Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry. This journal is © The Royal Society of Chemistry 2016

### **Supporting Information**

### Photo-cleavable Nucleotides for Primer Free Enzyme mediated DNA synthesis.

Anu Stella Mathews, <sup>a, b</sup> Haikang Yang, <sup>a, b</sup> Carlo Montemagno\* <sup>a, b</sup>

- a. Ingenuity Lab, 11421 Saskatchewan Drive, Edmonton, Alberta T6G 3M9, Canada
- b. Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta T6G 2V4, Canada
- \* Phone: +1 780-641-1617.
- \* E-mail: montemag@ualberta.ca

### **Table of Contents**

- I. General information
- II. Experimental procedures
- III. <sup>1</sup>H spectra of compounds
- IV. <sup>31</sup>P spectra of compounds
- V. <sup>13</sup>C spectra of DMNB-dNTPs
- VI. Mass Spectra of compounds.
- VII. High Resolution Mass Spectra of DMNB-dNTPs
- VIII. <sup>1</sup>H spectra of compounds after complete photodecomposition
- IX. Mass Spectra of compound after complete photodecomposition
- X. Gel electrophoresis Images showing stop-start DNA synthesis

### (I) General information

### Materials:

The starting materials for dNTPs were purchased from Berry Associates and Alfa Aesar. All the other reagents and solvents were purchased from Sigma Aldrich. Enzyme, Terminal Deoxynucleotidyl Transferase (TdT), Recombinant 2500 UN and 5XTdT Buffer was purchased from affymetrix. Blunt end 750 base pair DNA was prepared in our laboratory by molecular cloning and amplification using PCR and purification by a Wizard<sup>®</sup> Plus SV Minipreps DNA Purification System, according to the manufacture's protocol.

### **Characterization:**

<sup>1</sup>H and <sup>31</sup>P NMR spectra were recorded on a Varian (600 MHz) NMR spectrometer. HPLC (HEWALETT PACKARD Series 1100) measurements were done using c18 reverse phase column, with acetonitrile, water, triethyl ammonium bicarbonate Buffer (TEAB pH 8) as mobile phase. LCMS analysis was done in negative ion mode with water/methanol mobile phase on Agilent 1100 series. UV irradiation was done using New Port UV light source with 365 nm band pass filter (Thor Lab). 2% Agarose gel electrophoresis is used for the separation of DNA fragments and to monitor the stop-start DNA synthesis process. The images were taken by exposing the gel to ChemiDoc-It<sup>TS2</sup> Imager.

### (II) Experimental procedures

## 1. Synthesis of 3'-O-(2-nitrobenzyl)-dATP (NB-dATP) and 3'-O-(4,5-dimethoxy-2-nitrobenzyl)-dATP (DMNB-dATP)

### **1.2.** Synthesis of 9-[β-D-5'-O-(tert-butyldimethylsilyl)-2'-deoxyribofuranosyl]-6-chloropurine.

To a solution of 9-[ $\beta$ -D-5'-hydroxy-2'-deoxyribofuranosyl]-6-chloropurine **1.1** (2.00 g, 7.39 mmol) in anhydrous DMF (20 mL) imidazole (1.06 g, 15.5 mmol), followed by TBDMSCl (1.15 g, 7.63 mmol), was added. The reaction mixture was stirred at room temperature overnight, diluted with EtOAc (150 mL), washed with water (2x20 mL), brine (20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. After concentration of the filtrate, the residue was purified by flash column chromatography over silica gel using EtOAc/hexanes (1:1) to give **1.2** as a colorless oil (2.25 g, 79%). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$  8.73 (s, 2H, 2-H and 8-H), 6.54 (t, *J* = 6.0 Hz, 1H, 1'-H), 4.61 (dt, *J* = 4.2, 6.0 Hz, 1H, 3'-H), 4.07-4.04 (m, 1H, 4'-H), 3.95 (dd, *J* = 3.6, 11.4 Hz, 1H, one of 5'-H), 3.84 (dd, *J* = 3.6, 11.4 Hz, 1H, one of 5'-H), 2.81-2.86 (m, 1H, one of 2'-H), 2.54-2.60 (m, 1H, one of 2'-H), 0.86 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.06 (s, 3H, one of SiCH<sub>3</sub>), 0.05 (s, 3H, one of SiCH<sub>3</sub>).

### **1.3a.** Synthesis of 9-[β-D-5'-O-(tert-butyldimethylsilyl)-3'-O-(2-nitrobenzyl)-2'- deoxyribofuranosyl]-6-chloropurine.

To a solution of 9-[ $\beta$ -D-5'-O-(tert-butyldimethylsilyl)-2'-deoxyribofuranosyl]-6-chloropurine **1.2** (2.22 g, 5.77 mmol) in DCM (180 mL), tetrabutylammonium bromide (0.930 g, 2.89 mmol), 2-nitrobenzyl bromide (3.74 g, 17.3 mmol) and 40% aq. NaOH (88 mL) were added. The reaction mixture was stirred at room temperature for 1 h, diluted with EtOAc (500 mL) and then the organic layer was separated. The aqueous layer was extracted with EtOAc (2x150 mL). The combined

organic layers were washed with sat. NaHCO<sub>3</sub> (80 mL), brine (80 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. After concentration of the filtrate, the residue was purified by flash column chromatography over silica gel using EtOAc/hexanes (1:2) to give **1.3a** as a pale yellow oil (2.74 g, 91%). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.74 (s, 1H, 2-H), 8.50 (s, 1H, 8-H), 8.10 (dd, J = 1.2, 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.79 (d, J = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.69 (t, J = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.40 (t, J = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.56 (dd, J = 6.0, 7.8 Hz, 1H, 1'-H), 5.01-4.94 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.46-4.42 (m, 1H, 3'-H), 4.36-4.33 (m, 1H, 4'-H), 3.93 (dd, J = 4.2, 11.4 Hz, 1H, one of 5'-H), 3.86 (dd, J = 3.0, 11.4 Hz, 1H, one of 5'-H), 2.80-2.68 (m, 2H, 2'-H), 0.90 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.11 (s, 3H, one of SiCH<sub>3</sub>), 0.10 (s, 3H, one of SiCH<sub>3</sub>).

#### 1.4a. Synthesis of 3'-O-(2-nitrobenzyl)-2'-deoxyadenosine

To solution of 9-[B-D-5'-O-(tert-butyldimethylsilyl)-3'-O-(2-nitrobenzyl)-2'а deoxyribofuranosyl]-6-chloropurine 1.3a (2.74 g, 5.27 mmol) in THF (50 mL), 1.0 M tetrabutylammonium fluoride (TBAF) in THF solution (5.80 mL, 5.80 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1.5 h. After concentration, the residue was dissolved in a mixture of dioxane (25 mL) and 7 N NH<sub>3</sub> in MeOH (50 mL). The solution was stirred in a sealed flask at 85-90 °C for another 18 h. After evaporation, the residue was purified by flash column chromatography over silica gel using MeOH/DCM (1:20) to afford pure 1.4a as a white solid (0.88 g, 44% yield) and slightly impure product 1.4a (0.3 g). <sup>1</sup>H NMR (600 MHz, DMSO- $d_6$ )  $\delta$  8.34 (s, 1H, 2-H), 8.13 (s, 1H, 8-H), 8.06 (d, J = 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.82-7.76 (m, 2H, two of  $C_6H_4$ ), 7.62-7.58 (m, 1H, one of  $C_6H_4$ ), 7.32 (brs, 2H, 6-N $H_2$ ), 6.34 (dd, J = 6.0, 8.4 Hz, 1H, 1'-H), 5.34 (t, J = 5.4 Hz, 1H, 5'-OH), 4.95-4.88 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.38-4.35 (m, 1H, 3'-H), 4.14-4.11 (m, 1H, 4'-H), 3.66-3.53 (m, 2H, 5'-H), 2.88-2.82 (m, 1H, one of 2'-H), 2.55-2.49 (m, 1H, 2'-H, partly superimposed by solvent signal).

### NB-dATP. Synthesis of 3'-O-(2-nitrobenzyl)-2'-deoxyadenosine-5'-triphosphate.

Using the same preparation procedure of **DMNB-dATP**, compound **3'** -**O**- (**2-nitrobenzyl**) -**2'**- **deoxyadenosine** -**5'**- **triphosphate** was obtained as a colorless syrup of TEA salt (130 mg, 40%). <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  8.60 (brs, 1H, 2-H), 8.30 (brs, 1H, 8-H), 8.11 (d, *J* = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.83-7.77 (m, 2H, two of C<sub>6</sub>H<sub>4</sub>), 7.64-7.60 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.57-6.50 (m, 1H, 1'-H), 5.10-5.03 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.73-4.69 (m, 1H, 3'-H), 4.55-4.52 (m, 1H, 4'-H), 4.28-4.15 (m, 2H, 5'-H), 2.89-2.76 (m, 2H, 2'-H); <sup>31</sup>P NMR (161.9 MHz, D<sub>2</sub>O)  $\delta$  -10.3 (brs, 1P), -11.3 (brs, 1P), -23.2 (brs, 1P); MS (ES<sup>-</sup>) calcd for C<sub>17</sub>H<sub>20</sub>N<sub>6</sub>O<sub>14</sub>P<sub>3</sub><sup>-</sup> [(M+3H)]: 625.0, found: 625.2; HPLC: 95.0%.

### 2. Synthesis of 3'-O-(2-nitrobenzyl)-2'-deoxycytidine-5'-triphosphate (NB-dCTP) and 3'-O-(4,5-dimethoxy-2-nitrobenzyl)-dCTP (DMNB-dCTP)

**2.2.** Synthesis of 3'-O-(tert-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine. To a solution of 5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine **2.1** (4.00 g, 7.50 mmol) in DMF (15 mL), imidazole (1.90 g, 15.0 mmol) and TBDMSCl (1.80 g, 12.0 mmol) were added. The reaction

mixture was stirred for 5 h at room temperature, diluted with EtOAc (300 mL), washed with water (3x30 mL), brine (30 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. After concentration of the filtrate, the residue was purified by flash column chromatography over silica gel using EtOAc/hexanes (1:2) to give **2.2** as white foam (3.80 g, 79% yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.36 (s, 1H, NH), 7.92 (d, *J* = 8.4 Hz, 1H, 6-H), 7.39 (d, *J* = 8.4 Hz, 2H, two aromatic protons of DMTr), 7.36-7.25 (m, 7H, seven aromatic protons of DMTr), 6.86 (d, *J* = 8.4 Hz, 4H, four ortho protons to CH<sub>3</sub>O of DMTr), 6.30 (t, *J* = 6.0 Hz, 1H, 1'-H), 5.39 (dd, *J* = 2.4, 8.4 Hz, 1H, 5-H), 4.56-4.52 (m, 1H, 3'-H), 3.99-3.95 (m, 1H, 4'-H), 3.82 (s, 6H, two CH<sub>3</sub>O), 3.51 (dd, *J* = 3.0, 10.8, 1H, one of 5'-H), 2.41-2.36 (m, 1H, one of 2'-H), 2.23-2.17 (m, 1H, one of 2'-H), 0.86 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.05 (s, 3H, one of SiCH<sub>3</sub>), 0.00 (s, 3H, one of SiCH<sub>3</sub>).

### 2.3. Synthesis of 4-O-allyl-3'-O-(tert-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-2'- deoxyuridine.

To a solution of 3'-O-(tert-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine 2.2 (3.78 g, 5.86 mmol) in DCM (60 mL), triethylamine (1.80 mL, 12.9 mmol), DMAP (72.0 mg, 0.586 mmol) and 2-mesitylenesulfonyl chloride (2.56 g, 11.7 mmol) were added. The reaction mixture was stirred for 4 h at room temperature and then diluted with DCM (60 mL), washed with water (30 mL) and saturated aqueous NaHCO<sub>3</sub> solution (30 mL). The organic layer was separated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After evaporation, the residue was dissolved in DCM (25 mL) to which allyl alcohol (5.81 mL, 85.5 mmol) and triethylamine (7.80 mL, 56.0 mmol) were added, and the resulting mixture was stirred at 0 °C for 15 min. DBU (0.970 mL, 6.45 mmol) was then added. The reaction mixture was allowed to warm to room temperature and stirred overnight, diluted with DCM (50 mL) and washed with brine (30 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>. After evaporation, the crude product was purified by flash column chromatography over silica gel using hexanes/ethyl acetate (3:1) to afford 2.3 as white foam (2.81 g, 70% yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.30 (d, J = 7.8 Hz, 1H, 6-H), 7.45 (d, J = 8.4 Hz, 2H, two aromatic protons of DMTr), 7.35-7.26 (m, 7H, seven aromatic protons of DMTr), 6.85 (d, J = 8.4 Hz, 4H, four ortho protons to CH<sub>3</sub>O of DMTr), 6.32 (dd, J = 4.2, 6.6 Hz, 1H, 1'-H), 6.11- $6.05 \text{ (m, 1H, CH}_2=CHCH_2), 5.64, \text{ (d, } J = 7.2 \text{ Hz}, 1\text{H}, 5\text{-H}), 5.42 \text{ (dd, } J = 1.8, 17.4 \text{ Hz}, 1\text{H}, \text{ one of}$  $CH_2$ =CHCH<sub>2</sub>), 5.32 (dd, J = 1.2, 9.6 Hz, 1H, one of  $CH_2$ =CHCH<sub>2</sub>) 4.93 (d, J = 6.0 Hz, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>), 4.56-4.51 (m, 1H, 3'-H), 4.03-4.00 (m, 1H, 4'-H), 3.85 (s, 6H, two CH<sub>3</sub>O), 3.59 (dd, J = 2.4, 10.8 Hz, 1H, one of 5'-H), 3.37 (dd, J = 2.4, 10.8 Hz, 1H, one of 5'-H), 2.60-2.54 (m, 10.8 Hz, 1H, 10.8 H1H, one of 2'-H), 2.30-2.24 (m, 1H, one of 2'-H), 0.87 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.06 (s, 3H, one of SiCH<sub>3</sub>), 0.00 (s, 3H, one of SiCH<sub>3</sub>).

#### 2.4. Synthesis of 4-O-allyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine.

To a solution of 4-O-allyl-3'-O-(tert-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-2'deoxyuridine **2.3** (2.81 g, 4.11 mmol) in THF (35 mL) 1.0 M TBAF in THF solution (5.00 mL, 5.00 mmol) was added dropwise. The reaction mixture was stirred for 1 h at room temperature. After concentration, the residue was dissolved in ethyl acetate (150 mL) and then washed with brine (30 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After evaporation, the crude product was purified by flash column chromatography over silica gel using hexanes/ethyl acetate (1:1~1:3) to afford **2.4** as white foam (2.30 g, 98% yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.09 (d, J = 7.2 Hz, 1H, 6-H), 7.39 (d, J = 8.4 Hz, 2H, two aromatic protons of DMTr), 7.31-7.21 (m, 7H, seven aromatic protons of DMTr), 6.83 (d, J = 8.4 Hz, 4H, four ortho protons to CH3O of DMTr), 6.29 (dd, J = 5.4, 6.0 Hz, 1H, 1'-H), 6.05-5.98 (m, 1H, CH<sub>2</sub>=*CH*CH<sub>2</sub>), 5.65 (d, J = 6.6 Hz, 1H, 5-H), 5.37 (dd, J = 1.2, 16.8 Hz, 1H, one of  $CH_2$ =CHCH<sub>2</sub>), 5.27 (dd, J = 1.2, 10.8 Hz, 1H, one of  $CH_2$ =CHCH<sub>2</sub>), 4.87-4.84 (m, 2H, CH<sub>2</sub>=CHCH<sub>2</sub>), 4.54-4.48 (m, 1H, 3'-H), 4.09-4.03 (m, 1H, 4'-H), 3.79 (s, 6H, two CH<sub>3</sub>O), 3.50 (dd, J = 3.0, 10.8 Hz, 1H, 5'-H), 3.42 (dd, J = 3.6, 10.8 Hz, 1H, 5'-H), 2.67-2.61 (m, 1H, one of 2'-H), 2.45 (d, J = 3.6 Hz, 1H, OH), 2.28-2.21 (m, 1H, one of 2'-H).

### 2.5a. Synthesis of 4-O-allyl-5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-nitrobenzyl)-2'- deoxyuridine.

To a vigorously stirring mixture of 4-O-allyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyuridine 2.4 (2.84 g, 4.98 mmol), Bu<sub>4</sub>NOH (tetrabutylammonium hydroxide) (2.2 mL, 55-60% in water) and NaI (75.0 mg, 0.50 mmol) in DCM/water (15 mL/15 mL) was added 1.0 M NaOH solution (15 mL, 15 mmol). The mixture was stirred for 10 min at room temperature, a solution of 2-nitrobenzyl bromide (2.16 g, 10.0 mmol) in 15.0 mL of DCM was added over 5 min and the resulting reaction mixture was stirred for another 7 h at room temperature. The reaction mixture was diluted with DCM (200 mL) and then washed with brine (30 mL). The organic layer was dried over anhydrous  $Na_2SO_4$ . After evaporation, the crude product was then purified by flash column chromatography over silica gel using hexanes/ethyl acetate (3:1~1:1) to afford 2.5a as a white solid (3.2 g, 91%) yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.10-8.04 (m, 2H, 6-H and one of C<sub>6</sub>H<sub>4</sub>), 7.74 (d, J = 7.8 Hz, 1H, one of  $C_6H_4$ ), 7.66-7.61 (m, 1H, one of  $C_6H_4$ ), 7.48-7.36 (m, 3H, two aromatic protons of DMTr and one of  $C_6H_4$ ), 7.30-7.21 (m, 7H, seven aromatic protons of DMTr), 6.85-6.80 (m, 4H, four ortho protons to CH<sub>3</sub>O of DMTr), 6.33 (t, J = 6.6 Hz, 1H, 1'-H), 6.07-5.99 (m, 1H,  $CH_2 = CHCH_2$ ), 5.68 (d, J = 7.8 Hz, 1H, 5-H), 5.38 (dd, J = 1.2, 18.0 Hz, 1H, one of  $CH_2 = CHCH_2$ ), 5.28 (dd, J = 1.2, 10.2 Hz, 1H, one of  $CH_2$ =CHCH<sub>2</sub>), 4.93-4.82 (m, 4H, CH<sub>2</sub>=CHCH<sub>2</sub> and  $OCH_2C_6H_4$ ), 4.32-4.23 (m, 2H, 3'-H and 4'-H), 3.78 (s, 6H, two CH<sub>3</sub>O), 3.51 (dd, J = 3.6, 10.8Hz, 1H, one of 5'-H), 3.42 (dd, J = 3.6, 10.8 Hz, 1H, one of 5'-H), 2.80-2.75 (m, 1H, one of 2'-H), 2.22-2.16 (m, 1H, one of 2'-H).

### 2.6a. Synthesis of 3'-O-(2-nitrobenzyl)-2'-deoxycytidine.

To an ice/water cold solution of 4-O-allyl-5'-O-(4,4'-dimethoxytrityl)-3'-O-(2-nitrobenzyl)-2'deoxyuridine **2.5a** (3.20 g, 4.54 mmol) in DCM (45 mL) was added trifluoroacetic acid (4.10 mL, 52.4 mmol) was added slowly over 5 min. The resulting red solution was stirred for 2 h (long reaction time caused low yield, 10-20 min should be enough.) at room temperature and then quenched with saturated aqueous NaHCO<sub>3</sub> solution until pH = 8-9. The mixture was diluted with DCM (150 mL). The organic layer was separated, washed with saturated NaHCO<sub>3</sub> solution (2x30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and then concentrated. The residue was dissolved in 7 N NH<sub>3</sub> in methanol (100 mL) and stirred in a sealed tube for 20 h at 55 °C. After evaporation, the crude product was then purified by flash column chromatography over silica gel using MeOH/DCM (1:10) to afford **2.6a** as a white solid (0.63 g, 38% yield). <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.05 (d, *J* = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.78-7.73 (m, 3H, 6-H and two of C<sub>6</sub>H<sub>4</sub>), 7.60-7.56 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.15 and 7.08 (s, 2H, NH<sub>2</sub>), 6.15 (dd, *J* = 6.0, 8.4 Hz, 1H, 1'-H), 5.72 (d, J = 6.6 Hz, 1H, 5-H), 5.05 (t, J = 5.4 Hz, 1H, OH), 4.86 (s, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.18-4.16 (m, 1H, 3'-H), 4.02-4.00 (m, 1H, 4'-H), 3.58-3.55 (m, 2H, 5'-H), 2.35-2.31 (m, 1H, one of 2'-H), 2.05-2.02 (m, 1H, one of 2'-H).

#### NB-dCTP. Synthesis of 3'-O-(2-nitrobenzyl)-2'-deoxycytidine-5'-triphosphate.

Using the same preparation procedure of **DMNB-dATP**, compound **3'-O-(2-nitrobenzyl)-dCTP** was obtained as a colorless syrup of TEA salt (105 mg, 35%). <sup>1</sup>H NMR (600 Hz, D<sub>2</sub>O)  $\delta$  8.11 (d, J = 7.8 Hz, 1H, 6-H), 8.07 (d, J = 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.81-7.77 (m, 2H, two of C<sub>6</sub>H<sub>4</sub>), 7.64-7.61 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.34 (dd, J = 6.0, 8.4 Hz, 1H, 1'-H), 6.23 (d, J = 6.0 Hz, 1H, 5-H), 5.05-4.97 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.58-4.55 (m, 1H, 3'-H), 4.49-4.46 (m, 1H, 4'-H), 4.27-4.21 (m, 2H, 5'-H), 2.67-2.63 (m, 1H, one of 2'-H), 2.33-2.26 (m, 1H, one of 2'-H); <sup>31</sup>P NMR (242.7 MHz, D<sub>2</sub>O)  $\delta$  -8.8 (brs, 1P), -10.5 (brs, 1P), -21.2 (brs, 1P); MS (ES<sup>-</sup>) calcd for C<sub>16</sub>H<sub>20</sub>N<sub>4</sub>O<sub>15</sub>P<sub>3</sub><sup>-</sup> [(M+3H)]: 601.0, found: 601.2; HPLC: 92.0%.

### 3. Synthesis of 3'-O-(2-nitrobenzyl)-dGTP and 3'-O-(4,5-dimethoxy-2-nitrobenzyl)-dGTP.

### **3.2.** Synthesis of 2-Amino-6-chloro-9-[β-D-5'-O-(tert-butyldimethylsilyl)-2'-deoxyribo furanosyl] purine.

To a stirred solution of 2-amino-6-chloro-9-[ $\beta$ -D-2'-deoxyribofuranosyl] purine **3.1** (2.00 g, 7.00 mmol) in anhydrous DMF (20 mL) was added imidazole (1.25 g, 18.3 mmol) followed by TBDMSCl (1.17 g, 7.73 mmol). The reaction mixture was stirred at room temperature overnight, diluted with EtOAc (200 mL), washed with water (2x25 mL), brine (20 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. After evaporation of the filtrate, the residue was purified by flash column chromatography over silica gel using EtOAc/hexanes (2:1) to afford **3.2** as a white solid (2.13 g, 76%). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$  8.25 (s, 1H, 8-H), 6.34 (t, *J* = 6.6 Hz, 1H, 1'-H), 4.57-4.53 (m, 1H, 3'-H), 4.02-3.97 (m, 1H, 4'-H), 3.92-3.82 (m, 2H, 5'-H), 2.73-2.68 (m, 1H, one of 2'-H), 2.48-2.44 (m, 1H, one of 2'-H), 0.89 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.08 (s, 3H, one of SiCH<sub>3</sub>), 0.07 (s, 3H, one of SiCH<sub>3</sub>).

### **3.3.** Synthesis of 6-Chloro-2-[(dimethylaminomethylene)amino]-9-[β-D-5'-O-(tertbutyldimethylsilyl)-2'-deoxyribofuranosyl] purine.

To a solution of 2-amino-6-chloro-9-[ $\beta$ -D-5'-O-(tert-butyldimethylsilyl)-2'deoxyribofuranosyl]purine **3.2** (2.10 g, 5.26 mmol) in anhydrous THF (12.0 mL) was added *N*,*N*dimethylformamide dimethylacetal (4.20 mL, 31.6 mmol) and then the reaction mixture was stirred at 45 °C under argon atmosphere for 4 h. After evaporation, the resulting residue was purified by flash column chromatography over silica gel using MeOH/DCM (1:20) to afford **3.3** as a colorless solid (2.08 g, 87%). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD)  $\delta$  8.77 (s, 1H, CHN(CH<sub>3</sub>)<sub>2</sub>), 8.45 (s, 1H, 8-H), 6.47 (t, *J* = 6.6 Hz, 1H, 1'-H), 4.60-4.56 (m, 1H, 3'-H), 4.04-4.00 (m, 1H, 4'-H), 3.94-3.91 (m, 1H, one of 5'-H), 3.86-3.86 (m, 1H, one of 5'-H), 3.24 (s, 3H, one of N(CH<sub>3</sub>)<sub>2</sub>), 2.78-2.72 (m, 1H, one of 2'-H), 2.55-2.50 (m, 1H, one of 2'-H), 0.89 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.08 (s, 3H, one of SiCH<sub>3</sub>), 0.07 (s, 3H, one of SiCH<sub>3</sub>).

### **3.4a.** Synthesis of 6-Chloro-2-[(dimethylaminomethylene) amino]-9-[β-D-5'-O-(tertbutyldimethylsilyl)-3'-O-(2-nitrobenzyl)-2'-deoxyribofuranosyl] purine.

To an ice cold solution of 6-chloro-2-[(dimethylaminomethylene) amino]-9-[ $\beta$ -D-5'-O-(tertbutyldimethylsilyl)-2'-deoxyribofuranosyl] purine **3.3** (1.08 g, 2.39 mmol) in anhydrous THF (8 mL) 95% NaH powder (0.135 g, 5.34 mmol) was added in portions. After stirring for 50 min at room temperature, a solution of 2-nitrobenzyl bromide (1.14 g, 5.28 mmol) in THF (2.0 mL) was added, and then the reaction mixture was stirred for another 2.5 h at room temperature with exclusion of air and light. After concentration, the resulting residue was dissolved in ethyl acetate (150 mL), washed with saturated aqueous NaHCO<sub>3</sub> (20 mL), brine (20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After concentration, the crude product was purified by flash column chromatography over silica gel using EtOAc/hexanes (1:1-2:1) to afford **3.4a** (0.69 g, 49%) as a yellow foam. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.76 (s, 1H, *CH*N(CH<sub>3</sub>)<sub>2</sub>), 8.28 (s, 1H, 8-H), 8.11 (d, J = 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.83 (d, J = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.72-7.68 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.50-7.46 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.63 (dd, J = 6.0, 8.4 Hz, 1H, 1'-H), 4.95-4.92 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.39-4.36 (m, 1H, 3'-H), 4.28-4.26 (m, 1H, 4'-H), 3.92-3.84 (m, 2H, 5'-H), 3.18 (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>), 2.76-2.71 (m, 1H, one of 2'-H), 2.50-2.44 (m, 1H, one of 2'-H), 0.92 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.13 (s, 3H, one of SiCH<sub>3</sub>), 0.12 (s, 3H, one of SiCH<sub>3</sub>).

### 3.5a. Synthesis of 5'-O-(t-butyldimethylsilyl)-N<sup>2</sup>-[(dimethylamino) methylene]-3'-O-(2-nitrobenzyl)-2'-deoxyguanosine.

solution То а of 6-Chloro-2-[(dimethylaminomethylene)amino]-9-[β-D-5'-O-(tertbutyldimethylsilyl)-3'-O-(2-nitrobenzyl)-2'-deoxy ribofuranosyl] purine 3.4a (0.690 g, 1.17 mmol) in anhydrous DMF (10.0 mL) were added cesium acetate (0.674g, 3.51 mmol), 1,4diazabicyclo[2.2.2]octane (DABCO) (0.131 g, 1.17 mmol) and triethylamine (0.490 mL, 3.51 mmol) under argon and stirred overnight at room temperature with exclusion of air and light. Ac<sub>2</sub>O (3.5 mL) was added to the above reaction mixture and stirred for another 30 min. The reaction mixture was then quenched with water (20 mL) and extracted with ethyl acetate (3x50 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. After evaporation, the crude product was purified by flash column chromatography over silica gel using MeOH/DCM (1:20) to afford **3.5a** (0.354g, 53%) as a pale yellow foam. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) & 8.86 (s, 1H, NH), 8.64 (s, 1H, CHN(CH<sub>3</sub>)<sub>2</sub>), 8.07 (d, J = 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.88 (s, 1H, 8-H), 7.76 (d, J = 7.2 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.67 (dd, J = 7.2, 7.8 Hz 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.49 (dd, J = 7.2, 7.8 Hz, 1H, one of C6H4), 6.36 (dd, 1H, 4'-H), 3.85-3.75 (m, 2H, 5'-H), 3.18 (s, 3H, one of N(CH<sub>3</sub>)<sub>2</sub>), 3.10 (s, 3H, one of N(CH<sub>3</sub>)<sub>2</sub>), 2.66-2.50 (m, 2H, 2'-H), 0.90 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 0.09 (s, 6H, two of SiCH<sub>3</sub>).

#### 3.6a. Synthesis of N<sup>2</sup>-[(dimethylamino)methylene]-3'-O-(2-nitrobenzyl)-2'-deoxyguanosine.

To an ice cold solution of 5'-O-(t-butyldimethylsilyl)-N<sup>2</sup>-[(dimethylamino)methylene]-3'-O-(2nitrobenzyl)-2'-deoxyguanosine **3.5a** (0.40 g, 0.70 mmol) in THF (5.0 mL) was added 1.0 M TBAF in THF solution (1.4 mL, 1.4 mmol) over 1 min. The reaction mixture was allowed to warm to room temperature and stirred for 2.5 h with exclusion of air and light, diluted with EtOAc (100 mL), washed with water (15 mL), brine (15 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After concentration of the filtrate, the residue was purified by flash column chromatography over silica gel using MeOH/DCM (1: 20) to afford **3.6a** (0.293 g, 91%) as yellow solid. <sup>1</sup>H NMR (600 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.3 (s, 1H, NH), 8.56 (s, 1H, *CH*N(CH<sub>3</sub>)<sub>2</sub>), 8.04 (d, *J* = 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 8.03 (s, 1H, 8-H), 7.80-7.75 (m, 2H, two of C<sub>6</sub>H<sub>4</sub>), 7.60-7.56 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.21 (dd, *J* = 6.0, 8.4 Hz, 1H, 1'-H), 5.03 (t, *J* = 6.0 Hz, 1H, OH), 4.93-4.86 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.33-4.30 (m, 1H, 3'-H), 4.07-4.04 (m, 1H, 4'-H), 3.59-3.51 (m, 2H, 5'-H), 3.13 (s, 3H, one of N(CH<sub>3</sub>)<sub>2</sub>), 3.01 (s, 3H, one of N(CH<sub>3</sub>)<sub>2</sub>), 2.74-2.69 (m, 1H, one of 2'-H), 2.53-2.48 (m, 1H, one of 2'-H overlapped with DMSO signal).

#### NB-dGTP. Synthesis of 3'-O-(2-nitrobenzyl)-2'-deoxyguanosine-5'-triphosphate.

Using the same preparation procedure of **DMNB-dATP**, compound **3'-O-(2-nitrobenzyl)-dGTP** was obtained as a colorless syrup of TEA salt (112 mg, 36%). <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  8.19 (s, 1H, 8-H), 8.10 (d, *J* = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.81-7.77 (m, 2H, two of C<sub>6</sub>H<sub>4</sub>), 7.63-7.61 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.30 (d, *J* = 6.0, 9.0 Hz, 1H, 1'-H), 5.08-5.02 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.68-4.65 (m, 1H, 3'-H), 4.49-4.46 (m, 1H, 4'-H), 4.24-4.16 (m, 2H, 5'-H), 2.87-2.82 (m, 1H, one of 2'-H), 2.70-2.65 (m, 1H, one of 2'-H); <sup>31</sup>P NMR (242.7 MHz, D<sub>2</sub>O)  $\delta$  -0.96 (brs, 1P), -11.5 (brs, 1P), -22.6 (brs, 1P); MS (ES<sup>-</sup>) calcd for C<sub>17</sub>H<sub>20</sub>N<sub>6</sub>O<sub>15</sub>P<sub>3</sub><sup>-</sup> [(M+3H)<sup>-</sup>]: 641.0, found: 641.2; HPLC: 94.0%.

### 4. Synthesis of 3'-O-(2-nitrobenzyl)-dTTP and 3'-O-(4,5-dimethoxy-2-nitrobenzyl)-dTTP

# 4.3a, 4.4a. Synthesis of 3-N-benzoyl-5'-O-(tert-butyldimethyl silyl)-3'-O-(2-nitrobenzyl) thymidine and 5'-O-(tert-butyl dimethylsilyl)-3-N-(2-nitrobenzyl)-3'-O-(2-nitrobenzyl) thymidine

To an ice cold mixture of 3-N-benzoyl-5'-O-(tert-butyldimethylsilyl) thymidine **4.2** (1.33 g, 2.81 mmol) in DCM (10 mL), aqueous  $Bu_4NOH$  (60%, 0.9 mL), NaI (84 mg, 0.56 mmol), water (10 mL) and aqueous NaOH solution (1.0 M, 10 mL), a solution of 2-nitrobenzyl bromide (0.630 g, 2.92 mmol) in DCM (10 mL) was added dropwise. The reaction mixture was stirred at 0°C for 2 h and then stirred at room temperature for 6 h with exclusion of light. The reaction mixture was diluted with water (20 mL) and then extracted with DCM (3x50 mL). The organic layer was washed with brine (30 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration, the resulting crude product was purified by flash column chromatography over silica gel using 10-25% ethyl acetate in hexanes to afford a white foam mixture of **4.3a** and **4.4a** is about 85:15). Without further purification, the mixture of **4.3a** and **4.4a** was directly used in the next step.

### 4.5a. Synthesis of 5'-O-(tert-butyldimethylsilyl)-3'-O-(2-nitrobenzyl) thymidine.

To a mixture of 3-N-benzoyl-5'-O-(tert-butyldimethylsilyl)-3'O-(2-nitrobenzyl)thymidine **4.3a** and 5'-O-(tert-butyldimethylsilyl)-3-N-(2-nitrobenzyl)-3'-O-(2-nitrobenzyl)thymidine **4.4a** (0.77 g) in ethanol (10 mL) was added 30% ammonium hydroxide solution (1.00 mL, 8.56 mmol). The reaction mixture was stirred for 3 h at room temperature with exclusion of light, and then subjected

to evaporation. The residue was extracted with DCM (3x50 mL). The organic layers were combined and washed with brine (20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After concentration, the residue was purified by flash column chromatography over silica gel using ethyl acetate/hexanes (1:2) to afford **4.5a** as a white foam (0.51 g, 94% yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.20 (s, 1H, N*H*), 8.08 (d, *J* = 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.78 (d, *J* = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.67 (t, *J* = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.51 (s, 1H, 6-H), 7.47 (t, *J* = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.35 (dd, *J* = 5.4, 9.0 Hz, 1H, 1'-H), 4.95-4.88 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.27-4.24 (m, 1H, 3'-H), 4.22-4.20 (m, 1H, 4'-H), 3.94-3.90 (m, 1H, one of 5'-H), 3.83-3.79 (m, 1H, one of 5'-H), 2.54-2.50 (m, 1H, one of 2'-H), 2.08-1.98 (m, 1H, one of SiCH<sub>3</sub>), 0.11 (s, 3H, one of SiCH<sub>3</sub>), 0.10 (s, 3H, one of SiCH<sub>3</sub>).

#### 4.6a Synthesis of 3'-O-(2-nitrobenzyl)thymidine.

To a cooled (0 °C) solution of 5'-O-(tert-butyldimethylsilyl)-3'-O-(2-nitrobenzyl)thymidine **4.5a** (0.510 g, 1.04 mmol) in THF (8 mL) was added 1.0 M TBAF in THF solution (2.08 mL, 2.08 mmol) over 2 min. The solution was allowed to warm to room temperature and continue stirring for 2 h with exclusion of air and light. The mixture was poured into cold water (30 mL), and the resulting mixture was extracted with ethyl acetate (3x50 mL). The organic layers were combined, washed with brine (20 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After concentration, the residue was purified by flash column chromatography over silica gel using 3-5% MeOH in DCM to afford **4.6a** (0.38 g, 97% yield) as a white solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  8.15 (s, 1H, N*H*), 8.06 (d, J = 8.4 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.73 (d, J = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.66 (t, J = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.48 (t, J = 7.8 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.39 (s, 1H, 6-H), 6.15 (t, J = 6.6 Hz, 1H, 1'-H), 4.96-4.88 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.39-4.36 (m, 1H, 3'-H), 4.21-4.18 (m, 1H, 4'-H), 3.99-3.95 (m, 1H, one of 5'-H), 3.86-3.81 (m, 1H, one of 5'-H), 2.49-2.38 (m, 3H, 2'-H and OH), 1.93 (s, 3H, 5-CH<sub>3</sub>).

### NB-dTTP. Synthesis of 3'-O-(2-nitrobenzyl) thymidine-5'-triphosphate.

Using the same preparation procedure of **DMNB-dATP**, compound **3'-O-(2-nitrobenzyl)-dTTP** was obtained as a colorless syrup of TEA salt (95 mg, 33%). <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  8.09 (d, J = 7.2 Hz, 1H, one of C<sub>6</sub>H<sub>4</sub>), 7.82-7.74 (m, 3H, 6-H and two of C<sub>6</sub>H<sub>4</sub>), 7.63-7.59 (m, 1H, one of C<sub>6</sub>H<sub>4</sub>), 6.35 (dd, J = 5.4, 9.0 Hz, 1'-H), 5.04-4.99 (m, 2H, OCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 4.61-4.52 (m, 1H, 3'-H), 4.44-4.41 (m, 1H, 4'-H), 4.26-4.19 (m, 2H, 5'-H), 2.56-2.51 (m, 1H, one of 2'-H), 2.38-2.33 (m, 1H, one of 2'-H), 1.95 (s, 3H, 5-CH<sub>3</sub>); <sup>31</sup>PNMR (242.7 MHz, D<sub>2</sub>O)  $\delta$  -10.6 (brs, 1P), -11.7 (brs, 1P), -23.1 (brs, 1P); MS (ES<sup>-</sup>) calcd for C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O<sub>16</sub>P<sub>3</sub><sup>-</sup> [(M+3H)<sup>-</sup>]: 616.0, found: 616.2; HPLC: 95.1%.



Figure 4. <sup>1</sup>H NMR Spectra of NB-dTTP





### (IV) <sup>31</sup>P spectra of compounds



Figure 9. <sup>31</sup>P NMR Spectra of NB-dNTPs



Figure 10. <sup>31</sup>P NMR Spectra of DMNB-dNTPs

### (IV) <sup>13</sup>C spectra of DMNB-dN TPs



Figure 11. <sup>13</sup>C NMR Spectra of DMNB-dATP and DMNB-dCTP



Figure 12. <sup>13</sup>C NMR Spectra of DMNB-dGTP and DMNB-dTTP

### (VI) Mass spectra of compounds



Figure 13. 3D Mass Spectra of NB-dNTPs and DMNB-dNTPs obtained from LCMS analysis



### (VII) High Resolution Mass spectra of DMNB-dNTPs

Figure 14. HRMS of DMNB-dNTPs



(VIII) <sup>1</sup>H spectra of compounds after complete photodecomposition

Figure 15. <sup>1</sup>H NMR Spectra of NB-dNTPs after complete photodecomposition



Figure 16. <sup>1</sup>H NMR Spectra of DMNB-dNTPs obtained after complete photodecomposition

### (IX) Mass Spectra of compounds after complete photodecomposition



Figure 17. Mass Spectra of NB-dNTPs and DMNB-dNTPs obtained after complete photodecomposition

#### (X) Gel Electrophoresis Images



**Figure 18:** Gel electrophoresis showing the incorpoartion of dNTPs by TdT to 750bp-DNA causing the temperarory termination of sequencing and the reinitiation after photo-cleaving and incorporating nonprotected dNTP. (1) Standard Ladder, (2) 750 base pair band (3) Commercial dNTP mix, (4) NB-dATP, (5) NB-dCTP, (6) NB-dGTP, (7) NB-dTTP, (8) DMNB-dATP, (9) DMNB-dCTP, (10) DMNB-dGTP (11) DMNB-dTTP and samples after UV irradiation and reinitation with (12) Standard Ladder, (13) 750 base pair band (14) Commercial dNTP mix, (15) NB-dATP, (16) NB-dCTP, (17) NB-dGTP, (18) NB-dTTP, (19) DMNB-dATP, (20) DMNB-dCTP, (21) DMNB-dGTP (22) DMNB-dTTP