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

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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.[1] 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[2], similar to that observed in the triosephosphate isomerase (TIM)-catalyzed isomerization of dihydroxyacetone-phosphate to D-glyceraldehyde-phosphate,[3] while the hydride shift mechanism has been proposed to operate with some other isomerases, e.g. D-xylose isomerases.[4]

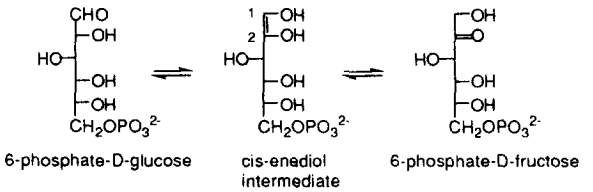

Figure 1. Isomerization reaction catalyzed by 6-phosphate-D-glucose isomerases.

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

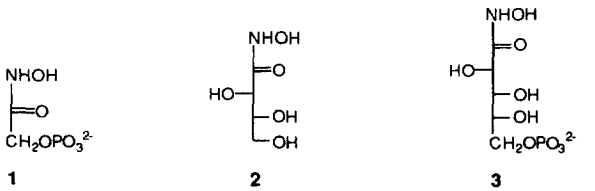

Figure 2. Selected hydroxamate-based inhibitors.

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.[6]

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.[7] PGI is involved in various and important pathologic processes,[8] 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[9] still need considerable refinement in order to identify active site residues involved in the isomerization mechanism, by contrast with other isomerases like TIM[10] or D-xylose isomerase.[4c-f]

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**).

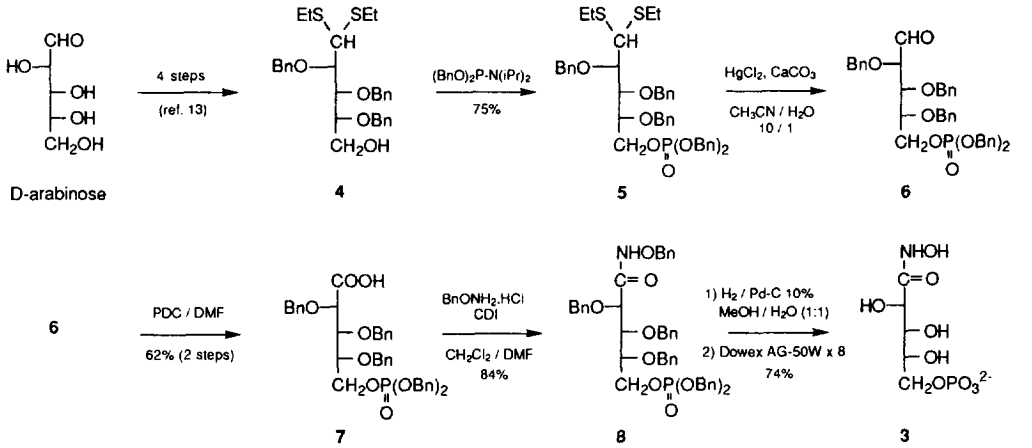

Figure 3. Synthesis of 5-phosphate-D-arabinohydroxamic acid **3**.

The starting product for the synthesis of **3** was D-arabinose, which has the same absolute configuration of carbon atoms C_2, C_3 and C_4. 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 C_5. Deacetalation followed by oxidation led to the protected 5-phosphate-D-arabinonic acid derivative **7**, the precursor of 5-phosphate-D-arabinohydroxamic acid **3**[11] (5-phosphate-D-arabinonic acid, a known PGI inhibitor,[8e,12] 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.[13] **4** was also obtained in three steps from β-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.[14] Phosphorylation of **4** was achieved using dibenzyloxy(diisopropylamino)phosphine[15] to give **5** in 75% yield. Dethioacetalation[13] of **5** with $HgCl_2$ in the presence of $CaCO_3$ gave the protected 5-phosphate-D-arabinose derivative **6**, which was converted into the corresponding acid **7** by oxidation with pyridinium dichromate (PDC)[16] with a yield of

62% (two steps). **7** was then reacted with O-benzylhydroxylamine in the presence of carbonyldiimidazole (CDI)[17] 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 $FeCl_3$.[18]

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.

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11. All new compounds gave spectroscopic data and elemental analysis in agreement with the assigned structure; selected data are given for the following compounds (δ in ppm, J_{ij} in Hz, *: exchangeable resonances): **7**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.93 (br q, 1H, H_4, J_{34}=5.9), 4.11 (d, 1H, H_2, J_{23}=5.9), 4.15 (ddd, 1H, H_5, $J_{55'}$=-11.1, J_{P5}=6.9, J_{45}=4.9), 4.27 (m, 1H, H_3), 4.29 (ddd, 1H, $H_{5'}$, $J_{P5'}$=6.4, $J_{45'}$=3.9), 4.41 (d, 1H, $COCH_AH_BPh$, J_{AB}=-11.5), 4.47 (d, 1H, $COCH_{A'}H_{B'}Ph$, $J_{A'B'}$=-11.4), 4.57 (d, 1H, $COCH_{A'}H_{B'}Ph$), 4.60 (d, 1H, $COCH_AH_BPh$), 4.95 (d, 2H, $POCH_2Ph$, J_{PH}=7.9), 4.97 (d, 2H, $POCH'_2Ph$, $J_{PH'}$=8.0), 5.10 (s, 2H, $COCH_2Ph$), 7.24-7.35 (m, 25H, Ph); ^{13}C BB NMR ($CDCl_3$, 62.9 MHz) δ: 65.62 (C_5, J_{PC}=4.5), 66.92 ($1COCH_2Ph$), 69.29 ($2POCH_2Ph$, J_{PC}=5.7), 72.70 and 72.84 ($2COCH_2Ph$), 77.46, 78.15 and 78.27 (C_2, C_3 and C_4)*, 127.46-128.58 (25CH Ph), 135.35, 135.73, 135.8, 136.85 and 137.51 (5Cq Ph), 170.39 (C_1); MS (flight-time/^{252}Cf) *m/z* : 697 $(M)^+$ (6), 607 (8), 576 (15), 312 (27), 278 (100), 237 (85), 223 (29), 221 (28), 187 (55). **8**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.00 (br q, 1H, H_4, J_{34}=5.9, J_{45}=5.9), 4.19 (d, 1H, H_2, J_{23}=5.9), 4.21 (m, 1H, H_5), 4.36 (m, 2H, H_3 and $H_{5'}$, $J_{55'}$=-11.0, $J_{P5'}$=6.0, $J_{45'}$=4.0), 4.48 (d, 1H, $COCH_AH_BPh$, J_{AB}=-11.5), 4.53 (d, 1H, $COCH_{A'}H_{B'}Ph$, $J_{A'B'}$=-11.3), 4.64 (d, 1H, $COCH_AH_BPh$), 4.67 (d, 1H, $COCH_{A'}H_{B'}Ph$), 4.80 (br d, 1H, $NHOCHH'Ph$, $J_{HH'}$=-12), 4.83 (br d, 1H, $NHOCHH'Ph$), 5.01 (d, 2H, $POCH_2Ph$, J_{PH}=7.5), 5.02 (d, 2H, $POCH'_2Ph$, $J_{PH'}$=7.7), 5.18 (s, 2H, $COCH_2Ph$), 7.29-7.40 (m, 30H, Ph), 7.82 (br s, 1H, NH); ^{13}C BB NMR ($CDCl_3$, 50.3 MHz) δ: 65.68 (C_5), 66.92 ($1COCH_2Ph$), 69.33 ($2POCH_2Ph$, J_{PC}=4.5), 72.73 and 72.86 ($2COCH_2Ph$), 77.54, 78.20, 78.37 and 78.75 (C_2, C_3, C_4 and $NHOCH_2Ph$)*, 127.92-128.68 (30CH Ph), 135.18, 135.38, 135.74, 135.8, 136.89 and 137.55 (6Cq Ph), 170.37 (C_1); MS (flight-time/^{252}Cf) *m/z* : 801.84 $(M)^+$ (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) υ: 3204 (br), 2927, 2855, 1609 (br), 1415, 1275, 1067, 988, 931, 795 cm^{-1}; ^{1}H NMR (D_2O, 400 MHz) δ: 3.6-4.1 (m, 5H); ^{13}C BB NMR (D_2O, 50.3 MHz) δ: 66.29 (C_5, J_{PC}=4.6), 72.22 and 73.91 (C_2 and C_3)*, 73.05 (C_4, J_{PC}=7.9), 164.60 (C_1, C(OH)=N-OH form), 178.83 (C_1, C(=O)-NHOH form); ^{13}C-{^{1}H} NMR (D_2O, 100.6 MHz) δ: 66.20 (C_5, J_{CH}=144), 72.30 (J_{CH}=144) and 74.01 (J_{CH}=147) (C_2 and C_3)*, 73.13 (C_4, J_{CH}=147), 164.60 (C_1, C(OH)=N-OH form), 178.94 (C_1, C(=O)-NHOH form); MS (CI-D/NH_3) *m/z* : 260 $(M+1)^+$ (9), 241 (38), 182 (11), 158 (41), 141 (100), 124 (18).
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