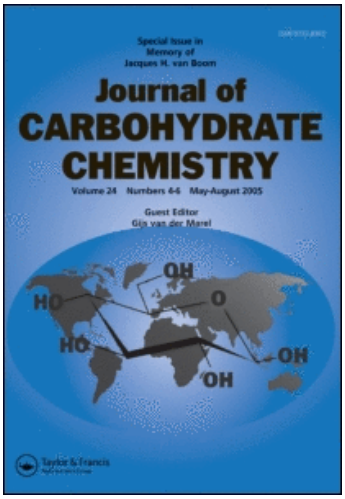


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

N-Acetylglucosaminyl Disaccharide and Trisaccharide Formation Through Lysozyme-Catalyzed Transfer Reaction

Yoshiharu Matahira[a]; Kyoko Ohno[b]; Mitsuaki Kawaguchi[b]; Hirokazu Kawagishi[b]; Taichi Usui[b]
[a] United Graduate School of Agricultural Science, Gifu University (Shizuoka University), Shizuoka, Japan [b] Department of Applied Biochemistry, Faculty of Agriculture, Shizuoka University, Shizuoka, Japan

To cite this Article Matahira, Yoshiharu , Ohno, Kyoko , Kawaguchi, Mitsuaki , Kawagishi, Hirokazu and Usui, Taichi(1995) 'N-Acetylglucosaminyl Disaccharide and Trisaccharide Formation Through Lysozyme-Catalyzed Transfer Reaction', Journal of Carbohydrate Chemistry, 14: 2, 213 — 225
To link to this Article: DOI: 10.1080/07328309508002064
URL: http://dx.doi.org/10.1080/07328309508002064

J. CARBOHYDRATE CHEMISTRY, 14(2), 213-225 (1995)

N-ACETYLGLUCOSAMINYL DISACCHARIDE AND TRISACCHARIDE FORMATION THROUGH LYSOZYME-CATALYZED TRANSFER REACTION

Yoshiharu Matahira,[a] Kyoko Ohno,[b] Mitsuaki Kawaguchi,[b] Hirokazu Kawagishi[b] and Taichi Usui[b]*

[a]United Graduate School of Agricultural Science, Gifu University (Shizuoka University), Ohya 836, Shizuoka 422, Japan
[b]Department of Applied Biochemistry, Faculty of Agriculture, Shizuoka University, Ohya 836, Shizuoka 422, Japan

Received June 23, 1994 - Final Form October 14, 1994

ABSTRACT

A hen egg-white lysozyme produced regioselectively 4-*O*-(2-acetamido--2-deoxy-β-D-glucopyranosyl)-D-mannose and *p*-nitrophenyl 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-mannopyranoside through a transglycosylation reaction from *N,N'*-diacetylchitobiose and respectively mannose and *p*-nitrophenyl β-D-mannopyranoside. These enzyme reactions were efficient enough to allow the one-pot preparation of the desired disaccharide. When *p*-nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranoside was the acceptor, the enzyme catalyzed the formation of a β-(1-3)-linked disaccharide glycoside (*p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranoside) with its β-(1-4)-linked isomer. This is also the case for the formation of *p*-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-maltoside with *p*-nitrophenyl α-maltoside acceptor. The results show that the anomeric configuration of the glycosidic linkage in the glycosyl acceptors had a pronounced effect on the position of transglycosylation.

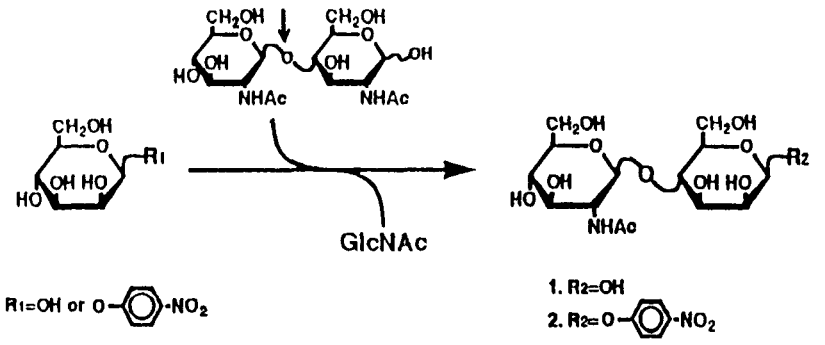
INTRODUCTION

It is well known that hen egg-white lysozyme [EC. 3.2.1.17] splits the polysaccharide chain of chitin and bacterial cell wall mucopeptides, both of which have β-(1-4)-2-acetamido-2-deoxy-D-glucopyranosidic linkages as their basic structures. The lysozyme has been also shown to catalyze a reaction of the oligosaccharides consisting either of alternating β-(1-4)-linked *N*-acetylmuramic acid and GlcNAc residues, with formation of higher oligosaccharides.[1] We have recently reported that the transferase activity of the lysozyme can be used for the preparative-scale synthesis of *N,N',N'',N''',N'''',N'''''*-hexaacetylchitohexaose $(GlcNAc)_6$ and *N,N',N'', N''',N'''',N''''',N'''''''*-heptaacetylchitoheptaose $(GlcNAc)_7$ from *N,N'*-di-acetylchitobiose $(GlcNAc)_2$.[2] Those results indicated that the enzyme is capable of transferring the *N*-acetylglucosaminyl group of $(GlcNAc)_2$ to acceptor molecule, although it has been shown that the dimer is known to be a competitive inhibitor of lysozyme.[3] Further study of this mode of oligosaccharide synthesis through a lysozyme-catalyzed transfer reaction was undertaken by using various glycoside acceptors. Our interest was also directed to an enzymatic approach to *N*-acetylglucosaminyl disaccharide units involved in glycoconjugate.

This report details an efficient synthesis of β-D-GlcNAc-(1-4)-D-Man and its disaccharide glycoside by utilizing hen egg-white lysozyme-catalyzed transglycosylation and also the capacity of the enzyme to catalyze the (1-3)-linked *N*-acetylglucosaminyl oligosaccharide with its (1-4)-linked isomer by using α-glycosyl acceptors.

RESULTS AND DISCUSSION

Enzymatic synthesis of 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose (1) and *p*-nitrophenyl 4-*O*-(2-acetamido-2-deoxy-β-D-gluco-pyranosyl)-β-D-mannopyranoside (2). The present study shows that the commercially available lysozyme can be used to produce the disaccharide **1** and its glycoside **2** with the desired β-(1-4)-linkage. Thus, the enzyme is capable of transferring an *N*-acetylglucosaminyl group from $(GlcNAc)_2$ exclusively to the 4-OH of mannose moiety through a route as in Scheme I.



Scheme 1

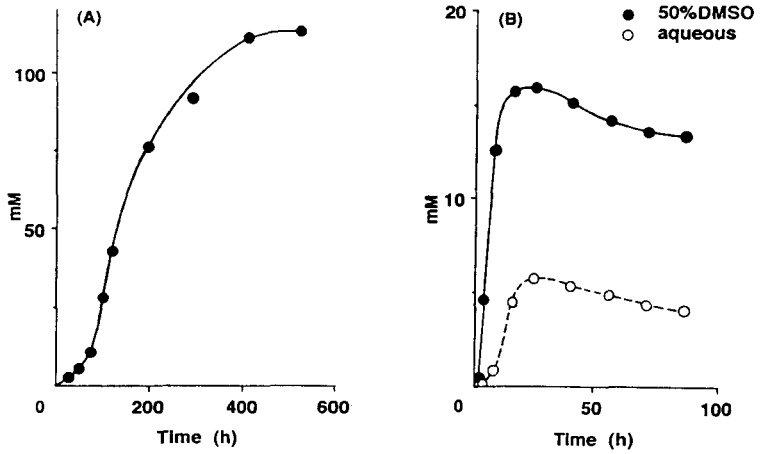
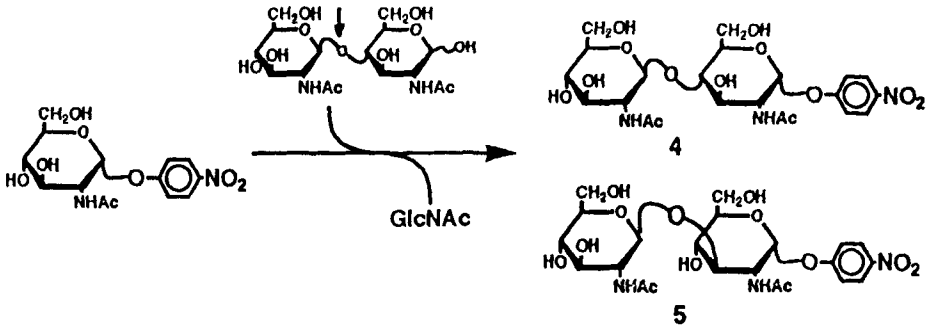


Fig. 1. Time course of lysozyme-mediated production. **A**, compound **1** production from $(GlcNAc)_2$ and mannose. **B**, compound **2** production from $(GlcNAc)_2$ and β-D-Man-OPhNO_2-*p* in an aqueous-organic system containing 50 % DMSO and in a reaction medium in the absence of DMSO. The amounts of products produced as a function of a time were examined on the 0.5 mL scale.

Compound **1** was obtained in a yield of 20.9 % based on the $(GlcNAc)_2$ acceptor added. The time-course of **1** production from $(GlcNAc)_2$ and mannose was examined by HPLC as in **Fig. 1A**. Formation of **1** was much slower and the time for its maximal production was ~500 h. When the acceptor was *N,N',N",N"'*-tetraacetylchitotetraose $(GlcNAc)_4$ instead of

$(GlcNAc)_2$, the time at which maximum concentration of **1** production was reached was ~80 h (data not shown). However, the present work is directed toward synthesizing **1** from a lower oligomer such as $(GlcNAc)_2$, obtainable enzymatically[4] or chemically [5, 6] in substantial quantities from low-price chitin. An increase of the molar ratio of the acceptor to donor was suited for the effective synthesis of **1**. When the molar ratio of $(GlcNAc)_2$ and mannose was changed from 1 : 2 to 1 : 4, the maximum concentration of **1** proposed was a 3-fold difference. When *p*-nitrophenyl β-D-mannopyranoside (β-D-Man-$OPhNO_2$-*p*) was the acceptor, compound **2** was obtained in a yield of 10.5 % based on the $(GlcNAc)_2$ added. In this case, the efficiency of lysozyme-catalyzed transglycosylation was greatly influenced by the solubility of the acceptor molecule in an aqueous-DMSO (dimethylsulfoxide) system. Thus, β-D-Man-$OPhNO_2$-*p* shows higher solubility (3%) in a medium containing 50 % DMSO than that (1 %) in the medium in the absence of DMSO. The maximum concentration of **2** production at 50 % DMSO was about 3 times of that in a reaction medium in the absence of DMSO (**Fig. 1B**). The effects of temperature on the transglycosylation were also examined. The maximum rate of **2** production increased markedly with rising temperature. The times for maximum **2** production at 30, 40, and 50 ℃ were ~ 240, 100, and 20 h, respectively. At 50 ℃ , once formation of **2** reached its maximum, the amount decreased significantly during the subsequent reaction. Furthermore, the efficiency of the transglycosylation was dependent on the molar ratio of β-D-Man-$OPhNO_2$-*p* acceptor to $(GlcNAc)_2$ donor. When the molar ratio of the acceptor to donor substrates was 1 : 2, it was best suited for efficient synthesis of **1**. Mannose and β-D-Man-$OPhNO_2$-*p* function as suitable acceptors for GlcNAc transfer and their yields (10-21 %) are sufficiently high to make the method attractive. The higher yield of product from mannose acceptor than from its β-glycoside might reflect a difference in solubility in the reaction system.[7] This enzymatic method not only provides a useful technique for the preparation of **1**, but also for that of chromogenic substance **2**.

Acceptor effect on lysozyme-mediated oligosaccharide glycoside formation from $(GlcNAc)_2$ donor. The regioselectivity of lysozyme-catalyzed formation of disaccharide glycoside is changed by using the corresponding α-glycoside acceptor. Thus, when *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (β-D-GlcNAc-$OPhNO_2$-*p*) was an



Scheme 2

acceptor, the enzymatic reaction gave exclusively the β-(1-4)-linked disaccharide glycoside (*p*-nitrophenyl 2-acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranoside, **3**) in a yield of 6.5 % based on the GlcNAc added. On the other hand, when *p*- nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranoside (α-D-GlcNAc- $OPhNO_2$-*p*) was the acceptor, the β-(1-3)-linked product (*p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranoside, **4**) is formed along with the β-(1-4)-linked isomer (*p*-nitrophenyl 2-acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranosyl)-α-D-glucopyranoside, **5**) as in Scheme 2. The yields of isomers **4** and **5** were 0.6 and 1.9 % based on the $(GlcNAc)_2$ added, respectively. This is also the case for the formation of β-(1-3)-linked product (*p*-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-maltoside **6**) with *p*-nitrophenyl α-maltoside acceptor. In this case, compound **6** predominated as its β-(1-4)-linked isomer (*p*-nitrophenyl 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-maltoside, **7**) during the entire course of reaction (data not shown) and the yields of isomers **6** and **7** were 8.3 and 4.2 % based on the acceptor added, respectively. The results suggest that the formation of β-(1-3) linkage is not only effected by configurational change of the aglycon moiety in the glycosyl acceptor, but also the chemical properties. Thus, the enzyme can be used to produce the (1-3)-linked *N*-acetylglucosaminyl oligosaccharide by using α-glycosyl acceptor. With α-D-GlcNAc-$OPhNO_2$-*p* acceptor, a significant lower yield than from the corresponding β-anomer was obtained, which could be attributed to the decrease in substrate affinity toward the enzyme subsite. GalNAc, *p*-nitrophenyl 2-acetamido-2-deoxy-

β-D-galactopyranoside and *p*-nitrophenyl α-D-mannopyranoside did not serve as acceptors under the present conditions. An interesting result from these studies is the formation of the β-(1-3)-linked *N*-acetylglucosaminyl-disaccharide and trisaccharide by lysozyme. We have already reported that a maltotetraose-forming amylase from *Pseudomonas stutzeri* transfers a maltotetraosyl group from maltopentaose exclusively to the 4-position of *p*-nitrophenyl α- and β-D-glucopyranoside acceptors, regardless of the anomeric configuration.[7,8] Nilsson has demonstrated that the regioselectivity of glycosidase-catalyzed formation of disaccharide could be changed by using α- or β-glycosyl acceptors with various acceptors.[9,10] While glycosidases do exhibit some regioselectivity for particular hydroxyl groups on an acceptor, this selectivity is less predictable and lower than that of such polysaccharide hydrolases as amylase,[7,8,11] cellulase,[12] β-mannanase,[13] and lysozyme.[2,3,14,15]

In conclusion, we have developed a practical route for the synthesis of **1** and its glycoside **2**, whose disaccharide sequence is a structural unit of *O*- and *N*-linked glycoproteins, by transglycosylation employing hen egg-white lysozyme. Further transglycosylation by the enzyme led to the formation of β-(1-3)-linked *N*-acetylglucosaminyl disaccharide and trisaccharide with β- (1-4)-linked isomers, by using α-glycoside acceptors.

EXPERIMENTAL

General Procedures. HPLC was performed with a YMC packed column Type AQ-312 (ODS) (6 mm i.d. × 15 cm) and an Asahipak packed column NH2P-50 (4.6 mm i.d. × 25 cm) in a Shimadzu LC-6A liquid chromatograph equipped with a SPD-6A ultraviolet detector. Elution of the latter was with water/acetonitrile (1 : 3, v/v). The flow rate was 0.8 mL/min at a pressure of 60 Kg/cm^2. ^{13}C NMR was determined with a JEOL EX-270 spectrometer, operating for ^{13}C at 67.5 MHz in the pulsed Fourier-transform mode with computer proton decoupling, and for ^{1}H at 270 MHz. Chemical shifts are expressed in ppm relative to 3-(trimethylsilyl) propanesulfonate sodium salt (TPS) as an internal standard. The FAB-MS spectra of oligosaccharides were recorded with a JEOL DX-303 HF mass spectrometer, operating at the full accelerating potential (3 KV) and coupled to a JEOL DX-500 mass data system. The sample in distilled water was

added to the glycerol matrix. The molecular weight of each sample was estimated from the *m/z* value of the quasimolecular-ion $(M + H)^+$ peak. Specific rotations were determined with a digital automatic polarimeter PM-101 apparatus (Union Giken Corp., Ltd).

Materials. The hen egg-white lysozyme used in the present experiments was a preparation that had been recrystallized six times (lot F83Z05) and was obtained from Seikagaku Kogyo. $(GlcNAc)_2$ and $(GlcNAc)_4$ was kindly provided from Yaizu Suisan Kagaku Industry. The charcoal-Celite used in the separation of transglycosylation products was prepared as follows: equal parts by weight of dry charcoal and Celite were slurried in water and packed into a glass column. All other chemicals were obtained from commercial sources.

Enzyme Assay. Lysozyme activity was assayed with ethylene glycol chitin (Seikagaku Kogyo) as a substrate. One mL of 50 mM acetate buffer (pH 5.5) containing 1 mg of the substrate and an appropriate amount of the enzyme was incubated at 40 ℃ for 20 min. The amount of reducing sugars produced were measured by a modification of Schales method.[16] One unit of the activity was defined as the amount of the enzyme releasing 1 μ mol of *N*-acetylglucosamine per min from the substrate.

Preparation of 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose (1). To a solution of $(GlcNAc)_2$ (4.1 g) and mannose (7 g) in 27.4 mL of 0.1 M acetate buffer (pH 4.5) was added lysozyme (370 U, 820 mg). The molar ratio of the former and latter compounds was 1 : 4 and the total substrate concentration was 40 %. After the mixture was incubated for 20 days at 40 ℃, the insoluble portion was removed by centrifugation. The solution was directly loaded onto a charcoal-Celite column as in **Fig. 2A**. The column was first eluted with water (1 L) and then with a linear gradient of 0 (2.5 L)-30 % (2.5 L) ethanol. The elution was monitored by measuring the absorbance at 210 nm (characteristic absorption of *N*-acetyl group) and at 485 nm (carbohydrate content, determined by the phenol-sulfuric acid method[17]). The chromatogram shows that peak F-1 (tubes 154-175), for which the absorption at 210 nm coincides with that at 485 nm, is contaminated by $(GlcNAc)_2$ judging from the overlapping of the absorption at 210 nm. Fraction 1 was concentrated to low volume (10 mL), and the 1/5 vol of the solution was applied to a Bio-Gel P-2 column as in **Fig. 2B**. The remaining aliquots were similarly treated. The F-1' fraction, eluted as

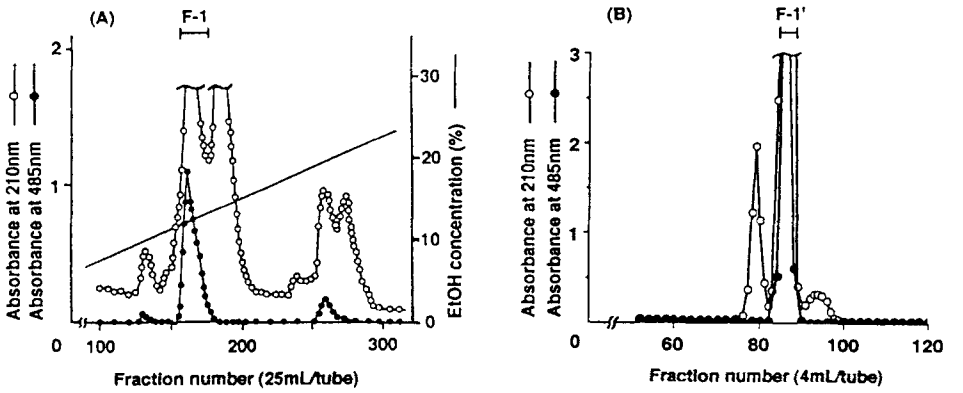


Fig. 2. Chromatographic separation of transglycosylation product by the action of lysozyme on $(GlcNAc)_2$ and mannose. **A**, chromatography of carbohydrate was carried out on a column (4.0 × 72 cm) of charcoal-Celite. **B**, further chromatography was performed on a column (2.6 × 95 cm) of Bio-Gel P-2 at 60 °C.

sharp peak (tubes 84-88), was combined, concentrated, and lyophilized to give compound **1** (857 mg). Compound **1** had $[\alpha]_D^{25} = +15.5°$ (*c* 1, H_2O) and *m/z* 384. NMR data (D_2O): δ ^1H, 5.14 (d, $J_{1,2}<1$ Hz, H-1α), 4.87 (d, $J_{1,2}<1$ Hz, H-1β), 4.53 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1') and 2.04 (s, 3H, Ac); ^{13}C, δ 177.36 (C=O of Ac), 104.35 (C-1'β), 104.28 (C-1'α), 96.49 (C-1α),96.37 (C-1β), 80.38 (C-4α), 79.96 (C-4β), 78.67 (C-5'), 77.45 (C-5β), 76.24 (C-3'), 74.59 (C-3β), 73.46 (C-5α), 73.39 (C-2β), 72.96 (C-2α), 72.56 (C-4'), 71.81 (C-3α), 63.41 (C-6'), 63.18 (C-6) and 58.36 (C-2').

Preparation of of *p*-nitrophenyl-4-*O*-(2-acetamido-2-deoxy-β-D-gluco-pyranosyl)-β-D-mannopyranoside (2). To a solution of $(GlcNAc)_2$ (511 mg) and β-D-Man-OPhNO_2-*p* (182 mg) in 3.5 mL of 0.2 M acetate buffer (pH 4.5) containing 50 % DMSO (3.5 mL) was added lysozyme (95 U, 210 mg). After the mixture was incubated for 21 h at 50 °C , the insoluble material was removed by centrifugation. The supernatant was directly loaded onto a Toyopearl HW-40S column. The elution pattern is shown in **Fig. 3**. The elution was monitored by measuring the absorbance at 300 nm (*p*-nitrophenyl group) and at 210 nm. The chromatogram showed one main peak (F-2; tubes 67-73) with one minor peak (F-1; 55-60) as transglycosylation products for which the absorption at 310 nm coincides with that at 210 nm. The F-2 fraction, eluted as a sharp peak, were combined, measuring the

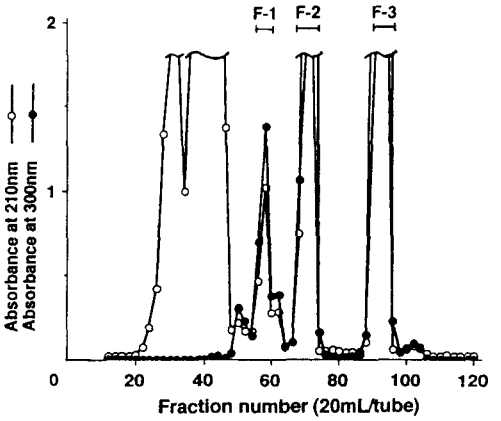


Fig. 3. Chromatographic separation of transglycosylation product by the action of lysozyme on $(GlcNAc)_2$ and β-D-Man-$OPhNO_2$-*p*. Chromatography was carried out on a column (4.5×90 cm) of Toyopearl HW-40S, eluted with water/methanol (v/v, 3 : 1) at flow rate of 150 mL/h.

absorbance at 300 nm (*p*-nitrophenyl group) and at 210 nm. The chromatogram showed one main peak (F-2; tubes 67-73) with one minor peak (F-1; 55-60) as transglycosylation products for which the absorption at 310 nm coincides with that at 210 nm. The F-2 fraction, eluted as a sharp peak, was concentrated and lyophilized to give compound **2** (53.4 mg). Peak 3 (tubes 89-95) contained β-D-Man-$OPhNO_2$-*p* (115 mg) used as the acceptor. Compound **2** had $[\alpha]_D^{25} = -57.2°$ (*c* 1, H_2O) and *m/z* 505. NMR data (D_2O):^1H, δ 8.23 (d, 2H, J=9.2 Hz, *m*-Ph), 7.18 (d, 2H, J=9.2 Hz, *o*-Ph), 5.49 (d, 1H, $J_{1,2}<1$ Hz, H-1), 4.56 (d, 2H, $J_{1',2'}=8.3$ Hz, H-1') and 2.07 (s, 3H, Ac);^{13}C, δ 177.41 (C=O of Ac), 164.20 (Ph carbon attached to the phenolic oxygen), 145.21 (*p*-Ph carbon), 128.86 (*m*-Ph carbon×2), 119.03 (*o*-Ph carbon×2), 104.40 (C-1'), 99.71 (C-1), 79.82 (C-4), 78.71 (C-5'), 77.81 (C-5), 76.21 (C-3'), 74.20 (C-3), 72.56 (C-4'), 72.43 (C-2), 63.41 (C-6'), 63.05 (C-6), 58.36 (C-2') and 24.91 (s, 3H, Ac).

Preparation of *p*-nitrophenyl 2-acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranoside (3). To a solution of $(GlcNAc)_2$ (558 mg) and β-D-GlcNAc-$OPhNO_2$-*p* (115 mg) in 5.0 mL of 0.1 M acetate buffer (pH 4.5) containing 50 % DMSO was added lysozyme (68 U, 150 mg). After the mixture was incubated for 24 h at 50 ℃, the insoluble material was removed by centrifugation. The supernatant was directly

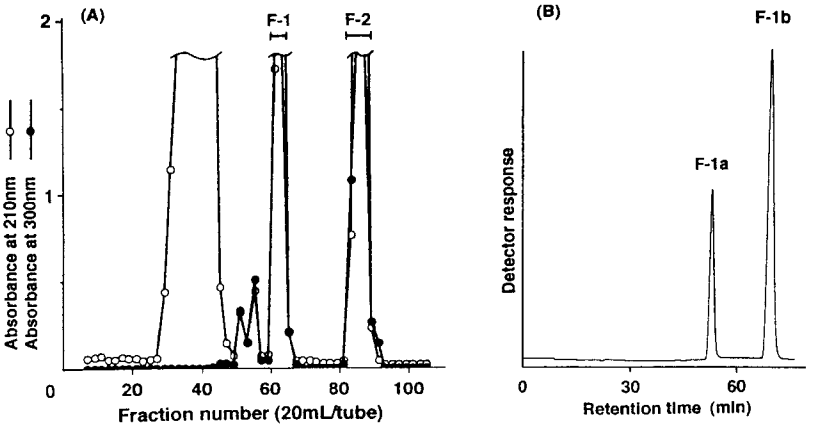


Fig. 4. Chromatographic separation of transglycosylation products by the action of lysozyme on $(GlcNAc)_2$ and α-D-GlcNAc-$OPhNO_2$-*p*. **A**, chromatographic conditions were the same as those in Fig. 3. **B**, HPLC was performed with a YMC-packed SH-345-5 (ODS) column (20 × 250 mm). Elution of the column was with water/methanol (v/v, 7 : 3).

loaded onto a Toyopearl HW-40S column. The chromatographic conditions were the same as those in **Fig. 3**. It showed one main peak (F-1, tube numbers 62-66) and one minor peak (tube numbers 83-90) as transglycosylation products. Fraction F-1 was concentrated and lyophilized to give compound **3**; yield 36.3 mg. The structural data for compound **3** was identical to those of β-D-GlcNAc-(1-4)-β-D-GlcNAc-$OPhNO_2$-*p* already reported.[18]

Preparation of *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranoside (4) and *p*-nitrophenyl 2-acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-gluco-pyranoside (5). To a solution of $(GlcNAc)_2$ (560 mg) and α-D-GlcNAc-$OPhNO_2$-*p* (150 mg) in 0.1 M acetate buffer (pH 4.5) containing 50 % DMSO (5 mL) was added lysozyme (68 U, 150 mg). The molar ratio of the former and latter compounds was 3 : 1 and the total substrate concentration was 14.2 %. After the mixture was incubated for 8 days at 50 °C, the solution was loaded onto a Toyopearl HW-40S column (**Fig. 4A**). The chromatogram showed one main peak (F-1, tube numbers 60-64) as transglycosylation product for which the absorption at 300 nm coincides with that at 210 nm. Fraction 1 was concentrated and lyophilized to give a yield of 22.5 mg. Peak

F-2 (tube numbers 82-89) contained α-D-GlcNAc-OPhNO_2-*p* used as the acceptor. F-1 was dissolved in 1 mL of water and 1/10 vol of the solution was applied to HPLC as in **Fig. 4B**. The fraction was separated into two main peaks (F-1a and F-1b). The remaining aliquots were similarly treated. The eluates corresponding to F-1a and F-1b were each combined, concentrated, and lyophilized to afford compound **4** (3.5 mg) and **5** (10.5 mg), respectively. Compound **4** had $[\alpha]_D^{25}$ = +158.5° (c 1, 50 % methanol) and *m/z* 546. NMR data [$(CD_3)_2SO$]:^{13}C, δ 171.43 and 171.05 (C=O of Ac), 163.23 (Ph carbon attached to the phenolic oxygen), 143.52 (*p*-Ph carbon), 127.37 (*m*-Ph carbon× 2), 118.60 (*o*-Ph carbon× 2), 102.57 (C-1'), 97.52 (C-1), 81.76 (C-3), 78.51 (C-5'), 75.61 (C-3'), 75.51 (C-5), 72.24 (C-4'), 69.65 (C-4), 62.66 (C-6'), 61.89 (C-6), 57.21 (C-2'), 53.33 (C-2), and 24.60 and 24.20 (Me of Ac). Compound **5** had $[\alpha]_D^{25}$ = +156.5° (*c* 1, H_2O) and *m/z* 546. NMR data [$(CD_3)_2SO$]:^{13}C, δ 171.28 and 170.78 (C=O of Ac), 163.16 (Ph carbon attached to the phenolic oxygen), 143.48 (*p*-Ph carbon), 127.35 (*m*-Ph carbon×2), 118.56 (*o*-Ph carbon×2), 103.61 (C-1'), 97.27 (C-1), 82.21 (C-4), 78.54 (C-5'), 75.49 (C-3'), 73.60 (C-5), 72.27 (C-4'), 70.30 (C-3), 62.66 (C-6'), 61.02 (C-6), 56.89 (C-2'), 54.23 (C-2), and 24.51 and 24.06 (Me of Ac).

Preparation of *p*-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-maltoside (6) and *p*-nitrophenyl 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl-α-maltoside (7). $(GlcNAc)_2$ (382 mg) and *p*-nitrophenyl α-maltoside (418 mg) dissolved in 2 mL of 0.1 M acetate buffer (pH 4.5) were incubated with lysozyme (66 U, 146 mg) for 5 days at 50 ℃. The insoluble material formed during the reaction was removed by centrifugation. The supernatant was directly loaded onto a column (2.2 ×95 cm). The eluate was collected in 10 mL fractions. The chromatogram showed only one main peak (F-1: tubes 54-65) as transglycosylation product with overlapping of the absorptions at 300 and 485 nm. The fraction was concentrated and lyophilized to give a yield of 55.6 mg. F-1 was dissolved in 1 mL of water and 1/5 vol of the solution was applied to HPLC. The chromatographic conditions were the same as those in **Fig. 4B**. The fraction was separated into two peaks of F-1a and F-1b (data not shown). The remaining aliquots were similarly treated. The eluates corresponding to F-1a and F-1b were each combined, concentrated, and lyophilized to afford compound **6** (31.6 mg) and **7** (15.9 mg), respectively. Compound **6** had $[\alpha]_D^{25}$ = +130.1° (*c* 1, H_2O) and *m/z* 667, NMR data (D_2O):^1H, δ 8.21 (d, 2H, J=9.2 Hz, *m*-Ph), 7.27

Table 1.

Carbon-13 chemical shifts of compounds **6** and **7** in D_2O solution

compound **6** ; ß-D-GlcNAc-(1-3)-α-D-Glc-(1-4)-α-D-Glc-OPhNO_2
III II I P

compound **7** ; ß-D-GlcNAc-(1-4)-α-D-Glc-(1-4)-α-D-Glc-OPhNO_2
III II I P

Compound		C-1	C-2	C-3	C-4	C-5	C-6	CH_3 (NHAc)	C=O (NHAc)	m-P	o-P	p-P	c-Pa
6	I	99.23	73.60	75.18	78.92	74.21	62.96						
	II	102.55	74.21	84.67	70.64	76.48	63.25						
	III	104.60	58.58	76.15	72.67	78.63	63.49	25.05	177.68				
	P									119.48	128.82	145.08	164.17
7	I	99.24	73.53	74.36	79.50	74.14	62.68						
	II	102.30	73.85	76.30	81.98	74.14	62.89						
	III	104.20	58.42	76.08	72.54	78.69	63.38	24.91	177.20				
	P									119.50	128.84	145.08	164.17

a. Phenyl carbon attached to the phenolic oxygen.

(d, 2H, J=9.2 Hz, *o*-Ph), 5.79 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.40 (d, 1H, $J_{1',2'}$=3.6 Hz, H-1'), 4.56 (d, 1H, $J_{1'',2''}$=8.6 Hz, H-1'') and 2.03 (s, 3H, Ac). Compound **7** had $[\alpha]_D^{25}$ = +148.2° (*c* 1, H_2O) and *m/z* 667. NMR data (D_2O): ^1H, δ 8.21 (d, 2H, $J_{1,2}$=9.2 Hz, *m*-Ph), 7.25 (d, 2H, J=9.2 Hz, *o*-Ph), 5.78 (d, 2H, $J_{1,2}$=3.6 Hz, H-1), 5.41 (d, 2H, $J_{1',2'}$=3.6 Hz, H-1'), 4.75 (d, 1H, $J_{1'',2''}$=8.6 Hz, H-1'') and 2.05 (s, 3H, Ac). ^{13}C NMR data of isomeric forms are shown in Table I.

ACKNOWLEDGEMENTS

This work was partly supported by a Grant-in-Aid for Scientific Research on Priority Areas No. 06240224 from the Ministry of Education, Science and Culture of Japan and also by a Grant-in-Aid (Glycotechnology programme) from the Ministry of Agriculture, Forestry and Fishers of Japan.

REFERENCES

1. D. M. Chipman, J. J. Pollock, and N. Sharon, *J. Biol. Chem.*, **243**, 467 (1968).
2. T. Usui, H. Matsui, and K. Isobe, *Carbohydr. Res.*, **203**, 65 (1990).
3. J. A. Rupley and Y. Gates, *Proc. Nat. Acad. Sci., USA* **57**, 496 (1967).
4. Y. Takiguchi and K. Shimahara, *Lett. Appl. Microbiol.*, **6**, 129 (1988).
5. J. A. Rupley, *Biochim. Biophys. Acta*, **83**, 245 (1964).
6. C. Bosso, J. Defaye, A. Doward, A. Gadelle and C. Pedersen, *Carbohydr. Res.*, **156**, 57 (1986).
7. K. Ogawa, T. Murata, and T. Usui, *Carbohydr. Res.*, **212**, 61 (1991).
8. T. Usui and T. Murata, *J. Biochem.*, **103**, 969 (1988).
9. K. G. I. Nilsson, *Biocat. Org. Med.*, 369 (1987).
10. K. G. I. Nilsson, *Carbohydr. Res.*, **167**, 95 (1987).
11. K. Ogawa, T. Nakakuki, and T. Usui, *J. Carbohydr. Chem.*, **10**, 877(1991).
12. S. Kobayashi, K. Kainuma, T. Kawasaki, and S. Shoda, *J. Am. Chem. Soc.*, **113**, 3079 (1991).
13. T. Usui, M. Suzuki, T. Sato, H. Kawagishi, K. Adachi and H. Sano, *Glycoconjugate J.*, **11**, 105 (1994).
14. T. Usui, Y. Hayashi, F. Nanjo and Y. Ishido, *Biochim. Biophys. Acta*, **953**, 179 (1988).
15. F. W. Ballardie, B. Capon, M. W. Cuthbert and M. W. Dearie, *Bioorganic Chem.*, **6**, 483 (1977).
16. T. Imoto and K.Yagishita, *Agric. Biol. Chem.*, **35**, 1154 (1971).
17. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 3560 (1956).
18. F. Nanjo, K. Sakai, T. Usui, I. Takai, and Y. Ishido, *J. Carbohydr. Chem.*, **7**, 67 (1988).