Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Convenient Synthesis of Pyrimidine 2'-Deoxyribonucleoside Monophosphates with Important Epigenetic Marks at the 5-Position

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Synthesis of 5'-*O*-(4,4'-dimethoxytrityl)-5-formyl-2'-deoxyuridine-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite and 5'-*O*-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite.

Synthesis of 5-formyl-2'-deoxyuridine.

Place 3',5'-*O*-di-*t*-butylsilyl-5-formyl-2'-deoxyuridine[1] (190 mg, 0.48 mmol) in a 50 mL polypropylene tube. Add 8 mL of anhydrous THF followed by HF-pyridine (12 μL, 0.48 mmol) under argon atmosphere. The mixture was stirred at room temperature for 1 h before 4 g of silica gel was added. Solvent was evaporated under reduced pressure to afford a silica gel mixed residue. Crude product was purified by column chromatography (CH_2Cl_2 with 6% to 12% methanol) to afford 5-formyl-2'-deoxyuridine[1] (100 mg, 0.39 mmol, 81% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO) δ: 9.76 (s. 1H), 8.71 (s, 1H), 6.09, (t, *J* = 6.3 Hz, 1H), 5.27 (br, 1H), 5.11 (br, 1H), 4.23 (dd, *J* = 4.2, 8.9 Hz, 1H), 3.85 (dd, *J* = 3.5, 6.9 Hz, 1H), 3.62 (m, 2H), 2.21 (m, 2H). HRMS (*m/z*): calculated for $C_{10}H_{13}N_2O_6$ (M+H): 257.0768; found: 257.0772. The ^{1}H NMR spectrum of 5-formyl-2'-deoxyuridine is consistent with CAS 4494-26-2.[1]

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-5-formyl-2'-deoxyuridine.

To a solution of 5-formyl-2'-deoxyuridine (60 mg, 0.23 mmol) in 3 mL of anhydrous pyridine was added 4,4'-dimethoxytrityl chloride (119 mg, 0.35 mmol) under argon atmosphere. The mixture was stirred overnight at room temperature before it was quenched with the addition of 0.5 mL of methanol. Solvent was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2, washed with 5% $NaHCO_3$, water, brine and dried over anhydrous Na_2SO_4. Solvent was removed *in vacuo*, crude product was purified by column chromatography (CH_2Cl_2 with 2.5% methanol) to afford 5'-*O*-(4,4'-dimethoxytrityl)-5-formyl-2'-deoxyuridine (115 mg, 0.21 mmol, 90% yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.65 (s, 1H), 8.42 (s, 1H), 7.24 (m, 2H), 7.22 (m, 7H), 6.80 (dd, *J* = 0.84, 9 Hz, 4H), 6.15 (t, *J* = 6.6 Hz, 1H), 4.42 (m, 1H), 4.10 (m, 1H), 3.73

(s, 6H), 3.38 (m, 2H), 2.59 (m, 1H), 2.25 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 185.3, 161.6, 158. 6, 149.3, 145.8, 144.3, 135.4, 135.3, 130.0, 127.9, 127.0, 113.3, 111.1, 87.06, 87.00, 86.8, 72.4, 63.4, 55.2, 41.5. HRMS (*m/z*): calculated for $C_{31}H_{31}N_2O_8$ (M+H): 559.2075; found: 559.2078. The ^{1}H and ^{13}C NMR spectra of 5'-*O*-(4,4'-dimethoxytrityl)-5-formyl-2'-deoxyuridine are consistent with CAS 157022-75-8.[2]

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-5-formyl-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite.

To a solution of 5'-*O*-(4,4'-dimethoxytrityl)-5-formyl-2'-deoxyuridine (40 mg, 0.072 mmol) in 1 mL of anhydrous CH_3CN were added diisopropylethylamine (28 mg, 0.215 mmol), tetrazole in CH_3CN (0.24 mL, 0.108 mmol) and 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (33 mg, 0.108 mmol). The reaction was kept at room temperature and monitored by TLC. Ten minutes later, precipitate appeared. Upon completion, the reaction was quenched with the addition of 0.5 mL of absolute ethanol. Reaction was diluted with ethyl acetate with 1% triethylamine, washed with 5% $NaHCO_3$, water, brine, and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure, crude product was purified by column chromatography (CH_2Cl_2 with 2% methanol and 1% triethylamine) to afford 5'-*O*-(4,4'-dimethoxytrityl)-5-formyl-2'-deoxyuridine-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (48 mg, 0.063 mmol, 88% yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.64 (d, J = 3.34 Hz, 1H), 8.46 (s, 0.53H), 8.42 (s, 0.47H), 7.99 (s, 1H), 7.21 (m, 10H), 6.81 (m, 4H), 6.14 (m, 1H), 5.86 (s, 1H), 4.49 (m, 1H), 4.15 (m, 1H), 3.76 (s, 6H), 3.61 (m, 8H), 2.72 (m, 2H), 2.58 (m, 1H), 2.42 (m, 1H), 1.23 (m, 12H). ^{13}C NMR (75 MHz, CD_3OD) δ: 187.5, 163.7, 160.4, 151.1, 149.0, 146.2, 137.1, 136.9, 131.53, 131.50, 129.4, 129.0, 128.1, 114.4, 112.2, 88.9, 88.8, 88.3, 72.7, 64.8, 55.9, 55.0, 41.7, 26.7, 26.4, 19.4, 19.0. ^{31}P NMR (120 MHz, $CDCl_3$) δ: 149.2, 148.8. HRMS (*m/z*): calculated for $C_{40}H_{48}N_4O_9P$ (M+H): 759.3153; found: 759.3158. The ^{1}H and ^{13}C NMR spectra of 5'-*O*-(4,4'-dimethoxytrityl)-5-formyl-

2'-deoxyuridine-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite are consistent with CAS 181421-94-3.[2]

Synthesis of 5-methoxycarbonyl-2'-deoxyuridine.

To a solution of 5-iodo-2'-deoxyuridine (100 mg, 0.282 mmol) in 4 mL of anhydrous DMF were added triphenylphosphine (45 mg, 0.17 mmol), triethylamine (57 mg, 0.566 mmol), $Pd_2(dba)_3$ (26 mg, 0.028 mmol) and 0.5 mL of methanol. The reaction flask was vacumm-flushed with carbon monoxide for three times. The reaction was then allowed to proceed at 70°C under CO atmosphere overnight. Reaction was filtered, filtrate was concentrated and purified by column chromatography (CH_2Cl_2 with 10% methanol) to afford 5-methoxycarbonyl-2'-deoxyuridine[3] (79 mg, 0.276 mmol, 98% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, DMSO) δ: 8.81 (s, 1H), 6.08 (t, *J* = 6.3 Hz, 1H), 5.23 (br, 1H), 5.03 (br, 1H), 4.23 (m, 1H), 3.83 (m, 1H), 3.68 (s, 3H), 3.58 (m, 2H), 2.20 (m, 2H). HRMS (*m/z*): calculated for $C_{11}H_{15}N_2O_7$ (M+H): 287.0874; found: 287.0871. The ^{1}H NMR spectrum of 5-methoxycarbonyl-2'-deoxyuridine is consistent with CAS 158021-27-3.[3]

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine.

To a solution of 5-methoxycarbonyl-2'-deoxyuridine (250 mg, 0.874 mmol) in 20 mL of anhydrous pyridine was added 4,4'-dimethoxytrityl chloride (355 mg, 1.049 mmol) under argon atmosphere. The mixture was stirred overnight at room temperature before it was quenched with the addition of 1 mL of methanol. Solvent was evaporated under reduced pressure. The residue was redissolved in CH_2Cl_2, washed with 5% $NaHCO_3$, water, brine and dried over anhydrous Na_2SO_4. Solvent was removed *in vacuo*, crude product was purified by column chromatography (CH_2Cl_2 with 3% methanol) to afford 5'-*O*-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine[4] (450 mg, 0.765 mmol, 88% yield) as a white foam. ^{1}H NMR (300 MHz, CD_3COCD_3) δ: 8.61 (s, 1H),

7.51 (m, 2H), 7.31 (m, 7H), 6.88 (m, 4H), 6.24 (t, *J* = 6.8 Hz, 1H), 4.47 (m, 1H), 4.11 (m, 1H), 3.79 (s, 6H), 3.42 (dd, *J* = 3.3, 10.5 Hz, 1H), 3.33 (dd, *J* = 4.3, 10.5 Hz, 1H), 3.31 (s, 3H), 2.37 (m, 2H). HRMS (*m/z*): calculated for $C_{32}H_{33}N_2O_9$ (M+H): 589.2181; found: 589.2189. The ^{1}H NMR spectrum of 5'-*O*-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine is consistent with CAS 158021-28-4.[4]

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine-3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite.

To a solution of 5'-*O*-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine (80 mg, 0.136 mmol) in 2 mL of anhydrous CH_3CN were added diisopropylethylamine (53 mg, 0.408 mmol), tetrazole in CH_3CN (0.45 mL, 0.204 mmol) and 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (61 mg, 0.204 mmol). The reaction was kept at room temperature and monitored by TLC. Ten minutes later, a white precipitate appeared. Upon completion, the reaction was quenched with the addition of 0.5 mL of absolute ethanol. Reaction was diluted with ethyl acetate with 1% triethylamine, washed with 5% $NaHCO_3$, water, brine, and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure, crude product was purified by column chromatography (CH_2Cl_2 with 1.5% methanol and 1% triethylamine) to afford 5'-*O*-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite[4] (105 mg, 0.133 mmol, 98% yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.69 (s, 0.52H), 8.65 (s, 0.48H), 7.39 (m, 2H), 7.23 (m, 7H), 6.79 (m, 4H), 6.22 (m, 1H), 4.48 (m, 1H), 4.20 (m, 1H), 3.74 (s, 6H), 3.54 (m, 5H), 3.21 (m, 1H), 3.18 (s, 1.5H), 3.17 (s, 1.5H), 2.72 (m, 2H), 2.57 (m, 1H), 2.22 (m, 1H), 1.23 (m, 12H). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 149.2, 148.6. HRMS (*m/z*): calculated for $C_{41}H_{50}N_4O_{10}P$ (M+H): 789.3259; found: 789.3264. The ^{1}H NMR spectrum of 5'-*O*-(4,4'-dimethoxytrityl)-5-methoxycarbonyl-2'-deoxyuridine-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite is consistent with CAS 158021-31-9.[4]

3’-dNMPs.

3’-^{ca}dUMP ^{1}H NMR (300 MHz, D_2O) δ: 8.45 (s, 1H), 6.35 (dd, *J* = 6.1, 7.4 Hz, 1H), 4.76 (m, 1H), 4.25 (m, 1H), 3.88 (m, 2H), 2.63 (m, 1H), 2.44 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 2.5. HRMS (*m/z*): calculated for $C_{10}H_{12}N_2O_{10}P$ (M-H): 351.0230; found: 351.0236.

3’-^{ca}dCMP ^{1}H NMR (300 MHz, D_2O) δ: 8.56 (s, 1H), 6.32 (dd, *J* = 6.1, 7.4 Hz, 1H), 4.78 (m, 1H), 4.29 (m, 1H), 3.88 (m, 2H), 2.67 (m, 1H), 2.36 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 2.7. HRMS (*m/z*): calculated for $C_{10}H_{13}N_3O_9P$ (M-H): 350.0389; found: 350.0390.

3’-^{hm}dCMP ^{1}H NMR (300 MHz, D_2O) δ: 7.92 (s, 1H), 6.29 (dd, *J* = 5.9, 8.0 Hz, 1H), 4.70 (m, 1H), 4.46 (s, 2H), 4.17 (m, 1H), 3.84 (m, 2H), 2.58 (m, 1H), 2.36 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 1.1. HRMS (*m/z*): calculated for $C_{10}H_{15}N_3O_8P$ (M-H): 336.0597; found: 336.0586.

3’-dCMP ^{1}H NMR (300 MHz, D_2O) δ: 7.90 (d, *J* = 7.6 Hz, 1H), 6.34 (t, *J* = 6.9 Hz, 1H), 6.11 (d, *J* = 7.6 Hz, 1H), 4.76 (m, 1H), 4.25 (m, 1H), 3.87 (m, 2H), 2.63 (m, 1H), 2.39 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 1.9. HRMS (*m/z*): calculated for $C_9H_{13}N_3O_7P$ (M-H): 306.0491; found: 306.0490.

3’-dUMP ^{1}H NMR (300 MHz, D_2O) δ: 7.87 (d, *J* = 8.1 Hz, 1H), 6.29 (t, *J* = 7.0 Hz, 1H), 5.90 (d, *J* = 8.1 Hz, 1H), 4.77 (m, 1H), 4.20 (m, 1H), 3.80 (m, 2H), 2.57 (m, 1H), 2.39 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 1.1. HRMS (*m/z*): calculated for $C_9H_{12}N_2O_8P$ (M-H): 307.0331; found: 307.0345.

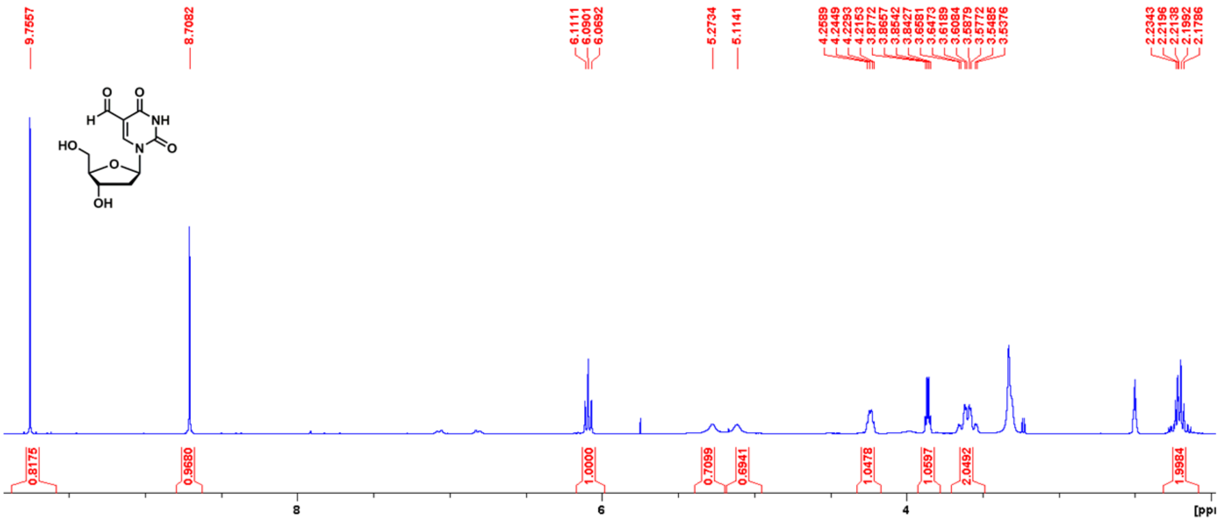
3’-^{hm}dUMP ^{1}H NMR (300 MHz, D_2O) δ: 8.04 (s, 1H), 6.34 (dd, *J* = 6.1, 7.3 Hz, 1H), 4.75 (m, 1H), 4.49 (s, 2H), 4.37 (m, 1H), 4.10 (m, 2H), 2.60 (m, 1H), 2.37 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 0.8. HRMS (*m/z*): calculated for $C_{10}H_{14}N_2O_9P$ (M-H): 337.0437; found: 337.0444.

3'-^{fo}dUMP ^{1}H NMR (300 MHz, D_2O) δ: 9.69 (s, 1H), 8.56 (s, 1H), 6.33 (dd, *J* = 6.0, 7.2 Hz, 1H), 4.73 (m, 1H), 4.27 (m, 1H), 3.93 (m, 2H), 2.75 (m, 1H), 2.49 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 3.2. HRMS (*m/z*): calculated for $C_{10}H_{12}N_2O_9P$ (M-H): 335.0280; found: 335.0289.

3'-^{m}dCMP ^{1}H NMR (300 MHz, D_2O) δ: 7.68 (s, 1H), 6.30 (t, *J* = 6.8 Hz, 1H), 4.73 (m, 1H), 4.18 (m, 1H), 3.85 (m, 2H), 2.57 (m, 1H), 2.33 (m, 1H), 1.97 (s, 3H). ^{31}P NMR (120 MHz, D_2O) δ: 1.0. HRMS (*m/z*): calculated for $C_{10}H_{15}N_3O_7P$ (M-H): 320.0648; found: 320.0643.

3'-dTMP ^{1}H NMR (300 MHz, D_2O) δ: 7.65 (s, 1H), 6.27 (t, *J* = 6.8 Hz, 1H), 4.76 (m, 1H), 4.14 (m, 1H), 3.81 (m, 2H), 2.52 (m, 1H), 2.39 (m, 1H), 1.88 (s, 3H). ^{31}P NMR (120 MHz, D_2O) δ: 1.5. HRMS (*m/z*): calculated for $C_{10}H_{14}N_2O_8P$ (M-H): 321.0488; found: 321.0493.

3'-^{fo}dCMP ^{1}H NMR (300 MHz, CD_3OD) δ: 9.58 (s, 1H), 9.06 (s, 1H), 6.24 (t, *J* = 5.9 Hz, 1H), 4.44 (m, 1H), 4.06 (m, 1H), 3.96 (m, 1H), 3.84 (m, 1H), 2.56 (m, 1H), 2.31 (m, 1H). ^{31}P NMR (120 MHz, D_2O) δ: 2.3. HRMS (*m/z*): calculated for $C_{10}H_{13}N_3O_8P$ (M-H): 334.0440; found: 334.0424.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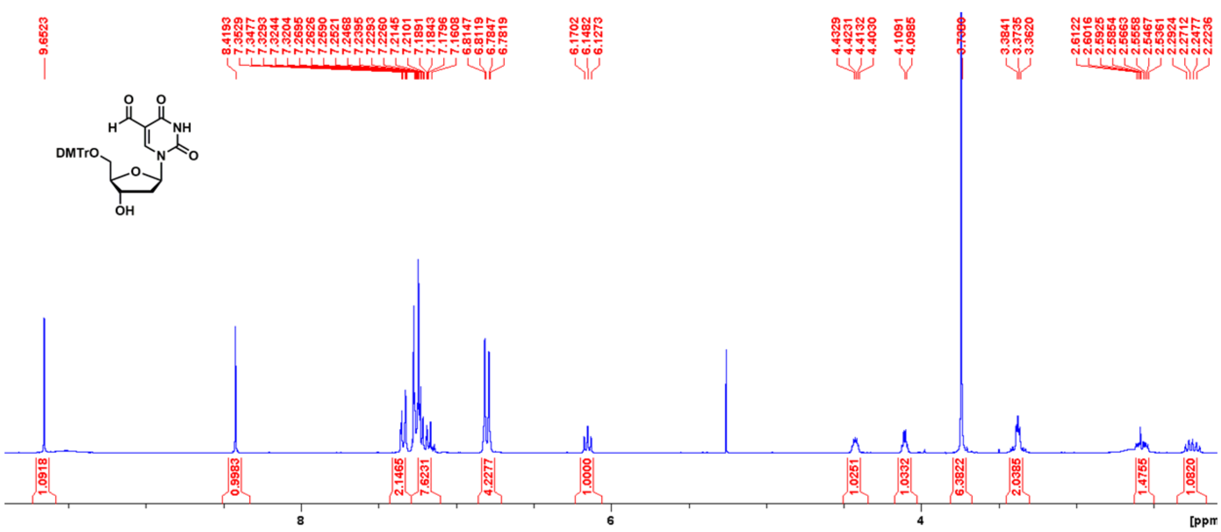
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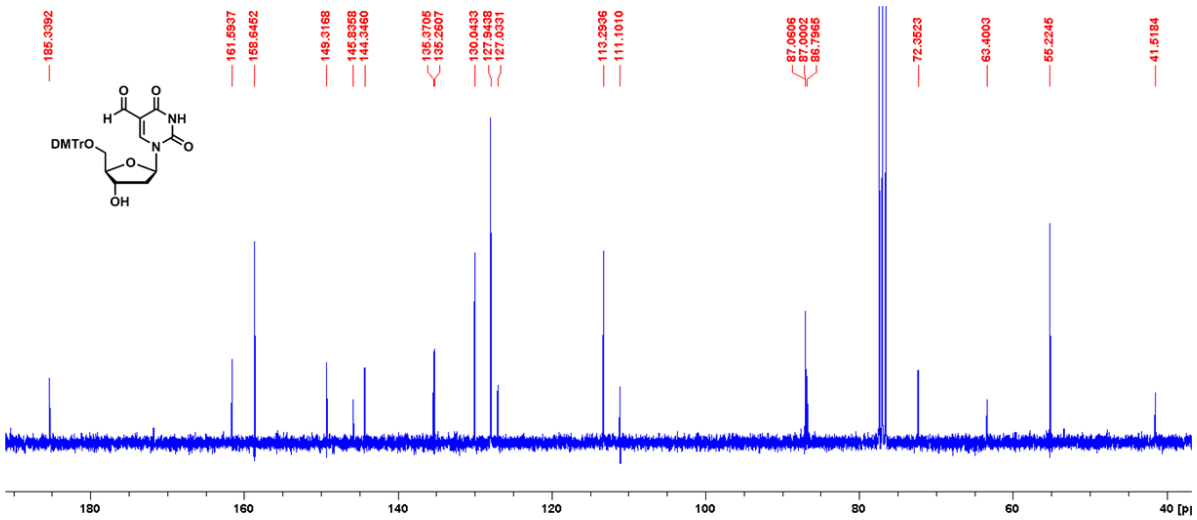
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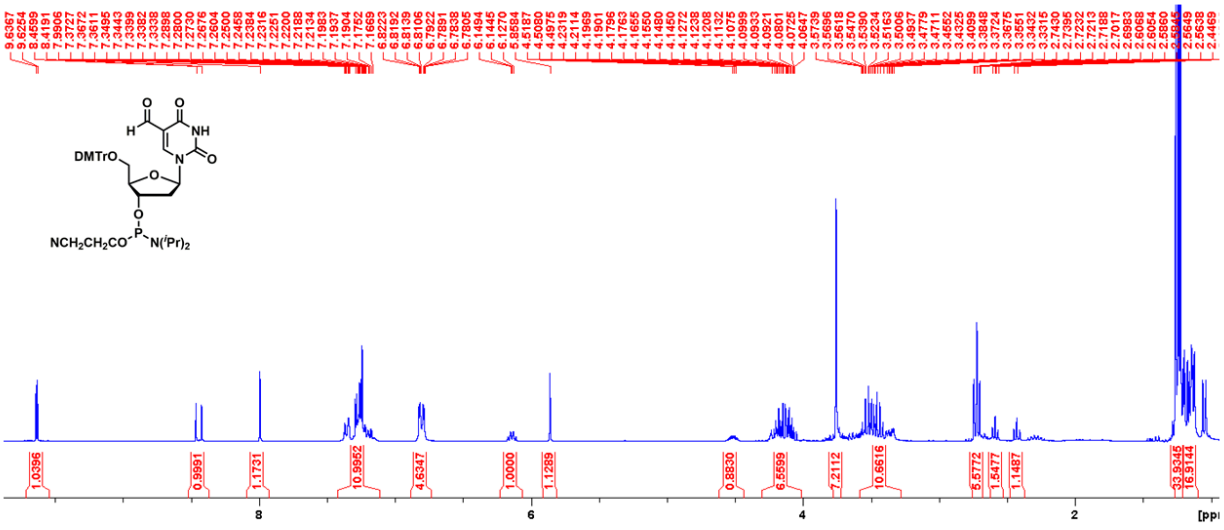
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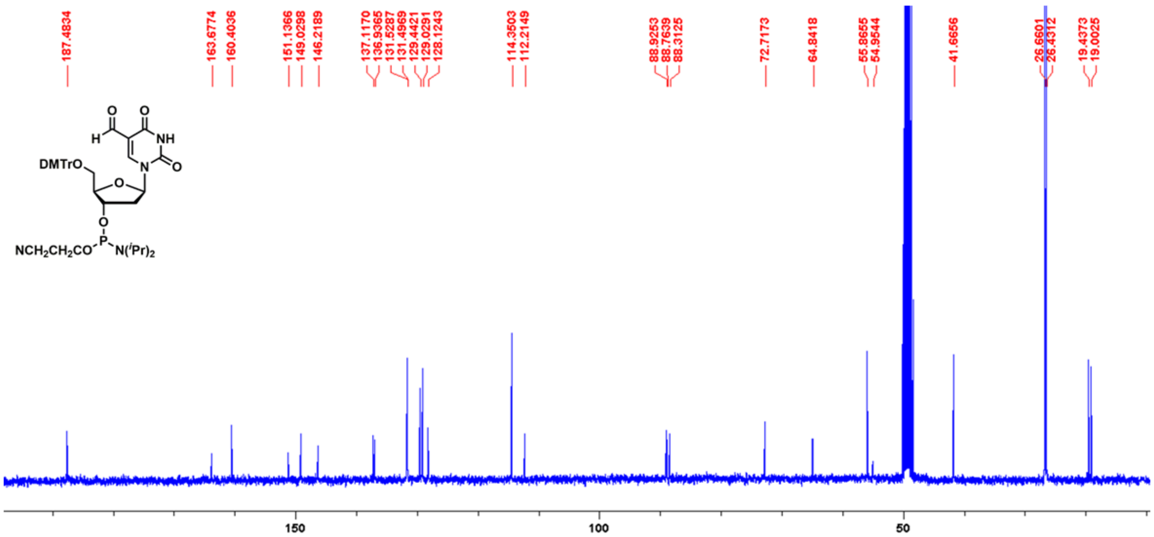
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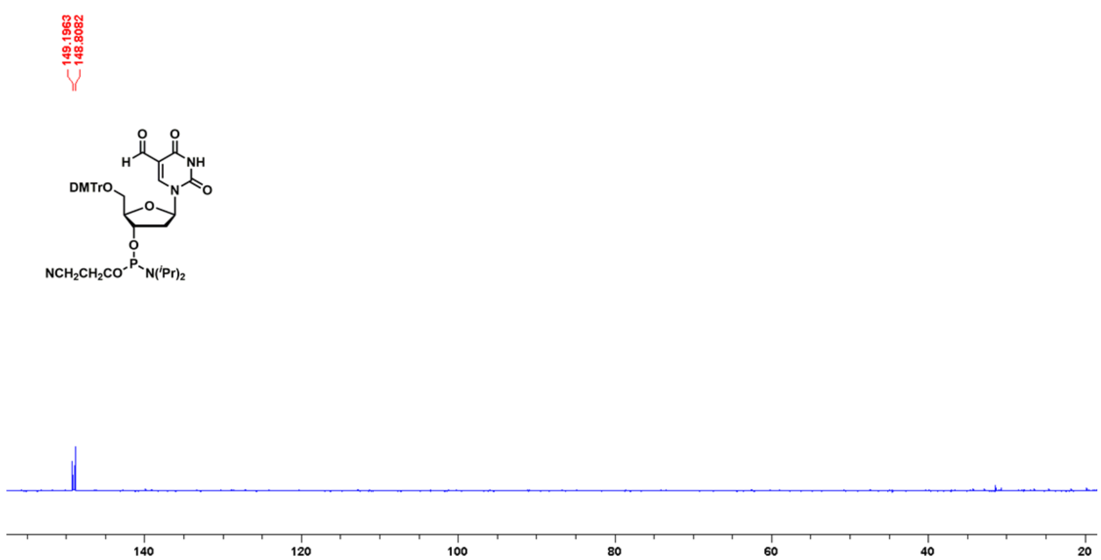
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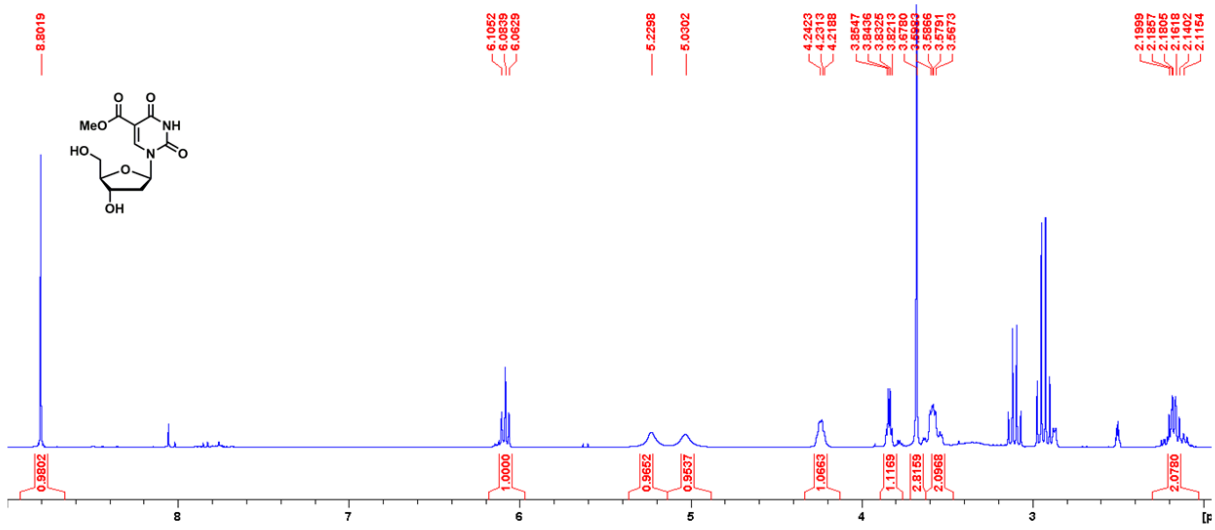
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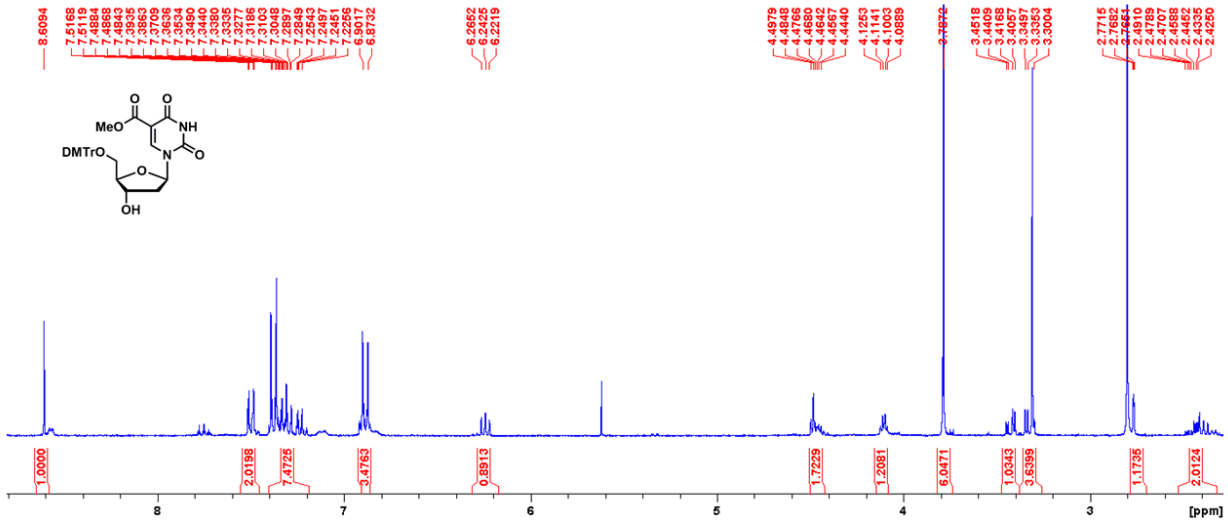
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2.4910
2.4789
2.4707
2.4588
2.4482
2.4335
2.4250
MeO
DMTrO
OH
NH
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2.0198
7.4725
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6.0471
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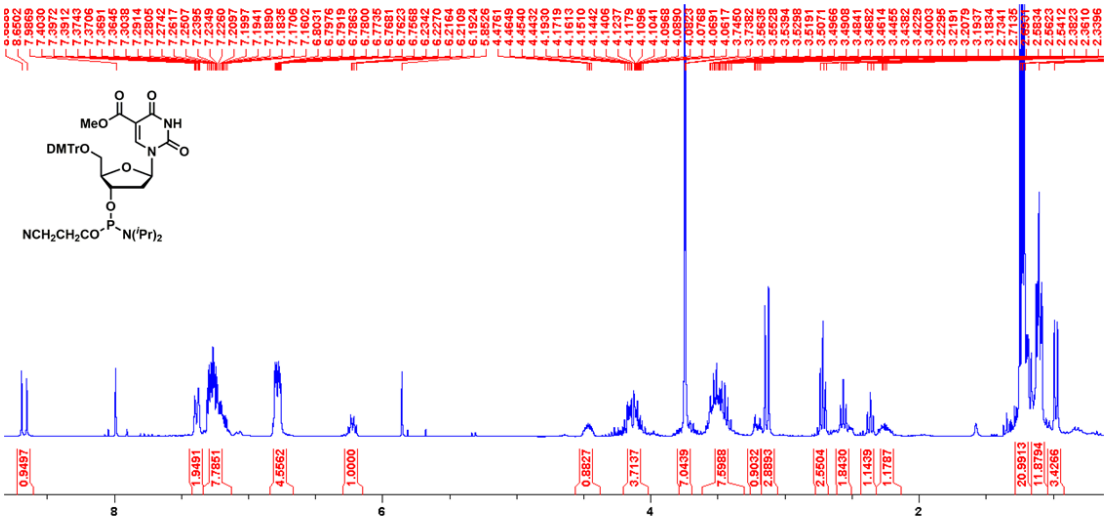
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N(iPr)2
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0.8827
3.7137
7.0439
7.5988
0.9032
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8
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4
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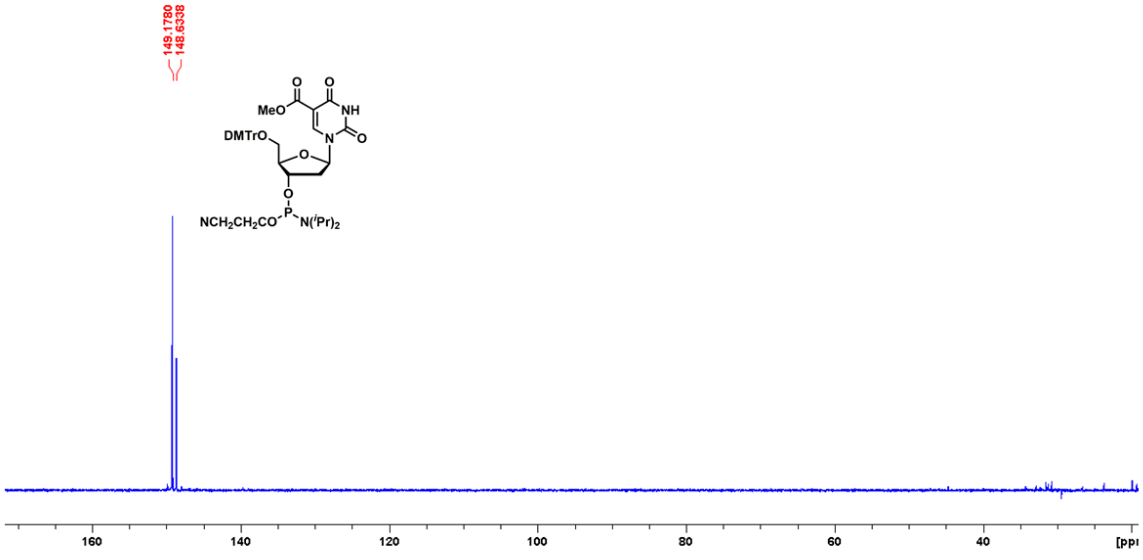
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N(iPr)2
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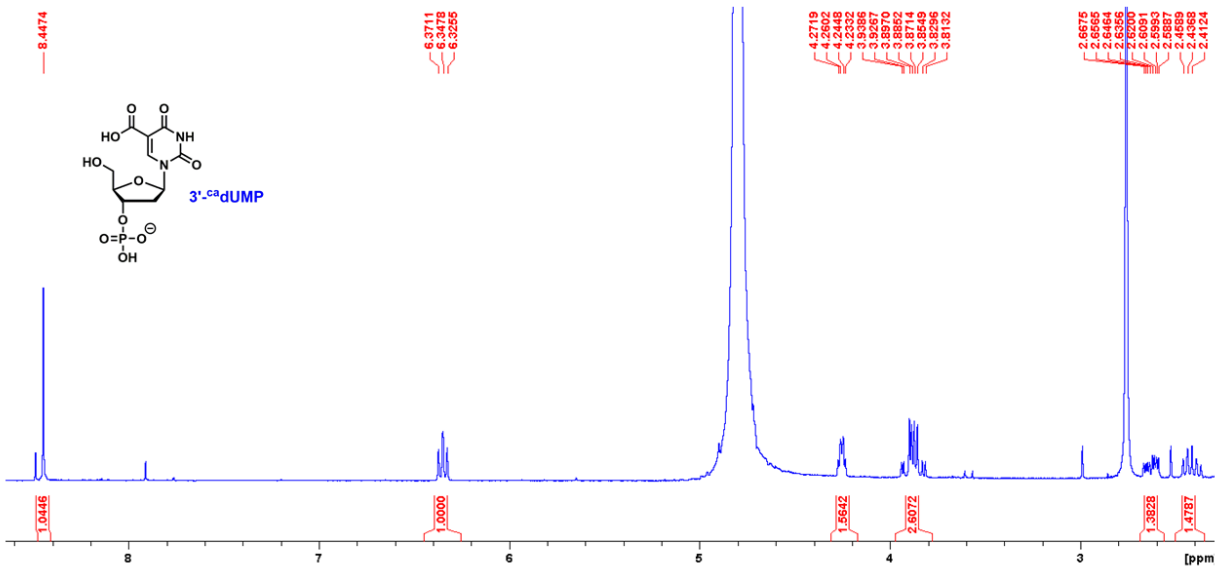
140
120
100
80
60
40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3.8970
3.8852
3.8714
3.8549
3.8296
3.8132
2.6675
2.6565
2.6464
2.6356
2.6200
2.6091
2.5953
2.4687
2.4589
2.4388
2.4124
3'-caDUMP
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4
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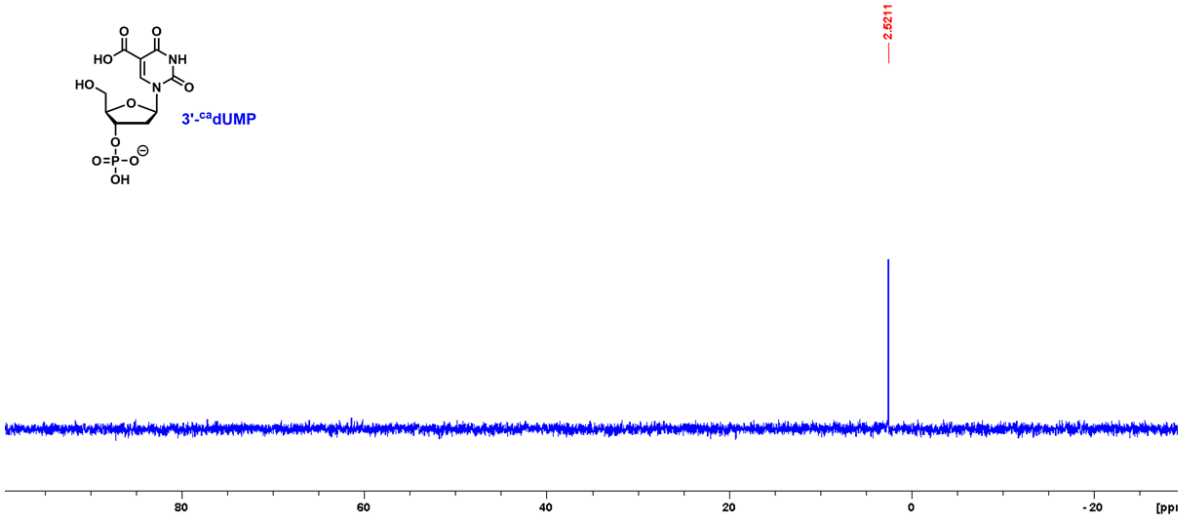
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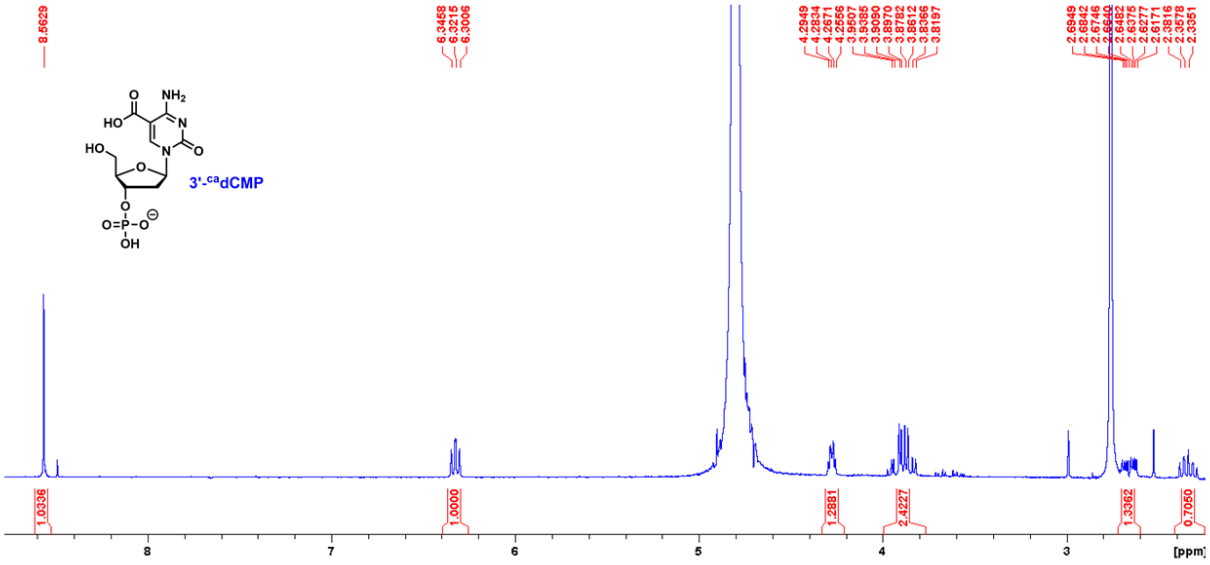
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4.2671
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3.9090
3.8970
3.8782
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3.8366
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2.6949
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2.6746
2.6540
2.6482
2.6375
2.6277
2.6171
2.3816
2.3578
2.3351
3'-cadCMP
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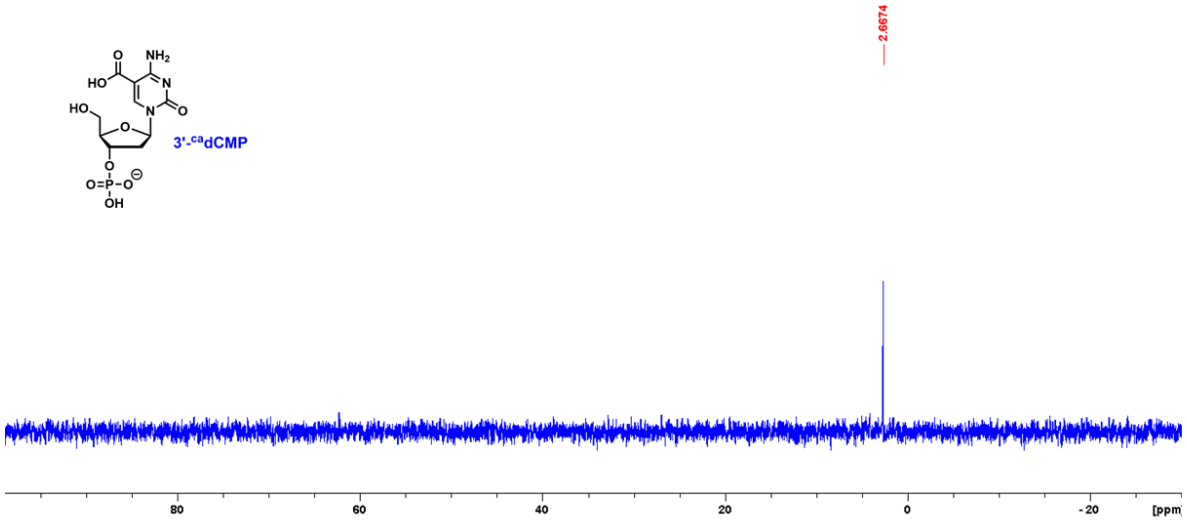
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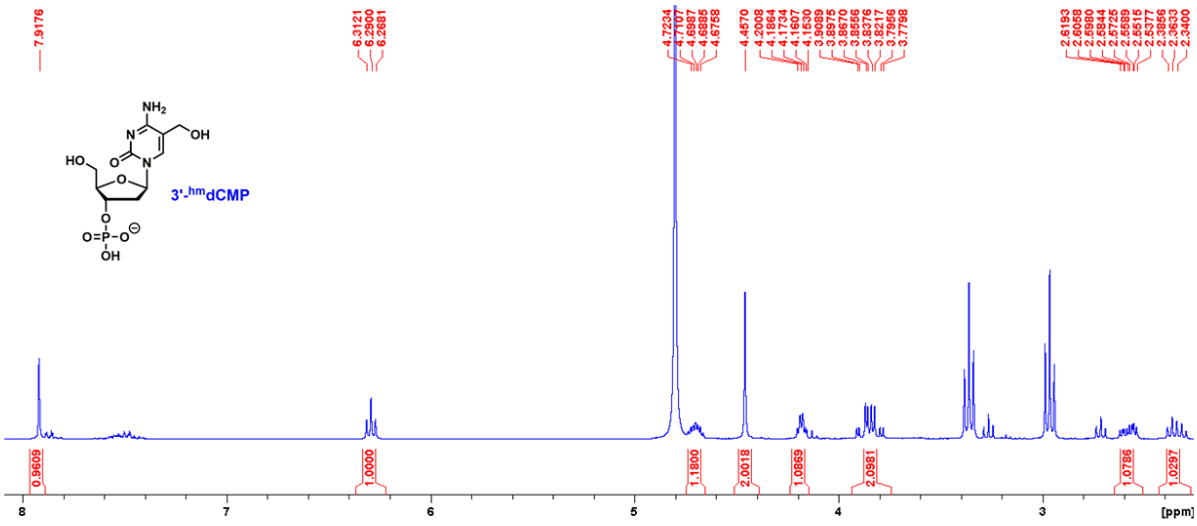
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4.2008
4.1864
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4.1607
4.1530
3.8699
3.8575
3.8570
3.8556
3.8376
3.8217
3.7956
3.7798
2.6193
2.6058
2.5980
2.5944
2.5725
2.5659
2.5515
2.5377
2.3856
2.3633
2.3400
0.9609
1.0000
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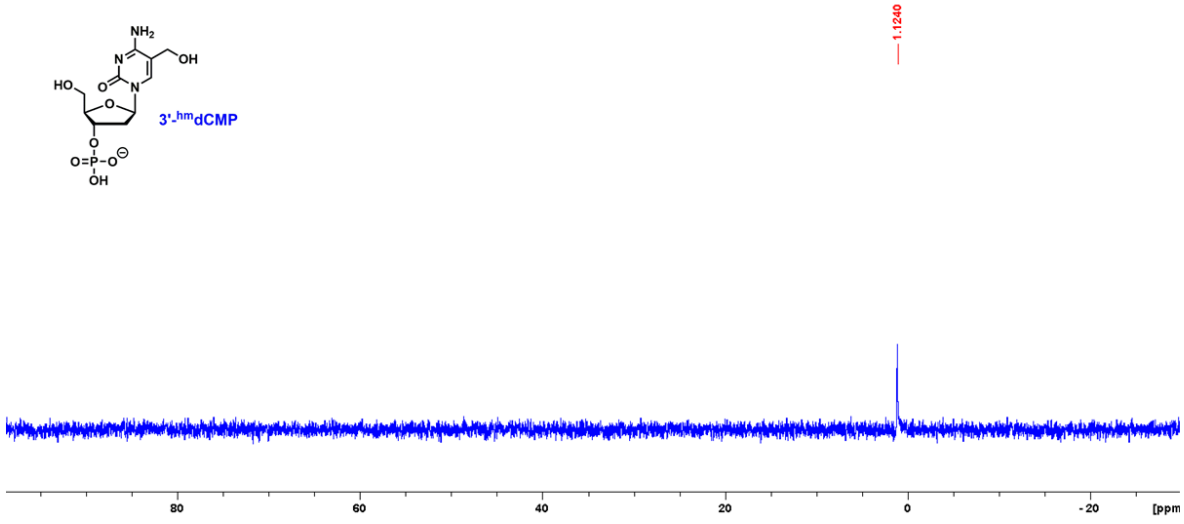
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0
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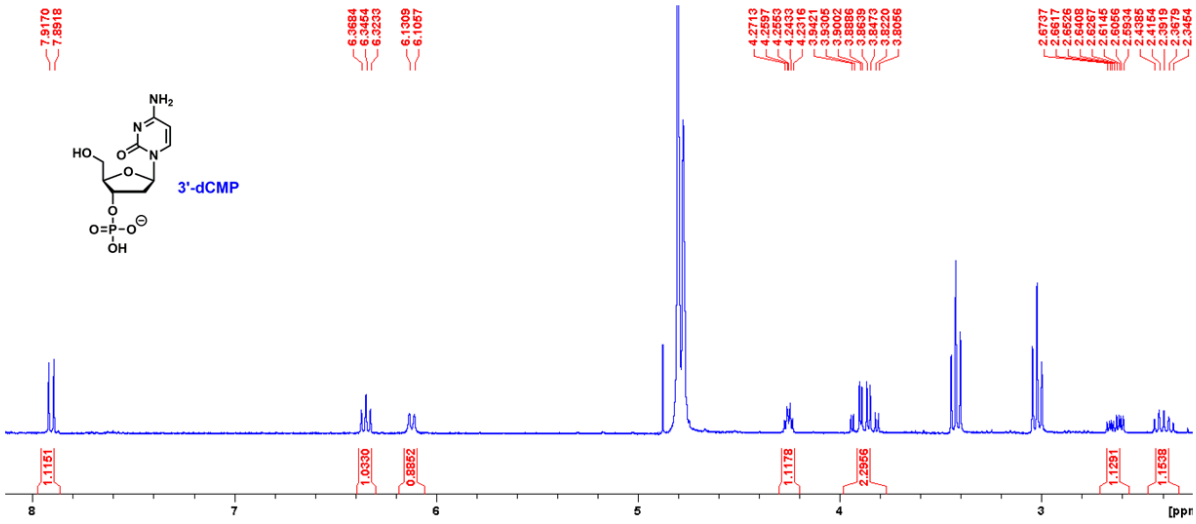
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6.1057
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4.2597
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4.2333
4.2316
3.9421
3.9305
3.9002
3.8886
3.8639
3.8473
3.8220
3.8056
2.6737
2.6617
2.6526
2.6408
2.6267
2.6145
2.6056
2.5934
2.4595
2.4154
2.3919
2.3679
2.3454
1.1151
1.0330
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8
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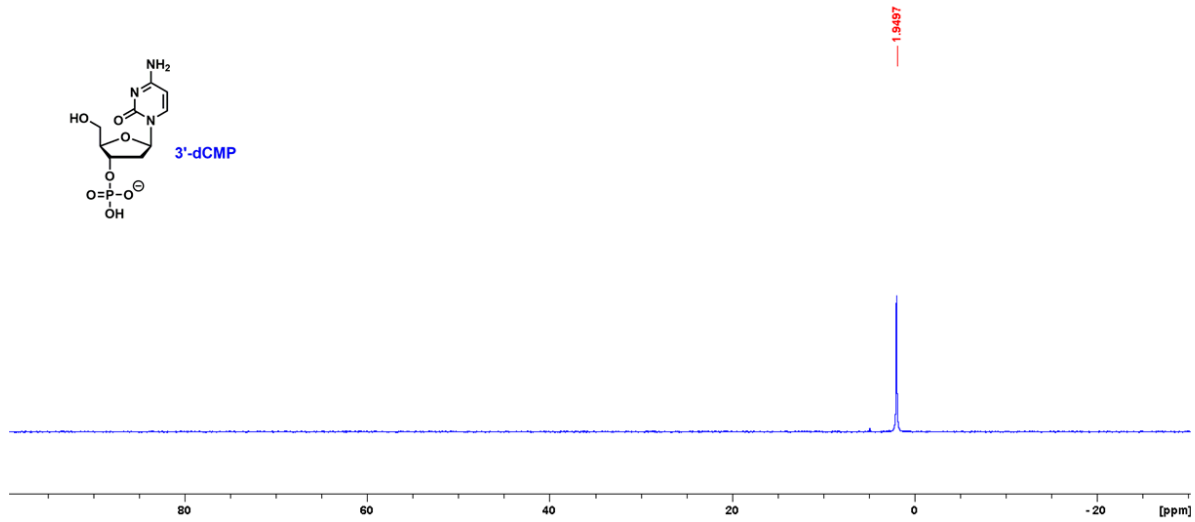
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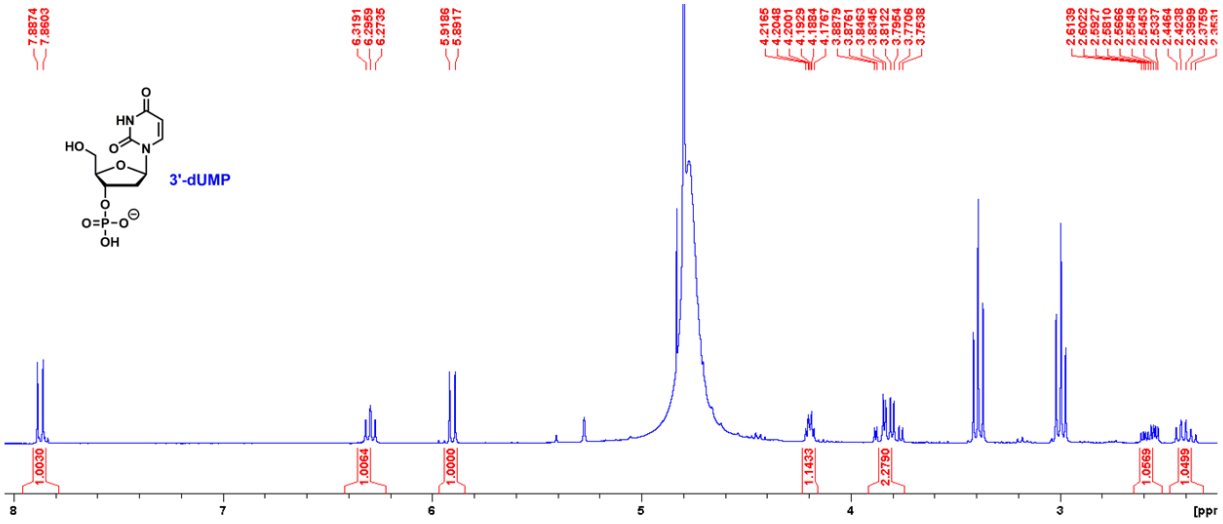
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5.8917
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4.2001
4.1929
4.1884
4.1767
3.8879
3.8761
3.8463
3.8345
3.8122
3.7954
3.7706
3.7538
2.6139
2.6022
2.5927
2.5810
2.5666
2.5549
2.5453
2.5337
2.4454
2.4338
2.3989
2.3759
HO
HN
O
N
O
O
O=P-O
OH
3'-dUMP
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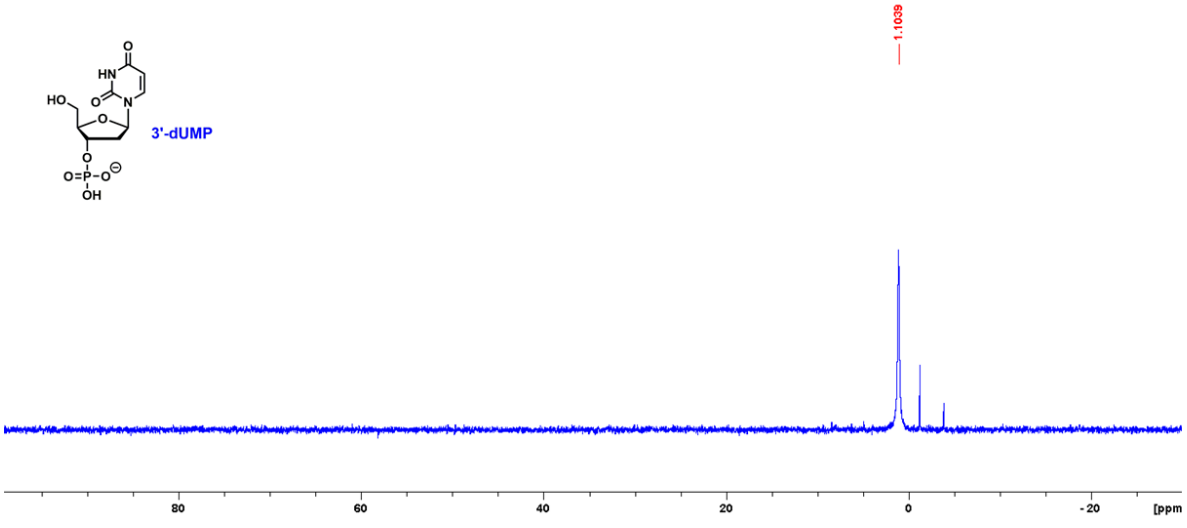
1.0569
1.0499
8
7
6
5
4
3
[ppr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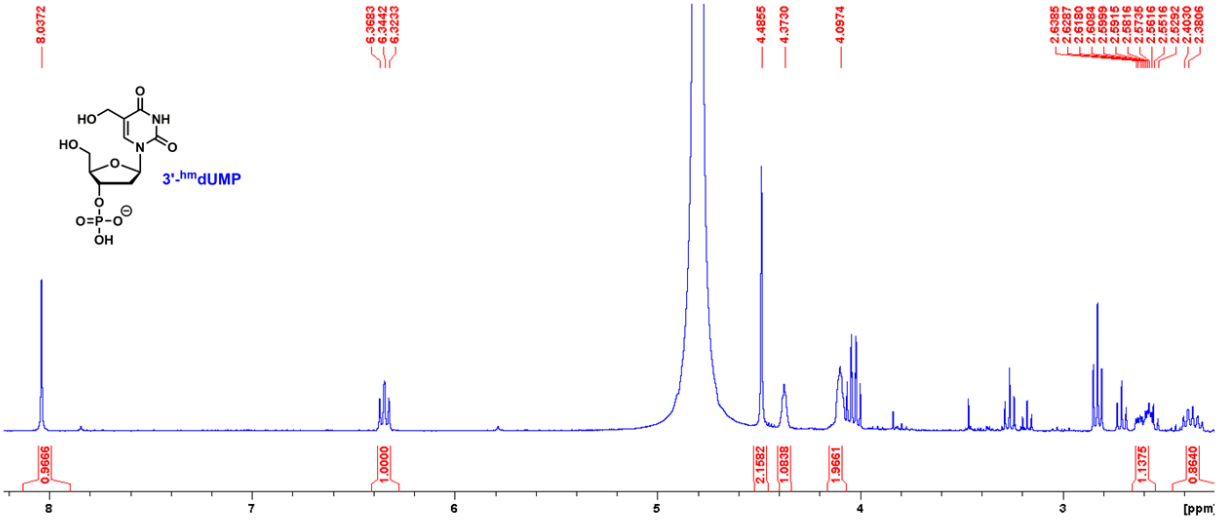
HO
HN
O
N
O
O
O=P-O
OH
3'-dUMP
80
60
40
20
0
-20
[ppm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4.3730
4.0974
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2.6287
2.6180
2.6084
2.5999
2.5915
2.5816
2.5735
2.5616
2.5511
2.5292
2.4030
2.3806
3'-hmdUMP
0.9666
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1.0838
1.9661
1.1375
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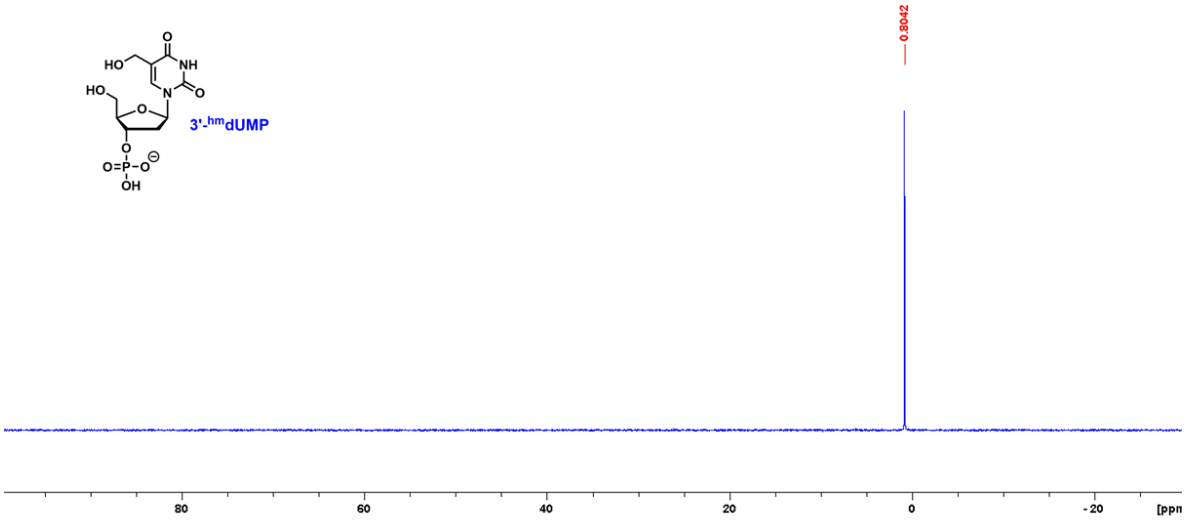
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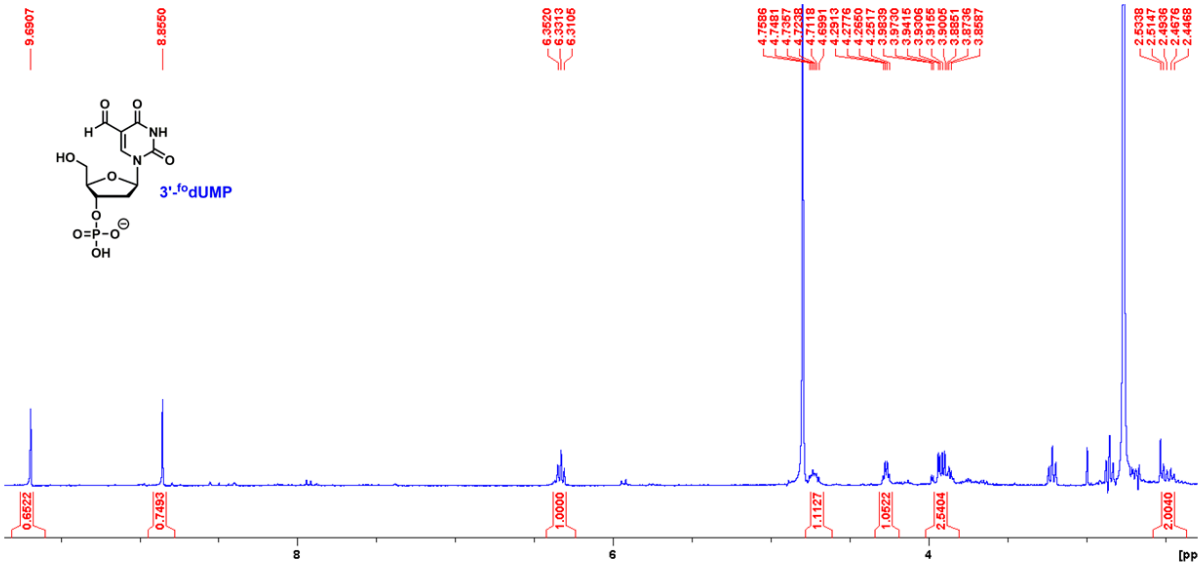
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6.3106
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4.6813
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4.2550
4.2517
3.9839
3.9730
3.9416
3.9286
3.9165
3.9005
3.8851
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2.5338
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2.0040
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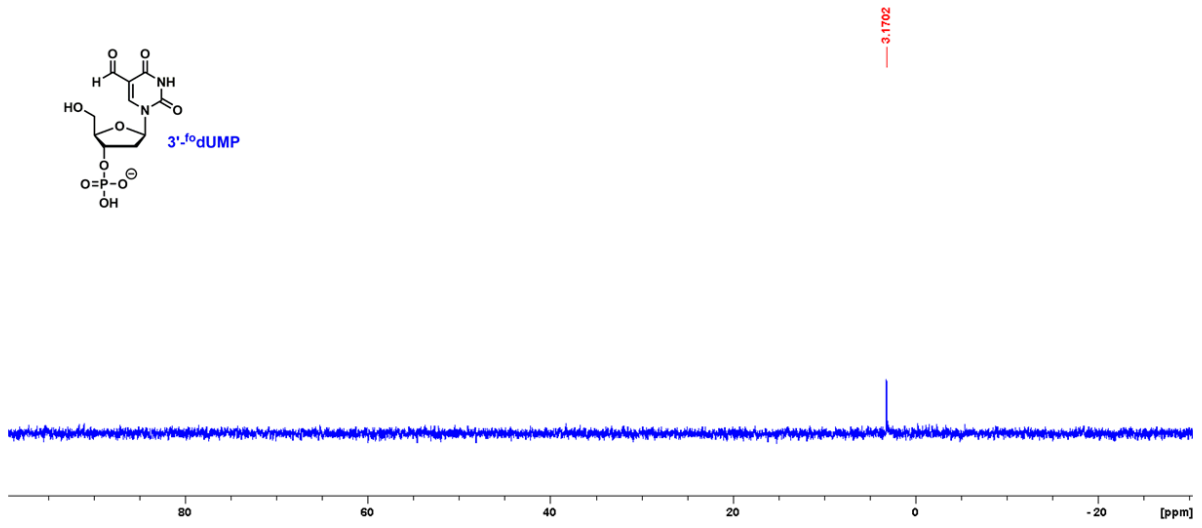
3'-fodUMP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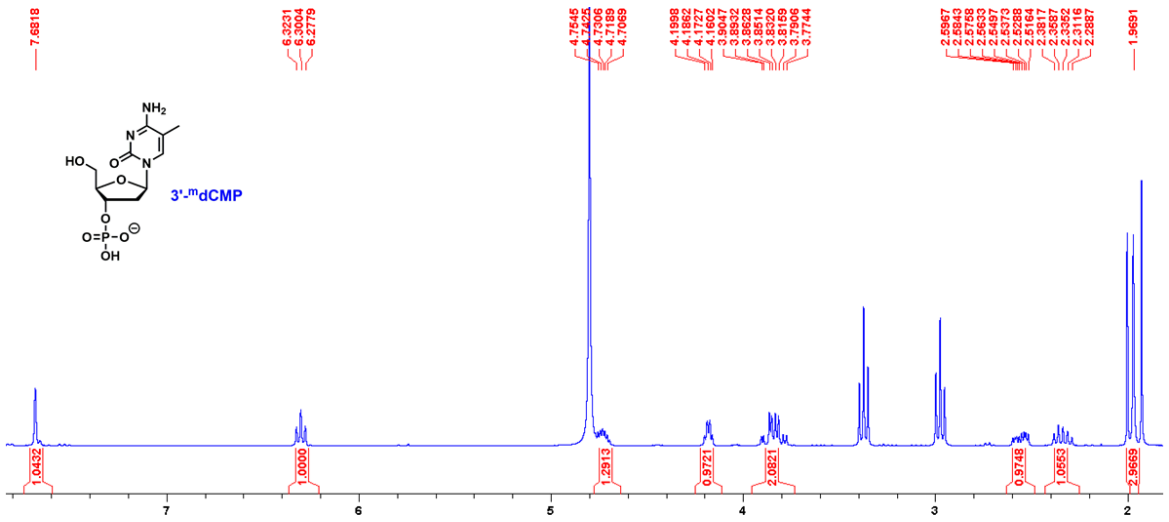
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4.1998
4.1862
4.1727
4.1602
3.9047
3.8932
3.8625
3.8514
3.8320
3.8169
3.7906
3.7744
2.5967
2.5843
2.5768
2.5633
2.5497
2.5373
2.5288
2.5164
2.3817
2.3587
2.3352
2.3116
2.2887
1.9691
3'-mdCMP
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2.9669
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5
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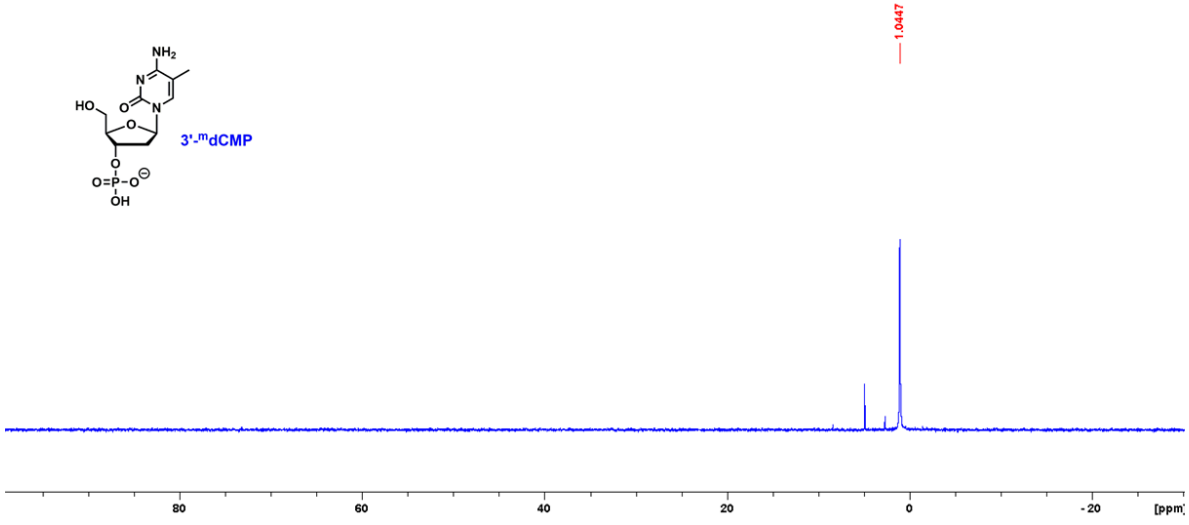
3
2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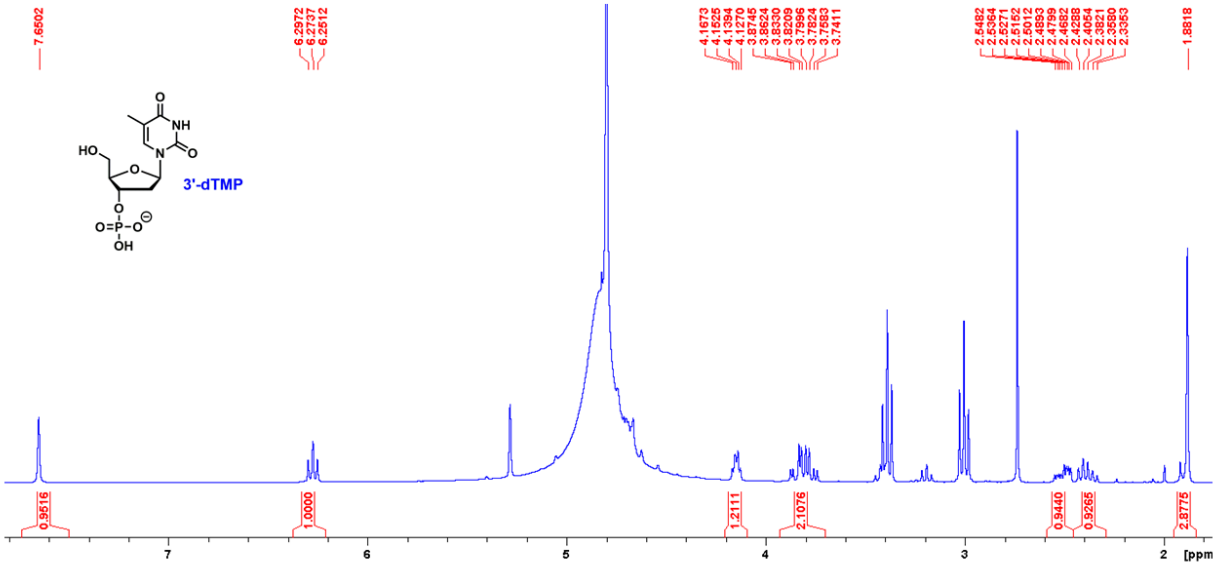
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3.8209
3.7996
3.7824
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3.7411
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2.5364
2.5271
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2.4789
2.4682
2.4288
2.4054
2.3821
2.3580
2.3353
1.8818
3'-dTMP
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0.9440
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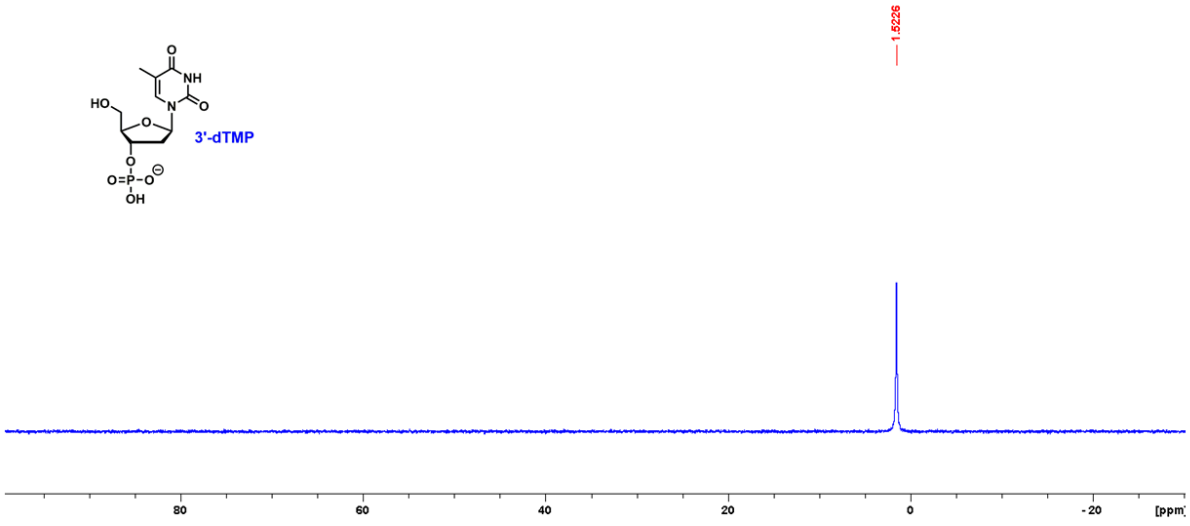
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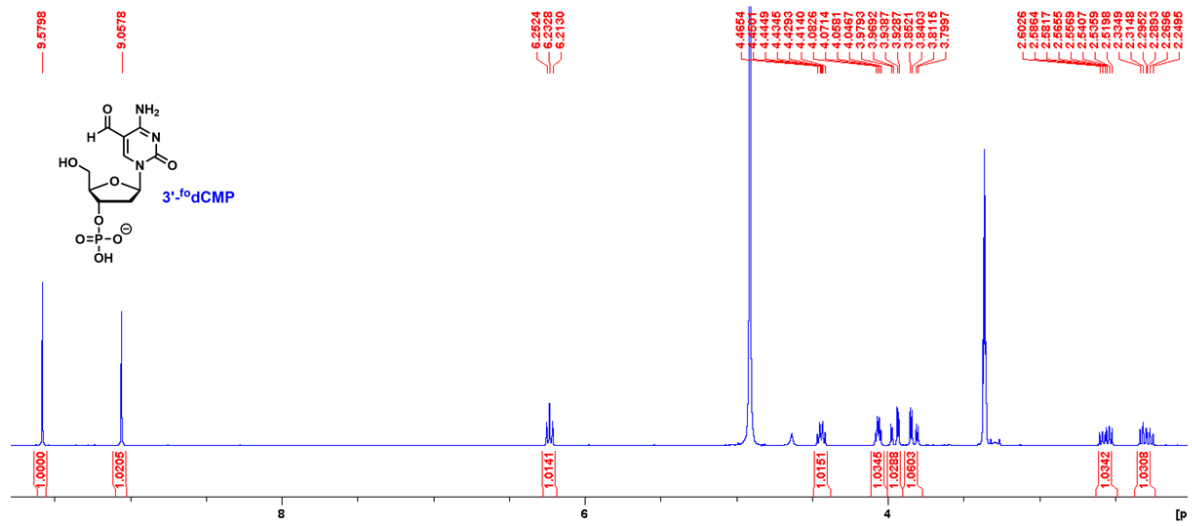
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6.2130
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4.4345
4.4293
4.4140
4.0826
4.0714
4.0551
4.0467
3.9793
3.9692
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3.9287
3.8521
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3.8115
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1.0603
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4
[p
3'-fodCMP

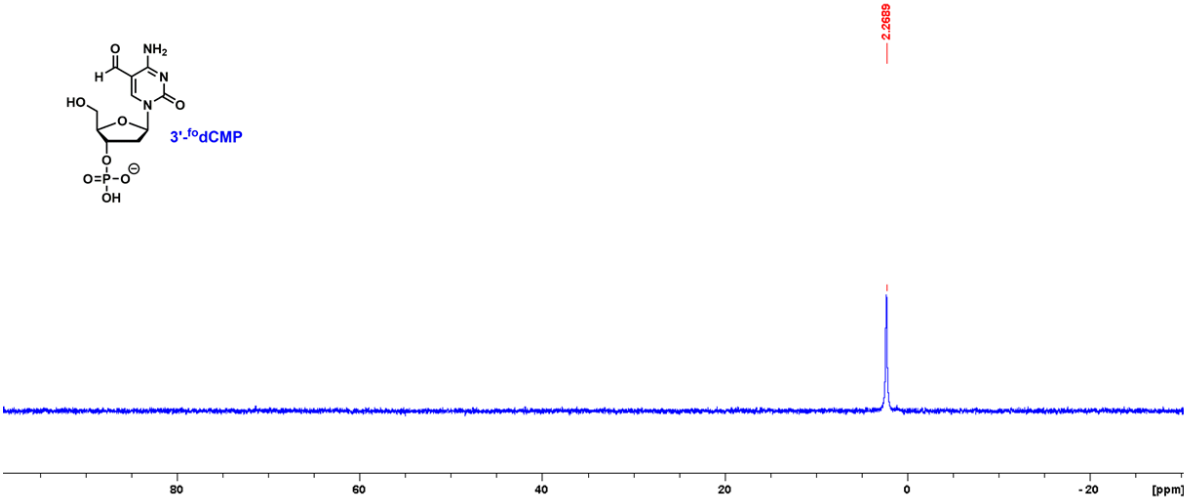
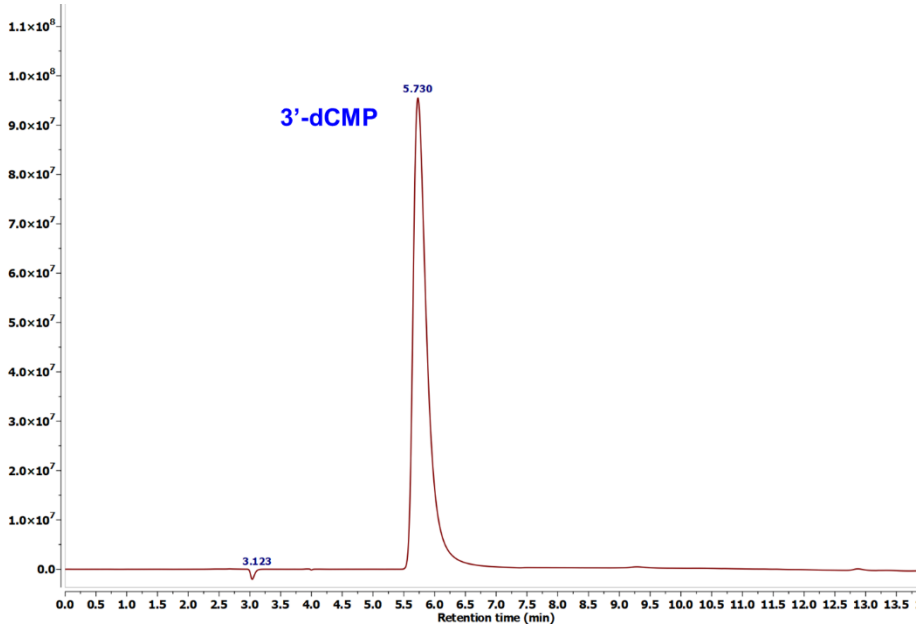
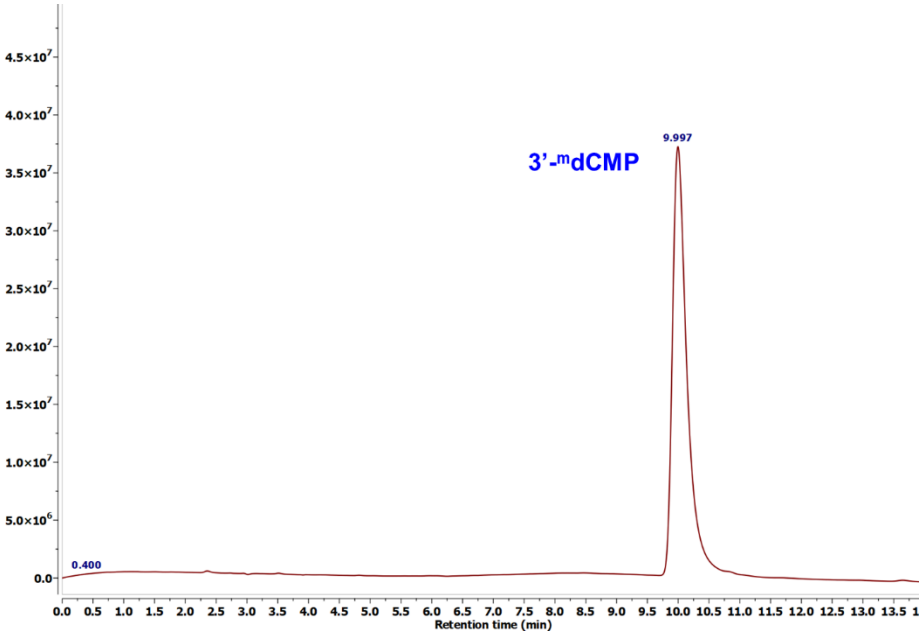
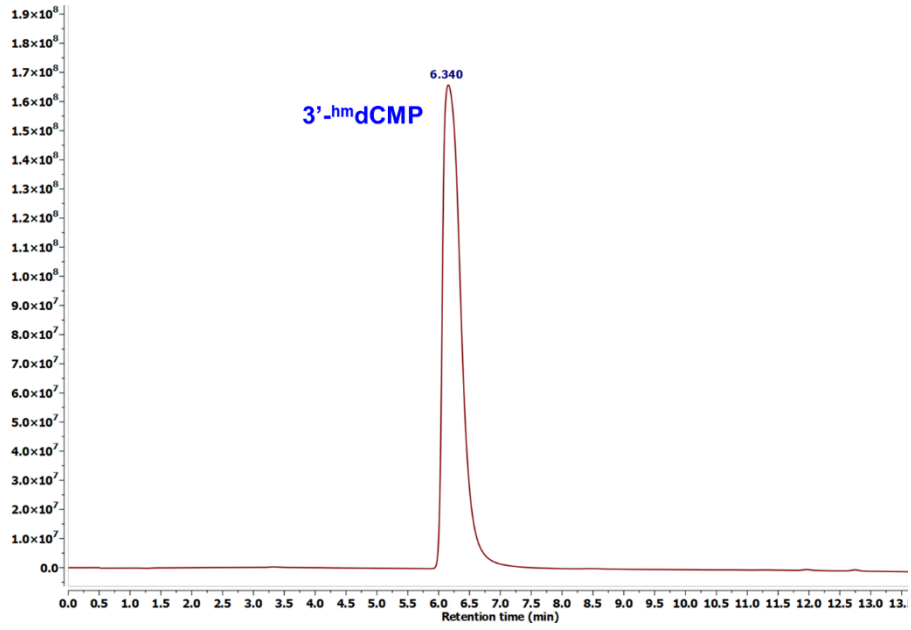
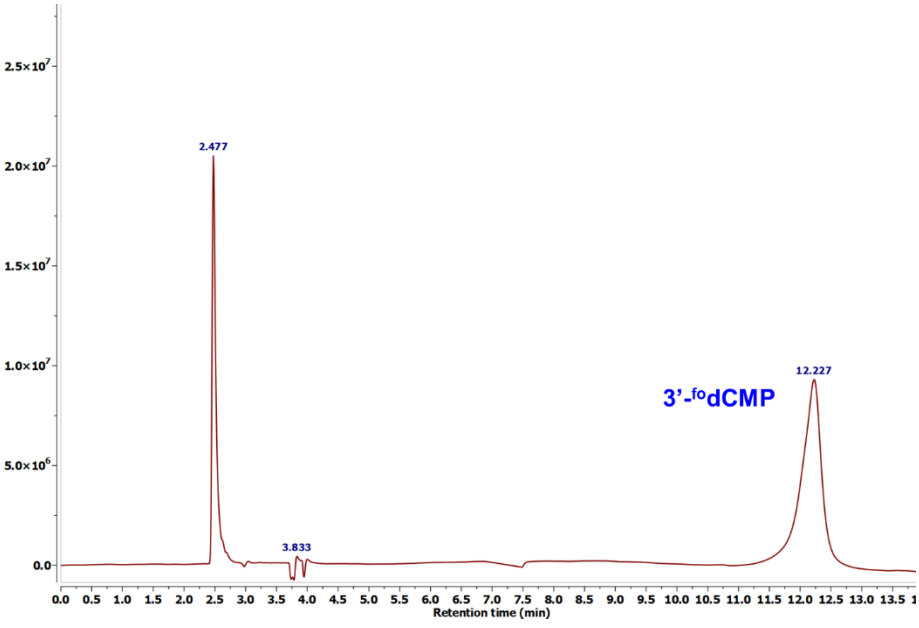

2.2689
3'-fodCMP
80
60
40
20
0
-20
[ppm]

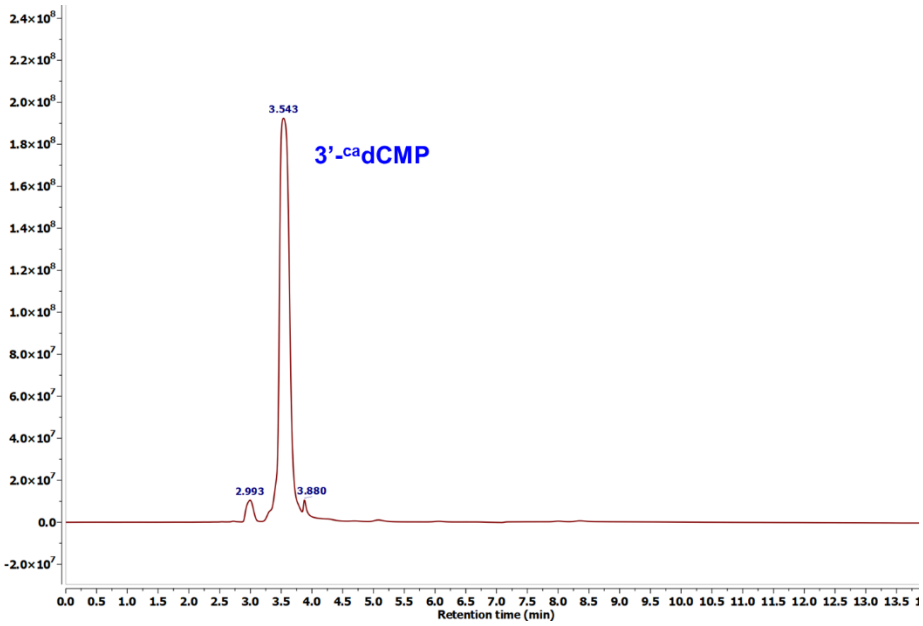
Figure S1. HPLC chromatograms of individual 3’-dNMP or 5’-dNMP

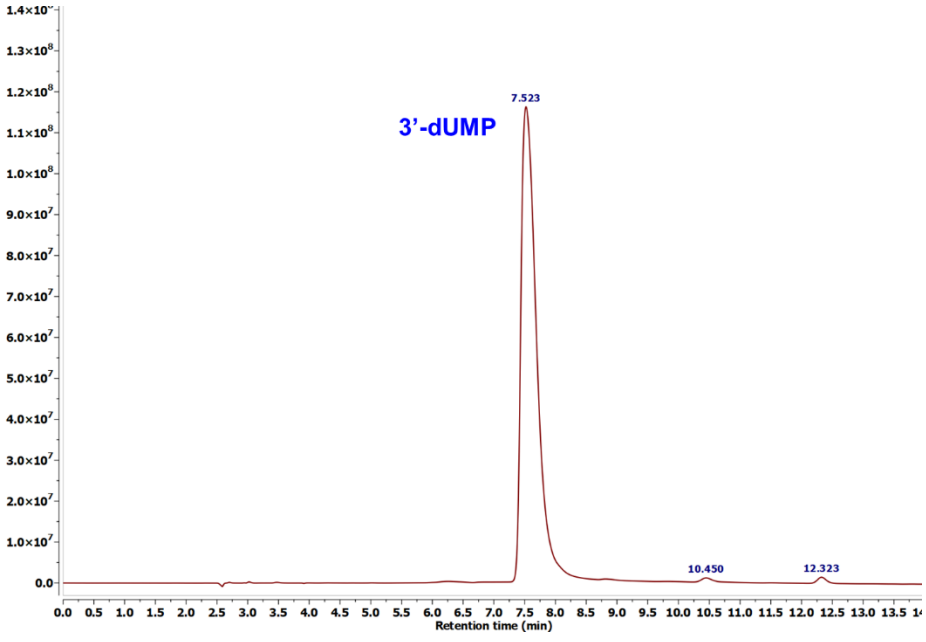


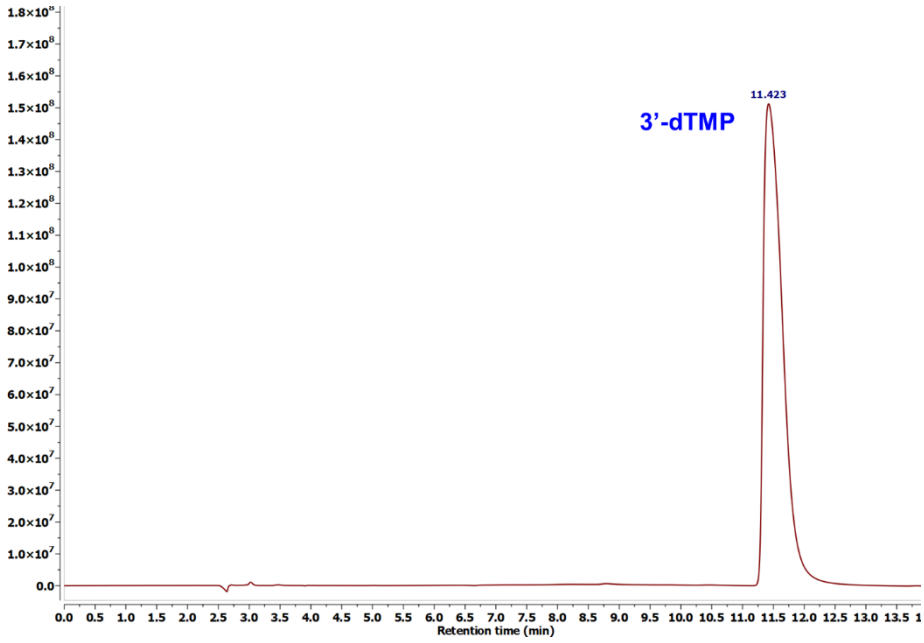


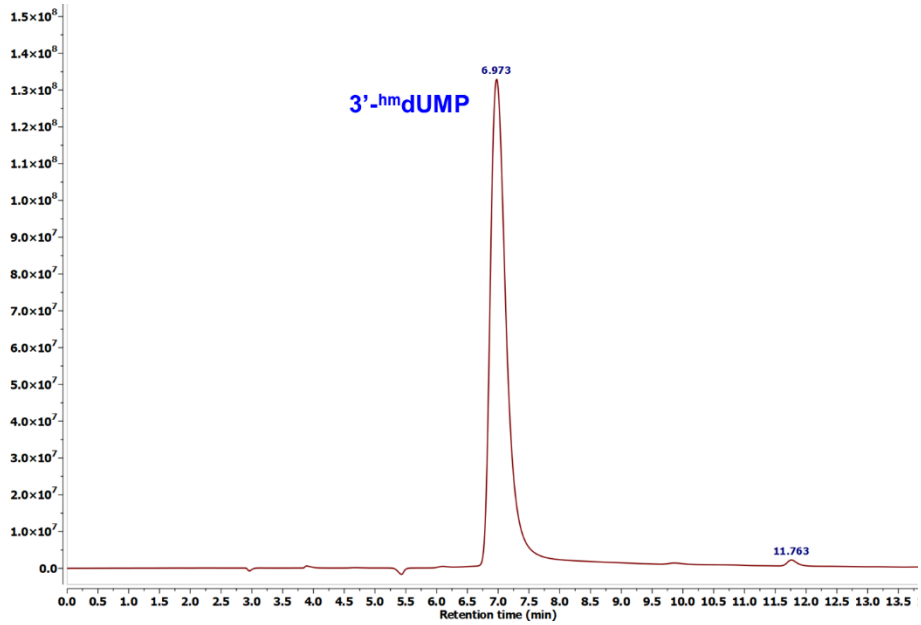

6.340
3'-hmdCMP
Retention time (min)

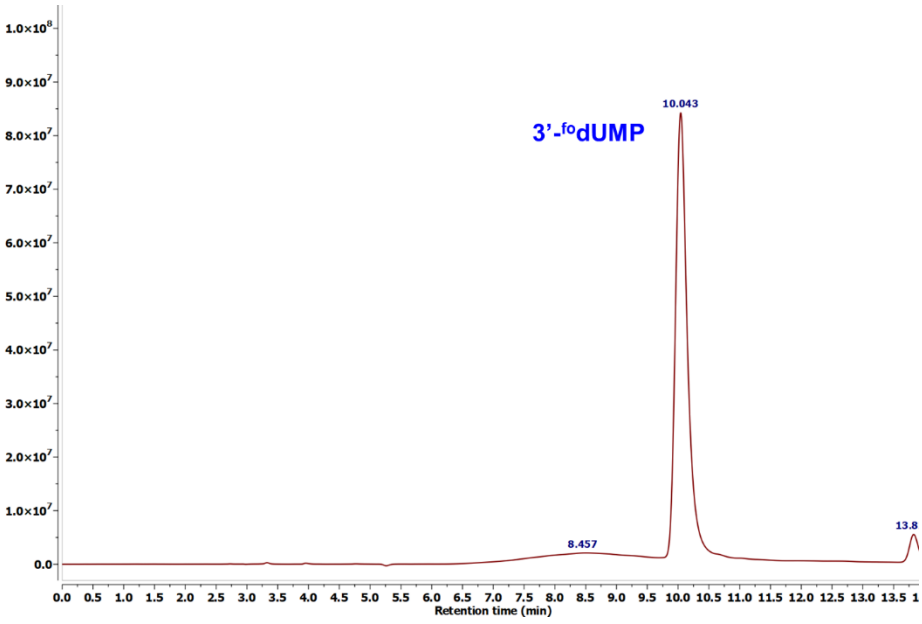

2.477
3.833
12.227
3'-fodCMP
Retention time (min)

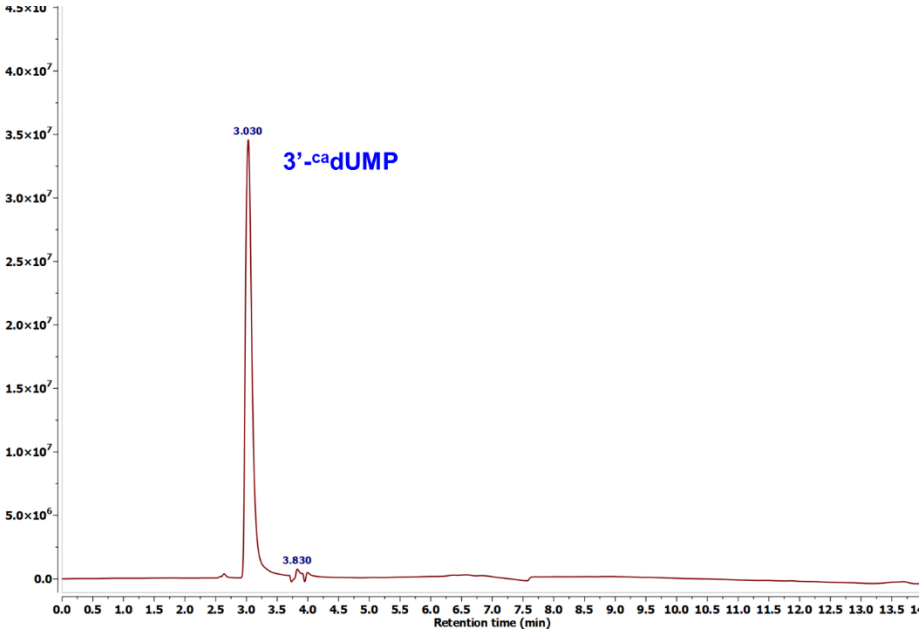

3.543
3'-caDCMP
2.993
3.880
Retention time (min)

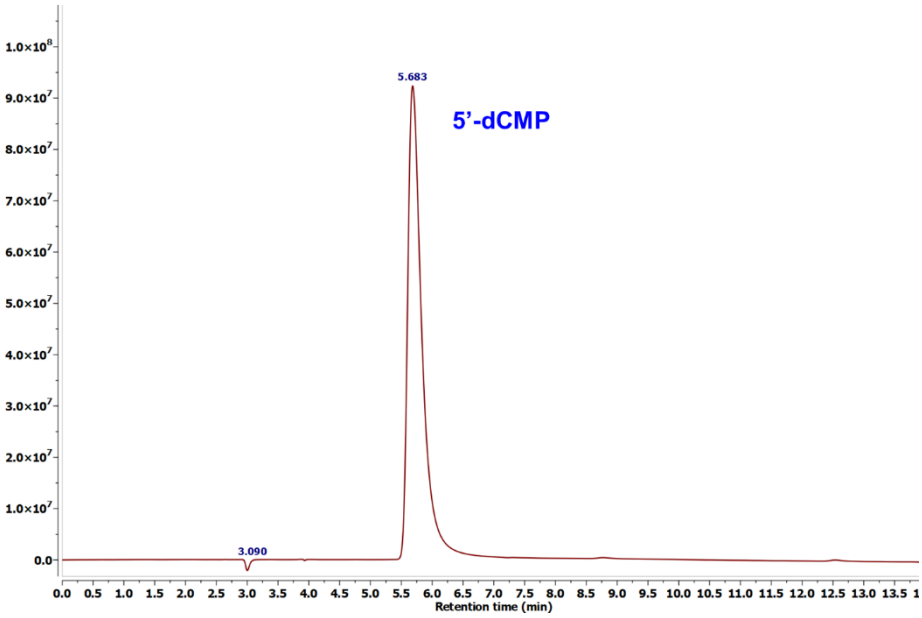

7.523
3'-dUMP
10.450
12.323
Retention time (min)

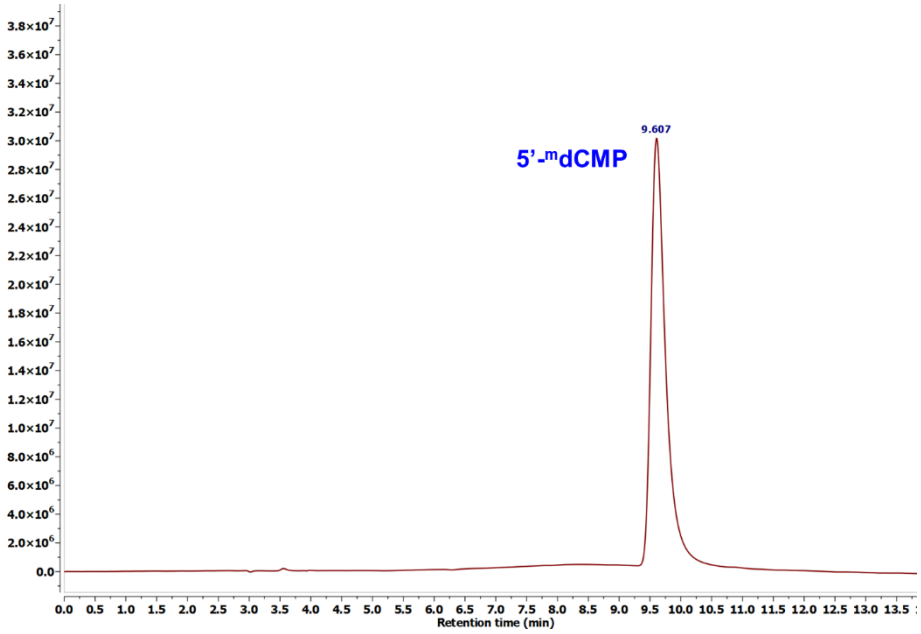

11.423
3'-dTMP
Retention time (min)

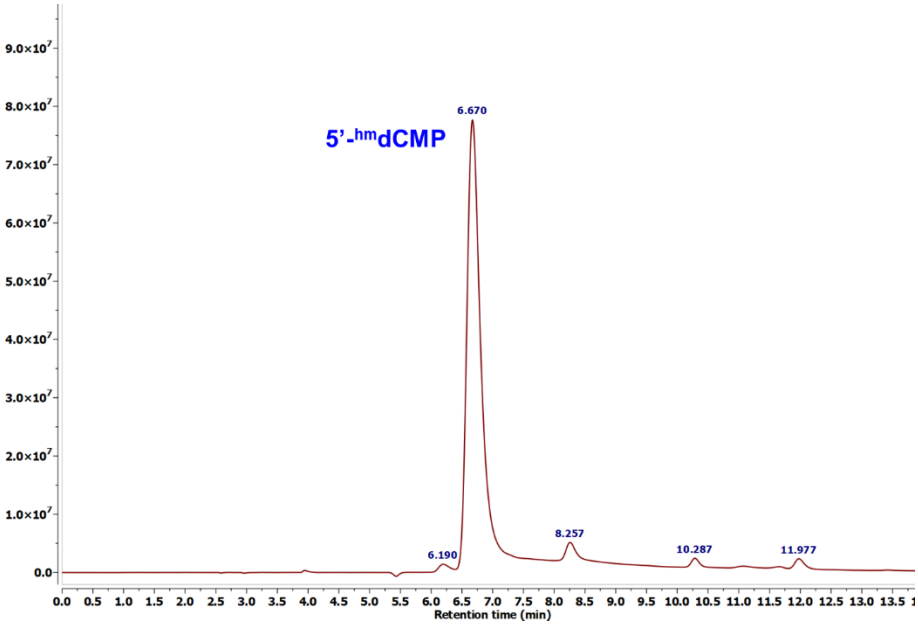

6.973
3'-hmdUMP
11.763
Retention time (min)

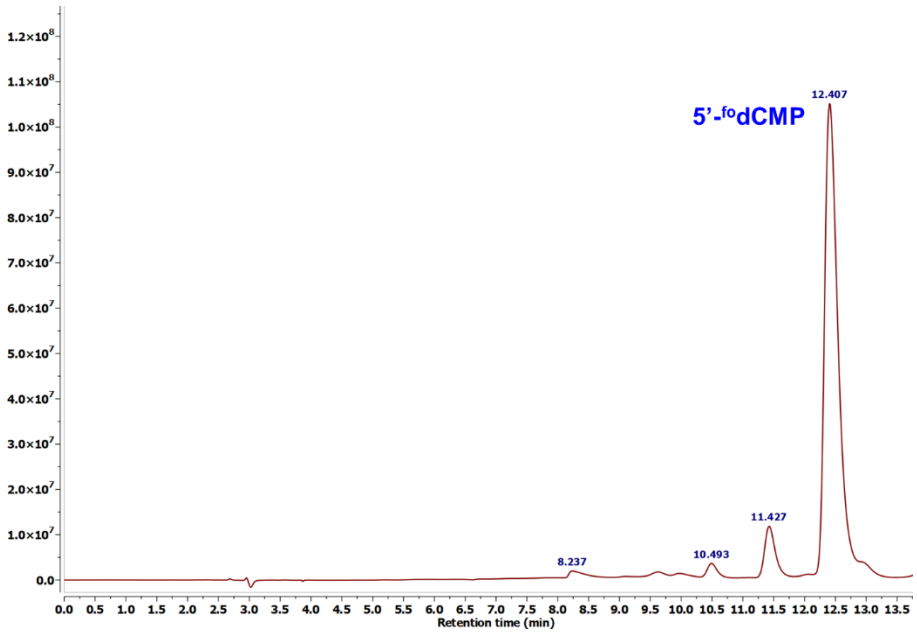

3'-fodUMP
10.043
8.457
13.8
Retention time (min)

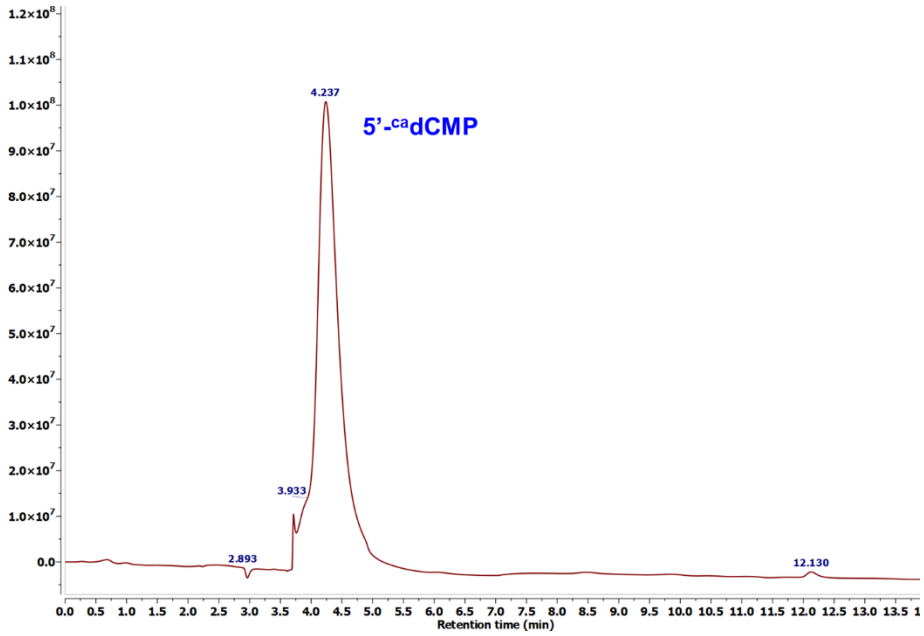

3'-cadUMP
3.030
3.830
Retention time (min)

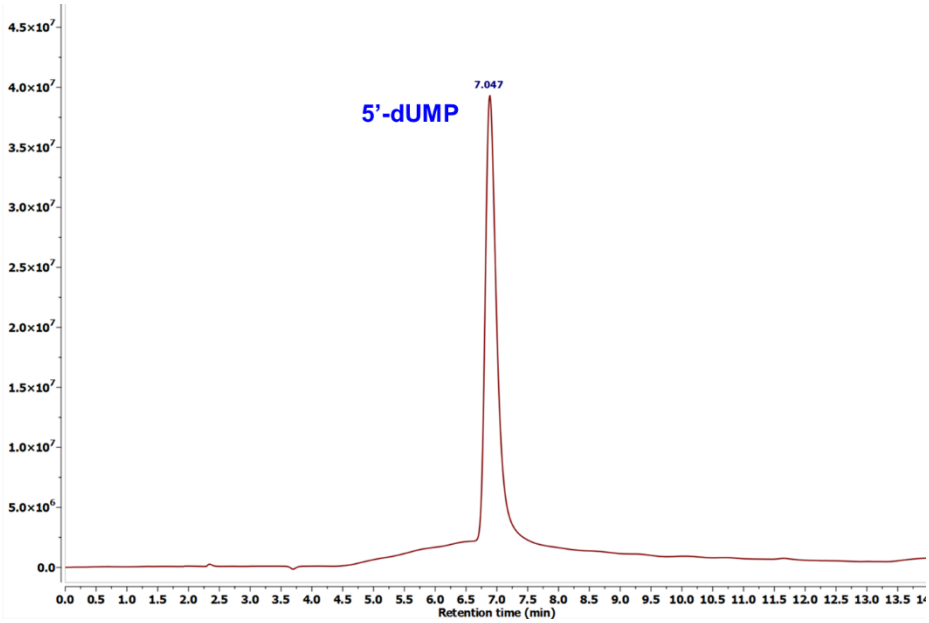

5.683
5’-dCMP
3.090
Retention time (min)

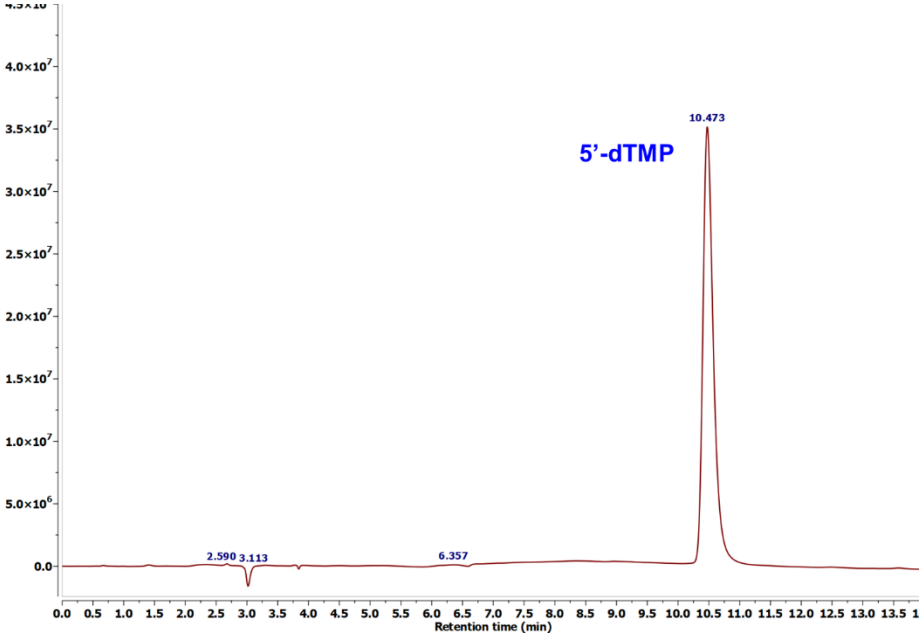

9.607
5’-mdCMP
Retention time (min)

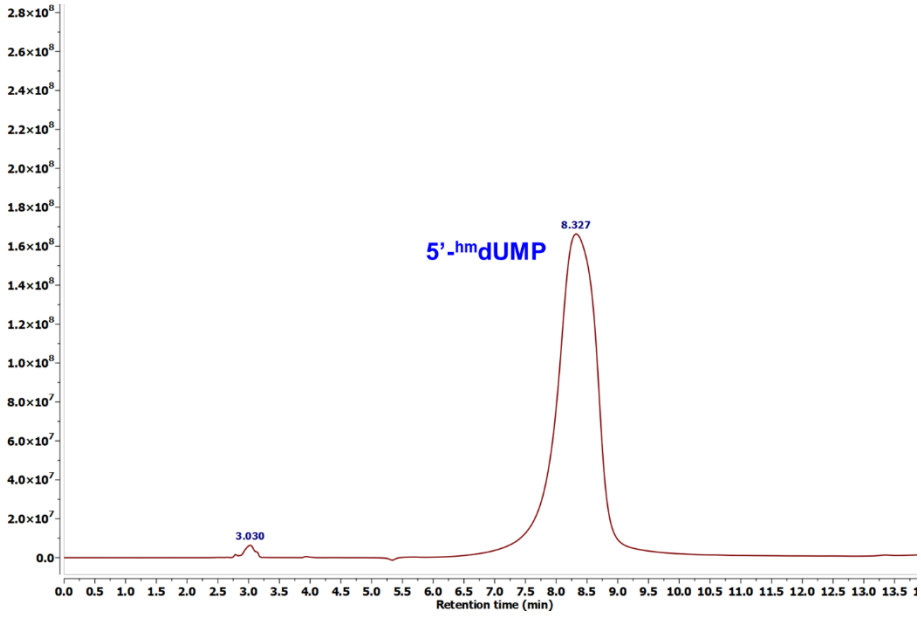

5'-hmdCMP
6.670
6.190
8.257
10.287
11.977
9.0×10^7
8.0×10^7
7.0×10^7
6.0×10^7
5.0×10^7
4.0×10^7
3.0×10^7
2.0×10^7
1.0×10^7
0.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5 12.0 12.5 13.0 13.5
Retention time (min)

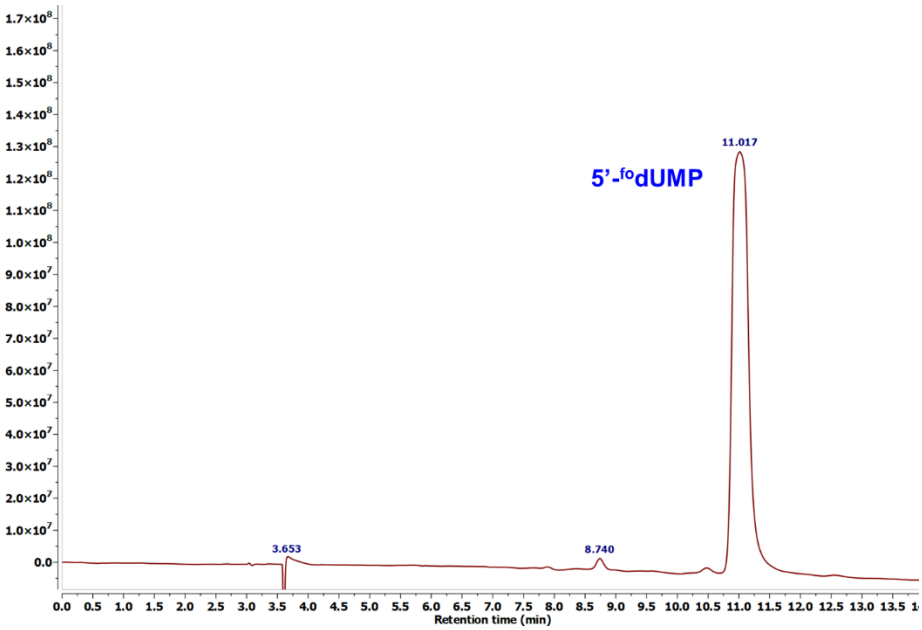

5'-fodCMP
12.407
11.427
10.493
8.237
1.2×10^8
1.1×10^8
1.0×10^8
9.0×10^7
8.0×10^7
7.0×10^7
6.0×10^7
5.0×10^7
4.0×10^7
3.0×10^7
2.0×10^7
1.0×10^7
0.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5 12.0 12.5 13.0 13.5
Retention time (min)

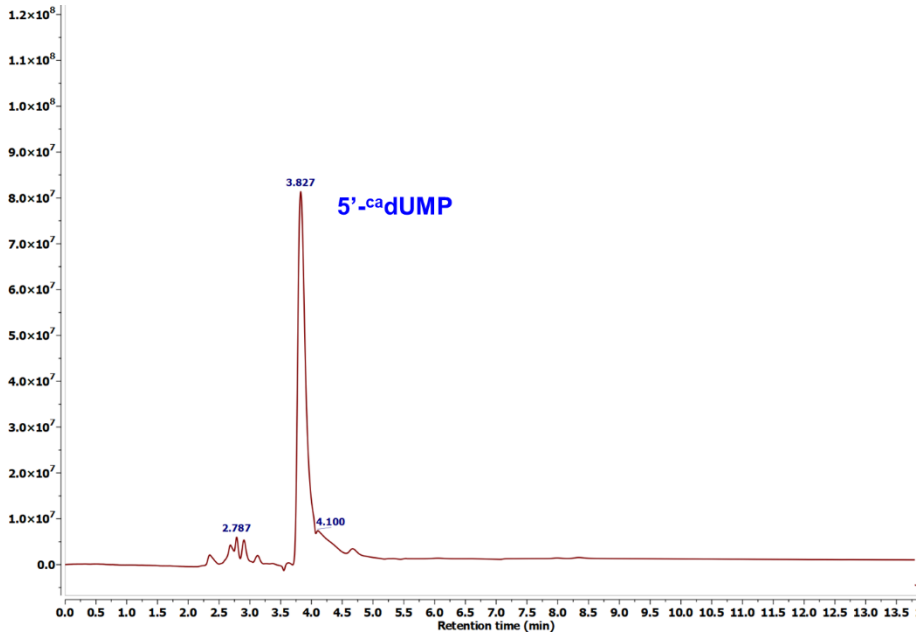

5'-caDCMP
4.237
3.933
2.893
12.130
Retention time (min)


5'-dUMP
7.047
Retention time (min)


5'-dTMP
10.473
2.590
3.113
6.357
Retention time (min)


5'-hmdUMP
8.327
3.030
Retention time (min)


11.017
5'-fodUMP
3.653
8.740
Retention time (min)


3.827
5'-cadUMP
2.787
4.100
Retention time (min)

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