

1,6,7,12-Tetramethoxy-4,9-dihydroxy-3,10-perylenequinone (26). A mixture of 23 (3.0 g, 4.73 mmol), sodium hydroxide (6.0 g), and water (2 mL) was heated in an oil bath. After the temperature was elevated gradually to 200 °C to remove water, it was further heated for 2 h at 250 °C. After cooling, water (50 mL) was added to dissolve the residue. Dimethyl sulfate (7.5 mL) was added dropwise over 30 min to the resulting solution. It was adjusted to pH 8-9 and stirred for 2 h at 50 °C. After cooling, the precipitate was collected and washed with water and acetone to afford 26 (450 mg, 21%): black amorphous; mp >300 °C dec; IR (KBr) 3450 (OH), 3140 (aromatic C—H), 1630 (quinone C=O), 1395 cm⁻¹. Anal. (C₂₄H₁₈O₈) C, H.

1,6,7,12-Tetraacetyl-4,9-dihydroxy-3,10-perylenequinone (27). Compound 25 (600 mg, 1.59 mmol) was suspended in acetic anhydride (30 mL) and pyridine (30 mL). The mixture was heated in an oil bath (100 °C) for 48 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (SiO₂, 20 g) using chloroform-methanol (100:1) as eluant to furnish 27 (115 mg, 13%): yellow amorphous; mp 200-201 °C; IR (KBr) 3150 (aromatic C—H), 1680 (C=O of ester), 1625 (C=O of quinone), 1590 (C=C), 1482, 1397, 1376 cm⁻¹; ¹H NMR (CDCl₃) δ 2.17, 2.18, 2.42 (all s, 6 H each, OAc × 6), 6.53 (s, 2 H, 2-H, 11-H), 7.14 (s, 2 H, 5-H, 8-H). Anal. (C₂₈H₁₈O₁₂) C, H.

Cytotoxic Assay. Assay for cytotoxicity in various tumor cells were carried out according to the procedures described previously.^{8,9,10} The tumor cells included in this study were A-549

lung carcinoma, HCT-8 colon carcinoma, RPMI-7951 melanoma, TE-671 medulloblastoma, KB epidermoid carcinoma of nasopharynx, and P-388 lymphocytic leukemia cells.

Protein Kinase C Assay. The assay was performed by a modification of the mixed micelle assay as described elsewhere.¹¹ The reaction mixture in a total volume of 250 μL contained 0.3% Triton X-100 with 6 mol% phosphatidylserine (Avanti) and 0.5 mol% dioleoylglycerol (Avanti) in 29 mM Hepes buffer, pH = 7.5, 10 mM magnesium chloride, 100 μM calcium chloride, and 20 μM [³²P]ATP. Compound was dissolved in DMSO, and 10 μL was added to the assay. DMSO alone was added in the control assay reactions. The reaction was started with 20 μL of protein kinase C (PKC) (1 μg/mL). The reaction was terminated with 0.5 mL ice-cold trichloroacetic acid followed by 100 μL of bovine serum albumin (1 mg/mL). The precipitate was collected by vacuum filtration using GF/C filters and quantified by counting in a β scintillation counter.

Anti HSV-1/HSV-2 Assay. Confluent Vero cells were infected with 3PFU/Cell of HSV-1 (Kos) or HSV-2 (333) in RPMI-1640 medium containing 2% FCS. Two-fold serial dilutions of the drugs (final concentration: 100, 50, 25, 12.5 μg/mL) were added during the inoculation phase. After 24 h, the CPE was observed under the microscope.

Acknowledgment. This investigation was supported by a grant from the National Cancer Institute CA-17625 (K.H. Lee).

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3'-Spiro Nucleosides, a New Class of Specific Human Immunodeficiency Virus Type 1 Inhibitors: Synthesis and Antiviral Activity of [2',5'-Bis-O-(tert-butyl dimethylsilyl)-β-D-xylo- and -ribofuranose]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-Dioxide] (TSAO) Pyrimidine Nucleosides

María-José Camarasa,*† María-Jesús Pérez-Pérez,† Ana San-Félix,† Jan Balzarini,† and Erik De Clercq†

Instituto de Química Médica, Juan de la Cierva, 3. 28006 Madrid, Spain, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000, Leuven, Belgium. Received January 27, 1992

A series of 3'-spiro nucleosides have been synthesized and evaluated as anti-HIV-1 agents. Reaction of O-mesyloxy nucleosides of furanos-3'-ulosyl nucleosides with base afforded [1-[2',5'-bis-O-(tert-butyl dimethylsilyl)-β-D-xylo- and -ribofuranosyl]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-dioxide] derivatives of thymine, uracil and 4-N-acetylcytosine 11 and 12. Desilylation of 11 and 12 gave the full deprotected 3'-spiro xylo- and ribofuranosyl nucleosides 13 and 14 or the partially 5'-O-deprotected-3'-spiro β-D-xylo- and -ribo-nucleosides 15 and 16, or 2'-O-deprotected-3'-spiro β-D-ribo-nucleoside 17. 2'-Deoxygenation of 17 afforded 2'-deoxy-3'-spiro β-D-erythro-pentofuranosyl derivative 18. These 3'-spiro derivatives were evaluated for their anti-HIV-1 activity. All 3'-spiro nucleosides having a xylo configuration did not show any anti-HIV-1 activity. 3'-Spiro ribo-nucleosides with none or only one silyl group at C-2' or C-5' or the 2'-deoxy derivative were also inactive at subtoxic concentrations. However, 3'-spiro ribo-nucleosides having two silyl groups at C-2' and C-5' were potent and selective inhibitors of HIV-1. None of the nucleoside analogues that showed anti-HIV-1 activity proved inhibitory to the replication of HIV-2 or SIV.

Introduction

Azidothymidine (AZT, Retrovir) and DDI (dideoxyinosine) are, so far, the only drugs approved for the clinical treatment of acquired immunodeficiency syndrome (AIDS).¹⁻⁴ However, they also induce bone marrow sup-

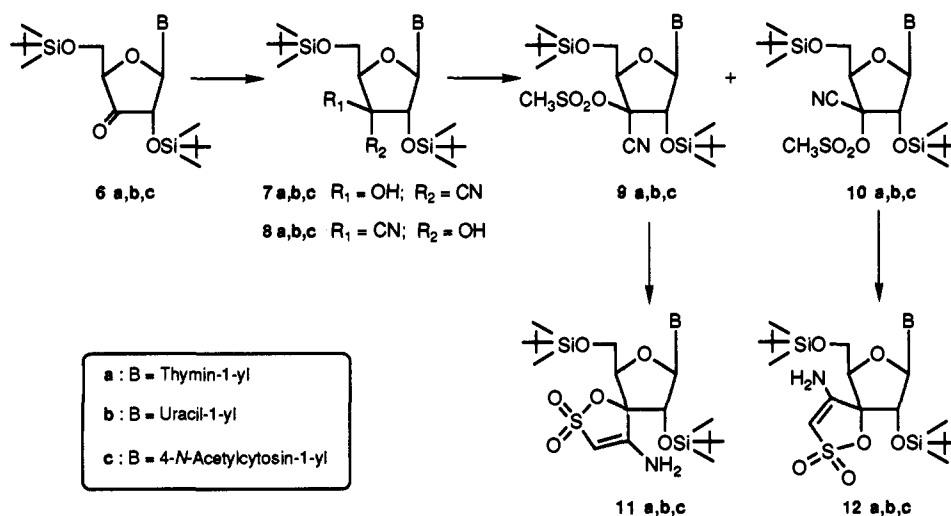
pression (AZT), peripheral neuropathy (DDI), pancreatitis (DDI), and other side effects.³⁻⁶ AZT and DDI have

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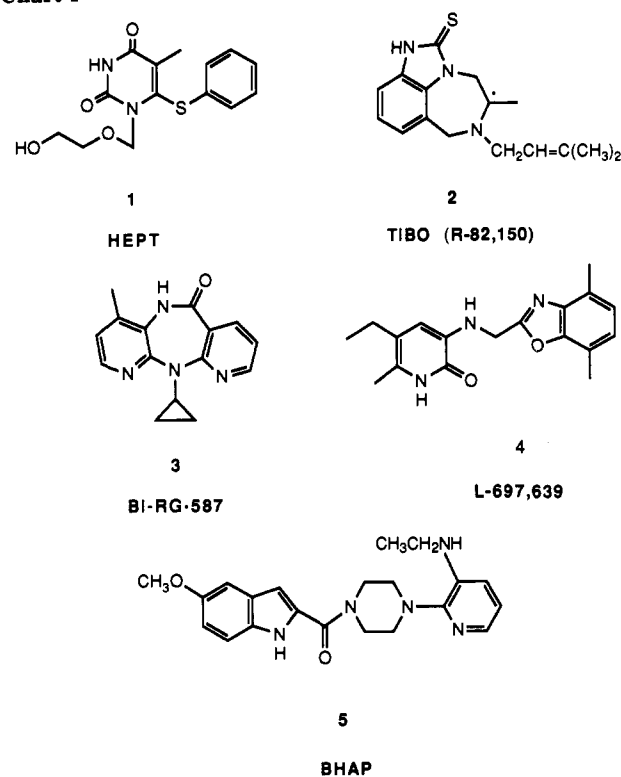
Scheme I



proved to be potent inhibitors of HIV reverse transcriptase.⁷⁻⁹ The emergence of drug-resistant virus strains¹⁰⁻¹² together with the severe side effects that have been observed, justify the search for new compounds which are more potent and selective in their anti-HIV activity.

Recently, five new lead compounds have been identified as highly specific inhibitors of HIV-1 replication, i.e. the

Chart I

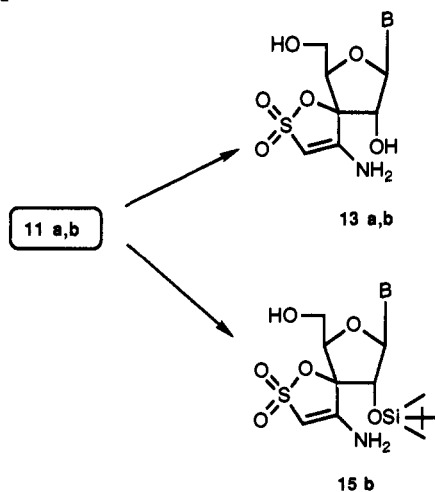


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acyclouridine 1 (HEPT),¹³⁻¹⁵ benzodiazepinone (TIBO) 2,^{16,17} dipyridodiazepinone 3,^{18,19} pyridinone 4,²⁰ and bis-

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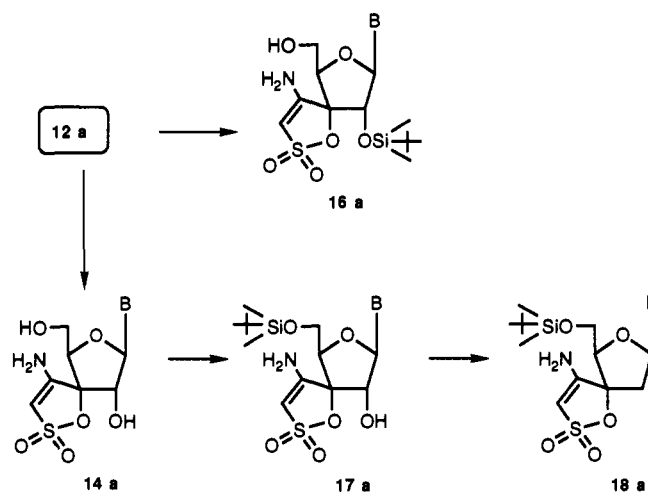
Scheme II



(heteroaryl)piperazine (BHAP) 5.²¹ All these compounds are highly specific for HIV-1. They do not inhibit HIV-2 or any other retroviruses. The specific HIV-1 inhibitors primarily interact with a nonsubstrate binding site at the HIV-1 reverse transcriptase (RT), but, in addition, their RT-inhibitory potency also depends on the type of the template used to monitor HIV-1 RT activity.²²⁻²⁵

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Scheme III



In a previous paper²⁶ we described the synthesis of furanose-3-spiro-5'-[4'-amino-1',2'-oxathiole 2',2'-dioxide] derivatives by reaction of *O*-mesylcyanohydrins of furanos-3-uloses with base. The method involves abstraction of one proton from the mesylate methyl group, followed by nucleophilic attack of the carbanion thus formed at the nitrile carbon atom. We have used this procedure for the synthesis of a variety of furanose- and pyranose-3-spiro sugar derivatives.²⁷ Similar behavior was observed with *O*-mesylcyanohydrins of furanosid-3'-ulosylthymine which under basic conditions afforded the corresponding 3'-spiro nucleosides.²⁸ In order to prepare a new kind of highly functionalized branched chain nucleosides, we now extend the method to 3'-*C*-cyanomesylates of pyrimidine nucleosides, and here we report on the synthesis and anti-HIV-1 activity of a novel class of compounds, the [2',5'-bis-*O*-(*tert*-butyldimethylsilyl)- β -D-pentofuranosyl]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-dioxide] (TSAO) pyrimidine derivatives.²⁹

These spiro branched-chain nucleosides are potent and selective inhibitors of HIV-1, but not HIV-2, replication. They represent a new lead in the development of specific anti-HIV-1 agents. The lead compound 12a is the first example of a nucleoside analogue which is highly specific for HIV-1.

Chemistry

Treatment of the 3'-ketonucleosides derived from thymine 6a,³⁰ uracil 6b,³¹ and 4-*N*-acetylcytosine 6c³² with

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sodium cyanide in a two phase ethyl ether/water system in the presence of sodium bicarbonate gave a mixture of the two epimeric nucleoside 3'-cyanohydrins **7** and **8**^{30,31} (Scheme I). These cyanohydrins, on standing in solution, reversed to the corresponding ketonucleosides **6** used as starting materials. Thus, they were not isolated, and used without further purification in the next step. Reaction of the cyanohydrin mixture (**7** and **8**) with mesyl chloride in pyridine gave the respective 3'-C-cyano-3'-O-mesyl-*xylo* and -*ribo* derivatives of thymine **9a** (53%) and **10a** (10%), uracil **9b** (52%) and **10b** (11%), and 4-*N*-acetylcytosine **9c** (53%) and **10c** (15%). Treatment of the *xylo*-cyanomesylates **9a** and **9b** with Cs₂CO₃ and of **9c** with DBU afforded the spiro derivatives **11** [**11a** (87%); **11b** (80%); **11c** (63% yield)]. Similarly, reaction of the *ribo*-cyanomesylates **10a-c** with Cs₂CO₃ afforded the spiro nucleosides **12** [**12a** (85%); **12b** (39%); **12c** (70% yield)].

Treatment of **11a**, **11b** (Scheme II) and **12a** (Scheme III) with tetrabutylammonium fluoride afforded the corresponding 3'-spiro 2',5'-O-deprotected nucleosides **13a**, **13b**, and **14a**. Attempts to deprotect the spiro derivatives of 4-*N*-acetylcytosine **11c** and **12c** gave complex reaction mixtures. Reaction of **11b** and **12a** with methanolic 0.1 N HCl gave the 5'-O-deprotected spiro derivatives **15b** and **16a**, respectively. Finally, treatment of **14a** with 1.1 equiv of *tert*-butyldimethylsilyl chloride gave the 5'-protected-2'-deprotected derivative **17a**. Deoxygenation³³ at the 2' position of **17a** by treatment with *N,N*-thiocarbonyldiimidazole followed by reaction with tributyltin hydride in the presence of α,α' -azobis(isobutyronitrile) afforded the 2'-deoxy derivative of thymine **18a** in 50% yield.

Since no epimerization has been observed during mesylation of cyanohydrins,^{26,27,34-36} the absolute configuration of the cyanomesylates **9** and **10** was assumed to be the same as that of the corresponding cyanohydrins **7** and **8**, and were assigned as *xylo* for the major compounds (**7a-c** and **9a-c**) and *ribo* for the minor compounds (**8a-c** and **10a-c**) based on the following criteria. Due to the deshielding effect of the adjacent mesyl group, H-2' in **9a-c** (δ 4.87-5.17) appeared at lower field than in **10a-c** (δ 4.51-4.62), while H-4' in compounds **10a-c** (δ 4.73-4.76) appeared at lower field than in compounds **9a-c** (δ 4.51-4.74). The stereochemical assignments are in agreement with the relative steric hindrance of the upper (β) or the lower (α) sides of the furanose ring. The *xylo* configuration of the major compounds **7a-c** and **9a-c** resulted from the approach of the CN⁻ ion to the uloses **6a-c** from the sterically less hindered α face of the furanose ring, opposite to the base and the 5'-O-substituent. This is in agreement with the stereochemistry observed for reactions of nucleophiles (RLi, RMgBr, and R₃Al) with **6b**.³⁷ The

assignments of cyanomesylates **9a-c** and **10a-c** as *xylo* and *ribo*, respectively, were confirmed by NOE experiments carried out on the corresponding spiro derivatives **11a**, **11c** and **12a**, **12c** as shown below.

The ¹H NMR of the spiro derivatives **11a-c** and **12a-c** showed no signal for a mesylate group, but there were two new singlets at δ 6.31-6.95 (NH₂) and δ 5.56-5.63 (H-3'') for **11a-c**, and at δ 6.44-6.61 (NH₂) and δ 5.71-5.79 (H-3'') for **12a-c**.³⁸

The absolute configurations at C-3' for spiro derivatives **11a**, **11c** and **12a**, **12c** and thus, for the *xylo* (**9a**, **9c**) and *ribo* (**10a**, **10c**) cyanomesylates were unequivocally determined by NOE.^{39,40} The signals of the NH₂ group of the compounds **11a**, **11c** and **12a**, **12c** were irradiated, and the magnitudes of NOE at all the protons were observed.⁴¹ Thus irradiation of the NH₂ group of **11a**, **11c** caused enhancements of the signals for H-1', H-4', and H-3''. Similarly, irradiation of the NH₂ group of **12a**, **12c** influenced the signals for H-2', H-5'a and H-3''.⁴¹ The NOE values indicated that in nucleosides **11a**, **11c** protons H-3'', H-4', H-1' and NH₂ are all "down", and thus, their structure is β -D-*xylo*. In compounds **12a**, **12c** protons H-3'', H-2', H-5'a and NH₂ are "up", thus, their structure is β -D-*ribo*.

The coupling constants of the furanose ring protons observed for the *xylo* derivatives **9a-c**, **11a-c**, **13a**, **13b**, and **15b** ($J_{1,2'} = 1.2-5.7$ Hz) are smaller than those observed for the *ribo*-pentofuranosyl nucleosides **10a-c**, **12a-c**, **14a**, **16a**, **17a**, and **18a** ($J_{1,2'} = 7.3-8.5$ Hz). These couplings constants are also in a good agreement with those reported in the literature for 3'-C-branched- β -D-*xylo*-nucleosides ($J_{1,2'} = 2.0-5.1$ Hz)⁴² and 3'-C-branched- β -D-*ribo*-nucleosides ($J_{1,2'} = 7.5-8.0$ Hz).⁴³⁻⁴⁵ This suggests that these coupling constant values could be used to assign the C-3'-configuration of an unknown 3'-C-cyanomesylate or 3'-spiro nucleoside.

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Biological Results

A number of *xylo*- and *ribo*-nucleoside analogues with a 3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-dioxide] substituent in either (*S*) (*xylo*) or (*R*) (*ribo*) configuration and substituted at C-5' and/or C-2' by a *tert*-butyldimethylsilyl (tBDMS) group, were evaluated for their anti-HIV-1 activity in human MT-4 lymphocyte cells.

Compounds with silyl groups at C-2' and C-5' and a 3'-spiro moiety in the (*S*) configuration (11a-c) did not show any anti-HIV-1 activity at subtoxic concentrations. Their 50% cytotoxic concentration (CC₅₀) ranged from 3.1 to 18 µg/mL. Also, those thymine and uracil derivatives that contained a 3'-spiro group in the (*S*) configuration and no silyl group at C-2' and C-5' of the ribose moiety were devoid of any anti-HIV-1 activity. However, when the 3'-spiro moiety was in the (*R*) configuration (compounds 12a-c), and silyl groups were present at C-2' and C-5', marked anti-HIV-1 activity was noted. The thymine derivative (12a) had an EC₅₀ (50% effective concentration) of 0.034 µg/mL, whereas the uracil (12b) and 4-*N*-acetylcytosine (12c) derivatives were 3-fold less effective than their thymine congener 12a. Interestingly, when only one silyl group was present, either at the C-2' position (16a) or C-5' position (17, 18), the compounds were completely inactive. Also, when only the 3'-spiro moiety was present, as in 14a, no inhibition was noted. The thymine derivative 14a was not cytotoxic at 100 µg/mL, which indicates that the toxicity observed for compounds 12a-c and 10a could be attributed to the silyl groups at C-2' or C-5' rather than to the 3'-spiro group.

None of the nucleoside analogues that showed activity against HIV-1 replication in MT-4 cells proved inhibitory to the replication of HIV-2 (ROD), HIV-2 (EHO), or SIV (MAC₂₅₁) in vitro (data not shown). The TSAO class of anti-HIV-1 compounds represents the first example of nucleoside analogues, containing an intact ribose moiety, that are highly specific and selective inhibitors of HIV-1. In this respect, these compounds behave similarly to the non-nucleoside derivatives HEPT,¹³⁻¹⁵ TIBO,^{16,17} BI-RG-587 (nevirapin),^{18,19} L-697,639, L697,661,²⁰ and BHAP.²¹ The TSAO derivatives should be considered as novel candidate drugs that deserve further investigations for their therapeutic potential in the treatment of HIV-1 infections.

Experimental Section

Chemical Procedures. Melting points were measured with a Reichert-Jung Kofler micro hot stage apparatus and are uncorrected. Microanalyses were obtained with a Heareus 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 90, 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 F₂₅₄ (Merck). Flash column chromatography was performed with silica gel 60 (230-400 mesh) (Merck).

Proximities were established conventionally on the basis of using NOE.

General Procedure for the Synthesis of 2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-cyano-3'-*O*-mesyl-β-*D*-pentofuranosyl Nucleosides (9a-c and 10a-c). A mixture of the 3'-ketonucleoside 6a-c (4 mmol), 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 16 h. 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, was dissolved in dry pyridine (8 mL). To this

Table I. Inhibitory Effects of the Test Compounds on HIV-1-Induced Cytopathicity in MT-4 Cells

compound ^a	EC ₅₀ ^b (µg/mL)	CC ₅₀ ^c (µg/mL)	SI ^d
10a	>1.6	4.3 ± 1.8	<2.7
11a	>4	18 ± 16	<4.5
11b	>1.6	3.1 ± 0.5	<2
11c	>4	7.1 ± 2.7	<1.8
12a	0.034 ± 0.015	7.7 ± 1.5	227
12b	0.114 ± 0.023	8.3 ± 0.55	73
12c	0.097 ± 0.043	7.5 ± 2.2	77
13a	>500	≥500	≤1
13b	>80	320 ± 40	<4
14a	>100	>100	-
15b	>100	221 ± 5.0	<2
16a	>40	106 ± 17	<2.6
17a	>20	45 ± 2.6	<2.2
18a	>0.8	1.5 ± 0.04	<1.9
HEPT	5.5 ± 0.1	>200	>36
TIBO (R82150)	0.021 ± 0.011	>20	>952
BI-RG-587	0.038 ± 0.015	>20	>526

^aData represent the mean values of at least three to five independent experiments. ^b50% effective concentration or compound concentration required to inhibit HIV-1-induced cytopathicity in MT-4 cells by 50%. ^c50% cytotoxic concentration or compound concentration required to reduce MT-4 cell viability by 50%. ^dSelectivity index or ratio of CC₅₀ to EC₅₀.

solution was added mesyl chloride (1.6 mL, 20 mmol). 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.

1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-cyano-3'-*O*-mesyl-β-*D*-xylofuranosyl]thymine and 1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-cyano-3'-*O*-mesyl-β-*D*-ribofuranosyl]thymine (9a and 10a). The residue was chromatographed with ethyl acetate/hexane (1:4). The fastest moving fractions afforded 1.18 g (53%) of 9a as a white foam: IR (KBr) 1375, 1185 cm⁻¹ (SO₂); ¹H NMR (CDCl₃, 90 MHz) δ 1.96 (s, 3 H, CH₃-5), 3.33 (s, 3 H, CH₃SO₂), 4.05 (m, 2 H, H-5'), 4.51 (m, 1 H, H-4'), 4.87 (d, 1 H, H-2', J_{1,2'} = 2 Hz), 6.03 (d, 1 H, H-1'), 7.28 (s, 1 H, H-6), 9.12 (bs, 1 H, NH-3); ¹³C NMR (CDCl₃, 20 MHz) δ 12.26 (CH₃-5), 40.24 (CH₃SO₂), 59.17 (C-5'), 81.51, 83.55 (C-2', C-4'), 82.51 (C-3'), 91.57 (C-1'), 111.87, 112.63 (CN, C-5), 134.68 (C-6), 150.22 (C-2), 163.55 (C-4). Anal. (C₂₄H₄₃N₃O₈SSi₂) C, H, N, S.

The slowest moving fractions afforded 0.22 g (10%) of 10a as a white foam: IR (KBr) 1375, 1180 cm⁻¹ (SO₂); ¹H NMR (CDCl₃, 300 MHz) δ 1.94 (s, 3 H, CH₃-5), 3.26 (s, 3 H, CH₃SO₂), 4.04 (m, 2 H, H-5', J_{5'a,5'b} = 12, J_{4',5'a} = 1.2, J_{4',5'b} = 2.1 Hz), 4.51 (d, 1 H, H-2', J_{1,2'} = 8.3 Hz), 4.73 (m, 1 H, H-4'), 6.24 (d, 1 H, H-1'), 7.38 (s, 1 H, H-6), 8.48 (bs, 1 H, NH-3); ¹³C NMR (CDCl₃, 20 MHz) δ 12.05 (CH₃-5), 40.33 (CH₃SO₂), 62.09 (C-5'), 80.34 (C-3'), 78.20, 84.10, 84.28 (C-2', C-4', C-1'), 112.24, 114.19 (C-5, CN), 133.68 (C-6), 150.42 (C-2), 163.18 (C-4). Anal. (C₂₄H₄₃N₃O₈SSi₂) C, H, N, S.

1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-cyano-3'-*O*-mesyl-β-*D*-xylofuranosyl]uracil and 1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-cyano-3'-*O*-mesyl-β-*D*-ribofuranosyl]uracil (9b and 10b). The residue was chromatographed with ethyl acetate/hexane (1:4). The fastest moving fractions afforded 0.26 g (11%) of 10b as a white foam: IR (KBr) 1375, 1180 cm⁻¹ (SO₂); ¹H NMR (CDCl₃, 90 MHz) δ 3.23 (s, 3 H, CH₃SO₂), 4.03 (m, 2 H, H-5'), 4.56 (d, 1 H, H-2', J_{1,2'} = 7.5 Hz), 4.73 (m, 1 H, H-4'), 5.76 (dd, 1 H, H-5), 6.26 (d, 1 H, H-1'), 7.80 (d, 1 H, H-6), 8.96 (bs, 1 H, NH-3); ¹³C NMR (CDCl₃, 20 MHz) δ 40.33 (CH₃SO₂), 62.46 (C-5'), 78.76, 80.50, 84.33, 84.93 (C-2', C-3', C-4', C-1'), 103.59 (C-5), 114.00 (CN), 138.73 (C-6), 150.27 (C-2), 162.55 (C-4). Anal. (C₂₃H₄₁N₃O₈SSi₂) C, H, N, S.

From the slowest moving fractions 9b (1.25 g, 52%) was isolated as a white foam: IR (KBr) 1375, 1185 cm⁻¹ (SO₂); ¹H NMR (CDCl₃, 90 MHz) δ 3.20 (s, 3 H, CH₃SO₂), 4.03 (m, 2 H, H-5'), 4.60 (m, 1 H, H-4'), 4.93 (d, 1 H, H-2', J_{1,2'} = 1.5 Hz), 5.73 (d, 1 H, H-5), 5.86 (d, 1 H, H-1'), 7.46 (d, 1 H, H-6), 9.73 (bs, 1 H, NH-3);

^{13}C NMR (CDCl_3 , 20 MHz) δ 40.01 (CH_3SO_2), 61.88 (C-5'), 77.71, 80.06, 82.51, 86.42 (C-2', C-3', C-4', C-1'), 105.10 (C-5), 114.33 (CN), 139.10 (C-6), 149.69 (C-2), 163.00 (C-4). Anal. ($\text{C}_{25}\text{H}_{41}\text{N}_3\text{O}_8\text{SSi}_2$) C, H, N, S.

4-*N*-Acetyl-1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-cyano-3'-*O*-mesyl- β -D-xylofuranosyl]cytosine and 4-*N*-Acetyl-1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-3'-*C*-cyano-3'-*O*-mesyl- β -D-ribofuranosyl]cytosine (9c and 10c). The residue was chromatographed with ethyl acetate/hexane (1:1). The fastest moving fractions afforded 1.3 g (53%) of 9c as a white foam: IR (KBr) 1375, 1185 cm^{-1} (SO_2); ^1H NMR (CDCl_3 , 90 MHz) δ 2.28 (s, 3 H, NAc), 3.17 (s, 3 H, CH_3SO_2), 4.10 (m, 2 H, H-5'), 4.76 (m, 1 H, H-4'), 5.17 (d, 1 H, H-2', $J_{1,2'} = 1.2$ Hz), 5.84 (d, 1 H, H-1'), 7.50 (d, 1 H, H-5), 7.82 (d, 1 H, H-6), 10.6 (bs, 1 H, NH-4); ^{13}C NMR (CDCl_3 , 20 MHz) δ 40.06 (CH_3SO_2), 59.11 (C-5'), 80.51 (C-3'), 81.57, 85.30 (C-2', C-4'), 93.29 (C-1'), 96.75 (C-5), 112.78 (CN), 143.20 (C-6), 154.87 (C-2), 163.67 (C-4), 171.45 (4-NCO). Anal. ($\text{C}_{25}\text{H}_{44}\text{N}_4\text{O}_8\text{SSi}_2$) C, H, N, S.

The slowest moving fractions gave 0.37 g (15%) of 10c as a white foam: IR (KBr) 1375, 1180 cm^{-1} (SO_2); ^1H NMR (CDCl_3 , 90 MHz) δ 2.30 (s, 3 H, NAc), 3.24 (s, 3 H, CH_3SO_2), 4.04 (m, 2 H, H-5'), 4.62 (d, 1 H, H-2', $J_{1,2'} = 8$ Hz), 4.74 (m, 1 H, H-4'), 6.42 (d, 1 H, H-1'), 7.45 (d, 1 H, H-5), 8.23 (d, 1 H, H-6), 10.28 (bs, 1 H, NH-4); ^{13}C NMR (CDCl_3 , 50 MHz) δ 40.36 (CH_3SO_2), 62.30 (C-5'), 80.70 (C-3'), 80.07, 84.46, 85.86 (C-1', C-2', C-4'), 97.81 (C-5), 114.10 (CN), 143.60 (C-6), 154.96 (C-2), 163.23 (C-4), 171.37 (4-NCO). Anal. ($\text{C}_{25}\text{H}_{44}\text{N}_4\text{O}_8\text{SSi}_2$) C, H, N, S.

General Procedure for the Synthesis of [2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)- β -D-pentofuranose]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] Pyrimidine Nucleosides (11a-c and 12a-c). To a solution of the 3'-*C*-cyano-3'-*O*-mesylpentofuranosyl nucleoside 9a-c or 10a-c (1 mmol) in dry acetonitrile (10 mL) was added either Cs_2CO_3 (325 mg, 1 mmol) or DBU (0.15 mL, 1 mmol). The mixture was stirred at room temperature for 1-24 h and then filtered and evaporated to dryness. The residue was purified by column chromatography.

1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)- β -D-xylofuranosyl]thymine]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (11a). 9a was treated with Cs_2CO_3 and stirred for 6 h at room temperature according to the general procedure. The residue was chromatographed with ethyl acetate/hexane (1:3) to give a white solid 11a which was crystallized from hexane (0.57 g, 87%): mp >230 °C; IR (KBr) 3420, 3350 cm^{-1} (NH_2), 1655 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{SO}$, 300 MHz] δ 1.81 (s, 3 H, CH_3 -5), 3.79 (m, 2 H, H-5'), $J_{5'a,5'b} = 11.7$ Hz), 4.43 (d, 1 H, H-2', $J_{1,2'} = 5.7$ Hz), 4.53 (dd, 1 H, H-4', $J_{4',5'a} = 6.4$, $J_{4',5'b} = 3.4$ Hz), 5.56 (s, 1 H, H-3''), 6.07 (d, 1 H, H-1'), 6.95 (bs, 2 H, NH_2), 7.44 (s, 1 H, H-6), 11.52 (bs, 1 H, NH-3); ^{13}C NMR [$(\text{CD}_3)_2\text{CO}$, 50 MHz] δ 12.53 (CH_3 -5), 61.53 (C-5'), 82.54, 84.35, 89.47, 90.05 (C-1', C-2', C-4', C-3'), 93.22 (C-3'), 112.31 (C-5), 135.83 (C-6), 151.42, 153.57 (C-2, C-4'), 163.95 (C-4). Anal. ($\text{C}_{24}\text{H}_{43}\text{N}_3\text{O}_8\text{SSi}_2$) C, H, N.

1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)- β -D-xylofuranosyl]uracil]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (11b). 9b was treated with Cs_2CO_3 and stirred at room temperature according to the general procedure for 8 h. The residue was chromatographed with chloroform/acetone (6:1) to give a solid 11b which was crystallized from hexane (0.31 g, 80%): mp 128-130 °C; IR (KBr) 3450, 3370 cm^{-1} (NH_2), 1650 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{CO}$, 300 MHz] δ 3.96 (d, 2 H, H-5', $J_{4',5'} = 5.1$ Hz), 4.58 (d, 1 H, H-2', $J_{1,2'} = 3.9$ Hz), 4.71 (t, 1 H, H-4'), 5.66 (s, 1 H, H-3''), 5.81 (dd, 1 H, H-5), 6.09 (d, 1 H, H-1'), 6.35 (bs, 2 H, NH_2), 7.69 (d, 1 H, H-6), 10.18 (bs, 1 H, NH-3); ^{13}C NMR (CDCl_3 , 75 MHz) δ 60.13 (C-5'), 82.66, 83.37, (C-2', C-4'), 90.29, 90.99, 91.50 (C-1', C-3', C-3''), 103.31 (C-5), 139.76 (C-6), 150.33, 151.62 (C-2, C-4''), 163.02 (C-4). Anal. ($\text{C}_{25}\text{H}_{41}\text{N}_3\text{O}_8\text{SSi}_2$) C, H, N.

4-*N*-Acetyl-1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)- β -D-xylofuranosyl]cytosine]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (11c). 9c was treated with DBU according to the general procedure for 3 h. The residue was chromatographed with ethyl acetate/hexane (1:1) to give a white solid 11c which was crystallized from hexane (0.22 g, 63%): mp >230 °C; IR (KBr) 3440, 3350 cm^{-1} (NH_2), 1650 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{CO}$, 300 MHz] δ 2.22 (s, 3 H, NAc), 4.03 (m, 2 H, H-5'), 4.60 (d, 1 H, H-2', $J_{1,2'} = 2.2$ Hz), 4.88 (t, 1 H, H-4', $J_{4',5'} = 5.3$ Hz), 5.63 (s, 1 H, H-3''), 6.04 (d, 1 H, H-1'), 6.31 (bs, 2 H, NH_2), 7.42 (d, 1 H,

H-5), 8.10 (d, 1 H, H-6), 9.90 (bs, 1 H, NH-4); ^{13}C NMR [$(\text{CD}_3)_2\text{CO}$, 50 MHz] δ 61.26 (C-5'), 83.52, 85.03, 89.57, 92.69 (C-2', C-4', C-1', C-3''), 92.45 (C-3'), 96.82 (C-5), 145.50 (C-6), 153.40, 155.61 (C-2, C-4''), 163.93 (C-4), 171.43 (4-NCO). Anal. ($\text{C}_{25}\text{H}_{44}\text{N}_4\text{O}_8\text{SSi}_2$) C, H, N.

1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)- β -D-ribofuranosyl]thymine]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (12a). According to the general procedure, 10a was treated with Cs_2CO_3 for 24 h. The residue was chromatographed with ethyl acetate/hexane (1:3) to give 0.11 g (85%) of 12a as an amorphous solid: IR (KBr) 3400, 3320 cm^{-1} (NH_2), 1645 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{CO}$, 300 MHz] δ 1.90 (s, 3 H, CH_3 -5), 4.06 (m, 2 H, H-5', $J_{5'a,5'b} = 12.2$ Hz), 4.31 (t, 1 H, H-4', $J_{4',5'} = 3.6$ Hz), 4.67 (d, 1 H, H-2', $J_{1,2'} = 8.0$ Hz), 5.75 (s, 1 H, H-3''), 6.00 (d, 1 H, H-1'), 6.47 (bs, 2 H, NH_2), 7.42 (s, 1 H, H-6), 10.32 (bs, 1 H, NH-3); ^{13}C NMR [$(\text{CD}_3)_2\text{CO}$, 50 MHz] δ 63.05 (C-5'), 72.12, 85.05, 87.62 (C-2', C-4', C-3''), 92.25, 92.31 (C-1', C-3'), 112.20 (C-5), 136.22 (C-6), 151.60, 152.28 (C-2, C-4''), 163.72 (C-4). Anal. ($\text{C}_{24}\text{H}_{43}\text{N}_3\text{O}_8\text{SSi}_2$) C, H, N.

1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)- β -D-ribofuranosyl]uracil]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (12b). According to the general procedure, 10b was treated with Cs_2CO_3 for 24 h. The residue was chromatographed with ethyl acetate/hexane (1:2) to give 0.24 g (39%) of 12b as an amorphous solid: IR (KBr) 3390, 3310, 3190 cm^{-1} (NH_2 , NH), 1645 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{CO}$, 300 MHz] δ 4.08 (m, 2 H, H-5', $J_{4',5'a} = 3.2$, $J_{4',5'b} = 2.9$, $J_{5'a,5'b} = 12.4$ Hz), 4.35 (dd, 1 H, H-4'), 4.59 (d, 1 H, H-2', $J_{1,2'} = 8.1$ Hz), 5.79 (s, 1 H, H-3''), 5.83 (d, 1 H, H-5), 6.08 (d, 1 H, H-1'), 6.44 (bs, 2 H, NH_2), 7.76 (d, 1 H, H-6), 10.30 (bs, 1 H, NH-3); ^{13}C NMR [$(\text{CD}_3)_2\text{SO}$, 75 MHz] δ 60.20 (C-5'), 74.50 (C-2'), 84.38, 84.58 (C-4', C-3'), 89.50 (C-1'), 92.81 (C-3'), 103.23 (C-5), 139.91 (C-6), 150.92, 151.32 (C-2, C-4''), 162.73 (C-4). Anal. ($\text{C}_{23}\text{H}_{41}\text{N}_3\text{O}_8\text{SSi}_2$) C, H, N.

4-*N*-Acetyl-1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)- β -D-ribofuranosyl]cytosine]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (12c). 10c was treated with Cs_2CO_3 for 1 h according to the general procedure. The residue was chromatographed with chloroform/methanol (50:1) to give 0.14 g (70%) of 12c as an amorphous solid: IR (KBr) 3410, 3320 cm^{-1} (NH_2), 1650 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{CO}$, 300 MHz] δ 2.24 (s, 3 H, NAc), 4.07 (m, 2 H, H-5'), 4.29 (dd, 1 H, H-5'), $J_{4',5'a} = 3.09$, $J_{4',5'b} = 4.66$ Hz), 4.83 (d, 1 H, H-2', $J_{1,2'} = 7.33$ Hz), 5.71 (s, 1 H, H-3''), 5.97 (d, 1 H, H-1'), 6.61 (bs, 2 H, NH_2), 7.46 (d, 1 H, H-5), 8.17 (d, 1 H, H-6), 9.86 (bs, 1 H, NH-4); ^{13}C NMR [$(\text{CD}_3)_2\text{CO}$, 50 MHz] δ 62.97 (C-5'), 75.94, 85.25 (C-2', C-4'), 91.12, 91.34, 91.50 (C-3', C-3''), 97.66 (C-5), 147.13 (C-6), 152.80, 155.80 (C-2', C-4''), 164.08 (C-4), 171.64 (4-NCO). Anal. ($\text{C}_{25}\text{H}_{44}\text{N}_4\text{O}_8\text{SSi}_2$) C, H, N.

General Procedure for Removal of the Protecting Groups of the 2',5'-Bis-*O*-silyl-protected 3'-Spiro β -D-xylo- and -ribofuranosyl Nucleosides (11a,b and 12a). To a solution of the protected nucleoside 11a,b or 12a (1 mmol) in THF (15 mL) was added tetrabutylammonium fluoride trihydrate (Bu_4NF) (0.63 g, 2 mmol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered through a column of silica gel using THF as the eluent. The filtrate was evaporated to dryness, and the residue was purified by column chromatography.

1-(β -D-Xylofuranosyl)thymine]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (13a). Following the general procedure, 11a was reacted with Bu_4NF for 24 h. The residue was chromatographed with chloroform/methanol (100:15) to afford 0.09 g (73%) of 13a as an amorphous solid: IR (KBr) 3420, 3400, 3320 cm^{-1} (NH_2 , NH, OH), 1620 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{SO}$, 300 MHz] δ 1.82 (s, 3 H, CH_3 -5), 3.62 (m, 2 H, H-5'), 4.31 (m, 1 H, H-2'), 4.50 (m, 1 H, H-4'), 5.10 (t, 1 H, OH-5') 5.57 (s, 1 H, H-3''), 5.89 (d, 1 H, H-1', $J_{1,2'} = 3.6$ Hz), 6.79 (d, 1 H, OH-2'), 6.83 (bs, 2 H, NH_2), 7.39 (s, 1 H, H-6), 11.47 (bs, 1 H, NH-3). Anal. ($\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_8\text{S}$) C, H, N.

1-(β -D-Xylofuranosyl)uracil]-3'-spiro-5'-[4'-amino-1'',2''-oxathiole 2'',2''-Dioxide] (13b). According to the general procedure, 11b was reacted with Bu_4NF for 24 h. The residue was chromatographed with chloroform/acetone (3:1) to give 0.11 g (62%) of 13b as a white solid: mp 240-242 °C dec; IR (KBr) 3480 cm^{-1} (OH), 3320, 3300, 3220 (NH_2 , NH), 1650 ($\text{C}=\text{C}-\text{N}$); ^1H NMR [$(\text{CD}_3)_2\text{SO}$, 300 MHz] δ 3.78 (dd, 1 H, H-5'a, $J_{5'a,5'b} = 11.5$, $J_{4',5'a} = 4.4$ Hz), 3.87 (dd, 1 H, H-5'b, $J_{4',5'b} = 6.2$ Hz), 4.24 (d, 1 H, H-2', $J_{1,2'} = 2.6$ Hz), 4.60 (dd, 1 H, H-4'), 5.55 (s, 1 H,

H-3''), 5.73 (d, 1 H, H-5), 5.82 (d, 1 H, H-1'), 6.81 (bs, 2 H, NH₂), 7.51 (d, 1 H, H-6). Anal. (C₁₁H₁₃N₃O₈S) C, H, N.

[1-(β-D-Ribofuranosyl)thymine]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-Dioxide] (14a). Following the general procedure 12a was reacted with Bu₄NF for 24 h. The residue was chromatographed with chloroform/methanol (10:1) to give 0.32 g (60%) of 14a as a white solid: mp 170 °C dec; IR (KBr) 3400, 3300, 3200 cm⁻¹ (NH₂, OH), 1660 (C=C-N); ¹H NMR [(CD₃)₂SO, 300 MHz] δ 1.80 (s, 3 H, CH₃-5), 3.54-3.72 (m, 2 H, H-5'), 4.16 (t, 1 H, H-4', J_{4',5'} = 3.1 Hz), 4.52 (dd, 1 H, H-2', J_{1,2'} = 8.5 Hz), 5.71 (s, 1 H, H-3''), 5.87 (d, 1 H, H-1'), 6.03 (t, 1 H, OH-5'), 6.17 (d, 1 H, OH-2'), 6.81 (bs, 2 H, NH₂), 7.77 (s, 1 H, H-6), 11.45 (bs, 1 H, NH-3); ¹³C NMR [(CD₃)₂SO, 50 MHz] δ 12.29 (CH₃-5), 60.21 (C-5'), 72.63 (C-2'), 84.24, 84.37, 88.92 (C-4', C-3'', C-1'), 93.23 (C-3'), 110.17 (C-5), 135.71 (C-6), 151.05, 152.07 (C-2, C-4''), 163.51 (C-4). Anal. (C₁₂H₁₆N₃O₈S) C, H, N.

[1-[2'-O-(tert-Butyldimethylsilyl)-β-D-xylofuranosyl]-uracil]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-Dioxide] (15b). The protected nucleoside 11b (0.13 g, 0.22 mmol) was stirred with methanolic 0.1 N HCl (15 mL) at room temperature for 30 min. The solution was neutralized with 1 N NaOH-MeOH, and the solvent was evaporated to dryness. The residue was purified by column chromatography using chloroform/acetone (3:1) to afford 0.09 g (86%) of 15b as an amorphous solid: IR (KBr) 3550, 3450, 3350 cm⁻¹ (OH, NH₂), 1645 (C=C-N); ¹H NMR [(CD₃)₂CO, 300 MHz] δ 3.80 (m, 2 H, H-5', J_{5'a,5'b} = 12.3, J_{4',5'a} = 6.5, J_{4',5'b} = 3.7 Hz), 4.13 (dd, 1 H, OH-5'), 4.60 (d, 1 H, H-2, J_{1,2'} = 4.2 Hz), 4.68 (dd, 1 H, H-4'), 5.68 (s, 1 H, H-3''), 5.81 (d, 1 H, H-5), 6.09 (d, 1 H, H-1'), 6.36 (bs, 2 H, NH₂), 7.72 (d, 1 H, H-6), 10.20 (bs, 1 H, NH-3). Anal. (C₁₇H₂₇N₃O₈SSi) C, H, N.

[1-[2'-O-(tert-Butyldimethylsilyl)-β-D-ribofuranosyl]-thymine]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-Dioxide] (16a). The protected nucleoside 12a (0.16 g, 0.27 mmol) was stirred with methanolic 0.1 N HCl (10 mL) at room temperature for 2 h. The solution was neutralized with 1 N NaOH-MeOH and the solvent was evaporated to dryness. The residue was purified by column chromatography using chloroform/acetone (4:1) to give 0.11 g (90%) of 16a as an amorphous solid: IR (KBr) 3400, 3350, 3200 cm⁻¹ (OH, NH₂, NH); ¹H NMR [(CD₃)₂CO, 200 MHz] δ 1.86 (s, 3 H, CH₃-5), 3.79-4.03 (m, 2 H, H-5', J_{5'a,5'b} = 12.8 Hz), 4.33 (dd, 1 H, H-4', J_{4',5'a} = 2.5, J_{4',5'b} = 1.3 Hz), 4.97 (d, 1 H, H-2, J_{1,2'} = 8.1 Hz), 5.74 (s, 1 H, H-3''), 5.79 (bs, 1 H, OH-5'), 5.84 (d, 1 H, H-1'), 6.61 (bs, 2 H, NH₂), 7.86 (s, 1 H, H-6), 10.24 (bs, 1 H, NH-3); ¹³C NMR [(CD₃)₂CO, 50 MHz] δ 12.36 (CH₃-5), 61.76 (C-5'), 74.92 (C-2'), 85.95, 89.70 (C-4', C-3''), 92.55 (C-1'), 94.62 (C-3'), 112.18 (C-5), 138.50 (C-6), 151.83, 152.12 (C-2, C-4''), 163.82 (C-4). Anal. (C₁₈H₂₉N₃O₈SSi) C, H, N.

[1-[5'-O-(tert-Butyldimethylsilyl)-β-D-ribofuranosyl]-thymine]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-dioxide] (17a). To a solution of 14a (0.33 g, 0.91 mmol) in dry pyridine (6 mL) tert-butyldimethylsilyl chloride (0.15 g, 1 mmol) was added. The resulting mixture was stirred at room temperature for 24 h and then evaporated to dryness. The residue was purified by column chromatography using chloroform/methanol (20:1) to yield 0.37 g (83%) of 17a as an amorphous solid: IR (KBr) 3410, 3330, 3200 cm⁻¹ (OH, NH₂), 1650 (C=C-N); ¹H NMR [(CD₃)₂CO, 300 MHz] δ 1.89 (s, 3 H, CH₃-5), 4.07 (m, 2 H, H-5', J_{4',5'} = 3.7 Hz), 4.31 (t, 1 H, H-4'), 4.79 (d, 1 H, H-2', J_{1,2'} = 8.3 Hz), 5.26 (bs, 1 H, OH-2'), 5.72 (s, 1 H, H-3''), 5.98 (d, 1 H, H-1'), 6.44 (bs, 2 H, NH₂), 7.55 (s, 1 H, H-6), 10.12 (bs, 1 H, NH-3); ¹³C NMR [(CD₃)₂CO, 50 MHz] δ 12.42 (CH₃-5), 63.02 (C-5'), 74.03 (C-2'), 84.95, 87.89 (C-4', C-3''), 91.31 (C-1'), 92.60 (C-3'), 111.64 (C-5), 136.85 (C-6), 151.87, 153.04 (C-2, C-4''), 164.02 (C-4). Anal. (C₁₈H₂₉N₃O₈SSi) C, H, N.

[1-[5'-O-(tert-Butyldimethylsilyl)-2'-deoxy-β-D-erythro-pentofuranosyl]thymine]-3'-spiro-5''-[4''-amino-1'',2''-oxathiole 2'',2''-Dioxide] (18a). To a solution of 17a (0.29 g, 0.61 mmol) in toluene/acetonitrile (8 mL) was added N,N'-thio-

carbonyldiimidazole (0.12 g, 0.67 mmol). The mixture was heated at 80 °C for 30 min, and the solvent was evaporated. The residue was dissolved in ethyl acetate (50 mL) and washed with (5%) HCl (25 mL), water, until the pH of the organic phase was neutral, and brine (50 mL). The organic phase was dried (Na₂SO₄), filtered, and evaporated to dryness. The residue was suspended in dry acetonitrile (10 mL), α,α'-azobis(isobutyronitrile) (0.02 g, 0.12 mmol) and tributyltin hydride (0.26 mL, 0.92 mmol) were added under argon, and the mixture was heated at 80 °C for 3 h. After cooling, the solvent was evaporated. The residue was washed with hexane and purified by column chromatography with chloroform/methanol (30:1) to give 0.14 g (50%) of 18a as an amorphous solid: IR (KBr) 3430, 3320, 3200 cm⁻¹ (NH₂, NH), 1640 (C=C-N); ¹H NMR [(CD₃)₂CO, 300 MHz] δ 1.85 (s, 3 H, CH₃-5), 2.69 (m, 1 H, H-2'b, J_{1,2'b} = 5.8, J_{2'a,2'b} = 14.3 Hz), 2.96 (m, 1 H, H-2'a, J_{1,2'a} = 9.0 Hz), 4.03 (m, 2 H, H-5'), 4.24 (dd, 1 H, H-4', J_{4',5'a} = 3.5, J_{4',5'b} = 5.3 Hz), 5.65 (s, 1 H, H-3''), 6.12 (dd, 1 H, H-1'), 6.50 (bs, 2 H, NH₂), 7.58 (s, 1 H, H-6), 10.21 (bs, 1 H, NH-3); ¹³C NMR [(CD₃)₂CO, 50 MHz] δ 12.43 (CH₃-5), 40.82 (C-2'), 62.90 (C-5'), 62.90 (C-5'), 86.42, 86.99, 90.03 (C-4', C-3'', C-1'), 93.02 (C-3'), 111.14 (C-5), 138.03 (C-6), 151.38, 154.77 (C-2, C-4''), 164.07 (C-4). Anal. (C₁₈H₃₀N₃O₈SSi) C, H, N.

Antiretrovirus Assays. HIV-1 (HTLV-III_B) was obtained from persistently HIV-infected H9 cells as described previously.⁴⁶ Virus stocks were prepared from the supernatants of HIV-1 (III_B)-infected MT-4 cells.

MT-4 cells were infected with HIV-1 as previously described.⁴⁷ Briefly, 5 × 10⁵ MT-4 cells/mL were infected with HIV-1 at 100 CCID₅₀ (50% cell culture infective dose) per mL cell suspension. Then, 100 μL of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 5 days, the number of viable cells was determined by trypan blue staining. The 50% effective concentration (EC₅₀) and 50% cytotoxic concentration (CC₅₀) were defined as the compound concentrations required to reduce by 50% the number of viable cells in the virus-infected and mock-infected cell cultures, respectively.

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