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Antiviral Research 50 (2001) 207–222

Antiviral Research

www.elsevier.com/locate/antiviral

Exploring the role of the 5′-position of TSAO-T. Synthesis and *anti*-HIV evaluation of novel TSAO-T derivatives

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Received 2 January 2001; accepted 3 April 2001

Abstract

Various 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) have been synthesized in which the 5′-TBDMS group has been replaced by alkyl-, alkenyl- or aromatic ether groups, substituted amines, carbamoyl or (thio)acyl groups. The compounds synthesized were evaluated for their inhibitory effect on HIV-1 and HIV-2 replication in cell culture. Replacement of the 5′-TBDMS group by an acyl, aromatic or a cyclic moiety markedly diminish or even eliminate the *anti*-HIV activity. However, the presence at that position of an alkyl or alkenyl chain, partially retain antiviral activity. These observations suggest that the 5′-TBDMS group of the TSAO molecule plays a crucial role.

Keywords: AIDS; Non-nucleoside HIV-1 RT inhibitors; Spironucleosides

1. Introduction

A key target enzyme in the search for effective drugs useful for AIDS therapy is the human immunodeficiency virus (HIV) encoded reverse transcriptase (RT) (Vaishnav and Wong-Staal, 1991; Kaltz and Skalka, 1993). An important class of RT inhibitors is the non-nucleoside RT inhibitors (NNRTIs) (De Clercq, 1993, 1996). Among them, TSAO derivatives represent a rather unique class of nucleoside analogues that have been identified as highly specific non-competitive inhibitors of the RNA-dependent DNA polymerase function of RT (Balzarini et al., 1992a,b,c; Camarasa et al., 1992; Pérez-Pérez et al., 1992). In spite of their structural similarities to nucleosides, and like the other NNRTIs, TSAO analogues target a nonsubstrate binding site (Balzarini et al., 1992c; Camarasa et al., 1992;

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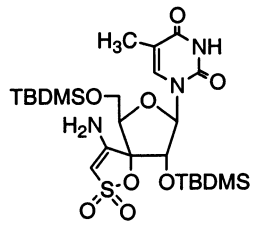
0166-3542/01/$ - see front matter
PII: S0166-3542(01)00145-0



1 (TSAO-T)

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

Pérez-Pérez et al., 1992; Camarasa et al., 2000). They seem to interfere at the interface between the p51 and p66 subunits of the RT heterodimer (Harris et al., 1998). Well-defined amino acids at both the p51 and the p66 RT subunits are needed for an optimum interaction of TSAO with the HIV-1 RT (Balzarini et al., 1994; Boyer et al., 1994; Jonckheere et al., 1994; Camarasa et al., 1995). Our experimental data strongly suggest a specific interaction of the 3′-spiro moiety of TSAO molecules with the glutamic acid residue at position 138 (Glu-138) of the p51 subunit of HIV-1 RT (Balzarini et al., 1993, 1994; Jonckheere et al., 1994; Alvarez et al., 1997, 1998). Recent biochemical evidence has also shown that both TSAO-T (**1**, Fig. 1) [the prototype of this family of compounds (Balzarini et al., 1992a; Camarasa et al., 1992)] and its *N*-3-ethyl derivative are able to destabilize the p66/p51 RT heterodimer in a concentration-dependent manner leading to a loss in its ability to bind DNA (Harris et al., 1998; Sluis-Cremer et al., 2000). This suggests a completely new and different mechanism of inhibition of HIV-RT by TSAO derivatives with regard to the other known NNRTIs.

Structure-activity relationship (SAR) studies within the TSAO family of compounds have revealed that the sugar part plays a crucial role in the interaction of the TSAO compounds with their target enzyme (Balzarini et al., 1992a,b; Camarasa et al., 1992; Pérez-Pérez et al., 1992; Camarasa et al., 2000). The bulky *tert*-butyldimethylsilyl groups (TBDMS) at both 2′ and 5′ positions of the sugar moiety seem to be important for activity (Balzarini et al., 1992c; Camarasa et al., 1992; Pérez-Pérez et al., 1992; Ingate et al., 1995; Camarasa et al., 2000). The role of these silyl moieties has always been very intriguing, since their removal, either at 2′, 5′ or at both positions, led to TSAO derivatives inactive at subtoxic concentrations (Camarasa et al., 1992, 2000). In our initial efforts to replace these groups by other lipophilic entities, it was found that the TBDMS group at position 2′ could be replaced by other moieties (i.e. benzoyl) although this resulted in a two- to 10-fold reduced *anti*-HIV-1 potency (Ingate et al., 1995). Whereas, similar substitutions at the 5′-position, resulted in antivirally inactive TSAO compounds (Pérez-Pérez et al., 1992; Ingate et al., 1995), both in cell culture and in inhibition studies against the enzyme. The only group that restores some activity is the *tert*-hexyldimethylsilyl group (Ingate et al., 1995). So, it was clear that both TBDMS groups (2′ or 5′) do not share the same role when interacting with their target enzyme and that the 5′-TBDMS group seems to have a 'peculiar' role in the interaction of TSAO derivatives with the HIV-1 RT.

Therefore, it seemed imperative to determine why the 5′-TBDMS group seem to be so important for activity. Thus, as part of a continuing effort to improve the activity/toxicity profile of the TSAO family of compounds, by exploring the effects of different substituents on TSAO derivatives and, in particular, the 'peculiar' role of the 5′-TBDMS substituent, various compounds have been synthesized in which this 5′-TBDMS group was replaced by alkyl-, alkenyl- or aromatic-ether groups, substituted amines, carbamoyl- or (thio)acyl groups. A preliminary account of this work was presented at the ECCDR-1 (First Euroconference on Carbohydrates in Drug Research, Sardinia-1999) (San-Félix et al., 2000). In this paper we report full details of the synthesis and *anti*-HIV-1 activity of these compounds.

2. Materials and methods: chemistry

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, a Varian XL-300 and a Bruker AM-200 spectrometer operating at 300 and at 200 MHz, respectively, with Me_4Si as internal standard. ^{13}C NMR spectra were recorded with a Bruker AM-200 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 2–4 ml/min. Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck).

2.1.2. [1-[5′-O-Acetyl-2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (3)

To a stirred solution (ice bath) of **2** (Ingate et al., 1995) (0.10 g, 0.20 mmol) in dry pyridine (5 ml), acetic anhydride (0.1 ml, 1.00 mmol) was added dropwise. The reaction mixture was allowed to reach room temperature, stirred for 17 additional hours and then evaporated to dryness. The residue was coevaporated successively with ethanol (3 × 10 ml) and toluene (3 × 10 ml) and then purified by CCTLC on the chromatotron (dichloromethane:acetone, 12:1) to give 0.07 g (65%) of **3** as a white amorphous solid. ^1H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.92 (s, 3H, CH_3-5), 2.05 (s, 3H, CH_3CO), 3.27 (s, 3H, N–CH_3), 4.45 (m, 3H, H-4′, H-5′), 5.03 (d, 1H, H-2′), 5.77 (s, 1H, H-3″), 5.88 (d, 1H, H-1′, $J_{1',2'} = 7.4$ Hz), 6.54 (bs, 2H, NH_2-4″), 7.65 (s, 1H, H-6). Anal. calcd. for $C_{21}H_{33}N_3O_9SSi$: C, 47.44; H, 6.26; N, 7.90; S, 6.03. Found: C, 47.38; H, 6.32; N, 7.98; S, 6.00.

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

To a stirred solution (ice bath) of **2** (Ingate et al., 1995) (0.10 g, 0.20 mmol) in dry pyridine (3 ml), isobutyryl chloride (0.024 ml, 0.22 mmol) was added. The reaction mixture was allowed to reach room temperature and stirred for 2 additional hours. Then, ethyl acetate (50 ml) was added and the mixture was washed with cold (4°C) 0.1 N HCl (2 × 25 ml), water (2 × 25 ml) and brine (25 ml). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by CCTLC on the chromatotron (hexane:ethyl acetate, 2:1) to yield 0.08 g (72%) of **4** as a white amorphous solid. ^1H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.12 (m, 6H, $(C\underline{H}_3)_2$–C), 1.89 (s, 3H, CH_3-5), 2.57 (m, 1H, $(CH_3)_2$–C$\underline{H}$), 3.25 (s, 3H, N–CH_3), 4.30–4.50 (m, 3H, H-4′, 2 H-5′), 5.02 (d, 1H, H-2′), 5.75 (s, 1H, H-3″), 5.89 (d, 1H, H-1′, $J_{1',2'} = 7.5$ Hz), 6.53 (bs, 2H, NH_2-4″), 7.60 (s, 1H, H-6). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ: 12.85 (CH_3-5), 18.79, 18.86 [$(\underline{C}H_3)_2$–CH], 27.93 (N–CH_3), 33.65 [$(CH_3)_2$–$\underline{C}$H], 61.62 (C-5′), 72.52 (C-2′), 80.12 (C-4′), 86.19 (C-3′), 88.34, 99.04 (C-1′, C-3″), 111.41 (C-5), 138.55 (C-6), 150.35, 152.95 (C-2, C-4″), 162.91 (C-4), 176.58 (CO). Anal. calcd. for $C_{23}H_{37}N_3O_9SSi$: C, 49.36; H, 6.66; N, 7.51; S, 5.73. Found: C, 49.40; H, 6.78; N, 7.45; S, 5.71.

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

To a stirred solution (−25°C) of **2** (Ingate et al., 1995) (0.10 g, 0.20 mmol) in dry dichloromethane (3 ml) and dry pyridine (0.045 ml), phenyl chloroformate (0.024 ml, 0.22 mmol) was added. The reaction mixture was allowed to reach room temperature and stirred for 1 additional hour. Then dichloromethane (50 ml) and water (10 ml) were added. The organic phase was separated and washed with brine (20 ml), dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude mixture was treated with dimethylamine (33 wt.% in ethanol, 3 ml) for 15 min. Volatiles were removed and the residue obtained was purified by CCTLC on the chromatotron (hexane:ethyl acetate, 1:3) to yield 0.08 g (70%) of **5** as a white amorphous solid. ^1H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.90 (s, 3H, CH_3-5), 2.86, 2.90 (2s, 6H, $(CH_3)_2N$), 3.26 (s, 3H, N–CH_3), 4.36–4.52 (m, 3H, H-4′, 2 H-5′), 4.96 (d, 1H, H-2′), 5.74 (s, 1H, H-3″), 6.00 (d, 1H, H-1′, $J_{1',2'} = 7.7$ Hz), 6.64 (bs, 2H, NH_2-4″), 7.67 (s, 1H, H-6). ^{13}C-NMR [$(CD_3)_2CO$, 50 MHz] δ: 13.00

(CH_3-5), 28.01 (N–CH_3), 36.06, 36.61 [$(CH_3)_2$N], 63.61 (C-5′), 74.24 (C-2′), 82.74 (C-4′), 90.25, 90.67, 91.32 (C-1′, C-3′, C-3″), 111.36 (C-5), 136.00 (C-6), 152.16, 152.83 (C-2, C-4″), 156.14 (OCON), 163.34 (C-4). Anal. calcd. for $C_{22}H_{36}N_4O_9SSi$: C, 47.13; H, 6.47; N, 9.99; S, 5.72. Found: C, 47.08; H, 6.58; N, 10.05; S, 5.70.

*2.1.5. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-O-(N,N-dimethyl)thiocarbamoyl-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**6**)*

To a solution of **2** (Ingate et al., 1995) (0.10 g, 0.20 mmol) in toluene: acetonitrile (1:1) (4 ml), *N*,*N*′-thiocarbonyldiimidazole (0.04 g, 0.22 mmol) was added. The reaction mixture was heated at 80°C for 3 h. After cooling to room temperature, volatiles were removed. The residue thus obtained was dissolved in ethyl acetate (50 ml) and washed with cold (4°C) 0.1 N HCl (25 ml), water (2 × 25 ml) and brine (25 ml). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was treated with dimethylamine (33 wt.% in ethanol, 3 ml) for 15 min, evaporated to dryness and purified by CCTLC on the chromatotron (dichloromethane: acetone, 7:1) to give 0.06 g (51%) of **6** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.89 (s, 3H, CH_3-5), 3.14 (s, 3H, N–CH_3), 3.26, 3.29 (2s, 6H, [$(CH_3)_2$N)], 4.61–4.81 (m, 3H, H-4′, 2H-5′), 5.03 (d, 1H, H-2′), 5.75 (s, 1H, H-3″), 5.98 (d, 1H, H-1′, $J_{1',2'} = 7.7$ Hz), 6.50 (bs, 2H, NH_2-4″), 7.62 (s, 1H, H-6). ^{13}C-NMR [$(CD_3)_2CO$, 50 MHz] δ: 13.08 (CH_3-5), 28.01 (N–CH_3), 38.00, 42.94 [$(CH_3)_2$N], 68.98 (C-5′), 74.07 (C-2′), 82.53 (C-4′), 89.82, 90.17, 91.24 (C-1′, C-3′, C-3″), 111.34 (C-5), 135.54 (C-6), 152.08, 152.70 (C-2, C-4″), 163.32 (C-4), 187.77 (C=S). Anal. calcd. for $C_{22}H_{36}N_4O_8S_2Si$: C, 45.82; H, 6.29; N, 9.71; S, 11.12. Found: C, 45.97; H, 6.46; N, 9.63; S, 11.00.

*2.1.6. [1-[5′-O-Allyloxycarbonyl-2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**7**)*

A solution of allyl chloroformate (0.032 ml, 0.30 mmol) in THF (0.50 ml) was added dropwise to a stirred solution of compound **2** (0.10 g, 0.20 mmol) in pyridine (0.024 ml, 0.30 mmol) and THF (0.10 ml) at 0°C. The reaction mixture was stirred at 4°C for 4 h and then at room temperature overnight. One additional portion (0.1 mmol) of allyl chloroformate and pyridine (0.1 mmol) was added. The reaction mixture was allowed to reach room temperature and stirred for 2 additional hours. Excess of allyl chloroformate was destroyed with water and volatiles were removed. Ethyl acetate (20 ml) was added to the residue and the solution was washed twice with water (10 ml). The dried (Na_2SO_4) organic layer was concentrated and coevaporated with toluene (2 × 10 ml), and ethanol (2 × 10 ml). The residue was purified by flash chromatography (dichloromethane:acetone, 20:1) to give 0.07 g (60%) of **7** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.90 (s, 3H, CH_3-5), 3.26 (s, 3H, N–CH_3), 4.54 (m, 3H, H-4′, 2H-5′), 4.64 (m, 2H, CH_2=CH–CH_2), 5.02 (d, 1H, H-2′), 5.29, 5.38 (2m, 2H, CH_2=CH), 5.78 (s, 1H, H-3″), 5.92 (d, 1H, H-1′, $J_{1',2'} = 7.5$ Hz), 5.93 (m, 1H, CH_2=CH), 6.51 (bs, 2H, NH_2-4″), 7.65 (s, 1H, H-6). Anal. calcd. for $C_{23}H_{35}N_3O_{10}SSi$: C, 48.15; H, 6.15; N, 7.32; S, 5.59. Found: C, 48.26; H, 6.33; N, 7.24; S, 5.38.

*2.1.7. [1-[5′-O-Allyl-2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide), [1-[2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(3″-C-allyl-4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[5′-O-allyl-2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(3″-C-allyl-4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**8**, **9** and **10**)*

To a solution of **7** (0.10 g, 0.18 mmol) in THF (10 ml, freshly distilled from lithium aluminium hydride) was added palladium tetrakis(triphenylphosphine) (2 mg), under an atmosphere of dry and oxygen-free nitrogen. After refluxing for 1.5 h, volatiles were removed and the residue was purified by CCTLC on the chromatotron (dichloromethane:acetone, 30:1). From the fastest running fractions 0.008 g (9%) of **10** was isolated as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$,

300 MHz] δ: ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.90 (s, 3H, CH_3-5), 3.15 (m, 2H, CH_2=CH–C$\underline{H}_2$C), 3.25 (s, 3H, N–CH_3), 3.83 (dd, 1H, H-5′a, $J_{4',5'a} = 1.8$, $J_{5'a,5'b} = 11.7$ Hz), 3.88 (dd, 1H, H-5′b, $J_{4',5'b} = 2.9$ Hz), 4.27 (m, 2H, CH_2=CH–C$\underline{H}_2$O), 4.41 (m, 1H, H-4′), 4.68 (d, 1H, H-2′), 5.05–5.46 (m, 4H, 2C$\underline{H}_2$=CH-), 5.93 (m, 4H, NH_2-4″, 2CH_2=C$\underline{H}$), 6.24 (d, 1H, H-1′, $J_{1',2'} = 8.0$ Hz), 7.80 (s, 1H, H-6). Anal. calcd. for $C_{25}H_{39}N_3O_8SSi$: C, 52.70; H, 6.90; N, 7.38; S, 5.63. Found: C, 52.78; H, 6.84; N, 7.49; S, 5.60.

From the intermediate moving band 0.02 g (24%) of **9** were isolated as a white amorphous solid.^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.89 (s, 3H, CH_3-5), 3.14 (m, 2H, CH_2=CH–C$\underline{H}_2$C), 3.26 (s, 3H, N–CH_3), 3.80 (m, 1H, H-5′a, $J_{4',5'a} = 1.0$, $J_{5'a,5'b} = 12.7$ Hz), 4.00 (m, 1H, H-5′b, $J_{4',5'b} = 2.5$ Hz), 4.33 (m, 1H, H-4′), 4.93 (d, 1H, H-2′), 5.06, 5.24 (2m, 2H, C$\underline{H}_2$=CH), 5.86 (m, 2H, OH-5′, CH_2=C$\underline{H}$), 5.94 (d, 1H, H-1′, $J_{1',2'} = 8.1$ Hz), 6.23 (bs, 2H, NH_2-4″), 7.91 (s, 1H, H-6). Anal. calcd. for $C_{22}H_{35}N_3O_8SSi$: C, 49.89; H, 6.66; N, 7.93; S, 6.05. Found: C, 49.86; H, 6.74; N, 7.79; S, 6.00.

From the slowest moving band 0.009 g (10%) of **8** were isolated as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.90 (s, 3H, CH_3-5), 3.25 (s, 3H, N–CH_3), 3.85 (dd, 1H, H-5′a, $J_{4',5'a} = 1.8$, $J_{5'a,5'b} = 11.7$ Hz), 3.91 (dd, 1H, H-5′b, $J_{4',5'b} = 2.9$ Hz), 4.29 (m, 2H, CH_2=CH–C$\underline{H}_2$O, $J = 5.6$ Hz), 4.43 (m, 1H, H-4′), 4.68 (d, 1H, H-2′), 5.32, 5.41 (2m, 2H, C$\underline{H}_2$=CH), 5.77 (s, 1H, H-3″), 6.08 (m, 1H, CH_2=C$\underline{H}$), 6.23 (d, 1H, H-1′, $J_{1',2'} = 7.9$ Hz), 6.36 (bs, 2H, NH_2-4″), 7.80 (s, 1H, H-6). Anal. calcd. for $C_{22}H_{35}N_3O_8SSi$: C, 49.89; H, 6.66; N, 7.93; S, 6.05. Found: C, 49.72; H, 6.67; N, 7.82; S, 5.98.

*2.1.8. General procedure for the reaction of **2** with alkyl and benzyl halides*

To a solution of **2** (Ingate et al., 1995) (0.10 g, 0.20 mmol) in dry DMF (5 ml), NaH 60% (0.009 g, 0.22 mmol) was added, and the mixture was stirred for 1 h at room temperature. The reaction mixture was cooled (0°C) and the corresponding alkyl or benzyl halide (0.3 mmol) was added. Then, the reaction mixture was stirred overnight. The mixture was neutralized with AcOH and evaporated. Ethyl acetate (20 ml) was added to the residue and the solution was washed twice with water (10 ml). The dried (Na_2SO_4) organic layer was concentrated and the residue was purified by CCLTC on the chromatotron. Several successive purifications were required to get pure compounds. In all cases, unreacted starting compound **2** was isolated. Chromatography eluent and yield of the isolated products are indicated below for each reaction.

*2.1.9. [1-[5′-O-Allyl-2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(3″-C-allyl-4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**8** and **9**)*

The general procedure was followed with allyl bromide (0.026 ml, 0.3 mmol). Two successive purifications first, with dichloromethane:methanol (20:1) and then with hexane:ethyl acetate (3:4) afforded from the fastest moving band 0.014 g (13%) of **9** as a white amorphous solid.

- The slowest moving band gave 0.03 g (28%) of **8** as a white amorphous solid.
- Recovered starting material **2** (0.03 g).

*2.1.10. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-O-(3,3-dimethylallyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-3″-C-(3,3-dimethylallyl)-1″,2″-oxathiole-2″,2″-dioxide) (**11** and **12**)*

The general procedure was followed with 3,3-dimethylallyl bromide (0.036 ml, 0.3 mmol). Two successive purifications, first with dichloromethane:methanol (20:1) and then with hexane:ethyl acetate (3:4) afforded from the fastest moving band 0.015 g (13%) of **12** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.68, 1.69 (2s, 6H, $(CH_3)_2$–C), 1.89 (s, 3H, CH_3-5), 3.23 (m, 2H, $(CH_3)_2$C=CH–C$\underline{H}_2$), 3.26 (s, 3H, N–CH_3), 3.80 (m, 1H, H-5′$_a$, $J_{4',5'a} = 1.4$, $J_{5'a,5'b} = 12.7$ Hz), 3.99 (m, 1H, H-5′$_b$, $J_{4',5'b} = 2.4$ Hz), 4.30 (dd, 1H, H-4′), 4.92 (d, 1H, H-2′),

5.25 (m, 1H, $(CH_3)_2C$=CH), 5.84 (bs, 1H, OH-5′), 5.94 (d, 1H, H-1′, $J_{1',2'} = 8.2$ Hz), 6.12 (bs, 2H, NH_2-4″), 7.91 (s, 1H, H-6). Anal. calcd. for $C_{24}H_{39}N_3O_8SSi$: C, 51.68; H, 7.05; N, 7.53; S, 5.75. Found: C, 51.61; H, 7.08; N, 7.45; S, 5.70.

The slowest moving band gave 0.03 g (26%) of **11** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.91, 1.92 (2s, 6H, $(CH_3)_2$–C), 1.97 (s, 3H, CH_3-5), 3.24 (s, 3H, N–CH_3), 3.81 (dd, 1H, H-5′a, $J_{4',5'a} = 1.7$ Hz, $J_{5'a,5'b} = 11.6$ Hz), 3.86 (dd, 1H, H-5′b, $J_{4',5'b} = 2.7$ Hz), 4.29 (d, 2H, $(CH_3)_2C$=CH$_2$, $J = 7.0$ Hz), 4.41 (m, 1H, H-4′), 4.66 (d, 1H, H-2′), 5.47 (m, 1H, CH =), 5.76 (s, 1H, H-3″), 6.23 (d, 1H, H-1′, $J_{1',2'} = 8.1$ Hz), 6.39 (bs, 2H, NH_2-4″), 7.86 (s, 1H, H-6). Anal. calcd. for $C_{24}H_{39}N_3O_8SSi$: C, 51.68; H, 7.05; N, 7.53; S, 5.75. Found: C, 51.80; H, 6.98; N, 7.75.

Recovered starting material **2** (0.03 g).

*2.1.11. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-O-methyl-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**13**)*

The general procedure was followed with metyl iodide (0.019 ml, 0.3 mmol). Three successive purifications with dichloromethane:methanol (50:1) gave 0.01 g (13%) of **13** as a white amorphous solid.^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.91 (s, 3H, CH_3-5), 3.24 (s, 3H, N–CH_3), 3.57 (s, 3H, OCH_3), 3.78 (m, 2H, 2 H-5′), 4.39 (m, 1H, H-4′), 4.70 (d, 1H, H-2′), 5.74 (s, 1H, H-3″), 6.21 (d, 1H, H-1′, $J_{1',2'} = 7.9$ Hz), 6.38 (bs, 2H, NH_2-4″), 7.87 (s, 1H, H-6). Anal. calcd. for $C_{20}H_{33}N_3O_8SSi$: C, 47.70; H, 6.60; N, 8.34; S, 6.37. Found: C, 47.50; H, 6.50; N, 8.10; S, 6.20.

Recovered starting material **2** (0.05 g).

*2.1.12. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-O-isobutyl-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**14**)*

The general procedure was followed with isobutyl bromide (0.034 ml, 0.3 mmol). The reaction mixture was stirred at 70°C overnight. Two successive purifications, first with dichloromethane:methanol (50:1) and then with hexane:ethyl acetate (1:1) afforded 0.01 g (10%) of **14** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.02 (m, 6H, $(CH_3)_2C$), 1.91 (s, 3H, CH_3-5), 2.07 (m, 1H, $(CH_3)_2$CH), 3.24 (s, 3H, N–CH_3), 3.42 (dd, 1H, $(CH_3)_2$CH–CH$_2$O), 3.56 (dd, 1H, $(CH_3)_2$CH–CH$_2$O, $J = 9.6$ Hz), 3.83 (dd, 1H, H-5′a, $J_{4',5'a} = 0.98$ Hz, $J_{5'a,5'b} = 11.7$ Hz), 3.88 (dd, 1H, H-5′b, $J_{4',5'b} = 2.8$ Hz), 4.41 (m, 1H, H-4′), 4.62 (d, 1H, H-2′), 5.79 (s, 1H, H-3″), 6.22 (d, 1H, H-1′, $J_{1',2'} = 8.1$ Hz), 6.39 (bs, 2H, NH_2-4″), 7.72 (s, 1H, H-6). Anal. calcd. for $C_{23}H_{39}N_3O_8SSi$: C, 50.62; H, 7.20; N, 7.70; S, 5.87. Found: C, 50.58; H, 7.10; N, 7.67.

Recovered starting material **2** (0.04 g).

*2.1.13. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-O-cyclopropylmethyl-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**15**)*

The general procedure was followed with cyclopropylmethyl bromide (0.034 ml, 0.3 mmol). The reaction mixture was stirred at 70°C overnight. Two successive purifications with dichloromethane:acetone (50:1) afforded 0.02 g (15%) of **15** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 0.32, 0.62, 1.27 (3m, 3H, cyclopropyl), 1.88 (s, 3H, CH_3-5), 3.24 (s, 3H, N–CH_3), 3.40 (dd, 1H, cyclopropyl–CH$_2$O), 3.80 (dd, 1H, cyclopropyl–CH$_2$O), 3.88 (m, 2H, 2 H-5′), 4.42 (m, 1H, H-4′), 4.69 (d, 1H, H-2′), 5.78 (s, 1H, H-3″), 6.24 (d, 1H, H-1′, $J_{1',2'} = 8.1$ Hz), 6.47 (bs, 2H, NH_2-4″), 7.85 (s, 1H, H-6). Anal. calcd. for $C_{23}H_{37}N_3O_8SSi$: C, 50.80; H, 6.86; N, 7.73; S, 5.90. Found: C, 50.78; H, 6.50; N, 7.50; S, 5.89.

Recovered starting material **2** (0.035 g).

*2.1.14. [1-[5′-O-Benzyl-2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-3″-C-benzyl-1″,2″-oxathiole-2″,2″-dioxide) (**16** and **17**)*

The general procedure was followed with benzyl bromide (0.036 ml, 0.30 mmol). Two successive purifications, first with dichloromethane:methanol (50:1) and then with hexane:ethyl acetate (1:2) afforded from the fastest moving band 0.005 g (5%) of **17** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.88 (s, 3H, CH_3-5), 3.25 (s, 3H, N–CH_3), 3.74 (m, 2H,

CH_2Ph), 3.81 (m, 1H, H-5′$_a$), 4.00 (m, 1H, H-5′$_b$), 4.37 (m, 1H, H-4′), 4.94 (d, 1H, H-2′), 5.85 (m, 1H, OH), 5.97 (d, 1H, H-1′, $J_{1',2'} = 8.3$ Hz), 6.28 (s, 1H, NH_2-4″), 7.30 (m, 6H, Ph, H-6). Anal. calcd. for $C_{25}H_{35}N_3O_8SSi$: C, 53.08; H, 6.24; N, 7.43; S, 5.67. Found: C, 53.00; H, 6.21; N, 7.42; S, 5.65.

The slowest moving band gave 0.04 g (47%) of **16** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.60 (s, 3H, CH_3-5), 3.22 (s, 3H, N–CH_3), 3.90 (dd, 1H, H-5′a, $J_{4',5'a} = 2.9$, $J_{5'a,5'b} = 11.7$ Hz), 3.96 (dd, 1H, H-5′b, $J_{4',5'b} = 1.7$ Hz), 4.46 (m, 1H, H-4′), 4.68 (d, 1H, H-2′), 4.83 (s, 2H, CH_2Ph), 5.78 (s, 1H, H-3″), 6.22 (d, 1H, H-1′, $J_{1',2'} = 8.1$ Hz), 6.40 (bs, 2H, NH_2-4″), 7.41 (m, 5H, Ph), 7.70 (s, 1H, H-6). Anal. calcd. for $C_{25}H_{35}N_3O_8SSi$: C, 53.08; H, 6.24; N, 7.43; S, 5.67. Found: C, 53.00; H, 6.21; N, 7.42; S, 5.65.

Recovered starting material **2** (0.04 g).

*2.1.15. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-O-(3-methylbenzyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**18**)*

The general procedure was followed with 3-methylbenzyl bromide (0.04 ml, 0.3 mmol). The reaction mixture was stirred at 60°C overnight. Two successive purifications, first with dichloromethane:methanol (100:1) and then with hexane:ethylacetate (1:1) afforded 0.05 g (50%) of **18** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.54 (s, 3H, CH_3-5), 2.29 (s, 3H, CH_3), 3.18 (s, 3H, N–CH_3), 3.84 (dd, 1H, H-5′$_a$, $J_{4',5'a} = 1.7$, $J_{5'a,5'b} = 11.7$ Hz), 3.94 (dd, 1H, H-5′$_b$, $J_{4',5'b} = 2.8$ Hz), 4.41 (m, 1H, H-4′), 4.63 (d, 1H, H-2′), 4.75 (d, 2H, CH_2Ph, $J = 1.4$ Hz), 5.75 (s, 1H, H-3″), 6.19 (d, 1H, H-1′, $J_{1',2'} = 8.1$Hz), 6.37 (bs, 2H, NH_2-4″), 7.22 (m, 4H, Ph), 7.66 (s, 1H, H-6). Anal. calcd. for $C_{27}H_{39}N_3O_8SSi$: C, 54.62; H, 6.62; N, 7.08; S, 5.40. Found: C, 54.10; H, 6.41; N, 6.92; S, 5.35.

Recovered starting material **2** (0.03 g).

*2.1.16. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-O-(4-methylbenzyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**19**)*

The general procedure was followed with 4-methylbenzyl bromide (0.04 ml, 0.3 mmol). The reaction mixture was stirred at 70°C overnight. Two successive purifications with dichloromethane:methanol (100:1) afforded 0.06 g (51%) of **19** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.56 (s, 3H, CH_3-5), 2.28 (s, 3H, CH_3), 3.17 (s, 3H, N–CH_3), 3.80 (dd, 1H, H-5′$_a$, $J_{4',5'a} = 1.7$, $J_{5'a,5'b} = 11.7$ Hz), 3.91(dd, 1H, H-5′$_b$, $J_{4',5'b} = 2.8$ Hz), 4.40 (m, 1H, H-4′), 4.63 (d, 1H, H-2′), 4.75 (s, 2H, CH_2Ph), 5.74 (s, 1H, H-3″), 6.19 (d, 1H, H-1′, $J_{1',2'} = 8.1$ Hz), 6.35 (bs, 2H, NH_2-4″), 7.28 (m, 4H, Ph), 7.66 (s, 1H, H-6). Anal. calcd. for $C_{27}H_{39}N_3O_8SSi$: C, 54.62; H, 6.62; N, 7.08; S, 5.40. Found: C, 54.40; H, 6.21; N, 7.02; S, 5.25.

Recovered starting material **2** (0.025 g).

*2.1.17. [1-[5′-O-(Benzyloxycarbonylmethyl)-2′-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**21**)*

A mixture of **2** (Ingate et al., 1995) (0.10 g, 0.2 mmol), benzyl bromoacetate (0.06 g, 0.4 mmol) and potassium carbonate (0.03 g, 0.22 mmol) in anhydrous DMF (2 ml) was heated at 70°C for 24 h. The solvent was removed in vacuo and water (5 ml) was added to the residue. The mixture was extracted twice with ethyl acetate (10 ml) and the organic layer was dried (Na_2SO_4), filtered and evaporated. The residue was purified by CCTLC on the chromatotron. Two successive purifications, first with dichloromethane:methanol (20:1) and then with hexane:ethyl acetate (2:1) afforded 0.06 g (53%) of **21** as a white foam. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.80 (s, 3H, CH_3-5), 3.23 (s, 3H, N–CH_3), 3.99 (m, 2H, H-5′), 4.44 (m, 1H, H-4′), 4.55 (dd, 2H, $COCH_2O$, $J = 12.4$ Hz), 4.77 (d, 1H, H-2′), 5.25 (dd, 2H, CH_2Ph, $J = 12.3$ Hz), 5.75 (s, 1H, H-3″), 6.25 (d, 1H, H-1′, $J_{1',2'} = 8.1$ Hz), 6.44 (bs, 2H, NH_2-4″), 7.37 (m, 5H, Ph), 7.85 (s, 1H, H-6). ^{13}C-NMR [$(CD_3)_2CO$, 50 MHz] δ: 13.17 (N–CH_3), 27.88 (CH_3-3), 67.39, 68.93, 70.64 ($COCH_2O$, CH_2Ph, CH_2-5′), 75.27 (C-2′), 83.89, 86.67 (C-3″, C-4′), 92.55 (C-1′), 94.33 (C-3′), 110.25 (C-5), 129.13, 129.33 (Ph) 134.41 (Ph, C-6), 151.26, 152.29 (C-2, C-4″), 163.45 (C-4), 170.53 (COO). Anal. calcd. for $C_{28}H_{39}N_3O_{10}SSi$: C, 52.73; H, 6.16; N, 6.59; S, 5.03. Found: C, 52.70; H, 6.14; N, 6.19; S, 5.00.

*2.1.18. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-deoxy-5′-phenylthio-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**22**)*

Tri-*n*-butylphosphine (0.18 ml, 0.72 mmol) was added to a suspension of **2** (Ingate et al., 1995) (0.10 g, 0.20 mmol) and diphenyl disulfide (0.10 g, 0.47 mmol) in dry pyridine (2 ml). The mixture was stirred at room temperature for 24 h and then evaporated to dryness. The residue was coevaporated successively with ethanol (3 × 10 ml) and dichloromethane (3 × 10 ml) and then purified by CCTLC on the chromatotron using dichloromethane:methanol (40:1) as eluent to give 0.11 g (91%) of **22** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.88 (s, 3H, CH_3-5), 3.26 (s, 3H, N–CH_3), 3.35 (dd, 1H, H-5′a, $J_{4',5'a} = 9.7$, $J_{5'a,5'b} = 14.4$ Hz), 3.51 (dd, 1H, H-5′b, $J_{4',5'b} = 3.5$ Hz), 4.28 (m, 1H, H-4′), 5.09 (d, 1H, H-2′), 5.66 (d, 1H, H-1′, $J_{1',2'} = 7.1$ Hz), 5.75 (s, 1H, H-3″), 6.65 (bs, 2H, NH_2-4″), 7.32 (m, 5H, Ph), 7.60 (s, 1H, H-6). Anal. calcd. for $C_{25}H_{35}N_3O_7S_2Si$: C, 51.61; H, 6.06; N, 7.22. Found: C, 51.86; H, 6.21; N, 7.33.

*2.1.19. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-deoxy-5′-(N,N-diethyl)amino-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′-O-(tert-butyldimethylsilyl)-5′-deoxy-β-D-eritro-pent-4′-ene-furanosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**23** and **24**)*

To a stirred solution of **20** (Ingate et al., 1995) (0.15 g, 0.23 mmol)in dry acetonitrile (5 ml) was added an excess of *N*,*N*-diethylamine (0.19 ml, 1.15 mmol). The solution was heated in a sealed tube at 70°C for 20 h. After evaporation of the solvent, the residue was purified by CCTLC on the chromatotron (dichloromethane:methanol, 90:1) to give, from the fastest moving band 0.02 g (15%) of **24** as a syrup. ^{1}H-NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.90 (s, 3H, CH_3-5), 3.26 (s, 3H, N–CH_3), 4.44 (d, 1H, H-5′$_a$, $J = 3.1$ Hz), 4.69 (d, 1H, H-5′$_b$), 5.03 (d, 1H, H-2′), 5.86 (s, 1H, H-3″), 6.32 (bs, 2H, NH_2-4″), 6.35 (d, 1H, H-1′, $J_{1',2'} = 7.9$ Hz), 7.65 (s, 1H, H-6). Anal. calcd. for $C_{19}H_{29}N_3O_7SSi$: C, 48.39; H, 6.20; N, 8.91; S, 6.79. Found C, 48.23; H, 6.28; N, 8.99; S, 6.75.

From the slowest moving band 0.04 g (32%) of **23** was isolated as an amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 0.99 (t, 6H, 2 C$\underline{H}_3$CH$_2$N, $J = 7.2$ Hz), 2.04 (s, 3H, CH_3-5), 2.60 (q, 4H, 2 CH$_3$–C$\underline{H}_2$N), 2.93 (m, 2H, H-5′), 3.26 (s, 3H, N–CH_3), 4.28 (m, 1H, H-4′, $J_{4',5'} = 2.8$ Hz), 4.96 (d, 1H, H-2′), 5.63 (d, 1H, H-1′, $J_{1',2'} = 7.0$ Hz), 5.64 (s, 1H, H-3″), 6.57 (bs, 2H, NH_2-4″), 7.71 (s, 1H, H-6). Anal. calcd. for $C_{23}H_{40}N_4O_7SSi$: C, 50.71; H, 7.40; N, 10.29; S, 5.89. Found: C, 50.83; H, 7.58; N, 10.25; S, 5.78.

*2.1.20. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-deoxy-5′-N-methylpiperazinyl-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′-O-(tert-butyldimethylsilyl)-5′-deoxy-β-D-eritro-pent-4′-ene-furanosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**25** and **24**)*

To a stirred solution of **20** (Ingate et al., 1995) (0.15 g, 0.23 mmol) in dry acetonitrile (5 ml) was added an excess of *N*-methylpiperazine (0.13 ml, 1.15 mmol). The solution was refluxed for 6 h. After evaporation of the solvent, the residue was purified by CCTLC on the chromatotron (dichloromethane:methanol, 5:1). From the fastest moving band 0.03 g (21%) of **24** was isolated.

From the slowest moving band 0.07 g (54%) of **25** was isolated as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz] δ: 1.89 (s, 3H, CH_3-5), 2.10 (s, 3H, C$\underline{H}_3$N–$(CH_2)_2(CH_2)_2$), 2.26, 2.39 (m, 8H, 4 CH_2N), 2.58 (dd, 1H, H-5′a, $J_{4',5'a} = 4.8$, $J_{5'a,5'b} = 13.4$ Hz), 2.72 (dd, 1H, H-5′b, $J_{4',5'b} = 7.9$ Hz), 3.16 (s, 3H, N–CH_3), 4.31 (dd, 1H, H-4′), 4.50 (d, 1H, H-2′), 5.67 (s, 1H, H-3″), 5.88 (d, 1H, H-1′, $J_{1',2'} = 8.2$ Hz), 7.01 (bs, 2H, NH_2-4″), 7.76 (s, 1H, H-6). Anal. calcd. for $C_{24}H_{41}N_5O_7SSi$: C, 50.42; H, 7.23; N, 12.25; S, 5.61. Found: C, 50.37; H, 7.15; N, 12.45; S, 5.40.

*2.1.21. [1-[5′-N-Benzylpiperazinyl-2′-O-(tert-butyldimethylsilyl)-5′-deoxy-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) and [1-[2′-O-(tert-butyldimethylsilyl)-5′-deoxy-β-D-eritro-pent-4′-ene-furanosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**26** and **24**)*

To a stirred solution of **20** (Ingate et al., 1995) (0.15 g, 0.23 mmol) in dry acetonitrile (5 ml) was added an excess of *N*-benzylpiperazine (0.2 ml, 1.15 mmol). The solution was refluxed for 48 h. After evaporation of the solvent, the residue was purified by CCTLC on the chromatotron (dichloromethane:methanol, 20:1). From the fastest moving band 0.01 g (10%) of **24** was isolated.

From the slowest moving band 0.12 g (78%) of **26** was isolated as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.89 (s, 3H, CH_3-5), 2.41, 2.52 (m, 8H, 4 CH_2N), 2.89 (m, 2H, H-5′), 3.26 (s, 3H, N–CH_3), 3.45 (s, 2H, CH_2Ph), 4.32 (dd, 1H, H-4′, $J_{4',5'} = 4.1$ Hz), 4.95 (d, 1H, H-2′), 5.64 (s, 1H, H-3″), 5.66 (d, 1H, H-1′, $J_{1',2'} = 7.2$Hz), 6.49 (bs, 2H, NH_2-4″), 7.25 (m, 5H, Ph), 7.60 (s, 1H, H-6). Anal. calcd. for $C_{30}H_{45}N_5O_7SSi$: C, 55.62; H, 7.00; N, 10.81; S, 4.95. Found: C, 55.53; H, 6.98; N, 10.95; S, 4.88.

*2.1.22. [1-[2′-O-(tert-Butyldimethylsilyl)-5′-deoxy-5′-piperazinyl-β-D-ribofuranosyl]-3-N-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**27**)*

A solution of **26** (0.10 g, 0.15 mmol) in methanol (5 ml) containing Pd/C (10%) (0.02 g, 20 wt.%) was hydrogenated at **2** atm at 30°C for 24 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness, under reduced pressure, to give 0.05 g (57%) of **27** as a white amorphous solid. ^{1}H-NMR [$(CD_3)_2CO$, 200 MHz] δ: 1.90 (s, 3H, CH_3-5), 2.34, 2.50 (2m, 4H, 2 CH_2N), 2.82 (m, 4H, 2H-5′, 2 CH_2N), 3.26 (s, 3H, N–CH_3), 4.31 (dd, 1H, H-4′, $J_{4',5'} = 4.1$ Hz), 4.96 (d, 1H, H-2′), 5.64 (s, 1H, H-3″), 5.66 (d, 1H, H-1′, $J_{1',2'} = 7.2$ Hz), 6.50 (bs, 2H, NH_2-4″), 7.62 (s, 1H, H-6). Anal. calcd. for $C_{23}H_{39}N_5O_7SSi$: C, 49.53; H, 7.05; N, 12.56; S, 5.75. Found: C, 49.67; H, 7.09; N, 12.46; S, 5.60.

2.2. Biological methods

2.2.1. Anti-HIV evaluation

Human immunodeficiency virus type 1 [HIV-1 (III_B)] was obtained from Dr R.C. Gallo (when at the National Cancer Institute, Bethesda, MD). HIV-2 (ROD) was provided by Dr L. Montagnier (when at the Pasteur Institute, Paris, France). 4×10^5 CEM or 3×10^5 MT-4 cells per milliliter were infected with HIV-1 or HIV-2 at ~ 100 $CCID_{50}$ (50% cell culture infective dose) per milliliter of cell suspension. Then 100 μl of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 μl of the appropriate dilutions of the test compounds. After 4 days giant cell formation (CEM) or HIV-induced cytopathicity (MT-4) was recorded microscopically (CEM) or by trypan blue dye exclusion (MT-4) in the HIV-infected cell cultures. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) of the test compounds were defined as the compound concentrations required to inhibit virus-induced cytopathicity (CEM) or to reduce cell viability (MT-4) by 50%, or to reduce by 50% the number of viable cells in mock-infected CEM and MT-4 cell cultures, respectively.

3. Results and discussion

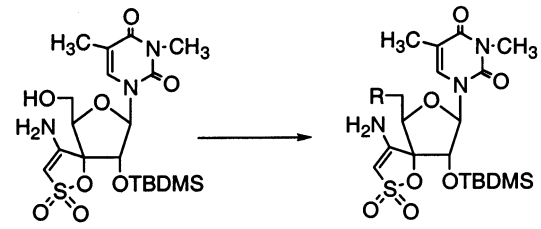
3.1. Chemical results

The synthesis of 5′-*O*-acyl derivatives **3** and **4** was carried out by reaction of the 5′-*O*-deprotected TSAO derivative **2** (Ingate et al., 1995) with acetic anhydride or isobutyryl chloride in pyridine (65 and 72% yields, respectively) (Scheme 1). Reaction of **2** with phenyl chloroformate (Fleming et al., 1973) followed by treatment with a 33% ethanolic solution of dimethylamine gave the 5′-*O*-carbamate TSAO derivative **5** (70%). Similarly, treatment of **2** with *N*,*N*′-thiocarbonyldiimidazole, and then with dimethylamine gave the corresponding 5′-*O*-thiocarbamate **6** in 51% yield.

The analytical and spectroscopic data of **3**–**6** were in agreement with the proposed structures. The ^{1}H-NMR spectra showed a downfield shift ($\Delta\delta$ 0.6–0.9 ppm) of the signals corresponding to the H-5′ protons, with respect to the same signals in the starting compound **2** (δ 3.91 ppm).

For the synthesis of TSAO derivative bearing a 5′-allyl group (**8**) we first assayed the neutral two-step allylation procedure reported by Guibe and Saint-M'Leux (1981) and further developed by Minami and Tsuji (1987)). This method involves the palladium (0) catalyzed decarboxylation of an allylcarbonate, obtained from the corresponding alcohol, to give an allyl ether derivative. This procedure has been successfully used in *O*-silyl-protected carbohydrates (Oltvoort et al., 1983; Boullanger et al., 1986). This prompted us to use it for the synthesis of the target 5′-*O*-allyl TSAO derivative **8** (Scheme 2). Thus, treatment of **2** (Ingate et al., 1995) with allyl chloroformate in the presence of pyridine gave the allylcarbonate **7** (60% yield). Reaction of **7** with a catalitic amount of palladium tetrakis(triphenylphosphine) gave the desired 5′-*O*-allyl derivative **8** in low yield (6%) together with the 3″-*C*-allyl TSAO derivatives **9** and **10**. Due to the low yield of the target 5′-*O*-allyl compound **8** and the side products obtained under these reaction conditions, we assayed the classical Williamson procedure for the synthesis of ethers (Stanek, 1990). Thus, reaction of **2** (Ingate et al., 1995) (Scheme 2) with an equimolar amount of NaH in DMF, followed by 'in situ' treatment with allyl bromide gave the 5′-*O*-allyl derivative **8** (28% yield) together with the 3″-*C*-allyl TSAO compound **9**. Several successive chromatographic purifications were required to get pure compounds. It should be noted that, as described in the literature for similar reactions (Benedit et al., 1979), unreacted starting material was recovered from the reaction. Addition of extra amounts of NaH (one or two additional equivalents), in order to consume all the starting material, led to extensive decomposition.

The synthesis of the 5′-*O*-ether TSAO derivatives **11**, **13**–**16**, **18** and **19** (Scheme 2) was performed following that above menctioned



Scheme 1. Synthesis of spironucleosides (**3**–**6**).

Williamson procedure. Thus, as described above, treatment of **2** (Ingate et al., 1995) with equimolar amounts of NaH in DMF followed by 'in situ' reaction with the appropriated alkenyl halide (3,3-dimethylallyl bromide) or alkyl halide (methyl iodide, isobutyl bromide, cyclopropylmethyl bromide) gave the corresponding 5′-*O*-ether substituted TSAO derivatives in moderate yields [**11** (26%), **13** (13%), **14** (10%), **15** (15%)]. Similarly, treatment of **2** with equimolar amounts of NaH in DMF followed by 'in situ' reaction with the appropriated benzyl halide (benzyl bromide, 3-methylbenzyl bromide or 4-methylbenzyl bromide) gave the 5′-*O*-benzyl ether substituted derivatives [**16** (47%), **18** (50%), **19** (51%)]. In the reactions with 3,3-dimethylallyl bromide or benzyl bromide the 5′-deprotected 3″-*C*-substituted compounds were also isolated [**12** (13%), **17** (5%)]. Again, in all of these reactions, varying amounts of starting material were recovered.

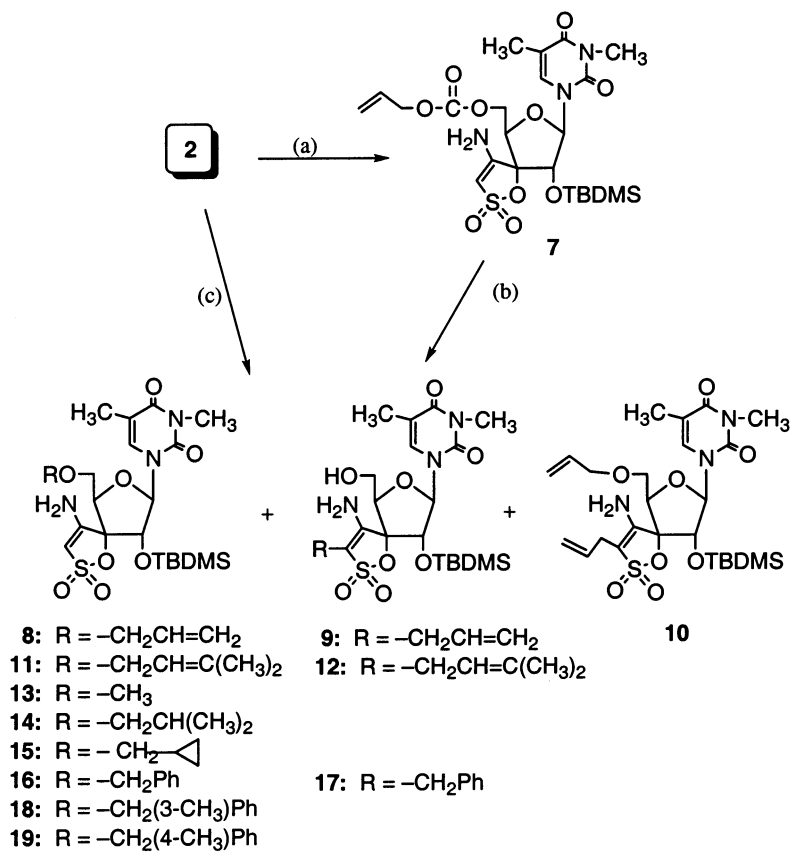
The 5′-*O*-ether TSAO derivatives **8**, **11**, **13**–**16**, **18** and **19** showed in their ^{1}H-NMR spectra the disappearance of the signal at 5.84 ppm assigned to the 5′-OH and the presence of a multiplet at δ 3.2–3.8 ppm corresponding to the O-CH_2 protons of the alkenyl, alkyl or benzyl moiety. No modification were observed at the signals of the protons of the spirooxathiol moiety (NH_2-4″ and H-3″) thus indicating that no alkylation had ocurred at this part of the molecule. On the other hand, formation of the 3″-*C*-alkenyl and 3″-*C*-benzyl compounds **9**, **12** and **17** was established by the disappearance of the signal of H-3″

(δ 5.77 ppm) and the presence of a multiplet at δ 3.14–3.74 ppm corresponding to the CH_2 protons of the alkenyl or benzyl moiety. No modification was observed at the signals corresponding to the 5′-OH and 4″-NH_2 with respect to those of the starting compound **2**.

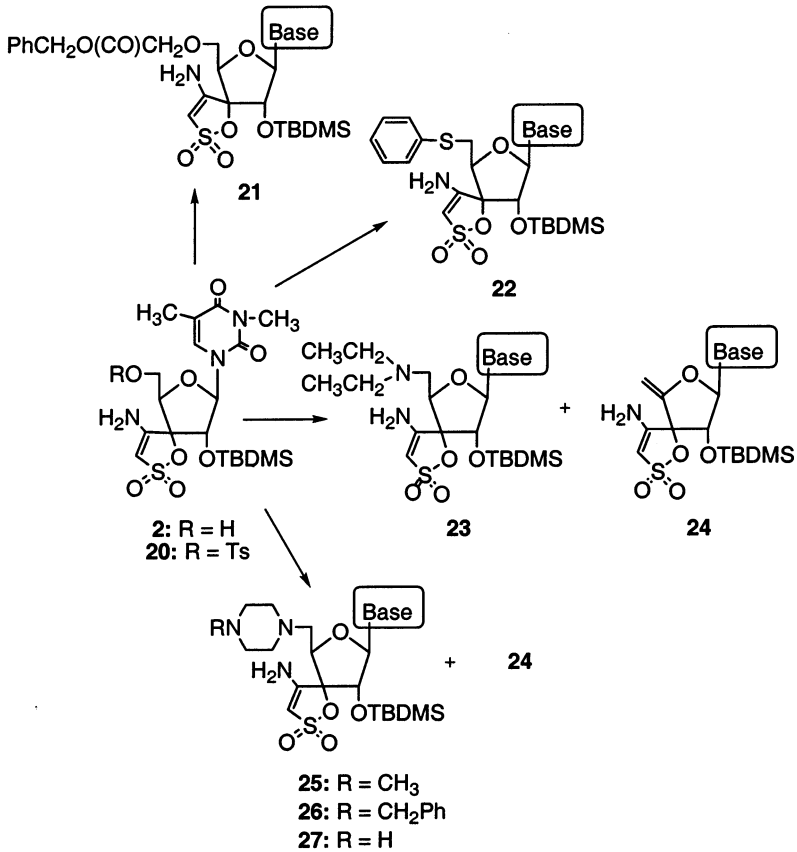
Other 5′-substituted TSAO analogues were prepared as follows. Treatment of **2** with benzyl bromoacetate, in the presence of potassium carbonate, in DMF at reflux (Scheme 3) gave the 5′-benzyloxycarbonylmethyl TSAO derivative **21** in 53% yield.

The phenylthio derivative **22** was obtained in 91% yield by reaction of **2** with diphenyl disulfide in the presence of tri-*n*-butylphosphine (Nakagawa and Hata, 1975; Nakagawa et al., 1983). However, reaction of **2** with dimethyl disulfide under the same reaction conditions failed to produce the desired 5′-methylthio derivative.

Finally, for comparative purposes we prepared a series of analogues possesing different hydrocarbon shapes capping an amine function at 5′-position. The 5′-*O*-tosyl derivative **20** (Ingate et al., 1995) (Scheme 3) was used as the common precursor for the synthesis of the target 5′-*N*-substituted TSAO compounds. Thus, treatment of **20** with excess of diethyl amine, methyl- and benzyl piperazine in refluxing acetonitrile, gave the corresponding 5′-*N*-substituted TSAO derivatives in good yields [**23** (32%), **25** (54%), **26** (78%)] together with the 4′,5′-didehydro nucleoside **24** (10–21%). A similar treatment of **20** with piperazine gave an unseparable mixture of compound **27** and *p*-toluensulfonic acid. Attempts to purify this



Scheme 2. Reaction conditions: (a) $ClCO_2CH_2CH{=}CH_2$, THF, Pyridine; (b) $Pd(PPh_3)_4$, THF; (c) NaH, RBr, DMF.



Scheme 3. Synthesis of spironucleosides (**21**–**27**).

mixture by different chromatographic systems were unsuccessful. However, pure compound **27** was obtained in 57% yield by catalytic hydrogenation (10% Pd/C) of the benzyl derivative **26**.

The new compounds **23**, **25**–**27** showed in their ^{1}H-NMR spectra the disappearance of the signals corresponding to the tosyl group and the strong upfield shift ($\Delta\delta$ 1.45–1.67 ppm) of the signals corresponding to the H-5′ protons with respect to the same signals in the starting compound **20** (δ 4.44 ppm).

The ^{1}H-NMR spectrum of the 4′,5′-didehydro nucleoside **24** showed the disappearance of the signals corresponding to the H-4′ and tosyl protons and the presence of a characteristic set of doublets ($J = 3.1$ Hz) centered at 4.5 ppm.

3.2. Biological results

Several TSAO derivatives were synthesized in which the 5′-TBDMS group was replaced by an acetyl (**3**), isobutyryl (**4**), (thio)carbamoyl (**5**, **6**) or allyloxycarbonyl (**7**) group. None of these compounds showed *anti*-HIV activity in CEM and MT-4 cell cultures at subtoxic concentrations, except for **3** that was not active in MT-4 cells, but showed some activity in CEM cells at a concentration that was five-fold lower than the toxicity of the compound. Their cellular toxicities ranged between 14 and 101 μM depending on the nature of the substituent. In contrast, several 5′-alkyl- (**13**, **14**), 5′-benzyl- (**16**, **18**, **19**) and 5′-alkenyl (**8**, **11**)-substituted TSAO derivatives were en-

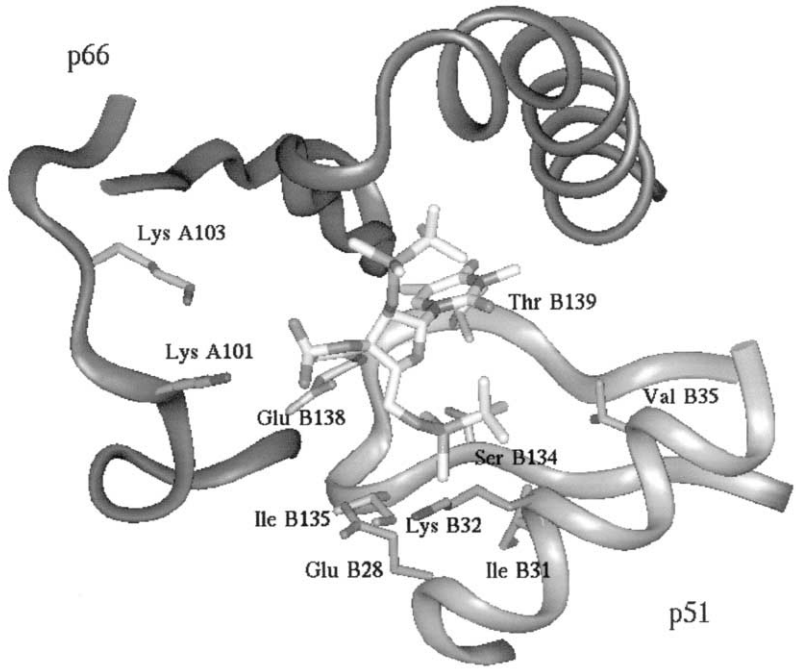


Fig. 2. Detail of the model proposed (Gago et al., 2000) for TSAO-T binding at the interface between the p66 and p51 subunits of HIV-1 reverse transcriptase. The C_α trace is displayed as a ribbon, colored dark for p66 and light for p51. The TSAO molecule and side chains of relevant amino acids are shown as sticks.

dowed with *anti*-HIV-1 activity at concentrations that were in most cases (i.e. **8**, **13**, **14**) well below the toxicity threshold. The most selective derivatives were the 5′-allyl (**8**) compound (selectivity index 10–25) and the 5′-isobutyl (**14**) derivative (selectivity index 5–30). The 5′-isopentenyl derivative **11** showed a selectivity of around 3. When substituted at the 5′-position by a piperazine entity (**25–27**), the TSAO derivatives lacked antiviral activity against HIV-1. Compound **27** showed activity against HIV-2 in MT-4 cells at a concentration that was three-fold lower of the CC_{50}, but no *anti*-HIV-2 activity was found in CEM cell cultures. Also, 5′-phenylthio TSAO (**22**) was not active. Interestingly, compound **24**, in which the 5′-*O*-TBDMS was removed leaving just a methylene at 4′ position of the sugar moiety, was inhibitory to HIV-1 at 3–3.5 μM, but poorly cytotoxic (CC_{50}: 85–97 μM). Consequently, the in vitro selectivity index of **24** increased to 30. We have also evaluated TSAO-m^3T and compounds **2–6** for their inhibitory activity against recombinant HIV-1 RT using poly rC.oligo dG as the template/primer and [^{3}H]dGTP as the radiolabeled substrate, to reveal whether the lack of antiviral activity of the 5′-acyl substituted **3–6** compounds was due to hydrolysis in cell culture to the inactive compound **2**. However, in contrast to TSAO-m^3T, which was inhibitory against HIV-1 RT at an IC_{50} of 3.5 μM, **2–6** were completely devoid of inhibitory activity at 500 μM. Thus, we concluded from these data that replacement of the 5′-TBDMS by an acyl moiety markedly diminishes or even annihiliates any *anti*-RT activity of the test compounds. We also concluded that introduction of an aromatic or hydrocarbon capped amine function at the 5′-position of TSAO seems to destroy *anti*-HIV-1 activity as well.

When tested against TSAO–m^3T-resistant HIV-1/138-Lys strain, in CEM cells, compounds

Table 1
Inhibitory activity of test compounds against HIV-1 and HIV-2 in MT-4 and CEM cell cultures

Comp.	EC_{50}[a,b] (μM)				CC_{50}[b,c] (μM)	
	MT-4		CEM		MT-4	CEM
	HIV-1	HIV-2	HIV-1	HIV-2		
1	0.06 ± 0.03	>4	0.06 ± 0.01	>4	14 ± 2.0	16 ± 1.0
3	>50	>50	17 ± 3.5	>50	75 ± 49	101 ± 0.7
4	>10	>50	≥10	>50	15 ± 6.2	65 ± 5.3
5	>50	>50	≥50	>50	75 ± 44	73 ± 22
6	>10	>10	>10	>10	14 ± 3.2	20 ± 1.6
7	>10	>10	>10	>10	16 ± 4.9	39 ± 2.2
8	11 ± 10	>50	4.0 ± 0.0	43.3 ± 11.5	97 ± 7.1	101 ± 32
11	6.5 ± 2.2	>10	8.5 ± 2.1	>10	22 ± 0.3	21 ± 0.5
12	>10	>10	>10	>10	16 ± 3.9	19 ± 1.0
13	12 ± 4	>50	6.2 ± 2.6	>50	50 ± 2.9	236 ± 20
14	4.5 ± 0.13	>10	0.60 ± 0.28	>10	20 ± 1.4	20 ± 1.7
15	8.5 ± 2.0	>10	3.0 ± 1.4	>10	15 ± 1.1	20 ± 1.8
16	5.2 ± 1.3	>10	>2	>2	5.3 ± 0.37	5.3 ± 0.72
18	15 ± 7.4	>10	4.0 ± 1.8	>2	5.8 ± 0.76	12 ± 5.8
19	>2	>10	>2	>2	5.7 ± 1.2	9.7 ± 4.7
21	>10	>10	>10	>10	31 ± 1.9	29 ± 1.2
22	>10	>10	>10	>10	23 ± 0.5	25 ± 2.1
23	≥50	>50	>50	>50	22 ± 3.3	53 ± 29
24	3.5 ± 1.6	>50	3.0 ± 0.0	>50	85 ± 12	97 ± 14
25	>50	>50	>50	>50	68 ± 46	71 ± 21
26	>10	>10	>10	>10	16 ± 9.0	18 ± 1.2
27	≥50	37 ± 6.9	≥50	>50	120 ± 6.4	69 ± 11

[a] 50% effective concentration.
[b] Results are the mean of 2–3 independent experiments.
[c] 50% cytostatic concentration.

1–6, **11**, **12**, **14**, **15**, **18**, **19**, **25** and **26** proved inactive, pointing to the importance of all compounds for their interaction with Glu-138 of the HIV-1 RT.

Only replacing the TBDMS moiety by an alkyl or alkenyl chain, or replacing the entire C-5′ group in the sugar molecule by a methylene group partially retained antiviral activity, although at a 20- to 100-fold lower potency than TSAO-m^3T or TSAO-T.

4. Discussion

As shown above, all our biological data indicated that replacement of the 5′-TBDMS group results in a marked decrease in the *anti*-HIV-1 activity.

In parallel to our work of synthesis and biological evaluation, the compounds synthesized were docked in our model of interaction of TSAO derivatives with the HIV-1 RT (Camarasa et al., 2000). Our working model located the 5′-TBDMS into the cavity formed by the aromatic rings of Tyr-A181, Tyr-A188 and Trp-A229. According to this model, the compounds described in this manuscript fitted in that aromatic cavity. However, the model failed to explain the large decrease observed in the *anti*-HIV-1 activity of these compounds. So, despite the relative success of this model in accounting for many experimental findings, the data here presented, and other observa-

tions of our laboratory (Camarasa and Balzarini, 2001) argued against it.

In the light of all these experimental data, we tried to find an alternative binding model. Results from docking studies on HIV-1 RT and molecular dynamics simulations of the complexes have led us to propose a binding mode for TSAO-T distinct from that of 'classical' NNRTIs (Gago et al., 2000), the new model of interaction is highlighted here. In the refined modeled complex TSAO-T binds at the interface between the two subunits (Fig. 2), in agreement with the experimental results. The spiro ring is oriented in the electrostatic field of the protein such that the amino group is close to the negatively charged Glu 138 in the p51 subunit and the sulfo group points to the positively charged side chains of Lys-101 and -103 in the p66 subunit. In this orientation, the bulky 5′-TBDMS substituent is placed in a large hydrophobic pocket made up by the side chains of a number of amino acid residues from the p51 subunit, most importantly Ile-135, Ile-31, Val-35, and the apolar part of Lys-32. The contribution of the 5′ substituent to the binding free energy is thus expected to depend largely on dispersion interactions and the hydrophobic effect resulting from the desolvation of apolar groups on both the protein and the ligand. Replacement of TSAO-m^3T with **14** in the same binding site results in a decrease of buried protein surface area of 15–25 $Å^2$, which can be translated into a free energy difference of about 1.5 kcal/mol, roughly equivalent to the decrease by one order of magnitude in binding constant or EC_{50} (Ajay and Murcko, 1995). The fact that our data revealed that TBDMS cannot be replaced by any other group without a significant loss of affinity (with the possible exception of the *tert*-hexyldimethylsilyl group (Ingate et al., 1995) indirectly supports the proposed binding model and underscores the stringent requirements for this group at this position Table 1.

Acknowledgements

We thank Ann Absillis and Lizette van Berckelaer for excellent technical assistance. We also thank the Ministry of Education of Spain for a grant to C.C. The Spanish CICYT (Project SAF 2000-0153-C02-01) the Comunidad de Madrid (Projects 08.2/0031.1/1999 and 08.2/0044/2000) and the European Commission (Project QLK2-CT-2000-00291) are also acknowledged for financial support.

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