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Synthesis and evaluation of six-membered GDP-iminocyclitol

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Abstract—Fructose-diphosphate aldolase was employed in the chemoenzymatic synthesis of six-membered GDP-iminocyclitols. These compounds were evaluated for activity against α-1,3-fucosyltransferases and found to be potent inhibitors.

Glycoconjugates are important mediators in many molecular recognition processes and thus have gained attention as possible targets for drug discovery.[1] An attractive and highly studied target for therapeutic intervention is the interaction of sialyl Lewis^X (SLe^X) with cell-surface receptors.[2,3] This binding event occurs early in the inflammation cascade, ultimately leading to the recruitment of leukocytes to damaged tissue.[4–6] The over-recruitment of leukocytes can damage healthy cells, but attenuation of the presented SLe^X ligand may provide a therapeutic benefit. Although development of SLe^X mimetics to block the SLe^X receptor interaction represents a viable approach, inhibition of SLe^X biosynthesis may be an effective alternative. The terminal step in the biosynthetic pathway of SLe^X is the addition of the fucose moiety by α-1,3-fucosyltransferase (FucT).[7–9] Ligands lacking the fucose moiety are unable to support the leukocyte rolling, a requisite event in the inflammatory cascade.[10–12] Thus, potent inhibitors of FucT may be novel anti-inflammatory agents.

The transition state of FucT is thought to exhibit a flattened half-chair conformation with substantial oxocarbenium ion character at the anomeric position (Fig. 1).[13] Iminocyclitols mimic the partial positive charge in the transition state and are good inhibitors of glycosi-

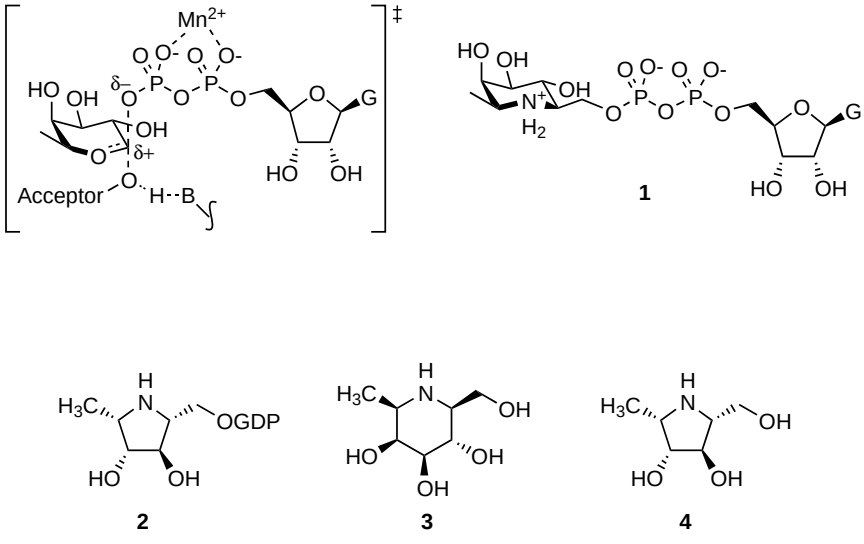

Figure 1. The proposed transition state of FucT exhibits a flattened half-chair conformation and oxocarbenium ion character.

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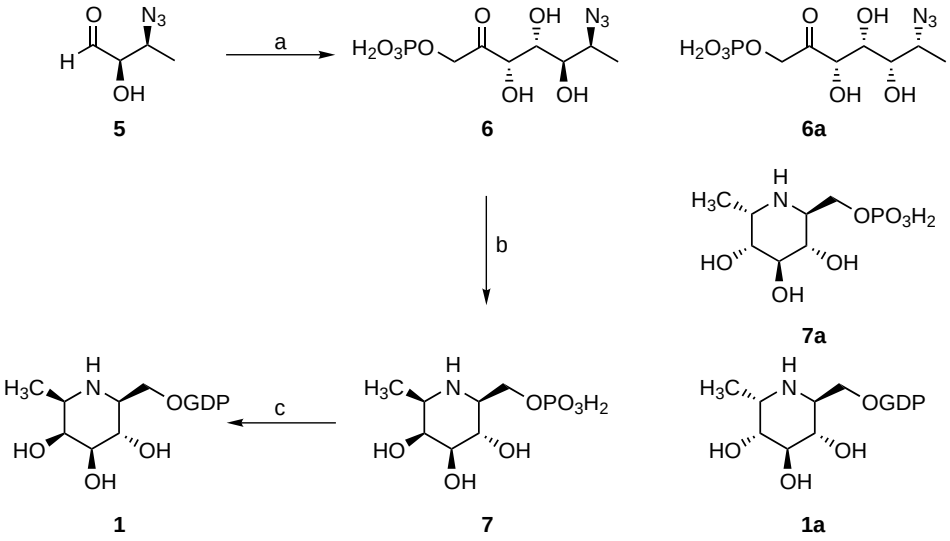

Scheme 1. (a) DHAP, FDP aldolase, pH 6.7, 70%; (b) H_2, Pd/C, H_2O/MeOH, 41%; (c) GMP-morpholidate, tetrazole, pyr, 13%.

dases,[14–16] but are only modest inhibitors of glycosyl transferases.[17,18] However, iminocyclitols exhibit synergistic inhibition with GDP likely due to the interaction of the positively charged iminocyclitol with the negatively charged GDP and the bound acceptor sugar which mimics the transition state.[18]

Recently, it was shown that covalently linking a five-membered iminocyclitol with GDP provided the transition state inhibitor **2** with a K_i of 45 μM.[19] It was therefore reasoned that the six-membered iminocyclitol covalently linked to GDP would be more potent because the parent six-membered iminocyclitol **3** exhibits better inhibition of FucT than the five-membered iminocyclitol **4**.[18] Herein we report the synthesis of GDP-iminocyclitol **1** as a general inhibitor of fucosyltransferases and evaluation of its inhibition against α-1,3-fucosyltransferases V and VI.

We envisioned an application of the aldolase/Pd-mediated reductive amination approach used in the preparation of homofuconojirimycin **3**.[18] Typically, aldolase catalyzed condensation of an azido-aldehyde with dihydroxyacetone phosphate (DHAP) and subsequent cleavage of the phosphate by acid phosphatase gives an azido-ketose. Catalytic hydrogenation[20–22] reduces the azide to the amine and further reduces the resulting cyclic imine to provide the iminocyclitol. However, if the phosphate group is not cleaved after the aldol reaction, hydrogenation of the phospho-ketose would give the phosphorylated iminocyclitol. GMP-morpholidate[23] coupling would then furnish the desired GDP-iminocyclitol **1**.

Thus, racemic 3-azido-2-hydroxy-butyraldehyde **5** underwent the aldol reaction with DHAP[24] catalyzed by 1,6-fructose-diphosphate aldolase (EC 4.1.2.13) to give a mixture of phospho-ketose **6** as a major product and another diastereomer **6a** as a minor product (Scheme 1). Typically, if diastereomers are obtained from the aldolase reaction, they can be separated after the phosphate cleavage. In our case, the phosphate group made separation of the diastereomers too difficult. Catalytic hydrogenation with palladium on carbon furnished iminocyclitol-phosphate **7** and **7a** in 41% overall yield (2:1 ratio by ^{1}H NMR), although the reductive cleavage of the phosphate was a competing side reaction. Reaction of the phosphate mixture with GMP-morpholidate afforded **1** and **1a** in a 2:1 ratio after purification.[25]

The GDP-iminocyclitol was evaluated using a fluorescence-based assay that couples the production of GDP to the consumption of NADH with pyruvate kinase (Sigma) and lactate dehydrogenase (Sigma).[13] The inhibition constants were determined for FucT V and VI (Calbiochem) at pH 7.4, 10 mM $MnCl_2$, and LacNAc ($2K_m$, 70 and 10 mM, respectively). GDP-iminocyclitol (**1+1a**) is one of the most potent inhibitors known for FucT V and VI,[26] with K_I values of 6 and 10 μM, respectively. The electrostatic interactions of the ring nitrogen and the pyrophosphate with active site residues are most likely responsible for the tight binding observed. The higher affinity of **1** versus **2** may be due to the higher affinity of the parent iminocyclitol. An asymmetric synthesis of diastereomerically pure **1** (K_i = 13 and 11 μM for FucT V and VI, respectively) will be reported in due course.

Acknowledgements

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25. Size exclusion chromatography of **1** using P-2 column resulted in an impure mixture. Flash chromatography on silica gel (7:1:2 IPA/30% NH_4OH/H_2O), followed by precipitation from 1:1 $MeOH/H_2O$ upon addition of acetone gave pure **1**.
Spectroscopic data for **1**: ^1H NMR (400 MHz, D_2O): 8.12 (br s, 2H), 5.88 (d, 2H, $J=3.24$ Hz), 4.51 (t, 1H, $J=3.52$ Hz), 4.45 (q, 2H, $J=3.24$ Hz), 4.41 (dd, 2H, $J=3.84$, 3.20 Hz), 4.36 (t, 1H, $J=3.52$ Hz), 4.34–4.25 (m, 7H), 4.22–4.10 (m, 9H), 4.11–4.03 (m, 6H), 3.90–3.94 (m, 2H), 1.33 (d, 2H, $J=4.40$ Hz), 1.32 (d, 2H, $J=5.0$ Hz); ^{13}C (100 MHz, D_2O): 159.0 (br), 154.43, 154.40, 154.1, 87.5, 87.4, 84.25, 84.18, 70.7, 70.60, 70.57, 70.0, 69.9, 65.30, 65.29, 64.8, 64.7, 59.03, 58.96, 55.1, 14.2. ESI: m/z ($M+H^+$) calcd 603, obsd 603; ($M+Na^+$) calcd 625, obsd 625; (MH^+) calcd 601, obsd 601.
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