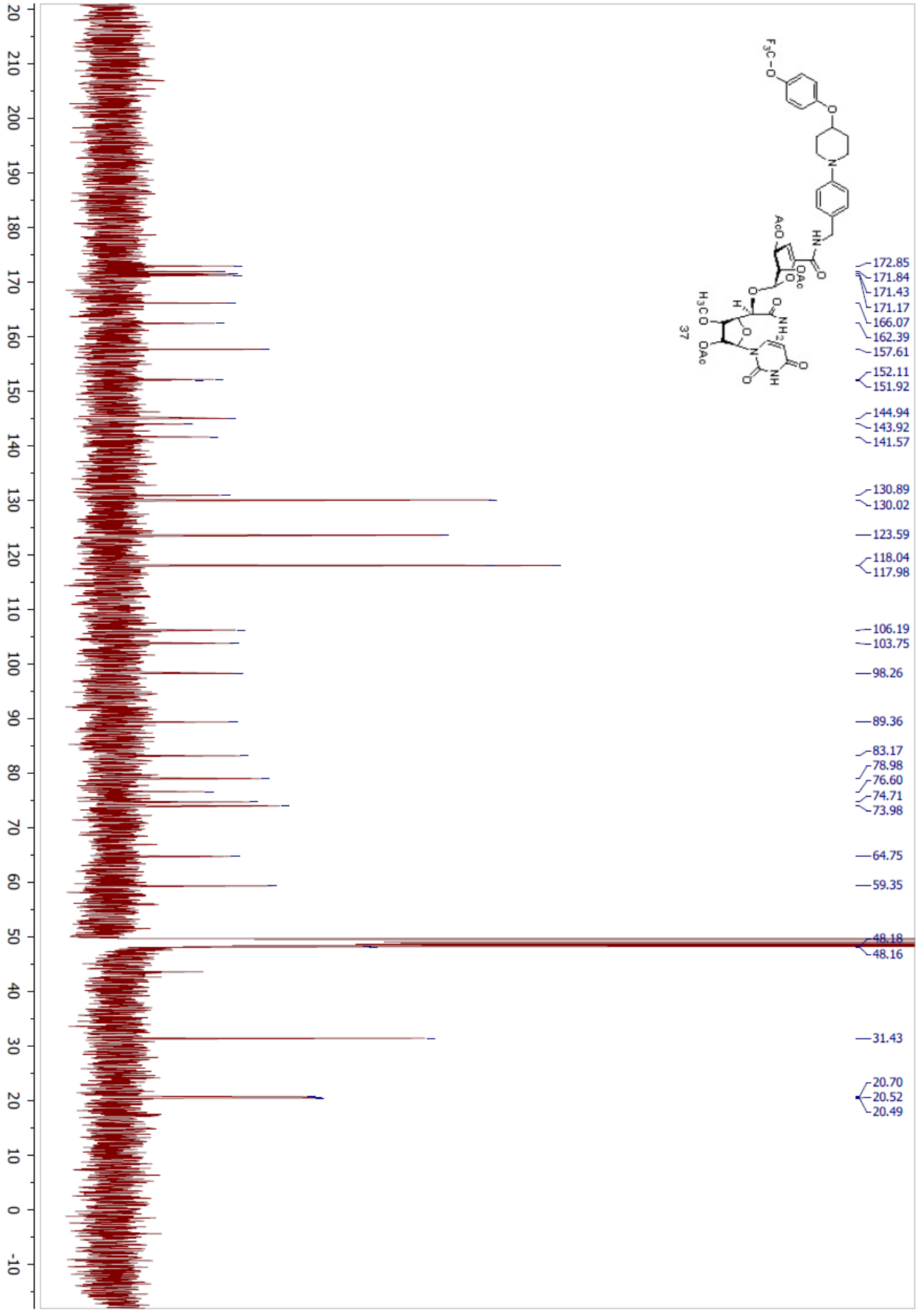
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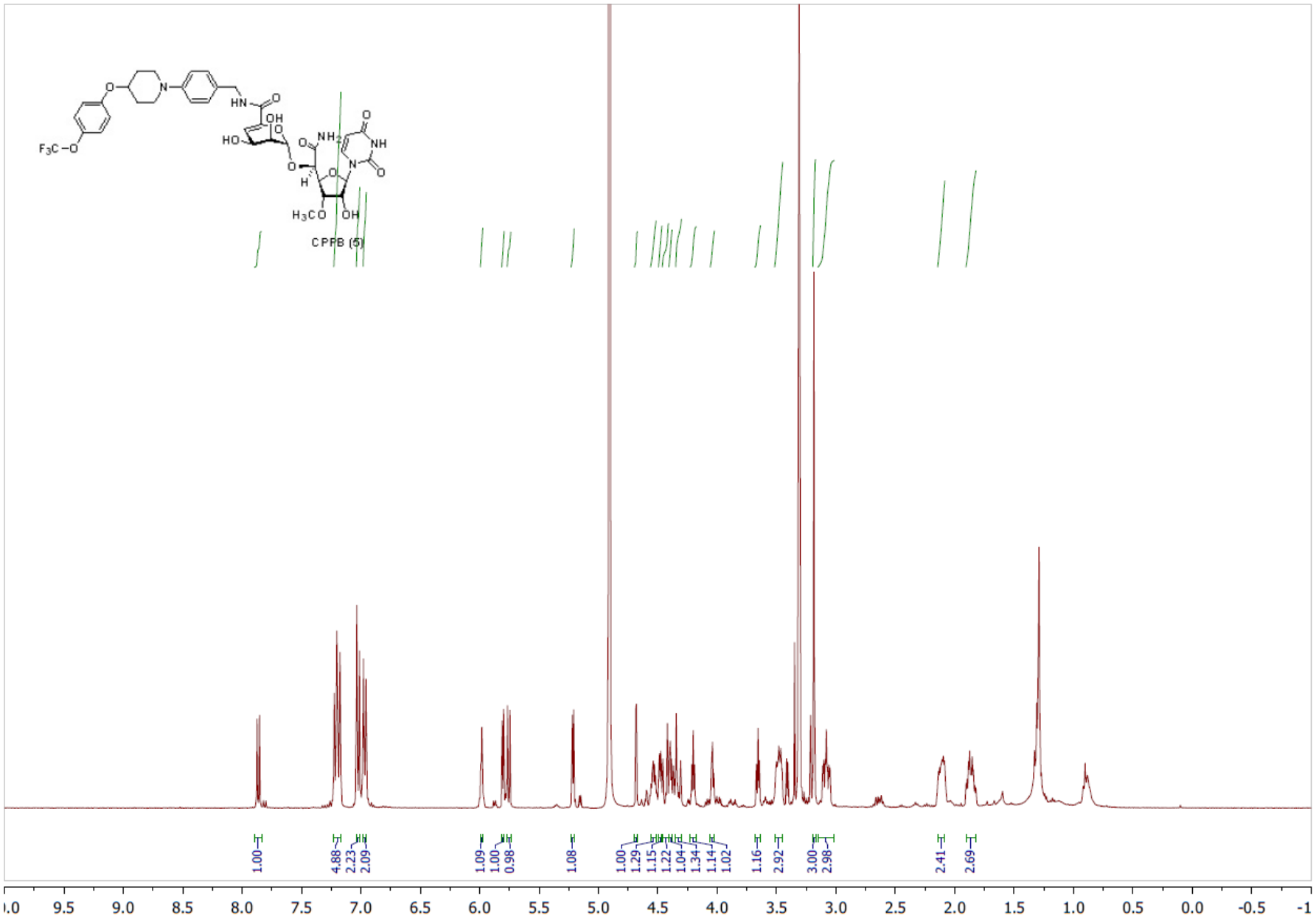
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1.02
1.16
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3.00
2.98
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7.5
7.0
6.5
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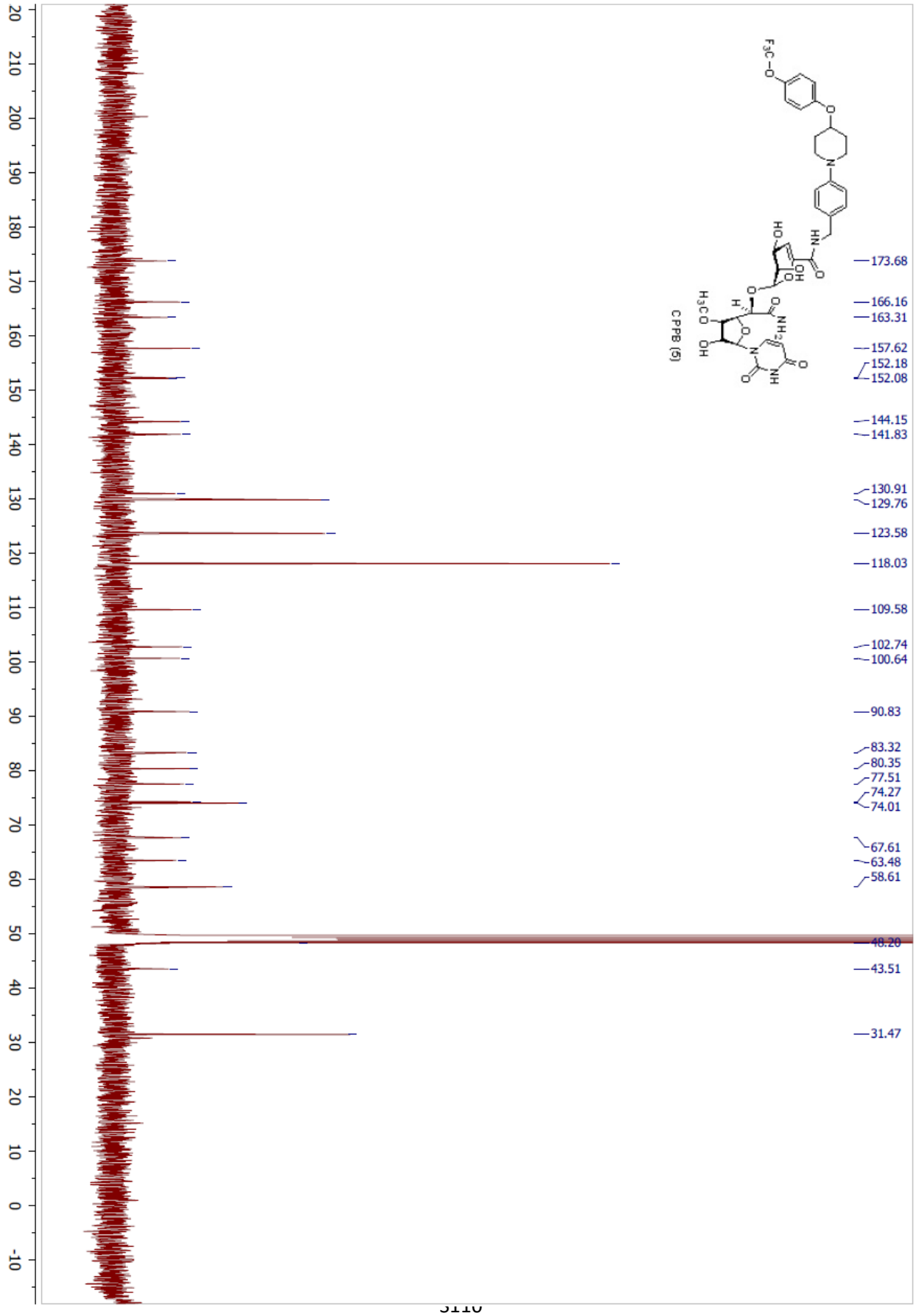
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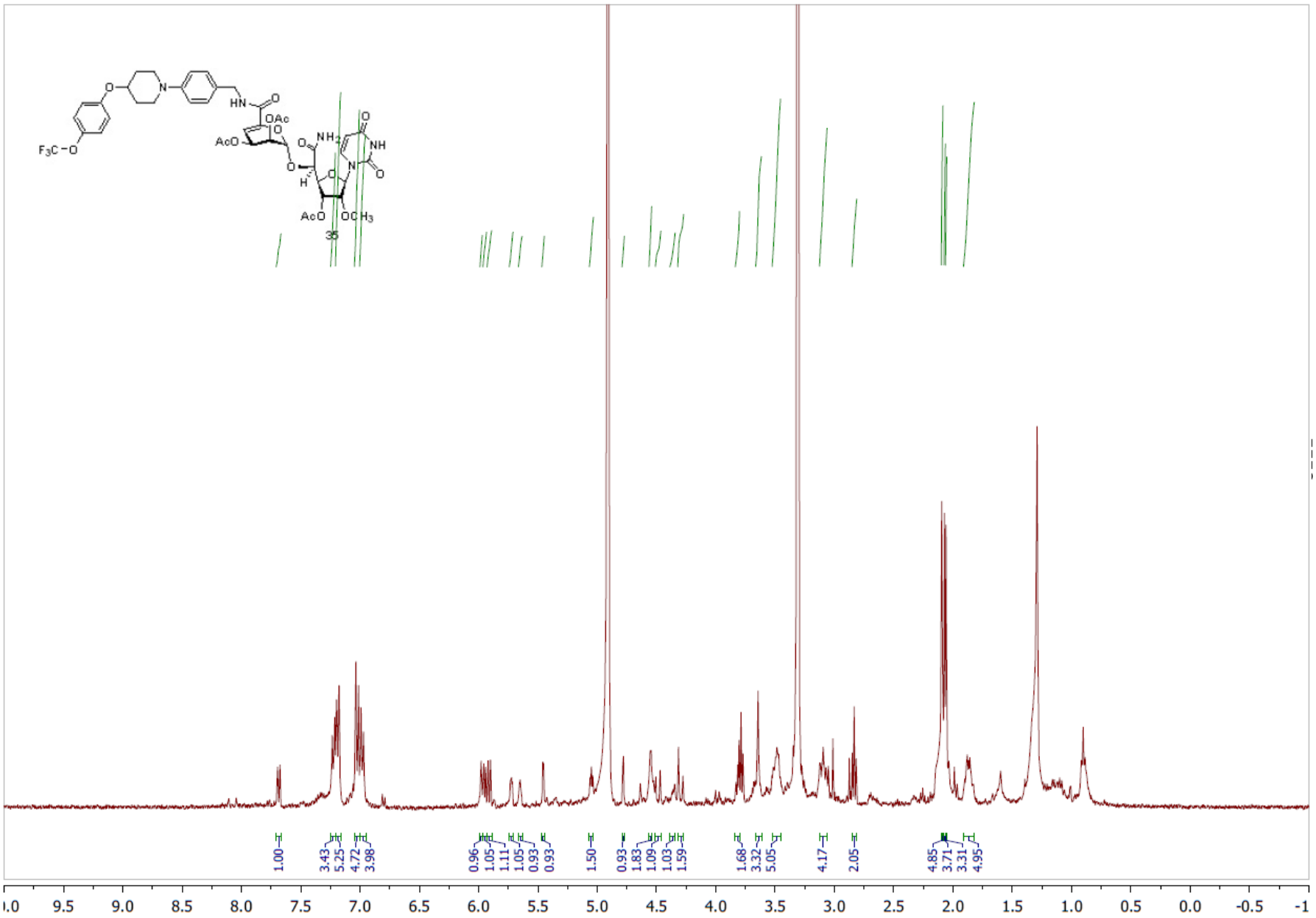
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1.59
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2.05
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3.71
3.31
4.95
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8.0
7.5
7.0
6.5
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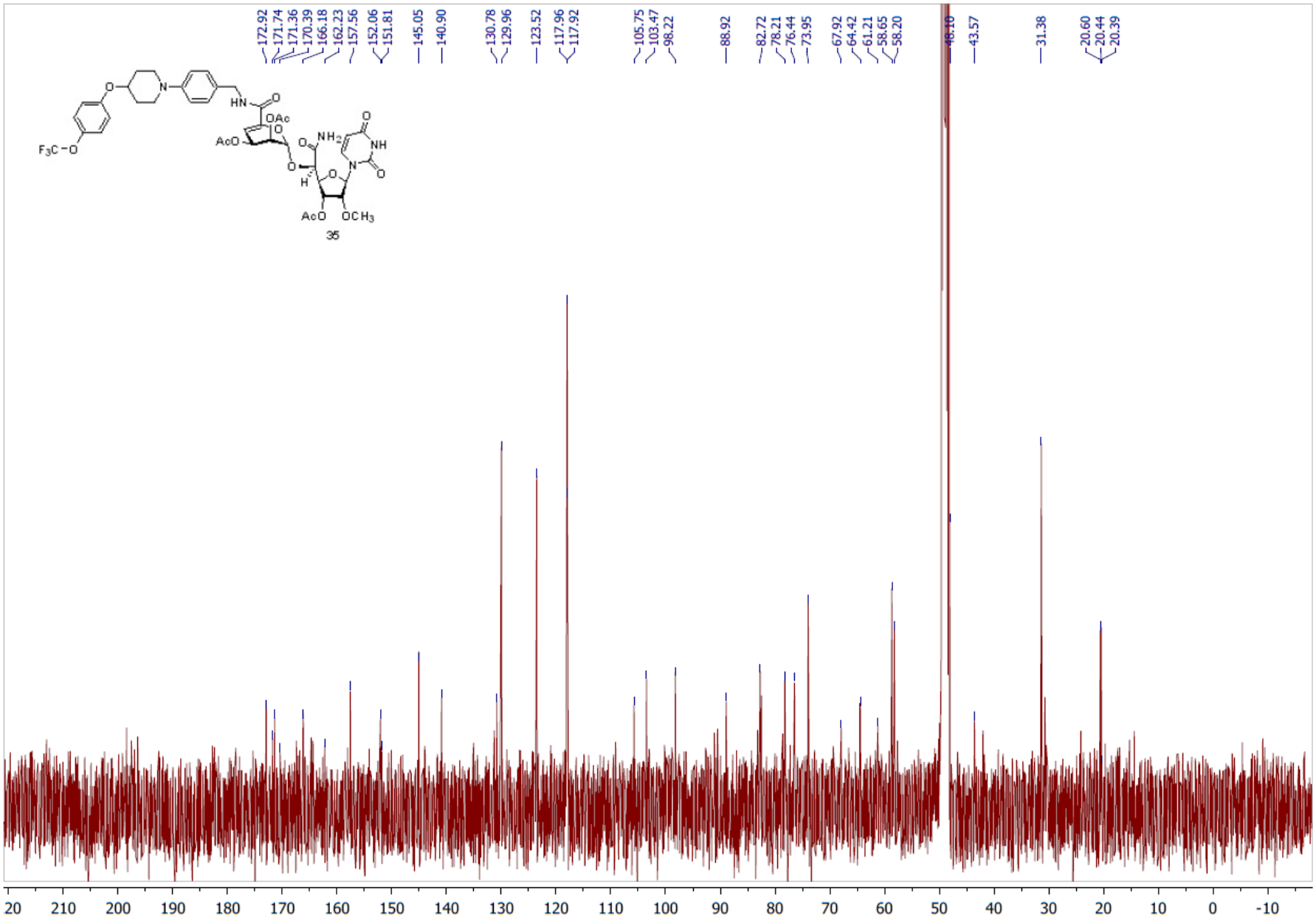
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98.22
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67.92
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20.60
20.44
20.39
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0
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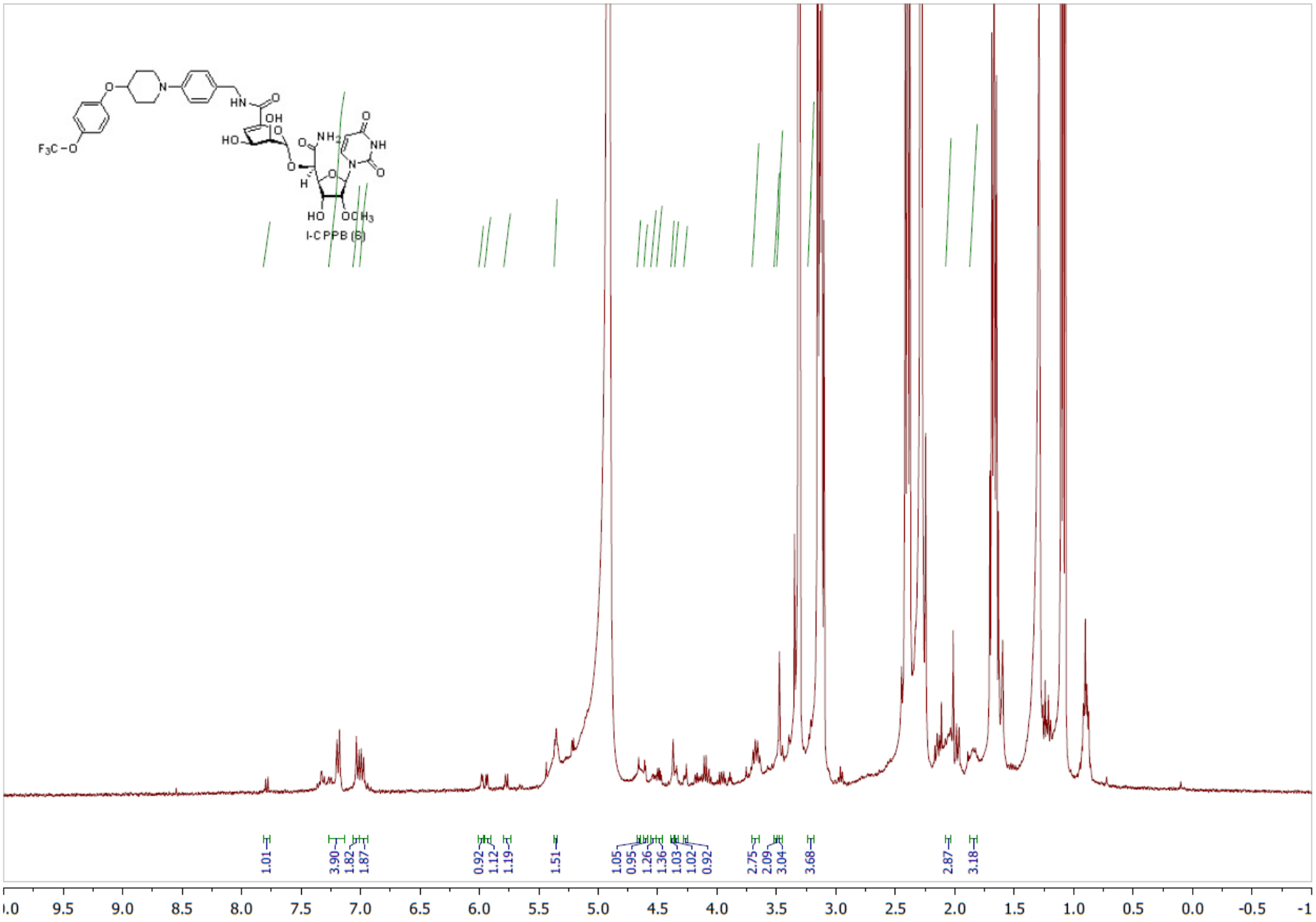
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1.26
1.36
1.03
1.02
0.92
2.75
2.09
3.04
3.68
2.87
3.18
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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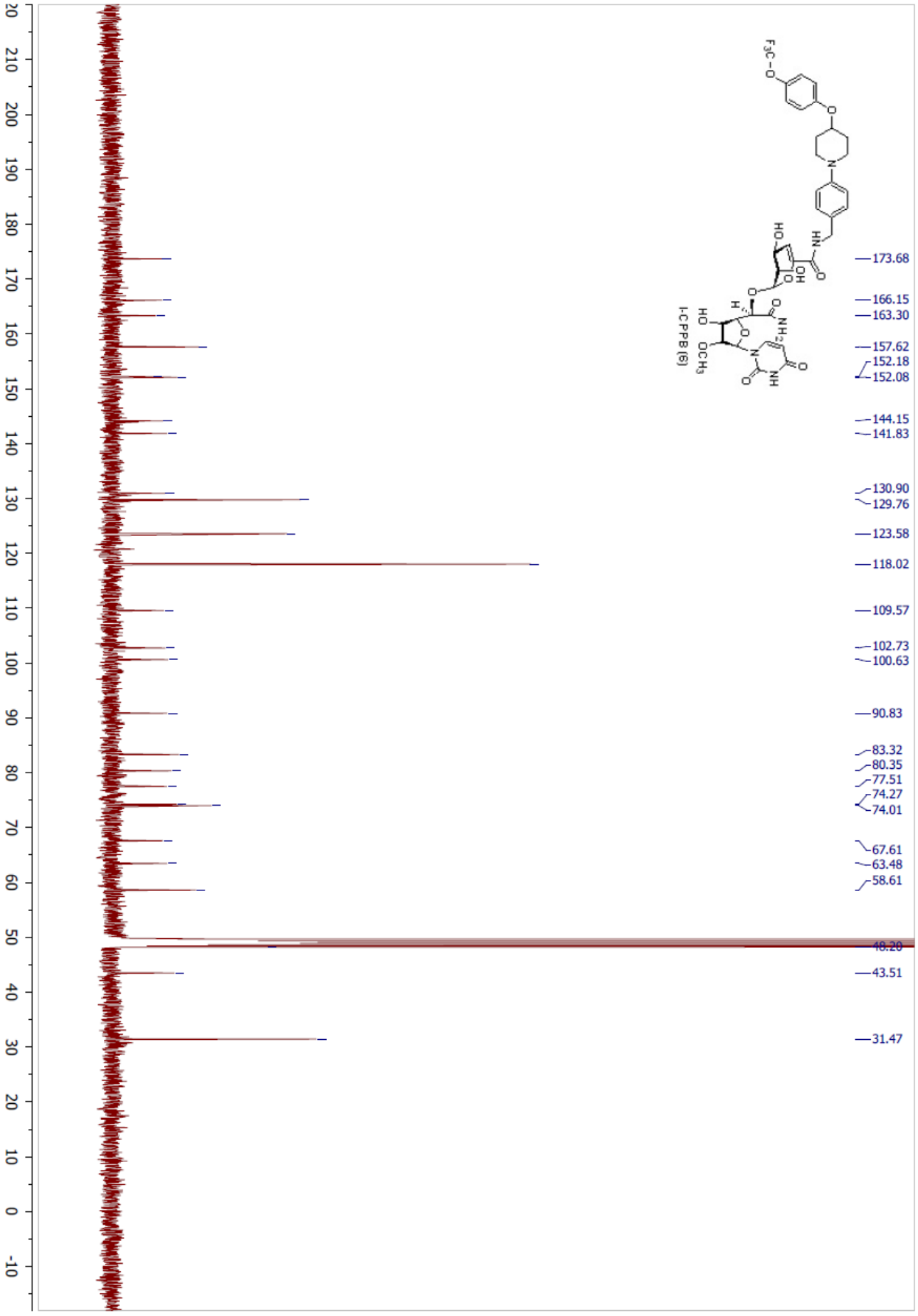
-1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10
0
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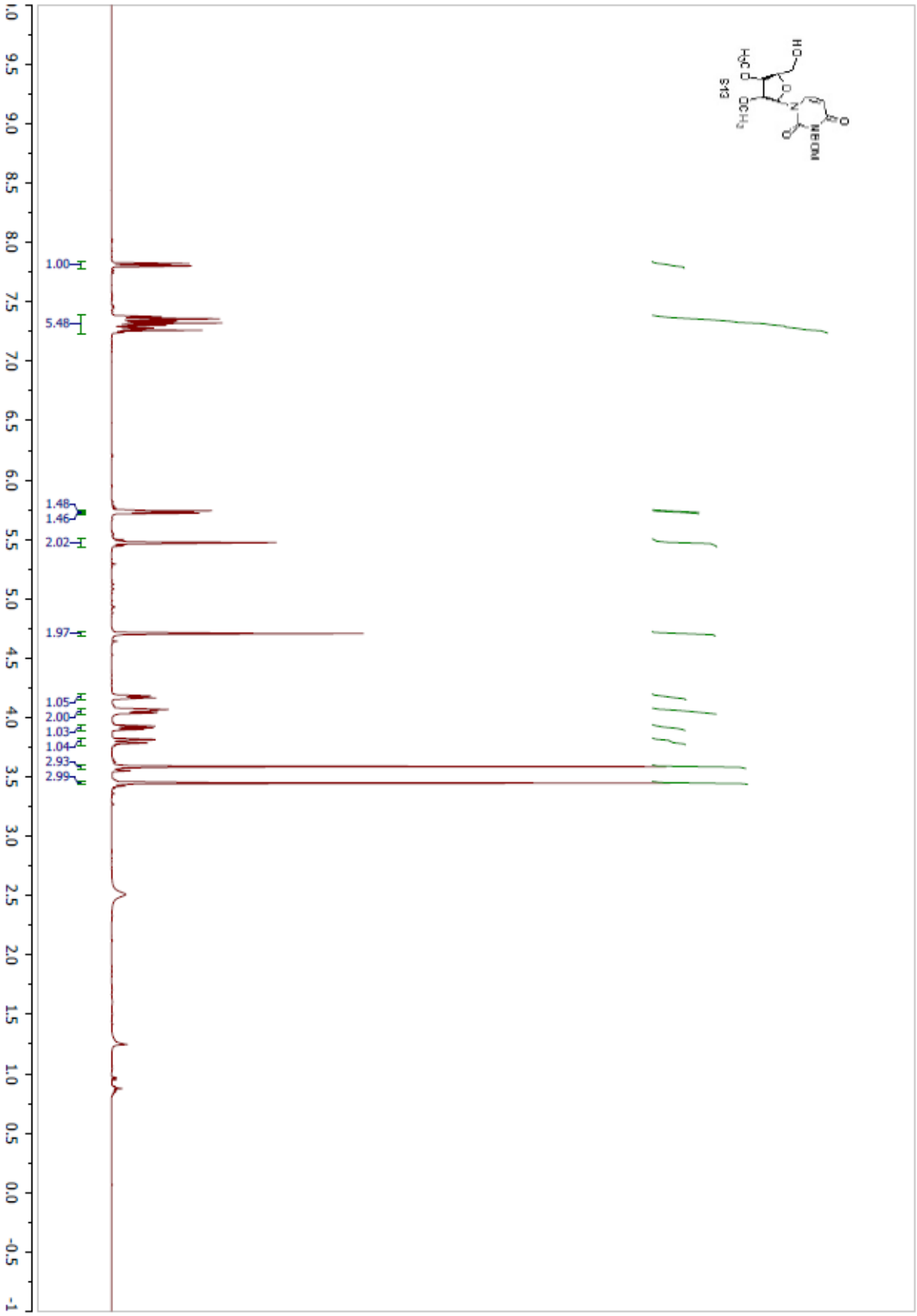
43.51
31.47
F3C
O
O
N
NH
O
OH
OH
O
H
O
HO
OCH3
N
O
NH
O
NH
ICPPB (6)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1.04
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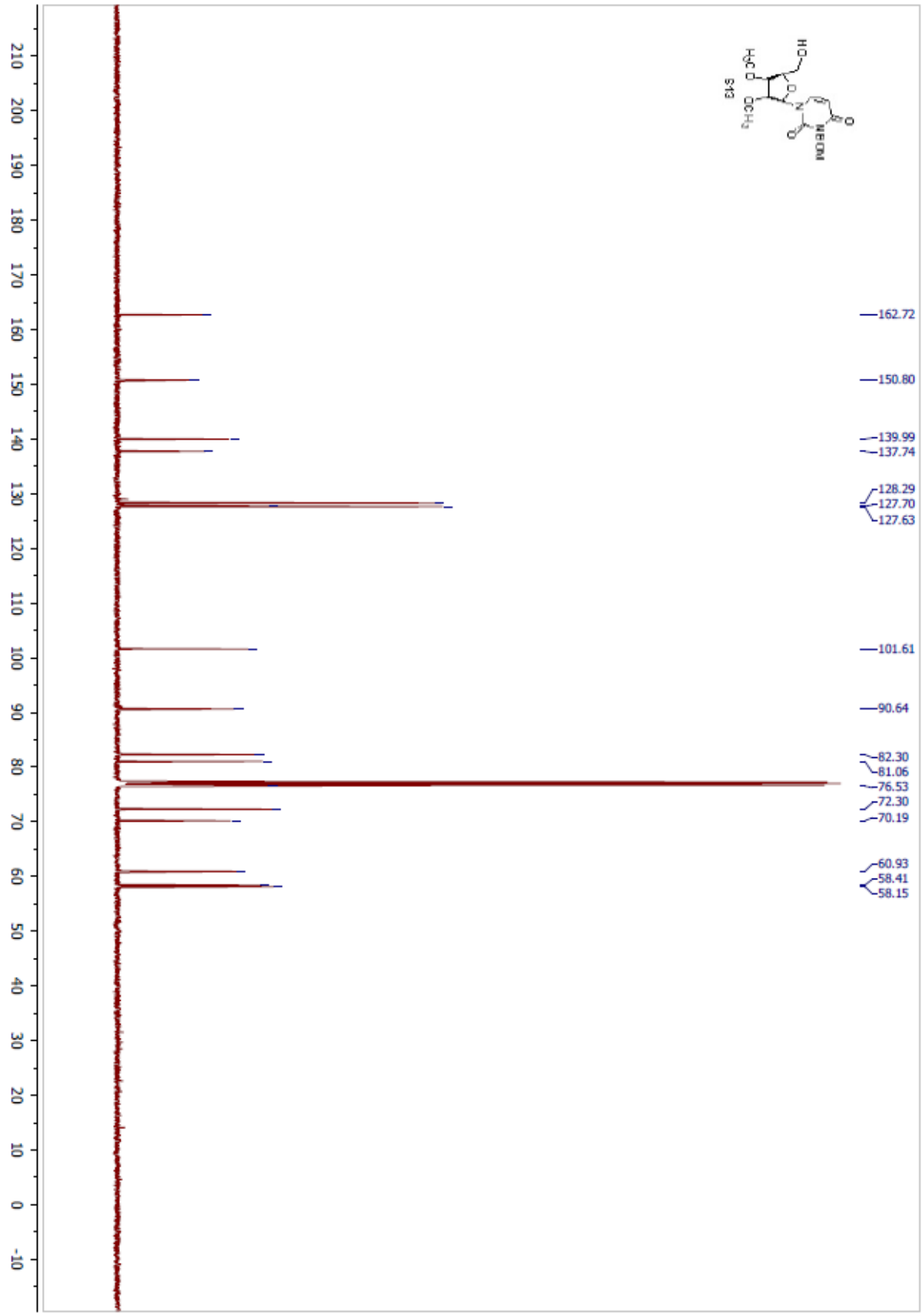
2.99
S13
MeBDM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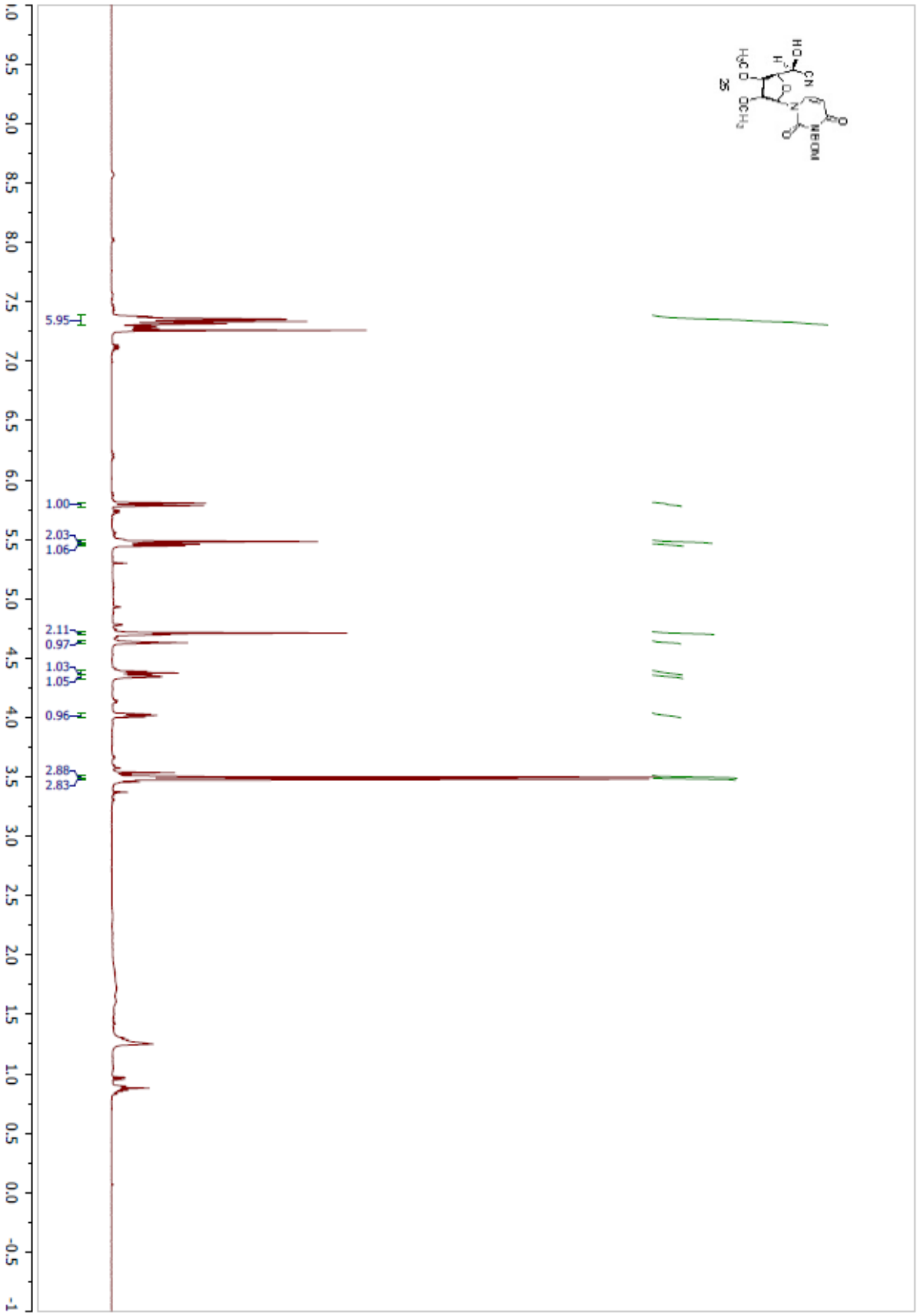
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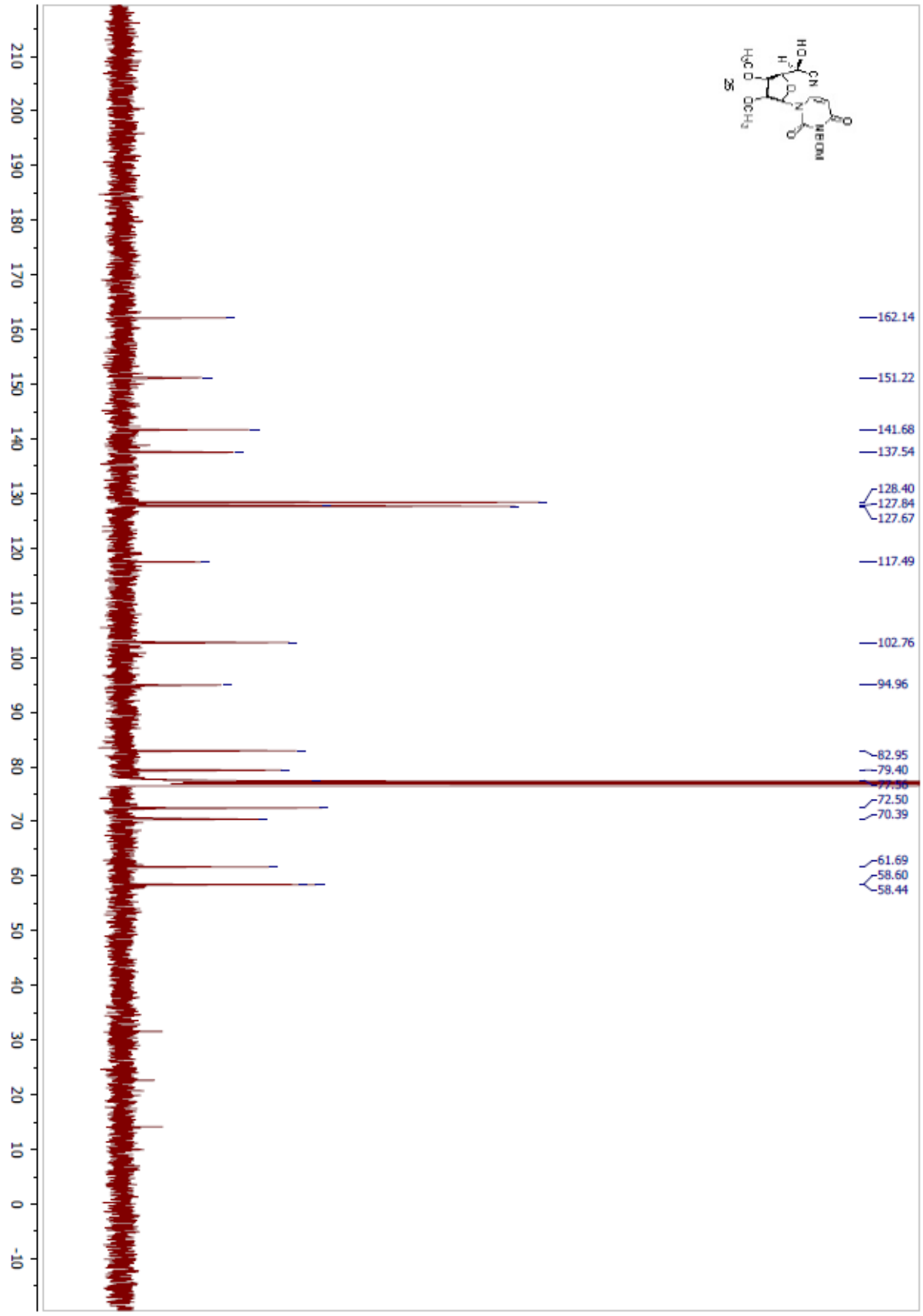
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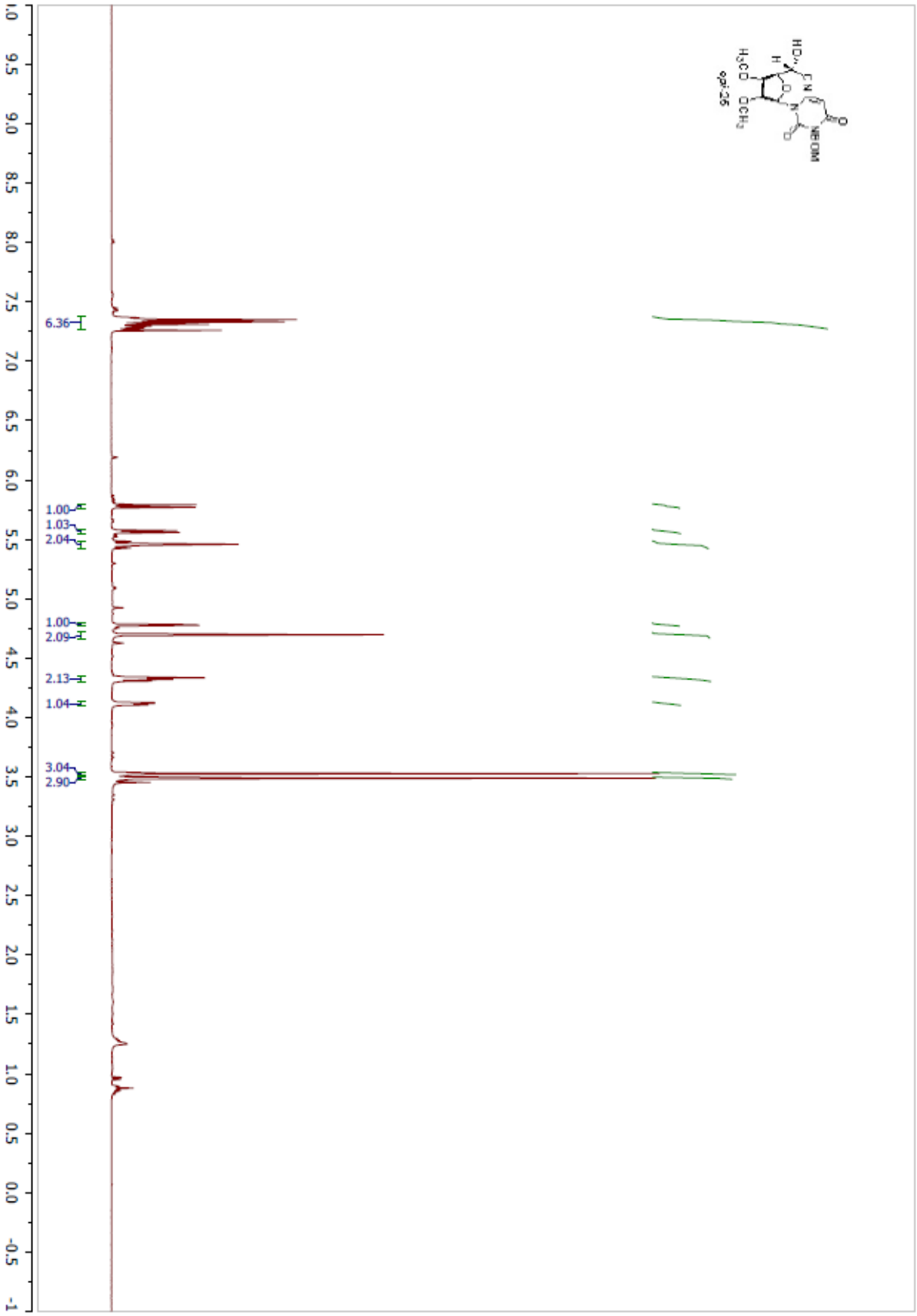
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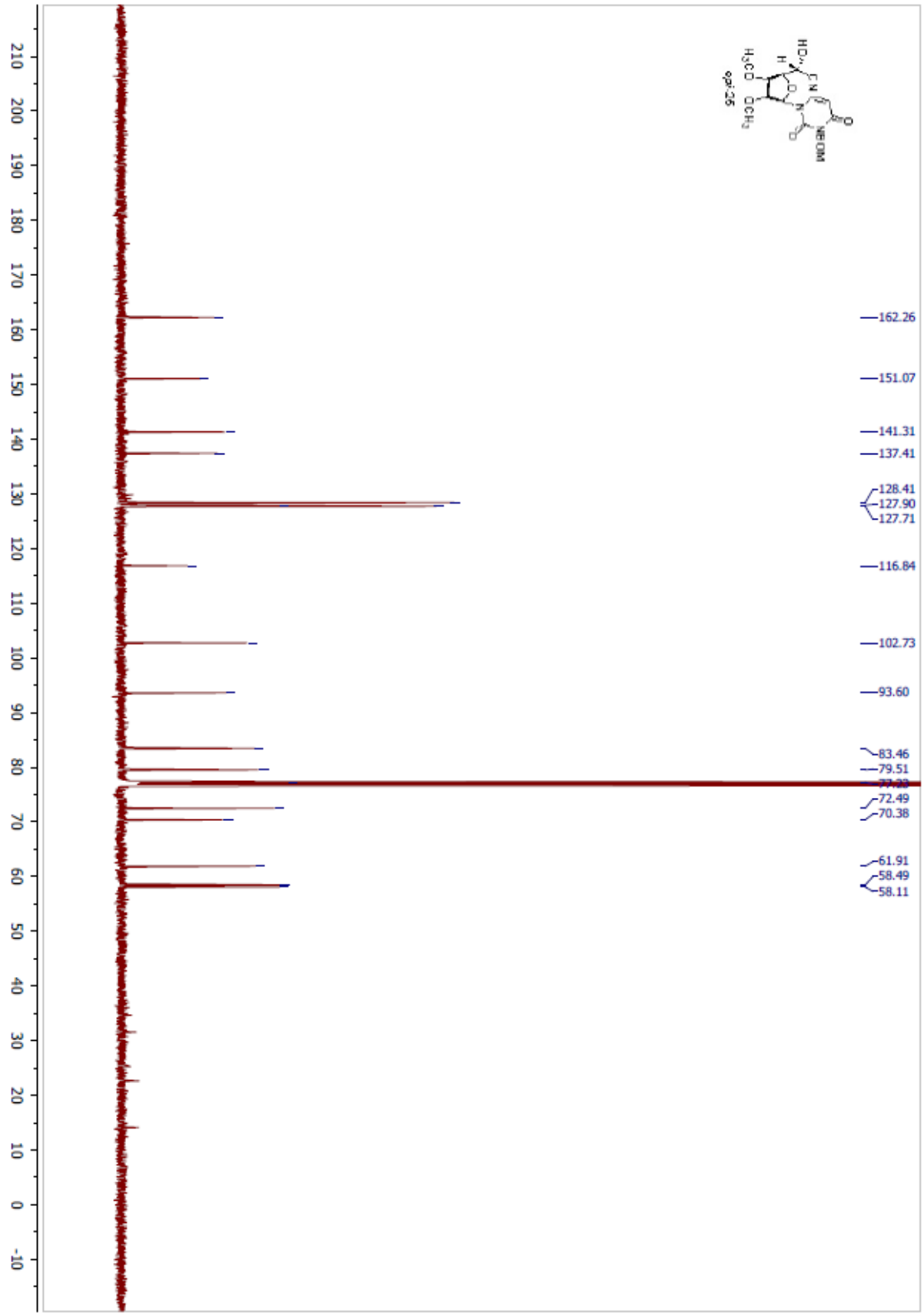
NBDM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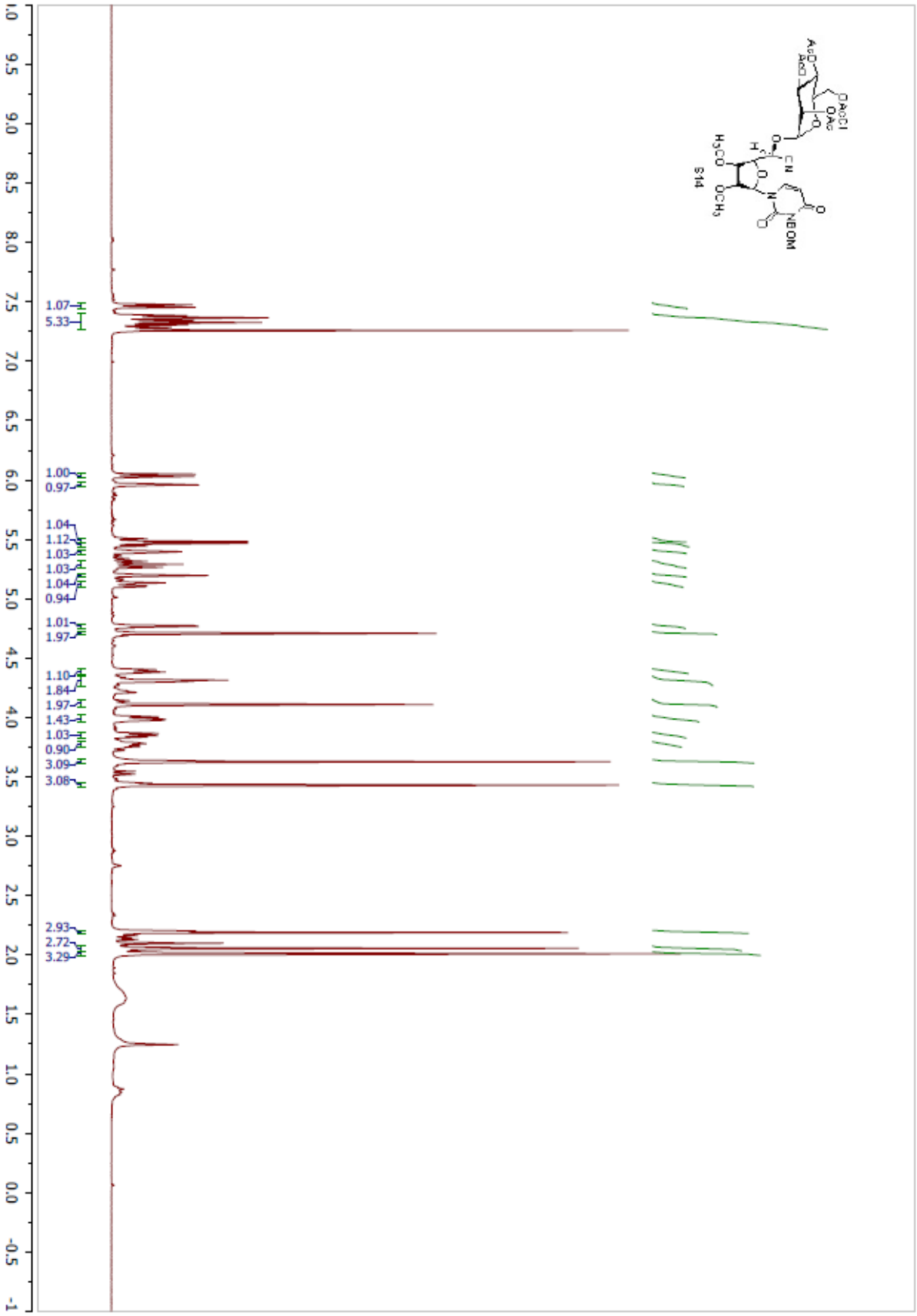
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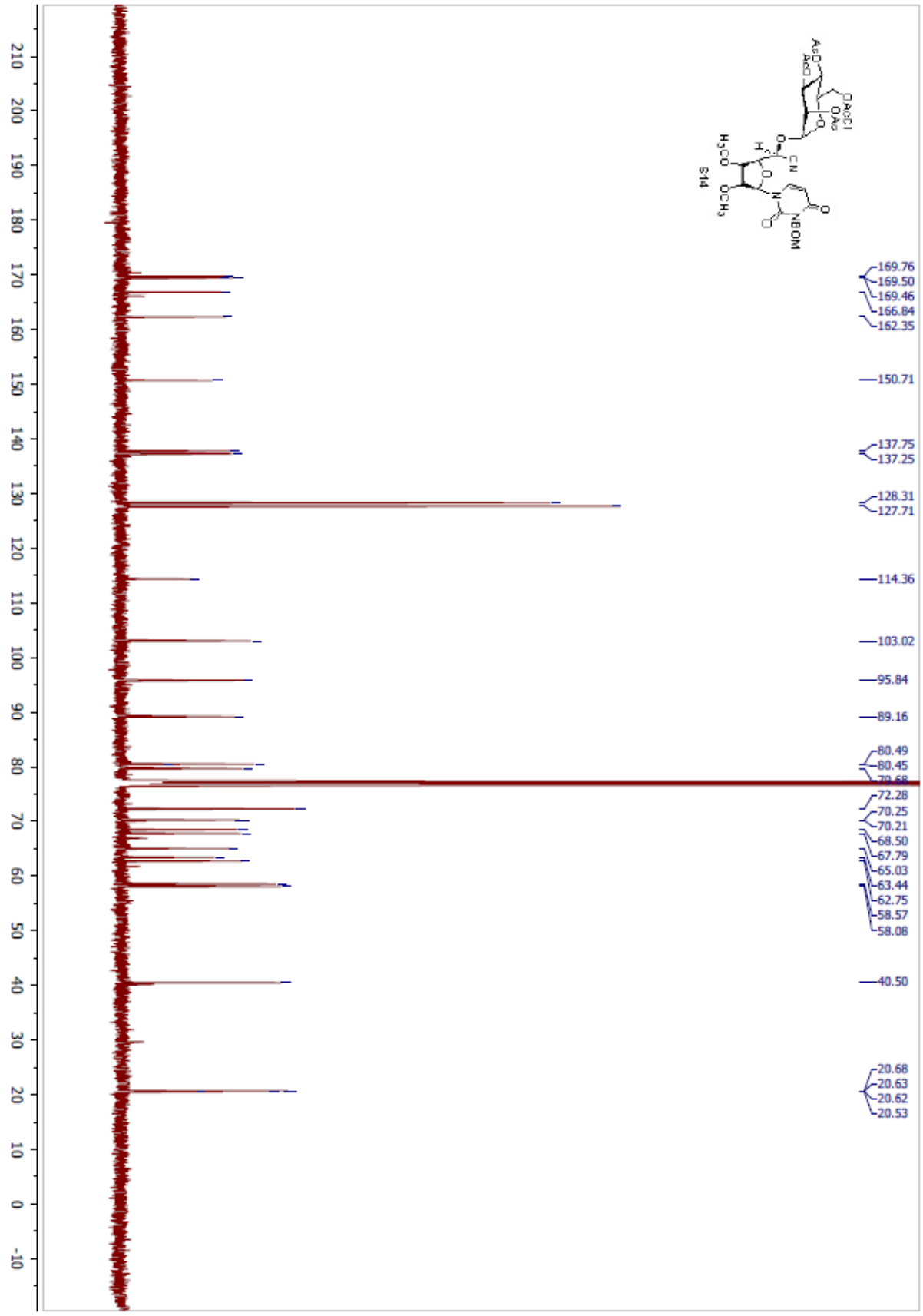
S14
NBOM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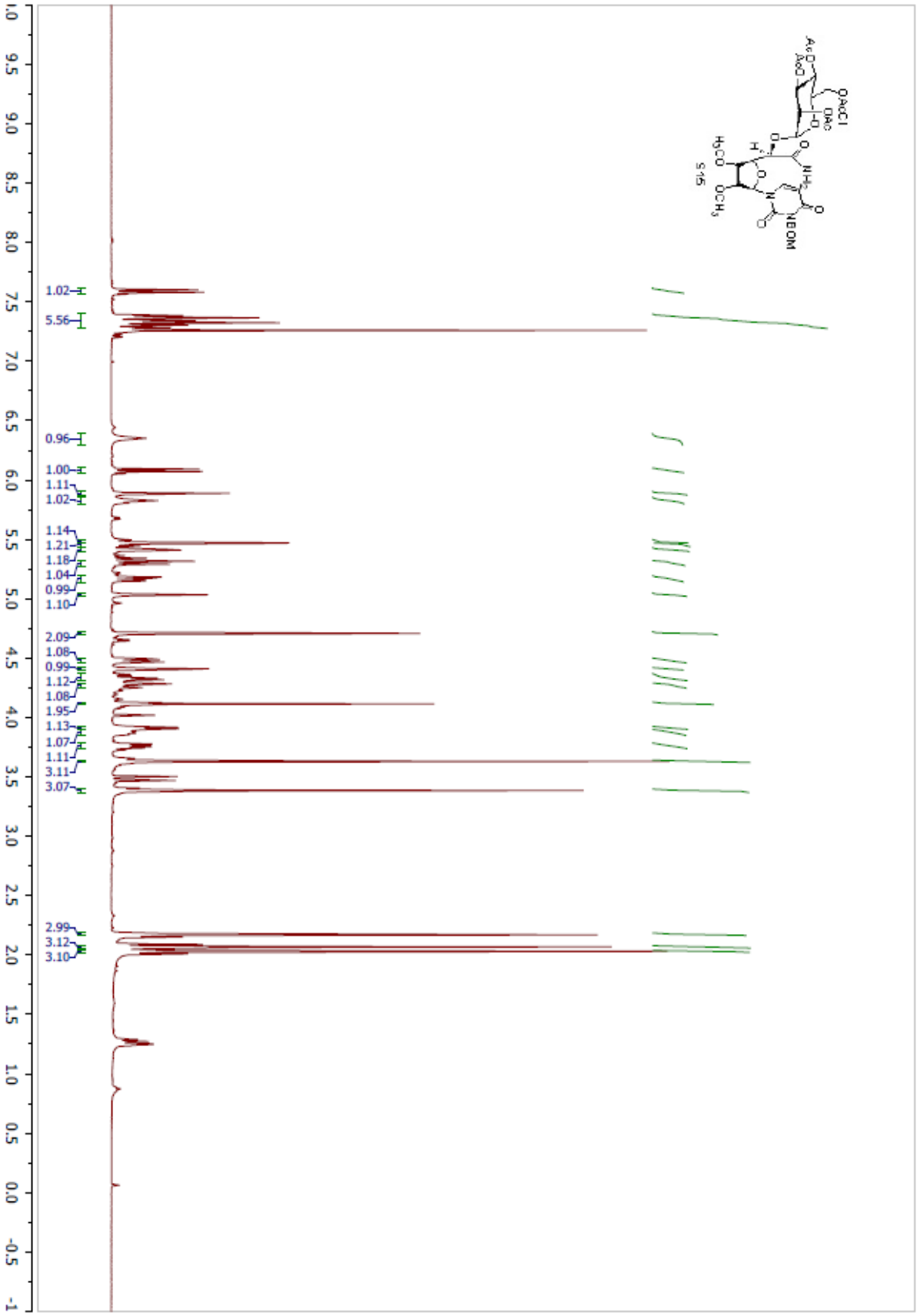
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2.09
1.08
0.99
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1.08
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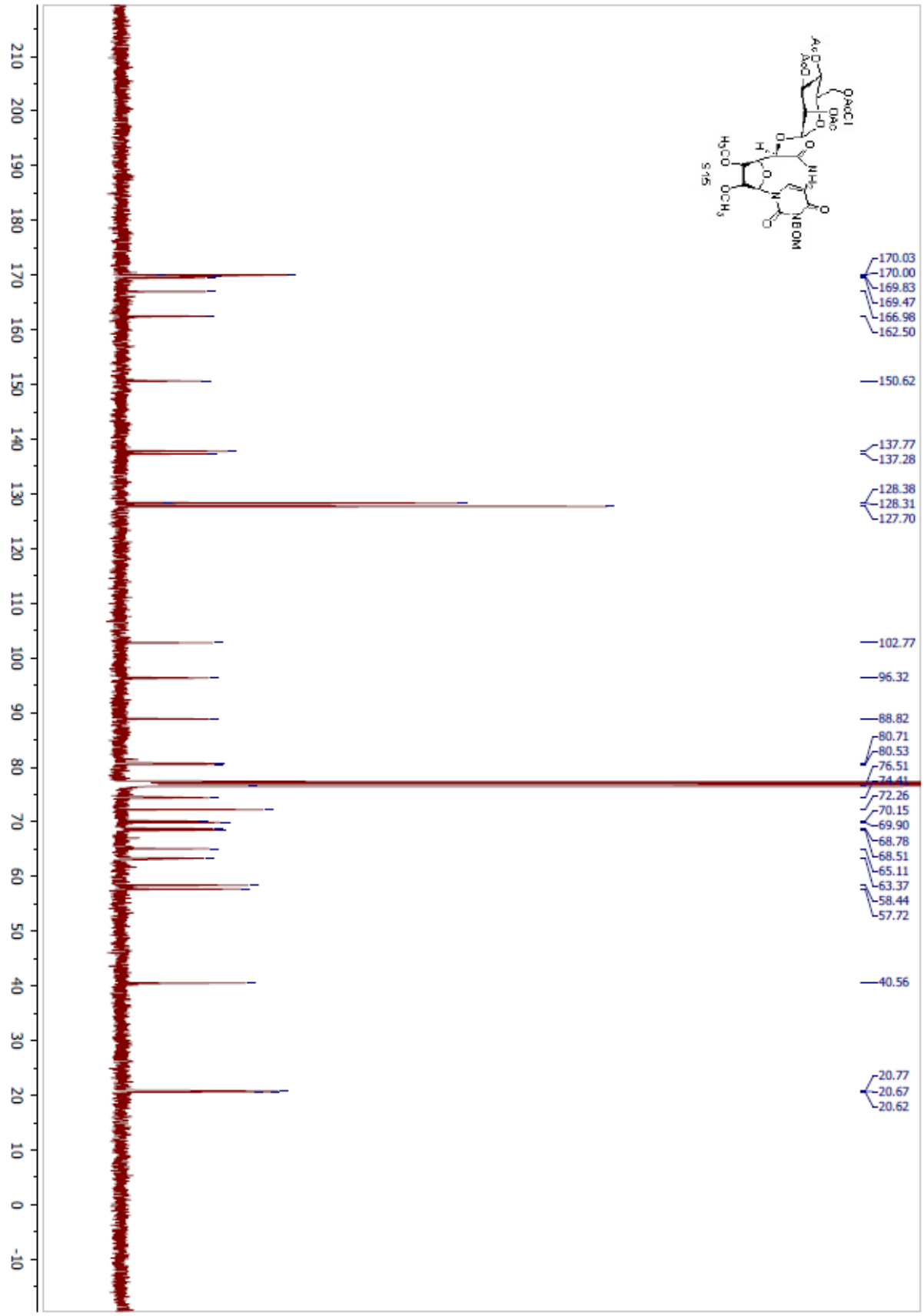
H3CO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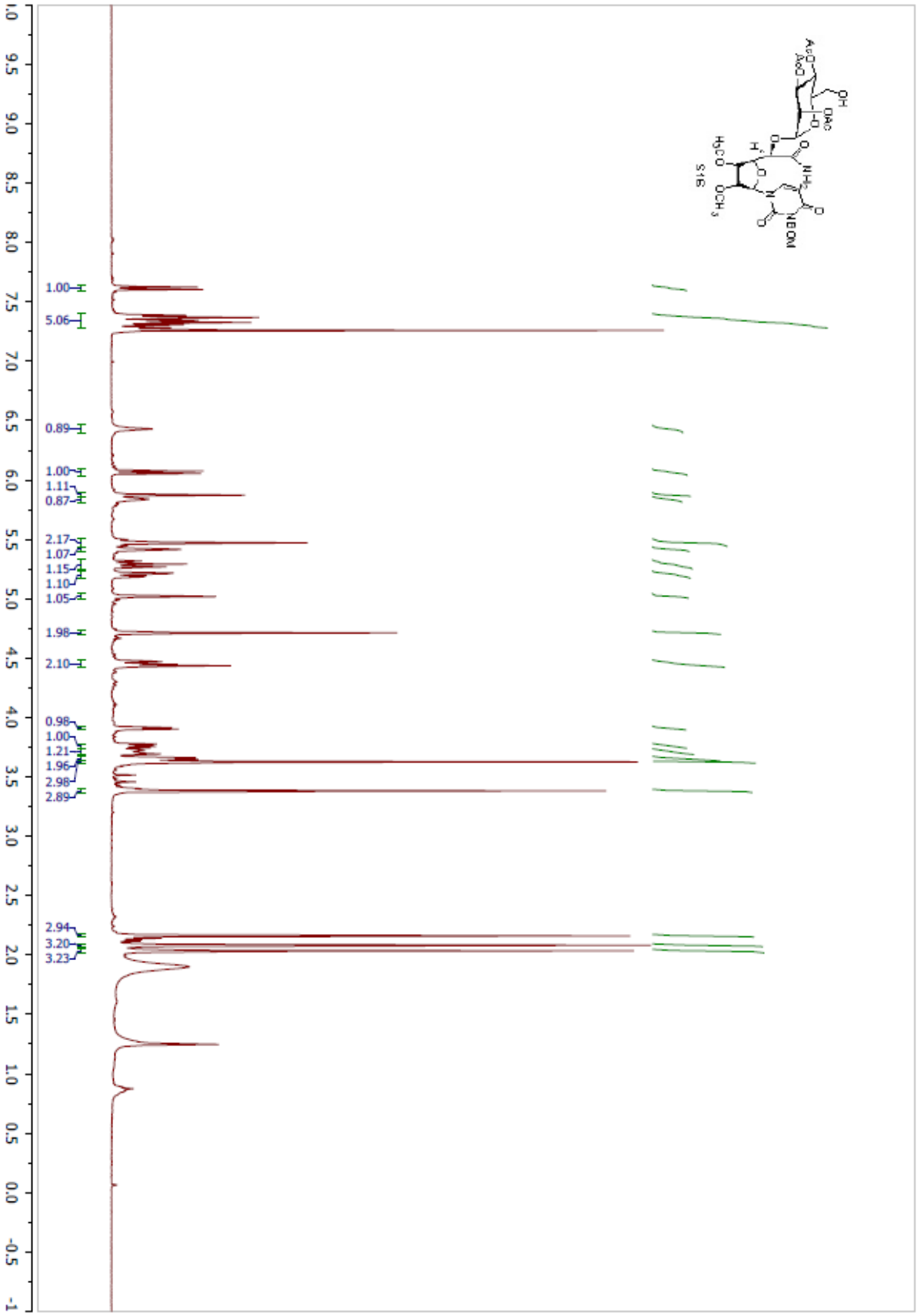
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S16
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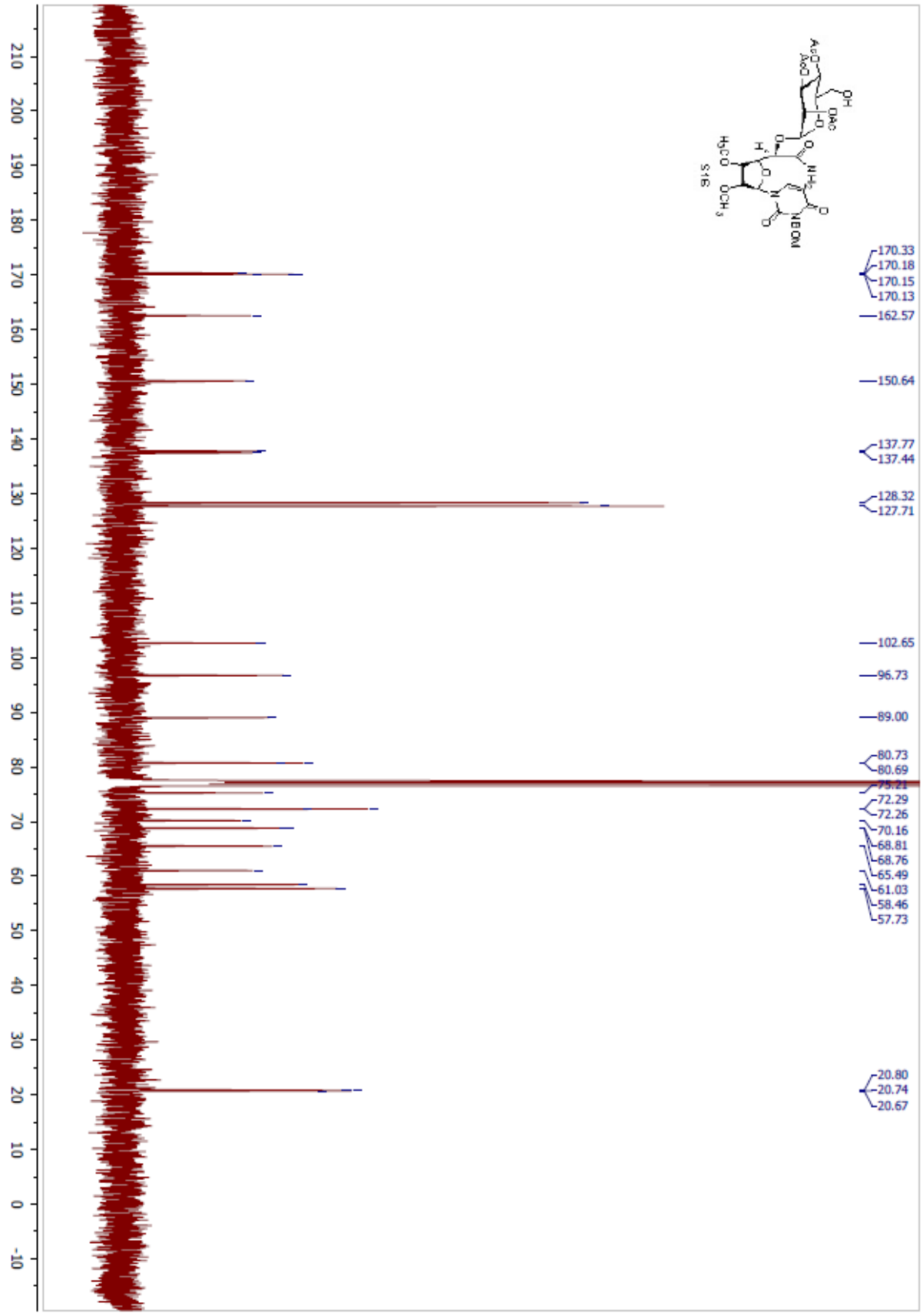
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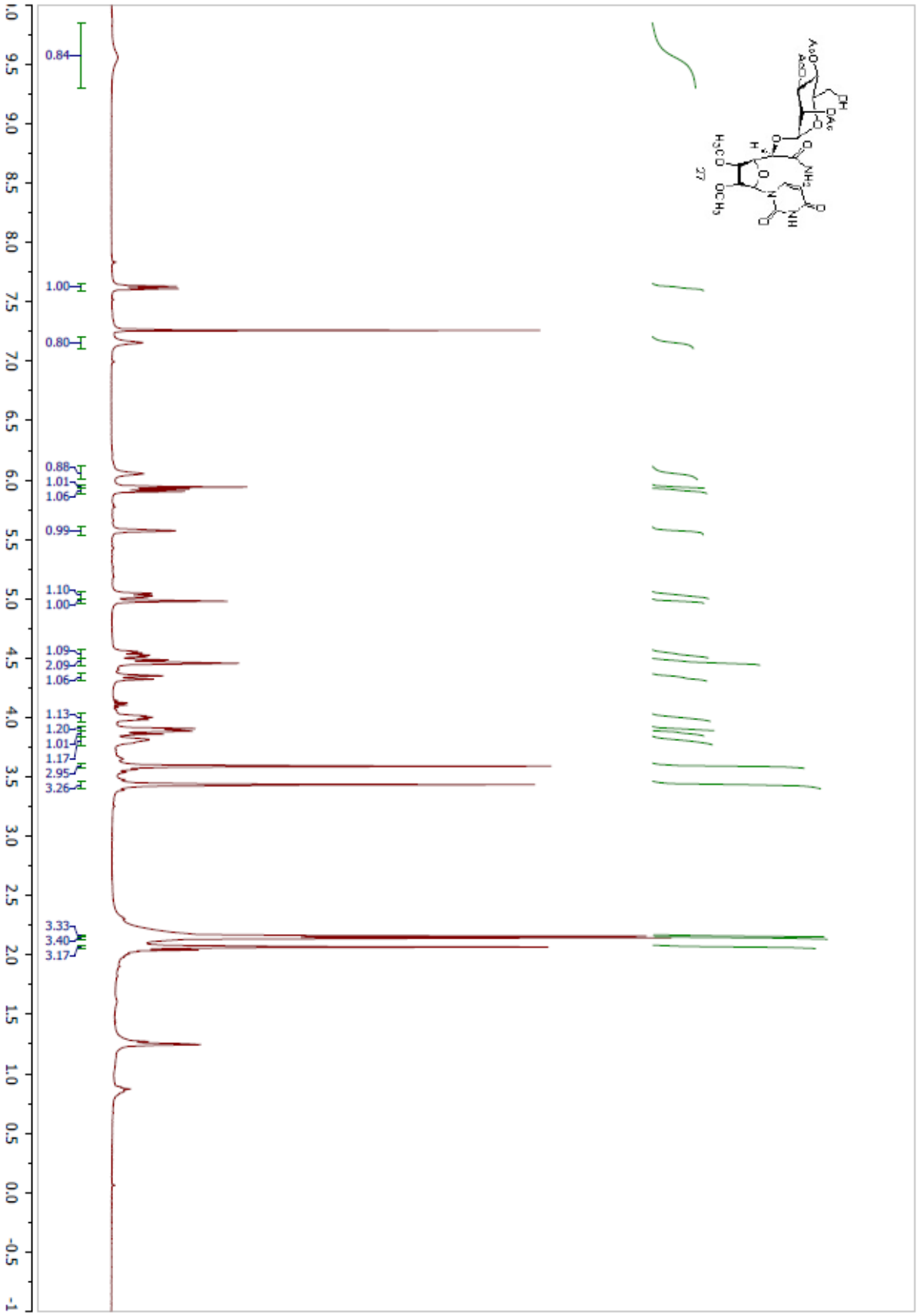
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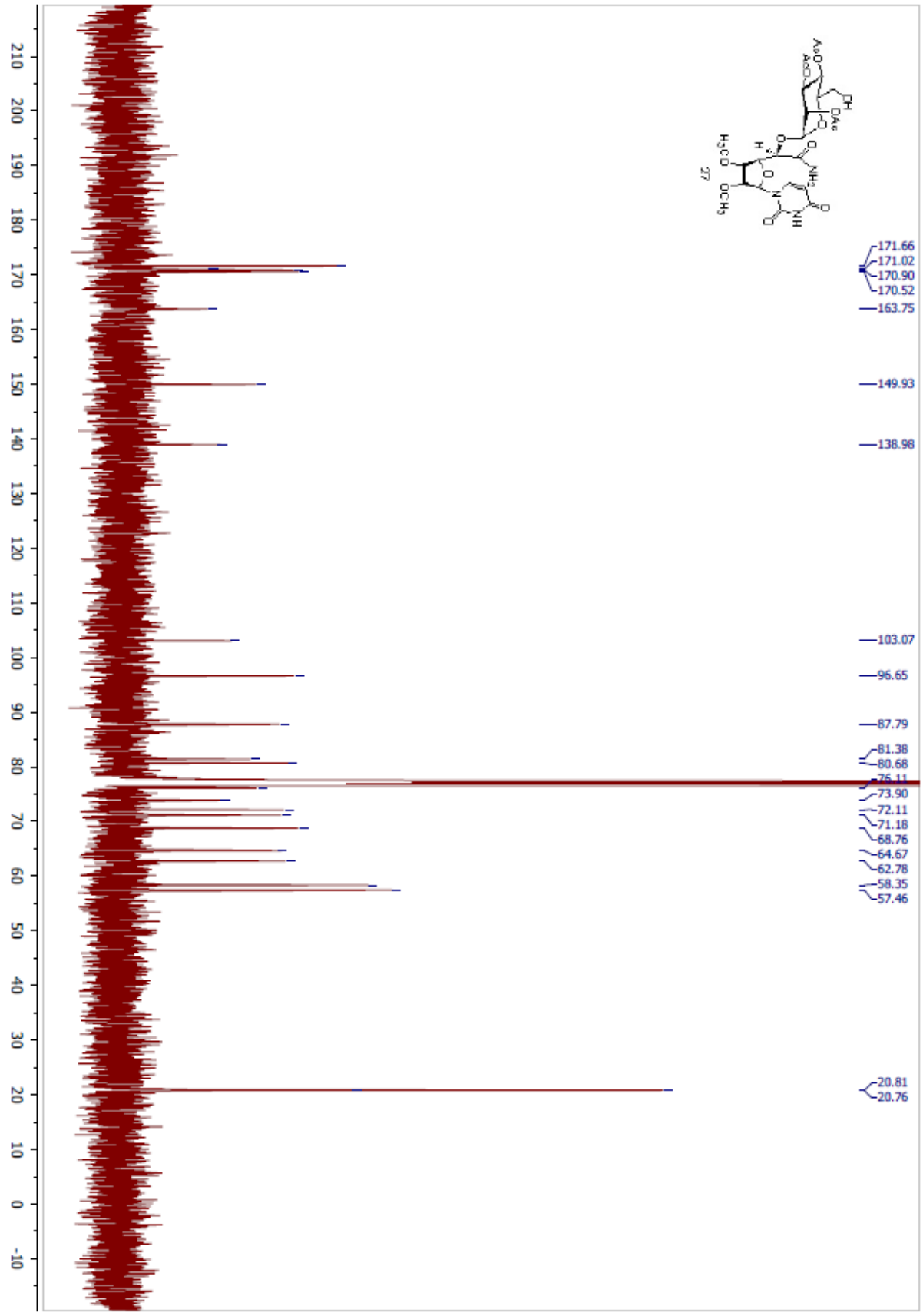
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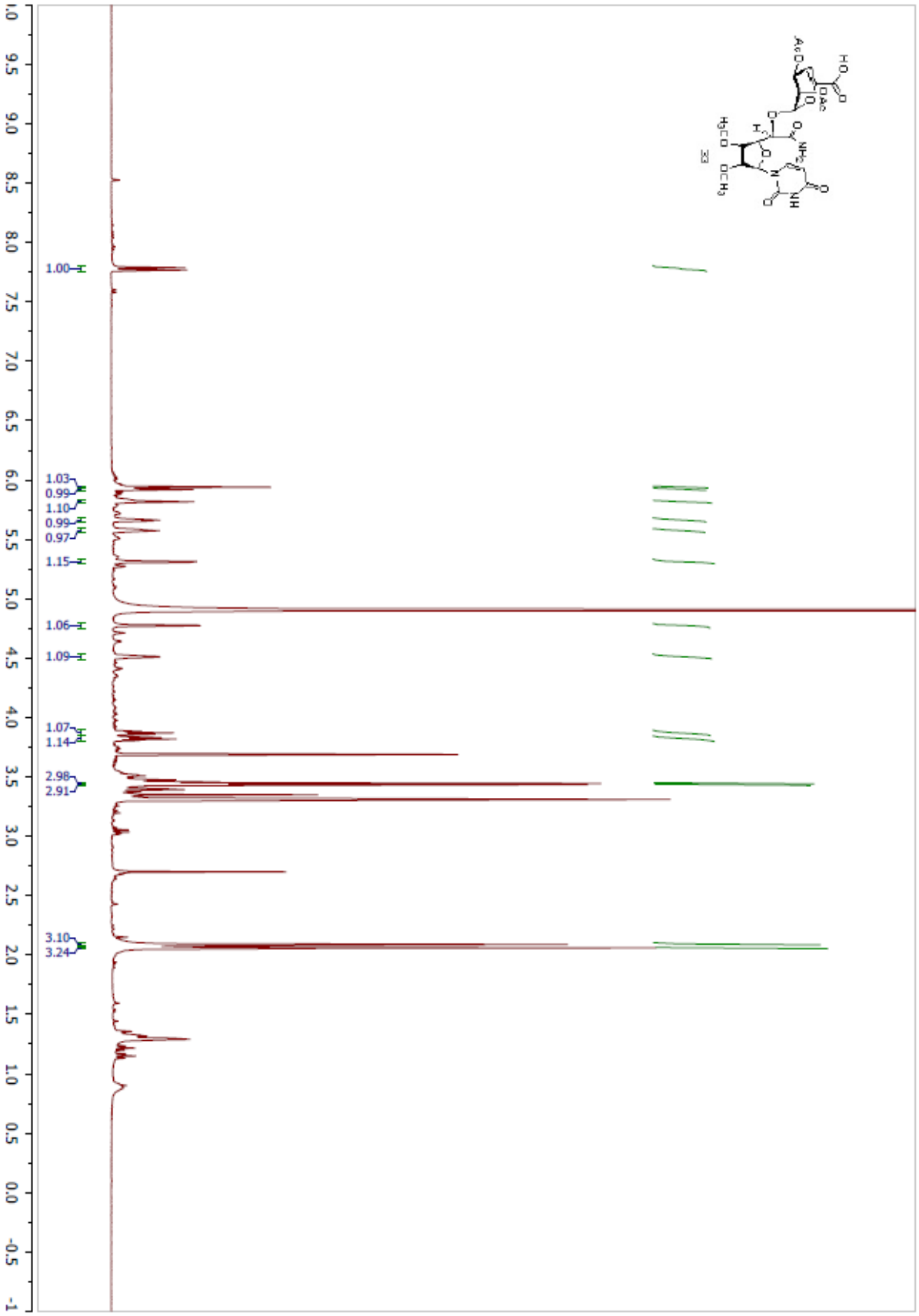
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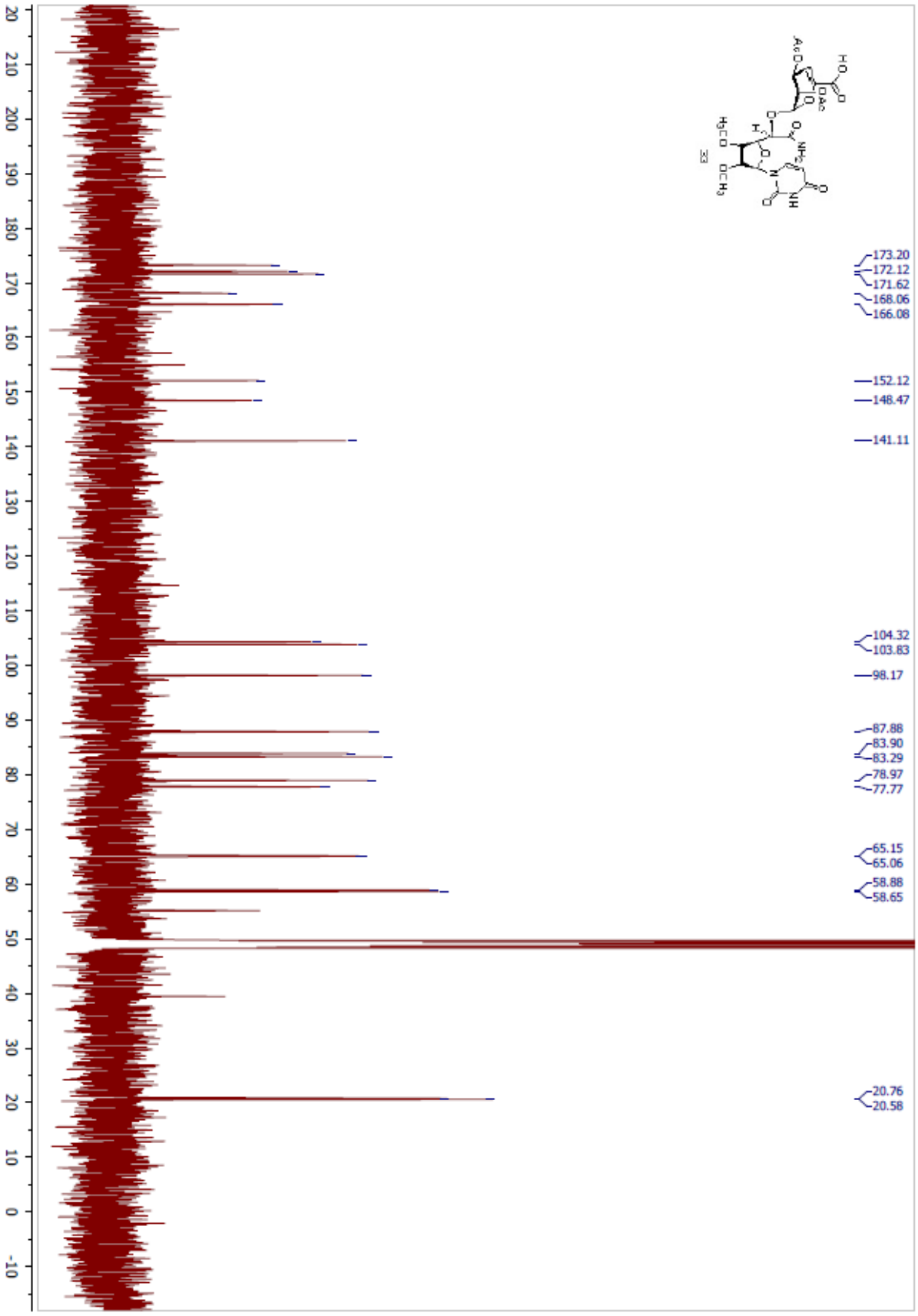
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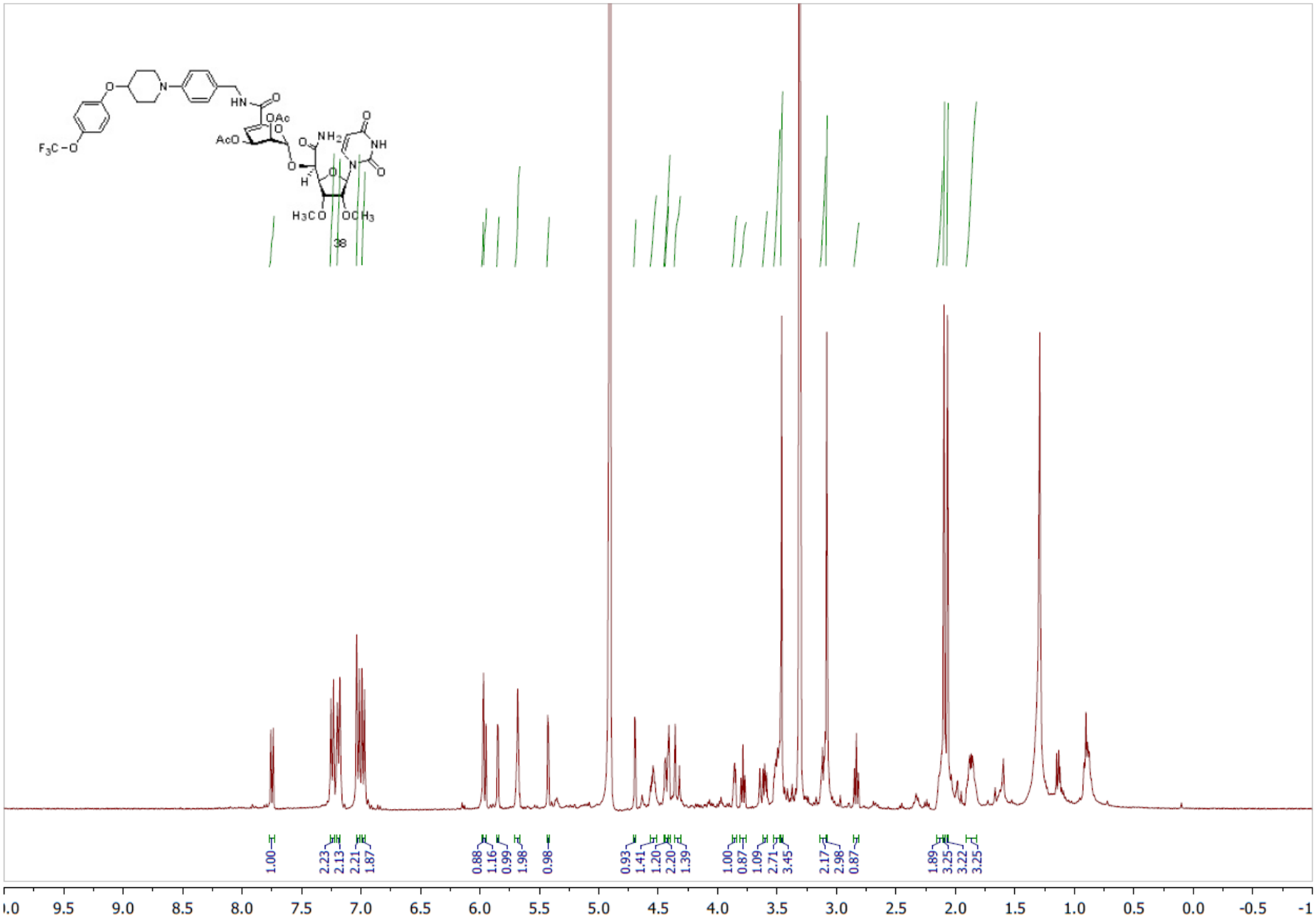
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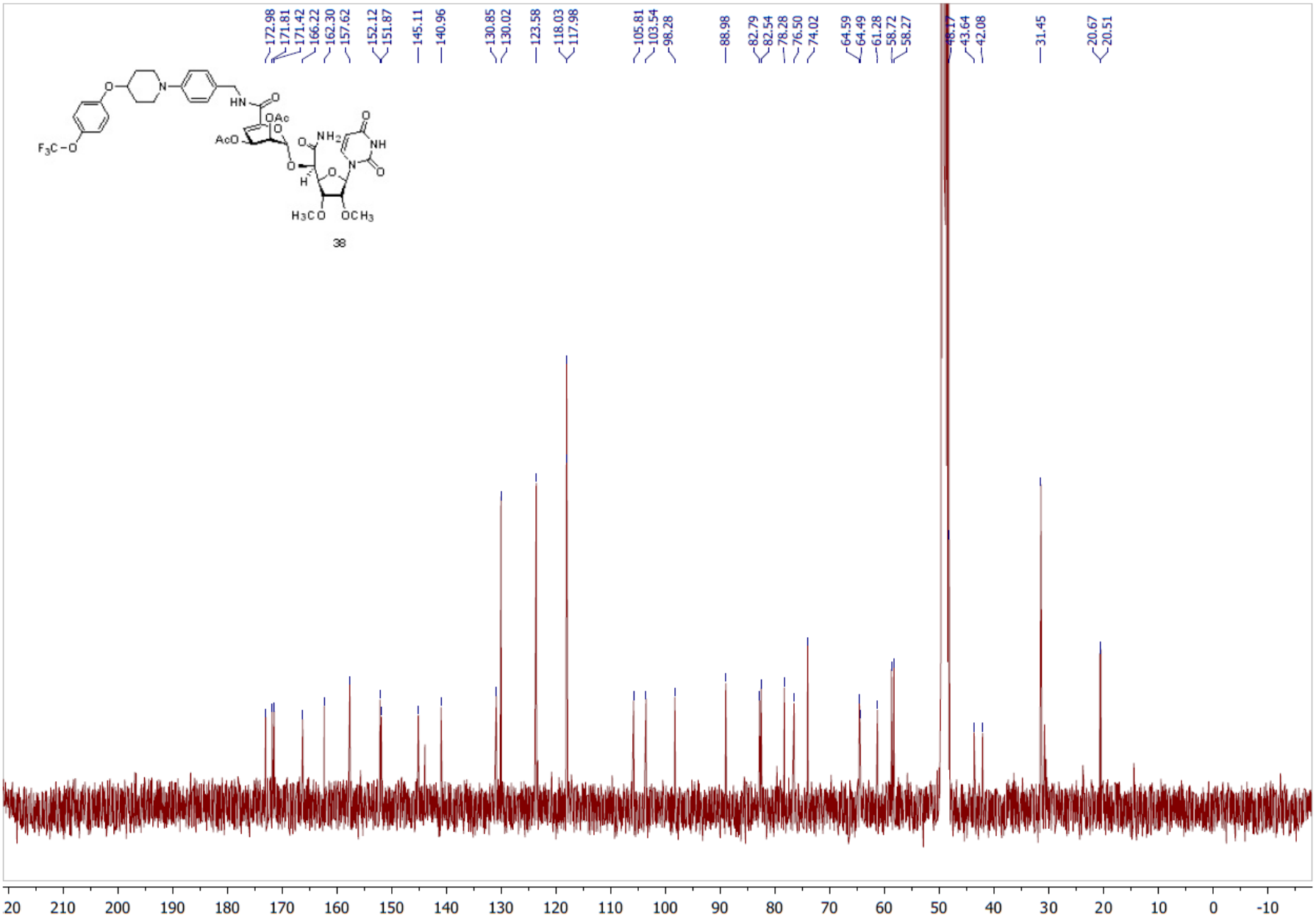
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61.28
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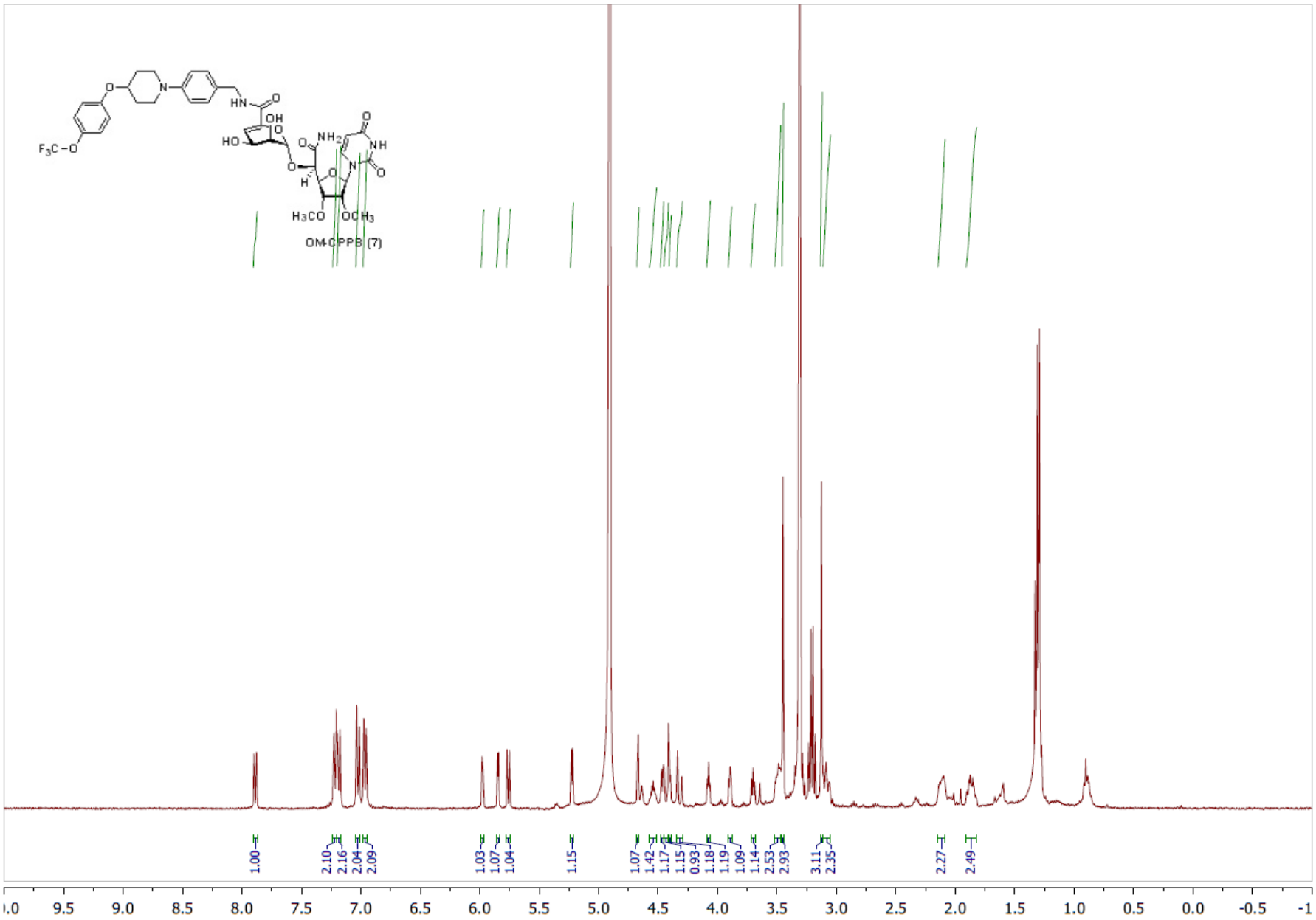
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1.19
1.09
1.14
2.53
2.93
3.11
2.35
2.27
2.49
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5.0
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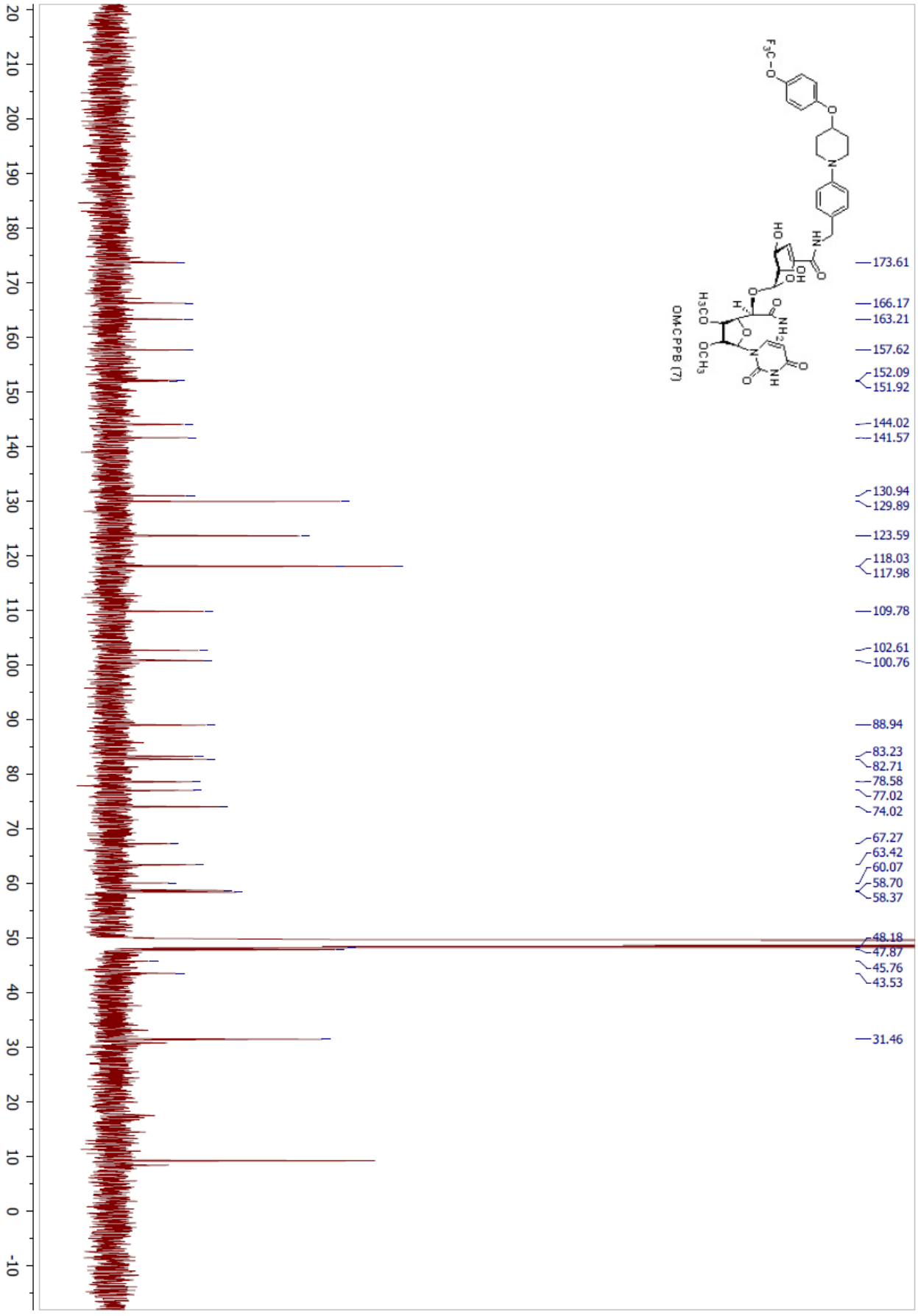
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20
10
0
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163.21
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152.09
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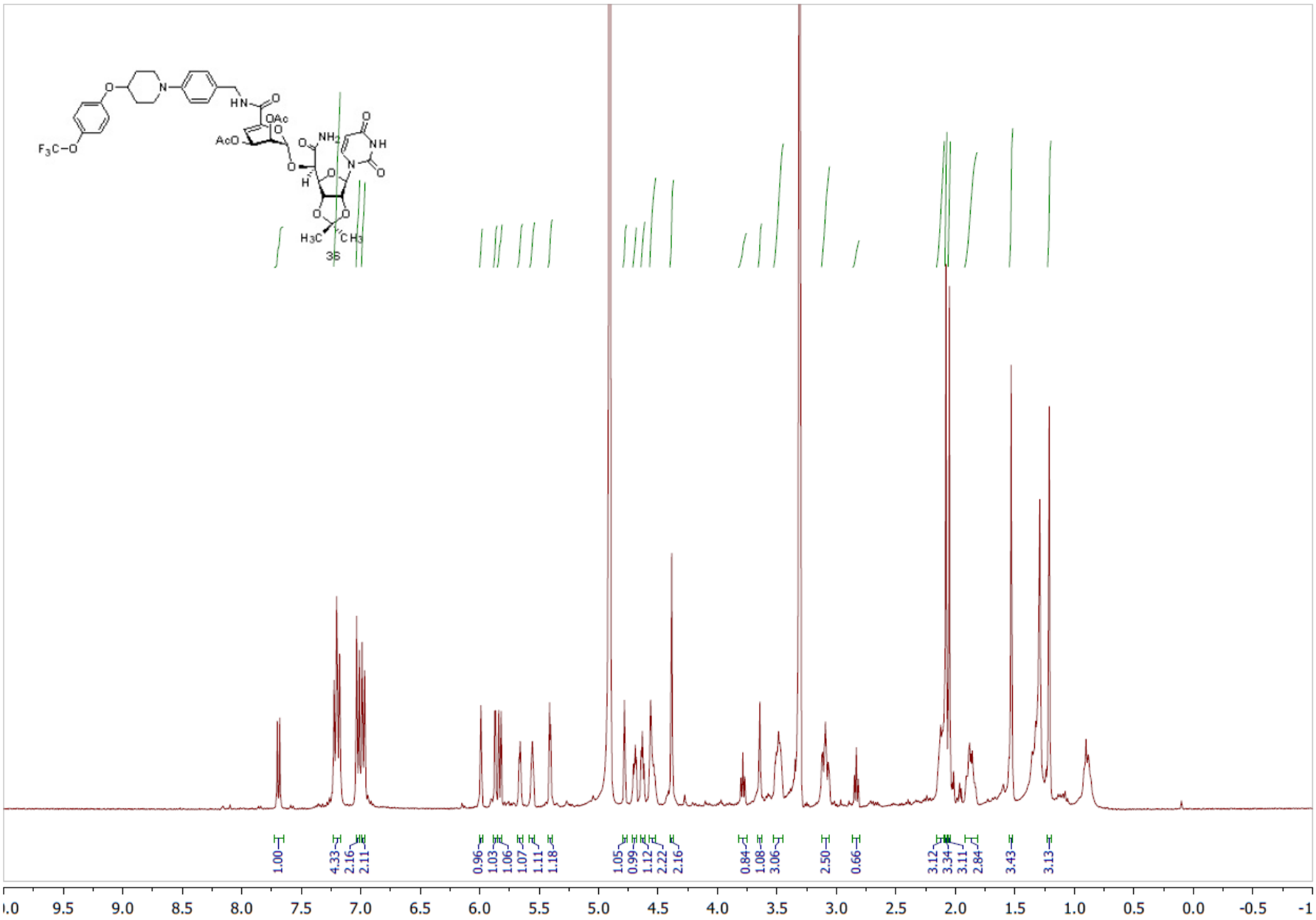
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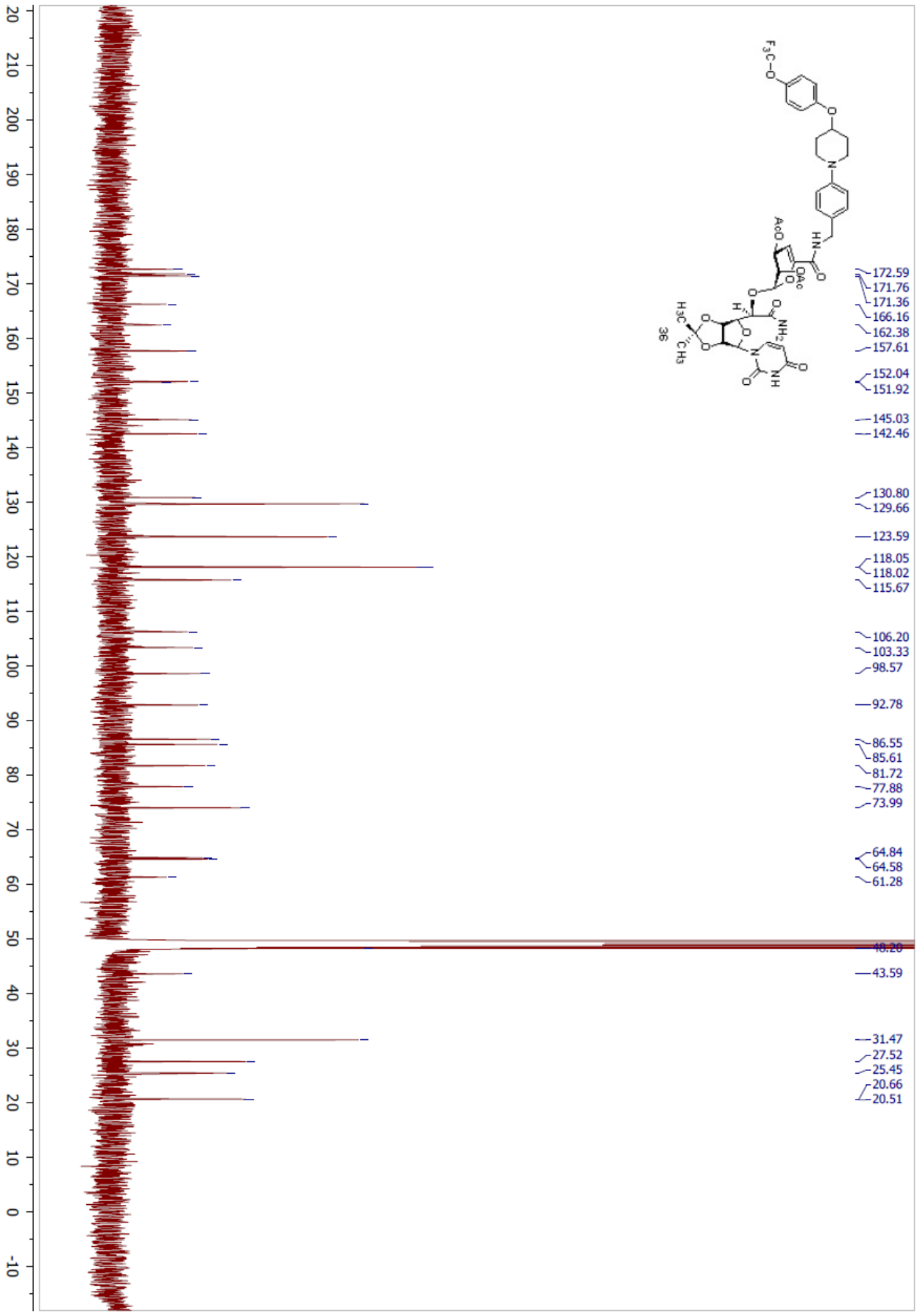
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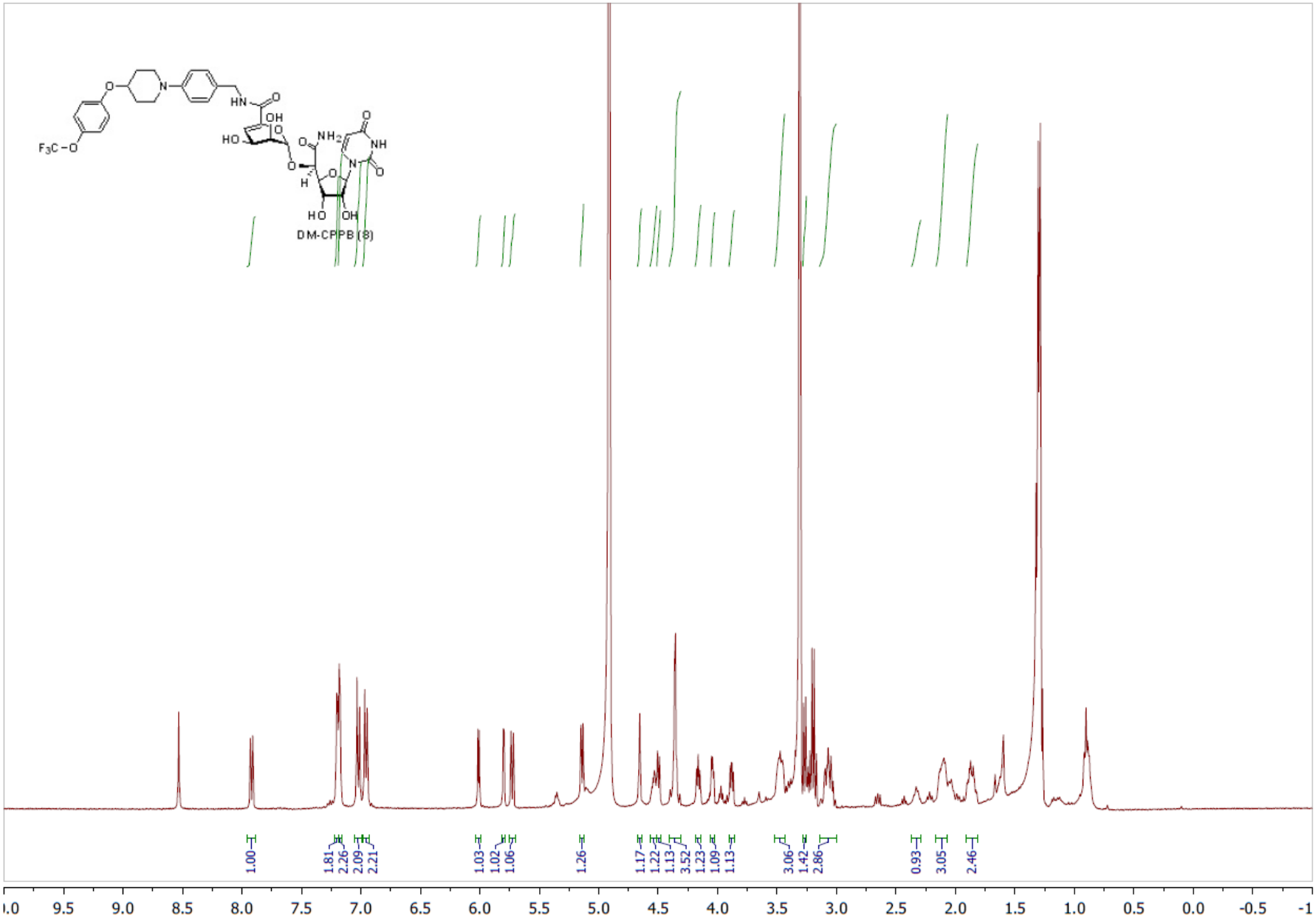
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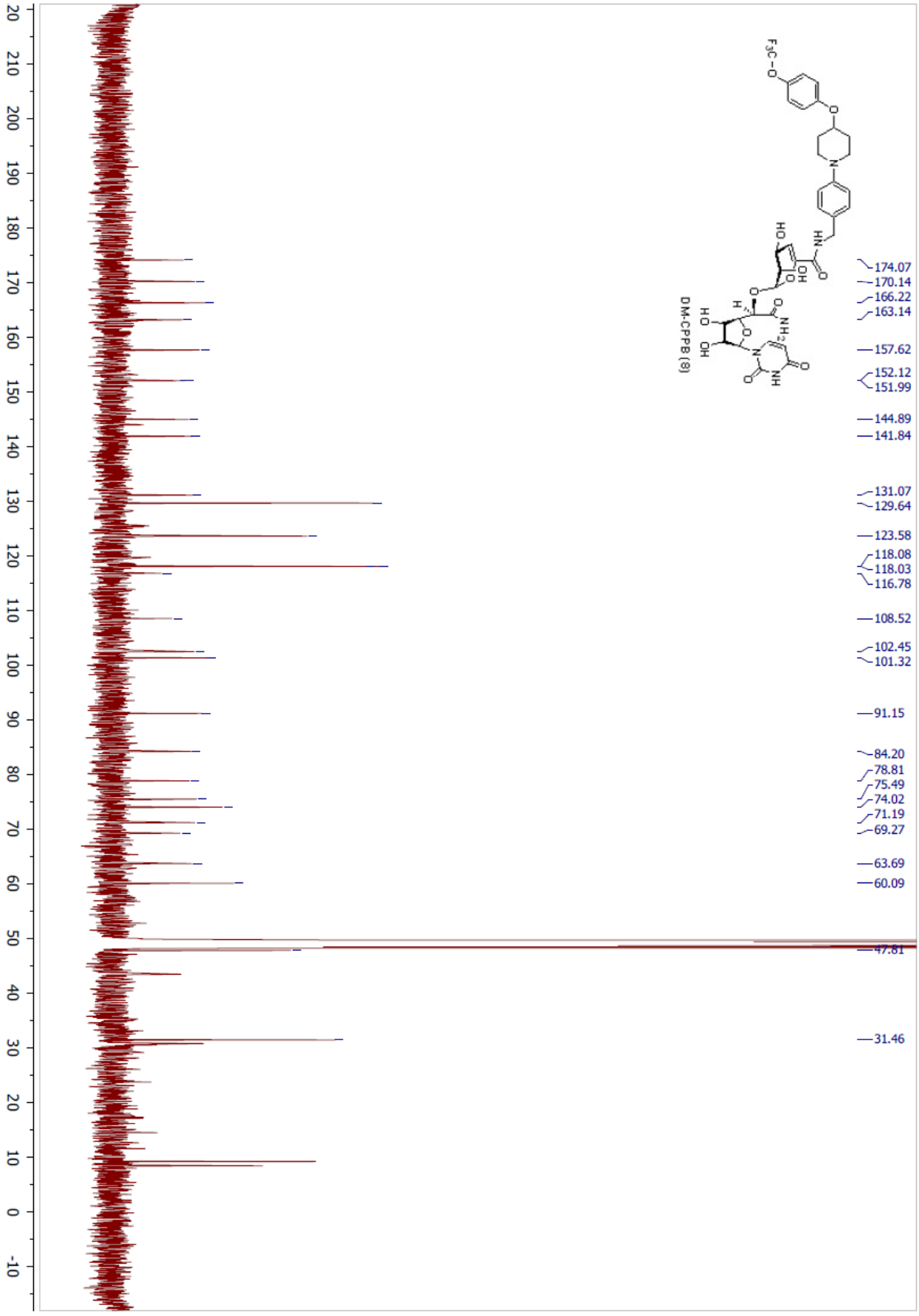
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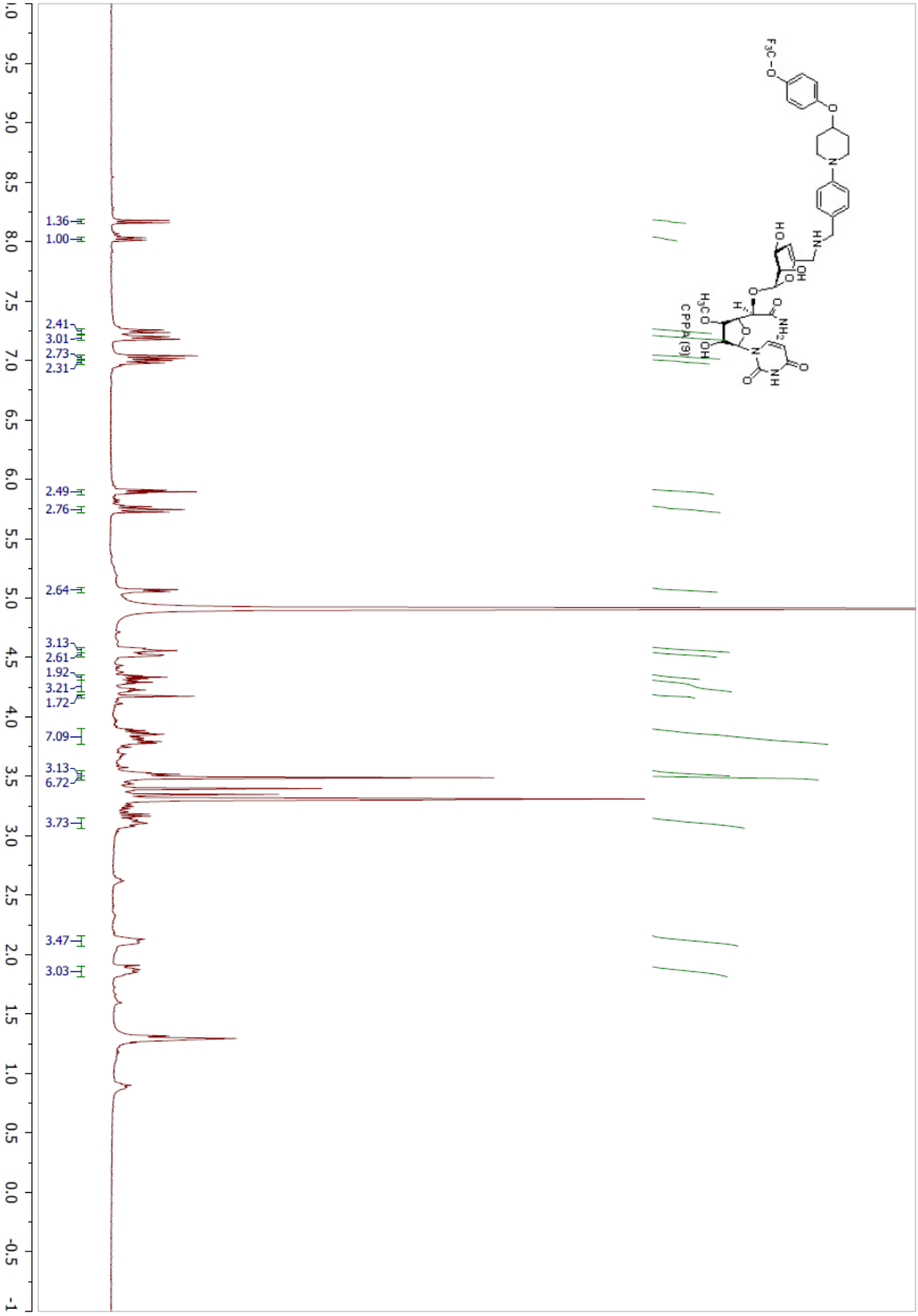
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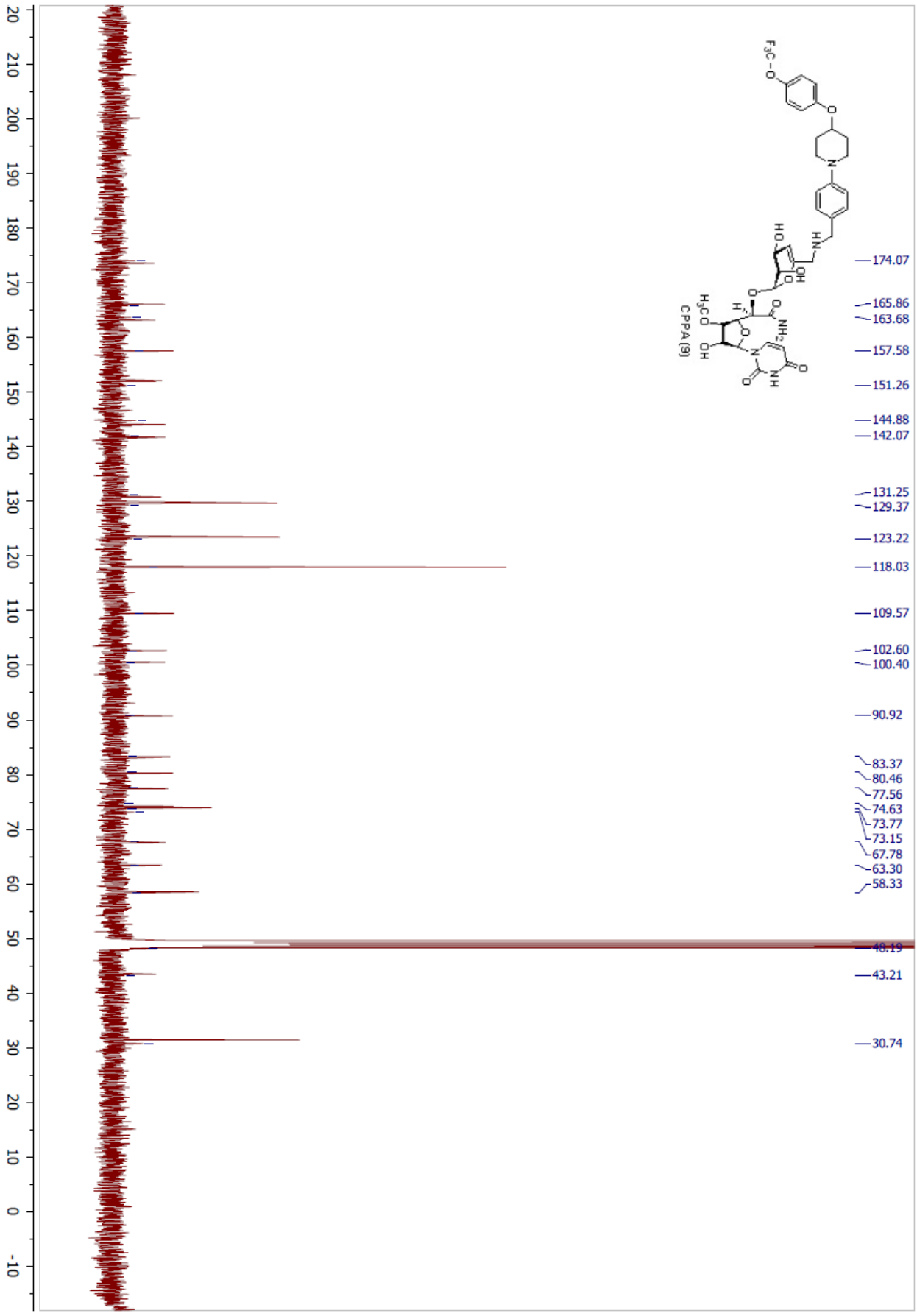
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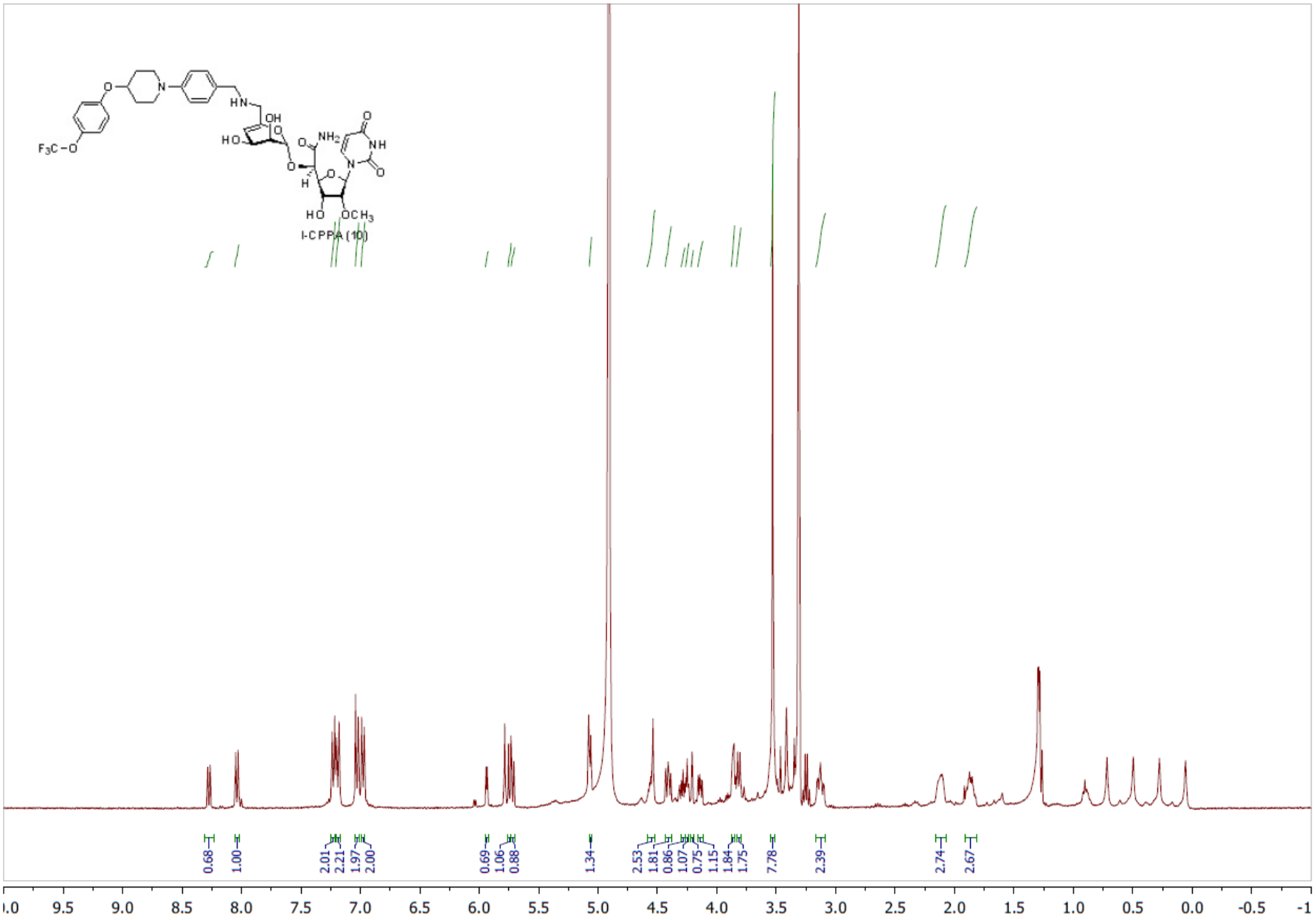
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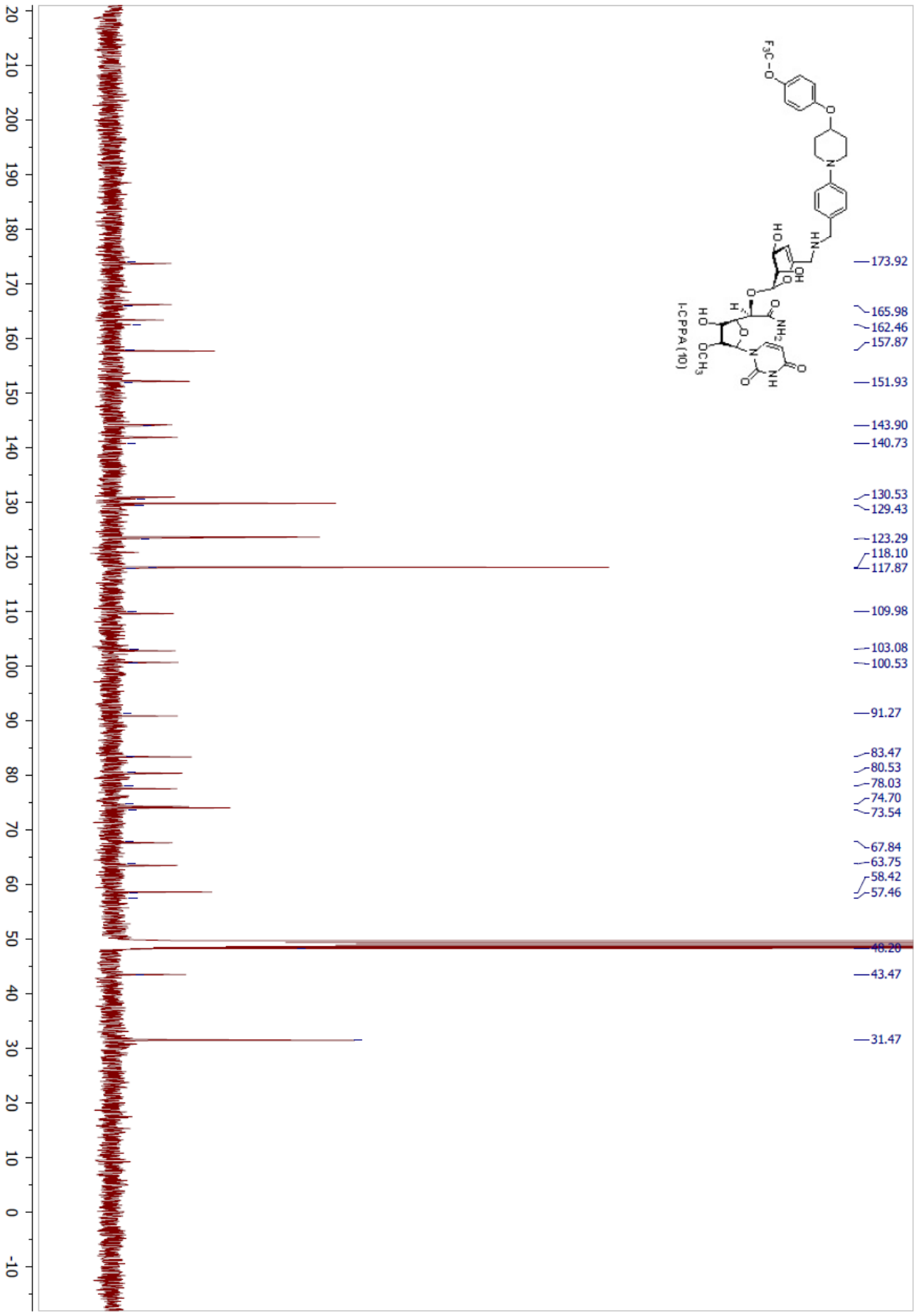
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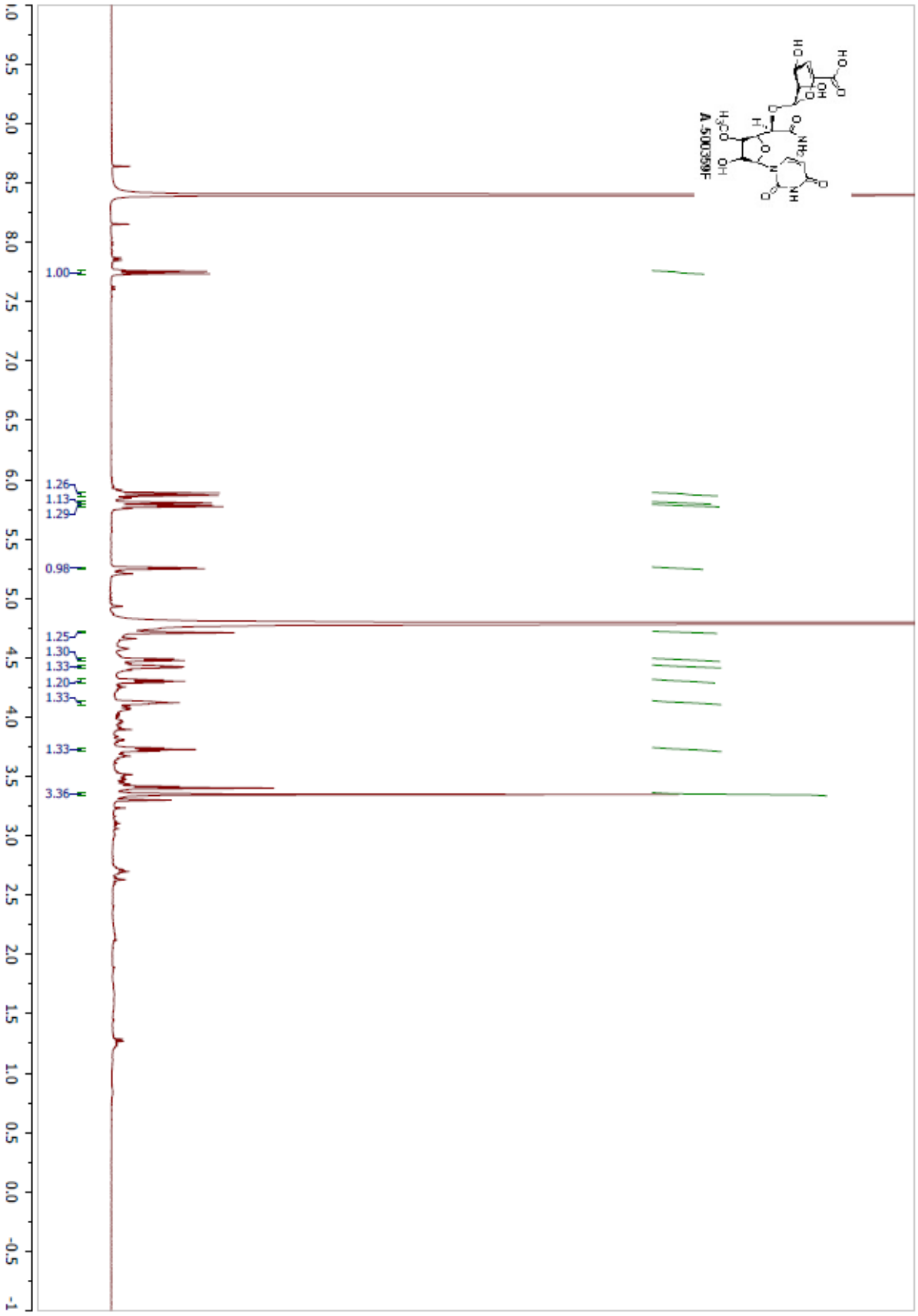
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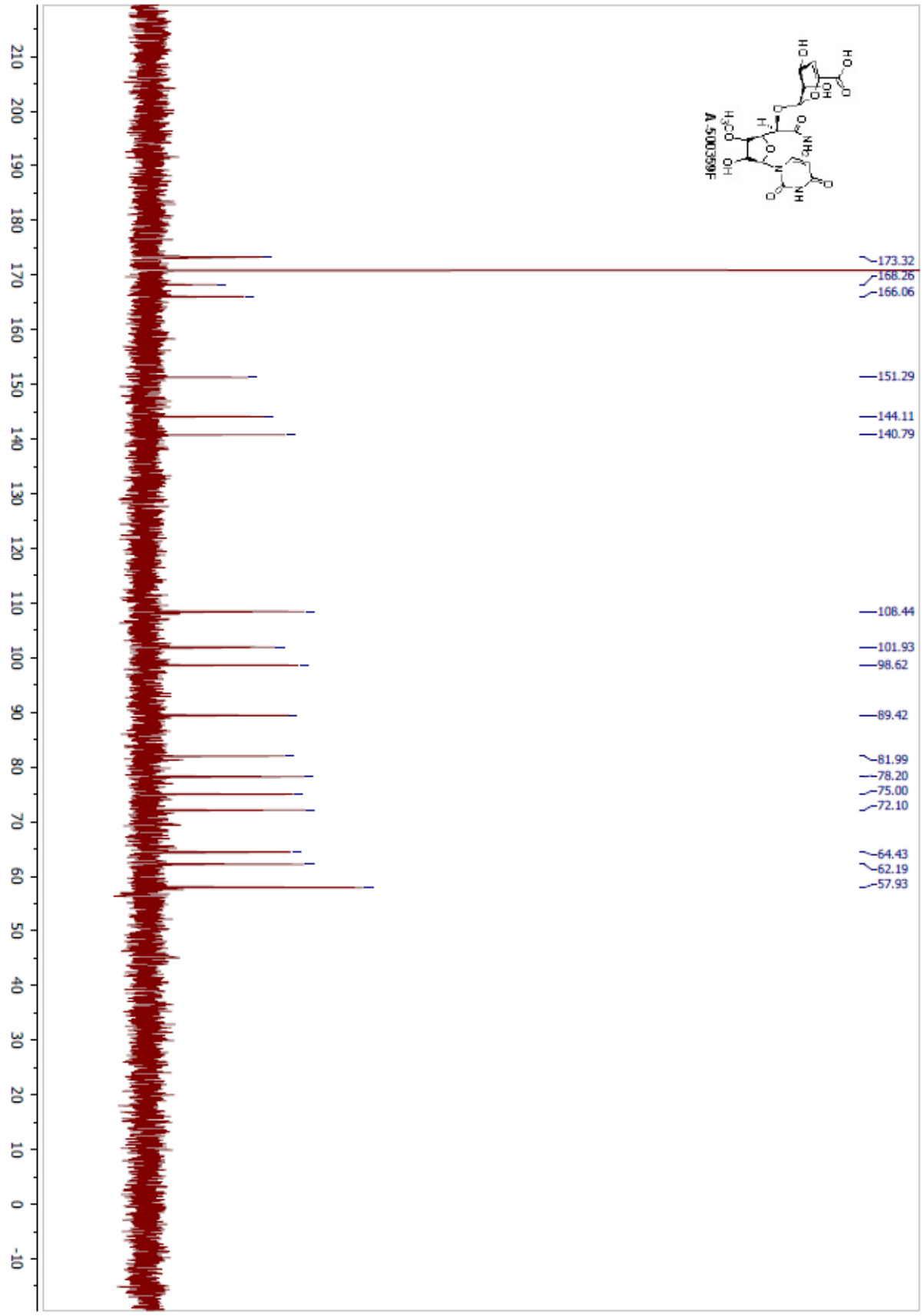
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