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Synthesis of 3'-azido/-amino-xylobicyclonucleosides

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Lipozyme® TL IM - mediates selective deacetylation of one of the two diastereotopic acetoxy groups in 4-*C*-acetoxymethyl-5-*O*-acetyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*furanose that led to the first efficient synthesis of 3'-azido/3'-amino-*xylo*bicyclonucleosides T, U, C and A in 30 to 35 % overall yields from diacetone-D-glucose. The single crystal X-ray study on the compound obtained by the tosylation of lipase-mediated monodeacetylated sugar unambiguously confirmed the point of diastereoselection on diacetoxy-sugar derivative. The synthesized bicyclic nucleosides have potential application in antisense/aptamer-based oligonucleotide therapeutics development.

Introduction

After the pioneering work of Zamecnik and Stephenson[1] RNA has been a very attractive terget in the medicinal chemistry. The RNA targeting drugs for therapeutic applications afforded the designing of potent analogues of bicyclic nucleosides and locked nucleic acid (LNA) involving them for antisense therapy.[2] The LNA monomers has a methylene bridge between 2'-oxygen and 4'-carbon of the ribose sugar, which forces it to adopt *C*3'- endo conformation suitable for efficient hybridization with complementary DNA and RNA-oligonucleotides (Figure 1).[3] The structurally rigid modifications have many advantages, such as increase in nuclease resistance, enhanced cellular uptake and significant enhancement of their duplex stability with the complimentary DNA/RNA strand.[4,5]

Various diastereomers of LNA have been synthesized and evaluated to optimize the drug like characteristics of the molecule.[6-8] It has been observed that *xylo*-LNA monomer gives significant results like LNA (Figure 1).[9] Encouraged by the

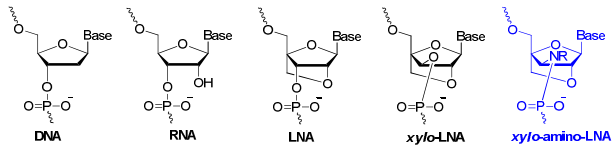

Figure 1. Structures of DNA, RNA, LNA, *xylo*-LNA and *xylo*-amino-LNA

current developments in the area, we have for the first time developed an efficient lipase-mediated convergent synthesis of 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-*xylo*nucleosides **1a-d** in 30-35 % overall yields from diacetone-D-glucose and have demonstrated that 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-*xylo*thymidine (**1a**) can easily be converted into its corresponding 3'-amino derivative **2** in quantitative yield, which is a precursor for the synthesis of *N*3'-*P*-*O*5'-oligonucleotides (Figures 1 and 2).

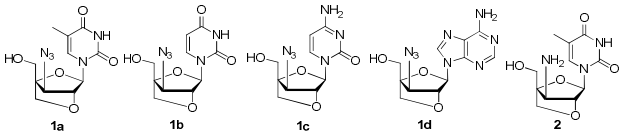

Figure 2. Structures of synthesized 3'-azido and 3'-amino-*xylo*bicyclonucleosides

Results and Discussion

The chemo-enzymatic approach has been envisioned for the synthesis of targeted azido/amino-*xylo*bicyclonucleosides **1a-d** and **2**. The key starting sugar derivative, *i.e.* 4-*C*-acetoxymethyl-5-*O*-acetyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*furanose (**5**) was synthesized from 4-*C*-acetoxymethyl-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*ribo*furanose (**3**) *via* debenzylation followed by triflation-azidation of the debenzylated sugar derivative **4** in 83 % overall yield. The diacetoxy sugar derivative **3** in turn was synthesized from diacetone-D-glucose by following literature procedure in 50 % overall yield.[10] Further, diastereoselective deacetylation of 4-*C*-acetoxymethyl group of diacetoxyazido sugar derivative **5** is required for the synthesis of azido/amino-*xylo*bicyclonuclesides. It is at this juncture the use of nature's catalyst LIPASE was sought. Four different lipases,[11] *viz.* *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM), *Candida antarctica* lipase-B immobilized on polyacrylate (Lewatit), commonly known as Novozyme®-435, *Porcine pancreatic* lipase (PPL) and *Candida rugosa* lipase (CRL)

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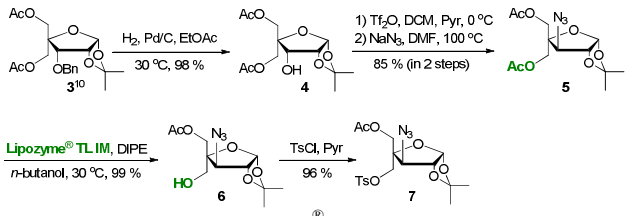
†Electronic Supplementary Information (ESI) available: [^{1}H- and ^{13}C NMR spectra of compounds **1a-d**, **2-7**, **8a-8b** and **9a-d**].

were screened in six set of organic solvents, *i.e.* diisopropyl ether (DIPE), dioxane, tetrahydrofuran (THF), toluene, acetone and acetonitrile using *n*-butanol as acetyl acceptor at 30, 40, 50 and 60 °C and at 250 rpm in an incubator shaker in order to identify a suitable lipase and the reaction condition for affecting selective deacetylation on compound **5** to afford 5-*O*-acetyl-3-azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*xylo*furanose (**6**). Among the four screened lipases, only Lipozyme® TL IM (20 % w/w of the substrate) in diisopropyl ether (DIPE) at 30 °C was found to exhibit exclusive selectivity for the deacetylation of 4-*C*-acetoxymethyl group over the 5-*O*-acetyl group in compound **5** (Scheme 1, Figure 3).



Scheme 1. Synthesis and Lipozyme® TL IM-mediated diastereoselective deacetylation studies on diacetoxy-azido-*xylo*furanose sugar derivative **5**

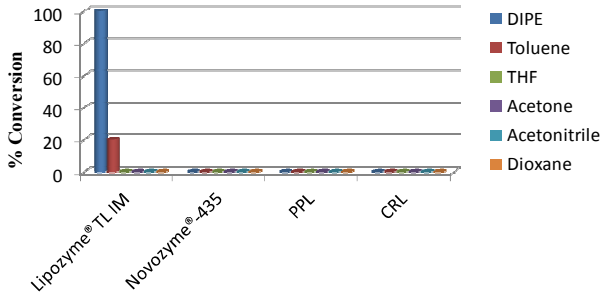


Figure 3. Screening of different lipases in organic solvents for selective deacetylation of diacetoxy-azido-*xylo*furanose suar derivative **5**. When the reaction was performed in the absence of Lipozyme® TL IM, no product was obtained. Other three lipases did not give any product under same reaction conditions.

In a typical enzymatic reaction, diacetoxy-azido-*xylo*furanose **5** was incubated with Lipozyme® TL IM in DIPE containing a small amount of *n*-butanol as acetyl accepter in an incubator shaker at 250 rpm and at 30 °C. On completion, the reaction was quenched by filtering off the enzyme and the solvent was removed under reduced pressure to afford the product which was purified by passing through a small pad of silica gel to obtain pure monodeacetylated azido-*xylo*furanose **6** in quantitative yield. The structure of monodeacetylated compound **6** was unambiguously established on the basis of its spectral data analysis, which was further confirmed by X-ray diffraction studies on the single crystal of its tosyl derivative, *i.e.* 5-*O*-acetyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-4-*C*-*p*-toluenesulphonyloxymethyl-α-D-*xylo*furanose (**7**) obtained by tosylation with TsCl-pyridine in 96 % yield (Scheme 1, Figure 4). The diastereoselectivity exhibited by Lipozyme® TL IM for the selective deacetylation of 4-*C*-acetoxymethyl over 5-*O*-acetyl group in compound **5** was found to be similar to our earlier finding on enzymatic deacetylation studies on tetracetylated nucleosides.[12]

The synthesis of azido-*xylo*bicyclonucleosides T, U, C and A **1a-d** was successfully achieved from monotosylated compound **7**. The acetolysis of monotosylated compound **7** with acetic acid-acetic anhydride-sulphuric acid (100:10:0.1) afforded the triacetoxy sugar derivative **8a-8b** in 95 % yield, which on Vorbrüggen coupling[13] with nucleobases thymine, uracil, cytosine and 6-*N*-benzoyladenine in the presence of

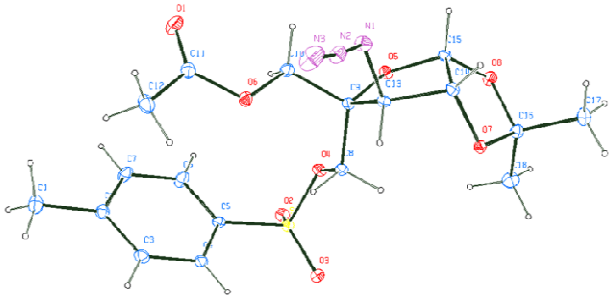
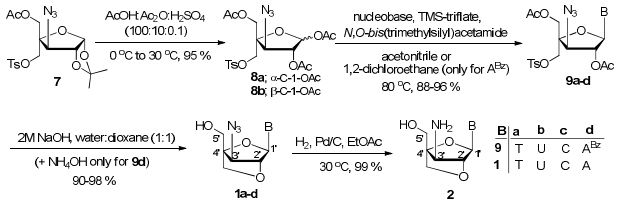


Figure 4. ORTEP diagram of tosylated sugar derivative **7**

N,O-bis(trimethylsilyl)acetamide and trimethylsilyltrifluoromethane sulfonate in acetonitrile (or in 1,2-dichloroethane only for 6-*N*-benzoyladenine) resulted in the formation of diacetylated nucleosides **9a-d** in 88-96 % yields. Treatment of nucleosides **9a-d** with 2M NaOH solution in water:dioxane (1:1) (+ 25 % NH_4OH, only for **9d**) resulted in the deacetylation followed by intramolecular cylization to afford 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-*xylo*nucleosides **1a-d** in 90-98 % yields. Further, it has been demonstrated as a model case that the azide group of 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-*xylo*thymidine (**1a**) can be reduced with Pd-C under hydrogen atmosphere in ethyl acetate to afford the corresponding amino-nucleoside, *i.e.* 3'-amino-3'-deoxy-2'-*O*,4'-*C*-methylene-*xylo*thymidine (**2**) in 99 % yield (Scheme 2).



Scheme 2. Convergent synthesis of 3'-azido/3'-amino-*xylo*bicyclonucleosides **1a-d** and **2**

The structure of all synthesized compounds **1a-d**, **2-7**, **8a-8b** and **9a-d** were unambiguously established on the basis of their spectral data (^{1}H- & ^{13}C-NMR, ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMQC NMR spectra, IR spectra and HRMS) analysis. The structure of known compound **3** was further confirmed on the basis of comparison of its physical and spectral data with those reported in the literature.[10] The single crystal X-ray diffraction analysis has been performed on 3'-azido-3'-deoxy-2'-*O*,4'-*C*-methylene-*xylo*uridine (**1b**) which

revealed important structural features of the synthesized 3'-azido/-amino-*xylo* LNA monomers where the sugar puckering was locked in *N*-type conformation (Figure 5A and 5B). The detailed crystallographic data of compounds **7** and **1b** has been deposited in the Cambridge Crystallographic Data Centre with CCDC nos. 1414989 and 1420474, respectively.

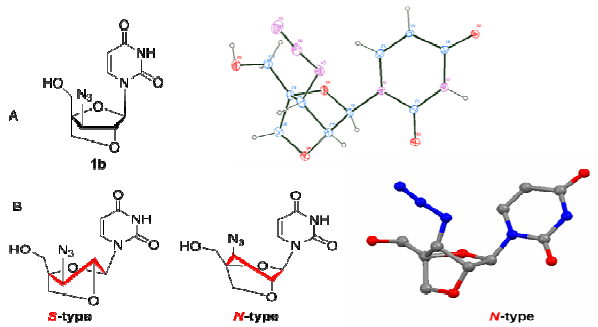


Figure 5. (A) ORTEP diagram of compound 3'-azido-*xylo*bicyclouridine **1b**; (B) Preferred *N*-type sugar puckering in compound **1b**

Experimental Section

The IR spectra were recorded by making KBr disc for solid samples and thin film for oils. The ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100.6 MHz, respectively using TMS as internal standard. The chemical shift values are on δ scale and the coupling constants (*J*) are in Hz. The HRMS analysis was done on a Q-TOF mass spectrometer. The optical rotations were measured using light of 589 nm wavelength. Analytical TLCs were performed on precoated silica-gel 60F_{254} plates; the spots were detected either under UV light or by charring with 4 % alcoholic H_2SO_4. Silica gel (100-200 mesh) was used for column chromatography. All solvents were distilled before use. The single crystal X-ray diffraction data was collected with graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at USIC, University of Delhi, Delhi. The activity of enzymes, Novozyme® 435, Lipozyme® TL IM, PPL and CRL screened for the deacetylation reaction as reported by manufacturers is 10000 IUN/g, 250 IUN/g, ≥200 IUN/mg and ≥ 900 IUN/g, respectively. For 100 % conversion of 1 g of substrate into the desired product, we used 200 mg of Lipozyme® TL IM, which means 20 % w/w of the substrate. Further, we can say that we used 50 units for converting 1 g of substrate into the desired product.

4-*C*-Acetoxymethyl-5-*O*-acetyl-1,2-*O*-isopropylidene-*α*-D-*ribo*furanose (**4**). Debenzylation of *ribo*furanose sugar **3** (5.0 g, 12.68 mmol) was achieved by its stirring with 10 % Pd-C (0.5g) in ethyl acetate (100 mL) under hydrogen atmosphere at 30 °C for 5 h. On completion, the reaction was quenched by filtering off the Pd-C through celite. Excess solvent was removed under reduced pressure and the residue thus obtained was purified by silica gel column chromatography using ethyl acetate in petroleum ether as eluent to afford compound **4** as colourless oil (3.78 g; 98 % yield). R_f = 0.6 (5 % methanol in chloroform); $[\alpha]_D^{29}$ = + 26.64 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3467, 1740, 1384, 1233, 1166 and 1020 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.67 (1H, d, *J* = 3.6 Hz), 5.52 (1H, d, *J* = 6.0 Hz), 4.58 (1H, t, *J* = 4.0 Hz), 4.49 (1H, d, *J* = 12.0 Hz), 4.21-4.17 (2H, m), 4.11 (1H, d, *J* = 12.4 Hz), 3.92 (1H, d, *J* = 11.6 Hz), 2.02 (3H, s), 2.00 (3H, s), 1.46 (3H, s) and 1.24 (3H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 170.82, 170.52, 113.86, 104.56, 84.78, 79.46, 72.79, 65.34, 63.21, 26.47, 26.32, 20.87 and 20.82; HR-ESI-TOF-MS: *m/z* 305.1236 ([M+H]$^+$), calcd. for [$C_{13}H_{20}O_8$+H]$^+$ 305.1231.

4-*C*-Acetoxymethyl-5-*O*-acetyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-*α*-D-*xylo*furanose (**5**). To a stirred solution of compound **4** (4.0 g, 13.15 mmol) in dry DCM:pyridine (3:1, 80 mL) was added trifluoromethanesulfonic anhydride (3.3 mL, 19.62 mmol) under nitrogen atmosphere at 0 °C. The resulting reaction mixture was stirred for 1 h and after completion; reaction was quenched by addition of ice cold water (200 mL). The resulting mixture was extracted with chloroform (3 x 150 mL), organic layer was washed with sodium bicarbonate (2 x 100 mL), brine (2 x 100 mL) and water (2 x 100 mL), and was dried over sodium sulphate. The solvent was removed in *vacuo* and the crude thus obtained was used further without any prior purification.

A mixture of crude product obtained in previous step (5.73 g, 13.13 mmol) and NaN_3 (3.42 g, 52.62 mmol) was suspended in anhydrous DMF (100 mL) and was stirred at 100 °C for 5 h. The reaction mixture was cooled to room temperature, extracted with ethyl acetate (3 x 150 mL) and washed with water (5 x 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using ethyl acetate in petroleum ether as gradient solvent system to give compound **5** (3.68 g, 85 % yield) as light yellow solid. R_f = 0.7 (5 % methanol in chloroform); M. Pt.: 70-72 °C; $[\alpha]_D^{30}$ = + 24.87 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 2113, 1748, 1374, 1221, 1163 and 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.91 (1H, d, *J* = 4.4 Hz), 4.71 (1H, dd, *J* = 2.4 & 4.4 Hz), 4.30 (2H, d, *J* = 11.6 Hz), 4.20 (1H, d, *J* = 11.6 Hz), 4.11 (1H, d, *J* = 1.6 Hz), 4.01 (1H, d, *J* = 12.4 Hz), 2.09 (3H, s), 2.08 (3H, s), 1.56 (3H, s) and 1.35 (3H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 170.22, 170.01, 114.07, 105.04, 86.04, 85.54, 68.95, 63.92, 63.50, 27.12, 26.80, 20.86 and 20.77; HR-ESI-TOF-MS: *m/z* 330.1310 ([M+H]$^+$), calcd. for [$C_{13}H_{19}N_3O_7$+H]$^+$ 330.1296.

5-*O*-Acetyl-3-azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-*α*-D-*xylo*furanose (**6**). To a solution of compound **5** (3.0 g, 9.11 mmol) in DIPE (60 mL), *n*-butanol (0.8 mL, 8.74 mmol) was added followed by the addition of *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM) (0.6 g, 20 % w/w of the compound **5**). The reaction mixture was stirred at 30 °C in an incubator shaker and the progress of the reaction was monitored periodically by TLC. On completion after 20 h, the reaction was quenched by filtering off the enzyme, the solvent was removed under reduced pressure and the residue thus obtained was purified by column chromatography using ethyl acetate in petroleum ether as gradient solvent system to afford the monodeacetylated compound **6** as a white solid (2.59 g, 99 %

yield). R_f = 0.4 (5 % methanol in chloroform); M. Pt.: 60-61 °C; $[\alpha]_D^{30}$ = + 10.81 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3447, 2927, 2113, 1749, 1457, 1375, 1240, 1163 and 1052 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 5.83 (1H, d, *J* = 3.6 Hz), 5.30 (1H, t, *J* = 6.4 Hz), 4.76 (1H, t, *J* = 4.0 Hz), 4.27 (1H, d, *J* = 3.2 Hz), 4.11 (1H, d, *J* = 11.2 Hz), 3.85 (1H, d, *J* = 12.0 Hz), 3.54 - 3.43 (2H, m), 2.03 (3H, s), 1.46 (3H, s) and 1.28 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 170.45, 113.62, 104.77, 87.72, 86.42, 68.56, 64.61, 62.53, 27.86, 27.76 and 21.26; HR-ESI-TOF-MS: *m/z* 310.1017 ($[M+Na]^+$), calcd. for $[C_{11}H_{17}N_3O_6+Na]^+$ 310.1010.

5-*O*-Acetyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-4-*C*-*p*-toluenesulphonyloxymethyl-α-D-*xylo*furanose (**7**). To a stirred solution of compound **6** (2.0 g, 6.96 mmol) in pyridine (20 mL), *p*-toluenesulfonyl chloride (1.59 g, 8.34 mmol) was added at 0 °C. The progress of the reaction was monitored by TLC and on completion after 2 h, the reaction mixture was neutralized by 10 % ice-cold hydrochloric acid solution (80 mL) and extracted with chloroform (3 x 100 mL). The combined organic extract was washed with saturated aqueous $NaHCO_3$ (2 x 100 mL), water (2 x 100 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue thus obtained was purified by silica gel column chromatography using ethyl acetate in petroleum ether as gradient solvent system to afford the tosylated compound **7** as white solid (2.95 g; 96 % yield). R_f = 0.6 (5 % methanol in choloroform); M. Pt.: 118-120 °C; $[\alpha]_D^{31}$ = + 35.06 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 2927, 2114, 1744, 1619, 1366, 1230, 1177, 1068, 838 and 668 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 7.77 (2H, d, *J* = 8.4 Hz), 7.47 (2H, d, *J* = 8.4 Hz), 5.84 (1H, d, *J* = 3.6 Hz), 4.78 (1H, d, *J* = 3.6 Hz), 4.41 (1H, s), 4.10 (2H, q, *J* = 10.0 Hz), 3.99 (1H, d, *J* = 11.6 Hz), 3.89 (1H, d, *J* = 11.6 Hz), 2.39 (3H, s), 1.86 (3H, s), 1.26 (3H, s) and 1.21 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 169.43, 145.48, 131.40, 130.32, 127.89, 112.67, 105.01, 85.34, 84.76, 68.12, 66.45, 61.86, 26.09, 26.05, 21.12 and 20.36; HR-ESI-TOF-MS: *m/z* 442.1278 ($[M+H]^+$), calcd. for $[C_{18}H_{23}N_3O_8S+H]^+$ 442.1279.

1,2,5-Tri-*O*-acetyl-3-azido-3-deoxy-4-*C*-*p*-toluenesulphonyloxymethyl-α,β-D-*xylo*furanose (**8a-8b**). Acetic anhydride (4.27 mL, 45.17 mmol) and concentrated sulphuric acid (0.024 mL, 0.451 mmol) was added to a stirred solution of compound **7** (2.0g, 4.53 mmol) in acetic acid (25.92 mL, 452.79 mmol) at 0 °C and mixture was stirred for 6 h. On completion, the reaction was quenched by addition of water (200 mL) and extracted with chloroform (3 x 100 mL). The combined organic layer was washed with sodium bicarbonate solution (2 x 100 mL), with cold water (2 x 100 mL) and then dried over sodium sulphate. The solvent was removed under reduced pressure and the residue thus obtained was purified on silica gel column chromatography using ethyl acetate in petroleum ether as gradient solvent system to afford an anomeric mixture **8a-8b** as colourless viscous oil (2.09 g, 95 % yield). R_f = 0.4 (5 % methanol in choloroform). IR (thin film) ν_{max}: 2925, 2116, 1751, 1368, 1227, 1178, 1048, 835 and 668 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.80, 7.39, 6.33 (d, *J* = 4.8 Hz), 6.10 (s), 5.22 (d, *J* = 2.4 Hz), 5.15 (q, *J* = 4.8 Hz), 4.43-3.93 (m), 2.47, 2.15, 2.14, 2.11, 2.09, 2.07, 2.05 and 1.99 (7s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 170.01, 169.96, 169.73, 169.33, 169.28, 145.80, 145.67, 132.16, 132.04, 130.23, 130.15, 128.15, 98.74, 91.95, 85.93, 81.41, 81.37, 75.00, 69.24, 68.43, 66.87, 63.99, 63.13, 63.03, 21.81, 21.08, 21.06, 20.83, 20.75 and 20.53; HR-ESI-TOF-MS: *m/z* 508.0994 ($[M+Na]^+$), calcd. for $[C_{19}H_{23}N_3O_{10}S+Na]^+$ 508.0996.

General procedure for the synthesis of 2',5'-Di-*O*-acetyl-3'-azido-3'-deoxy-4'-*C*-*p*-toluenesulphonyloxymethyl-*xylo*nucleosides **9a-d**. To the stirred solution of compound **8a-8b** (1.0 g, 2.06 mmol) and nucleobases, *viz.* thymine/uracil or cytosine (3.09 mmol) in anhydrous acetonitrile (25 mL), *N*,*O*-*bis*(trimethylsilyl)acetamide (2.0 mL, 8.18 mmol) was added dropwise. 1,2-Dichloroethane was used as solvent for coupling of 6-*N*-benzoyladenine (3.09 mmol) instead of acetonitrile. The reaction mixture was stirred at reflux for 1 h, and then cooled to 0 °C. In the cooled reaction mixture trimethylsilyltrifluoromethane sulfonate (0.63 mL, 3.48 mmol) was added dropwise under stirring and the reaction mixture was refluxed for 4-6 h. The reaction was quenched with a cold saturated aqueous solution of sodium hydrogen carbonate (200 mL) and the reaction mixture was extracted with chloroform (3 x 100 mL). The combined organic phase was washed with saturated aqueous solutions of $NaHCO_3$ (2 x 100 mL), brine (2 x 100 mL) and cold water (2 x 100 mL); and then was dried over anhydrous Na_2SO_4. 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 nucleosides **9a-d** in 88-96 % yields.

2',5'-Di-*O*-acetyl-3'-azido-3'-deoxy-4'-*C*-*p*-toluenesulphonyloxymethyl-*xylo*thymidine (**9a**). It was obtained as white solid (1.04 g, 92 % yield). R_f = 0.4 (10 % methanol in chloroform); M. Pt.: 88-90 °C; $[\alpha]_D^{31}$ = - 69.64 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3370, 2920, 2118, 1696, 1369, 1224, 1054, 998 and 814 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 9.03 (1H, brs), 7.82 (2H, d, *J* = 8.4 Hz), 7.40 (2H, d, *J* = 8.4 Hz), 7.28 (1H, s), 6.11 (1H, d, *J* = 6.0 Hz), 5.29 (1H, t, *J* = 6.4 Hz), 4.53 (1H, d, *J* = 12.4 Hz), 4.37 (1H, d, *J* = 6.4 Hz), 4.12 (2H, s), 4.00 (1H, d, *J* = 12.0 Hz), 2.47 (3H, s), 2.14 (3H, s), 2.09 (3H, s) and 1.95 (3H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 170.07, 169.69, 163.34, 150.49, 145.94, 134.40, 131.92, 130.30, 128.18, 112.46, 85.24, 82.64, 78.40, 68.15, 65.52, 63.02, 21.84, 20.86, 20.65 and 12.81; HR-ESI-TOF-MS: *m/z* 552.1407 ($[M+H]^+$), calcd. for $[C_{22}H_{25}N_5O_{10}S+H]^+$ 552.1395.

2',5'-Di-*O*-acetyl-3'-azido-3'-deoxy-4'-*C*-*p*-toluenesulphonyloxymethyl-*xylo*uridine (**9b**). It was obtained as white solid (1.06 g, 96 % yield). R_f = 0.4 (10 % methanol in chloroform); M. Pt.: 86-87 °C; $[\alpha]_D^{31}$ = - 60.53 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3370, 2920, 2118, 1696, 1369, 1224, 1177, 1054, 998 and 814 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.43 (1H, brs), 7.81 (2H, d, *J* = 8.4 Hz), 7.61 (1H, d, *J* = 8.4 Hz), 7.47 (2H, d, *J* = 8.0 Hz), 6.00 (1H, d, *J* = 6.8 Hz), 5.73 (1H, d, *J* = 8.4 Hz), 5.33 (1H, t, *J* = 7.6 Hz), 4.64 (1H, d, *J* = 7.6 Hz), 4.31 (2H, d, *J* = 2.4 Hz), 4.24 (1H, d, *J* = 12.0 Hz), 4.07 (1H, d, *J* = 12.0 Hz), 2.40 (3H, s), 2.04 (3H, s) and 1.98 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 169.78, 169.42, 162.86, 150.40, 145.42, 139.87,

131.74, 130.22, 127.86, 102.96, 83.85, 81.38, 76.43, 69.58, 64.25, 62.55, 21.16, 20.54 and 20.39; HR-ESI-TOF-MS: *m/z* 538.1253 ($[M+H]^+$), calcd. for $[C_{21}H_{23}N_5O_{10}S+H]^+$ 538.1238.

2',5'-Di-*O*-acetyl-3'-azido-3'-deoxy-4'-*C*-*p*-toluenesulphonyloxymethyl-*xylo*cytidine (**9c**). It was obtained as white solid (1.0 g, 91 % yield). R_f = 0.6 (10 % methanol in chloroform); M. Pt.: 90-91 °C; $[\alpha]_D^{31}$ = - 48.86 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3334, 2923, 2118, 1749, 1651, 1493, 1369, 1224, 1191, 1048, 909 and 789 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.80 (2H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 7.6 Hz), 7.38 (2H, d, *J* = 8.4 Hz), 6.99 (1H, brs), 6.16 (1H, d, *J* = 5.6 Hz), 5.80 (1H, d, *J* = 7.6 Hz), 5.60 (1H, brs), 5.27 (1H, t, *J* = 5.2 Hz), 4.52 (1H, d, *J* = 12.0 Hz), 4.29 (1H, d, *J* = 3.6 Hz), 4.11 (2H, s), 4.03 (1H, d, *J* = 12.4 Hz), 2.46 (3H, s), 2.12 (3H, s) and 2.03 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 169.86, 165.41, 155.39, 145.75, 140.35, 131.82, 130.16, 128.04, 95.46, 87.05, 83.33, 79.54, 67.59, 65.69, 62.64, 21.70, 20.72 and 20.65; HR-ESI-TOF-MS: *m/z* 537.1415 ($[M+H]^+$), calcd. for $[C_{21}H_{24}N_6O_9S+H]^+$ 537.1398.

2',5'-Di-*O*-acetyl-3'-azido-3'-deoxy-4'-*C*-*p*-toluenesulphonyloxymethyl-*xylo*-6-*N*-benzoyladenosine (**9d**). It was obtained as yellow solid (1.2 g, 88 % yield). R_f = 0.5 (10 % methanol in chloroform); M. Pt.: 70-72 °C; $[\alpha]_D^{31}$ = - 55.76 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3437, 2924, 2117, 1754, 1692, 1452, 1369, 1226, 1177, 1054, 996 and 815 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.27 (1H, brs), 8.76 (1H, s), 8.64 (1H, s), 8.03 (2H, d, *J* = 7.6 Hz), 7.85 (2H, d, *J* = 7.6 Hz), 7.64 (1H, t, *J* = 7.6 Hz), 7.54 (2H, d, *J* = 7.6 Hz), 7.47 (2H, d, *J* = 8.4 Hz), 6.30 (1H, d, *J* = 6.8 Hz), 6.16 (1H, t, *J* = 6.8 Hz), 4.82 (1H, d, *J* = 8.4 Hz), 4.38 - 4.35 (3H, m), 4.22 (1H, d, *J* = 11.2 Hz), 2.38 (3H, s), 2.02 (3H, s) and 1.93 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 169.58, 165.66, 152.11, 151.99, 150.63, 145.50, 142.99, 133.24, 132.62, 131.69, 130.30, 128.56, 127.92, 125.60, 83.67, 81.86, 76.18, 69.46, 64.88, 62.48, 21.14, 20.44 and 20.40; HR-ESI-TOF-MS: *m/z* 665.1771 ($[M+H]^+$), calcd. for $[C_{29}H_{28}N_8O_9S+H]^+$ 665.1773.

General procedure for the synthesis of 3′-azido-3′-deoxy-*xylo*bicyclonucleosides **1a-d**. To a stirred solution of diacetylated nucleosides 9a-d (1.5 mmol) in dioxane : water (1:1, 4 mL) was added 2M NaOH (4 mL) (+ 25 % NH_4OH, 2 mL for **9d** only) and the reaction mixture was stirred at RT for 2-10 h. On completion, the reaction mixture was neutralized with acetic acid and the solvent was removed under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford 3′-azido-3′-deoxy-*xylo*bicyclonucleosides **1a-d** in 90-98 % yields.

3'-Azido-3′-deoxy-2′-*O*,4'-*C*-methylene-*xylo*thymidine (**1a**). It was obtained as white solid (0.425 g, 96 % yield). R_f = 0.2 (10 % methanol in chloroform); M. Pt.: 122-124 °C; $[\alpha]_D^{31}$ = + 42.86 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3365, 3147, 2129, 1678, 1430, 1264 and 1107 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.37 (1H, brs), 7.55 (1H, s), 5.44 (1H, s), 5.26 (1H, t, *J* = 4.4 Hz), 4.64 (1H, d, *J* = 1.2 Hz), 4.36 (1H, d, *J* = 1.6 Hz), 3.99-3.76 (4H, m) and 1.78 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 164.48, 150.63, 136.18, 107.66, 89.87, 88.48, 77.97, 73.61, 62.74, 57.09 and 12.84; HR-ESI-TOF-MS: *m/z* 296.0991 ($[M+H]^+$), calcd. for $[C_{11}H_{13}N_5O_5+H]^+$ 296.0989.

3′-Azido-3′-deoxy-2′-*O*,4′-*C*-methylene-*xylo*uridine (**1b**). It was obtained as white solid (0.413 g; 98 % yield). R_f = 0.2 (10 % methanol in chloroform); M. Pt.: 248-249 °C; $[\alpha]_D^{32}$ = + 76.38 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3476, 3217, 2116, 1673, 1465, 1260, 1098 and 825 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.37 (1H, brs), 7.70 (1H, d, *J* = 7.6 Hz), 5.57 (1H, d, *J* = 7.6 Hz), 5.46 (1H, s), 5.29 (1H, brs), 4.64 (1H, s), 4.38 (1H, d, *J* = 1.6 Hz) and 3.99-3.76 (4H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 163.41, 150.20, 140.26, 99.69, 89.44, 88.03, 77.41, 73.11, 62.26 and 56.58; HR-ESI-TOF-MS: *m/z* 282.0837 ($[M+H]^+$), calcd. for $[C_{10}H_{11}N_5O_5+H]^+$ 282.0833.

3′-Azido-3′-deoxy-2′-*O*,4′-*C*-methylene-*xylo*cytidine (**1c**). It was obtained as white solid (0.391 g; 93 % yield). R_f = 0.3 (15 % methanol in chloroform); M. Pt.: 314-316 °C; $[\alpha]_D^{32}$ = + 24.16 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3372, 2126, 1666, 1523, 1486, 1284, 1191, 1049 and 779; ^1H NMR (DMSO-d_6, 400 MHz): δ 7.67 (1H, d, *J* = 8.0 Hz), 7.46 (1H, d, *J* = 8.4 Hz), 7.18-7.09 (2H, m), 5.69 (1H, d, *J* = 5.2 Hz), 5.43 (1H, s), 5.27 (1H, t, *J* = 6.0 Hz), 4.58 (1H, s), 4.32 (1H, d, *J* = 2.4 Hz) and 3.98-3.77 (3H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 165.94, 155.08, 140.87, 92.55, 89.11, 88.69, 77.49, 73.10, 62.36 and 56.71; HR-ESI-TOF-MS: *m/z* 281.0995 ($[M+H]^+$), calcd. for $[C_{10}H_{12}N_6O_4+H]^+$ 281.0993.

3′-Azido-3′-deoxy-2′-*O*,4′-*C*-methylene-*xylo*adenosine (**1d**). It was obtained as yellow solid (0.410 g; 90 % yield). R_f = 0.4 (15 % methanol in chloroform); M. Pt.: 210-212 °C; $[\alpha]_D^{32}$ = + 52.56 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3373, 2126, 1666, 1486, 1283, 1191 and 1049 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 8.15 (1H, s), 8.13 (1H, s), 7.29 (2H, brs), 5.98 (1H, s), 5.31 (1H, brs), 5.01 (1H, d, *J* = 2.4 Hz), 4.45 (1H, d, *J* = 2.4 Hz), 4.05 (1H, d, *J* = 8.4 Hz) and 3.95-3.81 (3H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 155.90, 152.66, 148.80, 138.57, 118.64, 88.77, 86.79, 77.72, 73.27, 69.80 and 62.72; HR-ESI-TOF-MS: *m/z* 305.1113 ($[M+H]^+$), calcd. for $[C_{11}H_{12}N_8O_3+H]^+$ 305.1105.

3'-Amino-3′-deoxy-2′-*O*,4'-*C*-methylene-*xylo*thymidine (**2**). The selective reduction of azido group of 3'-azido-3′-deoxy-2′-*O*,4'-*C*-methylene-*xylo*thymidine (**1a**, 1.0g, 3.39 mmol) was achieved by its stirring with 10 % Pd-C (0.1g) in ethyl acetate (30 mL) under hydrogen atmosphere at 30 °C. The progress of the reaction was monitored by analytical TLC. On completion after 2 h, the reaction was quenched by filtering off the Pd-C through celite, the solvent was removed under reduced pressure and the residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as eluent to afforded the aminonucleoside **2** as white solid (0.90 g; 99 % yield). R_f = 0.3 (10 % methanol in chloroform); M. Pt.: 218-220 °C; $[\alpha]_D^{32}$ = - 14.56 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3459, 1702, 1447, 1341, 1268, 1052, 942, 896 and 705 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.21 (1H, brs), 7.79 (1H, s), 5.37 (1H, s), 5.02 (1H, brs), 4.26 (1H, d, *J* = 2.4 Hz), 3.87-3.78 (4H, m), 3.68 (1H, d, *J* = 8.4 Hz), 3.31 (2H, d, *J* = 2.4 Hz) and 1.73 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 164.21, 150.24, 137.91, 105.58, 90.18, 88.65, 79.52, 73.50, 57.23, 56.56 and 12.43; HR-ESI-TOF-MS: *m/z* 270.1095 ($[M+H]^+$), calcd. for $[C_{11}H_{15}N_3O_5+H]^+$ 270.1084.

X-ray diffraction study on 5-*O*-acetyl-3-azido-3-deoxy-4-*C*-p-toluenesulphonyloxymethyl-1,2-*O*-isopropylidene-*α*-D-*xylo*furanose (7) and 3′-azido-3′-deoxy-2′-*O*,4′-*C*-methylene-*xylo*uridine (1b).

Single crystal suitable for X-ray diffraction study was grown by dissolving tosylated sugar derivative **7** and 3′-azido-3′-deoxy-2′-*O*,4′-*C*-methylene-*xylo*uridine (**1b**) in acetone and acetonitrile, respectively and allowing slow evaporation of the solution at room temperature. The X-ray diffraction data were collected with graphite monochromated Mo Kα radiation (λ = 0.71073 Å) at temperature 293 K. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squqres method on F2 (SHELXL-97).[14] All calculations were carried out using the WinGX package of the crystallographic programs.[15] For the molecular graphics, the program DIAMOND-2[16] and Mercury[17] were used. Molecular structure has been drawn using ORTEP as software as given in Figures 4 and 5. The selected bond lengths, bond angles, etc. are given in Table 1.

Table 1. Single crystal X-ray diffraction data of tosylated sugar derivative **7** and 3′-azido-3′-deoxy-2′-*O*,4′-*C*-methylene-*xylo*uridine (**1b**)

	Compound 7	**Compound 1b**
Empirical formula	$C_{18}H_{23}N_3O_8S$	$C_{10}H_{11}N_5O_5$
Formula weight	441.45	281.24
Temperature	293(2) K	293(2) K
Crystal system	Monoclinic	Monoclinic
Space group	P 2_1	P 2_1
Unit cell dimensions	a = 9.9041 (10) Å	a = 6.5121(16) Å
	b = 10.0867(9) Å β = 94.617(8)°	b = 6.8206(14) Å β = 93.72(3)°
	c = 10.6948(9) Å	c = 13.045(4) Å
Volume	1064.94(17) Å^3	578.2(3) Å^3
Z	2	2
Density	1.377 mg/m^3	1.615 mg/m^3
Absorption coefficient	0.201 mm^{-1}	0.132 mm^{-1}
F(000)	464	292
Index ranges	-12<=h<=12, -13<=k<=13, -14<=l<=14	-6<=h<=7, -7<=k<=8, -15<=l<=15
R(int)	0.0307	0.0518
GOF on F2	0.839	0.911
I>2sigma(I)	wR2 = 0.1339	wR2 = 0.1776
R indices	R1 = 0.0711	R1 = 0.0867
All data	wR2 = 0.1647	wR2 = 0.2016
CCDC	**1414989**	**1420474**

Conclusions

A convenient convergent methodology has been developed for the synthesis of 3'-azido-3′-deoxy-2′-*O*,4'-*C*-methylene-*xylo*bicyclonucleosides T, U, C and A and 3'-amino-3′-deoxy-2′-*O*,4'-*C*-methylene-*xylo*thymidine (**2**). The key step in the developed multi-step synthetic methodology is the Lipozyme® TL IM-mediated diastereoselective deacetylation of 4-*C*-acetoxymethyl over 5-*O*-acetyl group in 4-*C*-acetoxymethyl-5-*O*-acetyl-3-azido-3'-deoxy-1,2-*O*-isopropylidene-*α*-D-*xylo*furanose. Due to the presence of an extra ring between *C*-2' and *C*-4' in the synthesized 3'-azido/3'-amino-*xylo*bicyclonucleosides, the puckering of sugar moiety is locked in *N*-type confirmation, which enables these nucleosides to become a potential monomer for antisense oligonucleotide development

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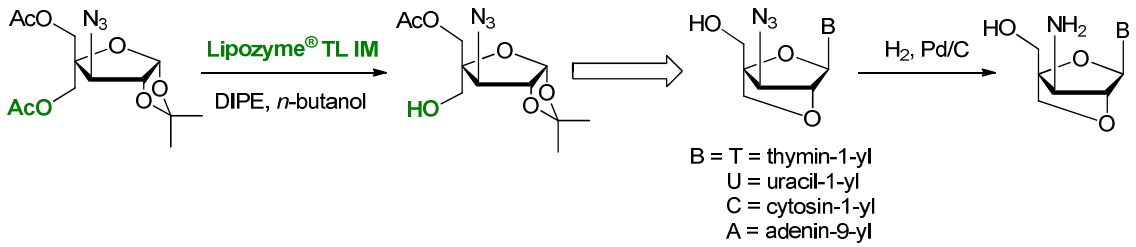

Lipozyme® TL IM - mediates selective deacetylation of one of the two diastereotopic acetoxy groups in 4-*C*-acetoxymethyl-5-*O*-acetyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*furanose that led to the first efficient synthesis of 3'-azido/3'-amino-*xylo*bicyclonucleosides T, U, C and A in 30 to 35 % overall yields from diacetone-D-glucose. The single crystal X-ray study on the compound obtained by the tosylation of lipase-mediated monodeacetylated sugar unambiguously confirmed the point of diastereoselection on diacetoxy-sugar derivative. The bicyclic nucleosides synthesized by environmentally benign enzymatic methodology have potential application in antisense/aptamer- based oligonucleotide therapeutics development.