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A short synthesis of highly soluble chemoselective chitosan derivatives via "click chemistry"

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ABSTRACT

A short synthesis of chemoselective chitosan derivatives was achieved by copper-catalyzed Huisgen cycloaddition, which is an ideal reaction for click chemistry, by using *N*-(4-azidophthaloyl)-chitosan. *N*-(4-azidophthaloyl)-chitosan was prepared through chemoselective *N*-bromophthaloylation of chitosan in acidic water and subsequent azidation. The obtained *N*-(4-bromopthaloyl)-chitosan had higher solubility in common solvents than conventional phthaloyl chitosan. *N*-(4-azidophthaloyl)-chitosan was successfully converted with ethynyl derivatives having functional groups (hydroxymethyl, phenyl, and methyl ester) in the presence of copper(II) sulfate, sodium ascorbate and/or trimethylamine. FT-IR spectra, elemental analyses, and ^{1}H and ^{13}C NMR spectra supported that the desired chitosan derivatives were chemoselectively transferred by these groups with a 1,4-triazole linker.

1. Introduction

Chitosan is obtained by deacetylation of chitin, which is the structural material in the exoskeleton of arthropods and the second most abundant polysaccharide in nature, after cellulose. Chitosan, the linear and partly acetylated (1–4)-2-amino-2-deoxy-β-D-glucan (Muzzarelli et al., 2012), has great potential for use in the design of advanced biomaterials (Muzzarelli, 2009). Chemical modification of chitosan is a typical way to design novel materials from chitosan. In particular, region- and/or chemo-selective modification with a controlled well-defined molecular structure is the most important key for the development of novel chitosan derivatives with useful properties (Kurita, 2001).

"Click chemistry" was first described by Bally Sharpless, and is tailored to generate substances quickly and reliably by joining small units together (Kolb, Finn, & Sharpless, 2001). One of the most popular reactions within the click chemistry concept is Cu(I) catalyst azide alkyne Huisgen cycloaddition, 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 great potential to build up several polymers with complex structures (Manetsch et al., 2004; Wu et al., 2004). In a previous report, we presented the first successful preparation of highly regioselective chitosan derivatives via click chemistry using a 6-azido-6-deoxy chitosan derivative (Ifuku, Wada, Morimoto, & Saimoto, 2011). Application of click chemistry with chitosan allows us to design various chitosan based materials via a one-step reaction from an azido-chitosan. Moreover, this synthetic strategy enables further chemical modification at the residual positions of chitosan, which leads to the creation of finely designed novel chitosan derivatives having several different functional groups at each position. However, the previously reported method requires 3 steps to provide an azido-chitosan derivative (Fig. 1). Protection of the amino group of chitosan using phthalic anhydride increases the number of reaction steps. Accordingly, in this study, we improved the synthetic strategy to reduce the number of reaction steps as shown in Fig. 2. That is, 4-bromophthalic anhydride was used for chemoselective bromination of chitosan. Also azido-chitosan derivative (**3**), the reactant for the Huisgen reaction, could be easily obtained by subsequent azidation. Thus, 4-bromophthalic anhydride was not used for protection, but as a one-step chemoselective bromination reagent for chitosan in this study.

2. Experimental

2.1. Materials

4-Bromophthalic anhydride (cat. no. B1693), 2-propyn-1-ol (cat. no. P0536), ethynylbenzene (cat. no. E0196), and methyl

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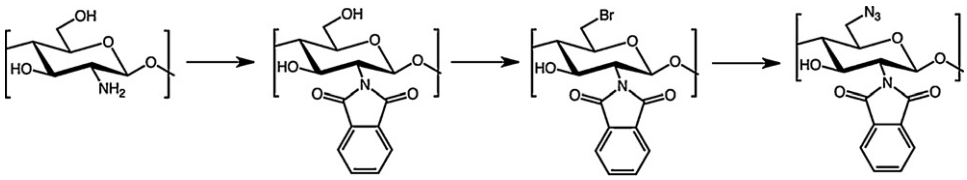

Fig. 1. Previously reported synthetic scheme for chemoselective azido chitosan derivative.

propiolate (P0528) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Sodium azide (cat. no. 197-11091), copper(II) sulfate pentahydrate (cat. no. 039-04412), sodium ascorbate (cat. no. 196-01252), and triethylamine (cat. no. 202-02641) were purchased Wako Pure Chemicals (Tokyo). All chemicals were used as received. Chitosan with a 99% degree of deacetylation (DDA) and a number average molecular weight (M_n) of 40,000 was prepared from the further deacetylation of chitosan (DDA = 90%, M_n = 55,300; Koyo Chemical Company, Osaka, Japan) using 40% NaOH and was thoroughly dried by freeze-drying prior to use.

2.2. Preparation of N-(4-bromophthaloyl)-chitosan (**2**)

N-(4-bromophthaloyl)-chitosan was prepared being referring to previously reported procedure (Ifuku, Miwa, Morimoto, & Saimoto, 2011). Fully deacetylated chitosan (**1**) (1.02 g, 6.4 mmol of anhydroglucosamine unit) was refluxed with 4-bromophthalic anhydride (4.22 g, 18.6 mmol) in 100 mL of AcOH/H_2O (1.0% (v/v)) for 48 h. After the reaction, the mixture was cooled to room temperature, and the precipitate was collected by centrifugation. The product was dispersed in methanol, filtered, washed thoroughly with methanol, and dried at 100 °C under a vacuum. Yield: 1.73 g (99%). DS_{BrPhth}: 1.01. Elemental analysis: Calcd.: C 45.43, H 3.27, N 3.78%; Found: C 43.82, H 3.79, N 3.63%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.97, 7.74 (phth), 5.04–2.93 (H-1–H-6). ^{13}C NMR (DMSO-d_6): δ (ppm) = 166.68, 166.13 (C=O_{phth}), 137.07–124.71 (phth), 97.38 (C-1), 79.01–68.68 (C-3–C-5), 59.50 (C-2), 56.78 (C-6). FT-IR (ATR): ν (cm^{-1}) = 3445 (OH), 2886 (C—H), 1775, 1708 (C=O_{imide}), 1150–1000 (pyranose), 742 (arom).

2.3. Preparation of N-(4-azidophthaloyl)-chitosan (**3**)

For *N*-(4-azidophthaloyl)-chitosan (**3**), *N*-(4-bromophthaloyl)-chitosan (**2**) (0.40 g, 1.5 mmol) was dissolved in 340 mL of *N*-methyl-2-pyrrolidone (NMP), and sodium azide (0.71 g, 10.9 mmol) was added to the solution. The mixture was stirred at 80 °C for 24 h under an argon atmosphere. The mixture was then filtered to remove the salts, and the filtrate was precipitated into ethanol. The precipitate was filtered, and washed with ethanol and acetone. Other washing solvents also will be available. After drying at 100 °C for 8 h under a vacuum, *N*-(4-azidophthaloyl)-chitosan (**3**) was obtained. Yield: 0.27 g (74%). DS_{N3}: 0.67. Elemental analysis: Calcd.: C 50.61, H 3.64, N 16.86%; Found: C 44.69, H 4.39, N 11.45%. ^{1}H NMR (DMSO-d_6): δ (ppm) = 8.01, 7.83, 7.47 (phth), 5.05–2.93 (H-1–H-6). ^{13}C NMR (DMSO-d_6): δ (ppm) = 168.31 (C=O_{phth}), 147.74–115.32 (phth), 99.23 (C-1), 81.05–70.52 (C-3–C-5), 61.29 (C-2), 58.32 (C-6). FT-IR (ATR): ν (cm^{-1}) = 3423 (OH), 2931 (C—H), 2121 (N_3), 1772, 1702 (C=O_{imide}), 1150–1000 (pyranose), 746 (arom).

2.4. Reaction of N-(4-azidophthaloyl)-chitosan (**3**) with terminal alkyne derivatives

The general procedure for the chitosan derivatives **4a** and **4b** was as follows: *N*-(4-azidophthaloyl)-chitosan (**3**) (0.10 g, 0.42 mmol) was dissolved in dimethyl sulfoxide (DMSO, 20 mL), and copper(II) sulfate pentahydrate (4.6 mg, in 0.2 mL of distilled water), sodium ascorbate (3.8 mg, in 0.1 mL of distilled water), triethyl amine (0.6 mL), and 0.84 mmol of terminal alkyne derivative (2-propyn-1-ol and ethynyl benzene) were added, and the mixture was stirred at 70 °C for 48 h. A mixture of diethyl ether-2-propanol (1/1 (v/v)), stirred at room temperature for 5 h, collected by filtration, washed with mixture of diethyl ether-2-propanol (1/1 (v/v)), and dried in a vacuum at 100 °C for 8 h. To prepare polymer **4c**, *N*-(4-azidophthaloyl)-chitosan (**3**) (0.10 g, 0.42 mmol) was dissolved in dimethyl sulfoxide (DMSO, 20 mL), and copper(II) sulfate pentahydrate (4.6 mg, in 0.2 mL of distilled water), sodium ascorbate (3.8 mg, in 0.1 mL of distilled water), and methyl propiolate (75.2 μL, 0.90 mmol) were added, and the mixture was stirred at 70 °C for 96 h. The mixture was washed and dried using the same procedure as above. Compound **4a**: Yield: 92 mg (80%). ^{1}H NMR (DMSO-d_6): δ (ppm) = 8.86 (triazole), 8.35–7.83 (phth), 5.20–3.72 (H-1–H-6), 4.64 (methylene). ^{13}C NMR (DMSO-d_6): δ (ppm) = 168.54 (C=O_{phth}), 151.54, 123.36 (C=$C_{triazole}$), 143.03–116.12 (phth), 95.58 (C-1), 81.28–70.90 (C-3–C-5), 61.65 (C-2), 58.89 (C-6), 56.81 (CH_2OH). FT-IR (KBr): ν (cm^{-1}) = 3379 (OH), 2934 (C—H), 1774, 1710 (C=O_{imide}), 1150–1000 (pyranose), 747 (arom). Compound **4b**: Yield: 101 mg (79%), ^{1}H NMR (DMSO-d_6): δ (ppm) = 9.47 (triazole), 8.41–7.82 (phth), 7.52–7.42 (phenyl), 5.29–3.71 (H-1–H-6), 4.35 (methylene). ^{13}C NMR (DMSO-d_6): δ (ppm) = 168.49 (C=O_{phth}), 149.64, 121.85 (C=$C_{triazole}$), 142.86–116.02 (phth), 131.75–127.31 (phenyl), 99.62 (C-1), 77.23–56.98 (C-2–C-5), 54.59 (methylene), 49.08 (C-6). FT-IR (KBr): ν (cm^{-1}) = 3381 (OH), 2933 (C—H), 1773, 1709 (C=O_{imide}), 1150–1000 (pyranose), 747–744 (phenyl), 746

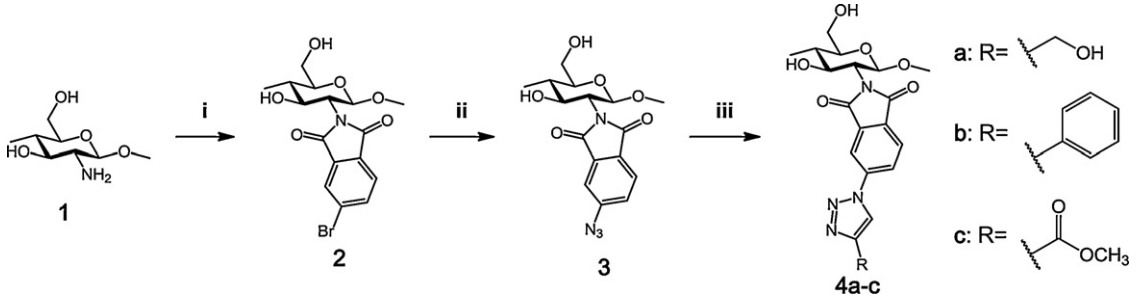

Fig. 2. Synthetic scheme for the preparation of chitosan derivatives **4**. Reagents and conditions: (i) 4-bromophthalic anhydride, 1.0 vol.% AcOH aq, 48 h, reflux; (ii) NaN_3, NMP, 80 °C, 24 h; (iii) alkyne derivatives, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, TEA, DMSO, 70 °C, 48 h.

(arom). Compound **4c**: Yield: 111 mg (91%), ^{1}H NMR (DMSO-d_6): δ (ppm) = 9.66 (triazole), 8.42–7.78 (phth), 5.09-3.75 (H-1–H-6), 3.91 (methyl). ^{13}C NMR (DMSO-d_6): δ (ppm) = 168.13 (C=O$_{phth}$), 161.96 (C=O$_{ester}$) 141.55, 129.58 (triazole), 142.13-116.80 (phth), 99.33 (C-1), 80.93–70.63 (C-3–C-5), 61.42 (C-2), 58.74 (C-6), 53.62 (OCH_3). FT-IR (KBr): ν (cm^{-1}) = 3440 (OH), 2951 (C—H), 1775, 1710 (C=O$_{imide}$), 1150–1000 (pyranose), 746 (arom).

2.5. Characterization

^{1}H and ^{13}C NMR spectra were recorded using a JEOL JNM-LA400 and Bruker AVANCE II600 spectrometer, respectively. Chemical shifts were referenced to tetramethyl silane (TMS; 0.0 ppm). Infrared spectra were recorded with a FT-IR spectrometer (Spectrum 65; Perkin-Elmer Japan Co. Ltd., Tokyo) equipped with an ATR attachment (Universal ATR; Perkin-Elmer Japan Co. Ltd.). All the spectra were obtained by accumulation of 16 scans, with resolution of 4 cm^{-1}, at 400–4000 cm^{-1}. The degrees of substitution (DS) 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, Hanau, Germany). X-ray diffraction profiles of the nanofibers were obtained with Ni-filtered CuKα from an X-ray generator (Ultima IV, Rigaku) operating at 40 kV and 30 mA. The diffraction profile was detected using an X-ray goniometer scanning from 5° to 40°.

3. Results and discussion

3.1. Preparation of N-(4-azidophthaloyl)-chitosan (3)

For application of the Huisgen reaction to chitosan, the *N*-(4-azidophthaloyl)-chitosan (**3**) was prepared according to the synthetic scheme in Fig. 2. It is well known that chemical modification of chitosan with a phthaloyl group can lead to *N*-chemoselective substitution. Recently, we reported that highly chemoselective *N*-phthaloylation of chitosan was achieved in acidic water (Ifuku, Miwa, et al., 2011). Thus, we applied this study for *N*-(4-bromophthaloylation) of chitosan in aqueous media. Fully deacetylated chitosan dissolved in AcOH/H_2O with a concentration of 1.0% (v/v) was treated with 4-bromophthalic anhydride under reflux for 48 h. After the reaction, the precipitate was collected by filtration. The DS value of *N*-(4-bromophthaloyl)-chitosan (**2**) determined by elemental analysis was approximately 1. This result suggests that the 4-bromophthaloyl group was successfully introduced into highly reactive amino groups in aqueous acetic acid. The FT-IR spectra of the chitosan (**1**) and *N*-(4-bromophthaloyl)-chitosan (**2**) are shown in Fig. 3a and b, respectively. The strong bands at 1775 and 1708 cm^{-1} correspond to the characteristic absorption of C=O stretching vibration bands of phthalimide groups (Fig. 3b). It should be emphasized that the bromophthaloyl group improved the solubility of chitosan in organic solvents. It is known that conventional phthaloyl chitosan is soluble in particular certain kinds of organic solvents, including *m*-cresol, dichloroacetic acid, and *N*,*N*-dimethylacetamide/8% LiCl, though it is insoluble in more common aprotic polar solvents (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002). On the other hand, *N*-(4-bromophthaloyl)-chitosan (**2**) is highly soluble in common solvents such as dimethylsulfoxide (DMSO), *N*,*N*-dimethyl formamide (DMF), *N*,*N*-dimethylacetamide (DMAc), and *N*-methylpyrrolidone (NMP). The difference in solubility is attributed to their crystallinities. Fig. 4 shows the X-ray diffraction profiles of *N*-phthaloyl-chitosan and the *N*-(4-bromophthaloyl)-chitosan (**2**). In Fig. 4a, multiple characteristic peaks with sharp contrast show that the conventional *N*-phthaloyl-chitosan had a clear crystal pattern. The high crystallinity resulted in low

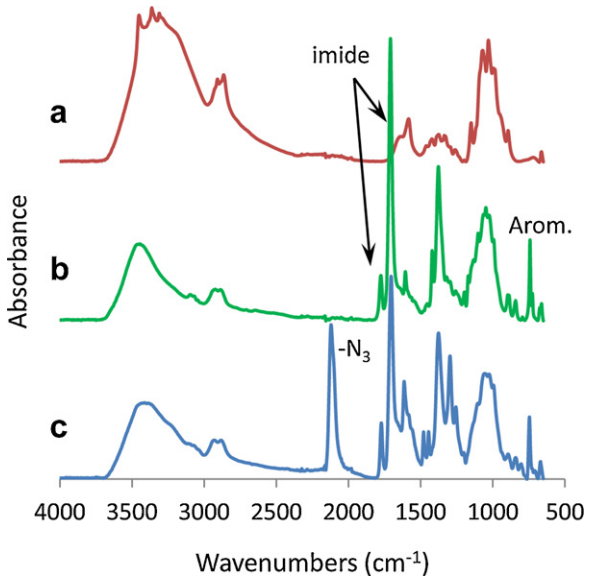

Fig. 3. FT-IR spectra of the chitosan (a), *N*-(4-bromophthaoyl)-chitosan (b), and *N*-(4-azidophthaloyl)-chitosan (c).

solubility. On the other hand, *N*-(4-bromophtaloyl)-chitosan (**2**) had much lower crystallinity than phthaloyl chitosan (Fig. 4b). The balky bromine group should interfere with the crystallization of the polymer **2**, resulting in high solubility. Although phthaloylation is known to be the most important reaction in chitosan chemistry for protecting its amino group, the highly chemoselective *N*-phthaloyl-chitosan has low solubility. Therefore, 4-bromophthaloylation in an aqueous solvent could be a novel protection reaction of chitosan, because highly soluble *N*-protected chitosan enables us further chemical modification in a homogeneous reaction system. Owing to the high solubility, the ^{13}C NMR spectrum of the polymer **2** was measurable in DMSO-d_6 (Fig. 5). Aromatic carbons were observed as six peaks at 124.7, 125.8, 127.9, 129.8, 132.8, and 137.1 ppm, and carbonyl carbons were observed as two peaks at 166.1 and 166.7 ppm. Moreover, pyranose ring carbons were clearly observed as six sharp peaks at δ = 56.8 ppm (C6), 59.5 ppm (C2), 68.7 ppm, 74.5 ppm, 79.0 ppm (C3–5), and 97.4 ppm (C1). Thus, the well-resolved ^{13}C spectrum strongly supports that the chitosan derivative (**2**) prepared in aqueous acetic acid has highly uniform *N*-(4-bromophthaloyl) glucosamine repeating units.

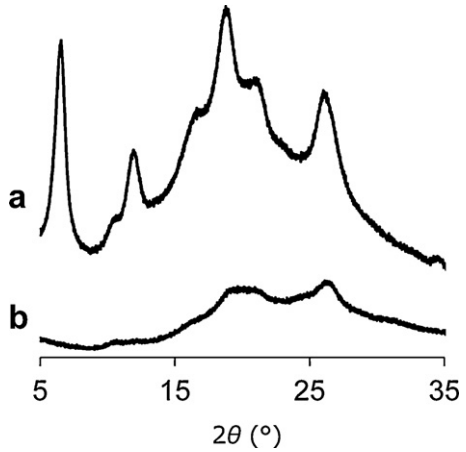

Fig. 4. X-ray diffraction profiles of (a) *N*-phthaloyl-chitosan and (b) *N*-(4-bromophthaoyl)-chitosan.

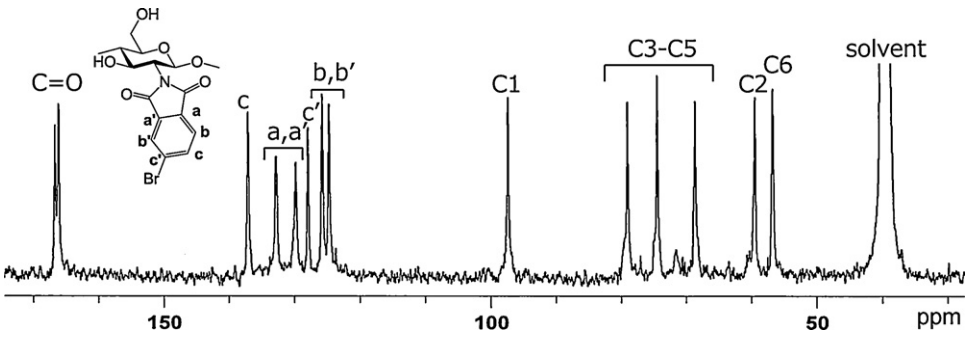

Fig. 5. ^{13}C NMR spectrum of *N*-(4-bromophthaoyl)-chitosan (**3**).

Azidation of the *N*-(4-bromophthaloyl)-chitosan (**2**) can be achieved by a nucleophilic displacement reaction with sodium azide for 48 h at 80 °C (Satoh et al., 2006). In the FT-IR spectrum of *N*-(4-azidophthaloyl)-chitosan (**3**), the azide moiety is evident from the presence of the significant absorption at around 2234 cm^{-1} (Fig. 3c). The DS value of the azide group was estimated to be 0.67 from the C and N contents as measured by elemental analysis. The ^{13}C NMR spectrum of the *N*-(4-azidophthaloyl)-chitosan (**3**) is shown in Fig. 6a. All of the peaks associated with the respective carbon atoms of the polymer **3** were well-resolved as sharp signals. The peak at 127.9 ppm (*C*-Br) was shifted to 115.3 ppm, supporting substitution of the azide moiety. Other aromatic ring carbons were also observed. Moreover, six pyranose ring carbons were also clearly observed.

3.2. Copper-catalyzed Huisgen cycloaddition

For preparation of chitosan derivatives via Huisgen cycloaddition, we selected three different types of terminal alkyne derivatives—2-propyn-1-ol, ethynylbenzene, and methyl propiolate—for reasons of their hydrophilicity, aromatic hydrophobicity, and high dipole moment, respectively, and also because of their availability and simple chemical structures. The coupling reactions between *N*-(4-azidophthaloyl)-chitosan (**3**) and terminal

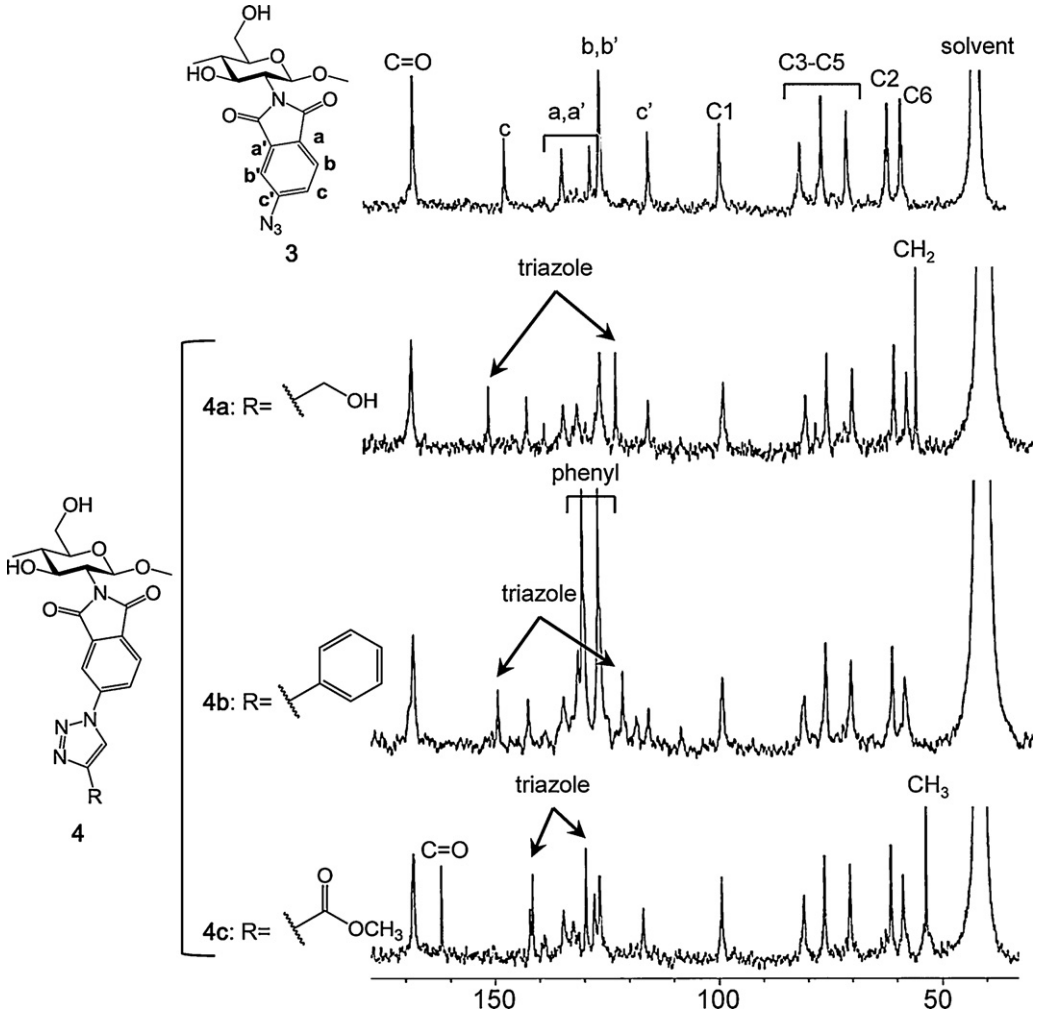

Fig. 6. ^{13}C NMR spectra of *N*-(4-azidophthaloyl)-chitosan (**3**) and the triazole derivatives (**4a**–**c**).

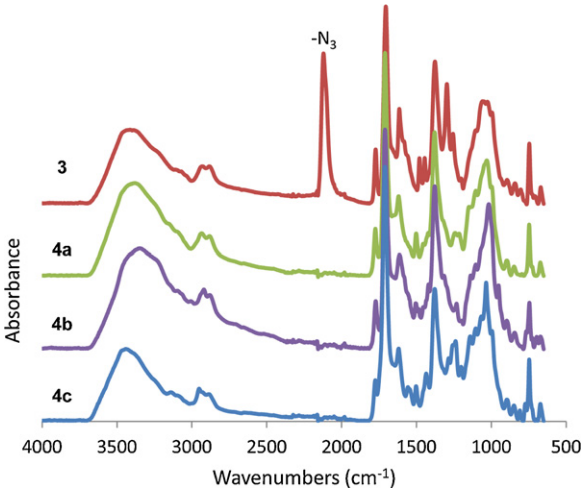

Fig. 7. FT-IR spectra of 4-azido-phthaloyl chitosan (**3**) and the triazole derivatives (**4a**–**c**).

alkynes were carried out in the presence of Cu(II) sulfate pentahydrate and sodium ascorbate at 70 °C. Since we have reported that amines accelerate the triazole formation, triethylamine (TEA) was added as a base for the coupling reactions (Ifuku, Wada, et al., 2011). Fig. 7 shows the FT-IR spectra of the coupling reaction between polymer **3** and 2-propyn-1-ol or ethynylbenzene in the presence of TEA. The azide peak at 2234 cm^{-1} completely disappeared after 48 h reaction (Fig. 7 (**4a** and **4b**)), indicating a quantitative conversion of the N_3 group. However, in the case of reaction with methyl propiolate, a residual peak at 2234 cm^{-1} was still observed in the presence of TEA. In contrast, without TEA, the azide peak completely disappeared after the 96 h reaction (Fig. 7 (**4c**)). The difference was probably due to the molecular interaction of TEA with the carbonyl group of methyl propiolate. The ^{13}C NMR spectra of the triazole chitosan derivatives **4a**, **4b**, and **4c** acquired in DMSO-d_6 are shown in Fig. 6. The spectrum clearly shows that the 4-azido-phthaloyl chitosan (**3**) was modified with the 1,4-triazole linker by Huisgen cycloaddition. All of the peaks associated with the respective carbon atoms of the derivatives were well-resolved as sharp signals. The C atoms of the 1,4-triazole linkers are clearly observed at δ = 123.4 and 151.5 (**4a**), 121.9 and 149.6 (**4b**), and 129.6 and 141.6 (**4c**) ppm, respectively. All of the anhydroglucosamine ring carbons are also assigned as sharp signals. Moreover, in polymer **4a**, the peak at 56.8 ppm can be assigned to the methylene moiety of the hydroxymethyl group. In polymer **4b**, the signals at 131.8–127.3 ppm are assigned to the introduced phenyl moiety. In polymer **4c**, the peaks at 53.6 and 162.0 ppm are assigned to the methyl and carbonyl moiety of the introduced methyl ester group. These spectra support that the desired structures of polymers **4a**, **4b**, and **4c** were achieved. All of the obtained chitosan derivatives **4a**, **4b**, and **4c** showed high solubility in typical aprotic polar solvents such as DMSO, DMF, DMAc, and NMP.

4. Conclusions

A short synthesis of chemoselective chitosan derivatives via click chemistry was achieved. The Huisgen cycloaddition between *N*-(4-azidophthaloyl)-chitosan and alkyne-terminated molecules was successfully carried out in the presence of Cu(I) catalyst and/or triethylamine. FT-IR, elemental analysis, ^{1}H NMR, and ^{13}C NMR spectra supported the desired structure of the chitosan derivatives. Since the desired functional groups with triazole linkers can be chemoselectively introduced into chitosan by shorter reaction steps, application of click chemistry to chitosan could be a useful and convenient method for designing novel chitosan materials with controlled and well-defined structures and promising properties. Since 4-bromophthalic anhydride was used for bromination of chitosan, a highly chemoselective azido chitosan derivative could be obtained by two reaction steps. *N*-(4-bromophthaloyl)-chitosan showed higher solubility than conventional phthaloylchitosan. Since *N*-phthaloylation is the most frequently used reaction to protect the amino group of chitosan, 4-bromophthaloylation in aqueous acetic acid could be an important alternative protection method.

References

Ifuku, S., Wada, M., Morimoto, M., & Saimoto, H. (2011). Preparation of highly regioselective chitosan derivatives via click chemistry. *Carbohydrate Polymers*, *85*, 653–657.

Ifuku, S., Miwa, T., Morimoto, M., & Saimoto, H. (2011). Preparation of highly N-phthaloyl chitosan in aqueous media. *Green Chemistry*, *13*, 1499–1502.

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.

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.

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.

Muzzarelli, R. A. A. (2009). Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. *Carbohydrate Polymers*, *76*, 167–182.

Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, *87*, 995–1012.

Rostovtsev, V. V., Green, L. G., Fokin, V. V., & Sharpless, K. B. (2002). A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective ligationof 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.