Regioselective and Quantitative Modification of Cellulose to Access Cellulose-based Advanced Materials: Cellulose-based Glycoclusters

Erika Yamashita,[1] Kiyomi Okubo,[1] Kaori Negishi,[1] and Teruaki Hasegawa*[1,2]
[1]*Department of Life Sciences, Faculty of Life Sciences, Toyo University, 1-1-1 Izumino, Itakura-machi, Ora-gun, Gunma 374-0193*
[2]*Bio-Nano Electronics Research Centre, Toyo University, 2100 Kujirai, Kawagoe 350-8585*

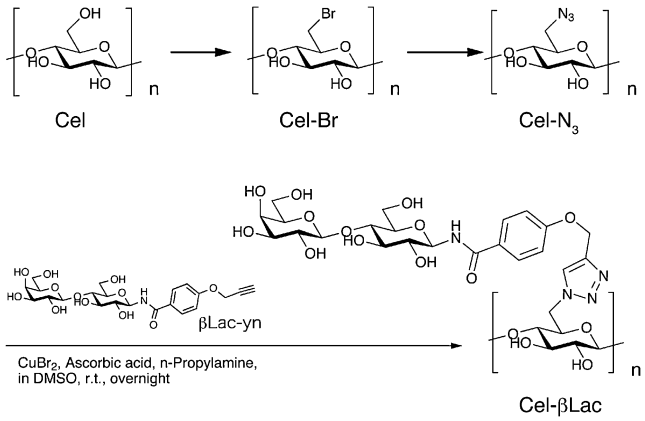
(Received November 12, 2008; CL-081069; E-mail: t-hasegawa@toyonet.toyo.ac.jp)

Regioselective and quantitative introduction of multiple β-lactoside modules onto the 6C-position of cellulose was achieved through regioselective bromination/azidation of cellulose and the following Cu^{+}-catalyzed chemoselective coupling with alkyne-functionalized β-lactoside. The resultant cellulose-based glycocluster has good water solubility and selective lectin affinity.

Cellulose (β-1,4-glucan) is one of the most promising candidates of ecomaterials, since it is abundant in nature and biodegradable. It should be also noted that the cellulose is also advantageous as a substrate for chiral materials and, for example, silica gels coated with cellulose derivatives are now widely used as stationary phase for chiral separation.[1] In spite of these great possibilities, chemical modification of cellulose, that is, the very first step to access cellulose-based fine materials, still contains tedious obstacles. Cellulose consists of many hydroxy functionalities having similar reactivity towards electrophiles; therefore, regioselective reaction is rarely accomplished. To the best of our knowledge, no regioselective/quantitative approach to access functionalized celluloses has been reported so far.[2]

Recently, a general synthetic approach toward functionalized curdlan (β-1,3-glucan) based on a two-step strategy was established: that is, (1) 6C-selective and quantitative bromination/azidation of curdlan to afford 6-azido-6-deoxycurdlan (CUR–N_3) and (2) the subsequent click chemistry-based functionalization of CUR–N_3 using alkyne-terminated functional modules.[3] This methodology is quite useful to develop curdlan-based cell-specific DNAs/RNAs carriers and unique carbon nanotubes solubilizers.[4] In this manuscript, we report the application of this synthetic methodology to cellulose chemistry to develop cellulose-based fine materials.

Cellulose (DP_n 280) was dissolved into *N*,*N*-dimethylacetoamide (DMA) containing LiCl by stirring at 80 °C for 24 h and then converted into 6-bromo-6-deoxycellulose (Cel–Br) through 6C-selective bromination using triphenylphosphine and carbon tetrabromide (Scheme 1). ^{13}C NMR spectrum of Cel–Br was too noisy to be assigned because of low solubility in DMSO-d_6. We, therefore, carried out the next reaction without detailed characterization of Cel–Br. The subsequent azidation was attained by treating Cel–Br with NaN_3 in a mixed DMA/DMSO solvent system at 85 °C for 40 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 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,



Scheme 1. 6C-Selective bromination/azidation of cellulose to affords Cel–N_3 and the subsequence introduction of β-lactoside-modules onto cellulose scaffold through click chemistry.

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. Quantitative and regioselective azidation was confirmed by ^{13}C NMR spectrum, in which the peaks assignable to 6C–Br (44.43 ppm) entirely disappear and that of 6C–N_3 (50.66 ppm) newly appears. Furthermore, no unassignable peak arising from random modification was observed, strongly indicating homogeneous chemical structure along the cellulose main chain. We would like to emphasize that this is the first example of regioselectively/quantitatively modified cellulose derivatives.

Although Cel–N_3 with homogeneous repeating units was obtained as mentioned above, the azide functionality has no practical (protein recognition, light harvesting, etc.) function, and, therefore, Cel–N_3 itself can find little practical application. On the contrary, Cel–N_3 can give full scope to its ability when it is used as a template for further modification toward functional cellulose-based materials. To establish easy and general methodology for the further modification, click chemistry, that is, Cu^{+}-catalyzed cycloaddition of alkyne-terminated functional modules onto Cel–N_3 was applied.[5] Since this reaction is exclusively chemoselective, tolerant for various coexisting functionalities, and applicable to various solvent systems, various cellulose-based advanced materials having desired functions can be developed from Cel–N_3.

The introduction of functional modules onto Cel–N_3 to develop advanced cellulose-based materials is exemplified by using an oligosaccharide as a functional module. Since the oligosaccharides are specific ligands for various carbohydrate-

binding proteins, various synthetic polymers having multiple copies of carbohydrate appendages (glycoclusters) developed so far show strong and specific affinity for these proteins. The cellulose derivatives having multiple copies of oligosaccharides appendages should find, therefore, potential applications in therapeutic purposes. In this work, we introduced β-lactoside, or a specific ligand for asiallo-glycoprotein receptor (AGPR), onto the cellulose scaffold to afford cellulose-based glycoclusters.

In a small vial, Cel–N_3 was dissolved into DMSO and alkyne-terminated β-lactoside (βLac–yn), $CuBr_2$, ascorbic acid, and *n*-propylamine were added. After being kept at room temperature overnight, the resultant reaction mixture was dialyzed (water, MWCO8000) and lyophilized to afford a cellulose derivative having β-lactoside-appendages at all 6C position along the cellulose main chain (Cel–βLac). It should be emphasized that this coupling procedure is quite easy and that no rigorous condition (inert atmosphere and/or anhydrous condition, etc.) are required. This advantage should remove a barrier for nonspecialists of organic synthesis and accelerate participation in development of cellulose-based functional materials. Furthermore, it is also of great advantage that this coupling reaction can be carried out in DMSO that is an excellent solvent for various polar and nonpolar substrates. This fact ensures the wide application of our synthetic procedure to various polar/nonpolar, organic/inorganic functional modules. The ^{13}C NMR spectrum of Cel–βLac shows peaks assignable to cellulose main chain, β-lactoside appendages, and linker moieties including a 1,4-triazole ring (Figure 1). No unassignable peak is observed in this spectrum, indicating quantitative conversion of Cel–N_3 to Cel–βLac with no side reaction.[6]

In contrast to native cellulose that is hardly soluble in water, Cel–βLac is well-soluble in water, and its lectin affinity can be assessed based on the fluorescence titration assay using fluorescein isothiocyanate (FITC)-labeled lectins. Fluorescent intensity of FITC–RCA_{120} (*Ricinus communis agglutinin*, β-Lac-specific) was suppressed by addition of Cel–βLac, and the dissociation constant can be estimated by computational curve fitting to 1.46×10^{-7} M, indicating strong binding. On the other hand, no such fluorescence spectral change was observed for FITC–ConA (Concanavalin A, α-Glc/Man-specific). These fluorescent spectroscopic data indicate strong and specific lectin affinity of

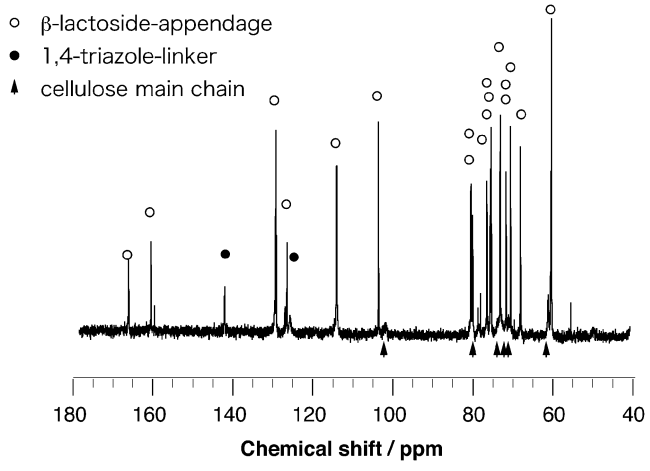


Figure 1. ^{13}C NMR spectrum of Cel–βLac: 300 MHz, DMSO-d_6, 60 °C.

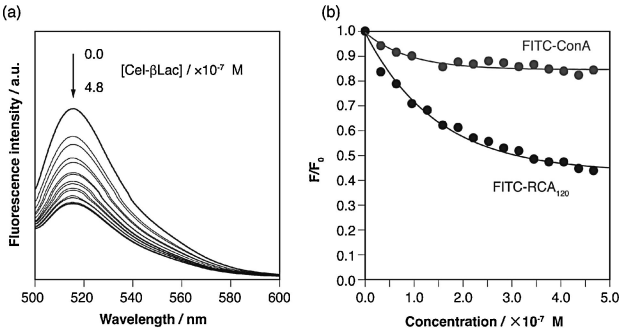


Figure 2. (a) Fluorescence spectral change of FITC–RCA_{120} on addition of Cel–βLac and (b) correlation between the fluorescence intensity and the concentration of Cel–βLac: 20 °C, $Ex = 490$ nm, $d = 10$ mm, Tris-HCl buffer (10 mM, pH 7.2), $[CaCl_2] = 0.1$ mM, $[MnCl_2] = 0.1$ mM.

Cel–βLac. It should be again noted that this specific interaction arises from the introduced β-lactoside modules (Figure 2).

In conclusion, we established a simple synthetic route toward 6-azide-6-deoxycellulose with perfect structural homogeneity along its main chain. The 6-azide-6-deoxycellulose can act as key substrate on the subsequent click chemistry-based introduction of functional modules onto the cellulose scaffold. Although we focused herein the β-lactoside as the functional module to develop cellulose-based glycoclusters, the introduced functional modules should not be limited to such an oligosaccharide. This assumption is reasonably supported by published data showing that various functional modules (porphyrin, ferrocene, pyrene, ammonium cation, etc.) can be readily introduced onto curdlan through the same strategy. We believe that our strategy is quite useful to develop cellulose-based eco-, bio-, and chiral materials.

This work was partially supported by Grant-in-Aid for Scientific Research (No. 18750103) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and Notes

1 a) T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, *Chem. Asian J.* **2008**, *3*, 1494. b) S. H. Kwon, Y. Okamoto, C. Yamamoto, W. Cheong, M. Moon, J. H. Park, *Anal. Sci.* **2006**, *22*, 1525.

2 a) Y. Wei, F. Cheng, *Carbohydr. Polym.* **2007**, *68*, 734. b) K. Furuhata, N. Aoki, S. Suzuki, M. Sakamoto, Y. Saegusa, S. Nakamura, *Carbohydr. Polym.* **1995**, *26*, 25.

3 a) G. Borjihan, G. Zhong, H. Baigude, H. Nakashima, T. Uryu, *Polym. Adv. Technol.* **2003**, *14*, 326. b) T. Hasegawa, M. Umeda, M. Numata, C. Li, A.-H. Bae, T. Fujisawa, S. Haraguchi, K. Sakurai, S. Shinkai, *Carbohydr. Res.* **2006**, *341*, 35. c) T. Hasegawa, M. Umeda, M. Numata, T. Fujisawa, S. Haraguchi, K. Sakurai, S. Shinkai, *Chem. Lett.* **2006**, *35*, 82.

4 a) M. Ikeda, T. Hasegawa, M. Numata, K. Sugikawa, K. Sakurai, M. Fujiki, S. Shinkai, *J. Am. Chem. Soc.* **2007**, *129*, 3979. b) T. Hasegawa, M. Numata, S. Okumura, T. Kimura, K. Sakurai, S. Shinkai, *Org. Biomol. Chem.* **2007**, *5*, 2404.

5 a) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057. b) B. Helms, J. L. Mynar, C. J. Hawker, J. M. J. Frechet, *J. Am. Chem. Soc.* **2004**, *126*, 15020.

6 Supporting Information is available electronically on the CSJ-Journal web site, http://www.csj.jp/journals/chem-lett/index.html.

Published on the web (Advance View) December 27, 2008; doi:10.1246/cl.2009.122