

A general glycomimetic strategy yields non-carbohydrate inhibitors of DC-SIGN†

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Shikimic acid can be transformed into monovalent and multivalent glycomimetics that target different members of the C-type lectin class, including DC-SIGN, a dendritic cell lectin that facilitates HIV transmission.

Carbohydrates act in conjunction with carbohydrate-binding proteins (lectins) to govern numerous cellular processes, including cell adhesion, recognition, and signaling.¹ Despite the importance of lectins, ligands that can be used to interrogate or mitigate their function are scarce.^{2–4} Carbohydrates themselves can serve as probes, but they have liabilities. First, they tend to bind with low affinity. Second, optimizing carbohydrate leads can be difficult because it typically requires the production of complex oligosaccharide analogs *via* multistep routes. Finally, since the specificity of lectins can overlap, natural oligosaccharides often bind multiple lectins, thereby complicating their use as probes.⁴ Non-carbohydrate antagonists are an appealing alternative, but few have been reported.^{2–5} Although screens of large compound libraries are beginning to yield inhibitors,^{2–5} general approaches to block lectins are lacking. Here, we describe a privileged glycomimetic scaffold that can give rise to effective and selective inhibitors of the C-type lectin class. Moreover, we show that such glycomimetics can be incorporated into multivalent displays to generate potent inhibitors. To date, the targeting of lectins with multivalent glycomimetics is underexplored;^{6,7} our data indicate it can serve as a powerful strategy.

C-type lectins are a large class of proteins that are integral to immune system function.⁸ Named for their dependence on calcium ions for carbohydrate complexation, these lectins often bind mannosides. In these complexes, the 2-, 3- and 4-hydroxyl groups of mannose contribute to binding (Fig. 1A).⁹ We therefore used scaffold **1**, which mimics this hydroxyl group arrangement (Fig. 1B), to afford glycomimetics. The natural product shikimic acid was transformed into compounds with the necessary arrangement of hydroxyl groups,¹⁰ from which inhibitors were identified of a prototype C-type lectin, mannose-binding protein A (MBP-A).

A key feature of the aforementioned approach is that it has the potential to be general. One attractive target for testing this possibility is dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN). DC-SIGN

resides on the surface of dendritic cells, which are critical antigen-presenting cells.¹¹ DC-SIGN facilitates dendritic cell–T cell interactions, but it is its involvement in the dissemination of infectious human pathogens that led us to seek inhibitors. DC-SIGN can interact with viruses, such as HIV-1 or Ebola virus, and bacterial species, such as *Mycobacterium tuberculosis*, to facilitate infection.¹¹ Moreover, several pathogens that bind DC-SIGN subvert normal immune system function. Thus, DC-SIGN ligands could serve as probes of the underlying mechanisms and as therapeutic leads. MBP-A and DC-SIGN are both mannose-binding C-type lectins; therefore, our objective—to generate agents that block DC-SIGN selectively—serves as a challenging test of our design strategy.

DC-SIGN binds weakly to monosaccharide ligands such as *N*-acetyl mannosamine (ManNAc, $K_d = 8.7$ mM) and L-fucose ($K_d = 6.7$ mM).¹² The affinity for oligosaccharides is slightly higher ($K_d = 0.21$ mM for Man₉GlcNAc).¹² To test whether glycomimetics with similar affinities could be found, we used solid-phase synthesis to assemble a collection of putative mannose mimics that vary at three positions (Fig. 2).

Aside from the triol-substituted 6-membered ring that we anticipate would mimic mannose, the glycomimetic scaffold differs structurally from the natural ligands. We envisioned substituents at the points of variation could endow ligands with lectin affinity and specificity. Our synthetic approach was designed to utilize building blocks that are readily accessible (*e.g.*, either commercially available or synthesized in a few steps). For example, we varied the amino acid substituent to explore how changes in R¹ influence binding. Glycine serves as a small, flexible amino acid, while phenylalanine is larger, more hydrophobic, and the aryl group can engage in a range

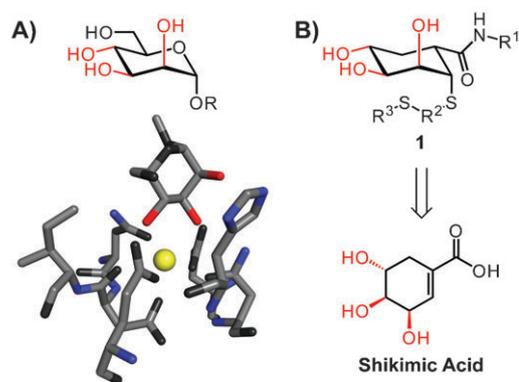


Fig. 1 Strategy for inhibitor design. (A) D-Mannose (top) and a substructure of the binding site of a complex of mannose and MBP-A (bottom) (PDB accession code 1kwy⁹). The hydroxyl groups necessary for lectin recognition and binding are shown in red, Ca²⁺ is yellow. (B) Glycomimetics **1** resemble mannose.

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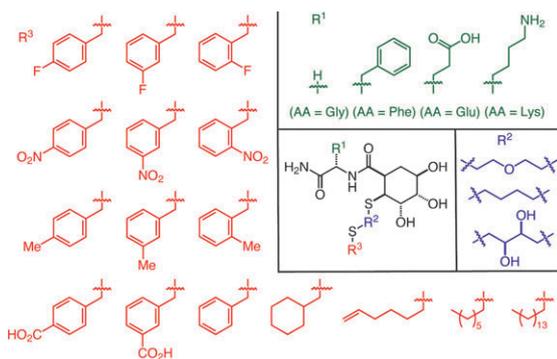


Fig. 2 Features of the glycomimetic library targeting DC-SIGN.

of interactions. Glutamic acid and lysine were chosen to test the influence of anionic or cationic substituents, respectively. The R^3 substituent was varied using a collection of alkylating agents. We tested some aliphatic R^3 groups, but we focused on benzyl substituents because aromatic side chains often line carbohydrate binding sites.⁴ The dithiol linkers (R^2) used are commercially available and capable of positioning the R^3 substituents near the carbohydrate or secondary binding sites.

These building blocks were employed¹⁰ to synthesize 192 compounds. The compounds were cleaved from resin and screened without further purification to estimate their activity. The screen involved a fluorescence-based high-throughput competition assay that assessed the ability of compounds to compete with immobilized mannan for binding to the fluorophore-labeled tetrameric extracellular domain of DC-SIGN (DC-SIGN/ECD). In this way, we identified several compounds that block DC-SIGN binding (Fig. 3). Trends in the inhibition data were readily apparent. The identity of the R^1 moiety affected binding most significantly; all of the hits contain glutamic acid at this position, but compounds with lysine at this position were largely ineffective. At the R^2 position, the DTT-derived linker was most prevalent, followed by the butanedithiol-derived linker. At the R^3 position, there was a preference for aromatic groups with electron withdrawing substituents. To confirm the validity of the initial binding data, we purified several library members and determined their IC_{50} values. The relative values parallel those determined from the initial screen.

Based on its activity in the initial screen, compound **2** (Fig. 3), was characterized further. To determine its IC_{50} value, we measured its ability to compete with probe, a fluorescein-labeled bovine serum albumin displaying 20–25 copies of mannose, for the immobilized extracellular domain of DC-SIGN.⁵ The IC_{50} value of **2** (3.2 ± 0.6 mM) is better than that of *N*-acetylmannosamine ($IC_{50} = 11.2 \pm 0.7$ mM). The MBP-A inhibitors identified previously were no more potent

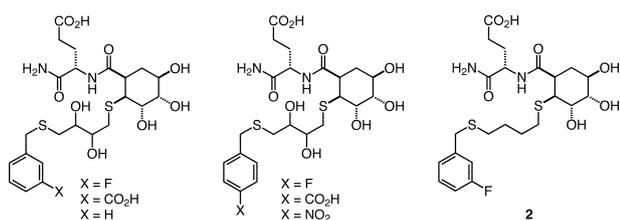


Fig. 3 The most potent hits identified from library screen.

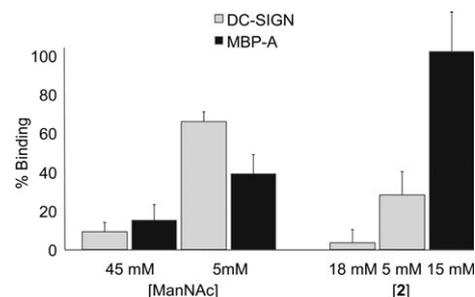


Fig. 4 Percent probe binding to DC-SIGN/ECD and MBP-A in the presence of ManNAc or **2** determined in fluorescence assay.

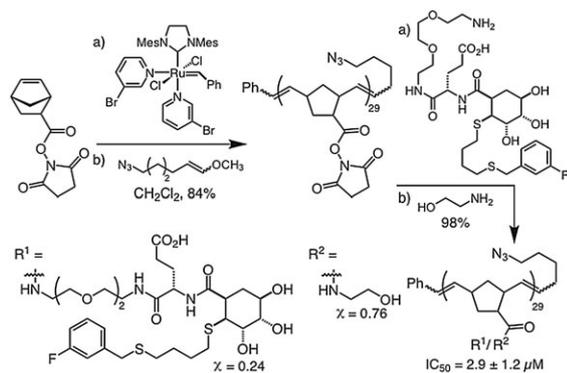
than the monosaccharide ligand α -methyl-D-mannopyranoside,¹⁰ so it is gratifying that the DC-SIGN inhibitors are superior to known monosaccharide ligands. The results, therefore, provide a starting point for optimizing ligand potency. Most significantly, they support the generality of our design—the same scaffold can be used to generate ligands for two lectins with different binding sites.

We postulated that the glycomimetics identified could exhibit selectivity for their protein target. Such selectivity is desirable for a probe yet difficult to achieve using natural glycans. A comparison of the results from our screens using MBP-A and DC-SIGN reveals that several of our best inhibitors of DC-SIGN exhibited no ability to block MBP-A.¹⁰ To examine this issue directly, we tested **2** for binding to MBP-A. Even at high concentration (15 mM) (Fig. 4), compound **2** was ineffective.

We envisioned augmenting the potency of our selective glycomimetic through multivalency. DC-SIGN is tetrameric, and multiple copies of the protein are displayed on the dendritic cell surface.^{11,12} As with other protein–carbohydrate interactions,⁴ DC-SIGN could bind antigens with the requisite functional affinity by exploiting multivalent binding.^{7,13–15} The majority of multivalent lectin inhibitors consist of scaffolds decorated with mono- or oligosaccharide epitopes. In these cases, selectivity is a concern because the carbohydrate epitope can interact with many lectins. Because glycomimetics can display higher selectivity for their target lectin,⁴ multivalent glycomimetics may serve as useful probes, although few have been described.

Our strategy for multivalent ligand synthesis involves the application of ring-opening metathesis polymerization (ROMP). This method can yield polymers of defined lengths that function as highly effective biological probes.^{4,16–20} Additionally, ROMP-generated polymers can promote receptor clustering,¹⁹ and this property could facilitate investigations of DC-SIGN-mediated internalization. Finally, ROMP can give rise to polymers of defined length with polydispersity indices (PDIs) near unity. In summary, ROMP affords products with valuable attributes.

The multivalent glycomimetics were generated from polymer backbones bearing succinimide esters, which could be modified post-polymerization²¹ to append the glycomimetic epitopes (Scheme 1). To generate polymers that bind avidly to DC-SIGN, the length of the polymer must be sufficient for it to engage in multivalent binding, *i.e.*, either occupy multiple binding sites within the tetrameric DC-SIGN or cluster



Scheme 1 Synthetic route to ROMP-derived multivalent scaffold **3**, with a degree of polymerization of 29. The ratio of ethanolamine spacer to DC-SIGN inhibitor is approximately 1 : 3.

multiple copies of DC-SIGN on the cell surface. We used the structure of the monomeric DC-SIGN carbohydrate-recognition domain (PDB accession code 1sl4²²) to estimate the distances between sub-units of DC-SIGN. The width of the DC-SIGN CRD is roughly 40 Å. When extended, we estimate that each monomer within a ROMP-derived polymer spans approximately 5 Å.²³ For polymers to engage multiple copies of DC-SIGN, our analysis indicates that they must have a minimum length of 40 Å. We generated polymers with a length of 25 monomeric units, as these should be capable of multivalent binding. Accordingly, the degree of polymerization (29) was controlled by using a ratio of ruthenium initiator to norbornene monomer of 1 : 25.²⁴

The ratio of glycomimetic to ethanolamine was chosen to balance epitope density, which has been shown to be important for biological function,¹⁹ with water solubility. The glycomimetic was conjugated to the polymer through reaction of the amine-terminated ethylene glycol linker with the succinimidyl ester moiety on each backbone unit. This process afforded a multivalent inhibitor displaying approximately seven glycomimetics per polymer. Like the corresponding monomer, the polymer also inhibits DC-SIGN/ECD binding; thus, the linker did not impair ligand binding. Intriguingly, the IC₅₀ value for the polymer is 2.9 ± 1.2 μM, which indicates the polymer is 1000-fold more potent than the monomeric inhibitor. These results illustrate the utility of the glycomimetic strategy for generating potent multivalent ligands for lectins.

In summary, the shikimic acid-derived glycomimetic scaffold can be used to generate ligands for C-type lectins. This approach can yield inhibitors that are selective and potent. Both MBP-A and DC-SIGN possess significantly different binding sites, revealing the general utility of our approach for targeting diverse lectins. Additionally, our conversion of a lead glycomimetic into a multivalent ligand yields a potent non-carbohydrate inhibitor of DC-SIGN. In this way, multivalency can be exploited to generate highly effective non-carbohydrate inhibitors of lectins. Thus, the strategy we have outlined will be useful in developing probes not only of DC-SIGN function but also of the many other C-type lectins whose function or dysfunction are critical for human health.

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