

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME 4'-C-(HYDROXYMETHYL)- α - AND - β -D-ARABINOFURANOSYL PYRIMIDINE AND ADENINE NUCLEOSIDES

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday.

A series of 4'-C-(hydroxymethyl) analogs of pyrimidine and purine nucleosides have been prepared utilizing standard methodologies, and the α and β anomers were separated. These analogs are part of our continuing efforts to identify new anticancer drugs as well as to explore the substrate specificities of these analogs with the initial activating enzymes in the metabolic pathway leading to nucleoside triphosphates. Although not cytotoxic to CCRF-CEM cells (an acute lymphoblastic leukemia of T-cell origin), many of these compounds were utilized as substrates for the various human nucleoside kinases, including deoxycytidine kinase, thymidine kinase 1, and thymidine kinase 2. Because the 4'-C-(hydroxymethyl) analog of arabinofuranosyl cytosine was identified as a good substrate with deoxycytidine kinase, its metabolism in CEM cells was evaluated. These results indicated that nucleosides with this modification could be activated in human cells without cytotoxicity, which suggested that they should be examined for antiviral activity.

Keywords: 4'-C-(Hydroxymethyl)nucleosides; 4'-C-Branched nucleosides; Deoxycytidine kinase; Cytotoxicity; Phosphorylation; Glycosidation; Pyrimidines; Purines.

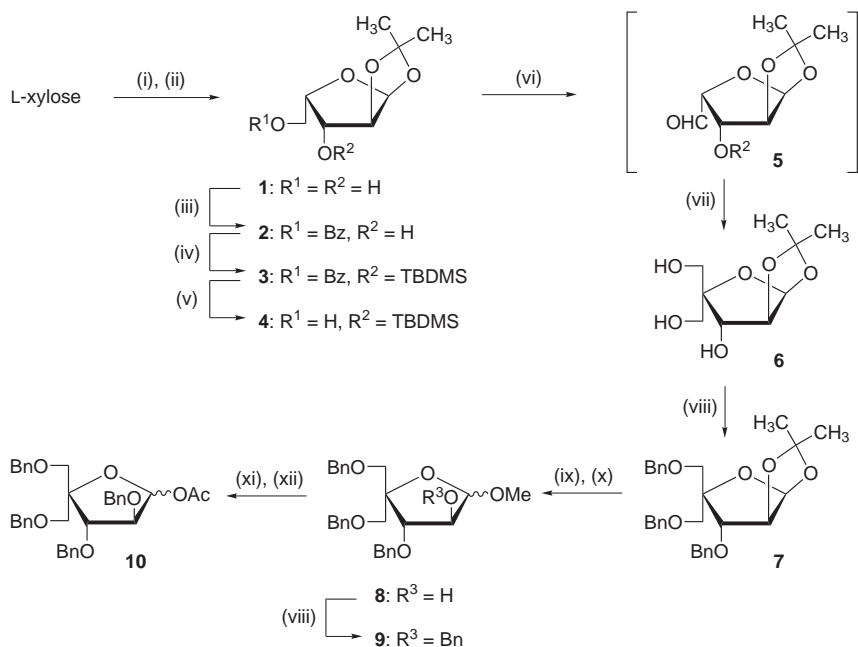
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Interest in 4'-substituted nucleosides dates back to the early work of Moffatt, Verheyden and others¹. Since that time a large number of 4'-substituted nucleosides have been prepared and some of them have been found to have interesting biological activity²⁻²¹. Compounds that have been found to have some cytotoxicity include 2'-deoxynucleosides with α -4'-C-methyl^{3,11}, cyano^{3,13}, ethynyl^{2,4,13}, ethenyl² and fluoromethyl¹⁹ groups. In our laboratory we have focused for many years on the development of new nucleosides with anticancer activity, including recently several series of compounds that have 4'-C-substitution. In addition, we have focused on other substitution patterns and other carbohydrate configurations as we explore what changes are allowed by the initial activating enzymes, typically deoxycytidine kinase, thymidine kinase and guanosine kinase. One of the first groups that was of interest was the α -4'-C-(hydroxymethyl) group because it had the potential of offering a second site for initial phosphorylation in the metabolism of a nucleoside analog up to the triphosphate level. Many 4'-C-(hydroxymethyl)nucleosides have been prepared, including analogs with β -D-*xylo*, ribo and 2'-deoxyribo configurations^{1,23-28}, as well as analogs of AZT, d4T, ddT prepared as potential antiviral agents^{29,30}. We report herein the synthesis of a series of 4'-C-(hydroxymethyl)- α - and - β -D-*threo*-pentofuranosyl pyrimidine and purine nucleosides³¹. These target compounds are additional examples of 4'-derivatives of arabinonucleosides. The newly synthesized compounds have been examined for their cytotoxicity as well as for their ability to serve as substrates for the appropriate initial activating kinases.

Chemistry

Our synthetic approach to the target hydroxymethyl-substituted nucleosides follows generally along standard lines. The suitably protected carbohydrate **10** was prepared in 13 steps from L-xylose (Scheme 1). 1,2-*O*-Isopropylidene- α -L-*xylo*-furanose intermediate **1** was prepared from L-xylose following the procedure described by Gosselin et al.³² in 94% yield. Selective benzylation of the 5-hydroxyl group of **1** with benzoyl chloride in dry pyridine at 0 °C was performed in 77% yield and was followed by the protection of the 3-hydroxy group by reaction with *tert*-butyldimethylsilyl chloride (TBDMSCl) in dry pyridine at room temperature to give compound **3** in quantitative yield. The 5-position of **3** was debenzoylated with sodium methoxide in a methanol/toluene mixture in 88% yield and subjected to an oxidation. Oxidation of the 5-position of **4** was performed under Pfizner-Moffatt conditions^{33,34}, with dimethyl sulfoxide (DMSO), dicyclo-

hexylcarbodiimide (DCC) and dichloroacetic acid and led to the pentodialdofuranose intermediate **5** which was subsequently converted to the 4-C-(hydroxymethyl) derivative **6**.



SCHEME 1

Our approach of the synthesis of **6** was based upon the precedent established by Schaffer^{35,36} and Moffatt and co-workers^{24,25}, utilizing an aldol condensation-crossed Cannizzaro reaction sequence between a suitably protected pentodialdofuranose and formaldehyde in aqueous sodium hydroxide. When the 3-*O*-(*tert*-butyldimethylsilyl)-1,2-*O*-isopropylidene- α -L-xyllo-pentodialdo-1,4-furanose intermediate **5** was subjected to the aldol condensation-crossed Cannizzaro reaction sequence, introduction of the hydroxymethyl group was accompanied by loss of the 3-*O*-*tert*-butyldimethylsilyl group² but without any epimerisation at C-3¹⁷. 4-*C*-(Hydroxymethyl)-1,2-*O*-isopropylidene- β -D-*arabino*-furanose **6** was obtained in 66% yield from **4**.

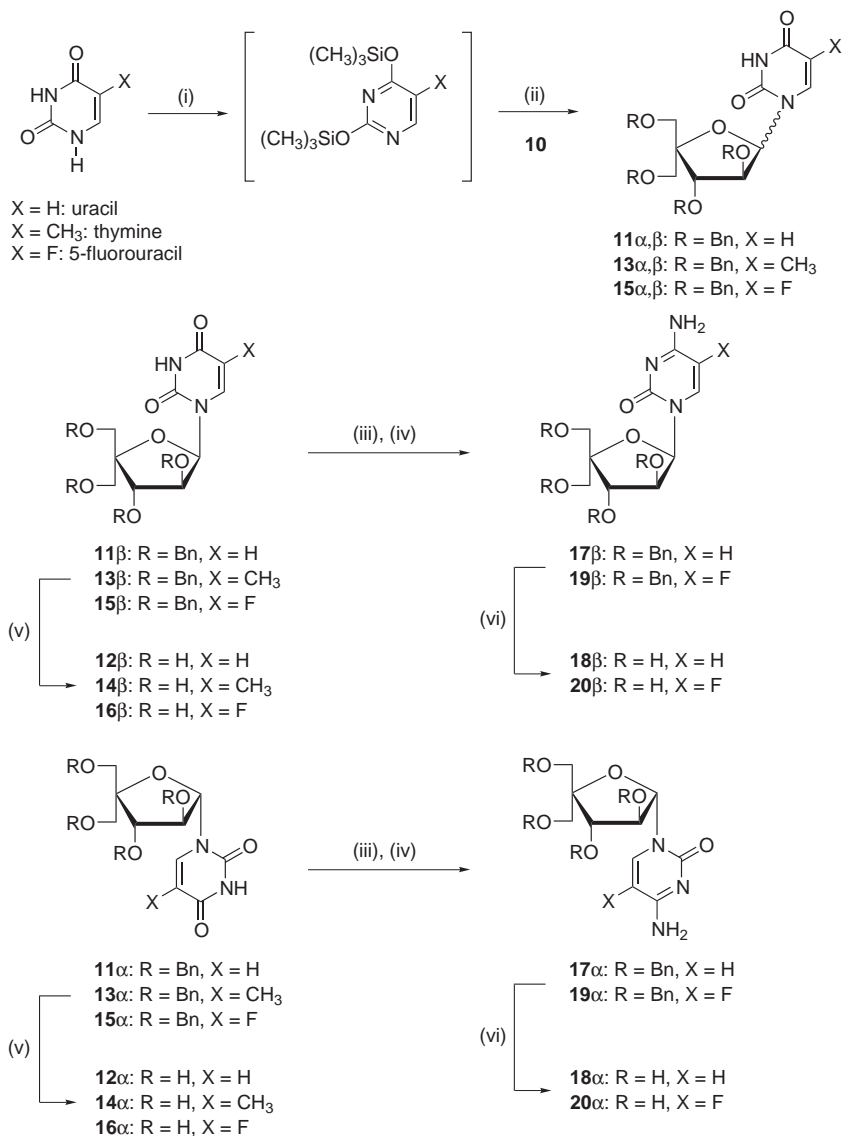
The three hydroxy groups of **6** were then protected by reaction with benzyl chloride in the presence of sodium hydride and tetrabutylammonium iodide in THF to afford compound **7** in 91% yield. Cleavage of the isopropylidene group of **7** with 85% acetic acid and concentrated sulfuric acid followed by methylation of the anomeric hydroxyl group with methanol and sulfuric acid afforded **8** in 95% yield. The last OH group of **8** was benzylated under the same condition described above to give the tetra-*O*-benzylated derivative **9** in 92% yield. Hydrolysis of the methyl glycoside **9** with 90% trifluoroacetic acid followed by acetylation of the anomeric OH with acetic anhydride in dry pyridine afforded the desired intermediate **10**.

As representative nucleoside targets, we chose **12**, **14**, **16**, **18**, **20**, and **22**, both the α and β anomers. Again, standard routes were utilized to prepare the twelve target compounds, though slightly different conditions were utilized for pyrimidines versus purines.

To prepare the 4-oxypyrimidine nucleosides (Scheme 2), blocked precursor **10** was respectively condensed with the commercially available uracil, thymine or 5-fluorouracil, under Vörbruggen³⁷ conditions using trimethylsilyltrifluoromethanesulfonate (TMSOTf) as a catalyst, to give the fully corresponding benzylated nucleosides as an α : β mixture, separable by silica gel chromatography, in 90% yield (α / β 1.8:1) for the uracil nucleosides **11 α** , **11 β** , 87% yield (α / β 3.3:1) for the thymine nucleosides **13 α** , **13 β** , and 88% yield (α / β 1.4:1) for 5-fluorouracil nucleosides **15 α** , **15 β** . Finally, benzyl ether protective groups of **11 α** , **13 α** , **15 α** and **11 β** , **13 β** , **15 β** were cleaved by catalytic hydrogenation in good yields to give target compounds **12 α** , **14 α** , **16 α** and **12 β** , **14 β** , **16 β** .

In order to generate cytosine nucleoside analogs (Scheme 2), conversion of benzylated uracil and 5-fluorouracil derivatives **11 α , β** and **15 α , β** into the corresponding cytosine and 5-fluorocytosine nucleosides **17 α , β** and **19 α , β** was carried out via a treatment with Lawesson's reagent^{38,39} which led to the 4-thioamide derivatives, followed by a treatment with methanolic ammonia at 100 °C in a stainless-steel bomb. This transformation was performed in quantitative yield for **17 α** , 82% yield for **17 β** and 90% yield for **19 α** , **19 β** . Finally, benzyl ether protective groups of **17 α** , **19 α** and **17 β** , **19 β** were cleaved with a boron trichloride solution in anhydrous dichloromethane in good yields to give target compounds **18 α** , **20 α** and **18 β** , **20 β** .

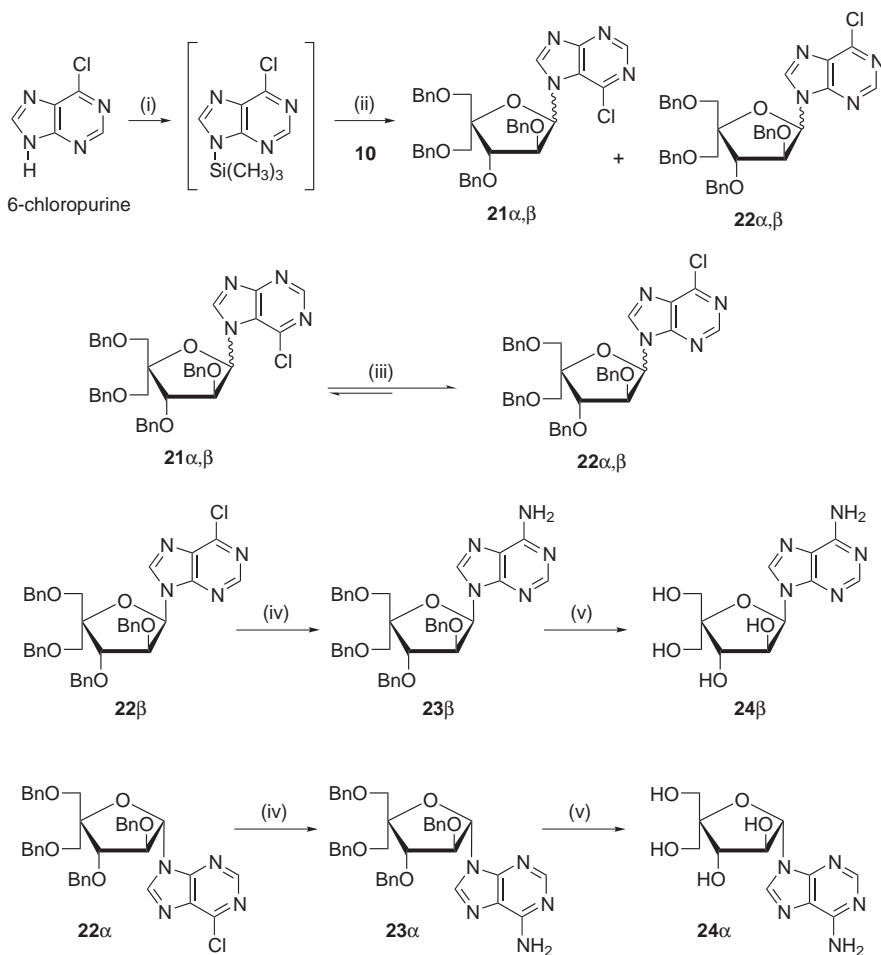
In order to prepare the two adenine nucleoside anomers **24 α** and **24 β** (Scheme 3), condensation of **10** with silylated 6-chloropurine was performed at room temperature in the presence of tin(IV) chloride in anhyd-



(i) HMDS, (NH₄)₂SO₄, reflux; (ii) TMSOTf, (CH₂Cl)₂, r.t.; (iii) Lawesson's reagent, (CH₂Cl)₂, reflux; (iv) MeOH/NH₃, 100 °C; (v) atm H₂, Pd/C, methanol/AcOH, r.t.; (vi) BCl₃, CH₂Cl₂, -78 °C, then -25 °C

SCHEME 2

rous acetonitrile and afforded in 74% yield a 1.8:1 mixture of N-7 and N-9 isomers (**21 $\alpha\beta$** and **22 $\alpha\beta$**). After separation by silica gel chromatography N-7 isomer (**21 $\alpha\beta$**) was converted to the N-9 isomer (**22 $\alpha\beta$**) in 54% yield by treatment with TMSOTf in acetonitrile at room temperature. Separation of **22 α** and **22 β** (α/β 0.8:1) was performed by silica gel chromatography. Displacement of the chlorine atom at the 6-position of compounds **22 α** and **22 β** was performed with ethanolic ammonia at 75 °C in a stainless-steel



Synthesis of compounds **24 α** and **24 β** . (i) BSA, reflux; (ii) SnCl_4 , CH_3CN , r.t.; (iii) TMSOTf, CH_3CN , r.t.; (iv) EtOH-NH_3 , 75 °C; (v) 50 Pa H_2 , $\text{Pd}(\text{OH})_2/\text{C}$, methanol/AcOH, r.t.

SCHEME 3

bomb in 94 and 82% yields, respectively. Finally, benzyl ether protective groups of **23 α** and **23 β** were cleaved by hydrogenation using Pd(OH)₂ as catalyst under 50 Pa of H₂ to give target compounds **24 α** and **24 β** in 66 and 49% yields, respectively.

Biological Results

The target compounds were prepared as 4'-substituted arabinonucleoside analogs, and they are referred to in that manner. Thus, the cytosine-containing target **18 β** can be considered to be 4'-C-(hydroxymethyl)-ara-C, abbreviated HM-araC below.

None of the 4'-C-(hydroxymethyl)-substituted target nucleosides was cytotoxic to CEM cells at a concentration of 40 $\mu\text{g/ml}$. The 4'-C-hydroxymethyl compounds were evaluated as appropriate as substrates with thymidine kinase 1, thymidine kinase 2, deoxycytidine kinase and deoxyguanosine kinase to determine whether or not they could be phosphorylated in human cells (Table I). The uracil, thymine and adenine analogs were poor substrates for their respective kinases, which suggested that their lack of cytotoxicity was due to their lack of conversion to cytotoxic nucleotides. However, the β isomers of the two cytosine analogs were phosphorylated by dCyd kinase. The K_m values for 2'-deoxycytidine (dCyd), HM-araC (**18 β**) and 5-F-HM-araC (**20 β**) with the recombinant enzyme were 1.0 ± 0.3 , 18 ± 6 , and 32 ± 19 μM , respectively, and K_{cat} values for these agents were 0.11 ± 0.02 , 0.07 ± 0.01 , and 0.11 ± 0.02 s^{-1} , respectively. The catalytic efficiency of HM-araC with this enzyme was 14% of that seen with dCyd.

Because of the activity of the cytosine analogs with the recombinant dCyd kinase, the metabolism [³H]HM-araC was studied in CEM cells. In our initial studies using our standard SAX HPLC system⁴⁰, there was a linear increase in a metabolite in CEM cells that eluted at 20 min (data not shown). This metabolite eluted with a retention time that was similar to that of dCTP and is therefore likely to be the 5'-triphosphate of HM-araC (HM-araCTP). Because there was considerable increase in radioactivity in the void of this SAX HPLC column, the samples were analyzed using BioBasic HPLC, which retains monophosphates better than our standard SAX HPLC system. Using this system it was clear that HM-araC was converted to 2 metabolites (Fig. 1). The addition of dCyd (100 μM) to the incubations with HM-araC completely inhibited the formation of both metabolites, and neither metabolite was formed in CEM cells that were deficient in dCyd kinase (data not shown). The peak eluting at 8 min was de-

terminated to be HM-araC by spiking the sample with [^3H]HM-araC. The 5'-phosphate of HM-araC (HM-araCMP) was created by incubating HM-araC with dCyd kinase that had been purified from CEM cells and ATP, and the peak of radioactivity that eluted at 24 min in Fig. 1 eluted with this HM-araCMP standard. The peak eluting at 36 min is HM-araCTP, based on its elution with dCTP in the first SAX HPLC column. The rate of conversion of 10 μM dCyd to dCTP in CEM cells was 0.91 pmol/ 10^6 cells/min, whereas the rate of conversion of 10 μM HM-araC to HM-araCMP and HM-araCTP in CEM cells was 0.01 pmol/ 10^6 cells/min, which indicated that the rate of activation of HM-araC in CEM cells was approximately 100-fold less than that of dCyd.

TABLE I

Phosphorylation of 4'-C-(hydroxymethyl)arabinofuranosyl analogs by recombinant dThd kinase 1, dThd kinase 2, or dCyd kinase

Base		dThd kinase 1	dThd kinase 2	dCyd kinase	dGuo kinase
		% of control activity			
Uracil	(α)	3.5 \pm 0.4	1.0 \pm 0.5	nd	nd
Uracil	(β)	3.0 \pm 0.1	1.0 \pm 0.5	nd	nd
Thymine	(α)	<1	3.1 \pm 0.8	nd	nd
Thymine	(β)	<1	3.7 \pm 0.9	nd	nd
5-Fluorouracil	(α)	<1	1.1 \pm 0.1	2.2 \pm 1.8	nd
5-Fluorouracil	(β)	<1	1.7 \pm 0.4	5.8 \pm 0.8	nd
5-Fluorocytosine	(α)	nd	<1	2.1 \pm 0.7	nd
5-Fluorocytosine	(β)	nd	<1	103 \pm 5	nd
Cytosine	(α)	nd	<1	<1	nd
Cytosine	(β)	nd	<1	72 \pm 0.9	nd
Adenine	(α)	nd	nd	<1	<1
Adenine	(β)	nd	nd	<1	<1

The phosphoryl transfer assays were performed as described in the Biological Materials and Methods. The final concentration of each nucleoside was 100 μM and the values (mean and standard deviations of three determinations) represent percent activity compared to either dThd or dCyd. nd, not done.

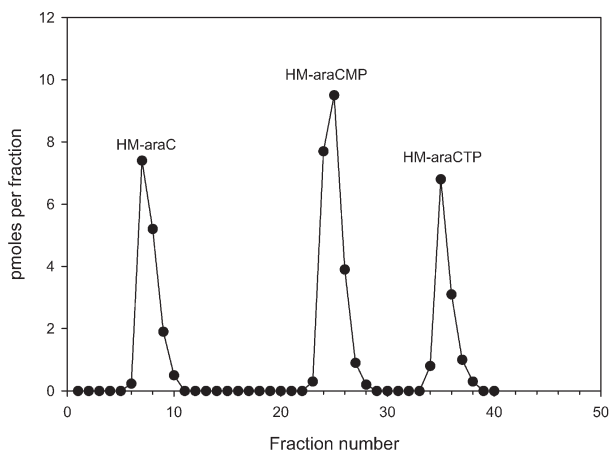


FIG. 1

Metabolism of HM-araC (**18β**) in CEM cells. CEM cells (1×10^6 cells/ml) were incubated with $10 \mu\text{M}$ [^3H]HM-araC ($1 \mu\text{Ci/ml}$) for 4 h. The cells from 15 ml cultures were collected by centrifugation, and an acid-soluble extract was prepared. The metabolites of HM-araC were separated using BioBasic AX ion exchange HPLC. Fractions were collected as they eluted from the column and counted for radioactivity

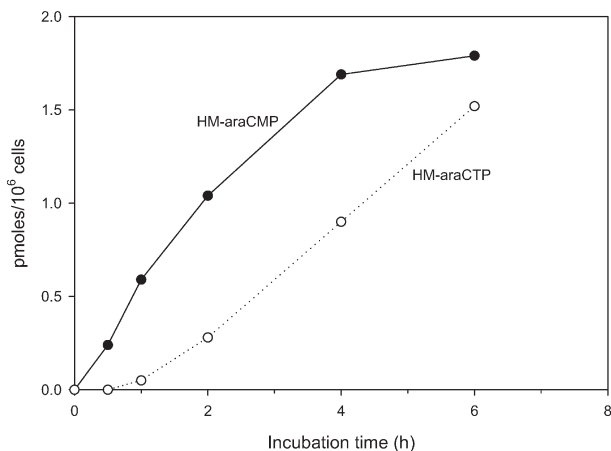


FIG. 2

Rate of production of HM-araCMP and HM-araCTP in CEM cells. CEM cells (1×10^6 cells/ml) were incubated with $10 \mu\text{M}$ [^3H]HM-araC ($1 \mu\text{Ci/ml}$). Cells were collected from 15 ml of culture and the amount of HM-araCMP and HM-araCTP were determined as described in the legend to Fig. 1

There was a considerable accumulation of HM-araCMP in CEM cells, which indicated that the rate of its synthesis was much greater than the rate of its use as a substrate by the monophosphate kinase (Fig. 2). At early time points the predominant intracellular metabolite was HM-araCMP. However, by 6 h the intracellular concentration of HM-araCTP was equal to that of the monophosphate (Fig. 2). This result indicated that HM-araCMP was a relatively poor substrate for CMP kinase.

With a partially pure preparation of dCyd kinase from CEM cells we compared the rate of phosphorylation of dCyd and HM-araC using both ATP and UTP as the phosphate donor (Table II). With ATP as the phosphate donor the HM-araC was phosphorylated at a rate that was 0.7% of that seen with dCyd, and with UTP the rate of HM-araC phosphorylation was 6% of that of dCyd. These results are in general agreement with the intact cells studies and indicate that HM-araC was only a modest substrate for dCyd kinase isolated from CEM cells. The kinetic data with the recombinant enzyme indicates that the phosphorylation with HM-araC at 10 μM would be 15% of that of dCyd. Previous studies in our laboratory⁴¹ indicate that the phosphorylation of 10 μM araC by dCyd kinase isolated from CEM cells would be similar to that of dCyd using both UTP and ATP as the phosphate donor. Therefore, these results indicate that HM-araC is approximately 100-fold less efficient as a substrate for dCyd kinase than araC.

HM-araC was not toxic to CEM cells. The concentration of HM-araC required to inhibit growth by 50% during a 96-h incubation was greater than 150 μM . Although the metabolism is not as great as that seen with araC, a considerable amount of HM-araCTP was formed in CEM cells, which suggested that HM-araCTP was poorly recognized by the human DNA polymerases involved in DNA replication. Since HM-araCTP was formed in

TABLE II
HM-araC (**18 β**) and dCyd as substrates for dCyd kinase purified from CEM cells

Substrate, 10 μM	Phosphate donor	Specific activity nmol/mg/min	Percent of activity with dCyd
dCyd	ATP	5.80	
	UTP	0.66	
HM-araC	ATP	0.04	0.7
	UTP	0.04	6.0

cells without toxicity, this result suggests that this compound should be examined for antiviral activity.

EXPERIMENTAL

Biological Materials and Methods

Determination of cytotoxicity. CCRF-CEM cells (obtained from the American Type Culture Collection) were grown in RPMI 1640 medium (Gibco-BRL) containing 10% of fetal bovine serum (Atlanta Biologicals), 10 U/ml of penicillin, 10 µg/ml of streptomycin, and 50 µg/ml of gentamycin. The cells were exposed continuously to various concentrations of the compounds at 37 °C for 72 h, and cell numbers were determined with a Coulter Counter.

Measurement of nucleoside kinase activity with recombinant nucleoside kinases. The recombinant human enzymes dThd kinase 1, dThd kinase 2, and dCyd kinase were expressed and purified from the bacterial expression system according to the procedures described previously⁴² without removal of the histidine tag sequences. The enzymes were at least 95% pure as judged by SDS-PAGE. dThd kinase 1 and dThd kinase 2 had specific activities of 17 and 70 nmol/min/mg protein, respectively, using dThd as substrate. The specific activity of dCyd kinase was 18 nmol/min/mg using dCyd as substrate. The assays were performed as described previously⁴² with minor modifications. The reaction mixtures contained final concentrations of 100 µM of nucleosides, 100 µM [³²P]ATP (with 0.0325 µM γ-[³²P]ATP from Amersham), 50 mM Tris-HCl (pH 7.6), 5 mM MgCl₂, 125 mM KCl, 10 mM dithiothreitol, 0.5 mg/ml bovine serum albumin and 2 µg/ml enzyme. After incubation at 37 °C for 20 min the samples were heated at 95 °C for 2 min followed by centrifugation. Five µl of each sample was applied onto PEI-cellulose TLC plates (Merck), which were developed overnight with a solvent system containing isobutyric acid/ammonium hydroxide/water (66:1:33). The radiolabeled spots were visualized by a phosphor-imager (Fuji Film, Science Lab., Image Gauge V3.3).

Measurement of [³H]HM-araC intracellular metabolites. CEM cells were incubated with [³H]HM-araC (obtained from Moravěk Biochemicals, Brea, CA, U.S.A.). At the end of the incubation periods, the cells were collected by centrifugation, washed twice with sterile phosphate buffered saline, and then treated with 0.5 M ice-cold perchloric acid as described⁴³. The perchloric extracts were centrifuged at 12 000 g for 10 min, and the supernatant fluid was neutralized with the appropriate amount of 4 M KOH and then buffered with 1 M tripotassium phosphate (pH 7.4). The KClO₄ precipitate was removed by centrifugation, and the extract was injected onto a strong anion exchange (SAX) HPLC system. A BioBasic ion exchange HPLC column (Thermo Electron Corp., Bellefonte, PA, U.S.A.) was used to analyze the phosphorylated metabolites of HM-araC, because of its superior retention of mononucleotides. Elution of nucleotides with this column was accomplished with 30-min linear salt and pH gradient from 6 mM ammonium phosphate (pH 2.8) to 900 mM ammonium phosphate (pH 6) with a flow rate of 2 ml/min. Radioactive metabolites of HM-araC were detected by counting 1-min fractions that eluted from the column. Natural nucleotides were detected by their absorbance at 260 nm.

Chemical Synthesis

Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. The UV absorption spectra (λ , nm; ϵ) were recorded on Perkin-Elmer Lambda 9 spectrophotometer in methanol. ^1H NMR spectra were run in CDCl_3 or $\text{DMSO}-d_6$ on a Nicolet NT-300 spectrometer operating at 300.625 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane. When necessary, deuterium exchange, selective decoupling experiments and NOE experiments were performed in order to confirm proton assignments. All J values are in Hz. Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer in the positive fast atom bombardment ion mode ($\text{FAB} > 0$) using 3-nitrobenzyl alcohol/lithium chloride matrix (NBA, LiCl) or on a PE Sciex API3 mass spectrometer in an electro-spray (ES) technique. HPLC analyses were carried out on a Hewlett-Packard 1100 series liquid chromatograph with a Phenomenex sphereclone 5 μ ODS(1) column (4.6 \times 250 mm) and UV monitoring (254 nm). Elemental analyses were carried out by the Spectroscopic and Analytical Laboratory of Southern Research Institute or by Atlantic Microlabs, Atlanta, GA, U.S.A. Thin layer chromatography (TLC) was performed on precoated aluminum sheets of Silica Gel 60 F_{254} (Merck, Art. 5554), visualization of products being accomplished by UV absorption followed by charring with a saturated ammonium sulfate solution. Column chromatography was carried out on Merck Silica Gel 60 (230–400 mesh) at atmospheric pressure.

1,2-*O*-Isopropylidene- α -L-xylofuranose (**1**)

To anhydrous acetone (1 l) were successively added 97% sulfuric acid (5 ml), anhydrous copper sulfate (100 g) and L-xylose (50 g, 0.33 mol). The mixture was stirred at room temperature for 24 h with exclusion of moisture and filtered. The filtrate was neutralized with 20% ammonium hydroxide (35 ml), filtered and evaporated to dryness under reduced pressure. The residue was then co-evaporated several times with absolute ethanol to afford 1,2:3,5-di-*O*-isopropylidene- α -L-xylofuranose as a syrup, only contaminated with a trace of **1**. To this syrup, aqueous 0.2% hydrochloric acid (440 ml) was added and the mixture was stirred at room temperature for 3 h and neutralized with solid sodium hydrogencarbonate. The solution was evaporated to dryness under reduced pressure and the crude material was dissolved in chloroform (200 ml), dried over anhydrous sodium sulfate and evaporated under reduced pressure to give pure **1** as a yellow syrup (59.1 g, 94%). ^1H NMR (CDCl_3): 5.99 (d, 1 H, H-1, $J_{1,2} = 3.6$); 4.53 (d, 1 H, H-2, $J_{2,1} = 3.6$); 4.34 (d, 1 H, H-3, $J_{3,4} = 2.4$); 4.2–4.0 (m, 3 H, H-4, H-5 and H-5'); 3.7–2.9 (br s, 2 H, OH-5 and OH-3); 1.49 (s, 3 H, CH_3); 1.33 (s, 3 H, CH_3). MS (matrix NBA, LiCl): $\text{FAB} > 0$ m/z 429 (2 M + Li + LiCl) $^+$, 387 (2 M + Li) $^+$, 239 (M + Li + LiCl) $^+$, 197 (M + Li) $^+$.

5-*O*-Benzoyl-1,2-*O*-isopropylidene- α -L-xylofuranose (**2**)

A solution of **1** (59.1 g, 0.31 mol) in anhydrous pyridine (400 ml) was stirred under argon and cooled to 0 °C. A solution of benzoyl chloride (37.8 ml, 0.327 mol) in anhydrous pyridine (100 ml) was added dropwise and the reaction mixture was stirred at 0 °C for 45 min. Then the reaction was quenched with water (5 ml) and the mixture was partially concentrated under reduced pressure and diluted in methylene chloride (500 ml). The organic layer was washed with a saturated aqueous sodium hydrogencarbonate solution (2 \times 300 ml), with water (2 \times 300 ml) and then dried over sodium sulfate and evaporated under reduced

pressure. The residue was purified by silica gel column chromatography (eluent: stepwise gradient of methanol, 0–8% in methylene chloride) to give pure **2** (70.3 g, 77%) as a syrup. $^1\text{H NMR}$ (CDCl_3): 8.1–7.4 (m, 5 H, $\text{C}_6\text{H}_5\text{CO}$); 5.96 (d, 1 H, H-1, $J_{1-2} = 3.6$); 4.81 (dd, 1 H, H-5, $J_{4-5} = 9.5$, $J_{5-5'} = 12.6$); 4.60 (d, 1 H, H-2, $J_{2-1} = 3.6$); 4.4–4.3 (m, 2 H, H-4 and H-5'); 4.18 (br t, 1 H, H-3, $J_{3-4} = 2.2$, $J_{3-\text{OH}} = 3.9$); 3.29 (d, 1 H, OH-3, $J_{\text{OH}-3} = 3.9$); 1.51 (s, 3 H, CH_3); 1.33 (s, 3 H, CH_3). MS (matrix NBA, LiCl): FAB > 0 m/z 301 (M + Li) $^+$, 295 (M + H) $^+$, 105 ($\text{C}_6\text{H}_5\text{CO}$) $^+$.

5-*O*-Benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-1,2-*O*-isopropylidene- α -L-xylofuranose (**3**)

To a solution of **2** (69.0 g, 0.23 mol) in dry pyridine (800 ml) under argon atmosphere were added *tert*-butyldimethylsilyl chloride (53.0 g, 0.35 mol) and imidazole (40.0 g, 0.59 mol), and the mixture was stirred at 30 °C for 24 h. Then the reaction was quenched with 10% aqueous sodium hydrogencarbonate solution (10 ml), partially concentrated under reduced pressure and diluted in chloroform (500 ml). The organic layer was washed with a 10% aqueous sodium hydrogencarbonate solution (2 \times 300 ml), with water (2 \times 300 ml) and then dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: stepwise gradient of methanol, 0–8% in chloroform) to give pure **3** (96.6 g, quantitative) as a colorless syrup. $^1\text{H NMR}$ (CDCl_3): 8.1–7.4 (m, 5 H, $\text{C}_6\text{H}_5\text{CO}$); 5.97 (d, 1 H, H-1, $J_{1-2} = 3.6$); 4.6–4.4 (m, 3 H, H-4, H-5 and H-5'); 4.40 (d, 1 H, H-2, $J_{2-1} = 3.6$); 4.30 (br d, 1 H, H-3, $J_{3-4} = 1.5$); 1.51 (s, 3 H, CH_3); 1.34 (s, 3 H, CH_3); 0.90 (s, 9 H, $(\text{CH}_3)_3\text{C-Si}$); 0.14 (s, 3 H, $(\text{CH}_3)\text{Si}$); 0.09 (s, 3 H, $(\text{CH}_3)\text{Si}$). MS (matrix NBA, LiCl): FAB > 0 m/z 425 (M + Li) $^+$, 409 (M + H) $^+$, 351 (M + H - $[(\text{CH}_3)_3\text{CH}]$), 115 (TBDMS) $^+$, 105 ($\text{C}_6\text{H}_5\text{CO}$) $^+$.

3-*O*-(*tert*-Butyldimethylsilyl)-1,2-*O*-isopropylidene- α -L-xylofuranose (**4**)

Sodium (5.37 g, 0.23 mol) was added at 0 °C to a solution of **3** (90.9 g, 0.22 mol) in a mixture of anhydrous toluene (225 ml) and methanol (450 ml). The resulting solution was stirred at 0 °C during 1 h and then neutralized at 0 °C by addition of Dowex 50WX2 (H $^+$ form). The resin was filtered and washed with warm methanol, and the combined filtrates were evaporated to dryness. Column chromatography of the residue on silica gel (eluent: stepwise gradient of methanol, 0–6% in chloroform) afforded **4** (59.4 g, 88%), which was recrystallized from cyclohexane, m.p. 67–68 °C. $^1\text{H NMR}$ (CDCl_3): 5.96 (d, 1 H, H-1, $J_{1-2} = 3.7$); 4.37 (d, 1 H, H-2, $J_{2-1} = 3.7$); 4.3–4.2 (m, 2 H, H-3 and H-4); 4.0–3.7 (m, 2 H, H-5 and H-5'); 2.01 (dd, OH-5, $J_{\text{OH}-5} = 8.8$, $J_{\text{OH}-5'} = 3.3$); 1.50 (s, 3 H, CH_3); 1.33 (s, 3 H, CH_3); 0.90 (s, 9 H, $(\text{CH}_3)_3\text{C-Si}$); 0.15 (s, 3 H, $(\text{CH}_3)\text{Si}$); 0.12 (s, 3 H, $(\text{CH}_3)\text{Si}$). MS (matrix NBA, LiCl): FAB > 0 m/z 657 (2 M + Li + LiCl) $^+$, 615 (2 M + Li) $^+$, 353 (M + Li + LiCl) $^+$, 311 (M + Li) $^+$.

4-*C*-(Hydroxymethyl)-1,2-*O*-isopropylidene- β -D-*threo*-pentofuranose (**6**)

Dicyclohexylcarbodiimide (DCC; 50.3 g, 0.24 mol) and dichloroacetic acid (4 ml, 48.7 mmol) were added to a solution of **4** (49.5 g, 0.16 mol) in anhydrous benzene (490 ml), DMSO (325 ml) and pyridine (13 ml). The resulting solution was stirred at room temperature under argon atmosphere for 23 h and diluted with ethyl acetate (650 ml). Oxalic acid (2.3 g, 25.5 mmol) dissolved in DMSO (30 ml) was added and the reaction mixture was stirred at room temperature during 1 h and then filtered to eliminate precipitated dicyclohexylurea (DCU). The filtrate was washed with brine (2 \times 500 ml), with a saturated aqueous sodium hydrogencarbonate solution (2 \times 500 ml) and finally with water (3 \times 500 ml) before

being dried over sodium sulfate and evaporated under reduced pressure. The presence of the aldehyde group was shown on TLC by a positive test after spraying with a solution of 2,4-dinitrophenylhydrazine in 1% ethanolic hydrochloric acid and heating. The crude residue was quickly chromatographed through a pad of silica gel (eluent: stepwise gradient of chloroform, 70–100% in hexanes). The collected material (46.7 g, 0.15 mol) was dissolved in a mixture of 37% aqueous formaldehyde (30.9 ml), water (155 ml) and dioxane (310 ml), and aqueous sodium hydroxide (154.5 ml of 1 M solution) was added. The mixture was stirred at room temperature during 23 h and then neutralized by addition of Dowex 50WX2 (H^+ form). The resin was filtered and washed with warm methanol, and the combined filtrates were evaporated to dryness. The residue was purified by silica gel column chromatography (eluent: stepwise gradient of methanol, 4–10% in chloroform) to give pure **6** (27.2 g, 76%), which was recrystallized from methylene chloride, m.p. 90–92 °C. 1H NMR (DMSO- d_6): 6.01 (d, 1 H, H-1, $J_{1-2} = 4.2$); 4.65 (d, 1 H, H-2, $J_{2-1} = 4.2$); 4.24 (d, 1 H, H-3, $J_{3-OH} = 5.3$); 3.89 (d, 2 H, H-5 and H-5', $J_{5-OH} = J_{5'-OH} = 6.2$); 3.82 (dd, 1 H, H-6, $J_{6-OH} = 3.7$, $J_{6-6'} = 11.6$); 3.71 (d, OH-3, $J_{OH-3} = 5.5$); 3.59 (dd, 1 H, H-6', $J_{6'-OH} = 8.8$, $J_{6-6'} = 11.6$); 2.52 (t, 1 H, OH-5, $J_{OH-5} = J_{OH-5'} = 6.2$); 2.26 (dd, 1 H, OH-6, $J_{OH-6} = 4.0$, $J_{OH-6'} = 8.8$); 1.54 (s, 3 H, CH_3); 1.32 (s, 3 H, CH_3). MS (matrix NBA, LiCl): FAB > 0 m/z 227 (M + Li) $^+$.

3,5-Di-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-1,2-*O*-isopropylidene- β -D-*threo*-pentofuranose (**7**)

To an ice-cold solution of **6** (26.8 g, 0.12 mol) in dry THF (730 ml) was added portionwise under argon sodium hydride (60% dispersion in mineral oil, 29.2 g, 0.73 mol), and the reaction mixture was stirred for 15 min under argon atmosphere. Solid tetrabutylammonium iodide (2.7 g, 7.3 mmol) was added to the reaction mixture, followed by a dropwise addition of benzyl bromide (72.4 ml, 0.61 mmol). The reaction mixture was stirred at room temperature for 24 h under argon atmosphere. After addition of methanol (25 ml), the solution was partially concentrated under reduced pressure and diluted with methylene chloride (500 ml). The organic layer was washed with brine (2 \times 400 ml), with water (400 ml) and then dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: chloroform/hexanes, 8:2) to give pure **7** (53.3 g, 91%) as a syrup. 1H NMR ($CDCl_3$): 7.3–7.2 (m, 15 H, 3 C_6H_5); 6.00 (d, 1 H, H-1, $J_{1-2} = 4.5$); 4.76 (dd, 1 H, H-2, $J_{2-1} = 4.6$, $J_{2-3} = 2.3$); 4.8–4.4 (m, 6 H, 3 $PhCH_2$); 4.12 (d, 1 H, H-3, $J_{3-2} = 2.3$); 3.7–3.5 (m, 4 H, H-5, H-5', H-6 and H-6'); 1.46 (s, 3 H, CH_3); 1.34 (s, 3 H, CH_3). MS (matrix NBA, LiCl): FAB > 0 m/z 497 (M + Li) $^+$, 91 ($C_6H_5CH_2$) $^+$.

Methyl 3,5-Di-*O*-benzyl-4-*C*-[(benzyloxy)methyl]- α,β -D-*threo*-pentofuranoside (**8**)

To an ice-cold solution of **7** (53.2 g, 92 mmol) in 85% acetic acid (185 ml) was added concentrated sulfuric acid (0.75 ml, 13.8 mmol). The temperature was raised to 50 °C and the reaction was stirred at this temperature during 3.5 h. Then the reaction mixture was cooled in an ice-cold bath, diluted with water (100 ml) and neutralized with solid sodium hydrogencarbonate. The organic layer was extracted with chloroform (2 \times 300 ml) and the combined extracts were successively washed with a saturated aqueous sodium hydrogencarbonate solution (2 \times 400 ml) and finally with water (2 \times 400 ml) before being dried over sodium sulfate and evaporated to dryness. The resulting crude residue was dissolved in dry methanol (550 ml) and cooled to 0 °C. To this ice-cold solution was added concentrated sulfuric acid (1.5 ml, 27.6 mmol) and the reaction was stirred at room temperature during 23 h, then neutralized with pyridine, concentrated under reduced pressure and

diluted with methylene chloride (300 ml). The organic layer was washed with water (2 × 300 ml) before being dried over sodium sulfate and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (eluent: chloroform/methanol, 0.5%) to give pure **8** as a pale yellow syrup (40.6 g, 95%) as a ~1:2 α : β mixture. $^1\text{H NMR}$ (CDCl_3): 7.3–7.2 (m, 15 H, 3 C_6H_5); 4.88 (br s, 1 H, H-1 α anomer); 4.85 (d, 1 H, H-1 β anomer, $J_{1,2} = 4.8$); 4.8–4.4 (m, 6 H, 3 PhCH_2); 4.4–4.3 (m, 1 H, H-2); 3.94 (m, 1 H, H-3); 3.7–3.5 (m, 4 H, H-5, H-5', H-6 and H-6'); 3.41 (s, 3 H, OCH_3); 2.43 (d, 1 H, OH-2, $J_{\text{OH-2}} = 9.9$). MS (matrix NBA, LiCl): FAB > 0 m/z 513 ($\text{M} + \text{Li} + \text{LiCl}$) $^+$, 471 ($\text{M} + \text{Li}$) $^+$, 91 ($\text{C}_6\text{H}_5\text{CH}_2$) $^+$.

Methyl 2,3,5-Tri-*O*-benzyl-4-*C*-[(benzyloxy)methyl]- α,β -D-*threo*-pentofuranoside (**9**)

To an ice-cold solution of **8** (10.2 g, 22.0 mmol) in dry THF (180 ml) was added portionwise under argon sodium hydride (60% dispersion in mineral oil, 2.63 g, 65.9 mol), and the reaction mixture was stirred for 15 min under argon atmosphere. Solid tetrabutylammonium iodide (162 mg, 0.44 mmol) was added to the reaction mixture followed by a dropwise addition of benzyl bromide (6.5 ml, 54.9 mmol). The reaction mixture was stirred at room temperature for 22 h under argon atmosphere. After the addition of methanol (9 ml), the solution was partially concentrated under reduced pressure and diluted with methylene chloride (400 ml). The organic layer was washed with brine (2 × 300 ml), with water (1 × 400 ml) and then dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: stepwise gradient of chloroform, 70–100% in hexanes) and then a stepwise gradient of methanol (0–1%) in chloroform to give pure **9** as a pale yellow syrup (11.3 g, 92%) as a ~1:2 α : β mixture. $^1\text{H NMR}$ (CDCl_3): 7.4–7.1 (m, 20 H, 4 C_6H_5); 4.94 (d, 1 H, H-1 α anomer, $J_{1,2} = 1.8$); 4.70 (d, 1 H, H-1 β anomer, $J_{1,2} = 4.4$); 4.7–4.4 (m, 8 H, 3 PhCH_2); 4.24 (d, 1 H, H-3 β anomer, $J_{3,2} = 8.1$); 4.12 (m, 2 H, H-2 β anomer and H-3 α anomer); 4.02 (dd, 1 H, H-2 α anomer, $J_{2,1} = 1.8$, $J_{2,3} = 3.5$); 3.8–3.4 (m, 4 H, H-5, H-5', H-6 and H-6'); 3.39 (s, 3 H, OCH_3 α anomer); 3.33 (s, 3 H, OCH_3 β anomer). MS (matrix NBA, LiCl): FAB > 0 m/z 561 ($\text{M} + \text{Li}$) $^+$, 91 ($\text{C}_6\text{H}_5\text{CH}_2$) $^+$.

1-*O*-Acetyl-2,3,5-tri-*O*-benzyl-4-*C*-[(benzyloxy)methyl]- α,β -D-*threo*-pentofuranose (**10**)

An ice-cold 90% aqueous trifluoroacetic acid solution (51 ml) was added to **9** (9.5 g, 17.1 mmol) and the reaction mixture was stirred between 3 and 10 °C for 4.5 h and at room temperature for 1.5 h. Then the reaction mixture was cooled in an ice-cold bath, diluted with water (50 ml) and neutralized with solid sodium hydrogencarbonate. The organic layer was extracted with chloroform (2 × 150 ml) and the combined extracts were successively washed with a saturated aqueous sodium hydrogencarbonate solution (2 × 200 ml) and finally with water (2 × 200 ml) before being dried over sodium sulfate and evaporated to dryness. The resulting residue was co-evaporated twice with anhydrous pyridine and diluted in this solvent (120 ml). To the solution was added acetic anhydride (16.2 ml, 171.3 mmol) and the reaction was stirred at room temperature during 5 h, then partially concentrated under reduced pressure and diluted with methylene chloride (300 ml). The organic layer was successively washed with a saturated aqueous sodium hydrogencarbonate solution (2 × 200 ml) and finally with water (2 × 200 ml) before being dried over sodium sulfate and evaporated to dryness and co-evaporated with toluene. The crude material was purified by silica gel column chromatography (eluent: stepwise gradient of chloroform, 70–100% in hexanes) to give pure **10** as a pale yellow syrup (8.1 g, 80%) as a 3:1 α : β mixture. $^1\text{H NMR}$ (CDCl_3): 7.4–7.2 (m, 20 H,

4 C₆H₅); 6.29 (d, 1 H, H-1 β anomer, $J_{1,2} = 4.4$); 6.21 (d, 1 H, H-1 α anomer, $J_{1,2} = 1.1$); 4.7–4.4 (m, 8 H, 3 PhCH₂); 4.36 (dd, 1 H, H-2 β anomer, $J_{2,1} = 4.4$, $J_{2,3} = 8.3$); 4.22 (d, 1 H, H-3 β anomer, $J_{3,2} = 8.3$); 4.16 (d, 1 H, H-3 α anomer, $J_{3,2} = 2.5$); 4.12 (dd, 1 H, H-2 α anomer, $J_{2,1} = 1.4$, $J_{2,3} = 2.9$); 3.8–3.3 (m, 4 H, H-5, H-5', H-6 and H-6'); 1.99 (s, 3 H, CH₃CO₂ α anomer); 1.99 (s, 3 H, CH₃CO₂ β anomer). MS (matrix NBA, LiCl): FAB > 0 m/z 589 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

1-(2,3,5-Tri-*O*-benzyl-4-*C*-[(benzyloxy)methyl]-α- and -β-*D*-*threo*-pentofuranosyl)uracil (**11α**) and (**11β**)

A mixture of uracil (276 mg, 2.46 mmol), hexamethyldisilazane (HMDS; 16 ml) and a catalytic amount of ammonium sulfate was heated during 18 h under reflux. After cooling to room temperature, the mixture was evaporated under reduced pressure, and the residue obtained as a colorless oil was diluted with anhydrous 1,2-dichloroethane (17 ml). To the resulting solution was added **10** (0.99 g, 1.70 mmol) in anhydrous 1,2-dichloroethane (10 ml), followed by addition of trimethylsilyl trifluoromethanesulfonate (TMSTf, 0.62 ml, 3.40 mmol). The solution was stirred at room temperature for 2.5 h under argon atmosphere, then diluted with chloroform (150 ml), washed with the same volume of a saturated aqueous sodium hydrogencarbonate solution and finally with water (2 × 100 ml). The organic phase was dried over sodium sulfate, then evaporated under reduced pressure. The resulting crude material, as a 1.8:1 α:β mixture, was purified by silica gel column chromatography (eluent: chloroform/methanol, 0.5%) to afford as pale yellow foams pure **11β** (295 mg, 27%), followed by a mixture of **11α** and **11β** (134 mg, 12%) and pure **11α** (550 mg, 51%).

11β: ¹H NMR (CDCl₃): 7.99 (br s, 1 H, NH); 7.69 (d, 1 H, H-6, $J_{6,5} = 8.2$); 7.4–7.1 (m, 20 H, 4 C₆H₅); 6.49 (d, 1 H, H-1', $J_{1',2'} = 6.1$); 5.33 (dd, 1 H, H-5, $J_{5,6} = 8.2$, $J_{5,NH} = 2.3$); 4.7–4.3 (m, 9 H, H-2' and 4 PhCH₂); 4.32 (d, 1 H, H-3', $J_{3',2'} = 6.3$); 3.64 (d, 1 H, H-6', $J_{6',6''} = 10.0$); 3.55 (d, 1 H, H-5', $J_{5',5''} = 10.2$); 3.41 (d, 1 H, H-5'', $J_{5'',5'} = 10.2$); 3.40 (d, 1 H, H-6'', $J_{6'',6'} = 10.0$). MS (matrix NBA, LiCl): FAB > 0 m/z 641 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

11α: ¹H NMR (CDCl₃): 8.05 (br s, 1 H, NH); 7.62 (d, 1 H, H-6, $J_{6,5} = 8.2$); 7.4–7.1 (m, 20 H, 4 C₆H₅); 6.08 (d, 1 H, H-1', $J_{1',2'} = 5.4$); 5.22 (dd, 1 H, H-5, $J_{5,6} = 8.2$, $J_{5,NH} = 2.3$); 4.7–4.3 (m, 8 H, 4 PhCH₂); 4.22 (d, 1 H, H-3', $J_{3',2'} = 5.6$); 4.17 (t, 1 H, H-2', $J_{2',1'} = J_{2',3'} = 5.5$); 3.75 (d, 1 H, H-6', $J_{6',6''} = 10.0$); 3.69 (d, 1 H, H-6'', $J_{6'',6'} = 10.0$); 3.49 (d, 1 H, H-5', $J_{5',5''} = 9.8$); 3.44 (d, 1 H, H-5'', $J_{5'',5'} = 9.8$). MS (matrix NBA, LiCl): FAB > 0 m/z 641 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

1-[4-*C*-(Hydroxymethyl)-β-*D*-*threo*-pentofuranosyl]uracil (**12β**)

A mixture of **11β** (280 mg, 0.441 mmol) and 67 mg of 10% Pd/C in a 1:1 solution of methanol/acetic acid (13 ml) was hydrogenated at room temperature and atmospheric pressure during 2 days. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: stepwise gradient of methanol, 10–20% in chloroform) to give pure **12β** (90 mg, 74%), which was recrystallized from ethanol, m.p. 182–184 °C. UV (methanol): λ_{max} 263 (11300), λ_{min} 242 (6200). HPLC 99.8%, 99:1 H₂O/MeCN. ¹H NMR (DMSO-*d*₆): 11.21 (s, 1 H, NH); 7.73 (d, 1 H, H-6, $J_{6,5} = 8.1$); 6.12 (d, 1 H, H-1', $J_{1',2'} = 5.8$); 5.55 (d, 1 H, H-5, $J_{5,6} = 8.1$); 5.44 (d, 1 H, OH-2', $J_{OH,2'} = 5.2$); 5.35 (d, 1 H, OH-3', $J_{OH,3'} = 5.1$); 5.03 (t, 1 H, OH-5', $J_{OH,5'} = J_{OH,5''} = 5.3$); 4.57 (t, 1 H, OH-6', $J_{OH,6'} = J_{OH,6''} = 5.8$); 4.28 (dd, 1 H, H-2', $J = 5.4$, $J = 10.9$); 4.03 (t, 1 H, H-3', $J_{3',2'} = J_{3',OH'} = 5.3$); 3.56 (dd, 1 H, H-6', $J_{6',6''} = 11.5$, $J_{OH,6'} = 5.6$);

3.48 (br d, 2 H, H-5' and H-5'', $J = 4.2$); 3.34 (dd, 1 H, H-6'', $J_{6''-6'} = 11.5$, $J_{OH-6'} = 5.9$). MS (matrix NBA, LiCl): FAB > 0 m/z 287 (M - H + 2 Li)⁺, 281 (M + Li)⁺. For C₁₀H₁₄N₂O₇ (274.2) calculated: 43.80% C, 5.15% H, 10.22% N; found: 43.83% C, 5.03% H, 10.09% N.

1-[4-C-(Hydroxymethyl)- α -D-*threo*-pentofuranosyl]uracil (**12 α**)

Hydrogenation of **11 α** (534 mg, 0.841 mmol) was performed following the same procedure as described for the deprotection of compound **11 β** , and provided, after purification by silica gel chromatography, pure **12 α** (218 mg, 94%), which was precipitated from a methanol/ethyl ether mixture, m.p. 79–85 °C (hygroscopic). UV (methanol): λ_{max} 262 (8000), λ_{min} 231 (1900). HPLC 99.0%, 99:1 H₂O/MeCN. ¹H NMR (DMSO-*d*₆): 11.26 (s, 1 H, NH); 8.07 (d, 1 H, H-6, $J_{6-5} = 8.1$); 5.76 (d, 1 H, H-1', $J_{1'-2'} = 6.4$); 5.69 (d, 1 H, H-5, $J_{5-6} = 8.1$); 5.57 (d, 1 H, OH-2', $J_{OH-2'} = 5.0$); 5.37 (d, 1 H, OH-3', $J_{OH-3'} = 4.9$); 4.89 (t, 1 H, OH-5', $J_{OH-5'} = J_{OH-5''} = 5.7$); 4.69 (t, 1 H, OH-6', $J_{OH-6'} = J_{OH-6''} = 5.4$); 4.1–4.0 (m, 2 H, H-2' and H-3'); 3.60 (dd, 1 H, H-6', $J_{6'-6''} = 11.7$, $J_{OH-6'} = 5.0$); 3.4–3.3 (m, 3 H, H-5', H-5'' and H-6''). MS (matrix NBA, LiCl): FAB > 0 m/z 287 (M - H + 2 Li)⁺, 281 (M + Li)⁺. For C₁₀H₁₄N₂O₇·H₂O (292.2) calculated: 41.10% C, 5.52% H, 9.59% N; found: 41.35% C, 5.11% H, 9.17% N.

1-(2,3,5-Tri-*O*-benzyl-4-C-[(benzyloxy)methyl]- α - and - β -D-*threo*-pentofuranosyl)thymine (**13 α**) and (**13 β**)

Condensation of thymine (311 mg, 2.46 mmol) with **10** (0.99 g, 1.70 mmol) was performed following the same procedure as described for the preparation of compounds **11 α** and **11 β** and provided a 3.3:1 α : β mixture of **13 α** and **13 β** , which was separable by silica gel chromatography (eluent: stepwise gradient of methanol, 0–0.5% in chloroform): **13 β** (213 mg, 19%), mixture of **13 α** and **13 β** (170 mg, 15%), and **13 α** (590 mg, 53%). Both compounds were pale yellow foams.

13 β : ¹H NMR (CDCl₃): 8.06 (br s, 1 H, NH); 7.47 (d, 1 H, H-6, $J_{6-Me} = 1.1$); 7.3–7.1 (m, 20 H, 4 C₆H₅); 6.51 (d, 1 H, H-1', $J_{1'-2'} = 6.2$); 4.7–4.3 (m, 9 H, 4 PhCH₂); 4.58 (t, 1 H, H-2', $J_{2'-1'} = J_{2'-3'} = 6.2$); 4.37 (d, 1 H, H-3', $J_{3'-2'} = 6.2$); 3.64 (d, 1 H, H-6', $J_{6'-6''} = 9.9$); 3.57 (d, 1 H, H-5', $J_{5'-5''} = 9.9$); 3.44 (d, 1 H, H-5'', $J_{5''-5'} = 10.1$); 3.40 (d, 1 H, H-6'', $J_{6''-6'} = 10.0$); 1.58 (d, 3 H, 5-CH₃, $J_{Me-6} = 1.1$). MS (matrix NBA, LiCl): FAB > 0 m/z 655 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

13 α : ¹H NMR (CDCl₃): 7.95 (br s, 1 H, NH); 7.39 (d, 1 H, H-6, $J_{6-Me} = 1.3$); 7.3–7.1 (m, 20 H, 4 C₆H₅); 6.11 (m, 1 H, H-1'); 4.7–4.4 (m, 8 H, 4 PhCH₂); 4.24 (m, 2 H, H-2' and H-3'); 3.73 (d, 1 H, H-6', $J_{6'-6''} = 10.1$); 3.69 (d, 1 H, H-6'', $J_{6''-6'} = 10.2$); 3.47 (d, 1 H, H-5', $J_{5'-5''} = 9.8$); 3.43 (d, 1 H, H-5'', $J_{5''-5'} = 9.8$); 1.42 (d, 3 H, 5-CH₃, $J_{Me-6} = 1.2$). MS (matrix NBA, LiCl): FAB > 0 m/z 655 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

1-[4-C-(Hydroxymethyl)- β -D-*threo*-pentofuranosyl]thymine (**14 β**)

Hydrogenation of **13 β** (213 mg, 0.328 mmol) was performed following the same procedure as described for the deprotection of compound **11 β** , and provided, after purification by silica gel chromatography, pure **14 β** (90 mg, 95%), which was lyophilized, m.p. 92–98 °C. UV (methanol): λ_{max} 268 (9900), λ_{min} 234 (2900). HPLC 98.9%, 99:1 H₂O/MeCN. ¹H NMR (DMSO-*d*₆): 11.18 (s, 1 H, NH); 7.65 (d, 1 H, H-6, $J_{6-Me} = 1.2$); 6.11 (d, 1 H, H-1', $J_{1'-2'} = 5.9$); 5.49 (d, 1 H, OH-2', $J_{OH-2'} = 5.5$); 5.32 (d, 1 H, OH-3', $J_{OH-3'} = 5.3$); 5.10 (t, 1 H, OH-5', $J_{OH-5'} = J_{OH-5''} = 5.3$); 4.59 (t, 1 H, OH-6', $J_{OH-6'} = J_{OH-6''} = 5.8$); 4.29 (dd, 1 H, H-2', $J = 5.8$, $J = 11.4$); 4.03 (t, 1 H, H-3', $J_{3'-2'} = J_{3'-OH} = 5.4$); 3.56 (dd, 1 H, H-6', $J_{6'-6''} = 11.5$, $J_{OH-6'} = 5.8$); 3.5–3.4

(m, 2 H, H-5' and H-5''); 3.4–3.3 (m, 1 H, H-6''); 1.75 (d, 3 H, 5-CH₃, $J_{\text{Me-6}} = 1.1$). MS (ES): m/z 599 (2 M + Na)⁺, 577 (2 M + H)⁺, 311 (M + Na)⁺, 289 (M + H)⁺. For C₁₁H₁₆N₂O₇·2/3H₂O (300.3) calculated: 43.99% C, 5.82% H, 9.33% N; found: 44.03% C, 5.78% H, 9.09% N.

1-[4-C-(Hydroxymethyl)- α -D-*threo*-pentofuranosyl]thymine (**14 α**)

Hydrogenation of **13 α** (309 mg, 0.476 mmol) was performed following the same procedure as described for the deprotection of compound **11 β** , and provided, after purification by silica gel chromatography, pure **14 α** (125 mg, 91%), which was lyophilized from water, m.p. 92–98 °C. UV (methanol): λ_{max} 266 (9300), λ_{min} 233 (2300). HPLC 99.9%, 99:1 H₂O/MeCN. ¹H NMR (DMSO-*d*₆): 11.26 (s, 1 H, NH); 7.93 (d, 1 H, H-6, $J_{\text{6-Me}} = 1.2$); 5.75 (d, 1 H, H-1', $J_{1'-2'} = 6.8$); 5.52 (d, 1 H, OH-2', $J_{\text{OH-2'}} = 5.1$); 5.32 (d, 1 H, OH-3', $J_{\text{OH-3'}} = 5.4$); 4.88 (t, 1 H, OH-5', $J_{\text{OH-5'}} = J_{\text{OH-5''}} = 5.7$); 4.7 (t, 1 H, OH-6', $J_{\text{OH-6'}} = J_{\text{OH-6''}} = 5.4$); 4.2–4.1 (m, 2 H, H-2' and H-3'); 3.60 (dd, 1 H, H-6', $J_{6'-6''} = 11.7$, $J_{\text{OH-6'}} = 5.0$); 3.41 (dd, 1 H, H-6'', $J_{6'-6''} = 11.7$, $J_{\text{OH-6''}} = 6.0$); 3.4–3.3 (m, 1 H, H-5' and H-5''); 1.78 (d, 3 H, 5-CH₃, $J_{\text{Me-6}} = 1.0$). MS (ES): m/z 599 (2 M + Na)⁺, 577 (2 M + H)⁺, 311 (M + Na)⁺, 289 (M + H)⁺. For C₁₁H₁₆N₂O₇·2/3H₂O (300.3) calculated: 43.99% C, 5.82% H, 9.33% N; found: 43.99% C, 5.53% H, 9.16% N.

5-Fluoro-1-{2,3,5-tri-*O*-benzyl-4-C-[(benzyloxy)methyl- α - and - β -D-*threo*-pentofuranosyl]uracil (**15 α**) and (**15 β**)

Condensation of 5-fluorouracil (324 mg, 2.49 mmol) with **10** (1.00 g, 1.72 mmol) was performed following the same procedure as described for the preparation of compounds **11 α** and **11 β** and provided a 1.4:1 α : β mixture, which was separable by silica gel chromatography (eluent: stepwise gradient of methanol, 0–0.2% in chloroform): **15 β** (258 mg, 23%), mixture of **15 α** and **15 β** (146 mg, 13%), and **15 α** (584 mg, 52%). Both compounds were pale yellow foams.

15 β : ¹H NMR (CDCl₃): 8.10 (br d, 1 H, NH, $J_{\text{NH-F}} = 5.0$); 7.95 (d, 1 H, H-6, $J_{6-F} = 6.5$); 7.4–7.1 (m, 20 H, 4 C₆H₅); 6.43 (dd, 1 H, H-1', $J_{1'-2'} = 6.2$, $J_{1'-F} = 1.7$); 4.7–4.3 (m, 9 H, 4 PhCH₂); 4.59 (t, 1 H, H-2', $J_{2'-1'} = 6.2$, $J_{2'-3'} = 6.4$); 4.34 (d, 1 H, H-3', $J_{3'-2'} = 6.4$); 3.61 (d, 1 H, H-6', $J_{6'-6''} = 9.9$); 3.54 (d, 1 H, H-5', $J_{5'-5''} = 10.2$); 3.36 (d, 1 H, H-5'', $J_{5'-5''} = 10.2$); 3.35 (d, 1 H, H-6'', $J_{6'-6''} = 9.9$). MS (matrix NBA, LiCl): FAB > 0 m/z 659 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

15 α : ¹H NMR (CDCl₃): 8.13 (br d, 1 H, NH, $J_{\text{NH-F}} = 4.1$); 7.80 (d, 1 H, H-6, $J_{6-F} = 6.4$); 7.4–7.1 (m, 20 H, 4 C₆H₅); 6.00 (dd, 1 H, H-1', $J_{1'-2'} = 5.9$, $J_{1'-F} = 1.8$); 4.7–4.4 (m, 8 H, 4 PhCH₂); 4.23 (d, 1 H, H-3', $J_{3'-2'} = 6.3$); 4.18 (t, 1 H, H-2', $J_{2'-1'} = 5.9$, $J_{2'-3'} = 6.3$); 3.49 (d, 1 H, H-6', $J_{6'-6''} = 10.1$); 3.89 (d, 1 H, H-6'', $J_{6'-6''} = 10.1$); 3.42 (d, 1 H, H-5', $J_{5'-5''} = 9.8$). MS (matrix NBA, LiCl): FAB > 0 m/z 659 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

5-Fluoro-1-[4-C-(hydroxyl)methyl- β -D-*threo*-pentofuranosyl]uracil (**16 β**)

Hydrogenation of **15 β** (215 mg, 0.329 mmol) was performed following the same procedure as described for the deprotection of compound **11 β** , and provided, after purification by silica gel chromatography, **16 β** (92 mg, 96%), which was lyophilized from water, m.p. 86–90 °C. UV (methanol): λ_{max} 271 (8100), λ_{min} 235 (1700). HPLC 99.3%, 98:2 0.01 M NH₄H₂PO₄ (pH 5.1)/MeOH. ¹H NMR (DMSO-*d*₆): 11.76 (br s, 1 H, NH); 8.14 (d, 1 H, H-6, $J_{6-F} = 7.4$); 6.09 (dd, 1 H, H-1', $J_{1'-2'} = 5.9$, $J_{1'-F} = 1.9$); 5.58 (d, 1 H, OH-2', $J_{\text{OH-2'}} = 5.5$); 5.38 (d, 1 H, OH-3', $J_{\text{OH-3'}} = 5.3$); 5.23 (t, 1 H, OH-5', $J_{\text{OH-5'}} = J_{\text{OH-5''}} = 5.0$); 4.63 (t, 1 H, OH-6', $J_{\text{OH-6'}} = J_{\text{OH-6''}} = 5.7$); 4.33 (dd, 1 H, H-2', $J = 5.8$, $J = 11.5$); 4.05 (t, 1 H, H-3', $J_{3'-2'} = J_{3'-\text{OH}} = 5.7$);

3.55 (dd, 1 H, H-6', $J_{6'-6''} = 11.7$, $J_{OH-6'} = 5.8$); 3.5–3.4 (m, 2 H, H-5' and H-5''); 3.28 (dd, 1 H, H-6'', $J_{6'-6''} = 11.7$, $J_{OH-6'} = 5.9$). MS (matrix NBA, LiCl): FAB > 0 m/z 293 (M + H)⁺. For C₁₀H₁₃FN₂O₇·1/2H₂O (301.2) calculated: 39.87% C, 4.69% H, 9.30% N; found: 39.65% C, 4.71% H, 8.92% N.

5-Fluoro-1-[4-C-(hydroxymethyl)- α -D-*threo*-pentofuranosyl]uracil (**16 α**)

Hydrogenation of **15 α** (226 mg, 0.346 mmol) was performed following the same procedure as described for the deprotection of compound **11 β** , and provided, after purification by silica gel chromatography, **16 α** (100 mg, 99%), which was lyophilized from water, m.p. 80–84 °C. UV (methanol): λ_{\max} 268 (8400), λ_{\min} 234 (2100). HPLC 98.1%, 98:2 0.01 M NH₄H₂PO₄ (pH 5.1)/MeOH. ¹H NMR (DMSO-*d*₆): 11.80 (br s, 1 H, NH); 8.43 (d, 1 H, H-6, $J_{6-F} = 7.3$); 5.75 (dd, 1 H, H-1', $J_{1'-2'} = 6.5$, $J_{1'-F} = 1.8$); 5.55 (d, 1 H, OH-2', $J_{OH-2'} = 4.9$); 5.40 (d, 1 H, OH-3', $J_{OH-3'} = 5.1$); 5.0–4.9 (m, 2 H, OH-5' and OH-6'); 4.2–4.0 (m, 2 H, H-2' and H-3'); 3.62 (dd, 1 H, H-6', $J_{6'-6''} = 11.7$, $J_{OH-6'} = 4.9$); 3.4–3.3 (m, 3 H, H-5', H-5'' and H-6''). MS (matrix NBA, LiCl): FAB > 0 m/z 293 (M + H)⁺. For C₁₀H₁₃FN₂O₇·5/4H₂O (315.3) calculated: 38.16% C, 4.96% H, 8.90% N; found: 38.10% C, 4.64% H, 8.82% N.

1-{2,3,5-Tri-*O*-benzyl-4-C-[(benzyloxy)methyl]- β -D-*threo*-pentofuranosyl}cytosine (**17 β**)

Lawesson's reagent (140 mg, 0.345 mmol) was added under argon to a solution of **11 β** (313 mg, 0.493 mmol) in anhydrous 1,2-dichloroethane (12 ml) and the reaction mixture was stirred under reflux overnight. The solvent was then evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: stepwise gradient of methanol, 0–1% in chloroform) to give the corresponding 4-thio intermediate which was treated by methanolic ammonia (12 ml previously saturated at –10 °C and tightly stoppered) at 100 °C in a stainless-steel bomb for 3 h, then cooled to room temperature. The solution was evaporated to dryness under reduced pressure and the residue coevaporated several times with methanol. The crude residue was purified by silica gel column chromatography (eluent: stepwise gradient of methanol, 0–4% in chloroform) to give pure **17 β** as a bright yellow foam (256 mg, 82%). ¹H NMR (CDCl₃): 7.98 (d, 1 H, H-6, $J_{6-5} = 7.4$); 7.3–7.1 (m, 20 H, 4 C₆H₅); 6.65 (d, 1 H, H-1', $J_{1'-2'} = 5.6$); 5.37 (d, 1 H, H-5, $J_{5-6} = 7.5$); 5.3–5.1 (br s, 2 H, NH₂); 4.6–4.3 (m, 9 H, H-2' and 4 PhCH₂); 4.25 (d, 1 H, H-3', $J_{3'-2'} = 5.1$); 3.66 (d, 1 H, H-6', $J_{6'-6''} = 10.1$); 3.58 (d, 1 H, H-5', $J_{5'-5''} = 10.1$); 3.53 (d, 1 H, H-5'', $J_{5''-5'} = 10.1$); 3.49 (d, 1 H, H-6'', $J_{6''-6'} = 10.1$). MS (matrix NBA, LiCl): FAB > 0 m/z 640 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

1-[4-C-(Hydroxymethyl)- β -D-*threo*-pentofuranosyl]cytosine (**18 β**)

A solution of **17 β** (250 mg, 0.394 mmol) in anhydrous dichloromethane (4 ml) was treated at –78 °C under argon with a 1 M boron trichloride solution in dichloromethane (6.3 ml). After stirring at –78 °C for 3 h and at –25 °C overnight, the reaction mixture was cooled at –78 °C, a 1:1 mixture of anhydrous methanol and pyridine was added dropwise and the solution was evaporated to dryness. The residue was purified by silica gel column chromatography (eluent: chloroform/methanol/ammonium hydroxide, 65:30:5) to give pure **18 β** (81 mg, 75%), which was recrystallized from methanol, m.p. 226–227 °C. UV (methanol): λ_{\max} 274 (6500), λ_{\min} 253 (4500), λ 218 sh (5900). HPLC 99.7%, 99:1 H₂O/MeCN. ¹H NMR (DMSO-*d*₆): 7.65 (d, 1 H, H-6, $J_{6-5} = 7.4$); 7.1–6.9 (br d, 2 H, NH₂); 6.15 (d, 1 H, H-1', $J_{1'-2'} = 5.0$); 5.64 (d, 1 H, H-5, $J_{5-6} = 7.3$); 5.37 (d, 1 H, OH-2', $J_{OH-2'} = 5.6$); 5.29 (d, 1 H, OH-3',

$J_{\text{OH}-3'} = 5.0$); 4.97 (t, 1 H, OH-5', $J_{\text{OH}-5'} = J_{\text{OH}-5''} = 5.3$); 4.49 (t, 1 H, OH-6', $J_{\text{OH}-6'} = J_{\text{OH}-6''} = 5.8$); 4.15 (dd, 1 H, H-2', $J = 5.3$, $J = 9.6$); 4.00 (t, 1 H, H-3', $J_{3'-2'} = J_{3'-\text{OH}''} = 4.5$); 3.57 (dd, 1 H, H-6', $J_{6'-6''} = 11.7$, $J_{\text{OH}-6'} = 5.6$); 3.52 (d, 2 H, H-5' and H-5'', $J = 5.4$); 3.41 (dd, 1 H, H-6'', $J_{6''-6'} = 11.5$, $J_{\text{OH}-6'} = 6.1$). MS (ES): m/z 547 (2 M + H)⁺, 274 (M + H)⁺, 112 (BH₂)⁺. For C₁₀H₁₅N₃O₆ (273.3) calculated: 43.96% C, 5.53% H, 15.38% N; found: 43.81% C, 5.33% H, 15.26% N.

1-{2,3,5-Tri-*O*-benzyl-4-*C*-[(benzyloxy)methyl]- α -D-*threo*-pentofuranosyl}cytosine (**17 α**)

Thionation of **11 α** (760 mg, 1.20 mmol) with Lawesson's reagent (339 mg, 0.83 mmol) in anhydrous 1,2-dichloroethane (12 ml), was performed following the same procedure as described for the thionation of compound **11 β** , and provided the corresponding 4-thio intermediate which was treated with methanolic ammonia following the same procedure as described above to give, after purification by silica gel chromatography (eluent: stepwise gradient of methanol, 0–4% in toluene), **17 α** (300 mg, 100%). ¹H NMR (CDCl₃): 7.69 (d, 1 H, H-6, $J_{6-5} = 7.5$); 7.3–7.1 (m, 20 H, 4 C₆H₅); 6.26 (d, 1 H, H-1', $J_{1'-2'} = 3.4$); 5.38 (d, 1 H, H-5, $J_{5-6} = 7.4$); 6.0–5.0 (br s, 2 H, NH₂); 4.8–4.3 (m, 8 H, 4 PhCH₂); 4.13 (t, 1 H, H-2', $J_{2'-1'} = J_{2'-3'} = 3.3$); 4.09 (d, 1 H, H-3', $J_{3'-2'} = 3.2$); 3.83 (d, 1 H, H-6', $J_{6'-6''} = 9.9$); 3.78 (d, 1 H, H-6'', $J_{6''-6'} = 10.2$); 3.69 (d, 1 H, 1 H, H-5', $J_{5'-5''} = 9.5$); 3.64 (d, 1 H, H-5'', $J_{5''-5'} = 9.6$). MS (matrix NBA, LiCl): FAB > 0 m/z 640 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

1-[4-*C*-(Hydroxymethyl)- α -D-*threo*-pentofuranosyl]cytosine (**18 α**)

Deprotection of **17 α** (695 mg, 1.10 mmol) with a 1 M boron trichloride solution in dichloromethane (17.5 ml), was performed following the same procedure as described for the deprotection of **17 β** , and provided, after purification by silica gel chromatography (eluent: chloroform/methanol/ammonium hydroxide, 65:30:5), pure **18 α** which was recrystallized from methanol, m.p. 205–206 °C. UV (methanol): λ_{max} 235 (7200), 272 (7600); λ_{min} 228 (7100), 255 (6400). HPLC 100%, 99:1 H₂O/MeCN. ¹H NMR (DMSO-*d*₆): 7.91 (d, 1 H, H-6, $J_{6-5} = 7.4$); 7.2–7.0 (br d, 2 H, NH₂); 5.78 (d, 1 H, H-1', $J_{1'-2'} = 6.0$); 5.73 (d, 1 H, H-5, $J_{5-6} = 7.4$); 5.48 (d, 1 H, OH-2', $J_{\text{OH}-2'} = 5.1$); 5.27 (d, 1 H, OH-3', $J_{\text{OH}-3'} = 5.3$); 4.83 (t, 1 H, OH-5', $J_{\text{OH}-5'} = J_{\text{OH}-5''} = 5.7$); 4.59 (t, 1 H, OH-6', $J_{\text{OH}-6'} = J_{\text{OH}-6''} = 5.5$); 4.1–4.0 (m, 2 H, H-2' and H-3'); 3.58 (dd, 1 H, H-6', $J_{6'-6''} = 11.6$, $J_{\text{OH}-6'} = 5.4$); 3.43 (dd, 1 H, H-6'', $J_{6''-6'} = 11.8$, $J_{\text{OH}-6''} = 5.8$); 3.37 (d, 2 H, H-5' and H-5'', $J = 5.6$). MS (ES): m/z 547 (2 M + H)⁺, 274 (M + H)⁺, 112 (BH₂)⁺. For C₁₀H₁₅N₃O₆·CH₃OH (305.3) calculated: 43.28% C, 6.27% H, 13.76% N; found: 43.29% C, 5.98% H, 13.53% N.

5-Fluoro-1-{2,3,5-tri-*O*-benzyl-4-*C*-[(benzyloxy)methyl]- α - and - β -D-*threo*-pentofuranosyl}cytosine (**19 α**) and (**19 β**)

A mixture of **15 α** and **15 β** (273 mg, 0.418 mmol) was treated with Lawesson's reagent (118 mg, 0.293 mmol) in anhydrous 1,2-dichloroethane (10.5 ml), following the same procedure as described for the thionation of compound **11 β** , and provided the corresponding 4-thio intermediates, which were treated with methanolic ammonia following the same procedure as described above to give, after several purifications by silica gel chromatography (eluent: stepwise gradient of methanol, 2–3% in chloroform), pure **19 β** (100 mg, 42%), a mixture of **19 α** and **19 β** (7 mg, 3%), and pure **19 α** (130 mg, 55%). Both compounds were pale yellow foams.

19 β : $^1\text{H NMR}$ (CDCl_3): 7.92 (d, 1 H, H-6, $J_{6,\text{F}} = 6.6$); 7.4–7.1 (m, 20 H, 4 C_6H_5); 6.57 (dd, 1 H, H-1', $J_{1',2'} = 5.7$, $J_{1',\text{F}} = 1.9$); 6.6–6.3 (br s, 1 H, NH_2); 6.4–6.1 (br s, 1 H, NH_2); 4.6–4.2 (m, 9 H, H-2' and 4 PhCH_2); 4.26 (d, 1 H, H-3', $J_{3',2'} = 5.5$); 3.63 (d, 1 H, H-6'', $J_{6'',6'''} = 10.1$); 3.58 (d, 1 H, H-5', $J_{5',5''} = 10.0$); 3.47 (d, 1 H, H-5'', $J_{5'',5'''} = 10.1$); 3.44 (d, 1 H, H-6'', $J_{6'',6'''} = 10.0$). MS (matrix NBA, LiCl): FAB > 0 m/z 658 (M + Li) $^+$, 91 ($\text{C}_6\text{H}_5\text{CH}_2$) $^+$.

19 α : $^1\text{H NMR}$ (CDCl_3): 7.80 (d, 1 H, H-6, $J_{6,\text{F}} = 6.5$); 7.4–7.1 (m, 20 H, 4 C_6H_5); 6.19 (br s, 1 H, H-1'); 5.5–5.1 (br s, 2 H, NH_2); 4.8–4.4 (m, 8 H, 4 PhCH_2); 4.13 (m, 2 H, H-2' and H-3'); 3.81 (d, 1 H, H-6'', $J_{6'',6'''} = 10.3$); 3.77 (d, 1 H, H-6'', $J_{6'',6'''} = 10.3$); 3.63 (d, 1 H, H-5', $J_{5',5''} = 9.7$); 3.54 (d, 1 H, H-5'', $J_{5'',5'''} = 9.6$). MS (matrix NBA, LiCl): FAB > 0 m/z 658 (M + Li) $^+$, 91 ($\text{C}_6\text{H}_5\text{CH}_2$) $^+$.

5-Fluoro-1-[4-C-(hydroxymethyl)- β -D-*threo*-pentofuranosyl]cytosine (**20 β**)

Deprotection of **19 β** (162 mg, 0.248 mmol) with a 1 M boron trichloride solution in dichloromethane (4.0 ml) was performed following the same procedure as described for the deprotection of **17 β** , and provided, after purification by silica gel chromatography (eluent: chloroform/methanol/ammonium hydroxide, 65:30:5), pure **20 β** (62 mg, 86%), which was precipitated from an ethanol/diethyl ether mixture, m.p. 83–88 °C (hygroscopic). UV (methanol): λ_{max} 239 (7000), 284 (6800); λ_{min} 226 (6300), 263 (4600). HPLC 98.3%, 99:1 $\text{H}_2\text{O}/\text{MeCN}$. $^1\text{H NMR}$ ($\text{DMSO}-d_6$): 7.93 (d, 1 H, H-6, $J_{6,\text{F}} = 7.3$); 7.7–7.3 (br d, 2 H, NH_2); 6.10 (dd, 1 H, H-1', $J_{1',2'} = 5.4$, $J_{1',\text{F}} = 2.0$); 5.45 (d, 1 H, OH-2', $J_{\text{OH}-2'} = 5.6$); 5.33 (d, 1 H, OH-3', $J_{\text{OH}-3'} = 5.1$); 5.14 (t, 1 H, OH-5', $J_{\text{OH}-5'} = J_{\text{OH}-5''} = 5.2$); 4.56 (t, 1 H, OH-6', $J_{\text{OH}-6'} = J_{\text{OH}-6''} = 5.9$); 4.22 (dd, 1 H, H-2', $J = 5.4$, $J = 10.8$); 4.00 (t, 1 H, H-3', $J_{3',2'} = J_{3',\text{OH}'} = 4.5$); 3.6–3.5 (m, 3 H, H-5', H-5'' and H-6); 3.34 (dd, 1 H, H-6'', $J_{6'',6'''} = 10.5$, $J_{\text{OH}-6''} = 6.1$). MS (ES): m/z 605 (2 M + Na) $^+$, 583 (2 M + H) $^+$, 314 (M + Na) $^+$, 292 (M + H) $^+$. For $\text{C}_{10}\text{H}_{14}\text{FN}_3\text{O}_6 \cdot 1.5\text{H}_2\text{O}$ (318.2) calculated: 37.74% C, 5.38% H, 13.20% N; found: 37.83% C, 4.90% H, 12.50% N.

5-Fluoro-1-[4-C-(hydroxymethyl)- α -D-*threo*-pentofuranosyl]cytosine (**20 α**)

Deprotection of **19 α** (212 mg, 0.325 mmol) with a 1 M boron trichloride solution in dichloromethane (5.2 ml) was performed following the same procedure as described for the deprotection of **17 β** , and provided, after purification by silica gel chromatography (eluent: chloroform/methanol/ammonium hydroxide, 65:30:5), pure **20 α** (94 mg, 99%), which was recrystallized from ethanol, m.p. 132–135 °C (hygroscopic). UV (methanol): λ_{max} 242 (8500), 283 (7200); λ_{min} 225 (6800), 265 (5900). HPLC 100%, 99:1 $\text{H}_2\text{O}/\text{MeCN}$. $^1\text{H NMR}$ ($\text{DMSO}-d_6$): 8.22 (d, 1 H, H-6, $J_{6,\text{F}} = 7.2$); 7.5–7.8 (br d, 2 H, NH_2); 5.78 (br d, 1 H, H-1', $J_{1',2'} = 5.1$, $J_{1',\text{F}} = 2.0$); 5.49 (d, 1 H, OH-2', $J_{\text{OH}-2'} = 4.4$); 5.33 (d, 1 H, OH-3', $J_{\text{OH}-3'} = 4.6$); 4.86 (t, 1 H, OH-5', $J_{\text{OH}-5'} = J_{\text{OH}-5''} = 5.3$); 4.77 (t, 1 H, OH-6', $J_{\text{OH}-6'} = J_{\text{OH}-6''} = 5.1$); 4.1–4.0 (m, 2 H, H-2' and H-3'); 3.61 (dd, 1 H, H-6'', $J_{6'',6'''} = 11.6$, $J_{\text{OH}-6''} = 4.9$); 3.41 (dd, 1 H, H-6'', $J_{6'',6'''} = 11.7$, $J_{\text{OH}-6''} = 5.6$); 3.34 (m, 2 H, H-5' and H-5''). MS (ES): m/z 605 (2 M + Na) $^+$, 583 (2 M + H) $^+$, 314 (M + Na) $^+$, 292 (M + H) $^+$. For $\text{C}_{10}\text{H}_{14}\text{FN}_3\text{O}_6 \cdot \text{H}_2\text{O}$ (309.2) calculated: 38.84% C, 5.22% H, 13.59% N; found: 39.04% C, 4.91% H, 13.83% N.

6-Chloro-9-{2,3,5-tri-*O*-benzyl-4-C-[(benzyloxy)methyl]- α - and β -D-*threo*-pentofuranosyl}purine (**22 α**) and (**22 β**)

A suspension of 6-chloropurine (3.60 g, 6.18 mmol) in anhydrous acetonitrile (40 ml) was treated with *N,O*-bis(trimethylsilyl)acetamide (BSA; 9.2 ml, 37.07 mmol) during 18 h under

reflux. To the resulting solution was added at 0 °C **10** (0.99 g, 1.70 mmol) in anhydrous acetonitrile (18.5 ml), followed by addition of tin(IV) chloride (0.795 ml, 6.80 mmol). The solution was stirred at room temperature for 7 h under argon atmosphere. To the reaction mixture was added carefully a saturated aqueous sodium hydrogencarbonate solution (7 ml) and the resulting mixture was filtered through a sintered funnel covered with Celite. The Celite was then triturated and washed several times with boiling chloroform (100 ml). The combined filtrates were concentrated under reduced pressure and the resulting residue was diluted with chloroform (150 ml), washed with water (3 × 100 ml). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. Column chromatography of the residue using a stepwise gradient of ethyl acetate (20–40%) in hexanes afforded 6-chloro-7-{2,3,5-tri-*O*-benzyl-4-*C*-[(benzyloxy)methyl]- α - and - β -D-*threo*-pentofuranosyl}-purines **21 α** and **21 β** (2.02 g, 48%), and **22 α** and **22 β** (1.07 g, 26%). To a solution of **21 α** and **21 β** (2.02 g, 2.98 mmol) in anhydrous acetonitrile (20 ml) was added at 0 °C trimethylsilyl trifluoromethanesulfonate (TMSOTf; 0.864 ml, 4.77 mmol). The solution was stirred at room temperature for 1.5 h under argon atmosphere, then diluted with chloroform (150 ml), washed with the same volume of a saturated aqueous sodium hydrogencarbonate solution, with brine (2 × 100 ml) and finally with water (1 × 100 ml). The organic phase was dried over sodium sulfate, then evaporated under reduced pressure. Column chromatography of the residue using a stepwise gradient of ethyl acetate (20–40%) in hexanes afforded **22 α** and **22 β** (1.09 g, 54%). Anomeric purification of the combined material obtained above as a 0.8:1 α : β mixture was performed by silica gel chromatography (eluent: hexanes/ethyl acetate, 8:2) and afforded as pale yellow foams pure **22 β** (1.00 g, 48%), a mixture of **22 α** and **22 β** (0.29 g, 14%), and pure **22 α** (0.81 g, 39%).

22 β : ¹H NMR (CDCl₃): 8.64 (s, 1 H, H-2); 8.46 (s, 1 H, H-8); 7.4–6.7 (m, 20 H, 4 C₆H₅); 6.60 (d, 1 H, H-1', J_{1',2'} = 6.1); 4.72 (t, 1 H, H-2', J_{2',1'} = J_{2',3'} = 6.3); 4.7–4.0 (m, 9 H, H-3' and 4 PhCH₂); 3.72 (d, 1 H, H-6', J_{6',6''} = 10.0); 3.53 (d, 1 H, H-5', J_{5',5''} = 9.7); 3.47 (m, 2 H, H-5'' and H-6''). ¹³C NMR (CDCl₃): 151.6 (2-C); 151.5 (4-C); 150.5 (6-C); 145.7 (8-C); 137.9–136.3 (C₆H₅); 131.3 (9-C); 128.5–127.4 (C₆H₅); 84.9 (4'-C); 83.0 (2'-C); 82.4 (1'-C); 81.7 (3'-C); 73.8–73.1 (PhCH₂); 70.0 (5'-C or 6'-C); 69.3 (5'-C or 6'-C). MS (matrix NBA, LiCl): FAB > 0 m/z 683 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

22 α : ¹H NMR (CDCl₃): 8.66 (s, 1 H, H-2); 8.37 (s, 1 H, H-8); 7.4–6.9 (m, 20 H, 4 C₆H₅); 6.20 (d, 1 H, H-1', J_{1',2'} = 5.4); 4.73 (t, 1 H, H-2', J_{2',1'} = J_{2',3'} = 5.6); 4.7–4.3 (m, 9 H, H-3' and 4 PhCH₂); 3.81 (d, 1 H, H-6', J_{6',6''} = 10.2); 3.72 (d, 1 H, H-6'', J_{6'',6'} = 10.2); 3.55 (br s, 2 H, H-5' and H-5). ¹³C NMR (CDCl₃): 151.7 (2-C); 151.3 (4-C); 150.6 (6-C); 144.0 (8-C); 137.7–136.7 (C₆H₅); 131.7 (9-C); 128.4–127.6 (C₆H₅); 87.0 (4'-C); 86.0 (1'-C and 2'-C); 83.3 (3'-C); 73.8–73.0 (PhCH₂); 70.7 (5'-C or 6'-C); 69.9 (5'-C or 6'-C). MS (matrix NBA, LiCl): FAB > 0 m/z 683 (M + Li)⁺, 91 (C₆H₅CH₂)⁺.

9-{2,3,5-Tri-*O*-benzyl-4-*C*-[(benzyloxy)methyl]- β -D-*threo*-pentofuranosyl}adenine (**23 β**)

Compound **22 β** (0.98 g, 1.45 mmol) was treated with ethanolic ammonia (29 ml previously saturated at -10 °C and tightly stoppered) at 75 °C in a stainless-steel bomb for 5.5 h, then cooled to room temperature. The solution was evaporated to dryness under reduced pressure. The resulting residue was diluted with chloroform (150 ml) and the organic layer was washed with brine (3 × 150 ml), dried over sodium sulfate and evaporated to dryness. The crude residue was purified by silica gel column chromatography (eluent: stepwise gradient of methanol, 0–4% in chloroform) to give pure **23 β** as a pale yellow foam (782 mg, 82%).

$^1\text{H NMR}$ (CDCl_3): 8.32 (s, 1 H, H-2); 8.17 (s, 1 H, H-8); 7.4–6.8 (m, 20 H, 4 C_6H_5); 6.64 (d, 1 H, H-1', $J_{1',2'} = 5.9$); 5.60 (br s, 2 H, NH_2); 4.7–4.0 (m, 10 H, H-2', H-3' and 4 PhCH_2); 3.72 (d, 1 H, H-6', $J_{6',6''} = 10.1$); 3.6–3.5 (m, 3 H, H-5', H-5'' and H-6''). MS (ES): m/z 658 ($\text{M} + \text{H}$) $^+$.

9-[4-C-(Hydroxymethyl)- β -D-*threo*-pentofuranosyl]adenine (**24 β**)

To a solution of **23 β** (617 mg, 0.938 mmol) in a 1:1 solution of methanol/acetic acid (20 ml) was added 375 mg of 20% $\text{Pd}(\text{OH})_2/\text{C}$ and the suspension was shaken on a Parr apparatus under 50 Pa of H_2 . After 1, 2 and 3 days 94 mg portions of 20% $\text{Pd}(\text{OH})_2/\text{C}$ were added. After 5 days the mixture was filtered through a pad of Celite and filtrate was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: chloroform/methanol/ammonium hydroxide, 80:18:2) to give pure **24 β** (136 mg, 49%), which was recrystallized from methanol, m.p. 240–242 °C. UV (methanol): λ_{max} 259 (13700), λ_{min} 237 (2600). HPLC 100%, 90:10 0.01 M $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 5.1)/MeOH. $^1\text{H NMR}$ ($\text{DMSO}-d_6$): 8.20 (s, 1 H, H-8); 8.11 (s, 1 H, H-2); 7.20 (s, 2 H, NH_2); 6.32 (d, 1 H, H-1', $J_{1',2'} = 6.0$); 5.48 (d, 1 H, OH-2', $J_{\text{OH}-2'} = 5.6$); 5.41 (d, 1 H, OH-3', $J_{\text{OH}-3'} = 5.3$); 5.12 (t, 1 H, OH-5', $J_{\text{OH}-5'} = J_{\text{OH}-5''} = 5.4$); 4.62 (t, 1 H, OH-6', $J_{\text{OH}-6'} = J_{\text{OH}-6''} = 5.8$); 4.47 (dd, 1 H, H-2', $J = 6.1$, $J = 12.0$); 4.32 (t, 1 H, H-3', $J_{3'-\text{OH}} = J_{3'-2'} = 5.6$); 3.61 (dd, 1 H, H-6', $J_{6',6''} = 11.6$, $J_{\text{OH}-6''} = 5.8$); 3.51 (d, 2 H, H-5' and H-5'', $J_{5'-\text{OH}} = J_{5''-\text{OH}} = 5.3$); 3.39 (dd, 1 H, H-6'', $J_{6',6''} = 11.7$, $J_{\text{OH}-6''} = 5.8$). MS (ES): m/z 320 ($\text{M} + \text{Na}$) $^+$, 298 ($\text{M} + \text{H}$) $^+$, 136 (BH_2) $^+$. For $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_5$ (297.3) calculated: 44.45% C, 5.09% H, 23.56% N; found: 44.16% C, 5.01% H, 23.38% N.

9-[2,3,5-Tri-*O*-benzyl-4-C-[(benzyloxy)methyl]- α -D-*threo*-pentofuranosyl]adenine (**23 α**)

Compound **22 α** (0.720 g, 1.06 mmol) was treated with ethanolic ammonia (21 ml), following the same procedure as described for amination of compound **22 β** , and provided, after purification by silica gel column chromatography (eluent: stepwise gradient of methanol, 0–4% in chloroform), pure **23 α** (657 mg, 94%). $^1\text{H NMR}$ (CDCl_3): 8.33 (s, 1 H, H-2); 8.05 (s, 1 H, H-8); 7.4–7.0 (m, 20 H, 4 C_6H_5); 6.21 (d, 1 H, H-1', $J_{1',2'} = 5.3$); 5.56 (br s, 2 H, NH_2); 4.72 (t, 1 H, H-2', $J_{2'-1'} = J_{2'-3'} = 5.4$); 4.7–4.4 (m, 8 H, 4 PhCH_2); 3.84 (d, 1 H, H-6', $J_{6',6''} = 10.2$); 3.73 (d, 1 H, H-6'', $J_{6',6''} = 10.1$); 3.60 (d, 1 H, H-5', $J_{5',5''} = 10.1$); 3.56 (d, 1 H, H-5'', $J_{5',5''} = 9.9$). MS (ES): m/z 658 ($\text{M} + \text{H}$) $^+$.

9-[4-C-(Hydroxymethyl)- α -D-*threo*-pentofuranosyl]adenine (**24 α**)

To a solution of **23 α** (650 mg, 0.988 mmol) in a 1:1 solution of methanol/acetic acid (20 ml) was added 395 mg of 20% $\text{Pd}(\text{OH})_2/\text{C}$ and the suspension was shaken on a Parr apparatus under 50 Pa of H_2 . After 1, 2 and 3 days 99 mg portions of 20% $\text{Pd}(\text{OH})_2/\text{C}$ were added. After 5 days the mixture was filtered through a pad of Celite and filtrate was concentrated under reduced pressure. The crude material was purified by silica gel column chromatography (eluent: chloroform/methanol/ammonium hydroxide, 80:18:2) to give pure **24 α** (194 mg, 66%), which was recrystallized from methanol, m.p. 246–248 °C. UV (methanol): λ_{max} 259 (15400), λ_{min} 228 (2600). HPLC 100%, 90:10 0.01 M $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 5.1)/MeOH. $^1\text{H NMR}$ ($\text{DMSO}-d_6$): 8.37 (s, 1 H, H-8); 8.14 (s, 1 H, H-2); 7.34 (s, 2 H, NH_2); 5.77 (d, 1 H, H-1', $J_{1',2'} = 6.9$); 5.67 (d, 1 H, OH-2', $J_{\text{OH}-2'} = 5.6$); 5.38 (d, 1 H, OH-3', $J_{\text{OH}-3'} = 6.3$); 5.15 (dd, 1 H, OH-6', $J_{\text{OH}-6'} = 7.1$, $J_{\text{OH}-6''} = 4.8$); 4.91 (t, 1 H, OH-5', $J_{\text{OH}-5'} = J_{\text{OH}-5''} = 5.8$); 4.67 (m, 1 H, H-2); 4.21 (t, 1 H, H-3', $J_{3'-\text{OH}} = J_{3'-2'} = 5.6$); 3.59 (dd, 1 H, H-6', $J_{6',6''} = 11.9$, $J_{\text{OH}-6''} = 7.2$); 3.47 (dd, 1 H, H-6'', $J_{6',6''} = 11.7$, $J_{\text{OH}-6''} = 4.8$); 3.40 (d, 2 H, H-5' and H-5'', $J_{5'-\text{OH}} = J_{5''-\text{OH}} = 6.0$). MS (ES):

m/z 320 (M + Na)⁺, 298 (M + H)⁺, 136 (BH₂)⁺. For C₁₁H₁₅N₅O₅ (297.3) calculated: 44.45% C, 5.09% H, 23.56% N; found: 44.30% C, 5.09% H, 23.23% N.

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