Electronic Supplementary Information

3-Phosphono-L-alanine as Pyrophosphate Mimic for DNA Synthesis Using HIV-1 Reverse Transcriptase

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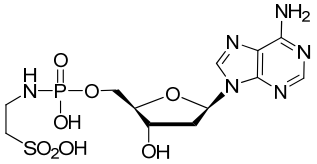
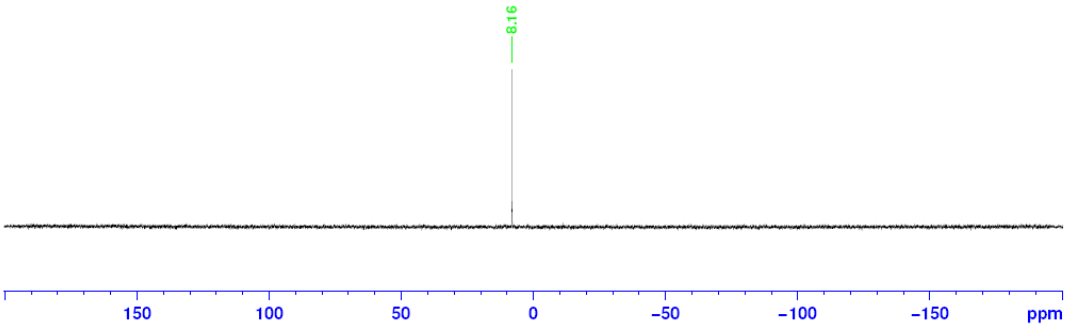
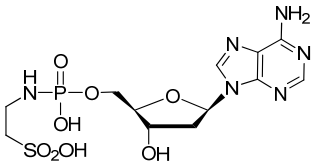
The compound is labile. Complete removal of Et_3N by preparative chromatography was not possible without disturb the compound. Therefore, the ^{1}H NMR was analyzed after Et_3N suppress.

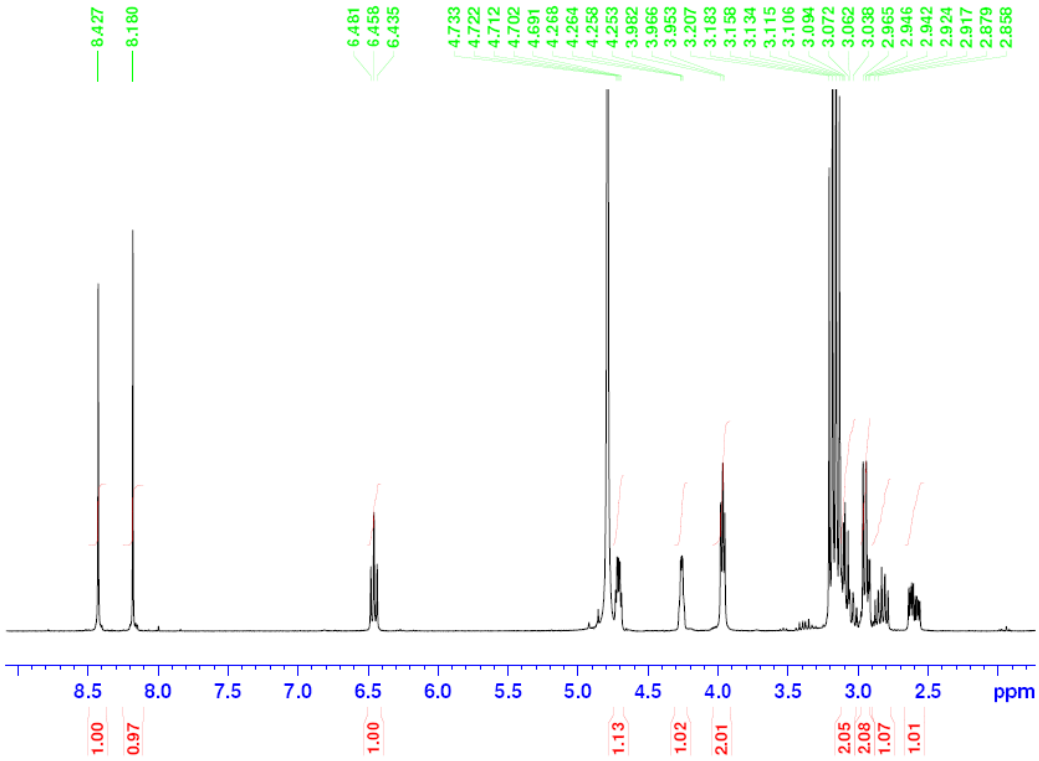
2′-Deoxyadenosine-5′-taurine phosphoramidate (**1**) ^{31}P NMR





2′-Deoxyadenosine-5′-taurine phosphoramidate (**1**) ^{1}H NMR





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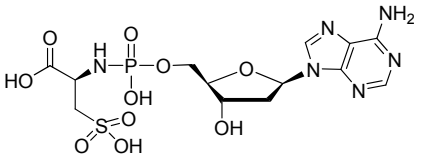
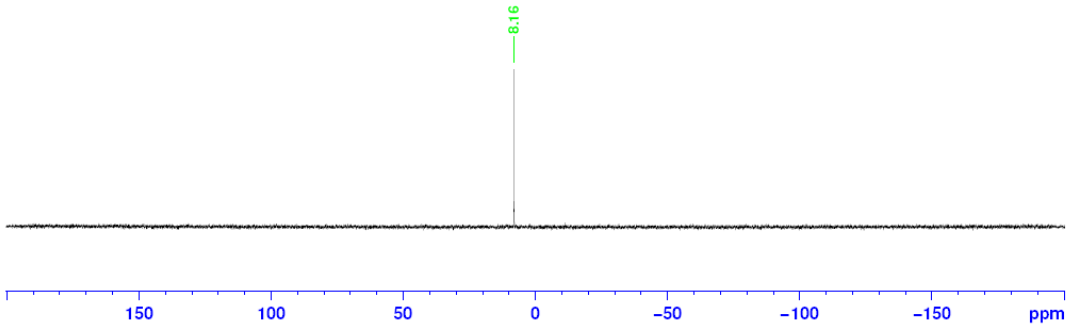
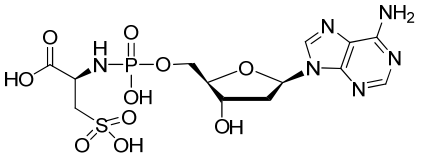
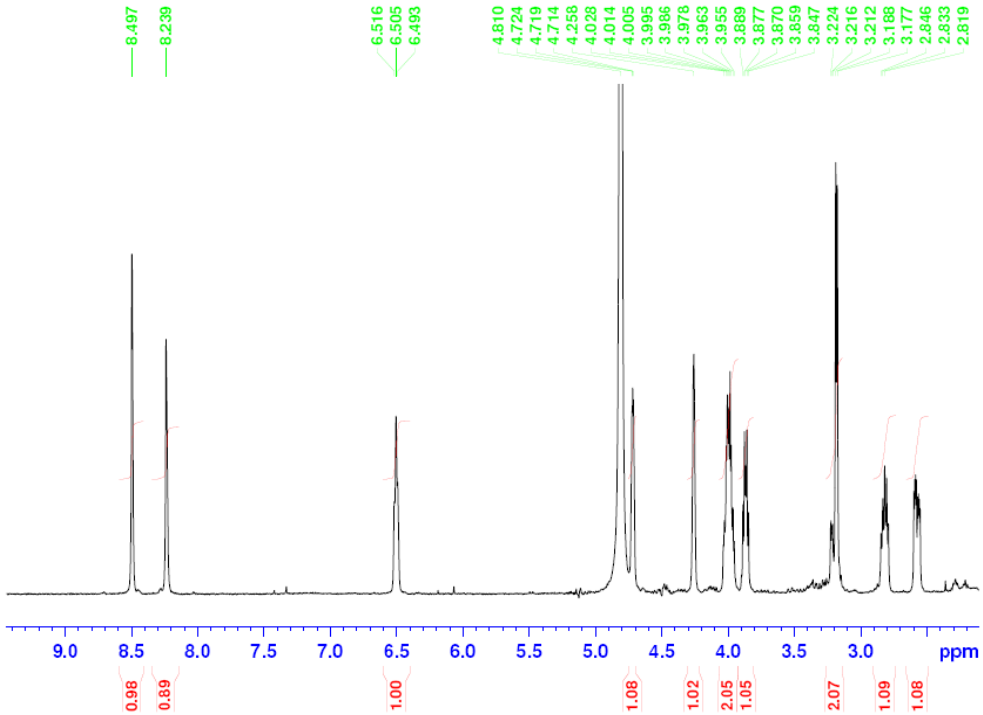


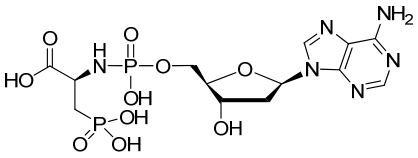


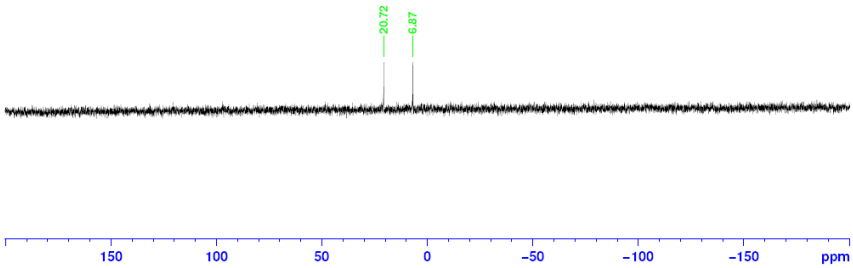
2′-Deoxyadenosine-5′-(L-cysteic acid) phosphoramidate (**2**) ^{1}H NMR





2′-Deoxyadenosine-5′-(3-phosphono-L-alanine) phosphoramidate (**3**) ^{31}P NMR





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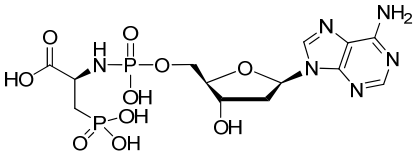

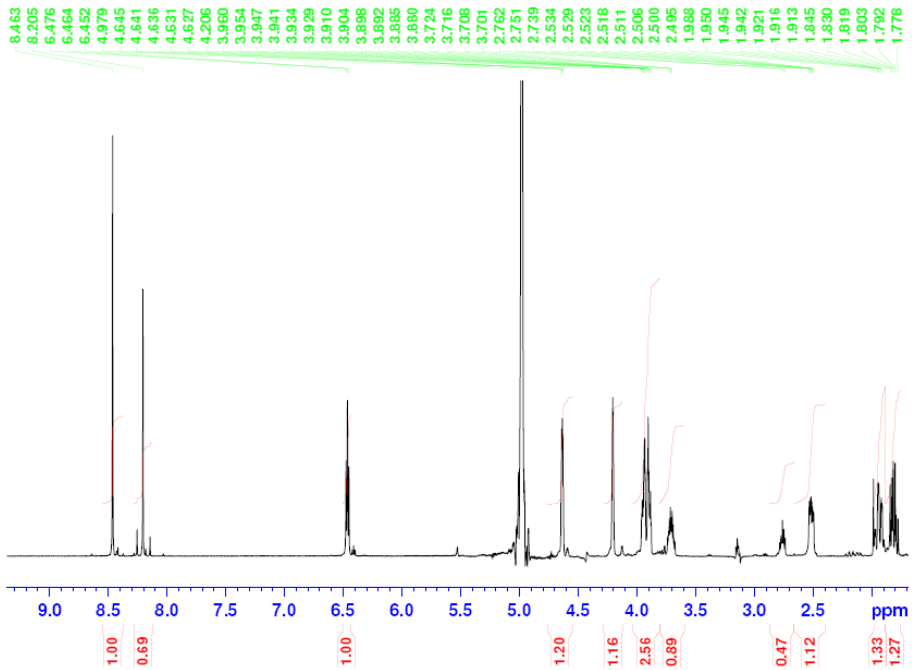

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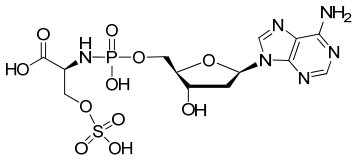

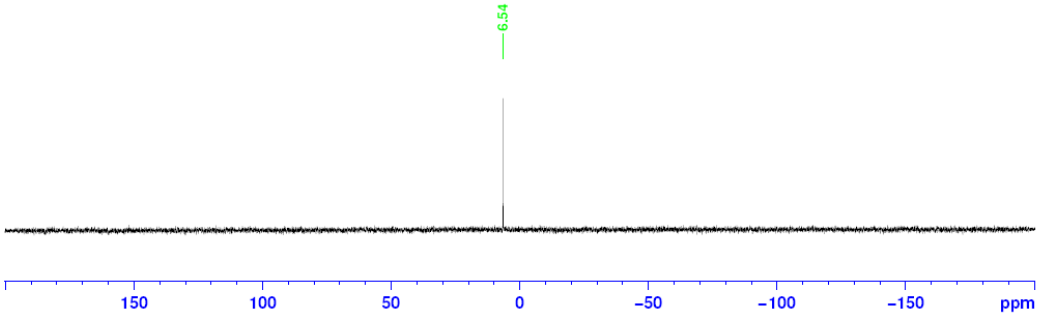

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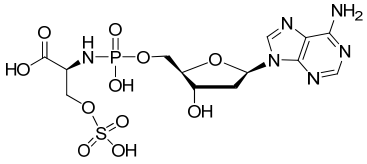

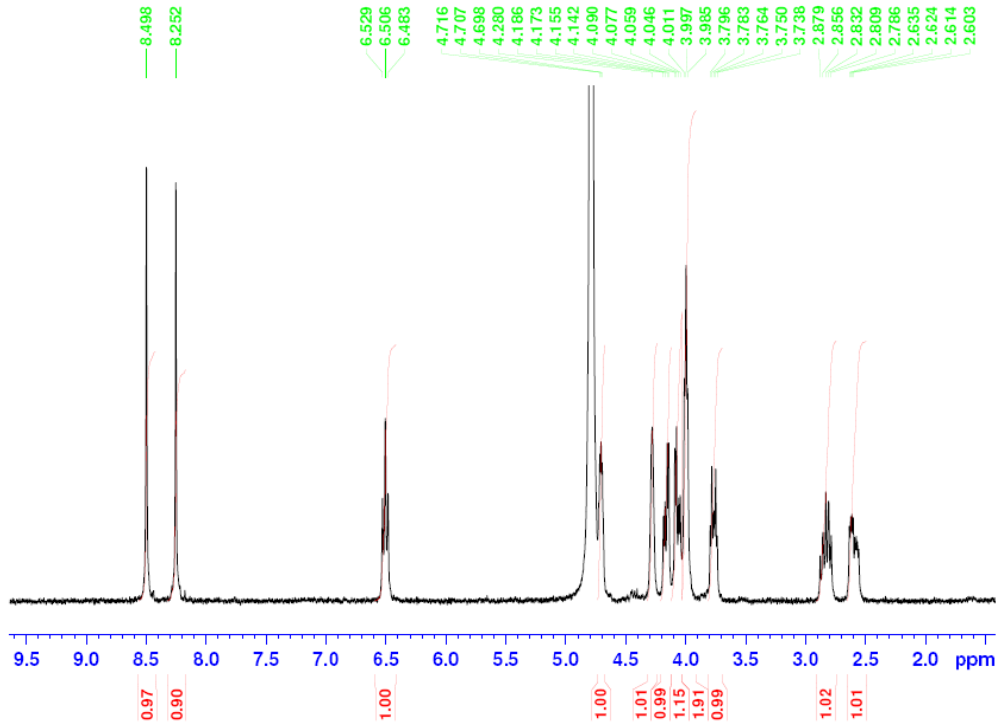

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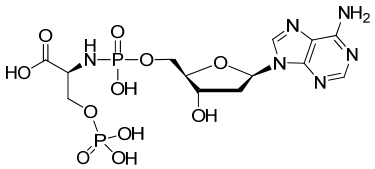

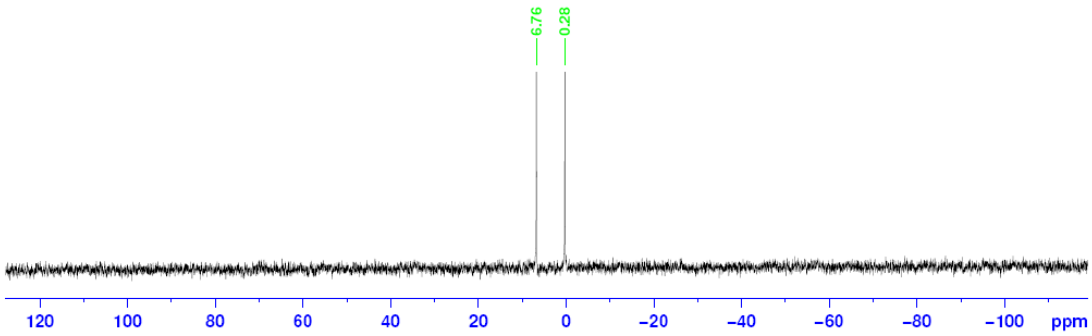

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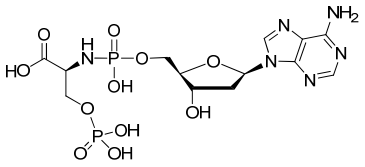
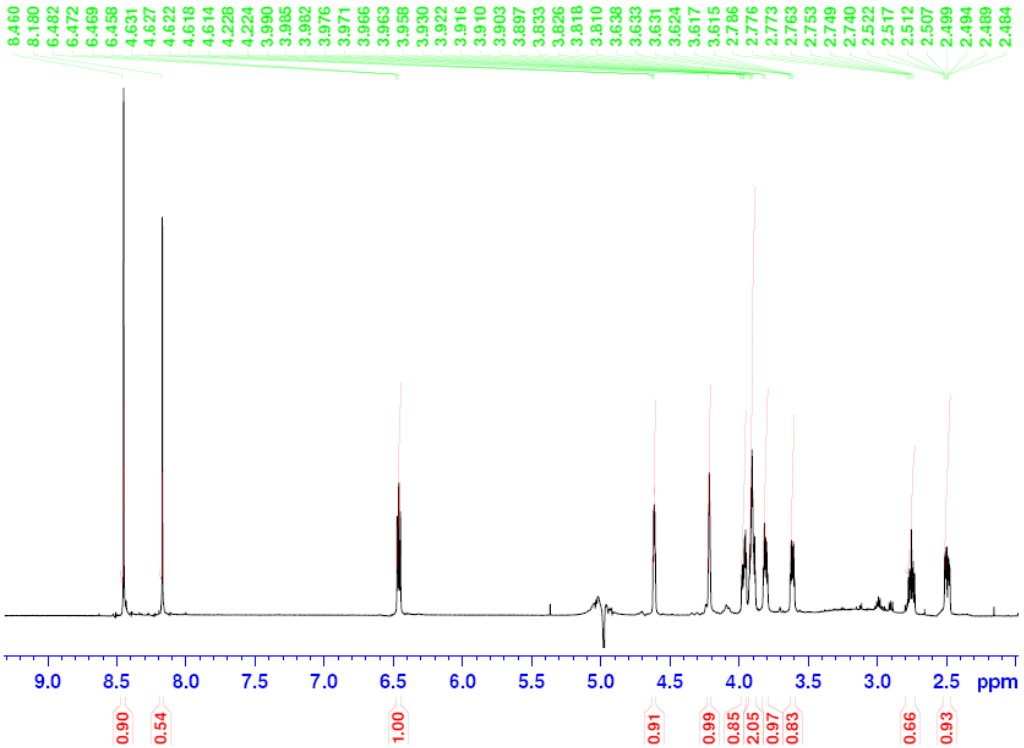
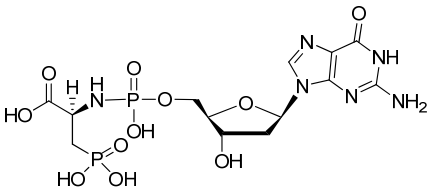
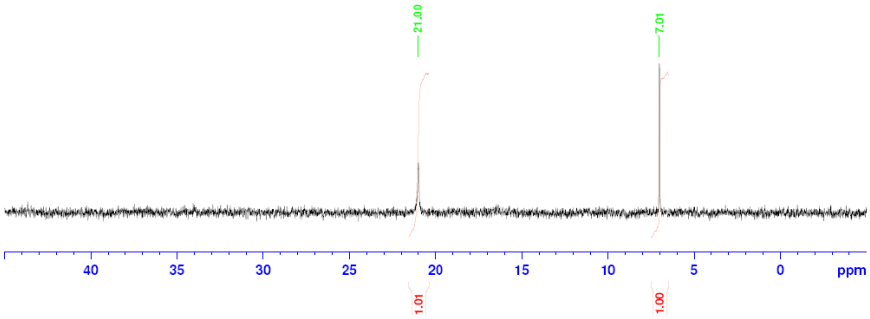


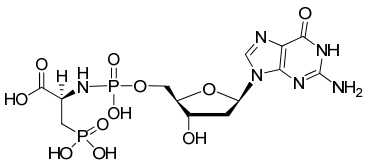


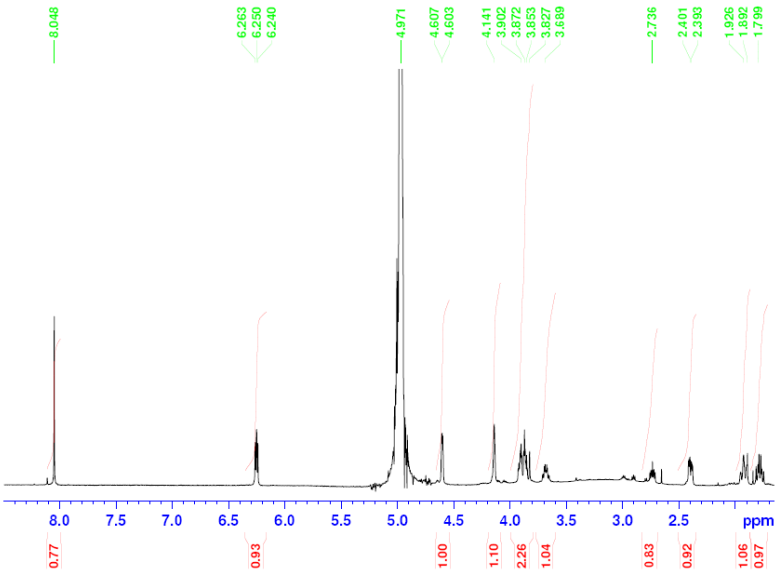
2′-Deoxyguanosine-5′-(3-phosphono-L-alanine) phosphoramidate (**6**) ^{31}P NMR



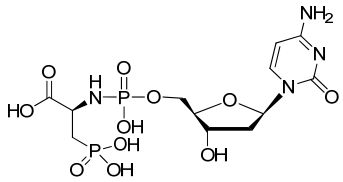


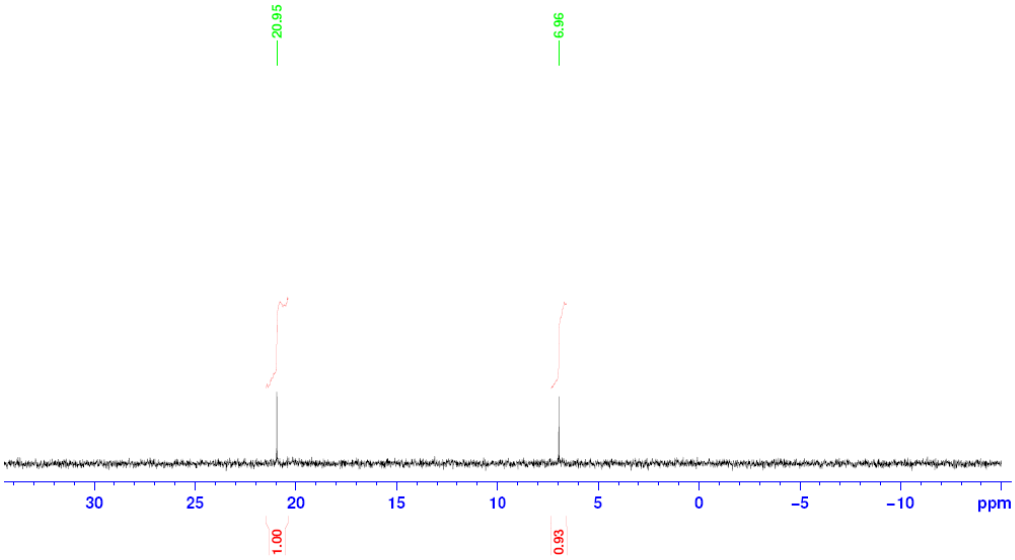
2′-Deoxyguanosine-5′-(3-phosphono-L-alanine) phosphoramidate (**6**) ^{1}H NMR[#]



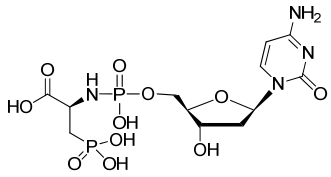


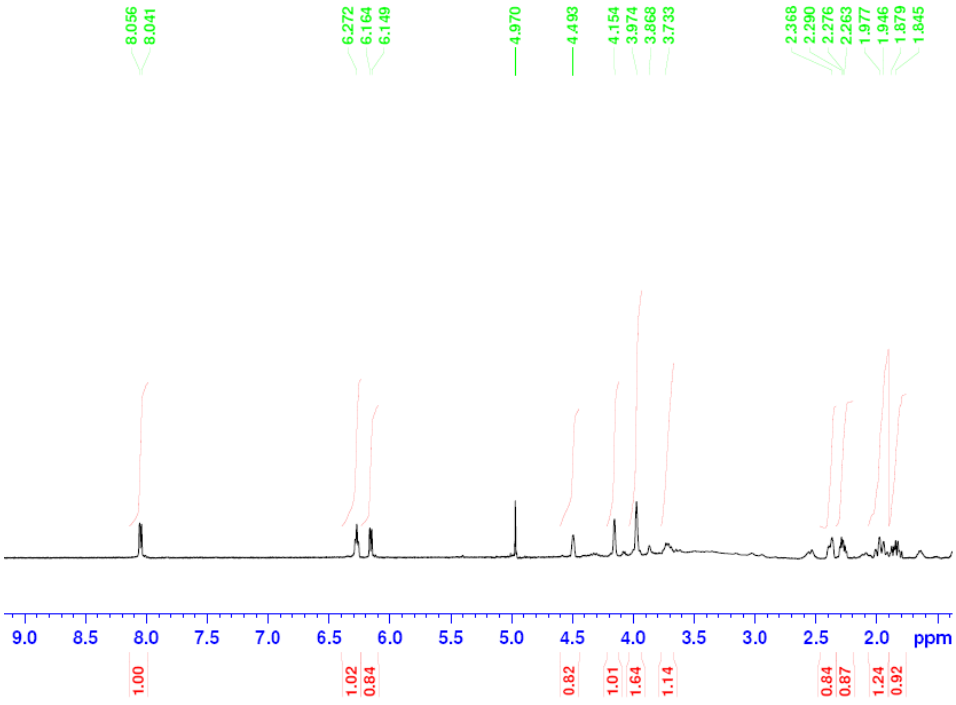
2′-Deoxycytidine-5′-(3-phosphono-L-alanine) phosphoramidate (**7**) ^{31}P NMR



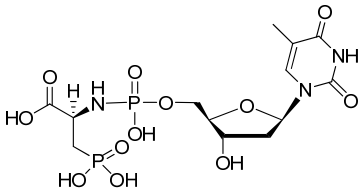


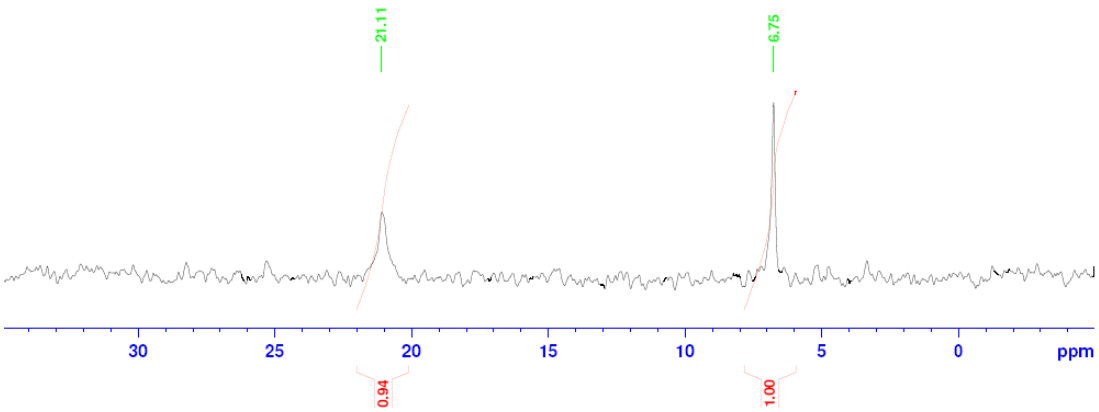
2′-Deoxycytidine-5′-(3-phosphono-L-alanine) phosphoramidate (**7**) ^{1}H NMR[#]



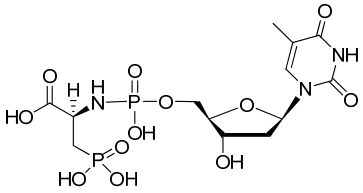


2′-Deoxythymidine-5′-(3-phosphono-L-alanine) phosphoramidate (**8**) ^{31}P NMR

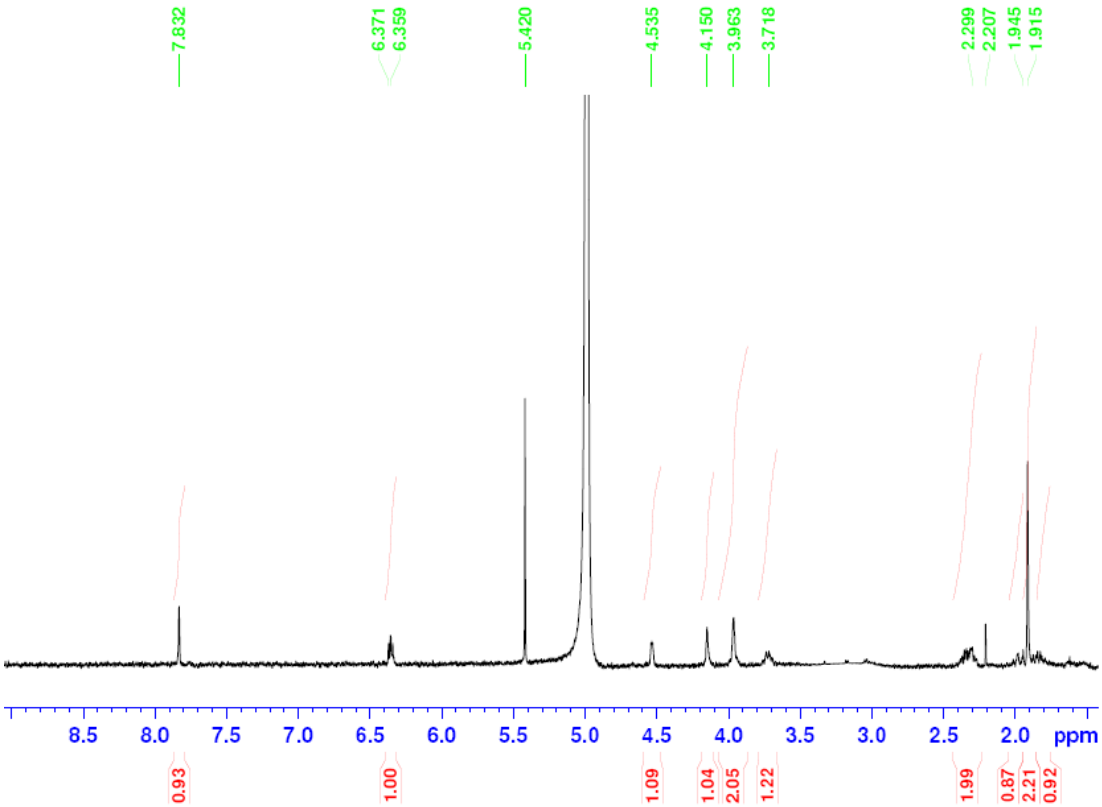




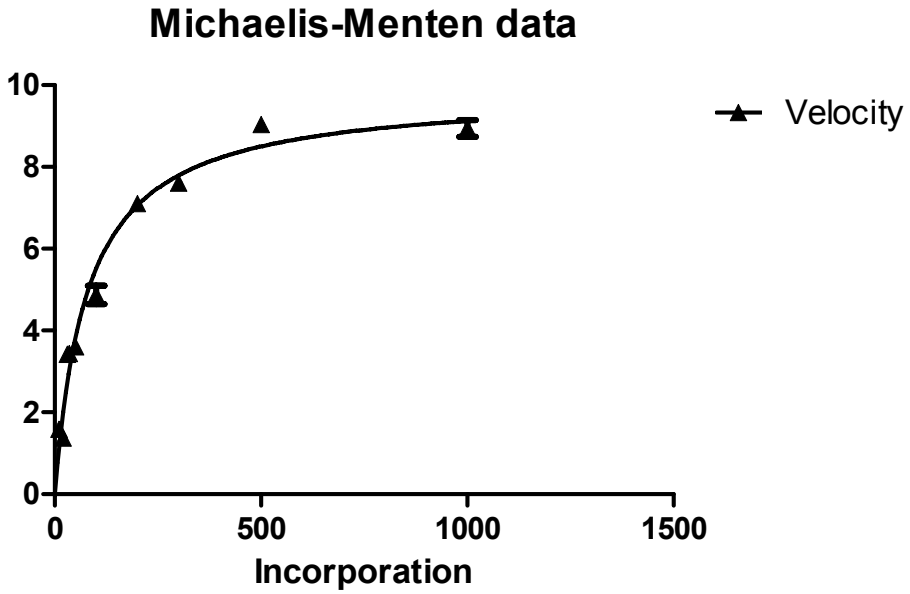
2′-Deoxythymidine-5′-(3-phosphono-L-alanine) phosphoramidate (**8**) ^{1}H NMR[#]



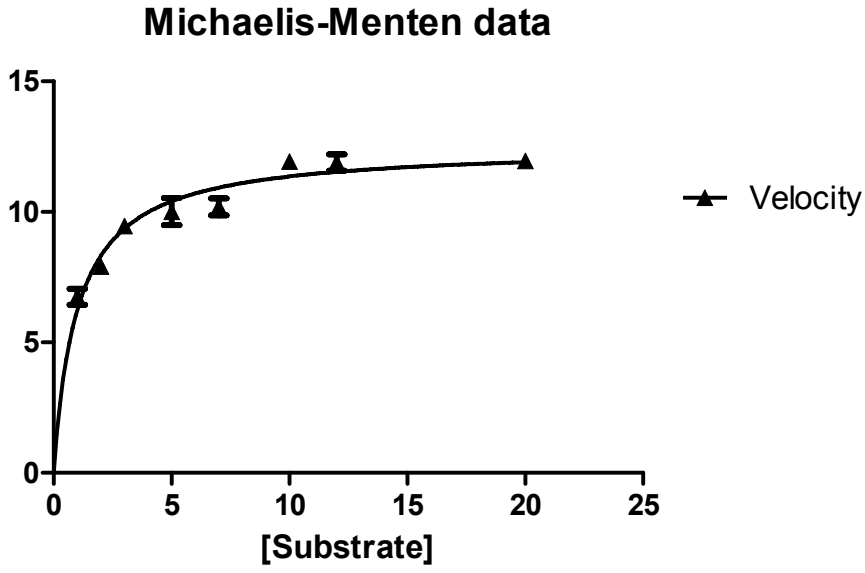
5.420 ppm: CH_2Cl_2 (for washing tube)



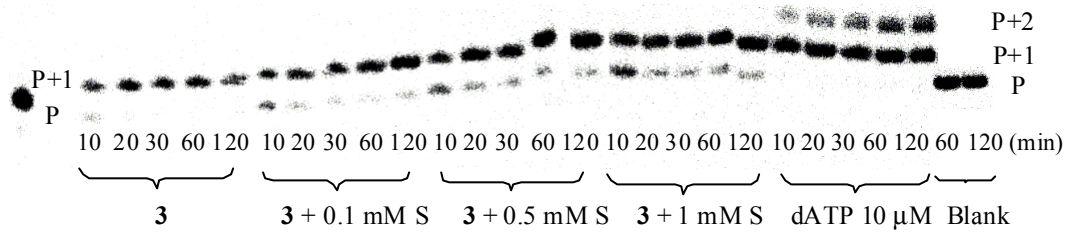
3-Phosphono-L-Ala-dAMP (**3**) steady-state kinetics of single nucleotide incorporation by HIV-1 RT



dATP steady-state kinetics of single nucleotide incorporation by HIV-1 RT:



Product inhibition experiment for compound **3** by HIV-1 RT:

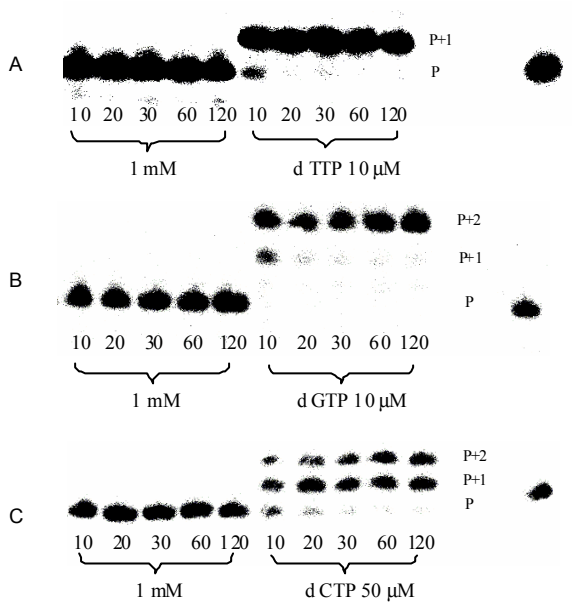


Primer (P_1) 5′-CAGGAAACAGCTATGAC-3′

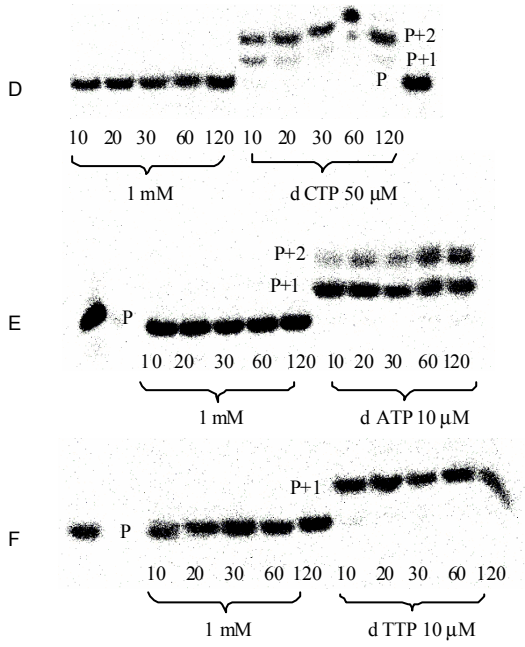
Template (T_1) 5′-CCC**T**GTCATAGCTGTTTCCTG-3′

Figure 1S. Single incorporation of the primer of P_1T_1 (125 nM) by HIV-1 RT with phosphoramidate substrate concentrations and time intervals (min) as indicated, [HIV-1 RT] = 0.025 U/µL; incorporation of **3** (50 µM) and (0.1 mM, 0.5 mM, 1 mM) 3-phosphono-L-alanine (S), respectively; Blank: 125 nM primer/template (P_1T_1), [HIV-1 RT] = 0.025 U/µL and no substrate; dATP (10 µM) is used as reference.

Compound **3** (A against A, C, G,)



Compound **6** (G against G, T, A)



Compound **8 (**T against C, T, G)

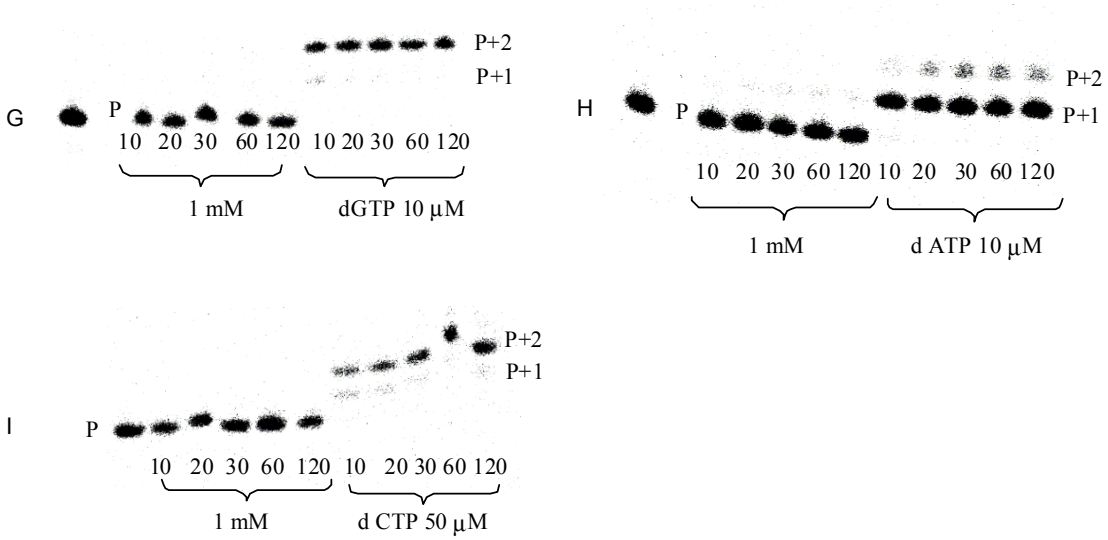


Figure 2S. Control experiments for compounds **3, 6** and **8** by HIV-1 RT with phosphoramidate substrate concentrations and time intervals (min) as indicated, [HIV-1 RT] =0.025 U/µL; 10 µM dATP, 10 µM 10 µM dTTP, 10 µM dGTP and 50 µM dCTP are used as reference.

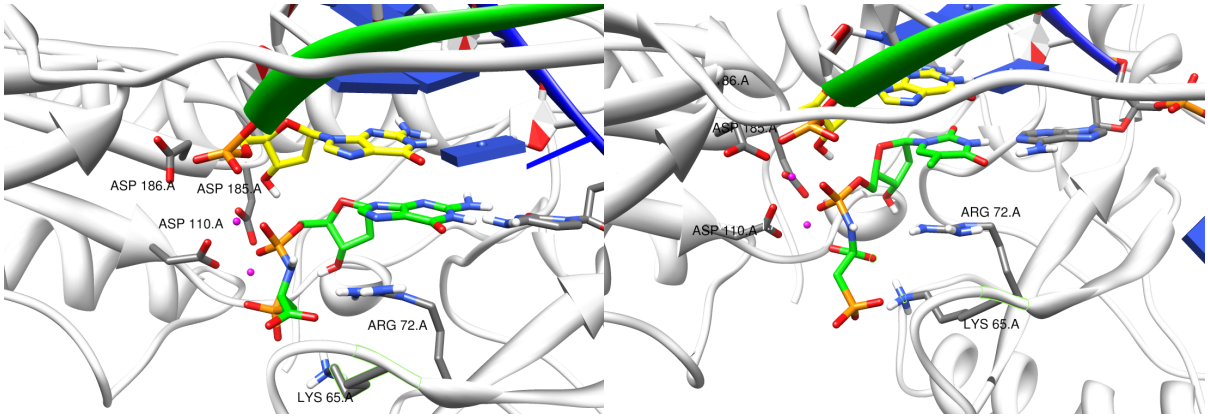


Figure 3S. Model structures of 3-phosphono-L-Ala-dNMP (N = G and T, respectively) in the RT dNTP pocket. The primer strand is drawn with a green ribbon; the template strand has a blue ribbon. The residues Asp 110, 185 and 186 anchor the 2 Mg^{2+} ions (purple balls). Some distances between charged atoms are indicated. The first nucleic acid of the primer strand (yellow carbons) and the complementary residue to 3-phosphono-L-Ala-dNMP is shown in stick representation. Possible stabilization of the carboxyl function and the phosphonate function in the leaving group by Arg 72 and Lys 65 is indicated. Figures are generated using Chimera.[16]