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Synthesis of [1-[3',5'-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-arabino- and β-D-ribofuranosyl]cytosine]-2'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide). Analogues of the Highly specific Anti-HIV-1 Agent TSAO-T

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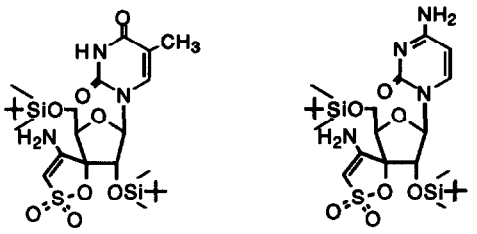
Abstract: Reaction of *O*-mesylcyanohydrins of furanos-2'-ulosyl cytosine with bases afforded β-D-arabino- and ribo-2'-spiro substituted nucleosides. Two of them exist, in solution, as mixtures of rotationally restricted *"syn-anti"* isomers.

INTRODUCTION

TSAO nucleoside analogues represent a structural class of compounds that inhibit the replication of human immunodeficiency virus type 1 (HIV-1), but not HIV-2, simian immunodeficiency virus (SIV) or other DNA or RNA viruses. They are targeted at the virus-encoded reverse transcriptase (RT) with which they interact at a non-substrate binding site.[1-7] Their mechanism of action and biological properties are similar to the non-nucleoside HIV-1-specific inhibitors (i.e. TIBO, HEPT, nevirapine, pyridinone, BHAP, α-APA, etc.).[8-16] However, TSAO derivatives represent the first molecules for which the active pharmacophore (i.e. the 4"-amino group at 3'-spiro of the sugar moiety) could be identified. Indeed, the 4"-amino group of TSAO strongly interacts with the COOH of glutamic acid (Glu) at position 138 of reverse transcriptase.[17] In contrast with the other HIV-1-specific inhibitors, the TSAO derivatives are structurally very closelly related to nucleoside analogs and, moreover, they are the first nucleoside analogs reported to show specificity for HIV-1. The prototype compound is [1-[2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine]-3'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (designated TSAO-T, **1**).[18]

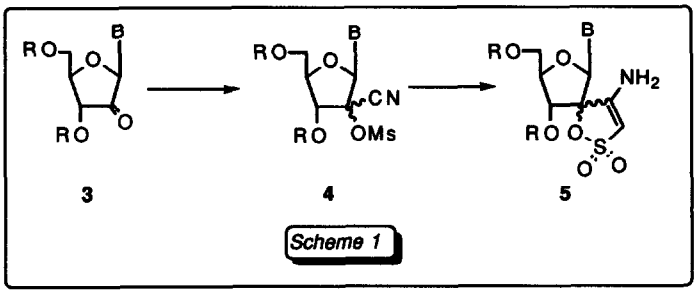
The prototype compound is the more active (EC_{50} 0.034 μg/mL) whereas the cytosine derivative TSAO-C (**2**) is 13-fold less antivirally effective than TSAO-T but markedly (~ 30-fold) less cytotoxic ($CC_{50} \geq 200$ μg/mL).[2,4] Therefore, due to its marked lower toxicity, the TSAO-cytosine derivative (**2**) is more selective as an anti-HIV-1 agent than the corresponding TSAO-T (**1**) (selectivity indices: ≥ 456 and 227, respectively).

Our approach for the synthesis of the TSAO derivatives involved the reaction of α-mesylcyanohydrins of furanosid-3'-ulosyl nucleosides with base to give furanose-3'-spiro-5"-(4"-amino-1",2"-oxathiole 2",2"-



1 (TSAO-T) 2 (TSAO-C)

dioxide)nucleosides by abstraction of one proton from the mesylate methyl group, followed by nucleophilic attack of the carbanion thus formed at the nitrile carbon atom. We have used this procedure for the synthesis of a variety of 3'-spironucleoside derivatives of pyrimidines and purines.[3,19,20] In the present paper we extend this procedure to α-mesyloxynitriles (**4**), prepared from 2'-ketonucleosides (**3**) (Scheme 1), and synthesize 2'-spiro-aminooxathiole nucleoside derivatives of cytosine.



3 **4** **5**

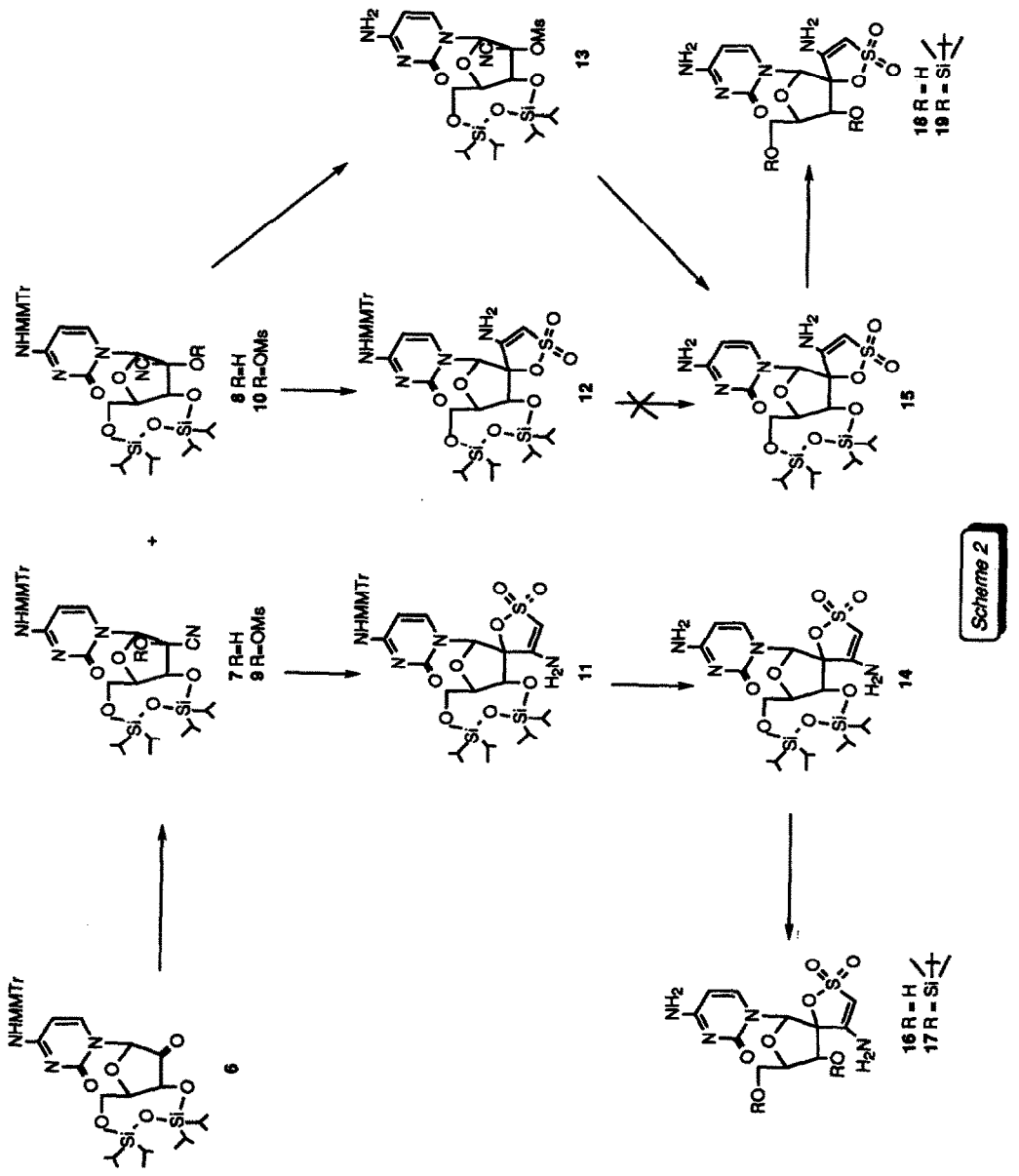
Scheme 1

RESULTS AND DISCUSSION

Treatment of the conveniently protected 2'-keto nucleoside **6**[21] (Scheme 2) with sodium cyanide, in a two-phase ethyl ether/water system, in the presence of sodium bicarbonate, afforded a mixture of the two possible epimeric nucleoside 2'-cyanohydrins **7** and **8**. These cyanohydrins, on standing in solution, reversed to the corresponding ketonucleoside **6**, used as starting material. Thus, they were not isolated, and used without further purification in the next step. Reaction of the cyanohydrin mixture (**7** and **8**) with mesyl chloride in pyridine gave the respective 2'-*C*-cyano-2'-*O*-mesyl-*arabino* and -*ribo* nucleosides **9** (19%) and **10** (25%).

The ^{1}H NMR spectra ($CDCl_3$) at room temperature of α-mesyloxynitriles **9** and **10** showed, a new singlet at 3.14-3.40 ppm (mesyl group) and a significant peak broadening for the signals of H-1' and H-3', suggesting a possible-dynamic process. The same effect was also observed when the spectra were registered in DMSO. At higher temperatures these signals became narrow and H-1' resonance appeared as a singlet and H-3' as a doublet. The dynamic process might be due to the existence of a mixture of two rotational isomers (*syn* and *anti*)[22] of **9** and **10**, due to restricted rotation about the glycosidic bond.

In order to study this process, and fully characterize the products, variable temperature ^{1}H NMR experiments of **9** and **10** were carried out using $CDCl_3$ and DMSO as solvents, the results are shown in Table 1.


NHMMTr
8 R=H
10 R=OMs
12
13
15
16 R = H
19 R = Si
7 R=H
9 R=OMs
11
14
6
16 R = H
17 R = Si

Scheme 2

***Table 1.** Selected ^1H NMR Spectral Data of Cyanomesylates **9** and **10**: Chemical Shifts (ppm), Multiplicity and Coupling Constants (Hz)*

Comp.	Temp. (K)	Solvent	H-1'	H-3' ($J_{3',4'}$)	H-4'	H-5'	H-5	H-6	OMs
	303	$CDCl_3$	6.48 (b signal)	4.84 (b signal)	4.00 (m)		5.08 (d)	7.30 (m)[a]	3.14 (s)
	233	$CDCl_3$	6.63 (s) *anti* 6.04 (s) *syn*	4.50 (d) *anti* (5.2) 5.85 (d) *syn*	4.00 (m)		5.06 (d)	7.25 (m)[a]	3.13 (s)
9	295	DMSO	6.25 (bs)	4.88 (b signal)	3.99 (2bm)		6.32 (d)	7.42 (d)	3.13 (s)
	323	DMSO	6.27 (bs)	4.89 (b signal)	3.99 (2bm)		6.32 (d)	7.42 (d)	3.13 (s)
	333	DMSO	6.27 (bs)	4.89 (b signal)	3.99 (2bm)		6.32 (d)	7.40 (d)	3.13 (s)
	363	DMSO	6.31 (s)	4.89 (d) (5.2)	3.96 (m)	4.06 (m)	6.32 (d)	7.43 (d)	3.13 (s)
	303	$CDCl_3$	6.21 (bs)	4.40 (b signal)	4.08 (m)	4.10 (m)	5.16 (d)	7.30 (m)[a]	3.41 (s)
10	233	$CDCl_3$	6.46 (s) *anti* 6.16 (s) *syn*	4.98 (d) *anti* (9.2) 4.24 (d) *syn* (9.3)	4.02 (m)	3.99 (m)	5.08 (d)	7.40 (d)	3.40 (s)
	363	DMSO	6.06 (s)	4.68 (d) (9.3)	3.99 (m)	4.08 (m)	6.34 (d)	7.48 (d)	3.40 (s)

[a] *Multiplet including MMTr.*

The spectra at low temperature (233 K) showed double signals corresponding to both conformers *syn* and *anti* for each compound (**9** and **10**). The signal corresponding to the anomeric proton (H-1') that appeared at lower field (δ 6.63 for **9** and 6.46 for **10**) was assigned to the *anti* conformers[23], as a result of the deshielding produced by the C=O group. The ratio of *syn:anti* conformers was estimated as (25:75) for **9** and (76:24) for **10**.

The spectra in DMSO at 363 K showed the average signals of the *syn* and *anti* protons H-1' as a sharp singlet (δ 6.31 for **9** and 6.06 for **10**) and H-3' as a doublet (δ 4.89 $J_{3',4'}$=5.2 Hz for **9** and δ 4.68 $J_{3',4'}$=9.3 Hz for **10**).

The absolute configuration at C-2' for the cyanomesylates **9** and **10** was tentatively assigned as *arabino* and *ribo*, respectively, based on the deshielding effect of the adjacent mesyl group over H-3' which appeared at lower field (Δδ=0.44 ppm) in **9** than in **10**. This suggested that H-3' and the mesyl group in **9** are at the same side of the furanose ring, and therefore, its stereochemistry is *arabino*. These deshieldings effects are in agreement with those observed for other 3'-α-mesyloxynitriles of this series.[3,4,19,20,24,25] The assignments of cyanomesylates **9** and **10** as *arabino* and *ribo*, respectively, were confirmed by NOESY experiments carried out on the corresponding spiroderivatives (**11** and **12**) as shown below.

Reaction of **9** and **10** with Cs_2CO_3 or DBU, in dry acetonitrile at room temperature, afforded 2'-spiro-derivatives **11** (67%) and **12** (55%), respectively. Selective deprotection of *arabino* spiro nucleoside (**11**) with 80% acetic acid gave the *N*-deprotected spiro nucleoside **14** (59%). However, a similar deprotection of the *ribo*-spironucleoside **12** lead to complex reaction mixtures from which the deprotected spiro nucleoside **15** could not

be identified. Compound **15** was obtained in 45% yield by a different synthetic strategy, which consisted of *N*-deprotection of cyanomesylate **10** with 80% acetic acid to give the deprotected cyanomesylate **13**, followed by treatment **13** with DBU.

Finally, deprotection of **14** and **15** with tetrabutylammonium fluoride, followed by reaction with *tert*-butyldimethylsilyl chloride yielded the 3',5'-bis-*O*-silylated nucleoside **17** (58%) and **19** (50%).

As described in previous reports in this series,[24,25] formation of the spiro-aminooxathiole ring in **11** and **14** was established by the disappearance in the ^{1}H NMR spectra of the signal corresponding to the mesyl group and the presence of two new singlets at δ 5.52-6.15 assigned to NH_2-4" and at δ 5.48-5.55 assigned to H-3". No dynamic effects were observed in the spectra.

The absolute configuration at C-2' for spiroderivatives **11** and **14** and thus, for the *arabino* cyanomesylate **9**, was unequivocally determined by NOESY experiments. Thus, appearance of correlation peaks between NH_2-4", H-1' and H-4' were only compatible with an *arabino* configuration of the furanose moiety.

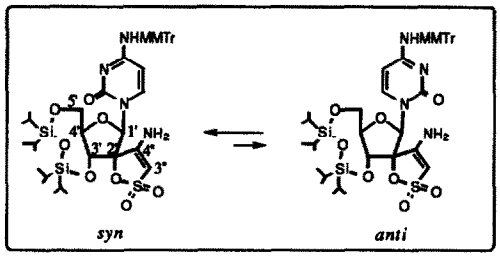
^{1}H NMR spectra of **12** and **15** in $CDCl_3$ and DMSO, at room temperature, showed a significant peak broadening for H-1', H-3' and NH_2-4". When we heated the samples at 343 K (**12**) or 363 K (**15**) in DMSO we observed averaged spectra with sharpened resonances for all of each compound (see Table 2) corresponding to the proposed structures.

In order to study this dynamic process, low variable temperature ^{1}H NMR experiments were performed on compound **12** in $CDCl_3$ (see Figure 1). At 233 K the spectrum showed the overlapping of two subspectra corresponding to both conformers *syn/anti*. The assignment of the resonances of each conformer was carried out by NOESY and COSY experiments (see table 2). The ratio of *syn:anti* conformers was established as 70:30 by integration of the signal of each conformer at 233 K.

***Table 2.** Selected Spectral Data for 2'-Spiro-nucleosides **12** and **15**: Chemical Shifts (ppm), Multiplicity and Coupling Constants (Hz)*

Comp.	Temp. (K)	Solvent	H-1'	H-3' ($J_{3',4'}$)	H-4'	H-5'	H-3"	NH_2-4"	H-5	H-6
	303	$CDCl_3$	—	—	4.00 (bm)[a]	4.25 (bm)	5.54 (s)	—	4.99 (bd)	7.00 (b signal)
	233	$CDCl_3$	6.18 (s) *anti* 5.27 (s) *syn*	4.08 (d) *anti* 5.38 (d) *syn (8.2)*	4.00 (bm)[a] *anti* 3.94 (m)[a] *syn*	3.86 (m) *anti* 4.18 (m) *syn*	5.68 (s) *anti* 5.56 (s) *syn*	4.80 (s) *anti* 3.74 (s) *syn*	5.12 (d) *anti* 5.02 (d) *syn*	7.56 (d) *anti* 6.84 (d) *syn*
12	302	DMSO	5.80 (bs)	—	4.00 (bm)[a]		5.57 (s)	6.12 (bs)	6.32 (d)	7.52 (d)
	323	DMSO	5.81 (bs)	4.63 (bd)	3.99 (bm)[a]	4.13 (m)	5.60 (s)	5.92 (bs)	6.30 (d)	7.48 (d)
	343	DMSO	5.82 (s)	4.70 (d) (8.2)	3.92 (m)[a]	4.14 (m)	5.62 (s)	5.82 (s)	6.30 (d)	7.47 (d)
	303	DMSO	5.98 (bs)	—	4.00 (bm)[a]	4.18 (m)	5.62 (s)	6.22 (bs)	5.78 (d)	5.58 (d)
15	363	DMSO	5.88 (s)	4.79 (d) (5.0)	4.00 (m)[a]	4.18 (m)	5.61 (s)	6.06 (s)	5.80 (d)	7.57 (d)

[a] *Broad multiplet including H-5'a.*



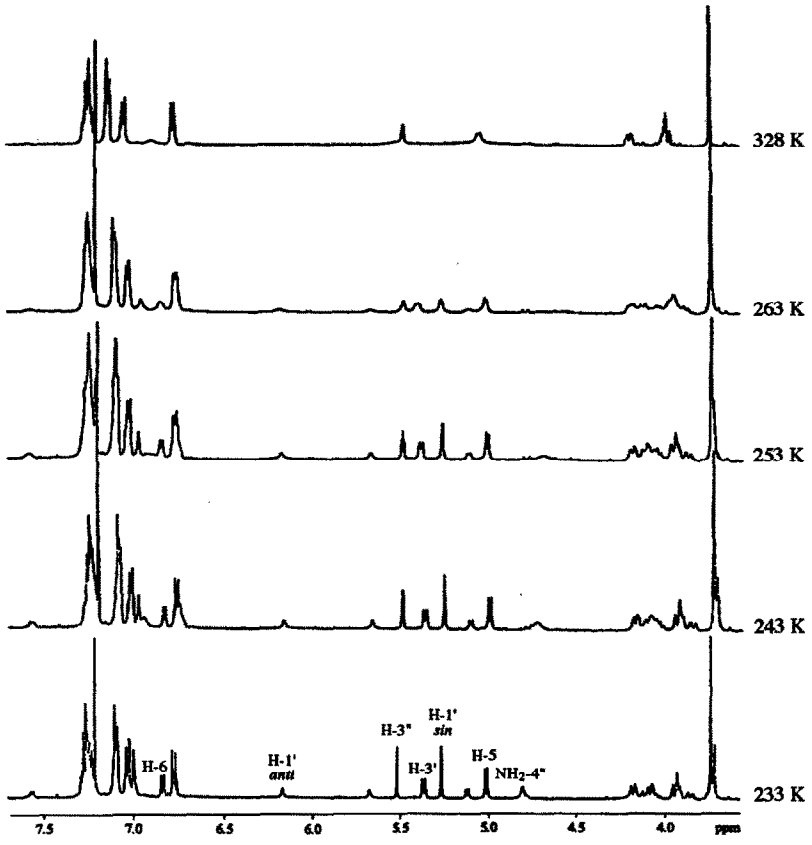


Figure 1.

Section of the 500-MHz ^1H NMR variable temperature spectra of spiroderivative **12** *in $CDCl_3$ at the temperatures indicated.*

The possible interaction between NH_2-4", located at the β-face of the *ribo*-spironucleosides (**12** and **15**) and the 2-C=O group of the nucleobase might be responsible for resticted rotation around the glycosidic bond, and therefore, of the dynamic process.

Compounds **9-12, 14, 15, 17, 19** were tested for their *in vitro* inhibitory effects on HIV-1 replication. Unfortunately none of them showed any significant anti-HIV-1 activity at subtoxic concentrations.

EXPERIMENTAL

Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer operating at 300 MHz, under standard conditions. ^{1}H NMR spectra of compounds **9**, **10**, **12** and **15** were recorded on a Varian Unity-500 spectrometer operating at 499.84 MHz using $CDCl_3$ or DMSO as solvent. The spectra were acquired using 4600 Hz spectral width digitized with 32 K data points with a pulse witdth of 7 μs (90° flip angle). Digital resolution for the real part was ± 0.3 Hz. Variable temperature experiments were carried out under the same conditions. The temperature was varied in the range 295-363 K. The phase-sensitive NOESY experiment of compound **14** was performed at 298 K. The spectral widths were 4602 Hz in both domains, a ralaxation delay of 2.0 s, a mixing time of 200 ms and 512 incremets with 2048 data points each. Zero filling of the F1 dimensions produced a 2048 x 2048 data matrix after 2D Fourier transform. The COSY experiment was performed at 233 K, in the same conditions to those for the NOESY spectrum. ^{13}C NMR spectra were recorded on a Bruker AM-200 and a Varian XL-300 spectrometer operating at 50 and 75 MHz, with Me_4Si as internal standard. IR spectra were recorded with a Shimadzu IR-435 spectrometer. Analytical TLC was performed on silica gel 60 F_{254} (Merck). 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 (1mm), flow rate (5 mL/min). Flash column chromatography was performed with silica gel 60 (230-400 mesh) (Merck).

1-[2'-*C*-Cyano-2'-*O*-mesyl-3',5'-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-arabinofuranosyl]-4-*N*-(monomethoxytrityl)cytosine and 1-[2'-*C*-cyano-2'-*O*-mesyl-3',5'-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-ribofuranosyl]-4-*N*-(monomethoxytrityl)cytosine (9 and 10).
A mixture of the 2'-ketonucleoside **6** (3.02 g, 4 mmol), water (16 mL), ethyl ether (32 mL), sodium bicarbonate (0.67 g, 8 mmol) and sodium cyanide (0.2 g, 4 mmol) was stirred vigorously at room temperature for 3 days. The organic phase was separated, and the aqueous phase was washed with ethyl ether (2x50 mL). The combined ethereal phases were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue, a mixture of the two epimeric cyanohydrins (**7** and **8**), was dissolved in dry pyridine (8 mL). To this solution mesyl chloride (1.6 mL, 20 mmol) was added. The mixture was stirred at 0-5°C for 72 h. The solvent was evaporated to dryness and the residue was treated with dichloromethane (50 mL) and washed with 1N HCl (2 x 25 mL) and water (2 x 25 mL), dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by column chromatography (hexane:ethyl acetate, 1:1). The faster moving fractions afforded 0.35 g (19%) of **9** as a yellow foam: IR (KBr) 1370, 1185 cm^{-1} (SO_2). Anal. calcd. for $C_{43}H_{56}N_4O_9SSi_2$: C, 59.97; H, 6.55; N, 6.51; S, 3.72. Found: C, 59.60; H, 6.49; N, 6.50; S, 3.43.

The slower moving fractions afforded 0.48 g (25%) of **10** as a yellow foam: IR (KBr) 1370, 1185 cm^{-1} (SO_2). Anal. calcd. for $C_{43}H_{56}N_4O_9SSi_2$: C, 59.97; H, 6.55; N, 6.51; S, 3.72. Found: C, 59.69; H, 6.42; N, 6.59; S, 3.57.

[1-[3',5'-*O*-(Tetraisopropyldisiloxan-1,3-diyl)-β-D-arabinofuranosyl]-4-*N*-(monomethoxytrityl)cytosine]-2'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (11).
To a solution of **9** (0.30 g, 0.35 mmol) in dry acetonitrile (10mL), Cs_2CO_3 (0.11 g, 0.35 mmol) was added. The mixture was stirred at room temperature for 24 h. and filtered. The filtrate was neutralized with acetic acid and evaporated to dryness. The residue was purified by column chromatography (hexane:ethyl acetate, 1:2) to give 0.20 g (67%) of **11** as a yellow foam. IR (KBr) 3400, 3350 cm^{-1} (NH_2), 1640 (C=C-N); UV (MeOH) λ_{max} nm

(log ε) 202 (4.38), 228 (4.26), 274 (3.79). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87-0.97 (m, 28H, isopropyl). 3.72 (s, 3H, OCH_3), 3.89 (m, 2H, 2H-5'), 3.93 (m, 1H, H-4'), 4.52 (d, 1H, $J_{3',4'}$=4.2 Hz, H-3'), 5.03 (d, 1H, H-5), 5.48 (s, 1H, H-3"), 5.52 (bs, 2H, NH_2-4"), 6.55 (s, 1H, H-1'), 6.70 (bs, 1H, NHMMTr), 6.77 (d, 2H, m-H of MMTr), 7.20-7.40 (m, 13H, Ar-H, H-6). Anal. calcd. for $C_{43}H_{56}N_4O_9SSi_2$: C, 59.97; H, 6.55; N, 6.51; S, 3.72 Found: C, 59.58; H, 6.76; N, 6.50; S, 3.40.

[1-[3',5'-*O*-(Tetraisopropyldisiloxan-1,3-diyl)-β-D-ribofuranosyl]-4-*N*-(monomethoxytrityl)cytosine]-2'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (12).

According to the method described for the synthesis of **11**, cyanomesylderivative **10** (0.20 g, 0.23 mmol) was treated vith Cs_2CO_3 (0.07 g, 0.23 mmol) for 24h. After the work-up, the residue was purified by column chromatography (hexane:ethyl acetate, 1:1) to give 0.11 g (55%) of **12** as a yellow foam. IR (KBr) 3450, 3380 cm^{-1} (NH_2), 1655 (C=C-N); UV (MeOH) λ_{max} nm (log ε) 201 (4.24), 228 (4.35), 274 (3.62). Anal. calcd. for $C_{43}H_{56}N_4O_9SSi_2$: C, 59.97; H, 6.55; N, 6.51; S, 3.72. Found: C, 59.52; H, 6.78; N, 6.50; S, 3.56.

1-[2'-*C*-Cyano-2'-*O*-mesyl-3',5'-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-ribofuranosyl] cytosine (13).

The protected cianomesylate **10** (0.25 g, 0.29 mmol) was treated with 80% aqueous acetic (10 mL) and the reaction mixture was heated at 80°C for 20 min. The solvent was evaporated to dryness (at a temperature below 30°C) and the residue was succesively co-evaporated with ethanol (2 x 25 mL) and toluene (1 x 5 mL). The residue was purified by CCTLC on chromatotron (dichloromethane: methanol, 20:1) to give 0.09 g (60%) of **13** as a white foam. IR (KBr) 3400 cm^{-1} (NH_2), 1370, 1185 (SO_2); ^{1}H NMR [$(CD_3)_2CO$, 300 MHz]: δ 1.11-1.17 (m, 28H, isopropyl). 3.47 (s, 3H, SO_2CH_3), 4.18 (m, 3H, H-4', 2H-5'), 4.75 (d, 1H, $J_{2',3'}$=7.8 Hz, H-3'), 6.02 (d, 1H, H-5), 6.19 (s, 1H, H-1'), 7.00 (bs, 2H, NH_2), 7.71 (d, 1H, H-6). ^{13}C NMR [$(CD_3)_2CO$, 50 MHz): δ 40.61 (SO_2CH_3), 58.91 (C-5'), 73.86 (C-4'), 81.18 (C-3'), 82.32 (C-2'), 90.31 (C-1'), 96.29 (C-5), 113.10 (CN), 139.18 (C-6), 155.21 (C-2), 165.47 (C-4). Anal. calcd. for $C_{23}H_{40}N_4O_8SSi_2$: C, 46.92; H, 6.85; N, 9.51; S, 5.44. Found: C, 46.48; H, 6.78; N, 9.50; S, 5.28.

[1-[3',5'-*O*-(Tetraisopropyldisiloxan-1,3-diyl)-β-D-arabinofuranosyl]-cytosine]-2'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (14).

The protected compound **11** (0.12 g, 0.12 mmol) was treated with 80% aqueous acetic (2.4 mL) . The reaction mixture was heated at 80°C for 20 min. The solvent was evaporated to dryness (at a temperature below 30°C) and the residue was succesively co-evaporated with ethanol (2 x 3 mL) and toluene (2 x 3 mL). The residue was purified by CCTLC on chromatotron (dichloromethane: methanol, 20:1) to give 0.042 g (59%) of **14** as an amorphous solid. IR (KBr) 3400, 3350 cm^{-1} (NH_2), 1655 (C=C-N). ^{1}H NMR [$(CD_3)_2CO$, 300 MHz]: δ 1.08-1.13 (m, 28H, isopropyl). 4.17 (m, 3H, H-4', 2H-5'), 4.74 (d, 1H, $J_{3',4'}$=4.5 Hz, H-3'), 5.55 (s, 1H, H-3"), 5.84 (d, 1H, H-5), 6.15 (bs, 2H, NH_2-4"), 6.70 (m, 3H, H-1', NH_2), 7.49 (d, 1H, H-6). Anal. calcd. for $C_{23}H_{40}N_4O_8SSi_2$: C, 46.92; H, 6.85; N, 9.51; S, 5.44. Found: C, 46.53; H, 6.75; N, 9.41: S, 5.19.

[1-[3',5'-*O*-(Tetraisopropyldisiloxan-1,3-diyl)-β-D-ribofuranosyl]-cytosine]-2'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (15).

According to the method described for the synthesis of **11**, cyanomesylderivative **13** (0.09 g, 0.15 mmol) was treated vith DBU (0.02 g, 0.15 mmol) for 1h. After the work-up, the residue was purified by by CCTLC on chromatotron (dichloromethane: methanol, 20:1) to give 0.02 g (45%) of **15** as a white foam. IR (KBr) 3400,

3350, 3250 cm^{-1} (NH_2), 1650 (C=C-N).Anal. calcd. for $C_{23}H_{40}N_4O_8SSi_2$: C, 46.92; H, 6.85; N, 9.51; S, 5.44. Found: C, 46.43; H, 6.74; N, 9.40; S, 5.40.

[1-[2',5'-Bis-*O*-(*t*-butyldimethylsilyl)-β-D-arabinofuranosyl]cytosine]-2'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (17).

The solution of the protected nucleoside **14** (0.05 g, 0.06 mmoles) in THF (5 mL) was added tetrabutylammonium fluoride trihydrate (Bu_4NF) (0.04 g, 0.12 mmoles), and the mixture was stirred at room temperature for 2 h. The reaction mixture was filtered through a column of silica gel using THF as eluent. The filtrate was evaporated to dryness. The residue (deprotected compound **16**) was suspended in dry acetonitrile (2 mL) and then 4-(dimethylamino)pyridine (0.06 g, 0.48 mmol) and *tert*-butyldimethylsilyl chloride (0.04 g, 0.24 mmol) were added. The mixture was heated to reflux for 7 h. The solvent was evaporated to dryness and the residue was treated with ethyl acetate (10 mL) and water (10 mL). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (10 mL). The combined organics were successively washed with cold (4°C) 0.1 NHCl (5 mL), water (5 mL), and brine (5 mL) and finally dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by CCTLC on chromatotron (ethyl acetate:methanol, 30:1) to give 0.02 g (58%) of **17** as a white foam. IR (KBr) 3450, 3300, cm^{-1} (NH_2), 1650 (C=C-N). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.89, 0.92 (2s, 18H, 2*t*-Bu), 3.83 (m, 2H, 2H-5'), 4.09 (m. 1H, H-4'), 4.40 (d, 1H, $J_{3',4'}$=3.4 Hz, H-3'), 5.42 (s, 1H, H-3"), 5.82 (d, 1H, H-5), 6.11 (bs, 2H, NH_2), 6.79 (s, 1H, H-1'), 7.64 (d, 1H, H-6). Anal. cald. for $C_{23}H_{42}N_4O_7SSi_2$: C, 48.06; H, 7.36; N, 9.75; S, 5.58. Found: C, 47.89; H, 7.30; N, 9.63; S, 5.21.

[1-[2',5'-Bis-*O*-(*t*-butyldimethylsilyl)-β-D-ribofuranosyl]cytosine]-2'-spiro-5"-(4"-amino-1",2"-oxathiole-2",2"-dioxide) (19).

According to the method described for the synthesis of **17**, protected nucleoside **15** (0.05 g, 0.06 mmoles) was treated with Bu_4NF (0.04 g, 0.12 mmoles) for 2 h. After the work-up, the residue was silylated with *tert*-butyldimethylsilyl chloride. The residue was purified by CCTLC on chromatotron (ethyl acetate:methanol, 30:1) to give 0.017 g (50%) of **19** as a white foam. IR (KBr) 3450, 3300, cm^{-1} (NH_2), 1650 (C=C-N). ^1H NMR ($CDCl_3$, 200 MHz): δ 0.88, 0.91 (2s, 18H, 2*t*-Bu), 3.99 (m, 3H, H-4', 2H-5'), 4.55 (d, 1H, $J_{3',4'}$=3.2 Hz, H-3'), 5.70 (bm, 3H, NH_2, H-3"), 5.83 (d, 1H, H-5), 6.64 (s, 1H, H-1'), 7.41 (d, 1H, H-6). Anal. cald. for $C_{23}H_{42}N_4O_7SSi_2$: C, 48.06; H, 7.36; N, 9.75; S, 5.58. Found: C, 47.76; H, 7.25; N, 9.61; S, 5.31.

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