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

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Several analogues of a new lead for anti-HIV-1 agents [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (TSAO) modified at positions N-3, O-4 and C-5 of the thymine moiety, have been prepared and evaluated as inhibitors of HIV-1 replication. A new stereoselective synthetic procedure is described. Reaction of 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-*C*-cyano-3-*O*-mesyl-D-ribofuranose with pyrimidine bases, followed by treatment with Cs_2CO_3 afforded stereoselectively, β-D-ribofuranosyl-3′-spiro nucleosides. 2′,5′-*O*-Deacylation and subsequent treatment with *tert*-butyldimethylsilyl chloride gave the TSAO derivatives. Only those analogues having a tBDMSi group at both the C-5′ and C-2′ positions of the ribose moiety showed potent anti-HIV-1 activity. The activity ranged from 0.060 μM to 1.0 μM. Introduction of an alkyl or alkenyl function at N-3 of the thymine ring markedly decreased cytotoxicity without affecting the antiviral activity. While markedly active against HIV-1, the TSAO derivatives had no activity against HIV-2 or SIV. They represent the first example of nucleoside analogues with an intact ribose moiety that discriminate between HIV-1 and other retroviruses.

Introduction

Several different kinds of nucleoside analogues have proved to be effective anti-HIV agents. Work in this field has mainly focused, on the one hand, on 2′,3′-dideoxynucleoside derivatives, in which the 3′-OH group is replaced by a 3′-substituent, a 2′,3′-double bond or a hydrogen[1-15] and, on the other hand, acyclic nucleoside phosphonates.[16-22] The mechanism of action of these types of compounds is similar to that of AZT:[1,19,23-26] they inhibit the HIV reverse transcriptase by acting as alternative substrates and thus terminate the growing DNA chain.

In search of new nucleoside derivatives as inhibitors of HIV replication, and as a part of our program on the stereospecific synthesis of branched-chain sugars and nucleosides, we synthesized[27,28] *xylo*- and *ribo*-3′-spiro nucleoside derivatives. Among these derivatives, [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide)[29] (TSAO), obtained as a minor product by the reported pathway, exhibited a potent and selective inhibition of HIV-1 replication in vitro.[28,30] This new lead compound appears to interact with the HIV-1 reverse transcriptase (RT), but not the HIV-2 RT, at a nonsubstrate binding site. The kinetics of HIV-1 RT inhibition by these compounds is now under investigation.

To direct the synthesis of these 3′-spiro nucleosides to the preparation of the active *ribo* derivatives, we have designed a new route which exclusively provides the desired *ribo*-spiro nucleosides. With the aim of establishing a detailed structure–activity relationship, this new synthetic route has been extended to base-modified analogues of the lead compound TSAO.

Chemistry

The spiro nucleosides were obtained stereoselectively by glycosylation of a tertiary cyano mesylate of ribose with heterocyclic bases followed by basic treatment of the cyano mesyl nucleosides to give exclusively the β-D-*ribo*-spiro nucleosides. The *ribo* configuration of the nucleosides was determined by the configuration of the starting cyanohydrin used in the preparation of the cyano mesylate of ribose, as clearly demonstrated in previous papers of this series.[31,32]

5-*O*-Benzoyl-1,2-*O*-isopropylidene-β-D-*erythro*-pentofuranos-3-ulose (1)[33] was prepared from D-xylose following

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(1) (a) Broder, S. Clinical Applications of 3′-Azido-2′,3′-Dideoxythymidine (AZT) and Related Dideoxynucleosides. *Med. Res. Rev.* **1990**, *10*, 419–439. (b) Mitsuya, H. Strategies for Antiviral Therapy in AIDS. *Nature* **1987**, *325*, 773–778.
(2) Mitsuya, H.; Broder, S. Inhibition of the in Vitro Infectivity and Cytopathic Effect of Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus (HTLV-III/LAU) by 2′,3′-Dideoxynucleosides. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911–1915.
(3) Herdewijn, P.; De Clercq, E. Dideoxynucleoside Analogues as Inhibitors of HIV Replication. In *Design of Anti-AIDS Drugs*; De Clercq, E., Ed.; Elsevier: Amsterdam, 1990; pp 141–174.
(4) Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E.; Desmyter, J.; Vandeputte, M. Both 2′,3′-Dideoxythymidine and its 2′,3′-Unsaturated Derivative (2′,3′-Dideoxythymidine) are Potent and Selective Inhibitors of Human Immunodeficiency Virus Replication in Vitro. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 128–134.
(5) Balzarini, J.; Kang, G. J.; Dalal, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; Johns, D. G. The Anti-HTLV-III (anti-HIV) and Cytotoxic Activity of 2′,3′-Didehydro-2′,3′-dideoxyribonucleosides: a Comparison with Their Parental 2′,3′-Dideoxyribonucleosides. *Mol. Pharmacol.* **1987**, *32*, 162–167.
(6) Fischl, M. A.; Richman, D. D.; Grieco, M. H. et al. and the AZT Collaborative Working Group. The Efficacy of Azidothymidine (AZT) in the Treatment of Patients with AIDS and AIDS-Related Complex. *N. Engl. J. Med.* **1987**, *317*, 185–191.
(7) Yarchoan, R.; Broder, S. Anti-Retroviral Therapy of AIDS and Related Disorders: General Principles and Specific Development of Dideoxynucleosides. *Pharm. Ther.* **1989**, *40*, 329–348.
(8) Yarchoan, R.; Mitsuya, H.; Thomas, R. V.; Pluda, J. M.; Hartman, N. R.; Perno, C. F.; Marczyk, K. S.; Allain, J. P.; Johns, D. G.; Broder, S. In vivo activity against HIV and favorable toxicity profile of 2′,3′-dideoxyinosine. *Science* **1989**, *245*, 412–415.
(9) Richman, D. D.; Fisch, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. I.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schooly, R. T.; Jackson, G. G.; Durack, D. T.; King, D. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. *N. Engl. J. Med.* **1987**, *317*, 192–197.
(10) De las Heras, F. G.; Camarasa, M. J.; Fiandor, J. Nucleosides: Potential drugs for AIDS therapy. In Recent Progress in the Chemical Synthesis of Antibiotics; Lukacs, G., Ohno, M., Eds.; Springer Verlag: Berlin–Heidelberg, 1990; pp 321–363.
(11) Balzarini, J.; Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E. Anti-retrovirus activity of 3′-fluoro- and 3′-azido-substituted pyrimidine 2′,3′-dideoxynucleoside analogues. *Biochem. Pharmacol.* **1988**, *37*, 2847–2856.

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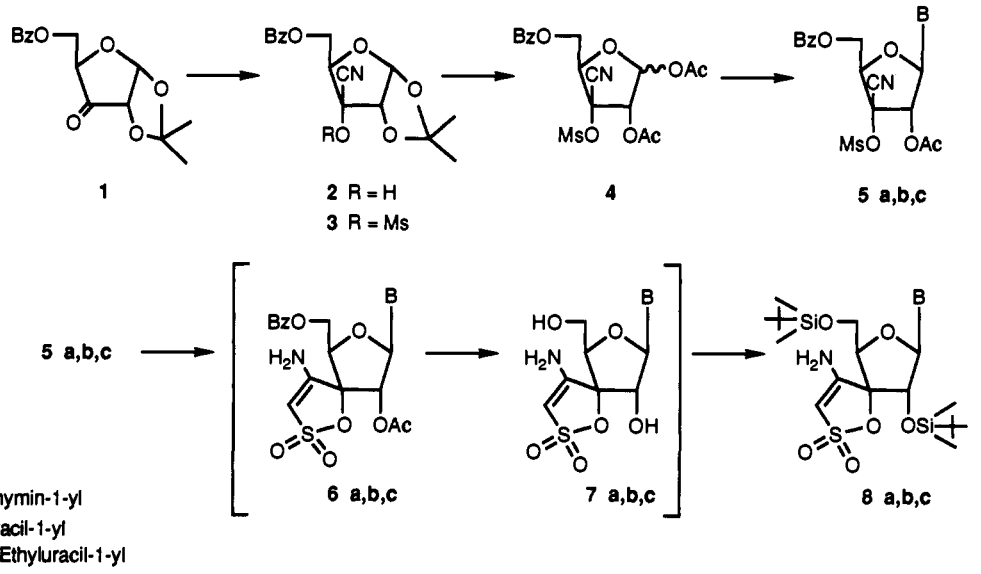
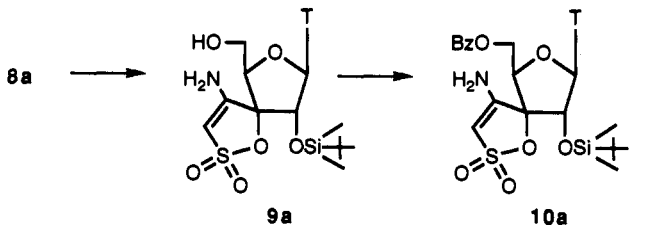
Scheme I



a well-established procedure.[33,34] Treatment of the ulose 1 with sodium cyanide in a two-phase ethyl ether/water system, in the presence of $NaHCO_3$, afforded the kinetically controlled[31,32] *ribo*-cyanohydrin 2 (Scheme I). For-

Scheme II



(12) Balzarini, J.; Van Aerschot, A.; Pauwels, R.; Baba, M.; Schols, D.; Herdewijn, P.; De Clercq, E. 5-Halogeno-3′-fluoro-2′,3′-dideoxyuridines as inhibitors of human immunodeficiency virus (HIV): potent and selective anti-HIV activity of 3′-fluoro-2′,3′-dideoxy-5-chlorouridine. *Mol. Pharmacol.* **1989**, *35*, 571–577.

(13) Chu, C. K.; Schinazi, R. F.; Ahn, M. K.; Ullas, G. U.; Gu, Z. P. Structure–activity relationship of pyrimidine nucleosides as antiviral agents for human immunodeficiency virus type 1 in peripheral blood mononuclear cells. *J. Med. Chem.* **1989**, *32*, 612–617.

(14) Lin, T. S.; Shen, Z. Y.; August, E. M.; Brankovan, V.; Yang, H.; Ghazzouli, I.; Prusoff, W. H. Synthesis and antiviral activity of several 2,5′-anhydro analogues of 3′-azido-3′-deoxythymidine, 3′-azido-2′,3′-dideoxyuridine, 3′-azido-2′,3′-dideoxy-5-halouridines, and 3′-deoxythymidine against human immunodeficiency virus and Rauscher-murine Leukemia virus. *J. Med. Chem.* **1989**, *32*, 1891–1895.

(15) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. 3′-Substituted 2′,3′-dideoxynucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents. *J. Med. Chem.* **1987**, *30*, 1270–1278.

(16) Pauwels, R.; Balzarini, J.; Schols, D.; Baba, M.; Desmyter, J.; Rosenberg, I.; Holy, A.; De Clercq, E. Phosphonylmethoxyethyl purine derivatives, a new class of anti-human immunodeficiency virus agents. *Antimicrob. Agents Chemother.* **1988**, *32*, 1025–1030.

(17) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. Marked in vivo anti-retrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine, a selective anti-HIV agent. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332–336.

(18) Balzarini, J.; Naesens, L.; Slachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holy, A.; Schellekens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) effectively inhibits retrovirus replication in vitro and simian immunodeficiency virus infection in rhesus monkeys. *AIDS* **1991**, *5*, 21–28.

(19) Balzarini, J.; Holy, A.; Jindrich, J.; Dvorakova, H.; Hao, Z.; Snoeck, R.; Herdewijn, P.; Johns, D. G.; De Clercq, E. 9-[(2RS)-3-fluoro-2-phosphonylmethoxypropyl]derivatives of purines: A class of highly selective antiretroviral agents in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4961–4965.

(20) Balzarini, J.; Naesens, L.; De Clercq, E. Anti-retrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) in vivo increases when it is less frequently administered *Int. J. Cancer* **1990**, *46*, 337–340.

(21) Naesens, L.; Balzarini, J.; Rosenberg, I.; Holy, A.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)-2,6-diaminopurine (PME-DAP): A Novel agent with anti-human immunodeficiency virus activity in vitro and potent anti-Moloney Murine Sarcoma virus activity in vivo. *Eur J. Clin. Microbiol. Infect. Dis.* **1989**, *8*, 1043–1048.

(22) Naesens, L.; Balzarini, J.; De Clercq, E. Single-dose administration of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) and 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) in the prophylaxis of retrovirus infection in vivo. *Antiviral Res.* **1991**, *16*, 53–64.

(23) Balzarini, J.; Hao, Z.; Herdewijn, P.; Johns, D. G.; De Clercq, E. Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1499–1503.

(24) Hao, Z.; Conney, D. A.; Farquhar, D.; Perno, C. F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N. R.; Balzarini, J.; Johns, D. G. Potent DNA chain termination activity and selective inhibition of human immunodeficiency virus reverse transcriptase by 2′,3′-dideoxyuridine-5′-triphosphate. *Mol. Pharmacol.* **1990**, *37*, 157–163.

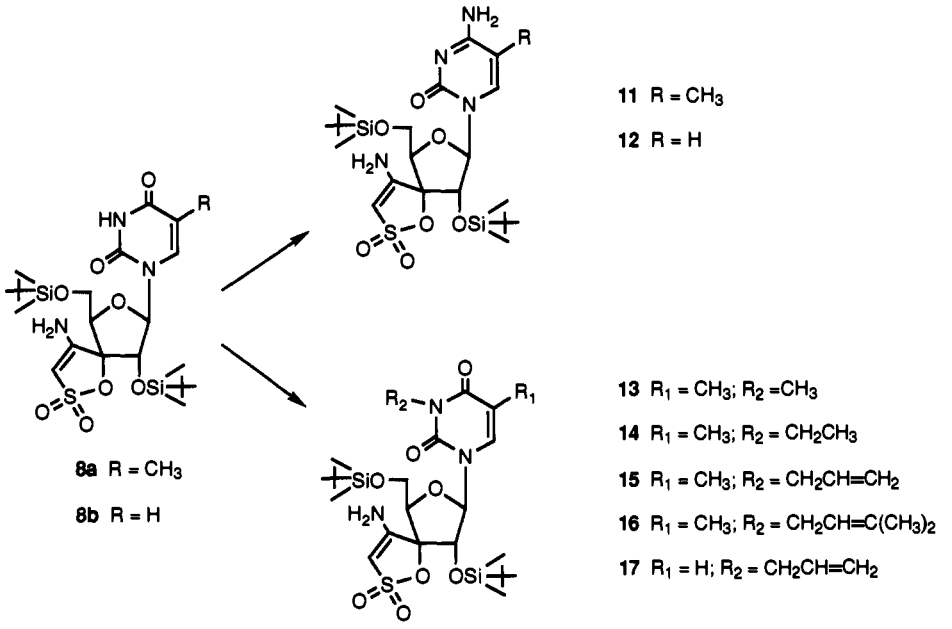
(25) Furman, P. A.; Fyle, J. A.; St Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Nusinoff-Lehrman, S.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W.; Phosphosylation of 3′-azido-3′-deoxythymidine and selective interaction of the 5′-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333–8337.

(26) St. Clair, M. H.; Richards, C. A.; Spector, T.; Weinhold, K. J.; Miller, W. H.; Langlois, A. J.; Furman, P. A. 3′-Azido-3′-deoxythymidine triphosphate as an Inhibitor and Substrate of purified human Inmunodeficiency Virus Reverse Transcriptase. *Antimicrob. Agents Chemother.* **1987**, *31*, 1972–1977.

(27) Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. *Tetrahedron Lett.* **1992**, *33*, 3029–3032.

(28) Camarasa, M. J.; Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E. *J. Med. Chem.*, in press.

Scheme III



mation of **2** is in agreement with the approach of the cyanide ion from the sterically less hindered β-face of the ulose **1**, opposite to the 1,2-*O*-isopropylidene group. The cyanohydrin **2** was not isolated. It was transformed without further purification to the cyano mesylate **3**. The absolute configuration of **3** was assumed to be identical to that of the corresponding cyanohydrin **2** since, in previous mesylations of cyanohydrins, no epimerization was observed.[31,32,35-37] Hydrolysis of the 1,2-*O*-isopropylidene group of **3**, with aqueous trifluoroacetic acid, followed by reaction with acetic anhydride/pyridine afforded a (1.5:1) mixture of the two anomeric forms (α and β) of the diacetate derivative **4** in 95% yield. Condensation of 1,2-bis-*O*-acetyl cyano mesylate **4** with silylated bases using trimethylsilyl triflate as condensing reagent, as described by Vorbrüggen,[38] resulted in 3′-cyano mesylate nucleosides **5** in good yields. Thus, the glycosylation of **4** with thymine, uracil, and 5-ethyluracil[39] afforded the 3′-cyano mesylates of thymine **5a** (77%), uracil **5b** (78%) and 5-ethyluracil **5c** (93%), respectively. Due to the presence of a 2-*O*-acyl participating group, the products obtained were exclusively β-anomers. Coupling constant values were, in the range of $J_{1'2'}$ = 6.0–7.0 Hz, which is in reasonably good agreement with literature data for other β-D-*ribo*-3′-*C*-branched nucleosides,[40,41] and further corroborates the β-anomeric configuration of cyano mesylates **5a–c**.

Treatment of cyano mesylate **5a** with Cs_2CO_3 afforded the spiro derivative **6a** in 65% yield. Deprotection of **6a** with saturated methanolic ammonia, gave the fully deprotected nucleoside **7a**[28] in 66% yield, which, by reaction with *tert*-butyldimethylsilyl chloride yielded the 2′,5′-bis-*O*-silylated nucleoside **8a**.[28] Selective cleavage of the 5′-*O*-silyl ether of **8a** (Scheme II) followed by benzoylation with benzoyl chloride/pyridine gave the 5′-*O*-benzoyl-2′-*O*-silyl spiro nucleoside **10a** (80%).

The spiro nucleosides **8b** and **8c** were prepared from the corresponding cyano mesylates **5b** and **5c**, in an overall yield of 24% and 32%, respectively. The reaction sequence was similar to that described for the synthesis of **8a**, and no attempts were made at isolating the spiro nucleoside intermediates **6b**, **6c**, **7b**, and **7c**.

Structures of the new compounds were assigned on the basis of the corresponding analytical and spectroscopic data, and by comparison of such data with those of the previously reported **8a** and **8b** whose structure had been

(29) Although the oxathiole ring has priority over the nucleoside system, double primes have been used in the numbering of the oxathiole ring in order to keep the same numbering system for all the nucleosides described in this paper.

(30) Balzarini, J.; Pérez-Pérez, M. J.; San-Félix, A.; Schols, D.; Perno, C. F.; Vandamme, A. M.; Camarasa, M. J.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4392–4396.

(31) Calvo-Mateo, A.; Camarasa, M. J.; Díaz-Ortiz, A.; De las Heras, F. G. Novel aldol-type cyclocondensation of O-mesyl (methylsulphonyl)cyanohydrins. Application to the stereospecific syntheses of branched-chain sugars. *J. Chem. Soc., Chem. Commun.* **1988**, 1114–1115.

(32) Pérez-Pérez, M. J.; Camarasa, M. J.; Díaz-Ortiz, A.; San Félix, A.; De las Heras, F. G. Stereospecific synthesis of branched-chain sugars by a novel aldol-type cyclocondensation. *Carbohydr. Res.* **1991**, *216*, 399–411.

(33) Hollemberg, D. H.; Klein, R. S.; Fox, J. J. Pyridinium chlorochromate for the oxidation of carbohydrates. *Carbohydr. Res.* **1978**, *67*, 491–494.

(34) Levene, P. A.; Raymond, A. L. Derivatives of monoacetone xylose. *J. Biol. Chem.* **1933**, *102*, 317–330.

(35) Bourgeois, J. M.; Synthese de sucres aminés ramifiés II. Synthèse et reactions de *spiro*-aziridines. *Helv. Chim. Acta* **1974**, *57*, 2553–2557.

(36) Thang, T. T.; Winternitz, F.; Lagrange, A.; Oleste, A.; Lukas, G. Stereospecific access to branched-chain carbohydrate synthons. *Tetrahedron Lett.* **1980**, *21*, 4495–4498.

(37) Yoshimura, J.; Aqeel, A.; Hong, N.; Sato, K.; Hashimoto, H. Syntheses of D-rubranitrose and methyl α-D-tetrorithose by cyanomesylation of hexopyranosid-3-uloses. *Carbohydr. Res.* **1986**, *155*, 236–246.

(38) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. Nucleoside Synthesis with Trimethylsilyl Triflate and Perchlorate as Catalysts. *Chem. Ber.* **1981**, *114*, 1234–1256.

(39) Kaul, R.; Kiefer, G.; Erhardt, S.; Hempel, B. 2-^{14}C-1(2′-Deoxy-β-D-ribofuranosyl)-5-ethyluracil: Synthesis and Biotransformation in Rats. *J. Pharm. Sci.* **1980**, *69*, 531–534.

(40) Beigelman, L. N.; Gurskaya, G. U.; Tsakina, E. N.; Mikhailov, S. N. Epimerization during the acetolysis of 3-O-acetyl-5-O-benzoyl-1,2-O-isopropylidene-3-C-methyl-β-D-ribofuranose. Synthesis of 3′-C-methylnucleosides with the β-D-*ribo* and β-D-*arabino*-configurations. *Carbohydr. Res.*, **1988**, *181*, 77–88.

(41) Walton, E.; Jenkins, S. R.; Nutt, R. F. et al. Branched-Chain Sugar Nucleosides. V. Synthesis and Antiviral Properties of Several Branched-Chain Sugar Nucleosides. *J. Med. Chem.* **1969**, *12*, 306–309.

unequivocally determined[28] in an earlier paper of this series.

As described in previous reports in this series,[28,31,32] the disappearance, in the ^{1}H NMR spectra, of the signal corresponding to the mesyl group and the presence of two singlets at δ 6.93–7.17 assigned to NH_2-4″ and at δ 5.60–5.74 assigned to H-3″ indicate the formation of the spiro aminooxathiole dioxide ring. The values of $J_{1',2'}$ = 6.0–8.1 Hz indicate a β-D-*ribo* configuration[40-43] of the spironucleosides, **6**, **7**, and **8**.

Spiro nucleosides of thymine **8a** and uracil **8b** were transformed (Scheme III) to the corresponding derivatives of 5-methylcytosine **11** and cytosine **12**, respectively, following a known method.[44] Thus, treatment of derivatives **8a** and **8b** with 2 equiv of phosphorous oxychloride and 4 equiv of 1,2,4-triazole for 4 h at room temperature, followed by treatment with an excess of concentrated ammonia afforded the cytidine analogues **11** and **12** in 67% and 84% yield, respectively.

Finally, selective N-3-alkylation[45] of the spiro nucleosides **8a** and **8b** afforded the N-3-substituted derivatives **13–17**. Reaction of **8a** with methyl iodide in the presence of potassium carbonate gave 3-methyl-3′-spiro derivative **13** in 55% yield.

Attachment of the methyl group to the N-3 and not to either the oxygen atoms of the C=O groups of thymine or to the NH_2-4″ or C-3″ of the spiro oxathiole moiety was established as follows: (a) the ^{1}H NMR spectrum showed the disappearance of the signal at 10.32 ppm assigned to the NH-3 and the presence of a new singlet at 3.26 ppm corresponding to the 3-*N*-CH_3. (b) No modification was observed at the signals of the protons of the spiro oxathiole moiety, thus indicating that no methylation had occurred at this moiety. (c) The ^{13}C NMR showed no changes in the chemical shifts of C-2 and C-4 with respect to those observed for **8a**, used as starting material.

These carbons appear in derivative **13** at 152 ppm (C-2) and at 163 ppm (C-4) and are in agreement with the values observed for C-2 and C-4 in thymidine,[46] confirming that the methyl group was not attached to them.

Similarly, reaction of **8a** with ethyl iodide, allyl bromide, and 4-bromo-2-methyl-2-butene and of **8b** with allyl bromide afforded the 3-*N*-ethyl-, 3-*N*-allyl-, and 3-*N*-(3-methyl-2-butenyl) spiro nucleosides of thymine, **14**, **15**, and **16** in 77%, 65%, and 70% yield, respectively, and the 3-*N*-allyl spiro derivative of uracil **17** in 89% yield. Their analytical and spectroscopic data were in agreement with the proposed structures and with those reported for other N-3-substituted pyrimidines.[45,47,48]

Table I. Inhibitory Effects of 3′-Spiro Oxathiole Dioxide Nucleoside Analogues on HIV-1-Induced Cytopathicity in MT-4 Cells

compd[a]	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]
6a	>200	>200	–
8a	0.060 ± 0.027	14 ± 2.0	227
8b	0.206 ± 0.042	15 ± 1.0	73
8c	0.066 ± 0.010	5.5 ± 1.0	82
9a	>80	229 ± 37	<2.5
10a	>15	36 ± 4.2	<2.5
11	0.127 ± 0.039	31 ± 1.0	246
12	1.0 ± 0.002	≥360	≥360
13	0.059 ± 0.010	240 ± 91	4088
14	0.123 ± 0.114	123 ± 7.4	1000
15	0.233 ± 0.162	≥330	≥1418
16	0.377 ± 0.057	69 ± 1.6	183
17	0.615 ± 0.348	8.9 ± 2.3	15
TIBO (R 82150)	0.076 ± 0.040	>80	>1053
BI-RG-587	0.128 ± 0.041	>80	>625

[a] Data represent the mean values of at least three to five independent experiments. [b] 50% Effective concentration or compound concentration required to inhibit HIV-1-induced cytopathicity in MT-4 cells by 50%. [c] 50% Cytotoxic concentration or compound concentration required to reduce MT-4 cell viability by 50%. [d] Selectivity index or ratio of CC_{50} to EC_{50}.

Biological Results

A number of pyrimidine 3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) pyrimidine nucleoside analogues were evaluated for their inhibitory activity against HIV-1 (III_B)-induced cytopathicity in human MT-4 lymphocyte cells (Table I). Only those nucleoside analogues that contain a *tert*-butyldimethylsilyl (tBDMS) group at both the C-5′ and C-2′ position of the ribose moiety showed potent anti-HIV-1 activity, irrespective of the nature of the pyrimidine base (i.e. thymine, uracil, or cytosine). The anti-HIV-1 activity ranged from 0.060 μM to 1.0 μM. The thymine derivative **8a** (EC_{50}: 0.060 μM) proved superior to the uracil derivative **8b** (EC_{50}: 0.206 μM) and cytosine derivative **12** (EC_{50}: 1.0 μM). However, due to its marked lower toxicity, the cytosine derivative **12** proved more selective as an anti-HIV-1 agent than the corresponding thymine and uracil derivatives (selectivity indexes: ≥360, 227, and 73, respectively).

Introduction of a methyl group at C-5 of the cytosine ring (**11**) enhanced the antiviral activity by 6-fold but also markedly increased the toxicity; thus the selectivity index of compound **11** was 246. Also, the 5-ethyluracil derivative **8c** showed an antiviral activity comparable to that of the parent thymine derivative **8a**, but was slightly more cytotoxic than **8a**. Interestingly, introduction of an alkyl moiety at 3-N of the thymine ring (as in compounds **13** and **14**) did not alter the antiviral potency of the parent compound, but markedly decreased the cytotoxicity. Consequently, the selectivity index went up to 4088 and 1000, respectively. Substitution of an alkenyl function at 3-N of the thymine ring [as in 3-*N*-allylthymine **15** and 3-*N*-(dimethylallyl)thymine **16**] decreased the toxicity of the parent compound but also weakened the antiviral activity by 4- to 7-fold. Surprisingly, the 3-*N*-allyluracil derivative

(42) Rosenthal, A.; Cliff, B. L. Synthesis of an Analog of the Nucleoside Moiety of Polyoxins. *Carbohydr. Res.* **1980**, *79*, 63–77.

(43) Townsend, L. B. Nuclear Magnetic Resonance Spectroscopy in the Study of Nucleic Acid Components and Certain Related Derivatives. In *Synthetic Procedures in Nucleic Acid Chemistry*; Lorbach, W. W., Tipson, R. S., Eds.; Wiley-Interscience: New York–London, 1973; Vol. 2, pp 267–398.

(44) (a) Van Aerschot, A.; Everaert, D.; Balzarini, J.; Augustyns, K.; Jie, L.; Janssen, G.; Peeters, O.; Blaton, N.; De Ranter, C.; De Clercq, E.; Herdewijn, P. Synthesis and Anti-HIV Evaluation of 2′,3′-Dideoxyribo-5-chloropyrimidine Analogues: Reduced Toxicity of 5-Chlorinated 2′,3-Dideoxynucleosides. *J. Med. Chem.* **1990**, *33*, 1833–1839. (b) Divakar, K. J.; Reese, C. B. 4-(1,2,4-Triazol-1-yl)- and 4-(3-nitro-1,2,4-triazol-1-yl)-1-(β-D-2,3,5-tri-*O*-acetylarabinofuranosyl)pyrimidin-2(1*H*)-ones. Valuable Intermediates in the Synthesis of Derivatives of 1-(β-D-Arabinofuranosyl)cytosine (Ara-C). *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171–1176.

(45) Sasaki, T.; Minamoto, K.; Suzuki, H. Elimination Reactions on the Di- and Trimesylated Derivatives of N-3-Benzyluridine. *J. Org. Chem.* **1973**, *38*, 598–607.

(46) Jones, A. J.; Grant, D. M.; Winkley, M. W.; Robins, R. K. Carbon-13 Magnetic Resonance. XVII. Pyrimidine and Purine Nucleosides. *J. Am. Chem. Soc.* **1970**, *92*, 4079–4087.

(47) Yamamoto, I.; Kimura, T.; Tateoka, Y.; Watanabe, K.; Ho, I. K. N-Substituted oxopyrimidines and Nucleosides: Structure–Activity Relationship for Hypnotic Activity as Central Nervous System Depresant. *J. Med. Chem.* **1987**, *30*, 2227–2231.

(48) Cook, A. F.; Moffat, J. G. Carbodiimide–Sulfoxide Reactions. VI. Syntheses of 2′- and 3′-Ketouridines. *J. Am. Chem. Soc.* **1967**, *89*, 2697–2705.

17 was slightly less active and more cytotoxic than its unsubstituted counterpart **8b**.

While markedly active against HIV-1, the silylated 3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) derivatives had no activity against HIV-2 (ROD) or HIV-2 (EHO) or simian immunodeficiency virus (SIV) (data not shown). They represent the first example of nucleoside analogues with an intact ribose moiety that discriminate between HIV-1 and other retroviruses. In this respect, the novel nucleoside analogues resemble the non-nucleoside analogues HEPT,[49-51] TIBO,[52,53] BI-RG-587 (nevirapin),[54,55] L-697,639,[56] and BHAP.[57] As noted for these compounds,[58-62] our data also indicate that the 2′,5′-silylated 3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) derivatives are inhibitory to HIV-1 reverse transcriptase but not HIV-2 reverse transcriptase.[63,64]

In conclusion, nucleoside analogues containing a silyl group at both C-2′ and C-5′, and a spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) group at C-3′, proved to be potent and selective anti-HIV-1 agents that should be further pursued for their therapeutic potential in the treatment of HIV-1 infections.

Experimental Section

Chemical Procedures. Melting points were measured with a Reichert-Junt Kofler micro hot stage apparatus and are uncorrected. Microanalyses were obtained with a Heareus 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 90, 300, and 200 MHz, and ^{13}C NMR spectra with a Bruker WP-80-SY, a Bruker AM-200, and a Varian XL-300 spectrometer operating at 20, 50, and 75 MHz, with Me_4Si as internal standard. IR spectra were recorded with a Shimadzu IR-435 spectrometer. Analytical TLC was performed on silica gel 60 F_{254} (Merck). Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck).

Proximities were established conventionally on the basis of using NOE effects.

5-*O*-Benzoyl-3-*C*-cyano-1,2-*O*-isopropylidene-3-*O*-mesyl-α-D-ribofuranose (3). A mixture of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranos-3-ulose (1)[33] (1.3 g, 4.5 mmol), water (13 mL), ethyl ether (26 mL), sodium hydrogen carbonate (0.85 g, 10.2 mmol), and sodium cyanide (0.85 g, 10.2 mmol) was stirred vigorously at room temperature for 4 h. Ethyl ether (50 mL) was added, the organic phase was separated, and the aqueous phase was washed with ethyl ether (2 × 30 mL). The combined ethereal phases were dried over Na_2SO_4, filtered, and evaporated to dryness. The residue (the cyanohydrin 2), was dissolved in dry pyridine (10 mL). To this solution was added mesyl chloride (1.9 mL, 25.5 mmol). The mixture was stirred at 8–10 °C for 16 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

(49) Baba, M.; Tanaka, H.; De Clercq, E.; Pauwels, R.; Balzarini, J.; Schols, D.; Nakashima, H.; Perno, C. F.; Walker, R. T.; Miyasaka, T. Highly Specific Inhibition of Human Immunodeficiency Virus Type 1 by a Novel 6-Substituted Acyclouridine Derivative. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1375–1381.

(50) Baba, M.; De Clercq, E.; Iida, S.; Tanaka, H.; Nitta, I.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Umezu, K.; Nakashima, H.; Shigeta, S.; Walker, R. T.; Miyasaka, T. Anti-human Immunodeficiency Virus Type 1 Activities and Pharmacokinetics of Novel 6-Substituted Acyclouridine Derivatives. *Antimicrob. Agents Chemother.* **1990**, *34*, 2358–2363.

(51) Tanaka, H.; Baba, M.; Hayakawa, H.; Sakamaki, T.; Miyasaka, T.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R. T.; Balzarini, J.; De Clercq, E. A New Class of HIV-1-Specific 6-Substituted Acyclouridine Derivatives: Synthesis and Anti-HIV-1 Activity of 5- or 6-Substituted Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT). *J. Med. Chem.* **1991**, *34*, 349–357.

(52) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Potent and Selective Inhibition of HIV-1 Replication in Vitro by a Novel Series of TIBO Derivatives. *Nature* **1990**, *343*, 470–474.

(53) Kukla, M. J.; Breslin, H. J.; Pauwels, R.; Fedde, C. L.; Miranda, M.; Scott, M. K.; Sherrill, R. G.; Raeymaekers, A.; Van Gelder, J.; Andries, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one (TIBO) Derivatives. *J. Med. Chem.* **1991**, *34*, 746–751.

(54) Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C. K.; Eckner, K.; Hattox, S.; Adams, J.; Rosenthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, R. A.; Sullivan, J. L. Inhibition of HIV-1 Replication by a Nonnucleoside Reverse Transcriptase Inhibitor. *Science* **1990**, *250*, 1411–1413.

(55) Koup, R. A.; Merluzzi, V. J.; Hargrave, K. D.; Adams, J.; Grozinger, K.; Eckner, R. J.; Sullivan, J. L. Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) Replication by the Dipyridodiazepinone BI-RG-587. *J. Infect. Dis* **1991**, *163*, 966–970.

(56) Goldman, M. E.; Nunberg, J. H.; O'Brien, J. A.; Quintero, J. C.; Schleif, W. A.; Freund, K. F.; Gaul, S. L.; Saari, W. S.; Wai, J. S.; Hoffman, J. M.; Anderson, P. S.; Hupe, D. J.; Emini, E. A.; Stern, A. M. Pyridinone Derivatives: Specific Human Immunodeficiency Virus Type 1 Reverse Transcriptase Inhibitors with Antiviral Activity. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6863–6867.

(57) Romero, D. L.; Busso, M.; Tan, C. K.; Reusser, F.; Palmer, J. R.; Poppe, S. M.; Aristoff, P. A.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G. Nonnucleoside Reverse Transcriptase Inhibitors that Potently and Specifically Block Human Immunodeficiency Virus Type-1 Replication. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8806–8810.

(58) Baba, M.; De Clercq, E.; Tanaka, H.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Umezu, K.; Nakashima, H.; Mori, S.; Shigeta, S.; Walker, R. T.; Miyasaka, T. Potent and Selective Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) by 5-Ethyl-6-phenylthiouracil Derivatives through Their Inaction with the HIV-1 Reverse Transcriptase. *Proc. Natl Acad. Sci. U.S.A.* **1991**, *88*, 2356–2360.

(59) Cohen, K. A.; Hopkins, J.; Ingraham, R. H.; Pargellis, C.; Wu, J. C.; Palladino, D. E. H.; Kinkade, P.; Warren, T. C.; Rogers, S.; Adams, J.; Farina, P. R.; Grob, P. M. Characterization of the Binding Site for Nevirapine (BI-RG-587), a Nonnucleoside Inhibitor of Human Immunodeficiency Virus Type-1 Reverse Transcriptase. *J. Biol. Chem.* **1991**, *266*, 14670–14674.

(60) Frank, K. B.; Noll, G. J.; Connell, E. V.; Sim, I. S. Kinetic Interaction of Human Immunodeficiency Virus Type 1 Reverse Transcriptase with the Antiviral Tetrahydroimidazo-[4,5,1-*jk*][1,4]-benzodiazepine-2(1*H*)-thione Compound, R. 82150. *J. Biol. Chem.* **1991**, *266*, 14232–14236.

(61) Debyser, Z.; Pauwels, R.; Andries, K.; Desmyter, J.; Kukla, M.; Janssen, P. A. J.; De Clercq, E. An Antiviral Target on Reverse Transcriptase of Human Immunodeficiency Virus Type 1 Revealed by Tetrahydroimidazo-[4,5,1-*jk*][1,4]-benzodiazepin-2-(1*H*)-one and -thione Derivatives. *Proc. Natl. Acad. Sci. U. S.A.* **1991**, *88*, 1451–1455.

(62) White, E. L.; Burkheit, R. W., Jr.; Ross, L. J.; Germany, J. M.; Andries, K.; Pauwels, R.; Janssen, P. A. J.; Shannon, W. M.; Chirigos, M. A. A TIBO derivtive, R 82913, is a Potent Inhibitor for HIV-1 Reverse Transcriptase with Heteropolymer Templates. *Antiviral Res.* **1991**, *16*, 257–266.

(63) Balzarini, J.; Pérez-Pérez, M. J.; San-Félix, A.; Camarasa, M. J.; Bathurst, I. C.; Barr, P. J.; De Clercq, E. Kinetics of Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) Reverse Transcriptase by the Novel HIV-1 Specific Nucleoside Analogue 2′,5′-Bis-*O*-(*tert*-Butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″.2″-dioxide) thymine (TSAO-T). *J. Biol. Chem.*, in press.

(64) Balzarini, J.; Pérez-Pérez, M. J.; San-Félix, A.; Velázquez, S.; Camarasa, M. J.; De Clercq, E. 2′,5′-bis-*O*-(*tert*-Butyldimethylsilyl)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″.2″-dioxide) (TSAO) Derivatives of Purine and Pyrimidine Nucleosides as Potent and Selective Inhibitors of Human Immunodeficiency Virus Type. *Antimicrob. Agents Chemother.* **1992**, *36*, 1073–1080.

mL), and brine (50 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexane/ethyl acetate (3:1) as the eluent, to give 1.4 g (78%) of **3** as a white solid: mp 102–103 °C (ethanol); $[\alpha]_D$ 39° (c 1, chloroform); IR (KBr) 1725 cm^{-1} (C=O), 1370, 1185 (SO_2); ^1H NMR ($CDCl_3$, 90 MHz) δ 1.40, 1.60 (2 s, 6 H, $MeSO_2$), 4.44–4.83 (m, 3 H, H-4, 2H-5), 5.18 (d, 1 H, H-2, $J_{1,2}$ = 4 Hz), 6.05 (d, 1 H, H-1), 7.35–8.21 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$, 20 MHz) 26.22, 26.79 (Me_2C), 40.36 ($MeSO_2$), 61.52 (C-5), 76.96, 81.74 (C-2, C-4), 79.60 (C-3), 104.20 (C-1), 113.73, 114.84 (CN, Me_2C), 128.43, 129.16, 129.85, 133.40 (Ph), 165.74 (C=O). Anal. ($C_{17}H_{19}NO_8S$) C, H, N, S.

1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-*C*-cyano-3-*O*-mesyl-D-ribofuranose (4). A solution of cyano mesylate **3** (4 g, 10 mmol) in 20 mL of a (9:1) mixture of trifluoroacetic acid/water was stirred at room temperature for 4 h. The solvent was evaporated to dryness, and the residue was acetylated with acetic anhydride (15 mL) and pyridine (35 mL) at room temperature overnight. The solvents were evaporated under reduced pressure, and the residue was purified by column chromatography with hexane/ethyl acetate (2:1) to afford **4** (4.2 g, 95%) as a syrup. The NMR spectrum showed that it was a mixture (1.5:1) of the α and β anomers: IR (film) 1755, 1720 cm^{-1} (C=O), 1370, 1180 (SO_2); ^1H NMR ($CDCl_3$, 90 MHz) δ 2.10, 2.19 (2 s, 6 H, 2 OAc), 3.23 (s, 3 H, $MeSO_2$), 5.71 (d, 1 H, H-2α), 5.77 (d, 1 H, H-2β), 6.22 (d, 1 H, H-1β, $J_{1,2}$ = 1 Hz), 6.56 (d, 1 H, H-1α, $J_{1,2}$ = 5 Hz). Anal. ($C_{18}H_{19}NO_{10}S$) C, H, N.

General Procedure for the Synthesis of 2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl Nucleosides 5. The heterocyclic base (1.2 mmol) was silylated with hexamethyldisilazane (6 mL) under reflux in the presence of ammonium sulfate (10 mg), and the reaction was refluxed until the solution became clear. The excess of HMDS was removed by distillation under reduced pressure. A solution of compound **4** (1 mmol) in dry acetonitrile (8 mL) was added to the syrupy silylated base, followed by the addition of trimethylsilyl triflate (1.1 mmol). The resulting mixture was heated to reflux. After 2 h, an additional portion of trimethylsilyl triflate (1.1 mmol) was added and the refluxing continued for 3 h. The reaction was allowed to cool to room temperature, dichloromethane (50 mL) was added, and poured into cold, saturated, aqueous $NaHCO_3$. The organic phase was separated, the aqueous phase was washed with dichloromethane (2 × 20 mL) and dried (Na_2SO_4), and the solvent was removed. The residue was purified by column chromatography.

1-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)thymine (5a). Thymine (0.52 g, 4.08 mmol), the sugar derivative **4** (1.52 g, 3.4 mmol), and trimethylsilyl triflate (1.46 mL, 7.4 mmol) yielded after chromatography (hexane/ethyl acetate, 1:2) 1.34 g (77%) of **5a** as a white foam: IR (KBr) 1760 cm^{-1} (C=O), 1375, 1180 (SO_2); ^1H NMR ($CDCl_3$, 90 MHz) δ 1.74 (s, 3 H, CH_3-5), 2.21 (s, 3 H, OAc), 3.26 (s, 3 H, $MeSO_2$), 5.13–5.43 (m, 3 H, H-4′, H-5′), 5.85, 6.30 (2 d, 2 H, H-1′, H-2′, $J_{1',2'}$ = 7.0 Hz), 7.10 (s, 1 H, H-6), 7.40–8.25 (m, 5 H, Ph), 9.44 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2SO$, 50 MHz] δ 11.65 (CH_3-5), 19.95 (OAc), 40.34 ($MeSO_2$), 62.31 (C-5′), 73.44, 80.82, 84.64 (C-2′, C-4′, C-1′), 76.32 (C-3′), 110.71 (C-5), 113.02 (CN), 128.72, 129.20, 133.65, 135.52 (C-6, Ph), 150.24 (C-2), 163.18, 164.95, 168.87 (C-4, C=O). Anal. ($C_{21}H_{21}N_3O_{10}S$) C, H, N, S.

1-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)uracil (5b). Uracil (0.43 g, 3.6 mmol) and the sugar derivative **4** (1.32 g, 3 mmol) afforded, after chromatography (hexane/ethyl acetate, 2:3), the product **5b** (1.15 g, 78%) as a white foam: IR (KBr) 1760 cm^{-1} (C=O), 1375, 1180 (SO_2); ^1H NMR ($CDCl_3$, 200 MHz) δ 2.20 (s, 3 H, OAc), 3.23 (s, 3 H, $MeSO_2$), 4.70–4.92 (m, 3 H, H-4′, H-5′), 5.6 (d, 1 H, H-5), 5.74 (d, 1 H, H-1′, $J_{1',2'}$ = 5.6 Hz), 6.13 (d, 1 H, H-2′), 7.27 (d, 1 H, H-6), 7.43–8.07 (m, 5 H, Ph), 8.80 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2CO$, 20 MHz] δ 20.27 (OAc), 40.95 ($MeSO_2$), 63.34 (C-5′), 75.63 (C-2′), 78.19 (C-3′), 82.43, 87.65 (C-1′, C-4′), 104.19 (C-5), 114.07 (CN), 129.57, 130.45, 134.43, (Ph), 140.63 (C-6), 151.11 (C-2), 163.04 (C-4), 166.24, 169.67 (C=O). Anal. ($C_{20}H_{19}N_3O_{10}S$) C, H, N, S.

1-(2′-*O*-Acetyl-5′-*O*-benzoyl-3′-*C*-cyano-3′-*O*-mesyl-β-D-ribofuranosyl)-5-ethyluracil (5c). 5-Ethyluracil[39] (0.5 g, 3.6 mmol) and the sugar derivative **4** (1.32 g, 3 mmol) afforded, after chromatography (hexane/ethyl acetate, 1:1), compound **5c** (1.45 g, 93%) as a white foam: IR (KBr) 1750 cm^{-1} (C=O), 1375, 1185 (SO_2); ^1H NMR ($CDCl_3$, 90 MHz) δ 1.88 (t, 3 H, CH_3CH_2), 2.12 (q, 2 H, CH_3CH_2), 2.18 (s, 3 H, OAc), 3.25 (s, 3 H, $MeSO_2$), 4.40–5.10 (m, 3 H, H-4′, H-5′), 5.83 (d, 1 H, H-1′, $J_{1',2'}$ = 6.0 Hz), 6.18 (d, 1 H, H-2′), 7.08 (s, 1 H, H-6), 7.40–8.22 (m, 5 H, Ph), 9.14 (bs, 1 H, NH-3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.34 (CH_3CH_2), 20.03 (CH_3CH_2), 20.20 (OAc), 40.50 ($MeSO_2$), 61.86 (C-5′), 75.88 (C-2′), 77.47 (C-3′), 80.88, 86.03 (C-1′, C-4′), 113.23 (CN), 118.86 (C-5), 128.69, 128.84, 129.74, 132.84, 133.93 (C-6, Ph), 150.03 (C-2), 162.65 (C-4), 165.57, 168.72 (C=O). Anal. ($C_{22}H_{23}N_3O_{10}S$) C, H, N, S.

[1-(2′-*O*-Acetyl-5′-*O*-benzoyl-β-D-ribofuranosyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (6a). To a solution of the cyano mesylate **5a** (1.2 g, 2.4 mmol) in dry acetonitrile (12 mL) was added Cs_2CO_3 (0.78 g, 2.4 mmol). The mixture was stirred at room temperature for 3 h and then filtered. The filtrate was neutralized with acetic acid and then evaporated to dryness. The residue was purified by column chromatography with chloroform/acetone (3:1) as the eluent to give 0.78 g (65%) of **6a** as a white solid: mp 156–159 °C (chloroform); IR (KBr) 3400, 3330 cm^{-1} (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2SO$, 300 MHz] δ 1.82 (s, 3 H, CH_3-5), 2.02 (s, 3 H, OAc), 4.59–4.70 (m, 3 H, H-4′, H-5′), 5.74 (s, 1 H, H-3″), 5.77 (d, 1 H, H-1′, $J_{1',2'}$ = 8.4 Hz), 6.11 (d, 1 H, H-2′), 7.17 (bs, 2 H, NH_2), 7.49–7.99 (m, 6 H, H-6, Ph), 11.57 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 12.22 (CH_3-5), 20.22 (OAc), 62.89 (C-5′), 72.03, 81.95 (C-2, C-4′), 87.46 (C-3′), 89.31, 91.19 (C-1′, C-3″), 112.18 (C-5), 139.52 (C-6), 151.74, 153.93 (C-2, C-4″), 163.90 (C-4). Anal. ($C_{21}H_{21}N_3O_{10}S$) C, H, N.

[1-(β-D-Ribofuranosyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (7a).[28] The protected nucleoside **6a** (0.70 g, 1.23 mmol) was treated with saturated methanolic ammonia (15 mL). After standing at room temperature overnight, the solvent was evaporated to dryness. The residue was chromatographed with chloroform/methanol (10:1) as the eluent, to yield compound **7a**[28] (0.29 g, 66%) as a white solid, mp 168–170 °C dec (lit. mp 170 °C dec).

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (8a).[28] To a suspension of **7a** (0.20 g, 0.55 mmol) in dry acetonitrile (15 mL), 4-(dimethylamino)pyridine (0.34 g, 2.76 mmol) and *tert*-butyldimethylsilyl chloride (0.41 g, 2.76 mmol) was stirred at room temperature for 24 h. The solvent was evaporated to dryness and the residue, dissolved in ethyl acetate (50 mL), was washed with cold (4 °C) 1 N HCl (25 mL) water (50 mL), and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was chromatographed with chloroform/acetone (8:1) as the eluent to give **8a**[28] (0.24 g, 74%) as an amorphous solid.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]uracil]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (8b).[28] To a solution of the cyano mesylate **5b** (0.98 g, 2.0 mmol) in dry acetonitrile (16 mL) was added Cs_2CO_3 (0.65 g, 2.0 mmol). The mixture was stirred at room temperature for 6 h and filtered. The filtrate was neutralized with acetic acid, and finally, evaporated to dryness. The residue (spiro derivative **6b**) 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 **7b**) was filtered and suspended in dry acetonitrile (15 mL) and then 4-(dimethylamino)pyridine (0.53 g, 4.4 mmol) and *tert*-butyldimethylsilyl chloride (0.66 g, 4.4 mmol) were added. The mixture was stirred at room temperature for 48 h, and evaporated to dryness. The residue was treated with ethyl acetate (100 mL) and water (50 mL). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organics were successively washed with cold (4 °C) 1 N HCl (25 mL), water (50 mL), and brine (50 mL), and, finally, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was chromatographed with chloroform/acetone (8:1) as the eluent to give **8b**[28] (0.28 g, 24%) as an amorphous solid.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-ethyluracil]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (8c). A solution of cyano mesylate **5c** (1.0

g, 1.91 mmol) in dry acetonitrile (16 mL) was treated with Cs_2CO_3 (0.62 g, 1.91 mmol) for 4 h at room temperature. A similar treatment to that described for the synthesis of 8a afforded a residue that was chromatographed with chloroform/acetone (10:1) to give 0.37 g (32%) of 8c as an amorphous solid: IR (KBr) 3500, 3420 cm^{-1} (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2SO$, 200 MHz] δ 1.05 (t, 3 H, CH_3CH_2-5), 2.26 (m, 2 H, CH_3CH_2), 3.87 (m, 2 H, H-5', $J_{5'a,5'b}$ = 11.4, $J_{4',5'a}$ = 4.8, $J_{4',5'b}$ = 7.5 Hz), 4.19 (dd, 1 H, H-4'), 4.52 (d, 1 H, H-2', $J_{1',2'}$ = 8.4 Hz), 5.73 (s, 1 H, H-3''), 5.89 (d, 1 H, H-1'), 6.93 (bs, 2 H, NH_2), 7.56 (s, 1 H, H-6), 11.55 (bs, 1 H, NH-3); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 14.17 (CH_3CH_2), 21.20 (CH_3CH_2), 62.99 (C-5'), 74.99 (C-2'), 85.07, 88.66 (C-4', C-3''), 91.85 (C-3'), 91.98 (C-1'), 118.20 (C-5), 136.56 (C-6), 151.55, 152.28 (C-2, C-4''), 163.23 (C-4). Anal. ($C_{25}H_{45}N_3O_8SSi_2$) C, H, N.

[1-[5'-*O*-Benzoyl-2'-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (10a). To a solution of the 5'-deprotected nucleoside **9a**[28] (0.085 g, 0.18 mmol) in dry pyridine (3 mL) was added benzoyl chloride (0.023 mL, 0.26 mmol). The resulting mixture was stirred at room temperature overnight, poured into ice and water, and extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with 1 N HCl (50 mL), water (50 mL), and brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography with chloroform/acetone (7:1) to give 10a (0.083, 80%) as an amorphous solid: IR (KBr) 3420, 3340 cm^{-1} (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2CO$, 200 MHz] δ 1.82 (s, 3 H, CH_3-5), 4.68 (m, 3 H, H-4', H-5'), 5.08 (d, 1 H, H-2', $J_{1',2'}$ = 7.6 Hz), 5.77 (s, 1 H, H-3''), 5.91 (d, 1 H, H-1'), 6.57 (bs, 2 H, NH_2), 7.46–8.07 (m, 6 H, H-6, Ph), 10.40 (bs, 1 H, NH-3). Anal. ($C_{25}H_{33}N_3O_9SSi$) C, H, N.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-5-methylcytosine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (11). To a solution of 8a (0.15 g, 0.25 mmol) in dry pyridine (2 mL) was added a solution of 1,2,4-triazole (0.069 g, 1.01 mmol) and $POCl_3$ (0.05 mL, 0.5 mmol) in dry pyridine (5 mL). The resulting solution was stirred at room temperature for 4 h and then chilled on ice, and aqueous ammonia (2 mL) was added. After stirring at room temperature for 30 min, the solvent was evaporated to dryness. The residue was treated with 20 mL of a (1:1) mixture of chloroform/methanol. The solid was filtered, and the filtrate, after evaporating to dryness, was purified by column chromatography with chloroform/acetone (1:1) as the eluent to give 11 (0.10 g, 67%) as an amorphous solid: IR (KBr) 3350, 3190 cm^{-1} (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 2.05 (s, 3 H, CH_3-5), 4.02 (dd, 1 H, H-5'a, $J_{5'a,5'b}$ = 11.7, $J_{4',5'a}$ = 6.8 Hz), 4.09 (dd, 1 H, H-5'b, $J_{4',5'b}$ = 2.5 Hz), 4.14 (dd, 1 H, H-4'), 5.09 (d, 1 H, H-2', $J_{1',2'}$ = 6.6 Hz), 5.51 (d, 1 H, H-1'), 5.60 (s, 1 H, H-3''), 6.92 (bs, 2 H, NH_2-4''), 7.64 (s, 1 H, H-6), 8.22 (bs, 2 H, NH_2-4); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 13.19 (CH_3-5), 74.38 (C-2'), 84.57, 88.98, 95.19 (C-4', C-3'', C-1'), 89.58 (C-3'), 104.06 (C-5), 138.42 (C-6), 154.63, 156.63 (C-2, C-4''), 167.10 (C-4). Anal. ($C_{24}H_{44}N_4O_7SSi_2$) C, H, N.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]cytosine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (12). Compound 8b (0.18 g, 0.031 mmol) was reacted with 1,2,4-triazole (0.085 g, 1.24 mmol) and $POCl_3$ (0.06 mL, 0.62 mmol) for 4 h and then treated with aqueous ammonia, following a similar procedure to that described for the synthesis of compound 11. The residue was chromatographed with chloroform/ethyl acetate (1:2) to yield 0.15 g (84%) of 12 as an amorphous solid: IR (KBr) 3410, 3350, 3200 cm^{-1} (NH_2), 1650 (C=CN); ^1H NMR [$(CD_3)_2SO$, 300 MHz] 3.87 (m, 2 H, H-5', $J_{4',5'}$ = 5.3 Hz), 4.15 (t, 1 H, H-4'), 4.60 (d, 1 H, H-2', $J_{1',2'}$ = 7.8 Hz), 5.73 (s, 1 H, H-3''), 5.87 (d, 1 H, H-5), 5.90 (d, 1 H, H-1'), 6.96 (bs, 2 H, NH_2-4''), 7.42 (bs, 2 H, NH_2-4), 7.74 (d, 1 H, H-6). Anal. ($C_{23}H_{42}N_4O_7SSi_2$) C, H, N.

General Procedure for the Synthesis of (3-*N*-Alkylnucleosides)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) 13–17. To a solution of the spiro nucleoside 8 (1 mmol) in acetone (12 mL) were added K_2CO_3 (0.5 mmol) and the corresponding alkyl halide (1.1–2.0 mmol). The reaction mixture was refluxed for 3–8 h. After removal of the solvent, the residue was purified by column chromatography.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (13). Compound 8a (0.15 g, 0.25 mmol) and methyl iodide (0.07 mL, 0.5 mmol) reacted according to the general procedure for 4 h. The residue was chromatographed with hexane/ethyl acetate (3:1) to give 13 (0.083 g, 55%) as a white foam: IR (KBr) 3390 cm^{-1} (NH_2), 1715, 1675 (C=O), 1645 (C=CN); ^1H NMR [$(CD_3)_2CO$, 200 MHz] δ 1.95 (s, 3 H, CH_3-5), 3.26 (s, 3 H, *N*-CH_3), 4.09 (m, 2 H, H-5', J_{gem} = 12.2, $J_{4',5'}$ = 3.5 Hz), 4.34 (t, 1 H, H-4'), 4.66 (d, 1 H, H-2', $J_{1',2'}$ = 8.1 Hz), 5.76 (s, 1 H, H-3''), 6.08 (d, 1 H, H-1'), 6.45 (bs, 2 H, NH_2), 7.50 (s, 1 H, H-6); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 13.10 (CH_3-5, CH_3N), 63.14 (C-5'), 75.43 (C-2'), 85.18, 88.27 (C-4', C-3''), 92.47 (C-1', C-3'), 111.33 (C-5), 134.47 (C-6), 152.17, 152.24 (C-2, C-4''), 163.37 (C-4). Anal. ($C_{25}H_{45}N_3O_8SSi_2$) C, H, N.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-ethylthymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (14). Compound 8a (0.15 g, 0.25 mmol) and ethyl iodide (0.077 mL, 0.5 mmol) reacted according to the general procedure for 8 h. The residue was chromatographed with hexane/ethyl acetate (3:1) to yield 0.12 g (77%) of 14 as an amorphous solid: IR (KBr) 3400, 3320 cm^{-1} (NH_2), 1715, 1670 (C=O), 1645 (C=CN); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.15 (t, 3 H, *N*-CH_2CH_3, J = 7.05 Hz), 1.94 (s, 3 H, CH_3-5), 3.95 (q, 2 H, *N*-CH_2CH_3), 4.11 (m, 2 H, H-5', J_{gem} = 12.2, $J_{4',5'a}$ = 3.6, $J_{4',5'b}$ = 3.5 Hz), 4.34 (dd, 1 H, H-4'), 4.66 (d, 1 H, H-2', $J_{1',2'}$ = 8.15 Hz), 5.77 (s, 1 H, H-3''), 6.10 (d, 1 H, H-1'), 6.48 (bs, 2 H, NH_2), 7.49 (s, 1 H, H-6); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 13.00 (CH_3-5, CH_3CH_2N), 36.98 (CH_3CH_2N), 63.14 (C-5'), 75.32 (C-2'), 85.14 (C-4'), 88.00 (C-3''), 92.52 (C-1', C-3'), 111.56 (C-5), 134.56 (C-6), 151.89, 152.20 (C-2, C-4''), 162.98 (C-4). Anal. ($C_{26}H_{47}N_3O_8SSi_2$) C, H, N.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-allylthymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (15). 8a (0.15 g, 0.25 mmol) was reacted with allyl bromide (0.023 mL, 0.27 mmol) according to the general procedure for 3 h. The residue was chromatographed with chloroform/acetone (10:1) to afford 15 (0.10 g, 65%) as an amorphous solid: IR (KBr) 3400, 3320 cm^{-1} (NH_2), 1710, 1670 (C=O), 1645 (C=CN); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.96 (s, 3 H, CH_3-5), 4.10 (m, 2 H, H-5', J_{gem} = 12.2, $J_{4',5'a}$ = 3.7, $J_{4',5'b}$ = 3.5 Hz), 4.35 (dd, 1 H, H-4'), 4.52 (d, 2 H, NCH_2), 4.70 (d, 1 H, H-2', $J_{1',2'}$ = 8.1 Hz), 5.12–5.25 (m, 2 H, CH_2=CH), 5.78 (s, 1 H, H-3''), 5.86 (m, 1 H, CH_2=CH), 6.10 (d, 1 H, H-1'), 6.49 (bs, 2 H, NH_2), 7.54 (s, 1 H, H-6); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 13.07 (CH_3-5), 43.88 (NCH_2), 63.14 (C-5'), 75.25 (C-2'), 85.17, 88.21 (C-4', C-3''), 92.50 (C-1', C-3'), 111.52 (C-5), 118.04 (CH_2=CH), 132.98 (CH_2=CH), 134.83 (C-6), 151.78, 152.22 (C-2, C-4''), 162.87 (C-4). Anal. ($C_{27}H_{47}N_3O_8SSi_2$) C, H, N.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(3-methyl-2-butenyl)thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (16). According to the general procedure, 8a (0.15 g, 0.25 mmol) and 4-bromo-2-methyl-2-butene (0.046 mL, 0.5 mmol) reacted for 2.5 h. The residue was chromatographed with chloroform/acetone (15:1) to give 16 (0.115 g, 70%) as a white solid: mp 194–195 °C (chloroform/hexane); IR (KBr) 3400, 3320 cm^{-1} (NH_2), 1710, 1675 (C=O), 1645 (C=CN); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 1.67, 1.79 [2 s, 6 H, $(CH_3)_2C$=C], 1.94 (s, 3 H, CH_3-5), 4.08 (m, 2 H, H-5', J_{gem} = 12.2, $J_{4',5'a}$ = 3.5, $J_{4',5'b}$ = 3.5 Hz), 4.34 (dd, 1 H, H-4'), 4.50 (d, 2 H, *N*-CH_2), 4.66 (d, 1 H, H-2', $J_{1',2'}$ = 8.1 Hz), 5.20 (m, 1 H, C=$CHCH_2$), 5.77 (s, 1 H, H-3''), 6.10 (d, 1 H, H-1'), 6.48 (bs, 2 H, NH_2), 7.49 (s, 1 H, H-6); ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ 39.90 (*N*-CH_2), 63.14 (C-5'), 75.34 (C-2'), 85.14, 88.07 (C-4', C-3''), 92.54 (C-1', C-3'), 111.55 (C-5), 119.06 (C=CH), 134.60 (C-6), 137.16 [$(CH_3)_2C$=CH], 151.84, 152.21 (C-2, C-4''), 162.98 (C-4). Anal. ($C_{29}H_{51}N_3O_8SSi_2$) C, H, N.

[1-[2',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-allyluracil]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole 2'',2''-dioxide) (17). According to the general procedure, 8b (0.15 g, 0.26 mmol) reacted with allyl bromide (0.045 mL, 0.5 mmol), for 6 h. The residue was chromatographed with chloroform/acetone (15:1) to give 17 (0.143 g, 89%) as an amorphous solid: IR (KBr) 3400, 3330 cm^{-1} (NH_2), 1720, 1675 (C=O), 1645 (C=CN); ^1H NMR [$(CD_3)_2CO$, 300 MHz] δ 4.07 (m, 2 H, H-5', J_{gem} = 12.4 Hz), 4.37 (dd, 1 H, H-4', $J_{4',5'a}$ = 2.7, $J_{4',5'b}$ = 3.2 Hz), 4.48 (m, 2 H, N-CH_2), 4.59 (d, 1 H, H-2', $J_{1',2'}$ = 8.1 Hz), 5.16 (m, 2 H, CH_2=CH), 5.80 (s, 1 H, H-3''), 5.83 (m, 1 H, CH_2=CH), 5.94

(d, 1 H, H-5), 6.15 (d, 1 H, H-1'), 6.45 (bs, 2 H, NH_2), 7.79 (s, 1 H, H-6). Anal. ($C_{26}H_{45}N_3O_5SSi_2$) C, H, N.

Antiretrovirus Assays. HIV-1 was originally obtained from the culture supernatant of the persistently HIV-infected H9 cell line (H9/HTLV-III_B),[65] which was kindly provided by R. C. Gallo and M. Popovic (National Institutes of Health, Bethesda, MD). Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells.

MT-4 cells are human T-lymphocyte cells transformed by HTLV-1 and highly susceptible to the cytopathic effect of HIV. The methodology of the anti-HIV assays has been described previously.[17,22] Briefly, MT-4 cells (5×10^5 cells/mL) were suspended in fresh culture medium and infected with HIV-1 at 100 times the 50% cell culture infective dose ($CCID_{50}$) per milliliter of cell suspension. Then 100 μL of infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of test compounds. After 5 days, the number of viable cells for both virus-infected and mock-infected cell cultures was determined in a blood-cell-counting chamber by trypan blue staining. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) 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.

(65) Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. G. Detection, Isolation, and Continuous Production of Cytopatic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS. *Science* 1984, *224*, 497–500.

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Template-Directed Design of a DNA–DNA Cross-Linker Based upon a Bis-Tomaymycin–Duplex Adduct

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A template-directed approach to the design of a DNA–DNA interstrand cross-linker based upon the structure of a bis-tomaymycin–duplex adduct has been carried out. Tomaymycin is a member of the pyrrolo[1,4]benzodiazepines antitumor antibiotics. In a previous study (F. L. Boyd et al., *Biochemistry* 1990, *29*, 2387–2403), we have shown that two tomaymycin molecules can be covalently bound to a 12-mer duplex molecule, where the drug molecules are on opposite strands six base-pairs apart, and the stereochemistry at the drug bonding site, and orientation in the minor groove, was defined by high-field NMR. This bis-tomaymycin 12-mer duplex adduct maintains the self-complementarity of the duplex and a B-type structure. In the present study we have shown using high-field NMR that this same 12-mer sequence can be truncated by two base pairs so that the two tomaymycin-modified guanines are now only four base-pairs apart, the two species of tomaymycin molecules are still bound with the same stereochemistry and orientation, and the 10-mer duplex adduct maintains its self-complementarity. In a second 10-mer duplex we have shown that changing the bonding sequence from 5'CGA to 5'AGC does not significantly affect the structure of the bis-tomaymycin–duplex adduct. However, when the sequence is rearranged so that the drugs point in a tail-to-tail orientation rather than in the previous head-to-head configuration, there are more than one species of tomaymycin bound to DNA, and, as a consequence, the bis-tomaymycin 10-mer duplex adduct loses its self-complementarity. Last, we have used the 10-mer duplex containing the 5'CGA sequence, in which the tomaymycin molecules are oriented head to head, to design an interstrand cross-linking species in which the two drug molecules are linked together with a flexible linker molecule.

Introduction

Anthramycin, tomaymycin (I), and sibiromycin are the best known examples of the naturally occurring pyrrolo-[1,4]benzodiazepines (P[1,4]B).[1-3] These antibiotics react covalently with DNA to form an N2-guanine adduct that lies within the minor groove of DNA (Figure 1).[4,5] The P[1,4]Bs are not only specific for N2 of guanine, but are only reactive with guanines in certain sequences, and therefore show sequence selectivity.[6,7] The most favored sequences for bonding are 5'PuGPu with 5'PyGPu and 5'PuGPy of intermediate reactivity (Pu = purine; Py = pyrimidine), while 5'PyGPy sequences show the least reactivity. In principle, there are four species of covalently bound adducts that can occur as the two 11*S* enantiomers, in which the aromatic ring of the drug lies either to the

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(1) Remers, W. A. In *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1988; Vol. 2, pp 28–92.
(2) Thurston, D. E.; Hurley, L. H. A Rational Basis for Development of Antitumor Agents in the Pyrrolo(1,4)benzodiazepine Group. *Drugs Future* 1984, *8*, 957–971.
(3) Remers, W. A.; Barkley, M. D.; Hurley, L. H. Pyrrolo(1,4)-benzodiazepines. Unraveling the Complexity of the Structures of the Tomaymycin-DNA Adducts in Various Sequences Using Fluorescence, ^{1}H-NMR, and Molecular Modeling. A chapter in *Nucleic Acid Targeted Drug Design*; Perun, T., Propst, C., Eds.; Marcel Dekker, Inc.: New York, 1992, in press.
(4) Hurley, L. H.; Petrusek, R. Proposed Structure of the Anthramycin-DNA Adduct. *Nature (London)* 1979, *282*, 529.

0022-2623/92/1835-2995$03.00/0