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Synthesis and NMR Conformational Studies of Stable Analogues of 2-Deoxy-α-D-ribose-1-Phosphate

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Abstract

Malonate ethers and phosphonate derivatives of 2-deoxyribose and 2-deoxy-2-fluoroarabinose have been synthesized, for the first time, as stable analogues of 2-deoxy-α-D-ribose-1-phosphate (**1**). In almost all the cases, the α-anomers have been obtained as the major isomers. The NMR conformational analysis performed indicate a similar conformational equilibria for the natural phosphate **1** and the here described analogues, with the exception of the glycosyl phosphonate **3α**. None of the compounds were inhibitory to purified E. coli thymidine phosphorylase at 250 μM. Also, when administered to intact CEM cells, no inhibitory effect was observed in hypoxanthine and inosine metabolising enzymes, including purine nucleoside phosphorylase.

1. Introduction

Glycosyl phosphates are crucial intermediates in the biosynthesis of nucleic acids and glycosides. However, they are easily degraded by the action of phosphatases. Due to the high biological interest associated to these molecules, the synthesis of new and stable analogues of the natural glycosyl phosphates is of great interest, since they could behave as inhibitors or regulators of carbohydrate and nucleoside processing enzymes. In addition, such compounds could also be helpful as mechanistic probes in enzymatic reactions [1].

2-Deoxy-α-D-ribose-1-phosphate (**1**) is a glycosyl phosphate involved in the biosynthesis of purines and pyrimidines 2'-deoxyribonucleosides, being a co-substrate for thymidine phosphorylase (TPase) (EC 2.4.2.4) and purine nucleoside phosphorylase (PNPase) (EC 2.4.2.1). Important therapeutic benefits have been suggested by the use of inhibitors of both enzymes. Indeed, TPase inhibitors could be of clinical benefit in the chemotherapy of solid tumors and metastasis, by inhibiting the angiogenic activity associated to TPase [2]. On the

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other hand, PNPase inhibitors might be useful as immunosuppresive agents with potential clinical utility in the treatment of human T-cell leukemia and autoimmune disorders [3]. Moreover, inhibitors of these enzymes may also be beneficial in protecting therapeutically useful nucleoside analogues, such as (*E*)-5-(2-bromovinyl)-2'-deoxyuridine [4], or 2',3'-dideoxyinosine [5], that are degraded by TPase and PNPase, respectively, to their (biologically) inactive bases.

Within this framework, and as part of our programme on the design and development of inhibitors of nucleoside processing enzymes, we have undertaken the synthesis of stable analogues, hitherto unknown, of 2-deoxyribose-1-phosphate, of structure **2-6** (Figure 1). Here we report in full detail [6] their synthesis and their inhibitory effect on several enzymes, including TPase and PNPase. We have also determined the conformational properties in solution by NMR techniques of the sugar moiety of the natural glycosyl phosphate **1** and its analogues (**2-6**).

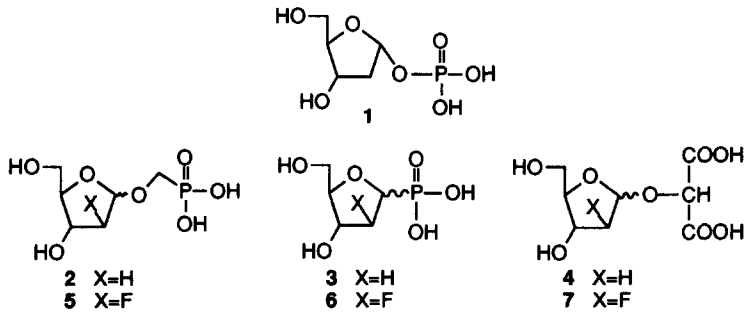


Figure 1

The most studied strategy when preparing stable analogues of the natural phosphate (O—P—O) is the synthesis of the corresponding phosphono derivatives (C—P—O), where the oxygen linkage is replaced by a carbon atom [7]. More recently, the phosphonomethoxy moiety (O—C—P—O) has been described as a better mimic of the monophosphate function [8]. It has been proposed that the electron withdrawing oxygen located β to the phosphorous atom makes the pKa value a more closely match of the phosphate [9]. This has been proven by its incorporation in acyclic [10], furanosyl [11] and pyranosyl [12] nucleosides resulting in stable analogues of the corresponding monophosphate nucleosides, compounds that are endowed with important biological and therapeutic properties [8,10]. Thus, the phosphonomethoxy moiety can be considered as a good surrogate of the monophosphate function. Therefore, it would be interesting to incorporate this moiety in 2-deoxyribose (compounds **2** and **3**). On the other hand, some recent reports described that a malonate ether moiety can effectively mimic a natural phosphate in different substrates [13-15], this replacement resulting in significant enzymatic inhibition. Thus, we considered worth testing whether this could also apply to the phosphate of **1**, and synthesized and tested the corresponding malonate ether **4**.

Besides the 2-deoxy derivatives **2-4**, we have undertaken the synthesis of the corresponding 2-fluorarabino derivatives (**5-7**), based, among other factors, on the well documented analogy between 2'-deoxyribofuranosylnucleosides and 2'-deoxy-2'-fluoro-arabinofuranosylnucleosides [16]. It has been established that the presence of a fluorine atom at position 2 increases the chemical and enzymatic stability of the glycosidic bond, without affecting the steric properties of the molecule, due to the similar Van der Waals radii of hydrogen and fluorine [16].

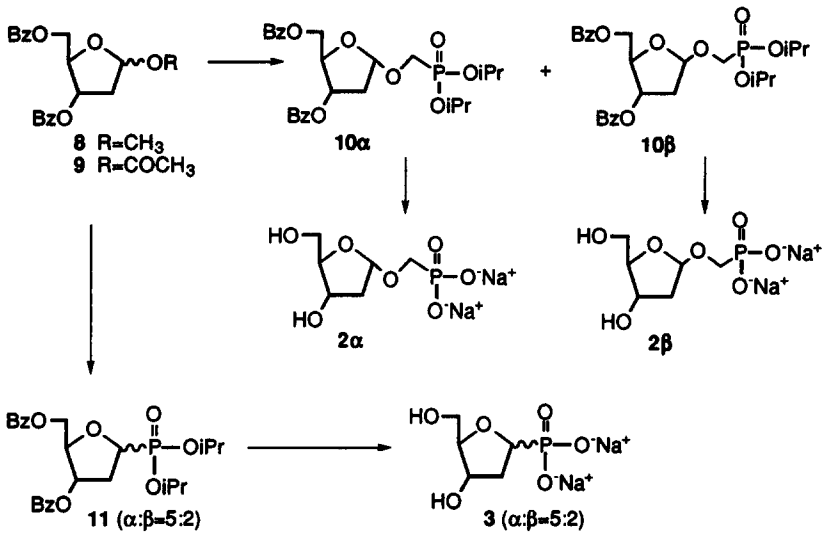
2. Results and Discussion

2.1. Synthesis

Our strategy for the synthesis of compounds **2**, **3** and **4** has been based on Lewis-acid promoted reaction of glycosyl donors of 2-deoxyribose (**8** or **9**) with the appropriate aglycone, followed by deprotection under smooth conditions. This procedure has allowed us to synthesize the proposed structures in 3 to 5 steps from commercially available 2-deoxyribose. Since the natural phosphate **1** shows the α-anomeric configuration, special attention has been paid in the glycosylation reaction to those conditions that lead preferentially to α-glycosides. In this respect, it has been reported that in glycosylation of 5-*O*-acyl-2-deoxyribose donors, acetonitrile in the solvent of choice to increase the ratio of the α-glycosides [17].

Thus, reaction of the 1-*O*-methyl derivative (**8**) [18] (Scheme 1) with diisopropyl hydroxymethylphosphonate [19] in dry acetonitrile in the presence of trimethylsilyltriflate (TMSOTf) as catalyst afforded the coupling derivates **10α** and **10β** in a 2:1 ratio (76% yield), that were separated by flash column chromatography. Deprotection of **10α** and **10β** was carried out in two steps: treatment with bromotrimethylsilane, in the presence of 2,6-lutidine [20] (deprotection of the phosphonate esters), followed by reaction with saturated methanolic ammonia (removal of the benzoyl esters) afforded compounds **2α** and **2β** that were isolated as their corresponding disodium salts by eluting through a Dowex 50WX4 (Na^+ form) (43 and 48%,from **10α** and **10β**, respectively).

The stereochemistry of the anomeric center was assigned as α for the major isomer and β for the minor based on NOESY experiments carried out in **10α** and **10β**. Thus, in the major isomer **10α** the signal corresponding to H-2a (δ 2.54) correlates with the signals of both H-1 and H-3, indicating that H-2a, H-1 and H-3 must be in the same side of the molecule, and therefore was assigned as the α-anomer. On the other hand, NOESY experiments in the minor isomer **10β** showed a correlation peak between H-2b (δ 2.60) and H-3, while H-2a (δ 2.35) correlates with the signal of H-1. This pattern indicates that H-1 and H-3 must be in opposite sides of the molecule, and therefore was assigned as the β-anomer. It should be mentioned that, between a couple of anomers (**10α** and **10β**, or **2α** and **2β**), the signal corresponding to H-3 in the β-anomers (**10β** and **2β**) appears downfield (0.2-0.3 ppm) when compared to this signal in the α-anomers (**10α** and **2α**). A similar pattern for these protons is observed in other structures of this series.



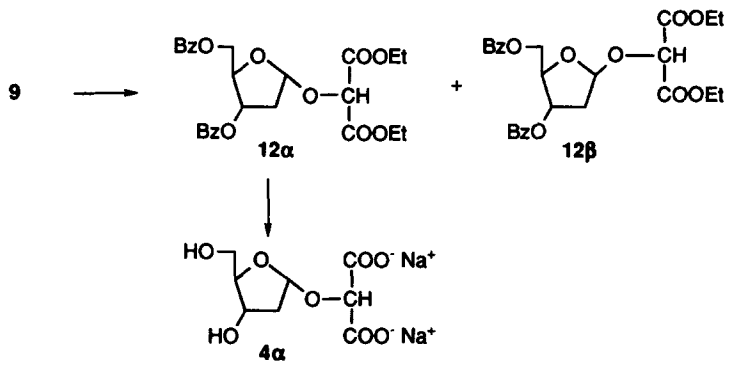
Scheme 1

The glycosyl phosphonates **3** were prepared by Lewis-acid-promoted reaction of glycosyl donors with trialkyl phosphites, as described by Vasella et al [21]. Thus, reaction of the 1-*O*-acetylderivative (**9**) [19] (Scheme 1) with triisopropylphosphite in dry acetonitrile in the presence of TMSOTf afforded the glycosyl phosphonates **11** as a mixture of α and β anomers (5:2 ratio, 70% yield) that could not be separated by chromatography. When $BF_3{\cdot}OEt_2$ was used as catalyst instead of TMSOTf, the global yield of the reaction was increased up to 86%, but the ratio of anomers remained unchanged. Deprotection of **11**, carried out in two steps as described above for compounds **10**, yielded the fully deprotected derivatives **3** (43%) as a mixture (5:2) of the α and β anomers.

Assignment of the anomeric configuration on the protected derivatives **11** was problematic, and therefore this was performed on the deprotected analogues **3**. A bidimensional COSY experiment allowed the complete assignment of the protons corresponding to each isomer. The values of the coupling constants for H2 protons in the major isomer ($J_{H1,H2a}$=7.9, $J_{H2a,H3}$=7.0, and $J_{H1,H2b}$=8.0, $J_{H2b,H3}$=6.1 Hz) suggest that both H2 protons must be *gauche* to H1 and H3. On the other hand, such coupling constants in the minor isomer ($J_{H1,H2a}$=6.6, $J_{H2a,H3}$=2.0, and $J_{H1,H2b}$=11.0, $J_{H2b,H3}$=5.6 Hz) seem to indicate that H1 is *anti* to H2b, while H3 is *gauche* to both H2. These results point to the assignment of the major isomer as the α-anomer. This was confirmed by NOE experiments.

The synthesis of the malonate derivatives **4** (Scheme 2) was performed as follows: 2-hydroxy diethylmalonate [22] was reacted with the 1-*O*-acetyl derivative (**9**) [19] in the presence of TMSiTf at -40°C to afford the *O*-glycosides **12α** and **12β** in 55% and 22% yield, respectively. Higher temperatures led to extensive decomposition of the reaction mixture. Removal of the protecting groups in compound **12α** was performed in a single step by treatment with 0.2N-NaOH in THF, followed by purification on DEAE-Sephadex A-25 ($^-HCO_3$ form) eluting with a gradient of 0-0.1M NH_4HCO_3, and then transformation

into the disodium salt. Thus, compound **4α** was obtained in 73% yield. However, when **12β** was subjected to the same procedure of deprotection and purification, extensive decomposition occured.

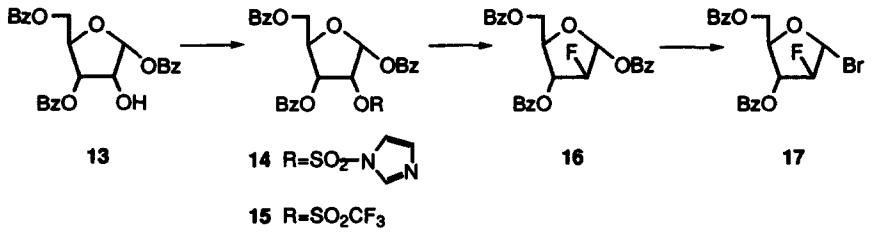


Scheme 2

The assignment of the anomeric configuration of the new compounds was performed by comparison of the chemical shifts and coupling constants observed for the sugar protons in the ^{1}H-NMR spectra of **12α** and **12β** with those observed for **10α** and **10β**, which allowed to assign the major isomer as the α-anomer. This was further confirmed by NOESY experiments.

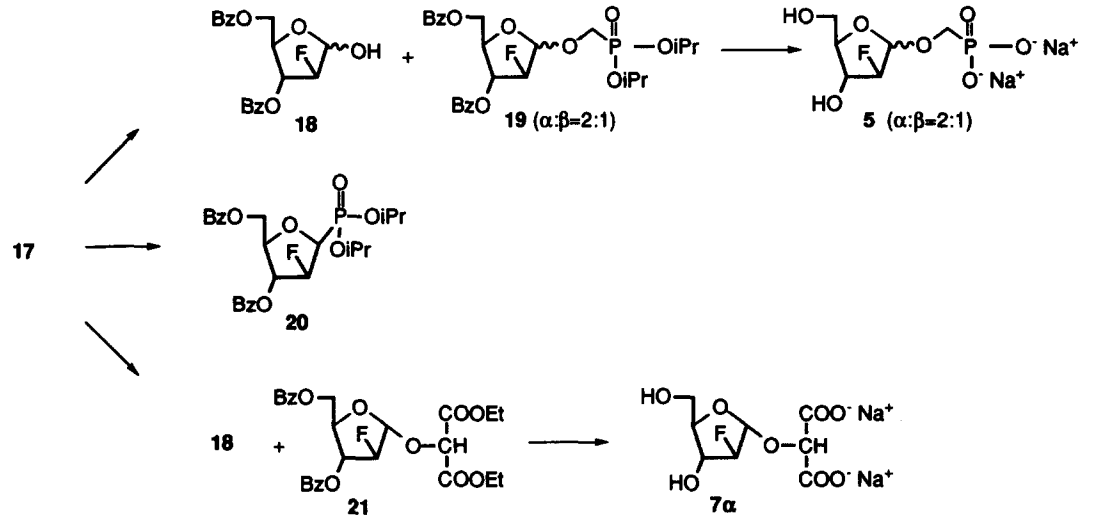
Regarding the synthesis of the corresponding series of 2-deoxy-2-fluoro-arabinofuranosylglycosides (**5-7**) (Figure 1), the common intermediate is 3,5-bis-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**17**) [23]. Again, special attention was paid to the preparation of the α-anomers, that show the same stereochemistry that the natural substrate (**1**). It has also been reported that in the glycosidation reaction of the bromide **17** the solvent can strongly affect the ratio of anomers [24]. Thus, low dielectric constants solvents (CCl_4) would favour the S_N2 products (β-anomers) while high dielectric constants solvents, such as acetonitrile, should favour the formation of the α-anomers.

The key step in the synthesis of the bromide **17** (Scheme 3) is the introduction of the fluorine atom at position 2. This was performed following a recently described non-corrosive method which uses $Et_3N{\cdot}3HF$ [25] as fluorinating agent to displace a good leaving group at position 2 such as the imidazosulfonyl group (**14**) [23]. We have used as leaving groups at position 2 both the imidazosulfonyl (**14**) and the triflate (**15**) [23,26]. Although this triflate has been reported to be unstable [26], in our hands it was stable enough to be isolated and also under the reaction conditions. Thus, reaction of the triflate **15** with $Et_3N{\cdot}3HF$ afforded the 2-fluorosugar **16** in 85% yield from 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (**13**). Bromination as described gave exclusively the α-anomer (**17**) in quantitave yield [23].



Scheme 3

Reaction of the 1-α-bromosugar (**17**) (Scheme 4) with diisopropylhydroxymethylphosphonate [19] in the presence of $AgClO_4$ as catalyst afforded very low yields of the coupling products (**19**), the major product being the hydrolysis derivative (**18**). We then tried different catalysts (TMSiTf, $BF_3.OEt_2$, $SnCl_4$, $AgClO_4/Ag_2CO_3$) in the presence of different drying agents (drierite, molecular sieves...). The combination that afforded better yields of the corresponding *O*-glycosides (**19**) was $SnCl_4$/4Å molecular sieves in refluxing acetonitrile (42% yield, α/β ratio: 2/1). Deprotection of **19** performed in a similar way to that described for compounds **10** afforded compounds **5** as a mixture of α/β anomers (2:1 ratio, 39% yield).



Scheme 4

The assigment of the anomeric configuration was performed by ^{1}H-NMR experiments on the deprotected derivatives **5**. A COSY experiment allowed the complete assignment of the sugar protons corresponding to both anomers and the determination of their coupling constants (^{1}H-^{1}H and ^{1}H-^{19}F). For the major isomer, the signal corresponding to H1 is a doblet with a $J_{H1,F}$=10.6 Hz, while no coupling was observed between H1 and H2. This strongly suggests that H1 is *cis* to the fluorine atom at position 2, and *trans* to the vicinal H2,

and this is in good agreement with the Jvalues reported for other α-anomers in the literature [27]. On the other hand, the signal corresponding to H1 for the minor isomer is also a doblet with a smaller coupling constant value ($J_{H1,H2}$=4.4 Hz) and almost no coupling with the fluorine ($J_{H1,F}$≈0 Hz). This seems to indicate that in the minor isomer H1 is located *trans* to the fluorine atom at 2 with a dihedral angle close to 90°, and *cis* to the vicinal H2. This is in agreement with a β-configuration. However it should be noted that this $^3J_{H1,F}$ value does not agree with values reported in the literature for a *trans* relationship between ^1H and ^{19}F, that show $^3J_{H,F}$ values in the range 15-30 Hz [27]. This point will be discussed in more detail in the NMR conformational analysis section.

The synthesis of the glycosyl phosphonates **6** (Figure 1) was attempted by reaction of the 1-bromosugar (**17**) with triisopropylphosphite in acetonitrile and in the presence of $SnCl_4$ or TMSTf and 4Å molecular sieves. However, under these reaction conditions, only extensive decomposition was observed. We then tried an Arbuzov-type reaction between the bromo derivative (**17**) and $P(OiPr)_3$ in toluene at 140°C, which afforded a complex reaction mixture, from where only the β-coupling derivative (**20**) could be isolated in 28% yield. The β-anomeric configuration of **20** was established from the observed coupling constants $J_{H,H}$ and $J_{H,F}$. To discriminate between $^3J_{H1,F}$ and $^3J_{H1,P}$, a ^{19}F-NMR spectrum was recorded. The observed $^3J_{H1,F}$=37 Hz suggests a *trans* disposition between H1 and the fluorine atom at 2, which is in agreement with a β-configuration. This was unequivocally confirmed by NOESY experiments.

The exclusive isolation of the β-anomer (**20**) from the 1-α-bromo sugar seems to indicate that the reaction of **17** with $P(OiPr)_3$ proceeds mostly through a S_N2 type reaction with inversion at the configuration of the stereogenic center C-1. Therefore, we reasoned that by inverting the configuration of the starting bromide **17** from α to β we should obtain the corresponding α-glycosyl phosphonate under the same reaction conditions. To attain this goal, we added tetrabutylammonium bromide to the reaction medium to favour the anomerization "in situ" of **17** as described by Lemieux for α-pyranosyl bromides [28]. However, when this strategy was applied to the bromo sugar **17**, only the β-anomer **20** was isolated in a similar poor yield than in the absence of the phase transfer catalyst.

Finally, treatment of the bromo sugar **17** with diethyl α-hydroxymalonate [22] in acetonitrile at 40°C and in the presence of $SnCl_4$ and 4Å molecular sieves afforded the coupling derivative **21** in 63% yield together with the hydrolysis product **18**. In this case the β-anomer could not be detected. The α-anomeric configuration of **21** was established by comparison of its coupling constant values ($J_{H1,F}$, $J_{H1,H2}$, $J_{H2,H3}$) with those of other α-anomers in this series. Deprotection of **21** was carried out in a single step as described for **4α**, to give **7α** in 70% yield that was isolated as the disodium salt.

2.2. *NMR Conformational Studies*

2.2.1. *Comparative studies of the conformation of the furan ring in* ***1, 2α, 3α, 4α, 5α and 7α.***

NMR studies provide insight into the conformational dynamics in solution. We have performed a conformational analysis of the furanose ring of the α-glycosides here described

(**2α**, **3α**, **4α**, **5α** and **7α**) in comparison with the natural substrate, 2-deoxy-α-D-ribose-1-phosphate (**1**). For such purpose, we have used the PSEUROT method developed by Altona and co-workers, based on the experimental values $^3J_{H,H}$ [29]. The method assumes the existence of an equilibrium of two possible interconverting conformers N (north) and S (south). Since each conformer is characterized by a value of P (phase of pseudorotation) and τ (puckering amplitude), the equilibrium is described by the values of P and τ for each conformer (P_N, P_S, τ_N, τ_S) and the mole fraction (χ_N), which represents the relative population of the N conformer. The experimental data ($^3J_{H,H}$) together with the pseudorotational parameters obtained from PSEUROT for the different α-glycosides, are collected in Table 1.

For the natural phosphate **1** it was found that PSEUROT converges towards a North-type conformation with a P=64.6° and τ=21.3°, corresponding to a ^0_4T conformation, and a South-type conformation with a P=157.7° and τ=29.4°, which corresponds to a ^2E conformation, both conformers almost equally contributing to the equilibrium (χ_N=0.47). A PLUTO representation of this conformational equilibrium for compound **1** is shown in Figure 2.

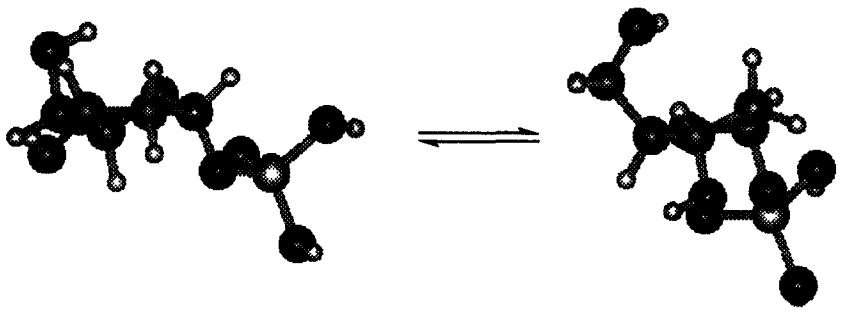
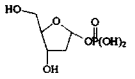
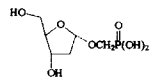
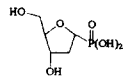
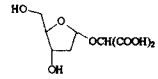
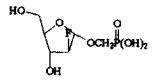
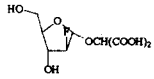


Figure 2

When comparing the data obtained for the 2-deoxy *O*-glycosides **2α** and **4α** with those of the natural phosphate **1**, no significant differences are observed. The phase angles P_N and P_S values obtained for these two analogues are grossly similar to those of compound **1** (86°<P_N<98° and 131°<P_S<142°). Also, no major differences were observed in the mole fraction (χ_N= 0.47-0.62). However, the situation changes when comparing the values of P_N and P_S obtained for the glycosyl phosphonate **3α** with those of compound **1**. For compound **3α** PSEUROT converges towards a North conformation (P=357.6°) corresponding to a ^3_2T conformation, and a South conformation (P=220.3°) corresponding to a ^4_3T conformation, the North conformation being predominant. The values obtained for **3α** are clearly distinct and far away in the pseudorotational circuit from the values obtained for the phosphate **1**.

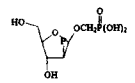
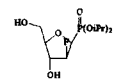
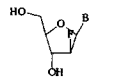
In that concerning the 2-fluoroarabinosides **5α** and **7α**, both compounds show a very similar conformation with phase angle values in the intervals 50°<P_N<60° and 114°<P_S<129°. There are some differences in the mole fraction (χ_N= 0.28-0.42), but in both

Table 1. Pseudorotational parameters determined from PSEUROT method for compounds **1-7α**.

Compound		$J_{H1,H2a}$ [a]	$J_{H1,H2b}$ [a]	$J_{H2a,H3}$ [a]	$J_{H2b,H3}$ [a]	$J_{H3,H4}$ [a]	P_N	τ_N	P_S	τ_S	X_N	rms
	**1**	6.4 (6.4)	1.5 (1.4)	7.4 (7.4)	2.5 (2.6)	3.6 (3.6)	64.6	21.3	157.7	29.4	0.47	0.05
	**2α**	5.2 (54)	1.0 (1.1)	7.5 (7.3)	2.1 (1.9)	3.6 (3.7)	97.7	25.9	141.2	46.5	0.62	0.21
	**3α**	8.0 (7.8)	7.9 (7.9)	6.1 (6.2)	7.0 (6.9)	5.2 (5.4)	357.6	38.5	220.3	40.0	0.63	0.15
	**4α**	5.6 (5.9)	1.1 (1.2)	7.9 (7.6)	2.1 (1.9)	3.5 (3.6)	86.4	17.0	131.4	37.6	0.48	0.22
	**5α**	1.2 (1.2)		2.1 (2.1)		5.5 (5.5)	59.1	40.0[b]	128.5	40.0[b]	0.42	0.01
	**7α**	1.2 (1.2)		1.6 (1.6)		5.9 (5.9)	50.8	40.0[b]	114.0	40.0[b]	0.28	0.01

[a] Experimental coupling constants in Hz (in parentheses values obtained from PSEUROT calculations). [b] Constrained values.

Table 2. Pseudorotational parameters determined from PSEUROT method for compounds **5β**, **20** and **22**.

Compound		$J_{H1,H2}$ [a]	$J_{H2,H3}$ [a]	$J_{H3,H4}$ [a]	P_N	τ_N	P_S	τ_S	X_N	rms
	**5β**	4.4 (4.6)	6.8 (6.8)	6.8 (6.8)	347.1	40[b]	142.4	40[b]	0.8	0.09
	**20**	3.1 (3.6)	0.0 (0.1)	3.0 (3.5)	-	-	123.8	40[b]	0.0	0.56
	**22**	4 - 5[c] (5.9)	4[c] (4.8)	6[c] (5.4)	336.8	25.4	-	-	1.0	1.00

[a] Experimental coupling constants in Hz (in parentheses values obtained from PSEUROT calculations). [b] Constrained values. [c] Experimental coupling constants obtained from ref 26.

Table 3. Calculated dihedral angles and vicinal coupling constants (Hz) for compounds **5β**, **20** and **22**.

Compound	**5β**			**20**			**22**		
Dihedral angle	$\phi_{PSEUROT}$ [a]	ϕ_{MM} [b]	^3J [c]	$\phi_{PSEUROT}$ [a]	ϕ_{MM} [b]	^3J [c]	$\phi_{PSEUROT}$ [a]	ϕ_{MM} [b]	^3J [c]
H1,H2	-33.5	-37.5	4.4	45.6	53.2	3.1	-18.8	-24.0	4-5
H1,F	90.0		~0	169.0		37.0	105.0		11
H2,H3	160.2	162.7	6.8	84.8	91.0	~0	146.7	145.6	4
F,H3	36.9		16.8	-36.8		15.0	24.2		20
H3,H4	-157.6	-153.7	6.8	-109.8	-127.0	3.0	-146.1	-	6

[a] Obtained from PSEUROT calculations. [b] Obtained from molecular mechanics calculations. [c] Experimental coupling constants obtained from ref 26.

cases the N-conformers predominate. Again these conformations are close in the pseudorotational circuit to those obtained for the phosphate **1**.

Therefore, it can be concluded that, with the exception of the glycosyl phosphonate **3α**, no important differences for the furanose conformational preferences in solution are observed between the natural phosphate **1** and the here described analogues.

2.2.2. Comparative studies of the conformation of the furan ring in 5β , 20 and 22

As mentioned before, the 2-fluoroarabinofuranoside **5β** showed a value for the coupling constant $^3J_{H1,F}\approx 0$ Hz, that is unusual for a *trans* relationship between ^{19}F and ^1H. According to the literature, typical values for such a *trans* relationship are in the range of 15-30 Hz [27]. On the other hand, the 2-fluoro-β-D-arabinofuranosyl phosphonate **20** showed a completely different value for this coupling constant ($^3J_{H1,F}$=37 Hz). A third example taken from the literature, 1-(2-fluor-2-deoxyarabinofuranosyl)-5-trifluoromethyl-uracil (**22**), shows an intermediate value for this coupling constant ($^3J_{H1,F}$=11 Hz). From all these data it is clear that among 2-deoxy-2-fluoro-β-D-arabinofuranosyl glycosides, the values observed for $^3J_{H1,F}$ can vary, at least between 0-37 Hz. Although it has been suggested that a fluorine atom at position 2 of a furanose can drive the sugar conformation, the different values just mentioned seem to indicate that this effect is not the only one determining the conformation in 2-deoxy-2-fluoro-β-D-arabinofuranosyl glycosides. From a structural point of view, the major differences among the three derivatives **5β**, **20** and **22** is the nature of the substituent at the anomeric position. Thus we considered of interest to perform a comparative study of the conformation of the three compounds that could allow us to determine the geometry of the sugar ring and the corresponding dihedral angles. From these data we could establish if the differences observed in the experimental data ($^3J_{H1,F}$) really account for differences in the values of the dihedral angles, and therefore differences in the sugar conformation. The data obtained could be helpful in gaining further insights into the forces (the presence of the fluorine atom at position 2 and/or the nature of the substituent at the anomeric position, among others) that drive the sugar conformation in 2-deoxy-2-fluoro-β-D-arabinofuranosyl glycosides.

We first performed a conformational analysis of the sugar conformation in solution of the above mentioned derivatives (**5β** , **20** and **22**) using PSEUROT from the ^1H-^1H coupling constants (Table 2). Compound **5β** showed a strong preference for a North-type conformation (P_N=347.1°, χ_N=0.8), conformation very close to a C-3'endo. However the data obtained for compound **20** indicate that the south conformers are highly favoured with a preferential conformation $_1E$ (P_S=123.8°). Finally, compound **22** was found to be in a "single" N-type conformation (P_N=336.8°).

It should be noted that PSEUROT uses exclusively the ^1H-^1H coupling constants. So it was important to determine if the experimental ^1H-^{19}F coupling constants were compatible with these conformational equilibria. Based on the P and τ values obtained, the molecular models of the three major conformers were constructed and these conformations were minimized by molecular mechanics (see Experimental section). The minima obtained showed the $\phi_{H,H}$ and $\phi_{H,F}$ values indicated in Table 3. The $\phi_{H,F}$ values obtained were graphically represented

versus the experimental $^3J_{H,F}$ (both with H-1 and H-3 of the sugar), showing a typical Karplus relationship, similar to the one described by Williamson and co-workers [30].

From these results we can conclude that among 2-deoxy-2-fluoro-β-D-arabinofuranosyl glycosides and taken into account the two factors studied, that is, the presence of the fluorine atom at position 2 or the nature of the substituent at the anomeric position, the later is the one that controls the sugar conformation, leading to almost opposite conformations as shown when comparing compounds **20** and **22**. Since the nature of the substituent at the anomeric position can strongly influence the sugar conformation and therefore the experimental value of the coupling constant $^3J_{H1,F}$, it is highly risky to assign the anomeric configuration relying exclusively on the values of this coupling constant.

2.3. Enzymatic evaluation

Compounds **2α**, **3α**, **4α**, **5α** and **7α** have been tested for their inhibitory effect on thymidine phosphorylase (TPase) from *E. coli* by evaluating the release of free thymine from thymidine in function of time (5', 10', 20' and 60'). The formation of thymine was measured by HPLC and spectrophotometric analysis. No significant inhibition of TPase was detected at compound concentrations up to 250 μM.

The compounds have also been tested for their inhibitory effect on tritium release from [2,8-^{3}H]inosine in intact CEM cells. In this assay, tritium is released from [2,8-^{3}H]inosine upon the subsequent conversions of inosine to [2,8-^{3}H]hypoxanthine by Purine Nucleoside Phosphorylase (PNPase), [2,8-^{3}H]hypoxanthine to [2,8-^{3}H]inosinate by Hypoxanthine-Guanine Phosphoribosyl transferase (HG-PRTase), and [2,8-^{3}H]inosinate to [8-^{3}H]xantosine-5'-monophosphate by Inosinate Dehydrogenase (IMP-D). No inhibitory activity was detected. However, it cannot be excluded that the test compounds could not be efficiently taken up by the intact cells. Still, when the HG-PRTase activity was measured in a cell free assay, using hypoxanthine as the radiolabelled substrate, no significant effect was detected.

3. Experimental

Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. Analytical TLC was performed on silica gel 60 F_{254} (Merck). Separations on silica gel were performed by preparative centrifugal circular thin layer chromatography (CCTLC) on a Chromatotron (Kiesegel 60 PF 254 gipshaltig (Merck), layer thickness (1mm or 2mm), flow rate (4 or 8 mL/min). Flash column chromatography was performed with silica gel 60 (230-400 mesh) (Merck). Sephadex A-25 ($HCO3^-$ form) was used for ion exchange chromatography. Analytical HPLC was performed in Waters Novapak C_{18} column using 65% CH_3CN and 35% H_2O, with UV detection at 254 nm and a flow rate of 1 mL/min. Liquid secondary mass spectra (LSIMS) were obtained in a Kratos Concept 1H mass spectrometer, using glycerol (GLY) or thioglycerol (THYGLY) as matrix.

NMR: Materials and methods.

^1H and ^{13}C-NMR spectra were recorded in a Varian INOVA-400 spectrometer operating at 400 and 100 MHz, respectively. Monodimensional spectra were obtained in standard conditions. Homonuclear 2D spectra (COSY and NOESY) were acquired in the phase sensitive mode. Data were collected in a 2048 x 512 matrix with a spectral width of 4300 Hz and 1.0 s of relaxation delay and then processed in a 2048 x 1024 matrix. The NOESY spectra were generated with a mixing time of 0.5 s.

For the conformational analysis, samples were dissolved in D_2O and the spectra recorded at 30°C, with presaturation of the water signal. 2-Deoxy-α-D-ribose-1-phosphate di-(monocyclohexylammonium)salt (**1**) was purchased from SIGMA. Spectra were simulated by using PANIC-86 [31] in order to obtain more accurate values of the coupling constants. Pseudorotational parameters were calculated from the experimental coupling constants by the programme PSEUROT 6.2 [29] using an improved generalized equation as modified by Donders et al. [32]. The geometries were optimized by the MM+ method as implemented in the programme HYPERCHEM [33].

Enzymatic evaluation: Materials and methods.

***E. coli* thymidine phosphorylase assay.** The inhibitory activity of the test compounds was evaluated against *E. coli* thymidine phosphorylase (Sigma Chemical Company, St. Louis, MO). The reaction mixture contained 50 µl test compound (10 mM in buffer) or buffer (control) (Tris.HCl 10 mM pH 7.6; potassium phosphate 2 mM pH 7.6; EDTA 1 mM and NaCl 150 mM), 50 µl α-deoxyribose-1-phosphate (1 mM), 100 µl thymine (500 µM), 12.5 µl enzyme (0.025 units) and buffer to reach a total volume of 500 µl. The reaction was started at room temperature upon addition of thymine, and 100 µl-samples were withdrawn at 0, 5, 10, 20 and 60 min after initiation of the reaction, heated at 95°C for 3 min, cooled on ice and analysed on a reversed phase C-8 column by HPLC. The gradient solvents consisted of potassium phosphate 10 mM 5.5 (buffer A) and potassium phosphate 10 mM pH 5.5 + methanol 80% (buffer B). The gradient started with 5% buffer A and 95% buffer B for 10 min, followed by a linear gradient during 5 min to 20% buffer A and 80% buffer B; a linear gradient during 5 min to 5% buffer A and 95% buffer B, and isocratic equilibration for 5 min at the same buffer ratios. The reaction [conversion of thymine (Rf range 3.56-3.74 min) to thymidine (Rf range 5.93-6.56 min)] was followed as a function of time at 267 nm.

Tritium release from [2,8-^{3}H]inosine in intact CEM cells. The inhibitory activity of the test compounds was evaluated against tritium release from [2,8-^{3}H]inosine in intact human lymphocyte CEM cells. The assay was performed as described previously [34]. Briefly, CEM cells were suspended at 2.5 x 10^6 cells/ml; 300 µl of this cell suspension was mixed with 60 µl RPMI-1640 medium and 40 µl (4 µCi) of radiolabelled [2,8-^{3}H]Ino (specific radioactivity: 25 Ci/mmol) (Moravek Pharmaceuticals, Brea, CA). At various times (0, 5, 10, 20 and 30 min), 100 µl of the reaction mixture was withdrawn and mixed with 250 µl of carbon black (UCB; Brussels, Belgium) (100 mg/ml) in 5% trichloroacetic acid (TCA). After centrigugation, 200 µl samples of the supernatants were analysed for radioactivity.

HG-PRTase activity assay. Murine leukemia L1210 cell extracts served as the source for HG-PRT. The reaction system (100 µl) consisted of 70 µl of a buffer mixture [120 µl (500 mM Tris.HCl pH 7.8, 5 mM $MgCl_2$, 20 mM DTT), 60 µl hypoxanthine 2 mM, 540 µl aqua distillata, and 120 µl [G-^{3}H]hypoxanthine (1.2 µCi)], 10 µl PRPP and 20 µl enzyme extract. The assay was started with the addition of enzyme, and incubation was for 30 min at 37°C, after which 200 µl methanol was added. The mixture was then kept on ice for 10 min, centrifuged at 13,000 rpm for 5 min, after which 250 µl of the supernatant was analysed on an anion exchange partisphere SAX column (Whatman, Clifton, NJ). The buffer were 5 mM $NH_4H_2PO_4$ pH 5.0 (buffer A) and 0.3 M $NH_4H_2PO_4$ pH 5.0 (buffer B). Following gradient was used: 5 min buffer A; 15 min linear gradient to 100% buffer B; 20 min isocratic 100% buffer B; 5 min linear gradient to 100% buffer A; 4 min equilibration with buffer A. The amount of radiolabelled IMP formed during the reaction was used to estimate the enzyme activity.

(Diisopropylphosphonyl)methyl 3,5-bis-*O*-benzoyl-2-deoxy-α- and -β-D-*erythro*-pentofuranoside (10α and 10β). To a solution of **8** [18] (1.30 g, 3.6 mmol) and diisopropylhydroxymethyl phosphonate [18] (2.13 g, 10.9 mmol) in dry acetonitrile (10 mL) at 0°C, TMSOTf (1.18 mL, 6.12 mmol) was added. After 4h, 200mL of CH_2Cl_2 and 50mL of $NaHCO_3$ solution were added. The organic phase was washed with water, brine, dried (Na_2SO_4), filtered and evaporated. The residue obtained was purified by flash column chromatography [CH_2Cl_2:acetone (20:1)]. The fastest moving fractions afforded 0.47 g (25%) of **10β** as a syrup. $[\alpha]_D$ -26.7 (*c* 1, methanol). ^{1}H-NMR ($CDCl_3$) δ 1.33 (m, 12H, CH_3), 2.38 (m, 1H, H-2a), 2.65 (m, 1H, H-2b), 3.85 (m, 2H, OCH_2P), 4.52 (m, 3H, H-4, H-5), 4.74 [m, 2H, $OCH(CH_3)_2$], 5.45 (dd, $J_{H1,H2a}$=3.3, $J_{H1,H2b}$=1.2 Hz, 1H, H-1), 5.61 (m, 1H, H-3), 7.35-8.20 (m, 10H, 2 Ph). ^{13}C-NMR ($CDCl_3$) δ 23.89, 23.98 [$OCH(CH_3)_2$], 39.12 (C-2), 61.67 (d, $J_{C,P}$=168 Hz, OCH_2P), 65.25 (C-5), 71.11 [d, $J_{C,P}$=6 Hz, $OCH(CH_3)_2$], 75.41 (C-3), 82.10 (C-4), 105.37 (d, $J_{C,P}$=11 Hz, C-1), 128.38 -133.29 (Ph), 165.95, 166.07 (C=O). Anal. Calcd. for $C_{26}H_{33}O_9P$: C, 60.00; H, 6.39. Found: C, 59.98; H, 6.53. The slowest moving fractions afforded compound **10α** (0.95g, 51% yield) that was isolated as a syrup. $[\alpha]_D$ +63.7 (*c* 1, methanol). ^{1}H-NMR ($CDCl_3$) δ 1.32 (m, 12 H, CH_3), 2.31 (dd, $J_{H2a,H2b}$=14.6, $J_{H2a,H3}$=1.5 Hz, 1H, H-2a), 2.54 (m, 1H, H2b), 3.99 (m, 2H, OCH_2P), 4.60 (m, 3H, H-4, H-5), 4.74 [m, 2H, $OCH(CH_3)_2$], 5.34 (d, $J_{H1,H2b}$=5.1 Hz, 1H, H-1), 5.45 (m, 1H, H-3), 7.35-7.81 (m, 10H, 2 Ph). ^{13}C-NMR ($CDCl_3$) δ 24.00, 24.07 [$OCH(CH_3)_2$], 39.22 (C-2), 61.70 (d, $J_{C,P}$=169 Hz, OCH_2P), 64.22 (C-5), 71.02 [d, $J_{C,P}$=6 Hz, $OCH(CH_3)_2$], 74.61 (C-3), 81.57 (C-4), 104.92 (d, $J_{C,P}$=12 Hz, C-1), 128.70-133.24 (Ph), 166.14, 166.40 (C=O). Anal. Calcd. for $C_{26}H_{33}O_9P$: C, 60.00; H, 6.39. Found: C, 60.25; H, 6.58.

Phosphonomethyl 2-deoxy-α-D-*erythro*-pentofuranoside disodium salt (2α). A solution of **10α** (0.21 g, 0.41 mmol) and 2,6-lutidine (0.71 ml, 6.15 mmol) in dry acetonitrile was treated with trimethylsilyl bromide (TMSBr) (0.62 g, 4.1 mmol) and the resulting mixture was stirred at rt for 24h. Then 3 mL of MeOH were added, and volatiles were removed. The residue obtained was treated with acetone (10 mL), filtered and the filtrate was purified by flash column chromatography [iPrOH:NH_4OH:H_2O, (8:1:1)]. Appropiate fractions were evaporated and coevaporated with methanol. The residue was

then treated with saturated methanolic ammonia for 48h. Volatiles were removed and the residue was treated with CH_2Cl_2:methanol to yield a white solid. This was transformed into the disodium salt by applying to a Dowex50WX4 (Na^+ form), eluting with water. Appropriate fractions were lyophilised to yield 0.043 g of **2α** (43% from **10α**). $[\alpha]_D$+69.4 (*c* 1, H_2O). ^{1}H-NMR (D_2O) δ 1.85 (dd, $J_{H2a,H2b}$=14.9, $J_{H2a,H3}$=1.3 Hz, 1H, H2a), 2.18 (ddd, $J_{H1,H2b}$=5.1, $J_{H2b,H3}$=7.7 Hz, 1H, H2b), 3.37 (dd, J=9.9, J=13.0 Hz, 1H, OCH_2P), 3.46-3.61 (m, $J_{H4,H5a}$=5.1, $J_{H4,H5b}$=3.6, $J_{H5a,H5b}$=12.3 Hz, 2H, H5), 3.70 (dd, 1H, OCH_2Ph), 3.97 (m, $J_{H3,H4}$=5.5 Hz, 1H, H4), 4.09 (m, 1H, H3), 5.11 (d, 1H, H1). ^{13}C-NMR (D_2O) δ 43.40 (C-2), 64.44 (C-5), 66.38 (d, $J_{C,P}$=156 Hz, OCH_2P), 74.00 (C-3), 88.80 (C-4), 108.50 (d, $J_{C,P}$=12 Hz, C-1). MS (THGLY) 227.1 $(M-H)^-$, 249.1 $(M-2H+Na)^-$. HRMS (GLY-THGLY) calc. for $C_6H_{12}O_7P$ 227.0320 $(M-H)^-$, found 227.0313.

Phosphonomethyl 2-deoxy-β-D-*erythro*-pentofuranoside disodium salt (2β). Compound **10β** (0.24 g, 0.46 mmol) was deprotected in a similar way to that described for **10α**, yielding 0.058 g (48%) of a white lyophilate that was identified as **2β**. $[\alpha]_D$-36.5 (*c* 1, H_2O). ^{1}H-NMR (D_2O) δ 2.11 (ddd, $J_{H2a,H2b}$=14.2, $J_{H1,H2a}$=5.2, $J_{H2a,H3}$=5.2 Hz, 1H, H2a), 2.25 (ddd, $J_{H1,H2b}$=2.2, $J_{H2b,H3}$=6.6 Hz, 1H, H2b), 3.46-3.72 (m, $J_{H4,H5a}$=4.0, $J_{H4,H5b}$=6.8 Hz, 4H, 2H5, OCH_2P), 3.91 (ddd, $J_{H3,H4}$=4.0 Hz,1H, H4), 4.31 (ddd, 1H, H3), 5.23 (dd, 1H, H1).^{13}C-NMR (D_2O) δ 43.31 (C-2), 65.63 (C-5), 66.68 (d, $J_{C,P}$=156 Hz, OCH_2P), 74.05 (C-3), 89.33 (C-4), 108.48 (d, $J_{C,P}$=12 Hz, C-1). MS (THGLY) 227. HRMS (THGLY) calcd. for $C_6H_{12}O_7P$ $(M-H)^-$ 227.0320, found 227.0310.

Diisopropyl (3,5-bis-*O*-benzoyl-2-deoxy-D-*erythro*-pentofuranoside) phosphonate (11). To a solution of **9** [18] (0.39 g, 1.01 mmol) and triisoproplyphosphite (0.96 g, 4.64 mmol) in dry acetonitrile (4 mL), $BF_3.OEt_2$ (0.35 g, 2.5 mmol) was added. After 2h, CH_2Cl_2 (200 mL) and a cold solution of $NaHCO_3$ (50 mL) were added. The organic phase was washed with water, brine, dried on Na_2SO_4, filtered and evaporated. The residue thus obtained was purified by flash column chromatography [CH_2Cl_2:acetone (15:1)]. The appropiate fractions were further purified by CCTLC on the Cromatotron [hexane:ethyl acetate (1:2)] to yield 0.43 g (88%) of **11**, as a mixture of α:β isomers (5:2 ratio). ^{1}H-NMR (Benzene-d_6) δ 1.34 [m, 12H, $OCH(CH_3)_2$], 2.10-2.58 (m, 2H, H-2), 4.19 (m, H-1α), 4.48-4.59 (m, H-5, H-1β, H-4β), 4.62-4.90 [m, $OCH(CH_3)_2$, H-4α], 5.28 (m, H-3α), 5.44 (d, J=7.6 Hz, H-3β), 6.96-8.30 (m, 10H, 2 Ph). ^{13}C-NMR (Benzene d_6) δ 24.06 [$OCH(CH_3)_2$], 33.88 (C-2α), 34.22 (C-2β), 64.09 (C-5α), 64.65 (C-5β), 70.46, 71.36 [d, $J_{C,P}$=7 Hz, $OCH(CH_3)_2$], 73.82 (d, J=168 Hz, C-1α), 74.20 (d, J=175.5 Hz, C-1β), 75.61 (d, J=1.5 Hz, C-3α), 76.90 (d, J=10 Hz, C-3β), 82.20 (d, J=6.5 Hz, C-4α), 83.96 (d, J= 9.5 Hz, C-4β), 129.87-133.12 (Ph), 165.58, 165.91, 166.04, 166.11 (C=O). Anal. Calcd. for $C_{25}H_{31}O_8P$: C, 61.22; H, 6.37. Found: C, 61.05; H, 6.67.

2-Deoxy-D-*erythro*-pentofuranosyl phosphonate disodium salt (3). Compound **11** (0.42 g, 0.85 mmol) was deprotected following a similar procedure to that describe for **10α**, to yield 0.037 g (43%) of **3** as a white lyophilate, consisting of a mixture of α:β anomers (5:2 ratio). ^{1}H-NMR (D_2O) δ 1.96 (m, $J_{H1,H2a}$=8.0, $J_{H2a,H3}$=6.1 Hz, 1H, H-2α), 2.07 (m, $J_{H1,H2a}$=6.6, $J_{H1,H2b}$=11.0, $J_{H2a,H3}$=5.6, $J_{H2b,H3}$=2.0 Hz, 2H, 2H-2β), 2.41 (m, $J_{H1,H2b}$=7.9, $J_{H2b,H3}$=7.0 Hz, 1H, H-2α), 3.45 (dd, $J_{H4,H5a}$=5.6, $J_{H5a,H5b}$=12.2 Hz, 1H, H-5β), 3.49 (dd,

$J_{H4,H5a}$=5.8, $J_{H5a,H5b}$=12.2 Hz, 1H, H-5α), 3.53 (dd, $J_{H4,H5b}$=4.1 Hz, 1H, H-5β), 3.59 (dd, $J_{H4,H5b}$=3,6 Hz, 1H, H-5α), 3.83 (ddd, $J_{H3,H4}$=5.2 Hz, 1H, H-4α), 3.85 (ddd, $J_{H3,H4}$=2.3 Hz, 1H, H-4β), 4.05 (dd, 1H, H-1α), 4.09 (dd, 1H, H-1β), 4.13 (ddd, 1H, H-3α), 4.24 (ddd, 1H, H-3β). ^{13}C-NMR (D_2O) δ 36.42 (C-2α), 37.10 (C-2β), 62.26 (C-5α), 63.25 (C-5β), 73.00 (d, J=6 Hz, C-3α), 73.51 (d, J=9 Hz, C-3β), 75.39 (d, J=150 Hz, C-1α) 75.55 (d, J=138 Hz, C-1β), 86.60 (d, J=4 Hz, C-4α), 88.9 (C-4β). MS (THGLY) 197.0 $(M-H)^-$. HRMS (GLY) calc. for $C_5H_{10}O_6P$ 197.0215, found 197.0222.

3,5-Bis-*O*-benzoyl-2-deoxy-1-*O*-(2-diethylmalonyl)-α- and β-D-*erythro*-pentofuranose (12α and 12β). To a solution of **9** (0.30 g, 0.78 mmol) and diethyl α-hydroxymalonate [22] (0.34 g, 1.9 mmol) in dry acetonitrile (4 mL) at -40°C, TMSOTf (0.075 ml, 0.39 mmol) was added. After 30 min, CH_2Cl_2 (100 mL) and a cold $NaHCO_3$ solution (50 mL) were added. The organic phase was washed with water (2x50 mL), brine (50 mL), dried (Na_2SO_4), filtered and evaporated. The residue obtained was purified by flash column chromatography [hexane:ethyl acetate (4:1)]. The fastest moving fractions afforded 0.21 g (55%) of **12α** as a syrup. $[\alpha]_D$ +124.3 (*c* 1, chloroform). ^{1}H-NMR (Benzene-d_6) δ 0.86 (m, 6H, OCH_2CH_3), 1.95 (ddd, $J_{H1,H2a}$=5.1, $J_{H2a,H2b}$=14.7, $J_{H2a,H3}$=7.7 Hz, 1H, H-2a), 2.23 (d, 1H, H-2b), 3.91 (m, 4H, OCH_2CH_3), 4.33 (m, 1H, H5a), 4.48 (m, 1H, H-5b), 4.57 (m, 1H, H-4), 4.88 [s, 1H, $CH(COOEt)_2$], 5.18 (d, $J_{H1,H2a}$=5.0 Hz, 1H, H-1), 5.31 (m, 1H, H-3), 7.06-8.19 (m, 10H, 2 Ph). ^{13}C-NMR (Benzene-d_6) δ 13.38 (OCH_2CH_3), 38.59 (C-2), 61.03, 61.12 (OCH_2CH_3), 63.86 (C-5), 74.30, 75.41 [C-3, $CH(COOEt)_2$], 82.44 (C-4), 103.81 (C-1), 127.02-132.55 (Ph), 165.34, 165.79, 166.08, 166.34 (C=O). Anal. Calcd. for $C_{26}H_{28}O_{10}$: C, 62.39; H, 5.64. Found: C, 62.25; H, 5.40. The slowest moving fractions afforded a syrup that was further purified by CCTLC on the Chromatotron [hexane:acetone (10:3)] to yield 0.107 g of **12β** (22% yield). ^{1}H-NMR (Benzene-d_6) δ 0.88 (m, 6H, OCH_2CH_3), 2.07 (m, $J_{H1,H2a}$=$J_{H2a,H3}$=5.5, $J_{H2a,H2b}$=14.6 Hz, 1H, H-2a), 2.54 (ddd, $J_{H1,H2b}$=2.1, $J_{H2b,H3}$=7.0 Hz, 1H, H-2b), 3.90 (m, 4H, OCH_2CH_3), 4.54 (m, 3H, H-4, H-5), 5.10 [s, 1H, $CH(COOEt)_2$], 5.42 (m, 1H, H-3), 5.54 (dd, 1H, H-1), 7.07-8.13 (m, 10H, 2 Ph). ^{13}C-NMR (Benzene-d_6) δ 13.83, 13.88 (OCH_2CH_3), 39.23 (C-2), 61.63, 61.82 (OCH_2CH_3), 65.54 (C-5), 75.71, 76.00 [$CH(COOEt)_2$, C-3], 83.38 (C-4), 104.46 (C-1), 127.52-133.08 (Ph), 165.65, 166.02, 166.16, 166.71 (C=O). Anal. Calcd. for $C_{26}H_{28}O_{10}$: C, 62.39; H, 5.64. Found: C, 62.21; H, 5.51.

2-Deoxy-1-*O*-(2-malonyl)-α-D-*erythro*-pentofuranose disodium salt (4α). To a solution of **12α** (0.186 g, 0.37 mmol) in THF (6.8 ml) at rt, 0.2 N-NaOH (10.2 mL) was added. The reaction was stirred at rt for 3 h. Then, it was diluted by addition of 10 mL of H_2O, and THF was removed at reduced pressure. The resulting solution was washed with ethyl acetate (2x10 mL), and then acidified until pH=3-4 by addition of a Dowex 50WX4 resin (H^+ form). It was then filtered and transformed into the ammonium salt by passing through a Dowex 50WX4 (NH_4^+ form). Appropiate fractions were collected and evaporated. The residue was finally purified by DEAE-Sephadex-A25 (HCO_3^- form) eluting with a gradient of 0-0.1M NH_4HCO_3. Appropiate fractions were collected, evaporated and co-evaporated with H_2O. The residue was then applied to a Dowex 50WX4 (Na^+ form), eluted with H_2O, evaporated and lyophilized to yield 0.071 g (73%) of **4α.** $[\alpha]_D$ +17.0 (*c* 0.5, H_2O). ^{1}H-NMR (D_2O) δ 1.94 (ddd, $J_{H1,H2a}$=1.2, $J_{H2a,H2b}$=14.5, $J_{H2a,H3}$=2.2 Hz, 1H, H-

2a), 2.20 (ddd, $J_{H1,H2b}$=5.2, $J_{H2b,H3}$=7.8 Hz, 1H, H-2b), 3.45 (dd, $J_{H5a,H5b}$=12.3, $J_{H4,H5a}$=5.1 Hz, 1H, H-5a), 3.55 (dd, $J_{H4,H5b}$=3.6 Hz, 1H, H-5b), 3.94 (ddd, $J_{H3,H4}$=3.7 Hz, 1H, H-4), 4.07 (ddd, 1H, H-3), 4.23 [s, 1H, C*H*(COO^-)$_2$], 5.12 (dd, 1H, H-1). ^{13}C-NMR (D_2O) δ 41.90 (C-2), 62.91 (C-5), 72.69 (C-3), 81.54 [*C*H(COO^-)$_2$], 87.48 (C-4), 104.84 (C-1), 176.47, 176.83 (C=O). HRMS (THGLY:1,3 DAP) calc. for $C_8H_{11}O_8$ $(M-H)^-$ 235.0453, found 235.0461.

2-Deoxy-2-fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose (16). To a cold solution (-10°C) of **13** (2.8g, 6.0 mmol) in dry dichloromethane:pyridine (6:1, 36 mL), a solution of trifluoromethanesulfonyl anhydride (4.9 mL, 30 mmol) in dry dichloromethane (15 mL) was added, as described by Smee et al. [26] to afford after work up the triflate **15** in quantitative yield. The triflate **15** was dissolved in ethyl acetate (6 mL) and $Et_3N{\cdot}3HF$ (14.6 mL) was added. The mixture was heated at 80°C. and the reaction was monitored by HPLC, in the conditions previously indicated. The retention times were 7.5 min for **16** and 11.5 min for **15**. After 24 h, the starting material dissapeared, volatiles were removed and the residue was purified by flash column chromatography (hexane:ethyl acetate, 4:1), to yield 2.39 g (85% from **13**) of **16**. ^{1}H-NMR was in agreement with that reported [23].

(Diisopropylphosphonyl)methyl 3,5-bis-*O*-benzoyl-2-deoxy-2-fluoro-D-*arabino*-furanoside (19). To a solution of **17** (0.37 g, 0.88 mmol) and diisopropylhydroxymethyl phosphonate (0.51 g, 2.64 mmol) in dry acetonitrile (4 mL) at 0°C, $SnCl_4$ (0.24 g, 0.96 mmol) was added. The reaction was allowed to reach rt and then heated at reflux for 5 h. Ethyl acetate (150 mL) and a solution of $NaHCO_3$ (50 mL) were added, and the resulting mixture was filtered through Celite. The organic phase was separated and washed with brine, dried (Na_2SO_4), filtered and evaporated. The resulting residue was purified by CCTLC on the Chromatotron [hexane:ethyl acetate (1:1); CH_2Cl_2:ethyl acetate (4:1)]. The fastest moving band afforded 0.07 g (23%) of **3,5-Bis-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose (18)** ^{1}H-NMR ($CDCl_3$) δ 3.90 (br s, 1H, OH), 4.69 (m, 3H, H-4, 2H-5), 5.14 (d, J=49.2 Hz, 1H, H-2), 5.48 (dd, $J_{H,F}$=21.9 $J_{H2,H3}$=4.2 Hz, 1H, H-3), 5.67 (d, $J_{F,H}$=9.3 Hz, 1H, H-1), 7.38-8.08 (m, 10H, 2 Ph). The slowest moving band afforded 0.20 g (42%) of **19**, as a mixture of α and β anomers in a 2:1 ratio. ^{1}H-NMR (Benzene-d_6) δ 0.98 [m, 12H, OCH(C*H*$_3$)$_2$], 3.88 (m, 2H, OCH_2P), 4.06 (m, 1H, H-4β), 4.50 (m, 1H, H-4α), 4.76 [m, 4H, H-5, OC*H*(CH_3)$_2$], 4.84 (ddd, $J_{H2,F}$=49, $J_{H1,H2}$=4.4, $J_{H2,H3}$=5.8 Hz, H2β), 5.02 (d, $J_{H,F}$=48.0 Hz, H-2α), 5.16 (d, J=10.0 Hz, H-1α), 5.30 (d, H-1β), 5,55 (dd, $J_{H,F}$=24.0, $J_{H3,H4}$=4.4 Hz, H-3α), 5.88 (ddd, $J_{H3,F}$=16.8, $J_{H2,H3}$=$J_{H3,H4}$=5.6 Hz, H-3β), 7.13-7.93 (m, 10H, 2 Ph). Anal. Calcd. for $C_{26}H_{32}FO_9P$: C, 57.99; H, 5.99; found: C, 57.70; H, 5.85.

Phosphonomethyl 2-deoxy-2-fluoro-D-*arabino*-furanoside disodium salt (5). Compound **19** (0.2 g, 0.37 mmol) was deprotected following a reaction sequence similar to that describe for the synthesis of **2α**. This afforded 0.042 g (39%) of **5** as a mixture of α:β anomers in a 2:1 ratio. ^{1}H-NMR (D_2O) δ 3.45-3.77 (m, 4H, H-5, OCH_2P), 3.81 (m, $J_{H3,H4}$=6.8, $J_{H4,H5b}$=3.6, $J_{H4,H5a}$=3.2 Hz, H-4β), 4.00 (m, $J_{H3,H4}$=5.6 Hz, H-4α), 4.08 (m, $J_{H,F}$=24.8 Hz, H_3α), 4.30 (ddd, $J_{H2,H3}$=6.8, $J_{H,F}$=18.4 Hz, H-3β), 4.86 (d, $J_{H,F}$=52.0 Hz, H-2α), 4.89 (ddd, $J_{H1,H2}$=4.4, $J_{H,F}$= 52.4 Hz, H-2β), 5.07 (d, H-1β), 5.16 (m, $J_{H,F}$=10.6 Hz, H-1α). MS (GLY) 245 $(M-H)^-$. HRMS (GLY) calc. for $C_6H_{11}FO_7P$ 245.0226, found 245.0243.

Diisopropyl (3,5-bis-*O*-benzoyl-2-deoxy-2-fluoro-β-D-*arabino*-furanosyl) phosphonate (20). A solution containing **17** (0.18 g, 0.43 mmol) and triisopropylphosphite (0.84 g, 4.0 mmol) in toluene (2 mL) was heated at 140°C for 4 h. The mixture was then evaporated to dryness and the residue purified by CCTLC on the Chromatotron [hexane:ethyl acetate (1:1)]. Appropiate fractions were collected evaporated and repurified in in the Chromatotron [CH_2Cl_2:ethyl acetate (4:1)] to yield 0.055 g (28%) of **20** as a syrup. $[\alpha]_D$ +17.0 (*c* 1, chloroform). ^{1}H-NMR (Benzene-d_6) δ 1.29 [m, 12H, OCH(CH_3)$_2$], 4.14 (m, $J_{H3,H4}$=3, $J_{H4,H5}$=5.5 Hz, 1H, H-4), 4.32 (ddd, $J_{H,F}$=37.0, $J_{H,P}$=11.0, $J_{H1,H2}$=3.1 Hz, 1H, H-1), 4.61 (d, 2H, H-5), 4.85 [m, 2H, OCH(CH$_3$)$_2$], 5.24 (dd, $J_{H,F}$=50.0 Hz, 1H, H-2), 5.52 (dd, $J_{H,F}$=15.0 Hz, 1H, H-3), 6.99-8.21 (m, 10H, 2 Ph). ^{13}C-NMR (Benzene-d_6) δ 23.53, 24.09 [OCH(CH_3)$_2$], 63.31 (C-5), 71.92 [d, J=7 Hz, OCH(CH$_3$)$_2$], 77.73 (dd, $J_{C,F}$=22.5, $J_{C,P}$= 173.0 Hz, C-1), 77.94 (dd, $J_{C,P}$=7.5, $J_{C,F}$=32.5 Hz, C-3), 83.31 (d, $J_{C,F}$=13.0 Hz, C-4), 90.09 (dd, $J_{C,P}$=5.0, $J_{C,F}$=187.0 Hz, C-2), 128.87-133.40 (Ph), 164.84, 165.95 (C=O). Anal. Calcd. for $C_{25}H_{30}FO_8P$: C, 59.05; H, 5.95. Found: C, 58.87; H, 5.85.

3,5-Bis-*O*-benzoyl-2-deoxy-1-*O*-(2-diethylmalonyl)-2-fluoro-α-D-*arabino*-pentofuranose (21). To a solution of dietyl α-hydroxydiethylmalonate [22] (0.4 g, 2.2 mmol) in dry acetonitrile (5 mL), previously treated with activated powdered 4 Å molecular sieves, compound **17** (0.415 g, 0.9 mmol) was added. The mixture was cooled to 0°C, and then $SnCl_4$ (0.26 g, 1.0 mmol) was added. Then, it was allowed to reach rt and further heated to 40°C for 45 min. After cooling to rt, ethyl acetate (150 ml) and a $NaHCO_3$ solution (50 mL) were added. The organic phase was washed with water, brine, dried (Na_2SO_4), filtered and evaporated. The resulting residue was purified by flash column chromatography [hexane, hexane:etyl acetate (1:1)]. Appropiate fractions were collected, evaporated and further purified by CCTLC on the Chromatotron [hexane:ethyl acetate (3:1)]. The fastest moving band afforded 0.025 g (8% yield) of compound **18**. The slowest moving fractions afforded 0.292 g (63%) of a syrup identified as **21**. $[\alpha]_D$ +75.5 (*c* 1, chloroform). ^{1}H-NMR (Benzene-d_6) δ 0.83 (m, 6H, OCH$_2$CH_3), 3.86 (m, 4H, OCH_2CH$_3$), 4.49 (m, 3H, H-4, H-5), 4.73 [s, 1H, CH(COOEt)$_2$], 5.16 (d, $J_{H2,F}$=48.9 Hz, 1H, H-2), 5.37 (d, $J_{H1,F}$=10.0 Hz, 1H, H-1), 5.55 (dd, $J_{H3,F}$=21.6, $J_{H3,H4}$=3.9 Hz, 1H, H_3), 7.06-8.15 (m, 10H, 2Ph). ^{13}C-NMR (Benzene-d_6) δ 13.64 (OCH$_2$$CH_3$); 61.61, 61.90 (O$CH_2CH_3$), 63.53 (C-5), 75.31 [$CH$(COOEt)$_2$], 77.47 (d, $J_{C,F}$=31 Hz, C-3), 83.09 (C-4), 98.12 (d, $J_{C,F}$=180 Hz, C-2), 105.40 (d, $J_{C,F}$=36 Hz, C-1), 127.55-133.40 (Ph), 165.57, 165.88, 166.33 (C=O). Anal. Calcd. for $C_{26}H_{27}FO_{10}$: C, 62.23; H, 5.25. Found: C, 62.01; H, 5.39.

2-Deoxy-1-*O*-(2-malonyl)-2-fluoro-α-D-*arabino*-pentofuranose disodium salt (7α). Compound **21** was deprotected following a procedure similar to that described for synthesis of **4α**, to give 0.077 g (70%) of **7α** as a white lyophilate. $[\alpha]_D$ +38.1 (*c* 0.5, H_2O). ^{1}H-NMR (D_2O) δ 3.59 (dd, $J_{H4,H5a}$=5.1 Hz, 1H, H-5a), 3.71 (dd, $J_{H4,H5}$=3.3, $J_{H5a,H5b}$=12.6 Hz, 1H, H-5b), 4.01 (ddd, $J_{H3,H4}$=5.7 Hz, 1H, H-4), 4.05 (ddd, $J_{H3,F}$=24.6, $J_{H2,H3}$=0.8 Hz, 1H, H-3), 4.35 [s, 1H, CH(COO$^-$)$_2$], 4.97 (dd, $J_{H2,F}$=50 Hz, 1H, H-2), 5.16 (d, $J_{H1,F}$=11.1 Hz, 1H, H-1). ^{13}C-NMR (D_2O) δ 63.68 (C-5), 77.99 (d, $J_{C,F}$=26.5 Hz, C-3), 82.31 [CH(COO$^-$)$_2$], 87.52 (d, $J_{C,F}$=3 Hz, C-4), 103.33 (d, $J_{C,F}$=178 Hz, C-2), 107.18 (d, $J_{C,F}$=36 Hz, C-1), 177.16, 177.62 (C=O). HRMS (THGLY) calc. for $C_8H_{10}FO_8$ $(M-H)^-$ 253.0359, found 253.0376.

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References

[1] Wong C.H, Halcomb R.L, Ichikawa Y, Kajimoto T. Angew. Chem. Int. Ed. Engl. 1995;34:521-546
[2] Griffiths L, Stratford IJ. Br. J. Cancer 1997;76:689-693.
[3] Gilbertsen RB, Sircar JC. Enzyme Cascades: Purine Metabolism and Immunosuppression. In: Sammes PG, editor. Comprehensive Medicinal Chemistry. Oxford: Pergamon Press, 1990; 2:443-480.
[4] Desgranges C, Razaka G, Rabaud M, Bricaud H, Balzarini J, De Clercq E. Biochem. Pharmacol. 1983;32:3583-3590.
[5] Weibel M, Balzarini J, Bernhardt A, Mamont P. Biochem. Pharmacol. 1994;48:245-252.
[6] For a preliminary communication, see: Rubira MJ, Pérez-Pérez MJ, Balzarini J, Camarasa MJ. Synlett 1998;177-179.
[7] Engel R. Chem. Rev. 1977;77:349-367.
[8] Holy A. Isopolar Phosphorous -Modified Nucleotide Analogues. In Advances in Antiviral Drug Design, Greenwich, CT: JAI Press, 1993:1:179-231.
[9] Bronson JJ, Kim CU, Ghazzouli I, Hitchcock MJM, Kern E, Martin JC. Synthesis and Antiviral Activity of Phosphonylmethoxyethyl Derivatives of Purine and Pyrimidine Bases. In: Martin J.C., editor. Nucleotide Analogues as Antiviral Agents. Washington, DC: American Chemical Society, 1989:72-87 .
[10] Naesens L, Snoeck R, Andrei G, Balzarini J, Neyts J, De Clercq E. Antivir. Chem. Chemother. 1997;8:1-23, and references therein.
[11] Kim CV, Luh BY, Martin JC. J. Org. Chem. 1991;56:2642-2647.
[12] Alexander P, Krishnamurthy VV, Prisbe EJ. J. Med. Chem. 1996;39:1321-1330.
[13] Miller MJ, Anderson KS, Braccolino DS, Clearly DG, Grys KJ, Han CY, Lin KC, Pansegrau PD, Ream JE, Sammons RD, Sikorski JA. Bioorg. Med. Chem. Lett. 1993;3:1435-1440.
[14] Ye B, Akamatsu M, Shoelson SE, Wolf G, Giorgetti-Peraldi S, Yan X, Roller PP, Burke TRJr. J. Med. Chem. 1995;38:4270-4275.
[15] Lindell SD, Earnshaw CG, Wright BJ, Carver DS, O'Mahony MJ, Saville-Stones EA. Bioorg. Med. Chem. Lett. 1996;6:547-552.
[16] Marquez VE; Tseng CKH, Mitsuya H, Aoki S, Kelley JA, Ford HJr, Roth JS, Broder S, Johns DG, Driscoll JS. J. Med Chem. 1990;33:978-985 and references therein.
[17] Watanabe KA, Hollenberg DH, Fox JJ. J. Carb. Chem. Nucleosides & Nucleotides 1974;1:1-37.
[18] Gold A, Sangaiah R. Nucleosides & Nucleotides 1990;9:907-912.
[19] Phillion D.P, Andrews J.J. Tetrahedron Lett. 1986;27:1477-1480.
[20] Pérez-Pérez MJ, Balzarini J, Rozenski J, De Clercq E, Herdewijn P. Bioorg. Med. Chem. Lett. 1995;5:1115-1118.
[21] Meuwly R, Vasella A. Helv. Chim. Acta 1986;69:25-34.
[22] Cohen SG, Khedouri E. J. Am. Chem. Soc. 1961;83:4228-4233.
[23] Tann CH, Brodfuehrer PR, Brundidge SP, Sapino CJr, Howell HG. J. Org. Chem. 1985;50:3644-3647.
[24] Howell HG, Brodfuehrer PR, Brundidge SP, Benigni DA, Sapino CJr. J. Org. Chem. 1988;53:85-88.
[25] Chou TS, Becke LM, O'Toole JC, Carr MA, Parker BE. Tetrahedron Lett. 1996;37:17-20.
[26] Smee DF, Chernow M, Kraft M, Okamoto PM, Prisbe EJ. Nucleosides & Nucleotides 1988;7:155-165
[27] Csuk R, Glänzer BI. N.M.R. Spectroscopy of Fluorinated Monosaccharides in Adv. Carbohydr. Chem. Biochem. 1988;46:73-177.
[28] Lemieux RU, Hendriks KB, Stick RV, James K. J. Am. Chem. Soc. 1975;97:4056-4062.
[29] PSEUROT version 6.2. Altona C. University of Leiden, Leiden, The Netherlands.
[30] Williamsom KL, Hsu Y-FL, Hall FH, Swager S. J. Am. Chem. Soc. 1968;90:6717-6722.
[31] PANIC 86. Brucker library. 1986. Germany
[32] Donders LA, de Leeuw F.A.A.M, Altona C. Magn. Res. Chem. 1989;27:556-563.
[33] HYPERCHEM. Hypercube, Inc. 1995. Waterloo, Canada.
[34] Balzarini J, De Clercq E. Biochem. J. 1992;287:785-790.