Carbohydrate Polymers 107 (2014) 209–213

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Use of chloromethylstyrene as a supporter for convenient preparation of carbohydrate monomer and glycopolymers

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ARTICLE INFO

Article history:
Received 3 December 2013
Received in revised form 14 January 2014
Accepted 21 January 2014
Available online 11 March 2014

Keywords:
Styrene
Glycopolymers
Radical polymerization
Lectins
Fluorescence spectrometry

ABSTRACT

A convenient access for glycopolymers was accomplished by using a styrene-modified glycomonomer, which was prepared from chloromethylstyrene as a key starting material and *N*-acetyl-D-glucosamine (GlcNAc) as a model carbohydrate. One-step conversion of the styrene derivative gave an azidostyrene, which was treated with a propargyl GlcNAc to afford a carbohydrate monomer after deprotection in good yield. The water-soluble GlcNAc monomer was polymerized with or without acrylamide to give the corresponding white powdery glycopolymers, which were evaluated for their interaction against wheat germ agglutinin (WGA) on the basis of fluorescence change of tryptophan in WGA.

1. Introduction

Huisgen cycloaddition is widely used for preparation of various functional molecules owing to the simple, convenient, and effective coupling reaction (Huisgen, 1984; Wu & Fokin, 2007). Considerable efforts have been made to enhance the efficiency of the cycloaddition reaction, and improvement of the reaction conditions was accomplished by using "Click chemistry" (Gil, Arévalo, & López, 2007; Kolb & Sharpless, 2003; Kolb, Finn, & Sharpless, 2001). Click chemistry is of great interest for the preparation of biologically important functional molecules because of the mild and efficient reaction conditions (Matsuoka et al., 2012).

In our ongoing studies on the synthesis of glycopolymers, the glycocluster effect induced by glycopolymers in carbohydrate–protein interactions is the most important function (Matsuoka & Nishimura, 1995; Matsuoka, Takita, et al., 2007; Matsuoka et al., 2009; Matsuoka, Yamaguchi, Koyama, Hatano, & Terunuma, 2010). A variety of glycopolymers as well as corresponding glycomonomers have been reported, but no Huisgen cycloaddition reaction has been applied to the synthesis of a glycomonomer (Ladmiral, Melia, & Haddleton, 2004; Varma, Kennedy, & Galgali, 2004; Vázquez-Dorbatt, Lee, Lin, & Maynard, 2012). Successful use of the reaction for preparation of glycomonomers is very attractive and has an advantage for reduction of the synthetic sequence and convenient introduction of the carbohydrate moiety into the precursor. In this paper, we describe a conventional way for construction of glycopolymers using commercially available reagents and 4-chloromethylstyrene as a starting material, and we also describe the biological properties of glycopolymers having *N*-acetyl-D-glucosamine (GlcNAc) moieties for wheat germ agglutinin (WGA) as a model protein.

2. Results and discussion

2.1. Synthesis of a glycomonomer via Huisgen cycloaddition as a key reaction

In our previous reports, we have described the preparation of various glycomonomers by means of ω-alkenyl alcohol or ω-acrylamido alcohol as a key polymerizable aglycon (Miyagawa et al., 2004; Nishimura et al., 1994). Since homopolymerization of a glycomonomer having ω-alkenyl aglycon in the presence of typical radical initiator systems has not been successful (Nishimura, Matsuoka, & Kurita, 1990), our attention turned toward the use of acrylamide derivatives (Matsuoka, Goshu, et al., 2007). Acrylamide-type alkene is known as a conjugated alkene and it can easily polymerize itself in high efficiency. However, preparation of a glycomonomer having an acrylamide moiety has been troublesome, when acryloylation for an amino moiety in the glycoside does not proceed smoothly in the case of poor solubility in the solvent and occurrence of acyl migration. In order to avoid the problems, a

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http://dx.doi.org/10.1016/j.carbpol.2014.01.104
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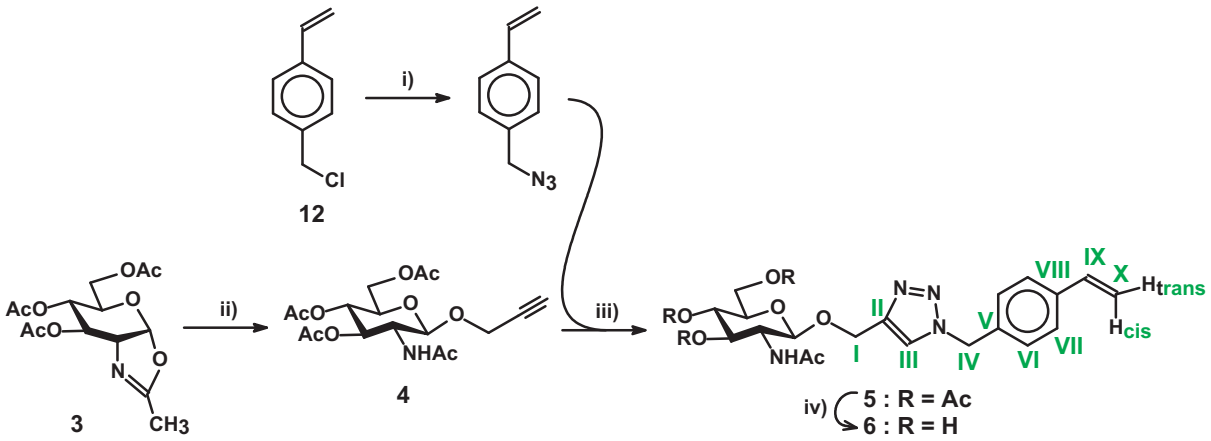


Scheme 1. *Reagents and conditions*: (i) NaN_3, DMF, 85 °C, 35 min, (ii) 2-propyn-1-ol, CSA, $ClCH_2CH_2Cl$, 80 °C, 4 h, (iii) 0.1 M aq $CuSO_4$ – 1 M aq Na ascorbate – MeOH, rt, 4 h, (iv) NaOMe, MeOH, rt.

styrene derivative is extremely attractive because of its availability and ease of handling. Therefore, we decided to use a commercially available 4-chloromethylstyrene as a versatile precursor for an azide compound that would be converted into the corresponding triazole derivative by Huisgen cycloaddition reaction. When the convenient preparation of a carbohydrate monomer by means of our proposed method is accomplished, various glycomonomers can be accessed by using known alkynyl glycosides.

A schematic diagram for convenient preparation of a glycomonomer **6** is shown in Scheme 1. Chloride of 4-chlolomethyl styrene **1** was replaced by azide by simple S_N2 reaction to afford the corresponding azide **2** in 89% yield. Preparation of GlcNAc derivatives was then started from a known oxazoline derivative **3** (Nakabayashi, Warren, & Jeanloz, 1986). The reactive oxazoline **3** was treated with propargyl alcohol in the presence of camphorsulfonic acid (CSA) (Nishimura, Matsuoka, Furuike, Ishii, & Kurita, 1991) to yield a colorless crystalline glycoside **4** (Rowan et al., 2009) in 59% yield.

Since an azide derivative and an alkynyl derivative were successfully prepared, our attention turned toward a coupling of each other by means of Huisgen cycloaddition reaction. Thus, an azide **2** was allowed to react with alkyne **4** in the presence of copper (II) sulfate and sodium ascorbate. The reaction proceeded smoothly to give the corresponding triazole derivative **5** as a white solid in 86% yield after chromatographic purification. Structural elucidation of the newly formed linkage in **5** was performed by means of ^1H and ^{13}C NMR spectroscopic analyses (Chabre et al., 2008). Since complete disappearance of the alkynyl proton signal at 2.50 ppm in **4** and appearance of the triazole proton signal at 7.46 ppm in **5** were observed, the progress of the Huisgen reaction was confirmed. In addition to the results of ^1H NMR, the combined results of ^{13}C NMR and HMQC experiments were also supported the adduct **5**. Especially, the changes of ^{13}C signals of the alkynyl carbon signal at 75.38 ppm in **4** into C-III carbon signal in the triazole ring at 122.81 ppm in **5** were characteristically observed. Removal of the protection in acetate **5** was performed by using the Zemplén method (Zemplén & Pacsu, 1929) to afford **6** as water-soluble material in 79% yield.

2.2. Polymerization of a glycomonomer by means of radical polymerization

Given the success of convenient preparation of a glycomonomer, our next objective was synthetic assembly of the glycomonomer by means of radical polymerization in order to produce novel glycoclusters. Polymerization ability of the glycomonomer **6** without a comonomer was evaluated by radical polymerization in aqueous media at room temperature. **6** underwent homopolymerization to yield white powdery **7a** in 91% yield after dialysis against water followed by lyophilization. The results of other polymerizations are summarized in Table 1. From the weight-average molecular weight ($\overline{Mw}$) of the glycopolymer **7a**, the degree of polymerization was estimated to be 84. The polymer compositions of the glycopolymers **7b** and **7c** were estimated on the basis of the results of ^1H NMR. All polymers (**7a**–**7c**) have water-soluble ability and appropriate molecular weight estimated by means of SEC analyses (Scheme 2).

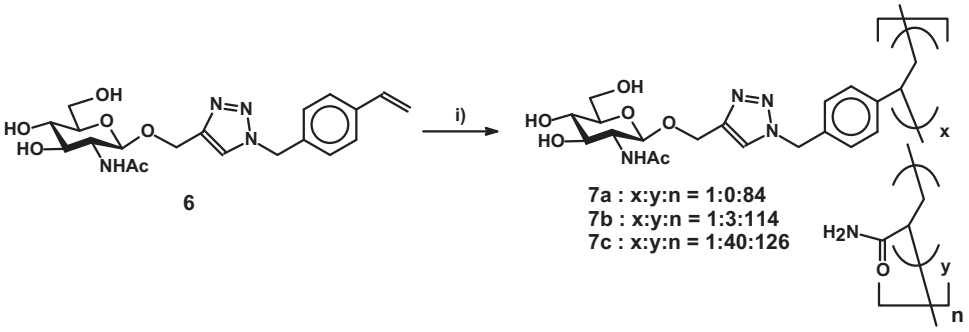
2.3. Biological evaluation of the glycopolymers for lectin

Since preparation of water-soluble glycopolymers had been accomplished, we focused on interaction between the glycopolymers and lectin (Lee, 1992). The glycopolymers having GlcNAc residues as pendant tops reminded us to use WGA, which is a plant protein and binds to GlcNAc residues with high specificity (Goldstein & Poretz, 1986). Thus, biological evaluation of the glycopolymer **7a**–**7c** against WGA was preliminarily examined on the basis of fluorescence measurement. The results of the measurement are summarized in Table 2. The results of fluorescence spectral

Table 1
Results of polymerizations of glycomonomer **5** with or without acryl amide (**AAm**).

Monomer ratio		Polymer	Total yield[a]	Polymer composition[b]			Sugar content	$\overline{Mw}$[c]	$\frac{\overline{Mw}}{\overline{Mn}}$
5	**AAm**		%	*X*	*y*	*n*	wt%	kDa	
1	0	**7a**	91	1	0	84	100	46	1.3
1	10	**7b**	59	1	3	1.1×10^2	66	72	1.5
1	20	**7c**	65	1	40	1.3×10^2	13	410	1.0

[a] Total yields were calculated on the basis of quantities of monomers used.
[b] Polymer compositions of sugar unit: acrylamide unit were estimated on the basis of the results of ^1H NMR.
[c] The weight-average molecular weights were estimated by size-exclusion chromatography in 0.3 M aq NaCl solution using a Shodex GF-510HQ column. Calibration curves were obtained using pullulan standards (5.9, 11.8, 22.8, 47.3, 112, 212, 404, and 788 kDa, Shodex P-82).



Scheme 2. *Reagents and conditions*: (i) TEMED, APS, Water, rt.

changes by addition of GlcNAc derivatives were analyzed on the basis of the Hill equation. Association constant K_a was estimated to 3.1–5.5 × 10^5 M^{-1} by using GlcNAc polymers. Interestingly, the difference in fluorescence intensity was largest when copolymer **7b** was used as a substrate. This phenomenon indicates a significant change around the tryptophan residue located at or near the binding sites of WGA. Bains, Lee, Lee, and Freire (1992) reported the results of a microcalorimetric study on WGA binding to GlcNAc and its oligomer, and the comparison of WGA-GlcNAc oligomer association was also reported. Since a polymeric effect of the sugar moieties could be displayed, our polymeric GlcNAc derivatives showed higher association constants than those in their study. Glycocluster effects (Lee et al., 1983; Lee, 1978; Lundquist & Toone, 2002), therefore, were confirmed when GlcNAc polymers **7a**–**7c** were used as substrates.

In summary, we have described a convenient preparation of a polymerizable GlcNAc derivative by means of click chemistry. Polymerization of the styrene derivative having a GlcNAc residue was performed in aqueous media and gave the corresponding glycopolymers. Binding activities of the glycopolymers to WGA were preliminarily examined on the basis of fluorescence measurement and showed higher affinity than GlcNAc itself due to a sugar-clustering effect. Since recent literature suggested attractive structure-activity relationships (SARs) between cluster-type GlcNAc derivatives and WGA (Schwefel et al., 2010), further transformation of the styrene derivative and the binding properties for WGA are now under investigation. The results of syntheses and the activities will be published elsewhere.

3. Experimental

3.1. Materials and methods

Unless otherwise stated, all commercially available solvents and reagents were used without further purification. *N*,*N*-Dimethylformamide (DMF) was stored over molecular sieves (4 Å MS), and methanol (MeOH) was stored over 3 Å MS before use. Acrylamide was recrystallized from benzene before use. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. The IR spectra were obtained using a Shimadzu IR Prestige-21 spectrometer. The ^{1}H NMR spectra were recorded at 200 MHz for ^{1}H with a Varian Gemini 2000 spectrometer, at 400 MHz for ^{1}H and 100 MHz for ^{13}C with a Bruker DRX-400 spectrometer, or at 500 MHz for ^{1}H and 125 MHz for ^{13}C with a Bruker AVANCE 500 spectrometer in chloroform-*d* ($CDCl_3$) or deuterium oxide (D_2O). Chemical shifts are expressed as parts per million (ppm, δ) and are relative to an internal tetramethylsilane (TMS) in $CDCl_3$ (δ 0.0) or HDO in D_2O (δ 4.78) for ^{1}H, and $CDCl_3$ (δ 77.0), MeOD (δ 49.0) or acetone (δ 29.8) for ^{13}C. Ring-proton assignments in the ^{1}H NMR spectra were made by first-order analysis of the spectra and are supported by the results of homonuclear decoupling experiments and H—H or HMQC experiments. Elemental analyses were performed with a Fisons EA1108 on samples extensively dried at 50–60 °C over phosphorus pentoxide for 4–5 h. Matrix-assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF-MS) were obtained using a Bruker AutoflexIII spectrometer. Reactions were monitored by thin layer chromatography (TLC) on a precoated plate of Silica Gel 60F254 (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). For detection of the intermediates, TLC sheets were dipped in (a) a solution of 85:10:5 (v/v/v) MeOH-*p*-anisaldehyde – concd H_2SO_4 and heated for a few minutes (for carbohydrate) or (b) an aq solution of 5 wt % $KMnO_4$ and heated similarly (for detection of C=C double bonds). Column chromatography was performed on silica gel (Silica Gel 60; 63–200 μm, E. Merck). Flush column chromatography was performed on silica gel (Silica Gel 60, spherical neutral; 40–100 μm, E. Merck). All extractions were concentrated below 45 °C under diminished pressure. Wheat germ agglutinin (WGA; a lectin from *Triticum valgaris*) was purchased from J-Oil Mils (Lot # 62015).

Table 2
Results of binding assay for WGA on the basis of fluorescence measurements.

Compounds	Δλ (nm)	$\left\|\frac{\Delta F'}{F'}\right\|$ (%)	K_a (M^{-1})	Reactive potency
GlcNAc	0	5.4	8.2×10^3	1
7a	1.8	6.5	3.1×10^5	37
7b	18.8	56	5.5×10^5	67
7c	1	22	3.7×10^5	45

3.1.1. 4-Azidomethylstyrene (2)

To a solution of styrene **1** (3.00 g, 19.6 mmol) in DMF (15 mL) was added sodium azide (3.82 g, 58.8 mmol) and the mixture was stirred at 85 °C for 35 min. After complete transformation of **1** judged by TLC, the reaction mixture was evaporated *in vacuo*. The residue was diluted with $CHCl_3$ and was then washed successively with water and brine, and dried over anhyd $MgSO_4$. The organic solution was filtered and evaporated *in vacuo*. The residual syrup was applied to a column of silica gel with 40:1 (v/v) hexane – EtOAc as the eluent to give a pure **2** (2.79 g, 89.1%) as a white powder: R_f 0.32 [40:1 (v/v) hexane – ethyl acetate]; IR (NEAT) 3086 (ν_{C-H}, Ph), 2926 (ν_{C-H}), 2099 ($\nu_{N=N=N}$), 1628 ($\nu_{C=C}$) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.41 (d, 2 H, J = 7.3 Hz, Ph), 7.26 (d, 2 H, J = 7.3 Hz, Ph), 6.70 (dd, 1 H, J_{cis} = 11.0 Hz, J_{trans} = 17.6 Hz, CH=), 5.75 [d, 1 H, trans of CH_2=], 5.26 [d, 1 H, cis of CH_2=], 4.30 (s, 2 H, PhCH_2).

3.1.2. Propargyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (4)

Oxazoline **3** was prepared by the method previously reported for 2-acetamido-1,3,4,6-tetra-*O*-acetyl-α-D-glucosamine (3.00 g, 7.71 mmol).[9] To a solution of oxazoline **3** and 2-propyn-1-ol (1.35 mL, 23.1 mmol) in dichloroethane (30 mL) was added CSA (0.10 g, 0.44 mmol) under Ar atmosphere and the reaction mixture

was stirred at 80 °C for 4 h. After cooling the reaction mixture to rt, $CHCl_3$ was added. The organic mixture was successively washed with water, satd aq $NaHCO_3$, and brine, dried over anhyd $MgSO_4$, filtered, and evaporated to yield the corresponding syrup, which was crystalized from EtOH to give a crystalline **4** (1.30 g). Chromatographic purification of the filtrate on silica gel with 1:2 (v/v) toluene – EtOAc as the eluent gave pure **4** (0.43 g) as a white solid. Total yield was 1.73 g in 58.2%: mp 192–193 °C [lit.[11] 189–190 °C]; R_f 0.23 [1:2 (v/v) toluene – EtOAc]; IR (KBr) 3285 (ν_{N-H}), 3246 ($\nu_{\equiv C-H}$), 3090 ($\nu_{C\equiv H}$, Ph), 2945 (ν_{C-H}), 2129 ($\nu_{C\equiv C}$), 1748 ($\nu_{C=O}$), 1659 ($\nu_{C=O}$, amide I), 1553 (δ_{N-H}, amide II), 1246 (ν_{C-O}), 1053 (ν_{C-O-C}) cm^{-1}; ^1H NMR δ (500 MHz, $CDCl_3$) 5.86 (d, 1 H, $J_{2,NH}$ = 9.0 Hz, N—H), 5.30 (dd, 1 H, $J_{2,3}$ = 10.6 Hz and $J_{3,4}$ = 9.4 Hz, H-3), 5.09 (t, 1 H, $J_{4,5}$ = 10.0 Hz, H-4), 4.87 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 4.38 (dd, 2 H, J_{gem} = 1.0 Hz and ^4J = 2.3 Hz, ≡CCH_2), 4.28 (dd, 1 H, $J_{5,6b}$ = 4.7 Hz and $J_{6a,6b}$ = 12.3 Hz, H-6b), 4.15 (dd 1 H, $J_{5,6a}$ = 2.4 Hz, H-6a), 3.97 (dt, 1 H, H-2), 3.75 (ddd, 1 H, H-5), 2.50 (t, 1 H, ≡CH), 2.09, 2.04, 2.03, and 1.97 (each s, 12 H, 4 $COCH_3$); ^{13}C NMR δ (125 MHz, $CDCl_3$) 170.91 (C═O), 170.70 (C═O), 170.36 (C═O), 169.36 (NC═O), 98.34 (C-1), 78.51 (C≡C—H), 75.38 (≡C—H), 72.43 (C-5), 71.95 (C-3), 68.52 (C-4), 61.96 (C-6), 55.89 (OCH_2), 54.23 (C-2), 23.32 ($NCOCH_3$), 20.74 (CH_3), 20.67 (CH_3), and 20.61 (CH_3); MALDI-TOF MS calcd for [M+Na^+]: 408.127. Found: *m*/*z* 408.144, [M+K^+]: 424.100. Found: *m*/*z* 424.115.

3.1.3. [1-(4-Vinylphenylmethyl)-1H-1,2,3-triazol-4-yl]methyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (5)

To a solution of alkyne **4** (500 mg, 1.30 mmol) in MeOH (16 mL) was added a solution of azide **2** (250 mg, 1.56 mmol) in MeOH (8 mL) at rt under Ar atmosphere. 0.1 M aq $CuSO_4$ (3.25 mL, 0.33 mmol) and 1 M aq sodium ascorbate (1.82 mL, 1.82 mmol) were successively added to the reaction mixture. After consuming the starting materials (~4 h), the reaction mixture was concentrated *in vacuo*. The residue was diluted with $CHCl_3$ and the organic solution was successively washed with water and brine, dried over anhyd $MgSO_4$, filtered, and evaporated. The residual syrup was chromatographed on silica gel with 40:1 (v/v) $CHCl_3$ – MeOH as the eluent to afford pure **5** (608 mg, 85.7%) as a white solid: R_f 0.29 [5:4:1 (v/v/v) $CHCl_3$ – EtOAc – MeOH], 0.16 [40:1 (v/v) $CHCl_3$ – MeOH]; $[\alpha]_D^{24}$ −40.6° (*c* 1.00, $CHCl_3$); IR (KBr) 3327 (ν_{N-H}), 3132 (ν_{C-H}, Ph), 3075 (ν_{C-H}, Ph), 2941 (ν_{C-H}), 2880 (ν_{C-H}), 1744 ($\nu_{C=O}$), 1668 ($\nu_{C=O}$, amide I), 1539 (δ_{N-H}, amide II), 1233 (ν_{C-O}), 1047 (ν_{C-O-C}) cm^{-1}; ^1H NMR δ (500 MHz, $CDCl_3$) 7.46 (s, 1 H, H-III), 7.41 (d, 2 H, *J* = 8.2 Hz, H-VII), 7.24 (d, 2 H, H-VI), 6.69 (dd, 1 H, J_{cis} = 10.9 Hz and J_{trans} = 17.6 Hz, H-IX), 5.76 (d, 1 H, J_{trans} = 17.6 Hz, H-Xcis), 5.77 (d, 1 H, $J_{2,NH}$ = 9.9 Hz, N-H), 5.49 (s, 2 H, H-IV), 5.29 (d, 1 H, J_{cis} = 10.9 Hz, H-Xtrans), 5.19 (dd, 1 H, $J_{2,3}$ = 10.5 Hz and $J_{3,4}$ = 9.5 Hz, H-3), 5.08 (t, 1 H, $J_{4,5}$ = 9.9 Hz, H-4), 4.89 (d, 1 H, J_{gem} = 13.0 Hz, H-Ib), 4.81 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 4.78 (d, 1 H, H-Ia), 4.25 (dd, 1 H, $J_{5,6b}$ = 4.7 Hz and $J_{6a,6b}$ = 12.3 Hz, H-6b), 4.12 (dd 1 H, $J_{5,6a}$ = 2.4 Hz, H-6a), 3.96 (dt, 1 H, H-2), 3.70 (ddd, 1 H, H-5), 2.07, 2.02, 2.01, and 1.75 (each s, 12 H, 4 $COCH_3$); ^{13}C NMR δ (125 MHz, $CDCl_3$) 171.05 (C═O), 170.83 (C═O), 170.41 (C═O), 169.49 (NC═O), 144.93 (C-II), 138.43 (C-V), 136.01 (C-IX), 133.85 (C-VIII), 128.57 (C-VI), 127.05 (C-VII), 122.81 (C-III), 115.22 (C-X), 100.53 (C-1), 72.76 (C-3), 72.10 (C-5), 68.55 (C-4), 62.78 (C-I), 62.15 (C-6), 54.46 (C-2), 54.09 (C-IV), 23.22 ($NCOCH_3$), 20.88 (CH_3), 20.79 (CH_3), and 20.75 (CH_3); MALDI-TOF MS calcd for [M+H^+]: 545.224. Found: *m*/*z* 545.114, [M+Na^+]: 567.206. Found: *m*/*z* 567.100, [M+K^+]: 583.180. Found: *m*/*z* 583.082.

Anal. Calcd for $C_{26}H_{32}O_9N_4S{\cdot}0.3\,H_2O$: C, 56.78; H, 5.97; N, 10.19. Found: C, 56.87; H, 5.90; N, 9.93.

3.1.4. [1-(4-Vinylphenylmethyl)-1H-1,2,3-triazol-4-yl]methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (6)

Sodium methoxide (8.89 mg, .13 mmol) was added to a solution of acetate **5** (600 mg, 0.55 mmol) in MeOH (6 mL) at rt under Ar atmosphere and the reaction mixture was stirred for 30 min. When complete disappearance of acetate **5** on TLC was observed, IR-120B (H^+) was added to the mixture. The suspension was filtered and the filtrate was evaporated *in vacuo* to yield the corresponding alcohol **6** (419 mg, 78.9%) as a white solid: R_f 0.85 [65:25:4 (v/v/v) $CHCl_3$ – MeOH – H_2O]; IR (KBr) 3281 (ν_{O-H}), 3092 (ν_{C-H}, Ph), 2932 (ν_{C-H}), 1651 ($\nu_{C=O}$, amide I), 1558 (δ_{N-H}, amide II) cm^{-1}; ^1H NMR δ (500 MHz, $CDCl_3$) 7.92 (s, 1 H, H-III), 7.43 (d, 2 H, *J* = 8.2 Hz, H-VII), 7.27 (d, 2 H, H-VI), 6.71 (dd, 1 H, J_{cis} = 11.0 Hz and J_{trans} = 17.7 Hz, H-IX), 5.80 (d, 1 H, J_{trans} = 17.6 Hz, H-Xcis), 5.51 (s, 2 H, H-IV), 5.29 (d, 1 H, J_{cis} = 11.0 Hz, H-Xtrans), 4.84 (d, 1 H, J_{gem} = 13.0 Hz, H-Ib), 4.73 (d, 1 H, H-Ia), 4.54 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 3.86 (dd, 1 H, $J_{5,6b}$ = 1.4 Hz and $J_{6a,6b}$ = 12.4 Hz, H-6b), 3.71 (dd 1 H, $J_{5,6a}$ = 5.3 Hz, H-6a), 3.63 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, H-2), 3.50 (dd, 1 H and $J_{3,4}$ = 9.1 Hz, H-3), 3.42 (m, 2 H, H-4 and H-5), and 1.77 (s, 3 H, $COCH_3$); ^{13}C NMR δ (125 MHz, $CDCl_3$) 174.42 (C═O), 144.11 (C-II), 138.04 (C-V), 136.05 (C-IX), 134.32 (C-VIII), 128.69 (C-VI), 126.90 (C-VII), 125.15 (C-III), 115.19 (C-X), 100.43 (C-1), 76.03 (C-5), 73.74 (C-3), 69.97 (C-4), 62.05 (C-I), 60.82 (C-6), 55.61 (C-2), 53.75 (C-IV), and 22.04 ($NCOCH_3$); MALDI-TOF MS calcd for [M+Na^+]: 441.174. Found: *m*/*z* 441.005, [M+K^+]: 457.148. Found: *m*/*z* 456.995.

3.1.5. Radical polymerization

A solution of carbohydrate monomer **6** and an appropriate amount of acrylamide in deionized water was deaerated under reduced pressure for a few minutes, and then TEMED (0.2 equivalent molar for **6**) and APS (0.1 equivalent molar for **6**) were added. The mixture was stirred at rt and diluted with 1 M aq pyridine – acetic acid buffer (pH 5). The viscous solution was dialyzed against distilled water, followed by direct lyophilization to give corresponding white powdery glycopolymer **7a**–**7c**. The results of radical polymerization are summarized in Table 1.

3.2. Biological evaluation of glycopolymers for WGA

Measurement of fluorescence emission spectra and excitation spectra was carried out at ambient temperature in a Teflon-stoppered cuvette (12.5 mm wide × 45 mm high) containing a 3.0-mL sample. The slit width of the excitation and the emission was 5.0 nm, and the scan speed was medium. The sensitivity was high, and the interval of sampling was 1 nm.

Emission spectra of WGA induced by excitation at 295 nm were uncorrected and were recorded with a Shimadzu RF-5300PC fluorescence spectrophotometer. The cuvette was mounted in a thermostated holder, and the measurement was carried out at 5 °C in order to eliminate the effect of nonspecific binding on the spectra. The concentration of WGA was estimated to be 0.65 μM by using absorption coefficient at 280 nm ($E_{280}^{1\%}$ = 15.0 in 50 mM Tris–HCl buffer, 0.15 M NaCl, pH 7.9) (Lotan & Sharon, 1973).

Acknowledgment

We are grateful to AGC Seimi Chemical Co., Ltd. for providing chloromethylstyrene (CMS) used in this study.

References

Bains, G., Lee, R. T., Lee, Y. C., & Freire, E. (1992). Microcalorimetric study of wheat germ agglutinin binding to *N*-acetylglucosamine and its oligomers. *Biochemistry, 31*, 12624–12628.

Chabre, Y. M., Contino-Pépin, C., Placide, V., Shiao, T. C., & Roy, R. (2008). *Expeditive synthesis of glycodendrimer scaffolds based on versatile TRIS and mannoside derivatives*.

Gil, M. V., Arévalo, M. J., & López, Ó. (2007). Click chemistry – What's in a name? Triazole synthesis and beyond. *Synthesis*, 1589–1620.

Goldstein, I. J., & Poretz, R. D. (1986). In I. E. Liener, N. Sharon, & I. J. Goldstein (Eds.), *The lectins* (pp. 103–115). Orlando, FL: Academic Press, Inc.

Huisgen, R. (1984). A. Padwa (Ed.), *1,3-Dipolar cycloaddition chemistry* (vol. 1) (pp. 1–176). New York: Wiley.
Kolb, H. C., Finn, M. G., & Sharpless, K. B. (2001). Click chemistry: Diverse chemical function from a few good reactions. *Angewante Chemie-International Edition*, *40*, 2004–2021.
Kolb, H. C., & Sharpless, K. B. (2003). The growing impact of click chemistry on drug discovery. *Drug Discovery Today*, *8*, 1128–1137.
Ladmiral, V., Melia, E., & Haddleton, D. M. (2004). Synthetic glycopolymers: An overview. *European Polymer Journal*, *40*, 431–449.
Lee, Y. C. (1978). Synthesis of some cluster glycosides suitable for attachment to proteins or solid matrices. *Carbohydrate Research*, *67*, 509–514.
Lee, Y. C., Townsend, R. R., Hardy, M. R., Lönngren, J., Arnarp, J., Haraldsson, M., et al. (1983). Binding of synthetic oligosaccharides to the hepatic Gal/GalNAc lectin – Dependence of fine structural futures. *Journal of Biological Chemistry*, *258*, 199–202.
Lee, Y. C. (1992). Biochemistry of carbohydrate–protein interaction. *FASEB Journal*, *6*, 3193–3200.
Lotan, R., & Sharon, N. (1973). The fluorescence of wheat germ agglutinin and of its complexes with saccharides. *Biochemical and Biophysical Research Communications*, *55*, 1340–1346.
Lundquist, J. J., & Toone, E. J. (2002). The cluster glycoside effect. *Chemical Reviews*, *102*, 555–578.
Matsuoka, K., & Nishimura, S.-I. (1995). Synthetic glycoconjugates, 5. Polymeric sugar ligands available for determining the binding specificity of lectins. *Macromolecules*, *28*, 2961–2968.
Matsuoka, K., Goshu, Y., Takezawa, Y., Mori, T., Sakamoto, J.-I., Yamada, A., et al. (2007). Practical synthesis of fully protected globotriaose and its glycopolymers. *Carbohydrate Polymers*, *69*, 326–335.
Matsuoka, K., Takita, C., Koyama, T., Miyamoto, D., Yingsakmongkon, S., Hidari, K. I. P. J., et al. (2007). Novel linear polymers bearing thiosialosides as pendant-type epitopes for influenza neuraminidase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *17*, 3826–3830.
Matsuoka, K., Kohzu, T., Hakumura, T., Koyama, T., Hatano, K., & Terunuma, D. (2009). Synthetic construction of a Le^x determinant *via* Gabriel amine synthesis and the glycopolymer involving highly clustered Le^x residues. *Tetrahedron Letters*, *50*, 2593–2596.
Matsuoka, K., Yamaguchi, H., Koyama, T., Hatano, K., & Terunuma, D. (2010). Synthetic construction of a fucosyl chitobiose as an allergen-associated carbohydrate epitope and the glycopolymer involving highly clustered trisaccharidic sequences. *Tetrahedron Letters*, *51*, 2529–2532.
Matsuoka, K., Yamaguchi, H., Kohzu, T., Sakamoto, J.-I., Koyama, T., Hatano, K., et al. (2012). Convenient assembly of trimeric Le^x determinants using carbosilane scaffolds by means of Huisgen cycloaddition. *Tetrahedron Letters*, *53*, 6793–6796.
Miyagawa, A., Kurosawa, H., Watanabe, T., Koyama, T., Terunuma, D., & Matsuoka, K. (2004). Synthesis of glycoconjugate polymer carrying globotriaose as artificial multivalent ligand for Shiga toxin-producing *Escherichia coli* O157: H7. *Carbohydrate Polymers*, *57*, 441–450.
Nakabayashi, S., Warren, C. D., & Jeanloz, R. W. (1986). A new procedure for the preparation of oligosaccharide oxazolines. *Carbohydrate Research*, *150*, C7–C10.
Nishimura, S., Matsuoka, K., & Kurita, K. (1990). Synthetic glycoconjugates: Simple and potential glycoprotein models containing pendant *N*-acetyl-D-glucosamine and *N*, N′-diacetylchitobiose. *Macromolecules*, *23*, 4182–4184.
Nishimura, S.-I., Matsuoka, K., Furuike, T., Ishii, S., & Kurita, K. (1991). Synthetic glycoconjugates. 2. *n*-Pentenyl glycosides as convenient mediators for the syntheses of new types of glycoprotein models. *Macromolecules*, *24*, 4236–4241.
Nishimura, S.-I., Furuike, T., Matsuoka, K., Maruyama, K., Nagata, K., Kurita, K., et al. (1994). Synthetic glycoconjugates. 4. Use of ω-(acrylamido)alkyl glycosides for the preparation of cluster glycopolymers. *Macromolecules*, *27*, 4876–4880.
Rowan, A. S., Nicely, N. I., Cochrane, N., Wlassoff, W. A., Claiborne, A. L., & Hamilton, C. J. (2009). Nucleoside triphosphate mimicry: A sugar triazolyl nucleoside as an ATP-competitive inhibitor of B. anthracis pantothenate kinase. *Organic & Biomolecular Chemistry*, *7*, 4029–4036.
Schwefel, D., Maierhofer, C., Beck, J. G., Seeberger, S., Diederichs, K., Möller, H. M., et al. (2010). Structural basis of multivalent binding to wheat germ agglutinin. *Journal of American Chemical Society*, *132*, 8704–8719.
Varma, A. J., Kennedy, J. F., & Galgali, P. (2004). Synthetic polymers functionalized by carbohydrates: A review. *Carbohydrate Polymers*, *56*, 429–445.
Vázquez-Dorbatt, V., Lee, J., Lin, E.-W., & Maynard, H. D. (2012). Synthesis of glycopolymers by controlled radical polymerization techniques and their applications. *ChemBioChem*, *13*, 2478–2487.
Wu, P., & Fokin, V. V. (2007). Catalytic azide-alkyne cycloaddition: Reactivity and applications. *Aldrichimica Acta*, *40*, 7–17.
Zemplén, G., & Pacsu, E. (1929). Übel die verseifung acetylierter zucker und verwandter substanzen. *Berichte*, *62*, 1613–1614.