A Modular Click Approach to Glycosylated Polymeric Beads: Design, Synthesis and Preliminary Lectin Recognition Studies

Gaojian Chen,[†] Lei Tao,[†] Giuseppe Mantovani,[†] J. Geng,[†] Daniel Nyström,[‡] and David M. Haddleton*,[†]

Department of Chemistry, The University of Warwick, Coventry CV4 7AL, United Kingdom, and Royal Institute of Technology, Fibre and Polymer Technology, Teknikringen 56-58, SE-100 44 Stockholm, Sweden

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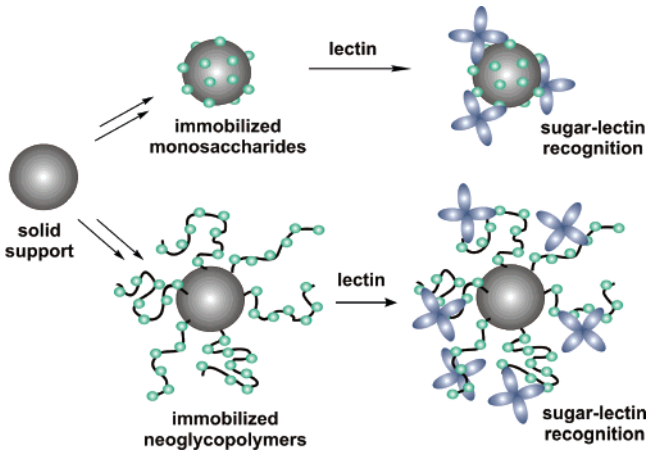
ABSTRACT: Covalent immobilization of a range of carbohydrate derivatives onto polymeric resin beads is described. Copper-catalyzed Huisgen [2 + 3] cycloaddition (often termed click chemistry) was used to graft mannose-containing azides to complementarily functionalized alkyne surfaces, namely (a) Wang resin or (b) Rasta particles consisting of a clickable alkyne polymer loose outer shell and a Wang resin inner core. For the second approach, Wang resin beads were first converted into immobilized living radical polymerization initiators with subsequent polymerization of trimethylsilanyl-protected propargyl methacrylate followed by deprotection with TBAF to yield the desired polyalkyne clickable scaffold. The appropriate α-mannopyranoside azide was then clicked onto the bead to give a mannose functionalized Rasta resin. IR, gel-phase ^{1}H NMR, and elemental analysis have been used to characterize the modified resins. The binding abilities of these D-mannose-modified particles were subsequently tested using fluorescein-labeled Concanavalin A (Con A), a lectin that binds certain mannose-containing molecules. Preliminary results indicated that the novel glyco-hybrid materials presented in this work are able to efficiently recognize mannose-binding model lectins such as Con A, opening the way for their potential application in affinity chromatography, sensors, and other protein recognition/separation fields.

Introduction

Thin organic films on solid substrates play a key role in many natural and non-natural processes. Organic/polymer-modified surfaces can be utilized in many fields, including adhesion and wetting, microfluidics, microfabrication, chemical sensing, and organic synthesis.[1−5] Recently, there has been advances in the microelectronics industry with block copolymer thin films leading to nanostructured inorganics with well-defined morphology.[6] In addition, many examples of biorelated applications, such as tissue engineering,[7] drug delivery, implants, cell adhesion,[8,9] and protein recognition,[10] have been reported. On the basis of the immobilization of molecular probes onto a variety of solid supports that exploits specific donor/receptor interactions in the presence of appropriate substrates (such as between antibodies and antigens, enzymes and inhibitors, and carbohydrates and lectins), affinity chromatography has evolved as a powerful and effective fractionation techniques for proteins purification.

In nature, sugars are information-rich molecules, and an increasingly large number of known lectins are able to recognize subtle variations of oligosaccharide structure, acting as decoders for carbohydrate-encoded information.[11] The considerable scientific effort spent in preparing immobilized sugar probes onto different surfaces led to the use of these functional materials in a number of different applications that include microarrays, microbeads, and biosensor chips.[12,13] Carbohydrate groups were bound to solid supports either noncovalently[14−16] or covalently.[17−19] Important examples of immobilized synthetic glycopolymers include controlled radical polymerization of sugar monomers from silica[20] and silicon wavers,[21] grafting of biotin-terminated polymers to (strept)avidin surfaces,[22−25] attachment of thiol-terminated materials prepared by RAFT to gold nanoparticles,[26,27] and functionalization of polypropylene microporous membranes.[28−30] Despite these advances, however, the use of immobilizes sugars as ligands in lectin-recognition studies has been less extensively investigated,[26,31,32] probably due to the difficulties in the synthesis of suitable carbohydrate probes.

Scheme 1. Immobilized Sugar Supports: Two General Approaches Employed in This Work



A modular approach to chemical functionalization, introduced by Sharpless and termed "click chemistry", covers a class of chemical transformations that share a number of attractive features including excellent functional-group tolerance, high yields, and good selectivity under mild experimental conditions.[33,34] Among these reactions, the Cu(I)-catalyzed version of the Huisgen 1,3-dipolar cycloaddition,[35,36] in which a terminal alkyne is "clicked" to an organic azide to give a 1,2,3-triazole, has been receiving increasing interest. This has led to its application in many processes, including the synthesis of therapeutics,[37,38] protein-based biohybrids,[39−40] functionalization

* Corresponding author. E-mail: D.M.Haddleton@warwick.ac.uk.
[†] Department of Chemistry, The University of Warwick.
[‡] Royal Institute of Technology, Fibre and Polymer Technology.

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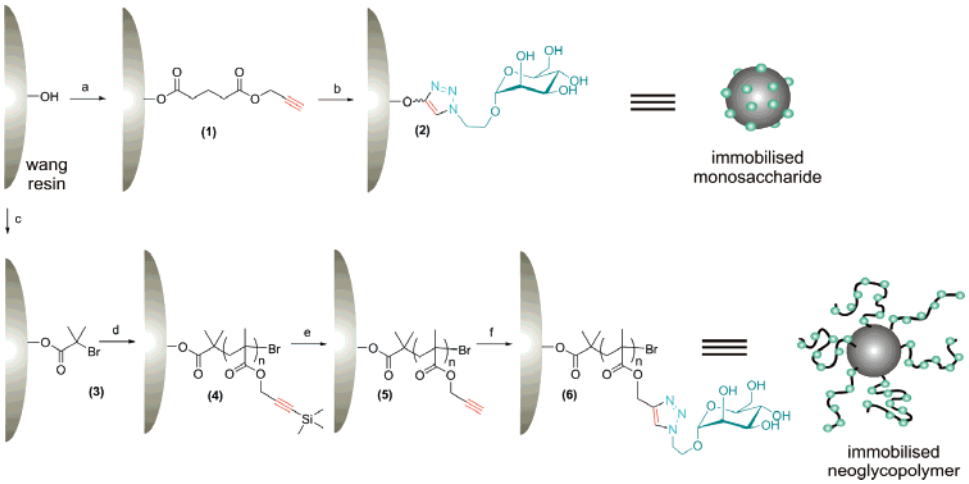
Scheme 2. Synthesis of Mannose Modified Wang Resins by a Click Reaction



Reagents and conditions: (a) 4-chlorocarbonyl-butyric acid prop-2-ynyl ester, DMAP, pyridine, 60 °C; (b) $(PPh_3)_3Cu(I)Br$, α-2-azidoethyl mannopyranoside, DMSO, 60 °C; (c) 2-bromoisobutyryl bromide, triethylamine, DMAP, dichloromethane; (d) trimethylsilyl-propargyl methacrylate, *N*-(ethyl)-2-pyridylmethanimine/Cu(I)Br, toluene, 70 °C; (e) TBAF·$3H_2O$, acetic acid, THF; (f) $(PPh_3)_3Cu(I)Br$, α-2-azidoethyl mannopyranoside, DIPEA, DMSO, 60 °C.

of alkyne functional polystyrene,[41] sugar-based materials,[42] dendrimers,[43,44] functional polymers,[45−51] and even nanotube functionalization.[52] In particular, it is now evident that this "click" process constitutes an extremely valuable tool for the synthesis of carbohydrate-based materials.

Recently, we developed a novel synthetic strategy that combines copper-catalyzed living radical polymerization and Huisgen 1,3-dipolar cycloaddition in which appropriate sugar azides are clicked onto preformed well-defined polyalkyne methacrylic scaffolds. One of the main advantages in using this protocol is the possibility of preparing libraries of different copolymers that only differ for the relative proportion of carbohydrate epitopes by simply employing appropriate mixtures of sugar azides in the reaction feed, starting from one single polyalkyne precursor (we termed this process "co-clicking"). This is of importance, as it allows for the study of the influence of one specific epitope density on the biological behavior of the glycopolymers, with all the other macromolecular features remaining unchanged. Another advantage is the avoidance of preparing and handling sugar methacrylic monomers that often tend to spontaneously self-polymerize.

Alkyne-azide cycloaddition has also been employed for the preparation of cross-linked polymer[53] and silica-based[32] stationary phase supports for chromatography. Most of the columns available for HPLC use stationary phases based on porous beads made of either silica or synthetic polymers. Polymeric supports displaying a broad range of functionalities are often prepared in a single step by copolymerization of appropriate functional monomers. For example, solid supports based on crosslinked functionalized polystyrene beads can be prepared by suspension copolymerization of monomers, such as styrene and divinylbenzene (DVB), with a functional comonomer.[54,55] Although simple, this approach requires reoptimization of the entire preparation process to control the ultimate properties of the beads each time a new monomer is included. A second strategy involves the functionalization of silica- or polymer-based preformed beads, either through modification with small functional molecules[56−59] or through grafting functional polymers from surface active site of the solid support to increase the density of the binding groups.[60−63] This is particularly attractive, as it enables the formation of numerous stationary phases starting from a single type of material with optimized size and porous properties.

This present work is focused on the synthesis of supports bearing covalently immobilized carbohydrates starting from preformed Wang resin beads and the use of these hybrid materials in a preliminary lectin-glycopolymer recognition study. Our synthetic approaches involved a combination of Cu-catalyzed living radical polymerization and Huisgen 1,3 dipolar cycloaddition. Recently, we and others reported that these two powerful synthetic tools can be successfully combined for preparing α-functional polymers,[64,65] glycopolymers,[50] and functional surfaces.[66]

Two distinct classes of immobilized carbohydrate displays were prepared. In the first one, mannose monosaccharide moieties were "clicked" onto alkyne-modified Wang resin; in the second case, polyalkyne polymers were grown from immobilized initiators, and mannose-azide units were subsequently "clicked" onto this macromolecular scaffold to give the desired glycopolymer-bead hybrid materials (Scheme 1). The latter materials will be also referred here as "Rasta" resins, general term indicating polymer chains grafted to crosslinked polymeric bead cores.[3]

Experimental

Reagents and Materials. Wang resin was provided by Avecia Ltd. (loading: 1 mmol g^{-1}). Copper(I) bromide (Aldrich, 98%) was purified according to the method of Keller and Wycoff.[67] *N*-(Ethyl)-2-pyridyl methanimine ligand was prepared as described earlier[68] and stored at 0 °C under a dinitrogen atmosphere. Triethylamine (Fischer, 99%) was stored over sodium hydroxide pellets. 2-Methyl-acrylic acid, 3-trimethylsilanyl-prop-2-ynyl ester, and 4-chlorocarbonyl-butyric acid prop-2-ynyl ester[66] were synthesized as described earlier. All other reagents and solvents were obtained at the highest purity available from Aldrich Chemical Co. and used without further purification unless stated.

Analysis. All reactions were carried out by using standard Schlenk techniques under an inert atmosphere of oxygen-free nitrogen, unless otherwise stated. Analytical TLC analysis was performed using precoated silica gel 60 F254 and developed in the solvent system indicated. Compounds were visualized by use of UV light (254 nm) or a basic solution (10% w/w K_2CO_3 in water) of $KMnO_4$. Merck 60 (230−400 mesh) silica gel was used for column chromatography. NMR spectra were obtained on Bruker DPX300 and Bruker DPX400 spectrometers. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of residual solvent resonances (^1H and ^{13}C).

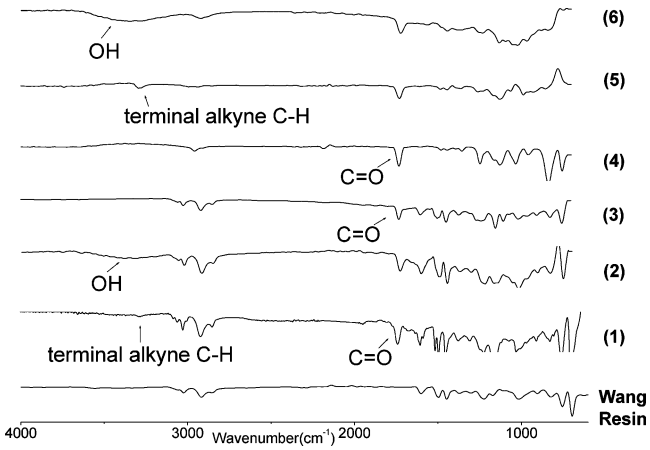


Figure 1. FTIR spectra of Wang resin, Wang initiator, and Wang mannose.

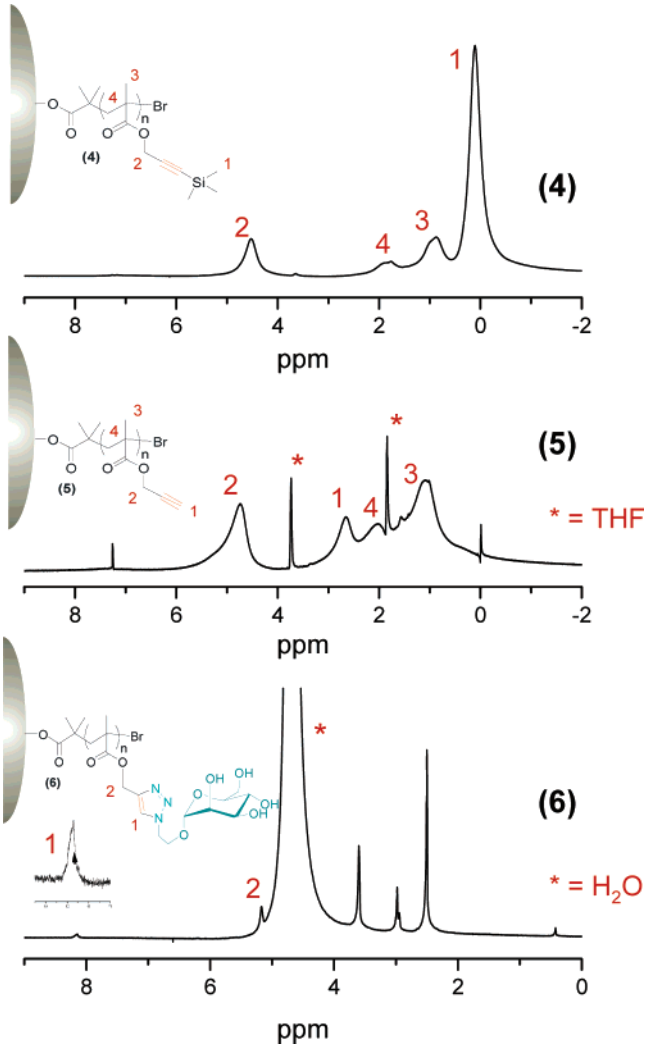


Figure 2. Gel-phase ^{1}H NMR spectra of Rasta polymers presented in this study. Beads were suspended either in $CDCl_3$ ((**4**) and (**5**)) or DMSO-d_6 ((**6**)).

Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell. Field-emission scanning electron microscopy (FE-SEM) and confocal microscopy have also been employed to image the surface of the resins. FE-SEM was measured using a Joel JSM 6100, with an accelerating voltage of 10 kV and equipped with an Oxford JSIS analytical system. Confocal microscopy experiments were performed on a Zeiss LSM 510 system. The 488 nm band of an argon-ion laser were used to excite the fluorescing materials. The filters in the experimental setup were chosen to allow the measurement of the fluorescence above 505 nm. HPLC-SEC and HPLC-FL spectra were determined by a HP 1050 UV-detector and a Hitachi L7480 FL detector. Elemental analyses were performed by the Warwick Analytical Service Ltd. (UK).

Table 1. Elemental Analysis of Functionalized Beads Prepared in This Work

product	value	C (%)	H (%)	N (%)
Wang resin	theoretical[a]	89.03	7.68	
	obtained	89.16	7.56	
(**2**)	theoretical[a]	77.9	7.21	3.10
	obtained	79.21	7.17	2.31
(**4**)	theoretical[b]	63.07	8.10	
	obtained	63.41	7.99	
(**5**)	theoretical[c]	69.03	6.55	
	obtained	69.98	6.82	
(**6**)	theoretical[d]	51.32	6.29	10.23
	obtained	51.64	6.32	6.57

[a] Assuming a styrene/[4-(4-vinyl-benzyloxy)-phenyl]-methanol molar ratio of 7/1. [b] The weight ratio between monomer and initiator (**3**) was 10:1 and the final monomer conversion was virtually 100%: C (%) = 82.01 × 1/11 + 61.18 × 10/11, H (%) = 7.04 × 1/11 + 8.21 × 10/11. [c] C (%) = 82.01 × 1/11 + 67.73 × 10/11, H (%) = 7.04 × 1/11 + 6.50 × 10/11.[d] C (%) = 82.01 × 1/11 + 48.25 × 10/11, H (%) = 7.04 × 1/11 + 6.21 × 10/11, N (%) = 11.25 × 10/11.

Synthesis of Wang Resin Alkyne (1). All the bead-related reactions in this work have been carried out using a FirstMate benchtop synthesizer and Wang resin (loading: 1 mmol g^{-1}, 1.0 g, 1.0 mmol) was added to a DMAP (10 mg, 0.082 mmol) solution in anhydrous pyridine (10 mL), and after 1 h, 4-chlorocarbonyl-butyric acid prop-2-ynyl ester (0.50 g, 3.0 mmol) in dichloromethane (10 mL) was added. The reaction mixture was subsequently heated to 60 °C and kept at this temperature for 20 h. The resin was then filtered and rinsed sequentially with acetone, distilled water, acetone, and dichloromethane. Residual traces of solvent were then removed under reduced pressure.

Synthesis of Wang Resin Initiator (3). Wang resin (loading 1 mmol g^{-1}, 2 g, 2 mmol of OH groups) was suspended in a triethylamine (2.8 mL, 20 mmol), DMAP (10 mg, 0.082 mmol), and 2-bromoisobutyryl bromide (1.2 mL, 10 mmol) solution in dichloromethane (30 mL). The reaction was carried out at ambient temperature for 12 h. The beads were subsequently filtered and rinsed thoroughly with dichloromethane, methanol, water, acetone, and dichloromethane and then dried in a vacuum oven overnight at ambient temperature.

Polymerization from Immobilized Initiator (3): Synthesis of Polymer (4). Wang resin initiator **(3)** (0.1 g, 0.9 mmol) and Cu(I)Br (14 mg, 0.10 mmol) were introduced into a reactor that was then deoxygenated by three vacuum/nitrogen fill cycles. A Schlenk tube was charged with 2-methyl-acrylic acid 3-trimethylsilanyl-prop-2-ynyl ester monomer (1.0 g, 5.1 mmol), *N*-(ethyl)-2-pyridyl-methanimine ligand (0.029 mL, 0.20 mmol), and toluene (20 mL). The resulting solution was degassed by five freeze/pump/thaw cycles, and the reactor was filled with nitrogen. The brown solution was then added to the reactor containing resin and Cu(I)Br via cannula. The reactor was heated to 70 °C under nitrogen and at the end of the polymerization the mixture was allowed to cool down to ambient temperature. The beads were isolated by filtration and washed sequentially with toluene, THF, diethyl ether, and finally dried under vacuum until a constant weight was obtained.

Preparation of Resin (6). (**4**) (0.2 g) was suspended in an acetic acid (1.5 mL)/THF (20 mL) mixture. Nitrogen was bubbled for approximately 10 min, and the suspension was cooled to −20 °C. A 0.20 M solution of TBAF·3H_2O in THF (1.5 mL) was added slowly via syringe (ca. 2−3 min). The resulting mixture was stirred at this temperature for 30 min and then warmed to ambient temperature. The reaction mixture was kept at ambient temperature overnight, the resins beads were isolated by filtration and washed sequentially with THF and diethyl ether, and then finally dried under

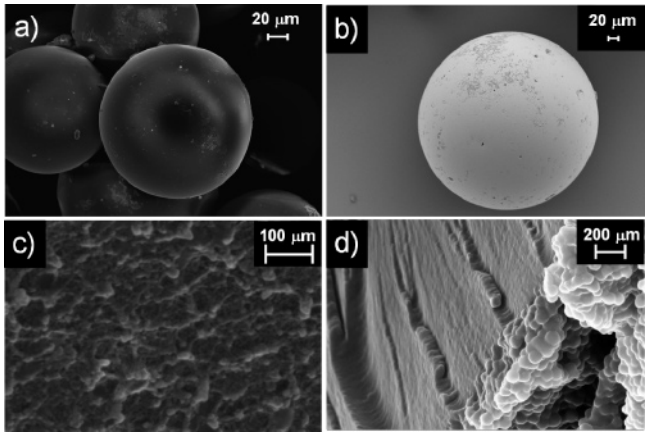


Figure 3. FE-SEM images of unmodified Wang (a) and Rasta (**4**) (b) beads. Images of cross sections of the same beads are also shown: (c) Unmodified Wang resin. Only the core is shown as there is no distinction between the core and the surface observed. (d) Rasta (**4**) beads. In the latter case, the loose grafted methacrylic outer shell (right) is clearly distinguishable from the core of the beads.

vacuum until a constant weight was obtained to give product Wang-(**5**) as a white powder.

(**5**) (0.1 g) was then added to a deoxygenated solution of $(PPh_3)_3$-Cu(I)Br (150 mg, 0.160 mmol) and α-2-azidoethyl mannopyranoside (424 mg, 1.60 mmol) in DMSO (20 mL). The mixture was heated to 60 °C under nitrogen and kept in the synthesizer for 48 h. The beads were then removed by filtration and rinsed thoroughly with DMSO, water, methanol, and dichloromethane and then dried under vacuum until a constant weight was obtained. (**6**) was isolated as an off-white solid.

Preparation of Resin (2). Similar procedures as above were used, reaction tubes containing Wang resin alkyne (0.33 g), $(PPh_3)_3$-Cu(I)Br (0.031 g 0.033 mmol), and α-2-azidoethyl mannopyranoside (87 mg, 0.33 mmol) in deoxygenated DMSO solution were heated to 60 °C under nitrogen and reacted for 48 h. The beads were then removed by filtration and rinsed thoroughly with DMSO, water, methanol, and dichloromethane and then dried under vacuum to a constant mass.

Lectin-Interaction Studies. (**6**) (100 mg) was suspended in PBS buffer (pH 7.4, 50 mM) and poured in a glass pipet equipped with a septum at the bottom end to form a sugar beads minicolumn cartridge. A solution of FITC-Con A (1 mL, 1 mg mL^{-1}) in 50 mM pH 7.4 PBS buffer was run through the cartridge. The average flow rate through the cartridge was approximately 0.3 mL min^{-1}. When all of the solution was eluted (the residual solution still present in the cartridge was eluted by applying a very gentle pressure of compressed air at the top of the column), 50 μL of the starting FITC−Con A solution and the sample solution eluted from the column were analyzed by SEC HPLC in a system equipped with a fluorescence detector (λ_{ex} 495 nm, λ_{em} 525 nm). The cartridge was subsequently washed with a further 1 mL of 50 mM PBS buffer (pH 7.4). The lectin bound to the column was finally eluted with a 4.0 M solution in 50 mM PBS buffer (pH 7.4) of α-methyl-D-mannopyranoside, a competitive monovalent ligand for Con A.

Results and Discussion

Synthesis of the Sugar Supports. Commercially available Wang resin (1 mmol g^{-1} OH groups loading) was chosen as the solid support starting material for the present study. In the past, we have reported the polymerization of methyl methacrylate[69,70] and *N*,*N*-dimethylacrylamide from living radical polymerization initiators immobilized to Wang resin via living radical polymerization using a Cu(I)Br/iminopyridine ligand catalyst. More recently, grafted copolymers, such as poly(*tert*-butyl acrylate-*b*-styrene)[71] and azalactone-functionalized copolymers,[72] have been synthesized by following similar strategies and the resulting materials employed for a number of different applications. Our general strategy here involved the clicking of appropriate azide sugar to preformed resin surfaces bearing terminal alkyne groups, affording either *immobilized monosaccharides* or *immobilized glycopolymers* (Scheme 2).

In the former case, each OH present at the bead surface (1 mmol g^{-1} loading) was first transformed into a monoalkyne handle in the presence of 4-chlorocarbonyl-butyric acid prop-2-ynyl ester, following a protocol that we have recently developed.[66] α-2-Azidoethyl mannopyranoside, prepared in two steps from unprotected D-mannose, was subsequently clicked onto this alkyne-functional support (**1**) to give the desired immobilized monosaccharide resin (**2**).

In the second approach, Wang resin was first converted into the immobilized living radical polymerization initiator (**3**) in the presence of 2-bromoisobutyryl bromide. Polymerization of trimethylsilyl-propargylmethacrylate[50] at 70 °C using Cu(I)Br/*N*-(ethyl)-2-pyridylmethanimine as the catalytic system in 51:0.9:1:2 (monomer/(**3**)/CuBr/ligand) molar ratio afforded the Rasta intermediate (**4**). Removal of the trimethylsilyl protecting groups with TBAF gave the polyalkyne scaffold (**5**) that was then clicked with α-2-azidoethyl mannopyranoside using $[(PPh_3)_3$-Cu(I)Br] as the catalyst to give the desired immobilized glycopolymer (**6**).

Characterization of the Sugar Hybrid Supports. The carbohydrate-based supports (**2**) and (**6**), as well as all the resin intermediates were characterized by FTIR, gel-phase NMR and CHN elemental analysis. The conversion of 1 g $mmol^{-1}$ Wang resin into the alkyne support (**1**) was confirmed by the appearance of an ester C═O and a weak alkyne C−H stretching in the IR spectrum at ca. 1730 and 3300 cm^{-1} respectively (Figure 1). Subsequent clicking of α-2-azidoethyl mannopyranoside led to the immobilized monosaccharide resin (**2**) in which a broad OH band centered at ca. 3400 cm^{-1}. For the synthesis of the immobilized glycopolymer (**6**), the same Wang resin starting material was first converted into the polyvalent initiator (**1**), which gave the typical ester C═O stretching band at 1730 cm^{-1}, which increased substantially after the subsequent polymerization of trimethylsilyl propargyl methacrylate to give the intermediate (**4**). Removal of the protective groups gave the polyalkyne scaffold (**5**), which showed a characteristic 1-alkyne C−H stretching band in the IR spectrum at ca. 3300 cm^{-1}. After clicking, the latter band disappeared, while a broad signal at ca. 3400 cm^{-1} corresponding to the sugar hydroxyl groups of (**6**) became evident.

The Rasta materials were also characterized by gel-phase ^1H NMR analysis. The spectrum of a suspension of (**4**) in $CDCl_3$ showed the presence of all the expected major signals, including the ester OCH_2 (ca 4.5 ppm) and the $Si(CH_3)_3$ (ca. 0 ppm) peaks (Figure 2). The latter signal was absent in the spectrum of (**5**) in the same solvent confirmed the complete removal of the silylated protecting group, with the C≡CH signal clearly visible at ca. 2.5 ppm. Analysis of mannose neoglycopolymer (**6**) resulted to be slightly more problematic, probably due to less efficient swelling of the beads in the very polar solvents required for solvating the pendant sugar polymer chains. However, analysis of a suspension of (**6**) in DMSO-d_6 allowed detection of both the OCH_2 signal at ca. 5.2 ppm and the 1,2,3-triazole proton at 8.2 ppm.

CHN analysis of the sugar beads and intermediates prepared in this work was carried out in an attempt to estimate their elemental composition (Table 1). Nitrogen content analysis of the immobilized monosaccharide beads (**2**) showed that ap-

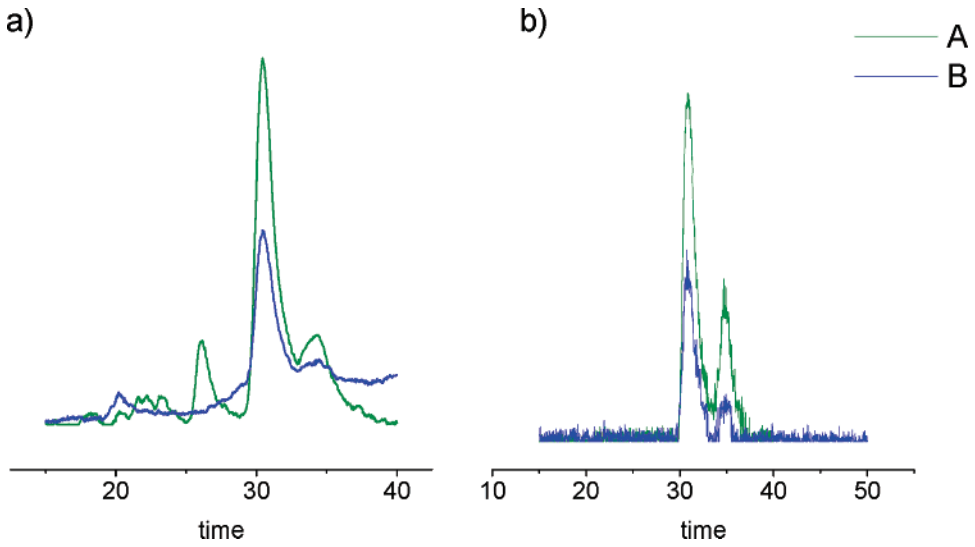


Figure 4. SEC-HPLC analysis of the FITC-Con A solution before (**A**, green solid line) and after (**B**, blue solid line) elution through the immobilized monosaccharide (**2**) cartridge: (a) UV detection, $\lambda = 280$ nm, (b) fluorescence detection ($\lambda_{ex} = 495$ nm, $\lambda_{em} = 525$ nm).

proximately 75% of the Wang resin hydroxyl groups have been converted into clicked mannose units.

All of the Rasta intermediates showed compositions very close to the theoretical values, calculated considering the alkyne monomer vs initiator (**3**) weight ratio of 10:1 employed for the synthesis of the Rasta intermediate (**4**) and monomer conversion very close to 100%. The loading in 1-alkyne in the resin (**5**) was therefore calculated to be approximately 7.3 mmol g^{-1}. Elemental analysis of the clicked immobilized glycopolymer (**6**) indicated the amount of nitrogen after grafting being 6.6%, corresponding to an efficiency of the cycloaddition step of ca. 65%.

FE-SEM analysis was also been used to evaluate the effect of the growth of polymer chains on the morphology and structure of our resins. Figure 3 shows an increase in beads size occurs by passing from unmodified Wang to Rasta resin (**4**). Interestingly, the image of a cross section of the latter material also suggests that polymerization may have also occurred inside the crosslinked beads.

Preliminary Lectin Recognition Experiments. The ability of these immobilized sugar displays to selectively recognize specific lectins was qualitatively investigated. Commercially available Concanavalin A (Con A) was chosen as the model D-mannose-binding lectin due the bulk of literature focusing on both its chemical and biological behavior.[73−76] Fluorescently labeled Con A (fluorescein isothiocyanate-labeled Concanavalin-A, FITC-Con A) was used in order to facilitate the detection and qualitative characterization of the sugar-lectin clusters by SEC-HPLC (system equipped with a fluorescence detector) and confocal microscopy.

First, slurry suspensions of 100 mg of sugar beads (**3**) and (**6**) in PBS buffer (pH 7.4, 50 mM) were packed into small cartridges. A 1.0 mL of a 1.0 mg mL^{-1} (9.6×10^{-3} μmol Con A tetramer mL^{-1}) solution of FITC-Con A solution was loaded onto the columns and allowed to elute by gravity. Approximately 3 min were required for the solution to pass through the cartridge. The residual solution still present in the column was forced to be eluted by applying a slight pressure at the top of the column. Aliquots (50 μL) of the starting FITC-Con A solution and of the sample collected after the column were then analyzed by SEC HPLC.

Figure 4 shows typical chromatograms obtained by SEC-HPLC, using both UV ($\lambda = 280$ nm) and fluorescence ($\lambda_{ex} = 495$ nm, $\lambda_{em} = 525$ nm) detection, for the case the immobilized monosaccharide resin (**2**). Con A, existing mainly as tetramer aggregates of 26 kDa monomer units at pH 7.4, tend to form dimers in more acidic environments.[77] The presence of two main peaks in the SEC-HPLC traces seems to indicate that two distinct aggregation states may coexist under the conditions employed for the analysis (mobile phase: water/acetonitrile/TFA 35:65:0.1 volume ratio). SEC-HPLC data allowed us to estimate that around 50% of the FITC-Con A had been retained by the immobilized monosaccharide (**2**) cartridge under the experimental conditions employed for this preliminary study.

An analogous protocol was followed using Rasta poly(mannose) beads (**6**) instead of the immobilized monosaccharide resin (**2**). (**6**) was expected to interact with lectins much more efficiently than (**2**) due to a number of reasons that include higher loading in mannose epitopes per bead mass unit and the presence of flexible glycopolymer chains able to span over multiple lectin actives sites, which is known for improving the binding ability of sugar multivalent ligands. A previous report indicated that, for the case of silica-based supports, a substantial increase in Con A binding ability was observed even by passing from immobilized monosaccharide to analogous tripodal ones.[32]

In our experiments, resin precursors with no sugar-binding epitopes were employed as control. As expected, only the resin (**6**) was able to specifically recognize Con A, with virtually no lectin eluted from the cartridge (Figure 5). The beads were then washed with 1 mL of 50 mM pH 7.4 PBS in order to remove physically absorbed Con A, and all the fractions were separately analyzed by SEC-HPLC.

These simple experiments suggested that unspecific interactions between the FITC-Con A and both the control materials, (**5**) and Wang resin starting material, were negligible. It should be noted here, for comparison, that the unspecific interactions between silica (another solid support commonly employed as stationary phase in affinity chromatography) and lectins (including Con A) can be in some cases relatively significant.[32,78] Interactions between conformationally flexible proteins and hydrophilic surfaces under conditions of electrostatic repulsion has also been predicted[32,79] and explained in terms of favorable entropic effects.

The rinsed Wang (**5**) and (**6**) supports were then analyzed by confocal microscopy. As expected, only (**6**) appeared to be highly fluorescent, in agreement with the HPLC results obtained

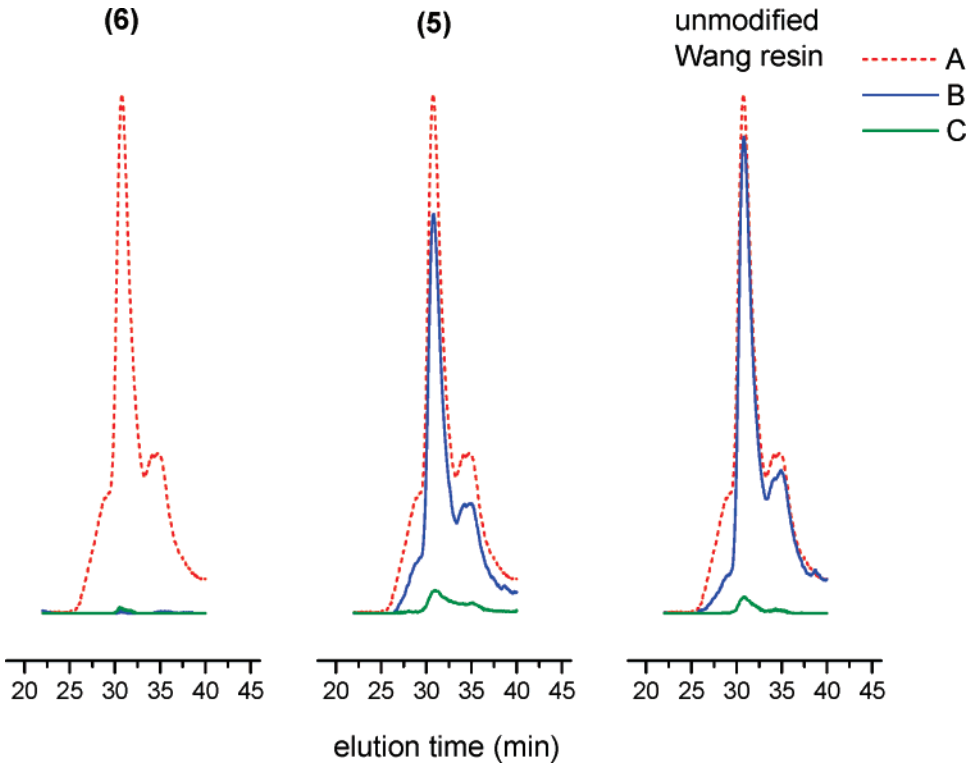


Figure 5. SEC-HPLC analysis (UV detection, $\lambda = 280$ nm) of the FITC-Con A solution before (**A**, red dot line line) and after (**B**, blue solid line) elution through the immobilized "rasta" neoglycopolymer resin (**6**) cartridge. Analogous minicolumns packed with non-sugar-functionalized supports (**5**) and Wang resin were used as controls and the solutions eluted from them were analyzed by SEC-HPLC under the same conditions employed for (**6**). The resin cartridges were then washed with 1 mL of 50 mM pH 7.4 PBS, and the eluted solution was analyzed by SEC HPLC (**C**, green solid line).

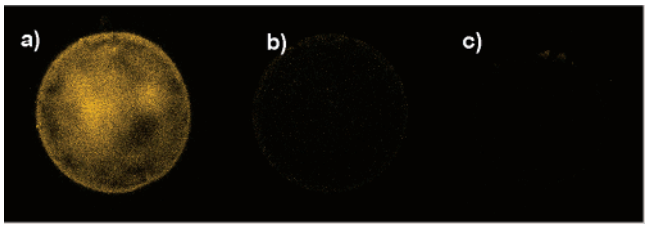


Figure 6. Confocal microscopy images of the (**6**) (a), (**5**) (b) and Wang resin (c) beads obtained after passing 1 mL of a 1 mg mL^{-1} FTIC-Con A solution in 50 mM pH 7.4 PBS through microcolumn cartridges packed with the resins. The resins were rinsed with 50 mM pH 7.4 PBS prior to confocal microscopy analysis.

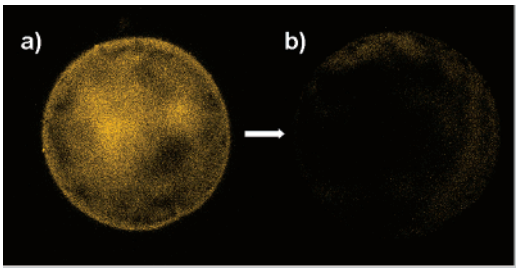


Figure 7. Confocal microscopy images of the resin (**6**)-FITC Con A cluster beads before (a) and after washing with a 4.0 M solution of α-methyl-D-mannopyranoside, a competitive monovalent ligand for Con A, in 50 mM pH 7.4 PBS.

previously, which indicated that only the resin (**6**) presenting pendant polymers with multiple copies of α-mannopyranoside epitopes could bind the fluorescently tagged Con A lectin (Figure 6).

In protein purification chromatography, the elution of the substrates can be promoted in various ways, i.e., by increasing the ionic strength of the mobile phase, by changing its pH, or by adding a competitive ligand for the protein analyte to the elution buffer.[80] In the present work, FITC-Con A was easily removed from the resin (**6**)-FITC clusters (still packed in the cartridge described previously) by adding a large excess (4.0 M) of α-methyl-D-mannopyranoside, a competitive monovalent ligand for Con A, to the mobile phase (Figure 7). Confocal microscopy analysis on the resulting beads revealed that the previously bound fluorescent lectin was successfully removed from the supported mannose glycopolymer (**6**), confirming the sugar epitope-lectin nature of the interactions in the (**6**)-Con A clusters.

Conclusions

Design and synthesis of two structurally distinct sugar supports is described. Both the synthetic protocols involve a key step in which an α-mannopyranose azide is clicked into immobilized alkyne moieties by Cu(I)-catalyzed Huisgen [2 + 3] cycloaddition. Immobilized monosaccharide resins were prepared by converting Wang resin hydroxyl groups into alkyne handles, followed by Cu(I)-catalyzed clicking of α-2-azidoethyl mannopyranoside moieties.

The second class of materials, termed Rasta-immobilized glycopolymers, consists of macromolecular chains displaying multiple copies of carbohydrate epitopes grafted onto a crosslinked polystyrene core. This time, Wang resin hydroxyl groups were converted into living radical polymerization initiators, then Cu-catalyzed polymerization in the presence of trimethylsilyl-protected propargyl methacrylate followed by TBAF-mediated deprotection afforded clickable beads consisting of a crosslinked polystyrene core and a loose polyalkyne outer shell.

The hybrid materials prepared in this work have shown the ability of recognizing Con A, a well-known mannose-binding lectin, and their use in the purification of complex mixtures of

mannose-binding biologically relevant lectins is already under investigation. We also believe that the strategy developed for mannose-functional beads is very general, and future research will focus on the synthesis and use of immobilized ligands bearing a plethora of different sugar epitopes, potentially opening the way for an application of these materials in a number of fields which include chemical sensing, responsive surfaces, and affinity chromatography.

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