Chitinase-Catalyzed Synthesis of an Alternatingly *N*-Sulfonated Chitin Derivative

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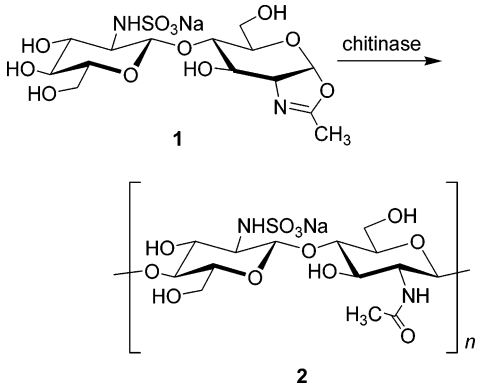
An alternatingly *N*-sulfonated chitin derivative (**2**) was synthesized via ring-opening polyaddition of an *N*-sulfonated chitobiose oxazoline derivative (**1**) catalyzed by chitinases from *Bacillus* sp. and *Serratia marcescens*. The polymerization proceeded homogeneously, providing **2** as a water-soluble polysaccharide in good yields with total control of regioselectivity and stereochemistry. M_n of **2** reached 1900 and 4180 by use of chitinases from *Bacillus* sp. and *Serratia marcescens*, which correspond to 8−10 (n = 4−5) and 18−20 (n = 9−10) saccharide units, respectively. These results indicate that M_n of **2** is controllable by selecting chitinases from different origins. It is considered that the C-2 position of the nonreducing unit in the oxazoline-type monomer is not deeply involved in the catalysis of chitinase.

Introduction

Chitin is a well-known polysaccharide consisting of β(1→4)-linked *N*-acetyl-D-glucosamine (GlcNAc), which is the most abundant polysaccharide in the animal world, found as skeletal material of crustaceans, insects, and so forth.[1] Chitin and its chemically modified derivatives exhibit a variety of physiological activities such as antimicrobial,[2] antitumor,[3] and wound-healing[4] activities in addition to excellent biocompatibility and biodegradability. Therefore, a large number of reports have been published, which describe applications of chitin and its derivatives in many fields such as medicine,[5] pharmaceutics,[6] and materials chemistry.[7] Particularly, sulfated chitin derivatives display potent blood anticoagulation[8] and anti-HIV-1 activities.[9] These activities are probably related to positions of the sulfate groups; that is, the 6-*O*-sulfonated derivatives have blood anticoagulation activity, whereas the derivative bearing 2-*N*- and 3-*O*-sulfo groups effectively inhibits HIV-1 infection of a human MT-1 cell. These results imply that biological activities of sulfated chitin and its derivatives are controllable by changing the position of the sulfate groups. However, chemically modified chitins have discrete structures due to randomly occurring chemical reactions. These indistinct structures become an obstacle to detailed investigation of their structure−activity relationships at a molecular level.

Synthetic polysaccharides with well-defined structure have been successfully prepared via enzymatic polymerization catalyzed by glycoside hydrolases.[10] Particularly, chitin and its derivatives have been produced by chitinase-catalyzed ring-opening polyaddition of sugar oxazoline derivatives as transition-state analogue substrate (TSAS) monomers with total control of regioselectivity and stereochemistry. For example, synthetic chitin was prepared from *N*,*N*′-diacetylchitobiose oxazoline derivative,[11] a cellulose−chitin hybrid polysaccharide from β-D-glucosyl-(1→4)-GlcNAc oxazoline,[12] a chitin−chitosan hybrid polysaccharide from β-D-glucosaminyl (GlcN)-(1→4)-GlcNAc oxazoline,[13] a chitin−xylan hybrid polysaccharide from β-D-xylosyl-(1→4)-GlcNAc oxazoline,[14] and 6-deoxyfluorinated chitin derivatives from 6-deoxyfluorinated *N*,*N*′-diacetylchitobiose oxazoline derivatives.[15] These results suggests that the C-2′ substitution of the nonreducing terminal monosaccharide unit in the monomer structures hardly affected the polymerizations catalyzed by chitinase, which belongs to glycoside hydrolase family 18 (GH18).[16] However, the GH18 chitinase has a cleftlike catalytic domain.[17] Therefore, it is considered that the molecular size of the monomer is critical for enzyme recognition. This important aspect of chitinase catalysis led us to explore synthesis of an alternatingly *N*-sulfonated chitin (**2**) by chitinase-catalyzed polymerization of a TSAS monomer bearing an *N*-sulfonate group at the C-2′ position (**1**) (Scheme 1). The C-2′ substituent sulfonamido group is sterically much bulkier than acetamido, hydroxy, and amino groups found on the monomer structures used in the previous studies.[12−14] Furthermore, the anionic sulfonamido group is electrically opposite to the cationic amino function in the structure of GlcNβ(1→4)GlcNAc oxazoline monomer, which provides a chitin−chitosan hybrid polysaccharide.[13] Thus, the present study is a challenge to realize such a function of GH18 chitinase catalysis.

Scheme 1



Experimental Section

Materials. Dichloromethane and 1,2-dichloroethane were purified by distillation from P_2O_5 and stored over activated molecular sieves

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4A prior to use. Molecular sieves AW300 was ground and activated by heating over 100 °C under reduced pressure. Other chemicals were used without further purification. Chitinase from *Bacillus* sp. was purchased from Wako Pure Chemicals Inc. (Lot CEE1689). Chitinase from *Aeromonas hydrophila* was obtained from Seikagaku Corp. (Lot DN1187). Chitinases from *Serratia marcescens* and *Streptomyces griseus* were acquired from Sigma (Lots 45H4117 and 104K4104, respectively). All enzymes were used without further purification. Chitooligosaccharides [from dimer to hexamer of $\beta(1\rightarrow4)$-linked GlcNAc] were purchased from Seikagaku Corp. (Lots 9612020, 9809100, 9812110, 9906280. and 9812110, respectively). Hyaluronan standards (M_n = 800, 2000, and 4000) were gifts from Denka.

Measurements. NMR spectra were recorded with a Bruker DPX-400 spectrometer. All assignments were based on correlation spectroscopy (COSY) experiments. High-resolution fast atom bombardment (HRFAB) mass spectra were obtained on a Jeol HX-110 spectrometer with 2,4-dinitrobenzyl alcohol or dithiothreitol−thioglycerol (1:1 v/v) as the matrix. Optical rotations were measured with a Jasco P-1010 polarimeter. Melting points were determined with Yamato MP-21. Yields and molecular weights of **2** were determined by size-exclusion chromatography (SEC) measurements on a Tosoh GPC8020 system equipped with Shodex OHpak SB-803 HQ column (8.0 × 300 mm; eluent 0.1 M aqueous sodium nitrate; flow rate 0.5 mL/min; 40 °C). The calibration curves were obtained by use of chitooligosaccharides and hyaluronan as the standards.

2-Acetamido-3,6-di-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-{[(2,2,2-trichloroethoxy)carbonyl]amino}-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranosyl Acetate (5). A mixture of compounds **3**[18] (540 mg, 0.84 mmol) and **4**[13] (200 mg, 0.58 mmol) in anhydrous dichloromethane (7.0 mL) was stirred in the presence of molecular sieves 4A (0.90 g) under argon atmosphere at room temperature for 1 h. After the reaction mixture was cooled to −20 °C, boron trifluoride diethyl ether complex (BF_3·OEt_2, 15 μL, 0.12 mmol) was added to the reaction mixture. The reaction mixture was stirred at −20 °C for 1 h and then at −10 °C for 13 h. After the reaction was completed, triethylamine (1.0 mL) was added dropwise and the reaction mixture was filtered through Celite. The mixture was extracted with $CHCl_3$ and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered through Celite, and concentrated under diminished pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate, 1:2 and 0:1, stepwise, v/v) to afford pure **5** (360 mg, 0.44 mmol, 77%) as a white amorphous powder. $[\alpha]_D^{21}$ +30° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 6.10 (1H, d, $J_{1,2}$ = 3.52 Hz, H-1), 5.52 (1H, d, $J_{2,NH}$ = 9.04 Hz, NH), 5.47 (1H, d, $J_{2',NH'}$ = 8.52 Hz, NH′), 5.26−5.21 (2H, m, H-3′, H-3), 5.05 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.80 Hz, H-4′), 4.78 (1H, d, *J* = 12.04 Hz, C*H*$_2$ of Troc), 4.67 (1H, d, *J* = 12.04 Hz, C*H*$_2$ of Troc), 4.58 (1H, d, $J_{1',2'}$ = 8.56 Hz, H-1′), 4.44−4.36 (3H, m, H-6a, H-6a′, H-2), 4.20 (1H, dd, $J_{5,6b}$ = 2.00 Hz, $J_{6a,6b}$ = 12.56 Hz, H-6b), 4.03 (1H, dd, $J_{5',6b'}$ = 2.00 Hz, $J_{6a',6b'}$ = 12.56 Hz, H-6b′), 3.85 (1H, m, H-5), 3.82 (1H, t, $J_{3,4} = J_{4,5}$ = 9.30 Hz, H-4), 3.64−3.60 (2H, m, H-5′, H-2′), 2.18−2.02 (18H, s, C*H*$_3$ of acetyl), 1.94 (3H, s, C*H*$_3$ of acetamido); ^{13}C NMR ($CDCl_3$) δ (ppm) 171.44−168.90 (*C*═O of acetyl and acetamido) 154.24 (*C*═O of Troc), 101.04 (C-1′), 95.58 (*C*Cl$_3$ of Troc), 90.47 (C-1), 75.32 (C-4), 74.38 (*C*H$_2$ of Troc), 71.91 (C-3′), 71.86 (C-5′), 70.75 (C-3), 70.48 (C-5), 68.27 (C-4′), 61.81 (C-6), 61.76 (C-6′), 56.34 (C-2′), 51.02 (C-2), 21.07−20.57 (*C*H$_3$ of acetyl), 14.18 (*C*H$_3$ of acetamido); HRMS (FAB) calcd for $C_{29}H_{40}O_{18}N_2Cl_3$ $[M + H]^+$ 809.1342, found 809.1433.

2-Methyl-4,5-dihydro-[3,6-di-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-{[(2,2,2-trichloroethoxy)carbonyl]amino}-β-D-glucopyranosyl)-1,2-dideoxy-α-D-glucopyranoso][2,1-*d*]-1,3-oxazole (6). Me_3-SiOTf (0.32 mL, 0.39 g, 1.77 mmol) under argon atmosphere at 55 °C was added to a solution of compound **5** (1.30 g, 1.60 mmol) in anhydrous 1,2-dichloroethane (10 mL). After it was stirred for 4 h, the reaction mixture was cooled to 0 °C, followed by the addition of triethylamine (1.0 mL) and evaporation under reduced pressure. The residue was immediately subjected to silica gel column chromatography (*n*-hexane/ethyl acetate, 1:1 then 1:2, stepwise, v/v) to afford pure **6** (1.0 g, 1.33 mmol, 83%) as a white amorphous powder. $[\alpha]_D^{20}$ +6.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 5.92 (1H, d, $J_{1,2}$ = 7.00 Hz, H-1), 5.64 (1H, d, $J_{2,3}$ = 1.52 Hz, H-3), 5.61 (1H, d, $J_{2',NH'}$ = 9.00 Hz, NH′), 5.26 (1H, t, $J_{2',3'} = J_{3',4'}$ = 10.04 Hz, H-3′), 5.07 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.54 Hz, H-4′), 4.77 (1H, d, $J_{1',2'}$ = 8.04 Hz, H-1′), 4.72 (1H, d, *J* = 12.04 Hz, C*H*$_2$ of Troc), 4. 71 (1H, d, *J* = 12.04 Hz, C*H*$_2$ of Troc), 4.29 (1H, dd, $J_{5',6a'}$ = 4.52 Hz, $J_{6a',6b'}$ = 12.56 Hz, H-6a′), 4.29 (1H, dd, $J_{5,6a}$ = 4.52 Hz, $J_{6a,6b}$ = 12.56 Hz, H-6a), 4.14−4.11 (3H, m, H-6b, H-6b′, H-2), 3.78 (1H, ddd, $J_{4',5'}$ = 10.04 Hz, $J_{5',6a'}$ = 4.04 Hz, $J_{5',6b'}$ = 2.52 Hz, H-5′), 3.69 (1H, dt, $J_{2',3'}$ = 10.52 Hz, $J_{1',2'} = J_{2',NH'}$ = 8.56 Hz, H-2′), 3.62 (1H, d, $J_{4,5}$ = 9.04 Hz, H-4), 3.46 (1H, d, $J_{4,5}$ = 9.00 Hz, $J_{5,6a}$ = 4.00 Hz, $J_{5,6b}$ = 2.00 Hz, H-5), 2.12−2.09 (12H, s, C*H*$_3$ of acetyl), 2.02 (3H, s, C*H*$_3$ of oxazoline); ^{13}C NMR ($CDCl_3$) δ (ppm) 171.01−169.35 (*C*═O of acetyl), 166.80 (CH_3*C*═N of oxazoline), 154.10 (*C*═O of Troc), 102.27 (C-1′), 99.17 (C-1), 95.52 (*C*Cl$_3$ of Troc), 77.60 (C-4), 74.40 (*C*H$_2$ of Troc), 72.25 (C-3′), 72.04 (C-5′), 70.51 (C-3), 68.45 (C-4′), 67.79 (C-5), 64.86 (C-2), 63.38 (C-6), 61.97 (C-6′), 56.13 (C-2′), 21.03−20.62 (*C*H$_3$ of acetyl), 13.97 (*C*H$_3$ of oxazoline); HRMS (FAB) calcd for $C_{27}H_{36}O_{16}N_2Cl_3$ $[M + H]^+$ 749.1130, found 749.1135.

Benzyl 2-Acetamido-3,6-di-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-{[(2,2,2-trichloroethoxy)carbonyl]amino}-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (7). Benzyl alcohol (0.50 mL, 0.50 g, 4.62 mmol) was added to the solution of compound **6** (1.00 g, 1.33 mmol) in 1,2-dichloroethane (7.0 mL), and the mixture was stirred at 50 °C under argon atmosphere for 10 min. Then, the pH of the reaction mixture was adjusted to 2−3 by addition of 10-camphorsulfonic acid (CSA). After it was stirred at 75 °C for 12 h, the mixture was diluted with chloroform and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered through Celite, and concentrated under diminished pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate, 1:1, 1:2, and 1:3, stepwise, v/v) to afford pure **7** (0.56 g, 0.65 mmol, 49%) as a white crystal. $[\alpha]_D^{22}$ −35° (*c* 1.0, $CHCl_3$/MeOH, 10/1 v/v), mp 229−230 °C; ^{1}H NMR ($CDCl_3$) δ (ppm) 7.35−7.27 (4H, m, aromatic), 6.66 (1H, d, $J_{2',NH'}$ = 9.00 Hz, NH′), 6.35 (1H, d, $J_{2,NH}$ = 9.52 Hz, NH), 5.26 (1H, t, $J_{2',3'} = J_{3',4'}$ = 10.04 Hz, H-3′), 5.07 (1H, t, $J_{2,3} = J_{3,4}$ = 9.52 Hz, H-3), 5.02 (1H, t, $J_{3',4'} = J_{4',5'}$ = 9.56 Hz, H-4′), 4.85 (1H, d, *J* = 12.04 Hz, C*H*$_2$ of benzyl), 4.71 (2H, s, C*H*$_2$ of Troc), 4.64 (1H, d, $J_{1',2'}$ = 8.00 Hz, H-1′), 4.58 (1H, d, *J* = 12.56 Hz, C*H*$_2$ of benzyl), 4.47 (1H, d, $J_{1',2'}$ = 8.04 Hz, H-1′), 4.42−4.37 (2H, m, H-6a, H-6a′), 4.20 (1H, d, $J_{5,6b}$ = 5.04 Hz, $J_{6a,6b}$ = 11.56 Hz, H-6b), 4.09−4.02 (2H, m, H-2, H-6b′), 3.82 (1H, t, $J_{3,4} = J_{4,5}$ = 9.04 Hz, H-4), 3.71 (1H, d, $J_{4',5'}$ = 9.52 Hz, H-5′), 3.64−3.57 (2H, m, H-2′, H-5), 2.14−1.98 (15H, s, C*H*$_3$ of acetyl), 1.91 (3H, s, C*H*$_3$ of acetamido); ^{13}C NMR ($CDCl_3$) δ (ppm) 171.10−169.45 (*C*═O of acetyl and acetamido), 154.35 (*C*═O of Troc), 136.74−127.81 (aromatic), 100.68 (C-1′), 99.20 (C-1), 95.47 (*C*Cl$_3$ of Troc), 75.98 (C-4), 74.24 (*C*H$_2$ of Troc), 72.56 (C-3), 72.47 (C-5), 71.87 (C-3′), 71.49 (C-5′), 70.23 (*C*H$_2$ of benzyl), 68.31 (C-4′), 62.67 (C-6), 61.66 (C-6′), 56.11 (C-2′), 53.41 (C-2), 22.88−20.41 (*C*H$_3$ of acetyl and acetamido); HRMS (FAB) calcd for $C_{34}H_{44}O_{17}N_2Cl_3$ $[M + H]^+$ 857.1706, found 857.1710.

Benzyl 2-Acetamido-3,6-di-*O*-acetyl-4-*O*-(2-amino-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (8). Compound **7** (0.60 g, 0.70 mmol) was dissolved in AcOH (18 mL), and freshly activated zinc dust (3.0 g) was added. After it was stirred at 50 °C for 1 h, the reaction mixture was filtered through Celite and extracted with $CHCl_3$. It was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered through Celite, and concentrated under diminished pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/methanol, 20:1 v/v) to afford pure **8** (0.39 g, 0.57 mmol, 81%) as a white amorphous powder. $[\alpha]_D^{22}$ −24° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 7.34−7.28 (4H, m, aromatic), 5.53 (1H, d, $J_{2,NH}$ = 9.04 Hz, N*H*), 5.07 (1H, dd, $J_{2,3}$ = 10.04 Hz, $J_{3,4}$ = 9.04 Hz, H-3), 4.96 (1H, t,

$J_{3',4'} = J_{4',5'} = 9.52$ Hz, H-4′), 4.91 (1H, t, $J_{2',3'} = J_{3',4'} = 9.52$ Hz, H-3′), 4.87 (1H, d, $J = 12.04$ Hz, CH_2 of benzyl), 4.66 (1H, dd, $J_{5',6a'} = 1.48$ Hz, $J_{6a',6b'} = 12.04$ Hz, H-6a′), 4.58 (1H, d, $J = 12.52$ Hz, CH_2 of benzyl), 4.47 (1H, d, $J_{1,2} = 8.04$ Hz, H-1), 4.35 (1H, dd, $J_{5,6a} = 4.52$ Hz, $J_{6a,6b} = 12.56$ Hz, H-6a), 4.30 (1H, dd, $J_{5',6a'} = 5.00$ Hz, $J_{6a',6b'} = 12.04$ Hz, H-6b′), 4.23 (1H, d, $J_{1',2'} = 7.52$ Hz, H-1′), 4.09 (1H, dt, $J_{1,2} = J_{2,NH} = 9.04$ Hz, $J_{2,3} = 10.04$ Hz, H-2), 4.03 (1H, dd, $J_{5,6b} = 2.00$ Hz, $J_{6a,6b} = 12.56$ Hz, H-6b), 3.82 (1H, t, $J_{3,4} = J_{4,5} = 9.56$ Hz, H-4), 3.64−3.61 (2H, m, H-5, H-5′), 2.83 (1H, t, $J_{1',2'} = J_{2',3'} = 9.00$ Hz, H-2′), 2.13−1.92 (18H, s, CH_3 of acetyl and acetamido); ^{13}C NMR ($CDCl_3$) δ (ppm) 171.09−169.76 (*C*═O of acetyl and acetamido), 136.95−127.95 (aromatic), 103.77 (C-1′), 99.46 (C-1), 75.59 (C-4), 75.11 (C-3′), 73.18 (C-5), 72.39 (C-3), 71.96 (C-5′), 70.33 (*C*H_2 of benzyl), 68.43 (C-4′), 62.44 (C-6′), 61.99 (C-6), 56.04 (C-2′), 53.78 (C-2), 23.29−20.64 (*C*H_3 of acetyl and acetamido); HRMS (FAB) calcd for $C_{31}H_{43}O_{15}N_2$ $[M + H]^+$ 683.2663, found 683.2660.

Triethylammonium Benzyl 2-Acetamido-3,6-di-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-sulfoamino-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (9). Triethylamine (0.7 mL) and $PySO_3H$ (0.31 g, 1.94 mmol) was added to the solution of compound **8** (0.38 g, 0.56 mmol) dissolved in dimethylformamide (7.0 mL), and the mixture was stirred for 24 h. After the addition of methanol (1.5 mL), the reaction mixture was evaporated and directly applied to silica gel column chromatography ($CHCl_3$/methanol, 50:1, 10:1, stepwise, v/v) to afford pure **9** (0.48 g, 0.56 mmol, quant) as a white amorphous powder. $[\alpha]_D^{21}$ −24° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 10.14 [1H, br s, SO_3H·N($CH_2CH_3)_3$], 7.28−7.26 (4H, m, aromatic), 5.93 (1H, d, $J_{2,NH} = 9.04$ Hz, NH), 5.16 (1H, t, $J_{2,3} = J_{3,4} = 9.52$ Hz, H-3), 5.10 (1H, t, $J_{2',3'} = J_{3',4'} = 8.04$ Hz, H-3′), 5.02 (1H, t, $J_{3',4'} = J_{4',5'} = 9.52$ Hz, H-4′), 4.87 (1H, d, $J = 12.04$ Hz, CH_2 of benzyl), 4.61 (1H, d, $J_{1,2} = 7.52$ Hz, H-1), 4.60−4.58 (2H, m, H-6a, H-6b), 4.58 (1H, d, $J = 12.56$ Hz, CH_2 of benzyl), 4.54 (1H, d, $J_{1',2'} = 8.00$ Hz, H-1′), 4.30 (1H, dd, $J_{5',6a'} = 5.00$ Hz, $J_{6a',6b'} = 12.52$ Hz, H-6a′), 4.08−4.03 (2H, m, H-2, H-6b′), 3.92 (1H, t, $J_{3,4} = J_{4,5} = 7.52$ Hz, H-4), 3.83−3.81 (1H, m, H-5), 3.62 (1H, ddd, $J_{4',5'} = 10.04$ Hz, $J_{5',6a'} = 4.48$ Hz, $J_{5',6b'} = 2.00$ Hz, H-6b′), 3.36 (1H, dd, $J_{1',2'} = 8.00$ Hz, $J_{2',3'} = 10.00$ Hz, H-2′), 3.14 [6H, q, $J = 7.04$ Hz, SO_3H·N(C$H_2CH_3)_3$], 2.12−1.93 (18H, s, CH_3 of acetyl and acetamido), 1.36 [9H, t, $J = 7.04$ Hz, SO_3H·N($CH_2CH_3)_3$]; ^{13}C NMR ($CDCl_3$) δ (ppm) 171.70−169.53 (*C*═O of acetyl and acetamido), 137.50−127.66 (aromatic), 101.85 (C-1′), 99.79 (C-1), 75.14 (C-4), 73.25 (C-3′), 72.95 (C-5), 72.54 (C-3), 71.52 (C-5′), 70.61 (*C*H_2 of benzyl), 68.57 (C-4′), 62.98 (C-6), 62.19 (C-6′), 58.99 (C-2′), 54.75 [SO_3H·N(*C*$H_2CH_3)_3$], 53.39 (C-2), 23.21−20.67 (*C*H_3 of acetyl and acetamido), 8.67 [SO_3H·N($CH_2CH_3)_3$]; HRMS (FAB) calcd for $C_{37}H_{58}O_{18}N_3S$ $[M + H]^+$ 864.3436, found 864.3436.

2-Methyl-4,5-dihydro-[triethylammonium 3,6-di-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-sulfoamino-β-D-glucopyranosyl)-1,2-dideoxy-α-D-glucopyranoso][2,1-*d*]-1,3-oxazole (10). $Pd(OH)_2$−C (50 mg) was added to the solution of compound **9** (100 mg, 0.12 mmol) in methanol (2.0 mL), and the reaction mixture was stirred vigorously under hydrogen atmosphere for 30 min. After filtration of $Pd(OH)_2$−C with Celite, the crude mixture was evaporated to dryness under diminished pressure. The residue was dissolved in dichloromethane (3.0 mL), followed by addition of tosyl chloride (27 mg, 0.14 mmol), DMAP (8.5 mg, 0.070 mmol), and triethylamine (16 μL, 12 mg, 0.12 mmol). After stirring the reaction mixture was stirred under Ar atmosphere for 24 h, tosyl chloride (27 mg, 0.14 mmol), DMAP (8.5 mg, 0.070 mmol), and triethylamine (16 μL, 12 mg, 0.12 mmol) were added to the reaction mixture again, and stirring continued for an additional 24 h. The reaction mixture was directly subjected to silica gel column chromatography ($CHCl_3$/methanol, 1:0 and 20:1, stepwise, v/v) and then purified by size-exclusion chromatography (LH-20, eluted with methanol) to afford pure **10** (54 mg, 0.072 mmol, 60%) as a white amorphous powder. $[\alpha]_D^{23}$ +21° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ppm) 5.91 (1H, d, $J_{1,2} = 7.52$ Hz, H-1), 5.71 (1H, d, $J_{2,3} = 1.52$ Hz, H-3), 5.46 (1H, dd, $J_{2',3'} = 10.52$ Hz, $J_{3',4'} = 9.04$ Hz, H-3′), 4.99 (1H, t, $J_{3',4'} = J_{4',5'} = 9.54$ Hz, H-4′), 4.92 (1H, d, $J_{1',2'} = 8.04$ Hz, H-1′), 4.54 (1H, dd, $J_{5,6a} = 2.00$ Hz, $J_{6a,6b} = 12.04$ Hz, H-6a), 4.42 (1H, dd, $J_{5,6b} = 5.52$ Hz, $J_{6a,6b} = 12.04$ Hz, H-6b), 4.30 (1H, d, $J_{2',NH'} = 5.52$ Hz, N*H*′), 4.28 (1H, dd, $J_{5',6a'} = 4.52$ Hz, $J_{6a',6b'} = 12.04$ Hz, H-6a′), 4.12 (1H, dd, $J_{5',6a'} = 2.00$ Hz, $J_{6a',6b'} = 12.04$ Hz, H-6b′), 4.09 (1H, m, H-2), 3.81 (1H, d, $J_{4,5} = 9.52$ Hz, H-4), 3.76 (1H, ddd, $J_{4',5'} = 10.04$ Hz, $J_{5',6a'} = 4.52$ Hz, $J_{5',6b'} = 2.52$ Hz, H-5′), 3.61 (1H, ddd, $J_{4,5} = 9.04$ Hz, $J_{5,6a} = 2.00$ Hz, $J_{5,6b} = 5.52$ Hz, H-5), 3.29 (1H, ddd, $J_{2',NH'} = 5.52$ Hz, $J_{1',2'} = 8.52$ Hz, $J_{2',3'} = 10.04$ Hz, H-2′), 3.10 [6H, q, $J = 7.00$ Hz, SO_3H·N(C$H_2CH_3)_3$], 2.09−2.03 (18H, s, CH_3 of acetyl) 1.99 (3H, s, CH_3 of oxazoline), 1.33 [9H, t, $J = 7.04$ Hz, SO_3H·N($CH_2CH_3)_3$]; ^{13}C NMR ($CDCl_3$) δ (ppm) 171.00−169.17 (*C*═O of acetyl), 166.50 (CH_3C═N of oxazoline), 102.86 (C-1′), 99.13 (C-1), 77.32 (C-4), 72.84 (C-3′), 71.39 (C-5′), 70.42 (C-3), 69.31 (C-4′), 68.23 (C-5), 64.82 (C-2), 64.04 (C-6), 62.21 (C-6′), 58.71 (C-2′), 46.02 [SO_3H·N(*C*$H_2CH_3)_3$], 21.03−20.62 (*C*H_3 of acetyl), 13.93 (*C*H_3 of oxazoline) 8.68 [SO_3H·N($CH_2CH_3)_3$]; HRMS (FAB) calcd for $C_{30}H_{50}O_{17}N_3S$ $[M + H]^+$ 756.2861, found 756.2862.

2-Methyl-4,5-dihydro-[sodium 1,2-dideoxy-4-*O*-(2-deoxy-2-sulfoamino-β-D-glucopyranosyl)-α-D-glucopyranoso][2,1-*d*]-1,3-oxazole (1). To a solution of compound **10** (0.10 g, 0.13 mmol) in anhydrous methanol (3.0 mL) was added dropwise sodium hydroxide (28 wt % methanol solution, 28 mg, 0.15 mmol). The reaction mixture was stirred at room temperature for 30 min under dry atmosphere. Then the mixture was concentrated to dryness under diminished pressure to afford **1** (62 mg, 0.13 mmol, purity 99%) as a white amorphous powder. ^{1}H NMR (D_2O) δ (ppm) 6.02 (1H, d, $J_{1,2} = 7.52$ Hz, H-1), 4.57 (1H, d, $J_{1',2'} = 8.52$ Hz, H-1′), 4.37 (1H, t, $J_{2,3} = J_{3,4} = 2.52$ Hz, H-3), 4.12 (1H, m, H-2), 3.86 (1H, dd, $J_{5,6a} = 1.52$ Hz, $J_{6a,6b} = 12.56$ Hz, H-6a), 3.86 (1H, dd, $J_{5',6a'} = 1.52$ Hz, $J_{6a',6b'} = 12.56$ Hz, H-6a′), 3.70−3.58 (4H, m, H-6b, H-6b′, H-4, H-3′), 3.47−3.35 (3H, m, H-5, H-5′, H-4′), 2.93 (1H, dd, $J_{1',2'} = 8.52$ Hz, $J_{2',3'} = 10.00$ Hz, H-2′), 2.00 (3H, s, CH_3 of oxazoline); ^{13}C NMR ($CDCl_3$) δ (ppm) 168.66 (CH_3C═N of oxazoline), 103.39 (C-1′), 100.23 (C-1), 79.50 (C-4), 76.04 (C-5′), 74.97 (C-3′), 71.16 (C-5), 70.18 (C-4′), 69.30 (C-3), 65.67 (C-2), 62.07 (C-6′), 61.07 (C-6), 60.45 (C-2′), 13.37 (*C*H_3 of oxazoline) 8.68 [SO_3H·N($CH_2CH_3)_3$]; HRMS (FAB) calcd for $C_{14}H_{23}O_{12}N_2Na_2S$ $[M + Na]^+$ 489.0767, found 489.0763.

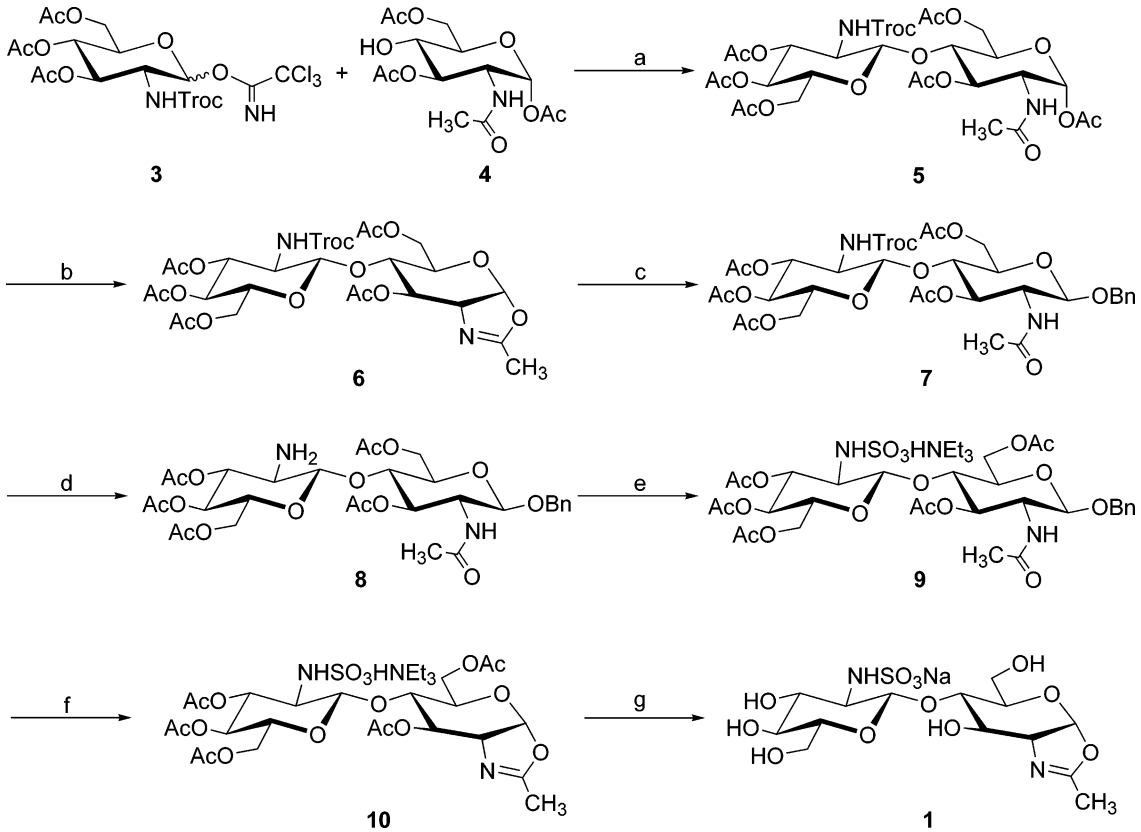
Typical Procedure for Monitoring Monomer Consumption. Monomer **1** (9.3 mg, 0.020 mol) was dissolved in a phosphate-buffered D_2O solution (50 mM, pD 8.5, 150 μL), and the solution was divided into two equal portions. To the control solution, only phosphate-buffered D_2O solution (50 mM, pD 8.5, 25 μL) was added. To another tube, *Bacillus* sp.-derived chitinase (0.23 mg) dissolved in a phosphate-buffered D_2O solution (50 mM, pD 8.5, 25 μL) was added. These two samples were kept standing at 30 °C in NMR tubes. The reaction time courses of **1** were calculated from ^{1}H NMR, and the integration values of the oxazoline ring combined H-1 proton signals were compared with those of methyl protons. After the consumption of monomer **1** was finished, the reaction mixture was heated at 90 °C for 10 min to inactivate the enzyme.

Typical Procedure for Enzymatic Polymerization. To a solution of monomer **1** (47 mg, 0.10 mmol) in phosphate buffer (50 mM, pH 8.0; 800 μL) was added chitinase from *Serratia marcescens* (2.3 mg) dissolved in a phosphate buffer (50 mM, pH 8.0; 200 μL), and the mixture was kept standing at 30 °C. Monomer consumption was monitored by TLC (solvent was $CHCl_3$/MeOH = 2/1, v/v). A spot derived from monomer was observed upon thin-layer chromatography (TLC) with $R_f = 0.2$. After 7.5 h, the mixture was heated at 90 °C for 10 min to inactivate the enzyme and subjected to Sephadex G-10 chromatography, eluted with distilled water to afford polymer **2** (28 mg, 60% as an isolated yield).

Results and Discussion

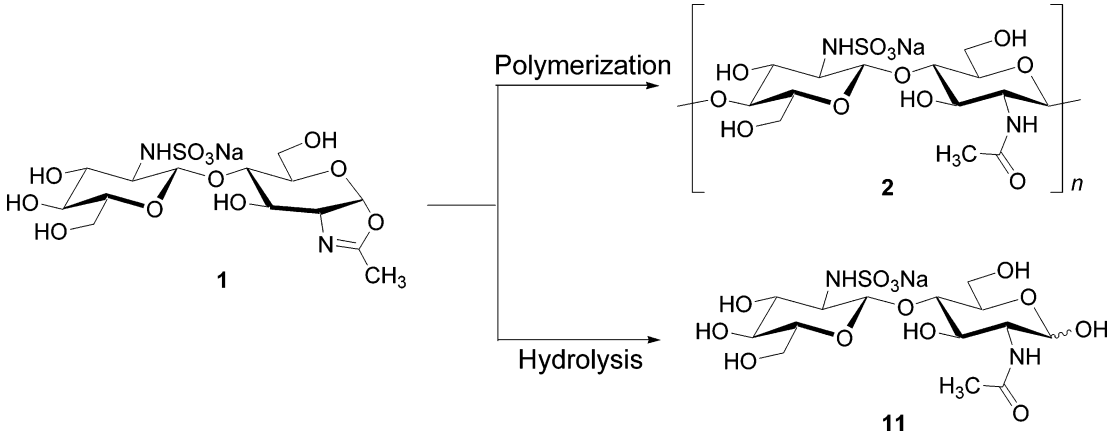
Synthesis of Monomer 1. Monomer **1** was synthesized according to the reactions outlined in Scheme 2. Glycosyl donor **3**[18] and glycosyl acceptor **4**[13] were prepared according to the

Scheme 2[a]



[a] Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, MSAW300/CH_2Cl_2, 77%; (b) TMSOTf/1,2-dichloroethane, 83%; (c) BnOH, CSA/1,2-dichloroethane, 49%; (d) Zn/AcOH, 81%; (e) $SO_3-C_6H_5N$/DMF, quant; (f) (i) $Pd(OH)_2-C$, H_2/MeOH, (ii) TsCl, Et_3N/CH_2Cl_2, 60% (two steps); (g) CH_3ONa/CH_3OH, quant (purity 99%).

Scheme 3. Two Kinds of Possible Reactions during Chitinase-Catalyzed Polymerization



literature. Glycosylation of **4** with **3** was carried out with boron trifluoride diethyl ether complex as a promoter to afford disaccharide **5**. Compound **5** was treated with (trimethylsilyl)-trifluoromethanesulfonate (TMSOTf) to give the corresponding oxazoline derivative **6**, followed by coupling with benzyl alcohol catalyzed by 10-camphorsulfonic acid, giving rise to compound **7**. The (2,2,2-trichloroethoxy)carbonyl (Troc) group of **7** was removed by use of zinc powder in the presence of acetic acid to give compound **8**. The resulting amino group of **8** was selectively sulfonated by treatment with sulfurtrioxide pyridine complex to afford compound **9**. The anomeric *O*-benzyl group of **9** was removed by hydrogenation by use of palladium(II) hydroxide−charcoal under hydrogen atmosphere followed by treatment with *p*-toluenesulfonyl chloride in dichloromethane containing triethylamine and 4-dimethylaminopyridine, giving rise to the corresponding oxazoline derivative **10**. Finally, all of the *O*-acetyl groups were deprotected by sodium methoxide in methanol to afford the target monomer **1**.

Enzymatic Polymerization of 1 Catalyzed by Chitinase from Various Origins. Monomer **1** bearing the oxazoline structure is a high-energy form of GlcNAc. Therefore, two kinds of reactions can occur during enzymatic polymerization: polymerization of **1** to provide polymer **2**, and hydrolysis of **1** enzymatically and nonenzymatically via oxazoline ring opening, leading to formation of hydrolysate **11** (Scheme 3).

There are four kinds of commercial enzymes containing GH18 chitinase from different origins: *Bacillus* sp. (abbreviated as chitinase Bs), *S. marcescens* (chitinase Sm), *S. griseus* (chitinase Sg), and *A. hydrophila* (chitinase Ah). Therefore, we examined the feasibility of monomer **1** polymerization catalyzed

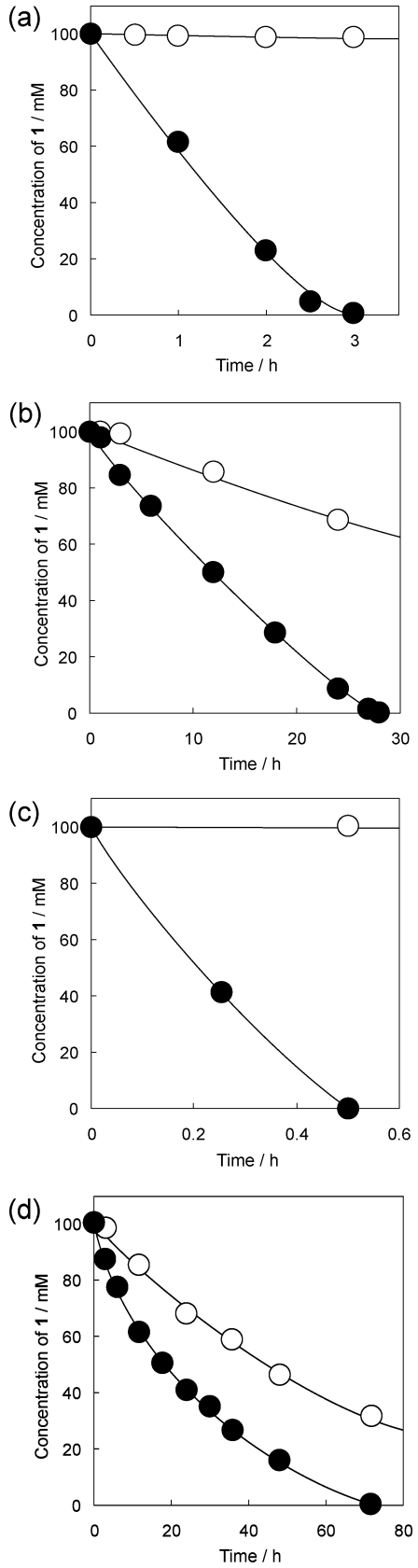


Figure 1. Reaction time courses of **1** with chitinase (●) and without enzyme (○): (a) chitinase Bs, (b) chitinase Sm, (c) chitinase Sg, and (d) chitinase Ah. Reaction conditions: phosphate buffer (10 mM), pD 8.5; amount of enzyme, 5 wt % for **1**; reaction temperature, 30 °C; initial concentration of **1**, 100 mM.

by these enzymes. Figure 1 indicates the time dependence of monomer **1** concentration during the polymerization. Concentration change of **1** was monitored by ^{1}H NMR spectroscopy measuring the integration of H-1 proton of the oxazoline at 30 °C. Chitinase Bs accelerated consumption of **1**, which disappeared within 3 h (Figure 1a). With chitinase Sm, monomer **1** was completely consumed within 28 h (Figure 1b). Monomer **1** was consumed quite rapidly by chitinase Sg, within 0.5 h (Figure 1c). In contrast, it took 72 h for complete consumption of **1** with chitinase Ah (Figure 1d). Without enzyme, monomer **1** was gradually decomposed; it remained at 98% (Figure 1a), 65% (Figure 1b), 99% (Figure 1c), and 32% (Figure 1d) at 3, 28, 0.5, and 72 h, respectively. These results indicate that all enzymes catalyze the oxazoline ring-opening reaction of **1**.

Table 1. Enzymatic Polymerization of **1** Catalyzed by Chitinases from Various Origins

	polymerization[a]		polymer **2**		
entry	chitinase	time,[b] h	yield,[c] %	M_n[d]	M_w[d]
1	Bs	3.0	39	1870	2180
2	Sm	28	58	3110	4100
3	Sg	0.5	11	1240	1250
4	Ah	72	0		

[a] In a phosphate buffer (10 mM, pD 8.5) at 30 °C with 5 wt % enzyme for **1**. [b] Time for complete consumption of **1**. [c] Determined by HPLC measurements containing products with molecular weight higher than that of tetrasaccharide. [d] Determined by SEC calibrated with chitooligosaccharides and hyaluronan standards.

After monomer **1** disappeared, the reaction was terminated by heating at 90 °C for 10 min. The reaction mixture was analyzed by HPLC and SEC measurements (Table 1). Chitinases Bs and Sm produced polymer **2** in 39% and 58% yields with molecular weights (M_n) 1870 (n = 4) and 3110 (n = 7), respectively (entries 1 and 2). The latter M_n was almost double compared with the former one, indicating that polymer **2** with a different molecular weight distribution can be obtained by appropriate enzyme selection. Furthermore, chitinase Sg provided oligosaccharide of **2** in a lower yield of 11% (entry 3); a single peak of the product was observed on the SEC chart, which corresponds to that from the tetrasaccharide of **2** (n = 2). Unexpectedly, chitinase Ah did not catalyze polymerization of **1** at all, exclusively providing hydrolysate **11**. Thus, monomer **1** was polymerized by chitinases Bs and Sm; in particular, chitinase Sm produced polymer **2** most effectively in terms of yields and M_n of **2**. Furthermore, it should be noted that these polymerizations proceeded homogeneously, indicating that polymer **2** is soluble in aqueous media.

Structural Characterization of 2. Polymer **2** obtained after purification by SEC was analyzed by IR spectroscopy (Figure 2). Compared with the spectrum of natural chitin (Figure 2a), that of **2** showed the specific absorption at 1250−1200 cm^{-1}, which is derived from S═O stretching of the sulfonate group.[19] Furthermore, the absorption around at 1350 cm^{-1} was assigned to N−S stretching.[19] These results indicate that the resulting polymer has sulfonated groups in the molecule.

Figure 3a shows the ^{1}H NMR spectrum of **2**. Two kinds of anomeric protons derived from internal GlcNAc and *N*-sulfonated GlcN (GlcNS) were observed at δ 4.6 as an overlapped signal. This signal became a doublet with a coupling constant of 7.04 Hz when measured at 70 °C, indicating that these saccharide units are connected through β-glycosidic linkage. It has been reported that the chemical shifts of the H-2 with sulfonamido group and that with free amino group were observed around at δ 3.1 and δ 2.7, respectively.[20] The signal from H-2′ was found at δ 3.1, whereas no signals were observed at δ 2.7. Therefore, the resulting polysaccharide **2** has *N*-sulfonate groups and no free amino group. Furthermore, the

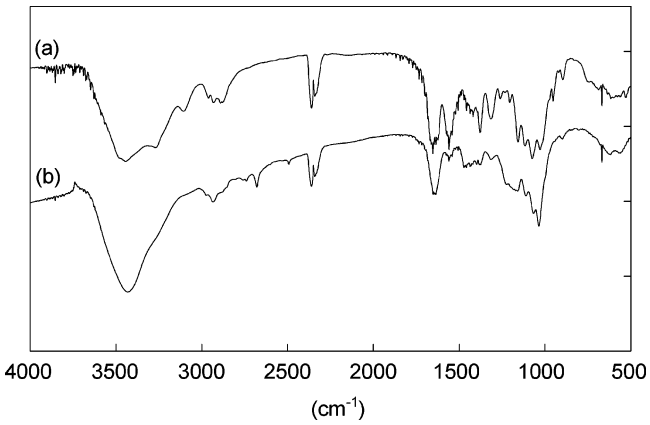


Figure 2. IR spectra of (a) natural chitin and (b) polymer **2**.

integration ratio of the signals at δ 4.6, 3.1, and 2.1 (CH_3 of GlcNAc) is 2:1:3. Figure 3b shows the ^{1}H NMR spectrum of **2** after a countercation of sulfonate group was exchanged from sodium to triethylamine by use of an ion-exchange resin (Dowex 50W-X4). At δ 1.3 and 3.2, triplet and quartet peaks derived from methyl and methylene protons of triethylamine salt were observed. The integration ratio of the methyl, the methylene, and H-2′ is 3:2:1, indicating that one sulfonate group is introduced in a repeating unit of **2**. ^{13}C NMR measurements also supported the structure of **2** (Figure 3c). The signals derived from C-1′ and C-1, two kinds of anomeric carbons, were observed at δ 101 and 102. The signals from C-4 of GlcNAc and GlcNS were found at δ 79 and 80. It is generally accepted that the signals derived from the carbon atom connecting to the glycosidic oxygen is characteristically shifted downfield (to a higher δ value) compared to those from other carbon atoms in the same pyranose unit.[21] Therefore, both the C-4 carbons of GlcNAc and GlcNS are adjacent to the glycosidic oxygen, indicating that polymer **2** has only a (1→4) glycosidic linkage. The results from IR and NMR measurements clearly confirmed the structure of **2**, which is alternatingly *N*-sulfonated chitin derivative.

Chitinase Bs-Catalyzed Polymerization of 1 under Various Reaction Conditions. To determine the optimal conditions for polymerization of **1** by chitinase Bs, reaction parameters of pH, enzyme amount, reaction temperature, and initial concentration of **1** were varied (Table 2). The polymerization proceeded at pH ranging from 6.5 to 11.0 (entries 5−14). M_n of **2** seemed to be bimodal, reaching 1760 at pH 8.0 (entry 8) and 1730 at pH 10.5. However, yield of **2** became higher with increasing pH until 10.5 (entry 13), at which the maximum yield of 56% was marked. Therefore, the optimum pH was 10.5. Next, the amount of enzyme was varied at the fixed pH value of 10.5. With 1 wt % enzyme, the polymerization took 168 h, providing **2** in a lower yield of 23% (entry 15). With an increase of the enzyme amount from 10 to 20 wt % (entries 16−18), yield of **2** decreased from 52% to 34%. However, M_n of **2** reached 1820 with 15 wt % enzyme (entry 17), whereas the yield was slightly lower (49%) than that in entry 13. The polymerization with 15 wt % enzyme proceeded at temperatures ranging from 10 to 40 °C (entries 17, 19−21), giving rise to polymer **2** with similar M_n values (1820−1900). In entry 19, polymer **2** with the highest M_n of 1900 was obtained. Initial concentration of **1** exerted little effect on yields and M_n of **2** (entries 17, 22, and 23). Thus, polymerization under the conditions in entry 13 was most effective in terms of the yield, and polymerization under the conditions in entry 19 provided polymer **2** with the highest M_n value.

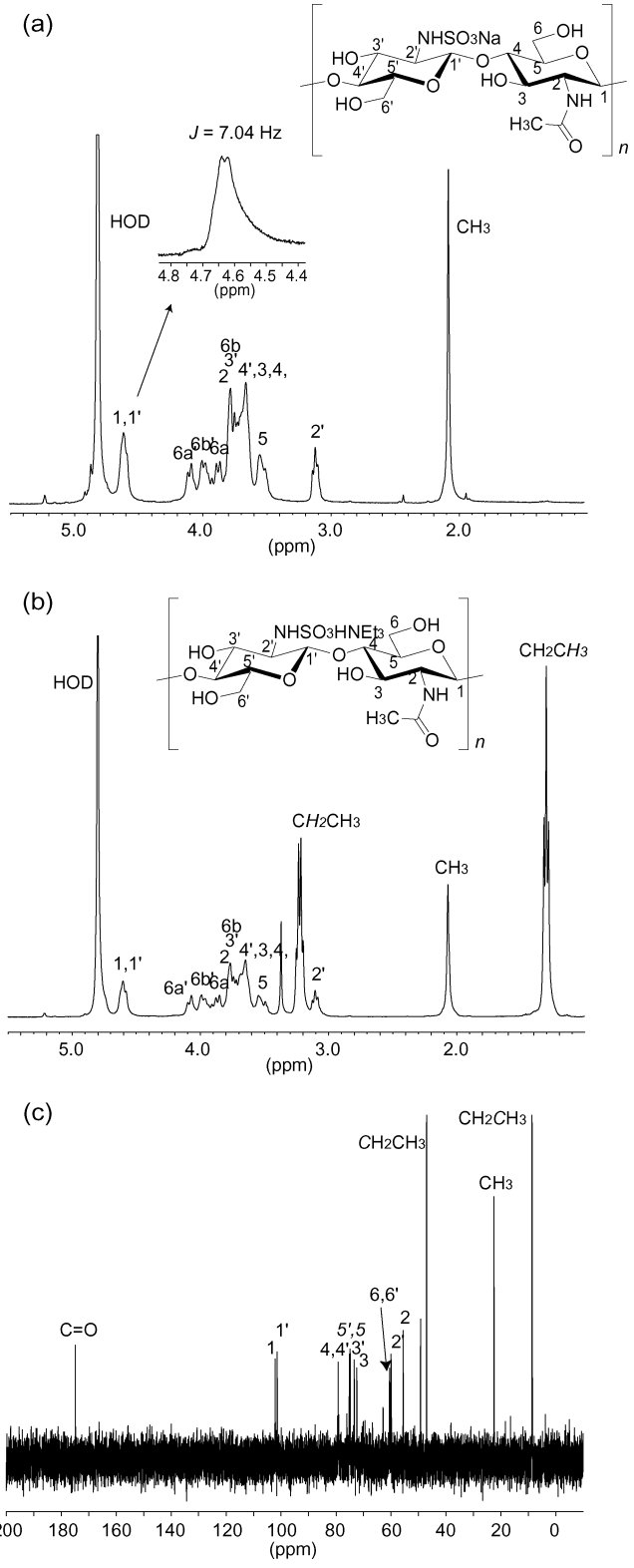


Figure 3. (a) ^{1}H NMR spectrum of polymer **2** in sodium salt form, and (b) ^{1}H and (c) ^{13}C NMR spectra of **2** in triethylamine salt form.

Chitinase Sm-Catalyzed Polymerization of 1 under Various Reaction Conditions. Polymerization of **1** catalyzed by chitinase Sm was also examined by varying reaction parameters of pH, enzyme amount, reaction temperature, and initial concentration of **1** (Table 3). With increasing pH value (entries 24−33), yield of **2** reached 62% at pH 8.0 (entry 27) and then drastically decreased to 7% at pH 10.5 (entry 32). At pH 11.0, the polymerization no longer proceeded (entry 33). M_n of **2**

Table 2. Chitinase Bs-Catalyzed Polymerization of **1** under Various Reaction Conditions

			polymerization[a]			polymer **2**		
entry	pH	enzyme, wt %	temp, °C	concn of **1**, mM	time,[b] h	yield,[c] %	M_n[d]	M_w[d]
5	6.5	5	30	100	0.2	28	1420	1510
6	7.0	5	30	100	0.2	38	1620	1770
7	7.5	5	30	100	1.5	38	1730	1930
8	8.0	5	30	100	1.5	40	1760	1970
9	8.5	5	30	100	2.5	38	1660	1810
10	9.0	5	30	100	3.5	34	1590	1710
11	9.5	5	30	100	4.0	35	1580	1680
12	10.0	5	30	100	11	42	1690	1810
13	10.5	5	30	100	42	56	1730	1870
14	11.0	5	30	100	72	17	1290	1330
15	10.5	1	30	100	168	23	1320	1350
16	10.5	10	30	100	20	52	1740	1870
17	10.5	15	30	100	13	49	1820	1960
18	10.5	20	30	100	7.0	34	1650	1780
19	10.5	15	10	100	48	36	1900	2050
20	10.5	15	20	100	24	37	1880	2040
21	10.5	15	40	100	6.0	36	1820	1970
22	10.5	15	30	50	12	35	1800	1940
23	10.5	15	30	200	15	50	1810	1960

[a] In 10 mM phosphate buffer (entries 5−10) and in 10 mM carbonate buffer (entries 11−23). [b] Time for the complete consumption of **1**. [c] Determined by HPLC measurements containing products with molecular weight higher than that of tetrasaccharide. [d] Determined by SEC calibrated with chitooligosaccharides and hyaluronan standards.

Table 3. Chitinase Sm-Catalyzed Polymerization of **1** under Various Reaction Conditions

			polymerization[a]			polymer **2**		
entry	pH	enzyme, wt %	temp, °C	concn of **1**, mM	time,[b] h	yield,[c] %	M_n[d]	M_w[d]
24	6.5	5	30	100	1.0	20	3010	3630
25	7.0	5	30	100	2.0	34	4180	5380
26	7.5	5	30	100	4.0	43	4120	5300
27	8.0	5	30	100	7.5	62	4070	4990
28	8.5	5	30	100	10	53	3990	4880
29	9.0	5	30	100	14	51	4000	4690
30	9.5	5	30	100	22	48	4010	4770
31	10.0	5	30	100	36	20	3900	4750
32	10.5	5	30	100	72	7	3930	5190
33	11.0	5	30	100	168	0		
34	8.0	1	30	100	48	37	3080	3530
35	8.0	10	30	100	2.5	45	3530	4210
36	8.0	15	30	100	1.5	35	3390	4080
37	8.0	5	10	100	27	43	3800	4490
38	8.0	5	20	100	15	59	3890	4530
39	8.0	5	40	100	7.0	46	3780	4570
40	8.0	5	30	50	6.0	34	3730	4500
41	8.0	5	30	200	11	55	3780	4500

[a] In 10 mM phosphate buffer (entries 24−29 and 34−41) and in 10 mM carbonate buffer (entries 30−33). [b] Time for the complete consumption of **1**. [c] Determined by HPLC measurements containing products with molecular weight higher than that of tetrasaccharide. [d] Determined by SEC calibrated with chitooligosaccharides and hyaluronan standards.

reached about 4000 (entries 25−32), excluding that in entry 24. Thus, polymer **2** was obtained effectively at pH 8.0 in terms of the yield, and the following reactions were carried out with the pH value fixed to 8.0. With 1, 10, and 15 wt % enzyme (entries 34−36), both yields and M_n of **2** decreased compared to those in entry 27. With increasing reaction temperature (entries 27, 37−39), reaction time was reduced, and yield and M_n of **2** reached maxima in entry 27. Initial concentration of **1** affected the polymer yield; polymer **2** was produced in a lower yield of 34% starting from 50 mM **1** (entry 40), and the yield reached the maximum of 62% with 100 mM **1** (entry 27) and then went down to 55% with 200 mM **1** (entry 41). Thus, polymer **2** was obtained most effectively under the conditions in entry 27.

Conclusion

In the present study, we demonstrated successfully the synthesis of alternatingly *N*-sulfonated chitin **2** via GH18 chitinase-catalyzed ring-opening polyaddition of a novel sugar oxazoline monomer **1**. This is the first example of a hybrid of natural (chitin) and unnatural (*N*-sulfonated chitosan) polysaccharides. The product polymer exhibited excellent solubility in aqueous media; the polymerization proceeded homogenously. Polymer **2** was obtained with different molecular weight distributions by appropriate use of enzyme; that is, chitinase Sm provided **2** with M_n about 4000, chitinase Bs produced **2** with M_n about 1700, and chitinase Sg afforded a tetrasaccharide of **2**. From the viewpoint of monomer structure, the sulfonamido

group in monomer **1** is sterically much bulkier and strongly anionic as the C-2′ substituent compared to that in other monomers employed for GH18 chitinase-catalyzed polymerizations. Therefore, the results obtained in this study strongly imply that the C-2′ position in the monomer is not deeply involved in the catalysis of GH18 chitinases, indicating a potential to create chitin derivatives alternatingly having various C-2 substituents via chitinase-catalyzed polymerization. An alternatingly *N*-sulfonated chitin derivative **2** prepared in this study for the first time is difficult to obtain by conventional chemical and biochemical methods. Thus the product polymer **2** is expected to become an important material for investigation of biological activities of *N*-sulfonate groups in chitin molecules at a molecular level.

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