Stereoselective Synthesis of the Isosteric Bisphosphono Analogue of β-D-Fructose 2,6-Bisphosphate

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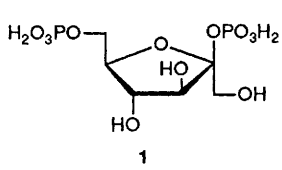
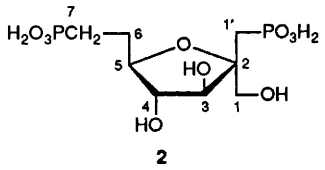
The isosteric bisphosphono analogue **2** of β-D-fructose 2,6-bisphosphate has been synthesized exploiting the *C*-fructofuranoside **5**, the free hydroxy group of which was converted into phosphonate *via* iodide; the methylphosphonic group at C-6 was introduced by Wittig–Horner reaction of the aldehyde **9**, prepared by selective deprotection and oxidation of the hydroxy group at C-6.

β-D-Fructose 2,6-bisphosphate **1** is a recently discovered important regulator of glycolysis and gluconeogenesis.[1] Its biological role has not completely been clarified owing to the lability of the anomeric phosphate towards enzymes and acids. Consequently, the synthesis of stable analogues of **1** has gained interest. In particular the interest has focused on the substitution of the phosphoric oxygen with a carbon atom, a concept which is well documented in the literature[2] dedicated to the search for antimetabolites of natural phosphates.

Some isosteric[3] and nonisosteric[4] phosphono analogues of



1



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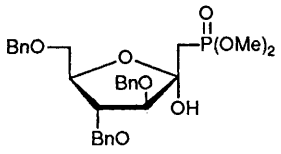

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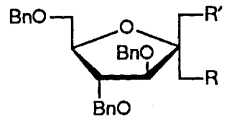
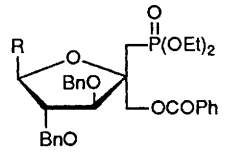


4 R = CH=CH$_2$, R' = OH
5 R = OCOPh, R' = OH
6 R = OCOPh, R' = I
7 R = OCOPh, R' = PO_3Et_2



8 R = CH_2OH
9 R = CHO
10 R = CH=$CHPO_3Et_2$
11 R = $CH_2CH_2PO_3Et_2$

1, have already been synthesized, in which the modification involved only the phosphate at C-2. In some cases, such as in yeast, fructose 2,6-bisphosphate undergoes the hydrolysis at the 6-phosphate to afford fructose 2-phosphate.[5] This observation makes interesting analogues of **1** in which the phosphate at C-6 is also substituted by a phosphonic group.

We now describe the synthesis of **2**, an isosteric bisphosphono analogue of β-D-fructose 2,6-bisphosphate **1**.

The main problem in the synthesis of the structure **2** is clearly the formation of the tetra-substituted C-2 with the correct stereochemistry. Compound **2** is a *C*-glycoside of D-fructose in which a β-oriented methylphosphonic group substitutes the anomeric hydroxy group. We initially took into account our results on the synthesis of *C*-fructosides,[6] which involves the introduction of an α-allyl group at C-2 of a D-fructofuranoside by treatment with allyltrimethylsilane in presence of a Lewis acid.

At first, we tried to obtain **2** by α-allylation of a structure such as **3** followed by conversion of the allyl substituent into a hydroxymethyl group. Unfortunately, despite the easy synthesis of **3** by reaction of 2,3,5-tri-*O*-benzyl-D-arabinolactone[7] with $LiCH_2PO_3Me_2$, we were unable to convert it into the corresponding allyl *C*-glycoside. So we turned to the allyl *C*-fructoside **4**,[6] the conversion of the hydroxy group of which into a phosphonate, through the corresponding iodide, proceeded in poor yields. Better results were obtained on **5**,[6] which on treatment with triphenylphosphine, iodine and imidazole[8] (under reflux in toluene–MeCN 2 : 1), afforded the iodide **6** in 95% yield. Compound **6**, refluxed with $P(OEt)_3$, gave the phosphonate **7** in 85% yield.

To introduce the second phosphonic group, we selectively deprotected the hydroxy group at C-6 of **7** (H_2–Pd/C, MeOH, 62% yield), we oxidized the free hydroxy group of **8** to the aldehydo group of **9** (pyridinium chlorochromate, 3 Å molecular sieves, CH_2Cl_2), and then we submitted **9** to a Wittig reaction to obtain the α,β-unsaturated phosphonate **10**. This last reaction was very troublesome; it was attempted under different experimental conditions, the best result (40% yield from **8**) was obtained using $CH_2[PO(OEt)_2]_2$, 1,8-diazobicyclo[5.4.0.] undec-7-ene (DBU) and LiCl in MeCN.[9] Compound **10** was hydrogenated on PtO_2 to afford **11** (96% yield), deprotection of which (Me_3SiBr; then H_2–Pd/C, and finally Dowex 50 × 8, H^+ form, 30% overall yield) gave **2** as the diammonium salt, after chromatographic purification on cellulose (n-C_3H_7OH–NH_4OH–H_2O 6 : 3 : 1).†

Preliminary tests carried out to check the effectiveness of the analogue as an activator of pyrophosphate dependent fructose-6-phosphate kinase from potato tubers and rabbit muscle fructose-6-phosphate kinase, and as an inhibitor of rabbit liver fructose-1,6-bisphosphatase, showed that the bisphosphonate was about three orders of magnitude less effective than fructose 2,6-bisphosphate. Thus, the replacement of the oxygen atoms in the phosphoester bonds with methylene groups dramatically decreases the affinity of the molecule for the binding sites of the aforementioned enzymes. This points to a crucial role of these atoms in determining the affinity constant of the binding process.

Thanks to its absolute stability against enzymatic attack, the bisphosphonate here described might also be employed as a tool to understand better the physiological effects of the natural bisphosphate sugar. Moreover, additional experiments with analogues carrying other substitutions might further elucidate the roles of different groups of the fructose 2,6-bisphosphate molecule in binding to those enzymes that are naturally modulated by this phosphorylated sugar.

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† All new compounds exhibited satisfactory spectroscopic and analytical data.