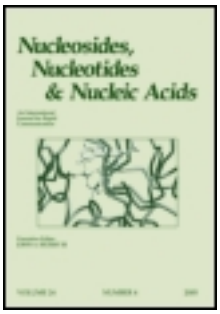

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D. Gavriliu [a] , C. Fossey [a] , G. Fontaine [a] , S. Benzaria [a] , A. Ciurea [a] , Z. Delbederi [a] , B. Lelong [a] , D. Ladurée [a] , A. M. Aubertin [b] & A. Kirn [b]

[a] Centre d'Etudes et de Recherche sur le Médicament de Normandie U. F. R. des Sciences, Pharmaceutiques, 5, Rue Vaubénard, 14032, Caen Cedex, France

[b] I.N.S.E.R.M., 3, Rue Koeberlé, 67000, Strasbourg, France

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF C-5 SUBSTITUTED ANALOGUES OF D4T BEARING METHYLAMINO- OR METHYLDIAMINO-LINKER ARMS

D. Gavriliu[1], C. Fossey[1], G. Fontaine[1], S. Benzaria[1], A. Ciurea[1], Z. Delbederi[1], B. Lelong[1], D. Ladurée*[1], A.M. Aubertin[2] and A. Kirn[2].

[1] *Centre d'Etudes et de Recherche sur le Médicament de Normandie U.F.R. des Sciences Pharmaceutiques, 5, Rue Vaubénard, 14032 Caen Cedex, France.*
[2] *I.N.S.E.R.M., 3, Rue Koeberlé, 67000 Strasbourg, France.*

Abstract: A general strategy is reported for the preparation of C-5-methylamino- or methyldiamino-d4T analogues of "different sizes". Reactions of the 2',3'-didehydro-2',3'-dideoxy-C-5 hydroxymethyl precursor (**7**) with either polymethylene diamines (n = 6, 8, 10 and 12) or propargylamine proceed regioselectively *via* subtitution reactions at the C-5 position of uracil. The compounds were evaluated for antiviral activity and cytotoxicity. No significant activity was observed for compounds **9**, **11**, and **13**, but **10** and **12** exhibited a weak activity against HIV-1.

INTRODUCTION

The detection of biomolecules at concentration in the subnanogram range is now routinely achieved with labels based on enzymes, fluorophores and luminophores.[1] Techniques based on luminescent labels are replacing those based on radioisotopes which have contributed greatly to the elucidation of biochemical mechanisms and the routine availability of immunoassays.[2] Detection systems generally consist of three components, the biomolecule, the spacer arm and the signal generator (Figure 1). Nucleosides

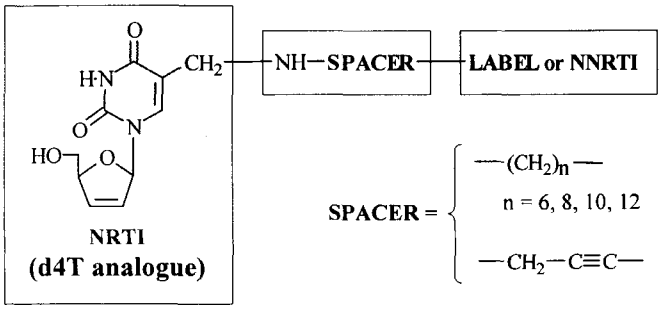

FIGURE 1. Heterodimers resulting from the linking of a NRTI and either a label or a NNRTI through a spacer.

covalently modified with functional labels such as fluorescent tags are commonly use in analytical applications (sequencing, hybridization assays) and therapeutic uses (antisense, antiviral pharmaceuticals).[3-8] Typically, the selective modification of the nucleobase occurs at the C-5 position of pyrimidines since this C-5 site of modification has been previously shown to tolerate a large number of different chemical functionalities without disfavoring duplex formation. The presence of a reporter or ligand groups in 2',3'-didehydro-2',3'-dideoxynucleosides makes it possible to monitor the diffusion of these compounds into cells and to observe their incorporation into viral DNA.

A second goal could be the design of "mixed heterodimer inhibitors" including a Nucleoside Reverse Transcriptase Inhibitor (NRTI) and a Non-Nucleoside RT Inhibitor (NNRTI) bound by a *linker* arm.[9-13] Knowledge of the HIV RT structure suggest a minimum distance of 10 Å between the two inhibitor binding sites. With such "mixed heterodimer inhibitors", we can hope an improvement of the antiviral properties and the delay of virus-drug emergence resistance.

Therefore, as part of our continuing efforts in the synthesis of nucleosides as antiviral agents, a series of d4T analogues bearing amino *linkers* arms at C-5 position were synthesized and evaluated *in vitro* for anti-HIV-1 activity. We have chosen a d4T analogue since various 2',3'-dideoxy-2',3'-didehydro nucleosides are known to be potent inhibitors of the human immunodeficiency virus (HIV) and to avoid several additional reactions. In fact, the 2',3'-dideoxy-2',3'-didehydro thymidine (d4T) has been already approved for the treatment of AIDS.[14-16]

The C-5 position of (deoxy)uridine is a popular position for introducing structural diversity when designing functional biopolymers for applications as for example sequence selective nucleic acid binding and/or damaging agents.[17-20] Part of the allure of utilizing C-5-substituted pyrimidines is that large substituents can be incorporated at this position without perturbating the syn/anti equilibrium about the glycosidic bond, or the overall structure of the biopolymer. 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 librairies of nucleic acid ligands.[17] As reported previously 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.[21-22]

RESULTS AND DISCUSSION

Chemical

The synthetic route for the d4T analogues bearing an amino-linker at C-5 position is described in Figure 2. The preparation of the required key C-5-hydroxymethyl intermediate (**7**) was developed starting from commercially available uridine. To introduce the hydroxymethyl group into the 5 position of uridine, the 2'- and 3'-hydroxyl groups of the uridine were first suitably protected by the isopropylidene group and subsequently treated with paraformaldehyde in alkaline media (aqueous KOH) at 60°C for formylation.[23] The resulting 5-hydroxymethyl-2',3'-isopropylidene uridine (**3**), obtained in 60% overall yield after chromatography purification, was then subjected to a deprotection of the isopropylidene group under mild conditions using 50% acetic acid affording **4** in 99% yield. This three step sequence (isopropylidene protection, formylation and deprotection of the isopropylidene group) provided the 5-hydroxymethyl-uridine (**4**) which was then subjected to a bromoacetylation reaction using acetyl bromide in dry acetonitrile to provide the bromoacetate (**5**) in 44% yield after chromatography.[24] The reductive β-elimination of the acetoxy-bromo nucleoside (**5**) proceeded by adding zinc dust in refluxing ethanol to afford the olefinic nucleoside (**6**) in 64% yield after chromatography.[25] Hydrolysis of the methyl esters of **6** was performed

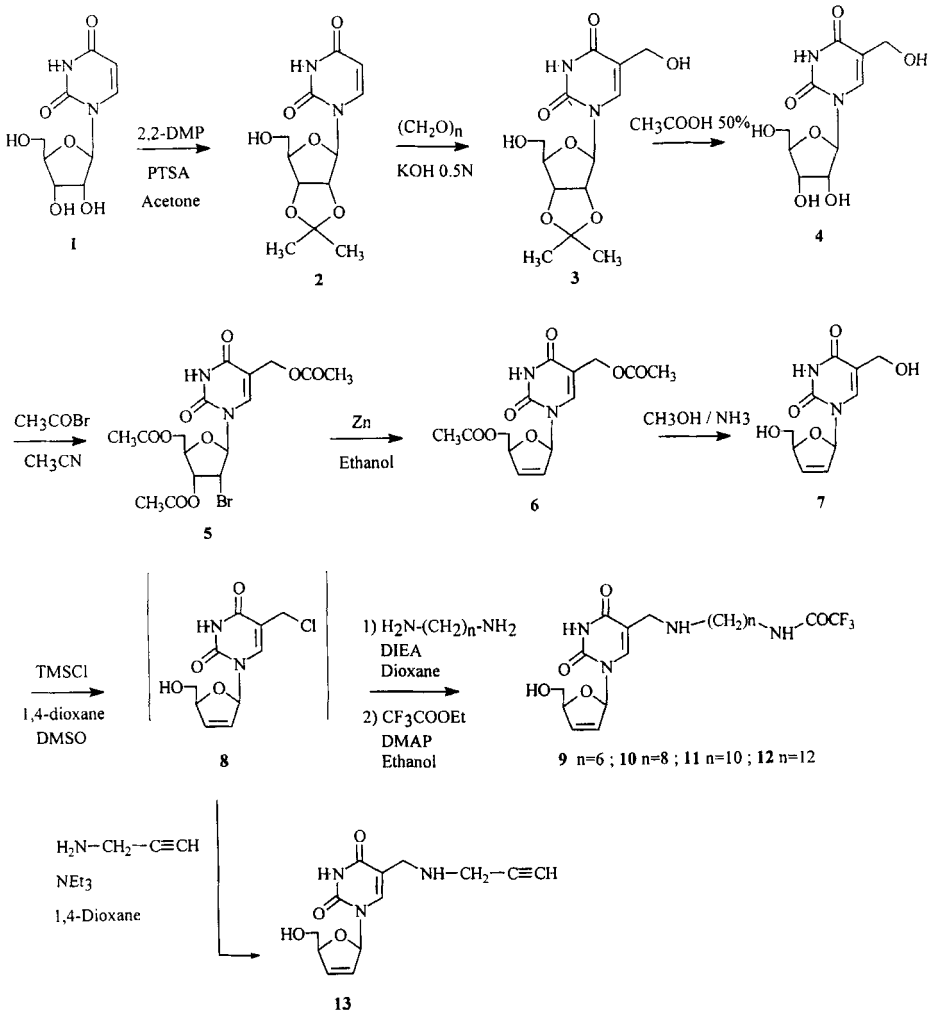

FIGURE 2. Synthesis of 2',3'-didehydro-2',3'-dideoxy-5-[N-(substituted)aminomethyl]-uridine (**9**) to (**13**).

using saturated methanolic ammonia at room temperature and the C-5-hydroxymethyl key intermediate (**7**) was isolated in 81% yield. The 5-hydroxymethyl-2',3'-didehydro-2',3'-dideoxy-nucleoside (**7**) was further converted into the corresponding 5-chloromethyl derivative (**8**) using chlorotrimethylsilane in the presence of dimethylsulfoxide in dry 1,4-dioxane at 60°C for 4h.[26] This intermediate was utilized without further purification to introduce an aminoalkyl group on C-5 position.

The 5-chloromethyl-nucleoside (**8**) was reacted with 1 Eq 1,*n*-diaminoalkanes (n=6, 8, 10 and 12) in the presence of *N,N*-diisopropylethylamine to introduce an amine-

linker at C-5 position of the uracil ring. The resulting compounds were unstable and very hygroscopic. To overcome this problem, the terminal amino group of these compounds was protected by trifluoroacetyl group (ethyl trifluoroacetate in the presence of DMAP) without further purification after removal of the excess solvent giving respectively the expected products **9** to **12** in 12 to 65% yield after chromatography. Similarly, **8** was treated with 7 Eq of propargylamine in the presence of triethylamine in the 1,4-dioxane. After 2h at 45°C, TLC analysis of the reaction indicated a complete consumption of the starting material and the expected 2',3'-didehydro-2',3'-dideoxy-5-[N-(prop-2-ynyl)aminomethyl]-uridine (**13**) was isolated in 13% yield after chromatography. To firmly establish the identity of the nucleosides, the structures have been examined in details using 2D NMR determination.

Biological

The aim of this work was focused on the development of a series of d4T analogues bearing amino *linker* arms at C-5 position of the uracil ring and the evaluation of their anti-HIV-1 activity. Therefore, the newly synthesized 2',3'-unsaturated-C-5-modified thymidine derivatives **9** to **13** were evaluated for inhibition of HIV-1 multiplication in cells of the lymphocytic lineage (CEM-SS and MT-4), and the results are summarized in Table 1. As shown in Table 1, the nucleosides **9**, **11** (containing respectively six and ten methylene units in the spacer) and **13** were unfortunately devoid of antiviral activity. Nevertheless, both compounds **10** and **12** bearing a tether containing eight and twelve methylene units displayed a weak activity depending on the nature of the cells, only in the CEM-SS cells (IC_{50} 7.4 μM and 2.8 μM respectively). These compounds **10** and **12** were also found the more cytotoxic (CC_{50} 18 μM and 12 μM respectively).

Perhaps, the weak antiviral activities of the analogues **10** and **12** are probably due to their poor phosphorylation in CEM-SS cells. These cell-dependent variations must probably lie 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 weak activities of these newly synthesized d4T analogues including the efficiency of phosphorylation, it is clear that introduction of a long tether on the C-5 position of the uracil ring leads unfortunately to a marked reduction of anti-HIV activity.

TABLE 1. Antiviral and cytotoxicity evaluation of β-D-d_4T analogues bearing linker arms **9**, **10**, **11**, **12**, and **13**.

Compd	CH_2 units	HIV-1_{LAI} in CEM-SS cells			HIV-1_{IIIB} in MT-4 cells		
		IC50 (μM)*	CC50 (μM)†	SI‡	IC50 (μM)	CC50 (μM)	SI
d_4T		0.059±0.016	>100	>1695	0.2 ± 0.08	>100	>500
9	6	>100	>100	>1	>100	>100	>1
10	8	7.4	18	2.43	>100	12	>0.12
11	10	≅100	>100	>1	>100	>100	>1
12	12	2.8	12	4.28	>100	16	>0.16
13		120	510	4.25	23	200	8.70

* 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

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. Moreover, coupling of the terminal alkyne of the C-5 linked d4T analogue **13** with an halide label is also ongoing.

EXPERIMENTAL SECTION

Chemical procedures

Uridine was purchased from Acros and was used without purification. Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were recorded on a Fourrier transform Mattson spectrometer Genesis DTGS using WinFIRST™ Macros and ApPro™ and only noteworthy absorptions are listed. ^1H 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). Thin layer chromatography (TLC) were performed on precoated plastic sheets, silica gel 60 F_{254}, 0.2 mm layer (Macherey-Nagel); products were visualized 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 1,4-dioxane was distilled from sodium-benzophenone; anhydrous acetonitrile was distilled from phosphorus pentoxide, anhydrous ethanol was prepared by using magnesium turnings, triethylamine and *N,N*-diisopropylethylamine were dried over calcium hydride.

2',3'-O-isopropylidene-5-hydroxymethyl-uridine (3). To a suspension of **2** (15 g, 0.053 mol) in 0.5 N KOH (95 mL) was added paraformaldehyde (3.45 g, 0.12 mol) and the reaction mixture was heated at 60°C for 2 days. After evaporation to dryness *in vacuo*, the oily residue was purified by silica gel column chromatography (dichloromethane-methanol 97:3). Fractions containing the same product were combined and concentrated to yield 13.5 g (81 %) of **3** as a white solid: R_f 0.49 [CH_2Cl_2-CH_3OH (90:10)]; mp 171-172°C (methanol); IR (KBr): 3489, 3361, 1702, 1675, 1638, 1497 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.42 (brs, 1H, NH), 7.68 (s, 1H, H-6), 5.84 (d, J = 2.9 Hz, 1H, H-1'), 5.05 (d, J = 2.9 Hz, 1H, H-2'), 4.91-4.73 (m, 3H, H-3' + 2 OH), 4.11-4.03 (m, 3H, H-4' + C-5-C<u>H</u>$_2$OH), 3.57 (m, 2H, H-5'), 1.43 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 162.8 (C-4), 150.3 (C-2), 138.4 (C-6), 114.3 (C-5), 113.1 (C), 91.0 (C-1'), 86.3 (C-4'), 83.5 (C-3'), 80.6 (C-2'), 61.3 (C-5'), 56.0 (C-5-<u>C</u>H_2OH), 27.1 (CH_3), 25.2 (CH_3). *Anal.* Calcd for $C_{13}H_{18}N_2O_7$: C, 49.68; H, 5.77; N, 8.91. Found: C, 49.52; H, 5.50; N, 8.71.

5-hydroxymethyl-uridine (4). A solution of **3** (9 g, 28.6 mmol) in 50% acetic acid was heated to reflux with stirring for 5 h. After cooling down to room temperature, the solvent was evaporated *in vacuo*. The oily residue was then co-evaporated with ethanol and the crude product was crystallised from diethyl ether to give 7.8 g (99%) of **4** as a white solid: R_f 0.24 [CH_2Cl_2-CH_3OH (80:20)]; mp 167°C (167°C)[28]; IR (KBr): 3360, 1710, 1670, 1460, 1250 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.32 (brs, 1H, NH), 7.76 (s, 1H,

H-6), 5.81 (d, J = 5.73 Hz, 1H, H-1'), 5.35 (s, 2H, OH), 5.04-4.91 (m, 2H, OH), 4.13 (s, 2H, C-5-C<u>H</u>$_2$OH), 4.02 (t, J = 5.43 Hz, 1H, H-2'); 3.94 (t, J = 4.88 Hz, 1H, H-3'), 3.83 (m, 1H, H-4'), 3.56 (m, 2H, H-5'); ^{13}C NMR (DMSO-d$_6$): δ 162.7 (C-4), 150.8 (C-2), 137.2 (C-6), 114.3 (C-5), 87.4 (C-1'), 84.9 (C-4'), 73.3 (C-3'), 70.1 (C-2'), 61.1 (C-5'), 56.0 (C-5-<u>C</u>H$_2$OH). *Anal.* Calcd for C_{10} H_{14} N_2 O_7: C, 43.80; H, 5.15; N, 10.22. Found: C, 43.77; H, 5.12; N, 10.19.

3',5'-di-O-acetyl-2'-bromo-2'-deoxy-5-acetoxymethyl-uridine (5). Under an argon atmosphere, a solution of acetyl bromide (4.3 mL, 58.2 mmol) in anhydrous acetonitrile (8 mL) was added dropwise to a boiling suspension of **4** (2 g, 7.29 mmol) in anhydrous acetonitrile (45 mL). The reaction mixture was stirred at 80°C for 30 min and allowed to cool to room temperature. After evaporation to dryness under reduced pressure, the residue was dissolved in dichloromethane (200 mL) and the solution was washed with water (5x200 mL). The organic layer was separated, dried (magnesium sulphate), and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel, using *n*-hexane-ethyl acetate (1:1) as eluent to yield 1.5 g (44%) of **5** as a white solid: R_f 0.40 [*n*-hexane-EtOAc (50:50)]; mp 88-90°C (diethyl ether); IR (KBr): 3200, 1750, 1745,1690, 1450, 1380, 1270 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 11.80 (brs, 1H, NH), 8.04 (s, 1H, H-6), 6.13 (d, J = 6.8 Hz, 1H, H-1'), 5.24 (t, J = 4.88 Hz, 1H, H-3'), 4.98 (t, J = 6.46 Hz, 1H, H-2'), 4.38-4.23 (m, 5H, H-4' + H-5' + C-5-C<u>H</u>$_2$OAc), 2.12 (s, 3H, CH$_3$), 2.07 (s, 3H, CH$_3$), 2.05 (s, 3H, CH$_3$); ^{13}C NMR (DMSO-d$_6$): δ 170.2 (CO), 169.3 (CO), 161.4 (C-4), 150.1 (C-2), 139.9 (C-6), 111.6 (C-5), 89.3 (C-1'), 79.7 (C-4'), 70.9 (C-3'), 62.7 (C-5'), 56.0 (C-5-<u>C</u>H$_2$OAc), 47.1 (C-2'), 20.6 (CH$_3$), 20.5 (CH$_3$). *Anal.* Calcd for C_{16} H_{19} N_2 O_9 Br: C, 41.49; H, 4.13; N, 6.05. Found: C, 41.10; H, 3.92; N, 5.92.

5'-O-acetyl-2',3'-didehydro-2',3'-dideoxy-5-acetoxymethyl-uridine (6). Under an argon atmosphere, zinc dust (0.97 g) was added with stirring to a boiling solution of **5** (4.4 g, 9.50 mmol) in anhydrous ethanol (150 mL). The heterogeneous reaction mixture was refluxed for 6 min (monitored by TLC) until no starting material remained, cooled to room temperature and filtered through a pad of celite. The filtrate was concentrated under reduced pressure and the residue was chromatographed on a silica gel column (ethyl

acetate). The fractions containing the desired product were pooled together, and the solvent was evaporated *in vacuo* to afford 1.95 g (64%) of the diacetate **6** as a white solid: R_f 0.53 [EtOAc]; mp 118-120°C (ethanol); IR (KBr) 3200, 1750, 1745, 1680, 1460, 1380, 1250 cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.84 (brs, 1H, NH), 7.39 (s, 1H, H-6), 6.82 (m, 1H, H-1'), 6.43 (d, J = 5.85 Hz, 1H, H-2'), 6.03 (d, J = 6.10 Hz, 1H, H-3'), 4.99 (m, 1H, H-4'), 4.25-4.09 (m, 4H, H-5' + C-5-CH_2OAc), 2.14 (s, 3H, CH_3), 2.08 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 170.1 (CO), 162.4 (C-4), 150.4 (C-2), 137.7 (C-2'), 133.7 (C-3'), 126.4 (C-6), 110.9 (C-5), 89.3 (C-1'), 83.6 (C-4'), 65.0 (C-5-CH_2OAc), 64.3 (C-5'), 20.6 (CH_3), 20.5 (CH_3). *Anal.* Calcd for $C_{14}H_{16}N_2O_7$: C, 51.85; H, 4.97; N, 8.64. Found: C, 51.75; H, 4.82; N, 8.43.

2',3'-didehydro-2',3'-dideoxy-5-hydroxymethyl-uridine (7). A solution of **6** (1 g, 3.1 mmol) in methanol saturated with ammonia (20 mL) was stirred at room temperature for 24 h. The reaction mixture was evaporated under reduced pressure and coevaporated several times with ethanol. The residue was chromatographed on a silica gel column with 11% methanol in chloroform as eluent. Concentration of the fractions gave a residue which was crystallized from diethyl ether to yield 0.6 g (81%) of the diol **7** as a white solid: R_f 0.60 [EtOAc-CH_3OH (90:10)]; mp 170-172°C; IR (KBr): 3450, 3420, 3200, 1680, 1700, 1635 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.39 (brs, 1H, NH), 7.76 (s, 1H, H-6), 6.82 (d, J = 1.1 Hz, 1H, H-1'), 6.42 (d, J = 5.61 Hz, 1H, H-2'), 5.94 (d, J = 5.61 Hz, 1H, H-3'), 4.98 (s, 1H, OH), 4.78 (s, 1H, H-4'), 4.10-3.99 (m, 3H, C-5-CH_2OH + OH), 3.58 (m, 2H, H-5'); ^{13}C NMR (DMSO-d_6): δ 162.5 (C-4), 150.6 (C-2), 139.1 (C-6), 135.1 (C-2'), 125.8 (C-3'), 110.8 (C-5), 89.1 (C-1'), 87.4 (C-4'), 64.2 (C-5-CH_2OH), 62.3 (C-5'). *Anal.* Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.95; H, 5.22; N, 11.86.

General procedure for the synthesis of 2',3'-didehydro-2',3'-dideoxy-5-[*N*-(substituted)aminomethyl]-uridine (9 to 12). A mixture containing **7** (300 mg, 1.25 mmol), 0.8 mL (6.33 mmol) chlorotrimethylsilane and dimethylsulfoxide (0.2 mL, 2.5 mmol) in dry 1,4-dioxane (20 mL) was stirred at 60-62°C for 4 h under argon atmosphere. The reaction solution was then evaporated and coevaporated with 1,4-dioxane, the residue was dissolved in anhydrous 1,4-dioxane (15 mL) and added successively with *N,N*-diisopropylethylamine (0.20 mL) and the corresponding *n*-

diaminoalkane (1.24 mmol). The mixture was stirred at 45°C for 6 h and the solvent was removed *in vacuo*. Then the terminal amino group was protected by trifluoroacetyl group without further purification. Ethyl trifluoroacetate (0.40 mL, 3.38 mmol) was added dropwise to a solution of the crude product in ethanol (15 mL) containing 4-dimethylaminopyridine (0.079 mmol). The reaction mixture was stirred at room temperature for 2 days and concentrated to dryness *in vacuo*. The purified products **9** to **12** were obtained by silica-gel column chromatography.

2',3'-didehydro-2',3'-dideoxy-5-[*N*-(6-trifluoroacetylaminohexyl)aminomethyl] -uridine (9). The crude product was purified by silica-gel column chromatography (silica pre-equilibrated with 1% Et_3N) using methanol in ethyl acetate (0-16%) as eluents to yield 350 mg (65%) of **9** as a white solid: R_f 0.33 [EtOAc-CH_3OH (70:30)]; ^{1}H NMR (DMSO-d_6): δ 9.41 (brs, 2H, NH), 7.63 (s, 1H, H-6), 6.82 (s, 1H, H-1'), 6.41 (m, 1H, H-2'), 5.91 (m, 1H, H-3'), 4.76 (m, 1H, H-4'), 3.56 (m, 4H, H-5' + C-5-$C\underline{H}_2$NH), 3.15 (m, 2H, $C\underline{H}_2NHCOCF_3$), 2.41 (m, 2H, C-5-$CH_2NHC\underline{H}_2$), 1.45-1.25 (m, 8H, $(CH_2)_4$); ^{13}C NMR (DMSO-d_6): δ 163.4 (C-4), 156.0 ($\underline{C}OCF_3$), 150.7 (C-2), 137.6 (C-6), 135.1 (C-2'), 125.7 (C-3'), 116.0 (CO$\underline{C}$F3), 111.7 (C-5), 89.1 (C-1'), 87.4 (C-4'), 62.5 (C-5'), 48.2 (C-5-$CH_2NH\underline{C}H_2$), 45.0 (C-5-$\underline{C}H_2NH$), 39.1 ($\underline{C}H_2NHCOCF_3$), 29.1-28.2-26.3-26.1 [$(CH_2)_4$]. *Anal.* Calcd for $C_{18}H_{25}N_4O_5F_3$: C, 49.77; H, 5.80; N, 12.90; F, 13.12. Found: C, 49.68; H, 5.79; N, 12.84; F, 13.09.

2',3'-didehydro-2',3'-dideoxy-5-[*N*-(8-trifluoroacetylaminooctyl)aminomethyl] -uridine (10). The crude product was purified by silica-gel column chromatography using dichloromethane-methanol (80:20) as eluent to yield 250 mg (43%) of **10** as a white solid: R_f 0.48 [CH_2Cl_2-CH_3OH (80:20)]; ^{1}H NMR (DMSO-d_6): δ 9.43 (brs, 2H, NH), 7.81 (s, 1H, H-6), 6.81 (s, 1H, H-1'), 6.42 (m, 1H, H-2'), 5.91 (m, 1H, H-3'), 4.77 (m, 1H, H-4'), 3.57 (m, 2H, H-5'), 3.46 (m, 2H, C-5-$C\underline{H}_2$NH), 3.15 (m, 2H, $C\underline{H}_2NHCOCF_3$), 2.58 (m, 2H, C-5-$CH_2NHC\underline{H}_2$), 1.45-1.23 (m, 12H, $(CH_2)_6$); ^{13}C NMR (DMSO-d_6): δ 163.2 (C-4), 156.3 ($\underline{C}OCF_3$), 150.6 (C-2), 140.3 (C-6), 135.5 (C-2'), 125.5 (C-3'), 115.8 (CO$\underline{C}$F3), 114.6 (C-5), 89.3 (C-1'), 87.6 (C-4'), 62.4 (C-5'), 47.2 (C-5-$CH_2NH\underline{C}H_2$), 43.6 (C-5-$\underline{C}H_2NH$), 39.1 ($\underline{C}H_2NHCOCF_3$), 28.6-28.5-28.2-26.3-26.1 [$(CH_2)_6$]. *Anal.* Calcd for $C_{20}H_{29}N_4O_5F_3$: C, 51.94; H, 6.32; N, 12.11; F, 12.32. Found: C, 51.89; H, 6.27; N, 12.08; F, 12.27.

2',3'-didehydro-2',3'-dideoxy-5-[*N*-(10-trifluoroacetylaminodecyl)amino methyl]-uridine (11). The crude product was purified by silica-gel column chromatography using chloroform-methanol (70:30) as eluent to yield 100 mg (17%) of **11** as a white solid: R_f 0.55 [$CHCl_3$-CH_3OH (70:30)]; ^1H NMR (DMSO-d_6): δ 9.43 (brs, 2H, NH), 7.87 (s, 1H, H-6), 6.81 (m, 1H, H-1'), 6.42 (d, J = 5.8 Hz, 1H, H-2'), 5.90 (d, J = 5.8 Hz, 1H, H-3'), 4.77 (m, 1H, H-4'), 3.58 (s, 2H, H-5'), 3.51 (s, 2H, C-5-CH_2NH), 3.16-3.12 (m, 2H, $CH_2NHCOCF_3$), 2.62 (m, 2H, C-5-CH_2NHCH_2), 1.46-1.23 (m, 16H, $(CH_2)_8$); ^{13}C NMR (DMSO-d_6): δ 163.4 (C-4), 156.1 ($COCF_3$), 150.7 (C-2), 141.0 (C-6), 135.5 (C-2'), 125.6 (C-3'), 116.0 (COCF3), 107.6 (C-5), 89.5 (C-1'), 87.7 (C-4'), 62.4 (C-5'), 47.2 (C-5-CH_2NHCH_2), 43.7 (C-5-CH_2NH), 39.1 ($CH_2NHCOCF_3$), 29.0-28.9-28.7-28.3-27.1-26.5-26.3 ($(CH_2)_8$). *Anal.* Calcd for C_{22} H_{33} N_4 O_5 F_3: C, 53.87; H, 6.78; N, 11.42; F, 11.62. Found: C, 53.84; H, 6.74; N, 11.39; F, 11.60.

2',3'-didehydro-2',3'-dideoxy-5-[*N*-(12-trifluoroacetylaminododecyl)amino methyl]-uridine (12). The crude product was purified by silica-gel column chromatography (silica pre-equilibrated with 1% Et_3N) using dichloromethane-methanol (90:10) as eluent to yield 50 mg (12%) of **12** as a white solid: R_f 0.68 [CH_2Cl_2-CH_3OH (80:20)]; ^1H NMR ($CDCl_3$): δ 7.91 (s, 1H, H-6), 7.02 (s, 1H, H-1'), 6.34 (d, J = 5.6 Hz, 1H, H-2'), 5.82 (d, J = 5.6 Hz, 1H, H-3'), 4.96 (s, 1H, OH), 4.88 (m, 1H, H-4'), 3.85-3.59 (m, 4H, H-5' + C-5-CH_2NH), 3.35 (m, 2H, $CH_2NHCOCF_3$), 2.63 (m, 2H, C-5-CH_2NHCH_2), 1.55-1.25 (m, 20H, $(CH_2)_{10}$); ^{13}C NMR ($CDCl_3$): δ 164.5 (C-4), 156.9 ($COCF_3$), 151.3 (C-2), 139.9 (C-6), 135.0 (C-2'), 126.0 (C-3'), 110.7 (C-5), 100.0 ($COCF_3$), 89.8 (C-1'), 87.8 (C-4'), 62.9 (C-5'), 49.1 (C-5-CH_2NHCH_2), 45.7 (C-5-CH_2NH), 39.9 ($CH_2NHCOCF_3$), 29.4-29.3-29.0-28.7-27.0-26.6 [$(CH_2)_{10}$]. *Anal.* Calcd for C_{24} H_{37} N_4 O_5 F_3: C, 55.59; H, 7.19; N, 10.80; F, 10.99. Found: C, 55.54; H, 7.17; N, 10.77; F, 10.92.

2',3'-didehydro-2',3'-dideoxy-5-[*N*-(prop-2-ynyl)aminomethyl]-uridine (13). A mixture containing **7** (200 mg, 0.83 mmol), 1.5mL (11.82 mmol) chlorotrimethylsilane and dimethylsulfoxide (0.2 mL, 2.83 mmol) in dry 1,4-dioxane (20 mL) was stirred at 60-62°C for 4 h under an argon atmosphere. The reaction solution was then evaporated and coevaporated with 1,4-dioxane, the residue was dissolved in anhydrous 1,4-dioxane

(20 mL) and added successively 0.75 mL fresh distilled triethylamine (5.38 mmol) and 0.4 mL propargylamine (5.76 mmol). The mixture was stirred at 45°C for 2 h and the solvent was removed *in vacuo*. The residue was chromatographed on a silica gel column with 3% methanol in ethyl acetate as eluent. Concentration of the fractions gave 30 mg (13%) of **13** as white crystals: R_f 0.34 [CH_2Cl_2-CH_3OH (90:10)]; IR (KBr): 3259, 1704-1681, 1466,1253, 1079 cm^{-1}; ^1H NMR (DMSO-d_6): δ 11.37 (brs, 1H, NH), 7.57 (s, 1H, H-6), 6.81 (s, 1H, H-1'), 6.41 (d, J = 5.7 Hz, 1H, H-2'), 5.90 (d, J = 5.7 Hz, 1H, H-3'), 4.96 (brs, 1H, OH), 4.75 (m, 1H, H-4'), 3.54 (m, 2H, H-5'), 3.31 (m, 2H, C-5-CH_2NH), 3.24 (m, 2H, CH_2CCH), 3.04 (s, 1H, CH_2CCH); ^{13}C NMR (DMSO-d_6): δ 163.5 (C-4), 150.9 (C-2), 137.7 (C-6), 135.4 (C-2'), 125.9 (C-3'), 111.7 (C-5), 89.3 (C-1'), 87.6 (C-4'), 82.9 (C), 74.0 (CCH), 62.7 (C-5'), 43.9 (C-5-CH_2NH), 36.9 (CH_2CCH). *Anal.* Calcd for C_{13} H_{15} N_3 O_4: C, 56.31; H, 5.45; N, 15.15. Found: C, 56.29; H, 5.42; N, 15.11.

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.[29] The 50% inhibitory concentration (IC_{50}) was derived from the computer generated median effect plot of the dose-effect data.[30] 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).[31] 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; [29,32] virus production was quantified by the RT activity associated to virus particles released from the cells in the culture medium. Conditions in which the inhibitory properties were measured on HIV-1 reverse transcriptase *in vitro* has also been described.[29] *In vitro* RT inhibition was also described.[29] 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).

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