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Novel L-*lyxo* and 5′-deoxy-5′-modified TSAO-T analogs: synthesis and anti-HIV-1 activity

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Abstract

Novel L-*lyxo*-TSAO-T analogs with an inverted configuration at the C-4′-position of the sugar moiety and 5′-deoxy-5′-modified TSAO-T derivatives have been prepared and evaluated for their inhibitory effect on human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) replication in cell culture. None of the compounds showed marked antiviral efficacy. The inactivity of the TSAO-T derivatives may most likely be explained by either their different 4′-configuration or their different chemical structure that may not allow an optimal interaction of the molecules with the lipophilic binding pocket of the HIV-1 reverse transcriptase.

Keywords: HIV; HIV-1-specific reverse transcriptase inhibitor; TSAO-T; L-*lyxo*-TSAO-T; 5′-Deoxy-5′-modified TSAO-T; 3′-Spironucleoside; Reverse transcriptase inhibitor; AIDS

1. Introduction

TSAO-T (**1**) or [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*ribo*-furanosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)[1] (Fig. 1), is the prototype compound of a series of potent and highly specific inhibitors of the human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT) (Balzarini et al., 1992a,b, 1993a). Unlike other nucleosides directed at HIV-1 (e.g. AZT, ddI, ddC, d4T and 3TC which act as DNA chain terminators), the TSAO derivatives are assumed to behave as 'non-nucleoside RT inhibitors' (NNRTIs), i.e. they bind to the enzyme at a hydrophobic pocket located in the vicinity of the polymerase active site, as do HEPT, TIBO,

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[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.

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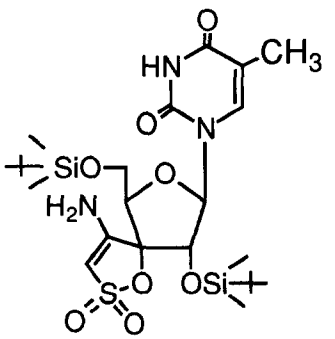
Nevirapine, BHAP, Quinoxaline and α-APA (Baba et al., 1989; Miyasaka et al., 1989; Pauwels et al., 1990, 1993; Merluzzi et al., 1990; Koup et al., 1991; Goldman et al., 1991; Romero et al., 1991; Kleim et al., 1993). The TSAO derivatives are interesting, since they are the largest molecules known so far that interact with the non-nucleoside binding site of HIV-1 RT.

Extensive structure–activity relationship (SAR) studies have shown that various features are required to maintain activity (Balzarini et al., 1993a; Camarasa et al., 1995). The nucleobase may be either pyrimidine (Balzarini et al., 1992b,c, 1993a; Camarasa et al., 1992; Pérez-Pérez et al., 1992; San-Félix et al., 1994), purine (Velázquez et al., 1993), or a substituted 1,2,3-triazole (Alvarez et al., 1994). Interestingly, alkylation of the N-3 of pyrimidines or N-1 of purines drastically reduces the cytotoxicity of the compounds without affecting their anti-HIV-1 activity (Balzarini et al., 1992c; Pérez-Pérez et al., 1992; Velázquez et al., 1993). The sugar part of the molecule has to have the unique spiro moiety at C-3′ and a D-*ribo* configuration, since compounds containing sugars with D-*xylo* or L-*ribo* configuration are inactive (Pérez-Pérez et al., 1992; Balzarini et al., 1992b,c; Ingate et al., 1995a,c). Also, compounds having the spiro moiety at C-2′ are inactive (Velázquez et al., 1994).



D-*ribo*-TSAO-T (**1**)

Fig. 1. Structure of TSAO-T (**1**).

Removal of the silyl groups either at the C-2′, C-5′, or both C-2′ and C-5′ positions results in inactive compounds (Camarasa et al., 1992; Balzarini et al., 1993a).

An investigation on the protecting groups of TSAO-T (Ingate et al., 1995b) showed that the 2′-silyl group may be replaced by other groups that mimic either the lipophilic or steric properties, without greatly affecting the antiretroviral activity. However, substitution of the 5′-silyl group, by groups bulkier than *tert*-butyldimethylsilyl (TBDMS) or by lipophilic groups lacking the silyl atom, resulted in inactive compounds. Attempts to prepare compounds with small silyl ether substituents at the 5′-position were unsuccessful, due to instability of the final products. It was deduced from this study that groups larger than TBDMS at the 5′-position would hamper the interaction of the NH_2 of the spiro moiety with RT. The spiro NH_2 group is believed to interact with a glutamic residue Glu-138 in the p51 subunit of HIV-1 RT (Balzarini et al., 1993a,b,c, 1994; Boyer et al., 1994; Jonckheere et al., 1994). This amino acid residue lies close to the polymerase active site and forms part of the non-nucleoside binding site (Smerdon et al., 1994; Tantillo et al., 1994; Ren et al., 1995). Therefore, in order to avoid the steric hindrance and to acquire deeper insight into the stereochemical requirements of the TSAO derivatives for anti-(retro)viral activity, it was of interest to prepare analogs of the prototype compound TSAO-T with an inverted configuration at C-4′ of the sugar moiety. This paper reports on the synthesis and anti-HIV-1 activity of α-L-*lyxo*-TSAO-T derivatives. In addition, since, as mentioned above, the protecting groups at both C-2′-and C-5′-positions of the sugar moiety are essential for activity but are prone to hydrolysis in vivo (Balzarini et al., 1993d), it was envisaged that perhaps this problem could be overcome by substituting the 5′-OTBDMS group by simpler lipophilic alkyl groups linked to the 5′-position by a C–C bond. Here, we describe the synthesis and anti-HIV-1 activity of 5′-deoxy-, 5′-deoxy-5′-cyclohexyl- and 5′-deoxy-5′-isobutyl-TSAO-T derivatives.

2. Materials and methods

2.1. Synthesis

2.1.1. General methods

Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. Melting points were measured with a Reichert-Jung Kofler hot-stage apparatus. ^{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 thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck). Optical rotations were recorded with a Perkin-Elmer 141 polarimeter. Separations on silica gel were performed by preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron® (Kiesegel 60 PF 254 gipshaltig (Merck)), layer thickness 1 mm, flow rate 5 ml/min. Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck).

2.1.2. *5-O-benzoyl-3-C-cyano-1,2-O-isopropylidine-3-O-mesyl-β-L-lyxofuranose* (**5**)

To a suspension of pyridinium dichromate (3.76 g, 10 mmol) and acetic anhydride (4.0 ml, 36.3 mmol) in dichloromethane (20 ml) was added a solution of **2** (Génu-Dellac et al., 1991) (3.40 g, 11.5 mmol) in dichloromethane (50 ml). After refluxing for 90 min, the solvent was removed, then the residue was taken up in ethyl acetate (20 ml) and filtered through a short (60 g) silica gel column, eluting with ethyl acetate. The solvent was removed and the residue was co-evaporated with ethanol (4 × 10 ml) and toluene (2 × 15 ml) to give a colorless syrup (the ulose **3**) which was used in the next step without further purification. A mixture of the crude product **3**, NaCN (0.49 g, 10.0 mmol), $NaHCO_3$ (1.65 g, 20.0 mmol), water (25 ml) and diethyl ether (35 ml) was stirred vigorously at room temperature for 4 h. The two phases were separated and the aqueous layer was extracted with ethyl ether (2 × 20 ml). The combined extracts were dried (Na_2SO_4) and evaporated to give a colorless syrup (the cyanohydrin **4**). This cyanohydrin was dissolved in pyridine (35 ml). To this solution mesyl chloride (3.0 ml, 37.8 mmol) was added. The mixture was stirred at 8–10°C for 48 h, poured into 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. Column chromatography (2:1, hexane:ethyl acetate) yielded cyanomesylate **5** (3.33 g, 73% from **2**) as a thick colorless syrup that crystallized upon standing (m.p. 119–121°C, from ethyl ether). ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.43, 1.49 (2s, 6H, Me_2C), 3.41 (s, 3H, MsO), 4.71 (m, 2H, 2H-5), 4.94 (t, 1H, H-4), 5.28 (d, 1H, H-2, $J_{1,2} = 4$ Hz), 6.17 (d, 1H, H-1), 7.5–8.1 (m, 5H, BzO). ^{13}C-NMR [$(CD_3)_2CO$]: δ 26.79, 26.97 (Me_2C), 40.76 (MsO), 62.42 (C-5), 78.42 (C-3), 82.39 (C-4), 84.0 (C-2), 106.6 (C-1), 116.7, 116.8 (CN, $Me_2\underline{C}$), 129.45, 130.4, 134.2 (BzO). Anal. calcd. for $C_{17}H_{19}NO_8S$: C, 51.38; H, 4.82; N, 3.52. Found: C, 51.29; H, 4.88; N, 3.51.

2.1.3. *1,2-Di-O-acetyl-5-O-benzoyl-3-C-cyano-3-O-mesyl-L-lyxofuranose* (**6**)

A solution of cyanomesylate **5** (3.00 g, 7.55 mmol) in trifluoroacetic acid (TFA) (27 ml) and water (3 ml) was stirred at room temperature for 1 h. The solvent was removed, and the residue was acetylated with acetic anhydride (4.0 ml, 38 mmol) and pyridine (25 ml) at room temperature overnight. The solvents were removed and the residue was purified by column chromatography (3:1, hexane:ethyl acetate) to give compound **6** (2.16 g, 65%) as a pale yellow syrup. The NMR spectrum showed that it was a (1:4) mixture of the β and α anomers. ^{1}H-NMR [$(CD_3)_2CO$]: δ 2.11, 2.16 (2s, 6H, 2AcO), 3.44 (s, 3H, MsO), 4.68 (d, 2H, 2H-5), 5.27 (t, 1H, H-4), 5.92 (d, H-2β, $J_{1\beta,2\beta} = 2.9$ Hz), 6.16 (d, H-1α, $J_{1\alpha,2\alpha} = 5.8$ Hz), 6.32 (d, H-2α), 6.26 (d, H-2β), 7.5–7.7 (m, 3H, BzO), 8.0–8.1 (m, 2H, BzO). ^{13}C-NMR [$(CD_3)_2CO$]: δ (β anomer) 20.50, 20.76 (2AcO), 40.62 (MsO), 61.15 (C-5), 78.0 (C-3), 78.97 (C-4), 83.25 (C-2), 99.06 (C-1), 113.2 (CN), 129.5, 130.5, 134.4 (BzO). Anal. calcd. for $C_{18}H_{19}NO_{10}S$: C, 48.98; H, 4.34; N, 3.17. Found: C, 48.96; H, 4.34; N, 3.20.

2.1.4.

(2′-O-acetyl-5′-O-benzoyl-3′-C-cyano-3′-O-mesyl-α-L-lyxo-furanosyl)thymine (7)

A solution of thymine (0.75 g, 6.0 mmol) and ammonium sulphate (10 mg) in hexamethyldisilazane (HMDS) (17 ml) was refluxed overnight. The excess HMDS was removed under reduced pressure. A solution of compound **6** (2.0 g, 4.53 mmol) in dry acetonitrile (30 ml) was added to the persilylated thymine, followed by the addition of trimethylsilyl triflate (TMS-OTfl) (1.0 ml). The reaction mixture was heated to reflux. After 3 h an additional portion of TMS-OTfl (1.5 ml) was added and the refluxing continued for 3.5 h. The solution was cooled to room temperature, ethyl acetate (100 ml) was added and the resulting solution was poured into cold, saturated aqueous $NaHCO_3$ (50 ml). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (2 × 15 ml). The combined ethyl acetate extracts were dried (Na_2SO_4) and evaporated. Purification by column chromatography (first 3:1 and then 1:1, hexane:ethyl acetate) yielded **7** (1.66 g, 72%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.82 (d, 3H, $J_{Me,6} = 1.2$ Hz), 2.13 (s, 3H, AcO), 3.46 (s, 3H, MsO), 4.70 (m, 2H, 2H-5′), 5.52 (q, 1H, H-4′), 6.07 (d, 1H, H-2′, $J_{1',2'} = 6.9$ Hz), 6.58 (d, 1H, H-1′), 7.5–8.1 (m, 6H, BzO, H-6), 10.25 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.30 (Me-5), 20.50 (AcO), 40.7 (MsO), 61.1 (C-5′), 75.9 (C-4′), 76.6 (C-3′), 83.5 (C-2′), 90.49 (C-1′), 112.0 (C-5), 113.5 (CN), 129.5, 130.5, 134.4 (BzO), 138.6 (C-6), 151.6, 164.0, 166.2, 169.4 (4C=O). Anal. calcd. for $C_{21}H_{21}N_3O_{10}S$: C, 49.70; H, 4.17; N, 8.28; Found: C, 49.56; H, 4.18; N, 8.26.

2.1.5.

[1-(2′-O-Acetyl-5′-O-benzoyl-α-L-lyxofuranosyl] thymine)-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (8)

To a solution of **7** (1.18 g, 2.35 mmol) in dry acetonitrile (20 ml) was added 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) (370 μl, 2.56 mmol), and the mixture was stirred at room temperature for 2 h. The solution was neutralized (acetic acid) and the solvent was evaporated to dryness. The residue was purified by CCTLC on chromatotron (10:1, chloroform:methanol) to give the spironucleoside **8** (0.77 g, 65%) as a white foam. $[\alpha]_D$ (c1, $CHCl_3$) = −124.5. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.80 (s, 3H, Me-5), 1.94 (s, 3H, AcO), 4.52 (m, 2H, 2H-5′), 5.37 (q, 1H, H-4′), 5.60 (s, 1H, H-3″), 6.03 (d, 1H, H-2′, $J_{1',2'} = 6.39$ Hz), 6.42 (d, 1H, H-1′), 6.44 (bs, 2H, NH_2-4″), 7.4–8.2 (m, 6H, BzO, H-6). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.36 (Me-5), 20.39 (AcO), 63.4 (C-5′), 75.25 (C-4′), 81.56 (C-2′), 89.97 (C-3″), 91.9 (C-1, C-3′), 111.7 (C-5), 129.2, 129.4, 130.4, 134.1 (BzO), 139.0 (C-6), 152.2 (C-6), 166.1 (C=O). Anal. calcd. for $C_{21}H_{21}N_3O_{10}S$: C, 49.70; H, 4.17; N, 8.28. Found: C, 49.71; H, 4.16; N, 8.15.

2.1.6.

[1-(5′-O-Benzoyl-α-L-lyxofuranosyl])thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (9)

Compound **8** (0.70 g, 1.38 mmol) was stirred for 1 h in a saturated methanolic ammonia solution (30 ml). Evaporation of the solvent and subsequent column chromatography (10:1, chloroform:methanol) yielded the nucleoside **9** (0.48 g, 75%) as a white foam. $[\alpha]_D$ (c1, $CHCl_3$) = −72.4. ^{1}H-NMR [$(CD_3)_2SO$]: δ 1.87 (s, 3H, Me-5), 4.35 (m, 2H, 2H-5′), 4.73 (d, 1H, 2′-OH, $J_{2',OH} = 8.3$ Hz), 5.02 (t, 1H, H-4′), 5.71 (m, 2H, H-2′, H-3″), 5.98 (d, 1H, H-1′, $J_{1',2'} = 8.46$ Hz), 7.11 (bs, 2H, NH_2-4″), 7.49–7.93 (m, 6H, BzO, H-6). ^{13}C-NMR [$(CD_3)_2SO$]: δ 12.30 (Me-5), 54.93 (C-5′), 77.25 (C-4′), 85.4 (C-3′), 85.67 (C-2′), 91.99 (C-1′, C-3″), 110.70 (C-6), 128.8, 129.2, 129.35, 134.0 (BzO), 151.0, 151.4 (C-4, C-4″), 163.4 (C-2). Anal. calcd. for $C_{19}H_{19}N_3O_9S$: C, 49.03; H, 4.11; N, 9.03. Found: C, 49.09; H, 4.08; N, 8.99.

2.1.7.

[1-[5′-O-Benzoyl-2′-O-(tert-butyldimethylsilyl)-α-L-lyxofuranosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (10)

To a suspension of **9** (0.45 g, 0.96 mmol) in dry acetonitrile (20 ml), were added *tert*-butyldimethylsilyl chloride (TBDMS-Cl) (0.75 g, 4.98 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.9 g, 5.92 mmol). The reaction mixture

was refluxed for 24 h. Standard work-up afforded, after purification, first by column chromatography (2:1, hexane:ethyl acetate) and then by CCTLC on chromatotron (25:1, dichloromethane:methanol), compound **10** (0.26 g, 46%) as a white solid (m.p. 173–175°C, from ethyl ether). ^{1}H-NMR [$(CD_3)_2CO$]: δ −0.02, 0.10 (2s, 6H, 2Me–Si), 0.85 (s, 9H, *t*-Bu–Si), 1.85 (d, 3H, Me-5, $J_{Me,6} = 1.2$ Hz), 4.52 (m, 2H, 2H-5′), 5.20 (t, 1H, H-4′, $J_{4',5a'} = J_{4',5b'} = 6.2$ Hz), 5.28 (d, 1H, H-2′, $J_{1',2'} = 7.8$ Hz), 5.77 (s, 1H, H-3″), 6.04 (d, 1H, H-1′), 6.51 (bs, 2H, NH_2-4″), 7.5–8.0 (m, 6H, BzO, H-6), 10.27 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ −5.53, −4.51 (Me–Si), 12.36 (Me-5), 18.43, 25.85 (*t*-Bu), 62.70 (C-5′), 75.79 (C-4′), 79.02 (C-2′), 90.78 (C-3″), 91.62 (C-1′), 92.97 (C-3′), 112.1 (C-5), 129.4, 130.4, 130.7, 134.1 (BzO), 137.6 (C-6), 151.4 (C-4″), 151.7, 163.7, 166.2 (C=O). Anal. calcd. for $C_{25}H_{33}N_3O_9SSi$: C, 51.80; H, 5.74; N, 7.25. Found: C, 51.78; H, 5.70; N, 7.22.

*2.1.8. [1-[2′,5′-Bis-O-(tert-butyldimethylsilyl)-α-L-lyxo-furanosyl]thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**12**)*

The protected nucleoside **8** (0.70 g, 1.37 mmol) was stirred in a saturated methanolic ammonia solution (50 ml) at room temperature overnight. The solvent was evaporated to dryness, and the crude residue (the fully deprotected nucleoside **11**) was refluxed for 10 h in acetonitrile (20 ml) containing TBDMS-Cl (1.8 g, 1.2 mmol) and DMAP (2.2 g, 18.0 mmol). After standard work-up, the residue was purified by flash column chromatography (1:1, hexane:ethyl acetate) to yield compound **12** (0.31 g, 39%) (m.p. > 230°C, ethyl ether:hexane) as a white foam. $[\alpha]_D$ (c1, $CHCl_3$) = −95.1. ^{1}H-NMR [$(CD_3)_2CO$]: δ −0.04, 0.05, 0.06, 0.08 (4s, 12H, 4Me–Si), 0.84, 0.88 (2s, 18H, 2*t*-Bu–Si), 1.85 (d, 3H, Me-5, $J_{Me,6} = 1.2$ Hz), 3.81 (m, 2H, 2H-5′), 4.86 (t, 1H, H-4′, $J_{4',5a'} = J_{4',5b'} = 5.7$ Hz), 5.17 (d, 1H, H-2′, $J_{1',2'} = 7.8$ Hz), 5.66 (s, 1H, H-3″), 5.95 (d, 1H, H-1′), 6.39 (bs, 2H, NH_2-4″), 7.58 (d, 1H, H-6), 10.23 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ −5.37, −5.08, −4.43 (Me–Si), 12.41 (Me-5), 18.42, 18.72, 25.84, 26.16 (*t*-Bu), 62.29 (C-5′), 76.15 (C-4′), 82.46 (C-2′), 90.23 (C-3′), 91.45 (C-1′), 93.13 (C-3″), 111.9 (C-5), 137.47 (C-6), 151.6 (C-4″, C-4), 163.9 (C-2). Anal. calcd. for $C_{24}H_{43}N_3O_8SSi_2$: C, 48.87; H, 7.35; N, 7.12. Found: C, 48.90; H, 7.32; N, 7.10.

*2.1.9. C-Cyano-5-deoxy-1,2-O-isopropylidine-3-O-mesyl-α-D-ribofuranose (**15a**)*

A procedure similar to that described for the synthesis of cyanomesylate **5** was followed with 3.62 g (20.8 mmol) of the sugar derivative **14a** (Hildebrandt et al., 1991), to give, after purification by column chromatography (3:1, hexane:ethyl acetate), compound **15a** (3.66 g, 63.5%) as white crystals (m.p. 112–114°C, from hexane:ethyl acetate). $[\alpha]_D$ (c1, $CHCl_3$) = +83.5. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.40, 1.57 (2s, 6H, Me_2C), 1.43 (d, 3H, Me-4, $J_{Me,4} = 6.2$ Hz), 3.39 (s, 3H, MsO), 4.31 (q, 1H, H-4), 5.21 (d, 1H, H-2, $J_{1,2} = 3.9$ Hz), 6.05 (d, 1H, H-1). ^{13}C-NMR [$(CD_3)_2CO$]: δ 14.31 (Me-4), 26.14, 26.81 (Me_2C), 40.71 (MsO), 75.88 (C-4), 81.96 (C-2), 104.9 (C-1), 114.7 (CN). Anal. calcd. for $C_{10}H_{15}NO_6S$: C, 43.31; H, 5.45; N, 5.05. Found: C, 43.28; H, 5.47; N, 5.00.

*2.1.10. 1,2-Di-O-acetyl-3-C-cyano-5-deoxy-3-O-mesyl-D-ribofuranose (**16a**)*

A solution of compound **15a** (3.72 g, 13.4 mmol) in 40 ml of a (9:1) mixture of trifluoroacetic acid and water was stirred at room temperature for 2 h. The solvent was removed and the residue was acetylated with acetic anhydride (4.8 ml) and pyridine (10 ml) at room temperature overnight. Evaporation of the solvents followed by co-evaporation with ethanol (3 × 30 ml) gave a brown syrup that was purified by column chromatography (2:1, hexane:ethyl acetate) to give a (3:2) mixture of the α and β anomers of **16a** (3.65 g, 84.6%) as a colorless syrup. ^{1}H-NMR [$(CD_3)_2CO$] δ (α and β anomers): 1.52, 1.55 (2d, Me-4, $J_{Me,4} = 6.3$ Hz), 2.17 (m, 6H, 2AcO), 3.42 (s, 3H, MsO), 4.63 (m, 1H, H-4), 5.60 (s, H-2β), 5.70 (d, H-2α, $J_{1\alpha,2\alpha} = 4.7$Hz), 6.11 (s, H-1β), 6.51 (d, H-1α).

Anal. calcd. for $C_{11}H_{15}NO_8S$: C, 41.12; H, 4.71; N, 4.36. Found: C, 41.16; H, 4.70; N, 4.33.

2.1.11.
(2'-O-Acetyl-3'-C-cyano-5'-deoxy-3'-O-mesyl-β-D-ribofuranosyl)thymine ***(17a)***

Following a glycosylation procedure similar to that described for the synthesis of compound **7**, persilylated thymine (0.5 g, 4.0 mmol), the sugar derivative **16a** (0.87 g, 2.71 mmol) and TMS-OTfl (2.0 ml) yielded after column chromatography (1:1, hexane:ethyl acetate), compound **17a** (0.85 g, 81%) as a white foam. $[\alpha]_D$ (c1, $CHCl_3$) = +45.4. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.63 (d, 3H, Me-4', $J_{Me,4'}$ = 2.9 Hz), 1.84 (d, 3H, Me-5), $J_{Me,6}$ = 1.18 Hz), 2.13 (s, 3H, MsO), 4.53 (q, 1H, H-4'), 5.84 (d, 1H, H-2', $J_{1',2'}$ = 2.9 Hz), 6.02 (d, 1H, H-1'), 7.54 (d, 1H, H-6), 10.21 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.39 (Me-5), 16.53 (Me-4'), 20.27 (AcO), 40.62 (MsO), 75.62 (C-4'), 80.09 (C-3'), 80.17 (C-2'), 87.58 (C-1'), 112.3 (C-5), 114.8 (CN), 136.4 (C-6), 163.8, 169.5 (C=O). Anal. calcd. for $C_{14}H_{17}N_3O_8S$: C, 43.41; H, 4.42; N, 10.85. Found: C, 43.42; H, 4.44; N, 10.79.

2.1.12.
[1-(5'-Deoxy-β-D-ribofuranosyl)thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'', 2''-dioxide) ***(19a)***

A solution of **17a** (0.37 g, 0.96 mmol) and Cs_2CO_3 (0.54 g, 1.66 mmol) in dry acetonitrile (20 ml) was stirred at room temperature for 4 h. The mixture was filtered, the filtrate was neutralized (acetic acid) and the solvent was evaporated. The residue (crude **18a**) was dissolved in a saturated methanolic ammonia solution (25 ml) and stirred at room temperature for 15 h. After removal of the solvent, the residue was purified by column chromatography (10:1, chloroform:methanol) to yield **19a** (0.17 g, 51%) as a slightly yellow foam. $[\alpha]_D$ (c1, MeOH) = −19.7. ^{1}H-NMR [$(CD_3)_2CO$]: δ 1.44 (d, 3H, Me-4'), $J_{Me,4'}$ = 6.8 Hz), 1.85 (s, 3H, Me-5), 4.24 (q, 1H, H-4'), 5.01 (d, 1H, H-2', $J_{1',2'}$ = 7.3 Hz), 5.59 (s,d, 2H, H-3'', H-1'), 6.56 (bs, 2H, NH_2-4''), 7.59 (s, 1H, H-5). Anal. calcd. for $C_{12}H_{15}N_3O_7S$: C, 41.73; H, 4.38; N, 12.17. Found: C, 41.68; H, 4.42; N, 12.13.

2.1.13.
[1-[2'-O-(tert-Butyldimethylsilyl)-5'-deoxy-β-D-ribo-furanosyl]thymine]-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide) ***(20a)***

Compound **19a** (0.07 g, 0.21 mmol) was suspended in dry acetonitrile (10 ml) and treated with TBDMS-Cl (104 mg, 0.7 mmol) and DMAP (0.14 g, 1.15 mmol). The reaction mixture was refluxed for 8 h. Standard work-up afforded, after purification by CCTLC on chromatotron (first, 6:1 and then 1:1, hexane:ethyl acetate), compound **20a** (0.02 g, 22%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.01, 0.10 (2s, 6H, 2Me–Si), 0.83 (s, 9H, *t*-Bu), 1.44 (d, 3H, Me-4', $J_{Me,4'}$ = 6.6 Hz), 1.86 (d, 3H, Me-5, $J_{Me,6}$ = 1.4 Hz), 4.25 (q, 1H, H-4'), 5.03 (d, 1H, H-2', $J_{1',2'}$ = 7.0 Hz), 5.58 (d, 1H, H-1'), 5.61 (s, 1H, H-3''), 6.53 (bs, 2H, NH_2-4''), 7.63 (d, 1H, H-6), 10.39 (bs, 1H, NH). ^{13}C-NMR [$(CD_3)_2CO$]: δ −4.55 (Me–Si), 12.24 (Me-5), 15.52 (Me-4'), 25.80 (*t*-Bu), 73.86 (C-4'), 80.31 (C-2'), 89.36 (C-1'), 90.31 (C-3'), 93.16 (C-3''), 112.24 (C-5), 139.39 (C-6). Anal. calcd. for $C_{18}H_{29}N_3O_7SiS$: C, 47.04; H, 6.36; N, 9.14. Found: C, 47.06; H, 6.37; N, 9.11.

2.1.14.
5-Deoxy-5-C-isobutyl-1,2-O-isopropylidine-α-D-xylofuranose ***(14b)***

Isobutyl bromide (0.3 ml, 2.8 mmol) was added to a suspension of magnesium turnings (0.5 g, 20.6 mmol) in freshly distilled tetrahydrofuran (THF) (20 ml) containing iodine (10 mg). Upon gentle heating the iodine color disappeared, indicating the start of the reaction. A further 2.5 ml (23 mmol) of isobutyl bromide were added at a rate to maintain a gentle reflux. After the addition was complete, the mixture was heated under reflux for 2 h. The flask containing the organomagnesium was flushed with argon, sealed with a septum and cooled to −78°C. Then, 1.0 ml of a 0.1 M solution of Li_2CuCl_4 in THF was added, and the mixture was stirred for 5 min. Change of the color from whitish grey to blue-black indi-

cated the formation of the copper-containing species. A solution of 1,2-*O*-isopropylidine-5-*O*-tosyl-α-D-*xylo*furanose **13** (Levene and Raymond, 1933) (1.19 g, 3.46 mmol) in THF (5 ml) was then added. The reaction mixture was allowed to reach room temperature and then was stirred overnight. The solution was poured into ice, neutralized with 1 N HCl and extracted with dichloromethane (3 × 40 ml). The extracts were dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (3:1, hexane:ethyl acetate) to yield 0.40 g (50.6%) of **14b** as an amorphous solid. $[\alpha]_D$ (c1, MeOH) = −22.7. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.85 (d, 6H, Me_2CH), 1.23, 1.38 (2s, 6H, Me_2C), 1.25–1.32 (m, 3H, CH_2CH), 1.41–1.69 (m, 2H, 2H-5), 3.97 (m, 2H, H-3, H-4), 4.10 (d, 1H, OH-3, $J_{3,OH}$ = 5.6 Hz), 4.45 (d, 1H, H-2, $J_{1,2}$ = 3.9 Hz), 5.78 (d, 1H, H-1). ^{13}C-NMR [$(CD_3)_2CO$]: δ 22.7 (Me_2CH), 26.20, 26.82 (Me_2C), 26.33 ($\underline{C}H_2CH$), 28.69 ($CH_2\underline{C}H$), 35.79 (C-5), 75.2 (C-4), 81.6 (C-3), 86.3 (C-2), 105.1 (C-1), 111.2 ($Me_2\underline{C}$). Anal. calcd. for $C_{12}H_{22}O_4$: C, 62.58; H, 9.63. Found: C, 62.55; H, 9.67.

2.1.15.

*C-Cyclohexyl-5-deoxy-1,2-O-isopropylidine-α-D-xylo-furanose (**14c**)*

To a suspension of magnesium turnings (2.20 g, 90.5 mmol) in THF (10 ml) containing iodine (10 mg), under argon, was added dropwise cyclohexyl bromide (12 ml, 96.8 mmol) whilst maintaining a gentle reflux. The resulting mixture was refluxed for 1.5 h. Then 4 ml of a 0.1-M solution of Li_2CuCl_4 in THF was added dropwise over 10 min, to the refluxing solution which immediately changed color (pale grey–black). Then, a solution of 1,2-*O*-isopropylidine-5-*O*-tosyl-α-D-*xylo*furanose **13** (Levene and Raymond, 1933) (2.50 g, 7.26 mmol) in THF (15 ml) was added. The reaction mixture was refluxed for 5 min. The solution was cooled, poured into ice, neutralized with 1 N HCl and extracted with dichloromethane. The extracts were dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (6:1, hexane:ethyl acetate) to give compound **14c** (1.52 g, 84.4%) as a white foam. $[\alpha]_D$ (c1, MeOH) = −25.0. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.8–1.8 (m, 13H, 2H-5, C_6H_{11}), 1.23, 1.39 (2s, 6H, Me_2C), 3.93 (q, 1H, H-3), 4.07 (d, 1H, OH), 4.15 (m, 1H, H-4), 4.44 (d, 1H, H-2, $J_{1,2}$ = 3.7 Hz), 5.78 (d, 1H, H-1). ^{13}C-NMR [$(CD_3)_2CO$]: δ 26.9, 27.0, 27.2, 33.9, 34.7 (CH_2), 26.4, 27.0 (Me_2C), 35.6 (CH), 36.1 (C-5), 76.2 (C-4), 78.9 (C-3), 86.6 (C-2), 105.3 (C-1), 111.2 ($Me_2\underline{C}$). Anal. calcd. for $C_{14}H_{24}O_4$: C, 65.60; H, 9.43. Found: C, 65.57; H, 9.42.

2.1.16.

*C-Cyano-5-deoxy-5-C-isobutyl-1,2-O-isopropylidine-3-O-mesyl-α-D-ribofuranose (**15b**)*

Following a procedure similar to that described for the synthesis of cyanomesylate **5** the sugar derivative **14b** (1.80 g, 7.81 mmol) was reacted first, with a suspension of pyridinium dichromate (1.90 g, 5.08 mmol) in dichloromethane (40 ml) containing acetic anhydride (2.4 ml, 22.0 mmol), then with NaCN (0.40 g, 8.17 mmol) and $NaHCO_3$ (1.45 g, 17.43 mmol) in a mixture of ethyl ether (30 ml) and water (15 ml), and finally with mesyl chloride (1.40 ml, 18.0 mmol) and pyridine (15 ml), to afford, after column chromatography (3:1, hexane:ethyl acetate), compound **15b** (0.69 g, 26.5%) as a yellow syrup. $[\alpha]_D$ (c1, MeOH) = +32.4. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.91 (d, 6H, Me_2CH), 1.2–1.4 (m, 9H, Me_2C, CH_2CH), 1.80 (m, 2H, 2H-5), 3.39 (s, 3H, MsO), 4.10 (q, 1H, H-4), 5.20 (d, 1H, H-2, $J_{1,2}$ = 3.9 Hz), 6.06 (d, 1H, H-1). ^{13}C-NMR [$(CD_3)_2CO$]: δ 22.6 (Me_2CH), 26.2, 26.9 (Me_2C), 28.1 ($\underline{C}H_2CH$), 28.6 ($CH_2\underline{C}H$), 35.27 (C-5), 40.8 (MsO), 78.6 (C-3), 79.95 (C-4), 82.2 (C-2), 104.9 (C-1), 100.9 ($Me_2\underline{C}$), 114.7 (CN). Anal. calcd. for $C_{14}H_{23}NO_6S$: C, 50.44; H, 6.95; N, 4.20. Found: C, 50.41; H, 6.91; N, 4.17.

2.1.17.

*C-Cyano-5-C-cyclohexyl-5-deoxy-1,2-O-isopropylidine-3-O-mesyl-α-D-ribofuranose (**15c**)*

Following a similar procedure as that described for the synthesis of cyanomesylate **5** and starting with sugar derivative **14c** (1.36 g, 5.30 mmol), the desired cyanomesylate **15c** (1.25 g, 66%) was isolated, after column chromatog-

raphy (3:1, hexane:ethyl acetate), as a pale yellow syrup. $[\alpha]_D$ (c1, MeOH) = +45.5. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.8–1.8 (m, 19H, 2H-5, Me_2C, C_6H_{11}), 3.39 (s, 3H, MsO), 4.26 (m, 1H, H-4), 5.19 (d, 1H, H-2, $J_{1,2}$ = 3.9 Hz), 6.06 (d, 1H, H-1). ^{13}C-NMR [$(CD_3)_2CO$]: δ 26.8, 26.9, 27.0, 33.3, 34.4 ($5CH_2$), 26.3, 26.7 (Me_2C), 35.3 (CH), 37.4 (C-5), 40.76 (MsO), 77.7 (C-4), 81.95 (C-2), 82.95 (C-3), 105.0 (C-1), 114.7 (CN). Anal. calcd. for $C_{16}H_{25}NO_6S$: C, 53.46; H, 7.01; N, 3.90. Found: C, 53.43; H, 7.02; N, 3.92.

2.1.18.

*1,2-Bis-O-benzoyl-3-C-cyano-5-deoxy-5-isobutyl-3-O-mesyl-D-ribofuranose (**16b**)*

A solution of compound **15b** (1.60 g, 4.80 mmol) in 30 ml of a (9:1) mixture of TFA and water was stirred at room temperature for 2 h. The solvent was removed and the residue was dissolved in pyridine (35 ml). The solution was cooled to 0°C, benzoyl chloride (1.40 ml, 12.0 mmol) was added and the reaction mixture was stirred at room temperature overnight. Evaporation of the solvent, followed by co-evaporation with ethanol (3 × 30 ml), gave a brown syrup that was purified by column chromatography (2:1, hexane:ethyl acetate) to give compound **16b** (2.11 g, 87%) as a white foam, which was shown to be a (1:1) mixture of the α and β anomers. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.86–0.95 (m, 6H, Me_2CH), 1.3–1.8 (m, 5H, 2H-5, CH_2CH), 4.7–4.9 (m, 1H, H-4), 6.11 (d, s, H-2α, H-2β), 6.66 (s, H-1α), 6.97 (d, H-1β, $J_{1\beta,2\beta}$ = 4.5 Hz), 7.40–8.20 (m, 10H, 2BzO). ^{13}C-NMR [$(CD_3)_2CO$]: δ 22.59 (Me_2CH), 28.5 ($Me_2\underline{C}H$), 29.5 (CH_2), 35.2 (C-5), 40.68 (MsO), 76.0 (C-4), 85.6 (C-3), 85.9, 94.4 (C-2α, C-2β), 99.0, 110.9 (C-1α, C-1β), 111.6 (CN), 129.3, 129.5, 129.6, 130.4, 130.8, 134.7, 134.9 (2BzO), 165.7 (C=O). Anal. calcd. for $C_{25}H_{27}NO_8S$: C, 59.87; H, 5.43; N, 2.79. Found: C, 59.88; H, 5.46; N, 2.82.

2.1.19.

*1,2-Bis-O-benzoyl-3-C-cyano-5-C-cyclohexyl-5-deoxy-3-O-mesyl-D-ribofuranose (**16c**)*

A solution of compound **15c** (1.08 g, 3.00 mmol) in 30 ml of a (9:1) mixture of TFA and water was stirred at room temperature for 2 h. After evaporation of the solvent and co-evaporation with ethanol (3 × 10 ml) the residue obtained was dissolved in pyridine (35 ml). The solution was cooled to 0°C, benzoyl chloride (1.40 ml, 12.0 mmol) was added dropwise, and the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was purified by column chromatography (4:1, hexane:ethyl acetate) to yield compound **16c** (1.35 g, 85%) as a white foam. This was identified by NMR as a (2:3) mixture of the α and β anomers. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.8–2.0 (m, 13H, 2H-5, C_6H_{11}), 3.44 (s, 3H, MsO), 4.75–4.98 (m, 1H, H-4), 6.10 (d, s, H-2α, H-2β), 6.67 (s, H-1α), 6.97 (d, H-1β, $J_{1\beta,2\beta}$ = 4.55 Hz), 7.40–8.20 (m, 10H, 2BzO). ^{13}C-NMR [$(CD_3)_2CO$]: δ 26.6, 26.8, 27.0, 32.8, 34.5 (CH_2), 34.9 (CH), 39.6 (C-5), 40.64 (MsO), 75.9 (C-4), 83.6 (C-2, C-3), 94.5 (C-1), 115.0 (CN), 129.5, 129.6, 130.6, 130.8, 133.6, 134.7, 134.9 (2BzO), 165.0, 168.1 (C=O). Anal. calcd. for $C_{27}H_{29}NO_8S$: C, 61.67; H, 5.54; N, 2.65. Found: C, 61.43; H, 5.50; N, 2.63.

2.1.20.

*1-(2′-O-Benzoyl-3′-C-cyano-5′-deoxy-5′-C-isobutyl-3′-O-mesyl-β-D-ribo-furanosyl)thymine (**17b**)*

Following a glycosylation procedure similar to that described for the synthesis of compound **8**, persilylated thymine (0.5 g, 4.0 mmol), the sugar derivative **16b** (0.8 g, 1.59 mmol) and TMS-Tfl (1.1 ml, 5.50 mmol) were reacted for 6 h, to yield, after column chromatography (1:1, hexane:ethyl acetate), compound **17b** (0.48 g, 60%) as a white foam. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.80–1.80 (m, 11H, 2H-5′, Me_2CHCH_2), 1.87 (d, 3H, Me-5, $J_{Me,6}$ = 1.2 Hz), 3.43 (s, 3H, MsO), 4.48 (dd, 1H, H-4′, $J_{4',5a'}$ = 9.2 Hz, $J_{4',5b'}$ = 4.1 Hz), 6.09 (d, 1H, H-2′, $J_{1',2'}$ = 5.7 Hz), 6.21 (d, 1H, H-1′), 7.5–8.2 (m, 6H, H-6, BzO), 10.19 (bs, 1H, NH-3). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.46 (Me-5), 22.6, 22.7 (Me_2CH), 28.5 (CH), 29.5 (CH_2), 35.2 (C-5′), 40.65 (MsO), 76.56 (C-4′), 83.6 (C-2′), 88.3 (C-1′), 112.3 (C-5), 129.5, 131.0, 135.0 (BzO, C-6). Anal. calcd. for $C_{23}H_{27}N_3O_8S$: C, 54.64; H, 5.38; N, 8.31. Found: C, 54.66; H, 5.44; N, 8.33.

2.1.21.
(2′-O-Benzoyl-3′-C-cyano-5′-C-cyclohexyl-5′-deoxy-3′-O-mesyl-β-D-ribofuranosyl)thymine ***(17c)***

Following a glycosylation procedure similar to that described for the synthesis of compound **7**, persilylated thymine (0.5 g, 4.0 mmol), the sugar derivative **16c** (0.8 g, 1.51 mmol) and TMS-OTfl (1.1 ml, 5.50 mmol) were reacted for 8 h, to yield, after column chromatography (first 2:1 then 1:1, hexane:ethyl acetate), compound **17c** (0.39 g, 49%) as a white foam. $[\alpha]_D$ (c1, MeOH) = +41.0. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.80–2.0 (m, 16H, 2H-5′, C_6H_{11}, Me-5), 3.43 (s, 2H, MsO), 4.64 (q, 1H, H-4′, $J_{4',5a'} = 8.0$ Hz, $J_{4',5b'} = 4.8$ Hz), 6.05 (d, 1H, H-2′, $J_{1',2'} = 5.5$ Hz), 6.24 (d, 1H, H-1′), 7.5–8.2 (m, 6H, H-6, BzO), 10.23 (s, 1H, NH-3). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.46 (Me-5), 26.65, 26.84, 27.0, 32.85, 34.52 (CH_2), 35.0 (CH), 38.96 (C-5′), 40.6 (MsO), 76.6 (C-4′), 80.0 (C-3′), 81.3 (C-2′), 88.46 (C-1′), 112.4 (C-5), 115.0 (CN), 129.5, 130.4, 131.0 (BzO), 136.6 (C-6), 163.9, 165.0, 167.2 (C=O). Anal. calcd. for $C_{25}H_{29}N_3O_8S$: C, 56.49; H, 5.50; N, 7.90. Found: C, 56.50; H, 5.52; N, 7.87.

2.1.22.
[1-(2′-O-Benzoyl-5′-deoxy-5′-C-isobutyl-β-D-ribofuranosyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) ***(18b)***

A solution of compound **17b** (100 mg, 0.30 mmol) and Cs_2CO_3 (87 mg, 0.267 mmol) in dry acetonitrile (15 ml) was stirred at room temperature for 8 h. The solution was filtered, the filtrate was neutralized (acetic acid) and the solvent was evaporated. The residue was purified by CCTLC on chromatotron (20:1, dichloromethane:methanol) to give **18b** (0.15 g, 75%) as a white foam. $[\alpha]_D$ (c1, MeOH) = −35.0. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.91 (s, 6H, Me_2CH), 1.20–1.70 (m, 5H, 2H-5′, CH_2CH), 1.83 (s, 3H, Me-5), 4.08 (q, 1H, H-4′), 5.62 (s, 1H, H-3″), 5.92 (d, 1H, H-2′, $J_{1',2'} = 6.7$ Hz), 6.32 (d, 1H, H-1′), 6.69 (bs, 2H, NH_2-4″), 7.4–7.8, 8.0–8.2 (2m, 6H, H-6, BzO), 10.37 (bs, 1H, NH-3). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.28 (Me-5), 22.5, 22.8 (Me_2CH), 27.3 (CH), 28.8 (CH_2), 35.9 (C-5′), 73.0 (C-4′), 76.2 (C-3′), 84.2 (C-3″), 88.68 (C-2′), 92.17 (C-1′), 111.9 (C-5), 129.8, 130.6, 134.5 (BzO, C-6), 151.8 (C-4″), 154.9, 168.2 (C=O). Anal. calcd. for $C_{23}H_{27}N_3O_8S$: C, 54.64; H, 5.38; N, 8.31. Found: C, 54.61; H, 5.41; N, 8.33.

2.1.23.
[1-(2′-O-Benzoyl-5′-C-cyclohexyl-5′-deoxy-β-D-ribofuranosyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) ***(18c)***

A solution of compound **17c** (0.33 g, 0.62 mmol) and Cs_2CO_3 (0.21 g, 0.61 mmol) in dry acetonitrile (10 ml) was stirred at room temperature for 4 h. The solution was filtered, the filtrate was neutralized (acetic acid) and the solvent was evaporated. The residue was purified by CCTLC on chromatotron (40:1, dichloromethane:methanol) to yield **18c** (0.12 g, 36%) as a white foam. $[\alpha]_D$ (c1, MeOH) = −41.9. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.80–2.0 (m, 16H, 2H-5′, Me-5, C_6H_{11}), 4.28 (dd, 1H, H-4′, $J_{4',5a'} = 10$ Hz, $J_{4',5b'} = 2.85$ Hz), 5.60 (s, 1H, H-3″), 5.90 (d, 1H, H-2′, $J_{1',2'} = 6.6$Hz), 6.29 (d, 1H, H-1′), 6.67 (bs, 2H, NH_2-3″), 7.5–8.1 (m, 6H, H-6, BzO), 10.35 (bs, 1H, NH-3). ^{13}C-NMR [$(CD_3)_2CO$]: δ 12.25 (Me-5), 26.68, 26.84, 27.05, 33.0, 34.71 (CH_2), 35.26 (CH), 36.48 (C-5′), 72.95 (C-4′), 81.62 (C-2′), 88.49 (C-3″), 92.69 (C-1′), 111.96 (C-5), 129.3, 130.7 (BzO), 134.4 (C-6), 151.2 (C-4″). Anal. calcd. for $C_{25}H_{29}N_3O_8S$: C, 56.49; H, 5.50; N, 7.90. Found: C, 56.49; H, 5.52; N, 7.88.

2.1.24.
[1-(2′-O-tert-Butyldimetylsilyl-5′-C-cyclohexyl-5′-deoxy-β-D-ribofuranosyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) ***(20c)***

The spiro derivative **18c** (0.06 g, 0.11 mmol) was treated with methylamine (33 wt.% solution in ethanol, 5 ml) and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated to dryness. The residue was purified by CCTLC (20:1,dichloromethane: methanol) to give a white foam (compound **19c**) which was dissolved in acetonitrile (5 ml) and treated with TBDMS-Cl (56 mg, 0.37 mmol) and 4-(dimethylamino)pyridine (0.12g, 0.92 mmol).

The mixture was stirred at room temperature overnight. The solvent was evaporated. The residue was dissolved in ethyl acetate (10 ml), washed successively with water (2 × 10 ml) and brine (2 × 10 ml), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by CCTLC on chromatotron (first 2:1, then 1:1, hexane:ethyl acetate) to give **20c** (0.053 g, 87%) as a white foam. $[\alpha]_D$ (c1, MeOH) = + 42.0. ^{1}H-NMR [$(CD_3)_2CO$]: δ 0.01, 0.10 (2s, 6H, 2Me–Si), 0.83 (s, 9H, *t*-Bu), 1.0–1.9 (m, 13H, 2H-5′, C_6H_{11}), 1.85 (d, 3H, Me-5, $J_{Me,6}$ = 1.2 Hz), 4.19 (dd, 1H, H-4′, $J_{4',5a'}$ = 10.5 Hz, $J_{4',5b'}$ = 2.9 Hz), 5.00 (d, 1H, H-2′, $J_{1',2'}$ = 6.9 Hz), 5.67 (d, 1H, H-1′), 5.61 (s, 1H, H-3″), 6.47 (bs, 2H, NH_2-4″), 7.60 (d, 1H, H-6), 10.36 (bs, 1H, NH-3). ^{13}C-NMR [$(CD_3)_2CO$]: δ 5.47 (Me–Si), 14.23 (Me-5), 17.62, 24.9 (*t*-Bu), 25.7, 25.9, 26.1, 32.0, 33.8 (CH_2), 34.2 (CH), 36.55 (C-5′), 73.14 (C-4′), 80.9 (C-2′), 88.4 (C-3″), 89.1 (C-3′), 92.8 (C-1′), 111.2 (C-5), 138.7 (C-6), 151.2 (C-4″), 154.0, 162.8 (C=O). Anal. calcd. for $C_{24}H_{39}N_3O_8SSi$: C, 53.21; H, 7.25; N, 7.76. Found: C, 53.20; H, 7.22; N, 7.71.

2.2. Biological methods

2.2.1. Cells and viruses

CEM cells were obtained from the American Type Culture Collection (Rockville, MD, USA). MT-4 cells were a kind gift of 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, USA) 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 post infection. 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 or HIV-2 was added at 100 $CCID_{50}$ (50% cell culture infective dose) to the cell cultures. 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 were 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 or 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% or to inhibit CEM cell proliferation by 59% (Table 1).

3. Results and discussion

3.1. Chemical results

3.1.1. Synthesis of TSAO-T analogs with an inverted configuration at C-4′

For the synthesis of α-L-*lyxo*-TSAO-T derivatives, we followed the method developed in our laboratory for the stereospecific synthesis of the prototype compound TSAO-T (Pérez-Pérez et al., 1992). The method involves the condensation of persilylated heterocyclic bases with the suitably functionalized and protected sugar intermediate **6**, using trimethylsilyl triflate (TMS-OTfl) as condensing reagent (Vorbrüggen et al., 1981), followed by basic treatment of the cyanomesyl nucleosides thus obtained, to give exclusively, α-L-*lyxo*-spironucleosides (**8**–**9**). As shown in Fig. 2, oxidation (pyridinium dichromate/Ac_2O) (Hollemberg et al., 1978) of the key intermediate **2** (Génu-Dellac et al., 1991) gave compound **3**, which was reacted immediately with sodium cyanide in the presence of sodium bicarbonate (Calvo-Mateo et al., 1988) to give kinetically controlled (Hollemberg et al., 1978; Levene and Raymond, 1933) *lyxo*-cyanohydrin **4** exclusively, which by mesylation

Table 1
Anti-HIV-1 and -HIV-2 activity of TSAO derivatives in CEM and MT-4 cell cultures

Compound	EC_{50} [a] (μg/ml)				CC_{50} [b] (μg/ml)	
	CEM		MT4		CEM	MT4
	HIV-1	HIV-2	HIV-1	HIV-2		
8	31 ± 25	>100	35 ± 26	>100	N.d.	86 ± 23
10	>100	>100	>100	>100	N.d.	88 ± 21
12	>20	>20	>100	>100	N.d.	83 ± 17
18b	>20	>20	>4	>4	27 ± 5.2	12 ± 3.8
18c	>4	>4	>4	>4	8.8 ± 0.5	6.4 ± 0.9
20a	>20	>20	54 ± 18	>100	96 ± 5.5	48 ± 8.6
20c	>4	>4	>4	>4	7.7 ± 1.3	7.4 ± 0.23
TSAO-T	0.02 ± 0.01	>4	0.03 ± 0.02	>4	N.d.	7.7 ± 1.5

N.d., not determined.
[a] 50% Effective concentration, or compound concentration required to inhibit HIV-1(III_B)- and HIV-2(ROD)-induced giant cell formation in CEM cell cultures, or HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in MT-4 cell cultures.
[b] 50% Cytostatic concentration, or compound concentration required to inhibit CEM cell proliferation, or MT-4 cell viability, by 50%.

(mesyl chloride/pyridine) gave the 3-*C*-cyano-3-*O*-mesyl derivative **5** in 73% yield. Hydrolysis of the 1,2-*O*-isopropylidine group of **5** with (9:1) TFA:H_2O, followed by acetylation with Ac_2O/pyridine, gave the intermediate **6** in 65% yield, as a 1:4 mixture of the β and α anomers, respectively. Glycosylation of **6** with persilylated thymine (Vorbrüggen et al., 1981) yielded the nucleoside **7** in 72% yield. The structure of **7** was assigned on the basis of the analytical and spectroscopic data. Since no epimerization occurred during mesylation of cyanohydrins (Calvo-Mateo et al., 1988; Pérez-Pérez et al., 1991; Bourgeois, 1974; Yoshimura et al., 1986; Thang et al., 1980), the absolute configuration of the cyanomesylate **7** was assumed to be the same as that of the corresponding cyanohydrin **5**, as clearly demonstrated in previous papers of this series (Calvo-Mateo et al., 1988; Pérez-Pérez et al., 1991). The presence in the starting sugar **6** of a 2-*O*-acyl participating group led exclusively to 1′,2′-*trans*-nucleosides (Vorbrüggen et al., 1981).

Ring closure to form the 3′-spiro moiety in **8** was performed in 65% yield by treatment of the cyanomesylate **7** with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (Pérez-Pérez et al., 1991). Deprotection of **8** with a solution of saturated methanolic ammonia at room temperature overnight, gave the fully deprotected nucleoside **11**, which, by reaction with TBDMS-Cl, yielded the 2′,5′-bis-*O*-silylated nucleoside **12** in 39% yield. Finally, when compound **8** was stirred in a methanolic ammonia solution for 1 h at room temperature, the 2′-*O*-deprotected compound **9** was obtained, which upon treatment with TBDMS-Cl, gave the 2′-*O*-silylated spironucleoside **10** in 46% yield.

3.1.2. *Synthesis of 5′-deoxy-TSAO-T derivatives*

The starting 5′-deoxy sugar **14a** was prepared (Fig. 3) from 5-*O*-*p*-toluenesulphonyl-1,2-*O*-isopropylidine-α-D-*xylo*-furanose (**13**) (Levene and Raymond, 1933) following a well-established procedure (Hildebrandt et al., 1991). Oxidation of **14a** with pyridinium dichromate/Ac_2O (Hollemberg et al., 1978), followed by treatment of the corresponding ulose with sodium cyanide in the presence of sodium bicarbonate and then with mesyl chloride in pyridine (Calvo-Mateo et al., 1988; Pérez-Pérez et al., 1991), gave the respective 3-*C*-cyano-3-*O*-mesyl derivative **15a** (63% overall yield from **14a**). Compound **15a** was converted to the corresponding diacetate **16a**, in 85% yield, by

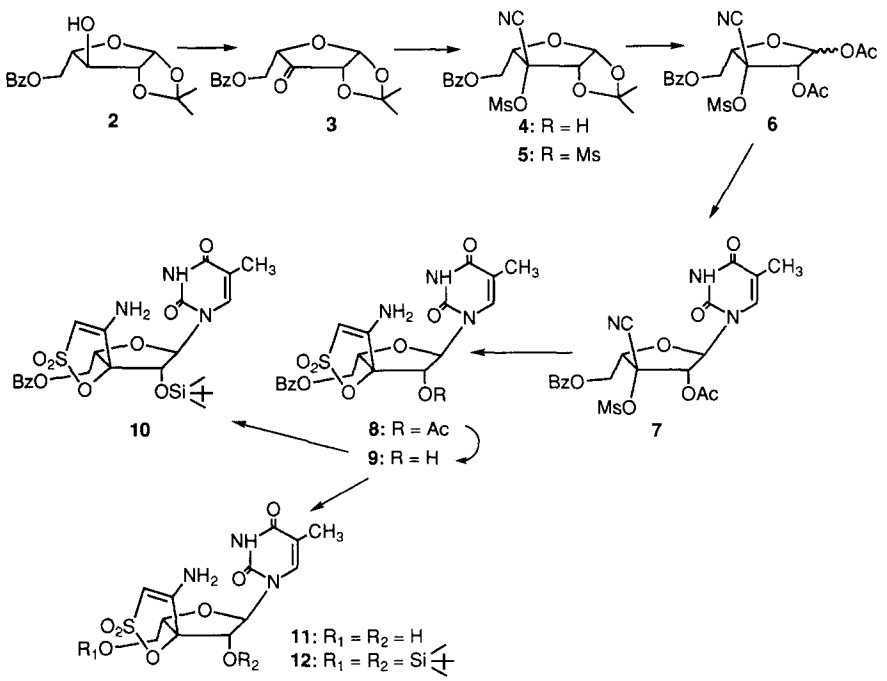


Fig. 2. Synthesis of L-*lyxo*-TSAO-T derivatives (**8**–**12**).

hydrolysis with a (9:1) mixture of TFA:H_2O, followed by acetylation with Ac_2O/pyridine. Furanoside **16a** was condensed with bis(trimethylsilyl)thymine under standard Vorbrüggen conditions (Vorbrüggen et al., 1981) to give the 3′-cyanomesyl nucleoside **17a** in 81% yield. Treatment of cyanomesylate **17a** with Cs_2CO_3 (Calvo-Mateo et al., 1988) afforded the spiro derivative **18a**, which, upon treatment with saturated methanolic ammonia, gave the fully deprotected nucleoside **19a** (51% yield over two steps). Reaction of **19a** with TBDMS-Cl yielded the 2′,5′-bis-*O*-silylated nucleoside **20a** in 22% yield.

The two isobutyl and cyclohexyl groups were introduced by nucleophilic attack of their respective organomagnesiums with copper catalysis (Pougny and Sinay, 1978; Tatsuta et al., 1981) on the 5-*O*-tosyl sugar intermediate **13** (Levene and Raymond, 1933) (Fig. 2). Thus, reaction of the sugar intermediate **13** with the corresponding organomagnesium reagent, prepared by reaction of isobutyl bromide or cyclohexyl bromide with magnesium turnings in the presence of iodine, gave the respective 5-*C*-isobutyl and 5-*C*-cyclohexyl sugar derivatives **14b** and **14c**, in 50 and 84% yield, respectively. Oxidation of these sugars **14b** and **14c** (pyridinium dichromate/Ac_2O) and subsequent reaction of the corresponding uloses with sodium cyanide, followed by reaction with mesyl chloride in pyridine gave the corresponding cyanomesylates **15b** and **15c** in 26% and 72% yield, respectively. Hydrolysis of the 1,2-*O*-isopropylidine group of the cyanomesylates (**15b**, **15c**) using (9:1) TFA:H_2O, followed by benzoylation of the resulting 1,2-diols with benzoyl chloride in pyridine gave the sugar derivatives **16b** and **16c** in 87% and 61% yield, respectively. The benzoyl group was chosen in this case, rather than acetyl as used in all our previous synthesis of TSAO derivatives, since in our previous studies it was found that the 2′-*O*-benzoyl protected TSAO-T derivatives gave active compounds (Ingate et al., 1995b). Hence, benzoylation at this stage would circumvent two synthetic steps (i.e. deprotection of the acetyl at 2′-position and subsequent

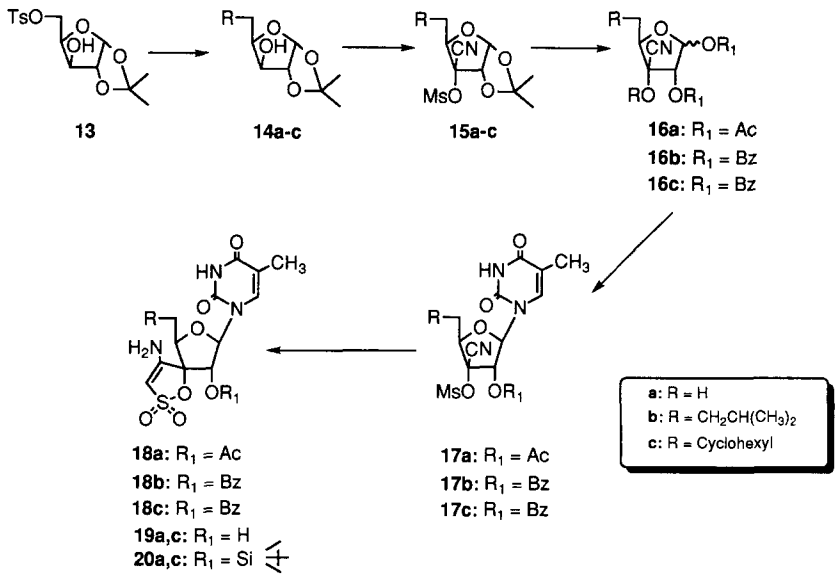


Fig. 3. Synthesis of 5′-deoxy-TSAO-T derivatives (**19**–**21**).

protection of the final product). Glycosylation of the sugar intermediates **16b** and **16c** with persilylated thymine (Vorbrüggen et al., 1981) afforded the 3′-cyanomesylates **17b** (60%) and **17c** (49%). The spiro derivatives were prepared from the corresponding cyanomesylates. Thus, treatment of **17b** and **17c** with Cs_2CO_3 gave the spiro derivatives **18b** and **18c** in good yield. Finally, deprotection of **18b** using methylamine in methanol, followed by reaction of the resulting deprotected product with TBDMS-Cl in the presence of 4-(dimethylamino)pyridine afforded the fully protected nucleoside **20c** in 87% yield.

3.2. Biological results

None of the α-L-*lyxo*-TSAO-T or 5′-deoxy-TSAO-T derivatives evaluated in this study proved inhibitory to HIV-1 at subtoxic concentrations, except for the α-L-*lyxo*-TSAO derivative **8**, whereas the 5′-deoxy-TSAO-T derivative **20a** showed marginal antiviral activity at its CC_{50}. It is questionable whether this activity is due to a specific antiviral effect or due to cellular toxicity. As expected, all compounds were inactive against HIV-2 in CEM and MT-4 cell cultures. The markedly decreased antiviral activity of the compounds suggest that most likely, these compounds do not fit in the lipophilic NNRTI pocket of the HIV-1 RT. Indeed, the fitting of an NNRTI into this pocket is obviously due to an appropriate interaction of the test compounds with the different amino acids of the pocket. Converting TSAO-T to its α-L-*lyxo* isomer most likely breaks up several of these crucial interaction points. Also, the nature of the lipophilic group at the 5′-position may be crucial to ensure the antiviral potency of the compound. Molecular modeling of the TSAO molecules in the RT would be of particular importance to understand the markedly decreased antiviral activity, and such studies are currently ongoing in our laboratories.

The compounds showing the greatest cytostatic activity were **18c** and **20c**, with a CC_{50} comparable to the CC_{50} of the parent compound TSAO-T. Both compounds represent 5′-cyclohexyl derivatives. Replacement of the cyclohexyl at C-5′ by smaller groups such as isobutyl (**18b**) or hydrogen (**20a**) progressively reduced the cytostatic potency of the TSAO derivatives. The nature of the lipophilic entity at the 2′-position of the ribose moiety did not seem to play a significant role in

the eventual cytostatic properties of the 5′-deoxy-TSAO-T compounds (benzoyl in **18c** or TBDMS in **20c**). The presence of a silyl or an acetyl at the C-2′ position of the sugar moiety resulted in a poor cytostatic activity of the α-L-*lyxo*-TSAO-T derivatives.

In conclusion, none of the α-L-*lyxo* or 5′-deoxy TSAO-T derivatives proved markedly inhibitory to HIV-1 replication in cell culture, most likely due to a conformational change of the molecule, thus preventing an optimal interaction with the binding pocket in the HIV-1 RT.

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