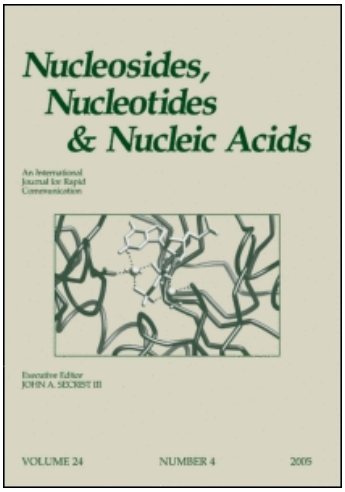

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SYNTHESIS AND ANTI-HIV ACTIVITY OF [D4U]-[TROVIRDINE ANALOGUE] AND [D4T]-[TROVIRDINE ANALOGUE] HETERODIMERS AS INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE

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SYNTHESIS AND ANTI-HIV ACTIVITY OF [D4U]-[TROVIRDINE ANALOGUE] AND [D4T]-[TROVIRDINE ANALOGUE] HETERODIMERS AS INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE

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ABSTRACT

A series of eleven heterodimers containing both a nucleoside analogue (d4U, d4T) and a non-nucleoside type inhibitor (Trovirdine analogue) were synthesized and evaluated for their ability to inhibit HIV replication. Unfortunately, the (*N-3*)d4U-Trovirdine conjugates (**9a–e**) and (*N-3*)d4T-Trovirdine conjugates (**10a–f**) were found to be inactive suggesting that the two individual inhibitor compounds do not bind simultaneously in their respective sites.

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INTRODUCTION

In addition to the Nucleoside Reverse Transcriptase Inhibitors (NRTIs) (AZT, d4T, ddC, ddI and 3TC) that are used clinically for the treatment of AIDS,[1–3] the structural diverse non-nucleoside RT inhibitors (NNRTIs) (HEPT, TIBO, nevirapine, pyridinone, BHAP, TSAO, α-APA, PETT, quinoxaline) have been identified as highly specific to HIV-1 RT.[4–8] In contrast to the NRTIs that interact to the substrate binding site after conversion to the triphosphate form, the NNRTIs do not need any metabolic conversion and are targeted at an allosteric non-substrate binding site of the HIV-1 RT which is functionally and spatially associated with the substrate binding site.

Combination chemotherapy of different anti-HIV agents has been widely used to treat AIDS patients to circumvent or delay the emergence of drug-resistant mutants.[9–12] Certain combinations of nucleoside and non-nucleoside RT inhibitors lead to a synergistic inhibition of HIV replication in vitro and in clinical trials [nevirapine/AZT/ddI,[13] Merck's pyridinone L-697,661/AZT,[14,15] delavirdine/AZT/ddI,[16,17] and MKC-442/AZT/ddI[18,19]]. The rationale for the NRTI-NNRTI combination therapy includes that a synergistic drug activity will lead to greater efficacy and that the emergence of resistance to individual agents will be forestalled.[12]

Furthermore, the cooperative interaction between the NNRTI-binding pocket and the substrate-binding site led Spence et al. [20] and Rittinger et al. [21]to postulate that an inhibitor combining the functionalities of a non-nucleoside inhibitor and a nucleotide analogue could bind very tightly and specifically to the HIV-RT and could be effective in the treatment of AIDS.

One alternative approach to combination therapy has been earlier suggested by Arnold and co-workers with the use of dimers resulting from the linking of a NRTI and a 2′,3′-dideoxynucleoside (ddN) through an appropriate spacer in an attempt to combine the inhibitory capacity of these two different classes of molecules.[22] The NNRTIs interact noncompetitively with the enzyme at an allosteric and highly hydrophobic nonsubstrate binding site that is distinct from, but functionally and also spatially associated with, the substrate binding site.[23] In 1995, Velázquez and co-workers reported for the first time novel [AZT]-[TSAO-T] and [AZT]-[HEPT] heterodimers linked through flexible polymethylene spacers between the N-3 positions of thymidine bases of both compounds.[24] The most active compound of this series was the [TSAO-T]-$(CH_2)_3$-[AZT] heterodimer (EC_{50} 0.10 μM against HIV-1 in CEM/0 cells) but nevertheless less potent than the parent compound from which it was derived.

With this aim, we also reported the synthesis and anti-HIV activity of a series of heterodimers of the general formula [d4T]-NH-$(CH_2)_n$-NH-[imidazo[1,5-*b*]pyridazine] (n = 6 to 12).[25] The in vitro inhibitory activities of these heterodimers were found to be comparable to that of d4T in

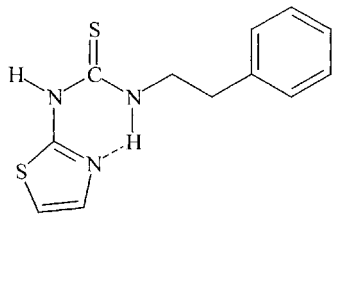
HIV-infected cells. The antiviral activities of d4T (IC_{50} 0.059 μM against HIV-1_{LAI} in CEM-SS cells; IC_{50} 0.28 μM against HIV-1_{IIIB} in MT-4 cells) and the heterodimers (IC_{50} 0.025 to 0.06 μM against HIV-1_{LAI} in CEM-SS cells; IC_{50} 0.09 to 0.36 μM against HIV-1_{IIIB} in MT-4 cells) were of the same magnitude. Moreover the [d4T]-NH-$(CH_2)_n$-NH-[imidazo[1,5-*b*]pyridazine] heterodimers showed pronounced activity against HIV-2 (IC_{50} 0.1 to 0.31 μM against HIV-2_{D194} in PBMC cells) by comparison to d4T (IC_{50} 0.18 μM against HIV-2_{D194} in PBMC cells). None of the heterodimers proved markedly cytotoxic to CEM-SS or MT-4 cells. In fact, cytotoxicity was not observed in either cell line at concentrations equal or below 10 μM.

Recently, Velázquez et al. reported novel [AZT]-[TSAO-T] and [d4T]-[TSAO-T] heterodimers modified in the linker and in the dideoxynucleoside moiety.[26] The [d4T]-$(CH_2)_3$-[TSAO-T] heterodimer was ~5- to 10-fold more inhibitory to HIV-1 in MT-4 and CEM cells cultures than the corresponding AZT heterodimer prototype and even 2-fold more potent than the unsubstituted TSAO-T parent compound. Furthermore, Pontikis et al. reported the [AZT]-[HEPT] and [ddC]-[HEPT] conjugates as inhibitors of HIV reverse transcriptase.[27] The [AZT]-[HEPT] conjugates displayed 2–5 μM anti-HIV activity, but they had no effect on the replication of HIV-2. The [ddC]-[HEPT] molecule displayed the same potency (EC_{50} 0.45 μM) against HIV-1 (wild type and the Y181C nevirapine-resistant strain) and HIV-2 in cell culture. No synergistic effect was observed for these bi-substrate inhibitors.

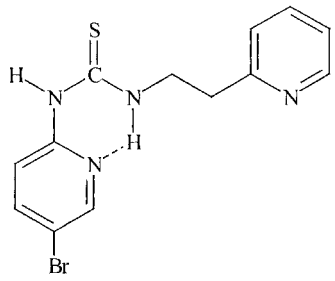
All these findings prompted us to report our results concerning the synthesis and the anti-HIV evaluation of a series of mixed inhibitors resulting from the linking of a known nucleoside analogue inhibitor (d4T) and a non-nucleoside RT inhibitor through a functionalized tethering arm. The tethering arm was varied in length and composition containing between 3 to 6 atoms in order to obtain a quite flexible dimer possessing an optimum distance between both active principles (NRTI and NNRTI). In fact, in the light of further structural studies it appears that, although the distance between the catalytic site and the hydrophobic binding pocket is relatively small (10–15 Å), the two sites are distinct.[28] Such a flexible molecule would be potentially have the right conformational freedom to interact with the RT.

For the NRTI, we have chosen a d4T analogue since d4T is used in the clinical treatment of AIDS. The choice of the attachment site of the tether on the N-3 position in the pyrimidine ring was based upon the observation that derivatives of AZT bearing a variety of N-3 side chains display potent anti-HIV-1 activity.[29]

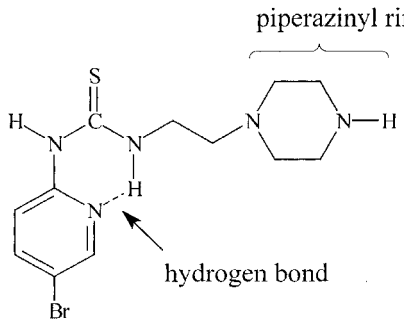
For the NNRTI, we have chosen a phenethyl thiazolyl thiourea (PETT) derivative (Sch. 1).[30–33] The representative compound of this series is Trovirdine. Indeed the *N*-[2-(1-piperazinyl)ethyl]-*N*′-2-(5-bromopyridyl)]thiourea (**4**) was found more active than AZT [$IC_{50} = 6$ nM for inhibition of HIV replication (measured by p24 production in HIV-infected human peripheral



PETT



Trovirdine (LY 300046)

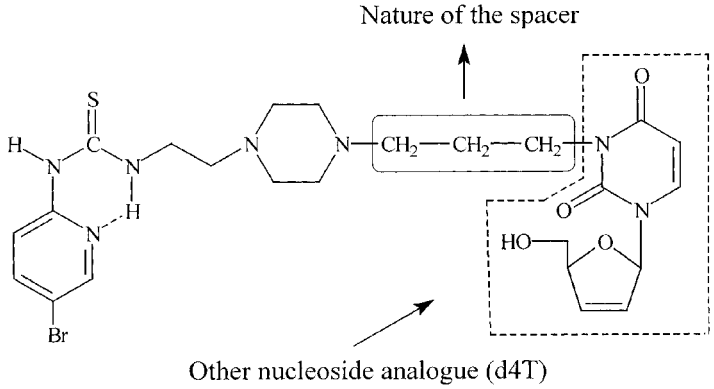


N-[2-(1-piperazinyl)ethyl]-*N'*-[2-(5-bromopyridyl)]thiourea (**4**)

Scheme 1. Phenethylthiazolylthiourea (PETT) derivatives.

blood mononuclear cells) and SI = 8000] or Trovirdine (IC_{50}[p24] = 7 nM in PBMC cells, SI > 10^4) (Sch. 1).[34] Furthermore, the Trovirdine derivative (**4**) inhibit HIV replication at nanomolar concentrations (IC_{50}[p24] = 2 nM) without evidence of cytotoxicity (no inhibition of cellular proliferation at concentrations as high as 100 μM).[34] In addition, the NH of the piperazine ring allowed further reactions with halogeno derivatives.

Since we have an ongoing program on the synthesis of many modified d4T analogues involving the formation of the key intermediate: the 5′-*O*-acetyl-2′,3′-didehydro-2′,3′-dideoxyuridine (5′-*O*-acetyl-d4U), the first objective of this project was to design and synthesize a series of covalently linked [d4U]-spacer-[Trovirdine derivative] heterodimers (**9a–f**) with an expanded range of spacers of different nature and conformational freedom (Sch. 2).[35] Then, we focused on the extension of the heterodimer approach with replacing the d4U by d4T leading to a series of [d4T]-spacer-[Trovirdine derivative] heterodimers (**10a–e**) in an attempt to combine the inhibitory capacity of these two different classes of molecules.



Scheme 2. Modifications carried out on the [d4U]-$(CH2)_3$-[Trovirdine derivative] heterodimer.

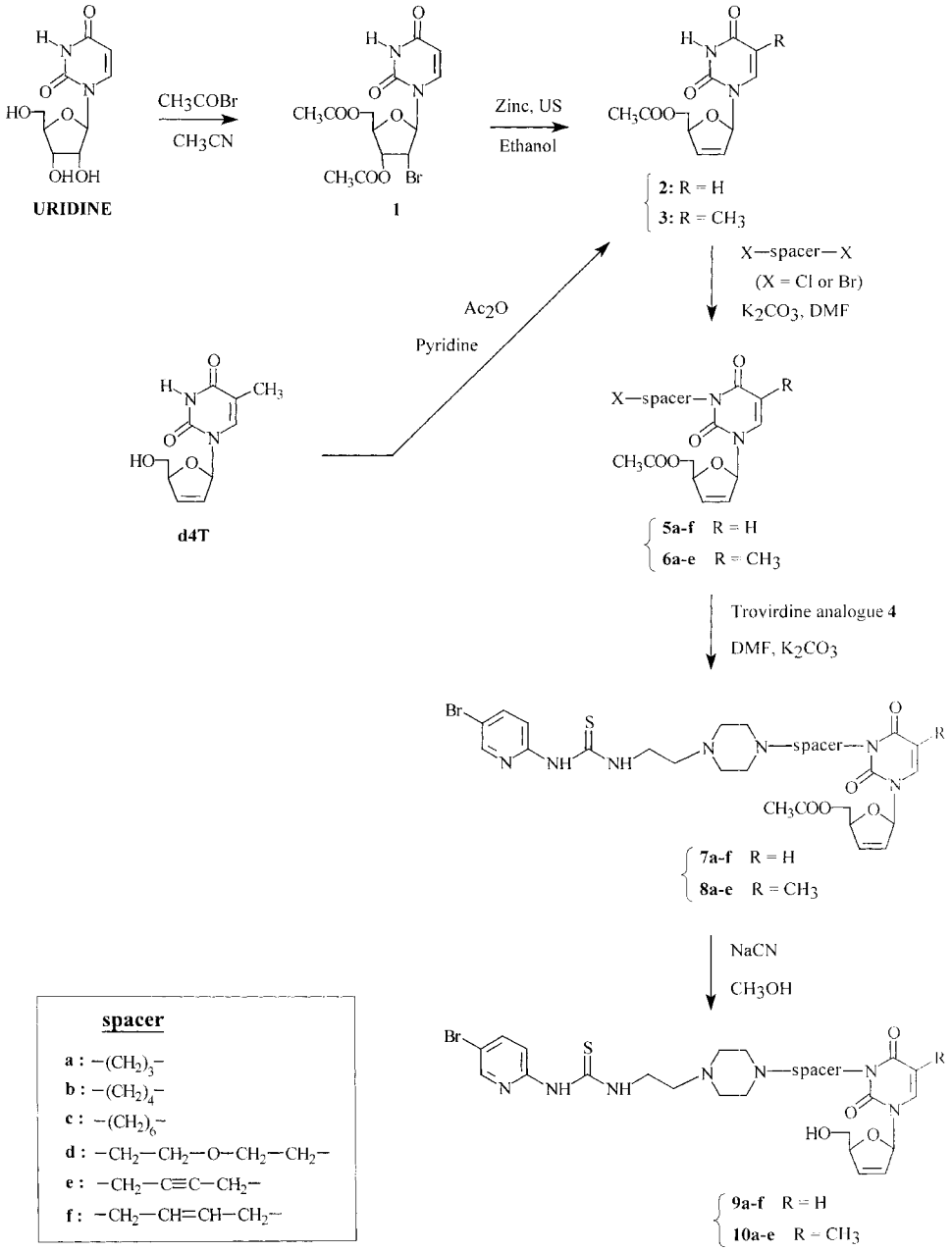
RESULTS AND DISCUSSION

Chemical

Our general strategy for the synthesis of the [d4U or d4T]N^3-spacer-$N^{piperazinyl}$[Trovirdine analogue] target heterodimers (**9a–f** and **10a–e**) was based on method previously described.[25]

Firstly, we have developed a two-step sequence to the versatile unsaturated nucleoside precursor **2** using a strategy similar to that previously used in our laboratory.[35] This intermediate **2** was also synthesized by Shiragami et al. according to a modified Eastwood deoxygenation procedure [mp 128–128.5°C (from ethanol); m/z 253 (MH^+)].[36] In order to develop a convenient synthetic route to the 2′,3′-didehydro-2′,3′-dideoxy-uridine (**2**), we have used the method described in the literature by Marumoto et al. for the conversion of uridine to acetoxybromo-nucleoside (**1**) (Sch. 3).[37] Treatment of the resulting bromoacetate **1** with zinc in anhydrous ethanol under irradiation of 35 KHz ultrasound (150 W) for 30 min provided the corresponding 5′-*O*-acetyl d4U (**2**) which was isolated as crystalline solid in 44% yield after silica gel chromatography.[35] This method was attractive since the olefinic nucleoside was obtained in neutral medium. In fact, the presence of acid promotes the decomposition of the unsaturated nucleosides.

Initial experiments were aimed at introducing the spacer moiety into the unsaturated precursor. Thus, reaction of **2** with commercially available dibromo- or dichloro-alkyl reagents [$(CH_2)_nBr_2$ (n = 3, 4 and 6), 2-bromoethyl ether, 1,4-dichlorobutyne and 1,4-dibromobutene] in anhydrous acetone, DMF or acetone:DMF (1:1) in the presence of potassium carbonate afforded respectively the d4U intermediates **5a–f**. After chromatography, these N-3-substituted derivatives **5a–e** were isolated with acceptable yields



Scheme 3. Synthesis of [d4U]-spacer-[Trovirdine analogue] (9a–f) and [d4T]-spacer-[Trovirdine analogue] (10a–e).

(39 to 80%) except for **5f** (spacer: $CH_2CH=CHCH_2$) which was isolated in a rather low yield (18%).

Subsequent reaction of these intermediates with the Trovirdine analogue **4** under basic conditions (K_2CO_3) gave the heterodimers **7a–f** [in 39 to 71% yield except for compound **17** [spacer: $(CH_2)_4$) which was isolated in 24% yield]. The Trovirdine analogue **4** was prepared as described previously by Vig et al.[33] Deacetylation of **7a–f** was performed using NaCN at room temperature and the [d4U]N^3-spacer-$N^{piperazinyl}$[Trovirdine analogue] final heterodimer targets **9a–f** were isolated in 74 to 98% yield as white crystalline solids after purification by silica gel column chromatography (dichloromethane/methanol 85:15).[38]

Next, we focused on modification on the nucleoside moiety in the model heterodimer. Thus, for comparative studies, the synthesis of a series of [d4T]N^3-spacer-$N^{piperazinyl}$[Trovirdine analogue] heterodimers (**10a–e**) was carried out in a similar way starting from the corresponding d4T which was provided by Bristol Myers Squibb (Sch. 3). In a first step, d4T was converted to its 5′-*O*-acetyl counterpart (**3**) by a standard procedure. This intermediate **3** was also synthesized by Shiragami et al. according to a reductive β-elimination method of the corresponding acetoxy-bromo nucleoside precursor.[39] Reaction of 5′-*O*-acetyl d4T (**3**) with the appropriate dibromo or dichloro reagent [$(CH_2)_nBr_2$ (n = 3, 4 and 6), 2-bromoethyl ether and 1,4-dichlorobutyne] in the presence of K_2CO_3 afforded the N-3 alkyl intermediates (**6a–e**) [73 to 97% yield except for **6a** (spacer: $(CH_2)_3$) isolated in 34% yield]. Treatment of these intermediates with the Trovirdine analogue (**4**) gave the corresponding heterodimers **8a–e** isolated in 23 to 48% yield. Finally, compounds **8a–e** were subjected to an ester hydrolysis to provide free heterodimers **10a–e** [89 to 98% yield except for **10d** (spacer: $(CH_2)_2$-O-$(CH_2)_2$) isolated in 40% yield].

Structure of the novel heterodimers **9a–f** and **10a–e** were assigned on the basis of their analytical and spectroscopic data. The full assignment of ^1H and ^{13}C NMR spectra for compounds **5a**, **5e**, **7a**, **7c**, **7d**, **7f**, **8e**, **9a** and **9b** were achieved by one and two-dimensional (1D and 2D) NMR techniques. Structures of the target heterodimers [d4T]-spacer-[Trovirdine analogue] (**10a–e**) were determined through comparison of their ^1H and ^{13}C NMR spectra to those of the model compound **9a**.

Biological

The novel [d4U]-[Trovirdine analogue] (**9a–f**) and [d4T]-[Trovirdine analogue] (**10a–e**) were evaluated by comparison to AZT for inhibition of HIV-1 multiplication in lymphocytic cell lines (CEM-SS and MT-4). As shown in Table 1, the heterodimers **9a–f** and **10a–e** were unfortunately devoid of antiviral activity at non toxic concentration. Overall, the activities

Table 1.

Compd	HIV-1_{LAI} in CEM-SS Cells			HIV-1_{IIIB} in MT-4 Cells			HIV-$1_{III\ B}$ in PBMC Cells		
	IC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]	IC_{50} (μM)	CC_{50} (μM)	SI	IC_{50} (μM)	CC_{50} (μM)	SI
d4T	0.059	> 100	> 1695	0.28	> 100	> 357	0.05	42	840
4	13	> 100	> 7.69	> 100	> 100	> 1	8.6	> 100	> 11.62
9a	> 100	> 100	> 1	> 100	> 100	> 1	ND[d]	ND	ND
9b	> 100	> 100	> 1	> 100	> 100	> 1	ND	ND	ND
9c	15	> 100	> 6.67	> 100	> 100	> 1	88	98	1.11
9d	> 100	> 100	> 1	> 100	> 100	> 1	ND	ND	ND
9e	> 73	> 100	> 1.37	> 100	> 100	> 1	ND	ND	ND
9f	> 100	> 100	> 1	> 100	> 100	1	ND	ND	ND
10a	> 100	> 100	> 1	> 100	> 100	> 1	ND	ND	ND
10b	19	> 100	> 5.26	> 100	> 100	> 1	ND	ND	ND
10c	10	> 100	> 10	> 100	> 100	> 1	70	> 100	> 1.43
10d	67	> 100	> 1.49	52	> 100	> 1.92	ND	ND	ND
10e	> 100	> 100	> 1	> 100	> 100	> 1	45	> 100	> 2.22

[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}.
[d] ND : not determined.
All data represent the mean values of three separate experiments ± SD.

observed for the conjugates **9a–f** and **10a–e** were inferior to the values for the parent nucleoside analogue and the NNRTI component. Indeed, the differences in activities may result in differences in cell membrane permeability and/or different levels of phosphorylation of the nucleoside subunit. In fact, the lack of activity of these N-3-substituted d4T derivatives is most likely due to the fact that the compounds are poorly recognized by cellular kinases, and therefore they will become much less phosphorylated than the parent d4T. Moreover, it is conceivable that the lack of activity for the heterodimers **9a–f** and **10a–e** is a consequence of a wrong positioning of either the NRTI or the NNRTI component with their respective sites.

EXPERIMENTAL SECTION

Chemical Procedures

Reagent grade acetonitrile was refluxed and distilled from phosphorus pentoxide. Anhydrous ethanol was prepared by using magnesium turnings. Anhydrous methanol p.a., acetone, *N*,*N*-dimethylformamide and pyridine were respectively purchased from E. Merck, Carlo Erba and Aldrich. Unless

otherwise stated, reactions were run under an atmosphere of 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 were 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 kept in a drying oven at 30°C over P_2O_5 for at least 24 h prior to analysis.

Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were recorded on a Fourier transform Mattson spectrometer Genesis DTGS using WinFIRST™ Macros and ApPro™ and only noteworthy absorptions are listed. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL Lambda 400 using TMS as an internal standard. NH and OH signals appeared as broad singlets exchangeable with D_2O (s = singlet, b = broad, d = doublet, t = triplet, q = quadruplet, m = multiplet). Mass spectra were determined with a JEOL GC mate apparatus. Only the peak due to the molecular ion H^+ is listed.

5′-*O*-Acetyl-2′,3′-didehydro-2′,3′-dideoxythymidine (3). Acetic anhydride (4 mL) was added dropwise to a stirred solution of d4T (500 mg, 2.23 mmol) in dry pyridine (50 mL) at 0°C (ice/water bath). The reaction mixture was stirred overnight and evaporated to dryness under reduced pressure. The residue was coevaporated with EtOH (3 × 20 mL) to afford 590 mg (99%) of crude product as a white foam which was used immediately for the next preparation without purification: R_f 0.52 [EtOAc (100%)]; ^{1}H NMR (DMSO-d_6): δ 1.76 (s, 3H, C*H*$_3$-5), 2.01 (s, 3H, C*H*$_3$COO), 4.16 (dd, 1H, *H-5′*, $J = 12.2$, $J = 2.7$ Hz), 4.21 (dd, 1H, *H-5″*, $J = 12.2$, $J = 4.1$ Hz), 4.96 (bs, 1H, *H-4′*), 5.99 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.40 (d, 1H, *H-3′*, $J = 5.8$ Hz), 6.80 (bs, 1H, *H-1′*), 7.25 (s, 1H, *H-6*), 11.38 (bs, 1H, N*H*); ^{13}C NMR (DMSO-d_6): δ 12.1 (*C*H$_3$-5), 20.6 (*C*H$_3$COO), 64.5 (*C-5′*), 83.6 (*C-4′*), 89.3 (*C-1′*), 109.5 (*C-5*), 126.6 (*C-2′*), 133.6 (*C-3′*), 135.8 (*C-6*), 150.7 (*C-2*), 163.8 (*C-4*), 170.0 (CH$_3$*C*OO).

***N*-[2-(1-Piperazinylethyl)]-*N′*-[2-(5-bromopyridyl)]thiourea (4).** The title compound was prepared by the method reported by Vig et al.[33] The remaining solid was purified by flash chromatography on silica gel with methanol in dichloromethane (from 0% to 10%) to give compound **4** as a white foam (overall yield:37%): R_f 0.20 [CH_2Cl_2-CH_3OH (7:3)]; mp 178–180°C, ^{1}H NMR (DMSO-d_6): δ 2.35 (m, 4H, 2 × NC*H*$_2$ *of piperazinyl*), 2.49 (m, 2H, NHCH$_2$C*H*$_2$N), 2.71 (m, 4H, 2 × C*H*$_2$NH *of piperazinyl*), 3.65 (m, 2H, CSNHC*H*$_2$), 7.12 (d, 1H, *H-III*, $J = 8.9$ Hz), 7.98 (dd, 1H, *H-IV*, $J = 8.9$ Hz, $J = 2.4$ Hz), 8.26 (d, 1H, *H-VI*, $J = 2.4$ Hz), 10.69 (bs, 1H, N*H*), 11.36 (bs, 1H, N*H*); ^{13}C NMR (DMSO-d_6): δ 41.9 (CSNH*C*H$_2$), 45.8 (*C*H$_2$NH *of piperazinyl*), 53.5 (2 × NCH$_2$ *of piperazinyl*), 55,8 (NHCH$_2$C*H*$_2$), 111.8 (*C-V*), 114.5 (*C-III*), 141.3 (*C-IV*), 145.8 (*C-VI*), 152.4 (*C-II*), 178.7

(CS); *Anal*. Calcd for C_{12} H_{18} Br N_5 S: C, 41.86; H, 5.23; N, 20.35. Found: C, 41.92; H, 5.35; N, 20.42; MS m/z 345 (MH^+).

General Procedure for the Synthesis of 5′-*O*-Acetyl-3-*N*-(*n*-bromo or *n*-chloro-spacer) Nucleosides Intermediates (5a–f) and (6a–e). To a solution of the nucleoside [5′-*O*-acetyl-d4U (**2**) or 5′-*O*-acetyl-d4T (**3**)] (1 equiv.) in acetone or DMF or acetone/DMF were added K_2CO_3 (1.1 equiv.) and 6 equiv. of the correspondant dibromoalkyl, alkenyl, aryl, or dichloroalkynyl reagent. The reaction mixture in acetone was refluxed for 19 h, in DMF heated at 80°C for 1–5 h and in the mixture acetone/DMF heated at 70°C for 12–24 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate (50 mL), washed with water (2 × 50 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by chromatography on a silica gel column (ethyl acetate). The fractions containing the desired product were pooled together, and the solvent was evaporated in vacuo. The reagent, solvent of the reaction mixture, reaction time, chromatography eluent, yield of the isolated products, and ^{1}H NMR and ^{13}C NMR data are indicated below each compound.

5′-*O*-Acetyl-3-*N*-(3-bromopropyl)-2′, 3′-didehydro-2′, 3′-dideoxyuridine (5a). reagent: 1,3-dibromopropane, solvent of the reaction mixture: acetone; reaction time 19 h; eluent of the chromatography: ethyl acetate; yield of **5a** (59%) as a syrup; R_f 0.67 [EtOAc (100%)]; ^{1}H NMR (DMSO-d_6): δ 2.00 (s, 3H, CH_3COO), 2.07 (s, 2H, CH_2CH_2N-3), 3.52 (t, 2H, CH_2Br, $J = 6.7$ Hz), 3.91 (t, 2H, CH_2N-3, $J = 7.1$ Hz), 4.18 (d, 2H, *H-5′* and *H-5′*, $J = 3.4$ Hz), 5.00 (bs, 1H, *H-4′*), 5.80 (d, 1H, *H-5*, $J = 8.0$ Hz), 6.01 (d, 1H, *H-2′*, $J = 6.1$ Hz), 6.44 (d, 1H, *H-3′*, $J = 6.1$ Hz), 6.84 (bs, 1H, *H-1′*), 7.49 (d, 1H, *H-6*, $J = 8.0$ Hz); ^{13}C NMR (DMSO-d_6): δ 20.6 (*C*H_3), 30.5 (*C*H_2CH_2N-3), 32.0 (*C*H_2Br), 39.4 (*C*H_2N-3), 64.5 (*C-5′*), 83.9 (*C-4′*), 90.4 (*C-1′*), 101.1 (*C-5*), 126.3 (*C-2′*), 134.0 (*C-3′*), 139.2 (*C-6*), 150.9 (*C-2*), 162.0 (*C-4*), 170.1 (CH$_3$*C*OO).

5′-*O*-Acetyl-3-*N*-(4-bromobutyl)-2′, 3′-didehydro-2′, 3′-dideoxyuridine (5b). reagent: 1,4-dibromobutane, solvent of the reaction mixture: acetone/DMF (1:1); reaction time 14 h; eluent of the chromatography: ethyl acetate in dichloromethane (from 0% to 10 %); yield of **5b** (39%) as a syrup; R_f 0.68 [EtOAc (100%)]; ^{1}H NMR ($CDCl_3$): δ 1.71 (m, 2H, CH_2CH_2Br), 1.80 (m, 2H, CH_2CH_2N-3), 1.96 (s, 3H, CH_3COO), 3.34 (t, 2H, CH_2Br, $J = 6.5$ Hz), 3.85 (t, 2H, CH_2N-3, $J = 6.9$ Hz), 4.15 (dd, 1H, *H-5′*, $J = 12.3$, $J = 3.2$ Hz), 4.22 (dd, 1H, *H-5″*, $J = 12.3$, $J = 3.8$ Hz), 4.97 (bs, 1H, *H*-4′), 5.64 (d, 1H, *H-5*, J = 8.1 Hz), 5.83 (d, 1H, *H-2′*, $J = 5.3$ Hz), 6.22 (d, 1H, *H-3′*, $J = 5.3$ Hz), 6.92 (bs, 1H, *H-1′*), 7.33 (d, 1H, *H-6*, $J = 8.1$ Hz); ^{13}C NMR ($CDCl_3$): δ 20.7 (*C*H_3), 26.2 (*C*H_2CH_2N-3), 29.9 (*C*H_2CH_2Br), 33.2 (*C*H_2Br),

40.1 (CH_2N-3), 64.5 (*C-5′*), 84.2 (*C-4′*), 90.6 (*C-1′*), 101.9 (*C-5*), 126.9 (*C-2′*), 133.5 (*C-3′*), 137.8 (*C-6*), 151.1 (*C-2*), 162.4 (*C-4*), 170.2 (CH_3COO).

5′-*O*-Acetyl-3-*N*-(6-bromohexyl)-2′, 3′-didehydro-2′, 3′-dideoxyuridine (5c). reagent: 1,6-dibromohexane, solvent of the reaction mixture: DMF; reaction time 4 h; eluent of the chromatography: ethyl acetate in dichloromethane (from 0 to 10%); yield of **5c** (41%) as a syrup; R_f 0.73 [EtOAc (100%)]; ^{1}H NMR ($CDCl_3$): δ 1.32 (m, 2H, $CH_2(CH_2)_2Br$), 1.41 (m, 2H, $CH_2(CH_2)_2$N-3), 1.58 (m, 2H, CH_2CH_2Br), 1.80 (m, 2H, CH_2CH_2N-3), 2.00 (s, 3H, CH_3COO), 3.33 (t, 2H, CH_2Br, $J = 6.8$ Hz), 3.85 (t, 2H, CH_2N-3, $J = 6.6$ Hz), 4.18 (dd, 1H, *H-5′*, $J = 12.3$, $J = 2.6$ Hz), 4.27 (dd, 1H, *H-5″*, $J = 12.3$, $J = 3.5$ Hz), 5.00 (bs, 1H, *H-4′*), 5.67 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.86 (d, 1H, *H-2′*, $J = 5.6$ Hz), 6.24 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.96 (bs, 1H, *H-1′*), 7.35 (d, 1H, *H-6*, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$): δ 20.6 (CH_3), 25.9 ($CH_2(CH_2)_2$N-3), 27.1 ($CH_2(CH_2)_2Br$), 27.6 (CH_2CH_2N-3), 32.4 (CH_2CH_2Br), 33.7 (CH_2Br), 40.9 (CH_2N-3), 64.5 (*C-5′*), 84.1 (*C-4′*), 90.5 (*C-1′*), 101.9 (*C-5*), 126.9 (*C-2′*), 133.5 (*C-3′*), 137.8 (*C-6*), 151.1 (*C-2*), 162.4 (*C-4*), 170.2 (CH_3COO).

5′-*O*-Acetyl-3-*N*-[2-(2-bromoethoxy)ethyl]-2′, 3′-didehydro-2′, 3′-dideoxyuridine (5d). reagent: 2-bromoethyl ether, solvent of the reaction mixture: acetone/DMF (7:2); reaction time 24 h; eluent of the flash chromatography: methanol in dichloromethane (from 0 to 3%); yield of **5d** (67%) as a syrup; R_f 0.57 [EtOAc (100%)]; ^{1}H NMR ($CDCl_3$): δ 2.07 (s, 3H, CH_3COO), 3.45 (t, 2H, CH_2Br, $J = 6.0$ Hz), 3.77 (t, 2H, $BrCH_2CH_2O$, $J = 6.0$ Hz), 3.82 (t, 2H, OCH_2CH_2N-3, $J = 6.0$ Hz), 4.18 (m, 2H, CH_2N-3), 4.25 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.7$ Hz), 4.34 (dd, 1H, *H-5″*, $J = 12.4$, $J = 3.6$ Hz), 5.07 (bs, 1H, *H-4′*), 5.75 (d, 1H, *H-5′*, $J = 8.0$ Hz), 5.94 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.32 (d, 1H, *H-3′*, $J = 5.7$ Hz), 7.02 (bs, 1H, *H-1′*), 7.44 (d, 1H, *H-6*, $J = 8.0$ Hz).

5′-*O*-Acetyl-3-*N*-(4-chloro-2-butynyl)-2′, 3′-didehydro-2′, 3′-dideoxyuridine (5e). reagent: 1,4-dichloro-2-butyne, solvent of the reaction mixture: acetone/DMF (1:1); reaction time 12 h; eluent of the chromatography: methanol in dichloromethane (from 0 to 2%); yield of **5e** (80%) as a syrup; R_f 0.68 [EtOAc (100%)]; ^{1}H NMR ($CDCl_3$): δ 1.96 (s, 3H, CH_3COO), 4.02 (bs, 2H, CH_2Cl), 4.15 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.6$ Hz), 4.23 (dd, 1H, *H-5′*, $J = 12.4$, $J = 3.6$ Hz), 4.63 (bs, 2H, CH_2N-3), 4.98 (bs, 1H, *H-4′*), 5.68 (d, 1H, *H-5*, $J = 8.1$ Hz), 5.85 (d, 1H, *H-2′*, $J = 5.6$ Hz), 6.24 (d, 1H, *H-3′*, $J = 5.6$ Hz), 6.93 (bs, 1H, *H-1′*), 7.39 (d, 1H, *H-6*, $J = 8.1$ Hz); ^{13}C NMR ($CDCl_3$): δ 20.7 (CH_3COO), 30.4 (CH_2Cl and CH_2N-3), 64.5 (*C-5′*), 77.0 ($ClCH_2C{\equiv}C$), 80.7 ($C{\equiv}CCH_2$N-3), 84.3 (*C-4′*), 90.7 (*C-1′*), 101.8 (*C-5*), 126.7 (*C-2′*), 133.7 (*C-3′*), 138.3 (*C-6*), 150.5 (*C-2*), 161.4 (*C-4*), 170.2 (CH_3COO).

5′-*O*-Acetyl-3-*N*-(4-bromo-2-(*E*)butenyl)-2′, 3′-didehydro-2′,3′-dideoxyuridine (5f). reagent: 1,4-dibromo-2-butene, solvent of the reaction mixture: DMF; reaction time 1 h 30 min; eluent of the chromatography: methanol in dichloromethane (from 0 to 1%); yield of **5f** (18%) as a syrup; R_f 0.77 [EtOAc (100%)]; ^{1}H NMR ($CDCl_3$): δ 2.07 (s, 3H, C*H*$_3$COO), 3.92 (d, 2H, C*H*$_2$Br, $J = 7.3$ Hz), 4.34 (dd, 1H, *H-5′*, $J = 12.4$, $J = 3.0$ Hz), 4.34 (dd, 1H, *H-5″*, $J = 12.4$, $J = 3.7$ Hz), 4.56 (d, 2H, C*H*$_2$N-3, $J = 6.0$ Hz), 5.07 (bs, 1H, *H-4′*), 5.76 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.93 (d, 1H, *H-2′*, $J = 6.0$ Hz), 5.95–6.00 (m, 2H, C*H*=C*H*), 6.31 (d, 1H, *H-3′*, $J = 6.0$ Hz), 7.03 (m, 1H, *H*-1′), 7.44 (d, 1H, *H-6*, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$): δ 20.7 (*C*H$_3$), 31.6 (*C*H$_2$Br), 41.7 (*C*H$_2$N-3), 64.6 (*C-5′*), 84.3 (*C-4′*), 90.7 (*C-1′*), 102.0 (*C-5*), 127.0 (*C-2′*), 128.3 (*C*HCH$_2$Br), 130.4 (*C*HCH$_2$N-3), 133.5 (*C-3′*), 137.9 (*C-6*), 151.0 (*C-2*), 162.1 (*C-4*), 170.2 (CH$_3$*C*OO).

5′-*O*-Acetyl-3-*N*-(3-bromopropyl)-2′, 3′-didehydro-2′, 3′-dideoxythymidine (6a). solvent of the reaction mixture: DMF; reaction time 5 h; eluent of the chromatography: ethyl acetate in dichloromethane (from 0 to 10%); yield of **6a** (34%) as a syrup; R_f 0.65 [EtOAc (100%)];^{1}H NMR ($CDCl_3$): δ 1.93 (s, 3H, C*H*$_3$-5), 2.10 (s, 3H, C*H*$_3$COO), 2.23 (t, 2H, C*H*$_2$CH$_2$N-3, $J = 6.9$ Hz), 3.44 (t, 2H, C*H*$_2$Br, $J = 6.9$ Hz), 4.09 (t, 2H, C*H*$_2$N-3, $J = 6.9$ Hz), 4.25 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.9$ Hz), 4.39 (dd, 1H, *H-5″*, $J = 12.4$, $J = 4.0$ Hz), 5.06 (bs, 1H, *H-4′*), 5.93 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.31 (d, 1H, *H-3′*, $J = 5.8$ Hz), 7.04 (bs, 1H, *H-1′*), 7.23 (s, 1H, *H-6*); ^{13}C NMR ($CDCl_3$): δ 13.1 (*C*H$_3$-5), 20.7 (*C*H$_3$COO), 30.3 (*C*H$_2$CH$_2$N-3), 30.8 (*C*H$_2$Br), 40.2 (*C*H$_2$N-3), 64.5 (*C-5′*), 84.0 (*C-4′*), 90.3 (*C-1′*), 109.9 (*C-5*), 127.1 (*C-2′*), 133.1 (*C-3′*), 133.5 (*C-6*), 151.1 (*C-2*), 163.0 (*C-4*), 170.1 (CH$_3$*C*OO).

5′-*O*-Acetyl-3-*N*-(4-bromobutyl)-2′, 3′-didehydro-2′, 3′-dideoxythymidine (6b). solvent of the reaction mixture: DMF; reaction time 5 h; eluent of the chromatography: ethyl acetate in dichloromethane (from 0 to 100%); yield of **6b** (73%) as a syrup; R_f 0.77 [EtOAc (100%)];^{1}H NMR ($CDCl_3$): δ 1.78–1.84 (m, 2H, C*H*$_2$CH$_2$Br), 1.88–1.96 (m, 2H, C*H*$_2$CH$_2$N-3), 1.93 (s, 3H, C*H*$_3$-5), 2.10 (s, 3H, C*H*$_3$COO), 3.45 (t, 2H, C*H*$_2$Br, $J = 6.6$ Hz), 3.99 (d, 2H, C*H*$_2$N-3, $J = 7.1$ Hz), 4.25 (dd, 1H, *H-5′*, $J = 12.3$, $J = 2.9$ Hz), 4.39 (dd, 1H, *H-5″*, $J = 12.3$, $J = 4.0$ Hz), 5.06 (bs, 1H, *H-4′*), 5.93 (d, 1H, *H-2′*, $J = 6.0$ Hz), 6.31 (d, 1H, *H-3′*, $J = 6.0$ Hz), 7.05 (bs, 1H, *H-1′*), 7.22 (s, 1H, *H-6*); ^{13}C NMR ($CDCl_3$): δ 13.1 (*C*H$_3$-5), 20.6 (*C*H$_3$COO), 26.1 (*C*H$_2$CH$_2$N-3), 29.9 (*C*H$_2$CH$_2$Br), 33.0 (*C*H$_2$Br), 40.2 (*C*H$_2$N-3), 64.5 (*C-5′*), 83.9 (*C-4′*), 90.3 (*C-1′*), 109.9 (*C-5*), 127.1 (*C-2′*), 133.0 (*C-3′*), 133.4 (*C-6*), 151.1 (*C-2*), 163.1 (*C-4*), 170.1 (CH$_3$*C*OO).

5′-*O*-Acetyl-3-*N*-(6-bromohexyl)-2′, 3′-didehydro-2′, 3′-dideoxythymidine (6c). solvent of the reaction mixture: DMF; reaction time 1 h 45 min; eluent of the chromatography: ethyl acetate in dichloromethane (from 0 to 10%);

yield of **6c** (87%) as a syrup; R_f 0.73 [EtOAc (100%)]; ^{1}H NMR ($CDCl_3$): δ 1.40 (m, 2H, C*H*$_2$(CH$_2$)$_2$Br), 1.49 (m, 2H, C*H*$_2$(CH$_2$)$_2$N-3), 1.65 (m, 2H, C*H*$_2$CH$_2$Br), 1.87 (m, 2H, C*H*$_2$CH$_2$N-3), 1.93 (s, 3H, C*H*$_3$-5), 2.10 (s, 3H, C*H*$_3$COO), 3.40 (t, 2H, C*H*$_2$Br, $J = 6.8$ Hz), 3.94 (t, 2H, C*H*$_2$N-3, $J = 7.5$ Hz), 4.25 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.9$ Hz), 4.38 (dd, 1H, *H-5″*, $J = 12.4$, $J = 4.0$ Hz), 5.06 (bs, 1H, *H-4′*), 5.92 (d, 1H, *H-2′*, $J = 6.0$ Hz), 6.30 (d, 1H, *H-3′*, $J = 6.0$ Hz), 7.05 (bs, 1H, *H-1′*), 7.21 (s, 1H, *H-6*); ^{13}C NMR ($CDCl_3$): δ 13.1 (*C*H$_3$-5), 20.7 (*C*H$_3$COO), 25.9 (*C*H$_2$(CH$_2$)$_2$N-3), 27.1 (*C*H$_2$(CH$_2$)$_2$Br), 27.6 (*C*H$_2$CH$_2$N-3), 32.4 (*C*H$_2$CH$_2$Br), 33.7 (*C*H$_2$Br), 41.1 (*C*H$_2$N-3), 64.5 (*C-5′*), 83.9 (*C-4′*), 90.3 (*C-1′*), 109.9 (*C-5*), 127.2 (*C-2′*), 133.0 (*C-3′*), 133.3 (*C-6*), 151.1 (*C-2*), 163.1 (*C-4*), 170.1 (CH$_3$*C*OO).

5′-*O*-Acetyl-3-*N*-[2-(2-bromoethoxy)ethyl]-2′, 3′-didehydro-2′, 3′-dideoxythymidine (6d). Solvent of the reaction mixture: DMF; reaction time 1 h 30 min; eluent of the chromatography: methanol in dichloromethane (from 0 to 15%); yield of **6d** (97%) as a syrup; R_f 0.63 [EtOAc (100%)];^{1}H NMR ($CDCl_3$): δ 1.78 (s, 3H, C*H*$_3$-5), 1.95 (s, 3H, C*H*$_3$COO), 3.30 (t, 2H, C*H*$_2$Br, $J = 5.6$ Hz), 3.62 (t, 2H, BrCH$_2$C*H*$_2$O, $J = 5.6$ Hz), 3.67 (m, 2H, OC*H*$_2$CH$_2$N-3), 4.06 (m, 2H, C*H*$_2$N-3), 4.09 (dd, 1H, *H-5′*, $J = 12.1$, $J = 2.2$ Hz), 4.23 (dd, 1H, *H-5″*, $J = 12.1$, $J = 3.5$ Hz), 4.91 (bs, 1H, *H-4′*), 5.78 (d, 1H, *H-2′*, $J = 5.2$ Hz), 6.16 (d, 1H, *H-3′*, $J = 5.2$ Hz), 6.89 (bs, 1H, *H-1′*), 7.08 (s, 1H, *H-6*); ^{13}C NMR ($CDCl_3$): δ 13.2 (*C*H$_3$-5), 20.7 (*C*H$_3$COO), 30.6 (*C*H$_2$Br), 39.7 (*C*H$_2$N-3), 64.5 (*C-5′*), 67.1 (O*C*H$_2$CH$_2$N-3), 70.1 (BrCH$_2$*C*H$_2$O), 83.9 (*C*-4′), 90.3 (*C-1′*), 109.8 (*C-5*), 127.1 (*C-2′*), 133.1 (*C-3′*), 133.6 (*C-6*), 151.2 (*C-2*), 163.1 (*C-4*), 170.1 (CH$_3$*C*OO).

5′-*O*-Acetyl-3-*N*-(4-chloro-2-butynyl)-2′, 3′-didehydro-2′, 3′-dideoxythymidine (6e). solvent of the reaction mixture: DMF; reaction time 1 h; eluent of the chromatography: ethyl acetate in dichloromethane (from 0 to 10%); yield of **6e** (76%) as a syrup; R_f 0.83 [EtOAc (100%)]; ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 3H, C*H*$_3$-5), 2.10 (s, 3H, C*H*$_3$COO), 4.13 (t, 2H, C*H*$_2$Cl, $J = 1.9$ Hz), 4.26 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.9$ Hz), 4.40 (dd, 1H, *H-5″*, $J = 12.4$, $J = 4.0$ Hz), 4.77 (t, 2H, C*H*$_2$N-3, $J = 1.9$ Hz), 5.08 (bs, 1H, *H-4′*), 5.95 (d, 1H, *H-2′*, $J = 6.0$ Hz), 6.34 (d, 1H, *H-3′*, $J = 6.0$ Hz), 7.05 (m, 1H, *H-1′*), 7.27 (s, 1H, *H-6*); ^{13}C NMR ($CDCl_3$): δ 12.9 (*C*H$_3$-5), 20.5 (*C*H$_3$COO), 30.2 (*C*H$_2$N-3), 30.4 (*C*H$_2$Cl), 64.5 (*C-5′*), 76.6 (ClCH$_2$*C*≡C), 80.7 (C≡*C*CH$_2$N-3), 83.9 (*C-4′*), 90.3 (*C-1′*), 109.8 (*C-5*), 126.9 (*C-2′*), 133.1 (*C-3′*), 133.8 (*C-6*), 150.3 (*C-2*), 162.0 (*C-4*), 170.0 (CH$_3$*C*OO).

General Procedure for the Synthesis of the Heterodimers [5′-*O*-acetyl-d4U or 5′-*O*-acetyl-d4T]-*N*^{3}-spacer-*N*^{piperazinyl}[Trovirdine analogue] (7a–f) and (8a–e). To a solution of the 5′-*O*-acetyl-*N*-(*n*-bromo or *n*-chloro-spacer)-2′, 3′-didehydro-2′, 3′-dideoxy-nucleoside **(5a–f and 6a–e)** (1 equiv.) in dry DMF were added K_2CO_3 (1.1 equiv.) and the Trovirdine analogue **(4)**

(1.1 equiv.). The reaction mixture was heated at 80°C for 8–16 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate (20 mL), washed with water (2 × 20 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. A flash chromatography of the residue was required to give the pure heterodimers.

The reaction time, chromatography eluent, yield of the isolated products, ^{1}H NMR and ^{13}C NMR data, and elemental analysis are indicated below each compound.

Heterodimer [5′-*O*-acetyl-d4U]N^3-$(CH_2)_3$-*N*^{piperazinyl}[Trovirdine analogue] (7a). reaction time 8 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **7a** (51%) as white crystals; R_f 0.50 [CH_2Cl_2-CH_3OH (90:10)]; mp 139°C; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2H, C*H*$_2$CH$_2$N-3, $J = 7.2$ Hz), 2.00 (s, 3H, C*H*$_3$COO), 2.32 (t, 2H, C*H*$_2$CH$_2$CH$_2$N-3, $J = 7.2$ Hz), 2.34–2.48 (m, 8H, C*H*$_2$ *of piperazinyl*), 2.49 (m, 2H, NHCH$_2$C*H*$_2$), 3.64 (q, 2H, NHC*H*$_2$, $J = 5.2$ Hz), 3.83 (t, 2H, C*H*$_2$N-3, $J = 7.2$ Hz), 4.17 (d, 2H, *H-5′* and *H-5″*, $J = 3.4$ Hz), 5.00 (m, 1H, *H-4′*), 5.79 (d, 1H, *H-5*, $J = 8.0$ Hz), 6.00 (d, 1H, *H-2′*, $J = 6.1$ Hz), 6.44 (d, 1H, *H-3′*, $J = 6.1$ Hz), 6.86 (m, 1H, *H-1′*), 7.12 (d, 1H, *H-III of pyridyl*, $J = 9.0$ Hz), 7.48 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.97 (dd, 1H, *H-IV of pyridyl*, $J = 9.0$, $J = 2.4$ Hz), 8.25 (d, 1H, *H-VI of pyridyl*, $J = 2.4$ Hz), 10.69 (bs, 1H, ArN*H*CS), 11.34 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR (DMSO-d_6): δ 20.6 (*C*H$_3$), 23.9 (*C*H$_2$CH$_2$N-3), 40.1 (*C*H$_2$N-3), 42.0 (NH*C*H$_2$), 52.4 (*C*H$_2$ *of piperazinyl*), 52.8 (*C*H$_2$ *of piperazinyl*), 55.2 (NHCH$_2$*C*H$_2$), 55.5 (*C*H$_2$CH$_2$CH$_2$N-3), 64.5 (*C-5′*), 83.9 (*C-4′*), 90.3 (*C-1′*), 101.2 (*C-5*), 111.8 (*C-V of pyridyl*), 114.5 (*C-III of pyridyl*), 126.4 (*C-2′*), 134.0 (*C-3′*), 139.0 (*C-6*), 141.6 (*C-IV of pyridyl*), 145.9 (*C-VI of pyridyl*), 150.4 (*C-2*), 152.4 (*C-II of pyridyl*), 162.0 (*C-4*), 170.1 (CH$_3$*C*OO), 178.7 (*C*S); *Anal.* Calcd for C_{26} H_{34} Br N_7 O_5 S: C, 49.06; H, 5.38; N, 15.40. Found: C, 48.88; H, 5.32; N, 15.37.

Heterodimer [5′-*O*-acetyl-d4U]N^3-$(CH_2)_4$-*N*^{piperazinyl}[Trovirdine analogue] (7b). reaction time 6 h; chromatography eluent: methanol in dichloromethane (from 0% to 4%); yield of **7b** (24%) as white crystals; R_f 0.37 [CH_2Cl_2-CH_3OH (90:10)]; mp 193°C; ^{1}H NMR ($CDCl_3$): δ 1.58 (m, 2H, C*H*$_2$(CH$_2$)$_2$N-3), 1.68 (m, 2H, C*H*$_2$CH$_2$N-3), 2.08 (s, 3H, C*H*$_3$COO), 2.40 (t, 2H, C*H*$_2$(CH$_2$)$_3$N-3, $J = 7.3$ Hz), 2.55 (m, 8H, C*H*$_2$ *of piperazinyl*), 2.65 (t, 2H, NHCH$_2$C*H*$_2$, $J = 5.7$ Hz), 3.81 (m, 2H, NHC*H*$_2$), 3.97 (t, 2H, C*H*$_2$N-3, $J = 6.6$ Hz), 4.26 (dd, 1H, *H-5′*, $J = 12.3$, $J = 3.0$ Hz), 4.35 (dd, 1H, *H-5″*, $J = 12.3$, $J = 3.4$ Hz), 5.07 (bs, 1H, *H-4′*), 5.76 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.93 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.30 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.88 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.04 (bs, 1H, *H-1′*), 7.42 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 1.8$ Hz), 8.24 (d, 1H, *H-VI of pyridyl*, $J = 1.8$ Hz), 9.38 (bs, 1H, ArN*H*CS), 11.50 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 20.7 (*C*H$_3$), 24.2 (*C*H$_2$CH$_2$N-3), 25.5 (*C*H$_2$(CH$_2$)$_2$N-3),

41.0 (CH_2N-3), 42.8 (NHCH_2), 52.6 (CH_2 *of piperazinyl*), 53.4 (CH_2 *of piperazinyl*), 55.4 (NHCH$_2$$CH_2$), 58.2 ($CH_2(CH_2)_3$N-3), 64.5 (*C-5′*), 84.2 (*C-4′*), 90.6 (*C-1′*), 102.0 (*C-5*), 112.6 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 127.0 (*C-2′*), 133.3 (*C-3′*), 137.5 (*C-6*), 141.0 (*C-IV of pyridyl*), 146.5 (*C-VI of pyridyl*), 151.2 (*C-2*), 151.8 (*C-II of pyridyl*), 162.5 (*C-4*), 170.2 (CH_3COO), 178.5 (*C*S); *Anal*. Calcd for C_{27} H_{36} Br N_7 O_5 S: C, 49.85; H, 5.58; N, 15.07. Found: C, 48.89; H, 5.49; N, 15.05.

Heterodimer [5′-*O*-acetyl-d4U]N^3-$(CH_2)_6$-$N^{piperazinyl}$[Trovirdine analogue] (7c). reaction time 14 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **7c** (43%) as white crystals; R_f 0.42 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.41 (m, 4H, (CH_2)$_2$(CH_2)$_2$N-3), 1.55 (m, 2H,$CH_2(CH_2)_5$N-3), 1.68 (m, 2H, CH_2CH_2N-3), 2.10 (s, 3H, CH_3COO), 2.38 (t, 2H, $CH_2(CH_2)_5$N-3, $J = 8.0$ Hz), 2.59 (m, 8H, CH_2 *of piperazinyl*), 2.68 (t, 2H, NHCH$_2$$CH_2$, $J = 6.3$ Hz), 3.84 (m, 2H, NHCH_2), 3.95 (m, 2H, CH_2N-3), 4.29 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.9$ Hz), 4.37 (dd, 1H, *H-5″*, $J = 12.4$, $J = 3.6$ Hz), 5.09 (m, 1H, *H-4′*), 5.78 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.95 (d, 1H, *H-2′*, $J = 6.1$ Hz), 6.32 (d, 1H, *H-3′*, $J = 6.1$ Hz), 6.84 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.07 (bs, 1H, *H-1′*), 7.44 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.75 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.2$ Hz), 8.28 (d, 1H, *H-VI of pyridyl*, $J = 2.2$ Hz), 9.13 (bs, 1H, ArN*H*CS), 11.50 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 21.0 (CH_3), 27.0 ($CH_2(CH_2)_2$N *of spacer*), 27.1 ($CH_2(CH_2)_2$N *of spacer*), 27.5 (CH_2CH_2N *of spacer*), 27.7 (CH_2CH_2N *of spacer*), 41.5 (CH_2N-3), 43.2 (NHCH_2), 53.0 (CH_2 *of piperazinyl*), 53.7 (CH_2 *of piperazinyl*), 55.8 (NHCH$_2$$CH_2$), 59.0 ($CH_2(CH_2)_5$N-3), 64.9 (*C-5′*), 84.5 (*C-4′*), 91.0 (*C-1′*), 102.4 (*C-5*), 113.0 (*C-V of pyridyl*), 113.7 (*C-III of pyridyl*), 127.4 (*C-2′*), 133.6 (*C-3′*), 137.8 (*C-6*), 141.4 (*C-IV of pyridyl*), 147.0 (*C-VI of pyridyl*), 151.5 (*C-2*), 152.0 (*C-II of pyridyl*), 162.8 (*C-4*), 170.6 (CH_3COO), 178.9 (*C*S); *Anal*. Calcd for C_{29} H_{40} Br N_7 O_5 S: C, 51.33; H, 5.94; N, 14.45. Found: C, 51.19; H, 5.81; N, 14.37.

Heterodimer [5′-*O*-acetyl-d4U]N^3-CH_2-CH_2-O-CH_2-CH_2-$N^{piperazinyl}$ [Trovirdine analogue] (7d). reaction time 6 h; chromatography eluent: methanol in dichloromethane (from 0% to 4%); yield of **7d** (39%) as white crystals; R_f 0.27 [CH_2Cl_2-CH_3OH (90:10)]; mp 125°C; ^{1}H NMR ($CDCl_3$): δ 2.07 (s, 3H, CH_3COO), 2.56 (m, 8H, CH_2 *of piperazinyl*), 2.60 (t, 2H, $CH_2CH_2O(CH_2)_2$N-3, $J = 5.7$ Hz), 2.64 (t, 2H, NHCH$_2$$CH_2$, $J = 6.1$ Hz), 3.66 (t, 2H, $CH_2O(CH_2)_2$N-3, $J = 5.7$ Hz), 3.71 (m, 2H, OC$H_2$$CH_2$N-3), 3.80 (m, 2H, NH$CH_2$), 4.19 (t, 2H, CH_2N-3, $J = 6.0$ Hz), 4.25 (dd, 1H, *H-5′*, $J = 12.3$, $J = 3.0$ Hz), 4.34 (dd, 1H, *H-5″*, $J = 12.3$, $J = 3.8$ Hz), 5.06 (m, 1H, *H-4′*), 5.74 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.91 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.29 (d, 1H, *H-3′*, $J = 5.8$ Hz), 6.65 (d, 1H, *H-III of pyridyl*, $J = 9.0$ Hz), 7.01 (m, 1H, *H-1′*), 7.41 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.73 (dd, 1H, *H-IV of pyridyl*, $J = 9.0$, $J = 2.4$ Hz), 8.26 (d, 1H, *H-VI of pyridyl*, $J = 2.4$ Hz), 8.41 (bs, 1H, ArN*H*CS), 11.41

(bs, 1H, CSN*H*CH_2); ^{13}C NMR ($CDCl_3$): δ 20.8 (*C*H_3), 39.8 (*C*H_2N-3), 43.1 (NH*C*H_2), 52.6 (*C*H_2 *of piperazinyl*), 53.9 (*C*H_2 *of piperazinyl*), 55.5 (NHCH$_2$*C*H_2), 58.0 (*C*H_2CH$_2$O(CH$_2$)$_2$N-3), 64.6 (*C-5′*), 67.5 (O*C*H_2CH$_2$N-3), 68.1 (*C*H_2O(CH$_2$)$_2$N-3), 84.3 (*C-4′*), 90.7 (*C-1′*), 102.0 (*C-5*), 112.8 (*C-V of pyridyl*), 113.1 (*C-III of pyridyl*), 127.1 (*C-2′*), 133.4 (*C-3′*), 137.7 (*C-6*), 141.2 (*C-IV of pyridyl*), 146.9 (*C-VI of pyridyl*), 151.4 (*C-2*), 151.5 (*C-II of pyridyl*), 162.6 (*C-4*), 170.5 (CH$_3$*C*OO), 178.8 (*C*S); *Anal*. Calcd for C_{27} H_{36} Br N_7 O_6 S: C, 48.65; H, 5.44; N, 14.71. Found: C, 48.59; H, 5.49; N, 14.60.

Heterodimer [5′-*O*-acetyl-d4U]N^3-CH$_2$-C≡C-CH$_2$-$N^{piperazinyl}$[Trovirdine analogue] (7e). reaction time 2 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **7e** (42%) as white crystals; R_f 0.46 [CH_2Cl_2-CH_3OH (90:10)]; mp 124°C; ^{1}H NMR ($CDCl_3$): δ 2.09 (s, 3H, C*H*$_3$COO), 2.59 (m, 8H, C*H*$_2$ *of piperazinyl*), 2.66 (t, 2H, NHCH$_2$C*H*$_2$, $J = 6.0$ Hz), 3.28 (bs, 2H, C*H*$_2$C≡CCH$_2$N-3), 3.83 (q, 2H, NHC*H*$_2$, $J = 5.6$ Hz), 4.27 (dd, 1H, *H-5′*, $J = 12.4$, $J = 3.0$ Hz), 4.36 (dd, 1H, *H-5″*, $J = 12.4$, $J = 3.8$ Hz), 4.77 (bs, 2H, C*H*$_2$N-3), 5.09 (bs, 1H, *H-4′*), 5.82 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.95 (d, 1H, *H-2′*, $J = 6.0$ Hz), 6.33 (d, 1H, *H-3′*, $J = 6.0$ Hz), 6.97 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.05 (m, 1H, *H-1′*), 7.47 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.4$ Hz), 8.27 (d, 1H, *H-VI of pyridyl*, $J = 2.4$ Hz), 9.67 (bs, 1H, ArN*H*CS), 11.51 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 20.6 (*C*H$_3$COO), 30.4 (*C*H$_2$N-3), 42.6 (NH*C*H$_2$), 47.0 (*C*H$_2$C≡CCH$_2$N-3), 52.2 (*C*H$_2$ *of piperazinyl*), 52.3 (*C*H$_2$ *of piperazinyl*), 55.3 (NHCH$_2$*C*H$_2$), 64.4 (*C-5′*), 78.9 (*C*≡*C*), 84.1 (*C-4′*), 90.5 (*C-1′*), 101.7 (*C-5*), 112.4 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.7 (*C-2′*), 133.3 (*C-3′*), 137.9 (*C-6*), 140.8 (*C-IV of pyridyl*), 146.3 (*C-VI of pyridyl*), 150.4 (*C-2*), 151.7 (*C-II of pyridyl*), 161.4 (*C-4*), 170.1 (CH$_3$*C*OO), 178.3 (*C*S); *Anal*. Calcd for C_{27} H_{32} Br N_7 O_5 S: C, 50.16; H, 4.99; N, 15.16. Found: C, 50.22; H, 5.02; N, 14.98.

Heterodimer [5′-*O*-acetyl-d4U]N^3-CH$_2$-CH = CH-CH$_2$-$N^{piperazinyl}$[Trovirdine analogue] (7f). reaction time 5 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **7f** (71%) as white crystals; R_f 0.36 [CH_2Cl_2-CH_3OH (90:10)]; mp 128°C; ^{1}H NMR ($CDCl_3$): δ 2.08 (s, 3H, C*H*$_3$COO), 2.55 (m, 8H, C*H*$_2$ *of piperazinyl*), 2.65 (t, 2H, NHCH$_2$C*H*$_2$, $J = 6.1$ Hz), 3.00 (d, 2H, C*H*$_2$CH=CHCH$_2$N-3, $J = 4.6$ Hz), 3.81 (q, 2H, NHC*H*$_2$, $J = 6.0$ Hz), 4.26 (dd, 1H, *H-5′*, $J = 12.4$, $J = 3.0$ Hz), 4.35 (dd, 1H, *H-5″*, $J = 12.4$, $J = 3.6$ Hz), 4.56 (d, 2H, C*H*$_2$N-3, $J = 3.9$ Hz), 5.07 (m, 1H, *H-4′*), 5.74 (m, 2H, C*H*=C*H*), 5.78 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.93 (d, 1H, *H-2′*, $J = 6.1$Hz), 6.31 (d, 1H, *H-3′*, $J = 6.1$ Hz), 6.88 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.04 (m, 1H, *H-1′*), 7.44 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.4$ Hz), 8.24 (d, 1H, *H-VI of pyridyl*, $J = 2.4$ Hz), 9.44 (bs, 1H, ArN*H*CS), 11.48 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 20.7 (*C*H$_3$), 42.1 (*C*H$_2$N-3), 42.8 (NH*C*H$_2$), 52.5 (*C*H$_2$ *of piperazinyl*), 53.2 (*C*H$_2$ *of*

piperazinyl), 55.4 ($NHCH_2CH_2$), 60.2 ($CH_2CH{=}CHCH_2N$-3), 64.5 (*C-5′*), 84.2 (*C-4′*), 90.6 (*C-1′*), 102.0 (*C-5*), 112.6 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.8 (*C-2′*), 126.9 (*C*H=CH), 129.9 (CH = *C*H), 133.3 (*C-3′*), 137.7 (*C-6*), 141.0 (*C-IV of pyridyl*), 146.5 (*C-VI of pyridyl*), 151.0 (*C-2*), 151.8 (*C-II of pyridyl*), 162.1 (*C-4*), 170.2 (CH_3COO), 178.5 (*C*S); *Anal.* Calcd for C_{27} H_{34} Br N_7 O_5 S: C, 50.00; H, 5.28; N, 15.12. Found: C, 49.87; H, 5.12; N, 15.17.

Heterodimer [5′-*O*-acetyl-d4T]N^3-$(CH_2)_3$-$N^{piperazinyl}$[Trovirdine analogue] (8a). reaction time 20 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **8a** (38%) as white crystals; R_f 0.33 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.86 (quint., 2H, CH_2CH_2N-3, $J = 7.0$ Hz), 1.93 (s, 3H, CH_3-5), 2.10 (s, 3H, CH_3COO), 2.48 (t, 2H, $CH_2CH_2CH_2N$-3, $J = 7.0$ Hz), 2.53 (m, 8H, CH_2 *of piperazinyl*), 2.64 (t, 2H, $NHCH_2CH_2$, $J = 5.9$ Hz), 3.81 (m, 2H, $NHCH_2$), 4.03 (t, 2H, CH_2N-3, $J = 7.0$ Hz), 4.25 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.7$ Hz), 4.39 (dd, 1H, *H-5″*, $J = 12.4$, $J = 3.8$ Hz), 5.05 (bs, 1H, *H-4′*), 5.92 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.29 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.85 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.06 (bs, 1H, *H-1′*), 7.20 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 2.1$, $J = 8.8$ Hz), 8.24 (d, 1H, *H-VI of pyridyl*, $J = 2.1$ Hz), 9.29 (bs, 1H, ArN*H*CS), 11.47 (bs, 1H, CSN*H*CH_2); ^{13}C NMR ($CDCl_3$): δ 13.3 (CH_3-5), 20.8 (CH_3COO), 24.5 (CH_2CH_2N-3), 39.9 (CH_2N-3), 42.8 ($NHCH_2$), 52.6 (CH_2 *of piperazinyl*), 53.2 (CH_2 *of piperazinyl*), 55.5 ($NHCH_2CH_2$), 55.8 ($CH_2CH_2CH_2N$-3), 64.7 (*C-5′*), 84.0 (*C*-4′), 90.4 (*C-1′*), 110.5 (*C-5*), 112.6 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 127.3 (*C-2′*), 133.0 (*C-3′*), 133.4 (*C-6*), 141.0 (*C-IV of pyridyl*), 146.6 (*C-VI of pyridyl*), 151.3 (*C-2*), 151.8 (*C-II of pyridyl*), 163.4 (*C-4*), 170.3 (CH_3COO), 178.6 (*C*S); *Anal.* Calcd for C_{27} H_{36} Br N_7 O_5 S: C, 49.85; H, 5.58; N, 15.07. Found: C, 49.74; H, 5.42; N, 14.99.

Heterodimer [5′-*O*-acetyl-d4T]N^3-$(CH_2)_4$-$N^{piperazinyl}$[Trovirdine analogue] (8b). reaction time 20 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **8b** (39%) as white crystals; R_f 0.25 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.56 (m, 2H, $CH_2(CH_2)_2N$-3), 1.68 (m, 2H, CH_2CH_2N-3), 1.93 (s, 3H, CH_3-5), 2.10 (s, 3H, CH_3COO), 2.40 (t, 2H, $CH_2(CH_2)_3N$-3, $J = 7.2$ Hz), 2.55 (m, 8H, CH_2 *of piperazinyl*), 2.65 (t, 2H, $NHCH_2CH_2$, $J = 5.7$ Hz), 3.82 (m, 2H, $NHCH_2$), 3.99 (t, 2H, CH_2N-3, $J = 6.5$ Hz), 4.25 (dd, 1H, *H-5′*, $J = 12.3$, $J = 2.0$ Hz), 4.39 (dd, 1H, *H-5″*, $J = 12.3$, $J = 3.5$ Hz), 5.05 (bs, 1H, *H-4′*), 5.92 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.29 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.91 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.07 (bs, 1H, *H-1′*), 7.20 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 1.8$ Hz), 8.24 (d, 1H, *H-VI of pyridyl*, $J = 1.8$ Hz), 9.49 (bs, 1H, ArN*H*CS), 11.50 (bs, 1H, CSN*H*CH_2); ^{13}C NMR ($CDCl_3$): δ 13.2 (CH_3-5), 20.7 (CH_3COO), 24.2 (CH_2CH_2N-3), 25.5 ($CH_2(CH_2)_2N$-3), 41.2 (CH_2N-3), 42.8 ($NHCH_2$), 52.6 (CH_2 *of piperazinyl*), 53.3 (CH_2 *of piperazinyl*), 55.4

($NHCH_2CH_2$), 58.2 ($CH_2(CH_2)_3$N-3), 64.6 (*C-5′*), 83.9 (*C-4′*), 90.3 (*C-1′*), 110.0 (*C-5*), 112.6 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 127.2 (*C-2′*), 133.0 (*C-3′*), 133.6 (*C-6*), 140.9 (*C-IV of pyridyl*), 146.5 (*C-VI of pyridyl*), 151.2 (*C-2*), 151.8 (*C-II of pyridyl*), 163.2 (*C-4*), 170.2 (CH_3COO), 178.4 (*C*S); *Anal.* Calcd for C_{28} H_{38} Br N_7 O_5 S: C, 50.60; H, 5.76; N, 14.75. Found: C, 50.49; H, 5.74; N, 14.69.

Heterodimer [5′-*O*-acetyl-d4T]N^3-$(CH_2)_6$-*N*^{piperazinyl}[Trovirdine analogue] (8c). reaction time 24 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **8c** (23%) as white crystals; R_f 0.30 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.38 (m, 4H, (CH_2)$_2$ (CH_2)$_2$N-3), 1.52 (m, 2H, $CH_2(CH_2)_4$N-3), 1.64 (m, 2H, CH_2CH_2N-3), 1.93 (s, 3H, CH_3-5), 2.10 (s, 3H, CH_3COO), 2.36 (t, 2H, $CH_2(CH_2)_5$N-3, $J = 7.6$ Hz), 2.57 (m, 8H, CH_2 *of piperazinyl*), 2.65 (t, 2H, $NHCH_2CH_2$, $J = 5.9$ Hz), 3.82 (m, 2H, $NHCH_2$), 3.95 (m, 2H, CH_2N-3), 4.25 (dd, 1H, *H-5′*, $J = 12.3$, $J = 2.7$ Hz), 4.39 (dd, 1H, *H-5″*, $J = 12.3$, $J = 3.9$ Hz), 5.05 (bs, 1H, *H-4′*), 5.92 (d, 1H, *H-2′*, $J = 5.9$ Hz), 6.28 (d, 1H, *H-3′*, $J = 5.9$ Hz), 6.87 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.07 (bs, 1H, *H-1′*), 7.20 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.2$ Hz), 8.25 (d, 1H, *H-VI of pyridyl*, $J = 2.2$ Hz), 9.35 (bs, 1H, ArN*H*CS), 11.49 (bs, 1H, CSN*H*CH_2); ^{13}C NMR ($CDCl_3$): δ 13.3 (CH_3-5), 20.8 (CH_3COO), 26.6 ($CH_2(CH_2)_2$N *of spacer*), 26.8 ($CH_2(CH_2)_2$N *of spacer*), 27.1 (CH_2CH_2N *of spacer*), 27.4 (CH_2CH_2N *of spacer*), 41.4 (CH_2N-3), 42.8 ($NHCH_2$), 52.6 (CH_2 *of piperazinyl*), 53.4 (CH_2 *of piperazinyl*), 55.4 ($NHCH_2CH_2$), 58.6 ($CH_2(CH_2)_5$N-3), 64.7 (*C-5′*), 84.0 (*C-4′*), 90.3 (*C-1′*), 110.1 (*C-5*), 112.6 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 127.4 (*C-2′*), 133.0 (*C-3′*), 133.3 (*C-6*), 141.0 (*C-IV of pyridyl*), 146.6 (*C-VI of pyridyl*), 151.2 (*C-2*), 151.8 (*C-II of pyridyl*), 163.2 (*C-4*), 170.3 (CH_3COO), 178.5 (*C*S); *Anal.* Calcd for C_{30} H_{42} Br N_7 O_5 S: C, 52.02; H, 6.11; N, 14.15. Found: C, 51.89; H, 6.14; N, 13.99.

Heterodimer [5′-*O*-acetyl-d4T]N^3-CH_2-CH_2-O-CH_2-CH_2-*N*^{piperazinyl} [Trovirdine analogue] (8d). reaction time 30 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **8d** (42%) as white crystals; R_f 0.37 [CH_2Cl_2-CH_3OH (90:10)]; mp 112°C; ^{1}H NMR ($CDCl_3$): δ 1.92 (s, 3H, CH_3-5), 2.10 (s, 3H, CH_3COO), 2.55 (m, 8H, CH_2 *of piperazinyl*), 2.59 (t, 2H, $CH_2CH_2O(CH_2)_2$N-3, $J = 5.7$ Hz), 2.64 (t, 2H, $NHCH_2CH_2$, $J = 6.0$ Hz), 3.66 (t, 2H, $CH_2O(CH_2)_2$N-3, $J = 5.7$ Hz), 3.72 (m, 2H, OCH_2CH_2N-3), 3.81 (q, 2H, $NHCH_2$, $J = 6.0$ Hz), 4.20 (t, 2H, CH_2N-3, $J = 5.8$ Hz), 4.24 (dd, 1H, *H-5′*, $J = 12.3$, $J = 2.6$ Hz), 4.38 (dd, 1H, *H-5′*, $J = 12.3$, $J = 3.9$ Hz), 5.04 (bs, 1H, *H-4′*), 5.90 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.28 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.80 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.03 (bs, 1H, *H-1′*), 7.20 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.2$ Hz), 8.25 (d, 1H, *H-VI of pyridyl*, $J = 2.2$ Hz), 9.04 (bs, 1H, ArN*H*CS), 11.45 (bs, 1H, CSN*H*CH_2); ^{13}C NMR ($CDCl_3$): δ 13.3 (CH_3-5),

20.8 (CH_3COO), 40.0 (CH_2N-3), 42.9 ($NHCH_2$), 52.5 (CH_2 *of piperazinyl*), 53.8 (CH_2 *of piperazinyl*), 55.5 ($NHCH_2CH_2$), 57.9 ($CH_2CH_2O(CH_2)_2N$-3), 64.7 (*C-5′*), 67.4 (OCH_2CH_2N-3), 68.0 ($CH_2O(CH_2)_2N$-3), 84.0 (*C-4′*), 90.4 (*C-1′*), 110.1 (*C-5*), 112.7 (*C-V of pyridyl*), 113.4 (*C-III of pyridyl*), 127.3 (*C-2′*), 133.1(*C-3′*), 133.5 (*C-6*), 141.1 (*C-IV of pyridyl*), 146.7 (*C-VI of pyridyl*), 151.3 (*C-2*), 151.7 (*C-II of pyridyl*), 163.3 (*C-4*), 170.2 (CH_3COO), 178.6 (*C*S); *Anal*. Calcd for C_{28} H_{38} Br N_7 O_6 S: C, 49.41; H, 5.63; N, 14.41. Found: C, 49.32; H, 5.67; N, 14.29.

Heterodimer [5′-*O*-acetyl-d4T]N^3-CH_2-C≡C-CH_2-$N^{piperazinyl}$[Trovirdine analogue] (8e). reaction time 16 h; chromatography eluent: methanol in dichloromethane (from 0% to 3%); yield of **8e** (48%) as white crystals; R_f 0.46 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3H, CH_3-5), 2.10 (s, 3H, CH_3COO), 2.59 (m, 8H, CH_2 *of piperazinyl*), 2.66 (t, 2H, $NHCH_2CH_2$, $J = 6.0$ Hz), 3.26 (bs, 2H, $CH_2C{\equiv}CCH_2N$-3), 3.82 (q, 2H, $NHCH_2$, $J = 5.6$ Hz), 4.25 (dd, 1H, *H-5′*, $J = 12.4$, $J = 2.9$ Hz), 4.39 (dd, 1H, *H-5″*, $J = 12.4$, $J = 4.1$ Hz), 4.78 (bs, 2H, CH_2N-3), 5.05 (m, 1H, *H-4′*), 5.92 (d, 1H, *H-2′*, $J = 6.0$ Hz), 6.29 (d, 1H, *H-3′*, $J = 6.0$ Hz), 6.85 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.06 (m, 1H, *H-1′*), 7.22 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.4$ Hz), 8.27 (d, 1H, *H-VI of pyridyl*, $J = 2.4$ Hz), 9.23 (bs, 1H, ArN*H*CS), 11.46 (bs, 1H, CSN*H*CH_2); ^{13}C NMR ($CDCl_3$): δ 13.3 (CH_3-5), 20.9 (CH_3COO), 30.9 (CH_2N-3), 43.0 ($NHCH_2$), 47.3 ($CH_2C{\equiv}CCH_2N$-3), 52.5 (CH_2 *of piperazinyl*), 52.6 (CH_2 *of piperazinyl*), 55.6 ($NHCH_2CH_2$), 64.7 (*C-5′*), 77.5 (*C*≡C), 79.4 (C≡*C*), 84.2 (*C-4′*), 90.6 (*C-1′*), 110.3 (*C-5*), 112.8 (*C-V of pyridyl*), 113.6 (*C-III of pyridyl*), 127.4 (*C-2′*), 133.2 (*C-3′*), 133.8 (*C-6*), 141.1 (*C-IV of pyridyl*), 146.7 (*C-VI of pyridyl*), 150.7 (*C-2*), 151.9 (*C-II of pyridyl*), 162.4 (*C-4*), 170.4 (CH_3COO), 178.8 (*C*S); *Anal*. Calcd for C_{28} H_{34} Br N_7 O_5 S: C, 50.91; H, 5.19; N, 14.84. Found: C, 50.84; H, 5.22; N, 14.87.

General Procedure for the Synthesis of the Heterodimers [d4U or d4T] N^3-spacer-$N^{piperazinyl}$[Trovirdine analogue] (9a–f) and (10a–e). To a solution of the protected heterodimers **(7a–f)** and **(8a–e)** in methanol was added a few crystals of NaCN. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the oily residue was purified by column chromatography (dichloromethane/methanol 85:15) to give the free heterodimers **(9a–f)** and **(10a–e)** as white crystals.

The yield of the isolated products, ^{1}H NMR and ^{13}C NMR data, and elemental analysis are indicated below each compound.

Heterodimer [d4U]N^3-$(CH_2)_3$-$N^{piperazinyl}$[Trovirdine analogue] (9a). Yield of **9a** (91%); R_f 0.18 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.78 (quint., 2H, CH_2CH_2N-3, $J = 7.0$ Hz), 2.40 (t, 2H, $CH_2CH_2CH_2N$-3, $J = 7.0$ Hz), 2.47 (m, 8H, CH_2 *of piperazinyl*), 2.58 (t, 2H, $NHCH_2CH_2$,

$J = 5.8$ Hz), 3.73–3.76 (m, 3H, NHCH_2 and *H-5′*), 3.83 (dd, 1H, *H-5″*, $J = 11.9$, $J = 2.2$ Hz), 3.91 (t, 2H, CH_2N-3, $J = 7.0$ Hz), 4.88 (bs, 1H, *H-4′*), 5.65 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.81 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.29 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.84 (d, 1H, *H-III of pyridyl*, $J = 8.9$ Hz), 6.97 (bs, 1H, *H-1′*), 7.64–7.68 (m, 2H, *H-6* and *H-IV of pyridyl*), 8.16 (d, 1H, *H-VI of pyridyl*, $J = 2.0$ Hz), 9.47 (bs, 1H, ArN*H*CS), 11.43 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$) δ 24.3 (*C*H_2CH$_2$N-3), 39.5 (*C*H_2N-3), 42.6 (NH*C*H_2), 52.4 (*C*H_2 *of piperazinyl*), 53.0 (*C*H_2 *of piperazinyl*), 55.4 (NHCH$_2$*C*H_2), 55.7 (*C*H_2CH$_2$CH$_2$N-3), 62.9 (*C-5′*), 87.2 (*C-4′*), 90.6 (*C-1′*), 101.6 (*C-5*), 112.6 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.1 (*C-2′*), 134.4 (*C-3′*), 138.8 (*C-6*), 140.9 (*C-IV of pyridyl*), 146.4 (*C-VI of pyridyl*), 151.3 (*C-2*), 151.7 (*C-II of pyridyl*), 162.8 (*C-4*), 178.4 (*C*S); *Anal*. Calcd for C_{24} H_{32} Br N_7 O_4 S: C, 48.49; H, 5.42; N, 16.49. Found: C, 48.47; H, 5.40; N, 16.41.

Heterodimer [d4U]N^3-$(CH_2)_4$-$N^{piperazinyl}$[Trovirdine analogue] (9b). Yield of **9b** (98%); R_f 0.20 [CH_2Cl_2-CH_3OH (85:15)]; mp 181°C; ^{1}H NMR ($CDCl_3$): δ 1.54–1.60 (m, 2H, CH_2(CH$_2$)$_2$N-3), 1.63–1.69 (m, 2H, CH_2CH$_2$N-3), 2.40 (t, 2H, CH_2(CH$_2$)$_3$N-3, $J = 7.4$ Hz), 2.55 (m, 8H, CH_2 *of piperazinyl*), 2.65 (t, 2H, NHCH$_2$CH_2, $J = 6.0$ Hz), 3.78–3.82 (m, 3H, *H-5′* and NHCH_2), 3.90–3.97 (m, 3H, *H-5″* and CH_2N-3), 4.94 (m, 1H, *H-4′*), 5.72 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.87 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.34 (d, 1H, *H-3′*, $J = 5.8$ Hz), 6.79 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.03 (m, 1H, *H-1′*), 7.63 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.4$ Hz), 8.25 (d, 1H, *H-VI of pyridyl*, $J = 2.4$ Hz), 9.02 (bs, 1H, ArN*H*CS), 11.46 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 24.1 (*C*H_2CH$_2$N-*3*), 25.5 (*C*H_2(CH$_2$)$_2$N-3), 41.0 (*C*H_2N-3), 42.9 (NH*C*H_2), 52.6 (*C*H_2 *of piperazinyl*), 53.4 (*C*H_2 *of piperazinyl*), 55.5 (NHCH$_2$*C*H_2), 58.2 (CH_2(CH$_2$)$_3$N-3), 63.5 (*C-5′*), 87.1 (*C-4′*), 90.8 (*C-1′*), 102.0 (*C-5*), 112.8 (*C-V of pyridyl*), 113.4 (*C-III of pyridyl*), 126.4 (*C-2′*), 134.4 (*C-3′*), 138.5 (*C-6*), 141.1 (*C-IV of pyridyl*), 146.7 (*C-VI of pyridyl*), 151.4 (*C-2*), 151.7 (*C-II of pyridyl*), 162.8 (*C-4*), 178.6 (*C*S); *Anal*. Calcd for C_{25} H_{34} Br N_7 O_4 S: C, 49.34; H, 5.63; N, 16.11. Found: C, 49.30; H, 5.60; N, 16.08.

Heterodimer [d4U]N^3-$(CH_2)_6$-$N^{piperazinyl}$[Trovirdine analogue] (9c). Yield of **9c** (90%); R_f 0.20 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.36 (m, 4H, (CH_2)$_2$(CH$_2$)$_2$N-3), 1.52 (m, 2H, CH_2(CH$_2$)$_4$N-3), 1.63 (m, 2H, CH_2CH$_2$N-3), 2.37 (t, 2H, CH_2(CH$_2$)$_5$N-3, $J = 7.7$ Hz), 2.58 (m, 8H, CH_2 *of piperazinyl*), 2.66 (t, 2H, NHCH$_2$CH_2, $J = 6.1$ Hz), 3.77–3.82 (m, 3H, *H-5′* and NHCH_2), 3.89–3.93 (m, 3H, *H-5″* and CH_2N-3), 4.94 (bs, 1H, *H-4′*), 5.72 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.87 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.34 (d, 1H, *H-3′*, $J = 5.8$ Hz), 6.83 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.04 (bs, 1H, *H-1′*), 7.67 (d, 1H, *H-6*, $J = 8.0$ Hz), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.2$ Hz), 8.25 (d, 1H, *H-VI of pyridyl*, $J = 2.2$ Hz), 9.21 (bs, 1H, ArN*H*CS), 11.47 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 26.3 (*C*H_2(CH$_2$)$_2$N *of spacer*), 26.7 (*C*H_2(CH$_2$)$_2$N *of spacer*), 27.1 (*C*H_2CH$_2$N *of spacer*), 27.3 (*C*H_2CH$_2$N *of spacer*), 41.1 (*C*H_2N-3), 42.8

($NHCH_2$), 52.4 (CH_2 *of piperazinyl*), 53.3 (CH_2 *of piperazinyl*), 55.4 ($NHCH_2CH_2$), 58.5 ($CH_2(CH_2)_5$N-3), 63.2 (*C-5′*), 87.2 (*C-4′*), 90.8 (*C-1′*), 101.9 (*C-5*), 112.7 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.3 (*C-2′*), 134.4 (*C-3′*), 138.6 (*C-6*), 141.1 (*C-IV of pyridyl*), 146.6 (*C-VI of pyridyl*), 151.4 (*C-2*), 151.7 (*C-II of pyridyl*), 162.8 (*C-4*), 178.6 (*C*S); *Anal*. Calcd for $C_{27}H_{38}BrN_7O_4S$: C, 50.94; H, 6.02; N, 15.40. Found: C, 50.90; H, 5.97; N, 15.37.

Heterodimer [d4U]N^3-CH_2-CH_2-O-CH_2-CH_2-$N^{piperazinyl}$[Trovirdine analogue] (9d). Yield of **9d** (91%); R_f 0.21 [CH_2Cl_2-CH_3OH (90:10)]; mp 120°C; ^{1}H NMR ($CDCl_3$): δ 2.48–2.53 (m, 10H, CH_2 *of piperazinyl* and CH_2CH$_2$O(CH$_2$)$_2$N-3), 2.58 (t, 2H, NHCH$_2$CH_2, $J = 5.5$ Hz), 3.58 (t, 2H, CH_2O(CH$_2$)$_2$N-3, $J = 4.6$ Hz), 3.64 (t, 2H, OCH_2CH$_2$N-3, $J = 5.2$ Hz), 3.69–3.75 (m, 3H, *H-5′* and NHCH_2), 3.82 (dd, 1H, *H-5′*, $J = 12.0$, $J = 2.9$ Hz), 4.12 (m, 2H, CH_2N-3), 4.86 (bs, 1H, *H-4′*), 5.64 (d, 1H, *H-5*, $J = 8.0$ Hz), 5.79 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.29 (d, 1H, *H*-3′, $J = 5.8$ Hz), and *H-IV of pyridyl*), 8.17 (bs, 1H, *H-VI of pyridyl*), 9.46 (bs, 1H, ArN*H*CS), 11.42 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 39.7 (*C*H$_2$N-3), 42.6 (NH*C*H$_2$), 52.3 (*C*H$_2$ *of piperazinyl*), 53.4 (*C*H$_2$ *of piperazinyl*), 55.4 (NHCH$_2$*C*H$_2$), 57.6 (*C*H$_2$CH$_2$O(CH$_2$)$_2$N-3), 62.8 (*C-5′*), 67.1 (O*C*H$_2$CH$_2$N-3), 68.1 (*C*H$_2$O(CH$_2$)$_2$N-3), 87.2 (*C-4′*), 90.6 (*C-1′*), 101.4 (*C-5*), 112.6 (*C-V of pyridyl*), 113.6 (*C-III of pyridyl*), 125.9 (*C-2′*), 134.6 (*C*-3′), 138.9 (*C-6*), 140.9 (*C-IV of pyridyl*), 146.4 (*C-VI of pyridyl*), 151.4 (*C-2*), 151.5 (*C-II of pyridyl*), 162.8 (*C-4*), 178.5 (*C*S); *Anal*. Calcd for $C_{25}H_{34}BrN_7O_5S$: C, 48.08; H, 5.49; N, 15.70. Found: C, 48.00; H, 5.47; N, 15.67.

Heterodimer [d4U]N^3-CH_2-C≡C-CH_2-$N^{piperazinyl}$[Trovirdine analogue] (9e). Yield of **9e** (82%); R_f 0.36 [CH_2Cl_2-CH_3OH (90:10)]; mp 150°C; ^{1}H NMR ($CDCl_3$): δ 2.59 (m, 8H, CH_2 *of piperazinyl*), 2.65 (t, 2H, NHCH$_2$CH_2, $J = 6.1$ Hz), 3.26 (bs, 2H, CH_2C≡CCH$_2$N-3), 3.76–3.84 (m, 3H, *H-5′* and NHCH_2), 3.91 (dd, 1H, *H-5″*, $J = 12.2$, $J = 2.5$ Hz), 4.74 (bs, 2H, CH_2N-3), 4.94 (bs, 1H, *H-4′*), 5.74 (d, 1H, *H-5*, $J = 8.1$ Hz), 5.87 (d, 1H, *H-2′*, $J = 5.9$ Hz), 6.35 (d, 1H, *H-3′*, $J = 5.9$ Hz), 6.82 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.03 (bs, 1H, *H-1′*), 7.72 (m, 2H, *H-6* and *H-IV of pyridyl*), 8.27 (d, 1H, *H-VI of pyridyl*, $J = 2.1$ Hz), 9.19 (bs, 1H, ArN*H*CS), 11.44 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 30.6 (*C*H$_2$N-3), 42.9 (NH*C*H$_2$), 47.1 (C*H*$_2$C≡C*C*H$_2$N-3), 52.3 (*C*H$_2$ *of piperazinyl*), 52.5 (*C*H$_2$ *of piperazinyl*), 55.5 (NHCH$_2$*C*H$_2$), 63.1 (*C-5′*), 79.3 (*C*≡*C*), 87.2 (*C-4′*), 90.8 (*C-1′*), 101.7 (*C-5*), 112.7 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.3 (*C-2′*), 134.5 (*C-3′*), 139.1 (*C-6*), 141.1 (*C-IV of pyridyl*), 146.6 (*C-VI of pyridyl*), 150.8 (*C-2*), 151.7 (*C-II of pyridyl*), 161.8 (*C-4*), 178.4 (*C*S); *Anal*. Calcd for $C_{25}H_{30}BrN_7O_4S$: C, 49.67; H, 5.00; N, 16.22. Found: C, 49.60; H, 4.94; N, 16.19.

Heterodimer [d4U]N^3-CH_2-CH=CH-CH_2-$N^{piperazinyl}$[Trovirdine analogue] (9f). Yield of **9f** (74%); R_f 069 [CH_2Cl_2-CH_3OH (80:20)]; mp 126°C;

^{1}H NMR ($CDCl_3$): δ 2.56 (m, 8H, CH_2 *of piperazinyl*), 2.64 (m, 2H, NHCH$_2$CH_2), 3.01 (m, 2H, CH_2CH=CHCH$_2$N-3), 3.80–3.95 (m, 4H, *H-5′*,*H-5″* and NHCH_2), 4.55 (m, 2H, CH_2N-3), 4.96 (m, 1H, *H-4′*), 5.77 (m, 3H, C*H*=C*H* and *H-5*), 5.89 (d, 1H, *H-2′*, $J = 6.8$ Hz), 6.37 (d, 1H, *H-3′*, $J = 6.8$ Hz), 6.83 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.04 (m, 1H, *H-1′*), 7.73 (m, 2H, *H-6* and *H-IV of pyridyl*), 8.26 (d, 1H, *H-VI of pyridyl*, $J = 2.2$ Hz), 9.23 (bs, 1H, ArN*H*CS), 11.49 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 42.1 (*C*H$_2$N-3), 42.8 (NH*C*H$_2$), 52.5 (*C*H$_2$ *of piperazinyl*), 53.2 (*C*H$_2$ *of piperazinyl*), 55.5 (NHCH$_2$*C*H$_2$), 60.2 (CH_2CH=CHCH$_2$N-3), 63.1 (*C-5′*), 87.2 (*C-4′*), 90.8 (*C-1′*), 101.8 (*C-5*), 112.7 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.2 (*C-2′*), 127.1 (*C*H=CH), 129.7 (CH=*C*H), 134.5 (*C-3′*), 138.8 (*C-IV of pyridyl*), 141.1 (*C-6*), 146.6 (*C-VI of pyridyl*), 151.2 (*C-2*), 151.7 (*C-II of pyridyl*), 162.5 (*C-4*), 178.7 (*C*S); *Anal*. Calcd for C_{25} H_{32} Br N_7 O_4 S: C, 49.51; H, 5.32; N, 16.17. Found: C, 49.45; H, 5.27; N, 15.98.

Heterodimer [d4T]*N*^{3}-(CH$_2$)$_3$-*N*^{piperazinyl}[Trovirdine analogue] (10a). Yield of **10a** (89%); R_f 0.21 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.85 (m, 2H, CH_2CH$_2$N-3), 1.88 (s, 3H, CH_3-5), 2.47 (t, 2H, CH_2CH$_2$CH$_2$N-3, $J = 7.1$ Hz), 2.54 (m, 8H, CH_2 *of piperazinyl*), 2.64 (t, 2H, NHCH$_2$CH_2, $J = 5.9$ Hz), 3.78–3.82 (m, 3H, NHCH_2 and *H-5′*), 3.91 (dd, 1H, *H-5″*, $J = 12.1$, $J = 2.5$ Hz), 4.00 (t, 2H, CH_2N-3, $J = 7.2$ Hz), 4.92 (bs, 1H, *H-4′*), 5.87 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.34 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.81 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.05 (bs, 1H, *H-1′*), 7.48 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.1$ Hz), 8.23 (d, 1H, *H-VI of pyridyl*, $J = 2.1$ Hz), 9.20 (bs, 1H, ArN*H*CS), 11.46 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 13.1 (*C*H$_3$-5), 24.5 (*C*H$_2$CH$_2$N-3), 39.8 (*C*H$_2$N-3), 42.8 (NH*C*H$_2$), 52.6 (*C*H$_2$ *of piperazinyl*), 53.1 (*C*H$_2$ *of piperazinyl*), 55.5 (NHCH$_2$*C*H$_2$), 55.8 (*C*H$_2$CH$_2$CH$_2$N-3), 63.5 (*C-5′*), 87.0 (*C-4′*), 90.6 (*C-1′*), 110.0 (*C-5*), 112.7 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.5 (*C-2′*), 134.2 (*C-3′*), 134.6 (*C-6*), 141.0 (*C-IV of pyridyl*), 146.6 (*C-VI of pyridyl*), 151.5 (*C-2*), 151.7 (*C-II of pyridyl*), 163.5 (*C-4*), 178.6 (*C*S); *Anal*. Calcd for C_{25} H_{34} Br N_7 O_4 S: C, 49.34; H, 5.63; N, 16.11. Found: C, 49.27; H, 5.58; N, 15.96.

Heterodimer [d4T]*N*^{3}-(CH$_2$)$_4$-*N*^{piperazinyl}[Trovirdine analogue] (10b). Yield of **10b** (98%); R_f 0.20 [CH_2Cl_2-CH_3OH (90:10)]; mp 186°C; ^{1}H NMR ($CDCl_3$): δ 1.52–1.57 (m, 2H, CH_2(CH$_2$)$_2$N-3), 1.60–1.66 (m, 2H, CH_2CH$_2$N-3), 1.88 (s, 3H, CH_3-5), 2.38 (t, 2H, CH_2(CH$_2$)$_3$N-3, $J = 7.2$ Hz), 2.55 (m, 8H, CH_2 *of piperazinyl*), 2.64 (t, 2H, NHCH$_2$CH_2, $J = 5.9$ Hz), 3.77–3.83 (m, 3H, *H-5′* and NHCH_2), 3.89–3.98 (m, 3H, *H-5″* and CH_2N-3), 4.93 (bs, 1H, *H-4′*), 5.86 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.34 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.86 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.06 (bs, 1H, *H-1′*), 7.45 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.1$ Hz), 8.24 (d, 1H, *H-VI of pyridyl*, $J = 2.1$ Hz), 9.29 (bs, 1H, ArN*H*CS), 11.47 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 13.1 (*C*H$_3$-5), 24.1 (*C*H$_2$CH$_2$N-3), 25.5 (*C*H$_2$(CH$_2$)$_2$N-3), 41.2 (*C*H$_2$N-3), 42.8 (NH*C*H$_2$), 52.6

(*C*H_2 *of piperazinyl*), 53.4 (*C*H_2 *of piperazinyl*), 55.5 (NHCH$_2$*C*H$_2$), 58.2 (*C*H$_2$(CH$_2$)$_3$N-3), 63.5 (*C-5′*), 87.0 (*C-4′*), 90.6 (*C-1′*), 110.0 (*C-5*), 112.7 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.5 (*C-2′*), 134.2 (*C-3′*), 134.4 (*C-6*), 141.0 (*C-IV of pyridyl*), 146.5 (*C-VI of pyridyl*), 151.4 (*C-2*), 151.8 (*C-II of pyridyl*), 163.4 (*C-4*), 178.5 (*C*S); *Anal*. Calcd for C_{26} H_{36} Br N_7 O_4 S: C, 50.16; H, 5.83; N, 15.75. Found: C, 50.10; H, 5.74; N, 15.67.

Heterodimer [d4T]*N*^{3}-(CH$_2$)$_6$-*N*^{piperazinyl}[Trovirdine analogue] (10c). Yield of **10c** (98%); R_f 0.15 [CH_2Cl_2-CH_3OH (90:10)]; ^{1}H NMR ($CDCl_3$): δ 1.36 (m, 4H, (C*H*$_2$)$_2$(CH$_2$)$_2$N-3), 1.52 (m, 2H, C*H*$_2$(CH$_2$)$_4$N-3), 1.62 (m, 2H, *C*H$_2$CH$_2$N-3), 1.88 (s, 3H, C*H*$_3$-5), 2.37 (t, 2H, *C*H$_2$(CH$_2$)$_5$N-3, $J = 7.6$ Hz), 2.58 (m, 8H, C*H*$_2$ *of piperazinyl*), 2.65 (t, 2H, NHCH$_2$C*H*$_2$, $J = 5.9$ Hz), 3.76–3.82 (m, 3H, *H-5′* and NHC*H*$_2$), 3.87–3.93 (m, 3H, *H-5″* and C*H*$_2$N-3), 4.92 (bs, 1H, *H-4′*), 5.85 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.33 (d, 1H, *H-3′*, $J = 5.8$ Hz), 6.79 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.05 (bs, 1H, *H-1′*), 7.46 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.0$ Hz), 8.24 (bs, 1H, *H-VI of pyridyl*), 9.15 (bs, 1H, ArN*H*CS), 11.45 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 13.1 (*C*H$_3$-5), 26.3 (*C*H$_2$(CH$_2$)$_2$N *of spacer*), 26.7 (*C*H$_2$(CH$_2$)$_2$N *of spacer*), 27.1 (*C*H$_2$CH$_2$N *of spacer*), 27.3 (*C*H$_2$CH$_2$N *of spacer*), 41.3 (*C*H$_2$N-3), 42.8 (NH*C*H$_2$), 52.3 (*C*H$_2$ *of piperazinyl*), 53.2 (*C*H$_2$ *of piperazinyl*), 55.4 (NHCH$_2$*C*H$_2$), 58.5 (*C*H$_2$(CH$_2$)$_5$N-3), 63.3 (*C-5′*), 87.0 (*C-4′*), 90.6 (*C-1′*), 110.0 (*C-5*), 112.7 (*C-V of pyridyl*), 113.5 (*C-III of pyridyl*), 126.5 (*C-2′*), 134.2 (*C-3′*), 134.5 (*C-6*), 141.1 (*C-IV of pyridyl*), 146.6 (*C-VI of pyridyl*), 151.5 (*C-2*), 151.7 (*C-II of pyridyl*), 163.5 (*C-4*), 178.7 (*C*S); *Anal*. Calcd for C_{28} H_{40} Br N_7 O_4 S: C, 51.69; H, 6.20; N, 15.07. Found: C, 51.60; H, 6.14; N, 14.92.

Heterodimer [d4T]*N*^{3}-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-*N*^{piperazinyl}[Trovirdine analogue] (10d). Yield of **10d** (40%); R_f 0.14 [CH_2Cl_2-CH_3OH (90:10)]; mp 130°C; ^{1}H NMR ($CDCl_3$): δ 1.87 (s, 3H, C*H*$_3$-5), 2.53 (m, 8H, C*H*$_2$ *of piperazinyl*), 2.57 (t, 2H, C*H*$_2$CH$_2$O(CH$_2$)$_2$N-3, $J = 5.3$ Hz), 2.63 (t, 2H, NHCH$_2$C*H*$_2$, $J = 6.1$ Hz), 3.64 (t, 2H, C*H*$_2$O(CH$_2$)$_2$N-3, $J = 5.2$ Hz), 3.71 (t, 2H, OC*H*$_2$CH$_2$N-3, $J = 5.7$ Hz), 3.76–3.82 (m, 3H, *H-5′* and NHC*H*$_2$), 3.90 (dd, 1H, *H-5″*, $J = 12.2$, $J = 2.1$ Hz), 4.20 (m, 2H, C*H*$_2$N-3), 4.92 (bs, 1H, *H-4′*), 5.85 (d, 1H, *H-2′*, $J = 5.8$ Hz), 6.33 (d, 1H, *H-3′*, $J = 5.8$ Hz), 6.79 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.03 (bs, 1H, *H-1′*), 7.44 (s, 1H, *H-6*), 7.72 (dd, 1H, *H-IV of pyridyl*, $J = 8.8$, $J = 2.2$ Hz), 8.25 (d, 1H, *H-VI of pyridyl*, $J = 1.9$ Hz), 8.99 (bs, 1H, ArN*H*CS), 11.43 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 13.1 (*C*H$_3$-5), 40.0 (*C*H$_2$N-3), 42.9 (NH*C*H$_2$), 52.6 (*C*H$_2$ *of piperazinyl*), 53.6 (*C*H$_2$ *of piperazinyl*), 55.6 (NHCH$_2$*C*H$_2$), 57.8 (*C*H$_2$CH$_2$O-(CH$_2$)$_2$N-3), 63.3 (*C-5′*), 67.3 (OCH$_2$CH$_2$N-3), 68.4 (*C*H$_2$O(CH$_2$)$_2$N-3), 87.0 (*C-4′*), 90.7 (*C-1′*), 109.9 (*C-5*), 112.8 (*C-V of pyridyl*), 113.4 (*C-III of pyridyl*), 126.5 (*C-2′*), 134.3 (*C-3′*), 134.6 (*C-6*), 141.1 (*C-IV of pyridyl*), 146.7 (*C-VI of pyridyl*), 151.4 (*C-2*), 151.7 (*C-II of pyridyl*), 163.3 (*C-4*), 178.6 (*C*S); *Anal*.

Calcd for $C_{26}H_{36}BrN_7O_5S$: C, 48.90; H, 5.68; N, 15.35. Found: C, 48.81; H, 5.74; N, 15.48.

Heterodimer [d4T]N^3-CH_2-C≡C-CH_2-$N^{piperazinyl}$[Trovirdine analogue] (10e). Yield of **10e** (95%); R_f 0.36 [CH_2Cl_2-CH_3OH (90:10)]; mp 119°C; ^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3H, C*H*$_3$-5), 2.60 (m, 8H, C*H*$_2$ *of piperazinyl*), 2.66 (t, 2H, NHCH$_2$C*H*$_2$, $J = 6.0$ Hz), 3.26 (bs, 2H, C*H*$_2$C≡CCH$_2$N-3), 3.78–3.83 (m, 4H, *H-5′* and NHC*H*$_2$), 4.76 (bs, 2H, C*H*$_2$N-3), 4.92 (m, 1H, *H-4′*), 5.86 (d, 1H, *H-2′*, $J = 5.7$ Hz), 6.36 (d, 1H, *H-3′*, $J = 5.7$ Hz), 6.87 (d, 1H, *H-III of pyridyl*, $J = 8.8$ Hz), 7.05 (bs, 1H, *H-1′*), 7.59 (s, 1H, *H-6*), 7.73 (dd, 1H, *H-IV of pyridyl*, $J = 8.7$, $J = 2.0$ Hz), 8.26 (bs, 1H, *H-VI of pyridyl*), 9.44 (bs, 1H, ArN*H*CS), 11.49 (bs, 1H, CSN*H*CH$_2$); ^{13}C NMR ($CDCl_3$): δ 13.2 (C*H*$_3$-5), 31.0 (*C*H$_2$N-3), 42.9 (NH*C*H$_2$), 47.3 (*C*H$_2$C≡CCH$_2$N-3), 52.4 (*C*H$_2$ *of piperazinyl*), 52.6 (*C*H$_2$ *of piperazinyl*), 55.7 (NHCH$_2$*C*H$_2$), 63.3 (*C-5′*), 77.3 (*C*≡C), 79.8 (C≡*C*), 87.4 (*C-4′*), 90.8 (*C-1′*), 110.1 (*C-5*), 112.9 (*C-V of pyridyl*), 113.9 (*C-III of pyridyl*), 126.5 (*C-2′*), 134.7 (*C-3′*), 135.3 (*C-6*), 141.3 (*C-IV of pyridyl*), 146.7 (*C-VI of pyridyl*), 151.0 (*C-2*), 152.1 (*C-II of pyridyl*), 163.0 (*C-4*), 179.0 (*C*S); *Anal*. Calcd for $C_{26}H_{32}BrN_7O_4S$: C, 50.49; H, 5.21; N, 15.85. Found: C, 50.29; H, 4.98; N, 15.73.

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 nm adsorption, free virus particles were washed out and cells were re-suspended in RPMI-1640 plus 10% foetal calf serum at a final concentration of 10^5 cells mL^{-1} in the presence of different concentrations of test compounds. After 5 days, virus production was measured by RT assay as already described.[40] The 50% inhibitory concentration (IC_{50}) was derived from the computer generated median effect plot of the dose-effect data.[41] 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).[42] 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;[40,43] 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.[40] In vitro RT inhibition was also described.[40] The CEM-SS cells were obtained from P. Nara through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (Bethesda, Md., USA).

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