Review

Preparation of Oligosaccharide Units Library and Its Utilization

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There is high current interest in developing synthetic routes to oligosaccharides involved in glycoconjugates. Significant attention has been focused on the application of glycosidase-catalyzed transglycosylation for practical synthesis of oligosaccharides. The enzymatic synthesis has become more practical by the use of several glycosidases available in sufficient quantities. This review describes convenient syntheses of di- and trisaccharide units, which are related to molecular recognition, by using regioselective transgalactosylation, trans-N-acetylgalactosaminylation, transfucosylation, and transmannosylation. The regioselectivity could be controlled to some extent by using the following techniques: (1) varying enzymes, (2) organic co-solvent system, (3) the configuration of the existing glycosidic linkage of the acceptor and (4) inclusion complex of acceptor glycoside with cyclodextrin. Furthermore, glycopolymers carrying a series of disaccharides containing β-D-galactosyl residues were synthesized and used as a model in oligosaccharide-lectin interaction analysis. These water-soluble glycopolymers were shown to be useful as probes of carbohydrate recognition.

Key words: oligosaccharide units library; glycosidase-catalyzed transglycosylation; glycoconjugate; glycopolymer

Oligosaccharides consist of glycoconjugates and glycolipids are information-rich molecules that guide many biological processes. Our purpose is to develop an efficient synthetic method to obtain oligosaccharide units in sufficient amounts to discover other functions of oligosaccharide units. Carbohydrate molecules are recognized as particularly challenging targets for regioselective glycosylation by either chemical or enzymatic methods because of their nature, having multiple hydroxyl groups. Chemical methods for obtaining oligosaccharide units have been extensively developed, but they involve various elaborate procedures for protection, glycosylation, and deprotection. The enzymatic approach has been done in part with the glycosyltransferase and glycosidase. The glycosidases usually hydrolyze glycosidic bonds, but they can be used for glycoside formation. From a practical viewpoint, the use of glycosidases is attractive for oligosaccharide synthesis, because the glycosidases are generally more available, less expensive than the glycosyltransferases, and do not require expensive sugar nucleotide donors. In general, glycosidase-catalyzed transglycosylation does have some regioselectivity for the hydroxyl linkage to the acceptor. For example, the β-D-galactosidase from Kluyveromyces lactis affords β-D-Gal-(1→6)-D-GlcNAc (N-acetyllactosamine, IsoLacNAc) as the major transglycosylation product of the reaction between lactose and GlcNAc, while the β-D-galactosidase from bovine or porcine testes produced the β-(1→3)-linked isomer. The enzymes isolated from different sources reflect on the product regioselectivity. On the other hand, if the regioselectivity on glycosidase-mediated formation of oligosaccharides is modified by changing the reaction conditions, it will be applicable to a wide range of glycosidases in the synthesis. Such an attempt of preparation of oligosaccharide units library following practical synthesis of biologically important oligosaccharides is essential for developing glyciobiology and glycotechnology. Several kinds of sugar-containing glycopolymers have also been prepared as biomedical materials such as cell-specific culture substrate, artificial antigens, and targeted drug delivery agents.

This review describes recent developments in glycosidase-catalyzed oligosaccharide synthesis, with particular focus on the regioselectivity. A convenient synthetic route to a new type of artificial glycopolymers was also designed to develop biomaterials, employing the synthetic oligosaccharides as recognition signals.

Disaccharide Containing β-D-Galactosyl Residue

Our interest was at first directed to an enzymatic approach to the synthesis of β-D-galactosyl-N-acetylgalactosamine units involved in glycoconjugates, because many D-galactose-containing oligosaccharide units are important in biological recognition events. Generally, the D-galactose residues are found at nonreducing positions in the sugar moieties of asialoglycoproteins. The most abundant linkage is the β-D-Gal-(1→4)-D-GlcNAc (N-acetyllactosamine, LacNAc) unit in sugar-chain components of glycoproteins. β-D-Gal-(1→3)-D-GlcNAc (lacto-N-biose I) is also an important constituent of sLeα in complex type carbohydrate chains and of mucin-type carbohydrate.

N-Acetyllactosamine and its related compounds

A β-D-galactosidase from Bacillus circulans induced predominant β-D-galactosyl transfer from lactose to the secondary hydroxyl group (OH-4) over the primary hydroxyl group of GlcNAc. Starting with 0.5 m lactose and 1 m GlcNAc with the enzyme, LacNAc (I) was readily

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Abbreviations: β-NHase, β-N-acetyl-d-glucosaminidase; IsoLacNAc, N-acetyllactosamine; LacNAc, N-acetyllactosamine; lacto-N-biose I, β-D-Gal-(1→4)-D-GlcNAc; lacto-N-tetrose II, β-D-GlcNAc-(1→2)-β-D-Gal-(1→3)-D-Glc; Glc3NAc, 3-acetamido-3-deoxy-d-glucose; (GlcNAc)n, N,N',N",N"'-tetraacetyllactotetraose; CD, cyclodextrin.
synthesized and isolated conveniently on a gram scale (a yield of 23.2% based on the GlcNAC added) by chromatography on a carbon–Cellite column. Thus, (1→4)-linked disaccharide was preferentially obtained over the (1→6)-linked compound. No (1→3)- or (1→1)-linkages were formed. When 3-acetamido-3-deoxy-d-glucose (Glc3NAC) was used as an acceptor, the enzyme catalyzed the β-d-galactosyl transfer to the anomeric position (OH-1) and OH-6 of this sugar to afford β,β,β-D-Gal-(1→3)-β-D-GlcNAc and β,β-D-Gal-(1→6)-β-D-GlcNAc, respectively.21 No galactosylation to O-2 and O-4 of the acceptor, which are adjacent to the 3-acetamido group, was observed in the reaction. This demonstrates that the regioselectivity of β-D-galactosyl transfer onto GlcNAc and Glc3NAC acceptors catalyzed by the enzyme is strongly affected by the position of the N-acetyl group. On the other hand, the galactosyl transfer to the 6-position of GlcNAc was efficiently done to give IsoLacNAc (2) by the use of the β-D-galactosidase from K. lactis.5

Further use of the transglycosylation by B. circulans β-D-galactosidase led to the syntheses of β-(1→4)-linked disaccharides containing a 2-acetamido-2-deoxy-d-hexose.13 Thus, β-D-Gal-(1→4)-β-D-GlcNAc-OC6H4NO2-p (3) was effectively synthesized from lactose and p-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside on a mmolar scale with its positional isomer β-D-Gal-(1→6)-β-D-GlcNAc-OC6H4NO2-p (4) by the action of the enzyme in an aqueous solution containing 50% acetone. Compound 3 was obtained in a yield of 21.0% based on the acceptor added and predominated over 4 during the entire course of the reaction. On the contrary, at 20% acetone, much more of 3 over 4 was found in the initial stage of the reaction, but the relation between this yield was reversed in the later stage of the reaction. As a result, 4 was obtained in a yield of 25.5% based on the acceptor added. The (1→4)-linked disaccharide was formed exclusively when the concentration of organic solvent was high, but the (1→6)-linked isomer was produced with a low concentration. When GalNAc and β-D-GalNAc-OC6H4NO2-p were used as acceptors, the galactosylation also led to the regioselective formation of β-D-Gal-(1→4)-β-D-GalNAc and β-D-Gal-(1→4)-β-D-GalNAc-OC6H4NO2-p. With the enzyme, β-D-galactosyl transfer occurred preferentially on the C-4 position of respective GlcNAc and GalNAc acceptors, regardless the configuration of the hydroxyl group.

Disaccharide α-glycoside containing β-(1→3)-galactosyl residue

Hedbys et al. have synthesized β-D-Gal-(1→3)-β-D-GalNAc and β-D-Gal-(1→3)-β-D-GlcNAc by transglycosylation, using the β-D-galactosidase from bovine testes.4,14 Our interest was directed to an enzymatic approach involving a 3-O-β-D-galactosylation of p-nitrophenol α-N-acetylgalactosaminide to form a mimic of mucin-type 1 core carbohydrate. When β-D-galactosidase from porcine testes instead of that from bovine testes was used, the enzyme induced transglycosylation from lactose regioselectively to the 3-position of α-D-GalNAc-OC6H4NO2-p.3 In this case, the p-nitrophenyl glycoside acceptor is only sparsingly soluble (0.1%) in aqueous medium. The problem of low solubility was partially solved by using β-cyclodextrin (β-CD), which is thought to form an inclusion complex with the p-nitrophenyl group of the substrate. When α-D-GalNAc-OC6H4NO2-p was dissolved in the presence of equimolar amounts of β-CD, the enzyme produced a 3:1 mixture of β-D-Gal-(1→3)-α-D-GalNAc-OC6H4NO2-p (5) and its (1→6) linked isomer (22% total yield, based on the acceptor added). The use of an inclusion complex of the glycoside acceptor with β-CD increased the efficiency of transglycosylation by increasing the solubility of the acceptor. These transfer products were conveniently isolated in one step by chromatography of a Toyopearl HW-40S column. Moreover, an excess of unreacted acceptor, which is quite expensive, could easily be recovered by the chromatography and reused for synthesis. Compound 5 was also shown to be a very sensitive substrate for the endo-α-N-acetylglactosaminidase from Clostridium perfringens.13 The Michaelis constant (Km) and kcat/Km were 0.041 mm and 2300 mm-1. In the same way, the use of β-D-GalNAc-OC6H4NO2-p as acceptor led to the preferential formation of β-D-Gal-(1→3)-β-D-GalNAc-OC6H4NO2-p over that of (1→6) linked isomer. β-D-Gal-(1→3)-β-D-GlcNAc-OC6H4NO2-p was also synthesized with β-D-GlcNAc-OC6H4NO2-p acceptor by the consecutive use of β-D-galactosidase from porcine testes and B. circulans. The synthesis of possible array of linkages of (1→3), (1→4), and (1→6) of β-D-Gal-D-GlcNAc and β-D-Gal-D-GalNAc except (1→1)-linkage has been done by using some readily available β-D-galactosidases as shown in Fig. 1 and the Table.

Di- and Trisaccharides Containing N-Acetylgalcosaminyl Residues

β-D-GlcNAc-(1→4)-β-D-Man and β-D-GlcNAc-(1→3)-β-D-Gal units are present in N-glycoprotein and characteristic of the lacto-series of glycolipids, respectively. The section concerns and enzymatic approach to such N-acetylgalcosaminyl oligosaccharide units involved in glycoconj-

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Table Glycosidase-mediated Oligosaccharide Synthesis

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a Yields were calculated based on acceptor substrates except for α-L-fucosidase. Yields for fucosyltransfer reaction were calculated based on donor substrate.

b Enzyme from bovine testes.

c Enzyme from Bacillus circulans.

d Enzyme from Kluyveromyces lactis.
ie Enzyme from Escherichia coli.
f Enzyme from porcine testes.
g Enzyme from hen egg-white.
h Enzyme from Nocardia orientalis.
i Enzyme from Alcaligenes sp.
j Enzyme from Aspergillus niger.

β-N-Acetamido-β-N-acetyl-β-N-hexosaminidase (β-N-AHase)

4-O-β-N-Acetylglucosaminyl-mannose and its glycoside

Commerically available lysozyme (hen egg-white) produced β-d-GlcNAc(1→4)-d-Mann (6) in a yield of 20.9% based on the (GlcNAc)2 donor added.16 Thus, the enzyme is capable of transferring an N-acetylglucosaminyl group from N,N'-diacetylchito-oligosaccharide (GlcNAc)2 exclusively to the OH-4 of the mannose moiety. When the donor was N,N',N''-tetraacetylchitotetraose (GlcNAc)4 instead of (GlcNAc)2, the rate of formation of 6 was 6–7 fold faster. However, our alternation was to synthesize 6 from a lower oligomer such as (GlcNAc)2, which is more available enzymatically or chemically in substantial quantities from low price chitin. When p-nitrophenyl β-d-mannopyranoside was used as the
acceptor, β-d-GlcNAc-(1→4)-β-d-Man-OC₆H₄(NO₂)₂-p (7) was obtained in a yield of 10.5% based on the donor added. The efficiency of lysozyme-catalyzed transglycosylation was greatly influenced by the solubility of the acceptor molecule in an aqueous-DMSO (dimethyl sulfoxide) system. The acceptor showed higher solubility (3%) in a medium containing 50% DMSO whereas 1% of solubility was observed in the absence of DMSO. The maximum production of 7 at 50% DMSO was about 3-fold higher than that obtained in a reaction done in the medium in its absence.

When χ-glycoside (χ-β-GlcNAc-OC₆H₄(NO₂)₂-p) was used as the acceptor, the enzyme catalyzed the formation of a β-(1→3)-linked disaccharide glycoside (β-β-GlcNAc-(1→3)-β-d-GlcNAc-OC₆H₄(NO₂)₂-p) with its β-(1→4)-linked isomer. This was also the case for the formation of ρ-nitrophenyl 3-O-α-N-acetylglucosaminyl-maltoside with the ρ-nitrophenyl χ-maltoside acceptor. The results showed that the anomic configuration of the glycosidic linkage in the glycosyl acceptors had a pronounced effect on the position of transglycosylation.

Lacto-N-triose II and its positional isomers

Lacto- and lactoneotetraglycosyleramides have the common structure lacto-N-triose II (β-β-GlcNAc-(1→3)-β-d-Gal-(1→4)-β-d-Glc unit). Our purpose is to develop a system for selective transfer of N-acetylglucosaminyl residues onto OH-3 of the Gal residue of the lactoside acceptor, based on the enzymatic approach. A β-NHase from Novocardiopsis orientals catalyzed the synthesis of lacto-N-triose II glycoside (β-β-GlcNAc-(1→3)-β-d-Gal-(1→4)-β-d-Glc-OMe, 8) with its isomers β-β-GlcNAc-(1→6)-β-d-Gal-(1→4)-β-d-Glc-OMe (9) and β-d-Gal-(1→4)-[β-β-GlcNAc-(1→6)]-β-d-Glc-OMe (10) through N-acetylglucosaminyl transfer from (GlcNAc)₂ to methyl β-lactoside. The enzyme formed the mixture of tri saccharides 8, 9, and 10 in 17% overall yield based on (GlcNAc)₂, in a ratio of 20: 21: 59. With ρ-nitrophenyl β-lactoside as an acceptor, the enzyme produced ρ-nitrophenyl β-lacto-N-triose II (β-d-GlcNAc-(1→3)-β-d-Gal-(1→4)-β-d-Glc-OC₆H₄(NO₂)₂-p, 11) with its isomers β-d-GlcNAc-(1→6)-β-d-Gal-(1→4)-β-d-Glc-OC₆H₄(NO₂)₂-p (12), and β-d-Gal-(1→4)-[β-β-GlcNAc-(1→6)]-β-d-Glc-OC₆H₄(NO₂)₂-p (13) in total yield of 2.9% (based on the donor) in a ratio of 14: 21: 65. The minor product (14%) was shown to be the desired β-(1→3) linked compound 11. This problem was partially solved by using β-CD as mentioned above. When ρ-nitrophenyl β-lactoside was dissolved in the presence of equimolar amounts of β-CD, the transfer products 11, 12, and 13 were obtained in 7% total yield and in a ratio of 27: 43: 30. The maximum production was in the absence of β-CD, about 6-fold higher than in its absence. This not only resulted in a significant improvement of the yield, but also in the higher proportion of 11 and 12 over 13. These three compounds were separated by chromatography on a ToyoPEARL HW-40S column and purified by HPLC with an ODS column. This was also the case for the reaction of LacNAcβ-OC₆H₄(NO₂)₂-p acceptor.

With the acceptor in the absence of β-CD, the enzyme formed a mixture of β-d-GlcNAc-(1→3)-β-d-Gal-(1→4)-β-d-GlcNAc-OC₆H₄(NO₂)₂-p (14), β-d-GlcNAc-(1→6)-β-d-Gal-(1→4)-β-d-GlcNAc-OC₆H₄(NO₂)₂-p (15), and β-d-Gal-(1→4)-[β-β-GlcNAc-(1→6)]-β-d-GlcNAc-OC₆H₄(NO₂)₂-p (16) in a ratio of 11: 33: 56. When an inclusion complex of LacNAcβ-OC₆H₄(NO₂)₂-p with χ-CD was used, transfer products 14, 15, and 16 were formed in a ratio of 24: 63: 13. A similar change with respect to regioselectivity was observed in the absence and presence of χ-CD. We further investigated the regioselectivity of the enzyme-catalyzed formation of trisaccharides containing an N-acetylglucosaminyl residue at different molar ratios of the acceptor to χ-CD as depicted in Fig. 2(A). The numbers on the ordinate show the percentage of three trisaccharide products formed by transglycosylation, based on the time at which the desired 14 production reached its maximum. A change of regioselectivity could be, to some extent, achieved by using an inclusion complex of acceptor with χ-CD. In the absence of χ-CD, the enzyme transfers O-6 of the acceptor moiety preferentially to O-6' and reacts only weakly with O-3'. On
the contrary, in the presence of \( \alpha \)-CD, the enzyme transfers much less to O-6 of the acceptor, while more to O-6' and O-3'. Furthermore, with an increased molar ratio of \( \alpha \)-CD to the acceptor, the production of 14 and 15 was increased, but that of 16 greatly decreased. It suggests that an excess of \( \alpha \)-CD to the acceptor greatly increases the probability of its complexation of \( \alpha \)-CD with the acceptor. This effect is thought to contribute to diminishing the \( N \)-acytglycosaminyl transfer onto O-6 at the GlcNAc residue of the acceptor, due to steric hindrance of the CD in an inclusion complex. A similar change with respect to regioselectivity was observed for the reaction of Lac\( \beta \)-OC\( \alpha \)C\( \alpha \)H\( \alpha \)NO\( \alpha \)\( -p \) in the absence and presence of \( \alpha \)-CD as in Fig. 2(B). The regioselectivity of \( \beta \)-NAHase-catalyzed formation of trisaccharides was modified by using a nature of hydrophobic \( p \)-nitrophenyl group in the glycosyl acceptor. We previously reported that the use of an organic solvent (50–60% DMSO, acetonitrile etc.) in transfer reactions using glycosidases not only ensured a sufficient solubility of \( p \)-nitrophenyl glycosides, but also affected the regioselectivity of the products.13–19 However, this concept was not applicable to this reaction system, because of the instability of the enzyme in organic co-solvent systems.

Trisaccharide containing \( N \)-acetylglucosaminyl residue as mucin-type carbohydrate

We have established a preparative synthetic method for obtaining enough amounts of 5, which is a mimic unit of the mucin type 1 core as mentioned above.4) The enzymatic approach was further extended to a convenient synthesis of \( \beta \)-d-Gal-(1→3)-[\( \beta \)-d-GlcNAc-(1→6)]-\( \alpha \)-d-GalNAC-OC\( \alpha \)C\( \alpha \)H\( \alpha \)NO\( \alpha \)\( -p \) (17), which is a carbohydrate structure of the mucin type 2 core, using 5, available in large enough amounts as an acceptor. A \( \beta \)-NAHase from \textit{N. orientalis} induced the synthesis of the desired compound 17 with its isomers \( \beta \)-d-GlcNAc-(1→6)-\( \beta \)-d-Gal-(1→3)-\( \alpha \)-d-GalNAc-OC\( \alpha \)C\( \alpha \)H\( \alpha \)NO\( \alpha \)\( -p \) (18) and \( \beta \)-d-GlcNAc-(1→3)-\( \beta \)-d-Gal-(1→3)-\( \alpha \)-d-GalNAc-OC\( \alpha \)C\( \alpha \)H\( \alpha \)NO\( \alpha \)\( -p \) (19) through \( N \)-acytglycosaminyl transfer from (GlcNAc)\( \alpha \) to 5.13 The molar ratio of the donor to acceptor was about 10:1, and the total substrate concentration was about 11.6%. The enzyme formed the trisaccharides 17, 18, and 19 in 13.7% overall yield based on the acceptor, and in a ratio of 44:32:24. These were separated by chromatography on a Toyopearl HW-40S column followed by HPLC with an ODS column. Moreover, the unreacted acceptor could be recovered by straightforward chromatography and reused. The \( N \)-acytglycosaminyl transfer favored O-6 of the acceptor rather than O-6', and occurred to a lesser extent at O-3'. In this way, consecutive use of transgalactosylation and trans-\( N \)-acytglycosaminylolation led to the formation of trisaccharide 17 as a mimic unit of mucin type 2, starting with \( \alpha \)-d-GalNAc-OC\( \alpha \)C\( \alpha \)H\( \alpha \)NO\( \alpha \)\( -p \) as shown in Fig. 3.
Trisaccharide Containing Fucosyl Residue

L-Fucose is also an important constituent of the carbohydrate chains of glycoconjugates involved in a variety of molecular recognition events. For example, the immunological determinants of the ABH(O) type are known to be oligosaccharides, which have the common structure of α-L-Fuc-(1 → 2)-β-D-Gal-R. Svensson and Thiem have reported that an α-L-fucosidase from porcine liver mediated the formation of (1 → 2)- and (1 → 6)-linked α-L-fucosyl-d-galactopyranoside (6.5 and 10%, respectively). The first purpose was to obtain 2'-O-α-L-fucosyl-N-acetyllactosamine unit (α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 4)-d-GlcNAc-OR) as the determinant of blood group antigen H (type 2), using glycosidase-catalyzed fucosylation. However, an α-L-fucosidase from Alcaligenes sp. induced highly regioselective transglycosylation from p-nitrophenyl α-L-fucopyranosyl to the 3-position of the Gal moiety on 1. Starting with p-nitrophenyl α-L-fucoside and LacNAc with the enzyme, α-L-Fuc-(1 → 3)-β-D-Gal-(1 → 4)-d-GlcNAc (20) was thus synthesized and isolated conveniently on a mmol scale by chromatography on a carbon–Celite column. In this case, the molar ratio of the donor to acceptor was 1:25 and the total substrate concentration was about 10%. This fucosylation reaction resulted in a high yield of 20 (48.8% yield based on the donor). Further use of the transglycosylation by Alcaligenes sp. α-L-fucosidase led to the synthesis of trisaccharides containing α-(1 → 3)-linked fucosyl residues, using other acceptors. When lactose and methyl β-lactoside were acceptors instead of 1, the enzyme formed regioselectively 3'-O-α-L-fucosyl-lactose (21) and methyl 3'-O-α-L-fucosyl-β-lactoside (22), respectively, in 31–42% yield based on the donor. These reactions were also efficient enough to allow us to perform the one-pot preparation of 20 and its analogues.

Trisaccharide Containing Mannosyl Residue

Trisaccharide β-D-Man-(1 → 4)-β-D-GlcNAc-(1 → 4)-GlcNAc (23) is known to be the common structural feature at the core region of most of the N-glycoproteins. Organic chemical methods for obtaining 23 have been developed, but it involves various elaborate procedure for protection, glycosylation, and deprotection. Especially, stereoselective glycosylation for the introduction of the β-D-Man residue is known as a difficult step of synthesis. We have developed several practical synthetic routes of biologically important di- and trisaccharides involved in glycoproteins and glycolipids. Such synthetic disaccharides containing β-D-galactosyl residue available in large enough amounts were used to synthesize glycopolymer according to the synthetic procedures.
to the methods previously reported. Our interest was at first directed to synthesize glycopolymers carrying a pendant LacNAc unit. Thus, $p$-nitrophenyl $N$-acetyl-$\beta$-lactosaminide (3) obtained enzymatically in large enough amounts was used as a starting substance for glycopolymer. Poly($N$-acetyl-$\beta$-lactosaminide-carrying acrylamide) (PAP-LacNAc) was obtained by the following three steps (Fig. 4): reduction of the nitro function to yield the $p$-amino-phenyl glycoside, introduction of an acryloyl function to give the $p$-acryloylaminophenyl glycoside monomer, and finally homopolymerization with azobisisobutyronitrile as initiator in DMSO to give a homopolymer with a number-average molecular weight ($M_n$) of $3.2 \times 10^5$. Other disaccharide-containing glycopolymers, carrying $\beta$-IsoLacNAc, $\alpha$-lactose, $\beta$-d-Gal-(1→3)-,$\beta$-d-Glc, $\beta$-Isolactose, and $\beta$-(GlcNAc)$_2$ moieties, which are abbreviated as PAP-IsoLacNAc, PAP-Lac, PAP-3Lac, PAP-IsoLac, and PAP-($\text{GlcNAc}_2$), respectively, were also prepared as reference polymers in similar manners. The resulting glycopolymers were soluble in DMSO, but their solubilities in water were somewhat low. The amphiphilic properties of glycopolymers are comparable to those of the corresponding glycopolymers of styrene derivatives previously reported.

Reactivity with lectin

A series of Gal and GlcNAc-containing glycopolymers were used as a model in oligosaccharide-lectin interaction analysis. Their interaction with various lectins was investigated by means of two-dimensional immunodiffusion test in agar and inhibition of hemagglutination activity. Several specific lectins were used: *Arachis hypogaea* (PNA, peanut, $\beta$-d-Gal-(1→3)-GalNAc), *Phytolacca americana* (PWM, pokeweed, $\beta$-d-GlcNAc), *Erythrina cristagalli* (ECA, LacNAc), *Triticum vulgaris* (WGA, wheat germ, $N$-acetyl-chitooligosaccharide), *Datura stramonium* (DSA, Jimson weed, LacNAc), and *Ricinus communis* (RCA$_{120}$, castor bean, $\beta$-d-Gal). In two-dimensional immunodiffusion test, PAP-LacNAc reacted with ECA, RCA$_{120}$, WGA, and DSA to produce a single precipitation band, respectively, as shown in Fig. 5. The latter two lectins showed sharper and stronger precipitation bands than the others. No precipitation band was observed between the combinations of the polymer with PNA and PWN. In other glycopolymers, PAP-IsoLacNAc and PAP-($\text{GlcNAc}_2$) reacted only with WGA and PAP-3Lac with PNA, whereas PAP-Lac reacted with RCA$_{120}$, PNA, and ECA. However, PAP-IsoLac showed no precipitation band with six lectins. Inhibitory effects of glycopolymers on the hemagglutination of human blood cells by lectins were also investigated. For example, inhibition was observed between the combinations of PAP-LacNAc with ECA, RCA$_{120}$, WGA, and DSA. Of these lectins, WGA and DSA were inhibited by the polymer much more strongly (about $10^{-3}$-$10^{-5}$ times) than that by the corresponding free sugar LacNAc. This is in accordance with the result, where WGA has been reported to be increased by the presence of hydrophobic aglycon and by the multi-antennary oligosaccharide chains. PAP-LacNAc, which also has a hydrophobic region and multi-ligands structural features, is thought to induce the strong binding with these lectins due to the clustered effect. Inhibition was also observed between PAP-Lac and the Gal-binding lectins (ECA, PNA, and RCA$_{120}$), however its inhibition activity was almost the same order as that of the corresponding sugar lactose. These disaccharide-substituted polymers were shown to interact specifically with the corresponding lectin, no matter how many sugar side chains of the polymers are short-chain length.

Conclusions and Perspectives

We prepared a biologically important di- and trisaccharide units library through glycosidase-mediated transglycosylation. In this process, the regioselectivity on trans-

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**Fig. 4.** Synthesis of PAP-LacNAc via a Chemo-enzymic Process.

**Fig. 5.** Double-diffusion Reaction of Glycopolymer with Lectin in Agar Gel.

Portions of the lectin solutions were added to the peripheral wells. Glycopolymer was added to the center well, 1, PNA; 2, PWM; 3, ECA; 4, WGA; 5, DSA; 6, RCA$_{120}$. 
glycosylation can be manipulated to some extent by devising the reaction system. The yields (10–49%) were considered to be sufficiently high for the practical method, because of the simplicity of enzymatic synthesis. Such enzymes are readily available in large amounts. Excesses of unreacted substrates are also recovered by straightforward column chromatography and reused for the synthesis. The approach described above should be applicable to a wide range of glycosidase-mediated oligosaccharide synthesis. Such well-defined oligosaccharides would be useful as substrates for exo- and endogenous glycosidases and glycosyltransferases involved in glycoconjugates processing, as probes for lectin or lectin binding, and as common synthetic intermediates of antigens. Furthermore, the synthetic oligosaccharides are also useful as starting substances for glycopolymers, which are valuable for investigating biological recognition phenomena using lectins. Accordingly, the supply of enough oligosaccharides offers a promising prospect to construct novel glycomaterials.

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References