ORGANIC LETTERS

2003
Vol. 5, No. 9
1407−1410

Parallel Synthesis of Glycomimetic Libraries: Targeting a C-Type Lectin

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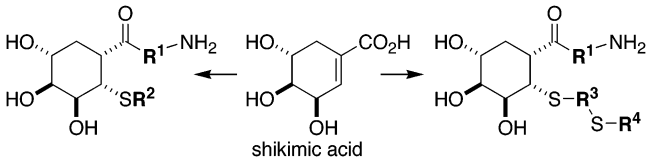
Received January 8, 2003

ABSTRACT



We have developed methods for the parallel synthesis of two libraries of non-carbohydrate-based analogues of mannose on a solid support. The natural product shikimic acid was used as a key building block. The ability of the compounds to block the binding of the C-type lectin MBP-A to a mannosylated surface was assessed in a high-throughput assay. Ten library members with inhibitory activities equivalent to that of α-methyl mannopyranoside were identified.

Carbohydrate−protein interactions are critical in physiological and pathological processes.[1] Consequently, synthetic glycomimetics and oligosaccharides designed to inhibit these interactions have been pursued as potential therapeutic agents.[2] Effective inhibitors have been identified for some carbohydrate−protein binding events, but most of these are derived from carbohydrate scaffolds.[3] The potential lability of synthetic carbohydrates toward in vivo glycosidases has prompted the search for stable carbohydrate analogues (glycomimetics) with potent biological activities.

We sought to develop non-carbohydrate-based glycomimetics targeted toward the C-type lectin family. The C-type lectins are a group of Ca^{2+}-dependent carbohydrate-binding proteins, many of which are found in mammals.[4] Human C-type lectins include the mannose-binding proteins (MBPs), the selectins (E-, L- and P-selectin), and DC-SIGN.[5] These proteins are all involved in immune system regulation.[6] Studies of ligand-bound MBP-A, MBP-C, E-selectin, and P-selectin by X-ray crystallography have revealed the importance of a vicinal axial−equatorial−equatorial display of hydroxyl groups on the carbohydrate ligand (Figure 1A).[7] We sought a core structure that was capable of providing a collection of diverse ligands and that could give rise to

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10.1021/ol0340383 CCC: $25.00
Published on Web 04/08/2003

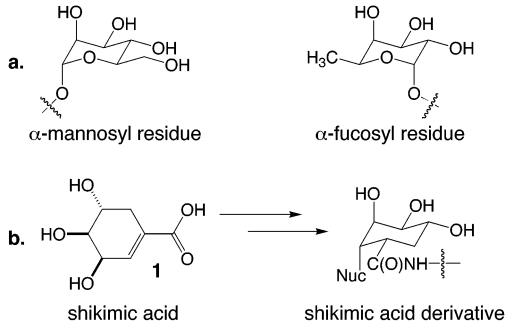


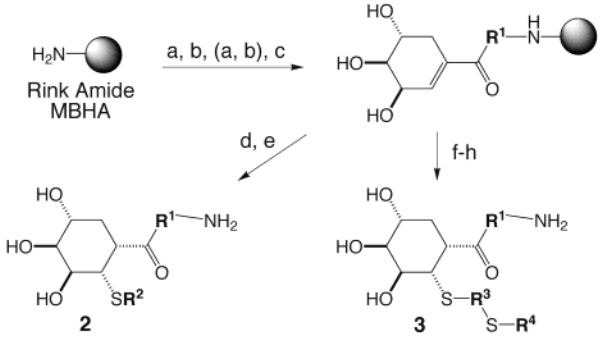
Figure 1. (a) Mannose and fucose residues participate in binding to C-type lectins via their axial−equatorial−equatorial vicinal hydroxyl group. (b) Transformation of shikimic acid affords products with the desired hydroxyl group arrangement.

ligands that target a range of lectins within the C-type lectin superfamily. A key criterion for our scaffold was that it should allow for the streamlined synthesis of libraries.[8]

We chose to build our library from the carbocycle shikimic acid. Shikimic acid possesses three hydroxyl groups that are displayed in a *pseudoaxial−pseudoequatorial−pseudoequatorial* configuration (Figure 1B). We envisioned that glycomimetics could be generated by the stereoselective conjugate addition of nucleophiles. Diversification could be achieved by using different thiol building blocks and by elaboration of the carboxylic acid. We chose to use thiolates as building blocks because they are excellent nucleophiles, and the corresponding conjugate addition reactions should proceed under mild conditions. With proper stereochemical control, conjugate addition could provide the requisite *axial−equatorial−equatorial* display of vicinal hydroxyl groups (Figure 1B).[9] Multivalent derivatives of shikimic acid itself have been used previously to inhibit a protein−carbohydrate interaction.[10] Because of the mode of C-type lectin ligand binding, we surmised that the proposed transformations of shikimic acid would yield more potent ligands.

To facilitate preparation of glycomimetic libraries, two related solid-phase, parallel syntheses were envisioned (Scheme 1). The key step in each strategy, the conjugate addition of a thiolate to a shikimic acid derivative, was tested in solution. At issue was the stereochemical outcome of the conjugate addition reaction. Specifically, we anticipated that

Scheme 1. Solid-Supported Synthesis of Libraries 1 and 2[a]



[a] Conditions: (a) FMOC-(R^1)-OH, HBTU, DIEA, DMF; (b) piperidine, DMF; (c) shikimic acid, DIC, C_6F_5OH, HOBt, DMF; (d) R^2SH, KO*t*-Bu, *t*-BuOH, DMF; (e) TFA, Et_3SiH, H_2O, $HSCH_2CH_2SH$; (f) HS-R^3-SH, KO*t*-Bu, *t*-BuOH, DMF; (g) R^4-Br, KI, Et_3N, DMF; (h) TFA, H_2O.

the incoming nucleophile would approach the unsaturated carbonyl compound on the *si* face opposite the allylic hydroxyl group. The stereochemical outcome from protonation of the incipient enolate, however, was more difficult to predict. We found that when the addition was conducted using a shikimic acid derivative with free hydroxyl groups, the desired isomer was obtained. Under the optimized conditions, thiolate addition occurs from the *si* face, followed by *axial* protonation to afford the desired isomer (Figure 2). With this knowledge, we set about to develop solid-phase routes to the target compounds.

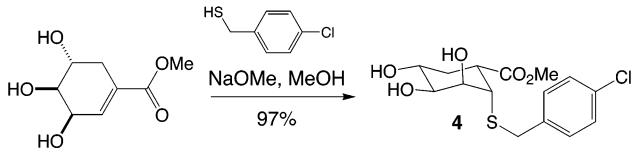


Figure 2. Desired stereochemical outcome was obtained from thiolate conjugate addition to shikimic acid methyl ester. Protonation of the incipient enolate on the other face affords a product that would exist in a conformer in which the hydroxyl groups are improperly oriented for C-type lectin binding.

In both of the synthetic routes developed, Rink amide 4-methylbenzhydrylamine polystyrene resin was utilized as the synthetic support. It is stable toward a variety of reaction conditions and delivers compounds with terminal amide groups.[11] To the immobilized amine was coupled either one or two commercially available amino acids via standard methods. Shikimic acid, which could be obtained via fermentation[12] or isolated from star anise, was then coupled to the free N-terminal amine.[13] Conveniently, shikimic acid could be added without protection of its secondary hydroxyl

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groups.[14] The coupling was carried out using diisopropyl carbodimide, hydroxybenzotriazole (HOBt), and pentafluorophenol. These conditions were employed to generate an activated shikimic acid derivative soluble under the reaction conditions. The key precursor for both synthetic routes was readily accessible.

The two synthetic strategies diverged at this stage, although both relied on nucleophilic addition of thiolate nucleophiles. In the first strategy, monothiols served as building blocks. Optimized reaction conditions involve incubation of the resin-bound substrate with the thiol in the presence of KO*t*-Bu. The stereochemical outcome for each addition reactions carried out on the resin was identical to that obtained under solution conditions.[13] Six thiols, three commercially available and three readily synthesized, were thus incorporated into the library.[13] Treatment of the resin-bound intermediates with trifluoroacetic acid in the presence of carbocation scavengers effected cleavage from the resin and removal of the protecting groups from the amino acid side chains.[15] A library of 72 compounds was synthesized using this route (Figure 3).

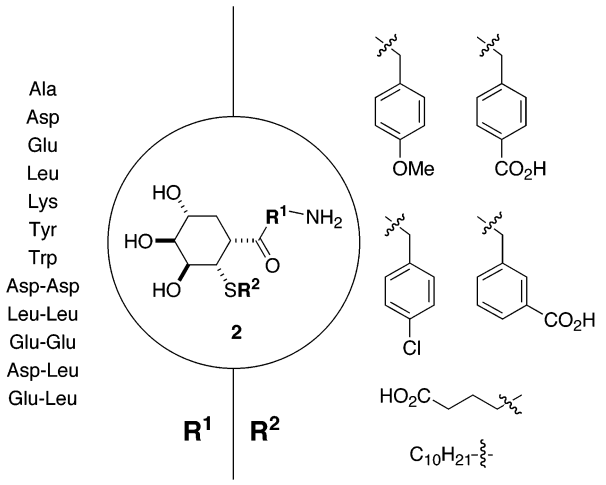

Figure 3. Target library derived from synthetic route 1.

A second library was synthesized using a modification of the original approach (Figure 2). Additional substituents were incorporated by introducing dithiol building blocks via conjugate addition. One thiol of the linker was used as the nucleophile in the conjugate addition, and the other was available for subsequent reaction with an alkyl bromide. The reaction conditions for the conjugate addition of the dithiols, dithiothreitol (DTT) or dithioerythritol (DTE), were identical to those described above. The alkyl bromides used in the synthesis of Library 2, or their direct precursors, were available commercially.[16] The thiolate alkylation reactions were effected in the presence of triethylamine and potassium iodide. Cleavage and amino acid side chain protecting group removal afforded the isomeric products. A total of 120 compounds were generated through this approach (Figure 4).

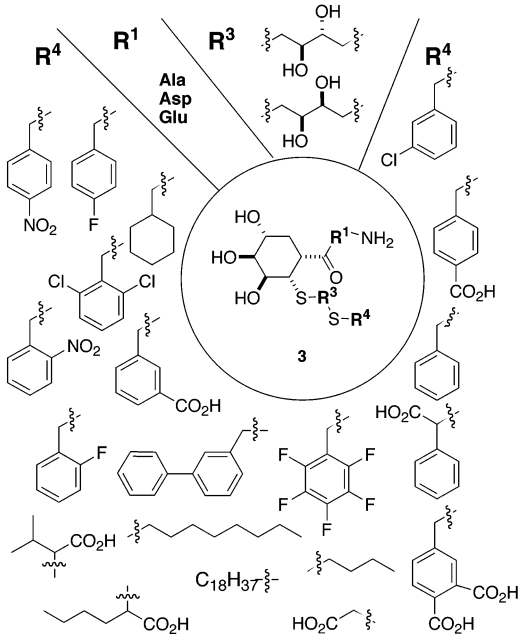

Figure 4. Target library derived from synthetic route 2. DTE and (±)-DTT were used as dithiols. The resulting diastereomeric mixtures were tested in inhibition assays.

We investigated the potency of the library members toward the well-studied and structurally characterized C-type lectin MBP-A. To this end, a bead-elution binding assay was developed.[17] This assay measures the ability of library members to compete with fluorescein-labeled MBP-A from a mannose-derivatized Sepharose resin. The MBP-A-bound resin is exposed to varying concentrations of a library member, and the amount of liberated fluorescein-MBP-A in solution is assessed by fluorescence detection. A major advantage of this method is that no radiolabels are required. Active library members were identified by comparing IC_{50} values for the library components to that of the MBP-A ligand, α-methyl mannopyranoside (α-MeMan).

A screen of the 192-member collection was carried out following cleavage of the compounds from the resin. Active compounds were purified by HPLC. From these samples, 10 compounds were identified with potencies comparable

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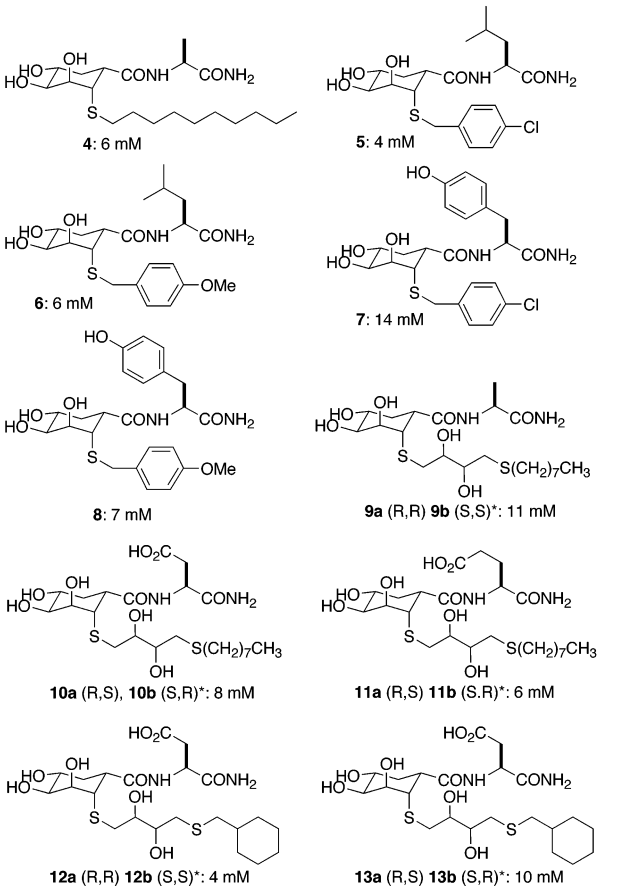

Figure 5. Compounds with activity in the MBP-A bead elution assay from Libraries 1 and 2. The reference IC_{50} for α-MeMan in this assay is 10 mM. An asterisk (*) indicates where compounds synthesized with DTT or DTE afforded diastereomers that were not separated.

to that of α-MeMan for MBP-A (Figure 5). Shikimic acid alone was inactive in the assay even at a concentration of 100 mM. These results indicate that members of the designed library have the key structural features required for interaction with the C-type lectins.

Analysis of the data reveals several trends. The amino acid substituent of the active ligands varies, suggesting that this position has only a minor influence on binding. The charged amino acid side chains in the active ligands, however, are all anionic; thus, they may engage in a Coulombic interaction or hydrogen bond. With regard to the thiol substituent, all of the active ligands possess a hydrophobic group at their terminus. In both libraries, none of the compounds bearing an anionic functional group at this position were found to have activity. The identification of MBP-A ligands that possess hydrophobic groups at this position suggests the presence of a hydrophobic pocket near the carbohydrate binding site. Significantly, ligands that bind as well as the carbohydrates that bind MBP-A have been identified from these libraries of modest size. These results indicate that shikimic acid is useful in glycomimetic synthesis.

The glycomimetics described here are effective inhibitors of a C-type lectin. Shikimic acid is a valuable building block because its carboxylic acid group and conjugated alkene allow for the incorporation of diversifying elements. These elements may be used to distinguish between members of the C-type lectin family. Thus, we anticipate that the synthetic strategy outlined here may serve as a general method for producing selective inhibitors of C-type lectins.

Acknowledgment. This research was supported by the NIH (GM49975) and the Mizutani and W. M. Keck Foundations. We thank Prof. J. Frost for suggesting shikimic acid as a template and Prof. J. Frost and Dr. K. Draths for a donation of shikimic acid. We thank Prof. K. Drickamer for the plasmids used for MBP-A production and S. Reddy for contributions to the assay. M.C.S. and T.J.B. were supported by the NIH (Chemistry−Biology Interface Training Grant, GM08505) and the Molecular Biosciences Training Grant (GM07215). K.M.J. was supported by a Hilldale Research Fellowship.

Supporting Information Available: Experimental procedures for the synthesis of library members, description and synthesis of thiol- and alkyl bromide library building blocks, and NMR spectra for select compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0340383