CHEMBIOCHEM

Supporting Information

6-Substituted 2-Aminopurine-2′-deoxyribonucleoside 5′-Triphosphates that Trace Cytosine Methylation

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cbic_201600245_sm_miscellaneous_information.pdf

Biochemical methods

Oligonucleotides were purchased from Biomers in HPLC grade and used directly for primer extension experiments.

Quantification of oligonucleotides

Quantification of oligonucleotides was conducted by measuring absorbance at 260 nm in water.

5´-Radioactive labelling of oligonucleotides

DNA oligonucleotide primers were radioactively labelled at the 5' terminus by usage of [γ-^{32}P]-ATP and T4 PNK. The reactions contained 0.4 µM primer, 1 x PNK reaction buffer, 0.8 µCi/µl [γ-^{32}P]-ATP and 0.4 U/µL T4 PNK in a total volume of 50 µL. The reaction mixture was incubated at 37 °C and stopped after 1 h by denaturation of the T4 PNK for 2 min at 95 °C. Buffer and excess [γ-^{32}P]-ATP were removed by gel fitration (MicroSpin Sephadex G-25). Addition of 20 µl of unlabelled primer (10 µM) led to a final concentration of 3 µM of diluted radioactive labelled primer.

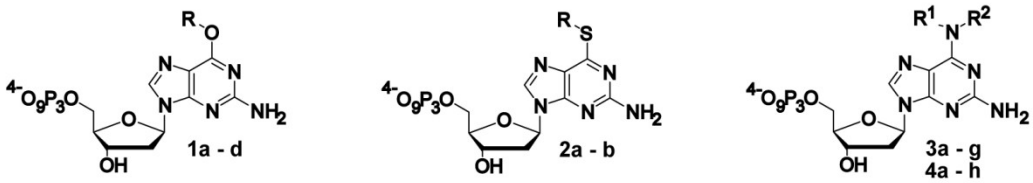
Primer extension assay

The mixture of 150 nM of a [γ-^{32}P]-labeled primer (5´-d(CGA AAT GAT CCC ATC CAG CTG C)-3´ and 200 nM of either template (5´-d(CCG CTG CCC ACC AGC CAT CAT GTC GGA CCC CGC GGT CAA CGX GCA GCT GGA TGG GAT CAT TTC GGA CT)-3´, X = C/5mC) in buffer (*KOD exo-*: 50 mM Tris-HCl pH 8.0, 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1 % Tween 20; *KlenTaq*: 50 mM Tris-HCl, pH 9.2, 16 mM $(NH_4)_2SO_4$, 1.75 mM $MgCl_2$, 0.1 % Tween 20; *9°North exo-*: 20 mM Tris-HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgCl_2$, 0.1 % Triton) was heated to 95 °C for 2 min and subsequently cooled to 4 °C for annealing. The respective polymerase was added and the reaction was started by addition of 50 µM of the respective dNTP at 55 °C. Reactions (10 µL) were stopped after the desired incubation time by addition of 10 µL stop solution (80 % (*v/v*) formamide, 20 mM EDTA, 0.25 % (*w/v*) Bromophenol Blue, 0.25 % (*w/v*) xylene cyanol) and analysed by 12 % or 15 % denaturing PAGE. Visualization was performed by phosphorimaging.

Gel electrophoresis

Denaturing polyacrylamide gels (12 or 15 %) were prepared by polymerization of a solution of bisacrylamide/acrylamide (12 % or 15 %) and urea (8.3 M) in TBE buffer using peroxodisulfate (APS, 0.08 %) and *N,N,N′,N′*-tetramethylethylene diamine (TEMED, 0.04 %). After initiation of polymerization, the solution was filled in a sequencing gel chamber (Bio-Rad) and left for polymerization for at least 30 min. After addition of TBE buffer (1 x) to the electrophoresis unit, the gel was prewarmed by electrophoresis at 100 W for 20 min before samples were applied to the gel. After electrophoresis at 100 W for approximately 2.5 h, the gel was transferred to Whatman filter paper and dried at 80 °C under reduced pressure using a gel dryer (Model 583, Bio-Rad). The dried gel was exposed to an imager screen overnight and read out was performed using a molecular imager (FX, Bio-Rad). Quantification was done using the Bio-Rad software Image Lab.ink.

a)



b)

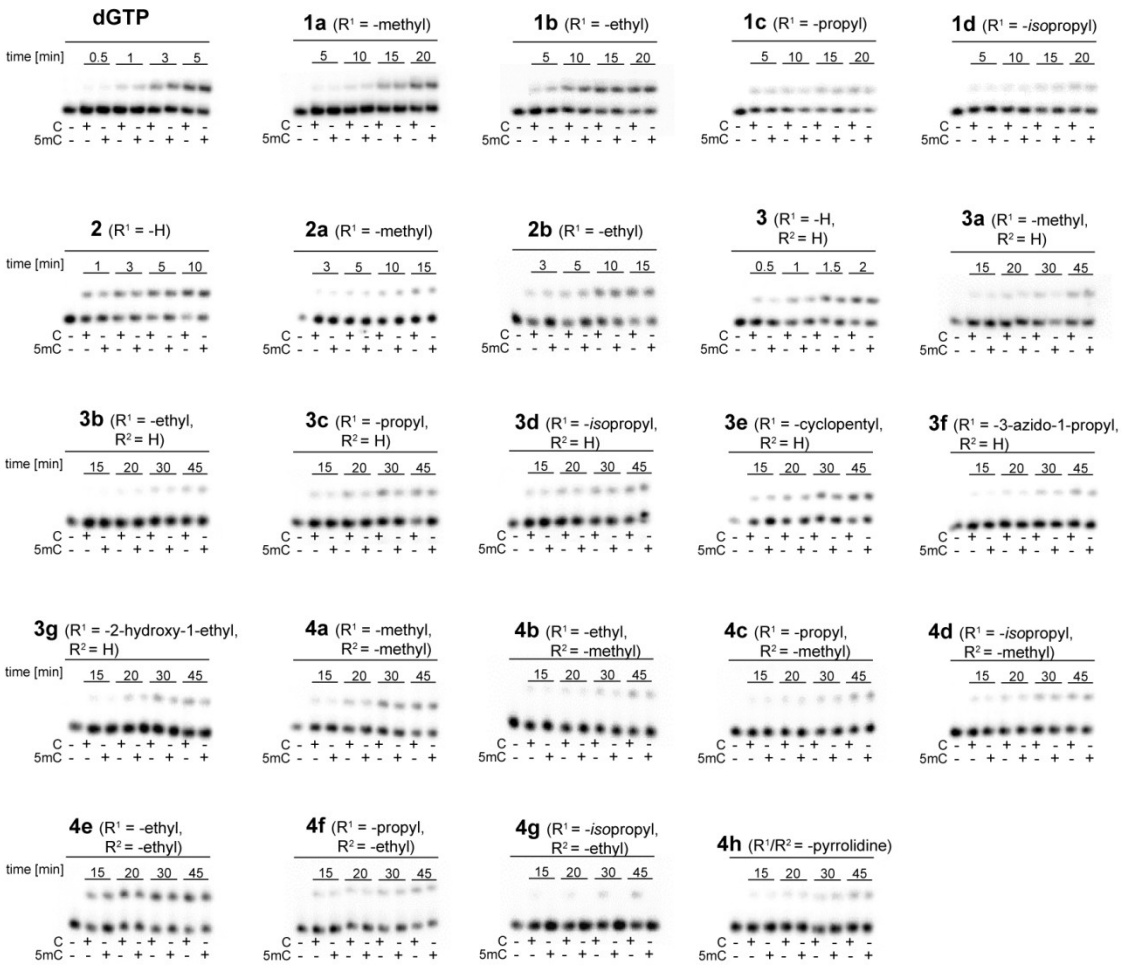
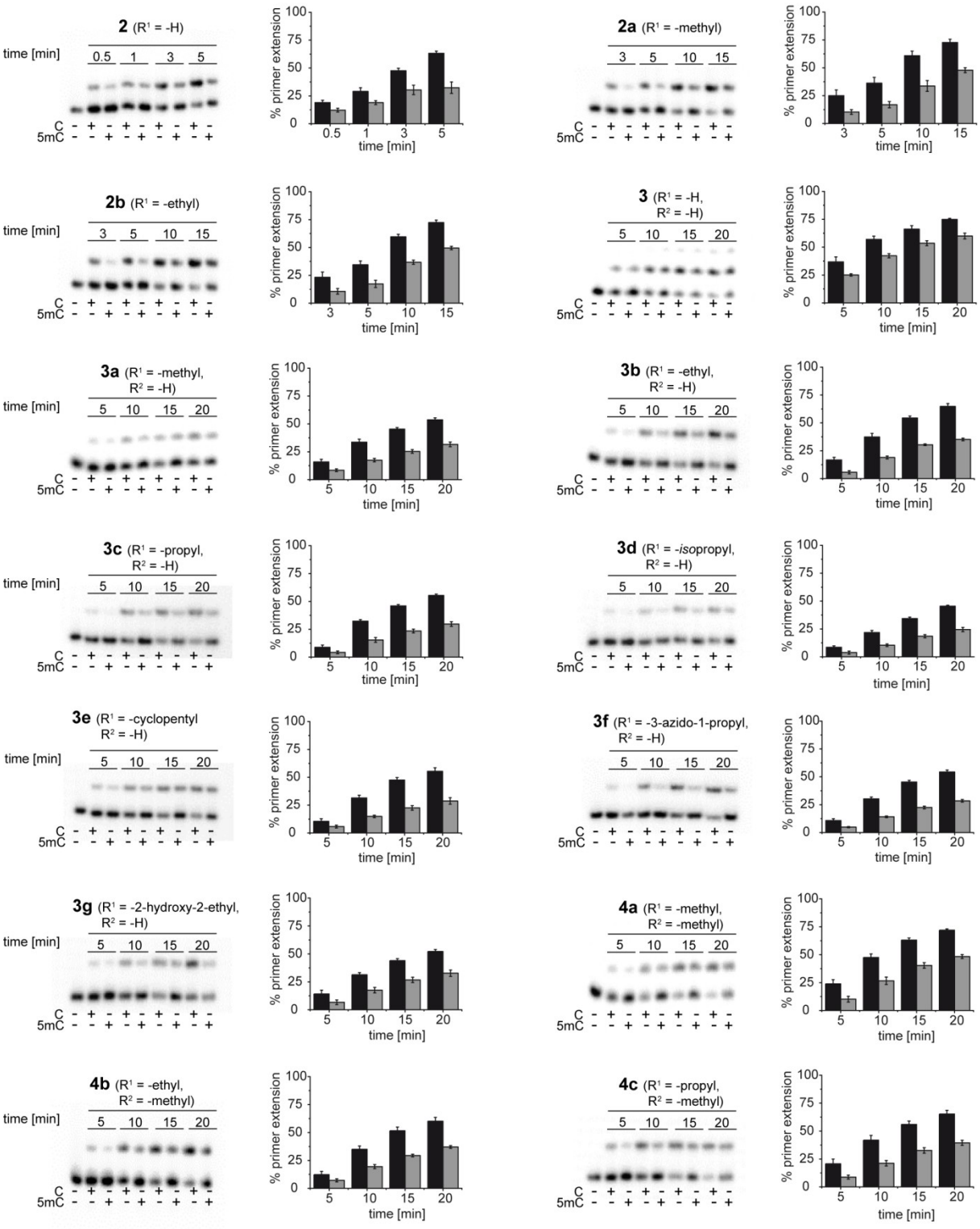


Figure S1: a) structures of modified nucleotides, b) PAGE analysis of single-nucleotide incorporation primer extension experiments of dGTP and modified nucleotides **1a - d, 2, 2a + b, 3, 3a - 3g** and **4a - h** opposite a template containing C in comparison to a template containing 5mC employing KlenTaq DNA polymerase. 100 µM dGTP or dG*TP and 0.1 nM KlenTaq were used, reactions were stopped after indicated timepoints.


2 (R¹ = -H)
time [min]
0.5 1 3 5
C - + - + - + - +
5mC - - + - + - + -
% primer extension
time [min]
2a (R¹ = -methyl)
3 5 10 15
2b (R¹ = -ethyl)
3 5 10 15
3 (R¹ = -H, R² = -H)
5 10 15 20
3a (R¹ = -methyl, R² = -H)
5 10 15 20
3b (R¹ = -ethyl, R² = -H)
5 10 15 20
3c (R¹ = -propyl, R² = -H)
5 10 15 20
3d (R¹ = -isopropyl, R² = -H)
5 10 15 20
3e (R¹ = -cyclopentyl R² = -H)
5 10 15 20
3f (R¹ = -3-azido-1-propyl, R² = -H)
5 10 15 20
3g (R¹ = -2-hydroxy-2-ethyl, R² = -H)
5 10 15 20
4a (R¹ = -methyl, R² = -methyl)
5 10 15 20
4b (R¹ = -ethyl, R² = -methyl)
5 10 15 20
4c (R¹ = -propyl, R² = -methyl)
5 10 15 20

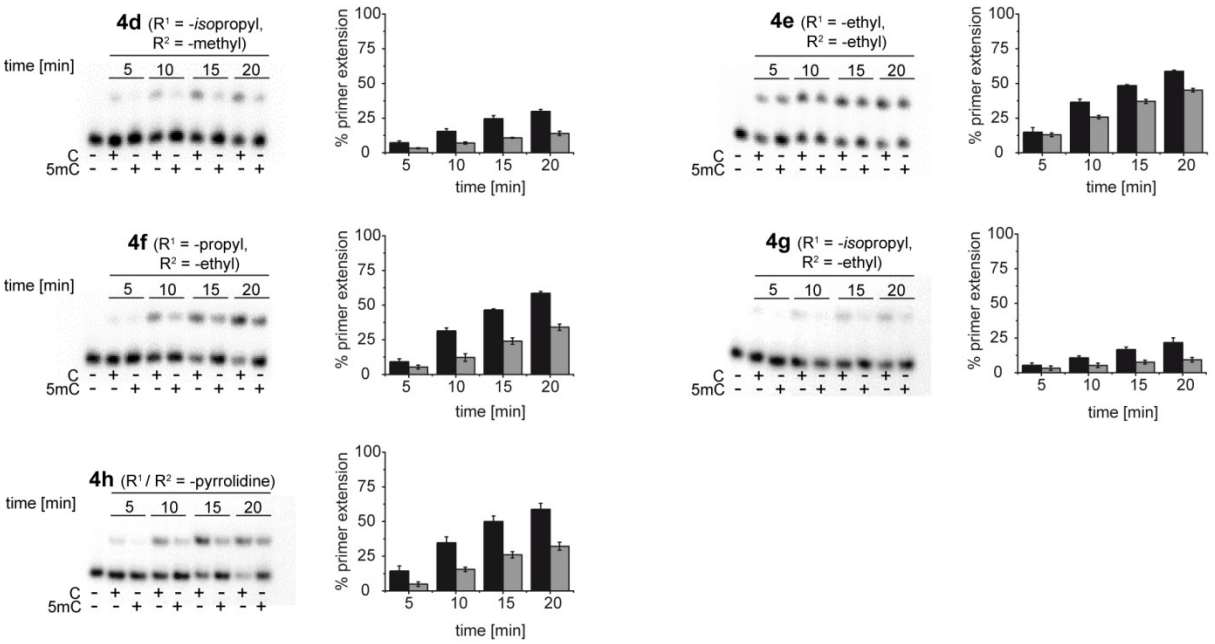


Figure S2: PAGE analysis of single-nucleotide incorporation primer extension experiments of dGTP and the modified nucleotides **2, 2a + b, 3, 3a - 3g** and **4a - h** opposite a template containing C in comparison to a template containing 5mC employing KOD exo- DNA polymerase. 100 µM dGTP or dG*TP and 10 nM KOD exo- were used, reactions were stopped after indicated timepoints. Data points derive from triplicates.

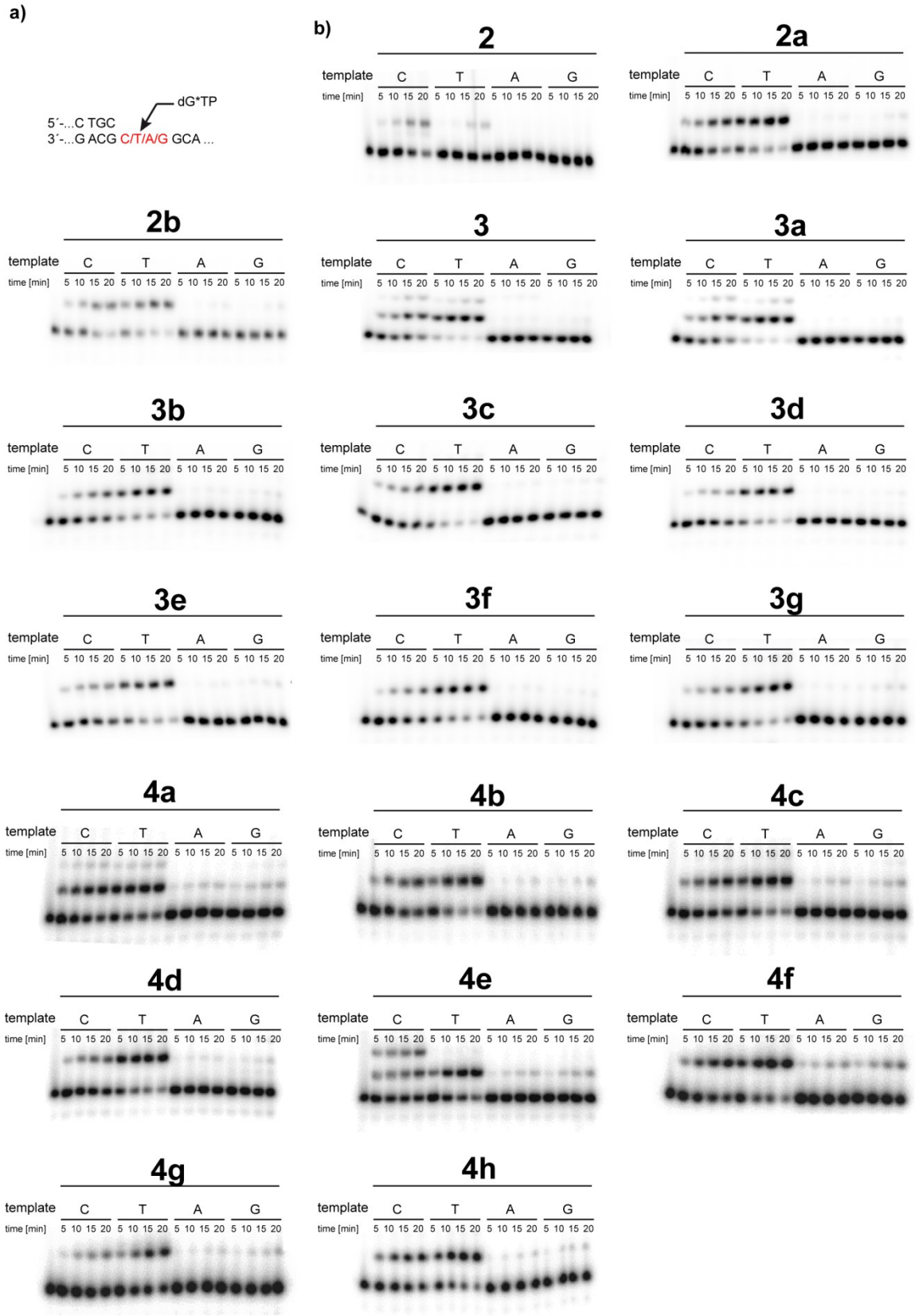
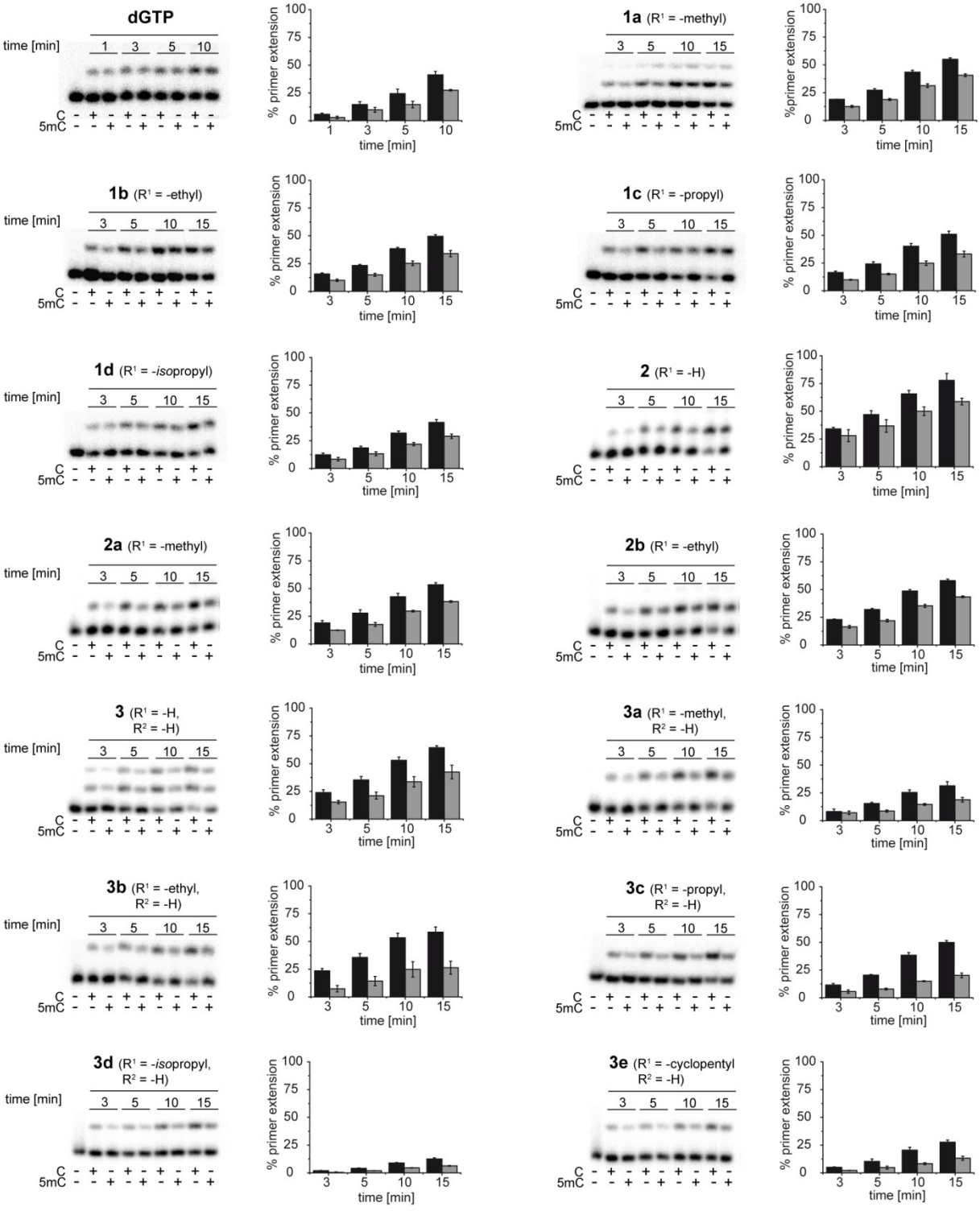


Figure S3: (a) Partial primer/ template sequence used (b) PAGE analysis of single-nucleotide incorporation primer extension experiments of nucleotides **2, 2a + b, 3, 3a - 3g** and **4a - h** opposite a template containing C, T, A or G employing KOD exo- DNA polymerase. 100 µM dG*TP and 10 nM KOD exo- were used, reactions were stopped after indicated timepoints.


dGTP
time [min]
1 3 5 10
C - + - + - + - +
5mC - - + - + - + -
% primer extension
time [min]
1a (R¹ = -methyl)
3 5 10 15
1b (R¹ = -ethyl)
1c (R¹ = -propyl)
1d (R¹ = -isopropyl)
2 (R¹ = -H)
2a (R¹ = -methyl)
2b (R¹ = -ethyl)
3 (R¹ = -H, R² = -H)
3a (R¹ = -methyl, R² = -H)
3b (R¹ = -ethyl, R² = -H)
3c (R¹ = -propyl, R² = -H)
3d (R¹ = -isopropyl, R² = -H)
3e (R¹ = -cyclopentyl R² = -H)

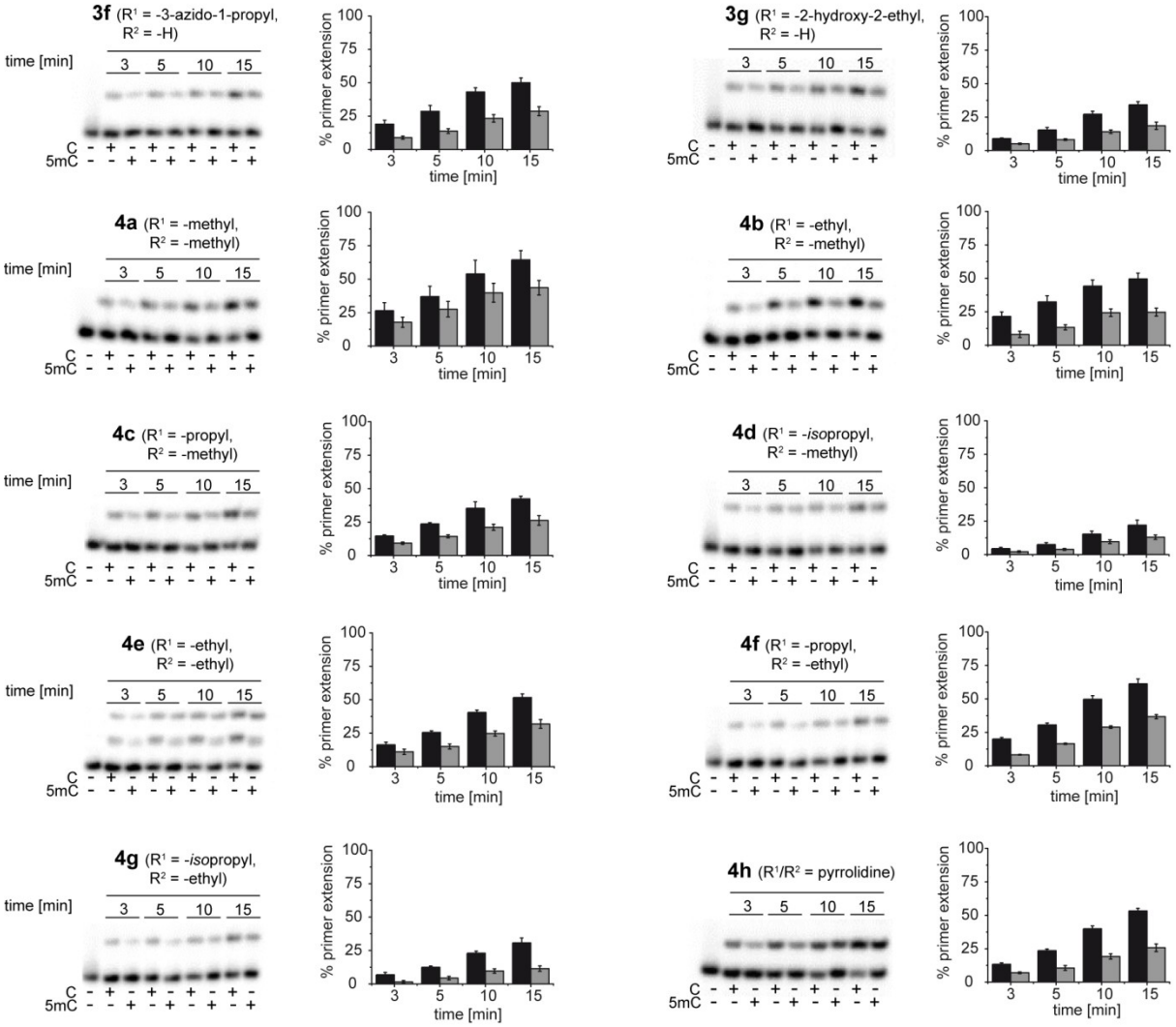
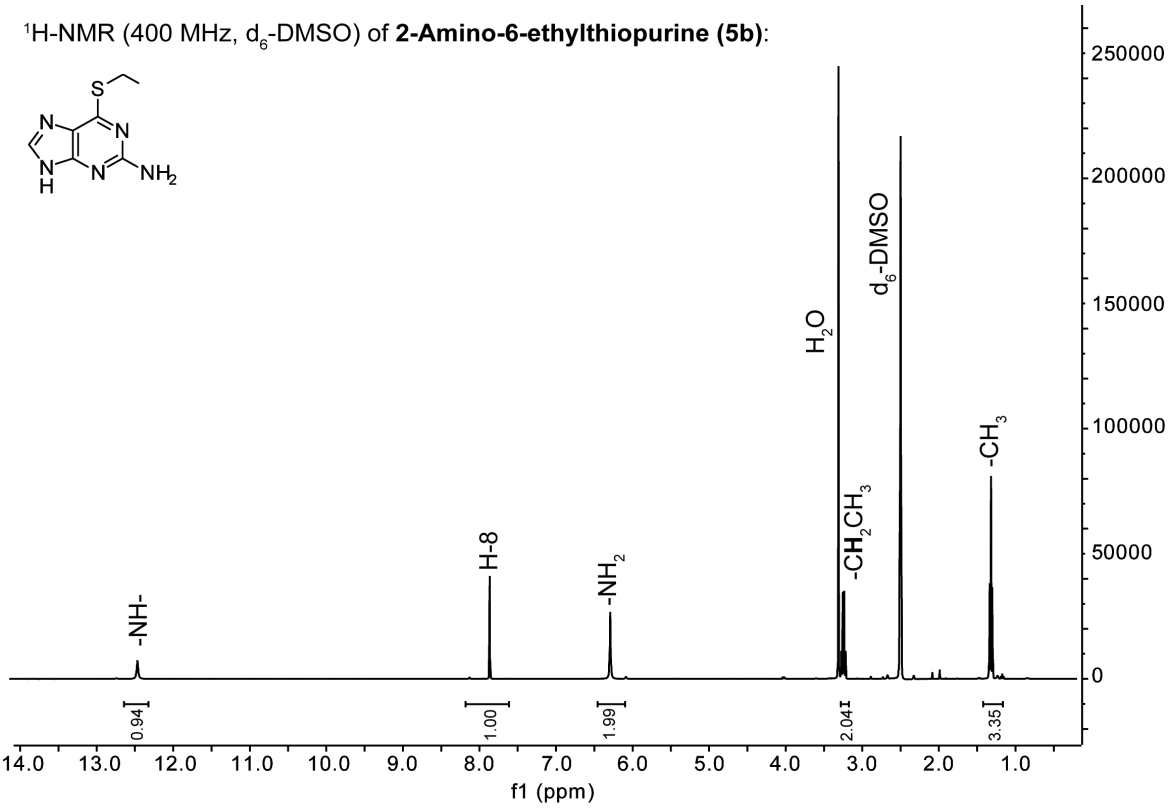


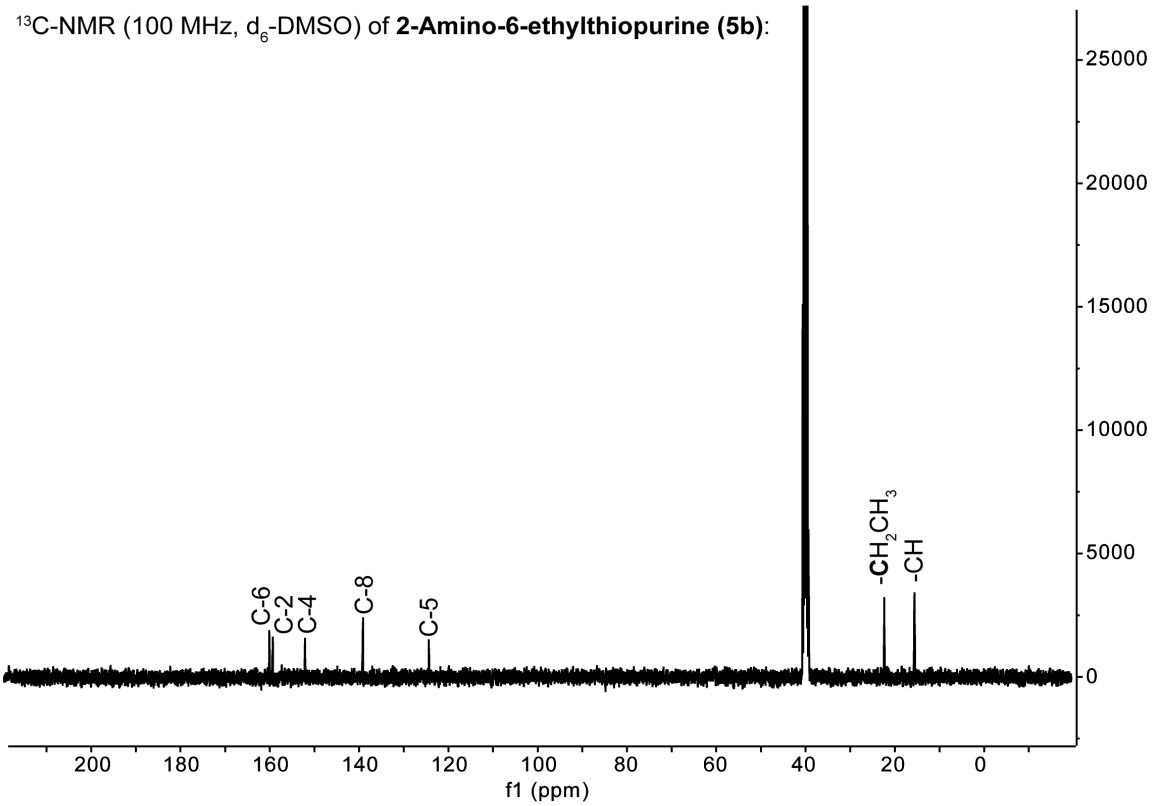
Figure S4: PAGE analysis of single-nucleotide incorporation primer extension experiments of nucleotides dGTP and modified nucleotides **1a - d, 2, 2a + b, 3, 3a - 3g** and **4a - h** opposite a template containing C in comparison to a template containing 5mC employing 9°North exo- DNA polymerase. 100 µM dGTP or dG*TP and 20 nM 9°North exo- were used, reactions were stopped after indicated timepoints. Data points derive from triplicates.

NMR – Spectra:

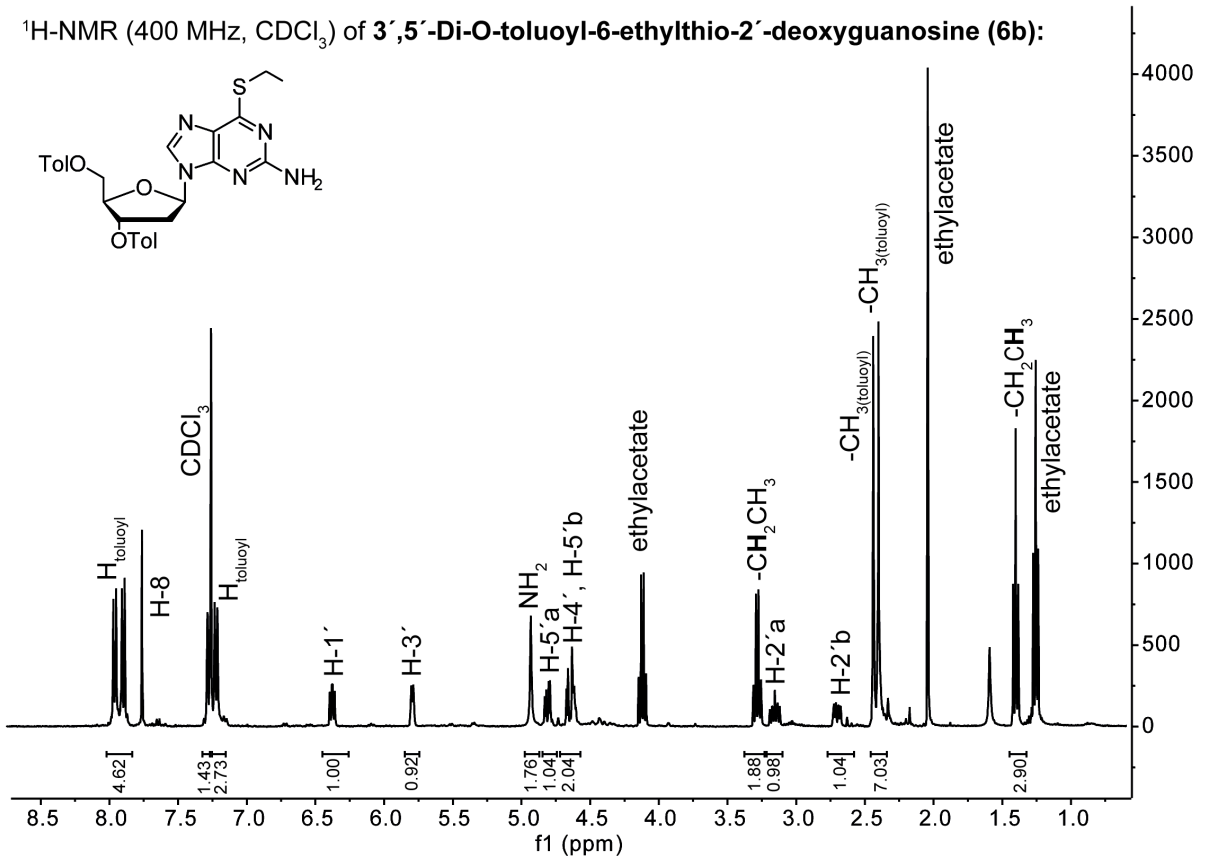
^{1}H-NMR (400 MHz, d_6-DMSO) of **2-Amino-6-ethylthiopurine (5b)**:



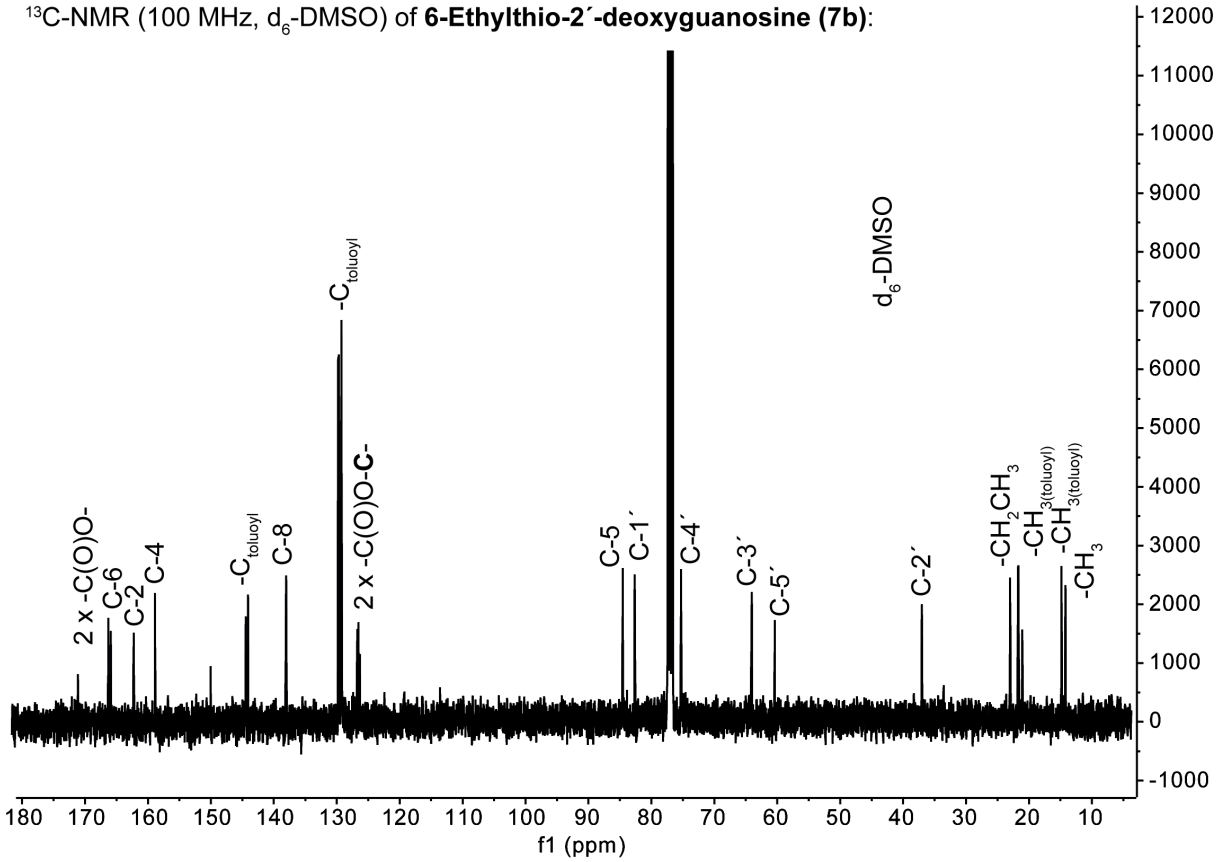
^{13}C-NMR (100 MHz, d_6-DMSO) of **2-Amino-6-ethylthiopurine (5b)**:



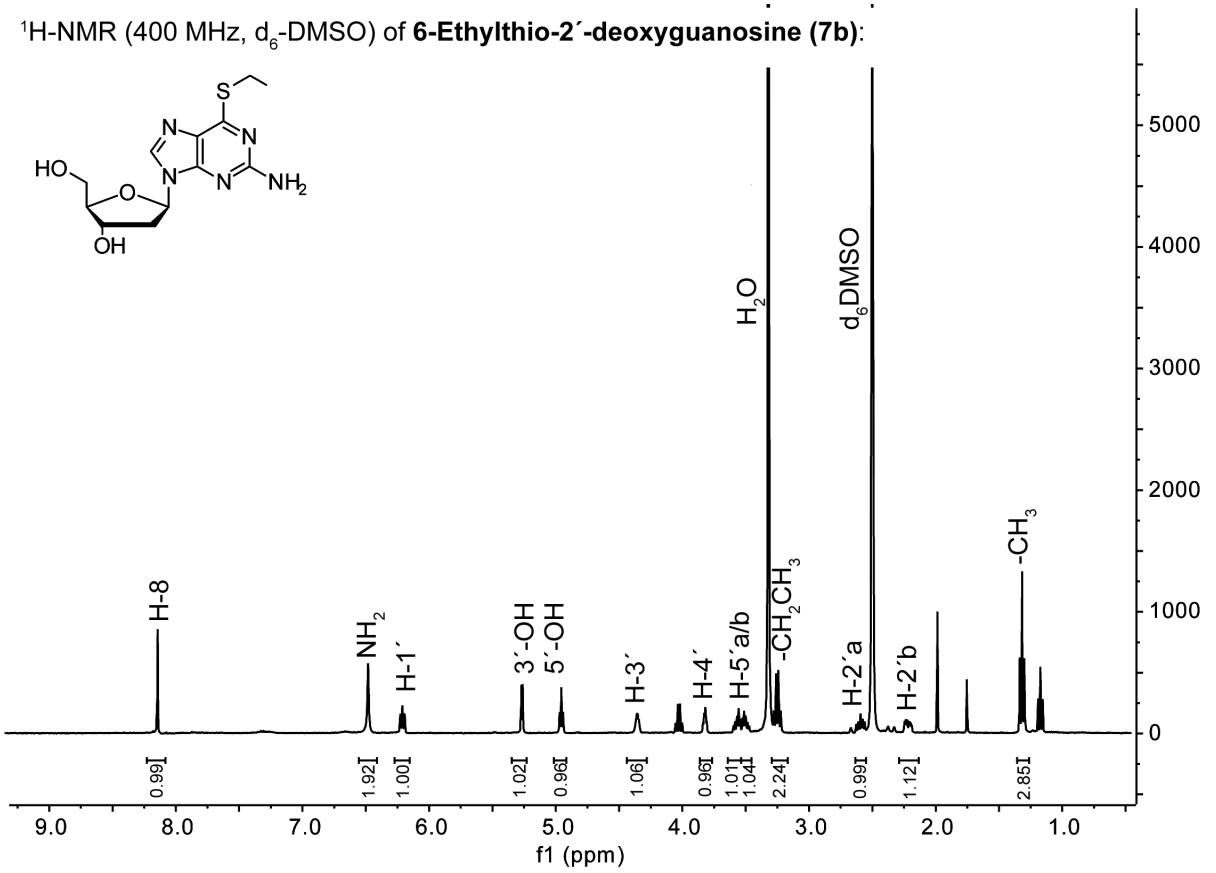
^{1}H-NMR (400 MHz, $CDCl_3$) of **3´,5´-Di-O-toluoyl-6-ethylthio-2´-deoxyguanosine (6b):**



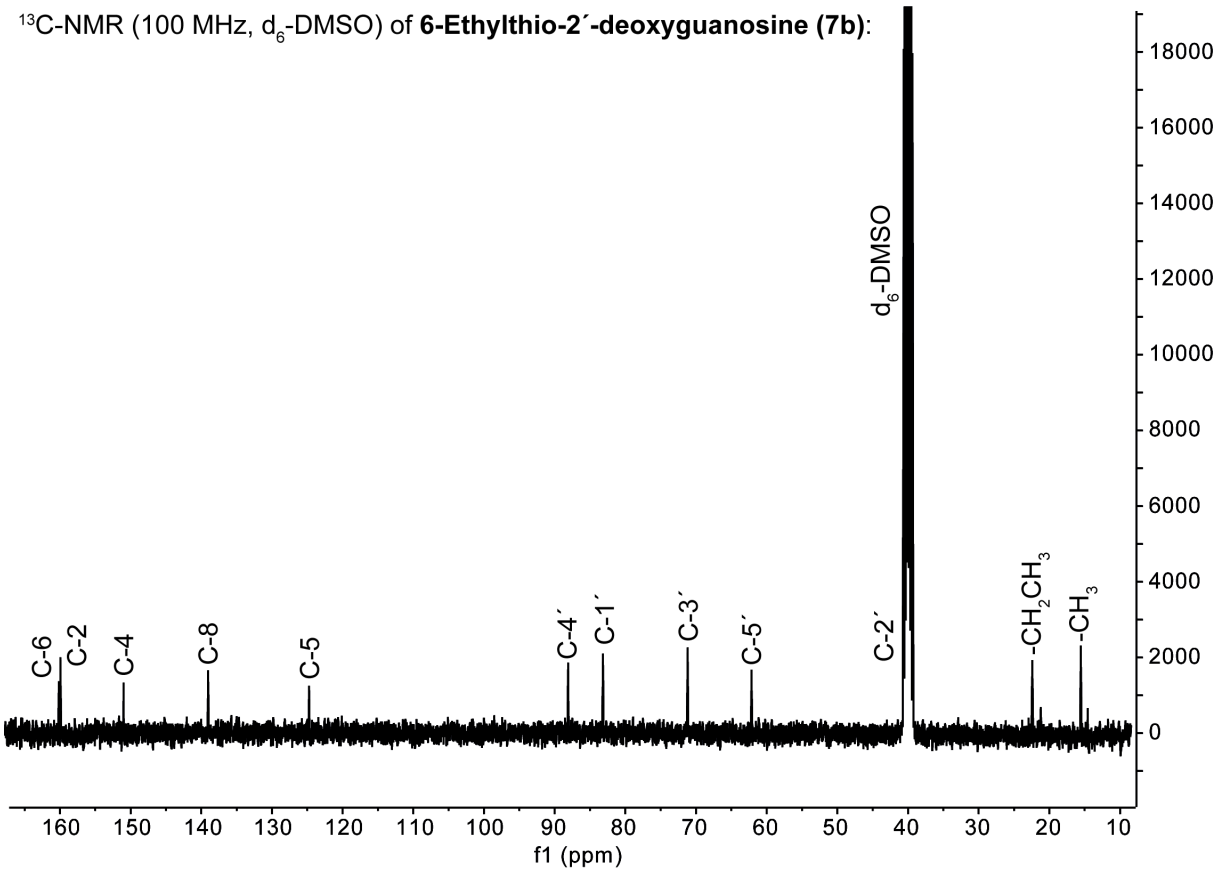
^{13}C-NMR (100 MHz, d_6-DMSO) of **6-Ethylthio-2´-deoxyguanosine (7b)**:



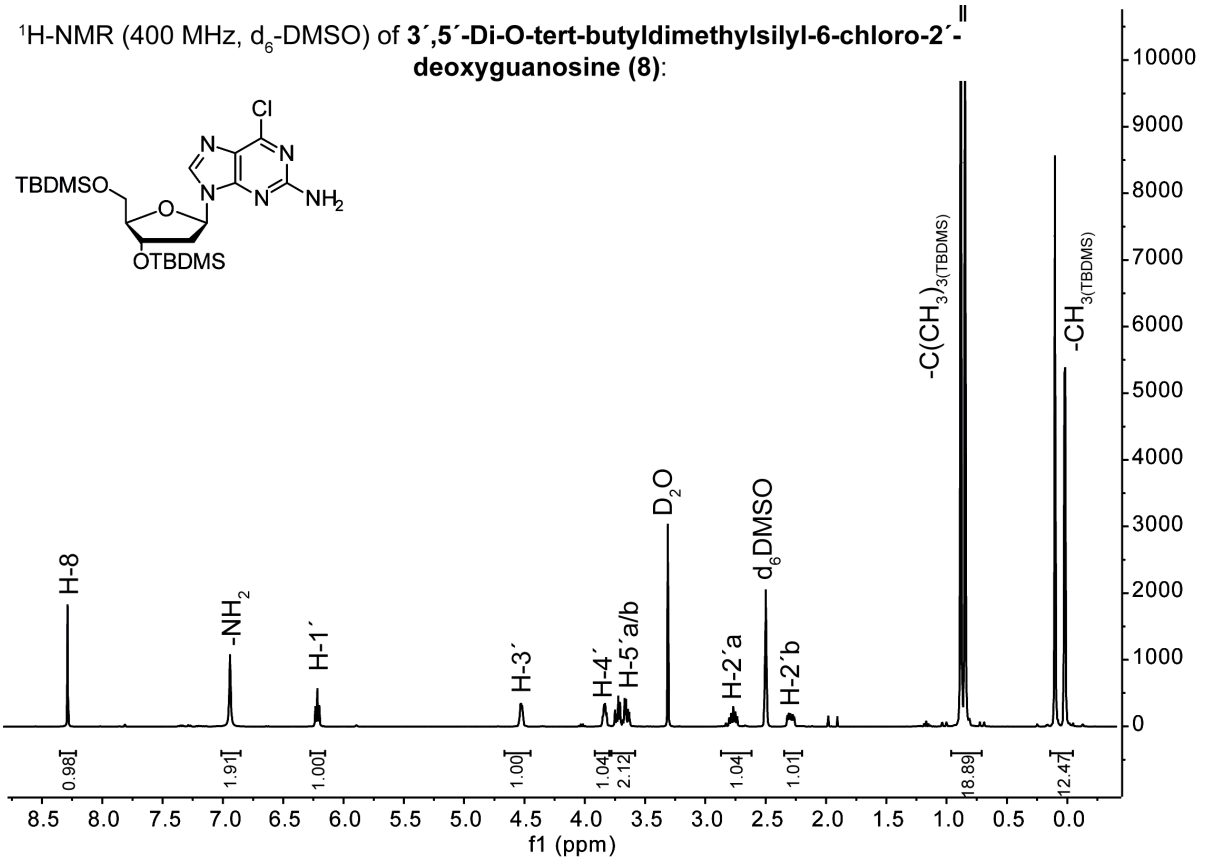
^{1}H-NMR (400 MHz, d_6-DMSO) of **6-Ethylthio-2´-deoxyguanosine (7b)**:



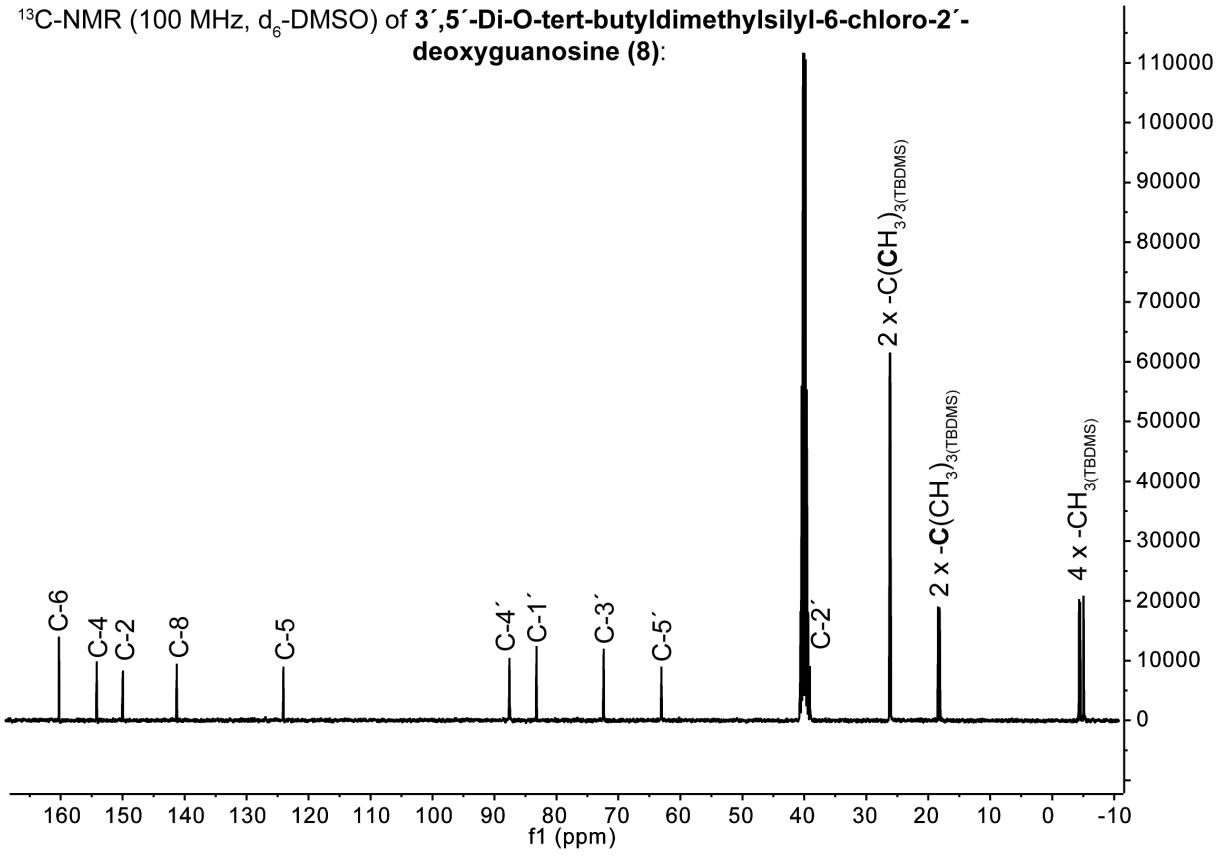
^{13}C-NMR (100 MHz, d_6-DMSO) of **6-Ethylthio-2´-deoxyguanosine (7b)**:



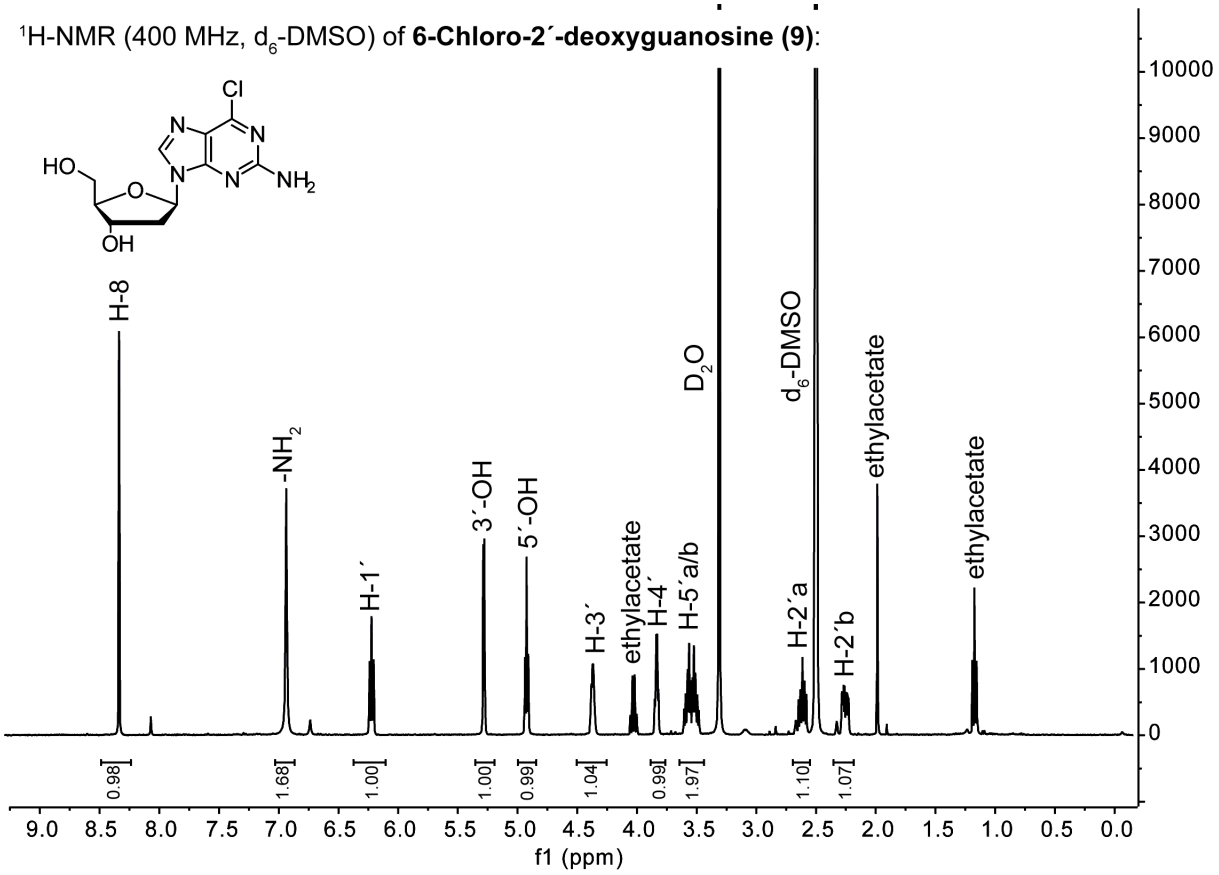
^{1}H-NMR (400 MHz, d_6-DMSO) of **3´,5´-Di-O-tert-butyldimethylsilyl-6-chloro-2´-deoxyguanosine (8)**:



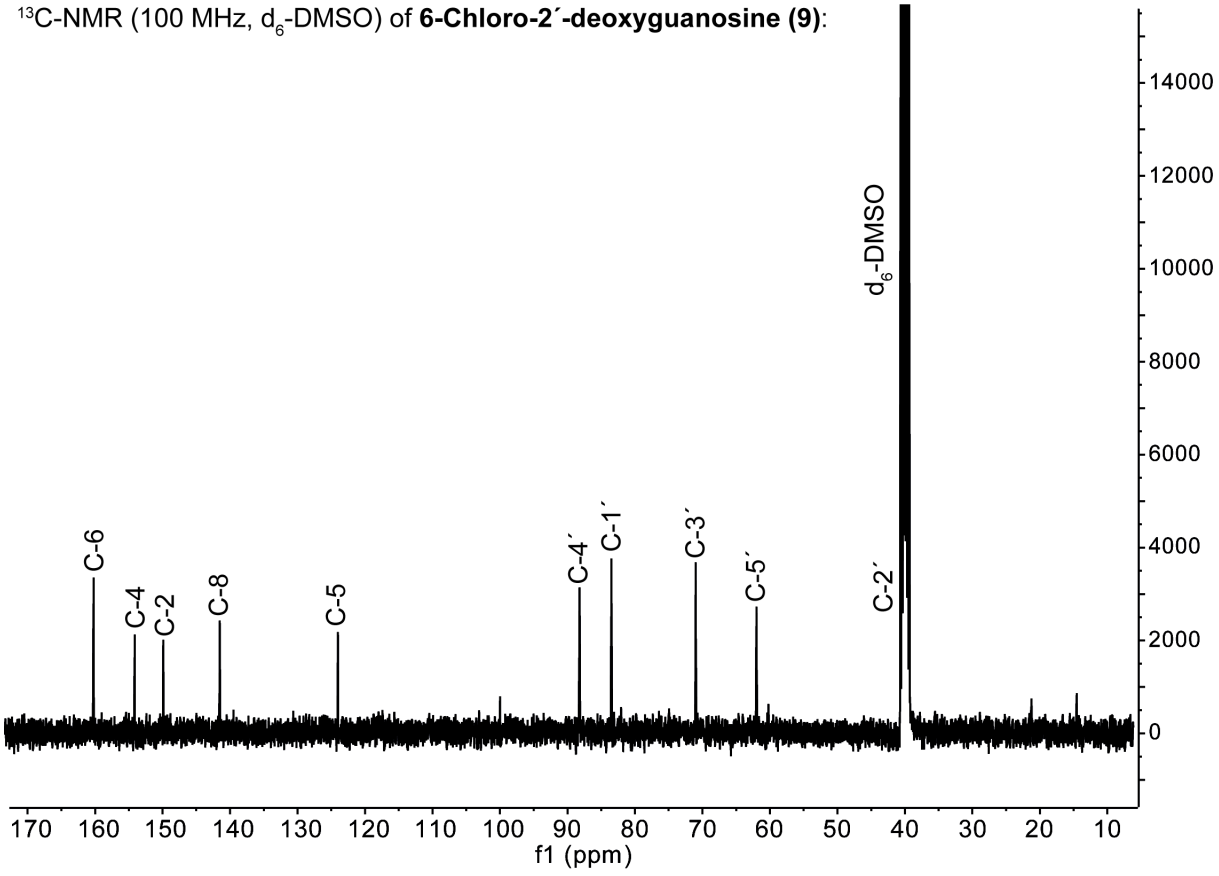
^{13}C-NMR (100 MHz, d_6-DMSO) of **3´,5´-Di-O-tert-butyldimethylsilyl-6-chloro-2´-deoxyguanosine (8)**:



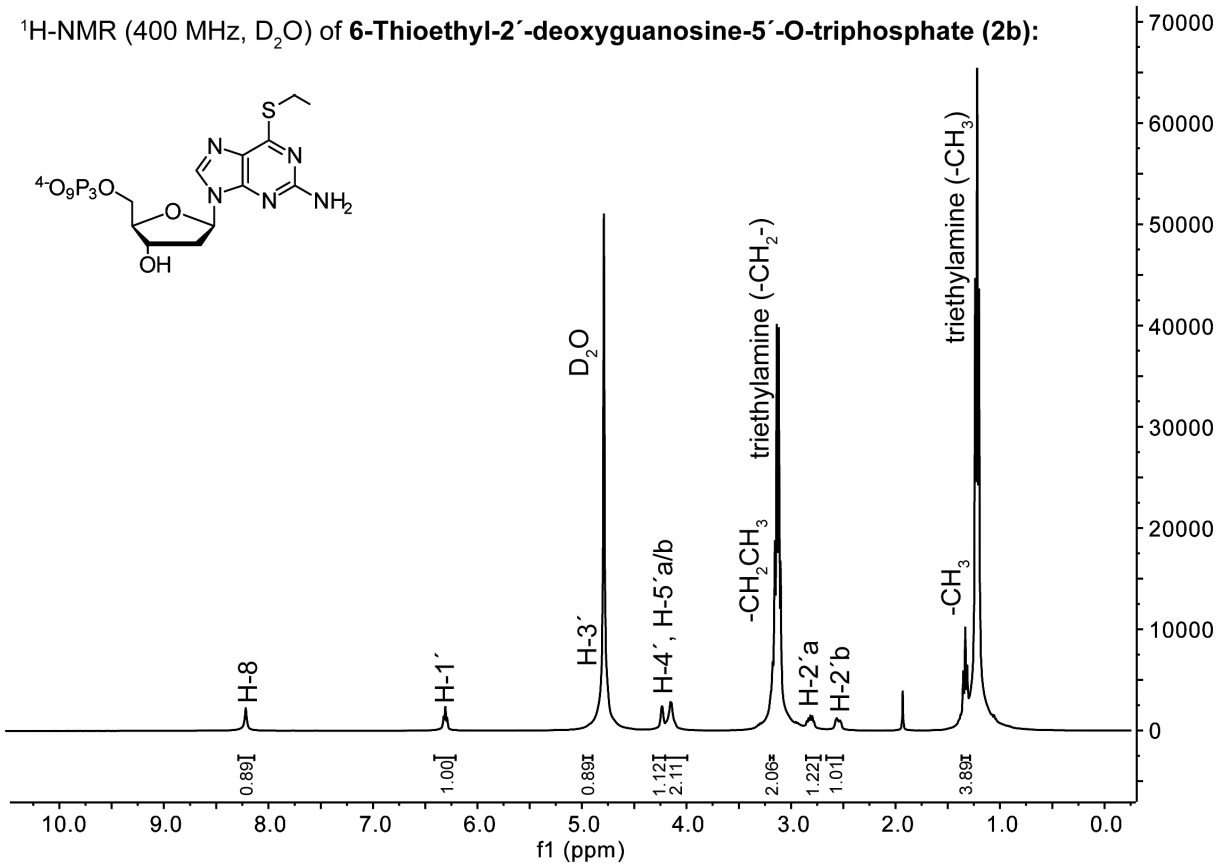
^{1}H-NMR (400 MHz, d_6-DMSO) of **6-Chloro-2´-deoxyguanosine (9)**:



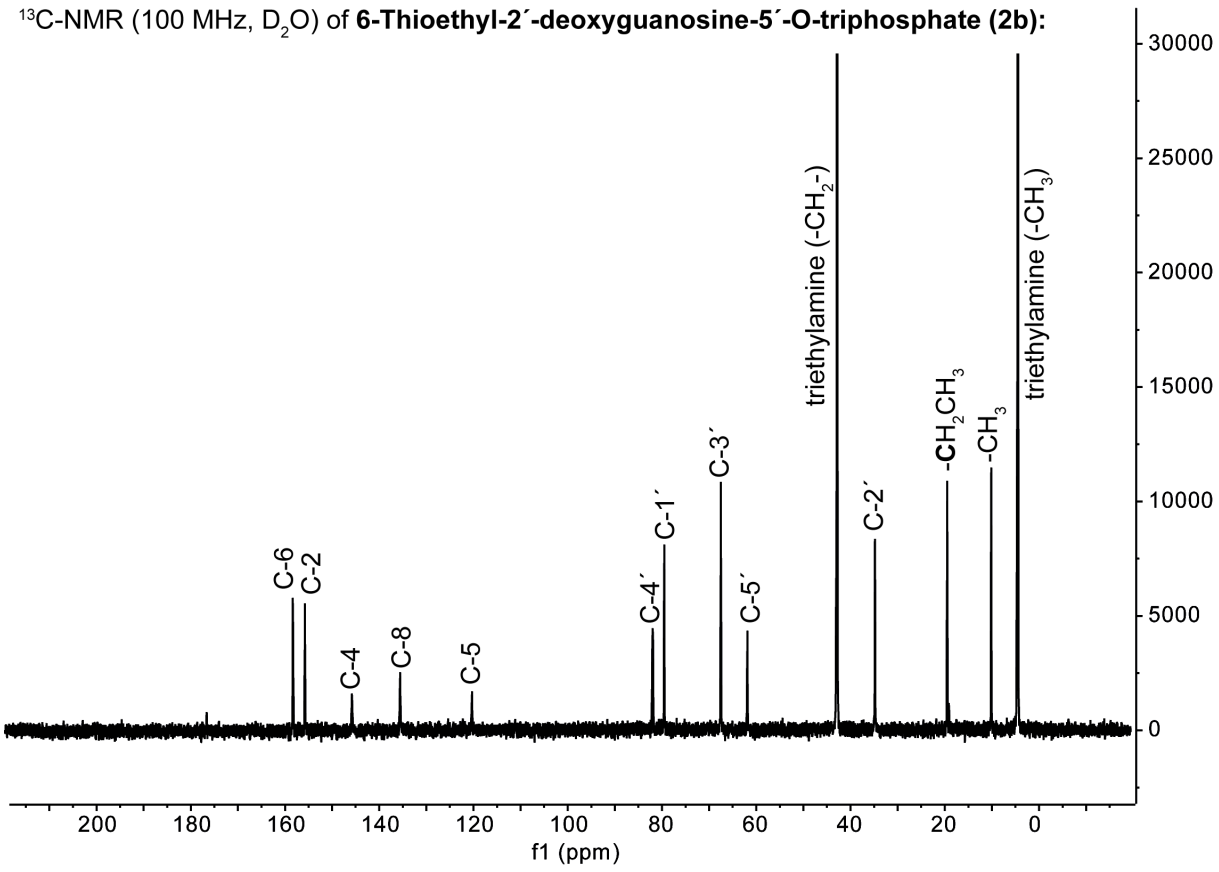
^{13}C-NMR (100 MHz, d_6-DMSO) of **6-Chloro-2´-deoxyguanosine (9)**:



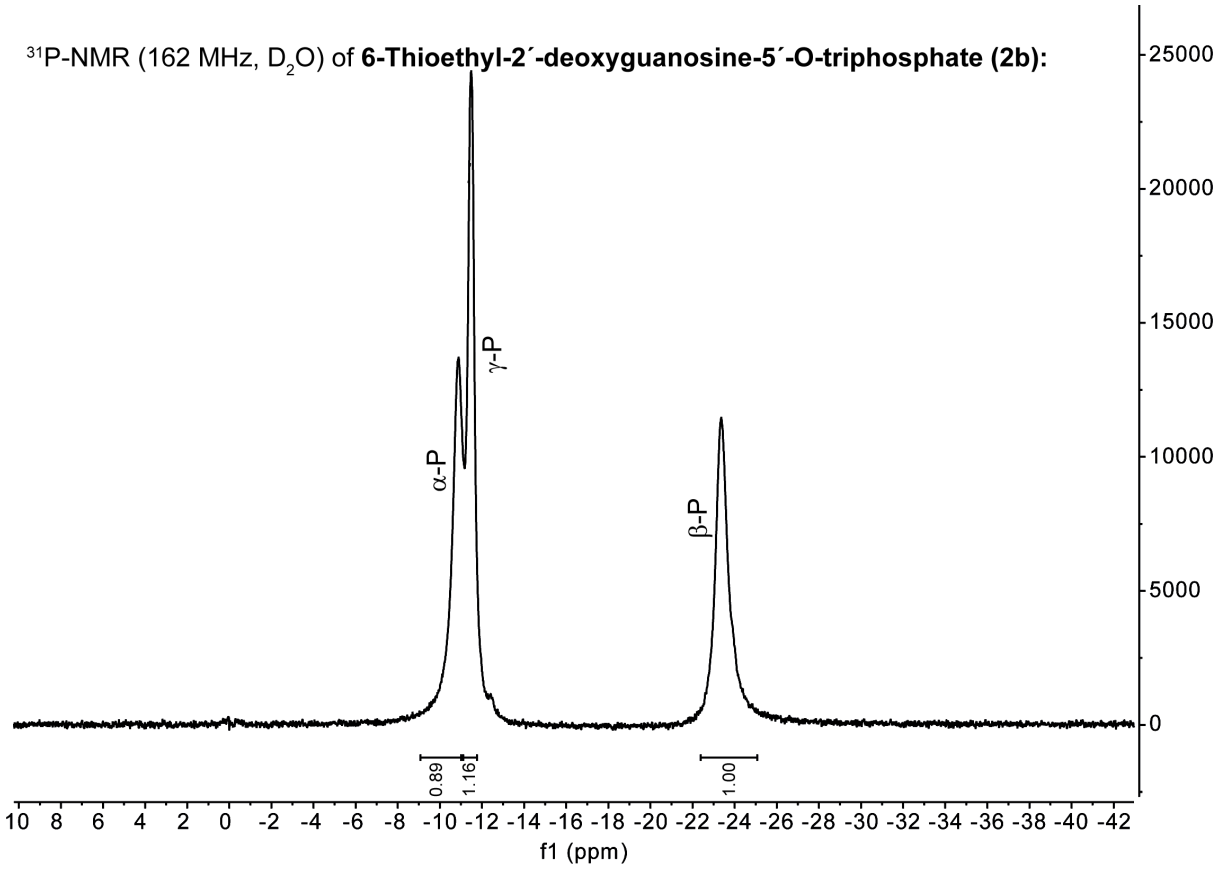
^{1}H-NMR (400 MHz, D_2O) of **6-Thioethyl-2´-deoxyguanosine-5´-O-triphosphate (2b):**



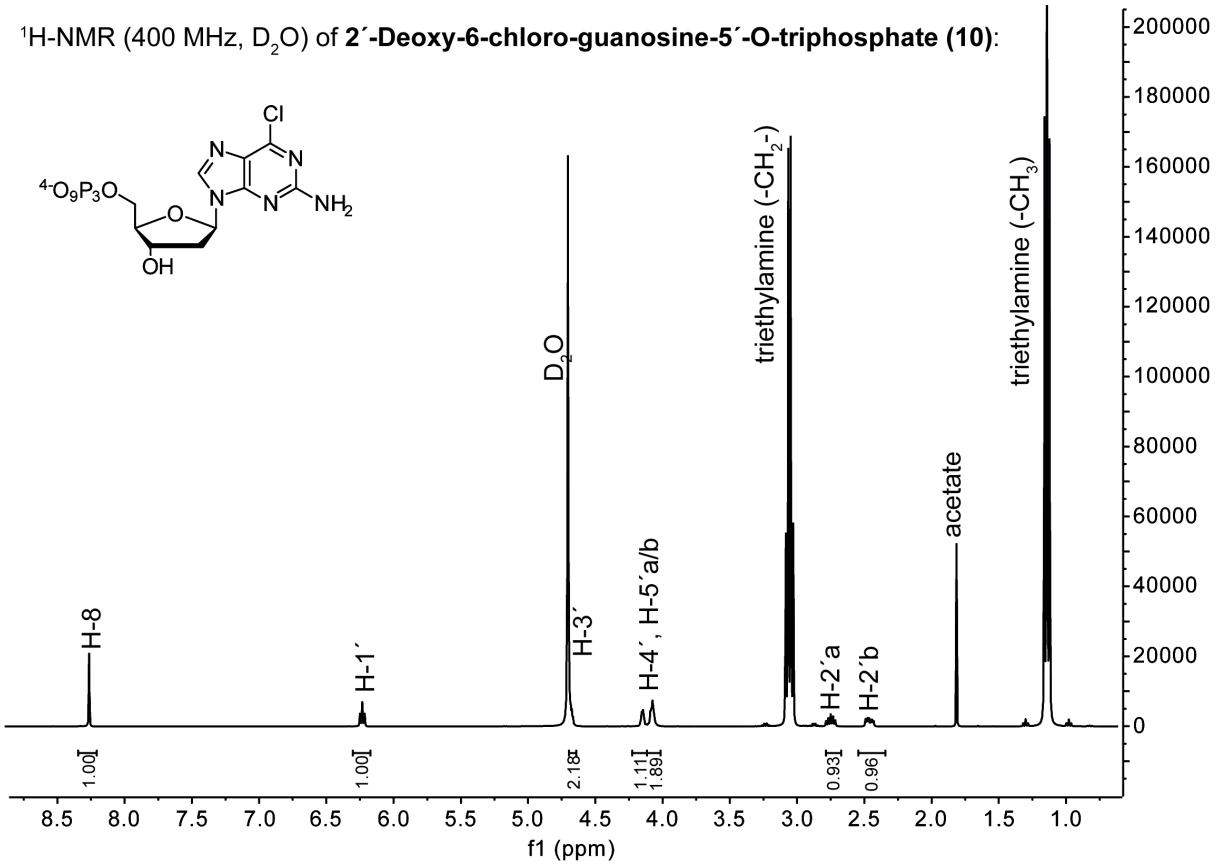
^{13}C-NMR (100 MHz, D_2O) of **6-Thioethyl-2´-deoxyguanosine-5´-O-triphosphate (2b):**



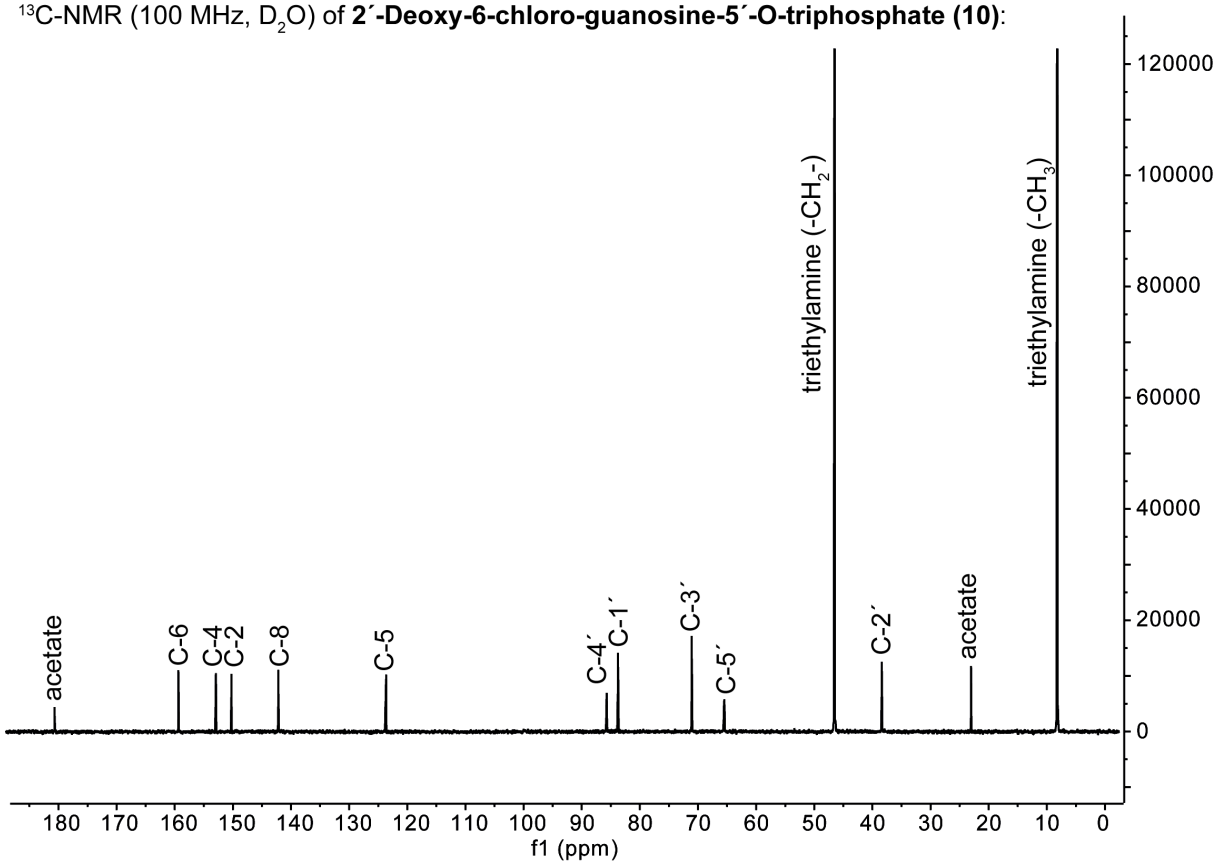
^{31}P-NMR (162 MHz, D_2O) of **6-Thioethyl-2´-deoxyguanosine-5´-O-triphosphate (2b):**



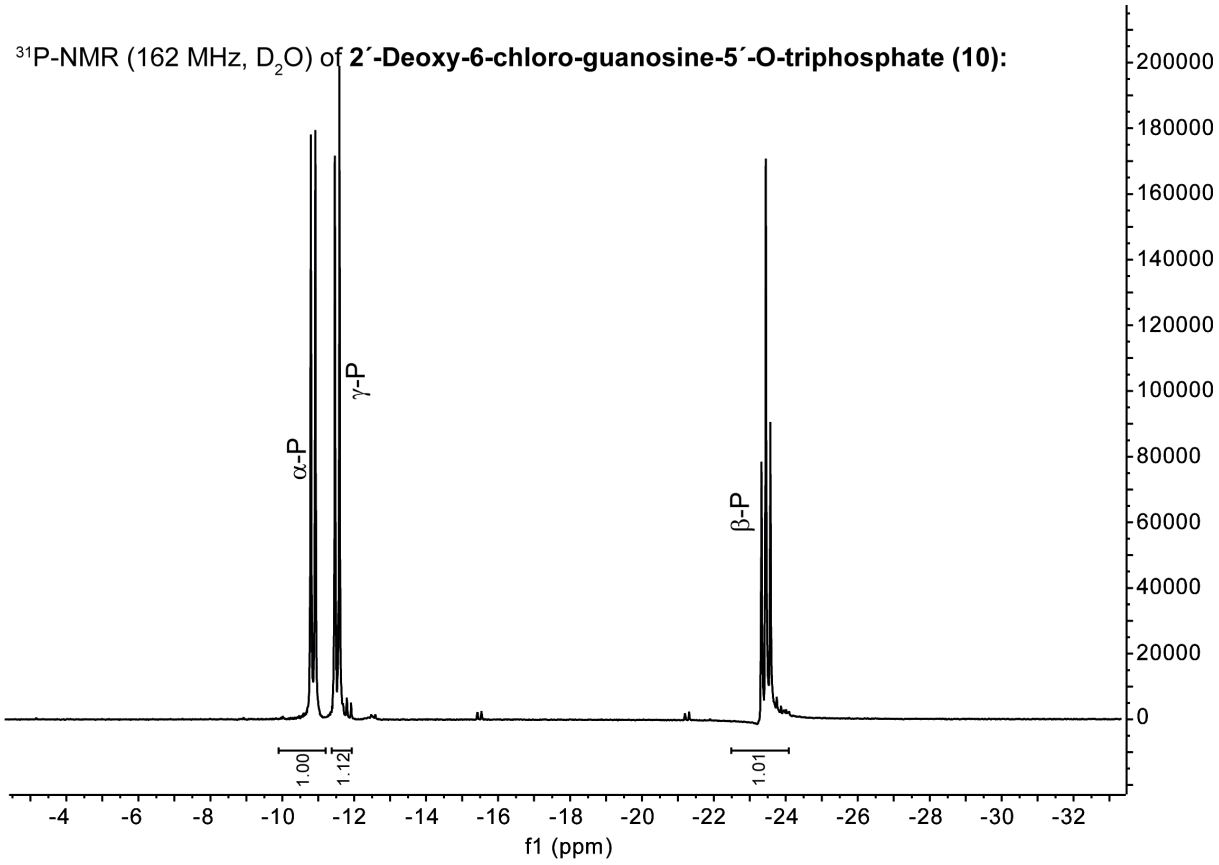
^{1}H-NMR (400 MHz, D_2O) of **2´-Deoxy-6-chloro-guanosine-5´-O-triphosphate (10)**:



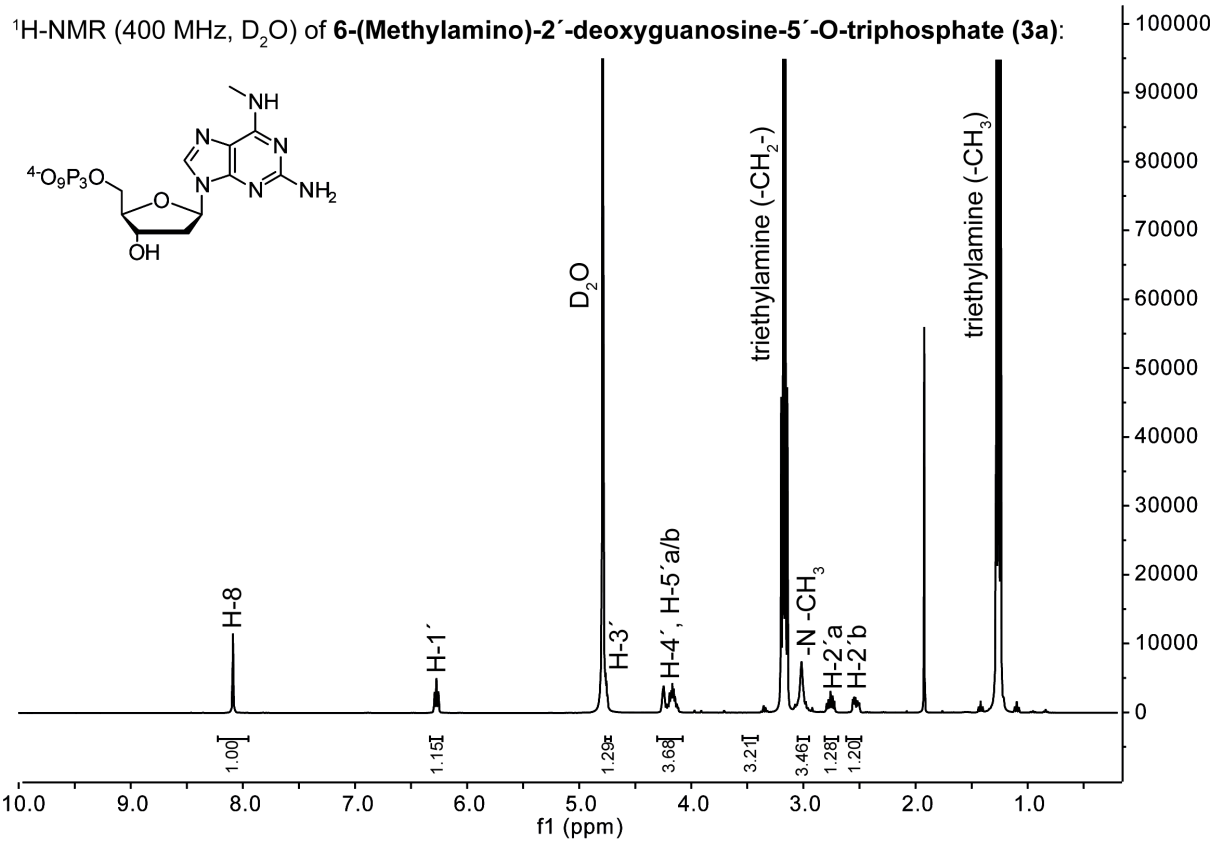
^{13}C-NMR (100 MHz, D_2O) of **2´-Deoxy-6-chloro-guanosine-5´-O-triphosphate (10)**:



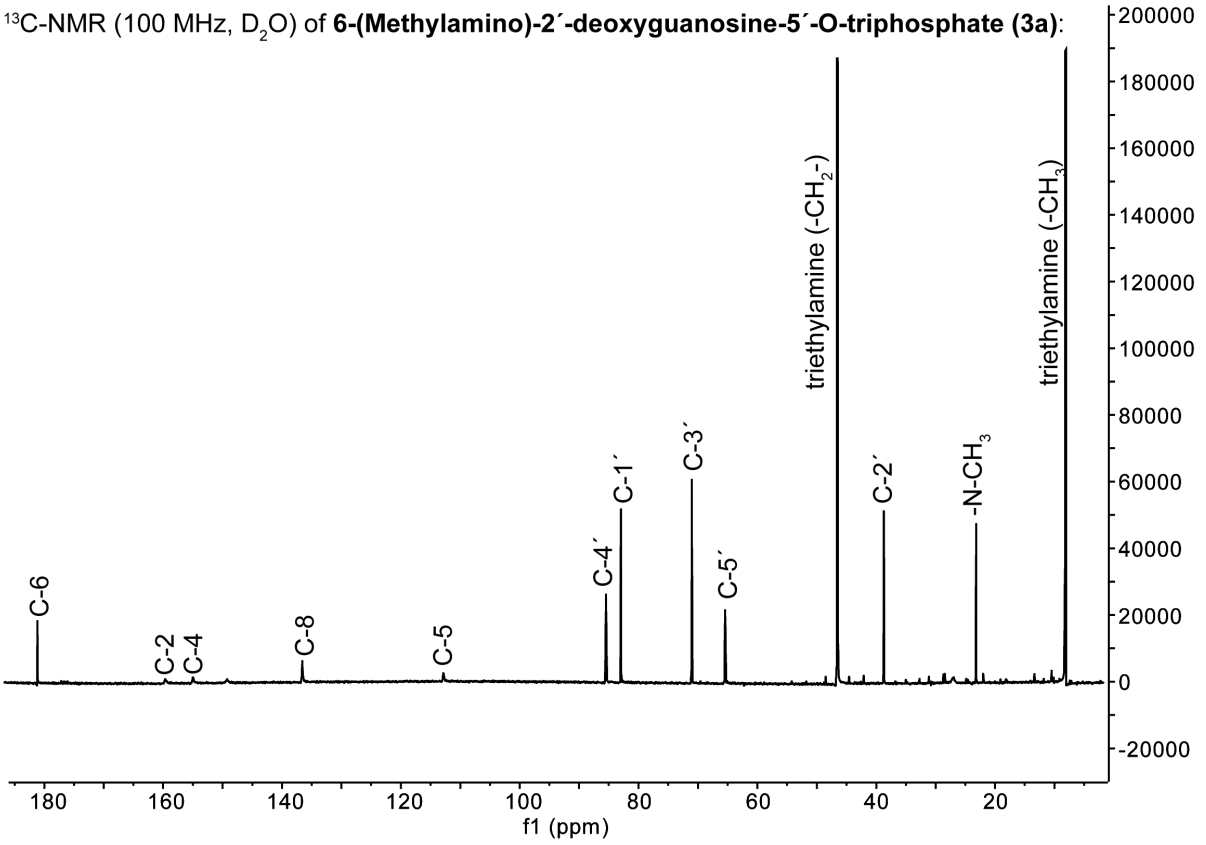
^{31}P-NMR (162 MHz, D_2O) of **2´-Deoxy-6-chloro-guanosine-5´-O-triphosphate (10):**



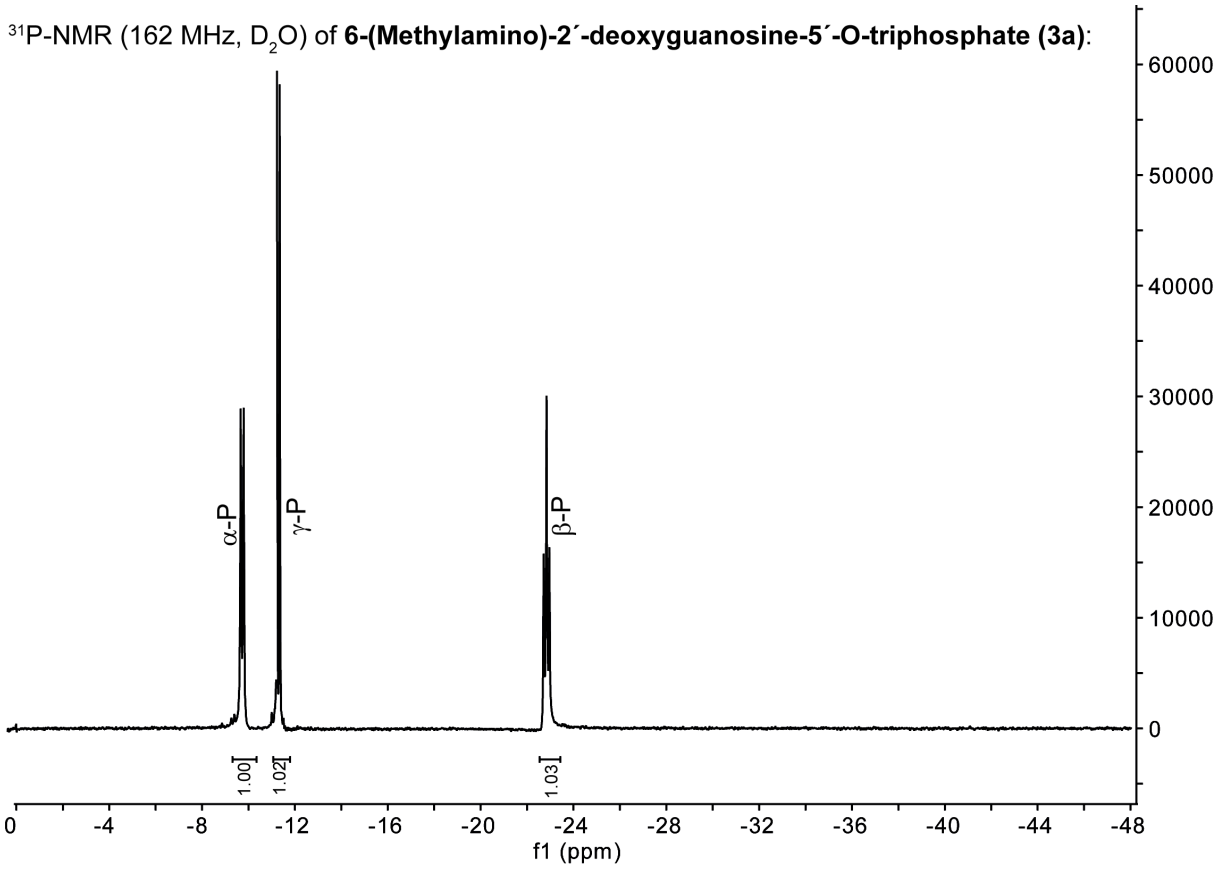
^{1}H-NMR (400 MHz, D_2O) of **6-(Methylamino)-2´-deoxyguanosine-5´-O-triphosphate (3a)**:



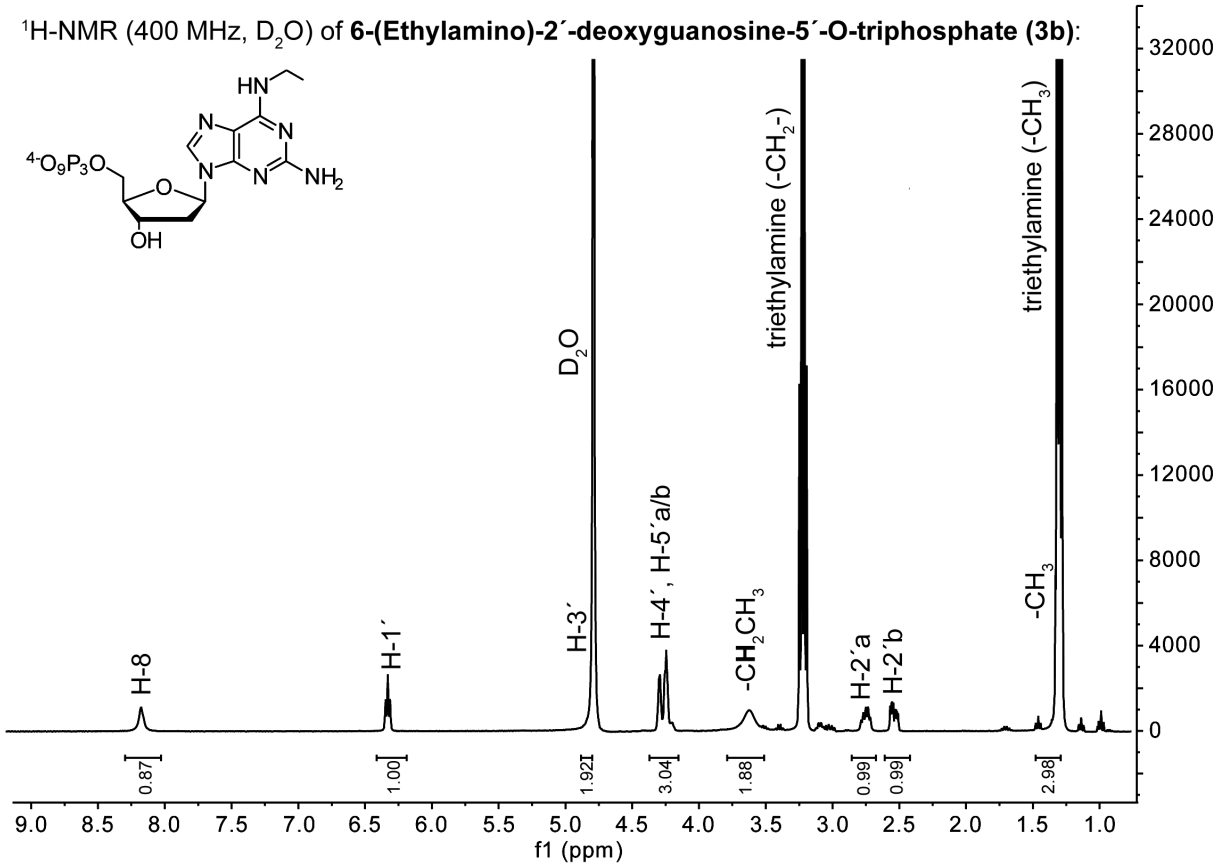
^{13}C-NMR (100 MHz, D_2O) of **6-(Methylamino)-2´-deoxyguanosine-5´-O-triphosphate (3a)**:



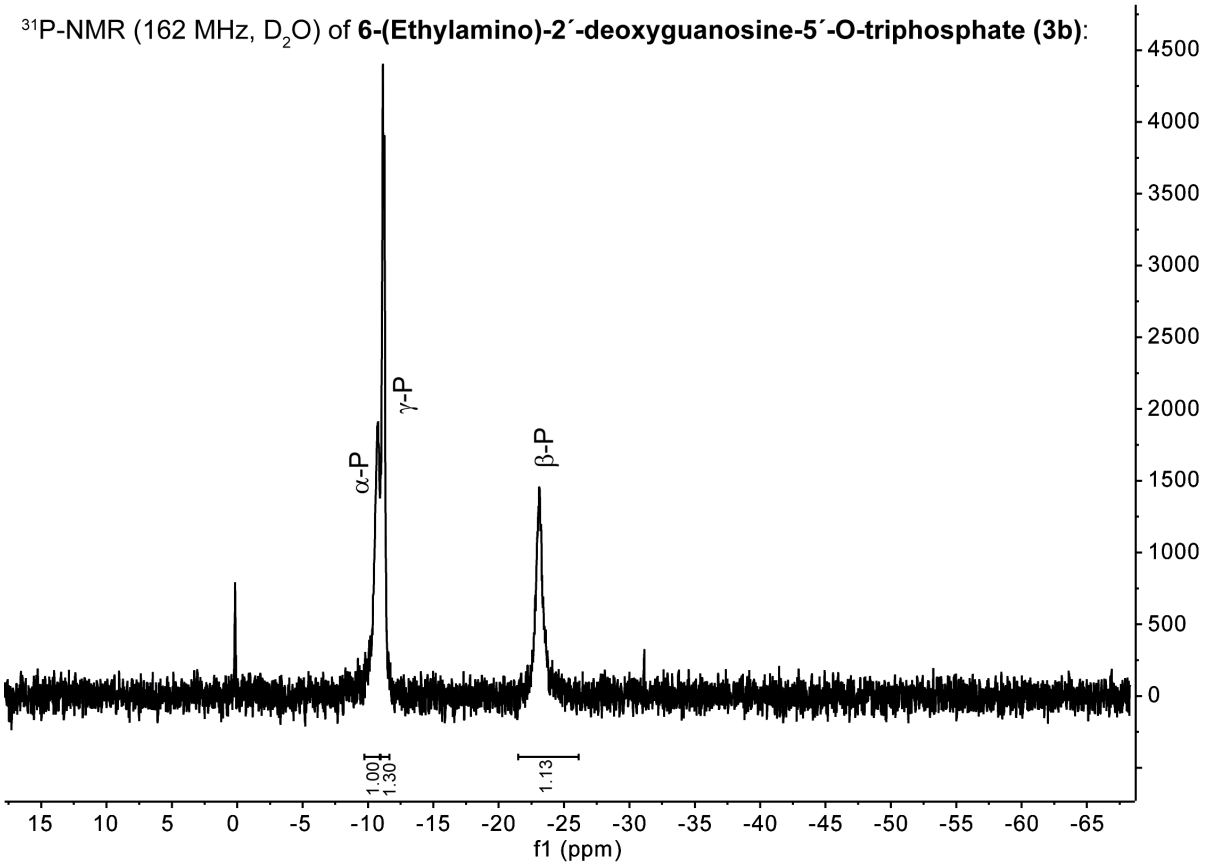
^{31}P-NMR (162 MHz, D_2O) of **6-(Methylamino)-2´-deoxyguanosine-5´-O-triphosphate (3a)**:



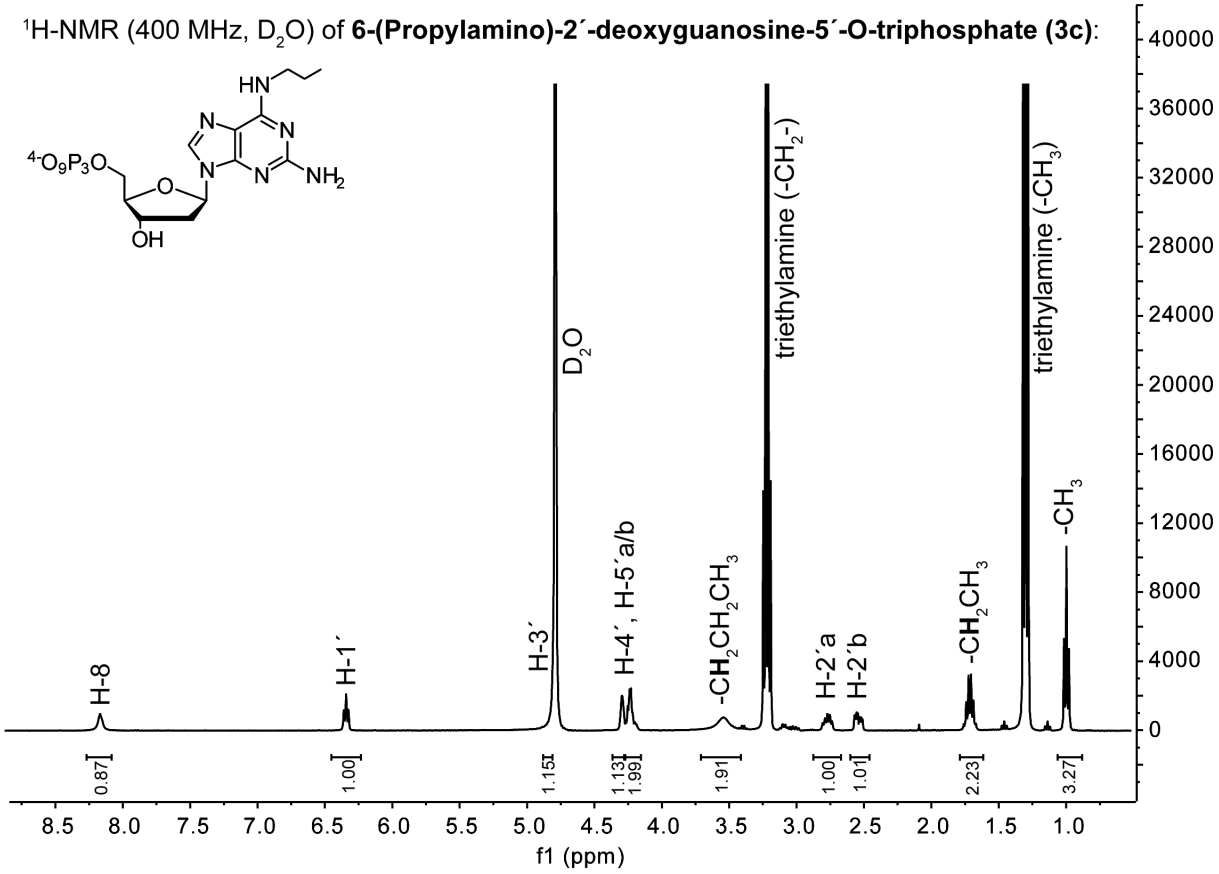
^{1}H-NMR (400 MHz, D_2O) of **6-(Ethylamino)-2´-deoxyguanosine-5´-O-triphosphate (3b)**:



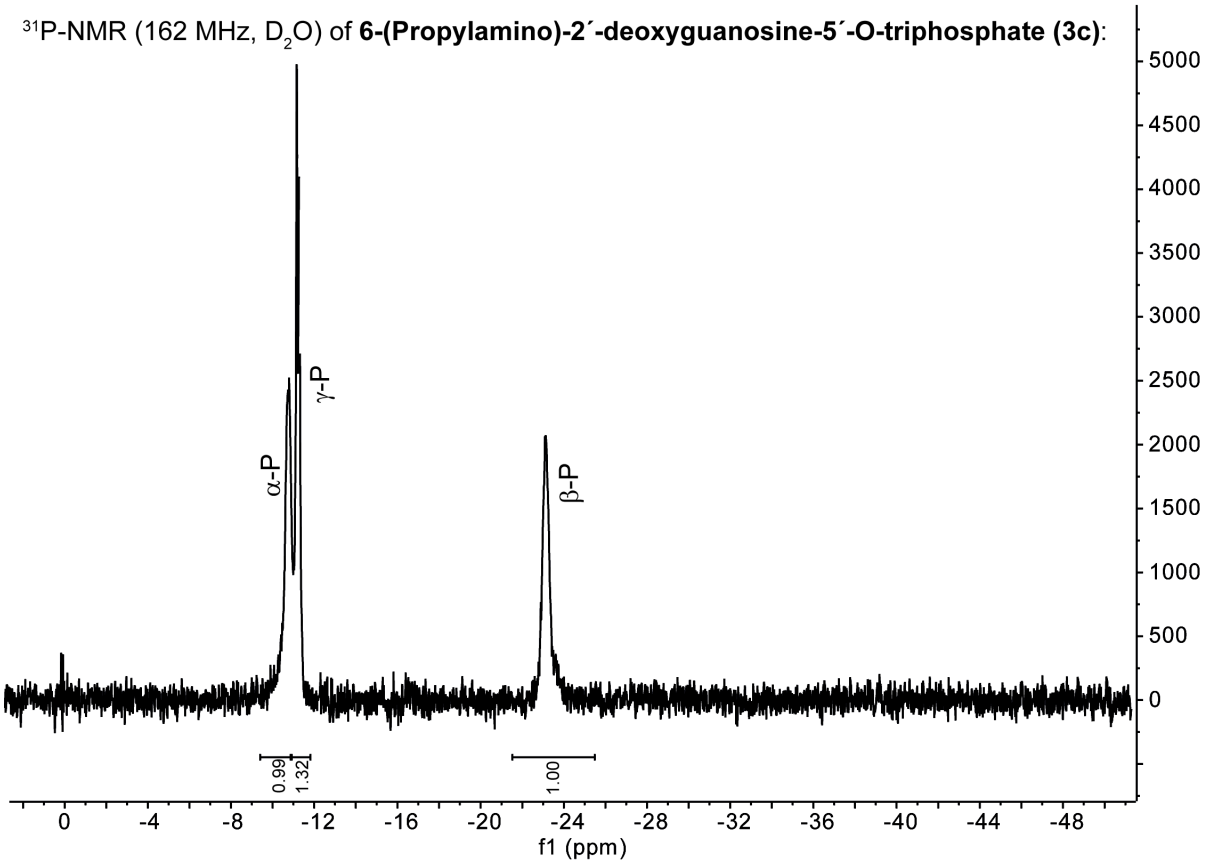
^{31}P-NMR (162 MHz, D_2O) of **6-(Ethylamino)-2´-deoxyguanosine-5´-O-triphosphate (3b)**:



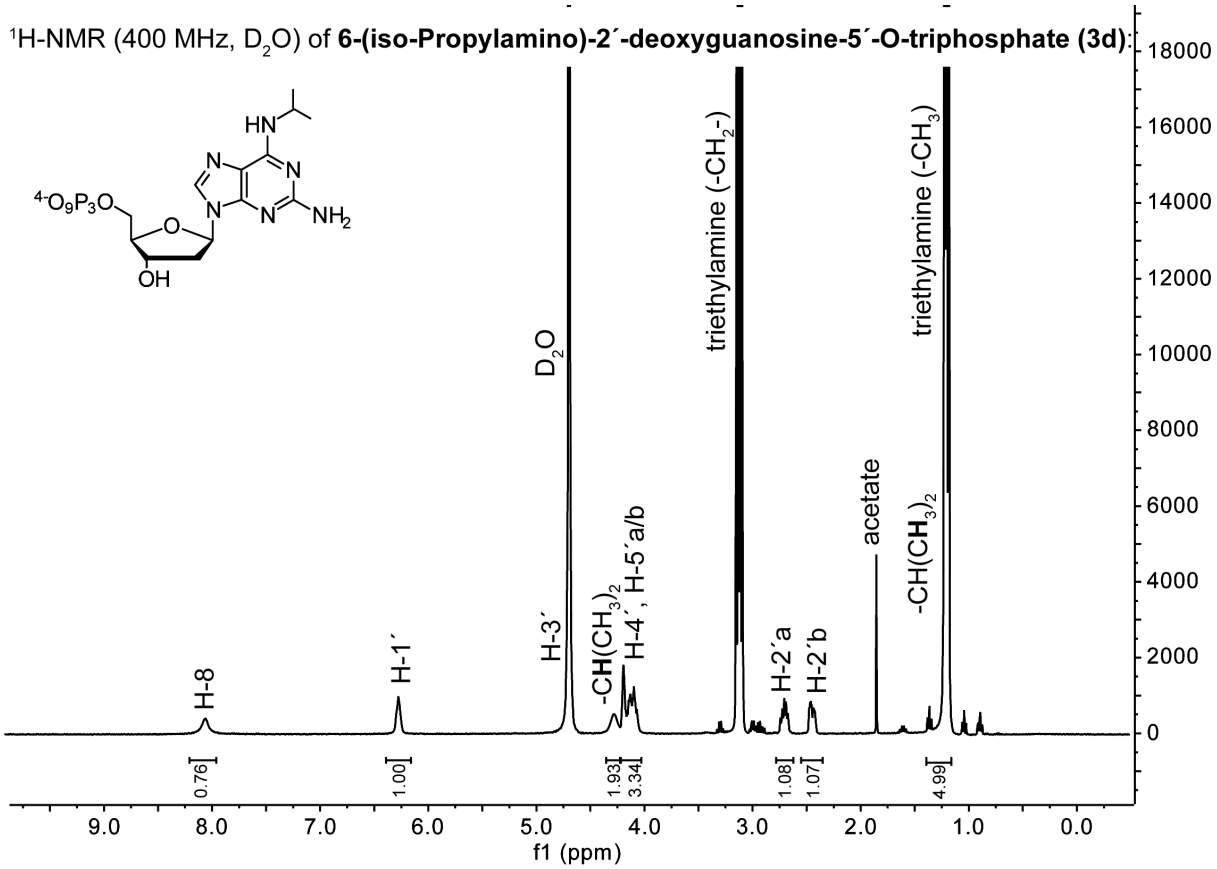
^{1}H-NMR (400 MHz, D_2O) of **6-(Propylamino)-2´-deoxyguanosine-5´-O-triphosphate (3c)**:



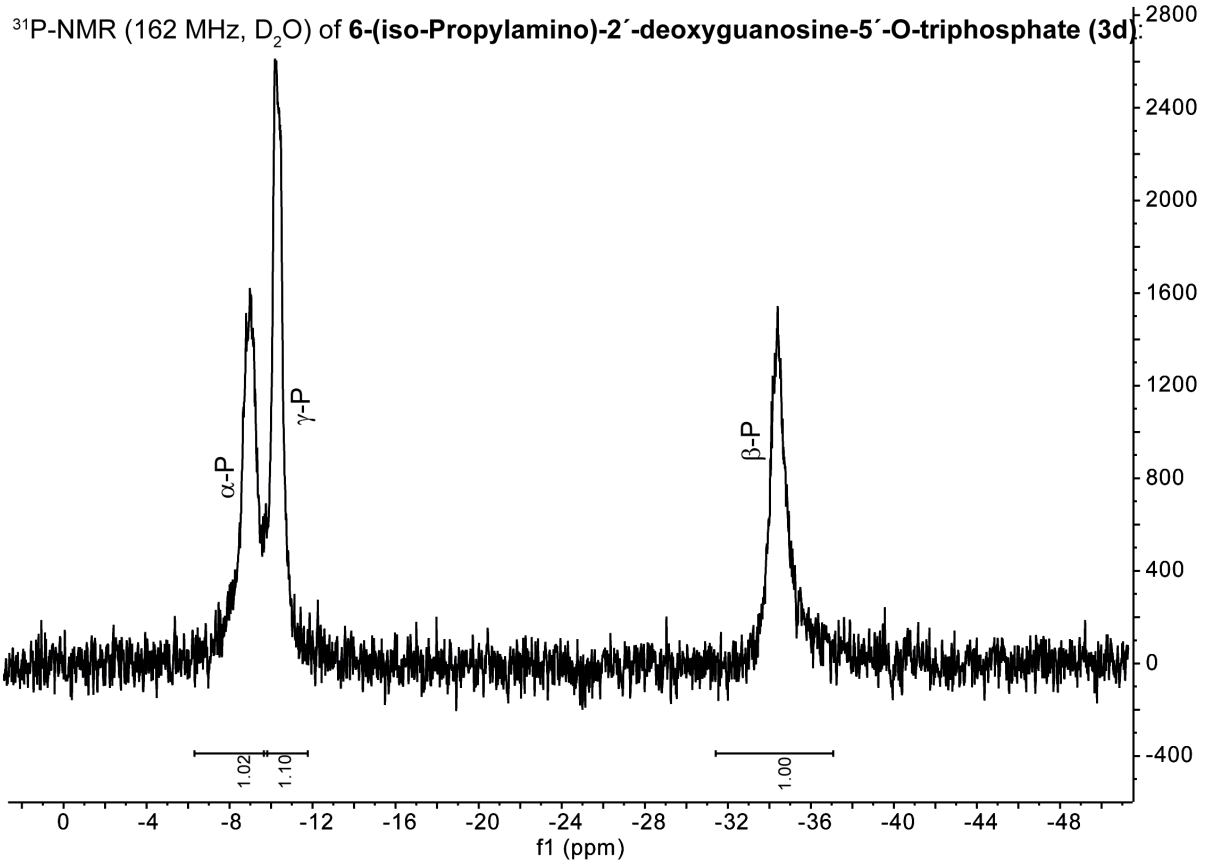
^{31}P-NMR (162 MHz, D_2O) of **6-(Propylamino)-2´-deoxyguanosine-5´-O-triphosphate (3c)**:



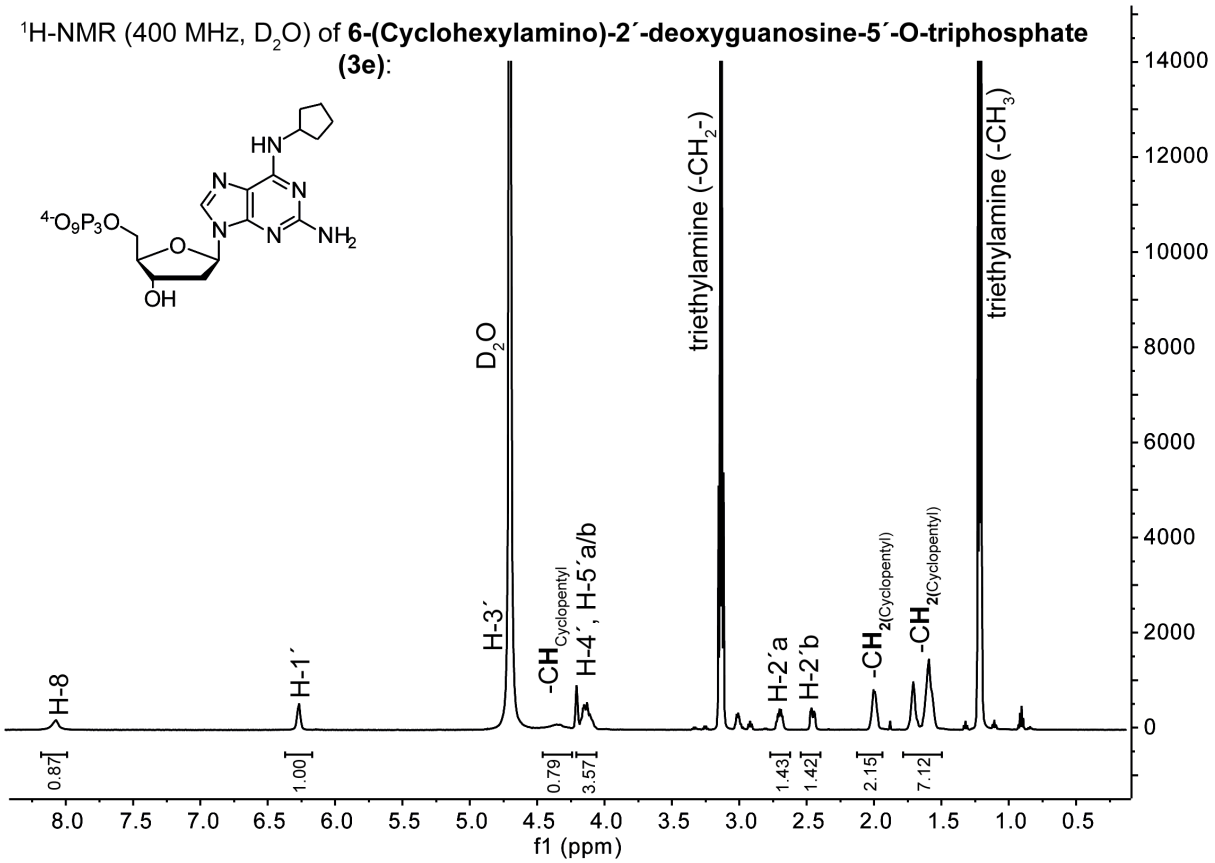
^{1}H-NMR (400 MHz, D_2O) of **6-(iso-Propylamino)-2´-deoxyguanosine-5´-O-triphosphate (3d)**:



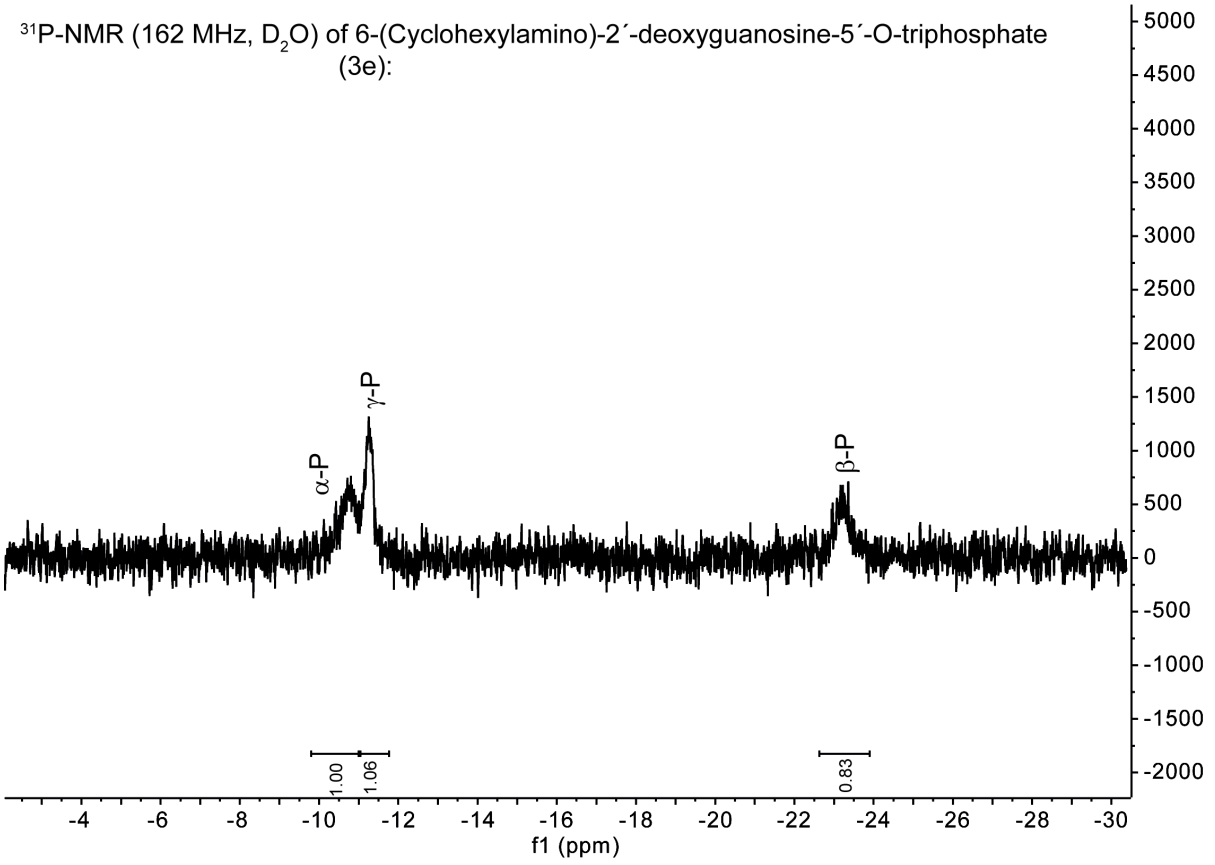
^{31}P-NMR (162 MHz, D_2O) of **6-(iso-Propylamino)-2´-deoxyguanosine-5´-O-triphosphate (3d)**:



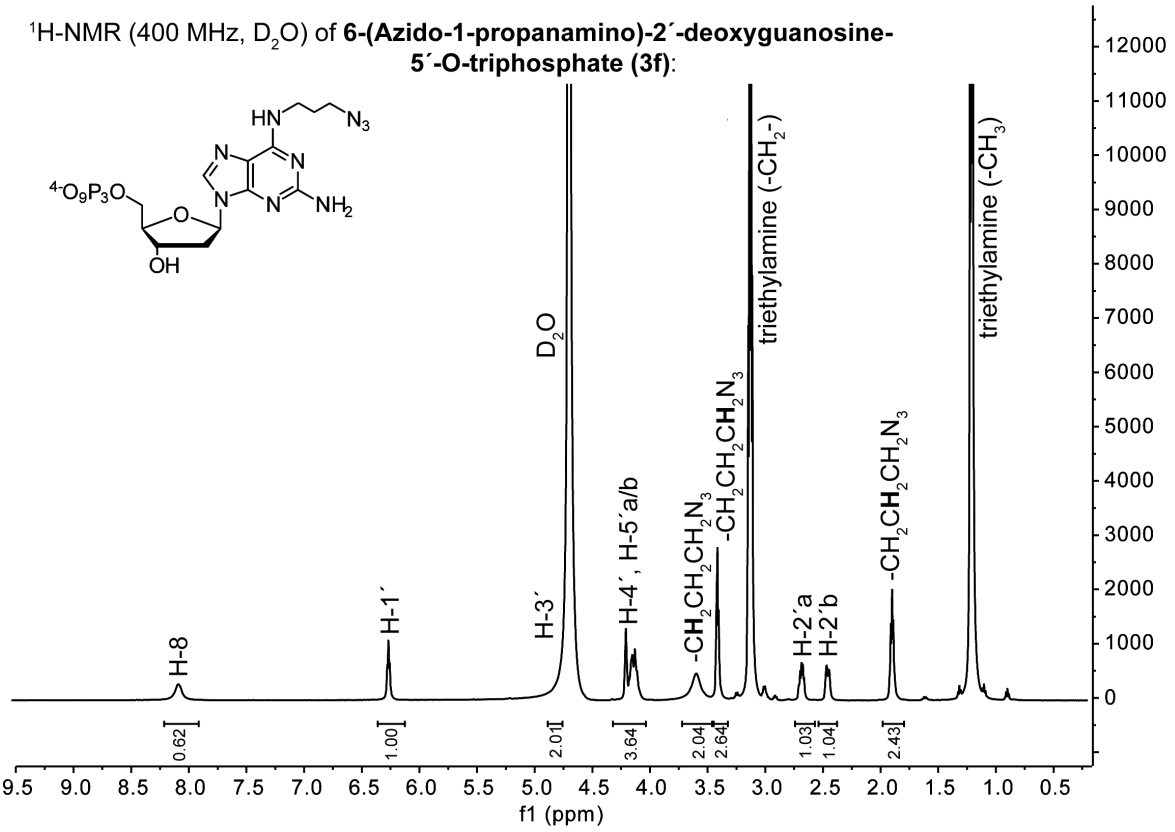
^{1}H-NMR (400 MHz, D_2O) of **6-(Cyclohexylamino)-2´-deoxyguanosine-5´-O-triphosphate (3e)**:



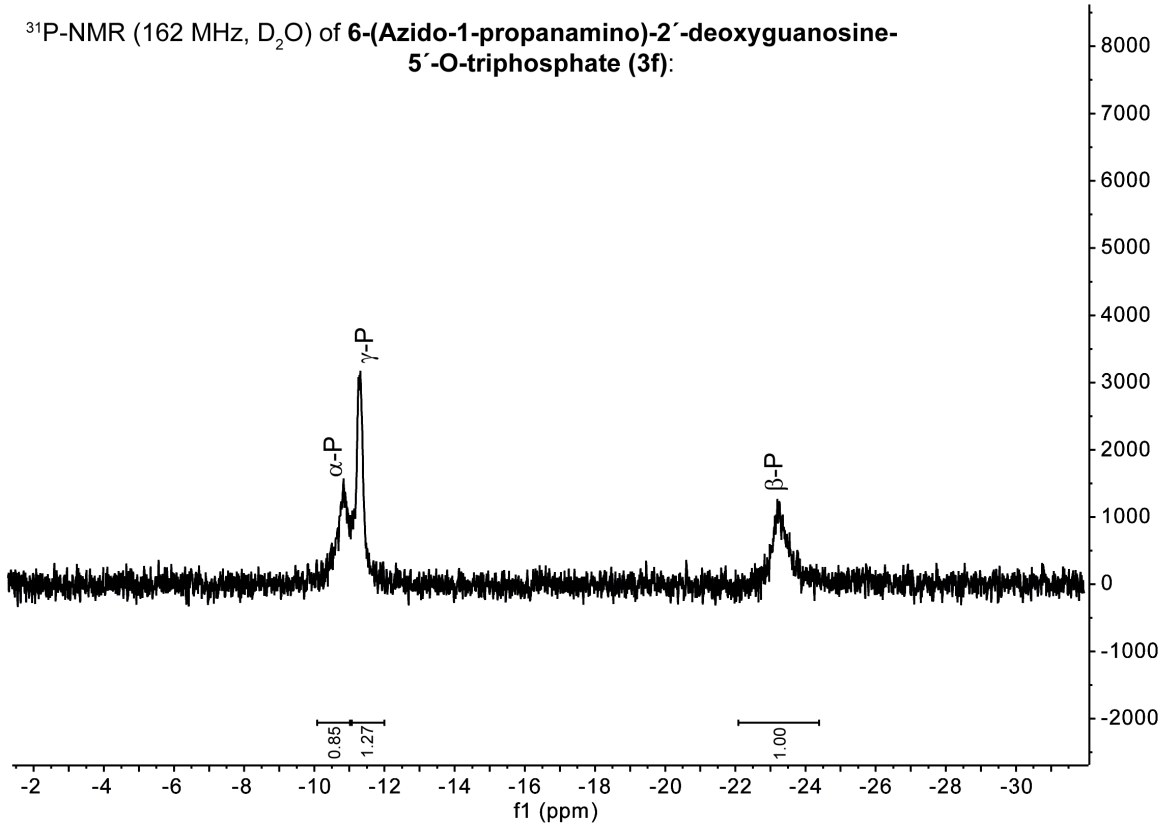
^{31}P-NMR (162 MHz, D_2O) of 6-(Cyclohexylamino)-2´-deoxyguanosine-5´-O-triphosphate (3e):



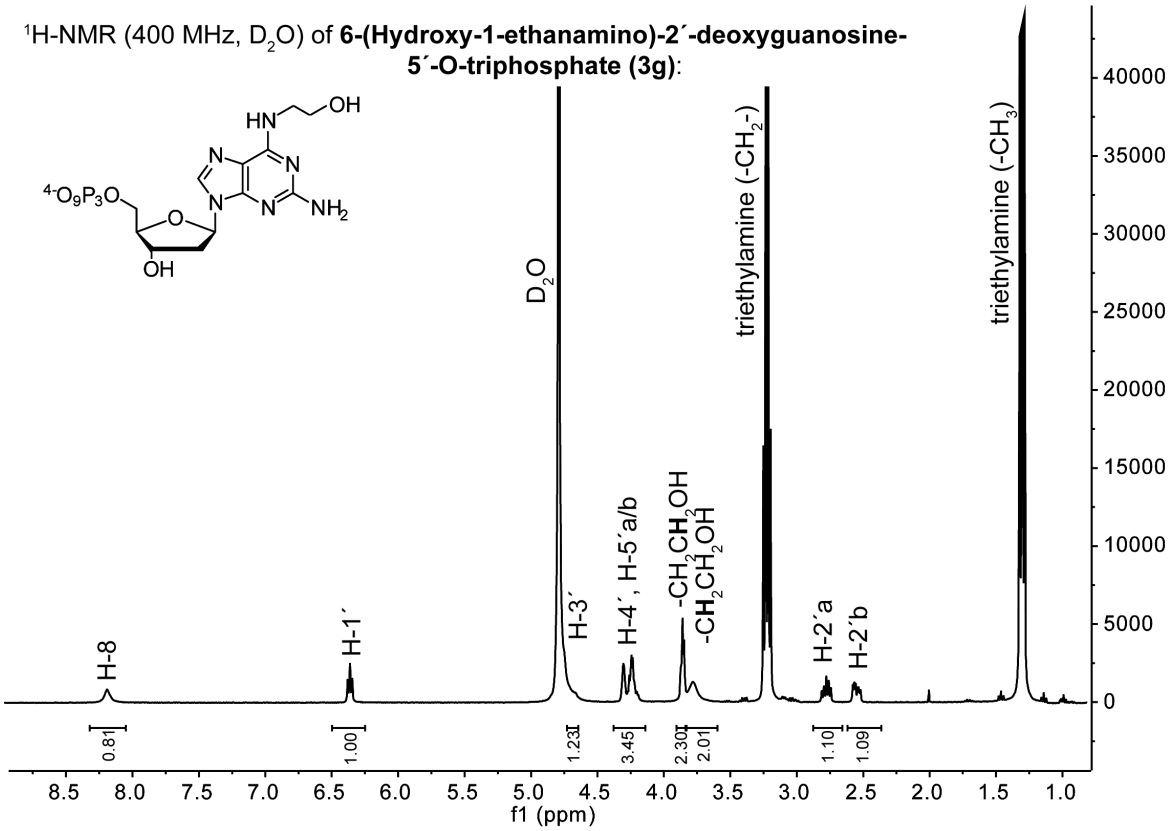
^{1}H-NMR (400 MHz, D_2O) of **6-(Azido-1-propanamino)-2´-deoxyguanosine-5´-O-triphosphate (3f)**:



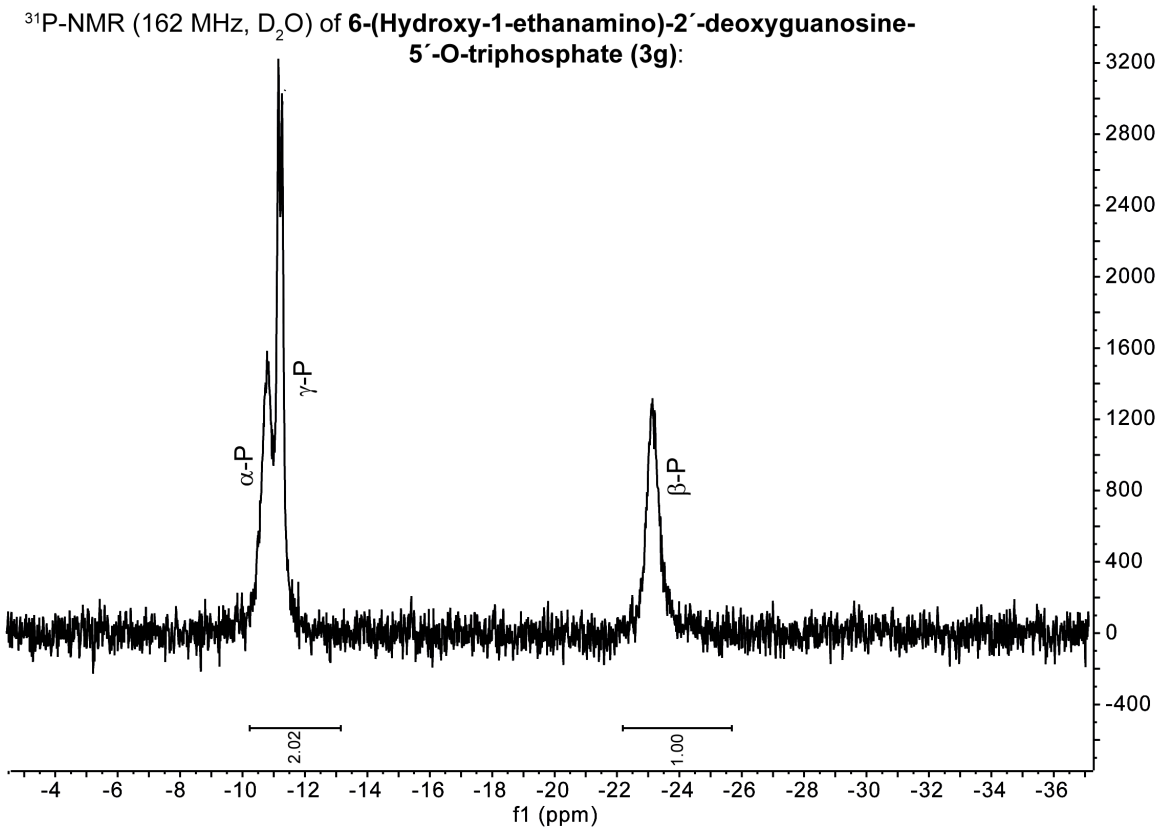
^{31}P-NMR (162 MHz, D_2O) of **6-(Azido-1-propanamino)-2´-deoxyguanosine-5´-O-triphosphate (3f)**:



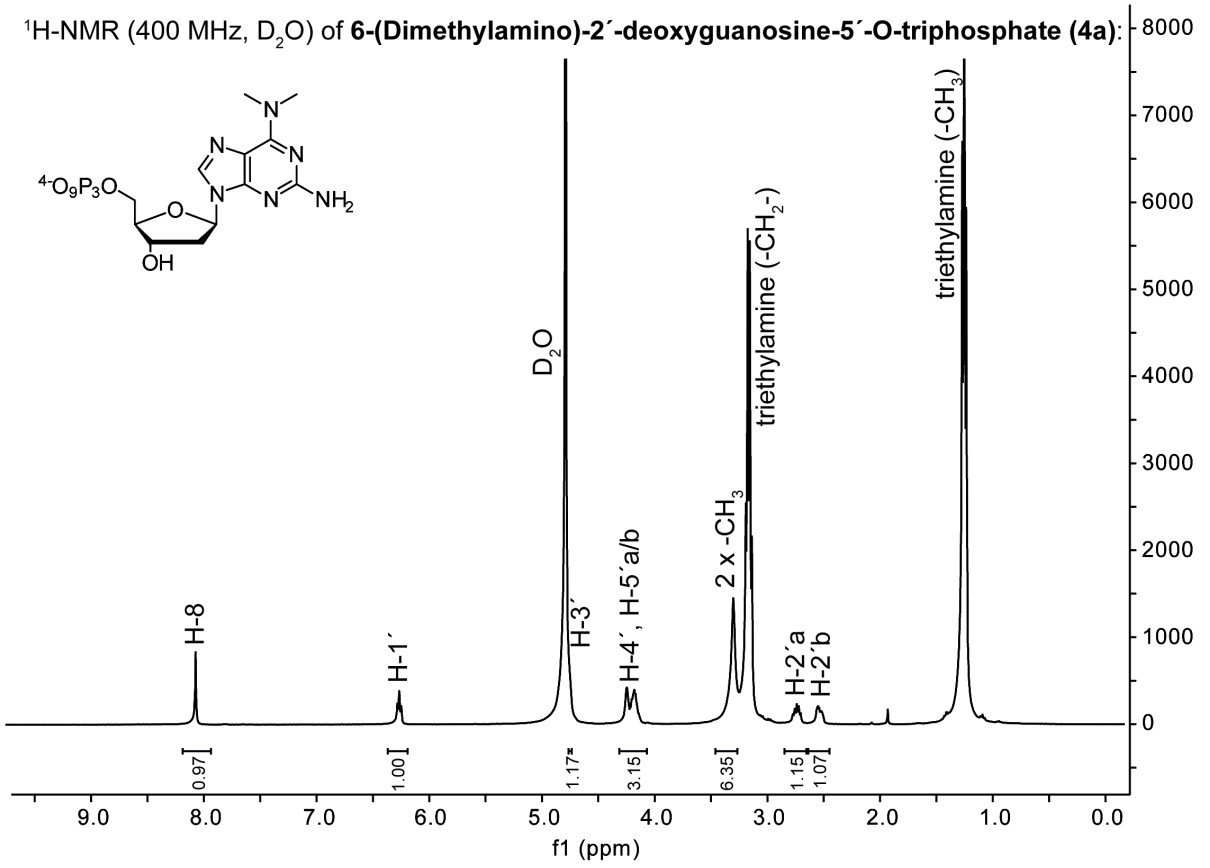
^{1}H-NMR (400 MHz, D_2O) of **6-(Hydroxy-1-ethanamino)-2´-deoxyguanosine-5´-O-triphosphate (3g)**:



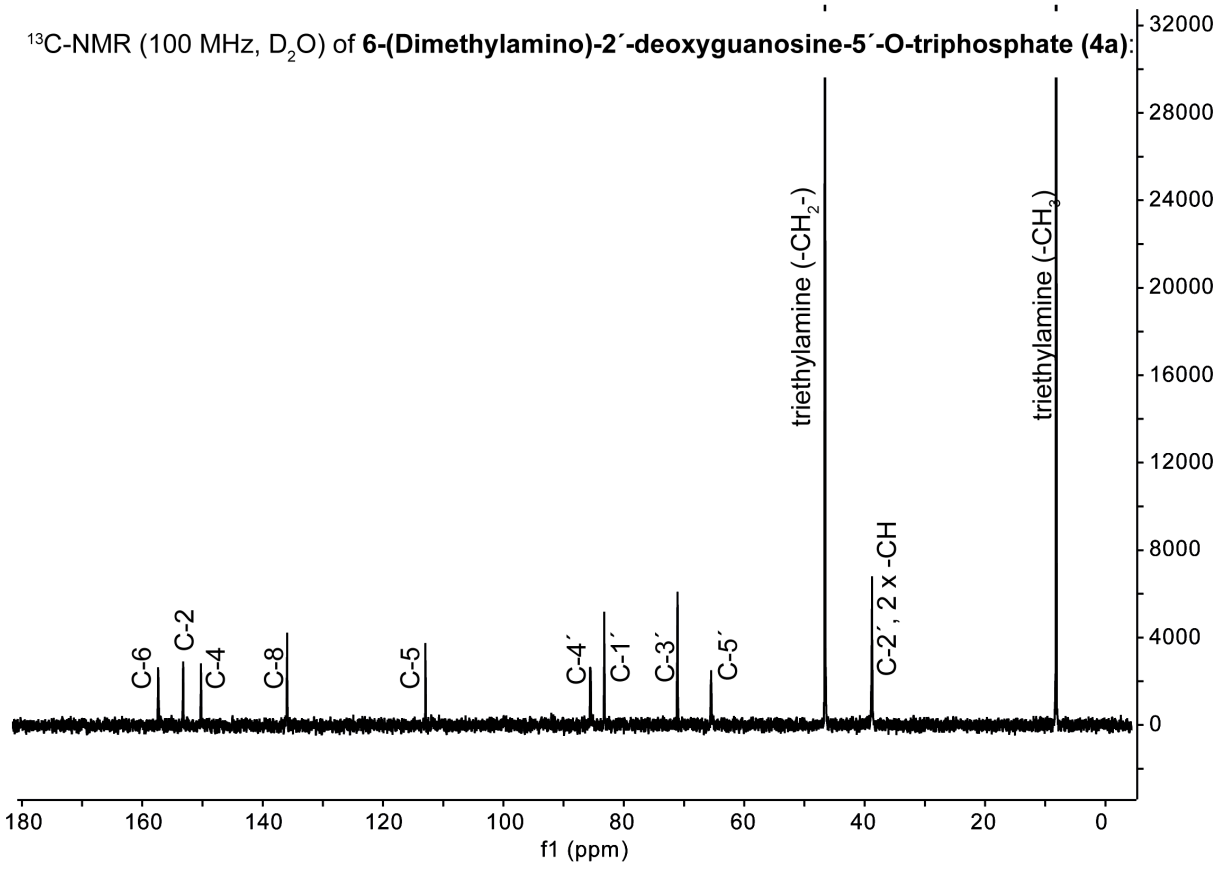
^{31}P-NMR (162 MHz, D_2O) of **6-(Hydroxy-1-ethanamino)-2´-deoxyguanosine-5´-O-triphosphate (3g)**:



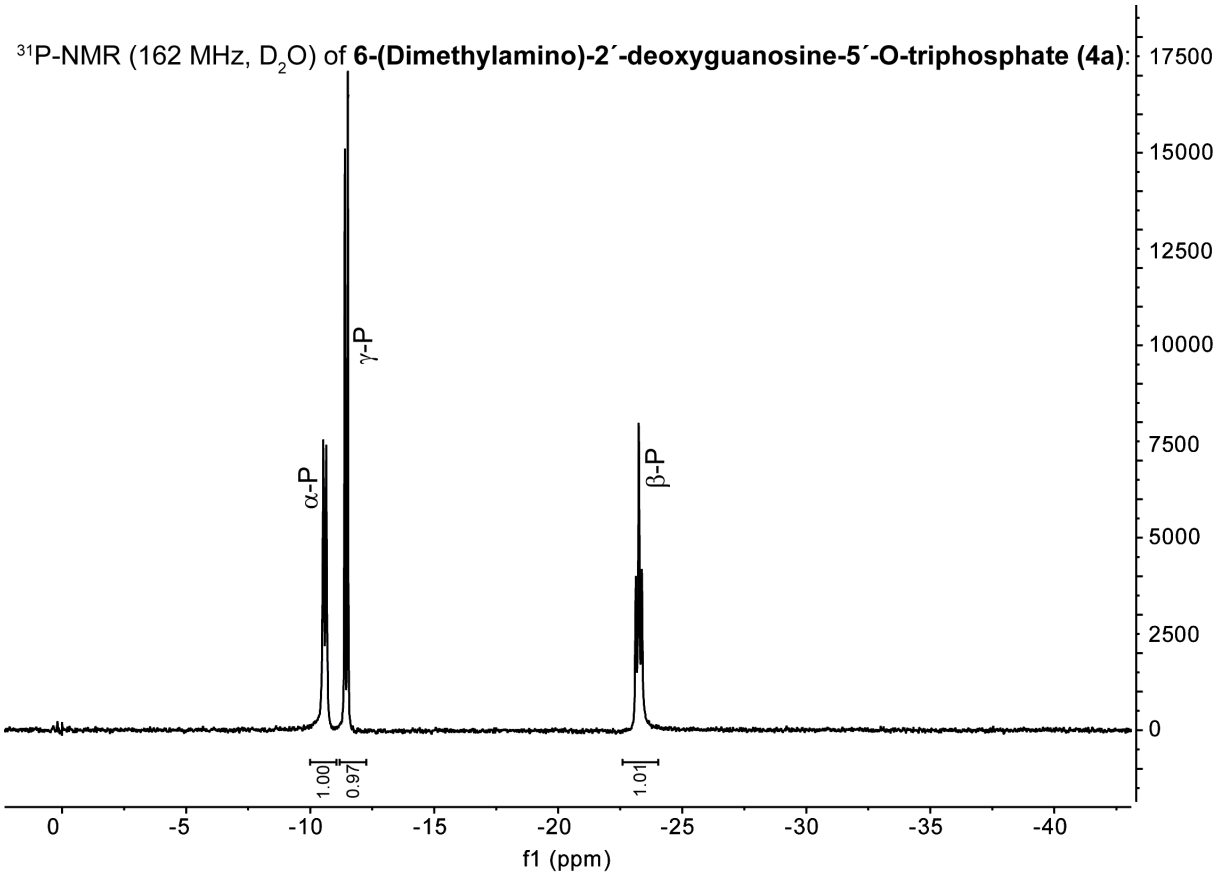
^{1}H-NMR (400 MHz, D_2O) of **6-(Dimethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4a)**:



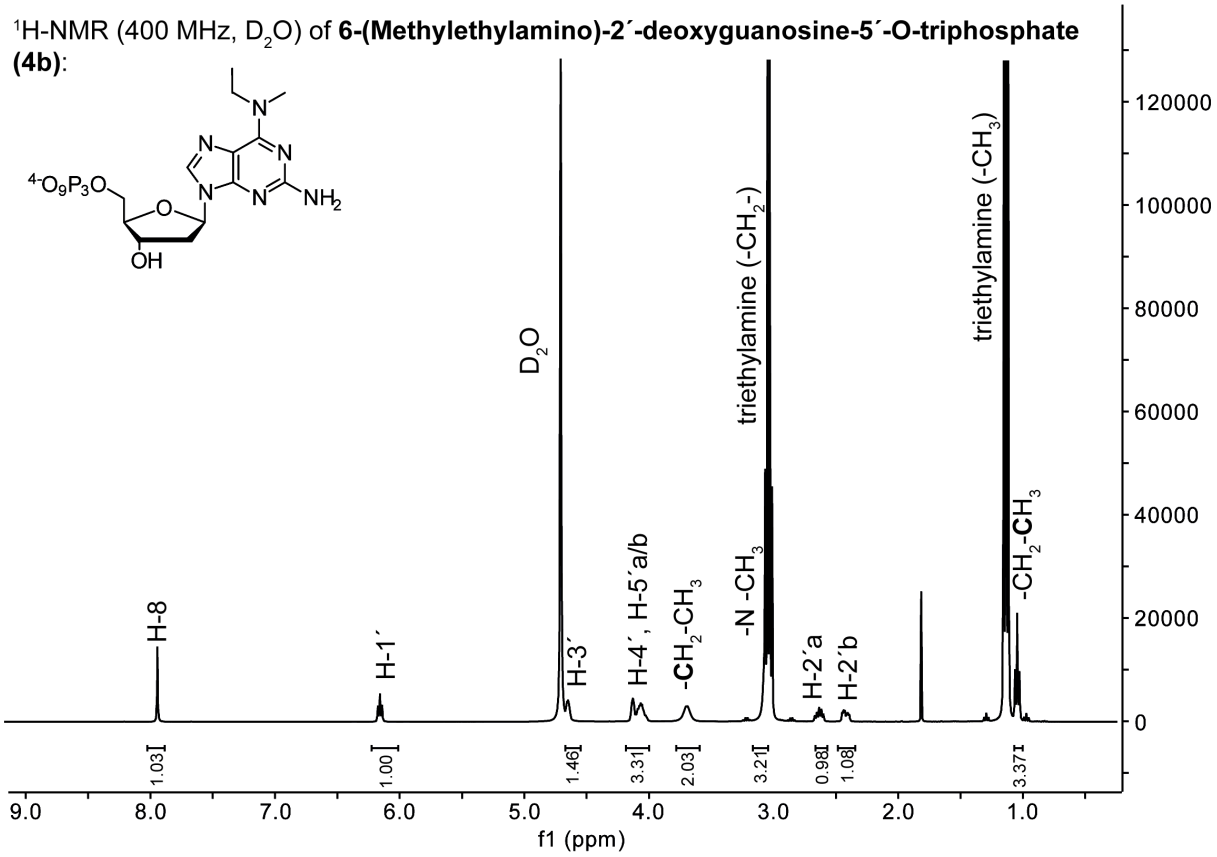
^{13}C-NMR (100 MHz, D_2O) of **6-(Dimethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4a)**:



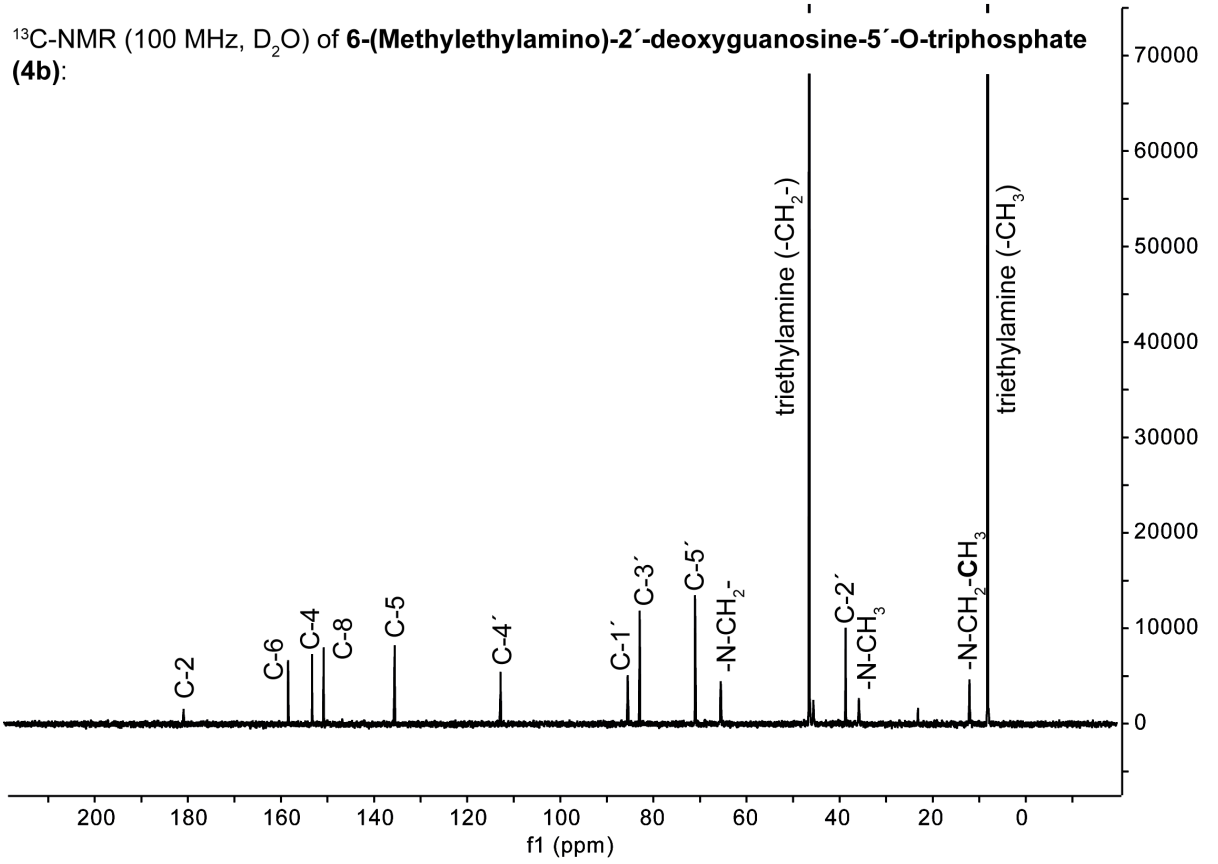
^{31}P-NMR (162 MHz, D_2O) of **6-(Dimethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4a)**:



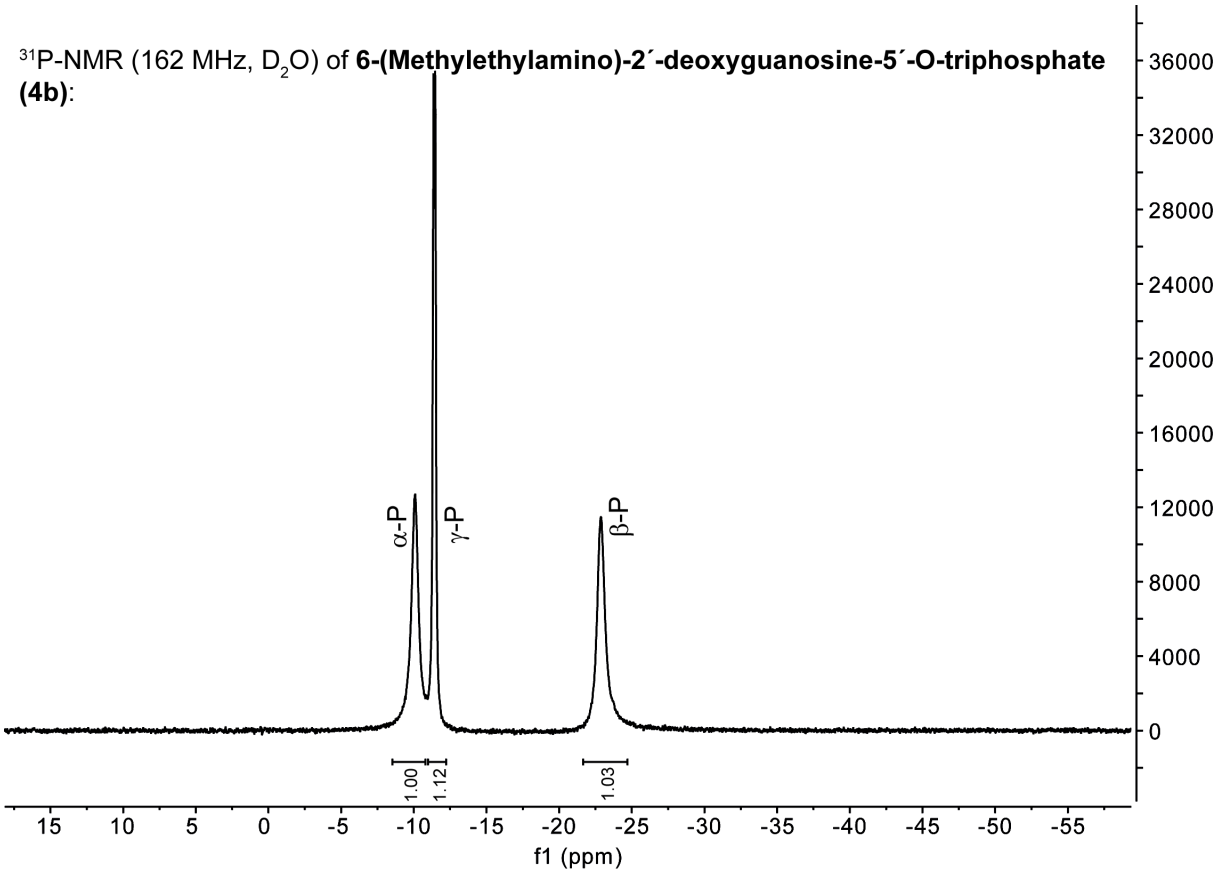
^{1}H-NMR (400 MHz, D_2O) of **6-(Methylethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4b)**:



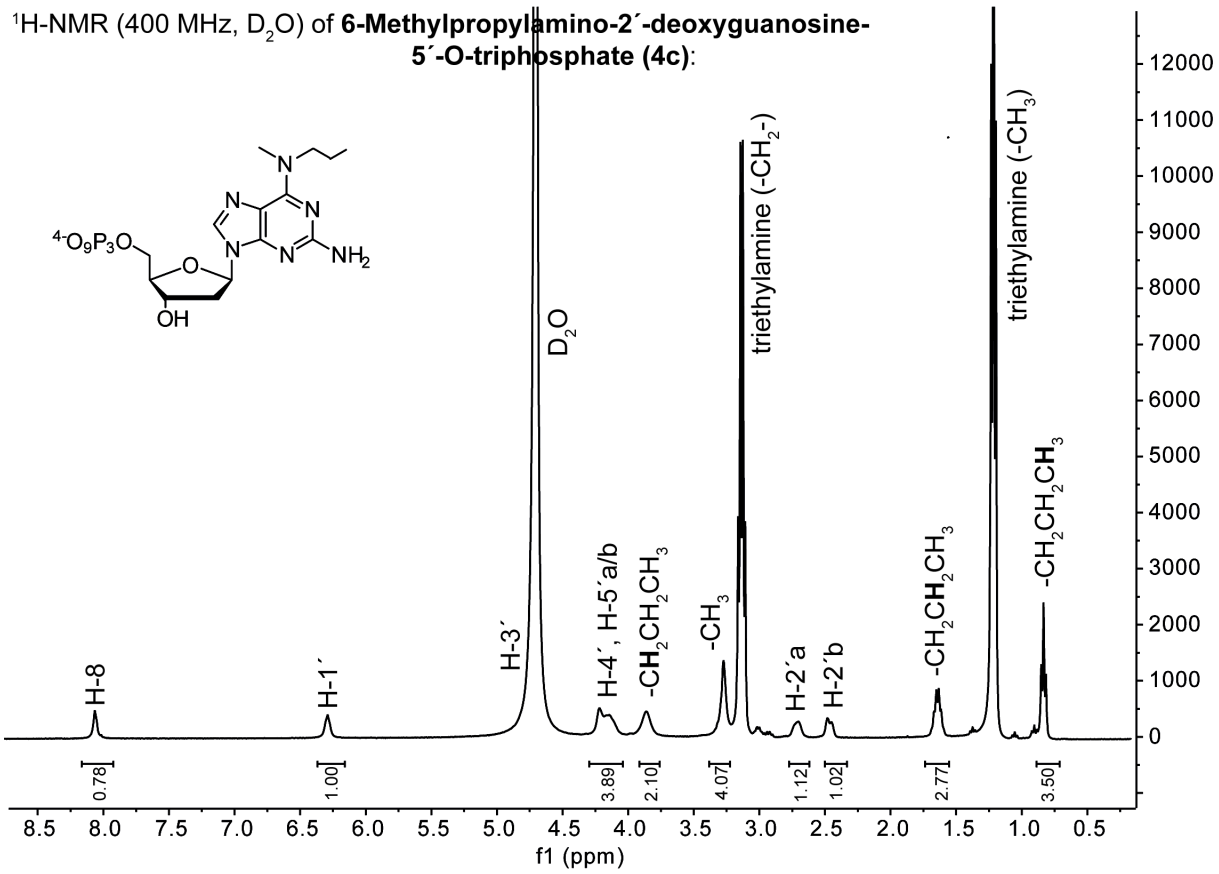
^{13}C-NMR (100 MHz, D_2O) of **6-(Methylethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4b)**:



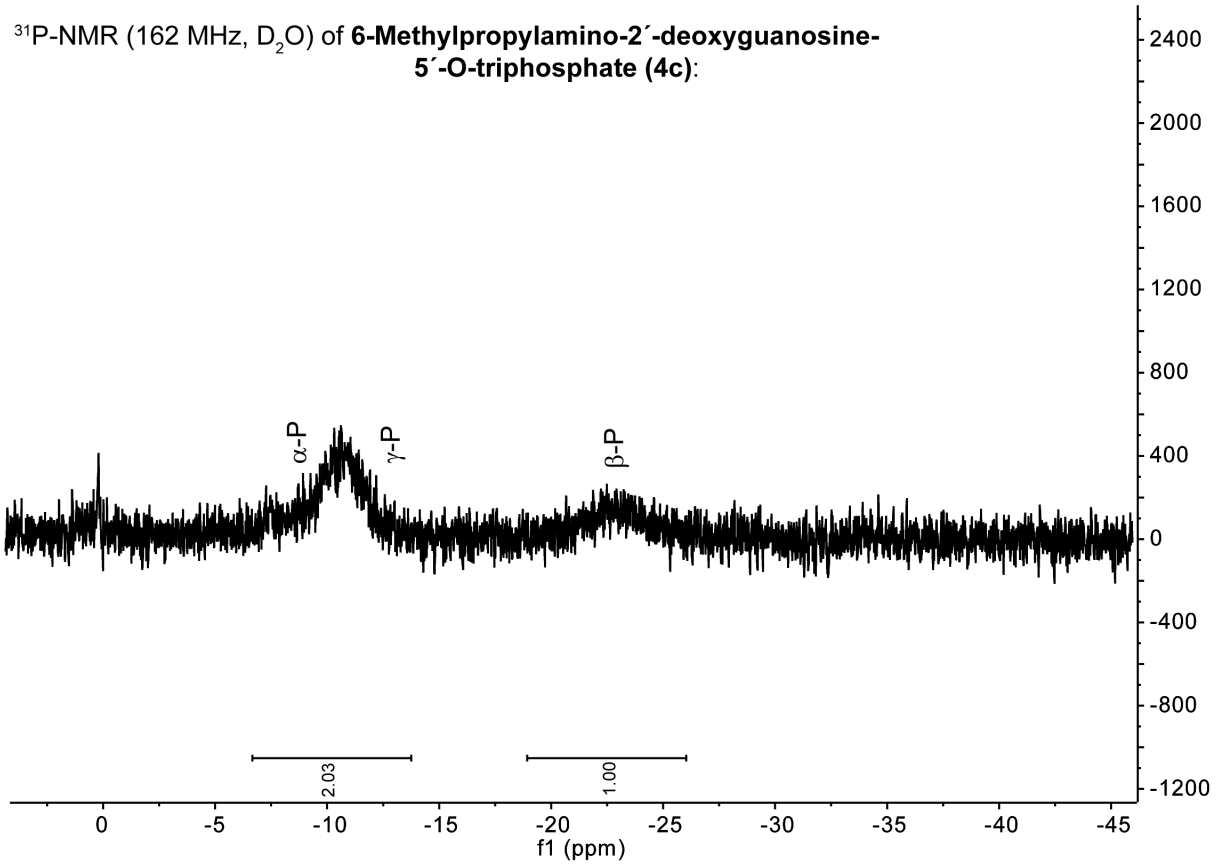
^{31}P-NMR (162 MHz, D_2O) of **6-(Methylethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4b)**:



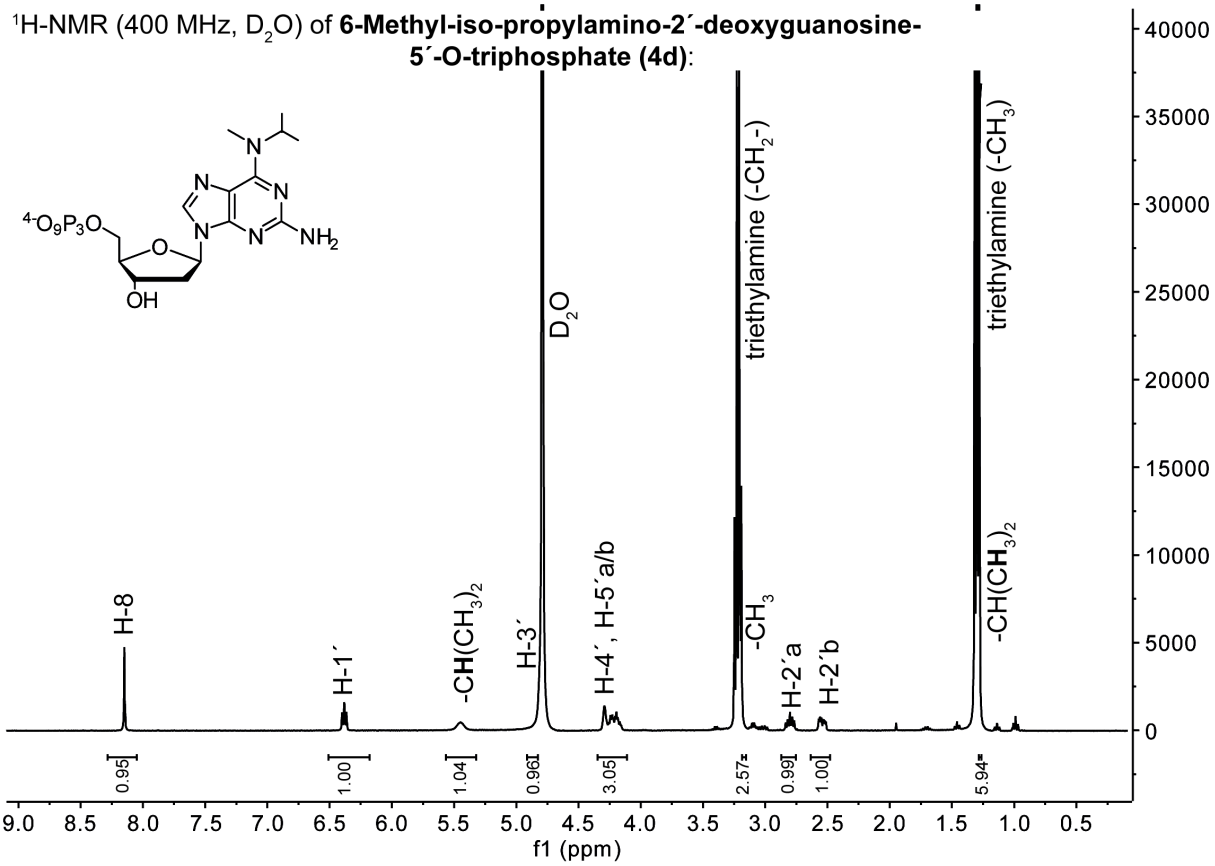
^{1}H-NMR (400 MHz, D_2O) of **6-Methylpropylamino-2´-deoxyguanosine-5´-O-triphosphate (4c)**:



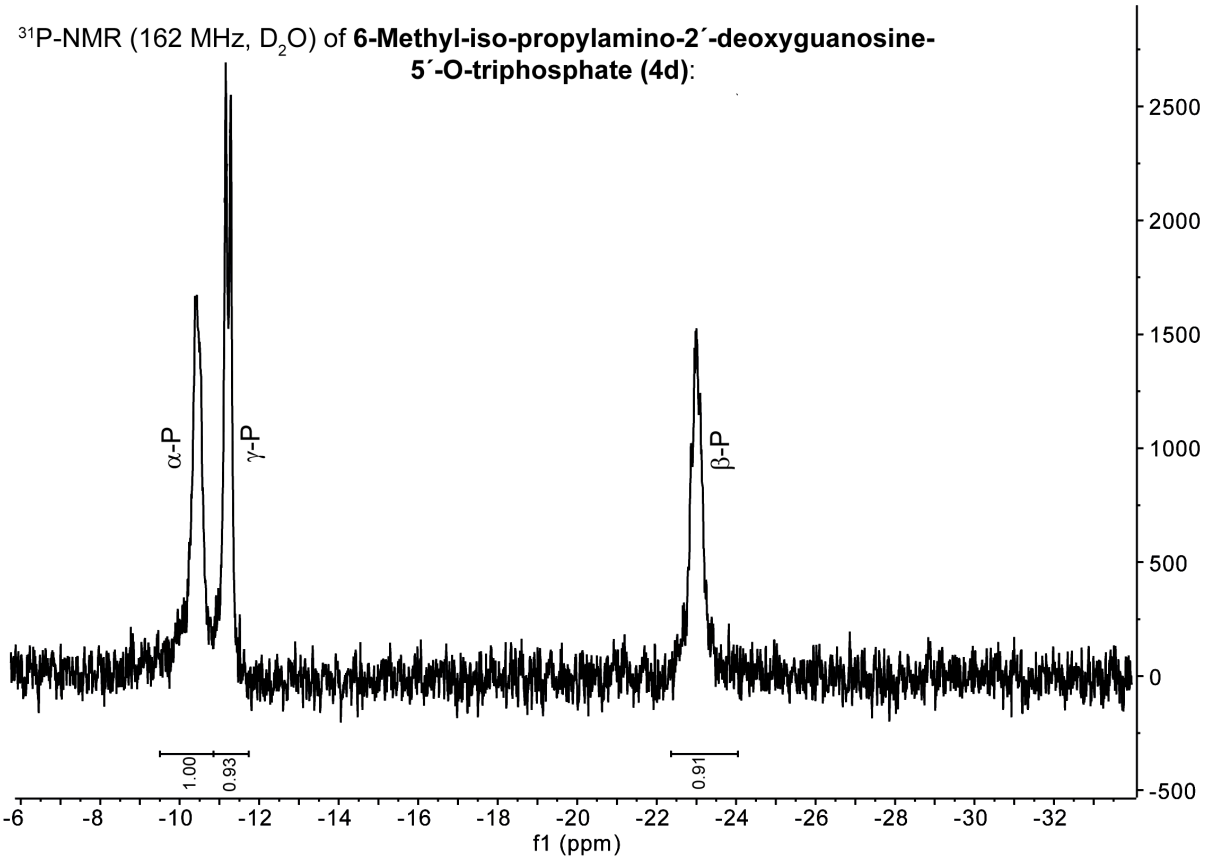
^{31}P-NMR (162 MHz, D_2O) of **6-Methylpropylamino-2´-deoxyguanosine-5´-O-triphosphate (4c)**:



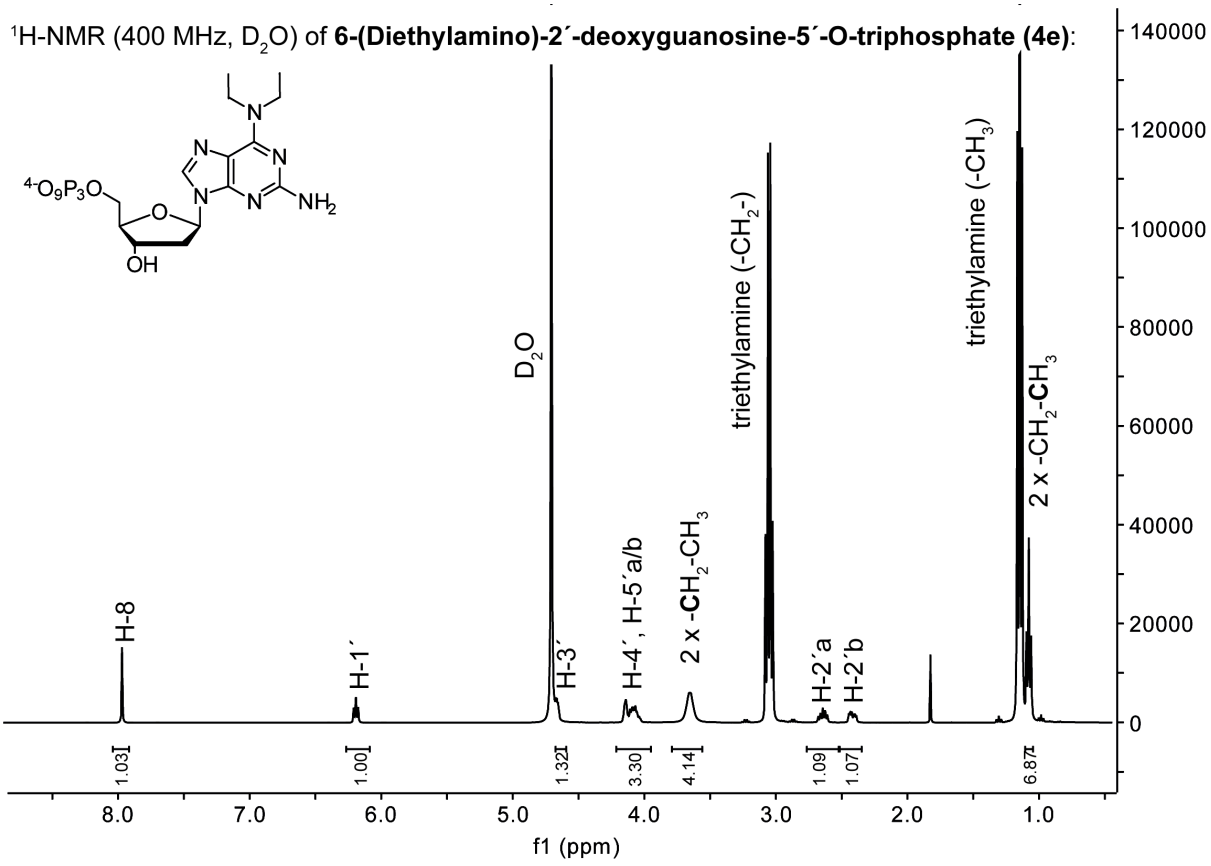
^{1}H-NMR (400 MHz, D_2O) of **6-Methyl-iso-propylamino-2´-deoxyguanosine-5´-O-triphosphate (4d)**:



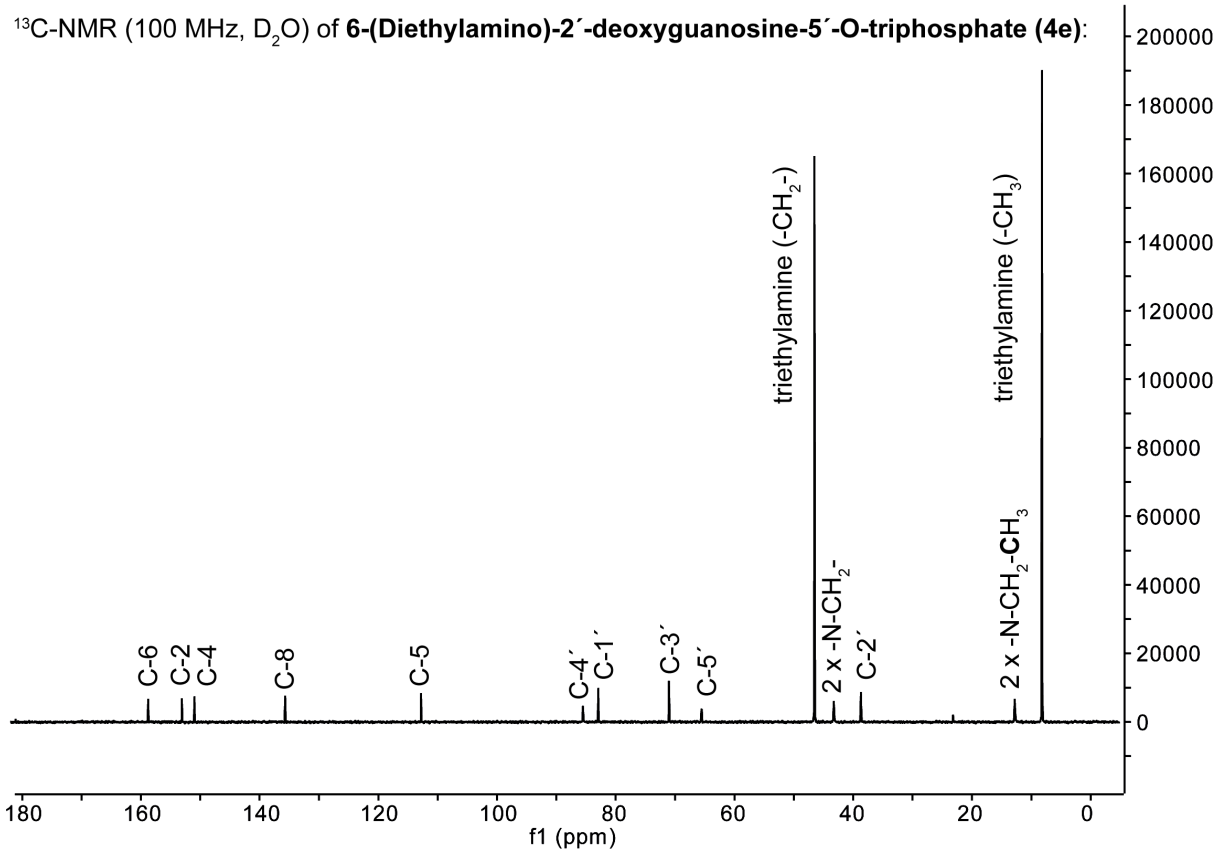
^{31}P-NMR (162 MHz, D_2O) of **6-Methyl-iso-propylamino-2´-deoxyguanosine-5´-O-triphosphate (4d)**:



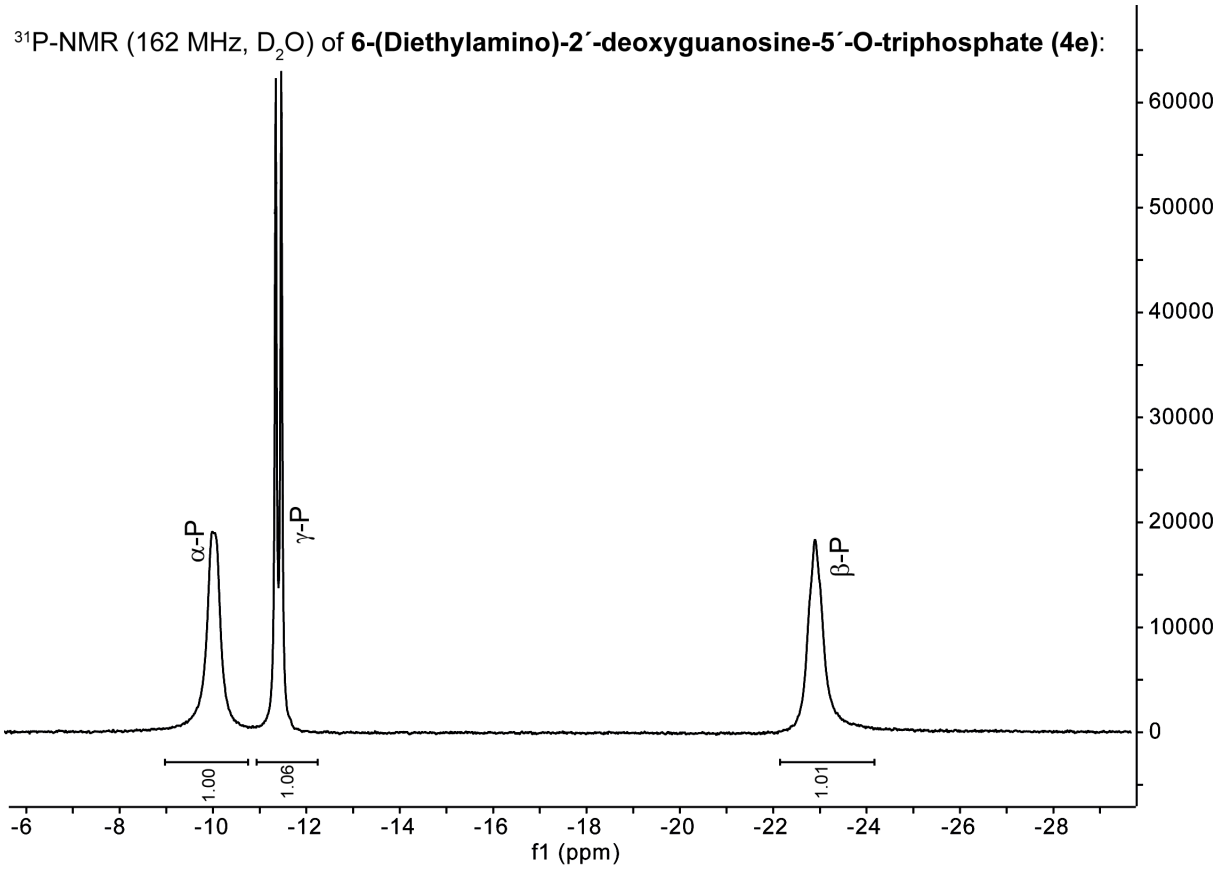
^{1}H-NMR (400 MHz, D_2O) of **6-(Diethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4e)**:



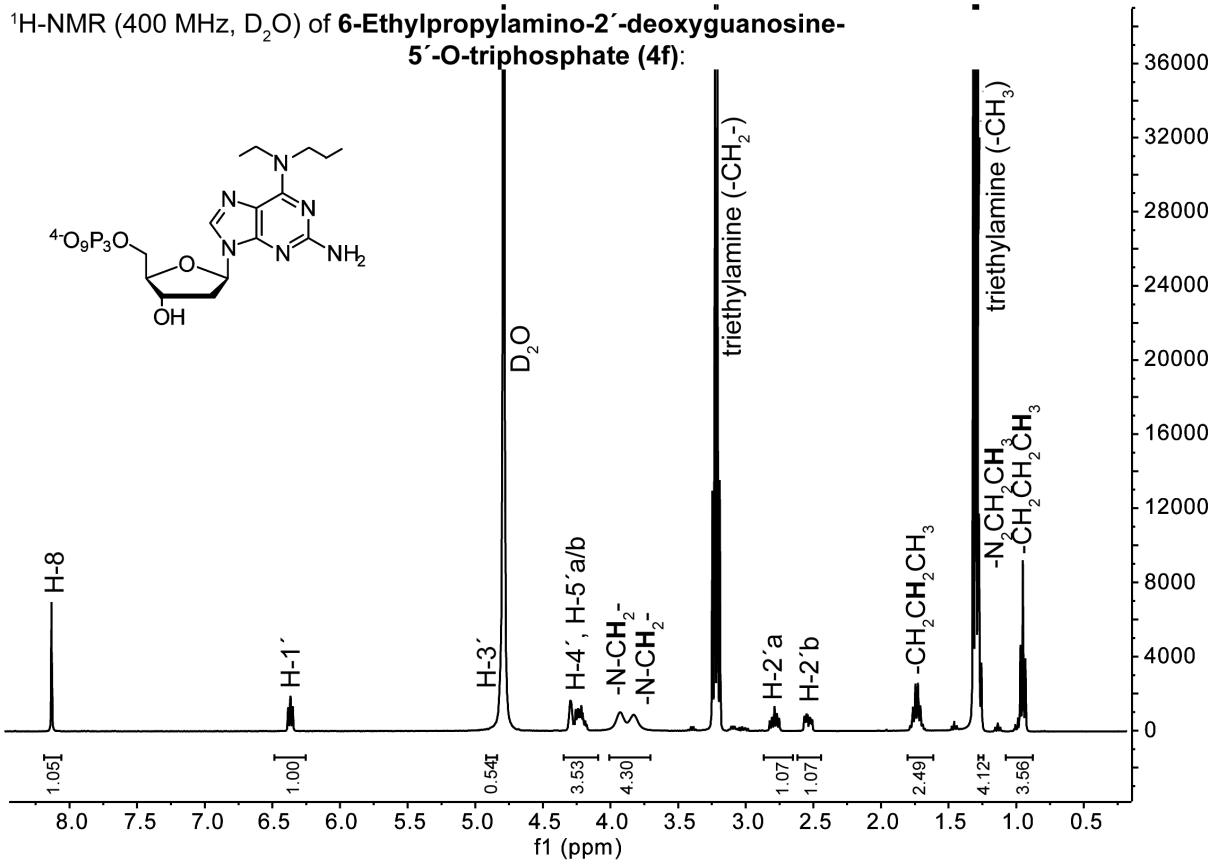
^{13}C-NMR (100 MHz, D_2O) of **6-(Diethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4e)**:



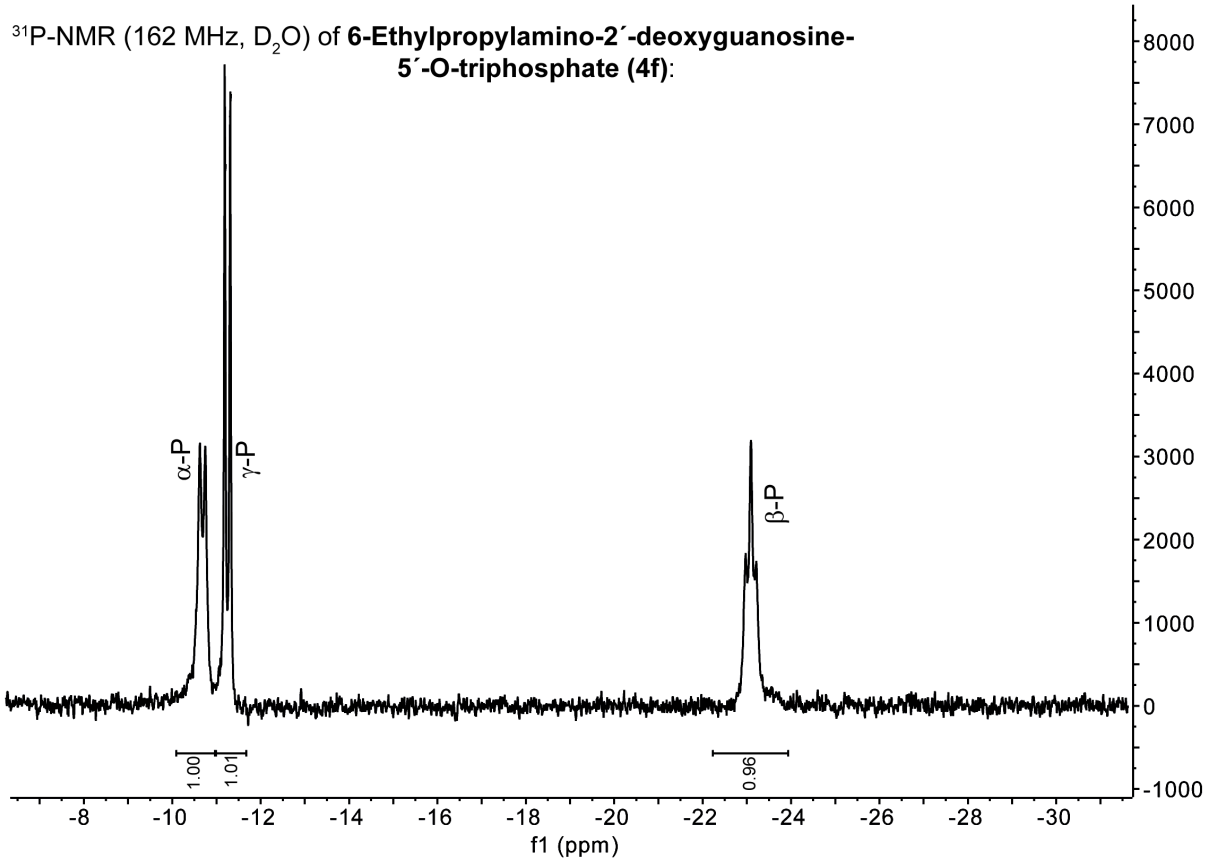
^{31}P-NMR (162 MHz, D_2O) of **6-(Diethylamino)-2´-deoxyguanosine-5´-O-triphosphate (4e)**:



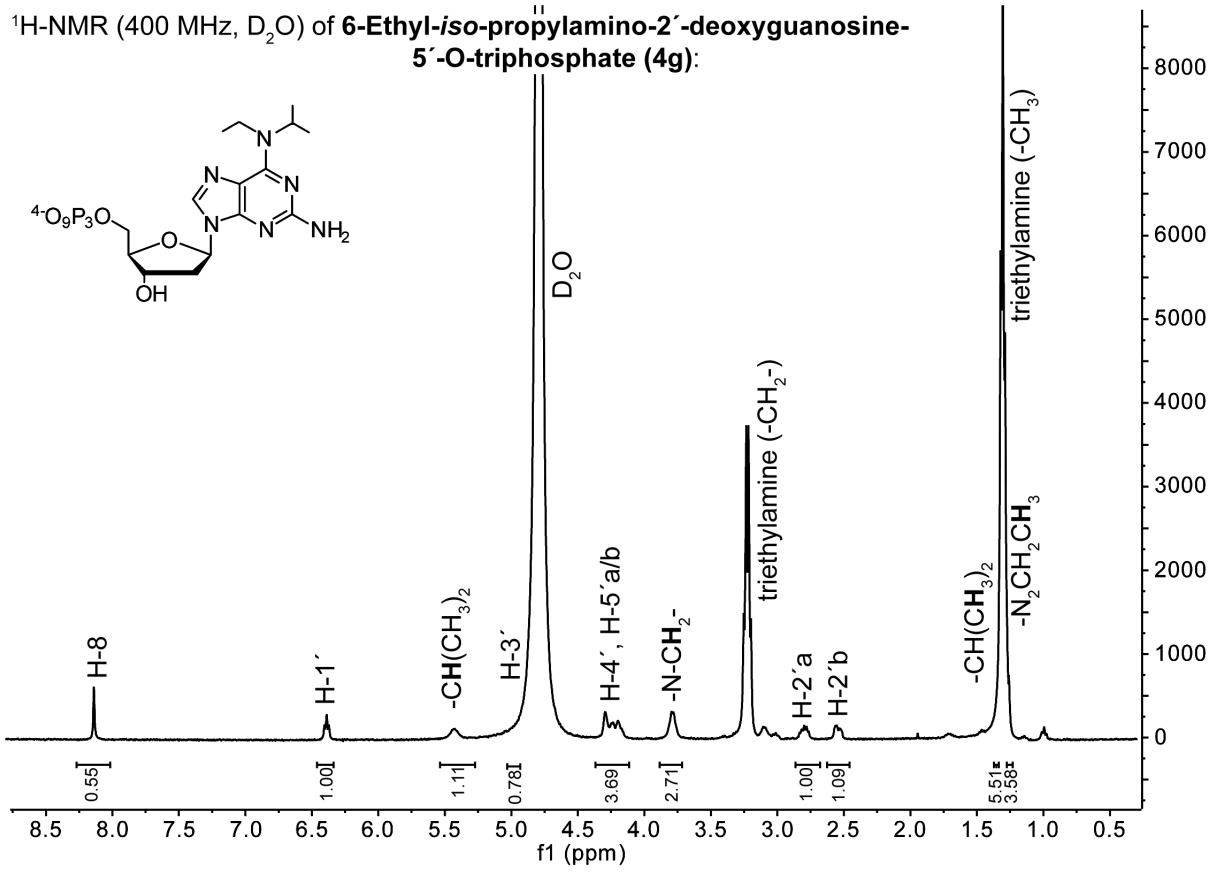
^{1}H-NMR (400 MHz, D_2O) of **6-Ethylpropylamino-2´-deoxyguanosine-5´-O-triphosphate (4f)**:



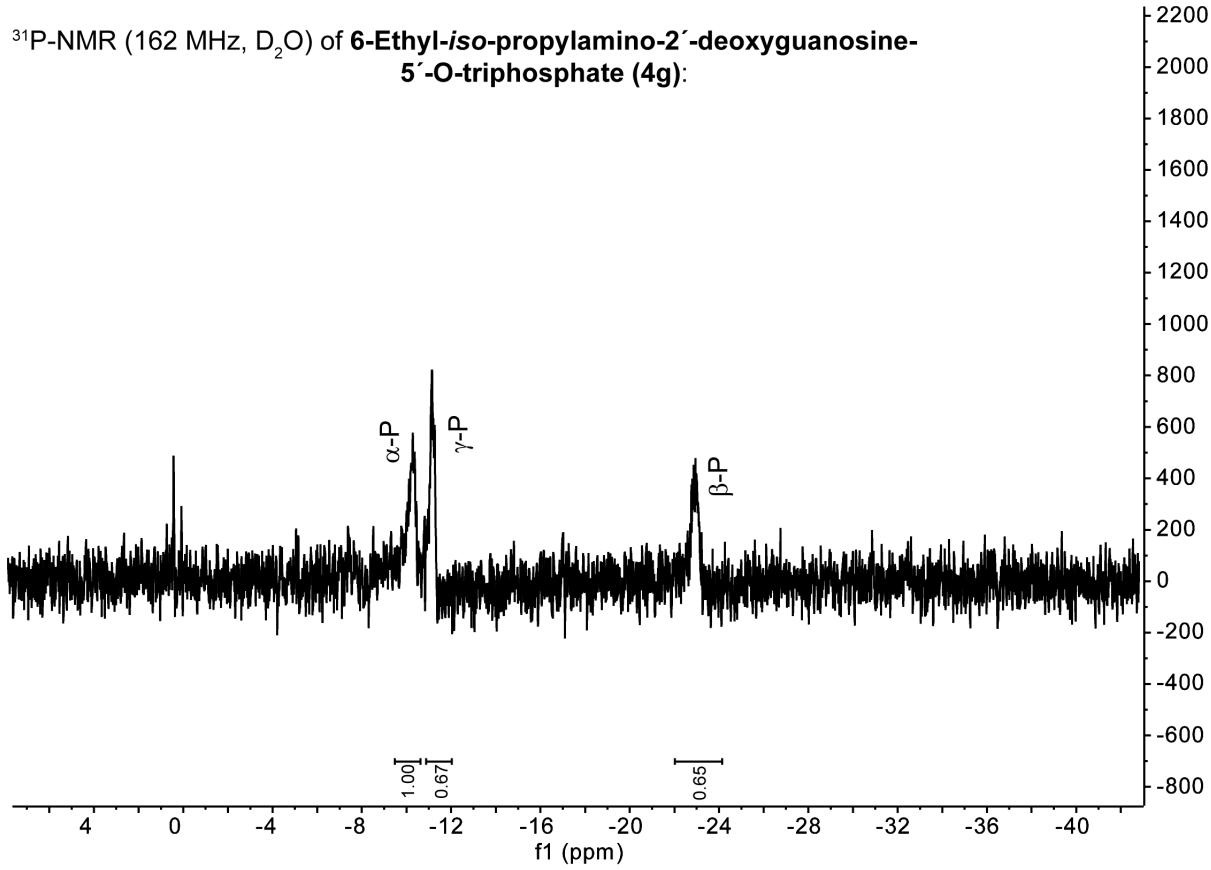
^{31}P-NMR (162 MHz, D_2O) of **6-Ethylpropylamino-2´-deoxyguanosine-5´-O-triphosphate (4f)**:



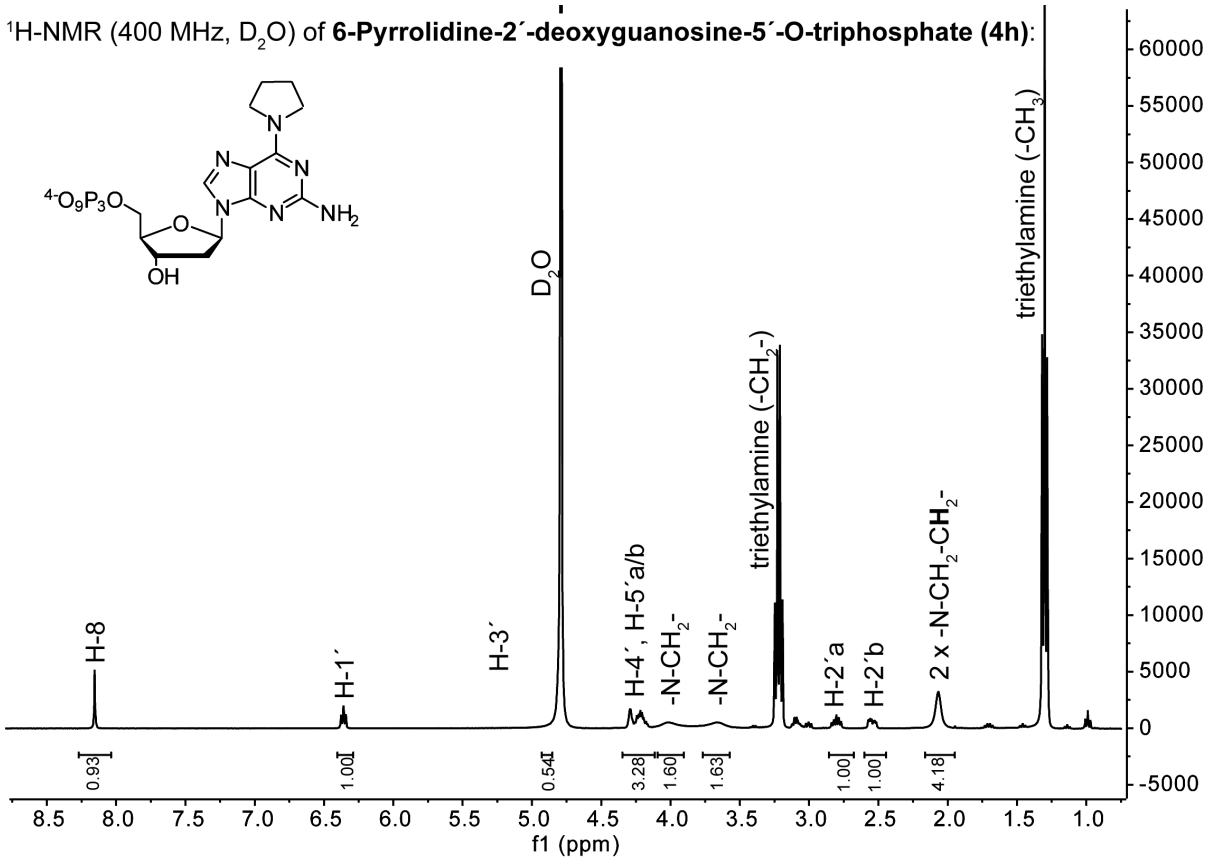
^{1}H-NMR (400 MHz, D_2O) of **6-Ethyl-*iso*-propylamino-2´-deoxyguanosine-5´-O-triphosphate (4g)**:



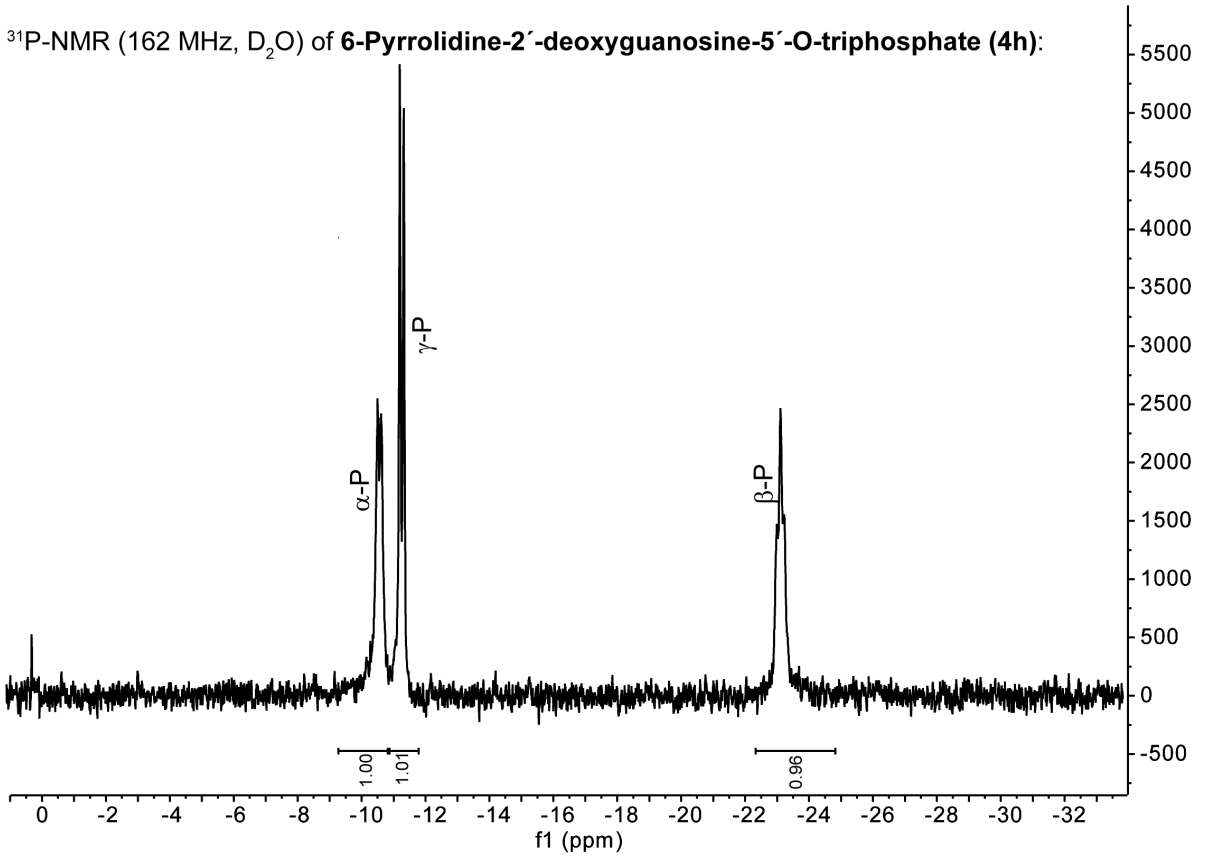
^{31}P-NMR (162 MHz, D_2O) of **6-Ethyl-*iso*-propylamino-2´-deoxyguanosine-5´-O-triphosphate (4g)**:



^{1}H-NMR (400 MHz, D_2O) of **6-Pyrrolidine-2´-deoxyguanosine-5´-O-triphosphate (4h)**:

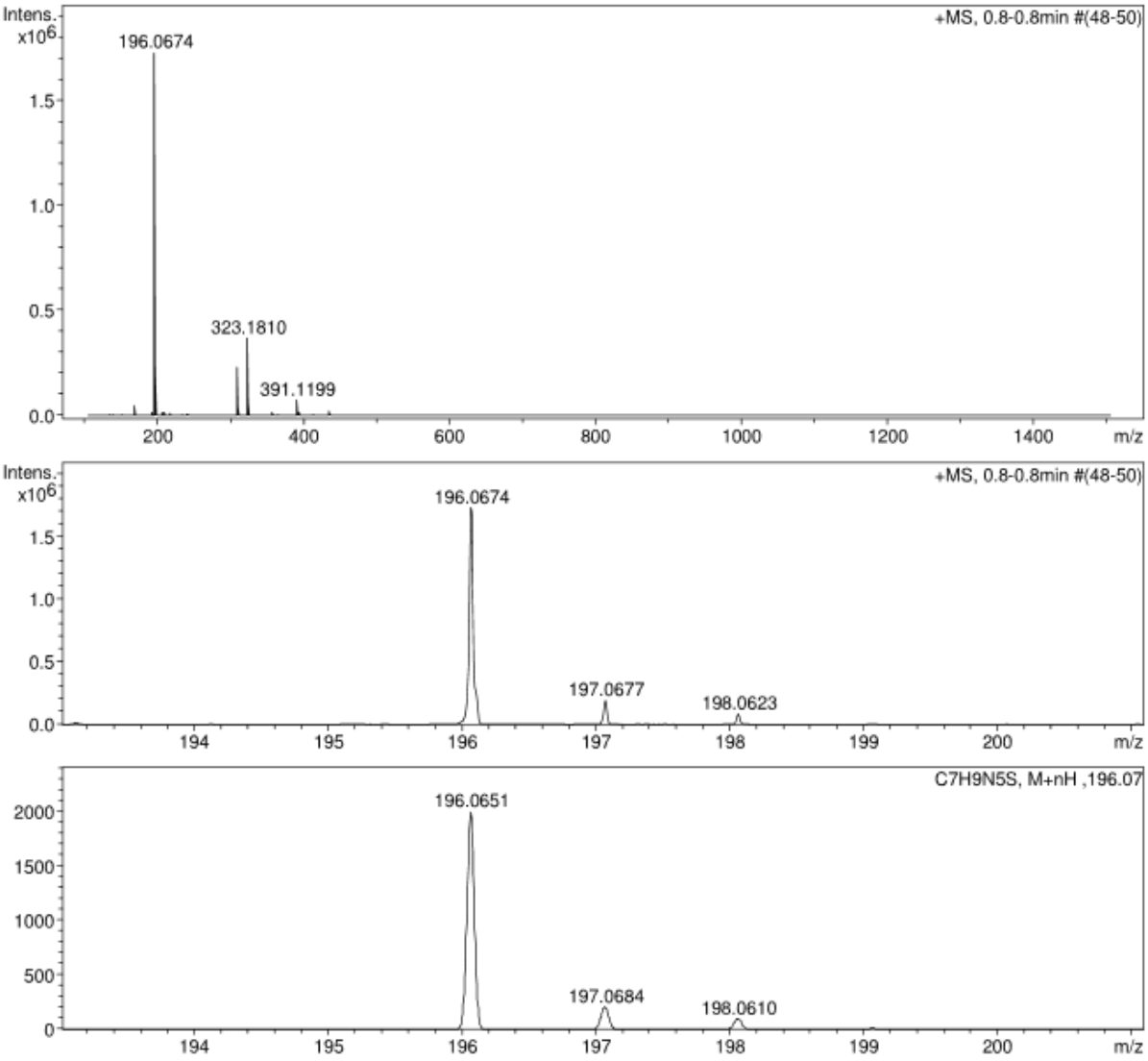


^{31}P-NMR (162 MHz, D_2O) of **6-Pyrrolidine-2´-deoxyguanosine-5´-O-triphosphate (4h)**:

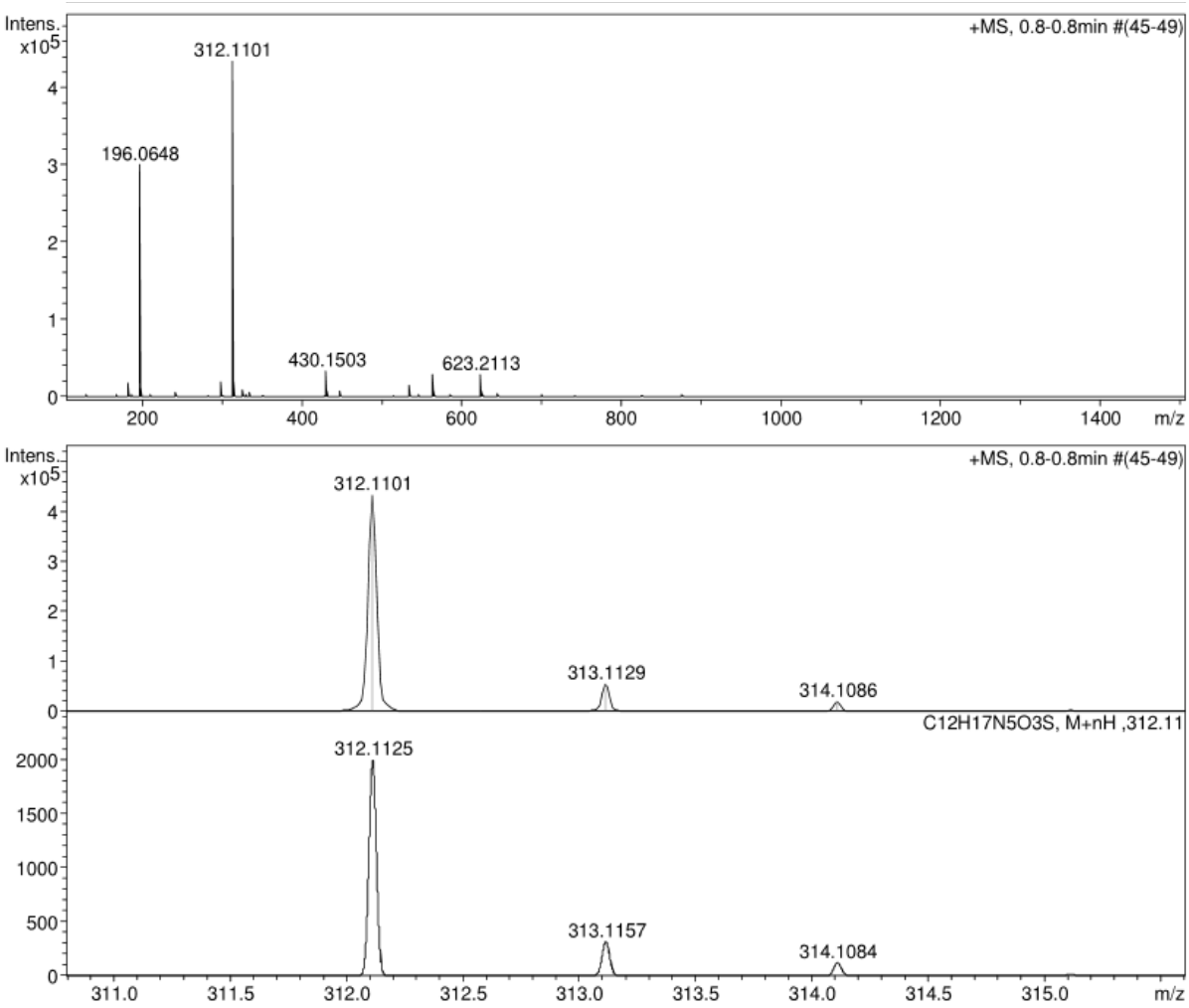


HR-MS Spectra:

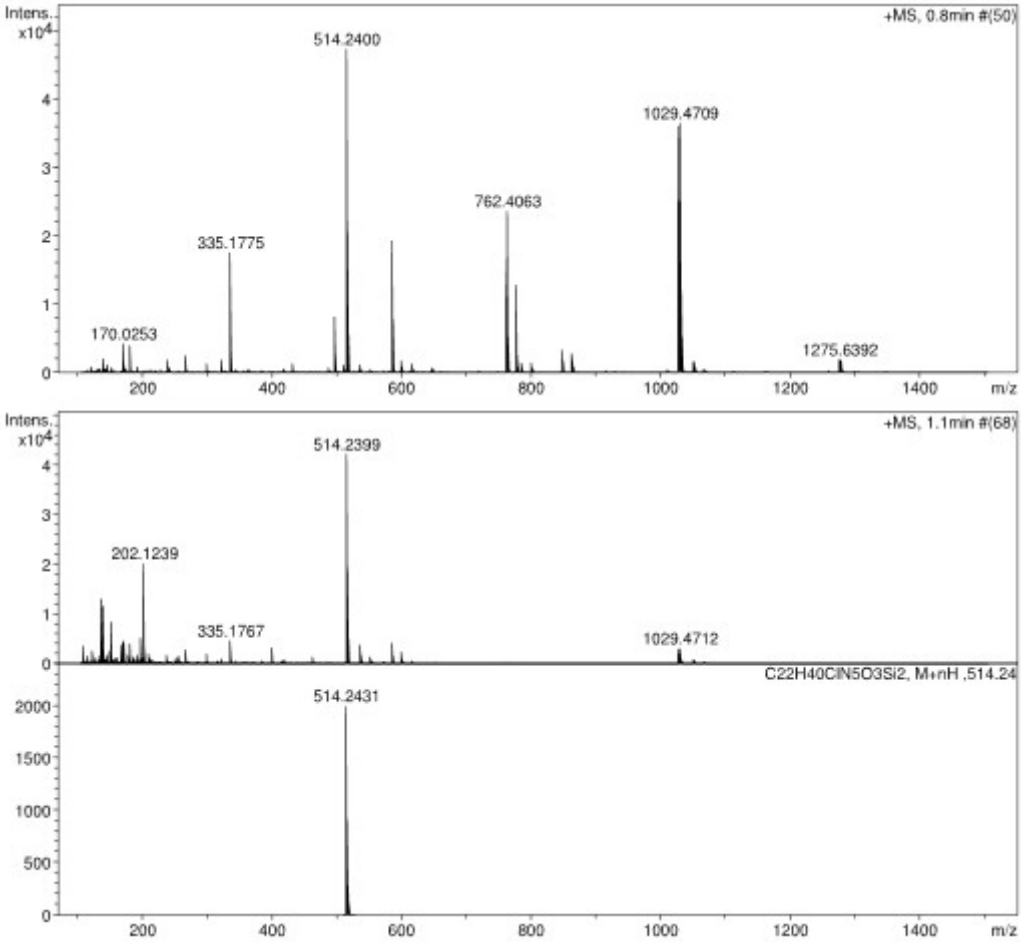
2-Amino-6-ethylthiopurine (5b):



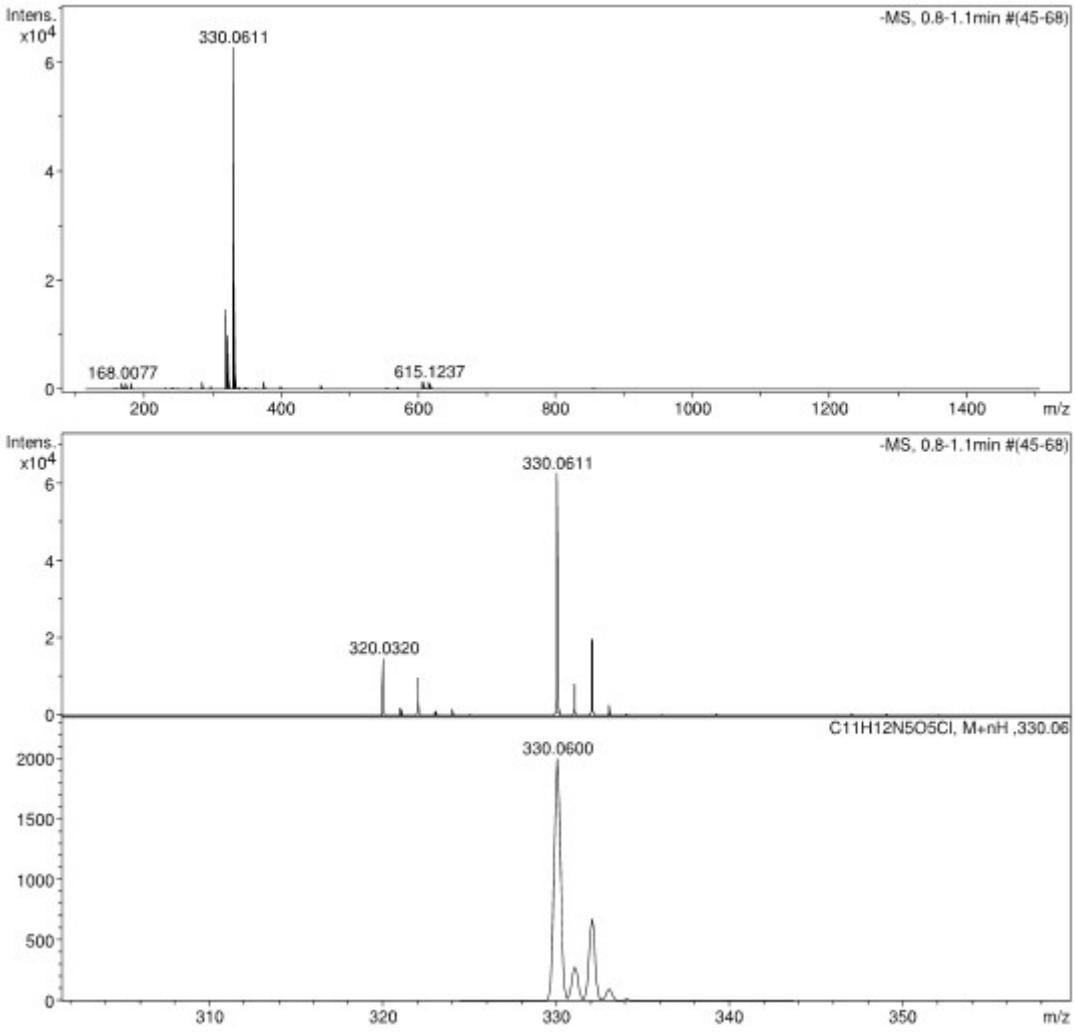
6-Ethylthio-2´-deoxyguanosine (7b):



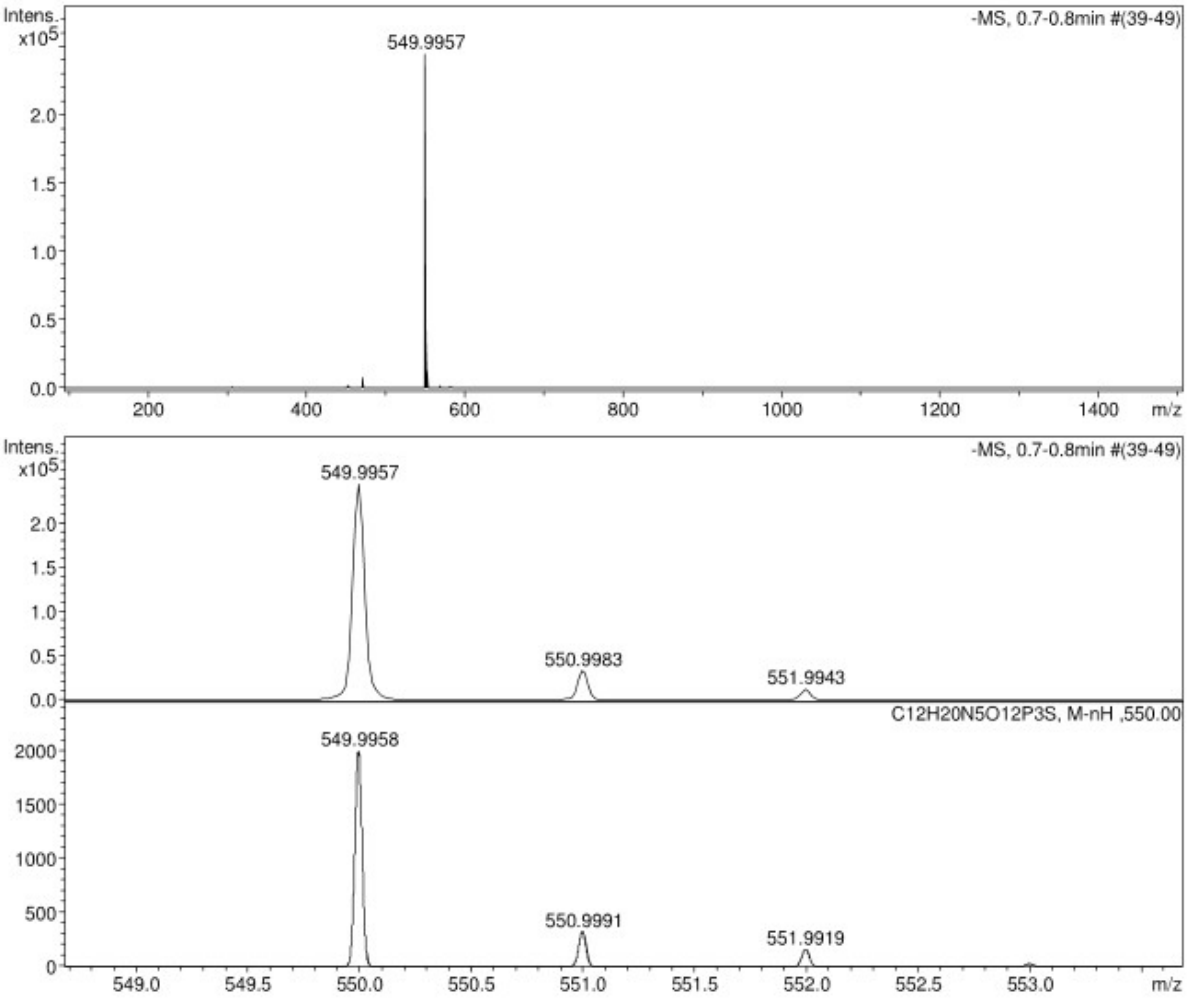
3´,5´-Di-*O-tert*-butyldimethylsilyl-6-chloro-2´-deoxyguanosine (8):



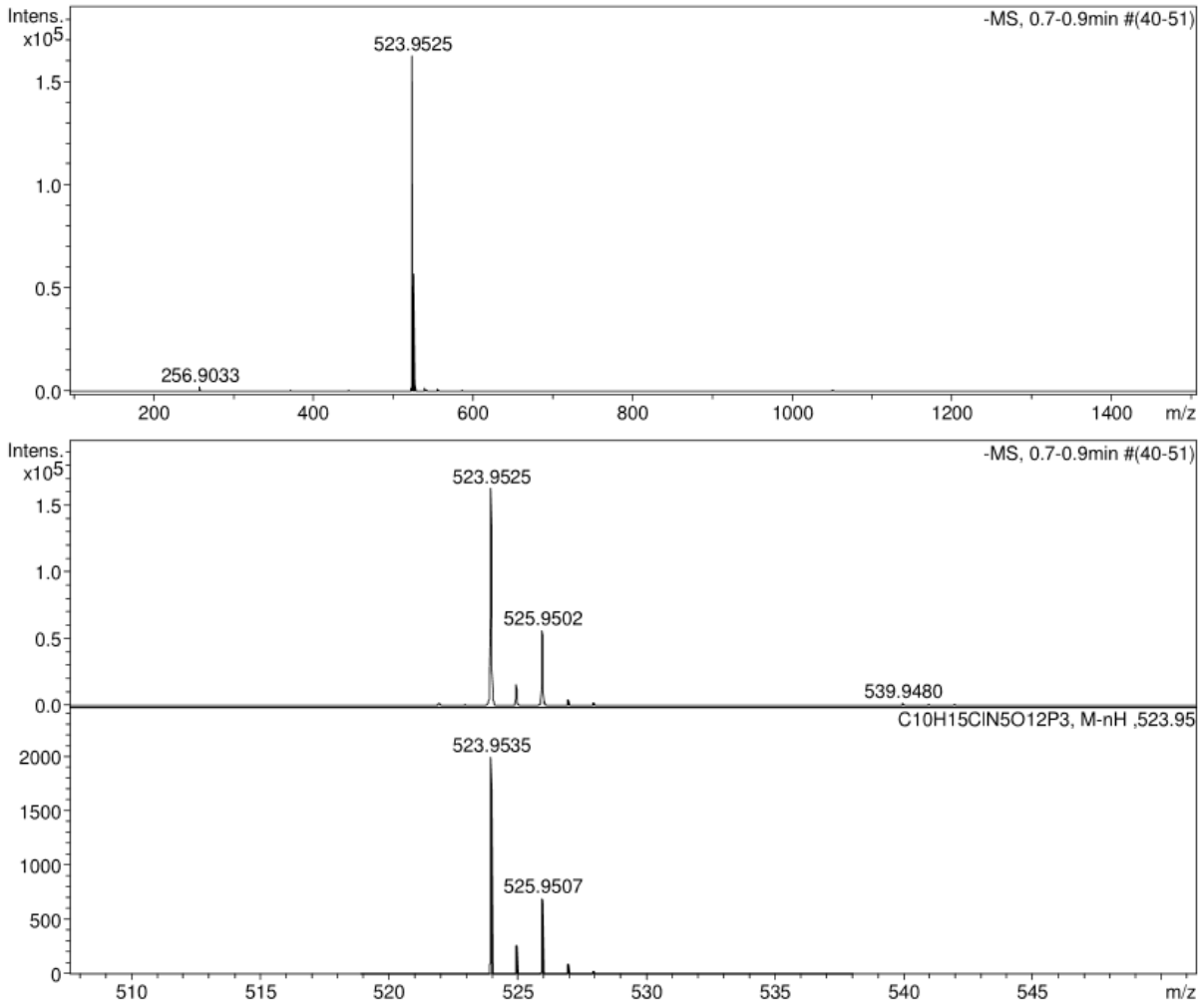
6-Chloro-2´-deoxyguanosine (9):



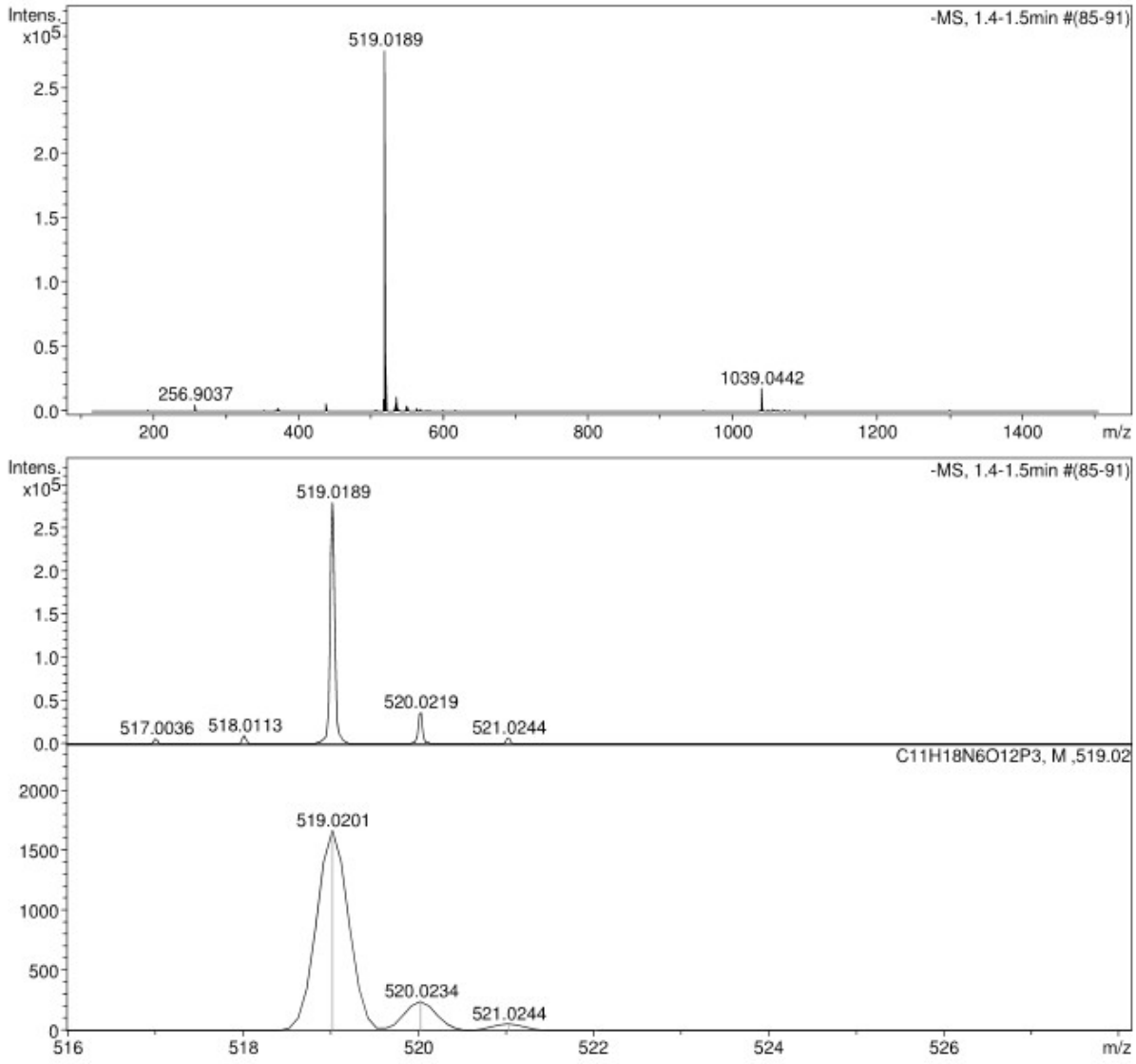
6-Thioethyl-2´-deoxyguanosine-5´-*O*-triphosphate (2b):



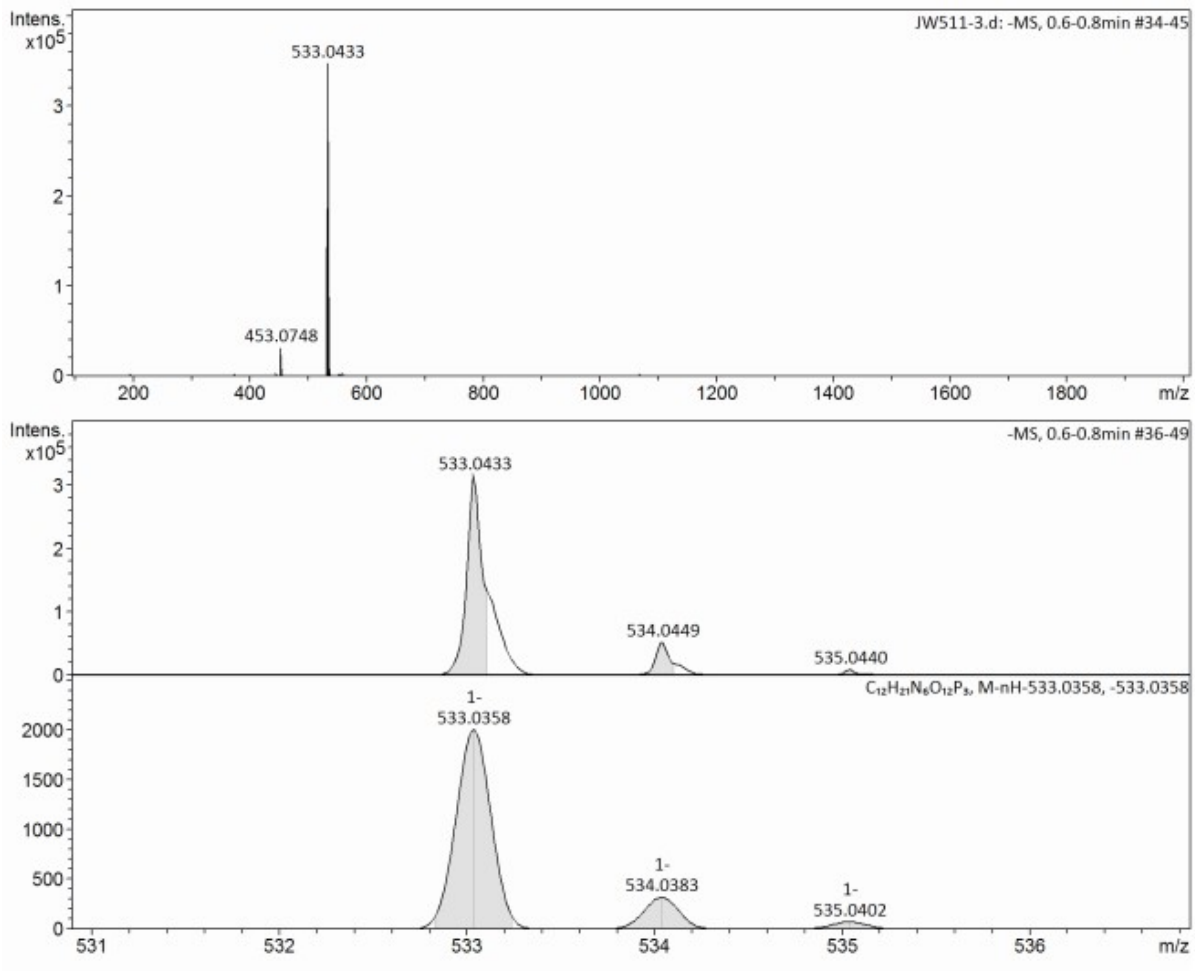
2´-Deoxy-6-chloro-guanosine-5´-*O*-triphosphate (10):



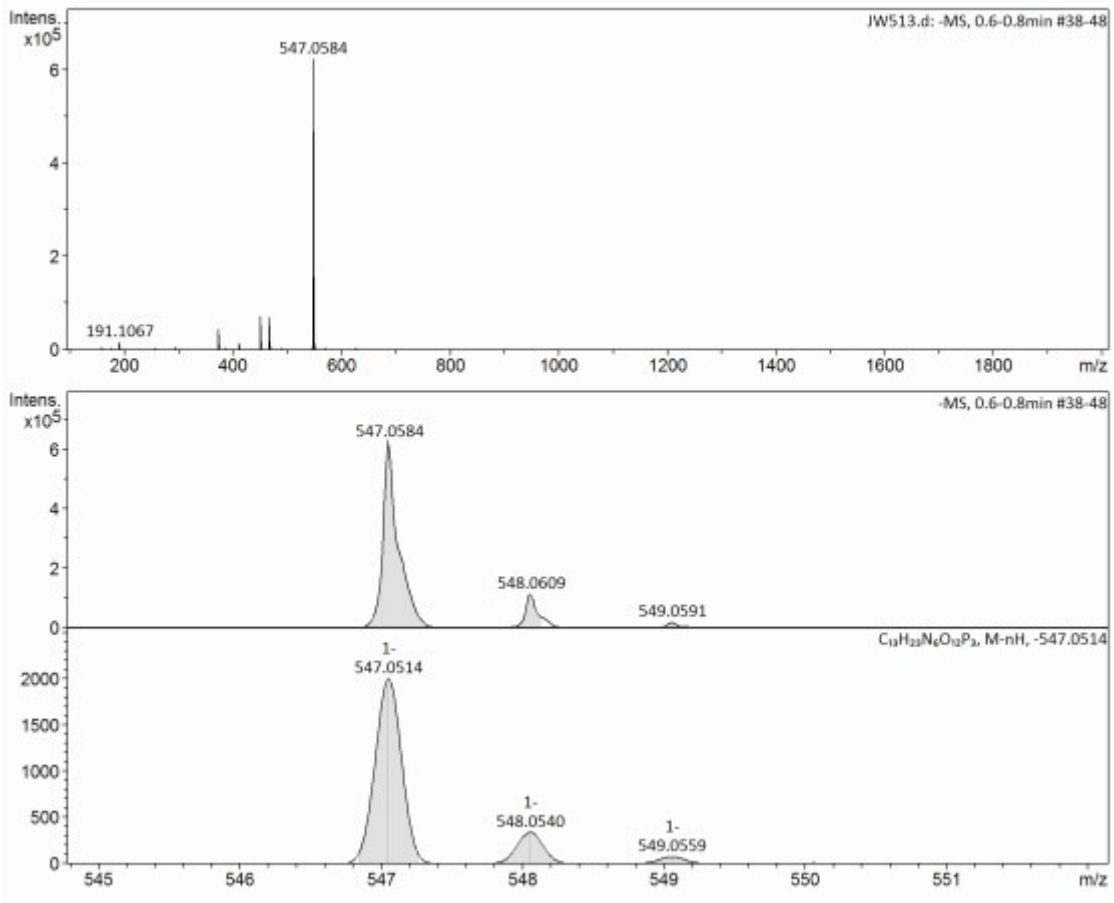
6-(Methylamino)-2´-deoxyguanosine-5´-*O*-triphosphate (3a):



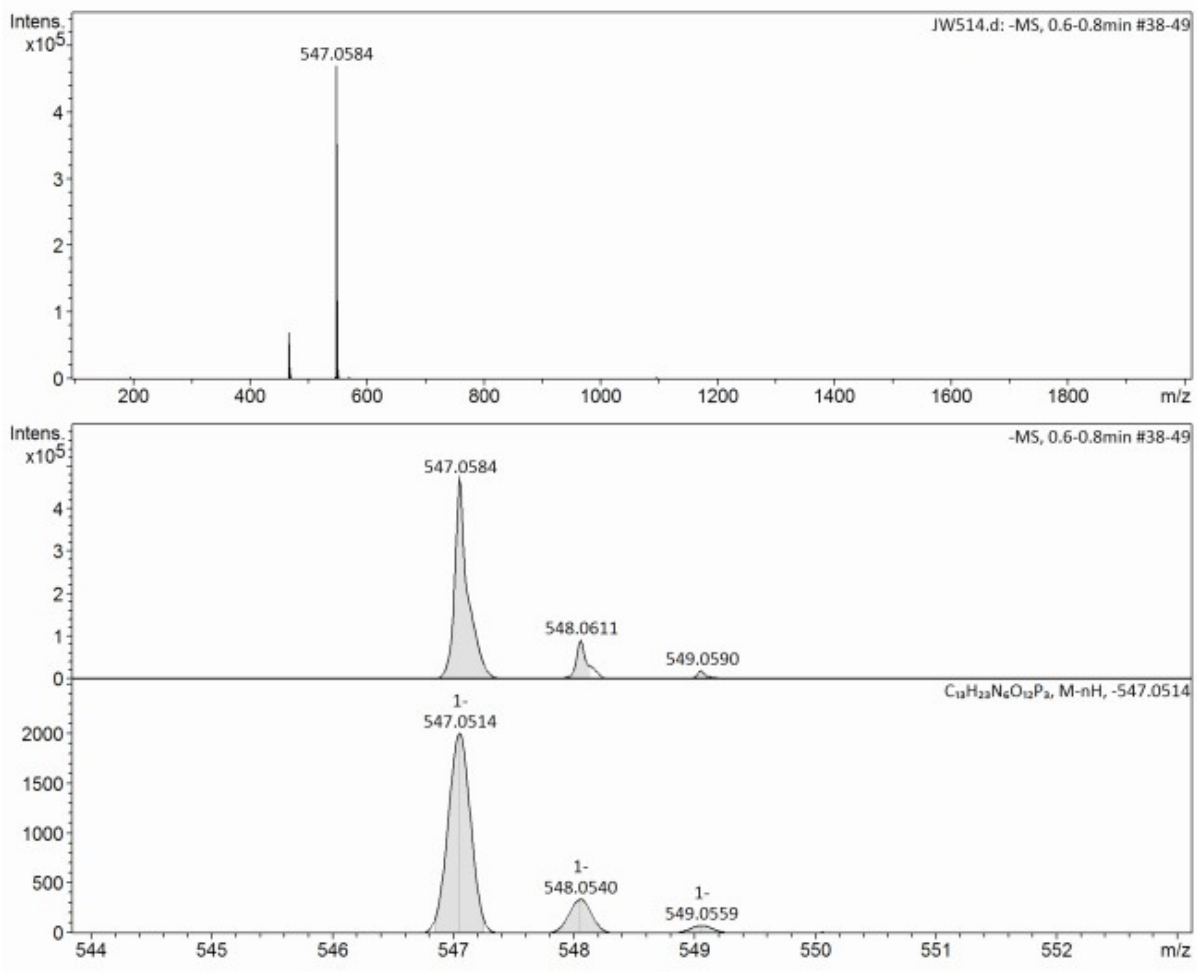
6-(Ethylamino)-2´-deoxyguanosine-5´-*O*-triphosphate (3b):



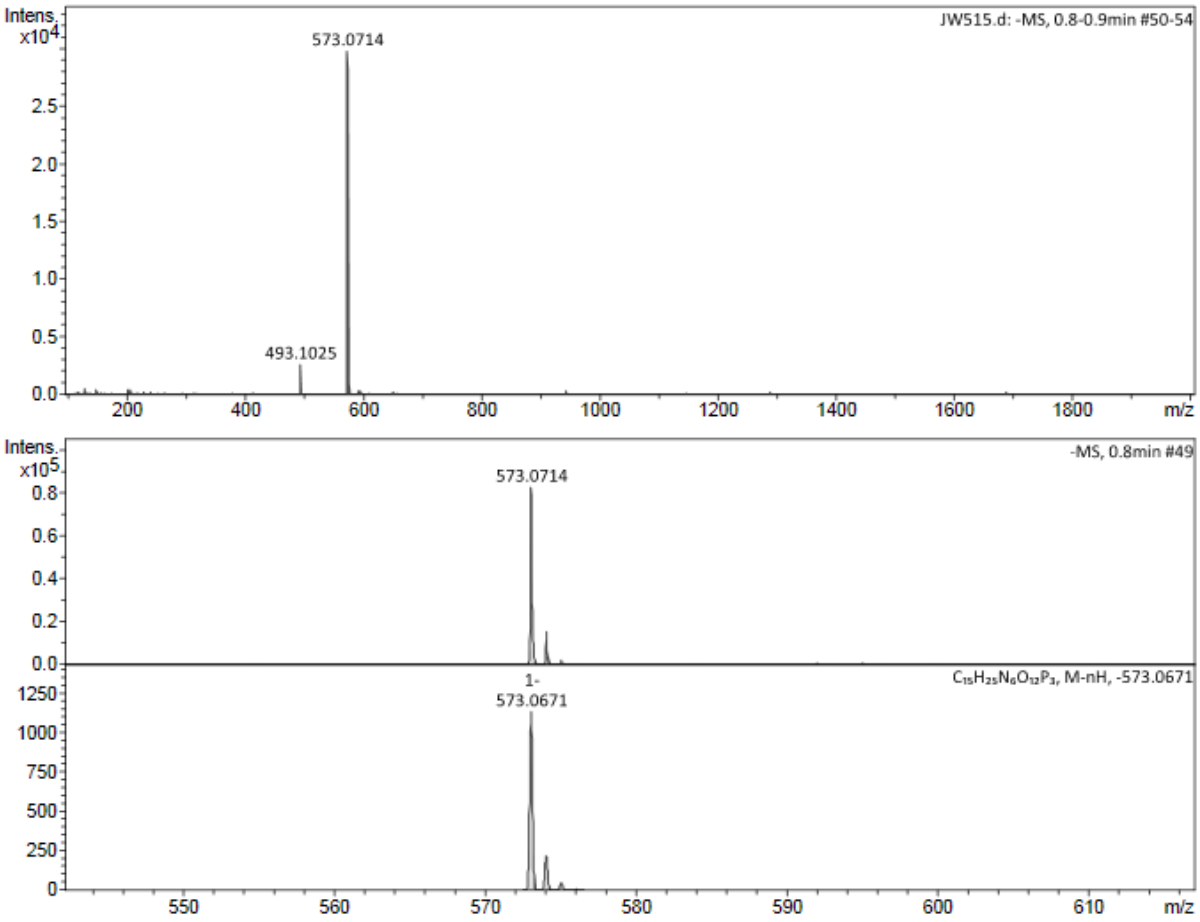
6-(Propylamino)-2´-deoxyguanosine-5´-*O*-triphosphate (3c):



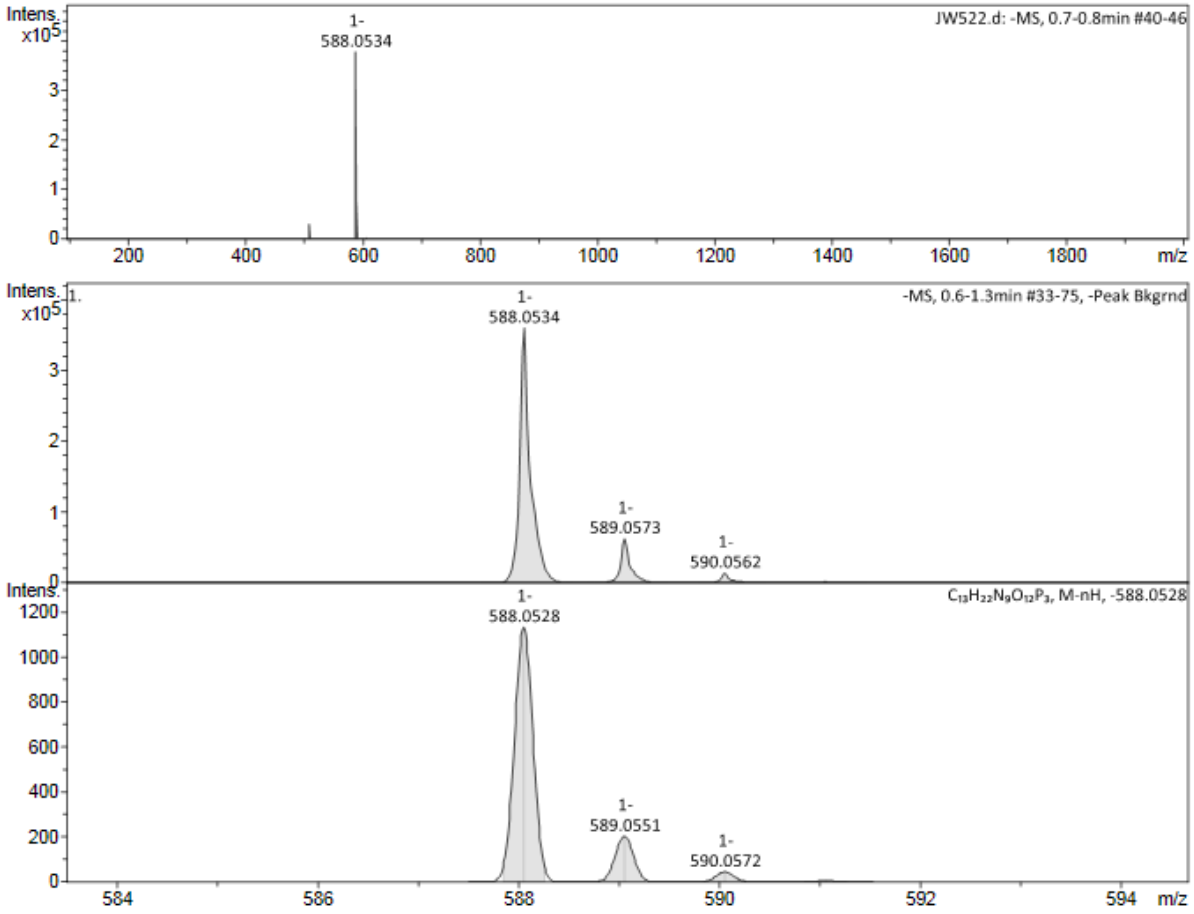
6-(*iso*-Propylamino)-2´-deoxyguanosine-5´-*O*-triphosphate (3d):



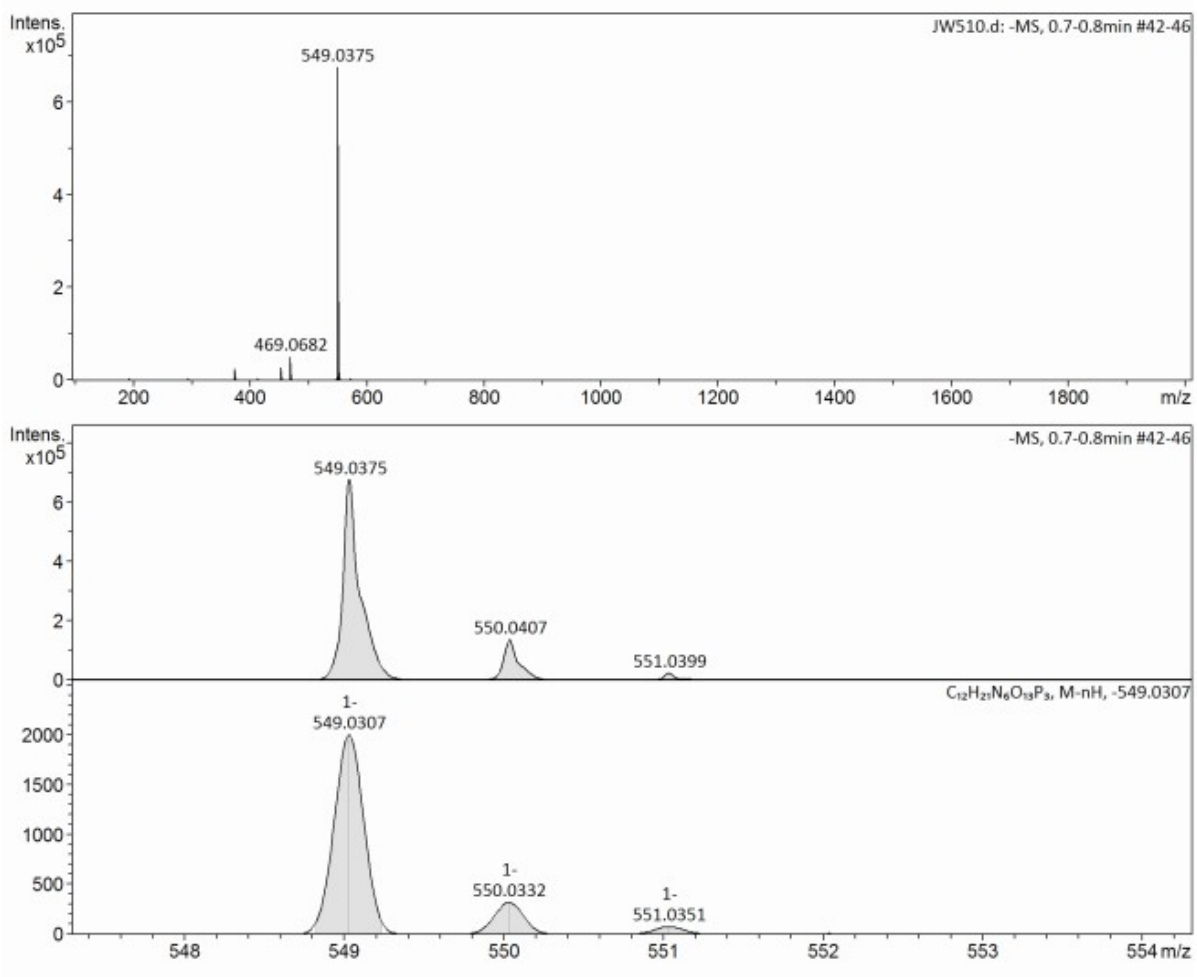
6-(Cyclohexylamino)-2´-deoxyguanosine-5´-*O*-triphosphate (3e):



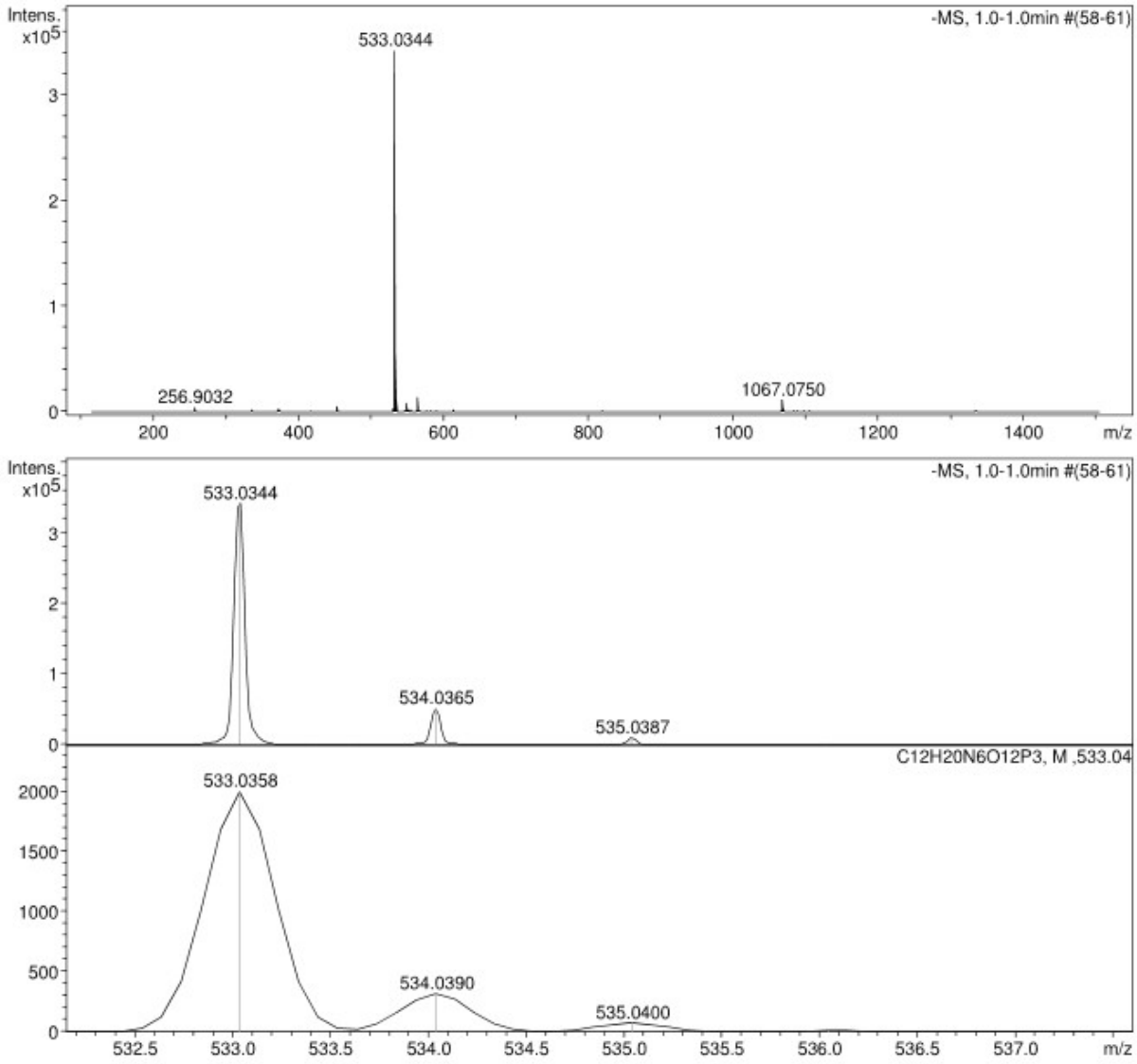
6-(Azido-1-propanamino)-2´-deoxyguanosine-5´-*O*-triphosphate (3f):



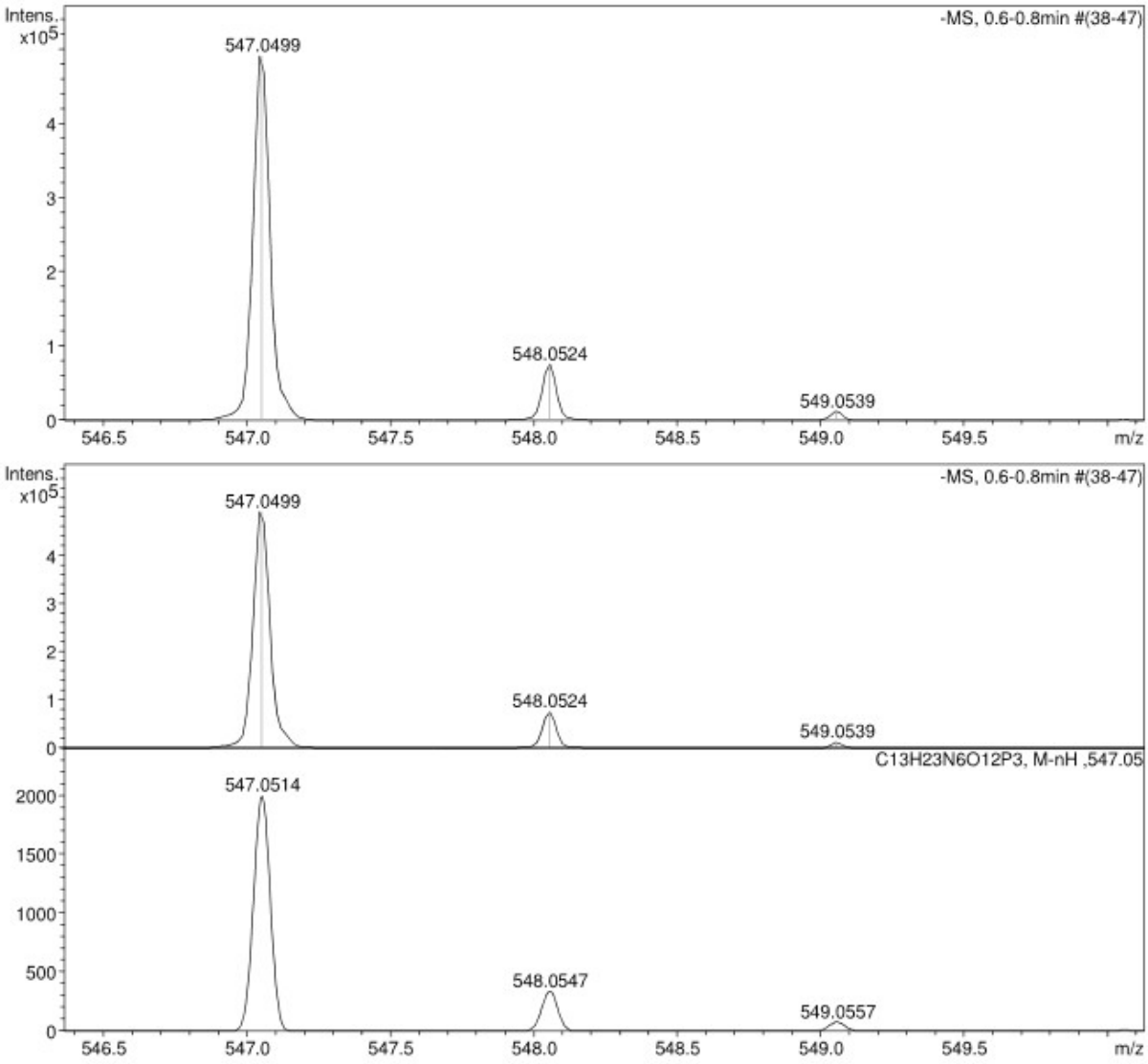
6-(Hydroxy-1-ethanamino)-2´-deoxyguanosine-5´-*O*-triphosphate (3g):



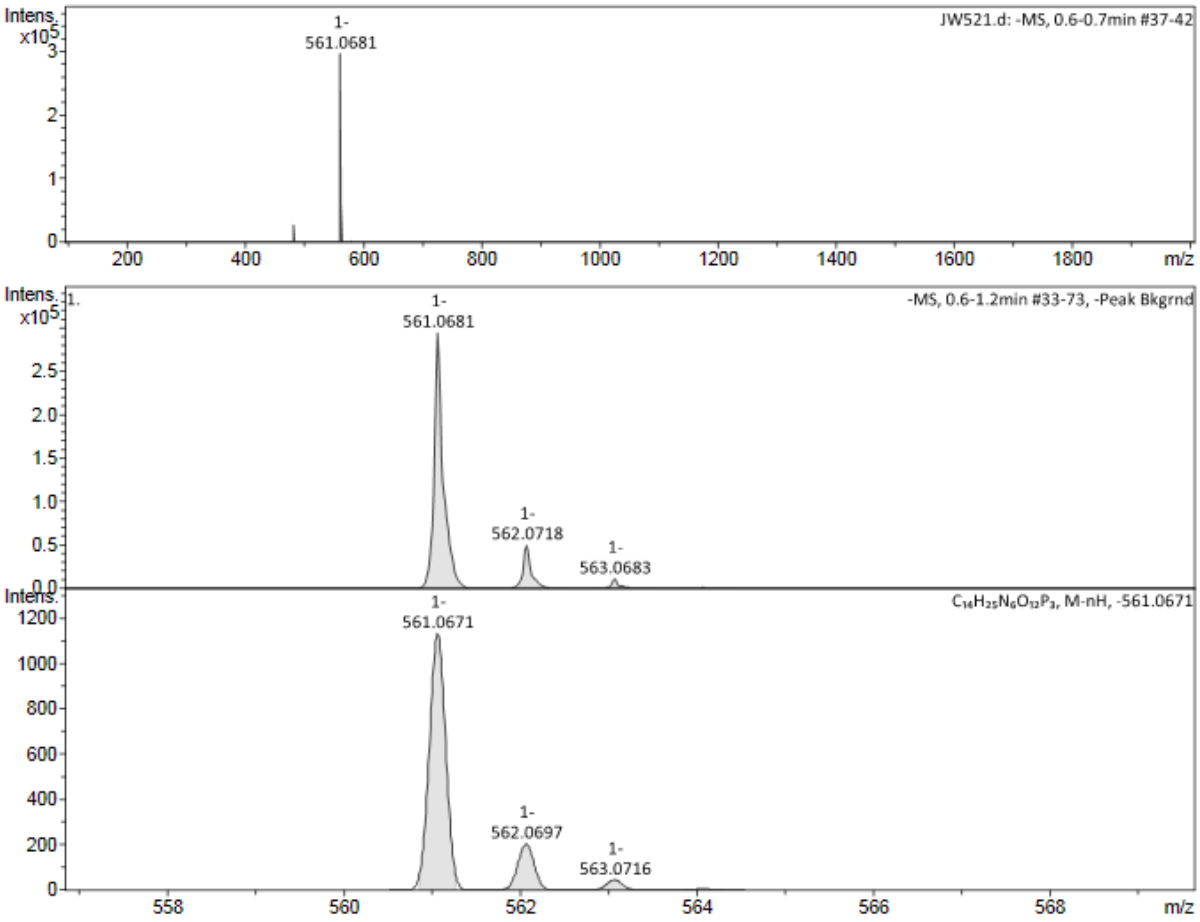
6-Dimethylamino-2´-deoxyguanosine-5´-*O*-triphosphate (4a):



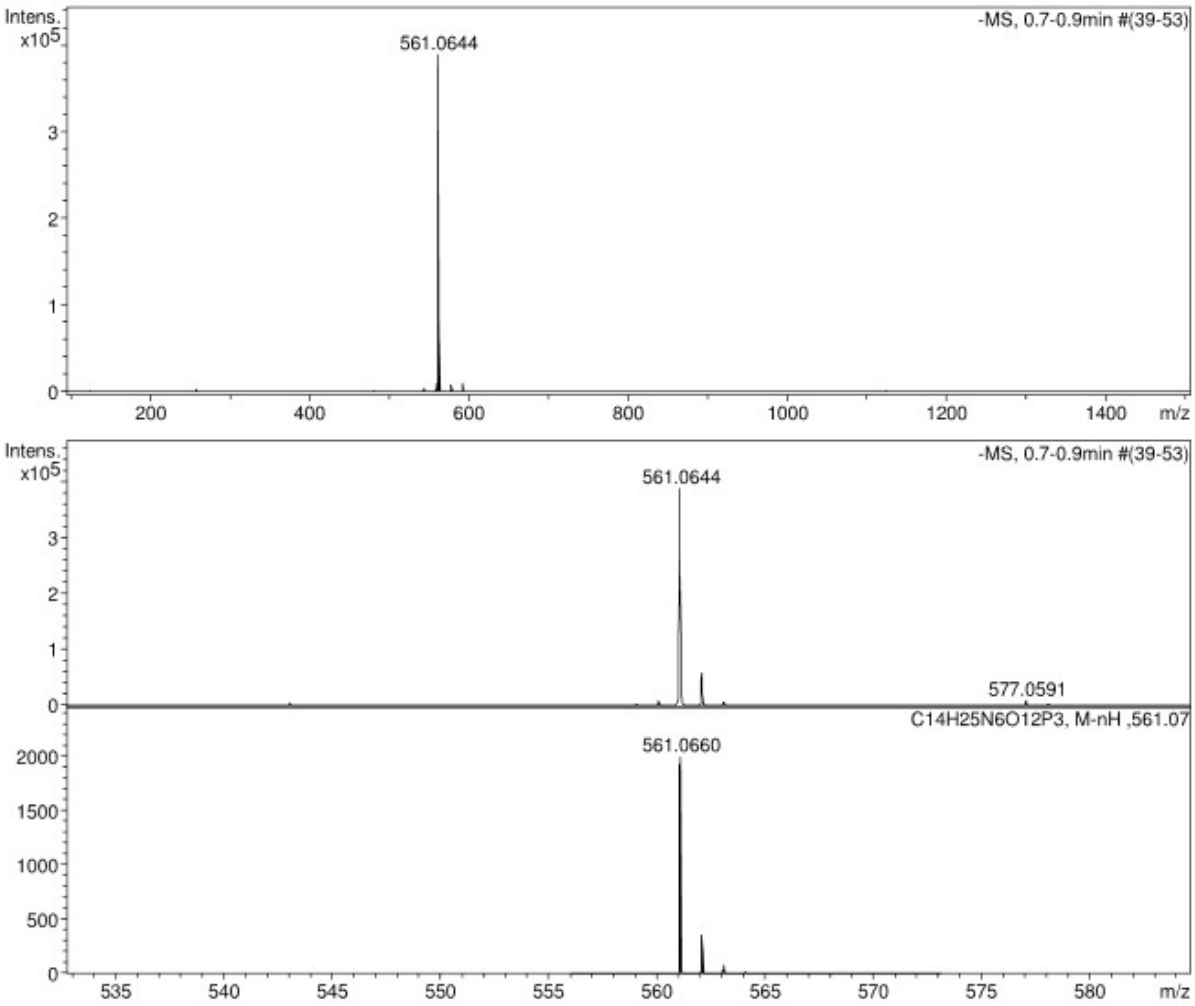
6-Methylethylamino-2´-deoxyguanosine-5´-*O*-triphosphate (4b):



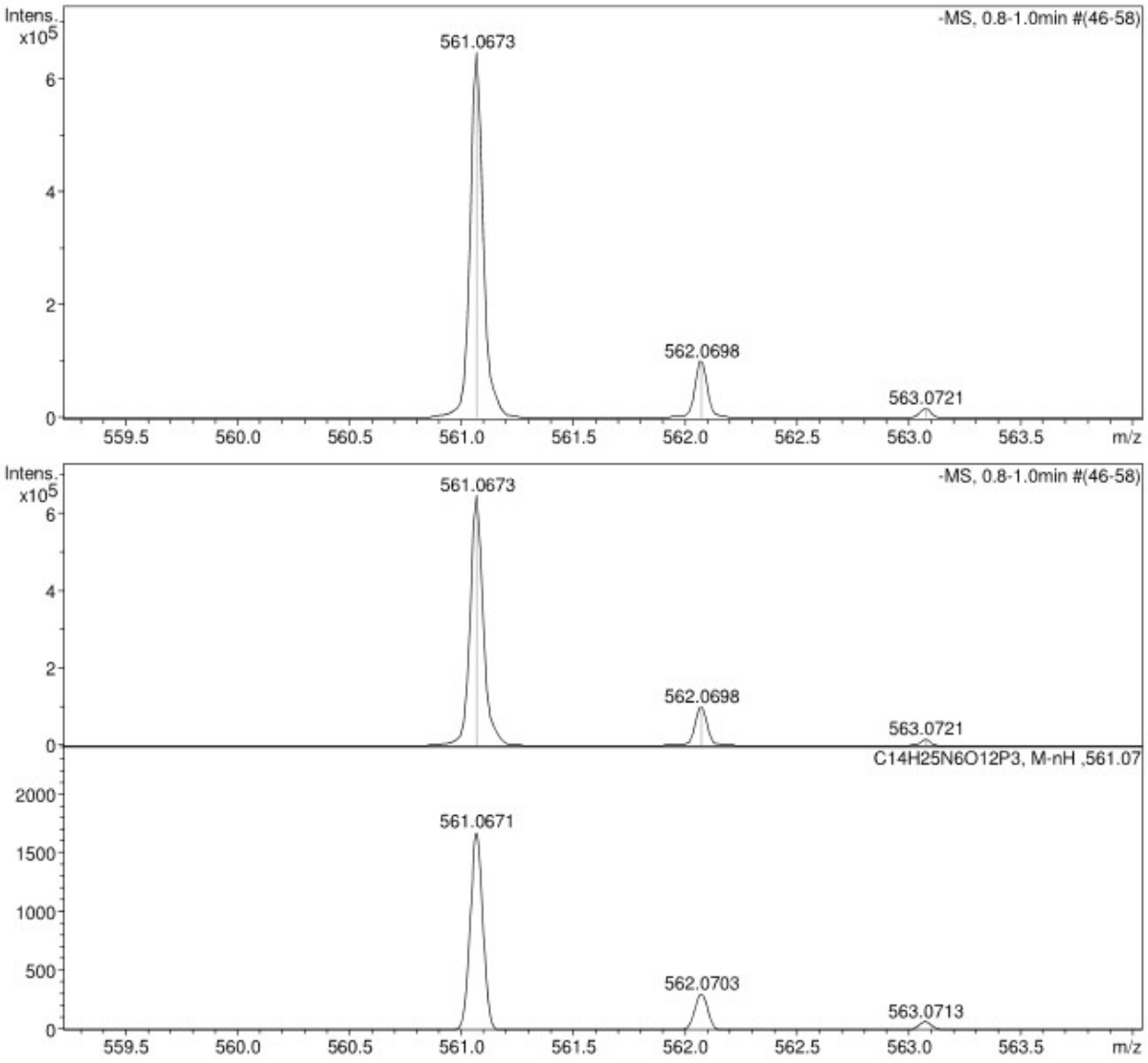
6-Methylpropylamino-2´-deoxyguanosine-5´-*O*-triphosphate (4c):



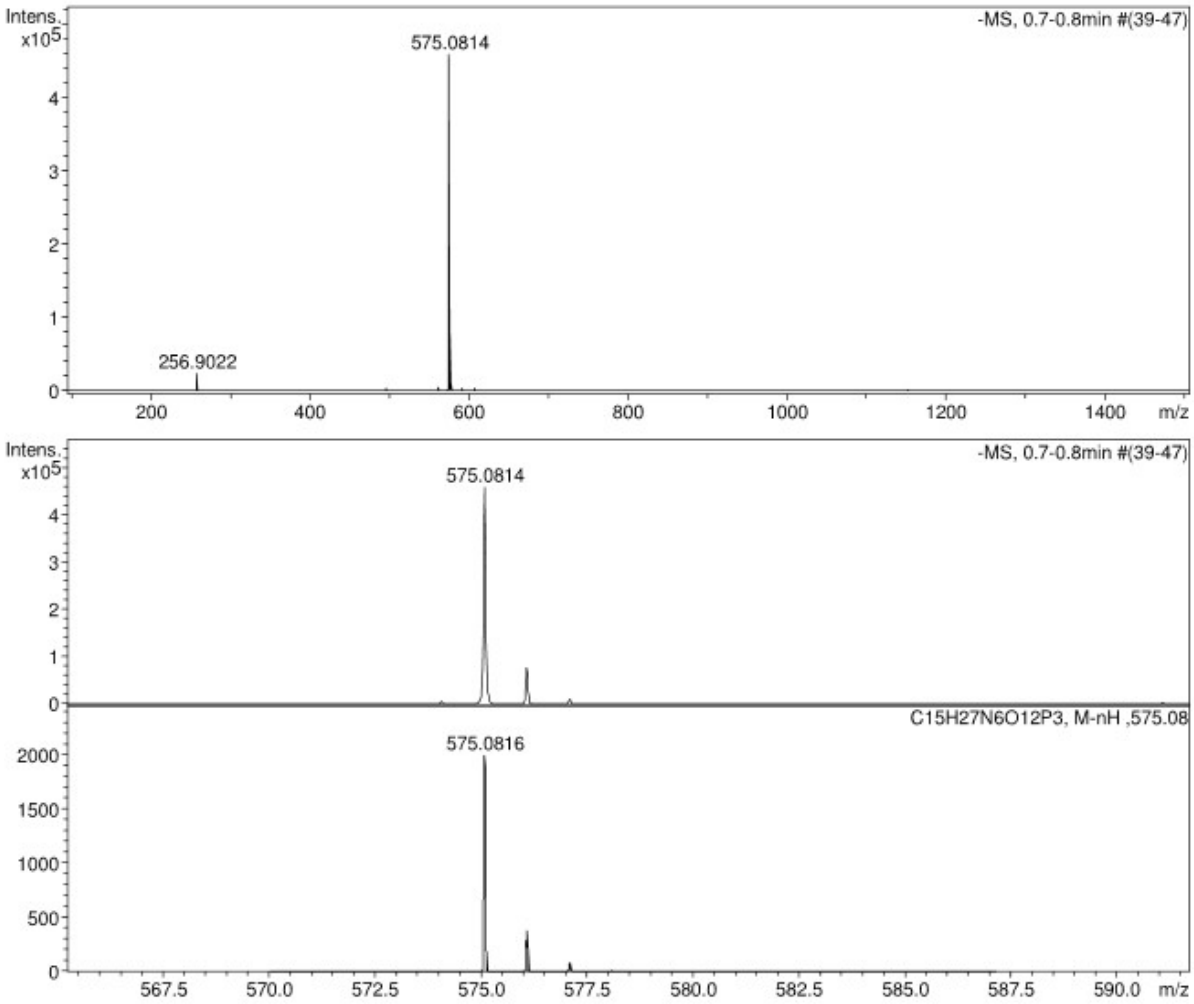
6-Methyl-*iso*-propylamino-2´-deoxyguanosine-5´-*O*- triphosphate (4d):



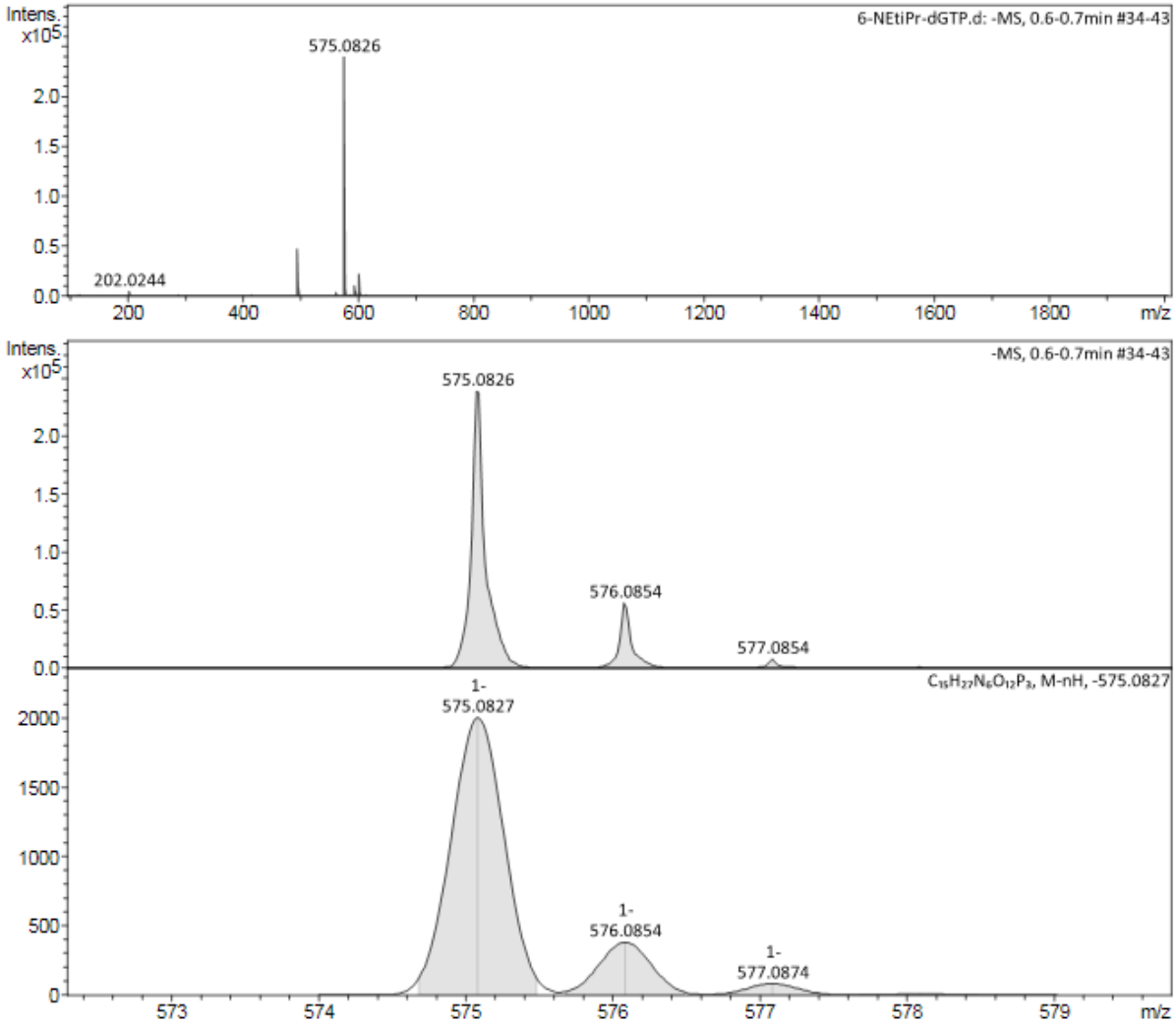
6-Diethylamino-2´-deoxyguanosine-5´-*O*-triphosphate (4e):



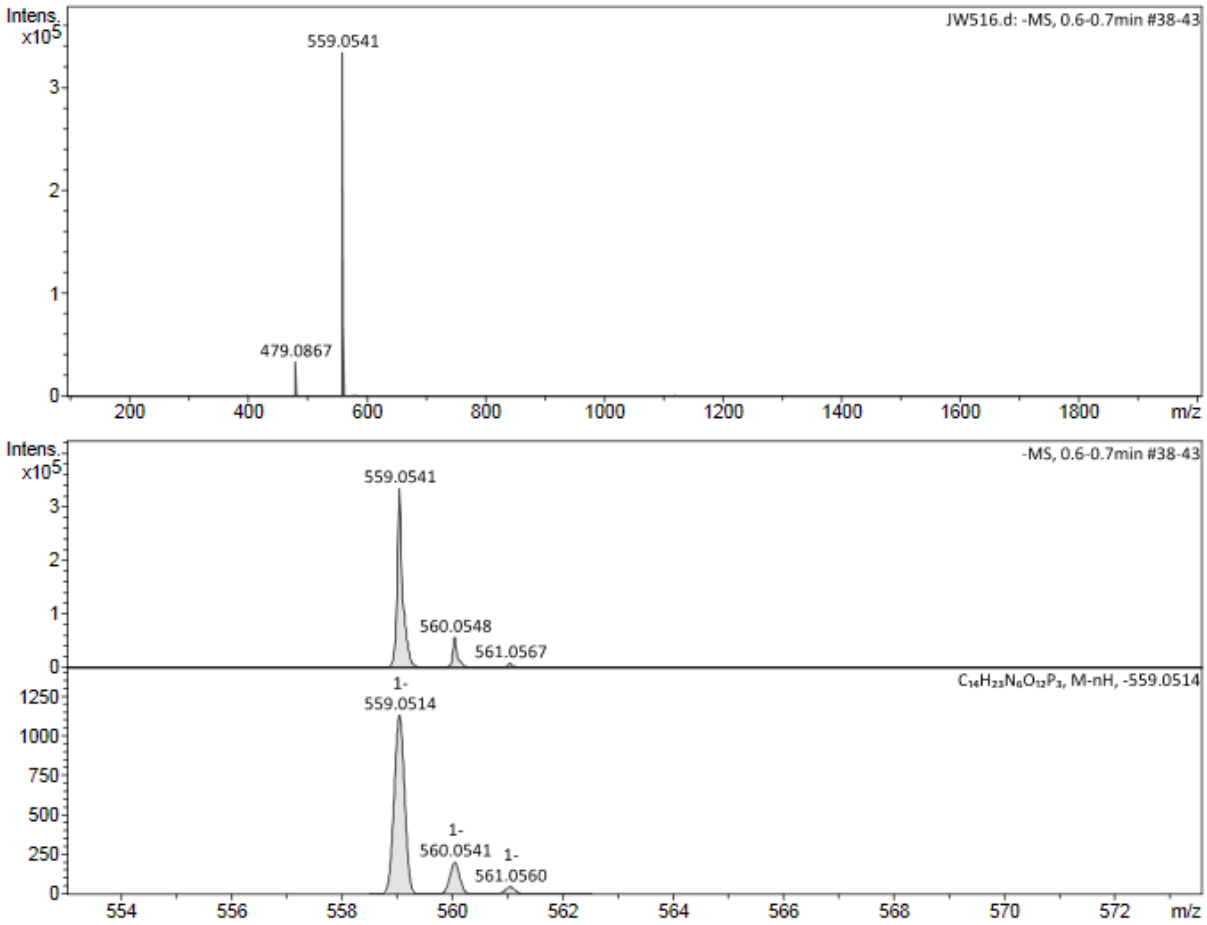
6-Ethylpropylamino-2´-deoxyguanosine-5´-*O*-triphosphate (4f):



6-Ethyl-iso-propylamino-2´-deoxyguanosine-5´-*O*-triphosphate (4g):



6-Pyrrolidine-2´-deoxyguanosine-5´-*O*-triphosphate (4h):



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