Biocatalysis and Biotransformation

ISSN: 1024-2422 (Print) 1029-2446 (Online) Journal homepage: http://www.tandfonline.com/loi/ibab20

Biocatalytic route to C-4'-spiro-oxetano-xylofuranosyl pyrimidine nucleosides

Pallavi Rungta, Priyanka Mangla, Vinod Khatri, Jyotirmoy Maity & Ashok K. Prasad

To cite this article: Pallavi Rungta, Priyanka Mangla, Vinod Khatri, Jyotirmoy Maity & Ashok K. Prasad (2018): Biocatalytic route to C-4′-spiro-oxetano-xylofuranosyl pyrimidine nucleosides, Biocatalysis and Biotransformation, DOI: 10.1080/10242422.2018.1438416

To link to this article: https://doi.org/10.1080/10242422.2018.1438416

BIOCATALYSIS AND BIOTRANSFORMATION, 2018
https://doi.org/10.1080/10242422.2018.1438416

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Biocatalytic route to *C*-4′-spiro-oxetano-xylofuranosyl pyrimidine nucleosides

Pallavi Rungta, Priyanka Mangla, Vinod Khatri, Jyotirmoy Maity and Ashok K. Prasad

Department of Chemistry, Bioorganic Laboratory, University of Delhi, Delhi, India

ABSTRACT

A facile access to *C*-4′-spiro-oxetano-xylofuranosyl nucleosides has been demonstrated for the first time through Lipozyme® TL IM-mediated regioselective acetylation of one of the primary hydroxyl group over the other primary and secondary hydroxyl groups in 3′-*O*-benzyl-4′-*C*-hydroxymethyl-β-D-xylofuranosyl nucleosides. Attempts to optimize a convergent route for these spironucleosides via selective manipulation of hydroxyl groups in 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose were unsuccessful. Nevertheless; the present linear biocatalytic route efficiently afforded the *C*-4′-spiro-oxetanoxylofuranosyl nucleosides T and U in 47 and 38% overall yields, respectively, starting from corresponding furanose diol.

ARTICLE HISTORY
Received 10 August 2017
Revised 9 January 2018
Accepted 1 February 2018

KEYWORDS
Biocatalysis; regioselective acetylation; spiro-nucleosides

1. Introduction

Chemical modifications of the sugar functionality in nucleosides have continuously reflected its supremacy for the treatment of cancer and viral infections over the past 50 years (De Clercq 2011; Prakash 2011; Sofia et al. 2012; Jordheim et al. 2013; Sharma et al. 2014a, 2014b). Among the studied sugar-modified nucleosides, spironucleosides had only little impact and progress until the recent finding that these are selective and potent inhibitors of Hepatitis C Virus (HCV) (Dang et al. 2014a, 2014b; Du et al. 2014; Jonckers et al. 2010, 2014). The presence of spiro-carbon in nucleosides imparts restriction to the conformational flipping of furanose ring and thus can modulate or enhance their selectivity and biological activity. This has prompted the synthesis of all possible classes of spironucleosides, e.g. *C*-1′-spiro-(Maza et al. 2009; Pal and Yashwant 2010), *C*-2′-spiro-(Du et al. 2014; Dang et al. 2014b; Jonckers et al. 2010, 2014), *C*-3′-spiro-(Camarasa et al. 1992; De Castro et al. 2007; Das et al. 2011) and *C*-4′-spironucleosides (Paquette et al. 2003; Paquette 2004; Paquette et al. 2004; Dong and Paquette 2005; Dong and Paquette 2006; Roy et al. 2006; Dang et al. 2014a; Sharma et al. 2014c; Kumar et al. 2015; Kumar et al. 2017); and many of them have shown excellent antiviral activity (Figure 1). Prominent among them are the *C*-4′-spironucleosides which display distinct sugar conformation (Paquette 2004). In order to scrutinize the presence of the spiro- and the oxetano-systems in a nucleoside, attempts have been made to install oxetano-spiro cyclization at *C*-4′-position in xylofuranosyl nucleosides ((Sharma et al. 2014a), Figure 1) that have earlier turned out to be futile (Roy et al. 2006). Our group has earlier demonstrated the synthesis of various *C*-4′-spiro-oxetano-ribonucleosides (Sharma et al. 2014c; Kumar et al. 2015, 2017). Kumar et al. optimized Novozyme®-435 mediated regio-selective deacetylation of *C*-5 acetoxy group in 5-*O*-acetyl-4-*C*-acetyloxymethyl-3-azido-1,2-*O*-isopropylidine-α-D-ribofuranose to establish a methodology for successful synthesis of *C*-4′-spiro-oxetano-β-D-ribonucleosides (Kumar et al. 2015). In another venture, Kumar et al. successfully used Lipozyme® TL IM-catalyzed acylation on *C*-4′-hydroxymethyl-β-D-xylofuranosyl nucleosides and synthesized structurally novel *C*-4′-spiro-oxetano-α-L-ribonucleosides (Kumar et al. 2017). In the present communication, we have reported the first synthesis of *C*-4′-spiro-oxetano-xylofuranosyl nucleosides that is devised by the mediation of Lipozyme® TL IM, an immobilised *Thermomyces lanuginosa* lipase.

2. Experimental section

Materials. Analytical TLCs were performed on precoated Merck silica gel $60F_{254}$ plates; the spots were detected either using UV light or by charring with 4% alcoholic sulphuric acid. The optical rotations were measured on Rudolph autopol II automatic polarimeter using light of 546 nm wavelegth. The melting points

CONTACT Ashok K. Prasad ashokenzyme@gmail.com Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi, India
Supplemental data for this article can be accessed here.

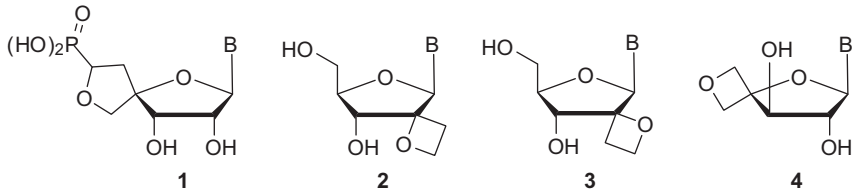


Figure 1. Some of the structures of biologically active spironucleosides **1**-**3** and the targeted *C*-4′-spiro-oxetano-xylofuranosyl nucleoside **4**.

were determined on Buchi M-560 instrument and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrometer by making KBr disc for solid samples and thin film for oils. The ^{1}H-,^{13}C- and 2D NMR spectra were recorded on a Jeol alpha-400 spectrometer 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 Hertz. The HRMS analyses were done on micro TOF-Q instrument from Bruker Daltonics, Bremen. The single crystal diffraction data were collected on an X'Calibur single crystal X-ray diffractor having CCD camera [Mo/Kα radiation ($\lambda = 0.71073$ Å)]. The *T. lanuginosus* lipase immobilized on silica (Lipozyme® TL IM) was obtained as a gift from Novozyme A/S Denmark. Other enzymes *Candida antarctica* lipase-B (Novozyme®-435), *C. rugosa* lipase (CRL) and porcine pancreatic lipase (PPL) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The enzyme was dried over P_2O_5 under vacuum for 24 h prior to use. For all the lipase-mediated reactions AR grade organic solvents were used, which were purchased from SD Fine-Chem Ltd, Mumbai, India.

2.1. General procedure for the synthesis of diacylated xylofuranose 7b-c

To a solution of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**5**) (Youssefyeh et al. 1979) (0.10 g, 0.32 mmol) in CH_2Cl_2 (10 mL), propionic (**6b**) or butyric anhydride (**6c**) (0.71 mmol) and catalytic amount of DMAP (0.03 mmol) were added. The reaction mixture was stirred at 25 °C for 2–4 h and then was poured into ice-water. The mixture was extracted with ethyl acetate (3 × 50 mL), the combined organic extract was washed with saturated aq. bicarbonate solution (2 × 25 mL), water (1 × 25 mL) and dried over anhydrous sodium sulphate. The excess of solvent was evaporated 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 the diacylated compounds **7b**-**c** in quantitative yields.

2.1.1. 3-O-Benzyl-1,2-O-isopropylidene-4-C-propanoyloxymethyl-5-O-propanoyl-α-D-xylofuranose (7b)

It was obtained as a colourless viscous oil (0.14 g) in 97% yield. $R_f = 0.5$ (30% ethyl acetate in petroleum ether); $[\alpha]_D^{28} = -48.23$ (*c* 0.1, $CHCl_3$); IR (thin film) ν_{max} cm^{-1}: 608, 700, 740, 807, 863, 1020, 1072, 1168, 1383, 1423, 1463, 1744, 2943, 2984; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (3H, t, $J = 7.6$ Hz, –OCOCH$_2$C*H*$_3$), 1.12 (3H, t, $J = 7.6$ Hz, –OCOCH$_2$C*H*$_3$), 1.36 and 1.55 (6H, 2s, 3H each, –C(C*H*$_3$)$_2$), 2.24 (2H, q, $J = 7.6$ Hz, –OCOC*H*$_2$CH$_3$), 2.33 (2H, q, $J = 7.6$ Hz, –OCOC*H*$_2$CH$_3$), 4.04 (1H, s, C-3H), 4.14 (1H, d, $J = 11.6$ Hz, C-1′H$_a$), 4.22 (2H, s, –OC*H*$_2$Ph), 4.38 (1H, d, $J = 11.6$ Hz, C-1′H$_b$), 4.51 (1H, d, $J = 12.4$ Hz, C-5H$_a$), 4.72–4.75 (2H, m, C-2H & C-5H$_b$), 5.97 (1H, d, $J = 4.8$ Hz, C-1H), 7.29–7.37 (5H, m, Ar*H*); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 8.88 and 9.02 (2 X –OCOCH$_2$C*H*$_3$), 26.68 and 27.09 (–C(C*H*$_3$)$_2$), 27.22 and 27.50 (2 X –OCOC*H*$_2$CH$_3$), 63.08 (C-1′), 63.35 (C-5), 72.24 (–OC*H*$_2$Ph), 83.63 (C-3), 85.37 (C-2), 85.89 (C-4), 105.20 (C-1), 113.42 (–*C*(CH$_3$)$_2$), 127.69, 128.02, 128.46 and 136.84 (Ar*C*), 173.72 and 173.82 (2 X –O*C*OC$_2$H$_5$); HR-ESI-TOF-MS: *m/z* 440.2279 ([M + NH$_4$]$^+$), calcd. for [C$_{22}$H$_{30}$O$_8$+NH$_4$]$^+$ 440.2279.

2.1.2. 3-O-Benzyl-4-C-butanoyloxymethyl-5-O-butanoyl-1,2-O-isopropylidene-α-D-xylofuranose (7c)

It was obtained as a colourless viscous oil (0.15 g) in 98% yield. $R_f = 0.5$ (20% ethyl acetate in petroleum ether); $[\alpha]_D^{27} = -18.74$ (*c* 0.05, MeOH); IR (thin film) ν_{max} cm^{-1}: 700, 744, 1016, 1075, 1167, 1379, 1458, 1739, 2876, 2966; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88–0.94 (6H, m, 2 X –OCOCH$_2$CH$_2$C*H*$_3$), 1.36 (3H, s, –C(C*H*$_3$)$_2$), 1.54–1.65 (7H, m, 2 X –OCOCH$_2$C*H*$_2$CH$_3$ and –C(C*H*$_3$)$_2$), 2.19 (2H, t, $J = 7.6$ Hz, –OCOC*H*$_2$CH$_2$CH$_3$), 2.29 (2H, t, $J = 7.6$ Hz, –OCOC*H*$_2$CH$_2$CH$_3$), 4.03 (1H, d, $J = 1.6$ Hz, C-3H), 4.14 (1H, d, $J = 12.0$ Hz, C-1′H$_a$), 4.21 (2H, s, –OC*H*$_2$Ph), 4.38 (1H, d, $J = 11.6$ Hz, C-1′H$_b$), 4.51 (1H, d, $J = 11.6$ Hz, C-5H$_a$), 4.72-4.75 (2H, m, C-5H$_b$ & C-2H), 5.98 (1H, d, $J = 4.8$ Hz, C-1H), 7.29-7.35 (5H, m, Ar*H*); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 13.60 (–OCOCH$_2$CH$_2$C*H*$_3$), 18.21 and 18.34 (2 X –OCOCH$_2$C*H*$_2$CH$_3$), 26.68 and 27.09 (–C(C*H*$_3$)$_2$), 35.83 and 36.11 (2 X –OCOC*H*$_2$CH$_2$CH$_3$), 63.04 (C-1′), 63.35 (C-5), 72.27 (–OC*H*$_2$Ph), 83.74 (C-3),

85.36 (C-2), 85.88 (C-4), 105.22 (C-1), 113.40 (-*C*$(CH_3)_2$), 127.69, 128.02, 128.46 and 136.85 (Ar*C*), 172.90 and 173.01 (2 X –O*C*OC$_3$H$_7$); HR-ESI-TOF-MS: *m/z* 468.2586 ([M + NH$_4$]$^+$), calcd. for [C$_{24}$H$_{34}$O$_8$+NH$_4$]$^+$ 468.2592.

2.2. 4-C-Acetoxymethyl-1,2,5-tri-O-acetyl-3-O-benzyl-α,β-D-xylofuranose (9a-9b)

Acetic anhydride (4.8 mL, 50.7 mmol) and concentrated sulphuric acid (0.02 mL, 0.50 mmol) were added to a stirred solution of compound **7a** (Youssefyeh et al. 1979) (2.0 g, 5.07 mmol) in acetic acid (28.9 mL, 507 mmol) at 0 °C and the resulting reaction mixture was stirred for 6 h at 25 °C. The reaction was quenched by the addition of cold water (200 mL) and stirring at 25 °C for 30 min. The crude compound was extracted from the reaction mixture with ethyl acetate (3 × 200 mL). The organic layer was washed with saturated aq. sodium bicarbonate solution (3 × 100 mL), brine solution (2 × 100 mL) and then dried over anhydrous sodium sulphate. The excess of 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 an anomeric mixture (α:β = ca. ~ 1:2, based on comparison of integration of anomeric proton) of **9a-9b** (2.16 g) in 97% yield as colourless viscous oil. R_f = 0.4 (20% ethyl acetate in petroleum ether); $[\alpha]_D^{27} = -37.08$ (*c* 0.1, MeOH); IR (thin film) ν_{max} cm^{-1}: 700, 745, 1011, 1216, 1371, 1741, 2937; ^{1}H NMR (CDCl$_3$, 400 MHz): δ 6.17 (s, C-1H$_\beta$), 6.37 (d, *J* = 4.8 Hz, C-1H$_\alpha$); ^{13}C NMR (CDCl$_3$, 100.6 MHz): δ 20.75, 20.94, 21.03, 21.14, 21.18, 21.21, 21.32, 21.42, 63.39, 63.54, 63.71, 65.60, 72.59, 73.64, 76.74, 77.54, 80.75, 81.45, 82.26, 83.11, 87.14, 92.56, 99.67, 127.84, 128.20, 128.37, 128.42, 128.77, 128.81, 137.26, 137.52, 169.49, 169.87, 169.93, 170.50, 170.58, 170.66, 170.81; HR-ESI-TOF-MS: *m/z* 456.1874 ([M + NH$_4$]$^+$), calcd. for [C$_{21}$H$_{26}$O$_{10}$+NH$_4$]$^+$ 456.1864.

2.3. General procedure for the synthesis of 4′-C-Acetoxymethyl-2′,5′-di-O-acetyl-3′-O-benzyl-β-D-xylofuranosyl nucleosides 10a-b

To the stirred solution of tetra-*O*-acetylated sugar derivative **9a-9b** (1.0 g, 2.28 mmol) and thymine/uracil (3.42 mmol) in anhydrous acetonitrile (40 mL), *N,O*-bis(-trimethylsilyl)acetamide (2.2 mL, 9.12 mmol) was added dropwise. The reaction mixture was stirred at reflux for 1 h, and then cooled to 0 °C. In the cooled reaction mixture trimethylsilyltrifluoromethane sulfonate (0.702 mL, 3.88 mmol) was added dropwise under stirring and the solution was heated at 70–80 °C for 4 h. The reaction was quenched with cold saturated aq. solution of sodium bicarbonate (100 mL) and extraction of the compound was performed with dichloromethane (3 × 100 mL). The combined organic phase was washed with saturated aq. sodium bicarbonate solution (2 × 100 mL), brine solution (2 × 100 mL) and was dried over anhydrous sodium sulphate. The excess of 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 afford nucleosides **10a-b** in 90 and 88% yields, respectively.

2.3.1. 4′-C-Acetoxymethyl-2′,5′-di-O-acetyl-3′-O-benzyl-β-D-xylofuranosyl thymine (10a)

It was obtained as sticky solid (1.03 g) in 90% yield. R_f = 0.3 (2% MeOH in CHCl$_3$); $[\alpha]_D^{31} = -16.79$ (*c* 0.1, MeOH); IR (thin film) ν_{max} cm^{-1}: 700, 749, 1041, 1214, 1369, 1455, 1687, 1736, 3027, 3092; ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.83 (3H, s, C-5C*H*$_3$), 2.02, 2.09 and 2.14 (9H, 3s, 3H each, –OCOC*H*$_3$), 4.04 (1H, d, *J* = 2.4 Hz, C-3′H), 4.12–4.23 (3H, m, C-5′H$_a$ and C-1″H$_2$), 4.57 (1H, d, *J* = 12.0 Hz, C-5′H$_b$), 4.61 (1H, d, *J* = 12.0 Hz, –OC*H*$_a$Ph), 4.76 (1H, d, *J* = 12.0 Hz, –OC*H*$_b$Ph), 5.28 (1H, t, *J* = 2.8 Hz, C-2′H), 6.25 (1H, d, *J* = 4.0 Hz, C-1′H), 7.28–7.37 (5H, m, Ar*H*), 7.45 (1H, d, *J* = 1.6 Hz, C-6H), 8.55 (1H, brs, NH); ^{13}C NMR (CDCl$_3$, 100.6 MHz): δ 12.50 (C-5C*H*$_3$), 20.66, 20.79 and 20.85 (3 X -OCO*C*H$_3$), 61.92 (C-1″), 62.31 (C-5′), 72.58 (–OCH$_2$Ph), 80.19 (C-2′), 81.00 (C-3′), 85.62 (C-4′), 87.17 (C-1′), 111.93 (C-5), 128.21, 128.50, 128.66 and 135.19 (Ar*C*), 136.21 (C-6), 150.20 (C-2), 163.23 (C-4), 169.90, 170.13 and 170.20 (3 X –O*C*OCH$_3$); HR-ESI-TOF-MS: *m/z* 505.1824 ([M + H]$^+$), calcd. for [C$_{24}$H$_{28}$N$_2$O$_{10}$+H]$^+$ 505.1817.

2.3.2. 4′-C-Acetoxymethyl-2′,5′-di-O-acetyl-3′-O-benzyl-β-D-xylofuranosyl uracil (10b)

It was obtained as sticky solid (0.98 g) in 88% yield. R_f = 0.3 (2% MeOH in CHCl$_3$); $[\alpha]_D^{30} = -7.88$ (*c* 0.1, MeOH); IR (thin film) ν_{max} cm^{-1}: 753, 1043, 1217, 1374, 1685, 1741, 3028, 3066; ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.98, 2.08, 2.15 (9H, 3s, 3H each, –OCOC*H*$_3$), 4.02 (1H, d, *J* = 2.4 Hz, C-3′H), 4.10–4.23 (3H, m, C-5′H$_a$ & C-1″H$_2$), 4.56 (1H, d, *J* = 12.0 Hz, C-5′H$_b$), 4.61 (1H, d, *J* = 12.0 Hz, –OC*H*$_a$Ph), 4.75 (1H, d, *J* = 11.6 Hz, –OC*H*$_b$Ph), 5.27 (1H, t, *J* = 3.2 Hz, C-2′H), 5.76 (1H, d, *J* = 7.6 Hz, C-5H), 6.23 (1H, d, *J* = 4.0 Hz, C-1′H), 7.28–7.37 (5H, m, Ar*H*), 7.65 (1H, d, *J* = 8.4 Hz, C-6H), 9.42 (1H, brs, NH); ^{13}C NMR (CDCl$_3$, 100.6 MHz): δ 20.59, 20.74 and 20.80 (3 X –OCO*C*H$_3$), 61.80 (C-1″), 62.20 (C-5′), 72.42 (–OCH$_2$Ph), 80.28 (C-2′), 80.58 (C-3′), 85.99 (C-4′), 87.56 (C-1′), 103.25 (C-5), 128.35, 128.48, 128.62 and 136.09 (Ar*C*),

139.50 (C-6), 150.29 (C-2), 162.99 (C-4), 169.87, 170.09 and 170.20 (3 X –OCOCH$_3$); HR-ESI-TOF-MS: *m/z* 513.1491 ([M + Na]$^+$), calcd. for [$C_{23}H_{26}N_2O_{10}$+Na]$^+$ 513.1480.

2.4. General procedure for the synthesis of 3′-O-Benzyl-4′-C-hydroxymethyl-β-D-xylofuranosyl nucleosides 11a-b

To a stirred solution of compound **10a-b** (2 mmol) in methanol (10 mL), methanolic NH_3 was added and the reaction mixture was stirred overnight at 25 °C. The reaction mixture was evaporated under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to give trihydroxynucleosides **11a-b** in 96 and 93% yields, respectively.

2.4.1. 3′-O-Benzyl-4′-C-hydroxymethyl-β-D-xylofuranosyl thymine (11a) (Rajwanshi et al. 1999)

It was obtained as sticky white solid (0.72 g) in 96% yield. R_f = 0.4 (10% MeOH in $CHCl_3$); $[\alpha]_D^{31} = -30.16$ (*c* 0.1, MeOH); IR (thin film) ν_{max} cm^{-1}: 770, 914, 1071, 1260, 1460, 1700, 2855, 2923, 3376; ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 1.75 (3H, s, C-5C*H*$_3$), 3.39 (2H, s, C-1′′H$_2$), 3.45 (1H, dd, *J* = 5.2 and 11.2 Hz, C-5′H$_a$), 3.65 (1H, dd, *J* = 4.4 and 11.6 Hz, C-5′H$_b$), 4.13 (1H, d, *J* = 6.4 Hz, C-3′H), 4.37 (1H, q, *J* = 6.4 Hz, C-2′H), 4.62 (1H, d, *J* = 11.6 Hz, –OC*H*$_a$Ph), 4.72 (1H, d, *J* = 12.8 Hz, –OC*H*$_b$Ph), 4.78 (1H, t, *J* = 5.2 Hz, OH), 5.05 (1H, t, *J* = 5.2 Hz, OH), 5.75 (1H, d, *J* = 4.8 Hz, OH), 5.81 (1H, d, *J* = 7.6 Hz, C-1′H), 7.27–7.37 (5H, m, Ar*H*), 7.89 (1H, s, C-6H), 11.30 (1H, s, NH); ^{13}C NMR (DMSO-d$_6$, 100.6 MHz): δ 12.29 (C-5CH$_3$), 61.24 (C-1′′), 62.68 (C-5′), 71.91 (-OCH$_2$Ph), 82.83 (C-2′), 85.20 (C-3′), 85.73 (C-4′), 99.50 (C-1′), 109.53 (C-5), 127.31, 127.39, 128.19 and 136.75 (Ar*C*), 138.56 (C-6), 150.96 (C-2), 163.76 (C-4); HR-ESI-TOF-MS: *m/z* 379.1503 ([M + H]$^+$), calcd. for [$C_{18}H_{22}N_2O_7$+H]$^+$ 379.1500.

2.4.2. 3′-O-Benzyl-4′-C-hydroxymethyl-β-D-xylofuranosyl uracil (11b)

It was obtained as white solid (0.68 g) in 93% yield. R_f = 0.4 (10% MeOH in $CHCl_3$); M. Pt.: 143–145 °C; $[\alpha]_D^{32}$ = -23.49 (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 738, 811, 1024, 1084, 1156, 1373, 1471, 1694, 2872, 3331; ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ 3.34 (2H, d, *J* = 5.6 Hz, C-1′′H$_2$), 3.43 (1H, d, *J* = 6.0 Hz, C-5′H$_a$), 3.65 (1H, dd, *J* = 4.8 and 11.6 Hz, C-5′H$_b$), 4.14 (1H, d, *J* = 7.2 Hz, C-3′H), 4.36 (1H, q, *J* = 6.8 Hz, C-2′H), 4.62 (1H, d, *J* = 16.4 Hz, –OC*H*$_a$Ph), 4.73 (1H, d, *J* = 11.6 Hz, –OC*H*$_b$Ph), 4.79 (1H, t, *J* = 5.2 Hz, OH), 5.05 (1H, t, *J* = 5.2 Hz, OH), 5.69 (1H, d, *J* = 8.4 Hz, C-5H), 5.77 (1H, d, *J* = 5.2 Hz, OH), 5.81 (1H, d, *J* = 7.6 Hz, C-1′H), 7.26–7.36 (5H, m, Ar*H*), 8.07 (1H, d, *J* = 8.0 Hz, C-6H), 11.32 (1H, brs, NH); ^{13}C NMR (DMSO-d$_6$, 100.6 MHz): δ 61.29 (C-1′′), 62.78 (C-5′), 72.02 (-OCH$_2$Ph), 77.44 (C-2′), 82.67 (C-3′), 85.23 (C-4′), 85.80 (C-1′), 102.13 (C-5), 127.33, 127.39, 128.20 and 138.57 (Ar*C*), 141.07 (C-6), 150.92 (C-2), 163.14 (C-4); HR-ESI-TOF-MS: *m/z* 387.1165 ([M + Na]$^+$), calcd. for [$C_{17}H_{20}N_2O_7$+Na]$^+$ 387.1163.

2.5. General procedure for selective biocatalytic monoacetylation: Synthesis of monoacetylated nucleosides 12a-b

To a solution of trihydroxy nucleoside **11a-b** (1.8 mmol) in MeCN (10 mL) was added vinyl acetate (1.98 mmol) followed by the addition of Lipozyme® TL IM (50% w/w). The reaction mixture was stirred at 45 °C in an incubator shaker at 200 rpm. On completion of the reaction after 1 h, the reaction mixture was quenched by filtering off the Lipozyme TL IM; 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 **12a-b** in 90 and 85% yields, respectively.

2.5.1. 4′-C-Acetoxymethyl-3′-O-benzyl-β-D-xylofuranosyl thymine (12a)

It was obtained as white solid (0.68 g) in 90% yield. R_f = 0.5 (8% MeOH in $CHCl_3$); M. Pt.: 86–88 °C; $[\alpha]_D^{31} = -44.84$ (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 699, 751, 917, 1044, 1105, 1234, 1376, 1470, 1683, 2927, 3029, 3064; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.84 (3H, d, *J* = 1.2 Hz, C-5C*H*$_3$), 2.07 (3H, s, –OCOC*H*$_3$), 2.72 (1H, dd, *J* = 5.2 and 9.2 Hz, C-5′OH), 3.72 (1H, dd, *J* = 9.2 and 12.4 Hz, C-5′H$_a$), 3.88 (1H, dd, *J* = 4.4 and 12.4 Hz, C-5′H$_b$), 4.11 (2H, q, *J* = 12.0 Hz, C-1′′H$_2$), 4.20 (1H, d, *J* = 5.2 Hz, C-3′H), 4.57 (1H, dd, *J* = 5.6 and 9.2 Hz, C-2′H), 4.66 (1H, d, *J* = 11.2 Hz) and 4.89 (1H, d, *J* = 12.0 Hz, –OC*H*$_2$Ph), 4.98 (1H, d, *J* = 3.6 Hz, C-2′OH), 5.96 (1H, d, *J* = 5.6 Hz, C-1′H), 7.32–7.36 (5H, m, Ar*H*), 7.72 (1H, d, *J* = 1.6 Hz, C-6H), 10.22 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.50 (C-5CH$_3$), 20.75 (–OCOCH$_3$), 63.36 (C-5′), 64.51 (C-1′′), 72.94 (–OCH$_2$Ph), 80.24 (C-2′), 83.84 (C-3′), 85.79 (C-4′), 88.33 (C-1′), 111.08 (C-5), 127.97, 128.30 and 128.63 (Ar*C*), 136.19 (C-6), 136.90 (Ar*C*), 151.62 (C-2), 164.30 (C-4), 170.69 (–OCOCH$_3$); HR-ESI-TOF-MS: *m/z* 421.1612 ([M + H]$^+$), calcd. for [$C_{20}H_{24}N_2O_8$+H]$^+$ 421.1605.

2.5.2. 4′-C-Acetoxymethyl-3′-O-benzyl-β-D-xylofuranosyl uracil (12b)

It was obtained as white solid (0.62 g) in 85% yield. $R_f = 0.5$ (8% MeOH in $CHCl_3$); M. Pt.: 82–83 °C; $[\alpha]_D^{30} = -32.41$ (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 699, 742, 841, 1040, 1066, 1260, 1385, 1458, 1687, 2947, 3037, 3408; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.05 (3H, s, –OCOC*H*$_3$), 2.75 (1H, dd, *J* = 4.8 and 8.4 Hz, C-5′OH), 3.66–3.71 (1H, m, C-5′H$_b$), 3.88 (1H, dd, *J* = 4.0 and 12.6 Hz, C-5′H$_a$), 4.09 (2H, dd, *J* = 12.0 and 22.2 Hz, C-1″H$_2$), 4.19 (1H, d, *J* = 6.0 Hz, C-3′H), 4.58 (1H, dd, *J* = 5.6 and 8.4 Hz, C-2′H), 4.64 (1H, d, *J* = 11.6 Hz) and 4.88 (1H, d, *J* = 12.0 Hz, –OC*H*$_2$Ph), 4.96 (1H, d, *J* = 2.8 Hz, C-2′OH), 5.71 (1H, d, *J* = 8.0 Hz, C-5H), 5.94 (1H, d, *J* = 5.6 Hz, C-1′H), 7.31–7.35 (5H, m, Ar*H*), 7.95 (1H, d, *J* = 8.0 Hz, C-6H), 10.37 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.72 (–OCOCH_3), 63.38 (C-5′), 64.55 (C-1″), 73.00 (–OCH_2Ph), 80.51 (C-2′), 83.84 (C-3′), 86.16 (C-4′), 88.82 (C-1′), 102.59 (C-5), 128.02, 128.33, 128.64 and 136.86 (Ar*C*), 140.61 (C-6), 151.51 (C-2), 163.95 (C-4), 170.63 (–OCOCH$_3$); HR-ESI-TOF-MS: *m/z* 407.1454 ($[M+H]^+$), calcd. for $[C_{19}H_{22}N_2O_8+H]^+$ 407.1449.

2.6. General procedure for the synthesis of 4′-C-Acetoxymethyl-3′-O-benzyl-5′-O-methanesulfonyl-β-D-xylofuranosyl nucleosides 13a-b

A solution of **12a-b** (1.5 mmol) and methanesulfonyl chloride (1.65 mmol) in anhydrous dichloromethane: pyridine (10 mL, 4:1) was stirred at 25 °C for 4 h. On completion, the reaction mixture was poured over 10% ice-cold hydrochloric acid solution (100 mL) and extracted with chloroform (3 × 100 mL). The combined organic extract was washed with saturated aq. sodium bicarbonate solution (2 × 100 mL) and dried over anhydrous sodium sulphate, and the excess of 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 the nucleosides **13a-b** in 92 and 90% yields, respectively.

2.6.1. 4′-C-Acetoxymethyl-3′-O-benzyl-5′-O-methanesulfonyl-β-D-xylofuranosyl thymine (13a)

It was obtained as white solid (0.69 g) in 92% yield. $R_f = 0.7$ (10% MeOH in $CHCl_3$); M. Pt.: 79–81 °C; $[\alpha]_D^{32} = +10.41$ (*c* 0.05, MeOH); IR (KBr) ν_{max} cm^{-1}: 753, 1047, 1176, 1230, 1357, 1709, 2363, 3451; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.81 (3H, s, C-5C*H*$_3$), 2.07 (3H, s, –OCOC*H*$_3$), 2.95 (3H, s, –OSO$_2$C*H*$_3$), 4.18–4.24 (3H, m, C-1″H$_2$ & C-3′H), 4.37 (1H, d, *J* = 10.8 Hz, C-5′H$_a$), 4.48 (1H, d, *J* = 10.4 Hz, C-5′H$_b$), 4.58–4.65 (2H, m, –OC*H*$_a$Ph & C-2′), 4.87 (1H, d, *J* = 11.6 Hz, –OC*H*$_b$Ph), 5.35 (1H, brs, C-2′OH), 6.07 (1H, d, *J* = 5.6 Hz, C-1′H), 7.29–7.36 (5H, m, Ar*H*), 7.56 (1H, d, *J* = 1.2 Hz, C-6H), 10.63 (1H, brs, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.16 (C-5*C*H$_3$), 20.68 (–OCOCH_3), 37.47 (–OSO$_2$CH_3), 63.48 (C-5′), 68.09 (C-1″), 72.83 (–OCH_2Ph), 79.07 (C-2′), 82.77 (C-3′), 83.91 (C-4′), 88.76 (C-1′), 111.64 (C-5), 128.16, 128.34, 128.60 and 135.87 (Ar*C*), 136.80 (C-6), 151.66 (C-2), 164.24 (C-4), 170.23 (–OCOCH$_3$); HR-ESI-TOF-MS: *m/z* 499.1390 ($[M+H]^+$), calcd. for $[C_{21}H_{26}N_2O_{10}S+H]^+$ 499.1381.

2.6.2. 4′-C-Acetoxymethyl-3′-O-benzyl-5′-O-methanesulfonyl-β-D-xylofuranosyl uracil (13b)

It was obtained as white solid (0.65 g) in 90% yield. $R_f = 0.7$ (10% MeOH in $CHCl_3$); M. Pt.: 69–70 °C; $[\alpha]_D^{32} = -17.98$ (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 752, 817, 965, 1045, 1174, 1235, 1355, 1683, 2938, 3029; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.76 (1H, brs, C-2′OH), 2.06 (3H, s, –OCOC*H*$_3$), 2.95 (3H, s, –OSO$_2$C*H*$_3$), 4.15 (1H, d, *J* = 4.0 Hz, C-3′H), 4.21 (1H, d, *J* = 12.0 Hz, C-1″H$_a$), 4.30 (1H, d, *J* = 11.6 Hz, C-1″H$_b$), 4.37 (1H, d, *J* = 10.4 Hz, C-5′H$_a$), 4.48 (1H, d, *J* = 10.4 Hz, C-5′H$_b$), 4.54 (1H, t, *J* = 4.4 Hz, C-2′H), 4.60 (1H, d, *J* = 11.6 Hz, –OC*H*$_a$Ph), 4.82 (1H, d, *J* = 10.8 Hz, –OC*H*$_b$Ph), 5.70 (1H, d, *J* = 8.4 Hz, C-5H), 5.96 (1H, d, *J* = 4.4 Hz, C-1′H), 7.30–7.36 (5H, m, Ar*H*), 7.68 (1H, d, *J* = 8.0 Hz, C-6H), 10.18 (1H, brs, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.67 (–OCOCH_3), 37.54 (–OSO$_2$CH_3), 63.09 (C-5′), 67.57 (C-1″), 72.86 (–OCH_2Ph), 79.75 (C-2′), 83.06 (C-3′), 85.00 (C-4′), 90.42 (C-1′), 102.78 (C-5), 128.23, 128.43, 128.65 and 136.63 (Ar*C*), 139.92 (C-6), 151.37 (C-2), 163.63 (C-4), 170.17 (–OCOCH$_3$); HR-ESI-TOF-MS: *m/z* 507.1049 ($[M+Na]^+$), calcd. for $[C_{20}H_{24}N_2O_{10}S+Na]^+$ 507.1044.

2.7. General procedure for the synthesis of 3′-O-Benzyl-C-4'-spiro-xylofuranosyl nucleosides 14a-b

To a stirred solution of compound **13a-b** (1.0 mmol) in dioxane:water (4 mL, 1:1), 2M NaOH (1.5 mL) was added and the reaction mixture was stirred at 25 °C for 48 h. The reaction mixture was neutralized with acetic acid 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 give **14a-b** in 76 and 70% yields, respectively.

2.7.1. 3′-O-Benzyl-5′-O,4′-C-methylene-β-D-xylofuranosyl thymine (14a)

It was obtained as white solid (0.28 g) in 76% yield. $R_f = 0.5$ (10% MeOH in $CHCl_3$); M. Pt.: 132–134 °C;

$[\alpha]_D^{31} = -24.19$ (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 763, 968, 1086, 1123, 1270, 1482, 1663, 1690, 2953, 3190, 3411; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.66 (3H, s, C-5CH_3), 4.31 (1H, s, C-3′H), 4.50 (1H, s, C-2′H), 4.53 (2H, s, –OCH_2Ph), 4.77 (2H, dd, $J = 7.2$ and 13.2 Hz, C-1′′H_2), 4.94 (1H, d, $J = 8.0$ Hz, C-5′H_a), 5.02 (1H, d, $J = 7.6$ Hz, C-5′H_b), 5.67 (1H, brs, C-2′-OH), 5.87 (1H, s, C-1′H), 7.11–7.30 (6H, m, Ar*H* & C-6H), 10.71 (1H, brs, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.32 (C-5CH_3), 72.22 (–OCH_2Ph), 76.66 (C-5′), 77.32 (C-2′), 82.29 (C-1′′), 83.89 (C-3′), 88.64 (C-4′), 93.35 (C-1′), 109.59 (C-5), 127.80, 128.32 and 128.62 (Ar*C*), 136.53 (C-6), 136.61 (Ar*C*), 150.68 (C-2), 164.72 (C-4); HR-ESI-TOF-MS: *m/z* 361.1403 ($[M + H]^+$), calcd. for $[C_{18}H_{20}N_2O_6+H]^+$ 361.1394.

2.7.2. 3′-O-Benzyl-5′-O,4′-C-methylene-β-D-xylofuranosyl uracil (14b)

It was obtained as white solid (0.24 g) in 70% yield. $R_f = 0.5$ (10% MeOH in $CHCl_3$); M. Pt.: 71–73 °C; $[\alpha]_D^{30} = +3.21$ (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 706, 762, 969, 1082, 1269, 1458, 1678, 2928, 3207, 3396; ^1H NMR ($CDCl_3$, 400 MHz): δ 4.29 (1H, s, C-3′H), 4.48 (1H, s, C-2′H), 4.53 (2H, s, –OCH_2Ph), 4.75 (2H, dd, $J = 7.6$ and 20.4 Hz, C-1′′H_2), 4.94 (2H, dd, $J = 7.2$ and 24.2 Hz, C-5′H_2), 5.43 (1H, brs, OH), 5.56 (1H, dd, $J = 1.6$ and 8.0 Hz, C-5H), 5.84 (1H, s, C-1′H), 7.15–7.17 (2H, m, Ar*H*), 7.30–7.32 (3H, m, Ar*H*), 7.36 (1H, d, $J = 8.4$ Hz, C-6H), 10.64 (1H, brs, NH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 72.32 (–OCH_2Ph), 76.57 (C-5′), 77.25 (C-2′), 82.13 (C-1′′), 83.67 (C-3′), 88.81 (C-4′), 93.50 (C-1′), 101.11 (C-5), 127.98, 128.43, 128.65 and 136.44 (Ar*C*), 140.62 (C-6), 150.71 (C-2), 164.20 (C-4); HR-ESI-TOF-MS: *m/z* 347.1241 ($[M + H]^+$), calcd. for $[C_{17}H_{18}N_2O_6+H]^+$ 347.1238.

2.8. General procedure for the synthesis of C-4′-spiro-oxetano-xylofuranosyl nucleosides 4a-b

To a solution of nucleoside **14a-b** (0.4 mmol) in anhydrous THF:MeOH (4 mL, 9:1, v/v) was added $Pd(OH)_2$-C (20 wt%, 0.03 g) and 88% formic acid (0.12 mL, 3.18 mmol). The reaction mixture was refluxed for 1 h whereupon it was cooled to 25 °C. The catalyst was carefully filtered off, washed with excess MeOH and the combined filtrate was concentrated. The crude product thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford spiro-nucleosides **4a-b** in 90 and 88% yields, respectively.

2.8.1. 5′-O,4′-C-Methylene-β-D-xylofuranosyl thymine (4a)

It was obtained as white solid (0.10 g) in 90% yield. $R_f = 0.2$ (10% MeOH in $CHCl_3$); M. Pt.: 253–255 °C; $[\alpha]_D^{31} = -39.93$ (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 754, 960, 1065, 1261, 1481, 1689, 2928, 3039, 3408, 3444; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.74 (3H, s, C-5CH_3), 4.05 (1H, s, C-3′H), 4.14 (1H, s, C-2′H), 4.52 (2H, dd, $J = 7.2$ and 10.8 Hz, C-1′′H_2), 4.64 (1H, d, $J = 7.6$ Hz, C-5′H_a), 4.85 (1H, d, $J = 7.6$ Hz, C-5′H_b), 5.68 (1H, d, $J = 2.4$ Hz, C-1′H), 5.80 (1H, brs, OH), 5.93 (1H, brs, OH), 7.42 (1H, s, C-6H), 11.33 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 12.29 (C-5CH_3), 76.43 (C-5′), 76.72 (C-2′), 78.68 (C-3′), 80.73 (C-1′′), 86.25 (C-4′), 90.26 (C-1′), 108.58 (C-5), 136.98 (C-6), 150.48 (C-2), 163.80 (C-4); HR-ESI-TOF-MS: *m/z* 271.0928 ($[M + H]^+$), calcd. for $[C_{11}H_{14}N_2O_6+H]^+$ 271.0925.

2.8.2. 5′-O,4′-C-Methylene-β-D-xylofuranosyl uracil (4b)

It was obtained as white solid (0.09 g) in 88% yield. $R_f = 0.2$ (10% MeOH in $CHCl_3$); M. Pt.: 203–205 °C; $[\alpha]_D^{31} = -44.12$ (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 962, 1075, 1128, 1273, 1470, 1682, 1705, 3018, 3299; ^1H NMR (DMSO-d_6, 400 MHz): δ 4.04-4.05 (1H, m, C-3′H), 4.15 – 4.17 (1H, m, C-2′H), 4.53 (2H, dd, $J = 7.2$ and 9.6 Hz, C-1′′H_2), 4.66 (1H, d, $J = 8.0$ Hz, C-5′H_a), 4.84 (1H, d, $J = 7.2$ Hz, C-5′H_b), 5.57 (1H, dd, $J = 2.0$ and 7.6 Hz, C-5H), 5.67 (1H, d, $J = 1.6$ Hz, C-1′H), 5.83 (1H, d, $J = 3.6$ Hz, C-2′-OH), 5.90 (1H, d, $J = 4.0$ Hz, C-3′-OH), 7.51 (1H, d, $J = 7.6$ Hz, C-6H), 11.35 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 76.23 (C-5′), 76.67 (C-2′), 78.97 (C-3′), 80.82 (C-1′′), 87.03 (C-4′), 90.99 (C-1′), 100.76 (C-5), 141.26 (C-6), 150.40 (C-2), 163.24 (C-4); HR-ESI-TOF-MS: *m/z* 257.0768 ($[M + H]^+$), calcd. for $[C_{10}H_{12}N_2O_6+H]^+$ 257.0768.

2.9. X-ray diffraction studies on 5′-O,4′-C-Methylene-β-D-xylofuranosyl thymine (4a)

Single crystals suitable for X-ray diffraction studies were grown by dissolving 5′-*O*,4′-*C*-methylene-β-D-xylofuranosyl thymine (**4a**) in 5% methanol in chloroform and allowing slow evaporation of the solution at room temperature. The X-ray diffraction data was collected on X′calibur CCD diffractometer with graphite monochromatized Cu/Kα radiation ($\lambda = 0.71073$ Å) at temperature 298 K. The structure was solved by direct methods using SHELXS-97, and refined by the full-matrix least-squares method on F^2 (SHELXL-97) (Sheldrick 2008). All calculations were carried out using the WinGX package of the crystallographic programs

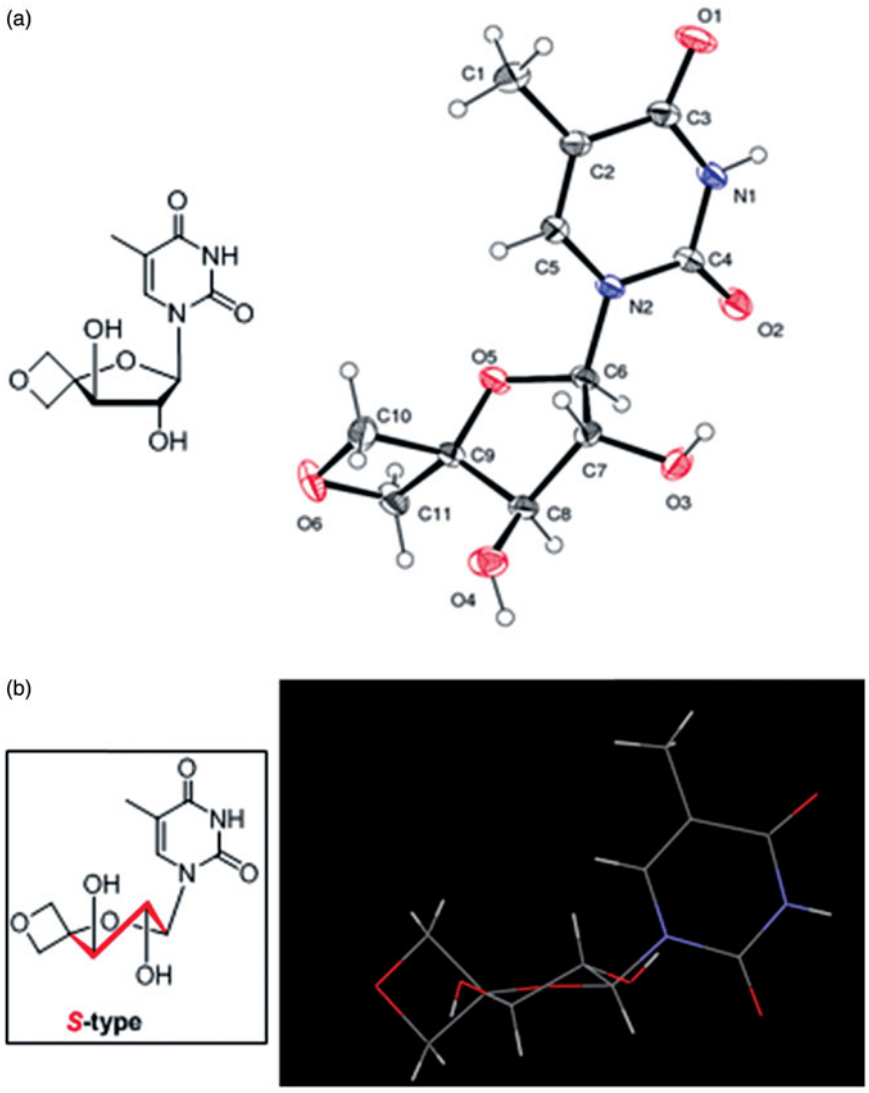


Figure 2. (a) ORTEP diagram of compound **4a** drawn in 20% thermal probability ellipsoids showing atomic numbering scheme. (b) Preferential *S*-type sugar ring puckering in compound **4a**.

(Farrugia 1999). For the molecular graphics, the program DIAMOND-2(Pennington 1999) and Mercury (Macrae et al. 2006) were used. Molecular structure was drawn, as given in Figure 2, using ORTEP software. The selected bond lengths, bond angles, etc. are given in Table 1.

3. Results and discussion

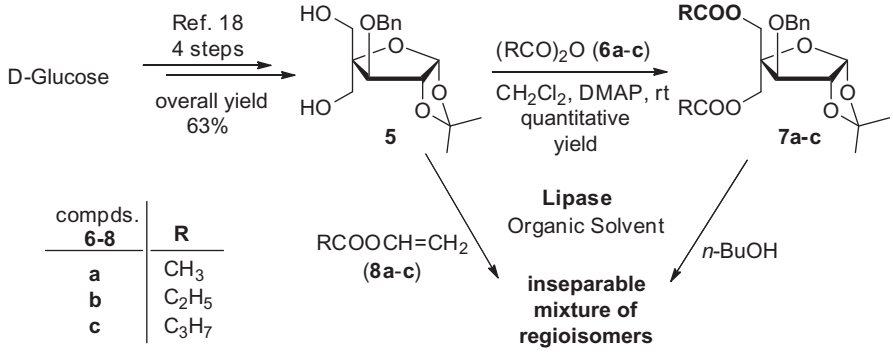
For the synthesis of targeted *C*-4′-spiro-oxetano-xylonucleosides **4**, it was envisaged that either the selective biocatalytic acylation of *C*-4-hydroxymethyl group in 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose (Youssefyeh et al. 1979) (**5**) or the selective biocatalytic deacylation of 5-*O*-acyl group in corresponding diacyl derivatives **7a-c** (Prasad et al. 2007) could provide a key precursor, 4-*C*-acyloxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose that would lead to the desired spironucleosides on subsequent chemical transformations. The diacylated xylofuranose **7a-c** were synthesized in quantitative yields by DMAP-catalyzed acylation of dihydroxy compound **5** with acetic- (**6a**), propionic- (**6b**) and butyric anhydride (**6c**); the dihydroxy compound **5** in turn was synthesized from D-glucose in 63% yield by following the literature procedure (Scheme 1) (Youssefyeh et al. 1979). On screening of different lipases for selective acylation/deacylation of dihydroxy- and diacyloxy- xylofuranoses **5** and **7a-c**, respectively, in organic solvents of varying polarity revealed the incapability of various lipases towards selective acylation/deacylation of these molecules (Scheme 1).

After the discouraging outcome with dihydroxyxylofuranose **5** and diacylated xylofuranoses **7a-c**, we focused our attention to probe selectivity for biocatalytic acetylation of 4′-*C*-hydroxymethyl group in 3′-*O*-benzyl-4′-*C*-hydroxymethyl-β-D-xylofuranosyl nucleosides **11a-b** or deacetylation of 5′-*O*-acetyl group in 4′-*C*-acetoxymethyl-2′,5′-di-*O*-acetyl-3′-*O*-benzyl-β-D-xylofuranosyl nucleosides **10a-b**. The desired diacetylated xylonucleosides **10a-b** were synthesized from 4-*C*-acetoxymethyl-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**7a**) (Scheme 2). Thus, acetolysis of compound **7a** led to the formation of tetra-*O*-acetylated glycosyl donor **9a-9b** in 97% yield, which on Vorbrüggen nucleobase coupling with thymine and uracil in the presence of *N,O*-bis(trimethylsilyl)acetamide and trimethylsilyltrifluoromethane sulfonate (TMS-triflate) afforded 4′-*C*-acetoxymethyl-2′,5′-di-*O*-acetyl-3′-*O*-benzyl-β-D-xylofuranosyl thymine (**10a**) and 4′-*C*-acetoxymethyl-2′,5′-di-*O*-acetyl-3′-*O*-benzyl-β-D-xylofuranosyl uracil (**10b**) in 90 and 88% yields, respectively. The complete deacetylation of nucleosides **10a-b** was effected with MeOH:NH_3 to give the corresponding 3′-*O*-benzyl-4′-*C*-hydroxymethyl-β-D-xylofuranosyl thymine (**11a**) and 3′-*O*-benzyl-4′-*C*-hydroxymethyl-β-D-xylofuranosyl uracil (**11b**) in 96 and 93% yields, respectively (Scheme 2).

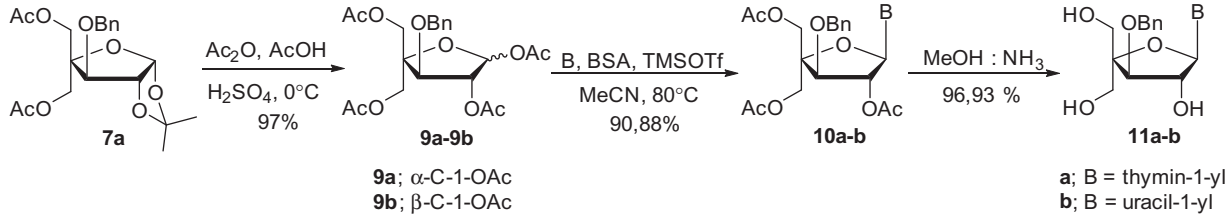
An attempt of selective deacetylation study on tri-*O*-acetylated nucleosides **10a-b** also led to similar intricate result as with diacylated xylofuranose derivatives **7a-c**. However, to establish the optimum reaction conditions, four different lipases, *viz. T. lanuginosus* lipase immobilized on silica (Lipozyme® TL IM), *C. antarctica* lipase-B immobilized on polyacrylate (Novozyme®-435), *C. rugosa* lipase (CRL) and porcine pancreatic lipase

Table 1. Crystal data and structure refinement for compound **4a**.

Empirical formula	$C_{11}H_{14}N_2O_6$
Formula weight	270.24
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 21
Unit cell dimensions	$a = 8.8875(11)$ Å, $\alpha = 90°$ $b = 6.9418(7)$ Å, $\beta = 111.685(15)°$ $c = 10.3533(13)$ Å, $\gamma = 90°$
Volume	593.54(12) $Å^3$
Z	2
Density (calculated)	1.512 mg/m^3
Absorption coefficient	0.124 mm^{-1}
F(000)	284.0
Crystal size	$0.20 \times 0.16 \times 0.12\ mm^3$
Theta range for data collection	3.62–25.00°.
Index ranges	$-10 \le h \le 7,\ -7 \le k \le 8,\ -12 \le l \le 9$
Reflections collected	3036
Independent reflections	1780 [R(int) = 0.0200]
Completeness to $\theta = 24.99°$	99.7%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9852 and 0.9755
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1780/1/185
Goodness-of-fit on F^2	1.098
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0317$, $wR2 = 0.0789$
R indices (all data)	$R1 = 0.0328$, $wR2 = 0.0799$
Absolute structure parameter	0.8(11)
Largest diff. peak and hole	0.159 and −0.190 e.$Å^{-3}$
CCDC	15,26,248



Scheme 1. Synthesis and biocatalytic acylation/deacylation studies on xylofuranose **5** and its diacylated derivatives **7a-c**.

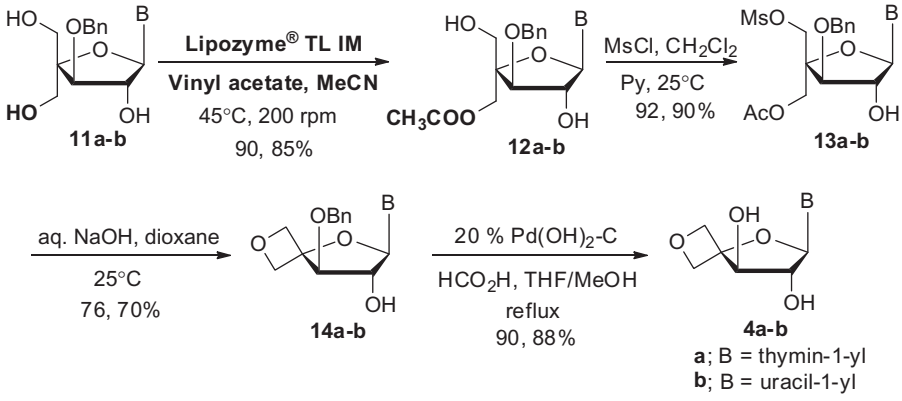


Scheme 2. Synthesis of 3′-*O*-benzyl-4′-*C*-hydroxymethyl-β-D-xylofuranosyl T and U.

(PPL) were screened for selective acetylation of *C*-4′-hydroxymethyl group over two other hydroxyl group present in nucleoside **11a** against four different solvents, i.e. tetrahydrofuran (THF), acetonitrile (MeCN), diisopropylether (DIPE) and dioxane at 45 °C and at 200 rpm using vinyl acetate as the acetylating agent. Notwithstanding, out of four screened lipases, Lipozyme® TL IM in acetonitrile was found to be selective for the acetylation of 4′-*C*-hydroxymethyl group in 3′-*O*-benzyl-4′-*C*-hydroxymethyl-β-D-xylofuranosyl nucleosides **11a-b** to afford the desired mono-acetylated products, 4′-*C*-acetoxymethyl-3′-*O*-benzyl-β-D-xylofuranosyl nucleosides **12a-b** in 90 and 85% yields, respectively (Scheme 3). Nevertheless, accomplishment of clean and efficient reactions with high yield of the products was associated with the presence of the nucleobase in the molecules. Previous endeavour for the selective acetylation of furanoside **5** or deacetylation of furanosides **7a-c** (Scheme 1) to afford regioselective product were not successful. This observation illustrates suitability of substrate molecules for the enzyme. Structural demarcation between the substrates dihydroxy xylofuranose **5** and nucleosides **11a-b** arises from the downward anomeric and C-2 hydroxy groups, connected by isopropylidene protection to form a five membered ring (α-configuration at anomeric position) and the downward C-2 hydroxy group and upward nucleobase (β-configuration at anomeric position). This result depicts the requirement of fittingness of the substrate with the enzyme. When the substrates are better fitted with enzyme, the acetylation was carried out regioselectively, whereas misfit of the substrate led to inseparable mixture of regioisomers.

The synthesis of targeted *C*-4′-spiro-oxetano-xylofuranosyl nucleosides **4a-b** was successfully achieved from nucleosides **12a-b**. Thus, mesylation of the lone primary hydroxyl group in nucleosides **12a-b** afforded monomesylated compounds **13a-b** which on deacetylation with aq. NaOH in dioxane and concomitant spirocylization at *C*-4′-position between 4′-*C*-hydroxymethyl and 5′-*O*-mesyl groups led to the formation of benzylated spironucleosides **14a-b** in 70 and 63% yields in two steps, respectively. The debenzylation of nucleosides **14a-b** with 20% $Pd(OH)_2$-C in the presence of formic acid afforded the desired compounds, 5′-*O*,4′-*C*-methylene-β-D-xylofuranosyl thymine (**4a**) and 5′-*O*,4′-*C*-methylene-β-D-xylofuranosyl uracil (**4b**) in 90 and 88% yields, respectively (Scheme 3, see Supplementary Material). The structure of one of the synthesized spironucleosides **4a** was confirmed by X-ray diffraction studies on its single crystal, which clearly indicated restriction on furanose ring flipping due to the introduction of spiro-oxetane ring. The presence of spiro-oxetane ring in the synthesized nucleosides compels the furanose ring to attain *S*-type puckering (Figure 2). It is worthy to mention here that earlier attempts to synthesize these nucleosides via chemical methodology by Mandal et al. (Roy et al. 2006) were unsuccessful.

The structures of all the synthesized compounds, i.e. **4a-b**, **5**, **7a-c**, **9a-9b**, **10a-b**, **11a-b, 12a-b, 13a-b** and **14a-b** were established on the basis of their spectral (IR, ^{1}H-, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and HRMS) data analysis. The structures of the known compounds **5, 7a, 11a** were further confirmed by the comparison of their physical and spectral data with those reported in the literature (Youssefyeh et al. 1979; Rajwanshi et al. 1999; Prasad et al. 2007). The structure of 5′-*O*,4′-*C*-methylene-β-D-xylofuranosyl thymine (**4a**) was confirmed by its X-ray data analysis that has been deposited in the Cambridge Crystallographic Data Centre with CCDC number 1526248.



Scheme 3. Biocatalytic route to 5′-*O*,4′-*C*-methylene-β-D-xylofuranosyl T and U **4a-b**.

4. Conclusions

In conclusion, novel *C*-4′-spiro-oxetanoxylofuranosyl thymine and uracil have been synthesized for the first time following a biocatalytic pathway. This methodology utilizes Lipozyme®-TL IM for the diastereoselective acetylation of one of the two diastereotopic hydroxymethyl functions of 3′-*O*-benzyl-4′-*C*-hydroxymethyl-β-D-xylofuranosyl nucleosides. The developed ecological method allows an easy access to these *C*-4′-spiro-xylonucleosides. The structure of one of the spironucleosides, i.e. 5′-*O*,4′-*C*-methylene-β-D-xylofuranosyl thymine was further confirmed by the single crystal X-ray diffraction analysis, which indicates that the presence of an extra spiro-ring in the xylonucleosides restricts the conformation of the sugar moiety by locking it in *S*-type conformation. These conformationally restricted spironucleosides can serve as potential drug candidates for viral disease, studies of which are under progress.

Acknowledgements

We are thankful to CIF-USIC University of Delhi, Delhi for providing NMR spectral recording facility. PR, VKS and VK thank CSIR, and PM thanks UGC for the award of Junior/Senior Research Fellowships.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

We are grateful to the University of Delhi for providing financial support under DU-DST Purse Grant and under scheme to strengthen research and development.

References

Camarasa MJ, Pérez-Pérez MJ, San-Félix A, Balzarini J, De Clercq E. 1992. 3′-Spiro nucleosides, a new class of specific human immunodeficiency virus type 1 inhibitors: synthesis and antiviral activity of [2′, 5′-bis-O-(tert-butyldimethylsilyl)-beta-D-xylo- and -ribofuranose]-3′-spiro-5″-[4″-amino-1″, 2″-oxathiole 2″, 2″-dioxide] (TSAO) pyrimidine nucleosides. J Med Chem 35:2721–2727.

Dang Q, Zhang Z, He S, Liu Y, Chen T, Bogen S, Girijavallabhan V, Olsen DB, Meinke PT. 2014a. Syntheses of 4′-spirocyclic phosphono-nucleosides as potential inhibitors of hepatitis C virus NS5B polymerase. Tetrahedron Lett 55:4407–4409.

Dang Q, Zhang Z, Tang B, Song Y, Wu L, Chen T, Bogen S, Girijavallabhan V, Olsen DB, Meinke PT. 2014b. Syntheses of nucleosides with 2′-spirolactam and 2′-spiropyrrolidine moieties as potential inhibitors of hepatitis C virus NS5B polymerase. Tetrahedron Lett 55:3813–3816.

Das K, Bauman JD, Rim AS, Dharia C, Clark AD Jr., Camarasa M-J, Balzarini J, Arnold E. 2011. Crystal Structure of tert-Butyldimethylsilyl-spiroaminooxathioledioxide-thymine (TSAO-T) in Complex with HIV-1 Reverse Transcriptase (RT) Redefines the Elastic Limits of the Non-nucleoside Inhibitor-Binding Pocket. J Med Chem 54:2727–2737.

De Castro S, Andrei G, Snoeck R, Balzarini J, Camarasa MJ, Velázquez S. 2007. Novel non-nucleoside human cytomegalovirus inhibitors based upon Tsao nucleoside derivatives: structure-activity relationships. Nucleosides Nucleotides Nucleic Acids 65:625–628.

De Clercq E. 2011. A 40-year journey in search of selective antiviral chemotherapy. Annu Rev Pharmacol Toxicol 51:1–24.

Dong S, Paquette LA. 2006. Highly Stereoselective beta-anomeric glycosidation of a 2′-deoxy syn-5′-configured 4′-spironucleoside. J Org Chem 71:1647–1652.

Dong S, Paquette LA. 2005. Stereoselective synthesis of conformationally constrained 2′-deoxy-4′-thia beta-anomeric spirocyclic nucleosides featuring either hydroxyl configuration at C5′. J Org Chem 70:1580–1596.

Du J, Chun BK, Mosley RT, Bansal S, Bao H, Espiritu C, Lam AM, Murakami E, Niu C, Micolochick-Steuer HM, Furman PA, Sofia MJ. 2014. Use of 2′-spirocyclic ethers in HCV nucleoside design. J Med Chem 57:1826–1835.

Farrugia LJ. 1999. WinGX suite for small-molecule single-crystal crystallography. J Appl Crystallogr 32:837–838.

Jonckers TH, Vandyck K, Vandekerckhove L, Hu L, Tahri A, Van-Hoof S, Lin TI, Vijgen L, Berke JM, Lachau-Durand S, et al. 2014. Nucleotide prodrugs of 2′-deoxy-2′-spirooxetane ribonucleosides as novel inhibitors of the HCV NS5B polymerase. J Med Chem 57:1836–1844.

Jonckers TH, Lin TI, Buyck C, Lachau-Durand S, Vandyck K, Van-Hoof S, Vandekerckhove L, Hu L, Berke JM, Vijgen L, et al. 2010. 2′-Deoxy-2′-spirocyclopropylcytidine revisited: a new and selective inhibitor of the hepatitis C virus NS5B polymerase. J Med Chem 53:8150–8160.

Jordheim LP, Durantel D, Zoulim F, Dumontet C. 2013. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. Nat Rev Drug Discov 12:447–464.

Kumar M, Sharma VK, Kumar R, Prasad AK. 2015. Biocatalytic route to C-3′-azido/-hydroxy-C-4′-spiro-oxetanoribonucleosides. Carbohydr Res Res 417:19–26.

Kumar R, Kumar M, Singh A, Singh N, Maity J, Prasad AK. 2017. Synthesis of novel C-4′-spiro-oxetano-α-L-ribonucleosides. Carbohydr Res Res 445:88–92.

Macrae CF, Edgington PR, McCabe P, Pidcock E, Shields GP, Taylor R, Towler M, Van de Steek J. 2006. Mercury: visualization and analysis of crystal structures. J Appl Crystallogr 39:453–457.

Maza S, Lopez O, Martos S, Maya I, Fernandez-Bolanos JG. 2009. Synthesis of the first selenium-containing acyclic nucleosides and anomeric spironucleosides from carbohydrate precursors. Eur J Org Chem 2009:5239–5246.

Pal APJ, Yashwant DV. 2010. Azidation of anomeric nitro sugars: application in the synthesis of spiroaminals as glycosidase inhibitors. Tetrahedron Lett 51:2519–2524.

Paquette LA, Seekamp CK, Kahane AL. 2003. Conformational restriction of nucleosides by spirocyclic annulation at C4′ including synthesis of the complementary dideoxy and didehydrodideoxy analogues. J Org Chem 68:8614–8624.

Paquette LA, Seekamp CK, Kahane AL, Hilmey DG, Gallucci J. 2004. Stereochemical features of Lewis acid-promoted glycosidations involving 4′-spiroannulated DNA building blocks. J Org Chem 69:7442–7447.

Paquette LA. 2004. Spirocyclic restriction of nucleosides. Aust J Chem 57:7–17.

Pennington WT. 1999. DIAMOND-visual crystal software information system. J Appl Crystallogr 32:1028–1029.

Prakash TP. 2011. An overview of sugar-modified oligonucleotides for antisense therapeutics. Chem Biodivers 8:1616–1641.

Prasad AK, Kalra N, Yadav Y, Singh SK, Sharma SK, Patkar S, Lange L, Olsen CE, Wengel J, Parmar VS. 2007. Selective biocatalytic deacylation studies on furanose triesters: a novel and efficient approach towards bicyclonucleosides. Org Biomol Chem 5:3524–3530.

Rajwanshi VK, Kumar R, Kofod-Hansen M, Wengel J. 1999. Synthesis and restricted furanose conformations of three novel bicyclic thymine nucleosides: a xylo-LNA nucleoside, a 3′-O,5′-C-methylene-linked nucleoside, and a 2′-O,5′-C-methylene-linked nucleoside. J Chem Soc Perkin Trans 1:1407–1414.

Roy A, Achari B, Mandal SB. 2006. An easy access to spiroannulated glyco-oxetane, -thietane and -azetane rings: synthesis of spironucleosides. Tetrahedron Lett 47:3875–3879.

Sharma VK, Kumar M, Sharma D, Olsen CE, Prasad AK. 2014c. Chemoenzymatic synthesis of C-4′-Spiro-oxetanoribonucleosides. J Org Chem 79:8516–8521.

Sharma V, Sharma RK, Singh SK. 2014a. Antisense oligonucleotides: modifications and clinical trials. Med Chem Comm 5:1454–1471.

Sharma VK, Rungta P, Prasad AK. 2014b. Nucleic acid therapeutics: basic concepts and recent developments. RSC Adv 4:16618–16631.

Sheldrick GM. 2008. A short history of SHELX. Acta Crystallogr A Found Crystallogr A64:112–122.

Sofia MJ, Chang W, Furman PA, Mosley RT, Ross BS. 2012. Nucleoside, nucleotide, and non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA-polymerase. J Med Chem 55:2481–2531.

Youssefyeh RD, Verheyden JPH, Moffatt JG. 1979. 4′-Substituted nucleosides. 4. Synthesis of some 4′-hydroxymethyl nucleosides. J Org Chem 44:1301–1309.