Stereoselective Synthesis of the 6-Phosphono Analogue of Fructose-6-phosphate

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The non-isosteric phosphono anlogue **2** of D-fructose-6-phosphate has been synthesized exploiting the reactivity of the fructose derivative **7** which is easily available on a large scale from saccharose; introduction of the phosphonic group by Arbuzov reaction using diphenyl ethyl phosphite followed by deprotection afforded the title compound.

Although D-fructose-2,6-biphosphate analogues have recently received much interest in the search for antimetabolites of the natural regulator of glycolysis,[1] reports on 1-deoxy and 6-deoxy-substituted fructose are scarce[2,3] and only enzymatic synthesis has allowed access to the isosteric phosphono analogue of **1**.[3]

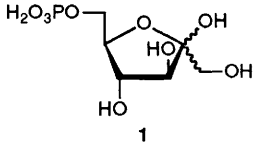
We now describe the synthesis of **2** and its evaluation as an inhibitor of *Escherichia coli* glucosamine-6-phosphate synthase (L-glutamine: D-fructose-6-phosphate amidotransferase).[4]

We selected saccharose **3** as the cheapest starting material. Reaction of **3** with Ph_3P–CBr_4 in pyridine under conditions similar to those for the synthesis of the chlorinated derivative[5] followed by acetylation (Ac_2O–pyridine) gave the peracetylated derivative of **4**† (m.p. 127–128 °C) in 10% yield. The acidic methanolysis of the glycosidic linkage gave the methylated 6-brominated fructofuranose and glucopyranose which could not be separated by chromatography.

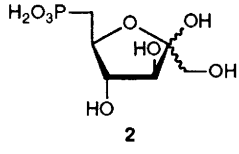
Selective functionalisation at the 6′ position of saccharose was then performed in two steps by benzoylation at the 6 position of **3**[6] followed by chlorination (Ph_3P–CCl_4) to afford **5**† in only 16% yield (m.p. 71 °C, as perbenzoylated derivative).

We finally opted for the selective functionalisation of saccharose at position 6′ using 2,4,6-triisopropylbenzenesulfonyl (tripsyl) chloride in pyridine[7] with the following modification: flash chromatography of the crude reaction products (silica gel, ethyl acetate–acetone–water, 10 : 10 : 1) followed by recrystallisation of the mixture of 6- and 6′-substituted derivatives from the same solvent gave **6** in 32% yield even in large-scale syntheses (up to 50 g). Hydrolysis of the glycosidic linkage (1% CF_3CO_2H in MeOH) followed by acetylation and tripsyl displacement by iodine (NaI–acetone) afforded iodide **8**† in 95% yield as an oily mixture of isomers (α : β 3 : 2). Compound **8** reacted with diphenyl ethyl phosphite[8] (5 equiv. 160 °C, 60 h) to afford **9** as a mixture of isomers separable by silica gel flash chromatography (cyclohexane–AcOEt, 3 : 1). The two isomers **9α**† and **9β**† were isolated as oils in 18 and 53% yields from their respective precursors **8** reflecting the much slower reaction of the phosphite with the α-isomer. They were separately hydrogenated in ethanol in the presence of platinum oxide: whereas the reaction with the α-isomer was complete after a few hours under 1 atm hydrogen pressure, hydrogenolysis of the β-isomer occurred to only 35% after 3 days under 5 atm. This result reflects the adsorption of **9** on the catalyst by its α-face which, in the case of the β-isomer, is masked by the CH_2OAc group. Deacetylation of **10**† (catalytic MeONa in MeOH, 10 min) followed by demethylation of the 2-OMe (CF_3CO_2H–water 1 : 2, 14 h) gave quantitatively the expected compound **2**† (m.p. 210 °C).

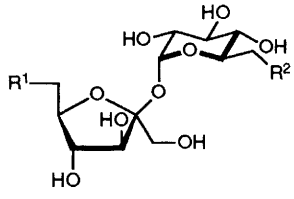
When tested as a substrate of glucosamine synthase, no transformation of **2** into 2-amino-2,6-dideoxy-6-phosphonato-fructose (glucosamine-6-phosphonate) could be detected



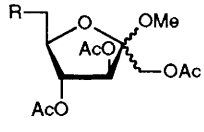
1



2



3 $R^1 = R^2 = OH$
4 $R^1 = R^2 = Br$
5 $R^1 = Cl$, $R^2 = Bz$
6 $R^1 = OTripsyl$, $R^2 = OH$



7 R = OTripsyl
8 R = I
9 $R = PO(OPh)_2$
10 $R = PO_3H_2$

† All new compounds exhibited satisfactory spectroscopic and analytical data.

using concentrations of enzyme tenfold higher than in the normal catalysis.[4] Compound **2** behaved as a poor competitive inhibitor *vs.* fructose-6-phosphate (K_i = 2.5 mmol dm^{-3}, K_m/K_i *ca.* 1). The oxime derivative, obtained in quantitative yield by incubation with aqueous hydroxylamine in tenfold excess (pH 4.5, 2 h) followed by precipitation of the barium salt, had a much higher affinity, however (K_i = 0.2 mmol dm^{-3}, K_m/K_i = 12). These data reflect the similarity between this inhibitor and the enolamine intermediate which has been postulated in the mechanism occurring at the fructose-6P binding site.[9] This result demonstrates that functionalization of the keto group of **2** is certainly an interesting avenue to generate stable and potent inhibitors of glucosamine-6P synthase.

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References

1 R. Meuwly and A. Vasella, *Helv. Chim. Acta*, 1986, **69**, 751; A. Dessingues and A. Vasella, *Carbohydr. Res.*, 1988, **174**, 47; F. Nicotra, L. Panza, G. Russo, A. Senaldi, N. Burlini and P. Tortora, *J. Chem. Soc., Chem. Commun.*, 1990, 1396.
2 D. Stribling, *Biochem. J.*, 1974, **141**, 725; J. C. Tang, B. E. Tropp and R. Engel, *Tetrahedron Lett.*, 1978, 723; M. C. Campbell and G. D. Hefferman, *Tetrahedron Lett.*, 1991, 1937.
3 D. Webster, W. R. Jondorf and H. B. F. Dixon, *Biochem. J.*, 1976, **155**, 433.
4 B. Badet, P. Vermoote, P. Y. Haumont, F. Lederer and F. Le Goffic, *Biochemistry*, 1987, **26**, 1940.
5 A. Kashem, M. Anisuzzaman and R. L. Whistler, *Carbohydr. Res.*, 1978, **61**, 511.
6 J. L. Navia, EP 0 352 048, 1989.
7 S. Singh, C. M. Maynard, R. J. Doyle and K. G. Taylor, *J. Org. Chem.*, 1984, **49**, 977.
8 B. S. Griffin and A. Burger, *J. Am. Chem. Soc.*, 1955, **78**, 2336.
9 B. Golinelli-Pimpaneau, F. Le Goffic and B. Badet, *J. Am. Chem. Soc.*, 1989, **111**, 3029.