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Synthesis of 5-alkynylated d4T Analogues as Potential HIV-1 Reverse Transcriptase Inhibitors

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A series of 2′,3′-didehydro-2′,3′-dideoxynucleosides substituted with an alkynylhydroxy- (6, 7, 12 and 13) and alkynylamino- (20) groups at the C-5 position were synthesized. All these five target modified nucleosides were tested for anti-human immunodeficiency virus type 1 activity in CEM-SS and MT-4 cells and unfortunately displayed no improvement in antiviral activity.

Keywords: HIV; Reverse Transcriptase; d4T Analogues; Alkynyl Tether; Sonogashira

INTRODUCTION

In connection with our ongoing investigations on d4T analogues as antiviral agents, we wanted to prepare C-5 alkynyl 2′,3′-dideoxy-2′,3′-didehydronucleosides bearing a tether potentially suitable for functionalization before attaching additional reporter groups. As for all bio-active nucleoside analogues, these C-5 alkynyl nucleosides need to be converted to their 5′-triphosphate form by metabolic phosphorylation.

Preliminary studies suggested that C-5-modified nucleosides did not significantly disrupt the normal base-pairing and helical conformation of the DNA duplex.[1,2] In fact, Rong *et al.* reported that tethering the 5-position by a flexible chain (10 Å) may permit the triphosphate to be generated and thereby their incorporation in nucleic acids.[3] Many C-5 substituted nucleoside analogues exerting biological properties have arisen in the literature incorporating a range of 5-alkynyl substituents in ribonucleosides,[4] 2′-deoxynucleosides[5] and 2′,3′-dideoxynucleosides.[6] In particular, the 1-(β-D-arabinofuranosyl)-5-prop-1-ynyluracil (netivudine, 882C) has emerged as a potent inhibitor of varicella-zoster virus (VZV) (Figure 1).[7] Among the chemical modifications carried out at the 5-position of the pyrimidine ring, alkynylamino[8,9] and alkynylhydroxy groups have been reported in the literature.[10–13]

Furthermore, dideoxynucleosides have been described as being useful as chain terminators in DNA sequencing using the Sanger method.[14] The pre-marking of the dideoxynucleosides could be accomplished by covalent attachment of a fluorescent reporter to a desired chain-terminating nucleoside. The reporter moiety can be bound covalently to the 5-position of the pyrimidine ring towards a linker arm.

Many oligonucleotides having tethers attached at the base have also been developed and used to introduce reporter groups and other functional groups. The structural chemical modifications, in particular those of the pyrimidine base at the 5-position have aroused a large amount of interest. Oligonucleotides including a 5-alkynyl modified nucleotide form stable duplex[15,16] or triplex structures with RNA and DNA target sequences. In particular, the triplex–helix complex is stabilized by substitution with 5-(1-propynyl)-2′-deoxyuridine and destabilized by substitution with 5-(1-propynyl)-2′-deoxycytidine, while the double-helix is stabilized by both substitutions.[17] This is likely to be due to the hydrophobicity of the propyne group which could displace highly ordered water molecules in the major groove and to the overlap of the π-system of the propyne group with the π-systems of the bases within the same strand.[17,18]

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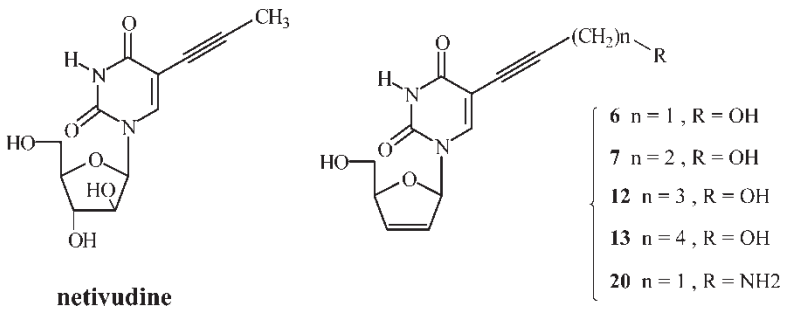


FIGURE 1 Structure of netivudine and the general structure of the new synthesized 5-alkynyl d4T analogues.

Recently, the use of 5-(1-propargylamino)-2′-deoxyuridine as a novel thymidine analogue for generating DNA triplexes with increased stability has been described.[19]

Since we are interested in 2′,3′-didehydro-2′,3′-dideoxynucleosides having anti-HIV properties, we report here the synthesis of new d4T analogues bearing 5-alkynyl type side-chains. These C-5 alkynyl-2′,3′-didehydro-2′,3′-dideoxynucleosides bearing a tether can be considered as chain terminators providing analogues available for attaching fluorescent dyes or other reporter groups.[20,21]

MATERIAL AND METHODS

Chemistry

Reagent grade acetonitrile was refluxed and distilled from phosphorus pentoxide. Anhydrous ethanol was prepared using magnesium turnings. Anhydrous methanol, acetone, *N*,*N*-dimethylformamide and pyridine were purchased from E. Merck, Carlo Erba and Aldrich respectively and were used as such. Unless otherwise stated, reactions were run under argon and monitored by thin-layer chromatography (TLC) using precoated silica gel 60 F_{254} sheets (0.2 mm layer) purchased from Macherey-Nagel, and compounds were detected by UV absorption at 254 nm. Column chromatography was achieved by using Merck silica gel 60 (0.063–0.200 nm). Silica gel Si-60 used for flash chromatography (40–63 μm) was supplied by Merck. All samples were kept in a drying oven at 30°C over P_4O_{10} for at least 24 h prior to analysis.

IR spectra were recorded on a Fourier transform Mattson spectrometer Genesis DTGS using WinFIRST™ Macros and ApPro™ 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, q = quadruplet, m = multiplet).

5′-O-Acetyl-5-[3-(tetrahydro-2H-pyran-2-yloxy)-prop-1-ynyl]-2′,3′-didehydro-2′,3′-dideoxyuridine (2)

To a deoxygenated solution of **1** (870 mg, 2.3 mmol) in anhydrous DMF (40 mL) were added successively tetrahydro-2-(2-propynyloxy)-2*H*-pyran (0.97 mL, 6.9 mmol, 3 equiv.), anhydrous triethylamine (8.0 mL, 57.5 mmol, 25 equiv.), copper(I)iodide (87.6 mg, 0.2 equiv.) and tetrakis(triphenylphosphine)palladium(0) (35.8 mg, 0.23 mmol, 0.1 equiv.). The reaction mixture was stirred at 25°C under an argon atmosphere for 3 h, filtered through celite and the filtrate evaporated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL), washed with saturated aqueous NaCl (2 × 100 mL), 5% EDTA (2 × 100 mL) and saturated aqueous NaCl (100 mL). The organic layer was separated, dried over anhydrous magnesium sulphate and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (CH_2Cl_2 to CH_2Cl_2/AcOEt = 50 : 50 as eluents) to yield 680 mg (76%) of **2**; TLC R_f (AcOEt) 0.63; ^{1}H-NMR (d_6-DMSO), *δ*; ppm; J, Hz: 1.42–1.48 (m, 4H, $OCH_2(CH_2)_2$ of the THP), 1.60–1.67 (m, 2H, $O(CH_2)_3CH_2$ of the THP), 2.05 (s, 3H, CH_3), 3.42–3.46 (m, 1H, OCH_{axial}), 3.66–3.72 (m, 1H, $OCH_{equatorial}$), 4.13 (dd, J = 12.6, J = 2.5, 1H, *H*-5′), 4.27 (dd, J = 16.1, J = 1.2, 1H, C ≡ C–*CH*–O), 4.31 (dd, J = 12.6, J = 2.7 Hz, 1H, *H*-5″), 4.38 (dd, C ≡ C–*CH*′–O, J = 16.1 Hz, J = 1.2 Hz), 4.72 (m, 1H, C*H* of the THP), 5.03 (s, 1H, *H*-4′), 6.01 (d, J = 5.8 Hz, 1H, *H*-2′), 6.42 (d, J = 5.8 Hz, 1H, *H*-3′), 6.79 (m, 1H, *H*-1′), 7.72 (s, 1H, *H*-6), 11.75 (bs, 1H, N*H*); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 18.7 ($O(CH_2)_2CH_2$ of the THP), 20.6 (CH_3), 24.8 (OCH_2CH_2 of the THP), 29.8 ($O(CH_2)_3CH_2$ of the THP), 54.0 (C ≡ C–CH_2–O), 61.1 (OCH_2 of the THP), 64.1 (*C*-5′), 77.8 (C ≡ C–CH_2), 84.1 (*C*-4′), 89.1 (C ≡ C–CH_2), 89.5 (*C*-1′), 96.2 (CH of the THP), 98.0 (*C*-5), 126.4 (*C*-2′), 134.1 (*C*-3′), 143.9 (*C*-6), 149.7 (*C*-2), 161.3 (*C*-4), 170.0 (CH_3COO). Anal. ($C_{19}H_{22}N_2O_7$) C, H, N.

5′-O-Acetyl-5-[4-(tetrahydro-2H-pyran-2-yloxy)-but-1-ynyl]-2′,3′-didehydro-2′,3′-dideoxyuridine (3)

Compound **1** (500 mg, 1.32 mmol) was converted to **3** using 2-(3-butynyloxy)tetrahydro-2*H*-pyran

(0.62 mL, 3.97 mmol) by the same procedure as that described for **2**, except that the reaction mixture was stirred at 25°C for 10 h. This product was purified by silica-gel chromatography (CH_2Cl_2 to CH_2Cl_2/AcOEt = 50 : 50 as eluents) to yield 370 mg (70%) of the title compound: TLC R_f (CH_2Cl_2/AcOEt = 30 : 70) 0.45; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 1.41–1.46 (m, 4H, $OCH_2(CH_2)_2$), 1.56–1.72 (m, 2H, $O(CH_2)_3CH_2$), 2.06 (s, 3H, CH_3), 2.59 (t, J = 6.7, 2H, C ≡ C–CH_2), 3.37–3.43 (m, 1H, OCH_{axial}), 3.44–3.48 (m, 1H, C ≡ C–CH_2–C*H*–O), 3.63–3.68 (m, 1H, C ≡ C–CH_2–C*H*′–O), 3.72–3.78 (m, 1H, $OCH_{equatorial}$), 4.13 (dd, J = 12.4, J = 2.3, 1H, *H*-5′), 4.30 (dd, J = 12.4, J = 2.6, 1H, *H*-5″), 4.60 (m, 1H, C*H* of the THP), 5.02 (s, 1H, *H*-4′), 6.01 (d, J = 5.9, 1H, *H*-2′), 6.41 (d, J = 5.9, 1H, *H*-3′), 6.78 (m, 1H, *H*-1′), 7.60 (s, 1H, *H*-6), 11.70 (bs, 1H, N*H*); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 19.1 ($O(CH_2)_2CH_2$ of the THP), 20.3 (C ≡ C–CH_2), 20.7 (CH_3), 25.0 (OCH_2CH_2 of the THP), 30.1 ($O(CH_2)_3CH_2$ of the THP), 61.3 (OCH_2 of the THP), 64.1 (*C*-5′), 64.7 (CH_2CH_2O), 73.2 (*C* ≡ C–CH_2), 84.1 (*C*-4′), 89.4 (*C*-1′), 91.2 (C ≡ *C*–CH_2), 97.9 (*C*H of the THP), 99.0 (*C*-5), 126.4 (*C*-2′), 134.2 (*C*-3′), 142.8 (*C*-6), 149.7 (*C*-2), 161.6 (*C*-4), 170.1 (CH_3COO). Anal. ($C_{20}H_{24}N_2O_7$) C, H, N.

5-(3-Hydroxyprop-1-ynyl)-5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (4)

A solution of **2** (150 mg, 0.384 mmol) in 15 mL of dichloromethane/methanol/trifluoroacetic acid (11:3:1) was stirred at room temperature for 30 min and then evaporated to dryness *in vacuo*. The oily residue was then co-evaporated with methanol (2 × 10 mL) and the crude product was crystallised from dichloromethane to give 90 mg (77%) of the title compound: TLC R_f (CH_2Cl_2/AcOEt = 20:80) 0.21; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 2.06 (s, 3H, CH_3), 4.13 (dd, J = 12.6, J = 2.4, 1H, *H*-5′), 4.18 (s, 2H, CH_2–OH), 4.31 (dd, J = 12.6, J = 2.6, 1H, *H*-5″), 5.03 (s, 1H, *H*-4′), 5.25 (bs, 1H, O*H*), 6.01 (d, J = 5.8, 1H, *H*-2′), 6.43 (d, J = 5.8, 1H, *H*-3′), 6.79 (m, 1H, *H*-1′), 7.68 (s, 1H, *H*-6), 11.73 (bs, 1H, N*H*). Anal. ($C_{14}H_{14}N_2O_6$) C, H, N.

5-(4-Hydroxybut-1-ynyl)-5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (5)

Method 1: Compound **1** (500 mg, 1.32 mmol) was converted to **5** using 3-butyn-1-ol (0.3 mL, 3.97 mmol, 3 equiv.) by the same procedure as that described for **2**, except that the reaction mixture was stirred at 25°C for 22 h. The product was purified by silica-gel chromatography using ethyl acetate as eluent to yield 190 mg (45%) of the title compound.

Method 2: Compound **3** (370 mg, 0.90 mmol) was converted to **5** by the procedure described for **4**. The product was purified by silica-gel chromatography (CH_2Cl_2 to CH_2Cl_2/AcOEt = 90 : 10 as eluents) to yield 216 mg (75%) of the title compound: TLC R_f (AcOEt) 0.28; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 2.06 (s, 3H, CH_3), 2.45 (t, J = 6.7, 2H, C ≡ C–CH_2), 3.47 (t, J = 6.7, 2H, CH_2–OH), 4.13 (dd, J = 12.5, J = 2.2, 1H, *H*-5′), 4.31 (dd, J = 12.5, J = 2.4, 1H, *H*-5″), 4.82 (bs, 1H, O*H*), 5.01 (s, 1H, *H*-4′), 6.00 (d, J = 5.7, 1H, *H*-2′), 6.41 (d, J = 5.7, 1H, *H*-3′), 6.79 (s, 1H, *H*-1′), 7.60 (s, 1H, *H*-6), 11.67 (bs, 1H, N*H*); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 20.7 (CH_3), 23.3 (C ≡ C–CH_2), 59.5 (CH_2OH), 64.1 (*C*-5′), 73.1 (*C* ≡ C–CH_2), 84.0 (*C*-4′), 89.4 (*C*-1′), 91.6 (C ≡ *C*–CH_2), 99.2 (*C*-5), 126.4 (*C*-2′), 134.2 (*C*-3′), 142.7 (*C*-6), 149.7 (*C*-2), 161.6 (*C*-4), 170.1 (CH_3COO). Anal. ($C_{15}H_{16}N_2O_6$) C, H, N.

5-(3-Hydroxyprop-1-ynyl)-2′,3′-didehydro-2′,3′-dideoxyuridine (6)

To a solution of 5-(3-hydroxyprop-1-ynyl)-5′-*O*-acetyl-nucleoside (**4**) (126 mg, 0.41 mmol) in dry methanol (8 mL) was added sodium methoxide (24 mg, 1.1 equiv.) and the reaction mixture was stirred at room temperature for 5.5 h. The solution was neutralized by addition of Amberlite IRN-77 (H^+). The mixture was filtered, and the resin was washed with methanol. The combined filtrate was evaporated to dryness *in vacuo*. The resulting powder was purified by silica-gel column chromatography (AcOEt to AcOEt/CH_3OH = 94 : 6 as eluents) to yield 70 mg (65%) of the title compound: TLC R_f (AcOEt/CH_3OH = 90:10) 0.43; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 3.59 (s, 2H, *H*-5′), 4.20 (s, 2H, C ≡ C–CH_2–OH), 4.80 (s, 1H, *H*-4′), 5.07 (bs, 1H, 5′–O*H*), 5.27 (bs, 1H, C ≡ C–CH_2–O*H*), 5.93 (d, J = 5.8, 1H, *H*-2′), 6.40 (d, J = 5.8, 1H, *H*-3′), 6.78 (s, 1H, *H*-1′), 8.06 (s, 1H, *H*-6), 11.60 (bs, 1H, N*H*); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 49.5 (C ≡ C–CH_2–OH), 61.8 (*C*-5′), 76.2 (*C* ≡ C–CH_2), 87.6 (*C*-4′), 89.4 (*C*-1′), 92.3 (C ≡ *C*–CH_2), 98.0 (*C*-5), 125.7 (*C*-2′), 135.5 (*C*-3′), 144.4 (*C*-6), 149.9 (*C*-2), 161.7 (*C*-4). Anal. ($C_{12}H_{12}N_2O_5$) C, H, N.

5-(4-Hydroxybut-1-ynyl)-2′,3′-didehydro-2′,3′-dideoxyuridine (7)

Compound **5** (100 mg, 0.31 mmol) was converted to **7** by the same procedure as that described for **6**, except that the reaction mixture was stirred at room temperature for 2.5 h. This product was purified by silica-gel chromatography (AcOEt to AcOEt/CH_3OH = 94:6 as eluents) to yield 65 mg (75%) of the title compound: TLC R_f (AcOEt/CH_3OH = 90 : 10) 0.45; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 2.46 (t, J = 6.8, 2H, C ≡ C–CH_2), 3.49 (t, J = 6.8, 2H, CH_2–CH_2–OH), 3.58 (s, 2H, *H*-5′), 4.79 (s, 1H, *H*-4′), 5.07 (bs, 1H, 5′–O*H*),

5.27 (bs, 1H, C ≡ C(CH$_2$)$_2$O*H*), 5.92 (d, J = 5.7, 1H, *H*-2′), 6.39 (d, J = 5.7, 1H, *H*-3′), 6.77 (s, 1H, *H*-1′), 8.01 (s, 1H, *H*-6), 11.31 (bs, 1H, N*H*); ^{13}C-NMR (d$_6$-DMSO),*δ*;ppm: 23.4 (C ≡ C−*C*H$_2$), 59.7 (CH$_2$−*C*H$_2$−OH), 61.8 (*C*-5′), 73.3 (*C* ≡ C−CH$_2$), 87.6 (*C*-4′), 89.4 (*C*-1′), 90.8 (C ≡ *C*−CH$_2$), 98.7 (*C*-5), 125.7 (*C*-2′), 135.5 (*C*-3′), 143.8 (*C*-6), 149.9 (*C*-2), 161.9 (*C*-4). Anal. (C$_{13}$H$_{14}$N$_2$O$_5$) C, H, N.

5-[3-(Tetrahydro-2H-pyran-2-yloxy)-prop-1-ynyl]-2′,3′-didehydro-2′,3′-dideoxyuridine (8)

Compound **2** (420 mg, 1.08 mmol) was converted to **8** by the same procedure as that described for **6**, except that the reaction mixture was stirred at room temperature for 2 h. This product was purified by silica-gel chromatography (Et$_2$O/AcOEt = 10 : 90 as eluent) to yield 230 mg (62%) of the title compound: TLC R$_f$ (Et$_2$O/AcOEt = 10 : 90) 0.33; ^{1}H-NMR (d$_6$-DMSO), *δ*;ppm; J, Hz: 1.44–1.49 (m, 4H, OCH$_2$(C*H*$_2$)$_2$ of the THP), 1.61–1.69 (m, 2H, O(CH$_2$)$_3$C*H*$_2$ of the THP), 3.41–3.46 (m, 1H, OC*H*$_{axial}$), 3.59 (s, 2H, *H*-5′), 3.66–3.73 (m, 1H, OC*H*$_{equatorial}$), 4.29 (d, J = 16.1, C ≡ C−C*H*−O), 4.39 (d, J = 16.1, C ≡ C−C*H*′−O), 4.74 (m, 1H, C*H* of the THP), 4.80 (s, 1H, *H*-4′), 5.07 (bs, 1H, O*H*), 5.93 (d, J = 5.5, 1H, *H*-2′), 6.40 (d, J = 5.5, 1H, *H*-3′), 6.78 (s, 1H, *H*-1′), 8.13 (s, 1H, *H*-6), 11.66 (bs, 1H, N*H*); ^{13}C-NMR (d$_6$-DMSO), *δ*;ppm: 18.7 (O(CH$_2$)$_2$*C*H$_2$ of the THP), 24.8 (OCH$_2$*C*H$_2$ of the THP), 29.8 (O(CH$_2$)$_3$*C*H$_2$ of the THP), 54.1 (C ≡ C−*C*H$_2$−O), 61.1 (O*C*H$_2$ of the THP), 61.7 (*C*-5′), 78.1 (*C* ≡ C−CH$_2$), 87.6 (*C*-4′), 88.4 (C ≡ *C*−CH$_2$), 89.4 (*C*-1′), 96.1 (*C*H of the THP), 97.5 (*C*-5), 125.6 (*C*-2′), 135.4 (*C*-3′), 144.9 (*C*-6), 149.8 (*C*-2), 161.6 (*C*-4). Anal. (C$_{17}$H$_{20}$N$_2$O$_6$) C, H, N.

5-[4-(Tetrahydro-2H-pyran-2-yloxy)-but-1-ynyl]-2′,3′-didehydro-2′,3′-dideoxyuridine (9)

Compound **3** (300 mg, 0.74 mmol) was converted to **9** by the same procedure as that described for **6**, except that the reaction mixture was stirred at room temperature for 2.5 h. This product was purified by silica-gel chromatography (Et$_2$O/AcOEt = 10:90 as eluent) to yield 180 mg (67%) of the title compound: TLC R$_f$ (Et$_2$O/AcOEt = 10 : 90) 0.40; ^{1}H-NMR (d$_6$-DMSO), *δ*;ppm; J, Hz: 1.41–1.46 (m, 4H, OCH$_2$(C*H*$_2$)$_2$ of the THP), 1.55–1.72 (m, 2H, O(CH$_2$)$_3$C*H*$_2$ of the THP), 2.59 (t, J = 6.8, 2H, C ≡ C−*C*H$_2$), 3.38–3.44 (m, 1H, OC*H*$_{axial}$), 3.45–3.53 (m, 1H, C ≡ C−CH$_2$−*C*H−O), 3.58 (s, 2H, *H*-5′), 3.63–3.72 (m, 1H, C ≡ C−CH$_2$−C*H*′−O), 3.73–3.79 (m, 1H, OC*H*$_{equatorial}$), 4.62 (s, 1H, C*H* of the THP), 4.79 (s, 1H, *H*-4′), 5.01 (bs, 1H, O*H*), 5.92 (d, J = 5.4, 1H, *H*-2′), 6,39 (d, J = 5.4, 1H, *H*-3′), 6.78 (s, 1H, *H*-1′), 7.99 (s, 1H, *H*-6), 11.55 (bs, 1H, N*H*); ^{13}C-NMR (d$_6$-DMSO), *δ*;ppm: 19.0 (O(CH$_2$)$_2$*C*H$_2$ of the THP), 20.3 (C ≡ C−*C*H$_2$), 25.0 (OCH$_2$*C*H$_2$ of the THP), 30.1 (O(CH$_2$)$_3$*C*H$_2$ of the THP), 61.3 (O*C*H$_2$ of the THP), 61.8 (*C*-5′), 64.8 (CH$_2$*C*H$_2$O), 73.4 (*C* ≡ C−CH$_2$), 87.5 (*C*-4′), 89.3 (*C*-1′), 90.2 (C ≡ C−CH$_2$), 97.9 (*C*H of the THP), 98.5 (*C*-5), 125.6 (*C*-2′), 135.3 (*C*-3′), 143.7 (*C*-6), 149.8 (*C*-2), 161.7 (*C*-4). Anal. (C$_{18}$H$_{22}$N$_2$O$_6$) C, H, N.

5-(5-Hydroxypent-1-ynyl)-5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (10)

Compound **1** (800 mg, 2.12 mmol) was converted to **10** using 4-pentyn-1-ol (0.58 mL, 6.35 mmol) by the same procedure as that described for **2**, except that the reaction mixture was stirred at 25°C for 24 h. This product was purified by silica-gel chromatography (CH$_2$Cl$_2$ to CH$_2$Cl$_2$/AcOEt = 80 : 20 as eluents) to yield 300 mg (43%) of the title compound: TLC R$_f$ (AcOEt) 0.31; ^{1}H-NMR (d$_6$-DMSO), *δ*;ppm; J, Hz: 1.57 (quint., J = 6.5,H, C*H*$_2$CH$_2$OH), 2.06 (s, 3H, C*H*$_3$), 2.35 (t, J = 6.5, 2H, C ≡ C−C*H*$_2$), 3.43 (q, J = 6.5, 2H, C*H*$_2$OH), 4.13 (dd, J = 12.5, J = 2.2, 1H, *H*-5′), 4.31 (dd, J = 12.5, J = 2.2, 1H, *H*-5′′), 4.49 (t, J = 6.5, 1H, O*H*), 5.02 (s, 1H, *H*-4′), 6.01 (d, J = 5.7, 1H, *H*-2′), 6.41 (d, J = 5.7, 1H, *H*-3′), 6.79 (m, 1H, *H*-1′), 7.60 (s, 1H, *H*-6), 11.66 (bs, 1H, N*H*); ^{13}C-NMR (d$_6$-DMSO), *δ*;ppm: 15.3 (C ≡ C−CH$_2$), 20.6 (*C*H$_3$), 31.3 (*C*H$_2$CH$_2$OH), 59.4 (*C*H$_2$OH), 64.1 (*C*-5′), 72.3 (*C* ≡ C−CH$_2$), 84.0 (*C*-4′), 89.4 (*C*-1′), 93.7 (C ≡ *C*−CH$_2$), 99.2 (*C*-5), 126.4 (*C*-2′), 134.2 (*C*-3′), 142.4 (*C*-6), 149.7 (*C*-2), 161.6 (*C*-4), 170.1 (CH$_3$COO). Anal. (C$_{16}$H$_{18}$N$_2$O$_6$) C, H, N.

5-(6-Hydroxyhex-1-ynyl)-5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (11)

Compound **1** (880 mg, 2.33 mmol) was converted to **11** using 5-hexyn-1-ol (0.77 mL, 6.98 mmol) by the same procedure as that described for **2**, except that the reaction mixture was stirred at 25°C for 24 h. The product was purified by silica-gel chromatography (CH$_2$Cl$_2$ to CH$_2$Cl$_2$/AcOEt = 50 : 50 as eluents) to yield 280 mg (35%) of the title compound: R$_f$ (AcOEt) 0.33; ^{1}H-NMR (d$_6$-DMSO), *δ*;ppm; J, Hz: 1.47 (m, 4H, (C*H*$_2$)$_2$CH$_2$OH), 2.06 (s, 3H, C*H*$_3$), 2.33 (t, J = 7.0, 2H, C ≡ C−C*H*$_2$), 3.36 (q, J = 6.0, 2H, C*H*$_2$OH), 4.13 (dd, J = 12.5, J = 2.2, 1H, *H*-5′), 4.31 (dd, J = 12.5, J = 2.7, 1H, *H*-5′′), 4.40 (t, J = 5.0, 1H, O*H*), 5.02 (s, 1H, *H*-4′), 6.01 (d, J = 5.7, 1H, *H*-2′), 6.41 (d, J = 5.7, 1H, *H*-3′), 6.79 (s, 1H, *H*-1′), 7.60 (s, 1H, *H*-6), 11.67 (bs, 1H, N*H*); ^{13}C-NMR (d$_6$-DMSO), *δ*;ppm: 18.5 (C ≡ C−*C*H$_2$), 20.6 (*C*H$_3$), 24.7 (*C*H$_2$(CH$_2$)$_2$OH), 31.6 (*C*H$_2$CH$_2$OH), 59.7 (*C*H$_2$OH), 64.1 (*C*-5′), 72.5 (*C* ≡ C−CH$_2$), 84.0 (*C*-4′), 89.4 (*C*-1′), 93.8 (C ≡ *C*−CH$_2$), 99.2 (*C*-5), 126.4 (*C*-2′), 134.2 (*C*-3′), 142.4 (*C*-6), 149.7 (*C*-2), 161.6 (*C*-4), 170.1 (CH$_3$COO). Anal. (C$_{17}$H$_{20}$N$_2$O$_6$) C, H, N.

5-(5-Hydroxypent-1-ynyl)-2′,3′-didehydro-2′,3′-dideoxyuridine (12)

Compound **10** (70 mg, 0.21 mmol) was converted to **12** by the same procedure as that described for **6**, except that the reaction mixture was stirred at room temperature for 2.5 h. The product was purified by silica-gel chromatography (AcOEt to AcOEt/CH_3OH = 92 : 8 as eluents) to yield 52 mg (85%) of the title compound: TLC R_f (AcOEt/CH_3OH = 90:10) 0.47; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 1.60 (quint., J = 6.5, 2H, C*H*$_2$CH$_2$OH), 2.36 (t, J = 6.5, 2H, C ≡ C–C*H*$_2$), 3.45 (t, J = 6.5, 2H, C*H*$_2$OH), 3.59 (s, 2H, *H*-5′), 4.49 (bs, 1H, (CH$_2$)$_3$O*H*), 4.79 (s, 1H, *H*-4′), 5.05 (bs, 1H, 5′-O*H*), 5.92 (d, J = 5.7, 1H, *H*-2′), 6.39 (d, J = 5.7, 1H, *H*-3′), 6.78 (m, 1H, *H*-1′), 8.00 (s, 1H, *H*-6), 11.32 (bs, 1H, N*H*); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 15.4 (C ≡ C–*C*H$_2$), 31.5 (*C*H$_2$CH$_2$OH), 59.4 (*C*H$_2$OH), 61.8 (*C*-5′), 72.6 (*C* ≡ C–CH$_2$), 87.5 (*C*-4′), 89.3 (*C*-1′), 92.9 (C ≡ *C*–CH$_2$), 98.8 (*C*-5), 125.8 (*C*-2′), 135.3 (*C*-3′), 143.5 (*C*-6), 150.0 (*C*-2), 162.1 (*C*-4). Anal. ($C_{14}H_{16}N_2O_5$) C, H, N.

5-(6-Hydroxyhex-1-ynyl)-2′,3′-didehydro-2′,3′-dideoxyuridine (13)

Compound **11** (160 mg, 0.46 mmol) was converted to **13** by the same procedure as that described for **6**, except that the reaction mixture was stirred at room temperature for 2.5 h. The product was purified by silica-gel chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH = 90:10 as eluents) to yield 100 mg (71%) of the title compound: TLC R_f (CH_2Cl_2/CH_3OH = 90:10) 0.52; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 1.49 (m, 4H, (C*H*$_2$)$_2$CH$_2$OH), 2.33 (t, J = 6.5, 2H, C ≡ C–C*H*$_2$), 3.37 (t, J = 6.0, 2H, C*H*$_2$OH), 3.59 (s, 2H, *H*-5′), 4.41 (bs, 1H, (CH$_2$)$_4$O*H*), 4.79 (s, 1H, *H*-4′), 5.04 (bs, 1H, 5′-O*H*), 5.92 (d, J = 5.7, 1H, *H*-2′), 6.39 (d, J = 5.7, 1H, *H*-3′), 6.78 (s, 1H, *H*-1′), 8.01 (s, 1H, *H*-6), 11.50 (bs, 1H, N*H*); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 18.6 (C ≡ C–*C*H$_2$), 24.9 (*C*H$_2$(CH$_2$)$_2$OH), 31.6 (*C*H$_2$CH$_2$OH), 60.2 (*C*H$_2$OH), 61.8 (*C*-5′), 72.7 (*C* ≡ C–CH$_2$), 87.6 (*C*-4′), 89.3 (*C*-1′), 93.1 (C ≡ *C*–CH$_2$), 98.8 (*C*-5), 125.8 (*C*-2′), 135.4 (*C*-3′), 143.5 (*C*-6), 149.9 (*C*-2), 161.9 (*C*-4). Anal. ($C_{15}H_{18}N_2O_5$) C, H, N.

5-[N-(Trifluoroacetyl)-3-aminoprop-1-ynyl]-5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (16)

Compound **1** (1 g, 2.64 mmol) was converted to **16** using *N*-(2-propynyl)-2,2,2-trifluoroacetamide (1.20 g, 7.93 mmol) by the same procedure as that described for **2**, except that the reaction mixture was stirred at 25°C for 6 h. The product was purified by silica-gel chromatography (CH_2Cl_2 to CH_2Cl_2/AcOEt = 50 : 50 as eluents) to yield 500 mg (47%) of the title compound: TLC R_f (CH_2Cl_2/AcOEt = 20:80) 0.38; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 2.02 (s, 3H, C*H*$_3$), 4.13 (dd, J = 2.2, J = 12.6, 1H, *H*-5′), 4.18 (s, 2H, C*H*$_2$NH), 4.31 (dd, J = 2.5, J = 12.6, 1H, *H*-5′′), 5.03 (s, 1H, *H*-4′), 6.01 (d, J = 5.8, 1H, *H*-2′), 6.43 (d, J = 5.8, 1H, *H*-3′), 6.78 (m, 1H, *H*-1′), 7.68 (s, 1H, *H*-6), 10.03 (bs, 1H, N*H*COCF$_3$), 11.75 (bs, 1H, CON*H*CO); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 20.6 (*C*H$_3$), 29.3 (*C*H$_2$NH), 64.1 (*C*-5′), 75.0 (*C* ≡ C–CH$_2$), 84.1 (*C*-4′), 88.0 (C ≡ *C*–CH$_2$), 89.5 (*C*-1′), 97.8 (*C*-5), 114.0 (q, J = 285 Hz, *C*F$_3$), 126.4 (*C*-2′), 134.2 (*C*-3′), 144.0 (*C*-6), 149.7 (*C*-2), 156.1(q, J = 45 Hz, *C*OOCF$_3$), 161.4 (*C*-4), 170.0 (CH$_3$*C*OO). Anal. ($C_{16}H_{14}F_3N_3O_6$) C, H, N.

5-[N-(tert-Butoxycarbonyl)-3-aminoprop-1-ynyl]-5′-O-acetyl-2′,3′-didehydro-2′, 3′-dideoxyuridine (17)

Compound **1** (400 mg, 1.06 mmol) was converted to **17** using *N-tert*-butoxycarbonyl-prop-2-ynylamine (488 mg, 3.17 mmol) by the same procedure as that described for **2**, except that the reaction mixture was stirred at 25°C for 20 h. This product was purified by silica-gel chromatography (CH_2Cl_2/AcOEt = 80 : 20 to CH_2Cl_2/AcOEt = 20 : 80 as eluents) to yield 227 mg (53%) of the title compound: TLC R_f (CH_2Cl_2/AcOEt = 20 : 80) 0.43; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 1.36 (s, 9H, C(C*H*$_3$)$_3$), 2.05 (s, 3H, C*H*$_3$COO), 3.88 (d, J = 5.7, 2H, C*H*$_2$NHBoc), 4.12 (dd, J = 12.6, J = 2.1, 1H, *H*-5′), 4.31 (dd, J = 12.6, J = 2.4, 1H, *H*-5″), 5.02 (s, 1H, *H*-4′), 6.01 (d, J = 5.8, 1H, *H*-2′), 6.42 (d, J = 5.8, 1H, *H*-3′), 6.79 (m, 1H, *H*-1′), 7.30 (t, J = 5.7, 1H, N*H*Boc), 7.65 (s, 1H, *H*-6), 11.73 (bs, 1H, CON*H*CO). Anal. ($C_{19}H_{23}N_3O_7$) C, H, N.

5-[N-(Trifluoroacetyl)-3-aminoprop-1-ynyl]-2′,3′-didehydro-2′,3′-dideoxyuridine (18)

Compound **16** (500 mg, 1.24 mmol) was converted to **18** by the same procedure as that described for **6**, except that the reaction mixture was stirred at room temperature for 10 h. The product was purified by silica-gel chromatography (silica pre-equilibrated with 1% Et_3N; CH_2Cl_2/CH_3OH = 70 : 30 as eluent) to yield 300 mg (67%) of the title compound: TLC R_f (CH_2Cl_2/CH_3OH = 70 : 30) 0.58; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 3.58 (s, 2H, *H*-5′), 4.19 (s, 2H, C*H*$_2$NH), 4.80 (s, 1H, *H*-4′), 5.04 (bs, 1H, O*H*), 5.93 (d, J = 5.5, 1H, *H*-2′), 6.40 (d, J = 5.5, 1H, *H*-3′), 6.78 (s, 1H, *H*-1′), 8.08 (s, 1H, *H*-6), 10.04 (bs, 1H, N*H*COCF$_3$), 11.45 (bs, 1H, CON*H*CO); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 29.4 (*C*H$_2$NH), 61.7 (*C*-5′), 75.2 (*C* ≡ C–CH$_2$), 87.3 (C ≡ *C*–CH$_2$), 87.7 (*C*-4′), 89.5 (*C*-1′), 97.4 (*C*-5), 117.2 (q, J = 285 Hz, *C*F$_3$), 125.6 (*C*-2′), 135.6 (*C*-3′), 145.1 (*C*-6), 149.8 (*C*-2), 155.9 (q, J = 45 Hz, *C*OOCF$_3$), 161.6 (*C*-4). Anal. ($C_{14}H_{12}F_3N_3O_5$) C, H, N.

5-(3-Aminoprop-1-ynyl)-5′-O-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine, Trifluoroacetate Salt (19)

To a solution of **17** (311 mg, 0.76 mmol) in dichloromethane (20 mL) was added 99% trifluoroacetic acid (2 mL, 34.2 equiv.) and the reaction mixture was stirred at room temperature for 20 min. After evaporation to dryness *in vacuo*, the oily residue was crystallized from dry diethyl ether to yield 221 mg (69%) of the title compound: TLC R_f (CH_2Cl_2/CH_3-OH = 80:20) 0.45; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 2.06 (s, 3H, C*H*$_3$), 3.93 (m, 2H, C*H*$_2$NH_3^+), 4.15 (dd, J = 12.6, J = 2.2, 1H, *H*-5′), 4.28 (dd, J = 12.6, J = 2.4, 1H, *H*-5′ ′), 5.04 (s, 1H, *H*-4′), 6.02 (d, J = 5.8, 1H, *H*-2′), 6.45 (d, J = 5.8, 1H, *H*-3′), 6.79 (m, 1H, *H*-1′), 7.72 (s, 1H, *H*-6), 8.27 (bs, 3H, N*H*$_3^+$), 11.83 (bs, 1H, CON*H*CO); ^{13}C-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 20.8 (*C*H$_3$), 29.1 (*C*H$_2$NH$_3^+$), 64.2 (*C*-5′), 78.5 (*C* ≡ C–CH$_2$), 84.2 (*C*-4′), 85.6 (C ≡ *C*–CH$_2$), 89.7 (*C*-1′), 97.3 (*C*-5), 120.5 (q, J = 285, *C*F$_3$), 126.3 (*C*-2′), 134.3 (*C*-3′), 144.6 (*C*-6), 149.7 (*C*-2), 155.5 (q, J = 42, CF$_3$*C*OO$^-$), 161.3 (*C*-4), 170.2 (CH$_3$*C*OO). Anal. ($C_{16}H_{16}F_3N_3O_7$) C, H, N.

5-(3-Aminoprop-1-ynyl)-2′,3′-didehydro-2′,3′-dideoxyuridine (20)

Method 1: Compound **18** (250 mg, 0.70 mmol) was converted to **20** by the same procedure as that described for **6**, except that the reaction mixture was stirred at 30°C for 4 h. This product was purified by silica-gel chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH = 60 : 40 as eluents) to yield 120 mg (65%) of the title compound.

Method 2: Compound **19** (100 mg, 0.24 mmol) was converted to **20** by the same procedure as that described for **6**, except that 2.2 equiv. of sodium methoxide was used and the reaction mixture was stirred at room temperature for 3 h. This product was purified by silica-gel chromatography (AcOEt to AcOEt/CH_3OH = 94 : 6 as eluents) to yield 37 mg (60%) of the title compound: TLC R_f (CH_2Cl_2/CH_3OH = 60 : 40) 0.22; ^{1}H-NMR (d_6-DMSO), *δ*;ppm; J, Hz: 1.22 (bs, 2H, N*H*$_2$), 3.41 (s, 2H, C*H*$_2$NH$_2$), 3.58 (s, 2H, *H*-5′), 4.80 (s, 1H, *H*-4′), 5.05 (bs, 1H, O*H*), 5.92 (d, J = 5.6, 1H, *H*-2′), 6.40 (d, J = 5.6,s 1H, *H*-3′), 6.78 (s, 1H, *H*-1′), 8.02 (s, 1H, *H*-6), 11.60 (bs, 1H, CON*H*CO); ^{13}C-NMR (d_6-DMSO), *δ*;ppm: 30.6 (*C*H$_2$NH$_2$), 61.8 (*C*-5′), 72.7 (*C* ≡ C–CH$_2$), 86.8 (C ≡ *C* – CH$_2$), 87.6 (*C*-4′), 89.3 (*C*-1′), 98.1 (*C*-5), 125.5 (*C*-2′), 135.4 (*C*-3′), 144.0 (*C*-6), 149.8 (*C*-2), 161.9 (*C*-4). Anal. ($C_{12}H_{13}N_3O_4$) C, H, N.

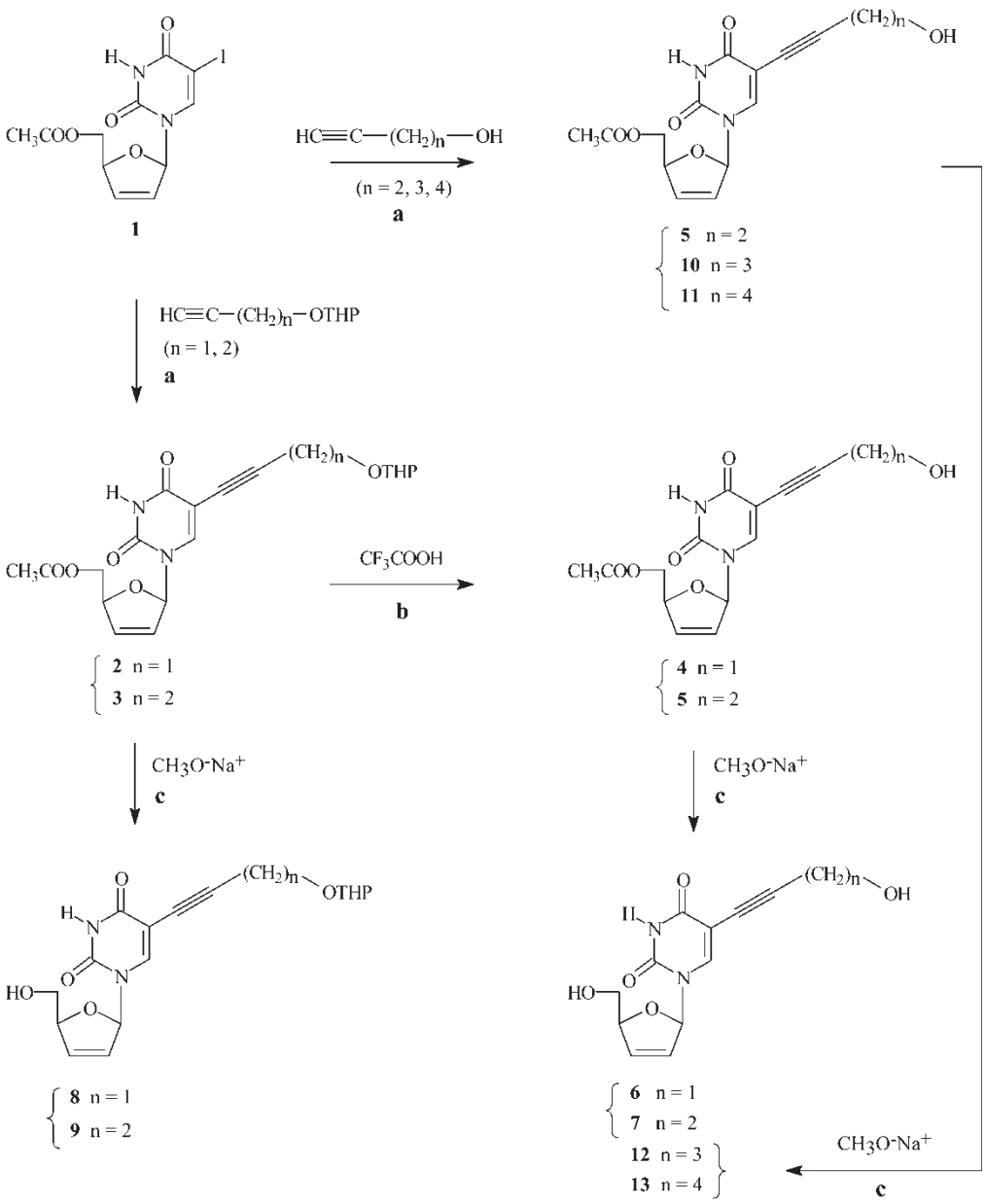
Antiviral Test Procedures

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 min 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 the RT assay as previously described.[22] The 50% inhibitory concentration (IC_{50}) was derived from the computer-generated median effect plot of the dose-effect data.[23] 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).[24] 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;[22,25] virus production was quantified by the RT activity associated to virus particles released from the cells in the culture medium. Conditions under which the inhibitory properties were measured on HIV-1 reverse transcriptase *in vitro* have also been described.[22] *In vitro* RT inhibition has also been described[22] 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).

RESULTS AND DISCUSSION

Chemistry

The synthetic method for the 5-alkynyl target nucleoside analogues **6, 7, 12, 13** and **20** involved the formation of the key precursor **1** (5′-*O*-acetyl-5-iodo-2′,3′-didehydro-2′,3′-dideoxyuridine or 5′-*O*-acetyl-5-iodo-d4U) as outlined in Scheme 1. This precursor, used as the starting material for the nucleoside analogues reported herein, was prepared from uridine in three steps

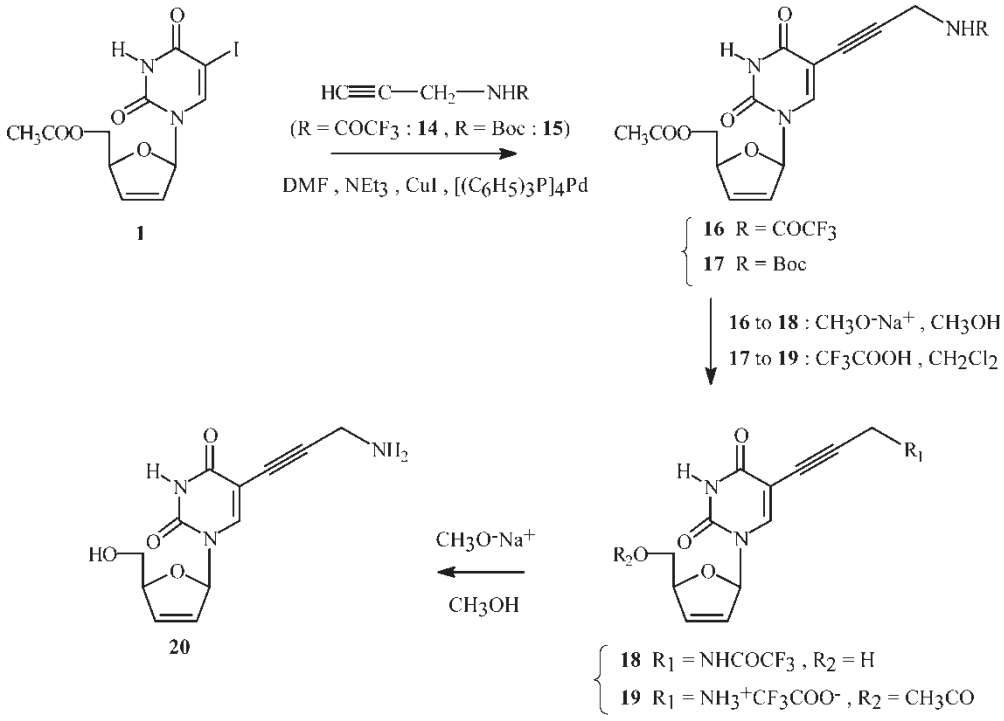


a: DMF , NEt_3 , CuI , $[(C_6H_5)_3P]_4Pd$; b: CH_2Cl_2 , CH_3OH ; c: CH_3OH

SCHEME 1 Synthesis of 5-(alkynylhydroxy)-2′,3′-didehydro-2′,3′-dideoxyuridine (**6**, **7**, **12** and **13**).

according to our previously published method.[26] The target nucleosides were synthesized *via* the smooth and efficient coupling of terminal alkynes with 5′-*O*-acetyl-5-iodo-d4U (**1**) in DMF under co-catalysis of Pd and Cu after adaptation of the general procedure described by Sonogashira *et al.*[27] Thus, similar treatment of **1** with conveniently protected hydroxy alkynes as tetrahydropyranyl (THP) acetals (n = 1, 2) in anhydrous and deoxygenated DMF/triethylamine at 25°C in the presence of tetrakis(triphenylphosphine) palladium(0) and copper(I)iodide under an argon atmosphere afforded the 5′-*O*-acetyl-5-[(tetrahydro-2*H*-pyran-2-yloxy)-alkynyl]-derivatives **2** and **3** after purification by silica-gel column chromatography in 76 and 70% yield, respectively (Scheme 1). Selective 5′-*O*-deacylation of **2** and **3** with sodium methoxide in methanol at room temperature afforded respectively the 2′,3′-didehydro-2′,3′-dideoxy-nucleosides **8** (62%) and **9** (67%).

Acid catalyzed removal of the THP group of **2** and **3** with TFA and subsequent 5′-*O*-deacylation with sodium methoxide in methanol provided respectively



SCHEME 2 Synthesis of 5-(3-aminoprop-1-ynyl)-2′,3′-didehydro-2′,3′-dideoxyuridine (**20**).

the target compounds 5-(3-hydroxyprop-1-ynyl)-d4U **6** (overall yield of 50%) and 5-(4-hydroxybut-1-ynyl)-d4U **7** (56%) after purification by silica-gel chromatography.

As shown in Scheme 1, 5-(4-hydroxybut-1-ynyl)-5′-*O*-acetyl-d4U **5** (45%), 5-(5-hydroxypent-1-ynyl)-5′-*O*-acetyl-d4U **10** (43%) and 5-(6-hydroxyhex-1-ynyl)-5′-*O*-acetyl-d4U **11** (35%) were also synthesized more conveniently by direct coupling of 3-butyn-1-ol, 4-pentyn-1-ol and 5-hexyn-1-ol with 5′-*O*-acetyl-5-iodo-d4U **1** according to a Sonogashira reaction. Finally, removal of the 5′-*O*-acetyl group of **10** and **11** was performed using sodium methoxide in methanol at room temperature to give the target compounds **12** (85%) and **13** (71%) as previously described.

For comparative studies, the corresponding 5-(3-aminoprop-1-ynyl)-d4U analogue **20** was prepared by a similar synthetic approach as indicated in Scheme 2.

Condensation of 5′-*O*-acetyl-5-iodo-d4U **1** with *N*-trifluoroacetyl propargylamine **14**[28] gave as major product the expected C-5 substituted d4U **16** isolated in 47% yield after purification by chromatography. Using the *N*-Boc propargylamine **15**[29], we similarly obtained compound **17** in 53% yield. Selective 5′-*O*-deacylation of **16** followed by removal of the *N*-trifluoroacetyl group of the intermediate **18** afforded the expected 5-(3aminoprop-1-ynyl)-d4U **20** isolated in an overall yield of 43%. Finally, removal of the *N*-Boc group of **17** with TFA and subsequent 5′-*O*-deacylation provided the target compound **20** in an overall yield of 41%.

All structures were confirmed by analytical and spectroscopic data (see chemical procedures in experimental section).

Biological Results

The newly synthesized 2′,3′-didehydro-2′,3′-dideoxy-C-5-modified nucleoside derivatives **6**, **7**, **8**, **9**, **12**, **13**, **18** and **20** were evaluated by comparison to AZT for inhibition of HIV-1 multiplication in cells of lymphocytic lineage (CEM-SS and MT-4), and the results are summarized in Table I.

The data clearly revealed that introduction of carbons into the side-chain possessing either a terminal hydroxy group or an amino group (compounds **6**, **7**, **12**, **13** and **20**) resulted in a reduction of anti-HIV activity compared to AZT. The results obtained here are disappointing as far as it was hoped that a highly lipophilic side-chain may lead to a further enhancement in antiviral potency. Unfortunately, the protected hydroxy alkynes compounds as tetrahydropyranyl (THP) acetals (compounds **8** and **9**) displayed no improvement in antiviral activity. Moreover, all compounds displayed no cytotoxicity to uninfected cells.

TABLE I Antiviral and cytotoxicity evaluation of β-D-d_4T analogues bearing C-5 hydroxy or C-5 amino alkynyl linker arms **6**,**7**,**8**,**9**,**12**,**13**,**18** and **20**

	HIV-1_{LAI} in CEM-SS cells			HIV-1_{IIIB} in MT-4 cells		
Compd	IC50 (M)*	CC50 (M) †	SI‡	IC50 (M)	CC50 (M)	SI
AZT	$1.4\ 10^{-8}$	$> 10^{-4}$	> 7142	$9.8\ 10^{-9}$	$> 10^{-6}$	> 102
6	$> 10^{-4}$	$9.5\ 10^{-5}$	> 0.95	$> 10^{-4}$	$6.4\ 10^{-5}$	> 0.64
7	$1.2\ 10^{-4}$	$3.6\ 10^{-4}$	3	$> 10^{-4}$	$7\ 10^{-5}$	> 0.7
8	$5\ 10^{-5}$	$> 10^{-4}$	> 2	$> 10^{-4}$	$6.8\ 10^{-5}$	> 0.68
9	$1.1\ 10^{-4}$	$3.9\ 10^{-4}$	3.54	$> 10^{-4}$	$7.1\ 10^{-5}$	> 0.71
12	$1.2\ 10^{-4}$	$3.6\ 10^{-4}$	3	$> 10^{-4}$	$7.1\ 10^{-5}$	> 0.71
13	$5\ 10^{-5}$	$2.4\ 10^{-4}$	4.8	$> 10^{-4}$	$7\ 10^{-5}$	> 0.7
18	$7\ 10^{-5}$	$4.3\ 10^{-5}$	0.61	$> 10^{-4}$	$3.7\ 10^{-5}$	> 0.37
20	$> 10^{-4}$	$8.3\ 10^{-5}$	> 0.83	$> 10^{-4}$	$> 10^{-4}$	> 1

* 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. ‡ Selective Index (SI) corresponds to the ratio CC_{50}/IC_{50}. All data represent the mean values of three separate experiments (± SD).

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