Click Chemistry on Curdlan: A Regioselective and Quantitative Approach to Develop Artificial β-1,3-Glucans with Various Functional Appendages

Teruaki Hasegawa,[1] Mariko Umeda,[1] Munenori Numata,[1] Tomohisa Fujisawa,[1]
Shuichi Haraguchi,[1] Kazuo Sakurai,[2] and Seiji Shinkai*[1]
[1]*Department of Chemistry, Graduate School of Engineering, Kyushu University, 6-10-1 Hakozaki, Fukuoka 812-8581*
[2]*Department of Chemical Processes and Environments, Faculty of Environmental Engineering, The University of Kitakyushu, 1-1 Hibikino, Wakamatsu-ku, Kitakyushu 808-0135*

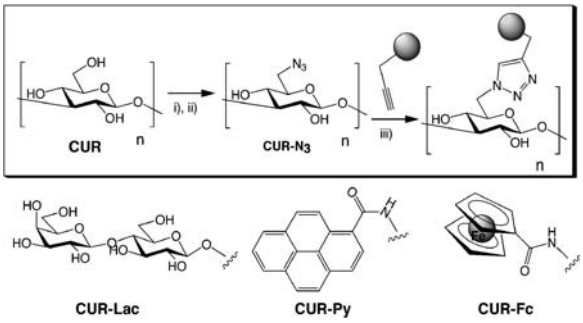
(Received September 26, 2005; CL-051223; E-mail: seijitcm@mbox.nc.kyushu-u.ac.jp)

β-1,3-Glucans having various functional appendages (lactoside, ferrocene, pyrene, etc.) can be prepared in an convenient, quantitative, and regioselective manner through chemo-selective [3 + 2]-cycloadditions between 6-azido-6-deoxy-curdlan and various functional modules with a terminal alkyne.

Polysaccharides are the most potential candidates for "eco-materials," since they are biodegradable and abundant in nature. Their inherent chiralities and resultant helical superstructures also emphasize their potential as scaffolds of "chiral-materials." The polysaccharides are also attractive research targets as "bio-materials" and many polysaccharides found in herbal medicines have been revealed to have strong pharmaceutical effects. The investigation on polysaccharide-based materials has, therefore, received substantial interest from researchers of wide scientific fields. However, chemical modifications of native polysaccharides face still troublesome obstacles, since hydroxy-groups of the polysaccharides have similar reactivity toward electrophiles and regio-selective and quantitative reactions are, therefore, hardly accomplished. Many research groups have placed their intense research efforts on exploiting chemical/enzymatic strategies to obtain polysaccharide-derivatives having various functionalities at desired positions. For example, enzymatic polymerization of chemically modified monosaccharides to the corresponding polysaccharides (bottom-up approach) is one of the most potential ones,[1,2] however, tedious synthetic routes for the modified monosaccharides as well as their lowered affinity (especially, that having large substituents) toward the enzymes (glycosynthase) strongly hinder their wide applications. Direct, convenient, general, quantitative, and regio-selective strategies are, therefore, strongly desired to convert native polysaccharides into polysaccharide-based fine materials.

We, herein, report a novel approach using "click chemistry"[3–7] (Cu(I)-catalyzed chemo-selective coupling between organic azides and terminal alkynes) to obtain β-1,3-glucans having various functional modules (β-lactoside, ferrocene, pyrene, and porphyrin) (Scheme 1). With the best of our knowledge, this is the first example of click chemistry applied to polysaccharide chemistry. Since β-1,3-glucans have interesting structural features (rigid and triple-stranded helical structure, etc.),[8] pharmaceutical effects (anticancer activity, etc.),[9] and binding properties (with polynucleotides, single-walled carbon nanotubes, etc.),[10] developments of these β-1,3-glucan derivatives should be useful not only to prepare various chiral-/bio-materials but also to reveal mechanisms of their pharmaceutical actions.

Native linear β-1,3-glucan (curdlan, CUR) was first converted into 6-bromo-6-deoxy-curdlan (CUR-Br) according to the literature, that is, activation of primary hydroxy-groups with triphenylphosphine followed by bromination with carbon tetrabromide (Scheme 1).[11] The subsequent azidation using sodium azide in DMSO (80 °C, 36 h) afforded 6-azido-6-deoxy-curdlan (CUR-N_3). Quantitative and exclusive conversion of primary hydroxy-groups into azido-groups was confirmed by the ^{13}C NMR spectrum of the product, in which a peak assignable to hydroxymethyl-group (–*C*H_2OH, 60.90 ppm) is entirely diminished and that of azidomethyl-group (–*C*H_2N_3, 50.72 ppm) newly appears (see Supporting Information).



Scheme 1. Chemo-selective coupling between 6-azido-6-deoxy-curdlan and alkyne-terminated functional modules: i) triphenylphosphine, DMF, LiCl, rt, 3 h, and then, carbon tetrabromide, 60 °C, 24 h, ii) sodium azide, DMSO, 80 °C, 36 h, iii) alkyne-terminated functional modules, $CuBr_2$, ascorbic acid, propylamine, rt, 12 h, DMSO.

The chemo-selective couplings between CUR-N_3 and alkyne-terminated functional modules were successfully carried out in DMSO containing $CuBr_2$, ascorbic acid, and propylamine at ambient temperature, where we used various alkyne-terminated modules having 1) β-lactoside (a strong ligand for asialo-glycoprotein receptors on hepatocytes), 2) ferrocene (a redox-active unit), and 3) pyrene (a chromophore with strong fluorescence). The subsequent dialysis (MWCO 8000) and lyophilization followed by washing with methanol afforded various CUR-derivatives (CUR-Lac, CUR-Fc, and CUR-Py, respectively). Along with their IR spectra showing no residual azido-peak (ESI), ^{13}C NMR spectra clearly showed quantitative conversion of CUR-N_3 into the resultant CUR-derivatives (Figure 1 for CUR-Lac as a representative, see Supporting Information for the others), which is clearly evidenced by 1) no residual signal of azidomethyl group, 2) new peaks assignable to the appended functional-modules, 3) two new peaks (145 and 124 ppm) assignable to 1,4-triazole-linker, and 4) no other unassignable peak. The peaks assignable to β-1,3-glucan mainchain are sig-

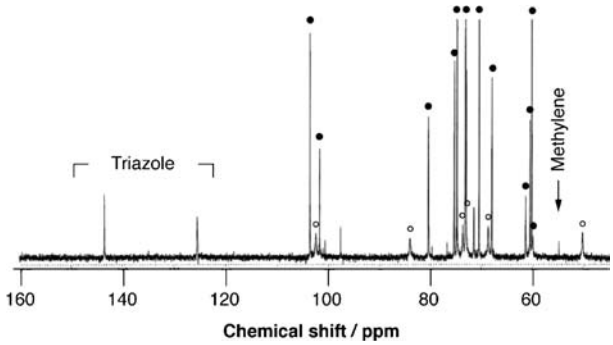


Figure 1. ^{13}C NMR spectra of CUR-Lac: DMSO-d_6, 60 °C. Peaks accompanied with open and closed circles are those of the β-1,3-glucan mainchain and the β-lactoside-appendages, respectively.

nificantly broadened, presumably owing to restricted molecular motion of these polysaccharides having the bulky appendages. It should be emphasized that these extremely bulky modules (Lac, Fc, and Py) can be never introduced into polysaccharides through the enzymatic bottom-up strategies. Furthermore, it should be noted that this reaction can be carried out in polar organic solvents, such as DMSO. This fact intensifies the advantages of our approach to develop various functionalized polysaccharide-derivatives, since they are excellent solvents for wide varieties of organic reagents and various functional modules can be, therefore, introduced through this approach.

Our approach has an additional great advantage, that is, an easy and in situ monitoring of the reaction by using attenuated total reflection-infrared (ATR-IR) spectroscopy, since azido-functionalities show a clear and strong infra-red (IR) peak at around 2100 cm^{-1} and no other functionalities show any obstructive signals around this wavenumber. This is clear advantage of our approach over conventional chemical modification on polysaccharides which is hardly monitorable. For example, the coupling between CUR-N_3 and alkyne-terminated lactoside can be easily monitored based on absorbance of the azido-peak using only 1 μL of the reaction mixture (see Supporting Information). This monitorability results in an easy tuning (or optimizing) of the reaction conditions. For example, we optimized the reaction conditions using various amines (aqueous ammonia, propylamine, triethylamine, etc.) to find that ammonia and propylamine strongly accelerate the reaction and the quantitative conversion was achieved within 1 h (see Supporting Information).

The CUR-derivatives have various potential applications in various scientific fields. For example, CUR-Lac would act as a hepatocyte-specific polynucleotide-carrier, since 1) the β-1,3-glucan mainchain interacts with certain polynucleotides to form stable macromolecular complexes[10] and 2) the β-lactoside-appendage has strong affinity toward hepatocytes. It should be noted that 2-OH of the β-1,3-glucan mainchain participate in hydrogen-bondings with the polynucleotides to stabilize the macromolecular complexes. Substitution on the 2-OH, therefore, strongly destabilizes the macromolecular complexes (unpublished data). The C6-selective modification should be useful to avoid this problem: In fact, our preliminary experiments show that CUR-Lac can interact with poly(C) to form the stable macromolecular complex. Furthermore, specific lectin-binding of CUR-Lac/poly(C) complex was clearly demonstrated by confocal microscopic observation using lectin-labeled agarose beads and rhodamin-labeled $(C)_{45}$: agarose bearing RCA_{120} (β-Lac specific) was stained by the CUR-Lac/Rho-$(C)_{45}$ complex (Figure 2), whereas agarose bearing ConA (α-Man/Glc specific) or WGA (β-GlcNAc-specific) was not, indicating specific interactions between CUR-Lac/Rho-$(C)_{45}$ complex and RCA_{120} on gel surfaces as well as a further potential application of CUR-Lac as a hepatocyte-specific polynucleotide-carrier.

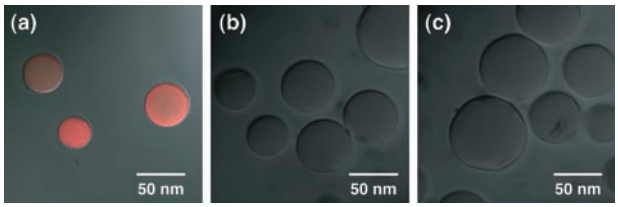


Figure 2. Confocal fluorescent microscopic images of (a) RCA_{120}- (b) ConA-, and (c) WGA-agarose beads stained by CUR-Lac/Rho-$(C)_{45}$: 25 °C, Tris-buffer (20 mM, pH 7.2) containing $CaCl_2$ (10 μM) and $MnCl_2$ (10 μM), Ex = 548.

In conclusion, linear β-1,3-glucan derivatives having various functional modules only at C6-positions of every glucoside-units were easily prepared through our protocol. A lot of advantages of this strategy, including quick, quantitative, regio-selective, and monitorable reaction as well as applicability for bulky modules, should open a new gate to access various polysaccharide-based advanced materials. Our research efforts are now focused on developments of cell-specific CUR-based polynucleotide-carriers as well as applications of our concept toward other polysaccharides.

This work was supported by the Japan Science and Technology Agency, SORST Program. We also thank Mitsui Sugar Co. (Japan) for providing native SPG.

References

1 S. Kobayashi, H. Uyama, S. Kimura, *Chem. Rev.* **2001**, *101*, 3793.
2 M. Jahn, D. Stoll, R. A. J. Warren, L. Szabo, P. Singh, H. J. Gilbert, V. M.-A. Ducros, G. J. Davis, S. G. Withers, *Chem. Commun.* **2003**, *33*, 1327.
3 L. V. Lee, M. L. Mitchel, S.-J. Huang, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 9588.
4 C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057.
5 M. C. Bryan, F. Fazio, H.-K. Lee, C.-Y. Huang, A. Chang, M. D. Best, D. A. Calarese, O. Blixt, J. C. Paulson, D. Burton, I. A. Wilson, C.-H. Wong, *J. Am. Chem. Soc.* **2004**, *126*, 8640.
6 B. Helms, J. L. Mynar, C. J. Hawker, J. M. J. Frechet, *J. Am. Chem. Soc.* **2004**, *126*, 15020.
7 J. A. Opsteen, J. C. M. van Hest, *Chem. Commun.* **2005**, 57.
8 a) T. Yanaki, T. Norisuye, H. Fujita, *Macromolecules* **1980**, *13*, 1462. b) C. T. Chuah, A. Sarko, Y. Deslandes, R. H. Marchessault, *Macromolecules* **1983**, *16*, 1375.
9 a) A. Bot, H. E. Smorenburg, R. Vreeker, M. Paques, A. H. Clark, *Carbohydr. Polym.* **2001**, *45*, 363. b) T. Harada, A. Harada, in *Polysaccharides in Medicinal Applications*, ed. by S. Dumitriu, MarcelDekker, Inc., New York, **1996**, p. 21.
10 K. Sakurai, K. Uezu, M. Numata, T. Hasegawa, C. Li, K. Kaneko, S. Shinkai, *Chem. Commun.* **2005**, 4383.
11 G. Borjihan, G. Zhong, H. Baigude, H. Nakashima, T. Uryu, *Polym. Adv. Technol.* **2003**, *14*, 326.

Published on the web (Advance View) December 10, 2005; DOI 10.1246/cl.2006.82