JOC The Journal of Organic Chemistry

Article

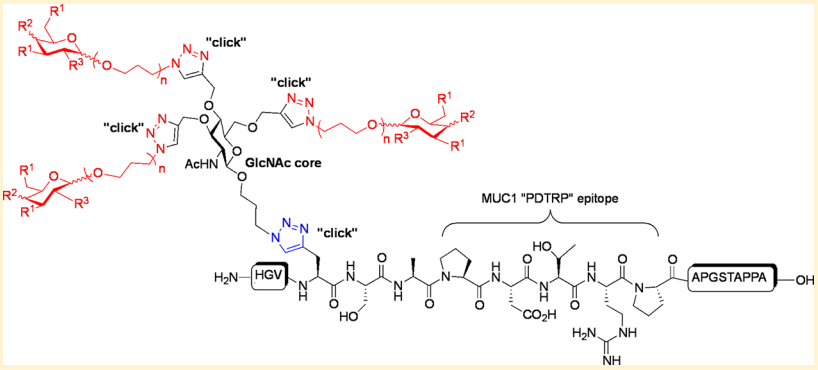
pubs.acs.org/joc

Synthesis of Multivalent Neoglyconjugates of MUC1 by the Conjugation of Carbohydrate-Centered, Triazole-Linked Glycoclusters to MUC1 Peptides Using Click Chemistry

Dong Jun Lee, Sung-Hyun Yang, Geoffrey M. Williams, and Margaret A. Brimble*

School of Chemical Sciences, The University of Auckland, 23 Symonds Street, Auckland, New Zealand

Supporting Information



ABSTRACT: The efficient synthesis of multivalent neoglycoconjugates of MUC1 is reported, which utilizes Cu(I)-catalyzed azide−alkyne 1,3-dipolar cycloaddition (CuACC) of azide-functionalized GlcNAc-centered neoglycotetrasaccharide clusters to the MUC1 peptide sequence that was equipped with a propargylglycine residue for "click chemistry". In turn the azido-GlcNAc-centered neoglycoclusters were assembled by reaction of a GlcNAc core containing peripheral propargyl functionalities with an appropriate azido-functionalized monosaccharide. The resulting suitably substituted tetrasaccharyl triazole cluster can be easily appended to a range of acetylene-functionalized peptides to produce neoglycoconjugates of biologically important glycopeptides. As proof of principle, the click neoglycoclusters prepared herein were ligated to the MUC1 peptide sequence.

INTRODUCTION

Cell-surface oligosaccharides play a vital role in biological processes such as cell adhesion, signal transduction, inflammation, cancer metathesis as well as bacterial and viral infection.[1−3] Carbohydrates mediate specific multivalent interactions with soluble or membrane proteins called lectins.[4] However, our understanding of the carbohydrate interactions with their lectin receptors is still limited, mainly due to the complexity and difficulty in obtaining homogeneous samples in sufficient quantities for biological testing and analysis. Total chemical synthesis of complex glycoconjugates also remains a major challenge, mainly hindered by the necessity to carry out extensive protection/deprotection of hydroxyl groups, and glycosylation reactions are also usually inconsistent and poor yielding. To further complicate matters, monovalent interactions between the carbohydrates and their receptors are usually weak (the K_d value being ca. 10^{-3}−10^{-6} M) and unspecific. In nature this weak monovalent binding is compensated by multiple interactions between "clusters" of glycans and receptors resulting in high selectivity and strong binding, a phenomenon now often referred to as the "glycoside cluster effect".[5] For these reasons, synthetic chemists have strived to prepare artificial multivalent carbohydrate models or glycomimics[6] by conjugation of the carbohydrate ligand moiety to different scaffolds to probe the specific interactions involved in these carbohydrate−protein bindings. A variety of multivalent architectures[7] have been explored such as polymers,[8] peptide dendrimers,[9] poly(amidoamine) (PAMAM) dendrimers,[10] cyclopeptides,[11] oligonucleotides,[12] cyclodextrins,[13] calixarenes,[14] silsesquioxanes,[15] fullerenes,[16] nanoparticles,[17] liposomes and beads.[6] These glycomimics offer exciting opportunities for biotechnology, pharmaceutical and medical applications[6] including the prevention of early adhesion of neutrophils to endothelial surfaces,[18] inhibitors of bacterial adhesion,[19] neutralization of viruses and toxins,[20] immunomodulators and angiogenesis inhibitors,[21] and carbohydrate-based anticancer therapy.[22]

Our research group[23] is interested in the synthesis of glycopeptide mimetics using Cu(I)-catalyzed azide−alkyne cycloaddition[24] ("click chemistry"). We recently reported the synthesis of a series of click analogues of the MUC1 glycopeptide.[23c] The membrane-bound tumor-associated MUC1 glycoproteins occur ubiquitously in epithelial tissues of most organs and are also excessively expressed on tumor tissue.[25] They are also involved in cell signaling events, and

Received: June 28, 2012
Published: August 9, 2012

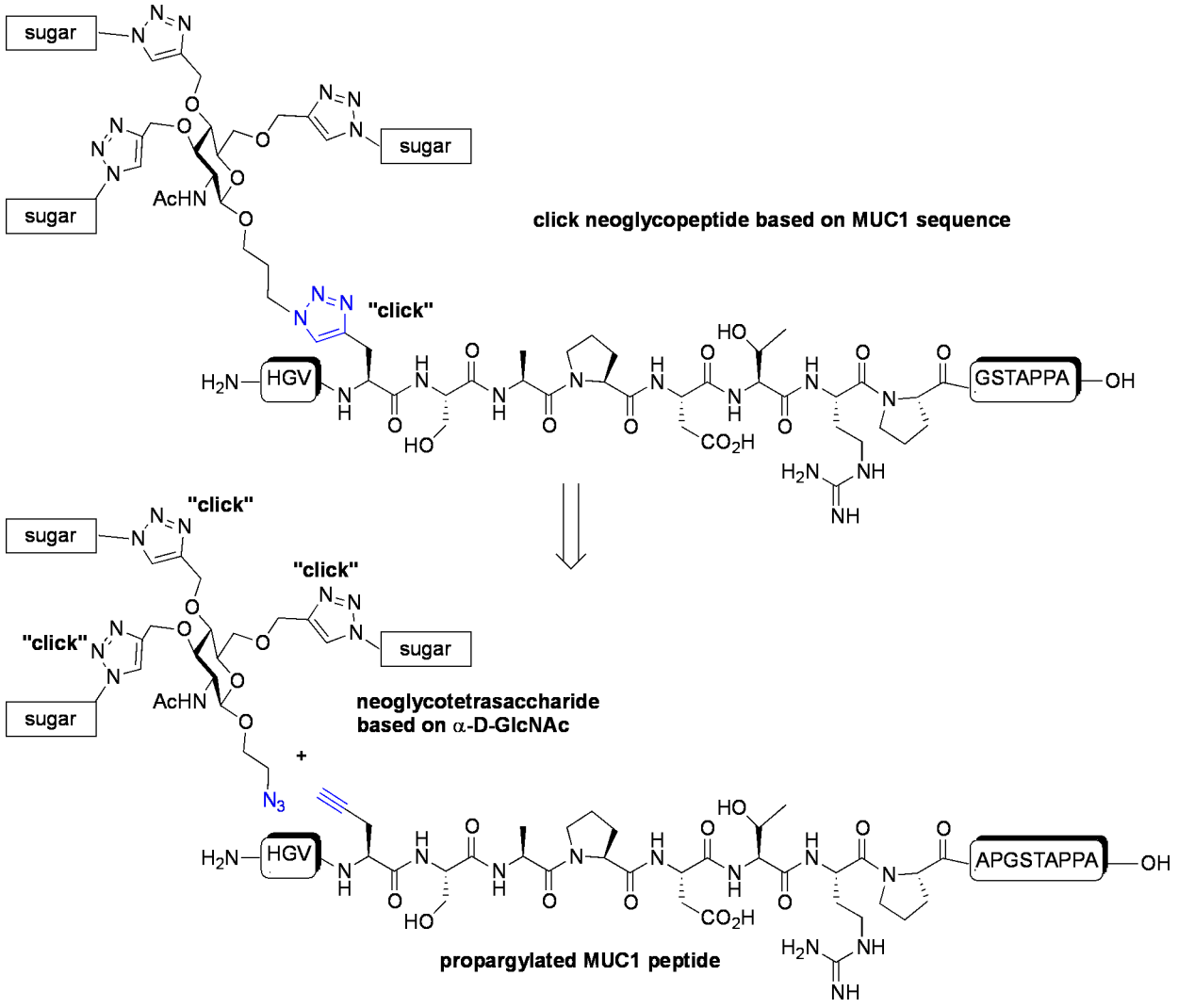


Figure 1. Structures of MUC1 neoglycopeptides prepared by appendage of different neoglycotetrasaccharides to the MUC1 peptide sequence.

their aberrant expression facilitates the metastatic spread of tumor cells. A number of excellent developments using MUC1 glycopeptides as immunotherapeutics have been made, notably by the Kunz and Payne groups.[26]

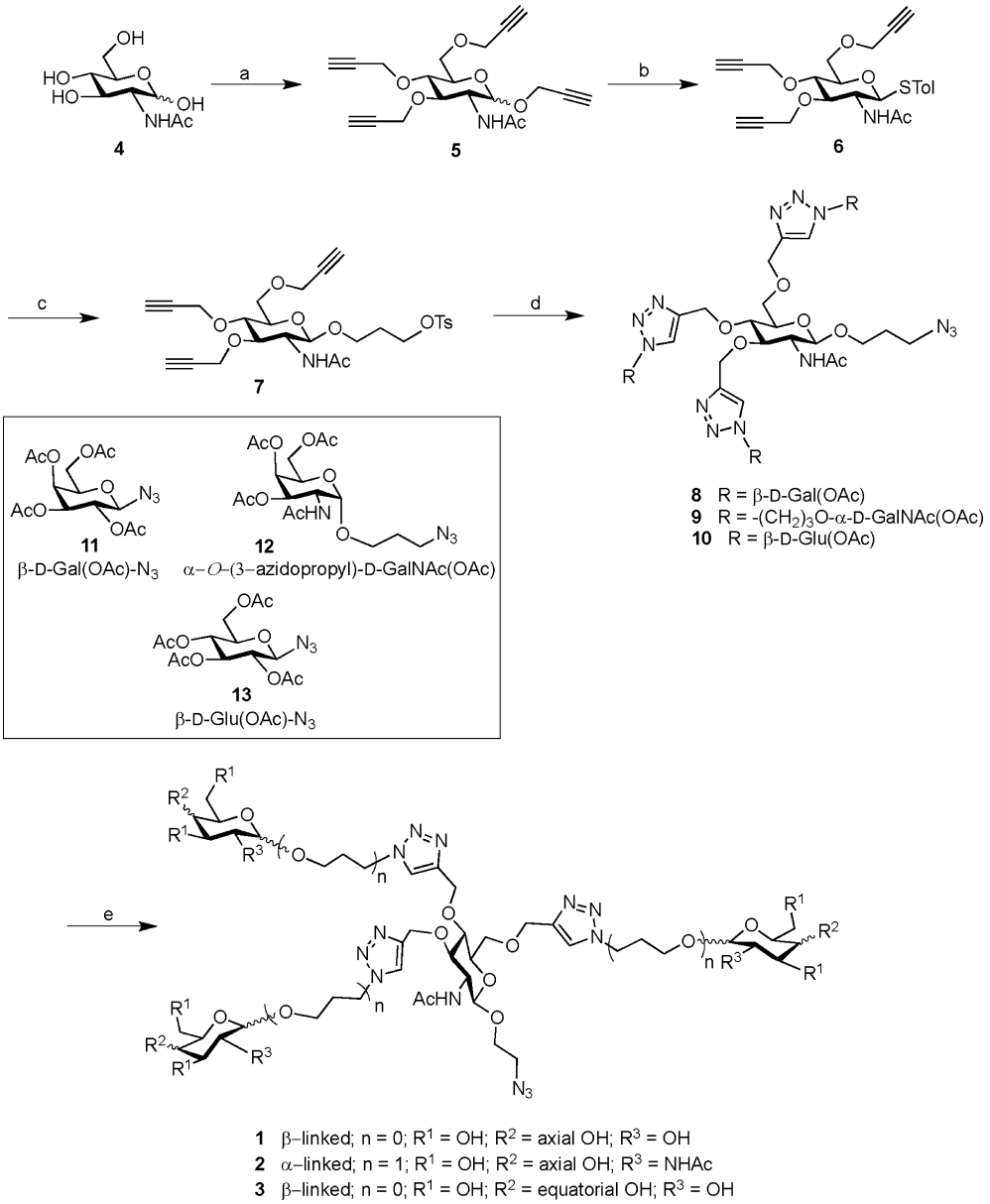
As part of our continued endeavor[23] to develop methods to obtain highly glycosylated MUC1 peptides more easily, our synthetic program resulted in the successful synthesis of a "penta-clicked" MUC1 neoglycopeptide assembled by addition of a pentapropargylglycine-substituted peptide to a monomeric sugar-azide mimic of the natural threonine or serine *O*-linked *N*-acetylgalactosamine (T_N antigen). Importantly, the click chemistry approach affords a focused library of neoglycopeptides of defined structures, thereby overcoming the purification problems of working with native glycoproteins that often exist as a variety of glycoforms. Furthermore, it is a chemoselective reaction, thus avoiding the necessary iterative deprotection/protection steps required for the synthesis of native glycopeptides.

Optimization of the binding affinity and/or potency of glycoclusters depends on the nature and number of the carbohydrate moieties. Accordingly, a wide variety of scaffolds of varying topology, valency and density for attaching appropriate carbohydrate motifs have been investigated.[7−16] However, tuning the carbohydrate structure still requires addressing problems associated with the inherent difficulties in synthesizing highly glycosylated molecules such as chemical incompatibility, poor regio- and stereoselectivity, incomplete reactions, and purification issues. One approach to achieve even stronger binding of glycoclusters to their native lectins is to increase the density of the monosaccharide units on the carbohydrate binding motif that is appended to a peptide scaffold thereby improving the chances of initiating the binding event. We therefore herein report our investigations into the synthesis of MUC1 neoglycopeptide analogues decorated with more complex "click neoglycotetrasaccharides" (Figure 1). In essence click chemistry is used to prepare both the carbohydrate ligand binding motifs and to append these motifs to a peptide scaffold thus demonstrating a powerful synthetic armory for the generation of structurally diverse complex neoglycopeptide clusters.

Several groups have used click chemistry for the synthesis of a diverse range of oligosaccharide glycoclusters. Examples include the facile synthesis and molecular recognition of carbohydrate-centered multivalent glycoclusters by a plant lectin RCA_{120}.[27] Santoyo-Gonzalez et al.[28] reported an extensive study on the synthesis of click multivalent neoglycoconjugates as activators in cell adhesion and stimulation of monocyte/macrophage cell lines. Linhardt and Bera[29] reported

dx.doi.org/10.1021/jo3013435 | *J. Org. Chem.* 2012, 77, 7564−7571

Scheme 1[a]



[a]Reagents and conditions: (a) NaH, DMF, rt, 2 h and then 0 °C, propargyl bromide, 10 min, 58% (α:β = 1:3); (b) *p*-thiocresol, CH_2Cl_2, reflux, 16 h, 71%; (c) 3-hydroxyprop-1-yl-*p*-toluenesulfonate, AgOTf, NIS, 4 Å MS, CH_2Cl_2, 0 °C → rt, 58%; (d) (i) sugar azide **11**, **12** or **13**, $CuSO_4{\cdot}5H_2O$, sodium-L-ascorbate, CH_2Cl_2:H_2O:^{t}BuOH (1:1:1, v/v/v), μW (100 W), 15 min; (ii) NaN_3, MeCN:H_2O (4:1, v/v), reflux, 5 h, **8** (89% over 2 steps), **9** (89% over 2 steps), **10** (85% over 2 steps); (e) 1 M NaOMe, MeOH, rt, 1 h and then Dowex resin, 0.5 h, **1** (95%), **2** (95%), **3** (95%).

the synthesis of unnatural heparosan and chondroitin building blocks using both linear and convergent click chemistry. Importantly, none of these glycoclusters were appended to a peptide scaffold using click chemistry.

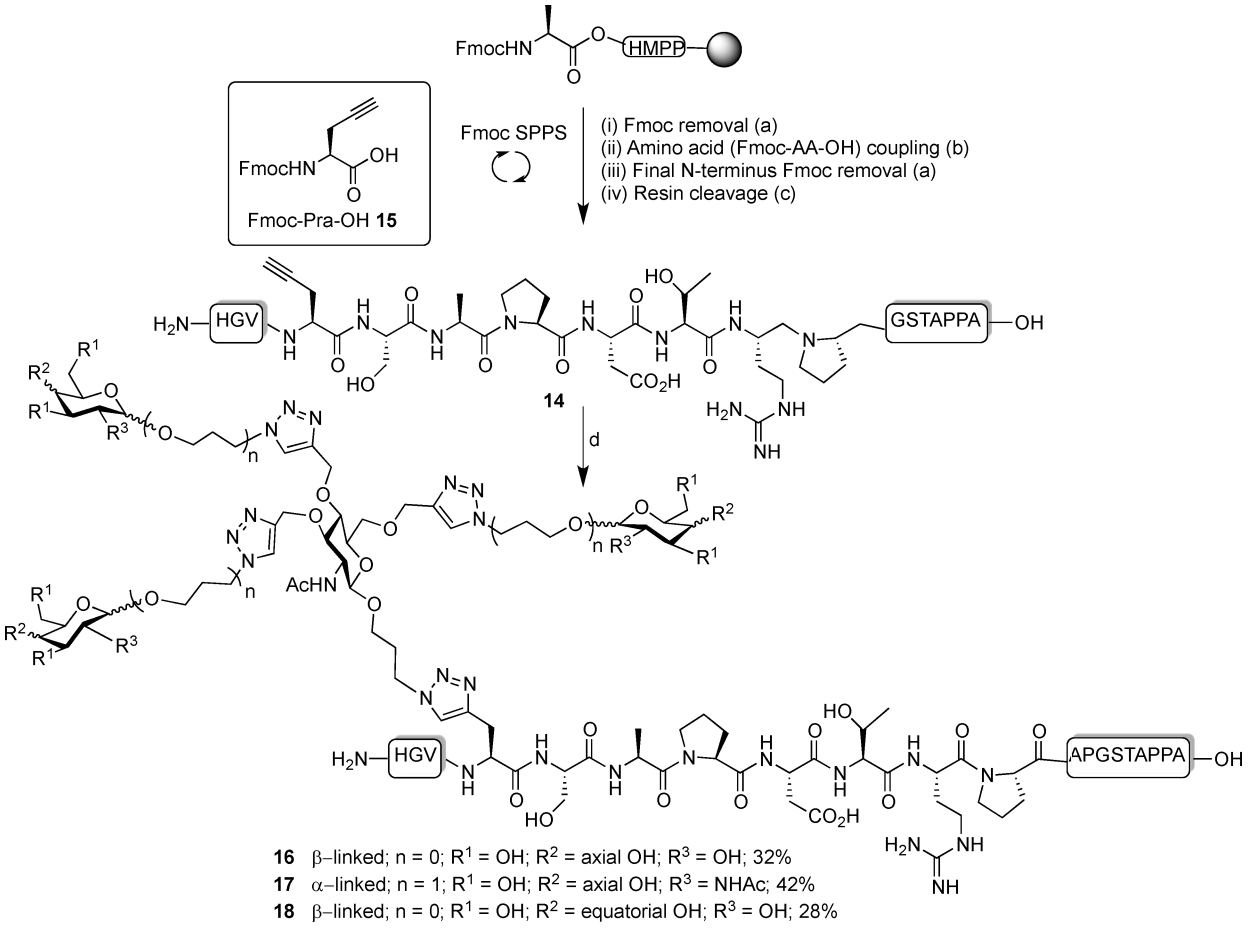
RESULTS AND DISCUSSION

As part of our continuous search for improved methods to prepare glycopeptide mimetics that exhibit similar or better biological activity than the native glycopeptides/glycoproteins, we designed an efficient method to not only synthesize complex tetrasaccharyl neoglycan moieties but also to append the tetrasaccharyl neoglycans to a peptide backbone. To showcase our concept, we herein describe the synthesis of MUC1 neoglycopeptides containing various carbohydrate-centered glycoclusters conjugated to the peptides via a triazole linkage. We also report an efficient method for the preparation of tetrasaccharyl neoglycoclusters that have different types of monosaccharides in the periphery, which is usually where receptor-recognition takes place.

The synthetic route to our carbohydrate-centered neoglycoclusters is depicted in Scheme 1. *N*-Acetyl-D-glucosamine (GlcNAc) armed with peripheral propargyl groups was used as the core molecule as most oligosaccharides present in glycopeptides extend from this core, which in turn is *N*-linked to the peptide backbone. Thus, three azido-functionalized unprotected neoglycoclusters **1**, **2**, and **3** each bearing three triazole-linked β-D-galactosyl, α-1-*O*-propyl-D-*N*-acetylgalactosyl, and β-D-glucosyl moieties respectively, were synthesized ready for direct click conjugation onto a propargylated MUC1 peptide sequence.

dx.doi.org/10.1021/jo3013435 | J. Org. Chem. 2012, 77, 7564−7571

Scheme 2[a]



[a]Reagents and conditions: (a) 20% (v/v) piperidine in DMF, μW (25 W); (b) Fmoc-Aaa–OH (incl. **15**), HBTU, DIPEA in DMF, μW (25 W); (c) TFA:TIS:H_2O (38:1:1, v/v/v), 2.5 h; (d) $CuSO_4{\cdot}5H_2O$ (20 mM), TCEP (20 mM), 6 M Gn·HCl/0.2 M Na_2HPO_4 (pH 7.2), μW (25 W), **1**, **2**, or **3**.

The synthesis of neoglycoclusters **1**, **2** and **3** started from readily available D-GlcNAc (**4**) (Scheme 1) that was reacted with sodium hydride and propargyl bromide in DMF at room temperature for 2 h to afford tetra-*O*-propargyl derivative **5** as a mixture of α:β anomers (1:3). Treatment of this mixture with *p*-thiocresol in dichloromethane under reflux for 16 h resulted in formation of β-thioglycoside (**6**) in 71% yield. Glycosylation of **6** with 3-hydroxyprop-1-yl-*p*-toluenesulfonate, activated by silver(I) triflate and *N*-iodosuccinimide in dichloromethane in the presence of 4 Å molecular sieves at 0 °C afforded tosylate (**7**) in 58% yield. The three propargyl groups in **7** then underwent smooth Cu(I)-catalyzed 1,3-dipolar cycloaddition with monosaccharyl azides **11**, **12**, or **13**, respectively, using $CuSO_4{\cdot}5H_2O$, sodium-L-ascorbate, CH_2Cl_2:H_2O:^{t}BuOH (1:1:1, v/v/v) using microwave irradiation (100 W) for 15 min followed by displacement of the tosylate using sodium azide in acetonitrile:H_2O (4:1) under reflux for 5 h to afford azides **8**, **9** and **10** in 85–89% yield over two steps. The previously reported monosaccharyl azides **11**,[30,31] **12**[23c] or **13**[32] in turn were readily prepared from the corresponding per-*O*-acylated sugars using known procedures. Finally global deprotection of the acetate groups on all of the three monosaccharides in neoglycoclusters **8**, **9** and **10** afforded the corresponding unprotected neoglycoside clusters **1**, **2** and **3** in 95% yield.

With neoglycoclusters **1**, **2** and **3** in hand, attention turned to their subsequent ligation with **14**, a propargylated peptide derivative of the MUC1 sequence (Scheme 2). The peptide **14** was synthesized on amino-functionalized polystyrene resin containing the acid labile hydroxymethylphenoxypropionic acid (HMPP) linker using microwave enhanced Fmoc SPPS.[23c] Using the conditions shown (Scheme 2), Fmoc-L-propargylglycine **15**[23d] was incorporated into the peptide chain as a replacement for the threonine residue, a site of glycosylation in the native MUC1 sequence.

Initial attempts to carry out click reactions between the azide-functionalized neoglycotetrasaccharides **1**, **2** and **3** and propargyl-containing MUC1 peptide **14** using catalytic amounts of $CuSO_4$ and sodium ascorbate in aqueous phosphate buffer were unsuccessful, even at elevated temperatures using microwave irradiation. Therefore these click reactions were carried out using protocols previously developed within our research group.[23c] $CuSO_4{\cdot}5H_2O$ (20 mM) and TCEP (20 mM) were premixed with heating before addition to the peptide **14** (3 mM) in 6 M guanidine hydrochloride (GnHCl)/0.2 M Na_2HPO_4 solution at pH 7.2. Click reactions on peptide **14** with neoglycosyl azides **1**, **2** and **3** proceeded cleanly under microwave conditions (25 W) at 50 °C for 5 h to afford the desired click neoglycopeptides **16**, **17** and **18** in excellent yields (Scheme 2 and Figure 2).

dx.doi.org/10.1021/jo3013435 | J. Org. Chem. 2012, 77, 7564–7571

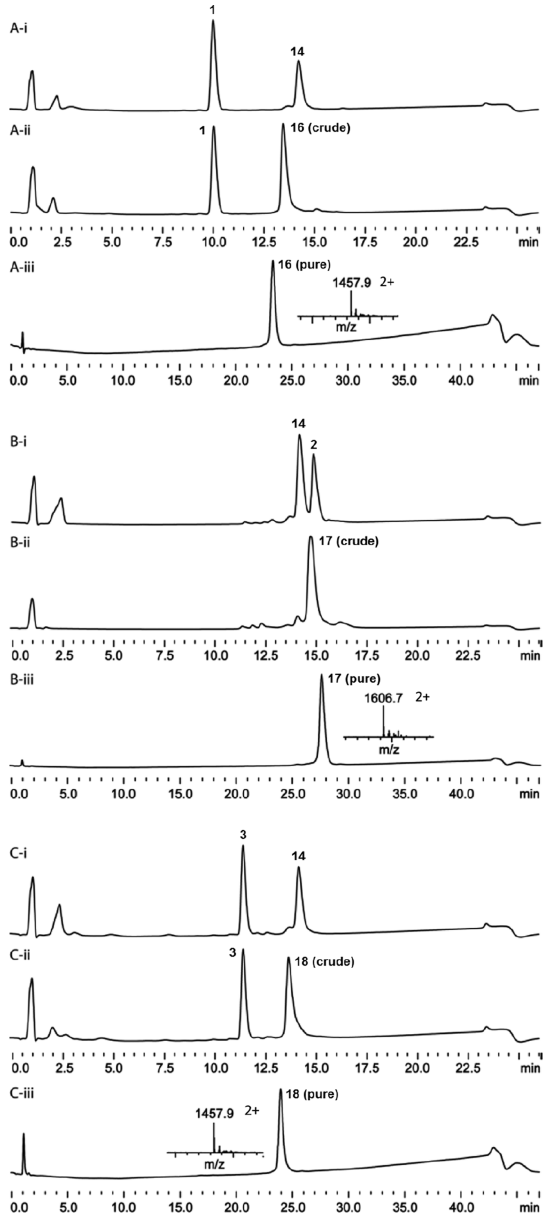


Figure 2. HPLC traces for click reactions between neoglycotetrasaccharides **1**, **2** and **3** and propargyl-containing MUC1 peptide **14**. (A) Click reaction between **1** and **14**: (i) t = 1 min; (ii) t = 180 min; (iii) purified (m/z (2^+): 1457.9). (B) Click reaction between **2** and **14**: (i) t = 1 min; (ii) t = 300 min; (iii) purified (m/z (2^+): 1606.7). (C) Click reaction between **3** and **14**: (i) t = 1 min; (ii) t = 180 min; (iii) purified (m/z (2^+): 1457.9). Column: Gemini C18 (5 μ; 2.0 × 50 mm) column (Phenomenex). Gradient: Ai–ii, Bi–ii and Ci–ii were run at 0.2 mL/min with a gradient of 5–65% B over 25 min, and Aiii, Biii and Ciii were run at 0.2 mL/min with a shallow gradient of 5–45% B over 40 min. The solvent system used was A (0.1% TFA in H_2O) and B (0.1% TFA in MeOH).

CONCLUSIONS

In conclusion, a full account of our synthetic efforts culminating in the successful completion of MUC1 neoglycopeptides **16**, **17** and **18** has been reported herein. The versatility and efficiency of this methodology using multiple applications of the Cu(I)-catalyzed 1,3-dipolar cycloaddition of alkynes and azides provides a useful synthetic strategy for convenient assembly of neoglycan containing peptides using a modular approach. Importantly, the neoglycoconjugates thus prepared have a well-defined architecture, and the nature of the saccharide epitope can also be varied. The flexibility and efficiency of the "click chemistry" used to prepare these well-defined and custom-made click multivalent neoglycoconjugates provide a powerful tool for the development of neoglycoconjugates with therapeutic and materials science applications.

EXPERIMENTAL SECTION

General Methods. Unless stated, all solvents and reagents were used as supplied from commercial sources. Aminomethyl polystyrene resin was synthesized using literature methods.[33] Analytical thin layer chromatography (TLC) was performed using Kieselgel F254 0.2 mm silica plates with visualization by ultraviolet irradiation (254 nm) followed by staining with potassium permanganate or vanillin. Flash chromatography was performed using Kieselgel S 63–100 μm silica gel. NMR spectra were recorded at room temperature in deuterated solvents operating at 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei. Unless otherwise noted the chemical shifts were referenced to δ 7.26 and 77.0 ppm from chloroform for ^{1}H and ^{13}C, respectively. The multiplicities of ^{1}H signals are designated by the following abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. All coupling constants J are reported in Hertz. All ^{13}C NMR spectra were acquired using broadband decoupled mode, and assignments were determined using heteronuclear correlation spectra. Infrared spectra were obtained as neat samples, and absorption maxima are expressed in wavenumbers (cm^{-1}) and recorded using a range of 450 to 4000 cm^{-1}. Melting points were recorded on an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured at 20 °C at λ = 598 nm and are given in units of deg cm^3 g^{-1} dm^{-1} with the concentration of the solution measured in grams per 100 mL. High resolution mass spectra (HRMS) were recorded on a mass spectrometer at a nominal accelerating voltage of 70 eV. High performance liquid chromatography (HPLC) was performed using an analytical 50 × 2.00 mm Phenomenex column with a guard column attached using the conditions outlined in the relevant experimental procedure. Semipreparative RH-HPLC was performed using 250 × 10.0 mm Phenomenex column.

General Procedure for SPPS of Peptides Following the Fmoc Strategy. Solid phase peptide synthesis was performed using a Liberty Microwave Peptide Synthesizer (CEM Corporation, Mathews, NC) using the Fmoc/*t*-Bu strategy. The Fmoc group was deprotected with 20% v/v piperidine in DMF for 30 s followed by a second deprotection for 3 min using a microwave power of 60 W for both deprotections. The maximum temperature for both deprotections was set at 75 °C. The coupling step was performed with 5 equiv of Fmoc protected amino acid in DMF (0.2 M), 4.5 equiv of HBTU in DMF (0.45 M) and 10 equiv of DIPEA in NMP (2 M). The building block Fmoc-Pra–OH **15** (0.11 g, 1.5 equiv) in DMF (2 mL) was manually added, and the following coupling conditions were used: 1.45 equiv of HATU, 1.5 equiv of HOAt, 5.0 equiv of 2,4,6-collidine and catalytic amounts of DMAP. All couplings were performed for 5 min at 25 W at a maximum temperature of 75 °C except for the following amino acids: Fmoc-Arg(Pbf)–OH was double coupled using a 25 min room temperature coupling followed by a 5 min period at 25 W; Fmoc-Pra–OH **15** couplings were performed for 20 min at 25 W, at a maximum temperature of 75 °C. Following completion of the sequence, peptides were released from the resin with concomitant removal of protecting groups by treatment with TFA/TIPS/H_2O (38/1/1, v/v/v) at room temperature for 2.0 h. The crude peptide was triturated with cold diethyl ether, isolated by centrifugation, washed with cold diethyl ether, dissolved in 1:1 (v/v) acetonitrile:H_2O containing 0.1% TFA, and then lyophilized.

1,3,4,6-Tetra-O-propargyl-2-acetamido-2-deoxy-α,β-D-glucopyranoside ***5***. To a suspension of D-GlcNAc **4** (1.0 g, 4.52 mmol) in DMF (20 mL) at room temperature was added sodium hydride (60% in mineral oil, 1.1 g, 27.1 mmol), and the mixture was left to stir for 2 h. The reaction mixture was cooled down to 0 °C followed by addition of propargyl bromide (80% in toluene, 4.7 mL, 31.6 mmol), and then

dx.doi.org/10.1021/jo3013435 | J. Org. Chem. 2012, 77, 7564–7571

stirring continued for a further 10 min. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with H_2O (3 × 20 mL). The organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. Purification by silica gel flash column chromatography using dichloromethane/methanol (98:2) as eluent afforded an inseparable anomeric mixture of compound **5** as a brown solid (0.98 g, 58%, α:β anomer 1:3). This compound was used in the next step without further purification of anomers.

p-Tolyl 3,4,6-tri-O-propargyl-2-acetamido-2-deoxy-β-D-1-thioglucopyranoside ***6***. To a solution of tetra-propargylated sugar **5** (2.5 g, 6.69 mmol) in dichloromethane (50 mL) at room temperature was added *p*-thiocresol (4.16 g, 33.4 mmol) in dichloromethane (25 mL), and the reaction mixture was heated under reflux for overnight. The reaction mixture was quenched by the slow addition of saturated aq $NaHCO_3$ (30 mL), and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. Purification by silica gel flash column chromatography using dichloromethane/methanol (99:1) as eluent afforded compound **6** as an off-white solid (2.1 g, 71%): mp 154.6−156.1 °C; $[\alpha]_D^{20}$ = +5.1 (*c* 0.53, MeOH); ν_{max} (neat)/cm^{-1} 3289, 3262, 3274, 3088, 2933, 2863, 2116, 1652, 1557, 1492; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.92 (s, 3 H), 2.33 (s, 3H), 3.29−3.37 (m, 1H), 3.44−3.52 (m, 4H), 3.57−3.74 (m, 3H), 3.76−3.84 (m, 1H), 4.17−4.25 (m, 2H), 4.35−4.46 (m, 4H), 4.82 (d, *J* = 9.90 Hz, 1H), 7.15−7.23 (m, 2H), 7.35−7.41 (m, 2H), 8.08 (d, *J* = 8.80 Hz, 1H, N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.4 (CH_3), 22.9 (CH_3), 53.2 (CH), 57.6 (CH_2), 59.0 (CH_2), 59.1 (CH_2), 68.2 (CH_2), 76.7 (CH × 3), 77.1 (CH), 77.2 (CH), 79.9 (quat.), 80.0 (quat.), 80.2 (quat.), 82.8 (CH), 85.7 (CH), 129.4 (CH), 130.1 (quat.), 130.6 (CH), 136.4 (quat.), 168.9 (quat); HRMS (ESI$^+$) $C_{24}H_{28}NO_5S$, [M + H$^+$] calcd 442.1683, found 442.1673.

3-[(3′,4′,6′-Tri-O-propargyl-2′-deoxy-β-D-glucopyranosyl)oxy]-p-toluenesulfonate ***7***. To a solution of compound **6** (0.5 g, 1.13 mmol), 3-hydroxyprop-1-yl-*p*-toluenesulfonate (1.0 g, 4.53 mmol) and preactivated 4 Å molecular sieve (0.5 g) in dichloromethane (10 mL) at 0 °C were added AgOTf (0.08 g, 0.34 mmol) and *N*-iodosuccinimide (0.3 g, 1.35 mmol). The reaction mixture was left to stir for 5 min at this temperature before being warmed to room temperature and stirred for further 2 h. The reaction mixture was quenched by addition of saturated aq $NaHCO_3$:saturated aq $Na_2S_2O_3$ (1:1, 10 mL), and the aqueous phase was extracted with dichloromethane (3 × 10 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. Purification by silica gel flash column chromatography using ethyl acetate/hexane (1:1) afforded compound **7** as an off-white solid (0.36 g, 58%): mp 99.8−100.5 °C; $[\alpha]_D^{20}$ = +3.7 (*c* 0.577, MeOH); ν_{max} (neat)/cm^{-1} 3265, 3246, 3092, 2872, 2116, 1653, 1552, 1354, 1169; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81−1.88 (m, 2H), 1.92 (s, 3H), 2.40 (s, 3H), 2.42−2.46 (m, 3H), 3.33−3.57 (m, 5H), 3.69−3.80 (m, 3H), 3.81−3.90 (m, 1H), 3.92−4.02 (m, 1H), 4.11−4.23 (m, 3H), 4.32−4.39 (m, 4H), 4.54 (d, *J* = 8.2 Hz, 1H), 6.16 (d, *J* = 8.2 Hz, 1H), 7.27−7.36 (m, 2H), 7.68−7.75 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4 (CH_3), 23.4 (CH_3), 28.8 (CH_2), 55.6 (CH), 58.4 (CH_2), 59.62 (CH_2) 59.66 (CH_2), 64.7 (CH_2), 67.2 (CH_2), 68.1 (CH_2), 74.0 (CH), 74.41 (CH), 74.46 (CH), 74.7 (CH), 77.7 (CH), 79.3 (quat.), 79.6 (quat.), 80.2 (quat.), 80.7 (CH), 100.5 (CH), 127.6 (CH), 129.8 (CH), 132.7 (quat.), 144.7 (quat.), 170.5 (quat.); HRMS (ESI$^+$) $C_{27}H_{33}NO_9SNa$, [M + Na$^+$] calcd 570.1768, found 570.1771.

General Procedure A for the Synthesis of Neoglycoclusters 8−10. A 10 mL microwave reaction vessel was charged with tosylate **7** (0.1 g, 0.18 mmol, 1.0 equiv) and sugar azides **11**−**13** (0.63 mmol, 3.5 equiv) in a mixture of dichloromethane:H_2O:^{t}BuOH (1.05 mL, 1:1:1 v/v/v) followed by the addition of a premixed solution of $CuSO_4$·5H_2O (37 μL, 1.0 M sol., 0.2 equiv) and sodium-L-ascorbate (73 μL, 1.0 M sol., 0.4 equiv). The reaction vessel was sealed with a cap containing a silicon septum and heated in the microwave reactor (Discover CEM) at 70 °C (100 W) for 15 min. After cooling, the reaction mixture was diluted with EtOAc (10 mL) and washed with saturated aq NH_4Cl (3 × 5 mL). The organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The resultant crude product was taken up in acetonitrile:H_2O (4:1, 30 mL), and sodium azide was added, which was then heated under reflux for 5 h. The reaction mixture was diluted with ethyl acetate (30 mL), washed with H_2O (3 × 20 mL), and the organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo.

1-Azidopropyloxy-3,4,6,-tri-O-[(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-1″H-1″,2″,3″-triazol-1″-ylmethyl]-2-acetamido-2-deoxy-β-D-glucopyranoside ***8***. Compound **8** was prepared according to the General procedure **A** using tosylate **7** and azide **11** (0.24 g, 0.64 mmol). Purification by silica gel flash column chromatography using dichloromethane/methanol (95:5) as eluent afforded compound **8** as an off-white solid (0.25 g, 89% over 2 steps): mp 217.7−218.5 °C; $[\alpha]_D^{20}$ = −22.5 (*c* 0.622, MeOH); ν_{max} (neat)/cm^{-1} 2916, 2100, 1745, 1674, 1368, 1211, 1041; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.78−1.85 (m, 11H), 1.94 (s, 3H), 1.97−2.03 (m, 18H), 2.17−2.23 (m, 9H), 3.28−3.38 (m, 2H), 3.40−3.66 (m, 4H), 3.71−3.84 (m, 3H), 3.84−3.93 (m, 1H), 4.08−4.28 (m, 9H), 4.54 (d, *J* = 8.2 Hz, 1H), 4.66−4.78 (m, 3H), 4.85−4.94 (m, 3H), 5.20−5.30 (m, 3H), 5.45−5.66 (m, 6H), 5.78−5.91 (m, 3H), 6.19 (d, *J* = 8.2 Hz, 1H), 7.88 (s, 1H), 7.89 (s, 1H), 7.97 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 20.1 (CH_3 × 2), 20.3 (CH_3), 20.41 (CH_3), 20.43 (CH_3 × 2), 20.54 (CH_3), 20.57 (CH_3 × 3), 20.60 (CH_3), 20.61 (CH_3), 23.3 (CH_3), 28.9 (CH_2), 48.0 (CH_2), 55.8 (CH), 61.0 (CH_2 × 3), 64.6 (CH_2), 64.7 (CH_2), 65.1 (CH_2), 65.7 (CH_2), 66.7 (CH), 66.8 (CH × 2), 67.7 (CH), 67.8 (CH), 68.1 (CH), 69.1 (CH_2), 70.5 (CH), 70.7 (CH), 70.8 (CH), 73.81 (CH), 73.85 (CH), 73.9 (CH), 74.6 (CH), 78.2 (CH), 79.3 (CH), 86.06 (CH), 86.09 (CH), 86.1 (CH), 100.6 (CH), 121.4 (CH), 121.7 (CH), 121.9 (CH), 145.1 (quat.), 145.4 (quat.), 145.7 (quat.), 168.8 (quat.), 168.9 (quat.), 169.3 (quat.), 169.72 (quat.), 169.77 (quat.), 169.79 (quat.), 169.8 (quat.), 170.00 (quat.), 170.03 (quat.), 170.23 (quat.), 170.26 (quat.), 170.28 (quat.), 170.3 (quat.); HRMS (ESI$^+$) $C_{62}H_{83}N_{13}O_{33}Na$, [M + Na$^+$] calcd 1560.5108, found 1560.5086.

1-Azidopropyloxy-3,4,6,-tri-O-{[(3′,4′,6′-tri-O-acetyl-2′-acetamido-2′-deoxy-α-D-galacto pyranosyl)oxy]propyl-1″H-1″,2″,3″-triazol-1″-ylmethyl}-2-acetamido-2-deoxy-β-D-glucopyranoside ***9***. Compound **9** was prepared according to the general procedure **A** using tosylate **7** and azide **12** (0.28 g, 0.65 mmol). Purification by silica gel flash column chromatography using dichloromethane/methanol (93:7) as eluent afforded compound **9** as an off-white solid (0.27 g, 85% over 2 steps): mp 204.5−205.9 °C; $[\alpha]_D^{20}$ = +79.4 (*c* 0.545, MeOH); ν_{max} (neat)/cm^{-1} 2914, 2098, 1746, 1662, 1542, 1372, 1234, 1132, 1048; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67−1.82 (m, 2H), 1.84−2.01 (m, 30H), 2.08 (s, 9H), 2.10−2.23 (m, 6H), 3.25−3.41 (m, 5H), 3.41−3.62 (m, 3H), 3.62−3.94 (m, 6H), 3.94−4.17 (m, 9H), 4.32−4.89 (m, 21H), 4.95−5.10 (m, 3H), 5.30 (br s, 3H), 6.40−6.49 (m, 1H, N*H*), 6.54−6.73 (m, 3H, N*H*), 7.68 (s, 1H), 7.74 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.52 (CH_3 × 6), 20.57 (CH_3 × 3), 22.9 (CH_3 × 3), 23.1 (CH_3), 28.7 (CH_2), 29.6 (CH_2), 29.7 (CH_2 × 2), 46.8 (CH_2), 47.0 (CH_2 × 2), 47.3 (CH), 47.4 (CH × 2), 47.9 (CH_2), 56.0 (CH), 61.7 (CH_2 × 2), 61.8 (CH_2), 64.4 (CH_2), 64.5 (CH_2), 64.61 (CH_2), 64.68 (CH_2), 65.1 (CH_2), 65.3 (CH_2), 65.9 (CH_2), 66.5 (CH × 3), 67.0 (CH × 3), 68.1 (CH × 3), 68.9 (CH_2), 74.4 (CH), 77.3 (CH), 80.9 (CH), 97.71 (CH), 97.75 (CH), 97.8 (CH), 100.6 (CH), 122.8 (CH), 123.4 (CH × 2), 144.6 (quat.), 145.0 (quat.), 145.2 (quat.), 170.20 (quat. × 2), 170.22 (quat.), 170.32 (quat.), 170.35 (quat. × 2), 170.51 (quat. × 2), 170.56 (quat.), 170.6 (quat. × 3), 170.7 (quat.); HRMS (ESI$^+$) $C_{71}H_{104}N_{16}O_{33}Na_2$, [M + 2Na^{2+}] calcd 877.3368, found 877.3362.

1-Azidopropyloxy-3,4,6,-tri-O-[(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-1″H-1″,2″,3″-triazol-1″-ylmethyl]-2-acetamido-2-deoxy-β-D-glucopyranoside ***10***. Compound **10** was prepared according to the General procedure **A** using tosylate **7** and azide **13** (0.24 g, 0.64 mmol). Purification by silica gel flash column chromatography using dichloromethane/methanol (95:5) as eluent afforded compound **10** as an off-white solid (0.25 g, 89% over 2 steps): mp 179.5−181.3 °C; $[\alpha]_D^{20}$ = −29.6 (*c* 0.54, MeOH); ν_{max} (neat)/cm^{-1} 2918, 2097, 1751, 1654, 1367, 1219, 1040; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 3H), 1.80 (s, 8H), 1.89−2.10 (m, 30H), 3.31−3.39 (m, 2H), 3.39−3.48 (m, 2H), 3.49−3.58 (m, 2H), 3.65−3.73 (m, 1H),

dx.doi.org/10.1021/jo3013435 | J. Org. Chem. 2012, 77, 7564−7571

3.85−3.97 (m, 3H), 3.98−4.07 (m, 2H), 4.08−4.19 (m, 4H), 4.23−4.35 (m, 3H), 4.59−4.71 (m, 2H), 4.75−4.94 (m, 4H), 5.19−5.30 (m, 1H), 5.37−5.46 (m, 4H), 5.46−5.56 (m, 2H), 5.56−5.68 (m, 2H), 5.98 (d, J = 9.3 Hz, 1H), 5.99 (d, J = 9.3 Hz, 1H), 6.07 (d, J = 7.8 Hz, N*H*, 1H), 6.15 (d, J = 9.5 Hz, 1H), 8.00 (s, 1H), 8.31 (s, 1H), 8.37 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 20.03 (CH_3), 20.09 (CH_3), 20.1 (CH_3), 20.4 ($CH_3 \times 4$), 20.5 ($CH_3 \times 2$), 20.6 ($CH_3 \times 3$), 23.5 (CH_3), 28.9 (CH_2), 48.1 (CH_2), 56.9 (CH), 61.4 (CH_2), 61.5 (CH_2), 61.6 (CH_2), 64.2 (CH_2), 64.5 (CH_2), 65.3 (CH_2), 65.9 (CH_2), 67.6 (CH), 67.7 (CH × 2), 69.4 (CH_2), 70.0 (CH × 2), 70.2 (CH), 72.7 (CH), 72.9 (CH), 73.1 (CH), 74.2 (CH), 74.7 (CH), 74.9 (CH × 2), 76.9 (CH), 78.2 (CH), 85.3 (CH), 85.4 (CH), 85.7 (CH), 100.1 (CH), 121.3 (CH), 122.6 (CH), 122.9 (CH), 144.7 (quat.), 145.1 (quat.), 145.6 (quat.), 168.7 (quat.), 168.9 (quat.), 169.0 (quat.), 169.2 (quat.), 169.4 (quat.), 169.6 (quat.), 169.94 (quat.), 169.98 (quat.), 170.0 (quat.), 170.4 (quat.), 170.51 (quat.), 170.57 (quat.), 170.7 (quat.); HRMS (ESI$^+$) $C_{62}H_{83}N_{13}O_{33}Na_2$, [M + $2Na^{2+}$] calcd 791.7500, found 791.7518.

General Procedure B for the Synthesis of Unprotected Neoglycoclusters 1−3. To a solution of acetylated neoglycoclusters **8**−**10** (0.16 mmol) in methanol (1.0 mL) at room temperature was added freshly prepared sodium methoxide (1.0 M, 3.0 mL), and the mixture was left to stir until analytical RP-HPLC analysis showed complete conversion to the corresponding product (approximately 1 h). The reaction mixture was diluted with methanol (30 mL) and quenched by stirring with acidified Dowex resins (0.5 g) for 30 min. The resin was then filtered and washed with methanol, and then combined organic extracts were concentrated in vacuo. The crude product was redissolved in H_2O (10 mL), lyophilized and used without further purification.

1-Azidopropyloxy-3,4,6,-tri-O-[(β-D-galactopyranosyl)-1″H-1″,2″,3″-triazol-1″-ylmethyl]-2-acetamido-2-deoxy-β-D-glucopyranoside ***1***. Compound **1** was prepared according to the General procedure **B** using acetylated neoglycocluster **8** (0.25 g, 0.16 mmol). Lyophilization of the reaction mixture afforded compound **1** as a white solid (0.16 g, 95%): mp 155.6−156.2 °C; $[\alpha]_D^{20}$ = −12.9 (c 0.400, H_2O); ν_{max} (neat)/cm^{-1} 3281, 2885, 2101, 1649, 1555, 1373, 1090, 1050; ^{1}H NMR (400 MHz, D_2O) δ 1.78−1.86 (m, 2H), 1.92 (s, 3H), 3.32−3.40 (m, 2H), 3.56−3.74 (m, 5H), 3.74−3.84 (m, 8H), 3.87−3.95 (m, 4H), 3.99−4.06 (m, 3H), 4.09−4.14 (m, 3H), 4.21−4.30 (m, 3H), 4.51 (d, J = 8.32 Hz, 1H), 4.71−4.90 (m, 6H), 5.69−5.77 (m, 3H), 8.27 (s, 1H), 8.33 (s, 1H), 8.35 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ 22.0 (CH_3), 28.0 (CH_2), 47.7 (CH_2), 54.7 (CH), 60.7 ($CH_2 \times 3$), 63.1 (CH_2), 64.5 (CH_2), 64.8 (CH_2), 67.2 (CH_2), 67.9 (CH_2), 68.51 (CH × 2), 68.54 (CH), 69.6 (CH × 3), 72.91 (CH × 2), 72.94 (CH), 73.3 (CH), 77.5 (CH), 78.2 (CH × 3), 81.4 (CH), 87.9 (CH × 3), 100.7 (CH), 124.0 (CH), 124.2 (CH × 2), 143.8 (quat.), 144.0 (quat.), 144.3 (quat.), 174.0 (quat.); HRMS (ESI$^+$) $C_{38}H_{59}N_{13}O_{21}Na$, [M + Na^+] calcd 1056.3841, found 1056.3860.

1-Azidopropyloxy-3,4,6,-tri-O-{[(2′-acetamido-2′-deoxy-α-D-galactopyranosyl)oxy]propyl-1″H-1″,2″,3″-triazol-1″-ylmethyl}-2-acetamido-2-deoxy-β-D-glucopyranoside ***2***. Compound **2** was prepared according to the general procedure **B** using acetylated neoglycocluster **9** (0.27 g, 0.16 mmol). Lyophilization of the reaction mixture afforded compound **2** as a white solid (0.2 g, 95%): mp 126.4−127.8 °C; $[\alpha]_D^{20}$ = +73.2 (c 0.492, H_2O); ν_{max} (neat)/cm^{-1} 3285, 2884, 2099, 1647, 1548, 1374, 1119, 1043; ^{1}H NMR (400 MHz, D_2O) δ 1.78−1.85 (m, 2H), 1.94 (s, 3H), 2.04−2.08 (m, 9H), 2.16−2.25 (m, 6H), 3.32−3.42 (m, 5H), 3.57−3.78 (m, 17H), 3.81−3.89 (m, 6H), 3.89−3.98 (m, 4H), 4.11−4.18 (m, 3H), 4.50−4.61 (m, 7H), 4.63−4.85 (m, 8H), 8.00 (s, 1H), 8.02 (s, 1H), 8.10 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ 21.9 ($CH_3 \times 3$), 22.0 (CH_3), 28.0 (CH_2), 29.0 (CH_2), 29.13 (CH_2), 29.16 (CH_2), 47.5 (CH_2), 47.62 (CH_2), 47.64 (CH_2), 47.7 (CH_2), 49.8 ($CH \times 3$), 54.9 (CH), 61.1 ($CH_2 \times 3$), 63.0 (CH_2), 64.3 (CH_2), 64.43 (CH_2), 64.46 (CH_2), 64.5 (CH_2), 64.9 (CH_2), 67.1 (CH_2), 67.6 ($CH \times 3$), 67.9 (CH_2), 68.4 ($CH \times 3$), 70.9 ($CH \times 3$), 73.3 (CH), 77.2 (CH), 81.7 (CH), 97.1 ($CH \times 3$), 100.7 (CH), 124.83 (CH), 124.85 (CH), 125.2 (CH), 143.5 (quat.), 143.6 (quat.), 143.9 (quat.), 173.9 (quat.), 174.42 (quat.), 174.43 (quat.), 174.44 (quat.); HRMS (ESI$^+$) $C_{53}H_{86}N_{16}O_{24}Na_2$, [M + $2Na^{2+}$] calcd 688.2893, found 688.2896.

1-Azidopropyloxy-3,4,6,-tri-O-[(β-D-glucopyranosyl)-1″H-1″,2″,3″-triazol-1″-ylmethyl]-2-acetamido-2-deoxy-β-D-glucopyranoside ***3***. Compound **3** was prepared according to the General procedure **B** using acetylated neoglycocluster **10** (0.25 g, 0.16 mmol). Lyophilization of the reaction mixture afforded compound **3** as a white solid (0.16 g, 95%): mp 179.1−181.5 °C; $[\alpha]_D^{20}$ = −6.6 (c 0.507, H_2O); ν_{max} (neat)/cm^{-1} 3274, 2882, 2101, 1645, 1557, 1374, 1094, 1045; ^{1}H NMR (400 MHz, D_2O) δ 1.77−1.85 (m, 2H), 1.91 (s, 3H), 3.31−3.37 (m, 2H), 3.54−3.70 (m, 8H), 3.70−3.83 (m, 11H), 3.85−3.94 (m, 4H), 3.96−4.06 (m, 3H), 4.49 (d, J = 7.95 Hz, 1H), 4.68−4.87 (m, 6H), 5.73−5.79 (m, 3H), 8.22 (s, 1H), 8.27 (s, 1H), 8.30 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ = 22.0 (CH_3), 28.0 (CH_2), 47.7 (CH_2), 54.7 (CH), 60.33 (CH_2), 60.37 ($CH_2 \times 2$), 63.1 (CH_2), 64.4 (CH_2), 64.8 (CH_2), 67.2 (CH_2), 67.9 (CH_2), 68.8 ($CH \times 3$), 72.2 ($CH \times 3$), 73.2 (CH), 75.8 ($CH \times 2$), 75.9 (CH), 77.4 (CH), 78.7 (CH), 78.8 ($CH \times 2$), 81.5 (CH), 87.4 ($CH \times 3$), 100.7 (CH), 124.1 (CH), 124.4 ($CH \times 2$), 143.7 (quat.), 144.0 (quat.), 144.3 (quat.), 174.0 (quat.); HRMS (ESI$^+$) $C_{38}H_{59}N_{13}O_{21}Na$, [M + Na^+] calcd 1056.3841, found 1056.3807.

General Procedure C for the CuAAC between Peptide 14 and Unprotected Neoglyclusters 1−3. To a degassed solution of 6.0 M GnHCl/0.2 M Na_2HPO_4 buffer at room temperature was added $CuSO_4 \cdot 5H_2O$ (20 mM):TCEP (20 mM), and pH was adjusted to approximately 7.1−7.2 by the addition of 1.0 M NaOH. The resultant solution was then incubated at 80 °C for 30 min and centrifuged prior to use. To this solution (177 μL) was then added peptide **14** (1.0 mg, 3 mM, 1.0 equiv) and unprotected neoglycoclusters **1**−**3** (4.5 mM, 1.5 equiv), and the reaction was carried out under nitrogen atmosphere using microwave reactor (Discover CEM) at 50 °C (25 W). The reaction progress was monitored by analytical RP-HPLC, and after 3−5 h the reaction was complete, and the crude product was isolated by solid phase extraction using a RP-C18 cartridge and then lyophilized.

Click Neoglycopeptide 16. Neoglycopeptide **16** was prepared according to the general procedure **C** using unprotected neoglycocluster **1** (0.82 mg) and peptide **14** (1.0 mg). Purification by semipreparative RP-HPLC afforded compound **16** as a white solid (0.54 mg, 32%).

Click Neoglycopeptide 17. Neoglycopeptide **17** was prepared according to the general procedure **C** using unprotected neoglycocluster **2** (1.06 mg) and peptide **14** (1.0 mg). Purification by semipreparative RP-HPLC afforded compound **17** as a white solid (0.72 mg, 42%).

Click Neoglycopeptide 18. Neoglycopeptide **18** was prepared according to the general procedure **C** using unprotected neoglycocluster **3** (0.82 mg) and peptide **14** (1.0 mg). Purification by semipreparative RP-HPLC afforded compound **18** as a white solid (0.43 mg, 28%).

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: m.brimble@auckland.ac.nz.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial assistance from the Maurice Wilkins Centre for Molecular Biodiscovery is gratefully acknowledged.

REFERENCES

(1) Chlubnova, I.; Sylla, B.; Nugier-Chauvin, C.; Daniellou, R.; Legentil, L.; Kralova, B.; Ferrieres, V. *Nat. Prod. Rep.* **2011**, *28*, 937−952.

(2) Mowery, P.; Yang, Z. Q.; Gordon, E. J.; Dwir, O.; Spencer, A. G.; Alon, R.; Kiessling, L. L. *Chem. Biol.* **2004**, *11*, 725−732.

(3) (a) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357−2364. (b) Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 477−488.

(4) *Carbohydrates in Chemistry and Biology, Part II, Biology of Saccharides*; Ernst, B., Hart, G. W., Sinay, P., Eds.; Wiley-VCH: Weinheim, 2000; Vol. *4*, Lectins and Saccharide Biology.

(5) (a) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327. (b) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794. (c) Lindquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578. (d) Dam, T. K.; Brewer, C. F. *Chem. Rev.* **2002**, *102*, 387−429. (e) Badjic, J. D.; Nelson, A.; Cantrill, S. J.; Turnbull, W. B.; Stoddart, J. F. *Acc. Chem. Res.* **2005**, *38*, 723−732.

(6) Choi, S. K. *Synthetic Multivalent Molecules: Concept and Biomedical Application*; John Wiley and Sons: Hoboken, NJ, 2005.

(7) (a) Grabosch, C.; Hartmann, M.; Schmidt-Lassen, J.; Lindhorst, T. K. *ChemBioChem* **2011**, *12*, 1066−1074. (b) Schierholt, A.; Hartmann, Mirja; Lindhorst, T. K. *Carbohydr. Res.* **2011**, *346*, 1519−1526. (c) Wehner, J. W.; Lindhorst, T. K. *Synthesis* **2010**, *18*, 3070−3082.

(8) (a) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431−449. (b) Voit, B.; Appelhaus, D. *Macromol. Chem. Phys.* **2010**, *211*, 727−735.

(9) (a) Niederhafer, P.; Sebestik, J.; Jezek, J. *J. Pept. Sci.* **2008**, *14*, 2−43. (b) Niederhafer, P.; Sebestik, J.; Jezek, J. *J. Pept. Sci.* **2008**, *14*, 44−65. (c) Kolomiets, E.; Swiderska, M. A.; Kadam, R. U.; Johansson, E. M. V.; Jaeger, K.-E.; Darbre, T.; Reymond, J.-L. *ChemMedChem* **2009**, *4*, 562−569. (d) Kadam, R. U.; Bergmann, M.; Hurley, M.; Garg, D.; Cacciarini, M.; Swiderska, M. A.; Nativi, C.; Sattler, M.; Smyth, A. R.; Williams, P.; Camara, M.; Stocker, A.; Darbre, T.; Reymond, J.-L. *Angew. Chem., Int. Ed.* **2011**, *50*, 10631−10635.

(10) (a) Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291−310. (b) Cloniger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742−748.

(11) (a) Renaudet, O. *Mini Rev. Org. Chem.* **2008**, *5*, 274−286. (b) Bossu, I.; Sulc, M.; Krenek, K.; Dufour, E.; Garcia, J.; Berthet, N.; Dumy, P.; Kren, V.; Renaudet, O. *Org. Biomol. Chem.* **2011**, *9*, 1948−1959.

(12) Gorska, K.; Huang, K.-T.; Chaloin, O.; Winssinger, N. *Angew. Chem., Int. Ed.* **2009**, *48*, 7695−7700.

(13) Fulton, D. A.; Stoddart, F. J. *Bioconjugate Chem.* **2001**, *12*, 655−672.

(14) (a) Baldini, L.; Casnati, A.; Sansone, F.; Ungaro, R. *Chem. Soc. Rev.* **2007**, *36*, 254−266. (b) Dondoni, A.; Marra, A. *Chem. Rev.* **2010**, *110*, 4949−4977.

(15) Gao, Y.; Eguchi, A.; Kakehi, K.; Lee, Y. C. *Org. Lett.* **2004**, *6*, 3457−3460.

(16) Nierengarten, J.-F.; Iehl, J.; Oerthel, V.; Holler, M.; Illescas, B. M.; Munoz, A.; Martin, N.; Rojo, J.; Sanchez-Navarro, M.; Cecioni, S.; Vidal, S.; Buffet, K.; Durka, M.; Vincent, S. P. *Chem. Commun.* **2010**, *46*, 3860−3862.

(17) (a) de la Fuente, J. M.; Penades, S. *Biochim. Biophys. Acta, Gen. Subj.* **2006**, *1760*, 636−651. (b) Marradi, M.; Matin-Lomas; Penades, S. *Adv. Carbohydr. Chem. Biochem.* **2010**, *64*, 211−290.

(18) Simanek, E. E.; McGarvey, G. J.; Jablonski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833−862.

(19) (a) Autar, R.; Khan, A. S.; Schad, M.; Hacker, J.; Liskamp, R. M. J.; Pieters, R. J. *ChemBioChem* **2003**, *4*, 1317−1325. (b) Sharon, N. *Biochim. Biophys. Acta, Gen. Subj.* **2006**, *1760*, 527−537. (c) Pieters, R. J. *Med. Res. Rev.* **2007**, *27*, 796−816.

(20) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669−672.

(21) Shaunak, S.; Thomas, S.; Gianasi, E.; Godwin, A.; Jones, E.; Teo, I.; Mireskandari, K.; Luthert, P.; Duncan, R.; Patterson, S.; Khaw, P.; Brocchini, S. *Nat. Biotechnol.* **2004**, *22*, 977−984.

(22) Roy, R.; Baek, M. G. *Rev. Mol. Biotechnol.* **2002**, *90*, 291−309.

(23) (a) Brimble, M. A.; Miller, N.; Williams, G. M. Recent Developments in Neoglycopeptide Synthesis. In *Amino Acids, Peptides and Proteins in Organic Chemistry*; Hughes, A. B., Ed.; Wiley-VCH: Weinheim, 2011; Vol. *4*, Protection Reactions, Medicinal Chemistry, Combinatorial Synthesis, pp 359−392; (b) Brimble, M. A.; Miller, N.; Williams, G. M. *Int. J. Pept. Res. Ther.* **2010**, *16*, 125−132. (c) Lee, D. J.; Harris, P. W. R.; Brimble, M. A. *Org. Biomol. Chem.* **2011**, *9*, 1621−1626. (d) Lee, D. J.; Mandal, K.; Harris, P. W. R.; Brimble, M. A.; Kent, S. B. H. *Org. Lett.* **2009**, *11*, 5270−5273. (e) Miller, N.; Williams, G. M.; Brimble, M. A. *Org. Lett.* **2009**, *11*, 2409−2412.

(24) (a) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057−3064. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599.

(25) (a) Hanisch, F.-G.; Muller, S. *Glycobiology* **2000**, *10*, 439−449. (b) Baldus, S. E.; Engelmann, K.; Hanisch, F.-G. *Crit. Rev. Clin. Lab. Sci.* **2004**, *41*, 189−231. (c) Hilkens, J.; Ligtenberg, M. J. L.; Vos, H. L.; Litvinov, S. V. *Trends Biochem. Sci.* **1992**, *17*, 359−363. (d) Sherblom, A. P.; Moody, C. E. *Cancer Res.* **1986**, *46*, 4543−4546.

(26) (a) Keil, S.; Claus, C.; Dippold, W.; Kunz, H. *Angew. Chem., Int. Ed.* **2001**, *40*, 366−369. (b) Dziadek, S.; Hobel, A.; Schmitt, E.; Kunz, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 7630−7635. (c) Dziadek, S.; Kowalczyk, D.; Kunz, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 7624−7630. (d) Westerlind, U.; Hobel, A.; Gaidzik, N.; Schmitt, E.; Kunz, H. *Angew. Chem., Int. Ed.* **2008**, *47*, 7551−7556. (e) Kaiser, A.; Gaidzik, N.; Westerlind, U.; Kowalczyk, D.; Hobel, A.; Schmitt, E.; Kunz, H. *Angew. Chem., Int. Ed.* **2009**, *48*, 7551−7555. (f) Kaiser, A.; Gaidzik, N.; Becker, T.; Menge, C.; Groh, K.; Cai, H.; Li, Y.-M.; Gerlitzki, B.; Schmitt, E.; Kunz, H. *Angew. Chem., Int. Ed.* **2010**, *49*, 3688−3692. (g) Hoffmann-Röder, A.; Kaiser, A.; Wagner, S.; Gaidzik, N.; Kowalczyk, D.; Westerlind, U.; Gerlitzki, B.; Schmitt, E.; Kunz, H. *Angew. Chem., Int. Ed.* **2010**, *49*, 8498−8503. (h) Chun, C. K. Y.; Payne, R. J. *Aust. J. Chem.* **2009**, *62*, 1339−1343. (i) Wilkinson, B. L.; Malins, L. R.; Chun, C. K. Y.; Payne, R. J. *Chem. Commun.* **2010**, *46*, 6249−6251. (j) Wilkinson, B. L.; Day, S; Malins, L. R; Apostolopoulos, V.; Payne, R. J. *Angew. Chem., Int. Ed.* **2011**, *50*, 1635−1639. (k) Wilkinson, B. L.; Chun, C. K. Y.; Payne, R. J. *Pept. Sci.* **2011**, *96*, 137−146.

(27) Gao, Y.; Eguchi, A.; Kakehi, K.; Lee, Y. C. *Bioorg. Med. Chem.* **2005**, *13*, 6151−6157.

(28) (a) Ortega-Munoz, M.; Morales-Sanfrutos, J.; Perez-Balderas, F.; Hernandew-Mateo, F.; Giron-Gonzalez, M. D.; Sevillano-Tripero, N.; Salto-Gonzalez, R.; Santoyo-Gonzalez, F. *Org. Biomol. Chem.* **2007**, *5*, 2291−2301. (b) Perez-Balderas, F.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Isac-García, J.; Santoyo-Gonzalez, F. *Eur. J. Org. Chem.* **2009**, 2441−2453.

(29) Bera, S.; Lindhardt, R. J. *J. Org. Chem.* **2011**, *76*, 3181−3193.

(30) Hong, S. Y.; Tobias, G.; Ballesteros, B.; El Oualid, F.; Errey, J. C.; Doores, K. J.; Kirkland, A. I.; Nellist, P. D.; Green, M. L. H.; Davis, B. G. *J. Am. Chem. Soc.* **2007**, *129*, 10966−10967.

(31) Gyorgydeak, Z.; Szilagyi, L. *Carbohydr. Res.* **1985**, *143*, 21−41.

(32) Soli, E. D.; Manoso, A. S.; Patterson, M. C.; DeShong, P.; Favor, D. A.; Hirschmann, R.; Smith, A. B. *J. Org. Chem.* **1999**, *64*, 3171−3177.

(33) Harris, P. W. R.; Yang, S. H.; Brimble, M. A. *Tetrahedron Lett.* **2011**, *52*, 6024−6026.