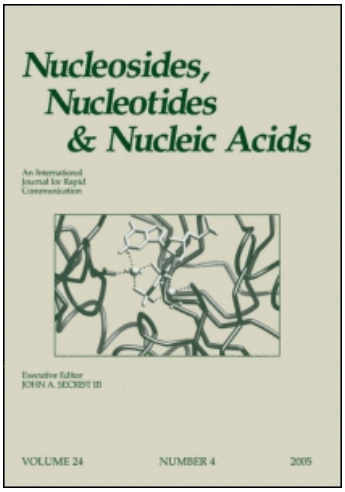

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NOVEL TSAO DERIVATIVES. SYNTHESIS AND ANTI-HIV-1 ACTIVITY OF ALLOFURANOSYL-TSAO-T ANALOGUES‡

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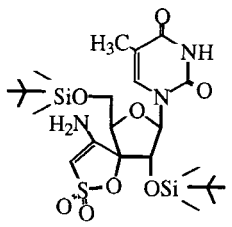
Abstract: *Novel TSAO-T analogues, in which the ribofuranosyl moiety has been replaced by an hexofuranosyl sugar moiety, have been prepared and evaluated for their inhibitory effect on HIV-1 replication in cell culture. In contrast to the prototype compound TSAO-T, the hexofuranosyl derivatives proved not active at subtoxic concentrations.*

TSAO derivatives are the first nucleoside analogues reported to show specificity for human immunodeficiency virus type 1 (HIV-1). They are potent and selective inhibitors of HIV-1, but not HIV-2 or other (retro)viruses,[1-7] and are specifically targeted at the HIV-1-encoded reverse transcriptase (RT) at a non-substrate binding site.[7] The prototype compound is [1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl] thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) designated as TSAO-T (**1**).[8] The amino group of the spiro moiety of TSAO molecules has been proposed as the active pharmacophore interacting with the COOH group of a glutamic acid residue at position 138 (Glu-138) of the p51 subunit of HIV-1-RT.[9-12] TSAO molecules are, at present, the only example among the HIV-1-specific RT inhibitors, that interact directly with the p51 subunit of the RT p66/p51 heterodimer.[11]



TSAO-T (1)

‡Dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday

Extensive structure-activity relationship studies have shown that the thymine moiety of TSAO-T can be replaced by other pyrimidines, purines, or 1,2,3-triazoles without marked decrease of antiretroviral efficacy.[3,4,8,13-15] However, the sugar part turns out to be very stringent in its structural requirements, and only those TSAO derivatives having the spiro moiety in nucleosides with a *ribo* configuration are endowed with antiviral activity.[1] Also, the presence of lipophilic silyl groups at both C-2' and C-5' of the ribose is a prerequisite for antiviral activity.[3,16]

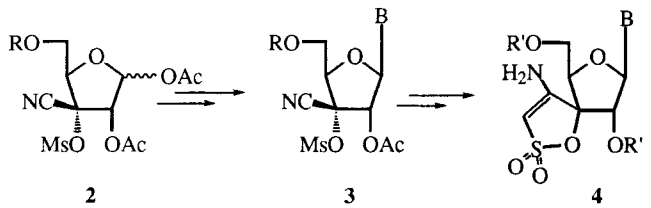
Here we report the synthesis and anti-HIV-1 activity of novel sugar-modified TSAO analogues. In these analogues the ribofuranosyl moiety has been replaced by an hexofuranosyl sugar moiety, in order to determine whether the presence of an extra carbon atom at C-5' position would play a significant role in the antiviral activity/toxicity profile of the TSAO derivatives. Our studies are aimed at further evaluating the structural features required for anti-HIV-1 activity, and improving the antiviral potency and /or selectivity of the TSAO derivatives.

RESULTS AND DISCUSSION

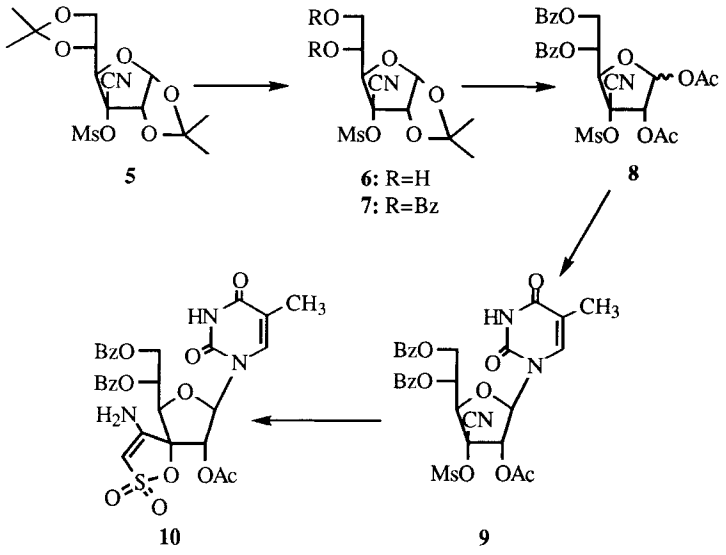
For the synthesis of the hexofuranosyl-TSAO analogues we followed the method developed in our laboratory for the stereospecific synthesis of TSAO derivatives.[4] The method (SCHEME 1) involves the condensation of persilylated heterocyclic bases with the suitably functionalized and protected sugar intermediate **2**, followed by basic treatment of the cyanomesyl nucleosides thus obtained, to give, exclusively, β-D-ribospironucleosides (**3**). We have used this procedure for the synthesis of a variety of 3'-spironucleosides of purines and pyrimidines.[4,13,14]

The starting material 3-*C*-cyano-1,2:5,6-di-*O*-isopropylidene-3-*O*-mesyl-α-D-allofuranose (**5**)[17] (SCHEME 2), was readily obtained from 1,2:5,6-di-*O*-isopropylidene-glucofuranose in three steps. Compound **5**, was hydrolyzed selectively using 70 % acetic acid to the corresponding 5,6-diol **6**, which by reaction with benzoyl chloride in pyridine gave the 5,6-dibenzoyl derivative **7** in 75 % yield. Compound **7** was converted to the corresponding 1,2-diacetate **8**, in 85 % yield, by hydrolysis of the 1,2-*O*-isopropylidene group with a (9:1) mixture of trifluoroacetic acid:water, followed by acetylation with acetic anhydride in pyridine.

Furanoside **8** was condensed with bis(trimethylsilyl)thymine in the presence of trimethylsilyl triflate (TMS-Tfl), under standard Vorbrüggen conditions,[18] to give 3'-cyanomesyl nucleoside **9** in 53 % yield. Structure of **9** was assigned on the basis of the corresponding analytical and spectroscopic data. Since, no epimerization occur during mesylation of cyanohydrins,[17,19, 20-23] the absolute configuration of cyano mesylate **9** was assumed to be the same as that of the corresponding cyanohydrin **8**, as clearly demonstrated in previous papers of this series.[19,20] The presence in the starting sugar (**8**) of a 2-*O*-acyl participating group led, exclusively, to β-anomers.[18]



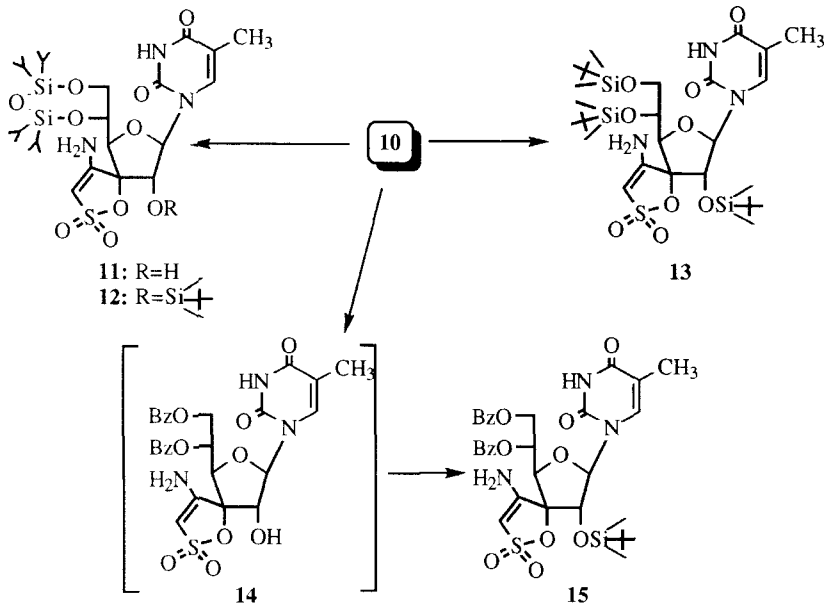
SCHEME 1



SCHEME 2

Treatment of **9** with DBU, in dry acetonitrile at room temperature, afforded 3'-spiroderivative **10** in 80 % yield. As described previously for other spironucleosides of this series,[3,19,20] formation of the spiroaminooxathiole dioxide ring 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 7.35 ppm assigned to NH_2-4" and at 5.40 ppm assigned to H-3".

Deprotection of **10** (SCHEME 3) using methylamine in ethanol followed by reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine, gave the 5,6-*O*-protected nucleoside **11** (60 % yield). Treatment of **11** with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) and pyridine afforded the fully protected nucleoside **12** in 70 %



SCHEME 3

yield. Similarly, deprotection of **10** (methylamine/ ethanol) followed by reaction with excess of TBDMS-Cl in the presence of 4-(dimethylamino)pyridine afforded the trisilylated nucleoside **13** (60 % yield).

Finally, selective deprotection of allospironucleoside **10** with methanolic ammonia, gave the 2'-*O*-deprotected compound **14** which by silylation with TBDMS-Cl and 4-(dimethylamino)pyridine yielded the 2'-*O*-silylated spironucleoside **15** (65 % yield, from **10**).

Compounds **11, 12, 13, 15** and the parental TSAO-T derivative have been evaluated on their anti-HIV-1 and -HIV-2 activity in CEM and MT-4 cell cultures (TABLE 1). In contrast with TSAO-T, none of the hexofuranosyl derivatives showed antiviral activity at subtoxic concentrations. Introduction of the *tert*-butyldimethylsilyl group at the 2'-position of the sugar decreased the cytotoxicity by 5 fold (compare compounds **11** and **12**), whereas **13** was devoid of any cytotoxicity at 100 μg/mL. Interestingly, replacement of the two silyl groups in **13** by two benzoyl groups, results in a pronounced increase of cytotoxicity.

TABLE 1. *Anti-HIV-1 and -HIV-2 activity of TSAO-T derivatives in cell culture*

	EC_{50}(μg/mL)[a]			CC_{50}(μg/mL)[b]	
	CEM	MT-4		CEM	MT-4
Compd.	HIV-1	HIV-1	HIV-2		
11	>4	>4	>4	7.3 ± 0.8	7.5
12	>20	>20	>20	39 ± 4.0	3.3
13	>100	>100	>100	>100	>100
15	>20	>4	>4	2.8 ± 0.5	5.9 ± 4.1
TSAO-T	0.03 ± 0.01	0.03 ± 0.02	>4	—	7.7 ± 1.5

[a] 50 % effective concentration
[b] 50 % cytotoxic concentration

EXPERIMENTAL

Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. ^{1}H NMR spectra were recorded with a Varian XL-300 spectrometer operating at 300 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^R (Kiesegel 60 PF 254 gipshaltig (Merck)), layer thickness (1mm), flow rate (5 mL/min). Flash column chromatography was performed with silica gel 60 (230-400 mesh) (Merck).

5,6-Di-*O*-benzoyl-3-*C*-cyano-1,2-*O*-isopropylidene-3-*O*-mesyl-α-D-allofuranose (7). A solution of 3-*C*-cyano-1,2:5,6-di-*O*-isopropylidene-3-*O*-mesyl-α-D-allofuranose (**5**)[17] (2 g, 5.5 mmol) in 70% aqueous acetic acid (30 mL) was heated at 80°C for 1 h. The solution was evaporated to dryness, and the residue was co-evaporated twice with ethanol (2 x 20 mL) and then with toluene (20 mL). The residue (deprotected derivative **2**) was benzoylated with benzoyl chloride (3.2 mL) and pyridine (20 mL) at room temperature overnight. The solvents were evaporated under reduced pressure. The residue was dissolved in ethyl acetate (40 mL), washed with water (2 x 50 mL) then, with 1N HCl (50 mL) and finally with brine (50 mL), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 3:1) to afford **7** (2.19 g, 75%) as a white foam. $[\alpha]_D$ = + 7.7° (c 1,

$CHCl_3$). IR (KBr) 1720 cm^{-1} (C=O), 1375, 1185 (SO_2); ^1H NMR ($CDCl_3$): δ 1.35, 1.55 (2s, 6 H, Me_2C), 2.84 (s, 3 H, SO_2Me), 4.55 (dd, 1 H, H-6b, $J_{5,6}$ = 4.5, J_{gem} = 12.3 Hz), 4.57 (d, 1 H, H-4, $J_{4,5}$ = 8.9 Hz), 4.85 (dd, 1 H, H-6a, $J_{5,6a}$ = 2.5 Hz), 5.10 (d, 1 H, H-2, $J_{1,2}$ = 3.6 Hz), 5.78 (m. 1 H, H-5), 5.94 (d, 1 H, H-1), 7.38, 7.52, 7.98 (3 m, 10 H, 2 OBz). Anal. Calcd. for $C_{25}H_{25}NO_{10}S$: C, 56.49; H, 4.74; N, 2.63; S, 6.03. Found: C, 56.19; H, 4.78; N, 2.54; S. 6.11.

1,2-Di-*O*-acetyl-5,6-di-*O*-benzoyl-3-*C*-cyano-3-*O*-mesyl-D-allofuranose (8). A solution of cyano mesylate **7** (2 g, 3.76 mmol) in 8 mL of a (9:1) mixture of trifluoroacetic acid/water was stirred at room temperature for 4 h. The solvent was evaporated to dryness, and the residue was acetylated with acetic anhydride (5.6 mL) and pyridine (13 mL), at room temperature overningh. The solvents were evaporated under reduced pressure. The residue was purified by column chromatography (hexane/ethyl acetate, 2:1) to give **8** (1.83 g, 85%) as slightly yellow syrup consisting of a (2:1) mixture of the α and β anomers (the relative proportions of the α+β anomers was determined from the integrals of the anomeric protons). A small portion of the mixture, was purified by CCTLC on chromatotron (hexane/ethyl acetate, 4:1) to separate the α and β anomers.

β-Anomer. $[\alpha]_D$ = –35.2° (c 1, $CHCl_3$). IR (KBr) 1760, 1720 cm^{-1} (C=O), 1375, 1185 (SO_2); ^1H NMR ($CDCl_3$): δ 2.06, 2.15 (2 s, 6 H, 2 OAc), 2.86 (s, 3 H, $MeSO_2$), 4.79 (d, 1 H, H-4, $J_{4,5}$ = 9,2 Hz), 4.50 (dd, 1 H, H-6a, J_{gem} = 12.6, $J_{5,6a}$ = 4.3 Hz), 4.91 (dd, 1 H, H-6b, $J_{5,6b}$ = 2.9 Hz), 5.62-5.67 (m, 2 H, H-2, H-5), 6.16 (s, 1 H, H-1), 7.36-7.65, 8.0 (2 m, 10 H, 2 OBz). Anal. Calcd. for $C_{26}H_{25}NO_{12}S$: C, 54.26; H, 4.38; N, 2.43; S, 5.57. Found: C, 54.13; H, 4.45; N, 2.60; S, 5.77.

α-Anomer. $[\alpha]_D$ = + 55.3° (c 1, $CHCl_3$). IR (KBr) 1720 cm^{-1} (C=O), 1370, 1185 (SO_2); ^1H NMR ($CDCl_3$): δ 1.97, 2.13 (2 s, 6 H, 2 OAc), 2.87 (s, 3 H, $MeSO_2$), 4.65 (dd, 1 H, H-6a, J_{gem} = 12.4, $J_{5,6a}$ = 5.0 Hz), 4.82-5.00 (m, 2 H, H-4, H-6b, $J_{4,5}$ = 8.1, $J_{5,6b}$ = 3.2 Hz), 5.70 (d, 1 H, H-2, $J_{1,2}$ = 4.7 Hz), 5.78-5.85 (m, 1 H, H-5), 6.48 (d, 1 H, H-1), 7.45, 7.59, 8.05 (3 m, 10 H, 2 OBz). Anal. Calcd. for $C_{26}H_{25}NO_{12}S$: C, 54.26; H, 4.38; N, 2.43; S, 5.57. Found: C, 54.33; H, 4.36; N, 2.52; S, 5.54.

1-(2'-*O*-Acetyl-5',6'-di-*O*-benzoyl-3'-*C*-cyano-3'-*O*-mesyl-β-D-allofuranosyl) thymine (9). Thymine (0.87 g, 6.94 mmol) was silylated with hexamethyldisilazane (HMDS) (15 mL) in the presence of ammonium sulfate (10 mg). The reaction mixture was heated at reflux until the solution became clear. The excess of HMDS was removed under reduce pressure. To the sirupy silylated base, were added, a solution of compound **8** (2 g, 3.47 mmol) in dry acetonitrile (20 mL) and trimethylsilyl triflate (TMS-Tfl) (0.8 mL, 4.33 mmol) and the resulting mixture was heated to reflux. After 2 h an

additional portion of TMS-Tfl (0.8 mL, 4.33) was added, and the refluxing continued for 2 h. The reaction was allowed to cool to room temperature, dichloromethane (30 mL) and cold water (50 mL) were added and the resulting solution was neutralized with $NaHCO_3$. The organic phase was separed and the aqueous phase was washed with dichloromethane (2 x 30 mL). The organic phases were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/ethyl acetate, 3:1). The fastest moving band gave 0.8 g of unreacted sugar (**8**). From the slowest moving band (1.17 g, 53% from reacted sugar) of **9** was obtained as a foam. IR (Nujol) 1760, 1720 cm^{-1} (C=O), 1375, 1180 (SO_2); ^1H NMR ($CDCl_3$): δ 1.90 (s, 3 H, Me-5), 2.21 (s, 3 H, OAc), 2.92 (s, 3 H, $MeSO_2$), 4.60 (dd, 1 H, H-6'b, J_{gem} = 12.5, $J_{5',6'b}$ = 6.5 Hz), 4.67 (d, 1 H, H-4', $J_{4',5'}$ = 8.3 Hz), 4.97 (dd, 1 H, H-6'a, $J_{5',6'a}$ = 3.0 Hz), 5.74 (d, 1 H, H-2', $J_{1',2'}$ = 4.4 Hz), 5.97 (m, 1 H, H-5'), 6.10 (d, 1 H, H-1'), 7.01 (s, 1 H, H-6), 7.55. 8.08 (2 m, 10 H, 2 OBz), 8.51 (bs, 1 H, NH-3). Anal. Calcd. for $C_{29}H_{27}N_3O_{12}S$: C, 54.29; H, 4.24; N, 6.55; S, 4.99. Found: C, 54.33; H, 4.32; N, 6.58; S, 4.93.

[1-(2'-*O*-Acetyl-5',6'-di-*O*-benzoyl-β-D-allofuranosyl)thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (10). To a solution of the cyano mesylate **9** (1 g, 1.56 mmol) in dry acetonitrile (32 mL) was added DBU (0.25 mL, 1.71 mmol). The reaction mixture was stirred at room temperature for 30 min. The solvent was evaporated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 1:2) to give (0.8 g, 80%) of **10** as a foam. ^1H NMR [$(CD_3)_2CO$]: δ 1.85 (s, 3 H, Me-5), 2.21 (s, 3 H, OAc), 4.48 (dd, 1 H, H-6'b, J_{gem} = 12.3, $J_{5',6'b}$ = 4.3 Hz), 4.92 (d, 1 H, H-4', $J_{4',5'}$ = 10.5 Hz), 4.99 (dd, 1 H, H-6'a, $J_{5',6'a}$ = 3.0 Hz), 5.40 (s, 1 H, H-3"), 5.98 (m, 1 H, H-5'), 6.16 (m, AB system, 2 H, H-1', H-2'), 7.35 (bs, 2 H, NH_2-4"), 7.40-7.61, 7.98 (2 m, 11 H, 2 OBz, H-6), 8.21 (bs, 1 H, NH-3). Anal. Calcd. for $C_{29}H_{27}N_3O_{12}S$: C, 54.29; H, 4.24; N, 6.55; S, 4.99. Found: 54.31; H, 4.26; N, 6.35; S, 5.11.

[1-[5',6'-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-allofuranosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (11). The spiro derivative **10** (0.3 g, 0.47 mmol) was treated with methylamine (33 wt % solution in EtOH, 30 mL) and the reaction mixture was stirred at 4°C overnight. The solvent was evaporated to dryness. The residue was dissolved in pyridine (12 mL) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.38 mL, 1.22 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated to dryness. The resulting residue was purified by column chromatography (chloroform/methanol, 50:1) to give **11** (0.18 g, 60%) as a white amorphous solid. ^1H NMR [$(CD_3OD$]: δ 1.1-1.25 (m, 28 H, isopropyl), 2.10 (s, 3 H, Me-5), 4.10 (dd, 1 H, H-6'b, J_{gem} = 12.7, $J_{5',6'b}$ = 4.9 Hz), 4.30 (dd, 1 H, H-6'a, $J_{5',6'a}$ = 1.5 Hz), 4.43 (d, 1 H, H-4', $J_{4',5'}$ = 8.8 Hz), 4.60 (m, 1 H,

H-5'), 4.75 (d, 1 H, H-2', $J_{1',2'}$ = 8.3 Hz), 4.81 (bs, 2 H, NH_2-4"), 5.69 (s, 1 H, H-3"), 6.05 (d, 1 H, H-1'), 7.76 (s, 1 H, H-6). Anal. Calcd. for $C_{25}H_{43}N_3O_{10}SSi_2$: C, 47.39; H, 6.79; N, 6.63; S, 5.06. Found: C, 47.33; H, 6.82; N, 6.42; S, 5.16.

[1-[2'-*O*-(*tert*-Butyldimethylsilyl)-5',6'-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-allofuranosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (12). A solution of **11** (0.15 g, 0.24 mmol) in dry acetonitrile (10 mL) was treated with 4-(dimethylamino)pyridine (0.12 g, 1 mmol) and *tert*-butyldimethylsilyl chloride (0.075 g, 0.5 mmol). The mixture was stirred at 70°C overnight, and then, evaporated to dryness. The residue was treated with ethyl acetate (5 mL), the solid was filtered and the filtrate was evaporated to dryness. The syrupy residue was purified by CCTLC on chromatotron to give **12** (0.13 g, 70%) as an amorphous solid. ^{1}H NMR ($CDCl_3$): δ 0.91-1.08 (m, 28 H, isopropyl), 1.95 (s, 3 H, Me-5), 3.92 (dd, 1 H, H-6'b, J_{gem} = 12.8, $J_{5',6'b}$ = 7.3 Hz), 4.01 (dd, 1 H, H-6'a, $J_{4',5'a}$ = 1.8 Hz) 4.29 (d, 1 H, H-4', $J_{4',5'}$ = 4.9 Hz), 4.41 (m, 1 H, H-5'), 4.77 (d, 1 H, H-2', $J_{1',2'}$ = 7.9 Hz), 5,34 (bs, 2 H, NH_2-4"), 5.57 (d, 1 H, H-2'), 5.63 (s, 1 H, H-3"), 7.08 (s, 1 H, H-6), 9.02 (bs, 1 H, NH-3). Anal. Calcd. for $C_{31}H_{57}N_3O_{10}SSi_3$: C, 49.79; H, 7.63; N, 5.62; S, 4.28. Found: C, 49.72; H, 7.73; N, 5.82; S, 4.31.

[1-[2',5',6'-Tri-*O*-(*tert*-butyldimethylsilyl)-β-D-allofuranosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (13). The spiroderivative **10** (0.3 g, 0.47 mmol) was treated with methylamine (33 wt % solution in EtOH, 30 mL) and the reaction mixture was stirred at 4°C overnight. The solvent was evaporated to dryness. The residue was suspended in a 1:1 mixture of dry acetonitrile and DMF and treated with 4-(dimethylamino)pyridine (0.58 g, 4.8 mmol) and *tert*-butyldimethylsilyl chloride (0,36 g, 2.4 mmol). The mixture was heated at 70°C overnight, and then, evaporated to dryness. The residue was dissolved in ethyl acetate (30 mL), washed successively with water (2 x 30 mL) and brine (2 x 30 mL), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 7:1) to afford **13** (0.20 g, 60%) as an amorphous solid. ^{1}H NMR [$(CD_3)_2CO$]: δ 0.82 (s, 9 H, t-Bu), 3.82 (dd, 1 H, H-6'b, J_{gem} = 11.7, $J_{5',6'b}$ = 4.7 Hz), 3.89 (dd, 1 H, H-6'a, $J_{5',6'a}$ = 5.5 Hz) 4.27 (m, 1 H, H-5'), 4,45 (d, 1 H, H-4', $J_{4',5'}$ = 4.8 Hz), 4.68 (d, 1 H, H-2', $J_{1',2'}$ = 8,4 Hz), 5,74 (s, 1 H, H-3"), 5.90 (d, 1 H, H-1'), 6.36 (bs, 2 H, NH_2-4"), 7.40 (s, 1 H, H-6), 10.15 (bs, 1 H, NH-3). Anal. Calcd. for $C_{31}H_{59}N_3O_9SSi_3$: C, 50.75; H, 8.05; N, 5.73; S, 4.36. Found: C, 50.65; H, 8.11; N, 5.75; S, 4.38.

[1-[5',6'-Di-*O*-benzoyl-2'-*O*-(*tert*-butyldimethylsilyl)-β-D-allofuranosyl] thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (15). The protected nucleoside **10** (0.2 g, 0.31 mmol) was treated with methanolic ammonia (8 mL). After stirring at room temperature for 3 h, the solvent was evaporated to dryness. The residue

(deprotected nucleoside **14**) was suspended in dry acetonitrile (15 mL) and then 4-(dimethylamino)pyridine (0.15 g, 1.25 mmol) and *tert*-butyldimethylsilyl chloride (0.09 g, 0.62 mmol) were added. The mixture was heated at 80°C overnight. The solvent was evaporated to dryness. The residue was treated with ethyl acetate (5 mL). The solid was filtered, and the filtrate was evaporated to dryness. The residue was purified by CCTLC on chromatotron (dichloromethane/methanol, 200:1) to give (.014 g, 65%) of **15** as an amorphous solid. ^{1}H NMR ($CDCl_3$): δ 0.82 (s, 9 H, t-Bu), 1.96 (s, 3 H, CH_3-5), 4.41 (dd, 1 H, H-6'a, J_{gem} = 13, $J_{5',6'a}$ = 4.1 Hz), 4.75 (dd, 1 H, H-6'a, $J_{4',5'a}$ = 2.5 Hz) 4.83 (d, 1 H, H-4', $J_{4',5'}$ = 10.1 Hz), 5.14 (d, 1 H, H-2', $J_{1',2'}$ = 6.2 Hz), 5.38 (s, 1 H, H-3"), 5.50 (d, 1 H, H-1'), 5.53 (bs, 2 H, NH_2-4"), 5.84 (m, 1 H, H-5'), 7.01 (s, 1 H, H-6), 7.30-7.62, 7.87 (2 m, 10 H, 2 OBz), 10.47 (bs, 1 H, NH-3). Anal. Calcd. for $C_{33}H_{39}N_3O_{11}SSi$: C, 55.53; H, 5.47; N, 5.89; S, 4.48. Found: C, 55.51; H, 5.52; N, 5.93; S, 4.50.

Antiretroviral Evaluation. CEM cells were obtained from the American Tissue Culture Collection (Rockville, MD). MT-4 cells were a kind gift of Dr. N. Yamamotto (Yamagushi University, Yamacushi Japan). HIV-1 (III_B) and HIV-2 (ROD) were generously provided by Dr. R.C. Gallo (National Cancer institute, NIH, Bethesda, M.D.) and Dr. L. Montaigner (Pasteur Institute, Paris, France), respectively.

The cells were suspended at 250,000 cells/mL of cell culture medium and infected with HIV-1 (III_B) or HIV-2 (ROD) at 100 $CCID_{50}$/mL. Then, 100 µL of the infected cell suspension were added to 200 µL microtiter plate wells containing 100 µL of an apropriate dilution of the test compounds. After 4 days (CEM) or 5 days (MT-4) of incubation at 37°C, the cell cultures were examined for syncytium formation (CEM) or cell lysis (MT-4). The EC_{50} was determined as the compound concentration required to inhibit syncytium formation (CEM) by 50%, or to reduce cell viability (MT-4) by 50%.

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