1,2,3-Triazole–[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (TSAO) Analogues: Synthesis and Anti-HIV-1 Activity

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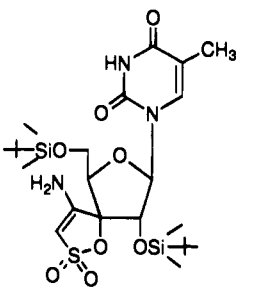
Several 4- or 5-monosubsituted and 4,5-disubstituted 1,2,3-triazole analogues of the anti-HIV-1 lead compound [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (TSAO-T) have been prepared and evaluated as inhibitors of HIV-1-induced cytopathicity. These analogues have been prepared by 1,3-diplar cycloaddition of [2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-spiro-5′-(4′-amino- and 4′-(*N*-acetylamino)-1′,2′-oxathiole 2′,2′-dioxide) (TSAO) azides to various substituted acetylenes. Several 4- and 5-substituted 1,2,3-triazole-TSAO analogues proved superior to the unsubstituted derivative by 1–2 orders of magnitude. In particular the 5-substituted amido-, (methylamido)-, and (dimethylamido)-1,2,3-triazole derivatives of TSAO were endowed with potent anti-HIV-1 activity (50% effective concentration: 0.056–0.52 μM). They show a similar resistance spectrum as previously noted for TSAO-T and related derivatives.

Introduction

In 1992, we reported on an entirely new class of HIV-1-specific agents, designated TSAO ([2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-spiro-5′-(4′-amino-1′,2′-oxathiole 2′,2′-dioxide)).[1-5] The prototype compound is TSAO-T ([1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide))[6] (Chart 1). TSAO derivatives are targeted at the HIV-1-encoded reverse transcriptase (RT) with which they interact at a non-substrate-binding site,[7,8] and they are not antivirally effect against HIV-2 and other (retro)viruses.[1-8] In this respect, they behave like the other HIV-1-specific RT inhibitors (also called nonnucleoside RT inhibitors or NNRTIs).[9-18] However, the TSAO derivatives are the first HIV-1-specific RT inhibitors for which a well-defined part of the molecule (i.e., the 4″-amino group at the 3′-spiro of the ribose moiety) has been identified as an essential pharmacophore interacting with a well-defined moiety (the -COOH group of Glu-138) of HIV-1 RT.[19]

Recently, crystallographic information regarding HIV-1 RT revealed that this amino acid at position 138 is located at the top of the finger domain of the p51 subunit of HIV-1 RT and closely approaches the binding pocket of the HIV-1-specific RT inhibitors at the p66 subunit, and may even be part of this pocket.[20-22] Moreover, our most recent data revealed that introduction of the Lys-138 mutation in the p66 subunit of the p66/p51 heterodimer does not result in resistance of this mutated RT against TSAO-T. However, introduction of the Lys-138 mutation in the p51 subunit of the p66/p51 heterodimer affords full resistance of this mutated RT to TSAO-T.[23,24] This phenomenon was not observed for other HIV-1-specific inhibitors such as TIBO R82913.[23,24]

Chart 1



TSAO-T

This observation clearly indicates that among the HIV-1-specific RT inhibitors the TSAO molecules represent a unique class of compounds for which the resistance due to mutation at position 138 of HIV-RT is specifically determined by the p51 subunit but not at the p66 subunit of the RT heterodimer.

So far, structure–activity studies have shown that the sugar part of the TSAO molecules plays a principal and crucial role in the interaction of TSAO compounds with their antiviral target enzyme (RT).[8] However, the role of the base part in this interaction is yet unclear. Thus while the sugar moiety in the TSAO molecules turns out to be very stringent with respect to its structural requirements to retain anti-HIV-1 activity, the structure of the base part can be considerably changed while keeping the antiviral activity. Also, the cytotoxicity can be modulated by introducing functional groups in the pyrimidine or purine base of the TSAO molecules.[1-5,8,25,26]

In order to gain insight in the interaction points of the TSAO derivatives with the HIV-1 RT and in particular to determine the role that the nucleobase may play in this interaction, we focused our attention on the modification of the base part in the prototype compound TSAO-T. We report herein the synthesis and anti-HIV-1 activity of a series of TSAO analogues in which the thymine moiety of the lead compound (TSAO-T) was

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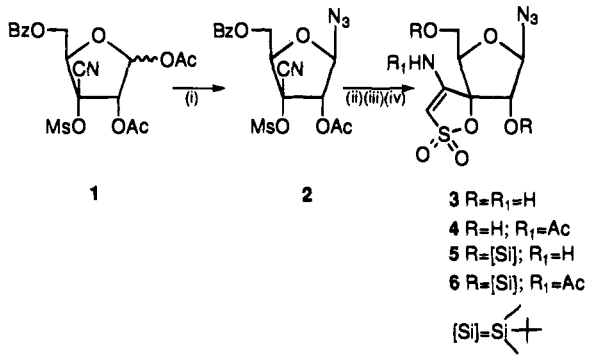
Scheme 1[a]



[a] (i) Trimethylsilyl azide; (ii) DBU; (iii) ammonia or methylamine; (iv) *tert*-butyldimethylsilyl chloride.

replaced by a series of 1,2,3-triazoles substituted with groups that may be involved in the interaction of the TSAO derivatives with functional groups in the RT enzyme. The nature of this interaction may be electrostatic, may be lipophilic, or may involve hydrogen bonding.

Chemistry

The 1,2,3-triazole 3′-spironucleosides were stereoselectively prepared by 1,3-dipolar cycloaddition of a suitably functionalized and protected ribofuranosyl azide intermediate, **5**, to differently substituted acetylenes to give, exclusively, β-D-*ribo*-spironucleosides. The *ribo* configuration of the nucleosides was determined by the configuration of the starting cyanohydrin used in the preparation of the cyanomesylate of ribose (1),[4] as clearly demonstrated in previous papers of this series.[4,27,28]

Ribosyl azide **5** was prepared as follows. Reaction of 1 (Scheme 1) with trimethylsilyl azide and stannic chloride gave the β-D-ribofuranosyl azide **2** (80%). The β-anomeric configuration of azide **2** was determined by the presence of a 2-*O*-acyl participating group in the starting sugar 1 that led, exclusively, to products with 1-N_3 and 2-*O*-Ac groups in *trans*,[29] thus β-anomers from compound 1.

Treatment of the cyanomesylate **2** with DBU followed by deprotection with saturated methanolic ammonia afforded the fully deprotected 3-spiroribofuranosyl azide **3** together with the *N*-acetyl derivative 4 (9% and 25% yield, respectively). Partial deprotection, upon treatment with DBU, of the base-sensitive 2-acetyl group and O → N migration may explain the formation of compound **4**. However, when the reaction was carried out using methylamine instead of methanolic ammonia, in the deprotection step, only compound **3** (56%) was obtained. Treatment of **3** and **4** with *tert*-butyldimethylsilyl chloride gave the 2′,5′-bis-*O*-silylated azide derivatives **5** and **6**.

As previously described,[7,27,28] formation of the spiroaminooxathiole dioxide ring in **3** and **5** was established by the disappearance in the ^{1}H NMR spectra of the signal corresponding to the mesyl group and the presence of two new singlets at δ 6.76−6.36 assigned to NH_2-4′ and at δ 5.68−5.74 assigned to H-3′. Attachment of the acetyl group to the NH_2-4′ group of the spirooxathiole moiety in **4** and **6** was established from ^{1}H NMR spectra by the disappearance of one of the NH_2-4′ protons and by the downfield shift of the signals corresponding to the protons of the spirooxathiole moiety NH-4′ (Δδ = 3.84−3.04 ppm) and H-3′ (Δδ = 1.83−1.78 ppm) with respect to the same signals in azides **3** and **5**.

4-Substituted or 5-substituted 1,2,3-triazole spironucleosides were prepared by 1,3-dipolar cycloaddition of azide **5** to unsymmetrical acetylenes. It is known in the literature that addition of azides to unsymmetrical acetylenes is determined by steric and electronic factors. In general, such addition tends to give mainly the isomers with electron-withdrawing groups at the 4-position and electron-releasing groups at the 5-position.[30−32] On the other hand, the sterically less hindered isomer tends to be the major isomer.[33−37]

Thus, cycloaddition of **5** (Scheme 2) with methyl propiolate, ethyl propiolate, 3-butyn-2-one, or propargyl bromide, in dry toluene under reflux, afforded a mixture of the two possible 4- and 5-substtitued nucleosides **7** [**7a** (65%), **7b** (67%), **7c** (62%), **7f** (49%)] and **8** [**8a** (19%), **8b** (25%), **8c** (17%), **8f** (24%)], respectively, in which **7** predominated. Similarly, cycloaddition of **5** with propioaldehyde diethyl acetal or methyl propargyl ether gave also a mixture of the corresponding 4- and 5-substituted isomers **7** [**7d** (24%), **7e** (17%)] and **8** [**8d** (64%), **8e** (26%)] in which the latter predominated.

However, cycloaddition of **5** with alkyl acetylenes (1-pentyne, 1-hexyne, or 5-chloro-1-pentyne) was more sluggish and required more severe reaction conditions to give almost equimolecular mixtures of the 4- and 5-isomeric triazoles **7** [**7g** (26%), **7h** (32%), **7i** (25%)] and **8** [**8g** (18%), **8h** (28%), **8i** (17%)] in moderate yields. Cycloaddition of **5** with (trimethylsilyl)acetylene gave, exclusively, the sterically less hindered 4-(trimethylsilyl)-1,2,3-triazole derivative **7j** (70%).

However, reaction of **5** with propiolic acid afforded the unsubstituted 1,2,3-triazole nucleoside **9**, resulting from decarboxylation of the intermediate adduct A (Scheme 2). Similar decarboxylation products from substituted 1,2,3-triazoles are described in the literature.[30]

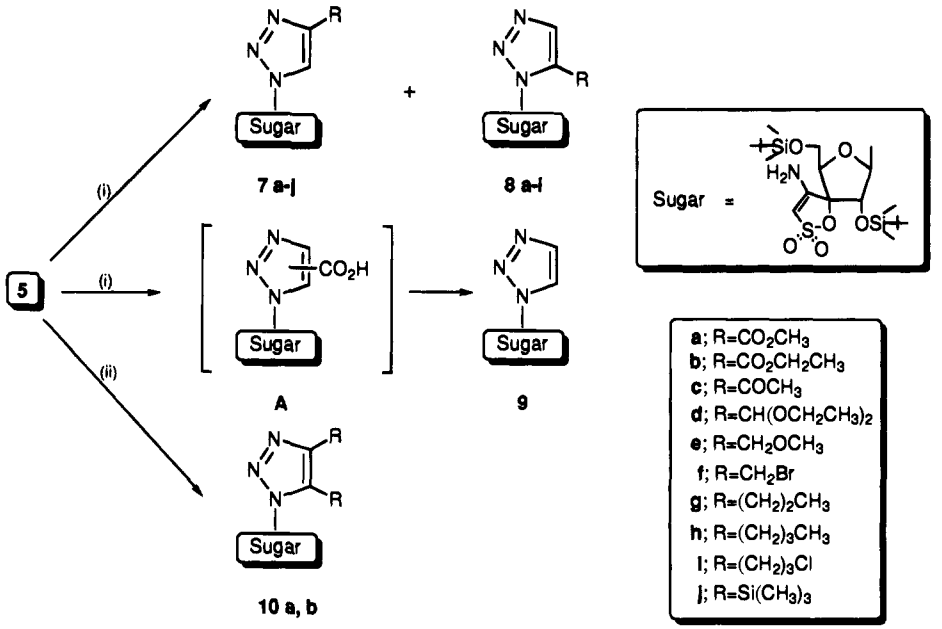
Finally, 1,3-dipolar cycloaddition of azide **5** to symmetric acetylenes (Scheme 2) gave difunctional 1,2,3-triazole nucleosides. Thus, cycloaddition of **5** to dimethyl- or diethylacetylenedicarboxylate afforded the disubstituted spironucleosides **10a** or **10b** in 86% and 82% yield, respectively.

Triazole derivatives **7a**, **8a**, and **7f** were utilized for the synthesis of other substituted triazole nucleosides such as **11**−**17** (Scheme 3). Thus, treatment of **7a** and **8a** with ammonia, methylamine, or dimethylamine afforded the 4- and 5-substituted carbamoyl (**11** and **12**), *N*-methylcarbamoyl (**13** and **14**), and *N*,*N*-dimethylcarbamoyl (**15** and **16**) derivatives, respectively. Catalytic hydrogenation of 4-(bromomethyl)triazole in methanol containing aqueous ammonia, in the presence of 10% palladium on charcoal, gave the 4-methyl-1,2,3-triazole derivative **17** in 57% yield (Scheme 4).

In order to study the effect on the antiretroviral activity of a blocking group on the NH_2-4″ function of the 3′-spirooxathiole moiety, azide **6** was reacted with methyl propiolate to give a mixture of the 4- and 5-substituted derivatives **18** and **19** from which only **18** could be obtained pure (Scheme 5).

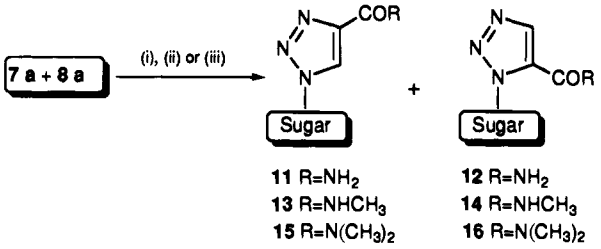
Structure of new compounds were assigned on the basis of the corresponding analytical and spectroscopic data. The β-anomeric configuration of nucleosides was established from ^{1}H NMR spectra by the coupling

Scheme 2[a]



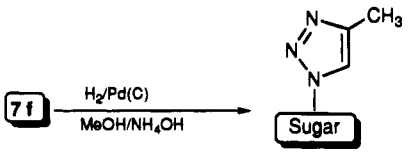
[a] (i) Monosubstituted acetylenes/dry toluene/reflux; (ii) disubstituted acetylenes/dry toluene/reflux.

Scheme 3[a]

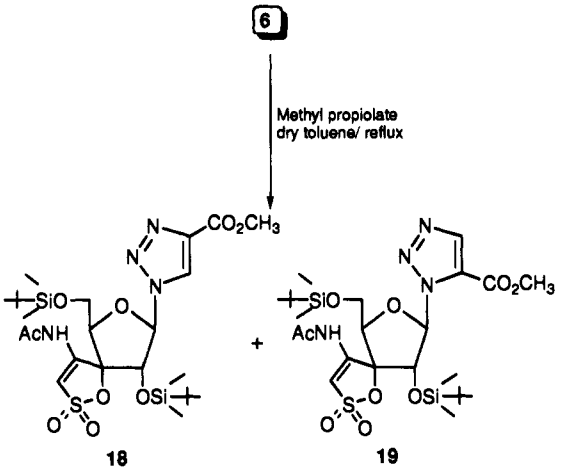


[a] (i) Ammonia; (ii) methylamine; (iii) dimethylamine.

Scheme 4



Scheme 5



constant values (Table 1) which were in the range of $J_{1',2'} = 7.6-7.9$ Hz. These values are in agreement with those observed for other β-D-*ribo*-3′-spironucleosides of this series[7,8,25,26] and with literature data for β-D-*ribo*-3′-*C*-branched nucleosides[38-40] and further corroborate the β-anomeric configuration of azides **5** and **6**, since no anomerization is expected in the cycloaddition reaction.[34,35,37,41-45] The 4- or 5-location of the substituent in 3′-spiro-1,2,3-triazole nucleosides **7** and **8** was determined on the basis of the differences in sensitivity of the chemical shifts (Δδ) of H-4 or H-5 to change in solvent.[32] It has been reported for 1-methyl- and 1-glycosyl-1,2,3-triazoles[32,34,37,41,42] that the chemical shift of the proton (H-5) adjacent to the substituted nitrogen is more sensitive to solvent changes than is H-4. Therefore, the isomers having the larger Δδ value (Table 1) were assigned as the 4-substituted triazoles **7**. Moreover, as seen from Table 1 due to the effect of the adjacent sugar the triazolic proton in the 4-substituted isomer **7** appeared at lower field than in the 5-substituted derivatives **8**. These differences in the chemical shifts between the isomers are in agreement with literature data for other 4- and 5-glycosyl-1,2,3,-triazole isomers.[34,37,41,42]

Biological Results

A series of 4- and 5-substituted 1,2,3-triazole-TSAO derivatives were evaluated for their inhibitory activity on HIV-1- and HIV-2-induced cytopathicity in MT-4 cells and syncytium formation in CEM cell cultures. None of the test compounds were active against HIV-2 (Table 2). The unsubstituted 1,2,3-triazole-TSAO derivative **9** had an EC_{50} for HIV-1 in MT-4 and CEM cells of 3.7 and 3.4 μM, respectively. The following substitutions were introduced at positions 4 and 5 of the 1,2,3-triazole moiety: alkyl ester groups in **7a,b** and **8a,b**, a diacetal group in **7d** and **8d**, an ether function in **7e** and **8e**, a ketone function in **7c** and **8c**, an alkyl group in **7g,h** and **8g,h**, and a haloalkyl function in **7i,f** and **8i,f**. As a rule, there were no striking differences in the antiviral activity of the substituted 1,2,3-triazole-TSAO derivatives with the substituent present in the C-4, as compared to the C-5, position of the triazole ring. In fact, most of the C-4- or C-5-substituted TSAO deriva-

Table 1. Selected ^{1}H NMR Spectral Data of Nucleosides: Chemical Shifts (ppm), Multiplicity, and Coupling Constants (Hz)[a]

compd	H-1′ ($J_{1',2'}$)	H-2′	H-4′ ($J_{4',5'}$)	H-5′	H-4/H-5[b]		Δδ[c]	others
7a	6.10 d (7.8)	5.58 d	4.44 t (2.3)	4.05 m	8.87 s	(8.28)	0.59	3.89 s, CO_2Me; 5.83 s, H-3″; 6.60 bs, NH_2-4″
7b[d]	6.11 d (7.8)	5.45 d	4.45 t (2.3)	4.05 m	8.85 s	(8.27)	0.58	1.35 t, 4.37 m, CO_2Et; 5.83 s, H-3″; 6.61 bs, NH_2-4″
7c	6.13 d (7.7)	5.46 d	4.47 t (2.4)	4.07 m	8.83 s	(8.27)	0.56	2.63 s, CO_2Me; 5.85 s, H-3″; 6.62 bs, NH_2-4″
7d	6.04 d (7.9)	5.34 d	4.41 t (2.3)	4.04 m	8.20 s	(7.77)	0.43	1.17 t, 3.58 m, 2 OEt; 5.74 s, 5.84 s, CH, H-3″; 6.59 bs, NH_2-4″
7e	6.00 d (7.8)	5.45 d	4.39 t (2.4)	4.02 m	8.23 s	(7.71)	0.52	3.32 s, OMe; 4.55 s, CH_2O; 5.80 s, H-3″; 6.59 bs, NH_2-4″
7f	6.00 d (7.7)	5.45 d	4.40 t (2.5)	4.02 m	8.34 s	(7.76)	0.38	4.75 s, CH_2Br; 5.79 s, H-3″; 6.55 bs, NH_2-4″
7g[d]	5.94 d (7.8)	5.44 d	4.37 t (2.3)	4.01 m	7.98 s	(7.44)	0.54	0.94 t, 1.68 m, 2.69 t, *n*-Pr; 5.79 s, H-3″; 6.58 bs, NH_2-4″
7h	5.93 d (7.8)	5.43 d	4.37 t (2.2)	4.01 m	7.97 s	(7.44)	0.53	0.91 t, 1.37 m, 1.64 m, 2.72 t, *n*-Bu; 5.79 s, H-3″; 6.58 bs, NH_2-4″
7i[e]	5.83 d (7.7)	5.39 d	4.40 m	3.88 m	7.52 s	(8.06)[f]	0.54	2.14 m, 2.90 t, 3.53 t, Cl-Pr; 5.63 s, H-3″; 5.67 bs, NH_2-4″
7j[d]	6.02 d (7.8)	5.39 d	4.39 t (2.3)	4.03 m	8.24 s	7.70	0.54	0.30 s, $SiMe_3$; 5.79 s, H-3″; 6.59 bs, NH_2-4″
8a	6.65 d (7.8)	5.75 d	4.42 t (2.1)	3.99 m	8.32 s	(8.16)	0.16	3.96 s, CO_2Me; 5.82 s, H-3″; 6.61 bs, NH_2-4″
8b[d]	6.68 d (7.9)	5.82 d	4.42 t (2.3)	4.01 m	8.32 s	(8.15)	0.17	1.37 t, 4.43 m, CO_2Et; 5.74 s, H-3″; 6.62 bs, NH_2-4″
8c	6.70 d (7.9)	5.73 d	4.42 m (2.2)	3.99 m	8.60 s	(8.17)	0.43	2.68 s, COMe; 5.83 s, H-3″; 6.62 bs, NH_2-4″
8d	6.08 d (7.8)	5.76 d	4.37 t (2.3)	3.98 m	7.74 s	(7.68)	0.05	1.21 m, 3.62 m, 2OEt; 5.80 s, 5.91 s, CH, H-3″; 6.59 bs, NH_2-4″
8e	5.96 d (7.7)	5.75 d	4.37 t (2.3)	3.97 m	7.77 s	(7.64)	0.13	3.34 s, OMe; 4.70 AB system, CH_2O; 5.80 s, H-3″; 6.60 bs, NH_2-4″
8f	6.02 d (7.6)	5.79 d	4.41 t (2.2)	3.97 m	7.89 s	(7.71)	0.18	4.93 m, CH_2Br; 5.81 s, H-3″; 6.56 bs, NH_2-4″
8g[d]	5.81 d (7.8)	5.75 d	4.36 t (2.4)	3.97 m	7.58 s	(7.46)	0.12	0.99 t, 1.73 m, 2.84 t, *n*-Pr; 5.79 s, H-3″; 6.61 bs, NH_2-4″
8h[d]	5.81 d (7.6)	5.75 d	4.37 t (2.2)	3.97 m	7.57 s	(7.45)	0.12	0.93 t, 1.42 m, 1.69 m, 2.86 t, *n*-Bu; 5.79 s, H-3″; 6.59 bs, NH_2-4″
8i[d]	5.85 d (7.7)	5.77 d	4.37 t (2.2)	3.97 m	7.65 s	(7.53)	0.12	2.19 m, 3.06 t, 3.70 m, Cl-Pr; 5.80 s, H-3″; 6.59 bs, NH_2-4″
9	6.03 d (7.8)	5.51 d	4.39 t (2.5)	4.02 m	7.80 d (H-4)	8.29 d (H-5)	–	5.79 s, H-3″; 6.60 bs, NH_2-4″
10a[e]	6.12 d (7.7)	5.64 d	4.45 m	3.85 m	–	–	–	3.97 s, 4.05 s, $2CO_2Me$; 5.66 s, H-3″; 5.77 bs, NH_2-4″
10b	6.17 d (7.7)	5.70 d	4.44 t (2.2)	4.01 m	–	–	–	1.36 t, 1.39 t, 4.40 m, 4.50 m, $2CO_2Et$; 5.84 s, H-3″; 6.62 bs, NH_2-4″
11	6.10 d (7.7)	5.39 d	4.44 t (2.3)	4.05 m	8.69 s	(8.29)	0.40	5.81 s, H-3″; 6.55 bs, NH_2-4″; 6.85 s, 7.45 s, $CONH_2$
12[e]	6.55 d (7.7)	5.69 d	4.47 m	3.86 m	8.06 s	(8.29)[f]	0.23	5.63 s, H-3″; 5.72 bs, NH_2-4″; 6.42 bs, $CONH_2$
13[g]	5.90 d (7.7)	5.22 d	4.39 m	3.83 m	8.22 s	(8.68)[f]	0.46	2.95 d, Me; 5.63 s, H-3″; 5.72 bs, NH_2-4″; 7.11 m, NHMe
14[e]	6.50 d (7.7)	5.70 d	4.46 t (1.9)	3.84 m	7.97 s	(8.18)[f]	0.21	3.60 d, CH_3; 5.62 s, H-3″; 5.73 bs, NH_2-4″; 6.46 m, NHMe
15	6.10 d (7.7)	5.43 d	4.44 m	4.04 m	8.65 s	(8.23)	0.42	3.05 s, 3.45 s, NMe_2; 5.82 s, H-3″; 6.59 bs, NH_2-4″
16	6.21 d (7.7)	5.72 d	4.36 m	3.97 m	8.04 s	(7.76)	0.28	3.08 s, 3.13 s, NMe_2; 5.78 s, H-3″; 6.58 bs, NH_2-4″
17[d]	5.93 d (7.7)	5.47 d	4.37 t (2.7)	4.02 m	8.02 s	–	–	2.31 s, Me; 5.78 s, H-3″; 6.62 bs, NH_2-4″
18	6.18 d (7.7)	5.57 d	4.47 m	4.19 m	8.96 s	–	–	2.27 s, NHAc; 3.90 s, CO_2Me; 7.62 s, H-3″; 9.85 bs, NH-4″

[a] [$(CD_3)_2CO$] at 200 MHz. [b] In parentheses are the chemical shifts in $CDCl_3$. [c] $\Delta\delta = \delta_{(CD_3)_2CO} - \delta_{CDCl_3}$. [d] At 300 MHz. [e] $CDCl_3$. [f] In parentheses are the chemical shifts in $(CD_3)_2CO$. [g] $CDCl_3$ at 300 MHz.

tives proved more inhibitory to HIV-1 than the parent compound **9**. The EC_{50} values for the ester derivatives **7a** and **8a** (methyl esters) and **7b** and **8b** (ethyl esters) against HIV-1 ranged from 0.134 to 1.32 μM in CEM cells and from 0.602 to 3.23 μM in MT-4 cells; this means a 5–10-fold greater activity than for the parent compound **9**. Compounds **7a,b** and **8a,b** were clearly superior to the ketone- and ether-substituted 1,2,3-triazole-TSAO derivatives **7c,e** and **8c,e**. Compound **18** which has a ketone substituent at the 4″-amino group of the 3′-spiro moiety lost antiviral activity by at least 2 orders of magnitude (as compared to compound **7a**). The C-4- and C-5-diacetal-substituted compounds **7d** and **8d** were devoid of significant antiviral activity (EC_{50}: 72.5–>150 μM).

There were no marked differences in the antiviral activity of the highly lipophilic C-4- and C-5-substituted propyl (**7g**, **8g**) and butyl (**7h**, **8h**) 1,2,3-triazole-TSAO derivatives (EC_{50}: 1.53–3.95 μM in CEM cells and 0.92–3.13 μM in MT-4 cells). Also, the C-4-methyl-substituted 1,2,3-triazole-TSAO derivative **17** and the highly lipophilic **7j** derivative fell within the same range

Table 2. Anti-HIV-1 and Anti-HIV-2 Activity of 1,2,3-Triazole-TSAO Derivatives in MT-4 and CEM Cell Lines

	EC_{50} (μM)[a]					
	MT-4		CEM		CC_{50} (μM),[b]	ratio CC_{50} (MT-4)/
compd	HIV-1	HIV-2	HIV-1	HIV-2	MT-4	EC_{50} (MT-4)
7a	0.60 ± 0.05	>150	0.18 ± 0.10	>40	≥150	≥249
7b	0.79 ± 0.13	≥165	0.54 ± 0.39	>165	>165	>209
7c	1.5 ± 0.6	>7	1.1 ± 0.4	>35	13 ± 1.6	8.6
7d	73 ± 56	>150	>150	>150	>150	>2.1
7e	27 ± 8.7	>35	2.3 ± 0.9	>70	73 ± 4.9	2.44
7f	≥6.5	>6.5	>1.3	>1.3	14 ± 0.9	<2.1
7g	0.92	>170	1.3 ± 0.2	>35	>170	>185
7h	2.6 ± 1.4	>170	1.5 ± 0.3	>7	107 ± 50	41
7i	0.58 ± 0.35	>170	0.66 ± 0.23	>33	53 ± 53	91.4
7j	≥6.6	>6.6	2.8 ± 2.5	>2.6	9.5 ± 3.8	≤1.44
8a	0.90 ± 0.47	>150	0.13 ± 0.03	>40	128 ± 52	142
8b	3.2 ± 0.3	>165	1.3 ± 0.0	>165	>165	>51
8c	2.3 ± 0.2	>35	1.1 ± 0.5	>70	69 ± 9.0	30
8d	>40	>40	>150	>150	>150	><1
8e	2.4 ± 0.3	>7	0.90 ± 0.33	>14	28 ± 14	11
8f	≥6	>6	>1.30	>6	13 ± 0.5	<2.1
8g	3.13	>350	3.9 ± 2.8	>35	>175	>56
8h	1.58 ± 1.2	>170	1.5 ± 0.2	>35	≥170	≥115
8i	1.9 ± 1.3	>164	0.82 ± 0.0	>7	79 ± 35	40.6
9	3.8 ± 0.0	>7.5	3.4 ± 0.1	>7.5	15 ± 1.1	4.1
10a	0.48 ± 0.11	>150	0.53 ± 0.11	>30	111 ± 61	231
10b	3.8 ± 0.2	>30	1.6 ± 0.71	>60	55 ± 4.8	14.5
11	1.2 ± 1.1	>7	0.90 ± 0.61	>7	15 ± 0.2	12.5
12	0.52 ± 0.07	>7	0.14 ± 0.02	>7	15 ± 1.1	28.1
13	0.41 ± 0.21	>7	1.2 ± 0.2	>7	12 ± 2.7	28.8
14	0.16 ± 0.06	>7	0.08 ± 0.0	>7	27 ± 21	171
15	0.42 ± 0.22	>7	1.2 ± 0.2	>7	12 ± 2.4	28.1
16	0.06 ± 0.06	>30	0.12 ± 0.012	>7	20 ± 7.5	335
17	2.8 ± 0.43	>35	1.4 ± 0.5	>6.9	23 ± 4.0	8.4
18	≥32	>32	28 ± 3.9	>32	56 ± 1.6	≤1.7
TSAO-T	0.06 ± 0.03	>20	0.06 ± 0.01	>20	14 ± 2	227
Nevirapine	0.12 ± 0.04	>80	0.11 ± 0.10	>80	>80	>625
AZT	0.002 ± 0.001	0.003 ± 0.0001	0.003 ± 0.002	0.004 ± 0.001	6.0 ± 0.1	3000

[a] 50% effective concentration or compound concentration required to reduce the number of viable HIV-infected MT-4 cells or to inhibit virus-induced giant cell formation in HIV-infected CEM cells by 50%. [b] 50% cytotoxic concentration or compound concentration required to reduce the number of viable mock-infected MT-4 cells by 50%. Data are the mean (± standard deviation) of at least two or three independent experiments.

of antiviral activities as the alkyl-substituted compounds. Interestingly, the derivatives **7i** and **8i**, containing a chloropropyl group at C-4 and C-5, respectively, showed a similar anti-HIV-1 activity as the C-4- and C-5-alkyl-substituted 1,2,3-triazole-TSAO derivatives. The C-4- and C-5-bromomethyl-substituted derivatives **7f** and **8f** were virtually devoid of antiviral activity at subtoxic concentrations (Table 2).

The bis-substituted 1,2,3-triazole-TSAO derivatives **10a,b**, which contain the methyl ester and ethyl ester functions of **7a,b** and **8a,b** simultaneously in both C-4- and C-5-positions of the 1,2,3-triazole ring, showed an antiviral activity which was not superior to the C-5-monosubstituted **8a,b** derivatives. Introduction of unsubstituted or methyl- and dimethyl-substituted amido functions at C-4 of 1,2,3-triazole-TSAO (**11**, **13**, **15**) resulted in comparable antiviral efficacies; these were 2–8-fold superior to that of the unsubstituted parent compound **9** (EC_{50}: 3.44–3.76 μM). When the unsubstituted and methyl- and dimethyl-substituted carbamoyl functions were introduced at C-5, instead of C-4, of the triazole moiety (**12**, **14**, **16**), the antiviral activities were increased by 10-fold, and thus these compounds proved at least 25–60-fold more inhibitory against HIV-1 than the parent compound **9**.

Compounds **7b,g**, **8a**, **14**, **16**, and TSAO-m^{3}T were evaluated on their antiviral activity against a clinical HIV-1 isolate designated HIV-1/HE[1] in CEM cells. Their EC_{50} values corresponded closely with those obtained for HIV-1/III$_B$ and were 0.28, 1.7, 0.45, 0.27, 0.32, and 0.05 μM, respectively. When the effect of **8a**, **14**, and the reference compound TSAO-m^{3}T was evaluated against p24 antigen expression in HIV-1/HE-infected CEM cell cultures, IC_{50} values of 0.8, 0.17, and 0.1 μM were obtained, respectively. Thus the EC_{50} values found for the 1,2,3-triazole-TSAO derivatives againsg HIV-1/III$_B$ in MT4 and CEM cells corresponded closely with their EC_{50} values against the clinical isolate HIV-1/HE, irrespective of the antiviral parameter measured (cytopathicity, p24 antigen expression).

Compounds **7b,g** and **16** that rank among the most active 1,2,3-triazole-TSAO derivatives evaluated were also examined for their anti-HIV-1/Ba-L activity in freshly prepared macrophages. As a rule, these test compounds were found to be equally inhibitory to HIV-1 replication in macrophages and T-lymphocytes (Table 3). This observation is important in view of the fact that monocytes/macrophages are a significant source of HIV particles in the intact organism, and any therapeutic modality should adequately address this issue. The 1,2,3-triazole-TSAO derivatives seem to fulfill this criterion.

The cytotoxicity of the C-4-substituted 1,2,3-triazole-TSAO derivatives did not markedly differ from that of the corresponding C-5-substituted derivatives. Generally, the ester- and the alkyl-substituted test compounds were markedly less toxic than the other compounds. The molecular target for the cytotoxicity of the test com-

Table 3. Inhibitory Effect of **7b,g** and **16** on HIV-1/Ba-L Replication in Macrophages

compd	concentration (μM)	percent of virus inhibition[a] (%)
7b	3.3	>99
	0.8	98
	0.16	20
	0.033	0
7g	3.4	67
	0.85	42
	0.17	12
	0.034	0
16	3.3	>99
	0.8	>99
	0.16	42
	0.033	0

[a] Virus production was tested after 14 days postvirus challenge by HIV-p24 ELISA. The position control contained 150.000 pg of p24/mL of supernatant. Data are the mean of two independent experiments.

pounds is unclear so far, but it has been suggested that the compounds may interfere with the cellular membrane function at the higher concentrations.[46]

We have previously demonstrated that TSAO-purine and TSAO-pyrimidine derivatives represent a novel class of HIV-1-specific inhibitors.[1-5] We also found that the structural requirements for anti-HIV-1 activity are much more stringent at the TSAO sugar part than at the base (purine or pyrimidine) moiety. Here we demonstrated that replacing the base moiety by an (un)-substituted 1,2,3-triazole ring results in a series of novel TSAO derivatives that are endowed with a potent inhibitory activity against HIV-1. These new TSAO derivatives retain specificity for HIV-1 (i.e., lack activity against HIV-2) and show high selectivity (i.e., CC_{50}/EC_{50} ratio). It should also be mentioned that replacement of the 1,2,3-triazole ring by an azido group annihilates the antiviral activity of the TSAO derivatives, suggesting that the electronic and/or conformational properties of the cyclic triazole system in the TSAO molecule play an important role in the antiviral activity of the compounds.

Our observations that the highly lipophilic 1,2,3-triazole-TSAO derivatives **7g,h** and **8g,h**, which are unable to form hydrogen bonds between the triazole C-4/C-5 substituents and the target enzyme (i.e., reverse transcriptase) show an antiviral activity comparable to that of the **7e,c**, **8e,c**, 11, **13**, and **15** derivatives (which should be able to form hydrogen bonds with RT) argue against a specific interaction, through hydrogen bonding, between these test compounds and HIV-1 RT. The moelcular basis for the markedly higher antiviral activities of compounds **12**, **14**, and **16** as compared to compounds **11**, **13**, and **15** is unclear. It is possible that the $CONH_2$, $CONH(CH_3)$, or $CON(CH_3)_2$ groups are involved in a specific interaction with HIV-1 RT and that this interaction is favored by their positioning at C-5 rather than at C-4.

The most active HIV-1 inhibitor, compound **16**, has also been used for selection of TSAO-resistant HIV-1 strains in CEM cell culture. When exposed to 1.5 μM **16**, that is, at a 10-fold higher concentration than its EC_{50} value, resistant virus breakthrough occurred at the third subcultivation (day 10 of the experiment) and full cytopathicity was recorded at day 14 postinitiation of the experiment. The virus that emerged from this study proved highly resistant to compound **16** and also to TSAO-m^{3}T but retained marked sensitivity to other HIV-1-specific RT inhibitors such as nevirapine, BHAP, and TIBO R82913. Sequencing of the RT gene of the mutant HIV-1 strain revealed a mutation in codon 138 of its RT, resulting in an amino acid change from glutamic acid (Glu) to lysine (Lys). This mutation is identical to that obtained previously for several other TSAO-pyrimidine and TSAO-purine derivatives.[8,19]

In conclusion, we have reported on a novel series of TSAO derivatives in which the pyrimidine/purine moiety of the molecule is replaced by a 1,2,3-triazole ring with various substitutions at C-4 or C-5. Several members of this class of compounds show potent anti-HIV-1 activities comparable to that of the TSAO prototype (thymine) derivative TSAO-T. The structural requirements of the triazole C-4 and C-5 substituents do not appear very stringent for the TSAO derivatives to show potent and selective anti-HIV-1 activity. Compound **16** emerged as the most active triazole-TSAO derivative. It selected for Glu-138→Lys-138 RT-mutated HIV-1 strains in cell culture and should be considered as a novel lead compound to develop further TSAO derivatives with potent anti-HIV-1 activity.

Experimental Section

Chemical Procedures. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. ^{1}H NMR spectra were recorded with a Varian Gemini, a Varian XL-300, and a Bruker AM-200 spectrometer operating at 300 and 200 MHz, and ^{13}C NMR spectra were recorded with a Bruker AM-200 spectrometer operating at 50 MHz, with Me_4Si as internal standard. IR spectra were recorded with a Shimadzu IR-435 spectrometer. Analytical TLC was performed on silica gel 60 F_{254} (Merck). Separations on silica gel were performed by preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron instrument (Kiesegel 60 PF 254 gipshaltig (Merck); layer thickness, 1 mm; flow rate, 5 mL/min). Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck).

2-*O*-Acetyl-5-*O*-benzoyl-3-*C*-cyano-3-*O*-mesyl-β-D-ribofuranosyl Azide (2). To a solution of **1**[4] (4.5 g, 10 mmol) in dry dichloromethane (100 mL) was added trimethylsilyl azide (3 mL) and stannic chloride (1.2 mL). The mixture was stirred at room temperature for 8 days and then concentrated to dryness. The residue was washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (chloroform/acetone, 80:1) to give 3.4 g (80%) of **2** as a white solid: mp 164–165 °C (ethyl ether); $[\alpha]_D$ −81.7° (*c* 1, $CHCl_3$); IR (Nujol) 2100 cm^{-1} (N_3), 1760, 1720 (C=O); ^{1}H NMR [$(CD_3)_2CO$, 200 MHz] δ 2.19 (s, 3H, CH_3CO), 3.43 (s, 3H, CH_3SO_2), 4.73 (dd, 1H, H-5a, $J_{4,5a}$ = 4.6, $J_{5a,5b}$ = 12.3 Hz), 4.88 (dd, 1H, H-5b, $J_{4,5b}$ = 4.5 Hz), 4.96 (t, 1H, H-4), 5.56 (d, 1H, H-2, $J_{1,2}$ = 3.1 Hz), 5.77 (d, 1H, H-1), 7.51–8.15 (m, 5H, Ph). Anal. ($C_{16}H_{16}N_4O_8S$) C, H, N, S.

β-D-Ribofuranosyl-3-spiro-5′-(4′-amino-1′,2′-oxathiole 2′,2′-dioxide) Azide (3). Method A. To a solution of **2** (2.8 g, 6.6 mmol) in dry acetonitrile (50 mL) was added DBU (1.1 mL, 7.26 mmol); the mixture was stirred at room temperature for 1 h. The solution was neutralized with acetic acid and then evaporated to dryness. The residue was treated with methylamine (33 wt % solution in EtOH, 300 mL), and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated to dryness, and the residue was purified by column chromatography (chloroform/methanol, 10:1) to give 0.99 g (56%) of **3** as an amorphous solid: IR (KBr) 3400 cm^{-1}, 3350, 3310 (NH_2, OH), 2100 (N_3), 1650 (C=CN), 1360, 1180 (SO_2); ^{1}H NMR (DMSO, 200 MHz) δ 3.40–3.70 (m, 2H, H-5), 4.16 (t, 1H, H-4, $J_{4,5}$ = 3 Hz), 4.30 (t, 1H, H-2, $J_{2,OH}$ = 7.1 Hz), 5.06 (d, 1H, H-1, $J_{1,2}$ = 7.1 Hz), 5.68 (s, 1H, H-3′), 5.82 (bs, 1H, OH-5), 6.19 (d, 1H, OH-2), 6.76 (bs, 2H, NH_2-4′). Anal. ($C_7H_{10}N_4O_6S$) C, H, N, S.

Method B. The procedure described in method A was followed except that methanol saturated with ammonia (350 mL) was used instead of methylamine, in the deprotection step. The solvent was evaporated to dryness, and the residue was purified by column chromatography (chloroform/methanol, 10:1). The fastest moving fractions afford 0.6 g (25%) of an amorphous solid which was identified as **β-D-ribofuranosyl-3-spiro-5′-[4′-(*N*-acetylamino)-1′,2′-oxathiole 2′,2′-dioxide] azide (4)**: IR (KBr) 3400 cm^{-1}, 3350, 3310 (NH_2, OH), 2100 (N_3), 1650 (C=CN), 1360, 1180 (SO_2); ^1H NMR (DMSO, 200 MHz) δ 2.12 (s, 3H, CH_3CO), 3.56 (dd, 1H, H-5a, $J_{4,5a}$ = 2.9, $J_{5a,5b}$ = 12.8 Hz), 3.70 (dd, 1H, H-5b, $J_{4,5b}$ = 4.0 Hz), 4.21 (dd, 1H, H-4), 4.47 (t, 1H, H-2, $J_{1,2} = J_{2,OH}$ = 7.0 Hz), 5.18 (d, 1H, H-1), 6.29 (d, 1H, OH-2), 7.51 (s, 1H, H-3′), 10.6 (bs, 1H, NHAc). Anal. ($C_9H_{12}N_4O_7S$) C, H, N, S.

The slowest moving fractions gave 0.19 g (9%) of an amorphous solid identified as **3**.

[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-spiro-5′-(4′-amino-1′,2′-oxathiole 2′,2′-dioxide) Azide (5). To a suspension of **3** (0.99 g, 3.56 mmol) in dry acetonitrile (100 mL) were added 4-(dimethylamino)pyridine (3.48 g, 28.48 mmol) and *tert*-butyldimethylsilyl chloride (2.14 g, 14.24 mmol). The reaction mixture was heated at 80 °C for 16 h and, then, evaporated to dryness. The residue was treated with ethyl acetate (20 mL). The solid was filtered, and the filtrate was evaporated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 1:1) to give 1.4 g (78%) of **5** as an amorphous solid: $[\alpha]_D$ −42.7° (*c* 1, $CHCl_3$); IR (KBr) 3400 cm^{-1}, 3310 (NH_2), 2100 (N_3), 1650 (C=CN), 1360, 1185 (SO_2); ^1H NMR (DMSO, 200 MHz) δ 3.91 (dd, 1H, H-5a, $J_{4,5a}$ = 1.5, $J_{5a,5b}$ = 12.7 Hz), 3.99 (dd, 1H, H-5b, $J_{4,5b}$ = 2.6 Hz), 4.28−4.30 (m, 1H, H-4), 4.31 (d, 1H, H-2, $J_{1,2}$ = 6.8 Hz), 5.34 (d, 1H, H-1), 5.74 (s, 1H, H-3′), 6.36 (bs, 2H, NH_2); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 63.29 (C-5), 78.54 (C-2), 86.69 (C-3′), 93.04, 94.09 (C-4, C-1), 94.78 (C-3), 151.36 (C-4′). Anal. ($C_{19}H_{38}N_4O_6SSi_2$) C, H, N, S.

[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-spiro-5′-[4′-(acetylamino)-1′,2′-oxathiole 2′,2′-dioxide) Azide (6). Via a procedure similar to that described above for the synthesis of azide **5**, compound **4** (0.065 g, 0.2 mmol) was treated with 4-(dimethylamino)pyridine (0.19 g, 1.62 mmol) and *tert*-butyldimethylsilyl chloride (0.12 g, 0.81 mmol). After the workup, the residue was purified by CCTLC on chromatotron (hexane/ethyl acetate, 3:1) to give 0.07 g (60%) of **6** as an amorphous solid: ^1H NMR [$(CD_3)_2CO$, 200 MHz] δ 2.76 (s, 3H, CH_3CO), 4.10 (d, 2H, H-5, $J_{4,5a} = J_{4,5b}$ = 2.4 Hz), 4.22 (d, 1H, H-2, $J_{1,2}$ = 6.9 Hz), 4.33 (t, 1H, H-4), 5.44 (d, 1H, H-1), 7.52 (s, 1H, H-3′), 9.40 (bs, 1H, NHAc). Anal. ($C_{21}H_{40}N_4O_7SSi_2$) C, H, N, S.

General Procedure for the Synthesis of [1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) Nucleosides 7−10. A mixture of axide **5** (1 mmol), the acetylene (1.5 mmol), and dry toluene (20 mL) was refluxed until the complete reaction of the azide, (18 h−3 days). The solution was evaporated to dryness, and the residue was purified by CCTLC on chromatotron (hexane/ethyl acetate, 3:1).

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-[(methyloxy)carbonyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) Nucleosides (7a and 8a). Via the general procedure, azide **5** (0.5 g, 1 mmol) reacted with methyl propiolate (0.13 mL, 1.5 mmol) for 18 h. The residue was purified by CCTLC to give, from the fastest moving band, 0.11 g (19%) of **8a** as a white foam: *IR* (KBr) *3400 cm^{-1}, 3310 (NH_2), 1740 (C=O)*, 1650 (*C=CN*); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 52.85 (CH_3O), 62.2 (C-5′), 75.36 (C-2′), 85.54 (C-3″), 88.17 (C-4′), 93.87, 93.79 (C-3′, C-1′), 129.75 (C-5), 138.49 (C-4), 150.23 (C-4″), 158.06 (CO). Anal. ($C_{23}H_{42}N_4O_8SSi_2$) C, H, N, S.

The slowest moving band gave 0.38 g (65%) of **7a** as an amorphous solid: IR (Nujol) 3400 cm^{-1} 3300 (NH_2), 1740 (C=O), 1650 (C=CN); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 52.31 (CH_3O), 62.25 (C-5′), 76.16 (C-2′), 85.65, 89.92 (C-3″, C-4′), 93.64, 93.93 (C-1′, C-3′), 129.18 (C-5), 140.10 (C-4), 149.73 (C-4″), 160.71 (CO). Anal. ($C_{23}H_{42}N_4O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-[(ethyloxy)carbonyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7b and 8b). Via the general procedure, azide **5** (0.1 g, 0.19 mmol) reacted with ethyl propiolate (0.03 mL, 0.29 mmol) for 24 h. The residue was chromatographed to give, from the fastest moving band, 0.03 g (25%) of **8b** as a white foam. Anal. ($C_{24}H_{44}N_4O_8SSi_2$) C, H, N, S.

The slowest moving band afforded 0.08 g (67%) of **7b** as an amorphous solid: IR (KBr) 3400 cm^{-1}, 3310 (NH_2), 1735 (C=O), 1650 (C=CN); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 61.54, 63.19 (C-5′, CH_2O), 76.98 (C-2′), 86.83 (C-3″), 90.63 (C-4′), 93.33 (C-1′), 94.28 (C-3′), 130.39 (C-5), 141.03 (C-4), 151.25 (C-4″), 162.00 (CO). Anal. ($C_{24}H_{44}N_4O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-(methylcarbonyl)-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7c and 8c). According to the general procedure, azide **5** (0.1 g, 0.19 mmol) was reacted with 3-butyn-2-one (0.02 mL, 0.29 mmol) for 24 h. Purification of the residue gave, from the fastest moving band, compound **7c** (0.07 g, 62%) as a white foam: IR (KBr) 3400 cm^{-1} (NH_2), 1710 (CO), 1650 (C=CN). Anal. ($C_{23}H_{42}N_4O_7SSi_2$) C, H, N, S.

The slowest moving band gave 0.02 g (17%) of **8c** as a white foam. Anal. ($C_{23}H_{42}N_4O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-[bis(ethyloxy)methyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7d and 8d). Via the general procedure, azide **5** (0.1 g, 0.19 mmol) reacted with propiolaldehydee diethyl acetal (0.04 mL, 0.29 mmol) for 48 h. Chromatography of the residue gave, from the fastest moving band, 0.08 g (64%) of **8d** as a white foam. Anal. ($C_{26}H_{50}N_4O_8SSi_2$) C, H, N, S.

The slowest moving band afforded 0.03 g (24%) of **7d** as a foam: IR (KBr) 3400 cm^{-1}, 3310 (NH_2), 2850 (CH_2O), 1650 (C=CN); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 15.48 ($2CH_3$), 61.34, 61.43 ($2CH_2O$), 63.29 (C-5′), 77.14 (C-2′), 86.46, 90.48, 93.27 (C-3″, C-4′, C-1′), 94.32 (C-3′), 97.25 (CHO_2), 124.19 (C-4), 148.28 (C-5), 151.48 (C-4″). Anal. ($C_{26}H_{50}N_4O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-[(methyloxy)methyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7e and 8e). Azide **5** (0.1 g, 0.19 mmol) was reacted with methyl propargyl ether (0.05 mL, 0.59 mmol) for 3 days, according to the general procedure. The residue was purified by CCTLC on chromatotron (dichloromethane/methanol, 100:1). The fastest moving band gave compound **8e** (0.03 g, 26%) as a white foam. Anal. ($C_{23}H_{44}N_4O_7SSi_2$) C, H, N, S.

The slowest moving band gave 0.02 g (17%) of **7e** as a foam. Anal. ($C_{23}H_{44}N_4O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-(bromomethyl)-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7f and 8f). Following the general procedure, azide **5** (0.1 g, 0.19 mmol) was reacted with propargyl bromide (0.025 mL, 0.29 mmol) for 3 days. The residue was purified by CCTLC on chromatotron (dichloromethane/methanol, 200:1). The fastest moving band gave compound **8f** (0.03 g, 24%) as an amorphous solid. Anal. ($C_{22}H_{41}BrN_4O_6SSi_2$) C, H, N, S.

The slowest moving band gave 0.06 g (49%) of **7f** as an amorphous solid. Anal. ($C_{22}H_{41}BrN_4O_6SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-propyl-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7g and 8g). Via the general procedure, azide **5** (0.1 g, 0.19 mmol) reacted with 1-pentyne (0.04 mL, 0.39 mmol) at 110 °C in a sealed tube for 2 days. The solvent was evaporated to dryness and the residue purified by CCTLC on chromatotron (dichloromethane/methanol, 100:1). The fastest moving band gave 0.03 g (26%) of **7g** as a white foam. Anal. ($C_{24}H_{46}N_4O_6SSi_2$) C, H, N, S.

The slowest moving band afforded 0.02 g (18%) of **8g** as a white foam. Anal. ($C_{24}H_{46}N_4O_6SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-butyl-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7h and 8h). Following the

general procedure, azide **5** (0.1 g, 0.19 mmol) was reacted with 1-hexyne (0.03 mL, 0.295 mmol) for 3 days. The residue was purified by CCTLC on chromatotron (dichloromethane/methanol, 200:1). The fastest moving band gave compound **8h** (0.026 g, 28%) as an amorphous solid. Anal. ($C_{25}H_{48}N_4O_6SSi_2$) C, N, N, S.

The slowest moving band yielded 0.03 g (32%) of **7h** as an amorphous solid. Anal. ($C_{25}H_{48}N_4O_6SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-(3-chloropropyl)-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7i and 8i). Azide **5** (0.1 g, 0.19 mmol) reacted with 5-chloro-1-pentyne (0.03 mL, 0.295 mmol) for 3 days, according to the general procedure. Purification of the residue (dichloromethane/methanol, 200:1) yielded, from the fastest moving band, 0.02 g (17%) of **8i** as a white foam. Anal. ($C_{24}H_{45}ClN_4O_6SSi_2$) C, H, N, S.

The slowest moving band gave 0.03 g (25%) of **7i** as a white foam. Anal. ($C_{24}H_{45}ClN_4O_6SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-(trimethylsilyl)-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7j). According to the general procedure, azide **5** (0.05 g, 0.09 mmol) was reacted with (trimethylsilyl)acetylene (0.02 mL, 0.15 mmol) for 48 h. The residue was chromatographed to give **7j** (0.04 g, 70%) as a syrup. Anal. ($C_{24}H_{48}N_4O_6SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (9). Following the general procedure described for the synthesis of 1,2,3-triazole nucleosides **7** and **8**, azide **5** (0.1 g, 0.19 mmol) was treated with propiolic acid (0.02 mL, 0.29 mmol), heated to reflux for 5 h, and then stirred at room temperature for 18 additional hours. After the workup, the residue was purified by CCTLC on chromatotron (hexane/ethyl acetate, 3:1) to yield 0.03 g (25%) of **9** as an amorphous solid: IR (Nujol) 3400 cm^{-1} 3310 (NH_2), 1650 (C=CN). Anal. ($C_{21}H_{40}N_4O_6SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,5-bis[(methyloxy)carbonyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (10a). The general procedure was followed with azide **5** (0.1 g, 0.19 mmol) and dimethyl acetylenedicarboxylate (0.05 mL, 0.39 mmol) to give, after purification of the residue, 0.11 g (86%) of **10a** as an amorphous solid: IR (KBr) 3400 cm^{-1} (NH_2), 1740 (CO), 1650 (C=CN). Anal. ($C_{25}H_{44}N_4O_{10}SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4,5-bis[(ethyloxy)carbonyl]-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (10b). Azide **5** (0.05 g, 0.09 mmol) reacted with diethyl acetylenedicarboxylate (0.05 mL, 0.14 mmol) for 24 h, following the general procedure. Purification of the residue yielded 0.05 g (82%) of **10b** as a white foam: IR (KBr) 3400 cm^{-1}, 3300 (NH_2), 1740 (CO), 1650 (C=CN). Anal. ($C_{27}H_{48}N_4O_{10}SSi_2$), C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-carbamoyl-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (11). Compound **7a** (0.1 g, 0.17 mmol) was treated with saturated methanolic ammonia (5 mL). The reaction mixture was stirred at room temperature for 7 h. The solvent was evaporated to dryness. The residue was purified by CCTLC on chromatotron (hexane/ethyl acetate, 1:1) to give compound **11** (0.05 g, 51%) as an amorphous solid: IR (KBr) 3400 cm^{-1} 3200 (NH_2), 1660 (CO), 1360, 1180 (SO_2); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 63.23 (C-5′), 77.14 (C-2′), 86.81 (C-3″), 90.71, 93.31 (C-4′, C-1′), 94.22 (C-3′), 128.12 (C-5), 144.74 (C-4), 151.36 (C-4″), 161.87 (CO). Anal. ($C_{22}H_{41}N_5O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-carbamoyl-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (12). Following the procedure described for the synthesis of compound **11**, nucleoside **8a** (0.04 g, 0.06 mmol) was treated with saturated methanolic ammonia (5 mL). The residue was purified by CCTLC on chromatotron (hexane/ethyl acetate, 1:1) to give compound **12** (0.02 g, 58%) as an amorphous solid. Anal. ($C_{22}H_{41}N_5O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-(*N*-methylcarbamoyl)-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (13). A solution of **7a** (0.1 g, 0.17 mmol) in methylamine (35 wt % in ethanol, 5 mL) was stirred at room temperature for 10 min. The solvent was evaporated to dryness and the residue was purified by CCTLC on chromatotron (hexane/ethyl acetate, 1:1) to give **13** (0.09 g, 90%) as a white foam. Anal. ($C_{23}H_{43}N_5O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-(*N*-methylcarbamoyl)-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (14). A similar procedure to that described for compound **13** carried out with **8a** (0.04 g, 0.06 mmol) and methylamine (5 mL) yielded nucleoside **14** (0.03 g, 85%) as a white foam. Anal. ($C_{23}H_{43}N_5O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-(*N*,*N*-dimethylcarbamoyl)-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (15 and 16). A solution of a 3:1 mixture of **7a** and **8a** (0.1 g, 0.17 mmol) in dimethylamine (33 wt % in ethanol, 5 mL) was stirred at room temperature for 8 h. The residue was purified by CCTLC on chromatotron (dichloromethane/methanol, 200:1). The fastest moving band afforded 0.02 g (19%) of **16** as a white foam. Anal. ($C_{24}H_{45}N_5O_7SSi_2$) C, H, N, S.

The slowest moving band gave 0.06 g (58%) of **15** as a white foam. Anal. ($C_{24}H_{45}N_5O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-methyl-1,2,3-triazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (17). A solution of spironucleoside **7f** (0.04 g, 0.064 mmol) in methanol (10 mL) containing concentrated NH_4OH (0.04 mL) and Pd/C (10%; 0.003 g) was hydrogenated at 30 psi at room temperature for 1 h. The reaction mixture was filtrated, and the filtrate was evaporated under reduced pressure. The residue was purified by CCTLC on chromatotron (dichloromethane/methanol, 200:1) to give 0.02 g (57%) of **17** as an amorphous solid. Anal. ($C_{22}H_{42}N_4O_6SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4- and 5-[(methyloxy)carbonyl]-1,2,3-triazole]-3′-spiro-5″-[4″-(acetylamino)-1″,2″-oxathiole 2″,2″-dioxide] (18 and 19). Following the general procedure described for the synthesis of triazole nucleosides **7**–**10**, azide **6** (0.04 g, 0.07 mmol) was reacted with methyl propiolate (0.13 mL, 1.5 mmol) for 18 h. The residue was purified by CCTLC (dichloromethane/methanol, 200:1). The fastest moving band yielded 0.03 g (67%) of **18** as a white foam: ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 24.64 (CH_3CO), 52.27 (CH_3O), 62.86 (C-5′), 76.22 (C-2′), 86.55 (C-3″), 90.14 (C-4′), 94.56 (C-1′), 108.42 (C-3′), 131.64 (C-5), 140.84, 140.91 (C-4, C-4″), 161.24, 169.82 (2CO). Anal. ($C_{25}H_{44}N_4O_9SSi_2$) C, H, N, S.

The slowest moving band afforded 0.01 g of a syrup that was identified by ^1H NMR as a 2:1 mixture of **18** and **19**: ^1H NMR [$(CD_3)_2CO$, 200 MHz] δ 2.27 (s, 6H, $2CH_3CO$), 3.90 (s, 3H, CH_3OCO **18**), 3.97 (s, 3H, CH_3OCO **19**), 4.15 (m, 4H, H-5′), 4.47 (m, 2H, H-4′), 5.57 (d, 1H, H-2′ **18**, $J_{1',2'}$ = 7.7 Hz), 5.69 (d, 1H, H-2′ **19**, $J_{1',2'}$ = 7.7 Hz), 6.18 (d, 1H, H-1′ **18**), 6.71 (d, 1H, H-1′ **19**), 7.61 (s, 2H, H-3″), 8.37 (s, 1H, H-4 **19**), 8.96 (s, 1H, H-5 **18**), 9.84 (s, 2H, NH-Ac). Anal. ($C_{23}H_{42}N_4O_8SSi_2$) C, H, N. S.

Antiretrovirus Activity Assays. HIV-1 was originally obtained from the culture supernatant of the persistently HIV-infected H9 cell line (H9/HTLV-III_B), which was kindly provided by R. C. Gallo and M. Popovic (National Institutes of Health, Bethesda, MD). HIV-1 (ROD) was obtained from L. Montagnier (Pasteur Institute, Paris, France). HIV-1/HE represents as HIV-1 strain isolated in our laboratory from an AIDS patient.[1] Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells.

The methodology of the anti-HIV assays has been described previously.[47] MT-4 cells or CEM cells (5×10^5 cells/mL) were suspended in fresh RPMI-1640 culture medium containing 10% fetal calf serum, 2 mM L-glutamine, and 0.075% $NaHCO_3$ and infected with HIV-1 or HIV-2 at 100 times the 50% cell culture infective dose ($CCID_{50}$) 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 test compounds (i.e., 100, 20, 4, 0.8, 0.16, 0.032, and 0.006 μg/mL). After 4 days, virus-induced syncytium formation was recorded microscopically in the HIV-

infected CEM cell cultures. In virus-infected control cell cultures, approximately 200 giant cells were recorded. After 5 days, the number of viable MT-4 cells for both virus-infected and mock-infected MT-4 cell cultures was determined by trypan blue staining. In mock-infected control cell cultures, approximately 150–200 cells were counted microscopically. Viability was usually more than 95%. The 50% effective concentration (EC_{50}) was defined as the compound concentration required to reduce by 50% the number of syncytia in the CEM cell cultures or the number of viable cells in the HIV-infected MT-4 cell cultures. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce by 50% the number of mock-infected MT-4 cells.

Human macrophages (M/M) were separated by 5-day adherence to plastic wells (10^6 cells/well) as described earlier.[48] More than 95% pure M/M preparations were obtained. Nonadherent cells were removed by extensive washing with a phosphate-buffered saline (PBS) solution. Purity of the M/M was ascertained according to nonspecific esterase activity determination and Giemsa staining. The M/M were then infected with 500 minimal infective doses per well of HIV-1/Ba-L and treated with various concentrations of the test compounds (i.e., 100, 20, 4, 0.8, 0.16, 0.032, and 0.0016 μM) starting from 30 min before virus challenge. Virus excess was removed at 48 h after challenge, and medium was replaced by fresh medium containing similar compound concentrations. Virus production was determined at day 14 postvirus challenge by HIV-p24 ELISA (Abbott Laboratories, Pomezia, Italy).

HIV-1 p24 core antigen was quantified in the CEM cell cultures infected with HIV-1/HE at 4 days after infection by an antigen-capture assay using a sandwich ELISA technique (DuPont) according to the company's manual description.

Selection of HIV-1 (III_B) Mutant Strains. HIV-1 (III_B) was subjected to several subpassages in 5-mL CEM cell cultures ($3-4 \times 10^5$ cells/mL) in the presence of 1.5 μM compound **16** in 25-cm^2 culture flasks (Falcon, Becton Dickinson). The multiplicity of the initial infection was approximately 200 times in $CCID_{50}$. Passages were performed every 3–4 days by addition of 0.5–1.0 mL of the infected culture supernatant to 4–5 mL of a suspension containing $3-4 \times 10^5$ uninfected CEM cells/mL. The virus that broke through was recovered from the third passage after full cytopathicity was observed.

Preparation of HIV-1-Infected Cell Cultures for PCR Analysis and Sequencing of the RT Gene. CEM cells (3×10^5 cells/mL) were infected with the mutant HIV-1 strain at 200 times the $CCID_{50}$ value and incubated in RPMI-1640 culture medium for 3 days at 37 °C. Then, cells were centrifuged and washed twice with phosphate-buffered saline in 1.5-mL Eppendorf tubes. To 10^6 CEM cells was added 100 μL containing 10 μL of PCR buffer [10 × concentrated: 100 mM Tris·HCl, pH 8.3, 500 mM $MgCl_2$, 0.01% (w/v) gelatin (Cetus-Vanderheyden, Brussels, Belgium)], 8 μL of $MgCl_2$ (25 mM), 72 μL of Milli-Q water, and 10 μL of proteinase K (10 μg; Calbiochem) in 0.5% Tween 20 and 0.5% Nonidet P-40 in H_2O. The cell suspension was then incubated at 56 °C for 1 h and subsequently heated at 95 °C for 10 min. The samples were stored at −20 °C before PCR analysis.

Amplification of proviral DNA was performed with an extract from 1×10^5 cells in 10 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 2.5 units of thermostable DNA polymerase (Dyna Zyme, Finnzymes Inc.), and 0.15 μM concentrations of each primer in a final volume of 100 μL. Oligonucleotides were chosen (sense primer, 5′-CCTGAAAATCCATACAATACTC-CAGTATTTG-3′; reverse complement primer, 5′-AGT-TGCTTTGGTTCCTCTAAGGAGTTTAC-3′) to give a 727-base pair fragment covering amino acids 50–270. The PCR product was purified from a 1% low-melting point agarose gel by MagicPCR Preps (Promega), directly sequenced with a Taq Dye Deoxy Terminator sequencing kit (Applied Biosystems), and analyzed on a model 373A DNA sequencer (Applied Biosystems).

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