Supporting Information

Catalytic Depolymerization of Chitin with Retention of *N*-Acetyl Group

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Experimental

Materials. Chitin, GlcNAc, H_2SO_4, methanol, distilled water, and diethyl ether were purchased from Wako Pure Chemical Industries. $(GlcNAc)_2$, $(GlcNAc)_3$, $(GlcNAc)_4$, and $(GlcNAc)_5$ were supplied by Tokyo Chemical Industry. α- and β-MeGlcNAc were obtained from Toronto Research Chemicals, and deuterium oxide was from Acros Organics.

Mechanocatalytic depolymerization of chitin. Chitin-H_2SO_4-BM was prepared as follows: chitin (5.0 g, 25 mmol based on number of GlcNAc unit) was dispersed in 15 mL of diethyl ether containing H_2SO_4 (0.30 g, 3.1 mmol, S/C = 8.1). After drying diethyl ether, 4.9 g of resulting powder was planetary ball-milled (Fritsch, Pulverisette 6) at 500 rpm using alumina balls (5 mm, 100 g) in a 250 mL of alumina pot. The milling time was 6 h with a 10 min interval after every 10 min of milling. The resulting powder contained 1.5 wt% of physisorbed water.

The solubility of a chitin sample was determined as follows. Chitin sample (430 mg, containing 406 mg of chitin and 24 mg of H_2SO_4) was added into 40 mL of distilled water. After stirring and sonication for 10 min, the suspension was filtered with a polytetrafluoroethylene (PTFE, 0.1 μm mesh) membrane. The solid phase was dried in an oven at 383 K overnight, and the solubility was calculated from the difference between weight of chitin sample used and that of dried residue. The water-soluble compounds were analyzed by high-performance liquid chromatography (HPLC, the conditions are shown below), LC/MS (Thermo Fischer Scientific, LCQ Fleet, the conditions are the same as those of HPLC), and NMR [JEOL, JNM-ECX-600, ^{1}H 600 MHz, ^{13}C 150 MHz, including ^{1}H NMR, proton-decoupled ^{13}C NMR, distortionless enhancement by polarization transfer (DEPT), ^{13}C–^{1}H heteronuclear multiple quantum coherence (HMQC), and ^{13}C–^{1}H heteronuclear multiple bond correlation (HMBC)]. The LC/MS and NMR spectra were shown in Figures S1 and S2. Chitin samples were also characterized by XRD (Rigaku, Ultima IV, CuKα, Figure S3). For the analysis of acetic acid, we used a Synergi 4 μm Hydro-RP 80Å column (Phenomenex, ø4.6 × 250 mm, mobile phase: 40 mM of potassium phosphate buffer solution 0.8 mL min^{-1}, 303 K); the yield of acetic acid was calculated from molar ratio of acetic acid produced to GlcNAc unit in chitin used.

Methanolysis of chitin. Chitin sample (430 mg, containing 406 mg of chitin and 24 mg of H_2SO_4) and methanol (40 mL) were charged into a SUS316 high-pressure reactor (OM-Lab Tech, MMJ-100, 100 mL). The temperature was raised from 298 K to 463 K in 16 min. After reaching the temperature, the reactor was rapidly cooled down to 298 K, named rapid heating-cooling condition (time course is shown in Figure S4). After the reaction, sorbitol (182 mg, 1.00 mmol) was added into the solution as an internal standard. The liquid and solid phases were separated by filtration using a PTFE membrane. Methyl acetate in liquid phase was quantified by GC (Shimadzu, GC-14B) with a ULBON HR-20M capillary column (Shinwa Chemical Industries, ø0.25 × 25 m, film thickness: 0.25 μm); the yield of methyl acetate was calculated from molar ratio of methyl acetate produced to GlcNAc unit in chitin used. After evaporating methanol, the reaction products were dissolved in 40 mL of distilled water. The solution was analyzed by HPLC [Shimadzu, LC10-AT*VP*, refractive index (RI) and ultraviolet (UV; 210 nm) detectors, equipped with a fraction collector] with a SUGAR SH-1011 column (Shodex, ø8 × 300 mm, mobile phase: H_2O 0.5 mL min^{-1}, 323 K) and a Rezex RPM-Monosaccharide Pb++ column (Phenomenex, ø7.8 × 300 mm, mobile phase: H_2O 0.6 mL min^{-1}, 343 K). We also conducted LC/MS, IR (PerkinElmer, Spectrum 100), NMR (JEOL, JNM-ECX-400, ^{1}H 400 MHz, ^{13}C 100 MHz), and elemental analysis to identify MeGlcNAc. The LC/MS, IR, and NMR spectra of MeGlcNAc are shown in Figures S6–S8.

Assignment of LC/MS analysis for water-soluble oligomers.
GlcNAc (M = $C_8H_{15}NO_6$): 204 ($[M + H - H_2O]^+$), 222 ($[M + H]^+$).

(GlcNAc)$_2$ (M = $C_{16}H_{28}N_2O_{11}$): 204 ($[M + H - C_8H_{15}NO_6]^+$), 407 ($[M + H - H_2O]^+$), 425 ($[M + H)^+$).

(GlcNAc)$_3$ (M = $C_{24}H_{41}N_3O_{16}$): 204 ($[M + H - C_{16}H_{28}N_2O_{11}]^+$), 407 ($[M + H - C_8H_{15}NO_6]^+$), 610 ($[M + H - H_2O]^+$), 628 ($[M + H]^+$).

(GlcNAc)$_4$ (M = $C_{32}H_{54}N_4O_{21}$): 204 ($[M + H - C_{24}H_{41}N_3O_{16}]^+$), 407 ($[M + H - C_{16}H_{28}N_2O_{11}]^+$), 610 ($[M + H - C_8H_{15}NO_6]^+$), 813 ($[M + H - H_2O]^+$), 831 ($[M + H]^+$).

(GlcNAc)$_5$ (M = $C_{40}H_{67}N_5O_{26}$): 204 ($[M + H - C_{32}H_{54}N_4O_{21}]^+$), 407 ($[M + H - C_{24}H_{41}N_3O_{16}]^+$), 610 ($[M + H - C_{16}H_{28}N_2O_{11}]^+$), 813 ($[M + H - C_8H_{15}NO_6]^+$), 1016 ($[M + H - H_2O]^+$), 1034 ($[M + H]^+$).

(GlcNAc)$_6$ (M = $C_{48}H_{80}N_6O_{31}$): 204 ($[M + H - C_{40}H_{67}N_5O_{26}]^+$), 407 ($[M + H - C_{32}H_{54}N_4O_{21}]^+$), 610 ($[M + H - C_{24}H_{41}N_3O_{16}]^+$), 813 ($[M + H - C_{16}H_{28}N_2O_{11}]^+$), 1016 ($[M + H - C_8H_{15}NO_6]^+$), 1219 ($[M + H - H_2O]^+$), 1238 ($[M + H]^+$).

(GlcNAc)$_7$ (M = $C_{56}H_{93}N_7O_{36}$): 204 ($[M + H - C_{48}H_{80}N_6O_{31}]^+$), 407 ($[M + H - C_{40}H_{67}N_5O_{26}]^+$), 610 ($[M + H - C_{32}H_{54}N_4O_{21}]^+$), 813 ($[M + H - C_{24}H_{41}N_3O_{16}]^+$), 1016 ($[M + H - C_{16}H_{28}N_2O_{11}]^+$), 1220 ($[M + H - C_8H_{15}NO_6]^+$), 1422 ($[M + H - H_2O]^+$), 1442 ($[M + H]^+$).

Assignment of NMR, IR, LC/MS, and elemental analysis for MeGlcNAc.
α-MeGlcNAc (standard). ^{1}H NMR (400 MHz, D_2O): δ 4.74 (d, *J* = 3.6 Hz, 1H, *H*–C1), 3.90 (dd, *J* = 11.2, 3.6 Hz, 1H, *H*–C2), 3.87 (dd, *J* = 12.4, 2.4 Hz, 1H, *H*–C6), 3.77 (dd, *J* = 12.4, 5.6 Hz, 1H, *H*–C6), 3.70 (dd, *J* = 10.4, 9.2 Hz, 1H, *H*–C3), 3.66 (ddd, *J* = 9.6, 5.6, 2.4 Hz, 1H, *H*–C5), 3.46 (dd, *J* = 10.0, 9.2 Hz, 1H, *H*–C4), 3.37 (s, 3H, *H*–C9), 2.02 (s, 3H, *H*–C7); ^{13}C NMR (100 MHz, D_2O): δ 175.4 (C, C8), 99.0 (CH, C1), 72.6 (CH, C5), 72.1 (CH, C3), 70.9 (CH, C4), 61.5 (CH_2, C6), 56.1 (CH_3, C9), 54.5 (CH, C2), 22.8 (CH_3, C7); IR (KBr pellet, cm^{-1}): 3393 [ν(O–H)], 3296 [ν(N–H, amide)], 2803–3027 [ν(C–H, alkyl)], 2954 [ν(C–H, alkyl)], 2932 [ν(C–H, alkyl)], 2904 [ν(C–H, alkyl)], 1650 [ν(C=O, amide I)], 1554 [δ(N–H, amide II)]; LC/MS (*m*/*z*): $[M + H]^+$ calcd. for $[C_9H_{17}NO_6 + H]^+$, 236; found, 236.

β-MeGlcNAc (standard). ^{1}H NMR (400 MHz, D_2O): δ 4.43 (d, *J* = 8.4 Hz, 1H, *H*–C1), 3.92 (dd, *J* = 12.4, 2.0 Hz, 1H, *H*–C6), 3.73 (dd, *J* = 12.4, 5.4 Hz, *H*–C6), 3.67 (dd, *J* = 10.4, 8.8 Hz, *H*–C2), 3.53 (d, *J* = 8.0 Hz, *H*–C3), 3.49 (s, 3H, *H*–C9), 3.39–3.47 (m, 2H, *H*–C4 and *H*–C5), 2.02 (s, 3H, *H*–C7); ^{13}C NMR (100 MHz, D_2O): δ 175.7 (C, C8), 102.9 (CH, C1), 76.9 (CH, C5), 74.9 (CH, C3), 70.9 (CH, C4), 61.7 (CH_2, C6), 58.0 (CH_3, C9), 56.4 (CH, C2), 23.1 (CH_3, C7); IR (KBr pellet, cm^{-1}): 3370 [ν(O–H)], 3292 [ν(N–H, amide)], 2790–3027 [ν(C–H, alkyl)], 1657 [ν(C=O, amide I)], 1554 [δ(N–H, amide II)]; LC/MS (*m*/*z*): $[M + H]^+$ calcd. for $[C_9H_{17}NO_6 + H]^+$, 236; found, 236.

MeGlcNAc (produced by methanolysis of chitin). Elemental analysis (calcd., found for $C_9H_{17}NO_6$): C (45.95, 45.74), H (7.28, 7.21), N (5.96, 5.96), O (40.81, 41.10); LC/MS (*m*/*z*): $[M + H]^+$ calcd. for $[C_9H_{17}NO_6 + H]^+$, 236; found, 236. The IR, ^{1}H NMR, and DEPT spectra indicated that this MeGlcNAc is a mixture of α- and β-MeGlcNAc in 5.0 : 1 (see Figures S6–S8).

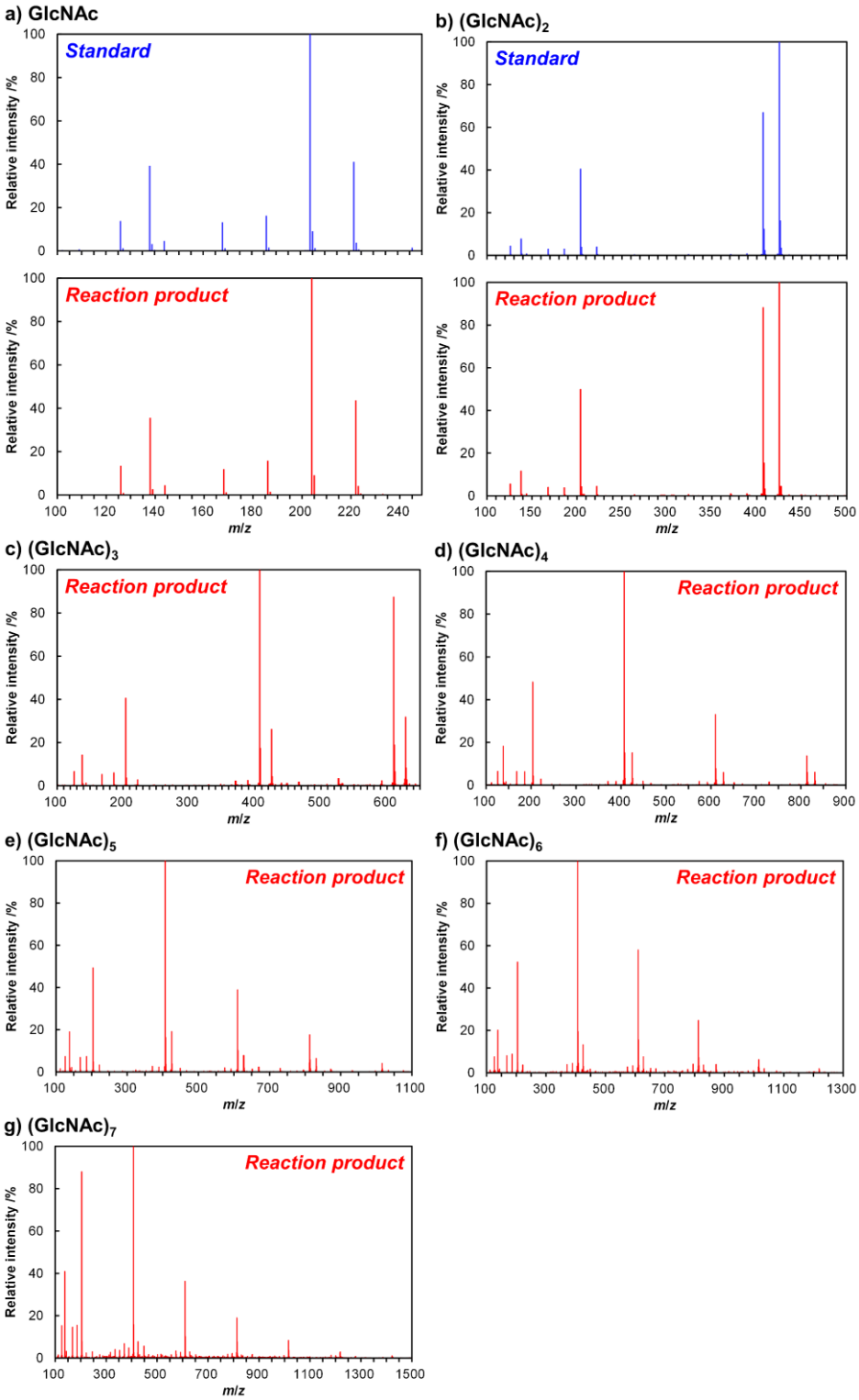


Figure S1. LC/MS spectra of reaction products of mechanocatalytic depolymerization, recorded by positive ion mode. a) GlcNAc, b) $(GlcNAc)_2$, c) $(GlcNAc)_3$, d) $(GlcNAc)_4$, e) $(GlcNAc)_5$, f) $(GlcNAc)_6$, and g) $(GlcNAc)_7$. A SUGAR SH-1011 column was used. The assignment of peaks is summarized above.

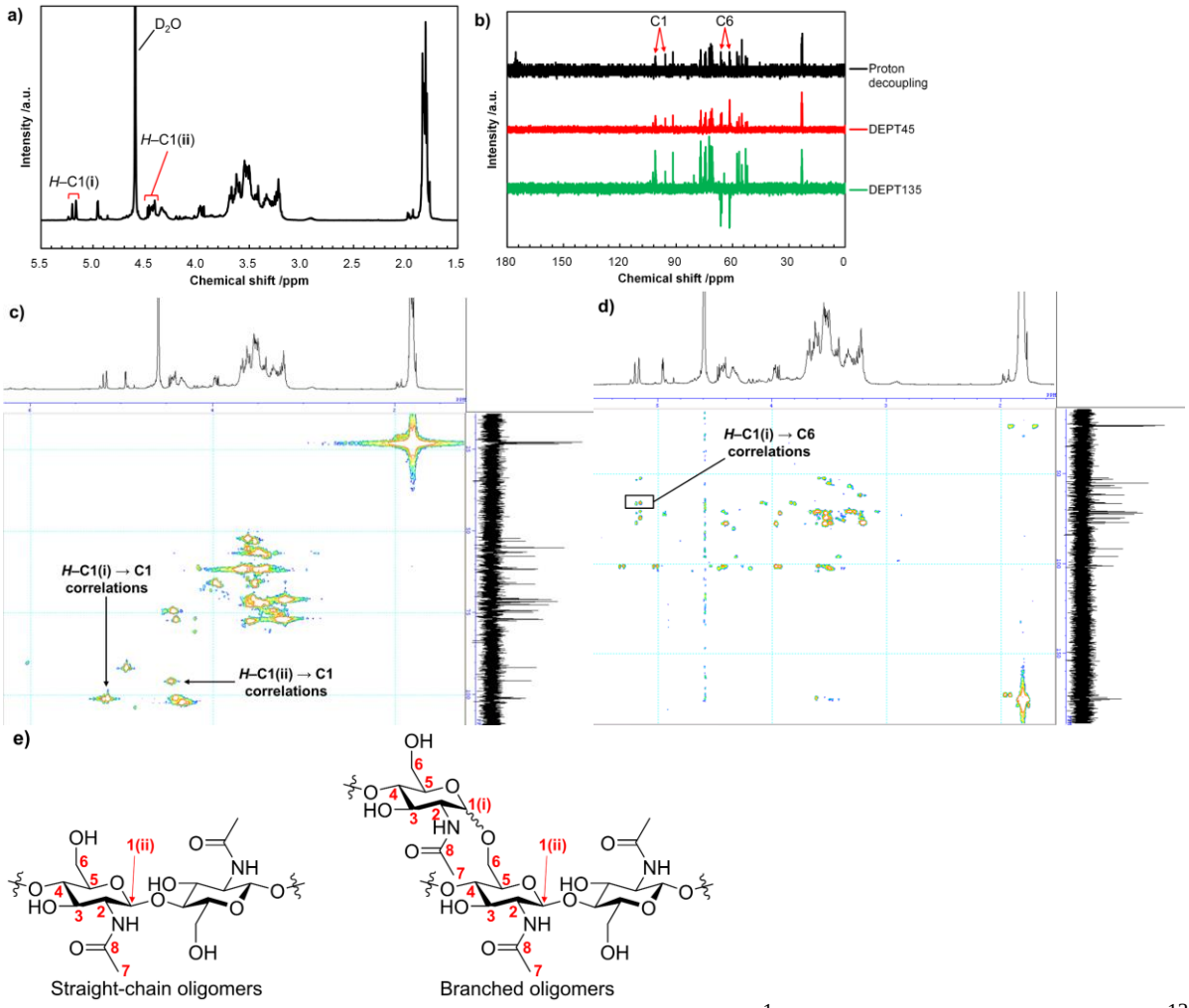


Figure S2. NMR spectra of Chitin-H_2SO_4-BM in D_2O. a) ^1H NMR, b) proton-decoupled ^{13}C NMR and DEPT, c) ^{13}C–^1H HMQC (horizontal axis: ^1H, vertical axis: ^{13}C), d) ^{13}C–^1H HMBC (horizontal axis: ^1H, vertical axis: ^{13}C), and e) possible structures of oligomers contained in Chitin-H_2SO_4-BM.

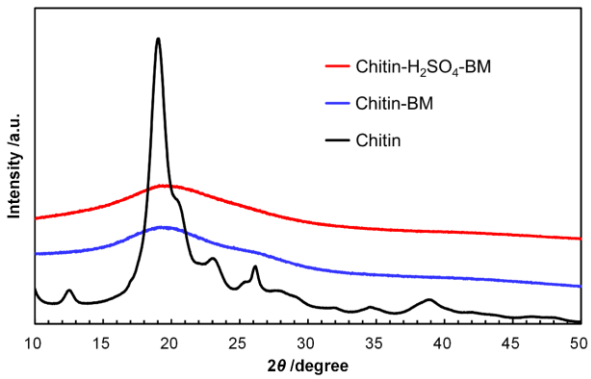


Figure S3. XRD patterns of chitin samples. The sharp peak at 19.8° is derived from the crystalline structure of chitin.[S1] Ball-milling chitin with/without H_2SO_4, corresponding to Chitin-H_2SO_4-BM/Chitin-BM, caused amorphization.

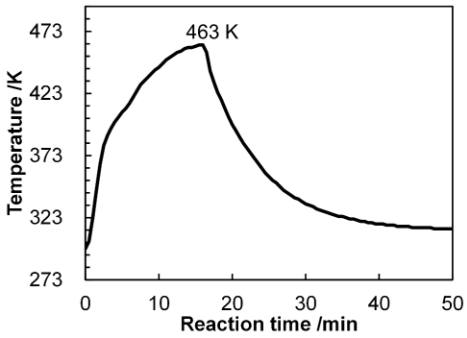


Figure S4. Temperature profile of rapid heating-cooling condition for solvolysis.

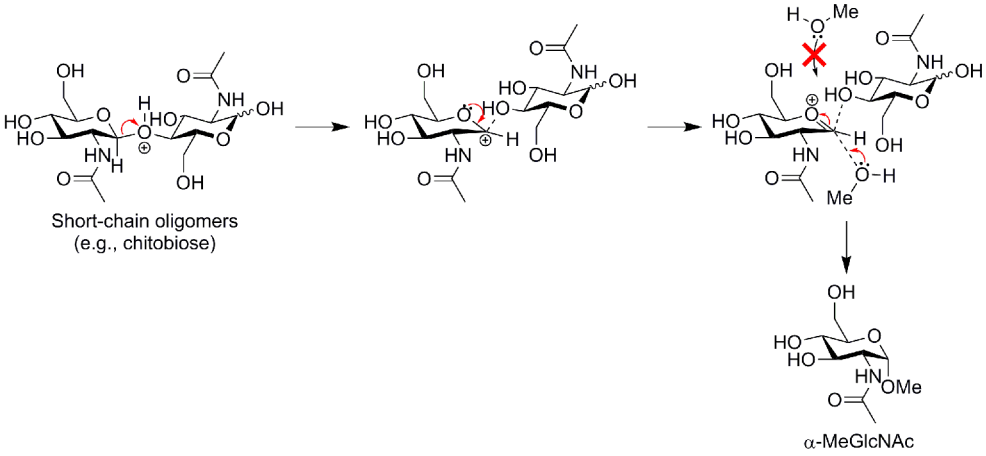


Figure S5. Proposed reaction mechanism of methanolysis to produce MeGlcNAc via oxocarbenium intermediates. Due to the presence of leaving group, the nucleophilic attack from the upper side is limited.

Reaction mechanism that provides α-MeGlcNAc in methanolysis of chitin is discussed (Figure S5). The ratio of α- to β-anomers of MeGlcNAc was 5.0 in our methanolysis reaction, as determined by ^{1}H NMR (Figure S8). This result shows an inversion of stereochemistry of chitin, which originally has β-1,4-glycosidic bond. The solvolysis of glycosidic bonds generally takes place by an S_N1 mechanism via oxocarbenium intermediates (Figure S5) except for enzymatic hydrolysis.[S2] The intermediates having sp^2 carbon lose the stereochemistry, possibly giving a mixture of α- and β-anomer products. However, in our reaction, a high concentration of nucleophile [methanol; 18 M at 463 K[S3]] probably attacks oxocarbenium ion from opposite side of leaving group (counterpart of chitin oligomer) before its diffusion.[S4] Possibility of anomeric effect can be excluded, since we have verified that both inversions of α to β and β to α take place in methanolysis reactions using related sugar compounds (Table S2).

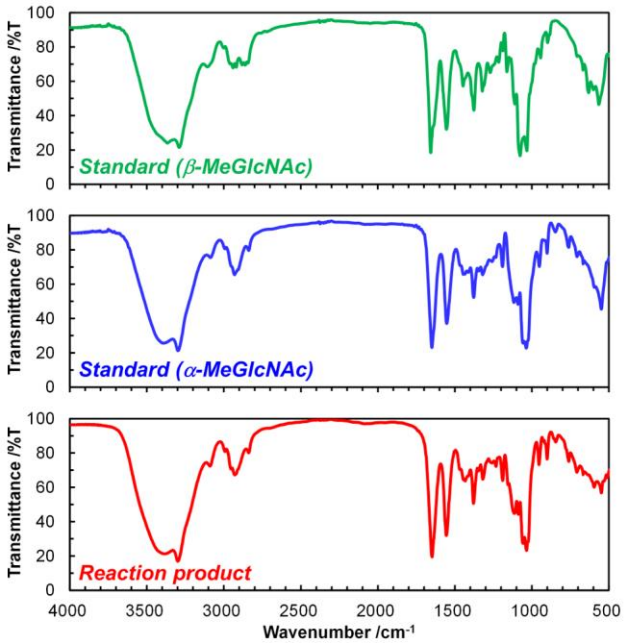


Figure S6. IR spectra of standard MeGlcNAc and the product that we identified as MeGlcNAc in the methanolysis of chitin. Transmission mode, KBr disk. The assignment of peaks is summarized above. The IR spectrum of reaction product is very similar to that of α-MeGlcNAc, since the ratio of α to β is 5.0 in the product (see Figure S8).

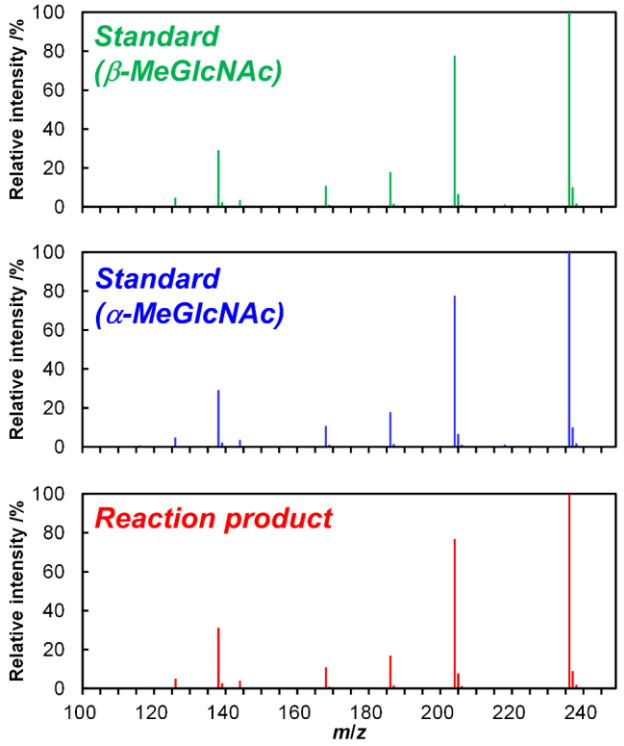


Figure S7. LC/MS spectra of MeGlcNAc, recorded by positive ion mode. A SUGAR SH-1011 column was used. The assignment of peaks is summarized above.

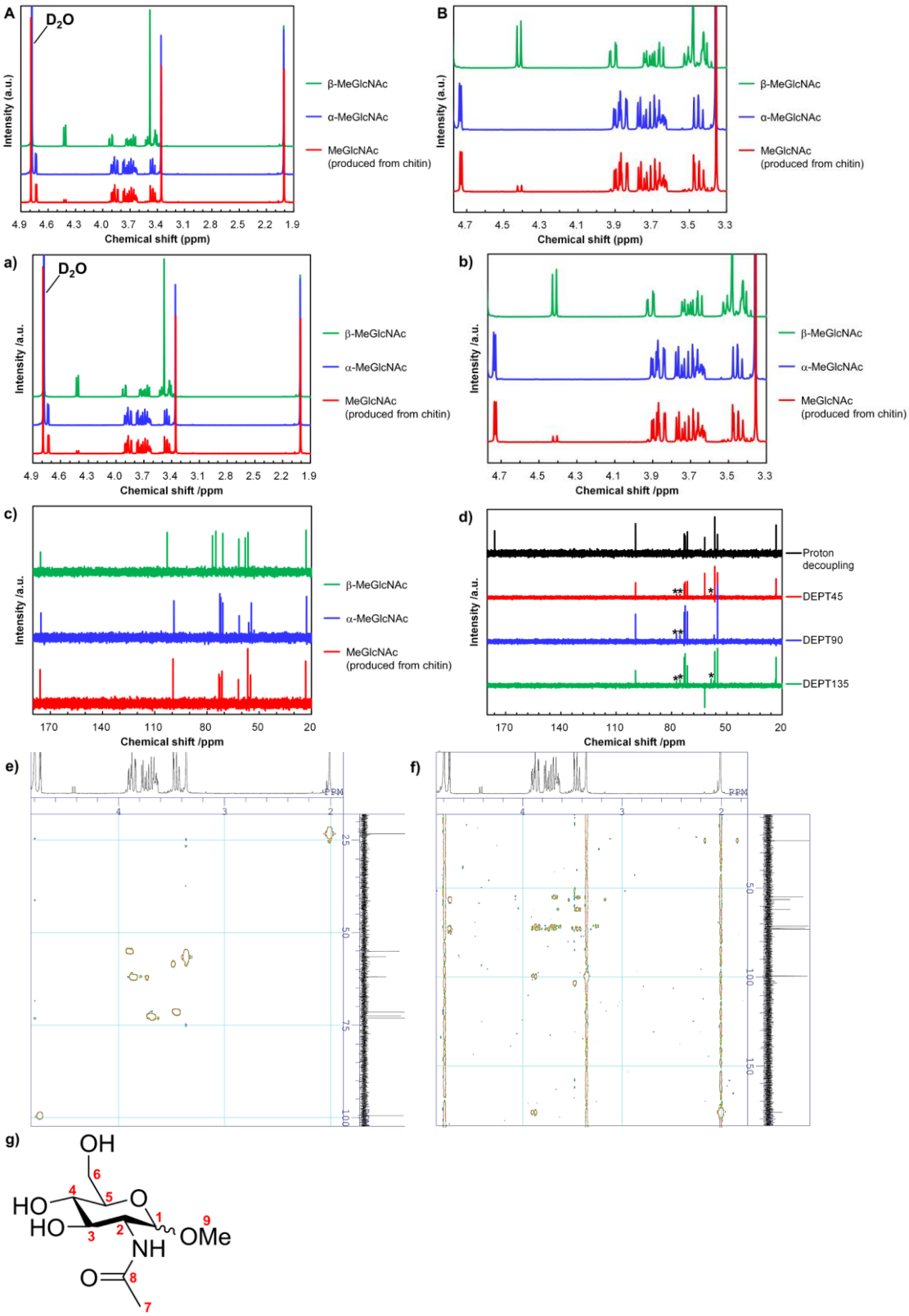


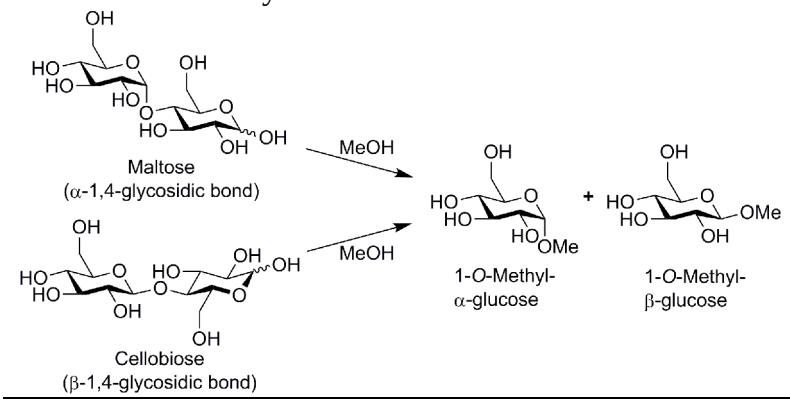
Figure S8. NMR spectra of MeGlcNAc produced from chitin in D_2O. a) ^{1}H NMR, b) expanded view of the ^{1}H NMR spectum (3.30–4.77 ppm), c) proton-decoupled ^{13}C NMR (comparison with standard samples), d) proton-decoupled ^{13}C NMR and DEPT, e) ^{13}C–^{1}H HMQC (horizontal axis: ^{1}H, vertical axis: ^{13}C), f) ^{13}C–^{1}H HMBC (horizontal axis: ^{1}H, vertical axis: ^{13}C), and g) structure of MeGlcNAc. The assignment of ^{1}H and ^{13}C NMR peaks is summarized above. Small peaks labeled as * in DEPT spectra are derived from β-MeGlcNAc. The ^{1}H NMR and DEPT spectra indicated that both α- and β-MeGlcNAc are produced by methanolysis of chitin. Based on the ^{1}H NMR spectrum, the ratio of α- to β-MeGlcNAc is 5.0.

Table S1. Stability test of monomers.

Entry	Monomer	Recovery (%)
S1[a]	GlcNAc	17
S2[b]	MeGlcNAc	94

[a] GlcNAc 442 mg (2.00 mmol), distilled water 40 mL, 463 K, rapid cooling-heating condition (see Figure S4). [b] MeGlcNAc 470 mg (2.00 mmol), methanol 40 mL, 463 K, rapid cooling-heating condition (see Figure S4).

Table S2. Methanolysis of disaccharides.[a]



Entry	Substrate	Yield (%)	
		1-*O*-Methyl-α-glucose	1-*O*-Methyl-β-glucose
S3[b]	Maltose	9.2	21
S4[c]	Cellobiose	46	23

[a] Substrate 342 mg (1 mmol), sulfuric acid 24 mg (0.24 mmol), methanol 40 mL. [b] 393 K, rapid heating-cooling condition (see Figure S4). [c] 413 K, rapid heating-cooling condition (see Figure S4).

References

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