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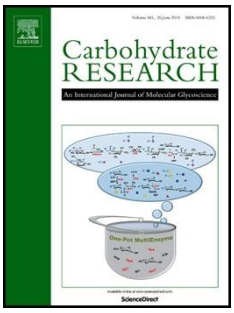

Title: Biocatalytic Route to *C*-3'-azido/-hydroxy-*C*-4'-spiro-oxetanoribonucleosides

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PII: S0008-6215(15)00252-9
DOI: http://dx.doi.org/doi: 10.1016/j.carres.2015.08.015
Reference: CAR 7059

To appear in: *Carbohydrate Research*

Received date: 4-8-2015
Revised date: 25-8-2015
Accepted date: 26-8-2015

Please cite this article as: Manish Kumar, Vivek K. Sharma, Rajesh Kumar, Ashok K. Prasad, Biocatalytic Route to *C*-3'-azido/-hydroxy-*C*-4'-spiro-oxetanoribonucleosides, *Carbohydrate Research* (2015), http://dx.doi.org/doi: 10.1016/j.carres.2015.08.015.

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Biocatalytic Route to *C*-3'-azido/-hydroxy-*C*-4'-spiro-oxetanoribonucleosides

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Abstract

The lipase, Novozyme®-435 exclusively deacetylates the 5-*O*-acetyl over 4-*C*-acetyloxymethyl group of almost identical reactivity in 5-*O*-acetyl-4-*C*-acetyloxymethyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-*α*-D-ribofuranose that led to the development of first and efficient synthesis of 3'-azido- / 3'-amino- *C*-4'-spiro-oxetanoribonucleosides T, U, C and A in 20 to 24 % overall yields. The X-ray study on the compound obtained by tosylation of lipase-mediated monodeacetylated product unambiguously confirmed the point of diastereoselective monodeacetylation on diacetoxy-azido-ribofuranose derivative. The capability of Novozyme®-435 for selective deacylation of 5-*O*-acetyl group in 5-*O*-acetyl-4-*C*-acetyloxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-*α*-D-ribofuranose recently discovered by us has been successfully used for the synthesis of *C*-4'-spiro-oxetanoribonucleosides A and C in good yields. These results clearly indicate that the broader substrate specificity and highly selective capability of Novozyme®-435 for carrying out acetylation/deacetylation reactions can be utilised for the development of environment friendly selective methodologies in organic synthesis.

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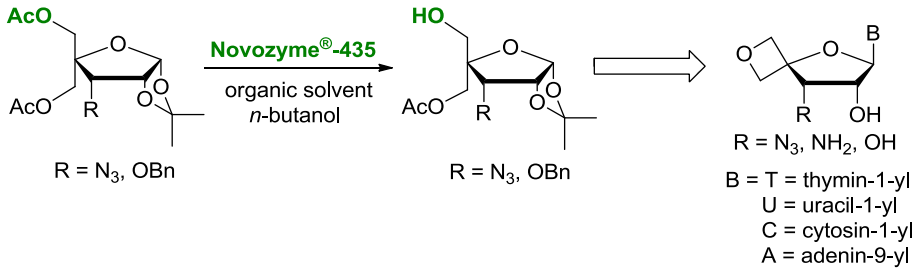
Graphical Abstract



Keywords: Biocatalysis, lipase, Novozyme®-435, diastereoselectivity, azido-spironucleosides.

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1. Introduction

The first naturally occurring spironucleoside (+)-hydantocidin (**1**) was isolated from the bacterial culture broth of *Streptomyces hygroscopicus* SANK 63584[1] and is known for its herbicidal and plant growth regulatory activities without exhibiting any mammalian toxicity.[2] Recently, it has been reported that the presence of a spiro-carbon in nucleosides restricts the conformational flipping of the furanose ring and thus enhances their biological activity.[3,4] This has led to the synthesis of different spironucleosides, such as *C*-1'-spiro-,[5] *C*-2'-spiro-,[4] *C*-3'-spiro-[6] and *C*-4'-spiro- nucleosides[7,8] **2** - **6** (**Figure 1**).

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The *C*-4'-spironucleosides imparts some distinct advantages including; (a) restriction in conformational flexibility to sugar moiety that may allow the molecule to attain the optimal puckering needed for drug action; (b) inhibition of the free radical-induced degradation of nucleos(t)ides by *C*-4'-proton abstraction and (c) serves as conformationally fixed models for elucidation of the glycosidic torsion angle in nucleosides.[9,10] Recently we have disclosed the first chemoenzymatic synthesis of *C*-4'-spiro-oxetanoribonucleosides U and T (**6**, **Figure 1**) by utilizing the lipase-mediated diastereoselective deacetylation of one of the two diastereotopic acetyl groups of 4-*C*-acetoxymethyl-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-*α*-D-ribofuranose.[8] Herein, we report the first synthesis of 3'-azido- / 3'-amino- *C*-4'-spiro-oxetanoribonucleosides T, U, C and A starting from lipase-mediated exclusive diastereoselective deacetylation of the required acetoxy group from 5-*O*-acetyl-4-*C*-acetyloxymethyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-*α*-D-ribofuranose along with the synthesis of *C*-4'-spiro-oxetanoribonucleosides A and C following our earlier developed chemo-enzymatic methodology in excellent to good yields.

2. Results and Discussion

The key starting sugar derivative, *i.e.* diacetoxy-azido-ribofuranose **8** was synthesized in 90 % yield by per-acetylation of the corresponding dihydroxy compound 3-azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**7**) which in turn was synthesized from D-glucose following literature procedure in 44 % overall yield.[11,12] For the synthesis of targeted azido-spironucleosides our aim was to find out a suitable lipase for diastereoselective deacetylation of 5-*O*-acetyl over 4-*C*-acetyloxymethyl group of diacetoxy-azido-ribofuranose **8**. On screening of different lipases,[13] *viz. Candida antarctica* lipase-B immobilized on polyacrylate (Lewatit), commonly known as Novozyme®-435, *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme® TL IM), *Candida rugosa* lipase (CRL) and porcine

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pancreatic lipase (PPL), the lipase Novozyme®-435 (35 % w/w of the substrate) in THF at 50 ^{o}C was found to be selective for efficient deacetylation of C-5 acetoxy over the other acetoxy group in compound **8** (Scheme 1). Thus, Novozyme®-435 mediated deacetylation of diacetoxy-azido-ribofuranose **8** in THF containing a small amount of *n*-butanol in an incubator shaker at 200 rpm and at 50 ^{o}C led to the formation of 4-*C*-acetoxymethyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (**9**) in 95 % yield (Scheme 1). The structure of monodeacetylated compound **9** was unambiguously established on the basis of its ^{1}H- & ^{13}C- NMR spectra, IR spectra and HRMS data analysis, which was further confirmed by X-ray diffraction analysis of single crystal of its tosyl derivative, 4-*C*-acetoxymethyl-3-azido-3-deoxy-5-*O*-(*p*-toluenesulphonyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (**10**) obtained by tosylation of compound **9** with TsCl-pyridine in 91 % yield (Scheme 1, Figure 2).

Recently, it has been revealed that the incubation of 3-azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**7**) with Novozyme®-435 in toluene in the presence of vinyl acetate leads to the exclusive formation of 5-*O*-acetyl-3-azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose by selective transfer of the acetyl group from acetylating agent to C-5 OH group of the dihydroxy sugar derivative. This enzymatic methodology of selective acetylation of dihydroxy sugar derivative **7** has been used for environment friendly lipase-mediated synthesis of azido-LNA monomers.[11] This indicates that during Novozyme®-435-mediated acetylation / deacetylation reaction the substrate is bound to the catalytic site of the lipase in such a way that only C-5 OH/OAc is exposed for the reaction.[8,11,14]

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Acetolysis of tosylated compound **10** with acetic acid-acetic anhydride-sulphuric acid (100:10:0.1) afforded the triacetoxy sugar derivative **11a-11b** in 94 % yield, which on coupling with nucleobases thymine, uracil, cytosine and 6-*N*-benzoyladenine under Vorbrüggen coupling condition[15] in the presence of *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 nucleoside **12a-d** in 81-85 % yields. Treatment of diacetylated nucleosides **12a-d** with 2M NaOH solution in water:dioxane resulted in the deacetylation followed by ring-closure through S_N^2 reaction between *C*-4'-hydroxymethyl and *C*-5'-*O*-tosyl group to afford 3'-azido-3'-deoxy-*C*-4'-spiro-oxetanoribonucleosides T, U, A and C **13a-d** in 80-93 % yields. Further, it has been demonstrated as a model case that the azido group of 3'-azido-3'-deoxy-*C*-4'-spiro-oxetanoribothymidine (**13a**) can be reduced with Pd-C under hydrogen atmosphere in ethyl acetate to afford the corresponding amino-nucleoside, *i.e.* 3'-amino-3'-deoxy-*C*-4'-spiro-oxetanoribothymidine (**14**) in 99 % yield (Scheme 2).

. Further in continuation of our recent report, two *C*-4'-spiro-oxetanoribonucleosides A and C were synthesized in good yields utilizing the capability of Novozyme®-435 for diastereoselective deacetylation of 5-*O*-acetyl group in 5-*O*-acetyl-4-*C*-acetyloxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose .[8] Thus the monotosylated triacetoxy glycosyl donor **16** was prepared from enzymatically monodeacetylated sugar derivative **15** according to the literature procedure in 92 % overall yields (Scheme 3). The coupling of the glycosyl donor **16** with nucleobases 6-*N*-benzoyladenine and cytosine under Vorbrüggen coupling condition[15] in the presence of *N,O-bis*(trimethylsilyl)acetamide and trimethylsilyltrifluoromethane sulfonate in acetonitrile or in 1,2-dichloroethane (for 6-*N*-

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benzoyladenine) resulted in the formation of 4'-*C*-acetoxymethyl-2'-*O*-acetyl-3'-*O*-benzyl-5'-*O*-*p*-toluenesulphonyl- 6-*N*-benzoyladenosine (**17a**) and cytidine (**17b**) in 74 and 81 % yields, respectively (Scheme 3). The treatment of tosylated nucleosides **17a** and **17b** with aq. NaOH-dioxane-NH_4OH and aq. NaOH-dioxane led to the hydrolysis of acetoxy group and concomitant cyclization *via* S_N^2 reaction between *C*-4'-hydroxymethyl and *C*-5'-*O*-tosyl group to afford 3'-*O*-benzyl- *C*-4'-spiro-oxetanoriboadenosine (**18a**) and 3'-*O*-benzyl- *C*-4'-spiro-oxetanoribocytidine (**18b**) in 68 and 71 % yields, respectively. The deprotection of 3′-*O*-benzyl group in nucleosides **18a** with $Pd(OH)_2$-C and $HCOONH_4$ in ethanol afforded *C*-4'-spiro-oxetanoriboadenosine **19** in 73 % yields (Scheme 3). However, several attempts to affect debenzylation on nucleoside **18b** using reagents, such as Pd-C in presence of H_2, 20 % $Pd(OH)_2$-C in presence of $HCOONH_4$, 20 % $Pd(OH)_2$-C in presence of HCOOH, 1M BCl_3 in dichloromethane, etc. led to the formation of inseparable mixture of compounds.

The structures of all synthesized compounds **7**, **8**, **9**, **10**, **11a-11b**, **12a-d**, **13a-d**, **14**, **15**, **16a-16b**, **17a** & **17b**, **18a** & **18b** and **19** were unambiguously established on the basis of their spectral data (^{1}H- & ^{13}C- NMR spectra, IR spectra and HRMS) analysis. The structure of known compounds **7**, **15** and **16a-16b** were further confirmed on the basis of comparison of their physical and spectral data with those reported in the literature.[8,11,12] Further, single crystal X-ray diffraction analysis has been performed on 3′-*O*-benzyl-*C*-4'-spiro-oxetanoribouridine (**20**) recently synthesized and reported by us.[8] The X-ray data analysis has revealed important structural features of 3'-hydroxy- / 3'-azido- *C*-4'-spiro-oxetanoribonucleosides, which is in consonance with the literature report.[3,4] The study clearly indicates that the presence of a spiro carbon restricts the conformational flipping of the furanose ring and the sugar puckering in *C*-4'-spiro-oxetanoribonucleosides attain *N*-type

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conformation (Figure 3). The detailed crystallographic data of compounds **10** and **20** has been deposited in the Cambridge Crystallographic Data Centre with CCDC nos. 951084 and 1032754, respectively.

3. Conclusion

In summary, Novozyme®-435 catalyzed deacetylation methodology has been optimised for exclusive diastereoselective monodeacetylation of *C*-5 acetoxy group in 5-*O*-acetyl-4-*C*-acetyloxymethyl-3-azido-3'-deoxy-1,2-*O*-isopropylidene-*α*-D-ribofuranose. The developed methodology has been successfully used for the efficient synthesis of 3'-azido- / 3'-amino- *C*-4'-spiro-oxetanoribonucleosides T, U, C and A. In continuation, recently developed enzymatic methodology for diastereoselective decaetylation of one of the two acetoxy group present in 5-*O*-acetyl-4-*C*-acetyloxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-*α*-D-ribofuranose has been used for the synthesis of *C*-4'-spiro-oxetanoribonucleosides A and C. The X-ray crystal data analysis on 3′-*O*-benzyl-*C*-4'-spiro-oxetanoribouridine revealed important structural features of 3'-hydroxy- / 3'-azido- *C*-4'-spiro-oxetanoribonucleosides, *i.e.* presence of spiro carbon restricts the conformational flipping of furanose ring and the sugar puckering in *C*-4'-spiro-oxetanoribonucleosides attains *N*-type conformation which is in consonance with the literature report. In addition, the broader substrate specificity and highly selective nature of Novozyme®-435 can be utilised for the development of environment friendly selective methodologies in organic synthesis.

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4. Experimental Section

Melting points 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- and ^{13}C- NMR spectras were recorded at 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 Hz. The mass spectra analyses were done on a microTOF-Q instrument from Bruker Daltonics, Bremen and 6520 Q-TOF instrument from Agilent Technologies. The optical rotations were measured on Rudolph Autopol II automatic polarimeter using light of 546 nm wavelength. Analytical TLCs were performed on precoated Merck 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. The single crystal X-ray diffraction data was collected on an Oxford Diffraction X'Calibur single crystal X-ray instrument having CCD camera [Cu Kα radiation (λ = 1.54184)] at USIC, University of Delhi, Delhi.

4-*C*-Acetoxymethyl-5-*O*-acetyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (8). To a solution of compound **7** (1.0 g, 4.08 mmol) in THF (10 mL) were added DMAP (97 mg, 0.79 mmol) and Ac_2O (1.15 mL, 12.17 mmol) and the reaction mixture was stirred at room temperature for 4 h. On completion, the mixture was diluted with cold water (25 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic layer was washed with cold water (2 x 50 mL), dried over sodium sulphate and concentrated under reduced pressure. The residue thus obtained was purified by column chromatography using ethyl acetate in petroleum ether as gradient solvent system to afford compound **8** as colourless oil (1.21 g, 90 % yield). R_f = 0.6 (40 % ethyl acetate in petroleum ether); $[\alpha]_D^{25}$ = + 48.78 (*c* = 0.1, MeOH); IR (thin film) ν_{max}: 2115, 1744, 1675, 1458, 1385, 1229, 1167, 1119, 1046 and 873

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cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.35 (3H, s), 1.64 (3H, s), 2.11 (6H, s), 4.10 (1H, d, *J* = 4.0 Hz), 4.12 (1H, d, *J* = 12.0 Hz), 4.23 (1H, d, *J* = 12.0 Hz), 4.34 (1H, d, *J* = 12.0 Hz), 4.54 (1H, d, *J* = 12.0 Hz), 4.81 (1H, dd, *J* = 4.0 and *J* = 5.2 Hz) and 5.83 (1H, d, *J* = 4.0 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.8, 20.8, 25.9, 26.2, 63.2, 64.4, 64.9, 79.9, 84.1, 104.3, 114.2, 170.3 and 170.4; HR-ESI-TOF-MS: *m/z* 352.1106 ($[M+Na]^+$), calcd. for $[C_{13}H_{19}N_3O_7+Na]^+$ 352.1115.

4-*C*-Acetoxymethyl-3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (9). To a solution of compound **8** (3.5 g, 10.63 mmol) in THF (70 mL), *n*-butanol (1 mL, 10.9 mmol) were added followed by the addition of Novozyme®-435 (3.5 g, 35 % w/w of the compound **8**). The reaction mixture was stirred at 50 °C in an incubator shaker and the progress of the reaction was monitored periodically by TLC. On completion after 24 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 **9** as a white solid (2.9 g, 95 % yield). R_f = 0.3 (40 % ethyl acetate in petroleum ether); M. Pt.: 72-74 ^oC; $[\alpha]_D^{26}$ = + 57.48 (*c* = 0.1, MeOH); IR (thin film) ν_{max}: 3351, 2924, 2114, 1738, 1458, 1384, 1240, 1119, 1045 and 873 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.26 (3H, s), 1.47 (3H, s), 2.02 (3H, s), 3.37-3.45 (2H, m), 4.10 (1H, d, *J* = 11.6 Hz), 4.34 (1H, d, *J* = 12.0 Hz), 4.36 (1H, d, *J* = 5.2 Hz), 4.86 (1H, dd, *J* = 3.6 and *J* = 4.8 Hz), 5.16 (1H, brs) and 5.76 (1H, d, *J* = 3.6 Hz); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 20.6, 25.8, 26.1, 62.2, 62.5, 63.2, 79.7, 85.4, 104.0, 112.5 and 170.2; HR-ESI-TOF-MS: *m/z* 310.1015 ($[M+Na]^+$), calcd. for $[C_{11}H_{17}N_3O_6+Na]^+$ 310.1010.

4-*C*-(Acetoxymethyl)-3-azido-3-deoxy-5-*O*-(*p*-toluenesulphonyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (10). To a stirred solution of compound **9** (3.5 g, 12.18 mmol) in pyridine

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(15 mL), *p*-toluenesulfonyl chloride (4.1 g, 21.51 mmol) was added in three portions at 0 ^{o}C. The progress of the reaction was monitored by TLC and on completion after 4 h, the reaction mixture was neutralized by 10 % ice-cold hydrochloric acid solution (100 mL) and extracted with chloroform (3 x 100 mL). The combined organic extract was washed with saturated aqueous $NaHCO_3$ (2 x 100 mL), dried over anhydrous Na_2SO_4, and the solvent was removed 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 afford the tosylated compound **10** as white solid (4.89 g; 91 % yield). R_f = 0.6 (40 % ethyl acetate in petroleum ether); M. Pt.: 116-118 ^{o}C; $[\alpha]_D^{27}$ = + 21.32 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 2115, 1743, 1598, 1458, 1368, 1234, 1177, 1033, 817 and 667 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.33 (3H, s), 1.60 (3H, s), 2.02 (3H, s), 2.47 (3H, s), 4.05-4.21 (4H, m), 4.47 (1H, d, *J* = 8.4 Hz), 4.78 (1H, t, *J* = 8.4 Hz), 5.74 (1H, d, *J* = 2.8 Hz), 7.38 (2H, d, *J* = 7.6 Hz) and 7.80 (2H, d, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.6, 21.7, 25.9, 26.2, 62.8, 63.4, 69.3, 79.9, 83.8, 104.4, 114.3, 128.0, 130.0, 132.2, 145.4 and 170.1; HR-ESI-TOF-MS: *m/z* 464.1089 ($[M+Na]^+$), calcd. for $[C_{18}H_{23}N_3O_8S+Na]^+$ 464.1098.

4-*C*-(Acetoxymethyl)-1,2-di-*O*-acetyl-3-azido-3-deoxy-5-*O*-(*p*-toluenesulphonyl)-α,β-D-ribofuranose (11a-11b). Acetic anhydride (10.68 mL, 112.98 mmol) and concentrated sulphuric acid (0.058 mL, 1.09 mmol) was added to a stirred solution of compound **10** (5.0g, 11.33 mmol) in acetic acid (64.84 mL, 1133.76 mmol) at 0 ^{o}C and mixture was stirred for 5 h. The reaction was quenched by addition of water (100 mL) and extracted with chloroform (3 x 100 mL). The combined organic layer was washed with bicarbonate solution (2 x 100 mL), with cold water (2 x 100 mL) and then dried over sodium sulphate. The excess of solvent was removed under reduced pressure, 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 (α:β = 1:9, based on comparison of integration of anomeric proton) of **11a-**

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11b as colourless oil (5.17 g, 94 %). R_f = 0.6 (40 % ethyl acetate in petroleum ether). IR (thin film) ν_{max}: 2121, 1748, 1598, 1438, 1371, 1229, 1178, 1095, 1020, 993, 816 and 667 cm^{-1}; Copy of ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) spectra are given in supporting information; 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 4′-*C*-(acetoxymethyl)-2′-*O*-acetyl-3′-azido-3′-deoxy-5′-*O*-(*p*-toluenesulfonyl)-ribonucleosides 12a-d. To the stirred solution of compound **11a-11b** (1.0 g, 2.0 mmol) and thymine (0.38 g, 3.0 mmol) / uracil (0.34 g, 3.0 mmol) or cytosine (0.34 g, 3.0 mmol) in anhydrous acetonitrile (20 mL), *N,O-bis*(trimethylsilyl)acetamide (1.97 mL, 8.06 mmol) was added dropwise. 1,2-Dichloroethane was used as solvent for coupling of 6-*N*-benzoyladenine (0.73 g, 3.0 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 (0.61 mL, 3.37 mmol) was added dropwise under stirring and the reaction was heated at 70-80 °C for 4-6 h. The reaction was quenched with a cold saturated aqueous solution of sodium hydrogen carbonate (50 mL) and extraction was performed with chloroform (3 x 100 mL). The combined organic phase was washed with saturated aqueous solutions of $NaHCO_3$ (2 x 100 mL) and brine (2 x 50 mL) and was dried over anhydrous Na_2SO_4. 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 gradient solvent system to afford nucleosides **12a-d** in 81-85 % yields.

4'-*C*-(Acetoxymethyl)-2'-*O*-acetyl-3'-azido-3'-deoxy-5'-*O*-(*p*-toluenesulphonyl)thymidine (12a). It was obtained as off white solid (0.89 g, 81 %). R_f = 0.6 (5 % methanol in chloroform); M. Pt.: 126-128 ^oC; $[\alpha]_D^{28}$ = + 25.28 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3199, 2927, 2120, 1696, 1370, 1228, 1049, 998, 815, 732 and 667 cm^{-1}; ^1H NMR ($CDCl_3$, 400

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MHz): δ 1.92 (3H, s), 2.06 (3H, s), 2.18 (3H, s), 2.47 (3H, s), 4.07 (1H, d, *J* = 12.0 Hz), 4.23 (2H, q, *J* = 8.4 Hz), 4.38 (1H, d, *J* = 12.4 Hz), 4.60 (1H, d, *J* = 6.4 Hz), 5.50 (1H, t, *J* = 5.6 Hz), 5.87 (1H, d, *J* = 5.6 Hz), 7.17 (1H, s), 7.40 (2H, d, *J* = 8.4 Hz), 7.84 (2H, d, *J* = 8.4 Hz) and 8.90 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 12.3, 20.4, 20.7, 21.7, 62.4, 62.5, 69.2, 74.2, 83.5, 88.7, 112.1, 128.0, 130.2, 131.9, 136.3, 145.8, 150.0, 163.4, 169.9 and 170.2; HR-ESI-TOF-MS: *m/z* 574.1212 ($[M+Na]^+$), calcd. for $[C_{22}H_{25}N_5O_{10}S+Na]^+$ 574.1214.

4'-*C*-(Acetoxymethyl)-2'-*O*-acetyl-3'-azido-3'-deoxy-5'-*O*-(*p*-toluenesulphonyl)uridine (**12b**). It was obtained as white solid (0.89 g, 83 %). R_f = 0.6 (5 % methanol in chloroform); $[\alpha]_D^{28}$ = + 34.60 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3221, 2925, 2121, 1747, 1696, 1373, 1229, 1178, 1049, 997, 913, 814, 733 and 667 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.94 (3H, s), 2.09 (3H, s), 2.40 (3H, s), 4.04 (1H, d, *J* = 12.0 Hz), 4.15 (1H, d, *J* = 12.0 Hz), 4.28 (2H, s), 4.81 (1H, d, *J* = 6.8 Hz), 5.56-5.65 (2H, m), 5.85 (1H, d, *J* = 5.2 Hz), 7.47 (2H, d, *J* = 8.4 Hz), 7.57 (1H, d, *J* = 8.4 Hz), 7.80 (2H, d, *J* = 8.0 Hz) and 11.46 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.4, 20.6, 21.7, 62.2, 62.5, 68.9, 74.3, 83.7, 89.5, 103.2, 128.0, 130.1, 131.9, 140.9, 145.8, 149.9, 162.8, 169.9 and 170.2; HR-ESI-TOF-MS: *m/z* 560.1059 ($[M+Na]^+$), calcd. for $[C_{21}H_{23}N_5O_{10}S+Na]^+$ 560.1058.

4'-*C*-(Acetoxymethyl)-2'-*O*-acetyl-3'-azido-3'-deoxy-5'-*O*-(*p*-toluenesulphonyl)-6-*N*-benzoyladenosine (**12c**). It was obtained as yellow solid (1.09 g, 82 %). R_f = 0.5 (5 % methanol in chloroform); M. Pt.: 75-77 ^{o}C; $[\alpha]_D^{29}$ = + 29.81 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3369, 2918, 2849, 2116, 1742, 1597, 1458, 1364, 1222, 1176, 1047, 815, 710 and 664 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.04 (3H, s), 2.17 (3H, s), 2.47 (3H, s), 4.15 (1H, d, *J* = 12.0 Hz), 4.31 (1H, d, *J* = 10.4 Hz), 4.39 (1H, d, *J* = 10.4 Hz), 4.54 (1H, d, *J* = 12.4 Hz), 5.01 (1H, d, *J* = 6.0 Hz), 6.12-6.16 (2H, m), 7.36 (2H, d, *J* = 7.6 Hz), 7.53-7.63 (3H, m), 7.79 (2H, d, *J* = 8.0 Hz), 8.03 (2H, d, *J* = 7.6 Hz), 8.07 (1H, s), 8.69 (1H, s) and 9.06 (1H, brs); ^{13}C

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NMR ($CDCl_3$, 100.6 MHz): δ 20.3, 20.7, 21.7, 62.2, 62.8, 68.1, 74.3, 84.2, 87.0, 123.7 127.9, 128.0, 128.9, 130.0, 132.0, 132.9, 133.3, 142.2, 145.6, 149.9, 151.2, 152.8, 164.5, 169.6 and 170.2; HR-ESI-TOF-MS: *m/z* 665.1748 ($[M+H]^+$), calcd. for $[C_{29}H_{28}N_8O_9S+H]^+$ 665.1773.

4'-*C*-(Acetoxymethyl)-2'-*O*-acetyl-3'-azido-3'-deoxy-5'-*O*-(*p*-toluenesulphonyl)cytidine (**12d**). It was obtained as white solid (0.91 g, 85 %). R_f = 0.3 (10 % methanol in chloroform); M. Pt.: 98-100 ^oC; $[\alpha]_D^{29}$ = + 43.25 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3345, 2962, 2121, 1746, 1654, 1492, 1371, 1231, 1177, 1048, 995, 913, 789, 733 and 668 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.01 (3H, s), 2.15 (3H, s), 2.45 (3H, s), 4.02 (1H, d, *J* = 12.0 Hz), 4.25 (2H, dd, *J* = 8.0 and *J* = 12.0 Hz), 4.41 (1H, d, *J* = 12.4 Hz), 4.78 (1H, d, *J* = 6.4 Hz), 5.56 (1H, d, *J* = 3.6 Hz), 5.71 (1H, dd, *J* = 2.8 and 7.0 Hz), 5.89 (1H, d, *J* = 7.2 Hz), 6.36 (1H, brs), 7.26 (1H, d, *J* = 6.0 Hz), 7.37 (2H, d, *J* = 8.0 Hz), 7.81 (2H, d, *J* = 8.0 Hz) and 8.10 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 22.6, 22.7, 23.7, 64.1, 65.1, 70.9, 77.0, 85.8, 95.2, 97.8, 130.0, 132.1, 134.0, 145.3, 147.6, 157.3, 168.3, 171.9 and 172.3; HR-ESI-TOF-MS: *m/z* 537.1381 ($[M+H]^+$), calcd. for $[C_{21}H_{24}N_6O_9S+H]^+$ 537.1398.

General procedure for the synthesis of 3′-azido-3′-deoxy-*C*-4'-spiro-oxetanoribonucleosides 13a-d. To a stirred solution of tosylated nucleosides **12a** (0.5g, 0.91 mmol), **12b** (0.5g, 0.93 mmol), **12c** (0.5g, 0.75 mmol) and **12d** (0.5g, 0.93 mmol) ($+NH_4OH$, 2 mL for **12c**) in dioxane : water (1:1, 4 mL) was added 2M, NaOH (4 mL) solution and the reaction mixture was stirred at RT for 24 h. On completion (analytical TLC), the reaction mixture was neutralized with acetic acid, 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-C-4'-spiro-oxetanoribonucleosides **13a-d** in 80-93 % yields.

3'-Azido-3′-deoxy-*C*-4'-spiro-oxetanoribothymidine (**13a**). It was obtained as white solid (0.244 g, 91 %). R_f = 0.3 (10 % methanol in chloroform); M. Pt.: 195-199 ^oC; $[\alpha]_D^{29}$ = +

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81.36 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3385, 2923, 2113, 1688, 1219, 1054 and 772 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.77 (3H, s), 4.44 (1H, d, *J* = 7.6 Hz), 4.52 (1H, d, *J* = 8.0 Hz), 4.60 (1H, d, *J* = 7.6 Hz), 4.69 (2H, d, *J* = 8.0 Hz), 4.81 (1H, dd, *J* = 4.0 Hz and *J* = 5.2 Hz), 5.63 (1H, d, *J* = 6.4 Hz), 6.21 (1H, s), 7.47 (1H, s) and 11.39 (1H, brs); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 12.0, 66.1, 71.9, 77.6, 81.1, 83.1, 89.7, 109.9, 137.8, 150.7 and 163.8; HR-ESI-TOF-MS: *m/z* 296.0987 ($[M+H]^+$), calcd. for $[C_{11}H_{13}N_5O_5+H]^+$ 296.0989.

3'-Azido-3′-deoxy-*C*-4'-spiro-oxetanoribouridine (**13b**). It was obtained as white solid (0.243 g; 93 %). R_f = 0.3 (10 % methanol in chloroform); M. Pt.: 90-92 °C; $[\alpha]_D^{30}$ = +79.22 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3431, 2926, 2854, 2116, 1654, 1440, 1095 and 555 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 4.44 (1H, d, *J* = 7.2 Hz), 4.51 (1H, d, *J* = 8.0 Hz), 4.59 (1H, d, *J* = 7.2 Hz), 4.67 (1H, d, *J* = 7.6 Hz), 4.69 (1H, d, *J* = 4.4 Hz), 4.81 (1H, s), 5.62 – 5.65 (2H, m), 6.22 (1H, brs), 7.62 (1H, d, *J* = 8.0 Hz) and 11.39 (1H, brs); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 65.9, 71.9, 77.5, 80.9, 83.2, 90.3, 102.1, 142.5, 150.6 and 163.1; HR-ESI-TOF-MS: *m/z* 282.0844 ($[M+H]^+$), calcd. for $[C_{10}H_{11}N_5O_5+H]^+$ 282.0833.

3'-Azido-3′-deoxy-*C*-4'-spiro-oxetanoriboadenosine (**13c**). It was obtained as yellow solid (0.205 g; 90 % yield). R_f = 0.3 (10 % methanol in chloroform); M. Pt.: 150-152 °C; $[\alpha]_D^{30}$ = + 44.28 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3392, 2922, 2117, 1640, 1261, 1125, 1097, 1012, 772 and 687 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.34 (1H, brs), 4.47 (1H, d, *J* = 8.4 Hz), 4.60 (1H, d, *J* = 8.0 Hz), 4.71 (2H, d, *J* = 8.0 Hz), 4.88 (1H, d, *J* = 4.4 Hz), 5.42 (1H, dd, *J* = 4.4 and *J* = 7.6 Hz), 5.90 (1H, d, *J* = 7.2 Hz), 7.32 (2H, brs), 8.11 (1H, s) and 8.32 (1H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 66.5, 72.5, 77.2, 81.2, 83.3, 87.6, 119.7, 140.7, 149.3, 152.6 and 156.2; HR-ESI-TOF-MS: *m/z* 305.1105 ($[M+H]^+$), calcd. for $[C_{11}H_{12}N_8O_3+H]^+$ 305.1105.

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3'-Azido-3′-deoxy-*C*-4'-spiro-oxetanoribocytidine (**13d**). It was obtained as white solid (0.208 g; 80 % yield). R_f = 0.3 (10 % methanol in chloroform); M. Pt.: 190-194 ^oC; $[\alpha]_D^{30}$ = + 48.53 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3351, 2921, 2115, 1648, 1497, 1219, 1125, 1034, 1012, 772 and 687 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 4.45 (1H, d, *J* = 7.6 Hz), 4.57 (2H, dd, *J* = 4.0 and *J* = 5.2 Hz), 4.63 (1H, d, *J* = 4.2 Hz), 4.67 (1H, d, *J* = 7.2 Hz), 4.89 (1H, dd, *J* = 4.0 Hz and *J* = 5.2 Hz) 5.56 (1H, d, *J* = 5.6 Hz), 5.72 (1H, d, *J* = 7.2 Hz), 6.11 (1H, d, *J* = 5.2 Hz), 7.24 (2H, d, 12.0 Hz) and 7.54 (1H, d, *J* = 7.2 Hz); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 66.1, 72.1, 77.5, 81.1, 83.2, 92.2, 94.3, 143.6, 155.1 and 165.7; HR-ESI-TOF-MS: *m/z* 281.1001 ($[M+H]^+$), calcd. for $[C_{10}H_{12}N_6O_4+H]^+$ 281.0993.

3'-Amino-3′-deoxy-*C*-4'-spiro-oxetanoribothymidine (**14**). The reduction of azido group of spironucleoside **13a** (0.5g, 1.69 mmol) was achieved by reaction with 10% Pd-C (0.05g) in ethyl acetate (15 mL) under hydrogen atmosphere at 28 ^oC. 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, 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 afford the spironucleoside **14** as white solid (0.450 g; 99 % yield). R_f = 0.2 (10 % methanol in chloroform); M. Pt.: 146-148 ^oC; $[\alpha]_D^{30}$ = + 52.09 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3413, 1700, 1458, 1268, 1054 and 970 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.77 (3H, s), 3.39 (2H, d, *J* = 4.0 Hz), 4.09 (1H, s), 4.35 (1H, d, *J* = 6.8 Hz), 4.62 (2H, s), 4.77 (2H, d, *J* = 6.8 Hz), 5.68 (1H, d, *J* = 2.4 Hz), 5.69 (1H, brs), 7.31 (1H, s) and 11.34 (1H, brs); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 12.1, 55.9, 72.9, 77.7, 79.2, 79.8, 89.9, 109.6, 136.6, 150.6 and 163.9; HR-ESI-TOF-MS: *m/z* 270.1083 ($[M+H]^+$), calcd. for $[C_{11}H_{15}N_3O_5+H]^+$ 270.1084.

General procedure for the synthesis of 4′-*C*-(Acetoxymethyl)-2′-*O*-acetyl-3′-*O*-benzyl-5′-*O*-(*p*-toluenesulfonyl)-ribonucleosides 17a & **17b**. To the stirred solution of tri-*O*-acetylated

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sugar derivative **16a-16b** (0.50 g, 0.91 mmol) and 6-*N*-benzoyladenine (1.36 mmol) in anhydrous dichloroethane (20 mL), *N,O-bis* (trimethylsilyl)acetamide (0.91 mL, 3.72 mmol) was added dropwise. Under the condition of coupling of 6-*N*-benzoyladenine, acetonitrile was used as solvent instead of dichloroethane for coupling of cytosine (1.36 mmol). The reaction mixture was stirred at reflux for 1 h, and then cooled to 0 ^{o}C. In the cooled reaction mixture trimethylsilyltrifluoromethane sulfonate (0.28 mL, 1.55 mmol) was added dropwise under stirring and the solution was heated at 70-80 ^{o}C for 6-10 h. The reaction was quenched with cold saturated aq. solution of sodium bicarbonate (100 mL) and extraction was performed with dichloromethane (3 x 100 mL). The combined organic phase was washed with saturated aq. sodium bicarbonate solution (2 x 100 mL), brine solution (2 x 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 **17a** & **17b** in 74 & 81 % yield.

4′-*C*-(Acetoxymethyl)-2′-*O*-acetyl-3′-*O*-benzyl-5′-*O*-(*p*-toluenesulfonyl)-6-*N*-benzoyladenosine (17a). It was obtained as white solid (0.49 g, 74 % yield). R_f = 0.4 (5 % methanol in chloroform); M. Pt.: 96-98 ^{o}C; $[\alpha]_D^{32}$ = + 13.3 (*c* 0.05, MeOH); IR (thin film) ν_{max}: 3327, 3066, 3031, 2927, 1747, 1706, 1610, 1584, 1483, 1456, 1367, 1238, 1177, 1097, 1050 and 756 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.00 (3H, s), 2.09 (3H, s), 2.43 (3H, s), 4.14 (1H, d, *J* = 12.4 Hz), 4.20 (1H, d, *J* = 10.0 Hz), 4.29 (1H, d, *J* = 10.8 Hz), 4.59 (2H, dd, *J* = 11.2 and 16.0 Hz), 4.65 (1H, d, *J* = 8.4 Hz), 4.93 (1H, d, *J* = 6.8 Hz), 5.96 (1H, dd, *J* = 3.6 and 6.0 Hz), 6.13 (1H, d, *J* = 3.6 Hz), 7.26-7.37 (7H, m), 7.52-7.55 (2H, m), 7.60-7.64 (1H, m), 7.68 (2H, d, *J* = 8.0 Hz), 8.03-8.08 (3H, m), 8.69 (1H, s), 9.11 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.6, 20.7, 21.6, 62.1, 68.1, 73.8, 74.6, 77.7, 84.5, 87.5, 123.5, 127.8, 127.9, 128.1, 128.3, 128.5, 128.8, 129.8, 132.2, 132.8, 133.4, 136.6, 142.2, 145.3, 149.8, 152.6, 164.7, 169.6 and 170.4; HR-ESI-TOF-MS: *m/z* 730.2175 ($[M+H]^+$), calcd. for $[C_{36}H_{35}N_5O_{10}S+H]^+$ 730.2177.

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4′-*C*-(Acetoxymethyl)-2′-*O*-acetyl-3′-*O*-benzyl-5′-*O*-(*p*-toluenesulfonyl)cytidine (17b). It was obtained as white solid (0.443 g, 81 % yield). R_f = 0.3 (10 % methanol in chloroform); M. Pt.: 205-206 ^{o}C; $[\alpha]_D^{32}$ = + 26.8 (*c* 0.05, MeOH); IR (thin film) ν_{max}: 3300, 3092, 1743, 1647, 1492, 1369, 1293, 1234, 1190, 1176, 1111, 1045, 989, 814, 790, 755 and 699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.95 (3H, s), 2.04 (3H, s), 2.41 (3H, s), 4.03 (1H, d, *J* = 12.4 Hz), 4.12 (1H, d, *J* = 11.2 Hz), 4.25 (1H, d, *J* = 10.8 Hz), 4.37 (1H, d, *J* = 11.2 Hz), 4.48-4.53 (3H, m), 5.53 (1H, dd, *J* = 2.8 and 6.4 Hz), 5.69 (1H, d, *J* = 2.4 Hz), 6.00 (1H, d, *J* = 8.0 Hz), 7.18-7.20 (2H, m), 7.27-7.38 (7H, m), 7.74 (2H, d, *J* = 8.0 Hz), 8.18 (1H, brs); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.7, 20.8, 21.7, 62.3, 68.7, 74.2, 74.4, 77.8, 84.3, 92.3, 95.7, 127.9, 128.0, 128.4, 130.1, 132.2, 137.0, 142.9, 145.5, 154.1, 164.6, 169.8 and 170.4; HR-ESI-TOF-MS: *m/z* 624.1632 ($[M+Na]^+$), calcd. for $[C_{28}H_{31}N_3O_{10}S+Na]^+$ 624.1622.

General procedure for the synthesis of 3′-*O*-benzyl-*C*-4'-spiro-oxetanoribonucleosides 18a & **18b**. To a stirred solution of compound **17a** & **17b** (0.67 mmol) in dioxane:water (2 mL, 1:1), 2M NaOH (1.5 mL) (+NH_4OH, 1ml for **17a** only) was added and reaction mixture was stirred at 28 ^{o}C for 30-45 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 **18a** & **18b** in 68 & 71 % yield.

3′-*O*-Benzyl-*C*-4′-spiro-oxetanoriboadenosine (18a). It was obtained as white solid (168 mg, 68 % yield). R_f = 0.4 (25 % MeOH in $CHCl_3$); M. Pt.: 96-98 ^{o}C; $[\alpha]_D^{32}$ = + 46.87 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3399, 2927, 1628, 1410, 1238, 1094, 966, 862 and 678 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 4.46-4.48 (2H, m), 4.65 (2H, dd, *J* = 8.0 and 20.8 Hz), 4.73-4.79 (2H, m), 4.98 (1H, d, *J* = 11.6 Hz), 5.04-5.07 (1H, m), 5.83 (1H, brs), 5.93 (1H, d, *J* = 6.0 Hz), 7.29-7.33 (3H, m), 7.36-7.40 (2H, m), 7.43-7.45 (2H, brs), 8.10 (1H, s), 8.29 (1H, s); ^{13}C

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NMR (DMSO-d_6, 100.6 MHz): δ 72.4, 72.8, 77.6, 80.5, 80.6, 84.0, 88.0, 119.5, 127.7, 127.9, 128.3, 138.5, 140.4, 149.2, 152.6 and 156.1; HR-ESI-TOF-MS *m/z* 370.1496 ($[M+H]^+$), calcd. for $[C_{18}H_{19}N_5O_4+H]^+$ 370.1510.

3′-*O*-Benzyl-*C*-4′-spiro-oxetanoribocytidine (18b). It was obtained as white solid (164 mg, 71 % yield). R_f = 0.4 (25 % methanol in chloroform); M. Pt.: 130-132 ^oC; $[\alpha]_D^{32}$ = + 80.72 (*c* 0.05, MeOH); IR (KBr) ν_{max}: 3422, 2928, 1641, 1484, 1282, 1123, 1103, 1054 and 787 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 4.09 (1H, d, *J* = 4.0 Hz), 4.41-4.43 (2H, m), 4.58-4.66 (3H, m), 4.77 (1H, d, *J* = 6.4 Hz), 4.87 (1H, d, *J* = 7.6 Hz), 5.61 (1H, brs), 5.65 (1H, d, *J* = 4.4 Hz), 5.70 (1H, dd, *J* = 1.6 and 7.2 Hz), 7.15 (1H, brs), 7.21 (1H, brs), 7.30 (1H, d, *J* = 7.2 Hz), 7.34-7.45 (5H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 71.9, 72.3, 78.0, 79.7, 80.0, 83.8, 91.8, 94.2, 127.6, 127.7, 128.3, 138.5, 142.2, 155.1 and 165.7; HR-ESI-TOF-MS: *m/z* 346.1402 ($[M+H]^+$), calcd. for $[C_{17}H_{19}N_3O_5+H]^+$ 346.1397.

***C*-4'-spiro-oxetanoriboadenosine (19)**. To a stirred solution of nucleoside **18a** (0.10 g, 0.27 mmol) in anhydrous EtOH (6 mL) was added 20 % $Pd(OH)_2$-C (0.03 g) and ammonium formate (0.09 g, 1.43 mmol). The reaction mixture was refluxed for 4 h whereupon it was cooled to rt. 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 spironucleoside **19** as sticky solid (0.055 g, 73 % yield). R_f = 0.2 (25 % MeOH in $CHCl_3$); $[\alpha]_D^{29}$ = - 5.13 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3166, 2886, 1713, 1628, 1418, 1234, 1044, 969 and 699 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 4.39 (1H, d, *J* = 4.4 Hz), 4.45 (1H, d, *J* = 7.2 Hz), 4.61 (2H, dd, *J* = 7.2 and 18.8 Hz), 4.79 (1H, d, *J* = 7.2 Hz), 4.90 (1H, m), 5.58 (1H, brs), 5.71 (1H, brs), 5.86 (1H, d, *J* = 6.8 Hz), 7.27 (2H, brs), 8.11 (1H, s), 8.29 (1H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 72.3, 73.5, 77.2, 80.8, 84.6, 87.6, 119.5, 140.4,

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149.3, 152.5 and 156.1; HR-ESI-TOF-MS: *m/z* 280.1052 ($[M+H]^+$), calcd. for $[C_{11}H_{13}N_5O_4+H]^+$ 280.1040.

X-Ray diffraction study of 4-*C*-(acetoxymethyl)-3-azido-3-deoxy-5-*O*-(*p*-toluenesulphonyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (10) and 3′-*O*-benzyl-*C*-4′-spiro-oxetanoribouridine (20).

Single crystal suitable for X-ray diffraction was grown by dissolving tosylated sugar derivative **10** and 3′-*O*-benzyl-*C*-4′-spiro-oxetanoribouridine **(20)** in toluene and chloroform respectively and allowing slow evaporation of the solution at room temperature. The X-ray diffraction data was collected on an Oxford Diffraction X'Calibur CCD diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54184 Å) at temperature 298 K. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squqres method on F^2 (SHELXL-97).[16] All calculations were carried out using the WinGX package of the crystallographic programs.[17] For the molecular graphics, the program DIAMOND-2[18] and Mercury[19] was used. Molecular structure have been drawn using ORTEP as software as given in **Figures 2** and **3**. The selected bond lengths, bond angles, *etc*. are given in **Tables 1** and **2**.

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Acknowledgement

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. We are also thankful to CIF-USIC University of Delhi, Delhi, for providing NMR spectral recording facility. M.K. and V.K.S. thank CSIR for the award of Junior/Senior Research Fellowships. R.K. thank UGC for the award of Junior/Senior Research Fellowships

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Supporting information

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Figure 1. Structures of natural and synthetic spirocyclic nucleosides

Figure 2. ORTEP diagram of compound **10** drawn in 20% thermal probability ellipsoids showing atomic numbering scheme.

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Figure 3a: ORTEP diagram of compound **20** drawn in 20 % thermal probability ellipsoids showing atomic numbering scheme.

Figure 3b: Preferential *N*-type sugar ring puckering in compound **20**.

Table 1. Single crystal X-ray diffraction data of tosylated sugar derivative **10**

Compound 10	
Empirical formula	$C_{18}\ H_{23}\ N_3\ O_8\ S$
Formula weight	441.45
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 21
Unit cell dimensions	a = 6.6548(4) Å α = 90°
	b = 16.0022(7) Å β = 99.431(6)°
	c = 10.3544(6) Å γ = 90°
Volume	1087.75(10) $Å^3$
Z	2
Density (calculated)	1.348 Mg/m^3
Absorption coefficient	0.197 mm^{-1}
F(000)	464
Crystal size	0.22 x 0.16 x 0.12 mm^3
Theta range for data collection	3.23 to 25.00°.

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Index ranges	-7<=h<=7, -19<=k<=19, -12<=l<=12
Reflections collected	8191
Independent reflections	3731 [R(int) = 0.0189]
Completeness to theta = 25.00 °	99.4 %
Max. and min. transmission	0.9767 and 0.9579
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3731 / 1 / 275
Goodness-of-fit on F^2	1.045
Final R indices [I>2sigma(I)]	R1 = 0.0366, wR2 = 0.0834
R indices (all data)	R1 = 0.0415, wR2 = 0.0859
Absolute structure parameter	-0.01(7)
Largest diff. peak and hole	0.173 and -0.169 e.$Å^{-3}$
CCDC	**951084**

Table 2: Single crystal X-ray diffraction data of 3′-O-benzyl-C-4′-spiro-oxetanoribouridine **(20)**.

Compound 20	
Empirical formula	$C_{17}H_{18}N_2O_6$
Formula weight	346.33
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic

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Space group	P 21
Unit cell dimensions	a = 6.4248(18) Å, α = 90°
	b = 7.327(2) Å, β = 92.47(3)°
	c = 18.217(5) Å, γ = 90°
Volume	856.7(4) $Å^3$
Z	2
Density (calculated)	1.405 mg / m^3
Absorption coefficient	0.111 mm^{-1}
F(000)	380.0
Crystal size	0.13 x 0.12 x 0.10 mm^3
Theta range for data collection	3.00 to 25.00°.
Index ranges	-7≤h≤7, -8≤k≤8, -20≤l≤21
Reflections collected	5047
Independent reflections	2971 [R(int) = 0.1457]
Completeness to theta = 25.00°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9890 and 0.9858
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2971 / 243 / 205
Goodness-of-fit on F^2	1.541
Final R indices [$I>2\sigma(I)$]	*R1* = 0.1735, *wR2* = 0.4136
R indices (all data)	*R1* = 0.2116, *wR2* = 0.4475
Absolute structure parameter	4(6)
Largest diff. peak and hole	0.552 and -0.515 e.$Å^{-3}$
CCDC	**1032754**

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Scheme 1. Lipase-mediated deacetylation studies on diacetoxy sugar derivative **8**

Scheme 2. Synthesis of 3'-azido- / 3'-amino-*C*-4'-spiro-oxetanoribonucleosides **13a-d** and **14**

Scheme 3. Synthesis of *C*-4'-spiro-oxetanoribonucleosides **18a** & **18b** and **19**

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