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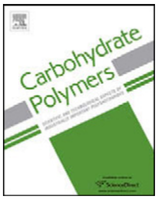

Preparation of highly regioselective chitosan derivatives via “click chemistry”

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ABSTRACT

Highly regioselective chitosan derivatives were achieved by copper-catalyzed Huisgen cycloaddition, known as an ideal reaction for click chemistry, using 6-azido-6-deoxy-chitosan derivative. The azide moiety introduced through regioselective bromination and azidation of chitosan was successfully converted with ethynyl compounds having functional groups (hydroxymethyl and phenyl groups) in the presence of copper(II) sulfate, sodium ascorbate and triethylamine. FTIR, elemental analysis, ^{1}H NMR, and ^{13}C NMR spectra showed that C-6 positions of the chitosan derivative were regioselectively transferred by these functional groups with 1,4-triazole linker, and the DS values were approximately 1.

1. Introduction

Chitosan is obtained by deacetylation of chitin, which is the second most abundant polysaccharide, next to cellulose, in nature. Chitosan is a linear polysaccharide composed of a β-(1-4)-linked D-glucosamine structure with reactive amino and hydroxyl groups per anhydroglucosamine unit and inherent chirality. Due to the unique macromolecular structure, chitosan has great potential for designing advanced materials. To this end, chemical modification of chitosan is a typical means of developing novel chitosan materials. In particular, regioselective and quantitative modification with a controlled well-defined macromolecular structure is the most promising candidate to provide novel chitosan derivatives with potentially useful properties (Kurita, 2001).

Recently, Sharpless et al. introduced “click chemistry”, which is tailored to generating substances quickly and reliably by joining small modular units (Kolb, Finn, & Sharpless, 2001). From the standpoint of the click chemistry philosophy, Cu(I)-catalyzed azide alkyne Huisgen cycloaddition is known to be the most ideal reaction due to its high regioselectivity, quantitative yield, and mild reaction conditions, without generation of by-products (Lee et al., 2003; Rostovtsev, Green, Fokin, & Sharpless, 2002). This efficient coupling reaction has widespread applications to build up several polymers with complex architectures (Manetsch et al., 2004; Wu et al., 2004).

Application of click chemistry with chitosan, especially Huisgen cycloaddition between regioselective azide chitosan derivatives and alkyne-terminated desired functional modules, allows us to design various chitosan-based materials via a one-step reaction. Despite the apparent utility of the reaction, there have been few reports of click chemistry for chitosan (Bao et al., 2010; Gao, Zhang, Chen, Gu, & Li, 2009; Kulbokaite, Ciuta, Netopilik, & Makuska, 2009; Zampano, Bertoldo, & Ciardelli, 2010). To our knowledge, the highly regioselective and quantitative modification of chitosan using Huisgen cycloaddition has not been reported. Accordingly, in this paper, we present the first successful preparation of highly regioselective chitosan derivatives via click chemistry using a 6-azido-6-deoxy chitosan derivative with two different kinds of terminal alkynes. This synthetic strategy will enable further regioselective modification at the residual C2 and C3 positions of chitosan by utilizing the difference in reactivity between amino and hydroxyl groups, which leads to creating finely designed novel chitosan derivatives having several different functional groups at each position.

2. Experimental

2.1. Materials

All chemicals were purchased from Kanto Chemical or Wako Pure Chemical and used without further purification. Chitosan with a 99% degree of deacetylation (DDA) and a number average molecular weight (M_n) of 40,000 was obtained from the complete deacetylation of chitosan (DDA = 90%, M_n = 55,300, Koyo Chemical)

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using sodium hydroxide and was thoroughly dried by freeze-drying prior to use.

2.2. Characterization

^{1}H and ^{13}C NMR spectra were recorded using a JEOL JNM-LA400 and Bruker AVANCEII600 spectrometer, respectively. Chemical shifts were referenced to tetramethyl silane (TMS; 0.0 ppm). Infrared spectra were recorded with a FT-IR spectrometer (Spectrum 65, PerkinElmer Japan Co., Ltd.) equipped with an ATR attachment (Universal ATR, PerkinElmer Japan Co., Ltd.). All the spectra were obtained by accumulation of 16 scans, with resolution of 4 cm^{-1}, at 400–4000 cm^{-1}. Degree of substitution (DS) values of the series of chitosan derivatives were calculated from the C and N contents in the elemental analysis (EA) data obtained using an elemental analyzer (Elementar Vario EL III, Elementar).

2.3. Preparation of 6-azido-6-deoxy-N-phthaloyl-chitosan (4)

N-phthaloyl chitosan (**2**) was prepared according to the previously reported procedure (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002). Fully deacetylated chitosan (2.9 g, 18 mmol of anhydro glucosamine unit) was dispersed in 60 mL of *N*,*N*-dimethylformamide (DMF) containing 5% (v/v) water, and phthalic anhydride (8.0 g, 54 mmol) was added at room temperature. After stirring for 8 h, at 120 °C under Ar atmosphere, the reaction mixture was allowed to cool to room temperature, and the product was purified by pouring it into ice water, filtering, and washing it with distilled water. Further purification was carried out by dispersing it in methanol and washing it thoroughly with methanol to afford a light brown powder of **2**. Yield: 3.5 g.

For the preparation of 6-bromo-6-deoxy-*N*-phthaloyl chitosan (**3**), *N*-phthaloyl chitosan (**2**) (3.4 g, 11.8 mmol) was dispersed in 337 mL of *N*-methyl-2-pyrrolidone (NMP) and *N*-bromosuccinimide (20.5 g, 115 mmol), and triphenylphosphine (30.3 g, 115 mmol) were added to an ice bath, and the mixture was heated to 80 °C and stirred for 2 h under an argon atmosphere. The reaction mixture was poured into ethanol, and the precipitate was collected by centrifugation. The polymer was precipitated into ethanol again, filtered, and washed with ethanol, and dried at 40 °C under vacuum. Yield: 3.6 g.

For 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**), 6-bromo-6-deoxy-*N*-phthaloyl chitosan (**3**) (3.5 g, 9.9 mmol) was dissolved in 349 mL of NMP, and sodium azide (6.4 g, 98 mmol) was added to the solution. The mixture was stirred at 80 °C for 4 h under an argon atmosphere. The mixture was filtered to remove the salts, and the filtrate was precipitated into ethanol. The precipitate was collected by centrifugation, and the product was dispersed into ethanol, filtered, and washed again with ethanol. After drying at 40 °C under vacuum, 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**) was obtained. Yield: 2.7 g. DS$_{azide}$ 0.95; Elemental analysis: Calcd.: C 53.17, H 3.82, N 17.71%; Found: C 52.14, H 4.29, N 16.72%. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 7.83 (phth), 5.20–2.97 (H-1–H-6). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 169.52 (C=O$_{phth}$), 136.24, 133.02, 124.77 (phth), 98.48 (C-1), 79.96, 74.44, 69.98 (C-3–C-5), 59.07 (C-2), 52.02 (C-6). FTIR (KBr): ν (cm^{-1}) = 3466 (OH), 2943 (C–H), 2108 (N$_3$), 1776, 1712 (C=O$_{imide}$), 723 (arom).

2.4. Reaction of 6-azido-6-deoxy-N-phthaloyl-chitosan (4) with terminal alkyne derivatives

The general procedure for azide–alkyne [3 + 2] dipolar cycloaddition to prepare the polymer **5a** and **5b** is as follows: 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**) (0.2 g, 0.64 mmol) was dissolved in dimethyl sulfoxide (DMSO, 40 mL), and copper(II) sulfate pentahydrate (8.2 mg, 0.033 mmol, in 0.2 mL of distilled water), sodium ascorbate (6.3 mg, 0.032 mmol in 0.1 mL of distilled water), triethylamine (1.1 mL, 7.9 mmol), and terminal alkyne derivative (2-propyn-1-ol or ethynyl benzene, 1.9 mmol) were added, and the mixture was stirred at 70 °C for 48 h. The mixture was precipitated in diethylether/2-propanol (1/1, v/v), collected by filtration, washed with diethylether/2-propanol (1/1, v/v), and dried in vacuum. Compound **5a**; Yield: 0.21 g. DS$_{CH_2OH}$ 1.01; EA: Calcd.: C 54.84, H 4.33, N 15.05%; Found: C 51.50, H 4.92, N 13.57%. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 7.5–8.0 (phth and triazole), 5.29–3.71 (H-1–H-6), 4.35 (C*H*$_2$OH). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 167.70 (C=O$_{phth}$), 147.38, 123.67 (C=C$_{triazole}$), 134.16, 131.17, 122.94 (phth), 95.75 (C-1), 77.23, 72.13, 67.42 (C-3–C-5), 56.98 (C-2), 54.59 (*C*H$_2$OH), 49.08 (C-6). FTIR (KBr): ν (cm^{-1}) = 3412 (OH), 2928 (C–H), 1777, 1713 (C=O$_{imide}$), 723 (arom). Compound **5b**; Yield: 0.23 g. DS$_{Ph}$ 0.93; EA: Calcd.: C 63.15, H 4.34, N 13.39%; Found: C 59.55, H 4.68, N 12.45%. ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 8.17 (triazole), 7.0–8.0 (phth, Ph), 5.33–3.62 (H-1–H-6). ^{13}C NMR (DMSO-d$_6$): δ (ppm) = 167.84 (C=O$_{phth}$), 146.28, 122.02 (C=C$_{triazole}$), 134.35, 131.32, 123.16 (phth), 130.61, 128.75, 127.60, 125.08 (Ph), 96.13 (C-1), 77.96, 73.03, 67.50 (C-3–C-5), 57.33 (C-2), 49.72 (C-6). FTIR (KBr): ν (cm^{-1}) = 3452 (OH), 2943 (C–H), 1777, 1713 (C=O$_{imide}$), 768, 696, 723 (arom).

2.5. Preparation of 6-hydroxymethyltriazole and 6-phenyltriazole-6-deoxy chitosan (6a and 6b)

Compound **5a** or **5b** (0.63 mmol) was dissolved in NMP (15 mL), 15 mL of 4 M aqueous hydrazine monohydrate was added, and the mixture was stirred at 100 °C for 4 h under Ar. The mixture was precipitated into EtOH, and the precipitate was collected by centrifugation and washed by EtOH. The product was dried by lyophilization. Compound **6a**: Yield: 0.10 g. ^{1}H NMR (D$_2$O with CD$_3$COOD): δ (ppm) = 8.06 (triazole), 5.12–3.21 (H-1–H-6). FTIR (KBr): ν (cm^{-1}) = 3441 (OH), 2931 (C–H). Compound **6b**: Yield: 0.062 g. ^{1}H NMR (D$_2$O with CD$_3$COOD): δ (ppm) = 8.15 (triazole), 7.54, 7.03 (Ph), 4.07–3.38 (H-1–H-6). FTIR (KBr): ν (cm^{-1}) = 3423 (OH), 2918 (C–H), 765, 694 (Ph).

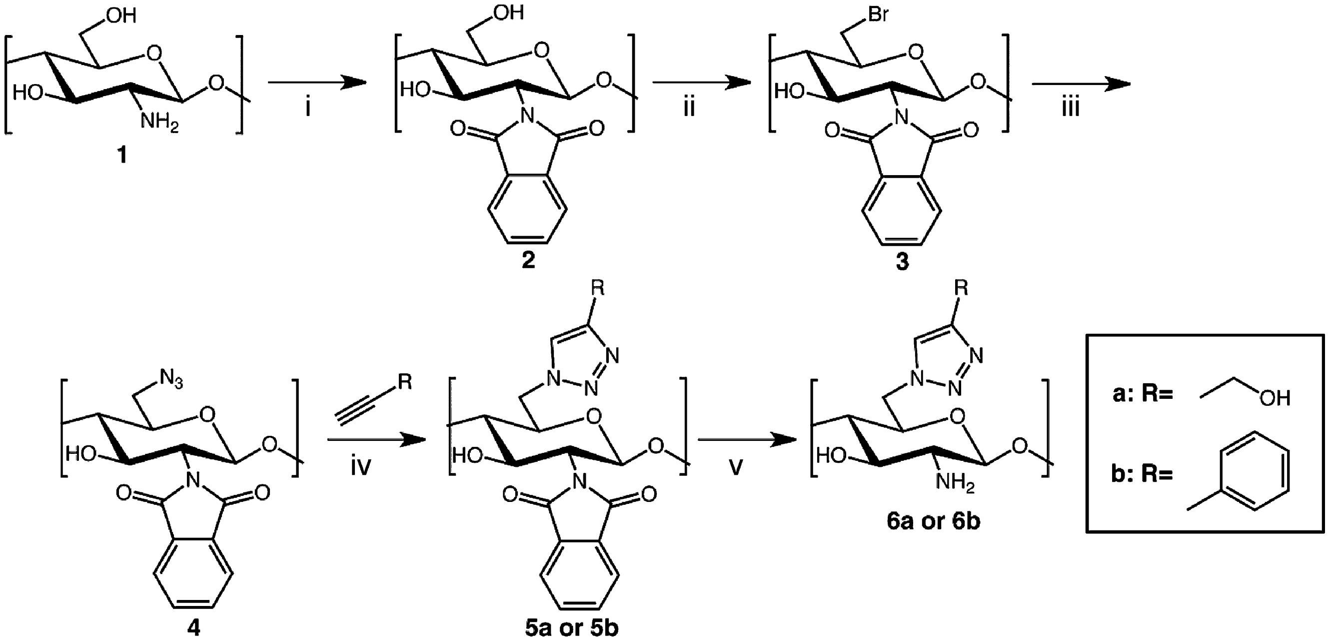
3. Results and discussion

3.1. Synthesis of 6-azido-6-deoxy-N-phthaloyl-chitosan (4)

For application of the Huisgen reaction to chitosan, 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**) was prepared according to the Scheme 1. For protection of the amino group of chitosan, it has been reported that chemical modification of chitosan with phthaloyl groups can lead to 2-*N* regioselective substitution (Kurita et al., 2002). Using phthalic anhydride in *N*,*N*-dimethylformamide (DMF) containing 5% (v/v) water, *N*-phthaloyl-chitosan (**2**) was formed.

Deoxyhalogenation was carried out according to the previously described method (Satoh et al., 2006). *N*-phthaloyl-chitosan (**2**) was brominated by reaction with *N*-bromosuccinimide and triphenylphosphine for 2 h at 80 °C in *N*-methyl-2-pyrrolidone (NMP). Although regioselective *N*-phthaloyl chitosan was insoluble in NMP due to the high crystallinity, the sample became soluble after bromination. It is known that a bulky *N*-phthaloyl group at the C-2 position of chitosan contributes to selective bromination at the C-6 primary hydroxyl group due to steric hindrance. In contrast, in the case of cellulose, the reaction proceeded at the C-3 position in addition to the C-6 position (Furuhata, Chang, Aoki, & Sakamoto, 1992).

Azidation of the 6-bromo-6-deoxy-*N*-phthaloyl chitosan (**3**) can be achieved by a nucleophilic displacement reaction with sodium azide (Satoh et al., 2006). In the FTIR spectrum of compound **4**, the azide moiety is evident from the presence of the significant



Scheme 1. Synthetic scheme for the preparation of chitosan derivatives **6a** and **6b**. Reagents and conditions: (i) phthalic anhydride, DMF/water (95:5), 120 °C, 8 h; (ii) NBS, TPP, NMP, 80 °C, 2 h; (iii) NaN_3, NMP, 80 °C, 4 h; (iv) 2-propyn-1-ol (a), or ethynyl benzene (b), $CuSO_4 \cdot 5H_2O$, sodium ascorbate, TEA, DMSO, 70 °C, 48 h; (v) $H_2NNH_2 \cdot H_2O$, NMP, 100 °C, 4 h.

absorption at around 2108 cm^{-1} (Fig. 1-a). The DS values of the azide group were estimated to be 0.95 from the C and N contents, as measured by elemental analysis, suggesting that an N_3 group was fully introduced at the C-6 position. The ^{13}C NMR spectrum of the 6-azido-6-deoxy chitosan derivative (**4**) is shown in Fig. 2. All of the peaks associated with the respective carbon atoms of the compound **4** were well-resolved as sharp signals. Although it has been reported that the C-6 carbon of bromodeoxy derivative **3** appeared at 33.5 ppm (Satoh et al., 2006), the peak was completely shifted downfield to 52.0 ppm as a single signal, supporting regioselective and complete C-6 substitution of the azide moiety. Other pyranose ring carbons (C-1–C-5) were also clearly observed as five sharp peaks. Furthermore, aromatic carbons are observed not as six but as three peaks, and a carbonyl carbon is observed as a single peak, indicating a symmetric structure of the *N*-phthalimide moiety at the C-2 position.

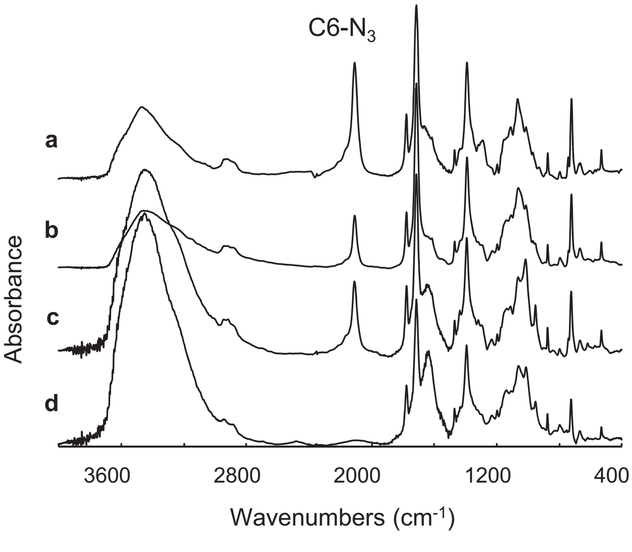


Fig. 1. FT-IR spectra of the 1,3-dipolar cycloaddition of **4** with 2-propyn-1-ol. (a) Original **4**, (b) 70 °C, (c) r.t. with TEA, (d) 70 °C with TEA.

3.2. Copper-catalyzed Huisgen cycloaddition

As a first trial for the successful preparation of highly regioselective chitosan derivatives via click chemistry, we selected hydrophilic 2-propyn-1-ol and hydrophobic ethynylbenzene as terminal alkynes for reasons of availability and simple chemical structure. We first tried the Huisgen cycloaddition reaction using 6-azido-6-deoxy-chitosan, prepared by removal of the *N*-phthaloyl protecting group of compound **4**. However, the amino group at the C-2 position of 6-azido-6-deoxy-chitosan was strongly complexed with copper catalyst, resulting in precipitate formation. Thus, *N*-phthaloylated 6-azido-6-deoxy-chitosan (**4**) was used for the reaction. The coupling reactions between 6-azido-6-deoxy-*N*-phthaloyl-chitosan (**4**) and alkyne-terminated functional moduli were carried out in the presence of Cu(II) sulfate pentahydrate and sodium ascorbate at 70 °C (Liebert, Hänsch, & Heinze, 2006). However, the FTIR spectrum showed that the coupling reaction was incomplete; a considerable azide peak at 2108 cm^{-1} remained even after the 24-h coupling reaction (Fig. 1-b). The remaining peak is probably due to the steric hindrance of the bulky phthaloyl group at the C-2 position of **4** with a triazole ring. It has been reported that amines accelerate the triazole formation (Hasegawa et al., 2006). Here, triethylamine (TEA) was added as base for the coupling reactions. Fig. 1 shows the FTIR spectra of the coupling reaction between **4** and 2-propyn-1-ol in the presence of TEA. Although a residual azide peak at 2108 cm^{-1} was still observed in the spectrum of the room-temperature reaction (Fig. 1-c), the peak completely disap-

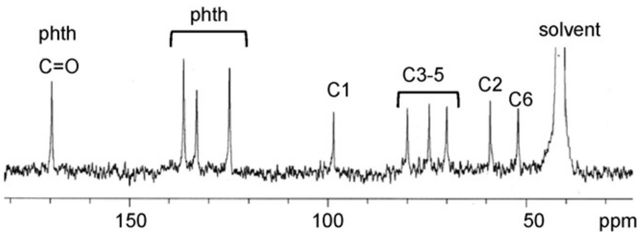


Fig. 2. ^{13}C NMR spectrum of chitosan derivative **4**.

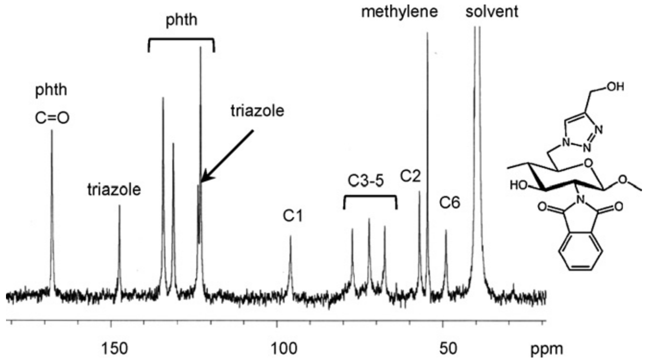


Fig. 3. ^{13}C NMR spectrum of chitosan derivative **5a**.

peared after the 70 °C reaction (Fig. 1-d). Moreover, the degree of substitution of the hydroxymethyl groups estimated by elemental analysis data was 1.01, suggesting a quantitative conversion of the N_3 group. The ^{13}C NMR spectrum of the hydroxymethyl triazole chitosan derivative **5a** acquired in DMSO-d_6 is shown in Fig. 3. The spectrum clearly shows that the C-6 position was regioselectively and quantitatively derivatized with 1,4-triazole linker. That is, all of the peaks associated with the respective carbon atoms of **5a** were well-resolved as sharp signals without residual signal. The C atom of the 1,4-triazole linker is clearly observed at $\delta = 123.7$ and 147.4 ppm as two new peaks, and the peak at 54.6 ppm can be assigned to the methylene moiety of the hydroxymethyl group. Moreover, all of the anhydroglucosamine ring carbons are assigned as follows: $\delta = 49.1$ (C-6), 57.0 (C-2), 67.4, 72.1, 77.2 (C-3, 4, and 5), and 95.7 ppm (C-1).

The coupling reaction between compound **4** and ethynyl benzene was also successfully carried out with TEA at 70 °C. The lack of an azide signal at 2108 cm^{-1} is shown in the FTIR spectra (Figs. 4-5b), and DS of the phenyltriazole moiety determined from elemental analysis was 0.93, suggesting successful conversion to 6-phenyltriazole chitosan derivative (**5b**). The ^{13}C NMR spectrum in DMSO-d_6 also clearly showed quantitative and regioselective conversion of the azide group at the C-6 position without hints of structural impurities, demonstrated as follows: (1) six well-resolved sharp peaks derived from anhydroglucosamine ring carbons with no residual signal; (2) two new peaks derived from the triazole moiety; (3) four new signals assigned to the introduced phenyl moiety (Fig. 5).

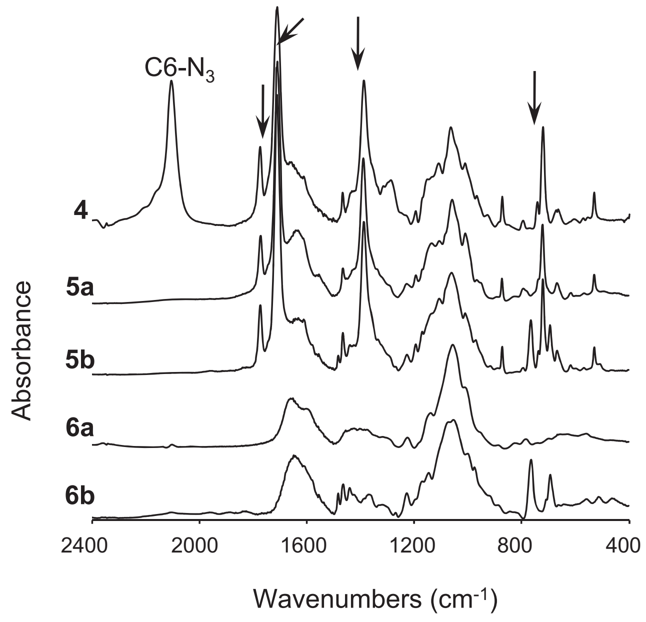


Fig. 4. FT-IR spectra of 6-azido-6-deoxy-*N*-phthaloyl chitosan (**4**) and the triazole derivatives (**5a** and **5b**), and the dephthaloylated derivatives (**6a** and **6b**) (arrows: phthaloyl group).

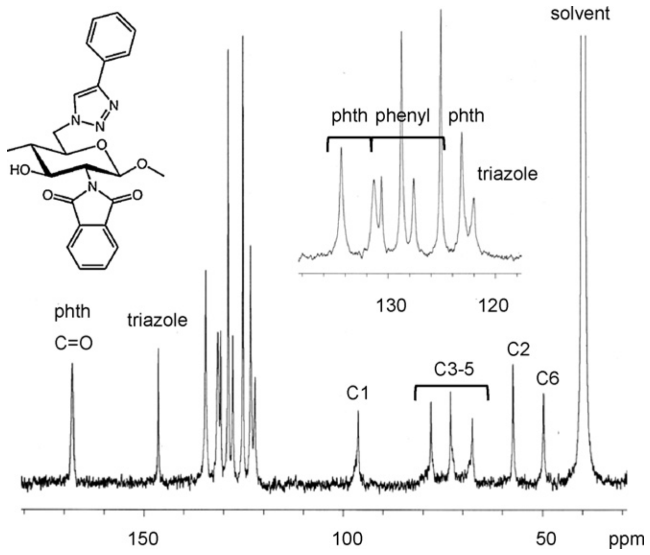


Fig. 5. ^{13}C NMR spectrum of chitosan derivative **5b**.

After the Huisgen cycloaddition reaction, the products **5a** and **5b** were treated with aqueous hydrazine monohydrate to remove the phthaloyl protecting group (Satoh et al., 2006). Deprotection of **5a** and **5b** was successfully achieved after 4 h at 100 °C. Four characteristic absorption bands of the phthalimide group at 723, 1389, 1713, and 1776 cm^{-1} were completely absent from the FTIR spectra shown in Fig. 4-6a and 6b. Since the products **6a** and **6b** had low solubility in several solvents even in acidic water and aprotic polar solvents after lyophilization, the ^{13}C NMR spectra could not be obtained. However, the characteristic peaks of the phthaloyl group at around 7.8 ppm were completely absent from the ^{1}H NMR spectra (data shown in supplementary contents), supporting the desired structure of **6a** and **6b**.

4. Conclusions

Highly regioselective 6-substituted chitosan derivatives were prepared via click chemistry for the first time. The Huisgen cycloaddition between 6-azido-6-deoxy chitosan derivative and alkyne-terminated molecules was successfully carried out in the presence of Cu(I) catalyst and triethylamine. FTIR, elemental analysis, ^{1}H NMR, and ^{13}C NMR spectra strongly supported the structural uniformity of the chitosan derivatives with no impurities. Since desired functional modules with triazole linker can be regioselectively and quantitatively introduced into chitosan by a one-step reaction, application of click chemistry to chitosan could be useful for designing novel chitosan materials with controlled and well-defined structures and promising properties. Further studies concerning correlation between chemical structures of the chitosan derivatives and properties are now in progress and will be reported elsewhere.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.03.030.

References

Bao, H., Li, L., Gan, L. H., Ping, Y., Li, L., & Ravi, P. (2010). Thermo- and pH-responsive association behavior of dual hydrophilic graft chitosan terpolymer synthesized via ATRP and click chemistry. *Macromolecules*, *43*, 5679–5687.

Furuhata, K., Chang, H., Aoki, N., & Sakamoto, M. (1992). Chlorination of cellulose with *N*-chlorosuccinimide-triphenylphosphine under homogeneous conditions in lithium chloride-*N*,*N*-dimethylacetamide. *Carbohydrate Research*, *230*, 151–164.

Gao, Y., Zhang, Z., Chen, L., Gu, W., & Li, Y. (2009). Synthesis of 6-*N*,*N*,*N*-trimethyltriazole chitosan via "click chemistry" and evaluation for gene delivery. *Biomacromolecules*, *10*, 2175–2182.

Hasegawa, T., Umeda, M., Numata, M., Li, C., Bae, A. H., Fujisawa, T., et al. (2006). 'Click chemistry' on polysaccharides: A convenient, general, and monitorable approach to develop (1 → 3)-β-D-glucans with various functional appendages. *Carbohydrate Research*, *341*, 35–40.

Kolb, H. C., Finn, M. G., & Sharpless, K. B. (2001). Click chemistry: Diverse chemical function from a few good reactions. *Angewandte Chemie International Edition*, *40*, 2004–2021.

Kulbokaite, R., Ciuta, G., Netopilik, M., & Makuska, R. (2009). *N*-PEG'ylation of chitosan via "click chemistry" reactions. *Reactive & Functional Polymers*, *69*, 771–778.

Kurita, K. (2001). Controlled functionalization of the polysaccharide chitin. *Progress in Polymer Science*, *26*, 1921–1971.

Kurita, K., Ikeda, H., Yoshida, Y., Shimojoh, M., & Harata, M. (2002). Chemoselective protection of the amino groups of chitosan by controlled phthaloylation: Facile preparation of a precursor useful for chemical modifications. *Biomacromolecules*, *3*, 1–4.

Lee, L. V., Mitchel, M. L., Huang, S.-J., Fokin, V. V., Sharpless, K. B., & Wong, C.-H. (2003). A potent and highly selective inhibitor of human α-1,3-fucosyltransferase via click chemistry. *Journal of the American Chemical Society*, *125*, 9588–9589.

Liebert, T., Hänsch, C., & Heinze, T. (2006). Click chemistry with polysaccharide. *Macromolecular Rapid Communications*, *27*, 208–213.

Manetsch, R., Krasiński, A., Radić, A., Raushel, J., Taylor, P., Sharpless, K. B., et al. (2004). In situ click chemistry: Enzyme inhibitors made to their own specifications. *Journal of the American Chemical Society*, *126*, 12809–12818.

Rostovtsev, V. V., Green, L. G., Fokin, V. V., & Sharpless, K. B. (2002). A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angewandte Chemie International Edition*, *114*, 2708–2711.

Satoh, T., Kano, H., Nakatani, M., Sakairi, N., Shinkai, S., & Nagasaki, T. (2006). 6-Amino-6-deoxy-chitosan. sequential chemical modifications at the C-6 positions of N-phthaloyl-chitosan and evaluation as a gene carrier. *Carbohydrate Research*, *341*, 2406–2413.

Wu, P., Feldman, A. K., Nugent, A. K., Hawker, C. J., Scheel, A., Voit, B., et al. (2004). Efficiency and fidelity in a click-chemistry route to triazole dendrimers by the copper(I)-catalyzed ligation of azides and alkynes. *Angewandte Chemie International Edition*, *43*, 3928–3932.

Zampano, G., Bertoldo, M., & Ciardelli, F. (2010). Defined chitosan-based networks byC-6-azide–alkyne "click" reaction. *Reactive & Functional Polymers*, *70*, 272–281.