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Synthesis of a Guanidino-Sugar as a Glycosyl Cation Mimic

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Abstract: The synthesis of a guanidine threose (**1a**) is described. Under basic conditions, compound **1a** equilibrates to tetrahydropyrimidine form **1b** which acts as a transition-state inhibitor for green coffee bean galactosidase.

Glycosidase inhibitors[1,2b,c] have been used to treat diabetes and other metabolic disorders,[2] and have been implicated in the blocking of viral infections.[3,4] Inhibitors for these enzymes[5–7] are usually designed to mimic the transition-state or transient intermediate present in the active-site during enzyme catalysis. Figure 1 illustrates one class of such inhibitors that were designed to mimic the galactosyl

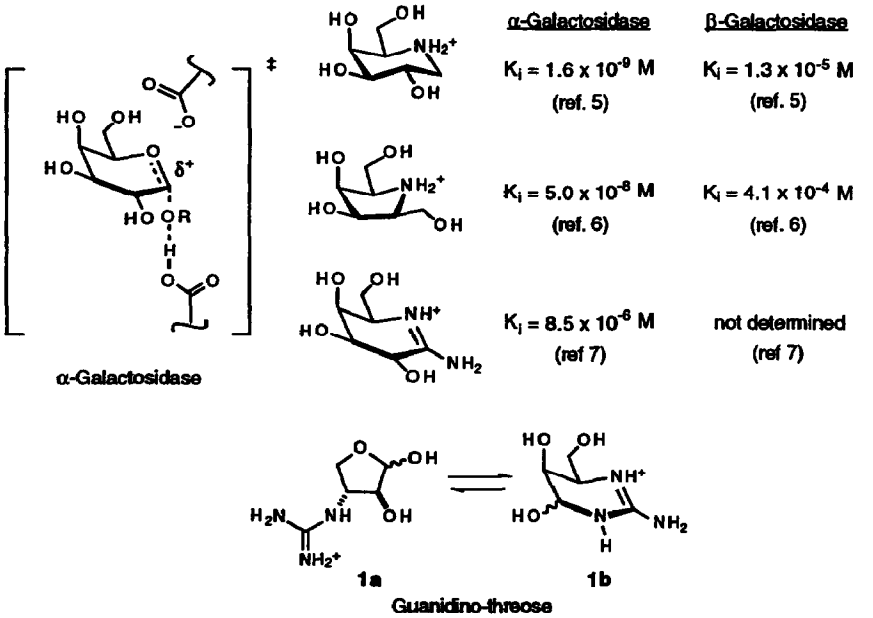
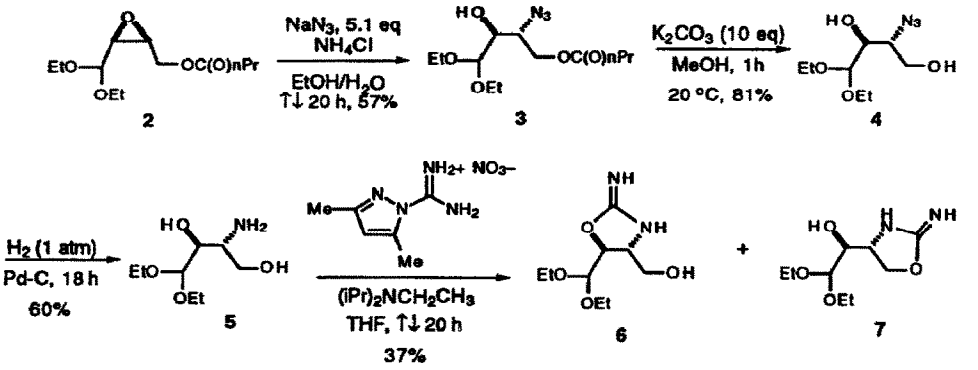


Figure 1

cation.[5–7] These inhibitors feature hydroxyl groups, a protonated heteroatom, and a ring conformation that either mimics the conformation of galactose or the half-chair conformation of the galactosyl cation. In almost all cases, the mimics for glycosyl cations have been pyrrolidine and piperidine "aza sugars"[1,8] or amidine derivatives of monosaccahrides.[7] In this report we wish to describe the synthesis of a new class of glycosyl cation mimics: the guanidino-tetroses. The proposed guanidine tetrose may exist in the furanose form **1a** (Figure 1) that itself may not be a good inhibitor since it does not reflect the structure of the galactosyl cation. However, the tetrahydropyrimidine form (**1b**, Figure 1) is a good mimic of the half-chair conformation present in the galactosidase transition state.

Synthesis of exo-guanidino hexoses have been described,[9] but a guanidino-tetrose and its use as a glycosidase inhibitor is heretofore unknown.[10] Because the guanidino-tetrose may exist in a conformation **1b**, the nitrogens are situated in such a way that they should interact favorably with the carboxylate resides[10] in the active-site of the glycosidase enzymes.

Synthesis of the guanidino-threose began with the non-carbohydrate precursor **2** which is readily available as the optically-active form[11,12] or a racemic mixture.[12] Epoxide **2** is regioselectively opened with NaN_3 to give 2-hydroxy-3-azido product **3**, and after removal of the butyrate, azido diol **4** is obtained. Reduction of the azide using H_2/Pd-C yielded amino compound **5** that was then treated with the guanidinlyating reagent 3,5-dimethylpyrazole-1-carboxamidinium nitrate (DPCN).[13] The only products obtained in this reaction were a mixture of imino carbamates **6** and **7** that apparently formed by both the hydroxyl and amino group adding to the activated C=N bond (Scheme 1).



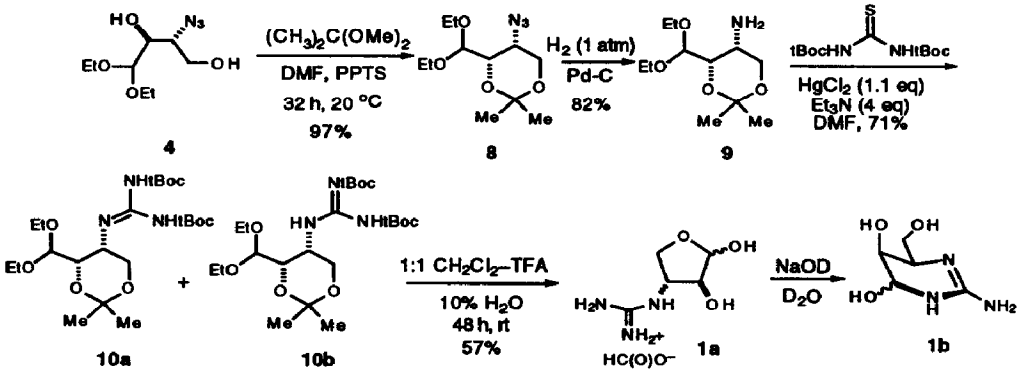
Scheme 1

An alternative route to the guanidine was carried out by first protecting the diol of **4** to give acetonide (**8**) (Scheme 2). Hydrogenation of the azide moiety provided amine **9** which was then treated with *N,N'*-bis-*tert*-butoxycarbonylthiourea[14,15] to give protected guanidine derivative **10**[16] as a mixture of tautomers (**10a** and **10b**). All of the protecting groups were then removed using trifluoroacetic acid (TFA) and the desired sample was isolated as the formate salt after chromatography (R_f = 0.5, 7:2:1 ethyl

acetate–formic acid–H_2O).[17] In D_2O, the ^{1}H-NMR indicates that **1** is a mixture of α and β furanose anomers (**1a**, pH 5). With the addition of NaOD the pH is raised to 11, and the neutral cyclic guanidine (**1b**) becomes the predominate form.

Inhibition studies carried out on green coffee bean α-galactosidase with **1**[18] showed that inhibition increased with an increase in pH. At pH 7.5, **1** exhibited an IC_{50} of 1.3 mM, but when the inhibitor was in pH 10.7 buffer and then added to the assay (still at pH 7.5), the IC_{50} dropped to 480 μM. As the pH of the solution is raised, the concentration of tetrahydropyrimidine **1b** must also increase as observed in the NMR study above. Therefore, inhibition may be due to the interaction of **1b** with the enzyme.

Guanidine compounds which exist solely as tetrahydropyrimidines should be better inhibitors of galactosidase, and methods for their prepartion are currently being examined.



Scheme 2

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14. Spectral data for (**9**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.56 (1H, d, J = 7.1 Hz, H-1), 4.09 (1H, d of d, J = 2, 12 Hz, H-4), 3.88 (1H, d of d, J = 1.8, 6.8 Hz, H-2), 3.78 (1H, d of q, J = 7.1, 9.2 Hz, $OC\underline{H}_2CH_3$), 3.68 (1H, d of d, J = 1.8, 12 Hz, H-4), 3.63 (2H, m, $OC\underline{H}_2CH_3$), 3.57 (1H, d of q, J = 7, 9.6 Hz, $OC\underline{H}_2CH_3$), 2.78 (1H, d of d, J = 2, 3.8 Hz, H-3), 1.45, 1.43 (6 H, 2s), 1.22, 1.21 (6H, 2t, J = 7 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 101.2, 98.66, 67.11, 62.74, 61.39, 45.36, 29.22, 18.19, 15.01, 14.94; IR (neat, FT) 3381 (br), 2975, 1380, 1067 cm^{-1}; HRMS (FAB, Cs^+) *m/z* 366.0680 (366.0681 calcd for $C_{11}H_{23}NO_4$+Cs^+).
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16. Spectral data for (**10a**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (1H, app d, J = 9.4 Hz, H-3), 4.41 (1H, d, J = 7.3 Hz, H-1), 4.03 (1H, app d, J = 11 Hz, H-4), 3.95 (1H, app d, J = 7.3 Hz, H-2), 3.79–3.41 (5H, m, H-4 and $OC\underline{H}_2CH_3$), 1.45 (18 H, s), 1.20 (6H, t, J = 7 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.0, 100.5, 99.12, 79.15, 71.05, 65.32, 64.16, 63.86, 60.48, 44.74, 29.58, 28.22,18.19, 15.47, 15.40, 14.84; (**10b**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.45 (1H, s, NH), 9.15 (1H, d, J = 8.8 Hz, NH), 4.39 (1H, d of d, J = 1.6, 8.8 Hz, H-3), 4.36 (1H, d, J = 7.3 Hz), 4.00 (1H, d of d, J = 1.4, 13 Hz, H-4), 3.98 (1H, d of d, J = 1.6, 7.6 Hz, H-2), 3.83 (1H, d of d, J = 1.7, 12 Hz, H-4), 3.73 (1H, d of q, J = 6.8, 9.1 Hz, $OC\underline{H}_2CH_3$), 3.64 (1H, d of q, J = 7.1, 9.2 Hz, $OC\underline{H}_2CH_3$), 3.56 (1H, d of q, J = 7.1, 8.8 Hz, $OC\underline{H}_2CH_3$), 3.43 (1H, d of q, J = 8.8, 9.1 Hz, $OC\underline{H}_2CH_3$), 1.50, 1.47 (6H, 2s), 1.19 (6H, t, J = 7 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 155.67, 152.66, 100.43, 99.26, 82.82, 78.81, 70.96, 64.55, 63.92, 60.31, 44.72, 29.47, 28.22, 27.97, 18.29, 15.35, 14.87; Both (**10a**) and (**10b**) IR (neat, FT) 2975, 1720, 1498, 1168, 1070 cm^{-1}; HRMS (FAB, Cs^+) *m/z* 608.1960 (608.1948 calcd for $C_{22}H_{41}N_3O_8$+Cs^+).
17. Spectral data for (**1a**): ^{1}H NMR (400 MHz, D_2O) (α-form) δ 5.04 (1H, app s, H-1), 4.59 (1H, d, J = 1.4 Hz, H-2), 4.41 (1H, d, J = 9 Hz, H-4), 4.24 (1H, d of d, J = 3.2, 9.2 Hz, H-4), 3.93 (1H, m, H-3); (β-form) 4.93 (1H, d, J = 3 Hz, H-1), 4.00 (1H, t, J = 2.2 Hz, H-2), 3.87 (1H, d of d, J = 4, 9.6 Hz, H-4), 3.75 (2H, m, H-4, H-3); ^{13}C NMR (101 MHz, D_2O, minor anomer in parentheses) δ 153.5 (154.9), 74.43 (83.09), 63.06 (77.65), 60.17 (70.34), 50.24 (53.93); (**1b**) ^{1}H NMR (400 MHz, D_2O+NaOD) δ 4.56 (1H, d, J = 2 Hz, H-1) 3.61 (2H, t, J = 6 Hz), 3.55 (1H, m); 3.36 (1H, d of t, J = 2, 6 Hz, H-5); ^{13}C NMR (101 MHz, D_2O+NaOD) δ 154.1, 80.24, 68.26, 62.93, 51.93; (**1**): IR (neat, FT) 3262 (br), 1673, 1202, 1136, 724 cm^{-1}; HRMS (FAB, Cs^+) *m/z* 162.0879 (162.0879 calcd for $C_5H_{11}N_3O_3$+Cs^+).
18. Inhibition studies were done in 100 mM HEPES-NaOH (pH 7.5) containing 1.5 mM α-D-*p*-nitrophenyl galactopyranoside and 56 Units/L green coffee bean α-galactosidase (Sigma). Liberation of *p*-nitrophenolate was monitored over 2 min at 410 nm.

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