5,6-Diaminocytidine a versatile synthon for pyrimidine based bicyclic nucleosides.

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Supporting Information:

6-Amino-2',3',5'-tri-O-benzoylcytidine (2): Compound **2** was synthesized from 4,6-diamino-2-hydroxypyrimidne (**1**) and 1–O–acetyl-2.3.5-ri-O-benzoyl-β-D-ribofuranose by following the reported procedure.[8] Compound **1** (6.0 g, 34.25 mmol) was trimethylsilylated in HMDS containing catalytic amount of TMS-Cl at reflux for 6 h. The silylated **1**was then coupled with: 1–O–acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (20 g, 39.6 mmol) in the presence of one equivalent of $SnCl_4$: (4.63 mL, 39.6 mmol) in dichloroethane for 24 h. After work-up, the product was purified by silica gel column (eluent: 6 % methanol in dichloromethane) to get **2** as a pale yellowish-white solid (17.0 g, 87 %). mp: 138-141 ^oC (dec.). ^1H NMR (DMSO-d_6 + D_2O): δ7.96-7.90 (m, 6H), 7.64-7.53(m, 3H), 7.47-7.33(m, 6H), 6.07-5.92(m, 3H, H1',H2', H3'), 4.93(s, 1H, H5), 4.63-4.56(bm, 3H,H4', H5', H5"). FAB-HRMS: Calc. for $C_{30}H_{27}N_4O_8$: 571.18020; Found: 571.18289.

6-Amino-5-nitroso-2',3',5'-tri-O-benzoylcytidine (3): Compound **2** (15.0 g, 26.32 mmol) was dissolved in acetic acid (100 mL) and 10 mL of water were added to the solution. The mixture was stirred below 10 ^oC and finely powdered sodium nitrite (3.64 g, 52.75 mmol, 2 eq.) was added over a period of 30 min. The color of the reaction mixture changed from pale yellow to intense purple with the addition of nitrite. Stirring was continued at a temperature below10 ^oC ; the reaction was complete after 4 h. Acetic acid was removed from the reaction mixture and the residue was neutralized with saturated sodium bicarbonate; the precipitated nitroso compound was thoroughly washed with water, filtered and dried over KOH under vacuum to get a purple solid 15.5 g (98 %). mp: 173-175 ^oC (dec.). ^1H NMR (DMSO-d_6 + D_2O): δ7.98-7.81(m, 6H), 7.64-7.57(m, 3H), 7.47-7.34(m, 6H), 6.37(s, 0.5H, H1'), 6.28(s, 0.5H, H1'), 6.09-6.00(bm, 2H,

H2', H3'), 4.65-4.50(m, 3H, H4', H5', H5"). FAB-HRMS: Calc. for $C_{30}H_{26}N_5O_9$: 600.17065; Found: 600.17305.

Note: The reaction took longer and required a higher temperature to go to completion when trace amounts of methanol were present in the reaction mixture.

5,6-Diamino-2',3',5'-tri-O-benzoylcytidine (4): Compound **3** (14.5 g, 24.21 mmol) was dissolved in 60 mL of DMF – water mixture (9:1) and finally powdered sodium hydrosulfite (8.4 g, 48.25 mmol, 2 eq.) was added. The reaction mixture was stirred at 50 ^oC for 4 h, at which time the reaction was complete. The intense purple color of the reaction mixture turned to pale yellowish green during the course of the reaction. DMF was removed under vacuum and the residue was thoroughly washed with water, filtered and dried over KOH under vacuum to yield compound **4** as a yellowish-green solid (13.8 g, 97.5 %). mp: 143-147 ^oC (dec.). ^1H NMR (DMSO-d_6 + D_2O): δ7.95-7.79(m, 6H), 7.63-7.55(m, 3H), 7.46-7.33(m, 6H), 6.09-5.93(m, 3H, H1', H2', H3'), 4.64-4.52(bm, 3H, H4', H5', H5"). FAB-HRMS: Calc. for $C_{30}H_{28}N_5O_8$ 586.19379; Found: 586.19230.

6-Amino-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine-2-one (5a): Compound **4** (590 mg, 1.008 mmol) was dissolved in 5 mL of DMF. $POCl_3$ (115.0 µL, 1.23 mmol) was added to the solution under an argon atmosphere and stirred at ambient temperature for 1 h. DMF was removed under vacuum and the residue was taken up in dichloromethane, then washed with saturated sodium bicarbonate solution followed by the standard workup. The desired product was purified by silica gel column chromatography; eluent: 5 % methanol in dichloromethane, and obtained as a pale white solid (530 mg, 89 %). A portion of the solid obtained was precipitated from methanol and its melting point was recorded. mp: 178-179 ^oC (Reported mp: 270-271 ^oC).[5] UV(Ethanol), $\lambda_{max}(\varepsilon_{max})$ = 282 nm($15.6x10^3$ $mol^{-1}cm^{-1}$); $\lambda_{shoulder}(\varepsilon)$ = 276 nm($14.0x10^3$ $mol^{-1}cm^{-1}$); [Reported UV(Ethanol), $\lambda_{max}(\varepsilon_{max})$ = 282.5 nm($16.1x10^3$ $mol^{-1}cm^{-1}$); $\lambda_{shoulder}(\varepsilon)$ = 276.5 nm($14.3x10^3$ mol^{-1} cm^{-1})][5] ^1H NMR (DMSO-d_6 + D_2O): δ7.96-7.79(m, 7H, H8 + 6H of Aromatic), 7.64-7.54(m, 3H), 7.45-7.32(m, 6H), 6.54-6.53(bd, 1H, J = 2.44 Hz), 6.36-6.30(m, 1H), 6.21-6.14(t, 1H, J' = 7.03, J" = 6.92 Hz), 4.67-4.49(m, 3H). FAB-HRMS: Calc. for $C_{31}H_{26}N_5O_8$: 596.17814; Found: 596.17732.

Note: During the course of the reaction, compound **4** gave an R_f value of around 0.4 in dichloromethane/methanol (9:1) and the product formed did not move on TLC plate under these conditions. After removing DMF, the residue was sparingly soluble in dichloromethane or chloroform, but after adding bicarbonate effervescence followed and the residue dissolved in the organic phase. A change in the R_f value of the product was noticed after the workup and the product was co-eluted with compound **4** The R_f value of the product and starting material were almost identical. Therefore, enough $POCl_3$ was added into the reaction in subsequent reactions to force compound **4** to react completely, giving a drastic improvement in yield from 60 % to 90 %; however, the excess of $POCl_3$ led to the formation of multiple products. It is recommended that phosphoryl chloride be carefully added just to the point that all starting material is consumed in order to maximize the yield and purity of the product.

6-Amino-8-methyl-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine-2-one (5b): The above reaction (one mmol scale) was repeated in DMAc under the same condition to obtain compound **5b** (500 mg, 82 %). mp: 175-176 ^oC UV(Ethanol), $\lambda_{max}(\varepsilon_{max})$ = 284 nm($15.9x10^3$ mol^{-1} cm^{-1}); $\lambda_{shoulder}(\varepsilon)$ = 276 nm($12.9x10^3$ $mol^{-1}cm^{-1}$) ^1H NMR (DMSO-d_6 + D_2O): δ7.96-7.82(m, 6H), 7.65-7.58(m, 3H), 7.46-7.36(m, 6H), 6.51(bs, 1H), 6.36-6.33(m, 1H), 6.24-6.20(t, 1H, J' = 7.5, J" = 7 Hz), 4.70-4.64 (m, 2H), 4.57-4.54(m, 1H), 2.38(s, 3H). FAB-HRMS: Calc. for $C_{32}H_{28}N_5O_8$: 610.19379; Found: 610.19157.

4-amino-2-oxo-3-(2,3,5-tri-O-benzoyl-β-D-riboforanosyl)-2,3-dihydropteridine (8a): Reaction of compound **4** (1.27 g, 2.17 mmol) with glyoxal (140 mg, 2.41 mmol, 0.28 mL of 40 % wt. percentage in water, d = 1.265) in DMF (10 mL) at ambient temperature for 1 h yielded a mixture of products. DMF was removed from the reaction mixture and standard workup followed. The faster eluting fraction on silica gel column was isolated as a white solid and characterized as compound **8a** (520 mg) and the slower eluting fraction was isolated and characterized as compound **9a** (560 mg). Overall yield: was 82 %. Compound **8a**: mp: 123-126 ^oC. ^1H NMR ($CDCl_3$): δ9.70(exchangeable with D_2O), 8.45-8.44(d, 1H, J = 2.5 Hz), 8.42-8.41(d, 1H, J = 2.5 Hz), 8.06-8.05(m, 2H), 7.98-7.91(m,

4H), 7.54-7.49(m, 3H), 7.37-7.30(m, 6H), 6.98(bs, 1H, H1'), 6.42-6.41(m, 1H), 6.29-6.26(t, 1H J', J" = 7.5 Hz), 4.82-4.66(m, 3H).

4-amino-2-oxo-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,2-dihydropteridine (9a): mp: 130-140 °C (Reported mp:105-120 °C)[6b] ^{1}H NMR ($CDCl_3$): δ8.56-8.55(d, 1H, J = 2.27 Hz), 8.40-8.39(d, 1H, J' = 2.30 Hz), 8.06-7.87(m, 6H), 7.55-7.47(m, 3H), 7.40-7.26(m, 6H), 7.12-7.11(d, 1H, H1', J = 2.22 Hz), 6.40-6.24(m, 2H, H2', H3'), 4.86-4.53(m, 3H, H4', H5', H5"). FAB-HRMS: Calc. for $C_{32}H_{26}N_5O_8$: 608.17814; Found: 608.17486.

4-amino-6,7-diemthyl-2-oxo-3-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2,3-dihydropteridine (8b): Reaction of compound **4** (300 mg, 0.5128 mmol) with butane-2,3-dione (50 μL, 0.5697 mmol) in DMF (5 mL) at ambient temperature for 1 h. yielded compound **8b** as a colorless solid (270 mg, 83 %). mp: 127-130 °C. ^{1}H NMR ($CDCl_3$): δ9.46(exchangeable with D_2O), 8.06-8.04(m, 2H), 7.97-7.91(m, 4H), 7.54-7.48(m, 3H), 7.37-7.30(m, 6H), 6.98(bs, IH, H1'), 6.40(bs, 1H), 6.30-6.27(t, 1H, J' = 7.5, J" = 7.0 Hz), 4.80-4.67(m, 3H), 2.57(s, 3H), 2.55(s, 3H).

6-Amino-3-(β-D-ribofuranosyl)purine-2-one (6a): Compound **5a** (170 mg, 0.2857 mmol) was suspended in methanol (10 mL),dichloromethane was added drop wise into the suspension to obtain a homogeneous solution, and a catalytic amount of sodium methoxide was added. The solution was stirred at ambient temperature for 4 h. Solvent was removed; the residue was taken in a 1:1 mixture of methanol and water (10 mL) and stirred with Dowex 50WX8-200 cation exchange resin (H^+ form) for 5 min. The resin was thoroughly washed with a 1:1 mixture of methanol-water. Finally the resin was washed with methanolic ammonium hydroxide (methanol/water 2:3), after removing ammonia and methanol the product was precipitated out from water, filtered and dried over KOH under vacuum to give the free nucleoside as a white solid Yield 75 mg, 92.7 %. mp: started turning brown >250 °C (Reported mp: slow decomp. >285 °C).[5] ^{1}H NMR (DMSO-d_6 + D_2O): δ7.86 (s, 1H, H8), 6.20-6.19(d, 1H, H1', J = 6.5 Hz), 4.70(bs, 1H,

H2'), 4.10-4.09(m, 1H, H4'), 3.88-3.87(d, 1H, H3'), 3.62-3.58(m, 1H, H5'), 3.50-3.48(bm, 1H, H5"). FAB-HRMS: Calc. for $C_{10}H_{14}N_5O_5$: 284.09949; Found: 284.09818

6-Amino-8-methyl-3-(β-D-ribofuranosyl)purine-2-one (6b): Compound **6b** was prepared from **5b** (140 mg, 0.2298 mmol)as above. Yield 60 mg, 87.8 %. mp: started turning brown >250 ^{o}C. ^{1}H NMR (DMSO-d_6 + D_2O): δ6.18-6.17(d, 1H, H1', J = 7 Hz), 4.67(bs, 1H, H2'), 4.08-4.06(m, 1H, H4'), 3.89(bs, 1H, H3'), 3.62-3.59(bm, 1H, H5'), 3.51-3.47(bm, 1H, H5"), 2.35(s, 3H, 8CH_3). FAB-HRMS: Calc. for $C_{11}H_{16}N_5O_5$: 298.11514; Found: 298.11569.

4-amino-2-oxo-3-(β-D-ribofuranosyl)-2,3-dihydropteridine (10a): Debenzoylation of **8a** (170 mg, 0.2801 mmol) was performed in aqueous methanolic sodium hydroxide and purified as above. Yield 70 mg 84 %. ^{1}H NMR (CD_3OD + D_2O): δ8.51-8.50(d, 1H, J = 2.43 Hz), 8.40-8.39(d, 1H, J = 2.39 Hz), 6.62-6.59(d, 1H, H1', J = 5.83 Hz), 5.02-4.96(t, 1H, H2'), 4.38-4.32(m, 1H, H4'), 4.04-3.99(m, 1H, H3'), 3.85-3.71 (m, 2H, H5', H5"). ^{13}C NMR (DMSO-d_6): δ156.7 (C2), 150.3(C4), 147.6(C7), 147.1(C8a), 138.6(C6), 125.8(C4a), 88.9 (C1'), 84.8(C3'), 70.4(C4'), 69.8(C2'), 62.4 (C5'). FAB-HRMS: Calc. for $C_{11}H_{14}N_5O_5$: 296.09949; Found: 296.09978.
Note: Debenzoylation of compound **8a** in methanolic sodium methoxide was unsuccessful. The major product isolated after 4 h treatment of **8a** with methanolic sodium methoxide was the 5'-O-benzoylated derivative (characterized by ^{1}H NMR. ^{1}H NMR (CD_3OD + D_2O): δ8.46-8.45(d, 1H, J = 2.47 Hz), 8.38-8.36(d, 1H, J = 2.55 Hz), 8.09-8.02(m, 2H), 7.62-7.40(m, 3H), 6.64-6.63(d, 1H, H1', J = 2.31 Hz), 4.80-4.62(m, 2H), 4.51-4.41(m, 1H), 4.17-4.08(m, 1H). Prolonged treatment of **8a** with methanolic sodium methoxide lead to partial deglycosylation.

4-amino-2-oxo-1-(β-D-ribofuranosyl)-2,3-dihydropteridine (11a): Debenzoylation of **9a** (190 mg, 0.3130 mmol) in methanolic sodium methoxide yielded 80 mg (86 %) of compound **11a** as a white solid. (DMSO-d_6): δ8.75-8.73(d, 1H, J = 2.31 Hz), 8.52-8.51(d, 1H, J = 2.26 Hz), 8.41-8.34(bd, 2H exchangeable with D_2O), 6.55-6.53(d, 1H, H1', J = 3.74 Hz), 5.05-5.03(d, 1H, exchangeable with D_2O), 4.88-4.85(d, 1H, exchangeable with

D_2O), 4.69-4.60(m, 1H, H2'), 4.28-4.18(m, 1H, H4'), 3.77-3.59(m, 2H, H3', H5'), 3.50-3.31(m. 1H, H5"). ^{13}C NMR (DMSO-d_6): δ162.0, 154.2, 148.5, 147.6, 138.6, 123.1, 88.7, 84.3, 70.9, 70.2, 62.4.