Chemistry & Biology, Vol. 9, 443–454, April, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00124-2

Carbohydrate Arrays for the Evaluation of Protein Binding and Enzymatic Modification

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Summary

This paper reports a chemical strategy for preparing carbohydrate arrays and utilizes these arrays for the characterization of carbohydrate-protein interactions. Carbohydrate chips were prepared by the Diels-Alder-mediated immobilization of carbohydrate-cyclopentadiene conjugates to self-assembled monolayers that present benzoquinone and penta(ethylene glycol) groups. Surface plasmon resonance spectroscopy showed that lectins bound specifically to immobilized carbohydrates and that the glycol groups prevented nonspecific protein adsorption. Carbohydrate arrays presenting ten monosaccharides were then evaluated by profiling the binding specificities of several lectins. These arrays were also used to determine the inhibitory concentrations of soluble carbohydrates for lectins and to characterize the substrate specificity of β-1,4-galactosyltransferase. Finally, a strategy for preparing arrays with carbohydrates generated on solid phase is shown. This surface engineering strategy will permit the preparation and evaluation of carbohydrate arrays that present diverse and complex structures.

Introduction

The development of biochip microarrays has had a major impact in biological research and in drug discovery programs. Microarrays combine the benefits of immobilized format assays, which simplify the isolation and detection of analytes, with the capability of analyzing thousands of analytes in parallel. Gene chips, which comprise patterned arrays of oligonucleotides or cDNAs, represent the prototype biochip. They have been commercialized and are now widely used in the global profiling of gene expression [1, 2]. This success has motivated significant efforts to develop functional peptide and protein chips that can more directly characterize cellular activities [3–5]. These chips still require further development and will likely be made available to the broader research community over the next several years. In this paper, we introduce a strategy for preparing a carbohydrate chip and demonstrate its utility for characterizing carbohydrate binding and modifying activities.

The functions of carbohydrates in biology have not been as extensively studied as those of proteins and nucleic acids. This discrepancy is due both to the more complex structures of oligosaccharides (which often are branched) and to a lack of general methods for synthesizing and analyzing these molecules [6–8]. This situation has changed significantly in the past ten years as carbohydrate-protein (and carbohydrate-carbohydrate) interactions have been characterized in a variety of settings, including cell adhesion [9], immunity [10], and development [11]. In parallel, recent important advances in chemical and enzymatic methods for the solid phase synthesis of complex carbohydrates permit many other studies of carbohydrate structure and function in biology [12–14].

Studies in both chemistry and biology will benefit from—and in time will require—chips that present arrays of many different carbohydrates. Several groups have prepared substrates that present immobilized carbohydrates and utilized these substrates to characterize protein-carbohydrate interactions [15–19], but there is still a need for substrates that present multiple carbohydrates in a homogeneous manner. These "carbochips" will be important for identifying proteins and enzymes that interact with carbohydrates, for mapping the substrate specificity of protein targets, and for identifying inhibitors of protein-carbohydrate or carbohydrate-carbohydrate interactions. One company has claimed such a chip, but no literature describing the composition, preparation, or use of this array has been published [20]. In this paper, we describe a flexible and chemically well-defined approach for the preparation of carbohydrate arrays, and we validate the utility of these carbochips for protein binding assays and enzyme activity assays.

The development of a carbochip must satisfy several requirements in order to provide both excellent selectivity and quantitative performance. Most importantly, the chip must prevent the nonspecific adsorption of proteins from a contacting solution. The use of substrates that are not inert will give adsorption of unwanted proteins and lead to false positive results. It will also obstruct the presentation of immobilized carbohydrates and thus give false negative results. The common strategy of treating the substrate with bovine serum albumin or other blocking proteins to passivate the surface and prevent this unwanted adsorption is not an effective solution because the blocking proteins often interfere with desired interactions on the substrate [21]. A second requirement is that carbohydrates are presented in a regular and homogeneous environment so that all immobilized ligands have equal activity toward soluble proteins and enzymes. This feature permits the quantitative evaluation of binding affinity and enzymatic activity. Third, it is vital that the density of carbohydrate ligands be controlled because many carbohydrate-protein and carbohydrate-carbohydrate interactions are polyvalent in nature [22, 23]. The affinities of these interactions are extremely sensitive to changes in the density and orientation of individual carbohydrates, so much so that binding affinities and specificities can change with the density of an immobilized carbohydrate [24, 25]. Finally, to be broadly useful, the carbochip should be compatible with several important detection methods, including phosphorimaging, fluorescence, and surface plasmon

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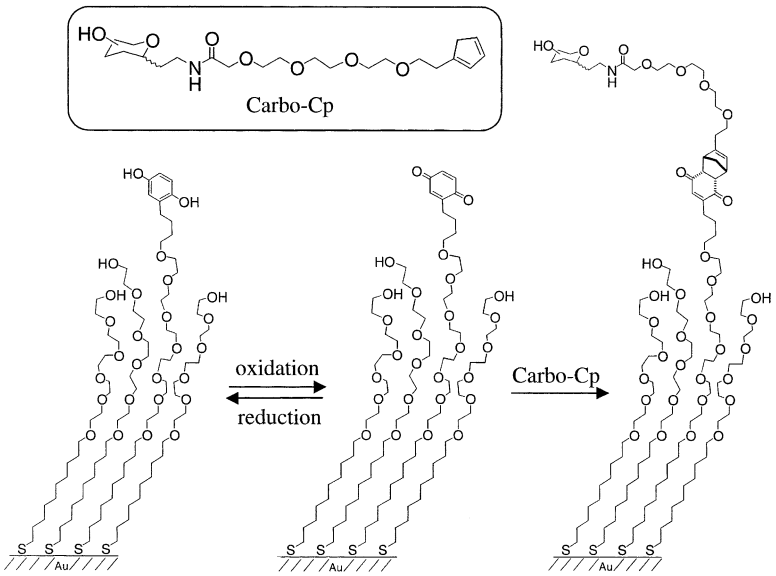

Figure 1. Chemical Approach for the Preparation of Carbohydrate Chips

The strategy is based on the Diels-Alder-mediated immobilization of carbohydrate-cyclopentadiene conjugates to a monolayer that presents benzoquinone groups. Because the Diels-Alder reaction is rapid, selective, and quantitative, all carbohydrates within the array are presented at a uniform density.

resonance spectroscopy. Below we describe an approach based on self-assembled monolayers of alkanethiolates on gold. This approach can produce carbohydrate chips that meet these requirements and that have the necessary characteristics to be broadly valuable in biological research and in applications to screening.

Results

Approach

We employed self-assembled monolayers of alkanethiolates on gold as a platform for immobilizing an array of carbohydrates. The monolayers were prepared by the immersion of gold-coated glass slides in a mixture of two alkanethiols, one substituted with a hydroquinone group and the other substituted with a penta(ethylene glycol) group (Figure 1). The relative concentration of the two alkanethiols was adjusted to give monolayers having hydroquinone groups at a density of 1% relative to total alkanethiolate. The hydroquinone groups were oxidized chemically or electrochemically to benzoquinone groups, which then reacted with carbohydrate-diene conjugates by way of a Diels-Alder reaction to covalently immobilize the ligand at a density of approximately 10 pmol/cm^2. This reaction is well suited for immobilization because it is rapid, quantitative, and selective [26–29]. A further benefit of this immobilization strategy derives from the reversible electrochemical reduction of the quinone, which can be used to quantitatively determine the density of quinone groups on the substrate [26]. This feature gives excellent control over the density of immobilized ligands. The penta(ethylene glycol) groups of the monolayer prevent the nonspecific adsorption of proteins to the substrate and ensure that only biospecific interactions between soluble proteins and the immobilized ligands are possible [30, 31]. The excellent control over unwanted adsorption with these monolayers has been validated in several applications [32–34].

Synthesis of Carbohydrate-Diene Conjugates

In order to prepare arrays that present several carbohydrates, we synthesized conjugates **1–10**, which contain a cyclopentadiene group for Diels-Alder-mediated immobilization to self-assembled monolayers. Each compound was prepared by the coupling of carboxylic acid **11** with the appropriate ω-aminoalkylglycoside derivative (Figure 2). Compound **11** was prepared in four steps and with a 19% overall yield from tetra(ethylene glycol) (Figure 3A). The protected *trans* aminoglycosides of glucose (**16a**), galactose (**16b**), N-acetylglucosamine (**16c**), fucose (**16d**), mannose (**16e**), and rhamnose (**16f**) were prepared by condensation of the corresponding peracetyl glycosyl bromides [35] with 5-(benzyloxycarbonylamino)pentanol (**15**) under Helferich conditions (Figure 3B). These derivatives were then deprotected by sequential treatment with catalytic sodium methoxide in methanol and catalytic hydrogenation to afford aminoglycosides **17a–f**. The *cis* aminoglycosides of glucose (**19a**), galactose (**19b**), N-acetylglucosamine (**19c**), and fucose (**19d**) were prepared from unprotected α-O-allyl glycosides **18a–d** by photochemical addition of cystamine hydrochloride to the olefin (Figure 3C) [36].

Characterization of Biospecific Association

We used surface plasmon resonance (SPR) spectroscopy [37] to show that monolayers presenting the carbohydrates described above could participate in biospecific association with soluble proteins but at the same time remain inert to the nonspecific adsorption of protein. To address the latter point, we characterized the adsorption of the "sticky" protein fibrinogen (1 mg/ml in DPBS) to the substrates before and after immobilization of each carbohydrate. Monolayers that presented hydroquinone groups at a density of 20% showed 35 ng/mm^2 of nonspecific protein adsorption, which corresponds to approximately 50% of a densely packed layer of adsorbed protein (Figure 4A, solid line) [38]. A separate monolayer was chemically oxidized and treated with mannose conjugate **9** (2 mM in H_2O for 2 hr at 25°C)

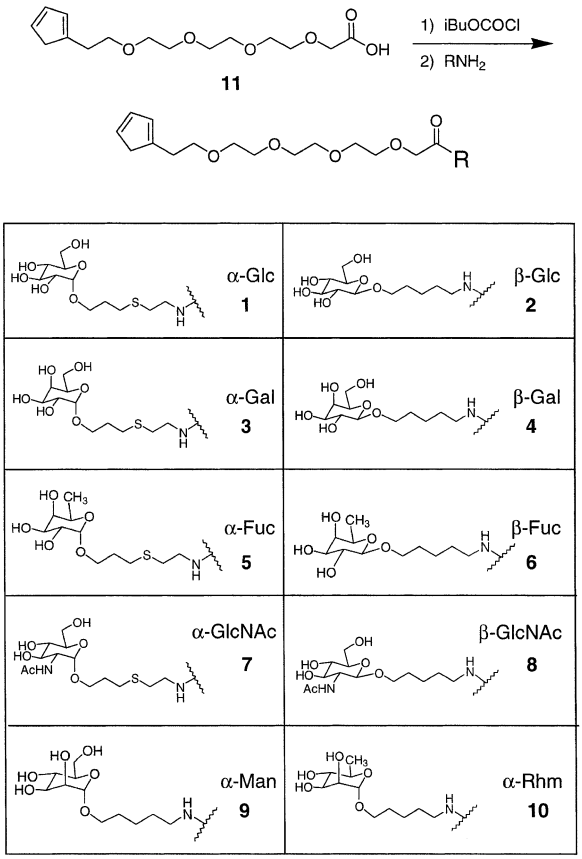

Figure 2. Strategy for Synthesis of Carbohydrate-Cyclopentadiene Conjugates

(A) Activation of cyclopentadienyl carboxylic acid **11** with isobutylchloroformate and coupling with an appropriate aminoglycoside affords the carbohydrate-cyclopentadiene conjugate.

(B) Structures of carbohydrate conjugates **1–10**.

to immobilize the carbohydrate to the substrate. SPR showed that this monolayer was completely inert to nonspecific adsorption of fibrinogen (Figure 4A, dashed line). Experiments using the nine other carbohydrate conjugates demonstrated that monolayers presenting these ligands were also inert to the nonspecific adsorption of fibrinogen (our unpublished data). The general finding that monolayers presenting carbohydrate groups, even at high densities, are inert to protein adsorption is consistent with previous work in which monolayers presenting N-acetylglucosamine [24], mannitol [39], and pentamethyl sorbitol [40] were shown to resist the nonspecific adsorption of fibrinogen.

To demonstrate specific association of protein with immobilized carbohydrates, we immobilized mannose conjugate **9** onto monolayers and repeated the SPR experiment with the lectin concanavalin A (Con A; 2 μM in DPBS). Figure 4B shows that Con A interacts with these substrates (solid line) and that this association could be completely blocked by preincubation of the lectin with soluble α-methyl mannose (2 mM in DPBS, dashed line). Together, these results confirm the presence of the immobilized carbohydrate on the monolayer and demonstrate that the carbohydrate can participate in biospecific association with soluble proteins.

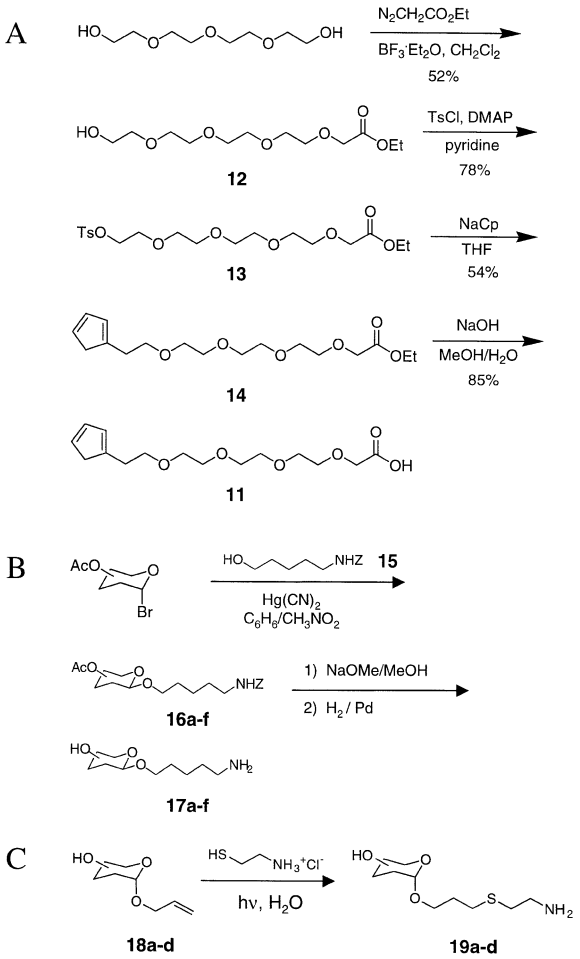

Figure 3. Synthesis of Aminoglycosides and Cyclopentadienyl Carboxylic Acid **11**

(A) Compound 11 was prepared in four steps by desymmetrization of tetra(ethylene glycol).

(B) *Trans* glycosides **16a–f** were synthesized by the condensation of a peracetyl glycosyl bromide with 5-(benzyloxycarbonylamino)-pentanol. Sequential deprotection of the acetyl and benzyloxycarbonyl groups provided aminoalkyl glycosides **17a–f** in good yield.

(C) *Cis* aminoalkyl glycosides **19a–d** were prepared by the photochemical addition of cysteamine hydrochloride to α-O-allyl glycosides **18a–d**.

Profiling Lectin Binding with the Carbochip

We next prepared an array presenting monosaccharide conjugates **1–10** and characterized the binding of several lectin proteins to the array. We did this by applying a drop of each carbohydrate conjugate (1 μl of a 2 mM solution in H_2O) to discrete locations on a single monolayer presenting benzoquinone groups (Figure 5A). The substrates were kept in a humidified chamber at 37°C for 2 hr, washed with water, and dried under a stream of nitrogen. These conditions permitted near-quantitative immobilization with minimal quantities of diene conjugate. Any remaining benzoquinone groups on the substrate were inactivated by treatment of the monolayer with tri(ethylene glycol)-cyclopentadiene conjugate **20** (2 mM in MeOH).

To investigate the lectin binding properties of the car-

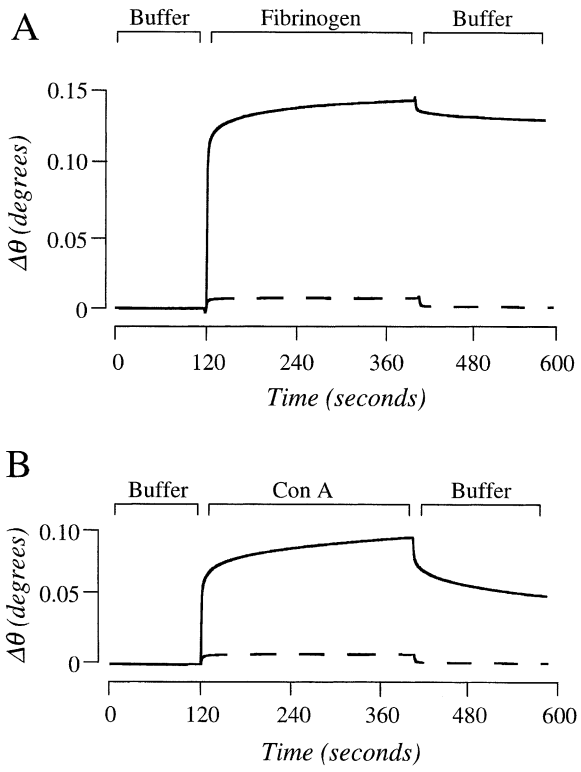

Figure 4. Monolayers that Present Carbohydrates Are Inert to the Nonspecific Adsorption of Protein

Substrates were mounted into the SPR flow cell, and the monolayer was treated with running buffer (DPBS) for 2 min. A solution of protein (fibrinogen or concanavalin A) was then introduced for 5 min. The protein solution was replaced with running buffer, and the binding of the protein to the monolayer was evaluated.

(A) Monolayers presenting 20% hydroquinone groups among penta(ethylene glycol) groups interact nonspecifically with fibrinogen (3 μM in DPBS, solid line). After the monolayer was oxidized and treated with mannose conjugate 9, there was essentially no nonspecific adsorption of fibrinogen (dashed line).

(B) Monolayers presenting mannose conjugate 9 interact with the lectin concanavalin A (5 μM in DPBS, solid line). Preincubation of the lectin with soluble α-methyl mannose (2 mM in DPBS) completely prevents binding to the monolayer, showing the specificity of the binding interaction (dashed line).

bochip, we treated identical arrays separately with each of five rhodamine-labeled lectins (2 μM in DPBS) for 30 min, rinsed them with DPBS, and then imaged them with a confocal array scanner (Figure 5B). The resulting images show that there is essentially no fluorescence in regions of the substrate that present no carbohydrate (signal-to-noise ratio > 50:1) and that each of the lectins binds to its known ligands. The carbohydrate array treated with Con A, for example, showed significant fluorescence intensity in the region presenting α-mannose, along with reduced binding to the α-glucose region and weak fluorescence in the α-GlcNAc region. Experiments with each of the four other lectins also gave the expected results: *B. simplicifolia I* bound to regions presenting α- and β-GlcNAc; *E. cristagalli* bound to regions presenting α- and β-Gal; *U. europaeus I* bound to the region presenting α-fucose; *G. nivalis* bound to the region presenting α-mannose. These experiments verify that the carbohydrate array is well suited for the selective identification of carbohydrate binding proteins.

Quantitative Analysis of Inhibitors with the Carbochip

The results reported above suggested that these carbohydrate arrays have the characteristics required for quantitative assays of protein-carbohydrate interactions. To assess the value of carbochips in quantitative assays, we measured the inhibition of Con A binding to the chip by α-methyl mannose (Figure 6A). A series of mixtures containing rhodamine-labeled Con A (2 μM in DPBS) and α-methyl mannose (0-4 mM in DPBS) were prepared and arrayed onto separate monolayers presenting α-mannose, α-glucose, or α-N-acetylglucosamine at a density of 1% total alkanethiolate. After incubation for 1 hr at 25°C, the substrates were rinsed with DPBS and analyzed with the fluorescence array scanner to quantitate the amount of bound lectin. In a typical experiment (in which solutions were spotted by hand), approximately 75 spots could be accommodated on a single chip with an area of 20 cm^2. The amount of lectin that bound to the chips for each concentration of soluble ligand is shown in Figure 6B. The concentration of α-methyl mannose required to inhibit 50% of Con A binding to the monolayer provides an IC_{50} value that can be used to compare the affinities of the lectin for the three immobilized carbohydrates. Monolayers presenting α-mannose (IC_{50} = 55 μM) competed more effectively with the soluble carbohydrate for Con A than did monolayers presenting α-glucose (IC_{50} = 23 μM) or α-glcNAc (IC_{50} = 8 μM). The relative binding affinities of these carbohydrates for Con A is consistent with those obtained in previous studies [41, 42].

Profiling Enzymatic Activities

Carbohydrate chips that can identify selective protein-carbohydrate interactions may also be well suited for characterizing the specificities of enzymes such as glycosyltransferases, glycosidases, kinases, and sulfotransferases that modify carbohydrates [43–45]. To demonstrate this principle, we treated the array with bovine β-1,4-galactosyltransferase (GalTase). Previous work has shown that monolayers presenting GlcNAc are good substrates for the enzyme-mediated galactosylation and that the resulting disaccharide (N-acetyllactosamine or LacNAc) can be characterized by its association with selective lectins [24]. We applied an aqueous HEPES buffer containing the enzyme, UDP-Gal, and $MnCl_2$ to two identical arrays and incubated the chips for 6 hr at 37°C. The substrates were then washed with buffer and incubated with rhodamine-labeled lectins from either *E. cristagalli* or *B. simplicifolia I*. Fluorescence images of chips reveal that after enzymatic modification, the GlcNAc-specific lectin *B. simplicifolia I* binds only to α-GlcNAc, suggesting the modification of β-GlcNAc on the array (Figure 7A). The lectin from *E. cristagalli* bound the β-GlcNAc, α-Gal, and β-Gal elements of an identical array (Figure 7B), demonstrating the addition of galactose to the β-GlcNAc element. This lectin binding profile is consistent with the substrate specificity of the enzyme and demonstrates the use of

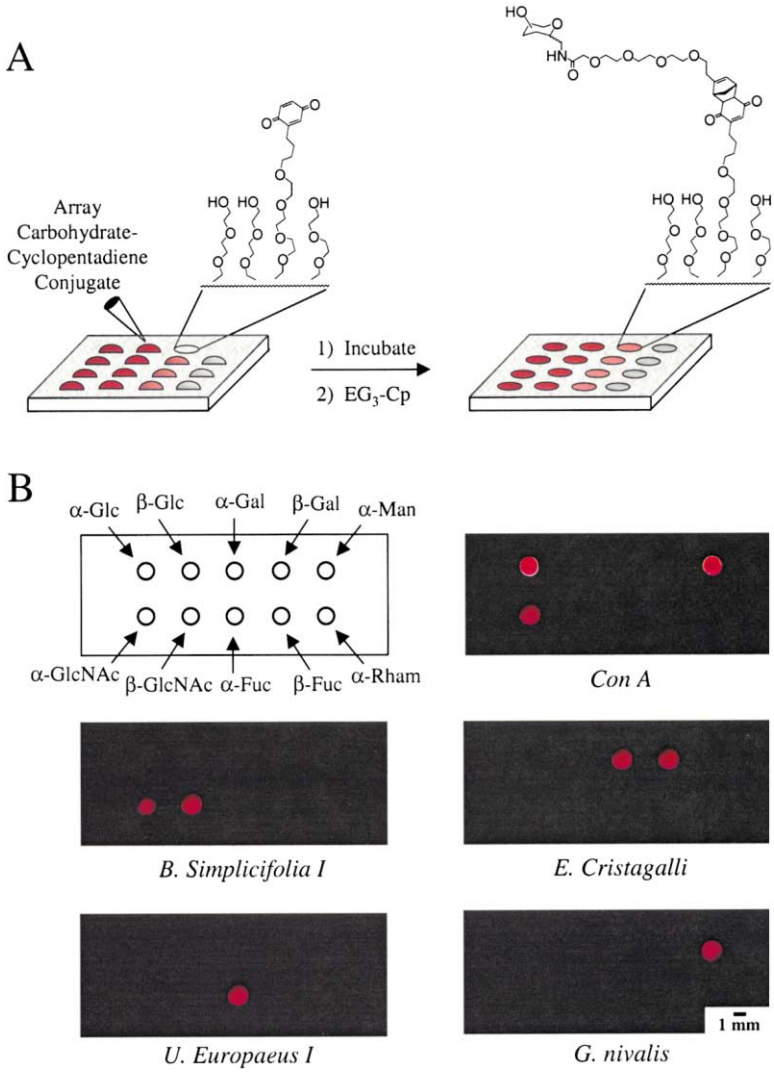

Figure 5. Profiling Lectin Binding Specificities with a Carbohydrate Array

(A) Carbohydrate arrays are prepared by spotting solutions of carbohydrate diene conjugates **1–10** onto discrete regions of a monolayer presenting benzoquinone groups. After the reaction is complete, benzoquinone groups in the remaining regions of the monolayer can be inactivated by treatment with tri(ethylene glycol)-cyclopentadiene conjugate 20 (EG_3-Cp).

(B) Identical carbohydrate chips were separately incubated with each of five rhodamine-labeled lectins (2 μM in DPBS) for 30 min, gently rinsed, and evaluated by confocal fluorescence microscopy. Fluorescent images of the resulting arrays are shown for each lectin. These images reveal that the proteins associate specifically with their known carbohydrate ligands on the array.

carbochips for characterizing enzymatic activities toward carbohydrate substrates.

Compatibility with Solid Phase Carbohydrate Synthesis

The extension of this chip to the study of more complex carbohydrate-protein interactions requires synthetic access to a large number of relevant oligosaccharide units. This challenge has recently been addressed by the development of methods for solid phase synthesis of carbohydrates [12–14, 46]. Seeberger and coworkers, for example, have described an automated method for the preparation of complex n-pentenyl glycosides [12, 46]. The terminal olefin of these glycosides can be readily converted to an aldehyde or carboxylic-acid group, which can be coupled with a variety of tags, including fluorescent dyes and biotin. We envisioned the use of this strategy for the synthesis of complex oligosaccharide-diene conjugates (Figure 8A). To validate this approach, we prepared hydrazide cyclopentadiene conjugate **21** and coupled this compound with 2-oxoethyl-α-mannose [42] (Figure 8B). The chemoselective ligation reaction proceeded rapidly to generate mannose conjugate **22** in 81% yield after only 30 min. This compound was then arrayed onto a benzoquinone-terminated monolayer under the conditions described above. Treatment of the array with rhodamine-labeled Con A yielded fluorescence only in regions where the conjugate was immobilized with an excellent signal-to-noise ratio (>50:1) (Figure 8C). Because the oxidation and chemoselective ligation reactions are compatible with carbohydates, this approach should be well suited for immobilizing complex structures generated on solid phase and should extend the utility of these carbochips.

Discussion

This paper describes a new class of carbohydrate chip that has several characteristics required for it to be broadly useful for studies of proteins and enzymes that interact with mono- and oligosaccharides. First, the immobilized carbohydrates can participate in binding interactions with protein partners and can serve as substrates for enzymes. Second, monolayers that present

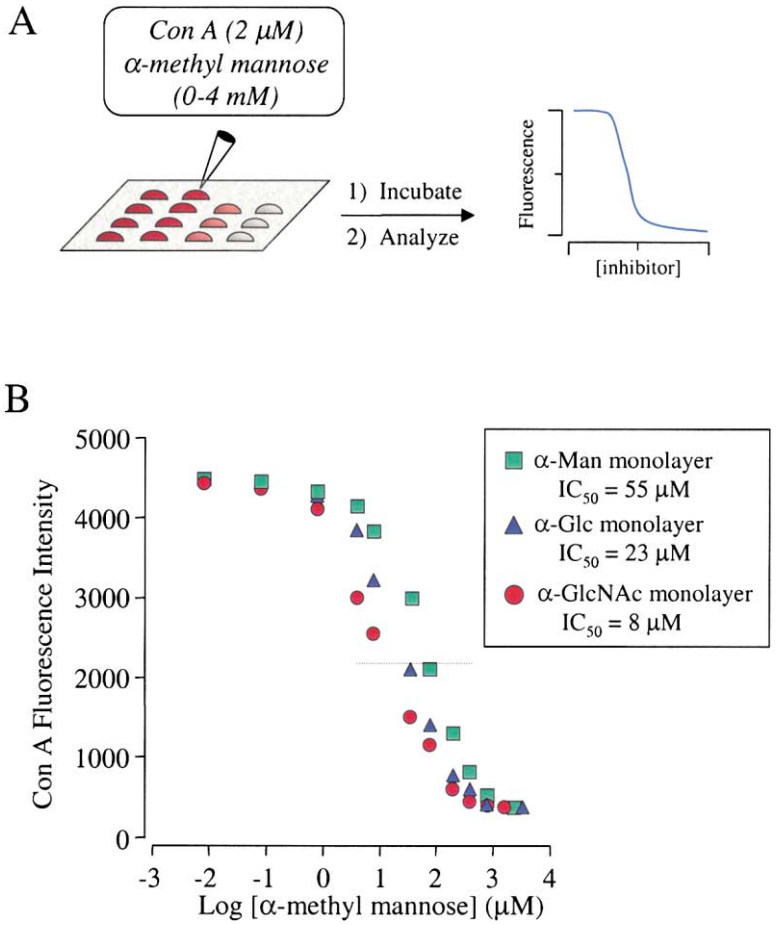

Figure 6. Using Carbochips for Quantitative Inhibition Assays

(A) A series of solutions containing rhodamine-labeled Con A (2 μM) and α-methyl mannose (with concentrations ranging from 0 to 4 mM) were arrayed onto single monolayers presenting α-glucose, α-glcNAc, or α-mannose. After 1 hr, each substrate was washed, and bound fluorescence was evaluated with the fluorescence array scanner.

(B) The intensity of fluorescence for each concentration of inhibitor is plotted against the concentration of inhibitor to give dose-dependent inhibition curves. The data represent one of three independent experiments that yielded similar results.

oligo(ethylene glycol) groups prevent nonspecific adsorption of proteins and other components that are present in sample solutions. These monolayers are currently the best available for preventing unwanted interactions and are critical to the performance of the carbohydrate chip. Third, the use of a Diels-Alder reaction to immobilize carbohydrates gives high yields and excellent selectivity for immobilization. Hence, the densities of immobilized carbohydrates can be controlled and kept constant for all carbohydrates in the array because they are determined by the density of benzoquinone groups in the film, not on the particular structure of carbohydrate. One can determine the absolute densities of benzoquinone groups with good accuracy by using cyclic voltammetry to quantitatively monitor the reversible reduction. These features are especially important with carbohydrate chips (as compared to DNA, peptide, and protein chips) because these ligands participate in multivalent interactions and are therefore exquisitely sensitive to the density and presentation of individual carbohydrates.

The method we describe here for preparing biochips is distinguished from other reports of gene chips, peptide chips, and protein chips in that it relies on a sophisticated application of surface engineering. Many approaches have used routine glass or plastic substrates and nonselective chemical reactions to immobilize biologically active molecules. Although the resulting chips have proven to be very important in biology and drug discovery, they still do not compete with quantitative performance and reproducibility of conventional solution-based assays. This limitation stems largely from two factors. First, the immobilized molecules are presented in a heterogeneous environment and therefore display different activities. Proteins, for example, are usually immobilized in a range of orientations and undergo denaturation to varying extents, both of which can compromise activity. Second, the substrates that are used to pattern arrays are often not very effective at blocking unwanted interactions, which can lead to high background levels and to interference with desired protein-ligand interactions. The use of blocking proteins or detergents can optimize the performance of an assay, but it introduces further heterogeneities that compromise the quantitative character of the chip. Our development of a carbohydrate chip avoids these factors by an appropriate engineering of the surface chemistry. We believe that these strategies will also be important for the preparation of peptide and protein chips [29, 47].

The utility of carbohydrate chips—particularly chips that present many distinct ligands—depends ultimately on the detection methods that can be adapted for reading the results of a chip-based assay. The monolayer substrates described here have the advantage that they are compatible with all of the principal techniques used for analyzing chips. In this paper we used fluorescence

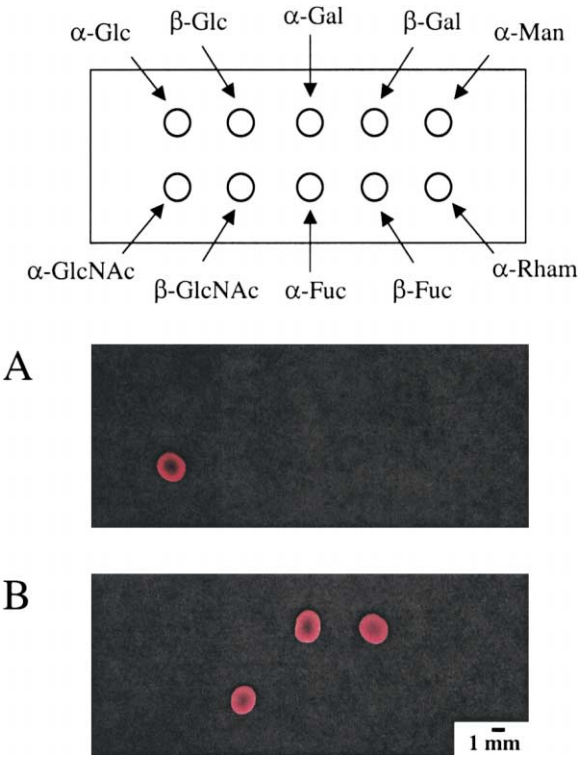

Figure 7. Enzymatic Modification of the Carbohydrate Array with β-1,4-Galactosyltransferase (GalTase)

(A) After treatment with GalTase, the *B. simplicifolia* lectin binds only to the α-GlcNAc element of the array, and this element is not a substrate for the enzyme.

(B) The lectin from *E. cristagalli* binds to the β-GlcNAc element that had converted to LacNAc, as well as the α- and β-galactose elements of the array.

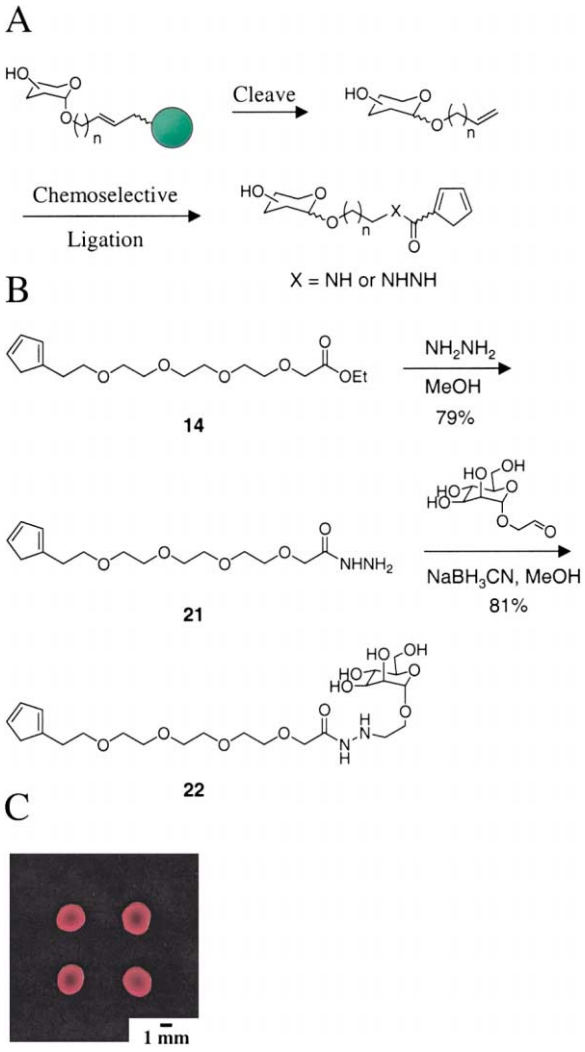

Figure 8. Adapting Carbohydrates Prepared by Solid Phase Synthetic Routes for Incorporation into Carbohydrate Arrays

(A) Allyl or n-pentenyl glycosides generated by solid phase methods can be oxidized and chemoselectively coupled with a nucleophilic diene.

(B) Synthesis of a mannose-diene conjugate, **22**, by chemoselective ligation. The conjugate was spotted onto a benzoquinone-terminated monolayer, and the resulting array was treated with rhodamine-labeled Con A (2 μM in DPBS) for 30 min.

(C) Fluorescence is observed only in substrate regions where the mannose conjugate is present.

microscopy to quantitate the binding of lectins to the substrate. This technique is currently the most widely used method for interrogating arrays. We also utilized SPR spectroscopy to measure the real-time interaction between immobilized carbohydrates and lectins. SPR is probably the best-suited method for measuring low-affinity ligand-receptor interactions because it does not require specially labeled reagents and does not require that the slide be rinsed before imaging. SPR has not yet been widely used for characterizing arrays, but the recent introduction of two-dimensional SPR imagers is likely to make this an important technique in the next several years [48]. In previous work with analogous chips, we demonstrated that radiolabel assays are compatible with the monolayer substrates [24, 29]. Finally, MALDI-ToF mass spectrometry has the sensitivity required to characterize ligands, even at densities as low as 0.1% (1 pmol/cm^2) [49]. This technique has an advantage over all other techniques in that it can identify enzymatic activities that are unanticipated. Because MALDI provides a mass, it is not necessary to have an antibody or reagent that recognizes a desired product. This feature promises to significantly extend the utility of all biochips.

We believe that the carbohydrate chip has a special relevance to studies and applications in microbiology because the adhesion of many microorganisms is carbohydrate-dependent [23, 50, 51]. In the case of the gram-negative bacterium *E. coli*, virulent strains have been shown to adhere more tightly to mannosides than their nonvirulent counterparts [52, 53]. Other bacteria and viruses use similar carbohydrate-protein interactions to adhere to and infect mammalian cells. These carbohydrate chips could be used both to identify the carbohydrate epitopes that promote adhesion and to screen for small-molecule inhibitors of adhesion. These applications require strict control of the densities of carbohydrate ligands to allow comparison of the activities of different carbohydrates across an array. These

arrays will also be useful for studies of other carbohydrate-dependent adhesion mechanisms, such as those of the pathogenic yeast *C. albicans* [54] and those mediated by the hepatic asialoglycoprotein receptor [55]. Experiments to evaluate each of these opportunities are currently underway.

Significance

The development of gene chips has profoundly changed the pace and scope of biological research and in turn has made it clear that other classes of biochips—principally peptide and protein chips—would have substantial value in research and applications for screening. This paper describes the first example of a carbohydrate chip for studies in glycobiology. The approach we describe here, which relies on the Diels-Alder mediated immobilization of carbohydrates to a self-assembled monolayer presenting benzoquinone groups, has several characteristics that make it well suited for a range of studies of proteins and enzymes that interact with carbohydrates. First, the immobilized carbohydrates participate in biospecific interactions with proteins and enzymes. Second, the use of a Diels-Alder reaction for immobilizing ligands ensures that the carbohydrate densities are well controlled and constant across the entire array. Third, the structural order of the monolayer substrates ensures that the carbohydrates are presented in a uniform microenvironment and therefore have equal activity toward soluble proteins, making quantitative assays possible. Fourth, arrays can be prepared with complex carbohydrates that are generated in solid phase schemes, thereby extending significantly the complexity and diversity of arrays. Finally, the monolayers are compatible with robotic tools for preparing arrays and with a range of detection technologies for interrogating the arrays [29]. We believe that the chemical approach for preparing carbochips described above will have broad utility both for research programs in fundamental glycobiology and for applications in drug discovery and diagnostics.

Experimental Procedures

General

All reagents for solution phase chemical synthesis were purchased from Aldrich Chemical Company (Milwaukee, WI). THF was distilled from sodium/benzophenone, and dichloromethane was distilled from calcium hydride. Anhydrous DMF was purchased from Aldrich and used without further purification. Flash chromatography was carried out with EM Science Kiselgel 60 (230–400 mesh). Dulbecco's phosphate-buffered saline was purchased from Gibco Life Sciences (Gaithersburg, MD). Lectins were purchased from EY Laboratories (San Mateo, CA) or Sigma (St. Louis, MO) and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 MHz spectrometer with chemical shifts reported in parts per million relative to tetramethylsilane. Fluorescence microscopy was performed on an AffyMetrix ArrayScanner. 5-(benzyloxycarbonylamino)pentyl-2,3,4-tri-O-acetyl-β-D-galactopyranoside (**16b**) and 5-(benzyloxycarbonylamino)pentyl-3,4-di-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside (**16c**) were prepared as described in reference [56]. 3-(2-aminoethylthio)propyl-α-L-glucopyranoside (**19a**), 3-(2-aminoethylthio)propyl-α-L-galactopyranoside (**19b**), and 3-(2-aminoethylthio)propyl-α-L-2-acetamido-2-deoxy-glucopyranoside (**19c**) were prepared according to reference [36]. The hydroquinone- and penta(ethylene glycol)-terminated alkanethiols were prepared according to references [27] and [57], respectively.

Preparation of Substrates

Titanium (5 nm) and then gold (15 nm) were evaporated onto glass coverslips. For SPR measurements, a 50 nm film of gold was used. Self-assembled monolayers were prepared by immersion of the coverslips in a methanolic solution containing hydroquinone-terminated alkanethiol and a penta(ethylene glycol)-terminated alkanethiol (10 μM in hydroquinone, 1 mM in total thiol). After 8 hr the substrates were rinsed extensively with absolute ethanol and dried under a stream of nitrogen.

Monolayers were treated with a saturated aqueous solution of 1,4-benzoquinone for 5 min to oxidize the immobilized hydroquinone groups to the corresponding benzoquinone. The substrates were then washed with water and dried under a stream of nitrogen. To prepare arrays, 1 μl of each conjugate (2 mM in H_2O) was applied to specified regions of the monolayer. Substrates presenting single carbohydrates were prepared by inversion of the monolayer onto parafilm presenting an aqueous solution of the appropriate diene conjugate (2 mM in H_2O). The substrates were kept in a humidified chamber at 37°C for 2 hr, washed with water and methanol, and dried under a stream of nitrogen.

Determination of Lectin Specificity

Monolayers presenting the carbohydrate array were inverted onto a solution of a rhodamine-lectin conjugate (100 μl of a 200 μg/ml solution in DPBS) on parafilm. After 30 min the substrates were gently washed with DPBS (3 × 5 min) and analyzed with an array scanner. For reproducible results, it is important that the substrates not dry during the analysis. For inhibition experiments, a solution of rhodamine-labeled Con A (2 μM in DPBS) was mixed with a series of concentrations of inhibitor. A 1 μl volume of each mixture was applied to a single monolayer presenting α-glucose, α-mannose, or α-N-acetylglucosamine. After 1 hr at 37°C, the substrates were rinsed with DPBS (3 × 5 min) and analyzed as described above. Fluorescence intensity was determined with the manufacturer's software.

Surface Plasmon Resonance Spectroscopy

SPR measurements were performed with a BIACore 1000 instrument. Monolayer substrates were incorporated into the BIACore cassettes by removing of the manufacturer's substrate and gluing the chip into the cassettes with a two-part epoxy (Devcon). Measurements were performed with Dulbecco's phosphate-buffered saline (pH 7.4) as the running buffer and are reported as changes in resonance angle ($\Delta\theta$), where 1° = 10 000 RU. The bulk refractive index of all protein solutions was measured by injection of the solution over a monolayer presenting penta(ethylene glycol) groups alone.

Enzymatic Modification of Arrays

Two drops of a solution (40 μl) containing GalTase (25 mU), $MnCl_2$ (10 mM), and UDP-Gal (0.1 mM) in HEPES buffer (50 mM, pH 7.5) were placed onto a piece of parafilm. Carbochips presenting an array of ten monosaccharides were inverted onto the solutions and incubated for 6 hr at 37°C. The chips were then rinsed extensively with distilled water and dried under a stream of nitrogen. The modified substrates were treated with rhodamine-labeled lectins from *E. cristagalli* and *B. simplicifolia* (100 μg/ml in DPBS) for 30 min, rinsed gently with DPBS, and analyzed with the confocal array scanner.

Synthesis of Tetra(Ethylene Glycol) Monoester (12)

Ethyl diazoacetate (2.1 ml, 20 mmol) was added dropwise to a solution of tetra(ethylene glycol) (28 ml, 160 mmol) and BF_3•Et_2O (0.25 ml, 2 mmol) in dichloromethane (50 ml) at 0°C. After the addition was complete, the reaction was allowed to proceed for 15 min at room temperature before saturated NH_4Cl (20 ml) was added. The solution was placed in a separatory funnel, and the aqueous phase was extracted with dichloromethane (2 × 50 ml). The combined organic phases were concentrated in vacuo to afford a yellow oil. Silica gel chromatography (3:1 EtOAc:hexanes → EtOAc) provided **12** as a clear oil (2.89 g, 52%). ^{1}H NMR ($CDCl_3$) δ 4.16 (q, 2H, J = 7.14), 4.10 (s, 2H), 3.62–3.70 (m, 14H), 3.56 (t, 2H, J = 4.24), 2.78 (br

s, 1H), 1.23 (t, 3H, $J = 7.14$); ^{13}C NMR ($CDCl_3$) δ 170.35, 72.39, 70.72, 70.47, 70.40, 70.18, 68.54, 61.54, 60.68, 14.07.

Synthesis of O-Toluenesulfonyl-Tetra(Ethylene Glycol) Ethyl Ester (13)

p-toluenesulfonyl chloride (1 g, 5.4 mmol) and DMAP (25 mg, 0.2 mmol) were added to a solution of **13** (1.25 g, 4.5 mmol) in 1:1 pyridine:dichloromethane (10 ml). After 5 hr, the solution was poured onto ice-cold water (50 ml), and the aqueous phase was extracted with dichloromethane (3 × 40 ml). The combined organic phases were washed with NH_4Cl (20 ml) and brine (20 ml), dried over $MgSO_4$, and concentrated. The yellow oil was chromatographed on silica gel (3:1 EtOAc:hexanes) to afford **13** as a clear oil (1.53 g, 78%). ^1H NMR ($CDCl_3$) δ 7.73 (d, 2H, $J = 8.30$), 7.28 (d, 2H, $J = 8.30$), 4.14 (q, 2H, $J = 7.14$), 4.08 (s, 2H), 3.52–3.68 (m, 14H), 2.39 (s, 3H), 1.22 (t, 3H, $J = 7.14$); ^{13}C NMR ($CDCl_3$) δ 170.07, 144.49, 132.64, 129.51, 127.59, 70.48, 70.32, 70.23, 70.20, 70.18, 70.15, 69.00, 68.28, 60.38, 21.26, 13.87.

Synthesis of Cyclopentadienylethyl-Tri(Ethylene Glycol) Ethyl Ester (14)

Sodium cyclopentadienylide (1 ml, 2 M solution in THF) was added dropwise to a solution of **13** (0.8 g, 1.85 mmol) in THF (15 ml) at −78°C. The reaction mixture was stirred at −78°C for 30 min and at room temperature for an additional 4 hr. The solution was filtered through a pad of celite, and the salts were washed thoroughly with THF. Evaporation of the filtrate and subsequent silica gel chromatography (3:1 EtOAc:hexanes) provided **14** as a clear oil (328 mg, 54%). ^1H NMR ($CDCl_3$) δ 6.43–6.36 (m, 1H), 6.23–6.21 (m, 1H), 6.17–6.03 (m, 1H), 4.18 (q, 2H, $J = 7.14$), 4.11 (s, 2H), 3.71–3.54 (m, 14H), 2.89 (dd, 2H, $J = 1.4, 9.1$), 2.65 (m,2H), 1.25 (t, 3H, $J = 7.14$); ^{13}C NMR δ 170.31, 145.86, 143.42, 134.57, 133.55, 132.24, 130.91, 127.42, 127.17, 71.02, 70.72, 70.52, 70.46, 70.01, 69.98, 68.56, 60.63, 43.56, 41.23, 30.82, 30.10, 14.07.

Synthesis of Cyclopentadienylethyl-Tri(Ethylene Glycol) Acetic Acid (11)

Sodium hydroxide (1.8 ml, 1 N solution) was added to a solution of **14** (280 mg, 0.85 mmol) in methanol (10 ml) at 0°C. The solution was allowed to warm to room temperature and was stirred for 2 hr. Ethyl acetate (30 ml) was added, and the aqueous phase was acidified to a pH of 2 with 1 N HCl. The organic phase was collected, and the aqueous layer was extracted with two portions of ethyl acetate (10 ml). The organic layers were washed with water (10 ml) and brine (10 ml), dried with $MgSO_4$, and concentrated to afford **11** as a yellow oil (217 mg, 85%). ^1H NMR ($CDCl_3$) δ 6.43–6.36 (m, 1H), 6.23–6.21 (m, 1H), 6.17–6.03 (m, 1H), 4.10 (s, 2H), 3.70–3.56 (m, 14H), 2.86 (dd, 2H, $J = 1.4, 9.8$), 2.63 (m,2H); ^{13}C NMR ($CDCl_3$) δ 173.16, 145.51, 143.12, 134.42, 133.44, 132.10, 130.81, 127.38, 127.03, 70.86, 70.38, 70.28, 70.19, 70.16, 70.13, 69.83, 69.80, 68.19, 43,40, 41,10, 30.54, 29.84.

Synthesis of 5-(Benzyloxycarbonylamino)Pentanol (15)

Benzyl chloroformate (50 ml, 294 mmol) was added dropwise over 15 min to a solution of 5-amino-1-pentanol (20 g, 194 mmol) and $NaHCO_3$ (54 g, 640 mmol) in water (800 ml). After the addition was complete, the reaction was allowed to stir at room temperature for 6 hr. The reaction mixture was extracted with ethyl acetate (3 × 200), and the combined organic phases were washed with water (100 ml) and brine (100 ml). The organic layer was dried over $MgSO_4$ and concentrated to afford clear oil that was crystallized from ethyl acetate/petroleum ether to afford the title compound (39.7 g, 86%). ^1H NMR ($CDCl_3$) δ 7.29–7.21 (m, 5H), 5.58 (br s, 1H), 5.00 (s, 2H), 3.51–3.48 (m, 2H), 3.09–3.05 (m, 2H), 1.49–1.38 (m, 4H), 1.31–1.26 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 156.37, 136.23, 127.96, 127.51, 127.46, 65.94, 61.60, 40.43, 31.63, 29.09, 22.45.

Synthesis of 5-(Benzyloxycarbonylamino)Pentyl Glycosides

A solution of 5-(benzyloxycarbonylamino)pentanol **15** (2.84 g, 12 mmol) and $Hg(CN)_2$ (3.02 g, 12 mmol) in benzene-nitromethane (1:1 v/v, 75 ml) was stirred under argon for 30 min before a solution of per-*O*-acetylglycosyl bromide [35] (10 mmol) in benzene-nitromethane (25 ml) was added by syringe. The reaction was allowed to stir for 24 hr, filtered to remove solid material, and concentrated to an oil. The residue was dissolved in dichloromethane (150 ml) and filtered once again. The organic phase was washed with brine (3 × 50 ml) and dried over $MgSO_4$. The organic layer was concentrated to afford the crude glycoside as an oil. Purification was performed by silica gel chromatography with hexanes/ethyl acetate as the eluent.

5-(Benzyloxycarbonylamino)Pentyl-2,3,4,6-Tetra-O-Acetyl-β-D-Glucopyranoside (16a)

Yield, 2.59 g (65%); ^1H NMR ($CDCl_3$) δ 7.30–7.22 (m, 5H), 5.18–5.14 (m, 2H), 5.05–5.00 (m, 4H), 4.95–4.90 (m, 1H), 4.45 (d, 1H, $J = 7.9$), 4.21–4.18 (m, 1H), 4.10–4.05 (m, 1H), 3.88–3.81 (m, 1H), 3.68–3.61 (m, 1H), 3.48–3.41 (m, 1H), 3.11–3.05 (m, 1H), 2.02 (s, 3H), 1.97 (s, 6H), 1.59 (s, 3H), 1.53–1.43 (m, 4H), 1.31–1.25 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.29, 169.87, 169.08, 168.96, 156.14, 136.43, 128.13, 127.70, 127.67, 100.34, 72.46, 71.33, 70.96, 69.43, 68.07, 66.05, 61.56, 40.51, 29.16, 28.59, 22.65, 20.35, 20.26, 20.23.

5-(Benzyloxycarbonylamino)Pentyl-2,3,4-Tri-O-Acetyl-β-L-Fucopyranoside (16d)

Yield, 2.35 g (61%); ^1H NMR ($CDCl_3$) δ 7.36–7.31 (5H), 5.22 (d, 1H, $J = 3.37$), 5.19–5.15 (m, 1H), 5.09 (s, 2H), 5.02–4.99 (m, 1H), 4.40 (d, 1H, $J = 7.93$), 3.91–3.88 (m, 1H), 3.79–3.75 (m, 1H), 3.45–3.42 (m, 1H), 3.21–3.16 (m, 2H), 2.16 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.62–1.47 (m, 4H), 1.40–1.31 (m, 2H), 1.21 (d, 3H, $J = 6.43$); ^{13}C NMR ($CDCl_3$) δ 170.51, 170.02, 169.33, 156.22, 136.48, 128.27, 127.84, 127.80, 100.84, 71.12, 70.07, 69.45, 68.81, 66.26, 40.66, 29.36, 28.75, 22.83, 20.56, 20.48, 20.43, 15.84.

5-(Benzyloxycarbonylamino)Pentyl-2,3,4,6-Tetra-O-Acetyl-α-D-Mannopyranoside (16e)

Yield, 2.68 g (67%); ^1H NMR ($CDCl_3$) δ 7.30–7.22 (m, 5H), 5.30–5.17 (m, 3H), 5.05–5.03 (m, 2H), 4.25–4.21 (m, 1H), 4.06–4.03 (m, 1H), 3.94–3.91 (m, 1H), 3.64–3.61 (m, 1H), 3.40–3.38 (m, 1H), 3.15–3.12 (m, 2H), 2.09 (s, 3H), 2.04 (s, 6H), 1.99 (s, 3H), 1.94 (s, 3H), 1.58–1.47 (m, 4H), 1.36–1.22 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.43, 169.85, 169.69, 169.52, 156.24, 136.48, 128.24, 127.83, 127.80, 97.28, 69.42, 68.88, 68.18, 68.00, 66.26, 66.00, 62.33, 40.64, 29.47, 28.62, 23.12, 20.66, 20.50, 20.46.

5-(Benzyloxycarbonylamino)Pentyl-2,3,4-Tri-O-Acetyl-α-L-Rhamnopyranoside (16f)

Yield, 2.41 g (63%); ^1H NMR ($CDCl_3$) δ 7.31–7.27 (m, 5H), 5.26-5.00 (m, 8H), 4.67 (s, 1H), 3.84-3.75 (m, 1H), 3.64–358 (m, 1H), 3.43–3.36 (m, 1H), 3.23–3.08 (m, 2H), 2.09 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H), 1.59–1.48 (m, 4H), 1.38–1.32 (m, 2H), 1.18 (d, 3H, $J = 6.29$); ^{13}C NMR ($CDCl_3$) δ 170.21, 169.92, 169.77, 156.26, 136.48, 128.19, 127.78, 127.75, 97.07, 71.01, 70.83, 70.32, 69.66, 68.91, 68.69, 67.70, 66.20, 66.01, 65.71, 40.61, 29.44, 28.63, 23.08, 20.63, 20.51, 20.45, 17.16.

Synthesis of 3-(2-Aminoethylthio)propyl-α-L-Fucopyranoside (19d)

A solution of allyl-α-L-fucopyranoside (500 mg, 2.45 mmol) and cysteamine hydrochloride (284 mg, 2.5 mmol) in deoxygenated water (10 ml) was irradiated at 254 nm for 12 hr. The solution was lyophilized to afford a waxy solid that was used without further purification (324 mg, 49%). ^1H NMR (400 MHz, D_2O) δ 4.75 (d, 1H, $J = 3.9$), 3.97–3.94 (m, 1H), 3.75–3.63 (m, 6H), 3.47–3.44 (m, 1H), 3.14–3.10 (t, 2H, $J = 6.7$), 2.78–2.74 (t, 2H, $J = 6.8$), 2.61–2.57 (t, 2H, $J = 7.1$), 1.83–1.80 (m, 2H), 1.10 (d, 3H, $J = 6.6$); ^{13}C NMR (D_2O) δ 99.50, 73.00, 70.77, 69.20, 67.78, 67.54, 39.62, 29.63, 29.29, 28.68, 16.74.

Preparation of 5-Aminopentyl Glycosides (17a–f)

To a solution of catalytic sodium methoxide in methanol (10 ml) was added a 5-(benzyloxycarbonylamino)pentyl glycoside (**16a–f**, 1 mmol). After the reaction was complete by TLC (4–12 hr), Rexyn 101 (H^+ form, prewashed with methanol) was added, and the solution was stirred for 15 min. The resin was removed by filtration and washed with methanol (5 ml). The combined filtrates were treated with Pd (50 mg, 10% on activated charcoal, DeGussa type) and hydrogenated for 3 hr at room temperature. The reaction mixture was filtered through a pad of celite, concentrated under reduced pressure, and coevaporated twice with dichloromethane to afford aminopentyl glycosides **17a–f** as white foams. The products were used directly without further purification.

Synthesis of Carbohydrate-Cyclopentadiene Conjugates (1–10)

A solution of compound **11** (150 mg, 0.5 mmol) in anhydrous DMF was cooled to 0°C under an atmosphere of argon. Isobutyl chloroformate (75 μl, 0.6 mmol) and tributylamine (250 μl, 1 mmol) were added, and the solution was stirred for 15 min at 0°C. An aminoglycoside conjugate (0.6 mmol) in DMF (3 ml) and then an additional portion of tributylamine (125 μl) were added. The reaction was allowed to proceed at room temperature for 8 hr, and the solvent was removed in vacuo at room temperature to afford a yellow oil. Silica gel chromatography of the residue via gradient elution (9:1 → 4:1 CH_2Cl_2/MeOH) provided the cyclopentadiene conjugates **1–10** as amorphous solids.

α-D-Glucopyranoside Conjugate (1)

Yield, 189 mg (69%); ^{1}H NMR (CD_3OD) δ 6.41 (br s, 1H), 6.34–6.32 (m, 1H), 6.19–6.14 (br s, 1H), 4.71 (d, 1H, J = 3.7), 3.93 (s, 2H), 3.79–3.72 (m, 2H), 3.63–3.43 (m, 24H), 3.39–3.31, (m, 2H), 3.26–3.21 (t, 2H, J = 9.86), 2.88–2.85 (m, 2H), 2.64–2.55 (m, 6H), 1.87–1.81 (m, 2H); ^{13}C NMR (CD_3OD) δ 173.52, 172.73, 147.23, 144.88, 135.66, 134.54, 133.26, 131.91, 128.27, 98.51, 73.78, 72.64, 72.25, 72.13, 71.92, 71.64, 71.456, 71.31, 71.16, 71.11, 67.16, 62.63, 44.45, 42.11, 39.50, 31.84, 31.17, 30.47, 29.08, 22.68.

β-D-Glucopyranoside Conjugate (2)

Yield, 176 mg (64%); ^{1}H NMR (CD_3OD) δ 6.41 (br s, 1H), 6.34–6.31 (m, 1H), 6.19–6.14 (br s, 1H), 4.18 (d, 1H, J = 7.78), 3.91 (s, 2H), 3.87–3.79 (m, 2H), 3.61–3.56 (m, 24H), 3.29–3.11 (m, 6H), 2.88–2.86 (m, 2H), 2.64–2.55 (m, 2H), 1.66–1.37 (m, 4H), 1.44–1.31 (m, 2H); ^{13}C NMR (CD_3OD) δ 172.58, 172.51, 153.11, 147.52, 135.64, 133.24, 131.89, 128.61, 128.26, 104, 27, 78.00, 77.91, 77.80, 75.01, 72.12, 71.85, 71.64, 71.54, 71.45, 71.27, 71.14, 71.10, 70.47, 62.70, 44.43, 42.09, 39.93, 39.80, 31.84, 31.15, 30.30, 30.21, 24.34.

α-D-Galactopyranoside Conjugate (3)

Yield, 192 mg (70%); ^{1}H NMR (CD_3OD) δ 6.41–6.39 (br s, 1H), 6.32–6.30 (m, 1H), 6.18–6.12 (br s, 1H), 4.72 (d, 1H, J = 3.19), 3.91 (s, 2H), 3.76–3.54 (m, 26H), 3.35–3.32, (m, 2H), 2.85–2.83 (m, 2H), 2.62–2.57 (m, 6H), 1.84–1.78 (m, 2H); ^{13}C NMR (CD_3OD) δ 172.71, 147.19, 144.86, 135.60, 134.49, 133.21, 131.86, 128.59, 128.24, 100.36, 72.46, 72.31, 72.11, 71.87, 71.62, 71.41, 71.26, 71.11, 71.05, 70.95, 19, 67.48, 62.63, 44.40, 42.06, 39.55, 39.50, 31.87, 31.80, 31.11, 30.57, 29.21.

β-D-Galactopyranoside Conjugate (4)

Yield 197 mg (72%); ^{1}H NMR (CD_3OD) δ 6.43–6.41 (br s, 1H), 6.34–6.31 (m, 1H), 6.20–6.15 (br s, 1H), 4.15 (d, 1H, J = 7.24), 3.91 (s, 2H), 3.87–3.79 (m, 2H), 3.61–3.56 (m, 24H), 3.44–3.38 (m, 2H), 3.29 (s, 2H), 3.21–3.17 (m, 2H), 2.88–2.86 (m, 2H), 2.64–2.55 (m, 2H), 1.61–1.46 (m, 4H), 1.40–1.34 (m, 2H); ^{13}C NMR (CD_3OD) δ 172.56, 172.49, 147.23, 144.86, 135.64, 133.24, 131.89, 128.60, 128.26, 104.88, 76.47, 74.92, 72.46, 72.12, 71.84, 71.64, 71.42, 71.26, 71.13, 71.08, 70.44, 70.16, 62.38, 44.43, 42.09, 39.93, 39.81, 31.82, 31.14, 30.35, 30.22, 24.36.

α-L-Fucopyranoside Conjugate (5)

Yield, 171 mg (64%); ^{1}H NMR (CD_3OD) δ 6.49–6.47 (br s, 1H), 6.32–6.30 (m, 1H), 6.18–6.12 (br s, 1H), 4.75 (d, 1H, J = 2.31), 4.00 (s, 2H), 3.97–3.94 (m, 1H), 3.82–3.62 (m, 22H), 3.53–3.42 (m, 3H), 3.15–3.11 (m, 1H), 2.94–2.92 (m, 2H), 2.70-2.62 (m, 6H), 1.93–1.86 (m, 2H), 1.73–1.69 (m, 1H), 1.46–1.41 (m, 1H), 1.22 (d, 3H, J = 6.56); ^{13}C NMR (CD_3OD) δ 172.66, 172.34, 147.16, 144.78, 135.74, 134.60, 133.34, 131.97, 128.66, 128.32, 100.44, 73.51, 72.15, 71.98, 71.67, 71.62, 71.51, 71.33, 71.21, 71.16, 69.96, 67.51, 67.36, 53.88, 44.54, 42.19, 39.58, 31.93, 31.24, 30.57, 29.27, 27.79, 20.94, 16.85, 14.09.

β-L-Fucopyranoside Conjugate (6)

Yield 161 mg (61%); ^{1}H NMR (CD_3OD) δ 6.42–6.40 (br s, 1H), 6.34–6.32 (m, 1H), 6.20–6.15 (br s, 1H), 4.11 (d, 1H, J = 7.54), 3.92 (s, 2H), 3.82–3.76 (s, 1H), 3.62–3.55 (m, 22H), 3.49–3.42 (m, 1H), 3.42–3.40 (m, 2H), 3.22–3.16 (m, 2H), 2.88–2.86 (m, 2H), 2.64–2.56 (m, 2H), 1.62–1.47 (m, 4H), 1.41–1.34(m, 2H), 1.21 (d, 3H, J = 6.44); ^{13}C NMR (CD_3OD) δ 172.56, 172.34, 147.24, 144.89, 135.66, 134.52, 133.24, 131.89.128.61, 128.26, 104.74, 75.09, 72.94, 72.23, 72.13, 53.91, 44.44, 42.09, 41.28, 39.95, 31.86, 31.18, 30.39, 30.26, 26.78, 24.39, 20.85, 16.81.

α-D-2-Deoxy-2-Acetamido-Glucopyranoside Conjugate (7)

Yield, 184 mg (62%); ^{1}H NMR (CD_3OD) δ 6.41 (br s, 1H), 6.34–6.32 (m, 1H), 6.19–6.14 (br s, 1H), 4.73 (d, 1H, J = 3.54), 3.93 (s, 2H), 3..83–3.71 (m, 3H), 3.65–3.50 (m, 22H), 3.44–3.36, (m, 3H), 3.32–3.27 (m, 1H), 2.88–2.85 (m, 2H), 2.64–2.55 (m, 6H), 1.93 (s, 3H), 1.82–1.79 (m, 2H); ^{13}C NMR (CD_3OD) δ 173.52, 172.73, 135.66, 134.54, 133.226, 131.91, 128.27, 98.51, 73.77, 72.65, 72.24, 72.13, 71.92, 71.64, 71.46, 71.31, 71.16, 71.11, 67.16, 62.63, 44.44, 42.11, 39.50, 31.84, 31.17, 30.47, 29.08, 22.68.

β-D-2-Deoxy-2-Acetamido-Glucopyranoside Conjugate (8)

Yield, 175 mg (59%); ^{1}H NMR (CD_3OD) δ 6.43–6.41 (br s, 1H), 6.34–6.31 (m, 1H), 6.19–6.14 (br s, 1H), 4.34 (d, 1H, J = 8.38), 3.92 (s, 2H), 3.86–3.80 (m, 2H), 3.65–3.54 (m, 24H), 3.44–3.38 (m, 2H), 3.29–3.16 (m, 6H), 2.88–2.86 (m, 2H), 2.64–2.56 (m, 2H), 1.93 (s, 3H), 1.53–1.44 (m, 4H), 1.36–1.30 (m, 2H); ^{13}C NMR (CD_3OD) δ 173.53, 172.45, 147.24, 144.88, 135.65, 134.54, 133.25, 131.91, 128.62, 128.27, 102.61, 77.87, 76.03, 72.13, 72.05, 71.84, 71.65, 71.41, 71.26, 71.14, 71.09, 70.24, 62.74, 57.30, 44.44, 42.11, 39.84, 31.84, 31.16, 30.19, 30.16, 24.37, 23.09.

α-D-Mannopyranoside Conjugate (9)

Yield, 152 mg (55%); ^{1}H NMR (CD_3OD) δ 6.42–6.40 (br s, 1H), 6.34–6.32 (m, 1H), 6.20–6.15 (br s, 1H), 4.67 (d, 1H, J = 1.42), 3.91 (s, 2H), 3.78–3.51 (m, 24H), 3.47–3.42 (m, 1H), 3.38–3.33 (m, 1H), 3.21–3.16 (t, 2H, J = 7), 2.88–2.86 (m, 2H), 2.64–2.55 (m, 2H), 1.57–1.46 (m, 4H), 1.39–1.33 (m, 2H); ^{13}C NMR (CD_3OD) δ 172.55, 172.48, 147.23, 144.89, 135.65, 134.52, 133.24, 131.89.128.62, 128.26, 101.44, 74.56, 72.60, 72.16, 72.13, 71.88, 71.65, 71.47, 71.44, 71.28, 71.16, 71.10, 68.56, 68.24, 62.88, 44.443, 42.09, 39.79, 31.85, 31.16, 30.27, 30.18, 24.65.

α-L-Rhamnopyranoside Conjugate (10)

Yield, 159 mg (60%); ^{1}H NMR (CD_3OD) δ 6.42–6.40 (br s, 1H), 6.34–6.32 (m, 1H), 6.20–6.15 (br s, 1H), 4.60 (s, 1H), 3.91 (s, 2H), 3.72–3.70 (s, 1H), 3.65–3.47 (m, 22H), 3.38–3.33 (m, 1H), 3.29–3.24 (m, 2H), 3.21–3.16 (m, 2H), 2.88–2.86 (m, 2H), 2.64–2.55 (m, 2H), 1.57–1.46 (m, 4H), 1.37–1.32 (m, 2H), 1.19 (d, 3H, J = 6.21); ^{13}C NMR (CD_3OD) δ 172.52, 172.39, 147.21, 144.88, 135.64, 134.50, 133.22, 131.88, 128.60, 128.24, 101.52, 73.91, 72.38, 72.23, 72.11, 71.87, 71.63, 71.44, 71.28, 71.16, 71.09, 69.67, 68.23, 44.41, 42.06, 39.77, 31.84, 31.16, 30.23, 24.65, 18.03.

Synthesis of Cyclopentadienylethyl-Tri(Ethylene Glycol) (20)

To a solution of tetra(ethylene glycol) (20 ml, 116 mmol) and pyridine (20 ml) in dichloromethane (50 ml) was added *p*-toluenesulfonyl chloride (2 g, 10.4 mmol). The reaction mixture was stirred for 4 hr and diluted with dichloromethane (100 ml). The solution was washed with 5% HCl (2 × 25 ml), H_2O (25 ml), and brine (25 ml). The organic phase was dried over sodium sulfate and concentrated in vacuo. Silica gel chromatography (5:1 ethyl acetate:hexane) provided the product as a clear oil (2.4 g, 66%). ^{1}H NMR (400 M, $CDCl_3$) δ 7.75 (d, 2H, J = 8.3), 7.30 (d, 2H, J = 8.3), 4.11 (t, 2H, J = 6.5), 3.66–3.55 (m, 14H), 2.65 (br s, 1H), 2.4 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 144.68, 132.75, 129.67, 127.77, 72.31, 70.51, 70.44, 70.26, 70.12, 69.13, 68.48, 61.47, 21.46.

To a solution of a compound of mono-toluenesulfonyl tetra(ethylene glycol) (1.3 g, 3.6 mmol) in THF (25 ml) at 0°C was added sodium cyclopentadienylide (1.9 ml, 2.0 M solution in THF). The reaction mixture was stirred at −78°C for 30 min and at room temperature for an additional 4 hr. The solution was filtered through a pad of celite, and the salts were extensively washed with THF. Evaporation of the filtrate and subsequent silica gel chromatography (5:1 ethyl acetate:hexane) provided the title compound as a clear oil (270 mg, 31%). ^{1}H NMR (400 M, $CDCl_3$) δ 6.42–6.37 (m, 1H), 6.24–6.17 (m, 1H), 6.03 (m, 1H), 3.70–3.53 (m, 14H), 2.91–2.83 (m, 3H), 2.70–2.61 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 145.78, 143.37, 134.56, 133.45, 132.24, 130.90, 127.44, 127.19, 72.38, 71.04, 70.55, 70.47, 70.42, 70.20, 69.95, 61.53, 43.54, 41.22, 30.76, 30.05.

Synthesis of Cyclopentadienylethyl-Tri(Ethylene Glycol) Hydrazide (21)

To a solution of ester **14** (200 mg, 0.67 mmol) in dry methanol (1 ml) was added anhydrous hydrazine (1 ml). The solution was stirred for 30 min at room temperature and concentrated in vacuo. The resulting oil was chromatographed on silica gel (5% MeOH/CH_2Cl_2) to afford hydrazide **35** as a clear oil (165 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 6.43–6.36 (m, 1H), 6.23–6.21 (m, 1H), 6.17–6.03 (m, 1H), 4.04 (s, 2H), 3.70–3.56 (m, 14H), 2.86 (dd, 2H, J = 1.4, 9.8), 2.63 (m,2H); ^{13}C NMR ($CDCl_3$) δ 160.54, 145.84, 143.42, 134.60, 133.67, 132.32, 130.99, 127.53, 127.29, 71.14, 71.07, 70.55, 70.44, 70.07, 43.62, 41.31, 30.83, 30.12.

Synthesis of Mannose-Hydrazide Conjugate (*22*)

To a solution of 2-oxoethylmannose [42] (100 mg, 0.45 mmol) in DMSO (1 ml) was added hydrazide **21** (125 mg, 0.4 mmol). After 5 min, $NaBH_3CN$ (28 mg, 0.45 mmol) was added and the reaction mixture was stirred for an additional two hours. The solution was concentrated *in vacuo* and chromatographed on silica gel using 20% MeOH/CH_2Cl_2 as the eluent to afford the title compound as a clear oil (189 mg, 81%). ^1H NMR (CD_3OD) δ 6.37–6.35 (m, 1H), 6.29–6.28 (m, 1H), 6.20–6.15 (m, 1H), 5.97 (m, 1H), 4.73 (s, 1H), 4.26–4.22 (m, 1H), 4.15–4.10 (m, 1H), 4.02 (s, 2H), 3.75–3.73 (m, 3H), 3.62–3.42 (m, 24H), 2.82–2.80 (m, 2H), 2.58–2.50 (m, 2H); ^{13}C NMR (CD_3OD) δ 166.35, 147.51, 144.21, 142.43, 132.60, 131.50, 130.20, 128.87, 125.58, 125.24, 98.58, 71.95, 69.37, 68.90, 68.85, 68.60, 68.39, 68.29, 68.21, 68.10, 68.05, 67.61, 65.38, 64.44, 59.80, 41.37, 39.04, 28.78, 28.09.

Acknowledgments

This work was funded by DARPA (N00173-01-1-G010). We thank Steven J. Kron for use of the Affymetrix ArrayScanner. B.T.H. is supported by MD/PhD Training Grant HD-09007.

Received: December 6, 2001
Accepted: January 17, 2002

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Note Added in Proof

An article describing a carbohydrate array appeared while our article was in press (Wang, D., et al., Nat. Biotechnol. *20*, 275).