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Phosphonic derivatives of carbohydrates: chemoenzymatic synthesis

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Abstract

A series of homologous racemic β-hydroxyaldehydes bearing a phosphonate group in the ω-position were synthesised and allowed to react with dihydroxyacetone phosphate (DHAP) via an enzymatic aldol addition catalysed by fructose 1,6-bisphosphate aldolase (FruA). After enzymatic dephosphorylation, a set of unnatural ω-phosphonic deoxysugars was obtained. © 2000 Elsevier Science Ltd. All rights reserved.

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Phosphonic acid derivatives, isosteric with natural and synthetic phosphates, in which the P–O bond of the phosphate is formally replaced with a more stable P–C bond, have attracted considerable attention due to their stability towards the action of phosphatases and to their potential bioactivity as inhibitors or regulators of metabolic processes. For example, phosphonate containing molecules have been shown to be inhibitors of EPSP synthase,[1] HIV protease,[2] renin,[3] farnesyl protein transferase[4] and PTPases.[5,6]

The synthesis of a large number of isosteric phosphonate analogues of phosphorylated sugars and their interaction with a number of enzymes has been described.[7] Most of the reported syntheses of phosphonic derivatives of carbohydrates employ natural sugar derivatives as the starting material and the phosphonic group is introduced in a late step; this strategy suffers from the need of a selective protection/deprotection scheme and from the limited availability of starting materials.

Enzymatic aldol addition of dihydroxyacetone phosphate (DHAP) has become a useful tool in the synthesis of natural carbohydrates and their analogues. The existence of a set of four stereocomplementary aldolases and the low substrate specificity of these enzymes, at least for the electrophilic component, has permitted the synthesis of a large number of carbohydrates and, in general, of polyhydroxylated compounds.[8–10] Fructose 1,6-bisphosphate aldolase from rabbit muscle (FruA, EC 4.1.2.13), catalyses

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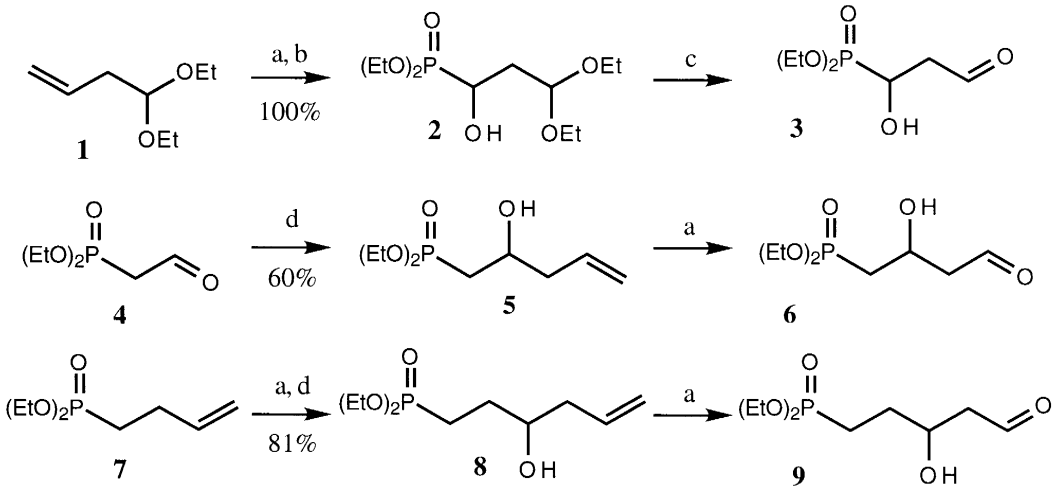
the addition of DHAP to a broad range of aldehydes to produce adducts of *threo*-3*S*,4*R*-configuration, and is the most widely used aldolase for synthetic purposes.

Few phosphonic aldehydes are reported to be substrates for DHAP aldol addition catalysed by FruA[11–13] and only in two cases have phosphonic sugars been obtained and characterised.[12] An alternative approach to sugar 1-phosphonates, bio-isosteric analogues of sugar 1-phosphates, based on the use of aldolases, employing a phosphonic analogue of DHAP, has been developed.[14,15] Here we present the synthesis of a set of sugar ω-phosphonates via aldol addition of DHAP to the homologous racemic β-hydroxyaldehydes **3**, **6** and **9** catalysed by FruA (Scheme 1).



Scheme 1. (a) O_3, CH_2Cl_2/MeOH, −78°C, then Me_2S; (b) diethylphosphite, KF, rt; (c) Dowex resins (H^+ form), H_2O, 50°C; (d) Zn, allyl-Br, H_2O, THF, rt

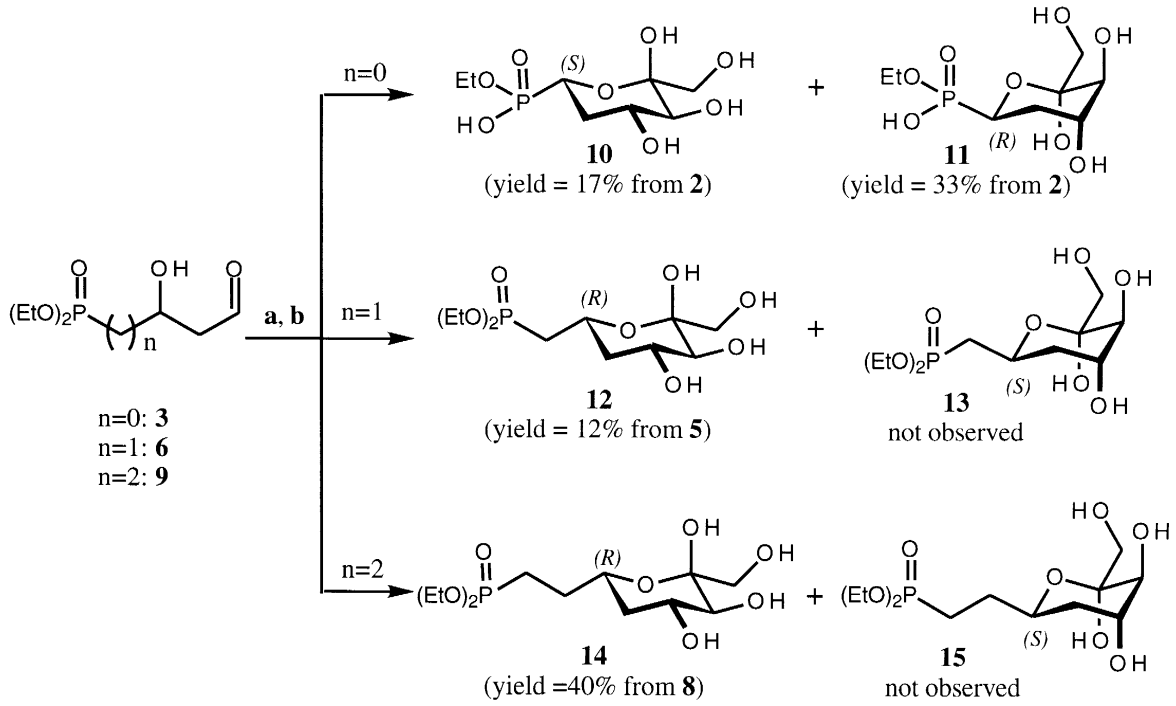
Hydroxyaldehyde **3** was synthesised as diethylacetal **2** following a modified Pudovic reaction[16] starting from diethylphosphite and 3,3-diethoxypropanal in the presence of KF as catalyst.[17] The latter aldehyde was easily obtained via ozonolysis followed by reductive work-up of 4,4-diethoxy-1-butene. After deprotection, the pH of the solution was adjusted to 7.0 by addition of 1 M NaOH and the resulting solution of crude aldehyde **3** was used directly in the enzymatic step.[18]

Hydroxyaldehyde **6** was synthesised in its masked form **5** via allylation of the known aldehyde **4**[19] using allyl bromide and zinc in H_2O–THF.[20] The allylation in a protic medium gave a cleaner reaction and a better overall yield in comparison with the Grignard addition using allyl magnesium bromide, probably because of the easy enolisation of **4**. Ozonolysis of **5** followed by reductive work-up gave the hydroxyaldehyde **6**. Formaldehyde, the by-product from the cleavage of the double bond, was quantitatively removed by evaporation in vacuo. The crude aldehyde **6** was taken up in H_2O, the pH of the solution adjusted to 7.0 by addition of NaOH and the resulting solution used directly in the enzymatic step.[18]

Aldehyde **9** was prepared from phosphonate **7**[21] following a similar procedure.[18]

The racemic mixtures of the aldehydes **3**, **6** and **9** in aqueous solution were subjected to the enzymatic aldol addition catalysed by FruA (Scheme 2).[22] DHAP was formed in situ from commercially available fructose 1,6-bisphosphate (FBP) by a combination of FruA and triose phosphate isomerase (TPI, EC 5.3.1.1).[23] In a typical procedure, an aqueous solution of crude hydroxyaldehyde (8 mmol) was treated with FBP (1 mmol), the pH of the solution was adjusted to 7.0 with NaOH, then FruA (50–100 U) and

TPI (1000 U) were added. After 3–5 days, the pH of the solution was adjusted to 4.8 with 1 M HCl and acid phosphatase (50 U) was added. Alternatively the pH of the solution was adjusted to 8.0 with 1 M NaOH and treated with alkaline phosphatase (200 U). The resulting adducts were purified by silica gel chromatography.



Scheme 2. (a) FBP, FruA, TPI (pH=7.0); (b) acid phosphatase (pH=4.8) or alkaline phosphatase (pH=8.0)

It is worth noting that, generally, in the FruA catalysed additions of DHAP to racemic β-hydroxy-aldehydes, the reaction of the enantiomer which produces the more stable adduct is thermodynamically favoured.[23] In this case it should be possible to produce exclusively the more stable aldol adduct by the use of an excess of aldehyde compared to the nucleophilic component. Nevertheless, when an excess of racemic aldehyde **3** was subjected to the enzymatic sequence of aldol addition/dephosphorylation,[24] two adducts were isolated in 50% global yield and in 1:2 ratio. After a careful chromatographic separation of the diastereomers, spectroscopic analysis showed that the major adduct corresponds to the monoester **11** (apparently the less stable one), while the minor adduct was the monoester **10** (apparently the more stable one). In fact, the adduct **11** shows $J_{3/4}$=1.8 Hz, diagnostic for a diequatorial relationship (*trans*), in a chair conformation $^{3}C_{6}$, while the adduct **10** shows $J_{3/4}$=9.4 Hz, diagnostic for a diaxial relationship (*trans*) in a chair conformation $^{6}C_{3}$, as shown in Scheme 2. This means that, surprisingly, the formation of the adduct **11** is favoured. Another unexpected result of this reaction is the isolation of a monoethyl phosphonate.[25]

When an excess of the racemic aldehydes **6** or **9** were subjected to the enzymatic sequence of aldol addition/dephosphorylation,[24] the adducts **12** and **14** were isolated in 12% and 40% yield, respectively. The spectroscopic analysis in both cases showed that the adduct anticipated to be the more stable was obtained. As expected, the formation of both **13** and **15** was not observed under these reaction conditions.

We have described the chemoenzymatic synthesis of a set of new sugar analogues bearing an unnatural substituent. The construction of complicated structures with three chiral centres in optically active form was achieved starting from easily accessible racemic phosphonates. Although the overall yields were modest, we believe that optimisation is possible (i.e. by the use of DHAP instead of FBP and by the use of more appropriate purification techniques). Further investigations in the chemoenzymatic synthesis of phosphonic derivatives of carbohydrates are in progress and will be published in due course.

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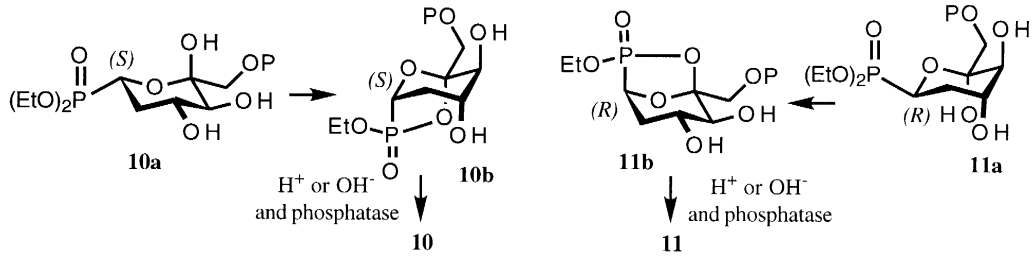
24. Approximately the same results (yield and diastereomeric ratio) were obtained by using acid or alkaline phosphatase.
25. A tentative explanation for the unexpected diastereomeric ratio and for the monohydrolysis of the phosphonic diethyl ester could be the formation of the bicyclic intermediates **10b** and **11b**, directly after the aldol addition. The formation of **11b** from **11a** is likely to be faster, when compared with the formation of **10b** from **10a**. The cyclic phosphonates **10b** and **11b** cannot undergo a retroaldolic addition, thus withdrawing the adducts from enzymatic equilibration. Then, the acidic or alkaline conditions used during the enzymatic dephosphorylation and/or the acidity of the silica gel during the chromatography,

affords ring opening. An alternative explanation could involve enzymatic hydrolysis of the diethylphosphonate catalysed by the phosphatase. Although this reaction is reported in the literature,[26] seems to be less likely because we did not observe this hydrolysis on similar phosphonates and because no differences were detected during the use of acidic and alkaline phosphatase.[24] As we did not isolate the intermediate monophosphate, it is not possible to decide definitively in which step such hydrolysis takes place.



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