First Synthesis and Evaluation of the Inhibitory Effects of Aza Analogues of TSAO on HIV-1 Replication

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Aza TSAO-T derivatives bearing a dihydroisothiazole dioxide ring instead of an oxathiole dioxide ring at the C-3′ position on the sugar moiety were prepared. We have synthesized four families of compounds depending on substitution at both N-3 and N-2″. Biological evaluation showed that these compounds are HIV-1(III_B)-specific and potent reverse transcriptase inhibitors with EC_{50} values between 0.13 and 3.5 μM in cell culture.

Introduction

Among the various viral human diseases, the acquired immune deficiency syndrome (AIDS) has been one of the major targets for the World Health Organization and the scientific community for the next decades. Since the identification of the human immunodeficiency virus (HIV) in 1983−84 as the etiological agent for AIDS, many therapeutic strategies have been proposed involving the viral enzymes that have critical roles in the life cycle of the virus as key targets in the search for effective drugs. Accordingly, different types of HIV inhibitors have been developed and studied with the aim of limiting the introduction of the virus into the host-cell (virus adsorption, virus−cell fusion, and virus uncoating) and inhibition of enzymes such as reverse transcriptase (RT) which is involved in the proviral DNA synthesis from genomic viral RNA, HIV protease, and HIV integrase (IN) which catalyzes the integration of viral DNA into the host DNA. The finding that dideoxynucleosides, such as ddC (2′,3′-dideoxycytidine), ddI (2′,3′-dideoxyinosine), and AZT (3′-azido-3′-deoxythymidine) are potentially effective therapeutic agents for the treatment of the AIDS has triggered explosive developments in the chemistry of the RT inhibitors. This heterodimeric enzyme consisting of p66 (560 amino acids) and p51 (440 amino acids) subunits has three enzymatic activities, a DNA-dependent and a RNA-dependent DNA polymerase activity and RNase-H activity, which play a direct role in the conversion of a single-stranded RNA genome into a double-stranded DNA. Because of the essential role of RT in viral replication, the polymerase activity had been a major target for anti-HIV drugs. Anti-RT drugs have been categorized as either nucleoside analogue RT inhibitors (NRTIs), nucleotide RT inhibitors (NtRTIs), or non-nucleoside RT inhibitors (NNRTIs). NRTIs and NtRTIs are analogues of natural nucleosides involved in viral DNA synthesis. They also act as DNA chain terminators when they are incorporated into the viral DNA because they lack a 3′-OH group. NNRTIs represent a particular group of compounds which bind to a hydrophobic pocket near, but not at, the polymerase active site. These induce restrictions in the dynamics of the enzyme, resulting in its being in an inactive conformation. Despite substantial advances in anti-HIV chemotherapy, notably with new treatment combinations, numerous mutations have been observed for therapies using NRTIs and NNRTIs. For these reasons, the search for new drugs is a priority in medicinal chemistry. [2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)thymine (TSAO-T) was first described in 1992 by Camarasa et al.[1] and Pérez-Pérez et al.[1] as a specific anti-HIV agent (Figure 1). This compound was the prototype of a new and unique class of nucleoside analogues with a specific inhibition against HIV-1, acting through a noncompetitive mechanism against substrate and template/primer (for an overview, see ref 1c). In contrast, no inhibitory effect was observed on HIV-2 strains, simian immunodeficiency virus (SIV), Moloney murine sarcoma virus, and a broad range of DNA and RNA viruses at subtoxic concentrations. With the aim of establishing structure−activity relationship studies (SAR), Camarasa et al. synthesized various congeners of TSAO compounds based on a variety of modifications to the silyl groups, nucleic acid base, and the sultone moiety. In addition, various heterodimers were synthesized where TSAO compounds were linked to NRTIs through various types of spacer groups, in an attempt to combine the inhibitory properties of both types of compounds.[2] The resulting SAR studies, by Camarasa and co-workers demonstrated that the sugar part played a crucial role in the interaction of the TSAO series of compounds with HIV-1 RT. Of particular importance was the presence of the SO_2 and 4″-NH_2 groups on the 3′-spiro sultone. Moreover, the oxathiole ring was shown to be a requirement on the sugar moiety with a *ribo* configuration and the *tert*-butyldimethylsilyl (TBDMS) group at both *O*-2′ and *O*-5′ positions (Figure 2). Recent molecular modeling studies have indicated that the methyl group of the N-3 methylated TSAO-T (TSAO-

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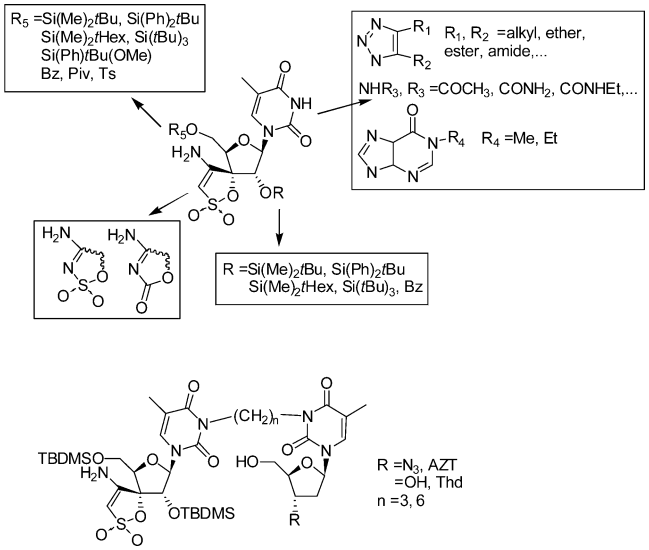


Figure 1. TSAO derivatives.

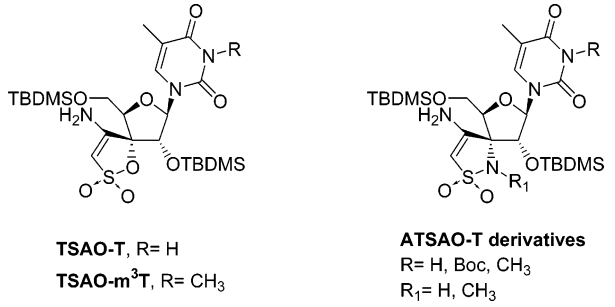
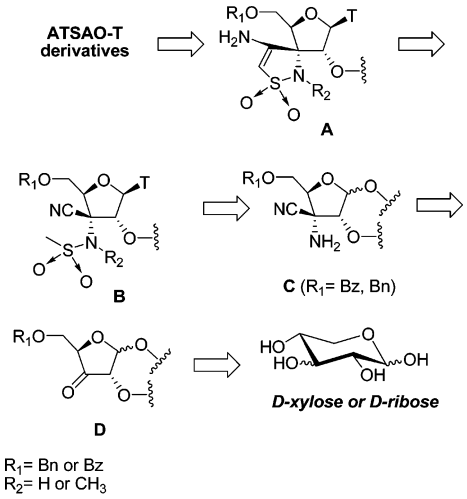


Figure 2. Aza analogues of TSAO.

m^{3}T) is relatively hydrophobic which induces a conformation with severely reduced flexibility which is preshaped to the geometry of its putative binding site, namely, the interface between the p66 and p51 subunits of RT.[3] In comparison with the other NRTIs and NNRTIs, resistance of HIV-1 RT to TSAO type compounds has also been observed, mainly due to the 138-Glu→Lys mutation. The phenomenon of resistance and eagerness for complete elucidation of the precise binding mode of drug to RT has prompted the exploration of further structural modifications of TSAO analogues. Among more than 600 analogues described by Camarasa and co-workers, the 3′-spiro ring moiety of such compounds has been most studied and modified by introduction of various substituents at C-3″ (nitro, halogen, alkenyl, alkynyl, allyl, and aromatic groups) and N-4″ (carbonyl, carboxylic acid, and ester). Analogues modified at the 3′-spiro moiety including the *xylo* and *ribo* form of 3′-spiro 4-amino-2-oxazolone and 4-amino-1,2,3-oxathiole-2,2-dioxide have also been synthesized. Surprisingly, no derivatives have been investigated in which the O-1″ has been substituted by a nitrogen to give the corresponding [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3′-spiro-3″-(4″-amino-1″,2″-dihydroisothiazole-1″,1″-dioxide)thymine analogues (Figure 2).

Our interest in carbohydrate chemistry, especially that pertaining to glyco-α-aminonitriles[4] and their derivatives,[5] encouraged us to extend our results obtained as part of our investigations into the carbanion-mediated sulfonamide intramolecular cyclization (CSIC reaction)[6] for the synthesis of the new family of aza-

Chart 1. Key Intermediates for the Access to ATSAOs



TSAO compounds, abbreviated as ATSAOs. Herein we report, in full, our studies on the synthetic routes for an easy access to ATSAOs with free, partially, or fully substituted N-3 and N-2″ starting from D-xylose.

Results and Discussion

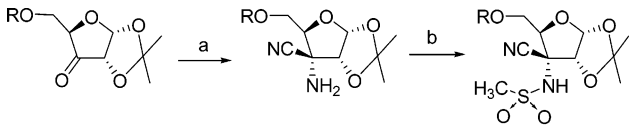
Retrosynthetic analysis demonstrates that glyco-α-aminonitrile formation, N-glycosylation, and carbanion-mediated sulfonamide intramolecular cyclization are key steps in the synthesis of ATSAO nucleosides (Chart 1).

Several glycoaminocyanation routes have been published. Most of them consist of catalytic (asymmetric) Strecker-type reactions using a Lewis acid as catalyst and various cyanogen reagents.[7a,b] Enantioselective approaches to the reaction generally use a preformed imine whereby the nitrogen atom bears a chiral inducer or chiral metallic complex as catalyst. However, few examples have been reported for the synthesis of α-aminonitriles of sugars and rarely for uloses involving a nonanomeric carbon atom of a sugar ring at C-α.

At the outset of our project we selected derivatives **C** (Chart 1), where the choice of 5-*O*-benzyl and 5-*O*-benzoyl protecting groups was made on the basis that such groups are well-known to be readily cleaved under mild conditions to facilitate introduction of the final TBDMS group on the O-5′. Such intermediates can be derived from the substrates **D**, which are readily available from either D-ribose or D-xylose (Chart 1).

Classical Strecker conditions applied to the protected *erythro*-pentofuranos-3-ulose derivative **1**[8a,b] and **1a**,[9a,b] respectively, which were each obtained from D-xylose using either PDC or a modified Swern oxidation, afforded the corresponding cyanohydrin. Several reaction conditions were examined, but the best results were obtained using NH_3−MeOH and $Ti(OiPr)_4$ as the Lewis acid. The 3-R glyco-α-aminonitriles **2** and **2a** were obtained stereoselectively in 70% and 82% yield, respectively. It should be noted that these conditions represent a convenient and versatile method for the formation of a large variety of N-substituted glyco-α-aminonitriles from either alkyl- or arylamines. Moreover, $Ti(OiPr)_4$ is a mild catalyst compatible with a large variety of acid-sensitive functional groups such as lactams, *tert*-butyldimethylsilyl ethers, and acetonides.

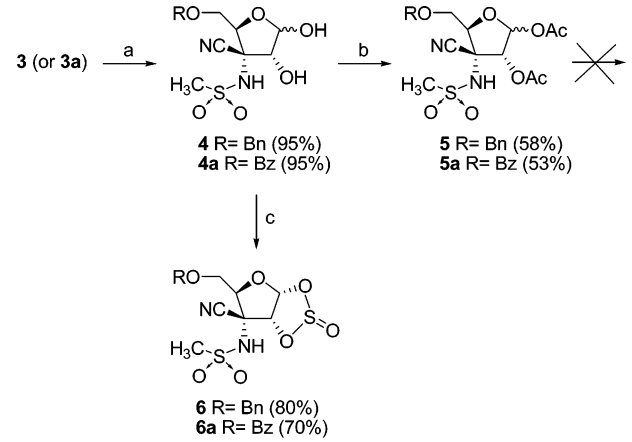
Scheme 1[a]



1 R= Bn (%) **1a** R= Bz (%) **2** R= Bn (70%) **2a** R= Bz (82%) **3** R= Bn (95 %) **3a** R= Bz (95 %)

[a] Reagents and conditions: (a) i. $Ti(OiPr)_4$ (1.2 equiv), NH_3, MeOH; ii. TMSCN (1.1 equiv); (b) CH_3SO_2Cl (3 equiv), C_5H_5N, DMAP (0.5 equiv).

Scheme 2[a]



4 R= Bn (95%) **4a** R= Bz (95%) **5** R= Bn (58%) **5a** R= Bz (53%) **6** R= Bn (80%) **6a** R= Bz (70%)

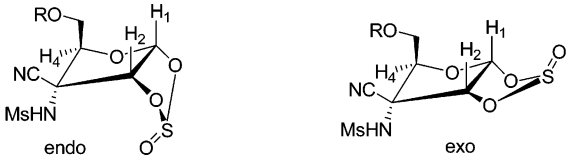
[a] Reagents and conditions: (a) TFA;H_2O (9:1); (b) Ac_2O, C_5H_5N; (c) $SOIm_2$, THF.

The compounds **2** and **2a** failed to react readily with RSO_2Cl−pyridine alone; however, upon addition of DMAP the key methanesulfonamidonitriles **3** and **3a** were each obtained in good yields (Scheme 1).

To achieve the N-glycosylation step for the introduction of the nucleic base, compounds **3** and **3a** were each deprotected in the anomeric position under classical acidic conditions (TFA:H_2O; 9:1) to give **4** and **4a**, which were converted into the corresponding diacetylated derivatives **5** and **5a** in 58% and 53%, respectively (Scheme 2). According to the TSAO synthesis reported by Camarasa, several glycosylations were attempted using the Vorbrüggen method to introduce the thymine moiety. However, it was demonstrated that such a methodology was ineffective for free sulfonamido derivatives **5** and **5a**. As a alternative approach, we chose to investigate the fusion procedure[10] based on the cyclic sulfite formation between C-1 and C-2 to effect substitution at the anomeric position. Thus, treatment of **4** and **4a** with $SO(Im)_2$ in THF (from thionyl chloride and imidazole) gave the corresponding sulfite derivatives **6** and **6a** (80 and 90%) as an endo and exo mixtures (endo/exo: 1/1 to 3/2), which were characterized by ^1H and ^{13}C NMR spectroscopy (Chart 2).

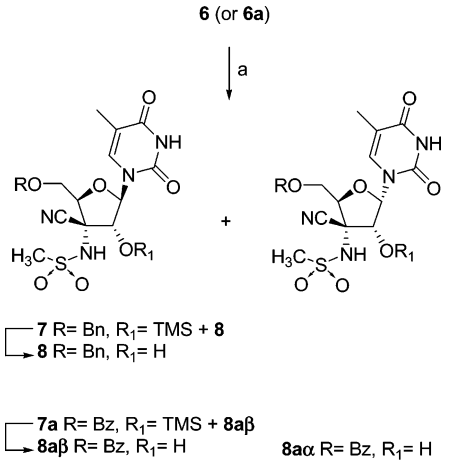
N-Glycosylation of **6** and **6a** was achieved at 125 °C using silylated thymine in dry conditions (Scheme 3) to give a mixture of 2′-O-silylated (**7, 7a**) and 2′-hydroxy-5-methyluridine derivatives (**8, 8a*β***) which, in turn, gave after desilylation (TBAF; MeOH) exclusively **8** (82%) and **8a*β*** (54.5%), respectively. In addition, the α N-glycosylated epimer **8aα** was obtained as the minor compound (13.5%) starting from the 5-O-benzoylated compound **6a**. The formation of α-substituted compounds via cyclic sulfites have been described in the literature[10] and their formation was found to be dependent upon the reaction temperature. In our case, several and various experimental conditions seemed to correlate this formation with the endo/exo ratio resulting in the cyclic sulfite synthesis. **8aα** could also be explained by anchimeric assistance of the benzoyl group. To control the stereospecificity of the N-glycosylation, our next investigations will only concern the 5-*O*-benzyl derivatives.

Chart 2. NMR Shift of Sulfite Derivatives (δ)



R	C-1 *endo*	C-1 *exo*	H-1 *endo*	H-1 *exo*	$J_{1,2}$ *endo* (Hz)	$J_{1,2}$ *exo* (Hz)
Bn	110.5	108.2	6.49	6.51	4.7	3.8
Bz	110.6	108.0	6.61	6.72	4.8	3.9

Scheme 3[a]



7 R= Bn, R_1= TMS + **8** R= Bn, R_1= H

7a R= Bz, R_1= TMS + **8aβ** R= Bz, R_1= H **8aα** R= Bz, R_1= H

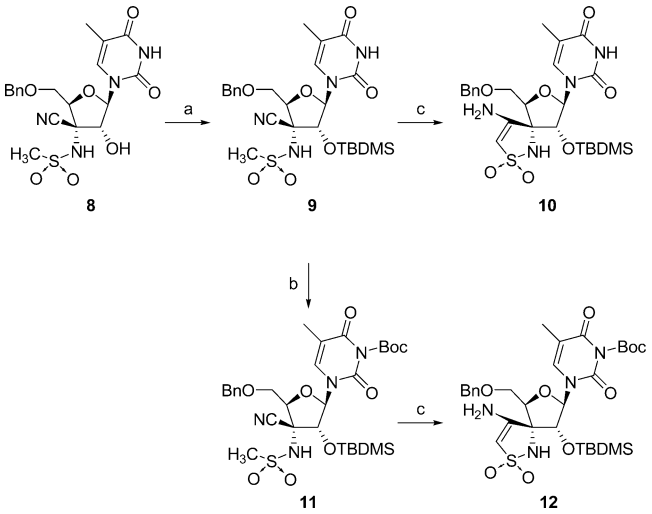
[a] Reagents and conditions: (a) i: Thymine, HMDS, $(NH_4)_2SO_4$, 135 °C; ii: MeOH, H_2O, reflux.

The *tert*-butyldimethylsilyl group was introduced in the 2′- position using TBDMSCl and imidazole in DMF to afford **9** in 89% (Scheme 4). Unfortunately, the CSIC reaction[6] of compound **9**, using LDA base, gave the dihydroisothiazolic derivative **10** in only 17% yield (Scheme 4). The poor yields obtained could be due to lithiation of the nucleic base. Therefore, to avoid such a side reaction, the N-3 of **9** was protected and isolated as the N-Boc derivative **11** in 88% yield (Scheme 4). Next, the dihydroisothiazolic derivative **12** was obtained efficiently using LDA in THF in 67% yield.

Several routes were followed to deprotect both O-5′ and N-3′ positions (Scheme 5).

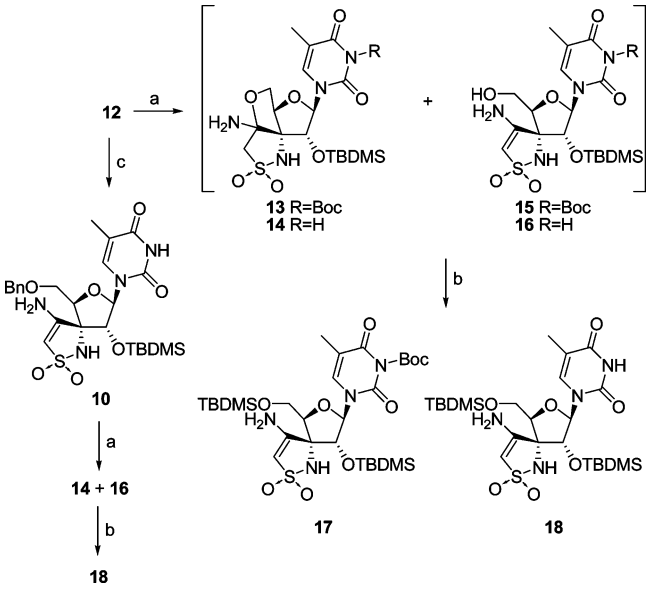
First, **12** was treated with $Pd(OH)_2$−cyclohexene in refluxing EtOH to give the debenzylated compounds **15** and **16**, the latter being the result of a decarbamoylation at N-3. Moreover, we also observed the polycyclic compounds **13** and **14** which resulted from a Michael intramolecular cyclization between the O-5′ and the corresponding enamine. Finally, introduction of the 5′-*O*-TBDMS group was achieved by treatment of the mixture of compounds **13**−**16** with TBDMSCl in DMF and imidazole as base to afford a mixture of 2′,5′-bis-

Scheme 4[a]



[a] Reagents and conditions: (a) TBDMSCl, imidazole, DMF; (b) Boc_2O, CH_2Cl_2, C_5H_5N; (c) LDA, THF, −78 °C.

Scheme 5[a]

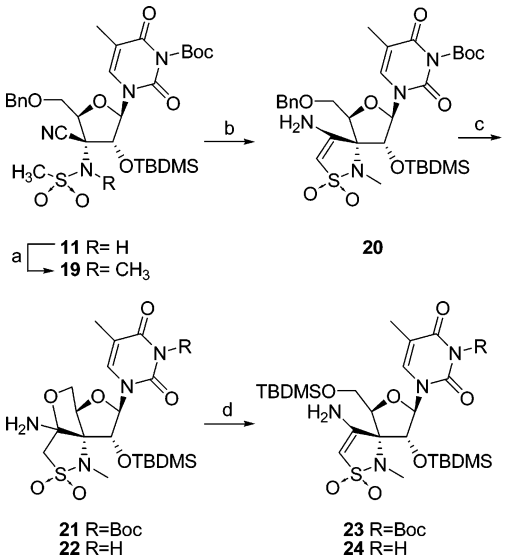


[a] Reagents and conditions: (a) $Pd(OH)_2$, cyclohexene, EtOH, Reflux; (b) TBDMSCl, imidazole, DMF, rt; (c) TFA, CH_2Cl_2, rt.

O-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3′-spiro-3″-(4″-amino-2″,3″-dihydroisothiazole-1″,1″-dioxide)-thymine (**18**), which is the first aza analogue of TSAO-T, and the corresponding *N*-3-Boc derivative **17**, which were separated by silica gel column chromatography and obtained in 35.5% and 11.5% yield, respectively. Compound **18** was also be obtained selectively from **12** in three steps involving acidic hydrolysis with TFA in CH_2Cl_2, to give **10** (80%), followed by hydrogenolysis to give a mixture of compounds **14** and **16** (80%) and finally silylation to give the product **18** in 74% yield.

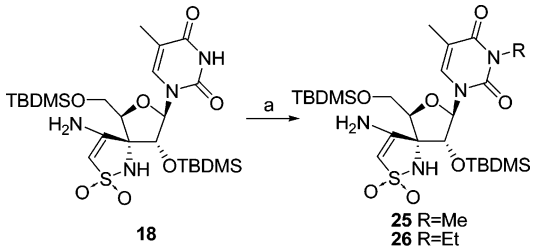
Access to the Deprotected N-3- and N-2″-Methylated ATSAO-T Derivatives. Compound **11** was the most appropriate choice of intermediate to proceed to the N-2″-methylated ATSAO derivative (**19**), which was achieved using MeI and K_2CO_3 in acetone with 95% yield. The cyclization step was performed using Cs_2CO_3 in accordance with our earlier work on N-alkylated glyco derivatives.[11] Among the several attempts made, the best resulted in **20**, being obtained in 55% yield using sonochemistry in MeCN as solvent. As previously reported, hydrogenolysis ($Pd(OH)_2$−cyclohexene; refluxing EtOH) gave a mixture of protected (**21**) and unprotected (**22**) N-3 hemiaminal derivatives in 40% and 26% yield, respectively. Finally, **23** and **24** were each obtained by treatment with TBDMSCl and imidazole in DMF from the isolated compounds **21** and **22**, in 68% and 71%, respectively (Scheme 6).

Scheme 6[a]



[a] Reagents and conditions: (a) CH_3I, K_2CO_3, acetone; (b) Cs_2CO_3, acetonitrile, 80 °C; (c). $Pd(OH)_2$, cyclohexene, EtOH, reflux; (d) TBDMSCl, imidazole, DMF, rt.

Scheme 7[a]

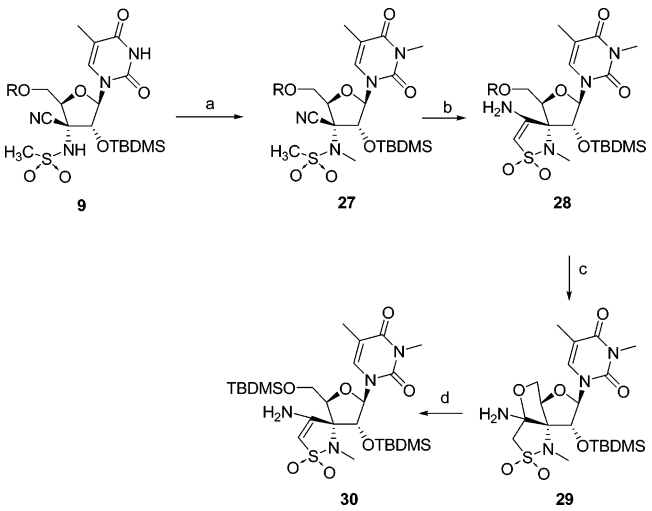


[a] Reagents and conditions: (a) CH_3I or C_2H_5I, K_2CO_3, acetone.

Access to the N-3 Methylated and Free N-2″-ATSAO-T Derivatives. Synthesis of the aza TSAO-m^3T analogues was investigated by a shorter route which consisted of treating ATSAO-T derivative **18** with the classical methylation reagents, MeI and K_2CO_3 in acetone. Several attempts were made using various amounts of MeI (1 to 3 equiv) but in each case a regioselective introduction of the Me group occurred at the N-3 position. This regiospecificity might be explained by the steric effect of the TBDMS group at O-2′ and the restricted conformation of the spiro ring leading to an unfavorable orientation of the N-2″ for effective alkylation. Thus an efficient synthesis of N-3-alkylated ATSAO derivatives was effected yielding the compounds **25** (87%) and **26** (81%), which are the aza analogues of TSAO-m^3T and TSAO-et^3T, respectively (Scheme 7).

Access to the N-3- and N-2″-Methylated ATSAO-T Derivatives. The lack of reactivity observed at N-2″ of **18** toward methylating reagents encouraged us to investigate the synthesis of derivatives ATSAO

Scheme 8[a]



[a] Reagents and conditions: (a) MeI, K_2CO_3, acetonitrile; (b) Cs_2CO_3, acetonitrile; (c) $Pd(OH)_2$, cyclohexene, EtOH, reflux; (d) TBDMSCl, imidazole, DMF, rt.

having both N-3 and N-2″ methylated starting from the acyclic methanesulfonamido derivative **9**.

Compound **9** was permethylated using MeI and K_2CO_3 in acetonitrile to give **27** in 87% yield. The deprotection step was achieved using hydrogenolysis to give the corresponding hemiaminal **29** in 50% yield. Finally, the silylation step, performed as described earlier, afforded the compound **30** in 74% (Scheme 8).

Biological Activity. Several aza derivatives of TSAO-m^{3}T (**30**), TSAO-T (**18** and **24**), and two ATSAO−Boc^{3}T derivatives (**17** and **23**) were evaluated for their inhibitory activity against HIV-1(III_B) and HIV-2(ROD) in human T-Lymphocyte MT-4 and CEM cell cultures (Table 1). Those ATSAO derivatives that did not contain a substituent (methyl) on the nitrogen atom in the spiro moiety were most inhibitory to HIV-1 in CEM and MT-4 cell cultures (EC_{50}: 0.13−0.53 μM). They were not inhibitory against HIV-2 at subtoxic concentrations. The methyl-substituted ATSAO derivatives (**23**, **24**, **30**) were ∼10-fold less inhibitory against HIV-1 than the unsubstituted ATSAO derivatives and also lacked any inhibitory activity against HIV-2. The ATSAO-T derivatives and also the ATSAO−Boc^{3}T derivatives were cytostatic at a CC_{50} that ranged between 19 and 105 μM. The ATSAO-m^{3}T and ATSAO-e^{3}T derivatives **25** and **26** were more cytotoxic (CC_{50}: 4.9−5.8 μM). Compound **30** (the only ATSAO-m^{3}T derivative of the series having a methyl group at both N-3 and N-2″) lacked significant cytostatic activity at 200−250 μM. The ATSAO derivatives showed the same HIV-1-specific activity spectrum as the TSAO derivatives published before.[1a,b] TSAO derivatives were found to be markedly less inhibitory against HIV-1 strains that contain NNRTI-specific mutations in the RT such as Lys103 → Asn, Glu138 → Lys, and Tyr181 → Cys.[13,14] None of the ATSAO derivatives were inhibitory against these mutant virus strains in CEM cell culture (Table 1), pointing to a similar mechanism of antiviral action as that of the prototype TSAO-T and TSAO-m^{3}T derivatives.

In conclusion, we have synthezised a new family of aza analogues of TSAO with diverse substitution on both N-3 and N-2″ positions. Some of them demonstrated HIV-1 specific reverse transcriptase inhibitory activity. Moreover, ATSAO-Boc^{3}T with the unsubstituted isothiazolic ring proved to be only 2- to 7-fold less active against HIV-1 replication in MT-4 and CEM cells than TSAO-T. Computational modeling studies are now in progress and will be reported in due course.

Experimental Section

Materials and Methods. Melting points were determined on a digital melting-point apparatus (Electrothermal) and are uncorrected. Optical rotations were recorded in $CHCl_3$, MeOH, acetone, or DMSO with a digital polarimeter DIP-370 (JASCO) using a 1 dm cell. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, acetone-d_6, Me_2SO-d_6, or MeOD-d_3 (internal Me_4-Si), respectively, at 300.13 MHz and at 75.47 MHz (Bruker Avance-300). TLC was performed on Silica F254 (Merck) and detection by UV light at 254 nm or by charring with phosphomolybdic acid−H_2SO_4 reagent. Column chromatography was effected on Silica Gel 60 (Merck, 230 mesh). Acetone, hexane, ethyl acetate, and diethyl ether were distilled before use. Bases and solvents were used as supplied. MeOH−NH_3 is methanol saturated (7 N) with ammonia gas at room temperature. Elemental analyses have been carried out in Madrid (IQOG, CSIC). ^{13}C NMR resonances have been assigned by using standard NMR (DEPT, COSY, HSQC) experiments. FTIR spectra were obtained on a AVATAR 320 neat using ATR and are reported in cm^{-1}. Mass spectral data were acquiered on a WATERS Micromass ZQ spectrometer or a WATERS Micromass Q-TOFF spectrometer.

General Method for Silylations (A). To a solution of nucleoside and imidazole (3 equiv) in DMF was added TBDMSCl (2.5 equiv). The reaction mixture was stirred at room-temperature overnight then evaporated to dryness. The residue was purified by flash chromatography.

General Method for Debenzylations (B). To a solution of spiranic derivative in absolute ethanol was added $Pd(OH)_2$/C (0.3 equiv) and cyclohexene (34 equiv). The reaction mixture was refluxed then filtered and evaporated to dryness. The residue was purified by flash chromatography or used in the next step without further purification.

General Method for the N-Alkylation of Sulfonamides (C). To a solution of sulfonamide and K_2CO_3 (1.5 equiv) in acetone was added MeI or EtI (2 equiv). The mixture was refluxed until complete reaction then filtered through a silica pad and evaporated to dryness. The residue was purified by flash chromatography.

General Method for the CSIC Reaction Using Cs_2CO_3 (D). Cs_2CO_3 (1 equiv) was added to a solution of the sulfonamidonitrile in CH_3CN. The mixture was refluxed until complete reaction and then was filtered through Celite and evaporated to dryness. The residue was purified by flash chromatography.

3-Amino-5-*O*-benzyl-3-*C*-cyano-3-deoxy-*N*-mesyl-D-ribofuranose (4). A 10 mL mixture of TFA and water (9/1; v/v) was added to the compound **3** (1.0 g, 2.92 mmol). The reaction mixture was stirred at room temperature for 2 h. Then, the solvent was evaporated to dryness and the residue was purified by flash chromatography (EtOAc/petroleum ether, 70:30) to yield compound **4** (0.85 g, 95%) as a mixture of the two anomeric epimers: IR (ATR) ν 3255, 1425, 1379, 1316, 1138, 929, 777, 750, 701 cm^{-1}; MS (ES): 365.4 [M + Na]$^+$. Anal. ($C_{14}H_{18}N_2O_6S$) C, H, N, S.

3-Amino-5-*O*-benzoyl-3-*C*-cyano-3-deoxy-*N*-mesyl-D-ribofuranose (4a). a 10 mL mixture of TFA and water (9/1; v/v) was added to the compound **3a** (4.048 g, 10.2 mmol). The reaction mixture was stirred at room temperature for 2 h. Then, the solvent was evaporated to dryness and the residue was purified by flash chromatography (EtOAc/petroleum ether, 70:30) to yield compound **4a** (3.607 g, 99%) as a mixture of the two anomeric epimers: IR (ATR) ν 1706, 1451, 1394, 1339, 1276, 1133, 1065, 983, 713, 666 cm^{-1}; MS (ES): 379.5 [M + Na]$^+$. Anal. ($C_{14}H_{16}N_2O_7S$) C, H, N, S.

Table 1. Inhibitory Activity of Test Compounds against HIV-1 and HIV-2 in CEM and MT-4 Cell Cultures

			EC_{50}[a] (μM)							CC_{50}[b] (μM)	
	group in		CEM					MT-4			
compound	N-2″	N-3	HIV-1 (IIIB)	Lys103→ Asn	Glu138→ Lys	Tyr181→ Cys	HIV-2 (ROD)	HIV-1 (III_B)	HIV-2 (ROD)	CEM	MT-4
30	CH_3	CH_3	2.5 ± 0.7	>250	>250	>250	>250	0.66 ± 0.1	>200	≥250	>200
23	CH_3	Boc	3.5 ± 0.7	>10	>10	>10	>10	1.8 ± 0.71	>20	20.6 ± 1.9	20 ± 1.3
18	H	H	0.33 ± 0.11	5.5 ± 0.7	>10	≥10	≥10	0.53 ± 0.04	>100	19.1 ± 0.4	105 ± 6.9
24	CH_3	H	3.5 ± 0.7	≥10	>10	>10	>10	1.6 ± 0.40	>20	21.6 ± 0.14	19 ± 0.71
25	H	CH_3	0.22 ± 0.0	4.0 ± 2.8	>10	≥10				5.8 ± 0.1	
26	H	C_2H_5	0.31 ± 0.13	>2	>2	>2				4.9 ± 0.1	
17	H	Boc	0.43 ± 0.25	5.0 ± 0.0	>10	≥10	>10	0.13 ± 0.03		21.2 ± 1.3	19 ± 0.73
TSAO-T (**1**)[c]	–	H	0.06 ± 0.01	0.4	4.6 ± 2.6	3.0 ± 0.0	>4	0.06 ± 0.03	>20	16 ± 2.0	14 ± 2.0

[a] 50% Effective concentration. [b] 50% Cytostatic concentration. [c] Data taken from refs 12, 13.

1,2-Di-*O*-acetyl-3-amino-5-*O*-benzyl-3-*C*-cyano-3-deoxy-3-*N*-mesyl-D-ribofuranose (5). A 125 mL mixture of pyridine and Ac_2O (4:1; v/v) was added to the compound **4** (0.95 g, 2.4 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature. Then, 50 mL of MeOH was added and stirred for an additional 1 h. The solvent was evaporated to dryness, and the residue was purified by flash chromatography (EtOAc/petroleum ether, 35:65) to give successively **5*β*** (1.03 g, 18%) as a pale yellow solid and **5α** (2.22 g, 40%) as a white solid. **5α**: mp 40−41 °C; $[\alpha]^{25}_D$ +67.88 (*c* 0.99, $CHCl_3$); IR (ATR) *ν* 1759, 1375, 1329, 1208, 1045, 977, 933, 891, 738, 700 cm^{-1}; MS (ES): 449.6 $[M + Na]^+$, 465.6 $[M + K]^+$. Anal. ($C_{18}H_{22}N_2O_8S$) calcd, C, 50.70; H, 5.20; N, 6.57; S,7.52 found, C, 49.13; H, 5.16; N, 6.41; S, 7.42. **5*β***: mp 114−116 °C; $[\alpha]^{25}_D$ −11.96 (*c* 1.18, $CHCl_3$); IR (ATR) *ν* 1768, 1730, 1341, 1248, 1203, 1155, 1099, 964, 891, 749, 703 cm^{-1}; MS (ES): 449.6 $[M + Na]^+$, 465.6 $[M + K]^+$. Anal. ($C_{18}H_{22}N_2O_8S$) C, H, N, S.

1,2-Di-*O*-acetyl-3-amino-5-*O*-benzoyl-3-*C*-cyano-3-deoxy-3-*N*-mesyl-D-ribofuranose (5a). A 27 mL mixture of pyridine and Ac_2O (3:1; v/v) was added to the compound **4a** (0.95 g, 2.4 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature. Then, 20 mL of MeOH was added and stirred for an additional 1 h. The solvent was evaporated to dryness, and the residue was purified by flash chromatography (EtOAc/petroleum ether, 40:60) to give successively **5a*β*** (0.11 g, 10%) and **5aα** (0.47 g, 43%) as white solids. **5a*β***: mp 60−62 °C; $[\alpha]^{25}_D$ +19.2° (*c* 1.25, $CHCl_3$); IR (ATR) *ν* 1760, 1728, 1372, 1336, 1207, 1098, 972, 889, 713 cm^{-1}; MS (ES): 463.5 $[M + Na]^+$, 479.5 $[M + K]^+$. Anal. ($C_{18}H_{20}N_2O_9S$) C, H, N, S. **5aα**: mp 68−70 °C; $[\alpha]^{25}_D$ +70.06 (*c* 4.66, $CHCl_3$); IR (ATR) *ν* 1764, 1725, 1333, 1272, 1228, 1108, 972, 893, 712 cm^{-1}; MS (ES): 463.5 $[M + Na]^+$, 479.5 $[M + K]^+$. Anal. ($C_{18}H_{20}N_2O_9S$) C, H, N, S.

3-Amino-5-*O*-benzyl-3-*C*-cyano-3-deoxy-3-*N*-mesyl-1,2-*O*-sulfinyl-α-D-ribofuranose (6). To a solution of imidazole (2.13 g, 31.36 mmol) in THF (15 mL) was added $SOCl_2$ (0.57 mL, 7.84 mmol) at 0 °C. After 1 h, the reaction mixture was filtered and additionned to a solution of compound **4** (1.34 g, 3.92 mmol) in THF (15 mL) at −15 °C and stirred for 45 min. The solvent was evaporated to dryness, and the residue was purified by flash chromatography (EtOAc/petroleum ether, 50:50) to give **6** (1.42 g, 93%) as an endo/exo mixture which was used in the next step without further purification. *exo*-**6**: mp 58−60 °C; $[\alpha]^{25}_D$ +3.1 (*c* 1.33, $CHCl_3$); *endo*-**6**: mp 127−129 °C; $[\alpha]^{25}_D$ +72.5 (*c* 1.50, $CHCl_3$).

3-Amino-5-*O*-benzoyl-3-*C*-cyano-3-deoxy-3-*N*-mesyl-1,2-*O*-sulfinyl-α-D-ribofuranose (6a). To a solution of imidazole (1.27 g, 18.64 mmol) in THF (10 mL) was added $SOCl_2$ (0.34 mL, 4.66 mmol) at 0 °C. After 1 h, the reaction mixture was filtered and added to a solution of compound **4a** (0.83 g, 2.33 mmol) in THF (10 mL) at −15 °C and stirred for 45 min. The solvent was evaporated to dryness, and the residue was purified by flash chromatography (EtOAc/petroleum ether, 50:50) to give **6a** (0.78 g, 83%) as an endo/exo mixture which was used in the next step without further purification.

1-(3′-Amino-5′-*O*-benzyl-3′-*C*-cyano-3′-deoxy-3′-*N*-mesyl-*β*-D-ribofuranosyl)thymine (8). A solution of thymine (0.33 g, 2.72 mmol) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (12 mL) was refluxed overnight. The excess of HMDS was removed under reduced pressure and then added to compound **6** (0.46 g, 1.36 mmol). The reaction mixture was heated at 125 °C. After 2 h, MeOH (10 mL) was added and stirred at room temperature for 10 min, followed by addition of water (1 mL), stirred at 65 °C for 5 min, and filtered through Celite to afford a mixture of silylated derivatives **7** and **8**. The solvent was evaporated under reduced pressure and the crude product solubilized with THF (10 mL) and TBAF·$3H_2O$ (514 mg, 1.63 mmol) for 30 min and filtered through Celite. After flash chromatography (EtOAc/petroleum ether, 50:50), compound **8** (0.5 g, 82%) was isolated as a white solid: mp 194−199 °C; $[\alpha]^{25}_D$ −5.68 (*c* 1.01, CH_3OH); IR (ATR) *ν* 1682, 1330, 1258, 1136, 978, 768, 699 cm^{-1}; MS (ES): 473.3 $[M + Na]^+$, 489.4 $[M + K]^+$. Anal. ($C_{19}H_{22}N_4O_7S$) C, H, N, S.

1-(3′-Amino-5′-*O*-benzoyl-3′-*C*-cyano-3′-deoxy-3′-*N*-mesyl-*β*-D-ribofuranosyl)thymine (8a*β*). A solution of thymine (0.37 g, 2.98 mmol) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (14 mL) was refluxed overnight. The excess of HMDS was removed under reduced pressure and then added to compound **6a** (0.60 g, 1.49 mmol). The reaction mixture was heated at 125 °C. After 2 h, MeOH (10 mL) was added and stirred at room temperature for 10 min, followed by addition of water (1 mL), stirred at 65 °C for 5 min, and filtered through Celite to afford a mixture of silylated derivative **7a, 8aα**, and **8a*β***. The solvent was evaporated under reduced pressure and the crude solubilized with THF (10 mL) and TBAF·$3H_2O$ (514 mg, 1.63 mmol) for 30 min and filtered through Celite. After flash chromatography (EtOAc/petroleum ether, 60:40), compound **8a*β*** (0.47 g, 68%) was isolated as a white solid: mp 170−175 °C; $[\alpha]^{25}_D$ +8.7 (*c* 1.21, $CHCl_3$); IR (ATR) *ν* 1701, 1383, 1331, 1272, 1136, 977, 786, 713 cm^{-1}; MS (ES): 487 $[M + Na]^+$, 503 $[M + K]^+$. Anal. ($C_{19}H_{20}N_4O_8S$) C, H, N, S.

1-(3′-Amino-5′-*O*-benzyl-2′-*O*-*tert*-butyldimethylsilyl-3′-*C*-cyano-3′-deoxy-3′-*N*-mesyl-*β*-D-ribofuranosyl)thymine (9). Following the general method (A), imidazole (0.17 g, 2.46 mmol) and TBDMSCl (0.31 g, 2.05 mmol) were added to the compound **8** (0.31 g, 2.05 mmol) in DMF (15 mL) overnight. After flash chromatography (EtOAc/petroleum ether, 40:60), compound **9** (0.36 g, 89%) was isolated as a white solid: mp 189−191 °C; $[\alpha]^{25}_D$ −10.82 (*c* 1.44, $CHCl_3$); IR (ATR) *ν* 1694, 1382, 1259, 1134, 842, 779 cm^{-1}; MS (ES): 587.3 $[M + Na]^+$, 603.2 $[M + K]^+$. Anal. ($C_{25}H_{36}N_4O_7SSi$) C, H, N, S.

[1-[5′-*O*-Benzyl-2′-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]thymine]-3′-spiro-3″-(2″-*H*-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole) (10). Method 1. To a solution of LDA (freshly prepared from 0.76 mmol of *n*BuLi and 0.78 mmol of diisopropylamine) in THF (2.5 mL) was added at −78 °C a solution of sulfonamidonitrile **9** (106 mg, 0.19 mmol) in dry THF (1.5 mL). After 1 h, water (5 mL) was added and the reaction mixture slightly acidified with aqueous HCl and extracted with EtOAc. The extracts were dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (EtOAc/petroleum ether, 50:50) to give compound **10** (17 mg, 16%) as a white solid: mp 139−141 °C; $[\alpha]^{25}_D$ −8.3 (*c* 0.44, $CHCl_3$); IR (ATR) *ν* 1690, 1641, 1464, 1378, 1278, 1255, 1129, 843, 775, 739 cm^{-1}; MS (ES): 587.20 $[M + Na]^+$, 603.17

$[M + K]^+$. Anal. ($C_{25}H_{36}N_4O_7SSi$) calcd. C, 53.17; H, 6.43; N, 9.92; S, 5.68, found: C, 52.65; H, 6.28; N, 8.99; S, 5.04.

Method 2. A 3 mL mixture of TFA and CH_2Cl_2 (2/3; v/v) was added to compound **12** (68 mg, 0.1 mmol). The reaction mixture was stirred at room temperature for 3 h 30 min. Then, the solvent was evaporated to dryness, and the residue was purified by flash chromatography (EtOAc/petroleum ether, 50: 50) to yield compound **10** (45 mg, 80%) as a white solid.

1-(3′-Amino-5′-*O*-benzyl-2′-*O*-*tert*-butyldimethylsilyl-3′-*C*-cyano-3′-deoxy-3′-*N*-mesyl-*β*-D-ribofuranosyl)-3-*N*-*tert*-butoxycarbonylthymine (11). A solution of compound **9** (2.79 g, 4.95 mmol) and Boc_2O (2.16 g, 9.9 mmol) in 30 mL of a (4:1) mixture of CH_2Cl_2 and pyridine was stirred at room temperature for 8 h 30 min. The solvent was removed and the residue flash chromatographed (EtOAc/petroleum ether, 20: 80) to give **11** (2.88 g, 88%) as a white solid: mp 88−90 °C; $[\alpha]^{25}_D$ −1.3 (*c* 0.72, $CHCl_3$); IR (ATR) *ν* 1786,1710, 1679, 1370, 1264, 1141, 841, 783 cm^{-1}; MS (ES): 687.44 $[M + Na]^+$. Anal. ($C_{30}H_{44}N_4O_9SSi$) C, H, N, S.

[1-[5′-*O*-Benzyl-2′-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(2″-*H*-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole) (12). To a solution of LDA (freshly prepared from 4.4 mmol of *n*BuLi and 4.51 mmol of diisopropylamine) in THF (10 mL) was added at −78 °C a solution of sulfonamidonitrile **11** (0.72 g, 1.1 mmol) in dry THF (5 mL). After 1 h, water (5 mL) was added and the reaction mixture slightly acidified with aqueous HCl and extracted with EtOAc. The extracts were dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (EtOAc/petroleum ether, 45:55) to give compound **12** (1.47 g, 67%) as a white solid: mp 145−146 °C; $[\alpha]^{25}_D$ −16.25 (*c* 0.61, $CHCl_3$); IR (ATR) *ν* 1786, 1715, 1666, 1370, 1255, 1140, 844, 644 cm^{-1}; MS (ES): 665.35 $[M + 1]^+$, 687.34 $[M + Na]^+$, 703.25 $[M + K]^+$. Anal. ($C_{30}H_{44}N_4O_9SSi$) C, H, N, S.

[1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(2″-*H*-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole) (17) and [1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-thymine]-3′-spiro-3″-(2″-*H*-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole) (18). Following the general method (B), **12** (468 mg, 0.7 mmol), $Pd(OH)_2/C$ (130 mg, 0.19 mmol), and cyclohexene (1.62 mL, 24 mmol) in absolute EtOH (8 mL) were refluxed for 14 h to give a complex mixture of compounds **13**, **14**, **15**, and **16**. The crude product was solubilized in DMF (5 mL), and TBDMSCl (264 mg, 1.75 mmol) and imidazole (143 mg, 2.1 mmol) were added and stirred at room temperature. After 20 h, the solvent was evaporated and the residue chromatographed (EtOAc/petroleum ether, 35:65) to give successively **17** (55 mg, 11.5%) and **18** (146 mg, 35.5%) as white solids. **17**: mp 159−160 °C; $[\alpha]^{25}_D$ +18 (*c* 0.1, acetone); IR (ATR) *ν* 2915, 1789,1721, 1681, 1371, 1256, 1146 839 cm^{-1}; MS (ES): 711.29 $[M + Na]^+$, 727.26 $[M + K]^+$. Anal. ($C_{29}H_{52}N_4O_9SSi_2$) C, H, N, S. **18**: mp 142−143 °C; $[\alpha]^{25}_D$ +16 (*c* 0.15, acetone); IR (ATR) *ν* 2967, 2891, 2356, 2338,1710, 1380, 1255, 1068, 840 cm^{-1}; MS (ES): 589.25 $[M + 1]^+$, 611.24 $[M + Na]^+$, 627.21 $[M + K]^+$. Anal. ($C_{24}H_{44}N_4O_7SSi_2$) C, H, N, S.

1-(3′-Amino-5′-*O*-benzyl-2′-*O*-*tert*-butyldimethylsilyl-3′-*C*-cyano-3′-deoxy-3′-*N*-mesyl-3′-*N*-methyl-*β*-D-ribofuranosyl)-3-*N*-*tert*-butoxycarbonylthymine (19). Following the general method (C), **11** (82 mg, 0.12 mmol), K_2CO_3 (26 mg, 0.18 mmol), and MeI (0.015 mL, 0.24 mmol) in acetone (3 mL) for 1 h 45 min gave, after flash chromatography (EtOAc/petroleum ether, 20:80), product **19** (67 mg, 81%) as a white solid: mp 67−69 °C; $[\alpha]^{25}_D$ +4.89 (*c* 0.63, $CHCl_3$); IR (ATR) *ν* 1785, 1718, 1671, 1346, 1143, 841, 774 cm^{-1}; MS (ES): 679.28 $[M + 1]^+$, 701.27 $[M + Na]^+$. Anal. ($C_{31}H_{46}N_4O_9SSi$) C, H, N, S.

[1-[5′-*O*-Benzyl-2′-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole) (20). Method 1. Following the general method (D), Cs_2CO_3 (31 mg, 0.1 mmol) was added to a solution of **19** (64 mg, 0.1 mmol) in CH_3CN. The reaction mixture was refluxed for 5 h and then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 40: 60) to give product **20** (20 mg, 32%) as a white solid: mp 121−122 °C; $[\alpha]^{25}_D$ +15 (*c* 0.2, acetone); IR (ATR) *ν* 1797, 1715, 1671, 1458, 1370, 1249, 1140, 833 cm^{-1}; MS (ES): 679.34 $[M + 1]^+$, 701.24 $[M + Na]^+$, 717.23 $[M + K]^+$. Anal. ($C_{31}H_{46}N_4O_9SSi$) C, H, N, S.

Method 2. Compound **19** (117 mg, 0.17 mmol) and Cs_2CO_3 (56 mg, 0.17 mmol) in CH_3CN (50 mL) were subjected to ultrasonic waves (10 s every 5 s at 60 °C) for 1 h 45 min. The mixture was filtered and evaporated. After flash chromatography, compound **20** (60 mg, 51%) was isolated.

***O*$^{5'}$,4″-Cyclo-[1-[2′-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]thymine]-3′-spiro-3″-[4″-amino-2″-*N*-methyl-1″,1″-dioxo-isothiazolidine] (22) and *O*$^{5'}$,4″-Cyclo-[1-[2′-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-[4″-amino-2″-*N*-methyl-1″,1″-dioxo-isothiazolidine] (21).** Following the general method (B), **20** (628 mg, 0.92 mmol), $Pd(OH)_2/C$ (170 mg, 0.23 mmol), and cyclohexene (2.11 mL, 31 mmol) in absolute EtOH (10 mL) for 12 h gave successively, after flash chromatography (EtOAc/petroleum ether, 45:55) compounds **21** (144 mg, 26%) and **22** (172 mg, 40%) as white solids. **21**: mp 126−128 °C; $[\alpha]^{25}_D$ +26 (*c* 0.15, acetone); IR (ATR) *ν* 1781, 1715, 1671, 1249, 1140, 778 cm^{-1}; MS (ES): 589.33 $[M + 1]^+$, 611.22 $[M + Na]^+$. Anal. ($C_{24}H_{40}N_4O_9SSi$) C, H, N, S. **22**: mp 108−109 °C; $[\alpha]^{25}_D$ +19 (*c* 0.1, acetone); IR (ATR) *ν* 1713, 1672, 1467, 1254, 1151, 836 cm^{-1}; MS (ES): 511.17 $[M + Na]^+$. Anal. ($C_{19}H_{32}N_4O_7SSi$) C, H, N, S.

[1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]thymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole) (24). Following the general method (A), imidazole (57 mg, 0.8 mmol) and TBDMSCl (106 mg, 0.70 mmol) were added to the compound **21** (137 mg, 0.28 mmol) in DMF (5 mL) for 15 h. After flash chromatography (EtOAc/petroleum ether, 35:65), compound **24** (120 mg, 71%) was isolated as a white solid: mp 167−168 °C; $[\alpha]^{25}_D$ +2.49 (*c* 0.7, $CHCl_3$); IR (ATR) *ν* 1702, 1647, 1464, 1262,1140, 1047, 833 cm^{-1}; MS (ES): 603.27 $[M + 1]^+$, 625.25 $[M + Na]^+$. Anal. ($C_{25}H_{46}N_4O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole) (23). Following the general method (A), imidazole (35 mg, 0.51 mmol) and TBDMSCl (64 mg, 0.42 mmol) were added to the compound **22** (100 mg, 0.17 mmol) in DMF (5 mL) for 14 h. After flash chromatography (EtOAc/petroleum ether, 30:70), compound **23** (81 mg, 68%) was isolated as a white solid: mp 146−148 °C; $[\alpha]^{25}_D$ −13.36 (*c* 0.87, $CHCl_3$); IR (ATR) *ν* 1786, 1715, 1671, 1260, 1140, 838, 773 cm^{-1}; MS (ES): 703.32 $[M + 1]^+$, 725.30 $[M + Na]^+$. Anal. ($C_{30}H_{54}N_4O_9SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-3″-(2″-*H*-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole) (25). Following the general method (C), **18** (51 mg, 0.09 mmol), K_2CO_3 (6 mg, 0.045 mmol), and MeI (0.011 mL, 0.18 mmol) in acetone (2 mL) for 6 h gave, after flash chromatography (EtOAc/petroleum ether, 30:70), product **25** (45 mg, 87%) as a white solid: mp 245−246 °C; $[\alpha]^{25}_D$ +4.87 (*c* 0.5, $CHCl_3$); IR (ATR) *ν* 2927, 2357, 1722, 1659, 1636, 1473, 1364, 1142, 839 cm^{-1}; MS (ES): 603.4 $[M + 1]^+$, 625.3 $[M + Na]^+$, 641.2 $[M + K]^+$. Anal. ($C_{25}H_{46}N_4O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl]-3-*N*-ethylthymine]-3′-spiro-3″-(2″-*H*-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole) (26). Following the general method (C), **18** (51 mg, 0.09 mmol), K_2CO_3 (6 mg, 0.045 mmol), and EtI (0.014 mL, 0.18 mmol) in acetone (2 mL) for 7 h gave, after flash chromatography (EtOAc/petroleum ether, 35:65), product **26** (43 mg, 81%) as a white solid: mp 108−109 °C; $[\alpha]^{25}_D$ +9 (*c* 0.065, acetone); IR (ATR) *ν* 2925, 1709, 1672, 1644, 1472, 1254, 1133, 836 cm^{-1}; MS (ES): 617.5 $[M + 1]^+$, 639.4 $[M + Na]^+$, 655.4 $[M + K]^+$. Anal. ($C_{26}H_{48}N_4O_7SSi_2$) calcd. C, 50.62; H, 7.84; N, 9.08; S, 5.20, found: C, 51.09; H, 7.67; N, 8.71; S, 4.93.

1-(3′-Amino-5′-*O*-benzyl-2′-*O*-*tert*-butyldimethylsilyl-3′-*C*-cyano-3′-deoxy-3′-*N*-mesyl-3′-*N*-methyl-β-D-ribofuranosyl)-3-*N*-methylthymine (27). Following the general method (C), **9** (0.2 g, 0.35 mmol), K_2CO_3 (0.03 g, 0.52 mmol), and MeI (0.02 mL, 0.70 mmol) in acetone (5 mL) for 9 h gave, after flash chromatography (EtOAc/petroleum ether, 35:65) product **27** (0.18 g, 87%) as a white solid: mp 81−83 °C; $[\alpha]^{25}_D$ 36 (*c* 0.1, acetone); IR (ATR) *ν* 1711, 1673, 1650, 1469, 1347, 1261, 1156, 1035, 840, 781, 658 cm^{-1}; MS (ES): 615.4, $[M + Na]^+$, 631.4 $[M + K]^+$. Anal. ($C_{27}H_{40}N_4O_7SSi$) calcd. C, 54.71; H, 6.80; N, 9.45; S, 4.74, found: C, 54.80; H, 6.68; N, 9.33; S, 5.31.

[1-[5′-*O*-Benzyl-2′-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole) (28). Following the general method (D), Cs_2CO_3 (0.54 g, 1.68 mmol) was added to a solution of **27** (1.0 g, 1.68 mmol) in CH_3CN (10 mL). The reaction mixture was refluxed for 15 h and then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 30:70) to give product **28** (0.30 g, 30%) as a slight yellow solid: mp 118−120 °C; $[\alpha]^{25}_D$ +2.1 (*c* 2.51, $CHCl_3$); IR (ATR) *ν* 1706, 1647, 1616, 1471, 1256, 1136, 1051, 838, 766 cm^{-1}; MS (ES): 615.4 $[M + Na]^+$, 631.3 $[M + K]^+$. Anal. ($C_{27}H_{40}N_4O_7SSi$) C, H, N, S.

***O*5′,4″-Cyclo-[1-[2′-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-3″-[4″-amino-2″-*N*-methyl-1″,1″-dioxo-isothiazolidine] (29).** Following the general method (B), **28** (53 mg, 0.09 mmol), $Pd(OH)_2$/C (14 mg, 0.02 mmol), and cyclohexene (0.2 mL, 2 mmol) in absolute EtOH (1 mL) for 2 h 30 min gave, after flash chromatography (EtOAc/petroleum ether, 60:40), product **29** (22 mg, 50%) as a white solid: mp 90−91 °C; $[\alpha]^{25}_D$ −14.2° (*c* 0.53, $CHCl_3$); IR (ATR) *ν* 1710, 1673, 1645, 1469, 1300, 1142, 841 cm^{-1}; HRMS: calcd. for $C_{20}H_{35}N_4O_7SSi$ [M +] 503.2013; found 503.1996.

[1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole) (30). Following the general method (A), imidazole (20 mg, 0.3 mmol) and TBDMSCl (40 mg, 0.25 mmol) were added to the compound **29** (50 mg, 0.1 mmol) in DMF (4 mL) for 4 h. After flash chromatography (EtOAc/petroleum ether, 30:70), compound **30** (40 mg, 65%) was isolated as a white solid: mp 126−127 °C; $[\alpha]^{25}_D$ +14.5 (*c* 0.81, $CHCl_3$); IR (ATR) *ν* 1702, 1644, 1621, 1471, 1255, 1138, 838, 785, 656 cm^{-1}; MS (ES): 639.4 $[M + Na]^+$. Anal. ($C_{26}H_{48}N_4O_7SSi_2$) calcd. C, 50.62; H, 7.84; N, 9.08; S, 5.20, found: C, 50.61; H, 8.02; N, 8.26; S, 4.95.

Biological Methods. 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). The selection and characterization of the mutant HIV-1(III_B) strains bearing the Lys103 → Asn, Glu138 → Lys and Tyr181 → Cys mutations in their RT was described previously.[13]

Anti-HIV Evaluation. 4×10^5 CEM or 3×10^5 MT-4 cells per milliliter were infected with HIV-1 or HIV-2 at ∼100 $CCID_{50}$ (50% cell culture infective dose) per milliliter of cell suspension. Then, 100 μL of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4 days, giant cell formation (CEM) or HIV-induced cytopathicity (MT-4) was recorded microscopically (CEM) or by the MTT method (MT-4) in the HIV-infected cell cultures.[13] The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) of the test compounds were defined as the compound concentrations required to inhibit virus-induced cytopathicity (CEM) or to reduce cell viability (MT-4) by 50%, or to reduce by 50% the number of viable cells in mock-infected CEM and MT-4 cell cultures, respectively.

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Supporting Information Available: ^1H and ^{13}C NMR chemical shift assignments and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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