

Article

Cellulose Chemistry Meets Click Chemistry: Syntheses and Properties of Cellulose-Based Glycoclusters with High Structural Homogeneity

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Abstract: β -1,4-Glucans having oligosaccharide appendages (*O*-/*N*-linked β -maltoside and *O*-/*N*-linked β -lactoside) at 6C positions of all repeating units can be readily prepared from cellulose through a two step strategy composed of: (1) regio-selective and quantitative bromination/azidation to afford 6-azido-6-deoxycellulose; and (2) the subsequent Cu^+ -catalyzed coupling with oligosaccharides having terminal alkyne. The resultant cellulose derivatives showed improved water solubility in comparison to native cellulose; they, however, bound to carbohydrate-binding proteins in a rather non-specific manner. Molecular dynamics calculations revealed that these properties are attributable to rigid sheet-like structures of the cellulose derivatives and the subsequent exposure of their hydrophobic moieties to solvents.

Keywords: cellulose; 6-bromo-6-deoxycellulose; 6-azide-6-deoxycellulose; glycopolymers; regio-selective modifications; lectins; molecular dynamics

1. Introduction

Cellulose (linear β -1,4-glucan) is the most promising candidate of eco-materials, since it is the most abundant biopolymer in nature. In addition, cellulose is advantageous as a chiral scaffold, since it has an enormous number of chiral centers. For example, silica gels coated with cellulose derivatives are now widely used as stationary phases for chiral separation [1-4]. Chemically modified celluloses find their application in pharmaceutical/medicinal areas because of their biocompatibility and biodegradability [5,6]. Artificial celluloses carrying multiple copies of carbohydrates are, in this respect, quite attractive bio-materials, since such cellulose derivatives should acquire enhanced affinity towards carbohydrate-binding proteins (lectins), toxins, and viruses, as well as excellent water solubility arising from the clustered carbohydrates [7-11]. A limited example of cellulose-based glycoclusters can be, however, found in the literature [12]. In spite of these great possibilities, chemical modification of cellulose, that is the very first step to access cellulose-based advanced materials, still contain tedious obstacles.

The traditional approach to develop chemically modified celluloses can be divided into two categories. The first one is direct modification of native cellulose, by which various cellulose derivatives have been developed so far [13-18]. This direct approach was adopted to develop cellulose-based glycoclusters [12]. Although this strategy is quite simple, it suffers from one serious problem: that is, random modification and the structural heterogeneity of resultant cellulose derivatives. Native cellulose has no reactive functionality except for hydroxy groups and therefore, modification onto cellulose is mainly based on nucleophilic substitution reactions between these hydroxy groups and electrophiles. The hydroxy groups of cellulose have, however, similar reactivity and therefore, regio-selective and quantitative reactions are hardly accomplished. To the best of our knowledge, no regio-selective/quantitative approach towards cellulose derivatives with perfect structural homogeneity has been established. The cellulose derivatives developed so far, in fact, have functional appendages at random positions along their cellulose main chains. Time and labor consuming characterization processes ($^1\text{H}/^{13}\text{C}$ NMR, HPLC analysis after digestion, *etc.*) are, therefore, inevitable to reveal their structural details. Furthermore, the structure-function relationship that is essential for tuning or refinement of their properties, is hardly obtained. These difficulties pose a serious barrier for non-specialists of organic chemistry to participate in cellulose chemistry.

The second approach to access cellulose derivatives is enzymatic polymerization of chemically modified glucoside monomers to the corresponding cellulose derivatives (bottom-up approach) [19-21]. The most potential advantage of this approach is perfect structural homogeneity of the resultant cellulose derivatives. Furthermore, if alternatively functionalized (AB-type) cellobiosides (β -1,4-linked diglucosides) are used as monomers, alternatively modified celluloses (...ABABAB...) can be obtained [22-24]. Although this chemo-enzymatic approach is quite powerful, it also suffers from some serious problems. Since preparation of the glucoside/cellobioside monomers requires tedious synthetic routes, research groups having specialists for organic synthesis can pursue this approach. Furthermore, the introduced functional modules are highly limited, since the monomers having large functional appendages cannot be polymerized because of their lowered affinities to the enzymes. In fact, the monomers having substituents larger than *O*-methyl group were reported to be hardly polymerized to the corresponding cellulose derivatives. Furthermore, hydrolysis (reverse

reaction of the enzymes) of the cellulose derivatives simultaneously occurs and usually molecular weight of the obtained cellulose derivatives is quite low. These disadvantages of the bottom-up approach strongly hinder their wide application. Convenient, general, quantitative and regio-selective methods for direct modifications of cellulose are, therefore, strongly desired to accelerate participation of researchers from wide scientific fields in cellulose chemistry.

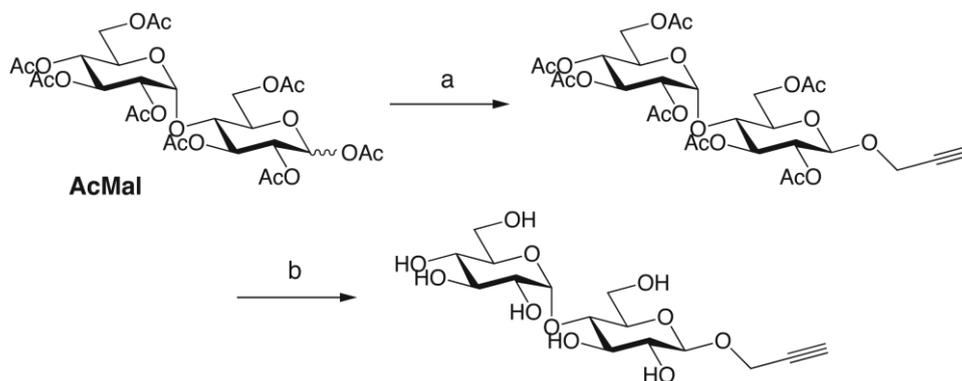
Recently, one of the authors with Shinkai's research group has developed an easy synthetic approach towards functionalized curdlan (β -1,3-glucan) through a unique two step strategy [25-27]. In this synthetic approach, native curdlan is firstly brominated with triphenylphosphine and carbon tetrabromide and then azidated with sodium azide to afford 6-azido-6-deoxycurdlan (CUR-N₃). The structural advantage of 6-azido-6-deoxycurdlan is its perfect homogeneity: that is, all 6-OH groups of curdlan are selectively and quantitatively converted into 6-N₃ groups without any side reactions at 2-OH or 3-OH groups. The subsequent coupling with alkyne-terminated functional modules (oligosaccharides, ferrocene, porphyrin, *etc.*) mediated by Cu⁺ (click chemistry) afforded curdlan derivatives having the functional modules exclusively at all 6C positions. This success encouraged us to apply this synthetic methodology towards cellulose chemistry, and recently, we reported a short communication in which artificial cellulose having *N*-linked β -lactosides (Cel-*N*-Lac) can be synthesized through a similar synthetic strategy [28]. Although Cel-*N*-Lac can be dissolved into water to some extent to show strong binding affinity towards *Recinis communis agglutinin* (RCA₁₂₀, β Gal specific), its water solubility and lectin specificity are not so high. We assumed that hydrophobic phenyl spacers between β -lactoside appendages and cellulose main chain, lower not only the water solubility but also the lectin specificity. In this respect, we newly synthesized three cellulose-based glycoclusters having *O*-/*N*-linked β -maltosides and *O*-linked β -lactosides and carried out detailed investigation on their structure-function relationship that is informative to develop practical cellulose-based glycoclusters. We herein report detailed synthetic protocols of these cellulose-based glycoclusters and their properties including water solubility and lectin affinity.

2. Results and Discussion

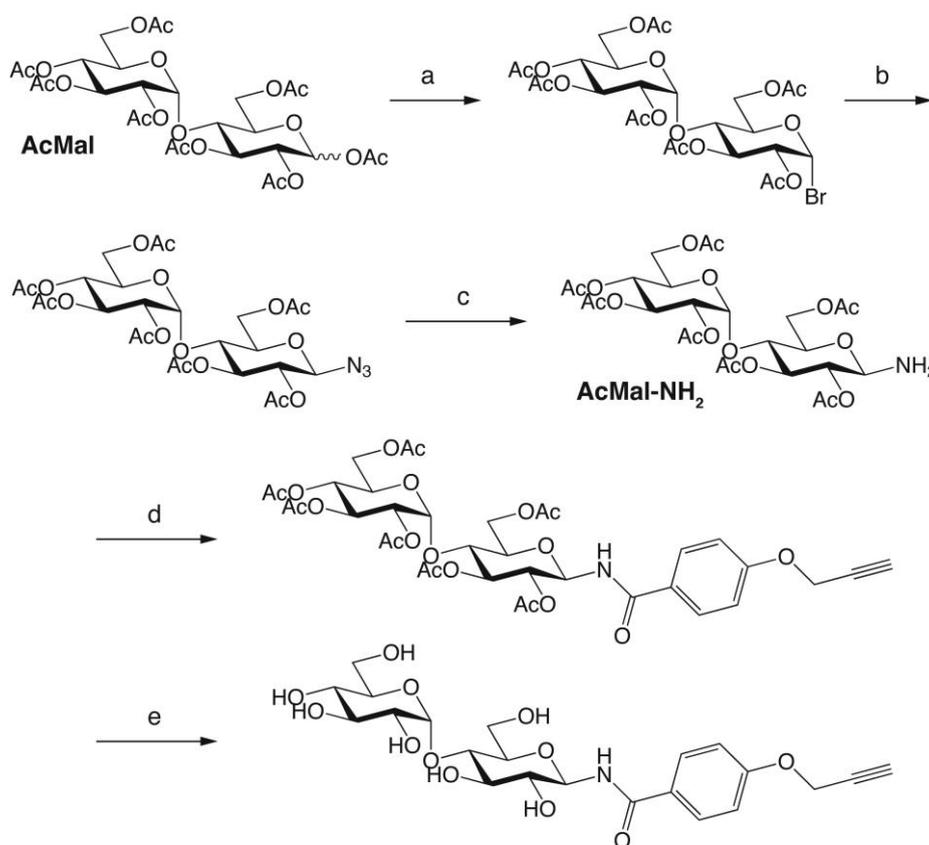
2.1. Synthesis of alkyne-terminated oligosaccharides

Oligosaccharides carrying terminal alkyne were prepared through two different synthetic routes. In the first one (Scheme 1), *per*-acetylated maltose (AcMal) and lactose (AcLac) were coupled with propargyl alcohol by treating with boron trifluoride. The following deacetylation attained by sodium methoxide in methanol gave *O*-linked β -maltoside and β -lactoside having terminal alkyne, respectively. On the other hand, in the second scheme (Scheme 2), AcMal and AcLac were firstly brominated with hydrogen bromide, azidated with sodium azide, and then hydrogenated to give the corresponding oligosaccharide derivatives having amino functionality at their anomeric positions (AcMal-NH₂ and AcLac-NH₂, respectively). The resultant oligosaccharides were coupled with a benzoic acid derivative having terminal alkyne and then, deacetylated to give *N*-linked β -maltoside and β -lactoside having terminal alkyne, respectively.

Scheme 1. Synthesis of the *O*-linked β -maltoside having terminal alkyne (*O*-Mal-yn):
 (a) BF_3OEt_2 , CH_2Cl_2 , propargyl alcohol, 2 day, (b) NaOMe, MeOH, r.t., 20 h, 68% (2 steps).



Scheme 2. Synthesis of the *N*-linked β -maltoside having terminal alkyne (*N*-Mal-yn):
 (a) HBr, Ac_2O , CH_2Cl_2 , 1 h, (b) NaN_3 , DMF, 70 °C, 3 h, 69% (2 steps) (c) H_2 , Pd/C, r.t., 24 h, 88%, (d) *p*-propargyloxybenzoyl chloride, Et_3N , r.t., 1 h, 42%, (e) NaOMe, MeOH/THF, r.t., 24 h, 99%.

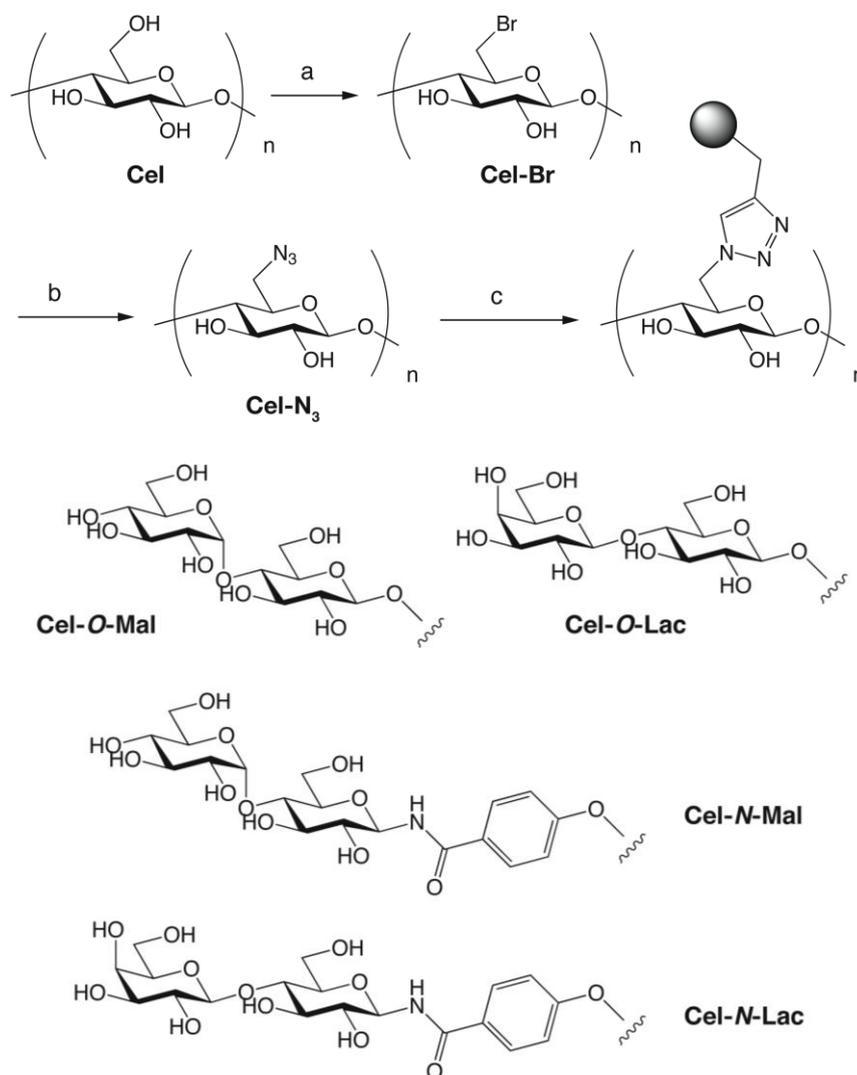


2.2. Preparation of 6-azido-6-deoxycellulose

Cellulose ($\text{DP}_n = 280$) was dissolved into *N,N*-dimethylacetamide (DMA) containing LiCl by stirring at 80 °C for 24 h and then, converted into 6-bromo-6-deoxycellulose (Cel-Br) through activation of its primary hydroxy groups with triphenylphosphine, followed by bromination with carbon tetrabromide (Scheme 3). It should be emphasized that the most popular and well studied

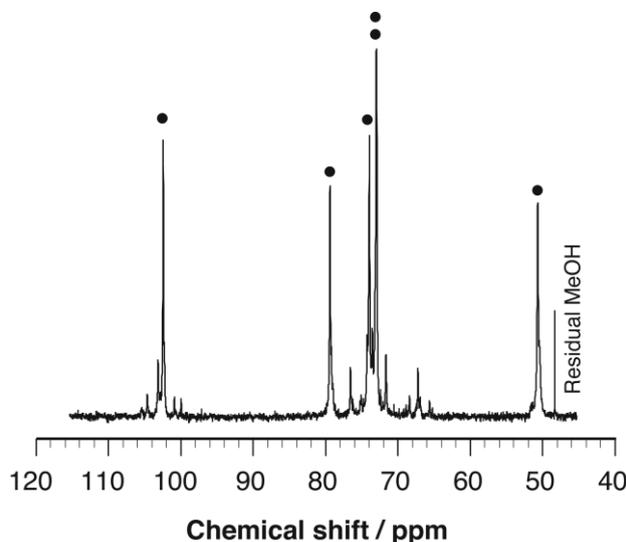
reagent for the bromination of cellulose includes *N*-bromosuccinimide (NBS) [13–18]. We, however, used carbon tetrabromide instead of NBS. Although we first carried out ^{13}C NMR measurement to reveal structural details of Cel-Br, its ^{13}C NMR spectrum was too noisy to be assigned, suffering from its low solubility in any deuterated solvents (e.g., $\text{DMSO-}d_6$). We therefore next carried out azidation reaction without full characterization of Cel-Br. The subsequent azidation was attained by treating Cel-Br with NaN_3 in a DMA/dimethylsulfoxide (DMSO) mixed solvent system at $85\text{ }^\circ\text{C}$ for 42 h to afford 6-azide-6-deoxycellulose (Cel- N_3). It should be noted that solubility of these cellulose derivatives drastically changes, that is, Cel-Br is relatively soluble in DMA but hardly soluble in DMSO, and Cel- N_3 is less soluble in DMA but well-soluble in DMSO. When we carried out this azidation in DMA solution throughout the reaction, partially azidated cellulose was precipitated and perfect conversion from Cel-Br to Cel- N_3 was never achieved. We therefore started the azidation in DMA solution and then suitably added DMSO to the reaction mixture to keep the mixture homogeneous.

Scheme 3. Synthesis of Cel- N_3 and the subsequent coupling with alkyne-terminated oligosaccharides to access cellulose-based glycoclusters: (a) triphenylphosphine, in DMA, r.t., 4 h, and then, carbon tetrabromide, $60\text{ }^\circ\text{C}$, 24 h, (b) NaN_3 in the DMA/DMSO mixed solvent system, $85\text{ }^\circ\text{C}$, 42 h, (c) alkyne-terminated oligosaccharides, CuBr_2 , ascorbic acid, propylamine, DMSO, r.t., 12 h, 65–79%.



Regio-selectivity and quantitativity of these reactions were assessed by ^{13}C NMR spectrum of Cel-N₃ (Figure 1). The peak assignable to hydroxymethyl groups ($-\text{CH}_2\text{OH}$, 60.90 ppm) entirely disappears and that of azidomethyl group ($-\text{CH}_2\text{N}_3$, 50.66 ppm) newly appears. Furthermore, the simple monosaccharide-like spectrum is composed of six predominant peaks which implies a highly homogeneous structure of Cel-N₃ along its main chain. It should, however, be noted that some small but unneglectable peaks accompany the predominant peaks, indicating that some undesired side reactions occur at the other positions, presumably 2-OH and 3-OH groups. This superfluous azidation was supported by elemental analysis, in which Cel-N₃ shows N/C ratio of 0.60567 ± 0.00059 . This N/C ratio means that an averaged number of azide group introduced onto the repeating unit, or degree of substitution (DS), is 1.0386 ± 0.0010 , slightly higher than that expected for Cel-N₃ with perfect homogeneity (DS = 1.0000). Although a limited side reaction was observed as mentioned above, it should be emphasized that the structural homogeneity (C6-selectivity and quantitativity) attained through our protocol is equal with, or higher than, that attained through conventional methodology using NBS as a bromination reagent [13-18].

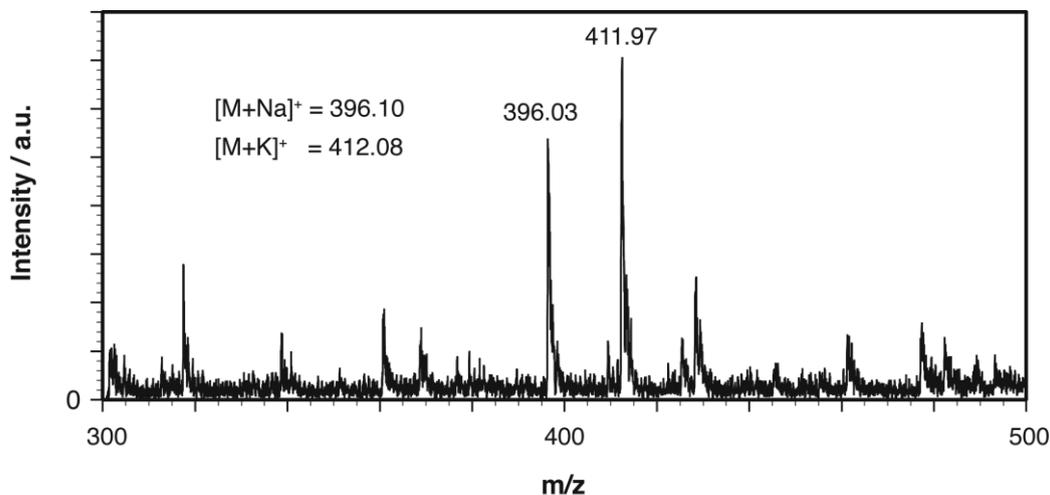
Figure 1. ^{13}C NMR spectrum of Cel-N₃, in which closed circles indicate main peaks assignable to the 6-azide-6-deoxyglucoside repeating unit: 300 MHz, DMSO-*d*₆, 60 °C.



The structural homogeneity of Cel-N₃ was also confirmed by acid-catalyzed hydrolysis of Cel-N₃ followed by tin layer chromatographic (TLC) and matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectral analysis of the hydrolysate. Briefly, we incubated Cel-N₃ in aqueous HCl (35 % v/v) at the ambient temperature for five days and the resultant hydrolysate was acetylated in a mixture of acetic anhydride and pyridine. The TLC analysis of the acetylated product showed only one main spot arising presumably from 1,2,3,4-tetra-*O*-acetyl-6-azide-6-deoxyglucose (Ac₄Glc-6-N₃) and no other spot attributable to the byproducts can be detected. The quantitative conversion was also supported by the MALDI-TOF mass spectral analysis showing two main peaks at $m/z = 396.03$ and 411.97 that can be attributed to $[\text{M} + \text{Na}]^+$ (calc. 396.10) and $[\text{M} + \text{K}]^+$ (calc. 412.08), respectively (Figure 2). Together with the aforementioned IR spectral, ^{13}C NMR

spectral, and elemental analysis data, these two additional data clearly indicate the high structural homogeneity of Cel-N₃.

Figure 2. MALDI-TOF-MS spectrum of the hydrolysate after the acetylation.



2.3. Cu⁺-catalyzed [3 + 2]-cycloadditions of the alkyne-terminated oligosaccharides onto Cel-N₃

Although Cel-N₃ with highly homogeneous repeating structure was obtained as mentioned above, the azide functionality has little function, such as protein recognition and light harvesting functions, and therefore, Cel-N₃ itself cannot find any practical applications. On the other hand, Cel-N₃ can give full scope to its ability when it is used as a substrate for further modification to access cellulose-based advanced materials. However, if the further modification reaction is accompanied by undesired side reactions and/or incomplete conversions, the resultant cellulose derivatives finally have heterogeneous repeating units. Exclusive chemoselectivity and perfect conversion yield are therefore highly required for the further modification reaction. To satisfy these criteria, we applied click chemistry for the further modification on Cel-N₃ [29-33].

A coupling of the alkyne-terminated oligosaccharides and Cel-N₃ can be achieved in DMSO containing CuBr₂, ascorbic acid, and propylamine. The resultant cellulose derivatives having *O*-/*N*-linked β-lactoside (Cel-*O*-Lac and Cel-*N*-Lac, respectively) and *O*-/*N*-linked β-maltoside (Cel-*O*-Mal and Cel-*N*-Mal, respectively) can be obtained as white powder after purification through dialysis (water, MWCO8000) and the subsequent lyophilization.

Characterization of these cellulose-based glycoclusters was achieved based on their IR and ¹³C NMR spectra. In their IR spectra, the strong azide peak (2,101 cm⁻¹) observed in that of Cel-N₃ was entirely diminished, suggesting perfect conversion of azidomethyl functionalities into oligosaccharides tethered with 1,4-triazole linkers (Figure 3). The quantitative conversion of Cel-N₃ into the corresponding cellulose-based glycoclusters was also supported by their ¹³C NMR spectra, in which the peak assignable to azidomethyl functionalities (–CH₂N₃) entirely disappeared and those of 1,4-triazole linkers (*ca.* 125 and 142 ppm) and oligosaccharide appendages newly appeared (Figure 4). Only a limited number of unassignable peaks were observed in the ¹³C NMR spectra, indicating that structural homogeneity is maintained through the coupling reaction taking full advantages of click chemistry. This effective conversion of the azidomethyl group to the triazole-tethered

oligosaccharides was also supported by elemental analysis. For example, Cel-*O*-Mal showed N/C ratio of 0.1700 ± 0.0014 indicating that an average number of β -maltoside units attached onto the repeating unit (DS) is 1.0198 ± 0.0084 , that is comparable to that of Cel- N_3 . It should be emphasized here that these extremely bulky carbohydrate modules can never be introduced into polysaccharides through the chemo-enzymatic bottom-up strategy. To the best of our knowledge, our approach is the only one to access β -1,4-glucan-based glycoclusters both with high structural homogeneity of the cellulose main chain and large multivalency of the oligosaccharide appendages.

Figure 3. IR spectra of (A) Cel- N_3 and (B) Cel-*O*-Mal: KBr.

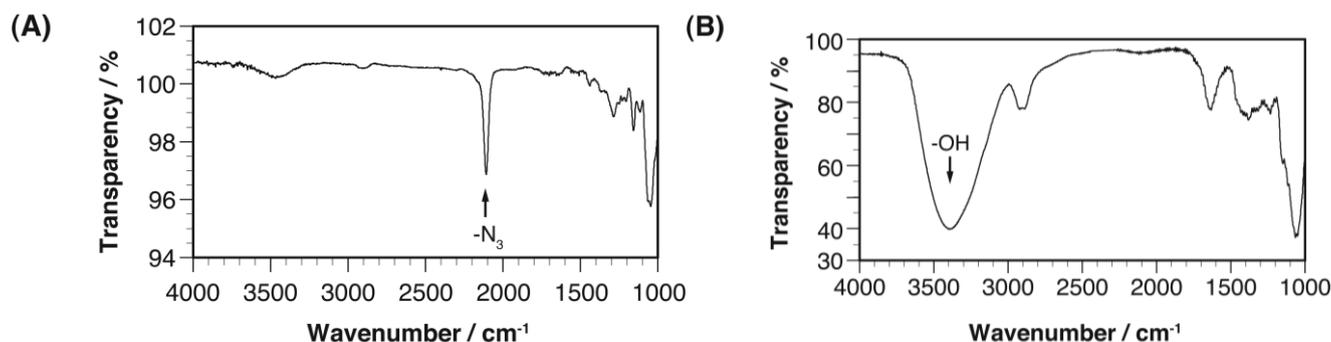
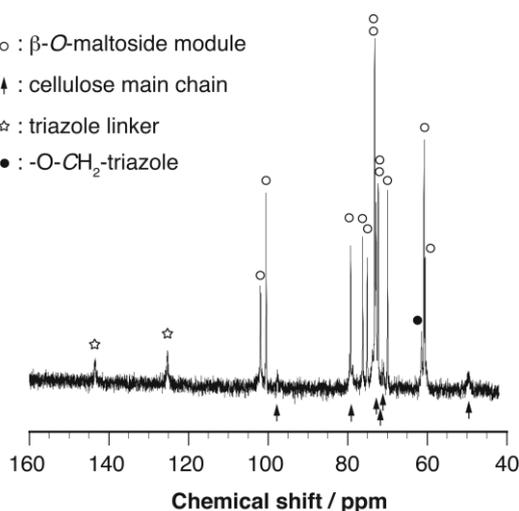


Figure 4. ^{13}C NMR spectrum of Cel-*O*-Mal, in which open circles, arrows, open stars, and a closed circle indicate main peaks assignable to the β -*O*-maltoside modules, the cellulose main chain, the triazole linkers, and methylene spacers ($-\text{O}-\text{CH}_2-\text{triazole}$), respectively: 300 MHz, $\text{DMSO}-d_6$, 60 °C.



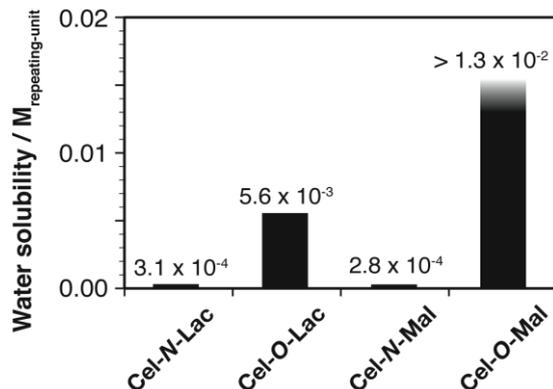
2.4. Water solubility of the cellulose derivatives

Cellulose itself is hardly soluble in water, mainly because of intramolecular hydrogen bonding networks (e.g., between O5 and 3-OH of the preceding repeating unit). The resultant rigid tape-like conformation induces enhanced packings of cellulose strands stabilized by intermolecular hydrogen bondings and hydrophobic interactions. Chemical modification of cellulose to increase its water solubility is quite important to develop cellulose-based advanced materials, especially those for pharmaceutical and medicinal purposes. To assess water solubility of the cellulose-based

glycoclusters, we added the cellulose-based glycoclusters into small amounts of water and then, the resultant turbid mixtures were kept at 60 °C for one day to prepare their saturated aqueous solutions. After cooling to an ambient temperature followed by centrifugations to remove precipitates, carbohydrate concentrations in their supernatants were assayed through a well-known phenol-sulfuric acid protocol.

Although cellulose ($DP_n = 280$) is hardly soluble in water, the cellulose-based glycoclusters, especially Cel-*O*-Lac and Cel-*O*-Mal, show enhanced water solubility arising from their bulky and water-soluble oligosaccharide appendages (Figure 5). The water solubility of the cellulose-based glycoclusters, however, drastically differ from each other depending on chemical structures of the appendages. Through comparison of these data, two important tendencies can be found in their structure-solubility relationship. First, one is a critical effect of hydrophobic spacers: that is, cellulose-based glycoclusters having *N*-linked oligosaccharides are less soluble in water than the corresponding *O*-linked counterparts. This low water solubility of *N*-linked glycoclusters is clearly attributable to hydrophobic phenyl spacers linking between the cellulose main chain and the oligosaccharide appendages. The other is the importance of its oligosaccharide structure: that is, Cel-*O*-Mal has much higher water solubility than that of Cel-*O*-Lac. The low water solubility of Cel-*O*-Lac probably arises from its hydrophobic α -face of non-reducing β -galactoside units.

Figure 5. Water solubility of the cellulose-based glycoclusters: water solubility of Cel-*O*-Mal is too high to be determined.

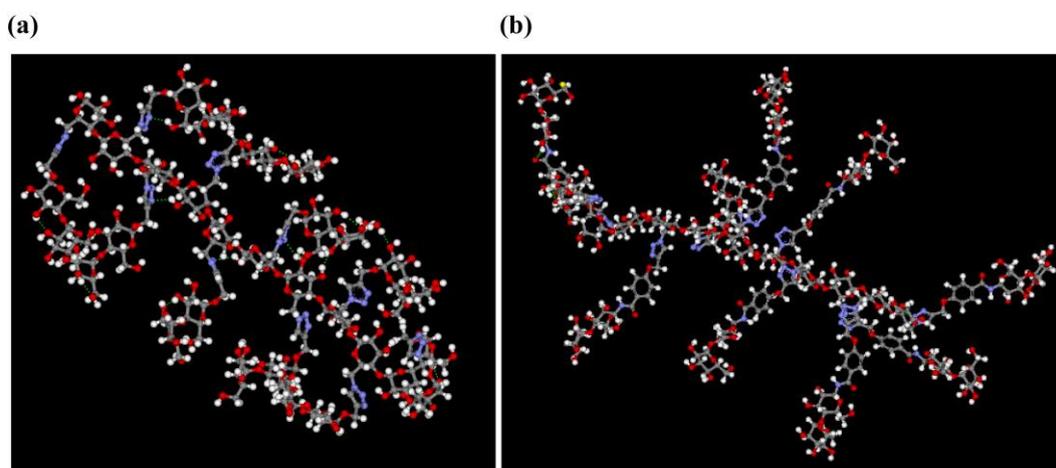


The observed negative effects arising from the hydrophobic spacers and the α -face on their water solubility are much more drastic in comparison with those observed for glycoclusters, based on flexible artificial polymers such as polystyrene [34-40] and polyacrylamide [41-46]. For example, polystyrenes having β -lactoside appendages still have excellent water solubility. In these glycoclusters, the flexible nature of the polymer main chain permits its structural rearrangement and therefore, these glycoclusters take unique cylindrical structures composed of hydrophobic cores and hydrophilic surfaces [47-49]. As a result, hydrophobic moieties interact with each other in an intrapolymer fashion, and intermolecular interactions to induce polymer aggregates are highly limited. Whereas, in the case of the cellulose-based glycoclusters, the rigid nature of the cellulose main chain should hinder their structural rearrangements and then, their hydrophobic moieties should still remain exposed to the solvent. Especially, in the case of Cel-*N*-Mal and Cel-*N*-Lac, the aforementioned

structural rigidity, and the resultant exposure of both phenyl and triazole spacers, critically lower the water solubility.

Our hypothesis was supported by molecular dynamics (MD) calculation, in which 10-mers of Cel-*O/N*-Mal were constructed and then, dynamics calculations (1,000,000 steps, CHARMM, 300 K, NVT, GBSW solvent model) were carried out after minimization, heating, and equilibration processes. The most stable conformations for these cellulose-based glycopolymers during the dynamics processes are shown in Figure 6, in which they both take sheet-like structures with their hydrophobic phenyl/triazole spacers exposed to the solvent. These sheet-like structures should result in enhanced intermolecular networks composed of hydrophobic interactions and hydrogen bondings to reduce water solubility. Since the most stable conformations of Cel-*O*-Mal and Cel-*N*-Mal take sheet-like forms and are not different from each other, the observed much lower water solubility of Cel-*N*-Mal in comparison to that of Cel-*O*-Mal should arise from the additional hydrophobic phenyl spacers in their structures.

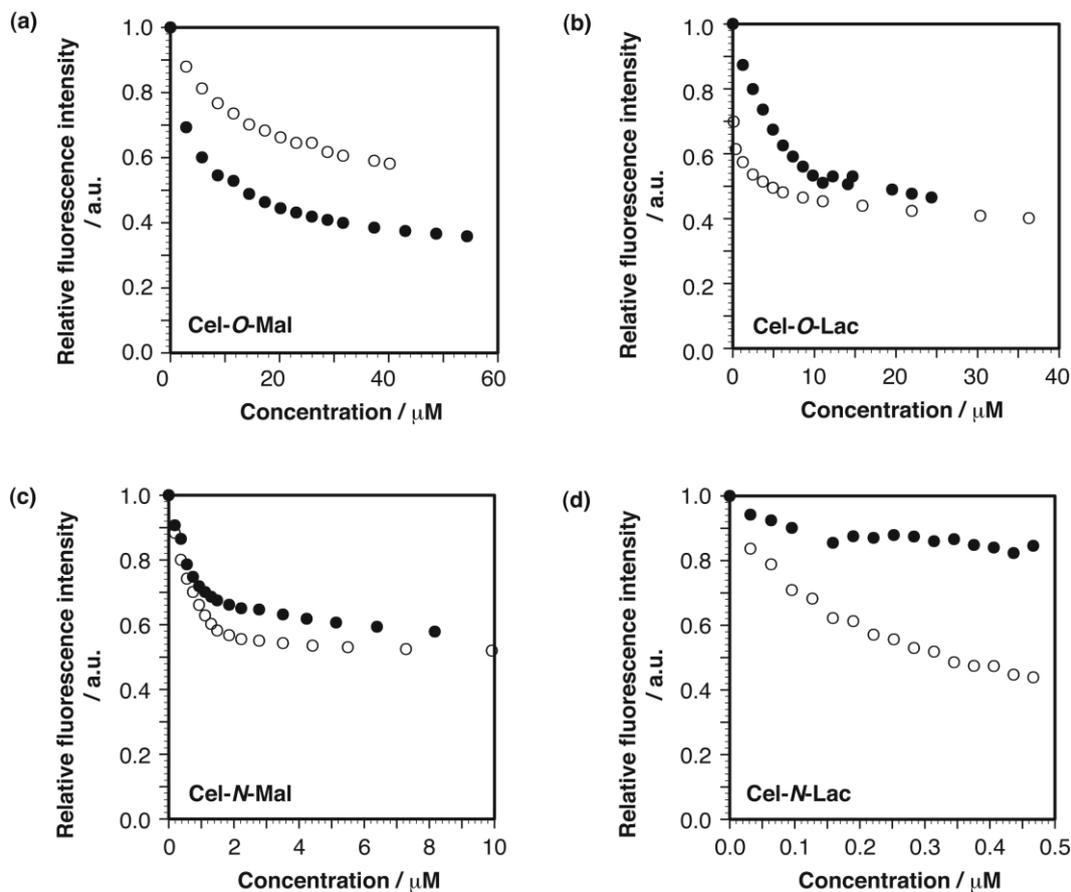
Figure 6. The most stable conformation of (a) Cel-*O*-Mal and (b) Cel-*N*-Mal (10-mer) during the dynamics (1 ns, CHARMM, 300K, NVT, GBSW).



2.5. Affinity between the cellulose-based glycoclusters and lectins

Affinity between the cellulose-based glycoclusters and lectins was assessed through fluorescence titration assay using fluorescein isothiocyanate (FITC) -labeled lectins [50,51]. In this assay, we used concanavalin A (ConA) and *ricinis communis agglutinin* (RCA₁₂₀) as lectins that specifically bind to α Man/ α Glc and β Gal, respectively [52]. We, therefore, expected that Cel-*O/N*-Mals specifically bound to FITC-ConA and Cel-*O/N*-Lacs to FITC-RCA₁₂₀. Results of the binding assays were, however, much different from our expectations: that is, little lectin specificity was observed for the cellulose-based glycoclusters. For example, as shown in Figure 7(a), an injection of Cel-*O*-Mal induced a drastic decrement in fluorescent intensity not only for FITC-ConA but also for FITC-RCA₁₂₀, indicating a non-specific binding. Such non-specific lectin bindings were also observed for the other cellulose derivatives, as shown in Figure 7(b–d). We assume that the non-specific lectin bindings arose from hydrophobic interactions between the cellulose-based glycoclusters and lectins. The aforementioned sheet-like structure and the resultant exposure of hydrophobic moieties should play negative roles not only for their water solubility but also for lectin specificity.

Figure 7. Relative fluorescence intensities (I/I_0) of (closed circle) FITC-ConA and (open circle) FITC-RCA₁₂₀ under various concentrations of (a) Cel-O-Mal, (b) Cel-O-Lac, (c) Cel-N-Mal, and (d) Cel-N-Lac: Tris-buffer (20 mM, pH 7.2), 20 °C, Ex = 490 nm, Em = 518 nm, $d = 10$ mm, $[CaCl_2]$ and $[MnCl_2] = 100$ μ M.



3. Experimental Section

3.1. General

^1H and ^{13}C NMR spectra were acquired on a JEOL AL300 (Jeol Datum, Ltd.) in CDCl_3 , CD_3OD , D_2O , or $\text{DMSO}-d_6$ at 300 MHz. The chemical shifts were reported in ppm (δ) relative to Me_4Si . IR spectra were recorded on a JASCO FT/IR-4100 fourier transform infrared spectrometer (Jasco Co., Ltd.). Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on SHIMAZU AXIMA-CFR+ (Shimazu, Ltd.). Fluorescence titration assay was carried out using JASCO FP-6200 spectrofluorometer (Jasco Co., Ltd.). Molecular dynamics calculation was carried out by using Discovery Studio 2.0 program (Accelrys Co., Ltd.). Elemental analysis was conducted by Chemical Analysis Center in Chiba University. Silica gel 60 N (particle size 63–210 μm) for column chromatography was purchased from Kanto Chemical Co. Inc. Thin layer chromatography (TLC) was carried out with Merck TLC aluminum sheets pre-coated with silica gel 60 F_{254} . Cellulose (Avicel microcrystalline cellulose, $\text{DP}_n = 280$) was kindly supplied from FMC Co. All other chemicals were purchased from Wako Pure Chemical Industries Ltd., Kishida Chemicals Co. Ltd., Kanto Chemical Co. Ltd. and Funakoshi Co. Ltd. and used without purification.

3.2. Synthesis of methyl *p*-propargyloxybenzoate

To methyl *p*-hydroxybenzoate (1.05 g) in DMF (50 mL), K₂CO₃ (3.41 g) was added and the resultant mixture was stirred at 60 °C for 1 h. After cooling down to an ambient temperature, propargyl bromide (1.28 mL) was added, and then stirring was continued at room temperature overnight. The reaction mixture was then diluted with ethyl acetate and washed with NaCl-saturated aqueous solution and deionized water repeatedly. The resultant organic layer was dried over anhydrous MgSO₄, and evaporated to give methyl *p*-propargyloxybenzoate as a white powder in 87% yield: [M + Na]⁺ = 213.14 (calc. 213.10); ¹H NMR (300 MHz, CDCl₃, TMS): 8.01 (d, *J* = 9.0 Hz, 2H), 7.00 (d, *J* = 9.0 Hz, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 3.89 (s, 3H), 2.56 (t, *J* = 2.4 Hz, 1H); ¹³C NMR (300 MHz, CDCl₃, TMS): 166.72, 161.14, 131.56, 123.43, 114.47, 77.81, 76.10, 55.81, 51.96; IR (KBr, cm⁻¹): 1703.

3.3. Synthesis of *p*-propargyloxybenzoic acid

To methyl *p*-propargyloxybenzoate (0.42 g) in THF (500 mL), KOH (1.20 g) in water (10 mL) was added and then, the mixture was refluxed for 14 h. After acidifying the resultant reaction mixture (*ca.* pH 2) by an addition of a proper amount of aqueous HCl, the mixture was evaporated to dryness and purified by chromatography on a silica gel column (30 cm long; 3 cm i.d.; chloroform-methanol = 9:1 in v/v) to give *p*-propargyloxybenzoic acid as a white powder (31%): [M + Na]⁺ = 169.98 (calc. 169.06); ¹H NMR (300 MHz, CD₃OD, TMS): 7.97 (d, *J* = 9.0 Hz, 2H), 7.03 (d, *J* = 9.0 Hz, 2H), 4.80 (d, *J* = 2.4 Hz, 2H), 2.98 (t, *J* = 2.4 Hz, 1H); ¹³C NMR (300 MHz, CDCl₃ containing a small amount of CD₃OD, TMS): 169.2, 161.8, 131.98, 124.31, 114.52, 77.38, 76.20, 55.90; IR (KBr, cm⁻¹): 1,680.

3.4. General procedure for the synthesis of *p*-propargyloxybenzamido-2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-maltoside/lactoside

A thionyl chloride solution (10 mL) containing *p*-propargyloxybenzoic acid (0.14 g) was refluxed for 5 h and evaporated to dryness. The resultant yellow solid was dissolved into THF and then, mixed with 2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-maltosylamine or -lactosylamine (0.53 g) in THF. Triethylamine was added to the reaction mixture immediately and the resultant mixture was stirred for 5 min, diluted with ethyl acetate, and washed with 0.5 N HCl aq and NaHCO₃ aq, repeatedly. The resultant organic layer was dried over anhydrous MgSO₄, evaporated, and purified by chromatography on a silica gel column (30 cm long; 3 cm i.d.; chloroform-methanol = 25:1 in v/v) to give *p*-propargyloxybenzamido-2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-maltoside or -lactoside as a white powder.

3.4.1. *p*-propargyloxybenzamido-2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-maltoside

Yield: 42%; [M + Na]⁺ = 817.32 (calc. 816.24); ¹H NMR (300 MHz, CDCl₃ containing a small amount of CD₃OD, TMS) δ 7.74 (d, *J* = 9.0 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 5.46 (t, *J* = 9.0 Hz, 1H), 5.47–5.40 (m, 2H), 5.38 (dd, *J* = 8.4 and 9.0 Hz, 1H), 5.07 (t, *J* = 9.9 Hz, 1H), 4.91 (d, *J* = 9.9 Hz, 1H), 4.87 (d, *J* = 5.4 and 6.3 Hz, 1H), 4.74 (d, *J* = 2.4 Hz, 2H), 4.46 (dd, *J* = 2.7 and 12.3 Hz, 1H), 4.30–4.20 (m, 2H), 4.08–3.87 (m, 4H), 2.56 (t, *J* = 2.4 Hz, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 6H), and 2.01 (s, 3H).

3.4.2. *p*-propargyloxybenzamido-2,3,6,2',3',4',6'-hepta-*O*-acetyl- β -lactoside

Yield: 48%; $[M + Na]^+ = 817.29$ (calc. 816.24); 1H NMR (300 MHz, $CDCl_3$, TMS) δ 8.11 (d, $J = 9.0$ Hz, 2H), 7.33 (d, $J = 9.0$ Hz, 2H), 6.92 (d, $J = 8.4$ Hz, 1H), 5.42 (t, $J = 9.0$ Hz, 1H), 5.37 (m, 2H), 5.12 (dd, $J = 8.1$ and 10.5 Hz, 1H), 5.00 (t, $J = 9.6$ Hz, 1H), 4.96 (dd, $J = 3.6$ and 10.5 Hz, 1H), 4.82 (d, $J = 2.4$ Hz, 1H), 4.50–4.45 (m, 1H), 4.19–4.15 (m, 2H), 4.10–4.06 (m, 1H), 3.92 (s, 3H), 3.89 (t, $J = 7.5$ and 7.5 Hz, 1H), 3.86–3.81 (m, 2H), 2.58 (t, $J = 2.4$ Hz, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), and 1.96 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$, TMS) δ 171.60, 170.35, 170.32, 170.12, 170.06, 169.35, 168.98, 165.31, 160.03, 130.68, 121.44, 113.99, 100.86, 79.13, 78.58, 77.93, 76.00, 74.57, 72.24, 71.10, 70.92, 70.67, 68.93, 66.56, 61.85, 60.81, 55.80, 20.83, 20.74, 20.70, 20.61, 20.60, 20.58, and 20.46; IR (KBr, cm^{-1}): 1,734, 1,639, and 1,577.

3.5. General procedure for the synthesis of *p*-propargyloxybenzamido- β -maltoside/lactoside

To *p*-propargyloxybenzamido-2,3,6,2',3',4',6'-hepta-*O*-acetyl- β -maltoside or -lactoside (0.01 g) in MeOH/THF mixture (1:1 v/v, 60 mL), aqueous ammonia (10 mL) was added. After stirring at an ambient temperature overnight, the resultant mixture was evaporated to dryness and the residue was re-dissolved into water and the resultant solution was lyophilized to give *p*-propargyloxybenzamido- β -maltoside or -lactoside as white powder.

3.5.1. *p*-propargyloxybenzamido- β -maltoside

Yield: 99%; $[M + Na]^+ = 523.05$ (calc. 522.47); 1H NMR (300 MHz, D_2O): 7.71 (d, $J = 9.0$, 2H), 7.01 (d, $J = 9.0$, 2H), 5.30 (d, $J = 3.9$, 1H), 5.05 (d, $J = 9.0$, 1H), 4.73 (d, $J = 2.4$, 2H), 3.75–3.19 (m, 12H), 2.82 (t, $J = 2.4$, 1H); IR (KBr, cm^{-1}): 3,343, 1,641, and 1,569.

3.5.2. *p*-propargyloxybenzamido-2,3,6,2',3',4',6'-hepta-*O*-acetyl- β -lactoside

Yield: 97%; $[M + Na]^+ = 522.89$ (calc. 522.47); 1H NMR (300 MHz, $DMSO-d_6$, TMS): 8.76 (d, $J = 8.7$, 1H), 7.89 (d, $J = 9.0$, 2H), 7.04 (d, $J = 9.0$, 2H), 5.14 (d, $J = 4.2$, 1H), 5.07 (d, $J = 5.1$, 1H), 4.97 (t, $J = 8.7$, 1H), 4.87 (d, $J = 2.4$, 2H), 4.81 (d, $J = 4.8$, 1H), 4.74 (s, 1H), 4.68 (t, $J = 4.8$, 1H), 4.57 (t, $J = 6.3$, 1H), 4.54 (d, $J = 4.5$, 1H), 4.23 (d, $J = 7.2$, 1H), 3.76–3.28 (br m, 12H), 2.49 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (300 MHz, $DMSO-d_6$, TMS): 165.88, 159.51, 129.14, 126.97, 114.13, 103.13, 80.46, 80.03, 78.64, 78.02, 76.45, 75.61, 75.37, 73.13, 71.62, 70.51, 68.03, 60.46, 60.28, 55.50; IR (KBr, cm^{-1}): 3,361, 1,639, and 1,577.

3.6. General procedure for the synthesis of 2,3,6,2',3',4',6'-hepta-*O*-acetyl-2''-propargyloxy- β -maltoside/lactoside

BF_3OEt_2 (5.0 mL) was added to 1,2,3,6,2',3',4',6'-octa-*O*-acetyl-maltose or -lactose (3.2 g) and propargyl alcohol (5.0 mL) in anhydrous CH_2Cl_2 (10 mL) at room temperature and stirring was continued for 45 h under nitrogen atmosphere. The resultant mixture was diluted with ethyl acetate and washed with $NaHCO_3$ saturated aqueous solution. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to dryness. In the case of the maltoside, MALDI-TOF-MS and TLC

analysis of the residue showed partially deacetylated product. The residue was, therefore, subjected to full deacetylation (next step) without purification and characterization at this step.

3.6.1. 2,3,6,2',3',4',6'-hepta-*O*-acetyl-2''-propargyloxy- β -maltoside

$[M+Na]^+ = 696.85$ (calc. 697.21).

3.6.2. 2,3,6,2',3',4',6'-hepta-*O*-acetyl-2''-propargyloxy- β -lactoside

Yield: 41%; 1H NMR (300 MHz, $CDCl_3$, TMS): 5.35 (d, $J = 2.7$ Hz, 1H), 5.23 (t, $J = 9.3$ Hz, 1H), 5.11 (dd, $J = 8.1$ and 10.2 Hz, 1H), 4.96 (dd, $J = 3.3$ and 7.8 Hz, 1H), 4.75 (d, $J = 7.8$ Hz, 1H), 4.34 (d, $J = 2.4$ Hz, 2H), 4.14–4.05 (m, 2H), 3.90–3.79 (m, 2H), 3.66–3.62 (m, 1H), 2.47 (t, $J = 2.1$ Hz, 1H), 2.18 (s, 3H), 2.13 (s, 3H), 2.07–2.05 (m, 12H), 1.97 (s, 3H); $[M+Na]^+ = 697.26$ (calc. 697.21); IR (KBr, cm^{-1}) 1,753 (acetyl).

3.7. General procedure for the synthesis of 2''-propargyloxy- β -maltoside/lactoside

A catalytic amount of sodium methoxide was added to crude Ac-Mal-yn in a MeOH (50 mL)/THF (50 mL) mixed solvent system and the resultant mixture was stirred for 6 h. The resultant mixture was neutralized on DOWEX and evaporated to dryness. The resultant residue was subjected to purification by silica-gel column chromatography ($CHCl_3/MeOH$ (2/1)) to obtain 2''-propargyloxy- β -maltoside (β Mal-yn) as white powder after lyophilization. In the case of 2''-propargyloxy- β -lactoside (β Lac-yn), pure Ac-Lac-yn was used as a substrate in this reaction. We, therefore, obtained pure β Lac-yn without any column chromatographic purification process.

3.7.1. 2''-propargyloxy- β -maltoside

Yield: 38% (2 steps); $[M+Na]^+ = 402.66$ (calc. 403.12); 1H NMR (300 MHz, D_2O , HOD): 5.26 (d, $J = 3.6$ Hz, 1H), 4.52 (d, $J = 8.1$ Hz, 1H), 4.33 (s, 2H), 3.78 (t, $J = 12.9$ Hz, 1H), 3.70–3.48 (m, 8H), 3.43 (dd, $J = 3.6$ and 9.9 Hz, 1H), 3.27 (t, $J = 9.3$ Hz, 1H), 3.19 (t, $J = 8.7$ Hz, 1H), 2.78–2.75 (m, 1H); ^{13}C NMR (300 MHz, D_2O): 101.016 (α Glc1), 100.200 (β Glc1), 79.390 ($-CH_2-CCH$), 77.231, 77.000, 76.843 ($-CH_2-CCH$), 76.643, 75.294, 73.448, 73.357, 72.294, 69.978, 61.308 (Glc6), 61.127 (Glc6), 57.204 ($-CH_2-CCH$); IR (KBr, cm^{-1}) 3,381.

3.7.2. 2''-propargyloxy- β -lactoside

Yield: 98%; $[M+Na]^+ = 403.09$ (calc. 403.12); 1H NMR (300 MHz, D_2O , HOD): 4.53 (d, $J = 7.93$ Hz, 1H), 4.35 (dd, $J = 1.90$ and 8.66 Hz, 1H), 4.32 (d, $J = 7.72$ Hz, 1H), 3.85 (d, $J = 11.5$ Hz, 1H), 3.80 (d, $J = 3.03$ Hz, 1H), 3.70–3.58 (m, 5H), 3.55–3.52 (m, 3H), 3.49–3.48 (m, 1H), 3.42 (t, $J = 8.11$ Hz, 1H), 3.32 (t, $J = 8.28$ Hz, 2H), 2.82 (s, 1H); ^{13}C NMR (300 MHz, D_2O): 103.282, 100.739, 79.136, 78.602, 76.769, 75.707, 75.206, 74.711, 72.959, 72.880, 71.311, 68.911, 61.403, 60.359, 56.958; IR (KBr, cm^{-1}) 3,397, 2,919.

3.8. Synthesis of 6-bromo-6-deoxycellulose (Cel-Br)

Cellulose (DPn = 280, 2.05 g) and LiCl (5.65 g) was added into toluene (10 mL) and then, evaporated to dryness. To the resultant white powder, DMA (150 ml) was added under N₂ atmosphere and the resultant mixture was stirred at 80 °C for 24 h. After cooling down to an ambient temperature, triphenylphosphine (12.2 g) in DMA (40 mL) was added and the stirring was continued at room temperature for 4 h. Carbon tetrabromide (10.5 g) in DMA (10 mL) was added, and then, the resultant reaction mixture was stirred at 60 °C overnight. The resultant reaction mixture was poured into water and then, the precipitate was washed with methanol and chloroform repeatedly to obtain Cel-Br as white powder in 71% yield. As mentioned in the manuscript, Cel-Br was hardly soluble in DMSO-*d*₆ and its ¹³C NMR spectrum was too noisy to be assigned. We, therefore, carried out next azidation reaction without full characterization.

3.9. Synthesis of 6-azide-6-deoxycellulose (Cel-N₃)

Cel-Br (2.00 g) was dissolved into DMA (150 mL) containing sodium azide (5.05 g) and then, the resultant mixture was stirred at 85 °C for 42 h. During the reaction, appropriate amounts of DMSO were suitably added to the reaction mixture to keep it homogeneous (the total amount of added DMSO was 150 mL). The resultant reaction mixture was poured into water and then, the precipitate was washed with cold water and methanol repeatedly to obtain Cel-N₃ as white powder in 80% yield: ¹³C NMR (DMSO-*d*₆, TMS): 101.76, 79.00, 73.92, 73.23, 73.20, 50.66; IR (ATR, cm⁻¹): 3,479 and 2,101.

3.10. General procedure for the chemo-selective coupling between Cel-N₃ and alkyne-terminated oligosaccharides

CuBr₂ (1.8 mg), ascorbic acid (7.5 mg), the alkyne-terminated oligosaccharides (*ca.* 400 mg) and propylamine (400 mL) were added to 6-azido-6-deoxycellulose (50 mg) in DMSO (0.80 mL) and the mixture was incubated at room temperature for 12 h. The mixture was dialyzed (water, MWCO8000) followed by lyophilization to give the cellulose-based glycoclusters (Cel-*N*-Mal, Cel-*N*-Lac, Cel-*O*-Mal and Cel-*O*-Lac).

3.10.1. Cel-*N*-Mal

¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 166.00, 160.43, 142.13, 129.26, 126.44, 125.87, 114.05, 100.55, 80.15, 79.39, 78.66, 77.10, 76.76, 73.75, 73.20, 73.20, 73.20, 73.01, 72.71, 72.34, 71.55, 69.97, 61.12, 60.81, 60.43; IR (ATR, cm⁻¹): 3,371, 1,645, and 1,542.

3.10.2. Cel-*N*-Lac

¹³C NMR (300 MHz, DMSO-*d*₆, TMS): 166.08, 160.45, 142.04, 129.17, 126.48, 125.73, 114.08, 103.55, 101.78, 80.47, 80.05, 79.88, 76.46, 75.63, 75.38, 75.37, 74.71, 73.15, 72.11, 71.64, 71.28, 70.55, 70.54, 68.07, 62.05, 60.50; IR (ATR, cm⁻¹): 3,402, 1,643, and 1,567.

3.10.3. Cel-*O*-Mal

^{13}C NMR (300 MHz, DMSO- d_6 , TMS): 143.45, 125.24, 101.91, 100.47, 97.66, 79.25, 78.75, 76.22, 75.07, 73.80, 73.21, 73.18, 72.90, 72.86, 72.64, 72.30, 69.95, 61.43, 60.81, 60.57, 49.74; IR (KBr, cm^{-1}): 3,389, 2,920, 1,632, and 1,064.

3.10.4. Cel-*O*-Lac

^{13}C NMR (300 MHz, DMSO- d_6 , TMS): 143.51, 125.44, 103.59, 101.79, 97.76, 79.81, 78.75, 75.22, 75.07, 73.80, 73.33, 72.90, 72.64, 71.30, 67.95, 61.43, 60.81, 60.57, 60.31, 59.61, 49.74; IR (ATR, cm^{-1}): 3,391, 2,930, 1,633, and 1,045.

4. Conclusions

We established a simple synthetic strategy to access cellulose-based glycoclusters with excellent structural homogeneity: that is, the carbohydrate modules are attached exclusively onto the C6 position of the each repeating unit. Such structural homogeneity could never be achieved through the conventional glycosylation on native polysaccharides (polymer-modification approach). The resultant cellulose-based glycoclusters, however, showed low lectin specificity, possibly arising from the hydrophobic interactions between their hydrophobic moieties and hydrophobic residues of lectins. The enhanced hydrophobic interactions are attributable to the rigid sheet-like structure of cellulose-based glycoclusters and the subsequent exposure of the hydrophobic moieties to the solvent. Whereas, cellulose derivatives obtained by the conventional random modification have carbohydrate units not only at the C6 position but also at C2 and C3. Comparison of these data suggests an importance of C3 glycosylation to develop cellulose-based glycoclusters with excellent water solubility and lectin specificity. Since it is widely recognized that the extended tape-like conformation of cellulose is stabilized mainly by intrastranded O5-HO3 hydrogen bondings [53], the introduction of large carbohydrate units, especially onto the C3 position, should break the intrapolymer hydrogen bonding network, change the original tape-like conformation to a random coiled one, and increase water solubility of the cellulose derivatives. Such structure-function relationships, obtained by using cellulose derivatives with high structural homogeneity, should help in developing practical cellulose-based fine materials.

The facts reported in this paper strongly underline the usefulness of our synthetic strategy to develop cellulose-based advanced materials maintaining the intrinsic tape-like conformations. We are now directing our research efforts to develop such tape-like cellulose derivatives with unique properties. One such example includes nucleotide-appended cellulose to solubilize single-walled carbon nanotubes. The result will soon be reported elsewhere.

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