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Synthesis of L-Analogues of 1-(2′,3′-Dideoxy-β-D-glycero-pent-2-enofuranosyl)thymine

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Abstract

β-L(−)-2′,3′-Dideoxy-3′-thiacytidine (3TC), its 5-fluoro derivative ((−)-FTC), 2′,3′-dideoxycytidine (β-L-ddC), and its 5-fluoro derivative (β-L-FddC) have been reported to have anti-HIV and anti-HBV activity. It was of particular interest therefore to develop a series of β-L-d4T analogues bearing several kinds of amino-linker arms at the C-5 position of the pyrimidine moiety in an attempt to find more potent and less toxic anti-HIV agents. In addition, modification of nucleosides with various functional molecules has been attracting wide interest in biological studies since the primary amino groups could be useful for the attachment of either fluorescent dyes or a non-nucleosidic reverse transcriptase inhibitor. These modified nucleosides were evaluated for antiviral activity against HIV-1_{LAI} in CEM-SS cells and HIV-1_{IIIB} in MT4 cells. Unfortunately, none of the compounds exhibited significant anti-HIV activity at the doses tested.

Considerable effort has been directed towards the search for novel nucleoside structures for use as antiviral agents. Most of these analogues are synthesized by modification of the naturally occurring nucleosides and, therefore, possess the β-D-configuration. Recently a number of nucleosides with L-configuration have been reported as chemotherapeutic agents against HIV. The first such compound, β-L(−)-2′,3′-dideoxy-3′-thiacytidine (3TC, Lamivudine) (Belleau et al 1990; Doong et al 1991; Soudeyns et al 1991) was recently approved as an anti-HIV agent, and its 5-fluoro derivative ((−)-FTC) (Furman et al 1992; Schinazi et al 1992) has also been found to exhibit antiviral activity against HIV, as well as HBV, in-vitro and in-vivo. 2′,3′-Dideoxycytidine (β-L-ddC) (Balzarini et al 1986) and its 5-fluoro derivative (β-L-FddC) (Gosselin et al 1994; Lin et al 1994) are additional L-nucleoside analogues. Interestingly, while these L-nucleosides have potent biological activity, some show lower toxicity profiles than their D-counterparts. It was of interest to synthesize the corresponding β-L-enantiomers of β-D-d4T analogues as potential anti-HIV and anti-HBV agents with resistance to the degradative enzymes.

We have also undertaken the synthesis of 2′,3′-didehydro-2′,3′-dideoxy-L-thymidine analogues bearing several kinds of amino-linker arms at the C-5 position of the pyrimidine moiety in order to discover more potent and less toxic anti-HIV agents. The choice of the attachment site of the tether on the C-5 position in the pyrimidine ring was made due to the low interference with base pairing in DNA. The rationale for the design and synthesis of such 5-tethered compounds resulted from preliminary studies (Rong & Soloway 1994; Rong et al 1995) which demonstrated that the binding to nucleosides was weak when the extension of the arms contained four or fewer methylene groups. However, when the tether increased to 10 Å by interposing additional methylene groups (6 to 12), there was a substantial increase in the strength of enzyme binding. In preliminary biochemical studies, tethering the 5-position by a flexible chain (10 Å) may permit the triphosphates to be generated and thereby their incorporation in nucleic acids. Finally, the β-L-nucleosides bearing *N*-aminoalkyl-linkers could have useful applications in biological studies with further derivations of the terminal

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primary amine with either a fluorescent dye or a non-nucleosidic reverse transcriptase inhibitor.

Materials and Methods

Chemical procedures

Melting points were measured on a Kofler apparatus and were uncorrected. IR spectra were recorded on a Unicam Mattson 1000 spectrometer and only noteworthy absorptions (reciprocal cm) are listed. ^{1}H and ^{13}C NMR spectra were obtained on a Jeol JNM-LA 400 spectrometer using tetramethylsilane as an internal standard. NH, NH_2 and OH signals appeared as broad singlets exchangeable with D_2O (s = singlet, b = broad, d = doublet, t = triplet, m = multiplet). Mass spectra were recorded with a Jeol FX 102 spectrometer with the Fast Atom Bombardment ionization (FAB Pos.). Thin-layer chromatography (TLC) was carried out on precoated F254 silica plates and column chromatography were performed with Merck silica gel 60 (230–400 mesh).

*2-Amino-β-*L*-arabinofurano[1′,2′:4,5]oxazoline (**1**)*

A mixture of L-arabinose (10 g, 66·6 mmol), crude cyanamide (6 g, 0·14 mol, 2 Eq), methanol (20 mL), and 6 M NH_4OH (3 mL) was heated at 50°C for 3 days. The reaction mixture was cooled to −10°C and kept at this temperature overnight. The product was collected with suction, washed with methanol (2 × 100 mL), diethyl ether (2 × 100 mL), and dried in-vacuo. White crystals (67%); mp = 228°C; IR (KBr) ν 3414 (NH_2), 3189–3148 (OH), 1666 (CN), 1608 (C=C), 1442; ^{1}H RMN (d_6-DMSO) δ (ppm) 6·32 (s, 2H, NH_2), 5·65 (d, J = 5·6 Hz, 1H, H1′), 5·45 (s, 1H, OH), 4·75 (s, 1H, OH), 4·52 (d, J = 5·4 Hz, 1H, H2′), 3·99 (s, 1H, H3′), 3·63 (m, 1H, H4′), 3·26 (m, 2H, H5′); ^{13}C NMR (d_6-DMSO) δ (ppm) 162·3 (C=N), 99·9 (C1′), 88·0 (C2′), 84·6 (C4′), 75·6 (C3′), 61·5 (C5′).

2,2′-Anhydro-1-(β- L*-arabinofuranosyl)-5-(methoxycarbonylmethyl)-uracil (**3**)*

Dimethyl-α-bromomethylfumarate (**2**) (4·6 g, 19·4 mmol) and triethylamine (2 mL) were added successively to a solution of **1** (2 g, 11·49 mmol) in methanol (25 mL). The reaction mixture was heated at 50°C for 1 h, and evaporated in-vacuo. The residual yellow oil was purified on a silica gel column prepacked in dichloromethane. Elution of the column with dichloromethane:methanol (100:0 to 85:15) gave **3** as beige crystals (TLC R_f = 0·3 in CH_2Cl_2:CH_3OH 85:15) (81%); mp = 100°C; IR (KBr) ν 3642–3250 (NH_2 and OH), 2961 (CH), 1730 (CO ester), 1668 (CO lactame), 1636 (CN), 1553, 1493, 1260, 1098, 1059, 1021; ^{1}H NMR (d_6-DMSO) δ (ppm) 7·83 (s, 1H, H6), 6·34 (d, J = 5·6 Hz, 1H, H1′), 5·89 (d, J = 4·4 Hz, 1H, OH), 5·20 (d, J = 5·6 Hz, 1H, H2′), 4·98 (t, J = 4·7 Hz, 1H, OH), 4·37 (broad s, 1H, H3′), 4·08 (broad s, 1H, H4′), 3·56 (s, 3H, OCH_3), 3·27 (broad s, 2H, 5-CH_2), 3·27 (s, 2H, H5′); ^{13}C NMR (DMSO-d_6) δ (ppm) 170·8 (CO ester and C2), 159·7 (C4), 134·6 (C6), 114·8 (C5), 90·2 (C1′), 89·1 (C4′), 88·8 (C2′), 74·6 (C3′), 60·7 (C5′), 51·6 (OCH_3), 32·8 (5-CH_2).

*1-(3′, 5′-Di-*O *-acetyl-2′-bromo-2′-deoxy-β-*L*-ribofuranosyl)-5-(methoxycarbonylmethyl)-uracil (**4**)*

Acetyl bromide (5·4 mL, 75 mmol) was added dropwise to a boiling suspension of **3** (2·5 g, 8·39 mmol) in anhydrous acetonitrile (50 mL). The reaction mixture was stirred at 80°C for 2 h and allowed to cool to room temperature. After evaporation to dryness under reduced pressure, the residue was dissolved in dichloromethane (100 mL) and the solution was washed with water (3 × 50 mL). The organic layer was separated, dried (magnesium sulphate), and evaporated in-vacuo to yield brown crystals (TLC R_f = 0·5 CH_2Cl_2:CH_3OH 90:10) (51%); mp = 76°C; IR (KBr) ν 1744 (CO ester), 1691 (CO lactame), 1463, 1379, 1226, 1038; ^{1}H NMR (DMSO) δ (ppm) 11·68 (s, 1H, NH), 7·67 (s, 1H, H6), 6·15 (d, J = 7·52 Hz, 1H, H1′), 5·26 (m, 1H, H2′), 4·93 (m, 1H, H3′), 4·25 (m, 3H, H4′, H5′), 3·59 (s, 3H, OCH_3), 3·29 (s, 2H, 5-CH_2), 2·13 (s, 3H, $COCH_3$), 2·05 (s, 3H, $COCH_3$); ^{13}C NMR (d_6-DMSO) δ (ppm) 170·7 (CO ester), 170·2 (CO ester), 162·7 (C4), 150·4 (C2), 137·9 (C6), 108·8 (C5), 88·6 (C1′), 79·6 (C4′), 71·2 (C3′), 62·9 (C5′), 51·7 (OCH_3), 47·4 (C2′), 31·6 (5-CH_2), 20·5 (CH_3).

*1-(5′-*O*-Acetyl-2′,3′-dideoxy-β-* L*-glycero-pent-2-enofuranosyl)-5-(methoxycarbonylmethyl)-uracil (**5**)*

Zinc dust (11·2 g) in 1 M NaOH (100 mL) was treated under irradiation of 35 kHz ultrasound (150 W) for 3 min and washed with water until neutralization. Zinc in 50% acetic acid (100 mL) was then treated under irradiation of ultrasound for 3 min, washed respectively with water until neutralization, ethanol (3 × 50 mL), acetone (3 × 50 mL), diethyl ether (3 × 50 mL), and dried in-vacuo at 78°C for 2 h.

The freshly activated zinc dust (0·5 g, 7·64 mmol, 4 Eq) was added with stirring to a solution of **4** (1 g,

2·16 mmol) in anhydrous ethanol (80 mL) and acetic acid (1 mL). The heterogeneous reaction mixture was treated under irradiation of 35 kHz ultrasound (150 W) under an argon atmosphere for 30 min (monitored by TLC CH_2Cl_2:CH_3OH 95:5) until no starting material remained, filtered on celite and the filtrate evaporated to dryness in-vacuo. The residual yellow oil was purified on a silica gel column prepacked in dichloromethane. Elution of the column with dichloromethane–methanol (100 : 0 to 95:5) gave **5** as yellow crystals (54%; TLC $R_f = 0{\cdot}48$ in CH_2Cl_2:CH_3OH 95:5) and ***5bis*** as white crystals (20%; TLC $R_f = 0{\cdot}33$ in CH_2Cl_2:CH_3OH 95:5); mp = 150°C; IR (KBr) 3036 (CH), 1752 (CO ester), 1739 (CO ester), 1697 (CO lactame), 1682 (CO lactame), 1466, 1344, 1264, 1227, 1085; ^{1}H NMR (d_6-DMSO) δ (ppm) 11·46 (s, 1H, NH), 7·42 (s, 1H, H6), 6·81 (s, 1H, H1′), 6·42 (d, J = 6·00 Hz, 1H, H2′), 6·00 (d, J = 6·00 Hz, 1H, H3′), 4·96 (s, 1H, H4′), 4·17 (m, 2H, H5′), 3·57 (s, 3H, OCH_3), 3·29 (s, 2H, 5-CH_2), 1·98 (s, 3H, $COCH_3$); ^{13}C NMR (d_6-DMSO) δ (ppm) 170·9 (CO), 170·2 (CO), 163·1 (C4), 150·7 (C2), 138·3 (C6), 133·8 (C2′), 126·4 (C3′), 108·0 (C5), 89·5 (C1′), 83·9 (C4′), 67·7 (C5′), 51·7 (OCH_3), 31·5 (5-CH_2), 20·5 (CH_3).

*5-(Methoxycarbonylmethyl)-uracil (**5bis**).* ^{1}H NMR (d_6-DMSO) δ (ppm) 11·06 (s, 1H, NH), 7·40 (s, 1H, H6), 3·57 (s, 3H, OCH_3), 3·21 (s, 2H, 5-CH_2); ^{13}C NMR (d_6-DMSO) δ (ppm) 170·7 (CO ester), 164·2 (C4), 151·4 (C2), 140·2 (C6), 106·3 (C5), 57·7 (OCH_3), 31·4 (5-CH_2).

*General procedure for the synthesis of 5-[*N*-(hexan-6-ol)carbamoylmethyl]- and 5-[*N*-(amino-alkyl)carbamoylmethyl]-1-(2′,3′-dideoxy-β- L-glycero-pent-2-enofuranosyl)-uracil (**6** and **7a–d**, respectively)*

To a solution of **5** (350 mg, 1 mmol) in methanol (50 mL) was added either 6-aminohexan-1-ol (1 Eq) or 1,*n*-diaminoalkane (1 Eq) in the presence of a catalytic amount of dimethylaminopyridine. The reaction mixture was heated at 50°C for 5 h, and evaporated to dryness in-vacuo.

*1-(2′,3′-dideoxy-β-L-glycero-pent-2-enofuranosyl)-5-[*N*-(hexan-6-ol)carbamoylmethyl]-uracil (**6**)*

Brown oil (75%); IR (KBr) 3300 (OH), 2930 (CH), 2858 (CH), 1706 (CO lactame), 1674 (CO lactame), 1605 (C=C), 1454, 1074, 1056; ^{1}H NMR (d_6-DMSO) δ (ppm) 7·60 (s, 1H, H6), 6·82 (s, 1H, H1′), 6·40 (d, J = 5·5 Hz, 1H, H2′), 5·90 (d, J = 5·5 Hz, 1H, H3′), 4·75 (m, 1H, H4′), 3·56 (m, 2H, H5′), 3·35 (s, 2H, 5-CH_2), 3·31 (m, 2H, linker-CH_2OH), 2·92 (m, 2H, NHCH_2-linker), 1·38–1·25 (m, 8H, CH_2-linker); ^{13}C NMR (d_6-DMSO) δ (ppm) 169·3 (CO amide), 163·5 (C4), 150·9 (C2), 139·0 (C6), 135·4 (C2′), 125·9 (C3′), 108·7 (C5), 89·4 (C1′), 87·6 (C4′), 62·6 (C5′), 60·8 (linker-CH_2OH), 32·4–29·3–26·5 (CH_2-linker).

*5-[*N*-(6-Aminohexyl)carbamoylmethyl]-1-(2′,3′-dideoxy-β-L-glycero-pent-2-enofuranosyl)-uracil (**7a**)*

The crude residue was crystallized from ethyl acetate (3 × 50 mL) as brown crystals (50%); mp = 92°C; IR (KBr) 3600–3114 (NH_2 and OH), 2931 (CH), 2858 (CH), 1658 (CO lactame), 1548; ^{1}H NMR (d_6-DMSO) δ (ppm) 7·60 (s, 1H, H6), 6·82 (s, 1H, H1′), 6·40 (d, J = 5·58 Hz, 1H, H2′), 5·88 (d, J = 5·58 Hz, 1H, H3′), 4·75 (m, 1H, H4′), 3·56 (m, 2H, H5′), 2·98 (s, 2H, 5-CH_2), 2·56 (m, 4H, NHCH_2-linker and linker-CH_2NH_2), 1·34–1·22 (m, 8H, CH_2-linker); ^{13}C NMR (d_6-DMSO) δ (ppm) 168·9 (CO amide), 163·0 (C4), 150·8 (C2), 138·6 (C6), 135·1 (C2′), 125·7 (C3′), 108·6 (C5), 89·1 (C1′), 87·5 (C4′), 62·5 (C5′), 42·0 (5-CH_2), 33·2–26·0 (CH_2-linker); MS m/z 367, 269, 133 (MH^+).

*5-[*N*-(8-Aminooctyl)carbamoylmethyl]-1-(2′,3′-dideoxy-β-L-glycero-pent-2-enofuranosyl)-uracil (**7b**)*

The crude residue was crystallized from ethyl acetate (3 × 50 mL) as brown crystals (61%); mp = 96°C; IR (KBr) 3643–3157 (NH_2 and OH), 2927 (CH), 2854 (CH), 1697 (CO lactame), 1660 (CO lactame), 1458, 1260, 1076; ^{1}H NMR (d_6-DMSO) δ (ppm) 7·80 (s, 1H, H6), 6·78 (s, 1H, H1′), 6·37 (d, J = 5·60 Hz, 1H, H2′), 5·85 (d, J = 5·60 Hz, 1H, H3′), 4·74 (m, 1H, H4′), 3·64 (m, 2H, H5′), 3·60 (broad s, 3H, NH_2, OH), 2·95 (s, 2H, 5-CH_2), 2·61–2·56 (m, 4H, NHCH_2-linker and linker-CH_2NH_2), 1·32–1·19 (m, 12H, CH_2-linker); ^{13}C NMR (d_6-DMSO) δ (ppm) 168·9 (CO amide), 163·0 (C4), 150·8 (C2), 138·6 (C6), 135·0 (C2′), 125·7 (C3′), 108·6 (C5), 89·1 (C1′), 87·4 (C4′), 62·5 (C5′), 40·9 (5-CH_2), 31·9–28·9–26·2 (CH_2-linker); MS m/z 395, 297 (MH^+).

*5-[*N*-(10-Aminodecyl)carbamoylmethyl]-1-(2′,3′-dideoxy-β-L-glycero-pent-2-enofuranosyl)-uracil (**7c**)*

The crude residue was crystallized from ethyl acetate (3 × 50 mL) as brown crystals (66%); mp = 92°C; IR (KBr) 3642–3357 (NH_2 and OH), 2923 (CH), 1705 (CO lactame), 1660 (CO lactame), 1558 (C=C), 1465, 1081; ^{1}H NMR (d_6-

DMSO) δ (ppm) 7·77 (s, 1H, NH), 7·57 (s, 1H, H6), 6·78 (s, 1H, H1′), 6·37 (d, J = 5·55 Hz, 1H, H2′), 5·86 (d, J = 5·55 Hz, 1H, H3′), 4·75 (m, 1H, H4′), 4·40 (m, 3H, NH_2 and OH), 3·56 (m, 2H, H5′), 2·98 (s, 2H, 5-CH_2), 2·51–2·49 (m, 4H, NHCH_2-linker and linker-CH_2NH_2), 1·33–1·21 (m, 16H, CH_2-linker); ^{13}C NMR (d_6-DMSO) δ (ppm) 168·9 (CO amide), 163,4 (C4), 150,8 (C2), 138,6 (C6), 135,1 (C2′), 125,7, (C3′), 108,6 (C5), 89,1 (C1′), 87,4 (C4′), 62·5 (C5′), 40·9 (5-CH_2), 31·9–28·9–26·3 (CH_2-linker); MS m/z 423, 325, 137 (MH^+).

*5-[N-(12-aminododecyl)carbamoylmethyl]-1-(2′,3′-dideoxy-β- L-glycero-pent-2-enofuranosyl)-uracil (**7d**)*

The crude residue was crystallized from ethyl acetate (3 × 50 mL) as brown crystals (88%); mp = 100°C; IR (KBr) 3642–3114 (NH_2 and OH), 2920 (CH), 2850, 1706 (CO lactame), 1644 (CO lactame), 1549, 1466, 1083; ^{1}H NMR (d_6-DMSO) δ (ppm) 7·78 (s, 1H, NH), 7·60 (s, 1H, H6), 6·82 (s, 1H, H1′), 6·40 (d, J = 5·67 Hz, 1H, H2′), 5·89 (d, J = 5·67 Hz, 1H, H3′), 4·75 (m, 1H, H4′), 4·50–4·37 (broad s, 3H, NH_2, OH), 3·56 (m, 2H, H5′), 2·94 (s, 2H, 5-CH_2), 2·52 (m, 4H, NHCH_2-linker and linker-CH_2NH_2), 1·33–1·22 (m, 20H, CH_2-linker); ^{13}C NMR (d_6-DMSO) δ (ppm) 168·9 (CO amide), 163·3 (C4), 150·8 (C2), 138·5 (C6), 135·0 (C2′) 125·7 (C3′), 108·6 (C5), 89·1 (C1′), 87·4 (C4′), 62·5 (C5′), 41·1 (5-CH_2), 32·3–28·9–26·3 (CH_2-linker); MS m/z 451, 353 (MH^+).

*1-(2′,3′-Dideoxy-β- L-glycero-pent-2-enofuranosyl)-5-(methoxycarbonylmethyl)-uracil (**8**)*

Compound **5** (400 mg, 1·23 mmol) was dissolved in methanolic sodium methoxide (50 mL, 1 Eq) and the reaction mixture was stirred for 45 min at room temperature. The solution was then cooled at 0°C and neutralized with 12 M hydrochloric acid, and evaporated to dryness in-vacuo. The oily residue was purified on a silica gel column prepacked in dichloromethane. Elution of the column with dichloromethane:methanol (100:0 to 95:5) afforded **8** as an oil (17%; TLC $R_f = 0·43$ in CH_2Cl_2:CH_3OH 90:10); IR (KBr) 3550 (OH), 2961 (CH), 1730 (CO ester), 1706 (CO lactame), 1681 (CO lactame), 1260, 1086, 1037; ^{1}H NMR (d_6-DMSO) δ (ppm) 11·46 (s, 1H, NH), 7·73 (s, 1H, H6), 6·82 (s, 1H, H1′), 6·41 (d, J = 6·09 Hz, 1H, H2′), 5·92 (d, J = 6·09 Hz, 1H, H3′), 4·99 (m, 1H, H4′), 4·76 (s, 2H, H5′), 3·57 (m, 3H, OCH_3), 3·21 (s, 2H, 5-CH_2); ^{13}C NMR (d_6-DMSO) δ (ppm) 167·8 (CO ester), 160·0 (C4), 147·6 (C2), 135·8 (C6), 132·1 (C2′), 122·7 (C3′), 104·5 (C5), 86·1 (C1′), 84·4 (C4′), 59·4 (C5′), 48·6 (OCH_3), 28·6 (5-CH_2).

Virology procedures

The cultures of CEM-SS and MT4 cells were maintained at 37°C in a 5% CO_2 atmosphere in RPMI-1640 medium supplemented with 10% complement-depleted foetal bovine serum. The antiviral HIV-1 activity of a given compound in CEM-SS cells was measured by quantification of the reverse transcriptase activity associated with particles released from HIV-1_{LAI}-infected cells in the culture medium. CEM-SS cells were infected with 100 TCDI50 (the virus stock was titrated under the same experimental conditions); after 30 min adsorption, free virus particles were washed out and cells were re-suspended in RPMI-1640 plus 10% foetal bovine serum at a final concentration of 10^5 cells mL^{-1} in the presence of different concentrations of test compounds. After 5 days, virus production was measured by reverse transcriptase assay as described by Moog et al (1994). The 50% inhibitory concentration (IC50) was derived from the computer-generated median effect plot of the dose–effect data (Chou & Chou 1985). The cytotoxicity of the drugs was evaluated in parallel by incubating uninfected cells in the presence of different concentrations of antiviral products. The cell viability was determined by a measure of mitochondrial dehydrogenase activity, enzymes reducing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into formazan (the quantity of which was measured by the absorbance at 540 nm) (Mosmann 1983). The 50% cytotoxic concentration (CC50) is the concentration of drug which reduces cell viability by 50% and was calculated with the program used in the determination of the IC50. The assays using different cells and virus isolates were done according to previously published protocols; virus production was quantified by the reverse transcriptase activity associated to virus particles released from the cells in the culture medium (Moog et al 1994). Conditions under which the inhibitory properties were measured on HIV-1 reverse transcriptase in-vitro, and in-vitro reverse transcriptase inhibition are described by Moog et al (1994). The CEM-SS cells were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (Bethesda, MD).

Results and Discussion

Chemistry

We report a simple synthetic method for 5-substituted uracil nucleosides, useful for attachment of various functional groups. The synthesis used L-arabinose as the starting material which on reaction with cyanamide in aqueous methanolic ammonia

gave 2-amino-β- L-arabinofurano[1′,2′:4,5]oxazoline (**1**) (Figure 1) (Holy 1972). The key step involved the addition reaction of dimethyl α-bromomethylfumarate to arabinoaminooxazoline followed by a subsequent cyclization in methanol under reflux in the presence of triethylamine in a one-flask reaction which afforded a crude product. Purification was achieved by column chromatography on silica gel using 15% methanol in dichloromethane as eluent to afford purified 2,2′ - anhydro-1-(β-L-arabinofuranosyl)-5-(methoxycarbonyl)-uracil (**3**) in 81% yield. The β-anomer was exclusively formed in this reaction. The ring opening of the 2,2′-anhydro linkage with a concomitant introduction of bromine atom at C-2′ seemed to be an attractive process (Marumoto & Honjo 1974). The reaction of **3** with acetyl bromide in anhydrous acetonitrile under reflux afforded crystalline 1-(3′,5′-di-*O*-acetyl-2′-bromo-2′-de-oxy-β- L-ribofuranosyl)-5-(methoxycarbonylmethyl)-uracil (**4**) in 51% yield. This reaction of fission of the ether linkage of 2,2′-anhydro nucleoside was realised with a concomitant esterification of the hydroxyl groups on 3′ and 5′ positions. The subsequent reductive β-elimination proceeded smoothly by adding freshly activated zinc powder in anhydrous ethanol in the presence of a catalytic amount of acetic acid to compound **4** (Shiragami et al 1996). The heterogeneous reaction mixture was submitted to ultrasound irradiation under an argon atmosphere at room temperature for 30 min and the reductive β-elimination reaction gave the expected olefinic nucleoside **5** (54%) with the nucleoside bond cleavage leading to the substituted uracil as the major by-product (***5bis***) (20%) purified after chromatography.

Various linker arms containing amino groups were then easily introduced to the 5 position of **5** via amide linkages by ester-amide exchange reactions of 5-carbonylmethyl esters (Shinozuka 1998). Thus the reaction of **5** with either 6-aminohexan-1-

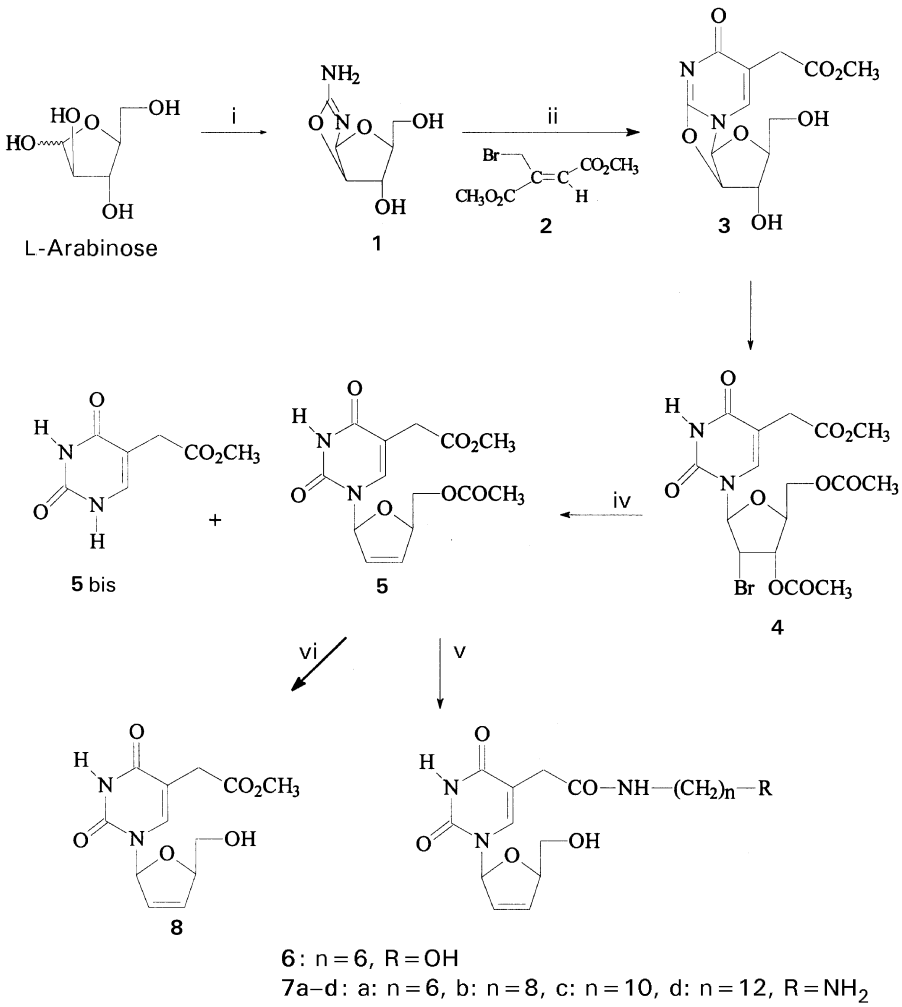

Figure 1. Synthesis of compounds **6**, **7a**–**d** and **8**. Reagents: i. NH_2CN, 6 M NH_4OH, CH_3OH; ii. **2**, triethylamine, methanol; iii. CH_3COBr, anhydrous CH_3CN; iv. zinc dust, anhydrous ethanol, acetic acid; v. 6-aminohexan-1-ol or 1,*n*-diaminoalkane, dimethylaminopyridine, methanol; vi. $CH_3O^-Na^+$, methanol.

Table 1. Inhibitory effect of β-L-d4T analogues against HIV-1_{LAI} replication in CEM-SS and HIV-1_{IIIB} in MT4 cells.

Compound	Number of CH_2 units	HIV-1_{LAI} in CEM-SS cells			HIV-1_{IIIB} in MT4 cells		
		IC50 (μM)	CC50 (μM)	CC50/IC50	IC50 (μM)	CC50 (μM)	CC50/IC50
d4T		0·059 ± 0·016	>100	>1695	0·28 ± 0·08	>100	>357
7a	6	>10	>10		>10	>10	
7b	8	>100	>100		>100	>100	
7c	10	25	86	3·44	>100	>100	
7d	12	4·4	36	8·18	>100	42	>0·42

IC50 is the drug concentration required to inhibit HIV-1 multiplication by 50%. CC50 is the drug concentration which causes 50% cytotoxicity to uninfected cells. All data represent the mean ± s.d of three separate experiments.

ol or 1, *n*-diaminoalkanes (n = 6, 8, 10 and 12) in methanol in the presence of dimethylaminopyridine gave the expected 5-[*N*-(hexan-6-ol)carbamoylmethyl]- and 5-[*N*-(aminoalkyl)carbamoylmethyl]-1-(2′,3′-dideoxy-β-L-glycero-pent-2-enofuranosyl)-uracil (**6** and **7a**–**d**, respectively).

Finally, the selective deprotection of the 5′-acetyl group of **5** was realised using methanolic sodium methoxide at room temperature yielding the 1-(2′,3′-Dideoxy-β-L-glycero-pent-2-enofuranosyl)-5-(methoxycarbonylmethyl)-uracil (**8**) as an oily compound.

Antiviral activity

The β- L-d4T analogues **7a**–**d** were evaluated for inhibition of HIV-1 multiplication in cells of the lymphocytic lineage (CEM-SS and MT4). The lack of anti-HIV activity of **7a**–**d** is in accordance with previously published results (Van Draanen et al 1992) (Table 1). We conclude that compounds **7a**–**d** are devoid of antiviral activity because they are not efficiently phosphorylated intracellularly in CEM-SS and MT4 cells.

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