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# Synthesis and evaluation of a new inhibitor of phosphoglucose isomerases: the enediolate analogue 5-phospho-D-arabinohydroxamate

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**Abstract**

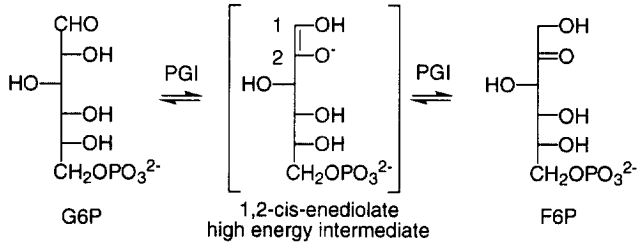
Designed as a high energy intermediate analogue inhibitor of the potent chemotherapeutic target phosphoglucose isomerases, 5-phospho-D-arabinohydroxamate was efficiently synthesized in a two steps procedure. To date, it proved to be the strongest competitive inhibitor with respect to substrate D-fructose-6-phosphate ($K_i$ down to 98 nM and $K_m/K_i$ values up to 513). A comparative inhibition study of this compound and other known strong inhibitors on phosphoglucose isomerases from three different sources is also reported.



*Keywords:* Carbohydrates; Enzyme inhibitors; Hydroxamic acids; Phosphoric acid and derivs.

## 1. Introduction

The phosphoglucose isomerases (PGI, phosphoglucose isomerases 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




Scheme 1. Isomerization reaction catalyzed by D-glucose-6-phosphate isomerases.

(G6P) and D-fructose-6-phosphate (F6P).[1,2] PGI are of interest for a number of reasons. First, these enzymes play a central role in the metabolism of phosphorylated sugars (glycolysis, gluconeogenesis and phosphate pentose pathways).[3] Second, PGI are involved in various and important pathological processes: (i) PGI deficiency is a well-known congenital disorder with the typical manifestation of nonspherocytic hemolytic anemia in humans,[4] (ii) since the observation of increased glycolytic activity in tumor cells,[5,6] numerous reports have described the use of glycolytic enzymes, e. g. PGI,[6] as tumor progression markers, (iii) very recently, PGI has been suggested to be closely related to the tumor cell autocrine motility factor,[7] a tumor-secreted cytokine which

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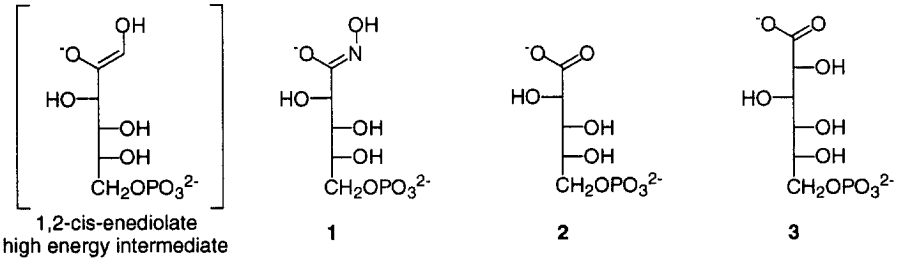
stimulates cell migration *in vitro* and metastasis *in vivo*, (iv) because parasites like *Trypanosoma brucei* and *Plasmodium falciparum* derive most of their energy from glycolysis,[8] PGI and other glycolytic enzymes are attractive targets for chemotherapeutic actions. Third, and surprisingly, the precise structural features of PGI remain unknown: (i) the primary sequences from a number of sources were determined only very recently,[7,9] (ii) only two X-ray diffraction studies of the enzyme have been reported which led to structures that still need refinement in order to identify active site residues involved in the isomerization mechanism.[10] Fourth, only a very few potent high energy intermediate (HEI) analogue inhibitors of the PGI-catalyzed reaction have been reported so far.[11] These reasons led us to undertake the synthesis and evaluation of the potent HEI analogue inhibitor 5-phospho-D-arabinohydroxamate **1**, for which we report a very convenient synthesis. Using identical experimental conditions, we also report a comparative study of its inhibitory properties with respect to the known competitive inhibitors 5-phospho-D-arabinonate **2**[12] and 6-phospho-D-gluconate **3**[2,13] (Scheme 2) on three PGI from yeast (YPGI), rabbit muscle (RmPGI) and *Bacillus stearothermophilus* (BsPGI).



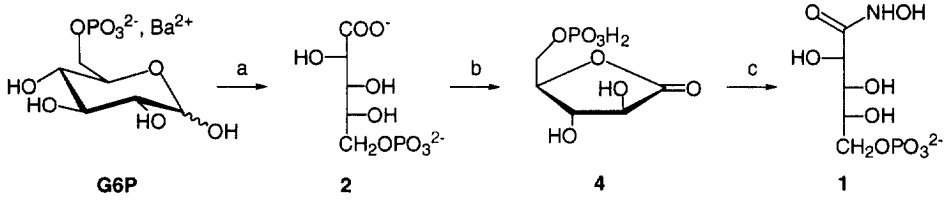

Scheme 2. Models of the cis-1,2-enediolate high-energy intermediate species of the PGI-catalyzed isomerization reaction.

## 2. Results and Discussion

The mechanism of isomerization of PGI has been postulated to involve a proton transfer through a 1,2-cis-enediolate HEI (Scheme 1),[1,12,14-16] similar to that observed in the triosephosphate isomerase (TIM) catalyzed isomerization of dihydroxyacetone-phosphate to D-glyceraldehyde-phosphate. The hydroxamate function closely mimics the 1,2-cis-enediolate HEI, with the atoms $O_1$, N, $C_2$ and $O_2$ coplanar (Scheme 2). This particular feature generally confers to hydroxamate-based inhibitors excellent inhibition properties, as shown for example with the TIM inhibitor phosphoglycolohydroxamate.[17] For xylose isomerase catalyzed reactions, where a hydride shift mechanism has been shown to operate, D-threonohydroxamate appears to have a close structural relationship to the proposed rearrangement transition state.[18] Because of their metal-complexing properties, numerous reports have described the use of hydroxamate-based inhibitors with metalloenzymes.[19]

### *2.1 Preparation of high energy intermediate analogues*

G6P, which has the same absolute configuration at carbon atoms C-3, C-4 and C-5 as compounds **1** and **2** have at carbons C-2, C-3 and C-4, was considered as suitable starting compound (Scheme 3). The synthesis of




Scheme 3. Conditions and reagents: a) 1-Dowex® 50WX8-100/$H^+$, 2-$O_2$/NaOH, 72 hr, 20°C, 3-HCl 1N, 4-$Ba(OH)_2$. b) 1-Dowex® 50WX8-100/$H^+$, 2-Freeze-drying, 78 % (2 steps). c) 1-MeONa/MeOH, $H_2NOH$.HCl, 20°C, 60 min, 2-Bio-Gel® P-2, 69 %.

**2** was achieved from the commercially available barium salt of G6P, using a modified procedure first reported for the synthesis of this product from the more expensive disodium salt of F6P.[12] The barium salt of **2** was converted to its sodium salt by ion exchange chromatography. D-Arabinolactone-5-dihydrogenophosphate **4** was simply obtained by freeze-drying of an aqueous solution of the acidic form of **2** (overall yield from G6P: 78%). Addition of an hydroxylamine methanolic solution to compound **4**, according to the strategy used for the synthesis of non-phosphorylated aldonohydroxamic acids from aldonolactones,[20] and subsequent desalting by size exclusion chromatography on Bio-Gel® P-2, afforded 5-phospho-D-arabinohydroxamate **1** in 69 % yield. In view of the spectroscopic data obtained for **1** which are in full agreement with the assigned structure,* it appears that the synthetic scheme we had previously reported did not lead to the same compound.[21] In this previous report, **1** was thought to have been obtained from hydrogenolysis of its perbenzylated precursor. A reexamination of the spectroscopic, kinetic and analytical data would indicate that during this last step, the hydroxamic group would have undergone a transformation into carboxylic acid and hydroxylamine.

*2.2 Comparative evaluation of **1** versus **2** and **3** for inhibition of PGI*

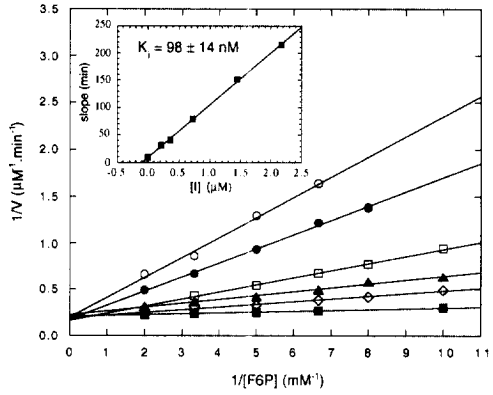



Figure 1. Inhibition of BsPGI (50 mM tris.HCl buffer, 30 °C, pH = 8.0). Double reciprocal plot of initial reaction velocity versus F6P concentration obtained at various concentrations of inhibitor 1 versus F6P concentration (Lineweaver-Burk graphical representation): ■, no inhibitor; ◇, [I] = 0.22 µM; ▲, [I] = 0.36 µM; □, [I] = 0.72 µM; ●, [I] = 1.45 µM; ○, [I] = 2.17 µM. Lines drawn obtained from a non-linear least squares fit to the observed data using Michaelis-Menten equation for competitive inhibition.

Figure 1 clearly demonstrates competitive inhibition of BsPGI by **1** with a $K_i$ value of 98 nM. Competitive inhibition was also observed for YPGI and RmPGI. In the experimental conditions we used, the $K_i$ values for **1** with YPGI, BsPGI and RmPGI are lower than the values for **2**, the strongest known PGI inhibitor so far, and *a fortiori* for **3** (Table 1). The order of the respective inhibition constants $K_i(\mathbf{1}) < K_i(\mathbf{2}) < K_i(\mathbf{3})$ remains identical

Table 1
Inhibitory effect of **1**, **2** and **3** on phosphoglucose isomerases from various origins[a]

| PGI | F6P[b] $K_m$ (µM) | **1** $K_i$ (µM) | $K_m/K_i$ | **2** $K_i$ (µM) | $K_m/K_i$ | **3**[b] $K_i$ (µM) | $K_m/K_i$ |
|---|---|---|---|---|---|---|---|
| YPGI | 69 ± 6 | 0.228 ± 0.018 | *303* | 2.1 ± 0.4 | *33* | 123 ± 12 | *0.6* |
| BsPGI | 44.7 ± 0.8 | 0.098 ± 0.014 | *456* | 1.0 ± 0.1 | *45* | 41 ± 6 | *1.1* |
| RmPGI | 100 ± 9 | 0.195 ± 0.006 | *513* | 1.47 ± 0.07[b] | *68* | 42 ± 5 | *2.4* |

[a] 50 mM tris.HCl buffer, pH=8.0, 30°C, ionic strength=17 mM (kinetic assays performed according to Noltmann[23]).
[b] Values in the order of the previously published ones determined in other experimental conditions.[2,12,15,22,24]

* Selected data: **4**: $^{13}C$ NMR ($D_2O$) δ 176.5 (C-1), 74.4 and 73.1 (C-2 and C-3), 80.1 (d, $J_{4P}$ 7.4 Hz, C-4), 64.1 (d, $J_{5P}$ 4.6 Hz, C-5); HR-MS (electrospray, negative ionization, TMS derivative): calcd for $C_{17}H_{41}O_8PSi_4$ (M): 516.1616, found: 516.1605. **1**: $^{13}C$ NMR ($D_2O$) δ 172.6 (C-1), 71.5 and 71.2 (C-2 and C-3), 70.4 (d, $J_{4P}$ 6.6 Hz, C-4), 66.3 (d, $J_{5P}$ 3.7 Hz, C-5); HR-MS (electrospray, negative ionization, TMS derivative): calcd for $C_{25}H_{65}NO_9PSi_7$ (M-$CH_3$): 750.2782, found: 750.2772.

whatever the enzyme source is. We can conclude, at least under our conditions, that **1** is the best competitive PGI inhibitor to date. The ratios $K_m/K_i$ for **1** reported in Table 1 ranging from 300 to 500 indicate that **1** rather behaves as a HEI analogue inhibitor of the PGI-catalyzed isomerization reaction than as a substrate analogue inhibitor. Similarly but to a lesser extend, **2** appears to also behave as a HEI analogue inhibitor with ratios $K_m/K_i$ in the order of 50. In the case of **3**, $K_m/K_i$ values in the order of 1 confirm that **3** acts more as a substrat analogue inhibitor. Hence, **1** appears as the best mimic ever synthesized of the 1,2-cis-enediolate HEI thought to be involved in the PGI-catalyzed isomerization of F6P.

Comparative evaluation of **1** for inhibition of YPGI, BsPGI and RmPGI in our conditions shows that this compound displays poor enzyme specificity: $K_i$ values do not vary that much among the three enzymes. The same conclusion stands for the other inhibitors **2** and **3**. These results are in agreement with the expected high degree of conservation of the active site of these enzymes.[22]

**1** seems to be a very promising compound, as it might also be a very good inhibitor of other enzymes, like for example D-mannose-6-phosphate isomerase[11] and D-glucosamine-6-phosphate synthase.[11,25] Our results confirm that hydroxamate-based compounds are the best analogues of the 1,2-cis-enediolate HEI proposed to be involved in many enzyme-catalyzed isomerizations, and that more specific derivatives might be of high chemotherapeutic interest. Our attention is now focused on biological evaluation of **1** and crystallographic studies of PGI and other related enzymes with this potent high energy intermediate analogue inhibitor at the active site.

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