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

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Abstract

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} In principle, 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 chemoenzymatic or chemical synthesis routes. Finally, since the specificity of lectins can overlap, natural oligosaccharides often bind multiple lectins, thereby complicating their use as probes of a target lectin in the presence of others.⁵ Non-carbohydrate antagonists are an appealing alternative, but few have been reported.^{2–4} Although screens of large compound libraries are beginning to yield inhibitors,^{6–10} 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;^{11–13} 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; they mediate pathogen recognition and processing as well as cell–cell interactions.¹⁴ C-type lectins, which are named for their dependence on calcium ions for carbohydrate complexation, often bind mannosides. In these complexes, the 2-, 3- and 4-hydroxyl groups of the sugar contribute to binding (Fig. 1A).¹⁵ We therefore hypothesized that scaffold **1**, which mimics the arrangement of the D-mannose 2-, 3-, and 4-hydroxyl groups (Fig. 1B), could afford glycomimetic probes of carbohydrate function. We demonstrated that the natural product shikimic acid¹⁶ could be transformed into compounds with the necessary arrangement of hydroxyl groups.¹⁸ From the resulting collections, inhibitors were identified of a prototype C-type lectin, mannose-binding protein A (MBP-A).

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†Electronic Supplementary Information (ESI) available: Additional figures, including binding curves, and detailed biochemical and synthetic experimental procedures.

A key feature of the aforementioned approach is that it has the potential to be general. Specifically, compounds represented by **1** might have the requisite attributes to bind C-type lectins other than MBP-A. 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.¹⁹ DC-SIGN is involved in pathogen recognition and 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.²⁰ Compounds that bind DC-SIGN and thereby prevent it from interacting with pathogens could serve as therapeutic leads. Moreover, several pathogens that bind DC-SIGN subvert normal immune system function, and DC-SIGN ligands could probe the underlying mechanisms. 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).²¹ The affinity for oligosaccharides is marginally higher ($K_d = 0.21$ mM for Man₉GlcNAc).²¹ We hypothesized that our strategy could yield effective glycomimetics with improved activity. To this end, 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. Accordingly, we wanted to test a range of functionality at each variable position. 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 of interactions. Glutamic acid and lysine were chosen to test the influence of anionic or cationic substituents, respectively. The R³ substituent was varied using a collection of alkylating agents. We tested some aliphatic R³ groups, but we focused on benzyl substituents because aromatic side chains often line carbohydrate binding sites.^{22, 23} Accordingly, aromatic rings with a range of functional groups were introduced, including those bearing halides, hydrogen bond donors or acceptors, and electron donating or withdrawing groups. The dithiol linkers (R²) used are commercially available and capable of positioning the R³ substituents near the carbohydrate or secondary binding sites.

These building blocks were employed¹⁸ to synthesize 192 compounds. The compounds were cleaved from resin and screened without further purification to get an estimate of their activity. The screen involved a fluorescence-based high-throughput competition assay that assessed the ability of compounds to compete against immobilized mannan for binding to the fluorophore-labeled tetrameric extracellular domain of DC-SIGN (DC-SIGN/ECD).^{10, 21} In this way, we identified several compounds that block DC-SIGN (Fig. 3). Trends in the inhibition data were readily apparent. The identity of the R¹ 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 R² position, the DTT-derived linker was most prevalent, followed by the butanedithiol-derived linker. At the R³ position, there was a preference for aromatic groups with electron withdrawing substituents. To confirm the validity of the inhibitory values upon which the trends were based, we purified several

library members and determined their IC₅₀ values. The relative values parallel those determined from the initial screen.

Based on its activity in the initial screen, compound **2** (Fig. 3), was chosen for further characterization. To determine its IC₅₀ 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.¹⁰ The IC₅₀ value of **2** (3.2 ± 0.6 mM) is superior to that of *N*-acetylmannosamine (IC₅₀ = 11.2 ± 0.7 mM). The MBP-A inhibitors identified previously were no more potent than the monosaccharide ligand α -methyl-D-mannopyranoside.¹⁸ Thus, our data indicate that this strategy can give rise to DC-SIGN inhibitors that are superior to known monosaccharide ligands. The results, therefore, provide a starting point for optimizing ligand potency. Most significantly, these results support the generality of our design—the same scaffold can be used to generate ligands for two lectins with different binding sites.

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.²¹ Its ability to detect antigens likely depends upon multivalent binding. As with other protein-carbohydrate interactions,^{24–27} DC-SIGN could exploit multivalent binding to achieve the necessary functional affinity.^{13, 28–31} The vast majority of multivalent lectin inhibitors consist of scaffolds decorated with the mono- or oligosaccharide binding epitopes recognized by the target lectin.³² In these cases, selectivity is a concern because the carbohydrate epitope can interact with many lectins, but glycomimetics can display higher selectivity for their target lectin.⁵ For this reason, multivalent glycomimetics may serve as useful probes,¹³ but few have been described.

Our strategy for multivalent ligand synthesis involves the application of ring-opening metathesis polymerization (ROMP).⁵ This method can yield polymers of defined lengths that function as highly effective biological probes.^{33–38} Additionally, ROMP-generated polymers can promote receptor clustering,³⁹ 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. These values are comparable to those obtained for the functionalization of higher generation dendrimers. In summary, ROMP affords products with valuable attributes.

The multivalent glycomimetics were generated from polymer backbones bearing succinimide esters, which could be modified post-polymerization⁴⁰ 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 multiple copies of DC-SIGN on the cell surface. We used the structure of the monomeric DC-SIGN carbohydrate-recognition domain (pdb accession code 1s14⁴¹) to estimate the distances between monomers 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.⁴² 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 1:25.⁴³

The ratio of glycomimetic to ethanolamine was chosen to balance epitope density, which has been shown to be important for biological function,⁴⁴ 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 highly potent non-carbohydrate inhibitor of DC-SIGN. Moreover, it demonstrates that 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 and dysfunction are critical for human health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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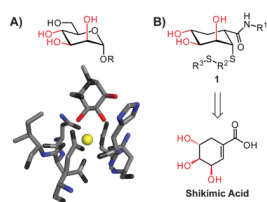


Fig. 1. Strategy for inhibitor design. A) D-Mannose (top) and a substructure of the binding site of a complex of mannose and MBP-A (bottom) (PDB accession code 1kwy¹⁷). The hydroxyl groups necessary for lectin recognition and binding are shown in red, Ca²⁺ is yellow. B) Glycomimetics **1** resemble mannose and can be obtained from the natural product shikimic acid.

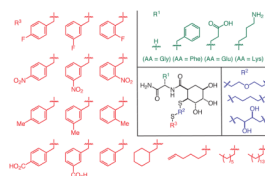


Fig. 2. Building blocks used in the synthesis of the glycomimetic library targeting DC-SIGN.

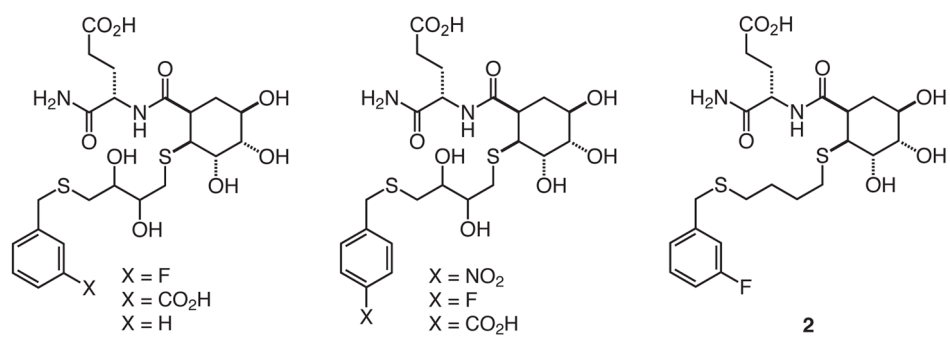


Fig. 3.
The most potent hits identified from library screen.

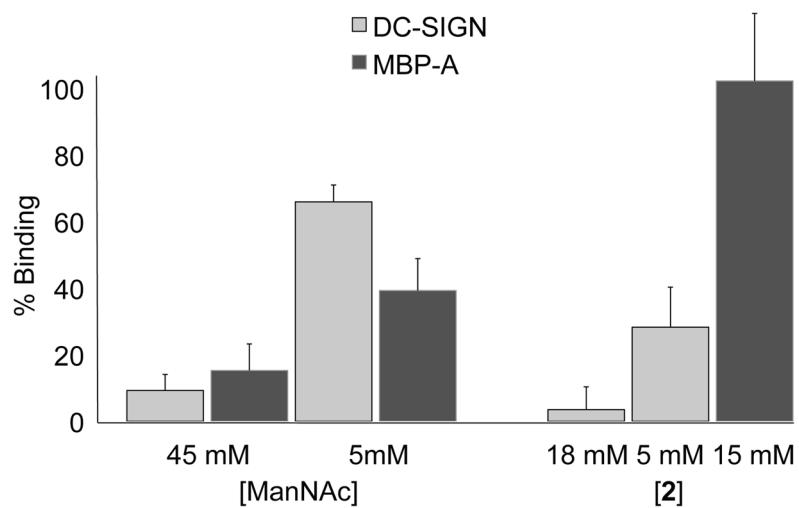
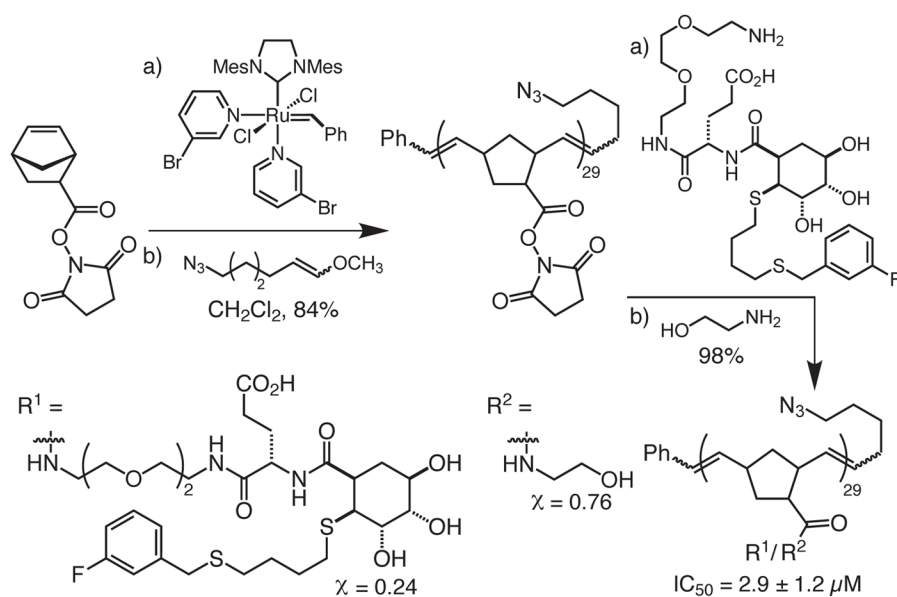


Fig. 4. Percent probe binding to DC-SIGN/ECD and MBP-A in the presence of ManNAc or **2** determined in fluorescence assay.

**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.