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Strategy for the Preparation of 2′ and 3′ Branched Nucleosides

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Abstract

1-(5-*O*-Benzyl-3-*C*-methylidene-2-oxo-3-deoxy-β-D-glycero-pentofuranosyl) thymine was prepared as the precursor of 2′,3′-dibranched nucleosides in six steps from 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose.

This enone is the key starting compound for the preparation of a large series of potential antiretroviral agents against HIV-1.

Recent progress in the medical management of HIV infection has shown that nucleoside reverse transcriptase inhibitors (AZT, ddI, ddC, 3TC and d4T), non-nucleoside reverse transcriptase inhibitors (nevirapine and delarvidine) and protease inhibitors (sequinavir, indinavir and ritonavir) all have antiretroviral activity (Morris-Jones et al 1997). Although the role of these new molecules is well documented (Huryn & Okabe 1992; De Clercq 1995a, b) the search for a new generation of antiretroviral agents continues because of growing concerns about resistance and long-term toxicity (De Clercq 1995a, b). One of the other nucleoside structure modifications explored, was the introduction of a double bond in the 2′,3′ position to give the 2′,3′-didehydro-2′,3′-dideoxyribonucleosides (d4Ns) (Balzarini et al 1987). Recent work on new 2′,3′-didehydro-3′-deoxythymidine (d4T) analogues described the preparation of 2′,3′-dibranched benzo (*c*) furan nucleoside in which the 2′,3′ double bond is incorporated into a benzene ring (Ewing et al 1996). We report the preparation of 1-(5-*O*-benzyl-3-*C*-methylidene-2-oxo-3-deoxy-β-D-glycero-pentofuranosyl) thymine (**8**) as a novel approach to obtain a new generation of 2′,3′-di-*C*-nucleosides such as the diene **9** and the 2′-*C*-3′-*C*-methylidene **10** (Figure 1).

Materials and Methods

Chemistry

Melting points were determined on a digital melting-point apparatus (Electrothermal) and are uncorrected. Optical rotations were recorded at 22°C in $CHCl_3$ solution with a digital polarimeter DIP-370 (Jasco) using a 1 dm cell. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ (internal standard: tetramethylsilane), respectively, at 300·11 MHz and at 75·47 MHz (Bruker AM WB-300). Key: s = singlet, bs = broad singlet, d = doublet, bd = broad doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet. TLC was performed on Silica F254 (Merck) and detection was by UV at 254 nm or by charring with phosphomolybdic–H_2SO_4 reagent. Column chromatography was effected on Silica Gel 60 (Merck, 230 mesh). Elemental analyses were performed by the Service Central de Micro-Analyse du Centre National de Recherche Scientifique (Vernaison, France).

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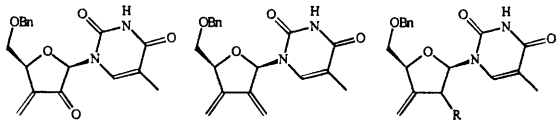
*5-*O*-Benzyl-1,2-*O*-isopropylidene-3-oxo-α-D-xylofuranose (***3***).* 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**2**) was added in one portion to a mixture of 3 Å molecular sieve powder (16·8 g) and pyridinium dichromate (16·8 g, 44·59 mmol) in anhydrous CH_2Cl_2 (35 mL). The solution was cooled at −15°C and acetic acid (1·8 mL, 32·11 mmol) was added dropwise. The mixture was stirred overnight at room temperature under N_2. Diethyl ether was added to the reaction mixture which was filtered through silica on a glass filter and the solvent was evaporated. H_2O (20 mL) and CH_2Cl_2 (50 mL) were added to the resulting brown residue. The organic phase was dried with $MgSO_4$ and evaporated. The crude product was purified by silica gel column chromatography (hexane–acetone, 9:1) to give **3** in 75% yield. ^{1}H NMR



8 9 10 R = alkyl, aryl...

Figure 1. 1-(5-*O*-Benzyl-3-*C*-methylidene-2-oxo-3-deoxy-β-D-glycero-pentofuranosyl) thymine (**8**) as precursor of 2′,3′-di-*C*-nucleoside **9** and 2′-*C*-3′-*C*-methylidene **10**.

($CDCl_3$) δ: 1·42 (3H, s, C*H*$_3$), 1·46 (3H, s, C*H*$_3$), 3·53 (1H, dd, J = 10·35 and 3·69, *H*-5a), 3·65 (1H, dd, J = 4·95, *H*-5b), 4·42 (1H, d, *H*-2), 4·56 (2H, s, C*H*$_2$), 4·57 (1H, bs, *H*-4), 6·16 (1H, d, J = 4·43, *H*-1), 7·26 (5H, bs, H_{arom}); ^{13}C NMR ($CDCl_3$) δ: 27·10 (1C, *C*H$_3$), 27·32 (1C, *C*H$_3$), 70·02 (1C, *C*-5), 73·03 (1C, *C*H$_2$), 76·73 (1C, *C*-4), 79·83 (1C, *C*-2), 103·50 (1C, *C*-1), 114·05 (1C, C_{iso}), 127·44, 127·78, 128·40 (5C, C_{arom}), 137·30 (1C, C_{ipso}), 209·86 (1C, *C*-3). Calculated for $C_{15}H_{18}O_5$: C, 64·74; H, 6·52. Found: C, 64·71; H, 6·53.

5-O-*Benzyl-1,2*-O-*isopropylidene-3*-C-*methylidene-3-deoxy-α-D-xylofuranose* (***4***). Lithium *n*-butyl (1·6 M in hexane, 13·5 mL, 21·56 mmol) was added dropwise to a solution of methyltriphenylphosphonium bromide (7·7 g, 21·56 mmol) in dry THF (20 mL) under N_2. After the solution became red, compound **3** (3·3 g, 10·78 mmol) in dry THF (20 mL) was added at 0°C. The mixture was stirred for 1 h at room temperature then poured into water (10 mL) and extracted twice with diethyl ether. The extract was worked up and the crude product was purified by silica gel column chromatography (hexane–acetone, 97:3) to give **4** in 50% yield. ^{1}H NMR ($CDCl_3$) δ: 1·35 (3H, s, C*H*$_3$), 1·48 (3H, s, C*H*$_3$), 3·53 (1H, dd, J = 10·35 and 3·69, *H*-5a), 3·65 (1H, dd, J = 4·95, *H*-5b), 4·86 (1H, d, *H*-4), 4·56 (2H, s, C*H*$_2$), 4·88 (1H, d, *H*-2), 5·16 (1H, s, C*H*), 5·39 (1H, s, C*H*), 5·85 (1H, d, J = 3·95, *H*-1), 7·26 (5H, bs, H_{arom}); ^{13}C NMR ($CDCl_3$) δ: 27·12 (1C, *C*H$_3$), 27·38 (1C, *C*H$_3$), 71·55 (1C, *C*-5), 73·41 (1C, *C*H$_2$), 78·58 (1C, *C*-4), 81·76 (1C, *C*-2), 104·57 (1C, *C*-1), 111·73 (1C, *C*H$_2$), 112·37 (1C, C_{iso}), 127·51, 127·59, 128·31 (5C, C_{arom}), 137·90 (1C, C_{ipso}), 146·70 (1C, *C*-3). Calculated for $C_{16}H_{20}O_4$: C, 69·55; H, 7·30. Found: C, 69·58; H, 7·31.

1,2-Di-O-*acetyl-5*-O-*benzyl-3*-C-*methylidene-3-deoxy-D-xylofuranose* (***5***). A mixture of **4** (1·45 g, 5·07 mmol), anhydride acetic acid (5 mL), and concentrated H_2SO_4 (43 μL) in acetic acid (46 mL) was stirred for 2·5 h at room temperature. The mixture was poured into ice-cooled water, stirred for 30 min, and extracted twice with $CHCl_3$, using saturated NH_4Cl water solution as an additive. The organic phase was washed with water, saturated $NaHCO_3$ solution, and brine. After drying ($MgSO_4$), the filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (hexane–acetone, 95:5) to give **5** in 77% yield. ^{1}H NMR ($CDCl_3$) **5α** δ: 1·89–2·10 (6H, s, C*H*$_3$), 3·56 (2H, bs, *H*-5a, *H*-5b), 4·52 (2H, s, C*H*$_2$), 4·78 (1H, bs, *H*-4), 5·18 (1H, s, C*H*), 5·22 (1H, s, C*H*), 5·59 (1H, d, *H*-2), 6·45 (1H, d, J = 4·47, *H*-1), 7·26 (5H, bs, H_{arom}); **5β** δ: 1·89–2·10 (6H, s, C*H*$_3$), 3·56 (2H, bs, *H*-5a, *H*-5b), 4·55 (2H, s, C*H*$_2$), 4·88 (1H, bs, *H*-4), 5·35 (1H, s, C*H*), 5·43 (1H, s, C*H*), 5·56 (1H, d, *H*-2), 6·15 (1H, s, *H*-1), 7·26 (5H, bs, H_{arom}); ^{13}C NMR ($CDCl_3$) **5α** δ: 21·00–20·43 (2C, *C*H$_3$), 72·05 (1C, *C*-5), 72·98 (1C, *C*H$_2$), 73·28 (1C, *C*-2), 79·31 (1C, *C*-4), 93·86 (1C, *C*-1), 109·06 (1C, *C*H$_2$), 127·55, 127·60, 128·31 (5C, C_{arom}), 137·85 (1C, C_{ipso}), 142·08 (1C, *C*-3), 169·17 (1C, *C*O); **5β** δ: 21·00–20·43 (2C, *C*H$_3$), 72·55 (1C, *C*-5), 73·29 (1C, *C*H$_2$), 77·74 (1C, *C*-2), 80·68 (1C, *C*-4), 98·95 (1C, *C*-1), 115·45 (1C, *C*H$_2$), 127·55, 127·60, 128·31 (5C, C_{arom}), 137·85 (1C, C_{ipso}), 142·08 (1C, *C*-3), 169·17 (1C, *C*O). Calculated for $C_{17}H_{20}O_6$: C, 63·74; H, 6·29. Found: C, 63·73; H, 6·31.

1-(2-O-*Acetyl-5*-O-*benzyl-3*-C-*methylidene-3-deoxy-β-D*-glycero-*pentofuranosyl) thymine* (***6***). A suspension of thymine (236 mg, 1·87 mmol) and a small crystal of ammonium sulphate in a mixture of 1,1,1,3,3,3-hexamethyldisilazane (4 mL) and trimethylchlorosilane (0·5 mL) was refluxed until a clear solution was obtained. Volatile matter was evaporated and the residue was repeatedly coevaporated with toluene. In the resulting syrup compound **5** (500 mg, 1·56 mmol) in dry CH_3CN (10 mL) was dissolved under N_2 and the mixture was cooled to −15°C. *tert*-Butyldimethylsilyl-trifluoromethanesulphonate (430 μL, 1·87 mmol) was added and the mixture left to warm slowly. After 2 h, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$, stirred for 30 min and extracted with CH_2Cl_2. The organic phase was dried with $MgSO_4$ and evaporated. The crude product was purified over silica gel column chromatography (hexane–ethyl acetate, 1:1) to give **6** in 55% yield. ^{1}H NMR ($CDCl_3$) δ: 1·59 (3H, s, C*H*$_3$), 2·10 (3H, s, C*H*$_3$), 3·60 (1H, dd, J = 10·10 and 2·27 *H*-5a), 3·85 (1H, bd, *H*-5b), 4·56 (1H, s, C*H*$_2$), 4·76 (1H, d, *H*-4), 5·21 (1H, s, C*H*), 5·33 (1H, s, C*H*), 5·83 (1H, d, *H*-2), 6·05 (1H, d, J = 5·64, *H*-1), 7·29 (5H, bs, H_{arom}), 7·49 (1H, d, *H*-6), 8·37 (1H, s, N*H*); ^{13}C NMR ($CDCl_3$) δ: 12·13 (1C, *C*H$_3$), 20·74 (1C, *C*H$_3$), 71·79 (1C, *C*-5), 73·65 (1C, *C*H$_2$), 75·81 (1C, *C*-4), 80·07 (1C, *C*-1), 86·44 (1C, *C*-2), 111·28 (1C, *C*H$_2$), 111·54 (1C, *C*-5),

127·60, 128·02, 128·57 (5C, C_{arom}), 135·65 (1C, *C*-6), 137·32 (1C, C_{ipso}), 142·79 (1C, *C*-3), 150·66 (1C, *C*-2), 163·79 (1C, *C*-4), 170·29 (1C, *C*O). Calculated for $C_{20}H_{22}O_6N_2$: C, 62·15; H, 5·74; N, 7·28. Found: C, 62·12; H, 5·75; N, 7·31.

*1-(5-*O*-Benzyl-3-*C*-methylidene-3-deoxy-β-D-*glycero*-pentofuranosyl) thymine (**7**).* Ammonia was bubbled into a solution of compound **6** in dry methanol for 1 h at 0°C. The mixture was left to warm to room temperature and stirred overnight. Volatile matter was evaporated and the residue was purified by silica gel column chromatography (ethyl acetate) to give **7** in quantitative yield. ^{1}H NMR ($CDCl_3$) δ: 1·45 (3H, s, C*H*$_3$), 3·59 (1H, bd, J = 10·18, *H*-5a), 3·83 (1H, bd, *H*-5b), 4·54 (1H, s, C*H*$_2$), 4·65 (1H, d, *H*-2), 4·76 (1H, bs, *H*-4), 5·15 (1H, s, C*H*), 5·43 (1H, s, C*H*), 5·89 (1H, d, J = 5·46, *H*-1), 6·92 (1H, s, N*H*), 7·29 (5H, bs, H_{arom}), 7·48 (1H, d, *H*-6); ^{13}C NMR ($CDCl_3$) δ: 11·99 (1C, *C*H$_3$), 72·28 (1C, *C*-5), 73·50 (1C, *C*H$_2$), 75·84 (1C, *C*-4), 79·98 (1C, *C*-1), 88·91 (1C, *C*-2), 109·61 (1C, *C*H$_2$), 110·87 (1C, *C*-5), 127·47, 127·81, 128·47 (5C, C_{arom}), 136·11 (1C, *C*-6), 137·59 (1C, C_{ipso}), 145·86 (1C, *C*-3), 151·64 (1C, *C*-2), 164·27 (1C, *C*-4). Calculated for $C_{18}H_{20}O_5N_2$: C, 62·76; H, 5·85; N, 8·17. Found: C, 62·75; H, 5·85; N, 8·14.

*1-(5-*O*-Benzyl-3-*C*-methylidene-2-oxo-3-deoxy-β-D-*glycero*-pentofuranosyl) thymine (**8**).* Pyridine (71 μL, 0·435 mmol) was added to stirred CrO_3 (43·5 mg, 0·435 mmol) at −15°C. After 2 min, the mixture became a yellow solid and anhydric acid (43 μL, 0·435 mmol) was added dropwise. Compound **7** (50 mg, 0·145 mmol) in CH_2Cl_2 (1 mL) was added to the pre-mixed complex, and the mixture was stirred at ambient temperature for 30 min. The resulting dark brown solution was filtered through silica gel (10 mL) and washed with ethyl acetate. The combined filtrate was concentrated (<25°C) under reduced pressure and precipitation gave compound **8** in 10% yield. ^{1}H NMR ($CDCl_3$) δ: 1·45 (3H, s, C*H*$_3$), 3·61 (1H, bd, J = 10·18, *H*-5a), 3·84 (1H, bd, *H*-5b), 4·53 (1H, s, C*H*$_2$), 4·72 (1H, bs, *H*-4), 5·22 (1H, s, C*H*), 5·34 (1H, s, C*H*), 5·89 (1H, d, J = 5·46, *H*-1), 6·90 (1H, s, N*H*), 7·28 (5H, bs, H_{arom}), 7·45 (1H, d, *H*-6); ^{13}C NMR ($CDCl_3$) δ: 11·99 (1C, *C*H$_3$), 72·28 (1C, *C*-5), 73·50 (1C, *C*H$_2$), 75·84 (1C, *C*-4), 79·98 (1C, *C*-1), 109·61 (1C, *C*H$_2$), 110·87 (1C, *C*-5), 127·47, 127·81, 128·47 (5C, C_{arom}), 136·11 (1C, *C*-6), 137·59 (1C, C_{ipso}), 145·86 (1C, *C*-3), 151·64 (1C, *C*-2), 164·27 (1C, *C*-4), 207·82 (1C, *C*-2). Calculated for $C_{18}H_{18}O_5N_2$: C, 63·13; H, 5·30; N, 8·22. Found: C, 63·11; H, 5·33; N, 8·18.

Results and Discussion

The 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**2**) was prepared by first converting D-xylose (**1**) into the 1,2-*O*-isopropylidene-α-D-xylofuranose, regio-selectively tosylating the primary hydroxyle and finally protecting the primary site by *O*-benzyl group (30% yield) (Kuzuhara & Emoto 1964). Pyridinium dichromate in dichloromethane in the presence of a 3 Å molecular sieve powder was used for the oxidation of **2** to give the desired 5-*O*-benzyl-1,2-*O*-isopropylidene-3-oxo-α-D-xylofuranose (**3**) in 75% yield.

Following Figure 2, application of the Wittig reaction by treatment of the ketone **3** with methylenetriphenyl phosphorane gave the 3′-*C*-methylidene **4** in 50% yield. The direct conversion of compound **4** to the diacetate **5** in 77% yield was achieved as noted by Kitano et al (1997) in an one-pot reaction by acetal deprotection and diacetylation, respectively, using acidic conditions. The glycoside **5** which was a mixture of α and β anomers (1:1) was converted regiospecifically to the β-nucleoside analogue **6** in 55% yield by standard Vorbruggen chemistry (Vorbruggen et al 1981; Wilson et al 1995) using *tert*-butyldimethylsilyltrifluoromethanesulphonate and silylated

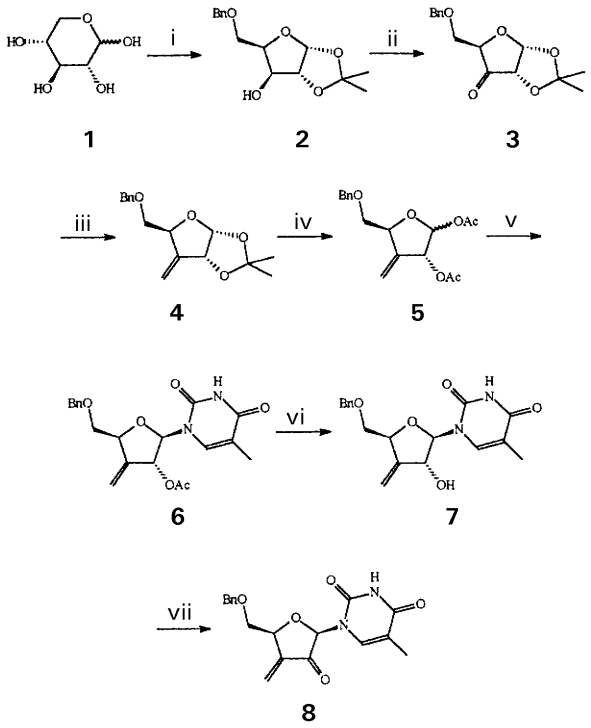


Figure 2. Preparation of 1-(5-*O*-benzyl-3-*C*-methylidene-2-oxo-3-deoxy-β-D-glycero-pentofuranosyl) thymine (**8**). Reagents: i. Kuzuhara & Emoto (1964); ii. PDC, 3Å molecular sieve powder, CH_2Cl_2; iii. $CH_3(C_5H_6)_3POBr$, *n*-BuLi, THF; iv. H_2SO_4, Ac_2O, pyridine; v. (*t*-$(CH_3)_3C$) $(CH_3)_2SiOSO_2CF_3$, CH_3CN, silylated thymine; vi. NH_3, CH_3OH; vii. CrO_3, Ac_2O, pyridine.

thymine. The β specificity was obtained by the participation of the 2-acetoxy group; this ester was created an oxonium bridge in 1,2-position which allowed the selective introduction of the nucleic acid in the β position. The anomeric configuration of the nucleoside **6** was assigned by detailed examination of the NMR spectra. Removal of the 2-*O*-acetyl by ammonia in methanol gave the pure nucleoside **7** in quantitative yield. The nucleoside **7** was transformed by oxidation with CrO_3, pyridine and anhydride acetic (Hansske et al 1984) to give the target enone **8** in moderate yield.

References

Balzarini, J., Kang, G. J., Dalal, M., Herdewijn, P., De Clercq, E., Broder, S., Johns, D. G. (1987) The anti-HTLV-III (anti-HIV) and cytotoxic activity of 2′,3′-didehydro-2′,3′-dideoxyribonucleosides: a comparison with their parental 2′,3′-dideoxyribonucleosides. Mol. Pharmacol. 32: 162–167

De Clercq, E. (1995a) Toward improved anti-HIV chemotherapy: therapeutic strategies for intervention with HIV infections. J. Med. Chem. 38: 2491–2517

De Clercq, E. (1995b) Antiviral therapy for human immunodeficiency virus infection. Clinical Microbiol. Rev. 8: 200–239

Ewing, D. F., Fahmy, N., Len, C., Mackenzie, G., Ronco, G., Villa, P., Shaw, G. (1996) Nucleoside analogues: glycones based on the benzo(*c*)furan core. Collect. Czech. Chem. Commun. 61: 145–147

Hansske, F., Madej, D., Robins, M. J. (1984) 2′ and 3′-ketonucleosides and their *arabino* and *xylo* reduction products. Tetrahedron 40: 125–135

Huryn, D. M., Okabe, M. (1992) AIDS-driven nucleoside chemistry. Chem. Rev. 92: 1745–1768

Kitano, K., Miura, S., Ohrui, H., Meguro, H. (1997) Synthesis of 4′-fluoromethylnucleosides as potential antineoplastic agents. Tetrahedron 53: 13315–13322

Kuzuhara, H., Emoto, S. (1964) D-xylose series (III) Synthesis of methyl 2-*O*-acetyl-5-*O*-benzyl-3-*O*-(methylsulfonyl)-D-xylofuranoside and solvolysis of its sulfonyl ester. Agr. Biol. Chem. 28: 900–907

Morris-Jones, S., Moyle, G., Easterbrook P. J. (1997) Clinical trials report, anti-infectives, antiretroviral therapies in HIV-1 infection. Exp. Opin. Invest. Drugs 6: 1049–1061

Vorbruggen, H., Krolikiewicz, K., Bennua, B. (1981) Nucleoside syntheses. XXII. Nucleoside synthesis with trimethylsilyl triflate and perchlorate as catalysts. Chem. Ber. 114: 1234–1255

Wilson, L. J., Hager, M. W., El-Kattan, Y. A., Liotta, D. C. (1995) Nitrogen glycosylation reactions involving pyrimidine and purine nucleoside bases with furanoside sugars. Synthesis 1465–1479