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Bioorganic & Medicinal Chemistry 16 (2008) 4733–4741
Bioorganic & Medicinal Chemistry

Synthesis and anti-HIV1 biological activity of novel 5″-ATSAO compounds

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Received 12 November 2007; revised 1 February 2008; accepted 8 February 2008
Available online 13 February 2008

Abstract—Aza TSAO-T derivatives bearing a substituted dihydroisothiazole dioxide ring with a phenyl group at 5″ position were prepared. Biological evaluation showed that phenyl group gives rise to a dramatical decrease of the inhibitory effect.

1. Introduction

Since the identification of the human immunodeficiency virus (HIV) in 1983–1984 as the etiological agent for AIDS, many therapeutic strategies have been proposed involving the viral enzymes that have critical roles in the life cycle of the virus as key targets in the search for effective drugs.

Because of the essential role of reverse transcriptase (RT) in the viral replication, this enzyme has been a key target in the development of novel anti-HIV drugs. This heterodimeric enzyme consisting of p66 (560 amino acids) and p51 (440 amino acids) subunits has three enzymatic activities: a DNA-dependent and a RNA-dependent DNA polymerase activity, and RNase-H activity, which plays a direct role in the conversion of a single-stranded RNA genome into the double-stranded proviral DNA. It has been recognized that the RT p66/p51 heterodimer is the biologically active form of the enzyme, while the monomeric subunits are inactive.[1] Structural analysis reveals three major contacts between the p66 and p51 subunits, with most of the interaction surfaces being largely hydrophobic.[2] The three contacts comprise an extensive dimer interface that includes the fingers subdomain of p51 with the palm of p66. In this sense, the β7–β8 loop in p51 subunit is essential for catalytic function on the p66 subunit.[3]

Non-nucleosides reverse transcriptase inhibitors (NNRTIs) represent a particular group of compounds which bind to a hydrophobic pocket near, but not at the polymerase active site of p66. These compounds induce restrictions in the dynamics of the enzyme, resulting in an inactive conformation. [2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)thymine (TSAO-T) was firstly described in 1992 by Camarasa et al.[4] as a specific anti-HIV agent (Fig. 1). This compound was the prototype of a new and unique class of nucleoside analogues with a specific inhibition against HIV-1, acting through a

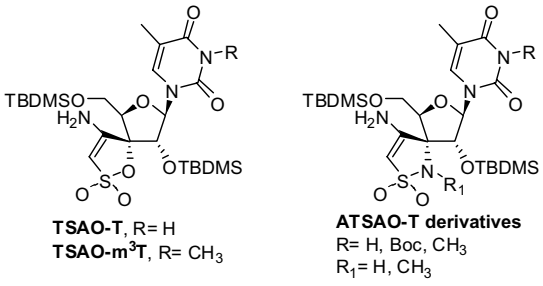

Figure 1. Aza analogues of TSAO.

Keywords: Glycoaminonitrile; CSIC reaction; TSAO; ATSAO; NNRTI.
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doi:10.1016/j.bmc.2008.02.026

non-competitive mechanism against the substrate and template/primer.[4c] Extensive structure–activity studies have been carried out to assess the structural requirements for their optimal interaction with HIV-1 RT. The *ribo* sugar plays an essential role in the interaction with the enzyme and the simultaneous presence of a 3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) moiety and TBDMS groups at both the 2′ and 5′ positions of the sugar moiety is a prerequisite for antiviral activity.[5] Modifications on the groups at 2′ and 5′ positions,[6] on the 3′-spiro moiety,[7] as well as on the base part,[8] have been performed and evaluated. In comparison with the NRTIs and NNRTIs, resistance of HIV-1 RT to TSAO type of compounds due to the Glu138 → Lys mutation has been observed, hence suggesting that they may interact with Glu138 residue, located in the β7–β8 loop in p51. The phenomenon of resistance and eagerness for complete elucidation of the precise binding mode of drug to RT has prompted the exploration of further structural modifications of TSAO analogues.

Recently, Camarasa et al. reported the synthesis and evaluation of several compounds bearing at the N-3 position a variety of polar, lipophilic or aromatic groups linked to that position through flexible polymethylene linkers. The TSAO derivative with a *N*-methylcarboxamidobutyl group at the N-3 position was 5- to 6-fold more active than the TSAO-T.[9]

Our interest in carbohydrate chemistry, especially that pertaining to glyco-α-aminonitriles,[10] and their derivatives,[11] encouraged us to extend our investigations into the carbanion-mediated sulfonamide intramolecular cyclizations (*CSIC* reaction)[12] to the synthesis of a new family of aza-TSAO compounds, abbreviated as ATSAOs. Recently, we reported the first synthesis of ATSAOs in which the 3′-spiro-oxathiazole ring is substituted with a spiro-isothiazole moiety (Fig. 1).[13] The evaluation of the inhibitory effects on HIV-1 replication demonstrated that these ATSAO derivatives showed the same HIV-1 specific activity spectrum as the TSAO derivatives published by Camarasa et al.[4] ATSAO derivatives that did not contain a methyl on the nitrogen atom in the spiro moiety were potent inhibitors of HIV-1 in CEM and MT-4 cell cultures (EC_{50}: 0.13–0.53 μM) but showed no inhibition activity against HIV-2 at subtoxic concentrations. The computational study revealed that the ATSAO compounds adopt a similar conformation at the p66–p51 interface than for the TSAO analogues.[14]

Continuing with our work in this area, and in order to gain a deeper insight into the structure-activity relationship of ATSAO derivatives, herein we report, in full, the synthesis and HIV-inhibition of new 5″-phenyl ATSAO-T derivatives **13**–**20**.

2. Results and discussion

2.1. Synthesis of the nucleosides

Based on the previous work from our laboratory,[13] in Chart 1 we have shown our planned retrosynthetic analysis for the synthesis of the new ATSAO nucleosides, based on a simple synthetic sequence that includes: (i) aminocyanation of the monosaccharidic substrate; (ii) N-glycosylation to give the nucleosidic scaffold; and (iii) the carbanion-mediated sulfonamide intramolecular cyclization (*CSIC* reaction) to introduce the spiro-dihydroisothiazolic ring at the 3′-position.[12]

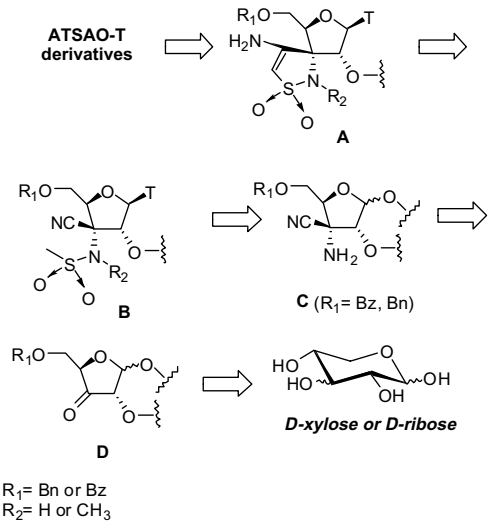
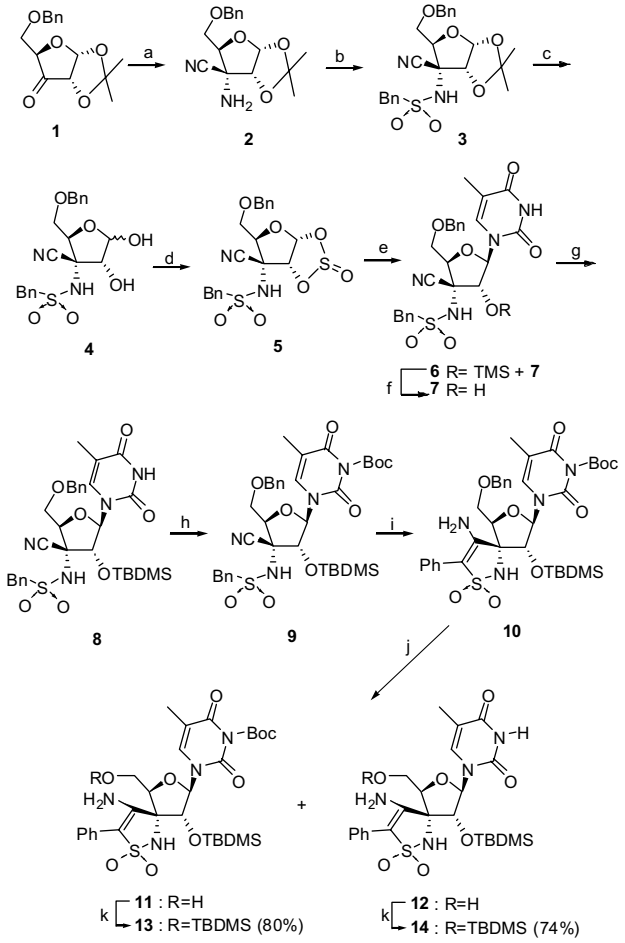


Chart 1. Key intermediates for the access to ATSAOs.

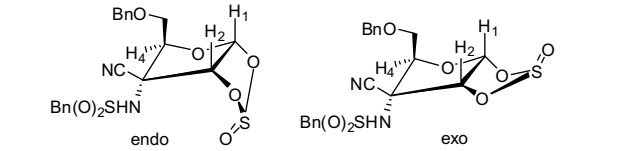
In our initial work, we selected derivatives **C** (Chart 1), bearing a 5-*O*-benzyl and 5-*O*-benzoyl as protecting groups, a choice based on such groups are well known to be readily cleaved under mild conditions to facilitate the incorporation of the TBDMS group at the oxygen at C-5′. Such intermediates can be derived from substrates **D**, which are readily available from either D-ribose or D-xylose (Chart 1). Starting from the 5-*O*-benzoyl derivative, the N-glycosylation step affords an epimeric mixture of thymine derivatives resulting from the probable anchimeric assistance of the benzoyl group. In order to control the stereospecificity of the N-glycosylation, our investigations targeted to accessing 5″-phenyl derivatives of ATSAO-T were successful starting from the 5-*O*-benzyl derivatives.

2.2. Synthesis of the 5″-phenyl ATSAO nucleosides (13–16, 20)

The 3-*R* glyco-α-aminonitrile **2** was obtained stereoselectively in 70%.[10] Subsequent treatment with $BnSO_2Cl$–pyridine–DMAP gave the key benzylsulfonamidonitrile **3** in 98% yield (Scheme 1). Like the methanesulfonamidonitrile derivatives,[13] the introduction of thymine or uracil using the N-glycosylation Vorbrüggen method on the substrate **3** was inefficient. Therefore, we investigated the fusion procedure,[15] by using silylated thymine. Thus, treatment of **4** with $SO(Im)_2$ in THF, prepared from thionyle chloride and imidazole, yielded the corresponding sulfite derivatives **5** in 75% as an *endo*/*exo* mixture, as was firmly established after detailed inspection of the NMR spectroscopic data (Chart 2). Subsequent N-glycosylation at 125 °C using silylated base in dry conditions, gave the 2′-*O*-silylated derivative



Scheme 1. Reagents and conditions: (a) i—$Ti(OiPr)_4$ (1.2 equiv), NH_3, MeOH; (ii) TMSCN (1.1 equiv) (70%); (b) $BnSO_2Cl$ (3 equiv), py, DMAP (0.5 equiv) (98%); (c) TFA/H_2O (9:1) (95%); (d) $SOIm_2$, THF (75%); (e) i—thymine, HMDS, $(NH_4)_2SO_4$, 135 °C; ii—MeOH, H_2O, reflux; (f) TBAF, MeOH (71%, for two steps); (g) TBDMSCl, imidazole, DMF (84%); (h) Boc_2O, CH_2Cl_2, py (75%); (i) LDA, THF, −78 °C (76%); (j) $Pd(OH)_2$, cyclohexene, EtOH, reflux (see the text); (k) TBDMSCl, imidazole, DMF, rt.



C-1 *endo*	C-1 *exo*	H-1 *endo*	H-1 *exo*	$J_{1,2}$ *endo* (Hz)	$J_{1,2}$ *exo* (Hz)
111.0	108.6	6.52	6.63	4.6	3.6

Chart 2. NMR shift of sulfite derivative (δ).

6 which upon treatment with TBAF in MeOH gave exclusively **7** in 71% yield.

The *tert*-butyldimethylsilyl group was introduced in the 2′-position using TBDMSCl and imidazole in DMF to afford **8** in 84% (Scheme 1). To avoid base lithiation during the CSIC step, the N-3 of **8** was protected and isolated as the *N*-Boc derivative **9** in 75% yield. Next, the dihydroisothiazolic derivative **10** was obtained efficiently using LDA in THF in 76% yield (Scheme 1).

Several routes were followed to deprotect both *O*-5′ and N-3′ positions (Scheme 1).

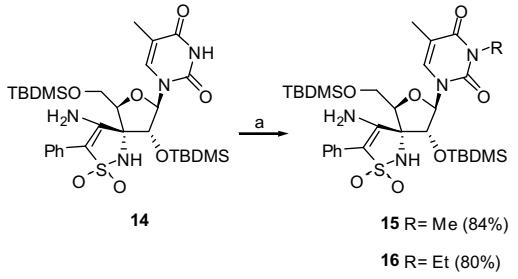
Unfortunately, mixtures of the debenzylated compounds **11** and **12** were obtained from **10** with $Pd(OH)_2$-cyclohexene in refluxing ethanol, in yields ranging from [**11** (80%), **12** (16%)] to [**11** (9%), **12** (89%)], depending on the reaction time, after 2 h 40 min or after 17 h, respectively. As shown, in the best reaction conditions, a small amount of the deprotected-Boc nucleoside **12** was isolated, but we also found conditions to obtain compound **12** as the major reaction product, a fact that allowed us to prepare aldo 3-*N*-alkyl, substituted derivatives (see below). Contrary to the alkylsulfonamide derivatives,[13,16] no polycyclic products were observed from Michael intramolecular cyclization between the *O*-5′ and the corresponding enamine. Finally, introduction of the 5′-*O*-TBDMS pharmacophore was achieved by treatment of the isolated derivatives **11** and **12** with TBDMSCl in DMF and imidazole to afford [1-(2′,5′-bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-5″-phenyl-1″,1″-dioxo-isothiazole) (**13**) (80%) and [1-(2′,5′-bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)]-3′-spiro-3″-(4″-amino-2″, 3″-dihydro-5″-phenyl-1″,1″-dioxo-isothiazole) (**14**) (74%), respectively (Scheme 1).

The regiospecificity of the N-3 alkylation step as observed previously[13] was applied to the synthesis of 5″-phenyl aza TSAO-m^{3}T and TSAO-et^{3}T.

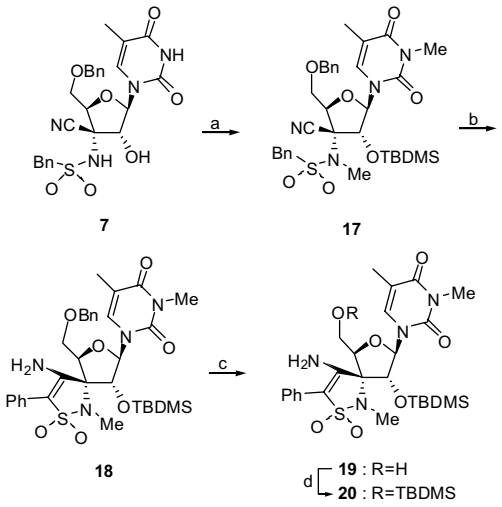
The introduction of the methyl and ethyl groups on **14** was achieved efficiently by a classical alkylation [MeI (2 equiv) or EtI (2 equiv) and K_2CO_3 (0.5 equiv)] in refluxing acetone to afford **15** (84%) and **16** (80%), respectively (Scheme 2).

The lack of reactivity previously observed in compounds with free sulfonamido groups at N-2″, towards methylating agents,[13] and the simultaneous one-pot CSIC reaction which we have also observed during the N-allylation, N-methylation and N-benzylation of monosaccharidic substrates,[16] encouraged us to investigate the synthesis of 5″-phenyl ATSAO derivatives having both N-3 and N-2″ methylated starting from the acyclic methanesulfonamido derivative **7**.

Compound **7** was permethylated using the system MeI/K_2CO_3, in acetonitrile to give **17** in 65% yield after



Scheme 2. Reagents: (a) CH_3I or C_2H_5I, K_2CO_3, acetone.



Scheme 3. Reagents and conditions: (a) CH_3I (2 equiv), K_2CO_3 (1.5 equiv), acetone, reflux, 24 h (65%); (b) Cs_2CO_3, (1 equiv), CH_3CN, reflux (90%); (c) $Pd(OH)_2$, cyclohexene, EtOH, reflux (99%); (d) TBDMSCl, imidazole, DMF, rt (75%).

24 h (Scheme 3). Improvements in the cyclization step from **17** using Cs_2CO_3 in refluxing acetonitrile resulted in the formation of **18** in 90% yield. It was interesting to note that, according to the results previously observed for the one-pot-N-alkylation plus CSIC reaction on phenylsulfonamido compounds,[16a] increasing the reaction time to 72 h afforded the cyclic derivative **18** in 40% overall yield from precursor **7**. It should be noted that the CSIC reaction provided a higher yield for the benzylsulfonamide derivative than for the methylated derivative.[13] Next, hydrogenolysis gave **19** quantitatively, which was silylated as described earlier, to afford **20** in 75% (Scheme 3).

3. Biological activity

The inhibitory activity against RT of HIV-1(III_B) and HIV-2(ROD) of compounds **13**–**16**, **20** was evaluated in CEM cell cultures (see Section 5). The results are shown in Table 1.

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

Compound	EC_{50} (μM)		CC_{50} (μM)
	HIV-1	HIV-2	
13	>50	30 ± 1.0	⩾10
14	⩾2	⩾2	⩾10
15	>50	>50	⩾10
16	>50	>50	⩾10
20	>2	>2	10.9 ± 4.53
ATSAO-T[a]	0.33 ± 0.11	>100	19.1 ± 0.4
TSAO-T	0.06 ± 0.010	>4	16 ± 1.0

EC_{50}, effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%. CC_{50}, cytotoxic concentration or concentration required to reduce CEM cell viability by 50%.

[a] (Fig. 1, R = R_1 = H).

4. Conclusion

In conclusion, various 5″-substituted ATSAO have been synthesized. Compounds **13**–**16**, **20** have been evaluated against HIV-1 and HIV-2 replication in CEM cells. According to the biological results, introduction of a phenyl group at the 5″-position clearly decreased the antiviral activity as previously described in the TSAO series.[17]

5. Experimental

5.1. Materials and methods

Melting points were determined on a digital melting-point apparatus (Electrothermal) and are uncorrected. Optical rotations were recorded in $CHCl_3$, MeOH, acetone, or DMSO with a digital polarimeter DIP-370 (JASCO) using a 1 dm cell. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, acetone-d_6, Me_2SO-d_6, or MeOD-d_3 (internal Me_4Si), respectively, at 300.13 MHz and at 75.47 MHz (Bruker Avance-300). TLC was performed on Silica F254 (Merck) and detection by UV light at 254 nm or by charring with phosphomolybdic acid–H_2SO_4 reagent. Column chromatography was effected on Silica Gel 60 (Merck, 230 mesh). Acetone, hexane, ethyl acetate, and diethyl ether were distilled before use. Bases and solvents were used as supplied. MeOH–NH_3 is methanol saturated (7 N) with ammonia gas at room temperature. Elemental analyses have been carried out in Madrid (IQOG, CSIC). ^{13}C NMR resonances have been assigned by using standard NMR (DEPT, COSY, HSQC) experiments. FTIR spectra were obtained on a AVATAR™ 320 neat using ATR and are reported in cm^{-1}. Mass spectral data were acquired on a WATERS Micromass ZQ spectrometer or a WATERS Micromass Q-TOFF spectrometer.

5.1.1. 3-Amino-5-*O*-benzyl-3-*C*-cyano-3-deoxy-3-*N*-phenylmethanesulfonyl-D-ribofuranose (4). TFA and water (100 mL, 9/1; v/v) were added to compound **3** (15.5 g, 34 mmol). The reaction mixture was stirred at room temperature for 1 h 30 min. Then, the solvent was evaporated to dryness and the residue was purified by flash chromatography (EtOAc/petroleum ether, 35:65) to yield compound **4** (12 g, 84%) as a mixture of the two anomeric epimers; IR (ATR) ν 1695, 1319, 1128, 1068, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.41–7.28 (m, $OCH_2C_6H_5$, $SO_2CH_2C_6H_5$), 5.34 (d, 1H, $J_{1,2}$ = 4.3 Hz, H-1β), 5.16 (s, 1H, H-1α), 4.61–4.36 (m, H-2α,β, H-4α,β, $OCH_2C_6H_5$, $SO_2CH_2C_6H_5$), 3.76 (m, H-5α,β); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 131.6, 131.5 (2C, Cip), 129.5–128.4 ($OCH_2C_6H_5$, $SO_2CH_2C_6H_5$), 117.6, 117.4 (2 CN), 102.7 (C-1α), 96.1 (C-1β), 81.0 (C-4α), 82.1 (C-4β), 81.4 (C-2α), 76.3 (C-2β), 74.6, 74.3 ($OCH_2C_6H_5$α,β), 70.2, 69.6 (C-5α,β), 60.9, 60.8 (C-3α,β), 60.3, 60.1 ($SO_2CH_2C_6H_5$α,β); MS (ES): 459.1 $[M+1]^+$. Anal. ($C_{20}H_{22}N_2O_6S$) C, H, N, S. Calcd C, 57.40; H, 5.30; N, 6.69; S, 7.66. Found: C, 55.83; H, 5.44; N, 6.52; S, 7.26.

5.1.2. 3-Amino-5-*O*-benzyl-3-*C*-cyano-3-deoxy-3-*N*-phenylmethanesulfonyl-1,2-*O*-sulfinyl-α-D-ribofuranose (5). To a solution of imidazole (3.58 g, 52.64 mmol) in THF (35 mL) was added $SOCl_2$ (0.85 mL, 13.16 mmol) at 0 °C. After 1 h, the reaction mixture was filtered and added to a solution of compound **4** (2.75 g, 6.58 mmol) in THF (30 mL) at −15 °C and stirred for 50 min. The solvent was evaporated to dryness and the residue was purified by flash chromatography (EtOAc/petroleum ether, 30:70) to give **5** (1.92 g, 63%, isolated as a mixture of *endo*/*exo* form which are used in the next step without further purification); IR (ATR) *ν* 1456, 1353, 1199, 1095, 898, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) *δ* 7.42–7.24 (m, 20H, $OCH_2C_6H_5$, $SO_2CH_2C_6H_5$), 6.63 (d, $J_{1,2}$ = 3.6 Hz, H-1$_{exo}$), 6.52 (5 d, $J_{1,2}$ = 4.6 Hz, H-1$_{endo}$), 5.82 (d, 1H, H-2$_{exo}$), 5.56 (d, 1H, H-2$_{endo}$), 4.52 (m, 9H, H-4, $OCH_2C_6H_5$, $SO_2CH_2C_6H_5$), 4.10 (m, 1H, H-4), 3.91 (m, 4H, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 136.5–128.1 (12C, $OCH_2C_6H_5$, $SO_2CH_2C_6H_5$), 15.5, 115.1 (2CN), 110.9 (C-1$_{endo}$), 108.6 (C-1$_{exo}$), 88.1 (C-2$_{endo}$), 84.7 (C-2$_{exo}$), 78.8, 78.6 (2C-4), 74.7, 74.6 (2 $OCH_2C_6H_5$), 68.2, 67.6 (2C-5), 63.2, 63.1 (2C-3), 61.3, 60.9 (2 $SO_2CH_2C_6H_5$); MS (ES): 487.1 $[M+Na]^+$.

5.1.3. 1-(3′-Amino-5′-*O*-benzyl-3′-*C*-cyano-3′-deoxy-3′-*N*-phenylmethanesulfonyl-β-D-ribofuranosyl)thymine (7). A solution of thymine (1.08 g, 8.54 mmol) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (25 mL) was refluxed overnight. The excess of HMDS was removed under reduced pressure then added to compound **5** (1.98 g, 4.27 mmol). The reaction mixture was heated at 125 °C. After 2 h, MeOH (10 mL) was added and stirred at room temperature for 10 min, followed by addition of water (1 mL), stirred at 65 °C for 5 min and filtered through Celite to afford a mixture of silylated derivative **6** and **7**. The solvent was evaporated under reduced pressure and the crude solubilized with MeOH (50 mL) and $NH_4F{\cdot}3H_2O$ (2 g, 54 mmol) for 2 h and filtered through Celite. After flash chromatography (EtOAc/petroleum ether, 40:60) compound **7** (1.6 g, 71%) was isolated as a white solid: mp 131–132 °C; $[\alpha]_D^{25}$ −33 (*c* 0.17, acetone); IR (ATR) *ν* 1690, 1667, 1644, 1472, 1384, 1244, 1142, 910, 752, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) *δ* 10.12 (br s, 1H, NH), 7.43 (m, 11H, H-6, $2C_6H_5$), 6.50 (s, 1H, NH), 6.16 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1′), 4.88 (d, 1H, H-2′), 4.80 (d, 1H, $J_{A,B}$ = 13.8 Hz, $SO_2CH_2C_6H_5$-A), 4.72 (d, 1H, $J_{A,B}$ = 10.8 Hz, $OCH_2C_6H_5$-A), 4.67 (d, 1H, $SO_2CH_2C_6H_5$-B), 4.62 (d, 1H, $OCH_2C_6H_5$-B), 4.27 (t, 1H, H-4′), 3.86 (dd, $J_{4',5'A}$ = 2.3 Hz, 1H, H5′-A), 3.76 (dd, $J_{4',5'B}$ = 1.7 Hz, $J_{5'A,5'B}$ = 11.2 Hz, 1H, H5′-B), 1.46 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 163.4 (C-4), 151.2 (C-2), 138.0 (Cip), 135.1 (C-6), 131.6, 129.6, 128.9, 128.6, 128.5 (9C, C_6H_5), 117.9 (CN), 111.5 (C-5), 85.4 (C-1′), 83.7 (C-4′), 77.4 (C-2′), 74.1 ($OCH_2C_6H_5$), 70.2 (C-5′), 60.3 ($SO_2CH_2C_6H_5$), 59.6 (C-3′), 11.7 (CH_3); MS (ES): 549.1 $[M+Na]^+$, 565.1 $[M+K]^+$. Anal. ($C_{25}H_{26}N_4O_7S$) C, H, N, S. Calcd C, 57.02; H, 4.98; N, 10.61; S, 6.09. Found: C, 53.58; H, 5.27; N, 9.84; S, 5.68.

5.1.4. 1-(3′-Amino-5′-*O*-benzyl-2′-*O*-*tert*-butyldimethylsilyl-3′-*C*-cyano-3′-deoxy-3′-*N*-phenylmethanesulfonyl-β-D-ribofuranosyl)thymine (8). To a solution of **7** (3.66 g, 6.95 mmol) and imidazole (1.42 g, 20.85 mmol) in DMF (55 mL) was added TBDMSCl (1.42 g, 20.85 mmol). The reaction mixture was stirred at room temperature overnight then evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 30:70) compound **8** (3.73 g, 84%) was isolated as a white solid: mp 148–151 °C; $[\alpha]_D^{25}$ −25 (*c* 0.18, $CHCl_3$); IR (ATR) *ν* 1710, 1683, 1656, 1397, 1264, 1152, 1072, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) *δ* 9.81 (s, 1H, NH), 7.37 (m, 11H, H-6, C_6H_5), 6.23 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1′), 5.91 (s, 1H, NH), 4.70 (d, 1H, $OCH_2C_6H_5$-A), 4.67 (d, 1H, H-2′), 4.57 (d, 1H, $SO_2CH_2C_6H_5$-A), 4.48 (d, 1H, $SO_2CH_2C_6H_5$-B), 4.45 (d, 1H, $OCH_2C_6H_5$-B), 4.02 (s, 1H, H-4′), 3.78 (s, 2H, H5′), 1.52 (s, 3H, CH_3), 0.78 (s, 9H, $[SiC(CH_3)_3]$), 0.1 (s, 3H, $SiCH_3$), −0.16 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 163.8 (C-4), 151.4 (C-2), 137.2 (Cip), 135.0 (C-6), 131.4, 129.5, 129.1, 129.0 (9C, C_6H_5), 117.5 (CN), 113.1 (C-5), 85.1 (C-1′), 83.5 (C-4′), 78.1 (C-2′), 74.8 ($OCH_2C_6H_5$), 70.3 (C-5′), 61.1 5 ($SO_2CH_2C_6H_5$), 59.6 (C-3′), 25.7 ($[SiC(CH_3)_3]$), 18.0 ($[SiC(CH_3)_3]$), 12.3 (CH_3), −4.2 ($SiCH_3$), −4.6 ($SiCH_3$); MS (ES): 663.4 $[M+Na]^+$, 679.3 $[M+K]^+$. Anal. ($C_{31}H_{40}N_4O_7SSi$) C, H, N, S. Calcd C, 58.10; H, 6.29; N, 8.74; S, 5.00. Found: C, 54.87; H, 5.84; N, 7.92; S, 4.62.

5.1.5. 1-(3′-Amino-5′-*O*-benzyl-2′-*O*-*tert*-butyldimethylsilyl-3′-*C*-cyano-3′-deoxy-3′-*N*-phenylmethanesulfonyl-β-D-ribofuranosyl)-3-*N*-*tert*-butoxycarbonylthymine (9). A solution of compound **8** (0.73 g, 1.15 mmol) and Boc_2O (0.53 g, 2.3 mmol) in 15 mL of a (4:1) mixture of CH_2Cl_2 and pyridine was stirred at room temperature for 15 h. The solvent was removed and the residue flash chromatographed (EtOAc/petroleum ether, 25:75) to give **9** (0.64 g, 75%) as a white solid: mp 91–92 °C; $[\alpha]_D^{25}$ −9 (*c* 0.13, acetone); IR (ATR) *ν* 1792, 1723, 1674, 1455, 1371, 1268, 1134, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) *δ* 7.40 (m, 11H, H-6, C_6H_5), 6.07 (d, $J_{1',2'}$ = 7.7 Hz, 1H, H-1′), 5.40 (s, 1H, NH), 4.71 (d, $J_{A,B}$ = 9.9 Hz, 1H, $OCH_2C_6H_5$-A), 4.63 (d, 1H, H-2′), 4.50 (s, 2H, $SO_2CH_2C_6H_5$), 4.48 (d, 1H, $OCH_2C_6H_5$-B), 4.25 (s, 1H, H-4′), 3.85 (dd, $J_{4',5'A}$ = 1.9 Hz, $J_{5'A,5'B}$ = 11.2 Hz, 1H, H-5′A), 3.79 (dd, $J_{4',5'B}$ = 1.3 Hz, 1H, H-5′B), 1.57 (s, 9H, $[OC(CH_3)_3]$), 1.49 (s, 3H, CH_3), 0.83 (s, 9H, $[SiC(CH_3)_3]$), 0.1 (s, 3H, $SiCH_3$), −0.14 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 161.2 (C-4), 149.0 (C-2), 147.8 (CO), 137.1 (Cip), 134.4 (C-6), 131.4, 129.7, 129.6, 129.2, 129.0 (8C, C_6H_5), 128.0 (Cip), 117.5 (CN), 112.4 (C-5), 87.1 $[OC(CH_3)_3]$, 85.6 (C-1′), 83.4 (C-4′), 78.6 (C-2′), 74.7 ($OCH_2C_6H_5$), 70.3 (C-5′), 61.2 ($SO_2CH_2C_6H_5$), 59.6 (C-3′), 27.8 $[OC(CH_3)_3]$, 25.8 $[SiC(CH_3)_3]$, 18.0 $[SiC(CH_3)_3]$, 12.3 (CH_3), −4.1 ($SiCH_3$), −4.9 ($SiCH_3$); MS (ES): 763.3 $[M+Na]^+$, 779.3 $[M+K]^+$. Anal. ($C_{36}H_{48}N_4O_9SSi$) C, H, N, S. Calcd C, 58.36; H, 6.53; N, 7.56; S, 4.33. Found: C, 57.93; H, 6.46; N, 7.28; S, 4.12.

5.1.6. [1-[5′-*O*-Benzyl-2′-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(2″-H-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole-

5″-phenyl) (10). To a solution of LDA (freshly prepared from 4 mmol of ^{n}BuLi and 4.4 mmol of diisopropylamine) in THF (24 mL) was added at −78 °C a solution of sulfonamidonitrile **9** (0.76 g, 1 mmol) in dry THF (10 mL). After 15 min, water (10 mL) was added and the reaction mixture slightly acidified with aqueous HCl and extracted with EtOAc. The extracts were dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (EtOAc/petroleum ether, 30:70) to give compound **10** (0.562 g, 76%) as a white solid: mp 132–133 °C; $[\alpha]_D^{25}$ +68 (*c* 0.17, acetone); IR (ATR) *v* 1778, 1723, 1681, 1370, 1258, 1151, 1091, 840, 696 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 7.53 (s, 1H, H-6), 7.40 (m, 10H, 2C_6H_5), 5.94 (d, $J_{1',2'}$ = 7.4 Hz, 1H, H-1′), 5.38 (s, 1H, NH), 5.29 (s, 2H, NH_2), 4.73 (d, 1H, H-2′), 4.66 (d, 2H, $CH_2C_6H_5$), 4.57 (m, 1H, H-4′), 3.95 (dd, $J_{4',5''A}$ = 2.8 Hz, $J_{5'A,5'B}$ = 11.4 Hz, 1H, H-5′A), 3.77 (dd, $J_{4',5'B}$ = 2.0 Hz, 1H, H-5′B), 1.61 (s, 3H, CH_3), 1.58 (s, 9H, [$OC(CH_3)_3$]), 0.88 (s, 9H, [$SiC(CH_3)_3$]), 0.1 (s, 3H, $SiCH_3$), 0.01 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 161.2 (C-4), 149.0 (C-2), 147.7 (CO), 146.7 (C-4″), 136.2 (Cip), 135.4 (C-6), 129.9, 129.5, 129.2, 129.1, 128.3, 127.8 (9C, C_6H_5), 112.6 (C-5″), 112.2 (C-5), 88.5 (C-1′), 87.2 [O*C*$(CH_3)_3$], 84.3 (C-4′), 74.4 (C-2′), 74.3 ($CH_2C_6H_5$), 70.2 (C-5′), 69.9 (C-3′), 27.8 [OC(CH_3)$_3$], 25.7 [SiC(CH_3)$_3$], 18.2 [Si*C*$(CH_3)_3$], 12.5 (CH_3), −4.5 ($SiCH_3$), −4.6 ($SiCH_3$); MS (ES): 763.3 $[M+Na]^+$. Anal. ($C_{36}H_{48}N_4O_9SSi$) C, H, N, S. Calcd C, 58.36; H, 6.53; N, 7.56; S, 4.33. Found: C, 57.70; H, 6.41; N, 7.38; S, 4.18.

5.1.7. [1-[2′-*O*-*tert*-Butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(2″-H-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole-5″-phenyl) (11) and [1-[2′-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3′-spiro-3″-(2″-H-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole-5″-phenyl) (12). *Method A*. To a solution of **10** (283 mg, 0.38 mmol) in absolute ethanol (7 mL) were added $Pd(OH)_2$/C (71 mg, 0.1 mmol) and cyclohexene (0.87 mL, 8.3 mmol). The reaction mixture was refluxed for 2 h 30 min to give a mixture of compounds **11** and **12**. The solvent was evaporated and the residue chromatographed (EtOAc/petroleum ether, 60:40) to give successively **11** (198 mg, 80%) and **12** (33 mg, 16%) as white solids. *Method B*. To a solution of **10** (502 mg, 0.68 mmol) in absolute ethanol (12.5 mL) were added $Pd(OH)_2$/C (127 mg, 0.18 mmol) and cyclohexene (1.55 mL, 14.8 mmol). The reaction mixture was refluxed for 17 h to give a mixture of compounds **11** and **12**. The solvent was evaporated and the residue chromatographed (EtOAc/petroleum ether, 60:40) to give successively **11** (38 mg, 9%) and **12** (334 mg, 89%) as white solids. **11**: mp 142 °C; $[\alpha]_D^{25}$ +49 (*c* 0.11, acetone); IR (ATR) *v* 1788, 1713, 1672, 1370, 1254, 1147, 1086, 1045, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 7.46 (m, 5H, C_6H_5), 7.26 (s, 1H, H-6), 5.78 (s, 2H, NH_2), 5.40 (s, 1H, NH), 5.38 (d, $J_{1',2'}$ = 7.7 Hz, 1H, H-1′), 5.29 (d, 1H, H-2′), 5.05 (d, 1H, OH-5′), 4.55 (s, 1H, H-4′), 3.94 (d, $J_{5'A,5'B}$ = 10.1 Hz, 1H, H-5′A), 3.60 (dd, 1H, H-5′B), 1.95 (s, 3H, CH_3), 1.60 (s, 9H, [$OC(CH_3)_3$]), 0.86 (s, 9H, [$SiC(CH_3)_3$]), 0.14 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 161.1 (C-4), 149.5 (C-2), 148.0 (C-4″), 147.4 (CO), 139.9 (C-6), 130.0, 128.9, 128.3, (4C, C_6H_5), 128.0 (Cip), 112.3 (C-5), 112.2 (C-5″), 95.5 (C-1′), 87.7 [O*C*$(CH_3)_3$], 87.1 (C-4′), 71.3 (C-3′), 70.5 (C-2′), 63.3 (C-5′), 27.8 [OC(CH_3)$_3$], 25.7 [SiC(CH_3)$_3$], 18.1 [Si*C*$(CH_3)_3$], 12.6 (CH_3), −4.4 ($SiCH_3$), −4.7 ($SiCH_3$); MS (ES): 673.4 $[M+Na]^+$, 689.3 $[M+K]^+$. Anal. ($C_{29}H_{42}N_4O_9SSi$) C, H, N, S. Calcd C, 53.52; H, 6.50; N, 8.61; S, 4.93. Found: C, 53.44; H, 6.48; N, 8.35; S, 4.78. **12**: mp 158–159 °C; $[\alpha]_D^{25}$ +24 (*c* 0.15, CH_3OH); IR (ATR) *v* 2922, 1685, 1643, 1471, 1257, 1150, 1043, 840, 783 cm^{-1}; ^{1}H NMR ($(CD_3)_2CO$, 300 MHz) *δ* 7.57–7.35 (m, 6H, H-6, C_6H_5), 6.39 (s, 2H, NH_2), 5.92 (d, $J_{1',2'}$ = 7.7 Hz, 1H, H-1′), 5.70 (q, 1H, OH5′), 5.60 (s, 1H, NH), 5.23 (d, 1H, H-2′), 4.43 (m, 1H, H-4′), 4.01 (dt, $J_{5'A,5'B}$ = 12.7 Hz, $J_{4',5'A}$ = 2.8 Hz, $J_{OH5',5'A}$ = 3.2 Hz, 1H, H-5′A), 3.82 (ddd, $J_{4',5'A}$ = 1.6 Hz, $J_{OH5',5'B}$ = 5.1 Hz, 1H, H-5′B), 1.88 (s, 3H, CH_3), 0.89 (s, 9H, [$SiC(CH_3)_3$]), 0.17 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$); ^{13}C NMR ($(CD_3)_2CO$, 75 MHz) *δ* 163.2 (C-4), 151.6 (C-2), 148.1 (C-4″), 137.9 (C-6), 129.4, 128.4, 128.1 (6C, C_6H_5), 111.6 (C-5), 112.2 (C-5″), 89.8 (C-1′), 86.5 (C-4′), 73.3 (C-2′), 70.7 (C-3′), 61.8 (C-5′), 25.3 [SiC(CH_3)$_3$], 17.9 [Si*C*$(CH_3)_3$], 11.9 (CH_3), −5.1 ($SiCH_3$), −5.4 ($SiCH_3$); MS (ES): 573.5 $[M+Na]^+$, 589.4 $[M+K]^+$. Anal. ($C_{24}H_{34}N_4O_7SSi$) C, H, N, S. Calcd C, 52.34; H, 6.22; N, 10.17; S, 5.82. Found: C, 56.40; H, 6.98; N, 8.66; S, 4.97.

5.1.8. [1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-*tert*-butoxycarbonylthymine]-3′-spiro-3″-(2″-H-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole-5″-phenyl) (13). To a solution of **11** (158 mg, 0.24 mmol) and imidazole (50 mg, 0.72 mmol) in DMF (5 mL) was added TBDMSCl (91 mg, 0.6 mmol). The reaction mixture was stirred at room temperature overnight then evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 25:65) compound **13** (149 mg, 80%) was isolated as a white solid: mp 145–148 °C; $[\alpha]_D^{25}$ +43 (*c* 0.15, acetone); IR (ATR) *v* 1783, 1718, 1676, 1370, 1254, 1147, 836 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 7.48 (m, 5H, C_6H_5), 7.26 (s, 1H, H-6), 5.60 (d, $J_{1',2'}$ = 7.3 Hz, 1H, H-1′), 5.38 (s, 3H, NH, NH_2), 4.86 (d, 1H, H-2′), 4.36 (s, 1H, H-4′), 4.05 (dd, $J_{5'A,5'B}$ = 12.3 Hz, $J_{4',5'A}$ = 2.0 Hz 1H, H-5′A), 3.93 (dd, $J_{4',5'B}$ = 3.6 Hz 1H, H-5′B), 2.00 (s, 3H, CH_3), 1.60 (s, 9H, [$OC(CH_3)_3$]), 0.95 (s, 9H, [$SiC(CH_3)_3$]), 0.89 (s, 9H, [$SiC(CH_3)_3$]), 0.18 (s, 3H, $SiCH_3$), 0.16 (s, 3H, $SiCH_3$), 0.13 (s, 3H, $SiCH_3$), 0.02 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 161.2 (C-4), 148.9 (C-2), 147.5 (2C, CO, C-4″), 136.5 (C-6), 130.0, 129.00, 128.5, 127.9 (6C, C_6H_5), 112.2 (C-5), 111.3 (C-5″), 91.1 (C-1′), 87.5 [O*C*$(CH_3)_3$], 86.2 (C-4′), 73.9 (C-2′), 68.4 (C-3′), 62.7 (C-5′), 27.8 [OC(CH_3)$_3$], 26.5 [SiC(CH_3)$_3$], 25.8 [SiC(CH_3)$_3$], 18.8 [Si*C*$(CH_3)_3$], 18.2 [Si*C*$(CH_3)_3$], 12.8 (CH_3), −4.4 ($SiCH_3$), −4.5 ($SiCH_3$), −4.6 ($SiCH_3$), −5.1 ($SiCH_3$); MS (ES): 787.2 $[M+Na]^+$, 803.1 $[M+K]^+$. Anal. ($C_{35}H_{56}N_4O_9SSi_2$) C, H, N, S. Calcd C, 54.95; H, 7.38; N, 7.32; S, 4.19. Found: C, 55.01; H, 7.19; N, 7.11; S, 3.99.

5.1.9. [1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]thymine]-3′-spiro-3″-(2″-H-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole-5″-phenyl) (14). To a solution

of **12** (313 mg, 0.57 mmol) and imidazole (117 mg, 1.71 mmol) in DMF (7 mL) was added TBDMSCl (217 mg, 1.42 mmol). The reaction mixture was stirred at room temperature overnight then evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 30:70) compound **14** (284 mg, 74%) was isolated as a white solid: mp 139–141 °C; $[\alpha]_D^{25}$ +28 (*c* 0.25, MeOH); IR (ATR) *v* 1695, 1644, 1249, 1170, 1040, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H, NH-3), 7.68 (s, 1H, H-6), 7.50 (m, 2H, C_6H_5), 7.45 (m, 2H, C_6H_5), 7.35 (m, 1H, C_6H_5), 6.19 (s, 2H, NH_2), 5.70 (d, $J_{1',2'}$ = 7.2 Hz, 1H, H-1′), 5.63 (s, 1H, NH), 5.12 (d, 1H, H-2′), 4.30 (t, 1H, H-4′), 4.12 (d, 2H, H-5′), 1.90 (s, 3H, CH_3), 0.93 (s, 9H, [$SiC(CH_3)_3$]), 0.91 (s, 9H, [$SiC(CH_3)_3$]), 0.17 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.3 (C-4), 151.3 (C-2), 148.3 (C-4″), 138.1 (C-6), 129.4, 128.9, 128.6, 128.2 (6 C, C_6H_5), 111.7 (C-5), 107.9 (C-5″), 91.6 (C-1′), 86.9 (C-4′), 73.6 (C-2′), 67.6 (C-3′), 62.8 (C-5′), 25.9 [$SiC(CH_3)_3$], 25.4 [$SiC(CH_3)_3$], 18.5 [$SiC(CH_3)_3$], 18.0 [$SiC(CH_3)_3$], 11.9 (CH_3), −5.1 ($SiCH_3$), −5.4 ($SiCH_3$), −5.5 ($SiCH_3$), −5.6 ($SiCH_3$); MS (ES): 687.3 $[M+Na]^+$, 703.3 $[M+K]^+$. Anal. ($C_{30}H_{48}N_4O_7SSi_2$) C, H, N, S. Calcd C, 54.19; H, 7.28; N, 8.43; S, 4.82. Found: C, 55.86; H, 7.54; N, 7.72; S, 4.33.

5.1.10. [1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-3″-(2″-H-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole-5″-phenyl) (15). To a solution of **14** (80 mg, 0.12 mmol) and K_2CO_3 (8 mg, 0.06 mmol) in acetone (3 mL) was added MeI (0.015 mL, 0.24 mmol). The mixture was refluxed until complete reaction then filtered through a silica pad and evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 20:80) compound **15** (70 mg, 84%) was isolated as a white solid: mp 159–160 °C; $[\alpha]_D^{25}$ +48 (*c* 0.12, acetone); IR (ATR) *v* 1709, 1672, 1644, 1254, 1156, 840, 775, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.51 (m, 5H, C_6H_5), 7.22 (s, 1H, H-6), 5.50 (s, 3H, H-1′, NH_2), 5.39 (s, 1H, NH), 4.93 (d, $J_{1',2'}$ = 7.1 Hz, 1H, H-2′), 4.30 (q, $J_{4',5'A}$ = 2.4 Hz, $J_{4',5'B}$ = 4.7 Hz, 1H, H-4′), 4.05 (dd, $J_{5'A,5'B}$ = 12.2 Hz, 1H, H-5′-A), 3.95 (dd, 1H, H-5′B), 3.36 (s, 3H, N-CH_3), 2.01 (s, 3H, CH_3), 0.95 {s, 9H, [$SiC(CH_3)_3$]}, 0.88 (s, 9H, {$SiC(CH_3)_3$]}), 0.17 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$), 0.13 (s, 3H, $SiCH_3$), −0.05 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.5 (C-4), 151.5 (C-2), 147.7 (C-4″), 135.9 (C-6), 130.0, 128.9, 128.5 (5C, C_6H_5), 127.9 (Cip), 111.6 (C-5), 110.1 (C-5″), 93.1 (C-1′), 86.4 (C-4′), 73.8 (C-2′), 67.9 (C-3′), 62.5 (C-5′), 28.5 (N-CH_3), 26.5 [$SiC(CH_3)_3$], 25.8 [$SiC(CH_3)_3$], 18.8 [$SiC(CH_3)_3$], 18.2 [$SiC(CH_3)_3$], 13.4 (CH_3), −4.4 ($SiCH_3$), −4.5 ($SiCH_3$), −4.7 ($SiCH_3$), −5.0 ($SiCH_3$); MS (ES): 701.3 $[M+Na]^+$, 717.3 $[M+K]^+$. Anal. ($C_{31}H_{50}N_4O_7SSi_2$) C, H, N, S. Calcd C, 54.84; H, 7.42; N, 8.25; S, 4.72. Found: C, 55.98; H, 7.55; N, 7.60; S, 4.36.

5.1.11. [1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-ethylthymine]-3′-spiro-3″-(2″-H-4″-amino-2″,3″-dihydro-1″,1″-dioxo-isothiazole-5″-phenyl) (16). To a solution of **14** (127 mg, 0.19 mmol) and K_2CO_3 (13 mg, 0.1 mmol) in acetone (5 mL) was added EtI (0.030 mL, 0.38 mmol). The mixture was refluxed until complete reaction then filtered through a silica pad and evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 15:85) compound **16** (108 mg, 80%) was isolated as a white solid: mp 93–95 °C; $[\alpha]_D^{25}$ +41 (*c* 0.165, acetone); IR (ATR) *v* 1705, 1699, 1643, 1473, 1255, 1157, 831 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.50 (m, 5H, C_6H_5), 7.21 (s, 1H, H-6), 5.50 (s, 3H, H-1′, NH_2), 5.37 (s, 1H, NH), 4.93 (d, $J_{1',2'}$ = 7.1 Hz, 1H, H-2′), 4.28 (q, $J_{4',5'A}$ = 2.3 Hz, $J_{4',5'B}$ = 4.7 Hz, 1H, H-4′), 4.06 (dd, $J_{5'A,5'B}$ = 12.2 Hz, 1H, H-5′-A), 4.00 (q, $J_{CH2,\ CH3}$ = 7 Hz, 2H, CH_2CH_3), 3.95 (dd, 1H, H-5′B), 1.99 (s, 3H, CH_3), 1.20 (t, 3H, CH_2CH_3), 0.93 (s, 9H, [$SiC(CH_3)_3$]), 0.87 (s, 9H, [$SiC(CH_3)_3$]), 0.16 (s, 3H, $SiCH_3$), 0.14 (s, 3 H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), −0.04 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.1 (C-4), 151.2 (C-2), 147.8 (C-4″), 136.0 (C-6), 129.9, 128.9, 128.5 (5C, C_6H_5), 127.9 (Cip), 111.7 (C-5), 110.0 (C-5″), 92.8 (C-1′), 86.3 (C-4′), 73.7 (C-2′), 67.9 (C-3′), 62.5 (C-5′), 37.1 (CH_2CH_3), 26.4 [$SiC(CH_3)_3$], 25.8 [$SiC(CH_3)_3$], 18.8 [$SiC(CH_3)_3$], 18.2 [$SiC(CH_3)_3$], 13.4 (CH_3), 13.1 (CH_2CH_3), −4.4 ($SiCH_3$), −4.5 ($SiCH_3$), −4.7 ($SiCH_3$), −5.0 ($SiCH_3$); MS (ES): 715.3 $[M+Na]^+$, 732.3 $[M+K]^+$. Anal. ($C_{32}H_{52}N_4O_7SSi_2$) C, H, N, S. Calcd C, 55.46; H, 7.56; N, 8.08; S, 4.63. Found: C, 56.62; H, 7.61; N, 7.51; S, 4.25.

5.1.12. 1-(3′-Amino-5′-*O*-benzyl-2′-*O*-*tert*-butyldimethylsilyl-3′-*C*-cyano-3′-deoxy-3′-*N*-benzylsulfonyl-3′-*N*-methyl-β-D-ribofuranosyl)-3-*N*-methylthymine (17). To a solution of **7** (600 mg, 0.94 mmol) and K_2CO_3 (195 mg, 0.47 mmol) in acetone (15 mL) was added MeI (0.117 mL, 1.87 mmol). The mixture was refluxed for 24 h and evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 20:80) compound **17** (408 mg, 65%) was isolated as a white solid: mp 71–72 °C; $[\alpha]_D^{25}$ +43 (*c* 0.24, acetone); IR (ATR) *v* 1709, 1662, 1644, 1463, 1351, 1151, 1128, 845, 780, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40 (m, 11H, H-6, $2C_6H_5$), 6.18 (d, $J_{1',2'}$ = 5.2 Hz, 1H, H-1′), 4.76 (d, $J_{A,B}$ = 11.1 Hz, 1H, $OCH_2C_6H_5$-A), 4.72 (d, 1H, H-2′), 4.54 (d, 1H, $OCH_2C_6H_5$-B), 4.53 (m, 1H, H-4′), 4.42 (m, 2H, $SO_2CH_2C_6H_5$), 4.00 (dd, $J_{4,5'A}$ = 4.0 Hz, $J_{5'A,5'B}$ = 10.1 Hz, 1H, H-5′A), 3.91 (dd, $J_{4,5'B}$ = 3.4 Hz, 1H, H-5′B), 3.32 (s, 3H, CH_3-N3), 2.85 (s, 3H, CH_3NSO_2), 1.74 (s, 3H, CH_3), 0.87 (s, 9H, [$SiC(CH_3)_3$]), 0.21 (s, 3H, $SiCH_3$), −0.09 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.6 (C-4), 151.6 (C-2), 137.2 (Cip), 133.2 (C-6), 131.5, 129.3, 128.0, (9C, C_6H_5), 117.5 (CN), 111.8 (C-5), 89.2 (C-1′), 82.2 (C-4′), 81.5 (C-2′), 74.6 (O-$CH_2C_6H_5$), 69.5 (C-5′), 64.8 (C-3′), 60.5 ($SO_2CH_2C_6H_5$), 35.9 (CH_3-N3), 28.5 (CH_3-NSO_2), 25.9 [$SiC(CH_3)_3$], 18.1 [$SiC(CH_3)_3$], 13.4 (CH_3), −3.97 ($SiCH_3$), −4.9 ($SiCH_3$); MS (ES): 691.3 $[M+Na]^+$, 707.2 $[M+K]^+$. Anal. ($C_{33}H_{44}N_4O_7SSi$) C, H, N, S. Calcd C, 59.26; H, 6.63; N, 8.38; S, 4.79. Found: C, 59.03; H, 6.49; N, 8.04; S, 4.67.

5.1.13. [1-[5′-*O*-Benzyl-2′-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-3″-(4″-ami-

no-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole-5″-phenyl) (18). A solution of **17** (342 mg, 0.51 mmol) and Cs_2CO_3 (189 mg, 0.51 mmol) in acetonitrile (5 mL), was refluxed for 25 min and evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 30:70) compound **18** (307 mg, 90%) was isolated as a white solid: mp 113–114 °C; $[\alpha]_D^{25}$ +31 (*c* 0.1, acetone); IR (ATR) *v* 1705, 1650, 1469, 1261, 1154, 1047, 840, 766 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.46 (m, 10H, $2C_6H_5$), 7.10 (s, 1H, H-6), 5.71 (br s, 2H, NH_2), 5.14 (d, $J_{1',2'}$ = 6.6 Hz, 1H, H-2′), 4.96 (d, 1H, H-1′), 4.57 (s, 2H, $CH_2C_6H_5$), 4.50 (dd, $J_{4',5'a}$ = 3.1 Hz, $J_{4',5'b}$ = 6.7 Hz, 1H, H-4′), 3.93 (m, 2H, H-5′), 3.33 (s, 3H, N-CH_3), 2.99 (s, 3H, CH_3NSO_2), 1.96 (s, 3H, CH_3), 0.80 (s, 9H, $SiC(CH_3)_3$), 0.14 (s, 3H, $SiCH_3$), 0.01 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.4 (C-4), 151.3 (C-2), 147.6 (C-4″), 139.1 (C-6), 129.9–128.9 (5C, C_6H_5), 127.7 (Cip), 111.5 (C-5), 104.0 (C-5″), 99.8 (C-1′), 80.0 (C-4′), 75.0 (C-2′), 68.4 (C-3′), 62.2 (C-5′), 28.3 (CH_3NSO_2), 27.3 (CH_3N-3), 26.4 [$SiC(CH_3)_3$], 25.8 [$SiC(CH_3)_3$], 18.9 [$SiC(CH_3)_3$], 18.5 [$SiC(CH_3)_3$], 13.3 (CH_3), −4.3 ($SiCH_3$), −4.8 ($SiCH_3$), −4.9 ($SiCH_3$), −5.6 ($SiCH_3$); MS (ES): 715.4 $[M+Na]^+$, 731.4 $[M+K]^+$. Anal. ($C_{32}H_{52}N_4O_7SSi_2$) C, H, N, S. Calcd C, 55.46; H, 7.56; N, 8.08; S, 4.63. Found: C, 56.43; H, 7.55; N, 7.61; S, 4.37.

5.1.14. [1-[2′-*O*-*tert*-Butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-ethylthymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole-5″-phenyl) (19). To a solution of **18** (279 mg, 0.42 mmol) in absolute ethanol (7.7 mL) were added $Pd(OH)_2/C$ (78 mg, 0.11 mmol) and cyclohexene (0.95 mL, 9 mmol). The reaction mixture was refluxed for 1 h 25 min, and the solvent was evaporated. The residue was chromatographed (EtOAc/petroleum ether, 70:30) to give **19** (240 mg, 99%) as a white solid: mp 130–132 °C; $[\alpha]_D^{25}$ +19 (*c* 0.15, acetone); IR (ATR) *v* 2925, 1704, 1644, 1463, 1258, 1151, 1035, 840, 761 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 300 MHz) δ 7.79 (s, H-6), 7.61–7.35 (m, 5H, C_6H_5), 6.35 (s, 2H, NH_2), 5.60 (d, $J_{1',2'}$ = 7.1 Hz, 1H, H-1′), 5.16 (d, 1H, H-2′), 4.45 (q, 1H, H-4′), 3.95 (m, 2H, H-5′), 3.28 (s, 3H, CH_3NSO_2), 2.99 (s, 3H, CH_3N-3), 1.92 (s, 3H, CH_3), 0.83 (s, 9H, [$SiC(CH_3)_3$]), 0.12 (s, 3H, $SiCH_3$), 0.01 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.9 (C-4), 151.7 (C-2), 148.0 (C-4″), 138.9 (C-6), 129.4–128.1 (5C, C_6H_5), 110.6 (C-5), 103.3 (C-5″), 96.4 (C-1′), 80.6 (C-4′), 75.6 (C-2′), 69.5 (C-3′), 61.0 (C-5′), 27.4 (CH_3), 26.3 [$SiC(CH_3)_3$], 25.4 [$SiC(CH_3)_3$], 18.1 [$SiC(CH_3)_3$], 12.4 (CH_3), −4.9 ($SiCH_3$), −6.1 ($SiCH_3$); MS (ES): 601.3 $[M+Na]^+$. Anal. ($C_{26}H_{38}N_4O_7SSi$) C, H, N, S. Calcd C, 53.96; H, 6.62; N, 9.68; S, 5.54. Found: C, 57.92; H, 7.49; N, 7.72; S, 4.45.

5.1.15. [1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-3″-(4″-amino-2″,3″-dihydro-2″-*N*-methyl-1″,1″-dioxo-isothiazole-5″-phenyl) (20). To a solution of **19** (200 mg, 0.35 mmol) and imidazole (130 mg, 0.87 mmol) in DMF (5 mL) was added TBDMSCl (71 mg, 1.05 mmol). The reaction mixture was stirred at room temperature overnight then evaporated to dryness. After flash chromatography (EtOAc/petroleum ether, 30:70) compound **19** (180 mg, 75%) was isolated as a white solid: mp 129–131 °C; $[\alpha]_D^{25}$ +22 (*c* 0.2, acetone); IR (ATR) *v* 1706, 1676, 1649, 1466, 1261, 1152, 1046, 838 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.56–7.36 (m, 5H, C_6H_5), 7.10 (s, 1H, H-6), 5.71 (s, 2H, NH_2), 5.13 (d, $J_{1',2'}$ = 6.5 Hz, 1H, H-2′), 4.93 (d, 1H, H-1′), 4.31 (q, $J_{4',5'A}$ = 3.5 Hz, $J_{4',5'B}$ = 6.5 Hz, 1H, H-4′), 4.04 (m, 2H, H-5′), 3.32 (s, 3H, CH_3NSO_2), 2.99 (s, 3H, CH_3N-3), 1.98 (s, 3H, CH_3), 0.90 (s, 9H, [$SiC(CH_3)_3$]), 0.81 (s, 9H, [$SiC(CH_3)_3$]), 0.14 (s, 3 H, $SiCH_3$), 0.06 (s, 6H, 2 $SiCH_3$), 0.01 (s, 3H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.4 (C-4), 151.3 (C-2), 147.6 (C-4″), 139.1 (C-6), 129.9–128.9 (5C, C_6H_5), 127.7 (Cip), 111.5 (C-5), 104.0 (C-5″), 99.8 (C-1′), 80.0 (C-4′), 75.0 (C-2′), 68.4 (C-3′), 62.2 (C-5′), 28.3 (CH_3NSO_2), 27.3 (CH_3N-3), 26.4 [$SiC(CH_3)_3$], 25.8 [$SiC(CH_3)_3$], 18.9 [$SiC(CH_3)_3$], 18.5 [$SiC(CH_3)_3$], 13.3 (CH_3), −4.3 ($SiCH_3$), −4.8 ($SiCH_3$), −4.9 ($SiCH_3$), −5.6 ($SiCH_3$); MS (ES): 715.4 $[M+Na]^+$, 731.4 $[M+K]^+$. Anal. ($C_{32}H_{52}N_4O_7SSi_2$) C, H, N, S. Calcd C, 55.46; H, 7.56; N, 8.08; S, 4.63. Found: C, 56.43; H, 7.55; N, 7.61; S, 4.37.

5.1.16. Biological methods. 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).

5.1.17. Anti-HIV evaluation. 4×10^5 CEM 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) was recorded microscopically in the HIV-infected cell cultures.[13] 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) by 50%, or to reduce by 50% the number of viable cells in mock-infected CEM cell cultures.

Acknowledgments

C.T. thanks the Ministère Français de la Recherche (France) for a fellowship. D.P. thanks the Conseil Régional de Picardie and the Ministère Français de la Recherche for financial support and G. Mackenzie for helpful discussions. D.P. and J.M.C. thank Miss Laura Domínguez for some preliminary experiments.

References and notes

1. Restle, T.; Muller, B.; Godoy, R. S. *J. Biol. Chem.* **1990**, *265*, 8986–8988.
2. Wang, J.; Smerdon, S. J.; Jager, J.; Kohlstaedt, L. A.; Rice, P. A.; Friedman, J. M.; Steitz, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7242–7246.
3. Pandey, P. K.; Kaushik, N.; Talele, T. T.; Yadav, P. N. S.; Pandey, V. N. *Biochemistry* **2001**, *40*, 9505–9512; Pandey,

P. K.; Kaushik, N.; Singh, K.; Sharma, B.; Upadhyay, A. K.; Kumar, S.; Harris, D.; Pandey, V. N. *BMC Biochem.* **2002**, *3*, 18–31.
4. (a) Camarasa, M.-J.; Pérez-Pérez, M.-J.; San-Félix, A.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 2721–2727; (b) Pérez-Pérez, M.-J.; San-Félix, A.; Camarasa, M.-J.; Balzarini, J.; De Clercq, E. *Tetrahedron Lett.* **1992**, *33*, 3029–3032; (c) Camarasa, M.-J.; San-Félix, A.; Velázquez, S.; Pérez-Pérez, M.-J.; Gago, F.; Balzarini, J. *Curr. Top. Med. Chem.* **2004**, *4*, 945–963.
5. Camarasa, M. J.; Pérez-Pérez, M. J.; Velázquez, S.; San-Félix, A., Alvarez, R.; Ingate, S.; Jimeno, M. L.; De Clercq, E.; Balzarini, J. An overview of TSAO-T derivatives, a 'peculiar' class of HIV-1 specific RT inhibitors. In *Antiinfectives. Recent Advances in Chemistry and Structure–Activity Relationships*, Bentley, P. H., O'Hanlon, P. J., Eds.; The Royal Society of Chemistry, 1997, Chapter 18, pp 259–268.
6. (a) Pérez-Pérez, M.-J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M.-J. *J. Med. Chem.* **1992**, *35*, 2988–2995; (b) Ingate, S.; Pérez-Pérez, M. J.; De Clercq, E.; Balzarini, J.; Camarasa, M. J. *Antiviral Res.* **1995**, *27*, 281–299; (c) Chamorro, C.; Pérez-Pérez, M. J.; Rodríguez-Barrios, F.; Gago, F.; De Clercq, E.; Balzarini, J.; San-Félix, A.; Camarasa, M. J. *Antiviral Res.* **2001**, *50*, 207–222.
7. (a) Alvarez, R.; Jimeno, M. L.; Gago, F.; Balzarini, J.; Perez-Perez, M. J.; Camarasa, M. J. *Antiviral Chem. Chemother.* **1998**, *9*, 333–340; (b) Lobatón, E.; Rodríguez-Barrios, F.; Gago, F.; Pérez-Pérez, M. J.; De Clercq, E.; Balzarini, J.; Camarasa, M. J.; Velázquez, S. *J. Med. Chem.* **2002**, *45*, 3934–3945.
8. (a) Velázquez, S.; San-Félix, A.; Pérez-Pérez, M. J.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. *J. Med. Chem.* **1993**, *36*, 3230–3239; (b) San-Félix, A.; Velázquez, S.; Pérez-Pérez, M. J.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 453–460.
9. Bonache, M. C.; Chamorro, C.; Velázquez, S.; De Clercq, E.; Balzarini, J.; Rodríguez-Barrios, F.; Gago, F.; Camarasa, M. J.; San-Félix, A. *J. Med. Chem.* **2005**, *48*, 6653–6660.
10. Postel, D.; Nguyen Van Nhien, A.; Pillon, M.; Villa, P.; Ronco, G. *Tetrahedron Lett.* **2000**, *41*, 6403–6406.
11. Postel, D.; Nguyen Van Nhien, A.; Villa, P.; Ronco, G. *Tetrahedron Lett.* **2001**, *42*, 593–595.
12. Postel, D.; Nguyen Van Nhien, A.; Marco, J. L. *Eur. J. Org. Chem.* **2003**, *19*, 3713–3726.
13. Nguyen Van Nhien, A.; Tomassi, C.; Len, C.; Marco-Contelles, J.; Balzarini, J.; Pannecouque, C.; De Clercq, E.; Postel, D. *J. Med. Chem.* **2005**, *48*, 4276–4284.
14. Soriano, S.; Marco-Contelles, J.; Tomassi, C.; Nguyen Van Nhien, A.; Postel, D. *J. Chem. Inf. Model.* **2006**, *46*, 1666–1677.
15. (a) Gagnieu, C. H.; Guiller, A.; Pacheco, H. *Carbohydr. Res.* **1988**, *180*, 223–231; (b) Gagnieu, C. H.; Guiller, A.; Pacheco, H. *Carbohydr. Res.* **1988**, *180*, 233–242.
16. (a) Domínguez, L.; Nguyen Van Nhien, A.; Tomassi, C.; Len, C.; Postel, D.; Marco-Contelles, J. *J. Org. Chem.* **2004**, *69*, 843–856; (b) Nguyen Van Nhien, A.; Domínguez, L.; Tomassi, C.; Rosario Torres, M.; Len, C.; Postel, D.; Marco-Contelles, J. *Tetrahedron* **2004**, *60*, 4709–4727.
17. Lobatón, E.; Rodríguez-Barrios, F.; Gago, F.; Pérez-Pérez, M.-J.; De Clercq, E.; Balzarini, J.; Camarasa, M.-J.; Velázquez, S. *J. Med. Chem.* **2002**, *45*, 3934–3945.