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Synthesis of 5-deoxy-5-phospho-D-ribonohydroxamic acid: a new competitive and selective inhibitor of type B ribose-5-phosphate isomerase from *Mycobacterium tuberculosis*

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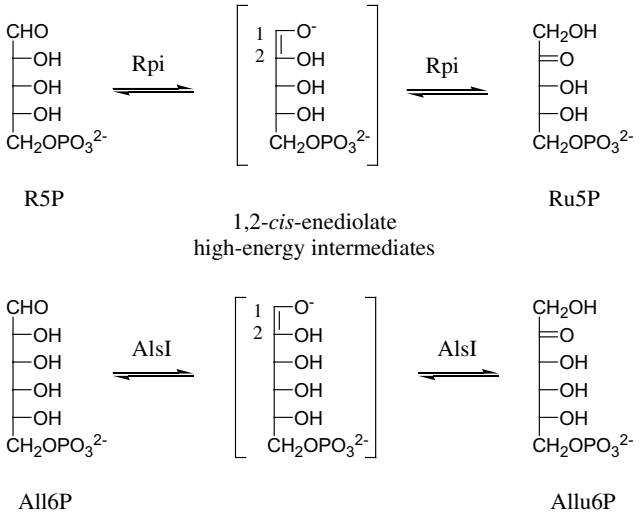
Abstract—5-Deoxy-5-phospho-D-ribonohydroxamic acid, a mimic of the 1,2-*cis*-enediolate high-energy intermediate species of the allose-6-phosphate isomerase reaction, was obtained by a six-step synthesis from D-erythronolactone. In contrast to the known competitive ribose-5-phosphate isomerase (Rpi) inhibitors 4-deoxy-4-phospho-D-erythronohydroxamic acid, 4-deoxy-4-phospho-D-erythronate, and 4-deoxy-4-phosphonomethyl-D-erythronate, the new hydroxamic acid selectively inhibits *Mycobacterium tuberculosis* RpiB (K_i = 0.40 mM, K_m/K_i = 4.5) versus *Spinacia oleracea* RpiA, and hence appears as a promising lead for the design of potent species-specific inhibitors of the bacterial enzyme.

Ribose-5-phosphate isomerase (Rpi, E.C. 5.3.1.6), an aldose–ketose isomerase involved in the pentose phosphate pathway, catalyzes the reversible isomerization of D-ribose 5-phosphate (R5P) and D-ribulose 5-phosphate (Ru5P) (Scheme 1).[1] The reaction is thought to proceed via a proton-transfer mechanism that involves a 1,2-*cis*-enediolate high-energy intermediate. Two completely distinct types of Rpi (A and B) have been differentiated.[2] Recently, the hypothesis was put forth that the RpiB type of enzyme might also catalyze the reversible isomerization of the six-carbon sugars D-allose 6-phosphate (All6P) and D-allulose 6-phosphate (Allu6P) (Scheme 1). Studies of D-allose catabolism in *Escherichia coli*[3,4] identified the product of *alsR* as the repressor for the *rpiB* gene, as well as for a neighboring operon of genes linked with allose transport and metabolism. Allose was a stronger inducer of these genes' expression than ribose. The *rpiB* gene was thus suggested to encode the isomerase in D-allose catabolism, and given the additional designation of *alsI*. Furthermore, the similarity of All6P and R5P gives substance to this hypothesis.



Scheme 1. Isomerization reactions catalyzed by D-ribose-5-phosphate isomerases (Rpi) and D-allose-6-phosphate isomerase (AlsI).

Keywords: Competitive inhibitor; Phosphate sugar; 5-Phosphate D-ribose isomerase; 6-Phosphate D-allose isomerase; RpiB.
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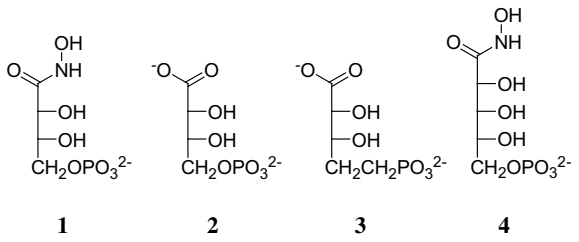


1 2 3 4

Scheme 2. Synthesized models of the 1,2-*cis*-enediolate high-energy intermediate species of the Rpi- and AlsI-catalyzed isomerization reactions.

The first three compounds depicted in Scheme 2, 4-deoxy-4-phospho-D-erythronohydroxamic acid (4PEH, **1**), 4-deoxy-4-phospho-D-erythronate (4PEA, **2**), and 4-deoxy-4-phosphonomethyl-D-erythronate (4PMEA, **3**), have been described as the best competitive inhibitors with regard to *Spinacia oleracea* (*So*) RpiA[5,6] and *M. tuberculosis* (*Mt*) RpiB[7] (Table 1). Compound **1** (4PEH), the strongest *Mt*RpiB competitive inhibitor ever reported, and compound **2** (4PEA), led recently to new information about the reaction mechanism through structural studies of the enzyme-inhibitor complexes.[7] All three inhibitors appear selective for the *So*RpiA catalyzed R5P isomerization, with selectivity factors for the type B enzyme (*R*, Table 1) ranging from 0.004 to 0.12. To date, no selective inhibitor of any type B Rpi has been reported. Such RpiB inhibitors would be valuable tools for structural and mechanistic studies of this type of Rpi, and for comparative studies of the A and B types. Furthermore, in some pathogenic organisms, such as *Trypanosoma cruzi* (the causative agent of Chagas disease) and *Mt* (which causes tuberculosis), the only representative of Rpi activity is the B-type enzyme. Therefore, a strong and selective competitive inhibitor of the RpiB versus the human RpiA enzyme might lead to molecules of therapeutic interest, and help determine whether the enzymes are viable targets for drug treatment. We report in this paper the synthesis of 5-deoxy-5-phospho-D-ribonohydroxamic acid (5PRH, **4**, Scheme 2), a reaction-intermediate analogue of the All6P to Allu6P isomerization reaction catalyzed by AlsI. A comparison of its inhibitory properties with those of 4PEH (**1**), 4PEA (**2**), and 4PMEA (**3**) for R5P to Ru5P isomerization reaction catalyzed by *So*RpiA and *Mt*RpiB is also presented.

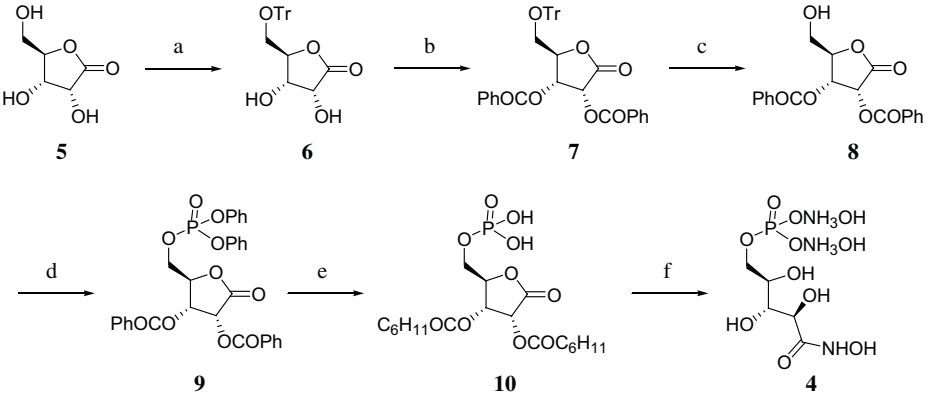
Our synthetic strategy to obtain compound **4** started from the commercially available D-ribono-1,4-lactone (**5**, Scheme 3). The primary alcohol position was tritylated according to reported procedures to give compound **6** in 81% yield.[8,9] Protection of the secondary hydroxyl groups of **6** was achieved with PhCOCl in pyridine to yield **7**[10] in 80% yield. Deprotection of the primary hydroxyl group through hydrogenolysis over 10% Pd/C gave compound **8**[11] in 96% yield, which was next phosphorylated with diphenylphosphochloridate in pyridine to yield **9**[12] in 95%. The product was finally converted quantitatively into **10**[13] by both hydrogenation and hydrogenolysis over PtO_2. As reported in our previous related synthesis,[5] the nucleophilic substitution at C-1 of the precursor **10** and deprotection of the hydroxyl groups on C-2 and C-3 were achieved in one step by reaction with hydroxylamine and gave 5-deoxy-5-phospho-D-ribonohydroxamic acid (5PRH, **4**) as the bis(hydroxylammonium) salt[14] in 91% yield.

The new hydroxamic acid **4** was evaluated as a potential inhibitor of the R5P to Ru5P isomerization reaction of both *So*RpiA (Aldrich) and *Mt*RpiB.[7] Kinetic constants (K_m, K_i) and IC_{50} values were obtained as previously described for *So*RpiA[5] and *Mt*RpiB.[7] Hydroxamic acid **4** behaves as a competitive inhibitor of both enzymes. With a K_i value similar to the K_m value of the substrate R5P, 5PRH (**4**) can be considered as a poor competitive inhibitor of the *So*RpiA-catalyzed reaction compared to the values previously obtained for compounds **1**, **2**, and **3** (Table 1).[5,6] For *Mt*RpiB, compound **4** appears as a good inhibitor with a K_m/K_i ratio of 4.5, a value between those obtained for **1** ($K_m/K_i = 32$) and **2** ($K_m/K_i = 1$). Such a feature makes compound **4** viable for biochemical and crystallographic studies, valuable in itself, considering that RpiB inhibitors are not widespread. The most interesting point for this compound, however, is its selectivity factor for the type B enzyme (Table 1): 5PRH (**4**) appears as the most selective inhibitor for *Mt*RpiB versus *So*RpiA ever reported, with an *R* value of 4.5, approximately 37-fold higher than the corresponding value for 4PEH (**1**) ($R = 0.12$). Our results thus lend strong support for the proposal that the *rpiB* gene

Table 1. Inhibitory effect of 4-deoxy-4-phospho-D-erythronohydroxamic acid **1** (4PEH), 4-deoxy-4-phospho-D-erythronate **2** (4PEA), 4-deoxy-4-phosphonomethyl-D-erythronate **3** (4PMEA), and 5-deoxy-5-phospho-D-ribonohydroxamic acid **4** (5PRH) on *Spinacia oleracea* and *Mycobacterium tuberculosis* ribose-5-phosphate isomerases (respectively *So*RpiA and *Mt*RpiB)

Inhibitor	*So*RpiA			*Mt*RpiB			R^c
	IC_{50} (mM)	K_i (mM)	K_m/K_i[a]	IC_{50} (mM)	K_i (mM)	K_m/K_i[b]	
1	0.018 ± 0.003[d]	0.029 ± 0.003[d]	260	0.040 ± 0.005[e]	0.057 ± 0.006[e]	32	0.12
2	0.010 ± 0.002[d]	0.028 ± 0.005[d]	270	6.0 ± 0.6[e]	1.7 ± 0.2[e]	1	0.004
3	0.15 ± 0.02	0.074 ± 0.009[f]	100	~12.0[e]	2.0 ± 0.2[e]	1	0.01
4	5.0 ± 0.5	6.2 ± 0.5	1	—	0.40 ± 0.04	4.5	4.5

[a] K_m (R5P) = 7.5 ± 0.8 mM.
[b] K_m (R5P) = 1.8 ± 0.2 mM.
[c] $R = (K_m/K_i)^{Mt}/(K_m/K_i)^{So}$.
[d] See Ref. 5.
[e] See Ref. 7.
[f] See Ref. 6.



Scheme 3. Reagents and conditions: (a) i. TrCl (1.1 equiv), pyridine, 4-DMAP (0.03 equiv), 45 °C, 12 h; ii. silica gel chromatography (AcOEt/pentane, 2:1, $R_f = 0.33$); iii. Crystallization ($CHCl_3$/*n*-hexane/acetone, 100/100/1), 81%; (b) i. PhCOCl (2.1 equiv), pyridine, 24 h, 25 °C; ii. Silica gel chromatography (AcOEt/pentane, 2:1, $R_f = 0.66$); iii. Crystallization upon MeOH addition ($F = 182$ °C), 80%; (c) H_2, 15 bar, Pd/C, CH_2Cl_2, 6 days, 96%; (d) $(PhO)_2POCl$ (1 equiv), pyridine, 12 h, 25 °C, 95%; (e) H_2, 25 bar, PtO_2, CH_2Cl_2, 6 days, 100%; (f) i. Solid NH_2OH, MeOH, 12 h, 4 °C; ii. Precipitation upon Et_2O addition; iii. Water dissolution, freeze-drying, 91%.

encodes both RpiB and AlsI activities.[3,4] The longer sugar in the All6P case could easily be accommodated in the active site, via small movements of the long flexible basic residues that interact with the phosphate group; the other end of the sugar, that which is isomerized, is expected to dock in a very similar manner in the two cases.[7] In conclusion, the synthesis of 5-deoxy-5-phospho-D-ribonohydroxamic acid **4** (5PRH), a new competitive and selective inhibitor of the R5P to Ru5P isomerization reaction catalyzed by *Mt*RpiB (vs *So*RpiA) appears to be very promising for the further kinetic, mechanistic, and structural investigations on the presumed second enzymatic activity of *Mt*RpiB, that is, AlsI activity. Such investigations will hopefully lead to the development of more selective and efficient inhibitors of *Mt*RpiB and other RpiBs of therapeutic interest.

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10. 2,3-Di-*O*-benzoyl-5-*O*-triphenylmethyl-D-ribono-1,4-lactone (**7**). A mixture of **6** (7.67 g, 19.6 mmol) and benzoyl chloride (4.9 mL, 42.5 mmol) in dry pyridine (35 mL) was stirred under argon for 19 h at 25 °C. After concentration of the residue and addition of water (30 mL), the product was extracted with chloroform (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated (10 mL). Upon addition of methanol (40 mL), 9.42 g (80% yield) of **7** were obtained as white crystals: mp = 182 °C. $R_f = 0.66$ (AcOEt/pentane 2/1). ^{13}C NMR (90 MHz, $CDCl_3$) δ (ppm) 171.9 (C-1), 166.0, 165.5 (C-11, C-11′), 143.4 (C-7), 134.4, 134.3 (C-12, C-12′), 130.7, 130.4 (C-14, C-14′), 129.3 (C-9), 129.2, 129.1 (C-15, C-15′), 129.1–128.9 (C-8, C-13, C-13′), 128.2 (C-10), 88,9 (C-6), 83.1 (C-4), 72.2 (C-2), 68.5 (C-3), 63.5 (C-5). Anal. Calcd for $C_{38}H_{30}O_7$ (598.6): C, 76.24; H, 5.05. Found C, 76.25; H, 5.03.
11. 2,3-Di-*O*-benzoyl-D-ribono-1,4-lactone (**8**). A mixture of **7** (6.65 g, 11.1 mmol) and 10% Pd/C (2.50 g) in anhydrous CH_2Cl_2 (130 mL) was hydrogenolyzed (15 bar) at 25 °C for 6 days. After filtration of the mixture and evaporation of the solvent, the residue was purified by silica gel chromatography (eluting triphenylmethane with $CHCl_3$, then the desired product with $CHCl_3$/AcOEt 1/1) to afford 3.82 g of **8** (96% yield) as a colorless oil: $R_f = 0.54$ ($CHCl_3$/AcOEt 1/1). ^{13}C NMR (62 MHz, $CDCl_3$) δ (ppm) 172.4 (C-1), 165.8, 165.3 (C-6, C-6′), 134.1 (C-7, C-7′), 130.2, 130.0 (C-9, C-9′), 128.8, 128.4 (C-10, C-10′), 128.7 (C-8, C-8′), 84.5 (C-4), 71.8 (C-2), 68.3 (C-3), 61.7 (C-5).
12. 2,3-Di-*O*-benzoyl-5-deoxy-5-diphenylphosphate-D-ribono-1,4-lactone (**9**). To a solution of compound **8** (3.82 g, 10.7 mmol) in anhydrous pyridine (15 mL) at 0 °C under argon was added diphenylphosphochloridate (2.22 mL, 10.7 mmol). Thereafter, the solution was stirred at 25 °C for 15 h. After evaporation of the solvent and addition of AcOEt (15 mL), the precipitated pyridinium hydrochloride was filtered off and the solvent evaporated. The residue was purified by silica gel chromatography ($CHCl_3$) to afford 6.00 g of **9** (95% yield) as a colorless oil: $R_f = 0.23$ ($CHCl_3$). ^{13}C NMR (90 MHz, $CDCl_3$) δ (ppm) 170.4

(C-1), 165.4, 165.0 (C-6, C-6′), 150.5 (C-11, C-11′), 134.2, 134.0 (C-7, C-7′), 130.4, 130.3 (C-9, C-9′), 130.0, 129.8 (C-10, C-10′), 129.7 (C-14, C-14′), 128.7, 128.4 (C-8, C-8′), 126.1 (C-13, C-13′), 120.3 (d, C-12, C-12′, $J = 4.8$ Hz), 81.3 (d, C-4, $J = 7.9$ Hz), 70.2 (C-2), 67.5 (d, C-5, $J = 5.6$ Hz), 67.4 (C-3).

13. 2,3-Di-*O*-cyclohexanoyl-5-deoxy-5-dihydrogenophosphate-D-ribono-1,4-lactone (**10**). A mixture of compound **9** (6.00 g, 10.2 mmol) and PtO_2 (250 mg) in anhydrous CH_2Cl_2 (100 mL) was placed under H_2 (25 bar) for 6 days. Removal of the catalyst by filtration and evaporation of the solvent afforded 4.57 g of **10** (100% yield) as a colorless oil: ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 175.3, 174.9 (C-6, C-6′), 171.7 (C-1), 82.1 (d, C-4, $J = 8.5$ Hz), 70.2 (C-2), 67.3 (C-3), 66.1 (C-5).
14. 5-Deoxy-5-phospho-D-ribonohydroxamic acid, bis-(hydroxylammonium) salt (5PRH, **4**). A solution of **10** (4.57 g, 10.2 mmol) in anhydrous methanol was stirred at 4 °C for 24 h with an excess of solid NH_2OH[15] (**CAUTION**, this very volatile and toxic product must be handled in a fume hood, wearing gloves; because hydroxamic acids are known to readily form stable metal complexes, a glass spatula was used to handle solid NH_2OH, and thereafter 5PRH). Upon addition of diethyl ether, compound **4** precipitated from the reaction mixture as the bis(hydroxylammonium) salt. Following water dissolution and freeze-drying, 3.04 g of **4** was recovered (91% yield) as a white solid (hygroscopic): ^1H NMR (250 MHz, D_2O) δ (ppm) 3.82–4.04 (m, 4 H, H-3, H-4, CH_2), 4.36 (d, $J = 3.8$ Hz, H-2). ^{13}C NMR (90 MHz, D_2O) δ (ppm) 171.2 (C-1), 72.8 (C-2), 72.3 (C-3), 71.0 (d, $J = 6.8$ Hz, C-4), 66.1 (C-5). ^{31}P NMR (90 MHz, D_2O) δ (ppm) 4.98. FT-IR ν (cm^{-1}) 3380, 3215 (NH, OH), 1653 (C=O), 1056, 966 (C–O, PO_3^{2-}). MS (negative ion electrospray) *m/z* (%) 260.1 $[MH]^-$ (78, MW = 260.0), 245.1 $[MH-NH]^-$ (21), 227.1 $[MH-NH_2OH]$ (100). Anal. Calcd for $C_5H_{18}N_3O_{11}P$ (327.2): C, 18.35; H, 5.55. Found: C, 18.41; H, 6.01.
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