Potential Multifunctional Inhibitors of HIV-1 Reverse Transcriptase. Novel [AZT]-[TSAO-T] and [d4T]-[TSAO-T] Heterodimers Modified in the Linker and in the Dideoxynucleoside Region

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In an attempt to combine the anti-HIV-inhibitory capacity of nucleoside reverse transcriptase (RT) inhibitors (NRTI) and non-nucleoside RT inhibitors (NNRTI), several heterodimer analogues of the previously reported [AZT]-$(CH_2)_3$-[TSAO-T] prototype have been prepared. In these novel series, other NRTIs, an expanded range of linkers with different conformational freedom and other attachment sites for these linkers on the base part of the NRTI analogue have been explored. Moreover, in order to circumvent the dependence of the NRTI moiety of the heterodimer on activation by cellular nucleoside kinases, novel heterodimers in which the NRTI is bearing a masked monophosphate group at the 5′-position are described. Among the novel heterodimers, several derivatives show a potent anti-HIV-1 activity, which proved comparable, or even superior, to that of the AZT heterodimer prototype. The nature of the NRTI was important for the eventual anti-HIV-1 activity. In particular, the d4T heterodimer derivative containing a propyl linker between the N-3 positions of the base of TSAO-T and d4T was ∼5- to 10-fold more inhibitory to HIV-1 than the corresponding AZT heterodimer prototype.

Introduction

Reverse transcriptase (RT) represents an attractive target for the chemotherapy of human immunodeficiency virus (HIV) infection because of its key role in virus replication.[1,2] Several members of different classes of HIV inhibitors that are targeted at the virus-encoded RT are currently approved for the treatment of HIV-infected individuals. Among them, the nucleoside RT inhibitors (NRTIs) such as 3′-azido-2′,3′-dideoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxycytidine (ddC), 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T), and (−)2′,3′-dideoxy-3′-thiacytidine (3TC) have been widely used to treat AIDS patients.[3,4] Another important group of RT inhibitors is the so-called non-nucleoside RT inhibitors (NNRTIs), i.e., nevirapine, delavirdine, efavirenz, emivirine, PETT, Quinoxaline, GW420-867X, TSAO, etc., which are structurally diverse but highly specific to HIV-1 RT.[5−7] Of the NNRTIs, nevirapine, delavirdine, and efavirenz have been formally approved for the treatment of HIV-1 infection in combination with NRTIs and/or protease inhibitors.[5−7] They 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.[8−10]

Among NNRTIs, the TSAO nucleosides[11,12] occupy a unique position in that they interfere at the interface between the p51 and p66 subunits of HIV-1 RT.[13a] Well-defined amino acids at both the p51 and p66 RT subunits are needed for an optimum interaction of TSAO with the HIV-1 RT.[12−15] Our experimental data strongly suggest a specific interaction of the 3′-spiro moiety of TSAO molecules with the glutamic acid residue at position 138 (Glu-138) of the p51 subunit of HIV-1 RT.[13b,14]

To obtain a sustained benefit from antiviral therapy, combination of different anti-HIV agents has become the standard clinical practice to prevent emergence of virus-drug resistance.[16−19] An interesting alternative approach to combination therapy, supported by structural[8−10] and biochemical studies,[20] would be the use of heterodimers resulting from the linking of a NNRTI and an NRTI through an appropriate spacer in an attempt to combine the inhibitory capacity of these two different classes of molecules. With this aim, in 1995, we reported for the first time[21,22] the synthesis and anti-HIV activity assessment of a series of heterodimers which combine in their structure an NRTI analogue such as AZT, or the natural substrate dThd, and an NNRTI such as TSAO-T or HEPT linked through flexible polymethylene spacers between the N-3 positions of the thymine bases of both compounds. The most active compound of this series was the [TSAO-T]-$(CH_2)_3$-[AZT] heterodimer (**1**). Polymethylene linkers [-$(CH_2)_n$-] with $n = 4-6$ showed good antiviral activity, while longer spacers $n > 7$ dramatically disminished activity. However, they were less potent inhibitors than the parent compounds from which they were derived.[21]

To obtain better insights in the feasibility of this heterodimer approach to increase the inhibitory efficacy of the test compounds against HIV-RT, this paper describes the synthesis and anti-HIV evaluation of a novel series of [NRTI]-spacer-[TSAO] heterodimers.[23] In

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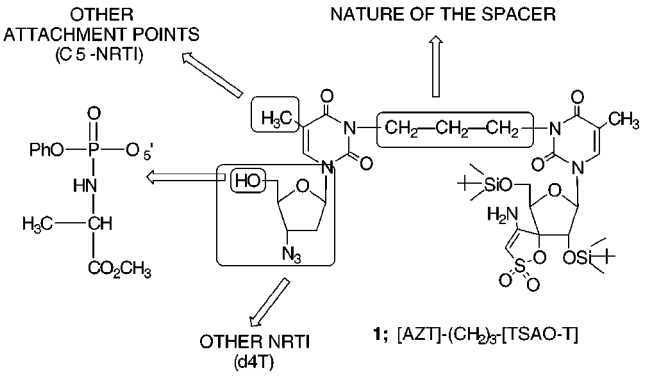


Figure 1. Modifications carried out on the TSAO-spacer-NRTI heterodimer molecule.

these novel series, several modifications in the spacer and in the NRTI moiety of the model heterodimer (**1**) were addressed (Figure 1).

First, we focused on the extension of the heterodimer approach to other approved NRTIs, and therefore, we replaced the AZT in the model heterodimer (**1**) by d4T.

Since NRTIs, and also dThd, interact with RT only after they have been converted to their 5′-triphosphate form, and since it is also likely that the [TSAO-T]-$(CH_2)_n$-[NRTI] heterodimers would not be recognized by the cellular kinases involved in the three phosphorylation steps, we prepared novel heterodimers in which the NRTI (AZT, d4T) or the natural substrate (dThd) bear at the 5′-position a masked monophosphate group. Among the most established and successful pro-nucleotide approaches the aryloxyaminoacyl-phosphoramidates introduced by McGuigan et al.[24] have been chosen based on the pronounced anti-HIV activity of the phenylphosphoramidate derivatives of AZT[25] and d4T.[26] We also prepared a series of [AZT]-spacer-[TSAO-T] heterodimers in which the flexible polymethylene spacer of the heterodimer prototype (**1**) was replaced by an expanded range of spacers of different nature and conformational freedom.

With the TSAO derivatives, we have ascertained that linkage of the methylene spacer to the N-3 position of the thymine base maintains the antiviral activity.[27] In contrast, attachment points of the spacer to the NRTI other than the N-3 position were explored to preserve potential base-pairing of the template with the heterodimer.

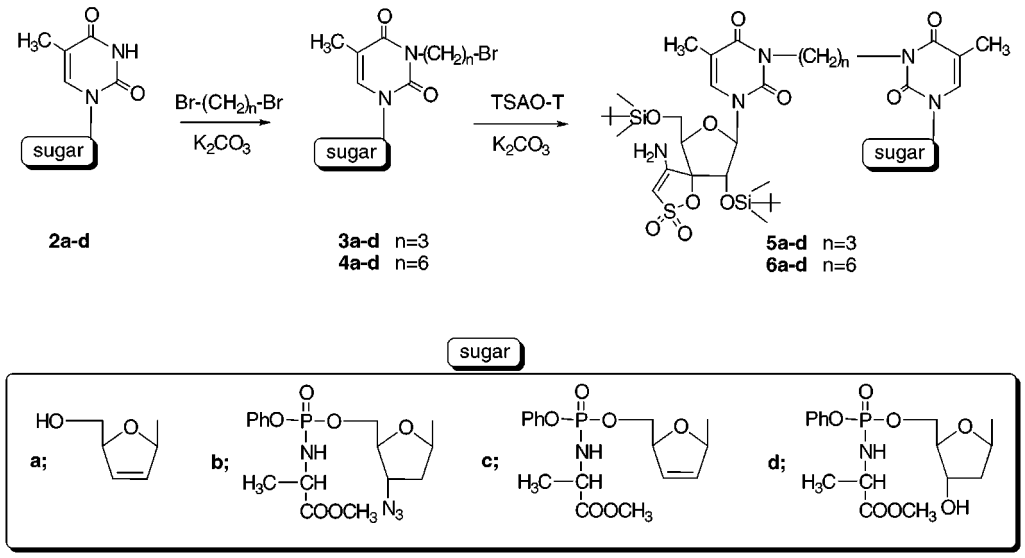
Chemistry

In an attempt to investigate the role of the NRTI in the activity of the heterodimers we first addressed our attention to the synthesis of [d4T]-$(CH_2)_n$-[TSAO-T] (**5a**, **6a**) heterodimers. The synthesis of these compounds was accomplished according to the method described in our previous paper.[21] Thus, as shown in Scheme 1, treatment of d4T (**2a**) with 2 equiv of 1,3-dibromopropane or 1,6-dibromohexane in dry acetone:DMF (1:1) and in the presence of potassium carbonate (K_2CO_3) gave the N-3-substituted derivatives **3a** (75%) and **4a** (75%). Subsequent reaction of these intermediates with TSAO-T, under basic conditions (K_2CO_3), gave heterodimers **5a** (50%) and **6a** (60%). The synthesis of a series of 5′-(phenylphosphoramidates) heterodimers **5b**−**d** and **6b**−**d** was carried out in a similar way starting from the corresponding 5′-phenylphosphoramidate derivative of AZT (**2b**),[25] d4T (**2c**),[26] and dThd (**2d**) (Scheme 1). Intermediates **2b** and **2c** were prepared as described.[25,26] Compound **2d** was prepared by reaction of thymidine with (phenylmethoxy)alaninyl chlorophosphate[28] in THF in the presence of *N*-methylimidazole. These starting materials were a mixture of two diastereoisomers, in an approximately 1:1 ratio, resulting from the mixed stereochemistry at the phosphate center and were used as isomeric mixtures in the synthetic sequence.

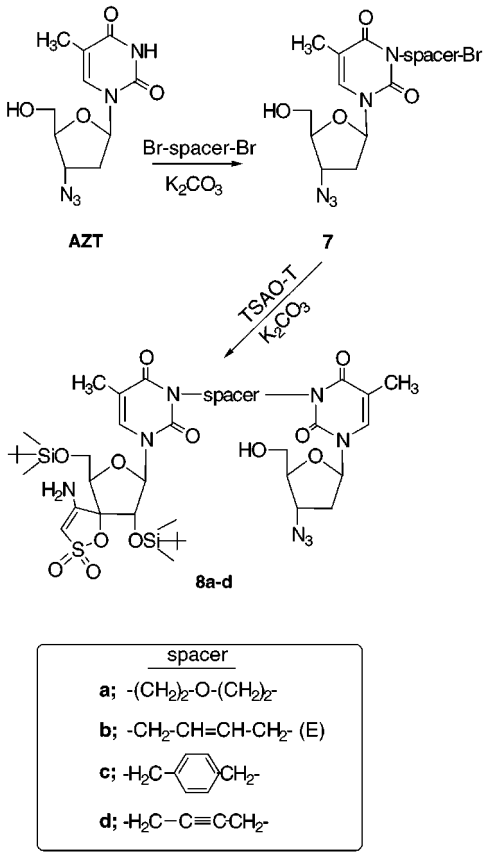
Next, we focused on modifications on the spacer in the model heterodimer. The synthesis of [AZT]N^3-spacer-N^3[TSAO-T] heterodimers **8a**−**d** was carried out as shown in Scheme 2. Spacers no longer than six atoms were introduced based on our previous biological results.[21] Reaction of AZT with commercially available 2-bromoethyl ether, 1,4-dibromo-2-butene (E), α,α′-dibromo-*p*-xylene, and 1,4-dichloro-2-butyne reagents, in the presence of K_2CO_3, yielded the *N*-3-bromo intermediates of AZT **7a**−**d** (92%, 80%, 75%, and 35%, respectively). Treatment of these intermediates with TSAO-T gave the corresponding heterodimers **8a**−**d** (75%, 73%, 92%, and 23%, respectively). The lower yields of **7d** and **8d** could be explained by the lower reactivity of the corresponding reagent (1,4-dichloro-2-butyne) that led to reaction mixtures of the final product together with unreacted starting material. Longer reaction times did not improve the yields due to partial deprotection of the *tert*-butyl-dimethylsilyl groups.

Finally, for the synthesis of heterodimers **11a**−**c**, where the position of the linker on the NRTI (AZT, d4T, or dThd) was changed from the N-3 to the C-5 position of the base moiety, we developed a facile and straightforward two-step procedure outlined in Scheme 3. The key step for the synthesis involves the palladium cross-coupling reaction[29−34] of terminal alkyne **9** with 5-iodo nucleosides **10a**−**c**. The requisite 5-iodonucleosides **10a**−**c** were prepared as follows. Compound **10a**[35] was synthesized by direct iodination of 3′-azido-2′-deoxyuridine (AZU)[36] which in turn was prepared in two steps—by direct conversion of 2′-deoxyuridine into 2,3′-anhydrouridine, under Mitsunobu conditions, followed by azidation at the 3′-position.[37] The synthesis of 5-iodonucleoside **10b**[38] was carried out following the procedure developed by Larsen et al.[39] but improved by using the easily synthesized[40] or commercially available 5-iodo-2′-deoxyuridine as starting material. The other coupling partner, the bulky highly functionalized N-3 propargyl TSAO analogue **9**, was easily prepared in excellent yield (90%) by reaction of TSAO-T with propargylbromide in the presence of K_2CO_3. The coupling reaction of terminal alkyne intermediate **9** with 5-iodoprecursors **10a**−**c**, performed in DMF with tetrakis(triphenylphosphine)-palladium(0), copper(I) iodide, and triethylamine under argon at room temperature, gave heterodimers **11a**−**c** (55%, 46%, and 61% yields, respectively) together with compound **12**, as a minor side product, due to partial self-dimerization of the alkyne **9** (Scheme 3). The catalyst $(Ph_3P)_4Pd(0)$ was found to be specific for the reaction. Other catalysts, i.e., bis(triphenylphosphine)-palladium(II) chloride, failed to give the coupled products. Under such mild conditions, the target heterodimers were obtained in moderate to good yields without hydroxyl group protection of the NRTI (or dThd)

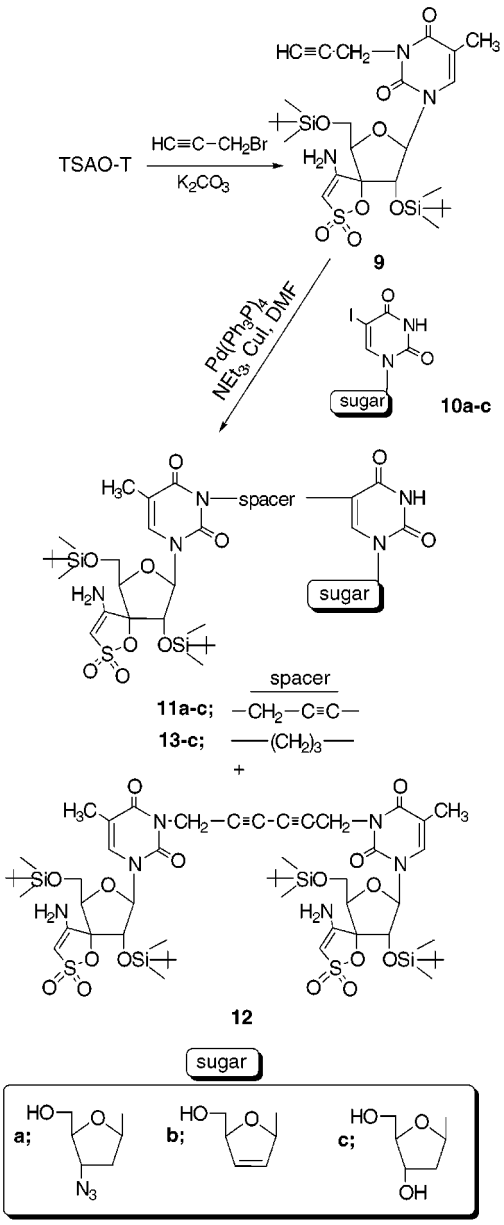
Scheme 1



Scheme 2



Scheme 3



moiety or deprotection of the labile *tert*-butyldimethylsilyl groups. Finally, compound **11c** was subjected to hydrogenolysis on Pd/C to provide heterodimer **13c** with a flexible polymethylene linker in 83% yield (Scheme 3).

Structures of the novel heterodimers **5a**−**d**, **6a**−**d**, **8a**−**d**, **11a**−**c**, and **13c** were assigned on the basis of their analytical and spectroscopic data. AZT heterodimers with different spacers **8a**−**d** and AZT and dThd heterodimers **11a**,**c** and **13** linked through the C-5 position of their base moiety were assigned by comparison of their ^{1}H NMR with the ^{1}H NMR of the previously

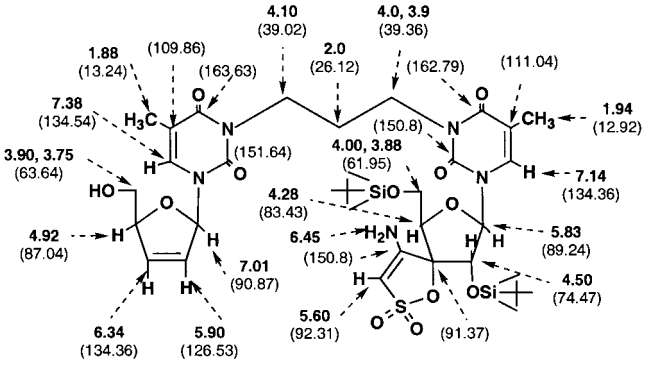


Figure 2. ^{1}H NMR (in bold) and ^{13}C NMR (in parentheses) chemical shifts (ppm) assignments for [d4T]-$(CH_2)_3$-[TSAO-T] heterodimer **5a**.

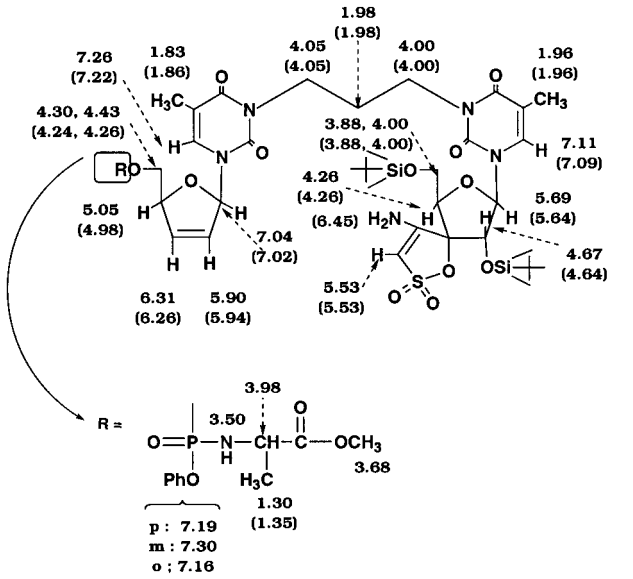


Figure 3. ^{1}H NMR chemical shifts (ppm) assignments for the [5′-MMP-d4T]-$(CH_2)_3$-[TSAO-T] heterodimer **5c**. This compound is a mixture of diastereoisomers that could not be separated. The chemical shifts for both diastereoisomers are shown.

reported [AZT or dThd]-$(CH_2)_n$-[TSAO-T] heterodimers whose structures were unequivocally determined.[21] For the assigment of d4T heterodimers **5a**, **6a**, and **11b** and 5′-(phenylphosphoramidates) heterodimers **5b**−**d** and **6b**−**d**, one- and two-dimensional (1D and 2D) NMR tecniques were used. The methodology followed is illustrated with compounds **5a** and **5c**, which were chosen as model compounds. Full asignment of 5′-(phenylphosphoramidate) heterodimer **5c** was not obvious because this compound was a mixture of the two diastereoisomers resulting from the mixed stereochemistry at the phosphate center, as was evident from the ^{31}P NMR, ^{1}H NMR, and ^{13}C NMR spectra. The full assignment of ^{1}H spectra were achieved by COSY and TOCSY 2D experiments and selective 1D-TOCSY experiments. The full assignment of ^{13}C spectra was achieved by HSQC and HMBC 2D experiments. ^{1}H NMR and ^{13}C NMR spectral data for compound **5a** are shown in Figure 2. ^{1}H NMR and ^{13}C NMR spectral data for nucleotide **5c** are shown in Figures 3 and 4, respectively. Structures of heterodimers **6a**, **11b**, **5b**,**d**, and **6b**−**d** were determined through comparison of their ^{1}H NMR spectra to those of the model compounds.

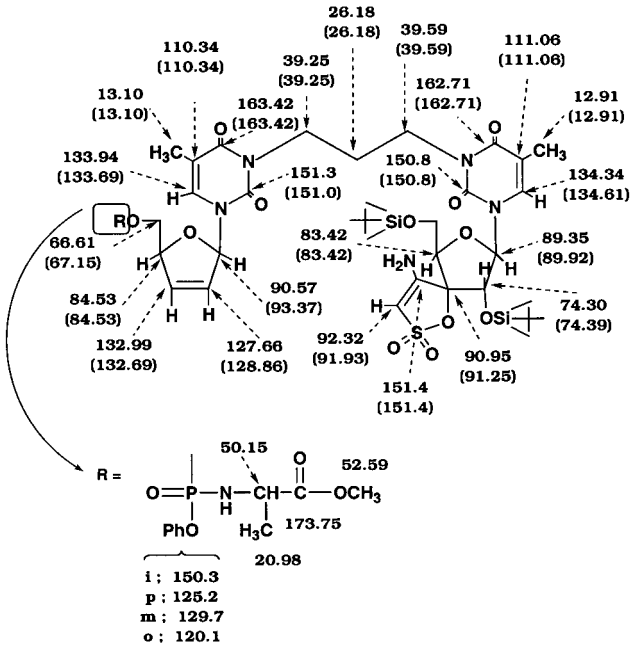


Figure 4. ^{13}C NMR chemical shifts (ppm) assignments for the [5′-MMP-d4T]-$(CH_2)_3$-[TSAO-T] heterodimer **5c**. This compound is a mixture of diastereoisomers that could not be separated. The chemical shifts for both diastereoisomers are shown.

Biological Results and Discussion

The inhibitory activity of the test compounds was explored against HIV-1 (IIIB) and HIV-2 (ROD) replication in MT-4 and CEM cell cultures (Table 1). TSAO-T was included as reference compound. The prototype heterodimer [AZT]-$(CH_2)_3$-[TSAO-T] **1** was inhibitory to HIV-1-induced cytopathicity at a 50% effective concentation (EC_{50}) ranging between 0.09 and 0.38 μM.

Replacement of AZT in the prototype heterodimer **1** by d4T (heterodimer **5a**) resulted in a 5- to 10- fold higher inhibitory effect against HIV-1 than against **1** and in a 2-fold more potent inhibitory effect than unsubstituted TSAO-T.[11a,b] Longer methylene linkers (i.e., n = 6, derivative **6a**) decreased the antiviral activity of **5a** by 6-fold.

In the prototype heterodimer **1** we also varied the nature of the spacer. No marked differences in anti-HIV-1 activity were observed when the spacer consisted of a butynyl (**8b**), butenyl (**8d**), or ethoxyethyl (**8a**) moiety (EC_{50}: 0.18−0.34 μM in CEM cells and 0.31−1.00 μM in MT-4 cells). Only when a benzylic spacer was introduced (**8c**) was a markedly lower antiviral activity noted.

Within the phenyloxy phosphoramidate series, the d4T (**5c**, **6c**) or thymidine (**5d**, **6d**) analogues showed marked anti-HIV-1 activity ranging between 0.17 and 0.33 μM for **5c** and **5d** and between 0.25 and 0.93 μM for **6c** and **6d**. In these series, the most potent derivative was the d4T analogue **5c**, but no improved antiviral potency could be noted when compared to the corresponding nonphosphorylated analogue **5a**. The AZT phenyloxy phosphoramidate heterodimers **5b** and **6b** were markedly less active than their corresponding d4T (**5c**, **6c**) and dThd (**5d**, **6d**) analogues. In contrast with the AZT phosphoramidate heterodimers **5b** and **6b**, the AZT phosphoramidate monomers **3b** and **4b** lacking the

Table 1. Anti-HIV and Cytostatic Activity of Test Compounds

	EC_{50}[a] (μM)				CC_{50}[b] (μM)	
	MT-4		CEM			
compd	HIV-1	HIV-2	HIV-1	HIV-2	MT-4	CEM
1	0.38 ± 0.07		0.09 ± 0.01		>100	>100
3b	>50	>50	>50	>50	44 ± 3.3	41 ± 4.9
4b	>10	>10	>10	>10	22 ± 1.9	22 ± 1.5
5a	0.04 ± 0.01	>50	0.02 ± 0.03	>50	16 ± 12	189 ± 64
5b	3.2 ± 2.1	>250	0.65 ± 0.1	>250	>250	>250
5c	0.18 ± 0.02	26 ± 8.1	0.17 ± 0.03	15 ± 7.1	28 ± 19	123 ± 0.7
5d	0.33 ± 0.14	>50	0.25 ± 0.15	>50	31 ± 15	>250
6a	0.23 ± 0.06	>250	0.12 ± 0.03	>50	116 ± 55	>250
6b	2−250[c]	>250	1.7 ± 1.1	>250	>250	>250
6c	0.57 ± 0.37	>50	0.4 ± 0.0	>50	37 ± 13	>250
6d	0.93 ± 0.08	>50	0.25 ± 0.04	>50	25 ± 4.6	104 ± 6.7
8a	0.31 ± 0.14	>250	0.18 ± 0.04	>50	>250	>250
8b	0.70 ± 0.15	>250	0.34 ± 0.09	57 ± 40	>250	>250
8c	1.7 ± 1.2	>50	5.3 ± 4.2	>250	>250	>250
8d	1.0 ± 0.01	>250	0.23 ± 0.14	>50	106 ± 9.7	>250
11a	0.33 ± 0.20	>10	0.20 ± 0.1		31 ± 19	>250
11b	0.18 ± 0.01	>10	0.11 ± 0.1	>10	8.0 ± 9.4	21 ± 1.1
11c	0.44 ± 0.31	>10	0.55 ± 0.21	>10	15 ± 2.9	21 ± 0.1
12	>50	>250	>50	>250	65 ± 20	>250
13	0.51 ± 0.43	>10	0.38 ± 0.28	>10	24	21 ± 1.3
TSAO-T[d]	0.06	>6	0.05	>6	12 ± 2.4	

[a] Effective concentration of 50% or the compound concentration required to inhibit HIV-induced cytopathicity. [b] Cytostatic concentration of 50%. [c] Within the concentration range of 2 and 250 μM, the inhibitory effect of the compound was close to 50%. Therefore, an exact EC_{50} value could not be determined. [d] Data taken from ref 11a,b.

TSAO moiety but containing the linker at the N-3 position of the base were devoid of anti-HIV activity. This observation suggests that the AZT moiety did not contribute to the antiviral activity of the heterodimers **5b** and **6b** and that the NH-3 fulfill a crucial role for enzyme recognition of AZT.

Given the above-mentioned considerations, other attachment points of the spacer to the NRTI have also been explored by anchoring the linker at the C-5 position of the pyrimidine base of the NRTI. The introduction of a propynyl spacer linked to the C-5 position (**11a**) showed comparable antiviral activity to that of the AZT heterodimer prototype (**1**), whereas in the corresponding d4T analogue (**11b**) a 5-fold decrease in anti-HIV potency was observed (compare **11b** with **5a**). However, the spacer rigidity in the C-5 series of compounds did not markedly influence the antiviral potency, and thus, the dThd heterodimer **13c** (bearing a more flexible propyl group as spacer) was endowed with anti-HIV-1 activity comparable to that of the corresponding propynyl analogue **11c** (EC_{50}s ranging between 0.38 and 0.55 μM).

With the exception of **5c**, none of the compounds investigated proved active against HIV-2 in MT-4 and CEM cell cultures, pointing to the predominant role of the NNRTI (TSAO-T) moiety in the eventual activity of the test compounds. It is intriguing why the phosphoramidate heterodimer **5c** (containing d4T as the nucleoside) had marginal, but significant, anti-HIV-2 activity, whereas the corresponding AZT derivative had not. This is the first TSAO derivative that was found to show some, albeit marginal, activity, against HIV-2.

Although most of the compounds were poorly cytotoxic to CEM cell cultures, the toxicity was in several cases more pronounced in MT-4 cell cultures. This difference could amount up to l0-fold (i.e., compounds **5a**, **5d**, **6c**, and **11a**). It is unclear which structural or functional determinants on the TSAO-T heterodimers play a role in the eventual toxicity.

Conclusions

In summary, in this paper we report on novel derivatives of the prototype heterodimer (**1**) by modifying the NRTI moiety, the conformational freedom of the linker, and the anchoring point of the linker to the NRTI moiety. Moreover, a series of arylphosphoramidate heterodimers (**5b**−**d** and **6b**−**d**), designed as membrane-soluble prodrugs of the corresponding 5′-monophosphate derivatives, have been prepared. Several members of this class of compounds show potent anti-HIV-1 activities comparable and even superior to those of the prototype heterodimer (**1**). The nature of the spacer and the position of the linker on the NRTI can be changed while keeping the antiviral activity. The phosphoramidate series of compounds had no improved anti-HIV-1 activity over the corresponding nonphosphorylated analogues. In contrast, the nature of the NRTI was shown to be important for increasing the inhibitory efficacy of the model heterodimer **1**. In particular, the [d4T]-$(CH_2)_3$-[TSAO-T] heterodimer analogue (**5a**) was 5- to 10-fold more inhibitory to HIV-1 in MT-4 and CEM cells cultures than the previously reported AZT counterpart **1**, and even 2-fold more potent than the unsubstituted TSAO-T parent compound.

Experimental Section

Chemical Procedures. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. HRMS spectra were registered in a VG autospec; the mode of ionization was fast atom bombardment (FAB) using MNOBA as matrix. ^{1}H and ^{13}C NMR spectra were recorded on a Varian UNITY 500 spectrometer operating at 499.84 MHz (^{1}H) and 125.71 MHz (^{13}C), respectively, using acetone-d_6 or $CDCl_3$ as solvent at 30 °C with TMS as internal standard. ^{31}P spectra were recorded on a Varian INOVA 400 spectrometer operating at 161.89 MHz, using acetone-d_6 or $CDCl_3$ as solvent at 30 °C with phosphoric acid as external standard. Monodimensional ^{1}H, ^{13}C, and ^{31}P spectra were obtained using standard conditions. Homonuclear 2D spectra (COSY and TOCSY) were acquired in the phase-sensitive mode. Data were collected in a 2048 ×

512 matrix with a spectral width of 3461 Hz and 1.2 s of relaxation delay and then processed in a 2048 × 1024 matrix. In the TOCSY spectra a mixing time of 0.128 s was used. 2D inverse proton detected heteronuclear one-bond shift correlation spectra were obtained using the pulsed field gradient HSQC pulse sequence. Data were collected in a 2048 × 512 matrix with a spectral width of 3460 Hz in the proton domain and 22500 Hz in the carbon domain and were processed in a 2048 × 1024 matrix. The experiment was optimized for a one-bond heteronuclear coupling constant of 150 Hz. 2D inverse proton detected heteronuclear long-range shift correlation spectra were obtained using the pulsed field gradient HMBC pulse sequence. The HMBC experiment was acquired in the same conditions of the HSQC experiment and optimized for long-range coupling constants of 7 Hz. 1D-selective TOCSY experiments were acquired using a selective pulse EBURP2-256 with a B1 corresponding to 10 Hz and a spin-lock time of 128 ms.

Analytical TLC was performed on silica gel 60 F_{254} (Merck). Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron (Kiesegel 60 PF_{254} gipshaltig (Merck)), layer thickness (1 mm), flow rate (5 mL/min). Flash column chromatography was performed with silica gel 60 (230−400 mesh) (Merck). Analytical HPLC was carried out on a Waters 484 system using a mBondapak C_{18} (3.9 × 300 mm: 10 mm). The systems used were as follows: (A) isocratic conditions, mobile phase CH_3CN/H_2O (0.05% TFA); (B) gradient conditions, mobile phase CH_3CN/H_2O (0.05% TFA); (C) linear gradient conditions, 2−22 min 95%−10% water, 22−32 min 10% water, 32−50 min 10%−95% water. Flow rate: 1 mL/min. Detection: UV (214 nm). All retention times are quoted in minutes.

The phosphoramidate intermediates and heterodimers (**2d**, **3b**−**d**, **4b**−**d**, **5b**−**d**, and **6b**−**d**) were isolated as mixtures of diastereoisomers, with the isomerism arising from mixed stereochemistry at the phosphate center. Many NMR peaks of the phosphoramidate compounds are split due to the presence of diastereoisomers in the sample. These compounds were noted to be hygroscopic and did not give useful microanalytical data but were found to be pure by high-field multinuclear NMR spectroscopy, mass spectrometry, and rigorous HPLC analysis.

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Triethylamine and acetonitrile were dried by refluxing over calcium hydride. Tetrahydrofuran was dried by refluxing over sodium/benzophenone. Anhydrous *N*,*N*′-dimethylformamide was purchased from Aldrich.

5′-(Phenylmethoxyalaninyl)phosphate Thymidine (2d). This synthesis was conducted in accordance with the procedure reported by McGuigan et al.[24,28] from thymidine and (phenylmethoxy)alaninyl phosphorochloridate. To a suspension of thymidine (0.6 g, 2.47 mmol) in dry THF (9 mL) was added *N*-methylimidazole (1.18 mL, 14.87 mmol). The resulting solution was cooled to −20 °C, and phenyl methoxyalaninylphosphorochloridate (4.95 mmol) dissolved in THF (5 mL) was slowly added. The clear solution became cloudy and slowly demixed. The solution was stirred for 2 h at −20 °C and overnight at room temperature. Water was added, and the solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (25 mL) and washed with saturated NH_4Cl (2 × 15 mL), water (15 mL), and brine (15 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified by flash chromatography on silica gel with dichloromethane/methanol, 10:1, to give compound **2d** as a white foam (0.49 g, 41%): HPLC Rt = 5.51 min (40:60); ^{31}P NMR (acetone-d_6) 4.18, 4.39 ppm; ^{1}H NMR (acetone-d_6) δ 1.32, 1.36 (d, 3H, Ala-CH_3, J = 7.1, 7.1 Hz), 1.80, 1.82 (d, 3H, CH_3-5, J = 1.1, 1.1 Hz), 2.15 (m, 2H, 2H-2′), 3.65 (s, 3H, Ala-OCH_3), 4.11−4.56 (m, 5H, H-3′, H-4′, 2H-5′, Ala-CH), 4.59, 5.00 (2m, 2H, OH, Ala-NH), 6.32 (m, 1H, H-1′), 7.24−7.37 (m, 5H, Ph), 7.57, 7.60 (d, 1H, H-6, J = 1.1, 1.1 Hz), 9.98 (bs, 1H, NH-3); MS *m*/*e* FAB 483.1414 (MH^+, $C_{20}H_{26}N_3O_9P$ requires 483.1406).

General Procedure for the Synthesis of 3-*N*-(bromospacer) Nucleoside Intermediates 3a, 4a, and 9a−d and 3-*N*-(Bromoalkyl)-5′-(phenylmethoxyalaninyl)phosphate Nucleotide Intermediates 3b−d and 4b−d. To a solution of the nucleoside [AZT or d4T] (1 equiv) in acetone/DMF (1:1) or the nucleotide (**2d**) (1 equiv) in acetone were added K_2CO_3 (1.1 equiv) and the corresponding dibromoalkyl, alkenyl, aryl, or dichloroalkynyl reagent (2.0−6.0 equiv). The reaction mixture was refluxed for 5−16 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate (14 mL), washed with water (2 × 14 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by CCTLC on the chromatotron. The reaction time, number of equivalents of the dibromo reagent, chromatography eluent, yield of the isolated products, and ^{1}H NMR data are indicated below for each compound.

***N*-(3-Bromopropyl)-2′,3′-didehydro-2′,3′-dideoxythymidine (3a):** reaction time 16 h; 6 equiv of 1,3-dibromopropane; eluent dichloromethane/methanol, 20:1; yield of **3a** (75%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 1.80 (d, 3H, CH_3-5, J = 1.2 Hz), 2.17 (m, 2H, CH_2), 3.51 (t, 2H, CH_2Br, J = 7.0 Hz), 3.79 (m, 2H, 2H-5′), 4.03 (t, 2H, CH_2N, J = 7.0 Hz), 4.12 (t, 1H, OH-5′), 4.46 (m, 1H, H-3′), 4.86 (m, 1H, H-4′), 5.92 (ddd, 1H, H-2′, $J_{2',3'}$ = 6.0, $J_{1',2'}$ = 1.3, $J_{2',4'}$ = 2.3 Hz), 6.42 (dt, 1H, H-3′, $J_{1',3'}$ = $J_{3',4'}$ = 1.7 Hz), 6.99 (m, 1H, H-1′), 7.78 (q, 1H, H-6). Anal. ($C_{13}H_{17}BrN_2O_4$) C, H, N.

3′-Azido-3-*N*-(3-bromopropyl)-3′-deoxythymidine-5′-(phenylmethoxyalaninyl)phosphate (3b): reaction time 9 h; 4 equiv of 1,3-dibromopropane; eluent dichloromethane/methanol, 20:1; yield of **3b** (74%) as a syrup; HPLC Rt = 5.70 min (system A, 45:55); ^{31}P NMR ($CDCl_3$) 3.14, 3.49 ppm; ^{1}H NMR ($CDCl_3$) δ 1.34, 1.37 (d, 3H, Ala-CH_3, J = 7.0 Hz), 1.88, 1.90 (s, 3H, CH_3-5), 2.05−2.48 (m, 4H, 2H-2′, CH_2), 3.39 (t, 2H, CH_2Br, J = 6.8 Hz), 3.60 (m, 1H, Ala-NH), 3.68, 3.69 (s, 3H, Ala-OCH_3), 3.92−4.40 (m, 7H, CH_2N, Ala-CH, H-3′, H-4′, 2H-5′), 6.21 (m, 1H, H-1′), 7.16−7.38 (m, 6H, H-6, Ph); MS *m*/*e* FAB 628.1040 (MH^+, $C_{23}H_{30}BrN_6O_8P$ requires 628.1045).

3-*N*-(3-Bromopropyl)-2′,3′-didehydro-2′,3′-dideoxythymidine-5′-(phenylmethoxyalaninyl)phosphate (3c): reaction time 7 h; 2 equiv of 1,3-dibromopropane; eluent dichloromethane/methanol, 10:1; yield of **3c** (48%) as a syrup; HPLC Rt = 5.51 min (system A, 40:60); ^{31}P NMR ($CDCl_3$) 3.13, 3.69 ppm; ^{1}H NMR ($CDCl_3$) δ 1.24, 1.28 (d, 3H, Ala-CH_3, J = 7.0, 7.0 Hz), 1.77, 1.81 (d, 3H, CH_3-5, J = 1.2, 1.2 Hz), 2.16 (m, 2H, CH_2), 3.36, 3.36 (t, 2H, CH_2Br, J = 7.1, 7.1 Hz), 3.42, 3.50 (m, 1H, Ala-NH), 3.63, 3.64 (s, 3H, Ala-OCH_3), 3.91 (m, 1H, Ala-CH), 4.06 (m, 2H, CH_2N), 4.17−4.34 (m, 2H, 2H-5′), 4.94, 4.97 (m, 1H, H-4′), 5.83 (m, 1H, H-2′), 6.21, 6.28 (dt, 1H, H-3′, $J_{2',3'}$ = 6.0, 6.0 Hz, $J_{1',3'}$ = $J_{3',4'}$ = 1.8, 1.6 Hz), 7.00, 7.01 (m, 1H, H-1′), 7.08−7.28 (m, 6H, H-6, Ph); MS *m*/*e* FAB 585.0877 (MH^+, $C_{23}H_{29}BrN_3O_8P$ requires 585.0875).

3-*N*-(3-Bromopropyl)thymidine-5′-(phenylmethoxyalaninyl)phosphate (3d): reaction time 30 h; 5 equiv of 1,3-dibromopropane; eluent dichloromethane/methanol, 10:1; yield of **3d** (55%) as a syrup; HPLC Rt = 3.45 min (system A, 45:55); ^{31}P NMR ($CDCl_3$) 3.66, 4.10 ppm; ^{1}H NMR ($CDCl_3$) δ 1.29, 1.29 (d, 3H, Ala-CH_3, J = 7.0, 7.1 Hz), 1.84 (d, 3H, CH_3-5, J = 0.9 Hz), 1.97 (m, 1H, H-2′a), 2.14 (m, 2H, CH_2), 2.27 (m, 1H, H-2′b), 3.35 (t, 2H, CH_2Br, J = 6.9 Hz), 3.63, 3.65 (s, 3H, Ala-OCH_3), 3.59−3.77 (m, 1H, Ala-NH), 3.89−4.44 (m, 8H, CH_2N, H-3′, H-4′, 2H-5′, Ala-CH, OH), 6.20, 6.24 (t, 1H, H-1′, $J_{1',2'a}$ = $J_{1',2'b}$ = 6.4, 6.3 Hz), 7.09−7.29 (m, 6H, Ph, H-6); MS *m*/*e* FAB 603.0987 (MH^+, $C_{23}H_{31}BrN_3O_9P$ requires 603.0980).

***N*-(6-Bromohexyl)-2′,3′-didehydro-2′,3′-dideoxythymidine (4a):** reaction time 10 h; 4 equiv of 1,6-dibromohexane; eluent dichloromethane/methanol, 20:1; yield of **4a** (75%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 1.36−1.63 (m, 6H, $3CH_2$), 1.79 (d, 3H, CH_3-5, J = 1.1 Hz), 1.85 (m, 2H, CH_2), 3.50 (t, 2H, CH_2-Br, J = 7.0 Hz), 3.83 (m, 4H, CH_2N, 2H-5′), 4.17 (t, 1H, 5′-OH, J = 5.8 Hz), 4.85 (m, 1H, H-4′), 5.92 (m, 1H, H-2′), 6.42 (m, 1H, H-3′), 6.99 (m, 1H, H-1′), 7.77 (d, 1H, H-6). Anal. ($C_{16}H_{23}BrN_2O_4$) C, H, N.

3′-Azido-3-*N*-(6-bromohexyl)-3′-deoxythymidine-5′-(phenylmethoxyalaninyl)phosphate (4b): reaction time 6 h; 3

equiv of 1,6-dibromohexane; eluent dichloromethane/methanol, 20:1; yield of **4b** (72%) as a syrup; ^{31}P NMR ($CDCl_3$) 3.09, 3.42 ppm; ^{1}H NMR ($CDCl_3$) δ 1.36, 1.37 (d, 3H, Ala-CH_3, J = 6.9, 6.8 Hz), 1.41−1.66 (m, 6H, 3CH_2), 1.85 (m, 2H, CH_2), 2.14 (m, 1H, H-2′a), 2.38 (m, 1H, H-2′b), 3.39 (t, 2H, CH_2Br, J = 6.8 Hz), 3.60 (m, 1H, Ala-NH), 3.70, 3.71 (s, 3H, Ala-OCH_3), 3.91 (t, 2H, CH_2N, J = 7.6 Hz), 3.99−4.37 (m, 5H, Ala-CH, H-3′, H-4′, 2H-5′), 6.18, 6.23 (t, 1H, H-1′, $J_{1',2'a} = J_{1',2'b}$ = 6.6 Hz), 7.15−7.36 (m, 6H, H-6, Ph); MS *m/e* FAB 670.1521 (MH^+, $C_{26}H_{36}BrN_6O_8P$ requires 670.1515).

3-*N*-(6-Bromohexyl)-2′,3′-didehydro-2′,3′-dideoxythymidine-5′-(phenylmethoxyalaninyl)phosphate (4c): reaction time 24 h; 4 equiv of 1,6-dibromohexane; eluent dichloromethane/methanol, 20:1; yield of **4c** (50%) as a syrup; HPLC Rt = 20.15 min (system A, 55:45); ^{31}P NMR ($CDCl_3$) 3.19, 3.73 ppm; ^{1}H NMR ($CDCl_3$) δ 1.27−1.86 (m, 14H, 4CH_2, Ala-CH_3, CH_3-5), 3.39 (t, 2H, CH_2Br, J = 6.8 Hz), 3.52 (m, 1H, Ala-NH), 3.69, 3.69 (s, 3H, Ala-OCH_3), 3.90 (m, 3H, CH_2N, Ala-CH), 4.12−4.38 (m, 2H, 2H-5′), 5.00 (m, 1H, H-4′), 5.89 (m, 1H, H-2′), 6.27, 6.34 (m, 1H, H-3′), 7.06−7.35 (m, 7H, H-1′, H-6, Ph); MS *m/e* FAB 627.1339 (MH^+, $C_{26}H_{35}BrN_3O_8P$ requires 627.1344).

3-*N*-(6-Bromohexyl)thymidine-5′-(phenylmethoxyalaninyl)phosphate (4d): reaction time 22 h; 4 equiv of 1,3-dibromohexane; eluent dichloromethane/methanol, 20:1; yield of **4d** (50%) as a syrup; ^{31}P NMR ($CDCl_3$) 3.56, 3.90 ppm; ^{1}H NMR ($CDCl_3$) δ 1.29−1.40 (m, 16H, Ala-CH_3, CH_3-5, 2H-2′, 4CH_2), 3.34 (t, 2H, CH_2Br, J = 6.7 Hz), 3.63, 3.65 (s, 3H, Ala-OCH_3), 3.81−4.43 (m, 9H, CH_2N, Ala-CH, Ala-NH, H-3′, H-4′, 2H-5′, OH), 6.24 (m, 1H, H-1′), 7.11−7.38 (m, 6H, H-6, Ph); MS *m/e* FAB 645.1450 (MH^+, $C_{26}H_{37}BrN_3O_9P$ requires 645.1450).

3′-Azido-3-*N*-(5-bromoethyl ether)-3′-deoxythymidine (7a): reaction time 24 h; 3 equiv of 2-bromoethyl ether; eluent dichloromethane/methanol, 10:1; yield of **7a** (92%) as a syrup; ^{1}H NMR (acetone-d_6) δ 1.90 (d, 3H, CH_3-5, J = 1.2 Hz), 2.39 (m, 1H, H-2′a), 2.50 (m, 1H, H-2′b), 2.65 (bs, 1H, 5′-OH), 3.40 (t, 2H, CH_2Br, J = 6.0 Hz), 3.80 (m, 5H, 2CH_2O, H-4′), 3.92 (m, 2H, 2H-5′), 4.15 (t, 2H, NCH_2, J = 5.9 Hz), 4.37 (m, 1H, H-3′), 6.01 (t, 1H, H-1′, $J_{1',2'a} = J_{1',2'b}$ = 6.4 Hz), 7.34 (d, 1H, H-6). Anal. ($C_{14}H_{20}BrN_5O_5$) C, H, N.

3′-Azido-3-*N*-(4-bromo-2-(E)butenyl)-3′-deoxythymidine (7b): reaction time 7 h; 2 equiv of 1,4-dibromo-2-butene (E); eluent hexane/ethyl acetate, 1:1; yield of **7b** (80%) as a syrup; ^{1}H NMR (acetone-d_6) δ 1.91 (d, 3H, CH_3-5, J = 1.2 Hz), 2.31−2.62 (m, 3H, 2H-2′, 5′-OH), 3.75−4.03 (m, 5H, H-4′, 2H-5′, CH_2Br), 4.39 (m, 1H, H-3′), 4.51, 4.54 (2s, 2H, CH_2N), 5.84 (m, 2H, CH=CH), 6.03 (t, 1H, H-1′, $J_{1',2'}$ = 6.5 Hz), 7.33 (d, 1H, H-6). Anal. ($C_{14}H_{18}BrN_5O_4$) C, H, N.

3′-Azido-3-*N*-(α-bromo-*p*-xylenyl)-3′-deoxythymidine (7c): reaction time 4 h; 2 equiv of α,α′-bromo-*p*-xylene; eluent dichloromethane/methanol, 20:1; yield of **7c** (75%) as a syrup; ^{1}H NMR ($CDCl_3$) δ 1.83 (d, 3H, CH_3-5, J = 1.1 Hz), 2.35 (m, 3H, 2H-2′, 5′-OH), 3.66−3.92 (m, 3H, H-4′, 2H-5′), 4.30 (m, 1H, H-3′), 4.36 (s, 2H, CH_2Br), 4.99 (s, 2H, CH_2N), 5.97 (t, 1H, H-1′, $J_{1',2'a} = J_{1',2'b}$ = 6.5 Hz), 7.27 (m, 5H, H-6, Ph). Anal. ($C_{18}H_{20}BrN_5O_4$) C, H, N.

3′-Azido-3-*N*-(4-chloro-2-butynyl)-3′-deoxythymidine (7d): reaction time 30 h; 4 equiv of 1,4-dichloro-2-butyne; eluent dichloromethane/methanol, 10:1; yield of **7d** (35%) as a syrup, recovered starting material (20%); ^{1}H NMR δ 1.85 (s, 3H, CH_3-5), 2.47 (m, 2H, 2H-2′), 3.82 (m, 2H, 2H-5′), 3.95 (m, 1H, H-4′), 4.28 (t, 2H, CH_2Cl, J = 1.9 Hz), 4.47 (m, 2H, H-3′, 5′-OH), 4.69 (d, 2H, CH_2N, J = 1.8 Hz), 6.24 (t, 1H, H-1′, $J_{1',2'a} = J_{1',2'b}$ = 6.3 Hz), 7.85 (s, 1H, H-6). Anal. ($C_{14}H_{16}ClN_5O_4$) C, H, N.

General Procedure for the Synthesis of the Heterodimers [TSAO-T]N^3-spacer-N^3[d4T or AZT] 5a, 6a, and 8a−d and [TSAO-T]N^3-spacer-N^3[5′-MMP-(AZT, d4T, or dThd)] 5b−d and 6b−d. To a solution of the key 3-*N*-(*n*-bromo-spacer) nucleoside (**3a**, **4a**, **7a**−**d**) or the 3-*N*-(*n*-bromoalkyl) nucleotide-5′-(phenylmethoxyalaninyl)phosphate (**3b**−**d**, **4b**−**d**) (1 equiv) in dry acetonitrile were added K_2CO_3 (1.1 equiv) and TSAO-T (1.1 equiv). The reaction mixture was refluxed for 8−16 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate (14 mL), washed with water (2 × 14 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. Repeated chromatography of the residue by preparative CCTLC on the chromatotron was required to give pure heterodimers.

The reaction time, chromatography eluent, and yield of the isolated compounds are indicated below for each compound.

Heterodimer [TSAO-T]N^3-(CH$_2$)$_3$-N^3[d4T] (5a).[41] TSAO-T and **3a** reacted for 5 h. The residue was chromatographed (dichloromethane/methanol, 20:1) to give **5a** (50%) as a white foam: HPLC Rt = 16.18 min (system A, 60:40); ^{1}H NMR and ^{13}C NMR are shown in Figure 2. Anal. ($C_{37}H_{59}N_5O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-(CH$_2$)$_3$-N^3[5′-MMP-AZT] (5b). TSAO-T and **3b** reacted for 7 h. The residue was chromatographed with hexane/ethyl acetate (1:1) and subsequently with dichloromethane/methanol (20:1) to give **5b** (65%) as a white foam: HPLC Rt = 5.70 min (system A, 45:55), Rt = 23.73 min (system B); MS *m/e* FAB 1137.4087 (MH^+, $C_{47}H_{72}N_9O_{16}P$ SSi_2 requires 1137.4093).

Heterodimer [TSAO-T]N^3-(CH$_2$)$_3$-N^3[5′-MMP-d4T] (5c). TSAO-T and **3c** reacted for 5 h. The residue was chromatographed (dichloromethane/acetone, 10:1) to give **5c** (75%) as a syrup: HPLC Rt = 12.48 min (system A, 55:45); ^{31}P NMR ($CDCl_3$) 3.20, 3.70 ppm; ^{1}H NMR and ^{13}C NMR are shown in Figures 3 and 4; MS *m/e* FAB 1094.3931 (MH^+, $C_{47}H_{71}N_6O_{16}P$ SSi_2 requires 1094.3922).

Heterodimer [TSAO-T]N^3-(CH$_2$)$_3$-N^3[5′-MMP-dThd] (5d). TSAO-T and **3d** reacted for 8 h. The residue was chromatographed (dichloromethane/ethyl acetate, 1:2) to give **5d** (81%) as a syrup: HPLC Rt = 12.68 min (system A, 55:45), Rt = 21.93 min (system B); MS *m/e* FAB 1112.4031 (MH^+, $C_{47}H_{73}N_6O_{17}P$ SSi_2 requires 1112.4028).

Heterodimer [TSAO-T]N$_3$-(CH$_2$)$_6$-N3[d4T] (6a). TSAO-T and **4a** reacted for 3 h. The residue was chromatographed (dichloromethane/methanol, 20:1) to give **6a** (60%) as a white foam: HPLC Rt = 18.25 min (system A, 55:45), Rt = 22.81 min (system B). Anal. ($C_{40}H_{65}N_5O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-(CH$_2$)$_6$-N^3[5′-MMP-AZT] (6b). TSAO-T and **4b** reacted for 12 h. The residue was chromatographed (dichloromethane/methanol, 20:1) to give **6b** (75%) as a white foam: HPLC Rt = 18.20 min (system A, 60:40), Rt = 24.82 min (system B); MS *m/e* FAB 1179.4561 (MH^+, $C_{50}H_{78}N_9O_{16}PSSi_2$ requires 1179.4562).

Heterodimer [TSAO-T]N^3-(CH$_2$)$_6$-N^3[5′-MMP-d4T] (6c). TSAO-T and **4c** reacted for 9 h. The residue was chromatographed (dichloromethane/acetone, 10:1) to give **6c** (70%) as a white foam: HPLC Rt = 19.80 min (system A, 60:40), Rt = 24.17 min (system B); MS *m/e* FAB 1136.4399 (MH^+, $C_{50}H_{77}N_6O_{16}PSSi_2$ requires 1136.4392).

Heterodimer [TSAO-T]N^3-(CH$_2$)$_6$-N^3[5′-MMP-dThd] (6d). TSAO-T and **4d** reacted for 16 h. The residue was chromatographed (dichloromethane/ethyl acetate, 1:2) to give **6d** (50%) as a syrup: HPLC Rt = 23.07 min (system A, 55:45); MS *m/e* FAB 1154.4492 (MH^+, $C_{50}H_{79}N_6O_{17}P$ SSi_2 requires 1154.4498).

Heterodimer [TSAO-T]N^3-(CH$_2$)$_2$-O-(CH$_2$)$_2$-N^3[AZT] (8a). TSAO-T and **7a** reacted for 15 h. The residue was chromatographed (dichloromethane/methanol, 20:1) to give **8a** (75%) as a white foam: HPLC Rt = 13.96 min (system A, 55:45). Anal. ($C_{38}H_{62}N_8O_{13}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-CH$_2$-CH=CH(E)-CH$_2$-N^3[AZT] (8b). TSAO-T and **7b** reacted for 4 h. The residue was chromatographed (hexane/ethyl acetate, 1:1) to give **8b** (73%) as a white foam: HPLC Rt = 16.28 min (system A, 55:45). Anal. ($C_{38}H_{60}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-CH$_2$-Ph-CH$_2$-N^3[AZT] (8c). TSAO-T and **7c** reacted for 3 h. The residue was chromatographed (dichloromethane/ethyl acetate, 20:1) to give **8c** (92%) as a white foam: HPLC Rt = 29.81 min (system A, 55:45). Anal. ($C_{42}H_{62}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-CH$_2$-C≡C-CH$_2$-N^3[AZT] (8d). TSAO-T and **7d** reacted for 30 h. The residue was chromatographed (dichloromethane/methanol, 10:1) to give **8d** (23%) as a white foam together with unreacted starting material

(36%): HPLC Rt = 15.72 min (system A, 55:45). Anal. ($C_{38}H_{58}N_8O_{12}SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(3-bromopropargyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (9). TSAO-T was reacted with 2 equiv of propargyl bromide for 7 h, according to the general procedure for the synthesis of 3-*N*-(bromo-spacer) nucleoside and nucleotide intermediates (**3a**−**d**, **4a**−**d**, and **7a**−**d**). The residue was purified by CCTLC on the chromatotron (hexane/ethyl acetate, 1:1) to give intermediate **9** (90%) as a white foam: HPLC Rt = 21.33 min (system A, 55:45); ^{1}H NMR ($CDCl_3$) δ −0.10, 0.05, 0.19, 0.21 (4s, 12H, $4CH_3$), 0.82, 0.97 (2s, 18H, 2t-Bu), 2.00 (d, 3H, CH_3-5, J = 1.2 Hz), 2.14 (t, 1H, C≡CH), 3.91 (m, 1H, H-5′a, $J_{5'a,\,5'b}$ = 12.4, $J_{4',5'a}$ = 2.6 Hz), 4.01 (m, 1H, H-5b′, $J_{4',5'b}$ = 2.8 Hz), 4.34 (t, 1H, H-4′), 4.54 (d, 1H, H-2′, $J_{1',2'}$ = 7.9 Hz), 4.71 (m, 2H, CH_2−C≡CH), 5.61 (bs, 2H, NH_2-4″), 5.63 (s, 1H, H-3″), 5.98 (d, 1H, H-1′), 7.23 (d, 1H, H-6). Anal. ($C_{27}H_{45}BrN_3O_8SSi_2$) C, H, N, S.

General Procedure for the Synthesis of Heterodimers [TSAO-T]N^3-CH$_2$-C≡C-C^5[AZT, d4T, or dThd] 11a−c. A solution of the corresponding 5-iodonucleoside **10a**−**c** (1 mmol) in 3−5 mL of dry DMF was deoxygenated under argon. The terminal alkyne **9** (2 mmol), $(Ph_3P)_4Pd$ (0.1 mmol), CuI (0.2 mmol), and NEt_3 (2 mmol) were added, and the mixture was stirred at room temperature under argon atmosphere. After 24 h the solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (50 mL), washed with 5% EDTA (2 × 25 mL) and saturated aqueous NaCl (25 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by CCTLC on the chromatotron. The apolar yellow impurities were first eluted with hexane. Further elution with hexane/ethyl acetate 1:1 and then with ethyl acetate gave, from the faster moving fractions, pure [TSAO-T]N_3-CH_2-C≡C-C≡C-CH_2-N_3[TSAO-T] dimer **12** as a white foam. The slower moving fractions were subjected to a second purification by CCTLC on the chromatotron (CH_2Cl_2/MeOH, 20:1, and then 10:1) to afford the corresponding pure heterodimer **11a**−**c**. The yield of the isolated compounds and analytical data are indicated below for each compound.

Heterodimer [TSAO-T]N^3-CH$_2$-C≡C-C^5[AZT] (11a): yield of dimer **12** (12%); yield of **11a** (55%) as a white foam. Analytical data of **12**: Anal. ($C_{54}H_{88}N_6O_{16}S_2Si_4$) C, H, N, S. Analytical data of **11a**: HPLC Rt = 7.67 min (system A, 55:45). Anal. ($C_{36}H_{54}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-CH$_2$-C≡C-C^5[d4T] (11b): yield of dimer **12** (16%); yield of **11b** (46%) as a white foam; HPLC Rt = 12.84 min (system A, 55:45). Anal. ($C_{36}H_{53}N_5O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-CH$_2$-C≡C-C^5[dThd] (11c): yield of dimer **12** (6%); yield of **11c** (61%) as a white foam; HPLC Rt = 9.35 min (system A, 45:55). Anal. ($C_{36}H_{55}N_5O_{13}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]N^3-CH$_2$-CH$_2$-CH$_2$-C^5[dThd] (13c). Compound **11c** (0.125 g, 0.15 mmol) was dissolved in ethanol (35 mL) and hydrogenated in the presence of 10% Pd/C (0.035 g) at 40 psi at 30 °C for 3 h. The mixture was filtered off and evaporated to give **13c** (0.106 g, 83%) as an amorphous solid. HPLC Rt = 2.95 min (system A, 55:45). Anal. ($C_{36}H_{59}N_5O_{13}SSi_2$) C, H, N, S.

Anti-HIV Evaluation. Human immunodeficiency virus type 1 [HIV-1 (III_B)] was obtained from Dr. R. C. Gallo (when at the National Cancer Institute, Bethesda, MD). HIV-2 (ROD) was provided by Dr. L. Montagnier (when at the Pasteur Institute, Paris, France). First, 4×10^5 CEM or 3×10^5 MT-4 cells per milliliter were infected with HIV-1 or HIV-2 at ∼100 $CCID_{50}$ (50% cell culture infective dose) per milliliter of cell suspension. Then 100 μL of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4 days giant cell formation (CEM) or HIV-induced cytopathicity (MT-4) was recorded microscopically in the HIV-infected cell cultures. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) of the test compounds were defined as the compound concentrations required to inhibit virus-induced cytopathicity (CEM) or cell viability (MT-4) by 50% or to reduce by 50% the number of viable cells in mock-infected cell cultures, respectively.

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Supporting Information Available: ^{1}H NMR chemical shift assignments of heterodimers **5b**,**d**, **6a**−**d**, **8a**−**d**, **11a**−**c**, and **13c** and dimer **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Katz, R. A.; Skalka, A. M. The retroviral enzymes. *Annu. Rev. Biochem.* **1994**, *63*, 133−173.
(2) Vaishnav, Y. N.; Wong-Staal, F. The biochemistry of AIDS. *Annu. Rev. Biochem.* **1991**, *60*, 577−630.
(3) Schinazi, R. F. Competitive inhibitors of human immunodeficiency virus reverse transcriptase. *Perspect. Drug Discovery Des.* **1993**, *1*, 151−180.
(4) For an overview, see: De Clercq, E. Toward improved anti-HIV chemotherapy: therapeutic strategies for intervention with HIV infections. *J. Med. Chem.* **1995**, *38*, 2491−2517.
(5) Young, S. D. Non-nucleoside inhibitors of HIV-1 reverse transcriptase. *Perspect. Drug Discovery Des.* **1993**, *1*, 181−192.
(6) De Clercq, E. HIV-1 specific RT inhibitors: Highly selective inhibitors of human immunodeficiency virus type 1 that are specifically targeted at the viral reverse transcriptase. *Med. Res. Rev.* **1993**, *13*, 229−258.
(7) (a) De Clercq, E. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of human immunodeficiency virus type 1 (HIV-1) infections: strategies to overcome drug resistance development. *Med. Res. Rev.* **1996**, *16*, 125−157. (b) De Clercq, E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. *Antiviral Res.* **1998**, *38*, 153−179.
(8) (a) Jacobo-Molina, A.; Ding, J.; Nanni, R. G.; Clark, A. D., Jr.; Lu, X.; Tantillo, C.; Williams, R. L.; Kamer, G.; Ferris, A. L.; Clark, P.; Hizi, A.; Hughes, S. H.; Arnold, E. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 Å resolution shows bent DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6320−4. (b) Tantillo, C.; Ding, J.; Jacobo-Molina, A.; Nanni, R. G.; Boyer, P. L.; Hughes, S. H.; Pauwels, R.; Andries, K.; Janssen, P. A. J.; Arnold, E. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. Implications for mechanisms of drug inhibition and resistance. *J. Mol. Biol.* **1994**, *243*, 369−387.
(9) Nanni, R. G.; Ding, J.; Jacobo-Molina, A.; Hughes, S. H.; Arnold, E. Review of HIV-1 reverse transcriptase three-dimensional structure: Implications for drug design. *Perspect. Drug Discovery Des.* **1993**, *1*, 129−150.
(10) Smerdon, S. J.; Jäger, J.; Wang, J.; Kohlstaedt, L. A.; Chirino, A. J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. Structure of the binding site for non-nucleoside inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3911−3915.
(11) (a) Balzarini, J.; Pérez-Pérez, M. J.; San-Félix, A.; Schols, D.; Perno, C. F.; Vandamme, A. M.; Camarasa, M. J.; De Clercq, E. 2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)pyrimidine (TSAO) nucleoside analogues: Highly selective inhibitors of human immunodeficiency virus type 1 that are targeted at the viral reverse transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4392−4396. (b) Camarasa, M. J.; Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E. 3′-Spironucleosides (TSAO derivatives), a new class of specific human immunodeficiency virus type 1 inhibitors: Synthesis and antiviral activity of 3′-spiro-5″-[4″-amino-1″,2″-oxathiole-2″,2″-dioxide]pyrimidine nucleosides. *J. Med. Chem.* **1992**, *35*, 2721−2727.
(12) (a) Camarasa, M. J.; Pérez-Pérez, M. J.; Velázquez, S.; San-Félix, A.; Alvarez, A.; Ingate, S.; Jimeno, M. L.; Karlsson, A.; De Clercq, E.; Balzarini, J. TSAO derivatives: Highly specific inhibitors of human immunodeficiency virus type-1 (HIV-1) replication.

Nucleosides Nucleotides **1995**, *14*, 585−594. (b) Balzarini, J.; Camarasa, M. J.; Karlsson, A. TSAO derivatives: Highly specific human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitors. *Drugs Future* **1993**, *18*, 1043−1055.

(13) (a) Jonckheere, H.; Taymans, J. M.; Balzarini, J.; Velázquez, S.; Camarasa, M. J.; Desmyter, J.; De Clercq, E.; Anné, J. Resistance of HIV-1 reverse transcriptase against [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)] (TSAO) derivatives is determined by the mutation $Glu_{138} \rightarrow Lys$ on the p51 subunit. *J. Biol. Chem.* **1994**, *269*, 25255−25258. (b) Harris, D.; Lee, R.; Misra, H. S.; Pandey, P. K.; Pandey, V. N. The p51 subunit of human immunodeficiency virus type 1 reverse transcriptase is essential in loading the p66 subunit on the template primer. *Biochemistry* **1998**, *37*, 5903−5908.

(14) Balzarini, J.; Kleim, J. P.; Riess, G.; Camarasa, M. J.; De Clercq, E.; Karlsson, A. Sensitivity of (138 GLU → LYS) mutated human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) to HIV-1-specific RT inhibitors. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 1305−1312.

(15) Boyer, P. L.; Ding, J.; Arnold, E.; Hughes, S. H. Subunit specificity of mutations that confer resistance to non-nucleoside inhibitors in human immunodeficiency virus type 1 reverse transcriptase. *Antimicrob. Agents Chemother.* **1994**, *38*, 1909−1914.

(16) De Clercq, E. HIV-1 resistance to reverse transcriptase inhibitors. *Biochem. Pharmacol.* **1994**, *47*, 155−169.

(17) Schinazi, R. F.; Larder, B. A.; Mellors, J. W. Mutations in HIV-1 reverse transcriptase and protease associated with drug-resistance. *Int. Antiviral News* **1996**, *4*, 95−107.

(18) Schmit, J. C.; Cogniaux, J.; Hermans, P.; Van Vaeck, C.; Sprecher, S.; Van Remoortel, B.; Witvrouw, M.; Balzarini, J.; Desmyter, J.; De Clercq, E.; Vandamme, A. M. Multiple drug resistance to nucleoside analogues and non-nucleoside reverse transcriptase inhibitors in an efficiently replicating human inmunodeficiency virus type 1 patient strain. *J. Infect. Dis.* **1996**, *174*, 962−968.

(19) Vandamme, A. M.; Van Vaerenbergh, K.; De Clercq, E. Anti-human inmunodeficiency virus drug combination strategies. *Antiviral Chem. Chemother.* **1998**, *9*, 187−203.

(20) Spence, R. A.; Kati, W. M.; Anderson, K. S.; Johnson, K. A. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. *Science* **1995**, *267*, 988−993.

(21) Velázquez, S.; Alvarez, R.; San-Félix, A.; Jimeno, M. L.; De Clercq, E.; Balzarini, J.; Camarasa, M. J. Synthesis and Anti-HIV Activity of [AZT]-[TSAO-T] and [AZT-[HEPT] Dimers as potential multifunctional inhibitors of HIV-1 reverse transcriptase. *J. Med. Chem.* **1995**, *38*, 1641−1649.

(22) For a recent paper, see: Renoud-Grappin, M.; Fossey, C.; Fontaine, G.; Ladurée, D.; Aubertin, A. M.; Kirn, A. Imidazo-[1,5-*b*]pyridazine-d4T conjugates: Synthesis and anti-human immunodeficiency virus evaluation. *Antiviral Chem. Chemother.* **1998**, *9*, 205−223.

(23) A preliminary account of this work was presented at the XIII International Round Table Conference on Nucleosides, Nucleotides and their biological Applications. Montpellier, France. September 6−10, 1998. Poster 155. *Nucleosides Nucleotides* **1999**, *18*, 1029−1030.

(24) McGuigan, C.; Devine, K. G.; O'Connor, T. J.; Galpin, S. A., Jeffries, D. J.; Kinchington, D. Synthesis and evaluation of some novel phosphoramidate derivatives of 3′-azido-3′-deoxythimidine (AZT) as anti-HIV compounds. *Antiviral Chem. Chemother.* **1990**, *1*, 107−113.

(25) McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E. Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.* **1993**, *36*, 1048−1052.

(26) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. Arylphosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. *J. Med. Chem.* **1996**, *39*, 1748−1753.

(27) Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. TSAO analogues. Stereospecific synthesis and anti-HIV-1 activity of 1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole-2″,2″-dioxide]pyrimidine and pyrimidine modified nucleosides. *J. Med. Chem.* **1992**, *35*, 2988−2995.

(28) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Devine, K. G.; Hay, A. J. Aryl phosphate Derivatives of AZT retain activity against HIV1 in cell lines which are resistant to the action of AZT. *Antiviral Res.* **1992**, *17*, 311−321.

(29) (a) Heck, R. F. Arylation, methylation and carboxyalkylation of olefins by group VIII metal derivatives. *J. Am. Chem. Soc.* **1968**, *90*, 5518−5526. (b) Heck, R. F. Palladium reagents in Organic Synthesis; Academic Press: New York, 1985. (c) Heck, R. F. Palladium-catalyzed reactions of organic halides. *Acc. Chem. Res.* **1979**, *12*, 146−151. (d) Heck, R. F. Palladium-catalyzed vinylation of organic halides. *Org. React.* **1982**, *27*, 345−390.

(30) Sonogashira, K.; Tohda, Y.; Hagihara; N. Convenient synthesis of acetylenes catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. *Tetrahedron Lett.* **1975**, 4467−4470.

(31) (a) Bergstrom, D. E.; Ruth, J. L. Synthesis of C-5 substituted pyrimidine nucleosides via organopalladium intermediates. *J. Am. Chem. Soc.* **1976**, *98*, 1587−1589. (b) Bergstrom, D. E.; Ogawa, M. K. C-5 substituted pyrimidine nucleosides. 2. Synthesis via olefin coupling to Organopalladium intermediates derived from uridines and 2′-deoxyuridines. *J. Am. Chem. Soc.* **1978**, *100*, 8106−8112. (c) Bergstrom, D. E.; Beal, P.; Jenson, J.; Lin, X. Palladium mediated synthesis of C-5 pyrimidines nucleoside thioethers from disulfides and mercurinucleosides. *J. Org. Chem.* **1991**, *56*, 5598−5602. (d) Bergstrom, D. E.; Lin, X.; Wang, G.; Rotsein, D.; Beal, P.; Norrix, K.; Ruth, J. L. *Synlett.* **1992**, 179−188.

(32) Robins, M. J.; Barr, P. J. Nucleic Acid Related Compounds. 39. Efficient conversion of 5-iodo to 5-alkynyl and derived 5-substituted uracil bases and nucleosides. *J. Org. Chem.* **1983**, *48*, 1854−1862.

(33) (a) Hobbs, F. W., Jr. Palladium-catalyzed synthesis of alkynylamino nucleosides. A universal linker for nucleic acids. *J. Org. Chem.* **1989**, *54*, 3420−3422. (b) Robins, M. J.; Vinayak, R. S.; Wood, S. G. Solvent not palladium oxidation state is the primary determinant for successful coupling of terminal alkynes with iodo-nucleosides. *Tetrahedron Lett.* **1990**, *31*, 3731−3734.

(34) (a) Kundu, N. G.; Dasgupta, S. K. Synthesis of 5-(acylethynyl)-uracils and their corresponding 2′-deoxyribonucleosides through Palladium-catalyzed reactions. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2657−2663. (b) Kundu, N. G.; Dasgupta, S. K.; Chaudhuri, L. N.; Mahanty, J. S.; Spears, C. P.; Shahinian, A. H. Synthesis and biological activities of (E)-5-(2-acylvinyl)uracils. *Eur. J. Med. Chem.* **1993**, *28*, 473−479.

(35) Lin, T. S.; Gao, Y. S.; Mancini, W. R. Synthesis and biological activity of various 3′-azido and 3′-amino analogues of 5-substituted pyrimidine deoxyribonucleosides. *J. Med. Chem.* **1983**, *26*, 1691−1696.

(36) Lin, T. S.; Mancini, W. R. Synthesis and antineoplastic activity of 3′-azido and 3′-amino analogues of pyrimidine deoxyribonucleosides. *J. Med. Chem.* **1983**, *26*, 544−548.

(37) Balagopala, M. I.; Ollapally, A. P.; Lee, H. H. An improved synthesis of azidothymidine. *Nucleosides Nucleotides* **1996**, *15*, 899−906.

(38) Joshi, B. V.; Rao, T. S.; Reese, C. B. Conversion of some pyrimidine 2′-deoxyribonucleosides into the corresponding 2′,3′-didehydro-2′,3′-dideoxynucleosides. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2537−2544.

(39) Larsen, E.; Kofoed, T.; Pedersen, E. B. Synthesis of 2′,3′-anhydro-2′-deoxyuridines and 2′,3′-didehydro-2′,3′-dideoxyuridines using polymer supported fluoride. *Synthesis* **1995**, 1121−1125.

(40) Robins, M. J.; Barr, P. J.; Giziewicz, J. Nucleic acid related compounds. 38. Smooth and high-yield iodination and chlorination at C-5 of uracil bases and *p*-toluyl-protected nucleosides. *Can. J. Chem.* **1982**, *60*, 554−557.

(41) For reasons of simplicity we have adopted this nomenclature for the whole set of heterodimers synthesized. A more correct name, for example, of the heterodimer [TSAO-T]N3-$(CH_2)_3$-N3-[d4T] (**5a**), chosen as a model, would be [1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-[(2‴,3‴-didehydro-2‴,3‴-dideoxythymidin-3‴-yl)propyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide). Although the oxathiole ring has priority over the TSAO nucleoside system, double primes have been used in the numbering of the oxathiole ring in order to keep the same numbering system as in the previous TSAO papers. Triple and quadruple primes have been used for the second nucleoside system chosen as radical.

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