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High-throughput identification of fucosyltransferase inhibitors using carbohydrate microarrays

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Abstract—A noncovalent carbohydrate microarray was used to screen for possible inhibitors to fucosyltransferases, which are critical to the synthesis of inflammation mediators like sialyl Lewis x (SLe^x). Inhibition was followed by observation of the transferred fucose on the carbohydrate array with the lectin *Tetragonolobus purpureas*. Of these compounds, four inhibitors with nanomolar K_is were discovered, with three of the top five inhibitors exhibiting a common architecture.

The inflammatory response in mammalian tissue involves neutrophils or leukocites present in the blood stream adhering to injured tissues followed by extravasation into the underlying tissue.[1–3] This process is mediated by the tetrasaccharide sialyl Lewis x (SLe^x), along with sialylated and sulfated Lewis a and Lewis x, found on glycolipids and glycoproteins at the surface of neutrophil or endothelial cells.[4–6] Inhibition of this interaction has proven to be an successful strategy for disrupting the inflammatory response[5,7] with the tetrasaccharide of SLe^x being the minimal ligand necessary for recognition.[1] α-(1,3)-Fucosyltransferase (FucT) mediates the final reaction in the biosynthesis of SLe^x by transferring a fucose from GDP-fucose (GDP-Fuc) to a sialyl lactosamine to form SLe^x and is therefore of keen interest to studies toward inhibiting the inflammation cascade.[1] Our laboratory has previously used carbohydrate arrays to study known inhibitors of this enzyme[8] and we chose to apply this same array technique to the screening of a library of possible FucT inhibitors.

In order for an assay to be applicable to libraries of compounds, the method must be rapid and require minimal materials. The screen must also be able to compare several compounds at a constant concentration that correlates to more in depth analysis trends. In order to analyze this type of inhibitor screen for high-throughput application, a series of compounds (**1**–**85**) were studied against FucT VI (Fig. 1).[9] FucT contains a hydrophobic pocket adjacent to the binding site for GDP, which is where the majority of the binding energy lies for the enzyme. These compounds had been synthesized with the aim of enhancing affinity of FucT for the GDP moiety by conjugating GDP to hydrophobic groups and had been previously studied against the enzyme using the pyruvate kinase/lactate dehydrogenase coupled-enzyme assay in solution.

FucT does not require the NeuAc residue to catalyze the fucosylation reaction.[2] Given that the unsialylated disaccharide is the commercially available *N*-acetyllactosamine (LacNAc), this was used for inhibition studies. LacNAc **86**[10] was displayed on the surface of microtiter plates via Cu(I)-catalyzed 1,3-dipolar cycloaddition with the lipid alkyne.[8,11] After incubation of the library with FucT and GDP-Fuc, the enzyme solution was incubated in the LacNAc-displaying wells (Fig. 2).[12] Transfer of fucose to LacNAc was then quantified using a fucose specific lectin from *Tetragonolobus purpureas* (TP) conjugated to a peroxidase. The results for the library screen with the peroxidase-conjugated TP are seen in Figure 3. From this plot, the compounds showing the five lowest absorbance readings were studied further to determine their IC_{50} and K_i values.

These compounds, **11**, **2**, **13**, **14**, and **66**, represent three different architectures, though **11**, **13**, and **14** all contain

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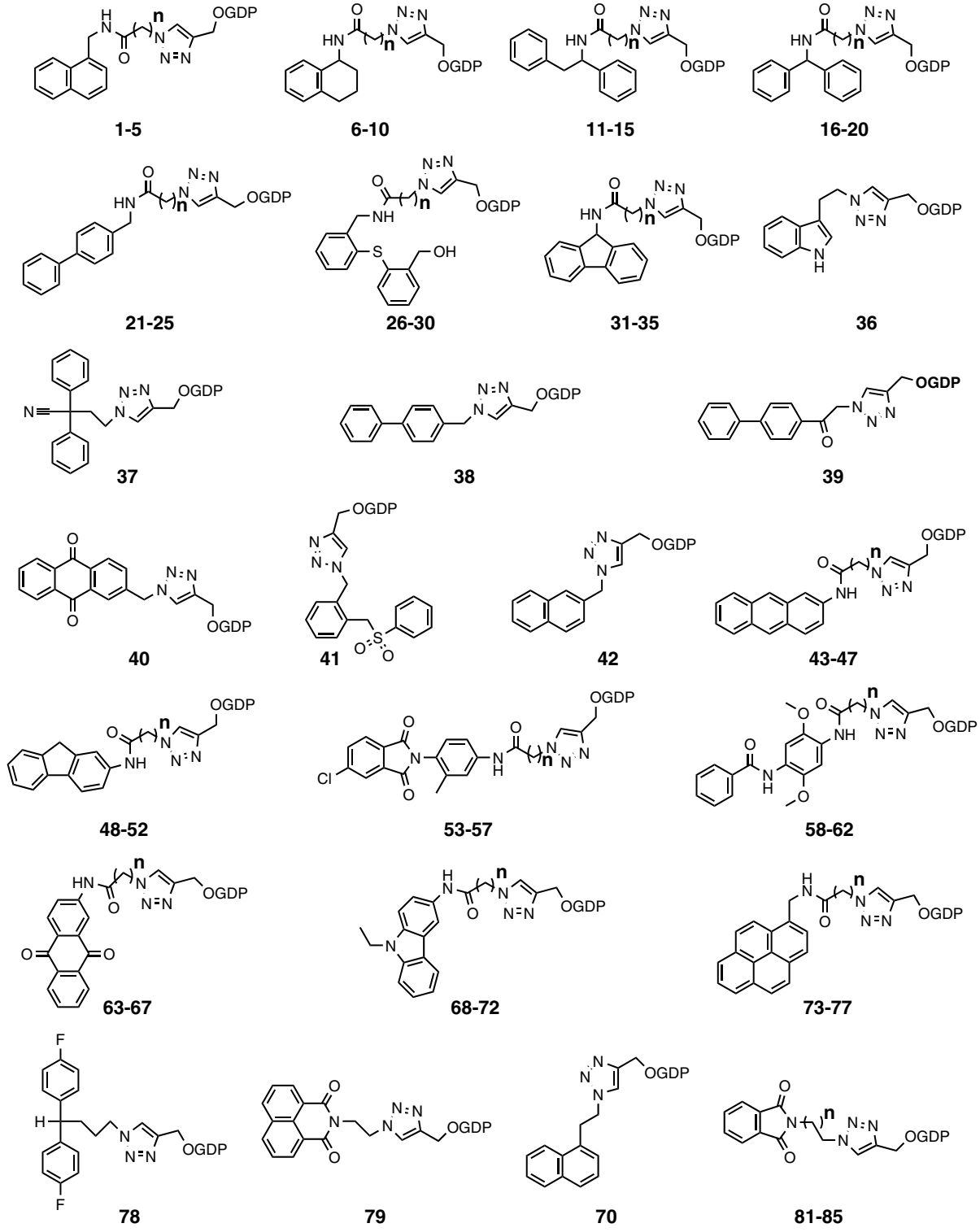

Figure 1. Triazole library used for inhibition studies with FucT; $n = 1$–5.

the same diphenyl substituent with varying distances between it and the GDP moiety. What is of greatest note from this initial screen is that the compounds selected as possible hits from this screen were not the compounds selected when the library was initially screened in solution using the fluorescence-based assay that coupled the production of GDP with the consumption of NADH previously described.[13] Compound **24** was isolated as the best hit based on percent relative rate and a K_i was determined to be 62 nM.[9] IC_{50} values were determined for the compounds against FucT VI using the previously described method (Fig. 4; Table 1).[14] Because the compounds were

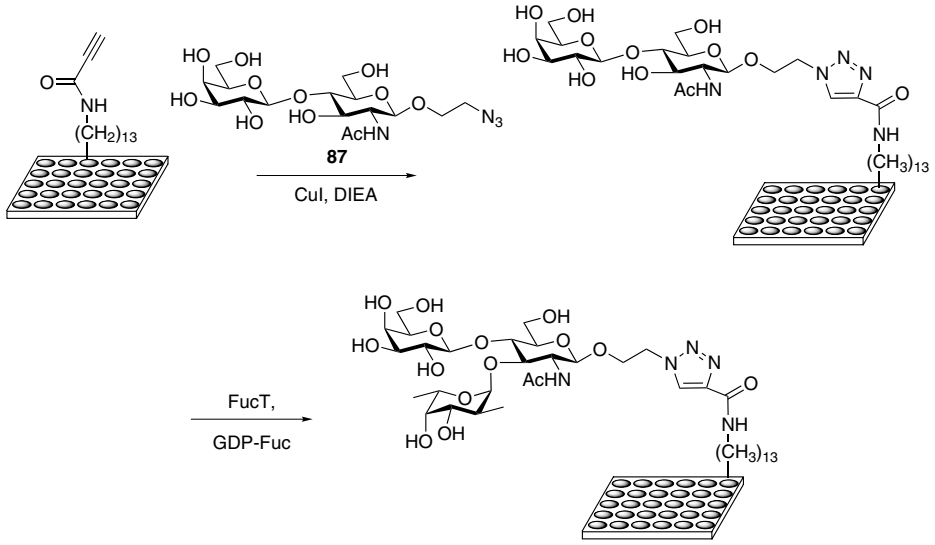

Figure 2. Coupling of LacNAc (**86**) to the lipid alkyne for noncovalent display and fucosylation of the displayed saccharide by FucT.

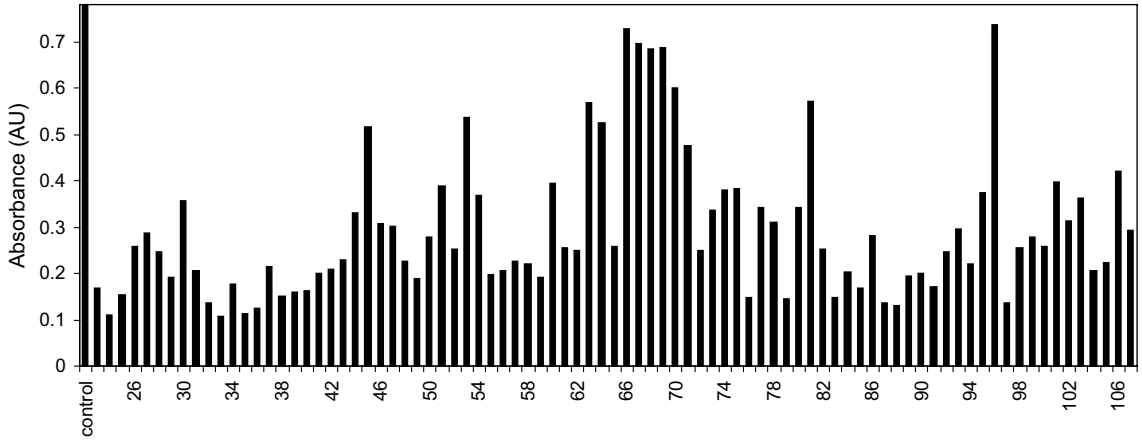

Figure 3. Absorbance based on fucose recognition by peroxidase-conjugated TP. Compounds are listed along the *X*-axis starting with the control/uninhibited reaction, which showed the highest absorbance.

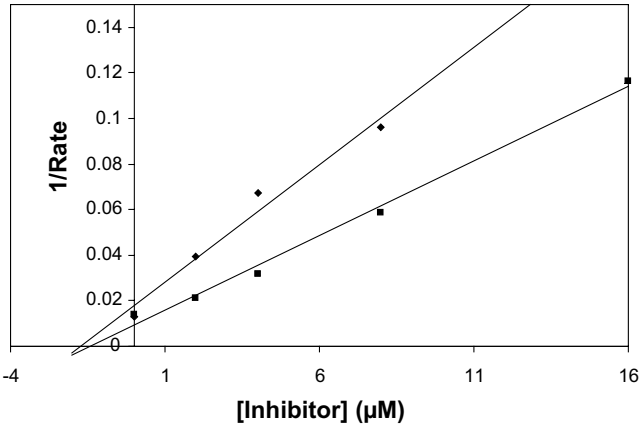

Figure 4. An example of the inhibition study for compounds **13** (■) and **14** (◆) against FucT VI used to determine IC_{50} and K_i values.

Table 1. IC_{50} and K_i values for compounds **3**, **12**, **14**, **15**, and **66**

Compound	IC_{50} (μM)	K_i (μM)
11	1.618	0.437
2	3.338	0.901
13	3.957	1.069
14	1.394	0.376
66	1.893	0.511

known to be competitively inhibit FucT,[14,15] K_i values were then determined from the following equation:

$$K_i = \frac{IC_{50}}{1 + \frac{S}{K_m}}$$

Based on the absorbance of the initial screen, the order of expected inhibition would be **11>2>13>14>66**. However, the best inhibitor was determined to be compound **14**, which has a K_i of 376 nM. Compound **11**

is the second best with a K_i of 437 nM followed by **66**, **2**, and **13** with 511, 901, and 1.069 μM, respectively.

None of the compounds determined as hits in this initial screen had a K_i value as low as the one determined for compound **24**. Also, compound **24** had 30% of the absorbance of the uninhibited reaction in this screen as compared to **11**, which showed only 14% of the absorbance of the uninhibited reaction (Fig. 3). Because the two strategies determined hits using different assays (relative rate vs inhibition at one time point), selection of different compounds is expected. It is therefore conceivable that observation at a different time point would have given different hits. However, the remaining hits that were determined from the previous screen all had higher K_is than those determined here[9] and this approach may be suitable for identifying architectures worthy of further exploration such as that of **11**, **13**, and **14**. Cost analysis of the two assays also benefits the screen presented here. The carbohydrate array method is 70% cheaper than the previously applied coupled-enzyme assay method. As cost is an important variable in high-throughput screening of inhibitors, this method would be more useful for large library screens such as preliminary screens to determine possible architectures for refined libraries. As such, this strategy is currently being applied to other transferases of interest.

Acknowledgements

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