Synthesis and Anti-HIV Activity of [AZT]-[TSAO-T] and [AZT]-[HEPT] Dimers as Potential Multifunctional Inhibitors of HIV-1 Reverse Transcriptase

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In an attempt to combine the HIV-inhibitory capacity of 2′,3′-dideoxynucleoside (ddN) analogues and non-nucleoside reverse transcriptase (RT) inhibitors (NNRTI), we have designed, synthesized, and evaluated for their anti-HIV activity several dimers of the general formula [ddN]-$(CH_2)_n$-[NNRTI]. These dimers combine in their structure a ddN such as AZT and a NNRTI such as TSAO-T and HEPT linked through an appropriate spacer between the N-3 of the thymine base of both compounds. The [TSAO-T]-$(CH_2)_n$-[AZT] dimers proved markedly inhibitory to HIV-1. Also, if AZT was replaced by thymidine in the dimer molecules, potent anti-HIV-1 activity was observed. However, although the compounds proved inhibitory to HIV-1, they were less potent inhibitors than the parent compounds from which they were derived. None of the dimers were endowed with anti-HIV-2 activity. In contrast with the TSAO-T monomers, none of the TSAO-T-containing dimers proved markedly cytotoxic to the cells. There was a clear trend toward decreased antiviral potency with lengthening the methylene spacer in the [TSAO-T]-$(CH_2)_n$-[AZT] dimers.

Introduction

Various compounds have been reported as potent and selective inhibitors of human immunodeficiency virus (HIV) targeted at the virus-encoded reverse transcriptase (RT).

Among them, 2′,3′-dideoxynucleosides (ddN) such as 3′-azido-2′,3′-dideoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), and 2′,3′-dideoxycytidine (ddC) have proved effective in treatment of HIV-infected patients. These compounds need to be converted to their 5′-triphosphate derivatives to become potent inhibitors of RT,[1-3] and they do not discriminate in their antiviral potency between HIV-1 and HIV-2. Unfortunately, AZT-resistant HIV strains emerge in patients that are on long-term therapy with AZT.[4,5]

Recently, a number of different structural classes of non-nucleoside analogues (also called non-nucleoside RT inhibitors (NNRTI)), i.e., HEPT, TIBO, nevirapine, pyridinone, BHAP, TSAO, α-APA, PETT, and quinoxaline, have been identified as potent and highly specific inhibitors of HIV-1 replication. They interact with HIV-1 RT at a nonsubstrate binding site.[6-16] The TSAO derivatives represent a unique structural class of non-nucleoside RT inhibitors, discovered in 1992 in our laboratories,[13,17-20] in that they specifically seem to interact with the p51 subunit of the RT heterodimer. Although they are structurally related to nucleoside analogues, TSAO derivatives behave like the other HIV-1-specific RT inhibitors since they are also targeted at a nonsubstrate-binding site of the HIV-1-encoded RT.[21,22] The TSAO derivatives are the first HIV-1-specific RT inhibitors for which a well-defined part of the molecule (i.e., the 4″-amino group at 3′-spiro of the ribose moiety) has been identified as an essential pharmacophore interacting with a well-defined moiety (the COOH group of Glu-138) of HIV-1 RT.[23,24] There is crystallographic evidence that Glu-138 is located at the top of the finger domain of the p51 subunit of HIV-1 RT, which closely approaches the binding pocket of the HIV-1-specific RT inhibitors at the p66 subunit, and/or may even be part of this pocket.[25,26]

Another common feature of the HIV-1-specific non-nucleoside RT inhibitors is that they lead to the rapid emergence of mutant HIV-1 strains that are resistant to these compounds. HIV-1 strains that are resistant to the non-nucleoside RT inhibitors are still sensitive to AZT or ddI, and vice versa, HIV-1 strains selected for resistance against AZT or ddI are still sensitive to the HIV-1-specific non-nucleoside RT inhibitors.[27,28] In fact, HIV-1 strains resistant to AZT or ddI show amino acid substitutions in the HIV-1 RT[5,29-31] that are clearly distinct from those reported in non-nucleoside-resistant HIV-1 strains.[28,32-36] Combination of anti-HIV agents is now being explored as therapeutic modalities to prevent emergence of virus–drug resistance.[37,38]

One approach to combination therapy, which has been suggested by Arnold and co-workers,[26] would be the use of dimers resulting from the linking of a NNRTI and a ddN through an appropriate spacer, in an attempt to combine the inhibitory capacity of these two different classes of molecules. Due to the NNRTI, the dimers might be highly specific to HIV-1 RT and might lower the speed of the emergence of virus–drug resistance.

With this aim, we have designed, synthesized, and evaluated on their anti-HIV-1 activity several dimers of the general formula **1** (Chart 1) which combine in their structure a ddN derivative and a NNRTI. As ddN inhibitor, we chose AZT (**2**) (the drug used in the clinical treatment of AIDS). Both TSAO-T (**4**) and HEPT (**5**) were used as non-nucleoside RT inhibitors. The two classes of inhibitors (ddN and NNRTI) were linked at the N-3 of the thymidine base of each compound by an appropriate spacer. As spacer, we used a carbon

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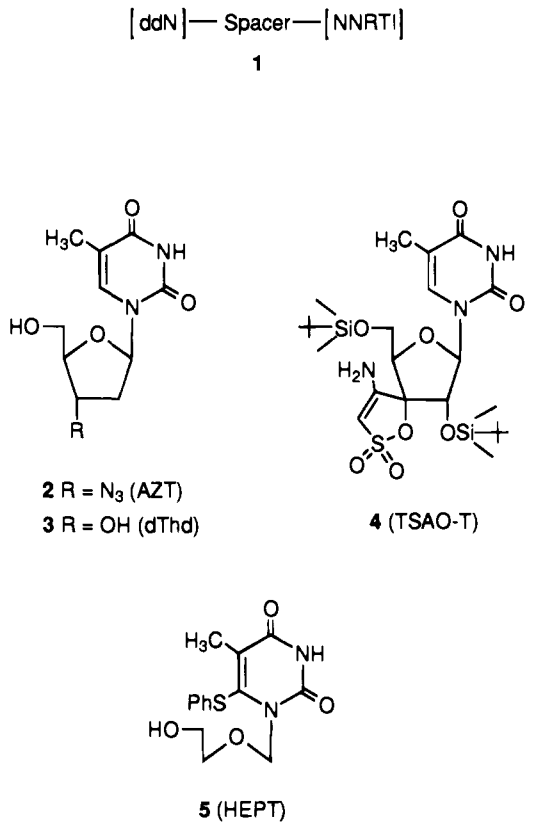
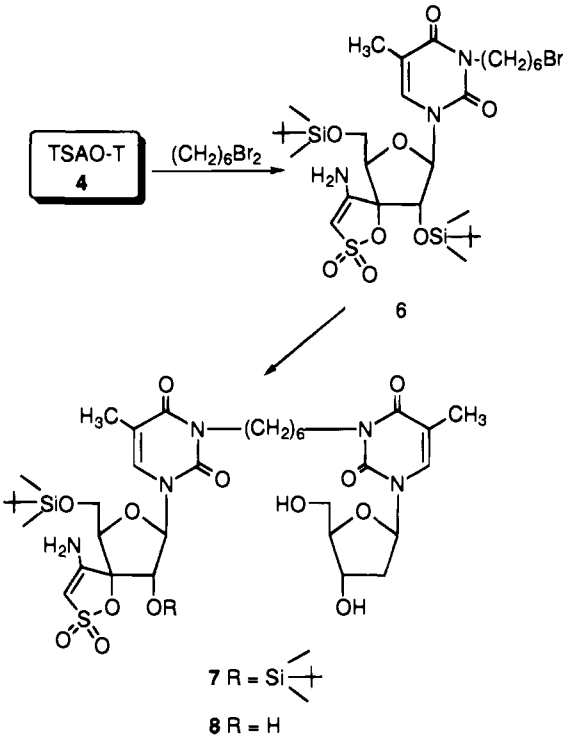
Chart 1



Scheme 1



aliphatic linkage of different lengths in order to obtain a dimer possessing an optimum distance betwene both active principles (ddN and NNRTI). Such an aliphatic spacer would give a quite flexible molecule. Some flexibility may be needed to permit penetration of the dimer through the enzyme reaching the binding pocket and also to avoid short contacts with protruding residues at the inner regions of the binding pocket.

It should be noted that ddN in their 5′-triphosphate forms strictly act as competitive inhibitors of RT with respect to the natural substrates **3**. Therefore we considered of interest to prepare dimers in which a NNRTI was linked to the natural substrate of the RT as well, and consequently, we prepared TSAO-$(CH_2)_n$-dThd and HEPT-$(CH_2)_n$-dThd heterodimers. Since we initially did not know (i) the optimum distance between both kind of inhibitors and (ii) the relative orientation of the binding sites for both types of inhibitors, we considered it best to initially test a quite flexible molecule with "some" chances to interact (even having the penalty of being less active) rather than a too rigid molecule, which in the case that it does not have the right conformation will never interact with the reverse transcriptase.

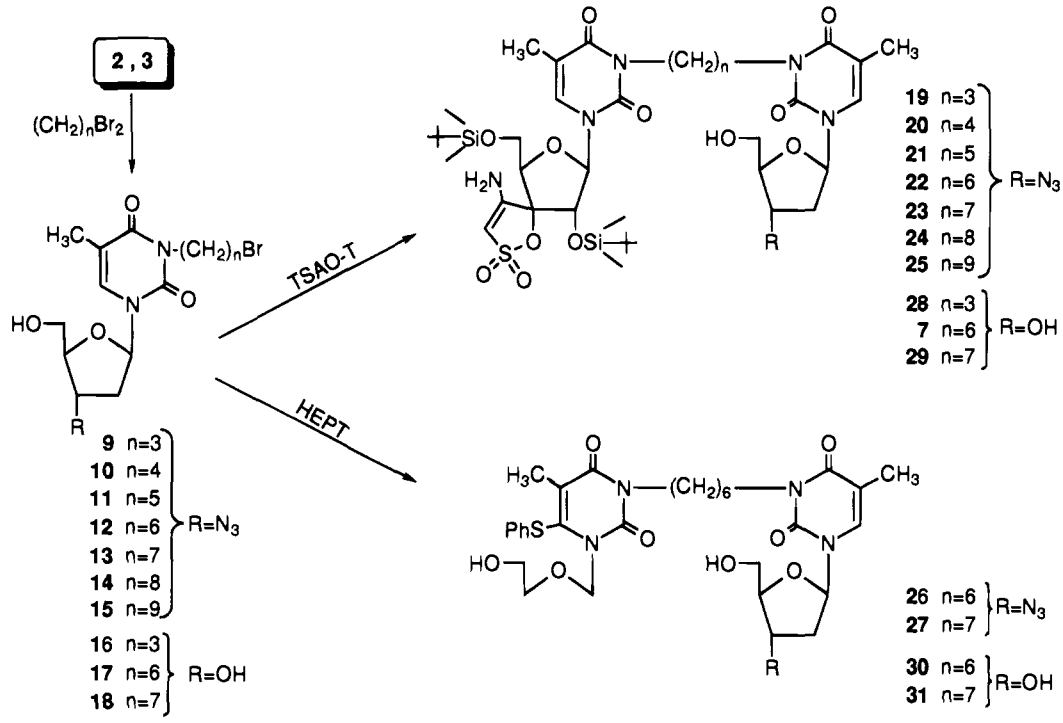
Chemistry

For the synthesis of the target heterodimers we designed a procedure based on the method used for selective N-3-alkylation of TSAO derivatives.[19] The method consisted on the basic treatment of the first key nucleoside with the appropriate dibromoalkyl reagent followed by reaction of the N-3-bromoalkyl nucleoside intermediate, thus obtained, with the second key nucleoside to give the N-3,N-3-alkyl heterodimers. Thus, reaction of 1 equiv of TSAO-T (**4**) (Scheme 1) with 2 equiv of 1,6-dibromohexane in dry acetone and in the presence of potassium carbonate (K_2CO_3) gave the N-3-substituted derivative **6** in 64% yield. Treatment of **6** with thymidine (**3**) and K_2CO_3, in acetonitrile:DMF (3:1), afforded a mixture of the [TSAO-T]-$(CH_2)_6$-[dThd] heterodimer (**7**) (30%) and the heterodimer deprotected at the 2′-position of the TSAO-T moiety (**8**) (13%), probably due to the traces of water or dimethylamine present in the DMF. This deprotection was avoided when the reaction conditions were changed, that is, initial treatment of thymidine (**3**) with 1,6-dibromohexane, in acetone:DMF (1:1), under basic conditions (K_2CO_3) followed by reaction with TSAO-T in dry acetonitrile (Scheme 2) gave, exclusively, the hexamethylene heterodimer **7** in 70% yield. Therefore, this second reaction sequence was followed to prepare the desired heterodimers.

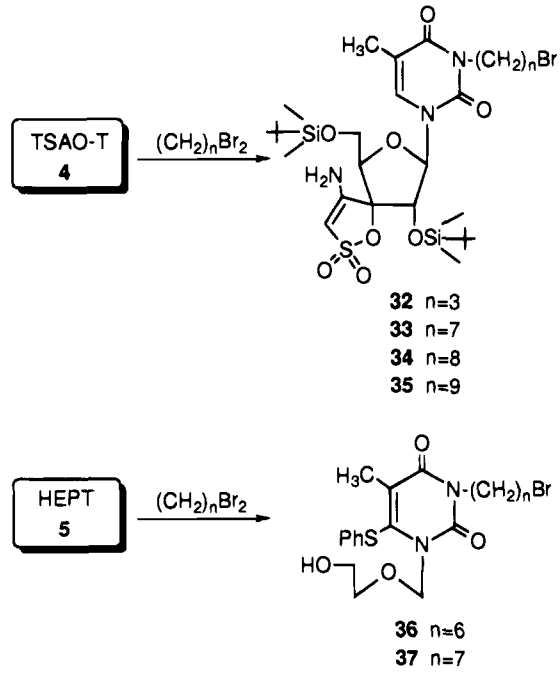
Thus, reaction of AZT (**2**) or thymidine (**3**) (Scheme 2) with a variety of dibromoalkyl reagents [$(CH_2)_nBr_2$, n = 3–9] in the presence of K_2CO_3 yielded the N-3-bromoalkyl derivatives of AZT [**9** (75%), **10** (84%), **11** (82%), **12** (70%), **13** (79%), **14** (71%), **15** (79%)] and thymidine [**16** (47%), **17** (64%), **18** (60%)]. Treatment of AZT intermediates **9**–**15** with TSAO-T (**4**) or HEPT (**5**) in the presence of K_2CO_3 gave the [AZT]-$(CH_2)_n$-[TSAO-T] (**19**–**25**) and [AZT]-$(CH_2)_n$-[HEPT] (**26**, **27**) heterodimers in high yields. Similarly, treatment of thymidine intermediates **16**–**18** with TSAO-T or HEPT in the presence of K_2CO_3 afforded the corresponding heterodimers [dThd]-$(CH_2)_n$-[TSAO-T] **28** and **29** and [dThd]-$(CH_2)_n$-[HEPT] **30** and **31**, respectively.

Finally, reaction of TSAO-T (Scheme 3) with several dibromoalkyl reagents in the presence of K_2CO_3 afforded the N-3-bromomethylene intermediates **32**–**35**. A similar reaction of HEPT with 1,6-dibromohexane or 1,7-dibromoheptane yielded N-3-bromohexa- and N-3-bromoheptamethylene intermediates **36** and **37** in 60% and 75% yields, respectively.

Scheme 2



Scheme 3



Structures of the heterodimers **7** and **19**–**31** were assigned on the basis of their analytical and spectroscopic data. One- and two-dimensional (1D and 2D) NMR techniques were used. From the whole set of compounds synthesized, one representative sample of each group of heterodimers (i.e., [TSAO]-$(CH_2)_n$-[AZT] (**19**–**25**), [TSAO]-$(CH_2)_n$-[dThd] (**7**, **28**, **29**), [HEPT]-$(CH_2)_n$-[AZT] (**26**, **27**), [HEPT]-$(CH_2)_n$-[dThd] (**30**, **31**)) was chosen as a model compound, with the assumption that the ^1H NMR spectra of each group of heterodimers differ only in the integral corresponding to the methylene groups of the spacer. Therefore, their structures were determined through comparison of their ^1H NMR spectra to those of the model compounds. These NMR data are included as supplementary material. Thus, we choose as model compounds the TSAO heterodimers **23** and **29** and the HEPT heterodimers **27** and **31**, which have a heptamethylene spacer in their structures (see Table 1). The methodology followed is illustrated with compound **23**. Thus, full assignment of all proton resonances was achieved by COSY and TOCSY experiments. The severe overlapping observed in the spectrum precluded the possibility of measuring chemical shifts and H–H vicinal coupling constants. Therefore, in order to unravel the spectrum and obtain precise values for these parameters, 1D selective TOCSY experiments were performed. Thus, by selective excitation of the anomeric protons (H-1′) of the AZT and TSAO moieties and H-4′ of the TSAO moiety and the $CH_2\beta$ of the linker (CH_2 at β-position to the nitrogen of the base), we obtained, with good digital resolution, four different subspectra (Figure 1) corresponding to the four main different coupling paths in the molecule without overlapping from other subsystems.

NOESY experiments allowed the tentative determination of some conformational features of this molecule. The conformations around the glycosidic bond of the AZT and TSAO moieties were analyzed by examining the correlation peaks in the NOESY spectrum between the base protons and those of the ribose moiety. Thus, the AZT moiety showed, in the NOESY spectrum, correlation peaks between the H-6 proton of the thymine base and Me-5 (1.831 ppm), H-1′ (6.234 ppm), H-2′ (2.497 ppm), H-3′ (4.480 ppm), and H-5′a (3.869 ppm). On the other hand, the H-6 proton of the thymine of the TSAO moiety showed correlation peaks with Me-5 (1.930 ppm), H-1′ (6.026 ppm), H-2′ (4.716 ppm), and H-5′ (4.04–4.11 ppm). Such NOE cross-peak patterns are compatible with a dynamic syn $\rightleftarrows$ anti equilibrium of the glycosidic bond of both moieties (AZT and TSAO). A similar conformational behavior around the glycosidic bond was observed with the other model heterodimers (**27**, **29**, **31**).

Table 1. ^{1}H NMR (500 MHz) Chemical Shifts (ppm) Assignments of Heterodimers 23, 27, 29, and 31 in Acetone-d_6 Solution, with TMS as Internal Reference

compd	moiety	H-1′	H-2′a	H-2′b	H-3′	H-4′	H-5′a	H-5′b	H-6	Me-5	CH_2 spacer	others
23	AZT	6.234	2.497	2.407	4.480	3.949	3.869	3.809	7.779	1.831	3.84–1.57	4.383 (OH-5′)
											1.30–1.40	
	TSAO-T	6.026	4.716	–	–	4.311	4.114	4.040	7.497	1.930	1.59–3.90	6.455 (NH_2-4″), 5.744 (H-3″)
27	AZT	6.233	2.493	2.405	4.480	3.948	3.868	3.807	7.780	1.829	3.86–1.57	4.410 (OH-5′)
											1.30–1.40	2.797 (OH), 7.2–7.4 (SPh)
	HEPT	5.625	–	–	–	–	–	–	–	1.976	1.61–3.93	3.624, 3.556 (OCH_2CH_2O)
29	dThd	6.333	2.245	2.223	4.478	3.916	3.776		7.801	1.829	3.85–1.57	4.359 (OH-3′), 4.192 (OH-5′)
											1.30–1.40	
	TSAO-T	6.017	4.719	–	–	4.309	4.111	4.033	7.499	1.929	1.60–3.90	6.471 (NH_2-4″), 6.471 (H-3″)
31	dThd	6.333	2.246	2.227	4.483	3.924	3.777		7.796	1.827	3.87–1.59	4.338 (OH-3′), 4.172 (OH-5′)
											1.30–1.40	7.2–7.4 (SPh), 2.794 (OH)
	HEPT	5.625	–	–	–	–	–	–	–	1.976	1.62–3.91	3.624, 3.556 (OCH_2CH_2O)

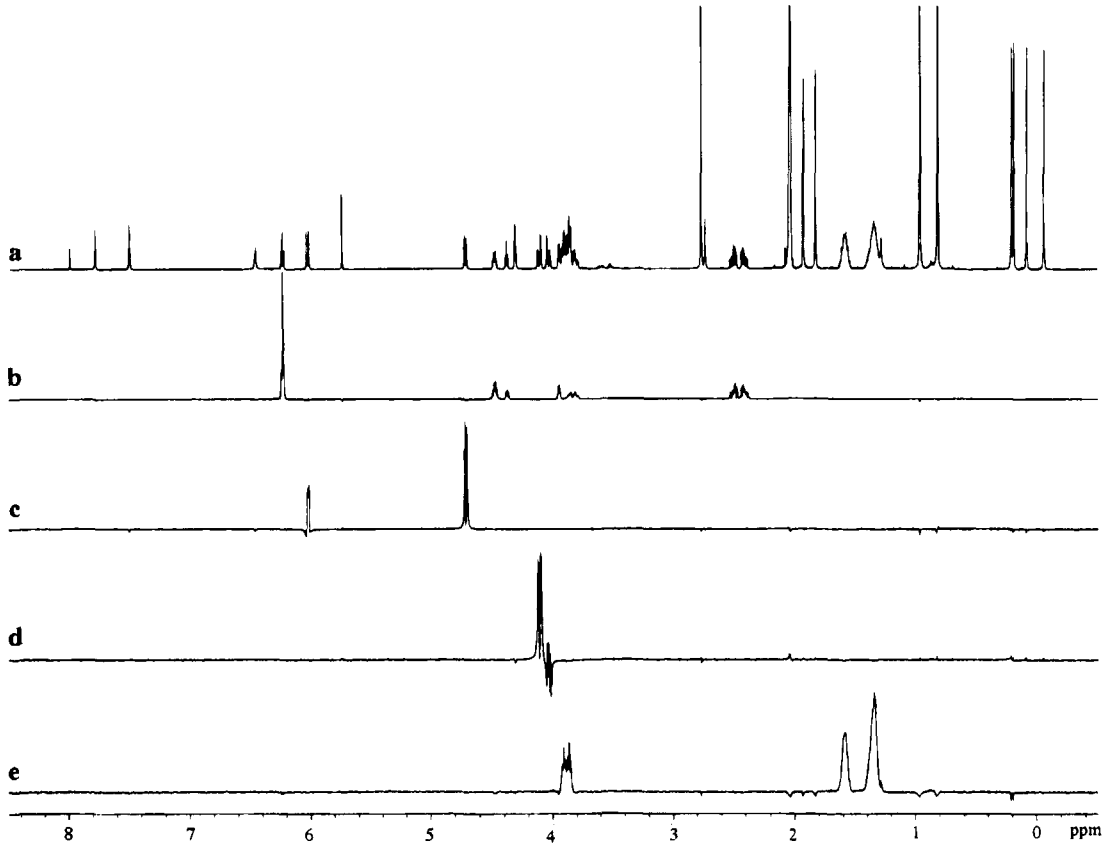


Figure 1. Selective TOCSY spectra for [TSAO-T]-$(CH_2)_7$-[AZT] heterodimer (**23**): (a) full spectrum, (b) subspectrum corresponding to AZT moiety, (c) subspectrum for H-1′ and H-2′ protons of TSAO-T moiety, (d) subspectrum for H-4′, H-5′a, and H-5′b protons of TSAO-T moiety, and (e) subspectrum corresponding to methylene protons of the linker.

Biological Results and Discussion

Anti-HIV Activity of (Un)substituted Nucleoside Monomers. The AZT, dThd, TSAO-T, and HEPT monomers containing different bromomethylene spacers on the N-3-position of the base moiety were evaluated against HIV-1- and HIV-2-induced cytopathicity in wild-type CEM/0 cells, as well as HIV-2-induced cytopathicity in thymidine kinase-deficient CEM/TK$^-$ cells (Table 2).

AZT proved exquisitely active against HIV-1 and HIV-2-induced cytopathicity in CEM/0 cells (EC_{50}: 0.011 μM). However, AZT virtually looses its antiviral potential when evaluated against HIV-2 in CEM/TK$^-$ cells, presumably due to the lack of cytosol thymidine kinase, the activating enzyme of AZT.

The N-3-substituted AZT derivatives **9–15** proved inactive at subtoxic concentrations irrespective of the chain length (n) of the methylene spacer (n = 3–9). The lack of activity of these N-3-substituted AZT 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 AZT. Also, the N-3-position of AZT might be required for hydrogen bonding with the complementary adenine base. Substituents on N-3 of AZT are expected to lower the efficacy of base pairing. Thymidine that is inactive on its own right (EC_{50}: >40 μM) is also inactive as an anti-HIV agent when a $Br(CH_2)_7$ spacer had been introduced at the N-3-position of its base (**18**). In contrast, TSAO-T kept part of its antiviral activity when carrying the bromomethylene linker. The longer the spacer, the more the anti-HIV-1 activity of TSAO-T decreased. The EC_{50} for HIV-1 gradually increased from 0.05 μM for the unsubstituted TSAO-T, to 0.11 μM for N-3-bromomethylene TSAO-T consisting of three methylene units, and to 0.47 and 0.79 μM for N-3-bromomethylene TSAO-T consisting of six and seven

Table 2. Inhibitory Effect of Nucleoside Monomers against HIV-1 and HIV-2 Replication in CEM Cells

compd	no. of methylene units	EC_{50}[a] (μM) CEM/0 HIV-1	CEM/0 HIV-2	CEM/TK⁻ HIV-2
AZT		0.011	0.011	>35
9	3	>50	>50	–
10	4	–	–	–
11	5	≥50	≥50	>10
12	6	–	–	>45
13	7	–	–	–
14	8	>8	>8	>8
15	9	>8	>8	>8
dThd		>40	>40	>40
18	7	>10	>10	>10
TSAO-T		0.05	>6	>6
32	3	0.11	>100	–
6	6	0.47	>25	–
33	7	0.79	>1	>5
34	8	>1	>1	>1
35	9	>1	>1	>1
HEPT			>100	>100
36	6	–	–	>8
37	7	>8	>8	>8

[a] 50% effective concentration.

Table 3. Inhibitory Effect of [ddN]-$(CH_2)_n$-[NNRTI] Dimers against HIV-1 and HIV-2 Replication in CEM Cells

compd	ddN	NNRTI	no. of methylene units	EC_{50}[a] (μM) CEM/0 HIV-1	CEM/0 HIV-2	CEM/TK⁻ HIV-2
7	dThd	TSAO	6	0.55	>4	–
28	dThd	TSAO	3	0.06	>4	>4
29	dThd	TSAO	7	0.22	>4	–
19	AZT	TSAO	3	0.10	>100	>100
20	AZT	TSAO	4	0.20	>100	>100
21	AZT	TSAO	5	0.22	>100	>100
22	AZT	TSAO	6	0.21	>100	>100
23	AZT	TSAO	7	1.58	>100	–
24	AZT	TSAO	8	1.56	>100	>100
25	AZT	TSAO	9	15.3	>100	>100
26	AZT	HEPT	6	>5	>5	>5
27	AZT	HEPT	7	>5	>5	–
30	dThd	HEPT	6	79	>30	–
31	dThd	HEPT	7	>30	>30	–

[a] 50% effective concentration.

methylene units, respectively. At higher spacer length (i.e., N-3-bromooctyl and N-3-bromononyl TSAO-T), no anti-HIV-1 activity was recorded at subtoxic concentrations. Interestingly, introduction of a short-length N-3-bromomethylene spacer on TSAO-T resulted in a markedly decreased cytotoxicity. Again, a gradual increase in toxicity was noted with increasing the spacer length. Finally, HEPT derivatives, linked with bromohexane and bromoheptane, were not inhibitory to HIV and proved also more cytotoxic than the unsubstituted HEPT.

Anti-HIV Activity of Dimers Between TSAO-T or HEPT and AZT or dThd. The shorter the methylene linker between both molecules the higher the activity of the [TSAO-T]-$(CH_2)_n$-[AZT] dimers (Table 3). In fact, [TSAO-T]-$(CH_2)_3$-[AZT] (**19**) was only 2-fold less inhibitory to HIV-1 than TSAO-T. Longer methylene linkers (n = 4–6) decreased the antiviral activity by 2-fold (Table 3).

The [TSAO-T]-$(CH_2)_n$-[AZT] derivatives containing heptane (**23**) or octane (**24**) as linker showed an EC_{50} of 1.6 μM, whereas the nonane-containing dimer **25** was at least 200-fold less inhibitory to HIV-1 than TSAO-T. Interestingly, none of the dimers proved markedly cytotoxic to the CEM cells at 100 μM, whereas the parent monomer TSAO-T had a CC_{50} of about 10 μM. None of the dimers was endowed with anti-HIV-2 activity in CEM/0 cells. They were also devoid of anti-HIV-2 activity in CEM/TK⁻ cells.

The [TSAO-T]-$(CH_2)_n$-[dThd] dimers **7**, **28**, and **29** also showed pronounced activity against HIV-1 but not HIV-2. In fact, the activity found for the propane dimer **28** (EC_{50}: 0.06 μM) closely corresponds with the EC_{50} (0.10 μM) found for [TSAO-T]-$(CH_2)_3$-[AZT]. Also, the EC_{50}'s (0.5 and 0.2 μM) found for the hexane and heptane dimers closely coincided with those found for the corresponding [TSAO-T]-$(CH_2)_n$-[AZT] dimers (EC_{50}'s: 0.21 and 1.6 μM). None of the [HEPT]-$(CH_2)_n$-[AZT] (**26**, **27**) or [HEPT]-$(CH_2)_n$-[dThd] (**30**, **31**) derivatives proved active against HIV-1 or HIV-2 (Table 3).

The concomitant decrease in the anti-HIV-1 activity of the [TSAO-T]-$(CH_2)_n$-[AZT], [TSAO-T]-$(CH_2)_n$-[dThd], and [TSAO-T]-$(CH_2)_n$Br derivatives with increasing methylene spacer length and the comparable antiviral activities of [TSAO-T]-$(CH_2)_n$-[AZT], [TSAO-T]-$(CH_2)_n$-[dThd], and [TSAO-T]-$(CH_2)_n$Br with identical spacer lengths clearly indicate that the AZT or dThd moieties did not contribute to the antiviral activity of the dimers. Moreover, since the [TSAO-T]-$(CH_2)_n$-[AZT] dimers were totally inactive against HIV-2, the inhibitory activity of the TSAO dimers against HIV-1 must reside only in the N-3-substituted TSAO-T monomer of the dimer.

Several reasons may explain the failure of this dimer approach to increase the inhibitory activity against HIV. (i) It is unknown which spacer length between the NNRTI (i.e., TSAO-T or HEPT) and the ddN analogue (i.e., AZT or dThd) is required to be optimal for antiviral activity. The spacer length should be able to position both the NNRTI and the ddN in close contact with their binding sites at HIV-1 RT. An increase or decrease of one methylene unit in the spacer may hinder one of the dimer components (presumably the nucleoside moiety) in its interaction with RT and, indirectly, also diminish the efficiency of interaction of the other part of the molecule (presumably the NNRTI moiety). (ii) Even if the spacer length between both monomers would be optimal, the monomers may be linked to each other in the wrong position. It is presently not known how the TSAO and HEPT derivatives exactly fit into the NNRTI-binding pocket of the RT. Therefore, it may well be possible that the nucleoside part of the dimer, which is linked to the NNRTI through the N-3-position of the thymine base, may be in the wrong position to reach its target site at RT. (iii) Since AZT (and also dThd) is known to interact with RT only after it has been converted to its 5′-triphosphate form, it may not be surprising that the nucleoside moiety of the dimer cannot interact with the RT without being phosphorylated, even if the linker may have the optimal length to let both components of the dimer fit into their interaction sites. It is highly unlikely that the [TSAO-T]-$(CH_2)_n$-[AZT] dimer would be phosphorylated inside the cells. We were unable to demonstrate any inhibitory effect of compounds **15**, **28**, and **22** on [^{3}H]thymidine phosphorylation by CEM cytosol thymidine kinase (data not shown). It is also doubtful that a 5′-triphosphorylated dimer would ever be able to efficiently cross the membrane of virus-infected cells in an intact form and, if taken up by the cells, would persist long enough to

reach its target enzyme RT. One possible way to circumvent premature breakdown of the phosphate groups at the [TSAO-T]-$(CH_2)_n$-[AZT-triphosphate] dimers may be the replacement of AZT-triphosphate by the AZT-α,β-methylene-5′-phosphonate analogue. Indeed, it has previously been shown that the phosphonate analogue of AZT-triphosphate is resistant to hydrolytic attack by phosphodiesterases and has proved stable in the presence of fetal calf serum.[39] In addition, although an α,β-methylene-AZT-triphosphonate derivative is a less potent inhibitor of HIV-1 RT than its parent compound AZT-triphosphate, it retains enough anti-RT activity (50% inhibitory concentration: 4.6 μM)[39] to be able to afford an inhibitory effect against RT when present with TSAO-T in the dimer molecule. With the TSAO derivatives, we have ascertained that linkage of the methylene spacer to the N-3-position of the thymine base maintains (at least partially) the antiviral activity. We found that for the NNRTI monomers, the larger the spacer arm, the lower the antiviral activity and the higher the cytotoxicity of the test compounds. In the dimers of TSAO with AZT, a similar trend of decreased antiviral activity with increasing spacer length was observed. This leads us to assume that the antiviral effect seen for [TSAO-T]-$(CH_2)_n$-[ddN] dimers is based upon residual activity of the TSAO-spacer monomer, without any contribution of the AZT or dThd part of the molecule. Interestingly, for the [TSAO-T]-$(CH_2)_n$-[ddN] dimers, no increased toxicity was found. Such increased toxicity was noted for the N-3-substituted monomers and may be due to the alkylating properties of the TSAO monomers containing a free bromoalkyl arm. The dimers should be devoid of alkylating properties due to the lack of the bromine group.

Our observations that the dimer derivatives were devoid of any appreciable activity against HIV-2 point to the stability of the dimers outside and inside the cells. Indeed, if any AZT should have been released extra- or intracellularly, one would expect an activity against HIV-2, and this was clearly not observed.

In conclusion, the antiviral activity of the TSAO dimers can be ascribed to the TSAO part of the molecule without any significant contribution from the nucleoside part (either AZT or dThd) of the dimer. Other linkers and attachment sites for these linkers on both the NNRTI and nucleoside analogues should be explored to obtain better insights in the feasibility of the dimer approach to increase the inhibitory efficacy of the test compounds against the reverse transcriptase of HIV.

Experimental Section

Chemical Procedures. Microanalyses were obtained with a Heraeus CHN-O-Rapid instrument. ^{1}H NMR spectra were recorded on a Varian Unity 500 spectrometer operating at 499.84 MHz, using acetone-d_6 as solvent, at 30 °C with Me_4Si as internal standard. Monodimensional spectra were obtained under standard conditions. Phase sensitive double-quantum-filtered (DQF) COSY experiments were performed using 512 increments in each phase with a 2048 × 1024 data matrix and a relaxation delay of 1.25 s. The experiments were performed with a spin–lock time of 128 ms. The NOESY spectra were generated with a mixing time of 0.55 s; other acquisition parameters were identical to those used in the DQF COSY. 1D-Selective TOCSY experiments were acquired using a EBURP2-256-4 selective pulse with γB1 of 2.5 Hz and a spin–lock time of 128 ms. The relaxation delay was 2 s and the digital resolution 0.5 Hz with Me_4Si as internal standard. IR spectra were recorded with a Shimadzu IR-435 spectrometer. 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 μBondapak C_{18} column (3.9 × 300 mm; 10 mm). Eluent: CH_3CN/H_2O (0.05% TFA). Flow rate: 1 mL/min. Detection: UV at 214 nm.

General Procedure for the Synthesis of 3-*N*-(*n*-Bromoalkyl) Nucleoside Intermediates 6, 9–18, and 32–37. To a solution of the nucleoside [AZT (**2**), TSAO-T (**4**), or HEPT (**5**)] (1 equiv) in acetone, or thymidine (**3**) (1 equiv) in acetone/DMF (1:1), were added K_2CO_3 (1.1 equiv) and the corresponding *n*-dibromoalkane (n = 3–9) (2.0 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 a chromatotron. The reaction time, chromatography eluent, yield of the isolated products, and ^{1}H NMR data are indicated below for each compound.

[1-[2′,5′-Bis[*O*-(*tert*-butyldimethylsilyl)]-β-D-ribofuranosyl]-3-*N*-(6-bromohexyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (6): reaction time 8 h; eluent hexane/ethyl acetate, 3:1; yield of **6** (64%) as a white foam; HPLC t_R = 5.12 min (50:50); ^{1}H NMR δ 1.50, 1.86 (2m, 8H, $4CH_2$), 1.93 (d, 3H, CH_3-5, J = 1.1 Hz), 3.48 (t, 2H, CH_2Br, J = 6.7 Hz), 3.91 (t, 2H, CH_2N, J = 7.1 Hz), 4.02 (m, 1H, H-5′, J_{gem} = 12.2 Hz, $J_{4',5'}$ = 3.7 Hz), 4.12 (m, 1H, H-5′, $J_{4',5'}$ = 3.3 Hz), 4.32 (t, 1H, H-4′), 4.68 (d, 1H, H-2′), 5.75 (s, 1H, H-3″), 6.07 (d, 1H, H-1′, $J_{1',2'}$ = 8.1 Hz), 6.43 (bs, 2H, NH_2-4″), 7.49 (s, 1H, H-6). Anal. ($C_{30}H_{54}BrN_3O_8SSi_2$) C, H, N, S.

3′-Azido-3-*N*-(3-bromopropyl)-3′-deoxythymidine (9): reaction time 8 h; eluent hexane/ethyl acetate, 2:1; yield of **9** (75%) as a syrup; HPLC t_R = 5.60 min (50:50); ^{1}H NMR δ 1.84 (d, 3H, CH_3-5, J = 1.1 Hz), 2.17 (m, 2H, CH_2), 2.45 (m, 2H, 2H-2′), 3.49 (t, 2H, CH_2Br, J = 7.0 Hz), 3.85 (m, 2H, 2H-5′), 3.95 (m, 1H, H-4′), 4.02 (t, 2H, CH_2N, J = 6.9 Hz), 4.30 (t, 1H, OH-5′), 4.46 (m, 1H, H-3′), 6.22 (t, 1H, H-1′, $J_{1',2'}$ = 6.3 Hz), 7.78 (d, 1H, H-6). Anal. ($C_{13}H_{18}BrN_5O_4$) C, H, N.

3′-Azido-3-*N*-(4-bromobutyl)-3′-deoxythymidine (10): reaction time 8 h; eluent dichloromethane/methanol, 20:1; yield of **10** (84%) as a syrup; HPLC t_R = 3.91 min (70:30); ^{1}H NMR δ 1.73, 1.84 (2m, 4H, $2CH_2$), 1.83 (d, 3H, CH_3-5, J = 1.8 Hz), 2.43 (m, 2H, 2H-2′), 3.52 (t, 2H, CH_2Br, J = 6.4 Hz), 3.83 (m, 3H, 2H-5′, OH-5′, J_{gem} = 12.1 Hz, $J_{4',5'}$ = 3.3, 3.4 Hz), 3.91 (t, 2H, CH_2N, J = 7.0 Hz), 3.95 (m, 1H, H-4′), 4.49 (m, 1H, H-3′), 6.24 (t, 1H, H-1′, $J_{1',2'}$ = 6.3 Hz), 7.82 (m, 1H, H-6). Anal. ($C_{14}H_{20}BrN_5O_4$) C, H, N.

3′-Azido-3-*N*-(5-bromopentyl)-3′-deoxythymidine (11): reaction time 16 h; eluent chloroform/ethyl acetate, 10:1; yield of 11 (82%) as a syrup; HPLC t_R = 7.80 min (50:50); ^{1}H NMR δ 1.44, 1.58, 1.91 (3m, 6H, $3CH_2$), 1.82 (d, 3H, CH_3-5, J = 1.2 Hz), 2.43 (m, 2H, 2H-2′), 3.49 (t, 2H, CH_2Br, J = 6.8 Hz), 3.80 (m, 5H, H-4′, 2H-5′, CH_2N), 4.46 (m, 2H, H-3′, OH-5′), 6.23 (t, 1H, H-1′, $J_{1',2'}$ = 6.3 Hz), 7.78 (d, 1H, H-6). Anal. ($C_{15}H_{22}BrN_5O_4$) C, H, N.

3′-Azido-3-*N*-(6-bromohexyl)-3′-deoxythymidine (12): reaction time 12 h; eluent dichloromethane/methanol, 50:1; yield of **12** (70%) as a syrup; HPLC t_R = 7.36 min (55:45); ^{1}H NMR δ 1.50, 1.80 (2m, 8H, $4CH_2$), 1.82 (d, 3H, CH_3-5, J = 1.2 Hz), 2.44 (m, 2H, 2H-2′), 3.48 (t, 2H, CH_2Br, J = 6.8 Hz), 3.92 (m, 5H, H-4′, 2H-5′, CH_2N), 4.39 (t, 1H, OH-5′), 4.47 (m, 1H, H-3′), 6.23 (t, 1H, H-1′, $J_{1',2'}$ = 6.3 Hz), 7.81 (d, 1H, H-6). Anal. ($C_{16}H_{24}BrN_5O_4$) C, H, N.

3′-Azido-3-*N*-(7-bromoheptyl)-3′-deoxythymidine (13): reaction time 5 h; eluent chloroform/methanol, 20:1; yield of **13** (79%) as a syrup; HPLC t_R = 10.20 min (55:45); ^{1}H NMR δ 1.30, 1.57, 1.80 (3m, 10H, $5CH_2$), 1.82 (d, 3H, CH_3-5, J = 1.2 Hz), 2.44 (m, 2H, 2H-2′), 3.49 (t, 2H, CH_2Br, J = 6.8 Hz), 3.85 (m, 4H, 2H-5′, CH_2N), 3.94 (m, 1H, H-4′), 4.47 (m, 2H, H-3′, OH-5′), 6.24 (t, 2H, H-1′, $J_{1',2'}$ = 6.3 Hz), 7.79 (d, 1H, H-6). Anal. ($C_{17}H_{26}BrN_5O_4$) C, H, N.

3'-Azido-3-*N*-(8-bromooctyl)-3'-deoxythymidine (14): reaction time 16 h; eluent chloroform/ethyl acetate, 10:1; yield of **14** (71%) as a syrup; HPLC t_R = 17.90 min (50:50); ^{1}H NMR δ 1.33, 1.48, 1.56, 1.84 (4m, 12H, 6CH$_2$), 1.82 (d, 3H, CH$_3$-5, J = 1.2 Hz), 2.40, 2.49 (2m, 2H, 2H-2', J_{gem} = 12.0 Hz, $J_{2',3'}$ = 5.2, 6.3 Hz), 3.48 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.80 (m, 4H, 2H-5', CH$_2$N), 3.94 (m, 1H, H-4'), 4.49 (m, 2H, H-3', OH-5'), 6.24 (t, 1H, H-1', $J_{1',2'}$ = 6.4 Hz), 7.80 (d, 1H, H-6). Anal. ($C_{18}H_{28}BrN_5O_4$) C, H, N.

3'-Azido-3-*N*-(9-bromononyl)-3'-deoxythymidine (15): reaction time 16 h; eluent chloroform/ethyl acetate, 10:1; yield of **15** (79%) as a syrup; HPLC t_R = 25.48 min (50:50); ^{1}H NMR δ 1.50, 1.91 (2m, 14H, 7CH$_2$), 1.83 (d, 3H, CH$_3$-5, J = 1.1 Hz), 2.44 (m, 2H, 2H-2'), 3.48 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.80 (m, 5H, H-4', 2H-5', CH$_2$N), 4.48 (m, 2H, H-3', OH-5'), 6.24 (t, 1H, H-1', $J_{1',2'}$ = 6.4 Hz), 7.79 (m, 1H, H-6). Anal. ($C_{19}H_{30}BrN_5O_4$) C, H, N.

3-*N*-(3-Bromopropyl)thymidine (16). Thymidine (**3**) was reacted according to the general procedure for 24 h. The residue was chromatographed (dichloromethane/methanol, 50:1). The slower moving fractions gave **16** (47%) as an amorphous solid: HPLC t_R = 3.61 min (50:50); ^{1}H NMR δ 1.83 (d, 3H, CH$_3$-5, J = 1.1 Hz), 2.15 (m, 2H, CH$_2$), 2.23 (m, 2H, 2H-2'), 3.49 (t, 2H, CH$_2$Br, J = 7.0 Hz), 3.70, 3.79 (2m, 2H, 2H-5'), 3.92 (q, 1H, H-4', $J_{3',4'} = J_{4',5'}$ = 3.3 Hz), 4.00 (t, 2H, CH$_2$N), 4.20 (t, 1H, OH-5'), 4.35 (d, 1H, OH-3'), 4.45 (m, 1H, H-3'), 6.30 (t, 1H, H-1', $J_{1',2'}$ = 6.9 Hz), 7.83 (m, 1H, H-6). Anal. ($C_{13}H_{19}BrN_2O_5$) C, H, N.

The faster moving factions gave 3-*N*-(3-bromopropyl)thymine (35%) as a syrup: ^{1}H NMR [(CD$_3$)$_2$CO, 200 MHz] δ 1.82 (d, 3H, CH$_3$-5, J = 1.2 Hz), 2.14 (m, 2H, CH$_2$), 3.50 (t, 2H, CH$_2$Br, J = 7.0 Hz), 3.98 (t, 2H, CH$_2$N, J = 6.8 Hz), 7.27 (m, 1H, H-6), 9.76 (bs, 1H, NH).

3-*N*-(6-Bromohexyl)thymidine (17): reaction time 12 h; eluent dichloromethane/methanol, 20:1; yield of **17** (64%) as a syrup; HPLC t_R = 4.91 min (50:50); ^{1}H NMR δ 1.45, 1.60, 1.84 (3m, 8H, 4CH$_2$), 1.82 (d, 3H, CH$_3$-5, J = 1.2 Hz), 2.22 (m, 2H, 2H-2'), 3.47 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.73 (m, 1H, H-5'), 3.75 (m, 1H, H-5'), 3.85 (dd, 2H, CH$_2$N, J = 6.6, 7.1 Hz), 3.91 (m, 1H, H-4', $J_{4',5'} = J_{3',4'}$ = 3.3 Hz), 4.30 (t, 1H, OH-5'), 4.43 (m, 1H, OH-3'), 4.50 (m, 1H, H-3'), 6.30 (t, 1H, H-1', $J_{1',2'}$ = 7.1 Hz), 7.82 (m, 1H, H-6). Anal. ($C_{16}H_{25}BrN_2O_5$) C, H, N.

3-*N*-(7-Bromoheptyl)thymidine (18): reaction time 8 h; eluent chloroform/methanol, 20:1; yield of **18** (60%) as a syrup; HPLC t_R = 5.86 min (50:50); ^{1}H NMR δ 1.40, 1.58, 1.85 (3m, 10H, 5CH$_2$), 1.81 (d, 3H, CH$_3$-5, J = 1.2 Hz), 2.21 (m, 2H, 2H-2'), 3.47 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.74 (m, 2H, 2H-5'), 3.85 (dd, 2H, CH$_2$N, J = 6.5, 7.0 Hz), 3.91 (m, 1H, H-4'), 4.35 (t, 1H, OH-5'), 4.44 (d, 1H, OH-3'), 4.45 (m, 1H, H-3'), 6.32 (t, 1H, H-1', $J_{1',2'}$ = 6.9 Hz), 7.82 (m, 1H, H-6). Anal. ($C_{17}H_{27}BrN_2O_5$) C, H, N.

[1-[2',5'-Bis[*O*-(*tert*-butyldimethylsilyl)]-β-D-ribofuranosyl]-3-*N*-(3-bromopropyl)thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (32): reaction time 4 h; eluent hexane/ethyl acetate, 3:1, and subsequently chloroform/ethyl acetate, 4:1; yield of **32** (67%) as a white foam; ^{1}H NMR δ 1.94 (d, 3H, CH$_3$-5, J = 1.2 Hz), 2.17 (m, 2H, CH$_2$), 3.50 (t, 2H, CH$_2$Br, J = 7.0 Hz), 4.05 (t, 2H, CH$_2$N), 4.04 (m, 1H, H-5'), 4.15 (m, 1H, H-5'), 4.33 (t, 1H, H-4', $J_{4',5'}$ = 3.5 Hz), 4.67 (d, 1H, H-2'), 5.76 (s, 1H, H-3''), 6.08 (d, 1H, H-1', $J_{1',2'}$ = 8.1 Hz), 6.44 (bs, 2H, NH$_2$-4''), 7.50 (s, 3H, CH$_3$-5). Anal. ($C_{27}H_{48}BrN_3O_8SSi_2$) C, H, N, S.

[1-[2',5'-Bis[*O*-(*tert*-butyldimethylsilyl)]-β-D-ribofuranosyl]-3-*N*-(7-bromoheptyl)thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (33): reaction time 7 h; eluent hexane/ethyl acetate, 2:1; yield of **33** (78%) as a white foam; HPLC t_R = 23.88 min (70:30); ^{1}H NMR δ 1.50 (m, 10H, 5CH$_2$), 1.93 (s, 3H, CH$_3$-5), 3.48 (t, 2H, CH$_2$Br, J = 6.9 Hz), 3.90 (t, 2H, CH$_2$N), 4.05 (m, 1H, H-5', J_{gem} = 12.0 Hz, $J_{4',5'}$ = 3.6 Hz), 4.18 (m, 1H, H-5', $J_{4',5'}$ = 3.5 Hz), 4.32 (t, 1H, H-4'), 4.68 (d, 1H, H-2'), 5.75 (s, 1H, H-3''), 6.07 (d, 1H, H-1', $J_{1',2'}$ = 8.1 Hz), 6.43 (bs, 2H, NH$_2$-4''), 7.48 (s, 3H, CH$_3$-5). Anal. ($C_{31}H_{56}BrN_3O_8SSi_2$) C, H, N, S.

[1-[2',5'-Bis[*O*-(*tert*-butyldimethylsilyl)]-β-D-ribofuranosyl]-3-*N*-(8-bromooctyl)thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (34): reaction time 8 h; eluent hexane/ethyl acetate, 3:1; yield of **34** (63%) as a white foam; HPLC t_R = 11.48 min (80:20); ^{1}H NMR δ 1.35, 1.45, 1.60, 1.85 (4m, 12H, 6CH$_2$), 1.93 (d, 3H, CH$_3$-5, J = 1.2 Hz), 3.48 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.90 (m, 2H, CH$_2$N), 4.08 (m, 1H, H-5', J_{gem} = 13.2 Hz, $J_{4',5'}$ = 3.7 Hz), 4.12 (m, 1H, H-5'), 4.33 (t, 1H, H-4'), 4.69 (d, 1H, H-2'), 5.75 (s, 1H, H-3''), 6.07 (d, 1H, H-1', $J_{1',2'}$ = 8.1 Hz), 6.44 (bs, 2H, NH$_2$-4''), 7.49 (d, 1H, H-6). Anal. ($C_{32}H_{58}BrN_3O_8SSi_2$) C, H, N, S.

[1-[2',5'-Bis[*O*-(*tert*-butyldimethylsilyl)]-β-D-ribofuranosyl]-3-*N*-(9-bromononyl)thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (35): reaction time 8 h; eluent hexane/ethyl acetate, 2:1; yield of **35** (65%) as a white foam; ^{1}H NMR δ 1.50, 1.80 (2m, 14H, 7CH$_2$), 1.93 (d, 3H, CH$_3$-5, J = 1.1 Hz), 3.52 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.89 (t, 2H, CH$_2$N, J = 7.3 Hz), 4.10 (m, 2H, 2H-5'), 4.35 (t, 1H, H-4'), 4.68 (d, 1H, H-2'), 5.79 (s, 1H, H-3''), 6.10 (d, 1H, H-1'), 6.48 (bs, 2H, NH$_2$-4''), 7.50 (d, 1H, H-6). Anal. ($C_{33}H_{60}BrN_3O_8SSi_2$) C, H, N, S.

3-*N*-(6-Bromohexyl)-1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (36): reaction time 12 h; eluent dichloromethane/methanol, 50:1; yield of **36** (60%) as a syrup; HPLC t_R = 10.46 min (55:45); ^{1}H NMR δ 1.60 (m, 8H, 4CH$_2$), 1.98 (s, 3H, CH$_3$-5), 3.48 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.55 (m, 5H, HO-CH$_2$-CH$_2$-O), 3.91 (t, 2H, CH$_2$N, J = 7.2 Hz), 5.62 (s, 2H, NCH$_2$O), 7.32 (m, 5H, SPh). Anal. ($C_{20}H_{27}BrN_2O_4S$) C, H, N, S.

3-*N*-(7-Bromoheptyl)-1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (37): reaction time 8 h; eluent dichloromethane/methanol, 20:1; yield of **37** (75%) as a syrup; HPLC t_R = 14.01 min (55:45); ^{1}H NMR δ 1.40, 1.61, 1.84 (3m, 10H, 5CH$_2$), 1.97 (s, 3H, CH$_3$-5), 3.49 (t, 2H, CH$_2$Br, J = 6.8 Hz), 3.58 (m, 5H, HO-CH$_2$-CH$_2$-O), 3.90 (t, 2H, CH$_2$N, J = 7.6 Hz), 5.62 (s, 2H, NCH$_2$O), 7.32 (m, 5H, SPh). Anal. ($C_{21}H_{29}BrN_2O_4S$) C, H, N, S.

General Procedure for the Synthesis of the Heterodimers 7, 8, and 19–31. To a solution of the key 3-*N*-(*n*-bromoalkyl) nucleoside (**9–18**) (1 equiv) in dry acetonitrile were added K_2CO_3 (1.1 equiv) and the corresponding nucleoside (TSAO-T or HEPT) (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 a chromatotron is 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]-(CH$_2$)$_6$-[dThd] (7). Method A. TSAO-T and **17** were reacted for 8 h. The residue was chromatographed (dichloromethane/methanol, 50:1) to give **7** (70%) as a syrup: HPLC t_R = 6.26 min (70:30). Anal. ($C_{40}H_{67}N_5O_{13}SSi_2$) C, H, N, S.

Method B. To a solution of **6** (1 equiv) in a 3:1 mixture of dry acetonitrile/DMF were added thymidine (**3**) (1.1 equiv) and K_2CO_3 (1.1 equiv). The mixture was refluxed for 8 h. After evaporation of the solvent, the residue was treated as described in the general procedure. Purification by CCTLC on a chromatotron (dichloromethane/acetone, 3:2, and subsequently dichloromethane/methanol, 50:1) gave from the faster moving fractions compound **7** (30%). From the slower moving fractions the 2'-deprotected compound **8** (13%) was obtained as a syrup. Anal. ($C_{34}H_{53}N_5O_{13}SSi$) C, H, N, S.

Heterodimer [TSAO-T]-(CH$_2$)$_3$-[AZT] (19). TSAO-T and **9** reacted for 12 h. The residue was chromatographed (hexane/ethyl acetate, 3:2) to give **19** (69%) as a white foam: HPLC t_R = 16.18 min (60:40). Anal. ($C_{37}H_{60}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]-(CH$_2$)$_4$-[AZT] (20). TSAO-T and **10** reacted for 12 h. The residue was chromatographed (hexane/ethyl acetate, 1:1, and subsequently dichloromethane/methanol, 20:1) to give **20** (86%) as a white foam: HPLC t_R = 7.78 min (70:30). Anal. ($C_{38}H_{62}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]-(CH$_2$)$_5$-[AZT] (21). TSAO-T and **11** reacted for **8** h. The residue was chromatographed (dichloromethane/methanol, 20:1) to give **21** (84%) as a white foam: HPLC t_R = 8.41 min (70:30). Anal. ($C_{39}H_{64}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]-$(CH_2)_6$-[AZT] (22). TSAO-T and **12** reacted for 12 h. The residue was chromatographed (dichloromethane/methanol, 50:1) to give **22** (60%) as a white foam: HPLC t_R = 10.91 min (70:30). Anal. ($C_{40}H_{66}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]-$(CH_2)_7$-[AZT] (23). TSAO-T and **13** reacted for 8 h. The residue was chromatographed (hexane/ethyl acetate, 2:1) to give **23** (64%) as a white foam: HPLC t_R = 13.20 min (70:30). Anal. ($C_{41}H_{68}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]-$(CH_2)_8$-[AZT] (24). TSAO-T and **14** reacted for 12 h. The residue was chromatographed (chloroform/ethyl acetate, 4:1) to give **24** (82%) as a white foam: HPLC t_R = 13.61 min (70:30). Anal. ($C_{42}H_{70}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]-$(CH_2)_9$-[AZT] (25). TSAO-T and **15** reacted for 12 h. The residue was chromatographed (dichloromethane/methanol, 20:1) to give **25** (75%) as a white foam: HPLC t_R = 16.58 min (70:30). Anal. ($C_{43}H_{72}N_8O_{12}SSi_2$) C, H, N, S.

Heterodimer [AZT]-$(CH_2)_6$-[HEPT] (26). HEPT and **12** reacted for 8 h. The residue was chromatographed (dichloromethane/methanol, 30:1) to give **26** (60%) as a syrup: HPLC t_R = 9.06 min (50:50). Anal. ($C_{30}H_{39}N_7O_8S$) C, H, N, S.

Heterodimer [AZT]-$(CH_2)_7$-[HEPT] (27). HEPT and **13** reacted for 10 h. The residue was chromatographed (dichloromethane/methanol, 20:1) to give **27** (75%) as a syrup: HPLC t_R = 6.10 min (60:40). Anal. ($C_{31}H_{41}N_7O_8S$) C, H, N, S.

Heterodimer [TSAO-T]-$(CH_2)_3$-[dThd] (28). TSAO-T and **16** reacted for 8 h. The residue was chromatographed (dichloromethane/methanol, 50:1) to give **28** (65%) as a syrup: HPLC t_R = 12.48 min (55:45). Anal. ($C_{37}H_{61}N_5O_{13}SSi_2$) C, H, N, S.

Heterodimer [TSAO-T]-$(CH_2)_7$-[dThd] (29). TSAO-T and **18** reacted for 7 h. The residue was chromatographed (dichloromethane/acetone, 20:1, and then 1:2) to give **29** (60%) as a syrup: HPLC t_R = 7.68 min (70:30). Anal. ($C_{41}H_{69}N_5O_{13}SSi$) C, H, N, S.

Heterodimer [dThd]-$(CH_2)_6$-[HEPT] (30). HEPT and **17** reacted for 8 h. The residue was chromatographed (dichloromethane/acetone, 3:2, and subsequently dichloromethane/methanol, 10:1) to give **30** (68%) as a syrup: HPLC t_R = 3.43 min (70:30). Anal. ($C_{30}H_{40}N_4O_9S$) C, H, N, S.

Heterodimer [dThd]-$(CH_2)_7$-[HEPT] (31). HEPT and **18** reacted for 7 h. The residue was chromatographed (dichloromethane/methanol, 10:1) to give **31** (78%) as a syrup: HPLC t_R = 6.10 min (60:40). Anal. ($C_{31}H_{42}N_4O_9S$) C, H, N, S.

Antiretroviral Evaluation. Human immunodeficiency virus type 1 [HIV-1 (HTLV-III_B)] was obtained from persistently HIV-infected H9 cells as described previously.[40] Virus stocks were prepared from the supernatants of HIV-1 (III_B)-infected MT4 cells. HIV-2 (ROD) was provided by Dr. L. Montaigner (Pasteur Institute, Paris, France). CEM/0 cells were obtained from the American Tissue Culture Collection (Rockville, MD), and CEM/TK$^-$ cells were a gift from Prof. S. Eriksson and Dr. A. Karlsson (Karolinska Institute, Stockholm, Sweden). CEM cells were infected with HIV as previously described.[41] Briefly, 4×10^5 CEM cells/mL were infected with HIV-1 or HIV-2 at ~100 $CCID_{50}$ (50% cell culture infective dose)/mL 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 was recorded microscopically in the HIV-infected cell cultures. The 50% effective concentration (EC_{50}) and the 50% cytotoxic concentration (CC_{50}) of the test compounds were defined as the compound concentrations required to inhibit virus-induced cytopathicity by 50% or to reduce by 50% the number of viable cells in mock-infected cell cultures, respectively.

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Supplementary Material Available: ^{1}H NMR chemical shift assignments of heterodimers **7**, **19**–**22**, **24**–**26**, **28**, and **30** (4 pages). Ordering information is given on any current masthead page.

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