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# Competitive inhibitors of yeast phosphoglucose isomerase: synthesis and evaluation of new types of phosphorylated sugars from the synthon D-arabinonolactone-5-phosphate

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**Abstract**

Designed as competitive inhibitors of the isomerization reaction catalyzed by the potential chemotherapeutic target phosphoglucose isomerases (PGI), D-arabinonamide-5-phosphate and D-arabinonhydrazide-5-phosphate were synthesized and fully characterized. These new types of phosphorylated sugar derivatives were easily and efficiently obtained in a one-step procedure from the promising synthon D-arabinono-1,4-lactone 5-phosphate. These two compounds proved to be new good competitive inhibitors of yeast PGI with the substrate D-fructose-6-phosphate, though not as strong as D-arabinonhydroxamic acid-5-phosphate. Overall, our results are in accord with the postulated 1,2-*cis*-enediolate species as a probable high-energy intermediate of the PGI-catalyzed reaction. 

*Keywords:* Enzyme inhibitors; Hydroxamic acids; Phosphoglucose isomerase; Sugar phosphates

## 1. Introduction

The phosphoglucose isomerases (PGI or D-glucose-6-phosphate isomerases, EC 5.3.1.9) catalyze the reversible isomerization reaction outlined in Scheme 1 between D-glucose-6-phosphate (G6P) and D-fructose-6-phosphate (F6P) [1]. In addition to isomerase activity, PGI have been shown to display anomerase activity between pyranose anomers of G6P [2], between furanose anomers of F6P [3] and between those of D-mannose-6-phosphate [4], as well as C-2-epimerase activity on G6P [5]. Recently, a side PGI-like activity was shown to be associated with the carboxyl-terminal domain of *Escherichia coli* D-glucosamine-6-phosphate synthase [6].

Phosphoglucose isomerases play a central role in the metabolism of phosphorylated sugars, since their two substrates, G6P and F6P, are not only intermediate species in the glycolytic and gluconeogenic metabolic pathways, but also in the pentose phosphate pathway (in which PGI is a key enzyme in the recycling of F6P), glycoprotein synthesis and inositol synthesis [7]. This key role explains the strong impact of PGI deficiency in humans

*Abbreviations:* F6P, D-fructose-6-phosphate; G6P, D-glucose-6-phosphate; HEI: high-energy intermediate; PGI, phosphoglucose isomerases; TIM, triosephosphate isomerases; TMS, trimethylsilyl; YPGI, yeast phosphoglucose isomerase.

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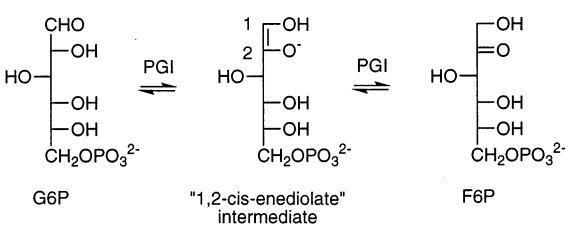
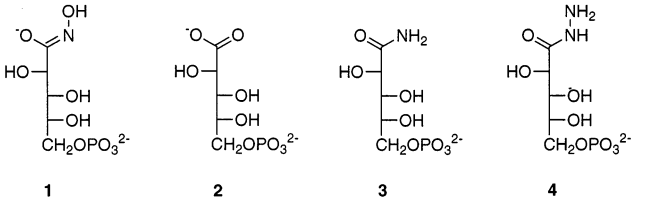
[8] as well as the interest as a therapeutic target in parasite metabolism [9–12]. Although the primary sequences from a number have been reported [13,14], the high-resolution 3D structural features of PGI remain unknown. Finally, only a very few potent high-energy intermediate (HEI) analogue inhibitors of the PGI-catalyzed reaction have been reported so far [15–17]. We have recently reported D-arabinonhydroxamic acid-5-phosphate (**1**) (depicted in Scheme 2 in its hydroximate form) as the best competitive inhibitor of yeast, rabbit muscle and *B. stearothermophilus* PGI-catalyzed reactions to date [16]. To our knowledge, D-arabinonate-5-phosphate (**2**) is the only other strong HEI analogue inhibitor of the PGI-catalyzed reaction. Both inhibitors have been postulated to be in anionic forms at the PGI active site, i.e., in their respective hydroximate and carboxylate forms (Scheme 2) [16,17]. In order to provide new tools to determine further which parameters are significant in the design of strong PGI inhibitors, we report in this paper the synthesis of two neutral analogues, D-arabinonamide-5-phosphate (**3**) and D-arabinonhydrazide-5-phosphate (**4**) (Scheme 2) derived from the synthon D-arabinono-1,4-lactone 5-phosphate (**5**) [16], as well as their inhibitory properties on yeast PGI (YPGI). We also give, in Section 3, details of the syntheses of compounds **1** and **5** not reported in our previous communication [16].

## 2. Results and discussion

*Preparation of HEI analogues.*—Our approach in this search for new PGI inhibitors was to develop synthetic strategies avoiding lengthy protection/deprotection steps. D-Arabinono-1,4-lactone 5-phosphate (**5**) [16], easily




Scheme 1. Isomerization reaction catalyzed by phosphoglucose isomerases (PGI).




Scheme 2. D-Arabinonhydroximate-5-phosphate (**1**), D-arabinonate-5-phosphate (**2**), D-arabinonamide-5-phosphate (**3**), and D-arabinonhydrazide-5-phosphate (**4**) as models of the postulated *cis*-1,2-enediolate high-energy intermediate species of the PGI-catalyzed isomerization reaction.

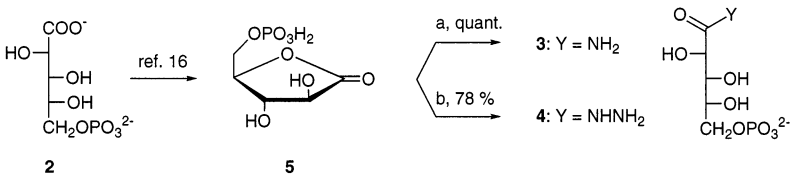
obtained from the barium salt of **2** [16,17], led us to synthesize in one-step procedures two new types of phosphorylated sugars, namely D-arabinonamide-5-phosphate **3** and D-arabinonhydrazide-5-phosphate (**4**), in addition to D-arabinonhydroxamic acid-5-phosphate (**1**) [16] previously obtained according to the same strategy (Scheme 3).

D-Arabinono-1,4-lactone 5-phosphate (**5**) was obtained by lyophilization of the acidic form of **2** in water according to the same procedure known in the case of non-phosphorylated aldonolactones [18]. Besides the NMR data, **5** exhibits a caracteristic C=O band at 1779 $cm^{-1}$. It should be noted that when lyophilization was not totally achieved, D-arabinonic acid-5-(dihydrogenophosphate) could be detected (but not isolated) through its characteristic C-1 resonance at 177.4 ppm (Table 1). D-Arabinonamide-5-phosphate (**3**) and D-arabinonhydrazide-5-phosphate (**4**) were directly obtained from **5** by treatment with, respectively, liquid ammonia and hydrazine, according to the respective procedures reported for the preparation of non-phosphorylated aldonamides [19] and aldonhydrazides [20] from aldonolactones. Each of the above products was fully characterized, as evidenced

Table 1
$^{13}C$ NMR data of D-arabinonic-5-phosphate derivatives [a]

| Cpd | C-1 | C-2 | C-3 | C-4 | C-5 | $J_{4,P}$ | $J_{5,P}$ |
|---|---|---|---|---|---|---|---|
| **2** [b] | 180.4 | 72.4 | 72.4 | 71.4 | 66.3 | 5.9 | 4.3 |
| **2** [c] | 177.4 | 71.0 | 71.7 | 69.8 | 68.1 | 8.1 | 5.2 |
| **3** | 179.7 | 71.7* | 71.5* | 70.7 | 66.1 | 6.2 | 4.3 |
| **4** | 174.7 | 71.8* | 71.3* | 70.6 | 65.9 | 5.5 | 3.7 |

[a] $^{13}C$ NMR data of **1** and **5** were previously reported [16].
[b] Trisodium salt.
[c] Acidic form (not isolated).




2 5

Scheme 3. Conditions and reagents: (a) 1-$NH_3$, −78 °C, 20 min. (b) 1-$H_2NNH_2$, 20 °C, 60 min, 2-Bio-Gel® P-2.

Table 2
Inhibitory effect of D-arabinonhydroximate-5-phosphate **1**, D-arabinonate-5-phosphate **2**, D-arabinonamide-5-phosphate **3**, and D-arabinonhydrazide-5-phosphate **4** on yeast phosphoglucose isomerase

| Compound | $K_i$ (μM) | $K_m/K_i$ [a] |
|---|---|---|
| **1** [b] | 0.23 ± 0.02 | 300 |
| **2** [b] | 2.1 ± 0.1 | 33 |
| **3** | 4.2 ± 0.9 | 16 |
| **4** | 8.0 ± 0.8 | 9 |

[a] $K_m$ (F6P) = 69 ± 6 μM.
[b] Ref. [16].

from their $^{13}C$ NMR data (Table 1) and high-resolution MS analysis of their per-trimethylsilyl (TMS) derivatives (see Section 3).

*Comparative evaluation of* **1**, **2**, **3** *and* **4** *for inhibition of yeast PGI.*—The evaluation of compounds **1**, **2**, **3** and **4** as inhibitors of yeast PGI-catalyzed isomerization of F6P was carried out in the same experimental conditions for comparative purpose. Apparent Michaelis constants ($K_m$) and inhibition constants ($K_i$) were determined (Table 2) from double reciprocal plots of the initial reaction velocity versus F6P concentration obtained at various concentrations of inhibitor (Lineweaver–Burk graphical representation) as described in Section 3.

Competitive inhibition of YPGI, previously reported for **1** and **2**, was also observed for **3** and **4**, which are to our knowledge the first amide and hydrazide inhibitors of an aldose–ketose isomerase-catalyzed reaction (Fig. 1). As shown from the $K_m/K_i$ ratios reported in Table 2, **1** remains by far the best PGI inhibitor reported to date. Despite its p$K_a$ in solution of 9.6, we had postulated that the anionic form of **1**, namely D-arabinonhydroximate-5-phosphate (Scheme 2), was the mimic of the 1,2-cis-enediolate high-energy intermediate [16]. The $K_m/K_i$ values for **3** and **4** were lower than that observed for **2** and a fortiori for **1** (Table 2). In view of these results, the anionic character of the HEI analogue appears to be a significant parameter in the design of strong PGI inhibitors. It seems likely that electrostatic stabilization of a 1,2-cis-enediolate HEI plays a significant role in the catalytic effect of PGI, as previously postulated in the case of TIM-catalyzed isomerization of dihydroxyacetonephosphate to D-glyceraldehyde-3-phosphate [21]. Furthermore, quantum mechanical studies are in accord with a 1,2-cis-enediolate species as a

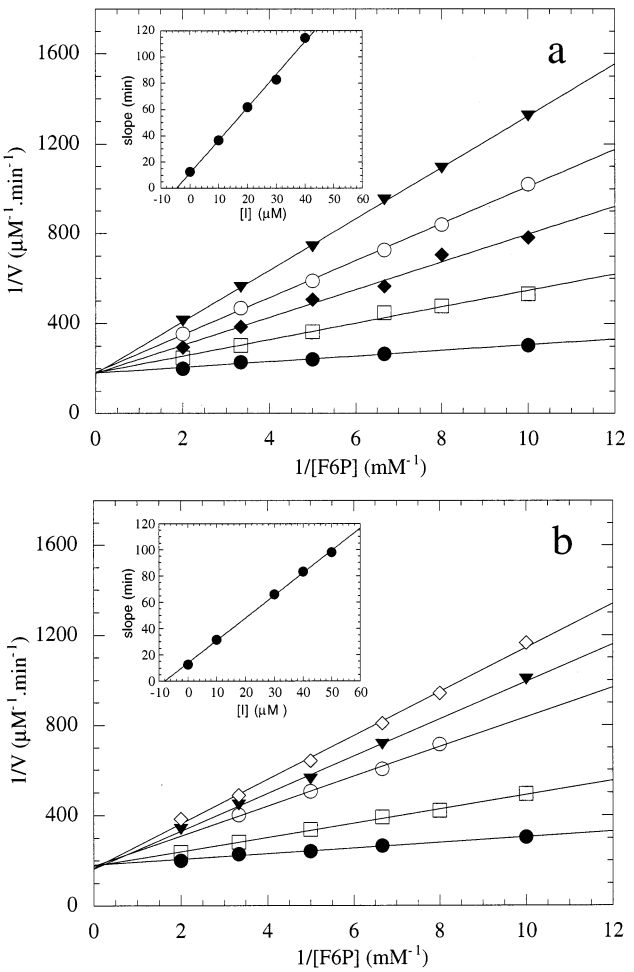



Fig. 1. Inhibition of YPGI. Double reciprocal plot of initial reaction velocity versus F6P concentration obtained at various concentrations of inhibitors **3** (a) and **4** (b): ●, no inhibitor; □, [I] = 10 μM; ◆, [I] = 20 μM; ○, [I] = 30 μM; ▼, [I] = 40 μM; ◇, [I] = 50 μM. See Section 3 for details on kinetic assay conditions.

very likely HEI of the TIM-catalyzed isomerization [22,23]. We postulate that the same conclusion stands in the case of PGI. In the case of the two anionic inhibitors, it is interesting to note that the ratio $K_i$(**1**)/$K_i$(**2**) is in the range of 10, which corresponds to a 1.4 kcal $mol^{-1}$ increase in the binding affinity of **1** versus **2** for the enzyme active site. This difference might appear relatively weak considering the much better analogy of **1** versus **2** with the postulated 1,2-cis-enediolate intermediate. Interactions of different natures are probably involved for the hydroximate and carboxylate functions with the enzyme active site residues.

In conclusion, these competitive inhibitors seem very promising tools in order to further develop the mechanistic, structural and therapeutic investigations on phosphoglucose isomerases. It clearly appears that other phosphoaldonolactones might be obtained following a similar approach, which could be potential synthons for easy access to new C-1-modified derivatives of phosphoaldoses, and consequently, to new inhibitors of the corresponding isomerases.

## 3. Experimental

*General methods.*—Optical rotations were determined on a Jasco model DIP-370 polarimeter in water solutions. NMR spectra were recorded in $D_2O$ with Bruker AC 200 or AC 250 spectrometers. $^1H$, $^{13}C$ and $^{31}P$ spectra were recorded at, respectively, 200 (or 250) MHz, 50.3 or (62.5) MHz, and 101.2 MHz. These spectra were referenced, respectively, to residual internal HOD (4.80 ppm), to internal 1,4-dioxane (67.40 ppm) and to an external 85% $Na_2HPO_4$ aq soln (0.00 ppm). For $CH_2$ groups, H-5 resonances appear arbitrarily at higher field than their respective geminal hydrogen H-5′. Chemical shifts are reported in $\delta$ units downfield from tetramethylsilane, and coupling contants $J_{i,j}$ in Hertz. Exchangeable resonances are designated by an asterisk. Infrared spectra were recorded with a FTIR Bruker IFS-66 spectrometer. Mass spectrometry (MS) analyses were performed by electrospray with negative ionization, using 1 mg $mL^{-1}$ of a 1:1 water–MeOH soln. High-resolution mass spectrometry analyses of the pertrimethylsilylated derivatives [24] were performed by electron impact (HR-EIMS) with negative ionization. Kinetic studies were performed with 1 mL quartz cuvettes on a UV–vis Safas 190 DES spectrophotometer, the sample compartment of which was thermostated at $30.0 \pm 0.1$ °C with a Heto circulating water bath. Evaporations were performed under diminished pressure below 35 °C. Unless otherwise stated, chemicals (used at their commercial purity) were obtained from Acros and solvents from SDS. Final products were stored at $-18$ °C.

D-*Arabinonhydroxamic acid-5-phosphate, monohydroxylammonium salt* (**1**) [16].—The following procedure was entirely achieved under inert atmosphere (argon), at room temperature (rt), and using freshly prepared anhydrous MeOH (distilled over Mg and $I_2$). A methanolic solution of NaOMe (2 M, 5.7 mmol) was prepared under argon from anhyd MeOH (3 mL) and sodium (131 mg, 5.7 mmol). This solution was then added to a methanolic solution (8.5 mL) of hydroxylamine hydrochloride (400 mg, 5.7 mmol) through an argon-purged syringe. A white solid precipitated (NaCl). Following decantation and taking care not to withdraw solid NaCl, the clear methanolic solution of hydroxylamine was then transferred dropwise to a MeOH solution (5 mL) of **5** (131 mg, 0.57 mmol) using a second argon-purged syringe. The reaction was allowed to proceed for 1 h at rt, and the solvent was evaporated under reduced pressure. Following purification by size-exclusion chromatography (water) on a Bio-Gel® P-2 resin from Bio-Rad (200–400 mesh, 25 g, $h = 95$ cm, $d = 1$ cm) and lyophilization, compound **1** was obtained in 69% yield as a white powder (159 mg, purity $= 72\%$, 0.39 mmol; although **1** was the only sugar obtained, it was still contaminated by the presence of salts): $[\alpha]_D^{28}$ $-21.4°$ ($c$ 1.1, water); positive $Fe^{3+}$ test ($\lambda_{max} = 481$ nm, $\varepsilon = 568$ $M^{-1}$ $cm^{-1}$); FTIR (KBr) $\nu$ 3340 (OH), 2922 (CH), 1654 (hy-

droxamic acid and $HONH_3^+$), and 1093 (OH and $PO_4^-$) $cm^{-1}$; $^1H$ NMR ($D_2O$): $\delta$ 4.46 (br s, 1 H, H-2), 3.71–3.99 (m, 4 H, H-3,4,5,5'); $^{31}P$ NMR ($D_2O$): $\delta$ 3.82; $^{13}C$ NMR and high-resolution MS data: see Ref. [16].

D-*Arabinono-1,4-lactone 5-(dihydrogenophosphate)* (**5**) [16].—Compound **2**, barium salt (50 mg, 0.11 mmol) obtained from either G6P [16] or F6P [17] was first solubilized in water (6 mL) by stirring with a Dowex® 50WX8-100 cation-exchange resin ($H^+$ form, 1 g). The resin was then removed by filtration on a glass funnel, and the solution was adsorbed on a Dowex® 50WX8-100 cation-exchange resin column ($H^+$ form, 3 g, $h = 14.5$ cm, $d = 1$ cm). Following elution of the product with water until the eluent reached neutral pH (approx. 60 mL), removal of most of the solvent under reduced pressure, and finally, lyophilization, the title compound was obtained as a colorless syrup in quantitative yield (26 mg, 0.11 mmol): $[\alpha]_D^{28}$ + 28.6° (*c* 3.2, water); FTIR (KBr) *v* 3382 (alcohol), 2919 (CH), 1778 (lactone), 1424–1384 (lactone), and 1130–1067 (OH and $PO_4^-$) $cm^{-1}$; $^1H$ NMR ($D_2O$): $\delta$ 4.48 (d, 1 H, $J_{2,3}$ 8.8 Hz, H-2), 4.19 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.32 (ddd, 1 H, $J_{4,5}$ 4.4, $J_{4,5'}$ 2.4 Hz, H-4), 4.00 (ddd, 1 H, $J_{5,5'}$ − 12.7, $J_{5,P}$ 6.8 Hz, H-5), 4.16 (ddd, 1 H, $J_{5',P}$ 5.8 Hz, H-5'); $^{31}P$ NMR ($D_2O$): $\delta$ 3.34; $^{13}C$ NMR and high-resolution MS data: see Ref. [16].

D-*Arabinonamide-5-phosphate*, *diammonium salt* (**3**).—In a round-bottom flask placed under argon, containing D-arabinolactone-5-phosphate **5** (88 mg, 0.39 mmol) and cooled in a dry-ice/isopropanol bath (− 78 °C), approximately 10 mL of anhyd liquid ammonia were condensed. The reaction mixture was left to proceed for 10 min. Then, the flask was taken off the dry-ice/isopropanol bath in order for the liquid ammonia to evaporate at room temperature. Complete removal of liquid ammonia was achieved with a stream of argon. Following drying over $P_2O_5$ under reduced pressure for 4 days, the title compound **3** (114 mg, quantitative yield) was obtained as a white solid: − 8.3° (*c* 1.1, water); FTIR (KBr) *v* 3201 (OH), 1671 (amide), 1401 (amide), and 1097 (OH and $PO_4^-$) $cm^{-1}$; $^1H$ NMR ($D_2O$): $\delta$ 4.24 (d, 1 H, $J_{2,3}$ 1.5 Hz, H-2), 3.80–3.56 (m, 4 H, H-3,4,5,5'); $^{31}P$ NMR ($D_2O$): $\delta$ 4.72; HR-EIMS (negative mode, TMS derivative): Anal. Calcd for $C_{23}H_{60}NO_8PSi_6$ $[M]^{\bullet -}$: 677.2672, Found: 677.2687.

D-*Arabinonhydrazide-5-phosphate*, *monohydrazinium salt* (**4**).—In a round-bottom flask placed under argon, equipped with a septum and containing D-arabinono-1,4-lactone 5-phosphate **5** (91 mg, 0.40 mmol) in anhydrous MeOH (5 mL), was added dropwise and through a syringe hydrazine, monohydrate (70 μL, 1.44 mmol). A white precipitate appeared immediately. The reaction mixture was stirred at rt for 1 h. After removal of the solvent under reduced pressure, the crude solid was purified by size-exclusion chromatography (water) on a Bio-Gel® P-2 resin from Bio-Rad (200–400 mesh, 25 g, $h = 95$ cm, $d = 1$ cm), and subsequently lyophilized to give the title compound **4** (76 mg, 78%) as a white solid: + 19.6° (*c* 1.6, water); FTIR (KBr) *v* 3323 (OH), 1631 (hydrazide), and 1097 (OH and $PO_4^-$) $cm^{-1}$; $^1H$ NMR ($D_2O$): $\delta$ 4.36 (d, 1 H, $J_{2,3}$ 1.5 Hz, H-2), 3.89–3.62 (m, 4 H, H-3,4,5,5'); $^{31}P$ NMR ($D_2O$): $\delta$ 4.40; HR-EIMS (negative mode, TMS derivative): Anal. Calcd for $C_{20}H_{53}N_2O_8PSi_5$ $[M]^{\bullet -}$: 620.2386, Found: 620.2384.

*Enzyme kinetic assays*.—Enzymes, β-nicotinamide adenine dinucleotide phosphate (NADP, sodium salt), F6P and G6P (disodium salts) were purchased from Sigma Chemical Company. Yeast PGI was assayed at 30 °C spectrophotometrically ($\lambda = 340$ nm) in the direction from F6P to G6P by coupling to yeast D-glucose-6-phosphate dehydrogenase (PGDH) and NADP [25,26]. The reaction mixture (1 mL) had the following composition (final concentrations): 50 mM TRIS, pH 8.0; 0.1–0.5 mM F6P; 1 mM EDTA; 0.4 mM NADP; 0.0–0.2 mM selected inhibitor. To ensure valid assay conditions, several precautions were taken. Sufficient PGDH (0.6 U)[1] was added and the reaction mixture was allowed to pre-incubate for 6–8 min. The reaction was then initiated by the addition of the PGI (0.0051 U). The ratio of activities in term of units of PGDH to PGI was kept at 120:1.

[1] 1 unit (U) is defined as that quantity of enzyme which will convert 1 mmol of substrate to product per min at 30 °C.

It was also ascertained that the compounds studied did not inhibit the dehydrogenase sufficiently to alter isomerase velocities. Kinetic data were analyzed by the graphical method of Lineweaver–Burk, and the values of the slopes of the straight lines in the primary graphs were plotted against the inhibitor concentrations. The inhibitor concentration obtained for the intercept corresponds to $-K_i$. Each $K_i$ value is based upon about 25 assays performed at four or five inhibitor concentrations.

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