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Published on Web 03/22/2006

Synthesis of Neoglycopolymers by a Combination of "Click Chemistry" and Living Radical Polymerization

Vincent Ladmiral, Giuseppe Mantovani, Guy J. Clarkson, Solene Cauet, Jacob L. Irwin, and David M. Haddleton*

Contribution from the Department of Chemistry, University of Warwick, Coventry CV4 7AL, United Kingdom

Received December 9, 2005; E-mail: D.M.Haddleton@warwick.ac.uk

Abstract: The synthesis of novel well-defined alkyne side chain functional polymers featuring narrow molecular weight distributions (PDI = 1.09−1.17) by living radical polymerization is described. Grafting of protected and unprotected carbohydrates is achieved via either a C-6 or an anomeric azide (α or β) onto these polymers by Cu(I)-catalyzed "click chemistry", providing a simple and efficient route to synthetic glycopolymers. The strategy provides an extremely powerful tool for the synthesis of libraries of materials that differ only in the nature of the sugar moiety presented on a well-defined polymer scaffold. A library of multivalent ligands were then prepared following a "coclicking" synthetic protocol, and the reactivity of these glycopolymers in the presence of concanavalin A and *Ricinus communis* agglutinin, model lectins able to selectively bind appropriate mannose and galactose derivatives, respectively, was assessed.

Introduction

"Click chemistry" is a term used to describe several classes of chemical transformations that share a number of important properties which include very high efficiency, in terms of both conversion and selectivity under very mild reaction conditions, and a simple workup.[1,2] The Cu(I)-catalyzed version of the Huisgen 1,3-cycloaddition[3,4] has been recently receiving attention as a highly efficient and stereoselective reaction coupled with excellent functional group compatibility. These important features (often simply referred to as "click") almost allow for the tailor-made synthesis of complex materials including dendrimers,[5−8] bioconjugates,[9−12] therapeutics,[13−15] functionalized polymers,[16−20] affinity chromatography supports,[21] and sugar derivatives.[22−28] In addition, click strategies have been used as an approach to synthetic cyclodextrins[29] and the decoration of cyclic peptides by glycosylation.[28] Synthetic glycochemicals have attracted increasing interest as carbohydrates are involved in a number of important biological processes involving highly specific events in cell−cell recognition, cell−protein interactions, and the targeting of hormones, antibodies, and toxins.[22,30−33] Sugars are information-rich molecules, and an increasingly large number of known lectins

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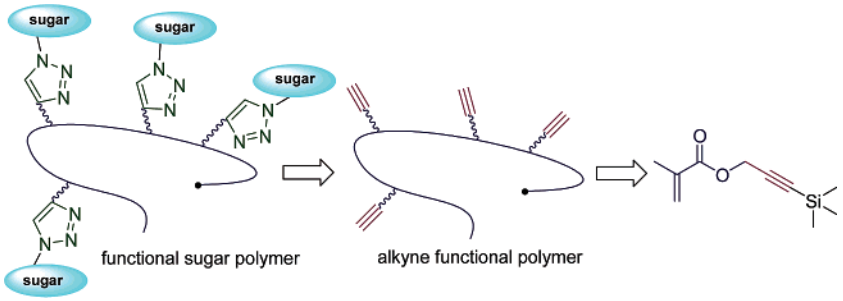

Figure 1. Retrosynthetic approach toward side chain carbohydrate functional polymers.

are able to recognize subtle variations of oligosaccharide structure and act as decoders for this carbohydrate-encoded information.[34] Gaining insight into the factors that control these phenomena may open the way for the development of new anti-infective, anti-inflammatory, and anticancer therapeutics and agents.[22,28,30,35,36]

Synthetic glycopolymers are receiving increasing attention as simple monosaccharides have weak interactions with protein receptors and thus only illicit a weak response to in vivo events mediated by carbohydrate−protein binding.[37−40] In nature, often both protein-binding carbohydrates and lectins exist in higher order oligomeric structures presenting multiple binding sites acting as "polydentate" donors which help to circumvent the intrinsic weak binding limitations related to the use of monovalent ligands.[40,41] The enhancement in activity that can be achieved with appropriate synthetic multivalent polymers as compared to the corresponding monovalent ligands is known as the "glycoside cluster effect".[34,42−44]

The synthesis of glycopolymers featuring well-defined macromolecular architectures (chain length, blocks, stars) is an interesting target in this field, and a number of synthetic techniques are being employed to achieve this. Controlled radical polymerization of carbohydrate-containing monomers offers one promising synthetic route to achieve new glycopolymers; however, only a relatively small number of examples have been reported in the literature thus far.[45−52] One contributing reason is the inherent difficulties in the synthesis of the requisite carbohydrate monomers, the compatibility of these highly functionalized monomers with the conditions of living radical polymerization, and in some cases subsequent deprotection to reveal the carbohydrate epitope.

Copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition is a particularly attractive route for the synthesis of new synthetic glycopolymers as the reaction conditions are compatible with unprotected sugar azides, as long as suitable "clickable" polymers are available.[53] The synthetic strategy developed in this current work is outlined in Figure 1. Since a large variety of carbohydrate-based materials can be obtained starting from the same alkyne containing clickable polymer, the method appears to be particularly useful for preparing libraries of sugar polymers featuring materials with identical size and structural architectures of the polymer backbone but differing only in the nature of the pendant sugar moieties to control the properties due to the multiple carbohydrates rather than the polymer backbone. The use of an alkyne monomer with azidosugars was also considered attractive over the reverse, an azide polymer with alkyne functional sugars, from a safety perspective as this reduces the number of azide groups in the same molecule and utilizes well-documented azido functional sugars.

Transition-metal-mediated living radical polymerization (TMM-LRP, often termed ATRP)[54,55] is well established and versatile, has good tolerance toward most functional groups, and allows excellent control over the polymer architecture. Thus, TMM-LRP was chosen as the polymerization technique for the synthesis of the required alkyne functional materials. A protected alkyne monomer was used, following the work of Van Hest and co-workers,[17] who recently reported the synthesis of α-functional alkyne polymers obtained by ATRP starting from a trimethylsilyl-*protected* alkyne initiator as the homopolymerization of unprotected propargyl methacrylate leads to polymers having a relatively broad molecular weight distribution.[20]

The synthetic strategy developed was employed for the preparation of a small representative library of glycopolymers that have been used as multivalent ligands for lectin binding studies. The interaction of various lectins with a number of synthetic glycopolymers has been previously reported.[56−59]

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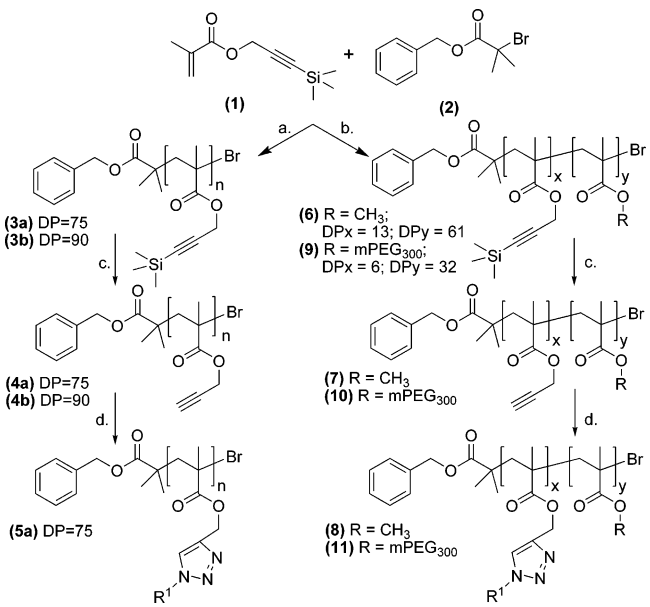
These materials have been employed for a range of potential applications covering lectin clustering studies[56,60−67] and for the evaluation of cell−surface interactions.[68−74] The possibility of using a combination of the two versatile synthetic methodologies click and TMM-LRP was attractive as excellent control over the multivalent ligand properties is associated with the advantages related to the robustness of both these processes (tolerance toward a number of functional groups and solvents, use of technical grade solvents, including water, and relatively inexpensive starting materials).

Results and Discussion

Synthesis of the Clickable Alkyne Polymers. Trimethylsilyl methacrylate monomer **1** was prepared in one step from commercially available 3-(trimethylsilyl)propyn-1-ol and methacryloyl chloride. *O*-Benzyl α-bromoester **2**[75] was chosen as the initiator since both the aromatic and the benzylic protons can be used as ^{1}H NMR internal standards for the determination of the number average molecular weight (M_n(NMR)) of the corresponding polymers (Scheme 1). Both homo- and copolymerization of **1** with MMA and mPEG$_{300}$MA in the presence of a Cu^{I}Br/*N*-(*n*-ethyl)-2-pyridylmethanimine catalyst[76] gave excellent first-order kinetic plots, indicating a good control over the polymer molecular weight and molecular weight distribution (Figure 2 and Table 1). For the homopolymerization of **1**, it is noted that the polydispersity index (M_w/M_n) of the purified polymers is as low as 1.15, even at relatively high monomer conversion (>80%). To verify the versatility of the synthetic strategy proposed, several polymers with very different solubilities in both organic and aqueous solvents were prepared. Methyl methacrylate (MMA) and methoxy(poly(ethylene glycol))$_{300}$ methacrylate (mPEG$_{300}$MA) were chosen as model comonomers as they furnish hydrophobic and hydrophilic copolymers, respectively. Moreover, poly(ethylene glycol)s have been shown not to illicit nonspecific protein binding, which is important in the interpretation of the lectin binding reported later.[77]

Scheme 1 [a]



[a] Reagents and conditions: (a) *N*-(*n*-ethyl)-2-pyridylmethanimine/CuBr, toluene, 70 °C; (b) *N*-(ethyl)-2-pyridylmethanimine/CuBr, MMA or (mPEG$_{300}$)MA, toluene, 70 °C; (c) TBAF, acetic acid, THF, −20 to +25 °C; (d) R^{1}N$_3$, (PPh$_3$)$_3$CuBr, DIPEA.

Interestingly, preliminary attempts using TBAF-mediated removal of the trimethylsilyl protecting group afforded polymers with a terminal alkyne content lower than expected. We suspected that the reason behind this behavior was related to the basicity of TBAF, and we were pleased to find that the addition of acetic acid as a buffering agent was sufficient to provide the expected terminal alkyne polymers in virtually 100% yields.[78] The complete removal of the trimethylsilyl groups was confirmed by both ^{1}H NMR, with the appearance of the C≡C*H* signal at 2.5 ppm along with the disappearance of the Si(C*H*$_3$)$_3$ signal at 0.2 ppm, and FT IR analysis with the alkyne C−H stretching frequency at 3291 cm^{-1}. The SEC analysis also revealed that, as expected, the hydrodynamic volume of the polymers decreased after deprotection while the polydispersity index remained almost unchanged. The use of a low-angle laser light scattering (LALLS) detector (5°) for SEC analysis allowed us to determine the absolute M_w values of the polymers before and after the removal of the trimethylsilyl group. The results obtained, M_w(**3b**) = 19800 and M_w(**4b**) = 13000, combined with the relative polydispersity indexes of these polymers, gave an indication of the number average molecular weights, M_n(**3b**) = 17300 and M_n(**4b**) = 11300, which agreed well with the data obtained by ^{1}H NMR analysis (M_n(NMR)(**3b**) = 17700 and M_n(NMR)(**4b**) = 11200).

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (**12**), 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide (**13**), and methyl α-D-6-azido-6-deoxymannopyranoside (**14**) were employed as model

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Table 1

polymer	monomer A	monomerB	M_n(NMR)	M_n(theor)	M_w/M_n(SEC)	notes
3a	**1**		14900	6000	1.16	homopolymer from **1**
4a	**1**		8500		1.15	obtained by deprotection of **3a**
3b	**1**		17600	7600	1.17	homopolymer from **1**
4b	**1**		11200		1.11	obtained by deprotection of **3b**
6	**1**	MMA[a]	8900	5700	1.09	statistical copolymer
7	**1**	MMA[a]	8200		1.09	obtained by deprotection of **6**
9	**1**	$mPEG_{300}MA$[b]	11900	7800	1.12	statistical copolymer
10	**1**	$mPEG_{300}MA$[b]	11100		1.15	obtained by deprotection of **9**

[a] MMA content in the polymer, 82% (mol/mol); MMA feed composition, 71%. [b] $mPEG_{300}MA$ content in the polymer, 84% (mol/mol); $mPEG_{300}MA$ feed composition, 83%.

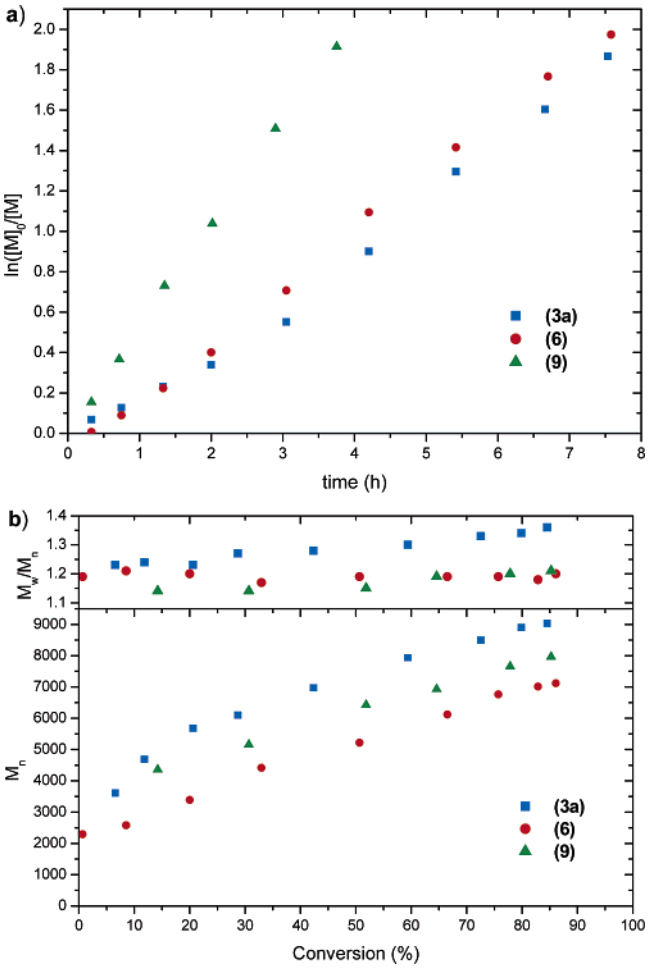


Figure 2. Homo- and copolymerization of **1** using **2** as the initiator with MMA or $mPEG_{300}MA$ as the comonomer in toluene solution at 70 °C. Reaction conditions: (**3a**) $[\mathbf{1}]_0:[\mathbf{2}]_0$:[CuBr]:[ligand] = 40:1:1:2; (**6**) $[\mathbf{1}]_0$:$[MMA]_0$:$[\mathbf{2}]_0$:[CuBr]:[ligand] = 20:50:1:1:2; (**9**) [**1**]:$[mPEG_{300}MA]$:[**2**]:[CuBr]:[ligand] = 5:25:1:1:2. (a) First-order kinetic plots. (b) Dependence of M_n and M_w/M_n on the monomer conversion.

sugar azide reagents for the click reaction to investigate the versatility of this approach to attach protected and unprotected carbohydrates via either a C-6 or an α or β anomeric azide following established synthetic protocols (Chart 1).

The conditions chosen for the click reaction of the sugar azides to the alkyne-containing polymers were modified from those reported by Hawker and co-workers for the synthesis of several dendritic libraries, with $[(PPh_3)_3CuBr]$ as the catalyst, in the presence of DIPEA.[6] Removal of the trimethylsilyl protecting group was accompanied by a decrease in the polymer molecular mass, while after conjugation of the azide sugar

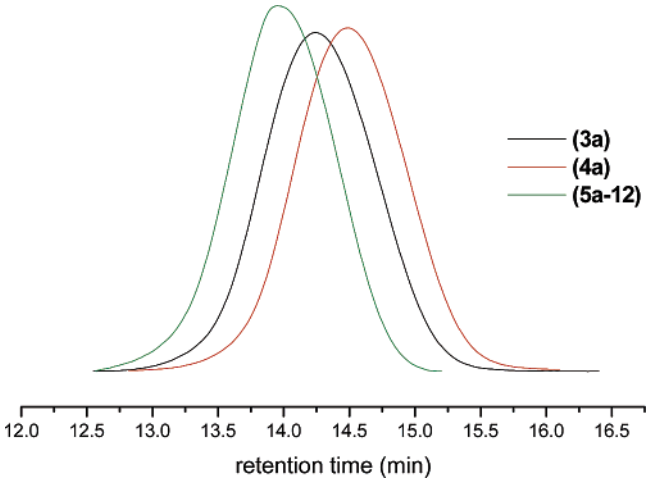
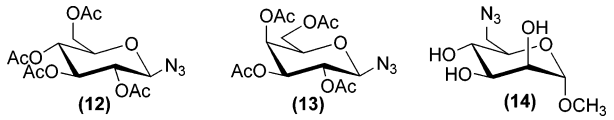


Figure 3. SEC analysis of the protected polymer **3a**, after removal of the trimethylsilyl group (**4a**), and after reaction with the glucose azide derivative **12**.

Chart 1. Azidosugar Derivatives Used in the Synthesis of Carbohydrate Comb Polymers



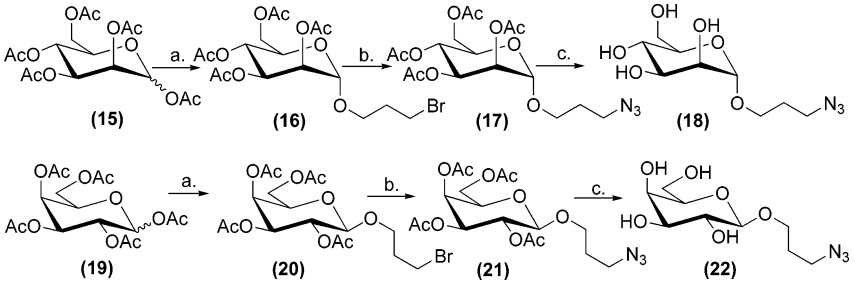
derivatives a substantial increase in the polymer hydrodynamic volume was observed (Figure 3). ^{1}H NMR and FT-IR analysis confirmed that the conversion of the alkyne groups into triazoles was achieved at close to 100% yield, while the molecular weight distribution of the polymers did not significantly change during both the deprotection and click reactions.

Table 2

polymer	precursor	azidosugar	M_n(NMR)	M_w/M_n(SEC)
5a−12	**4a**	**12**	27000	1.13
5a−13	**4a**	**13**	25500	1.13
8−12	**7**	**12**	10900	1.08
8−13	**7**	**13**	10800	1.08
11−14	**10**	**14**	12600	1.14

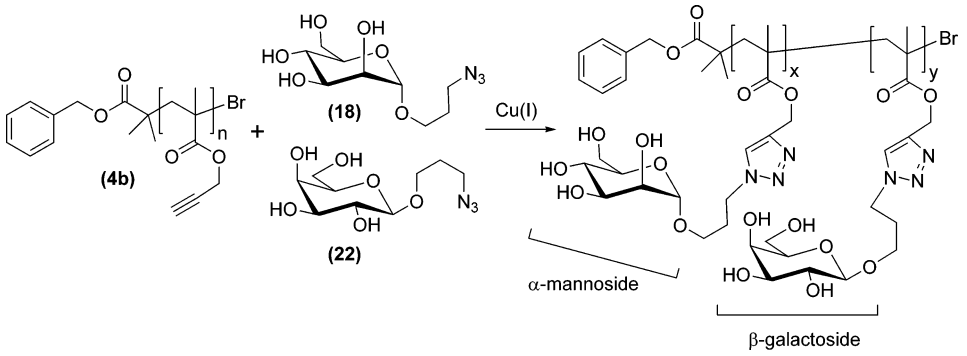
Synthesis of Multivalent Ligands and Preliminary Experiments in Lectin Conjugation Reactions. Subsequently, we implemented this synthetic strategy for the preparation of different classes of sugar polymers, and in particular, we focused our attention toward materials able to bind appropriate lectins. Concavalin A (Con A) was chosen as the model α-mannose-binding lectin, due to its involvement in a number of biological processes, with the bulk of literature focusing on both its chemical and its biological behavior.[79−83] Con A is an aggregate

Scheme 2 [a]



[a] Reagents and conditions: (a) 3-bromo-1-propanol, $BF_3{\cdot}OEt_2$, −20 °C to ambient temperature, (b) NaN_3, DMF, 100 °C, (c) CH_3ONa(cat.), CH_3OH, ambient temperature.

Scheme 3. Synthesis of the Polymers Employed for Con A Binding Studies, DP($x + y$) = 90



of 26 kDa monomeric units in higher order oligomeric structures. In the pH range 5.0−5.6, Con A exists exclusively as dimers, while at higher pH the dimers associate into tetramers, with the tetramer being the predominant form at pH 7.0.[44] Each monomeric unit possesses one coordination site with the ability to selectively bind α-gluco- and α-mannopyranoside derivatives, with the manno configuration at C2 preferred.[56,84]

The alkyne functional homopolymer **4b** was used as the starting material for the parallel synthesis of a library of polymers differing only in the amount of Con A-binding mannose ligand, obtained by "coclicking" reactions of appropriate mixtures of mannose- and galactose-based azides (Scheme 3). The purpose of this was to study the binding ability of these new materials with the Con A lectin. In particular, we were interested in deriving the influence the amount of mannopyranoside moieties has on the nature of polymer−protein interactions, in a study analogous to that described by Kiessling and co-workers for multivalent displays prepared by ROMP polymerization.[60]

The coclicking strategy is attractive as it allows for the preparation of a range of materials featuring identical macromolecular properties (polymer architecture, M_n, M_w/M_n) that only differ in their binding epitope density.[85] An added advantage related to the use of this approach is that this functionalization of the polyalkyne materials can be carried out under extremely mild conditions using nonexpensive starting materials easily obtainable on a multigram scale. β-Galactopyranoside units were employed to dilute the mannopyranoside epitopes present on the multivalent polymer ligands. Sugar azides are useful precursors normally employed for the synthesis of aminosugar derivatives and were prepared following the synthetic protocol shown in Scheme 2. Briefly, a peracetylated hexose, either manno- or galactopyranose, was treated with 3-bromo-1-propanol in the presence of $BF_3{\cdot}OEt_2$ to give the bromides **16** and **20**, respectively. The desired azide functional monomers **18** and **22** were obtained by conversion of the bromide intermediates into the corresponding azides **17** and **21** and subsequent removal of the acetate protecting groups.

Experimental conditions employed for the coclicking reactions were similar to those used for the synthesis of the polymers **5**, **8**, and **11** except that DMSO was employed as the solvent and triethylamine was used as the base.[86] SEC analysis of the product polymers in DMF eluent[87] showed that all of these polymers feature virtually identical molecular weights and molecular weight distributions. Again, the use of LALLS detection for the SEC analysis allowed us to determine the absolute weight average molecular weight (M_w) of the polydentate ligands (Table 3).[88] Again, the results obtained matched the theoretical values

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(86) DMSO was chosen as the solvent because of its ability to solubilize both the poly(propargyl methacrylate) starting material **4b** and the final clicked polymers. A number of many other different reaction conditions and catalysts [see, for example: (a) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6* (17), 2853−2855. (b) Lewis, W. G.; Magallon, F. G.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2004**, *126*, 9152−9153] could, in theory, be efficiently employed. Given the satisfactory results obtained with the $(PPh_3)_3CuBr$/triethylamine/DMSO catalyst system, no further investigation was carried out in this direction.
(87) All the multivalent ligands prepared are soluble in water, DMSO, and DMF.

Table 3. Multivalent Ligands: Composition and Macromolecular Features

polymer	[α-mannoside](%)	[β-galactoside] (%)	M_w/M_n[a]	M_w[b]	M_w(theor)[c]
23	100	0	1.10	37700	38600
24	75	25	1.10	39300	38600
25	50	50	1.10	37300	38600
26	25	75	1.10	37700	38600
27	0	100	1.10	38100	38600

[a] Obtained by SEC analysis using DMF as the eluent with DRI detection. [b] Obtained by SEC analysis using DMF as the mobile phase with LALLS detection. [c] M_w(theor) = $(M_w/M_n)M_n$(theor).

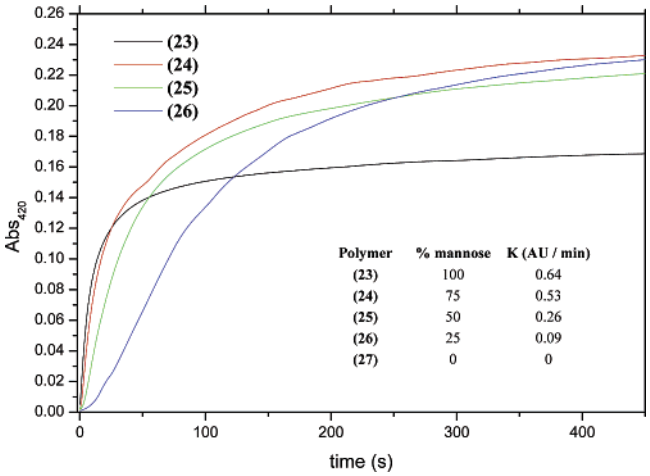

Figure 4. Turbidimetry assay results.

expected for the glycopolymer products. ^{1}H NMR analysis confirmed that the molar ratio of the two different sugar moieties in the polymers was essentially analogous to the **18**:**22** initial ratio employed in the coclicking reactions.

In biological processes the rate of the clustering events occurring at the cell surface is a crucial parameter, with a time scale that ranges from seconds to hours.[60] The influence of the epitope density on the rate of ligand−lectin aggregation was assessed by a turbidimetric assay,[60,89−91] and the results are shown in Figure 4. The clustering rates of Con A in the presence of an excess of different multivalent ligands was monitored by measuring changes in the absorbance at λ = 420 nm of appropriate solutions of the lectin and functional polymers in HEPES buffer at pH 7.4. In the case of the fully mannose-functionalized polymer **23** the absorbance reached a plateau and remained almost constant until the end of the measurement, indicating that this multivalent ligand was able to quickly precipitate virtually all of the Con A present in solution.

When polymers featuring a lower epitope density were used, the observed absorbance increased continuously with time, consistent with that previously described by Kiessling for macromolecular ligands obtained by ROMP which was ascribed to higher order aggregation of partially soluble conjugates formed in the early stages of measurement.[60] The initial clustering rates were employed for the determination of aggregation rate constants, expressed as arbitrary units per minute (AU/min). The values obtained indicate that under these experimental conditions the rate of the clustering process decreased with a decrease in epitope density. A control experiment using the multivalent ligand **27** revealed that the fully galactopyranose-functionalized ligand was unable to precipitate the Con A lectin. Aggregates formed in these experiments were then treated with a large excess of α-methyl mannopyranoside, a competitive monodentate ligand, and the decrease in the absorbance with time was monitored. The stability of the polymer−lectin conjugates was directly proportional to the polymer epitope density (see the Supporting Information).

Table 4. Quantitative Precipitation Assay Results for Con A with Mannose-Containing Polymers

polymer	[mannose] (%)	no. of Con A units per polymer chain	mannose:Con A
23	100	15	6.0
24	75	15	4.5
25	50	11	4.0
26	25	7	3.2

Quantitative precipitation (QP) experiments were carried out to determine the stoichiometry of the polymer−Con A conjugates. Measurement of the polymer concentration necessary to quantitatively precipitate the lectin from a solution with a known concentration of Con A allowed the determination of the average number of Con A tetramers bound by each polymer chain.[92] This was found to increase with an increase in the mannose content increasing from 0 to 75% (Table 4).[93] Beyond this value the number of bound lectin tetramers appeared to remain constant, presumably indicating that for high epitope density steric effects may hamper further lectin coordination.[60]

Synthesis of Fluorescent Multivalent Displays. The versatility of the strategy developed allows, in principle, addition of a number of further different functionalities into the polymer backbone, as long as the derivatives carrying the desired function contain the required azide group. The possibility of coclicking a visibly fluorescent tag in the polymeric scaffold appeared attractive, as the resulting multivalent ligands presenting both binding elements and a reporter unit are known for being extremely useful in protein−carbohydrate binding interaction studies.[94,95] Fluorescent glycopolymers have been employed in a range of applications including cell surface interactions,[96,97] anticancer therapy,[98] lectin recognition analysis,[99,100] PEGylation chemistry,[101] L-selectin binding,[94] and spermatozoa stability studies.[102] Living radical polymerization can be used to prepare visibly fluorescent polymers by employing either a fluorescent initiator or a monomer.[103]

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Scheme 4. Synthesis of Fluorescent Multivalent Ligands **29** and **30**

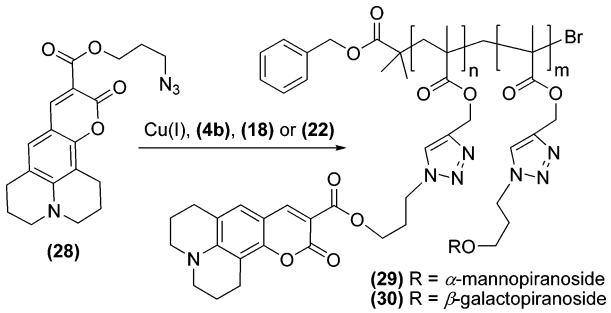

Table 5. Properties of the Fluorescent Polymers Prepared in This Study: Composition and Macromolecular Features

polymer	[α-mannoside](%)	[β-galactoside](%)	M_w/M_n[a]	M_w[b]	M_w(theor)[c]
29	100	0	1.15	38800	40400
30	0	100	1.16	38100	40700

[a] Obtained by SEC analysis using DMF as the eluent with DRI detection. [b] Obtained by SEC analysis using DMF as the mobile phase with LALLS detection. [c] $M_w(\text{theor}) = (M_w/M_n)M_n(\text{theor})$.

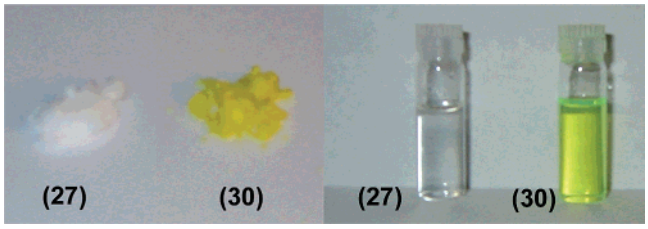

Figure 5. Galactose-containing multivalent displays: nonfluorescent (**27**) and fluorescent (**30**) polymers (0.5 mg mL^{-1} solutions in DMSO).

We chose to continue to use the benzyl bromoester **2** as the initiator, as it allowed us to determine the molecular mass M_n(NMR) of the corresponding polymers, and the visibly fluorescent tag was introduced via azide **28** (obtained in one step from coumarin 343 and 3-azido-1-propanol), 2.5% mol/mol in the reaction feed, along with the unprotected azidosugars **18** and **22**. An important advantage in using dye **28** is that only a relatively small percentage was required to confer high fluorescence to the multivalent ligands.

Polymers **29** and **30** (Scheme 4) showed a maximum absorbance at $\lambda = 436$ nm with maximum emission at $\lambda = 485$ nm, showing a Stokes shift of 49 nm. Grafting of azide **28** onto the polymer backbone occurred with virtually no change in the glycopolymer macromolecular features, with **29** and **30** being analogous, apart from their fluorescence behavior, to polymers **23** and **27**, respectively (Table 5 and Figure 5).

The potential of these fluorescent glycopolymers as multivalent ligands was then qualitatively assessed. Solutions of the fluorescent displays were analyzed by HPLC using a column packed with immobilized *Ricinus communis* agglutinin I (RCA I), a 120 kDa dimeric lectin isolated from *R. communis* (castor bean), as the stationary phase,[104] with a fluorescence HPLC detector. RCA I interacts selectively with β-D-galactose units

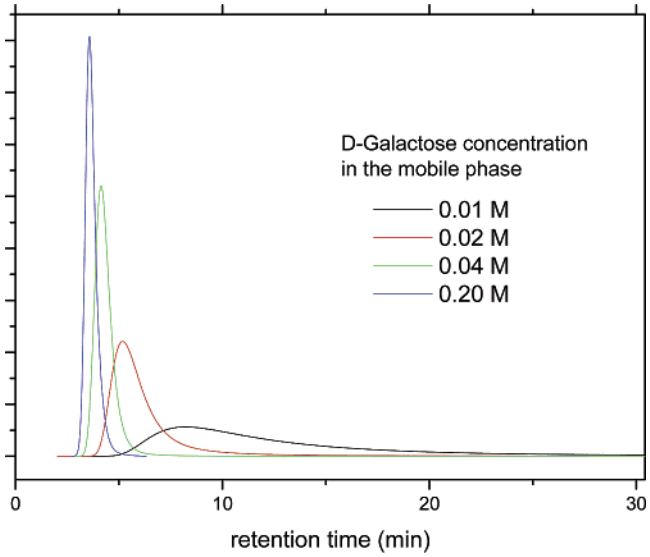

Figure 6. Affinity HPLC analysis of the ligand **30** using an immobilized RCA I lectin-packed column. Conditions: 66.7 M PBS (pH 7.4), 150 mM NaCl, ambient temperature. Different concentrations of D-galactose in the mobile phase were employed in each run. For comparative purposes the areas of all of the chromatograms have been normalized with respect to concentration.

and was therefore chosen as a conjugating substrate that could complement the results previously obtained using α-D-mannose-binding Con A.

Preliminary attempts using 0.067 M PBS (pH 7.4) and 0.15 M NaCl as the mobile phase showed that while the mannoside-based polymer **29** was not retained by the column, the ligand **30** interacted with the RCA I stationary phase sufficiently strongly that it was not eluted at all. Thus, several further mobile phases containing D-galactose in different concentrations were utilized. Due to its monotopic nature, D-galactose can only weakly interact with the RCA I receptors. However, if present in large excess, it competes with the multivalent display **30** for RCA I coordination, and the use of decreasing galactose concentrations in the mobile phase resulted in increasing retention times, with peaks featuring a typical broad shape with tailing (Figure 6). These results indicate that the galactose-containing display **30** is able to strongly interact with RCA I, and the use of similar synthetic glycopolymers as multidentate ligands will be a subject for further investigation.

Conclusion

In summary, a novel series of comb sugar polymers have been prepared by Huisgen 1,3 dipolar cycloaddition of appropriate sugar azides with poly(methacrylates) bearing terminal alkyne functionalities. These clickable materials have been prepared by TMM-LRP of (trimethylsilyl)propargyl methacrylate with excellent control over the polymer properties, with M_w/M_n of the purified products between 1.09 and 1.16. Removal of the TMS protecting groups was carried out under mild conditions with full retention of the terminal alkyne groups. The grafting of protected and unprotected carbohydrates via either a C-6 or an α or β anomeric azide onto the polymer backbone was successfully performed via a Cu(I)-catalyzed click reaction.

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A number of mannose- and galactose-containing multidentate ligands for lectin binding studies were prepared by simultaneously reacting different sugar azides onto a polyalkyne methacrylate backbone. This coclicking approach successfully coupled the advantages of controlled radical copolymerization with a highly efficient postfunctionalization process, leading to a library of multivalent displays that only differ in their epitope density. In addition, fluorescent ligands were prepared by simply adding a visibly fluorescent azide tag, namely, a coumarin 343 derivative, to the reaction mixture. Their behavior was then tested in the presence of model lectins able to selectively bind mannose (Con A) and galactose (RCA I) units. In the case of Con A this study showed that both the clustering rate and the stoichiometry of the polymer−protein conjugates depend on the epitope density of the displays employed.

The synthetic strategy proposed is quite general, as the protocol that had been successfully employed for the synthesis of sugar polymers can in principle be applied to a wide range of functional molecules, even ones containing functionalities that are not compatible with the conditions employed in TMM-LRP, opening the way for the synthesis of a wide range of precision materials. Moreover, in view of the biological application of carbohydrate polymers analogous to those described in the present work, the strategy appears to be an extremely powerful tool for the synthesis of libraries of materials that differ only in the nature of the sugar moiety presented on a well-defined polymer scaffold.

Acknowledgment. This research was supported by a Marie Curie Intra-European Fellowship within the 6th European Community Framework Programme (G.M., Grant MEIF-CT-2003-501305). We thank the University of Warwick (V.L.) for funding.

Supporting Information Available: Text and schemes giving the synthesis and characterization of sugar and fluorescent intermediates, polymerization procedures and polymer characterization, and lectin−polymer interaction study results. This material is available via the Internet at http://pubs.acs.org.

JA058364K