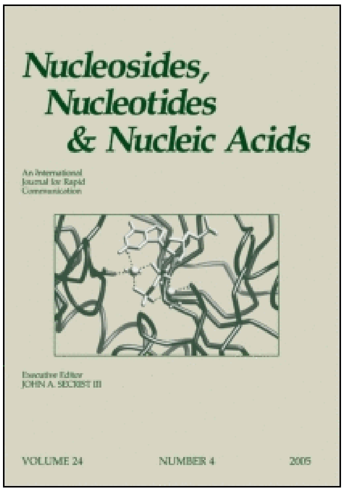

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Delbederi, Z. , Fossey, C. , Fontaine, G. , Benzaria, S. , Gavriliu, D. , Ciurea, A. , Lelong, B. , Ladurée, D. , Aubertin, A. M. and Kirn, A.(2000) 'Synthesis and Antiviral Activity of C-5 Substituted β-D- and β-L-D4T Analogues', Nucleosides, Nucleotides and Nucleic Acids, 19: 9, 1441 — 1461

To link to this Article: DOI: 10.1080/15257770008033853

URL: http://dx.doi.org/10.1080/15257770008033853

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(9), 1441–1461 (2000)

SYNTHESIS AND ANTIVIRAL ACTIVITY OF C-5 SUBSTITUTED β-D- and β-L-D4T ANALOGUES

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Abstract: A series of β-D-2',3'-didehydro-2',3'-dideoxy-nucleosides bearing a tether attached at the C-5 position and their β-L-counterparts was synthesized. Their inhibitory activities against human immunodeficiency virus (HIV) were investigated and compared to establish relationship(s) between compound structure and their antiviral activity. No significant activity was observed for β-D- and β-L-modified nucleosides respectively **7a-c** and **14a-c**, but **7d** and **14d** exhibited a weak activity against HIV-1.

INTRODUCTION

Intensive efforts in the search of effective therapies for treatment of human immunodeficiency virus (HIV) infection have led to the discovery of 2',3'-didehydro-2',3'-dideoxynucleosides (d4N) including 2',3'-didehydro-2',3'-dideoxythymidine (d4T) which has been already approved for the treatment of HIV infections.[1-3] Therefore, as a part of our continuing efforts in the synthesis of nucleosides as antiviral agents, a series of d4T analogues was synthesized and evaluated *in vitro* for anti-HIV-1 activity in various cells.

In recent years, there have been significant interests in the potential use of the C-5 substituted pyrimidine nucleosides in synthetic oligonucleotide probes as a tether site for linking reporter groups to nucleic acids. Therefore, the 5-position of pyrimidine nucleosides has been the target of extensive studies modifications.[4-6] Many nucleoside analogues with potent biological properties have arisen by introducing structural

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diversity at the C-5 position of the uracil base, particularly in the 2'-deoxyuridine series. The C-5 site of pyrimidine has been previously shown to tolerate a large number of different chemical functionalities without disfavoring duplex formation and large substituents can be incorporated at this position without perturbation the glycosydic bond. The absence of such perturbations often enables one to incorporate C-5-modified pyrimidine nucleotides enzymatically via their respective triphosphates. The compatibility of C-5-modified nucleotide triphosphates with polymerase enzymes has proven particularly useful in expanding the diversity of ligands.[7] As the C-5 position of pyrimidine is not involved in hydrogen bonding and faces outward in the major groove of the double helix when a double-stranded DNA helix is formed, the C-5 position of uridine is an appropriate site for the attachment of linker arms to functional groups. The rationale for the design and synthesis of such 5-tethered compounds resulted from preliminary studies which demonstrated that the binding to nucleosides was weak when the extension of the arms contained 4 or fewer methylene groups.[8,9] However, when the tether increased to 10Å 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 (10Å) may permit the triphosphates to be generated and thereby their incorporation in nucleic acids.[8,9]

We have an ongoing program on the synthesis of many modified nucleosides bearing an organic fluorophore chemically introduced (fluorescent dye), this label will be introduced at the C-5 position of pyrimidine with an aminoalkyl linker. These studies will be reported elsewhere. These modified nucleosides have been attracting wide interest in terms of applying to biological and biophysical studies. We herein report the synthesis of a series of d4T analogues bearing aminotether for the functionalization and to determine the optimum length of the linker before attaching additional reporter groups.

Moreover, a class of nucleosides with the unnatural L-configuration has recently drawn considerable attention by medicinal chemists due to their unique potency, mechanism, and toxicity profile.[10-14] The difference between a D-nucleoside and L-nucleoside in the 2',3'-didehydro-2',3'-dideoxy series is in fact not very pronounced. The ring oxygen function of the osidic moiety is situated on the backside in D-nucleosides and on the front side in L-nucleosides, opposite to the C-2'-C-3' bond. As the nucleosides have to be phosphorylated to become active, the presence of a hydroxymethyl group at position C-4' is likewise a prerequisite for biological activity. Thus, the configuration of the C-1' and C-4' atoms of a nucleoside is of crucial importance. In fact, a series of modified nucleosides with the unnatural L-configuration have been described recently as potent inhibitors of human immunodeficiency virus (HIV) replication including 2',3'-dideoxy-3'-thia-β-L-cytidine (3TC, Lamivudine)[15], 2',3'-dideoxy-3'-thia-β-L-5-fluorocytidine (L-FTC)[16], 2',3'-dideoxy-β-L-cytidine (β-L-ddC)[17,18], 2'-deoxy-2'-fluoro-5-methyl-β-L-arabinofuranosyl uracil (L-FMAU)[19], 2',3'-didehydro-2',3'-dideoxy-β-L-cytidine and 2',3'-didehydro-2',3'-dideoxy-β-L-5-fluorocytidine (β-L-FddC)[20]. For the most part, they display higher antiviral activity and lower cytotoxicity than their D-counterparts. In fact, the β-L-FddC and β-L-ddC showed no inhibition against mitochondrial DNA synthesis at a concentration of 100 μM whereas the ddC severely inhibited the mitochondrial DNA synthesis (IC_{50} 0.022 μM) of CEM cells. The dose-limiting toxicity of ddC is severe neuropathy which may be caused by the inhibition of the synthesis of mitochondrial DNA.[21] In addition, to the requirements necessary within these molecules for action as prodrugs such as appropriate structure, stereochemistry and efficiency of phosphorylation, a property that is also important is stability under physiological conditions. Dideoxynucleosides (ddNs), particularly those of the purine family, are very unstable with respect to hydrolytic cleavage of the glycosidic bond under acidic conditions.[22,23] In view of the above findings, it was of particular interest to study the antiviral activity of the β-D-analogues in comparison to the corresponding L-enantiomers.

RESULTS AND DISCUSSION

Chemical

The synthetic route of the β-D-d_4T analogues bearing an (aminoalkyl)carbamoyl methyl tether attached at the C-5 position is illustrated in Figure 1. The preparation of the modified nucleosides used commercially D-arabinose as starting material which on reaction with cyanamide in the presence of $NaHCO_3$ in DMF afforded the 2-amino-β-D-arabinofurano[1',2':4,5]oxazoline **1** according to the methodology reported by Holy in 80% yield.[24] Subsequent treatment of **1** with dimethyl-α-bromomethylfumarate **2** in the

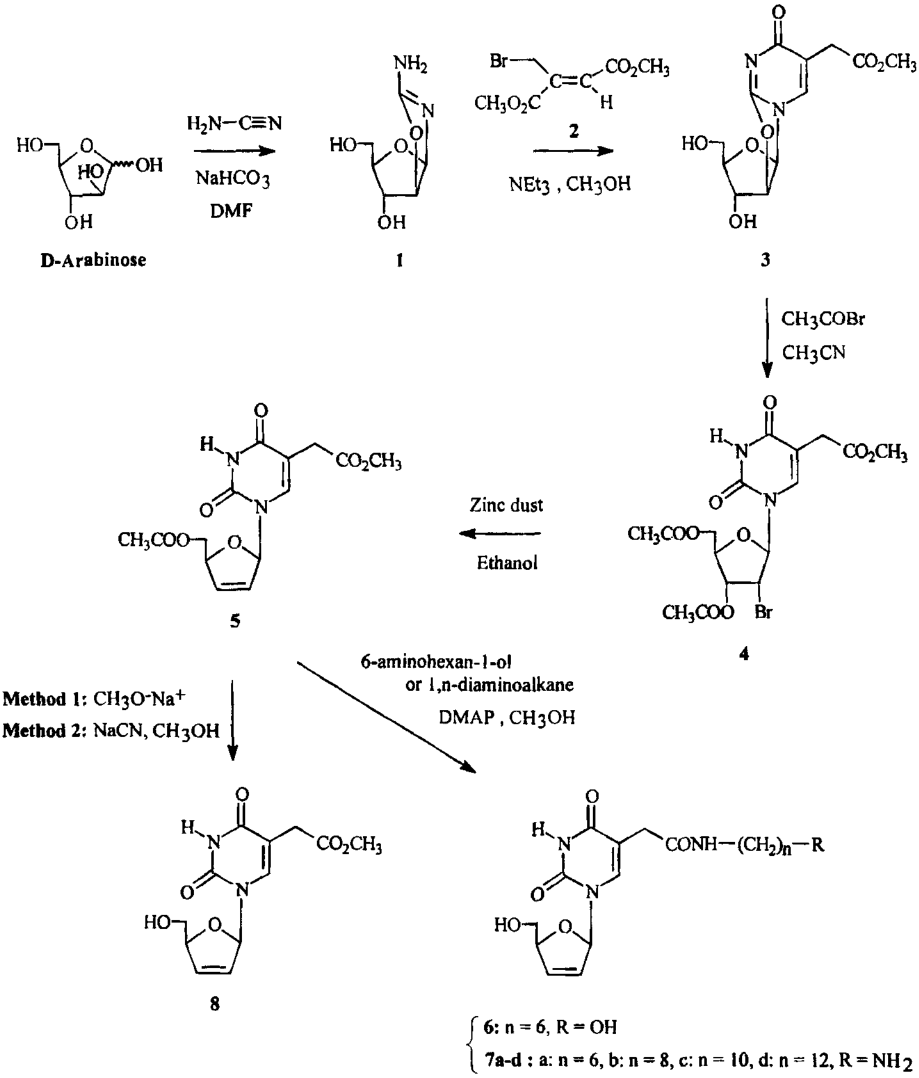

FIGURE 1. Synthesis of C-5 subsituted β-D-d4T analogues

presence of triethylamine in methanol yielded the 2,2'-anhydro-nucleoside **3** (61%) after purification by column chromatography.[25] Opening of the oxide bridge with a concomitant introduction of a bromine atom at the C-2' position of **3** was accomplished with acetyl bromide in anhydrous acetonitrile to afford the 1-(3',5'-di-O-acetyl-2'-bromo-2'-deoxy-β-D-ribofuranosyl)-5-(methoxycarbonyl methyl)-uracil **4** in 63% yield. The reductive β-elimination of this acetoxy-bromo intermediate proceeded by adding freshly activated zinc powder to **4** in anhydrous ethanol and 54% yield of the 5'-O-acetyl-*β*-D-d4T analogue **5** was isolated after chromatography. The target 2',3'-didehydro-2',3'-dideoxy-nucleosides **6** and **7a-d** bearing a *linker* at C-5 position were prepared by reaction of **5** with either 6-aminohexan-1-ol or 1,6- 1,8- 1,10 and 1,12-alkyldiamines in methanol in the presence of dimethylaminopyridine *via* amide linkages by ester-amide exchange reactions of 5-carbonylmethyl esters. This treatment resulted in the simultaneous conversion of the methoxycarbonyl group into an (aminoalkyl)carbamoyl group and deprotection. Finally, removal of the C-5'-0-acetyl protecting group of **5** with sodium cyanide in methanol afforded the 5-(methoxycarbonylmethyl)-d4T **8** in 83% yield after chromatography. This reaction was originally reported by Kozak and Johnson.[26]

For comparative studies, the corresponding 5-[N-(hexan-6-ol)carbamoylmethyl]- and 5-[N-(aminoalkyl)carbamoylmethyl]-*β*-L-d4T analogues **13** and **14a-d** (n=6, 8, 10 and 12) were prepared by a similar synthetic approach as summarized in Figure 2. Removal of the C-5'-O-acetyl group of **12** was carried out using methanolic sodium methoxide yielding the 5-(methoxycarbonyl methyl)-*β*-L-d4T analogue **15**.

The structures of the newly synthesized compounds were confirmed by proton and carbon nuclear magnetic resonance spectra. In addition, the compounds which were tested for biological activity **7a-d** and **14a-d** were analyzed either by mass spectrometry or elemental analysis.

Biological

The aim of this work was focused on the development of *β*-D- and *β*-L-enantiomers of 2',3'-unsaturated thymidine analogues respectively **7a-d** and **14a-d** bearing a tether on C-5 position of the uracil ring. These compounds evaluated for inhibition of HIV-1 multiplication in cells of the lymphocytic lineage (CEM-SS and MT-4) in order to appreciate the activity and toxicity between the enantiomers. Unfortunately, as shown in Table 1, the *β*-D- and *β*-L-d4T analogues **7a-c** and **14a-c** containing six to ten methylene units in the spacer were devoid of antiviral activity. Compounds **7b** and **14b** (n=8) are comparatively much more less cytotoxic than the corresponding analogues n=6 or n=12 with no explanation. Nevertheless, it is interesting to note that the enantiomers **7d** and **14d** bearing a tether containing twelve methylene units displayed a

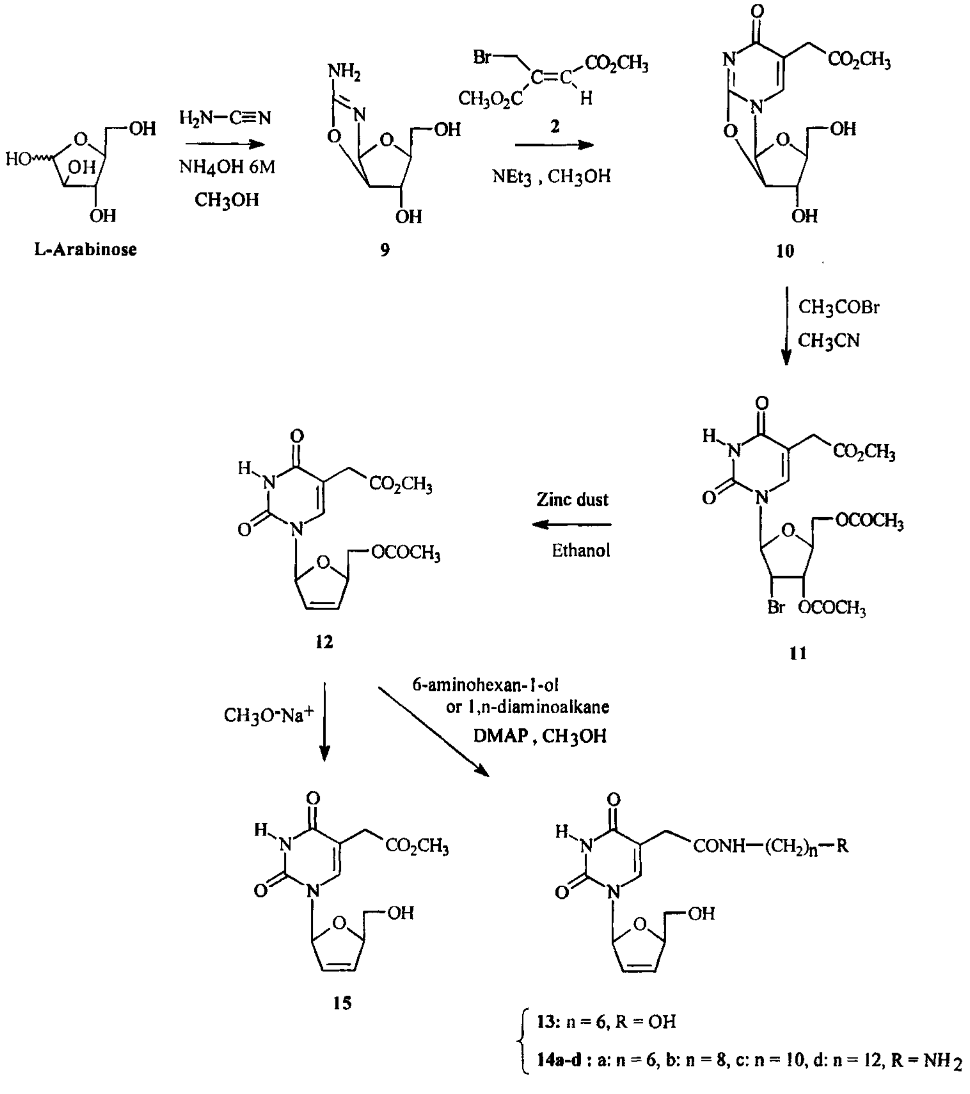

FIGURE 2. Synthesis of C-5 substituted *β*-L d4T analogues

weak activity depending on the nature of the cells, only in the CEM-SS cells (IC_{50} 2.3μM and 4.4μM respectively). These compounds **7d** and **14d** were also found the more cytotoxic (CC_{50} 22 μM and 36 μM respectively). Perhaps, the weak antiviral activities of the analogues **7d** and **14d** were probably due to their poor phosphorylation in CEM-SS cells. These cell-dependent variations must probably residue in the differential abilities of the cells to phosphorylate the nucleosides to their 5'-triphosphates, a process that is usually very slow for the 2',3'-dideoxynucleosides.[27]

While there may be different reasons for decrease in the activities of the D enantiomer compounds **7a-c** including the efficiency of phosphorylation, it is clear that introduction of a long tether on the C-5 position of the uracil ring including an amide linkage leads to a marked reduction of anti-HIV activity. On the other hand, the data found for the *β*-L-nucleosides **14a-c** were in accordance with previously published results since the *β*-L-d4T is devoid of anti-HIV activity [28,29] whereas the *β*-L-d4T 5'-triphosphate was shown to be a potent inhibitor of HIV reverse transcriptase [29]. Moreover, Furman reported that the fact that d4T is more active than the 1-*β*-L-enantiomer cannot be completely explained at the level of the reverse transcriptase alone.[30] The *β*-D-d4T is efficiently phosphorylated and has potent anti-HIV activity. On the other hand the *β*-L-d4T may not be well phosphorylated by human thymidine kinases.[29] Also the inactivity of the L-analogue may be due to insufficient phosphorylation. Differences in anti-HIV activity may occur due to differences in cell permeation and/or activation.[30] Only, the *β*-D (**7d**) and *β*-L (**14d**) d4T analogues bearing the longer tether on C-5 exhibited a weak activity, these nucleosides were more lipohilic and could have the best intracellular uptake.

In conclusion, these preliminary biological results are disappointing and the preparation of the phosphate prodrugs of these 2',3'-didehydro-2',3'-dideoxy-nucleosides bearing a tether on C-5 position are ongoing.

EXPERIMENTAL SECTION

Chemical procedures

Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were measured at room temperature using a Perkin-Elmer model 241 polarimeter (path length 10 cm) and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Fourier transform Mattson spectrometer Genesis DTGS using WinFIRST^{TM} Macros and ApPro^{TM} and only noteworthy absorptions are listed. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL Lambda 400 using TMS as an internal standard. NH 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) were performed on precoated silica gel 60 F_{254} sheets (0.2 mm layer, Macherey-Nagel), and compounds were detected by UV absorption at 254 nm. Column chromatography were effected by using Merck silica gel 60 (0.063-0.200 mm). All samples were kept in a drying oven at 50°C over P_2O_5 for at least 24 hours prior to analysis. Anhydrous acetonitrile was distilled from phosphorus pentoxide, anhydrous ethanol was prepared by using magnesium turnings.

2-amino-β-D-arabinofurano[1',2':4,5]oxazoline (1). A mixture of D-arabinose (36 g, 240 mmol), crude cyanamide (12.4 g, 296 mmol, 1.23 Eq), $NaHCO_3$ (1.2 g, 14.3 mmol, 0.06 Eq) in DMF (240 mL) was heated at 90°C for 3.5 h. The reaction mixture was cooled to room temperature, added dropwise with ethyl acetate (120 mL) and stirred for 30 min at room temperature and then 1 h at 0°C. The precipitate was collected by filtration, washed with a mixture of ethyl acetate-DMF (1:1) (100 mL), and dried *in vacuo* over P_2O_5, yielding 31.37 g of **1** as white crystals. The combined filtrate and washings were concentrated under reduced pressure and a second crop of crystals (2.14 g) was collected to give finally 33.5 g (80 %) of **1** after drying: mp decomposed from 108°C; IR (KBr): 3438 (NH_2), 3208 (OH), 1676 (C=N), 1446 (CH_2), 1063,1012,961, 885 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.29 (s, 2H, NH_2), 5.65 (d, J = 5.60 Hz, 1H, H-1), 5.40 (s, 1H, C-5-OH), 4.70 (m, 1H, H-3), 4.51 (d, J = 5.60 Hz, 1H, H-2), 3.99 (s, 1H, C-3-OH), 3.64-3.60 (m, 1H, H-4), 3.24 (m, 2H, H-5).

2,2'-anhydro-1-(β-D-arabinofuranosyl)-5-(methoxycarbonylmethyl)-uracil (3). Dimethyl-α-bromomethylfumarate **2** (20 g, 83.5 mmol, 1.67 Eq) and triethylamine (8 mL, 56.9 mmol, 1.14 Eq) were added successively to a solution of **1** (8.7 g, 50 mmol) in methanol (100 mL). The reaction mixture was heated at 50°C for 3 h, and evaporated *in vacuo*. The crude product was purified by silica-gel column chromatography using methanol in dichloromethane (0-8%) as eluents to yield 8.98 g (61%) of **3** as white crystals: R_f 0.3 [CH_2Cl_2-CH_3OH (85:15)]; mp decomposed from 100°C;.IR (KBr): 3361 (OH), 2953 (NH), 1738 (CO ester), 1676 (CO lactame), 1563, 1501, 1223, 1069 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.87 (s, 1H, H-6), 6.34 (d, J = 5.70 Hz, 1H, H-1'), 5.88 (d, J = 4.30 Hz, 1H, OH), 5.20 (d, J = 5.70 Hz, 1H, H-2'), 4.97 (t, J = 5.10 Hz, 1H, OH), 4.37 (d, J = 3.30 Hz, 1H, H-3'), 4.06 (t, J = 4.90 Hz, 1H, H-4'), 3.56 (s, 3H, OCH_3), 3.29 (dd, J_{gem} = 14.9 Hz, 2H, C-5-CH_2), 3.15 (d, J = 4.90 Hz, 2H, H-5'); ^{13}C NMR (DMSO-d_6): δ 170.8

($\underline{C}OCH_3$ and C-2), 159.6 (C-4), 134.6 (C-6), 114.8 (C-5), 90.2 (C-1'), 89.1 (C-4'), 88.8 (C-2'), 74.6 (C-3'), 60.7 (C-5'), 51.6 (OCH_3), 32.8 (C-5-$\underline{C}H_2$).

1-(3',5'-di-O-acetyl-2'-bromo-2'-deoxy-*β*-D-ribofuranosyl)-5-(methoxycarbonylmethyl)-uracil (4). Acetyl bromide (2 mL, 27 mmol, 4 Eq) was added dropwise to a boiling suspension of **3** (2 g, 6.73 mmol) in anhydrous acetonitrile (120 mL) under an argon atmosphere. The reaction mixture was stirred at 80°C for 1 h and allowed to cool to room temperature. After evaporation to dryness under reduced pressure, the residue was dissolved in a mixture of chloroform-dichloromethane 1:1 (100 mL) and the solution was washed with a saturated aqueous hydrogenocarbonate solution (100 mL) and then water (2 X 100 mL). The organic layer was separated, dried (magnesium sulphate), and evaporated *in vacuo* to yield 2.95 g (63%) of **4** as brown crystals which crystallized from diethyl ether: R_f 0.68 [CH_2Cl_2-CH_3OH (90:10)]; mp 62-64°C; IR (KBr): 3259 (NH), 1753 (CO), 1472, 1395, 1217, 1063, 808 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.55 (s, 1H, NH), 7.51 (s, 1H, H-6), 6.22 (d, J = 6.00 Hz, 1H, H-1'), 5.18 (dd, $J_{H2'\text{-}H3'}$ = 4.40 Hz, $J_{H3'\text{-}H4'}$ = 5.80 Hz, 1H, H-3'), 4.57 (t, J = 6.00 Hz, 1H, H-2'), 4.38 (m, 3H, H-4' and H-5'), 3.72 (s, 3H, OCH_3), 3.38 (dd, J_{gem} = 17.4 Hz, 2H, C-5-CH_2), 2.19 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.8-170.4-169.8 (CO ester), 162.2 (C-4), 49.8 (C-2), 137.5 (C-6), 109.1 (C-5), 91.0 (C-1'), 80.4 (C-4'), 71.3 (C-3'), 63.0 (C-5'), 52.5 (OCH_3), 40.3 (C-2'), 31.3 (C-5-$\underline{C}H_2$), 20.9 ($CO\underline{C}H_3$), 20.8 ($CO\underline{C}H_3$).

1-(5'-O-acetyl-2',3'-dideoxy-*β*-D-glycero-pent-2-enofuranosyl)-5-(methoxycarbonylmethyl)-uracil (5). Zinc dust (282 mg, 4.3 mmol, 4 Eq) freshly activated was added with stirring to a solution of 4 (500 mg, 1.08 mmol) in anhydrous ethanol (50 mL) under an argon atmosphere. The heterogeneous reaction mixture was heated for 30 min [monitored by TLC CH_2Cl_2-CH_3OH (90:10) until no starting material remained], filtered on celite and the filtrate evaporated to dryness *in vacuo*. The crude product was purified by silica-gel column chromatography using methanol in dichloromethane (0-5%) as eluents to yield 190 mg (54%) of **5** as white crystals: R_f 0.41 [EtOAc (100%)]; mp 138-139°C; ^1H NMR ($CDCl_3$): δ 8.57 (s, 1H, NH), 7.45 (s, 1H, H-6), 6.99 (dt, J = 8.60-1.70 Hz, 1H, H-1'), 6.29 (dt, J = 5.85-1.70 Hz, 1H, H-2'), 5.96 (ddd, J = 6.70-1.50 Hz, 1H, H-3'), 5.06 (m, 1H, H-4'), 4.31 (ddd, J_{gem} = 12.3 Hz, J =

4.70-3.30 Hz, 2H, H-5'), 3.71 (s, 3H, OCH_3), 3.38 (dd, J_{gem} = 17.2 Hz, 2H, C-5-CH_2), 2.09 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.7 (CO), 170.3 (CO), 162.8 (C-4), 150.5 (C-2), 137.9 (C-6), 133.1 (C-2'), 127.1 (C-3'), 108.3 (C-5), 90.1 (C-1'), 84.3 (C-4'), 64.7 (C-5'), 52.1 (OCH_3), 31.5 (C-5-$\underline{C}H_2$), 20.6 (CH_3); MS (CIMS, isobutane) m/z 323 $[M - H]^-$.

Zinc activation: Zinc dust (11.2 g) in 1N 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 x 50 mL), acetone (3 x 50 mL), diethyl ether (3 x 50 mL), and dried *in vacuo* at 78°C for 2 h.

General procedure for the synthesis of 5-[N-(hexan-6-ol)carbamoylmethyl]- and 5-[N-(aminoalkyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)-uracil (6) and (7a-d). To a solution of **5** (200 mg, 0.62 mmol) in methanol (2.5 mL) was added either 6-aminohexan-1-ol (10 Eq) or 1,*n*-diaminoalkane (10 Eq) in the presence of a catalytic amount of dimethylaminopyridine (DMAP).

1-(2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)-5-[N-(hexan-6-ol)carbamoylmethyl]-uracil (6). The reaction mixture was heated at 80°C for 26 h, and evaporated to dryness *in vacuo*. The crude product was purified by silica-gel column chromatography using methanol in dichloromethane (0-10%) as eluents to yield 180 mg (80%) of **6** as white crystals: R_f 0.47 [CH_2Cl_2-CH_3OH (85:15)]; IR (KBr): 3445 (OH), 3307 ($\underline{NH}$CO), 3178 (NH), 3039 (NH), 2931 (CH_2), 2852 (CH_2), 1700-1660 (CO lactame), 1566 (C-N), 1471, 1402, 1265, 1075, 1030, 826 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.77 (s, 1H, NH), 7.60 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.41 (d, J = 5.72 Hz, 1H, H-2'), 5.90 (d, J = 5.72 Hz, 1H, H-3'), 4.96 (bs, 1H, C-5'-OH), 4.75 (s, 1H, H-4'), 4.35 (bs, 1H, *linker*-$CH_2O\underline{H}$), 3.56 (s, 2H, H-5'), 3.35 (s, 2H, *linker*-$C\underline{H_2}OH$), 2.97 (m, 4H, C-5-CH_2 and NH-$C\underline{H_2}$-*linker*), 1.36-1.23 (m, 8H, $C\underline{H_2}$ of *linker*); ^{13}C NMR (DMSO-d_6): δ 168.9 (CO amide), 163.3 (C-4), 150.8 (C-2), 138.6 (C-6), 135.1 (C-2'), 125.7 (C-3'), 108.6 (C-5), 89.1 (C-1'), 87.4 (C-4'), 62.6 (C-5'), 60.7 (*linker*-$\underline{C}H_2OH$), 38.5 (C-5-$\underline{C}H_2$ and NH-$\underline{C}H_2$-*linker*), 32.5-29.2 ($\underline{C}H_2$ of *linker*); *Anal.* Calcd for $C_{17}H_{25}N_3O_5$: C, 58.11; H, 7.17; N, 11.96. Found: C, 58.02; H, 7.11; N, 11.44.

5-[N-(6-aminohexyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)-uracil (7a). The reaction mixture was stirred at room temperature for 3 days, and evaporated to dryness *in vacuo*. The oily residue was triturated with hexane (20 mL) and crystallized from diethyl ether to give 100 mg (45%) of **7a** as yellow crystals: mp 80-82°C; IR (KBr): 3073 (NH), 2936 (CH_2), 2859 (CH_2), 1650 (CO), 1555 (C-N), 1463, 1336 (C-N), 1074 (C-OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.78 (s, 1H, NH), 7.57 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.39 (d, J = 5.35 Hz, 1H, H-2'), 5.87 (d, J = 5.35 Hz, 1H, H-3'), 4.73 (s, 1H, H-4'), 3.74 (bs, 3H, NH_2 and OH), 3.56 (s, 2H, H-5'), 2.96 (s, 2H, C-5-CH_2), 2.49 (m, 4H, $NH\underline{CH_2}$-*linker* and *linker*-$\underline{CH_2}NH_2$), 1.32-1.22 (m, 8H, $\underline{CH_2}$ of *linker*); ^{13}C NMR (DMSO-d_6): δ 169.4 (CO amide), 163.8 (C-4), 151.2 (C-2), 139.0 (C-6), 135.5 (C-2'), 126.3 (C-3'), 109.1 (C-5), 89.6 (C-1'), 87.9 (C-4'), 65.4 (C-5'), 41.8-39.0-33.3-29.7-26.8 (C-5-$\underline{C}H_2$ and CH_2 of *linker*); *Anal.* Calcd for $C_{17}H_{26}N_4O_5$: C, 55.73; H, 7.15; N, 15.29. Found: C, 55.69; H, 7.09; N, 15.24.

5-[N-(8-aminooctyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)-uracil (7b). The reaction mixture was heated at 80°C for 24 h, and evaporated to dryness *in vacuo*. The oily residue was triturated successively with hexane (20 mL), ethyl acetate (20 mL) and crystallized from diethyl ether to give 100 mg (41%) of **7b** as pale yellow crystals: mp 82-85°C; $[\alpha]_D$ +21.0 (*c* 5 in DMSO); IR (KBr): 3291 (NH), 3073 (NH), 2926 (CH_2), 2853 (CH_2), 1658 (CO), 1552 (C-N), 1459, 1299 (C-N), 1076 (C-OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.78 (s, 1H, NH), 7.59 (s, 1H, H-6), 6.83 (s, 1H, H-1'), 6.40 (d, J = 5.31 Hz, 1H, H-2'), 5.89 (d, J = 5.31 Hz, 1H, H-3'), 4.75 (s, 1H, H-4'), 3.67 (bs, 3H, NH_2 and OH), 3.56 (s, 2H, H-5'), 2.98 (s, 2H, C-5-CH_2), 2.49 (m, 4H, $NH\underline{CH_2}$-*linker* and *linker*-$\underline{CH_2}NH_2$), 1.32-1.22 (m, 12H, $\underline{CH_2}$ of *linker*); ^{13}C NMR (DMSO-d_6): δ 168.9 (CO amide), 163.2 (C-4), 150.8 (C-2), 138.6 (C-6), 135.1 (C-2'), 125.7 (C-3'), 108.6 (C-5), 89.1 (C-1'), 87.4 (C-4'), 62.6 (C-5'), 41.2-38.9-32.7-29.0-26.3 (C-5-$\underline{C}H_2$ and CH_2 of *linker*); *Anal.* Calcd for $C_{19}H_{30}N_4O_5$: C, 57.85; H, 7.67; N, 14.20. Found: C, 57.79; H, 7.59; N, 14.14.

5-[N-(10-aminodecyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)-uracil (7c). The reaction mixture was heated at 80°C for 24 h, and evaporated to dryness *in vacuo*. The oily residue was triturated successively with hexane

(20 mL), ethyl acetate (20 mL) and crystallized from diethyl ether to give 150 mg (58%) of **7c** as pale yellow crystals: mp 77-78°C; $[\alpha]_D$ +22.8 (*c* 5 in DMSO); IR (KBr): 3288 (NH), 3075 (NH), 2924 (CH_2), 2852 (CH_2), 1660 (CO), 1549 (C-N), 1462, 1300 (C-N), 1076 (C-OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.77 (s, 1H, NH), 7.60 (s, 1H, H-6), 6.83 (s, 1H, H-1'), 6.40 (d, J = 5.49 Hz, 1H, H-2'), 5.89 (d, J = 5.49 Hz, 1H, H-3'), 4.75 (s, 1H, H-4'), 3.62 (bs, 3H, NH_2 and OH), 3.56 (s, 2H, H-5'), 2.98 (s, 2H, C-5-CH_2), 2.49 (m, 4H, $NHC\underline{H_2}$-*linker* and *linker*-$C\underline{H_2}NH_2$), 1.32-1.22 (m, 16H, $C\underline{H_2}$ of *linker*); ^{13}C NMR (DMSO-d_6): δ 168.8(CO amide), 163.2 (C-4), 150.8 (C-2), 138.6 (C-6), 135.0 (C-2'), 125.7 (C-3'), 108.6 (C-5), 89.1 (C-1'), 87.4 (C-4'), 62.6 (C-5'), 41.4-38.9-32.8-29.0-26.4 (C-5-$\underline{C}H_2$ and CH_2 of *linker*); *Anal.* Calcd for $C_{21}H_{34}N_4O_5$: C, 59.70; H, 8.11; N, 13.26. Found: C, 59.64; H, 8.09; N, 13.22.

5-[N-(12-aminododecyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)-uracil (7d). The reaction mixture was heated at 80°C for 9 h, and evaporated to dryness *in vacuo*. The oily residue was triturated successively with hexane (20 mL), ethyl acetate (20 mL) and crystallized from diethyl ether to give 210 mg (76%) of **7d** as pale yellow crystals: mp 81-83°C; $[\alpha]_D$ +13.4 (*c* 5 in DMSO); IR (KBr): 3289 (NH), 3070 (NH), 2920 (CH_2), 2850 (CH_2), 1659 (CO), 1555 (C-N), 1467, 1302 (C-N), 1076 (C-OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.77 (s, 1H, NH), 7.59 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.40 (d, J = 5.53 Hz, 1H, H-2'), 5.89 (d, J = 5.53 Hz, 1H, H-3'), 4.75 (s, 1H, H-4'), 3.56 (s, 2H, H-5'), 3.40 (bs, 3H, NH_2 and OH), 2.96 (s, 2H, C-5-CH_2), 2.49 (m, 4H, $NHC\underline{H_2}$-*linker* and *linker*-$C\underline{H_2}NH_2$), 1.31-1.22 (m, 8H, $C\underline{H_2}$ of *linker*); *Anal.* Calcd for $C_{23}H_{38}N_4O_5$: C, 61.31; H, 8.50; N, 12.43. Found: C, 61.29; H, 8.45; N, 12.39.

1-(2',3'-dideoxy-β-D-glycero-pent-2-enofuranosyl)-5-(methoxycarbonylmethyl)-uracil (8). *First method.* The protected nucleoside **5** (200 mg, 0.62 mmol) was treated with a solution of MeONa (33 mg, 0.62 mmol, 1 Eq) in methanol (20 mL) at 65°C for 8 h. The reaction mixture was neutralized by addition of Dowex 50X-X8 (H^+) resin and filtered. The filtrate was concentrated to dryness *in vacuo* and the residue was purified by silica-gel column chromatography using methanol in ethyl acetate (0-1%) as eluents to yield 190 mg (54%) of **8** as yellow crystals.

Second method. A solution of the protected nucleoside **5** (500 mg, 1.54 mmol) in methanol (14.5 mL) was added with a few crystals of NaCN. The reaction mixture was stirred at room temperature overnight and concentrated to dryness *in vacuo*. The oily residue was purified by silica-gel column chromatography using methanol in chloroform (0-5%) as eluents to yield 360 mg (83%) of **8** as a yellow crystals: R_f 0.50 [CH_2Cl_2-CH_3OH (90 :10)]; mp 114-116°C; IR (KBr): 3527 (OH), 3369 (NH), 3159 (NH), 3098, 3029, 2961 (CH_2), 2927, 2881, 2833 (CH_3O), 1737 (CO), 1687 (CONH), 1465, 1444 (CH_2), 1264, 1105 (C-C), 1030 (C-OH), 846 (C=C), 801 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.27 (s, 1H, NH), 7.72 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.40 (d, J = 5.85 Hz, 1H, H-2'), 5.92 (d, J = 5.85 Hz, 1H, H-3'), 5.00 (bs, 1H, OH), 4.76 (s, 1H, H-4'), 3.57 (s, 5H, H-5' and OCH_3), 3.23 (s, 2H, C-5-CH_2); ^{13}C NMR (DMSO-d_6): δ 170.8 (CO ester), 163.1 (C-4), 150.6 (C-2), 138.9 (C-6), 135.1 (C-2'), 125.7 (C-3'), 107.5 (C-5), 89.2 (C-1'), 87.5 (C-4'), 62.5 (C-5'), 51.7 (OCH_3), 31.6 (C-5-$\underline{C}H_2$); *Anal.* Calcd for $C_{12} H_{14} N_2 O_6$: C, 51.07; H, 5.00; N, 9.93. Found: C, 51.00; H, 4.88; N, 9.86.

2-amino-β-L-arabinofurano[1',2':4,5]oxazoline (9). A mixture of L-arabinose (10 g, 66.6 mmol), crude cyanamide (6 g, 0.14 mol, 2 Eq), methanol (20 mL), and 6M 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 x 100 mL), diethyl ether (2 x 100 mL), and dried *in vacuo* to afford 7.79 g (67%) of **9** as white crystals: mp 227-228°C; IR (KBr): 3414 (NH_2), 3189-3148 (OH), 1666 (CN), 1608 (C=C), 1442 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.32 (s, 2H, NH_2), 5.65 (d, J = 5.6 Hz, 1H, H-1), 5.45 (s, 1H, OH), 4.75 (s, 1H, OH), 4.52 (d, J = 5.4 Hz, 1H, H-2), 3.99 (s, 1H, H-3), 3.63 (m, 1H, H-4), 3.26 (m, 2H, H-5); ^{13}C NMR (DMSO-d_6): δ162.3 (C=N), 99.9 (C-1), 88.0 (C-2), 84.6 (C-4), 75.6 (C-3), 61.5 (C-5).

2,2'-anhydro-1-(β-L-arabinofuranosyl)-5-(methoxycarbonylmethyl)-uracil (10). Dimethyl-α-bromomethylfumarate **2** (4.6 g, 19.4 mmol) and triethylamine (2 mL, 14.2 mmol, 1.24 Eq) were added successively to a solution of **9** (2 g, 11.49 mmol) in methanol (25mL). The reaction mixture was heated at 50°C for 1 h, and evaporated *in vacuo*. The residual yellow oil was purified by silica-gel column chromatography using methanol in dichloromethane (0-15%) as eluents to yield 2.77 g (81%) of **10** as beige crystals: R_f 0.33 [CH_2Cl_2-CH_3OH (85:15)]; mp 99-101°C; IR (KBr): 3642-3250 (NH_2

and OH), 2961 (CH), 1730 (CO ester), 1668 (CO lactame), 1636 (CN), 1553, 1493, 1260, 1098, 1059, 1021 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.83 (s, 1H, H-6), 6.34 (d, J = 5.6 Hz, 1H, H-1'), 5.89 (d, J = 4.4 Hz, 1H, OH), 5.20 (d, J = 5.6 Hz, 1H, H-2'), 4.98 (t, J = 4.7 Hz, 1H, OH), 4.37 (bs, 1H, H3'), 4.08 (bs, 1H, H-4'), 3.56 (s, 3H, OCH_3), 3.27 (bs, 2H, C-5-CH_2), 3.27 (s, 2H, H-5'); ^{13}C NMR (DMSO-d_6): δ 170.8 (CO ester and C-2), 159.7 (C-4), 134.6 (C-6), 114.8 (C-5), 90.2 (C-1'), 89.1 (C-4'), 88.8 (C-2'), 74.6 (C-3'), 60.7 (C-5'), 51.6 (OCH_3), 32.8 (C-5-$\underline{C}H_2$).

1-(3',5'-di-O-acetyl-2'-bromo-2'-deoxy-β-L-ribofuranosyl)-5-(methoxycarbonylmethyl)-uracil (11). Acetyl bromide (5.4 mL, 75 mmol, 8.94 Eq) was added dropwise to a boiling suspension of **10** (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 X 50 mL). The organic layer was separated, dried (magnesium sulphate), and evaporated *in vacuo* to yield 1.98 g (51%) of **11** as brown crystals: R_f 0.5 [CH_2Cl_2-CH_3OH (90:10)]; mp 74-77°C; IR (KBr): 1744 (CO ester), 1691 (CO lactame), 1463, 1379, 1226, 1038 cm^{-1} ; ^{1}H NMR (DMSO-d_6): δ 11.68 (s, 1H, NH), 7.67 (s, 1H, H-6), 6.15 (d, J = 7.52 Hz, 1H, H-1'), 5.26 (m, 1H, H-2'), 4.93 (m, 1H, H-3'), 4.25 (m, 3H, H-4', H-5'), 3.59 (s, 3H, OCH_3), 3.29 (s, 2H, C-5-CH_2), 2.13 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$); ^{13}C NMR (DMSO-d_6): δ170.7 (CO ester), 170.2 (CO ester), 162.7 (C-4), 150.4 (C-2), 137.9 (C-6), 108.8 (C-5), 88.6 (C-1'), 79.6 (C-4'), 71.2 (C-3'), 62.9 (C-5'), 51.7 (OCH_3), 47.4 (C-2'), 31.6 (C-5-$\underline{C}H_2$), 20.5 ($CO\underline{C}H_3$).

1-(5'-O-acetyl-2',3'-dideoxy-β-L-glycero-pent-2-enofuranosyl)-5-(methoxycarbonylmethyl)-uracil (12). Zinc dust (0.5g, 7.64 mmol, 4 Eq) freshly activated was added with stirring to a solution of **11** (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 by silica-gel column chromatography using methanol in dichloromethane (0-5%)

as eluents to yield 0.38 g (54%) of **12** as yellow crystals: *R$_f$* 0.48 [CH_2Cl_2-CH_3OH (95:5)]; mp 149-151°C; IR (KBr): 3036 (CH), 1752 (CO ester), 1739 (CO ester), 1697 (CO lactame), 1682 (CO lactame), 1466, 1344, 1264, 1227, 1085 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.46 (s, 1H, NH), 7.42 (s, 1H, H-6), 6.81 (s, 1H, H-1'), 6.42 (d, J = 6.00 Hz, 1H, H-2'), 6.00 (d, J = 6.00 Hz, 1H, H-3'), 4.96 (s, 1H, H-4'), 4.17 (m, 2H, H-5'), 3.57 (s, 3H, OCH_3), 3.29 (s, 2H, C-5-CH_2), 1.98 (s, 3H, $COCH_3$); ^{13}C NMR (DMSO-d_6): δ 170.9 (CO), 170.2 (CO), 163.1 (C-4), 150.7 (C-2), 138.3 (C-6), 133.8 (C-2'), 126.4 (C-3'), 108.0 (C-5), 89.5 (C-1'), 83.9 (C-4'), 67.7 (C-5'), 51.7 (OCH_3), 31.5 (C-5-$\underline{C}H_2$), 20.5 ($CO\underline{C}H_3$).

General procedure for the synthesis of 5-[N-(hexan-6-ol)carbamoylmethyl]- and 5-[N-(aminoalkyl)carbamoylmethyl]-1-(2',3'-dideoxy-*β*-L-glycero-pent-2-enofuranosyl)-uracil (13) and (14a-d). To a solution of **12** (350 mg, 1 mmol) in methanol (50 mL) was added either with 6-aminohexan-1-ol or 1,*n*-diaminoalkane (1 Eq) in the presence of a catalytic amount of dimethylaminopyridine (DMAP). 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 (13). 300 mg (75%) of **13** as a brown oil: IR (KBr): 3300 (OH), 2930 (CH), 2858 (CH), 1706 (CO lactame), 1674 (CO lactame), 1605 (C=C), 1454, 1074, 1056 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.60 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.40 (d, J = 5.5 Hz, 1H, H-2'), 5.90 (d, J = 5.5 Hz, 1H, H-3'), 4.75 (m, 1H, H-4'), 3.56 (m, 2H, H-5'), 3.35 (s, 2H, C-5-CH_2), 3.31 (m, 2H, *linker*-$C\underline{H}_2OH$), 2.92 (m, 2H, $NHC\underline{H}_2$-*linker*), 1.38-1.25 (m, 8H, CH_2-*linker*); ^{13}C NMR (DMSO-d_6): δ 169.3 (CO amide), 163.5 (C-4), 150.9 (C-2), 139.0 (C-6), 135.4 (C-2'), 125.9 (C-3'), 108.7 (C-5), 89.4 (C-1'), 87.6 (C-4'), 62.6 (C-5'), 60.8 (*linker*-$\underline{C}H_2OH$), 32.4-29.3-26.5 ($\underline{C}H_2$-*linker*).

5-[N-(6-aminohexyl)carbamoylmethyl]-1-(2',3'-dideoxy-*β*-L-glycero-pent-2-enofuranosyl)-uracil (14a). The crude residue was crystallized from ethyl acetate to yield 198 mg (50%) of **14a** as brown crystals: mp 91-93°C; $[\alpha]_D$ -11.2 (*c* 5 in DMSO); IR (KBr): 3600-3114 (NH_2 and OH), 2931 (CH), 2858 (CH), 1658 (CO lactame), 1548 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.60 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.40 (d, J = 5.58 Hz, 1H, H-2'), 5.88 (d, J = 5.58 Hz, 1H, H-3'), 4.75 (m, 1H, H-4'), 3.56 (m, 2H, H-5'), 2.98

(s, 2H, C-5-CH_2), 2.56 (m, 4H, $NHC\underline{H}_2$-*linker* and *linker*-$C\underline{H}_2NH_2$), 1.34-1.22 (m, 8H, $C\underline{H}_2$-*linker*); ^{13}C NMR (DMSO-d_6): δ 168.9 (CO amide), 163.0 (C-4), 150.8 (C-2), 138.6 (C-6), 135.1 (C-2'), 125.7 (C-3'), 108.6 (C-5), 89.1 (C-1'), 87.5 (C-4'), 62.5 (C-5'), 42.0 (C-5-$\underline{C}H_2$), 33.2-26.0 ($\underline{C}H_2$-*linker*); MS m/z (FAB) 367, 269, 133 $(M+H)^+$.

5-[N-(8-aminooctyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-L-glycero-pent-2-enofuranosyl)-uracil (14b). The crude residue was crystallized from ethyl acetate to yield 260 mg (61%) of **14b** as brown crystals: mp 95-97°C; $[\alpha]_D$ -14.6 (*c* 5 in DMSO); IR (KBr): 3643-3157 (NH_2 and OH), 2927 (CH), 2854 (CH), 1697 (CO lactame), 1660 (CO lactame), 1458, 1260, 1076 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.80 (s, 1H, H-6), 6.78 (s, 1H, H-1'), 6.37 (d, J = 5.60 Hz, 1H, H-2'), 5.85 (d, J = 5.60 Hz, 1H, H-3'), 4.74 (m, 1H, H-4'), 3.64 (m, 2H, H-5'), 3.60 (bs, 3H, NH_2, OH), 2.95 (s, 2H, C-5-CH_2), 2.61-2.56 (m, 4H, $NHC\underline{H}_2$-*linker* and *linker*-$C\underline{H}_2NH_2$), 1.32-1.19 (m, 12H, CH_2-*linker*); ^{13}C NMR (DMSO-d_6): δ 168.9 (CO amide), 163.0 (C-4), 150.8 (C-2), 138.6 (C-6), 135.0 (C-2'), 125.7 (C-3'), 108.6 (C-5), 89.1 (C-1'), 87.4 (C-4'), 62.5 (C-5'), 40.9 (C-5-$\underline{C}H_2$), 31.9-28.9-26.2 (CH_2-*linker*); MS m/z (FAB) 395, 297 $(M+H)^+$.

5-[N-(10-aminodecyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-L-glycero-pent-2-enofuranosyl)-uracil (14c). The crude residue was crystallized from ethyl acetate (3 x 50 mL) to yield 300 mg (66%) of **14c** as brown crystals: mp 90-93°C; $[\alpha]_D$ -16.2 (*c* 5 in DMSO); IR (KBr): 3642-3357 (NH_2 and OH), 2923 (CH), 1705 (CO lactame), 1660 (CO lactame), 1558 (C=C), 1465, 1081 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.77 (s, 1H, NH), 7.57 (s, 1H, H-6), 6.78 (s, 1H, H-1'), 6.37 (d, J = 5.55 Hz, 1H, H-2'), 5.86 (d, J = 5.55 Hz, 1H, H-3'), 4.75 (m, 1H, H-4'), 4.40 (m, 3H, NH_2 and OH), 3.56 (m, 2H, H-5'), 2.98 (s, 2H, C-5-CH_2), 2.51-2.49 (m, 4H, $NHC\underline{H}_2$-*linker* and *linker*-$C\underline{H}_2NH_2$), 1.33-1.21 (m, 16H, $C\underline{H}_2$-*linker*); ^{13}C NMR (DMSO-d_6): δ 168.9 (CO amide), 163,4 (C-4), 150,8 (C-2), 138,6 (C-6), 135,1 (C-2'), 125,7, (C-3'), 108,6 (C-5), 89,1 (C-1'), 87,4 (C-4'), 62.5 (C-5'), 40.9 (C-5-$\underline{C}H_2$), 31.9-28.9-26.3 (CH_2-*linker*); MS m/z (FAB) 423, 325, 137 $(M+H)^+$.

5-[N-(12-aminododecyl)carbamoylmethyl]-1-(2',3'-dideoxy-β-L-glycero-pent-2-enofuranosyl)-uracil (14d). The crude residue was crystallized from ethyl acetate to yield 430 mg (88%) of **14d** as brown crystals: mp 99-101°C; $[\alpha]_D$ -18.2 (*c* 5 in DMSO);

Table 1. Antiviral and cytotoxicity evaluation of *β*-D-d$_4$T analogues bearing *linker* arms **7a-d** and their *β*-L-enantiomers **14a-d**.

Compd	No. CH_2	HIV-1_{LAI} in CEM-SS cells			HIV-1_{IIIB} in MT-4 cells		
	units	IC50 (μM)*	CC50 (μM)[†]	SI[‡]	IC50 (μM)	CC50 (μM)	SI
d$_4$T		0.059±0.016	> 100	> 1695	0.2 ±0.08	> 100	> 357
7a	6	>100	230	>2.3	>100	>100	>1
7b	8	>100	>100	>1	>100	>100	>1
7c	10	75	>100	>1.33	>100	>100	>1
7d	12	2.3	22	9.56	>100	40	>0.40
14a	6	>10	>10	>1	>10	>10	>1
14b	8	>100	>100	>1	>100	>100	>1
14c	10	33	57	1.73	>100	>100	>1
14d	12	4.4	36	8.18	>100	42	>0.42

* IC_{50} is the concentration required to inhibit HIV-1 multiplication by 50%

[†] CC_{50} is the concentration of drug which causes 50% cytotoxicity to uninfected cells

[‡] SI corresponds to the ratio CC_{50}/IC_{50}

All data represent the mean values of three separate experiments ±SD

IR (KBr): 3642-3114 (NH_2 and OH), 2920 (CH), 2850, 1706 (CO lactame), 1644 (CO lactame), 1549, 1466, 1083 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.78 (s, 1H, NH), 7.60 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.40 (d, J = 5.67 Hz, 1H, H-2'), 5.89 (d, J = 5.67 Hz, 1H, H-3'), 4.75 (m, 1H, H-4'), 4.50-4.37 (bs, 3H, NH_2, OH), 3.56 (m, 2H, H-5'), 2.94 (s, 2H, C-5-CH_2), 2.52 (m, 4H, $NH\underline{CH_2}$-*linker* and *linker*-$\underline{CH_2}NH_2$), 1.33-1.22 (m, 20H, CH_2-*linker*); ^{13}C NMR (DMSO-d_6): δ 168.9 (CO amide), 163.3 (C-4), 150.8 (C-2), 138.5 (C-6), 135.0 (C-2') 125.7 (C-3'), 108.6 (C-5), 89.1 (C-1'), 87.4 (C-4'), 62.5 (C-5'), 41.1 (C-5-$\underline{C}H_2$), 32.3-28.9-26.3 (*CH_2-linker*); MS m/z (FAB) 451, 353 $(M+H)^+$.

1-(2',3'-dideoxy-*β*-L-glycero-pent-2-enofuranosyl)-5-(methoxycarbonyl methyl)-uracil (15). The protected nucleoside **12** (40 mg, 1.23 mmol) was treated with a solution of MeONa (65 mg, 1.23 mmol, 1 Eq) in methanol (50 mL) at room temperature for 45 min. The reaction mixture was carefully neutralized by addition a solution of HCl in methanol and concentrated to dryness *in vacuo*. The crude product was purified by silica-gel column chromatography using methanol in dichloromethane (0-10%) as eluents to yield 60 mg (17%) of **15** as a brown oil: R_f 0.43 [CH_2Cl_2-CH_3OH (90:10]; IR (KBr):

3550 (OH), 2961 (CH), 1730 (CO), 1706 (CONH), 1681 (CONH), 1260, 1086, 1037 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 11.46 (s, 1H, NH), 7.73 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.41 (d, J = 6.09 Hz, 1H, H-2'), 5.92 (d, J = 6.09 Hz, 1H, H-3'), 4.99 (m, 1H, H-4'), 4.76 (s, 2H, H-5'), 3.57 (s, 3H, OCH_3), 3.21 (s, 2H, C-5-CH_2); ^{13}C NMR (DMSO-d_6): δ 167.8 (CO ester), 160.0 (C-4), 147.6 (C-2), 135.8 (C-6), 132.1 (C-2'), 122.7 (C-3'), 104.5 (C-5), 86.1 (C-1'), 84.4 (C-4'), 59.4 (C-5'), 48.6 (OCH_3), 28.6 (C-5-$\underline{C}H_2$).

Antiviral Test 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 (FBS). The antiviral HIV-1 activity of a given compound in CEM-SS cells was measured by quantification of the reverse transcriptase activity (RT) associated with particles released from HIV-1_{LAI}-infected cells in the culture medium. CEM-SS cells were infected with 100 $TCDI_{50}$ (the virus stock was titrated under the same experimental conditions); after 30 mn adsorption, free virus particles were washed out and cells were re-suspended in RPMI-1640 plus 10% foetal calf 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 RT assay as already described.[31] The 50% inhibitory concentration (IC_{50}) was derived from the computer generated median effect plot of the dose-effect data.[32] 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 (MTT) into formazan (whose quantity was measured by the absorbance at 540 nm).[33] The 50% cytotoxic concentration (CC_{50}) is the concentration of drug which reduces cell viability by 50% and was calculated with the program used in the determination of the IC_{50}. The assays using different cells and virus isolates were done according to previously published protocols; virus production was quantified by the RT activity associated to virus particles released from the cells in the culture medium.[31,34] Conditions in which the inhibitory properties were measured on HIV-1 reverse transcriptase *in vitro* has also been described.[31] *In vitro* RT inhibition was also described.[31] The CEM-SS cells were obtained from P. Nara through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (Bethesda, Md., USA).

ACKNOWLEDGMENT

We are indebted to S. Schmidt and G. Albrecht for excellent technical assistance in the antiviral and cytotoxic assays. The authors are deeply grateful to Ensemble Contre le SIDA (ECS grant) for financial support of this work.

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Received 7/11/00
Accepted 7/17/00