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Synthesis of D-Arabinohydroxamic Acid and D-Threonohydroxamic Acid, Potent Inhibitors of D-Xylose Isomerases

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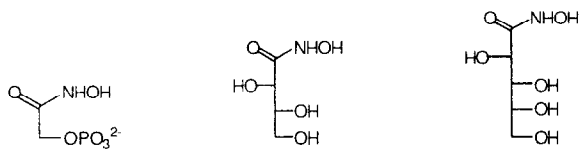
Abstract: Two potent inhibitors of D-xylose isomerases: D-threonohydroxamic acid **2** and D-arabinohydroxamic acid **3** have been synthesized by conversion of D-arabinose to a protected derivative of D-arabinonic acid and introduction of the hydroxamate group by coupling with O-benzylhydroxylamine.

Hydroxamic acids have been shown to inhibit various enzymes. Simple hydroxamic acids, e.g. benzohydroxamic acid have been shown to inhibit peroxidases.[1] Peptide-derived hydroxamates are efficient inhibitors of zinc proteases: e.g. thermolysin,[2] due in part to their metal-complexing properties. On the other hand, phosphoglycolohydroxamate **1** (**Fig. 1**) is a powerful inhibitor of triosephosphate isomerase (TIM)[3] and of yeast fructofuranose-1,6-diphosphate aldolase.[3a] With the former enzyme, the low K_i value of **1** (15 μM) is best explained by the structural similarity between the enediol(ate) intermediate of the isomerization reaction



1 2 3

Figure 1

and the hydroxamate anion. N-Hydroxy-N-isopropyloxamate was shown to be an exceptional potent inhibitor of the *E. coli* ketol-acid reductoisomerase:[4] again the very low K_i value (K_i = 22 pM) of this compound was rationalized by its structural similarity with the rearrangement transition state and by its ability to complex the divalent cation (Mg^{++}or Mn^{++}) which is present at the active site. In the case of aldose-ketose isomerases, two different mechanisms have been postulated: the enediol(ate) intermediate mechanism which is operating with TIM[5] and a hydride shift mechanism which has been proposed by several authors on the basis of X-ray structural data[6] and isotope exchange experiments[7] for the isomerization of D-xylose (or D-glucose) into D-xylulose (or D-fructose) by D-xylose isomerases. These two mechanisms have two important features in common: 1) the O_1-C_1-C_2-O_2 fragment must be planar in the transition state of the reaction and 2) a negative charge develops on O_1 and O_2 during the reaction. These considerations, plus the fact that the D-xylose isomerases possess two divalent metal cations (Mg^{++}, Mn^{++} or Co^{++}) bound to the O_1, O_2 and O_4 oxygen atoms of the substrate, led us to synthesize compounds **2** and **3** (**Fig. 1**) which should be transition state analog (TSA) inhibitors of these enzymes. Compound **3**, for which we report the first synthesis, could also

behave as a good inhibitor of sorbitol dehydrogenase[8] due to its close resemblance to the enzyme substrate, D-sorbitol, and to the ability of the hydroxamate group to bind to the Zn^{++} cation which is present at the active site. To our knowledge, only a very few sugar-derived hydroxamic acids have been synthesized to date.[3,9] While we were working on this project, an elegant synthesis of **2** was recently reported by Allen and coll.[9a]: **2** proves to be a powerful TSA inhibitor of D-xylose isomerase from *S. olivochromogenes* (Ki ≤ 100 nM).

The common starting product for the synthesis of both **2** and **3** was D-arabinose **4** (**Fig. 2**) which has the same absolute configurations at C_2, C_3 and C_4. D-arabinose was converted into the hydroxamic acid **9** which is the precursor of D-arabinohydroxamic acid **3**.[10] Selective removal of the isopropylidene group of **9** (**Fig. 3**), followed by periodic oxidation and $NaBH_4$ reduction of the oxidized intermediate led to compound **14**, the precursor of D-threonohydroxamic acid **2**.[11]

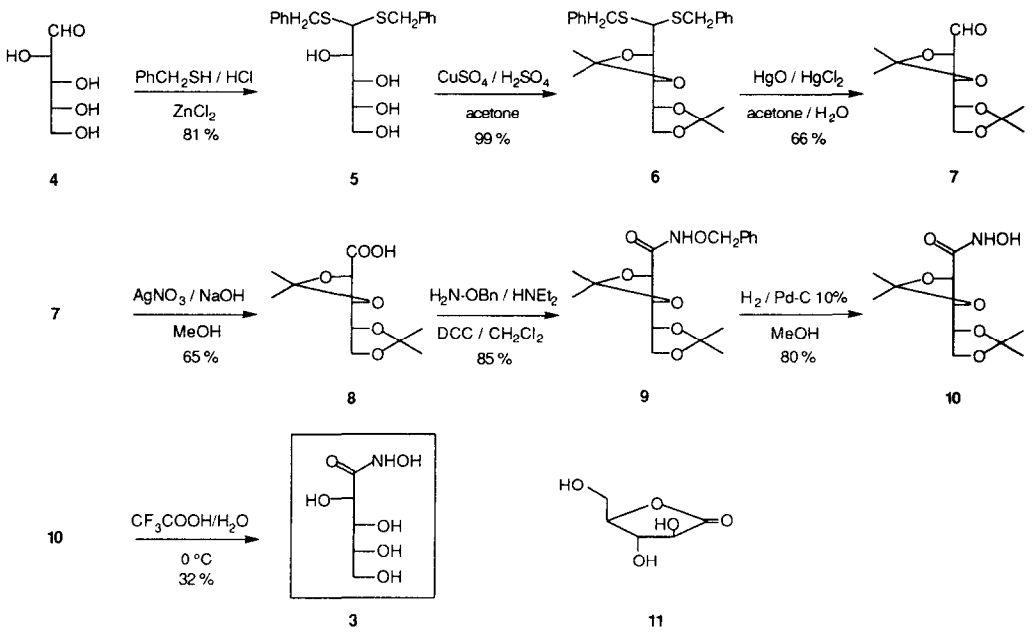


Figure 2. Synthesis of D-arabinohydroxamic acid **3**.

2,3-4,5-Di-O-isopropylidene-D-arabinose **7** (**Fig. 2**) was prepared from D-arabinose **4** by the procedure of Kochetkov and coll.[12]: D-arabinose was first converted into the dithioacetal **5** which by reaction with acetone in the presence of H_2SO_4 and $CuSO_4$ gives the diisopropylidene derivative **6**; treatment of **6** with a mixture of $HgCl_2$-HgO in the presence of water gives compound **7** with an overall yield of 50%. Several oxidizing reagents were used to convert **7** into the protected D-arabinonic acid **8**: the best results were obtained using $AgNO_3$ in alcaline solution.[13] Compound **8** was then reacted with O-benzylhydroxylamine[14] to give the protected hydroxamic acid derivative **9**. Hydrogenolysis of the benzyl group of **9** using Pd/C catalyst gave the hydroxamic acid **10** which by acid hydrolysis was converted into D-arabinohydroxamic acid **3**. Several experimental conditions were used to remove the isopropylidene groups. The best one in our hands proved to be CF_3COOH/H_2O (4:1) at 0°C: compound **3** was obtained together with D-arabino-1,4-lactone **11**[15] in a 1:2

ratio. Purification of **3** was simply achieved by complexation with copper(II) acetate in a water/methanol (1/1) solution, precipitation with acetone and decomplexation on Amberlite IR 718: the ^{1}H and ^{13}C NMR data of the purified product were in full agreement with the proposed structure. The presence of the hydroxamic acid function was further confirmed by its characteristic reaction with $FeCl_3$.[16]

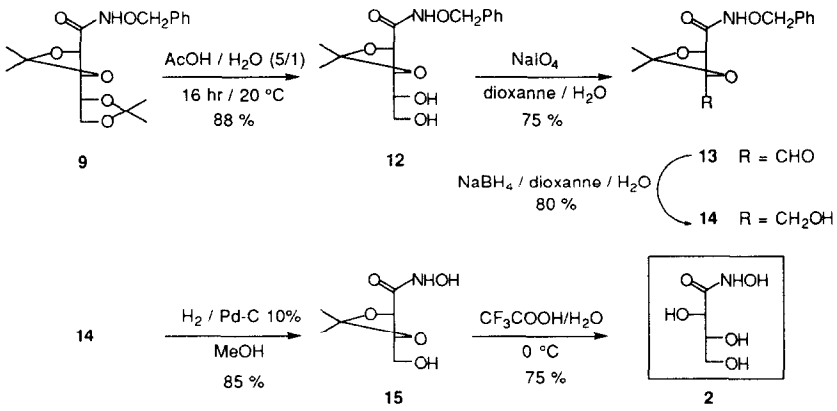


Figure 3. Synthesis of D-threonohydroxamic acid **2**.

Controlled hydrolysis of compound **9** (**Fig. 3**) afforded the diol **12** which by periodic oxidation followed by $NaBH_4$ reduction of the aldehyde **13** led to compound **14**. This product was deprotected first by hydrogenolysis and then by acid hydrolysis (CF_3COOH/H_2O=4/1 at 0°C) to give D-threonohydroxamic acid **2**. In this particular case, no lactone formation was detected.

The results of the inhibition study using **2** and **3** and *E. coli* D-xylose isomerase will soon be reported.

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10. All new compounds gave spectroscopic data in agreement with the assigned structure; selected data are given for the following compounds (δ in ppm, J_{ij} in Hz, *: exchangeable resonances): **9**: ^{1}H NMR ($CDCl_3$, 250 MHz): 1.31 and 1.37 (2s, 6H, $2CH_3$), 1.43 (s, 6H, $2CH_3$), 4.10 and 4.31 (2m, 5H, H_2, H_3, H_4, H_5 and $H_{5'}$) , 4.95 (s, 2H, $C\underline{H}_2Ph$), 7.39 (m, 5H, Ph), 8.86 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 62.9 MHz): 25.06, 25.73, 26.29 and 26.78 ($4CH_3$), 65.64 (C5), 75.64, 75.89, 78.74 and 79.14 (C2, C3, C4 and $\underline{C}H_2Ph$)*, 109.94 (2Cq isop), 128.54, 128.84, 129.14 and 134.94 (Ph), 167.94 (C1); MS (CI-D, NH_3): *m/z* 352 (M^+ + 1), 336 (M^+ - CH_3). **10**: ^{1}H NMR (CD_3OD, 250 MHz): 1.39 (s, 6H, $2CH_3$), 1.43 (s, 6H, $2CH_3$), 3.68 (m, 1H, H_5), 3.73 (m, 1H, H_4), 3.78 (m, 1H, $H_{5'}$), 4.15 (t, 1H, H_3, J_{23}=7.5, J_{34}=5.7), 4.45 (d, 1H, H_2); MS (EI): *m/z* 231 (M^+ + 2 - NHOH). **3** (sodium salt): ^{1}H NMR (D_2O, 250 MHz): 3.55-3.99 (m, 4H), 4.28 (s, 1H, H_2); ^{13}C NMR (D_2O, 62.9 MHz): 64.29 (C_5), 72.38, 72.78 and 73.41 (C_2, C_3 and C_4)*, 166.12 (C_1, C(OH)=NOH form), 180.68 (C_1, C(=O)-NHOH form); MS (CI-D, NH_3) tetraacetate derivative: *m/z* 366 (M + NH_3)$^+$, 289 (M - CONHOH)$^+$.
11. **12**: ^{1}H NMR ($CDCl_3$, 250 MHz): 1.27 and 1.42 (2s, 6H, $2CH_3$), 3.74 (m, 2H, H_5 and $H_{5'}$), 3.87 (m, 1H, H_4), 4.12 (t, 1H, H_3, J_{34}=6.1), 4.45 (d, 1H, H_2, J_{23}=7.6), 4.94 (s, 2H, $C\underline{H}_2Ph$), 7.38 (m, 5H, Ph), 9.31 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50.3 MHz): 25.32 and 26.58 ($2CH_3$), 62.94 (C5), 71.53, 75.80, 78.46 and 78.90 (C2, C3, C4 and $\underline{C}H_2Ph$)*, 111.09 (Cq isop), 128.66, 129.02, 129.30 and 134.52 (Ph), 168.91 (C1); MS (CI-D, NH_3): *m/z* 312 (M^+ + 1), 296 (M^+ + 1 - H_2O). **14**: ^{1}H NMR ($CDCl_3$, MHz): 1.31 and 1.45 (2s, 6H, $2CH_3$), 3.05 (br s, 1H, OH), 3.80 (dd, 1H, H_4, $J_{44'}$=-11.5, J_{34}=4.0), 3.92 (dd, 1H, $H_{4'}$, $J_{34'}$=4.0), 4.13 (m, 1H, H_3), 4.31 (d, 1H, H_2, J_{23}=7.8), 4.95 (s, 2H, $C\underline{H}_2Ph$), 7.38 (m, 5H, Ph), 9.56 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 50.3 MHz): 25.67 and 26.77 ($2CH_3$), 62.30 (C4), 76.28, 78.48 and 79.22 (C2, C3 and $\underline{C}H_2Ph$)*, 111.12 (Cq isop), 128.69, 129.01, 129.33 and 134.68 (Ph), 167.74 (C1); MS (CI-D, NH_3): *m/z* 282 (M^+ + 1), 281(M^+), 266 (M^+ - CH_3). **15**: ^{1}H NMR (CD_3OD, 200 MHz): 1.42 and 1.43 (2s, 6H, $2CH_3$), 3.68 (dd, 1H, H_4, $J_{44'}$=-12.0, J_{34}=4.0), 3.88 (dd, 1H, $H_{4'}$, $J_{34'}$=3.0), 4.14 (m, 1H, H_3), 4.24 (d, 1H, H_2, J_{23}=7.8); ($CDCl_3$, 200 MHz): 1.45 and 1.47 (2s, 6H, $2CH_3$), 3.88 (m, 2H, H_4 and $H_{4'}$), 4.14 (m, 1H, H_3), 4.36 (d, 1H, H_2, J_{23}=7.8); ^{13}C NMR ($CDCl_3$, 50.3 MHz): 26.07 and 27.04 ($2CH_3$), 62.51 (C4), 77.78 and 79.74 (C2 and C3)*, 111.45 (Cq), 168.11 (C1) ; (CD_3OD, 50.3 MHz): 25.97 and 27.11 ($2CH_3$), 62.96 (C4), 76.20 and 81.43 (C2 and C3)*, 112.12 (Cq), 169.64 (C1). **2**: ^{1}H NMR (D_2O, 250 MHz) δ: 3.63 (m, 1H, H_4), 4.02 (m, 1H, $H_{4'}$), 4.18 (br s, 1H, NH), 4.47 (m, 2H, H_2 and H_3); (CD_3OD, 250 MHz) δ: 3.65 (br s, 2H, H_4 and NH), 3.94 (dd, 1H, $H_{4'}$, $J_{44'}$=-8.4, $J_{34'}$=6.6), 4.31 (m, 2H, H_2 and H_3); ^{13}C NMR: (CD_3OD, 62.9 MHz) δ: 63.38 (C4), 72.16 and 73.94 (C_2 and C_3)*, 175.35 (C_1); (D_2O, 62.9 MHz) δ: 62.80 (C4), 71.61 and 72.59 (C_2 and C_3)*, 171.78 (C_1); lit.[9a]: (D_2O) δ: 63.0 (C4), 71.6 (C_3), 72.7 (C_2), 171.8 (C_1).
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