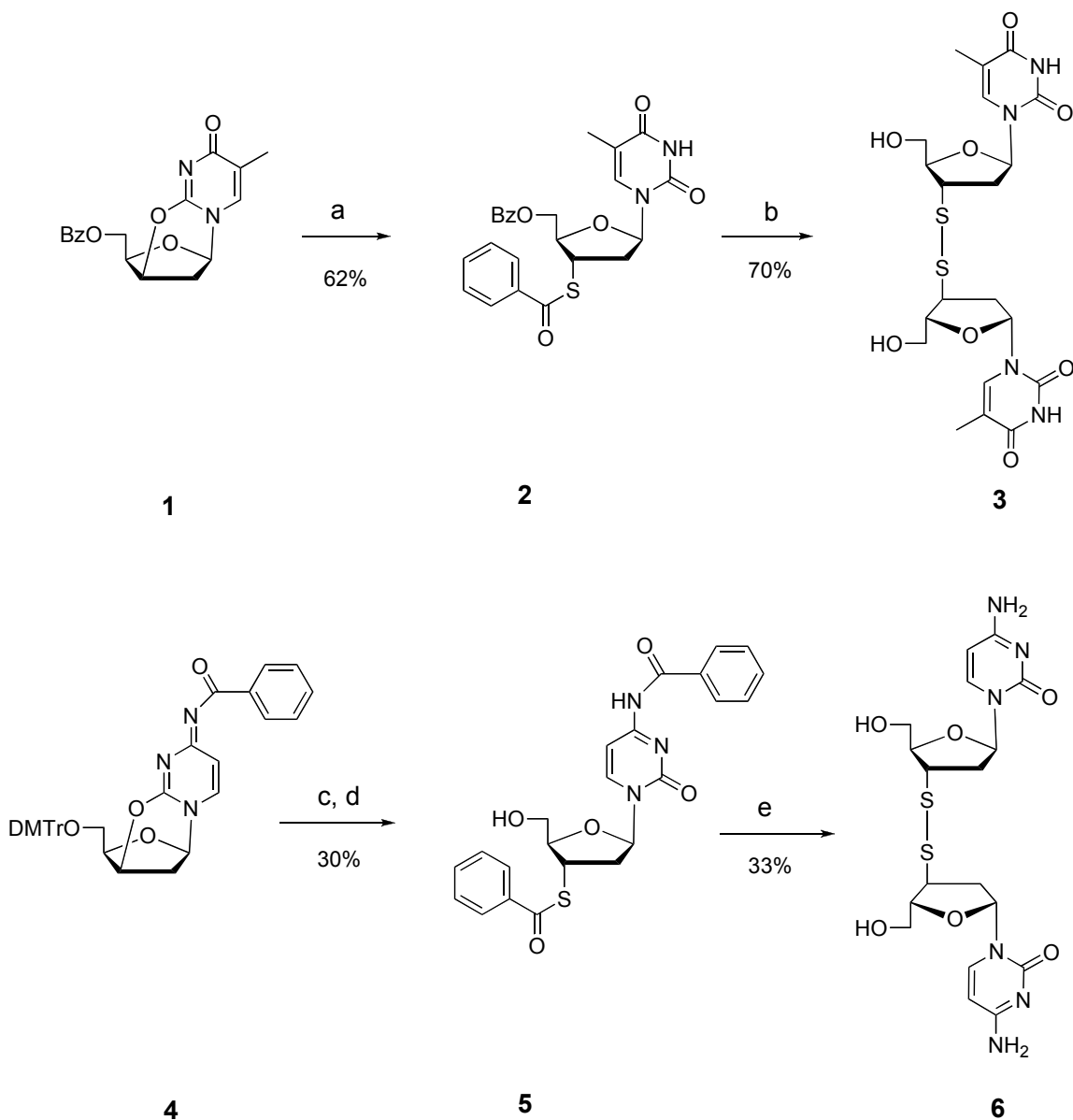


Synthesis of pyrimidine nucleoside precursors: Scheme 1



(a) C_6H_5COSH , NaH, DMF, reflux 2hrs (b) 1N NaOH, 1% I_2/Py : water (c) C_6H_5COS- +Cs, 18-crown-6, CH_3CN , reflux 1hr (d) p-TsOH/ CH_2Cl_2 , (e) 0.05N NaOH, $-10\text{ }^\circ C$, after completion of the reaction, 1M Citric acid to neutral, then 1% $I_2 Py$: water, NH_4OH $55\text{ }^\circ C$ 5hrs

General method for the preparation of 5'-substituted-2,3'-anhydro-2'-deoxypyrimidines

To a solution containing 5'-O-benzoyl-2'-deoxythymidine (7g, 20.23 mmol) or 5'-dimethoxytrityl-N6-benzoyl-2'-deoxycytidine¹ (12.8g, 20.23 mmol) and triphenylphosphine (Ph₃P, 6.3 g, 24.2 mmol) in ethyl acetate (100mL), diethylazodicarboxylate (DEAD, 4.4mL, 24.2 mmol) was added dropwise with stirring at 4 °C and the reaction was allowed to proceed for 30 min at 25 °C. The resulting precipitate was collected by filtration and washed with cold ethyl acetate to give the respective products. The product 1a was used without further purification while 1b was purified by column chromatography using dichloromethane and a stepwise gradient of methanol. Typical yields for both the anhydrides were close to 70%.

5'-O-Benzoyl-3'-S-benzoyl-2',3'-dideoxy-3'-thiothymidine (2)

To a suspension of NaH (125mg 60% suspension in oil, 3.05 mol) in *anhydrous* DMF was added thiobenzoic acid (0.4ml, 90% purity, 3.08mmol). When effervescence ceased and yellow clear solution appeared, the solution was added to a suspension of 2,3'-anhydro-5'-O-benzoyl-2'-deoxythymidine (1g, 3.05 mmol) in DMF (10ml) and the mixture was refluxed for two hours. TLC showed completion of reaction. The solvents were evaporated and the residue was extracted with ethyl acetate and water. The ethyl acetate layer was concentrated to obtain a dark brown residue, which was purified by column and was eluted in MeOH:CH₂Cl₂ (1:99) to obtain 900 mg of pure product (brown color). Yield = 62% R_f = 0.3 (Petroleum ether: CH₂Cl₂: 1:9)

¹H NMR (400 MHz; CDCl₃) 9.01 (1H, bs exchangeable with D₂O), 8.01-7.82 (m, 10H), 7.26 (1H, s) 6.19, 6.2 (1H, 2t, J=6.45), 4.7 (1H, m) 4.52 (1H, m) 4.29 (2H, m), 2.52 (2H, m), 1.55, 1.52 (3H, 2s)

¹ Szabo, I. E., Bruice, T. C. *Bioorg. and Med. Chem.* **2004**, 12, 4233-4244.

^{13}C NMR (100 MHz, CDCl_3) 190.3, 175.2, 166.2, 164.2, 162.9, 150.6, 136.2, 135, 134.2, 133.6, 129.7, 129, 128.8, 127.5, 111.4, 85.1, 83.3, 63.9, 40.0, 38.9, 12.4
 HRMS (ES+) 489.1075 [$\text{C}_{24}\text{H}_{22}\text{N}_2\text{O}_6\text{NaS}$ ($\text{M} + \text{Na}^+$) requires 489.1077]

2',3'-Dideoxy-3'-thiothymidine disulfide (3)

To a solution of crude 5'-O-benzoyl-3'-S-benzoyl-2',3'-dideoxy-3'-thiothymidine (2) (1.0g, 2.14mmol) in methanol (10 ml) was added 1N aq. sodium hydroxide solution dropwise while stirring at 4 °C. After 1 h of stirring TLC analysis showed the completion of hydrolysis (\rightarrow 4), I_2 solution (2 ml, I_2 : pyridine : water 1 : 90 :10) was then added dropwise and the mixture was stirred an additional 30 min. Methanol and other volatiles were removed by evaporation and the aqueous layer was extracted with dichloromethane to remove I_2 . The water was evaporated and the residue was purified by column. The silica gel was packed with MeOH: CHCl_3 (8:92) and the pure product was eluted with MeOH: CHCl_3 (10:90) yielding 770mg of a pale yellow powder. Yield = 70%

Rf = 0.4 (MeOH: CHCl_3 15:85)

^1H NMR (400 MHz, pyridine d_5) δ = (1H, bs exchangeable with D_2O), 7.61 (1H, s), 6.14 (1H, dd, $J_1=3.0$, $J_2 = 7.45$), 4.13-4.04 91H, m), 3.92-3.82 92H, m), 3.55 (1H, quintet, $J=9.5$), 1.88 (3H, s).

^{13}C NMR (100 MHz, pyridine d_5) 165.1, 152.0, 136.7, 110.58, 86.7, 85.0, 61.6, 47.4, 39.0, 13.0.

HRMS (ES+) 537.1114 [$\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_8\text{NaS}_2$ ($\text{M}+\text{Na}^+$) requires 537.1090]

2',3'-Dideoxy-3'-thiobenzoyl cytidine (5)

Fresh cesium thiobenzoate was prepared by adding cesium carbonate (2.5g, 15.4 mmol) to a mixture of thiobenzoic acid (2ml 90% pure, 15.4mmol) in acetonitrile: methanol (10ml, 9:1) and evaporated to dryness. The salt was co-evaporated with toluene before use. The suspension of freshly prepared cesium salt of thiobenzoic acid (2.28g, 2.54mmol) and 18-crown-6 (0.65g, 2.4mol) in anhydrous acetonitrile (15ml) was refluxed for 10 min. under argon atmosphere.

When the solution became clear, 5'-(dimethoxytrityl)-2,3'-anhydro-2'-deoxycytidine (1.42 g, 2.3 mmol) was added to it and the mixture was further refluxed for another hour. TLC showed the completion of reaction. The contents were cooled to room temperature and transferred to separating funnel with ethyl acetate, washed with water and dried over sodium sulfate. The product was purified by silica gel column chromatography and was eluted in EtOAc: petroleum ether; 3:7.

Rf = 0.4 (EtOAc: petroleum ether; 1:1)

3'-S-benzoyl-N⁶-benzoyl-5'-(dimethoxytrityl)-2',3'-dideoxycytidine² (1.2 g, 1.6 mmol) was dissolved in dichloromethane (20 ml) and *p*-toluenesulfonic acid monohydrate (0.5 g, 2.6 mmol) was added to it at 4 °C and the mixture was stirred for 20 min. Methanol (10 ml) was added and the mixture was further stirred for 10 min. TLC showed the completion of reaction. The mixture was concentrated to oil and stirred with petroleum ether. Petroleum ether was decanted, and the extraction was repeated; this process removed free trityl almost completely. The residue was further dissolved in methanol and titrated with aq. NaHCO₃ (5%) to pH 7.0. Methanol was evaporated and the product was extracted into ethyl acetate. The crude compound obtained by evaporating ethyl acetate was further purified by column and was eluted in MeOH: CH₂Cl₂; 1:99. Total yields obtained after both the reactions = 30%.

Rf = 0.3 (MeOH:CH₂Cl₂; 5:95)

¹H NMR (400 MHz, CDCl₃) δ= 9.33 (1H, bs exchangeable with D₂O), 8.3 (1H, d, J=5.6), 7.9-7.3 (11H, Ar + Cy-H), 6.31 (1H, t, J=5.98), 5.60 (1H, m), 4.27 (1H, m), 3.82 (1H, m), 3.71 (9H, m), 2.70 (1H, m), 2.58 (1H, m)

¹³C NMR (100MHz, Py-d₅) δ= 191.1, 168.4, 165.3, 161.4, 160.8, 134.3, 133.5, 132.9, 132.8, 130.1, 128.0, 127.9, 105.6, 83.9, 82.5, 60.6, 52.3, 46.4, 40.8

HRMS (ES⁺) 474.1092 [C₂₃H₂₁N₃O₅NaS (M+Na⁺) requires 474.1100]

² Sabbagh, G.; Fettes, K. J.; Gosain, R.; O'Neil, I. A.; Cosstick, R. *Nucleic Acids Res* **2004**, *32*, 495.

2',3'-Dideoxy-3'-thio-cytidine disulfide (6)

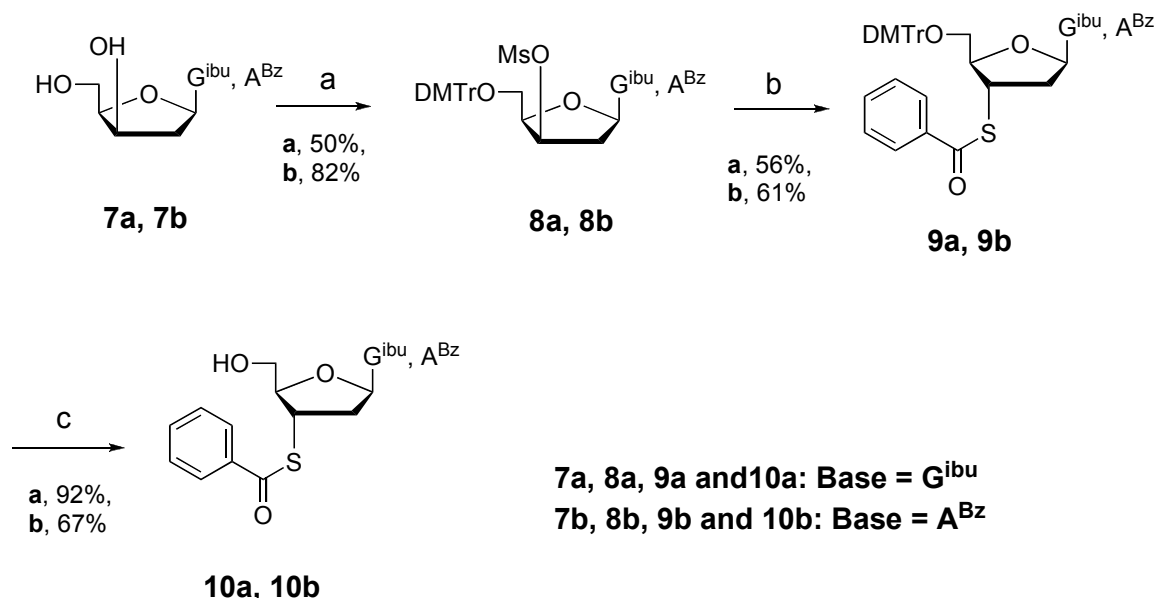
Simultaneous saponification of both the N6-benzoyl and the 3'-S-benzoyl groups yielded the elimination product (the 2',3'-alkene). To avoid the elimination side reaction, the 3'-thiobenzoyl group was first hydrolyzed under mild basic conditions, oxidized to disulfide and then N6-benzoyl was deprotected under strong basic conditions. 3'-S-benzoyl-N6-benzoyl-2',3'-dideoxy-3'-thiocytidine (200 mg, 0.44 mmol) was suspended in 10 ml of solvent (THF: methanol; H₂O; 5:4:1) and chilled to -10 °C, to which 0.5N aq. NaOH (2 ml) was added and the reaction was stirred for 20 min at -10 °C. The reaction was quenched with 1M citric acid, and a white precipitate appeared. A 1% I₂ solution (3 ml, pyridine: water; 98:2) was added and the mixture stirred for 10 min. TLC showed the formation of the desired dimer (MeOH: CH₂Cl₂; 7:93). Solvents were evaporated and the product was extracted using EtOAc and water. The ethyl acetate layer was evaporated and the crude product was further hydrolyzed using conc. NH₄OH solution at 55 ° for 5hrs. After evaporation of ammonium hydroxide the product was purified by silica gel chromatography using Et₃N: Methanol: DCM; 0.5:7.5:92 to obtain 70 mg of pure product. Yield = 33 %

R_f = 0.37 (MeOH:CH₂Cl₂; 10:90)

¹H NMR (400MHz, Py-d₅) δ= 8.54 (1H, d, J=6.65), 7.90 (2H, br), 7.55 (1H, d, J=7.3), 6.38 (1H, m), 5.85-5.63 (1H, m), 4.41-4.29 (2H, m), 3.01 (1H, m), 2.40-2.15 (2H, m)

HRMS (ES+) 507.1086 [C₁₈H₂₄N₆O₆NaS₂ (M+Na⁺) requires 507.1096]

Synthesis of purine nucleoside precursors: Scheme 2



(a) DMTrCl/pyridine 2 h; MsCl/pyridine 1 h (b) C₆H₅COSH, NaH, DMF (c) *p*-Toluenesulfonic acid monohydrate/ CH₂Cl₂/MeOH

General Procedure for the synthesis of xylo nucleosides (7a and 7b)³

The key step in the synthesis of 2'-deoxyxylo nucleosides is the inversion of configuration at 3'-position, which was achieved by using the reported method⁴. 5'-O-benzoyl purine nucleosides with the exocyclic amino functionalities of the bases protected by isobutyryl for guanine and benzoyl for adenine were treated with 1.5 equivalents of trifluoromethanesulfonic acid anhydride in 10% pyridine/CH₂Cl₂ followed by the addition of water to cleave the cyclic hemi-orthoester intermediate yielded the epimeric 3'-O-benzoyl product. The 3'-O-benzoate was quantitatively deprotected by selective alkaline hydrolysis.

Synthesis of N²-isobutyryl-5'-dimethoxytrityl-3'-mesyl-2',3'-dideoxy xyloguanosine (8a)

N²-isobutyryl-5'-O-benzoyl-2'-deoxyxyloguanosine (**7a**) (0.9 g, 2.66 mmol) was twice co-evaporated with anhydrous pyridine and then dissolved in anhydrous

³ Challa, H., Bruice, T. C. *Bioorg. Med. Chem.* **2004**, 12, 1475-1481.

⁴ Herdewijn, P., Van Aerschot, A. *Tetrahedron Letters* **1989**, 30, 855.

pyridine (20 ml). DMTrCl (0.99 g, 2.92 mmol) was dissolved in the pyridine and the reaction was stirred at ambient temperature for 2 h. TLC in MeOH: CH₂Cl₂; 12:88 showed the completion of the reaction. The flask was chilled to 4 °C and mesyl chloride (0.65 ml, 8 mmol) was added dropwise and the mixture was further stirred for 1 h. The solvents were evaporated, water was added and the crude product was extracted into ethyl acetate. The pure product (0.95 g) was obtained by column chromatography using Et₃N: MeOH: CH₂Cl₂; 0.5:2:98. Yield = 50% R_f = 0.4 (MeOH: CH₂Cl₂; 10:90)

¹H NMR (400MHz CDCl₃+ Py-D₅) δ=7.72 (1H, s), 7.29-7.10 (9H, m), 6.70 (4H, d, J=8.90), 6.05 (1H, dd, J₁=2.34, J₂=8.18), 5.25 (1H, t, J=4.02), 4.17 (1H, m), 3.66 (6H, s), 3.51 (1H, m), 3.21 (1H, m), 3.0 (1H, m), 2.66 (3H, s), 2.58 (1H, m).

¹³C NMR (100 MHz, CDCl₃+Py-d₅) δ=158.8, 156.8, 155.7, 149.5, 144.3, 135 .3, 130.0, 128.26, 127.2, 113.3, 87.0, 83.6, 81.7, 78.6, 60.8, 55.3, 46.4, 38.5, 36.3, 19.17, 11.8.

HRMS (ES⁺) 740.2394 [C₃₆H₃₉N₅O₉NaS (M+Na⁺) requires 740.2366]

Synthesis of N²-isobutyryl-5'-dimethoxytrityl-3'-S-benzoyl-2'.3'-dideoxy-3-thioguanosine (9a)

To a suspension of sodium hydride (50 mg 60% suspension in oil, 1.3mmol) in anhydrous DMF (10 ml), thiobenzoic acid (0.15 ml 11.6 mmol) was added while stirring and stirring continued for 10 min. A yellow solution was obtained, to which was added a DMF 10 mL solution containing N²-isobutyryl-5'-dimethoxytrityl-3'-mesyl-2'.3'-dideoxyxyloguanosine (**8a**) (0.7 g, 0.97 mmol). The mixture was heated at 100 °C for 2 h. TLC showed the completion of reaction. The mixture was cooled to ambient temperature and the solvents removed by rotary evaporation to yield a yellow solid. Water was added and the product was extracted with CH₂Cl₂. The organic layer was concentrated and purified by silica gel chromatography to obtain 410 mg of pure product. Yield = 56 % R_f = 0.5 (MeOH: CH₂Cl₂; 4:96)

^1H NMR (400MHz $\text{CDCl}_3 + \text{Py-d}_5$) $\delta =$ 8.15 (1H, d, $J=9.7$), 7.82 (2H, d, $J=6.82$), 7.76 (1H, s), 7.57 (1H, t, $J=7.39$), 7.41 (2H, t, $J=7.82$), 7.2-7.01 (9H, m), 6.56 (4H, dd, $J_1=3.12$, $J_2=8.93$), 5.47 (1H, dd, $J_1=10.16$, $J_2=17.64$), 4.04 (1H, dt, $J_1=3.42$, $J_2=9.64$), 3.59 (6H, s), 3.34-3.30 (1H, m), 3.19-2.8 (1H, m), 2.56-2.4 (1H, q, $J=6.15$), 1.14 (3H, d, $J=6.96$), 1.09 (2H, d, $J=6.93$).

HRMS (ES+) 782.2618 [$\text{C}_{42}\text{H}_{41}\text{N}_5\text{O}_7\text{NaS}$ ($\text{M} + \text{Na}^+$) requires 782.2624]

Synthesis of **N²-isobutyryl-3'-S-benzoyl-2'.3'-dideoxy-3'-thioguanosine (10a)**

To a cold solution of N²-isobutyryl-5'-dimethoxytrityl-3'-S-benzoyl-2'.3'-dideoxy-3'-thioguanosine (**9a**) (0.5 g, 0.66 mmol) in CH_2Cl_2 (20 ml), *p*-toluenesulfonic acid monohydrate (0.3 g, 1.5 mmol) was added. The reaction was stirred at ambient temperature for 20 min, quenched with methanol and evaporated to an oil. EtOAc was added to the residue and the organic solution washed with aq. 5% NaHCO_3 . Ethyl acetate was removed by evaporation and 210 mg of pure product was obtained after silica gel column purification. Yield = 92 %

R_f = 0.23 (MeOH: CH_2Cl_2 ; 5:95)

^1H NMR (400MHz, DMSO- d_6) $\delta =$ 11.80 (1H, bs), 8.23 (1H, s), 7.82 (2H, d, $J=7.77$), 7.61 (1H, t, $J=7.39$), 7.47 (2H, t, $J=7.81$), 6.13 (1H, t, $J=5.70$), 5.15 (1H, 4.25 (1H, q, $J=7.06$), 4.02 (1H, m), 3.64-3.45 (1H, m), 3.38-3.27 (1H, m), 2.91 (1H, m), 2.66 (1H, m), 2.49 (1H, m), 1.00 (3H, d, $J=1.29$), 0.99 (3H, d, $J=1.30$).

^{13}C NMR (100 MHz, Py- d_5) $\delta =$ 189.3, 178.7, 147.6, 136.4, 135.5, 132.9, 127.9, 126.2, 120.6, 85.3, 83.4, 60.9, 40.0, 38.4, 35.1, 18.2.

HRMS (ES+) 480.1322 [$\text{C}_{21}\text{H}_{23}\text{N}_5\text{O}_5\text{NaS}$ ($\text{M} + \text{Na}^+$) requires 480.1318]

Synthesis of N⁶-benzoyl-5'-dimethoxytrityl-3'-mesyl-2'.3'-dideoxyxyloadenosine (8b)

N⁶-benzoyl-2'-deoxyxyloadenosine (**7b**) (1.0 g, 2.8 mmol) was co-evaporated with anhydrous pyridine and then suspended in 50 ml anhydrous pyridine. DMTrCl (0.95 g, 2.8 mmol) was added to the solution and stirred for 2 h. The reaction mixture was then chilled in an ice bath and mesyl chloride (0.45 ml, 5.6 mmol) was added dropwise, and the reaction mixture stirred another 1 h. Pyridine was evaporated under vacuum, water was added and the product was extracted into ethyl acetate. Pure product (1.7 g) was obtained after silica gel column purification. Yield = 82%

R_f = 0.6 (Et₃N: MeOH: CH₂Cl₂; 0.5: 5; 99)

¹H NMR (400MHz, CDCl₃ + Py-d₅) δ=9.22 (1H, bs), 8.66 (1H, s), 7.90 (2H, d, J=7.92), 7.56-7.16 (12H, m), 6.71 (4H, d, J=8.91), 6.48 (1H, t, J=4.87), 5.37 (1H, m), 4.28 (1H, m), 3.67 (6H, s), 3.56 (1H, m), 3.23 (1H, m), 2.85 (2H, m), 2.62 (3H, s).

¹³C NMR (CDCl₃ + py-d₅) δ= 158.8, 155.6, 148.2, 144.2, 141.4, 137.3, 130.2, 128.2, 127.0, 113.3, 86.8, 84.8, 84.40, 63.4, 55.4, 40.9, 39.8

HRMS (ES⁺) 758.2250 [C₃₉H₃₇N₅O₈NaS (M+Na⁺) requires 758.2261]

Synthesis of N⁶-benzoyl-5'-dimethoxytrityl-3'-S-benzoyl-2'.3'-dideoxy-3'-thioadenosine (9b)

To a suspension of NaH (0.1 g, 60% suspension in oil, 2.6 mmol) in 10 ml anhydrous DMF, thiobenzoic acid (0.33 ml, 2.5 mmol) was added and stirred for 10 min. A solution of N⁶-benzoyl-5'-dimethoxytrityl-3'-mesyl-2'.3'-dideoxyxyloadenosine (**8b**) (1.54 g, 2.1 mmol) in 15 ml DMF was added to the sodium thiobenzoate solution. The mixture was heated at 100 °C for 2 h. DMF was evaporated, water was added and the product was extracted with ethyl acetate. Silica gel column purification resulted in a pure product (1.0g). Yield = 61% R_f = 0.4 (EtOAc)

^1H NMR (400MHz, CDCl_3 + Py-d5) δ = 9.022 (1H, bs), 8.71 (1H, s), 8.26 (1H, s), 7.94 (2H, d, J =7.82), 7.82 (2H, d, J =7.68), 7.57-7.08 (15H, m), 6.66 (2H, d, J =7.04), 6.42 (1H, m), 4.48 (1H, q, J =7.90), 4.23 (1H, m), 3.63 (6H, s), 3.39 (2H, m), 3.17 (1H, m), 2.62 (1H, m).

^{13}C NMR (100 MHz, CDCl_3 + Py-d5) δ = 190.2, 164.9, 158.6, 152.7, 151.3, 144.5, 141.34, 136.5, 135.6, 134, 132.6, 130.2, 128.9, 128.2, 128, 127.4, 127, 113.3, 86.8, 84.8, 84.4, 63.3, 55.3, 40.9, 39.8.

HRMS (ES+) 778.2675 [$\text{C}_{45}\text{H}_{40}\text{N}_5\text{O}_6\text{S}$ ($\text{M}+\text{H}^+$) requires 778.2699]

Synthesis of **N⁶-benzoyl-3'-S-benzoyl-2'.3'-dideoxy-3'-thioadenosine (10b)**

Treatment with p-TSA led to a depurination product. Instead we used the following procedure: To a cold solution of **N⁶-benzoyl-5'-dimethoxytrityl-3'-S-benzoyl-2'.3'-dideoxy-3'-thioadenosine** (0.5 g, 0.64 mmol) in DCM (20ml) was added 3%DCA/ CH_2Cl_2 (3 ml) and stirred for 10 minutes. The reaction was quenched with methanol, the solvent was evaporated and the residue was dissolved in ethyl acetate and washed with water and with 5% aq. NaHCO_3 solution. The crude residue was further purified by silica gel chromatography to obtain **10b** (0.2 g) Yield = 67%

Rf = 0.45 (MeOH: CH_2Cl_2 ; 5:95)

^1H NMR (400MHz, CDCl_3) δ = 9.06 (1H, bs), 8.72 (1H, s), 8.24 (1H, s), 7.94-7.35 (10H, m) 6.36 (1H, t, J =6.57), 5.92 (1H, bs), 4.51 (1H, m), 4.23 (1H, m), 3.99 (1H, d, J =11), 3.84 (1H, d, 11.87) 3.24 (1H, m), 2.52 (1H, m).

^{13}C NMR (100 MHz, CDCl_3) δ = 191.0, 165.2, 152.3, 151.0, 150.0, 142.3, 136.3, 134.1, 133.7, 132.8, 128.9, 128.8, 128.2, 127.4, 87.5, 86.2, 62.7, 40.9, 39.0.

HRMS (ES+) 498.1198 [$\text{C}_{24}\text{H}_{21}\text{N}_5\text{O}_4\text{NaS}$ ($\text{M}+\text{Na}^+$) requires 498.1212]

General Phosphorylation and Purification Procedure (11a, b and 12 a, b)

Nucleoside triphosphate syntheses were carried out according to method developed by Eckstein et. al⁵. All solvents were freshly distilled; DMF was distilled over P₂O₅, 1, 4-dioxane was distilled over sodium metal and tributylamine was fractionally distilled under vacuum. Starting materials were dried using two co-evaporations with freshly distilled pyridine followed by keeping for 2 hr under vacuum over P₂O₅ after which an argon atmosphere was introduced and maintained throughout the reaction. Typically, compounds (100 μmoles) dissolved in pyridine (0.5 ml) and dioxane (1.5 ml) (T and C) or pyridine (0.5 ml) and DMF (1.5 ml) (A and G) were treated with 1.1 equivalent of 2-chloro-4H-1,3,2- benzodioxane-phosphorin-4-one (1M in dioxane) and allowed to stir at ambient temperature for 15 minutes. 1.5 eq. of tributylammonium pyrophosphate (0.5 M in DMF) and tributylamine (10 eq.) were then added and the mixture was allowed to stir for 15 min. A 1% solution of I₂ in pyridine/ water 98/2 was added and allowed to react for 15 min. Solvents were removed and two diethyl ether washes were performed to remove excess iodine. In case of A and G water layers were then treated with concentrated NH₄OH for 5 hours at 60 °C followed by ammonia removal under vacuum to deprotect exocyclic amines as well as the 3'-S-benzoyl group.

The crude triphosphates were treated with tricarboxyethyl phosphine (TCEP, 100mg/ml) and heated at 50°C for 30 min before further purification to cleave disulfide bonds in case of T and C and to reduce the minor oxidation products in case of A and G. Ion exchange chromatography was then performed using a Mono Q HR 10/10 column eluting with a linear gradient from 0 to 1 M TEAB pH 7.5. Reverse phase chromatography was performed using a 50 mM TEAA/Water pH 7.0 to 50 mM TEAA/Water: methanol 50:50. Cation exchange was performed using Dowex 50WX8-100 resin in the Na⁺ form. Yields: ddtTTP 31%, ddtCTP 20%, ddtATP 35%, ddtGTP 35%.

⁵ Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, 54, 631

^{31}P NMR was carried out in D_2O .

A typical triphosphate ^{31}P NMR chemical shifts: -5.45 (d), -9.98 (d), -21.29 (t).

Synthesis of triphosphates: Scheme 3

