Novel Series of TSAO-T Derivatives.[1] Synthesis and Anti-HIV-1 Activity of 4-, 5-, and 6-Substituted Pyrimidine Analogues

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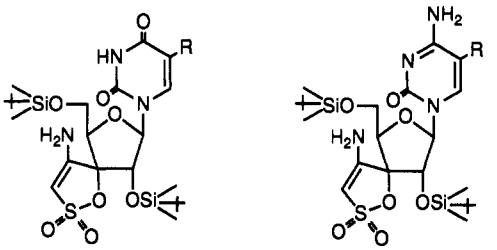
Several 4-, 5-, and 6-substituted pyrimidine analogues of the new anti-HIV-1 lead compound [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (TSAO-T) have been prepared and evaluated as inhibitors of HIV-1 and HIV-2 replication in cell cultures. Reaction of 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-*C*-cyano-3-*O*-mesyl-D-ribofuranose with 5-substituted pyrimidine bases, followed by treatment with Cs_2CO_3, afforded, stereoselectively, β-D-ribofuranosyl-3′-spironucleosides. 2′,5′-*O*-Deacylation and subsequent treatment with *tert*-butyldimethylsilyl chloride gave the TSAO-5-substituted pyrimidine derivatives. Reaction of 5-halogen-TSAO derivatives with nucleophiles gave 6-substituted-TSAO analogues. Treatment of TSAO-pyrimidine analogues with $POCl_3$/1,2,4-triazole and methylamine or dimethylamine afforded the 4-substituted pyrimidine compounds. Several substituted TSAO-thymine, TSAO-uracil, and TSAO-cytosine derivatives were found to be superior to their unsubstituted TSAO congeners with regard to their antiviral and/or cytotoxic properties.

Introduction

TSAO nucleoside analogues represent a structural class of highly specific and potent inhibitors of human immunodeficiency virus type 1 (HIV-1). They are not inhibitory to HIV-2, simian immunodeficiency virus (SIV), or other RNA or DNA viruses.[2-8] In this respect, they behave like the non-nucleoside HIV-1-specific TIBO,[9,10] HEPT,[11-15] nevirapine,[16,17] pyridinone,[18,19] BHAP,[20] and α-APA[21] derivatives. All these classes of compounds interact with the HIV-1 reverse transcriptase (RT) at a nonsubstrate binding site.[3-5,16,18,20,21] However, TSAO derivatives [whose prototype compound is [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide), designated TSAO-T (1)][22] are the first molecules for which one of the active pharmacophores (i.e. the 4″-amino group at 3′-spiro of the ribose moiety) could be identified.[23] To display antiviral efficacy, the TSAO derivatives have to fulfill stringent structural requirements with respect to the sugar part, but not the base part.[2-4,6-8] The thymine moiety of TSAO-T can be replaced by a number of other pyrimidines and purines without marked decrease of antiviral efficacy.[3,8,24] Thus, the prototype compound TSAO-T (1) is the more active (EC_{50} 0.034 μg/mL) whereas the cytosine derivative TSAO-C (2) is 13-fold less antivirally effective than TSAO-T but markedly (~30-fold) less toxic (CC_{50}≥ 200 μg/mL).[3,8] Therefore, due to its marked lower toxicity, the TSAO-cytosine derivative 2 is more selective as an anti-HIV-1 agent than the corresponding TSAO-thymine derivative 1 (selectivity indices: ≥456 and 227, respectively). The cytotoxicity of TSAO-T is markedly decreased (by 10–20-fold), whereas the antiviral activity is unaffected, upon introduction of an alkyl moiety at N-3 of the thymine ring.

To further improve the antiviral potency and/or selectivity of TSAO derivatives and to gain insight in the interaction points of the TSAO compounds with HIV-1 RT, we focused our attention on the modification of the most active (TSAO-T) and least cytotoxic (TSAO-C) TSAO-pyrimidine derivatives. In this paper we report the synthesis and anti-HIV-1 activity of a series of TSAO-T analogues in which the 5-methyl moiety is replaced by halogens and other groups in order to assess whether size and electronegativity of the C-5 substituent play a significant role in the biological activity of these compounds. In addition, 6-substituted TSAO-U analogues were prepared to determine the importance of the 6-substituent in the anti-HIV-1 activity of TSAO-U derivatives.



1 R = CH_3 (TSAO-T) **2** R = H (TSAO-C)
3 R = CH_2CH_3 **4** R = CH_3

It has previously been observed that introduction of an acetyl at N-4 of TSAO-C enhanced the anti-HIV-1 activity by ~4-fold but also markedly increased the toxicity.[3,4,8] In order to obtain TSAO-C derivatives with enhanced anti-HIV-1 activity and lower toxicity, a series of TSAO-C derivatives substituted with alkyl groups at the N-4 of the aglycon were also prepared.

Chemistry

The 3′-spironucleosides were stereoselectively prepared, following our previously reported method,[8] by glycosylation of trimethylsilylated heterocyclic bases with the suitably functionalized and protected ribofuranosyl sugar intermediate **5**,[8] followed by basic treatment of the cyanomesyl nucleosides thus obtained, to give, exclusively, β-D-ribospironucleosides. The *ribo* configuration of the

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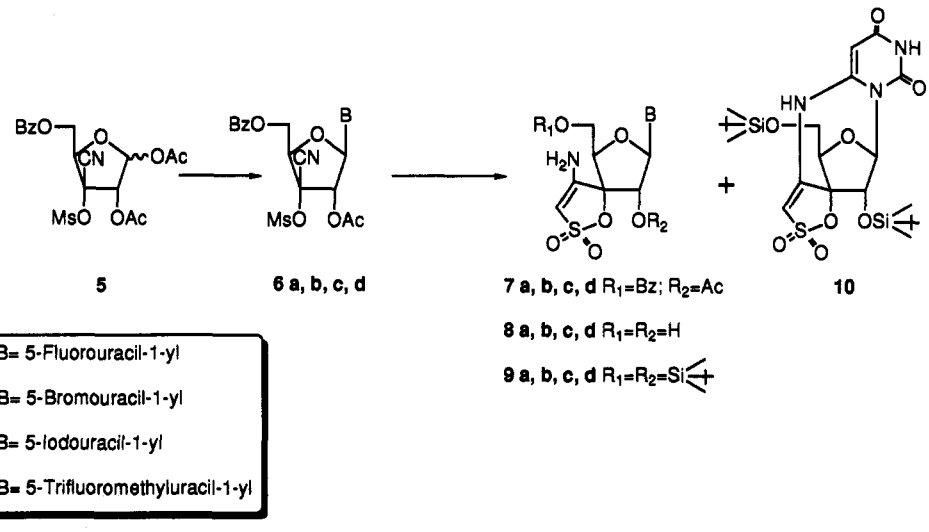
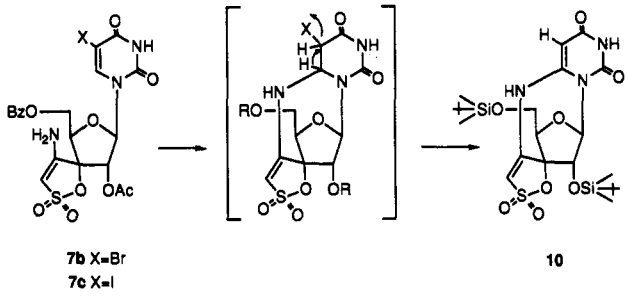
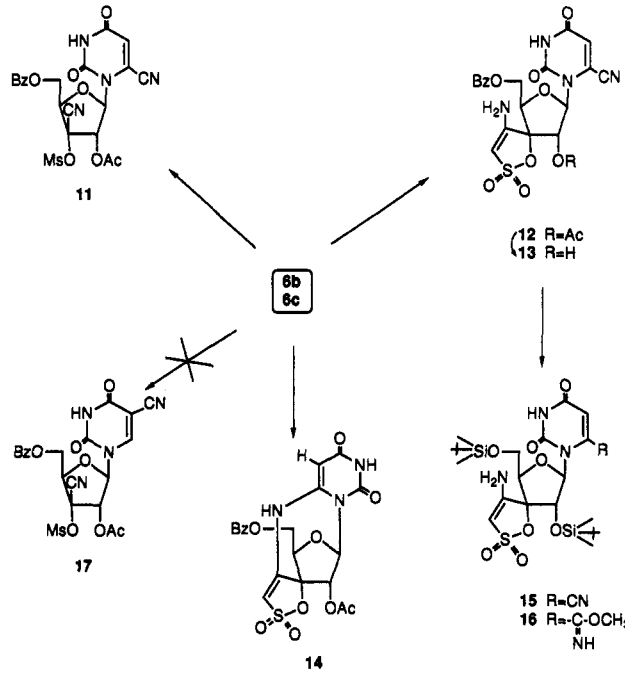
Scheme 1



Scheme 2



Scheme 3



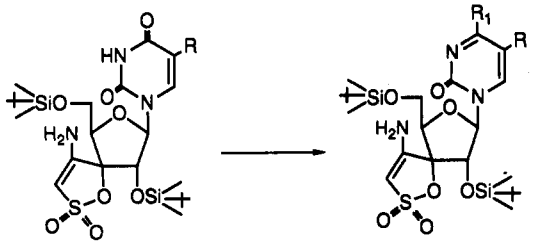
nucleosides was determined from the configuration of the starting cyanohydrin used in the preparation of the sugar intermediate **5**, as clearly demonstrated in previous papers of this series.[8,25,26] Thus, glycosylation of **5** with 5-fluoro-, 5-bromo-, 5-iodo-, or 5-(trifluoromethyl)uracil (Scheme 1), in the presence of trimethylsilyl triflate (TMS-Tfl) as condensing reagent,[27] gave the corresponding 3′-*C*-cyano-3′-*O*-mesyl nucleosides **6a** (36%), **6b** (78%), **6c** (51%), and **6d** (77%), respectively. Due to the presence of a 2-*O*-acyl participating group, the nucleosides obtained were exclusively β-anomers.[27] Coupling constant values were in the range of $J_{1',2'}$ = 7.0–7.1 Hz, which is in agreement with the values observed for other 3′-cyano-3′-*O*-mesyl-β-D-ribofuranosyl nucleosides of this series.[7,8,24] Treatment of 3′-cyanomesilates of 5-fluorouracil **6a** and 5-(trifluoromethyl)uracil **6d** with Cs_2CO_3 in dry acetonitrile gave the spiro derivatives **7a** and **7d**, which were used in the next step without further purification. Deprotection of **7a** and **7d** with saturated methanolic ammonia followed by silylation with an excess of *tert*-butyldimethylsilyl chloride (TBDMS-Cl) afforded the 2′,5′-bis-*O*-silylated nucleosides **9a** and **9d** in 46% and 25% yield, respectively. However, a similar reaction sequence with 3′-cyanomesyl nucleosides **6b** and **6c** gave the 3′-spironucleosides of 5-bromouracil **9b** and 5-iodouracil **9c** in very low yields (9% and 5%, respectively). These spiro derivatives were isolated as minor compounds of the reaction, the cyclospironucleoside **10** being the major compound in both reactions.

Formation of **10** could be explained by an intramolecular Michael addition of the enamine NH_2-4″ to the C-6 position of the pyrimidine base, followed by dehydrobromination or dehydroiodination (Scheme 2). Intramolecular Michael additions to the C-6 position of 5-bromouridine to give carbon-bridged nucleosides have been described.[28–33]

5-Bromo- or 5-Iodo derivatives **6b** and **6c** were used for the synthesis of other substituted pyrimidine 3′-spironucleosides such as **12–16** (Scheme 3). It has been reported that 6-cyanouridines can be obtained from 5-halogenouridines by treatment with potassium cyanide (KCN) at room temperature under mild conditions.[32–34] The reaction involves the initial nucleophilic addition of cyanide ion to the 5,6-double bond of the pyrimidine base and successive elimination of hydrogen bromide. Thus, treatment of 5-bromouracil derivative **6b** (Scheme 3) with 1.5 equiv of KCN in dimethylformamide (DMF) at room temperature for 64 h afforded a mixture of the 6-cyano-3′-cyanomesylate **11** and the 6-cyano-3′-spiro derivative **12** in 40% and 34% yield, respectively. Reaction of 5-iodo derivative **6c** with 1.5 equiv of KCN in DMF at room temperature for 4 h gave, exclusively, the 6-cyano-3′-spironucleoside **12** (80% yield).

On the other hand, it has also been described that heat treatment of 5-bromouridine or 6-cyanouridine with a large excess of cyanide affords the corresponding 5-cyanouridine analogue[35,36] by a double addition–elimination sequence

Scheme 4



18 R=H
1 R=CH_3 (TSAO-T)

19 R=H, R_1=$NHCH_3$
20 R=H, R_1=$N(CH_3)_2$
21 R=CH_3, R_1=$NHCH_3$
22 R=CH_3, R_1=$N(CH_3)_2$

through a 5,6-dicyanodihydro intermediate.[35] Attempts were made to prepare the 5-cyano-*ribo*-spiropyrimidine analogues following that method. Thus, reaction of **6b** (Scheme 3) with excess of KCN (6 equiv) in DMF at 80 °C for 6 h, afforded a mixture of the spiro derivative **12** (35%), the 2′-deprotected nucleoside **13** (11%) and the cyclospiro compound **14** (16%). A similar treatment of 5-iodo derivative **6c** [KCN (6 equiv), DMF, 80 °C] yielded a mixture of two compounds, the 3′-spiro-6-cyano nucleoside **12** (50%) and the cyclospiro derivative **14** (17%). Finally, when 6-cyanospiro derivative **12** was treated with 2 equiv of KCN in DMF at 80 °C, only the 2′-deprotected compound **13** was isolated. In none of the reactions described above was formation of the 5-cyano analogue **17** detected.

Deprotection of **12** with saturated methanolic ammonia (Scheme 3), followed by reaction with TBDMS-Cl, yielded the 2′,5′-bis-*O*-silylated nucleoside **15** together with the 6-carboximidate spironucleoside **16**.

Finally, 4-*N*-alkyl nucleoside analogues of TSAO-C **19**–**22** (Scheme 4) were prepared from the corresponding uracil and thymine-3′-spironucleosides **18**[8] and **1**[8] following the method described by Xu and Swan.[37] Thus, treatment of uracil-3′-spironucleoside **18** with 4 equiv of phosphorus oxychloride and 15 equiv of 1,2,4-triazole for 4 h at room temperature, in the presence of NEt_3, followed by treatment with an excess of methylamine or dimethylamine afforded the cytidine analogues **19** (51%) and **20** (47%), respectively. A similar reaction sequence with thymine-3′-spironucleoside **1** gave the 4-*N*-methyl- and 4-*N*-dimethyl-3′-spironucleosides **21** and **22** in 48% and 55% yield, respectively.

The structure of **16** was determined by the disappearance of the cyano function by ^{13}C-NMR and the presence of two signals at 54.29 and 162 ppm corresponding to the OCH_3 and C=NH carbons, respectively, and by the presence in the ^1H-NMR spectrum of methoxy and imino protons at 3.88 and 8.82 ppm, respectively.

The ^1H-NMR spectra of **10** and **14** showed a downfield shift of the signal corresponding to NH-4″ which appeared at ~10 ppm together with the NH-3 of the pyrimidine base and the presence of four singlets of 6.73, 4.87, 6.48, and 5.13 ppm for **10** and at 6.85, 5.85, 6.64, and 5.15 ppm for **14** which were unequivocally assigned by NOE difference[38,39] and NOESY[40] experiments as the signals corresponding to H-1′, H-2′, H-3″, and H-5, respectively. The anomeric proton signal of **10** and **14** as a singlet ($J_{1',2'}$ ~0 Hz) suggests a 3′-*endo*-envelope conformation for the sugar ring.[41] This coupling constant value is clearly different than those observed for other *ribo*-3′-spironucleosides of this series which are in the range of $J_{1',2'}$ = 5–8 Hz,[7,8] thus indicating these 3′-spironucleosides preference for an S-type sugar ring conformation.[42]

Biological Results

The TSAO-pyrimidine derivatives were evaluated for their inhibitory effect on HIV-1- and HIV-2-induced cytopathicity in MT-4 cells and syncytium formation in CEM cells (Table 1). None of the test compounds proved antivirally active against HIV-2 at subtoxic concentrations. The prototype compound TSAO-T (TSAO-thymine) (**1**) was inhibitory to HIV-1 induced cytopathicity at a 50% effective concentration (EC_{50}) of 0.06 μM in MT-4 cells and had an EC_{50} against syncytium formation of 0.04 μM in CEM cells, while being cytotoxic to MT-4 cells at 50% cytotoxic concentration (CC_{50}) of 13 μM. When thymine was replaced by cytosine (TSAO-C) (**2**), a 13–30-fold decrease in activiral activity was observed as compared with TSAO-T. Cytotoxicity was 25-fold decreased. Introduction of a methyl group at C-5 of the cytosine ring in the TSAO-C molecule (i.e. TSAO-m^5C) (**4**) restored almost completely the antiviral activity of the test compound to a potency comparable to that of the prototype compound TSAO-T. Cytotoxicity was even 3-fold lower than that observed for TSAO-T (Table 1). Similarly, the TSAO-uracil derivative **18**, that proved inhibitory to virus-induced cytopathicity at an ED_{50} of 0.19 μM in MT-4 cells, showed an increased antiviral potency when substituted at the C-5 position of the uracil ring with an alkyl group (i.e., TSAO-T (**1**) and TSAO-e^5U (**3**); EC_{50} for both compounds, 0.06 μM).

Introduction of a halogen atom at C-5 of the uracil ring in TSAO-U resulted in a progressive increase in antiviral activity. The lower the electronegativity and/or the larger the size of the substituent, the greater the increase in antiviral potency. The EC_{50} values of the 5-fluoro- (**9a**), 5-bromo- (**9b**) and 5-iodo-substituted (**9c**) TSAO-U derivatives in MT-4 cells were 0.50, 0.32, and 0.09 μM, respectively. The 5-(trifluoromethyl)uracil TSAO derivative **9d**, which contains a strong electron-withdrawing C-5 substituent of about the same size as the 5-methyl group in TSAO-T (**1**), was 4- and 14-fold less antivirally effective than TSAO-U and TSAO-T, respectively.

Compound **10** that represents the N-4″ 6-cyano derivative of TSAO-U showed significantly lower antiviral potency and selectivity than the parent compound. Clearly, the rather rigid structure and fixed sugar conformation of the TSAO derivative **10** does not allow pronounced antivirial activity (Table 1).

To investigate the role of the NH_2 group at C-4 of cytosine or 5-methylcytosine in the antiviral activity of the TSAO derivatives, one or two methyl groups at the amino function on C-4 of cytosine were introduced. The antiviral potency of TSAO-C (**2**) and TSAO-m^5C (**4**) remained unchanged if one or both hydrogens of the NH_2 group were replaced by a methyl group (compare compounds **19** and **20** with compound **2**, and compounds **21** and **22** with compound **4**). However, compound **19** proved markedly more cytotoxic to MT-4 cells than TSAO-C (**2**), whereas introduction of two methyl groups in the NH_2 function of TSAO-2 did not alter the cytotoxicity. Introduction of one or two methyl groups in the NH_2 function of TSAO-m^5C (**4**) did not markedly influence the cytotoxicity and thus resulted in a selectivity index comparable to that of TSAO-m^4C (Table 1).

Substitution of the C-6 position of the uracil ring in TSAO-U (**18**) by a cyano (**15**) or C(=NH)OCH_3 group

Table 1. Anti-HIV Activity of TSAO-Pyrimidine Derivatives

compd	EC_{50}[a] (μM) (CEM)		EC_{50}[a] (μM) (MT-4)		CC_{50}[b] (MT-4)	selectivity index (ratio CC_{50}/EC_{50}) (MT-4)
	HIV-1	HIV-2	HIV-1	HIV-2		
1	0.044 ± 0.008	>7.0	0.06 ± 0.02	>7.0	13 ± 2.5	217
2	1.39 ± 0.32	>350	0.76 ± 0.001	>350	≥350	460
3			0.06 ± 0.009	>2.6	5.3 ± 0.99	88
4	0.05 ± 0.03	>13.5	0.12 ± 0.037	>13.5	30 ± 0.85	250
9a	0.34 ± 0.23	>6.7	0.50 ± 0.03	>1.3	5.0 ± 3.5	10
9b			0.32 ± 0.23	>2.4	3.6 ± 2.6	11
9c	0.03 ± 0.0	>5.7	0.09 ± 0.03	>5.7	12 ± 0.51	133
9d	0.77 ± 0.21	>6.2	0.82 ± 0.26	>6.2	14 ± 0.0	17
10			5.9 ± 4.1	>12	16 ± 6.3	2.7
15	2.1 ± 1.5	≥6.6	3.6 ± 1.1	>6.6	11 ± 0.68	3.0
16	1.1 ± 0.36	>6.3	2.7 ± 0.13	>6.3	11 ± 1.6	4.1
18			0.19 ± 0.04	>6.9	14 ± 0.96	74
19	3.38 ± 0.84	>6.8	0.68	>6.8	12	18
20	0.75 ± 0.08	>166	0.66	>166	>166	252
21	0.11 ± 0.008	>33	0.12	>33	26	217
22			0.16 ± 0.07	>32	62 ± 8.1	388

[a] 50% effective concentration or compound concentration required to inhibit HIV-induced cytopathicity in MT-4 cells or syncytium formation in CEM cell cultures by 50%. [b] 50% cytotoxic concentration.

(16) decreased the antiviral potency of TSAO-U (18) by 10–20-fold, while leaving the cytotoxicity unaffected (Table 1).

In conclusion, the nature of the pyrimidine base (i.e., uracil, cytosine) and substitutions at C-5 or C-6 of uracil, or at the C-4 amino group of cytosine, by alkyl, halogen, or heteroatomic functions modulate the antiviral and cytotoxic properties in a different manner. As previously mentioned,[2-8,43] the sugar part of the TSAO molecule has to fulfill stringent structural requirements for any antiviral activity to be expressed. However, in achieving optimal anti-HIV-1 activity, the nature of the pyrimidine moiety, and the presence of additional functional groups in the pyrimidine moiety, also play an important role.

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 AM-200 and a Varian XL-300 spectrometer operating at 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 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 using NOE and NOESY. For the NOE difference spectra the signals were irradiated during 3 s with γB_2 = 20 Hz of decoupling power. NOESY experiments were carried out using the following conditions: spectral windows of 5852.2 Hz in both dimensions, 512 increments with 8 transients per increment, a relaxation delay of 1.5 s, and a mixing time of 250 ms and 2048 × 2048 final data point.

General Procedure for the Synthesis of 2′-*O*-acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl Nucleosides (6). The heterocyclic base (1.2 mmol) was silylated with hexamethyldisilazane (HMDS) (14 mL) under reflux in the presence of ammonium sulfate (25 mg). The reaction mixture was heated at reflux until the solution became clear, and excess HMDS was removed under reduced pressure. To the syrupy silylated base were added a solution of 1,2-bis-*O*-acetyl-5-benzoyl-3-C-cyano-3-*O*-mesyl-D-ribofuranose (5)[8] (1 mmol) in dry acetonitrile (8 mL) and TMS-Tfl (1.6 mmol), and the resulting mixture was heated to reflux. After 2 h an additional portion of TMS-Tfl (1.6 mmol) was added, and the refluxing continued for 2 h. The reaction mixture was allowed to cool to room temperature, dichloromethane (50 mL) and ice were added, and the mixture was neutralized with $NaHCO_3$. The organic phase was separated, and the aqueous phase was washed with dichloromethane (2 × 20 mL). The organic phases were combined, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography.

1-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)-5-fluorouracil (6a). 5-Fluorouracil (0.38 g, 4.08 mmol), the sugar derivative 5[8] (1.09 g, 2.47 mmol) and TMS-Tfl (1.56 mL, 8 mmol) yielded, after chromatography (hexane–ethyl acetate, 2:1), 0.45 g (36%) of **6a** as a white foam: IR (KBr, cm^{-1}) 1720 (C=O), 1375, 1180 (SO_2); UV (MeOH) λ_{max} nm (log ε) 260 (4.00), 226 (4.24), and 201 (4.20); ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.16 (s, 3 H, 1 OAc), 3.49 (s, 3 H, $MeSO_2$), 4.82 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 12.6, $J_{4',5'a}$ = 5.0 Hz), 4.95 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 4.4 Hz), 5.07 (t, 1 H, H-4′), 6.03 (d, 1 H, H-2′), 6.22 (dd, 1 H, H-1′, $J_{1',2'}$ = 7.1, $J_{1',F}$ = 1.3 Hz), 7.91 (d, 1 H, H-6, $J_{6,F}$ = 6.6 Hz), 7.50–8.14 (m, 5 H, Ph), 10.65 (bs, 1 H, NH-3). Anal. ($C_{20}H_{18}FN_3O_{10}S$) C, H, N, S.

1-(2′-*O*-Acetyl-5′-*O*-Benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)-5-bromouracil (6b). 5-Bromouracil (1.13 g, 5.92 mmol), the sugar derivative 5[8] (2.18 g, 4.94 mmol), and TMS-Tfl (3.12 mL, 16 mmol) gave, after chromatography (hexane-ethyl acetate, 2:1), 2.2 g (78%) of **6b** as a white foam: IR (KBr, cm^{-1}) 1720 (C=O), 1375, 1180 (SO_2); UV (MeOH) λ_{max} nm (log ε) 270 (4.00), 224 (4.21), and 202; ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.17 (s, 3 H, 1 OAc), 3.51 (s, 3 H, $MeSO_2$), 4.84 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 12.6, $J_{4',5'a}$ = 4.7 Hz), 4.97 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 4.3 Hz), 5.11 (t, 1 H, H-4′), 6.10 (d, 1 H, H-2′), 6.27 (d, 1 H, H-1′, $J_{1',2'}$ = 7.1 Hz), 8.08 (s, 1 H, H-6), 7.53–8.20 (m, 5 H, Ph), 10.55 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2CO$ 50 MHz)] δ 20.24 (OAc), 40.56 ($MeSO_2$), 61.92 (C-5′), 75.55 (C-2′), 77.53 (C-3′), 81.61, 86.40 (C-1′, C-4′), 99.03 (C-5), 112.86 (CN), 128.50–134.04 (Ph), 137.55 (C-6), 149.20 (C-2), 157.98 (C-4), 165.58, 168.87 (C=O). Anal. ($C_{20}H_{18}BrN_3O_{10}S$) C, H, N, S.

1-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)-5-iodouracil (6c). 5-Iodouracil (0.70 g, 2.96 mmol), the sugar derivative 5[8] (1.09 g, 2.47 mmol), and TMS-Tfl (1.56 mL, 8 mmol) yielded, after chromatography (hexane–ethyl acetate, 2:1), 0.78 g (51%) of **6c** as a white foam: IR (KBr, cm^{-1} 1720 (C=O), 1370, 1180 (SO_2); UV (MeOH) λ_{max} nm (log ε) 275 (4.95), 223 (4.22), and 203; ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.16 (s, 3 H, 1 OAc), 3.49 (s, 3 H, $MeSO_2$), 4.82 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 12.6, $J_{4',5'a}$ = 4.7 Hz), 4.96 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 4.2 Hz), 5.09 (t, 1 H, H-4′), 6.09 (d, 1 H, H-2′), 6.24 (d, 1 H, H-1′, $J_{1',2'}$ = 7.1 Hz), 8.11 (s, 1 H, H-6), 7.50–8.14 (m, 5 H, Ph), 10.55 (bs, 1 H, NH-3). Anal. ($C_{20}H_{18}IN_3O_{10}S$) C, H, N, S.

1-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)-5-(trifluoromethyl)uracil (6d). 5-(Trifluoromethyl)uracil (0.50 g, 2.80 mmol), the sugar derivative 5[8] (1.03 g, 2.30 mmol), and TMS-Tfl (0.72 mL, 3.7 mmol) gave, after workup and chromatography (chloroform–methanol 50:1), 1.0 g (77%) of **6d** as a white foam: IR (KBr, cm^{-1}) 1730 (C=O), 1375,

1185 (SO_2); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.04 (s, 3 H, 1 OAc), 3.49 (s, 3 H, $MeSO_2$), 4.84 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 12.6, $J_{4',5'a}$ = 5.0 Hz), 4.95 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 4.3 Hz), 5.10 (t, 1 H, H-4'), 6.18 (d, 1 H, H-2'), 6.29 (d, 1 H, H-1', $J_{1',2'}$ = 6.7 Hz), 8.26 (s, 1 H, H-6), 7.48–8.13 (m, 5 H, Ph), 10.50 (bs, 1 H, NH-3). Anal. ($C_{21}H_{18}F_3N_3O_{10}S$) C, H, N, S.

General Procedure for the Synthesis of 1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) Pyrimidine Nucleosides (9). A solution of cyanomesylate **6** (1 mmol) in dry acetonitrile (8 mL) was treated with Cs_2CO_3 (1.75 mmol), and the mixture was stirred at room temperature for 6 h. The reaction was filtered, the filtrate was neutralized with acetic acid and, finally, evaporated to dryness. The residue (spiro derivative **7**) was treated with saturated methanolic ammonia (10 mL). The solution was stirred at room temperature overnight. The solvent was evaporated to dryness, and the residue was treated with MeOH–ether to give **8** as an amorphous solid. This solid was suspended in dry acetonitrile (8 mL), and then 4-(dimethylamino)pyridine (3.36 mmol) and TBDMS-Cl (3.36 mmol) were added. The mixture was heated at 80 °C for 16 h. The solvent was evaporated to dryness, and the residue was treated with chloroform (2 × 50 mL) and water (25 mL). The organic phase was extracted with chloroform (2 × 25 mL). The combined organic layers were successively washed with cold (4 °C) 1 N HCl (13 mL), water (13 mL), and brine (13 mL) and, finally, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash column chromatography.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-fluorouracil]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (9a). The general procedure was followed with cyanomesylate **6a** (1.0 g, 1.95 mmol). The residue was chromatographed (chloroform–acetone, 50:1) to give 0.46 g (46%) of **9a** as an amorphous solid: IR (KBr, cm^{-1}), 3400, 3350, 3195 (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 4.03 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 12.4, $J_{4',5'a}$ = 2.7 Hz), 4.15 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 3.3 Hz), 4.38 (t, 1 H, H-4'), 4.53 (d, 1 H, H-2'), 5.81 (s, 1 H, H-3''), 6.09 (d, 1 H, H-1', $J_{1',2'}$ = 8.1 Hz), 6.42 (bs, 2 H, NH_2-4''), 7.87 (s, 1 H, H-6), 10.78 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz)] δ 26.10, 26.71 [$(CH_3)CSi$) 63.73 (C-5'), 76.42 (C-2'), 85.67, 86.53 (C-4', C-3''), 92.77 (C-1'), 93.73 (C-3'), 139.92 (C-5), 124.52 (C-6, $J_{C-6,F}$ = 33.50 Hz), 150.45, (C-4''), 144.59 (C-2), 151.71 (C-4). Anal. ($C_{23}H_{40}FN_3O_8SSi_2$) C, H, N, S.

[1-[-2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-bromouracil]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (9b). Cyanomesylate **6b** (1.1 g, 1.92 mmol) was reacted according to the general procedure. The residue was chromatographed (hexane–ethyl acetate, 2:1). The fastest moving fractions afforded 0.12 g (10%) of **9b** as a white foam: IR (KBr, cm^{-1}) 3400, 3320, 3195 (NH_2), 1645 (C=CN); UV (MeOH) λ_{max} nm (log ε) 272 (3.86) and 216 (3.97); ^1H NMR [$(CD_3)_2CO$, 200 MHz] δ 4.05 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 12.3, $J_{4',5'a}$ = 3.1 Hz), 4.14 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 3.3 Hz), 4.39 (t, 1 H, H-4'), 4.57 (d, 1 H, H-2'), 5.80 (s, 1 H, H-3''), 6.11 (d, 1 H, H-1', $J_{1',2'}$ = 8.1 Hz), 6.49 (bs, 2 H, NH_2-4''), 7.96 (s, 1 H, H-6), 10.40 (bs, 1 H, NH-3). Anal. ($C_{23}H_{40}BrN_3O_8SSi_2$) C, H, N, S.

The slowest moving fractions gave 0.40 g (30%) of N^6,4''-cyclo-**[1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyluracil]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (10)** as a white foam: IR (KBr, cm^{-1}) 3100, 2900, 2850 (NH), 1730 (C=O), 1640 (C=CN); UV (MeOH) λ_{max} nm (log ε) 348 (4.20), 300 (4.23), and 200 (3.88); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 3.91 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 12.0, $J_{4',5'a}$ = 5.1 Hz), 4.08 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 2.3 Hz), 4.52 (dd, 1 H, H-4'), 4.87 (s, 1 H, H-2'), 5.13 (s, 1 H, H-5), 6.48 (s, 1 H, H-3''), 6.73 (s, 1 H, H-1'), 10.19 (bs, 2 H, NH-3, NH-4''); ^{13}C NMR [$(CD_3)CO$, 50 MHz)] δ 26.00, 26.23 (CH_3CSi), 61.11 (C-5'), 76.39 (C-2'), 80.96 (C-4'), 86.60, 87.65 (C-1', C-3''), 88.10 (C-3'), 101.78 (C-5), 146.54 (C-6), 149.72 (C-4''), 150.64 (C-2), 162.44 (C-4). Anal. ($C_{23}H_{39}N_3O_8SSi_2$) C, H, N, S.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-iodouracil]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (9c). The general procedure was followed with cyanomesylate **6c** (0.76 g, 1.23 mmol). The residue was chromatographed (hexane–ethyl acetate, 5:1). The fastest moving fractions afford **9c** (0.04 g, 5%) as an amorphous solid: IR (KBr, cm^{-1}) 3400, 3320, 3190 (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2CO$, 200 MHz] δ 4.02 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 12.2, $J_{4',5'a}$ = 3.9 Hz), 4.12 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 3.2 Hz), 4.31 (t, 1 H, H-4'), 4.69 (d, 1 H, H-2'), 5.74 (s, 1 H, H-3''), 5.97 (d, 1 H, H-1', $J_{1',2'}$ = 8.0 Hz), 6.42 (bs, 2 H, NH_2-4''), 7.48 (s, 1 H, H-6), 10.20 (bs, 1 H, NH-3); ^{13}C NMR ([CD_3)CO, 50 MHz)] δ 25.79, 26.41, (CH_3CSi), 63.18 (C-5'), 76.75 (C-2'), 85.98, 86.96 (C-4', C-3''), 92.70 (C-2'), 93.13 (C-1'), 113.68 (CF_3), 148.24, 149.78 (C-5, C-6), 149.77, 151.29 (C-2, C-4''), 159.27 (C-4). Anal. ($C_{23}H_{40}IN_3O_8SSi_2$) C, H, N, S.

The slowest moving fractions gave the cyclospiro derivative **10** (0.30 g, 45%) as a white foam.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-(trifluoromethyl)uracil]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (9d). The general procedure was followed with cyanomesilate **6d** (0.75 g, 1.3 mmol). The residue was purified by CCTLC on chromatotron (dichloromethane–methanol, 100:1) to give 0.21 g (25%) of **9d** as an amorphous solid: IR (KBr, cm^{-1}) 3400, 3330, 3190 (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 4.07 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 12.4, $J_{4',5'a}$ = 5.0 Hz), 4.17 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 4.3 Hz), 4.44 (t, 1 H, H-4'), 4.60 (d, 1 H, H-2'), 5.82 (s, 1 H, H-3''), 6.13 (d, 1 H, H-1', $J_{1',2'}$ = 7.9 Hz), 6.44 (bs, 2 H, NH_2-4''), 8.37 (s, 1 H, H-6). Anal. ($C_{24}H_{40}F_3N_3O_8SSi_2$) C, H, N, S.

Reaction of 3'-*C*-Cyano-3'-*O*-mesyl Derivatives 6b, 6c or 3'-Spiro Derivative 12 with Potassium Cyanide. General Procedure. A mixture of **6b**, **6c**, or **12** (1 equiv) and KCN (1.5–6 equiv) was dissolved in DMF. The reaction mixture was stirred at room temperature or heated at 80 °C for 4–64 h. The solution was neutralized with 1 N HCl, and the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (14 mL), washed with water (2 × 14 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by CCTLC on chromatotron (chloroform–methanol, 200:1). The reaction conditions, yields, and products obtained are indicated below.

Reaction A. According to the general procedure, **6b** (0.20 g, 0.34 mmol) and KCN (34 mg, 0.52 mmol) in DMF (10 mL) was reacted at room temperature for 64 h. The residue was chromatographed. The fastest moving fractions gave **1-(2'-*O*-acetyl-5'-*O*-benzoyl-3'-*C*-cyano-3'-*O*-mesyl-β-D-ribofuranosyl)-6-cyanouracil (11)** (70 mg, 40%) as a white foam: IR (KBr, cm^{-1}) 1740 (C=O), 1375, 1180 (SO_2); UV (MeOH) λ_{max} nm (log ε) 272 (3.91), 227 (4.13), 202 (4.09); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.18 (s, 3 H, 1 OAc), 3.44 (s, 3 H, $MeSO_2$), 4.75 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 12.0, $J_{4',5'a}$ = 6.3 Hz), 4.92 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 4.9 Hz), 5.03 (dd, 1 H, H-4'), 6.14 (d, 1 H, H-2'), 6.33 (d, 1 H, H-1', $J_{1',2'}$ = 5.8 Hz), 6.66 (s, 1 H, H-5), 7.50–8.08 (m, 5 H, Ph), 10.95 (bs, 1 H, NH-3); ^{13}C NMR δ [$(CD_3)_2CO$, 50 MHz)] δ 20.29 (OAc), 40.88 ($MeSO_2$), 63.15 (C-5'), 75.01 (C-2'), 77.76 (C-3'), 82.40 (C-4'), 91.24 (C-1'), 111.81, 114.19 (2CN), 115.40 (C-5), 127.35 (C-6), 129.47–134.33 (Ph), 149.65 (C-2), 160.77 (C-4), 166.22, 170.00 (C=O). Anal. ($C_{21}H_{18}N_4O_{10}S$) C, H, N, S.

The slowest moving fractions gave **[1-(2'-*O*-acetyl-5'-*O*-benzoyl)-β-D-ribofuranosyl]-6-cyanouracil]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (12)** (60 mg, 34%) as a white foam: IR (KBr, cm^{-1}) 3400 (NH_2), 2220 (CN), 1710 (C=O), 1380, 1320 (SO_2). UV (MeOH) λ_{max} nm (log ε) 270 (3.71), 225 (4.19), 200 (4.14); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.09 (s, 3 H, 1 OAc), 4.62 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 3.7 Hz), 4.72 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 8.0 Hz), 4.82 (dd, 1 H, H-4'), 5.68 (s, 1 H, H-3''), 6.08 (d, 1 H, H-2'), 6.20 (d, 1 H, H-1', $J_{1',2'}$ = 6.5 Hz), 6.63 (bs, 2 H, NH_2-4''), 6.67 (s, 1 H, H-5), 7.45–8.03 (m, 5 H, Ph), 10.20 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz)] δ 20.19 (CH_3CO), 62.78 (C-5'), 72.17 (C-2'), 82.12 (C-4'), 86.56 (C-3'), 88.61 (C-3''), 91.95 (C-1'), 111.84 (CN), 115.57 (C-5), 128.57 (C-6), 129.39–134.20 (Ph), 150.65 (C-2), 154.30 (C-4''), 160.98 (C-4), 166.31, 169.54 (C=O). Anal. ($C_{21}H_{18}N_4O_{10}S$) C, H, N, S.

Reaction B. Via the general procedure, **6c** (0.20 g, 0.32 mmol) and KCN (32 mg, 0.48 mmol) in DMF (10 mL) were reacted at room temperature for 4 h. The residue was chromatographed to give spironucleoside **12** (0.13 g, 80%).

Reaction C. According to the general procedure, **6b** (0.20 g, 0.34 mmol), KCN (32 mg, 0.48 mmol), and DMF (10 mL) were heated at 80 °C for 6 h. The residue was chromatographed. The fastest moving fractions gave the spiro derivative **12** (62 mg, 35%).

The next moving fractions afforded **N^6,4″-cyclo-[1-(2′-*O*-acetyl-5′-*O*-benzoyl-β-D-ribofuranosyl)uracil]-3′-spiro)-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (14)** (26 mg, 16%) as an amorphous solid: UV (MeOH) λ_{max} nm (log ε) 350 (4.23), 298 (3.95), 226 (4.14), and 200 (4.01); ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.23 (s, 3 H, 1 OAc), 4.45 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 12.4, $J_{4',5'a}$ = 6.8 Hz), 4.75 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.7 Hz), 4.85 (dd, 1 H, H-4′), 5.15 (s, 1 H, H-5), 5.85 (s, 1 H, H-2′), 6.64 (s, 1 H, H-3″), 6.85 (s, 1 H, H-1′), 10.15 (bs, 2 H, NH-3, NH-4″); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz)] δ 20.50 (CH_3CO), 61.90 (C-5′), 74.79 (C-2′), 78.52 (C-4′), 85.12 (C-1′), 86.36 (C-3′), 87.67 (C-3″), 101.80 (C-5), 129.48–134.26 (Ph), 145.37 (C-6), 148.58 (C-4″), 150.43 (C-2), 161.97 (C-4), 166.00, 169.17 (C=O). Anal. ($C_{20}H_{16}N_3O_{10}S$) C, H, N, S.

The slowest moving fractions gave **[1-(5′-*O*-benzoyl-β-D-ribofuranosyl)-6-cyanouracil]-3′-spiro-5″-[4″-amino-1″-2″-oxathiole 2″,2″-dioxide) (13)** (18 mg, 11%): IR (KBr, cm^{-1} 3400, 3320 (NH_2), 2220 (CN), 1720 (CO), 1375, 1180 (SO_2); ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 4.67–4.74 (m, 4 H, 2 H-5′, H-4′, OH), 5.34 (d, 1 H, H-2′), 5.72 (s, 1 H, H-3″), 5.87 (d, 1 H, $J_{1',2'}$ = 6.6 Hz), 6.60 (bs, 2 H, NH_2-4″), 6.68 (s, 1 H, H-5), 7.48-8.07 (m, 5 H, Ph). Anal. ($C_{19}H_{15}N_4O_9S$) C, H, N, S.

Reaction D. Compound **6c** (0.10 g, 0.16 mmol), KCN (63 mg, 0.97 mmol), and DMF (5 mL) were heated at 80 °C for 7 h. The residue was chromatographed. The fastest moving fractions gave the spironucleoside **12** (41 mg, 50%).

The slowest moving fractions afforded the cyclospiro derivative **14** (13 mg, 17%).

Reaction E. According to the general procedure, compound **12** (0.10 g, 0.2 mmol) reacted with KCN (26 g, 0.4 mmol) in DMF (5 mL) at 80 °C for 7 h. After workup and purification of the residue, compound **13** (0.04 g, 40%) was obtained as a white foam.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-6-cyanouracil]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) and [1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-6-(methoxyimino)uracil]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (15 and 16). A solution of **12** (0.28 g, 0.54 mmol) in saturated methanolic ammonia (5 mL) was stirred at room temperature overnight. The solvent was evaporated to dryness. The residue was dissolved in methanol (1 mL) and then treated with ether (1 mL). The solid (2′,5′-*O*-deprotected nucleoside) was filtered and suspended in dry acetonitrile (4 mL), and then, 4-(dimethylamino)pyridine (0.22 g, 1.81 mmol) and TBDMS-Cl (0.27 g, 1.81 mmol) were added. The mixture was heated at 80 °C for 16 h. The solvent was evaporated to dryness, and the residue was treated with chloroform (2 × 25 mL) and water (25 mL). The organic phase was extracted with chloroform (2 × 25 mL). The combined organic layers were successively washed with cold (4 °C) 1 N HCl (13 mL), water (13 mL), and brine (13 mL) and, finally, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash column chromatography (chloroform–methanol, 20:1). The fastest moving fractions afforded **15** (0.06 g, 20%) as a white foam: ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 4.01 (dd, 1 H, H-5′a, $J_{5'a,5'b}$ = 11.9, $J_{4',5'a}$ = 6.8 Hz), 4.09 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 3.7 Hz), 4.33 (dd, 1 H, H-4′), 5.34 (d, 1 H, H-2′), 5.69 (s, 1 H, H-3″), 5.73 (d, 1 H, H-1′, $J_{1',2'}$ = 6.8 Hz), 6.43 (bs, 2 H, NH_2-4″), 6.77 (s, 1 H, H-5), 11.10 (bs, 1 H, NH_3); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz)] δ 25.87, 26.23 (CH_3CSi), 62.29 (C-5′), 73.39 (C-2′), 85.85 (C-4′), 89.20 (C-3′), 89.94 (C-3″), 94.45 (C-1′), 111.86 (CN), 115.60 (C-5), 128.71 (C-6), 150.00 (C-2), 153.74 (C-4″), 161.00 (C-4). Anal. ($C_{24}H_{40}N_4O_8SSi_2$) C, H, N, S.

The slowest moving fractions gave **16** (0.06 g, 17%) as a white foam: ^{1}H NMR [$(CD_3)_2CO$, 200 MHz] δ 3.88 (s, 3 H, 1 OAc), 3.96–4.12 (m, 3 H, H-4′, 2 H-5′), 5.19 (d, 1 H, H-2′), 5.36 (d, 1 H, H-1′, $J_{1',2'}$ = 6.4 Hz), 5.61 (s, 1 H, H-3″), 5.87 (s, 1 H, H-5), 6.47 (bs, 2 H, NH_2-4″), 8.82 (bs, 1 H, C=NH), 10.62 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz)] δ 28.63, 28.66 (CH_3CSi), 54.29 (CH_3-O), 62.19 (C-5′), 72.73 (C-2′), 84.83 (C-4′), 86.21 (C-3′), 89.05 (C-3″), 94.66 (C-1′), 104.66 (C-5), 128.31 (C-6), 150.27 (C-2), 151.50 (C-4″), 162.05 (C=NH), 154.61 (C-4). Anal. ($C_{25}H_{44}N_4O_9SSi_2$) C, H, N, S.

General Procedure for the Synthesis of (4-*N*-Alkylnucleosides)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (19–22). A suspension of 1,2,4-triazole (0.26 g, 3.7 mmol) and $POCl_3$ (0.08 mL, 0.8 mmol) in dry acetonitrile (2 mL) was stirred at 0 °C for 5 min. Then NEt_3 (0.6 mL)[39] was slowly added. The resulting mixture was stirred at 0 °C for 1 h, and then a solution of the 3′-spironucleoside **18**[8] or **1**[8] (0.25 mmol) in dry acetonitrile (1 mL) was added. The mixture was stirred at room temperature for 4 h and then filtered. The filtrate was diluted with ethyl acetate (25 mL), washed successively with aqueous $NaHCO_3$ (25 mL) and brine (25 mL), and finally, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was dissolved in dry dioxane (3 mL) and then treated with the corresponding amine (33 wt % solution in EtOH) (0.17 mL) for 30 min. The solvent was evaporated to dryness and purified by CCTLC on a chromatotron or by column chromatography.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-*N*-methylcytosine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2-dioxide) (19). Via the general procedure, compound **18**[8] (0.08 g, 0.14 mmol) was reacted with 1,2,4-triazole (0.14 g, 2 mmol) and $POCl_3$ (0.04 mL, 0.4 mmol) for 4 h and then treated with methylamine (33 wt % solution in EtOH) (0.08 mL). The residue was purified by CCTLC on a chromatotron (hexane–ethyl acetate, 1:2) to yield 39 mg (51%) of **19** as an amorphous sold: IR (KBr, cm^{-1}) 3400, 3325, 3190 (NH_2), 1650 (C=CN); ^{1}H NMR [$(CD_3)_2CO$, 200 MHz] δ 2.90 (s, 3 H, $NHCH_3$), 3.94–4.16 (m, 3 H, H-4′, 2 H-5′), 4.98 (d, 1 H, H-2′), 5.60 (s, 1 H, H-3″), 5.61 (d, 1 H, H-1′, $J_{1',2'}$ = 6.7 Hz), 5.96 (s, 1 H, H-5), 6.83 (bs, 2 H, NH_2-4″), 7.16 (s, 1 H, $NHCH_3$), 7.70 (d, 1 H, H-6). Anal. ($C_{24}H_{44}N_4O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-*N*,*N*-dimethylcytosine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2-dioxide) (20). According to the general procedure, **18**[8] (0.07 g, 0.12 mmol) was reacted with 1,2,4-triazole (0.12 g, 1.7 mmol) and $POCl_3$ (0.034 mL, 0.34 mmol) for 4 h and then treated with dimethylamine (33 wt % solution in EtOH) (0.08 mL). The residue was purified by CCTLC on a chromatotron (hexane–ethyl acetate, 1:2) to yield 34 mg (47%) of **20** as a white foam: IR (KBr, cm^{-1}) 3400, 3330, 3190 (NH_2), 1650 (C=CN); ^{1}H NMR [$(CD_3)_2CO$, 200 MHz] δ 3.15 [s, 6 H, $N(CH_3)_2$], 3.94–4.14 (m, 3 H, H-4′, 2 H-5′), 5.06 (d, 1 H, H-2′), 5.54 (d, 1 H, H-1′, $J_{1',2'}$ = 6.5 Hz), 5.58 (s, 1 H, H-3″), 6.21 (d, 1 H, H-5), 6.84 (bs, 2 H, NH_2-4″), 7.81 (d, 1 H, H-6). Anal. ($C_{25}H_{46}N_4O_7SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-*N*-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2-dioxide) (21). Compound **1**[8] (0.15 g, 0.25 mmol) was reacted with 1,2,4-triazole (0.26 g, 3.7 mmol) and $POCl_3$ (0.08 mL, 0.8 mmol) for 4 h and then treated with methylamine (33 wt % solution in EtOH) (0.17 mL) according to the general procedure. Purification of the residue by column chromatography (hexane–ethyl acetate, 1:2) gave 73 mg (48%) of **21** as an amorphous solid: IR (KBr, cm^{-1}) 3400, 3340, 3190 (NH_2), 1650 (C=CN); ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.97 (s, 3 H, CH_3-5), 2.93 (s, 3 H, $NHCH_3$), 4.02–4.09 (m, 3 H, H-4′, 2 H-5′), 5.11 (d, 1 H, H-2′), 5.39 (d, 1 H, H-1′, $J_{1',2'}$ = 6.4 Hz), 5.55 (s, 1 H, H-3″), 6.96 (bs, 2 H, NH_2-4″), 7.52 (s, 1 H, H-6). Anal. ($C_{25}H_{46}N_4O_7SSi_2$) C, H, N, S.

[1-[2′,5-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-4-*N*,*N*-dimethylthymine)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2-dioxide) (22). According to the general procedure, compound **1**[8] (0.15 g, 0.25 mmol) reacted with 1,2,4-triazole (0.26 g, 3.7 mmol) and $POCl_3$ (0.08 mL, 0.8 mmol) and then was treated with dimethylamine (33 wt % solution in EtOH) (0.17 mL). Purification of the residue by column chromatography (hexane–ethyl acetate, 1:2) yielded 85 mg (55%) of **22** as an amorphous solid: IR (KBr, cm^{-1}) 3400, 3320, 3200 (NH_2), 1655 (C=CN); ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.24 (s, 3 H, CH_3-5), 3.19 [s, 6 H, $N(CH_3)_2$], 3.97 (dd, 1 H, $J_{5'a,5'b}$ = 11.7, $J_{4',5'a}$ = 7.6 Hz), 4.06 (dd, 1 H, H-5′b, $J_{4',5'b}$ = 2.2 Hz), 4.08 (dd, 1 H, H-4′), 5.12 (d, 1 H, H-2′), 5.35 (d, 1 H, H-1′, $J_{1',2'}$ = 6.3 Hz), 5.54 (s, 1 H, H-3″), 6.98 (bs, 2 H, NH_2-4″), 7.56 (d, 1 H, H-6). Anal. ($C_{26}H_{48}N_4O_7SSi_2$) C, H, N, S.

Antiretrovirus Activity Assays. HIV-1 was originally obtained from the culture supernatant of the persistently HIV-infected H9 cell line (H9/HTLV-III_B), which was kindly provided by R. C. Gallo and M. Popovic (National Institutes of Health, Bethesda, MD). HIV-1 (ROD) was obtained from L. Montagnier

(Pasteur Institute, Paris, France). 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.[2,3,44] Briefly, MT-4 cells for CEM cells (5×10^5 cells/mL) were suspended in fresh culture medium and infected with HIV-1 or HIV-2 at 100 times the 50% cell culture infective dose ($CCID_{50}$) per milliliter of cell suspension. The 100 μL of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of test compounds. After 4 days, virus-induced syncytium formation was recorded microscopically in the HIV-infected CEM cell 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 cells in the MT-4 cell cultures. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce by 50% the number of mock-infected MT-4 cells.

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