Highly specific unnatural base pair system as a third base pair for PCR amplification

Rie Yamashige[1], Michiko Kimoto[1,2], Yusuke Takezawa[1], Akira Sato[1], Tsuneo Mitsui[2], Shigeyuki Yokoyama[1,3], Ichiro Hirao[1,2,]*

[1]RIKEN Systems and Structural Biology Center (SSBC), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan, [2]TagCyx Biotechnologies, 1-6-126 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan, and [3]Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

AUTHOR EMAIL ADDRESS. ihirao@riken.jp

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Biological experimental methods and data.

1. PCR amplification of DNA fragments involving the Ds–Px pair by several DNA polymerases

Supplementary table 1. Amplification folds and retention rates of the **Ds**–**Px** pair after 15 cycles of PCR in different template sequence contexts by several DNA polymerases, using d**Ds**TP and NH_2-hx-d**Px**TP.

DNA polymerase	3'→5' exonuclease activity	Natural base substrates	d**Ds**TP and NH_2-hx-d**Px**TP (μM)	DNA template	Amplification fold	Retention rate of **Ds**–**Px**[a] (%)
Deep Vent	+	300	50	**Ds**-temp 1	939	>97[b]
				Ds-temp 2	807	>97
				Ds-temp 3	814	>97
				Ds-temp 4	524	>97
				Cont-temp	1266	-
		500	50	**Ds**-temp 1	945	>97
				Ds-temp 2	814	>97
				Ds-temp 3	968	>97
				Ds-temp 4	766	96 ±1
				Cont-temp	1251	-
Deep Vent	-	300	50	**Ds**-temp 1	424	>97
				Ds-temp 2	433	>97
				Cont-temp	665	-
AccuPrime *Pfx*	mix	300	50	**Ds**-temp 1	1270	>97
				Ds-temp 2	1314	>97
				Ds-temp 3	1244	>97
				Ds-temp 4	531	92 ±1
				Cont-temp	1536	-
		400	50	**Ds**-temp 1	1774	>97
				Ds-temp 2	1735	>97
				Ds-temp 3	1841	>97
				Ds-temp 4	1503	93 ±2
				Cont-temp	1955	-
Pfx50	+	300	50	**Ds**-temp 1	1156	>97
				Ds-temp 2	1091	>97
				Ds-temp 3	1005	>97
				Ds-temp 4	381	94 ±1
				Cont-temp	1599	-
Phusion HF	+	300	50	**Ds**-temp 1	402	>97
				Ds-temp 2	174	>97
				Ds-temp 3	240	>97
				Ds-temp 4	103	94 ±1
				Cont-temp	1011	-
Pfu	+	300	50	**Ds**-temp 1	830	>97
				Ds-temp 2	697	>97
				Ds-temp 3	662	>97
				Ds-temp 4	221	86 ±2

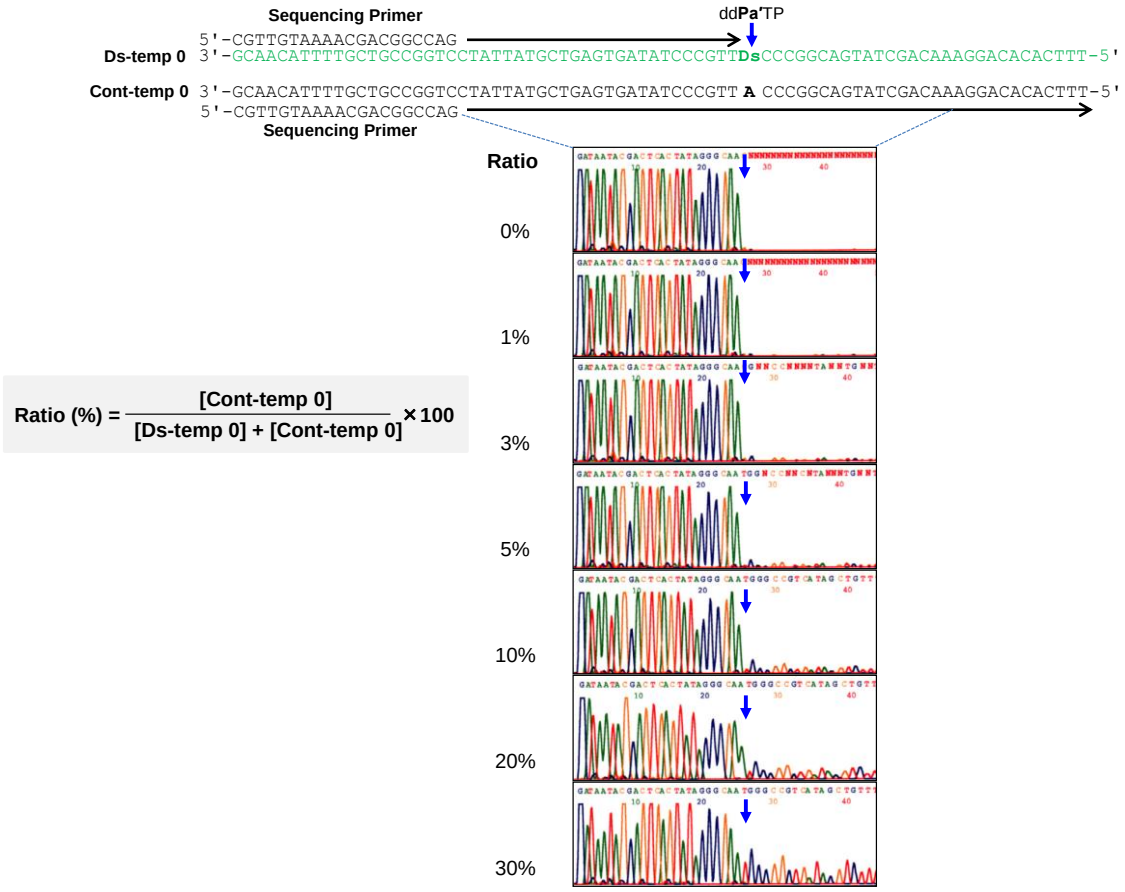
				Cont-temp	1113	-
Pwo SY	+	300	50	**Ds**-temp 1	939	>97
				Ds-temp 2	365	>97
				Ds-temp 3	544	>97
				Ds-temp 4	62	75 ±11
				Cont-temp	1403	-
Vent	+	300	50	**Ds**-temp 1	764	96 ±1
				Ds-temp 2	704	>97
				Cont-temp	898	-
N9°	(-)	300	50	**Ds**-temp 1	109	96 ±2
				Ds-temp 2	129	>97
				Cont-temp	133	-
TITANIUM Taq	-	300	50	**Ds**-temp 1	485	65 ±2
				Ds-temp 2	476	53 ±2
				Cont-temp	1039	-
Taq	-	300	50	**Ds**-temp 1	146	39 ±5
				Ds-temp 2	155	37 ±4
				Cont-temp	216	-

[a]Retention rates (%) of the **Ds**–**Px** pair in the amplified products after 15 cycles of PCR.

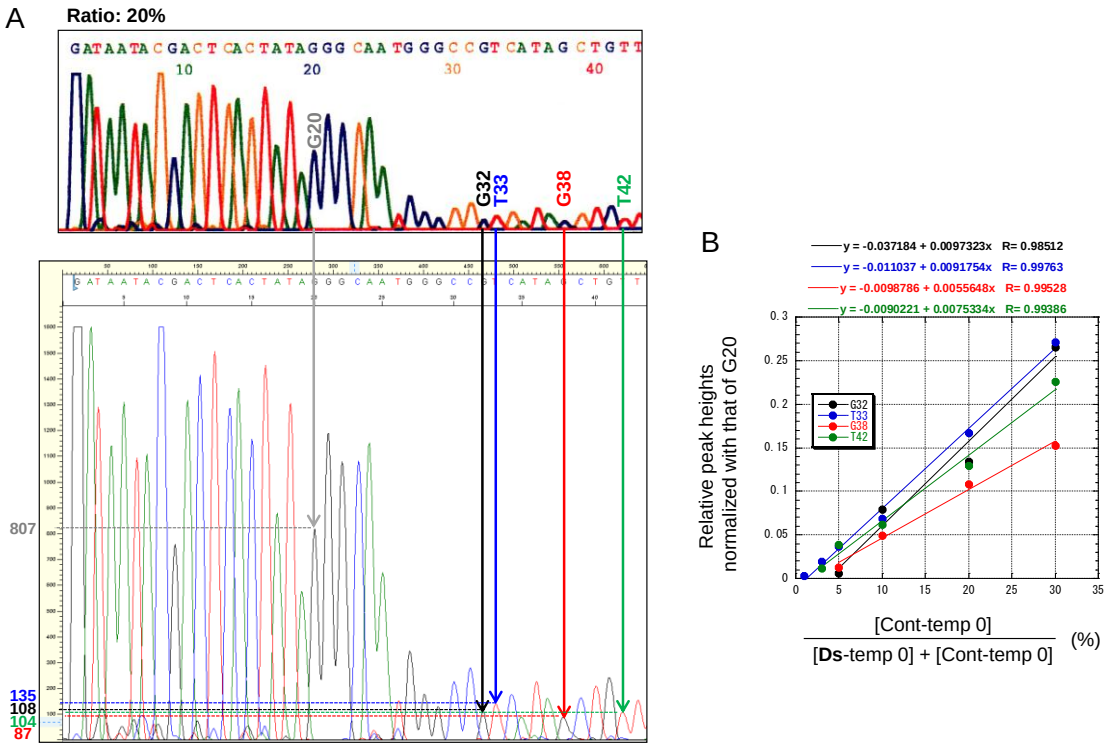
[b]>97% is the maximum detection limit by the assessment.

2. Analysis of the retention rates of the Ds−Px pair in the PCR-amplified DNAs

We calculated the retention rates of the **Ds−Px** pair in the amplified DNA fragments, by sequencing the DNA fragments in the presence of dd**Pa′**TP and comparing the sequencing peak patterns with those of authentic DNA samples (Figures S1 and S2). For authentic sequencing data, we used a DNA fragment containing one **Ds** base (75-mer, **Ds**-temp 0), mixed with the DNA fragment comprising natural bases only (Cont-temp 0, the **Ds** base in **Ds**-temp 0 was replaced by A), at ratios of 100:0, 99:1, 97:3, 95:5, 90:10, 80:20, and 70:30, as the template. The sequence data were analyzed by setting the start and stop points for the 55-nucleotide region, corresponding to the sequence, 5'-GATAATACGACTCACTATA**G**GGCAANGGGCC**GT**CATA**G**CTG**T**TTCCTGTGTGAAA-3' (N = the unnatural base position or T) (Figure S1). The sequencing peak heights of the G20, G32, T33, G38 and T42 positions (bold and underlined) were then determined with Sequence Scanner Version 1.0 (Applied Biosystems). An example is shown in panel A of Figure S2. The peak heights of the G32, T33, G38 and T42 positions, located after the unnatural base position, were normalized to that of the G20 position, located in the primer binding region for PCR. The normalized peak heights were plotted over the percentage of Cont-temp 0 in the DNA mixtures (corresponding to the pseudo percentage of the replacement of the **Ds** base with a natural base after PCR amplification), and the standard curves for the four nucleotide positions were obtained by fitting with a linear equation with correlation coefficients greater than 0.98 (Figure S2, B). We calculated the percentage of the replacement of the **Ds** base with a natural base, from the four standard curves and the normalized peak heights of each nucleotide position in the sequence peak patterns of the PCR-amplified DNA fragments. The retention rate of the **Ds−Px** pair was determined by averaging the four values, obtained from each standard curve. The limit of detection is 3% replacement of the **Ds** base with a natural base.

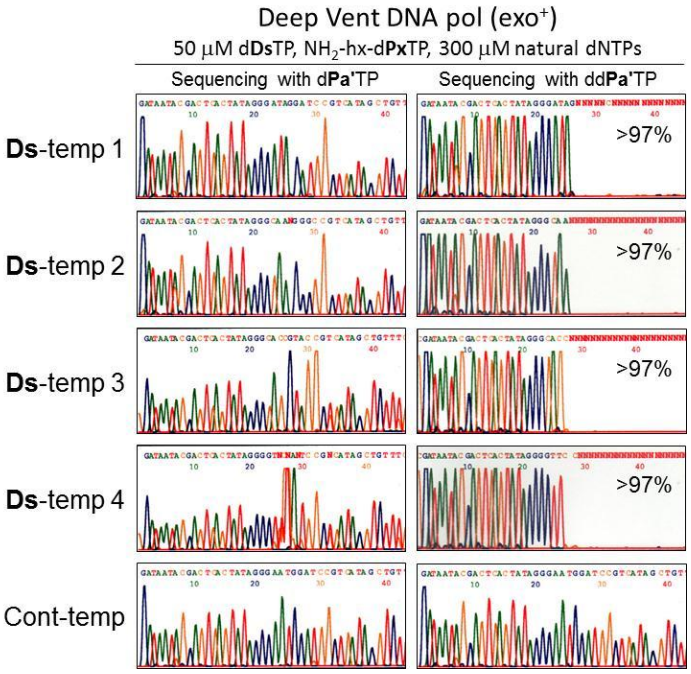


Supplementary figure 1. Dye-terminator sequencing, in the presence of ddPa'TP, of the mixture of Ds-temp 0 (75-mer) and Cont-temp 0 (75-mer), containing 0–30% Cont-temp 0. The blue arrow indicates the unnatural base position in Ds-temp 0.

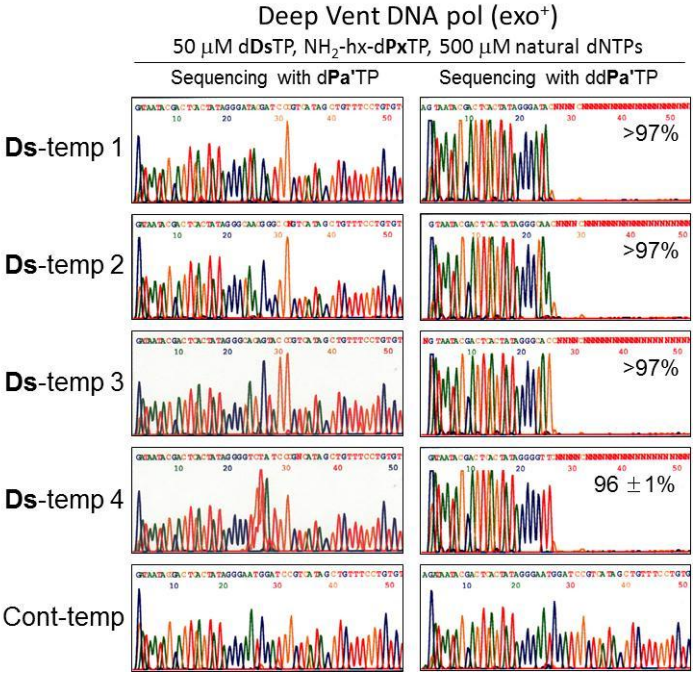


Supplementary figure 2. Representative data of peak height quantification at five nucleotide positions in a sequencing pattern (20% ratio in Figure S1) and standard curves used for the calculation of the retention rate of the Ds–Px pair. The sequencing peak heights at the G20 position and at the four-nucleotide positions, G32, T33, G38, and T42, in the sequencing data, obtained with the Applied Biosystems PRISM sequencing analysis software v3.2, were quantified by using an Applied Biosystems Sequence Scanner v1.0. The peak heights at the four nucleotide positions were divided by that at G20 to normalize each peak height, since the peak heights varied due to the different amounts of samples loaded on the sequencing gel.

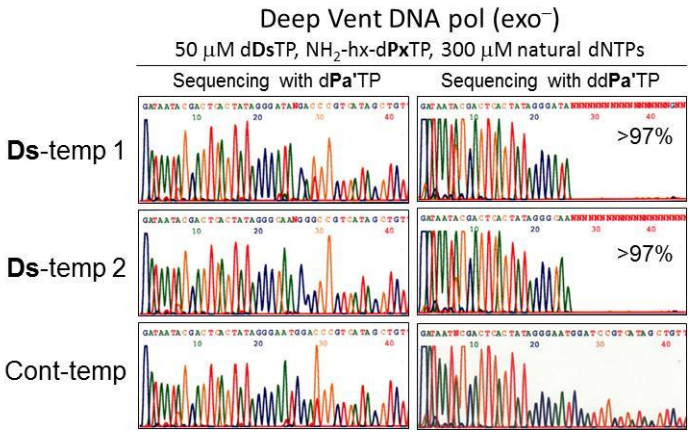
3. Sequencing analysis of the PCR products after 15 cycles of PCR by each DNA polymerase



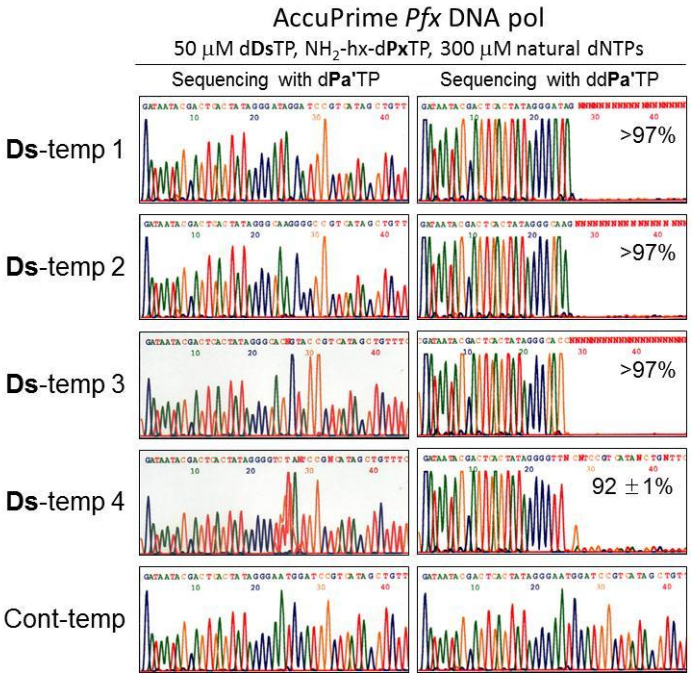
Supplementary figure 3. Sequencing analysis of the PCR products after 15 cycles of PCR by Deep Vent DNA pol (exo$^+$) (0.5 unit) in a buffer containing 2 mM $MgSO_4$ with 50 μM dDsTP, NH_2-hx-dPxTP and 300 μM natural dNTPs.



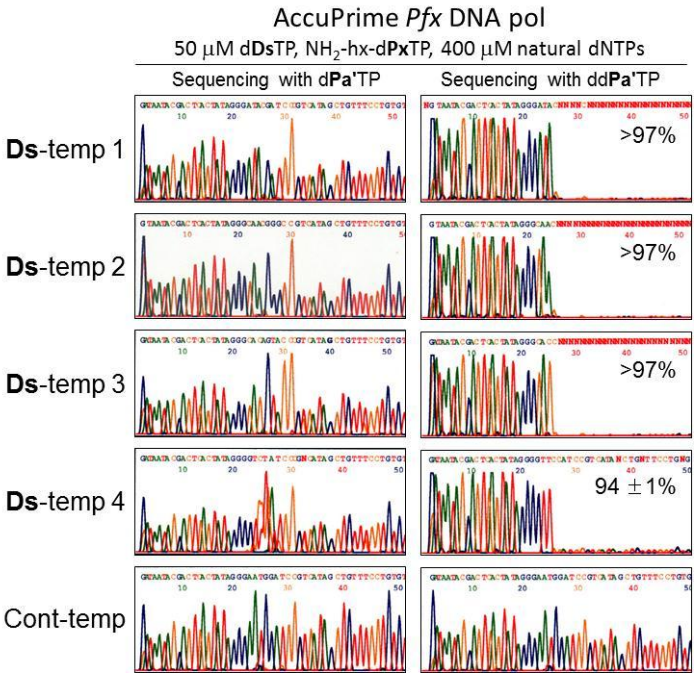
Supplementary figure 4. Sequencing analysis of the PCR products after 15 cycles of PCR by Deep Vent DNA pol (exo$^+$) (0.5 unit) in a buffer containing 4 mM $MgSO_4$ with 50 μM dDsTP, NH_2-hx-dPxTP and 500 μM natural dNTPs.



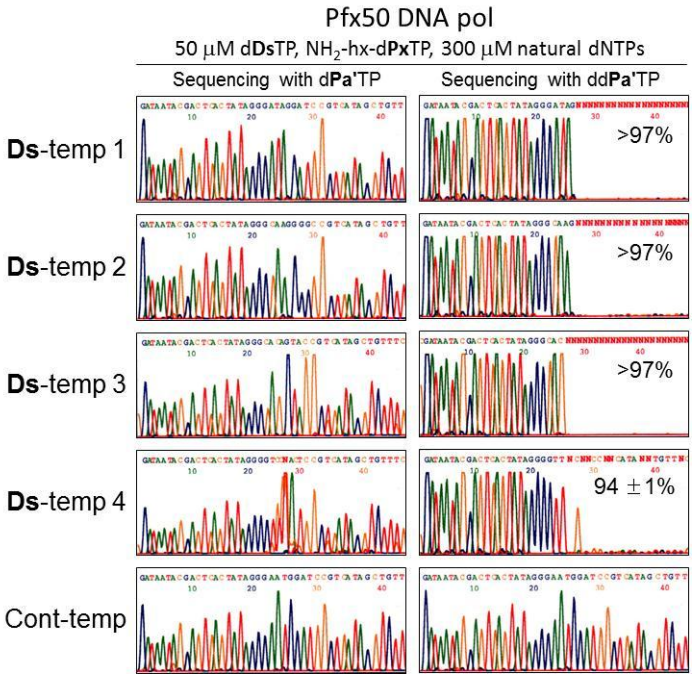
Supplementary figure 5. Sequencing analysis of the PCR products after 15 cycles of PCR by Deep Vent DNA pol (exo⁻) (0.5 unit) with 50 µM dDsTP, NH_2-hx-dPxTP and 300 µM natural dNTPs.



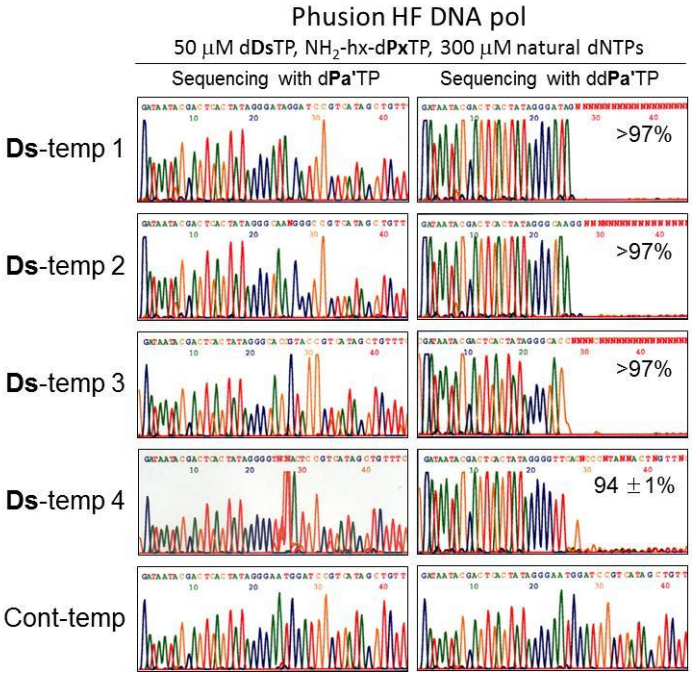
Supplementary figure 6. Sequencing analysis of the PCR products after 15 cycles of PCR by AccuPrime *Pfx* DNA pol (1.25 units) in a buffer containing 1 mM $MgSO_4$ with 50 µM dDsTP, NH_2-hx-dPxTP and 300 µM natural dNTPs.



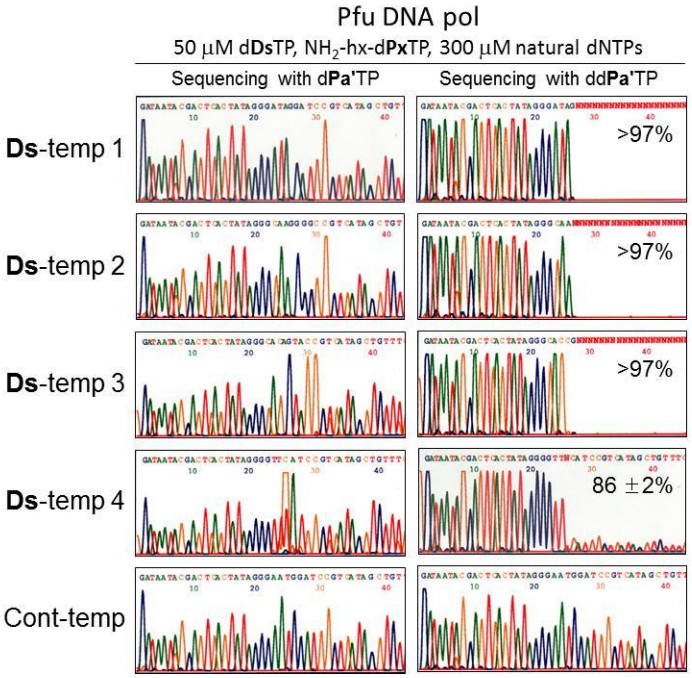
Supplementary figure 7. Sequencing analysis of the PCR products after 15 cycles of PCR by AccuPrime *Pfx* DNA pol (1.25 units) in a buffer containing 1.5 mM $MgSO_4$ with 50 μM dDsTP, NH_2-hx-dPxTP and 400 μM natural dNTPs.



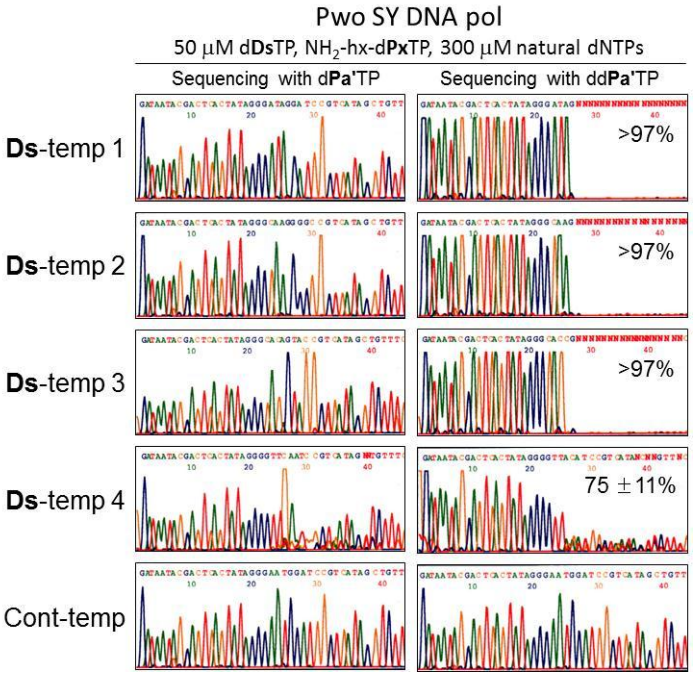
Supplementary figure 8. Sequencing analysis of the PCR products after 15 cycles of PCR by Pfx50 DNA pol (2.5 units) with 50 μM dDsTP, NH_2-hx-dPxTP and 300 μM natural dNTPs.



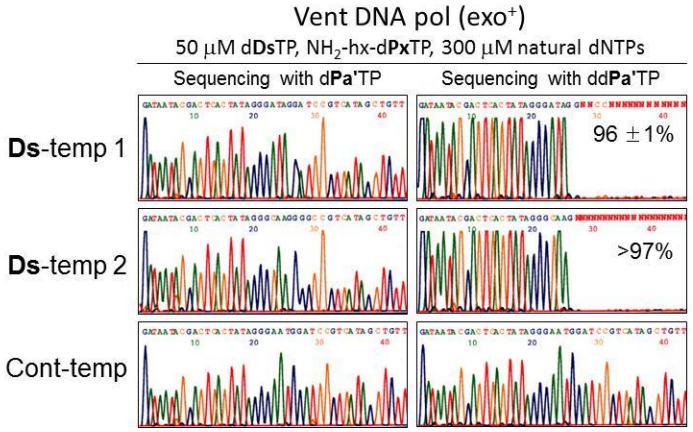
Supplementary figure 9. Sequencing analysis of the PCR products after 15 cycles of PCR by Phusion HF DNA pol (0.5 unit) with 50 μM dDsTP, NH_2-hx-dPxTP and 300 μM natural dNTPs.



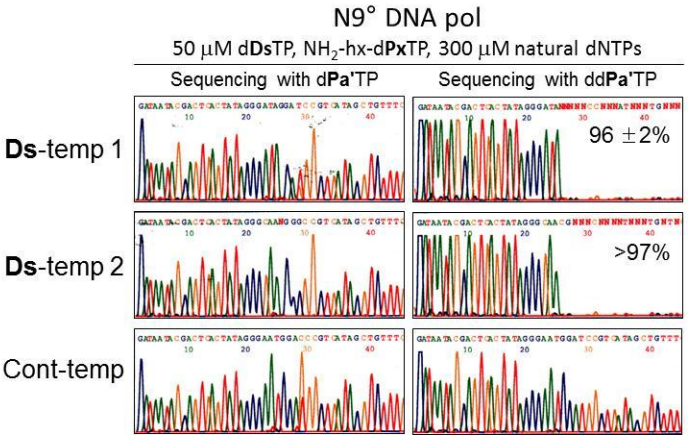
Supplementary figure 10. Sequencing analysis of the PCR products after 15 cycles of PCR by Pfu DNA pol (1.25 units) with 50 μM dDsTP, NH_2-hx-dPxTP and 300 μM natural dNTPs.



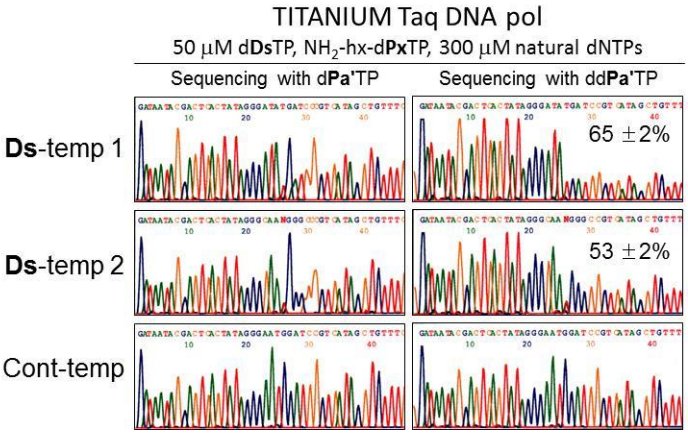
Supplementary figure 11. Sequencing analysis of the PCR products after 15 cycles of PCR by Pwo SY DNA pol (1.25 units) with 50 µM dDsTP, NH_2-hx-dPxTP and 300 µM natural dNTPs.



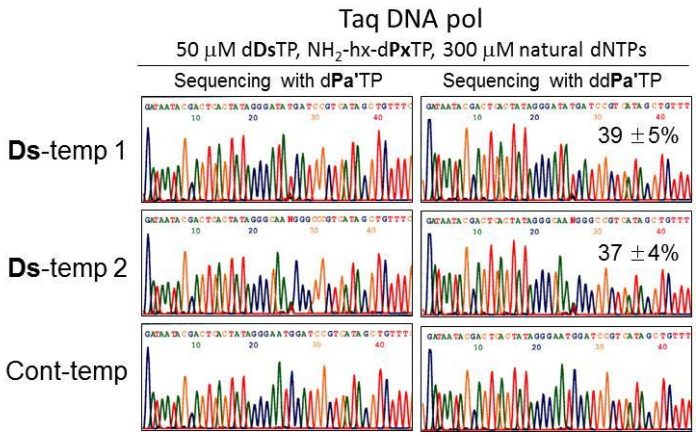
Supplementary figure 12. Sequencing analysis of the PCR products after 15 cycles of PCR by Vent DNA pol (exo$^+$) (0.5 unit) with 50 µM dDsTP, NH_2-hx-dPxTP and 300 µM natural dNTPs.



Supplementary figure 13. Sequencing analysis of the PCR products after 15 cycles of PCR by N9° DNA pol (exo$^+$) (0.5 unit) with 50 μM dDsTP, NH_2-hx-dPxTP and 300 μM natural dNTPs.



Supplementary figure 14. Sequencing analysis of the PCR products after 15 cycles of PCR by TITANIUM Taq DNA pol (1×) with 50 μM dDsTP, NH_2-hx-dPxTP and 300 μM natural dNTPs.



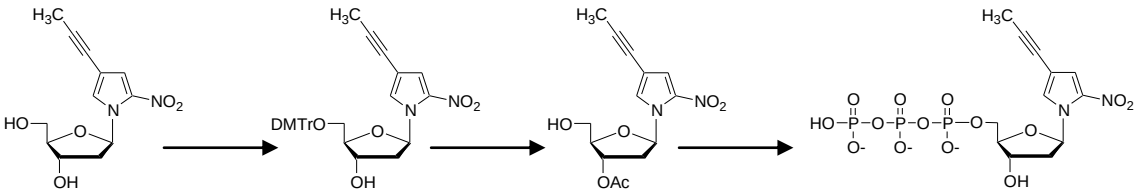
Supplementary figure 15. Sequencing analysis of the PCR products after 15 cycles of PCR by Taq DNA pol (0.5 unit) with 50 μM dDsTP, NH_2-hx-dPxTP and 300 μM natural dNTPs.

Chemical synthesis

1. General methods and materials

Reagents and solvents were purchased from standard suppliers and used without further purification. Reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm silica gel 60 plates impregnated with 254 nm fluorescent indicator (Merck). ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded on a Bruker (300-AVM) magnetic resonance spectrometer. Nucleoside purification was performed on a Gilson HPLC system with a preparative C18 column (Waters μ-BONDASPHERE, 150 × 19 mm). The triphosphate derivatives were purified with a DEAE-Sephadex A-25 column (300 × 15 mm) and C1, C8, and C18 columns (CAPCELL PAK, 250 × 4.6 mm, SHISEIDO). High resolution mass spectra (HRMS) and electrospray ionization mass spectra (ESI-MS) were recorded on a JEOL JM 700 or GC mate mass spectrometer and a Waters micromass ZMD 4000 equipped with a Waters 2690 LC system or a Waters UPLC-MS (H class) system, respectively.

2. Chemical synthesis of dPxTP



Reagents and abbreviations: (a) 4,4'-dimethoxytrityl chloride, pyridine (b) acetic anhydride, pyridine, then dichloroacetic acid, dichloromethane, 76% (c) 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, dioxane, pyridine, tri-*n*-butylamine, bis(tributylammonium)pyrophosphate, I_2/pyridine, water, NH_4OH, 31%. Ac: acetyl.

1-(2-Deoxy-3-*O*-acetyl-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole.1-(2-Deoxy-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole (200 mg, 0.75 mmol) was coevaporated to dryness with pyridine (three times). To the residue in pyridine (7.5 ml) was added 4, 4'-dimethoxytrityl chloride (280 mg, 0.83 mmol). The reaction mixture was stirred at room temperature for 1 h. The mixture was poured into 5% aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic phase was washed with water and

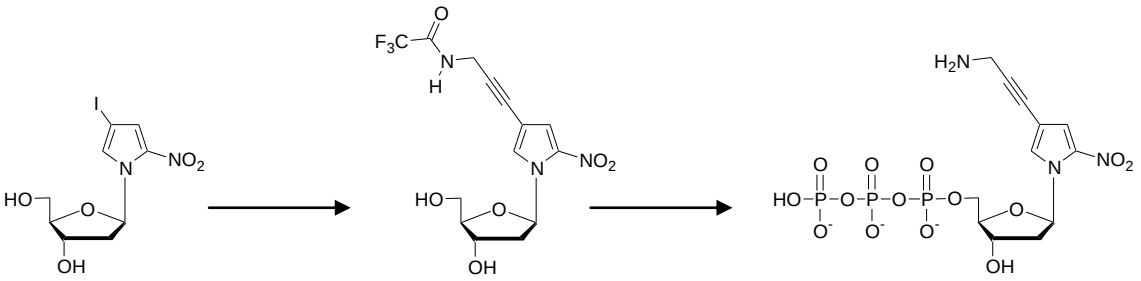
saturated aqueous NaCl, dried with Na_2SO_4, and evaporated *in vacuo*. The residue was subjected to silica gel column chromatography, using 0.5% methanol in CH_2Cl_2 as an eluent, to afford 365 mg of 1-(2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole in 86% yield.

1-(2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole (160 mg, 0.28 mmol) was coevaporated to dryness with pyridine three times. To the residue in pyridine (2.8 ml), acetic anhydride (53 μl, 0.56 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and was extracted by ethyl acetate and 5% aqueous $NaHCO_3$. The organic phase was dried with Na_2SO_4 and evaporated *in vacuo*. The residue was coevaporated to dryness with toluene. To the residue in CH_2Cl_2 (28 ml) was added dichloroacetic acid (280 μl) at 0°C. The reaction mixture was stirred at 0°C for 15 min. To the reaction mixture was added 5% aqueous $NaHCO_3$, and the organic phase was washed with 5% $NaHCO_3$ and saturated NaCl. After drying over Na_2SO_4, the solvent was evaporated *in vacuo*. The residue was subjected to silica gel column chromatography to afford 78 mg (89%) of 1-(2-deoxy-3-*O*-acetyl-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 7.90 (d, 1H, *J* = 2.1 Hz), 7.30 (d, 1H, *J* = 2.1 Hz), 6.60 (t, 1H, *J* = 6.4 Hz), 5.22 (m, 2H), 4.13 (m, 1H), 3.65 (m, 2H), 2.62 (ddd, 1H, *J* = 3.1, 6.1, 14.3 Hz), 2.43 (m, 1H), 2.08 (s, 3H), 2.00 (s, 3H). HRMS (FAB, 3-NBA matrix) for $C_{14}H_{17}N_2O_6$ $(M+H)^+$ calcd 309.1087, found 309.1066.

1-(2-Deoxy-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole 5'-triphosphate (dPxTP). 1-(2-Deoxy-3-*O*-acetyl-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole (31 mg, 0.1 mmol) was coevaporated with pyridine to dryness. The residue was dissolved in pyridine (100 μl) and dioxane (300 μl). A 1 M solution of 2-chloro-1,3,2-benzodioxaphosphorin-4-one in dioxane (110 μl, 0.11 mmol) was added. After 10 min, tri-n-butylamine (100 μl) and 0.5 M bis(tributylammonium)pyrophosphate in DMF (300 μl, 0.15 mmol) were added to the reaction mixture, which was stirred at room temperature for 10 min. A solution of 1% iodine in pyridine/water (98:2, v/v, 2.0 ml) was added. After 15 min, a 5% aqueous solution of $NaHSO_3$ (150 μl), followed by water (5 ml) was added to the reaction mixture, which was stirred at room temperature for 30 min, and then 28% NH_4OH (20 ml) was added.

Ammonolysis was performed at room temperature for 2 h. After the reaction mixture was concentrated *in vacuo*, the product was purified by DEAE Sephadex (A-25) column chromatography (eluted by a gradient of 50 mM to 1.0 M TEAB), and by C18-HPLC to give 1-(2-deoxy-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole 5'-triphosphate (d**Px**TP), (31 μmol, 31%). ^{1}H NMR (300 MHz, D_2O): δ 7.74 (d, 1H, *J* = 2.1 Hz), 7.35 (d, 1H, *J* = 2.1 Hz), 6.76 (t, 1H, *J* = 6.1 Hz), 4.63 (m, 1H), 4.24 (m, 3H), 3.21 (q, 20H, *J* = 7.3 Hz), 2.64 (ddt, 1H, *J* = 5.2, 13.9 Hz), 2.49 (ddt, 1H, *J* = 6.2, 14.0 Hz), 1.99 (s, 3H), 1.28 (t, 29H, *J* = 7.3 Hz). ^{31}P NMR (121 MHz, D_2O): δ -10.16 (d, 1P, *J* = 19.8 Hz), -10.66 (d, 1P, *J* = 20.0 Hz), -22.58 (t, 1P, *J* = 20.0 Hz). Electrospray ionization-mass spectroscopy (ESI-MS) for $C_{12}H_{17}O_{14}N_2P_3$; calcd, 505.18 $(M-H)^-$; found, 505.23 $(M-H)^-$. UV-vis: λ_{max} = 373 nm (ε 9500) in sodium phosphate buffer (pH 7.0).

3. Chemical synthesis of NH_2-C1-dPxTP



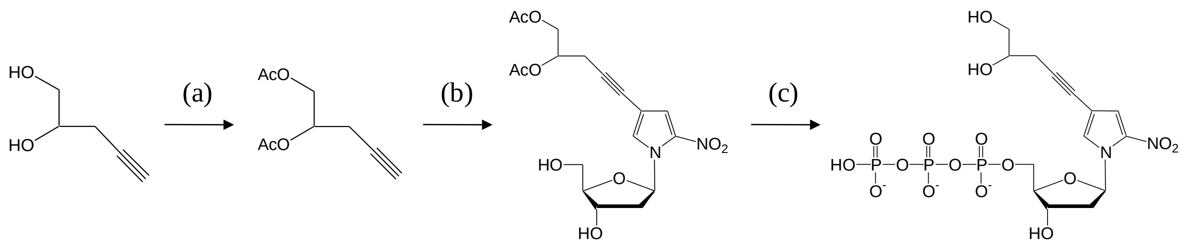
Conditions; (a) 1-(2-deoxy-β-D-ribofuranosyl)-4-iodo-2-nitropyrrole, TFA-NH-linker, CuI, I-dPn, $Pd(PPh_3)_4$, DMF (b) (i) $POCl_3$, proton sponge, tri-n-butylamine, bis(tributylammonium)pyrophosphate (ii) 28% NH_4OH

1-(2-Deoxy-β-D-ribofuranosyl)-4-[3-(trifluoroacetamido)-1-propynyl]-2-nitropyrrole (TFA-NH-C1-dPx). A solution of aceticanhydride (4.6 ml, 33 mmol) in CH_2Cl_2 (30 ml) was added to a solution of propargylamine (1.0 ml, 15 mmol), CH_2Cl_2 (30 ml), and pyridine (3.7 ml) at 0℃. The reaction mixture was stirred at room temperature for 12 h. The product was extracted with CH_2Cl_2/5% $NaHCO_3$, and the organic phase was washed with 5% $NaHCO_3$, and dried with Na_2SO_4. The organic phase was evaporated *in vacuo* to give the crude TFA-NH-linker as a pale yellow liquid (925 mg). A TFA-NH-linker (227 mg, 1.5 mmol) was added to a solution of 1-(2-deoxy-β-D-ribofuranosyl)-4-iodo-2-nitropyrrole (354 mg, 1.0 mmol), CuI (30 mg, 0.16 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol), and TEA (209 μl, 1.5 mmol) in DMF (5.0 ml). The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was evaporated *in vacuo*. The product was purified by chromatography on a silica gel column (eluted with 10% MeOH in CH_2Cl_2), and by RP-HPLC (35-50% CH_3CN in H_2O, 12 min) to give 330 mg (88%) of TFA-NH-C1-d**Px**. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 10.04 (s, 1H), 7.98 (s, 1H), 7.34 (s, 1H), 6.54 (t, 1H, *J* = 5.5 Hz), 5.27 (d, 1H, *J* = 4.4 Hz), 5.10 (t, 1H, *J* = 4.9 Hz), 4.22 (bs, 3H), 3.84 (m, 1H), 3.67-3.54 (m, 2H), 2.44 (m, 1H), 2.26 (m, 1H). HRMS (FAB, 3-NBA matrix) for $C_{14}H_{15}F_3N_3O_6$

$(M+H)^+$ calcd 378.0913, found 378.0882.

1-(2-Deoxy-β-D-ribofuranosyl)-4-(3-amino-1-propynyl)-2-nitropyrrole 5'-triphosphate (NH_2-C1-dPxTP). TFA-NH-C1-d**Px** nucleoside (75 mg, 0.2 mmol) was evaporated to dryness with pyridine and toluene, and then the nucleoside and proton sponge (66 mg, 0.3 mmol) was dissolved in $(CH_3O)_3PO$ (1.0 ml). $POCl_3$ (26 μl, 0.26 mmol) was added to the solution at 0 °C. After the reaction mixture was stirred at 0°C for 1 h, tri-n-butylamine (240 μl) and bis-tri-n-butylammonium pyrophosphate (2.0 ml, 0.5 M DMF solution) were added to the reaction mixture. After 30 min, 1.0ml of 0.5 M triethylamine bicarbonate and water (10 ml) were added to the reaction mixture, which was stirred at r.t. for 1 h. After lyophilization, the compound in H_2O (10 ml) was treated with 28% NH_4OH (40 ml) at room temperature for 1h. After the volatile components were removed, the product was purified by DEAE Sephadex A-25 column chromatography (eluted with a linear gradient of 50 mM to 1.0 M TEAB) and RP-HPLC (28% CH_3CN in 100 mM TEAA, 15 min) to give 54 μmol (27%) of NH_2-C1-d**Px**TP. ^{1}H NMR (300 MHz, D_2O) δ 7.96 (d, 1H, *J* = 2.1 Hz), 7.32 (d, 1H, *J* = 2.2 Hz), 6.67 (t, 1H, *J* = 6.4 Hz), 4.55 (m, 1H), 4.24-4.12 (m, 3H), 3.91 (s, 2H), 3.11 (q, 16H, *J* = 7.3 Hz), 2.58 (dt, 1H, *J* = 6.3 and13.8 Hz), 2.41 (ddd, 1H, *J* = 1.6, 4.8, and 14.0 Hz), 1.19 (t, 24H, *J* = 7.3 Hz). 31P NMR (121 MHz, D_2O) d -8.51 (bs, 1P), -10.70 (d, 1P, *J* = 19.4 Hz), -22.19 (t, 1P, *J* = 19.9 Hz). Electrospray ionization-mass spectroscopy (ESI-MS) for $C_{12}H_{18}O_{14}N_3P_3$; calcd, 520.20 $(M-H)^-$; found, 520.24 $(M-H)^-$. UV-vis: λ_{max} = 364 nm (ε 10,600) in sodium phosphate buffer (pH 7.0).

4. Chemical synthesis of Diol1-dPxTP



Conditions : (a) Ac_2O, pyridine, r.t., 14 h. (b) I-dPn, $Pd(PPh_3)_4$, CuI, TEA, DMF, r.t., 14h. (c) (i) $POCl_3$, proton sponge, tri-n-butylamine, bis-tri-n-butylammonium pyrophosphate (ii) 28%NH_3aq, r.t., 90 min.

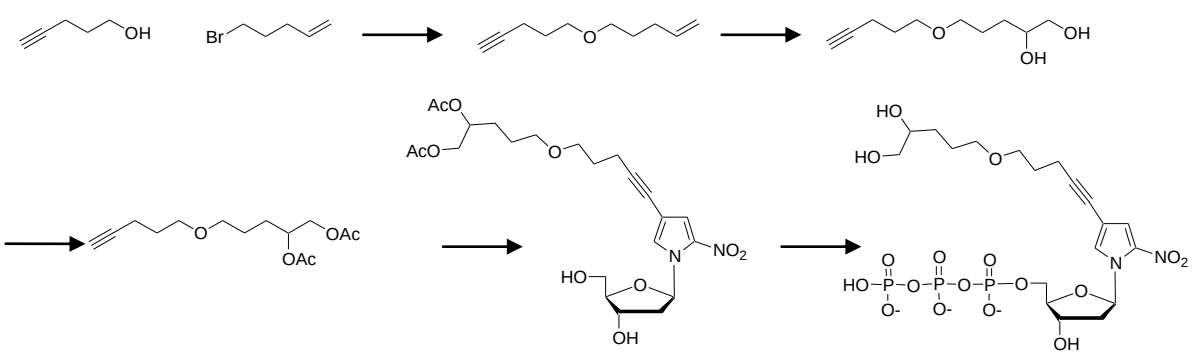
4-Pentyne-1,2-diacetate (Di(OAc)1 linker). 4-Pentyne-1,2-diol (13.5 mmol, crude) [reference *J. Org. Chem.* **2008**, *73*, 5965-5976] was co-evaporated with pyridine, and acetic anhydride (4.8 ml, 50.8 mmol) was added to the crude diol in pyridine (27 ml). The reaction mixture was stirred at room temperature for 14 h. The reaction mixture was extracted by ethylacetate and a 5% $NaHCO_3$ solution, and the organic phase was dried by Na_2SO_4 and evaporated *in vacuo*. 4-Pentyne-1,2-diacetate (Di(OAc)1 linker) (800 mg, 32%) was purified by silica gel column chromatography (50 % ethylacetate in CH_2Cl_2) as a clear liquid. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 5.05-4.98 (m, 1H), 4.24-4.07 (m, 2H), 2.91 (t, 1H, *J* = 2.7 Hz), 2.53 (dd, 1H, *J* = 2.6, 6.4 Hz), 2.01 (s, 6H).

1-(2-Deoxy-β-D-ribofuranosyl)-4-(4-pentyne-1,2-diacetate)-1-propynyl]-2-nitropyrrole (Di(OAc)1-dPx). A Di(OAc)1 linker (276 mg, 1.5 mmol) was added to a solution of 1-(2-deoxy-β-D-ribofuranosyl)-4-iodo-2-nitropyrrole (354 mg, 1 mmol), CuI (31 mg, 0.16 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol), and TEA (208 μl, 1.5 mmol) in DMF (5 ml). The reaction mixture was stirred at room temperature for 14 h, and was evaporated *in vacuo*. The product was purified by chromatography on a silica gel column (eluted with 7% MeOH in CH_2Cl_2), and by RP-HPLC (40-45% CH_3CN in H_2O, 9 min) to give 400 mg (97%) of (Di(OAc)1-d**Px**) as a yellow liquid. ^{1}H

NMR (300 MHz, DMSO-*d*6) δ 7.92 (d, 1H, *J* = 2.2 Hz), 7.28 (d, 1H, *J* = 2.2 Hz), 6.54 (t, 1H, *J* = 5.6 Hz), 5.27 (d, 1H, *J* = 4.5 Hz), 5.11-5.04 (m, 2H), 4.29-4.12 (m, 3H), 3.86-3.82 (m, 1H), 3.69-3.52 (m, 2H), 2.74 (d, 2H, *J* = 6.3 Hz), 2.47-2.38 (m, 1H), 2.27-2.19 (m, 1H), 2.04, 2.02 (s, s, 3H, 3H). HR-MS (FAB, NBA matrix) calcd. for $C_{18}H_{23}N_2O_9$ $(M+H)^+$ 411.1409, found 411.1403.

1-(2-Deoxy-β-D-ribofuranosyl)-4-(4-pentyne-1,2-diol)-1-propynyl]-2-nitropyrrole 5'-triphosphate (Diol1-dPxTP). The di(OAc)1-d**Px** nucleoside (41 mg, 0.1 mmol) was evaporated to dryness with pyridine and toluene. The nucleoside and proton sponge (33 mg, 0.15 mmol) were dissolved in $(CH_3O)_3PO$ (500 μl), and $POCl_3$ (13 μl, 0.13 mmol) was added to the solution at 0 °C. After the reaction mixture was stirred at 0°C for 1 h, tri-n-butylamine (120 μl) and bis-tri-n-butylammonium pyrophosphate (1.0 ml, 0.5 M DMF solution) were added to the reaction mixture. After 30 min, 500 μl of 0.5 M triethylamine bicarbonate and water (5.0 ml) were added to the reaction mixture, which was stirred at 0°C for 30 min. After lyophilization, the compound in H_2O (4.0 ml) was treated with 28% NH_4OH (20 ml) at room temperature for 90 min. After the volatile components were removed, the product was purified by DEAE Sephadex A-25 ion exchange column chromatography (eluted with a linear gradient of 50 mM to 1.0 M TEAB) and RP-HPLC (10-40% CH_3CN in 100 mM TEAA, 9 min) to give 24 μmol (24%) of Diol1-d**Px**TP. ^{1}H NMR (300 MHz, D_2O) δ 7.79 (d, 1H, *J* = 2.1 Hz), 7.39 (d, 1H, *J* = 2.1 Hz), 6.77 (t, 1H, *J* = 6.0 Hz), 4.66-4.61 (m, 1H), 4.27-4.22 (m, 3H), 3.98-3.91 (m, 1H), 3.74-3.60 (m, 2H), 3.20 (q, 22H, *J* = 7.3 Hz), 2.72-2.46 (m, 4H), 1.28 (t, 32H, *J* = 7.3 Hz). ^{31}P NMR (121 MHz, D_2O) δ -9.83 (d, 1P, *J* = 19.8 Hz), -10.66 (d, 1P, *J* = 20.0 Hz), -22.53 (t, 1P, *J* = 20.1 Hz). ESI-MS: calcd. for $C_{14}H_{21}N_2O_{16}P_3$ $(M-H)^-$ 566.24, found 565.04. UV-vis spectrum (10 mM sodium phosphate buffer pH 7.0) λ_{max} = 374 nm (ε 9,400).

5. Chemical synthesis of Diol3o3-dPxTP



Conditions: (a) KOH, benzene, reflux, 12h (b) OsO_4,N-methylmorpholine-*N*-oxide, acetone:H_2O:*t*BuOH (4:1:1, v/v/v), r.t., 1h (c) Ac_2O, pyridine, r.t., 12h, 24% 3 steps (d) CuI, I-dPn, $Pd(PPh_3)_4$, TEA, DMF, r.t., 12h, 24% (e) (i) $POCl_3$, proton sponge, TBA, bis(tributylammonium)pyrophosphate (ii) 28% NH_4OH, r.t., 1h

5-(4-Pentynyloxy)pentane-1,2-diacetate (Di(OAc)3o3 linker). A mixture of 4-pentyne-1-ol (4.65 ml, 50 mmol), 5-bromo-1-pentene (17.8 ml, 150 mmol), and KOH (12.6 g, 225 mmol) in benzene (50 ml) was refluxed for 12 h. After filtration, the reaction mixture was separated with ethyl acetate and 10% NH_4Cl. The organic phase was washed with 10% NH_4Cl and saturated NaCl, dried with Na_2SO_4, and evaporated *in vacuo*. The product was purified by silica gel chromatography (eluted with 10% EtOAc in hexane) to give 6.5 g of crude 5-(4-pentynyloxy)-1-pentene. OsO_4 (543 mg, 2.0 mmol) was added to a solution of crude 5-(4-pentynyloxy)-1-pentene (6.5 g) and N-methylmorpholine-N-oxide (10.0 g, 85.4 mmol) in acetone/H_2O/tBuOH (4:1:1, 214 ml). The reaction mixture was stirred at room temperature for 1 h. $NaHSO_3$ (1.5 g) was then added to the reaction mixture. After the resulting precipitate was removed by filtration and washed with MeOH, the filtered solution was concentrated *in vacuo*. The product was purified by silica gel column chromatography (eluted with 3% MeOH in CH_2Cl_2) to give crude 5-(4-pentynyloxy)pentane-1,2-diol (2.9 g). After the crude 5-(4-pentynyloxy)pentane-1,2-diol (2.9 g) was co-evaporated with pyridine, acetic anhydride (5.9 ml, 62.4 mmol) was added to the crude diol in

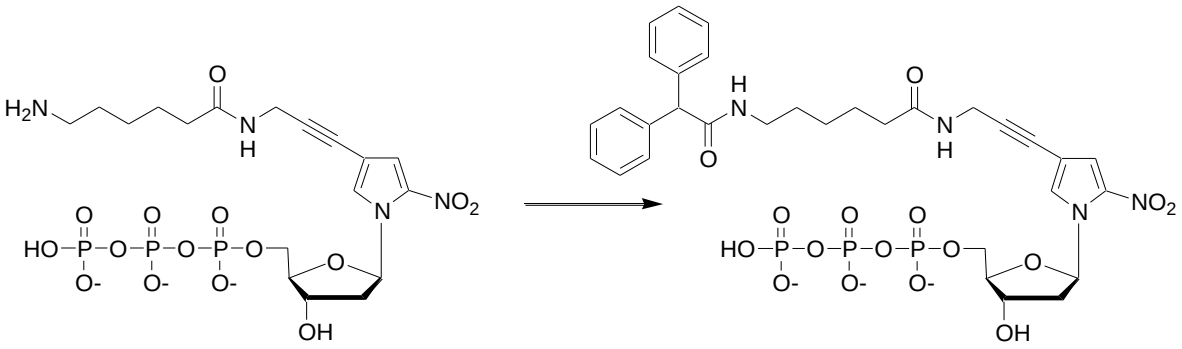
pyridine (78 ml). The reaction mixture was stirred at room temperature for 9 h. The reaction mixture was extracted by ethyl acetate and a 5% $NaHCO_3$ solution and the organic phase was dried by Na_2SO_4 and evaporated *in vacuo*. 5-(4-Pentynyloxy)pentane-1,2-diacetate (Di(OAc)3o3 linker), 3.27g, 24% 3 steps) was purified by silica gel column chromatography (20 % hexane in CH_2Cl_2) as a clear liquid. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 4.96 (m, 1H), 4.16 (dd, 1H, *J* = 3.3, 12.0 Hz), 4.01 (dd, 1H, *J* = 6.4, 11.9 Hz), 3.39 (t, 2H, *J* = 6.4 Hz), 3.33 (t, 2H, *J* = 6.2 Hz), 2.74 (t, 1H, *J* = 2.7 Hz), 2.18 (dt, 2H, *J* = 2.6, 7.2 Hz), 1.66-1.45 (m, 6H). HR-MS (FAB, NBA matrix) calcd. for $C_{14}H_{23}O_5$ $(M+H)^+$ 271.1545, found 271.1592.

1-(2-Deoxy-β-D-ribofuranosyl)-4-[5-(4-pentynyloxy)pentane-1,2-diacetate)-1-propynyl]-2-nitropyrrole (Di(OAc)3o3-dPx). A di(OAc)3o3 linker (180 mg, 0.7 mmol) was added to a solution of 1-(2-deoxy-β-D-ribofuranosyl)-4-iodo-2-nitropyrrole (177 mg, 0.5 mmol), CuI (19 mg, 0.1 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), and TEA (104 μl, 0.75 mmol) in DMF (2.5 ml). The reaction mixture was stirred at room temperature for 13 h, and then evaporated *in vacuo*. The product was purified by silica gel column chromatography (eluted with 5% MeOH in CH_2Cl_2), and RP-HPLC (50-55% CH_3CN in H_2O, 10 min) to give 60 mg (24%) of (Di(OAc)3o3-d**Px**) as a yellow liquid. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 7.91 (d, 1H, *J* = 2.2 Hz), 7.28 (d, 1H, *J* = 2.2 Hz), 6.55 (t, 1H, *J* = 5.7 Hz), 5.29 (d, 1H, *J* = 4.5 Hz), 5.10 (t, 1H, *J* = 5.2 Hz), 4.97 (m, 1H), 4.24 (m, 1H), 4.16 (dd, 1H, *J* = 3.3, 12.0 Hz), 4.01 (dd, 1H, *J* = 6.5, 11.9 Hz), 3.85 (m, 1H), 3.70-3.53 (m, 2H), 3.44 (t, 2H, *J* = 6.2 Hz), 3.36 (t, 2H, *J* = 6.1 Hz), 2.45-2.39 (m, 3H), 2.28-2.19 (m, 2H), 2.01, 2.00 (s, s, 3H, 3H), 1.76 -1.47 (m, 6H). HR-MS (FAB, NBA matrix) calcd. for $C_{23}H_{33}N_2O_{10}$ $(M+H)^+$ 497.2135, found 497.2110.

1-(2-Deoxy-β-D-ribofuranosyl)-4-[5-(4-pentynyloxy)pentane-1,2-diol)-1-propynyl]-2-nitropyrrole 5'-triphosphate (Diol3o3-dPxTP). A di(OAc)3o3-d**Px** nucleoside (50 mg, 0.1 mmol) was evaporated to dryness with pyridine and toluene, and the nucleoside and a proton sponge (33 mg, 0.15 mmol) were dissolved in $(CH_3O)_3PO$ (500 μl). $POCl_3$ (13 μl, 0.13 mmol) was added to the solution at 0 °C. After the reaction mixture was stirred at 0°C for 1.5 h, tri-n-butylamine (120 μl) and bis-tri-n-butylammonium

pyrophosphate (1.0 ml, 0.5 M DMF solution) were added to the reaction mixture. After 30 min, 500 μl of 0.5 M triethylamine bicarbonate and water (5.0 ml) were added to the reaction mixture, which was stirred at 0°C for 30 min. After lyophilization, the compound in H_2O (2.0 ml) was treated with 28% NH_4OH at room temperature for 1h. After the volatile components were removed, the product was purified by DEAE Sephadex A-25 ion exchange column chromatography (eluted with a linear gradient of 50 mM to 1.0 M TEAB) and RP-HPLC (5-50% CH_3CN in 100 mM TEAA, 12 min) to give 18 μmol (18%) of Diol3o3-d**Px**TP. ^{1}H NMR (300 MHz, D_2O) δ 7.73 (d, 1H, *J* = 2.1 Hz), 7.37 (d, 1H, *J* = 2.1 Hz), 6.76 (t, 1H, *J* = 6.1 Hz), 4.62 (m, 1H), 4.26-4.20 (m, 3H), 3.72-3.42 (m, 7H), 3.20 (q, 22H, *J* = 7.3 Hz), 2.64 (m, 1H), 2.53-2.44 (m, 3H), 1.89-1.41 (m, 6H), 1.28 (t, 32H, *J* = 7.3 Hz). ^{31}P NMR (121 MHz, D_2O) δ -10.07 (d, 1P, *J* = 19.7 Hz), -10.63 (d, 1P, *J* = 20.1 Hz), -22.55 (t, 1P, *J* = 20.0 Hz). ESI-MS: calcd. for $C_{19}H_{30}N_2O_{17}P_3$ $(M-H)^-$ 651.37, found 651.39. UV-vis spectrum (10 mM sodium phosphate buffer pH 7.0) λ_{max} = 374 nm (ε 9,200).

6. Chemical synthesis of DMP-hx-dPxTP



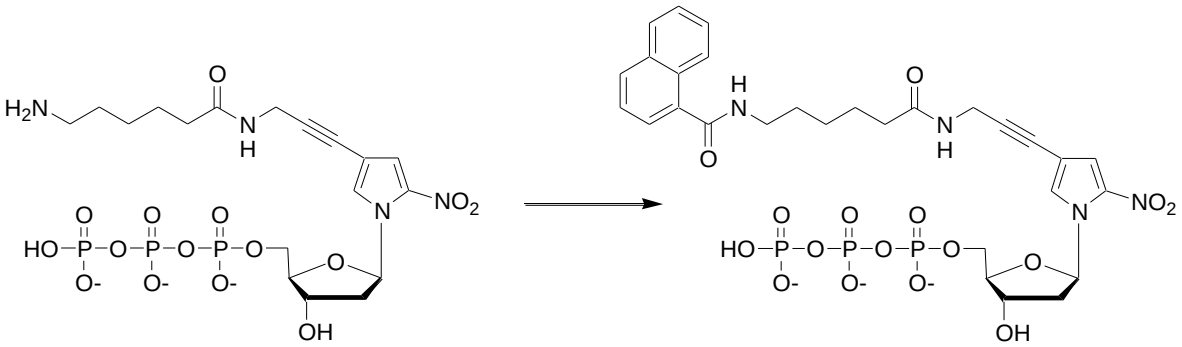
Condition: *N*-hydoroxysuccinimide ester, triethylamine, 70% DMF-H_2O, rt.

Diphenyl acetic acid *N*-hydroxysuccinimidyl ester. Diphenyl acetic acid (216 mg, 1.02 mmol), *N*-hydroxysuccinimide (177 mg, 1.53 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (290 mg, 1.51 mmol) were dissolved in dry CH_2Cl_2 (5 ml) and stirred at room temperature for 7 h. The reaction mixture was diluted with CH_2Cl_2 (20 ml) and washed with an aqueous solution of saturated $NaHCO_3$ (10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, and evaporated. The purification by the automated flash column chromatography system (Yamazen, AI-580, Hi-Flash Silica gel column, CH_2Cl_2-CH_3OH) afforded the title compound as a white solid (156 mg, 0.50 mmol, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.29 (m, 10H), 5.35 (s, 1H), 2.82 (s, 4H). (Ref. J. Med. Chem. 2000, 51, 8168.)

Diphenylmethane-modified dPxTP (DPM-hx-dPxTP). A solution of NH_2-hx-d**Px**TP (30 μmol) in 40% DMF-H_2O (3.0 ml) was reacted with diphenyl acetic acid *N*-hydroxysuccinimidyl ester (120 μmol) in DMF (3.0 ml) and triethylamine (6.2 μl) at room temperature. After 24 h, the reaction mixture was diluted with H_2O (18 ml) and filtered using a Steriflip (Millipore) to remove the precipitate thus generated. The filtrate was lyophilized, and then dissolved in TEAA buffer (2 ml). The product (15.4 μmol, 51%) was purified by C18 reverse phase column chromatography (Nacalai COSMOSIL 140C19-OPN, eluted by a linear gradient of 0–30% CH_3CN in 100 mM TEAA, pH 7.0) and C8 HPLC

(Shiseido, CAPCELLPAK C8, eluted by a linear gradient of 25–50% CH_3CN in 100 mM TEAA, pH 7.0). ^{1}H NMR (300 MHz, D_2O) δ 7.72 (d, 1H, *J* = 2.1 Hz), 7.43-7.33 (m, 6H), 7.26-7.21 (m, 5H), 6.64 (t, 1H, *J* = 5.9 Hz), 5.01 (s, 1H), 4.53 (m, 1H), 4.20 (m, 3H), 4.14 (m, 2H), 2.23-3.16 (m, 1H and $(CH_3CH_2)_3N$), 2.54 (m, 1H), 2.36 (m, 1H), 2.26 (t, 1H, *J* = 7.0 Hz), 1.63-1.58 (m, 2H), 1.53-1.49 (m, 2H), 1.30-1.35 (m, 1H and $(CH_3CH_2)_3N$). ^{31}P NMR (121 MHz, D_2O), δ -9.47 (d, 1P, *J* = 15.5 Hz), -10.70 (d, 1P, *J* = 19.8 Hz), -22.47 (t, 1P, *J* = 20.0 Hz). ESI-MS: calcd. for $C_{32}H_{37}N_4O_{16}P_3$ $(M-H)^-$ calcd. 827.16, found: 827.02. UV-vis spectrum (10 mM sodium phosphate buffer, pH 7.0) λ_{max} = 369 nm, ε_{260} = 2,400, ε_{369} = 10,700.

7. Chemical synthesis of NTP-hx-dPxTP.



Condition: *N*-hydoroxysuccinimide ester, triethylamine, 70% DMF-H_2O, rt.

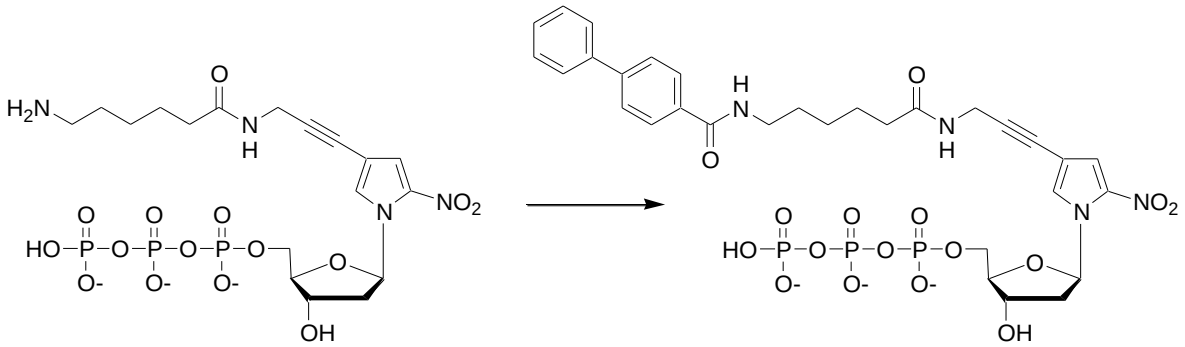
1-Naphthoic acid *N*-hydroxysuccinimidyl ester. 1-Naphthoic acid (176 mg, 1.02 mmol), *N*-hydroxysuccinimide (174 mg, 1.51 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (288 mg, 1.50 mmol) were dissolved in dry CH_2Cl_2 (5 ml) and stirred at room temperature for 18 h. The reaction mixture was extracted with CH_2Cl_2 (10 ml) and washed with an aqueous solution of saturated $NaHCO_3$ (10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, and evaporated. The purification by the automated flash column chromatography system (Yamazen, AI-580, Hi-Flash Silica gel column, CH_2Cl_2-CH_3OH) provided the title compound as a white solid (131 mg, 0.48 mmol, 48%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (dd, 1H, *J* = 7.7, 1.0 Hz), 8.40-8.35 (m, 4H), 8.13 (dd, 1H, *J* = 7.9, 0.7 Hz), 7.79-7.66 (m, 3H), 2.94 (s, 4H). Ref. Tetrahedron Letters 2003, 44(12) 2477-2480.

Naphthalene-modified dPxTP (NTP-hx-dPxTP). A solution of NH_2-hx-d**Px**TP (30 μmol) in 40% DMF-H_2O (3.0 ml) was reacted with 1-naphthoic acid *N*-hydroxysuccinimidyl ester (120 μmol) in DMF (3.0 ml) and triethylamine (6.2 μl) at room temperature. After 66 h, the reaction mixture was diluted with H_2O (18 ml) and filtered using a Steriflip (Millipore) to remove the precipitate thus generated. The filtrate was lyophilized, and then dissolved in TEAA buffer (3 ml). The product (16.4 μmol, 55%) was purified by C18 reverse phase column chromatography (Nacalai COSMOSIL 140C19-OPN, eluted by a

linear gradient of 0–30% CH_3CN in 100 mM TEAA, pH 7.0) and C8 HPLC (Shiseido, CAPCELLPAK C8, eluted by a linear gradient of 15–50% CH_3CN in 100 mM TEAA, pH 7.0).

^{1}H NMR (300 MHz, D_2O) δ 8.02-7.93 (m, 3H), 7.65-7.48 (m, 5H), 6.98 (d, 1H, *J* = 2.1 Hz), 6.50 (t, 1H, *J* = 5.9 Hz), 4.53 (m, 1H), 4.18 (m, 3H), 4.10 (s, 2H), 3.49 (t, 1H, J = 6.4 Hz), 2.58-2.49 (m, 1H), 2.37-2.26 (m, 3H), 1.79-1.67 (m, 4H), 1.54-1.46 (m, 2H). ^{31}P NMR (121 MHz, D_2O), δ 9.91 (d, 1P, *J* = 19.4 Hz), -10.77 (d, 1P, *J* = 20.0 Hz), -22.56 (t, 1P, *J* = 20.0 Hz). ESI-MS: calcd. for $C_{29}H_{34}N_4O_{16}P_3$ $(M\text{-}H)^-$ 787.13, found: 787.13. UV-vis spectrum (10 mM sodium phosphate buffer, pH 7.0) λ_{max} = 369 nm, ε_{260} = 4,900, ε_{369} = 9,350.

8. Chemical synthesis of BPh-hx-dPxTP.



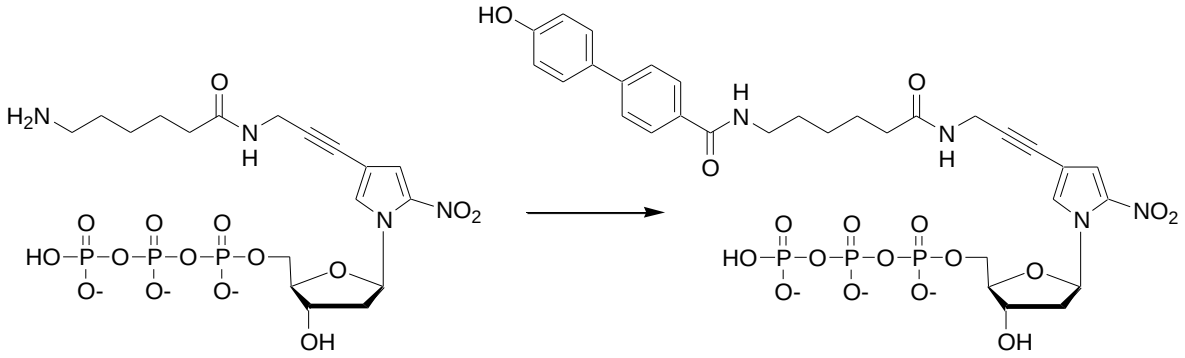
Condition: *N*-hydoroxysuccinimide ester, triethylamine, 70% DMF-H_2O, rt.

4-Biphenylcarboxylic acid *N*-hydroxysuccinimidyl ester. 4-Biphenylcarboxylic acid (200 mg, 1.00 mmol), *N*-hydroxysuccinimide (178 mg, 1.55 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (287 mg, 1.50 mmol) were dissolved in dry CH_2Cl_2 (5 ml) and stirred at room temperature for 21 h. The reaction mixture was diluted with CH_2Cl_2 (20 ml) and washed with an aqueous solution of saturated $NaHCO_3$ (10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, and evaporated. The crude product was purified by the automated flash column chromatography system (Yamazen, AI-580, Hi-Flash Silica gel column, CH_2Cl_2-CH_3OH). Subsequent reprecipitation in H_2O (50 ml) and DMF (2.5 ml) afforded the title compound as a white solid (139 mg, 0.47 mmol, 47%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (d, 2H, *J* = 8.6 Hz), 7.97 (d, 2H, *J* = 8.6 Hz), 7.82-7.78 (m, 2H), 7.57-7.44 (m, 3H), 2.91 (s, 4H). Ref. Russ. J. Bioorg. Chem. 2009, 35, 342.

Biphenyl-modified dPxTP (BPH-hx-dPxTP). A solution of NH_2-hx-d**Px**TP (30 μmol) in 40% DMF-H_2O (3.0 ml) was reacted with 4-biphenylcarboxylic acid *N*-hydroxysuccinimidyl ester (120 μmol) in DMF (3.0 ml) and triethylamine (6.2 μl) at room temperature. After 51 h, the reaction mixture was diluted with H_2O (18 ml) and filtered using a Steriflip (Millipore) to remove the precipitate thus generated. The filtrate was lyophilized, and then dissolved in TEAA buffer (3 ml). The product (10.0 μmol, 33%) was purified by C18 reverse phase column chromatography (Nacalai COSMOSIL

140C19-OPN, eluted by a linear gradient from 0–30% CH_3CN in 100 mM TEAA, pH 7.0) and C1 HPLC (Shiseido, CAPCELLPAK C1, eluted by a linear gradient of 20–50% CH_3CN in 100 mM TEAA, pH 7.0). ^{1}H NMR (300 MHz, D_2O) δ 7.76-7.68 (m, 7H), 7.61 (d, 1H, *J* = 2.1 Hz), 7.55-7.7.45 (m, 3H), 7.04 (d, 1H, *J* = 2.1 Hz), 6.38 (t, 1H, *J* = 5.9 Hz), 4.45 (m, 1H), 4.16-4.10 (m, 5H), 3.39 (t, 2H, *J* = 6.6 Hz), 2.42 (m, 1H), 2.32 (t, 1H, *J* = 6.5 Hz), 2.19 (m, 1H), 1.74-1.62 (m, 4H), 1.45 (m, 1H). ^{31}P NMR (121 MHz, D_2O), δ -10.33 (d, 1P, *J* = 19.7 Hz), -10.84 (d, 1P, *J* = 19.7 Hz), -22.75 (t, 1P, *J* = 19.8 Hz). ESI-MS: calcd. for $C_{31}H_{36}N_4O_{16}P_3$ $(M-H)^-$ 814.14, found: 813.43. UV-vis spectrum (10 mM sodium phosphate buffer, pH 7.0) λ_{max} = 368 nm, ε_{260} = 24,600, ε_{368} = 10,400.

9. Chemical synthesis of HBP-hx-dPxTP.



Condition: *N*-hydoroxysuccinimide ester, triethylamine, 70% DMF-H_2O, rt.

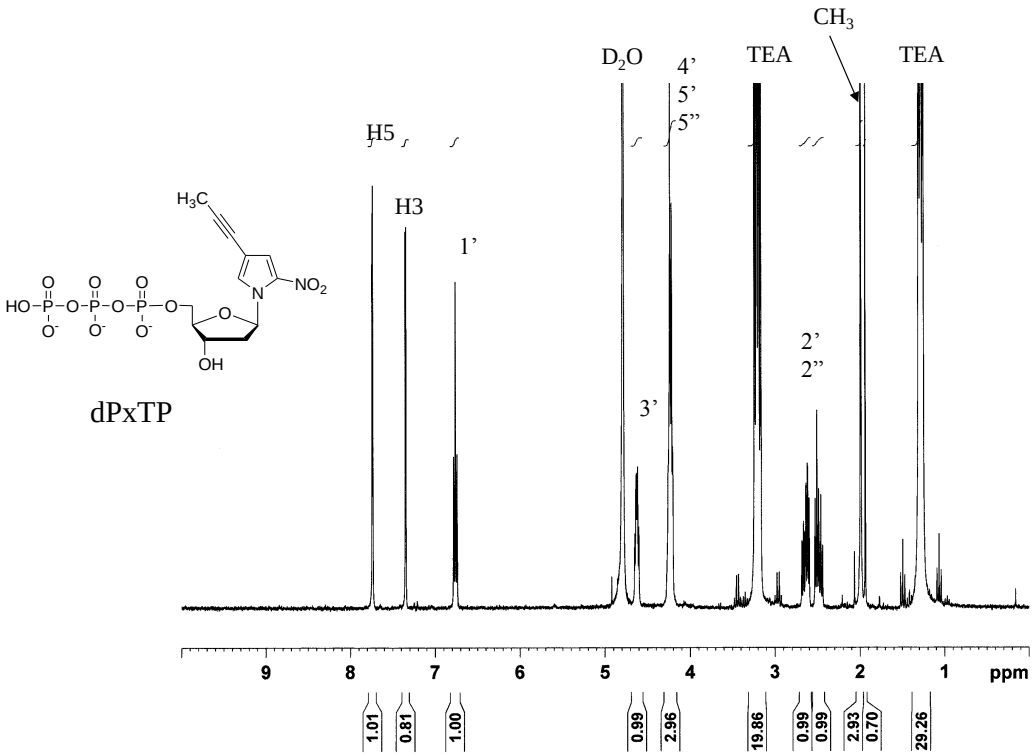
4'-Hydroxy-4-biphenylcarboxylic acid *N*-hydroxysuccinimidyl ester. 4'-Hydroxy-4-biphenylcarboxylic acid (214 mg, 1.0 mmol), *N*-hydroxysuccinimide (174 mg, 1.50 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (288 mg, 1.50 mmol) were dissolved in dry THF (10 ml) and dry DMF (4 ml), and stirred at room temperature. After 5 h, the solution was poured into H_2O (50 ml). The precipitate thus formed was filtered, washed with hexane, and dried in vacuo. The title compound was obtained as a white solid in a 67% yield (207 mg, 0.67 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.11 (dd, 2H, J = 6.8, 1.8 Hz), 7.88 (dd, 2H, J = 6.8, 1.8 Hz), 7.68-7.64 (m, 2H), 6.93-6.88 (m, 2H), 2.90 (s, 4H). Ref. J. Med. Chem. 2008, 51, 6665-6681.

4-hydroxybiphenyl-modified dPxTP (HBP-hx-dPxTP). A solution of NH_2-hx-d**Px**TP (30 μmol) in 40% DMF-H_2O (3.0 ml) was reacted with 4'-hydroxy-4-biphenylcarboxylic acid *N*-hydroxysuccinimidyl ester (120 μmol) in DMF (3.0 ml) and triethylamine (6.2 μl) at room temperature. After 45 h, the reaction mixture was diluted with H_2O (18 ml) and filtered using a Steriflip (Millipore) to remove the precipitate thus generated. The filtrate was lyophilized, and then dissolved in TEAA buffer (2 ml). The product (13.9 μmol, 46%) was purified by C18 reverse phase column chromatography (Nacalai COSMOSIL 140C19-OPN, eluted by a linear gradient of 0–30% CH_3CN in 100 mM TEAA, pH 7.0) and C8 HPLC (Shiseido, CAPCELLPAK C8, eluted by a linear gradient of 15–50% CH_3CN in 100 mM

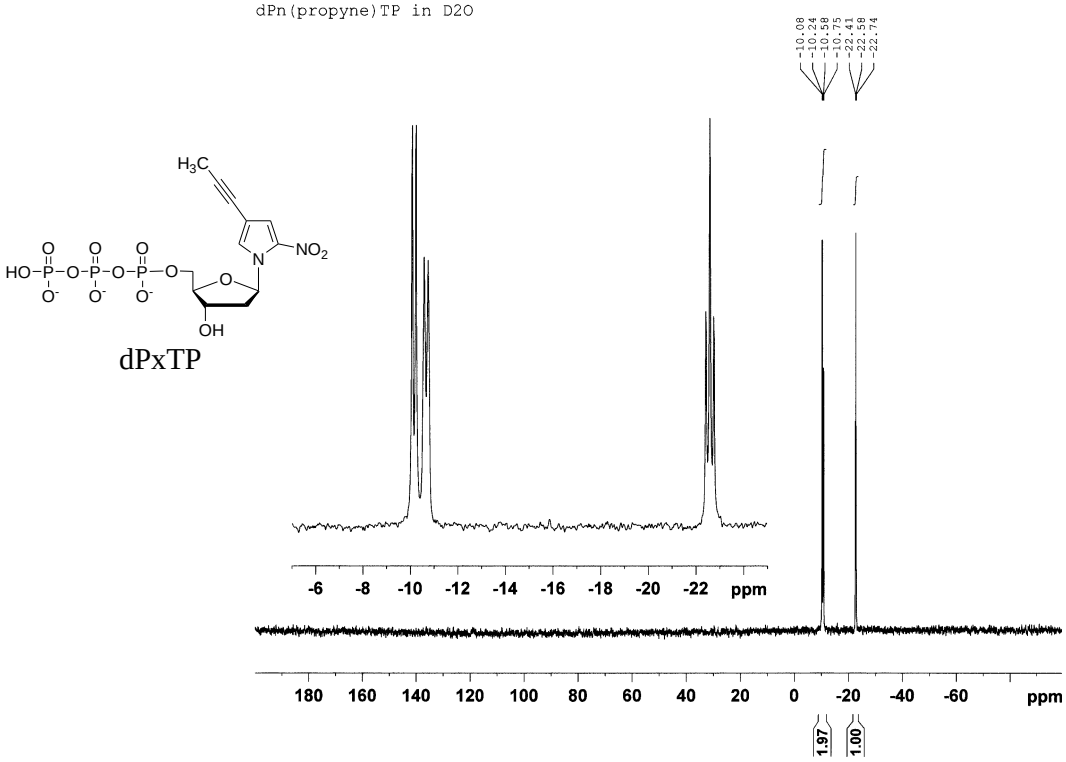
TEAA, pH 7.0). ^{1}H NMR (300 MHz, D_2O) δ 7.71 (d, 2H, *J* = 8.4 Hz), 7.62-7.56 (m, 5H), 6.99 (d, 2H, *J* = 7.5 Hz), 6.97 (s, 1H), 6.36 (t, 1H, *J* = 5.9 Hz), 4.44 (m, 1H), 4.15-4.09 (m, 5H), 3.39 (t, 2H, *J* = 6.5 Hz), 2.46-2.38 (m, 1H), 2.32 (t, 2H, *J* = 6.3 Hz), 2.18-2.12 (m, 1H), 1.73-1.61 (m, 4H), 1.29-1.24 (m, 2H). ^{31}P NMR (121 MHz, D_2O), δ -9.86 (d, 1P, *J* = 19.6 Hz), -10.75 (d, 1P, *J* = 19.8 Hz), -22.55 (t, 1P, *J* = 19.9 Hz). ESI-MS: calcd. for $C_{31}H_{36}N_4O_{17}P_3$ $(M-H)^-$ 829.14, found: 829.14. UV-vis spectrum (10 mM sodium phosphate buffer, pH 7.0) λ_{max} = 368 nm, ε_{260} = 14,100, ε_{368} = 10,200.

NMR and MS spectra of compounds.

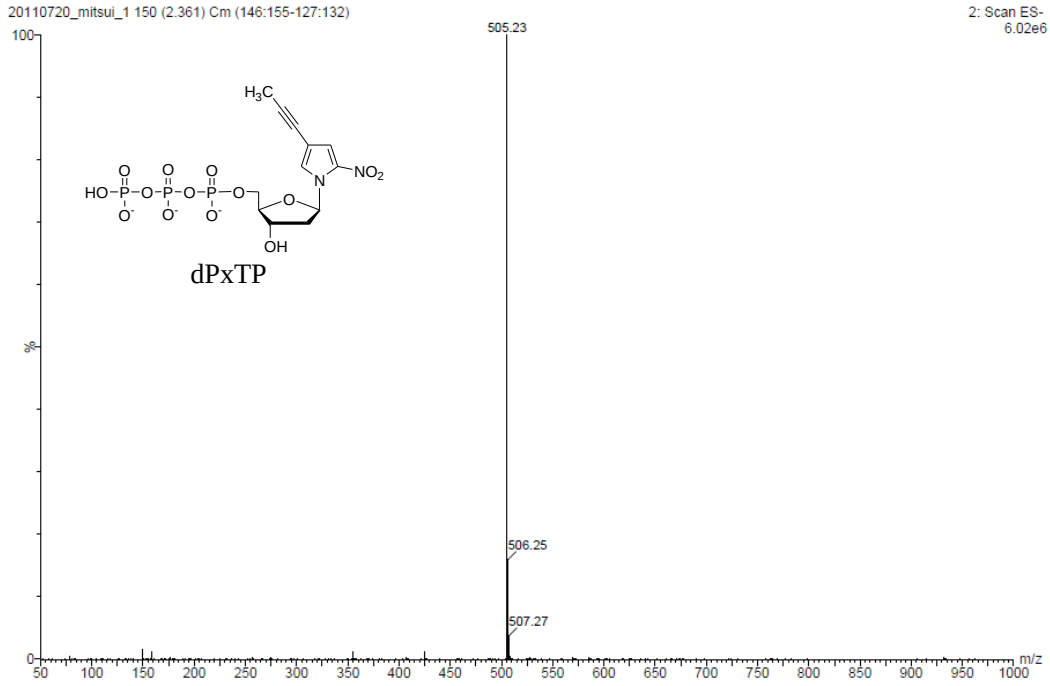
F1. ^{1}H NMR (300 MHz, D_2O) spectrum of d**Px**TP.



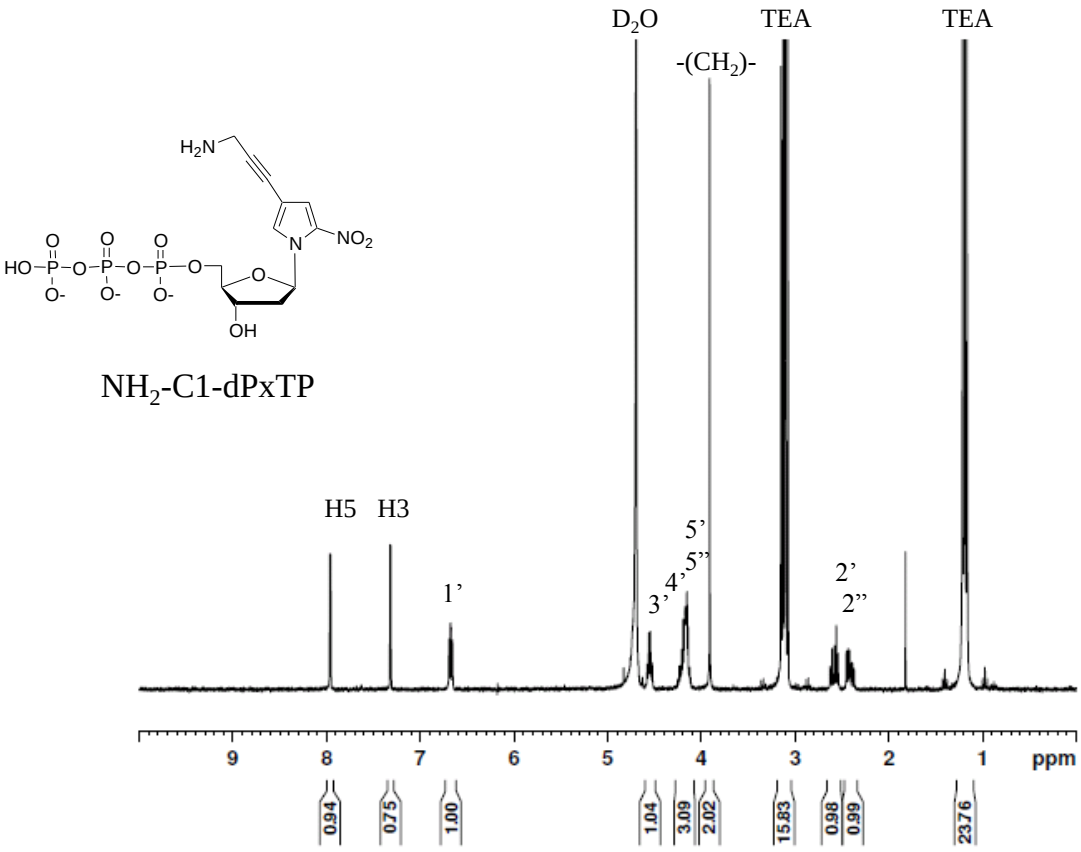
F2. ^{31}P NMR (121 MHz, D_2O) spectrum of d**Px**TP.



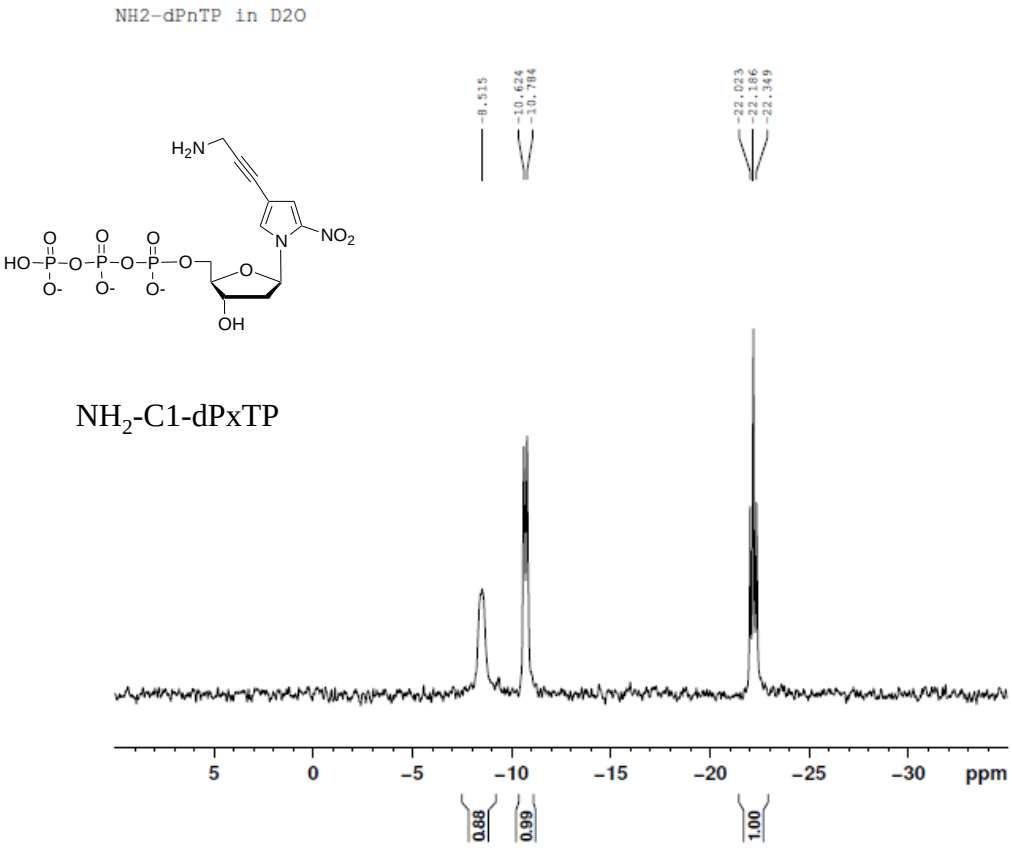
F3. ESI-MS spectrum of d**Px**TP.



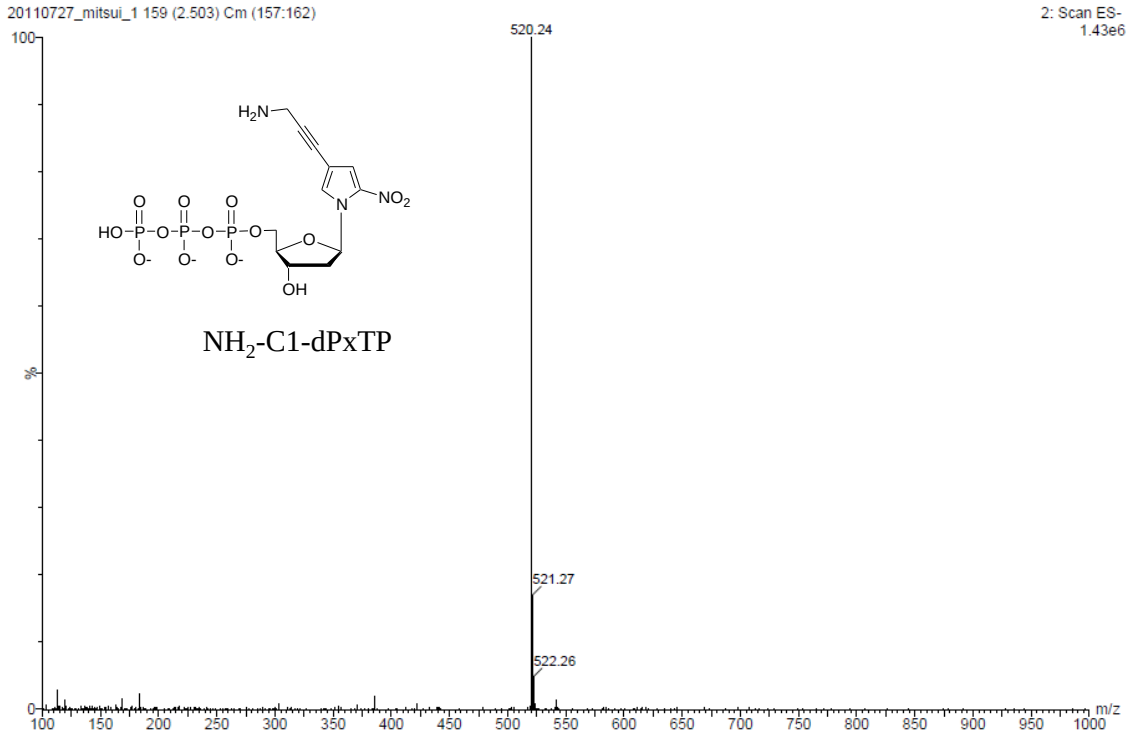
F4. ^{1}H NMR (300 MHz, D_2O) spectrum of NH_2-C1-d**Px**TP.



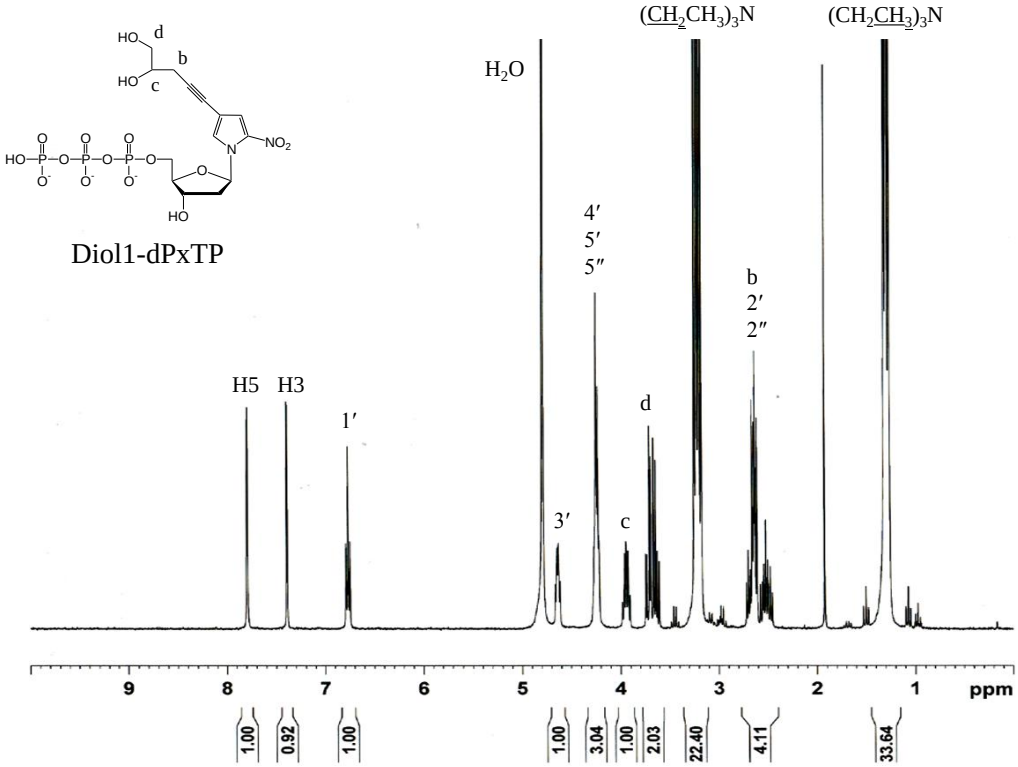
F5. ^{31}P NMR (121 MHz, D_2O) spectrum of NH_2-C1-d**Px**TP.



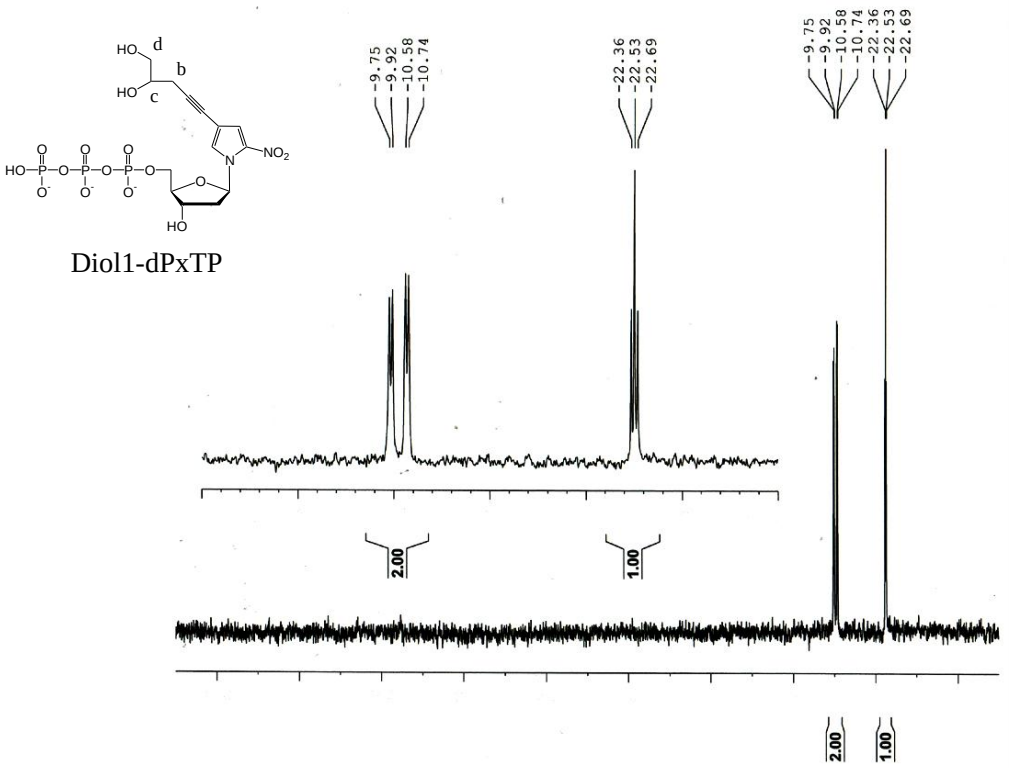
F6. ESI-MS spectrum of NH_2-C1-d**Px**TP.



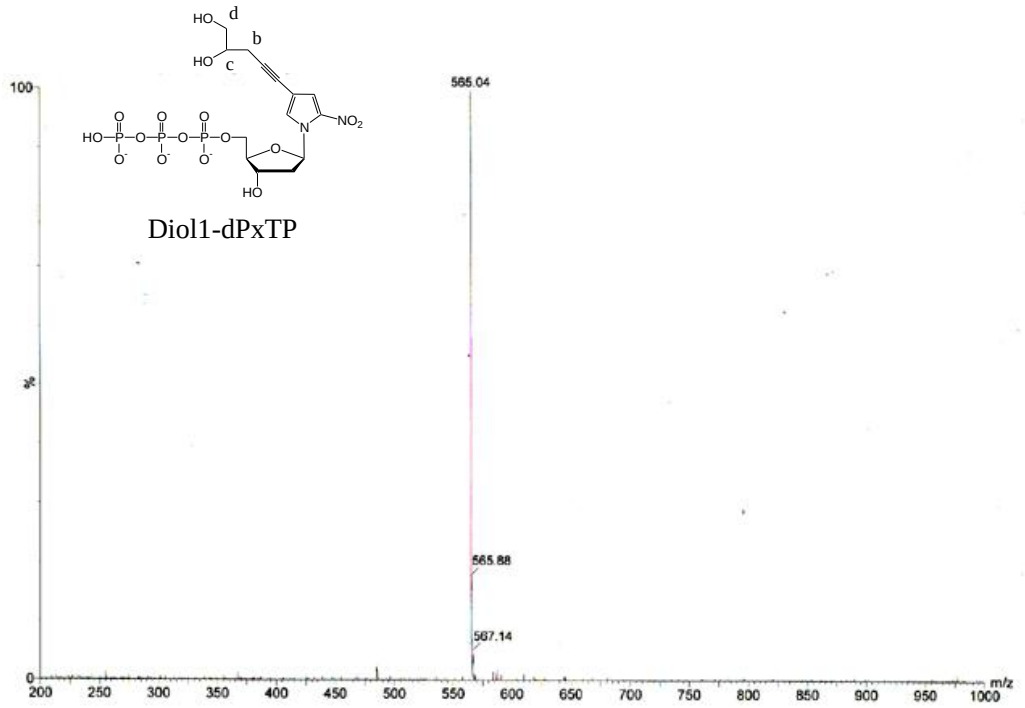
F7. ^{1}H NMR (300 MHz, D_2O) spectrum of Diol1-d**Px**TP.



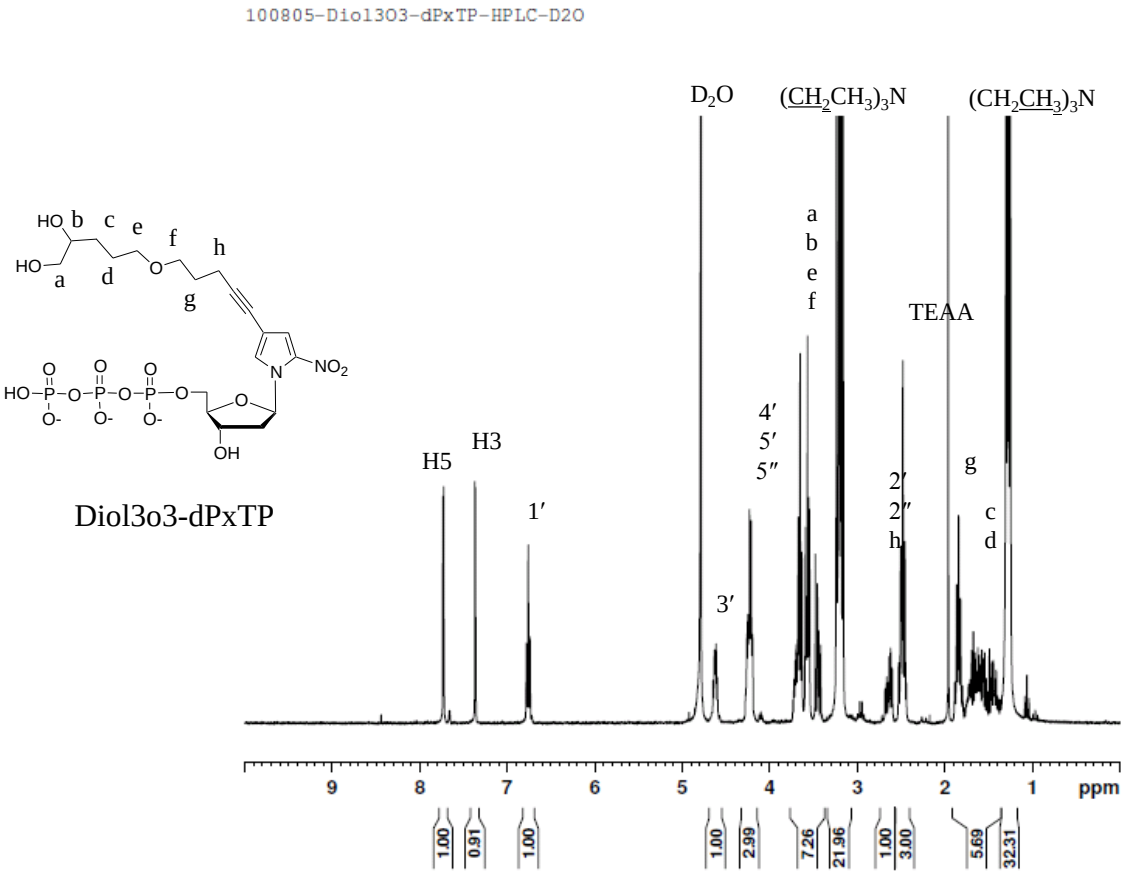
F8. ^{31}P NMR (121 MHz, D_2O) spectrum of Diol1-d**Px**TP.



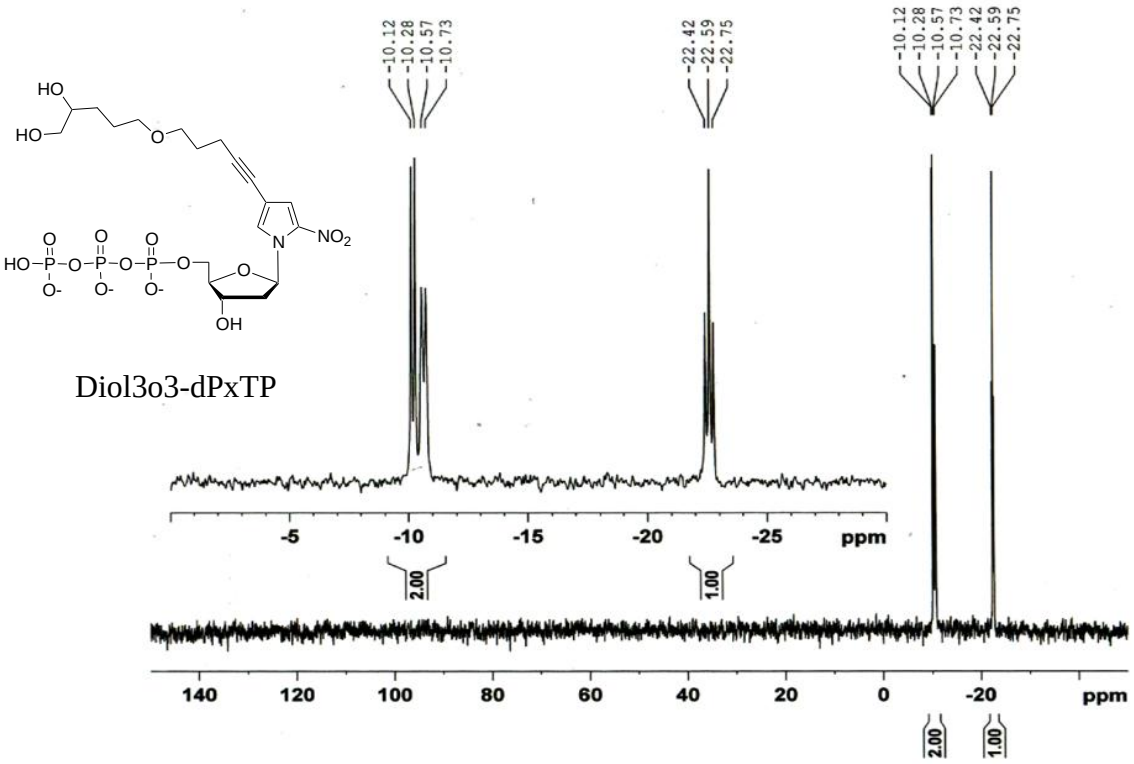
F9. ESI-MS spectrum of Diol1-d**Px**TP.



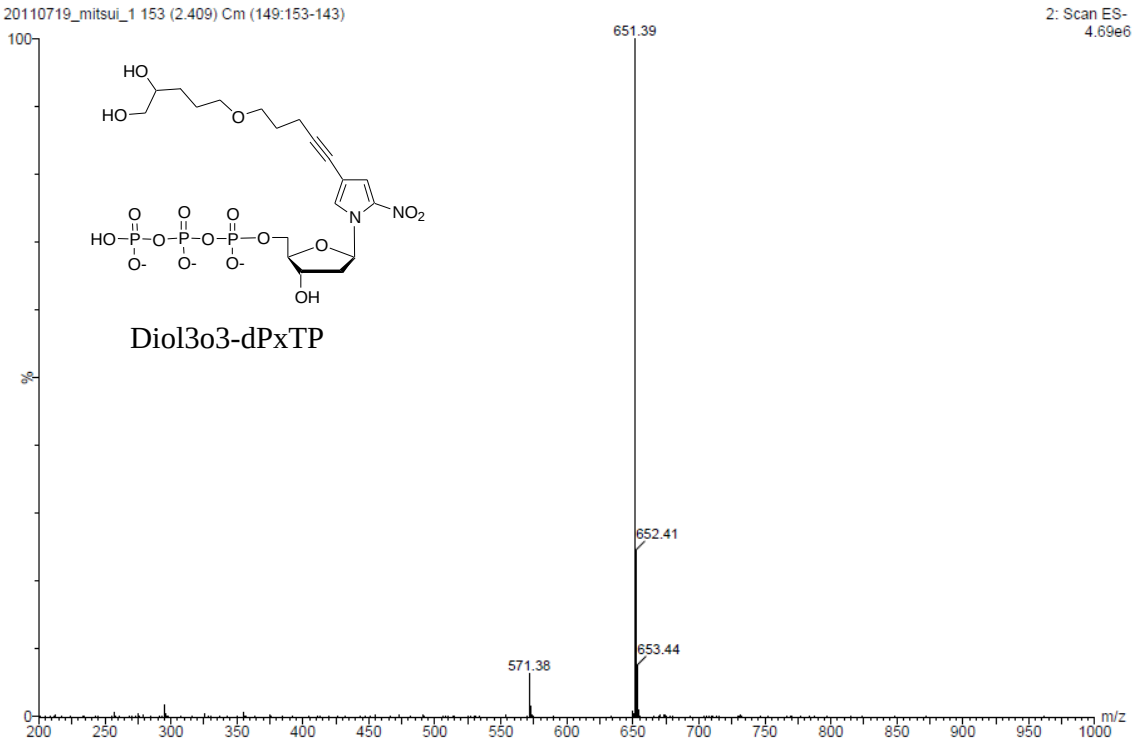
F10. ^{1}H NMR (300 MHz, D_2O) spectrum of Diol3o3-d**Px**TP.



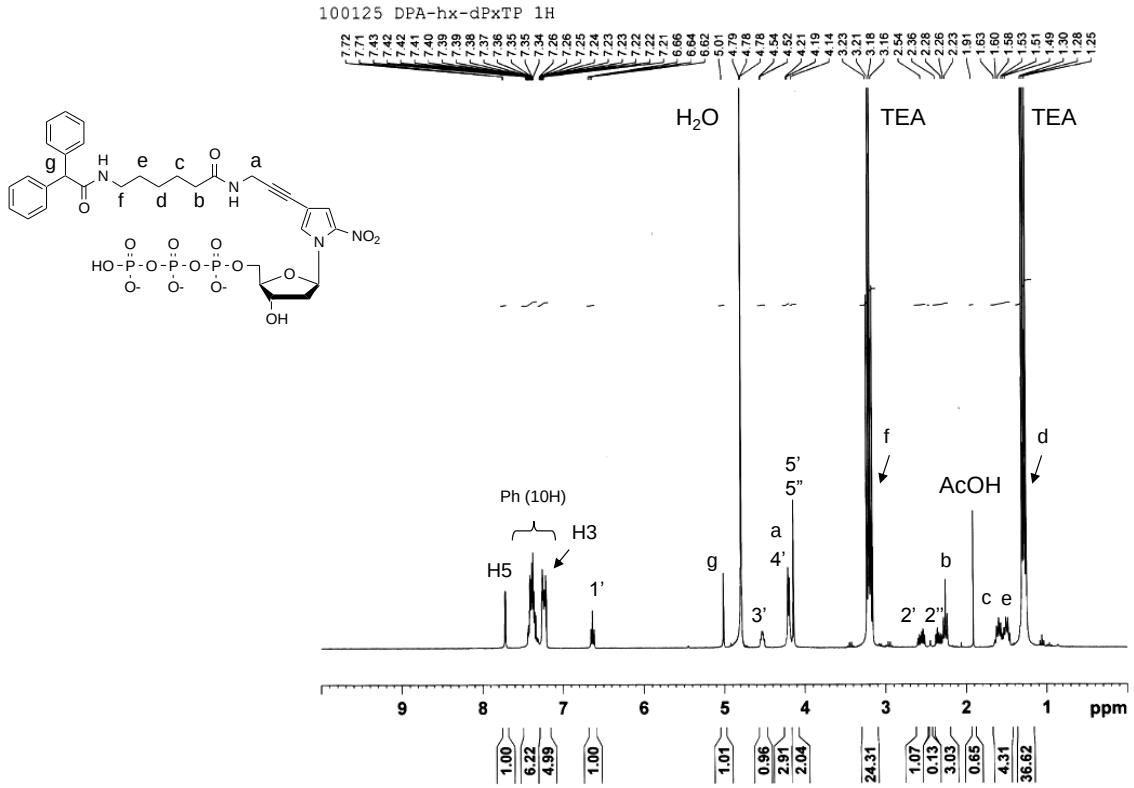
F11. ^{31}P NMR (121 MHz, D_2O) spectrum of Diol3o3-d**Px**TP.



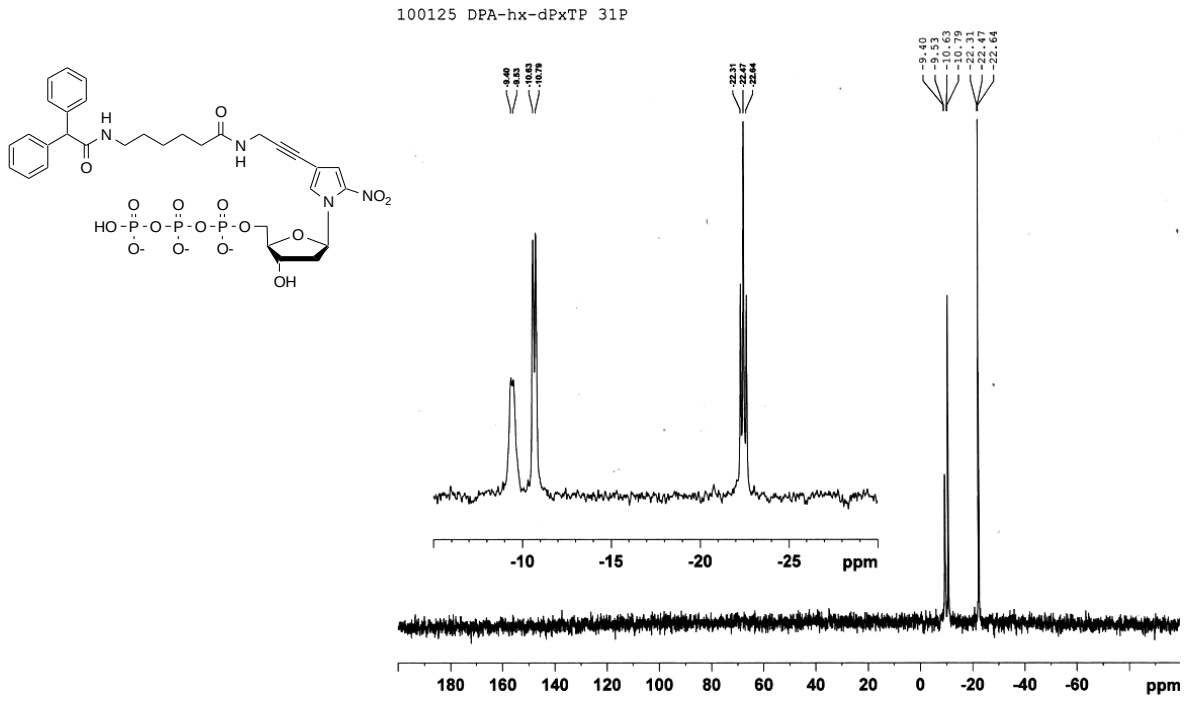
F12. ESI-MS spectrum of Diol3o3-d**Px**TP.



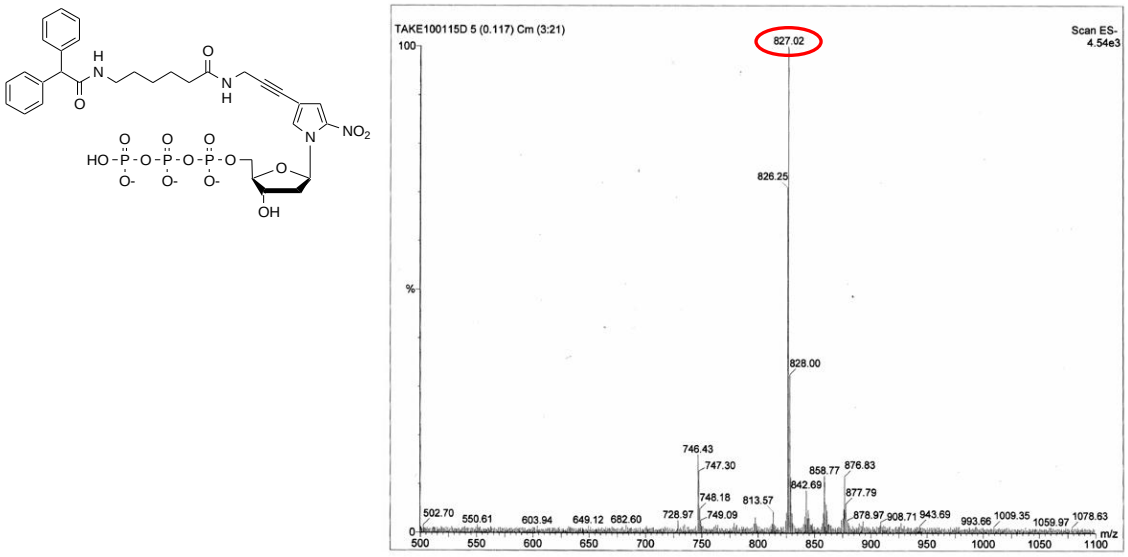
F13. ^{1}H NMR (300 MHz, D_2O) spectrum of DMP-hx-d**Px**TP.



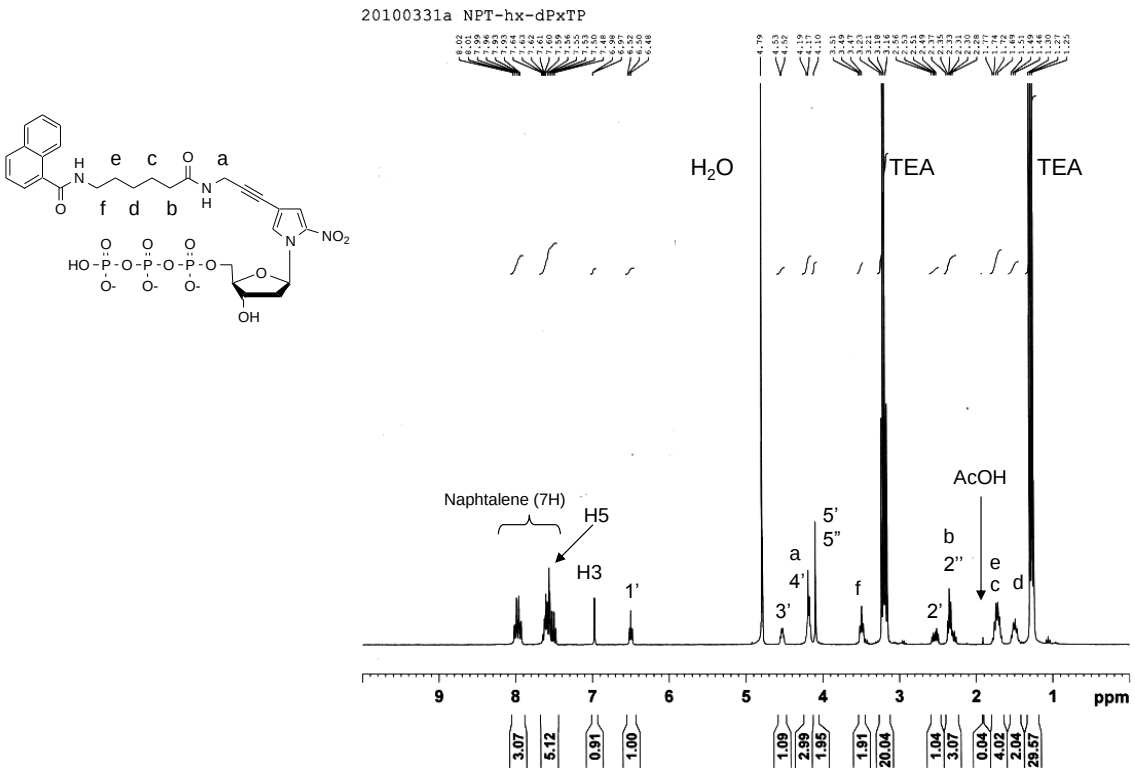
F14. ^{31}P NMR (121 MHz, D_2O) spectrum of DMP-hx-d**Px**TP.



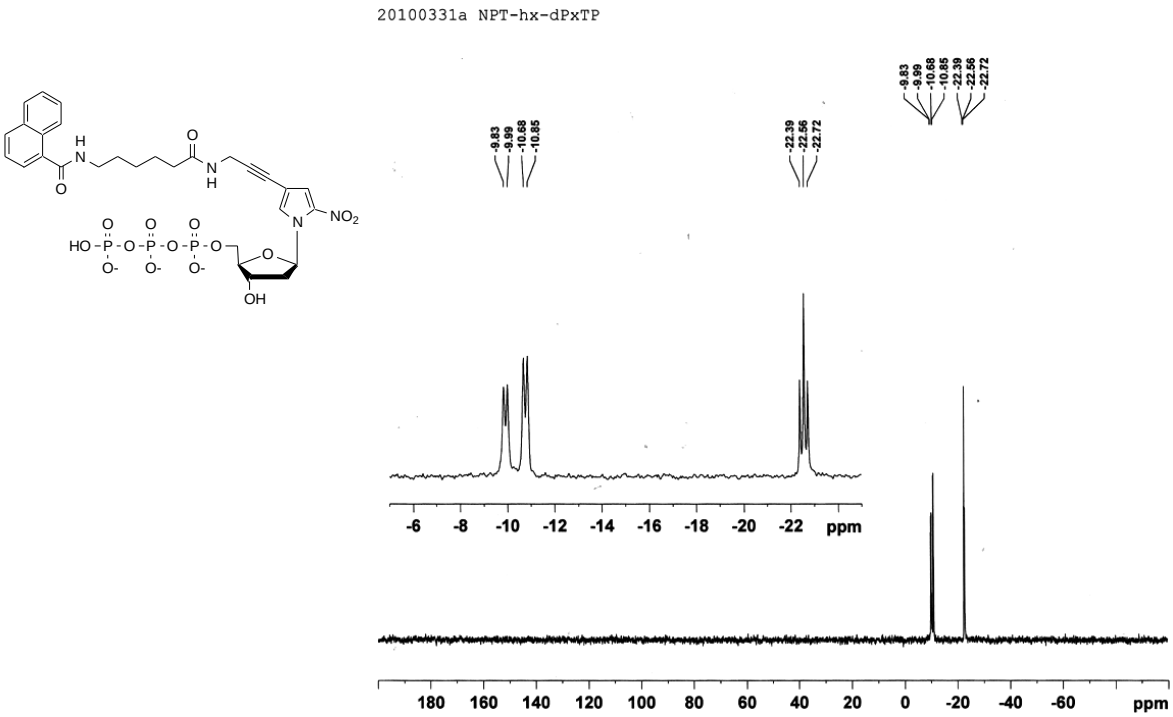
F15. ESI-MS spectrum of DMP-hx-d**Px**TP.



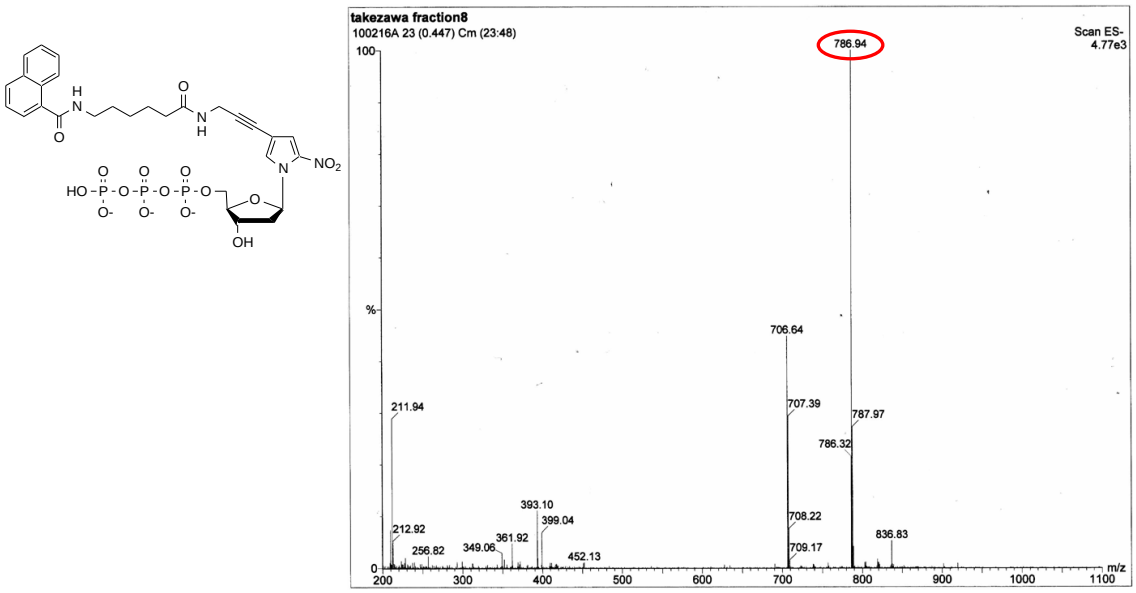
F16. ^{1}H NMR (300 MHz, D_2O) spectrum of NTP-hx-d**Px**TP.



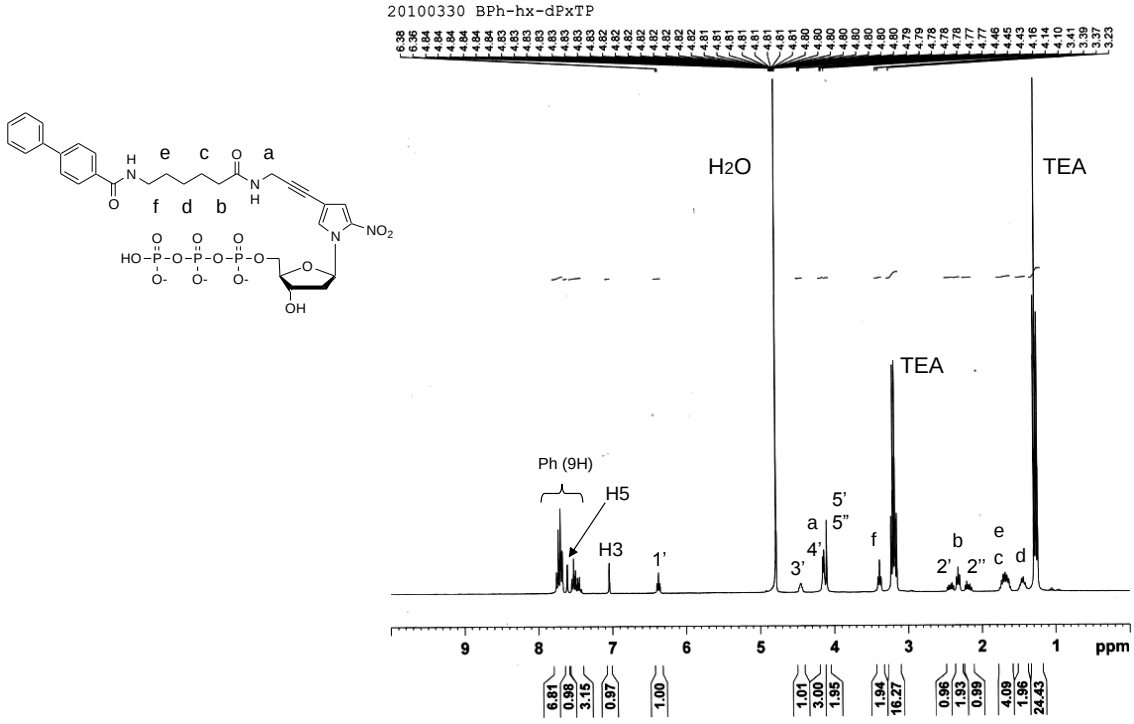
F17. ^{31}P NMR (121 MHz, D_2O) spectrum of NTP-hx-d**Px**TP.



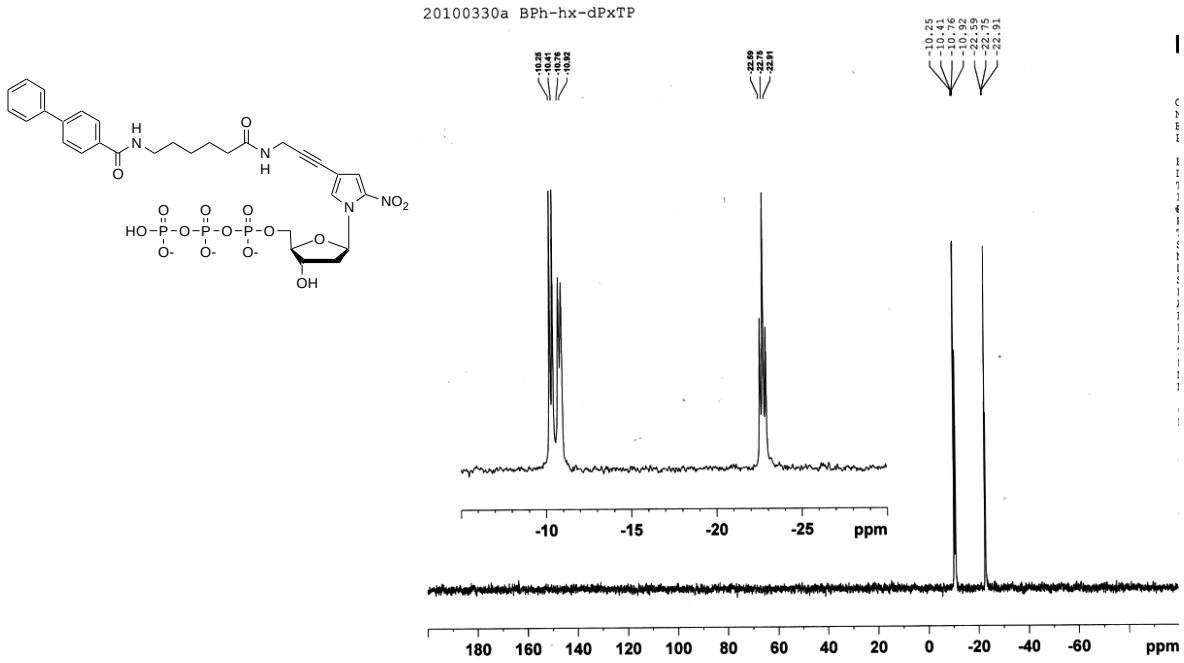
F18. ESI-MS spectrum of NTP-hx-d**Px**TP.



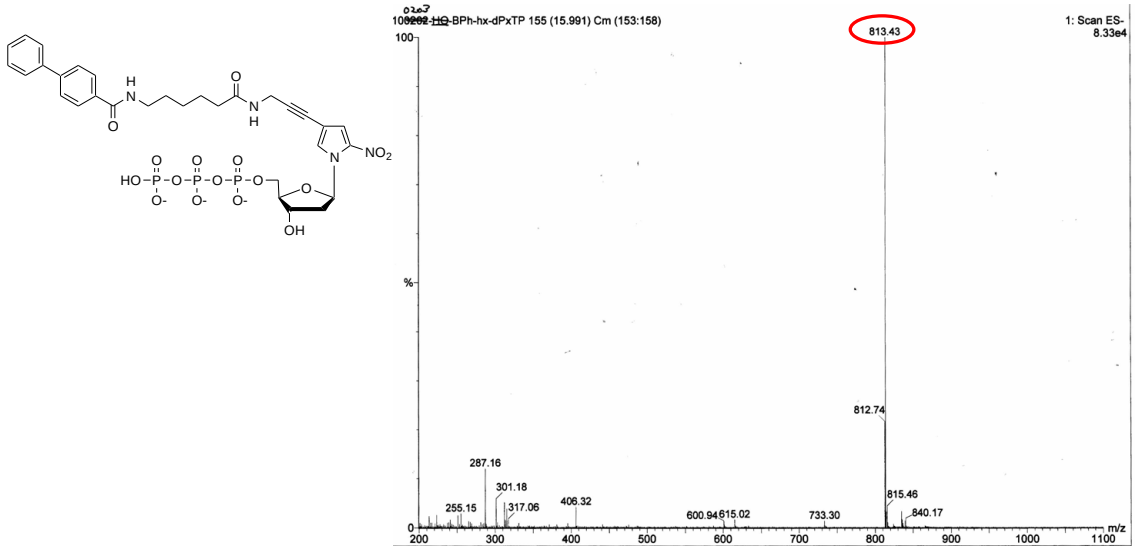
F19. ^{1}H NMR (300 MHz, D_2O) spectrum of BPh-hx-d**Px**TP.



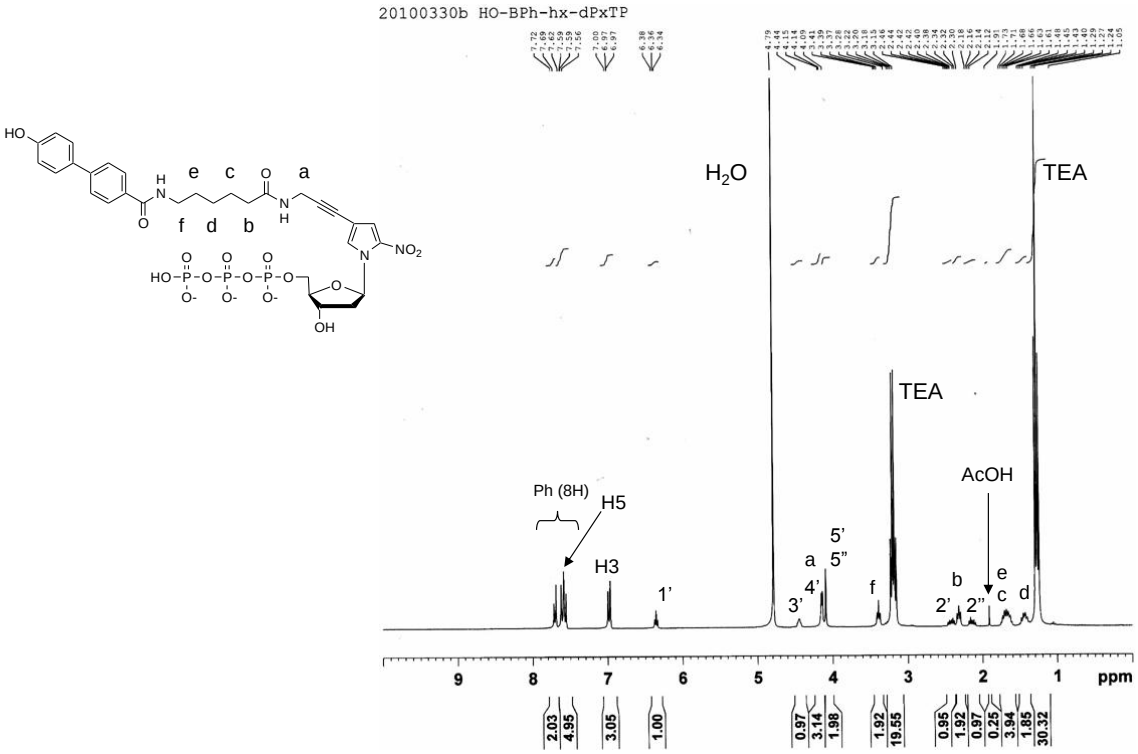
F20. ^{31}P NMR (121 MHz, D_2O) spectrum of BPh-hx-d**Px**TP.



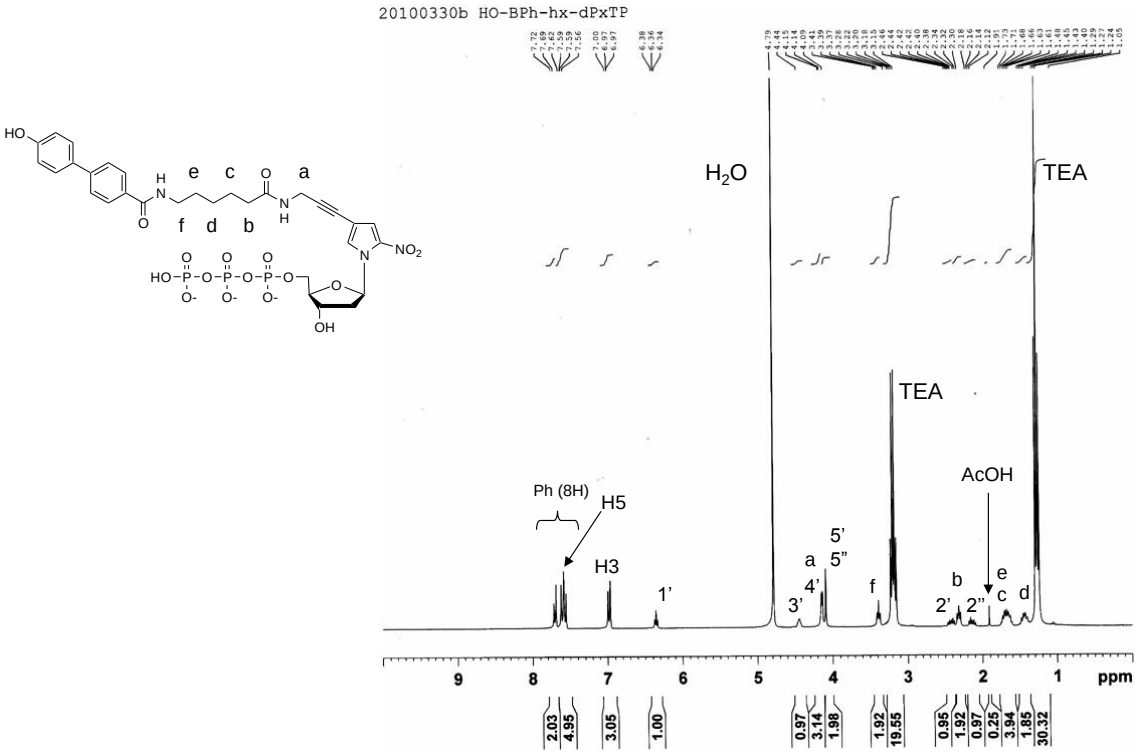
F21. ESI-MS spectrum of BPh-hx-d**Px**TP.



F22. ^{1}H NMR (300 MHz, D_2O) spectrum of HBP-hx-d**Px**TP.



F23. ^{31}P NMR (121 MHz, D_2O) spectrum of HBP-hx-d**Px**TP.



F24. ESI-MS spectrum of HBP-hx-d**Px**TP.