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Arabinose-5-Phosphate Oxime *vs* its Methylenephosphonate Mimetic as High Energy Intermediate of the Glucosamine-6P Synthase Catalyzed Reaction

Corentin Le Camus, Marie-Ange Badet-Denisot and Bernard Badet*

Institut de Chimie des Substances Naturelles-C.N.R.S.

91198 Gif-sur-Yvette, France,

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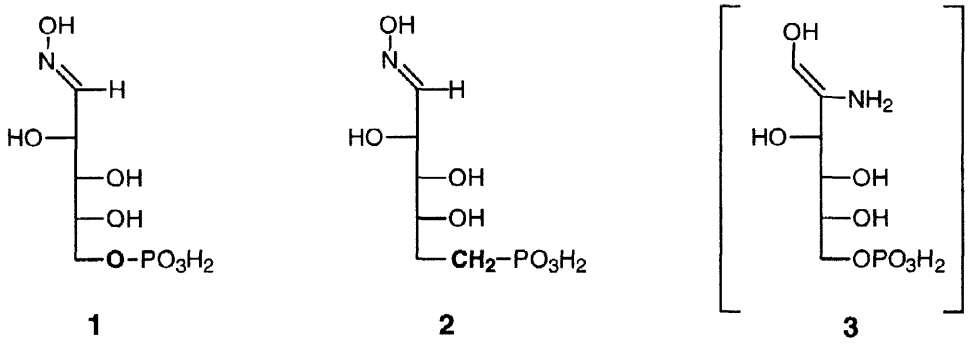
Abstract: Arabinose-5-phosphate oxime (**1**) was found to be the most potent competitive inhibitor of the glucosamine-6P synthase sugar site to date. Stereoselective synthesis of the hydrolytically stable methylenephosphonate analogue (**2**) gave, in 9% yield from D-arabinose, a compound exhibiting a 25 fold weaker inhibitory activity.

The reaction catalyzed by glucosamine-6-phosphate synthase (L-glutamine:D-fructose-6-phosphate amidotransferase, GlmS) is the first step committed to the amino-sugar biosynthetic pathway[1] of all living organisms. This is in particular the only endogeneous access to hexosamines which are absolutely required in the edification of microbial cell walls.

In the course of our search for transition state analogues of this reaction, the oxime **1** was considered to mimick reasonnably well the enolamine **3**, a postulated intermediate of the catalysis[2] (scheme 1). Because of the sensibility of the phosphate group towards hydrolysis[3], the phosphonate **2** was selected as a stable analogue of **1**.



1 **2** **3**

Scheme 1

Compound **1** was obtained quantitatively from the reaction of the commercially available arabinose-5-phosphate with hydroxylamine[4,5]. Compound **2** was synthesized in 6 steps from the previously described aldehyde **4**[6]. Reaction of **4** with tetraethyl methylenediphosphonate according to the Horner-Emmons methodology[7] gave the vinylphosphonate **5** (70% yield) quantitatively reduced into **6** using hydrogen and platinum oxide.

Deprotection of the anomeric hydroxyl group with 2M sulfuric acid in acetic acid afforded compound **7** in 55% yield. The phosphonic esters of **7** were hydrolyzed by reaction with trimethylsilyl bromide giving **8** (50% yield) which upon catalytic hydrogenation of the benzyl ethers yielded the arabinose-5-methylenephosphonate **9**.

* Fax: (33-1) 69 07 72 47; e-mail: Bernard.Badet@icsn.cnrs-gif.fr

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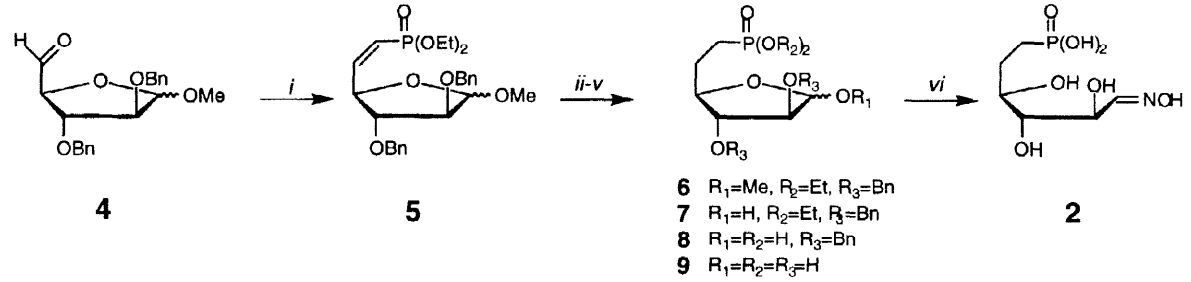

i) $[(EtO)_2PO]_2CHNa$/Et $_2O$-0°C; *ii)* H_2/PtO_2/EtOH-rt; *iii)* 2M H_2SO_4/AcOH-60°C; *iv)* Me_3SiBr neat-rt; *v)* H_2/Pd-C/EtOH-rt; *vi)* NH_2OH,HCl-MeONa/MeOH-60°C.

Scheme 2

Reaction of the latter compound with hydroxylamine gave quantitatively the desired oxime **2**[4,5] as a dicyclohexylamine monosalt after lyophilization.

When tested against purified *E.coli* GlmS[8], **1** behaved as a good competitive inhibitor *vs* fructose-6P with a K_i of 14.3 µM as determined from a Lineweaver-Burk plot. The value of K_m/K_i (≈28) is consistent with **1** being a high energy intermediate analogue of the GlmS-catalyzed keto/aldose isomerization[9]. However **2** exhibited a lower affinity for the enzyme (K_i= 0.36 mM, K_m/K_i ≈1). This 25 fold difference (0.36/0.0143) corresponds to a 2 kcal/mole decrease in the binding affinity of **2** *vs* **1** for the enzyme active site. Such a value might hardly correspond to the loss of a hydrogen bond (typically in the range 3-6 kcal/mole)[10] reflecting the change in the pK_{a2} from phosphate to methylenephosphonate (7.6 to 6.4). It is however consistent with the loss of one interaction between uncharged functions (typically 0.5-1.8 kcal/mole) and might reflect the replacement of the oxygen bridge by a methylene function. The evaluation of fluoromethylene- and difluoromethylenephosphonate equivalents of **1** shall help analyzing the origin of this difference.

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References and notes

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5. Selected analytical data: **1**: ^1H NMR (D_2O) δ 4.2-3.7 (m, 4H, H3 + H4 + 2H5), 5.2/4.6 (2dd, 1H, H2 Z/E), 7.7/7.0 (2d, 1H, H1 E/Z); **2** ^1H NMR (D_2O) δ 2.2-1.1 (m, 24H, dicycl. + H5-H6), 3.8-3.4 (m, 4H, H3 + H4 + 2H dicycl.), 5.2/4.5 (2dd, 1H, H2 Z/E), 7.6/6.9 (2d, 1H, H1 E/Z); ^{13}C NMR (CD_3OD): δ 25.5/24.0 (C6), 28.3 (C5), 54.3/30.2/25.8/25.2 (dicycl.), 69.8 (C4), 72.6 (C3), 76.3/75.6 (C2), 154.9/153.1 (C1); ^{31}P (D_2O): δ 26.3 (s); MS (FAB, NBA + LiCl): m/z= 425 (6, M + H^+), 413 (7, M - H_2O+ Li^+), 182 (100, dicycl.); Anal. for $C_{18}H_{37}N_2O_7P$ NaCl, $3H_2O$: (%) calculated: C 40.3, H 8.1, N 5.2, Cl 6.6, found: C 40.06, H 8.0, N 5.03, Cl 6.88
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