Supporting Information

for

A Semi-Synthesis of an Anticancer DPAGT1 Inhibitor from a Muraymycin Biosynthetic Intermediate

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General

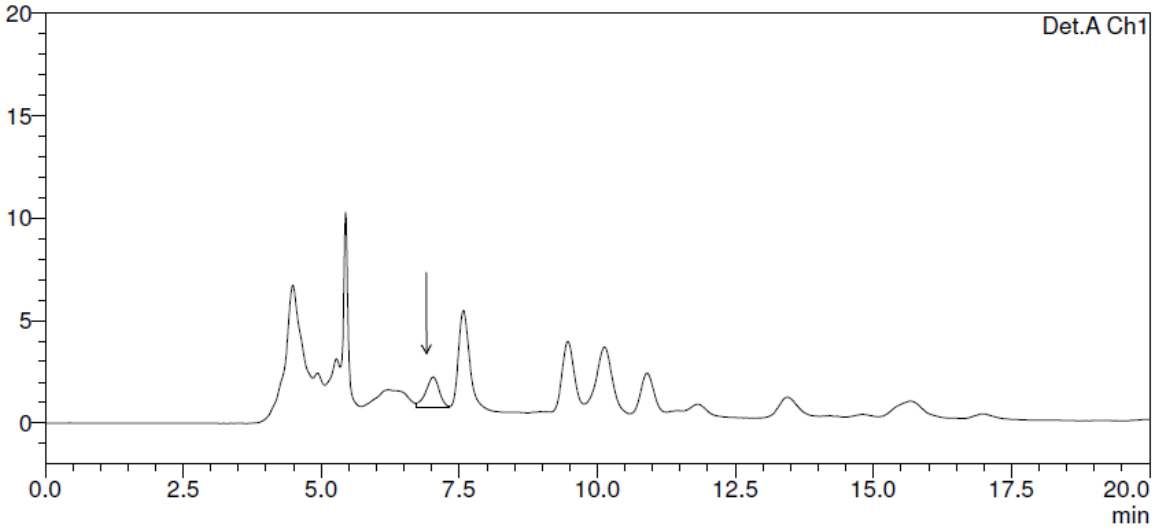
All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. THF, CH_2Cl_2, and DMF were purified via Innovative Technology's Pure-Solve System. All reactions were performed under an Argon atmosphere. All stirring was performed with an internal magnetic 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).

Isolation of muraymycin biosynthetic intermediate 3:

Streptomyces sp. NRRL 30471 (acquired from USDA) was cultured on a yeast malt extract agar plate (containing dextrose: 5 g, peptone: 2.5 g, malt extract: 1.5 g, yeast extract: 1.5 g, and agar: 10 g in water, 500 mL) at 27 ºC for 4 days. A single colony was isolated, and cultured in yeast malt extract (containing dextrose 5 g, peptone 2.5 g, malt extract 1.5 g, and yeast extract 1.5 g in water, 500 mL) in a 250 mL Erlenmeyer flasks at 27 ºC for 4 days to obtain a seed culture for fermentation.

An aliquot of the seed culture obtained above was inoculated into flasks (500 mL), each containing (glucose: 10 g, starch: 5 g, yeast extract: 4.5 g, peptone: 2.5 g, meat extract: 2.5 g, NaCl: 2.5 g, $CaCO_3$: 1.5 g, CB-442 (NOF Co., Ltd. Japan): (0.13% in water, 376 uL), pH 7.4). The fermentation (500 mL x3) was continued for 7 days at 23 °C on a rotary shaker (175 rpm). All culture flasks were combined and centrifuged at 4,700g for 20 min to separate the mycelium and water phase. The mycelial-cake portion was suspended in methanol (500 mL) and kept at -20 ºC for 0.5 h, and the cells were crushed by sonication (5 s on 2 s off for 5 min x2) at ice-water temperature. The organic phase was separated by centrifugation (4,700g for 5 min) and the precipitates were washed with MeOH. The combined MeOH extracts were evaporated to afford the brown solid (2.0 g wet). The water phase was adjusted ~pH 6.0 with 1 *N* HCl, and passed through a column of activated carbon. The column was washed with water (600 mL), 60% aq acetone (600 mL), and eluted with 0.3% NH_4OH in 60% aq acetone (500 - 1000 mL). The eluate was evaporated to provide the brown solid (1.5 g). The products obtained from intracellular and extracellular material were combined (~3.5 g) and purified by SiO_2 (^nBuOH:EtOH:$CHCl_3$:NH_4OH = 4:7:6:2 to 4:7:2:2 to 4:7:2:7); the crude products were separated into two groups (upper and lower spots), and the base-line materials were removed. The both fractions were separately dissolved in MeOH and the precipitates were filtered off. The MeOH extracts were concentrated and purified by Sephadex G25 (GH Healthcare) with MeOH; the first fractions (UV and nihydrin positive) were collected (350 mg). The obtained materials were absorbed on Dowex-22 (ammonium hydoxide-resin) in MeOH and washed with MeOH, and eluted with 0.1 *N* HCl in MeOH (1/10). The isolated materials were separated by reverse-HPLC (00G-4253-No, Luna 10 μm C18 100 Å, 250 x10 mm, 30% MeOH (15 min) to 60% MeOH (30 min) to 100% MeOH (10 min) flow rate 3 mL/min). The peak corresponding to the muraymycin biosynthetic intermediate **3** was collected. The purified **3** was identical to a synthetic sample in HPLC retention time. Due to low isolation yield of **3** under the current fermentation condition with the *Streptomyces sp*. NRRL 30471 strain, the reactions summarized in Table 1 were performed with synthetic **3**.[1]

HPLC analysis of **3** contained eluent.



HPLC analysis of synthetic **3**.

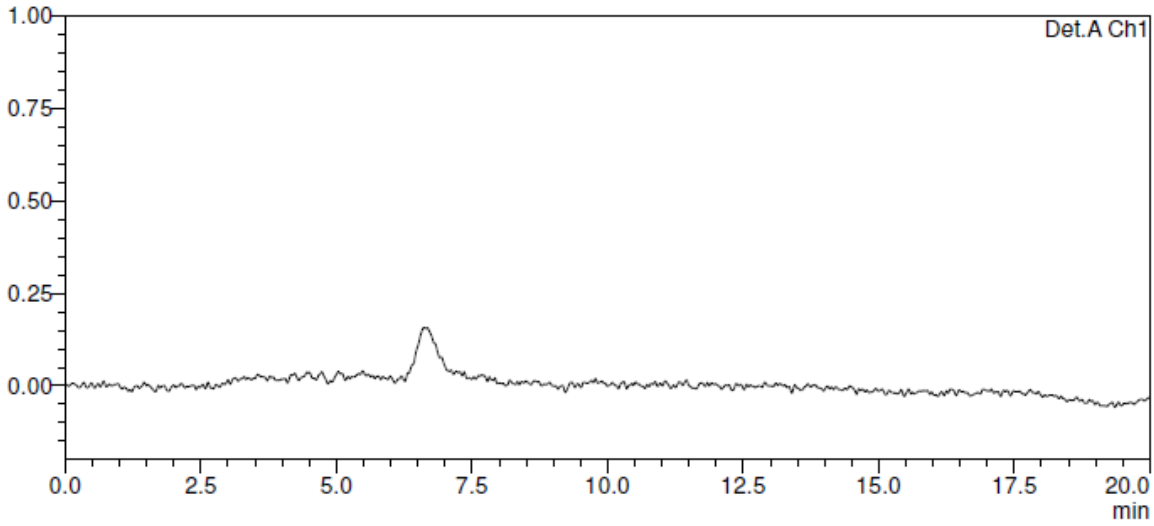
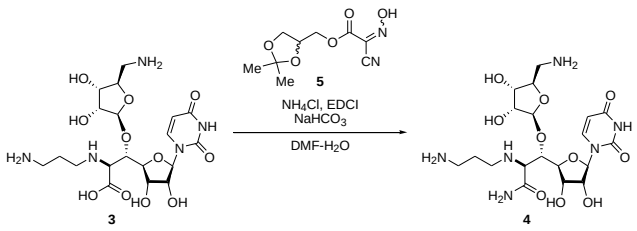


Figure S1: HPLC chromatograms of 3

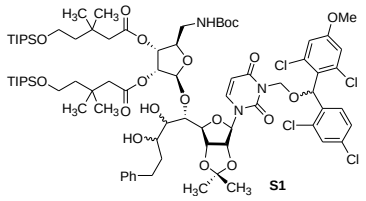
Amide-formation of 3.



(2*S*,3*S*)-3-(((2*S*,3*R*,4*S*,5*R*)-5-(Aminomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-2-((3-aminopropyl)amino)-3-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)propanamide (4). To a stirred solution

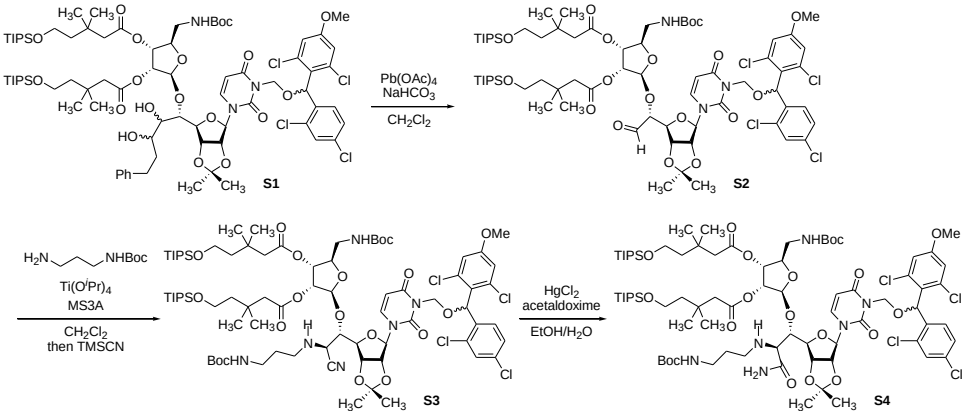
of **3** (32 mg, 0.06 mmol), NH_4Cl (0.17 g, 3.17 mmol), $NaHCO_3$ (80 mg, 0.95 mmol) and **5** [2] (72 mg, 0.32 mmol) in DMF/H_2O (9:1, 0.60 mL) was added EDCI (61 mg, 0.32 mmol). The reaction mixture was stirred for 8 h at rt, filtered and concentrated in vacuo. The crude mixture was purified by C18 reverse-phase HPLC [column: Luna® (100 Å, 10 μm, 250 x 10 mm), solvents: 5:95 MeOH:0.05 M NH_4HCO_3 in H_2O, flow rate: 3.0 mL/min, UV: 254 nm] to afford **4** (30 mg, 0.061 mmol, 95%, retention time: 6.7 min): TLC (n-butanol/ethanol/$CHCl_3$/28% aq ammonia 4:7:2:7) R_f = 0.10; $[\alpha]^{22}_D$ +0.168 (*c* = 0.26, methanol); IR (thin film) ν_{max} = 3298 (br), 2923, 2852, 1677, 1632, 1464, 1405, 1272, 1112, 1061 cm^{-1}; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.70 (d, *J* = 8.1 Hz, 1H), 5.84 (d, *J* = 8.0 Hz, 1H), 5.72 (d, *J* = 2.7 Hz, 1H), 5.18 (s, 1H), 4.37 (dd, *J* = 5.6, 2.8 Hz, 1H), 4.26 (t, *J* = 6.5 Hz, 2H), 4.20 (dd, *J* = 7.5, 4.8 Hz, 1H), 4.13 – 4.06 (m, 3H), 3.94 – 3.75 (m, 1H), 3.31 (d, *J* = 13.4 Hz, 1H), 3.12 – 3.06 (m, 1H), 3.01 (dt, *J* = 6.9, 3.4 Hz, 2H), 2.95 – 2.83 (m, 1H), 2.83 – 2.68 (m, 1H), 1.93 – 1.80 (m, 2H); ^{13}C NMR (101 MHz, D_2O) δ 166.19, 163.11, 162.76, 151.29, 142.38, 108.44, 101.89, 91.71, 83.25, 78.45, 74.44, 72.81, 71.54, 69.33, 62.08, 44.89, 42.07, 37.52; HRMS (ESI+) *m*/*z* calcd for $C_{19}H_{33}N_6O_{10}$ [M + H] 505.2258, found: 505.2272.

Chemical synthesis of muraymycin biosynthetic intermediate 3.



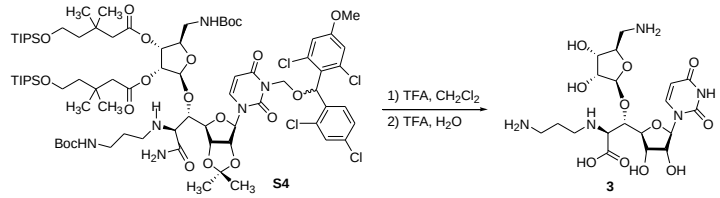
(2*R*,3*R*,4*R*,5*S*)-2-(((*tert*-Butoxycarbonyl)amino)methyl)-5-(((1*S*)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-2,3-dihydroxy-5-phenylpentyl)oxy)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (S1). The title compound was synthesized according to the reported procedure [1]: TLC (hexanes/EtOAc 67:33) R_f = 0.30; $[\alpha]^{22}_D$ 0.210 (*c* = 1.62, $CHCl_3$); IR (thin film) ν_{max} = 3444 (br), 2941, 2866, 1741, 1719, 1675, 1600, 1556, 1457, 1382, 1367, 1278, 1249, 1216, 1160, 1098, 1070, 1049, 1013, 998, 882, 867, 754, 681 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 8.4, 3.6 Hz, 1H), 7.31 (d, *J* = 2.0 Hz, 2H), 7.30 – 7.27 (m, 2H), 7.25 – 7.14 (m, 6H), 6.85 (d, *J* = 3.4 Hz, 2H), 6.50 (d, *J* = 5.9 Hz, 1H), 5.75 (dd, *J* = 17.6, 8.0 Hz, 1H), 5.63 (d, *J* = 22.1 Hz, 1H), 5.58 – 5.52 (m, 2H), 5.48 (d, *J* = 9.7 Hz, 1H), 5.21 (q, *J* = 7.3, 6.2 Hz, 2H), 5.11 (d, *J* = 6.8 Hz, 1H), 5.01 (dd, *J* = 8.4, 4.7 Hz, 1H), 4.85 – 4.78 (m, 2H), 4.25 (d, *J* = 5.6 Hz, 1H), 4.16 (dt, *J* = 8.6, 4.4 Hz, 1H), 4.03 (dd, *J* = 14.2, 5.1 Hz, 1H), 3.90 (d, *J* = 1.8 Hz, 1H), 3.78 (d, *J* = 1.8 Hz, 4H), 3.77 – 3.71 (m, 4H), 3.69 – 3.62 (m, 2H), 3.39 – 3.22 (m, 2H), 2.97 – 2.86 (m, 2H), 2.77 – 2.66 (m, 2H), 2.34 – 2.18 (m, 5H), 2.12 – 2.00 (m, 1H), 1.91 – 1.67 (m, 2H), 1.64 – 1.51 (m, 4H), 1.42 (s, 6H), 1.35 (d, *J* = 3.9 Hz, 3H), 1.13 – 0.99 (m, 41H), 0.99 – 0.94 (m, 6H), 0.86 (dtd, *J* = 9.1, 6.6, 2.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.85, 170.74, 170.70, 170.70, 162.10, 162.09, 159.44, 159.44, 156.05, 150.59, 150.53, 141.92, 141.89, 136.87, 136.84, 135.25, 135.09, 133.96, 133.77, 131.21, 131.17, 129.37, 129.32, 128.43 (2C), 128.38 (2C), 126.22, 126.14, 125.81, 125.36, 125.26, 115.27, 114.97, 80.36,

80.34, 79.85, 79.67, 79.58, 74.64, 74.62, 74.60, 73.82, 73.77, 73.72, 70.31, 70.31, 59.94, 59.90, 55.69, 46.13, 45.92, 44.72, 34.63, 34.50, 32.58, 32.57, 32.55, 32.54, 31.76, 29.69, 29.03, 28.35, 27.28, 27.22, 26.88, 25.32, 25.25, 20.68, 18.04 (1C), 11.88 (3C), 11.86 (3C), 11.43; HRMS (ESI+) *m*/*z* calcd for $C_{79}H_{120}Cl_4N_3O_{19}Si_2$ [M + H] 1610.6809, found: 1610.6827. Data for polar diastereomer: TLC (hexanes/EtOAc 67:33) R_f = 0.20; $[\alpha]^{22}_D$ 0.071 (*c* = 1.08, $CHCl_3$); IR (thin film) ν_{max} = 3413 (br), 2941, 2866, 1719, 1675, 1457, 1367, 1278, 1248, 1219, 1160, 1099, 1070, 1049, 882, 772 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.5 Hz, 1H), 7.31 – 7.29 (m, 2H), 7.28 (s, 2H), 7.24 – 7.11 (m, 6H), 6.84 (d, *J* = 1.4 Hz, 2H), 6.51 (d, *J* = 6.6 Hz, 1H), 5.90 (dd, *J* = 6.3, 2.7 Hz, 1H), 5.84 (t, *J* = 8.2 Hz, 1H), 5.61 – 5.41 (m, 2H), 5.23 – 5.10 (m, 2H), 5.04 (t, *J* = 5.8 Hz, 2H), 4.86 – 4.77 (m, 1H), 4.68 (ddd, *J* = 21.0, 6.3, 2.8 Hz, 1H), 4.57 (dt, *J* = 10.8, 3.8 Hz, 1H), 4.25 – 4.14 (m, 1H), 4.06 – 3.98 (m, 1H), 3.92 – 3.84 (m, 1H), 3.80 – 3.71 (m, 6H), 3.47 – 3.23 (m, 2H), 2.92 – 2.83 (m, 2H), 2.77 – 2.66 (m, 2H), 2.31 – 2.20 (m, 4H), 2.19 – 2.06 (m, 2H), 1.92 – 1.66 (m, 3H), 1.63 – 1.53 (m, 6H), 1.42 (d, *J* = 3.7 Hz, 2H), 1.36 (s, 6H), 1.10 – 0.94 (m, 50H), 0.91 – 0.81 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.04, 171.01, 170.93, 170.92, 162.00, 159.38, 150.79, 136.92, 136.91, 135.45, 131.30, 131.28, 129.29, 129.28, 128.46 (2C), 128.42 (2C), 126.09, 125.95, 125.93, 115.24, 81.03, 81.01, 79.95, 79.67, 75.03, 75.00, 74.98, 72.17, 70.38, 70.31, 69.52, 69.49, 59.95, 59.91, 55.69, 55.67, 46.13, 45.93, 44.86, 44.66, 35.27, 35.25, 34.64, 32.63, 32.59, 32.58, 31.95, 28.32, 27.38, 27.37, 27.36, 27.28, 27.27, 27.20, 26.89, 25.26, 18.05 (12C), 11.88 (3C), 11.87 (3C); HRMS (ESI+) *m*/*z* calcd for $C_{79}H_{120}Cl_4N_3O_{19}Si_2$ [M + H] 1610.6809, found: 1610.6831.



(2*S*,3R,4*R*,5*R*)-2-((1*S*,2*S*)-3-amino-2-((3-((*tert*-butoxycarbonyl)amino)propyl)amino)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-3-oxopropoxy)-5-(((*tert*-butoxycarbonyl)amino)methyl)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (S4). To a stirred suspension of **S1** (0.11 g, 0.065 mmol) and $NaHCO_3$ (55 mg, 0.65 mmol) in CH_2Cl_2 (1.3 mL) was added $Pb(OAc)_4$ (58 mg, 0.13 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and quenched with saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture of aldehyde **S2** was used for the next reaction without purification. To a stirred solution of **S2** (97 mg, 0.065 mmol) and *N*-Boc-1,3-propanediamine (46 μL, 0.26 mmol) in CH_2Cl_2 (1.3 mL)

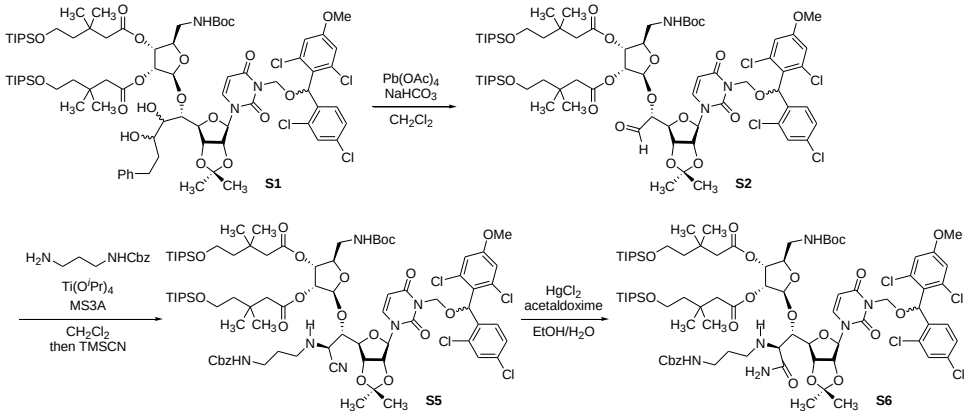
was added MS3A (0.21 g) followed by $Ti(OiPr)_4$ (0.19 mL, 0.65 mmol). After 9 h, the reaction was added TMSCN (82 μL, 0.65 mmol) and stirred for 12 h at rt. After completion, the reaction mixture was quenched with saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was passed through a silica gel pad (hexanes/EtOAc 75:25 to 60:40) to afford the crude mixture of **S3**. This mixture was used for next reaction without further purification. To a stirred solution of **S3** (0.11 g, 0.065 mmol) in EtOH/H_2O (9:1, 0.65 mL) were added $HgCl_2$ (36 mg, 0.13 mmol) and acetaldoxime (40 μL, 0.65 mmol) at rt. After being stirred for 8 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was quenched with saturated 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 50:50 to $CHCl_3$/MeOH 96:4) to afford **S4** (57 mg, 0.034 mmol, 52% for 3 steps): TLC ($CHCl_3$/MeOH 90:10) R_f = 0.50; $[\alpha]^{21}_D$ +0.193 (*c* = 2.26, $CHCl_3$); IR (thin film) ν_{max} = 3364 (br), 2942, 2866, 1718, 1676, 1599, 1556, 1521, 1462, 1366, 1277, 1250, 1170, 1099, 1070, 882 cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 7.57 – 7.51 (m, 1H), 7.31 (d, *J* = 1.9 Hz, 2H), 7.20 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.86 (d, *J* = 3.4 Hz, 2H), 6.52 (d, *J* = 9.8 Hz, 1H), 5.93 – 5.85 (m, 1H), 5.84 – 5.67 (m, 2H), 5.58 – 5.48 (m, 2H), 5.20 – 5.14 (m, 1H), 5.08 (s, 1H), 5.01 – 4.93 (m, 1H), 4.84 – 4.70 (m, 2H), 4.42 – 4.34 (m, 1H), 4.24 (dd, *J* = 7.2, 4.3 Hz, 1H), 4.17 – 4.09 (m, 1H), 3.78 (s, 3H), 3.73 (q, *J* = 7.4 Hz, 4H), 3.40 (ddd, *J* = 12.8, 7.7, 4.7 Hz, 1H), 3.36 – 3.20 (m, 3H), 3.20 – 3.11 (m, 1H), 2.75 – 2.66 (m, 2H), 2.25 – 2.17 (m, 4H), 1.69 – 1.51 (m, 11H), 1.43 (s, 18H), 1.36 (s, 3H), 1.12 – 1.03 (m, 42H), 1.02 (s, 3H), 0.96 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.05, 159.49, 156.10, 150.63, 136.92, 135.35, 135.19, 133.97, 133.76, 133.71, 131.27, 131.23, 129.39, 126.23, 126.15, 125.47, 125.36, 115.32, 115.16, 115.08, 105.71, 84.20, 80.93, 80.81, 79.37, 59.98, 59.92, 55.70, 46.14, 45.96, 44.81, 32.57, 32.50, 28.44 (6C), 27.25 (4C), 27.09, 25.23, 18.06 (12C), 11.92 (3C), 11.90 (3C); HRMS (ESI+) *m*/*z* calcd for $C_{79}H_{127}Cl_4N_6O_{20}Si_2$ [M + H] 1675.7398, found: 1675.7413.



(2*S*,3*S*)-3-(((2*S*,3*R*,4*S*,5*R*)-5-(aminomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-2-((3-aminopropyl)amino)-3-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)propanoic acid (3). To a stirred solution of **S4** (57 mg, 0.034 mmol) in CH_2Cl_2 (2.0 mL) was added TFA (1.0 mL). The reaction mixture was stirred for 2 h at rt, and all volatile were evaporated *in vacuo*. To a stirred solution of the crude mixture in H_2O (1.0 mL) was added TFA (1.5 mL). The reaction mixture was stirred for 20 h at 50 °C, and all volatile were evaporated *in vacuo*. The crude mixture was purified by DOWEX (50W x 4) ion exchange resin. The resin was washed with MeOH/H_2O (4:1) and MeOH. The crude product (TFA salt) was dissolved in MeOH (10 mL) and absorbed on DOWEX (50W x 4): the crude **3** was not detected by TLC ($CHCl_3$/MeOH/H_2O/50% aq ammonia 56:42:7:3). The resins were washed with MeOH

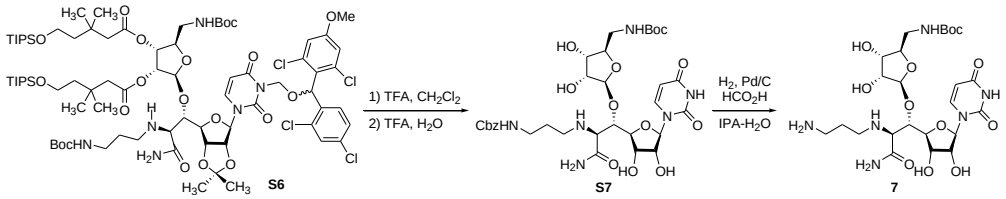
and eluted with MeOH/50% aq ammonia (10:1). The eluate was concentrated under reduced pressure and the resultant aqueous solution was lyophilize to afford **3** (5.7 mg, 6.4 μmol, 100%): TLC (n-butanol/ethanol/$CHCl_3$/28% aq ammonia 4:7:2:7) R_f = 0.10; ^{1}H NMR (400 MHz, D_2O) δ 7.78 (d, *J* = 8.1 Hz, 1H), 5.89 (d, *J* = 7.8 Hz, 1H), 5.78 (d, *J* = 3.1 Hz, 1H), 5.22 (s, 1H), 4.40 (dd, *J* = 5.5, 3.2 Hz, 1H), 4.29 (t, *J* = 6.3 Hz, 1H), 4.22 (dd, *J* = 6.8, 5.1 Hz, 1H), 4.18 (t, *J* = 4.5 Hz, 1H), 4.16 – 4.08 (m, 3H), 3.59 (d, *J* = 3.8 Hz, 1H), 3.28 (d, *J* = 12.6 Hz, 1H), 3.11 – 3.02 (m, 3H), 2.77 (dt, *J* = 12.8, 6.8 Hz, 1H), 2.64 (dt, *J* = 12.7, 7.0 Hz, 1H), 1.83 (quin, *J* = 7.1 Hz, 2H); ^{13}C NMR (101 MHz, D_2O) δ 172.95, 142.29, 108.25, 102.37, 89.42, 82.51, 79.03, 75.48, 74.44, 71.66, 71.52, 69.34, 66.57, 52.54, 42.50, 38.04, 37.12, 24.18; HRMS (ESI+) *m*/*z* calcd for $C_{19}H_{32}N_5O_{11}$ [M + H] 506.2098, found: 506.2113.

Synthesis of authentic sample of 7.



(2*S*,3R,4*R*,5*R*)-2-((1*S*,2*S*)-3-amino-2-((3-((benzyloxycarbonyl)amino)propyl)amino)-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(3-(((2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-3-oxopropoxy)-5-(((*tert*-butoxycarbonyl)amino)methyl)tetrahydrofuran-3,4-diyl bis(3,3-dimethyl-5-((triisopropylsilyl)oxy)pentanoate) (S6). To a stirred suspension of **S1** (0.11 g, 0.067 mmol) and $NaHCO_3$ (56 mg, 0.67 mmol) in CH_2Cl_2 (1.3 mL) was added $Pb(OAc)_4$ (59 mg, 0.13 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and quenched with saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture of aldehyde **S2** was used for the next reaction without purification. To a stirred solution of **S2** (99 mg, 0.067 mmol) and *N*-Cbz-1,3-propanediamine (56 mg, 0.27 mmol) in CH_2Cl_2 (1.3 mL) was added MS3A (0.22 g) followed by $Ti(OiPr)_4$ (0.20 mL, 0.67 mmol). After 10 h, the reaction was added TMSCN (84 μL, 0.67 mmol) and stirred for 7 h at rt. After completion, the reaction mixture was quenched with saturated aq $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was passed through a silica gel pad (hexanes/EtOAc 75:25 to 60:40) to afford the crude mixture of **S3**. This mixture was used for next reaction without further purification. To a stirred solution of **S5** (0.11 g, 0.067 mmol) in EtOH/H_2O (9:1, 0.67 mL) were added $HgCl_2$ (36 mg, 0.13 mmol) and acetaldoxime (41 μL, 0.67 mmol)

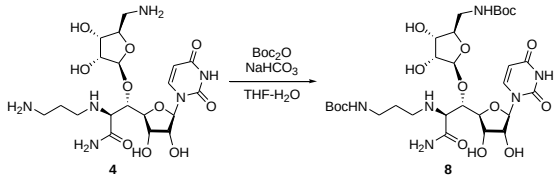
at rt. After being stirred for 15 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was quenched with saturated aq $NaHCO_3$, extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexanes/EtOAc 50:50 to $CHCl_3$/MeOH 96:4) to afford **S6** (55 mg, 0.032 mmol, 48% for 3 steps): TLC ($CHCl_3$/MeOH 90:10) R_f = 0.50; $[\alpha]^{20}_D$ +0.116 (*c* = 0.70, $CHCl_3$); IR (thin film) ν_{max} = 3348 (br), 2941, 2866, 1718, 1675, 1600, 1555, 1526, 1457, 1367, 1276, 1249, 1218, 1159, 1100, 1070, 1049, 882 cm^{-1}; ^1H NMR (400 MHz, Chloroform-*d*) δ 7.52 (d, *J* = 8.3 Hz, 1H), 7.37 – 7.29 (m, 6H), 7.22 – 7.16 (m, 2H), 6.86 (d, *J* = 2.6 Hz, 2H), 6.51 (d, *J* = 13.7 Hz, 1H), 6.01 – 5.88 (m, 1H), 5.83 – 5.63 (m, 3H), 5.60 – 5.45 (m, 3H), 5.20 – 4.92 (m, 6H), 4.84 – 4.73 (m, 2H), 4.36 – 4.07 (m, 3H), 3.79 – 3.69 (m, 7H), 3.44 – 3.15 (m, 4H), 2.76 – 2.63 (m, 2H), 2.34 – 2.15 (m, 5H), 1.76 – 1.46 (m, 6H), 1.41 (s, 9H), 1.36 – 1.23 (m, 4H), 1.12 – 0.99 (m, 46H), 0.96 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.91, 162.04, 159.45, 156.58, 156.05, 150.66, 136.86, 136.83, 136.50, 135.08, 133.95, 133.72, 131.24, 131.18, 129.36, 128.63, 128.47 (2C), 128.09 (2C), 126.21, 126.15, 125.35, 125.24, 115.28, 115.10, 105.87, 84.11, 79.41, 74.59, 66.64, 59.93, 59.88, 55.68, 46.10, 45.91, 44.77, 32.56, 32.54, 32.49, 28.43, 27.22 (3C), 27.02, 25.12, 18.03 (12C), 11.86 (3C), 11.85 (3C); HRMS (ESI+) *m/z* calcd for $C_{82}H_{125}Cl_4N_6O_{20}Si_2$ [M + H] 1709.7241, found: 1709.7260.



***tert*-Butyl (((2*R*,3*S*,4*R*,5*S*)-5-((1*S*,2*S*)-3-amino-2-((3-aminopropyl)amino)-1-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-oxopropoxy)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)carbamate (7).** To a stirred solution of **S6** (9.4 mg, 5.5 μmol) in CH_2Cl_2 (2.0 mL) was added TFA (1.0 mL). The reaction mixture was stirred for 2 h at rt, and all volatile were evaporated *in vacuo*. To a stirred solution of the crude mixture in H_2O (0.2 mL) was added TFA (0.8 mL). The reaction mixture was stirred for 2 h at rt, and all volatile were evaporated *in vacuo*. To a stirred solution of the crude mixture in THF-H_2O (1:1, 0.5 mL) were added $NaHCO_3$ (8.6 mg, 0.10 mmol) and Boc_2O (6.7 mg, 0.031 mmol). The reaction mixture was stirred for 2 h at rt, and all volatile were evaporated *in vacuo*. The crude mixture was passed through a silica gel pad (EtOAc to $CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) to afford the crude mixture of **S7**. To a stirred solution of the crude mixture of **S7** in IPA-H_2O-HCO_2H (100:10:1, 1.0 mL) was added Pd/C (10 wt % 6.8 mg). H_2 gas was introduced and the reaction mixture was stirred for 1 h under H_2. The solution was filtered through Celite and concentrated *in vacuo*. The crude mixture was purified by C18 reverse-phase HPLC [column: Luna® (100 Å, 10 μm, 250 x 10 mm), solvents: 25:75 MeOH:0.05 M NH_4HCO_3 in H_2O, flow rate: 3.0 mL/min, UV: 254 nm] to afford **7** (2.7 mg, 4.42 μmol, 81%, retention time: 13.0 min): TLC ($CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) R_f = 0.10; IR (thin film) ν_{max} = 3329 (br), 3312 (br), 2979, 2929, 1678, 1572, 1508, 1459, 1392, 1367, 1279, 1131, 1115, 1077, 1057, 1018 cm^{-1}; ^1H NMR (400 MHz, Deuterium

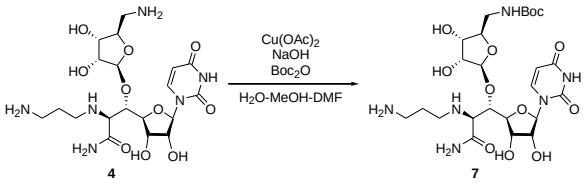
Oxide) δ 7.76 (d, *J* = 7.8 Hz, 1H), 5.79 (s, 1H), 5.76 (d, *J* = 7.8 Hz, 1H), 5.05 (s, 1H), 4.25 – 4.13 (m, 3H), 4.03 (dd, *J* = 10.5, 6.8 Hz, 3H), 3.52 (d, *J* = 6.4 Hz, 1H), 3.36 – 3.31 (m, 2H), 3.29 (s, 1H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.65 (t, *J* = 7.1 Hz, 2H), 1.82 – 1.73 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (101 MHz, D_2O) δ 176.10, 161.92, 161.73, 160.96, 146.67, 140.44, 109.60, 102.04, 90.59, 90.39, 82.37, 81.77, 80.86, 79.92, 75.26, 74.88, 41.07, 38.32, 38.19, 27.69 (3C); HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{41}N_6O_{12}$ [M + H] 605.2782, found: 605.2795.

Synthesis of authentic sample of 8.



***tert*-Butyl (((2*R*,3*S*,4*R*,5*S*)-5-((1*S*,2*S*)-3-amino-2-((3-((*tert*-butoxycarbonyl)amino)propyl)amino)-1-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-oxopropoxy)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)carbamate (8).** To a stirred solution of **4** (4.1 mg, 8.1 μmol) and $NaHCO_3$ (6.8 mg, 0.081 mmol) in THF-H_2O (1:1, 0.2 mL) was added Boc_2O (8.9 mg, 0.041 mmol). The reaction mixture was stirred for 12 h at rt, and all volatile were evaporated *in vacuo*. The crude mixture was passed through a silica gel column chromatography (EtOAc to $CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) to afford **8** (5.0 mg, 7.2 μmol, 88%): TLC ($CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) R_f = 0.55; $[\alpha]^{21}_D$ +0.558 (*c* = 0.45, methanol); IR (thin film) ν_{max} = 3331 (br), 2976, 2932, 1681, 1517, 1456, 1392, 1366, 1276, 1253, 1167, 1131, 1112, 1086, 1013 cm^{-1}; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.95 (d, *J* = 8.1 Hz, 1H), 5.83 (s, 1H), 5.76 (d, *J* = 8.2 Hz, 1H), 5.09 (s, 1H), 4.19 – 4.14 (m, 4H), 4.04 (d, *J* = 6.4 Hz, 1H), 4.00 – 3.93 (m, 4H), 3.45 (d, *J* = 6.3 Hz, 1H), 3.12 (t, *J* = 6.8 Hz, 2H), 2.62 (q, *J* = 6.0, 5.6 Hz, 2H), 1.69 – 1.63 (m, 2H), 1.45 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 166.19, 158.56, 152.19, 142.31, 110.64, 102.63, 91.56, 85.12, 83.90, 80.75, 80.30, 79.90, 76.69, 75.55, 72.89, 71.12, 65.24, 46.58, 43.79, 39.24, 31.10, 28.91 (3C), 28.82 (3C); HRMS (ESI+) *m*/*z* calcd for $C_{29}H_{49}N_6O_{14}$ [M + H] 705.3307, found: 705.3324.

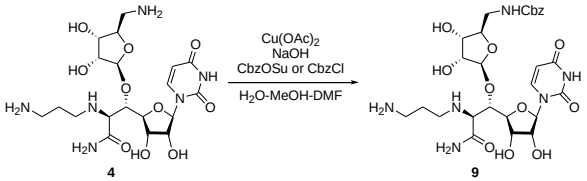
Selective Boc protection of 4.



***tert*-Butyl (((2*R*,3*S*,4*R*,5*S*)-5-((1*S*,2*S*)-3-amino-2-((3-aminopropyl)amino)-1-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-**

oxopropoxy)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)carbamate (7). To a stirred solution of **4** (8.1 mg, 0.016 mmol), $Cu(OAc)_2$ (1.0 M in H_2O, 0.048 mL, 0.048 mmol), and NaOH (1.0 M in H_2O, 0.048 mL, 0.048 mmol) in H_2O-MeOH-DMF (1:1:1, 0.6 mL) was added Boc_2O (8.7 mg, 0.040 mmol). The reaction mixture was stirred for 4 h at rt, filtered and concentrated *in vacuo*. The crude mixture was purified by C18 reverse-phase HPLC [column: Luna® (100 Å, 10 μm, 250 x 10 mm), solvents: 25:75 MeOH:0.05 M NH_4HCO_3 in H_2O, flow rate: 3.0 mL/min, UV: 254 nm] to afford **4** (8.8 mg, 0.015 mmol, 91%, retention time: 13.0 min): TLC ($CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) R_f = 0.10; IR (thin film) ν_{max} = 3329 (br), 3312 (br), 2979, 2929, 1678, 1572, 1508, 1459, 1392, 1367, 1279, 1131, 1115, 1077, 1057, 1018 cm^{-1}; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.76 (d, *J* = 7.8 Hz, 1H), 5.79 (s, 1H), 5.76 (d, *J* = 7.8 Hz, 1H), 5.05 (s, 1H), 4.25 – 4.13 (m, 3H), 4.03 (dd, *J* = 10.5, 6.8 Hz, 3H), 3.52 (d, *J* = 6.4 Hz, 1H), 3.36 – 3.31 (m, 2H), 3.29 (s, 1H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.65 (t, *J* = 7.1 Hz, 2H), 1.82 – 1.73 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (101 MHz, D_2O) δ 176.10, 161.92, 161.73, 160.96, 146.67, 140.44, 109.60, 102.04, 90.59, 90.39, 82.37, 81.77, 80.86, 79.92, 75.26, 74.88, 41.07, 38.32, 38.19, 27.69 (3C); HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{41}N_6O_{12}$ [M + H] 605.2782, found: 605.2795.

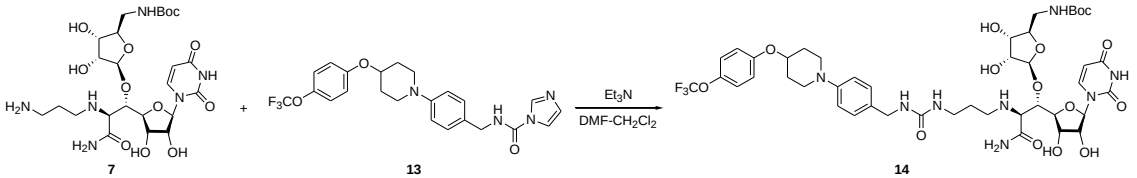
Selective Cbz protection of 4.



4 9

Benzyl (((2*R*,3*S*,4*R*,5*S*)-5-((1*S*,2*S*)-3-amino-2-((3-aminopropyl)amino)-1-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-oxopropoxy)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)carbamate (9). To a stirred solution of **4** (4.7 mg, 9.3 μmol), $Cu(OAc)_2$ (1.0 M in H_2O, 0.028 mL, 0.028 mmol), and NaOH (1.0 M in H_2O, 0.028 mL, 0.028 mmol) in H_2O-MeOH-DMF (1:1:1, 0.3 mL) was added CbzOSu (5.7 mg, 0.023 mmol) or CbzCl (3.3 μL, 0.023 mmol). The reaction mixture was stirred for 7 h at rt, filtered and concentrated *in vacuo*. The crude mixture was purified by C18 reverse-phase HPLC [column: Luna® (100 Å, 10 μm, 250 x 10 mm), solvents: 35:65 MeOH:0.05 M NH_4HCO_3 in H_2O, flow rate: 3.0 mL/min, UV: 254 nm] to afford **9** (5.5 mg, 8.7 μmol, 93%, retention time: 10.2 min): TLC (n-butanol/ethanol/$CHCl_3$/28% aq ammonia 4:7:2:7) R_f = 0.55; $[\alpha]^{20}_D$ +0.122 (*c* = 0.26, methanol); IR (thin film) ν_{max} = 3330 (br), 3125, 3037, 2958, 2925, 2852, 2808, 1686, 1677, 1542, 1443, 1399, 1268, 1114, 1057 cm^{-1}; ^1H NMR (400 MHz, Methanol-d_4) δ 7.94 (d, *J* = 8.1 Hz, 1H), 7.36 – 7.28 (m, 5H), 5.82 (d, *J* = 2.1 Hz, 1H), 5.75 (d, *J* = 8.1 Hz, 1H), 5.08 (s, 1H), 5.06 (s, 2H), 4.17 – 4.14 (m, 4H), 4.04 (dd, *J* = 6.6, 2.4 Hz, 1H), 3.97 – 3.94 (m, 4H), 3.44 (d, *J* = 6.3 Hz, 1H), 3.21 (t, *J* = 6.7 Hz, 2H), 2.67 – 2.59 (m, 2H), 1.69 (quin, *J* = 7.0 Hz, 2H); ^{13}C NMR (101 MHz, MeOD) δ 166.23, 158.96, 158.55, 152.22, 142.32, 138.46, 129.47 (2C), 128.82 (2C), 110.64, 102.59, 91.58, 85.19, 83.87, 80.79, 80.31, 76.70, 75.56, 72.90, 71.15, 67.38, 65.27, 46.47, 43.81, 39.70, 31.02; HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{39}N_6O_{12}$ [M + H] 639.2626, found: 639.2641.

Synthesis of APPU (2) from 7.



***tert*-Butyl (((2*R*,3*S*,4*R*,5*S*)-5-((1*S*,2*S*)-3-amino-1-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-oxo-2-((3-(3-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)ureido)propyl)amino)propoxy)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)carbamate (14).** To a stirred solution of **7** (4.3 mg, 7.1 μmol, prepared from **S7**) and **13** (9.8 mg, 0.021 mmol) in DMF-CH_2Cl_2 (1:1, 0.2 mL) was added Et_3N (4.7 μL, 0.036 mmol) at rt. The reaction mixture was stirred for 12 h at rt, and all volatile were evaporated *in vacuo*. The crude mixture was passed through a silica gel column chromatography (EtOAc to $CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) to afford **14** (6.4 mg, 6.4 μmol, 90%). The ^{1}H NMR spectral data was confirmed to be identical by comparison with **14** prepared via newly developed selective protection strategy: TLC ($CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) R_f = 0.55; $[\alpha]^{21}_D$ +0.086 (*c* = 0.17, methanol); IR (thin film) ν_{max} = 3325 (br), 2930, 2855, 1678, 1553, 1505, 1267, 1242, 1197, 1163, 1113, 1033 cm^{-1}; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.92 (d, *J* = 8.0 Hz, 1H), 7.21 – 7.16 (m, 4H), 7.01 – 6.95 (m, 6H), 5.82 (s, 1H), 5.75 (d, *J* = 8.1 Hz, 1H), 5.10 (s, 1H), 4.53 (dd, *J* = 7.8, 4.0 Hz, 2H), 4.34 (s, 1H), 4.26 (s, 2H), 4.22 (s, 1H), 4.19 – 4.15 (m, 2H), 4.06 (d, *J* = 4.5 Hz, 1H), 3.98 – 3.94 (m, 2H), 3.93 (s, 1H), 3.52 – 3.44 (m, 4H), 3.26 – 3.20 (m, 1H), 3.08 (ddd, *J* = 12.2, 8.6, 3.1 Hz, 4H), 2.15 – 2.06 (m, 2H), 1.91 – 1.82 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 173.09, 172.96, 158.76, 157.79, 157.63, 152.17, 152.09, 131.12, 130.54 (2C), 130.43, 129.63 (2C), 129.23, 123.85, 123.58, 121.89, 120.53, 120.32, 118.20, 118.11 (2C), 118.02, 117.15 (2C), 76.68, 74.01, 73.34, 71.10, 43.80, 31.62, 31.49, 28.89 (3C), 28.75, 23.77, 22.54; HRMS (ESI+) *m*/*z* calcd for $C_{44}H_{60}F_3N_8O_{15}$ [M + H] 997.4130, found: 997.4168.

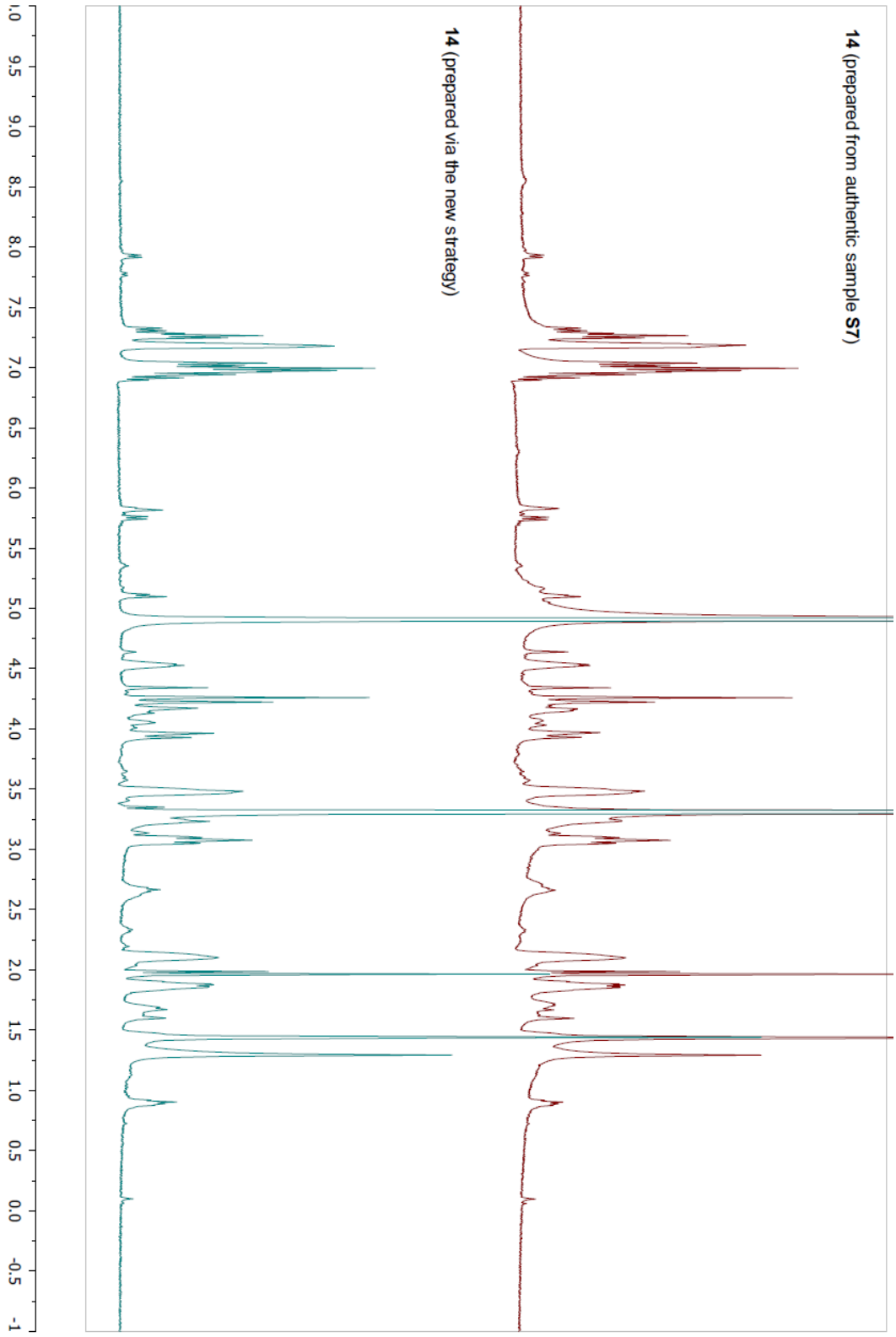
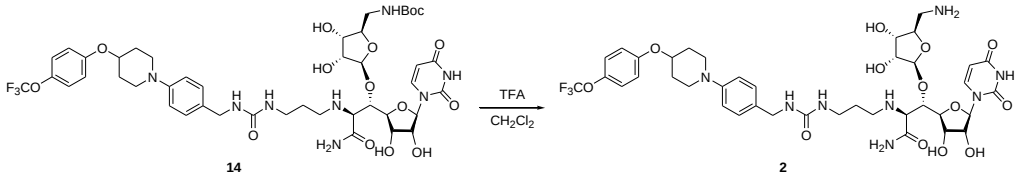


Figure S2: ^{1}H-NMR comparison between 14 synthesized from S7 in two steps (debenzylation and coupling with 13) and 14 synthesized via a new method described in this manuscript



(2*S*,3*S*)-3-(((2*S*,3*R*,4*S*,5*R*)-5-(Aminomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)oxy)-3-((2*S*,5*R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-2-((3-(3-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)benzyl)ureido)propyl)amino)propanamide (2). To a stirred solution of **14** (6.4 mg, 6.4 μmol) in CH_2Cl_2 (0.35 mL) was added TFA (0.15 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated *in vacuo*. The crude mixture was purified by DOWEX (50W x 4) ion exchange resin. The resin was washed with MeOH/H_2O (4:1) and MeOH. The crude product (TFA salt) was dissolved in MeOH (10 mL) and absorbed on DOWEX (50W x 4): the crude **2** was not detected by TLC ($CHCl_3$/MeOH/H_2O/50% aq ammonia 56:42:7:3). The resins were washed with MeOH and eluted with MeOH/50% aq ammonia (10:1). The eluate was concentrated under reduced pressure and the resultant aqueous solution was lyophilize to afford **2** (5.7 mg, 6.4 μmol, 100%): TLC ($CHCl_3$/MeOH/H_2O/28% aq ammonia 56:42:7:3) R_f = 0.35; $[\alpha]^{20}_D$ +0.037 (*c* = 0.05, methanol); IR (thin film) ν_{max} = 3310 (br), 3077, 2928, 2853, 1649, 1614, 1555, 1515, 1504, 1267, 1241, 1222, 1196, 1162, 1112, 1037, 1029 cm^{-1}; ^1H NMR (400 MHz, Methanol-d_4) δ 7.84 (d, *J* = 8.1 Hz, 1H), 7.22 – 7.15 (m, 4H), 7.06 – 6.96 (m, 6H), 5.79 (s, 1H), 5.74 (d, *J* = 8.1 Hz, 1H), 5.13 (s, 1H), 4.58 – 4.51 (m, 2H), 4.26 (s, 1H), 4.23 – 4.20 (m, 1H), 4.19 – 4.14 (m, 2H), 4.10 (d, *J* = 4.4 Hz, 1H), 3.97 – 3.94 (m, 1H), 3.47 (d, *J* = 10.7 Hz, 2H), 3.40 (d, *J* = 5.0 Hz, 1H), 3.23 (t, *J* = 6.7 Hz, 2H), 3.07 (ddd, *J* = 12.5, 8.5, 3.6 Hz, 2H), 2.90 (dd, *J* = 13.3, 3.8 Hz, 1H), 2.80 (dd, *J* = 13.3, 7.2 Hz, 1H), 2.70 – 2.55 (m, 2H), 2.17 – 2.05 (m, 2H), 1.92 – 1.82 (m, 2H), 1.69 – 1.60 (m, 2H); ^{13}C NMR (101 MHz, MeOD) δ 158.76, 157.63, 152.09, 143.91, 131.12, 130.54 (2C), 130.43, 129.63 (2C), 129.24, 123.85, 123.59, 121.89, 120.53, 120.32, 118.19, 118.11 (2C), 118.03, 117.16 (2C), 102.63, 76.69, 74.01, 73.35, 43.80, 31.62, 31.49, 28.92, 22.53; HRMS (ESI+) *m*/*z* calcd for $C_{39}H_{52}F_3N_8O_{13}$ [M + H] 897.3606, found: 897.3629.

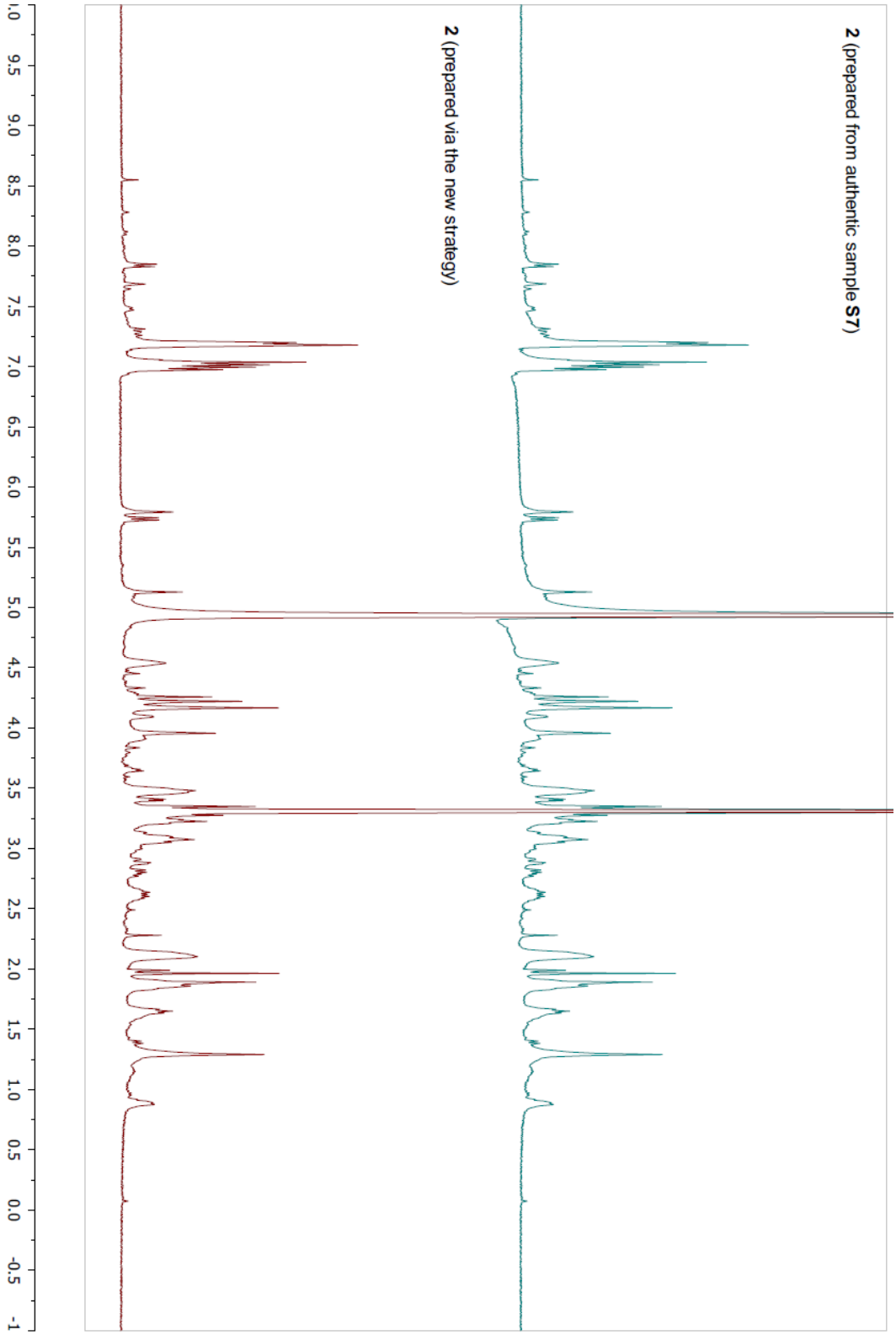


Figure S3: ^{1}H-NMR comparison of 2 with the authentic sample prepared via the previously reported method[1]

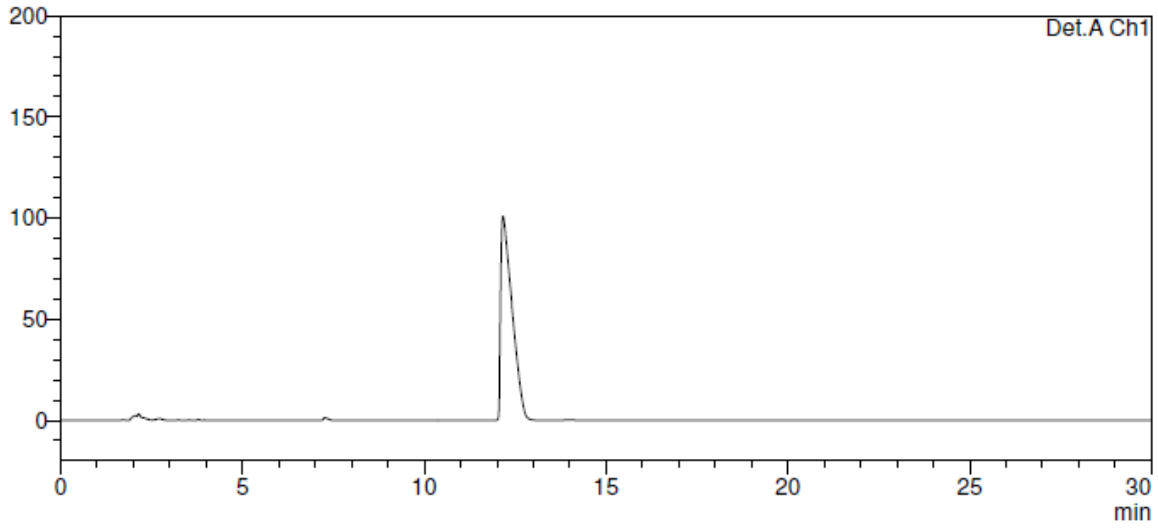
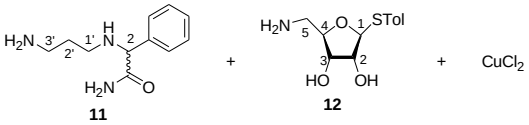


Figure S4: HPLC analysis of APPU (2)
Conditions: column: Phenomenex Luna® 10 µm C18 100 Å 250 x 10 mm column, solvents: 65:35 MeOH:0.05 M NH_4HCO_3 in water, flow rate: 3.0 mL/min, UV: 254 nm

NMR analyses of Cu complex.

A stirred solution of 1:1:1 mixture of **11** (5.2 mg, 0.025 mmol), **12** (6.4 mg, 0.025 mmol) and $CuCl_2$ (1.0 M in H_2O, 0.028 mL, 0.028 mmol) in H_2O-MeOH (1:1, 0.2 mL) was added NaOH (1.0 M in H_2O, 0.025 mL, 0.025 mmol). After 30 min, all volatiles were lyophilized. The crude mixture was dissolved in D_2O (0.5 mL).

Table S1: ^{1}H-NMR Chemical shifts for the Cu-complexes with 11 and 12



	H-2	H-1'	H-2'	H-3'
11	4.30	2.56	1.77	2.92
+Cu	5.03	3.06, ~3	2.03	2.97
Δδ	+0.73	+0.50, ~+0.5	+0.26	+0.05

	H-1	H-2	H-3	H-4	H-5
12	5.25	3.89	4.11	4.05	3.18, 2.83
+Cu	5.25	3.89	4.11	4.05	3.18, 2.83
Δδ	0	0	0	0	0

Cytotoxicity Assays

Selected molecules were tested for cytotoxicity (IC_{50}) in cancer and healthy cells via a MTT colorimetric assay.
For Vero cells: Vero cell was cultured in Complete eagle`s minimum essential growth medium (EMEM) containing L-glutamine, sodium pyruvate, minimum essential amino acids, penicillin-streptomycin and 10% fetal bovine serum. Inoculating number of cells were 400,000 cells/mL and a final 40,000 cells/well. After 72 h of exposure of molecules to this cell line at concentrations ranging from 0.78 to 200 μg/mL, the culture medium was changed to complete EMEM without phenol red before addition of yellow tetrazolium dye; MTT. Viability was assessed on the basis of cellular conversion of MTT into a purple formazan product. The absorbance of the colored formazan product was measured at 570 nm by BioTek Synergy HT Spectrophotometer. Linearity of the MTT response to the cell number was determined.
Each cell was cultured in recommended medium by ATCC.

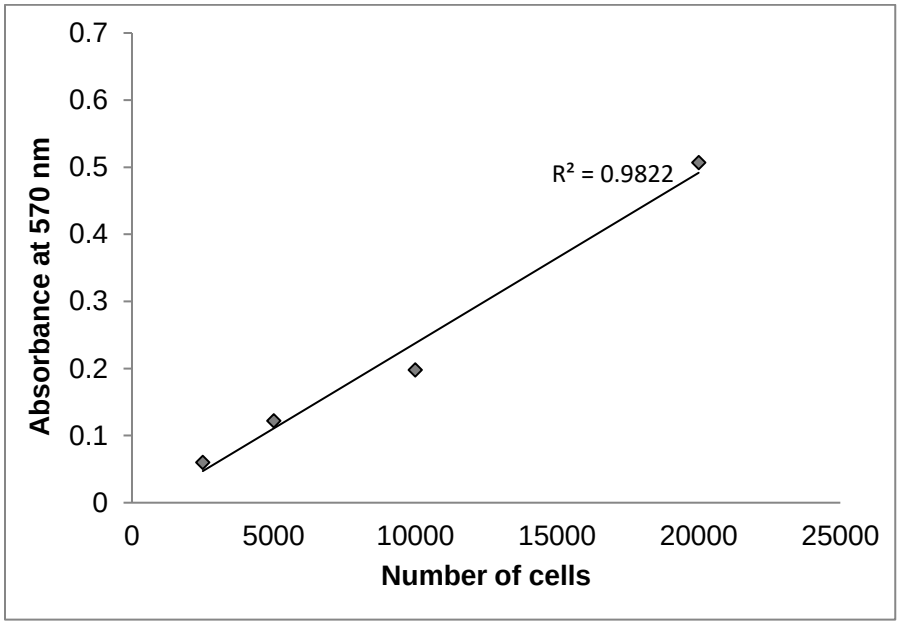


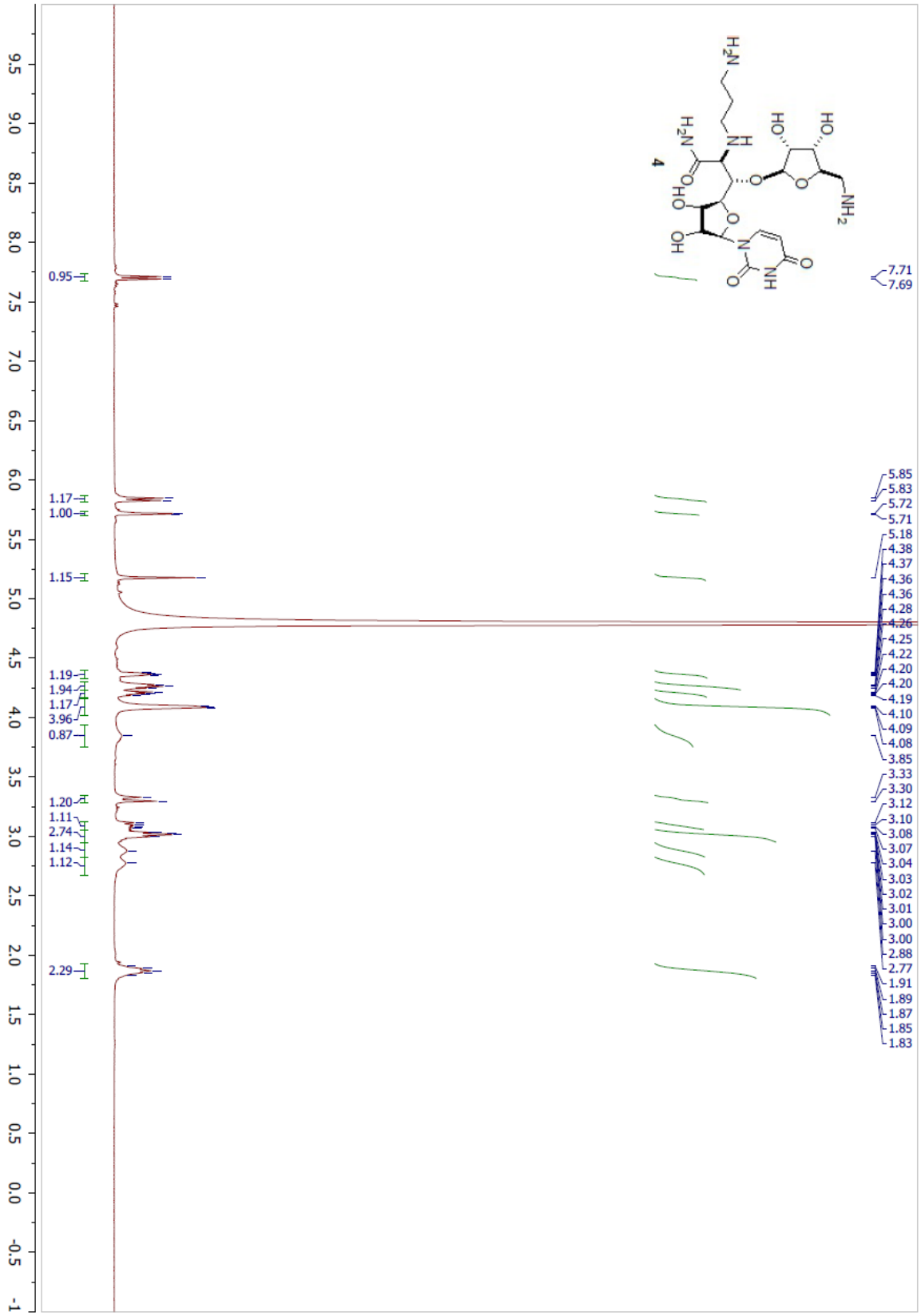
Figure S5: MTT response vs population of Vero cells

Table S2: Cytotoxicity of APPB and APPU against representative cell lines

Cells / Molecule	APPB (IC_{50} μM)	APPU (IC_{50} μM)
Panc-1	<0.098	0.098
AsPC-1	0.098	0.098
Vero	35-65	12.5-55

References

(1) Mitachi, K.; Yun, H. G.; Kurosu, S. M.; Eslamimehr, S.; Lemieux, M. R.; Klaić, L.; Clemons, W. M.; Kurosu, M. *ACS Omega* **2018**, *3*, 1726.
(2) Wang, Q.; Wang, Y.; Kurosu, M. *Org. Lett.* **2012**, *14*, 3372.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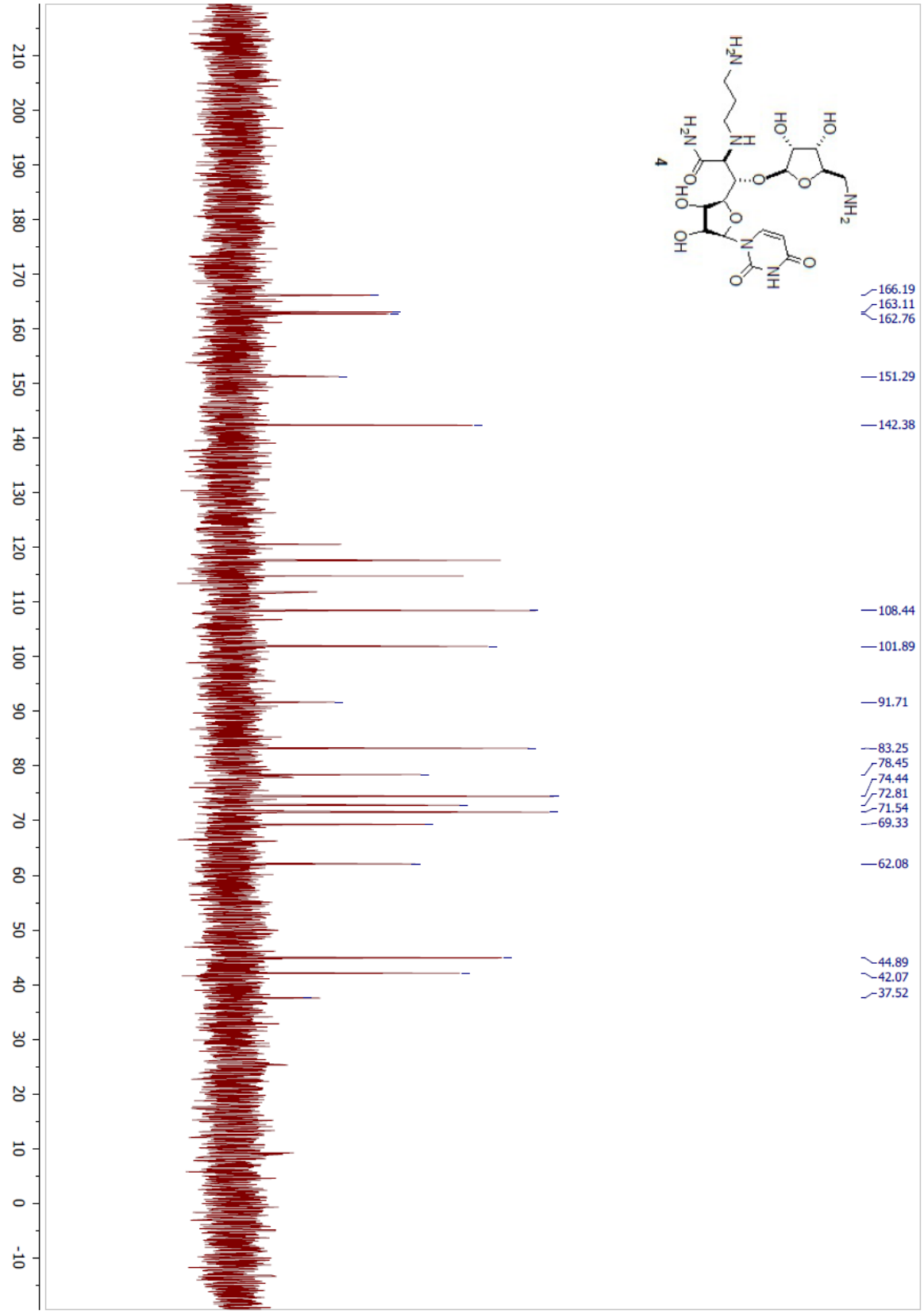
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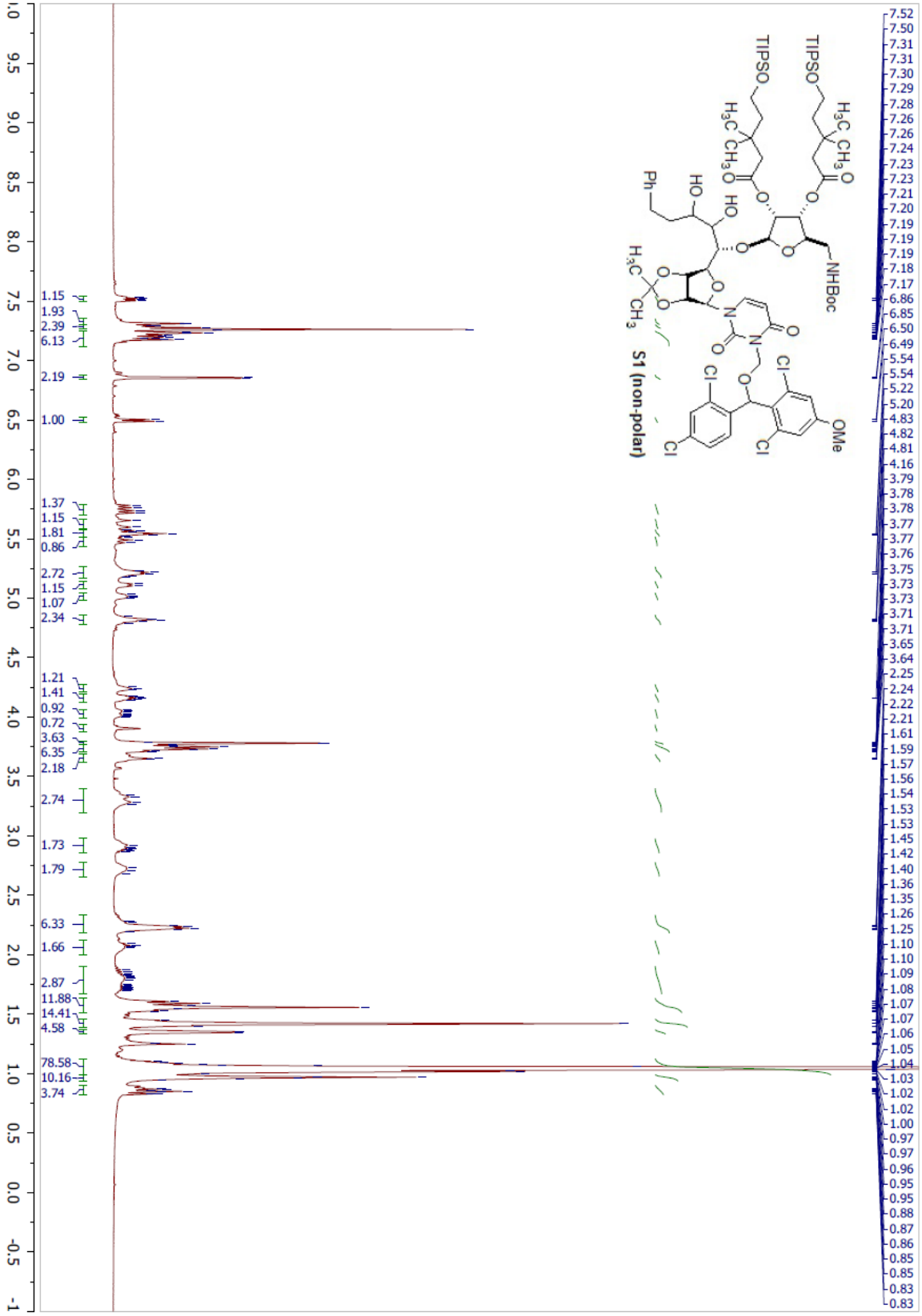
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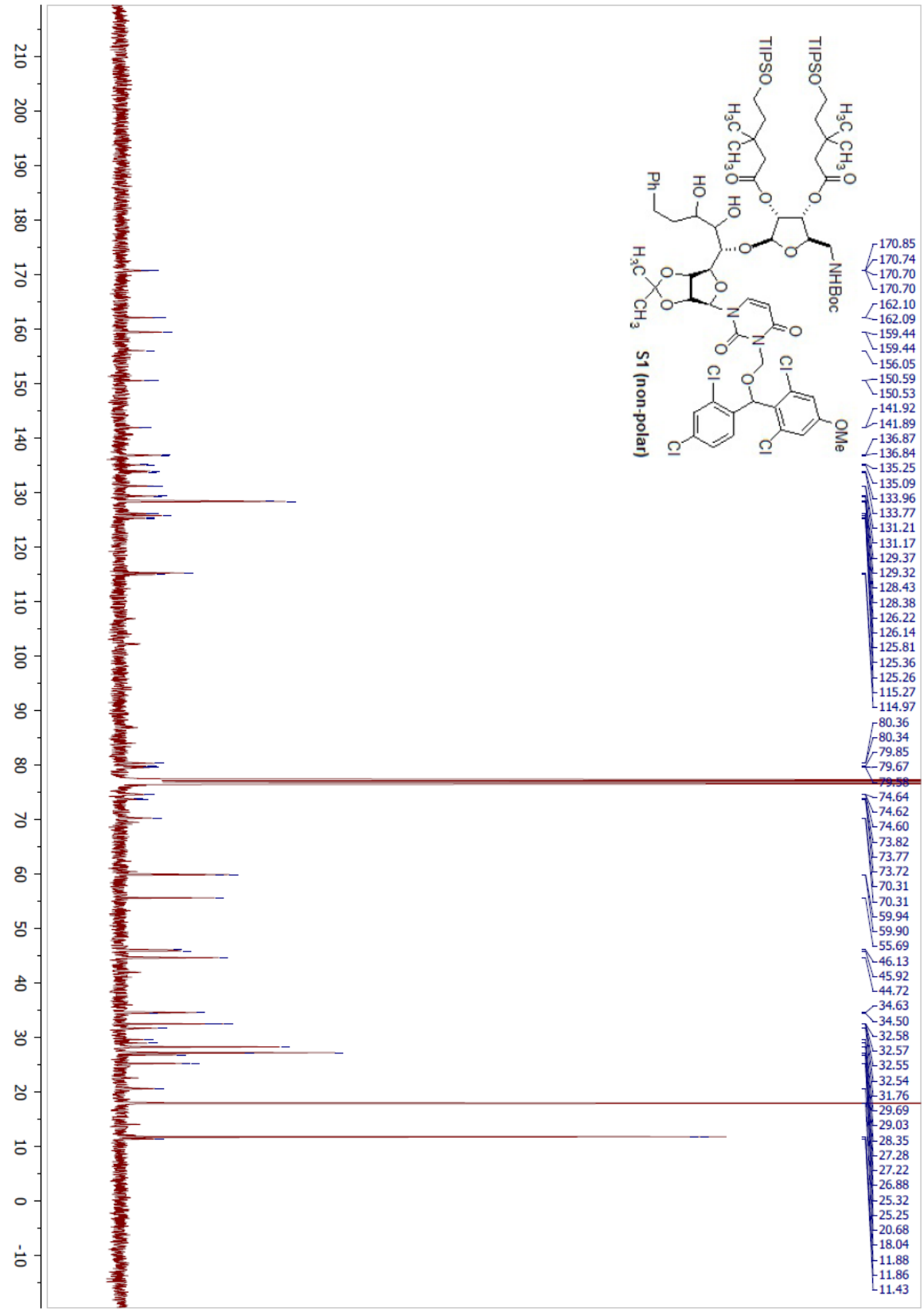
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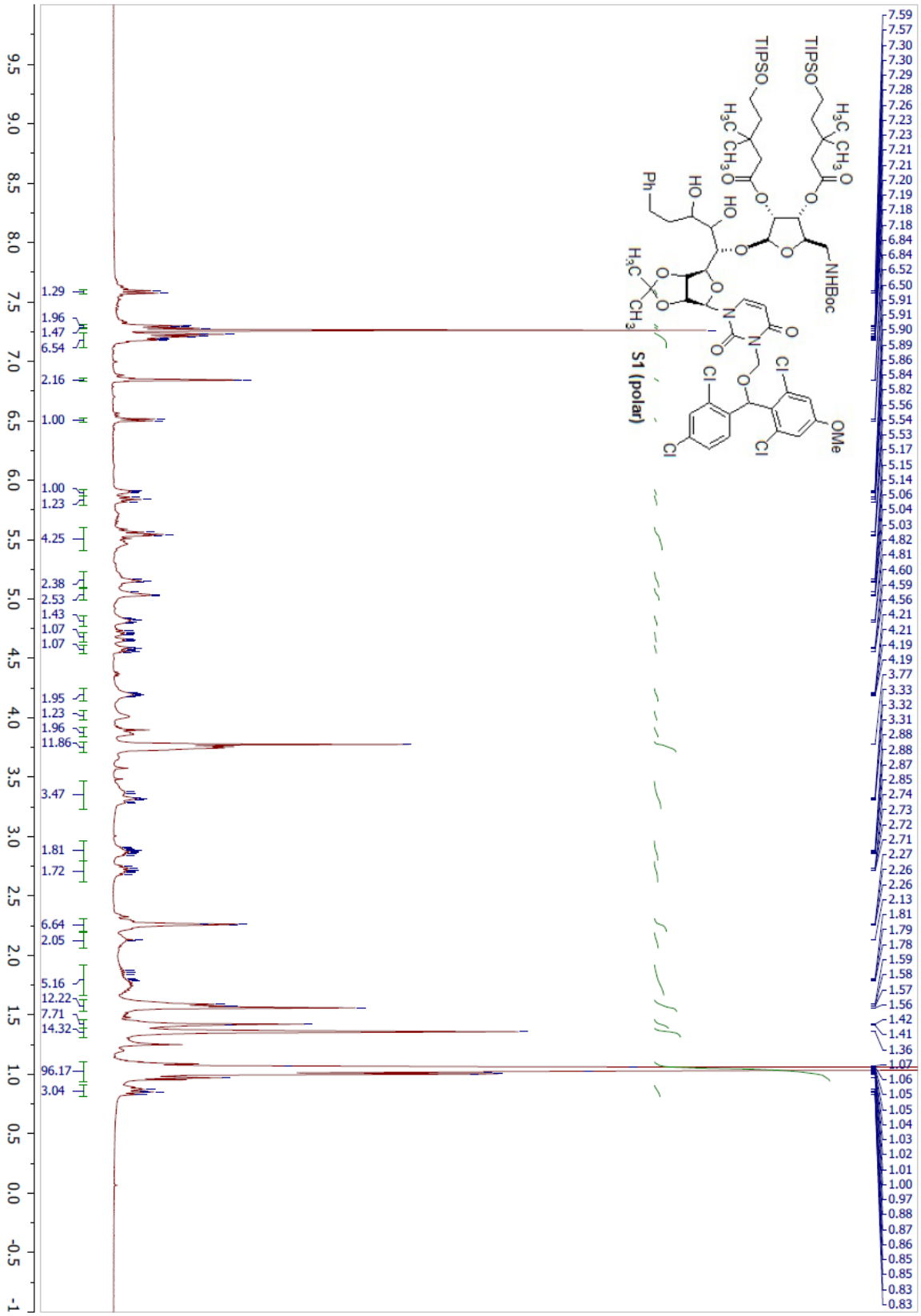
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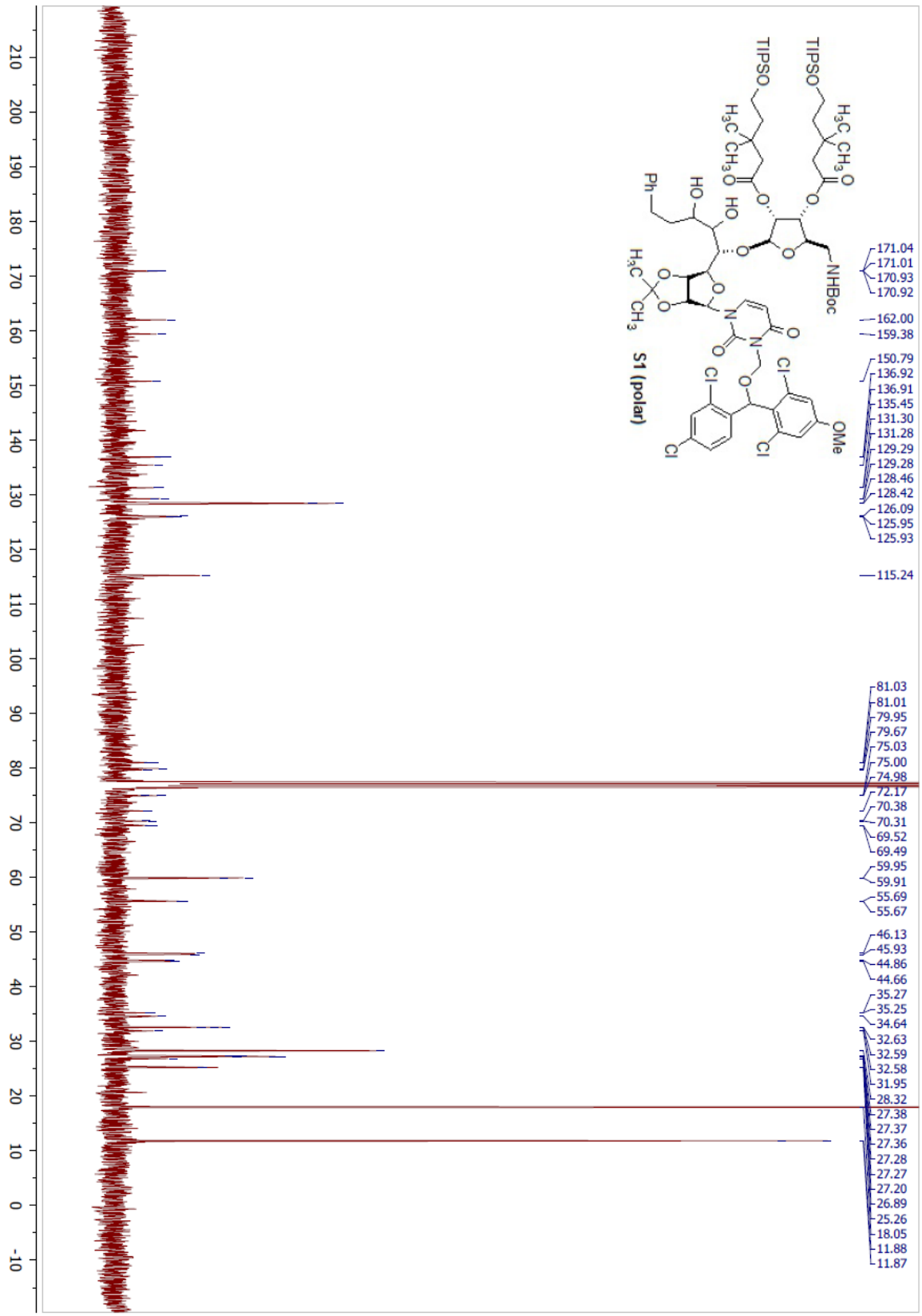
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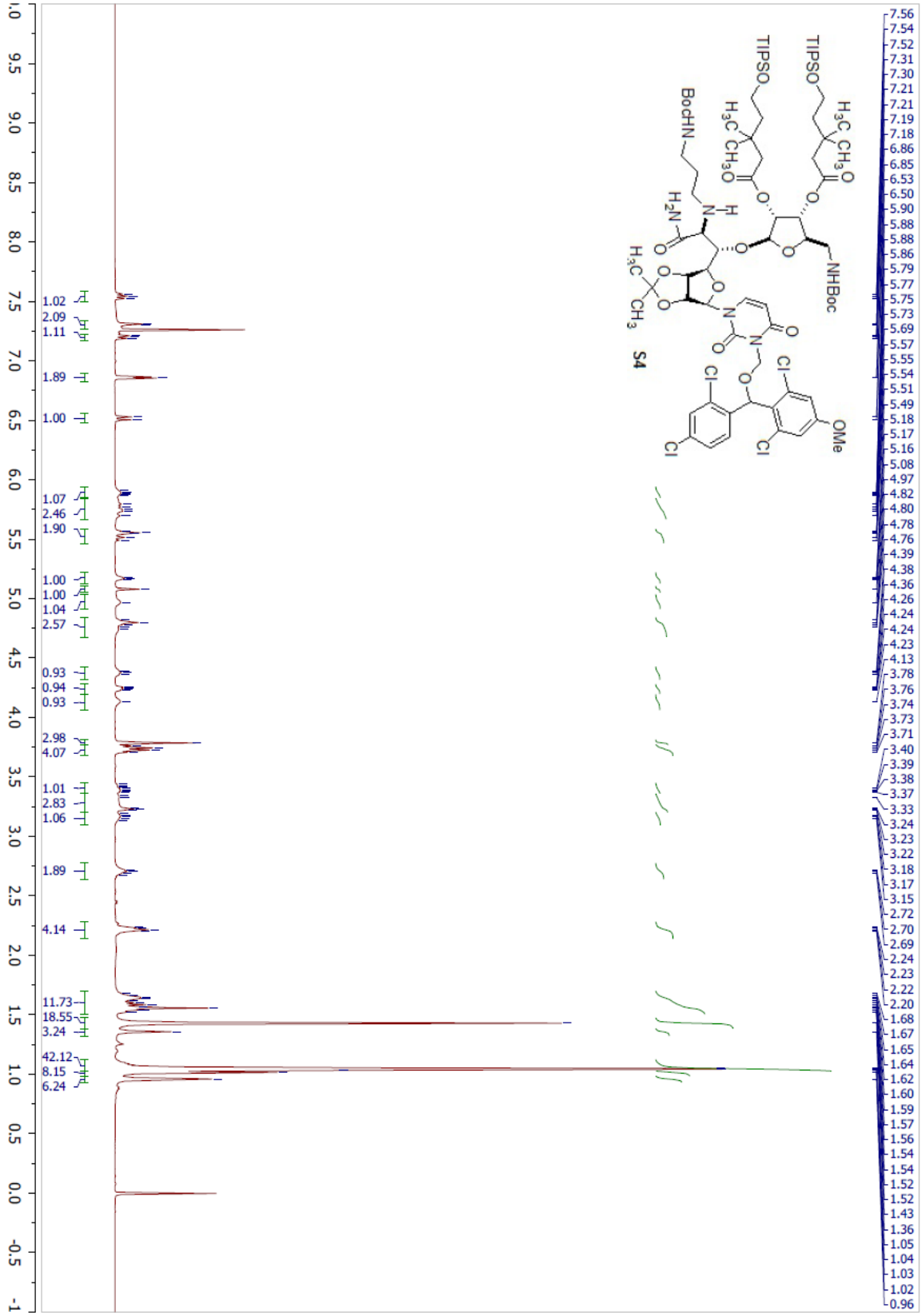
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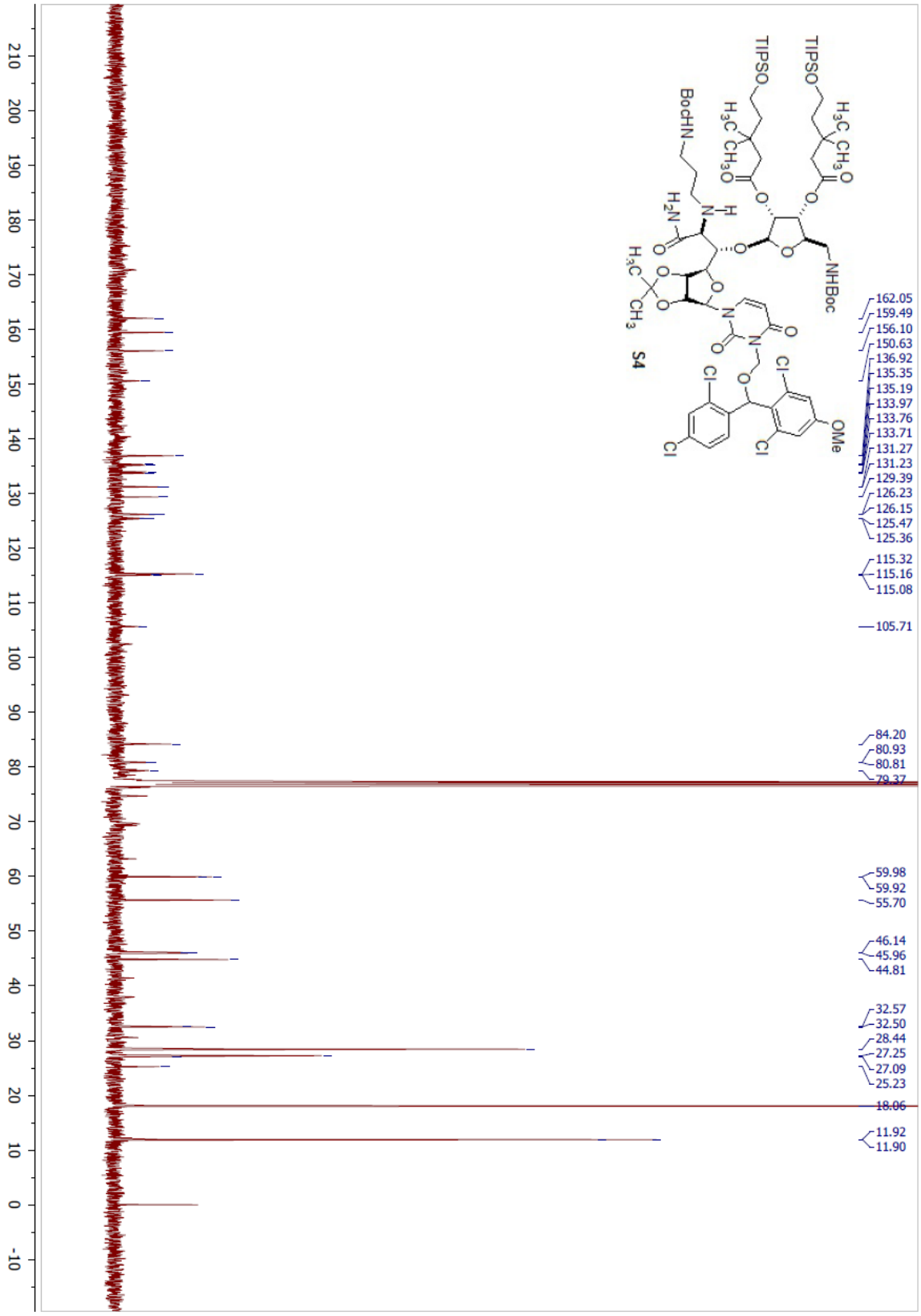
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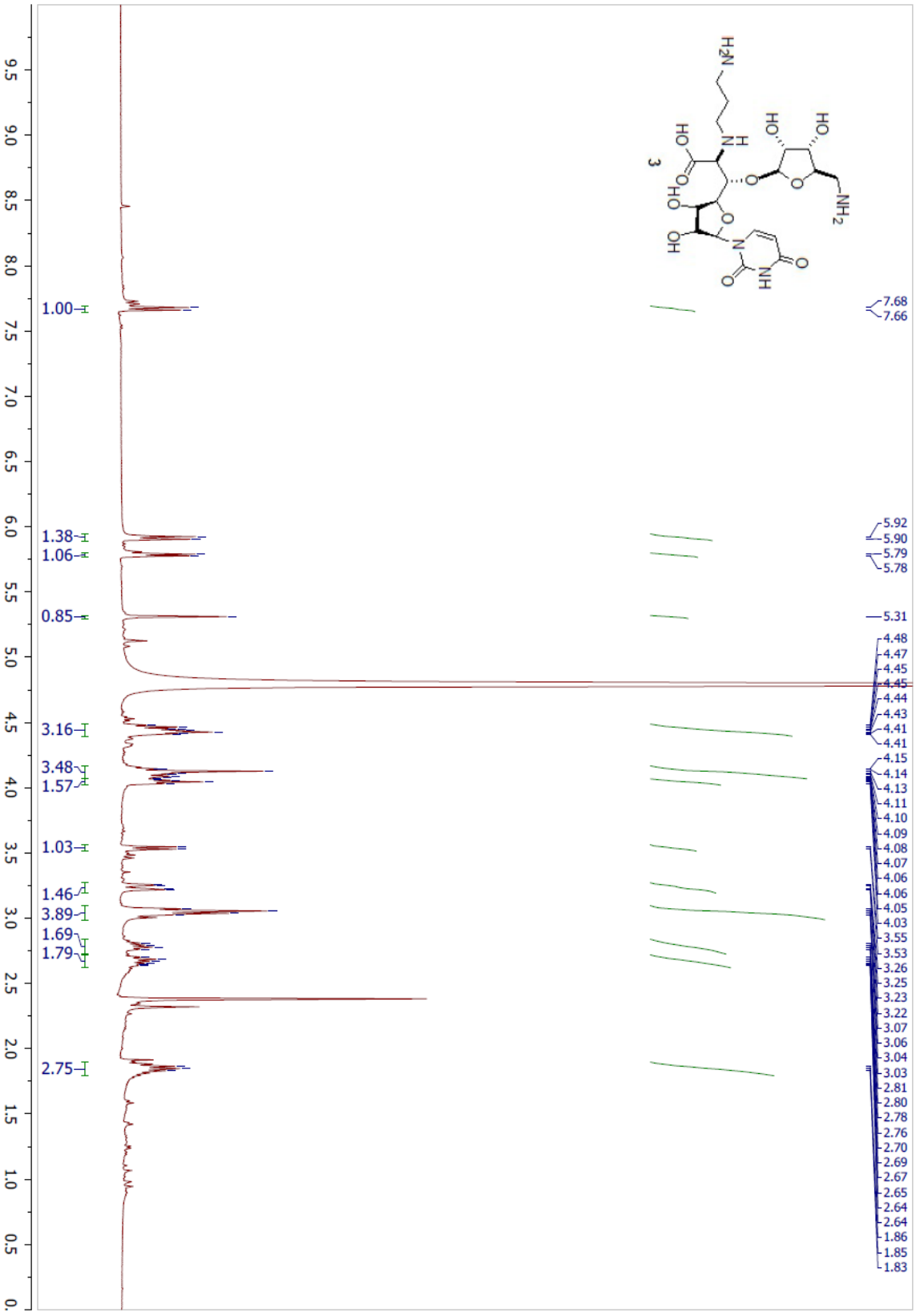
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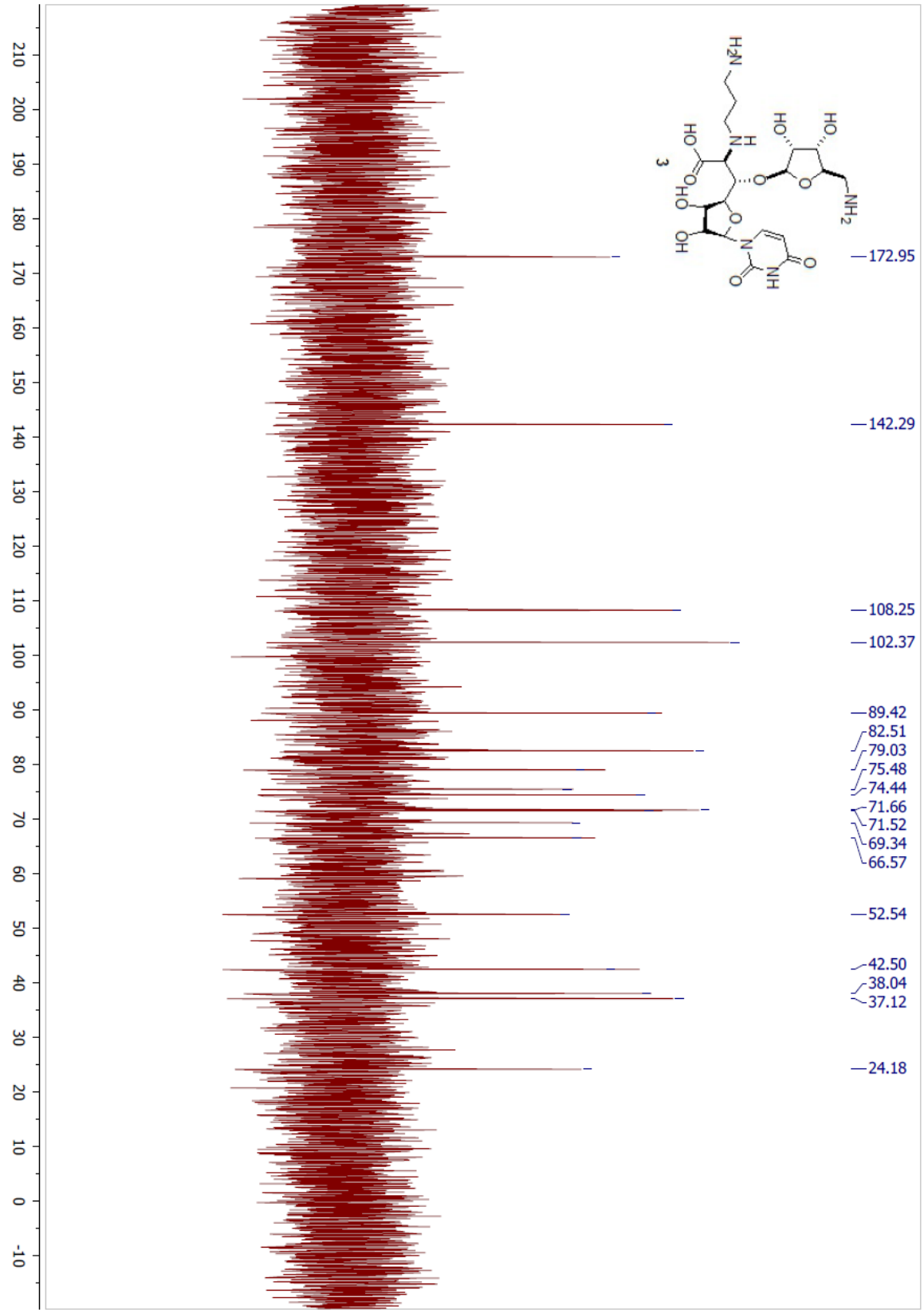
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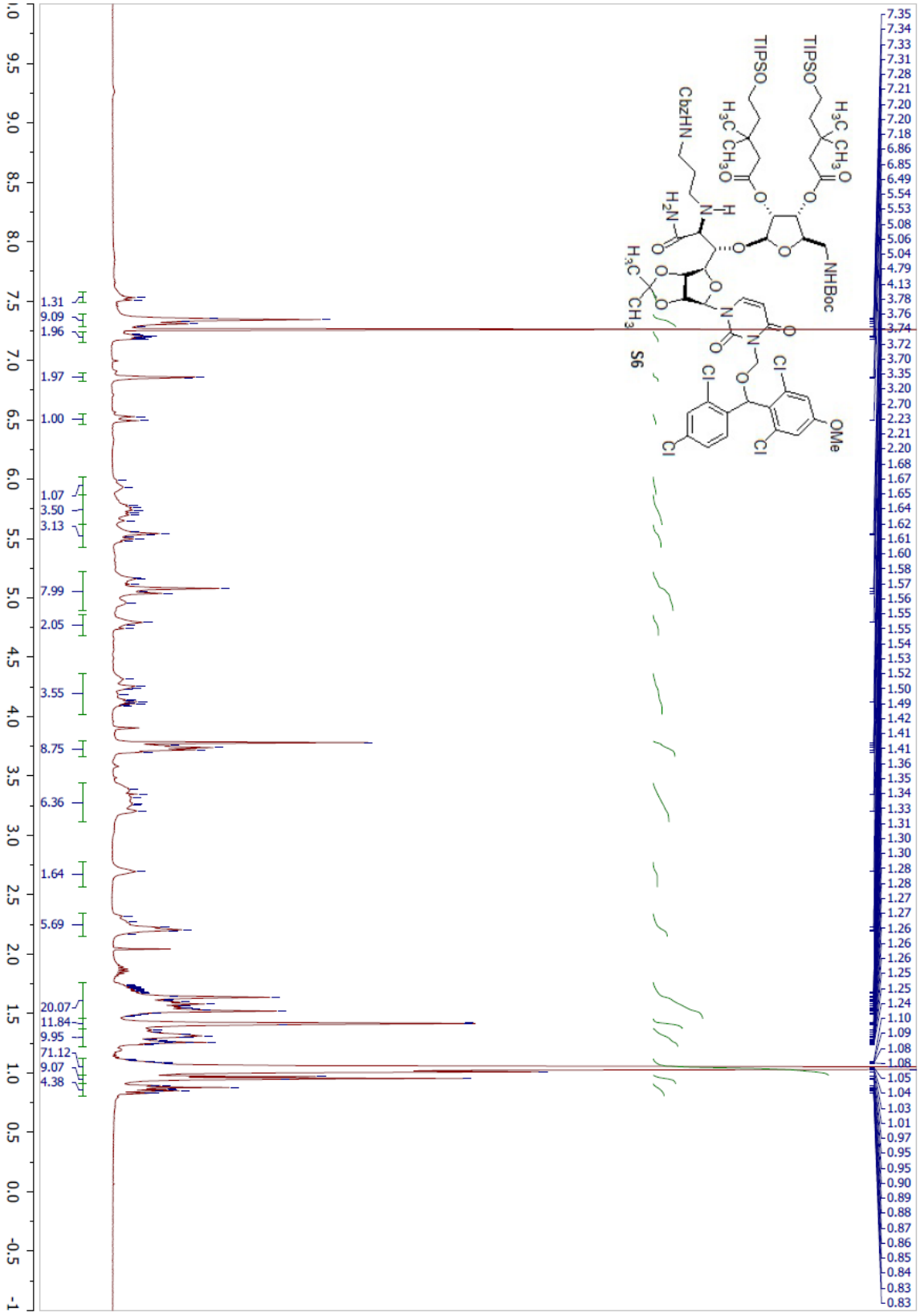
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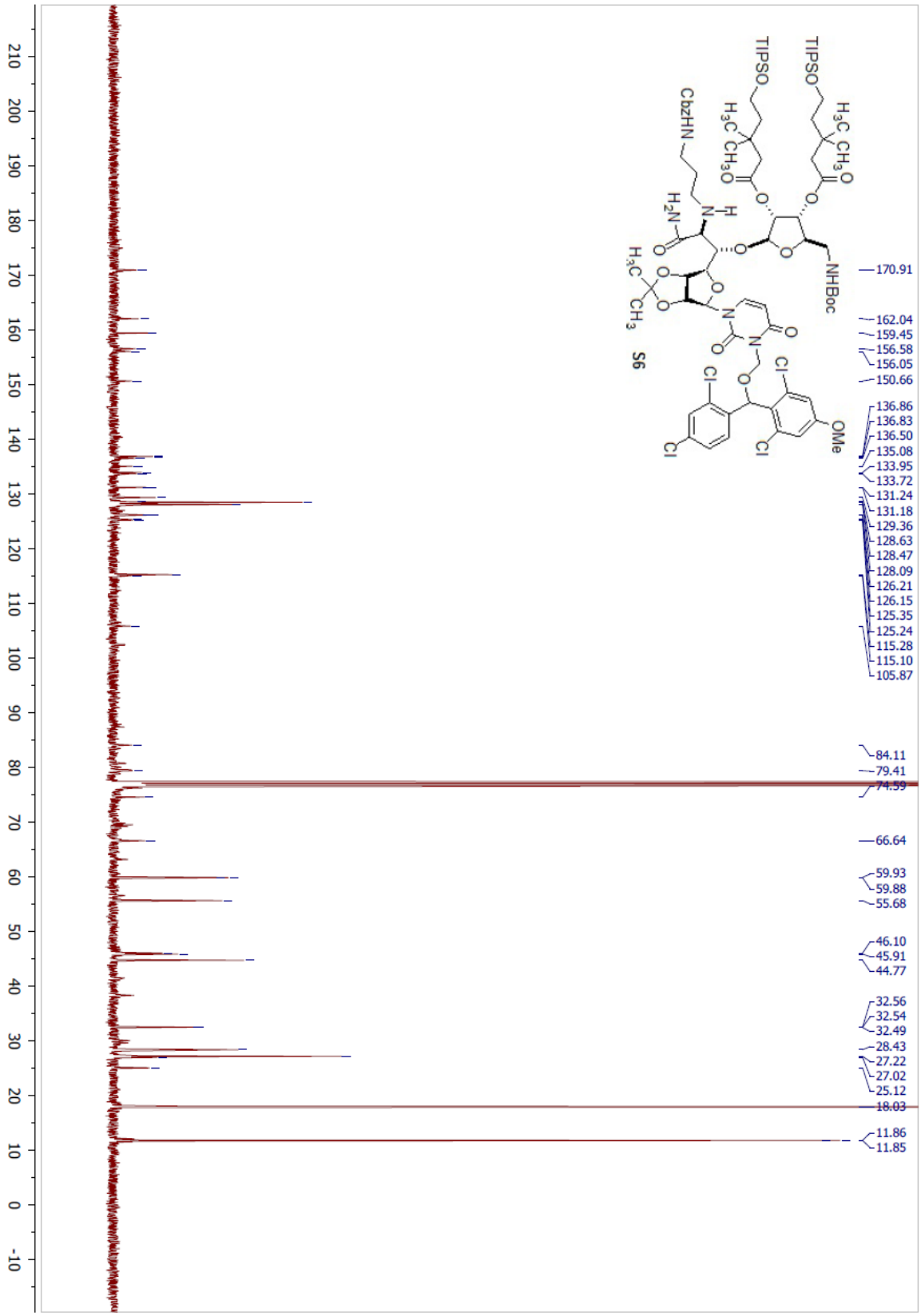
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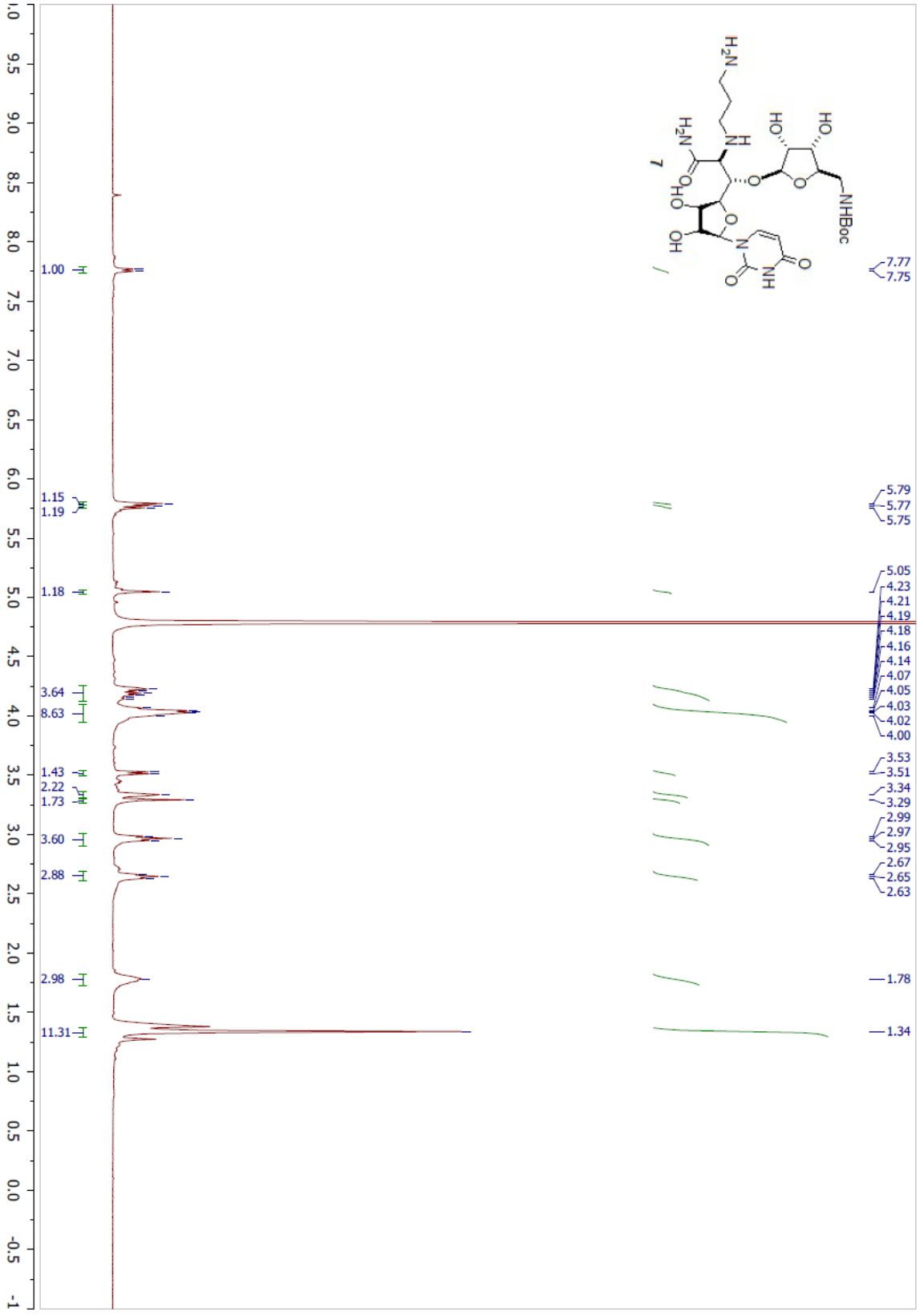
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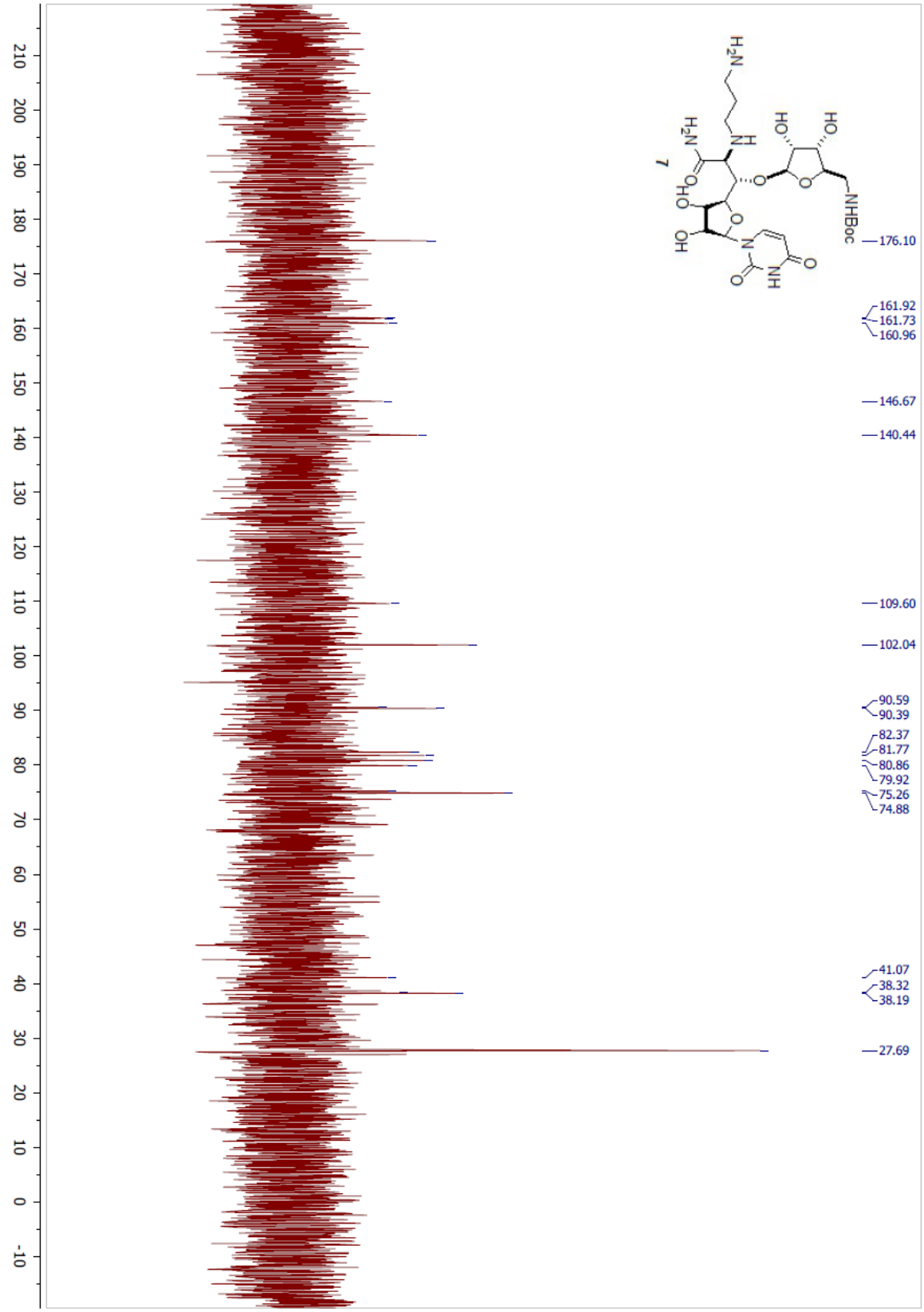
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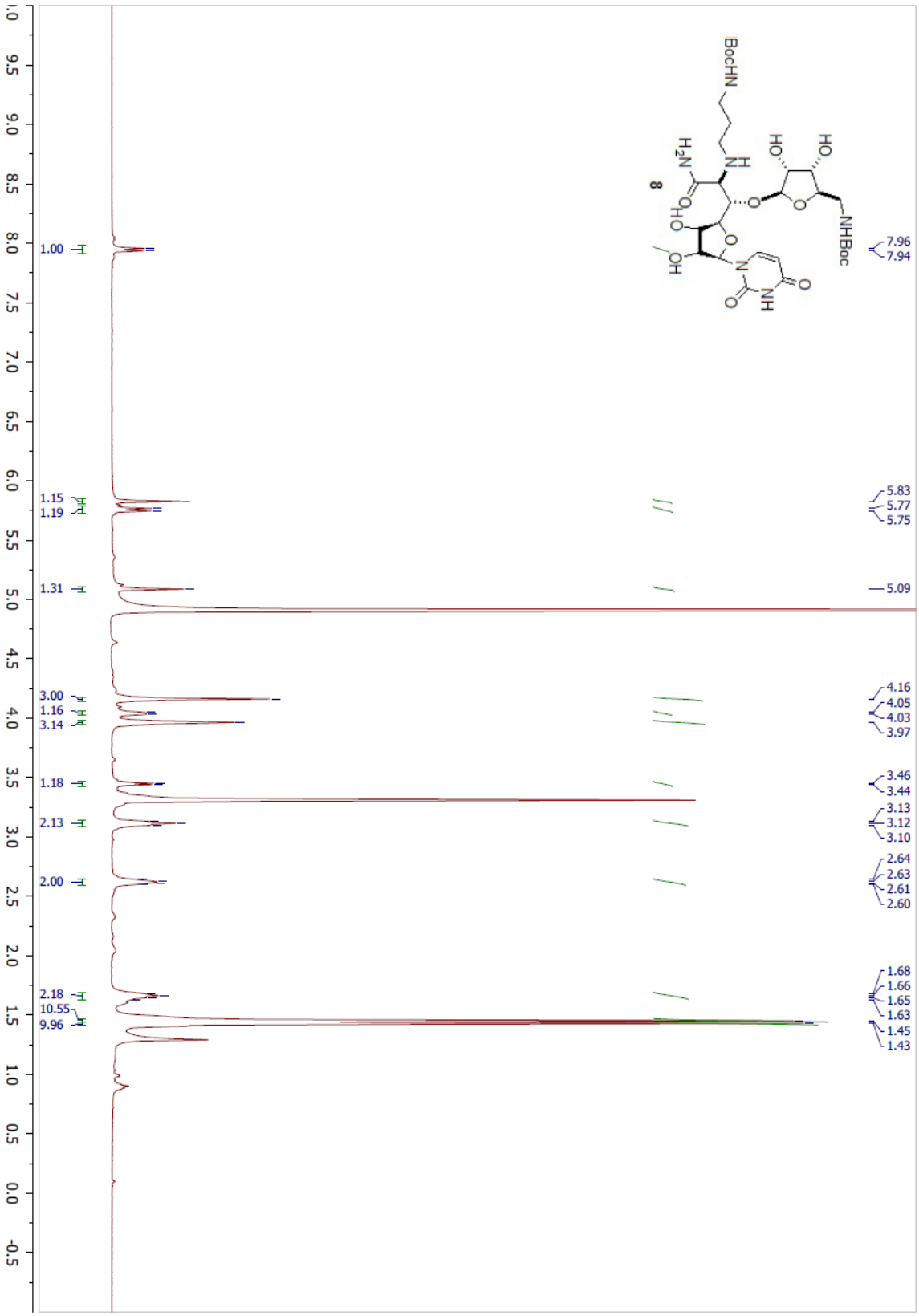
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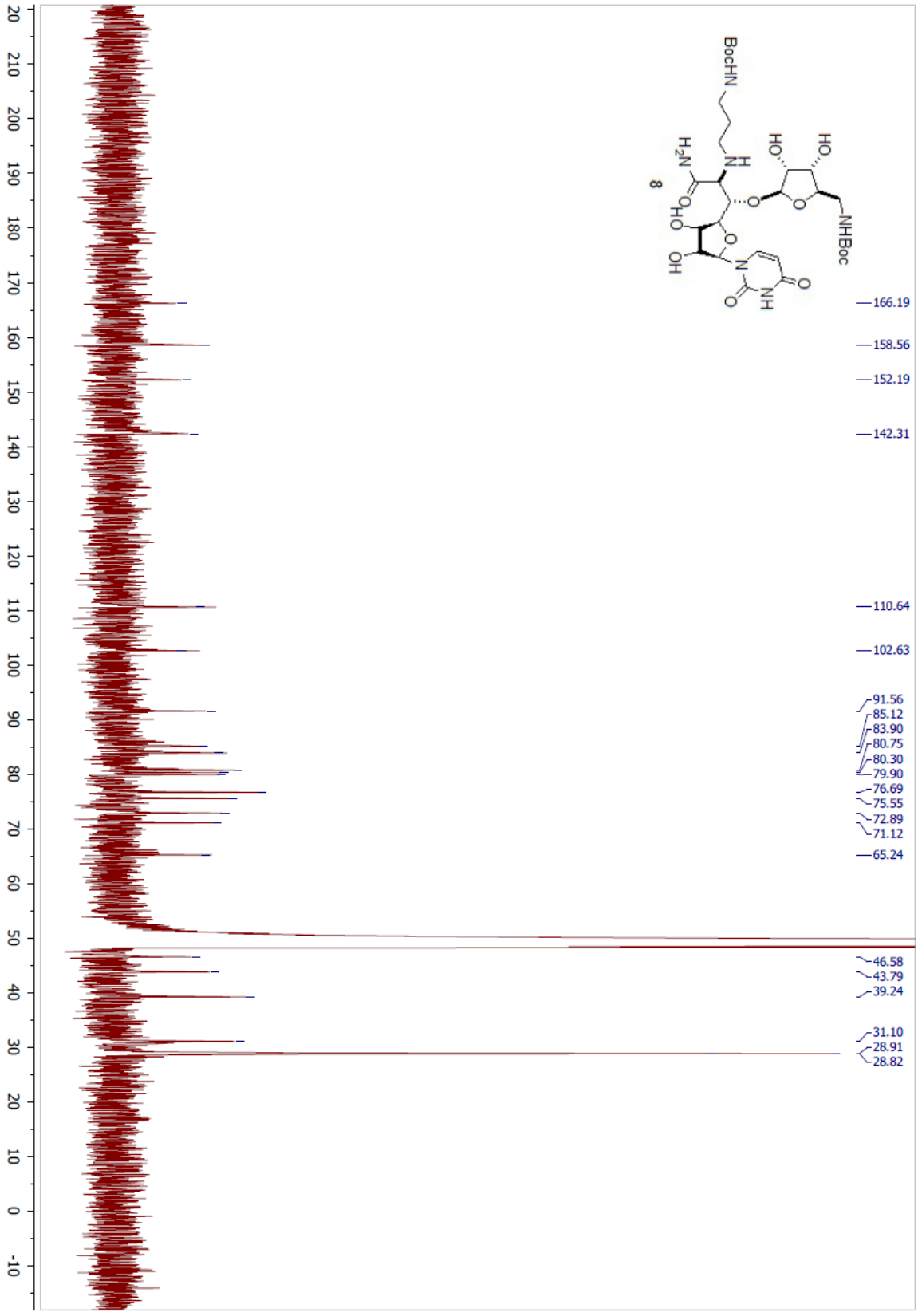
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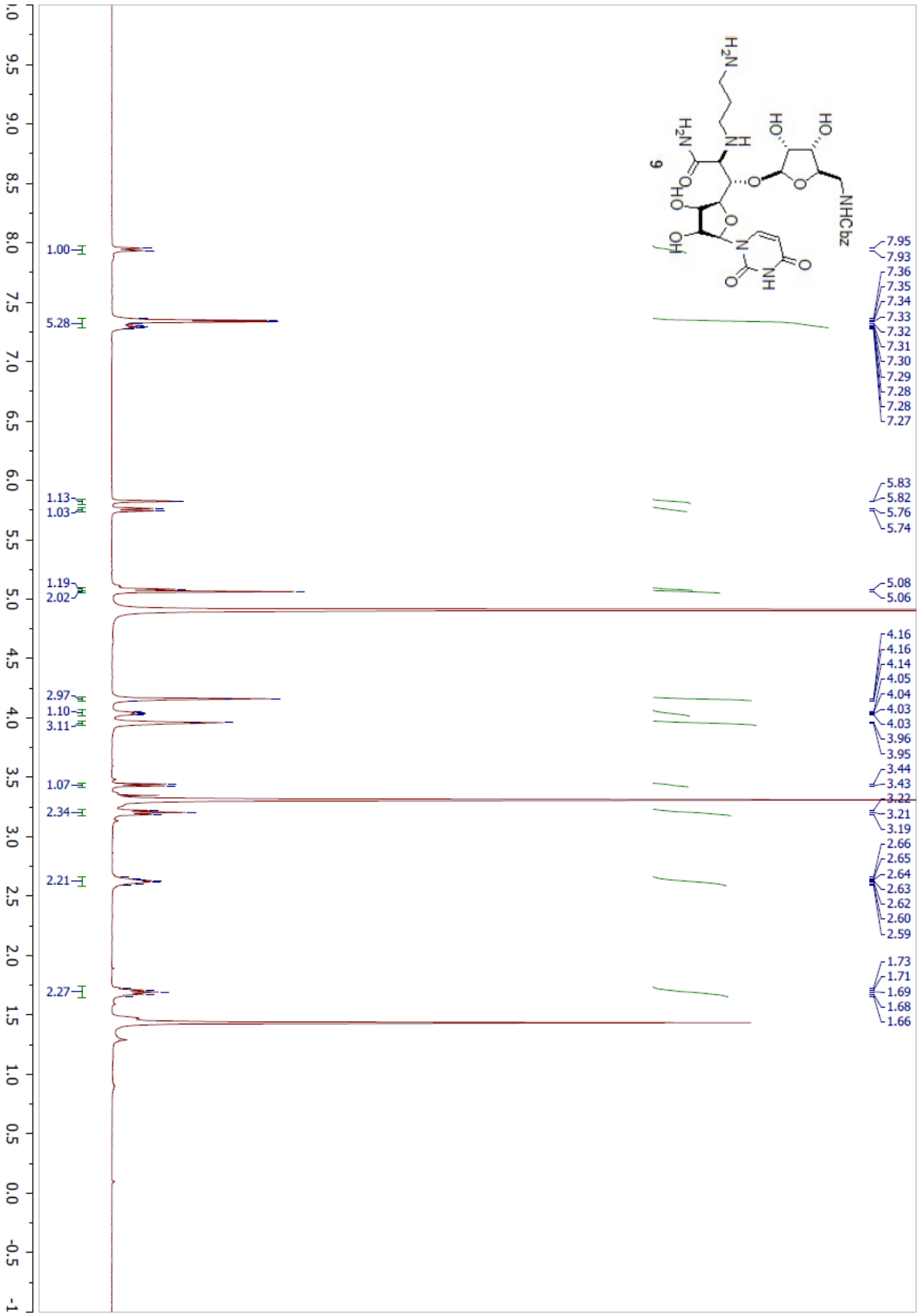
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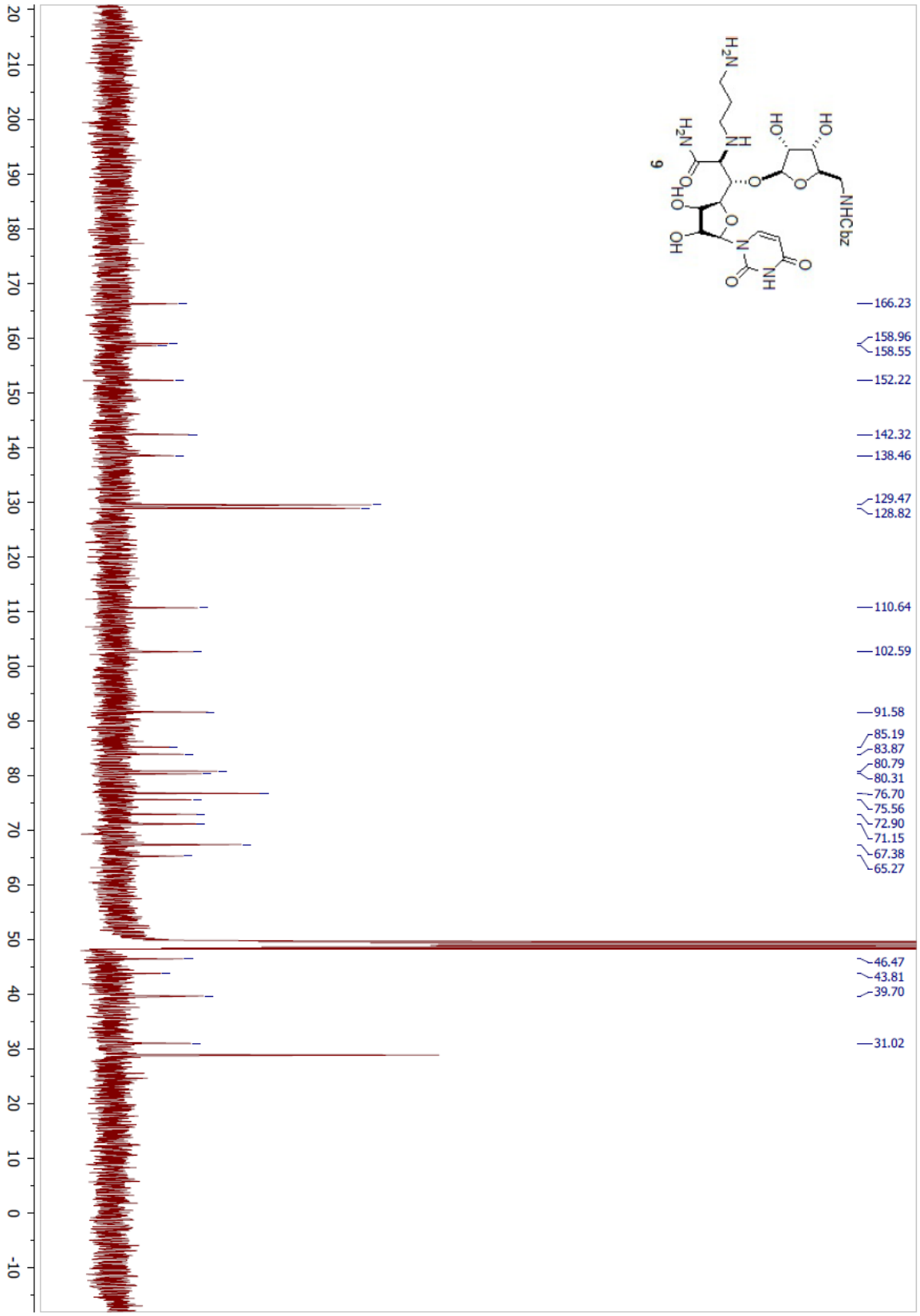
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31.02
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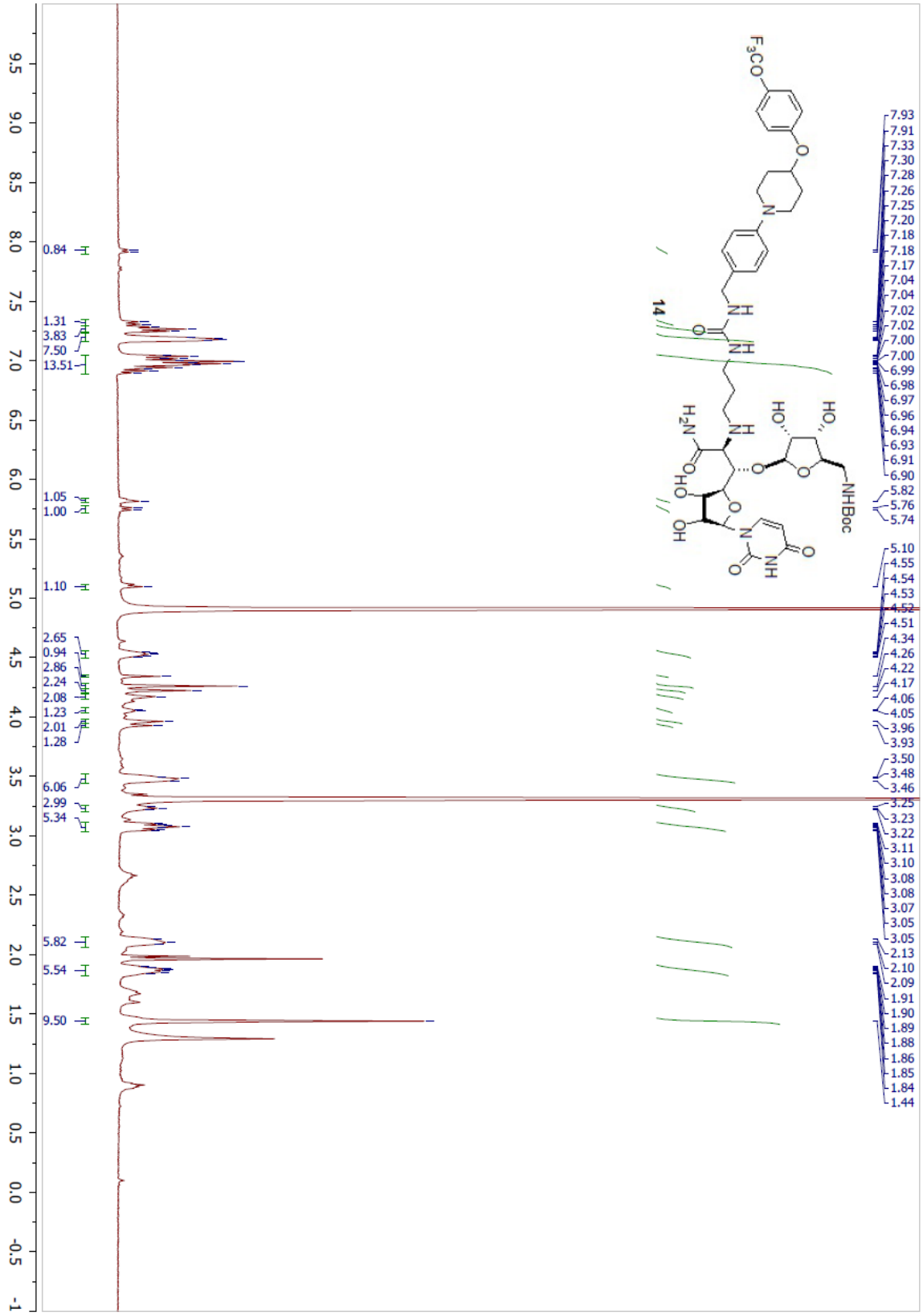
F3CO
14
H2N
HO
NHBoc
NH
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1.31
3.83
7.50
13.51
1.05
1.00
1.10
2.65
0.94
2.86
2.24
2.08
1.23
2.01
1.28
6.06
2.99
5.34
5.82
5.54
9.50
7.93
7.91
7.33
7.30
7.28
7.26
7.25
7.20
7.18
7.18
7.17
7.04
7.04
7.02
7.02
7.00
7.00
6.99
6.98
6.97
6.96
6.94
6.93
6.91
6.90
5.82
5.76
5.74
5.10
4.55
4.54
4.53
4.52
4.51
4.34
4.26
4.22
4.17
4.06
4.05
3.96
3.93
3.50
3.48
3.46
3.25
3.23
3.22
3.11
3.10
3.08
3.08
3.07
3.05
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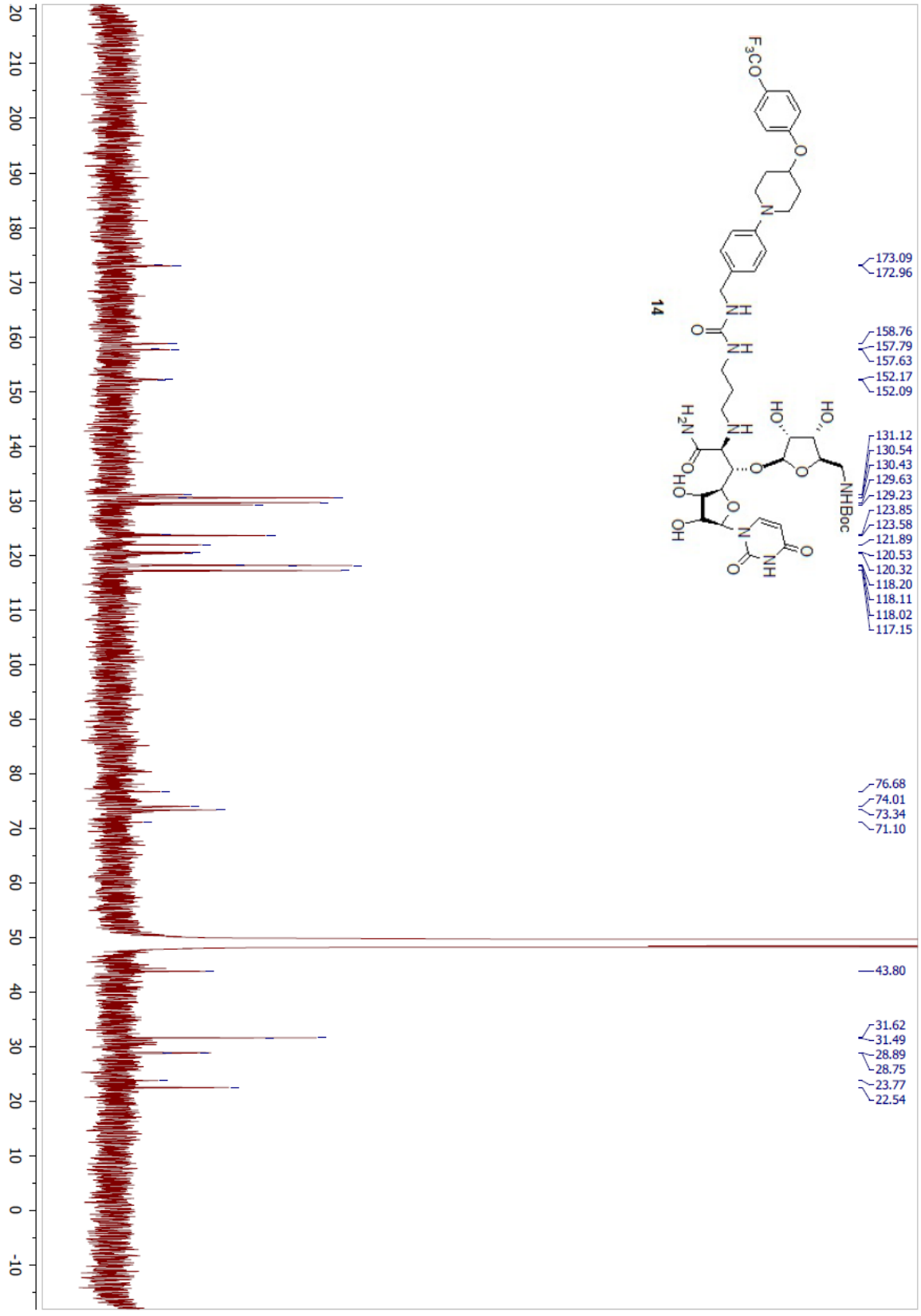
1.44


14
173.09
172.96
158.76
157.79
157.63
152.17
152.09
131.12
130.54
130.43
129.63
129.23
123.85
123.58
121.89
120.53
120.32
118.20
118.11
118.02
117.15
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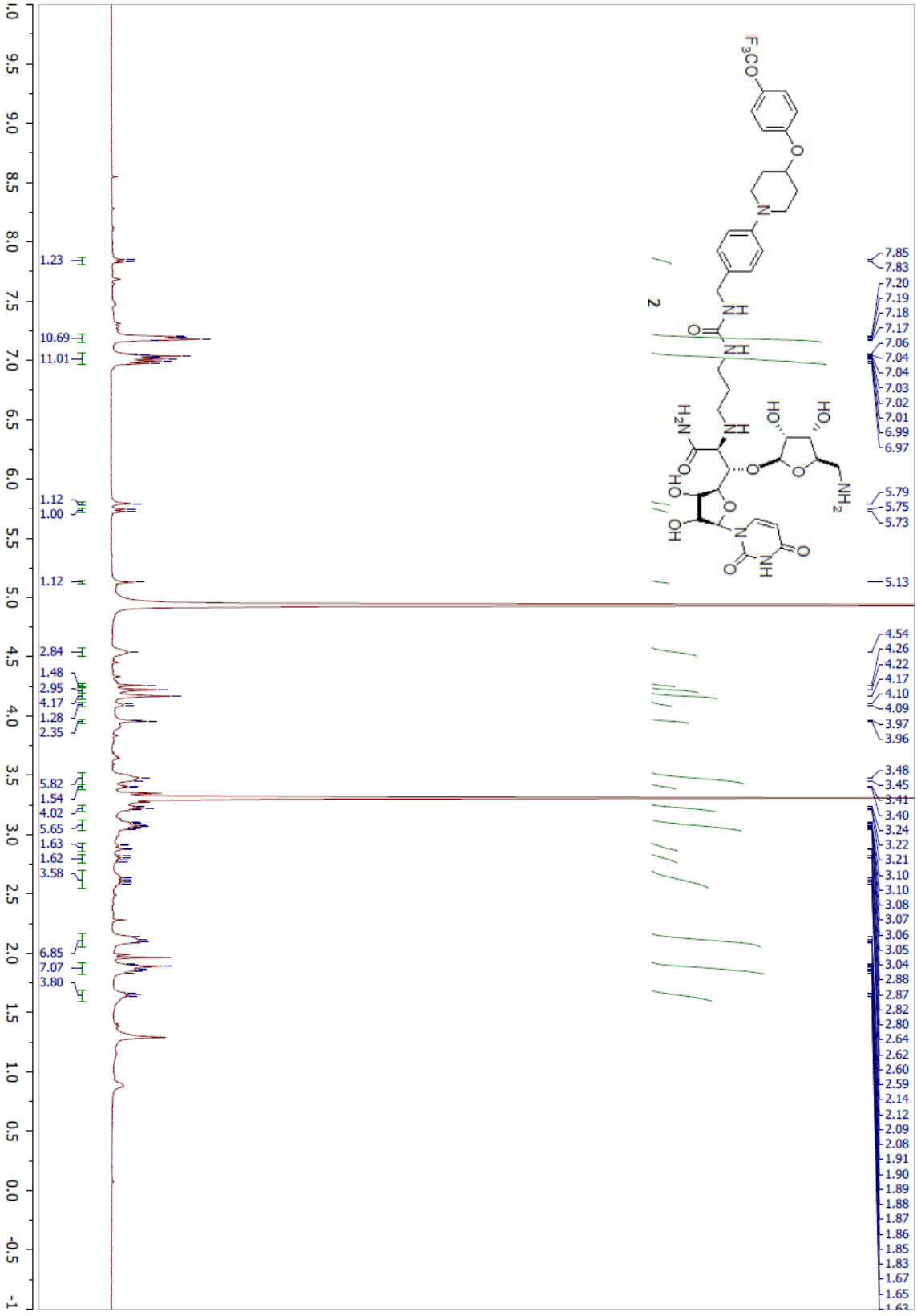
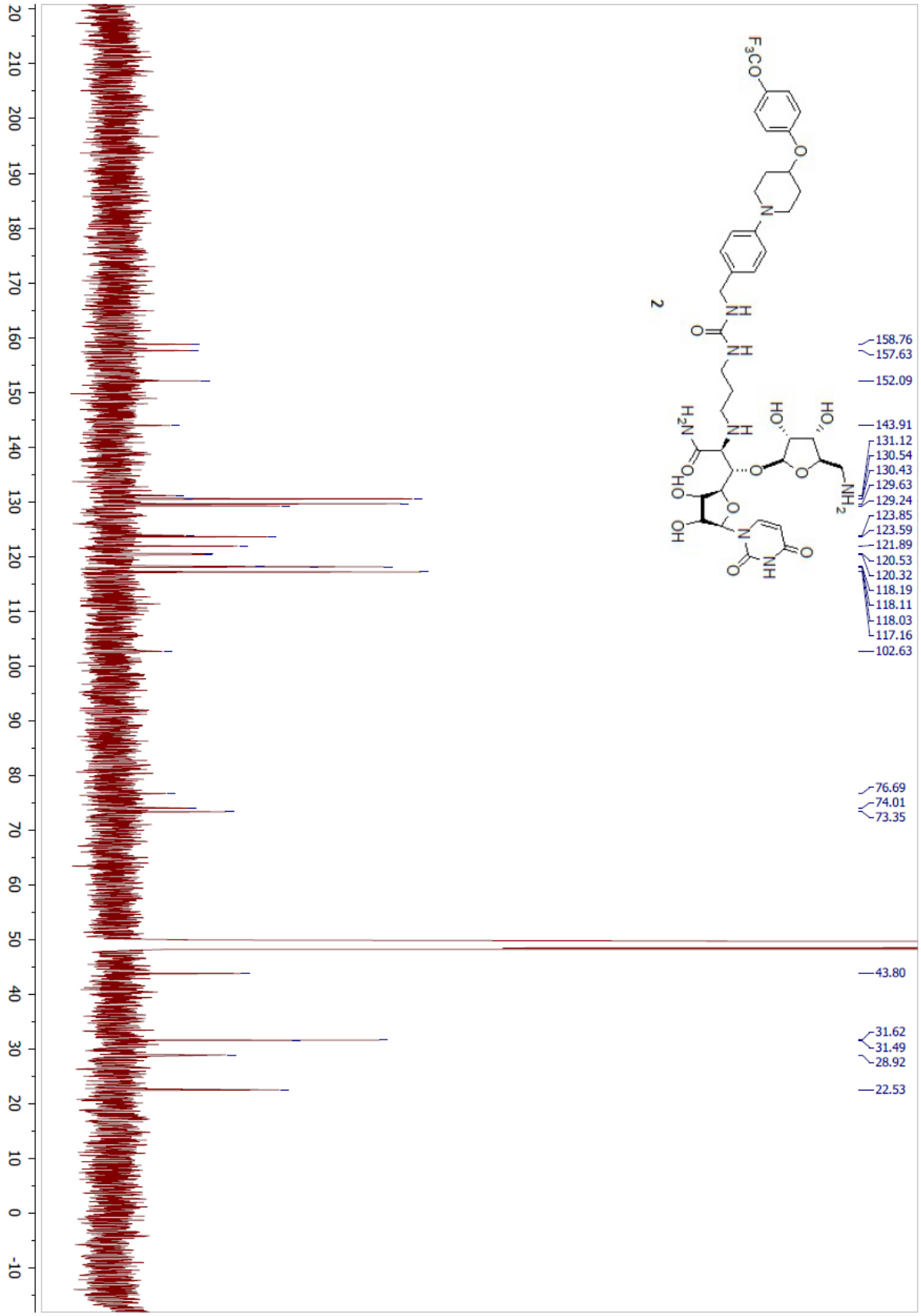
74.01
73.34
71.10
43.80
31.62
31.49
28.89
28.75
23.77
22.54

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157.63
152.09
143.91
131.12
130.54
130.43
129.63
129.24
123.85
123.59
121.89
120.53
120.32
118.19
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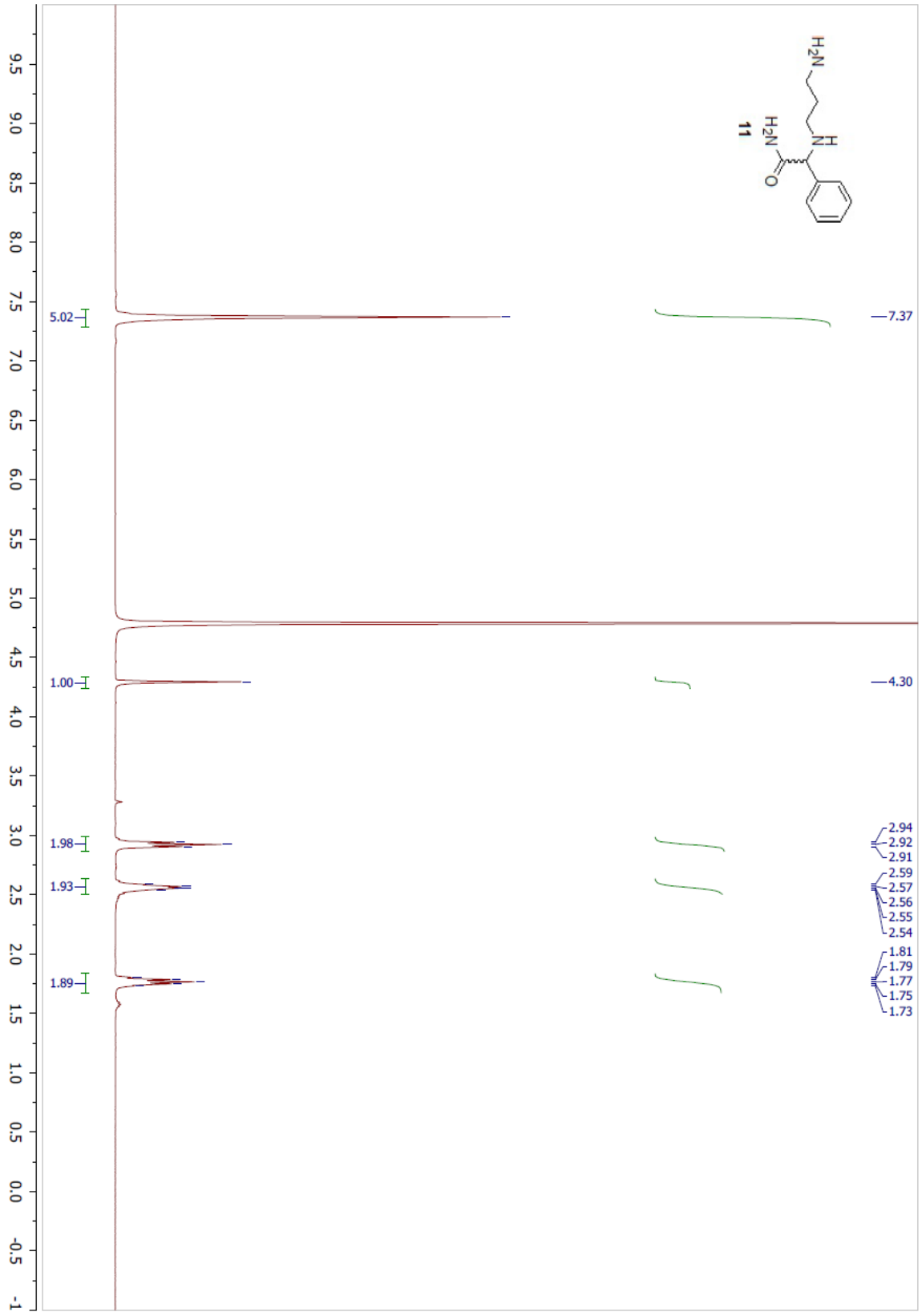
117.16
102.63
76.69
74.01
73.35
43.80
31.62
31.49
28.92
22.53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5.02
1.00
1.98
1.93
1.89
7.37
4.30
2.94
2.92
2.91
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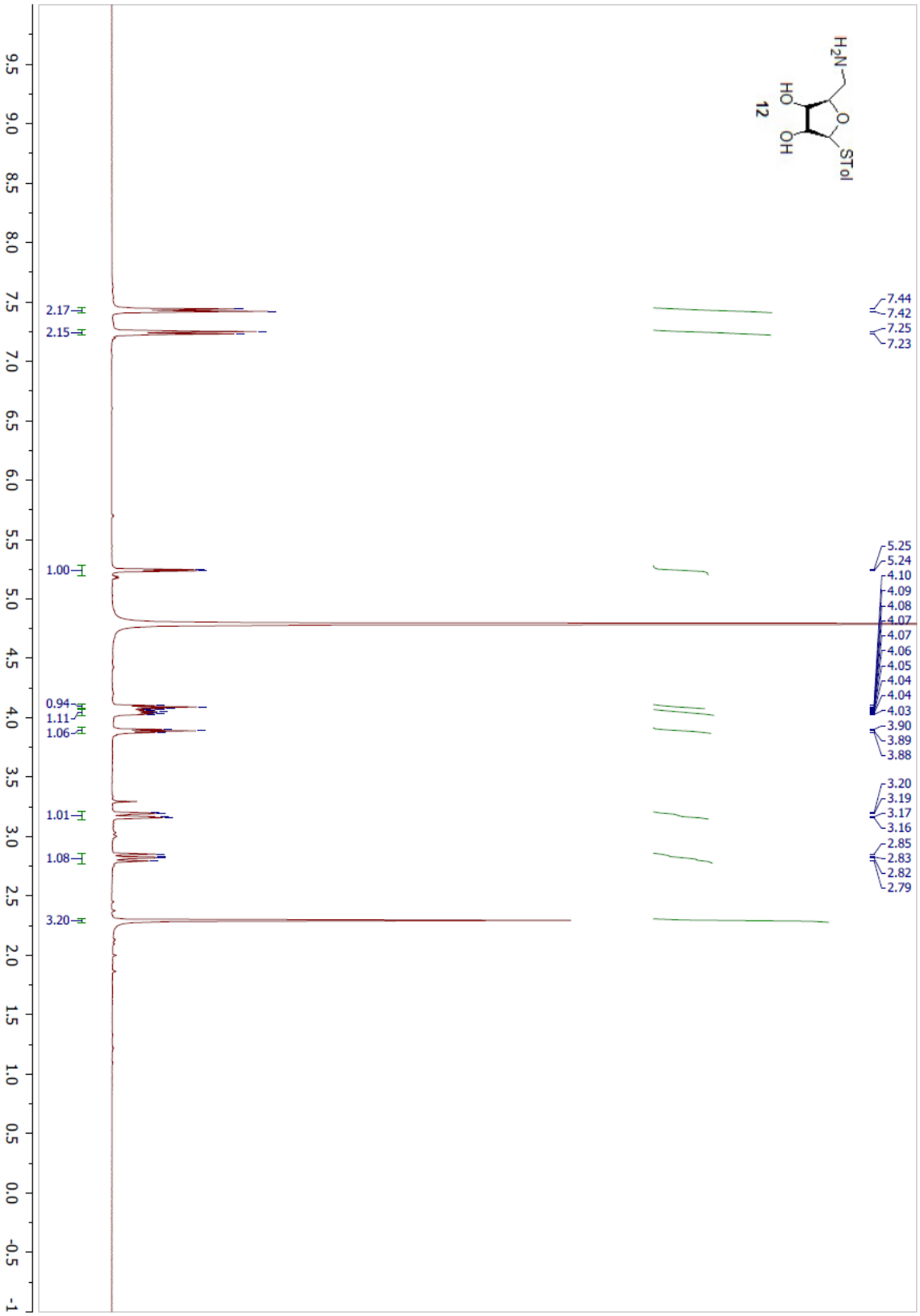
2.57
2.56
2.55
2.54
1.81
1.79
1.77
1.75
1.73


12
H2N
HO
OH
O
STol
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1
2.17
2.15
1.00
0.94
1.11
1.06
1.01
1.08
3.20
7.44
7.42
7.25
7.23
5.25
5.24
4.10
4.09
4.08
4.07
4.07
4.06
4.05
4.04
4.04
4.03
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3.88
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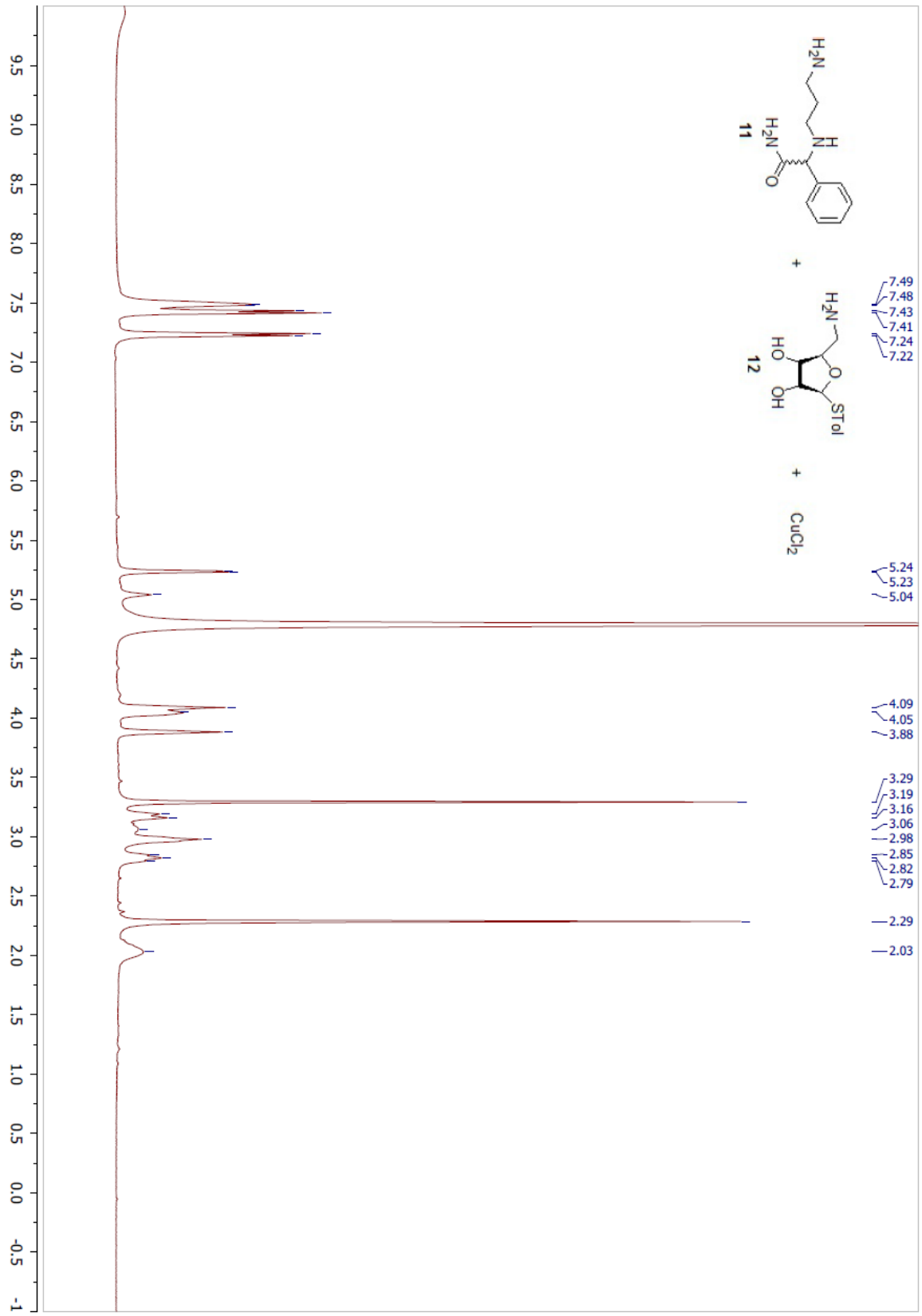
3.19
3.17
3.16
2.85
2.83
2.82
2.79


11
12
+
+
CuCl2
STol
7.49
7.48
7.43
7.41
7.24
7.22
5.24
5.23
5.04
4.09
4.05
3.88
3.29
3.19
3.16
3.06
2.98
2.85
2.82
2.79
2.29
2.03