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Synthesis and anti-HIV-1 activity of novel TSAO-T derivatives modified at the 2′- and 5′-positions of the sugar moiety

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Abstract

Novel analogues of the anti-HIV-1 agent TSAO-T, [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and its 3-methyl counterpart TSAO-m^{3}T were obtained by modifications at positions 2′ or 5′ of the sugar moiety. These compounds were evaluated for their inhibitory effect on HIV-1 and HIV-2 replication in cell culture. Introduction of new groups at the 5′-position (i.e. esters, benzylether and silylethers) resulted in compounds that were either inactive or less active than the parent compounds (TSAO-T and TSAO-m^{3}T). Attempts to introduce small silyl ether groups at this position were not successful since these products decomposed during purification. Similar modifications at the 2′-position had a much less pronounced influence on the anti-HIV-1 activity.

Keywords: HIV; HIV-1-specific RT inhibitor; TSAO-T; 3′-Spironucleoside; Reverse transcriptase inhibitor; AIDS

1. Introduction

The TSAO nucleoside analogues, their prototype being [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-di-

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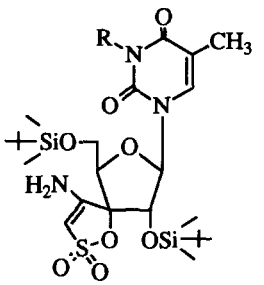


1a R=H (TSAO-T)
1b R=Me (TSAO-m^{3}T)

Fig. 1. Structures of TSAO-T (**1a**) and TSAO-m^{3}T (**1b**).

oxide) [1] (designated as TSAO-T, **1a**) (Fig. 1), are potent and highly specific inhibitors of the human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT) (Balzarini et al., 1992a–c; Camarasa et al., 1992, 1994; Pérez-Pérez et al., 1992a,b; Alvarez et al., 1994; San-Félix et al., 1994). Like the non-nucleoside HIV-1 RT inhibitors, i.e. HEPT, TIBO, nevirapine, pyridinone, BHAP, α-APA, PETT and quinoxaline, TSAO-T interacts with the enzyme at a non-substrate binding site (Baba et al., 1989; Miyasaka et al., 1989; Merluzzi et al., 1990; Pauwels et al., 1990, 1993; Goldman et al., 1991; Koup et al., 1991; Romero et al., 1991; Balzarini et al., 1992b,c; Camarasa et al., 1992, 1994; Kleim et al., 1993). The 4″-amino group of TSAO appears to interact with a glutamic acid residue at position 138 (Glu-138) of the p51 subunit of the RT (Balzarini et al., 1993a,b, 1994; Boyer et al., 1994; Jonckheere et al., 1994) that is located close to both the polymerase (pol) active site and the non-nucleoside binding pocket (Jacobo-Molina et al., 1993; Nanni et al., 1994; Smerdon et al., 1994). The activity of the TSAO derivatives depends upon several factors, of which the structural requirements with respect to the furanose ring are of greatest importance (Balzarini et al., 1992a–c; Camarasa et al., 1992, 1994; Pérez-Pérez et al., 1992a,b; Alvarez et al., 1994; San-Félix et al., 1994). Indeed, extensive SAR studies on the nucleobase of TSAO have been recently performed. The nucleobase may be replaced by either purines, pyrimidines or 1,2,3-triazoles without marked loss of antiretroviral activity (Balzarini et al., 1992a,b; Velázquez et al., 1993; Alvarez et al., 1994; San-Félix et al., 1994). Moreover, methylation of N-3 of thymine, as in TSAO-m^{3}T **1b** (Fig. 1), drastically reduces cytotoxicity and hence improves the selectivity index (SI = 227 and 4088 for **1a** and **1b**, respectively) (Balzarini et al., 1992a). However, previous structure–activity relationship (SAR) investigations have shown that only TSAO derivatives having the spiro moiety in nucleosides with a *ribo* configuration possess antiviral activity (Balzarini et al., 1992b).

Also, removal of one, or both, of the silyl protecting groups at the 5′- or 2′-positions that may occur during metabolism in the host, results in a complete loss of antiviral

[1] 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 accepted for TSAO derivatives in previous papers of this series.

activity (Camarasa et al., 1992; Balzarini et al., 1993c). Similarly the C-2′ deoxy derivative was found to be inactive (Camarasa et al., 1992). Thus, the protecting groups at the 2′-OH and 5′-OH of the ribose moiety may be of importance, either, because of their lipophilic nature, or due to their influence on the conformation of the furanose ring (Camarasa et al., unpublished results).

In this paper we report on the synthesis and anti-HIV activity of novel TSAO-T and TSAO-m^3T analogues in which the silyl ether protecting groups were replaced by other groups possessing different lipophilic and steric properties in order to assess the role of such substitutions in the antiviral activity/toxicity profile of the TSAO series.

2. Materials and methods

2.1. Synthesis

2.1.1. General methods

Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. ^{1}H-NMR spectra were recorded with a Varian Gemini spectrometer operating at 200 MHz, and ^{13}C-NMR spectra with a Varian Gemini spectrometer operating at 50 MHz, with Me_4Si as internal standard. Analytical TLC was performed on silica gel 60 F_{254} (Merck). Separations on silica gel were performed by preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron^R (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).

2.1.2. *[1-[2′,5′-Bis-O-(*tert*-butyldiphenylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**3a**)

A solution of **2a** (Camarasa et al., 1992) (71 mg, 0.20 mmol), 4-dimethylaminopyridine (DMAP) (126 mg, 1.03 mmol) and *tert*-butyldiphenylsilyl chloride (260 μl, 1.0 mmol) in acetonitrile (6 ml) was refluxed for 48 h. The solvent was removed, under reduced pressure, and the residue was purified by flash column chromatography (10:1, chloroform:methanol) to yield the required compound **3a** (36 mg, 22%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.00, 1.05 (2s, 18H, 2 tBu), 1.03 (d, 3H, CH_3-5, J = 1.2 Hz), 3.94 (dd, 1H, H-5′a, J_{gem} = −11.9, $J_{4',5'a}$ = 5.6 Hz), 4.13 (dd, 1H, H-5′b, $J_{4',5'b}$ = 3.6 Hz), 4.44 (q, 1H, H-4′), 5.00 (d, 1H, H-2′, $J_{1',2'}$ = 7.6 Hz), 5.07 (s, 1H, H-3″), 5.90 (d, 1H, H-1′), 6.38 (bs, 2H, NH_2-4″), 6.74 (d, 1H, H-6), 7.31–7.54, 7.60–7.69 (2m, 22H, 2OH, 4Ph). Anal. calcd. for C_{44} H_{51} N_3 O_8 Si_2 S: C, 63.05; H, 6.13; N, 5.01. Found: C, 63.40; H, 6.26; N, 4.89.

2.1.3. *[1-[2′-O-(*tert*-Butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**4b**)

A solution of **1b** (Pérez-Pérez et al., 1992b) (1.85 g, 3.06 mmol) in 0.1 N methanolic HCl was stirred at room temperature for 30 min. The mixture was neutralized with 0.1 N methanolic NaOH solution. The solvent was removed and the residue was purified by flash column chromatography (10:1, chloroform:methanol) to yield **4b** (1.54 g, 97%)

as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ −0.12, 0.06 (2s, 6H, 2 CH_3–Si), 0.81 (s, 9H, *t*-Bu), 1.89 (d, 3H, CH_3-5, J = 1.2 Hz), 3.26 (s, 3H, CH_3–N), 3.80–4.06 (m, 2H, 2H-5′), 4.36 (m, 1H, H-4′), 4.92 (d, 1H, H-2′, $J_{1',2'}$ = 8.1 Hz), 5.75 (s, 1H, H-3″), 5.84 (bs, 1H, OH), 5.94 (d, 1H, H-1′), 6.64 (bs, 2H, NH_2), 7.92 (d, 1H, H-6). Anal. calcd. for C_{19} H_{31} N_3 O_8 Si S: C, 46.61; H, 6.38; N, 8.58. Found: C, 46.65; H, 6.39; N, 8.70.

*2.1.4. [1-[5′-O-(*tert*-Butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**5b**)

A solution of **2a** (Camarasa et al., 1992) (1.40 g, 3.87 mmol) and *tert*-butyldimethylsilyl chloride (1.44 g, 9.49 mmol) in pyridine (50 ml) was stirred at room temperature for 2 days. The solution was evaporated and the residue was co-evaporated 3 times with ethanol. Purification by flash column chromatography (20 : 1, chloroform : methanol) yielded **5a** (Camarasa et al., 1992) (1.32 g, 73.2%). Methylation of the product (methyl iodide/K_2CO_3/acetone) afforded **5b** (1.12 g, 82%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.10, 0.16 (2s, 6H, $2CH_3$–Si), 0.94 (s, 9H, *t*-Bu), 1.91 (d, 3H, CH_3-5, J = 1.2 Hz), 3.26 (s, 3H, CH_3–N), 4.03 (m, 2H, 2H-5′), 4.29 (t, 1H, H-4′, $J_{4',5a'} = J_{4',5b'}$ = 3.6 Hz), 4.79 (m, 1H, H-2′), 5.22 (bd, 1H, OH), 5.71 (s, 1H, H-3″), 6.02 (d, 1H, H-1′, $J_{1',2'}$ = 8.4 Hz), 6.44 (bs, 2H, NH_2), 7.56 (d, 1H, H-6). Anal. calcd. for C_{19} H_{31} N_3 O_8 Si S: C, 46.62; H, 6.38; N, 8.58. Found: C, 46.71; H, 6.43; N, 8.59.

*2.1.5. General procedure for the synthesis of 5′-O-silylether-protected [1-[2′-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**6b–9b**)

To a solution of **4b** (0.07–0.14 mmol) in dry pyridine (4 ml) was added alkyl(aryl)silyl halide (0.38–0.45 mmol). The solution was stirred at room temperature for 48 h, the solvent was removed in vacuo and the residues were co-evaporated with ethanol (2 × 2 ml) and purified by CCTLC on the chromatotron.

*2.1.6. [1-[2′-O-(*tert*-Butyldimethylsilyl)-5′-O-(*tert*-butyldiphenylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**6b**)

According to the general procedure, **4b** (54 mg, 0.11 mmol) yielded, after chromatography (3 : 1, hexane : ethyl acetate), 53 mg (65%) of **6b** as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ −0.10, 0.09 (2s, 6H, $2CH_3$–Si), 0.87, 1.09 (2s, 18H, 2 *t*-Bu), 1.48 (s, 3H, CH_3-5), 3.24 (s, 3H, CH_3–N), 4.12 (dd, 1H, H-5a′, J_{gem} = −13 Hz, $J_{4',5a'}$ = 4.5 Hz), 4.25 (t, 1H, H-5′b), 4.49 (t, 1H, H-4′), 4.76 (d, 1H, H-2′, $J_{1',2'}$ = 8.1 Hz), 5.77 (s, 1H, H-3″), 6.10 (d, 1H, H-1′), 6.54 (bs, 2H, NH_2), 7.4–7.7 (m, 11H, H-6, 2 Ph–Si). Anal. calcd. for C_{35} H_{49} N_3 O_8 Si_2 S: C, 57.74; H, 6.78; N, 5.77. Found: C, 57.58; H, 6.83; N, 5.73.

*2.1.7. [1-[2′-O-(*tert*-Butyldimethylsilyl)-5′-O-(*tert*-hexyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**7b**)

Following the general procedure, compound **4b** (44 mg, 0.09 mmol) was silylated with *tert*-hexyldimethylsilyl chloride (88.5 μl, 0.45 mmol), to give, after purification (3 : 1, hexane : ethyl acetate), 39 mg (67%) of **7b**, as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.02, 0.18, 0.31, 0.34 (4s, 12H, 4 CH_3–Si), 0.95–1.0 (m, 24H, *t*-Bu, *t*-hexyl), 2.04 (s,

3H, CH_3-5), 3.36 (s, 3H, CH_3–N), 4.20 (m, 2H, 2H-5′), 4.42 (t, 1H, H-4′, $J_{4',5a'} = J_{4',5b'}$ = 3.8 Hz), 4.83 (d, 1H, H-2′, $J_{1',2'}$ = 8.0 Hz), 5.83 (s, 1H, H-3″), 6.13 (d, 1H, H-1′), 6.61 (bs, 2H, NH_2), 7.65 (s, 1H, H-6). ^{13}C-NMR [$(CD_3)_2CO$]: δ −5.15, −4.33, −3.14, −2.86 (4 CH_3–Si), 13.04 (CH_3 − 5), 18.43, 20.67 (*t*-Bu), 34.79 (CH_3–N), 62.90 (C-5′), 75.09 (C-2′), 85.36 (C-4′), 89.13 (C-1′), 91.74, 91.94 (C-3, C-3′), 111.3 (C-6), 135.1 (C-5), 152.1, 152.6 (C-2, C-4″), 163.4 (C-4). Anal. calcd. for C_{28} H_{52} N_3 O_8 Si_2 S: C, 51.98; H, 8.10; N, 6.49. Found: C, 52.07; H, 8.12; N, 6.46.

*2.1.8. [1-[2′-O-(*tert*-Butyldimethylsilyl)-5′-O-(tri-*tert*-butylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**8b**)*

Silylation of **4b** (37 mg, 0.07 mmol) with tri-*tert*-butylsilyl chloride (102 μl, 0.38 mmol) yielded, after purification by CCTLC (2 : 1, hexane : ethyl acetate) **8b** (41 mg, 83%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ −0.10, 0.06 (2s, 6H, 2 CH_3–Si), 0.80–0.99 (m, 36H, 4 *t*-Bu), 2.04 (s, 3H, CH_3-5), 3.26 (s, 3H, CH_3–N), 4.20 (dd, 1H, H-5a′, J_{gem} = −10.8 Hz, $J_{4',5'a}$ = 4.2 Hz), 4.20 (dd, 1H, H-5b′, $J_{4',5b'}$ = 3.3 Hz), 4.36 (dd, 1H, H-4′), 4.67 (d, 1H, H-2′, $J_{1',2'}$ = 7.9 Hz), 5.75 (s, 1H, H-3″), 6.02 (d, 1H, H-1′), 6.45 (bs, 2H, NH_2), 7.54 (s, 1H, H-6). Anal. calcd. for C_{31} H_{57} N_3 O_8 Si_2S: C, 54.11; H, 8.35; N, 6.10. Found: C, 54.21; H, 8.21; N, 5.89.

*2.1.9. [1-[2′-O-(*tert*-Butyldimethylsilyl)-5′-O-(*tert*-butylmethoxyphenylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**9b**)*

Compound **4b** (71 mg, 0.14 mmol) and *tert*-butyl-methoxy-phenylsilyl bromide (99 μl, 0.44 mmol) were reacted, according to the general procedure, to give after CCTLC purification (2 : 1 hexane : ethyl acetate) 71 mg (72%) of **9b**. This was a mixture of two isomers, due to the chiral silicon at the 5′-oxygen. ^{1}H-NMR [$(CD_3)_2CO$] of isomeric mixture. *Major isomer*: δ −0.6, 0.13 (2s, 6H, $2CH_3Si$), 0.93 (s, 9H, *t*-Bu), 0.96 (s, 9H, *t*-Bu), 1.26 (s, 3H, CH_3-5), 3.20 (s, 3H, CH_3–N), 3.59 (s, 3H, CH_3–O), 4.30–4.50 (m, 3H, 2H-5, H-4′), 4.67 (d, 1H, H-2′, $J_{1',2'}$ = 8.3 Hz), 5.86 (s, 1H, H-3″), 6.17 (d, 1H, H-1′), 6.57 (bs, 2H, NH_2), 7.37–7.75 (m, 6H, H-6, Ph). *Minor isomer*: δ −0.08, 0.08 (2s, 6H, 2 CH_3Si), 0.93, 0.96 (2 s, 18H, 2 *t*-Bu), 1.26 (s, 3H, CH_3-5), 3.26 (s, 3H, CH_3–N), 3.66 (s, 3H, CH_3–O), 4.30–4.50 (m, 3H, H-4′, 2H-5′), 4.73 (d, 1H, H-2′, $J_{1',2'}$ = 8.0 Hz), 5.79 (s, 1H, H-3″), 6.10 (d, 1H, H-1′), 6.54 (bs, 2H, NH_2), 7.37–7.75 (m, 6H, H-6, Ph). Anal. calcd. for C_{30} H_{47} N_3 O_9 Si_2 S: C, 52.84; H, 6.95; N, 6.16. Found: C, 52.88; H, 6.99; N, 6.18.

*2.1.10. [1-[2′-O-(*tert*-Butyldimethylsilyl)-5′-O-pivaloyl-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**11a**)*

Compound **4a** (44 mg, 0.09 mmol) was stirred with pivaloyl chloride (37 μl, 0.30 mmol) in dry pyridine (3 ml) at room temperature for 24 h. The mixture was poured over ice/water (10 ml) and extracted with ethyl acetate (3 × 30 ml). The combined organic extracts were washed twice with 1.0 N aqueous HCl (2 × 10 ml), once with saturated aqueous NaCl solution (30 ml), dried (Na_2SO_4) and evaporated. The residue yielded, upon chromatography, (8 : 1, chloroform : acetone) compound **11a** (35 mg, 68%), as a white solid (m.p. 142–143°C; hexane/ethyl ether). ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.00, 0.11 (2s, 6H, 2 CH_3–Si), 0.85 (s, 9H, *t*-BuSi), 1.17 (s, 9H, *t*-BuCO), 1.85 (s, 3H,

CH_3-5), 4.37–4.49 (m, 3H, H-4′, 2H-5′), 5.05 (d, 1H, H-2′, $J_{1',2'}$ = 7.7 Hz), 5.75 (s, 1H, H-3″), 5.86 (d, 1H, H-1′), 6.53 (bs, 2H, NH_2), 7.58 (s, 1H, H-6), 10.32 (bs, 1H, NH-3). Anal. calcd. for C_{23} H_{37} N_3 O_9 Si S: C, 49.36; H, 6.66; N, 7.51. Found: C, 49.26; H, 6.71; N, 7.47.

*2.1.11. [1-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-tosyl-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**12b**)

To a solution of **4b** (42 mg, 0.09 mmol) in pyridine (3 ml) was added *p*-toluenesulphonyl chloride (39 mg, 0.2 mmol). The mixture was stirred at room temperature for 24 h. The solvent was removed and the residue purified by CCTLC (3 : 1, hexane : ethyl acetate) to yield **12b** (26 mg, 47%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ −0.07, −0.03 (2 s, 6H, $2CH_3$–Si), 0.77 (s, 9H, *t*-BuSi), 1.88 (s, 3H, CH_3-5), 2.41 (s, 3H, CH_3–Ph), 3.23 (s, 3H, CH_3–N), 4.41 (s, 3H, 2H-5′, H-4′), 4.98 (d, 1H, H-2′, $J_{1',2'}$ = 7.2 Hz), 5.72 (s, 1H, H-3″), 5.80 (d, 1H, H-1′), 6.47 (bs, 2H, NH_2), 7.45, 7.79 (2d, 4H, Ph), 7.59 (s, 1H, H-5). Anal. calcd. for C_{25} H_{35} N_3 O_{10} Si S_2: C, 47.67; H, 5.60; N, 6.67. Found: C, 47.62; H, 5.61; N, 6.60.

*2.1.12. [1-[5′-*O*-Benzyl-2′-*O*-(tert-butyldimethylsilyl)-β-D-ribofuranosyl)-3-methylthymine]-3′-spiro-5″-(3″-*C*-benzyl-4″-*N*-benzylamino-1″,2″-oxathiole-2″,2″-dioxide)* (**13b**)

To a solution of **4b** (160 mg, 0.33 mmol) in dry THF (10 ml) was added freshly powdered KOH (150 mg) and benzyl bromide (200 μl, 1.67 mmol). The mixture was stirred at room temperature for 4 h, the solution was diluted with dichloromethane (45 ml) and was washed with water. The dried solvent (Na_2SO_4) was evaporated and the residue was purified by flash column chromatography (50 : 1 chloroform : methanol) to yield **13b** (157 mg, 62%) as a white foam. ^{1}H-NMR ($CDCl_3$): δ −0.18, −0.03 (2 s, 6H, $2CH_3$–Si), 0.84 (s, 9H, *t*-Bu) 1.40 (d, 1H, CH_3-5, J = 1.0 Hz), 3.25 (s, 3H, CH_3–N), 3.76 (AB system, 2H, 2H-5′), 3.91 (s, 2H, Ph–CH_2-3″), 4.20 (AB system, 2H, $PhCH_2O$, J_{gem} = −11.9 Hz), 4.30 (dq, 2H, $PhCH_2NH$, J_{gem} = −13 Hz, J_{NH,CH_2} = 4.5 Hz), 4.46 (t, 1H, H-4′), 4.56 (d, 1H, H-2′), 5.96 (t, 1H, NH), 6.07 (d, 1H, H-1′, $J_{1',2'}$ = 7.8 Hz) 6.87–7.36 (m, 16H, 3 Ph, H-6). ^{13}C-NMR [$(CD_3)_2CO$]: δ −4.8, −4.2 (2 CH_3Si), 12.7 (*t*-Bu), 28.66 (CH_3–N), 29.3 (Ph–CH_2–C-3″), 49.3 ($PhCH_2NH$), 69.82 (C-5′), 73.8 ($PhCH_2O$), 75.8 (C-2′), 83.9 (C-4′), 87.9 (C-1′), 92.8 (C-3′), 102.0 (C-3″), 111.4 (C-5), 134.5 (C-6), 137.4, 138.6, 140.1 (3 × quaternaries − aromatics), 140.5 (C-4″), 152.3 (C-2), 163.4 (C-4). Anal. calcd. for C_{40} H_{49} N_3 O_8 Si S: C, 63.22; H, 6.50; N, 5.53. Found: C, 63.34; H, 6.49; N, 5.52.

*2.1.13. General procedure for the synthesis of 2′-*O*-silylether-protected [1-[5′-*O*-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) analogues (**14b–16b**)*

To a solution of **5b** (0.09–0.12 mmol) and DMAP (0.86–1.08 mmol) in dry acetonitrile (10 ml) was added a substituted silyl chloride (0.45–0.58 mmol). The mixture was refluxed for 24 h. The solvent was removed, then, ethyl acetate (10 ml) was added and the mixture was filtered. The filtrate was evaporated and the residues were purified by CCTLC on the chromatotron (2 : 1, hexane : ethyl acetate).

*2.1.14. [1-[2′-O-(*tert*-Butyldiphenylsilyl)-5′-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* **(14b)**

From **5b** (57 mg, 0.12 mmol), diphenyl-*tert*-butylsilylchloride (120 μl, 0.46 mmol) and DMAP (114 mg, 0.928 mmol) compound **14b** (27 mg, 32%) was obtained as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.26, 0.23 (2s, 6H, $2CH_3$–Si), 0.87, 1.00 (2s, 18H, 2 *t*-Bu), 1.58 (s, 3H, CH_3-5), 3.08 (s, 3H, CH_3–N), 3.92–4.05 (dd, 2H, 2H-5′), 4.28 (t, 1H, H-4′), 4.64 (d, 1H, H-2′, $J_{1',2'} = 8.1$ Hz), 5.86 (s, 1H, H-3″), 6.15 (d, 1H, H-1′) 6.38 (bs, 2H, NH_2), 6.66 (s, 1H, H-6), 7.40–7.70 (m, 10H, 2Ph–Si). Anal. calcd. for C_{35} H_{49} N_3 O_8 Si_2S: C, 57.74; H, 6.78; N, 5.77. Found: C, 57.80; H, 6.81; N, 5.83.

*2.1.15. [1-[2′-O-(*tert*-Hexyldimethylsilyl)-5′-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* **(15b)**

According to the general procedure, compound **5b** (45 mg, 0.09 mmol) was reacted with *tert*-hexyldimethylsilyl chloride (106 μl, 0.58 mmol), in the presence of DMAP (132 mg, 1.08 mmol) to afford, after chromatography (2 : 1, hexane : ethyl acetate), compound **15b** (33 mg, 54%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ −0.03, 0.05, 0.13, 0.19 (4s, 12H, $4CH_3$–Si), 0.37–0.96 (m, 24H, *t*-hexyl and *t*-Bu), 1.93 (s, 3H, CH_3-5), 3.26 (s, 3H, CH_3–N), 4.05 (m, 2H, 2H-5′, $J_{gem} = -10.8$ Hz, $J_{4',5a'} = J_{4',5b'} = 3.8$ Hz), 4.32 (t, 1H, H-4′), 4.69 (d, 1H, H-2′, $J_{1',2'} = 8.1$ Hz), 5.76 (s, 1H, H-3″), 6.07 (d, 1H, H-1′) 6.49 (bs, 2H, NH_2), 7.50 (s, 1H, H-6). ^{13}C-NMR [$(CD_3)_2CO$]: δ 13.11 (CH_3–5), 18.64, 18.95, 18.99, 19.64, 20.05, 25.53, 26.36 (*t*-Bu and *t*-hexyl), 34.57 (CH_3–N), 63.12 (C-5′), 75.12 (C-2′), 85.10 (C-4′), 88.18 (C-3′), 92.28 (C-1′, C-3″), 111.30 (C-5), 134.5 (C-6), 152.3 (C-4″). Anal. calcd. for C_{28} H_{52} N_3 O_8 Si_2 S: C, 51.98; H, 8.10; N, 6.49. Found: C, 51.87; H, 8.14; N, 6.48.

*2.1.16. [1-[2′-O-(tri-isopropylsilyl)-5′-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* **(16b)**

Following the general procedure, **5b** (54 mg, 0.113 mmol) and tri-isopropylsilyl chloride (120 μl, 0.45 mmol), in the presence of DMAP (106 mg, 0.86 mmol), gave, after purification (2 : 1, hexane : ethyl acetate), compound **16b** (19.5 mg, 29%), and unreacted **5b** (27 mg). ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.14, 0.18 (2 s, 6H, $2CH_3$–Si), 0.94 (m, 30H, *t*-Bu, 3 *i*-propyl), 1.92 (s, 3H, CH_3-5), 3.26 (s, 3H, CH_3–N), 4.05 (ABq, 2H, 2H-5′), 4.24 (q, 1H, H-4′, $J_{4',5a'} = J_{4',5b'} = 3.0$ Hz), 5.02 (d, 1H, H-2′, $J_{1',2'} = 7.5$ Hz), 5.72 (s, 1H, H-3″), 5.88 (d, 1H, H-1′) 6.51 (bs, 2H, NH_2), 7.56 (s, 1H, H-6). Anal. calcd. for C_{28} H_{51} N_3 O_8 Si_2 S: C, 52.06; H, 7.96; N, 6.50. Found: C, 52.14; H, 8.02; N, 6.46.

*2.1.17. [1-[2′-O-Benzoyl-5′-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* **(17b)**

Compound **5b** (59 mg, 0.12 mmol) was stirred with benzoyl chloride (22 μl, 0.19 mmol) in dry pyridine (5 ml) at room temperature for 4 h. Evaporation of the solvent and chromatographic purification by CCTLC (2 : 1, hexane : ethyl acetate) gave **17b** (58 mg, 82%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.16, 0.18 (2s, 6H, $2CH_3$–Si), 0.92 (s, 9H, *t*-Bu), 2.00 (s, 3H, CH_3-5), 3.16 (s, 3H, CH_3–N), 4.13 (m, 2H, 2H-5′), 4.41 (dd,

1H, H-4′, $J_{4',5a'} = J_{4',5b'} = 3.9$ Hz), 5.71 (s, 1H, H-3″), 6.09 (d, 1H, H-2′, $J_{1',2'} = 7.7$ Hz), 6.24 (d, 1H, H-1′), 6.61 (bs, 2H, NH_2), 7.46, 7.62, 8.04 (m, 6H, H-6, Ph). Anal. calcd. for C_{26} H_{35} N_3 O_9 Si S: C, 52.58; H, 5.94; N, 7.08. Found: C, 52.60; H, 5.93; N, 7.11.

*2.1.18. [1-[2′-O-Benzoyl-5′-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**18a**)

To a solution of **5a** (49 mg, 0.10 mmol) in pyridine (5 ml) was added benzoyl chloride (20 μl, 0.19 mmol), the solution was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by CCTLC on the chromatotron (1 : 1, hexane : ethyl acetate) to give **18a** (47 mg, 81%) as a white solid (m.p. 126–128°C; hexane/ethyl ether). ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.16, 0.18 (2s, 6H, $2CH_3$–Si), 0.93 (s, 9H, *t*-Bu), 1.85 (s, 3H, CH_3-5), 4.13 (m, 2H, 2H-5′), 4.40 (t, 1H, H-4′, $J_{4',5a'} = J_{4',5b'} = 4.0$ Hz), 5.07 (s, 1H, H-3″), 6.07 (d, 1H, H-2′, $J_{1',2'} = 7.7$ Hz), 6.19 (d, 1H, H-1′), 6.59 (bs, 2H, NH_2), 7.46, 7.60, 8.03 (m, 6H, H-6, Ph), 10.24 (s, 1H, NH). Anal. calcd. for C_{25} H_{33} N_3 O_9 Si S: C, 51.80; H, 5.74; N, 7.25. Found: C, 51.90; H, 5.78; N, 7.20.

2.1.19. 5-O-Benzyl-1,2-O-isopropylidene-α-D-erythro-pentofuranos-3-ulose (**20**)

To a suspension of pyridinium dichromate (1.60 g, 4.23 mmol) in dichloromethane (30 ml) was added acetic anhydride (2.1 ml) and a solution of 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose **19** (Kuzuhara and Emoto, 1964) (1.70 g, 6.06 mmol) in dichloromethane (10 ml). After refluxing for 45 min, the solvent was removed, and the residues were taken up in ethyl acetate (30 ml) and filtered through a short (60 g) silica column, eluting with ethyl acetate. The eluate was concentrated and the residue was co-evaporated with toluene (3 × 25 ml), to give the product **20** (crude yield 1.50 g) which was used immediately without further purification.

2.1.20. 5-O-Benzyl-3-C-cyano-1,2-O-isopropylidene-3-O-mesyl-α-D-ribofuranose (**22**)

The crude ulose **20** (1.50 g), dissolved in diethyl ether (20 ml), was stirred at high speed with an aqueous solution of KCN (0.28 g, 5.68 mmol) and K_2CO_3 (0.91 g, 6.60 mmol) for 4 h. The two layers were separated and the aqueous phase was extracted with diethyl ether (3 × 20 ml). The combined extracts were dried (Na_2SO_4) and evaporated to give 1.12 g of a pale yellow syrup (the cyanohydrin **21**). This cyanohydrin was dissolved in pyridine (20 ml) and cooled to 0°C. Methane sulphonyl chloride (2 ml) was added dropwise, and the mixture was stirred at a temperature < 5°C overnight. The mixture was poured onto ice/water (10 ml) and extracted with chloroform (50 ml). The chloroform extracts were washed with 0.1 N HCl (2 × 10 ml), water (10 ml), dried (Na_2SO_4) and evaporated. CCTLC chromatography (3 : 1, hexane : ethyl acetate) yielded **22** (1.43 g, 69% from **19**) as a white solid. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.38, 1.55 (2s, 6H, $(CH_3)_2C$), 3.33 (s, 3H, CH_3SO_2), 3.72 (m, 2H, 2H-5), 4.65 (s, 3H, Ph–CH_2–O, H-4), 5.20 (d, 1H, H-2), 6.13 (d, 1H, H-1, $J_{1,2} = 3.7$ Hz). ^{13}C-NMR [$(CD_3)_2CO$]: δ 26.54, 26.62 [$(CH_3)_2C$], 40.4 (CH_3SO_2), 66.5 (C-5), 74.2 ($PhCH_2O$), 80.5 (C-2), 82.0 (C-3), 84.3 (C-4), 106.0 (C-1), 113.0 (CN), 114.5 [$(CH_3)_2C$], 128.3, 128.4, 129.1 (Ph). Anal. calcd. for C_{17} H_{21} N O_7 S: C, 53.25; H, 5.52; N, 3.65. Found: C, 53.31; H, 5.47; N, 3.67.

2.1.21. 1,2-Di-O-acetyl-5-O-benzyl-3-C-cyano-3-O-mesyl-D-ribofuranose **(23)**

Compound **22** (1.33 g, 3.47 mmol) in (9:1) trifluoroacetic acid:water (30 ml) was stirred at room temperature for 2 h. The solvent was removed and the residue was acetylated with acetic anhydride (2.85 ml) in pyridine (35 ml), at room temperature overnight. The solvents were removed in vacuo and the product was purified by flash column chromatography (3:1, hexane:ethyl acetate) to yield **23** (1.24 g, 84%) as a slightly yellow syrup consisting of a (1.75:1) mixture of the α- and β-anomers (the relative proportions of the $\alpha + \beta$-anomers was determined from the integrals of the anomeric protons). ^{1}H-NMR [$(CD_3)_2CO$]: δ 2.13, 2.17 (2s, 6H, 2OAc), 3.36 (s, 3H, CH_3SO_2), 3.82 (m, 2H, 2H-5), 4.60, 4.62 (2s, 2H, 2PhCH_2O ($\alpha + \beta$-anomers)), 4.79 (m, 1H, H-4), 5.83 (d, H-2α), 5.88 (s, H-2β), 6.14 (s, H-1β), 6.46 (d, H-1α, $J_{1\alpha,2\alpha} = 4.8$ Hz), 7.27–7.35 (m, 5H, Ph). ^{13}C-NMR [$(CD_3)_2CO$]: δ *α-anomer* 20.09, 20.59 (2OAc), 40.32 (CH_3SO_2) 67.15 (C-5), 74.1 (PhCH_2O), 76.9 (C-2), 80.74 (C-3), 83.2 (C-4), 93.8 (C-1), 113.5 (CN), 128.5, 129.2, 138.89 (Ph), 168.7, 169.2 (2C=O, 2OAc); *β-anomer* 20.46, 20.74 (2OAc), 40.48 (CH_3SO_2) 67.10 (C-5), 74.0 (PhCH_2O), 79.0 (C-4), 79.45 (C-3), 85.2 (C-2), 99.1 (C-1), 113.2 (CN), 128.5, 129.2, 138.89 (Ph), 169.0, 169.4 (2C=O, 2OAc). Anal. calcd. for C_{18} H_{21} N O_9 S: C, 50.58; H, 4.95; N, 3.28. Found: C, 50.55; H, 4.93; N, 3.26.

2.1.22. 1-(2′-O-Acetyl-5′-O-benzyl-3′-C-cyano-3′-O-mesyl-β-D-ribofuranosyl)thymine **(24a)**

A solution of thymine (0.62 g, 4.5 mmol) in hexamethyldisilazane (HMDS) (14 ml), in the presence of ammonium sulphate (10 mg), was refluxed overnight. The excess of HMDS was removed under reduced pressure and the residue was co-evaporated with toluene (2 × 10 ml). A solution of the sugar **23** (1.20 g, 2.80 mmol) in dry acetonitrile (40 ml) and trimethylsilyltriflate (0.6 ml) was added to the syrupy silylated thymine. The reaction mixture was refluxed for 2 h. An additional amount of trimethylsilyltriflate (0.5 ml) was added, and refluxing was continued for 7 h. After cooling, ethyl acetate (100 ml) and ice (10 g) were added and the resulting aqueous layer was neutralized ($NaHCO_3$). The mixture was filtered and the organic layer was washed with water (2 × 20 ml), dried (Na_2SO_4) and evaporated. Purification by flash column chromatography (first 3:1 and then 1:1, hexane:ethyl acetate) first gave unreacted sugar **23** (326 mg) and later compound **24a** (894 mg, 88.6% of reacted). ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.71 (s, 3H, CH_3-5), 2.19 (s, 3H, OAc), 3,45 (s, 3H, CH_3SO_2), 4.02 (m, 2H, 2H-5′), 4.71 (q, 2H, PhCH_2O), 4.83 (t, 1H, H-4′) 5.96 (d, 1H, H-2′, $J_{1',2'} = 3.9$ Hz), 6.16 (d, 1H, H-1′), 7.42 (m, 5H, Ph), 7.46 (s, 1H, H-6), 10.20 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.29 (CH_3-5), 20.34 (AcO), 40.39 (CH_3–SO_2), 66.84 (C-5′), 74.10 (Ph–CH_2–O), 78.57 (C-4′), 80.12 (C-3′), 83.10 (C-2′), 88.5 (C-1′), 112.1 (C-5), 113.0 (CN), 128.6, 129.2 (Ph), 135.5 (C-6), 139.0 (Ph), 151.0, 163.9, 168.8 (3 CO). Anal. calcd. for C_{21} H_{23} N_3 O_9 S: C, 51.11 H, 4.70; N, 8.51. Found: C, 51.07; H, 4.72; N, 8.44.

2.1.23. [1-[5′-O-Benzyl-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) **(27a)**

To a solution of **24a** (288 mg, 0.58 mmol) in dry acetonitrile (25 ml) was added Cs_2CO_3 (324 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for

3 h. The mixture was filtered, the filtrate was neutralized (acetic acid) and the solvent was evaporated. The residue was stirred with saturated methanolic ammonia solution (25 ml) overnight. The solvent was removed and the residue was purified by flash column chromatography (10 : 1, chloroform : methanol) to give **27a** (175 mg, 66.5%) as a white foam. ^{1}H-NMR [$(CD_3)_2SO$]: δ 0.87 (s, 3H, CH_3-5), 2.80 (m, 2H, H-4′, OH-2′), 3.41, 3.85 (m, 2H, 2H-5′), 3.64 (s, 2H, CH_2–Ph), 4.71 (s, 1H, H-3″), 4.99 (d, 1H, H-1′, $J_{1',2'} = 3.2$ Hz), 6.01 (bs, 3H, H-2′, NH_2), 6.40 (bs, 6H, Ph, H-6), 10.3 (bs, 1H, NH). Anal. calcd. for C_{19} H_{21} N_3 O_8 S: C, 50.55; H, 4.69; N, 9.31. Found: C, 50.70; H, 4.72; N, 9.33.

*2.1.24. [1-[5′-O-Benzyl-2′-O-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)* (**28a**)

Compound **27a** (40 mg, 0.09 mmol) in acetonitrile (10 ml) containing TBDMS chloride (45 mg, 0.3 mmol) and DMAP (73 mg, 0.6 mmol) was refluxed for 48 h. Standard work-up afforded, after purification by CCTLC (3 : 1, hexane : ethyl acetate) compound **28a** (27.5 mg, 55%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.06, 0.13 (2s, 6H, $2CH_3$–Si), 0.88 (s, 9H, *t*-Bu), 1.81 (s, 3H, CH_3-5), 3.82 (d, 2H, 2H-5′), 4.58 (s, 2H, Ph–CH_2–O), 4.60 (d, 1H, H-2′), 4.82 (t, 1H, H-4′, $J_{4',5a'} = J_{4',5b'} = 4.9$ Hz), 5.69 (s, 1H, H-3″), 6.11 (d, 1H, H-1′, $J_{1',2'} = 4.6$ Hz), 6.59 (bs, 2H, NH_2), 7.27–7.38 (m, 5-H, Ph), 7.49 (s, 1H, H-6), 10.2 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ −4.74, −5.11 ($2CH_3$–Si), 12.49 (CH_3-5), 18.59 (CH_3C–Si), 26.00 (tBu), 68.3 (C-5′), 73.75 (Ph–CH_2–O), 81.00 (C-2′), 84.00 (C-4′), 89.50 (C-3″), 89.90 (C-1′), 93.50 (C-3′), 112.30 (C-5), 128.30, 128.40, 129.00 (Ph), 135.80 (C-6), 139.10 (Ph), 151.40 (C-4), 153.40 (C-4″), 163.90 (C-2). Anal. calcd. for C_{25} H_{35} N_3 O_8 Si S: C, 53.08; H, 6.24; N, 7.43. Found: C, 53.04; H, 6.28; N, 7.33.

2.1.25. [1-[(2′-O-Benzoyl-5′-O-benzyl)-β-D-ribofuranosyl]thymine]-3′-spiro-5″-(4″-benzamido-1″,2″-oxathiole-2″,2″-dioxide) (**29a**)

To a solution of **27a** (25 mg, 0.055 mmol) and DMAP (25 mg, 0.166 mmol) in pyridine (2 ml) was added benzoyl chloride (30 μl, 0.25 mmol). The solution was stirred at room temperature overnight, the solvent was removed in vacuo. The residue was treated with ethyl acetate (2 ml) and was filtered through silica gel washing with further ethyl acetate. The solvent was removed and the remaining solid was purified by CCTLC (50 : 1, dichloromethane : methanol) to yield **29a** (22 mg, 60%) as a white solid (m.p. 129–130°C; hexane/ethyl ether). ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.90 (s, 3H, CH_3-5), 3.95 (d, 2H, 2H-5′), 4.56 (q, 2H, $PhCH_2O$), 5.36 (t, 1H, H-4′), 5.87 (d, 1H, H-2′, $J_{1',2'} = 4.6$ Hz), 6.50 (d, 1H, H-1′), 7.30–8.05 (m, 17H, 3 Ph, H-6, H-3″), 9.49 (s, 1H, NH-4″), 10.23 (s, 1H, NH-3). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.60 (CH_3-5), 67.11 (C-5′), 73.90 ($PhCH_2O$), 80.53 (C-2′), 83.26 (C-4′), 88.12 (C-1′), 92.58 (C-3′), 106.94 (C-3″), 112.50 (C-5), 128.20, 128.30, 128.60, 129.00, 129.30, 129.60, 130.80, 135.10, 135.30 (3 Ph), 133.6 (C-6), 138.90, 141.89 (Ph quaternaries), 151.30 (C-4″), 164.00, 165.00, 167.50 (C=O). Anal. calcd. for C_{33} H_{29} N_3 O_{10} S: C, 60.09; H, 4.43; N, 6.36. Found: C, 60.11; H, 4.48; N, 6.33.

2.1.26. [1-[(2'-O-Acetyl-5'-O-benzyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5''-(4''-acetamido-1'',2''-oxathiole-2'',2''-dioxide) (**30a**)

To a solution of **27a** (25 mg, 0.055 mmol) and DMAP (25 mg, 0.166 mmol) in pyridine (2 ml) was added acetic anhydride (60 μl). After stirring at room temperature for 24 h the solvent was removed and ethyl acetate was added. The mixture was passed through silica gel and the eluate was concentrated. Purification by CCTLC (40:1, dichloromethane:methanol) afforded **30a** (15 mg, 51%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.86 (s, 3H, CH_3-5), 2.16, 2.19 (2s, 6H, 2Ac), 3.85 (q, 2H, 2H-5'), 4.54 (q, 2H, $PhCH_2O$), 4.96 (t, 1H, H-4'), 5.47 (d, 1H, H-2', $J_{1',2'} = 3.8$ Hz), 6.14 (d, 1H, H-1'), 7.29 (bs, 5H, Ph), 7.45 (s, 1H, H-6), 7.57 (s, 1H, H-3''), 9.55 (bs, 1H, NHAc) [2], 10.23 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.55 (CH_3-5), 20.40 (AcO), 24.00 (AcNH), 67.06 (C-5''), 73.84 ($PhCH_2O$), 80.40 (C-2'), 82.20 (C-4'), 88.30 (C-1'), 92.60 (C-3''), 112.40 (C-5), 128.20, 128.30, 129.00 (Ph), 135.30 (C-6), 163.90, 169.00, 170.30 (C=O). Anal. calcd. for C_{23} H_{25} N_3 O_{10} S: C, 51.59; H, 4.71; N, 7.85. Found: C, 51.64; H, 4.72; N, 7.82.

2.2. Biological methods

2.2.1. Cells and viruses

CEM cells were obtained from the American Type Culture Collection (Rockville, MD). MT-4 cells were a kind gift from Dr. N. Yamamoto (Yamaguchi University, Yamaguchi, Japan). HIV-1(III_B) was provided by Dr. R.C. Gallo and Dr. M. Popovic (National Institutes of Health, Bethesda, MD) and HIV-2(ROD) was obtained from Dr. L. Montagnier (Pasteur Institute, Paris, France).

2.2.2. Antiviral assays

The anti-HIV-1 and -HIV-2 activity of the test compounds was examined in CEM cells at day 4 and in MT-4 cells at day 5 postinfection. Determination of antiviral activity was based on giant cell formation in CEM cells and cell viability (trypan blue dye staining) in MT-4 cells. HIV-1 and HIV-2 were added at 100 $CCID_{50}$ to the cell culture. Briefly, CEM and MT-4 cells were suspended at 250,000 cells per ml of RPMI 1640 culture medium and infected with HIV-1(III_B) or HIV-2(ROD). Then, 100 μl of the infected cell suspension was added to 200-μl microtiter plate wells containing 100 μl of an appropriate dilution of the test compounds. After 4 days (CEM cells) or 5 days (MT-4 cells) of incubation at 37°C, the cell cultures were examined for syncytium formation or cell viability, respectively. The 50% effective concentration (EC_{50}) was determined as the compound concentration required to inhibit syncytium formation by 50% in HIV-infected CEM and MT-4 cells, respectively. The 50% cytotoxic concentration (CC_{50}) was determined as the compound concentration required to reduce the cell viability of mock-infected MT-4 cells by 50%.

[2] These chemical shifts may be compared with those for the α- and β-anomers of (1,2,5-triacetyl-D-ribofuranose)-3-spiro-5-(4'-acetamido-1',2'-oxathiole-2',2'-dioxide) which are, respectively, 7.45 and 7.40 for the H-3'' protons and 8.30 and 8.40 for the NH protons (see Pérez-Pérez et al., 1991).

3. Results and discussion

3.1. Chemical results

Compound **3a** was produced by the silylation of **2a** (Camarasa et al., 1992) with *tert*-butyldiphenylsilyl chloride and 4-(dimethylamino)pyridine (DMAP) in refluxing acetonitrile (CH_3CN). The TSAO-T derivatives protected as silylethers (distinct from the *tert*-butyldimethylsilyl (TBDMS) group as in the parent compound) at either, the 5′- or 2′-positions (**6b–9b**) and (**14b–16b**), respectively, were prepared from the partially protected key intermediates **4b** and **5b** (Fig. 2). The intermediate **4b** was prepared, in 97% yield, by deprotection of the 5′-*O*-TBDMS group of compound **1b** (Pérez-Pérez et al., 1992a) with 0.1 N methanolic HCl (Wetter and Oertle, 1985). Reaction of **2a** with TBDMS chloride in pyridine gave **5a** (73.2%) (Camarasa et al., 1992), which after N-3 methylation, with CH_3I and K_2CO_3 in acetone, yielded the key intermediate **5b** in 82% yield.

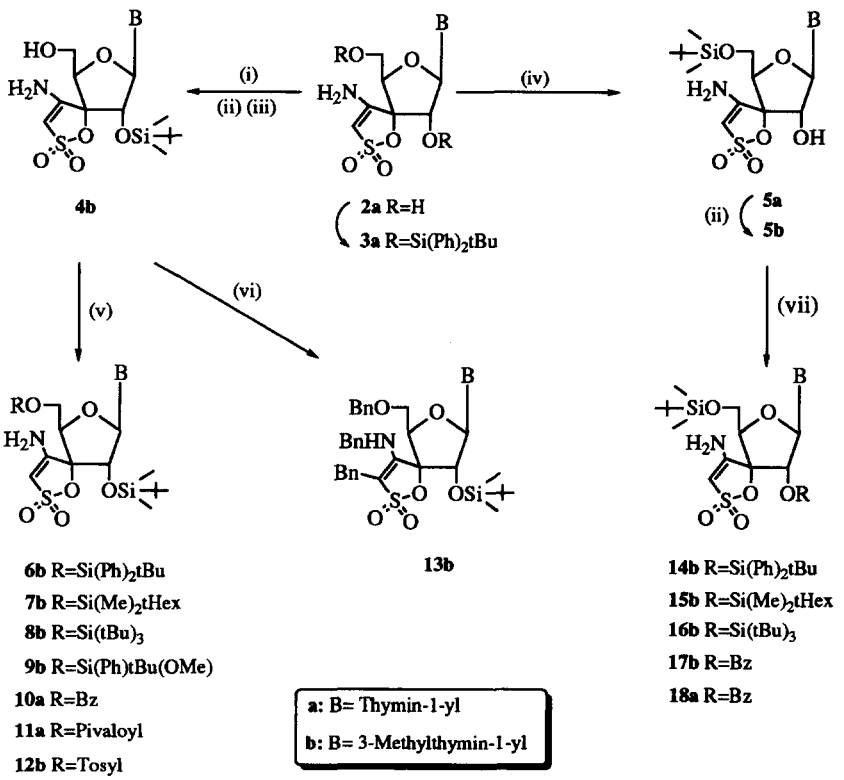
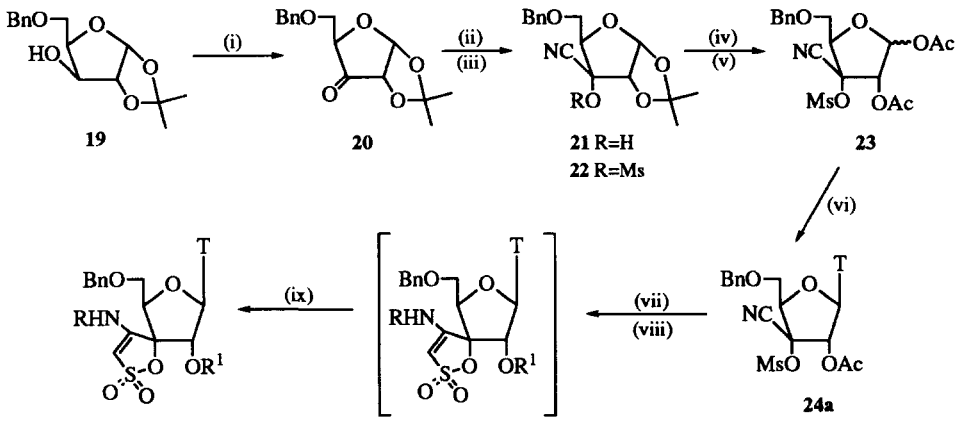


Fig. 2. Synthesis of spironucleosides (**2–18**).



19 20 21 R=H 22 R=Ms 23 24a

28a R=H; R1=Si(Me)2tBu
29a R=R1=Bz
30a R=R1=Ac

25a R=H; R1=Ac
26a R=Ac; R1=H
27a R=R1=H

(i) Pyridinium dichromate/Ac_2O; (ii) NaCN/$NaHCO_3$; (iii) MsCl/Py; (iv) 9:1, TFA/H_2O; (v) Ac_2O/Py; (vi) Thymine/TMStriflate; (vii) Cs_2CO_3/CH_3CN; (viii) NH_3/MeOH; (ix) Protection

Fig. 3. Synthesis of spironucleosides (**25–30**).

The 5′-*O*-silyl ether protected compounds (**6b–9b**) were prepared, in good yields (65–83%), by reaction of **4b** with silyl halides in pyridine. (Cunico and Bedell, 1980; Wetter and Oertle, 1985; Green and Wuts, 1991). Attempts to produce compounds with silyl ether protecting groups at 5′-position smaller than TBDMS (i.e. dimethylallylsilyl or trimethylsilyl) were unfruitful, due to the instability of the final products which decomposed during purification.

The DMAP/CH_3CN procedure was utilized to obtain the 2′-*O*-silyl ether protected compounds (**14b–16b**) from **5b** and silyl halides, the yields being rather low (29–54%) and the reaction times long.

The 5′-*O*- or 2′-*O*-ester protected derivatives (**10a** (Pérez-Pérez et al., 1992a), **11a** or **17b**, **18a**) and the 5′-*O*-tosyl protected compound **12b** were readily synthesized by treatment of **4b** or **5b** with acyl chlorides in pyridine. Attempts to produce the 2′-*O*-tosyl and 2′-*O*-pivaloyl compounds in pyridine or CH_3CN/DMAP were not successful.

The synthesis, via nucleophilic substitution, of compounds with 5′-*O*-ether groups using **4b** and alkyl/aryl halides was envisaged. Treatment of **4b**, with excess of benzyl bromide in dry THF and in the presence of KOH (Bessodes et al., 1988) led to the tribenzyl derivative **13b** (62% yield). Attempts to obtain the 5′-*O*-benzyl derivative using equimolar, or indeed sub-equimolar, amounts of benzyl bromide were not successful. Furthermore, prolonged exposure of **4b** to these basic conditions resulted in its decomposition.

Since only traces of the monobenzyl substituted compound **28a** (Fig. 3) were ever detected (by TLC), using direct alkylation of **4b**, an alternative synthetic route was devised beginning with 5-*O*-benzyl-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose **19** (Levene and Raymond, 1933) (Fig. 3).

Oxidation of **19** with pyridinium dichromate/acetic anhydride in dichloromethane (Hollemberg et al., 1987) gave the ulose **20**. This was treated with sodium cyanide, in a diethyl ether/water two phase system, in the presence of $NaHCO_3$, to yield the kinetically controlled *ribo*-cyanohydrin **21** (Calvo-Mateo et al., 1988; Pérez-Pérez et al., 1991) which was converted to the cyanomesylate **22** without isolation.

The 1,2-isopropylidene protecting group was cleaved by hydrolysis in (9:1) TFA:H_2O solution, subsequent acetylation (acetic anhydride/pyridine) converted the intermediate, 1,2-diols, to a (1.75:1) mixture of the α and β anomers of the 1,2-diacetate **23**. Glycosylation of **23** with persilylated thymine in refluxing acetonitrile with trimethylsilyltriflate catalysis (Vorbrüggen et al., 1981) gave the required nucleoside **24a** in 88% yield. The spirosultone moiety in **25a** was formed upon treatment of **24** with Cs_2CO_3 in acetonitrile (Calvo-Mateo et al., 1988; Pérez-Pérez et al., 1991). When DBU was used as a base, migration of the 2′-*O*-acetate group to give **26a** was observed (Calvo-Mateo et al., 1988). Deprotection of **25a** or **26a**, in methanolic ammonia solution, yielded compound **27a** (66.5% yield) which upon silylation with TBDMS chloride in CH_3CN with DMAP as an acid sink gave **28a** in 55% yield.

In order that comparisons of structure/activity could be made between 5′-*O*-benzyl derivatives and the 5′-*O*-TBDMS-2′-*O*-acyl derivatives (**17b** and **18a**), compounds protected as 5′-*O*-benzyl-2′-*O*-acyl were of interest. Treatment of **27a** with either benzoyl chloride or acetic-anhydride in pyridine did not give the expected compounds, and hence the addition of DMAP was investigated. This procedure resulted in the synthesis of the disubstituted ester derivatives **29a** and **30a** in 60 and 51% yield, respectively.

Structures of all new compounds were determined from their analytical and spectroscopic data.

3.2. Biological results

A series of TSAO-T and TSAO-m^3T analogues that contain other substituents than the *tert*-butyldimethylsilyl (TBDMS) groups at either the 2′-, 5′- or both 2′- and 5′-position were evaluated for anti-HIV-1 and anti-HIV-2 activity in human lymphocyte MT-4 and CEM cells (Table 1). When the TBDMS group at both 2′- and 5′-positions was replaced by an aromatic *tert*-butyldiphenylsilyl group (compound **3a**), antiviral activity was completely lost. Compound **3a** was also devoid of cytotoxic activity at 150 μM. When *tert*-butyldimethylsilyl was substituted only at the 5′-position of TSAO (**6b**), again antiviral activity was virtually lost, and, in addition, the compound proved more toxic than its parent compound TSAO-m^3T (**1b**). Also, the bulky 5′-tri-*tert*-butylsilyl substituted TSAO-m^3T derivative (**8b**) was devoid of antiviral activity and proved more toxic to the host cells than **1b**. Interestingly, the 5′-*tert*-hexyldimethylsilyl derivative **7b** was inhibitory to HIV-1 replication at a 50% effective concentration (EC_{50}) of 0.80–2.0 μM, that is at a 15- to 50-fold higher concentration than for the parent compound TSAO-m^3T. Compound **9b**, containing *tert*-butylphenylmethoxysilyl at the 5′-position, proved highly toxic to the cells and not antivirally active at subtoxic concentrations. The 5′-pivaloyl and 5′-tosyl derivatives of TSAO-T (**11a**) and TSAO-m^3T (**12b**) were virtually devoid of antiviral activity, whereas the 5′-benzyl substituted TSAO-m^3T

Table 1
Anti-HIV activity of TSAO derivatives in CEM and MT-4 cells

Compound	CEM EC_{50} [a,c] (μM)		MT-4 EC_{50} [a,c] (μM)		MT-4 CC_{50} [b,c] (μM)
	HIV-1	HIV-2	HIV-1	HIV-2	
3a	> 150	> 150	> 150	> 150	> 150
5a [d]	> 40	> 40	> 40	> 40	95
6b	> 60	> 60	18 ± 5.9	> 30	45 ± 22
7b	0.80 ± 0.43	> 60	2.0 ± 0.16	> 6	29 ± 23
8b	> 12	> 12	> 30	> 30	50 ± 19
9b	3.1 ± 2.5	> 6.6	3.4 ± 1.02	> 6.6	4.8 ± 3.3
10a [e]	–	–	> 15	> 15	36
11a	> 35	> 35	13 ± 6.4	> 35	85 ± 14
12b	> 6.2	> 6.2	24 ± 9.7	> 30	42 ± 41
13b	51 ± 36	> 130	5.2 ± 2.9	> 130	> 130
14b	0.99 ± 0.32	> 30	0.39 ± 0.19	> 150	133 ± 15
15b	0.11 ± 0.02	> 30	0.10 ± 0.006	> 30	62 ± 2.5
16b	0.45 ± 0.0	> 30	0.21 ± 0.02	> 150	77 ± 16
17b	0.50 ± 0.0	> 6.7	0.17 ± 0.05	> 6.7	15 ± 0.5
17a	0.27 ± 0.0	> 6.9	0.13 ± 0.01	> 6.9	14 ± 0.20
28a	> 6	> 6	> 6	> 6	13 ± 0.45
29a	⩾ 40	> 40	> 40	> 40	89 ± 2.3
30a	> 160	> 160	> 160	> 160	> 160
TSAO-T (**1a**)	0.06 ± 0.01	> 20	0.06 ± 0.03	> 20	14 ± 2
TSAO-m^3T (**1b**)	0.04 ± 0.01	> 250	0.06 ± 0.09	> 250	230 ± 7.3

[a] Compound concentration required to inhibit HIV-induced cell destruction (MT-4) or HIV-induced syncytium formation (CEM) by 50%.
[b] Compound concentration required to reduce MT-4 cell viability by 50%.
[c] Data are the mean of 2–3 independent experiments (± S.D.).
[d] See Camarasa et al. (1992).
[e] See Pérez-Pérez et al. (1992a).

derivative **13b** proved inhibitory at an EC_{50} of 5.2 μM in MT-4 cells, while being not toxic at 130 μM (Table 1).

The same substituents were also introduced at the 2′-position of TSAO-T (**1a**) and TSAO-m^3T (**1b**). The *tert*-butyldiphenylsilyl (**14b**), 2′-*tert*-hexyldimethylsilyl (**15b**) and the 2′-benzoyl-(**17a** and **17b**) TSAO derivatives showed a marked antiviral activity (EC_{50} ranging from 0.10 to 0.99 μM), the 2′-*tert*-hexyldimethylsilyl-TSAO-m^3T derivative (**15b**) (EC_{50} 0.10 and 0.11 μM) being almost as active as TSAO-m^3T itself (EC_{50} 0.04 and 0.06 μM). Toxicity was 3-fold higher than that observed for TSAO-m^3T. The 2′-tri-*tert*-butylsilyl-substituted TSAO-m^3T (**16b**) was equally active as the 2′-benzoyl-substituted TSAO derivative **17b**, but markedly less toxic. The presence of the 2′-benzoyl group in TSAO-T and TSAO-m^3T made these compounds equally cytotoxic. This is somewhat surprising in view of the markedly lower toxicity of TSAO-m^3T than of TSAO-T in cell culture (Table 1).

TSAO-T derivatives containing a benzyl group at the 5′-position (**28a**), and, in addition, a benzoyl group at both the 2′-position and the 4″-amino of the 3′-spiro moiety

(**29a**), or an acetyl group at both the 2′-position and the 4″-amino of the 3′-spiro moiety (**30a**), were virtually inactive against HIV-1 replication in CEM and MT-4 cells. Compound **28a** (containing a benzyl group at the 5′-position and TBDMS at 2′) was equally cytotoxic as **17a** (containing benzoyl at 2′ and TBDMS at 5′) (Table 1).

None of the test compounds showed activity at subtoxic concentrations against HIV-2 in either MT-4 or CEM cells (Table 1).

4. Discussion

Our previous observations have indicated that the TSAO molecules can be extensively modified in the nucleobase moiety without significant reduction of antiviral activity. More stringent structure–activity requirements have been noted for the sugar moiety. Indeed, most of the modifications carried out at the 5′-position of the TSAO molecule resulted in significant reduction or even total loss of antiviral activity. Still marked antiviral activity was observed with the 5′-*tert*-hexyldimethylsilyl derivative **7b**, which was inhibitory to HIV-1 at a concentration of 0.80–2.0 μM. In fact, this substituent closely mimics the original TBDMS group. In contrast, introduction of the *tert*-butyldiphenylsilyl group at the 5′-position of the TSAO molecule (compound **6b**) resulted in a complete loss of antiviral activity. This may be ascribed either to the introduction of an aromatic entity that by itself is not compatible with an adequate interaction of the molecule with its target enzyme (i.e. HIV-1 reverse transcriptase (RT)), and/or to the bulky nature of this substituent hampering interaction with RT through steric hindrance. Identical substituents in the 2′-position of the TSAO molecule gave compounds that were much more antivirally active than their 5′-substituted counterparts (compare **14b** with **6b**, and **15b** with **7b**). Also other lipophilic functional groups lacking the silyl entity, such as a benzoyl or benzyl, preserves the antiviral activity much better when introduced at the 2′- than at the 5′-position (compare **17a** with **10a** or **28a**). It would be interesting to decipher why modifications at the 5′-position of the TSAO sugar are much more deleterious to antiviral efficacy than similar modifications at the 2′-position. One possibility is that the HIV-1 RT amino acid(s) which interact with the lipophilic moiety of the 5′-position of TSAO easily sense any change in the size and/or electronic properties of the pharmacophore. Alternatively, the TBDMS group at the 5′-position of the TSAO molecule may help in bringing the 4″-amino group of the 3′-spiro moiety in the right position to interact with Glu-138 of the HIV-1 RT (Balzarini et al., 1993a,b). Changing the nature of the 5′-substituent may affect the positioning of the 4″-NH_2 function and thus compromise the interaction between the TSAO molecule and its target enzyme.

In conclusion, our data demonstrate that the nature of the lipophilic 5′-substituent of TSAO is a critical determinant for antiviral efficacy. The nature of the 2′-substituent appears to be much less stringent.

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