Communication

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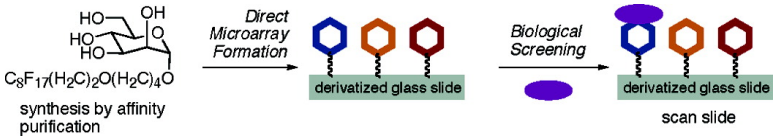

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Fluorous-Based Carbohydrate Microarrays

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The success of microarrays, such as DNA chips, for biosample screening with minimal sample usage has led to a variety of technologies for assays on glass slides.[1] Unfortunately, for small molecules, such as sugars, these methods usually rely on covalent bond formation, which requires unique functional handles and multiple chemical steps.[2] Herein, we present a new simpler concept in microarray formation that is based on noncovalent fluorous-based interactions and demonstrate the strength of these interactions in the direct formation of carbohydrate microarrays for biological screening.

Unlike comparable lipid tails interacting with hydrophobic solid phases,[3] a single C_8F_{17} tail is sufficient to bind biomolecules, such as peptides, to fluorinated solid supports.[4] The use of such fluorinated solid supports for affinity chromatography can substantially simplify the purification of synthetic carbohydrate intermediates in a process amenable to automation (Figure 1).[5] An additional

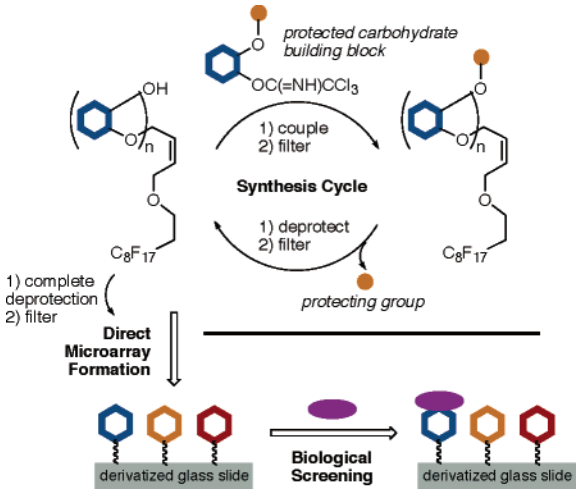
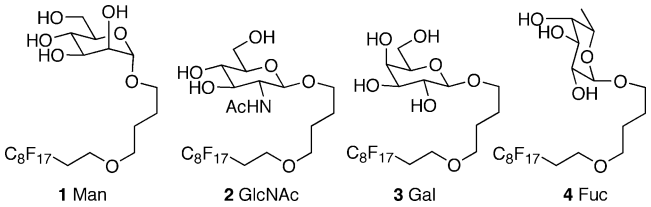


Figure 1. Strategy for the synthesis of carbohydrates and direct formation of fluorous-based carbohydrate microarrays.

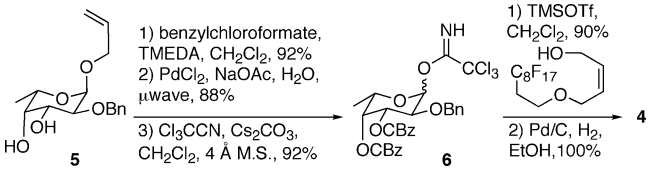
potential advantage of a fluorous-based approach is the direct formation of microarrays; the production of carbohydrate microarrays from compounds made on solid-phase still requires multiple solution-phase deprotection and derivatization steps.[2c]

We began our studies with the design of a suitable fluorous tag for carbohydrate synthesis that could survive the necessary sequential reaction conditions and also be removed if desired. An allyl group would be orthogonal to the trichloroacetimidate coupling conditions and other deprotection conditions. Therefore, to create a fluorous allyl protecting group as well as potential anchor for microarray formation, a fluorous tail was synthesized in one step by reaction of *cis*-1,4-butenediol with substoichiometric amounts of 1*H*,1*H*,2*H*,2*H*-perfluorodecyl iodide to produce an alcohol for use in glycosylations.

Scheme 1



Scheme 2



Sugars commonly found in plants were chosen as initial targets to test the feasibility of a noncovalent fluorous-based array for protein-binding studies (Scheme 1). Genome sequencing projects have revealed that the number of genes related to carbohydrate metabolism is far greater in plants, such as *Arabidopsis thaliana*, than in animals or fungi.[6] In addition to providing an understanding of plant biology, the study of carbohydrate−protein interactions in plants could lead to the discovery of new sugar-binding lectins for use as glycobiology tools.[7] The known trichloroacetimidates of peracylated mannose,[8] galactose,[9] and *N*-acetylglucosamine[10] were reacted with the fluorous-tagged allyl alcohol, and subsequent deacylation and hydrogenation of the double bond yielded the initial array components **1**−**3**.

Although neighboring-group participation could be used to control the stereochemistry of glycosylation for mannose, galactose, and *N*-acetylglucosamine, the synthesis of α-linked fucose required a different approach. Fucose glycosyl donors are usually built with nonparticipating benzyl protecting groups to provide predominantly the α-configured glycosylation products. Unfortunately, such electron-donating groups on a 6-deoxysugar also serve to make the resulting glycosidic linkage more acid sensitive. Several strategies alleviate this problem, for example, the use of more electron-withdrawing halobenzyl groups[11] or replacing the 3- and 4-OH protecting groups with an ester[2] that can potentially serve as a distant participating group. The latter approach is appealing, but requires an additional basic deprotection step. To avoid this extra step, we decided to test if a benzyl carbonate protecting group could serve the same purpose with the advantage of removal during the hydrogenation step. The requisite glycosyl donor was synthesized from the known allylated compound[13] **5** in three steps to provide the desired trichloroacetimidate **6** (Scheme 2). This donor was glycosylated with the fluorous-tagged alcohol using trimethylsilyl triflate to yield only the axially linked product in 90% yield. Indeed, the benzyl carbonate could serve to direct formation of the α-anomer and be removed by hydrogenation.

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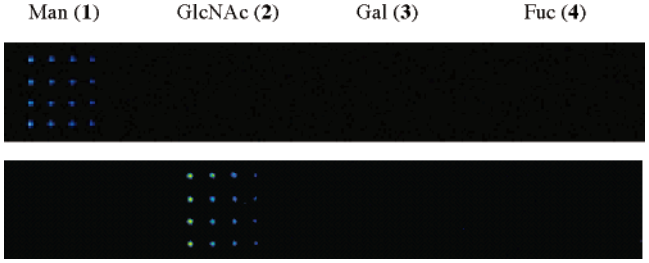

Figure 2. Fluorescence images of arrayed carbohydrates probed with FITC-labeled lectins. Columns of 4 spots each of 2, 1, 0.5, and 0.1 mM carbohydrates were incubated for 20 min with FITC−ConA (top) or FITC−ECA with 1% TWEEN-20 detergent (bottom) with BSA.

After synthesis of the requisite fluorous-tagged sugars, the next challenge was to find a suitable fluorinated surface. Solutions of each sugar were spotted onto a commercially available glass microscope slide coated with a Teflon/epoxy mixture employing a standard robot used for DNA arraying. The spots were dried, incubated with a solution of the fluorescein isothiocyanate-labeled jack bean lectin concanavalin A (FITC−ConA) for 20 min, rinsed repeatedly with assay buffer and distilled water, and then scanned with a standard fluorescent slide scanner. The scan clearly showed binding of FITC−ConA only to the mannose-containing spots. The anomeric position could be distinguished as the β-linked GlcNAc (**2**) was not recognized.

This lectin experiment demonstrated the ability of the C_8F_{17} tail to anchor the carbohydrates to the slide surface even after repeated washes. However, the slide was also intrinsically and unevenly fluorescent at 488 nM, a wavelength that is commonly used to detect labeled analytes. Clearly, a new approach was necessary to obtain an optically and fluorescently clear surface for the formation of compound microarrays. To this end, a glass microscope slide was reacted[14] with a fluoroalkylsilane to provide a clear coating on which water forms beads.

With the new microarray substrate in hand, we next probed the scope of a fluorous-based microarray approach for compound screening. Fluorous-tagged sugars were spotted on the coated slide using an arraying robot, and then the slides were incubated with FITC-labeled lectins (Figure 2). To test the reproducibility of the process, the same concentration of sugar was spotted repeatedly. In addition, several different concentrations of sugars were spotted. To test the ability of the array to withstand detergents often included in biological screens, the labeled plant lectin from the bush *Erythrina crystagalli* (FITC−ECA) was used to probe the microarrays with Tween-20. The array withstood the 20 min incubation time and repeated rinsing with this detergent-containing buffer.

A fluorous-based microarray method allows the facile formation of a range of carbohydrate chips for the plant and other sciences using synthetic sugars produced with the aid of fluorous-tagged synthesis. Efforts are underway to automate portions of the solution-phase fluorous-based synthetic process and to incorporate enzymatic steps to expand the scope of carbohydrates that can be easily arrayed for biological screening. Although not limited to carbohydrates, the approach should be especially valuable for the production of arrays containing compounds, such as glycosaminoglycan fragments, that contain nucleophiles that complicate current defined covalent attachment strategies.

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Supporting Information Available: Experimental details, including copies of ^{1}H NMR spectra, for the synthesis and production of the carbohydrate microarrays and the complete ref 2h. This material is available free of charge via the Internet at http://pubs.acs.org.

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