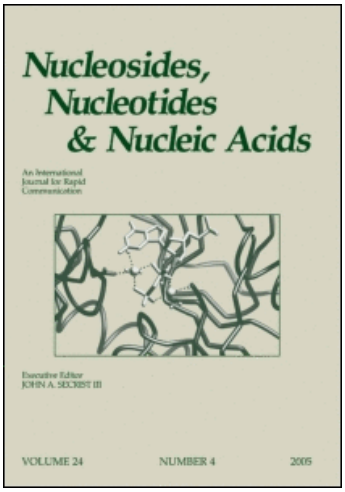

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Synthesis and Antiviral Evaluation of 2'-Deoxy-4'-thio-L-nucleosides and Their Phosphotriester Derivatives Bearing *S*-Acyl-2-thioethyl Bioreversible Phosphate-Protecting Groups

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SYNTHESIS AND ANTIVIRAL EVALUATION OF 2'-DEOXY-4'-THIO-L-NUCLEOSIDES AND THEIR PHOSPHOTRIESTER DERIVATIVES BEARING *S*-ACYL-2-THIOETHYL BIOREVERSIBLE PHOSPHATE-PROTECTING GROUPS.

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Abstract:
A new route to 2-deoxy-4-thio-L-ribofuranose and the synthesis of some 4'-thio-L-nucleosides are reported. Also, the bis(SATE) phosphotriester derivatives of 2'-deoxy-4'-thio-L-cytidine and -adenosine were synthesized and their biological activities are discussed.

Nucleosides containing a sulfur atom in place of the 4'-oxygen have been the focus of recent research because of their potential biological activity. A number of both D- and L-2'-deoxy-4'-thionucleosides have been synthesized[1] either starting from carbohydrate precursors [2-11] or by *de novo* approaches[12-15]. Some of them have shown anti-herpes (HSV) and anti-human cytomegalovirus (HCMV) activities as well as anticancer effects[4,9,16-18]. However, at the opposite of L-nucleosides, no L-2'-deoxy-4'-thionucleoside was reported to present an antiviral activity.

The antiviral activity of most nucleoside analogues is dependent on kinase-mediated activations to generate the bioactive triphosphate forms[19] which competitively inhibit the viral polymerase or terminate the newly synthesized viral DNA chain. These activations involve three successive cellular kinases, the first one being highly specific[20-24]. Some nucleoside analogues may not show biological response because they are not enzymatically transformed to their corresponding 5'-monophosphorylated nucleotide.

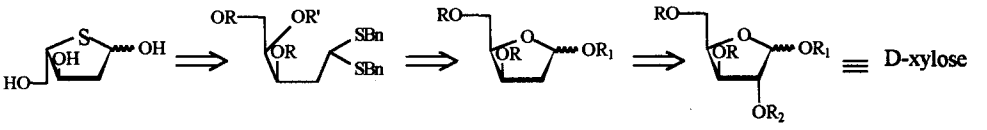
Various concepts have been proposed to by-pass the first phosphorylation step of nucleoside analogues[25-27]. These approaches consist in temporarily masking the 5'-phosphate negative charges of their corresponding mononucleotides with neutral bioreversible substituents, thereby forming more lipophilic derivatives which would be expected to revert back to the nucleoside monophosphate once inside the cell. For instance, (acyloxy)alkyl or (acyloxy)aryl groups have been already studied as esterase-mediated bioreversible phosphate protections[28-31], and among them the (pivaloyloxy)methyl (POM) has been proposed as an efficient transient protecting group for several bioactive nucleoside monophosphate or phosphonate analogues[32-34]. In our laboratory we have developped two other kinds of enzyme-labile phosphate protecting groups: the *S*-[(2-hydroxyethyl)sulfidyl]-2-thioethyl (DTE) group[35] and the *S*-acyl-2-thioethyl (SATE) group[36,37], the latter being a carboxylate esterase-labile transient phosphate group.

In this paper we report a new route to 2-deoxy-4-thio-L-ribofuranose starting from D-xylose, the synthesis of the L-nucleosides of uracil, cytosine and adenine, as well as the synthesis and antiviral evaluation of the bis(SATE)phosphotriester derivatives of 2'-deoxy-4'-thio-L-cytidine and adenosine.

CHEMISTRY

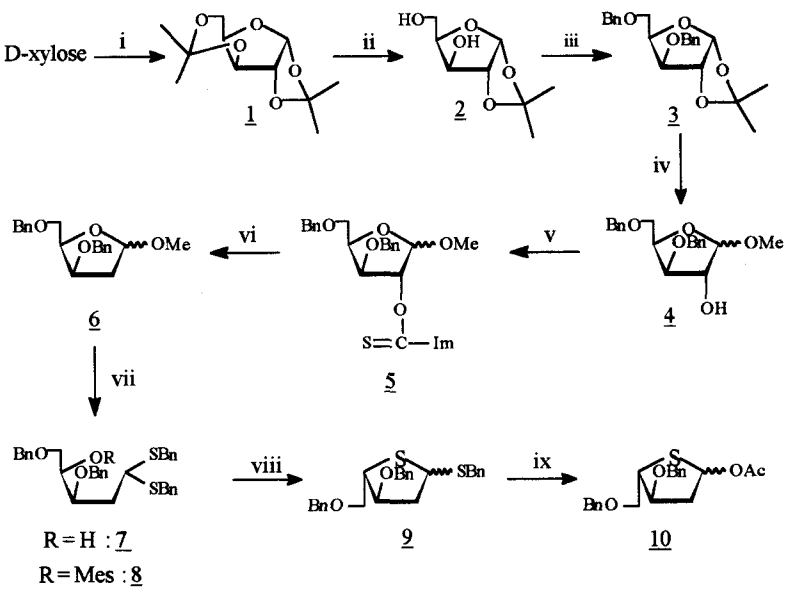
The 2-deoxy-4-thio-L-ribofuranose, a key intermediate in the synthesis of the corresponding 4'-thionucleosides, has been synthesized previously by Uenishi from propan-1,3-diol (23% overall yield in ten steps) under acyclic stereocontrol[13]. Furthermore, its D-enantiomer has been synthesized by several groups either starting from 2-deoxyribose (11% overall yield in seven steps as described by Walker[38]), or starting from L-arabinose as described by Bobek[39]. Another route from D-xylose[40] gave 2-deoxy-4-thio-D-ribofuranose derivative in 12 steps with an 2% overall yield, involving the formation and reduction of ketene dithioacetal derivatives.

The strategy we have developed for the preparation of 2-deoxy-4-thio-L-ribofuranose starts from D-xylose and affords an 17% overall yield in nine steps. This synthesis involves the deoxygenation of the 2-hydroxyl group of the protected D-xylofuranose, followed by a nucleophilic displacement of the previously activated 4-hydroxyl group with only one inversion of configuration (Scheme 1).



SCHEME 1.

Thus, 3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-D-xylofuranose 3 was prepared in three steps from D-xylose using a known methodology[41,42], (Scheme 2). The 3,5-isopropylidene group from 1 was efficiently removed in acidic conditions (HCl/H_2O) and the two free hydroxyl groups were benzylated to obtain 3. Then hydrolysis of the 1,2-isopropylidene group and acetalisation gave methyl-3,5-di-*O*-benzyl-D-xylofuranoside 4 in 57% yield from D-xylose.



i = Me_2CO, H_2SO_4, $CuSO_4$; ii = HCl/H_2O; iii = BnBr, KOH, THF; iv = CF_3COOH/H_2O, $MeOH/H_2SO_4$; v = $Im_2C{=}S$ (Im : imidazole), 1,2-dichloroethane; vi = $(Me_3Si)_3SiH$, AIBN, toluene; vii = BnSH, BF_3/Et_2O; viii = 1) MesCl, pyridine; 2) $BaCO_3$, NBu_4I; ix = $Hg(OAc)_2$, AcOH.

SCHEME 2.

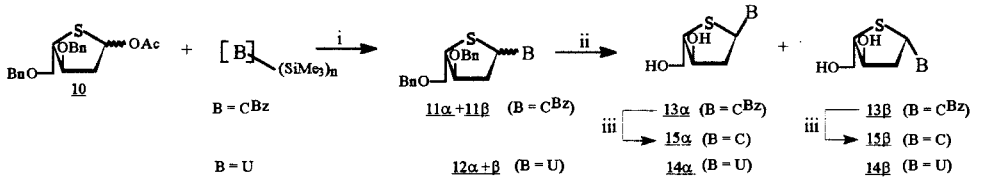
Deoxygenation[43,44] of 4 was performed according to the method of Barton[45], using 1,1'-thiocarbonyldiimidazole (TCDI) in refluxing 1,2-dichloroethane, *via* the intermediate thiourethane 5 (Scheme 2). A single-electron transfer chain reaction on 5 with

tris(trimethylsilyl)silane was initiated with α,α'-azo-isobutyronitrile (AIBN) in refluxing toluene leading to methyl 2-deoxy-3,5-di-*O*-benzyl-D-xylofuranoside 6 in 70% yield from 4. Dithioacetalisation of 6 was performed with benzyl mercaptan and boron trifluoride etherate, and afforded 2-deoxy-3,5-di-*O*-benzyl-1,1-dithiobenzyl acetal-D-xylose 7 in 66% yield. As previously described in the D-ribofuranose series[46], the treatment of 7 with mesyl chloride in pyridine, followed by the addition of barium carbonate and tetrabutylammonium iodide gave *S*-benzyl-2-deoxy-3,5-di-*O*-benzyl-4-thio-L-ribofuranoside 9 (89% yield) which was subsequently treated with $Hg(OAc)_2$ in acetic acid to give 10 (Scheme 2).

NUCLEOSIDE SYNTHESIS.

Synthesis of pyrimidine nucleosides.

Coupling reactions between the thio-sugar 10 and pyrimidine bases were performed by applying a modification of the Vorbrüggen[47,48] method. Thus, the 2,4-*bis*(trimethylsiloxy)derivative of uracil or N^4-benzoyl cytosine was coupled with 10 in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst (Scheme 3).



i = TMSOTf, CH_3CN; ii = BCl_3/CH_2Cl_2, -78°C; iii = MeONa, MeOH.

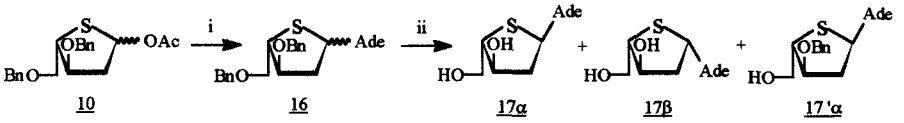
SCHEME 3.

The glycosylation reaction gave a 1:1 α/β anomeric mixture in 74% yield in the case of N^4-benzoyl-cytosine (11) and in 77% yield in the case of uracil (12). The two anomers of N4-benzoyl-2'-deoxy-3',5'-di-*O*-benzyl-4'-thio-L-cytidine 11 were separated by flash silica gel column chromatography. Then, the deprotection of the benzyl groups of each anomer 11α and 11β was performed using a solution of boron trichloride[5] in methylene chloride at -78°C and the N-benzoyl group was removed by MeONa to gave 15α and 15β in almost quantitative yield. On the other hand, separation by flash chromatography of the anomeric mixture of 2'-deoxy-3',5'-di-*O*-benzyl-4'-thio-L-uridine 12 failed. So, we

attempted the deprotection of the benzyl groups on the mixture 12α+β by the same method used for 2'-deoxy-4'-thiocytidine; the anomeric mixture of 2'-deoxy-4'-thio-L-uridine 14 was obtained in 60% yield. At this stage, a crystallization performed in absolute ethanol gave one anomer: 2'-deoxy-4'-thio-β-L-uridine 14β which was fully characterized. The spectroscopic data of these 2'-deoxy-4'-thio-L-nucleosides were consistent with those described by Uenishi[9].

Synthesis of purine nucleosides.

The syntheses of the 2'-deoxy-4'-thiopurine nucleosides in the L-series has never been reported. We describe here the synthesis of the α and β anomers of 2'-deoxy-4'-thio-L-adenosine by a methodology initially introduced by Saneyoshi[49] for natural ribofurano nucleosides. Coupling the 4-thiosugar 10 and adenine with stannic chloride in anhydrous acetonitrile[50] gave 16 as an anomeric mixture in 68% yield (Scheme 4). Separation of the two anomers by a variety of techniques was unsuccessful. So, at this stage the deprotection of the anomeric mixture was made with a large excess of boron trichloride in methylene chloride at -78°C, the reaction was not complete and two side-products were observed[4] : one 17'α (50%) resulted from the monodebenzylation on the primary hydroxyl, the other one was adenine due to the unstability of the glycosyl bond in acidic medium (Scheme 4). The purification of the obtained mixture by preparative HPLC afforded 14% of α-L-4'-S-dA (17α), 4% of β-L-4'-S-dA (17β) and 50% of 17'α.



i = Adenine, $SnCl_4$, CH_3CN; ii = BCl_3/CH_2Cl_2, or BBr_3/CH_2Cl_2 -78°C.

SCHEME 4.

In order to reduce the quantity of 17'α, we then performed the deprotection of the benzyl groups using the more reactive boron tribromide[17] in methylene chloride at -78°C because such procedure usually requires 3 fold less equivalents of the Lewis acid. In this case, we obtained 23% of 17α, 10% of 17β and only 10% of the monobenzylated nucleoside 17'.

TABLE 1.

Compound	$J_{1',2'a}+J_{1',2'b}$ (Hz)	$\delta H_{1'}$ (m) (ppm)	$\delta H_{4'}$ (ppm)
14α	12	6.20 (dd)	3.70
14β	15	6.09 (t)	3.50
15α	12	6.16 (dd)	3.50
15β	15	6.32 (t)	3.20
17α	11	6.20 (dd)	3.71
17β	14	6.17 (t)	3.34

Assignments of all anomeric configurations were made on the basis of proton NMR spectra, in analogy or comparison with 2'-deoxy-4'-thio D-nucleosides described previously in the pyrimidine series[9]. In this regard, it was demonstrated[2] in the 2'-deoxy-4'-thio-D-pyrimidine nucleoside series that several correlations allow the assignment of the anomeric configuration of the thionucleoside derivatives. For example: i) the sum ($J_{1',2'a}+J_{1',2'b}$) for the β anomer is greater than that for the α; ii) the chemical shift for the $H_{4'}$ of α-anomer of 4'-thiothymidine is downfield from $H_{4'}$ of the β-anomer of 4'-thiothymidine[15].

Applying these considerations to the 2'-deoxy-4'-thio-L-pyrimidine and purine nucleosides series (Table 1), we found NMR characteristics consistent with the earlier report of Secrist in the D series[2,15].

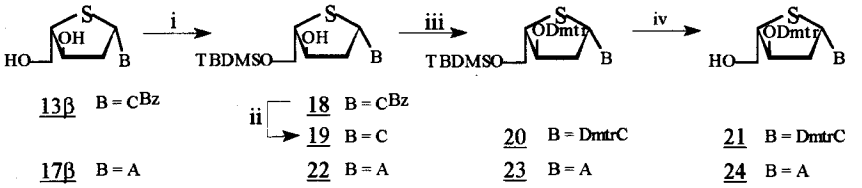
In order to confirm the anomeric configuration of the 2'-deoxy-4'-thio-adenosine 17α and 17β in the L-series, NOE difference spectroscopy experiments were also carried out (data not shown).

Pronucleotide syntheses.

The chemical syntheses of the SATE pronucleotides were performed through a P(III) chemistry, using phosphoramidite intermediates, an approach which was reported to

be the most efficient method to prepare such SATE phosphotriester derivatives[51].

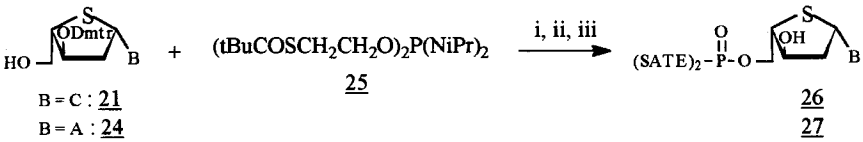
It requires the preparation of the phosphoramidite reagent 25 which was obtained according to the published procedure[37], as well as of the protected 4'-thio-β-L-nucleosides 21 and 24 (Scheme 5). The synthesis of 21 and 24 was performed firstly by selective silylation[52] of the primary hydroxyl of 13β and 17β with TBDMSCl to afforded 18 and 22 respectively in 90% and 97% yields. In the case of 18 we removed subsequently the N4 benzoyl protecting group in basic conditions. Then, treatment of 19 and 22 with DmtrCl in pyridine gave the intermediates 20 and 23. Finally, the deprotection of the 5'-silyl group by TBAF in THF afforded the desired protected 4'-thionucleosides 21 and 24 (Scheme 5).



i = TBDMSCl, Pyridine or DMF, imidazole; ii = NaOMe/MeOH; iii = DmtrCl, Pyridine; iv = TBAF, THF.

SCHEME 5

The coupling reaction of the protected 4'-thio nucleosides 21 and 24 with the phosphitylating agent 25 (Scheme 6) in presence of 1*H*-tetrazole, followed by subsequent *in situ* oxidation with tBuOOH gave the 4'-thio-β-L-nucleoside phosphotriesters 26 and 27 after acidic treatment and purification by silica gel column chromatography, with respectively 55 and 63% yields.



i = 1*H*-tetrazole, THF; ii = tBuOOH; iii = AcOH 80%.

SCHEME 6.

The prepared 4'-thionucleosides 4'-S-β-LdA 17β, 4'-S-α-LdA 17α, 4'-S-α-LdC 15α, 4'-S-β-LdC 15β and the bis(SATE)phosphotriester derivatives 26 and 27 were evaluated for

their ability to inhibit the replication of a variety of DNA and RNA viruses (including HIV and HBV). But they did not show significant antiviral activity and cytotoxicity at the higher concentration tested (usually 10 μM).

Since the pronucleotides 26, 27 are devoid of biological properties it appears that the lack of antiviral activity of their parent 4'-S-β-L-nucleosides is not due to poor phosphorylation.

EXPERIMENTAL SECTION.

General methods.

^{1}H NMR and ^{31}P NMR spectra were determined with a Bruker AC 250MHz with tetramethylsilane as internal standard, and the chemical shifts are quoted in ppm (s = singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet, br = broad signal). Electron mass spectra (70eV) were recorded on a Jeol JMS DX 300 mass spectrometer. Precoated Merck Silica gel F254 plates were used for TLC. Column Chromatography was performed on Merck silica gel (0.040-0.063mm). HPLC analyses and purifications of 4'-thionucleosides were carried out on a Prep Nova-Pak HR C18 6μm 60Å (40 x 100mm) column with system prep 4000 (Waters) and a model 481 UV variable detector.

All the solvents were distilled anhydrous according to the procedure given by D. D. Perrin, and W. L. F. Armarego, Purification of Laboratory Chemicals. Pergamon Press, London (1988).

Compounds 1 and 2 were synthesized following a previously reported procedure[41].

1,2-*O*-Isopropylidene-3,5-di-*O*-benzyl-D-xylofuranose 3 :

To a solution of 2 (10 g, 0.05 mol) in anhydrous tetrahydrofuran (70 ml) were added potassium hydroxide (28 g, 0.47 mol) and benzyl bromide (13 ml, 0.11 mol). The reaction mixture was refluxed with stirring for 24 h. The hot suspension was then filtered through celite and concentrated to dryness. The residue was diluted with methylene chloride (50 ml) then washed with water (2 x 35 ml). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by chromatography over silica gel (CH_2Cl_2) to give 3 (15.7g, 85%) as an oil.

Rf : 0.24 (CH_2Cl_2). ^1H NMR (250 MHz ; $CDCl_3$) : δ 1.15-1.3 (2s, 6H, 2 CH_3) ; 3.7 (m, 2H, H_5, $H_{5'}$) ; 4 (d, 1H, H_3, $J_{3,4}$ = 3.2) ; 4.4 (m, 1H, H_4, $J_{4,3}$ = 3.2) ; 4.5 (m, 4H, 2 CH_2Phe) ; 4.6 (d, 1H, H_2) ; 5.9 (d, 1H, H_1, $J_{1,2}$ = 3.8) ; 7.3 (m, 10H, Phenyls). MS FAB > 0, NBA, *m/z* 371 [M+H]$^+$.

Methyl 3,5-di-*O*-benzyl-D-xylofuranoside 4 :

Aqueous trifluoroacetic acid (80%) (75 ml) was added to 3 (15.7 g, 0.042 mol), and the mixture was stirred at 0°C for 3 h. Then the acidic solution was neutralized by solid sodium hydrogen carbonate. The aqueous layer was extracted with CH_2Cl_2 (70 ml). The organic solution was dried over Na_2SO_4 and evaporated to dryness to give 3,5-di-O-benzyl-D-xylofuranose as a oil. This brown oil was dissolved in dry methanol (100 ml) and concentrated sulphuric acid (0.036 mol) was added at 0°C. The reaction was monitored by TLC, and after 24 h the crude material was neutralized by pyridine (pH 7-8), evaporated to dryness, diluted by CH_2Cl_2 (50 ml), washed with water (30 ml), dried and evaporated to dryness.

The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH : 97/3) to give pure 4 as an anomeric mixture (α/β:53/47) (13 g, 90%).

Rf = 0.53 (diethyl ether). ^1H NMR (250 MHz ; $CDCl_3$) : δ 2.52 (2H, brs, $OH_{2\alpha}$ and $OH_{2\beta}$) ; 3.37-3.45 (2s, 6H, OCH_3 α and OCH_3 β) ; 3.69 (m, 4H, $H_{5\alpha}$, $H_{5\beta}$, $H_{5'\alpha}$ and $H_{5'\beta}$) ; 3.91 (m, H, $H_{3\alpha}$) ; 3.95 (q, 1H, $H_{3\beta}$) ; 4.17 (t, 1H, $H_{2\beta}$) ; 4.22 (m, 1H, $H_{2\alpha}$) ; 4.60 (m, 10H, 4 $C\underline{H}_2$Phe, $H_{4\alpha}$ and $H_{4\beta}$) ; 4.77 (d, 1H, $H_{1\beta}$) ; 4.95 (d, 1H, $H_{1\alpha}$) ; 7.25 (m, 20H, Phenyls). MS FAB > 0, NBA, m/z 391 [M+2Na+H]$^+$.

Methyl 2-deoxy-3,5-di-*O*-benzyl-2-(1-imidazoyl)thiocarbonyloxy-D-xylofuranoside 5 :

Solid 1,1'-thiocarbonyldiimidazole (8.75 g, 0.049 mol) was added to a solution of 4 (13 g, 0.038 mol) in 160 ml of 1,2-dichloroethane. The reaction mixture was heated at gentle reflux until TLC analysis confirmed the disappearance of starting material (3 h). After cooling, the solution was concentrated *in vacuo* and the product isolated by chromatography (diethyl ether/hexane : 70/30) to give 5 (19.5 g, 88%). This compound was used without more purification.

Methyl 2-deoxy-3,5-di-*O*-benzyl- D-xylofuranoside 6 :

Compound 5 (15 g, 0.03 mol) was dissolved in dry toluene (200 ml) and *tris*(trimethyl silyl)silane (11.5 ml, 0.037 mol) and AIBN (1.8 g, 0.01 mol) were added. The reaction mixture was boiled under reflux with stirring for 2 h. The solution was cooled and then concentrated in vacuo. The residue was extracted with methylene chloride. The organic layers were dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica gel column with CH_2Cl_2 to give 6 (8.6 g, 80%).

Rf = 0.52 (Diethyl ether/Hexane : 80/20). ^1H NMR (250 MHz ; $CDCl_3$) : δ 2.1 (m, 2H, H_2, $H_{2'}$) ; 3.35 (s, 3H, OCH_3) ; 3.72 (m, 2H, H_5, $H_{5'}$) ; 4.04 (m, 1H, H_3, $J_{3,4}$ = 5.1) ; 4.18 (m, 1H, H_4, $J_{4,3}$ = 5.2, $J_{4,5}$ = 7.2) ; 4.45 (m, 4H, 2 $C\underline{H}_2$Phe) ; 4.96 (dd, 1H, H_1, $J_{1,2}$ = 2.9) ; 7.25 (m, 10H, Phenyls). MS FAB > 0, NBA, *m/z* 297 [M-OMe]$^+$.

Compounds 7, 8 and 9 were prepared according to the procedure described by Bellon[46] in the D-ribofuranose series. The compound 8 was used without purification.

2-Deoxy-3,5-di-*O*-benzyl-1,1-dithiobenzylacetal-D-xylose 7 :

Yield : 66%. Rf = 0.55 (CH_2Cl_2/MeOH : 99/1). ^1H NMR (250 MHz ; $CDCl_3$) : δ 1.95 (m, 2H, H_2, $H_{2'}$) ; 2.2 (d, 1H, OH_4, $J_{4,OH}$ = 5.8) ; 3.3 (d, 2H, $SC\underline{H}_2$Phe) ; 3.65 (m, 7H, $SC\underline{H}_2$Phe, H_1, H_3, H_4, H_5, $H_{5'}$) ; 4.1 (q, 2H, $OC\underline{H}_2$Phe) ; 4.4 (s, 2H, $OC\underline{H}_2$Phe) ; 6.65 (m, 20H, Phenyl). MS FAB > 0, NBA, *m/z* 562 [M+H_2O]$^+$.

2-Deoxy-3,5-di-*O*-benzyl-1-thiobenzyl-4-thio-L-ribofuranose 9 :

Yield : 89%. Rf = 0.46 (CH_2Cl_2). ^1H NMR (250 MHz ; $CDCl_3$) : δ 2.17 (m, 2H, H_2, $H_{2'}$) ; 3.42 (m, 2H, H_5, $H_{5'}$) ; 3.78 (m, 3H, $SC\underline{H}_2$Phe and H_4) ; 4.2 (m, 1H, H_3) ; 4.43 (m, 5H, 2 $C\underline{H}_2$Phe and H_1) ; 7.23 (m, 15H, Phenyls). MS FAB > 0, NBA, *m/z* 313 [M+H-$PhCH_2SH$]$^+$.

1-Acetoxy-2-deoxy-3,5-di-*O*-benzyl-4-thio-L-ribofuranose 10 :

To a solution of 9 (6.6 g, 0.015 mol) in glacial acetic acid (85 ml) was added mercuric acetate (10.7 g, 0.03 mol). The solution was kept at room temperature with stirring for 1 h, then neutralised by aqueous 5% sodium hydrogen carbonate. The crude material was

extracted with CH_2Cl_2 (60 ml), water (40 ml) and 5% aqueous KCN (40 ml) ; the organic layers were dried, evaporated and the residue was purified by chromatography over silica gel column to give pure 10 (4.2 g, 75%).

Rf = 0.2 (CH_2Cl_2). ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 2.00 (s, 3H, OAc) ; 2.31 (m, 2H, H_2, $H_{2'}$) ; 3.3 (m, 2H, H_5, $H_{5'}$) ; 3.8 (m, 1H, H_4) ; 4.2 (m, 1H, H_3) ; 4.48 (m, 4H, 2 CH_2Phe) ; 6.08 (dd, 1H, H_1, $J_{1,2}$ = 1.7, $J_{1,2'}$ = 6) ; 7.25 (m, 10H, Phenyls). MS FAB > 0, NBA, *m/z* 373 [M+H]$^+$.

General coupling reaction of 10 and 2,4-bis(trimethylsilyloxy)pyrimidines.

To a suspension of uracil (89 mg, 0.795 mmol) or N^4-benzoylcytosine (170 mg, 0.795 mmol) in anhydrous acetonitrile (4 ml) was added BSA (780 µl, 3.18 mmol) and the mixture was boiled under reflux with stirring for 2 hours. To the resulting clear solution, was added 1-acetoxy-2-deoxy-3,5-di-*O*-benzyl-4-thio-L-ribofuranose 10 (200 mg, 0.53 mmol) and trimethylsilyl trifluoromethanesulfonate (115 µl, 0.636 mmol). The reflux was continued for 4 h and then the reaction mixture was diluted with methylene chloride (15 ml) and washed with 5% sodium bicarbonate and water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by chromatography over silica gel column (elution with CH_2Cl_2 for 11 and AcOEt:Hexane / 1:1 in the case of 12).

1-(2-Deoxy-3,5-di-*O*-benzyl-4-thio-L-ribofuranosyl)N^4-benzoyl-cytosine 11 :

α-anomer : Yield : 30%. Rf : 0.45 (AcOEt). ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 2.48 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.47 (m, 2H, $H_{5'}$, $H_{5''}$) ; 4.05 (m, 1H, $H_{3'}$) ; 4.35 (m, 1H, $H_{4'}$) ; 4.51 (m, 4H, 2 CH_2Phe) ; 6.38 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 1.6, $J_{1',2''}$ = 6.5) ; 7.35 (m, 10H, Phenyls) ; 7.60 (m, 3H, H_5, H *meta* and *para* of benzoyl) ; 7.92 (d, 2H, H *ortho* of benzoyl) ; 8.65 (d, 1H, H_6, $J_{6,5}$ = 7.5) ; 8.80 (brs, 1H, NH). MS FAB > 0, GT, *m/z* 528 [M+H]$^+$. UV (EtOH) λ_{max} 257 nm, λ_{min} 284 nm. **β-anomer** : Yield : 45%. Rf : 0.53 (AcOEt). ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 2.27 (m, 1H, $H_{2'}$) ; 2.60 (m, 1H, $H_{2''}$) ; 3.70 (m, 3H, $H_{5'}$, $H_{5''}$ and $H_{4'}$) ; 4.19 (m, 1H, $H_{3'}$) ; 4.50 (m, 4H, 2 CH_2Phe) ; 6.45 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 6) ; 7.37 (m, 11H, Phenyls) ; 7.58 (m, 3H, H_5, 2H of benzoyl) ; 7.85 (d, 2H, H *ortho* of benzoyl) ; 8.55 (brs, 1H, NH) ; 8.62 (d, 1H, H_6, $J_{5,6}$ = 7.2). MS FAB > 0, NBA, *m/z* 528 [M+H]$^+$. UV (EtOH) λ_{max} 259 nm, λ_{min} 286 nm.

2'-Deoxy-3',5'-di-*O*-benzyl-4'-thio-L-uridine 12 :

Yield : 77%. Rf = 0.21 (Diethyl ether). UV (EtOH) λmax 263 nm, λmin 232 nm ; UV (0.1 N HCl) λ_{max} 263 nm, λ_{min} 232 nm ; UV (0.1 N NaOH) λ_{max} 263 nm, λ_{min} 241.5 nm. α-anomer : Yield : 35%. ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 2.35 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.37 (m, 2H, $H_{5'}$, $H_{5''}$) ; 3.93 (m, 1H, $H_{4'}$) ; 4.27 (m, 1H, $H_{3'}$) ; 4.50 (m, 4H, 2 CH_2Phe) ; 5.50 (d, 1H, H_5, $J_{5,6}$ = 8.24) ; 6.26 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 1.8, $J_{1',2''}$ = 7.6) ; 7.20 (m, 10H, Phenyls) ; 8.10 (d, 1H, H_6, $J_{6,5}$ = 8.24) ; 8.47 (brs, 1H, NH). MS FAB > 0, NBA, *m/z* 425 [M+H]$^+$. β-anomer : Yield : 42%. ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 2.30 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.37 (m, 2H, $H_{5'}$, $H_{5''}$) ; 3.80 (m, 1H, $H_{3'}$) ; 4.23 (m, 1H, $H_{4'}$) ; 4.50 (m, 4H, 2 CH_2Phe) ; 5.30 (d, 1H, H_5, $J_{5,6}$ = 8.2) ; 6.43 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 7.5) ; 7.30 (m, 10H, Phenyls) ; 8.00 (d, 1H, H_6, $J_{6,5}$ = 8.2) ; 8.54 (brs, 1H, NH). MS FAB > 0, NBA, *m/z* 425 [M+H]$^+$.

General procedure for debenzylation.

To a solution of 1 M BCl_3 in CH_2Cl_2 (5.1 ml, 5.1 mmol), in a three necked round bottomed flask with a thermometer and magnetic stirrer, cooled to -78°C, was added dropwise a solution of the benzylated nucleoside (0.26 mmol) (11α, 11β or 12) in dry CH_2Cl_2 (5 ml). The reaction mixture was further stirred (-78°C) for 6 hours and then quenched with 5 ml of the 1:1 mixture of dry CH_2Cl_2 and dry CH_3OH. The solution was allowed to warm up to room temperature, neutralized with $NaHCO_3$ (5%) and evaporated to dryness. The product was purified by chromatography over silica gel column using CH_2Cl_2:CH_3OH / 90:10.

1-(2-Deoxy-4-thio-L-ribofuranosyl)N^4-benzoyl-cytosine 13 :

Yield : 82%. Rf : 0.24 (CH_2Cl_2:CH_3OH / 70:30). α anomer : ^{1}H NMR (250 MHz ; $DMSOd_6$) : δ 2.23 (m, 2H, $H_{2'}$, $H_{2''}$) ; 4.02 (m, 3H, $H_{4'}$, $H_{5'}$ and $H_{5''}$) ; 4.15 (m, 1H, $H_{3'}$) ; 5.16 (t, 1H, $OH_{5'}$) ; 5.41 (d, 1H, $OH_{3'}$) ; 6.15 (dd, 1H, $H_{1'}$) ; 7.35 (d, 1H, H_5) ; 7.59 (m, 3H, H *meta* and *para* of benzoyl) ; 8.36 (d, 2H, H *ortho* of benzoyl) ; 8.76 (d, 1H, H_6) ; 11.20 (brs, 1H, NH). MS FAB > 0, NBA, *m/z* 348 [M+H]$^+$. β anomer : ^{1}H NMR (250 MHz ; $DMSOd_6$) : δ 2.27 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.40 (m, 1H, $H_{4'}$) ; 3.62 (m, 2H, $H_{5'}$ $H_{5''}$) ; 4.39 (m, 1H, $H_{3'}$) ; 5.24 (t, 1H, $OH_{5'}$) ; 5.37 (d, 1H, $OH_{3'}$) ; 6.32 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 6.8) ;

7.37 (d, 1H, H_5, $J_{5,6}$ = 7.5) ; 7.57 (m, 3H, H *meta* and *para* of benzoyl) ; 8.03 (d, 2H, H *ortho* of benzoyl) ; 8.55 (d, 1H, H_6, $J_{6,5}$ = 7.5) ; 11.31 (brs, 1H, NH). MS FAB > 0, NBA, *m/z* 348 [M+H]$^+$.

2'-Deoxy-4'-thio-L-uridine 14 :

Rf = 0.26 (CH_2Cl_2/MeOH : 85/15). **α-anomer** : Yield : 60%. $[\alpha]_D^{24}$ -46° (c 0.69, MeOH). ^{1}H NMR (250 MHz ; D_2O) : δ 2.30 (m, 1H, $H_{2'}$) ; 2.65 (m, 1H, $H_{2''}$) ; 3.70 (m, 3H, $H_{4'}$, $H_{5'}$, $H_{5''}$) ; 4.50 (m, 1H, $H_{3'}$) ; 5.89 (d, 1H, H_5, $J_{5,6}$ = 8.14) ; 6.20 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 3.3, $J_{1',2''}$ = 8.2) ; 8.40 (d, 1H, H_6, $J_{6,5}$ = 8.14). MS FAB > 0, GT, *m/z* 245 [M+H]$^+$. **β-anomer** : Yield : 60%. $[\alpha]_D^{24}$ +28° (c 0.25, MeOH) [litt[9] [α]$_D^{24}$ +29.7° (c 1.0, MeOH)]. mp 190-192°C recrystallized from EtOH [litt[9] mp 186-187°C in the D-series]. ^{1}H NMR (250 MHz ; D_2O) : δ 2.35 (m, 1H, $H_{2'}$) ; 2.55 (m, 1H, $H_{2''}$) ; 3.50 (m, 1H, $H_{4'}$) ; 3.82 (m, 2H, $H_{5'}$, $H_{5''}$) ; 4.55 (m, 1H, $H_{3'}$) ; 5.90 (d, 1H, H_5) ; 6.36 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 8) ; 8.12 (d, 1H, H_6). MS FAB > 0, GT, *m/z* 245 [M+H]$^+$.

2'-Deoxy-4'-thio-L-cytidine 15 :

A solution of 13α or 13β (0.25 mmol) in anhydrous MeOH (5 ml) was stirred at room temperature with methanolic ammonia (5 ml). A TLC analysis showed complete consumption of the starting materiel after 12 h. The reaction mixture was evaporated to dryness and purified by chromatography over silica gel column (elution with CH_2Cl_2:CH_3OH / 90:10) to afford 15α or 15β.

α-anomer : Yield : 82%. Rf : 0.22 (CH_2Cl_2:CH_3OH / 70:30). mp 210-212°C. $[\alpha]_D^{24}$ -54.5° (c 0.11, MeOH). ^{1}H NMR (250 MHz ; DMSOd_6) : δ 1.95 (m, 1H, $H_{2'}$) ; 2.40 (m, 1H, $H_{2''}$) ; 3.35 (m, 1H, $H_{5'}$) ; 3.50 (m, 2H, $H_{4'}$, $H_{5''}$) ; 4.22 (m, 1H, $H_{3'}$) ; 4.98 (t, 1H, $OH_{5'}$, $J_{5,OH}$ = 5.2) ; 5.37 (d, 1H, $OH_{3'}$, $J_{3,OH}$ = 3.5) ; 5.69 (d, 1H, H_5, $J_{5,6}$ = 7.5) ; 6.16 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 4, $J_{1',2''}$ = 8) ; 7.05 (d, 2H, NH_2) ; 8.15 (d, 1H, H_6, $J_{6,5}$ = 7.5). MS FAB > 0, NBA, *m/z* 244 [M+H]$^+$.**β-anomer** : Yield : 70%. Rf : 0.16 (CH_2Cl_2:CH_3OH / 70:30). mp 212-214°C recrystallized from Et_2O [litt[9] mp 208-209°C in the D-series]. $[\alpha]_D^{24}$ +31.2° (c 0.096, MeOH) [litt[9] $[\alpha]_D^{24}$ +23.3° (c 1.0, MeOH)]. ^{1}H NMR (250 MHz ; DMSOd_6) : δ 2.10 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.20 (m, 1H, $H_{4'}$) ; 3.55 (m, 2H,

$H_{5'}$, $H_{5''}$) ; 4.30 (m, 1H, $H_{3'}$) ; 5.12 (t, 1H, $OH_{5'}$, $J_{5,OH}$ = 5.4) ; 5.22 (d, 1H, $OH_{3'}$, $J_{3,OH}$ = 3.7) ; 5.76 (d, 1H, H_5, $J_{5,6}$ = 7.4) ; 6.32 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 8) ; 7.17 (d, 2H, NH_2) ; 7.91 (d, 1H, H_6, $J_{6,5}$ = 7.4). MS FAB > 0, NBA, *m/z* 244 [M+H]$^+$.

2'-Deoxy-3',5'-di-*O*-benzyl-4'-thio-L-adenosine 16 :

A mixture of sugar 10 (281 mg, 0.754 mmol) and adenine (122 mg, 0.905 mmol) in 5 ml of acetonitrile was cooled to 0°C and stannic chloride (0.177 ml, 1.51 mmol) was added. Stirring was continued for 5 h at room temperature. The reaction mixture was quenched by 1.5 ml of satured $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed over silica gel column with CH_2Cl_2 to give pure 16 (230 mg, 68%).

Rf : 0.29 (CH_2Cl_2:CH_3OH / 95:5). MS FAB > 0, NBA, *m/z* 448 [M+H]$^+$. UV (EtOH) λ_{max} 260 nm, λ_{min} 228 nm ; UV (0.1 N HCl) λ_{max} 257 nm, λ_{min} 232 nm ; UV (0.1 N NaOH) λ_{max} 260 nm, λ_{min} 233 nm. **α-anomer** : ^{1}H NMR (400 MHz ; $CDCl_3$) : δ 2.49 (m, 2H, $H_{2'}$, $H_{2''}$, $J_{2',1'}$ = 3) ; 3.40 (m, 2H, $H_{5'}$,$H_{5''}$) ; 4.04 (m, 1H, $H_{4'}$) ; 4.34 (m, 2H, $H_{3'}$ and 1H of CH_2Phe) ; 4.48 (m, 3H, CH_2Phe) ; 5.50 (brs, 2H, NH_2) ; 6.26 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 3) ; 7.25 (m, 10H, Phenyls) ; 8.27 (s, 1H, H_2) ; 8.40 (s, 1H, H_8). **β-anomer** : ^{1}H NMR (400 MHz ; $CDCl_3$) : δ 2.60 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.67 (m, 2H, $H_{5'}$,$H_{5''}$) ; 3.71 (m, 1H, $H_{4'}$) ; 4.31 (m, 1H, $H_{3'}$) ; 4.50 (2s, 4H, 2 CH_2Phe) ; 5.60 (brs, 2H, NH_2) ; 6.26 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 6.6) ; 7.24 (m, 10H, Phenyls) ; 8.16 (s, 1H, H_2) ; 8.26 (s, 1H, H_8).

2'-Deoxy-4'-thio-L-adenosine 17 :

To a solution of 1 M BBr_3 in CH_2Cl_2 (4.69 ml, 4.69 mmol), in a three necked round bottomed flask with a thermometer and magnetic stirrer, cooled to -80°C, was added dropwise a solution of protected nucleoside (300 mg, 0.67 mmol) (16β) in dry CH_2Cl_2 (5 ml). The reaction mixture was further stirred (-78°C) for 6 hours and then quenched with 7 ml of the 1:1 mixture of dry CH_2Cl_2 and dry CH_3OH. The solution was allowed to warm up to room temperature, neutralized with $NaHCO_3$ (5%) and evaporated to dryness. The anomeric mixture was purified on a silica gel column using CH_2Cl_2:CH_3OH / 90:10 as eluant. Then the two anomers were separated by preparative HPLC on reverse phase (C18) with an isocratic elution of CH_3CN:H_2O(milliQ)/3:97 at a flow rate of 30ml/min : t_r (4'-S-βL-dA, 17β) = 22.5 min and t_r (4'-S-αL-dA, 17α) = 31.8 min.

α-anomer : Yield : 23.5%. Rf : 0.05 (CH_2Cl_2:CH_3OH / 90:10). $[\alpha]_D^{24}$ -103.7 ° (c 0.27, MeOH). ^1H NMR (250 MHz ; $DMSOd_6$) : δ 2.46 (m, 1H, $H_{2'}$, $J_{2',1'}$ = 3.5, $J_{2',2''}$ = 14) ; 2.68 (m, 1H, $H_{2''}$, $J_{2'',1'}$ = 7.9, $J_{2'',2'}$ = 14) ; 3.41 (m, 1H, $H_{5'}$, $J_{5',5''}$ = 11.1, $J_{5',4'}$ = 6.8) ; 3.55 (m, 1H, $H_{5''}$, $J_{5',5''}$ = 11.1) ; 3.71 (m, 1H, $H_{4'}$, $J_{4',5'}$ = 6.8) ; 4.46 (m, 1H, $H_{3'}$) ; 5.12 (t, 1H, $OH_{5'}$) ; 5.69 (d, 1H, $OH_{3'}$) ; 6.24 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 3.5, $J_{1',2''}$ = 7.9) ; 7.30 (brs, 2H, NH_2) ; 8.17 (s, 1H, H_2) ; 8.54 (s, 1H, H_8). MS FAB > 0, NBA, *m/z* 268 [M+H]$^+$. UV (EtOH) λ_{max} 260 nm, λ_{min} 230 nm. β-anomer : Yield : 10%. Rf : 0.05 (CH_2Cl_2:CH_3OH / 90:10). $[\alpha]_D^{24}$ +21.6 ° (c 0.18, MeOH). mp 205-208°C recrystallized from MeOH. ^1H NMR (250 MHz ; $DMSOd_6$) : δ 2.39 (m, 1H, $H_{2'}$) ; 2.61 (m, 1H, $H_{2''}$) ; 3.34 (m, 1H, $H_{4'}$) ; 3.54 (m, 1H, $H_{5'}$, $J_{5'',5'}$ = 11.5) ; 3.70 (m, 1H, $H_{5'}$) ; 4.48 (m, 1H, $H_{3'}$) ; 5.14 (t, 1H, $OH_{5'}$) ; 5.29 (d, 1H, $OH_{3'}$) ; 6.17 (t, 1H, $H_{1'}$) ; 7.24 (brs, 2H, NH_2) ; 8.11 (s, 1H, H_2) ; 8.39 (s, 1H, H_8). MS FAB > 0, NBA, *m/z* 268 [M+H]$^+$. Anal. Calcd for $C_{10}H_{13}O_2N_5S$: C, 44.9 ; H, 4.9 ; N, 26.2 ; S, 12. Found : C, 44.8 ; H, 5.1 ; N, 25.9 ; S, 11.8. UV (EtOH) λ_{max} 260 nm, λ_{min} 230 nm.

General procedure for the silylation for the 4'-thio-nucleosides.

The nucleoside 13β or 17β (2 mmol) was dissolved in the solvent (25 ml) (Pyridine or DMF), and TBDMS-Cl (2 mmol) was added (DMF reaction contained 2 equiv. of imidazole/mmol of TBDMS-Cl) to the stirred solution. After 18 h, the mixture was poured into water and extracted with CH_2Cl_2 (40 ml). The organic layer was dried over Na_2SO_4, then evaporated and purified on a silica gel column with CH_2Cl_2:CH_3OH / 95:5 as eluant to give the 5'-*O*-TBDMS-4'-thio-nucleosides.

1-(5-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-thio-β-L-ribofuranosyl)N^4-benzoyl-cytosine 18 :

Yield : 90%. Rf : 0.28 (CH_2Cl_2:CH_3OH / 93:7). ^1H NMR (250 MHz ; $CDCl_3$) : δ 0.09 (s, 6H, Si(CH_3)$_2$) ; 0.89 (s, 9H, Si*t*Bu) ; 2.31 (m, 1H, $H_{2'}$, $J_{2',1'}$ = 4.6) ; 2.59 (m, 1H, $H_{2''}$) ; 3.15 (brs, 1H, $OH_{3'}$) ; 3.46 (m, 1H, $H_{4'}$) ; 3.79 (m, 1H, $H_{5'}$) ; 3.99 (m, 1H, $H_{5''}$) ; 4.39 (m, 1H, $H_{3'}$) ; 6.36 (dd, 1H, $H_{1'}$, $J_{1',2'}$ = 4.6, $J_{1',2''}$ = 7) ; 7.52 (m, 4H, Phenyls) ; 7.87 (m, 2H, H_5 and 1H of benzoyl) ; 8.63 (d, 1H, H_6) ; 8.84 (brs, 1H, NH). MS FAB > 0, NBA, *m/z* 462 [M+H]$^+$.

9-(5-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-thio-β-L-ribofuranosyl)adenine 22 :

Yield : 97%. Rf : 0.34 (CH_2Cl_2:CH_3OH / 90:10). ^1H NMR (250 MHz ; $DMSOd_6$) : δ 0.03 (s, 6H, $Si(C\underline{H}_3)_2$) ; 0.83 (s, 9H, Si*t*Bu) ; 2.35 (m, 1H, $H_{2'}$) ; 2.70 (m, 1H, $H_{2''}$) ; 3.34 (m, 1H, $H_{4'}$) ; 3.72 (m, 1H, $H_{5'}$, $J_{5',5''}$ = 10.6) ; 3.95 (m, 1H, $H_{5''}$, $J_{5'',5'}$ = 10.6) ; 4.44 (m, 1H, $H_{3'}$) ; 5.33 (brs, 1H, $OH_{3'}$) ; 6.20 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 6.8) ; 7.25 (brs, 2H, NH_2) 8.08 (s, 1H, H_2) ; 8.36 (s, 1H, H_8). MS FAB > 0, NBA, *m/z* 382 [M+H]$^+$.

1-(5-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-thio-β-L-ribofuranosyl)cytosine 19 :

A solution of 18 (700 mg, 1.5 mmol) in anhydrous MeOH (25 ml) was stirred at room temperature with sodium methoxide (1.5 mmol). A TLC analysis showed complete consumption of starting material after 2 h. The reaction mixture was evaporated to dryness and purified by chromatography over silica gel column (elution with CH_2Cl_2:CH_3OH / 93:7) to afford 1-(2-deoxy-4-thio-5-*O*-*tert*-butyldimethylsilyl-β-L-ribofuranosyl)cytosine (460 mg, 84%).

Rf : 0.35 (CH_2Cl_2:CH_3OH / 85:15). ^1H NMR (250 MHz ; $CDCl_3$) : δ 0.09 (s, 6H, $Si(C\underline{H}_3)_2$) ; 0.89 (s, 9H, Si*t*Bu) ; 2.11 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.25 (m, 1H, $H_{4'}$) ; 3.74 (m, 2H, $H_{5''}$, $H_{5'}$, $J_{5',5''}$ = 10.8) ; 4.30 (m, 1H, $H_{3'}$) ; 5.27 (brs, 1H, $OH_{3'}$) ; 5.75 (d, 1H, H_5, $J_{5,6}$ = 7.4) ; 6.30 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 7.2) ; 7.15 (brs, 2H, NH_2) ; 7.95 (d, 1H, H_6, $J_{6,5}$ = 7.4). MS FAB > 0, NBA, *m/z* 358 [M+H]$^+$.

General procedure for dimethoxytritylation reaction :

The modified nucleosides 19 or 22 (1 mmol) were dissolved in dry pyridine (5 ml), DMAP (0.3 mmol) and DmtrCl (5 mmol) were added. The reaction mixture was stirred at room temperature overnight. The solution was poured into water and washed with methylene chloride (2 x 10 ml). The organic layer was dried and evaporated to dryness and purified by chromatography over silica gel column (elution with CH_2Cl_2/NEt_3 0.1% for 20 and hexane:diethyl ether/40:60/NEt_3 0.1% for 23).

1-[5-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-thio-3-*O*-(4,4'-dimethoxytrityl)-β-L-ribofuranosyl]N^4-(4,4'-dimethoxytrityl)-cytosine 20 :

Yield : 77%. Rf : 0.26 (CH_2Cl_2:CH_3OH / 97:3). ^1H NMR (250 MHz ; $CDCl_3$) : δ 0.03 (s, 6H, $Si(C\underline{H}_3)_2$) ; 0.63 (s, 9H, Si*t*Bu) ; 1.63 (m, 1H, $H_{2'}$) ; 2.19 (m, 1H, $H_{2''}$) ; 3.17 (m, 3H,

$H_{4'}$, $H_{5'}$ and $H_{5''}$) ; 3.74 (2s, 12H, Phe-O$\underline{CH_3}$) ; 4.08 (m, 1H, $H_{3'}$) ; 4.92 (d, 1H, H_5, $J_{5,6}$ = 7.6) ; 6.64 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 7.8) ; 6.77 (m, 7H, NH and Phenyls) ; 7.27 (m, 20H, Phenyls) ; 7.70 (d, 1H, H_6, $J_{6,5}$ = 7.6). MS FAB > 0, NBA, *m/z* 963 [M+H]$^+$.

9-[5-*O*-*tert*-Butyldimethylsilyl-2-deoxy-3-*O*-(4,4'-dimethoxytrityl)-4-thio-β-L-ribofuranosyl]adenine 23 :

Yield : 70%. Rf : 0.1 (Diethyl ether). ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 0.1 (s, 6H, Si($C\underline{H_3}$)$_2$) ; 0.91 (s, 9H, Si$\underline{t}$Bu) ; 2.60 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.29 (m, 1H, $H_{4'}$) ; 3.55 (m, 2H, $H_{5'}$, $H_{5''}$) ; 3.77 (s, 6H, Phe-O$\underline{CH_3}$) ; 4.56 (m, 1H, $H_{3'}$) ; 6.19 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 5) ; 6.73 (m, 4H, Phenyls) ; 7.21 (m, 9H, Phenyls) ; 8.05 (s, 1H, H_2) ; 8.16 (s, 1H, H_8). MS FAB > 0, GT, *m/z* 684 [M+H]$^+$.

General procedure for the desilylation reaction :

To a stirred solution of 5'-O-TBDMS-3'-Dmtr-2'-deoxy-4'-thio-β-L-nucleosides (0.5 mmol) in dry THF (20 ml) was added TBAF (1 M, 0.55 mmol). After 30 min the solvent was removed *in vacuo* and the mixture was chromatographed over silica gel column with CH_2Cl_2/NEt_3 0.1% as eluant to obtain pure 21 and 24.

1-[2-Deoxy-3-*O*-(4,4'-dimethoxytrityl)-4-thio-β-L-ribofuranosyl]N^4-(4,4'-dimethoxytrityl)-cytosine 21 :

Yield : 90%. Rf : 0.42 (CH_2Cl_2:CH_3OH / 95:5). ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 1.93 (m, 1H, $H_{2'}$) ; 2.23 (m, 1H, $H_{2''}$) ; 2.89 (m, 3H, $H_{4'}$) ; 3.27 (m, 2H, $H_{5'}$, $H_{5''}$) ; 3.72 (2s, 12H, Phe-O$\underline{CH_3}$) ; 4.15 (m, 1H, $H_{3'}$) ; 4.98 (d, 1H, H_5, $J_{5,6}$ = 7.61) ; 6.53 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 8.2) ; 6.76 (m, 8H, Phenyls) ; 7.18 (m, 19H, H_6, $J_{6,5}$ = 7.61, and Phenyls). MS FAB > 0, GT, *m/z* 848 [M+H]$^+$.

9-[2-Deoxy-3-*O*-(4,4'-dimethoxytrityl)-4-thio-β-L-ribofuranosyl]adenine 24 :

Yield : 80%. Rf : 0.1 (Diethyl ether). ^{1}H NMR (250 MHz ; $CDCl_3$) : δ 3.04 (m, 2H, $H_{2'}$, $H_{2''}$) ; 3.60 (m, 1H, $H_{4'}$) ; 3.72 (s, 6H, Phe-O$\underline{CH_3}$) ; 3.90 (m, 2H, $H_{5'}$, $H_{5''}$) ; 4.66 (m, 1H, $H_{3'}$) ; 6.20 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 7.4) ; 6.74 (m, 4H, Phenyls) ; 7.19 (m, 9H, Phenyls) ; 7.85 (s, 1H, H_2) ; 7.90 (s, 1H, H_8). MS FAB > 0, GT, *m/z* 570 [M+H]$^+$.

General procedure for the preparation of phosphotriester analogues :

1*H*-tetrazole (0.9 mmol) was added to a stirred solution of 21 or 24 (0.3 mmol) and the phosphoramidite[37] 25 (0.37 mmol) in tetrahydrofuran (5 ml) at room temperature. After 30 min, the reaction mixture was cooled to -40°C, and 3 M *tert*-butyl hydroperoxide in toluene was added ; the mixture was then allowed to warm at room temperature over 1 h. Sodium sulfite (4 ml) (10% aqueous solution) was added to the mixture which was diluted with CH_2Cl_2 and the organic layer was separated and the aqueous layer washed with dichloromethane (2 x 5 ml). The combined organic layers were dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure to afford the intermediate phosphotriester.

Each protected phosphotriesters was dissolved in 15 ml of AcOH:MeOH/80:20 and the solution was stirred at room temperature overnight. The reaction mixture was evaporated to dryness, coevaporated with ethanol (2 x 10 ml) and the residue was chromatographed on silica gel with a stepwise gradient of methanol (0-7%) in methylene chloride to afford pure 26 (55% yield from 21) or 27 (40% yield from 24).

2'-Deoxy-4'-thio-β-L-cytidin-5'-yl *bis*(*S*-pivaloyl-2-thioethyl)phosphate 26 :

Rf : 0.21 (CH_2Cl_2:CH_3OH / 90:10). ^1H NMR (250 MHz ; $CDCl_3$) : δ 1.18 (s, 18H, *t*Bu) ; 2.20 (m, 1H, $H_{2'}$) ; 2.54 (m, 1H, $H_{2''}$) ; 3.11 (t, 4H, $OCH_2\underline{CH_2}S$) ; 3.60 (m, 1H, $H_{4'}$) ; 4.07 (q, 4H, $POCH_2\underline{CH_2}S$) ; 4.22 (m, 2H, $H_{5'}$, $H_{5''}$) ; 4.48 (m, 1H, $H_{3'}$) ; 6.05 (d, 1H, H_5, $J_{5,6}$ = 7) ; 6.34 (t, 1H, $H_{1'}$) ; 8.01 (d, 1H, H_6, $J_{5,6}$ = 7). ^{31}P NMR (250 MHz ; $CDCl_3$) : δ -0.007. MS FAB > 0, GT, *m/z* 612 [M+H]$^+$.

2'-Deoxy-4'-thio-β-L-adenosin-5'-yl *bis*(*S*-pivaloyl-2-thioethyl)phosphate 27 :

Rf : 0.3 (CH_2Cl_2:CH_3OH / 90:10). ^1H NMR (250 MHz ; $CDCl_3$) : δ 1.24 and 1.25 (2s, 18H, *t*Bu) ; 2.69 (m, 1H, $H_{2'}$) ; 2.85 (m, 1H, $H_{2''}$) ; 3.18 (t, 4H, $OCH_2\underline{CH_2}S$) ; 3.76 (m, 1H, $H_{4'}$) ; 4.16 (q, 4H, $POCH_2\underline{CH_2}S$) ; 4.43 (m, 2H, $H_{5'}$, $H_{5''}$) ; 4.80 (m, 1H, $H_{3'}$) ; 5.91 (brs, 2H, NH_2) ; 6.34 (t, 1H, $H_{1'}$, $J_{1',2'}$ = 6.2) ; 8.21 (s, 1H, H_2) ; 8.36 (s, 1H, H_8). ^{31}P NMR (250 MHz ; $CDCl_3$) : δ -0.005. MS FAB > 0, GT, *m/z* 636 [M+H]$^+$.

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