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Synthesis and biological evaluation of prodrugs of 2-fluoro-2-deoxyribose-1-phosphate and 2,2-difluoro-2-deoxyribose-1-phosphate

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ABSTRACT
We report in this Letter the synthesis of prodrugs of 2-fluoro-2-deoxyarabinose-1-phosphate and 2,2-difluoro-2-deoxyribose-1-phosphate. We demonstrate the difficulty of realising a phosphorylation step on the anomeric position of 2-deoxyribose, and we discover that introduction of fluorine atoms on the 2 position of 2-deoxyribose enables the phosphorylation step: in fact, the stability of the prodrugs increases with the degree of 2-fluorination. Stability studies of prodrugs of 2-fluoro-2-deoxyribose-1-phosphate and 2,2-difluoro-2-deoxyribose-1-phosphate in acidic and neutral conditions were conducted to confirm our observation. Biological evaluation of prodrugs of 2,2-difluoro-2-deoxyribose-1-phosphate for antiviral and cytotoxic activity is reported.

Glycosyl-1-phosphates are important compounds in many life processes. They are essential constituents of larger biomolecules and are key intermediates in the metabolism of sugars and their transformation into nucleosides. Among them, 2-deoxy-α-D-ribose-1-phosphate is a catabolic product of thymidine phosphorylase (EC 2.4.2.4), an enzyme involved in the metabolism of pyrimidine nucleosides and which may promote angiogenesis. Thymidine phosphorylase is also involved in the degradation of antiviral agents such as BVDU. It is recognized that the therapeutic potential of drugs bearing a phosphate, phosphinate or phosphonate moiety is considerably reduced due to their low membrane permeation because each of these groups carry negative charges at physiological pH. Several prodrug technologies have been investigated to overcome this problem and, among them, the phosphoramidate ProTide technology has been successfully applied to various nucleoside analogues. Furthermore the phosphoramidate technology has been recently applied to sugars (mainly glucosamine) in order to improve their therapeutic potential. The synthesis of phosphoramidate prodrugs of 2-deoxy-α-D-ribose-1-phosphate is of great interest, as they could act as inhibitors of thymidine phosphorylase or regulators in associated metabolic processes.

Many papers have reviewed the introduction of a phosphate group at the anomeric position of a glycosyl unit. Three main strategies are observed: (a) the sugar, activated at the anomeric position, acts as an electrophilic compound and a nucleophilic displacement reaction is performed thanks to a phosphate anion. In this case, the electrophilic compounds are generally glycosyl halides, glycosyl-trichloroacetimidates, glycosyl nitrates, or glycosyl thioimidoyl derivatives; (b) the anomeric hydroxyl group of the sugar acts as the nucleophilic component and attacks an activating phosphate or phosphite in the presence of a base; (c) another alternative strategy consists in synthesising P(III) intermediates: the most famous approaches are the phosphoramidite methodology followed by oxidation of the intermediate phosphite, or the H-phosphonate approach which entails phosphorylation of the hydroxyl group followed by oxidation of the resulting H-phosphonate. However, these strategies are mainly applied to glycosyls in their pyranose form. In the case of the furanosyl family, the phosphorylation step has been successfully applied in the case of the ribo or arabinofuranosyl analogues, but it is hardly described in the case of 2-deoxyribose derivatives: due to the absence of a 2-hydroxyl group, the anemic phosphate is more labile, so its synthesis is quite difficult. In fact, to the best of our knowledge, only one strategy concerning the phosphorylation step at the anomeric position of 2-deoxyribose was described: under acidic conditions and with an excess of phosphoric acid, 2-deoxyribose-1-chloride 1 was phosphorylated in good yield to give compound 2 (Scheme 1).
The strategy used to synthesise prodrugs of 2-deoxyribose-1-phosphate is shown in Scheme 2: the anomeric position of 3,5-dibenzyl-2-deoxyribose is first protected as a methyl glycoside in order to remove the methyl ether was realised under acidic standard conditions. Protection of the diol was accomplished in 74% yield and removal of the methyl ether was realised under acidic standard conditions. The next step was the coupling of compound 4 with different suitable phosphorochloridates in order to obtain the desired phosphate. We were particularly interested to prepare C1-phosphoramidates as prodrugs of the free phosphate, following procedures we have widely reported for sugars describing their high potential and their broad field of applications.17

However it is well-known that the presence of a fluorine substituent adjacent to the anomeric centre stabilizes glycosyl phosphates or nucleosides15 and excellent reviews on fluorinated substituent adjacent to the anomeric centre stabilizes glycosyl nucleosides.5,18 This hypothesis is confirmed by the fact that the phosphorylation of a group at the 2 position destabilizes the resulting phosphate. We were particularly interested to prepare C1-phosphoramidates as prodrugs of the free phosphate, following procedures we have widely reported for nucleosides.15

The synthesis of prodrugs of 2-deoxyribose-1-phosphate. (a) CH3COCl, MeOH, rt, 1 h, 100%, (b) BnCl, KOH, THF, reflux, 31 h, 74%, (c) AcOH/H2O 8:2, 36 h, 50 °C, 75% (d) appropriate phosphorochloridate, tBuMgCl or NMI, THF.

We knew from our previous experience16b and from the literature16a that a phosphorylation reaction was possible on the 1-chloro-3,5-di-para-chlorobenzoyl-2-deoxyribose. Encouraged by this preliminary result, we decided to study the phosphorylation of 3,5-di-benzyl-2-deoxyribose. Results are summarized in Table 1 in the Supplementary data. The phosphorylation step was first tried in classical conditions using POCl3, phenylchlorophosphate19 or chlorodiphenyl phosphate20 in the presence of different bases and activating agents such as PyBOP.21 We also investigated the P(III) strategy: we tried to use the phosphoramidite,22 phosphoradiamidite23 and H-phosphonate24 methodologies. We decided to try the phosphorylation with charged phosphorylating agents such as methyl pyridinium dichlorophosphate25 or of the dibarium salt of 2-cyanoethylphosphate26 in order to stabilize the resulting phosphate. To finish our study, we decided to invert our strategy by placing the leaving group on the anomeric position of the sugar15a,b,27 and to use the phosphate reagent as the attacking agent. But all these attempts did not lead to the desired products, which lead to the conclusion that final compounds are not stable under the reaction conditions.

The strategy used to synthesize prodrugs of 2-deoxyribose-1-phosphate is shown in Scheme 2: the anomeric position of 2-deoxyribose 3 was first quantitatively protected as a methyl glycoside in standard conditions. Protection of the diol was accomplished in 74% yield and removal of the methyl ether was realised under acidic conditions. The next step was the coupling of compound 4 with different suitable phosphorochloridates in order to obtain the desired phosphoramidates. We tried this reaction under different conditions (~78 °C or rt) and with different bases (DMAP, NMI, tBuMgCl) but unfortunately this coupling reaction did not work.

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Stability study at pH 2
Stability study at pH 5
Stability study at pH 7
Stability study at ~20 °C

Table 1
Stability study of compound 14b and 25c

| Stability study at pH 2 | 2-Fluoro-2-deoxyarabinose family compound 14b | 2,2-Fluoro-2-deoxyribose family compound 25c |
|------------------------|-----------------------------------------------|-----------------------------------------------|
| Purity after 1 h: 88%  | No degradation observed after 2 days          | No degradation observed after 2 days          |
| Purity after 13 h: 68% | No degradation observed after 2 days          | No degradation observed after 2 days          |
| Purity after 4 months  | —                                             | —                                             |
| Purity after 20 h: 8%  | —                                             | —                                             |
used to remove it are compatible with the presence of the phosphoramidate group. So we decided to apply this group to our strategy of synthesis. Compound 9 was deprotected under basic conditions and the resulting diol was protected in the presence of benzyl chlorofor- 
mate to yield quantitatively compound 11 in a \(a/b\) ratio 1:1. Depro-
tection of the anomeric position was tried in several conditions (TBAF, NH4F, HCl, TFA) but the yields were low (0–20%) in all these 
conditions. Thus, we finally decided to perform the deprotection in 
the presence of HF-pyridine,\(^29\) and in this case the yield was quan-
titative and the \(a/b\) ratio changed to 1:0.2. Thanks to the literature,\(^31\) 
it was easy to discriminate the \(\alpha\) from the \(\beta\) diastereoisomers: the \(\alpha\) anomers had 
\(J_{1,2} = 0\) Hz, confirming that \(H1\) and \(H2\) were trans to 
each other, so the NMR signal of \(H2\) was a doublet with 
\(J_{2-F} = 49-50\) Hz. In the case of the \(\beta\) anomer, \(J_{1-F} = 0\) Hz, which confirmed that 
\(H1\) and \(2F\) were trans to each other, and the signal of \(H2\) was in this 
case a ddd (with a general range of \(J_{1,2} = 4\) Hz, \(J_{2-F} = 50\) Hz, \(J_{2,3} = 6\) Hz). Finally, coupling of compound 12 with the appropriate 
arloxy phosphorochloridates followed by deprotection of the 
benzyloxy carbonyl protecting groups\(^25\) yielded the desired 2-flu-
oro-2-deoxyarabinose-1-phosphate prodrugs 14a–h. Their success-
ful isolation highlights the change in stability upon 2-fluorination.

Surprisingly, only the \(\alpha\) prodrugs were isolated. However, 
during the purification of the final prodrugs, we experienced con-
siderable difficulty in isolating pure compound and we discovered 
that a second purification, either by preparative TLC, column chro-
matography or preparative HPLC, led to a degradation of the pro-
drugs. Thus, we conducted a preliminary stability study of 
compound 14b. One hour after purification by column chromatog-
raphy the purity of compound 14b was 68%, after one night at 
\(-20^\circ\)C, the purity of the compound decreased dramatically to 
30%, and after 20 h it was only 8%. At pH 2, the half-life of com-
ound 14b was only 64 min, while at pH 7 it was 120 min (Fig. 1).

Since all our 2-fluoro-2-deoxyarabinose-1-phosphate prodrugs 
were not stable, they could not be biologically evaluated but we 
were encouraged by these results: indeed by adding one fluorine 
atom on the 2 position of 2-deoxyribose, the phosphorylation was possible on the anomeric position, even if the resulting pro-
drugs were not stable enough to be tested. So we decided to ex-
plor the 2,2-difluoro-2-deoxyribose family. We hypothesized 
that the addition of a second fluorine atom at the 2 position would 
 further increase the stability of the resulting prodrugs.

The strategy used to synthesize prodrugs of 2,2-difluoro-2-
deoxyribose-1-phosphate was the same as the one previously used 
in the case of the 2-fluoro-2-deoxyribose family (Scheme 4).
Reduction of the lactone 15 was achieved in the presence of LiAl(OtBu)₃H and the resulting alcohol was protected as a silyl ether with 46% yield. We were also able to isolate a mixture of the monobenzoylated sugars 18 and 19 with 31% yield but this partial deprotection was acceptable as the next step consisted in removing all the benzoyl esters. Protection of the resulting 3 and 5 alcohols was achieved in the presence of benzyl chloroformate and deprotection of the silyl ether at the anomeric position was done with HF-Pyr and yielded compound 22 in a 1:0.6 α/β ratio. The coupling reaction of the compound 22 with the appropriate phosphorochloridates was done in the presence of tBuMgCl, and the final step consisted in the hydrogenolysis of the benzoyl groups which led to the desired prodrugs of 2,2-difluoro-2-deoxyribose-1-phosphate (with yields of between 24% and 68% over the two last steps). For each compound, α anomers 25 and β anomers 24 were isolated as a mixture of P₃ and P₆ diastereoisomers. In order to characterize the two anomers we performed different experiments. We first examined the NOE effects between the H1 and H4 protons, without success. So we based our study over the two last steps). For each compound, (a) LiAl(OtBu)₃H, THF/H₂O 4:1, –78 °C, 2 h, (b) TIPS-OTf, 2.6-lutidine, CH₂Cl₂, 0 °C, 2 h, 67% over 2 steps, (c) Et₂N, H₂O, MeOH, rt, 4 days, 70%, (d) CbzCl, DMAP, CH₂Cl₂, rt, 12 h, 100%, (e) HF-pyr, pyridine, rt, 100%, (f) appropriate phosphorochloridate, tBuMgCl, THF, rt, 15 h, (g) H₂, Pd/C, EtOH, rt, 2 h.

As expected, the 2,2-difluoro-2-deoxyribose-1-phosphate prodrugs are much more stable than the 2-fluoro-2-deoxyribose-1-phosphate prodrug: the difluoro phosphate group on the anomeric position of 2-deoxyribose is difficult due to the lack of a stabilizing group at the 2 position. However, by introducing one or two fluorine atoms on the 2 position, we increased the stability of the resulting prodrug: the higher the degree of fluorination on the 2 position, the more stable is the prodrug. We have confirmed this tendency by performing a pH stability study of the 2,2-difluoro-2-deoxyribose-1-phosphate prodrug and the 2-fluoro-2-deoxyarabinose-1-phosphate prodrug: the difluoro prodrugs are stable for 2 days under acidic conditions whereas the mono-fluorinated derivative had a half-life of 64 min. Moreover, the 2,2-difluoro prodrugs are stable at –20 °C for more than 4 months whereas the mono-fluorinated analogs were almost fully degraded within 24 h. Unfortunately, biological evaluation of the pro-drugs of the 2,2-difluoro-2-deoxyribose-1-phosphate showed that they had no inhibitory activity against a variety of virus infections and cancer cell proliferation.

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Supplementary data

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