

A Potent and Highly Selective Inhibitor of Human α -1,3-Fucosyltransferase via Click Chemistry

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Fucosyltransferases (Fuc-T) catalyze the final glycosylation step in the biosynthesis and expression of many important saccharides, such as sialyl Lewis x (sLe^x) and sialyl Lewis a (sLe^a) of cell-surface glycoproteins and glycolipids. These and other fucosylated oligosaccharide structures are central to cell–cell interactions and cell migration in connection with physiological and pathological processes such as fertilization, embryogenesis, lymphocyte trafficking, immune responses, and cancer metastasis.¹ The terminal step in the biosynthetic pathway of these fucose-containing saccharides is the transfer of the L-fucose from guanosine diphosphate β -L-fucose (GDP-fucose) to the corresponding glycoconjugate acceptor.¹

Different strategies have been employed to identify inhibitors of Fuc-T. Most rely on the design of acceptor, donor, and transition-state analogues.^{2–4} Several factors inherent to the chemistry of glycosyl-transfer reactions make rational design of Fuc-T inhibitors difficult. The enzymatic reaction involves a complex four-partner transition-state (sugar donor, acceptor, divalent metal, and nucleotide). Measurement of the catalytic proficiency for GDP-fucose hydrolysis shows that the Fuc-T has a low affinity for the transition-state of the GDP-fucose moiety.⁵ The affinity for the acceptor substrate is also low,^{5–7} and there is no structural data for Fuc-Ts. Hence, the rational development of potent inhibitors for Fuc-Ts has been difficult, and to date the best inhibitors are in the micromolar range.^{2–4}

As reported here, a substantially better Fuc-T inhibitor has been found. Success came quickly, using the click chemistry approach,^{8–10} to rapidly identify a new optimal binding component. In fact, the latest advance in this area, the Cu(I)-catalyzed triazole synthesis,^{10,11} made it possible to create the desired library of GDP-triazole candidates in water, without protecting groups (even for dianionic phosphate linkage), so that the crude aqueous reaction solutions were pure enough to test “as is.”

Since the majority of the binding energy of Fuc-T for its substrates lies at the GDP moiety^{4,5} (Figure 1), and the hydrophobic pocket adjacent to the binding site of the acceptor molecule enhances affinity of the acceptor molecule by 70-fold,¹² we have designed a library of compounds which retained the important GDP core, while the attached hydrophobic group and the linker length were varied. The carbohydrate moieties on the donor and acceptor substrates were omitted to simplify the synthetic challenge. An inhibitor that is derived from the screening of this library would constitute the optimal linker and hydrophobic moiety, and would result in the specificity for the target enzyme.

Thus, 85 azide compounds were synthesized, representing the diversity in the hydrophobic group and the tether (2 to 6 carbons; see Supporting Information). Using the new copper-catalyzed process, each of these azide molecules was linked to the GDP-alkyne core to give the 85 triazole candidates (Scheme 1). The GDP-

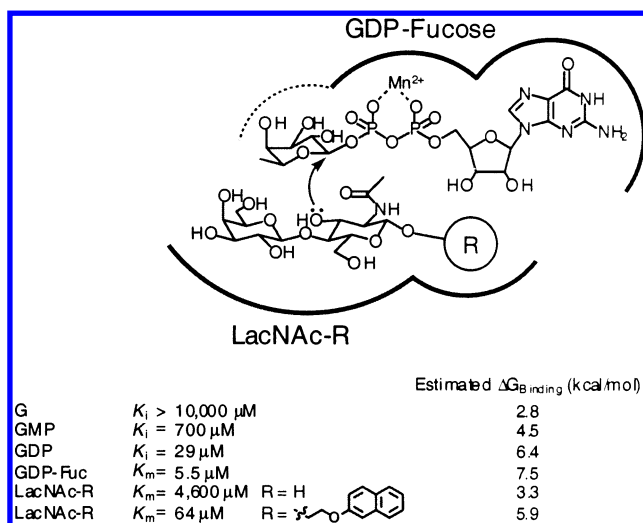
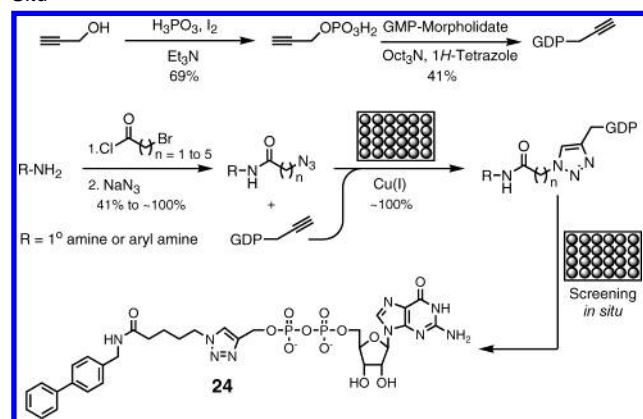


Figure 1. Fucosyltransferase-catalyzed reaction. The fucose moiety is transferred from GDP-fucose to an acceptor molecule, LacNAc-R. It is evident from the inhibition (K_i) and apparent dissociation (K_m) constants that the majority of the binding energies exist at the GDP moiety and to some extent the hydrophobic aglycon group.^{5,12} The design of the GDP-triazole library takes advantage of the binding energies for the GDP and hydrophobic aglycon moiety.

Scheme 1. Triazole Synthesis in Microtiter Plate for Screening in Situ



triazole compounds were screened for inhibitory activity directly in microtiter plates, using the pyruvate kinase/lactate dehydrogenase coupled-enzyme assay. For proof of principle, the library was screened against human α -1,3-fucosyltransferase VI (Fuc-T VI), although there exist six different human α -1,3-fucosyltransferases.¹³ Three “hits” emerged from this screen, and the measurement of the IC₅₀ values of these three crude compounds showed that molecule **24** had the highest affinity for Fuc-T VI (see Supporting Information).

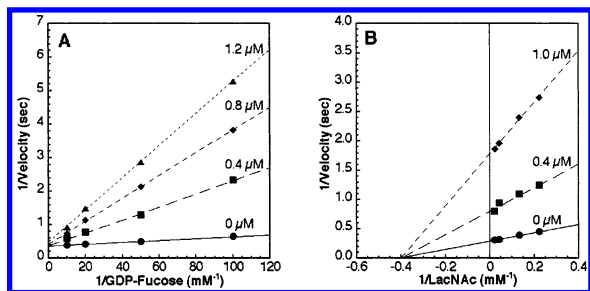


Figure 2. Inhibition study of human α -1,3-fucosyltransferase VI. (A) Double-reciprocal plot of GDP-fucose as the variable substrate and **24** as the inhibitor (0, 0.4, 0.8, 1.2 μ M). LacNAc concentration was held constant at 0.5 mM. (B) Double-reciprocal plot of LacNAc as the variable substrate and **24** as the inhibitor (0, 0.4, 1.0 μ M). GDP-fucose was held constant at 10 μ M.

Table 1. Inhibition Constants of **24** for Various Enzymes

enzyme	IC ₅₀ (μ M)	K _i (nM)
α -1,3-FucT III	1.0 \pm 0.2	—
α -1,3-FucT V	0.9 \pm 0.1	270 \pm 30
α -1,3-FucT VI	0.15 \pm 0.03	62 \pm 3
α -1,3-GalT	N.I.	—
β -1,4-GalT	N.I.	—
guanylate kinase	250 \pm 60	—
pyruvate kinase	N.I.	—

^a N.I. No Inhibition observed at 600 μ M.

Steady-state kinetic evaluation of purified **24** showed that it is a competitive inhibitor against GDP-fucose with $K_{i(\text{comp})} = 62$ nM (Figure 2A, Table 1), which would make this compound the first nanomolar and most potent inhibitor of Fuc-Ts. The inhibition constant of **24** represents an 800-fold improvement over GDP-alkyne ($K_i = 47$ μ M). Compound **24** is a noncompetitive inhibitor against the acceptor molecule, *N*-acetylglucosamine (LacNAc), with $K_{i(\text{noncomp})} = 221 \pm 8$ nM (Figure 2B), and is a mixed-type inhibitor against LacNAc- β -biphenyl, with $K_{i-\text{slope}} = 120 \pm 30$ nM and $K_{i-\text{intercept}} = 1000 \pm 400$ nM.¹⁴

The inhibition property of **24** was also tested against other glycosyltransferases and nucleotide binding enzymes (Table 1). As is evident from the table, **24** is a potent and highly selective inhibitor of Fuc-T VI, the enzyme from which the library was screened against. The molecule exhibited lower inhibition properties against other Fuc-Ts, and no inhibition was observed against two galactosyltransferases. Weak inhibition was observed for guanylate kinase, and no inhibition was observed against the catalytically promiscuous pyruvate kinase.

Potent inhibitors of fucosyltransferases, and glycosyltransferases in general, have been elusive due to the aforementioned difficulties surrounding the family of glycosyltransfer reactions. However, the problems of weak substrate affinity and low catalytic proficiency of the enzyme may be offset by recruiting additional binding features, such as hydrophobic interactions in this case.

The Cu(I)-catalyzed, stepwise variant^{10,11} of Huisgen's classic 1,3-dipolar cycloaddition process¹⁵ seems to be the best example of click chemistry reliability to date. It enabled a rapid synthesis of a GDP-triazole library of 85 compounds, among which one potent inhibitor of fucosyltransferases was identified. This method may be widely applicable for the identification of high-affinity inhibitors of other group-transfer targets that are of biological or medicinal interest.

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Supporting Information Available: ¹H and ¹³C NMR data of compound **24** and its components (azide and GDP-alkyne); procedure for the synthesis of azides and triazole compounds and measurements of IC₅₀ and K_i values (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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