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Chemo-enzymatic Synthesis of Five-membered Azasugars as Inhibitors of Fucosidase and Fucosyltransferase: An Issue Regarding The Stereochemistry Discrimination at Transition States

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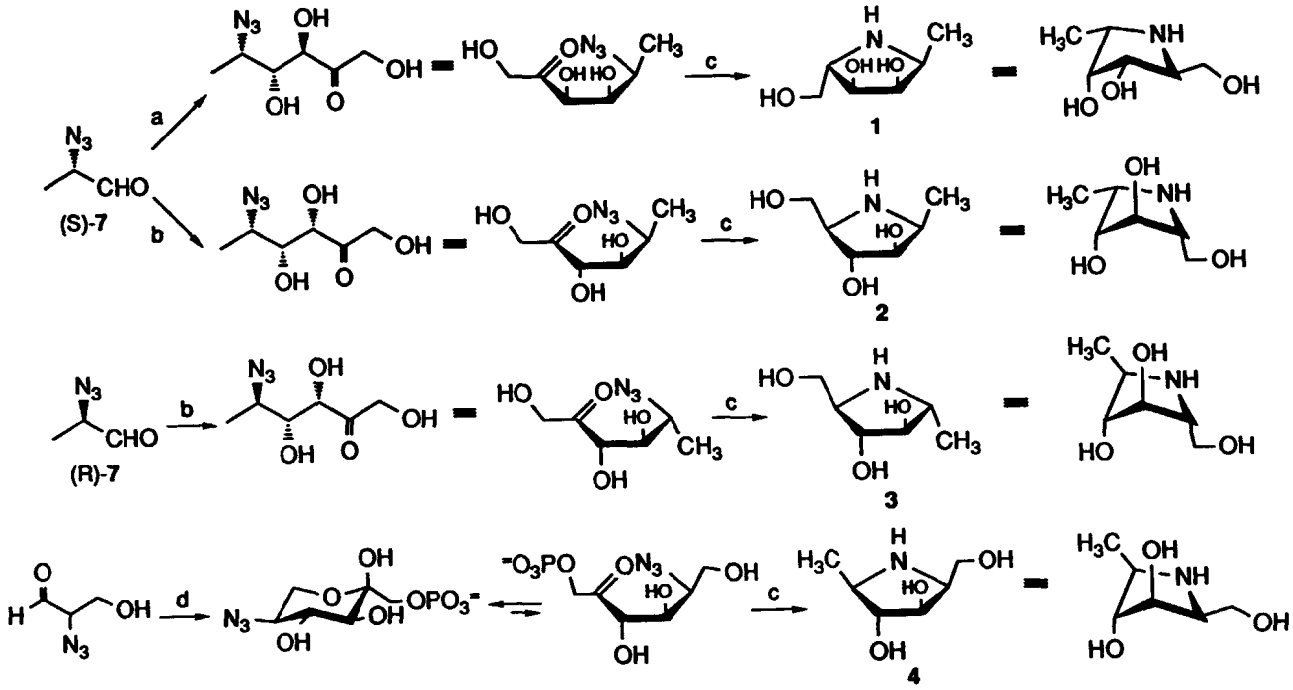
Abstract: Three new 5-membered aza sugars which inhibit bovine α-fucosidase with K_i values in the μM range have been prepared based on aldolase reactions.

Many natural and synthetic polyhydroxylated piperidines and pyrrolidines with potential therapeutic utility are inhibitors of glycosidase enzymes.[1] Although the mode of action of 6-membered aza sugars has been studied,[1,2] the inhibition of 5-membered aza sugars is not well understood. It has been suggested that the 6-membered aza sugars act as transition-state analog inhibitors[1,2] and that the stereochemistry discrimination of a half-chair like inhibitor interacting with the enzyme is not as significant as that of a chair-like inhibitor, as indicated in the broad spectrum inhibition activities of an aminidium ion and an amidrazone.[3] This lack of stereochemistry discrimination was attributed to the overriding electrostatic interaction between the enzyme carboxylate groups and the positive charge of the inhibitor or the flattened shape of the inhibitor. Our previous studies on the inhibition of glucosidases with five-membered half-chair-like and six-membered chair-like aza sugars reveal a similar behavior.[4] To further probe the stereochemistry-inhibition relationship of 5-membered half-chair aza sugars, we describe here the synthesis and evaluation of several diastereomeric five-membered azasugars as inhibitors of fucosyltransfer enzymes (Scheme 1).

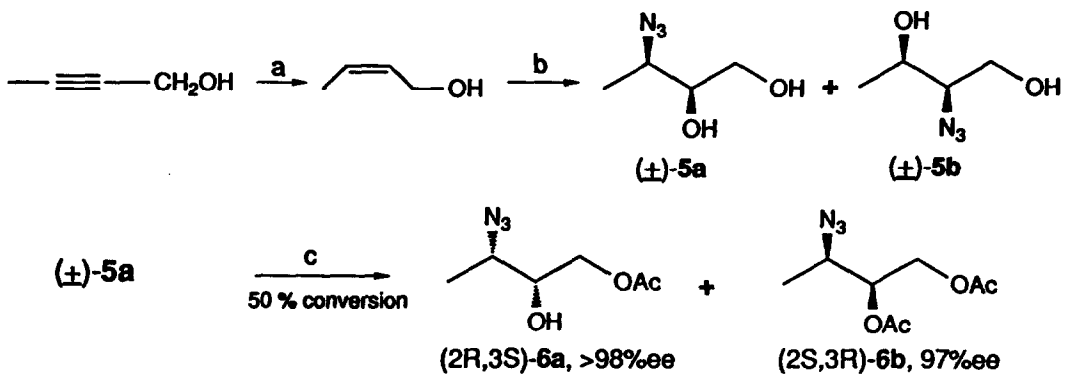
For the synthesis of compounds **1-3**, the azido-aldehydes (S)-**7** and (R)-**7** were chosen as acceptors for the aldolase-catalyzed reactions to create two additional steric centers in 3 and 4 positions. Compounds (S)-**7** and (R)-**7** were prepared from 2-butyn-1-ol via reduction with Lindlar catalyst followed by epoxidation and azide opening to give a 6:1 mixture of **5a** and **5b**. Resolution of **5a** was then carried out using a lipase from *Pseudomonas sp.* and vinyl acetate as acylating reagent[5] to obtain (2R,3S)-**6a** and (2S,3R)-**6b** in high optical purity (Scheme 2).[6] In this enzymatic transesterification the primary alcohol of both enantiomers of **5a** was first esterified to form monoacetates, but only the (2S,3R)-monoacetate product was further esterified to diacetate by the same enzyme.[7]

Compounds **6a** and **6b** were then transformed to (S)-**7** and (R)-**7** via hydrolysis and oxidative cleavage with sodium periodate. Reaction of (S)-**7** with dihydroxyacetone 3-phosphate (DHAP) catalyzed by fuculose 1-



Scheme 1 a: DHAP / Fuc-1-P aldolase, then acid phosphatase, 10 %; b: DHAP/ FDP aldolase, then acid phosphatase, 31 %; c: H_2 / Pd-C, 50 psi, 76 %; d: DHAP/ FDP-aldolase (see ref.11).

phosphate aldolase[4,8] (Fuc-1-P aldolase) followed by removal of the phosphate group and reductive amination[4] gave **1**.[9] Compounds **2** and **3** were prepared similarly from (S)-**7** and (R)-**7**, respectively, except rabbit muscle fructose-1,6-diphosphate aldolase instead of Fuc-1-P aldolase was used as the catalyst. It is worth noting that the reductive amination gave the product with a trans relation between C_4 and C_5, consistent with previous observations.[4]



Scheme 2 a: H_2 / Lindlar catalyst, >95 %; b: H_2O_2 / Benzonitrile then NaN_3/ pH 7.5, 78 %; c: vinyl acetate / lipase PSL, 46 % yield each.

Aza sugars **1**-**4** are competitive inhibitors of the α-fucosidase from bovine kidney at pH 5.5 with close K_i values of 1.4, 8, 22, and 4 μM,[4c,10] respectively. As expected, compound **1** is a slightly better inhibitor, perhaps because its shape is closer to the transition state of fucosidic cleavage. The difference in the inhibition activities among several six-membered chair-like aza sugars, however, is very significant;[4,10] epimerization of a stereogenic center often results in a complete loss of inhibition activity. These results suggest that half-chair, transition-state analog inhibitors of glycosidases are sterically less demanding than the chair-like inhibitors, and the relaxed stereochemistry discrimination is mainly due to the flatten-chain nature of the inhibitor, not the electrostatic interaction, as the pK_a values of the imine groups in both five-membered and six-membered aza sugars are essentially the same. Compounds **1** and **4** are also moderate inhibitors (IC_{50} = 80 and 34 mM vs LacNAc, respectively) of a recombinant human α-1,3-fucosyltransferase.[11] Interestingly, in the presence of GDP at its IC_{50} (0.05 mM, K_{ii} = 0.13 mM, K_{is} = 0.16 mM, non-competitive inhibitor vs LacNAc) a profound inhibition of the fucosyltransferase with **1** or **4** was observed. For example, approximately 90% of the enzyme activity was inhibited in the presence of 1 mM each of LacNAc acceptor and ^{14}C-GDP-Fuc, 0.05 mM GDP and 34 mM **4**. This synergistic effect may be due to an interaction between GDP and the aza sugar in the active site of enzyme to form a complex.[12] The results also suggest that many aza sugars used as glycosidase inhibitors may also act *in vivo* as glycosyltransferase inhibitors. The five-membered aza sugars described in this and previous[1,4] studies apparently are comparable to or better than six-membered aza sugars as glycosidase inhibitors. They seem to be a good mimic of the flattened glycosyl cation. They are easier to synthesize and

more stable than the half-chair like six-membered aza sugars.[3] Further investigation of the synergistic effect and development of new aza sugars based on aldolases as glycosyltransferase inhibitors are in progress.

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6. To determine the optical purity, **6** and racemic **5a** were acylated to the corresponding diacetates by treatment with acetic anhydride and the products were analyzed by ^{1}H NMR spectroscopy in the presence of Eu(hfc)$_3$ (diacetate: Eu(hfc)$_3$ = 12 mg: 24 mg). The relative intensities of methyl group at 1.82 (doublet, 2S,3R-isomer) and 1.84 ppm (doublet, 2R,3S-isomer) were used for ee determination.
7. To determine the absolute stereochemistry of **6b**, it was converted to N-benzyloxycarbonyl alanine via basic hydrolysis, catalytic hydrogenesis, protection of amino group, oxidative cleavage with sodium periodate and then Jones oxidation. The product has the R configuration based on the optical rotation, indicating the (2S,3R) configuration for **6b**.
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9. **1**:$[\alpha]_D^{25}$+21.8° (c=1.0, CH_3OH); Rf=0.20 ($CHCl_3/CH_3OH/H_2O/NH_4OH$=5/4/1/0.08); ^{1}H NMR (500 MHz, CD_3OD/TMS): δ 1.14 (3H, d, J=6 Hz, CH_3), 2.34-2.45 (1H, m, CHN), 2.47-2.55 (1H, m, CHN), 3.65 (1H, dd, J=4 Hz and 11 Hz, CH_aO), 3.74 (1H, dd, J=5 Hz and 11 Hz, CH_bO), 3.87 (1H, dd, J=5 Hz and 5 Hz, CHO), 4.26 (1H, dd, J=5 Hz and 8 Hz, CHO). ^{13}C NMR (125 MHz, CD_3OD): δ 12.98, 60.56, 65.24, 70.95, 72.14, 73.88. HRMS (M+H$^+$) calcd: 148.0974, found: 148.0968. **2**:$[\alpha]_D^{25}$ +22.7° (c=1.2, CH_3OH); Rf=0.19 ($CHCl_3/CH_3OH/H_2O/NH_4OH$=5/4/1/0.08); ^{1}H NMR (500 MHz, CD_3OD/TMS): δ 1.16 (3H, d, J=6.5 Hz, CH_3), 2.91 (1H, dt, J=4 and 4.5 Hz CHN), 3.21 (1H, dq, J=4 and 6.5 Hz, CHN), 3.65 (1H, dd, J=5 Hz and 11 Hz, CH_aO), 3.68 (1H, dd, J=5 Hz and 11 Hz, CH_bO), 3.741 (1H, dd, J=1.5 Hz and 4 Hz, CHO), 3.83 (1H, dd, J=1.5 Hz and 4 Hz, CHO). ^{13}C NMR (125 MHz, CD_3OD): δ 13.72, 57.85, 63.07, 68.60, 80.60, 81.54. HRMS (M+H$^+$) calcd: 148.0974, found: 148.0964. **3**:$[\alpha]_D^{25}$ +39.1° (c=0.8, CH_3OH); Rf=0.19 ($CHCl_3/CH_3OH/H_2O/NH_4OH$=5/4/1/0.08; ^{1}H NMR (500 MHz, CD_3OD/TMS): δ 1.19 (3H, d, J=6.5 Hz, CH_3), 2.92 (1H, dt, J=6.5 and 7.5 Hz, CHN), 2.98 (1H, ddd, J=4.5, 6.5 and 6.5 Hz, CHN), 3.50 (1H, dd, J=6.5 Hz and 7.5 Hz, CHO), 3.57 (1H, dd, J=6 Hz and 11 Hz, CH_aO), 3.64 (1H, dd, J=4.5 Hz and 11 Hz, CH_bO). 3.75 (1H, dd, J=6.5 Hz and 6.5 Hz, CHO). ^{13}C NMR (125 MHz, CD_3OD): δ 18.83, 58.07, 63.61, 64.36, 79.88, 84.88. HRMS (M+H$^+$) calcd: 148.0974, found: 148.0971.
10. For inhibition analysis, see 4c; For the synthesis of 6-membered deoxyfucojirimycin as inhibitor of intestinal α-fucosidase, see (a) Fleet, G.W.J.; Nicholas, S.J.; Smith, P.W.; Evans, S.V.; Fellows, L.E.; Nash, R.L. *Tetrahedron Lett.* **1985**, *26*, 3127. (b) Fleet, G.W.J.; Shaw, A.N.; Evans, S.V. and Fellows, L.E. *J. Chem. Soc. Chem. Commun.* **1985**, 841. (c) Winchester, B.; Barker, C.; Baines, S.; Jacob, G.S.; Namgoong, S.K.; Fleet, G.W.J. *Biochem. J.* **1990**, *265*, 277.
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