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Chemo-enzymatic route to bridged homolyxofuranosyl-pyrimidines

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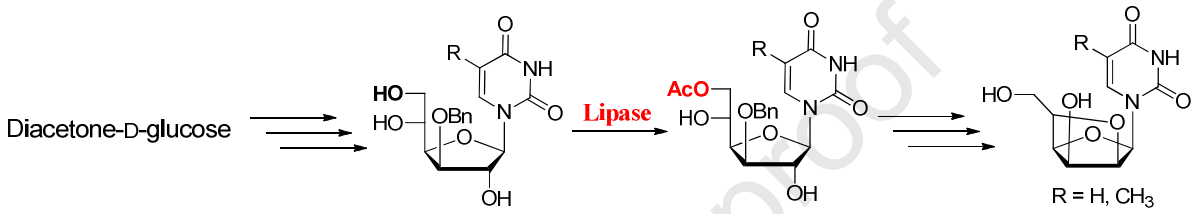
Graphical Abstract



Abstract

Synthesis of 2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl nucleosides U and T have been achieved starting from diacetone-D-glucose in overall yields 55.7 and 57.1%, respectively. Quantitative regioselective monoacetylation of the lone primary hydroxyl group in trihydroxy nucleoside intermediate, *i.e.* 3'-*O*-benzyl-*β*-D-glucofuranosyl nucleosides mediated by Novozyme®-435 has been utilized as the key step in the synthesis of homolyxofuranosyl nucleosides. The structure of the synthesized 2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl uracil and -thymine has been established on the basis of their spectral (IR, ^{1}H, ^{13}C NMR and HRMS) data analysis and the structure of earlier nucleoside was confirmed by its X-rays diffraction analysis which revealed that these 2'-*O*,5'-*C*-bridged homo-nucleosides are locked into *S*-type sugar puckering.

Keywords: Chemo-enzymatic pathway, Regioselective monoacetylation, Novozyme®-435, Bridged homolyxofuranosyl nucleosides.

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1. Introduction

Modified nucleosides and their analogues are of much importance due to their immense potential as key precursors for the synthesis of oligonucleotide based therapeutic agents for

RNA / gene targeting.[1,2] Most of the bicyclic sugar modified nucleosides possess constrained sugar puckering, which enable them to mimic a DNA or RNA type furanose ring conformation. The oligonucleotides derived from such nucleosides show high levels of complementarity with their corresponding DNA / RNA strands together with more stability towards nucleases. Quite a few sugar modified nucleosides have been found to exhibit excellent anti-tumour or antiviral activities.[3]

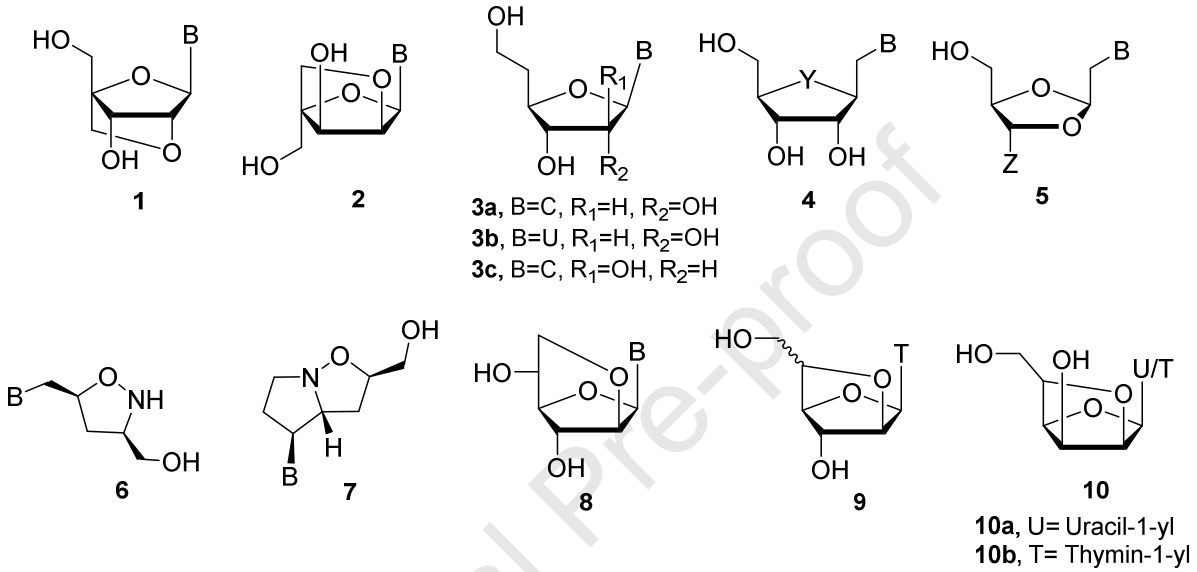


Figure 1. Representative bicyclic and homo-nucleosides.

Bicyclic nucleosides **1** and **2**, with extra methylene group in the sugar moiety are well known as locked nucleic acids (LNA) and used for the development of antisense / anti-gene oligonucleotides due to their restricted conformational structures (**Figure 1**).[4-6]

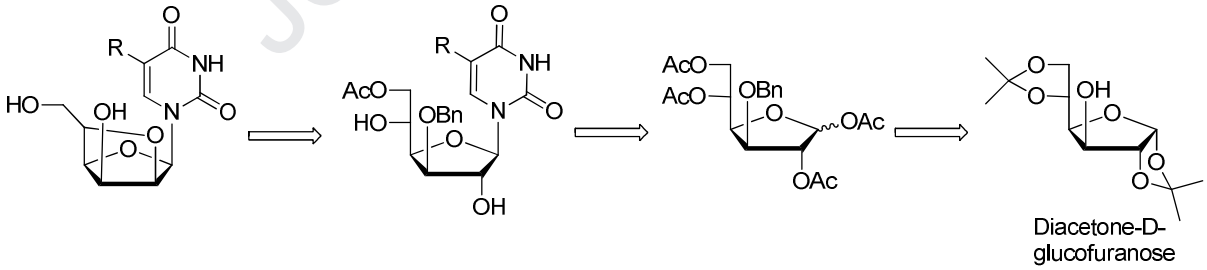
Likewise, homonucleoside monomers having an additional methylene group at the C-5' end as in nucleoside **3**[7-10] or between C-1' and nucleobase as in nucleoside **4**[11,12] have established their significance with diverse biological activities. Extensive modifications have been carried out in the sugar ring to synthesize homonucleoside analogues, such as 1,3-dioxolane nucleosides **5**,[13,14] homo-*N*,*O*-nucleosides **6**[15,16] and bicyclic-*N*,*O*-*iso*-homonucleosides **7**.[17] On the other hand, a 2'-*O*,5'-*C*-methylene-linked bicyclic nucleoside **8** was synthesized and its conformational studies revealed that it adopted *S*-type furanose configuration.[18] Two novel bicyclic nucleotide monomers **9** (5*R* & 5*S*) were synthesized with 3'-endo conformational restriction, where the sugar moiety had the D-arabinose conformation.[19] The 9-mer or 14-mer oligonucleotides with mono-, di- or tri-incorporation of these nucleoside monomers in

oligonucleotides showed decrease in binding affinity towards complementary DNA and RNA. Variation of the heterocyclic rings and bicyclic structures associated with these homonucleoside analogues has enabled researchers to enhance the physiological / biological properties of oligonucleotides involving them. Further, potential activities of homocytidine **3a**, homouridine **3b** and homoarabinofuranosylcytosine **3c** have been evaluated against herpes simplex virus type 1 along with their cytoxicity against HL-60, K-562, U-937 and human LY-PHA cell lines (**Figure 1**).[7] Similarly, antiviral activity of oxazolidine homonucleosides **6** were examined against variety of DNA and RNA viruses and these nucleosides were found to be non-toxic up to 250 μM concentration.[20]

We have reported the synthesis of several bicyclic and spiro-nucleosides where the bridging methylene group introduces conformational restriction to the sugar ring of the nucleoside.[21-24] Herein, we report a chemo-enzymatic synthesis of bicyclic 5'-homolyxofuranosyl nucleosides of U and T.

2. Results and Discussion

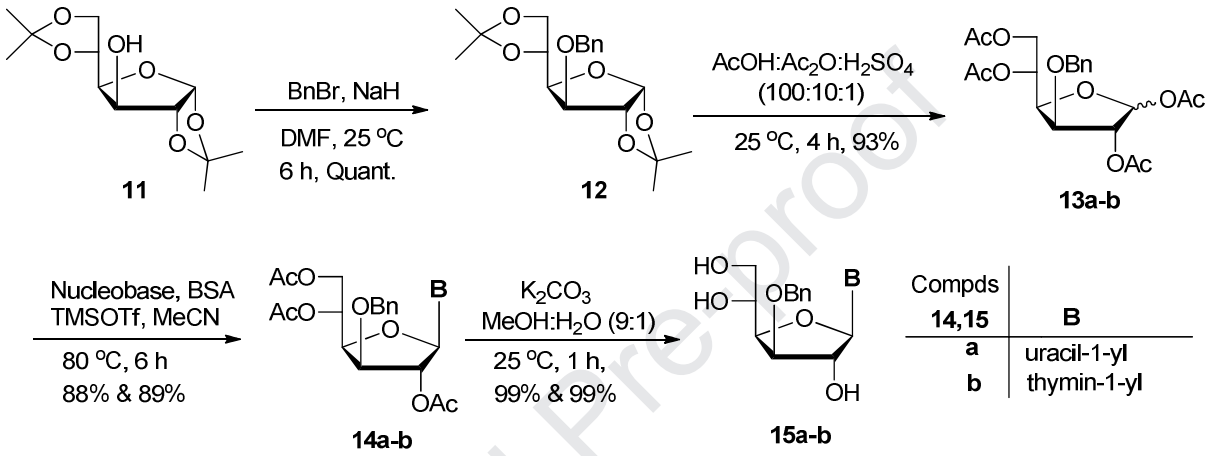
It was envisioned that the targeted bridged homo-nucleosides would be synthesized from diacetone-D-glucofuranose because this is an orthogonally protected substrate that will provide tetraacetate intermediate for nucleobase coupling and subsequently produce our desired nucleoside monomer (**Scheme 1**).



Scheme 1. Retrosynthetic analysis for synthesis of 2'-*O*,5'-*C*-bridged homolyxofuranosyl nucleosides.

Diacetone-D-glucofuranose (**11**) was converted into *O*-benzylated furanoside **12** using benzyl bromide in DMF in quantitative yield,[25] which on acetolysis with AcOH:Ac_2O:H_2SO_4 (100:10:0.1) afforded anomeric mixture of tetraacetate **13a-b** (α:β = 1:5, based on integration of anomeric proton in ^{1}H NMR). The Vorbrüggen nucleobase coupling of tetraacetate **13a-b** with uracil and thymine in presence of *N*,*O*-*bis*(trimethylsilyl)acetamide (BSA) and

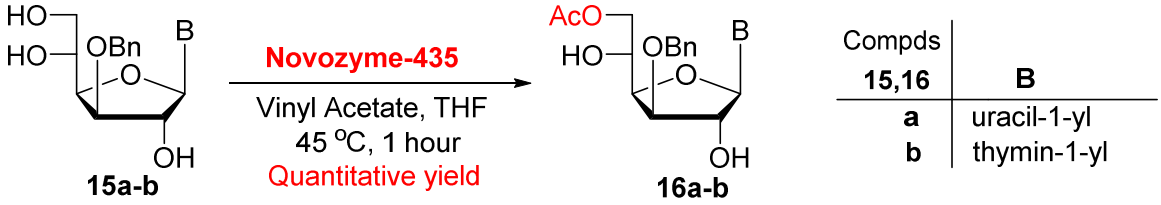
trimethylsilyltrifluoromethane sulfonate (TMS-triflate) in acetonitrile afforded 2',5',6'-tri-*O*-acetyl-3'-*O*-benzyl-*β*-D-glucofuranosyl-uracil (**14a**) and 2',5',6'-tri-*O*-acetyl-3'-*O*-benzyl-*β*-D-glucofuranosyl-thymine (**14b**) in 88% and 89% yields, respectively. The complete deacetylation of the nucleosides **14a** and **14b** was carried out efficiently with K_2CO_3 in methanol-water (9:1) to obtain trihydroxy nucleosides **15a** and **15b** in 99% yields (**Scheme 2**).



Scheme 2. Synthesis of 3'-*O*-benzyl-*β*-D-glucofuranosyl-uracil and -thymine (**15a-b**).

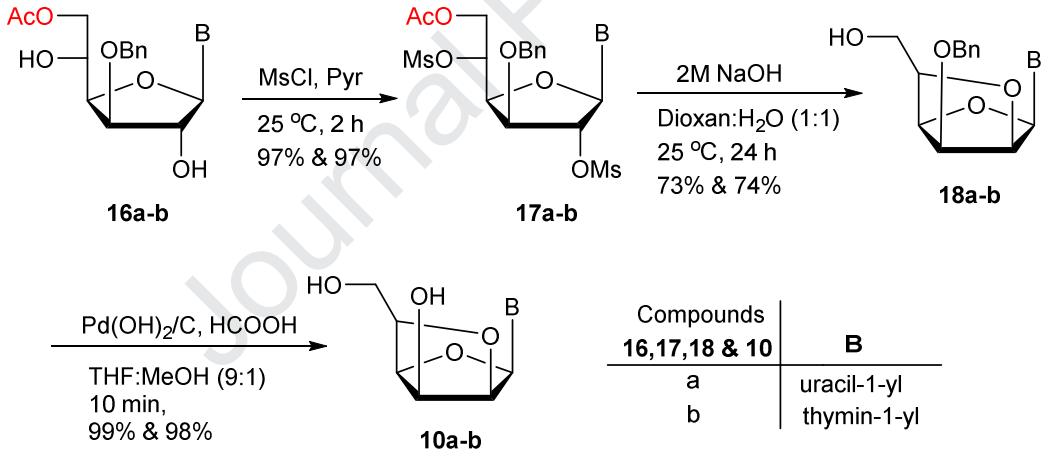
For the synthesis of targeted bridged homonucleosides the primary hydroxyl group of trihydroxy nucleosides **15a** and **15b** needs to be protected, which were achieved by the mediation of lipase.

Two lipases, Novozyme®-435 and lipozyme TL IM were screened for selective acetylation of primary hydroxyl group of nucleosides **15a** and **15b** in six different organic solvents, such as toluene, diisopropylether (DIPE), tetrahydrofuran (THF), 2-methyltetrahydrofuran (2-Me-THF), acetone and acetonitrile using vinyl acetate as acetyl donor in an incubator shaker at 25 to 45 °C. It was observed that Novozyme®-435 in THF and 2-Me-THF were able to carry out complete conversion of trihydroxy nucleosides **15a** and **15b** into monoacetylated nucleosides **16a** and **16b** in 98% and 99% yields, respectively. The reaction time for complete conversion of **15a** and **15b** into **16a** and **16b** in THF was found to be 1 hour, however the same reaction took 3 hours in 2-Me-THF. Thus, THF was found to be the solvent of choice (**Scheme 3**).



Scheme 3. Optimized condition for Novozyme-435 catalyzed monoacetylation of nucleosides **15a** and **15b**.

Monoacetylated nucleosides **16a** and **16b** was converted into bridged homonucleosides **10a** and **10b** in three steps (**Scheme 4**). First, the monoacetylated nucleosides **16a** and **16b** were permesylated to afford nucleosides **17a** and **17b** using methanesulfonyl chloride (MsCl) in pyridine at 25 °C in 97% yields, which on treatment with 2M aq. NaOH cyclises to afford 3'-*O*-benzyl-2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl nucleosides **18a** and **18b** in 74% and 73% yields, respectively. Finally debenzylation of nucleosides **18a** and **18b** was carried out using 20% $Pd(OH)_2$-C/HCOOH in a solvent mixture of THF:MeOH (9:1) to afford novel bridged homolyxofuranosyl nucleosides **10a** and **10b** in 98% and 99% yields, respectively.



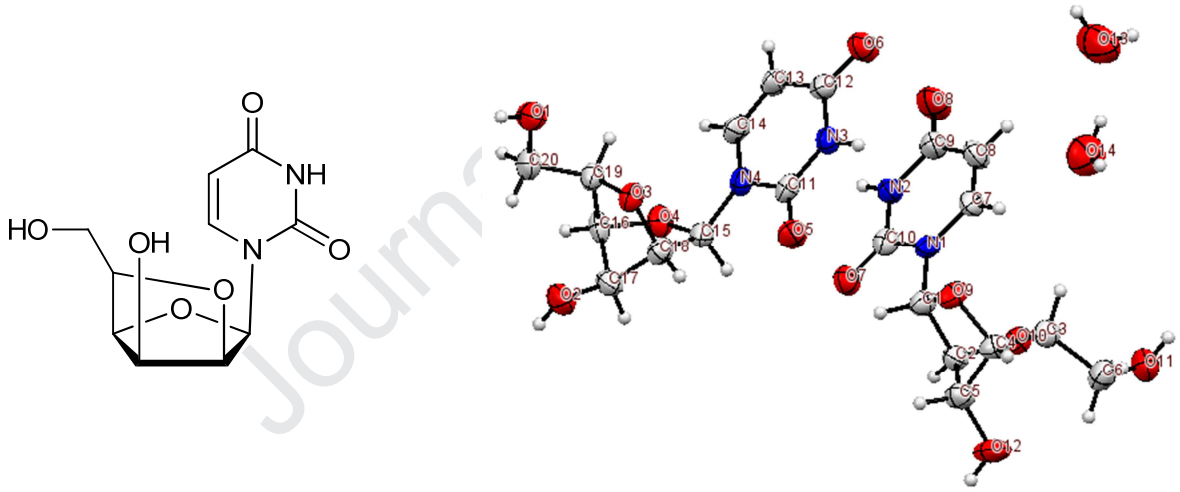
Scheme 4. Synthesis of 2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl nucleosides **10a-10b**.

The structures of all the synthesized compounds **12**, **13a-b**, **14a-b**, **15a-b**, **16a-b**, **17a-b**, **18a-b**, and **10a-b** were unambiguously established on the basis of their spectral (IR, ^{1}H-, ^{13}C-NMR, ^{1}H-^{1}H COSY NMR, ^{1}H-^{13}C HETCOR NMR, NOESY NMR and HRMS) data analysis. The structure of known compound **12** was further confirmed by the comparison of its physical and spectral data with those reported in literature.

The structure of 2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl nucleoside **10a** was further confirmed by single crystal X-rays diffraction analysis which revealed that sugar puckering

of this molecule is locked into *S*-type conformation (**Figure 2**). The conformational analysis of the furanoside ring was also supported by the dihedral and pseudorotational angle calculation.[26] The crystal structure consisted of two nucleoside monomers with pseudorotational phase angles (*P*) 193.74 and 201.13, and two crystal water molecules which confirmed that the sugar puckering of both the monomeric structures of **10a** to be C3'-exo *i.e.* *S*-type conformation. The calculated values of dihedral angles (ν_0 = -4.79, ν_1 = 41.61, ν_2 = -58.23, ν_3 = 56.51, ν_4 = -33.70 and ν_0 = 3.28, ν_1 = 34.91, ν_2 = -56.05, ν_3 = 58.46, ν_4 = -39.84), puckering amplitude (ν_{max} = 59.94 and 60.09), backbone angle (γ = 36.52 and 31.27) as well as the torsion describing the anomeric bond (χ = -161.00 and -175.40) support the inference. The detailed crystallographic data of compound **10a** have been deposited in the Cambridge Crystallopraphic Data Centre with CCDC no. 1978207 (**Figure 2**).

(a)



(b)

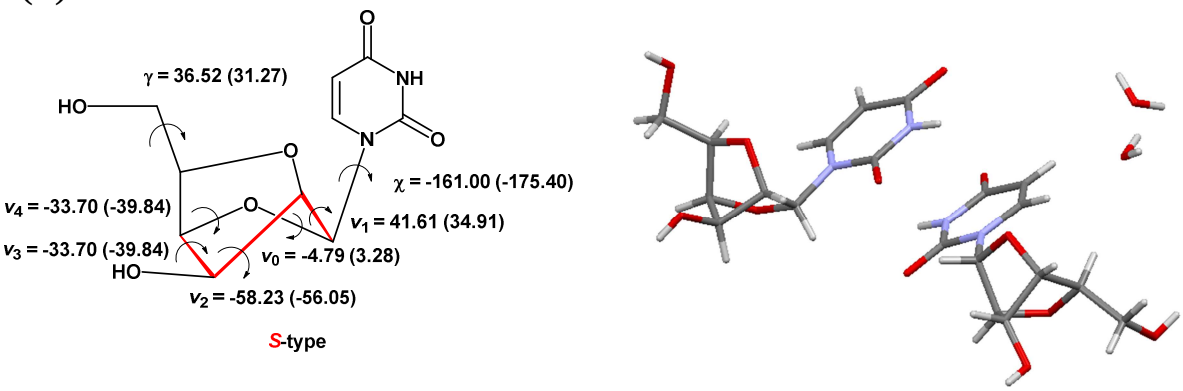


Figure 2: (a) ORTEP diagram of compound **10a** drawn in 50% thermal probability ellipsoids with atomic numbering scheme showing two crystallographically independent units (b) Exhibition of *S*-type puckering of furanose ring in nucleoside **10a**.

Table 1

Single crystal X-ray diffraction data of compound **10a**

Empirical formula	$C_{10}H_{12}N_2O_6.H_2O$
Formula weight	274.23
Temperature	293(2) K
Wavelength	0.71073 A
Crystal system	Monoclinic
Space group	P 21
Unit cell dimension	a = 5.4600(3) A
	α = 90 deg.
	b = 16.4865(8) A
	β = 99.402(4) deg.
	c = 13.2042(6) A
	γ = 90 deg.
Volume	1172.63(10) A^3
Z	4
Density (calculated)	1.553 mg/m^3
Absorption coefficient	0.133 mm^{-1}
F(000)	576
Theta range for data collection	3.363 to 25.345 deg.
Index ranges	-6≤h≤6
	-19≤k≤19
	-15≤l≤15
Reflections collected	13110
Independent reflections	4289 [R(int) = 0.0645]
Completeness to theta = 25.242	99.80 %
Max. and min. transmission	0.991 and 0.983
Refinement method	Full-matrix least-squares on F^2
Data/restrains/parameters	4289 / 1 / 353
Goodness-of-fit on F^2	1.057
Final R indices [1> 2sigma(I)]	R1 = 0.0575, wR2 = 0.0932
R indices (all data)	R1 = 0.1036, wR2 = 0.1187
Absolute structure parameter	0.5
Largest diff. peak and hole	0.218 and -0.239 $e.A^{-3}$
CCDC	1978207

Conclusion

A chemo-enzymatic pathway has been developed for the synthesis of 2'-*O*,5'-*C*-bridged-*β*-D-lyxofuranosyl-uracil and -thymine. In a pivotal step of this methodology, Novozyme®-435 was used for quantitative regioselective acetylation of one of the three hydroxy functional group present in the nucleoside. This process provided facile access to 2'-*O*,5'-*C*-bridged-*β*-D-lyxofuranosyl-uracil and -thymine with overall yields of 55.7% and 57.1%, respectively starting from diacetone-D-glucose. The synthesized nucleosides were found to be locked into *S*-type sugar puckering, which is a striking feature of our nucleoside monomer for the development of therapeutic oligonucleotides.

Experimental

All reagents were purchased from Sigma-Aldrich Chemicals Pvt. Limited, India and from local commercial sources and were used without any further purification unless otherwise specified. Melting points were determined on Buchi M-560 instrument and are uncorrected. The IR spectra of compounds were recorded on Perkin-Elmer model 2000 FT-IR spectrometer and are expressed as wavenumber (cm^{-1}). Specific rotation was measured on Rudolph Autopol II polarimeter. R_f values of compounds are reported for analytical TLC using the specified solvents and 0.25 mm silica gel 60 F_{254} plates that were visualized by UV irradiation or by charring with 5% alcoholic sulfuric acid solution. Solvents were removed under reduced pressure using rotary evaporator, followed by further removal of the residual solvent under high vacuum. Column chromatography was performed on silica gel (100-200 mesh). The ^{1}H, ^{13}C-NMR spectra were recorded on Jeol alpha-400 spectrometer at 400 MHz, 100.6 MHz, respectively by using tetramethylsilane (TMS) as internal standard. The chemical shift values are on δ scale and the coupling constant (*J*) are in Hz. HRMS analysis was carried out using Agilent G6530AA LC Q-TOF mass spectrometer using ESI method.

Synthesis of 3-*O*-benzyl-1,2,5,6-tetra-*O*-acetyl-α, *β*-D-glucofuranose (13a-b)

To the stirred solution of 3-*O*-benzyl-1,2:5,6-*O*-di-isopropylidene-α-D-glucofuranose (3.5 g, 9.98 mmol) in acetic acid (57.12 mL, 998 mmol) at 0° C, acetic anhydride (9.5 ml, 99.8 mmol) and concentrated sulphuric acid (0.053 mL, 0.998 mmol) were added. The reaction mixture was stirred for 3-4 hour at room temperature. After completion, reaction was quenched by adding cold water and neutralized by sodium bi-carbonate. The compound was extracted with ethyl acetate (3 x 100 mL), brine solution (2 x 100 mL) and combined organic layer was dried over

anhydrous sodium sulphate to afford the crude product. The solvent was removed under reduced pressure. The crude product thus obtained was purified by column chromatography using ethyl acetate in petroleum ether as gradient solvent system to afford an anomeric mixture (α:β = 1:5, based on integration of anomeric proton in ^{1}H NMR) of **13a-b** as colourless oil, 4.2 g in 96% yield, R_f = 0.38 (20% ethyl acetate in petroleum ether).

3-*O*-Benzyl-1,2,5,6-tetra-*O*-acetyl-*α, β*-D-glucofuranose (13a-b)

$[\alpha]_D^{24}$ = + 37.18 (*c* 0.1, MeOH). IR (thin film) ν_{max}: 1739, 1435, 1369, 1211, 1045, 881, 742, 700, 592, 501 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 7.30-7.37 (5H, m), 6.16 (1H, s), 5.35-5.39 (1H, m), 5.26 (1H, s), 4.78 (1H, d, *J* = 12.1 Hz), 4.66 (1H, dd, *J* = 12.3, 2.3 Hz), 4.56 (1H, d, *J* = 12.1 Hz), 4.45 (1H, dd, *J* = 7.8, 5.1 Hz), 4.12 (1H, dd, *J* = 12.3, 5.0 Hz) 4.02 (1H, d, *J* = 5.0 Hz), 2.12 (3H, s), 2.09 (3H, s), 2.05 (3H, s), 1.88 (3H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): *δ* 170.7, 169.6, 169.5, 136.9, 128.5, 128.0, 127.9, 99.3, 80.8, 79.0, 78.5, 71.7, 69.1, 63.0, 21.1, 20.9, 20.8, 20.7; HR-ESI-TOF-MS: *m/z* cal. for $C_{21}H_{30}NO_{10}$ $[M+NH_4]^+$: 456.1864; found: 456.1861.

Synthesis of 2',5',6'-tri-*O*-acetyl-3'-*O*-benzyl-*β*-D-glucofuranosyl pyrimidine nucleosides (14a-b).

To the stirred solution of tetra-*O*-acetylated sugar derivative **13a-b** (1.0 g, 2.28 mmol) and uracil / thymine (3.42 mmol) in anhydrous acetonitrile (40 mL), *N*,*O*-bis(trimethylsilyl)acetamide (2.23 mL, 9.12 mmol) was added dropwise. The reaction mixture was stirred at reflux for one hour, and then cooled to 0°C. In the cooled reaction mixture trimethylsilyltrifluromethane sulfonate (0.70 mL, 3.88 mmol) was added dropwise under stirring and the solution was refluxed for 6-8 hours. The reaction was quenched with cold saturated aq. sodium bicarbonate solution (150 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic layer was washed with saturated aq. sodium bicarbonate solution (2 x 100 mL), brine solution (2 x 100 mL), and the combined organic layer was dried over anhydrous sodium sulfate to afford the crude residue. The crude residue was purified by silica gel column chromatography using ethyl acetate in petroleum ether as eluent to afford pure nucleosides **14a** and **14b** in good yields.

2',5',6'-Tri-*O*-acetyl-3'-*O*-benzyl-*β*-D-glucofuranosyl uracil (14a)

It was obtained as white solid (0.98 g) in 88% yield. R_f = 0.23 (2% MeOH/chloroform); $[\alpha]_D^{24}$ = + 51.78 (*c* 0.1, MeOH); m/p: 112-115 °C; IR (KBr, cm^{-1}): 3064, 1739, 1685, 1454,

1369, 1325, 1215, 1107, 1045, 939, 916, 866, 812, 748, 700, 634, 601, 570, 545, 495, 418, 405; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.24 (1H, s), 7.56 (1H, d, J = 7.8 Hz), 7.29-7.34 (5H, m), 6.19 (1H, s), 5.66 (1H, d, J = 7.5 Hz), 5.43 (1H, d, J = 8.1 Hz), 5.17 (1H, s), 4.68 (2H, t, J = 12.0 Hz), 4.54 (1H, d, J = 11.3 Hz), 4.24 (1H, d, J = 7.8 Hz), 4.09 (1H, d, J = 9.6 Hz), 3.90 (1H, s), 2.19 (3H, s), 2.08 (3H, s), 1.86 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 169.8, 169.3, 162.9, 150.2, 140.1, 135.8, 129.0, 128.8, 128.6, 103.0, 88.8, 79.4, 79.3, 78.1, 71.6, 67.5, 62.6, 20.9, 20.8**;** HRMS (ESI): *m/z* calcd for $C_{23}H_{27}N_2O_{10}$ $[M+H]^+$: 491.1660; found: 491.1671.

2',5',6'-Tri-*O*-acetyl-3'-*O*-benzyl-*β*-D-glucofuranosyl thymine (14b)

It was obtained as white solid (1.02 g) in 89% yield. R_f = 0.24 (2% MeOH/chloroform); $[\alpha]_D^{24}$ = + 24.67 (*c* 0.1, MeOH); m/p: 118-120 °C; IR (KBr, cm^{-1}): 1741, 1691, 1460, 1371, 1219, 1049, 746, 700, 592, 480; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (1H, s), 7.30-7.36 (6H, m), 6.24 (1H, d, J = 1.4 Hz), 5.47-5.51 (1H, m), 5.15 (1H, s), 4.71 (1H, d, J = 11.2 Hz), 4.66 (1H, dd, J = 12.5, 2.2 Hz), 4.55 (1H, d, J = 11.2 Hz), 4.23 (1H, dd, J = 9.2, 3.2 Hz), 4.10 (1H, dd, J = 12.5, 4.4 Hz), 3.91 (1H, d, J = 3.2 Hz), 2.19 (3H, s), 2.09 (3H, s), 1.94 (3H, s), 1.68 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 169.9, 169.4, 163.6, 150.4, 136.0, 135.9, 128.7, 128.6, 111.8, 88.4, 79.5, 79.0, 78.8, 71.8, 67.7, 62.7, 20.9, 20.9, 12.4; HRMS (ESI): *m/z* calcd for $C_{24}H_{29}N_2O_{10}$ $[M+H]^+$: 505.1817; found: 505.1831.

Synthesis of 3'-*O*-benzyl-*β*-D-glucofuranosyl pyrimidines nucleosides 15a-b.

To the stirred solution of compound **14a** (1.2 g, 2.44 mmol) or **14b** (1.2 g, 2.38 mmol) in methanol:water (9:1, 120 mL), K_2CO_3 (1.01 g, 7.32 mmol for **14a**) or (0.98 g, 7.14 mmol for **14b**) was added at 0 °C and the reaction mixture was kept under stirring at 25 °C for 1h. On completion of the reaction, solvent was removed under reduced pressure. The residue thus obtained was purified by column chromatography with a gradient solvent system of methanol in chloroform to afford trihydroxy nucleosides **15a** and **15b** in quantitative yields.

3'-*O*-Benzyl-*β*-D-glucofuranosyl uracil (15a)

It was obtained as white solid (0.88 g) in 99% yield. R_f = 0.23 (10% MeOH/chloroform); $[\alpha]_D^{24}$ = + 45.13 (*c* 0.1, MeOH); m/p: 150-153 °C; IR (KBr, cm^{-1}): 3363, 3059, 2939, 1666, 1462, 1394, 1261, 1207, 1070, 1043, 896, 813, 740, 698, 640, 572, 547; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (1H, s), 7.56 (1H, d, J = 8.1 Hz), 7.27-7.34 (5H, m), 5.87 (1H, d, J = 4.0 Hz), 5.67 (1H, s), 5.46 (1H, dd, J = 8.1, 2.2 Hz), 4.85 (1H, s), 4.60 (2H, q, J = 11.8 Hz), 4.52

(1H, s), 4.21 (1H, d, *J* = 3.5 Hz), 4.07 (1H, dd, *J* = 9.2, 3.1 Hz), 3.91 (1H, s), 3.88 (1H, d, *J* = 3.1), 3.62 (1H, d, *J* = 11.2 Hz), 3.44 (1H, dd, *J* = 11.4, 5.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.2, 150.4, 140.7, 138.0, 128.2, 127.4, 127.3, 100.8, 91.4, 82.4, 82.2, 77.1, 71.1, 68.1, 63.7; HRMS (ESI): *m/z* calcd for $C_{17}H_{21}N_2O_7$ $[M+H]^+$: 365.1343; found: 365.1341.

3'-*O*-Benzyl-*β*-D-glucofuranosyl thymine (15b)

It was obtained as white solid (0.89 g) in 99% yield. R_f = 0.23 (10% MeOH/chloroform); $[\alpha]_D^{24}$ = + 40.99 (*c* 0.1, MeOH); m/p: 94-96 °C; IR (KBr, cm^{-1}): 3361, 3034, 2933, 1660, 1465, 1392, 1327, 1261, 1078, 1047, 902, 742, 696, 592, 478; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.30 (1H, s), 7.40 (1H, d, *J* = 1.2 Hz), 7.26-7.34 (5H, m), 5.88 (1H, d, *J* = 4.0 Hz), 5.75 (1H, d, *J* = 0.8 Hz), 4.90 (1H, d, *J* = 5.9 Hz), 4.58-4.64 (2H, m), 4.55 (1H, t, *J* = 5.6 Hz), 4.23 (1H, d, *J* = 3.8 Hz), 4.02 (1H, dd, *J* = 9.2, 3.0 Hz), 3.89 (2H, d, *J* = 3.0 Hz), 3.61 (1H, d, *J* = 11.3 Hz), 3.42 (1H, dt, *J* = 11.4, 5.8 Hz), 1.47 (3H, d, *J* = 1.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.6, 150.4, 138.0, 136.7, 128.2, 127.4, 108.6, 90.9, 82.5, 81.8, 77.3, 71.0, 68.2, 63.7, 12.0; HRMS (ESI): *m/z* calcd for $C_{18}H_{23}N_2O_7$ $[M+H]^+$: 379.1500; found: 379.1505.

Synthesis of monoacetylated pyrimidine nucleosides 16a-b.

To a solution of 2',5',6'-trihydroxy-3'-*O*-benzyl-*β*-D-glucofuranosyl nucleosides **15a** (1.0 g, 2.74 mmol) and **15b** (1.0 g, 2.64 mmol) in THF (80 mL) was added vinyl acetate (0.30 mL, 2.74 mmol for **15a**) / (0.29 mL, 3.17 mmol for **15b**) followed by addition of Novozyme®-435 (0.5 g, 50% w/w). The reaction mixture was stirred at 45 °C in an incubator shaker at 200 rpm for 50-60 minutes. On completion, the reaction was quenched by filtering off the Novozyme®-435; the solvent was removed under reduced pressure and the residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford the monoacetylated nucleosides **16a** and **16b** in quantitative yields.

6'-*O*-Acetyl-3'-*O*-benzyl-*β*-D-glucofuranosyl uracil (16a)

It was obtained as white solid (1.09 g) in 98% yield. R_f = 0.25 (5% MeOH/chloroform); $[\alpha]_D^{24}$ = + 26.27 (*c* 0.1, MeOH); m/p: 133-136 °C; IR (KBr, cm^{-1}): 3323, 3034, 2947, 2873, 1739, 1697, 1674, 1460, 1419, 1386, 1313, 1257, 1228, 1103, 1072, 1043, 972, 900, 831, 765, 734, 694, 634, 605, 572, 557, 418; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (1H, s), 7.54 (1H, d, *J* = 8.2 Hz), 7.27-7.35 (5H, m), 5.94 (1H, s), 5.68 (1H, s), 5.46 (1H, d, *J* = 8.1

Hz), 5.32 (1H, s), 4.59 (2H, q, *J* = 11.7 Hz), 4.29 (1H, dd, *J* = 11.4, 2.0 Hz), 4.24 (1H, s), 4.15 (1H, d, *J* = 12.8 Hz), 4.10 (1H, dd, *J* = 9.3, 2.9 Hz), 3.98 (1H, dd, *J* = 11.4, 5.7 Hz), 3.90 (1H, d, *J* = 2.8 Hz), 2.03 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.5, 163.2, 150.4, 140.7, 137.9, 128.2, 127.5, 127.4, 100.8, 91.5, 82.3, 82.0, 76.9, 71.1, 66.5, 64.9, 20.8; HRMS (ESI): *m/z* calcd for $C_{19}H_{23}N_2O_8$ $[M+H]^+$: 407.1449; found: 407.1458.

6'-*O*-Acetyl-3'-*O*-benzyl-*β*-D-glucofuranosyl thymine (16b)

It was obtained as white solid (1.09 g) in 99% yield. R_f = 0.26 (5% MeOH/chloroform); $[\alpha]_D^{24}$ = + 77.90 (*c* 0.1, MeOH); m/p: 144-146 °C; IR (KBr, cm^{-1}): 3414, 3157, 3084, 3005, 2953, 2904, 2827, 1697, 1633, 1487, 1423, 1386, 1240, 1080, 1039, 966, 904, 866, 758, 700, 597, 540, 499, 466, 422; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.32 (1H, s), 7.38 (1H, s),7.28-7.33 (5H, m), 5.93 (1H, d, *J* = 3.8 Hz), 5.75 (1H, s), 5.35 (1H, d, *J* = 5.9 Hz), 4.60 (2H, q, *J* = 11.2 Hz), 4.31 (1H, d, *J* = 11.1 Hz), 4.27 (1H, d, *J* = 3.3 Hz), 4.15 (1H, s), 4.06 (1H, dd, *J* = 9.3, 2.7 Hz), 3.95 (1H, dd, *J* = 11.5, 6.3 Hz), 3.91 (1H, d, *J* = 2.6 Hz), 2.04 (3H, s), 1.50 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.5, 163.7, 150.4, 137.9, 136.5, 128.2, 127.5, 127.5, 108.6, 91.1, 82.1, 81.9, 77.0, 71.1, 66.6, 65.0, 20.8, 12.1; HRMS (ESI): *m/z* calcd for $C_{20}H_{25}N_2O_8$ $[M+H]^+$: 421.1605; found: 421.1617.

Synthesis of 6'-*O*-acetyl-3'-*O*-benzyl-2',5'-di-*O*-methanesulfonyl-*β*-D-glucofuranosyl pyrimidine nucleosides 17a-b.

A solution of **16a** (1.0 g, 2.46 mmol) or **16b** (1.0 g, 2.37 mmol) and methanesulfonyl chloride (0.48 mL, 6.15 mmol for **16a**) and (0.46 mL, 5.92 mmol for **16b)** in anhydrous pyridine (10 mL) was stirred at 25 °C for 2 hours. On completion, the reaction mixture was poured over 10% ice cold hydrochloric acid solution (100 mL) to neutralize pyridine and extracted with ethyl acetate (3 x 100 mL). The combined organic layer was washed with saturated sodium bicarbonate solution (200 mL) and dried over sodium sulfate. The excess of solvent was removed under reduced pressure. The residue thus obtained was purified over silica gel column chromatography using methanol in chloroform as gradient solvent system to afford the nucleosides **17a** and **17b** in pure form.

6'-*O*-Acetyl-3'-*O*-benzyl-2',5'-di-*O*-methanesulfonyl-*β*-D-glucofuranosyl uracil (17a)

It was obtained as white solid (1.34 g) in 97% yield. R_f = 0.31 (5% MeOH/chloroform); $[\alpha]_D^{24}$ = + 139.11 (*c* 0.1, MeOH); m/p: 82-85 °C; IR (KBr, cm^{-1}): 3030, 2937, 2314, 1741, 1683, 1456, 1355, 1263, 1226, 1174, 1111, 1049, 1002, 964, 923, 879, 813, 742, 700, 638,

605, 522, 507, 418; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (1H, s), 7.37 (1H, d, J = 8.2 Hz), 7.27-7.31 (5H, m), 5.92 (1H, s), 5.58 (1H, d, J = 8.0 Hz), 5.37-5.40 (1H, m), 4.97 (1H, s), 4.80 (1H, s,), 4.78 (1H, s), 4.50 (1H, d, J = 10.7 Hz), 4.44 (1H, d, J = 9.2 Hz), 4.34 (1H, s), 4.16 (1H, dd, J = 12.8, 5.3 Hz), 3.24 (3H, s), 3.10 (3H, s), 2.15 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.5, 163.2, 150.5, 139.3, 136.1, 128.9, 128.8, 128.7, 102.3, 89.9, 83.1, 80.4, 79.8, 73.4, 72.9, 63.0, 39.3, 38.7, 20.8; HRMS (ESI): *m/z* calcd for $C_{21}H_{27}N_2O_{12}S_2$ $[M+H]^+$: 563.1000; found: 563.1011.

6'-*O*-Acetyl-3'-*O*-benzyl-2',5'-di-*O*-methanesulfonyl-*β*-D-glucofuranosyl thymine (17b)

It was obtained as white solid (1.33 g) in 97% yield. R_f = 0.31 (5% MeOH/chloroform); $[\alpha]_D^{24}$ = + 82.34 (*c* 0.1, MeOH); m/p: 96-98 °C; IR (KBr, cm^{-1}): 3172, 3030, 2941, 2823, 2314, 1732, 1683, 1645, 1463, 1417, 1355, 1328, 1263, 1240, 1176, 1089, 1041, 1002, 962, 918, 833, 792, 758, 702, 624, 586, 549, 518, 501, 422; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.01 (1H, s), 7.33 (5H, m), 7.21 (1H, d, J = 1.2 Hz), 6.10 (1H, s), 5.41-5.44 (1H, m), 5.13 (1H, s), 4.82 (1H, d, J = 10.6 Hz), 4.78 (1H, d, J = 12.8 Hz), 4.59 (1H, d, J = 10.6 Hz), 4.43 (1H, d, J = 2.9 Hz), 4.40 (1H, d, J = 4.3 Hz), 4.16 (1H, dd, J = 12.9, 6.1 Hz), 3.31 (3H, s), 3.12 (3H, s), 2.15 (3H, s), 1.54 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 163.5, 151.1, 136.3, 135.4, 128.7, 128.6, 128.5, 111.6, 89.1, 83.5, 79.7, 79.7, 73.1, 72.6, 63.1, 39.3, 38.7, 20.9, 12.2; HRMS (ESI): *m/z* calcd for $C_{22}H_{29}N_2O_{12}S_2$ $[M+H]^+$: 577.1156; found: 577.1170.

Synthesis of 3'-*O*-benzyl-2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl pyrimidine nucleosides 18a-b.

To a solution of compound **17a** (0.8 g, 1.42 mmol) or **17b** (0.8 g, 1.39 mmol) in dioxane:water (1:1, 20 mL), 2M NaOH (0.8 mL) was added at 0 °C and the reaction mixture was stirred for 24 h at 25 °C. On completion, acetic acid (10 mL) was added to neutralize the reaction mixture and co-evaporated with toluene under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford nucleosides **18a** and **18b** in pure form.

3'-*O*-Benzyl-2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl uracil (18a)

It was obtained as white solid (0.36 g) in 73% yield. R_f = 0.26 (5% MeOH/chloroform); $[\alpha]_D^{24}$ = + 265.42 (*c* 0.1, MeOH); m/p: 145-146 °C ; IR (KBr, cm^{-1}): 3334, 3039, 2924, 2812, 1772, 1670, 1620, 1460, 1371, 1315, 1273, 1207, 1178, 1145, 1099, 1041, 1024, 979, 933, 894, 850, 812, 752, 700, 636, 590, 555, 470, 420; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41

(1H, s), 7.80 (1H, d, *J* = 8.0 Hz), 7.31-7.38 (5H, m), 5.77 (1H, s), 5.62 (1H, d, *J* = 8.1 Hz), 4.80 (1H, t, *J* = 5.4 Hz), 4.71 (1H, s), 4.67 (1H, s), 4.61 (2H, q, *J* = 11.9 Hz), 4.41 (1H, s), 4.03 (1H, t, *J* = 6.1 Hz), 3.61 (1H, dt, *J* = 11.7, 6.0 Hz), 3.45 (1H, dt, *J* = 11.1, 5.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.2, 150.3, 140.2, 137.5, 128.4, 127.7, 127.6, 100.5, 87.5, 85.2, 79.2, 78.3, 76.1, 71.2, 60.3; HRMS (ESI): *m/z* calcd for $C_{17}H_{19}N_2O_6$ $[M+H]^+$: 347.1238; found: 347.1246.

3'-*O*-Benzyl-2'-*O*, 5'-*C*-bridged-*β*-D-homolyxofuranosyl thymine (18b)

It was obtained as white solid (0.37 g) in 74% yield. R_f = 0.28 (5% MeOH/chloroform); $[\alpha]_D^{24}$ = + 211.22 (*c* 0.1, MeOH); m/p: 155-157 °C ; IR (KBr, cm^{-1}): 3437, 3026, 2918, 2821, 1680, 1463, 1371, 1319, 1269, 1205, 1151, 1105, 1070, 1024, 852, 732, 694, 594, 559, 486; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.40 (1H, s), 7.65 (1H, s),7.31-7.40 (5H, m), 5.75 (1H, s), 4.78 (1H, s), 4.71 (1H, d, *J* = 2.0 Hz), 4.66 (1H, s), 4.61 (2H, q, *J* = 11.8 Hz), 4.41 (1H, d, *J* = 2.0 Hz), 4.08 (1H, t, *J* = 6.3 Hz), 3.61 (1H, dt, *J* = 10.6, 5.4 Hz), 3.46 (1H, dd, *J* = 10.5, 5.0 Hz), 1.82 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.9, 150.2, 137.5, 135.5, 128.4, 127.7, 127.6, 108.0, 87.5, 85.1, 79.2, 78.3, 76.2, 71.2, 60.3, 12.3; HRMS (ESI): *m/z* calcd for $C_{18}H_{21}N_2O_6$ $[M+H]^+$: 361.1394; found: 361.1408.

Synthesis of 2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl pyrimidine nucleosides 10a-b.

To a solution of compound **18a** (0.2 g, 0.58 mmol) or **18b** (0.2 g, 0.55 mmol) in anhydrous THF:MeOH (9:1, 10 mL) was added $Pd(OH)_2$-C (20 wt%, 0.04 g) and 88% formic acid (0.16 mL, 4.4 mmol). The reaction mixture was then refluxed for 10 min. After completion of reaction checked by TLC, catalyst was carefully filtered off and was washed with excess of MeOH. The combined filtrate was then concentrated under reduced pressure. The crude product obtained was purified on silica gel column chromatography with a gradient solvent system of methanol in chloroform to obtain bridged nucleosides **10a** and **10b** in pure form.

2'-*O*,5'-*C*-Bridged-*β*-D-homolyxofuranosyl uracil (10a)

It was obtained as white solid (0.15 g) in 99% yield. R_f = 0.32 (10% MeOH/chloroform); $[\alpha]_D^{24}$ = + 330.94 (*c* 0.1, MeOH); m/p: 221-223 °C; IR (KBr, cm^{-1}): 3153, 3034, 2927, 2816, 1770, 1676, 1620, 1462, 1398, 1363, 1313, 1269, 1203, 1139, 1093, 1041, 1018, 935, 893, 852, 810, 754, 696, 632, 553, 520, 460, 426; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (1H, s), 7.78 (1H, d, *J* = 8.1 Hz), 5.95 (1H, s), 5.74 (1H, s), 5.61 (1H, d, *J* = 8.1 Hz), 4.95 (1H, s), 4.45 (1H, d, *J* = 2.2 Hz), 4.39 (1H, d, *J* = 2.1 Hz), 4.37 (1H, s), 3.98 (1H, t, *J* = 5.8 Hz), 3.65

(1H, dd, *J* = 11.5, 6.1 Hz), 3.50 (1H, dd, *J* = 11.5, 5.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 150.4, 140.2, 100.4, 87.6, 85.2, 80.1, 78.5, 72.3, 60.3; HRMS (ESI): *m/z* calcd for $C_{10}H_{13}N_2O_6$ $[M+H]^+$: 257.0768; found: 257.0761.

2'-*O*,5'-*C*-Bridged-*β*-D-homolyxofuranosyl thymine (10b)

It was obtained as white solid (0.15 g) in 98% yield. R_f = 0.31 (10% MeOH/chloroform); $[\alpha]_D^{24}$ = + 140.99 (*c* 0.1, MeOH); m/p: 225-228 °C; IR (KBr, cm^{-1}): 3396, 3035, 2821, 1676, 1469, 1359, 1271, 1205, 1143, 1099, 1043, 1014, 983, 947, 894, 831, 756, 732, 692, 609, 574, 491, 420; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (1H, s), 7.63 (1H, d, *J* = 1.1 Hz), 5.96 (1H, s), 5.71 (1H, d, *J* = 0.9 Hz), 4.95 (1H, s), 4.45 (1H, d, *J* = 2.3 Hz), 4.39 (1H, d, *J* = 2.2 Hz), 4.36 (1H, s), 4.02 (1H, t, *J* = 5.8 Hz), 3.66 (1H, dd, *J* = 11.5, 6.1 Hz), 3.51 (1H, dd, *J* = 11.5, 5.5 Hz), 1.81 (3H, d, *J* = 0.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.9, 150.3, 135.5, 107.9, 87.5, 85.0, 80.1, 78.5, 72.2, 60.3, 12.3; HRMS (ESI): *m/z* calcd for $C_{11}H_{15}N_2O_6$ $[M+H]^+$: 271.0925; found: 271.0935.

Notes

The authors declare no competing financial interest.

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Highlights:

1. 2'-*O*,5'-*C*-Bridged-*β*-D-homolyxofuranosyl nucleosides U and T have been synthesized.
2. X-rays diffraction analysis revealed that 2'-*O*,5'-*C*-bridged homo-nucleosides are locked into *S*-type sugar puckering.
3. Environment friendly regioselective monoacetylation carried out by Novozyme®-435.
4. Overall yields for synthesis of 2'-*O*,5'-*C*-bridged-*β*-D-homolyxofuranosyl nucleosides U and T, starting from diacetone-D-glucose are 55.7 and 57.1%, respectively.

Notes

The authors declare no competing financial interest