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Inhibition of Fucosyltransferase V by a GDP–Azasugar

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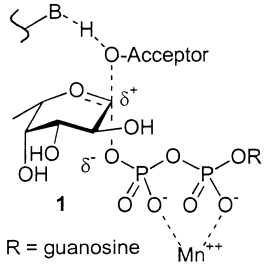
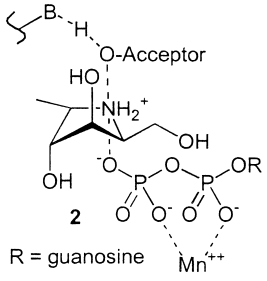
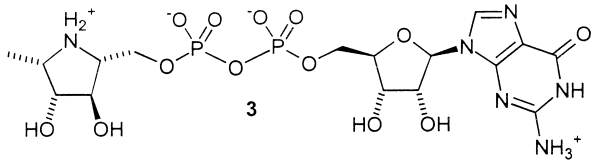
Abstract—A GDP–azasugar conjugate was synthesized starting from an enzymatically obtained phosphorylated azasugar. It inhibits human fucosyltransferase V at micromolar concentrations, which is discussed in terms of transition state analogy.

The specific binding of complex carbohydrates forms the basis of vital intercellular recognition processes.[1] Thus, the regional inflammatory response via leucocyte invasion relies on the binding of the tetrasaccharide SLe^x present on the leucocyte surface to members of the selectin family of adhesion glycoproteins expressed on the surface of endothelial cells.[2] The presence of a fucose residue introduced by an α-(1→3)fucosyltransferase (FucT) in the final step of the SLe^x biosynthesis is crucial for this particular recognition system. Accordingly, one approach towards antiinflammatory agents is based on the development of potent FucT inhibitors.[3] The design of transition state analogues has long been hampered by the limited knowledge of glycosyltransferase transition states in general. Nevertheless, mechanistic studies conducted during recent years have led to the postulation of a FucT transition state (**1**),[4] which is characterized by a positively charged fucose moiety adapting a half-chair conformation and the GDP leaving group binding a bivalent cation and taking over the developing negative charge. Thus far, only few inhibitors containing fucose analogues exhibiting flattened ring conformations, have been reported.[5] Wong and coworkers have reported a synergistic inhibition of FucT by GDP and five-membered azasugars.[6] This intriguing observation has been attributed to the non-covalent formation of transition state-like complexes such as **2** within the FucT active site.







We reasoned that a covalent linkage between GDP and suitable five-membered azasugars could yield highly potent transition state analogues and would allow further insight into the properties of the FucT transition state. Herein, we report about the inhibition of FucT by GDP-azasugar **3**. Recently, we have described a flexible enzyme-mediated approach towards five-membered phosphorylated azasugars such as **4**.[7] Reaction of **4** with 0.7 equiv GMP-morpholidate afforded **3** in 40% yield.[8]

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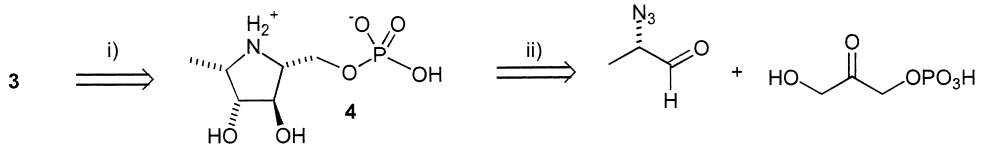
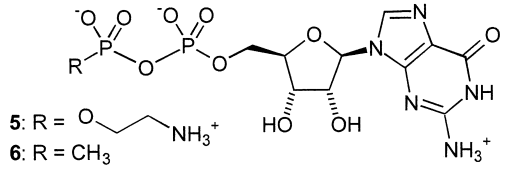


Scheme 1. Retrosynthesis of **3**: (i) coupling with GMP-morpholidate; (ii) (1) aldolase-catalyzed C,C, bond formation; (2) reductive amination.

The presence of a small amount of water, introduced by using two equivalents of wet tetrazole, proved crucial for the success of the coupling reaction. Compound **3** was obtained as a white solid, which is stable for several months.[9] Aqueous stock solutions showed no decomposition within one week even at room temperature (Scheme 1).

A coupled enzymatic assay[10] allowing the time-dependent spectroscopic detection of the amount of consumed donor substrate (GDP-Fuc) was used for inhibition studies. Using standard assays,[11] it was ensured that neither **3** nor the other compounds under investigation had any effect on individual enzymes present in the GDP-Fuc assay besides fucosyltransferase itself. We investigated the inhibition of FucT V (CALBIOCHEM) at pH 7.0 using 0.07 mM GDP-Fuc and 10 mM $MnCl_2$. Under these conditions **3** inhibited FucT V with IC_{50} values of 45 and 82 μM in the presence of 1 and 10 mM of the fucosyl acceptor *N*-acetyllactosamine, respectively. This is close to the IC_{50} of GDP, which was reported to be 67 μM under similar conditions.[6b] In order to investigate whether the inhibition of FucT V by **3** has to be attributed to its GDP moiety alone, GDP-ethanolamine **5** was synthesized as an minimized structural analogue of **3** and characterized as FucT inhibitor. The synthesis was achieved by coupling *N*-Cbz-protected ethanolamine-*O*-phosphate with GMP-morpholidate under standard conditions and subsequent removal of the Cbz group by catalytic hydrogenation. An IC_{50} of ≥ 1 mM was obtained for **5** applying the conditions described above. Since the positive charge of the protonated amine resembling the oxocarbenium character of the putative transition state of FucT can occupy the same position relative to the rest of the molecule in both **3** and **5**, the presence of the five-membered ring and its substituents apparently contribute to the overall binding of **3**. Possibly, the azasugar moiety resembles an additional key feature of **1**, the flattened conformation of the fucose ring. However, the question arises: *Why is **3** not a much more potent inhibitor than GDP, if the azasugar in fact interacts favourably with the enzyme?* By covalently linking GDP, one of the oxygens at the terminal phosphoryl group is masked and, thereby, one negative charge is eliminated. If this charge is indeed required to resemble the ion pair character of the transition state (see right-low part of **1**), then no GDP conjugate of this type can be a perfect transition state analogue. This notion is supported by our investigation of **6**, which was obtained by coupling GMP-morpholidate and methylphosphonic acid. This carbon analogue of GDP is a very weak inhibitor of FucT V ($IC_{50} > 0.5$ mM), thus, emphasizing the importance of the replaced hydroxyl group for the transition state analogy of GDP.



In summary, a conjugate of GDP with a five-membered azasugar has been synthesized and shown to inhibit FucT V in micromolar concentrations. The comparison of its IC_{50} with those of simplified analogues could be explained by a contribution of the five-membered azasugar residue to a transition state-like binding. This finding as well as the dramatic effect of the masking of one negative charge of the GDP moiety on the overall inhibition gives further support to the notion of a transition state exhibiting significant charge separation along the glycosidic bond.

Acknowledgements

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For the synthesis of **3**, wet solid tetrazole was used as a catalyst. An anhydrous solution of tetrazole (0.45 M in MeCN) was used in the synthesis of **5** and **6**. Proton-decoupled ^{31}P NMR was used to follow the reactions. Product formation is accompanied by the appearance of doublets with typical J^2 coupling constants. Products were purified by chromatography on Bio-Gel P2 (Bio-Rad) using 250 mM NH_4HCO_3 (pH 7) as the eluent.

9. Selected spectroscopic data for **3**: ^1H NMR (500 MHz, D_2O): δ 7.9 (sbr, 1H), 5.75 (d, 1H, $J=6.0$ Hz), 4.6–4.5 (m, 1H), 4.32 (dd, 1H, $J=5.0$, 3.0 Hz), 4.2–4.12 (m, 2H), 4.08–4.02 (m, 2H), 4.01–3.96 (m, 1H), 3.96–3.92 (m, 2H), 3.72 (dq, 1H, $J=6.5$, 3.5 Hz), 3.59–3.54 (m, 1H), 1.22 (d, 3H, $J=6.5$ Hz); ^{13}C NMR (125.8 MHz, D_2O): δ 158.9 (Cq), 153.9 (Cq), 86.8 (CH), 83.6 (d, CH, $J=8.8$ Hz), 76.0 (CH), 75.6 (CH), 73.7 (CH), 70.3 (CH), 65.6 (d, CH, $J=7.4$ Hz), 65.3 (d, CH_2, $J=5.0$ Hz), 63.4 (d, CH_2, $J=5.0$ Hz), 58.1 (CH), 10.2 (CH_3); ^{31}P NMR (202.5 MHz, D_2O): δ −15.1 (d, 1P, $J=21.3$ Hz), −15.5 (d, 1P, $J=21.3$ Hz); LC–MS (ESI): m/z $(M-H)^-$: calcd 571, obsd 571; $(M-2H)^{2-}$: calcd 285, obsd 285.

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