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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Received April 20, 1993®

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 Cs₂CO₃ 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 Cs₂CO₃, 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 $0.1-1 \,\mu$ M 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 -APA¹⁴) 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.



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 TSAOpyrimidine 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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Abstract published in Advance ACS Abstracts, September 1, 1993.

Scheme I



13 a 13 t 13 c BzO Suga Sugar Sugar MsÒ ÒAc 12 13 d 13 e 13 f 13 g B₇C MSÓ ÓA Suga 13 h 13 I 13 j

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 nitromethane²⁴ 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 ¹H-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 NH₂-4" 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-1,7diglycosyl 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

Scheme II

Table I. Selected ¹ H-NMR Spectral Data of Nucleosides: Chemical Shifts (ppm), Multiplicity, and Coupling Conf	stants (Hz)
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						,, ,		
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	
6ª	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 ₂ CH ₃ ; 8.00 bs, NH	
7ª	6.14 d	5.43 d	4.84 dd	4.14 dd	7.95 s	8.30 s	3.47 (s. SO ₂ CH ₃ : 8.00 bs. NH	
ga	6.18 d	5.06 d	4.79 t	4.00 d	7.95 s	8.32 s	5.68 s. H-3".6.40 bs. NH-4": 8.00 bs. NH	
Ŭ	(4.2)	0,000 u		(5451)	11000	0.020		
Qa	6 01 d	5 95 d	4 31 44	4 05 dd 4 19 dd	701 .	8 35 .	5.68 a H-3": 6.89 ba NH-4": 6.80 NH	
J-	(4.9)	0.20 u	4.01 uu	4.00 uu, 4.10 uu	1.01.6	0.00 8	0.00 8, 11-0 , 0.09 08, 14112-4 , 0.00 1411	
104	(4.2)	F 00 J	1 70 +	(0.1, 2.9)	0.07 0	01 -	5 CO - 11 OV- 0 41 0 54 h- NUT 4// NUT	
104	6.21 d	5.08 d	4.79 t	4.01 d	8.27, 8	.31 s	$5.69 \text{ s}, \text{H}-3^{\prime\prime}; 6.41, 6.74 \text{ Ds}, \text{NH}_2-4^{\prime\prime}, \text{NH}_2$	
	(4.2)			(5.2, 5.2)				
11ª	6.02 d	5.31 d	4.31 dd	4.07 dd, 4.16 dd	8.22, 8	.33 s	5.70 s, H-3"; 6.86, 7.06 NH ₂ -4", NH ₂	
	(6.7)			(6.4, 2.9)				
13 a ^b	6.49 d	6.62 d	5.08 t	4.65 dd, 4.92 dd	8.11 s	8.65 s	$3.57 \text{ s}, \text{SO}_2\text{CH}_3$	
	(7.8)			(5.8, 6.1)				
13b ^b	6.35 d	6.69 d	5.12 t	4.66 dd. 4.92 dd	7.79 s	8.38 s	3.59 s. SO ₂ CH ₃	
	(7.9)			(3.9, 4.7)				
$13e^{b}$	6.37 d. 6.61 s	6.61 s. 6.71 d	4.70	⊢5.04 m	868 9 866 9		3.22. 3.28 s. SO ₂ CH ₂	
1344	6 11	6 14 d	494d 466 m		0.00 5, 0.00 5		3.40 s, SO ₂ CH ₃	
104	0.11,	0.11, 0.14 U		(47)				
1904	6 49 4	6 60 4	5.00 +	1 80 44 5 00 44		8.02 -	2 40 a SO-CH. 0 07 10 66 ha 2NH	
196.	0.42 u (7 5)	0.00 u	0.09 1	4.00 uu, 5.00 uu		0.20 8	3.45 S, SO2CH3, 5.57, 10.00 DS, 214H	
10.67	(7.0)	7 00 1	4.00	(0.0, 0.0)		7.01	0.50 - CO OU - 10.10 (b. NU)	
131	6.51 a	7.03 a	4.93-	4.99 m 4.76 dd		7.91	3.50 8, SU ₂ CH ₃ ; 10.19 (D8, NH)	
	(7.0)			(6.7)				
13g ^a	6.49 s, 6.50 d	6.49 s, 6.87 d	4.75	-5.10 m		8.26 s	3.47, 3.49 s, SO ₂ CH ₃ ; 10.51 bs, NH	
	(6.7)	(6.7)						
13 h °	6.55 d	6.80 d	$5.22~{ m t}$	4.72 dd, 4.99 dd	8.49 s	8.99 s	3.63 s, SO ₂ CH ₃	
	(7.9)			(4.1, 4.2)				
13i ^b	6.36 d	6.77 d	$5.21 \mathrm{t}$	4.82 dd, 5.00 dd	8.89 s	9.23 s	$3.59 \text{ s}, \text{SO}_2\text{CH}_3$	
	(7.4)			(4.9, 5.5)				
13ja	6.22 d	6.54 d	5.14 t	4.88 dd, 5.06 dd	8.34 s		3.52 s, SO ₂ CH ₃ ; 7.09, 7.25–8.17 aromatics	
14h°	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-4"	
16aª	6.08 d	5.09 d	4.40 dd	4.08 dd. 4.16 dd	8.22 8	8.26 s	5.78 s. H-3": 6.67 bs. NH-4": 11.42 bs. NH-1	
	(7.7)	0.00 -		(4.7. 3.4)	•	0.20 2		
16hª	610 d	5 23 d	4 41 dd	4 05 dd 4 14 dd	8 91 e	8 53 9	5.66 s H-3" 6.68 bs NH-4" 11.66 bs NH-1	
100	(77)	0.20 u	7.11 uu	(1170)	0.218	0.00 8	0.00 5, 11-0 , 0.00 b5, 14112-4 , 11.00 b5, 1411-1	
16-4	6 00 J 6 07 J	514 4 546 4	(4.1, 7.0)		9.60 -	0.00 -	5 69 5 70 - 11 9//. 6 75 6 90 h- NIU 4//	
100-	0.09 0, 0.27 0	0.14 u, 0.40 u	4.14	-4.40 Ш	0.03 8	0.00 8	5.00, 5.19 S, 11-5 ; 0.15, 0.00 DS, 14112-4	
10-0	(7.4, 7.0)	F 10 J	4 00 33			0.00 -	5 07 - 11 04 0 55 h. NUL 44 10 00 h. ONUL	
166.	5.97 a	5.19 d	4.39 dd	4.03 aa, 4.13 aa		8.32 S	5.67 s, H-3"; 6.55 Ds, NH2-4"; 10.88 Ds, 2NH	
105	(7.6)	F 0F 1	, i a	(4.4, 6.7)				
16f ^a	6.27 d	5.35 d	4.0	4-4.10 m 4.21 dd		8.14 s	5.57 s, H-3"; 7.00 bs, NH_2 -4"; 10.25 bs, NH	
	(6.7)			(2.9)				
16j ^a	4.95 d	6.09 d	4.47 t	4.09 dd, 4.20 dd	8.42 s		$5.82 \text{ s}, \text{H-3''}; 5.60 \text{ bs}, \text{NH}_2-4''; 7.31-7.87 \text{ m},$	
	(7.1)			(3.9, 4.0)			aromatics	
19ª	6.15 d	5.29 d	4.39 dd	4.09 dd, 4.18 dd	8.50, 8	.55 s	4.16 s, OMe; 5.76 s, H-3"; 6.77 bs, NH ₂ -4"	
	(7.2)			(3.3, 5.3)				
21 ^a	6.00 d	5.30 d	4.29 dd	4.07 dd, 4.15 dd	8.29 s	8.29 s	3.13 s, NMe, 5.69 s, H-3"; 7.09 bs, NH ₂ -4"	
	(6.8)			(3.0, 6.3)				
23ª	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.	
				· · · · · · · · · · · · · · · · · · ·	,		NH2-4"	
25ª	6.24 d	5.35 d	4.45 dd	4.21 dd. 4.14 dd	9.00 s	8.75 s	5.82 hs. H-3": 6.76 hs. NHa-4": 9.22 s. H-6	
20	(7.5)	0.00 u		(3.4. 4.8)	2.00 5	0.10 5		
260	6107	4 70 A	4 47 +	3 92 44 4 04 44	8 05 9	08 e	368 NCH 570 H-3" 579 ha NH-4"	
40	(7 0)	7.10 U	7.7 I V	(9 9 9 A)	0.00, 0.		0.00 5, 110113, 0.10 5, 110 , 0.12 05, 1112-4	
97c	5064	5 00 4	A 47 +	(2·2, 2·2) 1 01	010 0	15 a	262 . NOW 5 57 . W 9// 5 90 L. NET 4//	
41° 994	0.00 U	5.00 0	4.40 77	тту. г.с. т.г. 4 6 90 V	0.14,0	54 0	1 29 + NOL OL, 4 90 ~ NOL OL . 5 00 -	
40-	0.1/ U	0.24 U	4.40 UU	4.1/00 uu, 4.1/00	0.40, 8	012 8	U 9//. C CA La NUL A//	
	(1.1)			(4.1, 0.0)			п-о ; 0.04 из, INП2-4"	

^a (CD₃)₂CO. ^b (CD₃)₂SO. ^c CDCl₃.

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 derivatives¹⁸ and with literature data for other β -D-ribo-3'-C-branched nucleosides³¹⁻³³ 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 ¹H- and ¹³C-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. ¹H-NMR of 13c showed two signals for H-1' at 6.37 and 6.61 ppm,

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

		<u> </u>				,
compd	C-6	C-4	C-2	C-8	C-5	others
6ª	155.11	149.49	152.66	139.37	122.05	113.80 (CN), 82.68 (C-3'), 40.44 (SO ₂ CH ₃)
7 •	155.34	150.19	152.91	139.84	122.26	115.24 (CN), 81.03 (C-3'), 40.94 (SO ₂ CH ₃)
8ª	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 ₂ CH ₃)
13b ^b	153.79	158.62	145.77	144.53	114.40	113.00 (CN), 75.72 (C-3'), 40.43 (SO ₂ CH ₃)
1 3c ^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 ₂ CH ₃)
13 d ^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 ₂ CH ₃)
1 3e ª	155.74 (s)¢	151.76 (d)	151.22 (s)	143.58 (dd)	106.86	113.65 (CN), 78.01 (C-3'), 40.65 (SO ₂ CH ₃)
13fa	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 ₂ CH ₃)
13g ^a	154.82 (s)°	150.28 (d)	150.60 (d)	142.93 (dd)	108.34	113.00, 114.40 (2CN), 75.72 (C-3'), 40.43 (SO ₂ CH ₃)
13h ^b	151.43	149.77	151.72	146.41	131.63	112.90 (CN), 76.49 (C-3'), 40.51 (SO ₂ CH ₃)
13i ^b	142.13	161.87	152.34	148.34	122.01	112.98 (CN), 76.16 (C-3'), 40.45 (SO ₂ CH ₃)
16 a ^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'')
16bª		161.02	145.8	4, 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")
1 9 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)
26ª	156.06		136.1	3, 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)
27ª		161.04	145.8	6, 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)
28ª		160.87	145.6	1, 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 ₂ CH ₃),
						$13.31 (NCH_2CH_3)$

^a (CD₃)₂CO. ^b (CD₃)₂SO. ^c Multiplicity in the coupled ¹³C-NMR spectra.





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 ¹³C-NMR spectra of 13c with those of N-9 and N-7 isomers (13a and 13b) showed good accordance with the ¹³C-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 ¹³C-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



experiments from the ¹³C-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 ¹H- and ¹³C-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 dimethylamine³⁸ 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 NH₂-4" or C-3" of the spirooxathiole moiety was established from analytical and spectroscopic data. ¹H-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 (NH₂-4" and H-3"), thus indicating that no methylation had occurred at this moiety. ¹³C-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

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

	E	C50 ^a (µM			
	MT-4			CCmb	selectivity index (ratio
compd	HIV-1	HIV-2	CEM	(μ M)	CC50/ÉC50)
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
16 a	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
16 f	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%.

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 (EC₅₀) of $0.278 \,\mu$ M, while being toxic to MT-4 cells at a 50% cytotoxic concentration (CC₅₀) of $13 \,\mu$ M. 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

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 TSAOpurine 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 stel 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 TSAOpyrimidine derivative TSAO-m³T 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-m¹Hx showed a single amino acid change (138-Glu \rightarrow Lys) that is identical to the amino acid change that has recently been observed in several HIV-1/TSAOpyrimidine 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. ¹H-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 ¹³C-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 Me₄Si 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'-Omesyl-\$-D-xylofuranosyl]-6-N-(monomethoxytrityl)adenine and 9-[2',5'-Bis-O-(tert-butyldimethylsilyl)-3'-C-cyano-3'-O-mesyl-\$-D-ribofuranosyl]-6-N-(monomethoxytrityl)adenine (6 and 7). Compound 2²³ (3.07 g, 4 mmol) was oxidized [CrO₃/pyridine/Ac₂O (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 (0.2g, 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 $(2 \times 50 \text{ mL})$. 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 \times 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 (SO₂). Anal. (C₄₄H₅₉N₆O₇SSi₂) C, H, N.

The slowest moving fractions afforded 0.35 g (10%) of 7 as a white foam: IR (KBr) 1370, 1180 cm⁻¹ (SO₂). Anal. (C₄₄H₅₆N₆O₇-SSi₂) 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 Cs₂CO₃ (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 (NH₂), 1650 (C=CN). Anal. (C₄₄H₅₉N₆O₇SSi₂) 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 Cs₂CO₃ 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 (NH₂), 1650 (C=CN). Anal. (C₄₄H₅₉N₆O₇SSi₂) 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 ZnBr₂ (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 (NH₂), 1650 (C=CN). Anal. (C₂₄H₄₂N₆O₆SSi₂) 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 ZnBr₂, 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 (NH₂), 1650 (C=CN). Anal. (C₂₄H₄₂N₆O₆SSi₂) C, H, N.

General Procedure for the Synthesis of 2'-O-Acetyl-5'-O-benzoyl-3'-C-cyano-3'-O-mesyl-\$-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 NaHCO₃. The organic phase was separated, the aqueous phase was washed with ethyl acetate $(2 \times 20 \text{ mL})$ 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 (SO₂). Anal. (C₃₇H₃₄N₆O₁₇S₂) C, H, N.

The following fractions afforded 0.66 g (22%) of 13b as a white foam: IR (KBr) 1375, 1180 cm⁻¹ (SO₂). UV (MeOH) λ_{max} nm (log ϵ) 224 (4.11), 259 (3.73). Anal. (C₂₁H₁₉N₅O₉S) C, H, N.

The slowest moving fractions gave 0.52 g (17%) of 13a as a white foam: IR (KBr) 1380, 1180 cm⁻¹ (SO₂); UV (MeOH) λ_{max} nm (log ϵ) 226 (4.09), 246 (3.77). Anal. (C₂₁H₁₉N₅O₉S) 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 (SO₂). Anal. (C₃₇H₃₄N₆O₁₈S₂). 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 (SO₂); UV (MeOH) λ_{max} nm (log ϵ) 208 (3.98), 227 (4.09), 264 (3.96). Anal. (C₂₁H₁₉N₅O₁₀S) C, H, N.

The next fractions gave 13d (0.04 g, 3%) as a white foam: IR (KBr) 1385, 1185 cm⁻¹ (SO₂); UV (MeOH) λ_{max} nm (log ϵ) 207 (3.95), 228 (4.19), 271 (3.57). Anal. (C₂₁H₁₉N₅O₁₀S) C, H, N.

The slowest moving fractions gave 0.50 g (27%) of 13e as a white foam: IR (KBr) 1385, 1185 cm⁻¹ (SO₂); UV (MeOH) λ_{max} nm (log ϵ) 208 (4.02) 226 (4.07), 272 (3.77). Anal. (C₂₁H₁₉N₅O₁₀S) 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 (SO₂); UV (MeOH) λ_{max} nm (log ϵ) 207 (3.94), 228 (3.90), 262 (3.68). Anal. (C₂₁H₁₈N₅O₈ClS) C, H, N.

The slowest moving fractions gave 0.10 g (5%) of 13i as a white foam: IR (KBr) 1375, 1185 cm⁻¹ (SO₂); UV (MeOH) λ_{max}

nm (log $\epsilon)$ 210 (4.07), 228 (4.14), 270 (3.75). Anal. (C_{21}H_{18}N_5O_8-ClS) 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 (SO₂). Anal. (C₂₃H₂₁N₃O₆S) 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 Cs₂CO₃ (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 (NH₂), 1650 (C=CN). Anal. (C₂₁H₁₈N₅O₈ClS) C, H, N.

General Procedure for the Synthesis of [[2',5'-Bis-O-(tertbutyldimethylsilyl)-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 $(2 \times 50 \text{ mL})$. 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 Na₂SO₄, 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 (NH₂), 1650 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 212 (4.15), 226 (4.14), 250 (3.79). Anal. (C₂₄H₄₂N₅O₇SSi₂) 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 (NH₂), 1645 (C==CN), UV (MeOH) λ_{max} nm (log ϵ) 212 (4.15), 229 (4.13), 262 (3.86). Anal. (C₂₄H₄₂N₅O₇SSi₂) 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 (NH₂), 1650 (C=CN). Anal. (C₄₃H₇₈N₆O₁₃S₂-Si₄) 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 0.16g (28%) of 16e as an amorphous solid: IR (KBr) 3500, 3420 cm⁻¹ (NH₂), 1650 (C=CN); UV (MeOH) λ_{mar} nm (log ϵ) 207 (4.14), 226 (4.22), 262 (4.12). Anal. (C₂₄H₄₂N₅O₈SSi₂) C, H, N.

 $[3-[2',5'-Bis-O-(tert-butyldimethylsilyl)-\beta-D-ribofurano$ syl]xanthine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''dioxide) (16f). 13f (0.2 g, 0.37 mmol) was reacted according to

TSAO Analogues

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 (NH₂), 1650 (C=CN). UV (MeOH) λ_{max} nm (log ϵ) 203 (3.99), 221 (3.96), 269 (3.65). Anal. (C₂₄H₄₂N₅O₉SSi₂) 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 (NH₂), 1645 (C=CN). Anal. (C₂₈H₄₄N₃O₆SSi₂) 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 $(2 \times 50 \text{ mL})$. 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 (Na₂SO₄), 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 H₂O) (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 (NH₂), 1645 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 204 (4.30), 219 (4.24), 252 (4.27). Anal. (C₂₅H₄₄N₆O₆-SSi₂) 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 (0.12g, 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 (NH₂), 1650 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 215 (4.16), 270 (4.16). Anal. (C₂₈H₄₆N₆O₆SSi₂) 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 NH₄OH (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 (TBDMSCI/ 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 (NH₂), 1650 (C—CN). Anal. (C₂₄H₄₁N₅O₆-SSi₂) 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 (0.1mmol) in acetone (1.5 mL) were added K₂CO₃ (0.05 mmol) andthe corresponding alkyl halide (1.1-2.0 mmol). The reactionmixture was refluxed for 3-6 h. After removal of the solvent, theresidue 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 (NH₂), 1715 (C=O), 1645 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 212 (4.12), 226 (4.13), 249 (3.75). Anal. (C₂₅H₄₃N₅O₇SSi₂) 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 (NH₂), 1715 (C=O), 1645 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 210 (4.10), 228 (4.14), 261 (3.84). Anal. (C₂₅H₄₃N₅O₇SSi₂) 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 (NH₂), 1710 (C=O), 1640 (C=CN); UV (MeOH) λ_{max} nm (log ϵ) 210 (4.12), 228 (4.14), 260 (3.80). Anal. (C₂₈H₄₅N₅O₇SSi₂) C, H, N.

Antiretrovirus Activity Assays. HIV-1 was originally obtained from the culture supernatant of the persistently HIVinfected 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 $(5 \times 10^5 \text{ cells/mL})$ were suspended in fresh culture medium and infected with HIV-1 at 100 times the 50% cell culture infective dose (CCID₅₀) per milliliter of cell suspension. Then 100 μ M of infected cell suspension was transferred to microtiter plate wells and mixed with 100 μ L 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 (EC₅₀) 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 (CC₅₀) was defined as the compound concentration required to reduce by 50% the number of mock-infected MT-4 cells.

Acknowledgment. We thank María Jesús Moreno and Ann Absillis, for excellent technical assistance, and Francisco Caballero, for editorial assistance. This research was supported in part by grants from the Spanish CICYT (Project FAR 91-0769) and from the Plan Regional de Investigación de la Comunidad de Madrid (Project C195/ 91), the AIDS Basic Research Programme of the European Community, and by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Projects 3.0097.87 and 3.0026.91), the Belgian National Fonds voor Wtenschappelijk Onderzoek (Project 3.3010.91), and the Belgian Geconcerteerde Onderzoeksacties (Project 90/94-2).

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