Pergamon

Tetrahedron Letters, Vol. 37, No. 13, pp. 2237-2240, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00254-7

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

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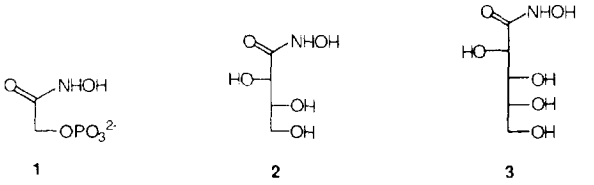
Abstract: Two potent inhibitors of D-xylose isomerases: D-threonohydroxamic acid **2** and D-arabinohydroxamic acid **3** have been synthesized by conversion of D-arabinose to a protected derivative of D-arabinonic acid and introduction of the hydroxamate group by coupling with O-benzylhydroxylamine.

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]

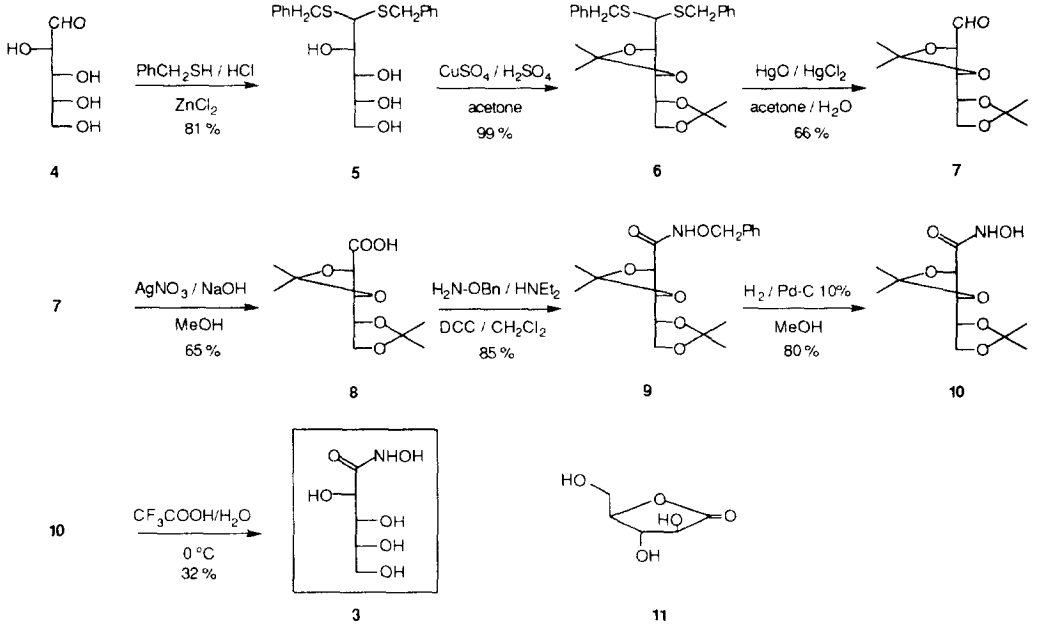


Figure 2. Synthesis of D-arabinohydroxamic acid **3**.

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]

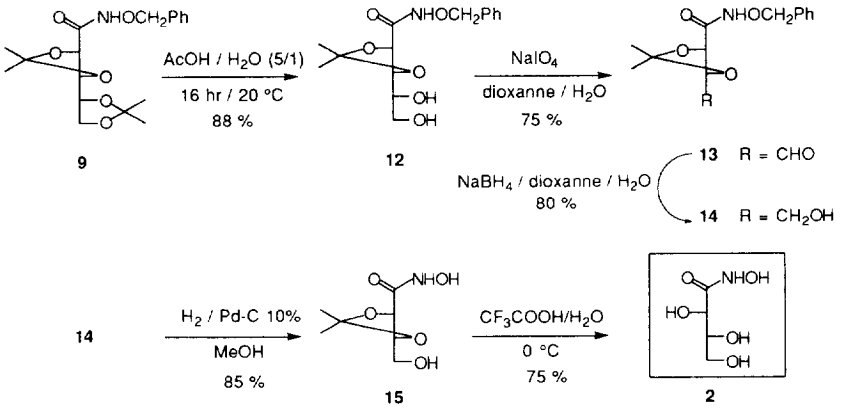


Figure 3. Synthesis of D-threonohydroxamic acid **2**.

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.

Acknowledgments: This research was supported by the Centre National de la Recherche Scientifique (C.N.R.S.) ant the Ministère de la Recherche et de l'Enseignement Supérieur (M.R.E.S.).

References and Notes

1. (a) Schonbaum, G. R. *J. Biol. Chem.* **1973**, *248*, 502-511. (b) Kimura, S.; Yamazaki, I. *Arch. Biochem. Biophys.* **1979**, *198*, 580-588. (c) Kitagawa, T.; Hashimoto, S.; Teraoka, J.; Nakamara, S.; Yajima, H.; Hosoya, T. *Biochemistry* **1983**, *22*, 2788-2792. (d) Thanabal, V.; de Ropp, J. S.; La Mar, G. N. *J. Am. Chem. Soc.* **1987**, *109*, 7516-7525. (e) Ikeda-Saito, M.; Shelley, D. A.; Lu, L.; Booth, K. S.; Caughey, W. S.; Kimura, S. *J. Biol. Chem.* **1991**, *266*, 3611-3616.
2. (a) Nishino, N.; Powers, J. C. *Biochemistry* **1978**, *17*, 2846-2850. (b) Nishino, N.; Powers, J. C. *Biochemistry* **1979**, *18*, 4340-4347. (c) Holmes, M. A.; Matthews, B. W. *Biochemistry* **1981**, *20*, 6912-6920. (d) Izquierdo-Martin, M.; Stein, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 325-331.
3. (a) Collins, K. D. *J. Biol. Chem.* **1974**, *249*, 136-142. (b) Davenport, R. C.; Bash, P. A.; Seaton, B. A.; Karplus, M.; Petsko, G. A.; Ringe, D. *Biochemistry* **1991**, *30*, 5821-5826.
4. Aulabaugh, A.; Schloss, J. V. *Biochemistry* **1990**, *29*, 2824-2830.
5. Rose, I. A. *Methods Enzymol.* **1982**, *87*, 84-97.
6. (a) Farber, G. K.; Glasfeld, A.; Tiraby, G.; Ringe, D.; Petsko, G. A. *Biochemistry* **1989**, *28*, 7289-7297. (b) Collyer, C. A.; Henrick, K.; Blow, D. M. *J. Mol. Biol.* **1990**, *212*, 211-235. (c) Whitlow,

M.; Howard, A. J.; Finzel, B. C.; Poulos, T. L.; Winborne, E.; Gilliland, G. L. *Proteins: Struct. Funct. Genet.* **1991**, *9*, 153-173.

7. (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.
8. Jeffery, J.; Jörnvall, H. *Adv. Enzymol.* **1988**, *61*, 47-106.
9. (a) Allen, K. N.; Lavie, A.; Petsko, G. A.; Ringe, D. *Biochemistry* **1995**, *34*, 3742-3749. (b) Park, J. -B.; Imamura, L.; Kobashi, K.; Itoh, H.; Miyazaki, T.; Horisaki, T. *Biol. Pharm. Bull.* **1995**, *18*, 208-213. (c) Bonnette, C.; Salmon, L.; Gaudemer, A. *Tetrahedron Lett.* **1996**, *37*, 0000.
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 (C_4), 72.16 and 73.94 (C_2 and C_3)*, 175.35 (C_1); (D_2O, 62.9 MHz) δ: 62.80 (C_4), 71.61 and 72.59 (C_2 and C_3)*, 171.78 (C_1); lit.[9a]: (D_2O) δ: 63.0 (C_4), 71.6 (C_3), 72.7 (C_2), 171.8 (C_1).
12. Kochetkov, N. K.; Dmitriev, B. A. D. *Tetrahedron* **1965**, *21*, 803-815.
13. Hampton, A.; Perini, F.; Harper, P. J. *Carbohyd. Res.* **1974**, *37*, 359-367.
14. Sheenan, J. C.; Preston, J.; Cruickshank, P. A. *J. Am. Chem. Soc.* **1965**, *87*, 2492-2493.
15. Angelotti, T.; Krisko, M.; O'Connor, T.; Serianni, A. S. *J. Am. Chem. Soc.* **1987**, *109*, 4464-4472.
16. Vogel, A. I. *Practical Organic Chemistry, 3rd ed.*; Longmans: London, 1964; 1062-1063.

(*Received in France* 19 *January* 1996; *accepted* 6 *February* 1996)