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Anti-AIDS compounds: A new synthesis of β-thymidine from D-xylose¶

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Abstract. β-thymidine is a key intermediate in the manufacturing of anti-AIDS agents – AZT and d_4 T. A practical and economically viable route to β-thymidine starting from inexpensive D-xylose has been described. This synthetic route is based on a new rearrangement leading to 2,2′-anhydro-formation with concomitant epimerisation at C-3′, observed for the first time in nucleoside chemistry.

Keywords. 3′-Azido-3′-deoxy-β-thymidine; β-thymidine; D-xylose; anti-AIDS agents.

1. Introduction

During the past few years, several hundred nucleosides lacking a 3′-hydroxyl group have been tested for anti-HIV activity (Mansuri and Hitchcock 1992). However, azidothymidine (AZT) (2) in which an azido group (N_3) replaces the 3′-hydroxyl of thymidine is undoubtedly the most effective one. Several synthetic approaches have been reported for AZT (2) (Huryn and Okabe 1992), the classical approach starting from β-thymidine (1) still being the most common one commercially practised (*Drugs of the Future* 1986). β-Thymidine (1) being the key intermediate, undoubtedly dictates the price of AZT and other nucleosides such as d_4T (3) (Joshi *et al* 1992). The most logical route to β-thymidine involves the coupling of suitably substituted 2-deoxy-D-ribofuranose and thymine (Hubbard *et al* 1984). This reaction invariably produces a mixture of α- and β-nucleosides whose separation needs extensive chromatography. With D-ribose as a precursor, the possibility of α- and β-nucleoside formation was avoided giving predominantly the β-isomer. However, expensive D-ribose as a raw material poses severe limitations for β-thymidine (1) manufacturing (Freskos and Senaratna 1990). As a part of our continued interest (Gurjar *et al* 1993), we herein describe a new rearrangement in nucleoside chemistry leading to the formation of 2,2′-anhydro-nucleoside with concomitant epimerisation at C-3′. The 2,2′-anhydro derivatives are versatile precursors in chemical transformations. For instance (Rama Rao *et al* 1994), this report describes the transformation of 2,2′-anhydro derivative 8, obtained from an inexpensive D-xylose (4), into β-thymidine (1).

¶Dedicated to Prof C N R Rao on the occasion of his 60th birthday

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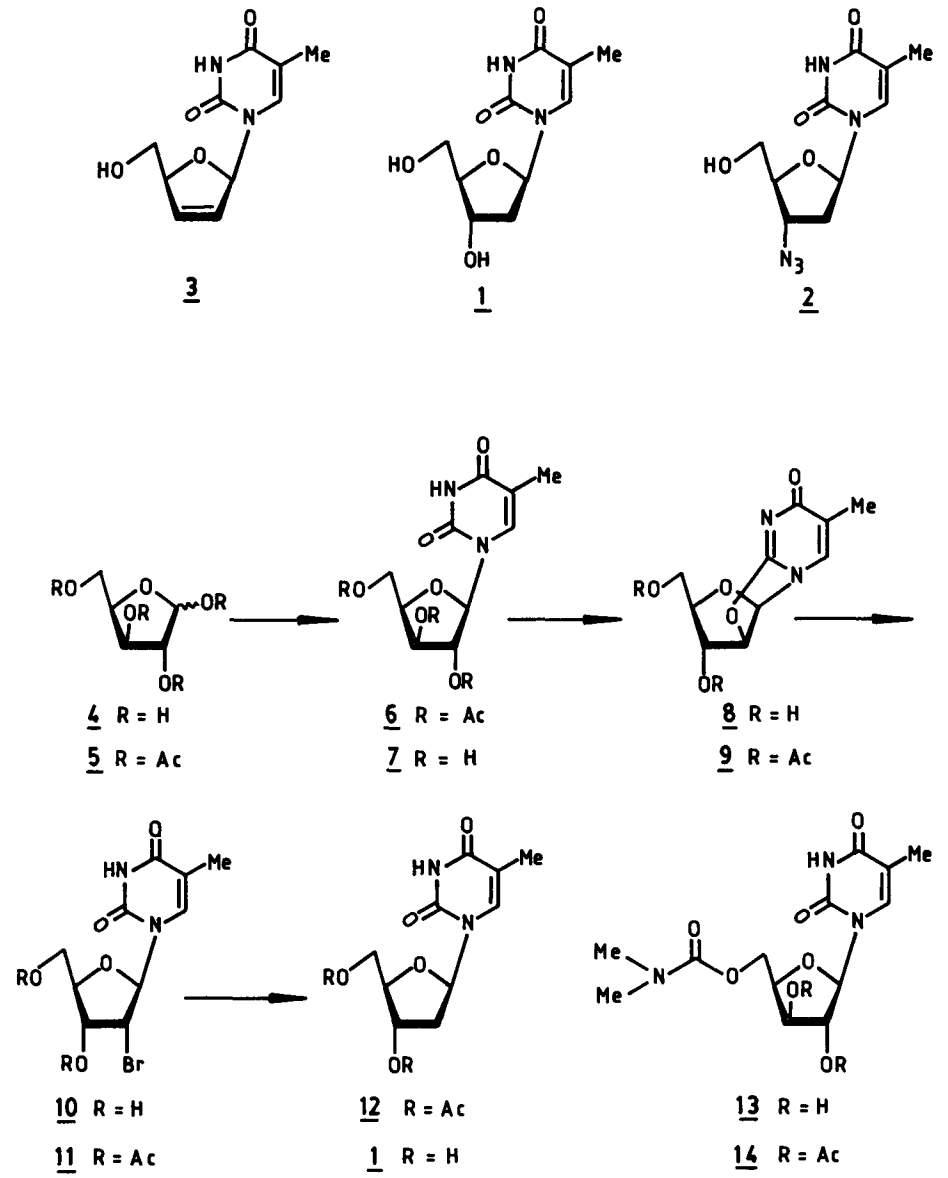


Scheme 1.

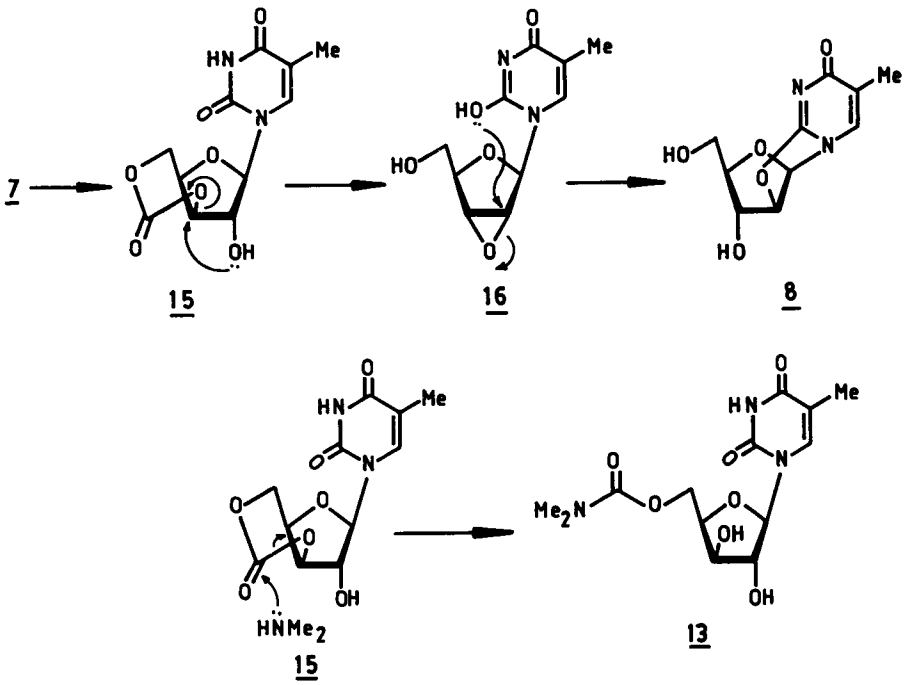
2. Results and discussion

D-Xylose (4) was converted into 1,2,3,5-tetra-O-acetyl-D-xylofuranose (5) (Reist and Goodmann 1964). Subsequent condensation of 5 with O,O-*bis*(trimethylsilyl)thymine in the presence of $SnCl_4$ in CH_2Cl_2 at room temperature, followed by deacetylation under Zemplen condition gave 7 in 77% overall yield (Gosselin *et al* 1986). Treatment of 7 with diphenylcarbonate in DMF containing a catalytic amount of $NaHCO_3$ at 150°C, gave two products after chromatography. The non-polar product was assigned the structure 13 based on the ^1H NMR and mass spectral analysis. Subsequently 13

was transformed into the corresponding diacetate derivative 14 by using Ac_2O and pyridine. The ^1H NMR and mass spectral analysis established the structure of 14.

The second fraction provided the 2,2′-anhydro-derivative (8) in 55% yield. Upon treatment with Ac_2O and pyridine, 8 gave the corresponding diacetate 9. Comparison of ^1H NMR, IR, mass spectra and optical rotation values with that of the authentic sample prepared from D-ribose, unequivocally confirmed the structure of 2,2′-anhydro derivatives (8 and 9).

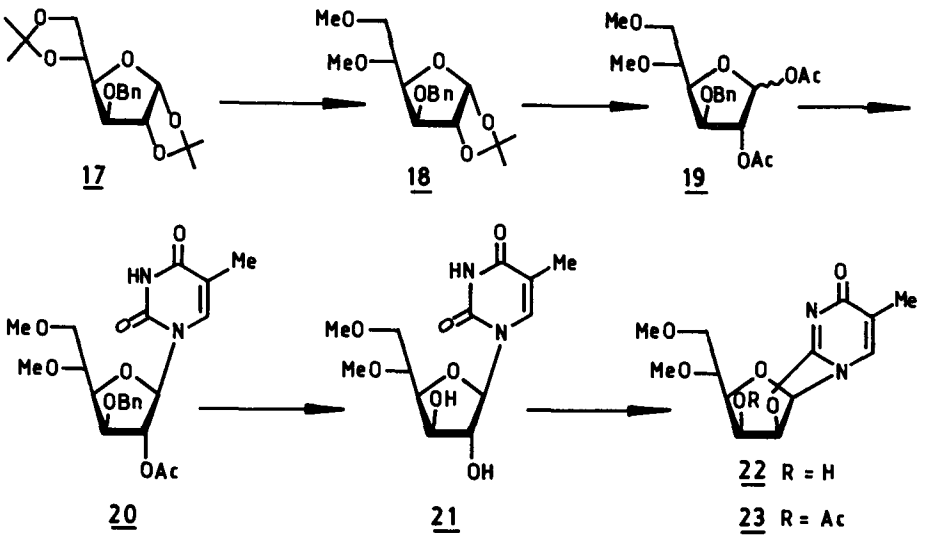
The formation of 2,2′-anhydro-derivative 8 with epimerisation at C-3′ is unusual in the field of nucleoside chemistry. The mechanism for the above rearrangement could be envisaged with the initial formation of 3′,5′-carbonate derivative 15. Subsequent rearrangement then formed the epoxide intermediate 16 which underwent ring opening reaction as indicated to form the product 8. The carbonate intermediate 15 could also explain the formation of 13 by the attack of dimethylamine, the latter perhaps formed as a result of DMF decomposition at elevated temperature.



Scheme 2.

In order to convert 8 or the corresponding diacetate (9) into β-thymidine, treatment with pyridinium-hydrobromide salt in pyridine or HBr in DMF was conducted to give the 2′-bromo-2′-deoxy derivatives 10 and 11 respectively. Subsequent hydrogenation over Raney Nickel and deacetylation of 11 gave β-thymidine (1). It is pertinent to mention that conversion of the diacetate (9) into β-thymidine (1) was practically more adaptable in our hands.

In order to substantiate our mechanism, we envisaged that if the 5′-position of the nucleoside is blocked, the anhydro formation may occur without C-3′ epimerisation. With this view, the synthesis of the precursor 21 became our first concern and its



Scheme 3.

synthesis from 3-O-benzyl-1,2-5,6-di-O-isopropylidene-α-D-glucofuranose (17) (Berry and Hall 1976) was sought. Compound 17 was converted into the dimethylether derivative 18 with initial hydrolysis of the 5,6-O-isopropylidine group with 0·8% H_2SO_4 in methanol followed by methylation with NaH-MeI. With 6N H_2SO_4 at 100°C, the 1,2-isopropylidine group in 18 was cleaved and then conventionally acetylated to give 19. Treatment of 19 with O,O-*bis*(trimethylsilyl)thymine in presence of $SnCl_4$ at room temperature furnished the nucleoside derivative 20. Removal of the protecting groups in 20 by successive deacetylation and hydrogenolysis furnished the required diol 21. Reaction of 21 with diphenylcarbonate/$NaHCO_3$ in DMF at 150°C was found to be sluggish. After 20 h, the reaction mixture was worked-up and the major product (22) isolated in 20% yield. It was then converted into the diacetate derivative 23 by employing Ac_2O and pyridine. On comparison of ^1H NMR data, particularly the chemical shift values and coupling constants for H-2′ and H-3′, the structures 22 and 23 were assigned. For example, H-2′ and H-3′ in 23 appeared as characteristic triplets with $J = 6{\cdot}3$ Hz indicating *cis* relationship between them. In addition the mass spectra of 22 and 23 revealed molecular ion peaks at 298 and 340 respectively. The above experiment revealed that the epimerisation at C-3′ had not occurred.

3. Experimental

2′,3′,5′-Tri-O-acetyl-β-D-xylofuranosylthymine (6)

To a slurry of 1,2,3,5-tetra-O-acetyl-D-xylofuranose (5) (10·0 g, 31·5 mmol) and O,O-*bis*(trimethylsilyl)thymine (10·2 g, 37·7 mmol) in dry CH_2Cl_2 (40 ml) under nitrogen was added dropwise a solution of $SnCl_4$ (9·8 g, 37·7 mmol) in dry CH_2Cl_2 (10 ml). After 18 h at room temperature, the reaction was quenched with aqueous $NaHCO_3$, filtered through celite, the filtrate dried and concentrated. The residue was

purified by column chromatography on silica gel using chloroform–methanol (98:2) as eluent to give 6 (9·9 g, 82%), as a syrup, $[\alpha]_D$ 11·3° (*c* 1·1, chloroform), ^{1}H NMR ($CDCl_3$): δ 1·89 (*s*, 3H), 2·05 (*bs*, 9H), 4·29 (*m*, 2H), 4·42 (*m*, 1H), 5·13 (*m*, 1H), 7·26 (*s*, 1H), 9·79 (*s*, 1H).

3′,5′-Di-O-acetyl-2,2′-anhydro-β-D-arabinofuranosylthymine (9) and 2′,3′-di-O-acetyl-5′-O-(N,N-dimethylaminocarbonyl)-β-D-xylofuranosylthymine (14)

To a solution of 6 (9·1 g, 28·8 mmol) in methanol (100 ml) was added sodium metal (0·14 g). The solution was stirred at room temperature for 8 h, neutralised with Amberlite IR 120 H^+ resin, filtered and concentrated to afford 7 (5·75 g, 94%).

A mixture of 7 (5·0 g, 19·4 mmol), diphenylcarbonate (4·6 g, 21·5 mmol) and $NaHCO_3$ (0·16 g) in DMF (10 ml) was heated to 150°C for 4 h. The reaction mixture was diluted with methanol and ether was added to precipitate the solid which was filtered and chromatographed on silica gel by eluting first with chloroform–methanol (96:4) to give 13 (1·24 g, 20%). ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1·93 (*s*, 3H), 2·96 (*s*, 6H), 4·07 (*bs*, 1H), 4·21 (*bs*, 1H), 3·24–3·41 (*m*, 2H), 4·52 (*m*, 1H), 5·00 (*t*, J = 2·8 Hz, 1H), 5·31 (*bs*, 1H), 5·82 (*s*, 1H), 7·59 (*s*, 1H), 10·23 (*bs*, 1H): MS:*m/z* 329 (M^+). 13 (1·0 g, 3·04 mmol) was treated with Ac_2O (2 ml) and pyridine (5 ml) for 2 h. After usual workup, the residue was chromatographed on silica gel using chloroform–methanol (99:1) to give 14 (1·33 g, 85%), as a syrup. $[\alpha]_D$ 14·2° (*c* 1·1, chloroform); ^{1}H NMR ($CDCl_3$): δ 1·93 (*s*, 3H), 2·12 (*s*, 6H), 2·91 (*s*, 6H), 4·33 (*m*, 2H), 4·48 (*m*, 1H), 5·12 (*t*, J = 2·4 Hz, 1H), 5·38 (*m*, 1H), 6·02 (*d*, J = 2·4 Hz, 1H), 7·29 (*s*, 1H), 8·71 (*bs*, 1H).

The second fraction isolated by using chloroform–methanol (92:8) gave 8 (2·55 g, 55%) which was treated with Ac_2O (3 ml) and pyridine (10 ml) for 1 h. After usual workup, the residue was chromatographed on silica gel using chloroform–methanol (96:4) to give 9 (2·8 g, 90%), m.p. 178°C; $[\alpha]_D$-75° (*c* 1·2, chloroform); ^{1}H NMR ($CDCl_3$): δ 1·89 (*s*, 3H), 1·92 (*s*, 3H), 2·13 (*s*, 3H), 3·96 (*dd*, 1H, J = 4·2, 12·7 Hz), 4·25 (*dd*, 1H, J = 3·8, 12·7 Hz), 4·44 (*bs*, 1H), 5·34 (*bs*, 1H), 5·40 (*d*, 1H, J = 6·3 Hz), 6·29 (*d*, 1H, J = 6·3 Hz), 7·20 (*s*, 1H); MS:*m/z* 324 (M^+).

β-Thymidine (1)

A solution of hydrobromic acid (1·5 g dissolved in 20 ml of DMF) and 9 (5·0 g, 15·4 mmol) was stirred at 100–110°C for 2 h under nitrogen. The mixture was then poured into ice water and extracted with chloroform. The chloroform layer was washed with aqueous $NaHCO_3$, water, dried and concentrated to give 2′-bromo-2′-deoxy-3′,5′-di-O-acetylthymidine (11) (5·7 g, 91%); ^{1}H NMR ($CDCl_3$): δ 1·95 (*s*, 3H), 2·16 (*s*, 3H), 2·19 (*s*, 3H), 4·39 (*bs*, 3H), 4·51 (*t*, J = 4·65 Hz, 1H), 5·19 (*m*, 1H), 6·23 (*d*, J = 4·65 Hz, 1H), 7·19 (*s*, 1H), 8·75 (*bs*, 1H): MS:*m/z* 406 (M^+ + 2), 404 (M^+).

The above product 11 (5·0 g, 12·3 mmol), with Raney-Ni (5 g) in methanol (30 ml), was hydrogenated at 40 psi for 5 h. The reaction mixture was filtered through celite, concentrated, redissolved in ethyl acetate (30 ml) and washed with water. The organic layer was dried and concentrated. The residue (3·5 g) was dissolved in methanol (20 ml) containing sodium (62 mg), stirred at room temperature for 8 h, neutralised with Amberlite IR 120 H^+ resin, filtered and concentrated to give β-thymidine (1) (2·3 g, 76% from 11); m.p. 182–184°C, lit. 186–187°C; $[\alpha]_D$ 18·8° (*c* 1·0, H_2O), lit. $[\alpha]_D$ 18·5° (*c* 1, H_2O).

3-O-Benzyl-1,2-di-O-isopropylidene-5,6-di-O-methyl-α-D-glucofuranose (18)

A mixture of 17 (6 g, 17·7 mmol) in methanol (60 ml) and 0·8% sulphuric acid (24 ml) was stirred at room temperature for 6 h. The reaction mixture was neutralised with barium carbonate, filtered through celite, washed with methanol and the combined filtrate concentrated. The crude diol (4·68 g) was added to a cooled slurry of sodium hydride (2·9 g, 50% suspension, 60·4 mmol) in dry THF (20 ml). It was stirred at room temperature for 3 h, cooled to 0°C and then MeI (5·35 g, 37·7 mmol) was introduced. After 18 h at room temperature, excess sodium hydride was quenched with methanol and concentrated. The residue in ethyl acetate was successively washed with water, dried and concentrated. The residue was purified by chromatography on silica gel by using chloroform–methanol (99:1) to give 18 (3·8 g, 75%); $[\alpha]_D$-20° (*c* 1·4, chloroform); ^{1}H NMR ($CDCl_3$): δ 1·29, 1·47 (2*s*, 6H), 3·35 (*s*, 6H), 3·4–3·8 (*m*, 3H), 3·96 (*d*, $J = 4·0$ Hz, 1 H), 4·14 (*dd*, $J = 4·0$ and 8·3 Hz, 1 H), 4·48 (*d*, $J = 4·0$ Hz, 1 H), 4·55 (*ABq*, 2H), 5·83 (*d*, $J = 4$ Hz, 1H).

2'-O-Acetyl-3'-O-benzyl-5',6'-di-O-methyl-β-D-glucofuranosylthymine (20)

Compound 18 (3 g, 8·88 mmol) and 3N H_2SO_4 (7·5 ml) in dioxan (18 ml) were heated at 95–100°C for 2 h, neutralised with barium carbonate, filtered through celite and concentrated. The residue was acetylated with Ac_2O (3·1 ml) and pyridine (5·5 ml) in $CHCl_3$ (15 ml) in the presence of N,N-dimethylaminopyridine (20 mg). After usual workup the crude diacetate was purified by chromatography using hexane–ethyl acetate (85:15) to give 19 (3·16 g, 88%) as a mixture of α- and β-anomers.

To a solution of 19 (3·16 g, 8·28 mmol) and O,O-*bis*(trimethylsilyl)-thymine (2·7 g, 9·9 mmol) in dry CH_2Cl_2 (15 ml) was added a solution of $SnCl_4$ (2·4 g, 9·1 mmol) in portions. The reaction mixture was stirred at room temperature for 18 h, quenched with aqueous $NaHCO_3$, filtered through celite and extracted with CH_2Cl_2. The combined extracts were dried, concentrated and purified by silica gel chromatography using hexane–ethyl acetate (65:35) to give 20 (3·5 g, 94%). $[\alpha]_D$-17° (*c* 1·6, chloroform). ^{1}H NMR ($CDCl_3$): δ1·66 (*s*, 3H), 2·15 (*s*, 3H), 3·42, 3·48 (2*s*, 6H), 3·78 (*m*, 2H), 4·03 (*d*, $J = 2·5$ Hz, 1H), 4·10 (*dd*, $J = 2·5$ and 10·0 Hz, 1H), 4·68 (*ABq*, 2H), 5·10 (*s*, 1H), 6·12 (*s*, 1H), 7·64 (*m*, 5H), 8·99 (*s*, 1H).

5',6'-di-O-methyl-β-D-glucofuranosylthymine (21)

Compound 20 (3·5 g, 7·8 mmol) was deacetylated in methanolic sodium methoxide (0·1 g of Na in 25 ml of methanol). The reaction mixture was neutralised with Amberlite IR 120 H^+ resin and filtered. The solution was hydrogenated in the presence of 10% Pd–C (0·1 g) at normal pressure and temperature for 12 h. The catalyst was filtered and the filtrate concentrated and then purified by chromatography using chloroform–methanol (95:5) to give 21 (1·86 g, 75%), $[\alpha]_D$-24·5° (*c* 1·3, chloroform). ^{1}H NMR ($CDCl_3$):δ1·81 (*s*, 3H), 3·40, 3·54 (2*s*, 6H), 3·74 (*dd*, $J = 2·8$ and 11·4 Hz, 1H), 3·91 (*bs*, 1H), 4·03 (*s*, 1H), 4·23 (*bs*, 1H), 4·37 (*s*, 1H), 5·69 (*s*, 1H), 7·61 (*s*, 1H), 10·63 (*s*, 1H).

3'-O-Acetyl-5',6'-di-O-methyl-2',2'-anhydro-β-D-mannofuranosylthymine (23)

A mixture of 21 (0·5 g, 1·6 mmol), diphenylcarbonate (0·51 g, 2·37 mmol) and $NaHCO_3$ (20 mg) in DMF (2 ml) was heated at 150°C for 20 h. The reaction mixture was diluted with methanol (1 ml) and ether was added for precipitation. The solid was filtered

and chromatographed on silica gel using chloroform–methanol (96:4) to give 22 (96 mg, 20%). $[\alpha]_D$-47° (*c* 1·2, chloroform); ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1·91 (*s*, 3H), 3·36, 3·38 (2*s*, 6H), 3·2–3·7 (*m*, 3H), 4·03 (*dd*, J = 4·25 and 8·5 Hz, 1H), 4·49 (*dd*, J = 6·38, 1H), 5·38 (*t*, J = 6·38 Hz, 1H), 6·04 (*d*, J = 6·38 Hz, 1H), 7·40 (*s*, 1H). MS:*m/z* 298 (M^+).

Compound 22 (58 mg, 0·019 mmol) was acetylated with Ac_2O (0·5 ml) and pyridine (2 ml). After usual workup, the residue was purified by chromatography using chloroform–methanol (98:2) to give 23 (60 mg, 91%), m.p. 219°C; $[\alpha]_D$-120·5° (*c* 1·4, chloroform); ^{1}H NMR ($CDCl_3$): δ 2·0 (*s*, 3H), 2·12 (*s*, 3H), 3·34, 3·40 (2*s*, 6H), 3·45 (*m*, 2H), 3·66 (*m*, 1H), 4·28 (*dd*, J = 4·2 and 6·3 Hz, 1H), 5·42 (*t*, J = 6·3 Hz, 1H), 5·64 (*t*, J = 6·3 Hz, 1H), 6·02 (*d*, J = 6·3 Hz, 1H), 7·22 (*s*, 1H); MS:*m/z* 340 (M^+).

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