Synthesis of 3′′-Substituted TSAO Derivatives with Anti-HIV-1 and Anti-HIV-2 Activity through an Efficient Palladium-Catalyzed Cross-Coupling Approach

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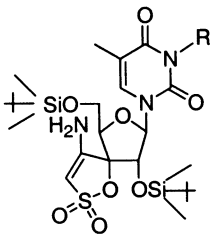
Various synthetic studies for the introduction of several functional groups at position 3′′ of the spiro moiety of TSAO derivatives have been explored. Among them, Stille cross-coupling of 3′′-iodo-TSAO derivatives with different stannanes provided an efficient and straightforward route for the direct and selective functionalization of the 3′′-position of the sultone spiro moiety via carbon−carbon bond formation. The compounds synthesized were evaluated for their inhibitory effect on HIV-1 and HIV-2 replication in cell culture. The introduction of a bromine and particularly an iodine at the 3′′-position conferred the highest anti-HIV-1 activity. In contrast, the presence at this position of (un)substituted vinyl, alkynyl, phenyl, or thienyl groups markedly diminished the anti-HIV-1 activity. Surprisingly, several of the 3′′-alkenyl-substituted TSAO derivatives also gained anti-HIV-2 activity at subtoxic concentrations, an observation that is very unusual for NNRTIs and never observed before for TSAO derivatives. Finally, the anti-HIV-1 activity of some of the 3′′-substituted TSAO derivatives is discussed in light of our recently proposed molecular model of interaction of TSAO derivatives with the interphase between the two subunits of HIV-1 reverse transcriptase.

Introduction

Among the different families of specific nonnucleoside reverse transcriptase inhibitors (NNRTIs) described so far, [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3′-spiro-5′′-(4′′-amino-1′′,2′′-oxathiole-2′′,2′′-dioxide) nucleosides (TSAO) represent a rather unique class of nucleoside analogues that have been identified as highly specific noncompetitive inhibitors of the RNA-dependent DNA polymerase function of reverse transcriptase.[1,2] The prototype compound is the thymine derivative designated as TSAO-T (**1**). The most selective compound is the 3-*N*-methyl-substituted derivative TSAO-m^{3}T (**2**) (Figure 1). There are several features of the "unique" character of the TSAO family of compounds. Despite their nucleosidic structure, TSAO analogues, like the other NNRTIs, are targeted at a non-substrate-binding site of the human inmunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT).[1,2b,3] TSAO derivatives are, so far, the only HIV-1 RT inhibitors that seem to interfere at the interface between the p51 and the p66 subunits of the RT heterodimer.[4] Well-defined amino acids at both the p51 and the p66 RT subunits are needed for optimum interaction of TSAO compounds with the HIV-1 RT.[5,6] 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.[5a,6b,7,8] Recent biochemical studies have revealed that both TSAO-T and its 3-*N*-ethyl derivative



1 R = H (TSAO-T)
2 R = CH_3 (TSAO-m^{3}T)

Figure 1. Chemical structures of TSAO-T and TSAO-m^{3}T.

are able to destabilize the p66/p51 RT heterodimer in a concentration-dependent manner, leading to a loss in its ability to bind DNA.[4,9] This suggests a completely new and different mechanism of inhibition of HIV-1 RT with regard to the other known NNRTIs.

Structure−activity relationship (SAR) studies within the TSAO family have revealed that the sugar part plays a crucial role in the interaction of the TSAO compounds with their target enzyme.[1−3] In particular, the presence of the unique 3′-spiro ring of 4-amino-1,2-oxathiole-2,2-dioxide in nucleosides having a ribo configuration is crucial for antiviral activity, and replacement of this amino sultone spiro ring by closely related analogues results in a 100-fold decrease of the anti-HIV-1 activity.[7] Moreover, the presence of bulky *tert*-butyldimethylsilyl (TBDMS) groups at both C-2′ and C-5′ positions is also a prerequisite for antiviral activity.[1,3,10]

As part of our program to further explore the importance of substituent effects on the anti-HIV-1 activity/toxicity of TSAO derivatives, we focused our attention

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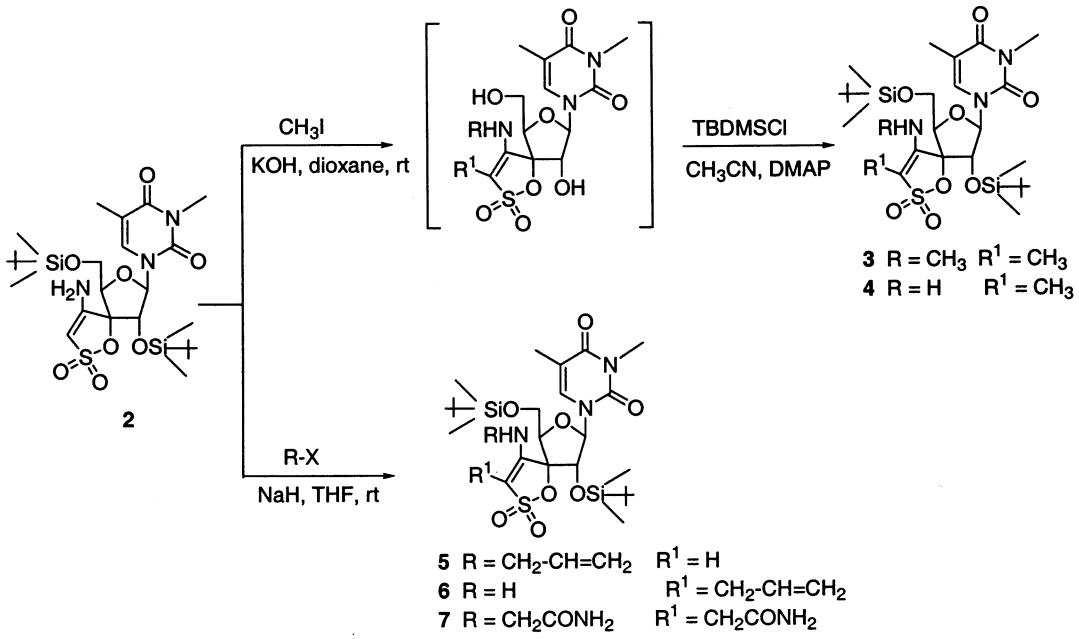
Scheme 1. Synthesis of Compounds **3**−**7** by Alkylation of TSAO-m^{3}T



on modifications of the 3′-spiro moiety. This represents the closest part of the molecule to the interface between the p51 and p66 subunit domains. On the basis of this hypothesis, we have now introduced different functional groups at position 3″ of the spiro ring of TSAO-T (**1**) and TSAO-m^{3}T (**2**) that may give additional interactions with amino acids adjacent to the Glu-B138 of the p51 subunit. In these novel analogues, the 4″-amino group of the sultone spiro moiety was maintained to allow the crucial interaction with the Glu-B138.

For the synthesis of the target compounds, two important issues have been considered. First, the chemical reactivity of the amino sultone heterocyclic system, first reported by us in 1988,[11] has been scarcely studied and poses a synthetic challenge.[12] Second, the presence of TBDMS at positions 2′ and 5′, sensitive to basic and acid media, respectively, but essential for the antiviral efficacy of the TSAO compounds, implies the selection of smooth reaction conditions compatible with such groups.

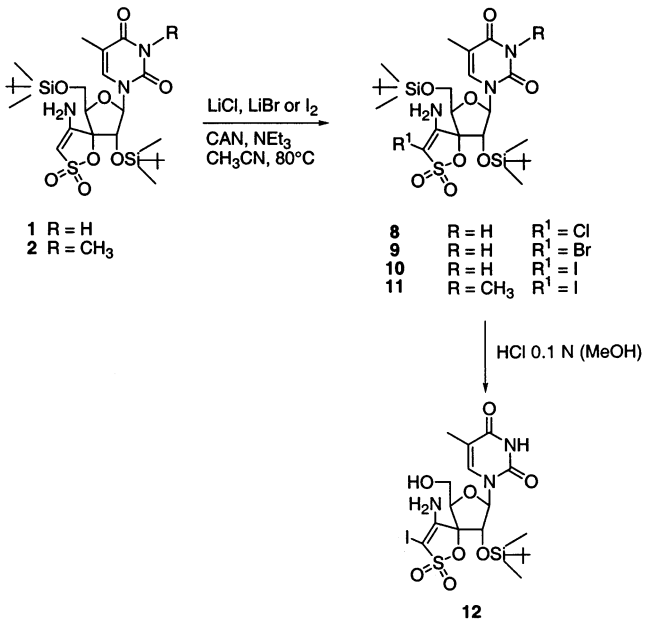
In this manuscript, we describe in detail a mild, efficient, and general route[13] for the synthesis of 3″-substituted TSAO analogues carrying differently substituted alkenyl, alkynyl, allyl, aromatic, and heteroaromatic groups. They were prepared from 3″-iodo compounds, as key intermediates, via Pd-catalyzed Stille cross-coupling reactions. To the best of our knowledge, there is no report in the literature for palladium cross-coupling reactions in this amino sultone heterocyclic system. Furthermore, the anti-HIV activity of these novel compounds is also reported and the results are discussed in the light of our recently proposed molecular model for the TSAO−HIV-1 RT interaction.[14]

Results

Synthesis. To perform modifications at the C-3″ position of the 4-amino-1,2-oxathiole-2,2-dioxide spiro ring of TSAO compounds, we first investigated alkylation reactions on the 3′-spiro moiety, following the procedures described in the literature for alkylation of enamines.[14] However, when we assayed the reaction of TSAO-m^{3}T (**2**) with alkyl halides, we noticed that the presence of strong bases (KOH or NaH) was required, in contrast to the alkylation of "classical" enamines where no base is needed.[15] Thus, reaction of **2** with CH_3I and KOH in dry dioxane followed by "in situ" silylation with an excess of *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of DMAP afforded the corresponding 3″-C-methylated derivative **4**, together with the N,C-dialkylated compound **3** in low yields (Scheme 1). On the other hand, reaction of **2** with allyl bromide and NaH yielded the 4″-N- and 3″-C-allylated derivatives **5** and **6** in a 1:1 ratio; under these reaction conditions no deprotection of the TBDMS groups was observed. Similarly, reaction of **2** with ICH_2CONH_2 in the presence of NaH afforded, exclusively, the N,C-dialkylated derivative **7**. In all these reactions, mixtures of N,C-alkylated products were obtained together with considerable amounts of unreacted starting material. Longer reaction times or increasing amounts of NaH (additional 1 or 2 equiv) led to complex reaction mixtures from which only 2′- and /or 5′-deprotected compounds were isolated.

In contrast to the lack of selectivity of the alkylation reaction, halogenation occurs exclusively at the C-3″ position. Thus, chloro, bromo, and iodo substituents were easily introduced into the 3″-position of the spiro moiety by using the mild ceric ammonium nitrate (CAN) mediated halogenation method,[16] which avoids the use of acidic and (or) high reaction temperature conditions that are not compatible with the labile TBDMS groups. When **1** was treated with lithium halides (LiCl or LiBr) and CAN in acetonitrile at 80 °C, 3″-chloro and 3″-bromo derivatives **8** and **9** were obtained in 50% and 80% yields, respectively (Scheme 2). Similarly, reaction of **1** or **2** with I_2 and CAN afforded the iodo derivatives **10** and **11** in excellent yields (93% and 90%, respectively). Addition of NEt_3 was always required to avoid deprotection of the TBDMS group at the 5′-position.

Scheme 2. Synthesis of 3′′-Halo-TSAO Nucleosides **8**−**12**



1 R = H
2 R = CH_3

8 R = H R^1 = Cl
9 R = H R^1 = Br
10 R = H R^1 = I
11 R = CH_3 R^1 = I

12

Finally, selective deprotection of iodide **10** with 0.1 N methanolic HCl yielded the 5′-deprotected iodo derivative **12** in 81% yield (Scheme 2). The position of halogenation was established by ^{1}H NMR and ^{13}C NMR spectroscopy. Thus, the vinylic proton (H-3′′) of **1** and **2** at 5.75 ppm (singlet) disappeared after halogenation. On the other hand, ^{13}C NMR spectra of halonucleosides **8**−**11** showed an upfield shift of the signal corresponding to C-3′′ ($\Delta\delta$ = 7−37 ppm) with respect to the same signal in the starting TSAO derivatives **1** and **2**.

At this point, taking into account the easy availability of 3′′-halo-TSAO derivatives, we decided to use them as key intermediates for the synthesis of the target 3′′-substituted TSAO compounds. The palladium-catalyzed cross-coupling reaction between organic electrophiles and alkenyl or alkynyl donors, among others, provides an extremely powerful synthetic tool for generating a carbon−carbon bond.[17] This versatile and mild reaction is tolerant to a wide variety of functional groups on each coupling partner, is stereospecific and regioselective, and gives high yields of the coupling products. Initial attempts of the cross-coupling of iodo derivative **10** and activated alkenes (i.e., methyl acrylate) using catalytic quantities of $Pd(OAc)_2$ and Ph_3P in dioxane in the presence of NEt_3 according to the original method of Heck et al.,[18] failed to give the coupled product. In the reaction only unreacted starting material **10** together with the corresponding deiodinated derivative **1** were isolated (Scheme 3). On the other hand, palladium catalyzed cross-coupling reactions of iodo derivative **10** with terminal alkynes (i.e., 5-chloro-1-pentyne) using the Sonogashira modification[19] in the presence of catalytic quantities of $(Ph_3P)_2PdCl_2$ and CuI in degassed NEt_3 at 50 °C also failed, and only 5′-deprotected derivatives **12** and **13** (Scheme 3) were observed. Similarly, when this reaction was carried out in the presence of CuI and tetrakis(triphenylphosphine)-palladium(0) as catalyst in DMF at room temperature

Scheme 3. Palladium Catalyzed Cross-Coupling Reactions of Iodo Compound **10** with Alkenes, Alkynes, and Trialkyltin Derivatives[a]

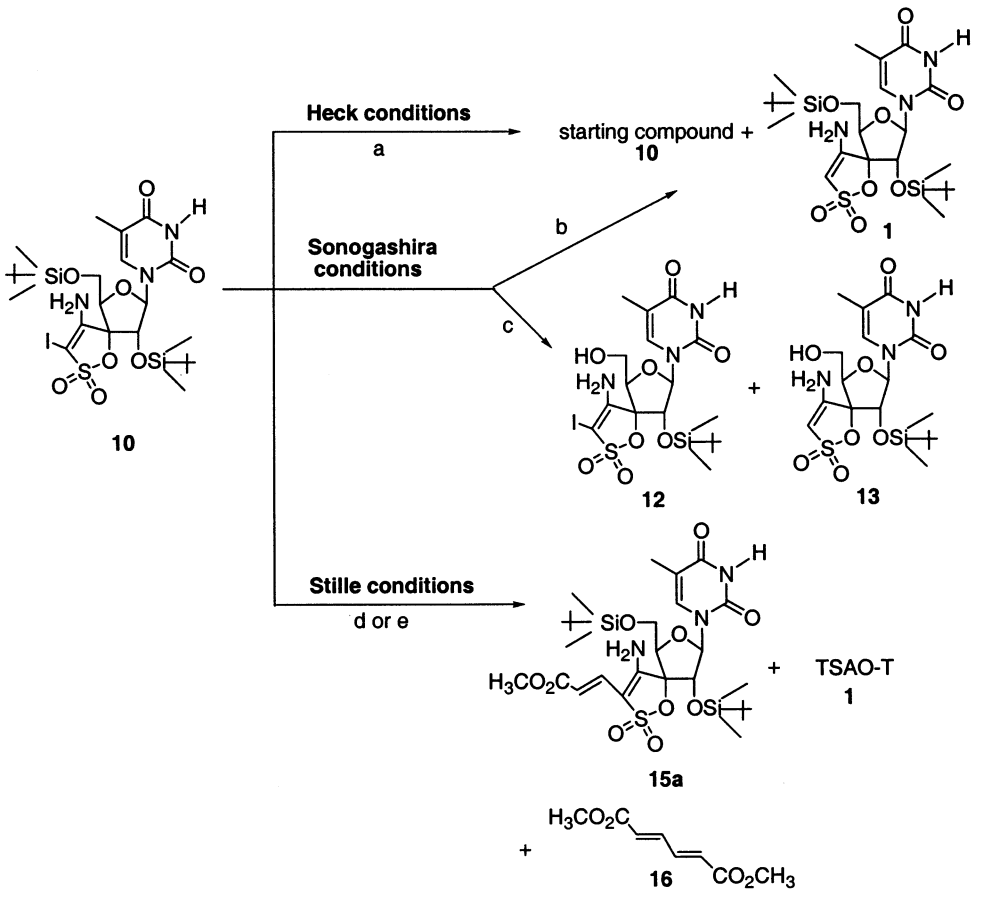
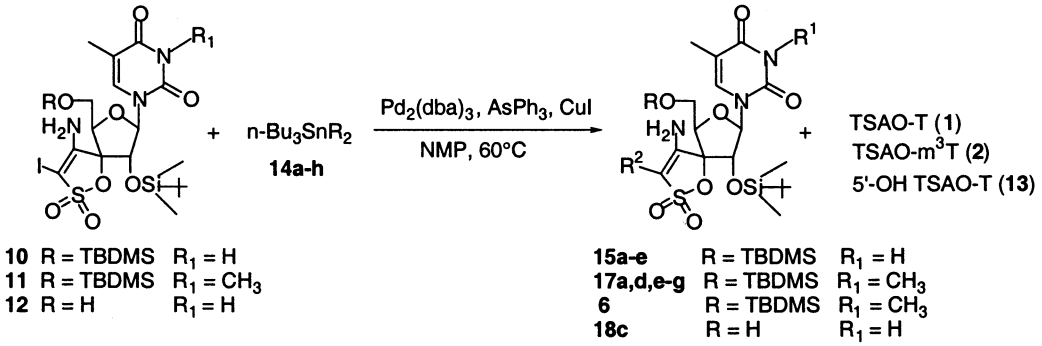


Table 1. Palladium-Catalyzed Cross-Coupling Reactions of Iodo Nucleosides **10**−**12** with Different *n*-Bu_3SnR_2 **14a**−**h** under Optimized Conditions[a]



10 R = TBDMS R_1 = H
11 R = TBDMS R_1 = CH_3
12 R = H R_1 = H

15a-e R = TBDMS R_1 = H
17a,d,e-g R = TBDMS R_1 = CH_3
6 R = TBDMS R_1 = CH_3
18c R = H R_1 = H

entry	coupling product	R_2	ratio coupling/reduction[b]	yield of coupling product (%)[c]
1	**15a**	−CH=CHCO$_2$CH$_3$ (*E*)	3:1	72
2	**15b**	−CH=CHCO$_2$CH$_2$CH$_3$ (*E*)	3:1	65
3	**15c**	−CH=CH$_2$	7:1	79
4	**15d**	−CH=CHCH$_2$OH (*E*)	9:1	82
5	**15e**	−Ph	2:1	60
6	**17a**	−CH=CHCO$_2$CH$_3$ (*E*)	3:1	66
7	**17d**	−CH=CHCH$_2$OH (*E*)	10:1	82
8	**17e**	−Ph	2:1	60
9	**17f**		3:2	50
10	**17g**	−C≡CCH$_3$	1:1	30
11	**6**	−CH$_2$CH=CH$_2$	1:1	30
12	**18c**	−CH=CH$_2$	7:1	70

[a] Most reactions were $>^1/_2$ complete after 1 h but were allowed to run for 12 h for convenience. [b] HPLC ratio. [c] Isolated yields after several chromatographic purifications.

with 2 equiv of NEt_3,[20] only unreacted starting material **10** and the corresponding dehalogenated derivative **1** were observed (Scheme 3).

Finally, the coupling process of the iodo **10** with organotin reagents under classical Stille coupling conditions[21] was studied. The readily available methyl-(*E*)-3-(tri-*n*-butylstannyl) acrylate **14a**[22] was chosen as a model reagent to couple with iodide **10** (Scheme 3) because of the ease of monitoring of the reaction by HPLC compared with commercially available vinylstannane. Initial attempts of coupling iodide **10** with stannane **14a** in DMF and in the presence of $Pd(PPh_3)_4$ as catalyst produced the desired cross-coupling product **15a** in low yield (<10%) together with small amounts of **1**, resulting by the reduction of the iodo precursor **10**, and compound **16** obtained by oxidative homocoupling of stannane **14a**. Homocoupling of stannanes and electrophile reductions are often common side reactions, described in the literature for Stille couplings.[21] It should be also noted that in the reaction conditions described above, 70% of the starting compound **10** was recovered unchanged. Thus, our system appears difficult to couple and required carefully optimized conditions. After several endeavors for optimizacion the reaction parameters, described in our preliminary communication,[13] an optimized procedure for the Stille coupling was elaborated. It was found that the yield and rate of the reaction were significantly affected by the addition of a catalyst obtained from the weakly coordinated Pd−dibenzylidene acetone complex ($Pd_2(dba)_3$) and triphenylarsine, as the ligand, and copper iodide as cocatalyst. It should be pointed out that when an increased number of equivalents of the stannane were used, the ratio coupling/reduction products was higher. Thus, the best conditions for coupling in our system were the following: *N*-methylpyrrolidinone (NMP) as solvent at 60 °C, 4 equivs of stannane and 2 mol % of $Pd_2(dba)_3$, 8 mol % of $AsPh_3$ and 4 mol % of CuI as catalyst systems.[13] It should be noted that under these optimal reaction conditions, the rate and overall yield of coupling, compared to the initially used $Pd(PPh_3)_4$, were significantly improved (<10% vs 72%) (Scheme 3). Thus, these results represent yet one more example of the beneficial effect of these palladium catalytic system [$Pd_2(dba)_3$/$AsPh_3$/Cu] in Stille cross-coupling reactions.[23,24]

Thus encouraged, we considered it to be of interest to study the scope and limitations of the cross-coupling reaction of iodide **10** with a broader range of stannanes, under the optimized reaction conditions. Moreover, to study the effect on the activity/toxicity of the resulting compounds, the method was also further extended to other electrophiles such as iodo-TSAO-m^{3}T **11** and the corresponding 5′-deprotected iodo-TSAO-T derivative **12**. The results are summarized in Table 1. The reaction tolerates TBDMS groups on the sugar moiety (entries 1−11), as well as an unprotected 5′-hydroxyl group (entry 12). In all cases moderate to good yields of the coupling products and variable amounts of the reduction products (**1**, **2**, and **13**) were obtained. It should be noted that separation of the desired coupling products from the reduction side products was rather laborious, and repeated chromatographic purifications were required to give the coupling products in the yields shown in Table 1. The ratio coupling/reduction products depends on the nature of the stannane. In general, the higher the transfer rate of the different groups from tin the higher the ratio coupling/reduction products.[20] Substituted or unsubstituted alkenyl, phenyl, and heteroaryl groups on tin were all transferred in moderate to good yields (entries 1−9). Among them, Stille coupling with

Table 2. Anti-HIV Activity of 3″-Substituted TSAO Derivatives in MT-4 and CEM Cell Cultures

	EC_{50}[a] (μM)					
	MT-4		CEM		CC_{50}[b] (μM)	
compd	HIV-1	HIV-2	HIV-1	HIV-2	MT-4	CEM
1 (TSAO-T)	0.06 ± 0.03	>10	0.05 ± 0.01	>10	12 ± 2.4	
2 (TSAO-m3T)	0.06 ± 0.09	>250	0.04 ± 0.01	>250	230 ± 7.3	
3	>250	>250	≥ 100	>250	>250	234 ± 23
4	0.25 ± 0.09	>250	2.2 ± 1.7	>50	≥250	10−250
5	>50	>50	>50	>50	25 ± 20	126 ± 12
6	>50	>50	>50	>50	82 ± 11	45 ± 53
7	>2	>2	>2	>2	3.2 ± 1.1	3.9 ± 0.2
8	>10	>10	>10	>10	5.4 ± 1.3	7.0 ± 1.2
9	0.93 ± 0.23	>10	0.85 ± 0.21	>10	18 ± 0.5	17 ± 2.0
10	0.12 ± 0.02	>2	0.06 ± 0.02	>2	4.3 ± 0.2	4.0 ± 0.04
11	0.11 ± 0.06	>10	0.05 ± 0.03	>2	3.9 ± 0.13	5.6 ± 2.0
15a	3.7 ± 0.01	9.9 ± 6.4	3.0 ± 1.4	7.3 ± 2.3	29 ± 7.8	39 ± 5.6
15b	10 ± 8.9	>50	3.5 ± 0.7	>10	40 ± 5.7	174 ± 95
15c	3.2 ± 2.2	3.3 ± 3.5	4.0 ± 1.4	4.0 ± 0.0	24 ± 3.0	36 ± 7.6
15d	6.6 ± 2.9	5.4 ± 0.2	≥2	1.2 ± 0.0	17 ± 0.5	18 ± 1.9
15e	6.7 ± 1.6	>50	2.5 ± 0.7	>2	55 ± 45	37 ± 23
17a	2.9 ± 1.2	>10	0.98 ± 0.87	>2	55 ± 45	37 ± 23
17d	5.9 ± 1.8	>10	2.8 ± 2.7	5.7 ± 0.58	13 ± 19	18 ± 2.1
17e			1.7 ± 1.1	>50	24 ± 12	65 ± 36
17f	2.3 ± 2.0	>2	>2	>2	3.9 ± 2.5	33 ± 14
17g	>2	>2	>2	>2	3.3 ± 2.3	5.0 ± 0.6
18c	23 ± 0.9	>50	15 ± 5	>50	47 ± 18	76 ± 11

[a] 50% effective concentration or compound concentration required to inhibit HIV-induced cytopathicity in cell culture by 50%. [b] 50% cytostatic concentration or compound concentration required to inhibit CEM cell proliferation by 50% or to reduce MT-4 cell viability in mock-infected cell cultures by 50%.

vinyl stannane **14c** (entries 3 and 12) or (*E*)-3-(tri-*n*-butylstannyl)-2-propen-1-ol[22] **14d** (entries 4 and 7) gave the highest yields (70−82%). The unexpected low yield observed in the coupling of iodide **11** with the highly reactive alkynyl stannane **14g** (entry 10) could be explained by the higher reactivity of this stannane, which leads to increasing amounts of the homocoupling side product. Under the optimized conditions described above, successful coupling was observed even in the more demanding couplings (i.e., allyl derivatives, entry 11). However, the above methodology did not work with tributylcyanostannane and tetramethylstannane. In the latter case, it is generally accepted that an sp^3 carbon directly attached to the metal is less reactive than carbons with lower hybridization in Pd-catalyzed reactions,[21] which likely accounts for the lack of reactivity observed.

The structures of compounds **15a**−**e**, **17a**,**d**,**e**−**g**, and **18c** were assigned on the basis of their corresponding analytical and spectroscopic data. The trans stereochemistry around the double bond of the vinyl-substituted tin reagents **14a**,**b** and **14d** was retained during the coupling reaction as shown by the values of $^3J_{H,H}$ = 15.6−15.9 Hz for the olefinic protons of the corresponding coupling products **15a**,**b**,**d** and **17a**,**d**. Stille couplings reaction of organic electrophiles with substituted vinyl tin reagent usually proceeds with retention of configuration.[21]

Biological Results. The anti-HIV activity of the 3″-substituted TSAO derivatives was evaluated in MT-4 and CEM cell cultures and compared with the prototype compounds TSAO-T and TSAO-m^3T (Table 2). Among the 3″-halogen-substituted TSAO derivatives, the chloro derivative (**8**) was devoid of antiviral activity whereas the bromo (**9**) and particularly the iodo derivatives (**10** and **11**) were endowed with pronounced anti-HIV-1 activity. The iodo derivatives **10** and **11** were virtually equally potent anti-HIV-1 agents as the prototype compounds TSAO-T and TSAO-m^3T. The novel TSAO derivatives described herein invariably contained a thymine moiety (as in TSAO-T) or a N^3-methyl-substituted thymine moiety (as in TSAO-m^3T). Cellular toxicity of the 3″-iodo-substituted TSAO-T and TSAO-m^3T derivatives (CC_{50} = 3.9−5.6 μM) was comparable with that of TSAO-T but substantially more pronounced than for TSAO-m^3T (CC_{50} ≥ 100 μM). The most active anti-HIV-1 3″-halogen derivatives **9**, **10**, and **11** and the 3″-methyl derivative **4** proved to be inactive (>2−10 μM for **9**−**11** and >500 μM for **4**) against TSAO-resistant HIV-1/138Lys mutant virus strains.[25] Substitution of the 3″-spiro position by alkenyl derivatives containing allyl (**6**) or an acetamide (**7**) moiety annihilates the antiviral activity. In contrast, a variety of other alkenyl-substituted TSAO derivatives showed anti-HIV-1 activity at EC_{50} concentrations between 1 and 10 μM, which are 20- to 200-fold higher than those of the unsubstituted prototype compounds. Surprisingly, several of these 3″-substituted TSAO derivatives also gained anti-HIV-2 activity at subtoxic concentrations, an observation that is very unusual for NNRTIs and never observed before for TSAO derivatives. Usually, the anti-HIV-1 activity was of the same order of magnitude as their anti-HIV-2 activity. The structure−activity relationship for having both anti-HIV-1 and anti-HIV-2 activity is currently unclear. For example, the methyl ester of the propionic acid derivative (**15a**) shows both anti-HIV-1 and anti-HIV-2 activity, whereas the ethyl ester (**15b**) has only anti-HIV-1 activity. Also, the 3″-vinyl (**15c**) and hydroxypropenyl (**15d**, **17d**) derivatives show activity against both viruses, but the 3″-phenyl-substituted compounds again show selectivity for only HIV-1. 3″-Propynyl-TSAO (**17g**) is inactive at subtoxic concentrations. To exclude HIV-2 RT as a potential target for those TSAO derivatives that gained antiviral activity against HIV-2 in addition to anti-HIV-1 activity, compounds **15a**, **15c**, and **15d** were evaluated on their

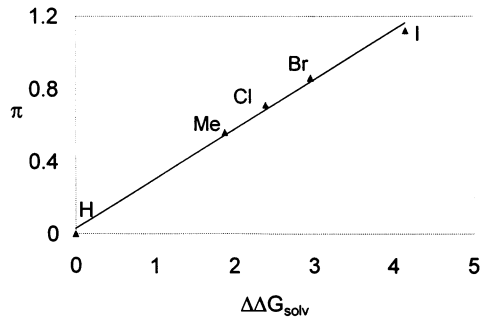


Figure 2. Effect of replacing hydrogen by halogen or methyl on the calculated electrostatic solvation energies of the spiro systems ($\Delta\Delta G_{ele}^{solv}$ in kcal mol^{-1}) vs hydrophobic constants (π) of the halogen substituents experimentally determined for halobenzenes.[28]

inhibitory activity against recombinant HIV-2 RT and found to be inactive at 500 μM. In addition, the most active anti-HIV-2 compounds **15a**, **15c**, and **15d** but also **17a** and **17d** were also shown to be inactive (>500 μM) against a mutant HIV-1 RT that contains the TSAO-characteristic Glu138Lys resistance mutation. Taken together, our data point to a different mechanism of action of some of the 3″-substituted TSAO derivatives. Recently, Buckheit[26] reported on the NNRTI SJ-3366 that inhibits both HIV-1 and HIV-2 replication and that inhibits HIV-1 but not HIV-2 RT. This compound was found to interfere with entry of HIV as a second mechanism of action. Investigations on the mechanism of action of the TSAO derivatives against HIV-2 is currently ongoing.

Computational Results and Discussion

To rationalize the biological results obtained in cell culture assays,[27] a theoretical investigation was undertaken with the 3″-halogenated and methyl-substituted TSAO compounds. Halogen or methyl substitution at the 3″ position of the spiro moiety of **1** or **2** brings about an increase in the electrostatic desolvation energy that closely parallels the hydrophobicity increments expected from the π constant values calculated from differences in octanol−water partition coefficients for toluene and halobenzenes with respect to benzene (Figure 2).

In our recently proposed model for **1** and **2** binding to heterodimeric RT,[14] the binding site is found at the subunit interface in the vicinity of the β7−β8 loop of p51. The spiro group of TSAO appears to play a dominant role in aligning the molecule in the electrostatic field created by Lys-A101 and Lys-A103 in the p66 subunit and Glu-B138 in the p51 subunit. In fact, the most prominent interaction detected in our analysis was between TSAO-T and Glu-138B, the residue that is found mutated to lysine under the selective pressure of TSAO derivatives.[25] When the electrostatic component of the interaction energy between the halogenated analogues and these protein residues in the equilibrated complexes was calculated, the favorable interaction energy with Glu-138B was similar for compounds **2** (H) and **10** (I) but steadily decreased for **9** (Br) and **8** (Cl). On the other hand, the interaction with Lys-A101 and Lys-A103 fluctuated more because of variations in the orientation of the side chain with respect to the amino-oxathiol-dioxide (Table 3). Adaptation of the binding site to the presence of the halogen also brings about slight changes in the orientation of the thymine ring that are translated into differential interactions with Arg-A172, which turned out to be particularly unfavorable for **8**, the least potent compound in this series.

Table 3. Electrostatic Component of the Interaction Energy (kcal mol^{-1})[a] between TSAO-m^{3}T and Its Halogenated Analogues with Selected RT Residues[b] during the Molecular Dynamics Simulations Calculated Using a Continuum Method[14]

	Glu-B138	Lys-A101	Lys-A103	Arg-A172
TSAO-m^{3}T (**2**)	−4.7 ± 0.2	−0.4 ± 0.2	0.1 ± 0.1	−0.05 ± 0.1
Cl-TSAO-m^{3}T (**8**)	−2.9 ± 0.6	1.2 ± 0.2	0.1 ± 0.03	1.6 ± 0.1
Br-TSAO-m^{3}T (**9**)	−3.8 ± 0.2	−0.7 ± 0.1	−0.9 ± 0.1	0.9 ± 0.04
I-TSAO-m^{3}T (**10**)	−5.0 ± 0.5	−0.4 ± 0.2	0.6 ± 0.3	0.4 ± 0.4

[a] Average ± root-mean-square deviation. [b] Differences in interaction of the different TSAO derivatives with other protein residues fell within the standard errors of our measurements.

The increase in hydrophobicity in going from 3″-H to methyl, Cl, Br, and I, as assessed from the incremental π constants derived from log $P_{o/w}$ values for benzene derivatives,[28] was not directly correlated with the decrease in activity of the present TSAO derivatives, although it is in very good agreement with the differences in solvation free energies calculated for these compounds (Figure 2). Therefore, this physicochemical property alone is unlikely to be responsible for the observed changes in activity. No correlation with activities was found either when the cost in electrostatic desolvation energy upon complex formation was calculated (data not shown).

When the dynamic behavior of the complexes of the Br, Cl, and I derivatives of TSAO-T, taken as representative of the series, in the putative binding site of HIV-1 RT was simulated and the interaction energies were calculated, the largest differences with respect to the complex with TSAO-m^{3}T were detected in their interaction with Glu-B138 in the p51 subunit and Arg-A172 in the p66 subunit (Table 3). These differences could account for the observed differences in activity (Table 2). The presence of the halogen atom in the vicinity of the exocyclic amino group also appeared to have an influence on the interaction with the lysine residues that are proposed to face the sulfoxide group providing additional electrostatic steering for TSAO binding.[14] The net electrostatic interaction with Lys-A101 and Lys-A103, however, is rather variable along the simulation time (Table 3) because the side chains of these two more distant residues are very exposed to the solvent. As a result, their position relative to the amino sultone spiro ring is not permanently fixed as is the case with the buried carboxylate group of Glu-B138, and the overall interaction fluctuates over time. This electrostatic interaction appears to be comparatively more favorable for **9** and **10**, which could also account for the greater inhibitory potency of these two compounds relative to **8**.

Conclusions

In conclusion, various synthetic studies toward 3″-substituted TSAO nucleosides have been explored. Among them, Stille cross-coupling of 3″-iodo TSAO derivatives with different stannanes provides an efficient and straightforward route for the direct and selective functionalization of the 3″-position of the sultone spiro moiety via carbon−carbon bond formation. Documented herein is an efficient method for the direct

attachment of (un)substituted vinyl, alkynyl, phenyl, thienyl, and allyl groups to the sultone core. Surprisingly, several of these 3″-substituted TSAO derivatives showed anti-HIV-1 and anti-HIV-2 activity at subtoxic concentrations, an observation that is very unusual for NNRTIs and never observed for TSAO derivatives. Furthermore, among the 3″-halogen-substituted TSAO derivatives, particularly the 3″-iodo derivatives showed a potent anti-HIV-1 activity, which proved comparable to that of the prototype compound TSAO-T. However, there was a clear trend toward decreased antiviral potency in going from iodine to bromine to chlorine. Theoretical studies revealed that differences in the electrostatic component of the interaction energy between the halogenated analogues and the HIV-1 RT could account for the observed changes in activity.

Experimental Section

Chemical Procedures. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. ^{1}H NMR spectra were recorded with a Varian Gemini, a Varian XL-300, and a Bruker AM-200 spectrometer operating at 300 and 200 MHz with Me_4-Si as the internal standard. ^{13}C NMR spectra were recorded with a Varian XL-300 and a Bruker AM-200 spectrometer operating at 75 and 50 MHz with Me_4Si as the internal standard. Analytical thin-layer chromatography (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 or 2 mm, flow rate 5 mL/min). Preparative TLC was performed on 20 cm × 20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck). Analytical HPLC was carried out on a Waters 484 system using a μBondapak C_{18} (3.9 mm × 300 mm, 10 mm). Eluent: CH_3CN/H_2O (0.05% TFA). Flow rate: 1 mL/min. Detection: UV (214 nm).

Triethylamine, dioxane, and acetonitrile were dried by refluxing over calcium hydride. Tetrahydrofuran was dried by refluxing over sodium/benzophenone. Anhydrous *N*-methylpyrrolidinone (NMP) was purchased from Aldrich.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methylamino-3″-methyl-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-3″-methyl-1″,2″-oxathiole-2″,2″-dioxide) (3 and 4). To a solution of TSAO-m^{3}T (**2**) (0.1 g, 0.17 mmol) in dry dioxane (5 mL), KOH (0.014 g, 0.25 mmol) was added and the mixture was stirred for 15 min at room temperature. Then, methyl iodide (48 μL, 0.34 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. The mixture was neutralized with AcOH, filtered through silica gel, and evaporated to dryness. The residue was dissolved in dry acetonitrile (5 mL), and then 4-(dimethylamino)pyridine (0. 83 g, 0.68 mmol) and TBDMSCl (0.10 g, 0.68 mmol) were added. The mixture was stirred at room temperature for 24 h and evaporated to dryness. The residue was treated with ethyl acetate (20 mL) and water (20 mL). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 20 mL). The combined organics were succesively washed with cold (4 °C) 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL) and finally were dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1). From the fastest running fractions, 0.02 g (20%) of **3** was isolated as a white foam. ^{1}H NMR [$CDCl_3$, 200 MHz] δ: 0.81, 0.98 (s, 18H, 2*t*-Bu), 1.98 (d, 3H, J = 1.1 Hz, CH_3-5), 2.23 (s, 3H, CH_3-3″), 3.08 (d, 3H, J = 5.1 Hz, CH_3-4″), 3.35 (s, 3H, CH_3-3), 3.80 (dd, 1H, $J_{4',5'a}$ = 2.4 Hz, $J_{5'a,5'b}$ = 12.2 Hz, H-5′a), 3.96 (dd, 1H, $J_{4',5'b}$ = 2.8 Hz, H-5′b), 4.26 (t, 1H, H-4′), 4.41 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-2′), 5.97 (m, 1H, NH-4″), 6.01 (d, 1H, H-1′), 7.26 (d, 1H, H-6). Anal. ($C_{27}H_{49}N_3O_8SSi_2$) C, H, N, S.

The intermediate moving band gave 0.035 g (35%) of unreacted starting material (TSAO-m^{3}T).

The slowest moving band gave 0.018 g (18%) of **4** as a white foam. ^{1}H NMR [$CDCl_3$, 200 MHz] δ: 0.78, 0.97 (s, 18H, 2*t*-Bu), 1.91 (s, 3H, CH_3-3″), 1.98 (d, 3H, J =1.1 Hz, CH_3-5), 3.34 (s, 3H, CH_3-3), 3.84 (dd, 1H, $J_{4',5'a}$ = 2.1 Hz, $J_{5'a,5'b}$ = 12.4 Hz, H-5′a), 3.97 (dd, 1H, $J_{4',5'b}$ = 2.9 Hz, H-5′b), 4.30 (dd, 1H, H-4′), 4.45 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 5.59 (bs, 2H, NH_2), 6.09 (d, 1H, H-1′), 7.22 (d, 1H, H-6). Anal. ($C_{26}H_{47}N_3O_8SSi_2$) C, H, N, S.

1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-allylamino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-3″-allyl-1″,2″-oxathiole-2″,2″-dioxide) (5 and 6). To a solution of TSAO-m^{3}T (0.1 g, 0.17 mmol) in dry THF (5 mL), NaH 60% (0.25 mmol) was added, and the mixture was stirred for 15 min at room temperature. Then, allyl bromide (30 μL, 0.34 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The mixture was neutralized with AcOH, filtered through silica gel, and evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 5:1). From the fastest running fractions 0.02 g (19%) of **5** were isolated as a white foam. ^{1}H NMR [$(CD_3)_2CO$, 200 MHz] δ: 0.80, 0.96 (s, 18H, 2*t*-Bu), 2.01 (s, 3H, CH_3-5), 3.26 (s, 3H, CH_3-3), 3.82−3.88 (m, 2H, CH_2−CH=CH_2), 4.00 (dd, 1H, $J_{4',5'a}$ = 4.2 Hz, $J_{5'a,5'b}$ = 12.4 Hz, H-5′a), 4.11 (dd, 1H, $J_{4',5'b}$ = 3.6 Hz, H-5′b), 4.30 (dd, 1H, H-4′), 4.69 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-2′), 5.28 (dd, 1H, J = 1.4 Hz, J_{cis} = 10.2 Hz, CH_2−CH=CH_2), 5.40 (dd, 1H, J_{trans} = 17.2 Hz, CH_2−CH=CH_2), 5.79 (s, 1H, H-3″), 5.91 (ddd, 1H, CH_2−CH=CH_2), 5.99 (d, 1H, H-1′), 6.60 (m, 1H, NH-4″), 7.46 (d, 1H, H-6). Anal. ($C_{28}H_{49}N_3O_8SSi_2$) C, H, N, S.

The intermediate moving band gave 0.03 g (28%) of **6** as a white foam. ^{1}H NMR [$(CD_3)_2CO$, 200 MHz] δ: 0.81, 0.98 (s, 18H, 2*t*-Bu), 1.95 (d, 3H, J =1.2 Hz, CH_3-5), 3.13−3.17 (m, 2H, CH_2−CH=CH_2), 3.27 (s, 3H, CH_3-3), 4.03 (dd, 1H, $J_{4',5'a}$ = 3.6 Hz, $J_{5'a,5'b}$ = 12.2 Hz, H-5′a), 4.11 (dd, 1H, $J_{4',5'b}$ = 3.4 Hz, H-5′b), 4.34 (t, 1H, H-4′), 4.65 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 5.08 (dd, 1H, J = 1.6 Hz, J_{cis} = 8.3 Hz, CH_2−CH=CH_2), 5.25 (dd, 1H, J_{trans} = 17.0 Hz, CH_2−CH=CH_2), 5.80−5.91 (m, 1H, CH_2−CH=CH_2), 6.11 (bs, 2H, NH_2), 6.13 (d, 1H, H-1′), 7.50 (d, 1H, H-6). Anal. ($C_{28}H_{49}N_3O_8SSi_2$) C, H, N, S.

The slowest moving band afforded 0.040 g (40%) of unreacted starting material (TSAO-m^{3}T).

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-carbamoylmethylamino-3″-carbamoylmethyl-1″,2″-oxathiole-2″,2″-dioxide) (7). Following the procedure described for the synthesis of derivatives **5** and **6**, TSAO-m^{3}T (0.1 g, 0.17 mmol) was reacted with iodoacetamide (0.062 g, 0.34 mmol) in THF (5 mL) at room temperature for 2 h. The final residue was purified by CCTLC on the Chromatotron first with hexane/ethyl acetate, 5:1, followed by a second purification on the Chromatotron using dichloromethane/methanol, 20:1, as the eluent. The fastest moving band gave compound **7** (0.047 g, 40%) as a white foam. ^{1}H NMR [$(CD_3)_2CO$, 200 MHz] δ: 0.78, 0.97 (s, 18H, 2*t*-Bu), 1.95 (d, 3H, J = 1.2 Hz, CH_3-5), 3.22−3.25 (m, 7H, CH_3-3, $2CH_2$), 4.08 (dd, 1H, $J_{4',5'a}$ = 4.6 Hz, $J_{5'a,5'b}$ = 12.0 Hz, H-5′a), 4.11 (dd, 1H, $J_{4',5'b}$ = 4.0 Hz, H-5′b), 4.32 (dd, 1H, H-4′), 4.67 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.12 (d, 1H, H-1′), 6.55 (bs, 4H, $2CONH_2$), 7.31 (s, 1H, NH-4″), 7.54 (d, 1H, H-6). Anal. ($C_{29}H_{51}N_5O_{10}SSi_2$) C, H, N, S.

The slowest moving band gave 0.048 g (48%) of unreacted starting material (TSAO-m^{3}T).

General Procedure for the Synthesis of 3″-Halo-TSAO and Halo-TSAO-m^{3}T Derivatives (8−11). To a solution of TSAO-T (**1**) (1 mmol) or TSAO-m^{3}T (**2**) (1 mmol) in dry acetonitrile (12 mL) under argon, LiCl (3.6 mmol), LiBr (2 mmol), or I_2 (0.6 mmol), CAN (0.5 mmol), and NEt_3 (0.5 mmol) were added. The reaction mixture was heated at 80 °C until there was complete reaction of the starting material (1−48 h). The final residue obtained after the appropiate workup was

purified by CCTLC on the Chromatotron. The workup conditions, chromatography eluent, yield of the isolated products, and analytical and spectroscopic data are indicated below for each reaction.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-3″-chloro-1″,2″-oxathiole-2″,2″-dioxide) (8). Via the general halogenation procedure, TSAO-T (0.20 g, 0.34 mmol) was treated with LiCl (0.03 g, 0.68 mmol) for 48 h and then concentrated to dryness. The residue was treated with cold (4 °C) ethyl acetate (30 mL) and cold (4 °C) brine (15 mL). The organic phase was separated, and the aqueous phase was extracted with cold (4 °C) ethyl acetate (3 × 30 mL). The combined organics were washed with cold (4 °C) brine (30 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane.ethyl acetate, 2:1) to give compound **8** (0.11 g, 50%) as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.81, 0.97 (s, 18H, 2*t*-Bu), 1.90 (d, 3H, J =1.3 Hz, CH_3-5), 4.08 (dd, 1H, $J_{4',5'a}$ = 3.3 Hz, $J_{5'a,5'b}$ = 9.5 Hz, H-5′a), 4.13 (dd, 1H, $J_{4',5'b}$ = 4.3 Hz, H-5′b), 4.39 (dd, 1H, H-4′), 4.69 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.09 (d, 1H, H-1′), 6.67 (bs, 2H, NH_2), 7,45 (d, 1H, H-6), 10.30 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.41 (CH_3-5), 18.44, 18.96 [$(CH_3)_3$-*C*−Si], 25.74, 26.43 [(*C*$H_3)_3$−C−Si], 62.83 (C-5′), 75.44 (C-2′), 84.82, 86.63 (C-4′, C-1′), 92.50 (C-3′), 93.92 (C-3″), 112.44 (C-5′), 135.75 (C-6), 145.57 (C-4″), 151.62 (C-2), 163.66 (C-4). Anal. ($C_{24}H_{42}ClN_3O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-3″-bromo-1″,2″-oxathiole-2″,2″-dioxide) (9). TSAO-T (0.20 g, 0.34 mmol) was reacted with LiBr (0.06 g, 0.68 mmol) for 2 h according to the general halogenation procedure, and then the mixture was concentrated to dryness. The residue was treated with cold (4 °C) ethyl acetate (30 mL) and cold (4 °C) brine (15 mL). The organic phase was separated, and the aqueous phase was extracted with cold (4 °C) ethyl acetate (3 × 30 mL). The combined organics were washed with cold (4 °C) brine (30 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. Purification of the residue by CCTLC on the Chromatotron (hexane/ethyl acetate, 3:1) afforded compound **9** (0.17 g, 75%) as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.81, 0.97 (s, 18H, 2*t*-Bu), 1.90 (d, 3H, J = 1.1 Hz, CH_3-5), 4.07 (dd, 1H, $J_{4',5'a}$ = 3.4 Hz, $J_{5'a,5'b}$ = 10.5 Hz, H-5′a), 4.10 (dd, 1H, $J_{4',5'b}$ = 4.1 Hz, H-5′b), 4.37 (dd, 1H, H-4′), 4.68 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.06 (d, 1H, H-1′), 6.61 (bs, 2H, NH_2), 7,45 (d, 1H, H-6), 10.30 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.40 (CH_3-5), 18.42, 18.96 [$(CH_3)_3$−*C*−Si], 25.73, 26.43 [(*C*$H_3)_3$−C−Si], 62.83 (C-5′), 75.47 (C-2′), 79.92 (C-3″), 86.52, 86.72 (C-4′, C-1′), 93.47 (C-3′), 112.40 (C-5′), 135.79 (C-6), 148.00 (C-4″), 151.60 (C-2), 163.61 (C-4). Anal. ($C_{24}H_{42}BrN_3O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-3″-iodo-1″,2″-oxathiole-2″,2″-dioxide) (10). Following the general procedure, TSAO-T (0.20 g, 0.34 mmol) was treated with I_2 (0.05 g, 0.20 mmol) for 1 h and then concentrated to dryness. The residue was treated with cold (4 °C) ethyl acetate (30 mL), cold (4 °C) brine (15 mL), and cold (4 °C) $NaHSO_3$ 5% (10 mL). The organic phase was separated, and the aqueous phase was extracted with cold (4 °C) ethyl acetate (3 × 30 mL). The combined organics were washed with cold (4 °C) brine (30 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 1:1) to give compound **10** (0.23 g, 93%) as a white amorphous solid. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.81, 0.96 (s, 18H, 2*t*-Bu), 1.90 (d, 3H, J =1.0 Hz, CH_3-5), 4.06 (dd, 1H, $J_{4',5'a}$ = 3.6 Hz, $J_{5'a,5'b}$ = 12.2 Hz, H-5′a), 4.09 (dd, 1H, $J_{4',5'b}$ = 3.7 Hz, H-5′b), 4.37 (t, 1H, H-4′), 4.67 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.06 (d, 1H, H-1′), 6.56 (bs, 2H, NH_2), 7.46 (d, 1H, H-6), 10.30 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.41 (CH_3-5), 18.41, 18.95 [(CH_3)−*C*−Si], 25.76, 26.46 [(*C*$H_3)_3$−C−Si], 47.84 (C-3″), 62.89 (C-5′), 75.56 (C-2′), 84.77, 86.71 (C-1′, C-4′), 94.39 (C-3′), 112.34 (C-5′), 135.86 (C-6), 151.60 (C-4″), 152.53 (C-2), 163.63 (C-4). Anal. ($C_{24}H_{42}IN_3O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-3″-iodo-1″,2″-oxathiole-2″,2″-dioxide) (11). According to the general procedure, TSAO-m^{3}T (0.20 g, 0.34 mmol) was reacted with I_2 (0.05 g, 0.20 mmol) for 1 h and then concentrated to dryness. The residue was treated with cold (4 °C) ethyl acetate (30 mL), cold (4 °C) brine (15 mL), and cold (4 °C) $NaHSO_3$ 5% (10 mL). The organic phase was separated, and the aqueous phase was extracted with cold (4 °C) ethyl acetate (3 × 30 mL). The combined organics were washed with cold (4 °C) brine (30 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 1:1) to give compound **10** (0.22 g, 90%) as a white amorphous solid. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.79, 0.98 (s, 18H, 2*t*-Bu), 1.94 (d, 3H, J =1.2 Hz, CH_3-5), 3.26 (s, 3H, CH_3-3), 4.06 (dd, 1H, $J_{4',5'a}$ = 3.8 Hz, $J_{5'a,5'b}$ = 10.2 Hz, H-5′a), 4.10 (dd, 1H, $J_{4',5'b}$ = 4.0 Hz, H-5′b), 4.37 (t, 1H, H-4′), 4.67 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-2′), 6.13 (d, 1H, H-1′), 6.55 (bs, 2H, NH_2), 7.48 (d, 1H, H-6). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.41 (CH_3-5), 18.41, 18.95 [$(CH_3)_3$−*C*−Si], 25.76, 26.46 [(*C*$H_3)_3$−C−Si], 28.10 (CH_3-3), 47.84 (C-3″), 62.89 (C-5′), 75.55 (C-2′), 86.71, 84.77 (C-1′, C-4′), 94.39 (C-3′), 112.34 (C-5′), 135.86 (C-6), 151.60 (C-4″), 152.53 (C-2), 163.63 (C-4). Anal. ($C_{25}H_{44}IN_3O_8SSi_2$) C, H, N, S.

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-β-D-ribofuranosyl]-thymine]-3′-spiro-5″-(4″-amino-3″-iodo-1″,2″-oxathiole-2″,2″-dioxide) (12). Iodo compound **10** (0.10 g, 0.14 mmol) was stirred with methanolic 0.1 N HCl (8 mL) at room temperature for 45 min. The solution was neutralized with 1 N NaOH/MeOH, and the solvent was evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 1:2) and afforded compound **12** (0.075 g, 90%) as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.79 (s, 9H, *t*-Bu), 1.89 (d, 3H, J =1.2 Hz, CH_3-5), 3.84 (m, 1H, H-5′a), 4.01 (m, 1H, H-5′b), 4.39 (m, 1H, H-4′), 4.96 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 5.96 (m, 2H, H-1′, 5′-OH), 6.72 (bs, 2H, NH_2), 7.91 (d, 1H, H-6), 10.20 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.48 (CH_3-5), 17.69 [$(CH_3)_3$−*C*−Si], 25.09 [(*C*$H_3)_3$−C−Si], 27.36 (CH_3-3), 47.40 (C-3″), 61.03 (C-5′), 74.87 (C-2′), 84.84 (C-4′), 89.04 (C-3′), 95.69 (C-1′), 110.72 (C-5′), 135.77 (C-6), 151.32 (C-4″), 151.84 (C-2), 162.75 (C-4). Anal. ($C_{18}H_{28}IN_3O_8SSi$) C, H, N, S.

General Procedure for the Synthesis of 3″-Substituted TSAO and TSAO-m^{3}T Derivatives (15a−e, 17a,d−g, 18c, 6) via Stille Coupling Conditions. A solution of the iodonucleoside **10**, **11**, or **12** (1 mmol) in dry NMP (5 mL) was treated with $AsPh_3$ (0.08 mmol), $Pd_2(dba)_3$ (0.02 mmol), CuI (0.04 mmol), and after 10 min the corresponding stannanne (2 mmol). The resulting solution was stirred under argon at 60 °C. After 30 min and 1 h, two additional portions of the corresponding stannane (2 × 1 mmol) were added and the reaction was continued for 12 h at 60 °C. The reaction mixture was allowed to cool to room temperature, and water (15 mL) and ethyl acetate (25 mL) were added. The organic phase was separated and washed several times with water (4 × 15 mL) to remove NMP completely, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was redissolved in acetonitrile (20 mL) and washed with hot hexane (4 × 15 mL) to remove the tin iodide. The final residue was purified by CCTLC on a Chromatotron or by preparative TLC. In general, several chromatographic purifications were required to give pure compounds in the yields shown below. The chromatography eluents, yield, and analytical and spectroscopic data of the isolated products are indicated below for each reaction.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-[4″-amino-3″-(*E*)-(2-methoxyacryloil)-1″,2″-oxathiole-2″,2″-dioxide] (15a). According to the general procedure, iodo nucleoside **10** (0.1 g, 0.14 mmol) was treated with methyl (*E*)-3-(tri-*n*-butylstannyl)acrylate **14a**[20] (0.16 g, 0.56 mmol). The final residue was purified first by CCTLC (dichloromethane/methanol, 50:1) followed by preparative TLC (dichloromethane/methanol, 20:1) developed three times. The fastest moving fractions gave 0.016 g (20%) of the

reduction product TSAO-T (**1**). The slowest moving band gave compound **15a** (0.068 g, 72%) as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.80, 0.95 (s, 18H, 2*t*-Bu), 1.90 (d, 3H, J = 1.2 Hz, CH_3-5), 3.70 (s, 3H, OCH_3), 4.01 (dd, 1H, $J_{4',5'a}$ = 4.2 Hz, $J_{5'a,5'b}$ = 8.6 Hz, H-5′a), 4.13 (dd, 1H, $J_{4',5'b}$ = 4.1 Hz, H-5′b), 4.39 (t, 1H, H-4′), 4.78 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-2′), 6.03 (d, 1H, H-1′), 6.08 (d, 1H, J_{trans} = 15.6 Hz, C*H*=CH−CO_2Me), 7.49−7.57 (m, 4H, CH=C*H*−CO_2Me, NH_2, H-6), 10.40 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.93 (CH_3-5), 18.35, 18.92 [$(CH_3)_3$−*C*−Si], 25.68, 26.23 [(*C*H$_3$)$_3$−C−Si], 54.3 (OCH_3), 62.92 (C-5′), 74.80 (C-2′), 83.23, 88.90 (C-1′, C-4′), 92.42 (C-3′), 110.31 (C-3″), 111.28 (C-5), 129.22 (*C*H=CH−CO_2Me), 132.12 (CH=*C*H−CO_2Me), 134.2 (C-6), 151.11 (C-4″), 152.11 (C-2), 160.11(*C*O_2Me), 163.33 (C-4). Anal. ($C_{28}H_{47}N_3O_{10}SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-[4″-amino-3″-(*E*)-(2-ethoxyacryloil)-1″,2″-oxathiole-2″,2″-dioxide] (15b). The general procedure was followed with iodo derivative **10** (0.1 g, 0.14 mmol) and ethyl (*E*)-3-(tri-*n*-butylstannyl)acrylate **14b**[21] (0.21 g, 0.56 mmol). After the workup, the final residue was purified twice by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1). From the fastest moving band, TSAO-T (**1**) was isolated (0.019 g, 23%). From the slowest moving band, compound **15b** (0.063 g, 65%) was isolated as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.80, 0.95 (s, 18H, 2*t*-Bu), 1.24 (t, 3H, J = 7.1 Hz, OCH_2CH_3), 1.90 (d, 3H, J = 1.1 Hz, CH_3-5), 3.06 (s, 3H, OCH_3), 4.09−4.22 (m, 4H, 2H-5′, OCH_2CH_3), 4.39 (t, 1H, $J_{4',5'}$ = 4.0 Hz, H-4′), 4.76 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-2′), 6.04 (d, 1H, H-1′), 6.06 (d, 1H, J_{trans} = 15.9 Hz, C*H*=CH−CO_2Et), 7.46 (bs, 2H, NH_2), 7.50 (m, 2H, H-6, CH=C*H*−CO_2Et), 10.47 (bs, 1H, NH-3). Anal. ($C_{29}H_{49}N_3O_{10}SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-3″-vinyl-1″,2″-oxathiole-2″,2″-dioxide) (15c). The general procedure was followed with iodo derivative **10** (0.1 g, 0.14 mmol) and tri-*n*-butylvinylstannane **14c** (0.18 g, 0.56 mmol). The residue obtained after the workup was purified twice by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1). From the fastest moving fractions, compound **15c** (0.068 g, 79%) was identified as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.79, 0.80 (s, 18H, 2*t*-Bu), 1.97 (d, 3H, J = 1.2 Hz, CH_3-5), 4.05 (dd, 1H, $J_{4',5'a}$ = 3.5 Hz, $J_{5'a,5'b}$ = 12.5 Hz, H-5′a), 4.10 (dd, 1H, $J_{4',5'b}$ = 3.8 Hz, H-5′b), 4.32 (dd, 1H, H-4′), 4.67 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 5.17 (dd, 1H, J_{cis} = 11.4 Hz, J = 0.6 Hz, CH=CH_2), 5.45 (dd, 1H, J_{trans} = 17.5 Hz, CH=CH_2), 6.05 (d, 1H, H-1′), 6.49 (bs, 2H, NH_2), 6.53 (dd, 1H, C*H*=CH_2), 7,49 (d, 1H, H-6), 10.32 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.53 (CH_3-5), 18.38, 18.93 [$(CH_3)_3$−*C*−Si], 25.68, 26.37 [(*C*H$_3$)$_3$−C−Si], 63.02 (C-5′), 75.94 (C-2′), 84.92, 85.33 (C-1′, C-4′), 91.24 (C-3′), 111.01 (C-3″), 112.32 (C-5), 121.94 (CH=*C*H_2), 133.70, 136.42 (*C*H=CH_2, C-6), 145.51 (C-4″), 151.61 (C-2), 163.73 (C-4). Anal. ($C_{26}H_{45}N_3O_8SSi_2$) C, H, N, S.

From the slowest moving band, TSAO-T (**1**) (0.008 g, 10%) was isolated.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-[4″-amino-3″-(*E*)-(1-hydroxy-2-propenyleno)-1″,2″-oxathiole-2″,2″-dioxide] (15d). Iodo compound **10** (0.10 g, 0.14 mmol) was reacted with (*E*)-3-(tri-*n*-butylstannyl)-2-propen-1-ol **14d**[21] (0.19 g, 0.56 mmol) according to the general procedure. Two consecutive purifications of the residue by CCTLC on the Chromatotron (dichloromethane/methanol, 25:1) gave TSAO-T (**1**) (0.008 g, 10%) from the fastest moving fractions. The slowest moving fractions afforded 0.074 g (82%) of compound **15d** as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.81, 0.97 (s, 18H, 2*t*-Bu), 1.90 (d, 3H, J = 1.2 Hz, CH_3-5), 4.01 (dd, 1H, $J_{4',5'a}$ = 3.5 Hz, $J_{5'a,5'b}$ = 8.9 Hz, H-5′a), 4.08 (dd, 2H, J = 1.6 Hz, $J_{CH2,OH}$ = 4.7 Hz, CH_2), 4.10 (dd, 1H, $J_{4',5'b}$ = 3.4 Hz, H-5′b), 4.32 (t, 1H, H-4′), 4.70 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.06 (d, 1H, H-1′), 6.12 (dt, 1H, J_{trans} = 16.0, $J_{CH,CH2}$ = 4.6 Hz, CH=C*H*−CH_2OH), 6.35 (dt, 1H, C*H*=CH−CH_2OH), 6.48 (bs, 2H, NH_2), 7,49 (d, 1H, H-6), 10.31 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 13.03 (CH_3-5), 18.38, 18.95 [$(CH_3)_3$-*C*-Si], 25.68, 26.33 [(*C*H$_3$)$_3$−C−Si], 63.22 (C-5′), 75.40 (C-2′), 85.23, 88.20 (C-1′, C-4′), 92.42 (C-3′), 111.31 (C-3″), 111.48 (C-5′), 129.32 (*C*H=CH−CH_2OH), 134.42 (CH=*C*H−CH_2OH, C-6), 152.11 (C-4″), 152.31 (C-2), 163.03 (C-4). Anal. ($C_{27}H_{47}N_3O_9SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-3″-phenyl-1″,2″-oxathiole-2″,2″-dioxide) (15e). Via the general procedure, iodo nucleoside **10** (0.1 g, 0.14 mmol) was treated with tri-*n*-butylphenylstannane **14e** (0.18 g, 0.56 mmol). The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) twice using the same eluent. The fastest moving fractions afforded 0.057 g (60%) of **15e** as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.83, 0.96 (s, 18H, 2*t*-Bu), 1.91 (d, 3H, J =1.3 Hz, CH_3-5), 4.13 (dd, 1H, $J_{4',5'a}$ = 3.5 Hz, $J_{5'a,5'b}$ = 12.3 Hz, H-5′a), 4.15 (dd, 1H, $J_{4',5'b}$ = 3.7 Hz, H-5′b), 4.45 (dd, 1H, H-4′), 4.78 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.11 (d, 1H, H-1′), 6.45 (bs, 2H, NH_2), 7.37 (m, 1H, Ph), 7.45 (m, 2H, Ph), 7.52 (d, 1H, H-6), 7.57 (m, 1H, Ph), 7.59 (m, 1H, Ph), 10.32 (bs, 1H, NH). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.43 (CH_3-5), 18.43, 18.93 [$(CH_3)_3$−*C*−Si], 25.72, 26.41 [(*C*H$_3$)$_3$−C−Si], 63.11 (C-5′), 75.35 (C-2′), 85.20, (C-4′), 86.80 (C-1′), 91.35 (C-3′), 105.40 (C-3″), 112.26 (C-5), 128.28, 128.94, 129.13, 129.97 (Ph), 135.77 (C-6), 145.48 (C-4″) 151.62 (C-2), 163.64 (C-4). Anal. ($C_{30}H_{47}N_3O_8SSi_2$) C, H, N, S.

The slowest moving fractions gave 0.041 g (20%) of TSAO-T (**1**).

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-amino-3″-(*E*)−(2-methoxyacryloil)-1″,2″-oxathiole-2″,2″-dioxide] (17a). Following the general procedure, iodo nucleoside **11** (0.1 g, 0.14 mmol) was reacted with (*E*)-methyl 3-(tri-*n*-butylstannyl)-acrylate **14a**[21] (0.16 g, 0.55 mmol). The residue was purified first by CCTLC (dichloromethane/methanol, 25:1) followed by preparative TLC (dichloromethane/methanol, 25:1), developed three times. From the fastest moving band, TSAO-m^{3}T (**2**) (0.015 g, 16%) was isolated. From the slowest moving band, compound **17a** (0.063 g, 66%) was isolated as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.82, 0.91 (s, 18H, 2*t*-Bu), 1.90 (s, 3H, CH_3-5), 3.27 (s, 3H, CH_3-3), 3.71 (s, 3H, OCH_3), 4.01 (dd, 1H, $J_{4',5'a}$ = 3.8 Hz, $J_{5'a,5'b}$ = 12.0 Hz, H-5′a), 4.13 (dd, 1H, $J_{4',5'b}$ = 4.0 Hz, H-5′b), 4.42 (t, 1H, H-4′), 4.76 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.08 (d, 1H, J_{trans} = 15.8 Hz, C*H*=CH−CO_2Me), 6.13 (d, 1H, H-1′), 6.42 (bs, 2H, NH_2), 7.51 (s, 1H, H-6), 7.54 (d, 1H, CH=C*H*−CO_2Me). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 12.93 (CH_3-5), 18.38 (CH_3), 18.95, 18.92 [$(CH_3)_3$−*C*−Si], 25.68, 26.23 [$(CH_3)_3$−C−Si], 54.0 (OCH_3), 62.72 (C-5′), 74.80 (C-2′), 83.23, 88.90 (C-1′, C-4′), 92.44 (C-3′), 110.33 (C-3″), 111.18 (C-5′), 129.23 (*C*H=CH−CO_2Me), 132.12 (CH=*C*H−CO_2Me), 134.2 (C-6), 151.11, 152.11 (C-4″, C-2), 160.13 (*C*O_2Me), 163.35 (C-4). Anal. ($C_{29}H_{49}N_3O_{10}SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-d-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-amino-3″-(*E*)-(1-hydroxy-2-propenyleno)-1″,2″-oxathiole-2″,2″-dioxide] (17d). Iodo nucleoside **11** (0.1 g, 0.14 mmol) was treated with (*E*)-3-(tri-*n*-butylstannyl)-2-propen-1-ol **14d**[21] (0.19 g, 0.56 mmol) according to the general procedure. The final residue was purified twice by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1). The fastest moving fractions gave 0.009 g (10%) of TSAO-m^{3}T (**2**). The slowest moving band afforded compound **17d** (0.075 g, 82%) as a white foam. ^{1}H NMR [$(CD_3)_2$CO, 300 MHz] δ: 0.79, 0.27 (s, 18H, 2*t*-Bu), 1.95 (d, 3H, J = 1.4 Hz, CH_3-5), 3.27 (s, 3H, CH_3-3), 3.99 (m, 1H, OH), 4.07 (dd, 1H, $J_{4',5'a}$ = 3.3 Hz, $J_{5'a,5'b}$ = 8.0 Hz, H-5′a), 4.13 (dd, 1H, $J_{4',5'b}$ = 3.3 Hz, H-5′b), 4.20 (m, 2H, CH_2), 4.36 (t, 1H, H-4′), 4.65 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.13 (dt, 1H, J_{trans} = 16.0 Hz, $J_{CH,CH2}$ = 4.6 Hz, CH=C*H*−CH_2OH), 6.16 (d, 1H, H-1′), 6.35 (dt, 1H, J = 1.8 Hz, C*H*=CH−CH_2OH), 6.54 (bs, 2H, NH_2), 7.52 (d, 1H, H-6). ^{13}C NMR [$(CD_3)_2$CO, 75 MHz] δ: 13.09 (CH_3-5), 18.41, 18.98 [$(CH_3)_3$−*C*−Si], 26.34, 26.38 [$(CH_3)_3$−C−Si], 28.06 (CH_3-3), 61.7 (CH_2), 63.13 (C-5′), 75.40 (C-2′), 85.17, 88.23 (C-1′, C-4′), 92.42 (C-3′), 111.31 (C-3″), 112.48 (C-5), 114.77 (*C*H=CH−CH_2OH), 129.27 (CH=*C*H−CH_2OH), 134.45 (C-6), 152.14, 152.22 (C-2, C-4″), 163.34 (C-4). Anal. ($C_{28}H_{49}N_3O_9SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-3″-phenyl-1″,2″-oxathiole-2″,2″-dioxide) (17e). According to the general procedure, iodo nucleoside **11** (0.1 g, 0.14 mmol) was reacted with tri-*n*-butylphenylstannane **14e** (0.18 g, 0.56 mmol). Purification of the final residue by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) gave compound **17e** (0.057 g, 60%) as a white foam from the fastest moving fractions. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ: 0.81, 0.89 (s, 18H, 2*t*-Bu), 1.95 (d, 3H, J =1.2 Hz, CH_3-5), 3.29 (s, 3H, CH_3-3), 4.01 (dd, 1H, $J_{4',5'a}$ = 4.3 Hz, $J_{5'a,5'b}$ = 12.3 Hz, H-5′a), 4.15 (dd, 1H, $J_{4',5'b}$ = 4.8 Hz, H-5′b), 4.45 (t, 1H, H-4′), 4.78 (d, 1H, $J_{1',2'}$ = 6.5 Hz, H-2′), 6.20 (d, 1H, H-1′), 6.54 (bs, 2H, NH_2), 7.33−7.60 (m, 6H, Ph, H-6). ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ: 13.15 (CH_3-5), 18.39, 18.93 [$(CH_3)_3-C-$Si], 25.65, 26.41 [$(CH_3)_3-$C−Si], 29.03 (CH_3-3), 63.13 (C-5′), 75.61 (C-2′), 85.29, 87.40 (C-1′, C-4′), 91.45 (C-3′), 108.2 (C-3″), 111.33 (C-5), 128.93, 129.12, 129.96 (Ph), 134.01 (C-6), 145.44 (C-4″), 152.12 (C-2), 163.30 (C-4). Anal. ($C_{31}H_{49}N_3O_8SSi_2$) C, H, N, S.

The slowest moving fractions afforded 0.027 g (15%) of TSAO-m^{3}T (**2**).

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-amino-3″-(2-thienyl)-1″,2″-oxathiole-2″,2″-dioxide] (17f). Iodo nucleoside **11** (0.1 g, 0.14 mmol) was treated with tri-*n*-butyl-2-thienylstannane **14f** (0.21 g, 0.56 mmol) according to the general procedure. After the workup, the residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1). From the fastest moving band, compound **17f** (0.048 g, 50%) was isolated as a white amorphous solid. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ: 0.80, 0.96 (s, 18H, 2*t*-Bu), 1.94 (d, 3H, J =1.2 Hz, CH_3-5), 3.26 (s, 3H, CH_3-3), 4.03 (dd, 1H, $J_{4',5'a}$ = 3.5 Hz, $J_{5'a,5'b}$ = 12.3 Hz, H-5′a), 4.15 (dd, 1H, $J_{4',5'b}$ = 3.7 Hz, H-5′b), 4.44 (t, 1H, H-4′), 4.73 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-2′), 6.19 (d, 1H, H-1′), 6.54 (bs, 2H, NH_2), 7.16 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ = 5.1 Hz, H-4$_{thiopheno}$), 7.25 (dd, 1H, $J_{3,5}$= 2.3 Hz, $J_{3,4}$ = 3.6 Hz, H-3$_{thiopheno}$), 7.52 (s, 1H, H-6), 7.61 (dd, 1H, H-5$_{thiopheno}$). ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ: 13.01 (CH_3-5), 18.47, 19.02 [$(CH_3)_3-C-$Si], 26.51, 28.11 [$(CH_3)_3-$C−Si], 28.63 (CH_3-3), 63.15 (C-5′), 75.80 (C-2′), 85.36 (C-4′), 87.02 (C-1′), 92.00 (C-3′), 101.51 (C-3″), 111.45 (C-5), 127.67, 128.08, 128.28, 128.49 (thiopheno), 134.92 (C-6), 146.34 (C-4″), 152.24 (C-2), 163.40 (C-4). Anal. ($C_{29}H_{47}N_3O_8S_2Si_2$) C, H, N, S.

From the slowest moving fractions, 0.047 g (26%) of TSAO-m^{3}T (**2**) was identified.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-amino-3″-(1-propynyl)-1″,2″-oxathiole-2″,2″-dioxide] (17g). Following the general procedure, iodo derivative **11** (0.1 g, 0.14 mmol) was reacted with tri-*n*-butyl-1-propynylstannane **14g** (0.18 g, 0.56 mmol). The final residue was purified first by CCTLC (hexane/ethyl acetate, 3:1) followed by preparative TLC (hexane/ethyl acetate, 4:1). The fastest moving fractions afforded 0.025 g (30%) of TSAO-m^{3}T (**2**). The slowest moving fractions gave 0.027 g (30%) of compound **17g** as a white foam. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ: 0.84, 0.95 (s, 18H, 2*t*-Bu), 1.92 (d, 3H, J =1.2 Hz, CH_3-5), 2.11 (s, 3H, $CH_3-C\equiv C$), 3.28 (s, 3H, CH_3-3), 3.92 (dd, 1H, $J_{4',5'a}$ = 4.2 Hz, $J_{5'a,5'b}$ = 12.4 Hz, H-5′a), 4.12 (dd, 1H, $J_{4',5'b}$ = 4.3 Hz, H-5′b), 4.33 (t, 1H, H-4′), 4.67 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-2′), 6.15 (d, 1H, H-1′), 6.81 (bs, 2H, NH_2), 7.49 (d, 1H, H-6). ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ: 5.07 ($CH_3-C\equiv C$), 12.98 (CH_3-5), 17.79, 18.39 [$(CH_3)_3-C-$Si], 25.14, 26.02 [$(CH_3)_3-$C−Si], 28.15 (CH_3-3), 62.04 (C-5′), 75.25 (C-2′), 77.22 ($CH_3-C\equiv$C), 83.42 (C-4′), 87.55 (C-1′), 91.27 (C-3′), 93.71 ($CH_3-C\equiv C$), 98.83 (C-3″), 110.89 (C-5′), 133.36 (C-6), 150.05 (C-2), 151.07 (C-4″), 163.15 (C-4). Anal. ($C_{28}H_{47}N_3O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-3″-allyl-1″,2″-oxathiole-2″,2″-dioxide) (6). Iodo nucleoside **11** (0.1 g, 0.14 mmol) was reacted with tri-*n*-butylallylstannane **14h** (0.18 g, 0.56 mmol) according to the general procedure. After the workup, the residue was purified by CCTLC on the Chromatotron twice (hexane/ethyl acetate, 5:1) followed by preparative TLC (hexane/ethyl acetate, 4:1). From the fastest moving band, compound **6** (0.026, 30%) was isolated as a white amorphous solid. From the slowest moving fractions, 0.016 g (20%) of TSAO-m^{3}T (**2**) was isolated.

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-*β*-D-ribofuranosyl]-thymine]-3′-spiro-5″-(4″-amino-3″-vinyl-1″,2″-oxathiole-2″,2″-dioxide) (18c). According to the general procedure, iodo nucleoside **12** (0.1 g, 0.17 mmol) was reacted with tri-*n*-butylvinylstannane **14c** (0.21 g, 0.67 mmol). Purification of the final residue by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) twice gave compound **18c** (0.06 g, 70%) as a white foam from the fastest moving fractions. ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ: 0.86 (s, 9H, *t*-Bu), 1.85 (d, 3H, J = 1.2 Hz, CH_3-5), 3.87 (dd, 1H, $J_{4',5'a}$ = 2.5 Hz, H-5′a), 4.01 (dd, 1H, $J_{4',5'b}$ = 2.4 Hz, H-5′b), 4.37 (t, 1H, H-4′), 5.00 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-2′), 5.19 (dd, 1H, J_{cis} = 11.4 Hz, J = 0.8 Hz, CH=CH_2), 5.49 (dd, 1H, J_{trans} = 17.6 Hz, CH=CH_2), 5.87 (d, 1H, H-1′), 5.91 (m, 1H, OH), 6.56 (dd, 1H, CH=CH$_2$), 6.79 (bs, 2H, NH_2), 7,89 (d, 1H, H-6), 10.35 (bs, 1H, NH-3). Anal. ($C_{20}H_{31}N_3O_8SSi$) C, H, N, S.

From the slowest moving fractions, 0.014 g (15%) of [1-[2′-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)[1b] (**13**) was isolated.

Biological Methods. a. Cells and Viruses. 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).

b. Activity Assay for Test Compounds against HIV-1 and HIV-2 in Cell Cultures. An amount of 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. Giant cell formation (CEM) or HIV-induced cytopathicity (MT-4) was recorded microscopically (CEM) or by trypan blue dye exclusion (MT-4) in the HIV-infected cell cultures after 4 days (CEM) or 5 days (MT-4). The 50% effective concentration (EC_{50}) of the test compounds was defined as the compound concentration required to inhibit virus-induced cytopathicity (CEM) or to reduce cell viability (MT-4) by 50%. The 50% cytostatic or cytotoxic concentration (CC_{50}) was defined as the compound concentration required to inhibit CEM cell proliferation by 50%, or to reduce the number of viable MT-4 cells in mock-infected cell cultures by 50%.

c. Reverse Transcriptase Assay. For determination of the 50% inhibitory concentration (IC_{50}) of the test compounds against HIV-2 RT, the RNA-dependent DNA polymerase assay was performed as follows. The reaction mixture (50 μL) contained 50 mM Tris-HCl (pH 7.8), 5 mM DTT, 300 μM glutathione, 500 μM EDTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 μg of bovine serum albumin, a fixed concentration of the labeled substrate [^{3}H]dGTP (5.6 μM, 1 μCi; specific radioactivity, 3.6 Ci/mmol; Amersham Pharmacia Biotech), a fixed concentration of the template/primer poly(rC)·oligo(dG)$_{12-18}$ (0.1 mM; Amersham Pharmacia Biotech), 0.06% Triton X-100, 5 μL of inhibitor solution [containing various concentrations (10-fold dilutions) of the compounds], and 5 μL of the RT preparations. The reaction mixtures were incubated at 37 °C for 30 min, at which time 200 μL of yeast RNA (2 mg/mL) and 1 mL of trichloroacetic acid (5% v/v) in saturated phosphate buffer were added. The solutions were kept on ice for at least 15 min, after which the acid-insoluble material was filtered over Whatman GF/C glass-fiber filters and washed with 5% trichloroacetic acid in H_2O and ethanol. The filters were then analyzed for radioactivity in a liquid scintillation counter (Canberra Packard, Zellik, Belgium).

The IC_{50} for each test compound was determined as the compound concentration that inhibited HIV RT activity by 50%.

Computational Methods. The spiro systems of **1** and **2**, their 3″-methylated derivative **4**, and the corresponding halogenated analogues **8** (Cl−), **9** (Br−), and **10** (I−) were

model-built in Insight-II.[29] Their geometries were fully optimized with the ab initio quantum mechanical program Gaussian 94[30] using the 6-31+G(d) basis set except for the iodo derivative for which a polarized electric property 3-21+G basis set[31] was employed. Atomic point charges were derived by fitting the molecular electrostatic potential calculated on each optimized geometry to a monopole−monopole expression.[32] The electrostatic contribution to the solvation free energy of each spiro system was obtained by solving the linearized Poisson equation by means of a finite difference method,[33] as implemented in the DelPhi module of Insight II. The total electrostatic free energy in vacuo (exterior dielectric = 1) was subtracted from the total electrostatic free energy in water (exterior dielectric = 80) using the same interior dielectric for the solute ($\epsilon = 4$) and the same grid definitions in both cases.[34] A solvent-accessible surface,[35] calculated using a probe radius of 1.4 Å, defined the boundary between each solute molecule and the exterior. Cubic grids with a resolution of 1.0 Å were centered on the molecular systems considered, leaving a separation of 15 Å between any solute atom and the borders of the box. The potentials at the grid points delimiting the box were calculated analytically by treating each charge atom as a Debye−Hückel sphere. The accuracy of the calculated electrostatic potentials was subsequently improved by defining a smaller box (10 Å separation) with a lower grid resolution (0.5 Å spacing) so that the new boundary potentials were linearly interpolated from those calculated in the previous run (*focusing*).

To explore the effect of halogen substitution on the binding affinity of TSAO-T, our previously reported reduced model of RT comprising the NNRTI binding site and all those residues within 30 Å of residue 138 of the p51 subunit was used, which included a spherical "drop" of about 370 TIP3P water molecules centered on the C_ of Glu-B138.[14] A molecular dynamics trajectory spanning 1 ns was then simulated for the complexes of RT with **2**, **8**, **9**, and **10** under the same conditions reported previously, and the electrostatic interactions between each of these ligands and individual RT amino acids were calculated by solving the Poisson−Boltzmann equation as recently described.[14] Coordinates from the last 300 ps were used for the analysis.

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