# Novel [2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) Derivatives with Anti-HIV-1 and Anti-Human-Cytomegalovirus Activity

Sonia de Castro,[†] Esther Lobatón,[†] María-Jesús Pérez-Pérez,[†] Ana San-Félix,[†] Alessandra Cordeiro,[†] Graciela Andrei,[‡] Robert Snoeck,[‡] Erik De Clercq,[‡] Jan Balzarini,[‡] María-José Camarasa,*[,†] and Sonsoles Velázquez*[,†]

*Instituto de Química Médica (C.S.I.C.), Juan de la Cierva 3, E-28006 Madrid, Spain, and Rega Institute for Medical Research, K. U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

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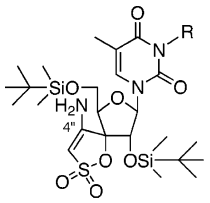
New [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (TSAO) derivatives substituted at the 4″-amino group of the spiro moiety with different carbonyl functionalities have been designed and synthesized. Various synthetic procedures, on the scarcely studied reactivity of the 3′-spiroaminooxathioledioxide moiety, have been explored. The compounds were evaluated for their inhibitory effect on both wild-type and TSAO-resistant HIV-1 strains, in cell culture. The presence of a methyl ester (**10**) or amide groups (**12**) at the 4″-position conferred the highest anti-HIV-1 activity, while the free oxalyl acid derivative (**11**) was 10- to 20-fold less active against the virus. In contrast, the presence at this position of (un)substituted ureido or acyl groups markedly diminished or annihilated the anti-HIV-1 activity. Surprisingly, some of the target compounds also showed inhibition of human cytomegalovirus (HCMV) replication at subtoxic concentrations. This has never been observed previously for TSAO derivatives. In particular, compound **26** represents the first TSAO derivative with dual anti-HIV-1 and -HCMV activity.

## Introduction

Reverse transcriptase (RT) is a key enzyme that plays an essential and multifunctional role in the replication of the human immunodeficiency virus (HIV) and thus represents an attractive target for the development of new drugs useful in AIDS therapy.[1]

A class of inhibitors targeted at the viral RT, the so-called nonnucleoside RT inhibitors (NNRTIs), are compounds used in the treatment of HIV infections in combination with nucleoside analogue RT inhibitors (NRTIs) and/or HIV protease inhibitors (PIs) and/or the fusion inhibitor enfuvirtide.[2−4] Currently, only three NNRTIs (namely, nevirapine, delarvidine, and efavirenz) are available in clinical practice. The virus can be markedly suppressed for a relatively long period of time when exposed to multiple-drug combination therapy (highly active antiretroviral therapy, HAART).[5] Although NNRTIs generally exhibit low toxicity and favorable pharmacokinetic properties, their clinical utility is adversely affected by the emergence of drug-resistant HIV-1 variants and by the problem of cross-resistance to other NNRTIs.[6] Therefore, the synthesis of additional NNRTIs for use in the multidrug (cocktail) approaches that may also be effective against mutant HIV strains remains a high priority for medical research.

Among the NNRTIs, [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) nucleosides (TSAOs), developed in the past decade in our research group, represent a rather unique class of compounds[7,8] that seem to interact at the interface between the p51 and p66 subunits of HIV-1 RT.[9] The prototype compound of this family is the thymine derivative designated as TSAO-T (**1**), and the most selective compound is its 3-*N*-methyl-substituted derivative TSAO-m$^3$T (**2**) (Figure 1). More than 700 TSAO derivatives have been prepared, and extensive structure activity (SAR), resistance, and metabolic studies have been performed. These studies have revealed the structural requirements of this unique family of compounds for their optimal interaction with the enzyme (HIV-1 RT). A comprehensive review of all the work performed within this family of compounds has been recently published.[10] Although the pharmacological profile of TSAO molecules was unfavorable to their clinical development,[10,12] further biochemical studies have shown that both TSAO-T and its 3-*N*-ethyl derivative are able to destabilize the p66/p51 RT heterodimer in a concentration-dependent manner resulting in a loss in the ability of the RT to bind to DNA.[12,13] This suggests




**1** R = H (TSAO-T)
**2** R = $CH_3$ (TSAO-m$^3$T)

**Figure 1.** Chemical structures of TSAO-T (**1**) and TSAO-m$^3$T (**2**).

* To whom correspondence should be addressed. For M.-J.C.: telephone, (+34)-915622900; fax, (+34)-915644853; e-mail, mj.camarasa@iqm.csic.es. For S.V.: (+34)-915622900; fax, (+34)-91 5644853; e-mail, iqmsv29@iqm.csic.es.
† Instituto de Química Médica.
‡ Rega Institute for Medical Research.

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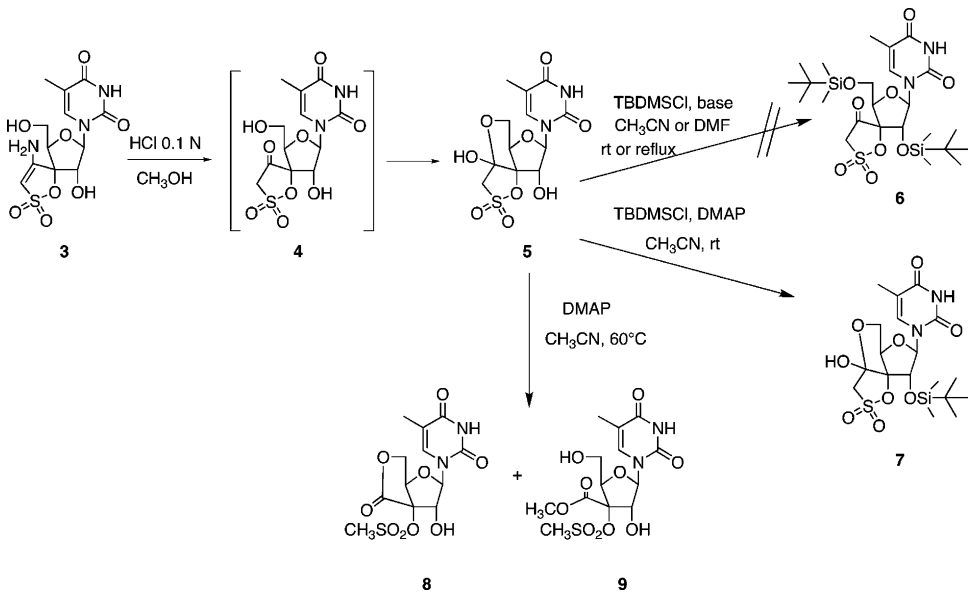
**Scheme 1.** Synthesis of Bicyclic Nucleosides **5**−**8** and 3′-C-branched Nucleoside **9**




a new and different mechanism of inhibition of HIV-1 RT with regard to the other known NNRTIs, which deserves further exploration.

HIV-1 resistance to NNRTIs is primarily associated with mutations of amino acids at the lipophilic NNRTIs binding pocket in the p66 subunit.[4,14] However, resistance to TSAO compounds is determined by a single mutation (Glu-138-Lys) located in the p51 subunit of HIV-1 RT, which does not generally result in cross-resistance to other NNRTIs.[15]

Our experimental data strongly suggest a specific interaction of the 4″-amino group of the 3′-spiro-aminooxathioledioxide moiety of TSAO molecules with the carboxylic group of the glutamic acid residue at position 138 (Glu-138) of the p51 subunit of HIV-1 RT.[8−10]

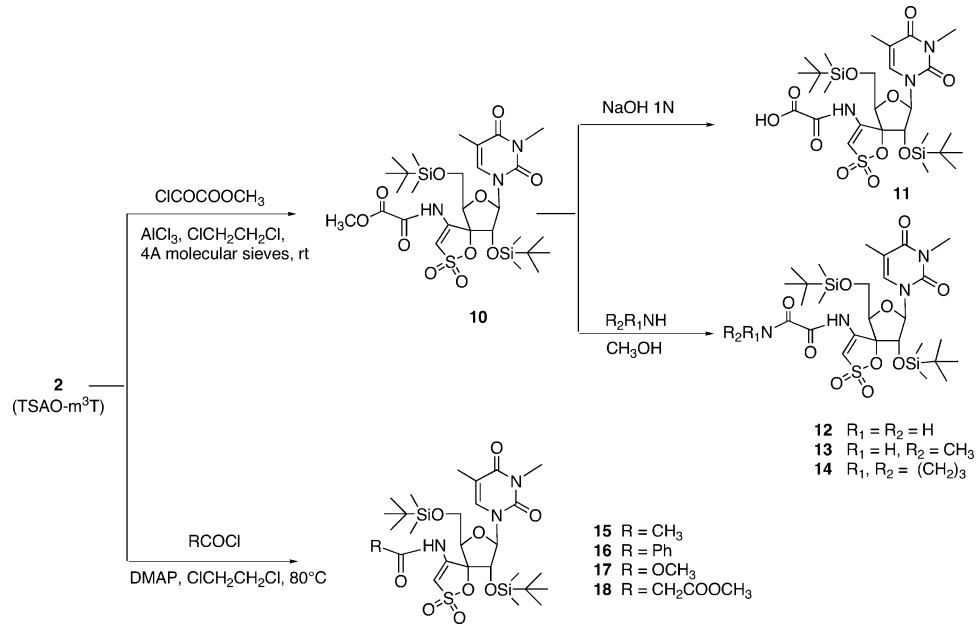
Aimed at obtaining novel derivatives more resilient to drug resistance development by the virus than the "first generation" of TSAO molecules, we designed a series of TSAO compounds bearing at the 4″-position different carbonyl functionalities that may interact with the amino group of the Lys-138 in the TSAO-resistant viral strains. Moreover, the presence of amide or urea groups at this 4"-position may stabilize interactions with highly conserved residues of the enzyme close to the Glu-B138/Lys-B138 of the p51 subunit such as the Asn-B136. This paper reports on the synthesis and in vitro anti-HIV-1 activity of these novel compounds against both wild-type HIV-1 and TSAO-resistant HIV-1 strains.

## Results and Discussion

**Synthesis.** The preparation of the target compounds represents a synthetic challenge because the chemical reactivity of the 3′-spiroaminooxathioledioxide moiety (aminosultone heterocyclic system) of TSAO compounds has been scarcely studied.[16,17] In this paper three types of modifications of the 4″-amino group of the 3′-spiro moiety were addressed: (i) acid hydrolysis of this enamino type system, (ii) acylation reactions of the amino group with different electrophiles, and (iii) substitution of this amino group by other conveniently functionalized amines via transamination reactions.

To get compounds able to interact with the amino group of the Lys-B138 (TSAO-resistant strains), the direct replacement of the enamino group by a ketone through acid hydrolysis (compound **6** in Scheme 1) was envisaged. In view of the known acid lability of the 5′-*tert*-butyldimethylsilyl group (TBDMS), the suitable TSAO-deprotected nucleoside precursor **3**[18] was used as starting material. However, although compound **3** showed high reactivity toward acid hydrolysis (1 N HCl in methanol), the corresponding keto derivative **4** exhibited a strong tendency toward its 5′-cyclic hemiacetal form, and therefore, the isolated compound was identified as **5** (70% yield) (Scheme 1). Further attempts of silylation of **5** to give the target compound **6** failed in all the experimental conditions investigated [a large escess of TBDMSCl either in pyridine or in the presence of (dimethylamino)pyridine (DMAP) or of imidazole in acetonitrile or dimethylformamide]. No bis-silylated derivative **6** was observed in any of the conditions tested, and only the monosilylated 5′-cyclic hemiacetal **7** was isolated (62% yield). Interestingly, when the 5′-cyclic hemiacetal compound **5** was reacted with DMAP in dry acetonitrile at 60 °C, the novel highly functionalized bicyclic nucleoside **8** was obtained in a 49% yield together with minor quantities of the 3′-C-branched nucleoside **9** (9%). Further work performed in this reaction clarified that compound **9** was obtained from **8** during the purification step that involved the use of methanol. Therefore, the formation of **9** can be avoided if methanol is excluded as solvent in the purification step. The structure of compounds **5**, **7**, and **8** were unequivocally assigned on the basis of their corresponding spectroscopic data, using a combination of mono- and bidimensional $^1$H and $^{13}$C NMR (g-HMBC, g-HSQC) and IR techniques. The $^1$H NMR spectra for **5** and **7** showed the disappearance of the characteristic signals of the spiroaminooxathioledioxide moiety (a singlet at $\delta$ 5.56 ppm assigned to H-3″ and a broad singlet at $\delta$ 6.95 ppm assigned to $NH_2$-4″) and the presence of an AB system at $\delta$ 3.62−4.05 ppm corresponding to the new H-3″ protons and a new broad singlet at $\delta$ 8.41 and 7.44 ppm corresponding to the hemiacetalic OH. In the g-HMBC

**Scheme 2.** Synthesis of 4″-*N*-Oxalyl Derivatives **10**−**14** and 4″-*N*-Acyl TSAO Compounds **15**−**18**



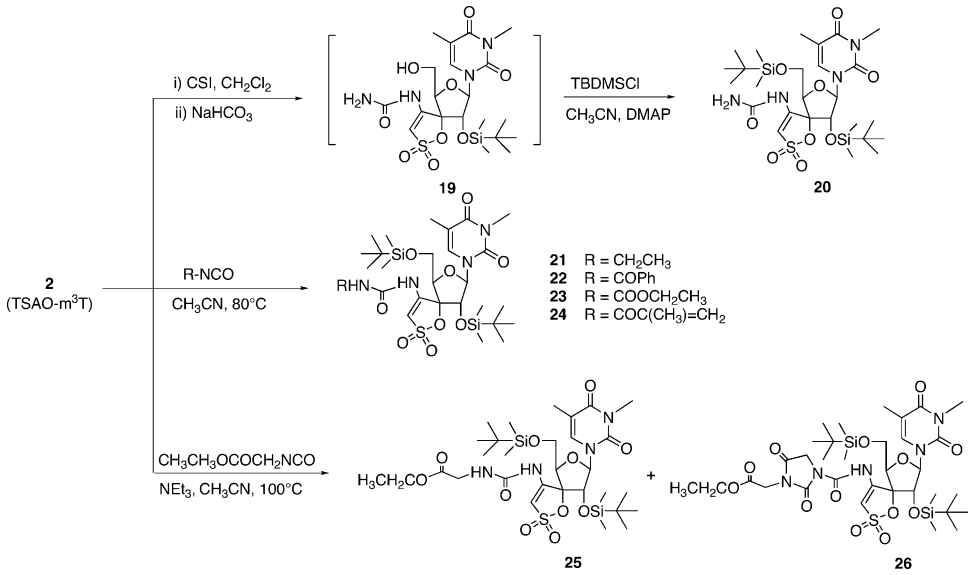

experiments of **5** and **7**, a correlation peak was observed between C-4″ and H-5′, which is only compatible with the hemiacetal structure. Compounds **5** and **7** exist exclusively as a single 5′-cyclic hemiacetal diastereomer. Selective NOE experiments showed that irradiation of the hemiacetal-OH-4″ caused enhancements of the signals for H-2′ and one of the H-3″ protons. This observation allowed us to unequivocally assign the C-4″ configuration of the only isolated diastereomer as *S*. On the other hand, the IR spectra of the lactone bicyclic nucleoside **8** showed a band at 1770 $cm^{-1}$ characteristic of five-membered ring lactones. The $^1H$ NMR spectra for this compound showed the disappearance of the signals corresponding to the AB system of the sultone ring and to the hemiacetalic OH and the presence of a singlet at $\delta$ 3.38 ppm corresponding to the methyl of the mesyl group. The $^{13}C$ NMR spectra showed a downfield shift of the C-4″ ($\delta$ 170.92 ppm) corresponding to the carbonyl of the lactone moiety. In the g-HMBC experiments, the H-4′ and the H-5′ protons showed long-range correlations with the carbonyl of the lactone moiety, which confirmed the proposed structure.

Next, we focused on the synthesis of compounds bearing carbonyl groups on the 4″-amino of the spiro-oxathioledioxide moiety. This was performed by reaction of TSAO-m$^3$T (**2**) with differently substituted acid chlorides (Scheme 2). Ab initio theoretical calculations of the amino sultone system[19] showed that the nitrogen atom of the amino sultone ring has mainly $sp^2$ character and the HOMO energy ($E_{HOMO}$) of the amine was very low (less than −9.73 eV), which corresponds to amines with very low reactivity toward electrophiles.[20] Because of this expected low reactivity of the 4″-amino group, reaction of TSAO-m$^3$T (**2**) with the highly reactive methyloxalyl chloride reagent was first attempted. Thus, when TSAO-m$^3$T (**2**) was treated with methyloxalyl chloride in the presence of DMAP as an acid scavenger at room temperature, compound **10** was isolated in 35% yield together with unreacted starting material. A higher temperature in the reaction led to complex reaction mixtures of unidentified products. However, when the reaction of **2** and methyloxalyl chloride was performed at room temperature in the presence of stoichiometric amounts of $AlCl_3$ and 4 Å molecular sieves, the N-acylated compound **10** was obtained in higher yield (66%). In contrast to the lack of selectivity observed in previous alkylation reactions of TSAO-m$^3$T,[17] acylation of **2** with methyloxalyl chloride occurs exclusively at the amino of the 4″-position. Attachment of the acyl residue to the amino group of the sultone moiety and not to the 3″-C-position in compound **10** was established from their $^1H$ NMR spectra by the disappearance of the broad singlet at 6.45 ppm assigned to the 4″-$NH_2$ and by the appearance of one 4″-NH proton at 10.60 ppm, due to the deshielding effect of the C=O of the acyl group attached to this NH. This deshielding effect was also observed in the signal corresponding to H-3″, which showed a downfield shift ($\Delta\delta$) with respect to the same signal in the starting TSAO derivative **2**. The $^{13}C$ NMR spectra of the compound also confirmed the structural assignments.

The nucleoside **10** was used as a starting compound to synthesize other *N*-oxalyl-substituted derivatives (Scheme 2) bearing either carboxylic acid groups that may interact with the Lys-B138 (TSAO-resistant strains) or amido groups that may stabilize interactions with highly conserved residues close to Glu-B138/Lys-B138 such as Asn-B136. Thus, the methyl ester group of oxalyl nucleoside **10** was further transformed into the free acid **11** (72% yield) by treatment with 1 N NaOH. Alternatively, reaction of the methyl ester **10** with appropriate amides afforded the amides **12**−**14** in 52%, 50%, and 60% yield, respectively (Scheme 2). The methyl ester derivative **10** and the amides **12**−**14** were endowed with potent anti-HIV-1 activity similar to or even slightly higher than that of TSAO-m$^3$T as will be discussed later.

These results prompted us to explore the role that each or both of the two carbonyl groups of the *N*-oxalyl derivatives may play in the anti-HIV-1 activity. To determine the importance of the carbonyl directly attached to the 4″-amino group, novel acyl TSAO com-

**Scheme 3.** Synthesis of of (Un)Substituted 4″-Ureido TSAO Compounds **19**−**25** and the Cyclic Compound **26**




pounds **15**−**17** (Scheme 2) bearing one carbonyl group were designed. Also, compound **18** with two carbonyl groups linked through a methylene spacer was prepared (Scheme 2). Thus, treatment of **2** with acetyl chloride in the presence of $AlCl_3$ and 4 Å molecular sieves (optimized acylation conditions to get compound **10** from **2**) or in the presence of different bases (pyridine, triethylamine, or DMAP) and solvents (acetonitrile or THF) at room temperature was unsuccessful. When the reaction was performed in dichloroethane at 80 °C in the presence of an excess of DMAP in a pressure reaction vessel, the *N*-acyl derivative **15** was obtained in moderate yield (42%). A similar treatment of **2** with the corresponding acyl chlorides yielded the *N*-acyl derivatives **16**−**18** (44%, 11%, and 51% yield, respectively).

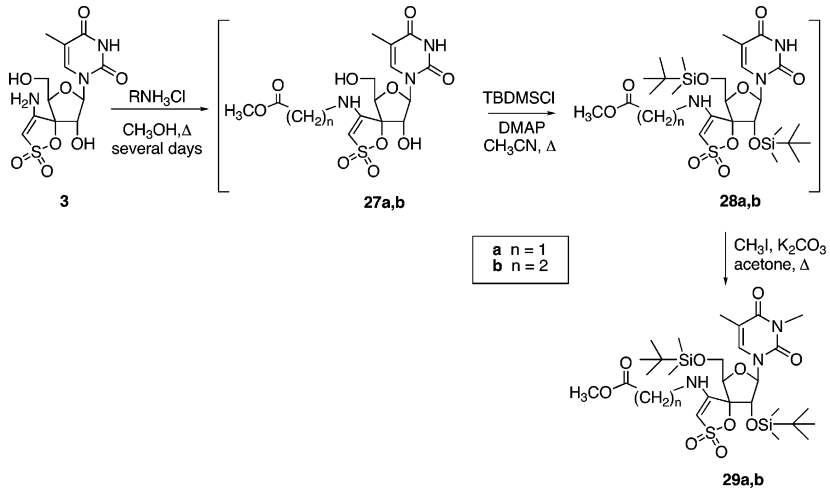
Next, 4″-ureido-(un)substituted TSAO-m$^3$T derivatives bearing only one carbonyl group at the 4″-position (**20**, **21**) or two carbonyl groups separated by different spacers (**22**−**25**) were prepared by reaction of **2** with differently substituted isocyanates (Scheme 3). Reaction of **2** with chlorosulfonyl isocyanate, followed by treatment with aqueous $NaHCO_3$, gave the 5′-O-deprotected 4″-unsubstituted ureido derivative **19** that was silylated "in situ" (TBDMSCl/DMAP) to give the bisilylated 4″-ureido compound **20** in 40% overall yield. Similarly, reaction of **2** with an excess of ethyl, benzoyl, ethoxycarbonyl, or methacryloyl isocyanate, in dry acetonitrile at 80 °C, afforded the corresponding 4″-*N*-alkyl- and acyl-substituted ureido derivatives **21** (50%), **22** (90%), **23** (80%), and **24** (75%). When ethoxycarbonylmethyl isocyanate was reacted with TSAO-m$^3$T (**2**) under similar reaction conditions, only the starting material was recovered. However, when this reaction was carried out in a sealed pressure tube in the presence of a catalytic amount of triethylamine at 100 °C, the corresponding *N*-substituted ureido derivative **25** was isolated in 52% yield together with the unexpected cyclic compound **26** (30%). Formation of this side product could be explained by attack of a second molecule of the isocyanate on the initially formed *N*-ureido derivative **25** and subsequent cyclization to give the dioxoimidazoline ring. Scarce reports on this type of cyclization have been previously described.[21]

Finally, compounds **29a**,**b** (Scheme 4), **31a**,**b**, and **32a**,**b** (Scheme 5), in which a carboxylic ester, carboxylic acid, or amide, respectively, were attached to the 4″-amino group of the sultone ring through one or two methylenes, were prepared. Compounds **29a**, **31a**, and **32a** would allow us to study the influence of the $\beta$-carbonyl group of the *N*-oxalyl derivatives **10**−**12** on the anti-HIV-1 activity. Compounds **29a**,**b** were obtained from the TSAO-deprotected derivative **3**[18] in a 20% and 28% overall yield by a three-step protocol (Scheme 4), which is transamination reaction of **3** with the corresponding amines, followed by "in situ" silylation of the 2′- and 5′-hydroxyl groups and finally N-3 selective methylation of the thymine ring using standard conditions.[18] The transamination reactions were carried out by treatment of **3** with excess corresponding methoxycarbonylalkylamine hydrochlorides in refluxing methanol in a sealed tube for several days.

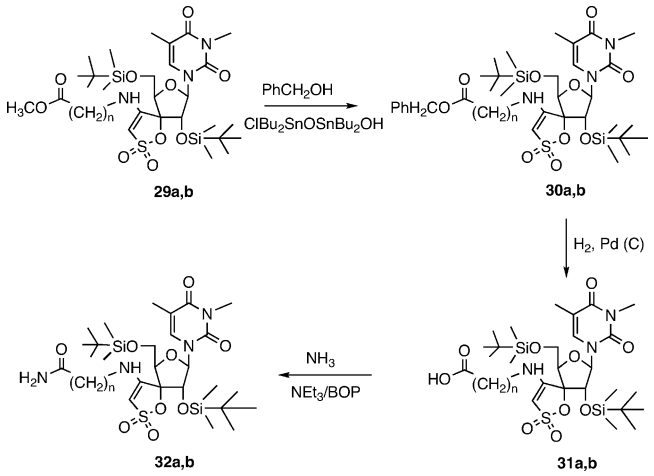
We also prepared the corresponding acid (**31a**,**b)** and amide (**32a**,**b)** derivatives (Scheme 5). Thus, saponification of **29a**,**b** with 1 N NaOH to obtain **31a**,**b** led to complex reaction mixtures from which only 2′- and/or 5′-deprotected compounds were isolated. A milder alternative that consisted of transesterification to the benzyl esters (**30a**,**b)** followed by hydrogenolysis of the benzyl moiety was attempted. Thus, transesterification of **29a**,**b** was readily achieved (Scheme 5) by heating the corresponding nucleosides and benzyl alcohol in the presence of a catalytic amount of 1,3-disubstituted tetrabutyldistannoxane in toluene.[22,23] Benzyl esters thus obtained (**30a**,**b**) were hydrogenated ($H_2$, Pd/C) to afford the corresponding free acids **31a**,**b** in 84% and 83% yield, respectively. Finally, treatment of **31a**,**b** with ammonia (2 M in methanol) in the presence of BOP as coupling reagent and triethylamine as a base gave the amide derivatives **32a**,**b** in 44% and 82% yield, respectively.

**Biological Evaluation.** The compounds were evaluated for their inhibitory activity against HIV-1 and

**Scheme 4.** Synthesis of 4″-*N*-Alkyl TSAO Compounds **27**−**29**




**Scheme 5.** Synthesis of 4″-*N*-Alkyl TSAO Compounds **30**−**32**




HIV-2 in MT-4 and CEM cell cultures and compared with the prototype TSAO-T and TSAO-m$^3$T compounds (Table 1).

The 5′-cyclic hemiacetal and bicyclic lactone derivatives **5**, **7**, and **8** were devoid of anti-HIV activity but also lacked any significant cytostatic activity at 100 $\mu$M in CEM and MT-4 cell cultures. Whereas the methyl ester nucleoside **10** and the amide **12** proved exquisitely inhibitory against HIV-1 in both MT-4 and CEM cell cultures, the free acid derivative **11** was 10- to 20-fold less active against the virus. Also, the azetidine derivative **14** showed decreased antiviral activity compared to the amide **12**. In contrast, compounds containing *N*-acyl groups on the 4″-amino group of the spiro moiety of TSAO (**15**−**18**) were devoid of antiviral activity at subtoxic concentrations. Also, the ureido derivatives (**20**, **21**) or those nucleosides containing two carbonyl groups separated by different spacers (**22**−**25**) were inactive. Compound **26**, in which the two carbonyl functions were separated by a cyclic entity, showed surprising antiviral activity ($EC_{50}$ = 0.2−0.9 $\mu$M) although it was very close to its cytostatic activity ($CC_{50}$ = 1.4 $\mu$M). Interestingly, whereas **29a** was markedly inhibitory against HIV-1 and relatively nontoxic, **29b**, in which the 4″-$NH_2$ group was separated from the methyl ester by two methylenes instead of one methylene, lacked any antiviral efficacy.

Drug-resistant HIV-1 strains were selected under pressure of escalating drug regimens of **11** and **12**. Two virus strains independently selected in the presence of compound **11** were found to have the E138K mutation in its reverse transcriptase. This mutation is a characteristic amino acid change in the RT of TSAO-resistant virus strains. When virus strains were selected in the presence of **12**, one strain acquired the V106A mutation in its RT whereas another strain also contained, besides E138K, L228V in its RT. There is only one article in the literature in which the latter mutation has been reported to appear under NRTI drug pressure.[24] This mutation is due to a transversion mutation of the first nucleotide in codon 228 (CTT → GTT). Compound **11** was 12- to 15-fold resistant to the E138K and E138K + L228V RT HIV-1 strains, whereas compound **12** was ∼40-fold resistant to E138K and ≥50-fold resistant to the double mutant virus strains. These levels of resistance are less pronounced than the resistance level observed for TSAO-m$^3$T against the mutant virus strains (>1000-fold). The NNRTIs nevirapine and UC-781 and the NtRTI tenofovir virtually fully kept their inhibitory potential against both E138K and E138K + L228V RT virus mutants (data not shown).

When the compounds were evaluated against replication of human CMV in cell culture, several compounds showed pronounced antiviral activity at concentrations in the lower micromolar range (i.e., 0.29−2.0 $\mu$M for compounds **16**, **20**, **22**, **23**, **26**, that is, at a concentration well below their toxicity threshold). In particular, compound **22** showed the highest anti-human-cytomegalovirus (anti-HCMV) activity lacking marked cytotoxicity or antiproliferative activity against HEL cell cultures (selectivity index $CC_{50}/EC_{50}$ or $MCC/EC_{50}$ ≥ 100). It should be mentioned, however, that this compound was rather cytostatic against the lymphocytic CEM and MT-4 cell cultures. It should also be noted that compound **26** showed marked inhibitory activity against HCMV at 0.29−0.32 $\mu$M, 10-fold lower than the cytotoxicity threshold. Interestingly, this compound was also very active against HIV-1. Therefore, it could be considered as the first TSAO derivative with dual activity against HIV-1 and HCMV. The antiviral activity profile of this molecule deserves further investigation.

The highly modified structures of these TSAO molecules point to a different mechanism of inhibition of HCMV than that of currently used anti-HCMV nucleo-

**Table 1.** Antiviral Activity of TSAO Derivatives in Cell Culture

| | HIV | | | | | | HCMV in human embryonic lung (HEL) cells | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $EC_{50}$ [a] ($\mu$M) | | | | | | | | cytotoxicity ($\mu$M) | |
| | MT-4 | | CEM | | $CC_{50}$ [b] ($\mu$M) | | $EC_{50}$ [c] ($\mu$M) | | | |
| compd | HIV-1 ($III_B$) | HIV-2 (ROD) | HIV-1 ($III_B$) | HIV-2 (ROD) | MT-4 | CEM | HCMV (AD-169) | HCMV (Davis) | cell morphology (MCC)[d] | cell growth ($CC_{50}$)[e] |
| **1** (TSAO-T) | 0.06 ± 0.03 | >10 | 0.05 ± 0.01 | >10 | 12 ± 2 | | | | | |
| **2** (TSAO-$m^3$T) | 0.06 ± 0.09 | >250 | 0.04 ± 0.01 | >250 | 230 ± 7 | | | | | |
| **5** | >100 | >100 | >100 | >100 | >100 | >100 | | | | |
| **7** | >100 | >100 | >100 | >100 | >100 | >100 | | | | |
| **8** | >50 | >250 | >50 | >50 | ≥250 | 160 ± 39 | 179 | 164 | ≥400 | 36 |
| **10** | 0.08 ± 0.006 | >4 | 0.06 ± 0.02 | >4 | 10.3 ± 2.6 | 5.57 ± 2.21 | 253 | 179 | >400 | >200 |
| **11** | 1.30 ± 0.45 | >10 | 0.23 ± 0.04 | >50 | 31.1 ± 20.4 | 72.6 ± 6.5 | >80 | 36 | 400 | 146 |
| **12** | 0.057 ± 0.008 | >2 | 0.028 ± 0.017 | >10 | 9.59 ± 0.87 | 9.96 ± 1.48 | 6.5 | 6.5 | ≥16 | 14.5 |
| **14** | 0.8 ± 0.1 | >50 | 0.7 ± 0.1 | >10 | 17.4 ± 3.1 | 21.6 ± 3.1 | 10 | 10 | 65 | 84.4 |
| **15** | >5 | >5 | >25 | >25 | 10.7 ± 0.6 | 25.7 ± 5.0 | 40 | >16 | ≥80 | >200 |
| **16** | >5 | >5 | >5 | >5 | 4.9 ± 1.5 | 4.91 ± 1.54 | 1.3 | 1.2 | ≥3.2 | 9.7 |
| **17** | >20 | >20 | >20 | >20 | 29.9 ± 7.3 | 30.7 ± 0.3 | ≥20 | 25 | ≥50 | >200 |
| **18** | 19.5 ± 7.8 | >5 | ≥5 | >25 | 11.9 ± 0.3 | 29.7 ± 3.5 | 9 | 10 | 400 | 29 |
| **20** | >2 | >2 | >2 | >2 | 3.7 ± 0.4 | 4.36 ± 0.13 | >3.2 | 2.0 | 16 | 11 |
| **21** | >2 | >2 | >2 | >2 | | 4.26 ± 0.30 | 1.8 | 2.0 | ≥3.2 | 10 |
| **22** | >2 | >10 | >2 | >2 | 3.33 ± 0.04 | 2.29 ± 0.07 | 1.2 | 0.8 | ≥80 | >200 |
| **23** | >2 | >2 | >2 | >2 | | 3.0 ± 0.35 | 2.3 | 1.8 | 16 | 14 |
| **24** | >0.8 | >0.8 | >0.8 | >0.8 | 1.19 ± 0.59 | 1.20 ± 0.52 | >20 | >4 | 20 | 1.2 |
| **25** | >2 | >2 | >2 | >2 | 3.78 ± 1.60 | 5.02 ± 0.62 | >3.2 | >3.2 | 16 | 13 |
| **26** | 0.88 ± 0.02 | >2 | 0.24 ± 0.02 | >2 | 1.43 ± 0.50 | 1.42 ± 0.51 | 0.29 | 0.32 | ≥3.2 | 2.5 |
| **29a** | 1.61 ± 0.89 | >125 | 0.6 ± 0.0 | >125 | 105 ± 29 | 96 ± 41 | >400 | >400 | ≥400 | 160 |
| **29b** | >250 | >250 | >250 | >250 | >250 | >250 | >400 | >400 | ≥400 | 94 |
| **30a** | | >250 | 6.7 ± 3.1 | >250 | >250 | >250 | >400 | >400 | ≥400 | >200 |
| **30b** | >250 | >250 | >250 | >250 | >250 | >250 | >400 | >400 | ≥400 | >200 |
| **31a** | >10 | >10 | >10 | >10 | 30 ± 1 | 65 ± 16 | ≥50 | 32 | 300 | 136 |
| **31b** | >50 | >50 | >10 | >10 | 11.5 ± 3.2 | 19.4 ± 0.5 | >16 | 8.0 | 80 | 34 |
| **32a** | >10 | >10 | >2 | >2 | 14.9 ± 3.5 | 14.6 ± 2.0 | >3.2 | >3.2 | 16 | 13.5 |
| **32b** | >10 | >10 | 5 | >10 | 20.0 ± 4.5 | 21.0 ± 1.2 | >16 | 29 | ≥80 | 14.8 |

[a] 50% effective concentration required to inhibit HIV-induced cytopathogenicity by 50%. [b] 50% cytostatic concentration to inhibit MT-4 or CEM cell proliferation by 50%. [c] 50% effective concentration to inhibit HCMV-induced plaque formation by 50%. [d] Minimal cytotoxic concentration required to cause a microscopically visible alteration of cell morphology. [e] 50% cytostatic concentration required to inhibit HEL cell proliferation by 50%.

side analogues. However, although it has been shown that TSAO derivatives in general interact in a specific manner with the virus-encoded reverse transcriptase, it is currently unclear whether the TSAO derivatives that are inhibitory to HCMV are also targeted against HCMV DNA polymerase (acting as a nonnucleosidic inhibitor) or inhibit another viral (or cellular) function necessary for virus replication.

## Conclusions

In conclusion, several TSAO derivatives substituted, at the 4″-amino group of the spiromoiety, with different carbonyl functionalities have been synthesized. Compounds **10** and **12** were among the more potent and selective inhibitors of HIV-1. Surprisingly, several of these 4″-substituted TSAO derivatives also gained activity against anti-HCMV at subtoxic concentrations. To date, all TSAO compounds reported by us showed complete selectivity for HIV-1, with no antiviral activity against a range of other (DNA or RNA) viruses. Compound **26** represents the first example of an NNRTI with dual anti-HIV-1 and -HCMV activity. This dual activity seems interesting because opportunistic infections caused by HCMV are common among AIDS patients.

## Experimental Section

**Chemical Procedures.** Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. Mass spectra were measured on a quadropole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MS HP 1100). $^1$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 $Me_4Si$ 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 of 1 mm, flow rate of 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 $\mu$Bondapak $C_{18}$ (3.9 mm × 300 mm, 10 mm). Isocratic conditions were used: mobile phase $CH_3CN/H_2O$ (0.05% TFA); flow rate, 1 mL/min; detection, UV (254 nm). All retention times are quoted in minutes.

Triethylamine, dioxane, 1,2-dichloromethane, 1,2-dichloroethane, toluene, and acetonitrile were dried by refluxing over calcium hydride.

The name of the bicyclic nucleoside **8** is given according to the von Baeyer nomenclature. However, for easy comparison, the assignments of the signals of the NMR spectra follow standard carbohydrate/nucleoside numbering (i.e., the furanose skeleton numbered 1′-5′) with the thymine moiety having the highest priority.

**(4″*S*)-4″,*O*-5′-Cyclic Hemiacetal of [1-(β-D-Ribofuranosyl)thymine]-3′-spiro-5″-(4″-oxo-1″,2″-oxathiolane-2″,2″-dióxide) (5).** To a solution of nucleoside **3** (0.2 g, 0.55 mmol) in methanol (5 mL), a 0.1 N HCl solution in methanol (30 mL) was added, and the mixture was stirred at room temperature for 24 h. The mixture was neutralized to pH 5 with a solution of 1 N NaOH in methanol, and the solvent was evaporated to dryness. The residue was treated with isobutanol (30 mL) and

water (30 mL). The organic phase was separated, and the aqueous phase was extracted several times with isobutanol (3 × 20 mL). The combined organics were dried over $Na_2SO_4$, filtered, and evaporated to dryness. The final residue was purified by column chromatography (dicloromethane/methanol, 6:1) to give 0.15 g (70%) of **5** as an amorphous white solid. $^1$H NMR spectral analysis of compound **5** proved that it exists as a single 5′-cyclic hemiacetal diastereomer in $(CD_3)_2SO$ solution. $^1$H NMR [200 MHz, $(CD_3)_2SO$] $\delta$: 1.80 (s, 3H, $CH_3$-5), 3.65 (d, 1H, $J_{3\text{“a},3\text{”b}}$ = 14.0 Hz, H-3″a), 4.03 (dd, 1H, $J_{4',5'a}$ = 3.6 Hz, $J_{5'a,5'b}$ = 10.4 Hz, H-5′a), 4.05 (d, 1H, H-3″b), 4.13 (dd, 1H, $J_{4',5'b}$ = 5.2 Hz, H-5′b), 4.64 (dd, 1H, $J_{2',OH}$ = 6.8 Hz, $J_{1',2'}$ = 7.2 Hz, H-2′), 4.58 (dd, 1H, H-4′), 5.87 (d, 1H, H-1′), 6.03 (d, 1H, OH), 7.59 (m, 1H, H-6), 8.41 (bs, 1H, OH), 11.47 (bs, 1H, NH-3). $^{13}$C NMR [50 MHz, $(CD_3)_2SO$] $\delta$: 11.87 ($CH_3$-5), 54.85 ($CH_2$-3″), 68.92 (C-5′), 69.39 (C-2′), 82.72 (C-4′), 89.75 (C-1′), 96.36 (C-4″), 107.67 (C-3′), 110.14 (C-5), 135.71 (C-6), 150.40 (C-2), 163.35 (C-4). MS (ES$^+$) *m/z* 363.1 (M + 1)$^+$. Anal. ($C_{12}H_{14}N_2O_9S$) C, H, N, S. Long-range correlations observed in a $^1$H-$^{13}$C g-HMBC experiment were hemiacetal-C-4″/5′-$CH_2$ for compound **5**. Selective NOE experiments showed that irradiation of the hemiacetal-OH-4“ caused enhancements of the signals for H-2 and one of the H-3” protons.

**(4″*S*)-4″,*O*-5′-Cyclic Hemiacetal of [1-[2′-*O*-(*tert*-Butyldimethylsilyl)-*β*-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-oxo-1″,2″-oxathiolane-2″,2″-dióxide) (7).** Compound **5** (0.1 g, 0.28 mmol) was dissolved in dry acetonitrile (5 mL) and then 4-(dimethylamino)pyridine (DMAP) (0.17 g, 1.4 mmol) and *tert*-butyldimethylsilyl chloride (TBDMSCl) (0.21 g, 1.4 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), filtered over silica gel and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (dichloromethane:methanol, 20:1) to give 0.082 g (62%) of **7** as an amorphous white solid.$^1$H NMR [200 MHz, $(CD_3)_2CO$] $\delta$: 0.88 (s, 9H, *t*-Bu), 1.85 (d, 3H, $J$ = 1.2 Hz, $CH_3$-5), 3.62 (d, 1H, $J_{3''a,3''b}$ = 13.9 Hz, H-3″a), 4.03 (d, 1H, H-3″b), 4.20 (dd, 1H, $J_{4',5'a}$ = 2.9, $J_{5'a,5'b}$ = 10.7 Hz, H-5′a), 4.15 (dd, 1H, $J_{4',5'b}$ = 4.9 Hz, H-5′b), 4.79 (dd, 1H, H-4′), 4.93 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-2′), 6.02 (d, 1H, H-1′), 7.44 (bs, 1H, OH), 7.58 (d, 1H, H-6), 10.25 (bs, 1H, NH-3). $^{13}$C NMR [50 MHz, $CDCl_3$] $\delta$: 12.27 ($CH_3$-5), 18.04 [$(CH_3)_3$-*C*-Si], 25.55 [($CH_3$)$_3$−C−Si], 56.08 ($CH_2$-3″), 70.09 (C-5′), 71.91 (C-2′), 83.48 (C-4′), 95.38 (C-1′), 98.17 (C-4″), 109.8 (C-5), 111.93 (C-3′), 130.93 (C-6), 150.35 (C-2), 163.76 (C-4). MS (ES$^+$) *m/z* 477.2 (M + 1)$^+$. Anal. ($C_{18}H_{28}N_2O_9SSi$) C, H, N, S. Long-range correlations observed in a $^1$H−$^{13}$C gHMBC experiment were hemiacetal-C-4″/5′-$CH_2$ for compound **7**. Selective NOE experiments showed that irradiation of the hemiacetal-OH-4″ caused enhancements of the signals for H-2 and one of the H-3″ protons.

**1-([1′*S*,3′*R*,4′*R*,5′*R*]-4′-Hydroxyl-5′mesyl-6′-oxo-2′,7′-dioxabicyclo[3.3.0]octan-3′-yl)thymine (8).** To a suspension of the cyclic hemiacetal **5** (0.1 g, 0.28 mol) in dry acetonitrile (10 mL), DMAP (0.068 g, 0.56 mmol) was added. The resulting mixture was stirred at 60 °C for 24 h and then acetonitrile (25 mL) was removed by rotary evaporation under reduced pressure. The residue was treated with ethyl acetate (25 mL) and filtered through silica gel. The final residue was purified by CCTLC on the Chromatotron using dichloromethane/methanol, 20:1, as eluent. The fastest moving fractions afforded 0.050 g (49%) of **8** as an amorphous white solid. IR (KBr) = 1770 cm$^{-1}$. $^1$H NMR [300 MHz, $(CD_3)_2CO$] $\delta$: 1.82 (d, 3H, $J$ = 1.3 Hz, $CH_3$-5), 3.38 (s, 3H, $OSO_2CH_3$), 4.45 (dd, 1H, $J_{4',5'a}$ = 2.4 Hz, $J_{5'a,5'b}$ = 10.5 Hz, H-5′a), 4.73 (m, 2H, H-2′, H-5′b), 5.21 (dd, 1H, $J_{4',5'a}$ = 2.4 Hz, $J_{4',5'b}$ = 6.3 Hz, H-4′), 5.80 (bs, 1H, 4″-OH), 6.04 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1′), 7.70 (d, 1H, H-6), 10.21 (bs, 1H, NH-3). $^{13}$C NMR [100 MHz, $(CD_3)_2CO$] $\delta$: 11.59 ($CH_3$-5), 40.54 ($OSO_2CH_3$), 71.27 (C-5′), 74.91 (C-2′), 79.90 (C-4′), 84.81 (C-3′), 88.14 (C-1′), 111.36 (C-5), 135.74 (C-6), 150.95 (C-2), 163.24 (C-4), 170.92 (C-4″). MS (ES$^+$) *m/z* 363.4 (M + 1)$^+$. Anal. ($C_{12}H_{14}N_2O_9S$) C, H, N, S.

From the slowest moving fractions 0.01 g (9%) of [1-[3′-*O*-mesyl-3′-*C*-methoxycarbonyl-*β*-D-ribofuranosyl]thymine] **(9)** as an amorphous white solid was isolated. $^1$H NMR [300 MHz, $(CD_3)_2CO$] $\delta$: 1.83 (d, 3H, $J$ = 1.3 Hz, $CH_3$-5), 3.33 (s, 3H, $OSO_2CH_3$), 3.73 (m, 1H, H-5′a), 3.81 (s, 3H, $OCH_3$), 3.85 (m, 1H, H-5′b), 4.29 (t, 1H, $J_{4',5a'}$ = $J_{4',5b'}$ = 3.2 Hz, H-4′), 4.42 (bt, 1H, OH-5′), 4.99 (t, 1H, $J_{1',2'}$ = $J_{2',OH}$ = 6.6 Hz, H-2′), 5.56 (bd, 1H, OH-2′), 6.04 (d, 1H, H-1′), 7.83 (d, 1H, H-6), 10.18 (bs, 1H, NH-3). MS (ES$^+$) *m/z* 395.1 (M + 1)$^+$. Anal. ($C_{13}H_{18}N_2O_{10}S$) C, H, N, S.

When chromatographic purification of the final residue by CCTLC on the Chromatotron was carried out with dichloromethane/ethyl acetate (2:1) as eluent, 0.082 g of compound **8** (62%) was the only isolated product in this reaction.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (10). Method A.** To a solution of TSAO-m$^3$T (**2**) (0.2 g. 0.33 mmol) in dry 1,2-dichloroethane (3 mL), DMAP (0.121 g, 0.99 mmol) and methyloxalyl chloride (0.022 mL, 0.47 mmol) were added. The reaction mixture was stirred at room temperature for 12 h. After the mixture was cooled, the solvent was removed and the residue was treated with dichloromethane (20 mL) and water (20 mL). The organic phase was successively washed with with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried ($Na_2SO_4$), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (dichloromethane/ethyl acetate, 10:1). The fastest moving fractions gave 0.08 g (35%) of **10** as a white amorphous solid. HPLC: $t_R$ = 12.29 min (65:35). $^1$H NMR [300 MHz, $(CD_3)_2CO$] $\delta$: 0.70, 0.79 (2s, 18H, 2*t*-Bu), 1.97 (d, 3H, $J$ = 1.1 Hz, $CH_3$-5), 3.28 (s, 3H, $CH_3$-3), 3.92 (s, 3H, $CO_2CH_3$), 4.01 (dd, 1H, $J_{4',5'a}$ = 2.3, $J_{5'a,5'b}$ = 13.0 Hz, H-5′a), 4.11 (dd, 1H, $J_{4',5'b}$ = 2.2 Hz, H-5′b), 4.48 (t, 1H, H-4′), 4.97 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-2′), 6.07 (d, 1H, H-1′), 7.77 (s, 1H, H-3″), 7.94 (d, 1H, H-6), 10.60 (bs, 1H, NH-4″). $^{13}$C NMR [50 MHz, $(CD_3)_2CO$] $\delta$: 12.92 ($CH_3$-5), 18.33, 19.05 [$(CH_3)_3$-*C*−Si], 25.68, 26.23 [(*C*$H_3$)$_3$−C−Si], 28.11 ($CH_3$-3), 54.26 ($OCH_3$), 61.86 (C-5′), 73.78 (C-2′), 83.23, 88.92 (C-1′, C-4′), 92.43 (C-3′), 110.26 (C-3″), 111.19 (C-5), 134.01 (C-6), 139.43 (C-4″), 151.12 (C-2), 155.59 (CO-*C*$O_2CH_3$), 159.12 (*C*O−$CO_2CH_3$), 163.01 (C-4). Anal. ($C_{28}H_{47}N_3O_{11}SSi_2$) C, H, N, S. From the slowest moving fractions 0.108 g (36%) of starting material was recovered.

**Method B.** A solution of methyloxalyl chloride (0.13 mL, 4.2 mmol) and aluminum chloride (0.19 g, 4.2 mmol) in dry 1,2-dichloroethane (1 mL) was added dropwise to a solution of TSAO-m$^3$T (0.7 g, 1.19 mmol) in dry 1,2- dichloroethane (2 mL) in the presence of 4 Å molecular sieves (0.9 g). The reaction mixture was stirred for 24 h at room temperature and then was quenched with ice/water (10 mL). The mixture was stirred for an additional hour and filtered through a Celite pad. The organic layer was separated, washed several times with water (3 × 10 mL), dried ($Na_2SO_4$), filtered, and evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) to give 0.50 g (66%) of **10** as a white amorphous solid. From the slowest moving fractions 0.054 g (18%) of starting material was recovered.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-oxaloamino-1″,2″-oxathiole-2″,2″-dioxide) (11).** To a solution of the oxalyl nucleoside **10** (0.1 g, 0.14 mmol) in 1,4-dioxane (2 mL), a solution of 1 N NaOH in dioxane (0.16 mL, 0.16 mmol) was added, and the mixture was stirred for 1 h at room temperature. Then the mixture was neutralized with 0.1 N HCl. The resulting mixture was dissolved in ethyl acetate and was successively washed with water (2 × 10 mL) and brine (2 × 10 mL). Finally, the organic phase was dried ($Na_2SO_4$), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 15:1) to give 0.10 g (72%) of **11** as a white amorphous solid. Anal. ($C_{27}H_{45}N_3O_{11}SSi_2$) C, H, N, S.

**General Procedure for the Synthesis of 4″-Oxamoyl TSAO-m$^3$T Derivatives (12−14).** A solution of oxalyl nucleoside **10** (0.1 g, 0.14 mmol) and the corresponding amine was stirred at room temperature until complete reaction of the starting material (45 min to 4 h). Then the reaction mixture

was neutralized with AcOH and evaporated to dryness to give the crude product. The product was redissolved in dichloromethane (20 mL), and the solution was washed with water (2 × 20 mL) and brine (1 × 20 mL), dried ($Na_2SO_4$), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron. The chromatography eluent and yield are indicated below for each reaction.

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-oxamoylamino-1″,2″-oxathiole-2″,2″-dioxide) (12).** According to the general procedure, nucleoside **10** (0.1 g, 0.14 mmol) was reacted with methanol saturated with ammonia (5 mL) at room temperature for 45 min. The residue was purified by CCTLC (dichloromethane/ethyl acetate, 10:1) to give 0.049 g (52%) of **12** as a white amorphous solid. $^1$H NMR [300 MHz, $(CD_3)_2CO$] δ: 0.78, 0.97 (2s, 18H, 2*t*-Bu), 1.97 (s, 3H, $CH_3$-5), 3.28 (s, 3H, $CH_3$-3), 4.10 (dd, 1H, $J_{4',5'a}$ = 2.2 Hz, $J_{5'a,5'b}$ = 12.9 Hz, H-5′a), 4.31 (dd, 1H, $J_{4',5'b}$ = 3.7 Hz, H-5′b), 4.43 (dd, 1H, H-4′), 4.72 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-2′), 6.25 (d, 1H, H-1′), 7.64 (bs, 1H, NH), 7.50 (s, 1H, H-6), 7.78 (s, 1H, H-3″), 8.15 (bs, 1H, NH), 10.65 (bs, 1H, NH). $^{13}$C NMR [75 MHz, $(CD_3)_2CO$] δ: 13.01 ($CH_3$-5), 18.30, 19.73 [$(CH_3)_3$-*C*−Si], 25.57, 26.89 [(*C*$H_3$)$_3$−C−Si, 28.64 ($CH_3$-3), 62.70 (C-5′), 74.98 (C-2′), 84.69, 86.95 (C-1′, C-4′), 94.49 (C-3′), 110.71, 111.59 (C-3″, C-5), 133.76 (C-6), 140.56 (C-4″), 152.19 (C-2), 160.58 (CO*C*ONH$_2$), 160.81 (*C*O-CONH$_2$), 163.31 (C-4). Anal. ($C_{27}H_{46}N_4O_{10}SSi_2$) C, H, N, S. Recovered starting material was 0.021 g (21%).

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methyloxamoylamino-1″,2″-oxathiole-2″,2″-dioxide) (13).** Via the general procedure, nucleoside **10** (0.1 g, 0.14 mmol) was treated with methylamine (8 M solution in ethanol, 10 mL) at room temperature for 4 h. After the workup, the residue was purified by CCTLC (hexane/ethyl acetate 3:1) to yield 0.048 g (50%) of compound **13** as a white amorphous solid. HPLC: $t_R$ = 12.26 min (65:35). MS (ESI$^+$): *m/z* 689.1 (M$^+$). Anal. ($C_{28}H_{48}N_4O_{10}SSi_2$) C, H, N, S. From the slowest moving fractions 0.016 g (19%) of TSAO-m$^3$T was isolated.

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-(1-azetidinyl)-oxalylamino-1″2″-oxathiole-2″,2″-dioxide] (14).** To a solution of compound **10** (0.1 g, 0.14 mmol) in MeOH (2 mL) azetidine (0.01 mL, 0.15 mmol) was added. The reaction mixture was stirred at 0 °C for 4 h. After the workup according to the general procedure, the residue was purified by CCTLC (hexane/ethyl acetate, 2.1) to give **14** (0.060 g, 60%) as a white foam. HPLC: $t_R$ = 15.91 min (65:35). MS (ESI$^+$): *m/z* 715.2 (M$^+$). Anal. ($C_{29}H_{50}N_4O_{10}SSi_2$) C, H, N, S. From the slowest moving fractions 0.014 g (17%) of TSAO-m$^3$T was isolated.

**General Acylation Procedure for the Synthesis of 4″-Substituted TSAO-m$^3$T Derivatives (15−18) by Reaction with Acid Chlorides.** To a solution of TSAO-m$^3$T (**2**) (1 mmol) in dry 1,2-dichloroethane (14 mL), the corresponding acid chloride (1.5−4 equiv) and DMAP (6−12 equiv) were added. The mixture was stirred in an Ace pressure tube for 0.5−24 h at 80 °C. The reaction mixture was allowed to cool to room temperature. Then dichloromethane was added and the solution was successively washed with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried ($Na_2SO_4$), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1).

The yields of the isolated products are indicated below for each reaction. In all cases, unreacted starting compound was isolated.

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-acetylamino-1″,2″-oxathiole-2″,2″-dioxide) (15).** According to the general procedure, TSAO-m$^3$T (**2**) (0.1 g, 0.17 mmol) was reacted with acetyl chloride (0.048 mL, 0.68 mmol) and DMAP (0.25 g, 2.04 mmol) for 24 h. The final residue was purified by CCTLC on the Chromatotron to give 0.046 g (42%) of **15** as a white amorphous solid. HPLC: $t_R$ = 10.53 min (65:35). $^1$H NMR [300 MHz, $(CD_3)_2CO$] δ: 0.80, 0.81 (2s, 18H, 2*t*-Bu), 1.92 (s, 3H, $CH_3$-5), 2.25 (s, 3H, *C*$H_3$−CO), 3.31 (s, 3H, $CH_3$-3), 3.95 (dd, 1H, $J_{4',5'a}$ = 6.3 Hz, $J_{5'a,5'b}$ = 12.2 Hz, H-5′a), 4.07 (dd, 1H, $J_{4',5'b}$ = 3.7 Hz, $J_{5'a,5'b}$ = 12.2 Hz, H-5′b), 4.21 (dd, 1H, H-4′), 5.01 (d, 1H, $J_{1',2'}$ = 6.7 Hz, H-2′), 5.60 (d, 1H, H-1′), 7.44 (s, 1H, H-3″), 7.63 (s, 1H, H-6), 9.95 (s, 1H, NH). MS (ESI$^+$): *m/z* 646.3 (M$^+$). Anal. ($C_{27}H_{47}N_3O_9SSi_2$) C, H, N, S. Recovered starting material was 0.028 g (28%).

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-benzoylamino-1″,2″-oxathiole-2″,2″-dioxide) (16).** According to the general procedure, TSAO-m$^3$T (0.1 g, 0.17 mmol) was reacted with benzoyl chloride (0.28 mL, 0.24 mmol) and DMAP (0.13 g, 1.02 mmol) for 2 h. The final residue was purified by CCTLC on the Chromatotron to give 0.053 g (44%) of **16** as a white amorphous solid. HPLC: $t_R$ = 27.24 min (65:35). MS (ESI$^+$): *m/z* 708.3 (M$^+$). Anal. ($C_{32}H_{49}N_3O_9SSi_2$) C, H, N, S. Recovered starting material was 0.035 g (35%)

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxycarbonylamino-1″,2″-oxathiole-2″,2″-dioxide) (17).** The general procedure was followed with TSAO-m$^3$T (0.1 g, 0.17 mmol), methyl chloroformate (0.037 mL, 0.48 mmol), and DMAP (0.124 g, 1.02 mmol) for 1 h. Purification of the final residue by CCTLC on the Chromatotron gave compound **17** (0.012 g, 11%) as a white amorphous solid. HPLC: $t_R$ = 13.93 min (65:35). Anal. ($C_{27}H_{47}N_3O_{10}SSi_2$) C, H, N, S. Recovered starting material: 0.07 g (70%).

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxymalonylamino-1″,2″-oxathiole-2″,2″-dioxide) (18).** Following the general procedure, TSAO-m$^3$T (0.1 g, 0.17 mmol) was treated with methoxymalonyl chloride (0.069 mL, 0.65 mmol) and DMAP (0.25 g, 2.04 mmol) for 24 h. Purification of the final residue by CCTLC on the Chromatotron gave compound **18** (0.060 g, 51%) as a white amorphous solid. HPLC: $t_R$ = 11.43 min (65:35). MS (ESI$^+$): *m/z* 704.3 (M$^+$). Anal. ($C_{29}H_{49}N_3O_{11}SSi_2$) C, H, N, S. Recovered starting material: 9 mg (9%)

**[1-[2′,5′-Bis-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-ureido-1″,2″-oxathiole-2″,2″-dioxide) (20).** To a cooled (−30 °C) solution of TSAO-m$^3$T (**2**) (0.2 g, 0.33 mmol) in dry dichloromethane (5 mL), previously degassed under an argon atmosphere, chlorosulfonyl isocyanate (0.19 g, 1.32 mmol) was added. The resulting mixture was stirred at −30 °C for 15 min. Then the reaction was quenched ($NaHCO_3$), and the organic layer was separated. The aqueous layer was extracted several times with ethyl acetate (3 × 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried ($Na_2SO_4$), filtered, and evaporated to dryness. The residue (compound **19**) was dissolved in dry acetonitrile (2 mL) and treated with TBDMSCl (0.098 g, 0.66 mmol) and DMAP (0.08 g, 0.66 mmol). The resulting reaction mixture was stirred at room temperature for 5 h, and the solvent was removed. The residue was redissolved in dichloromethane (20 mL) and was successively washed with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried ($Na_2SO_4$), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 3:1), yielding 0.085 g (40%) of compound **20** as a white foam. MS (ESI$^+$) *m/z* 669.3 (M + Na)$^+$. Anal. ($C_{26}H_{46}N_4O_9SSi_2$) C, H, N, S.

**General Acylation Procedure for the Synthesis of 4″-Ureido Substituted TSAO-m$^3$T Derivatives (21−24) by Reaction with Isocyanates.** To a solution of TSAO-m$^3$T (0.1 g, 0.17 mmol) in dry acetonitrile (2 mL) the appropiate isocyanate (4−20 equivalents) was added. The reaction mixture was stirred at 80 °C for 3−96 h. After evaporation of the solvent, the residue was purified by CCTLC on the Chromatotron. The chromatography eluent, 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]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-(3-ethyl-**

**ureido)-1″,2″-oxathiole-2″,2″-dioxide] (21).** The general procedure was followed with TSAO-m$^3$T (**2**) (0.1 g, 0.17 mmol) and ethyl isocyanate (0.26 mL, 3.30 mmol) for 96 h. Chromatography of the final residue with dicloromethane/methanol (200:1) afforded 0.058 g (50%) of **21** as a white foam. $^1$H NMR [300 MHz, $(CD_3)_2CO$] $\delta$: 0.83, 0.89 (2s, 18H, 2*t*-Bu), 1.15 (t, 3H, $J$ = 7.2 Hz, $CH_2CH_3$), 1.93 (d, 3H, $J$ = 1.2 Hz, $CH_3$-5), 3.25 (m, 2H, $J_{CH2,NH}$ = 5.6 Hz, $CH_2CH_3$), 3.32 (s, 3H, $CH_3$-3), 3.88 (dd, 1H, $J_{4',5'a}$ = 6.7 Hz, $J_{5'a,5'b}$ = 11.9 Hz, H-5′a), 4.08 (dd, 1H, $J_{4',5'b}$ = 4.5 Hz, H-5′b), 4.18 (dd, 1H, H-4′), 5.04 (d, 1H, $J_{1',2'}$ = 6.6 Hz, H-2′), 5.50 (d, 1H, H-1′), 6.18 (bt, 1H, NH), 7.12 (s, 1H, H-3″), 7.67 (d, 1H, H-6), 8.87 (bs, 1H, NH-4″). $^{13}$C NMR [75 MHz, $(CD_3)_2CO$] $\delta$: 12.91 ($CH_3$-5), 15.36 (NH−$CH_2$−*C*H$_3$), 18.39, 18.81 [$(CH_3)_3$−*C*−Si], 25.72, 26.18 [(*C*H$_3$)$_3$−C−Si, 28.03 ($CH_3$-3), 35.61 (NH−*C*H$_2$−CH$_3$), 61.60 (C-5′), 73.55 (C-2′), 84.38 (C-4′), 89.25 (C-3′), 96.45 (C-1′), 100.06 (C-3″), 111.63 (C-5), 138.89 (C-6), 145.41 (C-4″), 152.34 (C-2), 153.67 (NH*C*ONH), 163.17 (C-4). Anal. ($C_{28}H_{50}N_4O_9SSi_2$) C, H, N, S. Recovered starting material was 0.048 g (48%).

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (22).** TSAO-m$^3$T (**2**) (0.1 g, 0.17 mmol) was reacted with benzoyl isocyanate (0.12 mL, 1.02 mmol) for 26 h. Chromatography of the final residue on the Chromatotron (hexane/ethyl acetate 3:1) yielded 0.12 g (90%) of **22** as a white foam. Anal. ($C_{33}H_{50}N_4O_{10}SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-(3-ethoxycarbonylureido)-1″,2″-oxathiole-2″,2″-dioxide] (23).** According to the general procedure, TSAO-m$^3$T (**2**) (0.1 g, 0.17 mmol) was treated with ethoxycarbonyl isocyanate (0.041 mL, 0.51 mmol) for 3 h. The final residue was purified on the Chromatotron (dicloromethane/methanol, 200:1) to give 0.095 g (80%) of **23** as a white foam. Anal. ($C_{29}H_{50}N_4O_{11}SSi_2$) C, H, N, S. Recovered starting material was 0.005 g (5%).

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-(3-methacryloylureido)-1″,2″-oxathiole-2″,2″-dioxide] (24).** Following the general procedure, TSAO-m$^3$T (**2**) (0.1 g, 0.17 mmol) was reacted with methacryloyl isocyanate (0.12 g, 1.02 mmol) for 72 h. Purification of the final residue on the Chromatotron (dicloromethane/methanol, 60:1) afforded 0.09 g (75%) of **24** as a white foam. Anal. ($C_{30}H_{50}N_4O_{10}SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-[4″-(3-ethoxycarbonylmethylureido)-1″,2″-oxathiole-2″,2″-dioxide] (25).** TSAO-m$^3$T (**2**) (0.10 g, 0.17 mmol) was treated with ethoxycarbonylmethyl isocyanate (0.034 mL, 0.34 mmol) in the presence of $NEt_3$ (0.010 mL, 0.07 mmol. The mixture was stirred in an Ace pressure tube for 12 h at 100 °C. After the reaction mixture was allowed to cool to room temperature, the solvent was evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 4:1). The fastest moving fractions afforded 0.06 g (52%) of **25** as a white foam. $^1$H NMR [300 MHz, $(CD_3)_2CO$] $\delta$: 0.84, 0.88 (2s, 18H, 2*t*-Bu), 1.25 (t, 3H, $J$ = 7.1 Hz, $CH_2CH_3$), 1.93 (d, 3H, $J$ = 1.2 Hz, $CH_3$-5), 3.33 (s, 3H, $CH_3$-3), 3.92 (dd, 1H, $J_{4',5'a}$ = 7.0 Hz, $J_{5'a,5'b}$ = 11.9 Hz, H-5′a), 4.24−4.40 (m, 6H, $CH_2CO_2CH_2CH_3$, H-4′, H-5′b), 5.07 (d, 1H, $J_{1',2'}$ = 6.5 Hz, H-2′), 5.53 (d, 1H, H-1′), 6.53 (m, 1H, NH), 7.13 (s, 1H, H-3″), 7.69 (d, 1H, H-6), 9.30 (bs, 1H, NH-4″). $^{13}$C NMR [75 MHz, $(CD_3)_2CO$] $\delta$: 14.35, 14.56 (*C*H$_3$−CH$_2$, $CH_3$-5), 18.55, 18.79 [$(CH_3)_3$−*C*−Si], 25.84, 26.23 [(*C*H$_3$)$_3$−C−Si], 29.03 ($CH_3$-3), 42.55 ($CH_2CO_2CH_2CH_3$), 61.62 (C-5′), 73.50 (C-2′), 84.45 (C-4′), 89.21 (C-3′) 97.44 (C-1′), 100.67 (C-3″), 111.75 (C-5), 139.01 (C-6), 145.51 (C-4″), 152.52 (C-2), 153.95 (NH*C*ONH), 163.18 (C-4), 170.42 ($CO_2CH_2CH_3$). MS (ESI$^+$) $m/z$ 733.3 (M + 1)$^+$. Anal. ($C_{30}H_{52}N_4O_{11}SSi_2$) C, H, N, S.

The next moving fractions gave 0.042 g (30%) of **26** as a white foam. $^1$H NMR [400 MHz, $(CD_3)_2CO$] $\delta$: 0.79, 0.87 (2s, 18H, 2*t*-Bu), 1.27 (t, 3H, $J$ = 7.0 Hz, $CH_2CH_3$), 1.93 (d, 3H, $J$ = 1.1 Hz, $CH_3$-5), 3.26 (s, 3H, $CH_3$-3), 4.03 (dd, 1H, $J_{4',5'a}$ = 5.0 Hz, $J_{5'a,5'b}$ = 12.3 Hz, H-5′a), 4.10 (dd, 1H, $J_{4',5'b}$ = 5.7 Hz, H-5′b), 4.22 (m, 2H, $J$ = 1.6, $J$ = 7.0 Hz, $CH_2CH_3$), 4.44 (m, 3H, H-4′, $CH_2N$), 4.60 (m, 2H, $J$ = 17.9 Hz, $J$ = 15.6 Hz, $CH_2$), 4.84 (d, 1H, $J_{1'2'}$ = 7.8 Hz, H-2′), 6.02 (d, 1H, H-1′), 7.43 (s, 1H, H-3″), 7.55 (d, 1H, H-6), 10.25 (bs, 1H, NH-4″). $^{13}$C NMR [100 MHz, $(CD_3)_2CO$] $\delta$: 13.01 ($CH_3$-5), 14.39 ($CH_3CH_2O$), 18.34, 19.01 [$(CH_3)_3$−*C*−Si], 25.69, 26.22 [(*C*H$_3$)$_3$−C−Si], 28.05 ($CH_3$-3), 40.61 ($CH_2$−N), 48.70 ($CH_2$), 62.03 (C-5′), 62.67 (*C*H$_2$-OOC), 74.70 (C-2′), 85.01 (C-4′), 89.34 (C-1′), 91.37 (C-3′), 107.23 (C-3″), 111.49 (C-5), 135.09 (C-6), 142.38 (C-4″), 148.30 ($CH_2CON$), 151.96 (C-2), 156.64 (NCON), 163.34 (C-4), 167.09 (*C*OOCH$_2$CH$_3$), 167.50 (NHCON). MS (ESI$^-$) $m/z$ 814.5 (M − 1)$^-$. Anal. ($C_{33}H_{53}N_5O_{13}SSi_2$) C, H, N, S. The slowest moving fractions yielded 0.01 g (10%) of starting material.

**General Procedure for the Synthesis of 4″-Substituted TSAO-m$^3$T Derivatives 29a,b via Transamination Reaction.** TSAO deprotected nucleoside **3**[18] (1 equiv) and the corresponding amine hydrochloride (3 equiv) in methanol was refluxed for 2−10 days. The solvent was removed, and the residue was filtered through silica gel using ethyl acetate/methanol, 8:1, as eluent. The residue (compounds **27a**,**b**) was dissolved in dry acetonitrile (4 mL) and treated with TBDMSCl (0.15 g, 1 mmol) and DMAP (0.12 g, 1 mmol). The resulting reaction mixture was heated at 80 °C for 5 h, and the solvent was removed. The residue was redissolved in ethyl acetate (20 mL) and was successively washed with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried ($Na_2SO_4$), filtered, and evaporated to dryness. The residue (compounds **28a**,**b**) was dissolved in dry acetone (4 mL) and $K_2CO_3$ (0.028 g, 0.2 mmol), and methyl iodide (0.05 mL, 0.8 mmol) was added. The mixture was heated to 50 °C overnight. The solvent was removed, and the final residue was purified by CCTLC on the Chromatotron.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxycarbonylmethylamino-1″,2″-oxathiole-2″,2″-dioxide) (29a).** According to the general procedure, compound **3** (0.5 g, 1 mmol) was treated with H-Gly-OMe·HCl (0.414 g, 3 mmol) in methanol (20 mL) at reflux for 10 days. After silylation and N-3 methylation, the final residue was purified on the chromatotron with hexane/ethyl acetate (4:1), which afforded 0.146 g (20%) of **29a** as a white foam. HPLC: $t_R$ = 10.53 min (65:35). $^1$H NMR [300 MHz, $(CD_3)_2CO$] $\delta$: 0.80, 0.96 (2s, 18H, 2 *t*-Bu), 1.96 (s, 3H, $CH_3$-5), 3.27 (s, 3H, $CH_3$-3), 3.79 (s, 3H, O*C*H$_3$), 4.02−4.26 (m, 4H, H-5′, *C*H$_2$−N), 4.35 (dd, 1H, $J_{4',5'a}$ = 3.2 Hz, $J_{4',5'b}$ = 2.93 Hz, H-4′), 4.65 (d, 1H, $J_{1',2'}$ = 8.1, Hz, H-2′), 5.92 (s, 1H, H-3″), 6.10 (d, 1H, H-1′), 6.85 (bt, 1H, $J_{NH-CH2}$ = 5.7 Hz, *NH*−CH$_2$), 7.49 (s, 1H, H-6).$^{13}$C NMR [75 MHz, $(CD_3)_2CO$] $\delta$: 13.05 ($CH_3$-5), 18.38, 19.16 [$(CH_3)_2$−*C*−Si], 25.62, 26.43 [(*C*H$_3$)$_2$−C−Si], 28.09 ($CH_3$-3), 46.78 ($OCH_3$), 52.78 ($CH_2$), 62.97 (C-5′), 75.64 (C-2′), 85.09 (C-4′), 87.89 (C-3″), 91.98 (C-1′), 92.52 (C-3′), 111.32 (C-5), 134.23 (C-6), 151.94, 152.11 (C-4″, C-2), 163.34 (*C*OCH$_2$), 170.10 (C-4). MS (ESI$^+$) $m/z$ 676.3 (M$^+$). Anal. ($C_{28}H_{49}N_3O_{10}SSi$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxycarbonylethylamino-1″,2″-oxathiole-2″,2″-dioxide) (29b).** Compound **3** (0.50 g, 1 mmol) was reacted with H-β-Ala-OMe.HCl (4.2 g, 3 mmol) in methanol (20 mL) at reflux for 10 days. After silylation and N-3 methylation, the final residue was purified on the Chromatotron with hexane/ethyl acetate (4:1), which afforded 0.212 g (28%) of **29b** as a white foam. HPLC: $t_R$ = 10.21 min (65:35). MS (ESI$^+$): $m/z$ 690.1 (M$^+$). Anal. ($C_{29}H_{51}N_3O_{10}SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-benzyloxycarbonylmethylamino-1″,2″-oxathiole-2″,2″-dioxide) (30a).** A toluene solution (20 mL) of 1-hydroxy-3-chlorotetrabutyldistannoxane[22] (0.164 g, 0.16 mmol), benzyl alcohol (0.208 mL, 2.0 mmol), and **29a** (0.143 g, 0.20 mmol) was refluxed for 24 h. The toluene and excess benzyl alcohol were evaporated in vacuo. The residue was purified by flash column chromatography (hexane/ethyl acetate, 3:1) to give 0.125 g (82%) of **30a** as a white foam. MS (ESI$^+$): $m/z$ 752.2 (M$^+$). Anal. ($C_{34}H_{53}N_3O_{10}SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-benzyloxycarbonylethylamino-1″,2″-oxathiole-2″,2″-dioxide) (30b).** Compound **29b** (0.212 g, 0.30 mmol) was reacted with 1-hydroxy-3-chlorotetrabutyldistannoxane[22] (0.247 g, 0.45 mmol) and benzyl alcohol (0.310 mL, 3 mmol) in toluene (40 mL), as described for **30a**. Purification of the residue by flash column chromatography (hexane/ethyl acetate, 3:1) yielded 0.15 g (65%) of **30b** as a white foam. HPLC: $t_R = 24.20$ min (65:35). MS (ESI$^+$): *m/z* 766.3 ($M^+$). Anal. ($C_{35}H_{55}N_3O_{10}SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5"-(4″-carboxymethylamino-1″,2″-oxathiole-2″,2″-dioxide) (31a).** A solution of compound **30a** (0.100 g, 0.125 mmol) in ethyl acetate (15 mL) containing 0.036 g of 10 % Pd/C (40 wt %) was hydrogenated at 30 psi at 30 °C for 2 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure to give 0.068 g (84%) of **31a** as a white amorphous solid. MS (ESI$^+$): *m/z* 663.1 $(M + 1)^+$. Anal. ($C_{27}H_{47}N_3O_{10}SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-carboxyethylamino-1″,2″-oxathiole-2″,2″-dioxide) (31b).** Compound **30b** (0.095 g, 0.12 mmol) was hydrogenated in ethyl acetate (12 mL) in the presence of 0.038 g of 10% Pd/C (40 wt %) at 30 psi at 30 °C for 1 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure to give 0.070 g (83%) of **31b** as a white amorphous solid. HPLC: $t_R = 4.39$ min (65:35). MS (ESI$^+$): *m/z* 676.3 ($M^+$). Anal. ($C_{28}H_{49}N_3O_{10}SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-carbamoylmethylamino-1″,2″-oxathiole-2″,2″-dioxide) (32a).** To a solution of the acid derivative **31a** (0.06 g, 0.09 mmol) in dry dichloromethane (6 mL) at −20 °C, $NEt_3$ (0.013 mL, 0.09 mmol) and a 2 M solution of ammonia in methanol (0.112 mL, 0.225 mmol) were added. After 15 min at −20 °C, BOP (0.04 g, 0.09 mmol) was added and the reaction mixture was stirred at room temperature overnight and the solvent was removed. The residue was dissolved in ethyl acetate (20 mL) and was successively washed with a 10% citric acid solution (1 × 20 mL), 10% sodium bicarbonate (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried ($Na_2SO_4$), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1) to give compound **32a** (0.027 g, 44%) as a white amorphous solid. HPLC: $t_R = 4.06$ min (65:35). MS (ESI$^+$): *m/z* 661.3 ($M^+$). Anal. ($C_{27}H_{48}N_4O_9SSi_2$) C, H, N, S.

**[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-carbamoylethylamino-1″,2″-oxathiole-2″,2″-dioxide) (32b).** According to the procedure described for **32a**, the acid derivative **31b** (0.06 g, 0.09 mmol) was reacted with triethylamine (0.013 mL, 0.09 mmol), 2 M solution of ammonia in methanol (0.112 mL, 0.225 mmol), and BOP (0.04 g, 0.09 mmol) in dry dichloromethane (6 mL). Purification of the final residue by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1) yielded compound **32b** (0.051 g, 82%) as a white amorphous solid. HPLC: $t_R = 3.72$ min (65:35). MS (ESI$^+$): *m/z* 675.3 ($M^+$). Anal. ($C_{28}H_{50}N_4O_9SSi_2$) C, H, N, S.

**Biological Methods. a. Cells and Viruses.** Human immunodeficiency virus type 1 [HIV-1 (IIIB)] 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).

The laboratory HCMV strains Davis and AD-169 were used in the CPE (cytopathic effect) reduction assays. Virus stocks consisted of cell-free virus obtained from the supernatant of infected cell cultures that had been clarified by low-speed centrifugation. The virus stocks were stored at −80 °C.

**b. Activity Assay of Test Compounds against HIV-1 and HIV-2 in Cell Cultures.** A total number of $4 \times 10^5$ CEM or $3 \times 10^5$ 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 an amount of 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. Selection of Drug-Resistant Virus Strains and Identification of Mutations in HIV-1 Reverse Transcriptase.** Drug resistance was selected for compounds **11** and **12** against HIV-I (IIIB) in 1 mL cell cultures (48-well plates) under escalating drug regimens. The compound concentrations that were added at initiation of the drug selection were about 2−3 times their $EC_{50}$ value. Then, as soon as full cytopathicity appeared, the concentrations were gradually increased by 2- or 3-fold. The cultures were passaged every 3−4 days by adding ∼900 μL of fresh cell culture medium to 50−100 μL from the virus-infected cell cultures. The mutations that appeared in the RT genes of the drug-exposed virus strains were determined according to previously published procedures.[25]

**d. Activity Assay of Test Compounds against HCMV in Cell Culture.** Confluent HEL cells grown in 96-well microtiter plates were inoculated with HCMV at an input of 100 PFU (plaque forming units) per well. After a 1−2 h incubation period, residual virus was removed, and the infected cells were further incubated with MEM (supplemented with 2% inactivated FCS, 1% l-glutamine, and 0.3% sodium bicarbonate) containing varying concentrations of the compounds (in duplicate).

After 7 days of incubation at 37 °C in a 5% $CO_2$ atmosphere, the cells were fixed with ethanol and stained with 2.5% Giemsa solution. Virus plaque formation or viral cytophatic effect [virus input: 100 PFU] was monitored microscopically. The antiviral activity is expressed as $IC_{50}$, which represents the compound concentration required to reduce virus plaque formation or cytopathicity by 50%. $IC_{50}$ values were estimated from graphic plots of the number of plaques (percentage of control) or percentage of cytopathicity as a function of the concentration of the test compounds. A variety of test compound concentrations were used and differed 5-fold from each other. The $IC_{50}$ was calculated from the graphic plots using the compound concentrations that were just above and just below the $IC_{50}$ value. Data are the mean of two independent experiments.

Confluent HEL fibroblasts, grown in 96-well microtiter plates, were infected with HCMV Davis strain at 100 PFU/well. After a 2 h adsorption period, residual virus was removed and the infected cells were further incubated with medium. A concentration of the test compounds at 5 μg/mL was added at different time points after infection: 2 h (control) or 6, 14, 24, 30, 36, 48, or 72 h. The cultures were further incubated at 37 °C in a 5% $CO_2$ atmosphere and fixed at day 7. The percentage of CPE was then calculated for each point. The last time point for which an activity comparable to that of the control was recorded corresponds to the stage of the virus cycle at which the test compound interacts.

**e. Cytotoxicity Assays.** Cytotoxicity measurements were based on the inhibition of HEL cell growth. HEL fibroblasts were seeded at a rate of $5 \times 10^3$ cells/well in 96-well microtiter plates and allowed to proliferate for 24 h. Different concentrations of the test compounds were then added (in duplicate), and after 3 days of incubation at 37 °C in 5% $CO_2$ atmosphere, the cell number was determined with a Coulter counter. Cytotoxicity is expressed as $CC_{50}$, which represents the

compound concentration required to reduce cell growth by 50%. As a second parameter of cytotoxicity, the minimum cytotoxic concentration (MCC) to cause a microscopically detectable change in morphology of normal cells treated with the compounds was determined.

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**Supporting Information Available:** $^{1}H$ and $^{13}C$ NMR chemical shift assignments of compounds **11**, **13**, **14**, **16**−**18**, **20**, **22**−**24**, **29b**, and **30a**,**b**−**32a**,**b** and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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