Supplementary Information

Synthesis of Nucleoside 5'-*O*-α,β-Methylene-β-Triphosphates and Evaluation of Their Potency Towards Inhibition of HIV-1 Reverse Transcriptase

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1. General Information. All solid-phase reactions were carried out in Bio-Rad polypropylene columns by shaking and mixing using a Glass-Col small tube rotator in dry conditions at room temperature unless otherwise stated. Real-time monitoring of loading of compounds on resin beads was carried out with a Thermo-Nicolet 550 FT-IR spectrophotometer using OMNIC software. The chemical structures of final products were characterized by nuclear magnetic resonance spectrometry (^{1}H NMR, ^{13}C NMR, ^{31}P NMR) determined on a Bruker NMR spectrometer (400 MHz). ^{13}C NMR spectra are fully decoupled. Chemical shifts are reported in parts per millions (ppm). The chemical structures of final products were confirmed by a high-resolution PE Biosystems Mariner API time-of-flight electrospray mass spectrometer and quantitative phosphorus analysis. The substitution of the resins for each step was estimated from the weight gain of the resin. Total isolated yields for final products were calculated based on the loading of aminomethyl polystyrene resin-bound α,β-methylene-β-triphosphitylating reagent **8** and the amount of nucleoside 5′-*O*-α,β-methylene-β-triphosphates products. The polymer-bound *p*-acetoxybenzyl alcohol (**7**) was synthesized according to the previously reported procedure.[24] The synthesis of phosphitylating reagents **6** was carried out under extremely dry conditions and nitrogen. C_{18} Sep-Pak columns were purchased from Altech (Cat. No. 20924).

2. Preparation of methylene[bis(2-cyanoethoxy)phosphite]-*O*-[2-cyanoethoxychlorophosphite]-*N*,*N*-diisopropylaminophoramidite (6). Bis(dichlorophosphino)methane (**1**, 979 µL, 7.2 mmol) and 2,6-lutidine (839 µL, 7.2 mmol) were added to anhydrous THF (25 mL). Then diisopropylamine (1,008 µL, 7.2 mmol) was added dropwise in 5 min to the solution and the mixture was stirred for 45 min at 0 °C to yield dichlorophosphino *N*,*N*-diisopropylaminechlorophosphino methane (**2**). 2,6-Lutidine (1,678 µL, 14.4 mmol) and 3-hydroxypropionitrile (984 µL, 14.4 mmol) were added dropwise in 10 min to the solution of **2** and the mixture was stirred for 20 min at 0 °C to yield bis(2-cyanoethoxy)phosphino *N*,*N*-diisopropylaminechlorophosphino methane (**3**). Water (130 µL, 7.2 mmol) and 2,6-lutidine (839 µL, 7.2

mmol) were added dropwise in 5 min period to the solution. The mixture was stirred for 10 min at 0 °C to yield bis[2-cyanoethoxy]phosphino *N*,*N*-diisopropylaminehydroxyphosphino methane (**4**). In a separate reaction vessel, phosphorus trichloride (630 μL, 7.2 mmol) and 2,6-lutidine (839 μL, 7.2 mmol) were added to anhydrous THF (25 mL). 3-Hydroxypropionitrile (492 μL, 7.2 mmol) was added dropwise in 10 min to the solution and the mixture was stirred for 25 min at 0 °C to yield 2-cyanoethoxyphosphorodichloridite (**5**). The contents of reaction vessel containing **4** and 2,6-lutidine (839 μL, 7.2 mmol) were added dropwise in 5 min to the solution of **5** and the mixture was stirred for 30 min at 0 °C to yield **6**, which was immediately used in coupling reactions with polymer-bound *p*-acetoxybenzyl alcohol **7**. HR-MS (ESI-TOF) (*m/z*): calcd. for $C_{16}H_{28}ClN_4O_4P_3$ 469.1012; found, 469.1674 $[M + H]^+$; *Anal.* Calcd, P 19.82%; found 19.97%.

3. Preparation of polymer-bound α,β-methylene-β-triphosphitylating reagent: Polymer-bound methylene[bis(2-cyanoethoxy)phosphite]-*O*-[2-cyanoethoxyphosphite]-*N*,*N*-diisopropylaminophoramidite (8). The prepared reaction mixture containing **6** in THF (~7.2 mmol) was added to a swollen solution of polymer-bound *p*-acetoxybenzyl alcohol **7** (9.231 g, 0.65 mmol/g) and 2,6-lutidine (839 μL, 7.2 mmol). The mixture was shaken for 24 h at room temperature. The resin was collected by filtration, washed with THF (3 × 25 mL), DCM (3 × 25 mL), and MeOH (3 × 25 mL), respectively, and was dried overnight under vacuum to give **8** (11.658 g, 93%, 0.48 mmol/g). IR (cm^{-1}): 1757 (C=O ester), 1028 (P-O-C), 2261 (CN).

4. Solid-phase α,β-methylene-β-triphosphorylation of unprotected nucleosides using polymer-bound α,β-methylene-β-triphosphitylating reagent (8).

4.1. Preparation of polymer-bound nucleoside 5′-*O*-α,β-methylene-β-triphosphite derivatives (9a–f). Unprotected nucleosides (**a–f**, 4.0 mmol) and 5-(ethylthio)-1*H*-tetrazole (260 mg, 2.0 mmol) were added to **8** (1943 mg each, 0.48 mmol/g) in anhydrous THF (2 mL) and DMSO (3 mL) in case of 3′-azido-3′-deoxythymidine, 3'-fluoro-3'-deoxythymidine, 2',3'-didehydro-2',3'-dideoxythymidine, and inosine or in anhydrous DMSO (5 mL) in case of adenosine and cytidine. The mixtures were shaken for 60 h at room temperature. The resins were collected by filtration, washed with DMSO (3 × 30 mL), THF (3 × 30 mL), and MeOH (3 × 35 mL), respectively, and dried under vacuum to give **9a–f** (2053-2092 mg), IR (cm^{-1}): **9a**: 3338 (OH), 2258 (CN), 1755 (C=O ester), 1028 (P-O-C); **9b**: 2258 (CN and N_3), 1760 (C=O ester), 1028 (P-O-C); **9c**: 2251 (CN), 1753 (C=O ester), 1028 (P-O-C); **9d**: 2252 (CN), 1753 (C=O ester), 1028 (P-O-C); **9e**: 3325 (OH), 2257 (CN), 1759 (C=O ester), 1027 (P-O-C); **9f**: 3333 (OH), 2252 (CN), 1759 (C=O ester), 1028 (P-O-C).

4.2. Oxidation of polymer-bound nucleoside 5′-*O*-α,β-methylene-β-triphosphite triester derivatives (9a–f) to polymer-bound nucleoside 5′-*O*-α,β-methylene-β-triphosphosphate triester derivatives (10a–f). *t*-Butyl hydroperoxide in decane (5-6 M, 2.2 mL) was added to the swollen resins **9a–f** (2053-2092 mg) in THF (5 mL). After 2.5 h of shaking at room temperature, the resins were collected by filtration, washed with THF (3 × 25 mL) and MeOH (3 × 25 mL), respectively, and were dried overnight at room temperature under vacuum to give **10a–f** (2094-2132 mg).

4.3. Preparation of polymer-bound nucleosides 5′-*O*-α,β-methylene-β-triphosphosphate diester derivatives (11a–f). DBU (1.79 mL, 12.0 mmol) was added to the swollen resins **10a–f** (2094-2132 mg) in THF (5 mL). After 48 h of shaking the mixtures at room temperature, the resins were collected by filtration, washed with THF (3 × 35 mL) and MeOH (4 × 35 mL), respectively, and were dried overnight at room temperature under vacuum to give **11a–f** (1956-1998 mg). IR (cm^{-1}): **11a**: 3328 (OH),

1752 (C=O ester), 1028 (P-O-C); **11b**: 2256 (N_3), 1757 (C=O ester), 1028 (P-O-C); **11c**: 1755 (C=O ester), 1027 (P-O-C); **11d**: 1746 (C=O ester), 1028 (P-O-C); **11e**: 3339 (OH), 1756 (C=O ester), 1028 (P-O-C); **11f**: 3326 (OH), 1747 (C=O ester), 1028 (P-O-C).

4.4. Preparation of nucleoside 5′-*O*-α,β-methylene-β-triphosphosphates (15a–f). The resins **11a–f** (1956-1998 g) were swollen in anhydrous DCM (20 mL) and filtered. To the swollen resins was added DCM/TFA/water/EDT (72.5:23:2.5:2 v/v/v/v, 5 mL). After 35 min of shaking the mixtures at room temperature, the resins were collected by filtration and washed with DCM (10 mL), THF (10 mL), and MeOH (10 mL), respectively. The solvents of filtrate solutions were evaporated at -20 °C. The residues were mixed with Amberlite AG-50W-X8 (100-200 mesh, hydrogen form, 1.0 g, 1.7 meq/g) in water/dioxane (75:25 v/v, 5 mL) for 25 min. After filtration, the solvents were removed using lyophilization and the crude products were purified on C_{18} Sep-Pak using appropriate solvents. In general, the crude products were eluted on 1-in. (2.5-cm) C_{18} Sep-Pak columns with 85:15 to 45:55 (v/v) water/methanol and then 30:70 to 75:25 (v/v) DCM/methanol. The solvents were evaporated and the residues were dried under vacuum at –20 °C to yield **15a–f**. The purity and total isolated yields for **15a–f** are shown in Table 1. The compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, high resolution mass spectrometer (ESI-TOF), and quantitative phosphorus elemental analysis. Resin **13** was reacted with a solution of potassium hydroxide in water/dioxane (65:35v/v, 5 mL, 1N) in 45 min at room temperature to yield **14**. The sharp peak of ~1750 (C=O ester) was disappeared. IR (cm^{-1}): 3325 (broad, OH).

4.5. Characterization of nucleoside 5′-*O*-α,β-methylene-β-triphosphosphates (15a–f).

Adenosine-5'-*O*-α,β-methylene-β-triphosphate (15a). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.14 (t, $J_{H,P}$ = 20.0 Hz, C$H_{2\alpha,\beta}$, 2H), 3.48–3.61 (m, H-5', 1H), 3.62–3.73 (m, H-5", 1H), 3.91–4.01 (m, H-4', 1H), 4.11–4.22 (m, H-3', 1H), 4.54–4.68 (m, H-2', 1H), 5.15–5.24 (m, O*H*, 1H), 5.38–5.52 (m, O*H*, 2H), 5.83–5.96 (m, H-1', 1H), 7.30–7.42 (br s, 6-NH_2, 2H), 8.17 (s, H-2, 1H), 8.38 (s, H-8, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 29.98 (dd, $J_{CP\beta}$ = 130.0 Hz, $J_{CP\alpha}$ = 125.0 Hz, CH$_{2\alpha,\beta}$), 62.54 (C-5'), 71.54 (C-3'), 74.31 (C-2'), 86.77, 88.80 (C-4', C-1'), 120.22 (C-5), 140.82 (C-8), 149.90 (C-4), 153.25 (C-2), 157.01 (C-6); ^{31}P NMR (in DMSO-d_6 and H_3PO_4 85% in water as external standard, 162 MHz, δ ppm): 3.14 (d, $J_{\beta,\gamma}$=29.2 Hz, OP_γ), 7.19 (dd, $J_{\beta,\gamma}$ = 29.2 Hz, $J_{\alpha,\beta}$ = 6.5 Hz, P_β), 14.00 (d, $J_{\alpha,\beta}$ = 6.5 Hz, CH$_2P_\alpha$); HR-MS (ESI-TOF) (*m/z*): calcd. for $C_{11}H_{18}N_5O_{12}P_3$, 505.0165; found, 506.0005 $[M + H]^+$; *Anal.* Calcd. P 18.39 %; found 18.24%.

3′-Azido-3′-deoxy-5′-thymidine-5'-*O*-α,β-methylene-β-triphosphate (15b). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.71–1.83 (br s, 5-CH_3, 3H), 2.24 (t, $J_{H,P}$ = 20.0 Hz, C$H_{2\alpha,\beta}$, 2H), 2.18–2.31 (m, H-2', 1H), 2.32–2.44 (m, H-2", 1H), 3.50–3.69 (m, H-5' and H-5", 2H), 3.71–3.88 (m, H-4', 1H), 4.29–4.48 (m, H-3', 1H), 5.10–5.27 (br s, O*H*, 1H), 6.01–6.16 (m, H-1', 1H), 7.62–7.80 (m, H-6, 1H), 11.21–11.37 (br s, N*H*, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 13.06 (5-CH$_3$), 28.13 (dd, $J_{CP\beta}$ = 136.5 Hz, $J_{CP\alpha}$ = 130.0 Hz, CH$_{2\ \alpha,\beta}$), 37.09 (C-2'), 61.01 (C-3'), 61.65 (C-5'), 84.29 (C-4'), 84.87 (C-1'), 110.38 (C-5), 136.89 (C-6), 151.27 (C-2 C=O), 164.58 (C-4 C=O); ^{31}P NMR (in DMSO-d_6 and H_3PO_4 85% in water as external standard, 162 MHz, δ ppm): 3.54 (d, $J_{\beta,\gamma}$ = 29.2 Hz, OP_γ), 6.33 (dd, $J_{\beta,\gamma}$ = 29.2 Hz, $J_{\alpha,\beta}$ = 6.5 Hz, P_β), 14.98 (d, $J_{\alpha,\beta}$ = 6.5 Hz, CH$_2P_\alpha$); HR-MS (ESI-TOF) (*m/z*): calcd. for $C_{11}H_{18}N_5O_{12}P_3$, 505.0165; found, 506.0041 $[M+H]^+$; *Anal.* Calcd. P 18.39 %; found 18.58%.

3'-Fluoro-3'-deoxythymidine-5'-*O*-α,β-methylene-β-triphosphate (15c). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.75–1.90 (br s, 5-CH_3, 3H), 2.18 (t, $J_{H,P}$ = 20.0 Hz, C$H_{2\alpha,\beta}$, 2H), 2.24–2.62 (m, H-2' and

H-2", 2H), 3.52–3.81 (m, H-5' and H-5", 2H), 4.07–4.36 (m, H-4', 1H), 5.20–5.59 (m, H-3′, O*H*, 2H), 6.18–6.43 (br s, H-1', 1H), 7.60–7.80 (br s, H-6, 1H), 11.31–11.59 (br s, N*H*, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 13.10 (5-*C*H$_3$), 30.43 (dd, $J_{CP\beta}$ = 136.5 Hz, $J_{CP\alpha}$ = 130 Hz, *C*H$_{2\ \alpha,\beta}$), 37.61 (C-2'), 61.69 (C-5'), 84.44 (C-4'), 85.52 (d, $J_{1',F}$ = 23 Hz, C-1'), 95.58 (d, $J_{3',F}$ = 172 Hz, C-3'), 110.47 (C-5), 136.41 (C-6), 151.12 (C-2 *C*=O), 164.26 (C-4 *C*=O); ^{31}P NMR (in DMSO-d_6 and H_3PO_4 85% in water as external standard, 162 MHz, δ ppm): 4.28 (d, $J_{\beta,\gamma}$ = 29.2 Hz, O*P*$_\gamma$), 7.59 (dd, $J_{\beta,\gamma}$=29.2 Hz, $J_{\alpha,\beta}$= 6.5 Hz, *P*$_\beta$), 16.08 (d, $J_{\alpha,\beta}$ = 6.5 Hz, CH$_2$*P*$_\alpha$); HR-MS (ESI-TOF) (*m/z*): calcd. for $C_{11}H_{18}FN_2O_{12}P_3$, 482.0057; found, 483.0013 $[M + H]^+$; *Anal.* Calcd. P 19.27%; found 19.04%.

2',3'-Didehydro-2',3'-dideoxythymidine-5'-*O*-α,β-methylene-β-triphosphate (15d). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.61–1.85 (br s, 5-C*H*$_3$, 3H), 2.16 (t, $J_{H,P}$ = 20.0 Hz, C*H*$_{2\alpha,\beta}$, 2H), 3.49–3.73 (m, H-5' and H-5", 2H), 4.61–4.84 (m, H-4', 1H), 4.88–5.13 (m, O*H*, 1H), 5.75–6.05 (m, H-2', 1H), 6.30-6.51 (m, H-3', 1H), 6.69–6.97 (m, H-1', 1H), 7.52–7.79 (m, H-6, 1H), 11.11–11.45 (br s, N*H*, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 13.01 (5-*C*H$_3$), 28.36 (dd, $J_{CP\beta}$ = 133 Hz, $J_{CP\alpha}$ = 127 Hz, *C*H$_{2\ \alpha,\beta}$), 63.16 (C-5'), 88.22 (C-4'), 89.80 (C-1'), 109.93 (C-5), 126.94 (C-2'), 135.92 (C-3'), 137.75 (C-6), 151.79 (C-2 *C*=O), 164.87 (C-4 *C*=O); ^{31}P NMR (in DMSO-d_6 and H_3PO_4 85% in water as external standard, 162 MHz, δ ppm): 3.44 (d, $J_{\beta,\gamma}$ = 29.2 Hz, O*P*$_\gamma$), 5.59 (dd, $J_{\beta,\gamma}$=29.2 Hz, $J_{\alpha,\beta}$= 6.5 Hz, *P*$_\beta$), 15.10 (d, $J_{\alpha,\beta}$ = 6.5 Hz, CH$_2$*P*$_\alpha$); HR-MS (ESI-TOF) (*m/z*): calcd. for $C_{11}H_{17}N_2O_{12}P_3$, 461.9994; found, 461.0448 $[M - H]^-$; *Anal.* Calcd. P 20.10%; found 19.94%.

Cytidine-5'-*O*-α,β-methylene-β-triphosphate (15e). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.09 (t, $J_{H,P}$ = 20.0 Hz, C*H*$_{2\alpha,\beta}$, 2H), 4.01–4.26 (m, H-5', H-5", 2H), 4.27–4.38 (m, H-4', 1H), 4.39–4.52 (m, H-2', H-3', 2H), 5.25–5.46 (br s, 2H, O*H*), 5.53–5.69 (br s, 1H, O*H*), 5.85–6.04 (m, H-1', H-5, 2H), 7.07–7.28 (br s, N*H*$_2$, 2H), 7.47–7.62 (m, H-6, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 27.76 (dd, $J_{CP\beta}$ = 124

Hz, $J_{CP\alpha}$ = 106.0 Hz, $CH_{2\ \alpha,\beta}$), 61.88 (C-5'), 70.74 (C-2'), 75.23 (C-3'), 85.39 (C-4'), 90.23 (C-1'), 95.69 (C-5), 142.79 (C-6), 157.02 (C-2 *C*=O), 166.76 (C-4 *C*=O); ^{31}P NMR (in DMSO-d_6 and H_3PO_4 85% in water as external standard, 162 MHz, δ ppm): 2.73 (d, $J_{\beta,\gamma}$= 29.2 Hz, OP_γ), 6.43 (dd, $J_{\beta,\gamma}$=29.2 Hz, $J_{\alpha,\beta}$= 6.5 Hz, P_β), 14.25 (d, $J_{\alpha,\beta}$= 6.5 Hz, CH_2P_α); HR-MS (ESI-TOF) (*m/z*): calcd. for $C_{10}H_{18}N_3O_{13}P_3$, 481.0052; found, 482.0183 $[M + H]^+$; *Anal.* Calcd. P 19.31%; found 19.47%.

Inosine-5'-*O*-α,β-methylene-β-triphosphate (15f). ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.11 (t, $J_{H,P}$ = 24.0 Hz, C$H_{2\alpha,\beta}$, 2H), 3.50–3.75 (m, H-5', H-5'', 2H), 3.95–4.04 (m, H-4', 1H), 4.11–4.22 (m, H-3', 1H), 4.48–4.53 (m, H-2', 1H), 5.05–5.14 (m, O*H*, 1H), 5.14–5.33 (m, O*H*, 1H), 5.48–5.61 (m, O*H*, 1H), 5.82–5.94 (m, H-1', 1H), 8.12 (s, H-2, 1H), 8.37 (s, H-8, 1H), 12.37-12.46 (br s, O*H*, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 30.11 (dd, $J_{CP\beta}$ = 122.0 Hz, $J_{CP\alpha}$ = 112.0 Hz, $CH_{2\ \alpha,\beta}$), 62.31 (C-5'), 71.37 (C-2'), 75.21 (C-3'), 86.68 (C-4'), 88.57 (C-1'), 125.39 (C-5), 139.90 (C-8), 147.01 (C-4), 149.21 (C-2), 157.70 (C-6); ^{31}P NMR (in DMSO-d_6 and H_3PO_4 85% in water as external standard, 162 MHz, δ ppm): 2.80 (d, $J_{\beta,\gamma}$= 29.2 Hz, OP_γ), 6.29 (dd, $J_{\beta,\gamma}$=29.2 Hz, $J_{\alpha,\beta}$= 6.5 Hz, P_β), 14.43 (d, $J_{\alpha,\beta}$= 6.5 Hz, CH_2P_α); HR-MS (ESI-TOF) (*m/z*): calcd. for $C_{11}H_{17}N_4O_{13}P_3$, 506.0005; found, 505.0034 $[M - H]^-$; *Anal.* Calcd. P 18.36%; found 18.19%.

Table S1. Overall Isolated Yields and Purity of Crude Products for **15a-f**.

no.	Overall yield (%) calculated from **8**	Weight of pure products (mg)	Purity of crude products
15a	65	307	77
15b	70	331	83
15c	47	212	68
15d	59	255	78
15e	67	302	76
15f	73	344	81

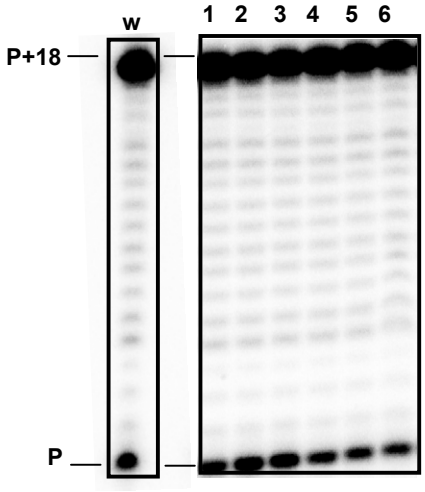
5. Purification of HIV-1 Reverse Transcriptase (RT) and Enzyme Assays. Enzyme p66/p51 HIV-1 RT was purified according to the protocol described in Le Grice *et al.*[26] RNase H assay was performed as previously described[27] using recombinant RT and the previously described RNA/DNA hybrids. Briefly, substrates were generated by 5' end-labeling the 30 nt RNA with γ-^{32}ATP and annealing it to a 40 nt DNA. Hydrolysis was initiated in the presence or absence of the compounds by adding wild type p66/p51 HIV-1 RT to the RNA-DNA hybrids in 10 mM Tris-HCl (pH 8.0), 80 mM NaCl, 5 mM DTT and 6 mM $MgCl_2$, with enzyme and substrate present at final concentrations of 50 and 200 nM, respectively at 37˚C. Reactions were terminated after 10 min by adding an equal volume of a formamide-based gel-loading buffer (95% (v/v) formamide containing 0.1% (w/v) bromophenol blue and xylene cyanol), and the hydrolysis products fractionated by high voltage electrophoresis through 15% (w/v) polyacrylamide gels containing 7 M urea. Products were visualized by autoradiography and/or phosphor-imaging and quantified using Quantity One software (Bio-Rad).

For Kinetic analysis, RNase H activity of the HIV-1 RT was estimated using the substrate at 200 nM and 400 nM in presence of increasing concentrations of compound **15e**. The assays are performed as described above. Dixon plot analysis is performed as described in Figure 2 legend.

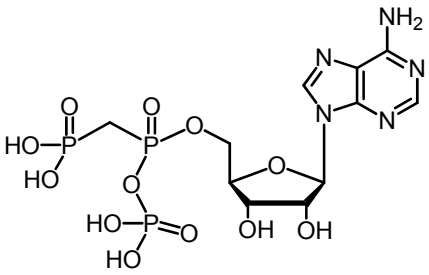
DNA-synthesis was measured on 40 nt DNA template annealed to a 5' end-labeled 22 nt DNA primer as described previously.[28] Reactions were initiated in the presence or absence of the compounds by adding 10 nM enzyme to a mixture containing 50 nM template/primer, 200 μM dNTPs, 10 mM Tris-HCl (pH 8.0), 80 mM NaCl, and 6 mM $MgCl_2$, and terminated after 10 min by adding an equal volume of a formamide-based gel-loading buffer at 37˚C. Reaction products were fractionated by high-voltage electrophoresis through 10% (w/v) polyacrylamide gels containing 7 M urea in Tris/borate/EDTA buffer. After drying, gels were subjected to autoradiography and/or phosphorimaging analysis using a Molecular Imager FX phosphorimager (BioRad, Hercules, CA).

The compounds did not show any inhibition against the polymerase activity. The results of the polymerase assay are presented in Figure S1. Lanes 1-6 represent the polymerase activity in the presence of the compounds **15a-f**, respectively. In comparison to the wild type enzyme (lane w), the polymerase activity was not affected in the presence of these compounds **15a-f** (lanes 1-6).

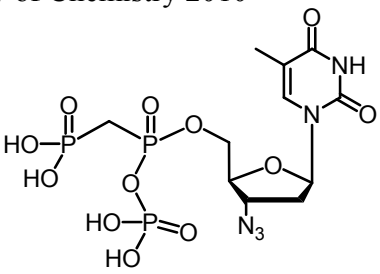
Figure S1. DNA polymerase activity of HIV-1 RT in presence of compounds **15a-e**. Lane w represents no inhibitor, lane 1 (**15a**), lane 2 (**15b**), lane 3 (**15c**), lane 4 (**15d**), lane 5 (**15e**), and lane 6 (**15f**), at 1 mM concentrations. The radiolabeled primer is marked as P, whereas the fully extended product is marked as P+18.



6. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra of nucleoside α,β-methylene-β-triphosphates 15a-f.



15a



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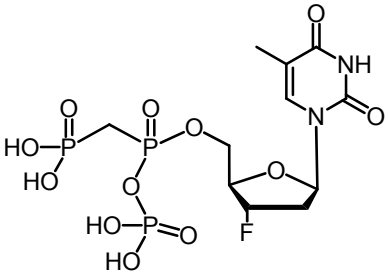

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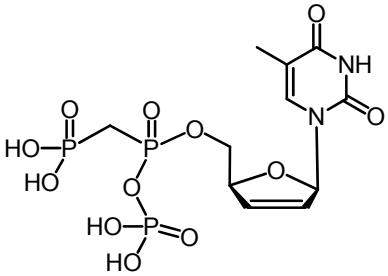

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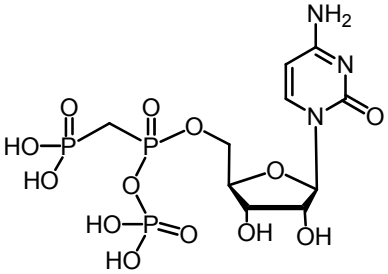
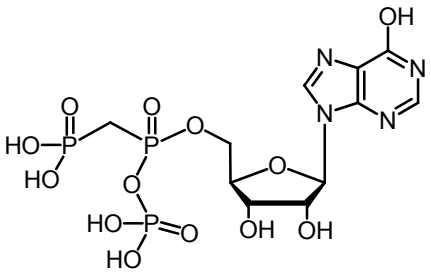
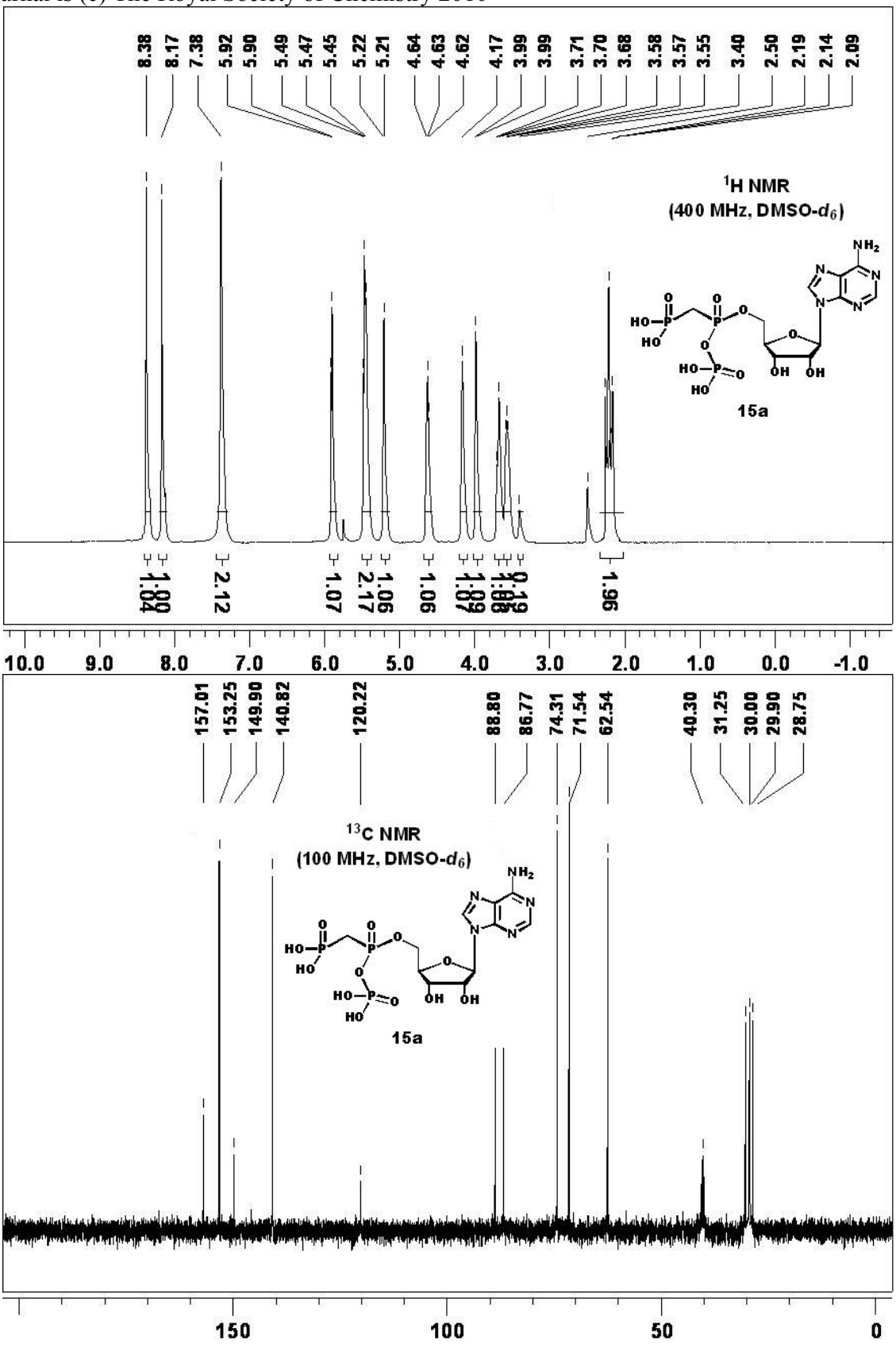
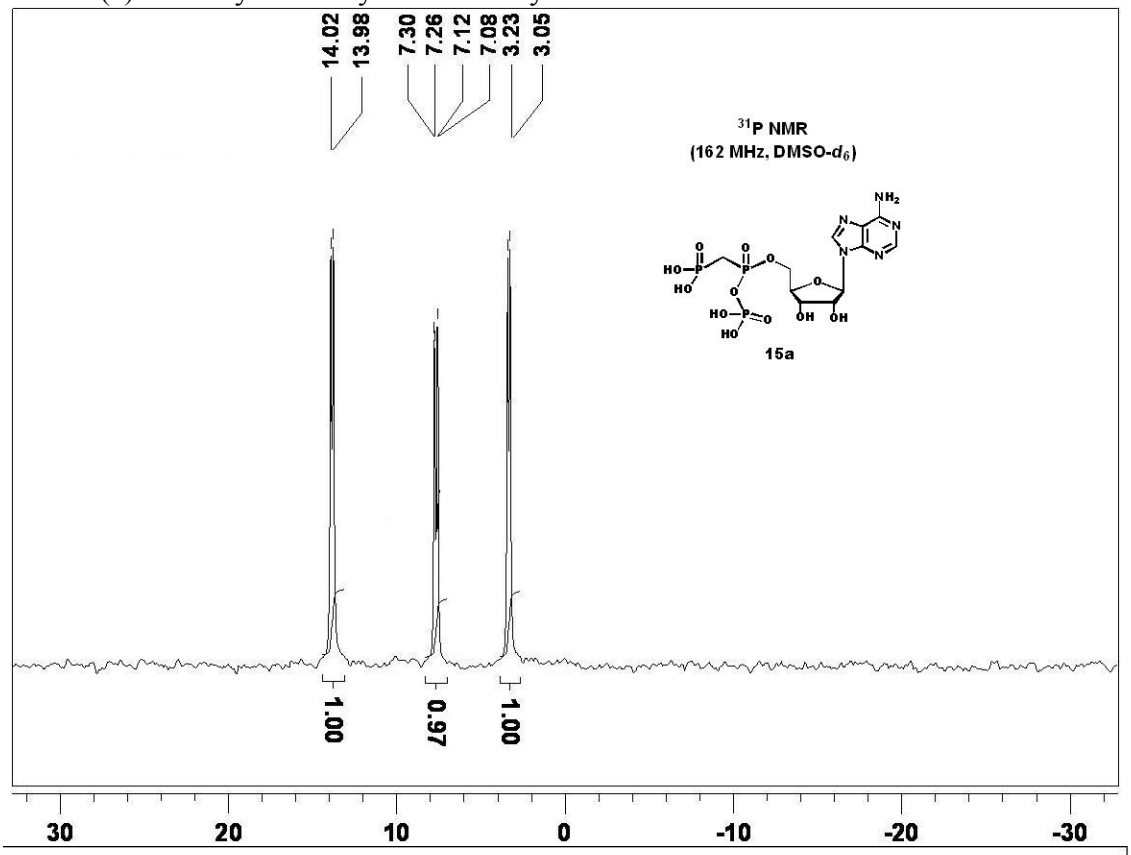


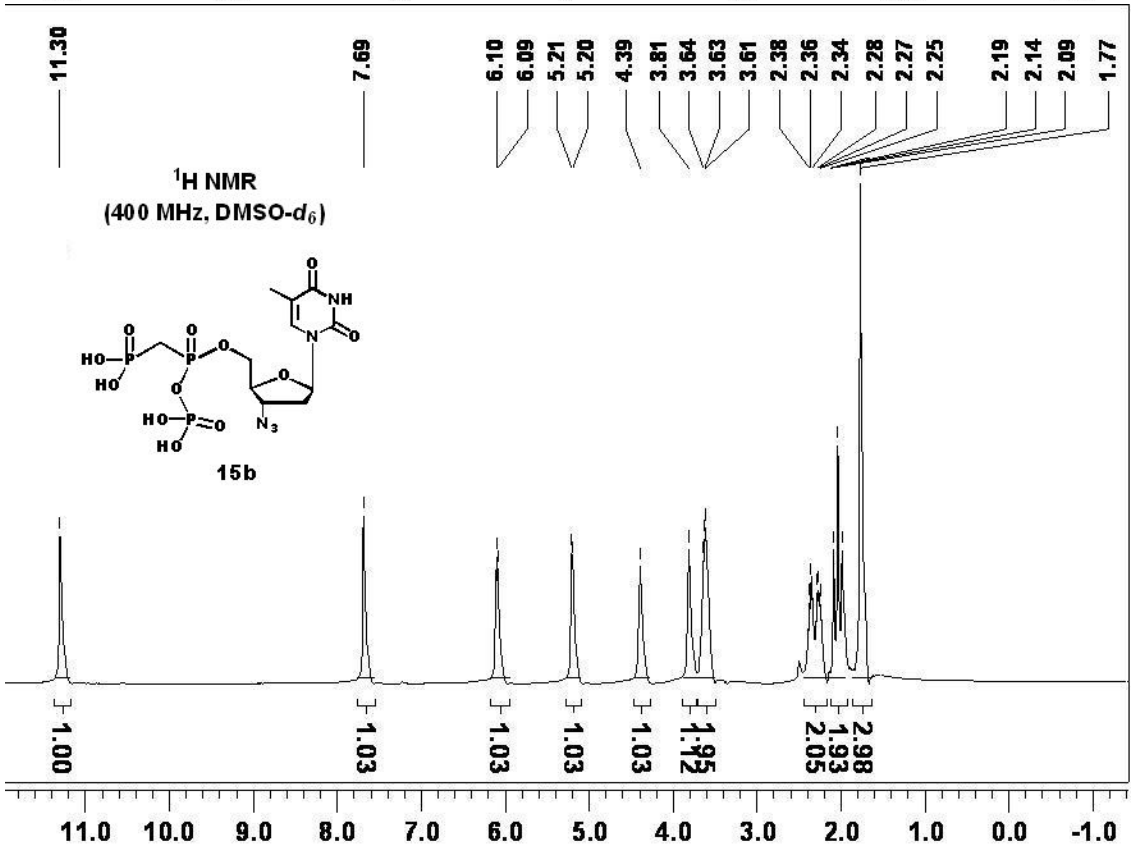
15e

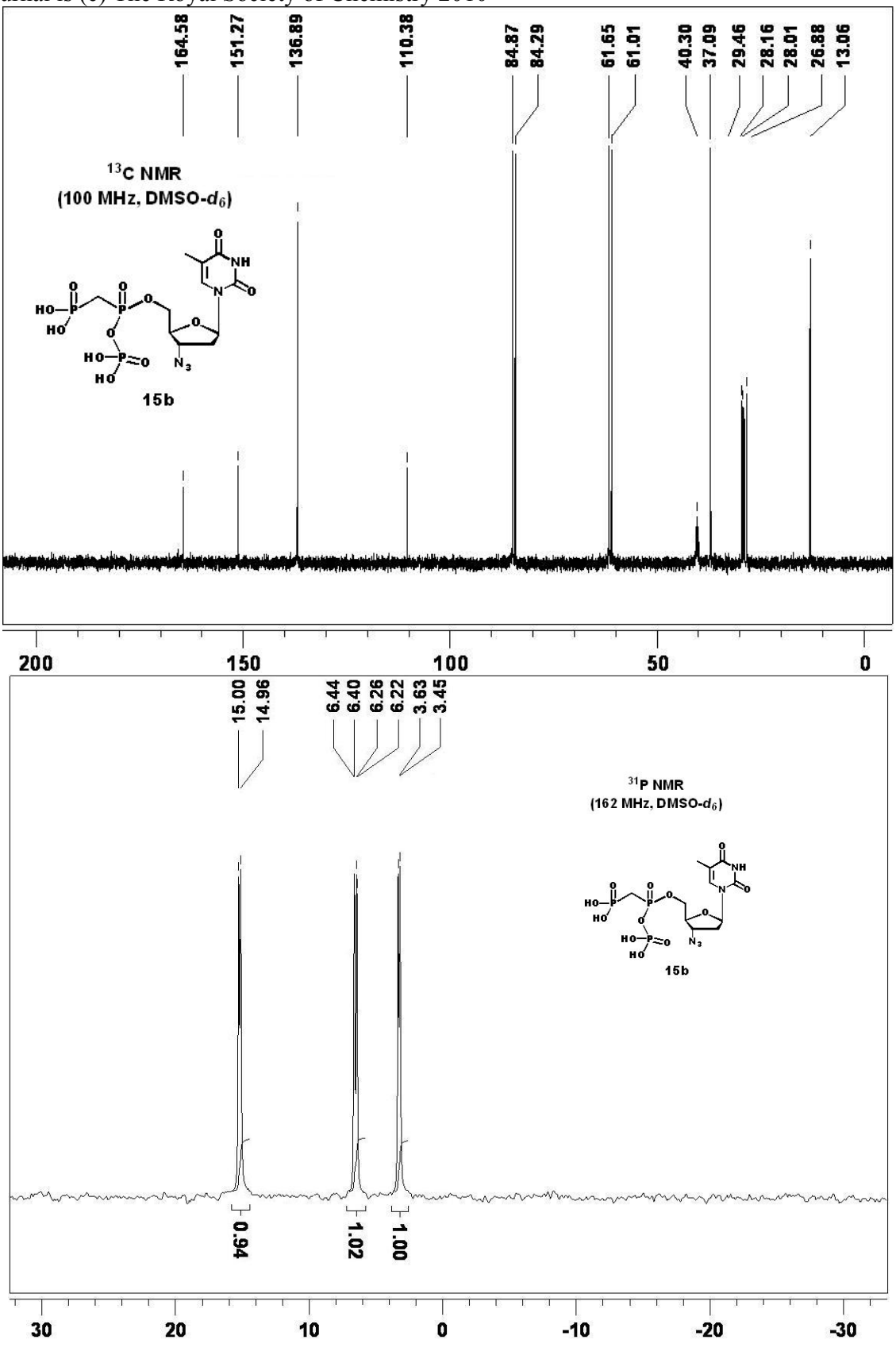


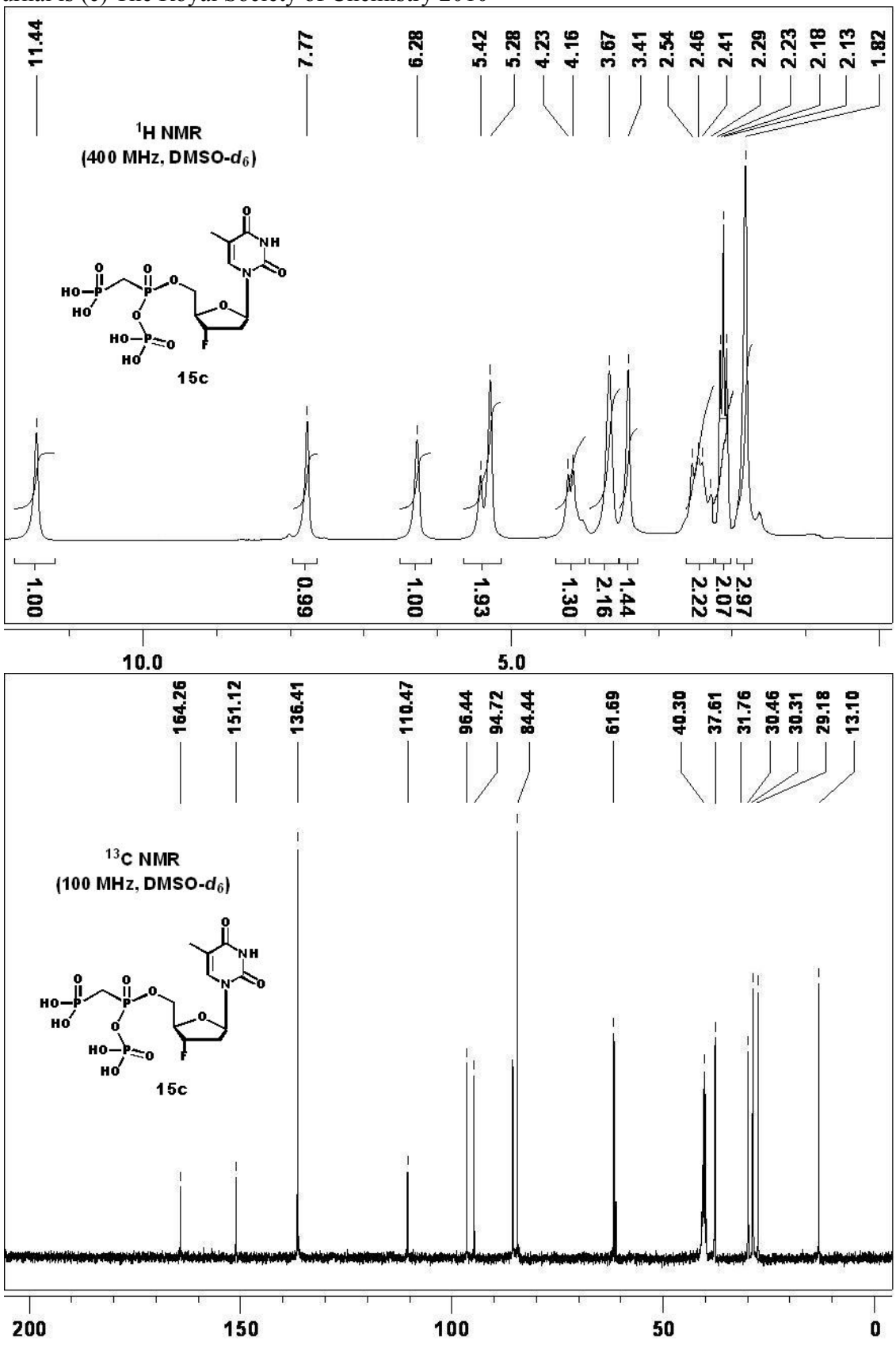
15f

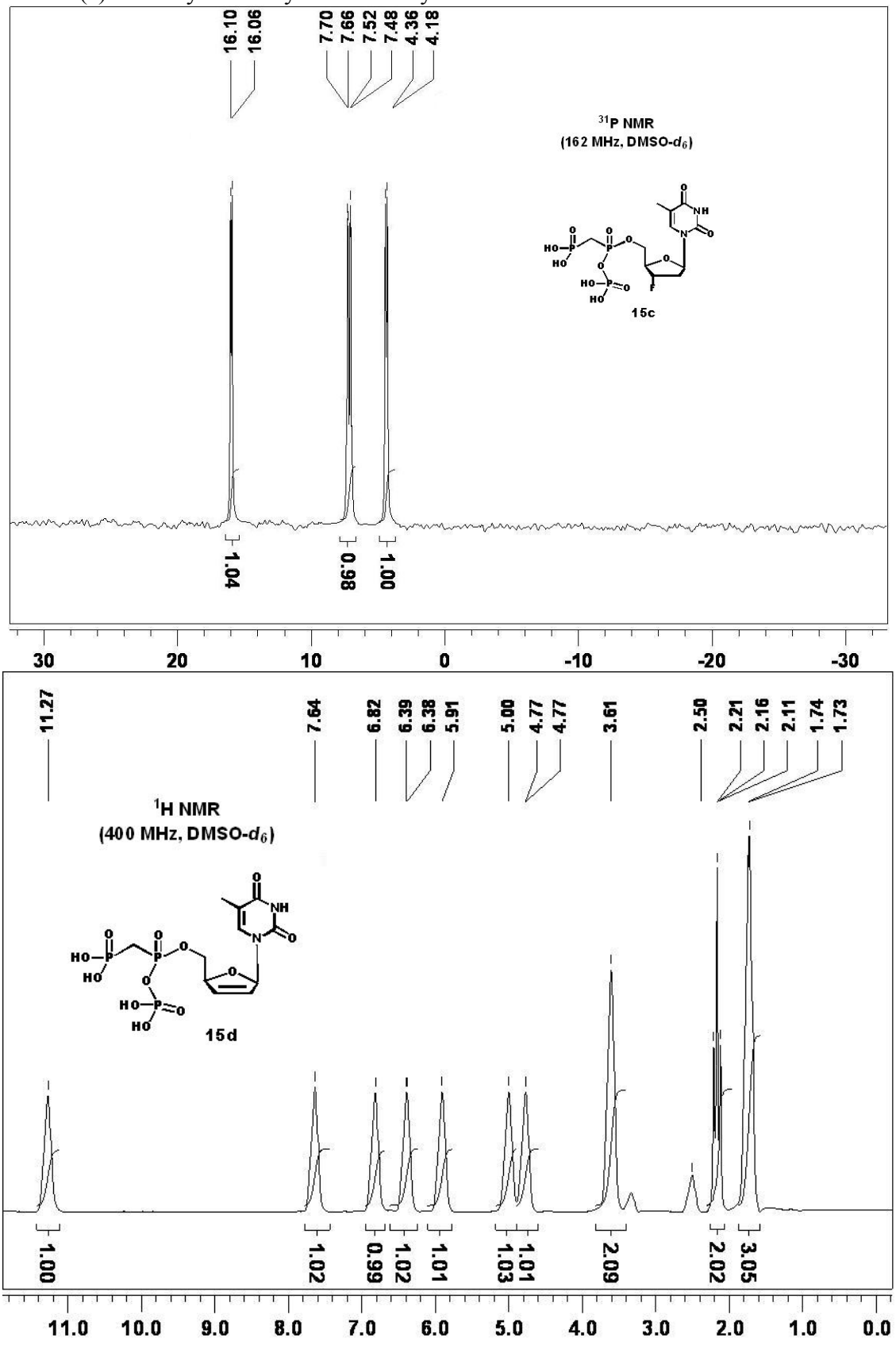











16.10
16.06
7.70
7.66
7.52
7.48
4.36
4.18
31P NMR
(162 MHz, DMSO-d6)
15c
1.04
0.98
1.00
30
20
10
0
-10
-20
-30
11.27
7.64
6.82
6.39
6.38
5.91
5.00
4.77
4.77
3.61
2.50
2.21
2.16
2.11
1.74
1.73
1H NMR
(400 MHz, DMSO-d6)
15d
1.00
1.02
0.99
1.02
1.01
1.03
1.01
2.09
2.02
3.05
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0