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# Carbohydrate Coatings via Aryldiazonium Chemistry for Surface Biomimicry

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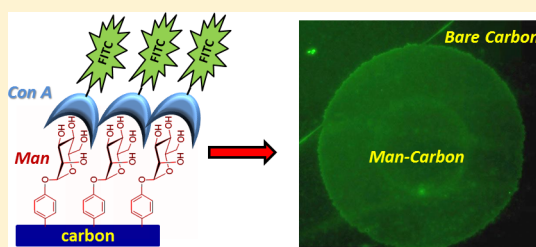
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## S Supporting Information

**ABSTRACT:** Carbohydrates are extremely important biomolecules and their immobilization onto solid surfaces is of interest for the development of new biomimetic materials and of new methods for understanding processes in glycobiology. We have developed an efficient surface modification methodology for the functionalization of a range of materials with biologically active carbohydrates based on aryldiazonium chemistry. We describe the synthesis and characterization of carbohydrate reagents, which were subsequently employed for the one-step, solution-based modification of carbon, metals, and alloys with monosaccharides. We used a combination of spectroscopic and nanogravimetric methods to characterize the structure of the carbohydrate layers; we report an average surface coverage of  $7.8 \times 10^{-10}$  mol cm<sup>-2</sup> under our experimental conditions. Concanavalin A, a mannose-binding lectin, and Peanut Agglutinin, a galactose-binding lectin, were found to bind from solution to their respective monosaccharide binding partners immobilized at the surface. This result suggests that the spontaneous chemisorption of aryldiazonium monosaccharide precursors leads to the formation of monosaccharide layers that retain the biological recognition specificity of the parent carbohydrate molecule. Finally, we carried out measurements using fluorescently labeled Bovine Serum Albumin (BSA) and found that these carbohydrate coatings reduce unspecific adsorption of this protein at carbon surfaces. These results suggest that aryldiazonium-derived carbohydrate coatings may offer a promising strategy for preventing undesirable protein accumulation onto surfaces.

**KEYWORDS:** carbohydrate, saccharide, diazonium, carbon, coatings



## 1. INTRODUCTION

Carbohydrates are extremely important biomolecules that are involved in a diverse array of biological functions including fertilization, cell–cell communication, and inflammatory responses.<sup>1–4</sup> The surfaces of eukaryotic cells and proteins are often heavily glycosylated, and this ‘glycocalyx’ modulates the early stages of many biological processes and interactions, usually through binding interactions between carbohydrates and proteins.<sup>5,6</sup> Glycosylated surfaces have emerged as extremely important materials and as tools for tailoring the response of organisms to solid surfaces, for improving our understanding of carbohydrate–protein interactions and for studying fundamental processes in glycobiology. Therefore, there has been great interest in developing new strategies for the preparation of functional glycosylated surfaces. Glycosylation alters the surface characteristics and biology of materials and has allowed for the development of new materials that can mimic the glycosylation pattern of cells.<sup>7</sup> These materials have many important applications in medical devices, diagnostics, and sensors. Surface glycosylation reactions have found applications in the fabrication of carbohydrate microarrays, which have been used to screen for carbohydrate binding

proteins and have identified high affinity ligands for lectin binding, antigenic carbohydrate structures, and compounds that inhibit carbohydrate–protein interactions.<sup>8–13</sup>

Due to the emergence of glycosylated materials as an important class of biomaterial, there has been significant focus on the development of new methodologies for the functionalization of surfaces and materials.<sup>11,12,14</sup> Sophisticated ligation methodologies and patterning techniques have been employed for the preparation of glycosylated surfaces.<sup>15</sup> The three most commonly employed methodologies for direct immobilization of carbohydrates on metal surfaces are thiol chemistry,<sup>16</sup> amide bond formation, and copper catalyzed [3 + 2] cycloaddition reactions.<sup>11,12,14</sup> Thiol chemistry on gold and silver surfaces is well established as a methodology for preparing carbohydrate-bearing monolayers; however, the bond that anchors the alkylthiol to the surface is labile and can easily be broken. Both the amide bond formation and copper catalyzed ‘click’ reaction necessitate prefunctionalization of the surface material with a

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'chemical handle' suitable for the ligation reaction with the glycan. With the aim of developing an efficient, one-step procedure for the direct glycosylation of untreated surfaces, we turned our attention toward diazonium grafting chemistry as an alternative to traditional carbohydrate immobilization methodologies. Diazonium chemistry has been widely employed for the functionalization of carbon with various aromatic groups.<sup>17,18</sup> The methodology is advantageous in that it forms strong stable covalent bonds with carbon surfaces and can also be used to directly functionalize metals such as copper, gold and alloys.<sup>19–23</sup> The methodology can be carried out in the presence of deprotected carbohydrates without any degradation of the glycan structure, and the grafting methodology can be carried out in aqueous conditions which are ideal for solubilizing the hydrophilic sugar groups. The diazotization conditions are extremely mild, and diazonium chemistry has successfully been employed for the functionalization of proteins specifically at tyrosine.<sup>24</sup> These reactions can be carried out without any denaturing of the protein structure. However, to the best of our knowledge, the use of diazonium chemistry has not previously been employed for the preparation of glycosylated surfaces. A one-pot, general grafting methodology for carbohydrates onto surfaces offers exciting potential for the preparation of a range of glycosylated materials with applications in glycobiology. Herein we describe the results of our studies into the development of aryl-diazonium grafting of carbohydrates onto a range of materials including metals and alloys.

## 2. EXPERIMENTAL SECTION

**Chemicals and Materials.** Sulfuric acid ( $\text{H}_2\text{SO}_4$ , concentrated), hydrogen peroxide ( $\text{H}_2\text{O}_2$ , 30%), phosphate buffered saline buffer (PBS, pH 7.4, 0.01 M), acetone (HPLC), methanol ( $\text{Me}-\text{OH}$ , semiconductor grade), sodium nitrite ( $\text{NaNO}_2$ ), fluoroboric acid ( $\text{HBF}_4$ ), nitric acid ( $\text{HNO}_3$ ), and acetonitrile (CAN, HPLC grade) were purchased from Sigma Aldrich and were used without further purification. Lectins from *Arachis hypogaea* (peanut agglutinin, PNA), FITC conjugate, and Concanavalin A from *Canavalia ensiformis* (Jack bean, ConA), FITC conjugate, Type IV, were purchased as lyophilized powder from Sigma Aldrich. Bovine Serum Albumin–fluorescein isothiocyanate conjugate was also purchased from Sigma Aldrich.

Deionized water was used for all aqueous solutions. All glassware, quartz crystals, and cells were cleaned with piranha solution before use (3:1,  $\text{H}_2\text{SO}_4$  to  $\text{H}_2\text{O}_2$ ; WARNING: Piranha solution should be handled with caution; it is a strong oxidant and reacts violently with organic materials. It also presents an explosion danger. All work should be performed under a fume hood).

### Synthesis of the Diazonium Tetrafluoroborate Derivative.

The respective diazonium salts were freshly prepared on treatment of the relevant 4-aminophenyl glycoside with a solution of sodium nitrite and fluoroboric acid (Scheme 1). The 4-aminophenyl glycosides (compounds 1–8) were dissolved in 20.0 mL of 1.25 mM  $\text{HBF}_4$  and kept in a beaker of ice. 5.0 mL of 5.0 mM  $\text{NaNO}_2$  that was also kept in

ice was then added dropwise over a period of 10 min to the precursor solution in order to form the diazonium salt at a final concentration of 1.0 mM. Functionalization of carbon substrates with precursor 7 required the use of a small amount of acetonitrile in the aqueous solution (approximately 1:10 v/v) due to the limited solubility of peracetylated rhamnose in water. Surface modification reactions were carried out by immersing the substrate into thus prepared solutions of diazoniated precursors for 60 min, at concentrations varying in the range 0.010–1.0 mM. Surface deprotection of peracetylated carbohydrates chemisorbed at surfaces was carried out by immersing samples in a freshly made sodium methoxide solution ( $\text{Na}^0$  and  $\text{CH}_3\text{OH}$ ) for 60 min followed by neutralization with dilute HCl.

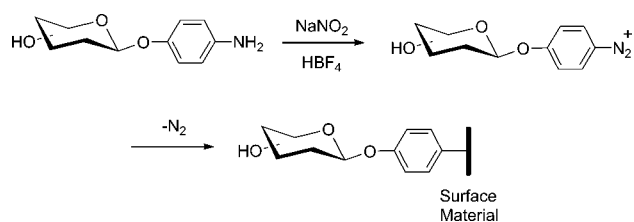
**Substrate Preparation.** Amorphous carbon (a-C) substrates were prepared by magnetron sputtering of Ti followed by a-C as previously described;<sup>25</sup> samples were stored under Ar and used as deposited in functionalization reactions. Metal and alloy substrates were cleaned prior to in situ surface functionalization with aryl diazonium salts. Copper foil (pipe grade) was cleaned in 0.1 M  $\text{HNO}_3$ , rinsed with copious amount of deionized water, and dried in Ar. Brass foil substrates (machining grade) were polished with 0.3  $\mu\text{m}$  alumina slurry, sonicated in methanol three times, and dried in Ar. Both Cu and brass substrates were used immediately after cleaning. Gold substrates were prepared via electroplating using a commercial electroplating solution (Spa Plating Ltd.) and stainless steel foil electrodes; after Au deposition, substrates were rinsed with deionized water and dried under Ar.

**Characterization Methods.** Infrared Reflectance Absorption spectroscopy (IRRAS) was carried out on Ti/a-C samples and on Au, Cu, and brass samples using a Fourier Transform Infrared (FTIR) Spectrometer (Tensor 27, Bruker) equipped with a Mercury Cadmium Telluride (MCT) detector, a specular reflectance accessory (VeeMax II), and a ZnSe polarizer. Spectra were taken at 80° incidence using p-polarized light; 256 spectra were collected at 4  $\text{cm}^{-1}$  resolution using a bare substrate as a background sample; IRRAS data was obtained in triplicates as a minimum.

Quartz crystals with 10 MHz resonant frequency were used for Quartz Crystal Microbalance (QCM) measurements crystals used in this study; crystals were coated with 100 nm thick vapor deposited gold electrodes (International Crystal Manufacturing). Carbon coated crystals were obtained via DC-magnetron sputtering of Au electrodes with 10 nm thick Ti adhesive layer followed by a 50 nm a-C film, as previously described.<sup>25</sup> The QCM setup has been previously described; briefly, it consists of a static Teflon reaction cell, a lever oscillator, and a frequency counter connected to a computer for data recording.<sup>23</sup> The crystal was clamped in the static cell with O-rings on both sides resulting in only one face, with a geometric area of 0.205  $\text{cm}^2$ , being immersed in the liquid. The frequency of a dry a-C coated crystal, stable over a period of 60 min at 20 °C before insertion into the Teflon cell, is taken as reference frequency. Surface functionalization and rinsing of QCM crystals was carried out following the same procedure as that of substrates for spectroscopic analysis.

**Biomolecule-Layer Interactions.** Lectin binding studies were carried out on a-C surfaces coated using precursors 4 and 6, two precursors bearing galactose and mannose units, respectively. FITC labeled Concanavalin A (FITC-Con A), a plant lectin that is known to bind  $\alpha$ -D-mannoside structures and FITC labeled Peanut Agglutinin (FITC-PNA), a plant lectin that is known to bind to galactoside units were used in this study. Mannose-coated surfaces were incubated for 2 h in a 0.5 mg/mL solution of FITC-ConA in 0.010 M PBS buffer at pH 7.4 with added 0.1 mM  $\text{CaCl}_2$  and  $\text{MgCl}_2$ .<sup>26,27</sup> Galactose-coated surfaces were incubated for 2 h in a 0.5 mg/mL solution of FITC-PNA in the same buffer solutions. Surfaces were rinsed with PBS buffer in order to remove excess unbound protein prior to imaging. Protein adsorption studies were carried on a-C surfaces modified using precursor 4; the galactose-coated carbon surface was incubated for 2 h with 200  $\mu\text{L}$  of a solution of Bovine Serum Albumin fluorescein-conjugate (FITC-BSA), 0.2 mg/mL in PBS at pH 7.4.<sup>28–30</sup> The surface was washed with the PBS solution to remove the unbound protein prior to imaging.

**Scheme 1. Formation of Glycosyl Diazonium Intermediate and Subsequent Net Chemisorption Reaction at Surfaces**



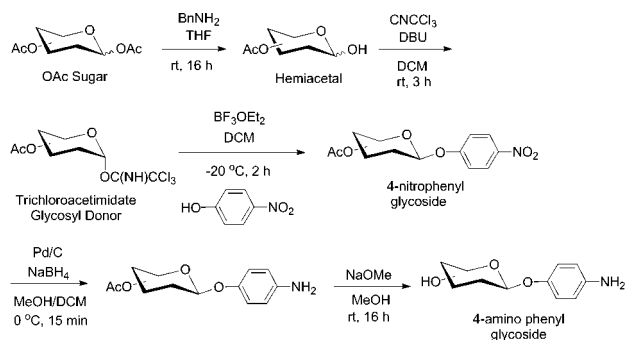
Fluorescence images were acquired with an Olympus BX51 inverted fluorescent microscope using a cellSense digital image processing software; no digital filters were used, only brightness and contrast were adjusted in all images presented. The images were acquired with a cube filter set having an excitation filter at 470–495 nm, a dichroic filter at 505 nm, and a barrier filter at 510–550 nm. Analysis of emission intensity was carried out using image analysis software (Gwyddion) from 1600 × 1200 px images, each covering a sample area of approximately 4 mm<sup>2</sup>. Intensity values were calculated by defining a mask (314 × 263 px) in carbohydrate-modified and unmodified regions of the carbon surface. The mean emission intensities were ratioed in order to compare the amount of adsorbed fluorophore on both types of sample regions; standard deviations were calculated over three samples.

### 3. RESULTS AND DISCUSSION

**3.1. Carbohydrate Synthesis and Surface Modification.** Diazonium grafting has emerged as a highly efficient and general strategy for the functionalization of carbon materials but has not previously been applied to the modification of surfaces with carbohydrates. The reactive diazonium species were generated *in situ* on treatment of 4-aminophenyl glycosides with a solution of sodium nitrite and fluoroboric acid (Scheme 1). The reactive diazonium species reductively adds to carbon, as well as metallic surfaces, through robust covalent bonds as shown in previous work.<sup>18,22,25,31</sup>

A series of both protected and fully unprotected 4-aminophenyl glycosides were prepared through the general synthetic pathway outlined in Scheme 2. In the first step the

**Scheme 2. General Synthetic Strategy for the Preparation of 4-Aminophenyl Glycosides**

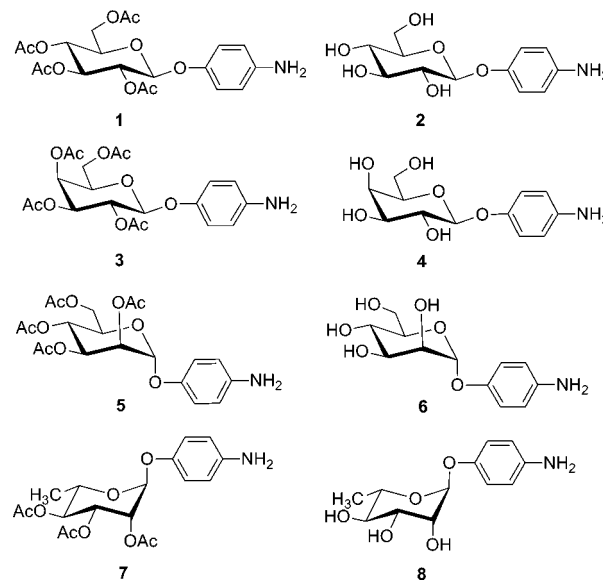


commercially available peracetylated sugar was treated with a stoichiometric quantity of benzylamine to selectively hydrolyze the anomeric acetate and release the free hemiacetal. The hemiacetal was treated with an excess of trichloroacetonitrile in the presence of 1,8-diazabicycloundec-7-ene (DBU), to furnish the trichloroacetimidate donor. Glycosylation reactions with 4-nitrophenol were carried out in the presence of a Lewis acid (BF<sub>3</sub>OEt<sub>2</sub>), to furnish the 4-nitrophenyl glycoside.

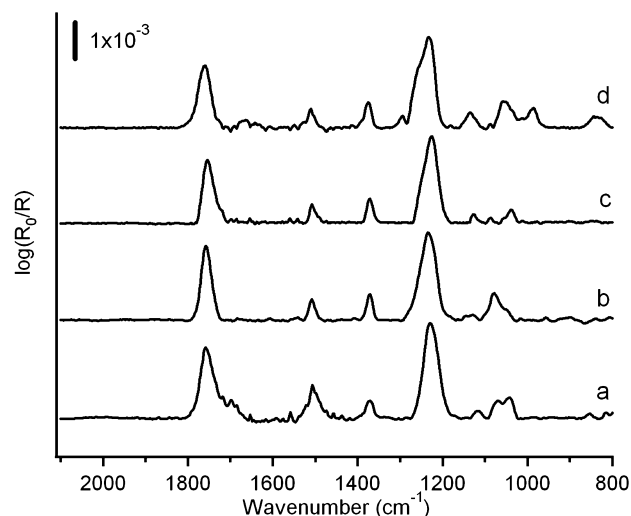
Stereochemistry of the glycoside bond was defined by neighboring group participation of the acetyl group at the 2-OH position. For the <sup>4</sup>C<sub>1</sub> sugars, glucose (Glc), and galactose (Gal), only the beta anomers were formed, for mannose (Man) the expected alpha anomer was formed exclusively. The <sup>1</sup>C<sub>4</sub> sugar, rhamnose (Rha), furnished the alpha anomer. The nitro compounds were treated under reducing conditions with sodium borohydride in the presence of palladium on charcoal to furnish the desired 4-aminophenyl glycoside in good yields. Subsequent treatment of the protected amines under zémpfen conditions furnished the fully deprotected 4-aminophenyl

glycoside in quantitative yields. Scheme 3 shows the 4-aminophenyl glycosides prepared using the general strategy.

**Scheme 3. Protected and Unprotected 4-Aminophenyl Glycosides Synthesized: Peracetylated 4-Aminophenyl Glucoside (1), Galactoside (3), Mannoside (5), and Rhamnoside (7) and the Corresponding Unprotected 4-Aminophenyl Glucoside (2), Galactoside (4), Mannoside (6), and Rhamnoside (8)**



Following purification and characterization of the 4-aminophenyl glycosides (see the Supporting Information for details) the compounds were used in the surface functionalization reactions. Infrared Reflectance Absorption Spectroscopy (IRRAS) was used in order to confirm that surface functionalization takes place after immersion of the substrates in aqueous solutions of the *in situ* generated aryldiazonium salts. Figure 1 shows IRRAS spectra obtained after surface modification of amorphous carbon coatings using the



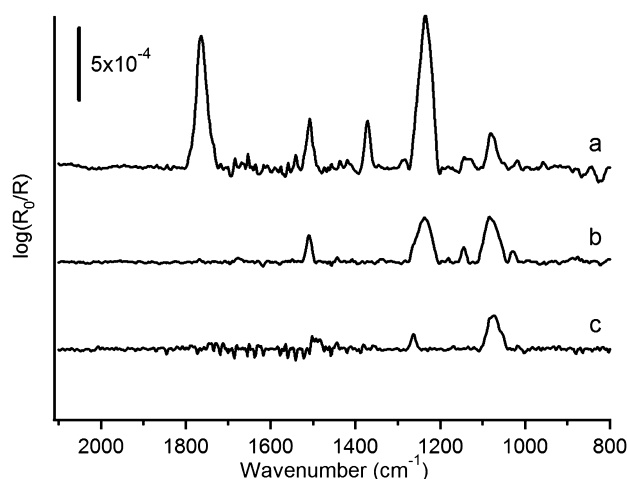
**Figure 1.** IRRAS spectra of carbohydrate coated carbon substrates obtained via surface functionalization from solutions of aryldiazonium salts of compounds 1 (trace a), 3 (trace b), 5 (trace c), and 7 (trace d) from 1.0 mM aqueous solutions.



aryldiazonium derivative of precursor compounds **1**, **3**, **5**, and **7**, which bear Glc, Gal, Man, and Rha monosaccharides, respectively. All four IRRAS spectra show the characteristic infrared absorbances of the parent peracetylated glycoside which are observed in the reference infrared spectra of bulk powders (see the Supporting Information). Absorbance peaks at  $1757\text{ cm}^{-1}$  and  $1371\text{ cm}^{-1}$  can be attributed to carbonyl and methyl groups of the acetyl moiety, respectively. Strong absorbances at  $\sim 1230\text{ cm}^{-1}$  and in the region  $1025\text{--}1090\text{ cm}^{-1}$  are associated with C–O stretching modes arising from the carbohydrate ring and the acetyl protecting groups.<sup>32</sup> Peaks in the region  $1500\text{--}1550\text{ cm}^{-1}$  arise from C–C skeletal vibrations of phenyl rings;<sup>32</sup> in particular, it was possible to observe in all spectra the presence of a peak at  $1508\text{ cm}^{-1}$  which can be attributed to the strong 19a stretching mode of phenyl rings.<sup>33</sup>

Control experiments carried out by immersing the substrates in aqueous solutions of 4-aminophenyl glycosides alone, without carrying out diazotization reactions, yielded no IRRAS signatures, thus indicating that formation of aryldiazonium cations is necessary in order to observe the formation of monosaccharide adlayers. These results indicate that peracetylated carbohydrate coatings were obtained in a single step procedure from aqueous solutions via diazotization of acetal-protected precursors followed by chemisorption to carbon substrates. Importantly, this functionalization methodology was found to be general to all of the monosaccharide-derivatives tested.

Carbohydrate coatings obtained with acetal-protected precursors could be deprotected at the surface to yield the corresponding native monosaccharides. Figure 2 shows a



**Figure 2.** IRRAS spectra on carbon substrates carbohydrate layers. Trace a: peracetylated galactose layers obtained via functionalization using precursor **3** at 1.0 mM concentration after exposure to methanol only. Trace b: galactose layers obtained after deacetylation of the sample in trace a, via immersion in Na/methanol solutions. Trace c: galactose layer obtained using Gal-bearing precursor **4** at 1.0 mM concentration.

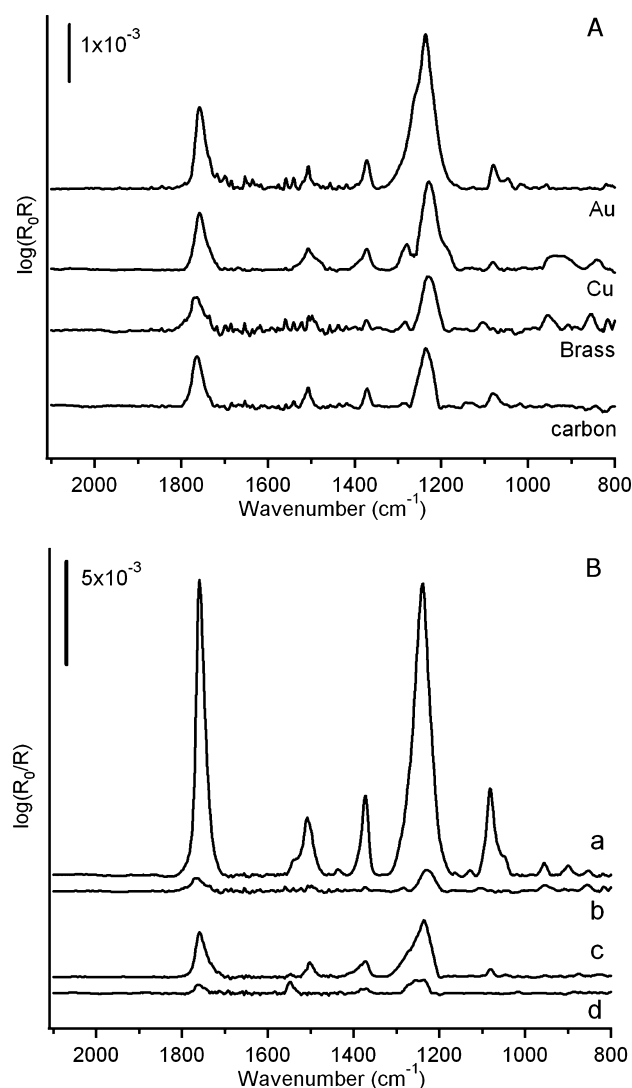
comparison of IRRAS spectra of a peracetylated-Gal coating obtained from **3** after immersion in methanol (trace a) and the same coating after deacetylation reactions via immersion in Na/methanol solutions (trace b). After sonication in methanol, in the absence of sodium methoxide, the integrated intensity of IRRAS peaks is reduced by approximately 50% with respect to peaks obtained after sonication in water alone (comparison of

Figure 2-trace a vs Figure 1-trace b). This observation is consistent with previous studies of aryldiazonium layer composition and indicates that adlayers consist of both strongly chemisorbed molecules and of more weakly bound species that can be removed via organic extraction.<sup>25</sup> After immersion in Na/methanol solutions, we observed the complete disappearance of carbonyl and methyl peaks associated with acetyl protecting groups from the IRRAS spectra. Peaks associated with C–O stretching modes remain however present after deprotection and rinsing. Although the IRRAS peak intensities might depend on monosaccharide orientation at the surface, it is possible to conclude from this result that the core carbohydrate ring system remains intact and covalently bound to the surface after deacetylation.

Surface modifications carried out via diazotization of the fully deprotected precursor **4** (Gal-bearing precursor) yielded IRRAS spectra similar to those obtained after functionalization with **3** followed by deacetylation. This is illustrated in trace c (Figure 2), which shows the IRRAS spectrum of carbon substrates after immersion in solutions of the diazonium salt of compound **4**. Peaks in the IRRAS spectrum obtained after functionalization using compound **4** closely match those of the parent compound obtained from bulk powders (see the Supporting Information). A comparison of traces b and c in Figure 2 suggests that similar carbohydrate layers are obtained on reaction with fully hydroxylated precursors (e.g., **4**) as from peracetylated precursors after deprotection (e.g., via deprotection of **3** at the surface). The relative ratios of C–O stretching peaks at  $1234$  and  $1084\text{ cm}^{-1}$  are however different in traces b and c; this can arise from differences in packing or orientation at the surface as discussed below. In summary, these results confirm that both protected and deprotected carbohydrates can be bound to the surface using this methodology and that effective deprotection chemistry can be carried out at surface bound monosaccharides.

The carbohydrate functionalization methodology was found to be also general with respect to the type of substrate material used. Figure 3A shows IRRAS spectra obtained after functionalization of metals and alloys via diazotization of the peracetylated Gal-bearing precursor, compound **3**. The IRRAS signature of carbohydrate coatings at Au (trace a), Cu (trace b), and brass (trace c) substrates was found to be similar; absorbance peaks match those of the parent compound as well as those observed for layers obtained at carbon substrates (see Figure 1, trace a). Control experiments in aqueous solutions of 4-aminophenyl glycosides alone in the absence of diazotization reactants yielded no IRRAS signatures, thus indicating that formation of aryldiazonium cations is necessary in order to observe the formation of monosaccharide adlayers. Analogous results were obtained for precursors **1**, **5**, and **7** on Au, Cu, and brass substrates as shown in the Supporting Information.

We observed that in the case of metals and alloys there was a strong dependence of peak intensities on the concentration of aryldiazonium precursor used for the functionalization reactions. Figure 3B shows IRRAS spectra obtained from reactions of aryldiazonium cations of precursor **3** at 1.0 mM and 0.25 mM concentrations at brass substrates after sonication in water and after sonication in organic solvents (acetonitrile and methanol). Peak absorbances in IRRAS spectra are determined by a combination of molecular surface density, molecular orientation, and surface enhancement of the electric field perpendicular to the surface.<sup>34</sup> However, assuming an isotropic orientation of acetal groups within the layer, net



**Figure 3.** (A) IRRAS spectra of Au (trace a), Cu (trace b) and brass (trace c) substrates coated with peracetylated galactose via diazotization of compound 3 in aqueous solutions. (B) Effect of precursor concentration and of rinsing protocols on surface coverage of adlayers of peracetylated galactose obtained via diazotization of compound 3.

absorbances provide a good estimate of relative coverage at a given substrate.<sup>35,36</sup> Therefore, a comparison of spectra obtained from 1.0 mM and 0.25 mM solutions indicates (traces a vs c) that the surface density of adlayers formed at 1.0 mM concentrations is higher. Subsequent sonication of these layers in organic solvents, however, drastically reduces the overall intensity of the spectrum (traces b and d) by more than 90%, thus suggesting that a larger proportion of adsorbed species than at carbon surfaces adsorbs via weak interactions. The fact that no physisorption was observed in the absence of diazotization conditions suggests, however, that species more weakly bound to the surface still result from reactions of aryldiazonium derivatives of monosaccharide precursor compounds.

Quantitative information on the molecular surface density of carbohydrate films obtained at solid surfaces from our one-step methodology was obtained via *ex situ* QCM measurements. Surface density was determined by calculating the mass change

at the quartz crystal from the change in resonance frequency using the Sauerbrey equation<sup>37</sup>

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\mu\rho}}\Delta m \quad (1)$$

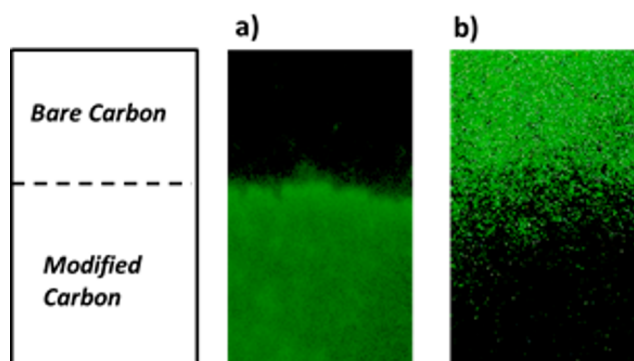
where  $f_0$  is the resonance frequency of the fundamental mode of the QCM in air,  $A$  is the effective surface area of the electrodes, and  $\mu$  and  $\rho$  are the density and shear modulus of quartz. The resonant frequency of a-C coated crystals prior to surface modification was considered as the baseline in determining frequency shifts and thereby the mass shifts for all of our experiments. Normalization of mass shifts by the molecular weight of the chemisorbed compound after nitrogen elimination yielded surface molar densities. The surface density of peracetylated monosaccharides obtained via spontaneous aryldiazonium adsorption at carbon substrates from 1.0 mM 4-aminophenyl glycoside solutions was found to be  $(8.1 \pm 0.5) \times 10^{-10} \text{ mol cm}^{-2}$  for compound 1 (Glc),  $(8.0 \pm 0.6) \times 10^{-10} \text{ mol cm}^{-2}$  for compound 3 (Gal),  $(7.5 \pm 0.4) \times 10^{-10} \text{ mol cm}^{-2}$  for compound 5 (Man), and  $(7.6 \pm 0.3) \times 10^{-10} \text{ mol cm}^{-2}$  for compound 7 (Rha). These values are lower than those reported for the spontaneous attachment of small aryldiazonium salts at the same carbon substrate (e.g., *p*-nitrobenzene diazonium salt),<sup>25</sup> as expected based on the much larger geometrical cross-section of compounds 1, 3, 5, and 7. In the case of deprotected monosaccharides, measurements of surface densities yielded values of  $(12 \pm 7) \times 10^{-10} \text{ mol cm}^{-2}$  for compound 4 (Gal) and  $(19 \pm 2) \times 10^{-10} \text{ mol cm}^{-2}$  for compound 8 (Rha). Previous studies of glycolipid monolayers of simple mono- and disaccharides report that, when perpendicularly oriented in cylindrical conformation, the surface area per molecule of saccharide headgroups is in the range 37–45 Å<sup>2</sup>,<sup>38,39</sup> so that 1 ML  $\approx 4 \times 10^{-10} \text{ mol cm}^{-2}$ . The surface density obtained after deposition of fully deprotected precursors 4 and 8 suggests that the spontaneous modification reaction leads to assembly of 1–4 ML at carbon surfaces. This coverage range compares well both with estimates of fully packed monosaccharide layers and with quantitative determinations of limiting coverage of spontaneous aryldiazonium reactions.<sup>22,25</sup> The molar surface density was found to be lower when the reaction was carried out using fully acetylated precursor compounds; this is also consistent with the final molar density being influenced by the magnitude of the molecular cross-section.

In summary, these results indicate that a one-step, solution-based, carbohydrate functionalization methodology is successful at carbon, metal, and alloy substrates; surface coverage can be determined quantitatively and is comparable to typical surface densities observed at aryldiazonium adlayers.

**3.2. Interaction of Modified Surfaces with Biomolecules.** Glycosylated surfaces find numerous applications, notably in glycoarrays,<sup>40</sup> nanoparticle based sensing<sup>41</sup> and delivery,<sup>42,43</sup> and recently in new approaches of eliciting specific immune responses.<sup>44,45</sup> All of the above applications rely on biomolecule-carbohydrate recognition processes that must take place at the solid/liquid interface. Carbohydrate conformation, orientation, and crowding at the surface are critical in determining whether these molecules can effectively elicit a specific biological response once bound at the surface, with the specific choice of linker and surface density often affecting biorecognition. Therefore, we decided to investigate whether monosaccharide layers prepared via spontaneous chemisorption of aryldiazonium derivatives of 4-aminophenyl glycosides

display biomolecule–substrate interactions typical of carbohydrates, by carrying out surface binding experiments using lectins.

Binding experiments with fluorescently labeled lectins were carried out via fluorescence microscopy. Figure 4a shows a



**Figure 4.** Fluorescence microscopy images of carbon surfaces after binding experiments using fluorescently labeled biomolecules; the lower half of the carbon surface has been modified with a monosaccharide, whereas the upper half remains bare. (a) Binding experiment on Man-modified carbon surfaces using FITC-ConA lectin (image width = 400  $\mu\text{m}$ ). (b) Adsorption experiment on Gal-modified carbon surfaces using FITC-BSA protein (image width = 400  $\mu\text{m}$ ).

fluorescence microscopy image of a carbon coated surface, where only the lower half of the area shown was modified with  $\alpha$ -mannose using compound **6** as a precursor as described in section 3.1. The sample was incubated for 2 h in a 0.5 mg mL<sup>-1</sup> solution of Concanavalin A fluorescein-conjugate (FITC-ConA) in phosphate buffer (PBS); the specific interaction of mannose toward Con A lectin has been widely explored and established.<sup>40</sup> Figure 4a shows that, after rinsing in PBS, the area of the sample that had undergone surface functionalization with  $\alpha$ -mannose displays stronger FITC emission than the area covered by bare amorphous carbon. The emission intensity was found to be  $17 \pm 1$  times greater over regions of the carbon sample coated with mannose than over bare carbon, thus indicating that ConA displays affinity toward surface-bound mannose groups. A similar result was obtained by using galactose-modified surfaces prepared using compound **4** as a precursor and Peanut Agglutinin fluorescein-conjugate (FITC-PNA), a lectin that displays binding specificity toward Gal units.<sup>40</sup> Also in this case, FITC-PNA was found to preferentially bind to the Gal-modified areas (see the Supporting Information). These experiments suggest that the spontaneous chemisorption of aryldiazonium monosaccharide precursors leads to the formation of monosaccharide layers that retain the biological recognition specificity of the parent carbohydrate molecule in solution.

In addition to investigating lectin affinity toward surface-bound carbohydrates we were also interested in assessing the potential of these aryldiazonium coatings as antifouling surfaces. It has been shown that galactose-rich surfaces display resistance toward nonspecific protein binding.<sup>27,46</sup> Therefore, we compared the level of nonspecific protein adsorption at both Gal-modified and unmodified carbon surfaces using Bovine Serum Albumin FITC conjugate (FITC-BSA). Figure 4b shows a fluorescence microscopy image of a carbon coated surface, where only the lower half of the area shown was modified with  $\beta$ -galactose using compound **4** as a precursor as described in

section 2.1. The entire sample was incubated for 2 h in a 0.2 mg mL<sup>-1</sup> solution of FITC-BSA in PBS at pH 7.4<sup>28–30</sup> and then washed with the PBS solution to remove unbound protein prior to imaging. Figure 4b clearly shows that the area of the sample that had undergone modification with Gal units displays much lower FITC emission than the unmodified area: the emission intensity from physisorbed FITC-BSA was found to be  $4.1 \pm 0.3$  times greater at bare carbon surfaces compared to Gal-modified carbon surfaces. This result suggests that the carbohydrate coating may be able to impart the ability to prevent undesirable albumin accumulation on surfaces.

## 4. CONCLUSIONS

In conclusion, we have demonstrated that aryldiazonium chemistry offers a viable approach for the modification of surfaces with carbohydrates. We have shown that monosaccharides can be spontaneously chemisorbed at carbon, metals, and alloys under mild conditions. The resulting carbohydrate layers retain the biological recognition properties of the parent monosaccharides used in the functionalization. Furthermore, we show that these layers can potentially impart antifouling properties onto surfaces. This functionalization methodology is mild and efficient and should find general applications in glycoarray technology and antifouling coatings and as a general strategy for the synthesis of a wide range of materials with biomimetic surfaces.

## ■ ASSOCIATED CONTENT

### Supporting Information

Detailed synthesis, purification and characterization of glycosides, NMR and FTIR data of precursor compounds, IRRAS spectra of carbohydrate organic thin films, Peanut Agglutinin binding experiments at Gal-modified surfaces. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare the following competing financial interest(s): A patent has been filed on carbohydrate coatings with E.M.S., P.E.C., D.M.M., and J.B. as inventors.

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