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Synthesis and Anti-HIV Activity of Some Heterodimers [NRTI]-Glycyl-Succinyl-[Trovirdine Analogue] of Known HIV-1 Reverse Transcriptase Inhibitors

ELENA SUGEAC[a], CHRISTINE FOSSEY[a], DANIEL LADURÉE[a,*], SYLVIE SCHMIDT[b], GERALDINE LAUMOND[b] and ANNE-MARIE AUBERTIN[b]

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

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Expected for their ability to inhibit HIV replication, four heterodimers with a Nucleoside Reverse Transcriptase Inhibitor (NRTI) and a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) bound by a linker arm were designed and synthesized. For the NRTIs, d_4U, d_2U, d_4T and 5′-*O*-acetyl-5-(3-hydroxypropynyl)d_2U were chosen. For the NNRTI, a Trovirdine Analogue (belonging to the phenethylthiazolylthiourea class) was chosen. The conjugation of the two different inhibitors (NRTI and NNRTI) was performed using the succinyl-glycine moiety as a spontaneously cleavable linker.

Keywords: HIV; NRTI; NNRTI; Cleavable linker; Heterodimer

INTRODUCTION

The primary aetiological agent for AIDS is human immunodeficiency virus type 1 (HIV-1).[1] This virus causes progressive destruction of the immune system. The reverse transcriptase (RT) of HIV-1 is essential for its replication. Most of the antiviral drugs approved for the treatment of HIV infection inhibit this enzyme. Among the NRTIs, zidovudine,[2–4] didanosine,[5] zalcitabine,[6] stavudine[7–12] and lamivudine[13–15] are clinically used for the treatment of AIDS. These agents act competitively at the catalytic site of the enzyme, after conversion to their triphosphate form.

Several classes of compounds have been identified as NNRTIs: TIBO derivatives,[16] HEPT,[17] nevirapine,[18] pyridinone derivatives,[19] BHAP,[20] α-APA,[21] and PETT.[22] In contrast to the NRTIs, the NNRTIs do not need any metabolic conversion and they bind to an allosteric site; generally they are selective inhibitors of HIV-1.

The introduction of combination therapy for HIV infection has transformed the prognosis for most patients receiving it. Here, the synthesis and anti-HIV activity of some heterodimers of the general formula [NRTI]-Glycyl-Succinyl-*N-piperazinyl*-[Trovirdine Analogue] including both a NRTI: d_4U, d_2U, d_4T or [5-(3-hydroxypropynyl)-2′,3′-dideoxyuridine] and a NNRTI [Trovirdine Analogue (belonging to the phenethylthiazolylthiourea class)] connected through the succinyl-glycine moiety, as a spontaneously cleavable linker, are reported.

MATERIALS AND METHODS

General

Reagent grade acetonitrile was refluxed and distilled from phosphorus pentoxide. Anhydrous ethanol was prepared by using magnesium turnings. Dioxane was distilled from sodium/benzophenone immediately prior to use. Anhydrous dichloromethane was prepared by using molecular sieves. Anhydrous methanol, acetone, *N*,*N*-dimethylformamide were purchased from E. Merk, Carlo Erba and Aldrich, respectively. Unless otherwise stated, reactions were run under an atmosphere of argon and monitored by thin-layer chromatography (TLC)

*Corresponding author. Tel.: +33-0-2-31-93-64-58. Fax: +33-0-2-31-93-34-73. E-mail: laduree@baligan.unicaen.fr

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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 effected by using Merck silica gel 60 (0.063–0.200 mm) and silica gel Si-60 for flash chromatography (40–63 μm) was supplied by Merck. All samples were dried over P_2O_5 for at least 24 hours prior to analysis.

Melting points were determined on a Kofler apparatus. IR spectra were recorded on a Fourier transform Mattson spectrometer Genesis DTGS using WinFIRST™ Macros and ApPro™; 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, br = broad, d = doublet, t = triplet, q = quadruplet, m = multiplet).

Synthesis

3′,5′-Di-O-acetyl-2′-bromouridine 2

A solution of uridine **1** (15.0 g; 61.4 mmol) in anhydrous acetonitrile (250 ml) was heated at reflux. To this solution was added dropwise acetyl bromide (37.5 ml; 0.51 mol) and the mixture was refluxed for 0.5 h. The mixture was then cooled at room temperature and concentrated *in vacuo*. The resulting oil was dissolved in CH_2Cl_2 (300 ml), washed with H_2O (2 × 300 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The nucleoside **2** was obtained as yellow crystals (20.75 g); Yield 87%; m.p. 70–73°C; TLC R_f (EtOAc 100%) 0.76; IR (KBr), cm^{-1}: 3212 (NH), 1749–1696 (4 × CO), 637 (C-Br); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 2.06 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 4.22–4.33 (m, 3H, H-4′, H-5′), 4.99 (dd, 1H, H-2′, 7.3, 5.9), 5.23 (dd, 1H, H-3′, 5.9, 3.6), 5.76 (dd, 1H, H-5, 8.2, 2.1), 6.13 (d, 1H, H-1′, 7.3), 7.67 (d, 1H, H-6, 8.2), 11.54 (d, 1H, NH, 2.1); ^{13}C-NMR (d_6-DMSO), δ, ppm: 20.5 (CH_3), 47.5 (C-2′), 62.8 (C-5′), 71.1 (C-3′), 79.6 (C-4′), 88.8 (C-1′), 102.9 (C-5), 140.0 (C-6), 150.5 (C-2), 162.8 (C-4), 169.3 (MeCOO), 170.1 (MeCOO).

5′-O-Acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine 3

To a solution of compound **2** (5.0 g; 12.78 mmol) in anhydrous ethanol (100 ml) was added zinc powder (2.5 g; 38.34 mmol) previously activated. The heterogeneous mixture was irradiated in a ultrasound tank (35 kHz; 150 W) under an atmosphere of argon for 0.5 h. The mixture was then filtered using a Celite pad, and the celite was washed with ethanol (2 × 20 ml). The combined filtrate was evaporated and the residue was dissolved in EtOAc (100 ml); the resulting precipitate (uracil) was isolated by filtration. The filtrate was concentrated *in vacuo*. Purification of the product by silica gel column flash chromatography (EtOAc 100%) gave the title compound **3** as white crystals (1.57 g); Yield 49%; m.p. 80°C; TLC R_f (EtOAc 100%) 0.44; IR (KBr), cm^{-1}: 3194 (NH), 1743 (CH_3OCO), 1700 (CO), 1684 (CO); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 2.00 (s, 3H, CH_3), 4.17 (d, 2H, H-5′, 3.4), 4.97 (s, 1H, H-4′), 5.66 (d, 1H, H-5, 8.1), 6.00 (d, 1H, H-2′, 5.9), 6.42 (d, 1H, H-3′ 5.9), 6.79 (s, 1H, H-1′), 7.44 (d, 1H, H-6, 8.1), 11.37 (br s, 1H, NH); ^{13}C-NMR (d_6-DMSO), δ, ppm: 20.6 (CH_3), 64.5 (C-5′), 83.8 (C-4′), 89.4 (C-1′), 101.9 (C-5), 126.4 (C-2′), 133.9 (C-3′), 140.6 (C-6), 150.8 (C-2), 163.2 (C-4), 170.1 (MeCOO).

2′,3′-Didehydro-2′,3′-dideoxyuridine 4

To a solution of **3** (1.5 g; 5.95 mmol) in anhydrous methanol (75 ml) was added sodium methoxide (0.642 g; 11.9 mmol) and the mixture stirred for 1 h at room temperature. The reaction mixture was neutralised with Amberlite IRN-77 resin (H^+). After filtration of the resin, the solvent was removed *in vacuo*. Purification of the product by silica gel column chromatography (EtOAc:MeOH) gave the title compound **4** as a white solid (1.09 g); Yield 87%; m.p. 120°C; TLC R_f (EtOAc : MeOH = 9:1) 0.43; IR (KBr), cm^{-1}: 3445 (OH), 3190 (NH), 1701 (*CO*), 1685 (CO); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 3.57 (s, 2H, H-5′), 4.76 (s, 1H, H-4′), 4.96 (br s, 1H, OH), 5.57 (d, 1H, H-5, 8.1), 5.91 (d, 1H, H-2′, 5.9), 6.38 (d, 1H, H-3′, 5.9), 6.80 (s, 1H, H-1′), 7.72 (d, 1H, H-6, 8.1), 11.28 (br s, 1H, NH); ^{13}C-NMR (d_6-DMSO), δ, ppm: 62.2 (C-5′), 87.3 (C-4′), 89.0 (C-1′), 101.5 (C-5), 125.7 (C-2′), 135.0 (C-3′), 141.0 (C-6), 150.8 (C-2), 163.3 (C-4).

5′-O-Acetyl-2′,3,-dideoxyuridine 5

A solution of compound **3** (1.0 g; 3.97 mmol) in anhydrous methanol (15 ml) was hydrogenated in the presence of catalyst (wet 10% palladium on carbon). The mixture was stirred for approximately 1.5 h. The catalyst was removed by filtration, and the filtrate concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH) to give compound **5** (0.89 g); Yield 89%; TLC R_f (CH_2Cl_2 : MeOH = 5 : 1) 0.80; IR (KBr), cm^{-1}: 3196 (NH), 1742 (CH_3OCO), 1698 (CO),1682 (CO); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.72–1.81 (m, 1H, H-3′), 1.95–2.06 (m, 2H, H-3′ and H-2′), 2.03 (s, 3H, CH_3COO), 2.22–2.34 (m, 1H, H-2′), 4.15–4.21 (m, 3H, H-5′ and H-4′), 5.62(d, 1H, H-5, 8.05), 5.95–5.98 (m, 1H, H-1′), 7.64 (d, 1H, H-6, 8.05), 11.3 (br s, 1H, NH); ^{13}C-NMR (d_6-DMSO), δ, ppm: 20.6 (CH_3), 25.5 (C-3′), 30.8 (C-2′), 64.9 (C-5′), 77.9 (C-4′), 85.2 (C-1′), 101.5 (C-5), 140.4 (C-6), 150.4 (C-2), 163.1 (C-4), 170.2 (MeCOO).

2′,3′-Dideoxyuridine 6

Compound **6** was prepared from compound **5** in a similar manner to that described for compound **4**; Yield 70%; m.p. 101°C; TLC R_f (EtOAc : MeOH = 9 : 1) 0.45; IR (KBr), cm^{-1}: 3449 (OH), 3157 (NH), 1684 (CO), 1654 (CO); ^{1}H-NMR ($CDCl_3$), δ, ppm, J, Hz: 1.77–1.96 (m, 3H, H-3′ and H-2′), 2.19–2.29 (m, 1H, H-2′), 3.47–3.51 (m, 1H, H-5′), 3.62–3.66 (m, 1H, H-5′), 3.97–4.00 (m, 1H, H-4′), 5.04 (br s, 1H, OH), 5.55 (d, 1H, H-5, 8.05), 5.90–5.94 (m, 1H, H-1′), 7.91 (d, 1H, H-6, 8.05), 11.22 (br s, 1H, NH); ^{13}C-NMR ($CDCl_3$), δ, ppm: 25.2 (C-3′), 32.2 (C-2′), 62.4 (C-5′), 81.9 (C-4′), 85.5 (C-1′), 101.4 (C-5), 141.0 (C-6), 150.8 (C-2), 163-7 (C-4).

5′-O-Acetyl-5-iodo-2′,3′-dideoxyuridine 7

A solution of compound **5** (1.0 g; 3.93 mmol) and ICl (1.91 g; 11.79 mmol) in anhydrous CH_2Cl_2 (70 ml) was heated at reflux for 2 h and then cooled. The stirred mixture was treated with the minimum quantity of 2% $NaHSO_3/H_2O$ necessary to reduce the excess of ICl. The organic phase was separated and washed with H_2O (2 × 50 ml), dried ($MgSO_4$) and evaporated. Purification of the product by silica gel column chromatography (CH_2Cl_2:MeOH) gave the title compound **7** as yellow crystals (0.94 g); Yield 63%; m.p. 127°C; TLC R_f (CH_2Cl_2 : MeOH = 95 : 5) 0.38; IR (KBr), cm^{-1}: 1733 (CH_3OCO), 1700 (CO), 1667 (CO), 604 (C-I); ^{1}H-NMR (d_6-DMSO), δ, ppm: 1.72–1.78 (m, 1H, H-3′), 1.93–2.02 (m, 2H, H-3′ and H-2′), 2.11 (s, 3H, CH_3COO), 2.23–2.28 (m, 1H, H-2′), 4.18–4.24 (m, 3H, H-5′ and H-4′), 5.89–5.92 (m, 1H, H-1′), 7.93 (s, 1H, H-6); ^{13}C-NMR (d_6-DMSO), δ, ppm: 17.3 (CH_3), 22.3 (C-3′), 27.5 (C-2′), 64.9 (C-5), 71.2 (C-5′), 73.2 (C-4′), 81.8 (C-1′), 144.1 (C-6), 154.2 (C-2), 160.0 (C-4), 171.0 (MeCOO).

5′-O-Acetyl-5-[3-(tetrahydro-2H-pyran-2-xyloxy)prop-1-ynyl]-2′,3′-dideoxyuridine 8

To a solution of compound **7** (0.5 g; 1.32 mmol) in anhydrous and deoxygenated DMF (20 ml), under an atmosphere of argon, was added, respectively: THPOP alkyne (0.56 ml; 3.96 mmol), CuI (0.05 g; 0.26 mmol), $(Ph_3P)_4Pd$ (0.152 g; 0.13 mmol) and anhydrous deoxygenated TEA (4.6 ml; 33.0 mmol). The mixture was stirred at room temperature for 22 h under argon. The mixture was then filtered using a Celite pad. The filtrate was concentrated *in vacuo*. The residue was dissolved in CH_2CL_2 (150 ml), washed with saturated NaCl (100 ml), 10% disodium EDTA/H_2O (2 × 80 ml), and saturated NaCl (100 ml), dried ($MgSO_4$) and evaporated. Purification of the product by chromatography was effected by using a column of silica gel packed in CH_2Cl_2 and eluting with CH_2Cl_2:EtOAc (from 100% to about 60:40). Appropriately pooled fractions were evaporated to give the title compound **8** as yellow crystals (0.34 g); Yield 67%; m.p. 63°C; TLC R_f (CH_2CL_2 : EtOAc = 30 : 70) 0.42; IR (KBr), cm^{-1}: 3194 (NH), 2236 ($C_{sp}C_{sp}$), 1745 (CH_3OCO), 1720 (CO), 1698 (CO); ^{1}H-NMR (d_6-DMSO), δ, ppm: 1.42–1.48 (m, 4H, $OCH_2(CH_2)_2$:THP), 1.60–1.67 (m, 2H, $O(CH_2)_3CH_2$:THP), 1.91–1.95 (m, 1H, H-3′), 2.03–2.15 (m, 2H, H-3′ and H-2′), 2.09 (s, 3H, CH_3COO), 2.25–2.33 (m, 1H, H-2′), 3.42–3.45 (m, 1H, OCH_{axial}:THP), 3.68–3.73 (m, 1H, $OCH_{equarorial}$:THP), 4.21–4.29 (m, 3H, H-5′ and H-4′), 4.30–4.43 (m, 2H, CH_2OTHP), 4.72–4.75 (m, 1H, CH:THP), 5.90–5.93 (m, 1H, H-1′), 7.97 (s, 1H, H-6); ^{13}C-NMR (d_6-DMSO), δ, ppm: 18.7 ($O(CH_2)_2CH_2$:THP), 20.6 (CH_3), 24.6 (C-3′), 24.7 (OCH_2CH_2:THP), 29.8 ($O(CH_2)_3CH_2$:THP), 31.6 (C-2′), 54.1 ($C_{sp}C_{sp}CH_2$), 61.1 (OCH_2:THP), 64.0 (C-5′), 78.6 (C-4′), 85.9 (C-1′), 88.4 ($C_{sp}C_{sp}CH_2$), 96.1 ($C_{sp}C_{sp}CH_2$), 96.1 (CH:THP), 97.2 (C-5), 143.5 (C-6), 149.7 (C-2), 162-1 (C-4), 170.2 (MeCOO).

5′-O-Acetyl-5-(3-hydroxypropynyl)-2′,3′-dideoxyuridine 9

A solution of compound **8** (0.34 g; 0.87 mmol) in 16 ml of CH_2Cl_2:MeOH:CF_3COOH (10:5:1) was stirred at room temperature for 1 h and then evaporated. Several portions of MeOH were added and evaporated. The residue was dissolved in the minimum quantity of MeOH and the CH_2Cl_2 was added dropwise. The title compound **9** was precipitated as white crystals (0.17 g); Yield 64%; m.p. 86°C; TLC R_f (EtOAc : MeOH = 95 : 5) 0.53; IR (KBr), cm^{-1}: 3431 (OH), 2236 ($C_{sp}C_{sp}$), 1733 (CH_3OCO), 1713 (CO), 1688 (CO); ^{1}H-NMR (d_6-DMSO), δ, ppm: 1.92–1.96 (m, 1H, H-3′), 1.98–2.13 (m, 2H, H-3′ and H-2′), 2.08 (s, 3H, CH_3COO), 2.26–2.32 (m, 1H, H-2′), 4.17–4.27 (m, 3H, H-5′ and H-4′), 4.20 (s, 2H, $C_{sp}C_{sp}CH_2$), 5.27 (br s, 1H, OH), 5.91–5.94 (m, 1H, H-1′), 7.91 (s, 1H, H-6), 11.5 (br s, 1H, NH); ^{13}C-NMR (d_6-DMSO), δ, ppm: 20.6 (CH_3), 24.7 (C-3′), 31.5 (C-2′), 49.4 (CH_2OH), 64.1 (C-5′), 78.4 (C-4′), 85.7 (C-1′), 92.1 ($C_{sp}C_{sp}CH_2$), 97.7 ($C_{sp}C_{sp}CH_2$), 128.7 (C-5), 142.8 (C-6), 150.0 (C-2), 162.6 (C-4), 170.2 (MeCOO).

N-{2-[4-(succin-1-yl)piperazin-1-yl]ethyl}-N′-(5-bromopyridin-2-yl)thiourea 12

To a solution of compound **11** (1.0 g; 2.90 mmol) in anhydrous CH_2Cl_2 (100 ml) were added DIEA (2.02 ml; 11.6 mmol) and succinic anhydride (0.58 g; 5.80 mmol) at room temperature and the mixture was stirred for 1.5 h. The solution was then washed with saturated NaCl and the aqueous phase was extracted several times with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the product by silica gel

column chromatography (EtOAc:MeOH) gave the title compound **12** as white crystals (0.82 g); Yield 64% m.p. 167°C; TLC R_f (EtOAc : MeOH = 60 : 40) 0.64; IR (KBr), cm^{-1}; 3276 (NH), 3168 (NH), 1733 (C═O:carboxyl), 1636 (C═O:carbonyl), ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 2.42–2.47 (m, 4H, $CH_2N_{pip}CH_2$), 2.49–2.57 (m, 2H, $NHCH_2CH_2N$), 2.58–2.64 (m, 4H, $CH_2N_{pip}CH_2$), 3.53 (s, 4H, $COCH_2$ CH_2COOH), 3.75 (m, 2H, $CSNHCH_2$), 7.20 (d, 1H, H-3:pyridine, 9.0), 8.04 (dd, 1H, H-4:pyridine, 2.4, 6.6), 8.38 (d, 1H, H-6:pyridine, 2.4), 9.84 (br s, 1H, COO*H*), 10.77 (br s, 1H, NHCS), 11.42 (br s, 1H, CSNH); ^{13}C-NMR (d_6-DMSO), (δ, ppm: 27.3 (C_{pip}), 28.9 (C_{pip}), 41.3 ($N_{pip}COCH_2$), 41.9 ($CSNHCH_2$), 44.8 ($N_{pip}COCH_2CH_2$), 52.1 (C_{pip}), 52.5 (C_{pip}), 55.2 ($NHCH_2CH_2$), 111.8 (C-5:pyridine), 114.4 (C-3:pyridine), 141.3 (C-4:pyridine), 146.0 (C-6:pyridine), 152.3 (C-2:pyridine), 169.4 ($N_{pip}CO$), 173.8 (COOH), 178.8 (CS).

5′-O-Glycyl(Boc)d4U 14a

To a solution of Boc-Gly-OH (0.416 g; 2.38 mmol) in DMF (6 ml) were added D_4U **(4)** (0.5 g; 2.38 mmol), DCC (0.490 g; 2.38 mmol) and DMAP (0.029 g; 0.238 mmol) at 0°C, and the mixture was stirred at room temperature for 17 h. After removal of the solvent *in vacuo*, the residue was dissolved in EtOAc (50 ml), washed with 10% citric acid (50 ml), 5% $NaHCO_3$ (50 ml) and saturated NaCl (2 × 50 ml), dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the product by silica gel column chromatography (CH_2Cl_2:MeOH) gave the title compound **14a** as white crystals (0.48 g); Yield 55%; m.p. 72°C; TLC R_f (CH_2Cl_2 : MeOH = 92 : 8) 0.52; IR (KBr), cm^{-1}; 3345 (NH), 3210 (NH), 1759 (CO), 1715 (CO), 1698 (CO), 1248 (ester), 1163 (ester); ^{1}H-NMR ($CDCl_3$), δ, ppm, J, Hz: 1.38 (s, 9H, 3 × CH_3), 3.41 (d, 2H, CH_2NHBoc, 5.1), 4.23 (dd, 1H, H-5′, 9.8, 2.4), 4.38 (dd, 1H, H-5′, 9.8, 2.4), 5.0 (s, 1H, H-4′), 5.72 (d, 1H, H-5, 8.1), 5.86 (d, 1H, H-2′, 5.9), 6.23 (d, 1H, H-3′, 5.9), 6.93 (s, 1H, H-1′), 7.38 (d, 1H, H-6, 8.1), 9.0 (br s, 1H, BocNH); ^{13}C-NMR ($CDCl_3$), δ, ppm: 28.2 (3 × CH_3), 42.2 (CH_2NHBoc), 65.2 (C-5′), 80.3 ($C(CH_3)_3$), 84.1 (C-4′), 89.9 (C-1′), 102.8 (C-5), 126.9 (C-2′), 133.4 (C-3′), 139.9 (C-6), 150.6 (C-2), 155.6 (CO:Boc), 162.9 (C-4), 170.1 ($COCH_2$NHBoc).

5′-O-Glycyl(Boc)d2U 14b

Compound **14b** was prepared from compound **6** and Boc-Gly-OH in a similar manner to that described for compound **14a**; Yield 79%; m.p. 84°C; TLC R_f (CH_2Cl_2 : MeOH = 92 : 8) 0.47; IR (KBr), cm^{-1}: 3399 (NH), 3177 (NH), 1765 (CO), 1710 (CO), 1693 (CO), 1243 (ester), 1162 (ester); ^{1}H-NMR ($CDCl_3$), δ, ppm, J, Hz: 1.45 (s, 9H, 3 × CH_3), 1.80–1.88 (m, 1H, H-3′), 2.06–2.12 (m, 2H, H-3′ and H-2′), 2.42–2.48 (m, 1H, H-2′), 3.95 (d, 2H, CH_2NHBoc, 5.8), 4.28–4.39 (m, 3H, H-4′ and H-5′), 5.78 (d, 1H, H-5, 8.0), 6.04–6.06 (m, 1H, H-1′), 7.57 (d, 1H, H-6, 8.0), 8.93 (br s, 1H, N*H*Boc); ^{13}C-NMR ($CDCl_3$), δ, ppm: 25.7 (C-3′), 28.2 (3 × CH_3), 32.3 (C-2′), 42.4 (CH_2NHBoc), 65.4 (C-5′), 78.4 (C-4′), 80.3 ($C(CH_3)_3$), 86.5 (C-1′), 102.2 (C-5), 139.5 (C-6), 150.1 (C-2), 155.7 (CO:Boc), 163.0 (C-4), 170.2 ($COCH_2$NHBoc).

5′-O-Glycyl(Boc)d4T 14c

Compound **14c** was prepared from **13** and Boc-Gly-OH in a similar manner to that described for compound **14a**; Yield 78%; m.p. 120°C; TLC R_f (CH_2Cl_2 : MeOH = 92 : 8) 0.49; IR (KBr), cm^{-1}: 3396 (NH), 3172 (NH), 1759 (CO), 1712 (CO), 1693 (CO), 1252 (ester), 1162 (ester); ^{1}H-NMR ($CDCl_3$), δ, ppm. J, Hz: 1.45 (s, 9H, 3 × CH_3), 1.94 (s, 3H, CH_3), 3.49 (d, 2H, CH_2NHBoc, 5.1), 4.29 (dd, 1H, H-5′, 9.7, 2.4), 4.46 (dd, 1H, H-5′, 9.7, 2.4), 5.05 (s, 1H, H-4′), 5.91 (d, 1H, H-2′, 6.0), 6.29 (d, 1H, H-3′, 6.0), 6.98–7.01 (m, 1H, H-1′), 7.19 (s, 1H, H-6), 8.35 (br s, 1H, N*H*Boc); ^{13}C-NMR ($CDCl_3$), δ, ppm: 12.6 (CH_3), 28.2 (3 × CH_3), 42.3 (CH_2NHBoc), 65.4 (C-5′), 80.3 ($C(CH_3)_3$), 83.9 (C-4′), 89.8 (C-1′), 111.3 (C-5), 127.3 (C-2′), 133.2 (C-3′), 135.3 (C-6), 150.5 (C-2), 155.6 (CO:Boc), 163.3 (C-4), 170.1 ($COCH_2$NHBoc).

5′-O-Glycyld4U.HCl 15a

To a solution of the resulting product **14a** (0.4 g; 1.09 mmol) in 4 M HCl/dioxane (10 ml) at 0°C was added anisole (0.10 ml; 0.95 mmol), and the mixture was stirred at room temperature for 1 h. After removal of the solvent *in vacuo*, the residue was precipitated from diethyl ether to give the title compound **15a** as a green solid (0.32 g); Yield 97%; IR (KBr), cm^{-1}: 3352 (NH), 3238 (NH), 1751 (CO), 1713 (CO), 1682 (CO), 1267 (ester), 1232 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 3.67–3.74 (m, 2H, CH_2NH_2·HCl), 4.31 (s, 2H, H-5′), 5.0 (s, 1H, H-4′), 5.63 (d, 1H, H-5, 7.9), 6.03 (d, 1H, H-2′, 5.8), 6.45 (d, 1H, H-3′, 5.8), 6.8 (s, 1H, H-1′), 7.46 (d, 1H, H-6, 7.9), 8.46 (br s, 3H, NH_2·HCl), 11.39 (s, 1H, NH); ^{13}C-NMR (d_6-DMSO), δ, ppm: 38.6 (CH_2NH_2·HCl), 65.8 (C-5′), 83.5 (C-4′), 89.3 (C-1′), 101.4 (C-5), 126.2 (C-2′), 133.7 (C-3′), 140.6 (C-6), 151.1 (C-2), 163.7 (C-4), 167.8 ($COCH_2NH_2$·HCl).

5′-O-Glycyld2U.HCl 15b

Compound **15b** was prepared from **14b** in a similar manner to that described for compound **15a**; Yield 97%; IR (KBr), cm^{-1}: 3426 (NH), 3192 (NH), 1755 (CO), 1715 (CO), 1685 (CO), 1265 (ester), 1229 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.47–1.57 (m, 1H, H-3′), 1.97–2.02 (m, 2H, H-3′ and H-2′), 2.27–2.34 (m, 1H, H-2′), 3.14 (s, 2H, CH_2NH_2·HCl), 3.82

(d, 2H, H-5′, 3.4), 4.18–4.22 (m, 1H, H-4′), 5.62 (d, 1H, H-5, 8.0), 5.98–6.01 (m, 1H, H-1′), 7.65 (d, 1H, H-6, 8.0), 8.44 (br s, 3H, NH_2·HCl), 11.30 (s, 1H, NH); ^{13}C-NMR (d_6-DMSO), (δ, ppm: 25.5 (C-3′), 30.5 (C-2′), 48.5 (CH_2NH_2·HCl), 66.2 (C-5′), 77.4 (C-4′), 85.1 (C-1′), 101.7 (C-5),140.7 (C-6), 150.4 (C-2), 163.1 (C-4), 167.6 (*C*OCH_2NH_2·HCl).

5′-O-Glycyld4T.HCl 15c

Compound **15c** was prepared from **14c** in a similar manner to that described for compound **15a**; Yield 87%; IR (KBr), cm^{-1}: 3423 (NH), 3211 (NH), 1749 (CO), 1719 (CO), 1696 (CO), 1245 (ester), 1212 (ester): ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.78 (s, 3H, CH_3), 3.49–3.59 (m, 2H, C*H*$_2$$NH_2$·HCl), 4.32 (d, 2H, H-5′, 3.5), 4.98 (s, 1H, H-4′), 6.02 (d, 1H, H-2′, 5.4), 6.43 (d, 1H, H-3′, 5.4), 6.80 (s, 1H, H-1′), 7.26 (s, 1H, H-6), 8.46 (br s, 3H, NH_2·HCl), 11.40 (s, 1H, NH); ^{13}C-NMR (d_6-DMSO), δ, ppm: 12.1 (CH_3), 38.8 (CH_2NH_2·HCl), 66.0 (C-5′), 83.2 (C-4′), 89.4 (C-1′), 109.7 (C-5), 126.7 (C-2′), 133.4 (C-3′), 136.0 (C-6), 150.8 (C-2), 163.8 (C-4), 167.6 (*C*OCH_2NH_2·HCl).

5′-O-[(5-Bromopyridin-2-yl)-thioureidoethylpiperazinylsuccinylglycyl]d4U 16a

To a solution of **12** (0.22 g; 0.49 mmol) in DMF (4 ml) were added compound **15a** (0.180 g; 0.59 mmol), HOBt (0.166 g; 1.23 mmol), TEA (0.14 ml; 1.02 mmol) and BOP (0.324 g; 0.73 mmol) at 0°C, and the solution was stirred for 24 h at room temperature. After removal of the solvent *in vacuo*, the purification of the product by silica gel column chromatography (CH_2Cl_2:MeOH) gave the title compound as a yellow solid (0.119 g); Yield 35%; m.p. 148°C; TLC R_f (CH_2Cl_2 : MeOH = 85 : 15) 0.58; IR (KBr), cm^{-1}: 3426 (NH), 3256 (NH), 3167 (NH), 3052 (NH), 1753 (CO), 1690 (CO), 1638 (CO), 1467 (amide), 1247 (ester), 1183 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 2.36–2.53 (m, 8H, 4 × CH_2 : piperazine), 2.51 (t, 2H, NHCH_2C*H*$_2$, 6.1), 3.35–3.46 (m, 4H, N_{pip}COCH_2 and N_{pip}COCH_2C*H*$_2$), 3.69–3.74 (m, 2H, CSNHC*H*$_2$), 3.77–3.88 (m, 2H, NHC*H*$_2$CO), 4.20 (s, 2H, H-5′), 4.97 (s, 1H, H-4′), 5.67 (d, 1H, H-5, 8.0), 5.99 (d, 1H, H-2′, 5.6), 6.40 (d, 1H, H-3′, 5.6), 6.79 (s, 1H, H-1′), 7.12 (d, 1H, H-3:pyridine, 9.0), 7.46 (d, 1H, H-6, 8.0), 7.97 (dd, 1H, H-4:pyridine, 9.0, 2.4), 8.33 (d, 1H, H-6: pyridine, 2.4), 8.92 (br s, 1H, CON*H*CH_2), 10.73 (br s, 1H, ArNHCS), 11.35 (br s, 1H, CSN*H*CH_2), 11.38 (br s, 1H, NH-3); ^{13}C-NMR (d_6-DMSO), δ, ppm: 27.6 (NHCOCH_2*C*H_2), 30.0 (NHCO*C*H_2), 41.3 (CSNH*C*H_2), 45.7 (NH*C*H_2CO), 52.1 (2 × *C*H_2 : piperazine), 52.5 (2 × *C*H_2 : piperazine), 59.7 (NHCH_2*C*H_2), 64.9 (C-5′), 83.7 (C-4′), 89.3 (C-1′), 102.0 (C-5), 111.9 (C-5:pyridine), 114.4 (C-3:pyridine), 126.4 (C-2′), 133.7 (C-3′), 140.6 (C-6), 141.3 (C-4:pyridine), 146.0 (C-6:pyridine), 150.7 (C-2), 152.4 (C-2:pyridine), 163.1 (C-4), 169.5 (NHCH_2*C*O), 169.8 (N_{pip}*C*O), 172.1 (*C*ONHCH_2), 178.8 (CS).

5′-O-[(5-Bromopyridin-2-yl)-thioureidoethylpiperazinylsuccinylglycyl]d2U 16b

Compound **16b** was prepared from **15b** in a similar manner to that described for compound **16a**; Yield 40%; m.p. 161°C; TLC R_f (CH_2Cl_2 : MeOH = 85 : 15) 0.62; IR (KBr), cm^{-1}: 3426 (NH), 1675–1639 (5 × CO), 1466 (amide), 1262 (ester), 1092 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.75–1.82 (m, 1H, H-3′), 1.94–1.99 (m, 2H, H-3′ and H-2′), 2.26–2.31 (m, 1H, H-2′), 2.37–2.56 (m, 8H, 4 × CH_2 : piperazine), 2.40 (t, 2H, NH CH_2C*H*$_2$, 6.3), 3.12–3.15 (m, 2H, NHC*H*$_2$CO), 3.35 (t, 2H, NHCOC*H*$_2$, 6.1), 3.45 (t, 2H, NHCOCH_2C*H*$_2$, 6.1), 3.82–3.85 (m, 2H, CSNHC*H*$_2$), 4.12–4.27 (m, 3H, H-5′ and H-4′), 5.64 (d, 1H, H-5, 8.0), 5.96–5.99 (m, 1H, H-1′), 7.13 (d, 1H, H-3:pyridine, 8.7), 7.63 (d, 1H, H-6, 8.0), 7.97 (dd, 1H, H-4:pyridine, 6.8, 2.2), 8.31 (d, 1H, H-6:pyridine, 2.2), 8.38 (br s, 1H, CON*H*CH_2), 10.76 (br s, 1H, ArNHCS), 11.29 (br s, 1H, CSN*H*CH_2), 11.36 (br s, 1H, NH-3); ^{13}C-NMR (d_6-DMSO), δ, ppm: 25.4 (C-3′), 27.5 (NHCOCH_2*C*H_2), 29.9 (NHCO*C*H_2), 30.8 (C-2′), 40.6 (CSNH*C*H_2), 48.5 (NH*C*H_2CO), 51.2 (2 × *C*H_2 : piperazine), 52.1 (2 × *C*H_2 : piperazine), 54.9 (NHCH_2*C*H_2), 65.2 (C-5′), 77.7 (C-4′), 85.0 (C-1′), 101.7 (C-5), 111.9 (C-5:pyridine), 114.4 (C-3:pyridine), 140.4 (C-6), 141.4 (C-4:pyridine), 146.0 (C-6:pyridine), 150.4 (C-2), 152.2 (C-2:pyridine), 163.1 (C-4), 170.0 (NHCH_2CO), 169.9 (N_{pip}CO), 172.0 (CONHCH_2), 178.8 (CS).

5′-O-[(5-Bromopyridin-2-yl)-thioureidoethylpiperazinylsuccinylglycyl]d4T 16c

Compound **16c** was prepared from **15c** in a similar manner to that described for compound **16a**; Yield 49%; m.p. 147°C; TLC R_f (CH_2Cl_2 : MeOH = 85 : 15) 0.69; IR (KBr), cm^{-1}: 3425 (NH), 3210 (NH), 1748 (CO), 1699 (CO), 1655 (CO), 1468 (amide), 1184 (ester), 1081 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.76 (s, 3H, CH_3), 2.37–2.56 (m, 8H, 4 × CH_2 : piperazine), 2.44 (t, 2H, NHCH_2C*H*$_2$, 6.3), 3.14–3.17 (m, 2H, NHC*H*$_2$CO), 3.35 (t, 2H, NHCOC*H*$_2$, 6.2), 3.45 (t, 2H, NHCOCH_2C*H*$_2$, 6.2), 3.69–3.75 (m, 2H, CSNHC*H*$_2$), 4.20 (dd, 1H, H-5′, 12.3, 3.0), 4.23 (dd, 1H, H-5′, 12.3, 3.0), 4.95 (s, 1H, H-4′), 5.99 (d, 1H, H-2′, 5.7), 6.39 (d, 1H, H-3′, 5.7), 6.79 (s, 1H, H-1′), 7.12 (d, 1H, H-3:pyridine, 6.8), 7.27 (s, 1H, H-6), 7.97 (dd, 1H, H-4:pyridine, 6.8, 2.2), 8.30 (d, 1H, H-6:pyridine, 2.2), 9.07 (br s, 1H, CON*H*CH_2), 10.71 (br s, 1H, ArNHCS), 11.35 (br s, 1H, CSN*H*CH_2), 11.39 (br s, 1H, NH-3); ^{13}C-NMR (d_6-DMSO), δ, ppm: 12.1 (CH_3), 27.6 (NHCOCH_2*C*H_2), 30.0 (NHCO*C*H_2), 38.8 (NH*C*H_2 CO), 45.6 (CSNH*C*H_2), 51.2 (2 × *C*H_2 : piperazine),

52.1 ($2\times CH_2$: piperazine), 55.1 ($NHCH_2CH_2$), 65.0 (C-5′), 83.5 (C-4′), 89.2 (C-1′), 109.6 (C-5), 111.9 (C-5:pyridine), 114.4 (C-3:pyridine), 126.6 (C-2′), 133.5 (C-3′), 135.9 (C-6), 141.3 (C-4:pyridine), 146.0 (C-6:pyridine), 150.8 (C-2), 152.4 (C-2:pyridine), 163.7 (C-4), 169.6 ($NHCH_2CO$), 169.8 ($N_{pip}CO$), 172.1 ($CONHCH_2$), 178.8 (CS).

5′-O-Acetyl-5-{3-[glycyl(Boc)]prop-1-ynyl}d2U 17

To a solution of Boc-Gly-OH (0.147 g; 0.84 mmol) in DMF (5 ml) were added NRTI **9** (0.26 g; 0.844 mmol), DCC (0.174 g; 0.84 mmol) and DMAP (0.010 g; 0.084 mmol) at 0°C, and the mixture was stirred at room temperature for 20 h. After removal of the solvent *in vacuo*, the residue was dissolved in EtOAc (80 ml), washed with 10% citric acid (50 ml), 5% $NaHCO_3$ (50 ml) and saturated NaCl (2×50 ml), dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the product by silica gel column chromatography (CH_2Cl_2:MeOH) gave the title compound **17** as a white solid (0.16 g); Yield 42%; m.p. 90°C; TLC R_f (EtOAc 100%) 0.62; IR (KBr), cm^{-1}: 3314 (NH), 2234 ($C_{sp}C_{sp}$), 1752 (CO), 1715 (CO), 1699 (CO), 1283 (ester), 1160 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.45 (s, 9H, $3\times CH_3$), 1.96–2.02 (m, 1H, H-3′), 2.14–2.21 (m, 2H, H-3′ and H-2′), 2.18 (s, 3H, CH_3COO), 2.33–2.39 (m, 1H, H-2′), 3.78 (d, 2H, $COCH_2NH$, 6.0), 4.28–4.38 (m, 3H, H-5′ and H-4′), 5.00 (s, 2H, $C_{sp}C_{sp}CH_2$), 5.97–6.0 (m, 1H, H-1′), 7.36 (t, 1H, NHBoc, 5.9), 8.10 (s, 1H, H-6), 11.8 (br s, 1H, NH-3); ^{13}C-NMR (d_6-DMSO), δ, ppm: 20.8 (CH_3OCO), 24.6 (C-3′), 28.3 ($3\times CH_3$), 32.0 (C-2′), 42.0 ($COCH_2NH$), 52.9 ($C_{sp}C_{sp}CH_2$), 64.1 (C-5′), 78.8 (C-4′), 79.2 ($C_{sp}C_{sp}CH_2$), 79.5 ($C(CH_3)_3$), 86.3 (C-1′), 86.8 ($C_{sp}C_{sp}CH_2$), 96.9 (C-5), 144.5 (C-6), 149.6 (C-2), 156.1 (CO:Boc), 161.9 (C-4), 170.1 (CH_3OCO), 170.6 ($COCH_2NH$).

5′-O-Acetyl-5-[3-(glycyl)prop-1-ynyl]d2U.HCl 18

To a solution of the resulting product **17** (0.10 g; 0.21 mmol) in 4 M HCl/dioxane at 0°C was added anisole (14 μl; 0.19 mmol), and the mixture was stirred at room temperature for 1 h. After removal of the solvent *in vacuo*, the residue was precipitated from diethyl ether to give the title compound **18** as a white solid (0.0846 g); Yield 98%; IR (KBr), cm^{-1}: 3425 (NH), 2239 ($C_{sp}C_{sp}$), 1758 (CO), 1714 (CO), 1683 (CO), 1280 (ester), 1167 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm: 1.71–1.80 (m, 1H, H-3′), 1.95–2.15 (m, 2H, H-3′ and H-2′), 2.02 (s, 3H, CH_3COO), 2.27–2.39 (m, 1H, H-2′), 3.76 (s, 2H, $CH_2NH_2\cdot HCl$), 4.21–4.33 (m, 3H, H-5′ and H-4′), 4.98 (s, 2H, $C_{sp}C_{sp}CH_2$), 6.09–6.13 (m, 1H, H-1′), 7.79 (s, 1H, H-6), 8.46 (br s, 3H, $NH_2\cdot HCl$), 11.59 (br s, 1H, NH-3); ^{13}C-NMR (d_6-DMSO), δ, ppm: 20.5 (CH_3OCO), 22.7 (C-3′), 31.3 (C-2′), 62.1 ($C_{sp}C_{sp}CH_2$), 64.6 ($COCH_2NH_2\cdot HCl$), 66.3 (C-5′), 78.5 (C-4′), 78.6 ($C_{sp}C_{sp}CH_2$), 86.2 (C-1′), 86.3 ($C_{sp}C_{sp}CH_2$), 109.0 (C-5), 141.0 (C-6), 149.6 (C-2), 160.3 (C-4), 168.0 (CH_3OCO), 170.2 ($COCH_2NH_2\cdot HCl$).

5′-O-Acetyl-5-[(5-Bromopyridin-2-yl)-thioureidoethylpiperazinylsuccinylglycylprop-1-ynyl]d2U 19

To a solution of **12** (0.069 g; 0.15 mmol) in DMF (3 ml) were added compound **18** (0.075 g; 0.19 mmol), HOBt (0.052 g; 0.38 mmol), TEA (0.044 ml; 0.32 mmol) and BOP (0.102 g; 0.23 mmol) at 0°C, and the solution was stirred for 22 h at room temperature. After removal of the solvent *in vacuo*, the purification of the product by silica gel column chromatography (CH_2Cl_2: MeOH) gave the title compound as a pale yellow solid (0.059 g); Yield 48%; m.p. 131°C; TLC R_f (CH_2Cl_2 : MeOH = 85 : 15) 0.53; IR (KBr), cm^{-1}: 3431 (NH), 2360 ($C_{sp}C_{sp}$), 1752 (CO), 1742 (CO), 1686 (CO), 1468 (amide), 1271 (ester), 1185 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.65–1.80 (m, 1H, H-3′), 1.92–2.12 (m, 2H, H-3′ and H-2′), 2.02 (s, 3H, CH_3COO), 2.32–2.50 (m, 1H, H-2′), 2.45–2.54 (m, 8H, $4\times CH_2$: piperazine), 2.48–2.64 (m, 2H, $NHCH_2CH_2$), 3.30–3.38 (m, 4H, $COCH_2CH_2CO$), 3.44–3.51 (m, 2H, $COCH_2NH$), 3.71–3.74 (m, 2H, $CSNHCH_2$), 4.20–4.25 (m, 3H, H-5′ and H-4′), 4.50 (s, 2H, $C_{sp}C_{sp}CH_2$), 5.94–5.97 (m, 1H, H-1′), 7.12 (d, 1H, H-3:pyridine, 8.8), 7.79 (s, 1H, H-6), 7.98(dd, 2.3 Hz, 1H, H-4:pyridine, 8.8), 8.31 (d, 1H, H-6:pyridine,2.3), 8.90 (br s, 1H, $CONHCH_2$), 10.72 (br s, 1H, ArNHCS), 11.35 (br s, 1H, $CSNHCH_2$), 11.63 (br s, 1H, NH-3); ^{13}C-NMR (d_6-DMSO), δ, ppm: 20.4 (CH_3COO), 25.0 (C-3′), 27.5 ($NHCOCH_2CH_2$), 30.0 ($NHCOCH_2$), 31.5 (C-2′), 40.5 ($COCH_2NH$), 45.7 ($CSNHCH_2$), 52.0 ($2\times CH_2$: piperazine), 52.4 ($2\times CH_2$: piperazine), 55.1 ($C_{sp}C_{sp}CH_2$), 61.2 ($NHCH_2CH_2$), 64.5 (C-5′), 78.5 (C-4′), 78.6 ($C_{sp}C_{sp}CH_2$), 86.0 (C-1′), 86.6 ($C_{sp}C_{sp}CH_2$), 109.1 (C-5), 111.9 (C-5:pyridine), 114.4 (C-3:pyridine), 140.8 (C-6), 141.3 (C-4:pyridine), 146.0 (C-6:pyridine), 149.6 (C-2), 152.3 (C-2:pyridine), 160.5 (C-4), 169.7 (CH_3OCO), 169.8 ($COCH_2NH$), 170.2 ($N_{pip}CO$), 172.0 (CH_2NHCO), 178.9 (CS).

5-[(5-Bromopyridin-2-yl)thioureido-ethylpiperazinylsuccinylglycylprop-1-ynyl]d2U 20

To a solution of the protected heterodimer **19** (0.120 g; 0.15 mmol) in methanol was added a few crystals of NaCN. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH) to give the final heterodimer **20** as pale green crystals (0.080 g); Yield 38%; m.p. 169°C; TLC R_f (CH_2Cl_2 : MeOH = 90 : 10) 0.14; IR (KBr), cm^{-1}: 3426 (NH), 2366 ($C_{sp}C_{sp}$), 1729 (CO), 1466 (amide), 1286

(ester), 1127 (ester); ^{1}H-NMR (d_6-DMSO), δ, ppm, J, Hz: 1.47–1.52 (m, 1H, H-3′), 1.59–1.70 (m, 2H, H-3′ and H-2′), 2.12–2.33 (m, 1H, H-2′), 2.45–2.54 (m, 8H, 4 × CH_2 : piperazine), 2.48–2.59 (m, 2H, $NHCH_2CH_2$), 2.29–3.35 (m, 4H, $COCH_2CH_2$-CO), 3.42–3.51 (m, 2H, $COCH_2NH$), 3.69–3.76 (m, 2H, $CSNHCH_2$), 4.19–4.23 (m, 1H, H-5′), 4.29–4.32 (m, 1H, H-5′), 4.37–4.41 (m, 1H, H-4′), 4.49 (s, 2H, $C_{sp}C_{sp}CH_2$), 5.92–5.96 (m, 1H, H-1′), 7.12 (d, 1H, H-3:pyridine, 8.8), 7.82 (s, 1H, H-6), 7.93 (dd, 2.3 Hz, 1H, H-4:pyridine, 8.8), 8.31 (d, 1H, H-6:pyridine, 2.3), 8.90 (br s, 1H, $CONHCH_2$), 10.70 (br s, 1H, ArNHCS), 11.36 (br s, 1H, $CSNHCH_2$), 11.63 (br s, 1H, NH-3); ^{13}C-NMR (d_6-DMSO), δ, ppm: 22.1 (C-3′), 27.6 ($NHCOCH_2CH_2$), 27.8 ($NHCOCH_2$), 38.2 (C-2′), 42.3 ($COCH_2NH$), 49.9 ($CSNHCH_2$), 52.3 (2 × CH_2 : piperazine), 53.0 (2 × CH_2 : piperazine), 53.4 ($C_{sp}C_{sp}CH_2$), 58.8 ($NHCH_2CH_2$), 67.3 (C-5′), 76.4 (C-4′), 84.4 ($C_{sp}C_{sp}CH_2$), 84.7 (C-1′), 88.9 ($C_{sp}C_{sp}CH_2$), 103.0 (C-5), 108.7 (C-5:pyridine), 108.9 (C-3:pyridine), 141.2 (C-6), 141.6 (C-4:pyridine), 149.0 (C-6:pyridine), 154.1 (C-2), 158.6 (C-2:pyridine), 160.0 (C-4), 169.2 ($COCH_2NH$), 171.3 ($N_{pip}CO$), 172.5 (CH_2NHCO), 178.6 (CS).

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 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 RT assay as previously 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

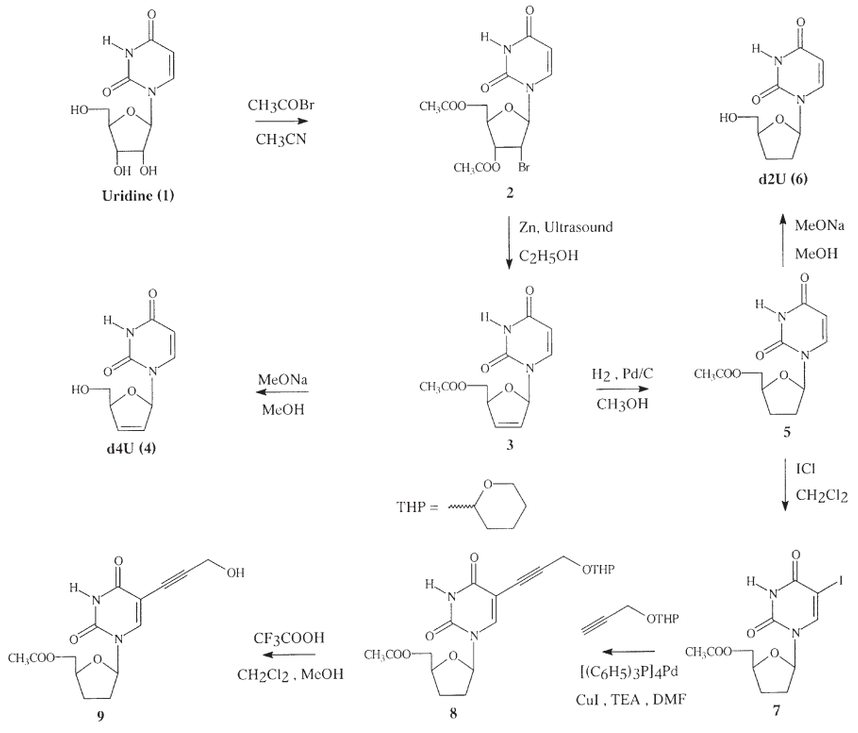


FIGURE 1 Synthesis of the NRTIs: d4U (**4**), d2U (**6**) and 5′-*O*-acetyl-5-(3-hydroxypropynyl)-2′,3′-dideoxyuridine (**9**).

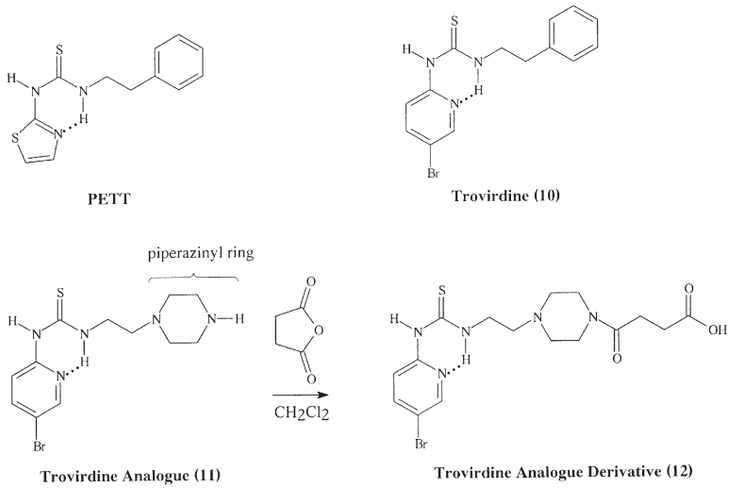


FIGURE 2 Phenethylthiazolylthiourea (PETT) derivatives; Synthesis of the Trovirdine Analogue Derivative (**12**).

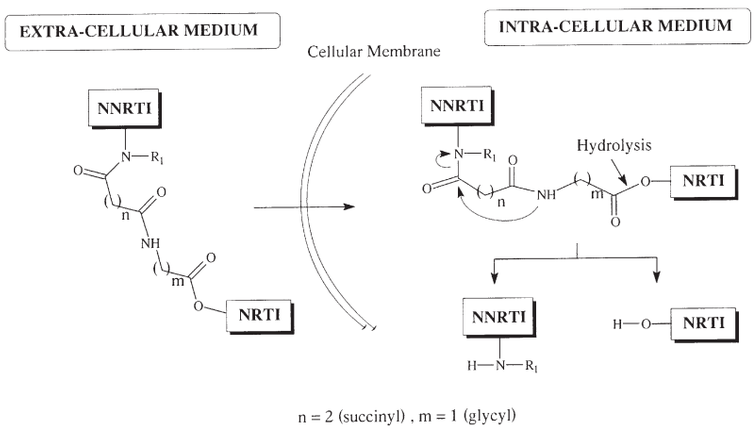
were done according to previously published protocols;[31,34] virus production was quantified by the RT activity associated with virus particles released from the cells in the culture medium. Conditions under which the inhibitory properties were measured on HIV-1 reverse transcriptase *in vitro* has 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).

RESULTS

Chemical

Synthesis of the NRTIs

Firstly, the synthesis of the NRTIs was performed. The synthetic route for the compounds d_4U, d_2U and the derivative of 2′,3′-dideoxyuridine (d_2U) bearing an alkynyl linker at the "C-5" position was developed starting from the commercially available uridine **1**. The required key intermediary, 5′-*O*-acetyl-d_2U **5**, was obtained in three steps starting from the uridine **1** (Figure 1). It was subjected to a bromoacetylation reaction using acetyl bromide in dry acetonitrile to provide the nucleoside **2** in 87% yield.[23] The reductive elimination of the acetoxybromo nucleoside **2** proceeded by adding activated zinc dust in anhydrous ethanol affording the olefinic nucleoside **3**, 5′-*O*-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine, in 49% yield after chromatography. The compound 5′-*O*-acetyl-d_4U **3** was then hydrogenated in the presence of wet 10% palladium on carbon into the 5′-*O*-acetyl-d_2U **5**, isolated in 89% yield.[24] Deacetylation of **3** and **5** was performed using sodium methoxide at room temperature and the NRTIs **4** (d_4U) and **6** (d_2U) were isolated in 87% and 70% yields respectively.



SCHEME 1 Concept of anti-HIV double-drugs containing spontaneously cleavable linker.

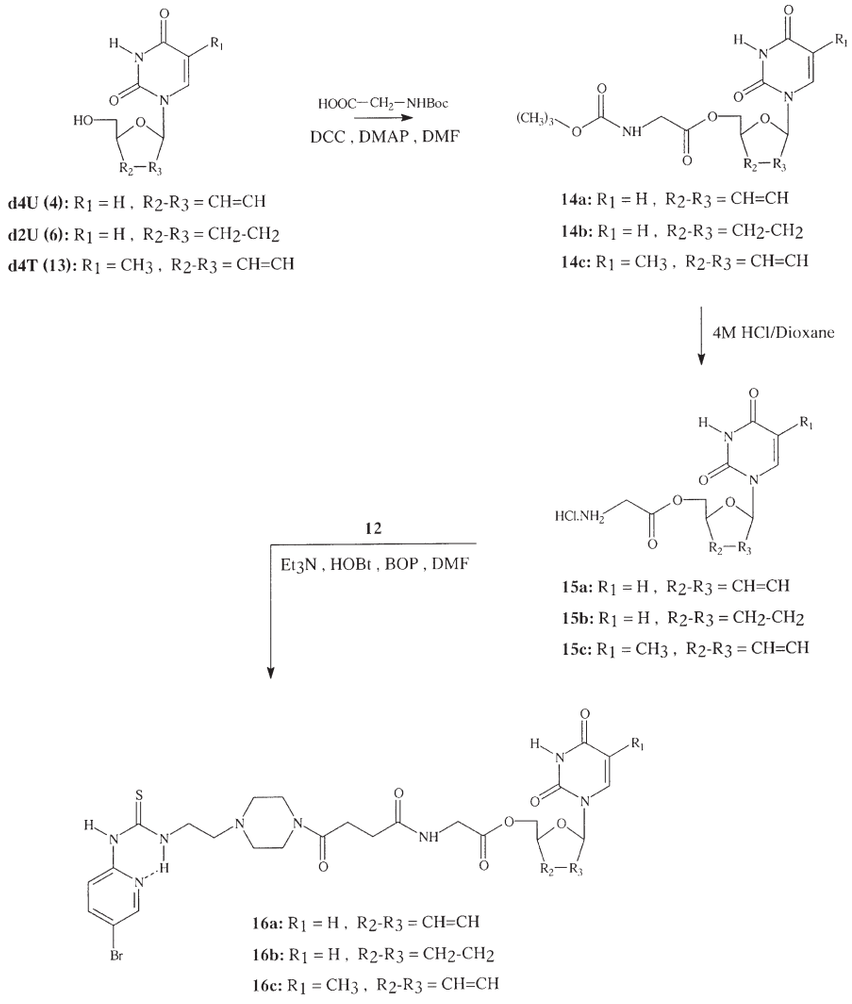


FIGURE 3 Conjugation in position C-5′ of the NRTIs; Synthesis of the heterodimers **16a–c**.

In the literature, it has been shown that 5-iodouracil derivatives undergo high-yield coupling with terminal alkynes to give 5-alkynyluracil nucleosides exhibiting antiviral activity, and that such products can be transformed into biological interesting compounds.[25,26] Moreover, the 5-halopyrimidine nucleosides have been used as intermediaries for a variety of synthetic transformations. In this way, the treatment of 5′-*O*-acetyl-d_2U **5** with iodine monochloride (ICl) in anhydrous dichloromethane at reflux gave the corresponding 5-iodonucleoside, 5′-*O*-acetyl-5-iodo-d_2U **7**, in 63% yield. This intermediate was used to introduce an alkynyl linker at the "C-5" position by a *Sonogashira* reaction.[27] In this end, the nucleoside **7** was condensed with an alkyne: the tetrahydro-2-(2-propynyloxy)-2*H*-pyrane (THPOP) in dimethylformamide as solvent and in the presence of: copper (I) iodide, anhydrous triethylamine and tetrakis(triphenylphosphine)palladium (0) to give the corresponding 5-propynyl derivative **8** isolated in 67% yield. The final deprotection of the "THP" group of the nucleoside **8** by CF_3COOH afforded the NRTI **9**.

The NNRTI

For the NNRTI, a Trovirdine Analogue **11** was chosen and was prepared as described previously by Vig *et al.* (Figure 2).[28] This compound contains the NH final group which is essential for connection with the hydroxyl group of NRTIs (**4, 6, 13, 9**) through the spontaneously cleavable linker.

Conjugation Strategy

For the conjugation, the succinyl-glycine moiety was chosen as a spontaneously cleavable linker. The essential criteria in the choice of this linker is based on the following premises: firstly, the heterodimer must be stable outside the target cell; secondly, after

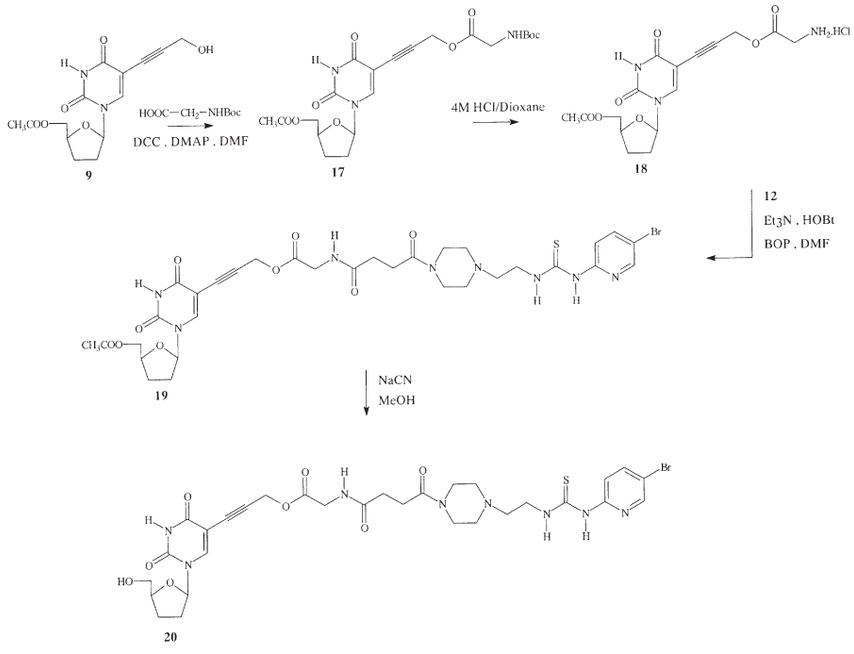


FIGURE 4 Conjugation in position C-5 of the NRTI **9**; Synthesis of the heterodimer **20**.

the penetration across the cell membrane, the heterodimer must regenerate the parent inhibitors (see Scheme 1).[29,30]

This concept of anti-HIV double-drugs containing a spontaneously cleavable linker was applied to the synthesis of the heterodimers including a NRTI (**4, 6, 13 or 9**) conjugated with a NNRTI **11**. To this end, after the penetration across the cell membrane, the disintegration of the heterodimer should regenerate the NRTI and the NNRTI.

Synthesis of the Heterodimers 16a–c and 20

The synthesis of a few heterodimers of the general formula: [NRTI]-Glycyl-Succinyl-*N-piperazinyl*-[Trovirdine Analogue] is reported. For the NRTIs: d_4U (**4**), d_4U (**6**) and d_4T (**13**), the hydroxyl group of the "C-5" position was connected with the NNRTI (**11**) through the "succinyl-glycine" linker. At the same time, for the NRTI **9**, the conjugation with the NNRTI (**11**) was performed on the "C-5" position. Initially compound **11** (NNRTI) was coupled with succinic anhydride in anhydrous dichloromethane to afford the Trovirdine Analogue Derivative **12** in 64% yield (Figure 2).

Figure 3 illustrates the synthetic procedure for conjugation on position "C-5′" of the NRTIs: d_4U, d_2U, d_4T (**4, 6, 13**). These NRTIs were coupled with Boc-Gly-OH using dicyclohexylcarbodiimide (DCC) in the presence of *N*,*N*-dimethylaminopyridine (DMAP) in dimethylformamide to give the corresponding compounds **14a–c**. Final deprotection of the Boc group using 4M HCl/dioxane afforded the amine hydrochlorides **15a–c**.

Condensation of compounds **15a–c** with the Trovirdine Analogue Derivative **12** using

TABLE I Antiviral and Cytotoxicity Evaluation of Trovirdine Analogue **11** and Heterodimers [NRTI]-*C5′* or *C5*-Glycyl-Succinyl-*N-piperazinyl*-[Trovirdine Analogue] **16a-c** and **20**

	HIV-1_{LAI} in CEM-SS cells			HIV-1_{IIIB} in MT-4 cells		
Compd	IC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]	IC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
11	13	>100	>7.69	>100	>100	>1
16a	6.9	94	13.62	>100	>100	>1
16b	>100	>100	>1	>100	>100	>1
16c	0.17	>100	>588	0.49	>100	>204
20	>10	>10	>1	>10	>10	>1
AZT	0.0033			0.01		

[a] IC_{50} is the concentration required to inhibit HIV-1 multiplication by 50%. [b] CC_{50} is the concentration of drug which causes 50% cytotoxicity to uninfected cells.
[c] SI corresponds to the ratio CC_{50}/IC_{50}.

benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate/1-hydroxybenzotriazole (BOP/HOBt) in the presence of triethylamine in dimethylformamide afforded the heterodimers **16a–c** in 35–45% yield.

The conjugation of the NNRTI on the "C-5" position of NRTI **9** is shown in Figure 4 and gives the corresponding compound **17**. After deprotection of the Boc group using 4M HCl/dioxane, the intermediate **18** was subjected to a condensation reaction with the Trovirdine Analogue Derivative **12** to provide the heterodimer **19**, isolated in 48% yield. The final heterodimer **20** was obtained after deprotection of the acetyl group of the nucleosidic moiety of **19** using sodium cyanide in anhydrous methanol (38% yield).

Biological

The heterodimers [NRTI]-Glycyl-Succinyl-[Trovirdine Analogue] (**16a–c and 20**) were evaluated by comparison with AZT for inhibition of HIV-1 multiplication in lymphocytic cell lines (CEM-SS and MT4). As shown in Table I, the heterodimers **16a–c** and **20** were unfortunately devoid of antiviral activity at non toxic concentrations. Overall, the activities observed for these heterodimers were inferior to the values obtained with the parent nucleosides. In fact, the lack of activity of these heterodimers is probably the consequence of a wrong positioning of either the NRTI or the NNRTI component with their respective sites on the Reverse Transcriptase.

Acknowledgement

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