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Highly efficient diastereoselective biocatalytic acylation of a diastereotopic furanose diol and synthesis of key intermediates for amino derivatized bicyclonucleosides

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Dedicated to the cherished memory of our collaborator and friend (Late) Professor Sukant K. Tripathy

Abstract—The selectivity of *Candida antarctica* lipase has been demonstrated and employed in the manipulation of a diastereotopic furanose diol as the key step in the synthesis of a novel bicyclo 3-amino-3-deoxy furanose derivative, which is an important intermediate for the synthesis of bicyclic analogs of AZT. The asymmetrization of the diol has been achieved with preferred formation of a monoacylated product with 100% diastereoselectivity. An efficient synthesis of an intermediate in the synthesis of amino derivatized bicyclonucleosides is also described, two such novel compounds containing cycloamino residues have been prepared.

1. Introduction

Recent years have seen the emergence of sugar modified nucleoside analogs as promising chemotherapeutic agents.[1] The importance of anti-sense and anti-gene oligonucleotides (ON's) as potential and selective inhibitors of gene expression along with the discovery of 2′,3′-dideoxy nucleosides, like AZT, ddC and d4T as anti-HIV agents have proved to be significant developments in this field. Reports have shown the anti-inhibitory activity of conformationally locked AZT analogs against HIV-1 reverse transcriptase.[2] In addition, a family of related molecules named locked nucleic acids (LNAs) have elicited much interest due to having enhanced duplex stability and higher binding affinity to complementary single stranded DNA and RNA.[3,4] The modified nucleosides have also attracted much attention as potential antiviral and anticancer agents.[5,6] Of these, particularly amino sugar nucleosides have long been known to possess strong antibacterial and anticancer properties.[7] Further, amine-modified oligonucleotides have displayed increased thermal affinities towards complementary ON's and excellent hybridization properties.[8–10]

Although there has been a growing demand for the development of nucleoside-based therapeutics, this class of compounds remains to some extent unexplored because the manipulation of polyhydroxy functionalities in carbohydrate moieties is a major limitation using conventional methods. In our studies, directed towards the synthesis of 4′-*C*-branched AZT analogs, the selective protection of a single hydroxyl group in the prochiral intermediate 3-azido-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-ribofuranose (**1**), gave unsatisfactory results using the classical chemical methods. Our continued interest[11] in regio- and stereoselective reactions on different types of compounds using enzymes led us to embark on a novel chemo-enzymatic strategy to overcome this problem.

2. Results and discussion

The synthetic route designed by us for the preparation of the

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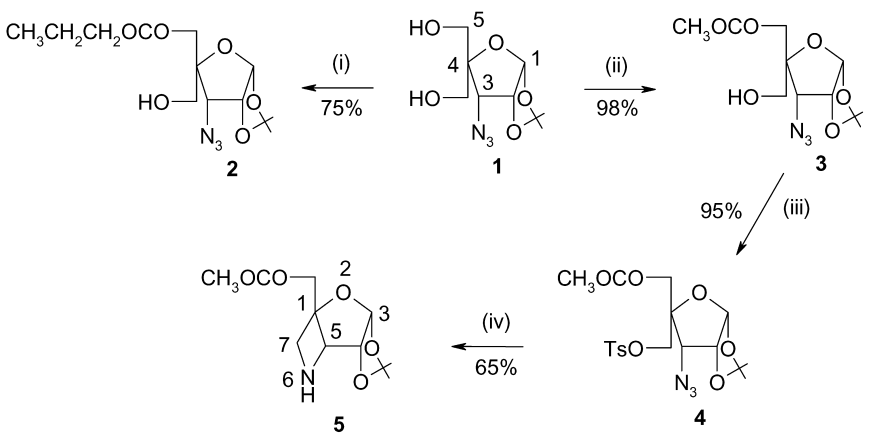
target intermediate compounds useful in the synthesis of bicyclic analogs of AZT starts with **1**, which was prepared from D-glucose by a multiple step sequence.[12,13] As per our scrutiny of literature, there has been no report on the successful monoprotection of the diol **1** other than a report involving its stereoselective protection by reaction with TBDPSCl in the presence of triethylamine and dichloromethane. This reaction, however yielded the monosilyl ether in a low 62% yield.[13] Our previous results have shown that the asymmetrization of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (**6**)[14,15] can be easily carried out in organic solvents by two enzymes, namely *Pseudomonas cepacia* and *Candida antarctica* lipase.[14] The latter lipase was seen to exhibit high diastereoselectivity (de) optimized to 79% de yielding the 5-*O*-acetyl-α-D-xylofuranose derivative, while the former lipase yielded the epimeric 4-*C*-(acetoxy)methyl-α-D-xylofuranose derivative in relatively lower diastereoselectivity. We describe here a similar enzymatic strategy for the selective protection of **1** as part of our chemosynthetic approach targeted towards the formation of modified nucleosides. A screening of lipases, e.g. *C. antarctica* lipase (CAL), *Candida rugosa* lipase (CRL), porcine pancreatic lipase (PPL) and Amano PS lipase in different organic solvents was employed for the optimization of the reaction conditions; CAL in toluene was found to be the most suitable system for selective acetylation of compound **1**. It was observed that *C. antarctica* lipase catalysed the preferential acetylation of the C-5 hydroxy group of **1** giving rise to the 5-*O*-acetyl-α-D-ribofuranose derivative **3** in 98% yield and in impressive 100% de (Scheme 1). We thus report here a promising, convenient and practical route for the monoacetylation of the diastereotopic diol **1** in a single step.

In a successful reaction, a solution of compound **1** (100 mg) and an equimolar amount of vinyl acetate in toluene (4 mL) was incubated with *C. antarctica* lipase (20 mg) at 25–28°C and the progress of the reaction was monitored by TLC. On completion of the reaction, the enzyme was filtered off, the solvent removed under reduced pressure and the product was purified by column chromatography on silica gel using a gradient solvent system of petroleum ether–ethyl acetate to afford compound **3** as an yellow oil. The formation of a single epimer was confirmed from its ^{1}H NMR spectrum wherein the anomeric proton appeared as a single doublet at δ 5.76 (J=3.7 Hz).

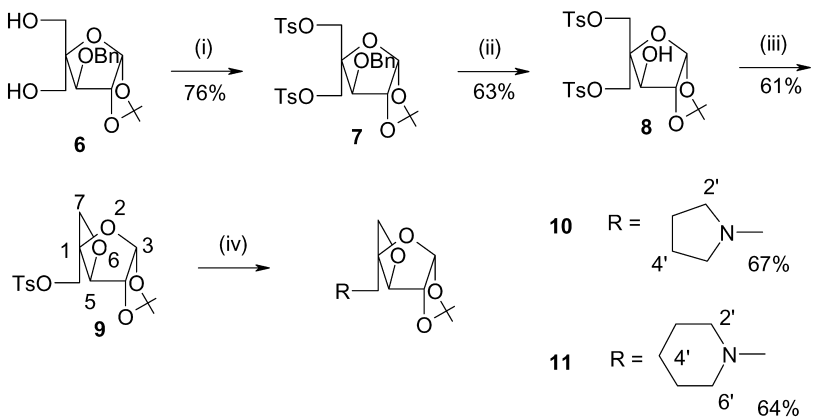
Repeated attempts to pinpoint the position of acetylation, however, gave unsatisfactory results using NOE and COSY experiments. The structure of the monoacetylated epimer was therefore established on the basis of further chemical transformations starting with the tosylation of the remaining hydroxyl group in **3** to yield **4** in 95% yield. This was followed by a one pot reduction and cyclization, achieved by refluxing the tosyl derivative **4** with 10% Pd–C in ethyl acetate in an atmosphere of hydrogen to afford the novel cyclized derivative **5** in 65% yield, whose structure was ascertained unambiguously on the basis of its spectral data. The bicyclic compound **5** is important as it could serve as a valuable synthon for the synthesis of AZT analogs and other sugar modified nucleosides and oligonucleotides.

We had earlier reported a reversal of stereoselectivity in the CAL-catalyzed acylation reaction on 3-*O*-benzyl-4-*C*-hydroxymethyl-1, 2-*O*-isopropylidene-β-L-*threo*-pentofuranose (**6**)[14,15] by changing the acylating agent from vinyl acetate to the more bulky 2,2,2-trifluoroethyl butyrate. This factor however, was not observed in the enzymatic butanoylation of **1** with 2,2,2-trifluoroethyl butyrate, this reaction gave the monobutyrate **2** involving the same site of esterification as in the formation of the monoacetate **3** (Scheme 1). This observation was concluded based on the similar splitting pattern of the C-5 methylene protons of the butanoyloxymethyl moiety in the proton NMR spectrum of the monobutanoylated **2** as observed for the corresponding protons of the acetyloxymethyl moiety in the ^{1}H NMR spectrum of the monoacetylated compound **3**. We speculate that the non-occurrence of reversal of selectivity in the biocatalytic acylation of **1** by change of the acylating agent is due to the polar nature and opposite configuration of the azido substituent at the C-3 position in **1** as compared to the benzyloxy substituent at the C-3 position in **6**, which perhaps is able to allow the reversal in the site of acylation in the latter compound by change of the acylating agent due to its bulk, non-polar nature and preferred stereochemistry appropriate for the active site of the *Candida antactica* lipase.

A set of chemical reactions was also carried out starting



Scheme 1. *Reagents and conditions*: (i) *C. antarctica* lipase, toluene, 2,2,2-trifluoroethyl butyrate, 25–28°C; (ii) *C. antarctica* lipase, toluene, vinyl acetate, 25–28°C; (iii) *p*-toluene sulphonyl chloride, pyridine, 0°C; (iv) H_2, 10% Pd–C, ethyl acetate, 60°C.



Scheme 2. *Reagents and conditions*: (i) *p*-toluene sulphonyl chloride, pyridine, 0°C; (ii) H_2, 10% Pd–C, ethyl acetate, 25–28°C; (iii) sodium hydride, dimethylformamide, toluene 42–45°C; (iv) piperidine/pyrrolidine, triethylamine, methanol, reflux.

with **6** towards the synthesis of key synthons for amino-derivatized bicyclonucleosides (Scheme 2). Treatment of **6** with *p*-toluene sulphonyl chloride gave the ditosylate **7** in 76% yield. The next step involved debenzylation by hydrogenolysis in the presence of palladium on activated charcoal to yield **8** in 63% yield. Subsequent cyclization of **8** was carried out in the presence of sodium hydride giving rise to the oxabicyclo compound **9** with a pendant tosyl group in 61% yield. This tosyl group could then be used as a handle for the introduction of various heterocyclic amines at the 4-*C*-hydroxymethyl position. We report here two different novel bicyclo compounds **10** and **11** thus synthesized having pyrrolidine and piperidine moieties, respectively as attached pendant amino functionalities.

3. Conclusion

We have explored complementary synthetic and chemo-enzymatic routes for the synthesis of key synthons towards novel modified bicyclonucleosides, including the synthesis of nine novel compounds, hitherto unknown in literature. It can thus be seen in the present study that biocatalytic transformations can be used to tailor the course of the reaction to yield desired products. Further, this approach also has advantages with respect to high stereoselectivity and better yields obtained through eco-friendly routes. Studies on the synthetic transformations and biological activity evaluation of these compounds are now in progress.

4. Experimental

4.1. General

Melting points were determined on a Mettler FP62 instrument and are uncorrected. The IR spectra were recorded either on a Perkin-Elmer model 2000 FT-IR or RXI FT-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance-300 spectrometer at 300 and at 75.5 MHz, respectively, using TMS as internal standard. The chemical shift values are on δ scale and the coupling constants (J) are in Hz. The FAB-HRMS spectra of all the compounds to measure their accurate masses were recorded on a JEOL JMS-AX505W high resolution mass spectrometer in positive mode using the matrix HEDS (bis-hydroxyethyldisulfide) doped with sodium acatate, except for compound **5** for which the matrix NBA (5-nitrobenzyl alcohol) was used. The *C. antarctica* lipase immobilized on accurel was gifted by Novozymes Inc. and used as such. The enzymes porcine pancreatic lipase (PPL, Type II) and *C. rugosa* lipase (CRL, Type VII) were purchased from Sigma Chemical Co. (USA) and used after storing in vacuo over P_2O_5 for 24 h. The lipase 'Amano PS' was a gift from Amano Co., Japan and used as such. The organic solvents used were distilled over activated molecular sieves (4 Å). Analytical TLCs were performed on precoated Merck silica gel $60F_{254}$ plates; the spots were detected either by UV light or by charring with 4% alcoholic H_2SO_4. Reactions were monitored at λ_{254} nm on a Shimadzu LC-10AS HPLC instrument with SPD-10A UV-VIS detector and Shimpack CLC-ODS (4.6×150 mm^2) reverse phase column; solvent system used was methanol–water (3:2) at a flow rate of 0.50 mL/min.

4.1.1. 3-Azido-5-butanoyl-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-erythro-ribofuranose (2). A solution of compound **1**[12,13] (100 mg, 0.4 mmol) and an equimolar amount of 2,2,2-trifluoroethyl butyrate (TFEB) in toluene (4 mL) was incubated with *C. antarctica* lipase (20 mg) at 25–28°C and the progress of the reaction was monitored by TLC. On completion, the enzyme was filtered off, the solvent removed under reduced pressure and the product was purified by column chromatography on silica gel using a gradient solvent system of petroleum ether–ethyl acetate (3:2) to afford compound **2** as an yellow oil (95 mg) in 75% yield. ^{1}H NMR (300 MHz, acetone-d_6) δ: 0.93 (3H, t, J=8.4 Hz, $CO(CH_2)_2CH_3$), 1.32 and 1.54 (6H, 2s, 3H each, 2×CH_3), 1.63 (2H, m, $COCH_2CH_2CH_3$), 2.35 (2H, t, J=7.5 Hz, $COCH_2CH_2CH_3$), 2.93 (1H, br s, OH), 3.75 and 3.90 (2H, dd, J=11.3 Hz each, CH_2OH), 4.15 and 4.22 (2H, dd, J=11.4 Hz each, $CH_2OCO(CH_2)_2CH_3$), 4.35 (1H, d, J=5.6 Hz, C-3H), 5.00 (1H, m, C-2H) and 5.86 (1H, d, J=3.8 Hz, C-1H); ^{13}C NMR (75.5 MHz, acetone-d_6) δ: 13.86 ($COCH_2CH_2CH_3$), 19.02 ($COCH_2CH_2CH_3$), 26.49 and 26.82 ($C(CH_3)_2$), 36.44 ($COCH_2CH_2CH_3$), 62.63 (C-3), 64.26 (CH_2OH), 65.65 ($CH_2OCO(CH_2)_2CH_3$), 81.55 (C-2), 87.10 (C-4), 105.52 (C-1), 114.19 ($C(CH_3)_2$) and 173.32 (CO); IR (neat film): 802, 873, 1029, 1168, 1261, 1385,

1458, 1740, 2114, 2965, 3502 cm^{-1}; FAB-HRMS: *m/z* 338.1347 ($[M+Na]^+$, $C_{13}H_{21}N_3O_6Na$ calcd 338.1328).

4.1.2. 3-Azido-5-acetyl-3-deoxy-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-erythro-ribofuranose (3). A solution of compound **1** (245 mg, 1 mmol) and an equimolar amount of vinyl acetate in toluene (10 mL) was incubated with *C. antarctica* lipase (50 mg) at 25–28°C and the progress of the reaction was monitored by TLC. On completion of the reaction, the enzyme was filtered off and the solvent removed under reduced pressure to afford compound **3** as an yellow oil (240 mg) in 98% yield. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.19 and 1.59 (6H, 2s, 3H each, 2×CH_3), 2.05 (3H, s, $COCH_3$), 3.36 (2H, br s, CH_2OH), 4.00 and 4.11 (2H, dd, J=11.2 Hz each, CH_2-OCOCH_3), 4.27 (1H, d, J=5.6 Hz, C-3H), 4.79 (1H, m, OH), 4.85 (1H, m, C-2H) and 5.76 (1H, d, J=3.7 Hz, C-1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 20.83 (CO*C*H_3), 26.34 and 26.51 (C(*C*H_3)$_2$), 61.00 (CH_2OH), 62.65 (C-3), 64.95 (*C*H_2OCOCH_3), 80.3 (C-2), 86.10 (C-4), 104.10 (C-1), 112.90 (*C*(CH_3)$_2$) and 170.21 (CO); IR (neat film): 605, 875, 1028, 1120, 1167, 1234, 1386, 1457, 1747, 2114, 2942, 2986, 3502 cm^{-1}; FAB-HRMS: *m/z* 310.0997 ($[M+Na]^+$, $C_{11}H_{17}N_3O_6Na$ calcd 310.1015).

4.1.3. 3-Azido-5-acetyl-3-deoxy-4-*C*-(*p*-toluenesulphonyloxymethyl)-1,2-*O*-isopropylidene-α-D-erythro-ribofuranose (4). Compound **3** (230 mg, 0.8 mmol) was dissolved in a minimum amount of pyridine and the solution allowed to stir at 0°C, tosyl chloride (84 mg) was added portion-wise into the stirred solution. The reaction was monitored by TLC and at completion, the reaction mixture was neutralized by pouring into acidified water. The solid that separated out was purified by column chromatography (petroleum ether–ethyl acetate 4:1) to yield compound **4** (335 mg) as a white solid in 95% yield, mp 122–13°C; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.29 and 1.38 (6H, 2s, 3H each, 2×CH_3), 2.05 (3H, s, $COCH_3$), 2.44 (3H, s, C$H_3C_6H_4$), 4.05 (2H, m, C-3H and CH_αOAc), 4.18 (1H, d, J=11.6 Hz, CH_βOAc), 4.28 and 4.38 (2H, dd, J=10.4 Hz each, CH_2OTs), 4.75 (1H, m, C-2H), 5.77 (1H, d, J=3.7 Hz, C-1H), and 7.34 and 7.82 (4H, 2d, 2H each, J=8.2 Hz each, C-2′H, C-3′H, C-5′H and C-6′H); ^{13}NMR (75.5 MHz, $CDCl_3$) δ: 21.59 (CO*C*H_3), 22.51 (*C*$H_3C_6H_4$), 26.71 and 26.85 (C(*C*H_3)$_2$), 65.48 (*C*H_2OTs), 65.75 (*C*H_2OAc), 68.93 (C-3), 80.72 (C-2), 84.65 (C-4), 105.34 (C-1), 115.06 (*C*(CH_3)$_2$), 129.08 (C-2′ and C-6′), 130.73 (C-3′ and C-5′), 133.61 (C-1′), 145.84 (C-4′) and 170.94 (CO); IR (KBr): 554, 666, 789, 840, 873, 984, 1033, 1097, 1121, 1178, 1191, 1225, 1310, 1455, 1495, 1598, 1750, 2117, 2932, 2959 and 2989 cm^{-1}; FAB-HRMS: *m/z* 464.1110 ($[M+Na]^+$, $C_{18}H_{23}N_3O_8SNa$ calcd 464.1103).

4.1.4. 1-Acetoxymethyl-6-amino-3,4-*O*-isopropylidene-2-oxabicyclo [3.2.0] heptane (5). The tosylated compound **4** (220 mg, 0.5 mmol) was dissolved in ethyl acetate in a reaction flask degassed with hydrogen and half mol equiv 10% Pd–C (65 mg) was added and the reaction was heated up to 60°C in an atmosphere of hydrogen and monitored by TLC. At completion, the catalyst was filtered off and from the filtrate, the solvent was evaporated under reduced pressure to yield the crude product. The pure bicyclo compound **5** was obtained as a colorless oil (79 mg) in 65% yield by column chromatography on elution with petroleum ether–ethyl acetate (3:1).; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.32 and 1.56 (6H, 2s, 3H each, 2×CH_3), 2.09 (3H, s, OCOCH_3), 3.71 (2H, s, C-7H), 3.89 and 4.08 (2H, dd, J=9.3 Hz each, CH_2OCOCH_3), 4.32 (1H, m, C-5H), 4.65 (1H, m, C-4H) and 6.03 (1H, d, J=4.1 Hz, C-3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 21.20 (CO*C*H_3), 27.03 and 27.83 (C(*C*H_3)$_2$), 55.30 (C-5), 65.03 (C-7), 66.51 (*C*H_2OAc), 79.80 (C-4), 88.80 (C-1), 110.50 (C-3), 113.50 (–*C*(CH_3)$_2$) and 171.51 (CO); IR (neat film): 1020, 1040,1080, 1140, 1230, 1370, 1642, 1742 (OCOCH_3), 2850, 2928 and 3335(NH) cm^{-1}; FAB-HRMS: *m/z* 487.2303 ($[M2+H]^+$, 2×($C_{11}H_{17}NO_5$)+H calcd 487.2292).

4.1.5. 3-*O*-Benzyl-5-(*O*-*p*-toluenesulphonyl)-4-*C*-(*p*-toluenesulphonyloxymethyl)-1,2-*O*-(isopropylidene)-β-L-threo-xylofuranose (7). Diol **6**[14,15] (245 mg, 1 mmol) was dissolved in minimum amount of pyridine and 2 mol equiv. tosyl chloride was added, the reaction mixture was stirred at 0°C and the progress monitored by TLC. On completion of the reaction, the contents were poured over ice-cold water, neutralization of aqueous solution with dilute hydrochloric acid yielded a white solid which was chromatographed over silica gel using petroleum ether–ethyl acetate (4:1) as eluent to yield the pure compound **7** as a white solid (495 mg) in 76% yield, mp 78–79°C; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.25 and 1.35 (6H, 2s, 3H each, 2×CH_3), 2.44 and 2.51 (6H, 2s, 3H each, 2×C$H_3C_6H_4$), 4.00 (1H, br s, C-3H), 4.08 (4H, m, 2×CH_2OTs), 4.47 (1H, d, J=11.6 Hz, C$H_\alpha C_6H_5$), 4.50 (2H, m, C$H_\beta C_6H_5$ and C-2H), 5.80 (1H, d, J=4.2 Hz, C-1H), and 7.30 and 7.70 (13H, 2m, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 23.65 (2×*C*$H_3C_6H_4$), 28.10 and 28.80 (C(*C*H_3)$_2$), 69.51 and 69.60 (2×*C*H_2OTs), 74.13 (O*C*H_2Ph), 85.44 (C-2), 86.38 (C-3), 87.69 (C-4), 107.40 (C-1), 115.15 (*C* (CH_3)$_2$), and 129.71, 130.01, 130.05, 130.16, 130.56, 131.83, 131.98, 134.24, 134.39, 138.55, 146.92 and 147.18 (aromatic carbons); IR (Nujol): 992, 1097, 1178, 1211, 1267, 1307, 1372, 1455, 1598 and 2923 cm^{-1}; FAB-HRMS: *m/z* 641.1516 ($[M+Na]^+$, $C_{30}H_{34}O_{10}S_2Na$ calcd 641.1491).

4.1.6. 5-(*O*-*p*-Toluenesulphonyl)-4-*C*-(*p*-toluenesulphonyloxymethyl)-1,2-*O*-(isopropylidene)-β-L-threo-xylofuranose (8). Compound **7** (310 mg, 0.5 mmol) was dissolved in dry ethyl acetate (10 mL) and half molar equiv. of palladium on activated charcoal was added to the reaction mixture. The contents were degassed with hydrogen and allowed to stir for 24 h at 25–28°C in the presence of hydrogen gas. The reaction was monitored by TLC and at completion, the charcoal was filtered off and the residue obtained on evaporation of the solvent was purified by column chromatography using petroleum ether–ethyl acetate (2:3) as eluent to afford the debenzylated compound **8** as a white solid (172 mg) in 63% yield, mp 164–65°C; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.21 and 1.28 (6H, 2s, 3H each, 2×CH_3), 2.47 (6H, s, 2×C$H_3C_6H_4$), 4.15 (5H, m, 2×CH_2OTs and C-3H), 4.56 (1H, m, C-2H), 5.86 (1H, d, J=3.8 Hz, C-1H), 7.52 and 7.80 (8H, 2m, aromatic protons); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 21.55 (2×*C*$H_3C_6H_4$), 25.83 and 26.53 (C(*C*H_3)$_2$), 68.18 and 68.75 (2×*C*H_2OTs), 76.35 (C-3), 86.97 (C-4), 88.03 (C-2), 106.57 (C-1), 112.93 (*C*(CH_3)$_2$), and 128.35, 128.77, 128.80, 129.11, 130.51, 130.83, 131.00, 131.34, 133.36,

133.55, 145.96 and 146.22 (aromatic carbons); IR (Nujol): 853, 897, 976, 992, 1179, 1365, 1456, 1598, 2925 and 3551 cm^{-1}; FAB-HRMS: *m/z* 551.1063 ($[M+Na]^+$, $C_{23}H_{28}O_{10}S_2Na$ calcd 551.1022).

4.1.7. 2,6-Dioxo-3,4-isopropylidene-1-(*p*-toluenesulphonyloxymethyl)-bicyclo [3.2.0] heptane (9). Sodium hydride (2 mol equiv., 20 mg) was taken in a three-necked flask and stirred with a few drops of DMF, followed by addition of toluene (10 mL) and compound **8** (160 mg, 0.3 mmol) and the reaction mixture allowed to stir under an atmosphere of dry nitrogen for 30 min. The temperature was slowly increased to 42–45°C and the reaction monitored by TLC. The reaction was quenched by adding methanol and extracted with ethyl acetate to yield the crude product, which was purified by column chromatography using petroleum ether–ethyl acetate (4:1) as eluent to yield **9** as a white solid (70 mg) in 61% yield, mp 80–81°C; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.33 and 1.40 (6H, 2s, 3H each, 2×CH_3), 2.46 (3H, s, $CH_3C_6H_4$), 4.19 (2H, s, CH_2OTs), 4.29 and 4.63 (2H, dd, J=7.7 Hz each, C-7H), 4.66 (1H, d, J=3.1 Hz, C-5H), 4.98 (1H, m, C-4H), 6.20 (1H, d, J=3.2 Hz, C-3H), and 7.36 and 7.81 (4H, 2d, 2H each, J=8.2 Hz each, C-2′H, C-3′H, C-5′H and C-6′H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.00 ($CH_3C_6H_4$), 26.70 and 28.00 ($C(CH_3)_2$), 68.42 (CH_2OTs), 78.70 (C-7), 84.60 (C-4), 86.00 (C-1), 87.80 (C-5), 108.20 (C-3), 115.20 ($C(CH_3)_2$), 128.30 and 130.30 (C-2′, C-3′, C-5′, C-6′), 132.64 (C-4′) and 145.71 (C-1′); IR (Nujol): 831, 1176, 1368, 1454, 1597 and 2929 cm^{-1}; FAB-HRMS: *m/z* 379.0811 ($[M+Na]^+$, $C_{16}H_{20}O_7SNa$ calcd 379.0827).

4.1.8. 2,6-Dioxo-3,4-*O*-isopropylidene-1-(*N*-pyrrolidinylmethyl)-bicyclo [3.2.0] heptane (10). Compound **9** (35 mg, 0.1 mmol) was dissolved in methanol (6 mL) and a few drops of triethylamine were added to the reaction mixture with continuous stirring. An equimolar amount of pyrrolidine was then added and the solution refluxed for 24 h. The reaction was worked-up by evaporation of the solvent under reduced pressure and the crude product was purified by column chromatography with a solvent system of petroleum ether–ethyl acetate (1:3) to yield compound **10** as an yellow oil (19 mg) in 67% yield. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.36 and 1.52 (6H, 2s, 3H each, 2×CH_3), 1.42 and 1.57 (4H, 2m, 2H each, C-3′H and C-4′H), 2.44 and 2.52 (4H, 2m, 2H each, C-2′H and C-5′H), 2.64 and 2.85 (2H, dd, J=13.7 Hz each, CH_2N), 4.35 and 4.76 (2H, 2d, J=7.2 Hz each, C-7H), 4.64 (1H, d, J=3.2 Hz, C-5H), 4.92 (1H, m, C-4H) and 6.21 (1H, d, J=3.2 Hz, C-3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 27.79 (C-3′ and C-4′), 28.82 and 29.93 ($C(CH_3)_2$), 57.56 (C-2′ and C-5′), 63.96 (CH_2N), 79.10 (C-7), 86.13 (C-4), 90.45 (C-1), 90.80 (C-5), 109.72 (C-3) and 116.36 ($C(CH_3)_2$); IR (neat film): 782, 852, 876, 959, 1013, 1067, 1096, 1173, 1228, 1320, 1371, 1381, 1454, 2852, 2932 cm^{-1}; FAB-HRMS: *m/z* 278.1379 ($[M+Na]^+$, $C_{13}H_{21}NO_4Na$ calcd 278.1368).

4.1.9. 2,6-Dioxo-3,4-*O*-isopropylidene-1-(*N*-piperidinylmethyl)-bicyclo [3.2.0] heptane (11). Compound **9** (35 mg, 0.1 mmol) was dissolved in methanol (6 mL) and a few drops of triethylamine were added to the reaction mixture with continuous stirring. An equimolar amount of piperidine was then added and the solution refluxed for 24 h. The reaction was worked-up by evaporation of the solvent under reduced pressure and the crude product was purified by column chromatography with a solvent system of petroleum ether–ethyl acetate (1:3) to yield compound **11** as an yellow oil (16 mg) in 64% yield. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.36 and 1.52 (6H, 2s, 3H each, 2×CH_3), 1.83 (6H, br s, C-3′H, C-4′H and C-5′H), 2.74 (4H, br s, C-2′H and C-6′H), 2.84 and 3.21 (2H, dd, J=13.2 Hz each, NCH_2), 4.40 (1H, d, J=7.4 Hz, C-7H_α), 4.66 (1H, d, J=3.3 Hz, C-5H), 4.83(1H, d, J=7.4 Hz, C-7H_β), 4.94 (1H, m, C-4H) and 6.23 (1H, d, J=3.3 Hz, C-3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 23.57 (C-4′), 27.13 and 27.93 ($C(CH_3)_2$), 29.40 and 29.74 (C-3′ and C-5′), 55.39 (C-2′ and C-6′), 59.08 (CH_2N), 80.81 (C-7), 84.25 (C-4), 87.81 (C-1), 88.95 (C-5), 108.5 (C-3) and 114.52 ($C(CH_3)_2$); IR (neat film): 849, 959, 1014, 1068, 1090, 1163, 1229, 1372, 1379, 1461, 2854, 2925 cm^{-1}; FAB-HRMS: *m/z* 292.1540 ($[M+Na]^+$, $C_{14}H_{23}NO_4Na$ calcd 292.1525).

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References

1. Cool, E. T. *Chem. Rev.* **1997**, *97*, 1473–1488.
2. Marquez, V. E.; Ezzitouni, A.; Russ, P.; Siddiqui, M. A.; Ford, H., Jr.; Feldman, R. J.; Mitsuya, H.; George, C.; Barchi, J. J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 2780–2789.
3. Wengel, J. *Acc. Chem. Res.* **1999**, *32*, 301–310.
4. Babu, B. R.; Prasad, A. K.; Trikha, S.; Thorup, N.; Parmar, V. S.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2509–2519.
5. Vince, R.; Hua, M. *J. Med. Chem.* **1990**, *33*, 17–21.
6. Ozaki, S.; Akiyama, T.; Ike, Y.; Mori, H.; Hoshi, A. *Chem. Pharm. Bull.* **1990**, *37*, 3405–3417.
7. Suhaldonik, R. J. *Nucleoside Antibiotics*; Wiley Interscience: New York, 1970.
8. Singh, S. K.; Kumar, R.; Wengel, J. *J. Org. Chem.* **1998**, *63*, 10035–10039.
9. Singh, I.; Hecker, W.; Prasad, A. K.; Parmar, V. S.; Seitz, O. *Chem. Commun.* **2002**, 500–501.
10. Beuck, C.; Singh, I.; Bhattacharya, A.; Hecker, W.; Parmar, V. S.; Seitz, O.; Weinhold, E. *ChemBioChem*, Under Communication.
11. (a) Hu, S.; Gupta, P.; Prasad, A. K.; Gross, R. A.; Parmar, V. S. *Tetrahedron Lett.* **2002**, *43*, 6763–6766. (b) Prasad, A. K.; Himanshu,; Bhattacharya, A.; Olsen, C. E.; Parmar, V. S. *Bioorg. Med. Chem.* **2002**, *10*, 947–951. (c) Poonam,; Prasad, A. K.; Azim, A.; Kumar, R.; Jain, S. C.; Parmar, V. S.; Olsen, C. E.; Errington, W. *Tetrahedron* **2001**, *57*, 7395–7402. (d) Kumar, R.; Azim, A.; Kumar, V.; Sharma, S. K.; Prasad, A. K.; Howarth, O. W.; Olsen, C. E.; Jain, S. C.; Parmar, V. S. *Bioorg. Med. Chem.* **2001**, *9*, 2643–2652. (e) Mukherjee, S.; Prasad, A. K.; Parmar, V. S.; Howarth, O. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2117–2121. (f) Parmar, V. S.; Prasad, A. K.; Pati, H. N.; Kumar, R.; Azim, A.; Roy, S.; Errington, W.

Bioorg. Chem. **1999**, *27*, 119–134. (g) Parmar, V. S.; Kumar, A.; Bisht, K. S.; Mukherjee, S.; Prasad, A. K.; Sharma, S. K.; Wengel, J.; Olsen, C. E. *Tetrahedron* **1997**, *53*, 2163–2176. (h) Parmar, V. S.; Singh, A.; Bisht, K. S.; Kumar, N.; Belokon, Y. N.; Kochetkov, K. A.; Ikonnikov, N. S.; Orlova, S. A.; Tararov, V. I.; Saveleva, T. F. *J. Org. Chem.* **1996**, *61*, 1223–1227. (i) Parmar, V. S.; Bisht, K. S.; Singh, A.; Jha, A. *Proc. Indian Acad. Sci. (Chem. Sci.)* **1996**, *108*, 575–583. (j) Prasad, A. K.; Sorensen, M. D.; Parmar, V. S.; Wengel, J. *Tetrahedron Lett.* **1995**, *36*, 6163–6166. (k) Parmar, V. S.; Sinha, R.; Bisht, K. S.; Gupta, S.; Prasad, A. K.; Taneja, P. *Tetrahedron* **1993**, *49*, 4107–4116. (l) Bisht, K. S.; Parmar, V. S.; Crout, D. H. G. *Tetrahedron: Asymmetry* **1993**, *4*, 957–958. (m) Parmar, V. S.; Prasad, A. K.; Sharma, N. K.; Vardhan, A.; Pati, H. N.; Sharma, S. K.; Bisht, K. S. *Chem. Commun.* **1993**, 27–29. (n) Parmar, V. S.; Prasad, A. K.; Sharma, N. K.; Singh, S. K.; Pati, H. N.; Gupta, S. *Tetrahedron* **1992**, *48*, 6495–6498.

12. Surzhykov, S. A.; Krayevsky, A. A. *Nucleosides Nucleotides* **1994**, *13*, 2283–2305.
13. (a) Obika, S.; Andoh, J.; Sugimoto, T.; Miyashita, K.; Imanishi, T. *Tetrahedron Lett.* **1999**, *40*, 6465–6468. (b) Olsen, A. G.; Rajwanshi, V. K.; Nielsen, C.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3610–3614.
14. Sharma, S. K.; Roy, S.; Kumar, R.; Parmar, V. S. *Tetrahedron Lett.* **1999**, *40*, 9145–9146.
15. Youssefyeh, R. D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1979**, *44*, 1301–1309.