

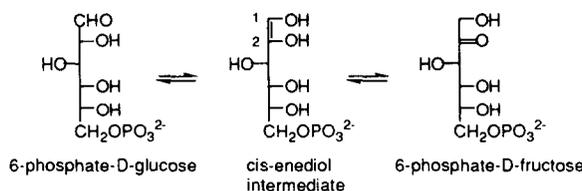
## Synthesis of 5-Phosphate-D-arabinohydroxamic Acid, a Potent Transition State Analogue Inhibitor of 6-Phosphate-D-glucose Isomerases

Corinne Bonnette, Laurent Salmon\* and Alain Gaudemer

Laboratoire de Chimie Bioorganique et Bioinorganique associé au CNRS,  
 Institut de Chimie Moléculaire d'Orsay, Université de Paris-Sud,  
 Bât. 420, 91405 Orsay, France

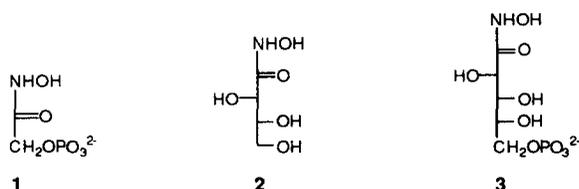
**Abstract:** The first hydroxamate-based and potent transition state analogue (TSA) inhibitor of 6-phosphate-D-glucose isomerases, 5-phosphate-D-arabinohydroxamic acid **3**, has been synthesized by conversion of D-arabinose to a protected derivative of 5-phosphate-D-arabinonic acid and introduction of the hydroxamate group by coupling with O-benzylhydroxylamine.

Phosphoglucose isomerases (PGI's, or 6-phosphate-D-glucose isomerases, EC 5.3.1.9), which catalyze the first isomerization step in D-glucose fermentation pathway, are present in most organisms.<sup>1</sup> The enzyme interconverts 6-phosphate-D-glucose and 6-phosphate-D-fructose (**Fig. 1**). PGI isomerization mechanism, through a probable proton transfer, involves a cis-enediol(ate) intermediate<sup>2</sup>, similar to that observed in the triosephosphate isomerase (TIM)-catalyzed isomerization of dihydroxyacetone-phosphate to D-glyceraldehyde-phosphate,<sup>3</sup> while the hydride shift mechanism has been proposed to operate with some other isomerases, e.g. D-xylose isomerases.<sup>4</sup>



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

By virtue of their structural similarity to the rearrangement transition state, hydroxamate-based inhibitors<sup>3,5</sup> have been shown to exhibit exceptional inhibition properties, e.g. phosphoglycolhydroxamate **1** and D-threono-hydroxamic acid **2** (**Fig. 2**), which are TSA inhibitors of TIM<sup>3</sup> and D-xylose isomerase,<sup>5a</sup>



**Figure 2.** Selected hydroxamate-based inhibitors.

respectively. Numerous reports have described the use of hydroxamate-based inhibitors with various other enzymes and proteins due in part to their metal-complexing properties.<sup>6</sup>

PGI plays a central role in the metabolism of phosphorylated sugars, since its substrates, 6-phosphate-D-glucose and 6-phosphate-D-fructose, are not only intermediate species in the glycolytic and gluconeogenic metabolic pathways, but also in the pentose phosphate pathway.<sup>7</sup> PGI is involved in various and important pathologic processes,<sup>8</sup> in particular in the development of parasitic diseases like malaria and sleeping sickness. Consequently, PGI is an attractive target for chemotherapeutic action.

The reported enzyme structures<sup>9</sup> still need considerable refinement in order to identify active site residues involved in the isomerization mechanism, by contrast with other isomerases like TIM<sup>10</sup> or D-xylose isomerase.<sup>4c-f</sup>

The need for a very good TSA inhibitor for PGI led us to undergo the synthesis of 5-phosphate-D-arabinohydroxamic acid **3** (Fig. 3) which, in addition to its structural similarity to the enediol(ate) intermediate, has the same stereochemistry as 6-phosphate-D-glucose (or 6-phosphate-D-fructose). To our knowledge, no hydroxamate-based phosphorylated sugar has ever been reported to date (except **1**).

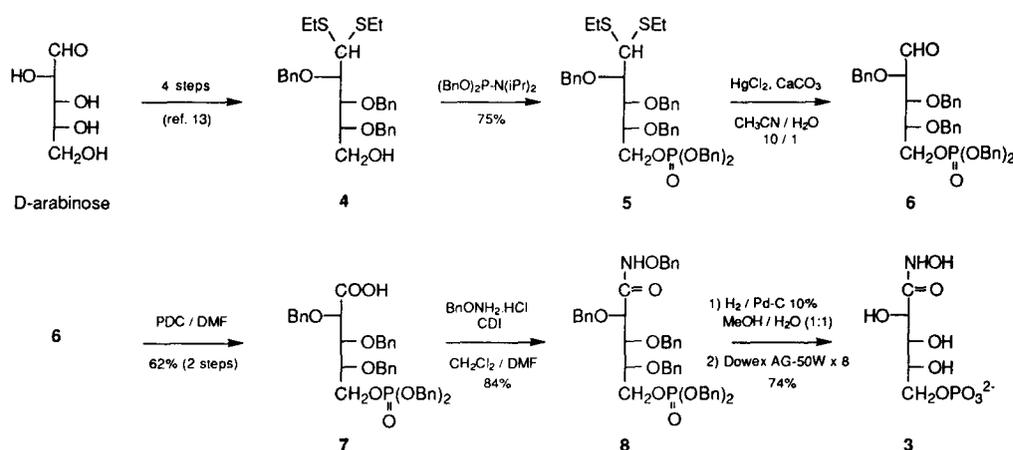


Figure 3. Synthesis of 5-phosphate-D-arabinohydroxamic acid **3**.

The starting product for the synthesis of **3** was D-arabinose, which has the same absolute configuration of carbon atoms C2, C3 and C4. Our strategy involved successive introduction of the phosphate group, and then of the hydroxamate group. D-Arabinose was first converted into the protected derivative **4**, which was selectively phosphorylated at C5. Deacetalation followed by oxidation led to the protected 5-phosphate-D-arabinonic acid derivative **7**, the precursor of 5-phosphate-D-arabinohydroxamic acid **3**<sup>11</sup> (5-phosphate-D-arabinonic acid, a known PGI inhibitor,<sup>8e,12</sup> might also probably be obtained from **7**).

**2, 3, 4-Tri-O-benzyl-D-arabinose diethyl dithioacetal 4** was prepared from D-arabinose in four steps according to the reported procedure.<sup>13</sup> **4** was also obtained in three steps from  $\beta$ -methyl-D-arabinopyranoside, which was first benzylated, then deacetalated and finally thioacetalated: however, the low overall yield (37%) and the high cost of the starting product led us to turn down this procedure.<sup>14</sup> Phosphorylation of **4** was achieved using dibenzoyloxy(diisopropylamino)phosphine<sup>15</sup> to give **5** in 75% yield. Dethioacetalation<sup>13</sup> of **5** with  $\text{HgCl}_2$  in the presence of  $\text{CaCO}_3$  gave the protected 5-phosphate-D-arabinose derivative **6**, which was converted into the corresponding acid **7** by oxidation with pyridinium dichromate (PDC)<sup>16</sup> with a yield of

62% (two steps). **7** was then reacted with O-benzylhydroxylamine in the presence of carbonyldiimidazole (CDI)<sup>17</sup> to give the protected phosphorylated hydroxamic acid derivative **8** in 84% yield. Hydrogenolysis of **8** using Pd/C 10 % catalyst in aqueous MeOH, followed by ion-exchange chromatography gave the disodium salt of 5-phosphate-D-arabinohydroxamic acid **3** in 74% yield. The spectroscopic data of **3** were in full agreement with the proposed structure. The presence of the hydroxamic function was further confirmed by its characteristic reaction with FeCl<sub>3</sub>.<sup>18</sup>

The results of the inhibition studies using **3** and known inhibitors with 6-phosphate-D-glucose isomerases from *Plasmodium falciparum* and other sources will soon be reported. **3** might also be a very good inhibitor of other enzymes, e.g. 6-phosphate-D-mannose isomerase and 6-phosphate-D-glucosamine synthase, which makes **3** a very promising compound.

**Acknowledgement:** This research was supported by the Centre National de la Recherche Scientifique (C.N.R.S.), the Ministère de la Recherche et de l'Enseignement Supérieur (M.R.E.S.) and the Institut de Formation Supérieure BioMédicale (I.F.S.B.M., Villejuif).

#### References and Notes

- Walch, C. *Enzymatic Reaction Mechanisms*; Freeman & Co.: San Francisco, 1979; pp. 586-590.
- (a) Liemans, V.; Malaisse-Lagae, F.; Willem, R.; Malaisse, W. J. *Biochim. Biophys. Acta* **1989**, *998*, 111-117. (b) Willem, R.; Malaisse-Lagae, F.; Ottinger, R.; Malaisse, W. J. *Biochem. J.* **1990**, *265*, 519-524. (c) Willem, R.; Biesemans, M.; Hallenga, K.; Lippens, G.; Malaisse-Lagae, F.; Malaisse, W. J. *J. Biol. Chem.* **1992**, *267*, 210-217. (d) Seeholzer, S. H. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 1237-1241.
- Collins, K. D., *J. Mol. Biol.* **1974**, *249*, 136-142.
- (a) Rose, I. A.; O'Connell, E. L.; Mortlock, R. P. *Biochim. Biophys. Acta* **1969**, *178*, 376-379. (b) Bock, K.; Meldal, M.; Meyer, B.; Wiebe, L. *Acta Chem. Scand.* **1983**, *B37*, 101-108. (c) Farber, G. K.; Glasfeld, A.; Tiraby, G.; Ringe, D.; Petsko, G. A. *Biochemistry* **1989**, *28*, 7289-7297. (d) Collyer, C. A.; Henrick, K.; Blow, D. M. *J. Mol. Biol.* **1990**, *212*, 211-235. (e) Whitlow, M.; Howard, A. J.; Finzel, B. C.; Poulos, T. L.; Winborne, E.; Gilliland, G. L. *Proteins: Struct. Funct. Genet.* **1991**, *9*, 153-173. (f) Jenkins, J.; Janin, J.; Rey, F.; Chiadmi, M.; van Tilbeurgh, H.; Lasters, I.; De Meyer, M.; Van Belle, D.; Wodak, S. J.; Lauwereys, M.; Stanssens, P.; Mrabet, N. T.; Snauwaert, J.; Matthyssens, G.; Lambear, A.-M. *Biochemistry* **1992**, *31*, 5449-5458.
- (a) Allen, K. N.; Lavie, A.; Petsko, G. A.; Ringe, D. *Biochemistry* **1995**, *34*, 3742-3749. (b) Hamilton, D. S.; Creighton, D. J. *J. Biol. Chem.* **1992**, *267*, 24933-24936. (c) Aulabaugh, A.; Schloss, J. V. *Biochemistry* **1990**, *29*, 2824-2830.
- (a) Izquierdo-Martin, M.; Stein, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 325-331. (b) Odake, S.; Okayama, T.; Obata, M.; Morikawa, T.; Hattori, S.; Hori, H.; Nagai, Y. *Chem. Pharm. Bull.* **1990**, *38*, 1007-1011. (c) Ikeda-Saito, M.; Shelley, D. A.; Lu, L.; Booth, K. S.; Caughey, W. S.; Kimura, S. *J. Biol. Chem.* **1991**, *266*, 3611-3616.
- (a) Trinquier, M.; Périé, J.; Callens, M.; Opperdoes, F.; Willson, M. *Bioorg. Med. Chem.* **1995**, *3*, 1423-1427. (b) Morgan, M. J. *FEBS Lett.* **1981**, *130*, 124-126.
- (a) Merkle, S.; Pretsch, W. *Blood* **1993**, *81*, 206-213. (b) Fillela, X.; Molina, R.; Jo, J.; Mas, E.; Ballesta, A. M. *Tumor Biol.* **1991**, *12*, 360-367. (c) Newton, H. B.; Fleisher, M.; Schwartz, M. K.; Malkin, M. G. *Neurology* **1991**, *41*, 395-397. (d) Srivastava, I. K.; Schmidt, M.; Grall, M.; Certa, U.;

- Garcia, A. M.; Perrin, L. H. *Mol. Biochem. Parasitol.* **1992**, *54*, 153-164. (e) Shapiro, T. A.; Talalay, P. *Exp. Parasitol.* **1982**, *54*, 196-201.
9. (a) Shaw, P. J.; Muirhead, H. *J. Mol. Biol.* **1977**, *109*, 475-485. (b) Achari, A.; Marshall, S. E.; Muirhead, H.; Palmieri, R. H.; Noltmann, E. A. *Phil. Trans. R. Soc. Lond. B.* **1981**, *293*, 145-157.
  10. Lolis, E.; Petsko, G. A. *Biochemistry* **1990**, *29*, 6619-6625.
  11. All new compounds gave spectroscopic data and elemental analysis in agreement with the assigned structure; selected data are given for the following compounds ( $\delta$  in ppm,  $J_{ij}$  in Hz, \*: exchangeable resonances): **7**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 3.93 (br q, 1H,  $\text{H}_4$ ,  $J_{34}=5.9$ ), 4.11 (d, 1H,  $\text{H}_2$ ,  $J_{23}=5.9$ ), 4.15 (ddd, 1H,  $\text{H}_5$ ,  $J_{55'}=-11.1$ ,  $J_{p5}=6.9$ ,  $J_{45}=4.9$ ), 4.27 (m, 1H,  $\text{H}_3$ ), 4.29 (ddd, 1H,  $\text{H}_5'$ ,  $J_{p5'}=6.4$ ,  $J_{45'}=3.9$ ), 4.41 (d, 1H,  $\text{COCH}_A\text{HBPh}$ ,  $J_{AB}=-11.5$ ), 4.47 (d, 1H,  $\text{COCH}_A'\text{H}_B'\text{Ph}$ ,  $J_{A'B'}=-11.4$ ), 4.57 (d, 1H,  $\text{COCH}_A'\text{H}_B'\text{Ph}$ ), 4.60 (d, 1H,  $\text{COCH}_A\text{HBPh}$ ), 4.95 (d, 2H,  $\text{POCH}_2\text{Ph}$ ,  $J_{PH}=7.9$ ), 4.97 (d, 2H,  $\text{POCH}'_2\text{Ph}$ ,  $J_{PH'}=8.0$ ), 5.10 (s, 2H,  $\text{COCH}_2\text{Ph}$ ), 7.24-7.35 (m, 25H, Ph);  $^{13}\text{C}$  BB NMR ( $\text{CDCl}_3$ , 62.9 MHz)  $\delta$ : 65.62 ( $\text{C}_5$ ,  $J_{PC}=4.5$ ), 66.92 ( $1\text{COCH}_2\text{Ph}$ ), 69.29 ( $2\text{POCH}_2\text{Ph}$ ,  $J_{PC}=5.7$ ), 72.70 and 72.84 ( $2\text{COCH}_2\text{Ph}$ ), 77.46, 78.15 and 78.27 ( $\text{C}_2$ ,  $\text{C}_3$  and  $\text{C}_4$ )\*, 127.46-128.58 ( $25\text{CH Ph}$ ), 135.35, 135.73, 135.8, 136.85 and 137.51 ( $5\text{Cq Ph}$ ), 170.39 ( $\text{C}_1$ ); MS (flight-time/ $^{252}\text{Cf}$ )  $m/z$ : 697 ( $\text{M}^+$ ) (6), 607 (8), 576 (15), 312 (27), 278 (100), 237 (85), 223 (29), 221 (28), 187 (55). **8**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 4.00 (br q, 1H,  $\text{H}_4$ ,  $J_{34}=5.9$ ,  $J_{45}=5.9$ ), 4.19 (d, 1H,  $\text{H}_2$ ,  $J_{23}=5.9$ ), 4.21 (m, 1H,  $\text{H}_5$ ), 4.36 (m, 2H,  $\text{H}_3$  and  $\text{H}_5'$ ,  $J_{55'}=-11.0$ ,  $J_{p5}=6.0$ ,  $J_{45'}=4.0$ ), 4.48 (d, 1H,  $\text{COCH}_A\text{HBPh}$ ,  $J_{AB}=-11.5$ ), 4.53 (d, 1H,  $\text{COCH}_A'\text{H}_B'\text{Ph}$ ,  $J_{A'B'}=-11.3$ ), 4.64 (d, 1H,  $\text{COCH}_A\text{HBPh}$ ), 4.67 (d, 1H,  $\text{COCH}_A'\text{H}_B'\text{Ph}$ ), 4.80 (br d, 1H,  $\text{NHOCCH}'\text{Ph}$ ,  $J_{HH'}=-12$ ), 4.83 (br d, 1H,  $\text{NHOCCH}\text{Ph}$ ), 5.01 (d, 2H,  $\text{POCH}_2\text{Ph}$ ,  $J_{PH}=7.5$ ), 5.02 (d, 2H,  $\text{POCH}'_2\text{Ph}$ ,  $J_{PH'}=7.7$ ), 5.18 (s, 2H,  $\text{COCH}_2\text{Ph}$ ), 7.29-7.40 (m, 30H, Ph), 7.82 (br s, 1H, NH);  $^{13}\text{C}$  BB NMR ( $\text{CDCl}_3$ , 50.3 MHz)  $\delta$ : 65.68 ( $\text{C}_5$ ), 66.92 ( $1\text{COCH}_2\text{Ph}$ ), 69.33 ( $2\text{POCH}_2\text{Ph}$ ,  $J_{PC}=4.5$ ), 72.73 and 72.86 ( $2\text{COCH}_2\text{Ph}$ ), 77.54, 78.20, 78.37 and 78.75 ( $\text{C}_2$ ,  $\text{C}_3$ ,  $\text{C}_4$  and  $\text{NHOCCH}_2\text{Ph}$ )\*, 127.92-128.68 ( $30\text{CH Ph}$ ), 135.18, 135.38, 135.74, 135.8, 136.89 and 137.55 ( $6\text{Cq Ph}$ ), 170.37 ( $\text{C}_1$ ); MS (flight-time/ $^{252}\text{Cf}$ )  $m/z$ : 801.84 ( $\text{M}^+$ ) (2), 668.0 (2), 612.6 (6), 563.5 (23), 535.5 (5), 355.2 (8), 281 (67), 221 (26), 207 (15), 147 (56), 91 (91), 73 (100). **3**: FT-IR (ATR, solid film)  $\nu$ : 3204 (br), 2927, 2855, 1609 (br), 1415, 1275, 1067, 988, 931, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 400 MHz)  $\delta$ : 3.6-4.1 (m, 5H);  $^{13}\text{C}$  BB NMR ( $\text{D}_2\text{O}$ , 50.3 MHz)  $\delta$ : 66.29 ( $\text{C}_5$ ,  $J_{PC}=4.6$ ), 72.22 and 73.91 ( $\text{C}_2$  and  $\text{C}_3$ )\*, 73.05 ( $\text{C}_4$ ,  $J_{PC}=7.9$ ), 164.60 ( $\text{C}_1$ ,  $\text{C}(\text{OH})=\text{N}-\text{OH}$  form), 178.83 ( $\text{C}_1$ ,  $\text{C}(\text{O})=\text{NHOH}$  form);  $^{13}\text{C}-\{^1\text{H}\}$  NMR ( $\text{D}_2\text{O}$ , 100.6 MHz)  $\delta$ : 66.20 ( $\text{C}_5$ ,  $J_{\text{CH}}=144$ ), 72.30 ( $J_{\text{CH}}=144$ ) and 74.01 ( $J_{\text{CH}}=147$ ) ( $\text{C}_2$  and  $\text{C}_3$ )\*, 73.13 ( $\text{C}_4$ ,  $J_{\text{CH}}=147$ ), 164.60 ( $\text{C}_1$ ,  $\text{C}(\text{OH})=\text{N}-\text{OH}$  form), 178.94 ( $\text{C}_1$ ,  $\text{C}(\text{O})=\text{NHOH}$  form); MS ( $\text{Cl}-\text{D}/\text{NH}_3$ )  $m/z$ : 260 ( $\text{M}+1$ ) $^+$  (9), 241 (38), 182 (11), 158 (41), 141 (100), 124 (18).
  12. Chirgwin, J. M.; Noltmann, E. A. *J. Biol. Chem.* **1975**, *250*, 7272-7276.
  13. Tadano, K.-I.; Maeda, H.; Hoshino, M.; Iimura, Y.; Suami, T. *J. Org. Chem.* **1987**, *52*, 1946-1956.
  14. Bonnette, C.; Salmon, L.; Gaudemer, A. *unpublished results*.
  15. (a) Perish, J. W.; Johns, R. B. *Tetrahedron Lett.* **1987**, *28*, 101-102. (b) Thompson, W.; Nicholls, D.; Irwin, W. J.; Al-Mushadani, J. S.; Freeman, S.; Karpas, A.; Petrick, J.; Nashmood, N.; Hay, A. J. *J. Chem. Soc. Perkin Trans. I* **1993**, 1239-1245.
  16. Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, *20*, 399-402.
  17. Sharma, S. K.; Miller, M. J.; Payne, S. M. *J. Med. Chem.* **1989**, *32*, 357-367.
  18. Vogel, A. I. *Practical Organic Chemistry*, 3<sup>rd</sup> ed.; Longmans: London, 1964; pp. 1062-1063.

(Received in France 7 December 1995; accepted 22 December 1995)