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Synthesis of prodrug-type anti-HIV agents conjugating a REVERSE transcriptase inhibitor to a HIV-1 integrase inhibitor by a spontaneously cleavable linker

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Abstract

Based on the prodrug concept as well as the combination of two different classes of anti-HIV agents, we have designed and synthesized a series of anti-HIV double-drugs consisting of a nucleoside reverse transcriptase inhibitor (NRTI) conjugated with an integrase inhibitor (INI) through a spontaneously cleavable linker in an effort to enhance the antiviral activity. These conjugates combined in their structure a dideoxy-didehydro-nucleoside (ddN) such as d4T and an INI such as α,γ-diketo acid (DKA) analogues of L-708,906 and L-731,988 linked through an appropriate self-immolative spacer. Among these novel bis-substrate inhibitors, several conjugates exhibited antiviral activity but this effect was accompanied for some of them by an increased cytotoxicity by comparison to d4T, DKA or even some precursors. These compounds are nevertheless interesting candidates for further investigations.

Keywords: *HIV, reverse transcriptase, integrase, nucleoside inhibitor*

Introduction

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS) [1]. The *pol* gene of HIV encodes for three enzymes, reverse transcriptase (RT), protease (PR) and integrase (IN). Drug discovery directed at inhibitors of RT and PR has produced clinically useful compounds for the treatment of AIDS [2–3]. Currently, the standard treatment strategy for AIDS is referred to the highly active antiretroviral therapy (HAART) which is a combination of three or more anti-HIV drugs inhibiting two viral enzymes (reverse transcriptase and protease) and virus fusion (interaction with glycoprotein 41) [4–6]. However, HAART strategy still suffers from issues of patient compliance, cost, deliterious side effects, and the ever-increasing emerging drug resistance [7–9].

Therefore, HIV-1 integrase, one of the three constitutive viral enzymes required for replication, has recently emerged as an attractive target for chemotherapeutic intervention in the treatment of AIDS since no human counterpart of the enzyme is known [10–14]. The viral integrase mediates the integration of the proviral DNA into the genome of the host cell. Integration is a multistep process which includes three different biochemical processes: assembly of proviral DNA on integrase, endonucleolytic processing of the proviral DNA, and strand transfer of the proviral DNA into host chromosomal DNA. Several classes of HIV IN inhibitors (INIs) have been developed, including dinucleotides [15], hydroxylated aromatics [16–20], and α,γ-diketo acids compounds (DKAs) [21–27]. While a number of structurally diverse compounds have been reported to be inhibitors of HIV integrase, only four drugs have reached the clinical trials [28–29]: S-1360 designed by Shionogi-GSK companies, L-870,810 and L-870,812 and 8-hydroxynaphthyridines designed by Merck Research laboratories, and JTK-303 designed

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by Japan Tobacco and developed by Gilead (Figure 1). In fact, the interest in studying DKAs started with reports of the selective inhibitory properties of L-708,906 and L-731,988 toward strand transfer and the crystal structure of the catalytic domain of HIV-1 IN with 5-CITEP [21,22]. Also, the α,γ-diketo acids represent the most convincing, biologically validated inhibitors of this viral enzyme [21,25]. The DKAs compete with host DNA for the binding sites on HIV integrase, thus selectively inhibiting the strand transfer process [22]. This inhibition is dependent on the presence of divalent magnesium ion, or manganese ion *in vitro* assays [25]. The L-731,988 compound showed good potency against integrase, particularly in the strand transfer step with an IC_{50} of 0.08 μM compared with 6 μM in the 3′-processing step [22].

However, the antiviral efficacy of inhibitors depends not only on the enzyme inhibitory activity, but also on their intracellular concentrations closely related to the membrane permeability. Also, the presence of a free carboxyl group on DKAs could lead to insufficient cell membrane permeability. To improve antiviral activity, we have suggested «double-drug» strategy that combined an effective anti-HIV agent such as a nucleoside RT inhibitor (NRTI) used for masking the free carboxylic acid of the HIV IN inhibitor in a single molecule. This strategy has been advocated for three main reasons: (1) the low membrane permeability of IN inhibitors would be improved while the cell membrane has affinity for nucleosides [30–31]; (2) the conjugation of HIV IN inhibitor with the nucleoside RT inhibitor would facilitate the penetration through the biological membrane mediated by the nucleoside transporter [32–33]; (3) once the prodrugs avoided the extracellular hydrolysis, the intracellular hydrolysis would regenerate the parent inhibitors, which could act on two separate targets and exhibit synergistic anti-HIV efficacy. Also, there are several attractive features to such an approach since the "double-drug" could act on two separate targets and exhibit synergistic anti-HIV efficacy (Figure 2). One essential criteria in the design of such prodrugs is the linker that must be stable outside the target cell and regenerate the parent compounds in the cytoplasm to act on their respective targets.

With this aim based on the "double-drug" strategy, we reported in 2004 the synthesis and the anti-HIV activity assessment of a series of heterodimers which combine in their structure an NRTI analogue [2′,3′-didehydro-2′,3′-dideoxyuridine (d4U) or 2′,3′-dideoxyuridine (d2U) or 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T)] and an INI (belonging to the β-diketo acids class) connected through an amino acid (glycine or β-alanine) as a cleavable linker [34]. The most active

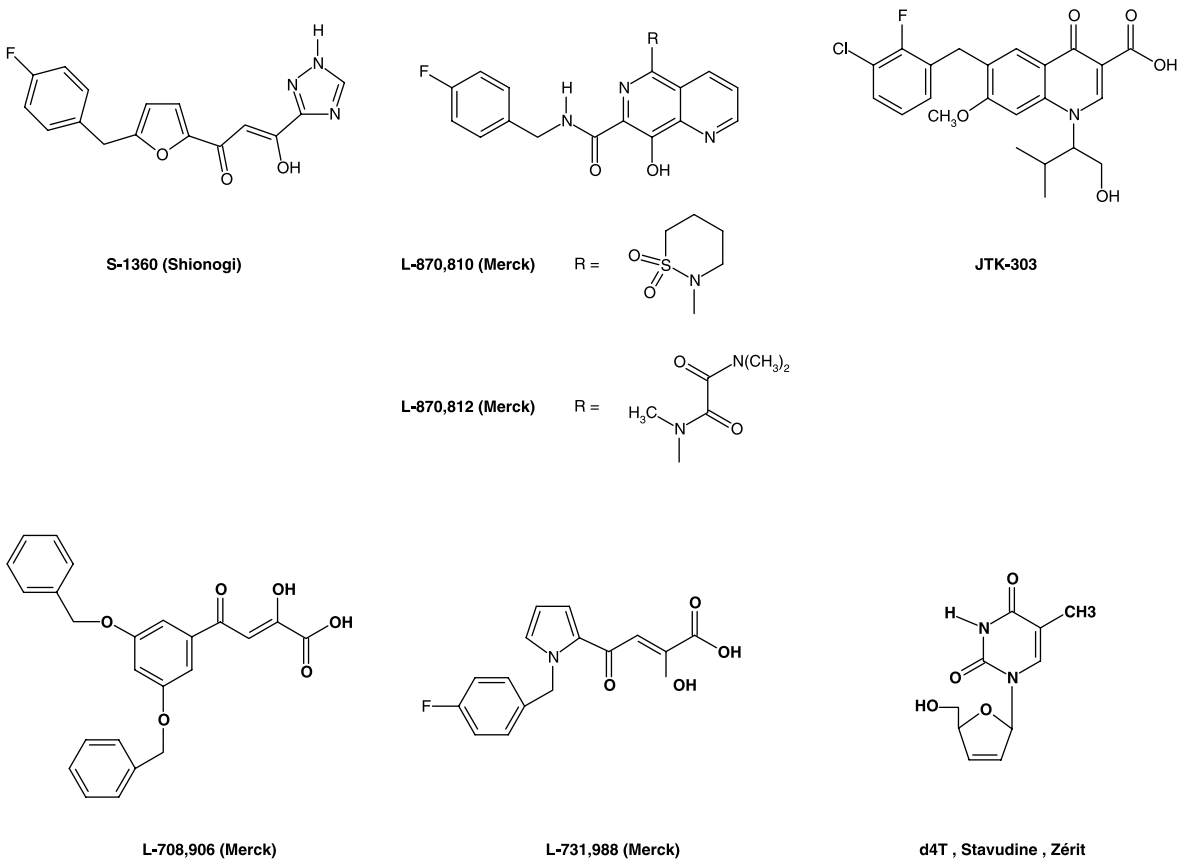


Figure 1. Chemical structures of representative α,γ-Diketo-Based integrase inhibitors and the nucleoside reverse transcriptase inhibitor (the 2′,3′,didehydro-2′,3′-dideoxythymidine).

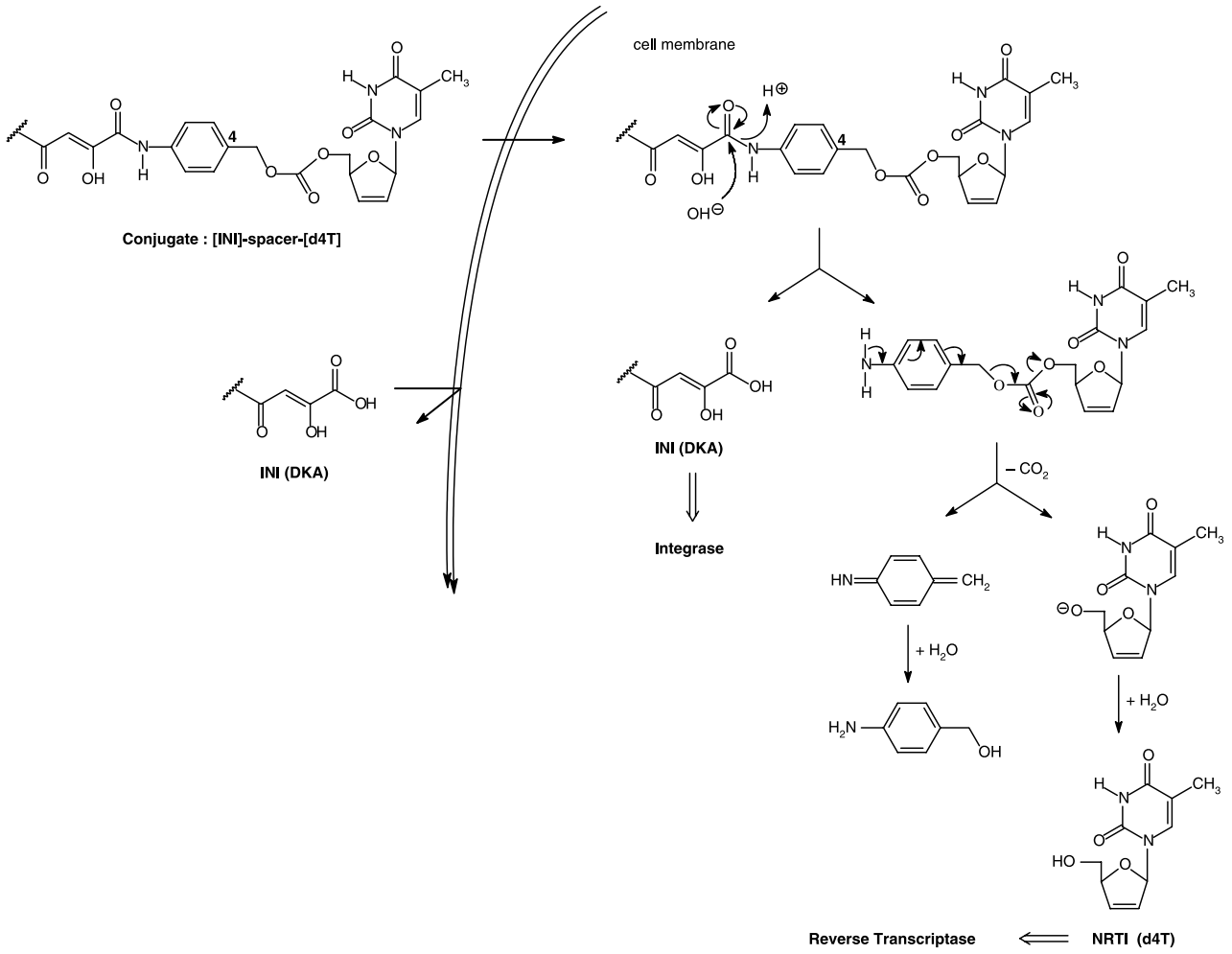


Figure 2. Design and proposed mechanism of conversion of double-drugs to d4T and IN inhibitor.

compound in this series was the [4-[1-(2-fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic]-GLY-CYL-C5′[d4T] heterodimer (IC_{50} 3 μM, HIV-1_{LAI} / CEM-SS cells and IC_{50} 0.73 μM, HIV-1_{IIIB} / MT4 cells). However, they were less potent inhibitors than the parent compounds from which they derived in lymphocytic cell lines (CEM-SS and MT-4).

Taking into account our previous studies focused on prodrugs containing conventional spacers, we decided to prepare new prodrug-type conjugates including a linker which has the ability to cleave spontaneously in physiological environment between the NRTI (the 2′,3′-didehydro-2′,3′-dideoxythymidine, d4T, Stavudine, Zerit® [35–39]) and the INI, taking into consideration the affinity of nucleosides towards cell membrane [30]. The most prominent example of an electronic cascade spacer is the 1,6-elimination spacer developed by Carl et al.[40]. The *p*-aminobenzyl alcohol (PAB) moiety was designed in the literature to hydrolytically decompose upon enzymatic deacylation releasing the free drug. After unmasking the aromatic amine, the amine group becomes electron-donating and initiates virtually instantaneously an electronic cascade that leads to the expulsion of the leaving group, which release the free drug after elimination of carbon dioxide (Figure 2) This type of spacer has been largely studied in preceding investigations in cancer chemotherapy for enhanced drug release by Scheeren et al. [41–43], Monneret et al. [44–46], Senter et al. [47], Dubowchick et al. [48] and Deny et al. [49].

Two types of prodrugs were synthesized bearing either an ortho- or para-hydroxybenzylcarbonate linkage, both spacers expected to spontaneously decompose after enzymatic cleavage. The linking group was conjugated to the DKA through amide bond formation and to the 5′-hydroxyl group of d4T by a carbonate group. This arrangement should cleave by amide hydrolysis followed by degradation of the remaining para- or ortho-aminobenzylcarbonate as proposed mechanism described in Figure 2.

Materials and methods

Chemistry

Instrumentation. Commercial reagents (acetone /VWR and toluene/Aldrich) were used as received without

additional purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use. Reagent grade dichloromethane (CH_2Cl_2) was refluxed and distilled from phosphorus pentoxide. 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 mm). All samples were kept in a drying oven at 30°C over P_4O_{10} for at least 24 h prior to analysis.

Melting points were determined using a Kofler melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer Spectrum BX FT-IR and only noteworthy absorptions are listed. ^{1}H and ^{13}C-NMR spectra were recorded on a JEOL NMR LA 400 (400 MHz) spectrometer using TMS as an internal standard. Chemical shifts were reported as δ values in parts per million units, downfield from TMS. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, br = broad, m = multiplet. Coupling constants J are given in hertz (Hz). NH and OH signals appeared as broad singlets exchangeable with D_2O. Elemental analysis of new compounds was performed at the Institute de Recherche en Chimie Organique Fine (Rouen, France).

*1-(3,5-Dibenzyloxyphenyl)ethanone (**2a**) [34]*

*1-(3-Benzyloxyphenyl)ethanone (**2b**)*. To a stirred and cooled (0°C) solution of 3-hydroxyacetophenone (10 g, 73.45 mmol) **1b** in anhydrous acetone (140 mL) was added, respectively: anhydrous sodium carbonate (15.22 g, 137 mmol) and benzyl bromide (10.48 mL, 88 mmol). The reaction mixture was stirred at room temperature for 24 h before the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (150 mL) and the resulting solution was washed with water (150 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The aryl methyl ketone **2b** was isolated as a brown oil (15.9 g) that was taken on to the next step without further purification; Yield: 96%; TLC R_f (n-hexane: acetone = 70: 30) 0.50; IR υ_{max} (KBr)/cm^{-1} 3065, 3033, 1683 (C=O), 1581, 1437, 1270, 1026, 739; ^{1}H-NMR δ (d_6-DMSO) 7.54-7.20 (m, 9H, H-Bn and H-Ph), 5.08 (s, 2H, CH_2), 2.57 (s, 3H, CH_3).

*1-(4-Benzyloxyphenyl)ethanone (**2c**)*. Compound **2c** was prepared from 4-hydroxyacetophenone **1c** (10 g, 73.45 mmol) by the same procedure as that described for compound **2b**. Yield: 80%, white solid (13.3 g); TLC R_f (n-hexane: acetone = 60: 40) 0.71; mp = 92°C; IR υ_{max} (KBr)/cm^{-1} 2940, 1676 (C=O), 1598, 1248, 1009, 759; ^{1}H-NMR δ (d_6-DMSO) 7.94 (d, 2H, J 8.7 Hz, H-Ph), 7.44-7.32 (m, 5H, H-Bn), 7.01 (d, 2H, J 8.7 Hz, H-Ph), 5.13 (s, 2H, CH_2), 2,55 (s, 3H, CH_3).

*Ethyl 4-(3,5-dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoate **(3a)** [34].*

*Ethyl 4-(3-benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoate (**3b**)*. To a stirred and cooled (0°C) solution of aryl methyl ketone **2b** (11.3 g, 50 mmol) in anhydrous toluene (80 mL) under an argon atmosphere was added respectively: sodium hydride (60% dispersion in mineral oil, 2.5 g, 62 mmol), diethyloxalate (7 mL, 51 mmol) and a drop of ethanol. The reaction mixture was heated under reflux for 2 h, then cooled at room temperature and concentrated to dryness *in vacuo*. The residue was dissolved in ethyl acetate (500 mL) and the resulting solution was washed respectively with 1N HCl/H_2O (200 mL), water (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was crystallized from diethyl ether to give the ketoenol ester **3b** (5.0 g) as a yellow powder; Yield: 31%; mp = 82°C; TLC R_f (n-hexane: acetone = 60: 40) 0.70; IR υ_{max} (KBr)/cm^{-1} 1749 (C=O), 1577, 1271, 1204, 1072, 1025, 771, 734, 696, 677; ^{1}H-NMR δ (d_6-DMSO) 7.66-7.33 (m, 9H, H-Bn and H-Ph), 7.10 (s, 1H, C*H* = C(OH)), 5.19 (s, 2H, CH_2-Bn), 4.30 (q, 2H, J 7.1 Hz, C*H*$_2$-CH_3), 1.30 (t, 3H, J 7.1 Hz, CH_2-C*H*$_3$).

*Ethyl 4-(4-benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoate (**3c**)*. Compound **3c** was prepared from precursor **2c** (5.9 g, 26.1 mmol) by the same procedure as that described for compound **3b**. Yield: 90%, yellow solid (7.7 g); TLC R_f (dichloromethane: methanol = 97: 3) 0.86; mp = 188°C; IR υ_{max} (KBr)/cm^{-1} 1743 (C=O), 1593, 1250, 1174, 1021, 988, 852, 778, 699; ^{1}H-NMR δ (d_6-DMSO) 8.08 (d, 2H, J 9.0 Hz, H-Ph), 7.47-7.34 (m, 5H, H-Bn), 7.18 (d, 2H, J 9.0 Hz, H-Ph), 7.09 (s, 1H, C*H*=C(OH)), 5.23 (s, 2H, CH_2-Bn), 4.30 (q, 2H, J 7.1 Hz, C*H*$_2$-CH_3), 1.30 (t, 3H, J 7.1 Hz, CH_2-C*H*$_3$).

*4-(3,5-Dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic acid (**4a**) [34]*

*4-(3-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic acid (**4b**)*. To a solution of ketoenol ester **3b** (5 g, 15.3 mmol) in 1:1 tetrahydrofuran/methanol (100 mL) was added a solution of 1N NaOH/H_2O (25 mL) and the mixture was stirred at room temperature for 2 h. Then, the reaction mixture was washed with diethyl ether (100 mL), acidified to pH = 2 with 1N HCl/H_2O and extracted several times with ethyl acetate (3 × 100 mL). The combined organic phases were washed successively with 1N HCl/H_2O (100 mL) and

water (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was crystallized from diethyl ether to give the ketoenol acid **4b** (3.5 g) as a yellow crystals; Yield: 77%, mp = 180°C; TLC R_f (dichloromethane: methanol = 97: 3) 0.56; IR υ_{max} (KBr)/cm^{-1} 3039-2635 (OH), 1708 (C=O), 1637, 1582, 1439, 1260, 1018, 772; ^{1}H-NMR δ ($CDCl_3$) 7.66-7.32 (m, 9H, H-Bn and H-Ph), 7.09 (s, 1H, C*H* = C(OH)), 5.19 (s, 2H, CH_2); ^{13}C-NMR δ ($CDCl_3$) 190.7 (C-4), 170.5 (C-1), 163.6 (C-2), 159.1 (C-3:phenyl), 137.1 (C-1:phenyl), 136.5 (C-1:Bn), 130.8 (C-5:phenyl), 128.9 (C-3 and C-5:Bn), 128.4 (C-4:Bn), 128.2 (C-2 and C-6:Bn), 121.4 (C-6:phenyl), 121.0 (C-4:phenyl), 113.6 (C-2:phenyl), 98.5 (*C*H=C(OH)), 69.9 (CH_2).

*4-(4-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic acid (**4c**)*. Compound **4c** was prepared from precursor **3c** (3.84 g, 11.8 mmol) by the same procedure as that described for compound **4b**. Yield: 94%, yellow powder (3.3 g); TLC R_f (n-hexane: ethyl acetate = 60: 40) 0.40; mp = 202°C; IR υ_{max} (KBr)/cm^{-1} 3034-2635 (OH), 1712 (C=O), 1595, 1404, 1243, 1174, 1003, 833; ^{1}H-NMR δ (d_6-DMSO) 8.06 (d, 2H, *J* 8.5 Hz, H-Ph), 7.47-7.34 (m, 5H, H-Bn), 7.16 (d, 2H, *J* 8.5 Hz, H-Ph), 7.05 (s, 1H, C*H*=C(OH)), 5.22 (s, 2H, CH_2); ^{13}C-NMR δ (d_6-DMSO) 190.0 (C-4), 169.9 (C-1), 163.3, 163.1, 136.3, 130.4 (C-2 and C-6:phenyl), 128.5, 128.1, 127.8, 127.4, 115.2 (C-3 and C-5:phenyl), 97.6 (*C*H=C(OH)), 69.6 (CH_2).

*1-[1-(4-Fluorobenzyl)-1H-pyrrol-2-yl]ethanone (**6a**) [34]*
*1-[1-(2-Fluorobenzyl)-1H-pyrrol-2-yl]ethanone (**6b**)*. To a stirred and cooled (0°C) solution of 2-acetylpyrrole (13.08 g, 120 mmol) in dimethylformamide (120 mL) under an argon atmosphere wad added respectively: sodium hydride (60% dispersion in oil, 5.75 g, 144 mmol), 2-flurobenzyl bromide (17.24 mL, 144 mmol). The reaction mixture was stirred at room temperature overnight, poured into a saturated solution of $NaHCO_3/H_2O$ (200 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic phases were washed with water (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The title compound **6b**, obtained as a clear yellow oil (26.34 g), was taken on to the next step without purification; Yield: 84%; TLC R_f (hexane: ethyl acetate = 80: 20) 0.55; IR υ_{max} (KBr)/cm^{-1} 1652 (C=O), 1491, 1407, 1328, 1229, 1086, 945, 754; ^{1}H-NMR δ (d_6-DMSO) 7.30-7.24 (m, 2H, H-5:pyrrole and H-4:Bn), 7.20-7.16 (m, 2H, H-3:pyrrole and H-5:Bn), 7.06 (t, 1H, *J* 7.5 Hz, H-3:Bn), 6.61 (t, 1H, *J* 7.5 Hz, H-6:Bn), 6.22 (t, 1H, *J* 3.3 Hz, H-4:pyrrole), 5.60 (s, 2H, CH_2-Bn), 2.32 (s, 3H, CH_3); ^{13}C-NMR δ (d_6-DMSO) 187.6 (C=O), 159.4 (d, $^1J_{C\text{-}F}$ 244.5 Hz, C-2:Bn), 131.5 (C-2:pyrrole), 129.7 (C-5:pyrrole), 129.0 (d, $^3J_{C\text{-}F}$ 8.2 Hz, C-6:Bn), 127.8 (d, $^3J_{C\text{-}F}$ 4.1 Hz, C-4:Bn), 126.1 (d, $^2J_{C\text{-}F}$ 14.9, C-1:Bn), 124.5 (d, $^4J_{C\text{-}F}$ 3.3 Hz, C-5:Bn), 120.7 (C-3:pyrrole), 115.0 (d, $^2J_{C\text{-}F}$ 21.5 Hz, C-3:Bn), 108.5 (C-4:pyrrole), 45.7 (d, $^3J_{C\text{-}F}$ 4.9 Hz, CH_2-Bn), 27.0 (CH_3).

*Ethyl 4-[1-(4-fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoate (7**a**) [34]*
*Ethyl 4-[1-(2-fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoate (7**b**)*. To a stirred and cooled (0°C) solution of compound **6b** (13.02 g, 60 mmol) in anhydrous dimethoxyethane (120 mL) under an argon atmosphere was added respectively: sodium hydride (60% dispersion in oil, 2.88 g, 72 mmol), diethyl oxalate (9.78 mL, 72 mmol) and a drop of ethanol. The reaction mixture was heated at reflux for 18 h, stirred overnight at room temperature, poured into a saturated into a solution of 1N $NaHCO_3/H_2O$ (200 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic phases were washed with water (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was crystallized with diethyl ether to give the title compound **7b** as yellow crystals (5.20 g); Yield: 28%; TLC R_f (hexane: ethyl acetate = 80: 20) 0.39; mp = 98°C; IR υ_{max} (KBr)/cm^{-1} 1734 (C=O), 1613, 1381, 1330, 1264, 1223, 1086, 1065, 754; ^{1}H-NMR δ (d_6-DMSO) 7.48 (m, 2H, H-5:pyrrole and H-4:Bn), 7.30-7.18 (m, 2H, H-3:pyrrole and H-5:Bn), 7.08 (t, 1H, *J* 7.6 Hz, H-3:Bn), 6.87 (s, 1H, C*H*=CHOH), 6.64 (t, 1H, *J* 7.1 Hz, H-6:Bn), 6.35 (t, 1H, *J* 3.4 Hz, H-4:pyrrole), 5.69 (s, 2H, CH_2-Bn), 4.25 (q, 2H, *J* 7.1 Hz, CH_2CH$_3$), 2.32 (t, 3H, *J* 7.1 Hz, CH_3); ^{13}C-NMR δ (d_6-DMSO) 183.7 (C-4), 161.8 (C-1), 160.3 (C-2), 159.4 (d, $^1J_{C\text{-}F}$ 243.7 Hz, C-2:Bn), 134.8 (C-2:pyrrole), 129.2 (d, $^3J_{C\text{-}F}$ 8.2 Hz, C-6:Bn), 128.6 (C-5:pyrrole), 127.6 (d, $^3J_{C\text{-}F}$ 4.2 Hz, C-4:Bn), 125.7 (d, $^2J_{C\text{-}F}$ 14.1, C-1:Bn), 124.6 (d, $^4J_{C\text{-}F}$ 3.3 Hz, C-5:Bn), 122.4 (C-3:pyrrole), 115.1 (d, $^2J_{C\text{-}F}$ 21.5 Hz, C-3:Bn), 110.2 (C-4:pyrrole), 101.1 (C-3), 61.9 (*C*H$_2$CH$_3$), 45.7 (d, $^3J_{C\text{-}F}$ 4.9 Hz, CH_2-Bn), 13.8 (CH_3).

*4-[1-(4-Fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic acid (**8a**) [34]*
*4-[1-(2-Fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic acid (**8b**)*. To a solution of compound **7b** (5.20 g, 17 mmol) in 1:1 tetrahydrofuran / methanol (120 mL) was added a solution of 1N NaOH / H_2O (80 mL) and the mixture was stirred at room temperature for 4 h. Then, the reaction mixture was washed with diethyl ether (150 mL), acidified to pH = 2 with 1N HCl / H_2O and extracted several times with ethyl acetate (3 × 150 mL). The combined organic phases were washed with 1N HCl / H_2O (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*.

The residue was crystallized with diethyl ether to give the title compound **8b** as yellow crystals (2.70 g); Yield: 96%; TLC R_f (dihloromethane: methanol = 97: 3) 0.56; mp = 162°C; IR υ_{max} (KBr)/cm^{-1} 2360, 1712 (C=O), 1623 (C=O), 1410, 1323, 1262, 1067, 755; ^{1}H-NMR δ (d_6-DMSO) 7.45-7.41 (m, 2H, H-5:pyrrole and H-4:Bn), 7.31-7.26 (m, 2H, H-3:pyrrole and H-5:Bn), 7.08 (t, 1H, *J* 7.6 Hz, H-3:Bn), 6.82 (s, 1H, C*H*=CHOH), 6.64 (t, 1H, *J* 7.8 Hz, H-6:Bn), 6.33 (dd, 1H, *J* 4.4 Hz *J* 2.4 Hz, H-4:pyrrole), 5.68 (s, 2H, CH_2-Bn); ^{13}C-NMR δ (d_6-DMSO) 183.9 (C-4), 163.3 (C-1), 161.4 (C-2), 159.4 (d, $^1J_{C\text{-}F}$ 244.5 Hz, C-2:Bn), 134.5 (C-2:pyrrole), 129.2 (d, $^3J_{C\text{-}F}$ 8.3 Hz, C-6:Bn), 128.7 (C-5:pyrrole), 127.7 (d, $^3J_{C\text{-}F}$ 4.1 Hz, C-4:Bn), 125.7 (d, $^2J_{C\text{-}F}$ 14.0, C-1:Bn), 124.6 (d, $^4J_{C\text{-}F}$ 3.3 Hz, C-5:Bn), 122.0 (C-3:pyrrole), 115.1 (d, $^2J_{C\text{-}F}$ 20.7 Hz, C-3:Bn), 110.1 (C-4:pyrrole), 100.8 (C-3), 46.4 (d, $^3J_{C\text{-}F}$ 4.9 Hz, CH_2-Bn).

*4-(3,5-Dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-PAB-OH (**9a**)*. To a stirred and cooled (0°C) solution of **4a** (404 mg, 1 mmol) in anhydrous tetrahydrofuran (8 mL) under an argon atmosphere were added 4-aminobenzyl alcohol (148 mg, 1.2 mmol, 1.2 equiv.) and 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ, 260 mg, 1.1 mmol, 1.05 equiv.), and the mixture was stirred at room temperature for 20 h. After removal of the solvent *in vacuo*, the residue was dissolved in ethyl acetate (50 mL), washed with 10% citric acid / H_2O (40 mL), and water (40 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was crystallized with diethyl ether to give the title compound **9a** as yellow crystals (361 mg); Yield: 71%; TLC R_f (chloroform: methanol = 95: 5) 0.47; mp = 154°C; IR υ_{max} (KBr)/cm^{-1} 3357 (OH and NH), 1695 (C=O), 1584, 1532, 1164, 1013, 823, 698; ^{1}H-NMR δ (d_6-DMSO) 10.64 (s, 1H, NH), 7.78 (d, 2H, *J* 7.6 Hz, H-PAB), 7.46-7.00 (15H, m, H-Bn and H-Ph and H-PAB), 6.77 (s, 1H, C*H*=C(OH)), 5.20 (s, 4H, 2xCH_2-Bn), 5.17 (s, 1H, PAB-O*H*), 4.61 (s, 1H, CH=C(O*H*)), 4.47 (s, 2H, CH_2-PAB).

*4-(3-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-PAB-OH (**9b**)*. Compound **9b** was prepared from precursor **4b** (600 mg, 2.01 mmol) by the same procedure as that described for compound **9a**; Yield: 60%, yellow crystals (486 mg); mp = 150°C; TLC R_f (chloroform: methanol = 95: 5) 0.39; IR υ_{max} (KBr)/cm^{-1} 3355 (OH and NH), 1696 (C=O), 1575, 1531, 1258, 1189, 1023, 819, 774; ^{1}H-NMR δ (d_6-DMSO) 10.68 (s, 1H, NH), 7.83 (d, *J* 8.3 Hz, 2H, H-PAB), 7.72-7.38 (m, 9H, H-Bn and H-Ph), 7.35 (d, *J* 8.3 Hz, 2H, H-PAB), 7.27 (s, 1H, C*H*=C(OH)), 5.26 (s, 2H, CH_2-Bn), 5.23 (s, 1H, PAB-OH), 4.67 (s, 1H, CH=C(O*H*)), 4.51 (s, 2H, CH_2-PAB).

*4-(4-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-PAB-OH (**9c**)*. Compound **9c** was prepared from precursor **4c** (587 mg, 2.03 mmol) by the same procedure as that described for compound **9a**; Yield: 69%, yellow crystals (565 mg); mp = 162°C; TLC R_f (chloroform: methanol = 95: 5) 0.41; IR υ_{max} (KBr)/cm^{-1} 3362 (OH + NH),1697 (C=O), 1605, 1588, 1527, 1508, 1255, 1181, 1014, 822, 773; ^{1}H-NMR δ (d_6-DMSO) 10.58 (s, 1H, NH), 8.08 (d, *J* 8.6 Hz, 2H, H-Ph), 7.77 (d, *J* 8.3 Hz, 2H, H-PAB), 7.48-7.34 (m, 5H, H-Bn), 7.29 (d, *J* 8.3 Hz, 2H, H-PAB), 7.19 (d, *J* 8.6 Hz, 2H, H-Ph), 7.16 (s, 1H, C*H*=C(OH)), 5.23 (s, 2H, CH_2-Bn), 5.16 (s, 1H, PAB-OH), 4.56 (s, 1H, CH=C(O*H*)), 4.45 (s, 2H, CH_2-PAB); ^{13}C-NMR δ (d_6-DMSO) 185.5 (C-4), 178.4, 163.1, 159.9, 150.5, 138.9, 136.3, 136.0, 130.0, 129.5, 128.9, 128.5, 128.1, 127.8, 126.8, 126.5, 121.4, 120.4, 120.1, 115.3 (C-3 and C-5:phenyl), 93.7 (*C*H=C(OH)), 69.7 (CH_2-Bn), 62.6 (CH_2-PAB).

*4-(3,5-Dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-OAB-OH (**10a**)*. Compound **10a** was prepared from precursor **4a** (400 mg, 0.99 mmol) by the same procedure as that described for compound **9a**, except that the reaction mixture was added with 2-aminobenzyl alcohol (1.2 equiv.) instead of 4-aminobenzyl alcohol; Yield: 71%, yellow powder (358 mg); mp = 170°C; TLC R_f (ethyl acetate: n-hexane = 1: 1) 0.41; IR υ_{max} (KBr)/cm^{-1} 3345 (OH), 3319 (NH), 1679 (C=O), 1589, 1524, 1455, 1167, 1056, 1020, 697; ^{1}H-NMR δ (d_6-DMSO) 10.77 (1H, s, NH), 8.03 (1H, d, *J* 8.0 Hz, H-OAB), 7.48-7.16 (16H, m, H-Bn and H-Ph and H-OAB), 7.01 (1H, s, C*H*=C(OH)), 5.21 (4H, s, 2xCH_2-Bn), 5.17 (1H, s, PAB-OH), 4.62 (2H, s, CH_2-PAB).

*4-(3-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-OAB-OH (**10b**)*. Compound **10b** was prepared from precursor **4b** (642 mg, 2.15 mmol) by the same procedure as that described for compound **9a**, except that the reaction mixture was added with 2-aminobenzyl alcohol (1.2 equiv.) instead of 4-aminobenzyl alcohol; Yield: 78%, yellow powder (633 mg); mp = 144°C; TLC R_f (chloroform: methanol = 97: 3) 0.64; IR υ_{max} (KBr)/cm^{-1} 3413 (OH and NH), 3115 (NH), 1674 (C=O), 1587, 1531, 1388, 1264, 1026, 759, 694, 572; ^{1}H-NMR δ (d_6-DMSO) 10.31 (s, 1H, NH), 8.20 (d, 2H, *J* 8.3 Hz, H-OAB), 7.56-7.06 (m, 12H, H-Ph and H-Bn and H-OAB and C*H*=C(OH)), 5.06 (2H, s, CH_2-Bn), 4.74 (2H, s, CH_2-OAB).

*4-(4-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-OAB-OH (**10c**)*. Compound **10c** was prepared from

precursor **4c** (600 mg, 2.01 mmol) by the same procedure as that described for compound **9a**, except that the reaction mixture was added with 2-aminobenzyl alcohol (1.2 equiv.) instead of 4-aminobenzyl alcohol; Yield: 78%, yellow powder (632 mg); mp = 180°C; TLC R_f (ethyl acetate: n-hexane = 1: 1) 0.50; IR υ_{max} (KBr)/cm^{-1} 3437 (OH), 3259 (NH), 1670 (C=O), 1600, 1583, 1506, 1455, 1258, 1230, 1171, 1007, 824, 777, 756, 716; ^{1}H-NMR δ (d_6-DMSO) 10.76 (s, 1H, NH), 8.08 (d, 2H, *J* 9.0 Hz, H-Ph), 7.20 (s, 1H, C*H*=C(OH)), 7.48-7.31 (m, 7H, H-Bn and H-OAB), 7.19 (d, 2H, *J* 9.0 Hz, H-Ph), 7.15 (d, 1H, *J* 6.8 Hz, H-OAB), 5.83 (s, 1H, OAB-OH), 5.23 (s, 2H, CH_2-Bn), 4.62 (s, 2H, CH_2-OAB), 4.58 (s, 1H, CH=C(O*H*)); ^{13}C-NMR δ (d_6-DMSO) 185.5 (C-4), 177.6, 163.2, 159.3, 136.3, 135.9, 132.3, 130.2 (C-2 and C-6:Ph), 128.5 (C-II and C-VI:Bn), 128.2, 128.1, 127.9 (C-III and C-V:Bn), 127.8, 124.8 (C-2:OAB), 115.4 (C-3 and C-5:Ph), 93.5 (*C*H=C(OH)), 69.6 (CH_2-Bn), 61.9 (CH_2-OAB).

*4-(3,5-Dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-PABC-PNP (**11a**)*. To a stirred and cooled (0°C) solution of **9a** (403 mg, 0.79 mmol) in anhydrous tetrahydrofuran (10 mL) under an argon atmosphere was added respectively: N-N-diisopropylethylamine (DIEA, 1.2 equiv., 0.16 mL, 0.95 mmol), bis-p-nitrophenyl carbonate (2 equiv., 481 mg, 1.58 mmol) and molecular sieves (3 g). The reaction mixture was stirred at room temperature for 2 days and washed respectively with 10% citric acid / H_2O (50 mL), water (50 mL), brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (n-hexane: dichloromethane: = 40: 60) yielding the title compound **11a** as yellow crystals (144 mg); Yield: 27%; TLC R_f (dichloromethane: methanol = 92: 8) 0.87; mp = 212°C; IR υ_{max} (KBr)/cm^{-1} 3363 (NH), 1760 (C=O), 1594, 1348, 1266, 1216, 1163, 1045; ^{1}H-NMR δ ($CDCl_3$) 9.06 (s, 1H, NH), 8.28 (d, 2H, *J* 9.0 Hz, H-PNP), 7.75 (d, 2H, *J* 8.3 Hz, H-PABC), 7.51-7.37 (m, 18H, H-Bn and H-Ph and H-PABC and H-PNP), 7.23 (s, 1H, C*H*=C(OH)), 6.85 (s, 2H, CH_2O(C=O)O), 5.10 (s, 4H, 2xCH_2-Bn); ^{13}C-NMR δ ($CDCl_3$) 185.5 (C-4), 179.5 (C-2), 159.1 (C-1), 155.5 (C-3:Ph), 154.8 (OC(=O)O), 152.4, 137.4, 135.5, 131.0, 129.9, 128.7, 128.3, 127.6, 126.2, 125.6, 125.3 (C-PNP), 121.8, 121.7, 120.1 (C-PABC), 115.6, 107.8, 106.7, 100.6, 94.2 (*C*H=C(OH)), 70.5 (*C*H_2OC(= O)O), 70.3 (CH_2-Bn).

*4-(3-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-PABC-PNP (**11b**)*. Compound **11b** was prepared from precursor **9b** (584 mg, 1.45 mmol) by the same procedure as that described for compound **11a**; Yield: 17%, yellow crystals (140 mg); mp = 108°C; TLC R_f (dichloromethane: methanol = 92: 8) 0.86; IR υ_{max} (KBr)/cm^{-1} 3342 (OH and NH), 1758 (C=O), 1682 (C=O), 1530, 1277, 1217, 1111, 1015, 678; ^{1}H-NMR δ ($CDCl_3$) 9.06 (s, 1H, NH), 8.28 (d, 1H, *J* 9.1 Hz, H-PNP), 7.76 (d, 1H, *J* 8.1 Hz, H-PABC), 7.63 (m, 2H, H-Ph), 7.50-7.29 (m, 11H, H-Bn and H-Ph and H-PABC and H-PNP), 7.23 (s, 1H, C*H*=C-OH), 5.29 (s, 2H, CH_2OC(=O)O), 5.15 (s, 2H, CH_2-Bn); ^{13}C-NMR δ ($CDCl_3$) 185.4 (C-4), 179.7 (C-2), 159.2 (C-1), 155.5 (C-3:Ph), 154.8 (OC(=O)O), 152.4, 145.9, 145.4, 137.4, 136.3, 134.8, 131.0, 130.0, 129.9, 128.7, 128.2, 127.5, 125.5 (C-PNP), 125.3 (C-PNP), 121.8, 121.6, 120.9, 120.5 (C-Ph), 120.1 (C-PABC), 113.3 (C-2:Ph), 94.0 (*C*H=C(OH)), 70.5 (*C*H_2OC(= O)O), 70.3 (CH_2-Bn).

*4-(4-Benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic-PABC-PNP (**11c**)*. Compound **11c** was prepared from precursor **9c** (592 mg, 1.47 mmol) by the same procedure as that described for compound **11a**; Yield: 20%, yellow crystals (167 mg); mp = 120°C; TLC R_f (dichloromethane: methanol = 92: 8) 0.88; IR υ_{max} (KBr)/cm^{-1} 3365 (OH + NH), 1755 (C=O), 1696 (C=O), 1613, 1527, 1266, 1231, 776; ^{1}H-NMR δ (d_6-DMSO) 10.70 (s, 1H, NH), 8.31 (d, 2H, *J* 9.1 Hz, H-PNP), 8.07 (d, *J* 8.8 Hz, 2H, H-Ph), 7.88 (d, 2H, *J* 8.5 Hz, H-PABC), 7.57 (d, 2H, *J* 9.1 Hz, H-PNP), 7.47 (d, 2H, *J* 8.5 Hz, H-PABC), 7.42-7.34 (m, 5H, H-Bn), 7.19 (d, 2H, *J* 8.8 Hz, H-Ph), 7.15 (s, 1H, C*H*=C(OH)), 5.25 (s, 2H, CH_2OC(= O)O), 5.24 (s, 2H, CH_2-Bn); ^{13}C-NMR δ (d_6-DMSO) 185.4 (C-4), 182.2 (C-2), 160.1 (C-1), 155.2 (C-3:Ph), 151.9 (OC(=O)O), 145.1, 138.2, 136.3, 129.9 (C-Ph), 129.2 (C-PABC), 128.4, 128.0, 127.8, 126.1, 125.3 (C-PNP), 122.6 (C-PNP), 120.4 (C-PABC), 115.7, 115.2, 114.8, 94.0 (CH=C(OH)), 70.1 (*C*H_2OC(=O)O), 69.6 (CH_2-Bn).

*5′-PNPC-2′,3′-didehydro-2′,3′-dieoxythymidine (**12**)*. To a stirred and cooled (0°C) solution of **d4T** (500 mg, 2.23 mmol) in anhydrous tetrahydrofuran (30 mL) under an argon atmosphere was added respectively: pyridine (0.27 mL, 3.34 mmol) and a solution of 4-nitrophenyl chloroformate (675 mg, 3.34 mmol) in anhydrous tetrahydrofuran (8 mL). The reaction mixture was stirred at room temperature and after 2 h TLC (silica, chloroform: methanol = 97: 3) indicated completion. The mixture was diluted with ethyl acetate (100 mL) and washed respectively with water (100 mL), 5% $NaHCO_3$ / H_2O (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was crystallized with diethyl ether to give the title compound **12** as white crystals (685 mg); Yield: 79%; TLC R_f

(chloroform: methanol = 97: 3) 0.57; mp = 190°C; IR υ_{max} (KBr)/cm^{-1} 3039 (NH), 1770 (C=O), 1694 (C=O), 1524, 1466, 1351, 1270, 1216, 1084, 859; ^{1}H-NMR δ ($CDCl_3$) 8.77 (s, 1H, NH), 8.30 (d, 2H, *J* 8.5 Hz, H-III:PNP and H-V:PNP), 7.39 (s, 1H, H-6), 7.35 (d, 2H, *J* 8.5 Hz, H-II:PNP and H-VI:PNP), 7.10 (s, 1H, H-1′), 6.38 (d, 1H, *J* 6.1 Hz, H-2′), 5.98 (d, 1H, *J* 6.1 Hz, H-3′), 5.13 (s, 1H, H-4′), 4.54 (s, 2H, H-5′a and H-5′b), 1.87 (s, 3H, CH_3); ^{13}C-NMR δ ($CDCl_3$) 163.6 (C-4), 155.0 (C-I:PNP), 152.3 (OC=O), 150.1 (C-2), 145.7 (C-IV:PNP), 135.9 (C-6), 132.7 (C-2′), 128.0 (C-3′), 125.5 (C-II and C-V:PNP), 121.7 (C-II and C-VI:PNP), 111.3 (C-5), 89.6 (C-1′), 83.5 (C-4′), 68.8 (C-5′), 12.4 (CH_3).

*Heterodimer [4-(3,5-dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic]-PABC-C5′[d4T] (**13a**)*. To a stirred and cooled (0°C) solution of **10a** (228 mg, 0.45 mmol) in anhydrous dichloromethane (10 mL) under an argon atmosphere was added respectively: 4-dimethylaminopyridine (1.3 equiv., 71 mg, 0.58 mmol), 5′-PNPC-d4T (**12**, 1 equiv.) and a catalytic amount of *N,N*-diisopropylethylamine. The reaction mixture was stirred at room temperature and after 3 days TLC (silica, ethyl acetate: n-hexane = 80: 20) indicated completion. The mixture was diluted with ethyl acetate: water (1/1, 200 mL). The organic layer was separated, washed with a solution of 10% citric acid/H_2O (200 mL), then water (200 mL), dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was crystallized with diethyl ether to give the title compound **13a** as yellow crystals (163 mg); Yield: 48%, mp = 156°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.45; IR υ_{max} (KBr)/cm^{-1} 3310 (OH and NH), 1753 (C=O), 1691 (C=O), 1583, 1264, 1159, 1080, 1058, 775, 736, 697; ^{1}H-NMR δ ($CDCl_3$) 9.80 (s, 1H, NH-d4T), 8.28 (s, 1H, NH-PAB), 7.81 (d, 1H, *J* 8.6 Hz, H-PAB), 7.46-7.34 (m, 16H, H-Bn and H-Ph and H-PAB and C*H*=C(OH)), 7.02 (s, 1H, H-6:d4T), 6.84 (s, 1H, H-1′:d4T), 6.28 (d, 1H, *J* 5.8 Hz, H-2′:d4T), 5.86 (d, 1H, *J* 5.8 Hz, H-2′:d4T), 5.18 (s, 4H, CH_2-Bn), 5.01 (s, 2H, $CH_2OC(=O)O$), 5.00 (s, 1H, H-4′:d4T), 4.31 (s, 2H, H-5′a and H-5′b:d4T), 1.55 (s, 3H, CH_3); Anal. Calcd. For C42H37N3O11: C, 66.40; H, 4.91; N, 5.53. Found: C, 66.34; H, 4.87; N, 5.46%.

*Heterodimer [4-(3-benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic]-PABC-C5′[d4T] (**13b**)*. Compound **13b** was prepared from precursor **10b** (200 mg, 0.50 mmol) by the same procedure as that described for compound **13a**; Yield: 36%, yellow crystals (117 mg); mp = 130°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.51; IR υ_{max} (KBr)/cm^{-1} 3309 (OH and NH), 1749 (C=O), 1694 (C=O), 1525, 1264, 777, 697; ^{1}H-NMR δ ($CDCl_3$) 11.32 (s, 1H, NH-d4T), 10.73 (s, 1H, NH-PAB), 7.63-7.11 (m, 15H, H-Bn and H-Ph and H-PAB and C*H*=C(OH) and H-6:d4T), 6.90 (s, 1H, H-1′:d4T), 6.19 (d, 1H, *J* 5.8 Hz, H-2′:d4T), 5.74 (d, 1H, *J* 5.8 Hz, H-2′:d4T), 5.03 (s, 4H, CH_2-Bn and $CH_2OC(=O)O$), 4.93 (br s, 1H, H-4′:d4T), 4.39 (dd, 1H, *J* 2.4 Hz, *J* 12.3 Hz, H-5′a:d4T), 4.28 (dd, 1H, *J* 2.4 Hz, *J* 12.3 Hz, H-5′b:d4T), 1.66 (s, 3H, CH_3); Anal. Calcd. For C35H31N3O10: C, 64.31; H, 4.78; N, 6.43. Found: C, 64.29; H, 4.74; N, 6.39%.

*Heterodimer [4-(4-benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic]-PABC-C5′[d4T] (**13c**)*. Compound **13c** was prepared from precursor **10c** (251 mg, 0.62 mmol) by the same procedure as that described for compound **13a**; Yield: 45%, yellow crystals (183 mg); mp = 162°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.32; IR υ_{max} (KBr)/cm^{-1} 3327 (OH and NH), 1749 (C=O), 1695 (C=O), 1591, 1499, 1337, 1286, 1169, 1112, 852, 754, 630; ^{1}H-NMR δ (d_6-DMSO) 11.42 (s, 1H, NH-d4T), 10.77 (s, 1H, NH-PAB), 8.19-6.98 (m, 15H, H-Bn and H-Ph and H-PAB and C*H*=C(OH) and H-6:d4T), 6.89 (s, 1H, H-1′:d4T), 6.47 (d, 1H, *J* 5.8 Hz, H-2′:d4T), 6.04 (d, 1H, *J* 5.8 Hz, H-3′:d4T), 5.30 (s, 2H, CH_2-Bn), 5.17 (s, 2H, $CH_2OC(=O)O$), 5.06 (br s, 1H, H-4′:d4T), 4.44 (dd, 1H, *J* 2.7 Hz, *J* 12.2 Hz, H-5′a:d4T), 4.34 (dd, 1H, *J* 2.7 Hz, *J* 12.2 Hz, H-5′b:d4T), 1.62 (s, 3H, CH_3); ^{13}C-NMR δ (d_6-DMSO) 185.3 (C-4), 177.9, 163.7 (C-4:d4T), 163.6, 162.9, 159.9, 154.1, 150.6 (C-2:d4T), 137.8, 136.2 (C-6:d4T), 135.8, 133.1 (C-2′:d4T), 131.0, 130.8, 129.9, 129.1, 128.4, 127.9, 127.6, 126.9 (C-3′:d4T), 126.0, 120.4, 120.1, 115.6, 115.2, 114.7, 109.4 (C-5:d4T), 93.6 (C-3), 88.7 (C-1′:d4T), 83.4 (C-4′:d4T), 69.5 ($CH_2OC(=O)O$), 68.9 (CH_2-Bn), 67.7 (C-5′:d4T), 11.7 (CH_3); Anal. Calcd. For C35H31N3O10: C, 64.31; H, 4.78; N, 6.43. Found: C, 64.25; H, 4.72; N, 6.35%.

*Heterodimer [4-(3,5-dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic]-OABC- C5′[d4T] (**14a**)*. Compound **14a** was prepared from precursor **11a** (226 mg, 0.44 mmol) by the same procedure as that described for compound **13a**; Yield: 49%, yellow crystals (165 mg); mp = 138°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.40; IR υ_{max} (KBr)/cm^{-1} 3365 (OH), 3262 (NH), 1733 (C=O), 1697(C=O), 1585, 1526, 1453, 1280, 1163, 1056, 936, 912; ^{1}H-NMR δ (d_6-DMSO) 9.80 (s, 1H, NH-d4T), 8.28 (s, 1H, NH-OAB), 8.06 (d, 1H, *J* 8.0 Hz, H-OAB), 7.46-7.34 (m, 16H, H-Bn and H-Ph and H-OAB), 7.25 (s, 1H, C*H*=C(OH), 7.06 (s, 1H, H-6:d4T), 6.84 (s, 1H, H-1′:d4T), 6.28 (d, 1H, *J* 6.0 Hz, H-2′:d4T), 5.86

(d, 1H, $\mathcal{J}$ 6.0 Hz, H-3′:d4T), 5.21 (m, 2H, $CH_2OC(=O)O$), 5.10 (s, 4H, CH_2-Bn), 5.00 (s, 1H, H-4′:d4T), 4.50 (br s, 1H, H-5′a:d4T), 4.3 (br s, 1H, H-5′b:d4T), 1.78 (s, 3H, CH_3); Anal. Calcd. For C42H37N3O11: C, 66.40; H, 4.91; N, 5.53. Found: C, 66.32; H, 4.84; N, 5.47%.

*Heterodimer [4-(3-benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic]-OABC-C5′[d4T] (**14b**)*. Compound **14b** was prepared from precursor **11b** (178 mg, 0.44 mmol) by the same procedure as that described for compound **13a**; Yield: 41%, yellow crystals (118 mg); mp = 147°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.48; IR υ_{max} (KBr)/cm^{-1} 3369 (OH), 3065 (NH), 1748 (C=O), 1694 (C=O), 1582, 1526, 1454, 1264, 1080, 1025, 757, 697; ^{1}H-NMR δ (d_6-DMSO) 11.39 (s, 1H, NH-d4T), 10.45 (s, 1H, NH-OAB), 7.67-7.28 (m, 14H, H-Bn and H-Ph and H-OAB and C*H* = C(OH), 7.24 (s, 1H, H-6:d4T), 6.87 (s, 1H, H-1′:d4T), 6.44 (d, 1H, $\mathcal{J}$ 5.6 Hz, H-2′:d4T), 6.04 (d, 1H, $\mathcal{J}$ 5.6 Hz, H-3′:d4T), 5.27 (s, 2H, CH_2-Bn), 5.22 (s, 2H, $CH_2OC(=O)O$), 5.04 (s, 1H, H-4′:d4T),), 4.43 (d, 1H, $\mathcal{J}$ 12.2 Hz, H-5′a:d4T), 4.33 (d, 1H, $\mathcal{J}$ 12.2 Hz, H-5′b:d4T), 1.60 (s, 3H, CH_3); Anal. Calcd. For C35H31N3O10: C, 64.31; H, 4.78; N, 6.43. Found: C, 64.27; H, 4.70; N, 6.37%.

*Heterodimer [4-(4-benzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic]-OABC-C5′[d4T] (**14c**)*. Compound **14c** was prepared from precursor **11c** (88 mg, 0.22 mmol) by the same procedure as that described for compound **13a**; Yield: 42%, yellow crystals (60 mg); mp = 17°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.39; IR υ_{max} (KBr)/cm^1 3373 (OH), 3035 (NH), 1754 (C=O), 1694 (C=O), 1454, 1252, 1174, 1068, 775, 759; ^{1}H-NMR δ (d_6-DMSO) 9.78 (s, 1H, NH-d4T), 8.38 (s, 1H, NH-OAB), 8.02 (d, 2H, $\mathcal{J}$ 9.0 Hz, H-Ph), 7.46-7.18 (m, 11H, H-Bn and H-OAB and C*H*=C(OH) and H-6:d4T), 7.07 (d, 2H, $\mathcal{J}$ 9.0 Hz, H-Ph), 7.03 (s, 1H, H-1′:d4T), 6.28 (d, 1H, $\mathcal{J}$ 5.6 Hz, H-2′:d4T), 5.87 (d, 1H, $\mathcal{J}$ 5.6 Hz, H-3′:d4T), 5.30 (s, 2H, $CH_2OC(=O)O$), 5.16 (s, 2H, CH_2-Bn), 5.04 (br s, 1H, H-4′:d4T),), 4.50 (dd, 1H, $\mathcal{J}$ 2.7 Hz $\mathcal{J}$ 12.2 Hz, H-5′a:d4T), 4.38 (dd, 1H, $\mathcal{J}$ 2.7 Hz $\mathcal{J}$ 12.2 Hz, H-5′b:d4T), 1.78 (s, 3H, CH_3); Anal. Calcd. For C35H31N3O10: C, 64.31; H, 4.78; N, 6.43. Found: C, 64.25; H, 4.71; N, 6.38%.

*4-[1-(4-Fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic-PAB-OH (**15a**)*. Compound **15a** was prepared from precursor **8a** (300 mg, 1.04 mmol) by the same procedure as that described for compound **10a**; Yield: 24%, yellow crystals (100 mg); mp = 170°C; TLC R_f (chloroform: methanol = 95: 5) 0.32; IR υ_{max} (KBr)/cm^{-1} 3405 (OH and NH), 1695 (C=O), 1639 (C=O), 1591, 1525, 1414, 1326, 1242, 1222, 1086, 1065, 824; ^{1}H-NMR δ (d_6-DMSO) 10.35 (s, 1H, NH), 7.71 (d, 2H, $\mathcal{J}$ 8.4 Hz, H-PAB), 7.55 (s, 1H, H-5:pyrrole), 7.45 (s, 1H, H-3:pyrrole), 7.27 (d, 2H, $\mathcal{J}$ 8.4 Hz, H-PAB), 7.15-7.06 (m, 4H, H-Bn), 6.90 (s, 1H, C*H*=C(OH)), 5.62 (s, 2H, CH_2-Bn), 4.44 (s, 2H, CH_2-PAB); ^{13}C-NMR δ (d_6-DMSO) 184.7 (C-4), 167.1 (C-1), 160.6 (d, $^1\mathcal{J}_{C\text{-}F}$ 202.2 Hz, C-4:Bn), 160.4 (C-2), 139.6, 137.2, 135.5, 133.5, 130.2, 129.2, 128.5, 127.7, 126.7, 126.6, 125.6, 122.9, 121.3, 120.9, 116.0 (d, $^2\mathcal{J}_{C\text{-}F}$ 20.7 Hz, C-3:Bn), 111.2 (C-4:pyrrole), 98.6 (*C*H=C(OH)), 63.4 (CH_2-Bn), 47.4 (CH_2-PAB).

*4-[1-(2-Fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic-PAB-OH (**15b**)*. Compound **15b** was prepared from precursor **8b** (300 mg, 1.04 mmol) by the same procedure as that described for compound **10a**; Yield: 24%, yellow crystals (100 mg); mp = 140°C; TLC R_f (chloroform: methanol = 95: 5) 0.33; IR υ_{max} (KBr)/cm^{-1} 3390 (OH and NH); 1690 (C=O), 1628 (C=O), 1592,1526, 1412, 1327, 1230, 1086, 1368, 1009, 754,677; ^{1}H-NMR δ (d_6-DMSO) 10.48 (s, 1H, NH), 7.71 (d, J 8.5 Hz, 2H, H-PAB), 7.47-7.04 (m, 8H, H-5 and H-3:pyrrole and H-Bn and H-PAB), 6.91 (s, 1H, C*H*=CHOH), 6.64 (t, 1H, $\mathcal{J}$ 7.8 Hz, H-6:Bn), 6.37 (dd, 1H, $\mathcal{J}$ 3.6 Hz $\mathcal{J}$ 2.9 Hz, H-4:pyrrole), 5.72 (s, 2H, CH_2-Bn), 4.45 (s, 2H, CH_2-PAB).

*4-[1-(4-Fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic-OAB-OH (**16a**)*. Compound **16a** was prepared from precursor **8a** (500 mg, 1.73 mmol) by the same procedure as that described for compound **10a**, except that the reaction mixture was added with 2-aminobenzyl alcohol (1.2 equiv.) instead of 4-aminobenzyl alcohol; Yield: 34%, yellow powder (230 mg); mp = 190°C; TLC R_f (chloroform: methanol = 95: 5) 0.69; IR υ_{max} (KBr)/cm^{-1} 3390 (OH + NH), 1690 (C=O), 1628 (C=O), 1592, 1526, 1412, 1327, 1230, 1086, 1368, 1009, 754, 677; ^{1}H-NMR δ (d_6-DMSO) 10.57 (s, 1H, NH), 7.99 (d, 2H, $\mathcal{J}$ 7.4 Hz, H-PAB), 7.57 (s, 1H, H-5:pyrrole), 7.46 (s, 1H, H-3:pyrrole), 7.99 (d, 2H, $\mathcal{J}$ 7.4 Hz, H-PAB), 7.16-7.14 (m, 4H, H-Bn), 6.95 (s, 1H, C*H*=C(OH)), 6.34 (s, 1H, H-4:pyrrole); 5.62 (s, 2H, CH_2-Bn), 4.58 (s, 2H, CH_2-PAB); ^{13}C-NMR δ (d_6-DMSO) 183.2 (C-4), 165.5 (C-1), 160.9 (d, $^1\mathcal{J}_{C\text{-}F}$ 244.5 Hz, C-2:Bn), 159.1 (C-2), 136.0, 134.7, 134.5, 132.3, 128.9, 128.8, 128.0, 127.7, 124.8, 122.2 (C-3:pyrrole), 115.1 (d, $^2\mathcal{J}_{C\text{-}F}$ 21.5 Hz, C-3:Bn), 110.4 (C-4:pyrrole), 97.7 (C-3), 61.9 (CH_2-Bn), 51.4 (CH_2-PAB).

*4-[1-(2-Fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic-OAB-OH (**16b**)*. Compound **16b** was prepared from precursor **8b** (579 mg, 2.00 mmol) by the same procedure as that described for compound **10a**, except that the reaction mixture was added with 2-aminobenzyl alcohol (1.2 equiv.) instead of 4-aminobenzyl alcohol; Yield: 40%, yellow powder (316 mg); mp = 172°C; TLC R_f (chloroform: methanol = 95: 5) 0.52; IR υ_{max} (KBr)/cm^{-1} 3298 (OH and NH),1678 (C=O),1633, 1591, 1537, 1454, 1325, 1244, 1230, 1084, 1064, 1010, 754; ^{1}H-NMR δ (d_6-DMSO) 10.53 (s, 1H, NH), 7.99-7.03 (m, 10H, H-5:pyrrole and H-3:pyrrole and H-Bn and H-OAB), 6.94 (s, 1H, C*H*=CHOH), 6.36 (s, 1H, H-4:pyrrole), 5.72 (s, 2H, CH_2-Bn), 4.57 (s, 2H, CH_2-OAB); ^{13}C-NMR δ (d_6-DMSO) 183.6 (C-4), 165.7 (C-1), 159.2 (d, $^1\mathcal{J}_{C\text{-}F}$ 217.9 Hz, C-2:Bn), 158.2 (C-2), 157.8, 135.9, 135.5, 134.5, 132.3, 129.3; 129.2, 128.8, 128.0, 127.8, 127.6, 126.5, 125.6,124.7, 124.6, 124.5, 124.4, 122.0, 121.5, 115.0 (d, $\mathcal{J}_{CIII\text{-}F}$ 20.6 Hz, C_{III}-OFP), 114.8, 113.8, 111.7, 110.2 (C_3-pyrrole), 109.1 (C-4:pyrrole), 97.3 (C-3), 61.8 (CH_2-OAB), 46.4 (CH_2-Bn).

*Heterodimer [4-[1-(4-fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic]-PABC-C5′[d4T] (**17a**)*. Compound **17a** was prepared from precursor **15a** (198 mg, 0.50 mmol) by the same procedure as that described for compound **13a**; Yield: 33%, yellow crystals (107 mg); mp = 142°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.38; IR υ_{max} (KBr)/cm^{-1} 3310 (OH), 3075 (NH), 1750 (C=O), 1693 (C=O), 1525, 1266, 1241, 1085, 1068, 750, 675; ^{1}H-NMR δ (d_6-DMSO) 11.29 (s, 1H, NH-d4T), 10.41 (s, 1H, NH-PAB), 7.78 (d, 2H, $\mathcal{J}$ 8.3 Hz, H-PAB), 7.52 (s, 1H, H-5:pyrrole), 7.43 (s, 1H, H-3:pyrrole), 7.35 (d, 2H, $\mathcal{J}$ 8. 3 Hz, H-PAB), 7.26 s, 1H, H-6:d4T), 7.16-7.05 (m, 4H, H-PFP), 6.91 (s, 1H, C*H* = C(OH)), 6.81 (s, 1H, H-1′:d4T), 6.39 (d, 1H, $\mathcal{J}$ 5.4 Hz, H-2′:d4T), 6.34 (s, 1H, H-4:pyrrole), 5.96 (d, 1H, $\mathcal{J}$ 5.4 Hz, H-3′:d4T), 5.63 (s, 2H, CH_2-PFP), 5.50 (s, 1H, CH=C(O*H*)), 5.10 (s, 2H, $CH_2OC(=O)O$), 4.99 (s, 1H, H-4′:d4T), 4.37 (d, 1H, $\mathcal{J}$ 11.7 Hz, H-5′a:d4T), 4.27 (d, 1H, $\mathcal{J}$ 11.7 Hz, H-5′b:d4T), 1.56 (s, 3H, CH_3); ^{13}C-NMR δ (d_6-DMSO) 183.8 (C-4), 183.3 (C-1), 164.9 (d, $^1\mathcal{J}_{C\text{-}F}$ 237.8 Hz, C-IV:PFP), 159.8, 154.2, 150.7 (C-2), 138.0, 135.9 (C-6:d4T), 134.3 (C-5:pyrrole), 133.2 (C-2′:d4T), 131.1, 127.0 (C-3′:d4T), 129.1 (C-2 and C-6:PAB), 128.7(d, $^3\mathcal{J}_{C\text{-}F}$ 8.3 Hz, C-II and C-VI:PFP), 127.0 (C-3pyrrole), 120.6 (C-3 and C-5:PAB), 115.2(d, $^2\mathcal{J}_{C\text{-}F}$ 21.5 Hz, C-III and C-V PFP), 110.3 (C-4:pyrrole), 97.8 (*C*H=C(OH)), 88.9 (C-1′:d4T), 83.5 (C-4′:d4T), 69.0 (*C*$H_2OC(=O)O$), 67.8 (C-5′:d4T), 51.3 (CH_2-PFP), 11.8 (CH_3); Anal. Calcd. For C33H29FN4O9: C, 61.49; H, 4.53; N, 8.69. Found: C, 61.42; H, 4.49; N, 8.64%.

*Heterodimer [4-[1-(2-fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic]-PABC-C5′[d4T] (**17b**)*. Compound **17b** was prepared from precursor **15b** (130 mg, 0.33 mmol) by the same procedure as that described for compound **13a**; Yield: 53%, yellow crystals (113 mg); mp = 140°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.43; IR υ_{max} (KBr)/cm^{-1} 3298 (OH), 3070 (NH),1749 (C=O),1693 (C=O), 1525, 1414, 1266, 1085, 1068, 755, 675; ^{1}H-NMR δ (d_6-DMSO) 11.31 (s, 1H, NH-d4T), 10.44 (s, 1H, NH-PAB), 7.77 (d, 2H, $\mathcal{J}$ 8.5 Hz, H-PAB), 7.45 (s, 1H, H-5:pyrrole), 7.35 (d, 2H, $\mathcal{J}$ 8.5 Hz, H-PAB), 7.33-7.10 (m, 8H, H-OFP and H-3:pyrrole and H-PAB and H-6:d4T), 6.91 (s, 1H, C*H*=C(OH)), 6.81 (s, 1H, H-1′:d4T), 6.37 (br s, 2H, H-2′:d4T and H-4:pyrrole), 5.97 (br s, 3H, H-3′:d4T and CH_2-OFP), 5.09 (s, 2H, $CH_2OC(=O)O$), 4.99 (s, 1H, H-4′:d4T), 4.37 (d, 1H, $\mathcal{J}$ 12.2 Hz, H-5′a:d4T), 4.27 (d, 1H, $\mathcal{J}$ 12.2 Hz, H-5′b:d4T), 1.54 (s, 3H, CH_3); ^{13}C-NMR δ (d_6-DMSO) 183.3 (C-4), 166.1, 163.7, 154.2, 150.7, 135.9 (C-6:d4T), 133.2, 131.1 (C-2′:d4T), 129.1 (C-2 and C-6:PAB), 127.7 (C-3′:d4T), 127.0 (C-3:pyrrole), 124.6, 122.0, 120.6 (C-3 and C-5:PAB), 120.2, 115.2, 115.0, 110.3 (C-4:pyrrole), 109.5, 97.8 (*C*H=C(OH)), 88.9 (C-1′:d4T), 83.5 (C-4′:d4T), 69.0 (*C*$H_2OC(=O)O$), 67.8 (C-5′:d4T), 30.6 (CH_2-OFP), 11.8 (CH_3); Anal. Calcd. For C33H29FN4O9: C, 61.49; H, 4.53; N, 8.69. Found: C, 61.44; H, 4.47; N, 8.62%.

*Heterodimer [4-[1-(4-fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic]-OABC-C5′[d4T] (**18a**)*. Compound **18a** was prepared from precursor **16a** (195 mg, 0.49 mmol) by the same procedure as that described for compound **13a**; Yield: 22%, yellow crystals (70 mg); mp = 145°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.54; IR υ_{max} (KBr)/cm^{-1} 3384(OH), 3044 (NH), 1753 (C=O), 1693 (C=O), 1510, 1267, 1241, 1084, 1068, 756, 682; ^{1}H-NMR δ (d_6-DMSO) 11.32 (s, 1H, NH-d4T), 10.19 (s, 1H, NH-OAB), 7.55-7.07 (m, 11H, H-PFP and H-5:pyrrole and H-3:pyrrole and H-POB and H-6:d4T), 6.90 (s, 1H, C*H*=C(OH)), 6.80 (s, 1H, H-1′:d4T), 6.34 (br s, 2H, H-4:pyrrole and H-2′:d4T), 5.94 (s, 1H, H-3′:d4T), 5.62 (s, 2H, CH_2-PFP), 5.18 (m, 2H, $CH_2OC(=O)O$), 4.06 (s, 1H, H-4′:d4T), 4.35 (d, 1H, $\mathcal{J}$ 11.9 Hz, H-5′a:d4T), 4.30 (d, 1H, $\mathcal{J}$ 11.9 Hz, H-5′b:d4T), 1.52 (s, 3H, CH_3); ^{13}C-NMR δ (d_6-DMSO) 183.3 (C-4), 166.4, 163.7, 160.1, 154.2, 150.7, 135.9 (C-5:pyrrole), 134.9, 134.4, 129.9, 129.6, 129.4, 129.0, 128.96, 128.9, 128.8, 128.7, 128.0, 127.0 (C-3:pyrrole), 126.4, 125.9, 122.2, 115.4, 115.1, 110.4 (C-4:pyrrole), 109.6, 97.9 (C-6:d4T), 88.9 (C-1′:d4T), 83.5 (C-4′:d4T), 68.0 (*C*$H_2OC(=O)O$), 66.0 (C-5′:d4T), 51.4 (CH_2-PFP), 11.7 (CH_3); Anal. Calcd. For C33H29FN4O9: C,

61.49; H, 4.53; N, 8.69. Found: C, 61.43; H, 4.49; N, 8.62%.

*Heterodimer [4-[1-(2-fluorobenzyl)-1H-pyrrol-2-yl]-2-hydroxy-4-oxobut-2-enoic]-OABC-C5′[d4T] (**18b**).* Compound **18b** was prepared from precursor **16b** (196 mg, 0.50 mmol) by the same procedure as that described for compound **13a**; Yield: 19%, yellow crystals (61 mg); mp = 135°C; TLC R_f (ethyl acetate: n-hexane = 80: 20) 0.46; IR υ_{max} (KBr)/cm^{-1} 3492 (OH), 3292 (NH), 1747 (C═O), 1695 (C═O), 1590, 1526, 1457, 1411, 1263, 1244, 1086, 756; ^{1}H-NMR δ (d_6-DMSO) 11.31 (s, 1H, NH-d4T), 10.17 (s, 1H, NH-OAB), 7.49-7.10 (m, 11H, H-OFP and H-5:pyrrole and H-3:pyrrole and H-OAB and H-6:d4T), 6.90 (s, 1H, C*H*═C(OH)), 6.80 (s, 1H, H-1′:d4T), 6.37 (s, 1H, H-4:pyrrole), 6.33 (d, 1H, *J* 5.8 Hz, H-2′:d4T), 5.93 (d, 1H, *J* 5.8 Hz, H-3′:d4T), 5.72 (s, 2H, CH_2-OFP), 5.15 (m, 2H, CH_2OC(═O)O), 4.96 (s, 1H, H-4′:d4T), 4.34 (d, 1H, *J* 10.7 Hz, H-5′a:d4T), 4.25 (d, 1H, *J* 10.7 Hz, H-5′b:d4T), 1.52 (s, 3H, CH_3); Anal. Calcd. For C33H29FN4O9: C, 61.49; H, 4.53; N, 8.69. Found: C, 61.40; H, 4.47; N, 8.62%.

Antiviral Test Procedures

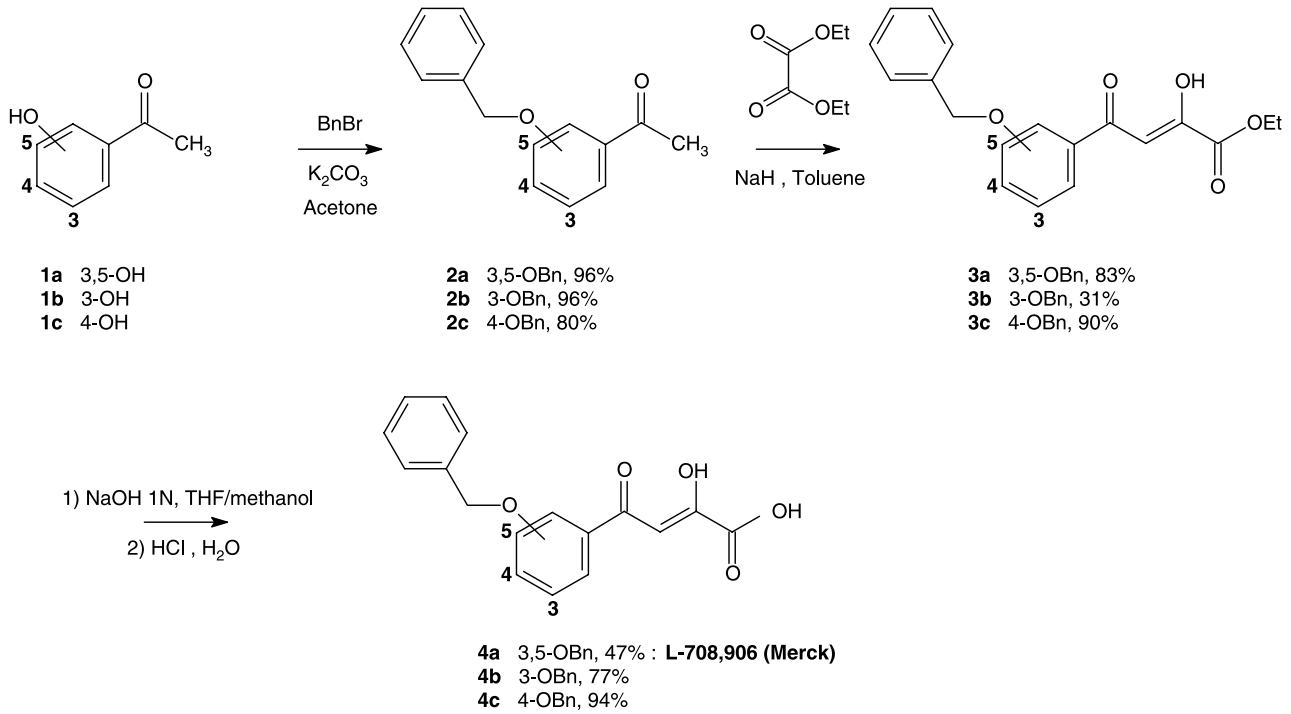
The cultures of CEM-SS and MT4 cells were maintained at 37°C in a 5% CO_2 atmosphere in RPMI-1640 medium supplemented with 10% complement-depleted foetal bovine serum (FBS). The antiviral HIV-1 activity of a given compound in CEM-SS cells was measured by quantification of the Reverse Transcriptase activity (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% calf foetal 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 [50]. The 50% inhibitory concentration (IC_{50}) was derived from the computer generated median effect plot of the dose-effect data [program: therapeutic and safety indexes, 51]. 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) [52]. 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 [50,53]; 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 [50]. 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 target prodrugs of the general formula [INI]-PABC-[d4T] (**13a-c**, **17a-b**) and [INI]-OABC-[d4T] (**14a-c**, **18a-b**) described in the present paper were prepared by attaching the nucleoside inhibitor moiety to the spacer end of the IN inhibitor-spacer moieties. The synthetic method for these target compounds involved the formation of the key precursors: the NRTI (d4T) and the INIs (DKAs). The synthesis of the d4T was achieved according to a method previously reported in the literature [54]. For the INIs, we have developed the synthesis of the α,γ-diketo acids **4a-c** and **8a-b** respectively as outlined in Scheme 1 and 2. The synthesis of the 4-(3,5-dibenzyloxyphenyl)-2-hydroxy-4-oxobut-2-enoic acid (**4a**) was described in a preceding paper [34]. Since the DKAs **4b** and **4c** have not been previously reported, we developed the synthesis of these compounds using a synthetic approach outlined in a preliminary account by oxalylation of the corresponding aryl methyl ketones in the presence of base followed by alkaline hydrolysis (Scheme 1) [34]. Thus, the DKAs **4b** and **4c** were obtained in three steps starting respectively from commercially available 3-hydroxyacetophenone (**1b**) and 4-hydroxyacetophenone (**1c**). In the first step, the acetophenone was subjected to a benzylation reaction using benzyl bromide in acetone in the presence of anhydrous sodium carbonate. The resulting aryl methyl ketones **2b-c** were then converted to the corresponding ketoenol esters **3b-c** using diethyl oxalate in the presence of NaH (in toluene) respectively in 31% and 90% yields. In the last step, hydrolysis of the ester intermediates **3b-c** provided the corresponding ketoenol acids **4b-c**, isolated respectively in 77% and 94% yields.

For comparative studies, the DKAs **8a-b** were prepared by a similar synthetic approach as indicated in Scheme 2. Firstly, 2-acetylpyrrole (**5**) was alkylated with the appropriate benzyl bromide in alkaline medium (sodium hydride), obtaining the N-alkylated derivatives **6a–b** that underwent condensation with diethyl oxalate in the presence of sodium hydride.



1a 3,5-OH
1b 3-OH
1c 4-OH

2a 3,5-OBn, 96%
2b 3-OBn, 96%
2c 4-OBn, 80%

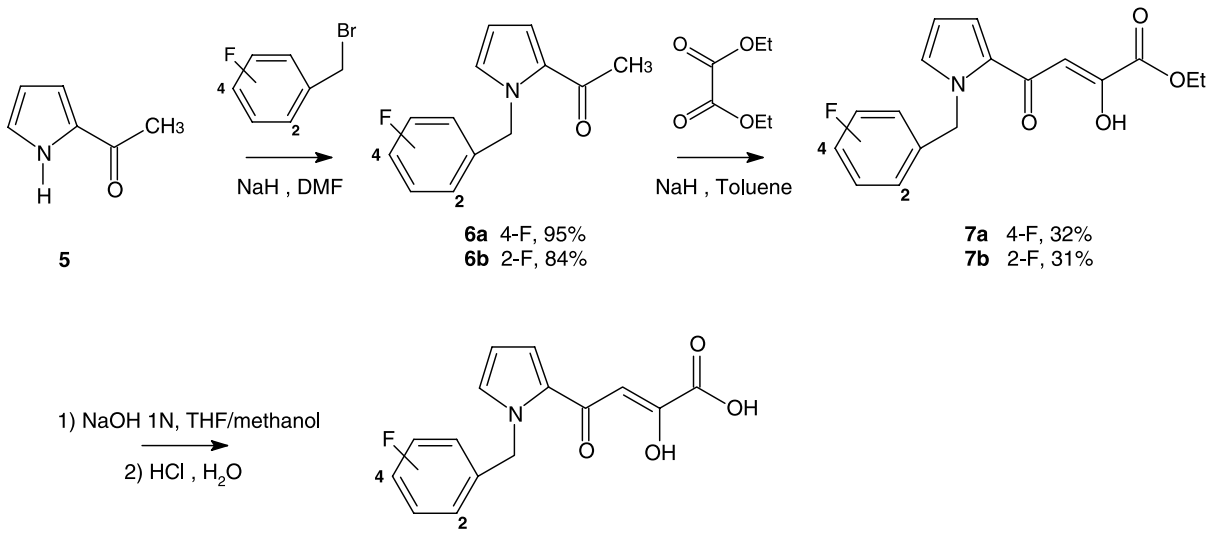
3a 3,5-OBn, 83%
3b 3-OBn, 31%
3c 4-OBn, 90%

4a 3,5-OBn, 47% : **L-708,906 (Merck)**
4b 3-OBn, 77%
4c 4-OBn, 94%

Scheme 1. Preparation of ketoenol acids **4a–c**.

Then, the ketoenol esters **7a–b** that formed were easily hydrolyzed in 1N sodium hydroxide to afford the required α,γ-diketo acids **8a–b** respectively in 20% and 22% overall yields.

The second part was devoted to the insertion of a *p*- or *o*-aminobenzyl alcohol (PAB-OH and OAB-OH) spacer between the α,γ-diketoacids **4a–c** and **8a–b** and the NRTI. Thus, the selective coupling reactions of **4a–c** with PAB-OH or OAB-OH were carried out in anhydrous THF under an argon atmosphere in the presence of EDDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) inducing the formation of peptide linkage and the expected products **9a–c** and **10a–c** were isolated (60 to 78%) (Scheme 3) [55]. Following this, the linker of compounds **9a–c** was activated toward coupling with d4T as the corresponding *p*-nitrophenyl carbonate derivatives **11a–c**. The benzylic alcohols **9a–c** were then converted subsequently into the corresponding activated analogues **11a–c** by reacting them with bis-(4-nitrophenyl)



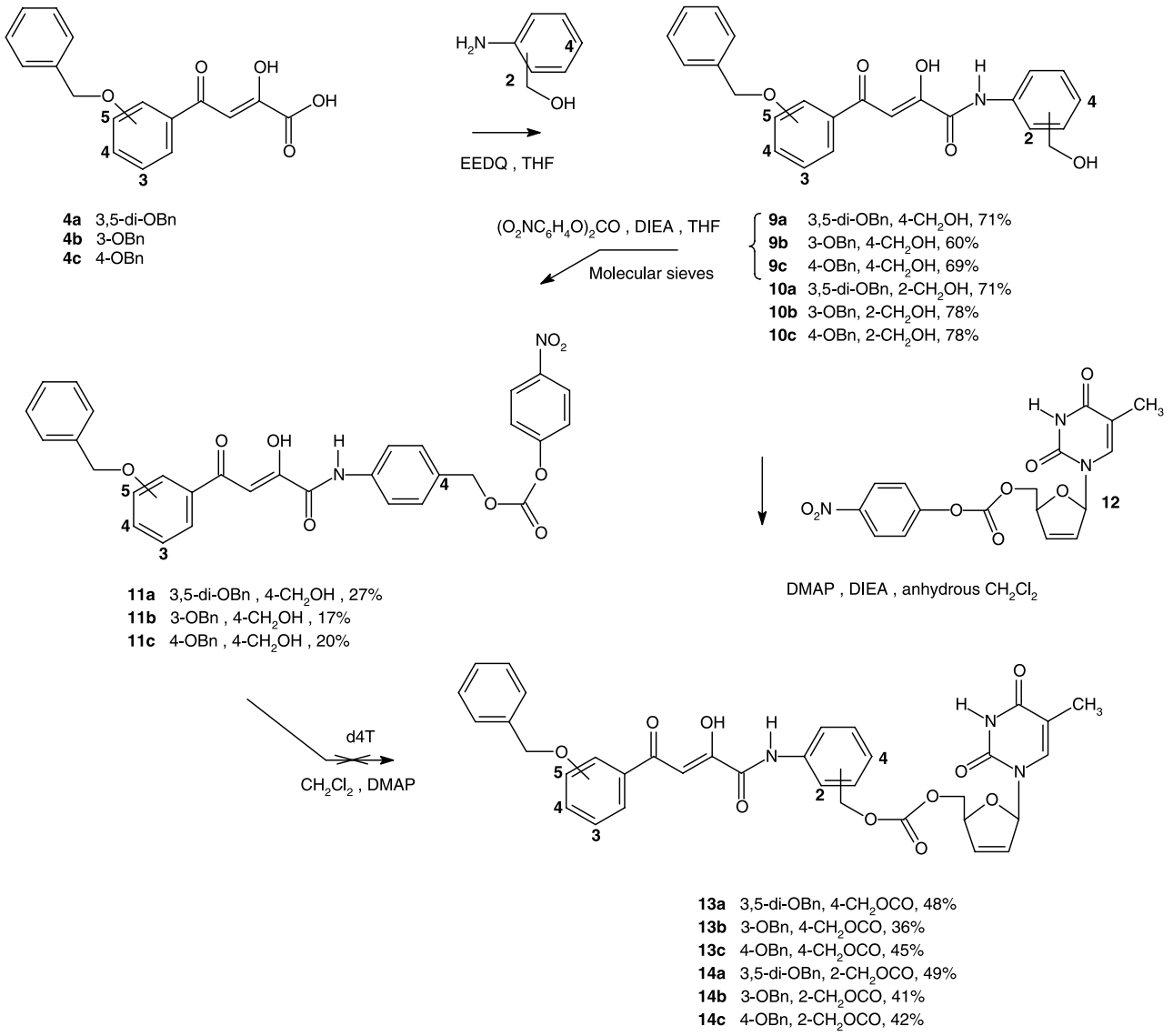
5

6a 4-F, 95%
6b 2-F, 84%

7a 4-F, 32%
7b 2-F, 31%

8a 4-F, 67% : **L-731,988 (Merck)**
8b 2-F, 96%

Scheme 2. Preparation of ketoenol acids **8a–b**.

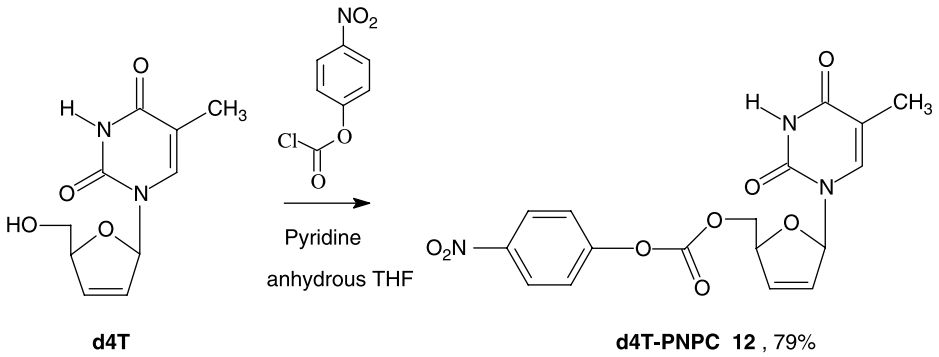


Scheme 3. Synthesis of [INI]-PABC-[d4T] (**13a–c**) and [INI]-OABC-[d4T] (**14a–c**) conjugates.

carbonate in anhydrous THF in the presence of *N,N*-diisopropylamine and were isolated in low yields (17 to 27%) after purification by silica-gel chromatography [56]. In a final step, reaction of 5′-hydroxyl of d4T with these activated carbonate derivatives **11a–c** in the presence of dimethylaminopyridine (DMAP) led unfortunately to a mixture consisting mainly of the unreacted products **11a–c**.

These data prompted us to devise a different strategy of linking the drugs, as depicted in Scheme 3. In an alternative approach, the activated carbonate d4T **12** is linked directly to the DKAs functionalized with respectively the *p*- and *o*-aminobenzyl alcohol spacers **9a–c** and **10a–c**. Thus, when d4T was treated with 1.5 equiv. of 4-nitrophenyl chloroformate in the presence of pyridine, the desired activated d4T-carbonate **12** was formed in a reasonable yield (79%) (Scheme 4) [57]. The final coupling step was performed by addition of activated d4T-carbonate **12** to a solution of the intermediates **9a–c** and **10a–c** in anhydrous dichloromethane in the presence of DMAP to yield the expected target prodrugs **13a–c** and **14a–c** (36 to 49%) (Scheme 3).

Finally, for comparative studies, we focused on modifications on the INI in the target heterodimers with the introduction of the L-731,988 compound based on our previous biological results [34]. The synthesis of the prodrugs **17a–b** and **18a–b** was carried out in a similar way starting from the corresponding DKAs **8a–b** as shown in Scheme 5. Reaction of DKAs **8a–b** with commercially available PAB-OH and OAB-OH in the presence of EEDQ yielded the intermediates **15a–b** (24%) and **16a–b** (34 and 40%). Treatment of these intermediates with activated d4T-carbonate **12** gave the corresponding heterodimers **17a–b** and **18a–b** (19 to 53%).



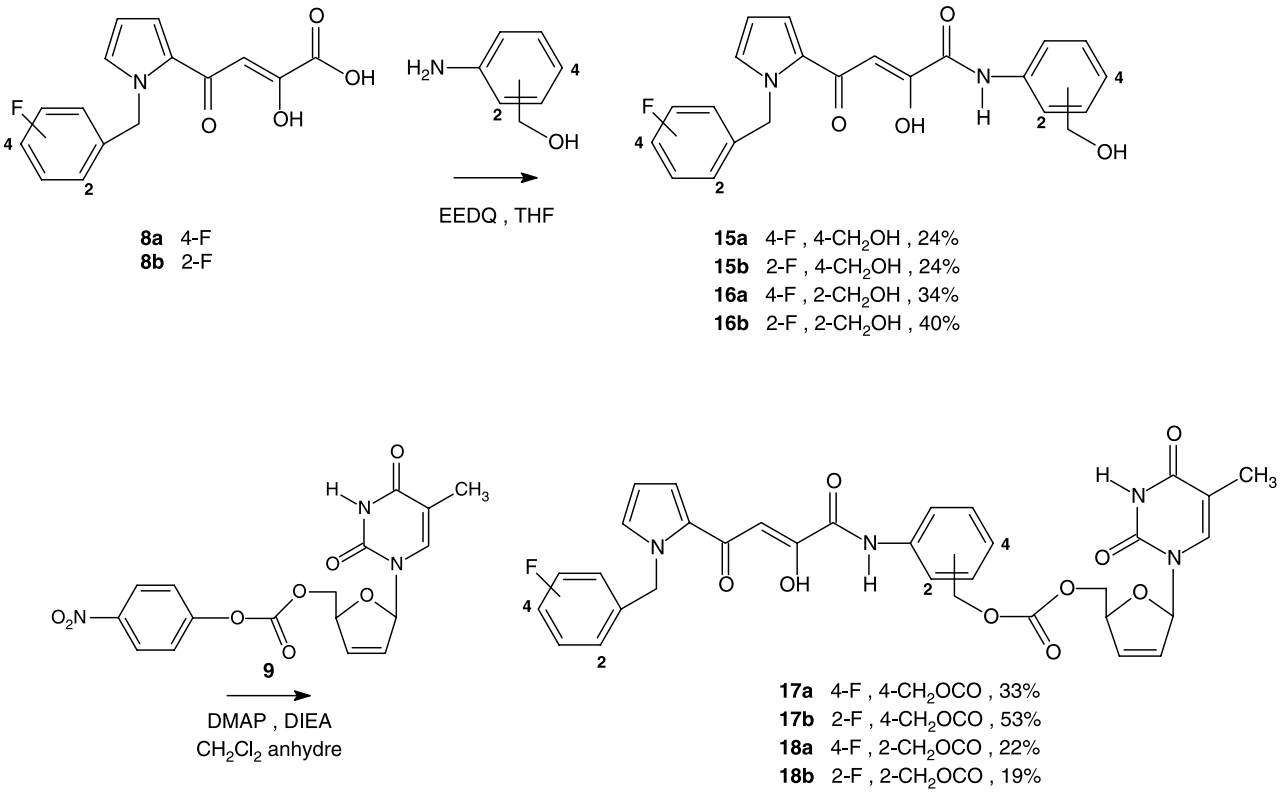
Scheme 4. Preparation of the 5′-PNPC-d4T **12**.

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

Biological

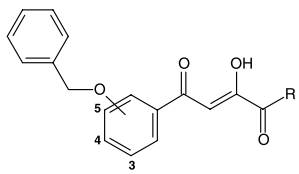
The synthesized target bis-substrate molecules [INI]-PABC-[d4T] (**13a–c**, **17a–b**) and [INI]-OABC-[d4T] (**14a–c**, **18a–b**) were evaluated *in vitro* for their ability to inhibit replication of HIV-1 in two human T-4 lymphoblastoid cell lines (CEM-SS and MT-4) and HIV-2 in PBMC cells (Table I). The clinically used nucleoside analogues AZT and d4T were included as reference materials. For the purpose of reference, data for parental DKAs (**4a–c**, **8a–b**) and the d4T analogue precursor (**12**, d4T-PNPC) were also displayed. We also included corresponding data for the [INI]-PAB-OH (**9a–c**, **15a–b**) and [INI]-OAB-OH (**10a–c**, **16a–b**) precursors.

Central to the idea of constructing bis-substrate inhibitors connected *via* an amide and a carbonate functionalities was to determine to what extent the individual NRTI and INI components in each molecule retain their capacity to inhibit HIV-1 replication once the spacers were introduced. As the data displayed in Table I, all active compounds proved to be more potent in the CEM model system also in order to rationalize the analysis, we concentrated on these results. Relative to d4T (IC_{50} 0.273 μM) and parental DKAs (**4a:** IC_{50} 0.58 μM and **8a:** IC_{50} 0.44

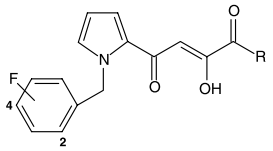


Scheme 5. Synthesis of [INI]-PABC-[d4T] (**17a–b**) and [INI]-OABC-[d4T] (**18a–b**) conjugates.

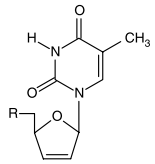
Table I. Antiviral and cytotoxicity evaluation of the DKAs, the modified DKAs, the d4T-PNPC, and the conjugates [INI]-spacer-[d4T] against selected HIV strains.



4a-c , 9a-c, 10a-c, 13a-c, 14a-c



8a-b, 15a-b, 16a-b, 17a-b, 18a-b



d4T , 12

			CEM-SS		M4	MT-4		PBMC	
			HIV LAI			HIV-1 IIIB		HIV-2 D194	
Compd		R	IC_{50} (μM)*	CC_{50} (μM)†		IC_{50} (μM)	CC_{50} (μM)	IC_{50} (μM)	CC_{50} (μM)
4a	3,5-di-OBn	OH	0.58 ± 0.15	> 10		> 10	> 10		
4b	3-OBn	OH	1.49 ± 0.71	> 100		20 ± 7	> 100		
4c	4-OBn	OH	> 100	> 100		> 100	> 100		
8a	4-F	OH	0.44 ± 0.33	> 100		4.3 ± 1.4	> 100		
8b	2-F	OH	18.5 ± 4.5	100		> CC50	92 ± 4		
9a	3,5-di-OBn	NH-PAB-OH	> 10	> 10		> 10	> 10		
9b	3-OBn	NH-PAB-OH	> 10	> 10		> CC50	8.5 ± 1.5		
9c	4-OBn	NHPAB-OH	39 ± 21	44.5 ± 18.5		> CC50	85 ± 8		
10a	3,5-di-OBn	NH-OAB-OH	2.35 ± 0.65	5.1 ± 1.3		> CC50	6.7 ± 1.3	4.65 ± 0.05	5.9 ± 0.8
10b	3-OBn	NH-OAB-OH	> 10	> 10		> 10	> 10	> 10	> 10
10c	4-OBn	NH-OAB-OH	> 10	> 10		> 10	> 10	> 10	> 10
12		PNPC	0.45 ± 0.33	> 100		0.57 ± 0.13	> 100	4.75 ± 2.05	55 ± 3
13a	3,5-di-OBn	NH-PABC-d4T	0.43 ± 0.21	5.8 ± 1.6		> CC50	3.47 ± 0.93	> CC50	5.2 ± 1
13b	3-OBn	NH-PABC-d4T	1.60 ± 0.99	> 10		4.65 ± 1.25	> 10	> 10	> 10
13c	4-OBn	NH-PABC-d4T	0.61 ± 0.58	> 10		1.85 ± 0.75	> 10	6.65 ± 4.35	> 10
14a	3,5-di-OBn	NH-OABC-d4T	1.47 ± 0.27	3.4 ± 0.2		> CC50	2.5 ± 0.8	1.25 ± 0.05	2.8 ± 0.3
14b	3-OBn	NH-OABC-d4T	4.67 ± 2.67	7.83 ± 1.43		> 10	> 10	> CC50	8.55 ± 0.45
14c	4-OBn	NH-OABC-d4T	1.85 ± 1.53	6.25 ± 1.75		> CC50	5.57 ± 1.17	2.23 ± 1.57	5.55 ± 0.75
15a	4-F	NH-PAB-OH	5.85 ± 0.55	> 10		> 10	> 10		
15b	2-F	NH-PAB-OH	> 10	> 10		> 10	> 10		
16a	4-F	NH-OAB-OH	2.65 ± 1.35	5.5 ± 1		> CC50	4.1 ± 1.0	1.55 ± 0.45	5.2 ± 1.1
16b	2-F	NH-OAB-OH	1.85 ± 0.15	> 10		> 10	> 10	5.5 ± 2.5	> 10
17a	4-F	NH-PABC-d4T	0.41 ± 0.06	5.5 ± 1.5		> CC50	4.25 ± 0.65	2.3 ± 0.4	5 ± 1.1
17b	2-F	NH-PABC-d4T	0.82 ± 0.27	6.0 ± 1.5		> CC50	5 ± 1.5	5.15 ± 0.75	6.55 ± 0.25
18a	4-F	NH-OABC-d4T	0.97 ± 0.13	5.75 ± 1.25		> CC50	4.85 ± 0.95	3.85 ± 0.35	5.3 ± 1.1
18b	2-F	NH-OABC-d4T	> 10	> 10		> 10	> 10	6.85 ± 3.15	> 10
d4T		OH	0.273 ± 0.143	> 100		0.541 ± 0.241	34.83 ± 20.83	0.331 ± 0.258	42 ± 6
AZT			0.0045 ± 0.0040			0.0148 ± 0.0077		0.13	

All data represent the mean values of three separate experiments (± SD). *IC_{50} is the concentration required to inhibit HIV-1 multiplication by 50%. †CC_{50} is the concentration drug which causes 50% cytotoxicity to uninfected cells.

μM), the corresponding heterodimers **13a, 17a** and **18a** were also active at submicromolar concentrations (IC_{50} 0.41 to 0.97 μM); the heterodimer **14a** only exhibited a weak activity (IC_{50} 1.47 μM) close to the toxicity (CC_{50} 3.4 μM). Relative to the parental DKA **4b** (IC_{50} 1.49 μM), the heterodimer **13b** had a similar activity (IC_{50} 1.60 μM) whereas **14b** displayed a poor or no specific activity in the CEM-SS cells (IC_{50} 4.67 μM, CC_{50} 7.83 μM). The results for the heterodimers **13c** (IC_{50} 0.61 μM) and **14c** (IC_{50} 1.85 μM) were remarkable since the parental DKA **4c** ($IC_{50} > 100$ μM) exhibited no significant anti-HIV activity, suggesting a contribution of d4T. Finally, relative to d4T (IC_{50} 0.273 μM) and parental DKA **8b** (IC_{50} 18.5 μM), the heterodimer **17b** was active at submicromolar concentrations (IC_{50} 0.82 μM) but **18b** exhibited no measurable anti-HIV activity ($IC_{50} > 10$ μM). Overall, all prodrugs showed a much higher cytotoxicity in CEM-SS cells in comparison with the corresponding free parent drugs.

Concerning the [INI]-spacer compounds, evaluation of the [INI]-PAB-OH (**9a–c**, **15a–b**) and [INI]-OAB-OH (**10a–c**, **16a–b**) precursors revealed that their antiviral activity dramatically decreased in comparison with the corresponding parent compounds or even was lost; this effect was accompanied, for some of the precursors, by an increase in cytotoxicity. The lack of antiviral activity for several precursors indicates that the parental DKA was not released neither extracellularly (the molecules are stable outside the cell) nor intracellularly, possibly in the latter case as a result of non internalization into the cell.

The d4T analogue precursor **12** (d4T-PNPC) was also evaluated against HIV-1 in CEM-SS cells and exhibited nearly equal antiviral activity (IC_{50} 0.45 μM) as d4T and low cytotoxicity (therapeutic index > 222). As all antiviral nucleoside drugs require metabolic activation in their target cells to the bioactive triphosphorylated forms by kinase-mediated phosphorylation for the expression of their antiviral activity [58] this result suggested the effective cleavage of the carbonate bond of compound **12**. The d4T-PNPC analogue (**12**) either would penetrate the cell membrane and then the d4T generated could, after phosphorylation, bind the RT target in the infected cell or the parent compound **12** might disintegrate outside the cell resulting in the release of d4T intermediate which may penetrate into the cell to inhibit the RT.

Looking next at the bis-substrate inhibitors, the conjugates [INI]-PABC-[d4T] (**13a,c** and **17a–b**) and [INI]-OABC-[d4T] (**18a**) retained antiviral activity but this effect was accompanied by an increased in cytotoxicity by comparison to d4T, DKA or even some precursors. At this stage, it is not known if the activity results from an incomplete liberation of both parental molecules. The prodrugs were more cytotoxic; this result led us to presume that this increase in toxicity may be due to the partial release of the [INI]-PAB-OH or [INI]-OAB-OH moieties with an uncleaved spacer. In exhaustive studies, it would have been interesting to compare the cytotoxicity of the spacers PAB-OH and OAB-OH alone. Possible interpretations for these results are that the conjugates would penetrate (according to our hypothesis) / or not the cell membrane and might disintegrate inside / or outside the cell, resulting in the release of [INI]-PAB-OH or [INI]-OAB-OH moieties and d4T, this latter could inhibit the RT target in infected cells. Consequently, the liberation of active d4T would be (apparently) due to the simple spontaneous hydrolysis of the carbonate bond connecting the spacer and d4T and the cytotoxicity of the conjugates might be probably due to the stability of [INI]-PAB-OH or [INI]-OAB-OH precursors in physiological conditions which prevents the disintegration to their parent INIs. These observations would lead us to presume that the amide bond connecting the INI and the spacer did not spontaneously hydrolyzed under physiological conditions and perhaps not released enough active INIs.

From this analysis, we cannot conclude that the nucleoside and the INI subunits of our molecules bound simultaneously with their respective sites so as to produce a synergistic effect as expected in our hypothesis. This latter study is however pertinent in view of this work is being pursed in further investigations with new hybrid-type prodrugs conjugating HIV INIs with d4T by self-cleavable spacers containing an amino acid residue.

In conclusion, based on the prodrug concept, novel bipharmacophore drugs associating in a single molecule a reverse transcriptase inhibitor covalently linked to an integrase inhibitor were developed. The antiviral efficacy of conjugated compounds depends on many factors, such as enzyme inhibition, cell membrane permeability, extracellular stability, intracellular disintegration and the correlation between them is very complex.

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