Supporting Information

Design, Synthesis, and Evaluation of Acyclic *C*-Nucleoside and *N*-Methylated Derivatives of the Ribitylaminopyrimidine Substrate of Lumazine Synthase as Potential Enzyme Inhibitors and Mechanistic Probes

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6-[1-Fluoro-(3S,4R,5R)-3,4,5,6-tetrahydroxyhexyl]-5-nitropyrimidine-2,4-dione (20a). To a suspension of Amberlite-IR-120 (H^+) (3 g) in H_2O (3 mL) was added **19a** (30 mg) in MeOH (3 mL). The reaction mixture was heated at reflux for 4 h. After it was cooled down, the reaction mixture was filtered and washed with MeOH (3 × 5 mL). Solvent was removed under reduced pressure. Reprecipitation from MeOH and ether gave **20a** as a white amorphous solid (18 mg, 80%). ^{1}H NMR (300 MHz, CD_3OD) δ 6.00 (dt, *J* = 5.52, 46.89 Hz, 1 H), 4.05-3.39 (m, 5 H), 2.76-2.07 (m, 2 H). Anal. Calcd. for $C_{10}H_{14}FN_3O_8 \cdot 0.2\ Et_2O$: C, 38.37; H, 4.77. Found C, 38.24; H, 4.98.

6-[1-Fluoro-(3S,4R,5R)-3,4,5,6-tetrahydroxy-hexyl]-5-nitropyrimidine-2,4-dione (20b). To a suspension of Amberlite-IR-120 (H^+) (1.5 g) in a mixture of MeOH (3 mL) and H_2O (3 mL) was added **19b** (20 mg) in MeOH (3 mL). The reaction mixture was heated at reflux for 4 h. After it was cooled down, the reaction mixture was filtered and washed with MeOH (3 × 5 mL). The solvent was removed under reduced pressure. Reprecipitation from MeOH and ether gave **20b** as a white amorphous solid (12 mg, 80%). ^{1}H NMR (300 MHz, CD_3OD) δ 6.20-5.95 (m, 1 H), 3.95 (m, 1 H), 3.70-3.45 (m, 4 H), 2.20-1.90 (m, 2 H); ^{19}F NMR (282 MHz, CD_3OD solvent with CF_3COOD as external standard) δ -113.19; ESIMS *m/z* 322 ($M\text{-}H^-$, 100). Anal. Calcd. for $C_{10}H_{14}FN_3O_8$: C, 37.16; H, 4.37. Found C, 37.35; H, 4.77.

6-E-(2,3:4,5-Di-*O*-isopropylidene-D-ribitylidenemethylene)-2,4-dimethoxy-5-nitropyrimidine (22). A solution of **14** (1.29 g, 2.81 mmol) and **21** (0.659 g, 2.86 mmol) in dry toluene (30 mL) was heated under reflux under Ar for 24 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (20 g, 3 × 40 cm column of SiO_2, hexane-ethyl acetate 3:1), to afford **22** as a glass (1.036 g, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 4.64, 15.02 Hz, 1 H), 6.78 (dd, *J* = 1.14, 15.02 Hz, 1 H) 4.91 (t, *J* = 5.52 Hz, 1 H), 4.17 (dd, *J* = 7.09, 8.25 Hz, 1 H), 4.05 (s, 3 H), 4.04 (m, 1 H), 4.03 (s, 3 H), 3.95 (m, 1 H), 3.87 (m, 1 H), 1.47 (s, 3 H), 1.38 (s, 3

H), 1.36 (s, 3 H), 1.26 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7, 162.8, 156.2, 140.6, 128.2, 121.7, 109.7, 109.4, 79.0, 77.0, 73.9, 67.4, 55.4, 55.3, 27.3, 26.6, 25.2, 25.1; EIMS (MH^+) *m*/*z* 412. Anal. Calcd for $C_{18}H_{25}N_3O_8 \cdot 1.7H_2O$: C, 48.91; H, 6.48; N, 9.51. Found: C, 48.93; H, 6.40; N, 9.32.

6-[(1R,2S,3S,4R,5R)-1,2,3,4,5,6-hexahydroxyhexyl]-5-nitropyrimidine-2,4-dione (24). Compound **23** (0.150 g, 0.337 mmol) and Amberlite-IR-120 (H^+) resin (5.0 g) were heated in refluxing 50% aq MeOH (24 mL) for 4.5 h. After the mixture was cooled to room temperature, it was filtered, and the solvent was removed under reduced pressure. The residue was reprecipitated from MeOH-ether. The precipitate was dried over P_2O_5 under vacuum to give **24** as an amorphous solid (0.070 g, 62%): ^{1}H NMR (500 MHz, D_2O) δ 5.47 (s, 1 H), 4.01 (d, *J* = 8.73 Hz, 1 H), 3.84 (d, *J* = 8.78 Hz, 1 H), 3.75 (s, 2 H), 3.66 (d, *J* = 11.87 Hz, 1 H), 3.50 (dd, *J* = 4.99, 11.48 Hz, 1 H). Anal. Calcd for $C_{10}H_{15}N_3O_{10}$: C, 35.61; H, 4.48. Found: C, 35.44; H, 4.43.

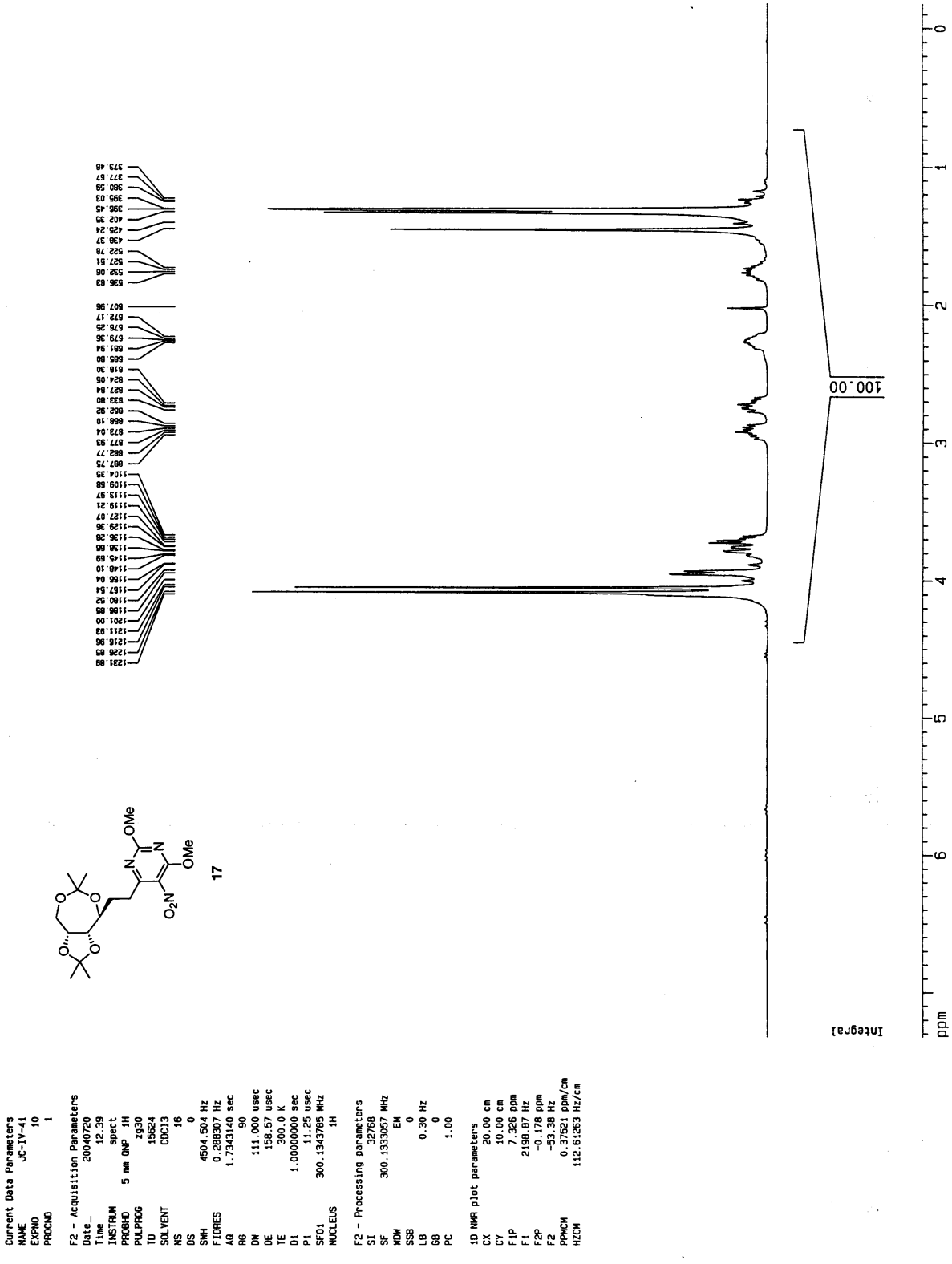
(1S,2R)-1-(2,6-Dimethoxy-5-nitro-pyrimidin-4-yl)-2-(2,2,6,6-tetramethyl-tetrahydro-[1,3]dioxolo[4,5-e][1,3]dioxepin-4-yl)-ethane-1,2-diol (25). After a mixture of water (5 mL), *tert*-butyl alcohol (5 mL) and AD-mix-β (1.54 g) was stirred at room temperature for 15 min, it appeared as two clear phases. Methanesulfonamide (0.104 g) was added and the mixture was cooled to 0 °C. Compound **16** (0.454, 1.10 mmol) was added at once. The reaction mixture was vigorously stirred at 0 °C for 12 h and then at 15 °C for 72 h. The reaction mixture was cooled to 0 °C and solid sodium sulfite (1.5 g) was added. The mixture was warmed to room temperature and stirred for 1 h. It was extracted with CH_2Cl_2 (5 × 10 mL). The organic layer was washed with 1 N NaOH solution (3 × 10 mL) and water (10 mL), and then dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (30 g, 3 × 30 cm column of SiO_2, hexanes-EtOAc 3:2) to give **25** as a glass (0.264 g, 52%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.08 (d, *J* = 8.35 Hz, 1 H), 4.11 (d, *J* = 5.42 Hz, 1 H), 4.08 (m, 1 H), 4.06 (s, 3 H), 4.03 (s, 3 H), 4.02 (m, 1 H), 3.96 (m, 3 H), 3.90 (m, 2 H), 1.51 (s, 3 H), 1.40 (s, 3 H),

1.35 (s, 3 H), 1.31 (s, 3 H); EIMS (MH^+) *m/z* 446. Anal. Calcd for $C_{18}H_{27}N_3O_{10}$·0.75 EtOAc: C, 49.31; H, 6.50. Found: C, 49.13; H, 6.51.

6-[(1S,2R,3S,4R,5R)-1,2,3,4,5,6-hexahydroxyhexyl]-5-nitro-pyrimidine-2,4-dione (26). Compound **25** (0.240 g, 0.539 mmol) and Amberlite-IR-120 (H^+) resin (7.0 g) were heated in refluxing 50% aq MeOH (30 mL) for 4.5 h. After the mixture was cooled to room temperature, it was filtered and the solvent was removed under reduced pressure. The residue was reprecipitated from MeOH-ether. The precipitate was dried over P_2O_5 under vacuum to give **26** as an amorphous solid (0.080 g, 44%). ^{1}H NMR (300 MHz, D_2O) δ 5.46 (s, 1 H), 4.00 (d, *J* = 8.73 Hz, 1 H), 3.83 (dd, *J* = 2.62, 8.81 Hz, 1 H), 3.75 (s, 2 H), 3.65 (m, 1 H), 3.50 (m, 1 H). Anal. Calcd for $C_{10}H_{15}N_3O_{10}$: C, 35.61; H, 4.48. Found: C, 35.32; H, 4.43.

***N*-Methylribitylamine (28)**.[28] Methylamine (10.0 mL, 2.0 M in MeOH, 20 mmol) was added to a solution of D-ribose **(27)** (3.0 g, 18.3 mmol) in MeOH (40 mL). After the solution was stirred at room temperature for 4 h, PtO_2 (200 mg) was added. The mixture was degassed and hydrogenated at 40 psi for 12 h. The mixture was filtered and solvent was removed to give **28** as a yellow glass (3.0 g, 100%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.88-3.51 (m, 5 H), 2.85-2.80 (m, 1 H), 2.70-2.63 (m, 1 H), 2.40 (s, 3 H).

5-Amino-6-(*N*-methyl)ribityl-pyrimidine-2,4-dione hydrochloride (32). Concd HCl (0.5 mL) and Pd/C (20 mg, 10%) were added to a solution of **31** (50 mg, 0.156 mmol) in MeOH (10 mL). The mixture was degassed and stirred under H_2 (1 atm) for 12 h. The catalyst was filtered off and the solvent was removed under reduced pressure. Reprecipitation from MeOH (0.2 mL) and ether (5 mL) gave **32** as pale yellow amorphous solid (35 mg, 70%). ^{1}H NMR (300 MHz, D_2O) δ 4.14-3.84 (m, 2 H), 3.70-3.45 (m, 3 H), 3.20-3.28 (m, 2 H), 2.61 (s, 3 H). Anal. Calcd for $C_{10}H_{19}ClN_4O_6$·4.5 H_2O: C, 29.45; H, 6.92. Found: C, 29.16; H, 6.69.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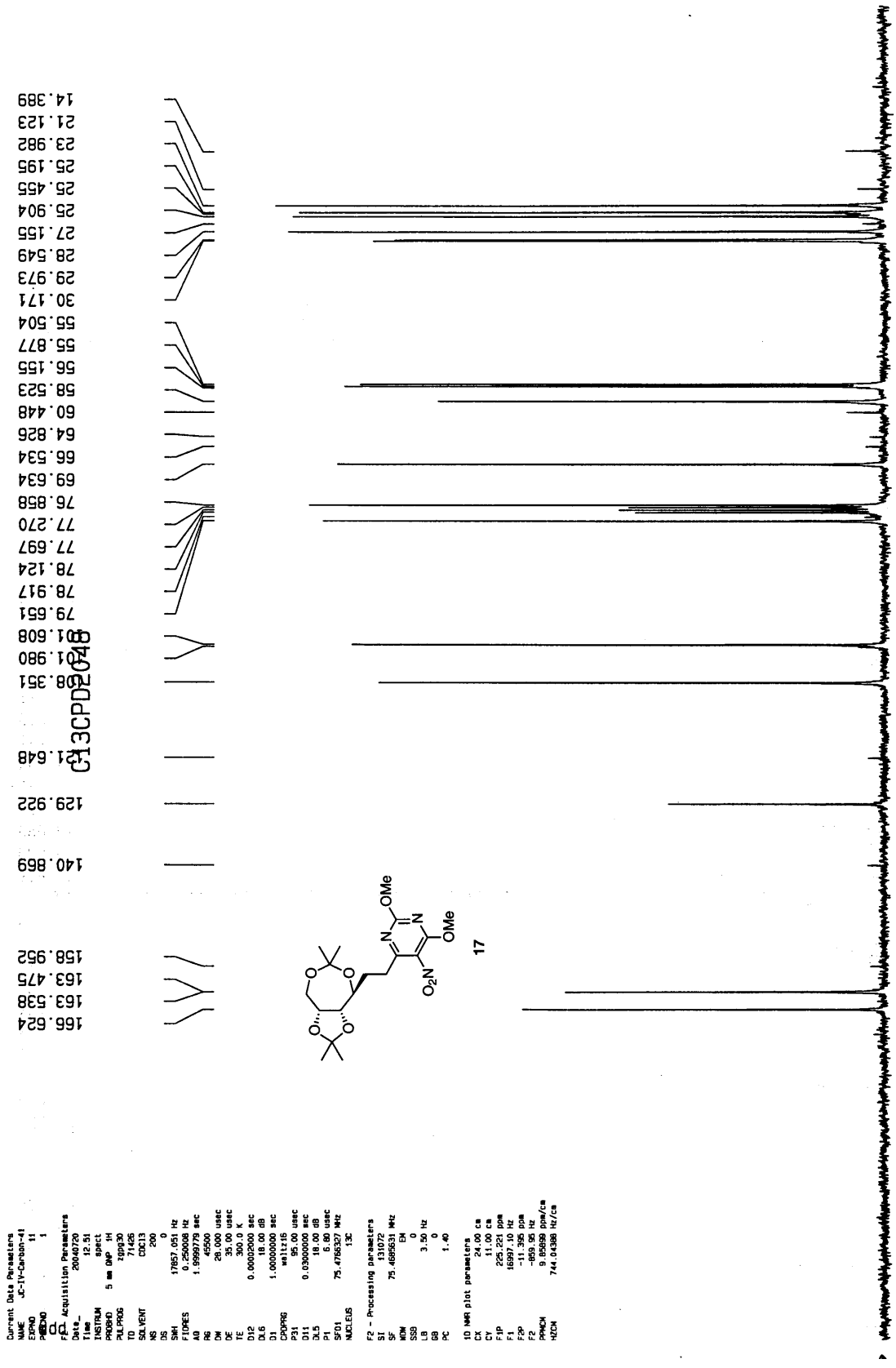
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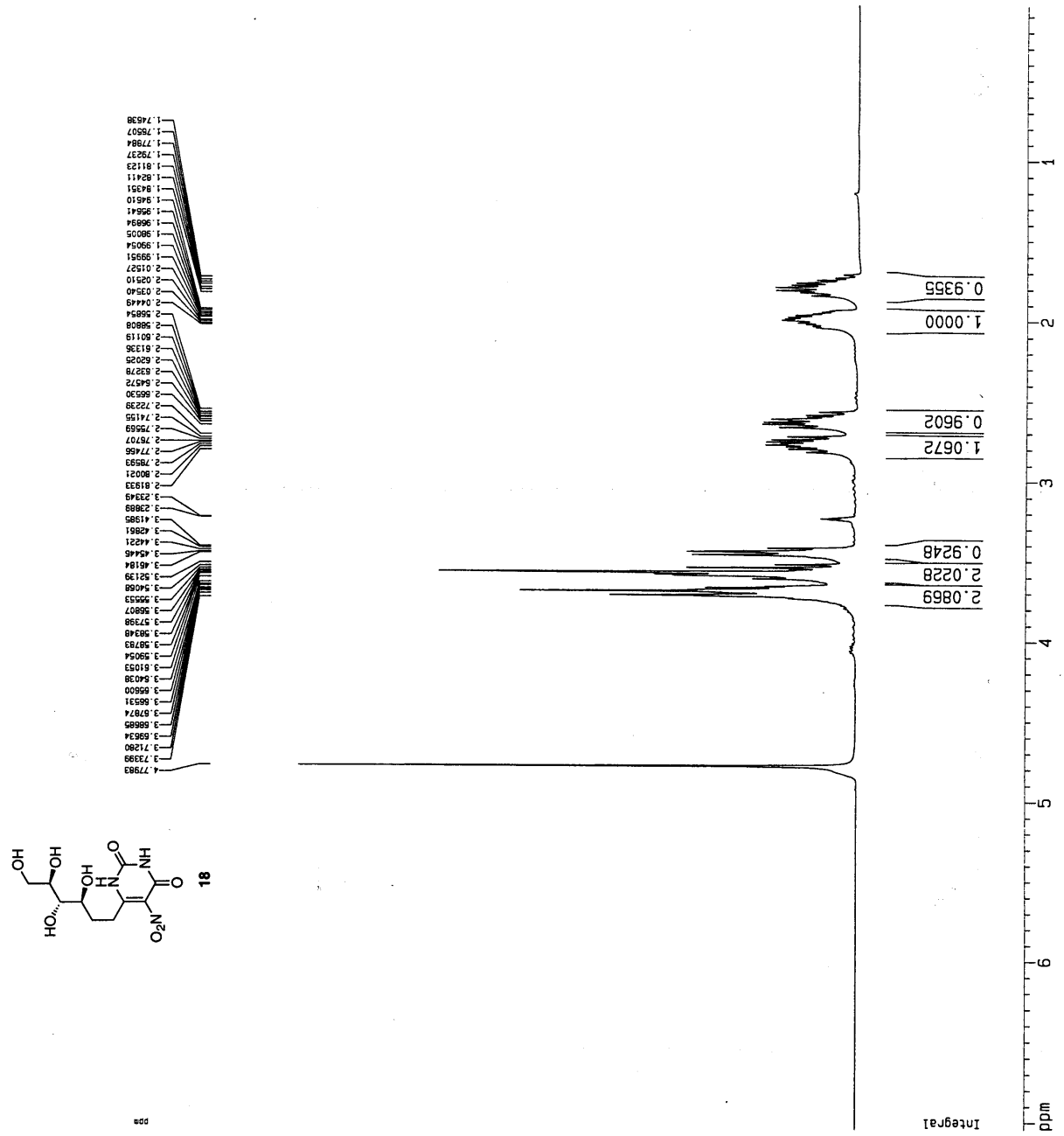


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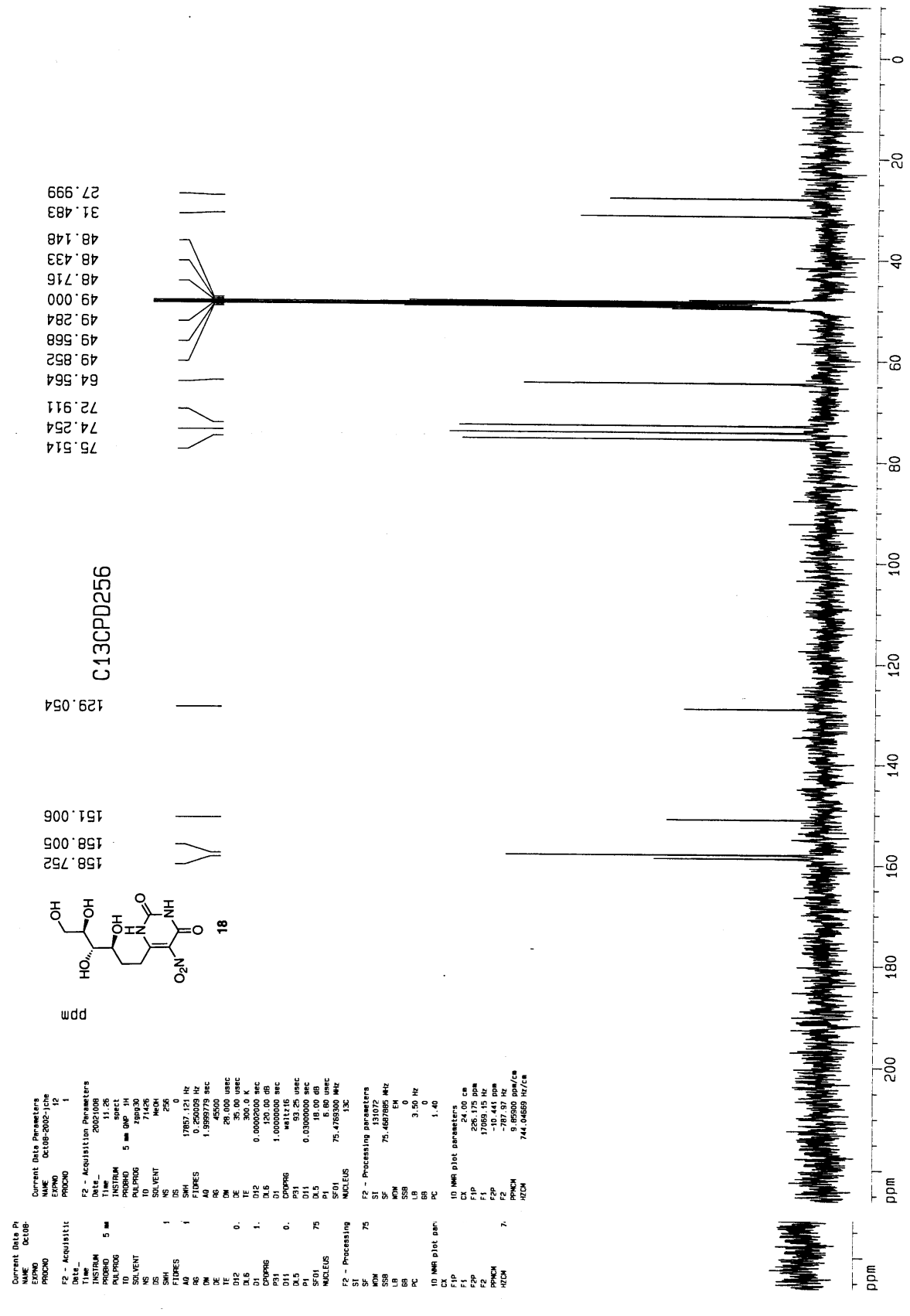
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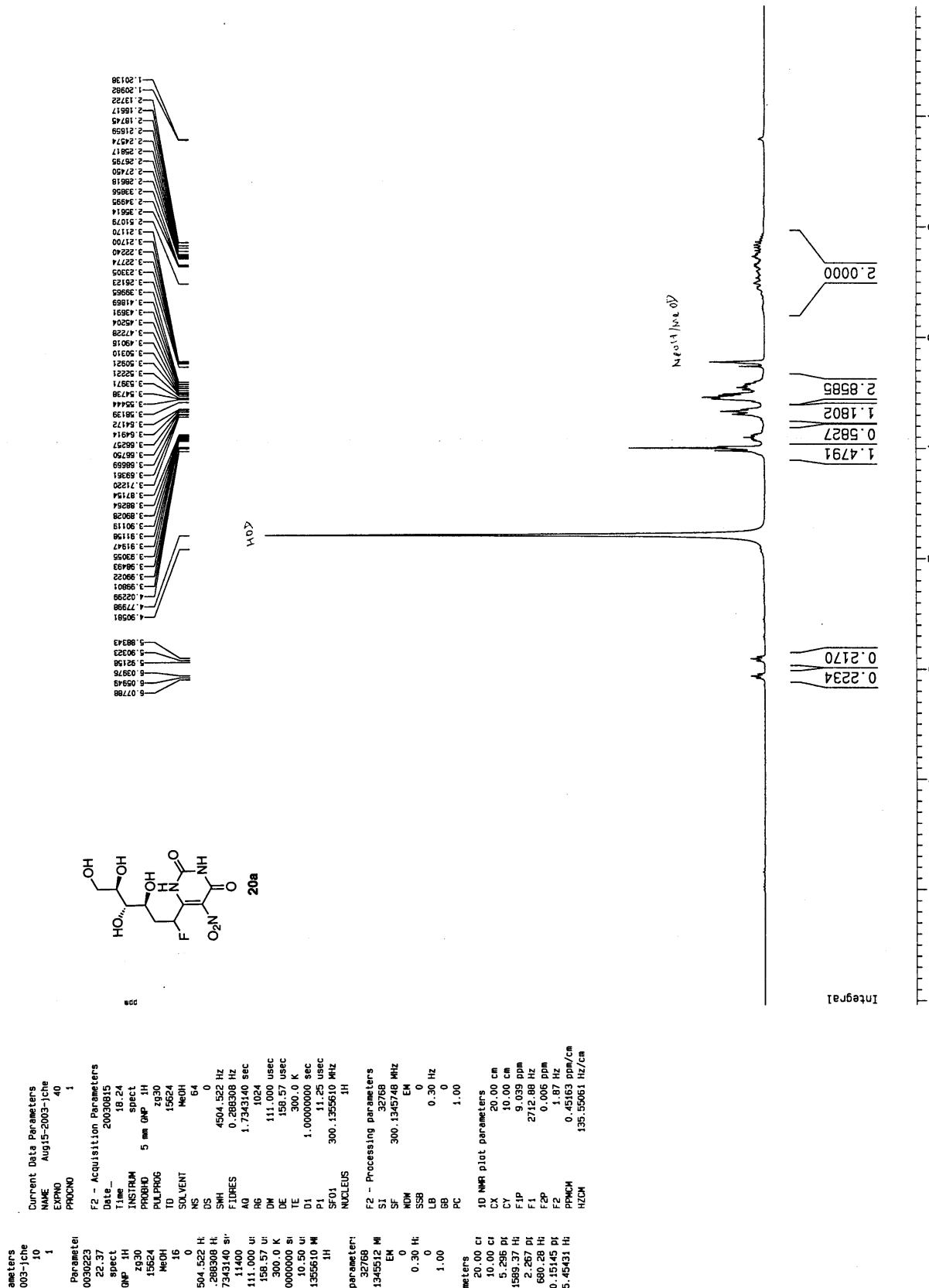
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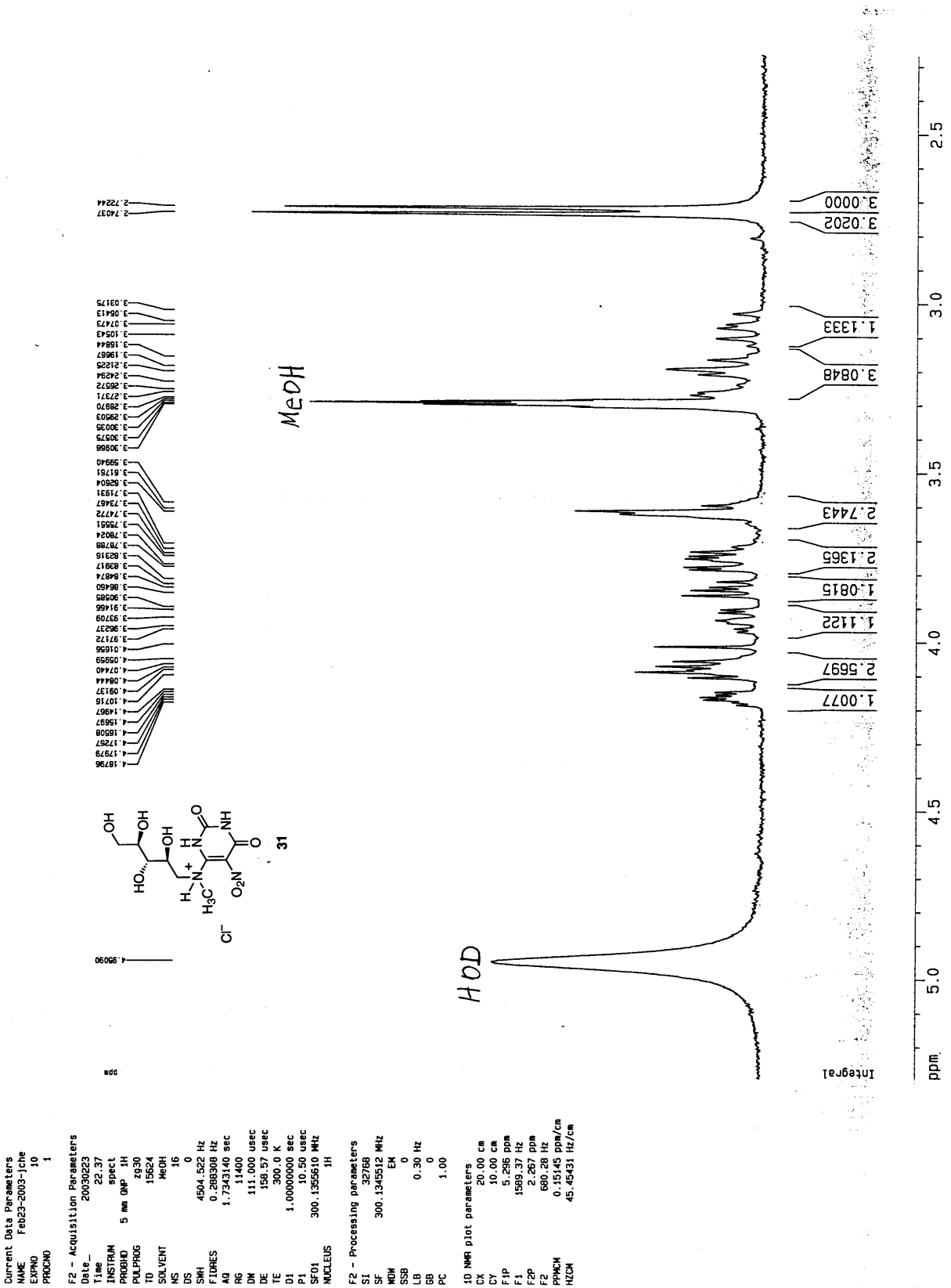
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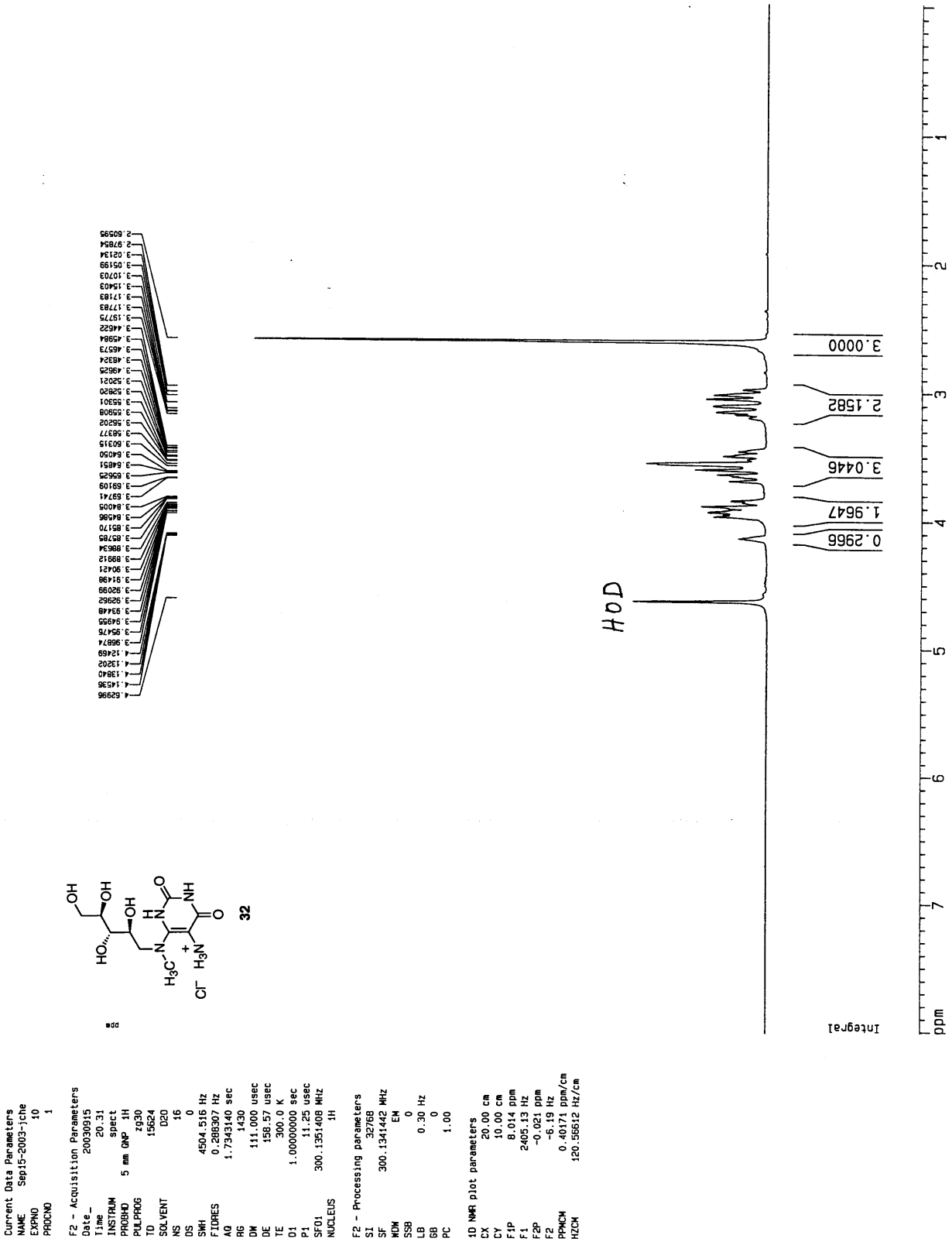
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