TSAO Analogues. 3.[1] Synthesis and Anti-HIV-1 Activity of 2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl 3'-Spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) Purine and Purine-Modified Nucleosides

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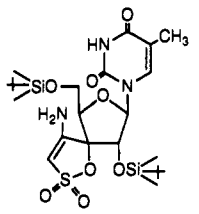
Several purine and purine-modified analogues of the new lead anti-HIV-1 agent [[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (TSAO-T) have been prepared and evaluated as inhibitors of HIV-1-induced cytopathicity. Reaction of *O*-mesylcyanohydrins of furanos-3'-ulosyladenine with Cs_2CO_3 afforded β-D-xylo- and ribofuranosyladenine 3'-spiro nucleosides. Reaction of 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-*C*-cyano-3-*O*-mesyl-D-ribofuranose with purine bases, followed by treatment with Cs_2CO_3, stereoselectively afforded β-D-ribofuranosyl 3'-spiro nucleosides. 2',5'-*O*-Deacylation and subsequent treatment with *tert*-butyldimethylsilyl chloride gave the required TSAO derivatives. The 3'-spiro nucleosides with a *xylo* configuration did not show any anti-HIV activity. However, the purine *ribo* 3'-spiro nucleosides were potent and selective inhibitors of HIV-1 with a 50% effective concentration in the range of 0.1–1 μM and a selectivity index ranging from 2 to 3 orders of magnitude. Introduction of an alkyl function at N-1 of the purine moiety markedly decreased cytotoxicity without affecting antiviral activity.

Introduction

In the last two years, different nonstructurally related non-nucleoside analogues (i.e., HEPT,[2-6] TIBO,[7,8] nevirapine,[9,10] pyridinones,[10-12] BHAP,[13] and α-APA[14]) have been reported as potent and highly specific inhibitors of human immunodeficiency virus type 1 (HIV-1), but not HIV-2, simian immunodeficiency virus (SIV), or other RNA viruses or DNA viruses. They are targeted at the HIV-1 reverse transcriptase (RT) with which they interact at a nonsubstrate binding site.[9,11,13,14] We have recently discovered a novel class of nucleoside derivatives that is highly specific in its antiviral action against HIV-1.[15-18]

The prototype compound is [[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide)[19] (designated as TSAO-T, 1, Figure 1). The TSAO analogues selectively inhibit HIV-1 replication, but not HIV-2, SIV, or other retroviruses;[15-18] they interact with HIV-1 RT at a nonsubstrate binding site.[20,21] To inhibit HIV-1 replication, TSAO-T has to fulfill stringent structural requirements, including (i) the presence of *tert*-butyldimethylsilyl groups at both C-2' and C-5' of the ribose and (ii) the presence of the unique 3'-spiro group [3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide)] in the *R* (*ribo*) configuration. However, the thymine of the TSAO-T molecule can be substituted by other pyrimidines without marked decrease of antiviral efficacy.[15,17,18] Introduction of an alkyl group at N-3 of the thymine moiety markedly decreases cytotoxicity without affecting antiviral activity.[15,17,18] Consequently, the 3-alkyl-substituted TSAO-T derivatives possess the highest antiviral selectivity in cell culture.



1
TSAO-T

Figure 1.

We report herein the synthesis and anti-HIV-1 activity of a series of purine and purine-modified TSAO analogues. Our studies were 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. The N-1 alkyl analogues of the most active purine derivatives were prepared in order to determine whether a similar alkylation as noted for the TSAO–pyrimidine analogues might also decrease the cytotoxicity of the TSAO–purine compounds. In addition, the *xylo* TSAO-adenine derivative was prepared to confirm the importance of the configuration of the spiro substituent at *C*-3'.

Chemistry

The xylo- and ribofuranosyl adenine nucleosides **10** and **11** were prepared following our reported procedure for the synthesis of the xylo- and ribofuranosyl TSAO-pyrimidine nucleosides.[16,17] Thus, treatment of the 3'-keto nucleoside **3** (Scheme I), obtained by oxidation (CrO_3/pyridine/Ac_2O)[22] of the 6-*N*-monomethoxytrityl (MMTr) adenine nucleoside **2**,[23] with sodium cyanide in the presence of sodium bicarbonate afforded, after mesylation (mesyl chloride/pyridine) of the two epimeric 3'-cyanohydrins (**4** and **5**), a mixture of the respective 3'-*C*-cyano-3'-*O*-mesyl-

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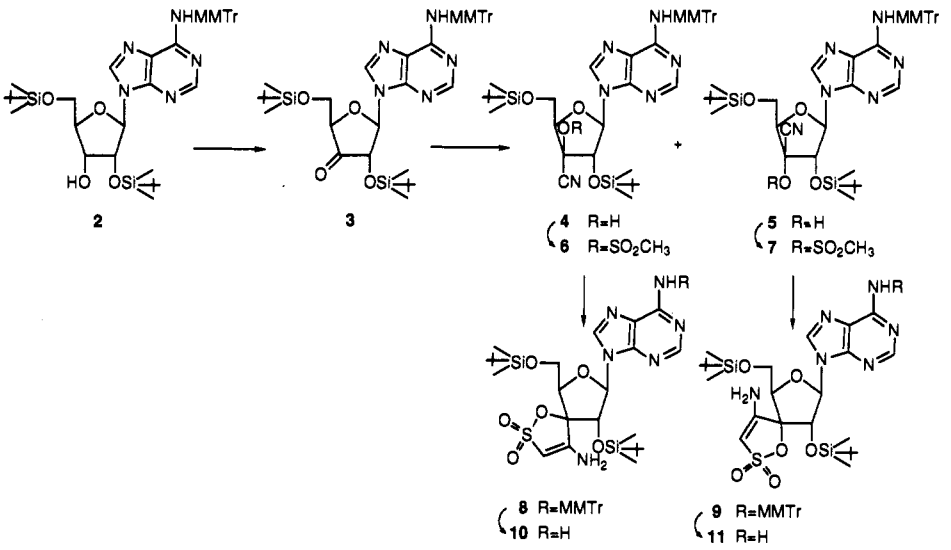
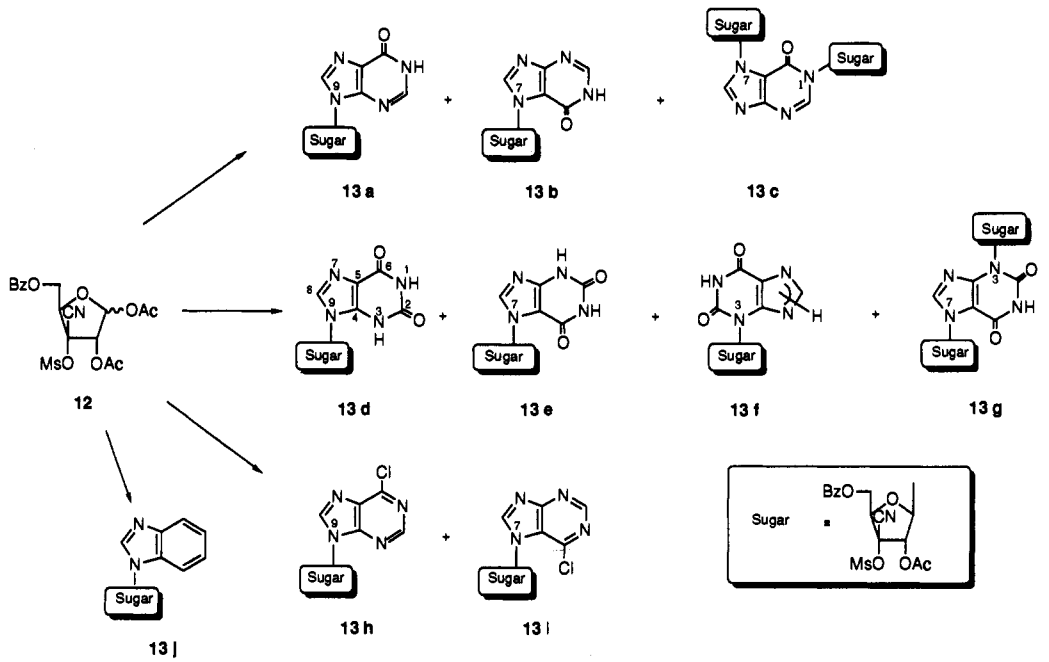
Scheme I



Scheme II



xylo and *ribo* nucleosides **6** (67%) and **7** (10%). Treatment of **6** and **7** with Cs_2CO_3 gave the 3′-spiro derivatives **8** (82%) and **9** (45%). Finally, deprotection of the 6-*N*-MMTr groups of **8** and **9** with $ZnBr_2$ in nitromethane[24] gave adenine nucleosides **10** (50%) and **11** (59%).

Since no epimerization has been observed during mesylation of cyanohydrins,[17,25-29] the absolute configuration of the cyanomesylates **6** and **7** was assumed to be the same as that of the corresponding cyanohydrins **4** and **5** and were assigned as *xylo* for the major compound **6** and *ribo* for the minor compound **7** by comparison of their spectroscopic data with those of the previously reported *xylo* and *ribo* 3′-cyanomesyl pyrimidine nucleosides, whose structure has been unequivocally determined in an earlier paper of this series.[17]

As previously described,[17,18,25,26] formation of the spiro-(aminooxathiole dioxide) ring in **8-11** was established by the disappearance in the ^{1}H-NMR spectra (Table I) of the signal corresponding to the mesyl group and the presence of two new singlets at δ 6.40–6.89 assigned to NH_2-4″ and at δ 5.68–5.79 assigned to H-3″.

The *ribo* spironucleosides **16a–j** were stereoselectively synthesized by condensation of the trimethylsilylated purine base with a suitably functionalized and protected ribofuranosyl derivative **12**,[18] using trimethylsilyltriflate (TMS-Tfl) as condensing reagent,[30] followed by basic treatment of the cyanomesyl nucleosides thus obtained, to give, exclusively, β-D-ribo spiro nucleosides (Schemes II and III). The *ribo* configuration of the nucleosides was determined by the configuration of the starting cyanohydrin used in the preparation of sugar intermediate **12**.[18,25,26] The β-anomeric configuration was determined by the presence in the sugar **12** of a 2-*O*-acyl participating group that led, exclusively, to β-anomers.[30] Thus, glycosylation of **12** with hypoxanthine (Scheme II) in the presence of an excess of TMS-Tfl [ratio of the reagents (mol) 1:1.2:2.2] gave a mixture of the *N*-9- and *N*-7-glycosyl derivatives **13a** (17%) and **13b** (22%) and the *N*-1,7-diglycosyl compound **13c** (19%). It should be emphasized that formation of **13c** was minimized when higher concentrations of the purine base (2.5 mol) and TMS-Tfl (3–4 mol) were used, thus resulting in higher total yield of

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

compd	H-1′ ($J_{1',2'}$)	H-2′	H-4′	H-5′ ($J_{4',5'a}$, $J_{4',5'b}$)	H-2	H-8	others
6[a]	6.17 d (2.0)	5.47 d	4.74 t	4.17 dd, 4.22 dd (6.3, 6.1)	7.95 s	8.19 s	3.35 s, SO_2CH_3; 8.00 bs, NH
7[a]	6.14 d	5.43 d	4.84 dd	4.14 dd	7.95 s	8.30 s	3.47 (s, SO_2CH_3; 8.00 bs, NH
8[a]	6.18 d (4.2)	5.06 d	4.79 t	4.00 d (5.4, 5.1)	7.95 s	8.32 s	5.68 s, H-3″,6.40 bs, NH_2-4″; 8.00 bs, NH
9[a]	6.01 d (4.2)	5.25 d	4.31 dd	4.05 dd, 4.13 dd (6.1, 2.9)	7.91 s	8.35 s	5.68 s, H-3″; 6.89 bs, NH_2-4″; 6.80 NH
10[a]	6.21 d (4.2)	5.08 d	4.79 t	4.01 d (5.2, 5.2)	8.27, 8.31 s		5.69 s, H-3″; 6.41, 6.74 bs, NH_2-4″, NH_2
11[a]	6.02 d (6.7)	5.31 d	4.31 dd	4.07 dd, 4.16 dd (6.4, 2.9)	8.22, 8.33 s		5.70 s, H-3″; 6.86, 7.06 NH_2-4″, NH_2
13a[b]	6.49 d (7.8)	6.62 d	5.08 t	4.65 dd, 4.92 dd (5.8, 6.1)	8.11 s	8.65 s	3.57 s, SO_2CH_3
13b[b]	6.35 d (7.9)	6.69 d	5.12 t	4.66 dd, 4.92 dd (3.9, 4.7)	7.79 s	8.38 s	3.59 s, SO_2CH_3
13c[b]	6.37 d, 6.61 s	6.61 s, 6.71 d	4.70–5.04 m		8.68 s, 8.66 s		3.22, 3.28 s, SO_2CH_3
13d[a]	6.11, 6.14 d		4.94 d	4.66 m (4.7)			3.40 s, SO_2CH_3
13e[a]	6.42 d (7.5)	6.60 d	5.09 t	4.80 dd, 5.00 dd (5.5, 5.6)		8.23 s	3.49 s, SO_2CH_3; 9.97, 10.66 bs, 2NH
13f[a]	6.51 d (7.0)	7.03 d	4.93-4.99 m	4.76 dd (6.7)		7.91	3.50 s, SO_2CH_3; 10.19 (bs, NH)
13g[a]	6.49 s, 6.50 d (6.7)	6.49 s, 6.87 d (6.7)	4.75–5.10 m			8.26 s	3.47, 3.49 s, SO_2CH_3; 10.51 bs, NH
13h[b]	6.55 d (7.9)	6.80 d	5.22 t	4.72 dd, 4.99 dd (4.1, 4.2)	8.49 s	8.99 s	3.63 s, SO_2CH_3
13i[b]	6.36 d (7.4)	6.77 d	5.21 t	4.82 dd, 5.00 dd (4.9, 5.5)	8.89 s	9.23 s	3.59 s, SO_2CH_3
13j[a]	6.22 d	6.54 d	5.14 t	4.88 dd, 5.06 dd	8.34 s		3.52 s, SO_2CH_3; 7.09, 7.25–8.17 aromatics
14h[c]	6.27 d	6.06 d	4.64-4.86 m		8.25 s	8.69 s	5.60 s, H-3″; 6.22 bs, NH_2-4″
16a[a]	6.08 d (7.7)	5.09 d	4.40 dd	4.08 dd, 4.16 dd (4.7, 3.4)	8.22 s	8.26 s	5.78 s, H-3″; 6.67 bs, NH_2-4″; 11.42 bs, NH-1
16b[a]	6.10 d (7.7)	5.23 d	4.41 dd	4.05 dd, 4.14 dd (4.1, 7.0)	8.21 s	8.53 s	5.66 s, H-3″; 6.68 bs, NH_2-4′; 11.66 bs, NH-1
16c[a]	6.09 d, 6.27 d (7.4, 7.6)	5.14 d, 5.46 d	4.14–4.46 m		8.69 s	8.83 s	5.68, 5.79 s, H-3″; 6.75, 6.80 bs, NH_2-4″
16e[a]	5.97 d (7.6)	5.19 d	4.39 dd	4.03 dd, 4.13 dd (4.4, 6.7)		8.32 s	5.67 s, H-3″; 6.55 bs, NH_2-4″; 10.88 bs, 2NH
16f[a]	6.27 d (6.7)	5.35 d	4.04–4.10 m	4.21 dd (2.9)		8.14 s	5.57 s, H-3″; 7.00 bs, NH_2-4″; 10.25 bs, NH
16j[a]	4.95 d (7.1)	6.09 d	4.47 t	4.09 dd, 4.20 dd (3.9, 4.0)	8.42 s		5.82 s, H-3″; 5.60 bs, NH_2-4″; 7.31–7.87 m, aromatics
19[a]	6.15 d (7.2)	5.29 d	4.39 dd	4.09 dd, 4.18 dd (3.3, 5.3)	8.50, 8.55 s		4.16 s, OMe; 5.76 s, H-3″; 6.77 bs, NH_2-4″
21[a]	6.00 d (6.8)	5.30 d	4.29 dd	4.07 dd, 4.15 dd (3.0, 6.3)	8.29 s	8.29 s	3.13 s, NMe, 5.69 s, H-3″; 7.09 bs, NH_2-4″
23[a]	6.01 d	5.31 d	4.29 dd	4.07 dd, 4.14 dd	8.23, 8.29 s		3.30–3.80 bs, $N(CH_3)_2$, 5.68 s, H-3″; 7.13 bs, NH_2-4″
25[a]	6.24 d (7.5)	5.35 d	4.45 dd	4.21 dd, 4.14 dd (3.4, 4.8)	9.00 s	8.75 s	5.82 bs, H-3″; 6.76 bs, NH_2-4″; 9.22 s, H-6
26[c]	6.10 d (7.9)	4.79 d	4.47 t	3.92 dd, 4.04 dd (2.2, 2.4)	8.05, 8.08 s		3.68 s, NCH_3; 5.70 s, H-3″; 5.72 bs, NH_2-4″
27[c]	5.96 d	5.00 d	4.47 t	4.01 m	8.12, 8.15 s		3.63 s, NCH_3; 5.57 s, H-3″; 5.80 bs, NH_2-4″
28[a]	6.17 d (7.7)	5.24 d	4.43 dd	4.08 dd, 4.17 dd (4.1, 6.5)	8.40, 8.54 s		1.38 t, NCH_2CH_3; 4.22 q, NCH_2CH_3; 5.69 s, H-3″; 6.64 bs, NH_2-4″

[a] $(CD_3)_2CO$. [b] $(CD_3)_2SO$. [c] $CDCl_3$.

nucleosides **13a** and **13b** (59%). The condensation of persilylated xanthine with acetate **12** gave a mixture of the N-7 and N-3 isomers **13e** (27%) and **13f** (10%), as the major compounds, together with the N-9 isomer **13d** (3%) and the diglycosyl compound **13g** (8%). Finally, glycosylation of **12** with 6-chloropurine (Scheme II) afforded the N-9 and N-7 6-chloropurine 3′-cyanomesylates **13h** (56%) and **13i** (5%). Similarly, glycosylation of **12** with benzimidazole gave the benzimidazole nucleoside **13j** (59% yield).

Structures of new cyanomesylates **13a-j** were assigned on the basis of the corresponding analytical and spectroscopic data, and by comparison with those of the previously reported cyanomesylates of pyrimidines.[17,18] Coupling constant values (Table I) were in the range of $J_{1',2'}$ = 7.0–7.9 Hz, which is in agreement with those observed for thymine derivatives[18] and with literature data for other β-D-ribo-3′-*C*-branched nucleosides[31–33] and further corroborates the β-anomeric configuration of cyanomesylates **13a–j**.

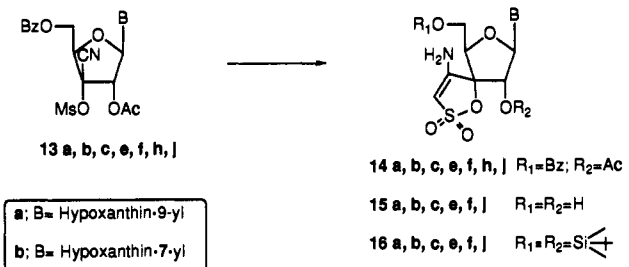
The site of glycosylation of hypoxanthine (**13a,b**) and 6-chloropurine (**13h,i**) nucleosides was determined through comparison of their ^{1}H- and ^{13}C-NMR spectra (Tables I and II) to those of the pairs of related N-9 and N-7 isomers of known structure.[34–39] H-8 and H-1′ signals and the C-4, C-8, and C-1′ carbon signals of the N-9 nucleosides **13a** and **13h** were shifted upfield relative to the corresponding resonances of the N-7 isomers **13b** and **13i**, whereas C-5 carbon signals were shifted downfield. The sites of glycosylation of diglycosyl compound **13c** were assigned as N-7 and N-1 based on the following criteria. ^{1}H-NMR of **13c** showed two signals for H-1′ at 6.37 and 6.61 ppm,

Table II. Selected ^{13}C-NMR Spectral Data of Nucleosides: Chemical Shifts (ppm)

compd	C-6	C-4	C-2	C-8	C-5	others
6[a]	155.11	149.49	152.66	139.37	122.05	113.80 (CN), 82.68 (C-3′), 40.44 (SO_2CH_3)
7[a]	155.34	150.19	152.91	139.84	122.26	115.24 (CN), 81.03 (C-3′), 40.94 (SO_2CH_3)
8[a]	155.10	149.96	152.71	139.63	121.72	153.78 (C-4″), 93.19 (C-3′), 89.32, 89.57 (C-1′, C-3″)
9[a]	155.57	149.27	152.37	141.33	122.78	154.12 (C-4″), 89.99 (C-3′), 89.48, 89.57 (C-1′, C-3″)
13a[b]	156.23	148.14	146.19	139.58	129.94	113.14 (CN), 76.50 (C-3′), 40.51 (SO_2CH_3)
13b[b]	153.79	158.62	145.77	144.53	114.40	113.00 (CN), 75.72 (C-3′), 40.43 (SO_2CH_3)
13c[b]	146.10	160.81	147.31	142.41		113.12, 113.67, 112.91 (C-5, 2CN), 76.36, 75.70 (2C-3′), 40.47, 40.41 ($2SO_2CH_3$)
13d[b]	166.23 (s)[c]	151.33 (d)	154.68 (s)	140.73 (dd)	120.00	115.41 (CN), 81.00 (C-3′), 40.74 (SO_2CH_3)
13e[a]	155.74 (s)[c]	151.76 (d)	151.22 (s)	143.58 (dd)	106.86	113.65 (CN), 78.01 (C-3′), 40.65 (SO_2CH_3)
13f[a]	154.74 (s)[c]	148.70 (dd)	151.33 (d)	140.74 (d)	108.89	114.49 (CN), 78.54 (C-3′), 40.89 (SO_2CH_3)
13g[a]	154.82 (s)[c]	150.28 (d)	150.60 (d)	142.93 (dd)	108.34	113.00, 114.40 (2CN), 75.72 (C-3′), 40.43 (SO_2CH_3)
13h[b]	151.43	149.77	151.72	146.41	131.63	112.90 (CN), 76.49 (C-3′), 40.51 (SO_2CH_3)
13i[b]	142.13	161.87	152.34	148.34	122.01	112.98 (CN), 76.16 (C-3′), 40.45 (SO_2CH_3)
16a[b]	156.27	148.52	146.61	134.07	124.07	151.69 (C-4″), 90.08 (C-3′), 84.41, 85.28, 88.70 (C-1′), C-2′, C-3″)
16b[a]		161.02	145.84, 145.94		115.61	153.72, 154.95 (C-6, C-4″), 89.88 (C-3′), 87.86, 90.12, 90.37 (C-1′, C-3″)
19[a]	162.33	153.36	153.00	142.84	123.05	153.36 (C-4″), 90.65 (C-3′), 85.75, 86.64, 91.12 (C-1′, C-2′, C-3″), 54.61 (OMe)
26[a]	156.06		136.13, 137.93		124.17	147.99, 151.63 (C-4, C-4″), 90.09 (C-3′), 84.60, 85.08, 88.69 (C-1′, C-2′, C-3″), 13.20 (NCH_3)
27[a]		161.04	145.86, 145.97		115.70	153.73, 155.03 (C-6, C-4″), 89.98 (C-3′), 87.56, 90.43, 90.58 (C-1′, C-2′, C-3″), 13.31 (NCH_3)
28[a]		160.87	145.61, 146.00		114.97	153.70, 155.01 (C-6, C-4″), 89.97 (C-3′), 87.55, 90.38, 90.61 (C-1′, C-2′, C-3″), 36.90 (NCH_2CH_3), 13.31 (NCH_2CH_3)

[a] $(CD_3)_2CO$. [b] $(CD_3)_2SO$. [c] Multiplicity in the coupled ^{13}C-NMR spectra.

Scheme III



the downfield signal suggested an N-1 glycosylation site due to the deshielding effect of the adjacent 6-C=O of the base. Comparison of the ^{13}C-NMR spectra of **13c** with those of N-9 and N-7 isomers (**13a** and **13b**) showed good accordance with the ^{13}C-NMR data of the N-7 isomer **13b**.

Due to the lack of literature data of a series of model compounds, the site of glycosylation of xanthine derivatives (**13d–g**) was established as follows. From the ^{13}C-NMR spectra of **13d–f** (Table II), approximate assignments of the carbons of xanthine base were made. C-8 is a protonated carbon, which was easily identified by DEPT as the signal appearing at 140–143 ppm; C-2 and C-6 were expected around 150–170 ppm, C-6 being the lower field signal.[40,41] C-4 is between two nitrogen atoms; it should be found around 150 ppm, whereas C-5, adjacent to only one nitrogen, is expected upfield around 120 ppm.[42] Therefore, the signal appearing at higher field (106-114 ppm) was assigned to C-5. The two signals that appeared around 150 should correspond to C-2 and C-4. Unequivocal assignment of those signals and of the glycosylation site for **13d,e,f** was made by selective proton decoupling

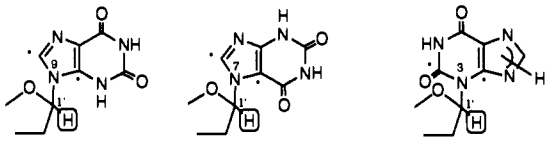


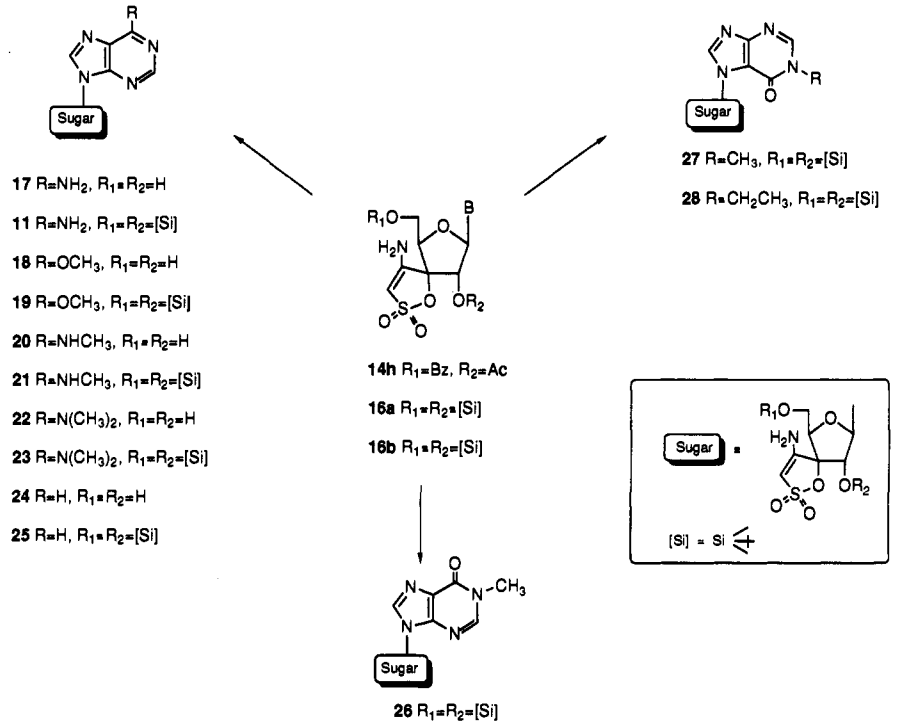
Figure 2.

experiments from the ^{13}C-NMR spectra by selective irradiation of the anomeric (H-1′) proton. In those experiments only the carbons within three bonds of that proton gave a simplification of the multiplicity of their signals (these carbons are depicted in Figure 2 with an asterisk), which clearly established the glycosylation site as N-9 for **13d**, N-7 for **13e**, and N-3 for **13f**. Finally, the glycosylation sites of diglycosyl derivative **13g** were established as N-7 and N-3 by comparison of its ^{1}H- and ^{13}C-NMR spectra with those of the N-9 (**13d**), N-7 (**13e**), and N-3 (**13f**) (Tables I and II).

The spiro derivatives were prepared from the corresponding cyanomesylates. Thus, treatment of the 3′-cyanomesylates of hypoxanthine **13a–c**, xanthine **13e,f**, and benzimidazole **13j** (Scheme III) with Cs_2CO_3 afforded the spiro derivatives **14a-c,e,f,j** which were used in the next step without further purification. Deprotection with saturated methanolic ammonia followed by silylation with *tert*-butyldimethylsilyl chloride (TBDMSCl) yielded the 2′,5′-bis-*O*-silylated spiro nucleosides **16** [**16a** (24%); **16b** (31%); **16c** (17%); **16e** (28%); **16f** (24%); **16j** (20%) overall yield].

A similar reaction sequence with 6-chloropurine cyanomesylate **13h** afforded a mixture of adenine spiro nucleoside **11** (15%) and the 6-methoxy purine derivative **19** (20%) (Scheme IV), resulting from nucleophilic displacement of 6-Cl of the purine base. Compound **19** was also obtained when deprotection of the spiro derivative intermediate **14h** was carried out with Amberlyst A-26 (OH^-)[43] in methanol followed by silylation. 6-Chloropurine spiro intermediate **14h** was utilized for the synthesis of

Scheme IV



other 6-substituted purine nucleosides such as **21**, **23**, and **25** (Scheme IV). Thus, nucleophilic displacement of the 6-Cl of **14h** with methylamine and dimethylamine[38] gave the 6-*N*-methyl and 6-*N*-dimethyl-2′,5′-deprotected nucleosides **20** and **22**, which were silylated with TBDMSCl/DMAP to give the fully protected nucleosides **21** and **23** in 20% and 19% overall yield, respectively. Catalytic hydrogenation of **14h** in methanol containing aqueous ammonia,[44] in the presence of 10% palladium on charcoal, gave the dechlorinated compound that was deprotected (methanolic ammonia) (**24**) and silylated (TBDMSCl) to afford **25** (18%).

Finally, spiro nucleosides of hypoxanthine **16a** and **16b** were transformed (Scheme IV) to the corresponding 1-*N*-alkyl nucleosides **26–28**. Thus, selective alkylation[45,46] of **16a** and **16b** with methyl iodide in the presence of potassium carbonate gave 1-methyl 3′-spiro derivatives **26** and **27** in 55% and 66% yield, respectively. Similarly, reaction of **16b** with ethyl iodide afforded the 1-*N*-ethyl spiro nucleoside **28** in 71% yield.

Attachment of the alkyl group to the N-1 and not to either the oxygen atoms of the 6-C=O of the hypoxanthine or the NH_2-4″ or C-3″ of the spirooxathiole moiety was established from analytical and spectroscopic data. ^{1}H-NMR spectra (Table I) showed the disappearance of the signal assigned to 1-NH (11.42, 11.66 ppm) and the presence of new signals corresponding to the alkyl groups. No modification was observed at the signals of the protons of the spirooxathiole moiety (NH_2-4″ and H-3″), thus indicating that no methylation had occurred at this moiety. ^{13}C-NMR spectra showed no changes in the chemical shift of C-6 with respect to those observed for **16a** and **16b**, used as starting materials, which indicated that the alkyl group was not attached to the C-6 carbonyl oxygen atoms.

Biological Results

The TSAO–purine derivatives were evaluated for their inhibitory effect against HIV-1- and HIV-2-induced cytopathicity in MT-4 cells (Table III). None of the test compounds were inhibitory against HIV-2 at subtoxic concentrations. The prototype compound *ribo* TSAO-A (TSAO-adenine) (**11**) proved inhibitory to virus-induced cytopathicity at a 50% effective concentration (EC_{50}) of 0.278 μM, while being toxic to MT-4 cells at a 50% cytotoxic concentration (CC_{50}) of 13 μM. The *xylo* counterpart (**10**) had no antiviral activity. Also, introduction of a monomethoxytrityl group at the amino group of C-6 of adenine (**9**) resulted in annihilation of the antiviral activity. However, when adenine was replaced by hypoxanthine [linked *via* its N-9 (**16a**) or N-7 (**16b**) atom to the ribose moiety], a slight increase in antiviral activity was observed as compared with TSAO-A. When adenine was replaced by xanthine, linked *via* its N-7 atom to the ribose moiety (**16e**), antiviral and cytotoxic effects were comparable to those of TSAO-A. However, linkage of xanthine *via* its

Table III. Anti-HIV Activity of TSAO–Purine Derivatives

	EC_{50}[a] (μM)				
	MT-4				
compd	HIV-1	HIV-2	CEM	CC_{50}[b] (μM)	selectivity index (ratio CC_{50}/EC_{50})
8	>100	>100		191 ± 153	<1.9
9	>100	>100		192 ± 55	<1.9
10	>2.7	>2.7		7.3 ± 0.84	<2.7
11	0.278 ± 0.05	>14		13 ± 5.8	47
16a	0.158 ± 0.104	>7		14 ± 1.7	89
16b	0.173 ± 0.005	>7		15 ± 0.22	86
16c	>150	>150		>150	<1
16e	0.358 ± 0.163	>7	0.126 ± 0.059	13 ± 0.08	36
16f	2.7 ± 0.18	>7	2.3 ± 3.2	14 ± 0.23	5.2
16j	3.1 ± 0.32	>7		15 ± 0.87	4.8
19	0.141 ± 0.040	>30	0.469 ± 0.351	21 ± 3.4	149
21	0.146 ± 0.076	>7	0.161 ± 0.127	14 ± 1.2	96
23	0.389 ± 0.068	>30	0.646 ± 0.300	15 ± 2.7	39
25	0.177	>7	0.074 ± 0.058	13 ± 1.8	73
26	0.514 ± 0.044	>150	0.101 ± 0.074	>150	>292
27	0.201 ± 0.122	>100	0.067 ± 0.041	156 ± 21	776
28	0.604 ± 0.279	>150	0.172 ± 0.086	>150	>248

[a] 50% effective concentration, or compound concentration required to inhibit HIV-induced cytopathicity in MT-4 and HIV-induced giant cell formation in CEM cells by 50%. [b] 50% cytotoxic concentration, or compound concentration required to reduce the viability of MT-4 cells by 50%.

N-3 atom to the ribose moiety (**16f**), or replacement of adenine by benzimidazole (**16j**), decreased the antiviral activity by 10-fold.

Introduction of one or two methyl groups at the amino group on C-6 of adenine (compounds **21** and **23**, respectively) did not markedly affect the antiviral or cytotoxic potency of TSAO-A. Also, removal of this amino group (compound **25**) or replacement of the amino group by a methoxy group (compound **19**) preserved the antiviral activity. The fact that the TSAO–benzimidazole derivative **16j** was clearly less active than the other TSAO–purine derivatives, including compound **25** which does not contain any functional groups on the purine moiety, suggests that the purine skeleton itself, rather than the functional groups on the purine moiety, determines the antiviral activity of the compounds.

Introduction of an alkyl group at N-1 of the hypoxanthine linked *via* its N-9 to ribose (compound **26**), or *via* its N-7 to ribose (compounds **27** and **28**), resulted in a markedly decreased cytotoxicity, whereas the antiviral activity was only slightly decreased (up to 3-fold). Therefore, compounds **26**, **27**, and **28** had substantially increased selectivity indices (>292, 776, and >248, respectively), as compared to those found for the parent compounds **16a** and **16b** (selectivity indices of 89 and 86, respectively). In this respect, the TSAO–purine derivatives behaved similarly to the TSAO–pyrimidine derivatives, where introduction of an alkyl or alkenyl group at N-3 of thymine also leads to a substantial increase of the selectivity index, due to reduction of toxicity.[18]

For a number of TSAO–purine derivatives (i.e. **16e,f**, **19**, **21**, **23**, **25–28**) the anti-HIV-1 activity was also evaluated in CEM cells. As a rule, the antiviral potencies of the test compounds in these cells were comparable to those found in the MT-4 cells (Table III).

Recently, a mutant HIV-I/III$_B$ strain was selected for resistance to compound **26**. It proved to be cross-resistant to TSAO–pyrimidine and TSAO–purine derivatives (i.e. compounds **11**, **16e**, **23**, and **27**).[50] Also, a mutant HIV-I/III$_B$ strain selected for resistance against the TSAO–pyrimidine derivative TSAO-m^{3}T proved cross-resistant to compounds **11**, **16a**, **16b**, **16e**, **23**, **26**, and **27** (data not shown). However, the TSAO-resistant virus strains kept full sensitivity to other HIV-1-specific compounds (i.e. nevirapine, BHAP). The reverse transcriptase of HIV-1/TSAO-m^{1}Hx showed a single amino acid change (138-Glu → Lys) that is identical to the amino acid change that has recently been observed in several HIV-1/TSAO-pyrimidine mutant strains.[51]

In conclusion, TSAO–purine derivatives represent a particular class of nucleoside analogues with potent and selective activity against HIV-1. The presence of an intact purine skeleton seems more important for antiviral activity than the nature of the functional groups on the purine moiety. Also, introduction of an alkyl group at N-1 of the purine moiety of TSAO–hypoxanthine renders the molecule considerably less cytotoxic.

Experimental Section

Chemical Procedures. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. ^{1}H-NMR spectra were recorded with a Varian EM-390, a Varian XL-300, and a Bruker AM-200 spectrometer operating at 300 and 200 MHz, and ^{13}C-NMR spectra with a Bruker WP-80-SY, a Bruker AM-200 and a Varian XL-300 spectrometer operating at 20, 50, and 75 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 [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).

Proximities were established conventionally on the basis of NOE.

9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-3′-*C*-cyano-3′-*O*-mesyl-β-D-xylofuranosyl]-6-*N*-(monomethoxytrityl)adenine and 9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl]-6-*N*-(monomethoxytrityl)adenine (6 and 7). Compound **2**[23] (3.07 g, 4 mmol) was oxidized [CrO_3/pyridine/Ac_2O (1:2:1 equiv)][22] to give after workup the 3′-ketonucleoside **3** [IR (KBr) 1780 cm^{-1} (C=O, ulose)] as a white foam, which was used immediately in the next step without further purification. Thus, a mixture of **3**, water (16 mL), ethyl ether (32 mL), sodium bicarbonate (0.64 g, 8 mmol), and sodium cyanide (0.2 g, 4 mmol) was stirred vigorously at room temperature for 3 days. The organic phase was separated, and the aqueous phase was washed with ethyl ether (2 × 50 mL). The combined ethereal phases were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue, a mixture of the two epimeric cyanohydrins (**4** and **5**), was dissolved in dry pyridine (8 mL). To this solution mesyl chloride (1.6 mL, 20 mmol) was added. The mixture was stirred at 8–10 °C for 48 h, poured into ice and water, and extracted with chloroform (2 × 50 mL). The combined extracts were washed with 1 N HCl (50 mL), aqueous sodium hydrogen carbonate (50 mL), and brine (50 mL), dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 5:1). The fastest moving fractions afforded 2.33 g (67%) of **6** as a white foam: IR (KBr) 1380, 1180 cm^{-1} (SO_2). Anal. ($C_{44}H_{59}N_6O_7SSi_2$) C, H, N.

The slowest moving fractions afforded 0.35 g (10%) of **7** as a white foam: IR (KBr) 1370, 1180 cm^{-1} (SO_2). Anal. ($C_{44}H_{59}N_6O_7SSi_2$) C, H, N.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xilofuranosyl]-6-*N*-(monomethoxytrityl)adenine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (8). To a solution of **6** (0.75 g, 0.86 mmol) in dry acetonitrile (10 mL) was added Cs_2CO_3 (0.31 g, 0.95 mmol). The mixture was stirred at room temperature for 70 min and filtered. The filtrate was neutralized with acetic acid and evaporated to dryness. The residue was purified by column chromatography (hexane/ethyl acetate, 2:1) to give 0.61 g (82%) of **8** as an amorphous solid: IR (KBr) 3350, 3330 cm^{-1} (NH_2), 1650 (C=CN). Anal. ($C_{44}H_{59}N_6O_7SSi_2$) C, H, N.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-6-*N*-(monomethoxytrityl)adenine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (9). According to the method described for the synthesis of **8**, cyanomesyl derivative **7** (0.18 g, 0.20 mmol) was treated with Cs_2CO_3 for 3 h. After the workup, the residue was purified by CCTLC on the Chromatotron (chloroform/methanol, 100:1) to give 0.08 g (45%) of **9** as a white foam: IR (KBr) 3350, 3330 cm^{-1} (NH_2), 1650 (C=CN). Anal. ($C_{44}H_{59}N_6O_7SSi_2$) C, H, N.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xilofuranosyl]adenine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (10). To a solution of the *N*-protected compound **8** (0.24 g, 0.26 mmol) in dry nitromethane (12 mL) was added $ZnBr_2$ (0.3 g, 1.4 mmol), and the mixture was stirred at room temperature for 10 min. The solvent was evaporated to dryness and the residue was purified by column chromatography (chloroform/methanol, 10:1) to afford 0.08 g (50%) of **10** as a white foam: IR (KBr) 3450, 3430 cm^{-1} (NH_2), 1650 (C=CN). Anal. ($C_{24}H_{42}N_6O_6SSi_2$) C, H, N.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]adenine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (11). Spiro derivative **9** (0.07 g, 0.076 mmol) was deprotected with $ZnBr_2$, following the method described for the synthesis of **10**. The residue obtained after the workup, was purified by CCTLC on the Chromatotron (dichloromethanemethanol, 20:1) to give 0.026 g (59%) of **11** as a white foam: IR (KBr) 3450, 3400 cm^{-1} (NH_2), 1650 (C=CN). Anal. ($C_{24}H_{42}N_6O_6SSi_2$) C, H, N.

General Procedure for the Synthesis of 2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl Nucleosides (13). To a suspension of the heterocyclic base (1.2 mmol) in dry dichloroethane (12 mL), was added *N*,*O*-bis(trimethylsilyl)-acetamide (0.65 mL) and the mixture was refluxed until the solution became clear. The solvent was evaporated under reduced pressure. A solution of compound **12** (0.44 g, 1 mmol) in dry acetonitrile (10 mL) was added to the syrupy silylated base, followed by the addition of TMS-Tfl (0.22 mL, 1.1 mmol). The resulting mixture was heated to reflux. After 2 and 4 h, two additional portions of TMS-Tfl (0.11 mL) were added and the refluxing continued for 2-4 h. The reaction mixture was allowed to cool to room temperature, ethyl acetate (50 mL) was added, and the solution was poured into cold, saturated, aqueous $NaHCO_3$. The organic phase was separated, the aqueous phase was washed with ethyl acetate (2 × 20 mL) and dried (Na_2SO_4), and the solvent was removed. The residue was purified by column chromatography.

9-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)hypoxanthine and 7-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)hypoxanthine (13a and 13b). Method A. Hypoxanthine (0.96 g, 7.06 mmol) was reacted with **12** (2.60 g, 5.88 mmol) for 8 h. The residue was chromatographed (ethyl acetate). The fastest moving fractions afforded 0.84 g (19%) of a white foam which was identified as **1,7-bis(2′-*O*-acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)hypoxanthine (13c)**: IR (KBr) 1380, 1185 cm^{-1} (SO_2). Anal. ($C_{37}H_{34}N_6O_{17}S_2$) C, H, N.

The following fractions afforded 0.66 g (22%) of **13b** as a white foam: IR (KBr) 1375, 1180 cm^{-1} (SO_2). UV (MeOH) λ_{max} nm (log ε) 224 (4.11), 259 (3.73). Anal. ($C_{21}H_{19}N_5O_9S$) C, H, N.

The slowest moving fractions gave 0.52 g (17%) of **13a** as a white foam: IR (KBr) 1380, 1180 cm^{-1} (SO_2); UV (MeOH) λ_{max} nm (log ε) 226 (4.09), 246 (3.77). Anal. ($C_{21}H_{19}N_5O_9S$) C, H, N.

Method B. Compound **12** (0.44 g, 1 mmol) was glycosidated according to the general procedure, except that 2.5 mmol (0.34 g) of hypoxanthine and 0.6 mL (3.0 mmol) of TMS-Tfl were used. The resulting mixture was refluxed for 24 h. The residue was chromatographed (ethyl acetate). The fastest moving fractions afforded 0.18 g (35%) of **13b**. The slowest moving fractions gave 0.12 g (24%) of **13a**.

9-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)xanthine, 7-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)xanthine, and 3-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)xanthine (13d, 13e, and 13f). According to the general procedure, xanthine (0.66 g, 4.32 mmol) was reacted with **12** (1.58 g, 3.6 mmol) for 6 h. The residue was chromatographed (hexane/ethyl acetate, 1:2). The fastest moving fractions gave **3,7-bis(2′-*O*-acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)xanthine (13g)** (0.26 g, 8%) as a white foam: IR (KBr) 1380, 1185 cm^{-1} (SO_2). Anal. ($C_{37}H_{34}N_6O_{13}S_2$). C, H, N.

The column was then eluted with ethyl acetate. The fastest moving fractions afforded **13f** (0.2 g, 10%) as a white foam: IR (KBr) 1380, 1185 cm^{-1} (SO_2); UV (MeOH) λ_{max} nm (log ε) 208 (3.98), 227 (4.09), 264 (3.96). Anal. ($C_{21}H_{19}N_5O_{10}S$) C, H, N.

The next fractions gave **13d** (0.04 g, 3%) as a white foam: IR (KBr) 1385, 1185 cm^{-1} (SO_2); UV (MeOH) λ_{max} nm (log ε) 207 (3.95), 228 (4.19), 271 (3.57). Anal. ($C_{21}H_{19}N_5O_{10}S$) C, H, N.

The slowest moving fractions gave 0.50 g (27%) of **13e** as a white foam: IR (KBr) 1385, 1185 cm^{-1} (SO_2); UV (MeOH) λ_{max} nm (log ε) 208 (4.02) 226 (4.07), 272 (3.77). Anal. ($C_{21}H_{19}N_5O_{10}S$) C, H, N.

9-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)-6-chloropurine and 7-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)-6-chloropurine (13h and 13i). According to the general procedure, 6-chloropurine (0.072 g, 4.68 mmol) was reacted with **12** (1.72 g, 3.90 mmol) for 6 h. The residue was chromatographed (dichloromethane/methanol, 50:1). The fastest moving fractions afforded 1.16 g (56%) of **13h** as a white foam: IR (KBr) 1375, 1185 cm^{-1} (SO_2); UV (MeOH) λ_{max} nm (log ε) 207 (3.94), 228 (3.90), 262 (3.68). Anal. ($C_{21}H_{18}N_5O_8ClS$) C, H, N.

The slowest moving fractions gave 0.10 g (5%) of **13i** as a white foam: IR (KBr) 1375, 1185 cm^{-1} (SO_2); UV (MeOH) λ_{max} nm (log ε) 210 (4.07), 228 (4.14), 270 (3.75). Anal. ($C_{21}H_{18}N_5O_8ClS$) C, H, N.

1-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)benzimidazole (13j). Following the general procedure, benzimidazole (0.48 g, 1.2 mmol) reacted with **12** (1.50 g, 3.39 mmol) for 8 h. The residue was chromatographed (hexane/ethyl acetate, 1:2) to give 0.99 g (59%) of **13j** as a white foam: IR (KBr) 1380, 1185 cm^{-1} (SO_2). Anal. ($C_{23}H_{21}N_3O_8S$) C, H, N.

[9-(2′-*O*-Acetyl-5′-*O*-benzoyl-β-D-ribofuranosyl)-6-chloropurine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (14h). To a solution of the cyanomesylate **13h** (1.08 g, 2 mmol) in dry acetonitrile (20 mL) was added Cs_2CO_3 (0.72 g, 2.2 mmol). The mixture was stirred at room temperature for 4 h and then filtered. The filtrate was neutralized with acetic acid and then evaporated to dryness. The residue was purified by column chromatography (chloroform/methanol, 30:1) to give 0.76 g (70%) of **14h** as a white foam: IR (KBr) 3400, 3330 cm^{-1} (NH_2), 1650 (C═CN). Anal. ($C_{21}H_{18}N_5O_8ClS$) C, H, N.

General Procedure for the Synthesis of [[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) Nucleosides (16). To a solution of the cyanomesylate **13** (1 mmol) in dry acetonitrile (8 mL) was added Cs_2CO_3 (0.36 g, 1.1 mmol). The mixture was stirred at room temperature for 1–8 h and filtered. The filtrate was neutralized with acetic acid and evaporated to dryness. The residue (spiro derivative **14**) was deprotected with saturated methanolic ammonia (20 mL). After standing at room temperature overnight, the solvent was evaporated to dryness. The residue was dissolved in methanol (2 mL) and then treated with chloroform. The solid (deprotected nucleoside **15**) was filtered and suspended in dry acetonitrile (15 mL), and then DMAP (0.58 g, 4.8 mmol) and TBDMSCl (0.72 g, 4.8 mmol) were added. The mixture was heated to 80 °C for 4–16 h and evaporated to dryness. The residue was treated with dichloromethane (50 mL) and water (50 mL). The organic phase was separated and the aqueous phase was extracted with dichloromethane (2 × 50 mL). The combined organics were successively washed with cold (4 °C) 1 N HCl (25 mL), water (50 mL) and brine (50 mL), and, finally, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography or by CCTLC on the Chromatotron, to give **16**.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]hypoxanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (16a). The general procedure was followed with **13a** (0.5 g, 0.97 mmol). The residue was purified by column chromatography (chloroform/methanol, 20:1) to yield 0.14 g (24%) of **16a** as a white foam: IR (KBr) 3450, 3410 cm^{-1} (NH_2), 1650 (C═CN); UV (MeOH) λ_{max} nm (log ε) 212 (4.15), 226 (4.14), 250 (3.79). Anal. ($C_{24}H_{42}N_5O_7SSi_2$) C, H, N.

[7-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]hypoxanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (16b). **13b** (0.6 g, 1.16 mmol) was reacted according to the general procedure to yield, after column chromatography (chloroform/methanol, 30:1), 0.21 g (31%) of **16b** as a white foam: IR (KBr) 3450, 3400 cm^{-1} (NH_2), 1645 (C═CN), UV (MeOH) λ_{max} nm (log ε) 212 (4.15), 229 (4.13), 262 (3.86). Anal. ($C_{24}H_{42}N_5O_7SSi_2$) C, H, N.

[1,7-Bis-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]hypoxanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (16c). The general procedure was followed with **13c** (0.6 g, 0.66 mmol). The residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 100:1) to give 0.12 g (17%) of **16c** as a yellow foam: IR (KBr) 3450, 3400 cm^{-1} (NH_2), 1650 (C═CN). Anal. ($C_{43}H_{78}N_6O_{13}S_2Si_4$) C, H, N.

[7-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]xanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (16e). The general procedure was followed with **13e** (0.5 g, 0.94 mmol). The residue was purified by column chromatography (chloroform/methanol, 50:1) to give 0.16 g (28%) of **16e** as an amorphous solid: IR (KBr) 3500, 3420 cm^{-1} (NH_2), 1650 (C═CN); UV (MeOH) λ_{max} nm (log ε) 207 (4.14), 226 (4.22), 262 (4.12). Anal. ($C_{24}H_{42}N_5O_8SSi_2$) C, H, N.

[3-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]xanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (16f). **13f** (0.2 g, 0.37 mmol) was reacted according to

the general procedure. The residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 50:1) to give 0.05 g (24%) of **16f** as an amorphous solid: IR (KBr) 3500, 3400 cm^{-1} (NH_2), 1650 (C=CN). UV (MeOH) λ_{max} nm (log ϵ) 203 (3.99), 221 (3.96), 269 (3.65). Anal. ($C_{24}H_{42}N_5O_8SSi_2$) C, H, N.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]benzimidazole]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (16j). **13j** (0.5 g, 1.0 mmol) was reacted according to the general procedure. The residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 150:1) to give 0.10 g (20%) of **16j** as a white foam: IR (KBr) 3450, 3350 cm^{-1} (NH_2), 1645 (C=CN). Anal. ($C_{26}H_{44}N_3O_6SSi_2$) C, H, N.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-6-methoxypurine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (19). Method A. Spiro derivative **14h** (0.27 g, 0.5 mmol) was treated with saturated methanolic ammonia (10 mL). After standing at room temperature overnight, the solvent was evaporated to dryness. The residue was dissolved in methanol (20 mL) and then treated with chloroform. The solid was filtered and suspended in dry acetonitrile (20 mL), and then DMAP (0.27 g, 2.25 mmol) and TBDMSCl (0.34 g, 2.25 mmol) were added. The mixture was heated to reflux for 16 h and evaporated to dryness. The residue was treated with dichloromethane (50 mL) and water (50 mL). The organic phase was separated, and the aqueous phase was extracted with dichloromethane (2 × 50 mL). The combined organics were successively washed with cold (4 °C) 1 N HCl (25 mL), water (50 mL), and brine (50 mL), and, finally, dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1). The fastest moving fractions gave 0.06 g (20%) of **19** as a white foam: IR (KBr) 3500, 3450 cm^{-1} (NH_2), 1650 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 201 (4.15), 242 (4.27). Anal. ($C_{25}H_{43}N_5O_7SSi_2$) C, H, N.

The slowest moving fractions afforded 0.045 g (15%) of the adenine derivative **11**.

Method B. To a solution of **14h** (0.12 g, 0.22 mmol) in methanol (10 mL) was added Amberlyst A-26 (OH^-)[43a] (0.025 g). The mixture was heated at 60 °C for 8 h and then filtered. The solvent was evaporated and the residue was silylated with TBDMSCl/DMAP as indicated in method A. The residue obtained after the workup, was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1) to give 0.04 g (31%) of **19**.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-6-*N*-methylpurine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (21). A solution of **14h** (0.12 g, 0.22 mmol) and methylamine (40 wt % solution in H_2O) (0.9 mL) in methanol (4.5 mL) was stirred at room temperature for 2.5 h. The solvent was evaporated and the residue was silylated (TBDMSiCl/DMAP), as indicated for the synthesis of **19**. After the workup, the residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 1:1) to give 0.05 g (37%) of **21** as a white foam: IR (KBr) 3500, 3380 cm^{-1} (NH_2), 1645 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 204 (4.30), 219 (4.24), 252 (4.27). Anal. ($C_{25}H_{44}N_6O_6SSi_2$) C, H, N.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-6-*N*-dimethylpurine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (23). A solution of **14h** (0.12 g, 0.22 mmol) and dimethylamine (33 wt % solution in EtOH) (1.5 mL) in ethanol (5 mL) was stirred at 30 °C for 8 h. The solvent was evaporated and the residue was silylated (TBDMSCl/DMAP), according to the procedure described for **19**. Purification of the crude product by CCTLC on the Chromatotron (dichloromethane/methanol 50:1) gave 0.052 g (38%) of **23** as an amorphous solid: IR (KBr) 3500, 3400 cm^{-1} (NH_2), 1650 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 215 (4.16), 270 (4.16). Anal. ($C_{26}H_{46}N_6O_6SSi_2$) C, H, N.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (25). A solution of **14h** (0.12 g, 0.22 mmol) in methanol (48 mL), containing concentrated NH_4OH (0.14 mL) and Pd/C (10%) (0.011 g), was hydrogenated at 15 psi at room temperature for 8 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was treated with saturated methanolic ammonia (30 mL) to give the deprotected nucleoside **24**, which was silylated (TBDMSCl/DMAP) according to the procedure described for **19**. The residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 50:1) to give 0.041 g (18%) of **25** as a white foam: IR (KBr) 3500, 3400 cm^{-1} (NH_2), 1650 (C=CN). Anal. ($C_{24}H_{41}N_5O_6SSi_2$) C, H, N.

General Procedure for the Synthesis of (1-*N*-Alkylnucleosides)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (26–28). To a solution of the spiro nucleoside **16a,b** (0.1 mmol) in acetone (1.5 mL) were added K_2CO_3 (0.05 mmol) and the corresponding alkyl halide (1.1–2.0 mmol). The reaction mixture was refluxed for 3–6 h. After removal of the solvent, the residue was purified by CCTLC on the Chromatotron.

[9-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-1-*N*-methylhypoxanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (26). Compound **16a** (0.05 g, 0.08 mmol) and methyl iodide (0.023 mL, 0.16 mmol) were reacted according to the general procedure for 6 h. The residue was chromatographed with hexane/ethyl acetate (1:1) to give **26** (0.027 g, 55%) as an amorphous solid: IR (KBr) 3390 cm^{-1} (NH_2), 1715 (C=O), 1645 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 212 (4.12), 226 (4.13), 249 (3.75). Anal. ($C_{25}H_{43}N_5O_7SSi_2$) C, H, N.

[7-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-1-*N*-methylhypoxanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (27). Compound **16b** (0.06 g, 0.01 mmol) and methyl iodide (0.028 mL, 0.2 mmol) were reacted, according to the general procedure, for 3 h. The residue was chromatographed with hexane/ethyl acetate (1:1) to yield 0.04 g (66%) of **27** as an amorphous solid: IR (KBr) 3400, 3320 cm^{-1} (NH_2), 1715 (C=O), 1645 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 210 (4.10), 228 (4.14), 261 (3.84). Anal. ($C_{25}H_{43}N_5O_7SSi_2$) C, H, N.

[7-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-1-*N*-ethylhypoxanthine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (28). Compound **16b** (0.04 g, 0.066 mmol) was reacted with ethyl iodide (0.02 mL, 0.132 mmol), according to the general procedure, for 5 h. The residue was chromatographed with hexane/ethyl acetate (1:1) to afford **28** (0.029 g, 71%) as an amorphous solid: IR (KBr) 3400 cm^{-1} (NH_2), 1710 (C=O), 1640 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 210 (4.12), 228 (4.14), 260 (3.80). Anal. ($C_{26}H_{45}N_5O_7SSi_2$) C, H, N.

Antiretrovirus Activity Assays. HIV-1 was originally obtained from the culture supernatant of the persistently HIV-infected H9 cell line (H9/HTLV-III_B),[47] which was kindly provided by R. C. Gallo and M. Popovic (National Institutes of Health, Bethesda, MD). HIV-2(ROD) was obtained from L. Montagnier (Pasteur Institute, Paris, France). 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.[48,49] Briefly, MT-4 or CEM cells (5 × 10^5 cells/mL) were suspended in fresh culture medium and infected with HIV-1 at 100 times the 50% cell culture infective dose ($CCID_{50}$) per milliliter of cell suspension. Then 100 μM of infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of test compounds. After 4 days, virus-induced syncytium formation was recorded microscopically in the HIV-infected CEM cell cultures. 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. The 50% effective concentrations (EC_{50}) were defined as the compound concentrations required to reduce by 50% the number of syncytia in the CEM cell cultures or the number of viable MT-4 cells in the virus-infected 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.

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