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Synthesis and evaluation of new 4-phospho-D-erythronic acid derivatives as competitive inhibitors of spinach ribose-5-phosphate isomerase

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Abstract—This paper reports the synthesis of new 4-phospho-D-erythronic acid derivatives, namely 4-phospho-D-erythronohydroxamic acid (**1**), 4-phospho-D-erythronohydrazide (**2**), and 4-phospho-D-erythronamide (**3**), and their kinetic evaluation as new competitive inhibitors of the isomerization reaction between D-ribose 5-phosphate and D-ribulose 5-phosphate catalyzed by spinach ribose-5-phosphate isomerase (RPI). By comparison to the only known RPI inhibitor, 4-phospho-D-erythronate (**4**, $K_i = 28\,\mu M$, $K_m/K_i = 270$), the hydroxamic acid **1**, obtained by an eight-step synthesis from D-arabinose, appears as a new potent high-energy intermediate analogue inhibitor of the isomerization reaction ($K_i = 29\,\mu M$, $K_m/K_i = 260$).

Ribose-5-phosphate isomerase (RPI, E.C. 5.3.1.6), an aldose–ketose isomerase involved in the pentose phosphate pathway, catalyzes the isomerization reaction of D-ribose 5-phosphate (R5P) and D-ribulose 5-phosphate (Ru5P) (Fig. 1).[1] The reaction is thought to proceed through a proton transfer mechanism and to involve a high-energy 1,2-*cis*-enediolate intermediate analogous to that reported previously for triosephosphate isomerase (TIM)[2] and glucose-6-phosphate isomerase (GPI).[3,4] During the last 3 years, site-directed mutagenesis[5,6] and X-ray diffraction studies[6,7] gave new insights into the nature and possible role of some of the active site residues. Nevertheless, unlike for TIM and GPI, little is known about RPI. For example, only a limited number of inhibitors have been reported in the literature,[8] with only one reaction intermediate analogue inhibitor available to date.[1] Consequently, it appeared important to us to provide new, strong competitive inhibitors of RPI to the scientific community, so that detailed kinetic and crystallographic studies could be pursued. This could then lead to the design of strong and species-specific RPI inhibitors of therapeutic interest, e.g. against the pathogenic human African trypanosomiasis where the pentose phosphate pathway has been shown to play a crucial role in the survival and development of the parasite *Trypanosoma brucei*.[9] As part of our program dedicated to the study of aldose–ketose isomerases and by analogy to our previous finding of strong GPI inhibitors,[10,11] we report in this study the synthesis of three original 4-phospho-D-erythronic acid derivatives, namely 4-phospho-D-erythronohydroxamic acid **1**, 4-phospho-D-erythronohydrazide **2**, and 4-phospho-D-erythronamide **3** (Fig. 2), as new reaction intermediate analogues of the R5P to Ru5P isomerization reaction catalyzed by spinach RPI. A comparison of their

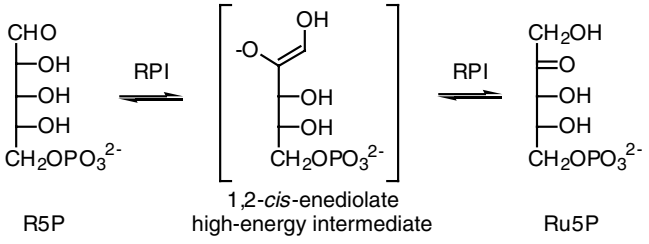

Figure 1. Isomerization reaction catalyzed by D-ribose-5-phosphate isomerases.

Keywords: Phosphate sugars; Enzyme inhibitors; Hydroxamic acids; Hydroxamates.

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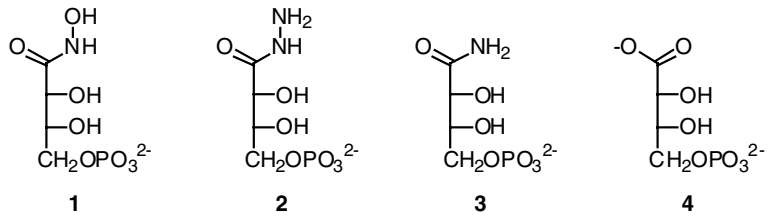
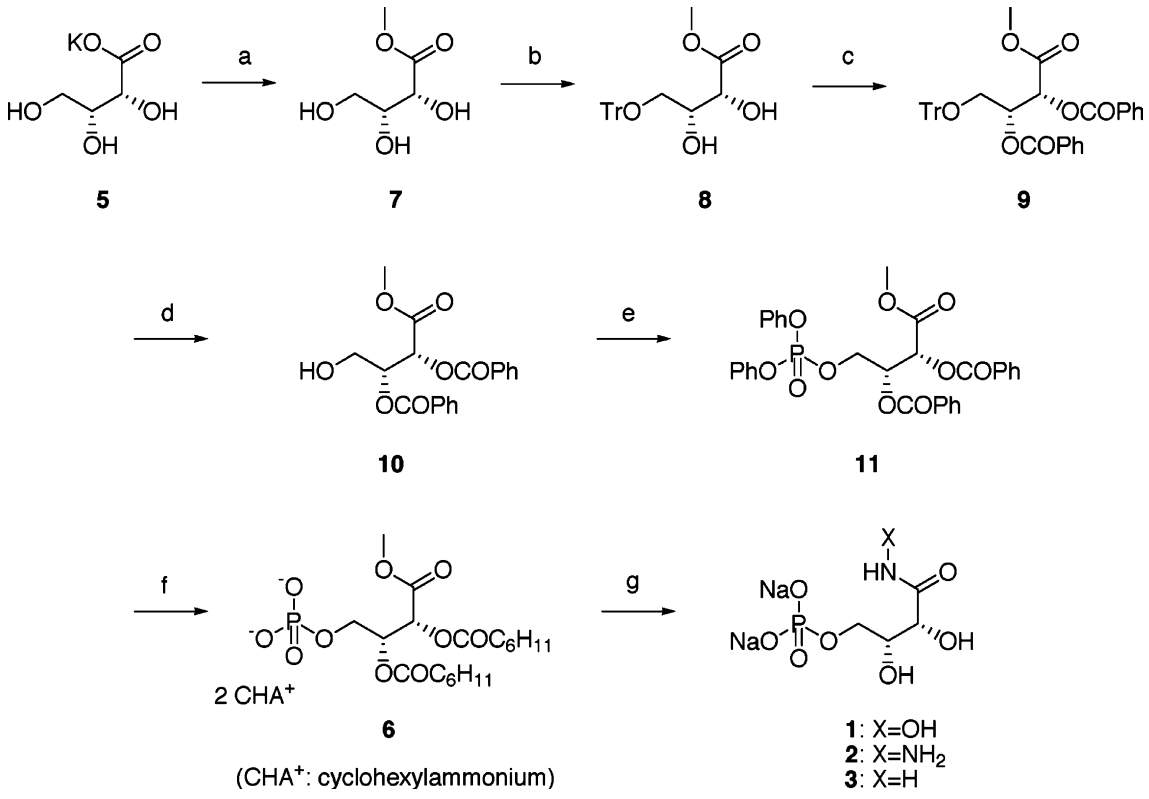


Figure 2. Synthesized models of the 1,2-*cis*-enediolate high-energy intermediate species of the RPI-catalyzed isomerization reaction.



Scheme 1. Reagents and conditions: (a) i. Dowex® 50X4-400 (H^+ form), MeOH, −20 °C, 15 min; ii. CH_2N_2, Et_2O,−20 °C, 25%; (b) TrCl (1.1 equiv), pyridine, DMAP (0.03 equiv), 45 °C, 12 h, 94%; (c) PhCOCl (2.1 equiv), pyridine, 24 h, 25 °C, 84%; (d) H_2, 25 bar, Pd/C 10%, CH_2Cl_2/MeOH (4/1), 12 h, 100%; (e) $(PhO)_2POCl$ (1 equiv), pyridine, 12 h, 25 °C, 94%; (f) i. H_2, 25 bar, PtO_2, 3 days; ii. cyclohexylamine, recrystallization (MeOH/*n*-hexane, 1/50), 70%; (g) **1** (X = OH): i. solid NH_2OH, MeOH, 12 h, 4 °C; ii. Dowex® 50X4-400 (Na^+ form); freeze-drying, 95%; **2** (X = NH_2): i. $NH_2NH_2{\cdot}H_2O$, MeOH, 12 h, 4 °C; ii. Dowex® 50X4-400 (Na^+ form); freeze-drying, 95%; **3** (X = H): i. NH_3, MeOH, 12 h, 4 °C; ii. NH_3–H_2O, MeOH, 12 h, 4 °C; iii. $Ba(OH)_2$; iv. Dowex® 50X4-400 (H^+ form); v. Dowex® 50X4-400 (Na^+ form); freeze-drying, 95%.

inhibitory properties with that of 4-phospho-D-erythronate **4** (Fig. 2), the only known efficient inhibitor of the enzyme, is also reported.

Our synthetic strategy to obtain compounds **1**, **2**, and **3** started from potassium D-erythronate **5**,[12] and led in six steps to methyl 4-phospho-(bis-cyclohexylammonium)-2,3-di-*O*-cyclohexanecarbonyl-D-erythronate **6**, the precursor of the targeted compounds (Scheme 1). Reaction of diazomethane with the acid form of **5** (Dowex® 50X4-400, H^+ form) in methanol at −20 °C led to a mixture[13] of methyl D-erythronate **7**, D-erythronolactone, and other methylated derivatives. Pure **7** was obtained by silica gel chromatography (acetone/toluene 1:1, $R_f = 0.16$) and recrystallization (AcOEt) in 25% yield. We did not succeed in purifying **7** by distillation because it easily equilibrates to erythronolactone upon heating. In order to prevent the lactone formation, **7** was tritylated to give compound **8** in 94% yield. Protection of the secondary hydroxyl groups of **8** was achieved with PhCOCl in pyridine. The resulting product **9** (84% yield) was then hydrogenolyzed (25 bar) over 10% Pd/C to give compound **10** (quantitative yield), which was next phosphorylated with diphenyl phosphorochloridate in pyridine to yield **11** (94%). Direct synthesis of the inhibitors from **11** (or its dibenzylphosphate analogue) led to complex, unexploitable mixtures, probably because of the phosphoester reactivity with nucleophiles. The product was finally converted into the precursor **6**[14] by both hydrogenation and hydrogenolysis (25 bar) over PtO_2 for 3 days. Following neutralization with cyclohexylamine and crystallization (MeOH/*n*-hexane, 1/50), pure **6** was obtained in 70% yield.

An interesting feature of this strategy lies in the fact that nucleophilic substitution at C-1 of the precursor **6** and

Table 1. Inhibitory effect of 4-phospho-D-erythronohydroxamic acid **1**, 4-phospho-D-erythronohydrazide **2**, 4-phospho-D-erythronamide **3**, 4-phospho-D-erythronate **4**, D-erythronate, and D-erythronohydroxamic acid on spinach ribose-5-phosphate isomerase (RPI)

Inhibitor	IC_{50} (mM)	K_i (mM)	K_m/K_i[a]
1	0.018 ± 0.003	0.029 ± 0.003	260
2	1.8 ± 0.2	1.8 ± 0.2	4
3	1.8 ± 0.2	2.5 ± 0.5	3
4	0.010 ± 0.002	0.028 ± 0.005	270
D-Erythronate	24 ± 2	—	—
D-Erythronohydroxamic acid	32 ± 3	—	—

[a] $K_m(R5P) = 7.5 \pm 0.8$ mM.

deprotection of the hydroxyl groups on C-2 and C-3 are achieved in one-step. The three new molecules **1** (X = OH), **2** (X = NH_2), and **3** (X = H) depicted in Scheme 1 were obtained by reaction in dry methanol with, respectively, solid NH_2OH[15] (**CAUTION**, this very volatile and toxic product must be handled in a fume hood, wearing gloves[†]), $NH_2NH_2{\cdot}H_2O$ and NH_3 at 4 °C. The initial fraction of **1**, bis(hydroxylammonium) salt was insoluble in the reaction mixture and could be collected (30%). A second fraction (65%) further precipitated from the mother liquor upon addition of diethyl ether (attempted precipitation of **1** with barium hydroxide led to precipitation of both barium salts of **1** and cyclohexanehydroxamate formed upon deprotection of the hydroxyl groups at C-2 and C-3, which could not be separated). Following elution (H_2O) on Dowex® 50X4-400 (Na^+ form) and freeze-drying, pure **1** (disodium salt)[16] was obtained in 95% overall yield from **6**. Compound **2**, precipitated as the bis(hydrazonium) salt from the reaction mixture and was rinsed with methanol, eluted (H_2O) on Dowex® 50X4-400 (Na^+ form) and freeze dried to afford pure **2** as the disodium salt[17] in 95% yield. Compound **3**, as the bis(ammonium) salt, did not precipitate from the reaction mixture. However, upon addition of barium hydroxide, the barium salt of **3**, could be collected upon precipitation. It was then successively eluted (H_2O) on Dowex® 50X4-400 (H^+ form) and Dowex® 50X4-400 (Na^+ form). Following lyophilization, pure **3** as the disodium salt,[18] was recovered in 95% yield.

The new compounds **1**, **2**, and **3**, the only known potent RPI inhibitor **4**,[1] as well as the nonphosphorylated analogues of **1** and **4**, respectively D-erythronohydroxamic acid[19] and D-erythronate, were all evaluated against spinach RPI (Aldrich) as potential inhibitors of the R5P to Ru5P isomerization reaction. Enzymatic activities were determined by following the change in ultraviolet absorbance that accompanies conversion of R5P to Ru5P,[20] ($\lambda = 282$ nm, $\varepsilon = 58.6\ M^{-1}\,cm^{-1}$) at 25 °C in 50 mM TRIS.HCl buffer (pH 7.5). Apparent Michaëlis constants (K_m) and inhibition constants (K_i) were determined (Table 1) from double reciprocal plots of the initial reaction velocity versus R5P concentration obtained at various concentrations of inhibitors (Lineweaver–Burk graphical representation) with 0.5 U/mL of RPI (and replots of apparent K_m/V_{max} values vs inhibitor concentration). IC_{50} determinations (Table 1) were achieved using a 3.2 mM R5P concentration. Compounds **1**–**4** all behave as competitive inhibitors of the isomerization reaction of R5P to Ru5P catalyzed by spinach RPI. Comparison of the IC_{50} values of **1** and **4** with those of the respective nonphosphorylated analogues confirms the primary importance of the phosphate group for achieving effective inhibition. Both the hydrazide **2** and amide **3** neutral derivatives behave as weak competitive inhibitors with K_i values close to the K_m value of the substrate R5P. The K_i value we obtained for the anionic compound **4** (28 μM, Table 1), the known strong competitive RPI inhibitor, is about 6-fold higher than the reported value for the same enzyme (4.4 μM),[1] a difference likely due to the different conditions used for the kinetic study (lit.[1]: 40 mM phosphate buffer, pH 6.4). With a K_i value of 29 μM and a K_m/K_i ratio of 260, the hydroxamic acid **1**, mostly neutral in solution owing to its probable pK_a of 9.5, appears to be a new potent competitive inhibitor of the reaction catalyzed by spinach RPI.[21] However, although 4-phospho-D-erythronohydroxamic acid **1** is a much better structural mimic of the 1,2-*cis*-enediolate reaction intermediate than 4-phospho-D-erythronate **4**, both inhibitors display about the same inhibition constant values. In view of our results, we suggest the hypothesis that the anionic character of the high-energy intermediate analogue is a significant parameter in the design of strong RPI inhibitors. In the case of the hydroxamic acid **1**, kinetic measurements at a higher pH value where the enzyme is still active and the inhibitor is partially ionized will have to be run in order to show whether or not the ionized form of **1** is the true inhibitor of the enzyme. In addition, a study of K_i as a function of the inhibitor's pK_a (from a series of fluoro analogues of **1** for instance) would be valuable for addressing this question. By comparison to our reported inhibition studies on GPI achieved in about the same conditions, the hydroxamic acid higher homologue of **1**, 5-phospho-D-arabinonohydroxamic acid, proved to be a stronger inhibitor by an order of magnitude than the carboxylate higher homologue of **4**, 5-phospho-D-arabinonate.[10] Also, higher K_m/K_i ratios were reported for the GPI inhibitors 5-phospho-D-arabinonohydrazide and 5-phospho-D-arabinonamide than for the corresponding RPI inhibitors **2** and **3**.[11] As previously reported in the case of TIM and GPI, and in view of the recently reported 3D high-resolved structures of RPI[6,7] and GPI,[22,23] our results are in accordance with the fact that RPI and GPI do not share, at least in part, an identical architecture of

[†] Because hydroxamic acids are known to readily form stable metal complexes, a glass spatula was used to handle solid NH_2OH, and thereafter **1**.

the active site. Thus, the synthesis of a new potent competitive inhibitor of the RPI-catalyzed isomerization reaction such as 4-phospho-D-erythronohydroxamic acid **1** appears to be very promising in order to further develop kinetic, mechanistic, and structural investigations on ribose-5-phosphate isomerases.

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References and notes

1. Woodruff, W. W.; Wolfenden, R. *J. Biol. Chem.* **1979**, *254*, 5866–5867.
2. Rieder, S. V.; Rose, I. A. *J. Biol. Chem.* **1959**, *234*, 1007–1010.
3. Bruns, F.; Okada, S. *Nature* **1956**, *177*, 87–88.
4. Topper, Y. J. *J. Biol. Chem.* **1957**, *225*, 419–423.
5. Jung, C.-H.; Hartman, F. C.; Lu, T.-Y.S.; Larimer, F. W. *Arch. Biochem. Biophys.* **2000**, *373*, 409–417.
6. Ishikawa, K.; Matsui, I.; Payan, F.; Cambillau, C.; Ishida, H.; Kawarabayasi, Y.; Kibuchi, H.; Roussel, A. *Structure* **2002**, *10*, 877–886.
7. Zhang, R.-G.; Andersson, C. E.; Savchenko, A.; Skarina, T.; Evdokimova, E.; Beasley, S.; Arrowsmith, C. H.; Edwards, A. M.; Joachimiak, A.; Mowbray, S. H. *Structure* **2003**, *11*, 31–42.
8. Burger, A.; Tritsch, D.; Biellmann, J.-F. *Carbohydr. Res.* **2001**, *332*, 141–149.
9. Dardonville, C.; Rinaldi, E.; Hanau, S.; Barrett, M. P.; Brun, R.; Gilbert, I. H. *Bioorg. Med. Chem.* **2003**, *11*, 3205–3214.
10. Hardré, R.; Bonnette, C.; Salmon, L.; Gaudemer, A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3435–3438.
11. Hardré, R.; Salmon, L. *Carbohydr. Res.* **1999**, *318*, 110–115.
12. Hardegger, E.; Kreis, K.; El Khadem, H. *Helv. Chim. Acta* **1951**, *34*, 2343–2348.
13. Barker, R.; Wold, F. *J. Org. Chem.* **1963**, *28*, 1847–1851.
14. Selected data for compound **6**: $[\alpha]_D^{28}$ −3.9° (*c* 1.17, $CDCl_3$). IR ν_{max} (cm^{-1}) (KBr): 3447 (NH_3^+), 2934, 2854 (CH_2), 1740 (CO), 1086, 1066 (OH, PO). ^1H NMR (250 MHz, $CDCl_3$) $\delta = 5.36$–5.30 (m, 2H), 3.90–3.80 (m, 2H), 3.71 (s, 3H), 2.39–1.20 (m, 50H). ^{13}C NMR (50 MHz, CD_3OD) $\delta = 176.2$ (C-1), 169.2 (CO, C′O), 73.2 (d, C-3, $J = 9.2$ Hz), 72.3 (C-2), 63.2 (d, C-4, $J = 4.6$ Hz), 53.0 (OMe), 51.2 (Cα CHA), 44.2–43.9 (Cα CH, Cα′ CH), 32.9 (Cβ CHA), 30.3–29.8 (Cβ CH, Cβ′ CH, Cγ CH, Cγ′ CH), 26.9–26.5 (Cδ CH, Cδ′ CH), 26.2 (Cδ CHA), 25.6 (Cγ CHA). ^{31}P NMR ($CDCl_3$) $\delta = -0.85$. MS (negative-ion electrospray): *m*/*z* (%) 321 [H_2PO_4] (29), 449 [M+H^+] (100). Anal. Calcd for $C_{31}H_{57}O_{10}PN_2 \cdot H_2O$ (666.8): C, 55.84; H, 8.92; N, 4.20; P, 4.65. Found C, 56.11; H, 8.93; N, 4.17; P, 4.60.
15. Brauer, G. *Handbook of Preparative Inorganic Chemistry*, 2nd ed.; Academic: NY, 1963; Vol. I, p 504.
16. Selected data for compound **1**: $[\alpha]_D^{28}$ −14.1° (*c* 1.17, H_2O). IR ν_{max} (cm^{-1}) (KBr): 3420 (OH), 1653 (CO), 1086 (OH, PO). ^1H NMR (250 MHz, D_2O) $\delta = 4.17$–4.14 (m, 1H), 3.90–3.87 (m, 3H). ^{13}C NMR (50 MHz, D_2O) $\delta = 170.7$ (C-1), 72.5 (d, C-3, $J = 6.1$ Hz), 71.1 (C-2), 64.8 (d, C-4, $J = 4.7$ Hz). ^{31}P NMR (D_2O) $\delta = 7.58$. MS (negative-ion electrospray): *m*/*z* (%) 97 [H_2PO_4] (5), 230 [M+H^+] (100), 252 [M+Na^+] (40). HRMS (negative-ion electrospray): calcd for $C_4H_9NO_8P$ (M+H^+) 230.0065, found 230.0066.
17. Selected data for compound **2**: $[\alpha]_D^{28}$ −14.2° (*c* 1.75, H_2O). IR ν_{max} (cm^{-1}) (KBr): 3387 (OH), 1674 (CO), 1091 (OH, PO). ^1H NMR (250 MHz, D_2O) $\delta = 4.22$–4.20 (m, 1H), 3.94–3.85 (m, 3H). ^{13}C NMR (50 MHz, D_2O) $\delta = 173.4$ (C-1), 72.8 (d, C-3, $J = 6.4$ Hz), 71.9 (C-2), 64.9 (d, C-4, $J = 4.6$ Hz). ^{31}P NMR (D_2O) $\delta = 7.59$; MS (negative-ion electrospray): *m*/*z* (%) 97 [H_2PO_4] (5), 229 [M+H^+] (100), 251 [M+Na^+] (40). HRMS (negative-ion electrospray): calcd for $C_4H_{10}N_2O_7P$ (M+H^+) 229.0225, found 229.0225.
18. Selected data for compound **3**: IR ν_{max} (cm^{-1}) (KBr): 3421 (OH), 1675 (CO), 1092 (OH, PO). ^1H NMR (250 MHz, D_2O) $\delta = 4.23$–4.20 (m, 1H), 3.91–3.89 (m, 3H). ^{13}C NMR (50 MHz, D_2O) $\delta = 177.9$ (C-1), 72.8 (d, C-3, $J = 7.4$ Hz), 72.5 (C-2), 65.3 (d, C-4, $J = 4.6$ Hz). ^{31}P NMR (D_2O) $\delta = 6.45$; MS (negative-ion electrospray): *m*/*z* (%) 97 [H_2PO_4] (4), 214 [M+H^+] (100), 236 [M+Na^+] (17). HRMS (negative-ion electrospray): calcd for $C_4H_9NO_7P$ (M+H^+) 214.0116, found 214.0114.
19. Salmon, L.; Prost, E.; Merienne, C.; Hardré, R.; Morgant, G. *Carbohydr. Res.* **2001**, *335*, 195–204.
20. Knowles, F. C.; Pon, M. K.; Pon, N. G. *Anal. Biochem.* **1969**, *29*, 40–47.
21. Qualitative observation on **1** with RPI showed that the enzyme did not hydrolyze the hydroxamic acid function (positive Fe^{3+} test after 12 h incubation time).
22. Davies, C.; Muirhead, H. *Proteins* **2002**, *50*, 577–579.
23. Arsenieva, D.; Hardré, R.; Salmon, L.; Jeffrey, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5872–5877.