Electronic supplementary information (ESI)

Multiple-turnover Single Nucleotide Primer Extension Reactions to Detect of 8-Oxo-2'-Deoxyguanosine in DNA

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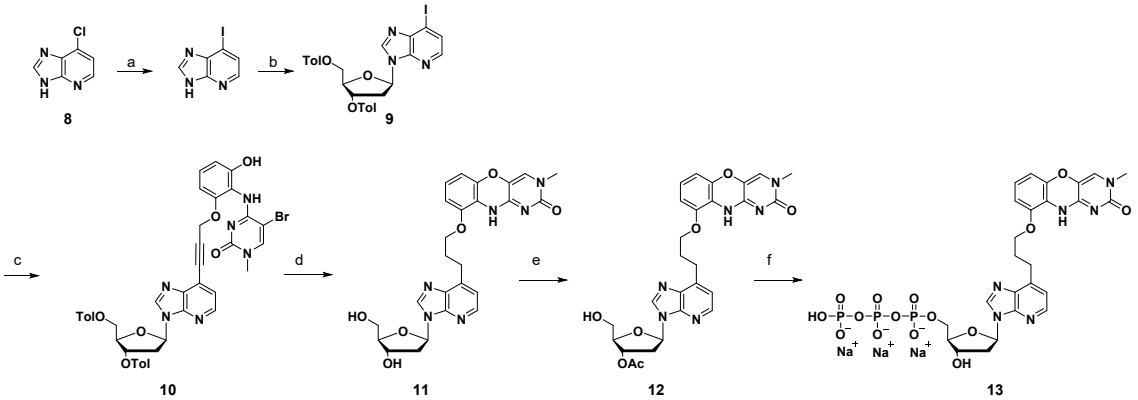
Contents

1. General. Most of reagents were purchased from TCI Co., Ltd. and Nacalai tesque, Inc. The ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded by Varian UNITY-400, INOVA-500 and Bruker AVANCE-III-500 spectrometers. The mass spectra were recorded by Bruker micrOTOF II system. All labelled primer and template ODNs were purchased from Gene Design Inc. or Japan Bio Services Co., Ltd, Japan.

2. Synthesis of dPdapTP (7) and 1-deaza-dPdapTP (13)

We synthesized the nucleoside triphosphate of dPdapTP (**7**) (Scheme 1) and 1-deaza-dPdapTP (**13**) (Scheme S1). The 5′-hydroxyl group was modified using a conventional triphosphate synthesis method. After purification by HPLC and conversion of the counter cation to sodium ion, the structure was identified by NMR and ESI mass spectrometry.



Scheme S1. Synthesis of 1-deaza-dPdapTP. Conditions and Reagents: (a) 60% HI, refluxed; (b) NaH, hoffer's chlorosugar, CH_3CN, 0°C, 41% for 2 steps; (c) $(Ph_3P)_4Pd$, CuI, compound **2**, DIPEA, DMF, 99%; (d) 1) Pd/Fib, MeOH, THF, 2) Ammonia in Methanol, 34% for 2 steps; (e) 1) DMTrCl, DMSO, then AcO_2, 2) CCl_3COOH, CH_2Cl_2, 55% for 2 steps; (f) 1) 2-Chloro-4-*H*-1,3,2-denzodioxaphosphrin-4-one, tributyl ammonium pyrophosphate, tributylamine, 1,4-dioxane, pyridine, 2) 1.0 % I_2, pyridine, H_2O, 3) 28% ammonium solution, HPLC purification and ion exchange treatment, 9.7% for 3 steps.

2-1. Synthesis of 2

Diisopropyl Azodicarboxylate (1.9 M in toluene, 4.0 mL, 7.7 mmol) was added to the mixture of compound **1** (2.0 g, 6.4 mmol), triphenylphosphine (2.9 g, 11.1 mmol) and propargyl alcohol (0.44 ml, 12.8 mmol) in CH_2Cl_2 (20 mL) at 0°C. After stirring for 8 hrs, the reaction was treated with saturated NH4Cl solution and extracted with EtOAc. The solvent was dried over Na_2So_4 and removed under reduced pressure, then the residue was purified by silica-gel column chromatography (Fuji Silysia NH, chloroform) to give **2** (1.8 g, 5.1 mmol, 79%) as pale yellow caramel.

IR (cm^{-1}): 2970.5, 2342.8, 1736.1, 1657.0. ^{1}H-NMR (400 MH_Z, DMSO-d_6): δ (ppm) 9.75 (1H, s), 8.16 (1H, s), 7.96 (1H, s), 7.07 (1H, t, J = 8.8, 8.5 Hz), 6.61 (1H, d, J = 8.8 Hz), 6.55 (1H, s , J = 8.5

Hz), 4.72 (2H, s), 3.52 (1H, s), 3.23 (3H.s). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 159.7, 154.8, 154.3, 147.9, 127.9, 115.2, 110.7, 104.6, 86.1, 79.8, 78.7, 56.9, 37.2. HR-ESIMS (*m/z*): calcd for $C_{14}H_{13}BrN_3O_3$ $(M+H)^+$ 350.0135, 352.0155; found, 350.0122, 352.0106.

2-2. Synthesis of 4

After addition of *N*,*N*-Diisopropylethylamine (0.59 mL, 3.44 mmol) to the mixture of compound **2** (440 mg, 1.26 mmol), compound **3** (746 mg, 1.67 mmol), copper(I) iodide (43 mg, 0.23 mmol) and $Pd(PPh_3)_4$ (131 mg, 0.11 mmol) in DMF (8.0 mL), the reaction mixture was heated at 60°C for 2 hrs. The reaction mixture was evaporated under reduced pressure, then the residue was purified by silica-gel column chromatography (Kanto 60N, chloroform/MeOH = 100/1) to give **4** (445 mg, 0.67 mmol, 53%) as a yellow powder.

IR (cm^{-1}): 3025.4, 2635.1, 1534.0, 1557.1. ^{1}H-NMR (400 MH_Z, $CDCl_3$): δ (ppm) 8.90 (1H, s), 8.28 (1H, s), 8.14 (1H, bs), 7.53 (1H, s), 7.03 (1H, t, *J* = 8.2 Hz), 6.73 (1H, d , *J* = 8.2 Hz), 6.61 (1H, d, *J* = 8.2 Hz), 6.47 (1H, t, *J* = 6.1 Hz), 5.44 (1H,m), 5.10 (2H,s), 4.36 (3H, m), 3.43 (3H,s), 2.98 (1H,m), 2.67 (1H,m), 2.12 (3H,s), 2.06 (3H,s). ^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm): 170.4, 170.33, 156.9, 153.3, 152.6, 151.1, 150.4, 149.4, 146.4, 143,9, 140.7, 135.1, 127.0, 116.3, 114.9, 103.3, 92.3, 87.8, 84.9, 82.8, 82.2, 74.3, 63.7, 57.9, 37.9, 37.5, 21.0, 20.9. HR-ESIMS (*m/z*): calcd. for $C_{28}H_{26}N_7O_8Na$ $(M+Na)^+$ 690.0919, 692.0898; found, 690.0924, 692.0910.

2-3. Synthesis of 9

The suspension of compound **8** (1.0 g, 6.51 mmol) in 60% of hydroiodic acid (10 mL) was refluxed for 4 hrs. The reaction mixture was poured into water with ice, and the precipitate was filtered off to give the iodine compound (950 mg) as a black solid. This product was used in the next reaction without further purification. The mixture of compound (950 mg, ca. 3.80 mmol) and sodium hydride (60% of oil suspension, 309 mg, 7.73 mmol) in acetonitrile (35 mL) were stirring for 30 minutes at room temperature. After addition of hoffer's chlorosugar (1.97 g, 5.94 mmol) at 0°C, the reaction mixture was stirred for 1 hr at same temperature. The reaction was treated with sat. $NaHCO_3$ solution and extracted with EtOAc. The organic solvent was evaporated under reduced pressure, then the residue was purified by silica-gel column chromatography (Kanto 60N, EtOAc/Hexane = 1/2) to give **9** (932 mg, 1.56 mmol, 41%) as a white powder

IR (cm^{-1}): 2926.0, 2331.8, 1716.8. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.56 (1H, s), 8.01 (1H, d, *J* = 4.9 Hz), 7.90 (2H, d, *J* = 7.0 Hz), 7.86 (2H, d, *J* = 7.0 Hz), 7.73 (1H, d, *J* = 4.9 Hz), 7.26 (2H, d, *J* = 7.9 Hz), 7.19 (2H, d, *J* = 7.9 Hz), 6.66 (1H, t, *J* = 6.7 Hz), 5.80 (1H, m), 4.75-4.66 (3H, m), 3.14 (1H, m), 2.91 (1H,m), 2.42 (3H, s), 2.38 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 166.2, 166.1, 166.0, 165.8, 144.5, 144.4, 144.2, 142.1, 139.2, 129.8, 129.7, 129.6, 129.3, 129.2, 128.4, 126.7, 126.5, 98.7, 85.1, 83.0, 75.2, 64.1, 37.9, 21.8, 21.7. HR-ESIMS (*m/z*): calcd. for $C_{27}H_{24}IN_3O_5Na$ $(M+Na)^+$

620.0653; found 620.0637.

2-4. Synthesis of 10

After addition of *N*,*N*-Diisopropylethylamine (0.60 mL, 3.53 mmol) to the mixture of compound **2** (400 mg, 1.14 mmol), compound **9** (1.0 g, 1.71 mmol), copper(I) iodide (44 mg, 0.23 mmol) and $Pd(PPh_3)_4$ (131 mg, 0.11 mmol) in DMF (8.0 mL), the reaction mixture was heated at 60°C for 2 hrs. The reaction mixture was evaporated under reduced pressure, then the residue was purified by silica-gel column chromatography (Kanto 60N, chloroform/MeOH = 50/1) to give **10** (925 mg, 1.13 mmol, 99%) as an orange foam.

IR (cm^{-1}): 3005.9, 2342.3.6, 1716.0, 1688.4. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 8.57 (1H,bs), 8.32 (1H, d, *J* = 5.0 Hz), 7.96 (2H, d, *J* = 8.0 Hz), 7.88 (2H, d, *J* = 8.0 Hz), 7.56 (1H,s), 7.27 (2H, d, *J* = 8.0 Hz), 7.26 (1H, s), 7.20 (2H, d, *J* = 8.0 Hz), 7.06 (1H, t, *J* = 8.5 Hz), 6.74 (1H, d, *J* = 8.5 Hz), 6.68 (1H, t, *J* = 6.0 Hz), 6.63 (1H, d, *J* = 8.0 Hz), 5.82-5.80 (1H, m), 5.10 (2H, s), 4.76-4.74 (1H, m), 4.69-4.64 (2H, m), 3.41 (3H, s), 3.20 (1H, m), 2.88 (1H, m), 2.42 (3H, s), 2.37 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 166.2, 166.0, 156.9, 153.0, 150.9, 149.9, 146.7, 144.6, 144.2, 142.5, 129.8, 129.7, 127.4, 126.6, 126.4, 121.9, 121.5, 114.6, 104.0, 87.6, 85.5, 83.3, 75.1, 64.0, 58.2, 53.8, 37.8, 37.7, 29.8, 21.8, 21.7. HR-ESIMS (*m/z*): calcd. for $C_{41}H_{37}BrN_6O_8$ $(M+H)^+$ 819.1700, 821.1679; found 819.1701, 821.1686.

2-5. General procedure of synthesis of 5 or 11

The solution of **4** (400 mg, 0.60 mmol) or **10** (900 mg, 1.10 mmol) in THF/methanol (50 mL, 5/1) was treated with 2.5% Palladium-Fibroin (400 mg) under hydrogen atmosphere. After stirring for 3 hrs, the reaction mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (Kanto 60N, chloroform/MeOH). The obtained compound was dissolved in 7M ammonia methanol solution (50 mL) and stirred for 48 hrs at room temperature, then chloroform was added to the reaction mixture and the precipitate was filtered off.

Pdap (**5**) as a white powder, 161 mg, 0.32 mmol, 53% for two steps. IR (cm^{-1}): 3385.4, 2355.1, 1674.0, 1562.1. 1H-NMR (400 MHZ, DMSO-d_6): δ (ppm) 9.81 (1H, s), 8.86 (1H,s), 8.85 (1H, s), 7.42 (1H,bs), 6.77 (1H, t, *J* = 8.2 Hz), 6.61 (1H, d, *J* = 8.2 Hz), 6.47 (1H, t, *J* = 6.4 Hz), 6.40 (1H, d, *J* = 8.2 Hz), 5.32 (1H, br), 5.10 (1H, br), 4.44-4.42 (1H, m), 4.04 (2H, t, *J* = 6.1 Hz), 3.89-3.87 (1H, m), 3.52-3.36 (2H, m), 3.18 (3H, s), 2.75-2.73 (1H, m), 2.38-2.30 (1H, m), 2.27-2.25 (2H, m). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 161.7, 152.3, 150.5, 147.9, 144.8, 132.9, 123.5, 108.3, 108.2, 88.5, 84.2, 71.2, 68.5, 62.0, 37.1, 36.9, 29.5, 29.3, 27.3, 23.0, 22.6, 14.4. HR-ESIMS (*m/z*), calcd. for: $C_{24}H_{25}N_7O_6Na$ $(M+Na)^+$: 530.1759; found 530.1762.

1-Deaza-Pdap (**11**) as a white powder, 187 mg, 0.37 mmol, 34% for two steps. IR (cm^{-1}): 3319.4,

2926.0, 2342.9, 1755.3, 1672.9. ^{1}H-NMR (500MHz, DMSO-d_6): δ (ppm) 8.82 (1H, s), 8.26 (1H, d, J = 5.0 Hz), 7.46 (1H, bs), 7.21 (1H, d, J = 5.1 Hz), 6.77 (1H, t, J = 8.0, 8.5Hz), 6.60 (1H, d, J = 8.5Hz), 6.51 (1H, t J = 6.0 Hz), 6.41 (1H, d, J = 8.5 Hz), 5.33-5.30 (2H, m), 4.43-4.40 (1H, m), 3.98 (2H, t , J = 6.0 Hz), 3.90-3.88 (1H, m), 3.64-3.50 (2H, m), 3.19-3.15 (5H, m), 2.76-2.60 (1H, m), 2.31-2.20 (1H, m), 2.17 (2H, q, J = 6.0 Hz). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 146.4, 144.2, 143.8, 143.2, 135.0, 126.7, 123.5, 118.8, 108.1, 108.0, 88.3, 84.2, 71.5, 68.1, 62.3, 56.5, 49.0, 37.0, 30.0, 29.1, 26.7, 19.0. HR-ESIMS (*m/z*), calcd. for $C_{25}H_{26}N_6O_6Na$ $(M+Na)^+$: 529.1806; found 529.1809.

2-6. General procedure of synthesis of 3'-OAc Pdap (6) or 3'-OAc 1-deaza-Pdap (12)

DMTrCl (0.57 mmol) was added to a solution of corresponding diol compound **5** or **11** (0.2 mmol) in DMSO (6.0 mL) at 50°C, and the mixture was stirred for 1 h. And then pyridine (2.0 mL) and acetic anhydride (0.4 mL, 0.41 mmol) were added to the reaction mixture at room temperature. After stirring for 1 h, the reaction mixture was evaporated under reduced pressure. The residue was dissolved in methanol (2.0 mL), dichloromethane (2.0 mL) and treated with 3% trichloroacetic acid in CH_2Cl_2 (1.0 mL). After stirring for 5 min at room temperature, the reaction mixture was neutralized by the addition of $NaHCO_3$ powder. The solvent was removed under reduced pressure, and the residue was purified by silica-gel column chromatography (Fuji Silysia NH, chloroform /methanol = 20:1).

6 as pale yellow foam, 71.4 mg, 0.13 mmol, 65%. IR (cm^{-1}): 3319.4, 2926.0, 2342.9, 1755.3, 1672.9. ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 9.94 (1H, bs), 8.89 (1H, s), 8.87 (1H, s). 7.45 (1H, bs), 6.76 (1H, t, J = 8.5 Hz), 6.61 (1H, d, J = 8.5 Hz), 6.50 (1H, dd, J = 6.0, 6.5 Hz), 6.40 (1H, d, J = 8.5 Hz), 5.39-5.37 (1H, m), 5.35 (1H, t, J = 5.5 Hz), 4.12-4.09 (1H, m), 4.03 (2H, t, J = 6.5 Hz), 3.70-3.59 (2H, m), 3.31 (2H, t, J = 7.5 Hz), 3.18 (3H, s), 3.02-2.98 (1H, m), 2.55 (1H, s), 2.27 (2H, q, J = 6.5 Hz), 2.09 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 170.52, 161.86, 152.32, 150.53, 144.68, 132.87, 123.52, 108.23, 108.16, 85.79, 84.3, 75.5, 68.4, 62.0, 56.3, 55.4, 37.1, 30.0, 29.3, 27.2, 21.4. HR-ESIMS (*m/z*), calcd. for $C_{26}H_{27}N_7O_7Na$ $(M+Na)^+$: 572,1864; found, 572.1873.

12 as a white foam, 60.3 mg, 0.11 mmol, 55%. IR (cm^{-1}): 3273.8, 2339.1, 1731.0, 1672.4. ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 10.42 (1H, bs), 9.32 (1H, s), 8.25 (1H, d, J = 5.0 Hz), 7.06 (1H, d, J = 5.0 Hz), 6.81 (1H, s), 6.75 (1H, t, J = 8.5 Hz), 6.67 (2H, m), 6.46 (1H, d, J = 8.5 Hz), 6.37 (1H, d, J = 8.5 Hz), 5.56 (1H, d, J = 5.5 Hz), 4.03-3.95 (4H, m), 3.35 (3H, s). 3.28-3.20 (1H, m), 3.08-2.88 (2H, m), 2.18-2.07 (7H, m). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 170.5, 156.5, 154.8, 146.4,146.2, 145.1, 143.6, 143.4, 143.0, 135.4, 128.2, 127.0, 123.1, 118.5, 116.7, 108.0, 106.6, 86.6, 86.1, 66.6, 62.9, 38.8, 37.7, 29.7, 29.3, 26.1, 21.2. HR-ESIMS (*m/z*): calcd. for $C_{27}H_{28}N_6O_7Na$ $(M+Na)^+$: 571.1912; found 571.1900.

2-7. General procedure of synthesis of dPdapTP (7) or 1-deaza-dPdapTP (13)

2-Chloro-1,3,2-benzodioxaphosphorin-4-one (19.2 mg, 0.09 mmol) in 1,4-dioxane (0.4 mL) was added to the solution of **6** or **12** (0.05 mmol) in pyridine/1,4-dioxane (50/50, 0.5 mL), and then the mixture was stirring for 30 minutes at room temperature. The reaction mixture was treated with 0.38 M solution of tributylammonium pyrophosphate in DMF (0.24 mL, 0.09 mmol) and tributylamine (54 μL, 0.35 mmol) at room temperature for 30 minutes. The reaction mixture was treated with 1% iodine in pyridine-water (98/2, 2.0 mL) for 5 minutes, which was treated with 5% $NaHSO_3$ solution (1.3 mL) for 30 minutes. The solvent was evaporated under reduced pressure, and then the residue was dissolved in 28% ammonium solution (15 mL). After stirring for 12 h, the solvent was removed under reduced pressure. The residue was washed with 75% ethanol in water including 0.07 M NaCl (1.3 mL), and the precipitate was dissolved in water and purified by HPLC. (HPLC conditions: Column (Shiseido CAPCELL PAK C18-MG), Buffer (A: 20 mM TEAA, B: CH_3CN, B conc. 10 to 50%/20 min. linear gradient.), Flow rate (1.0 mL/min), UV-detector (254 nm), Column oven (35°C).) After lyophilization of the fraction, the residue was dissolved in deionized water. The resulting pale yellow solution was treated with Dowex Resins (Na^+ form) to convert the counter cation to sodium ion, whose purity and concentration was determined by NMR measurements.

dPdapTP (**7**) was obtained as pale yellow solution (5.0 μmol, 10%). ^{1}H-NMR (D_2O, 500 MHz): δ (ppm) 8.64 (1H, bs), 8.46 (1H, s), 7.20 (1H, bs), 6.69 (1H, t, J = 8.5 Hz), 6.39-6.35 (2H, m), 6.25 (1H, d, J = 8.5 Hz), 4.26 (2H, bs), 4.20 (1H, bs) 4.13-4.05 (2H, m), 3.29(5H, bs), 3.24-3.22 (1H, m), 2.67 (1H, m) 2.49-2.47 (1H, m), 2.40 (2H, bs). ^{31}P-NMR (D_2O, 162 MHz): δ (ppm) -10.1, -11.3, -23.0. HR-ESIMS (*m/z*), calcd. for $C_{24}H_{27}N_7O_{15}P_3$ $(M-H)^-$: 746.0856, found 746.0849.

1-Deaza-dPdapTP (**13**) was obtained as pale yellow solution (4.9 μmol, 9.7%). ^{1}H-NMR (D_2O, 500 MHz): δ (ppm) 8.29 (1H,s), 8.08 (1H,d, J = 5.0 Hz), 7.21 (1H, d. J = 5.0 Hz), 7.12 (1H, s), 6.63 (1H,t, J = 8.5, 8.0 Hz), 6.37 (1H, t, J = 7.5 Hz), 6.33 (1H, d, J = 8.5 Hz), 6,18 (1H, d, J = 8.0 Hz), 4.17 (2H, bs), 4.11-4.02 (2H, m), 3.24 (3H, s), 3.03 (1H, m), 2.68-2.63 (1H, m), 2.48-2.43 (1H, m), 2.36 (1H, bs), 2.21 (1H, bs). ^{31}P-NMR (D_2O, 162 MHz): δ (ppm) -7.96, -10.9, -20.9. HR-ESIMS (*m/z*), calcd. for $C_{25}H_{28}N_6O_{15}P_3$ $(M-H)^-$: 745.0831; found 745.0881.

Reference: J. Ludwig, F. Eckstein, *J. Org. Chem*. **1989**, *54*, 631-635.

3. General procedure of single nucleotide primer extension and steady-state kinetics study

The mixture of FAM labeled primer (1.0 μM, 15 mer) and template DNA, Template(**X**) (**X** = 8-oxo-dG, dG, dA, dC and T, 1.0 μM, 25 mer), in buffer containing 10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$ and 1 mM DTT, pH 7.9 was annealed at 90°C for 5 min, and the Klenow fragment (exo^-) (NEB BioLabs Japan Inc.) (0.01 – 0.1 unit) were added to the mixture at 37°C. The mixture of primer and template in the buffer containing 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 150 mM KCl, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8 was annealed at 90°C for 5 min, and the Bst DNA polymerase (0.1 – 0.2 unit) were added to the mixture at 37°C. The reaction was initiated by the addition an identical volume of the dATP, dPdapTP or 1-deaza-dPdapTP solution (0.1 – 35 μM) in the same buffer at 37°C. The reaction times (1 – 20 min) were adjusted in the different dNTP reactions to achieve 1 – 20 % incorporation, and the reactions were then quenched with loading buffer and analyzed by 15% denaturing polyacrylamide gel electrophoresis. The bands were visualized and quantified using a fluorescence imager (LAS4000). The relative velocity v was calculated from the ratio of the extended product (I_{ext}) to remaining primer (I_{pri}) as follows: $v = I_{ext}/I_{pri}$ t, where t represents the reaction time, which was normalized to the lowest enzyme concentration used. The apparent K_m and V_{max} values were obtained from Hanes-Woolf plots using the data points of at least five dNTP concentrations.

4. Table S1. Steady-state kinetic data for the primer extension of dNTPs opposite nucleosides in template DNAs.[a]

Entry	dNTP and DNA polymerase	Template X	V_{max} (% min^{-1})	K_M (μM)	Efficiency (% min^{-1} M^{-1})
1	dAdapTP Klenow [b, e]	oxodG	2.35 (0.37)	0.80 (0.02)	3.02×10^6
2		dG	0.26 (0.01)	3.11 (0.25)	0.08×10^6
3		dA	0.99 (0.26)	6.35 (0.34)	0.16×10^6
4		dC	0.79 (0.01)	5.40 (0.47)	0.18×10^6
5		T	5.23 (0.43)	0.48 (0.14)	10.9×10^6
6	dPdapTP Klenow [b]	oxodG	1.04 (0.23)	0.59 (0.02)	1.82×10^6
7		dG	0.17 (0.01)	0.88 (0.10)	0.19×10^6
8		dA	0.40 (0.03)	3.52 (0.87)	0.11×10^6
9		dC	0.70 (0.11)	5.65 (1.52)	0.12×10^6
10		T	0.98 (0.29)	0.31 (0.01)	3.14×10^6
11	dAdapTP Bst pol [c]	oxodG	0.25 (0.02)	11.82 (3.92)	2.08×10^4
12		dG	0.02 (0.01)	2.06 (0.58)	0.98×10^4
13		dA	n.d.[d]	n.d.[d]	n.d.[d]
14		dC	n.d.[d]	n.d.[d]	n.d.[d]
15		T	0.52 (0.04)	7.27 (1.63)	7.23×10^4
16	dPdapTP Bst pol [c]	oxodG	0.09 (0.01)	2.31 (0.57)	3.90×10^4
17		dG	n.d.[d]	n.d.[d]	n.d.[d]
18		dA	n.d.[d]	n.d.[d]	n.d.[d]
19		dC	n.d.[d]	n.d.[d]	n.d.[d]
20		T	0.13 (0.01)	3.32 (0.14)	3.94×10^4

[a] Conditions: 1.0 μM of the 15-mer/25-mer FAM-labeled Primer-Template (X) duplex, DNA polymerase, the corresponding reaction buffer, and 0.1–35 μM dNTPs incubated at 37°C for 1–20 min in a 10-μL reaction volume. Velocity was normalized for the lowest enzyme concentration used. [b] 0.01–0.1 unit/μL Kf(exo^-) and 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT. [c] 0.1–0.2 unit/μL Bst DNA polymerase, and 20 mM Tris-HCl (pH 8.8), 10 mM $(NH_4)_2SO_4$, 150 mM KCl, 2 mM MgSO4, 0.1% Tween 20. [d] n.d.: not determined because of the lack of an incorporation reaction under this condition. [e] ref. 17. Standard deviations are given in parentheses (three independent experiments).

5. Thermal denaturing study of duplex DNA containing primers and templates

The melting temperatures (T_m values) of the duplex DNA containing template DNAs and primers were identified by performing thermal denaturing studies (Figure S1 and S2). Two micromolar of duplex DNA containing corresponding primers and templates was annealed in buffer containing 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 150 mM KCl, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8. UV melting curves were monitored at 260 nm from 35 to 75°C with a rate of 1.0°C/min, and the data were analysed using a V-730BIO instrument manufactured by JASCO Corporation. The melting curves of 15-mer primer or 13-mer primer and dG template duplex are orange curve or green curve, respectively (Figure S1). Furthermore, the melting curves of blocking primer and dG -template or 8-oxo-dG template duplex are red curve or blue curve, respectively (Figure S2).

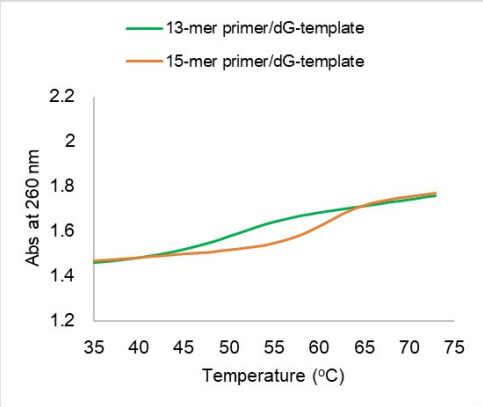


Figure S1. Melting curves of the duplexes. The orange curve shows the result of the duplex containing 15-mer primer and dG template. The green curve shows the result of the duplex containing 13-mer primer and dG template.

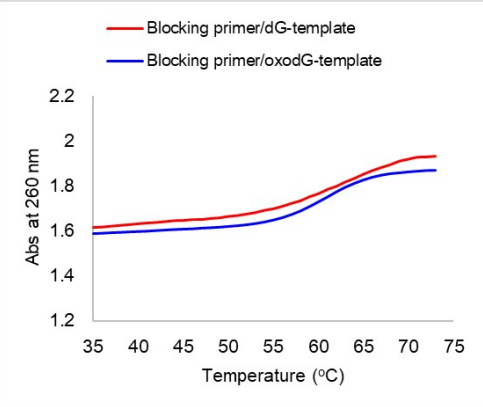


Figure S2. Melting curves of the duplexes. The red curve shows the result of the duplex containing blocking primer and dG template. The blue curve shows the result of the duplex containing blocking primer and oxodG template.

6. Table S2. Sequences of the blocking primer and normal primers and their T_m values[a]

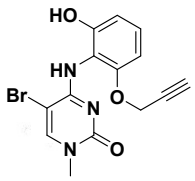
	Sequence	T_m (°C)
Blocking primer	5'-GCATAACCCTAACC-ddC	
dG template	3'-GCGTATTGGGATTGG**G**ATTGACAGC	65.2 ± 0.3
oxodG template	3'-GCGTATTGGGATTGG^{oxo}**G**ATTGACAGC	60.9 ± 0.2

[a] Conditions: 2.0 μM of duplex DNA (primer-template) in buffer containing 20 mM Tris-HCl (pH 8.8), 10 mM $(NH_4)_2SO_4$, 150 mM KCl, 2 mM $MgSO_4$, and 0.1% Tween 20. Samples were warmed at a rate of 1.0°C/min from 35 to 75°C, and T_m values were the average of three or more independent experiments.

7. Procedure of single nucleotide primer extension amplification reaction

Single nucleotide primer extension amplification reaction for 25-mer template: The mixture of template (1.0 μM of dG template with or without 1.0 nM of oxodG template) and primer (1.0 μM of 13-mer FAM labeled primer) and blocking primer (0.95 μM of 15-mer DNA) in buffer containing 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 150 mM KCl, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8. dPdapTP (20 μM) and Bst 3.0 DNA polymerase (1.0 unit/μM) were added, and the mixture was incubated at 37°C for 12 hours. The reaction was quenched with loading buffer and analyzed by 15% denaturing polyacrylamide gel electrophoresis. The bands were visualized using a fluorescence imager (LAS4000).

8. ^{1}H-NMR and ^{13}C-NMR spectrum of 2



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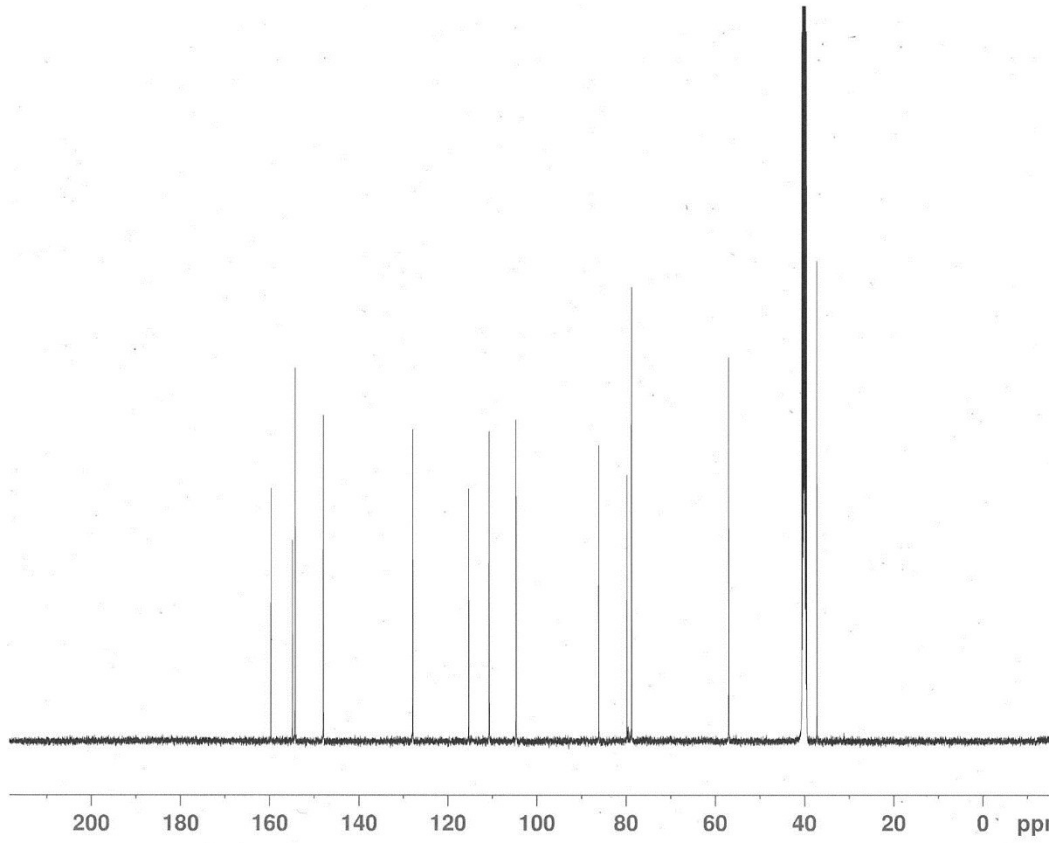
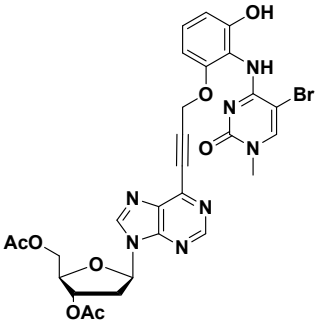


^{1}H-NMR and ^{13}C-NMR spectrum of 4



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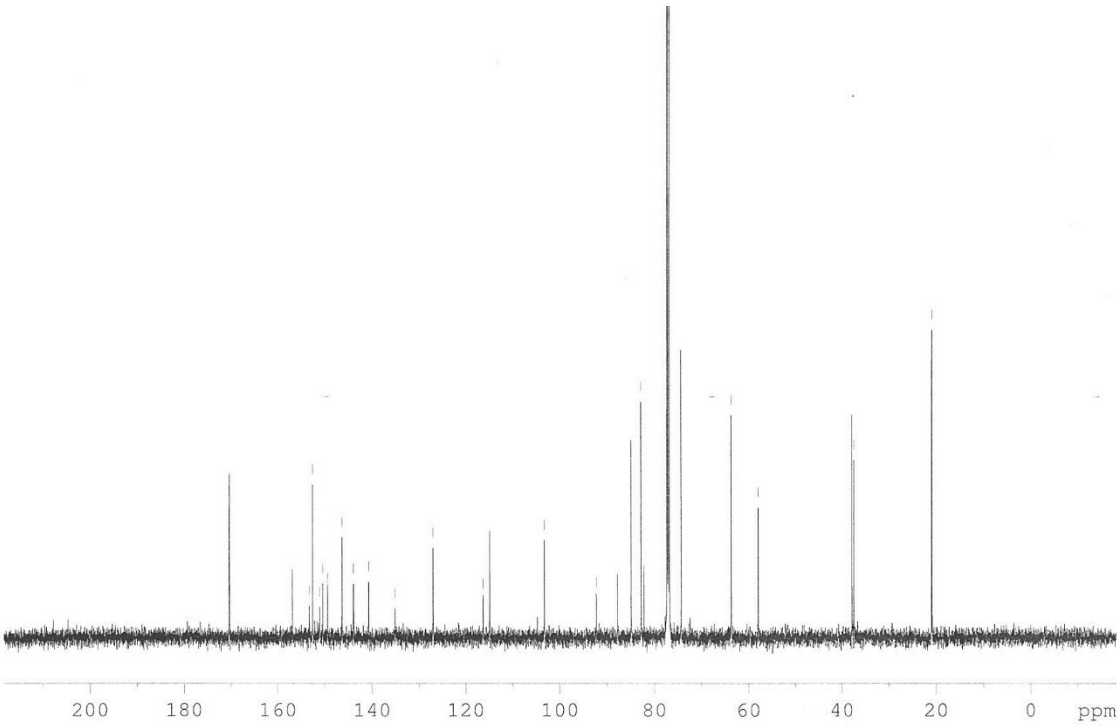
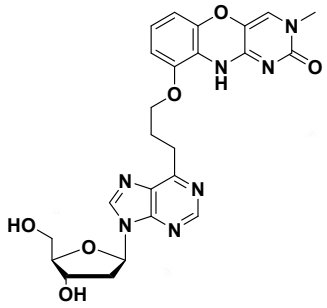


^{1}H-NMR and ^{13}C-NMR spectrum of 5



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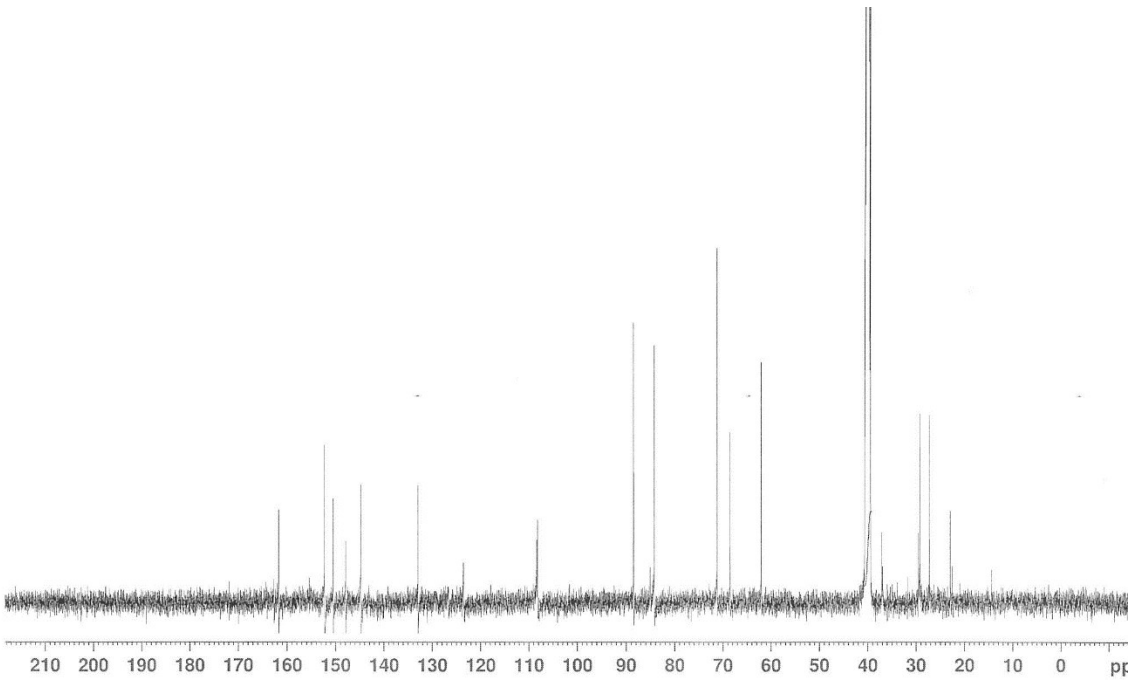
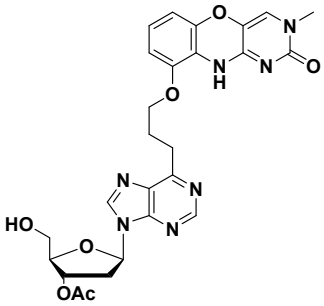


^{1}H-NMR and ^{13}C-NMR spectrum of 6



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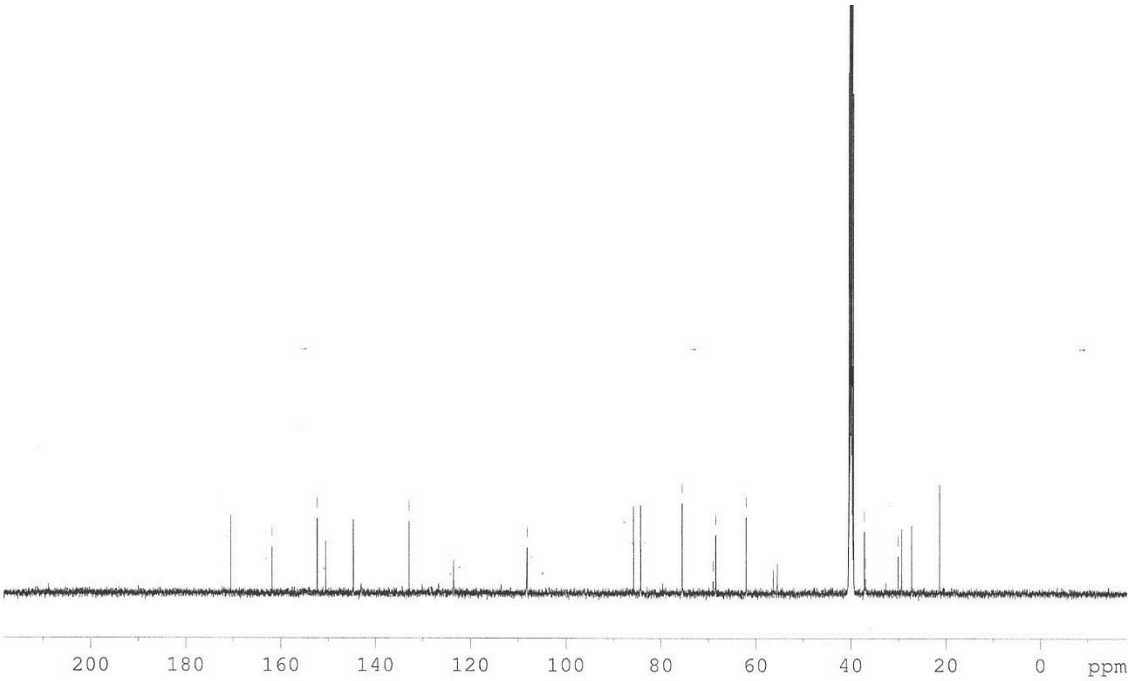
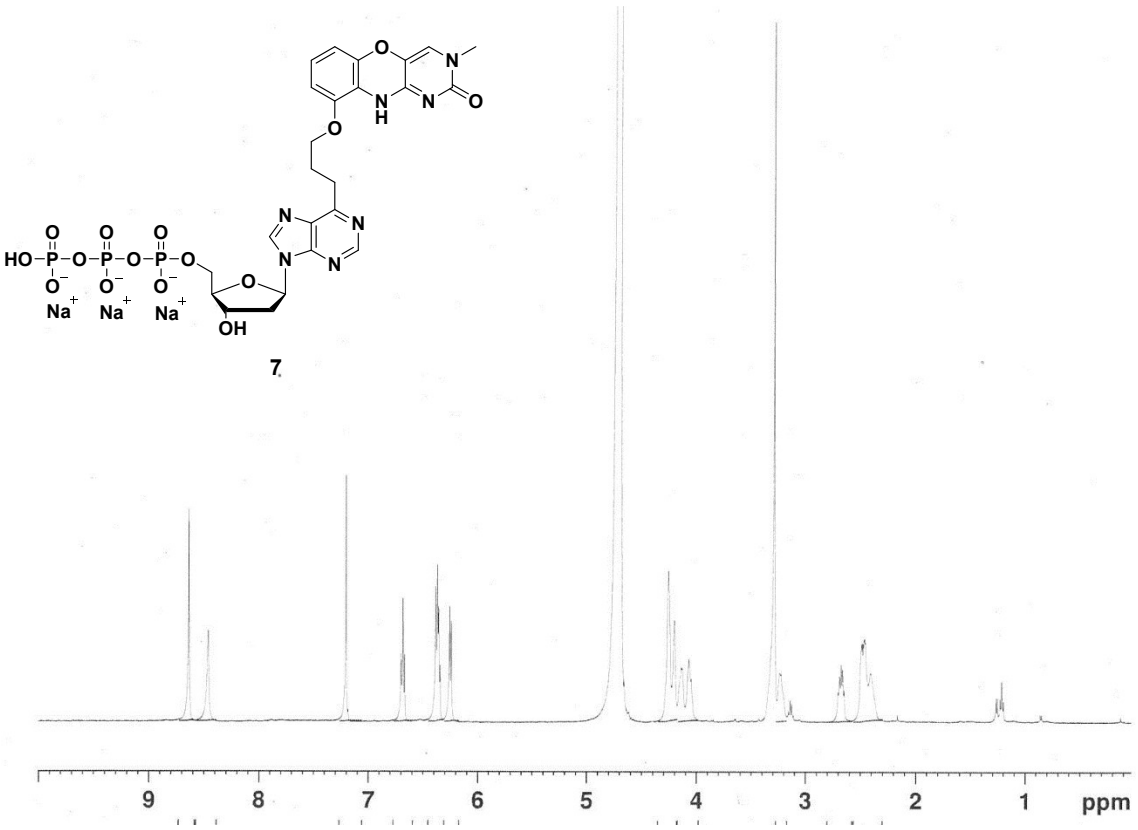
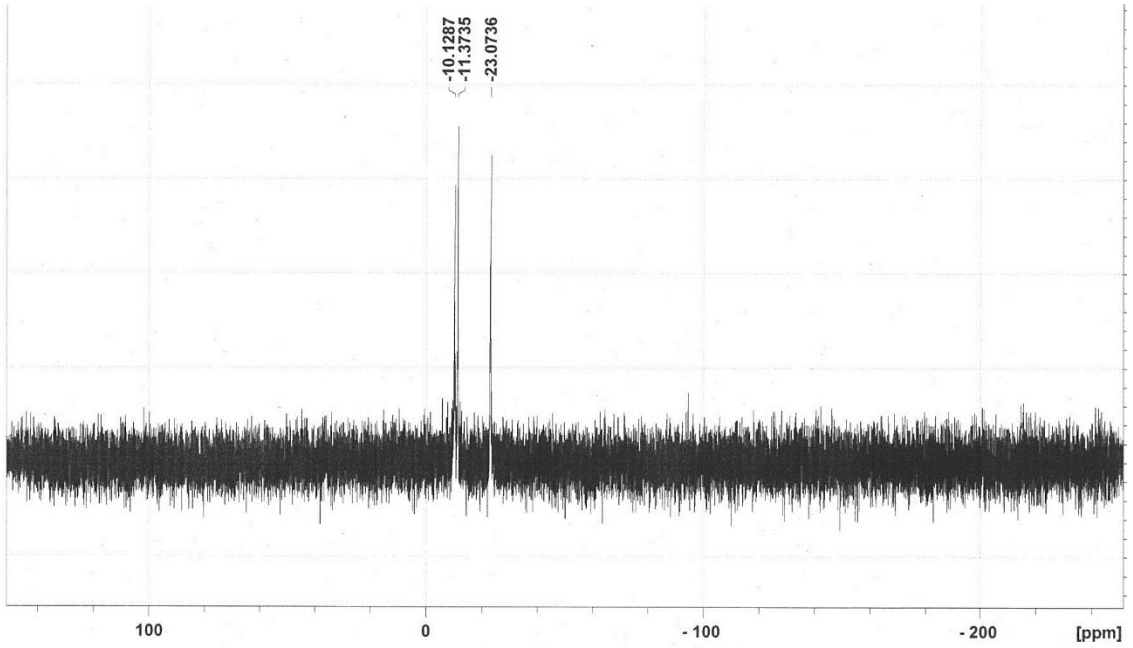
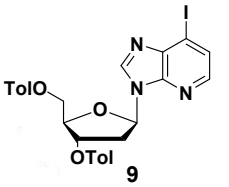


^{1}H-NMR and ^{31}P-NMR spectrum of 7





^{1}H-NMR and ^{13}C-NMR spectrum of 9



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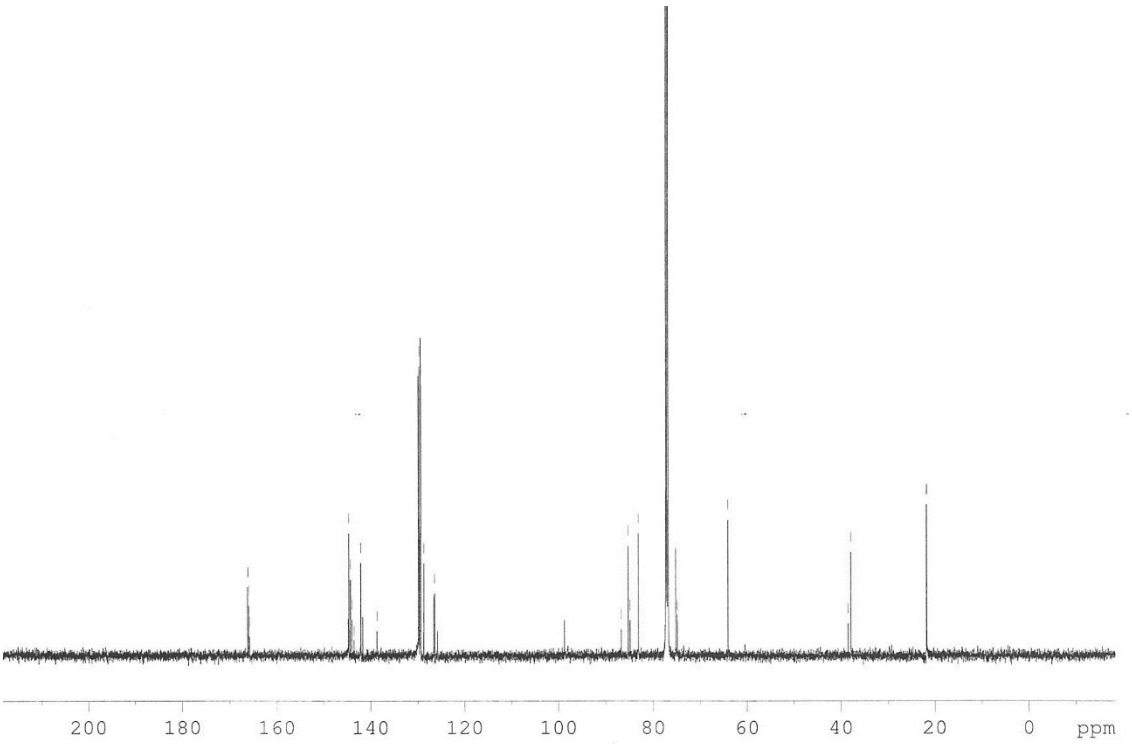
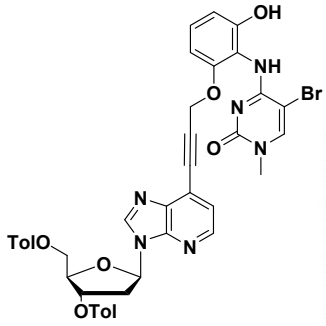


^{1}H-NMR and ^{13}C-NMR spectrum of 10



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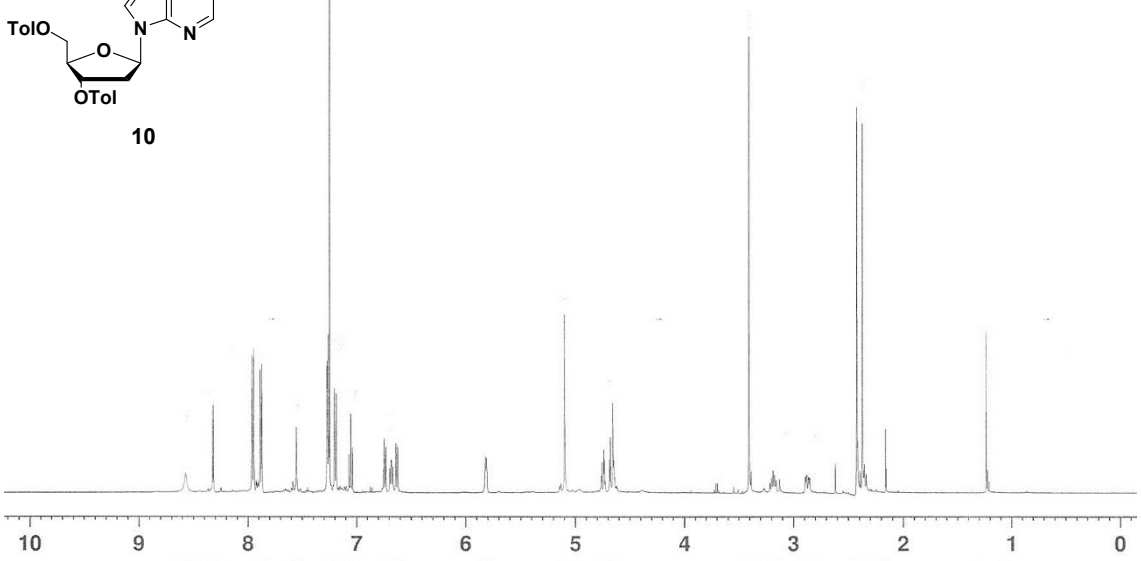
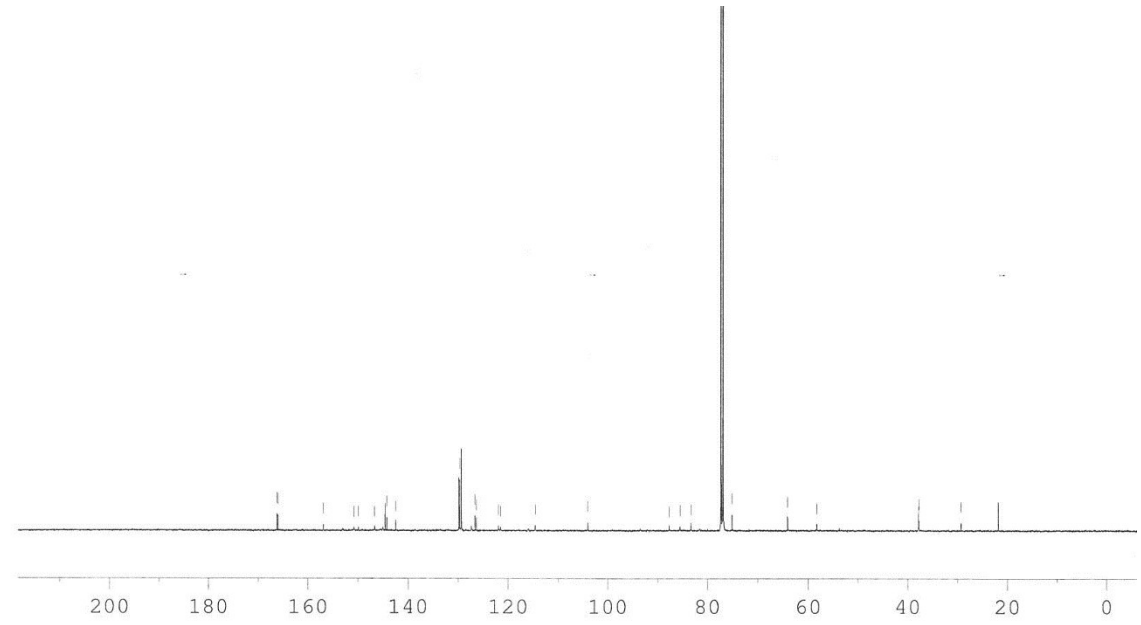
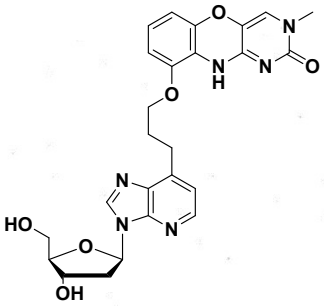




^{1}H-NMR and ^{13}C-NMR spectrum of 11



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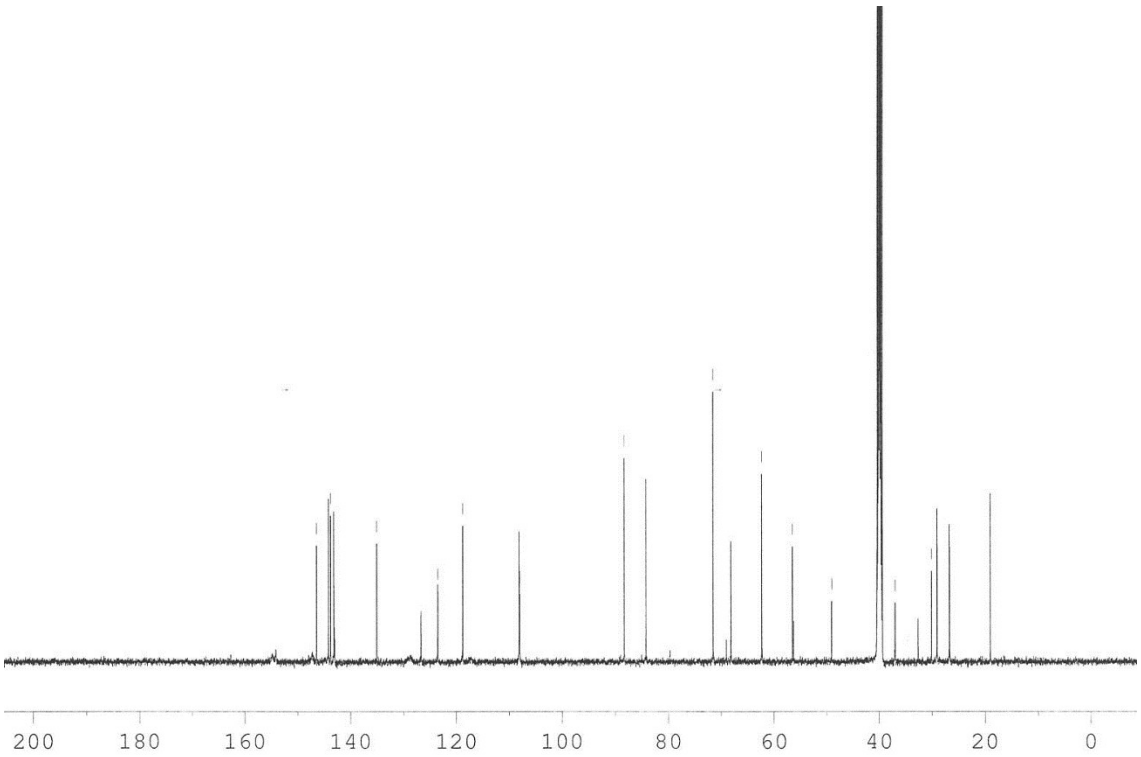
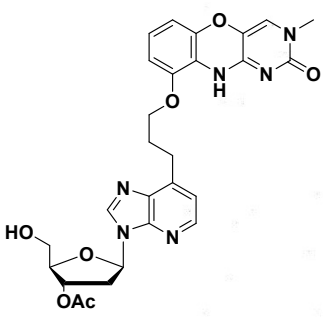


^{1}H-NMR and ^{13}C-NMR spectrum of 12



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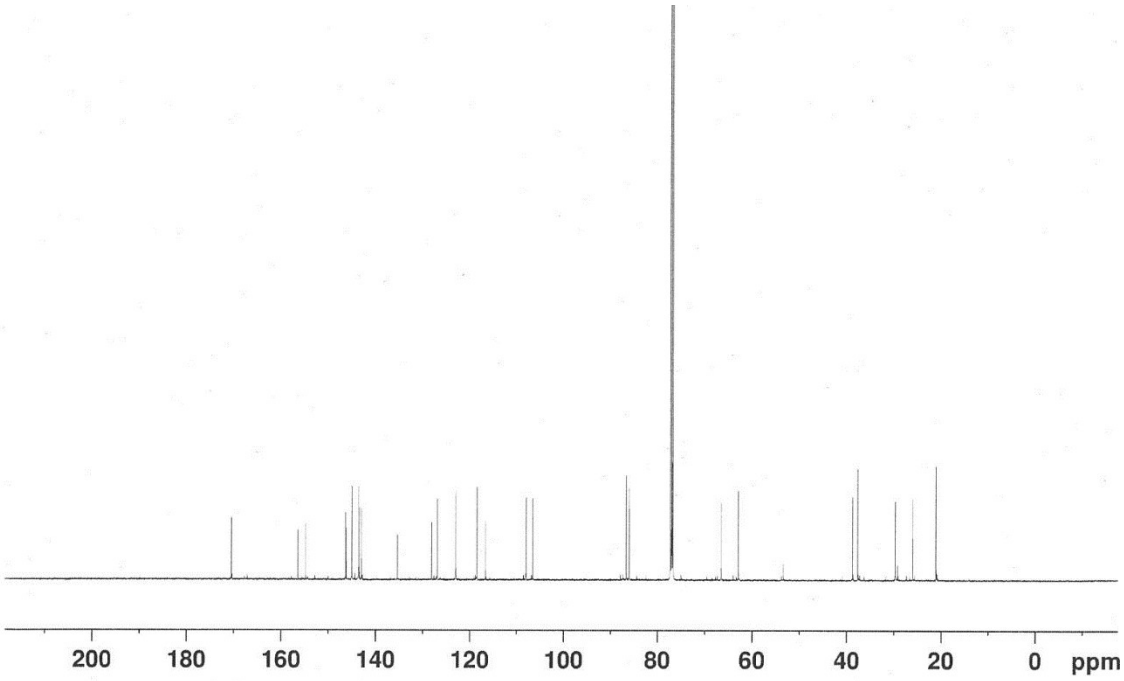
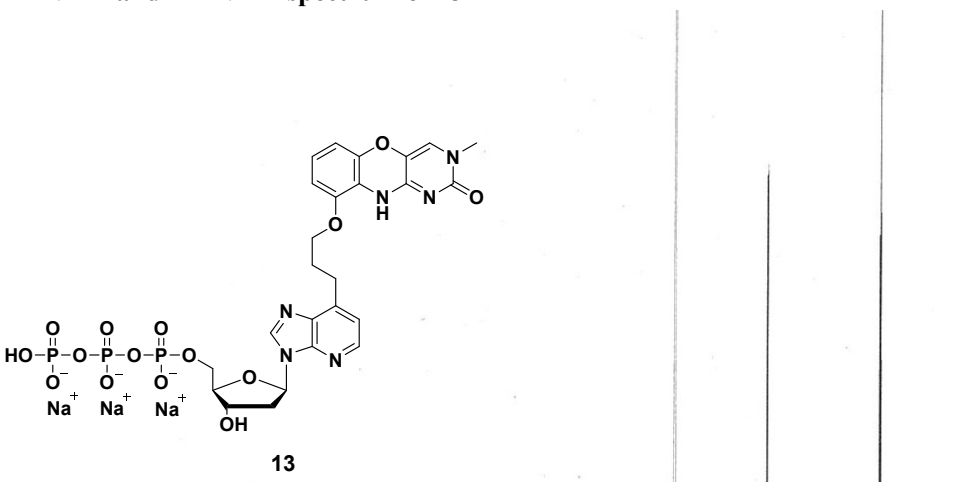


^{1}H-NMR and ^{31}P-NMR spectrum of 13



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