論 文

Synthetic Studies of Cello-oligosaccharides by a Linear Synthetic Method

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直線型合成法によるセロオリゴ糖の合成研究

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Abstract

A completely organic synthetic method for a series of cello-oligosaccharides from D-glucose was investigated.

Firstly, a basic synthetic design in which a glucose derivative, having two kinds of temporary protective groups at 1-O and 4-O, and a persistent protective group at 2-O, 3-O and 6-O, to be used as a starting material was indicated on. After surveying available protective groups for the above purpose, allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside was selected as the starting material, and the synthetic route for the starting material was established (eight steps from D-glucose; total yield was 60%).

Next, using the imidate method, a cellobiose derivative (having the same protective system as the starting material) was synthesized (84%) by the glycosylation reaction between a glycosyl acceptor, prepared from the starting material by the deprotection of the *p*-methoxybenzyl group, and a glycosyl donor, prepared from the starting material by the deprotection of the allyl group. Repeating the set of two reactions above, deprotection and glycosylation reactions, a series of cello-oligosaccharide derivatives up to octamer was successfully synthesized by the linear synthetic method (each glycosylation reaction was accomplished over 70%).

At the final stage of the synthesis, all protective groups were removed to synthesize cellooctaoseacetate. This organic synthetic method is thought to be excellent and widely applicable in the synthesis of (1→2)β-, (1→3)β- and (1→6)β-glycans.

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CONTENTS

ABBREVIATIONS

Å	angstrom
Ac	acetyl
All	allyl
ax	axial
Bzl	benzyl
CAN	cerium (IV) ammonium nitrate
δ	chemical shift
DBU	diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMSO	dimethyl sulfoxide
D.P.	degree of polymerization
eq	equatorial
eq.	equivalent
FABMS	fast atom bombardment mass spectrometry
J	coupling constant
Me	methyl
m.p.	melting point
m/z	mass-to-charge ratio
n	normal (e.g. *n*-hexane)
NMR	nuclear magnetic resonance
p	para (e.g. *p*-methoxybenzyl)
Pd/C	palladium on activated carbon
pMB	*p*-methoxybenzyl
R_f	divided by distance traveled by solvent front
R_S	divided by distance traveled by standard compound
SEM	scanning electron microscopy
S_N2	bimolecular nucleophilic substitution
t	tertiary (e.g. *t*-butoxide)
TCA	trichloroacetonitrile
THF	tetrahydrofuran
TLC	thin layer chromatography
VPO	vapor pressure osmometry
UV	ultraviolet

INTRODUCTION

The chemistry of cellulose began in 1838 with the work of Payen[51]. He tried to extract the plants under relatively severe conditions and finally arrived at the conclusion that the fibrous tissue of all young plant cells consists of one uniform chemical substance, a carbohydrate comprised of glucose residues and isomeric with starch, which he named cellulose.

In the early stage of cellulose chemistry, extensive researches on the chemical structure of cellulose have been carried out in various points of view. One of them is concerned with acid hydrolysis of cellulose including its derivatives and identification of the hydrolyzate. Through this kind of investigation, cello-oligosaccharide derivatives of relatively low degree of polymerization (D.P.) were isolated and confirmed the chemical structure by the classical methods of organic chemistry, especially on their methylated derivatives. Recently, through the development of isolation method such as a liquid chromatographic technique, the preparation of up to cellononaose (D.P.= 9) acetate from the acetolysis of cellulose in relatively pure state was reported by Buchanan[6] *et al.*

Although there is an enzymatical synthetic method to obtain cellulose[40,41], the most sure method to prepare cello-oligosaccharides having monodispersity in D.P. is that of stepwise organic synthesis. Circumstances of the development of synthetic method in carbohydrate chemistry will be described in details in the chapter 1 of this thesis. It is true that the chemically synthesized cello-oligosaccharides may play an important role as a model compound in cellulose investigations, and that, during the synthetic study, new information on reactions and reactivities of sugar derivatives would be obtained.

For these reasons, a preparation method of a series of cello-oligosaccharides from D-glucose was investigated based on the modern organic chemistry.

Chapter 1 contains a basic synthetic design for cello-oligosaccharides, a synthesis of starting material from D-glucose, and conversion method from the starting material into glycosyl donor and glycosyl acceptor.

In chapter 2, possibilities of the synthesis of a series of cello-oligosaccharide derivatives by a linear synthetic method and a trial for the synthesis of cellotetraose and cellooctaose derivatives by a convergent synthetic method are discussed.

In chapter 3, the removal of the protective groups from the synthesized cello-oligosaccharides and the characterization of the resulting cellooctaose acetate are discussed.

The detailed experimental methods and data of compounds are summarized in experimental part.

CHAPTER 1 Selection and synthesis of starting material for cello-oligosaccharides synthesis

Synthetic studies on cellobiose were appeared in 1930's, in which glycosyl acceptor, 2,3,6-tri-*O*-acetyl-β-D-glucopyranoside[28] or levoglucosan[14], was glycosylated with a glycosyl donor, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, by Köigs-Knorr method. In 1970's, synthesis of cellotriose was tried by the similar methods as that of cellobiose synthesis but using 2,3,4,6,2',3',6'-hepta-*O*-acetyl-α-D-cellobiosyl bromide as a glycosyl donor[23,24,60]. Although these earlier studies suggest how to achieve the β-1,4 glucosidic bond formation by regiospecific and by stereospecific controls, the remained problem is how to elongate the oligosaccharide chain.

In 1980's, Schmidt and co-worker developed a novel glycosylation reaction named imidate method[53] and synthesized cellotetraose by their method conducting glycosylation reaction of 1,6-anhydro-2,3-di-*O*-benzyl-D-glucopyranoside with trichloroacetimidoyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside[54]. Takeo *et al.* also synthesized cellotetraose by Köigs-Knorr method in 1983[61].

These two synthetic methods would suggest the possibilities for the elongation of oligosaccharide chain. However, their methodologies need a number of chemical reaction steps for conversion of the synthesized oligosaccharide into glycosyl donor and/or glycosyl acceptor for the subsequent glycosylation reaction, which decreases the total yield and makes it difficult to elongate the oligosaccharide chain.

Hence, the new basic synthetic design which makes it possible to convert the synthesized oligosaccharide intermediate into glycosyl donor and/or glycosyl acceptor by fewer steps, is indispensable.

In this chapter, a basic synthetic design for cello-oligosaccharide is proposed, and selection of a suitable starting material is discussed.

1 — 1 Basic synthetic design for cello-oligosaccharide

In principle, there are two problems to be solved for the synthesis of a cello-oligosaccharides: (1) regiospecific control: glycosylation of the hydroxyl group at the C-4 position of the glucopyranosyl residue has to be performed, (2) stereospecific control: β-glucosidic linkage between glucopyranosyl residues has to be made.

The first problem could be solved by the selection of 2,3,6-tri-*O*-substituted glucopyranose derivative as a starting material and the second by the stereospecific β-glycosylation method[50,70].

Considering the above problems, a newly developed synthetic design is thought[48] as shown in Figs. 1-1, 1-2 and 1-3.

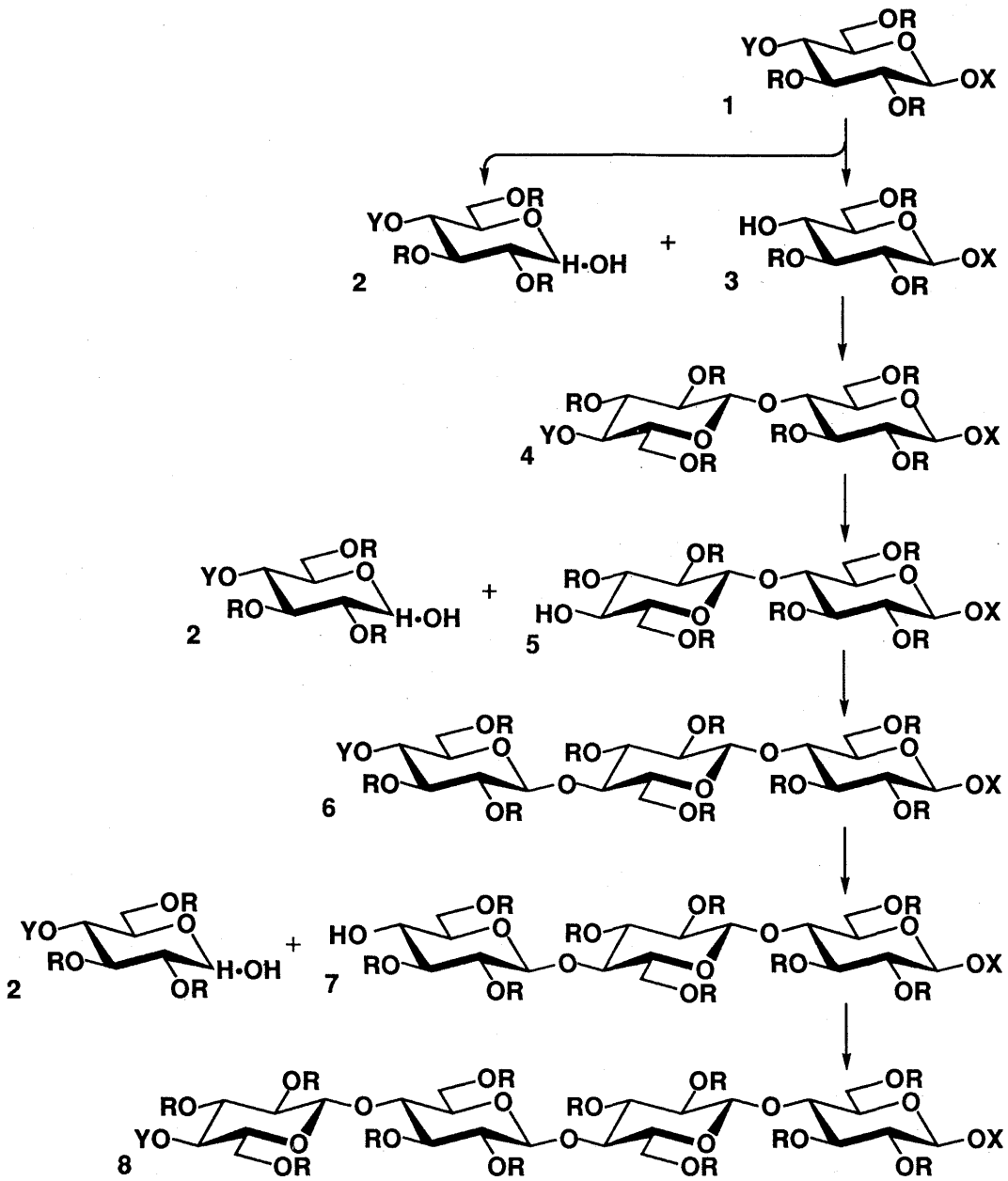


Fig. 1-1. Linear synthetic method (a) for cello-oligosaccharide derivatives.

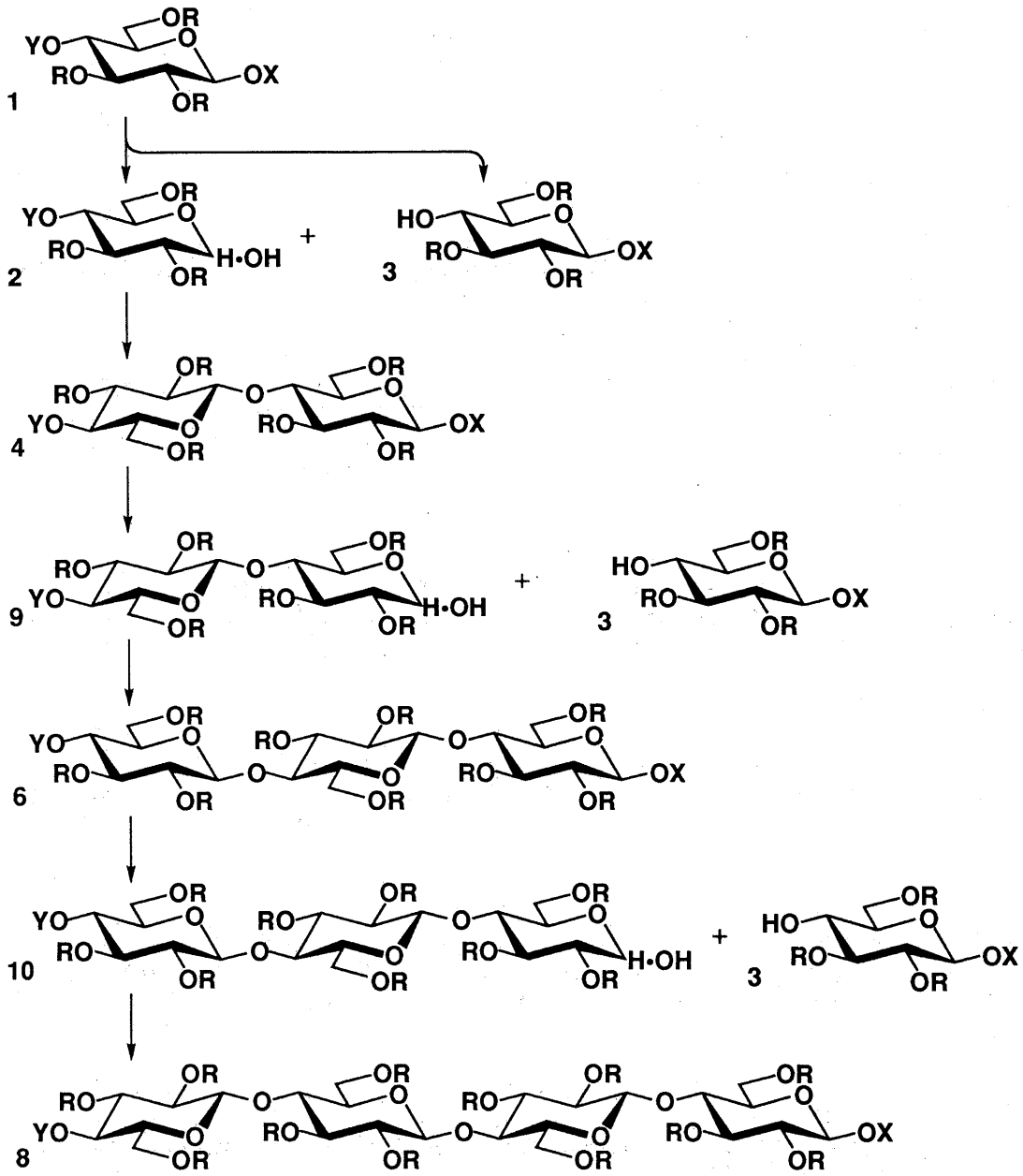


Fig. 1-2. Linear synthetic method (b) for cello-oligosaccharide derivatives.

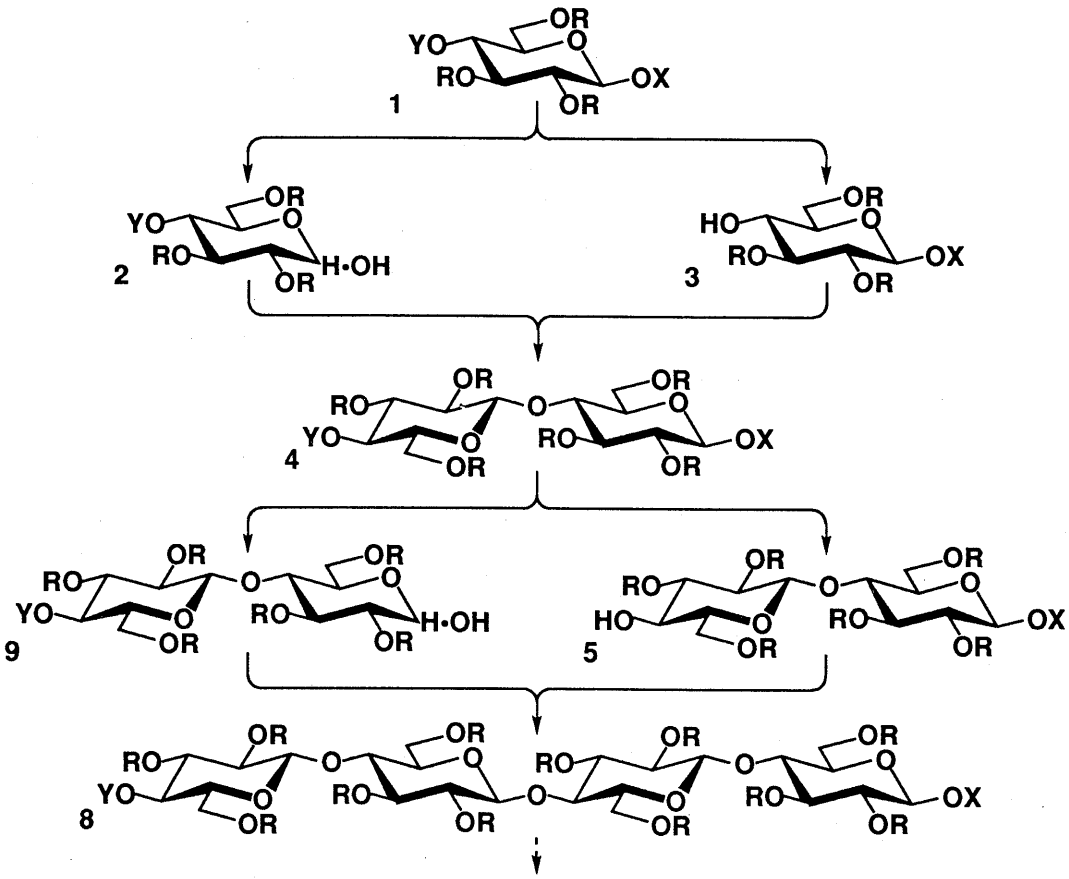


Fig. 1-3. Convergent synthetic method for cello-oligosaccharide derivatives.

For these synthetic designs, the substituted glucose derivative **1**, which has three kinds of protective groups, X-, Y- and R-groups, is reasonable as a starting material for the regulation of regiospecificity. Here, X- and Y-groups are "temporary" protective groups, and R-group is a "persistent" protective group[15,21]. The temporary protective groups should be removed independently before glycosylation reaction without any influence on the remaining protective groups. Thus, the glucose derivative having Y- and R-groups or X- and R-groups could be obtained. The persistent protective group will be removed at the final step of the synthesis to form the expected cello-oligosaccharide. Therefore, the R-group should be fairly stable under various reaction conditions, but the removal conditions of R-group must not cause decomposition of the hydroxyl groups and β-glucosidic bonds in the synthesized cello-oligosaccharides. It should be noted that the selection of the R-group is closely related to

those of X- and Y-groups and *vice versa*.

Here, two major synthetic routes are conceivable for the synthesis of cello-oligosaccharides: a linear synthetic method (Figs. 1-1 and 1-2) and a convergent synthetic method (Fig. 1-3) [63,64]. In these synthetic routes, the synthesis of cello-oligosaccharides is initiated by two reactions: deprotection of X- or Y-group from the starting material **1** and β-glycosylation. The removal of X- or Y-group from starting material **1** gives glycosyl donor **2** or glycosyl acceptor **3**, respectively. β-Glycosylation between glycosyl donor **2** and glycosyl acceptor **3** leads to the formation of cellobiose derivative **4** which has the same protective system as starting material **1**. In the linear synthetic method in Fig. 1-1 compound **2** derived from starting material **1** always is used as a sole glycosyl donor for further elongation of a sugar chain, and the method in Fig. 1-2 always compound **3** as a sole glycosyl acceptor. That is, in these linear methods, cellobiose derivative **4** can be converted into glycosyl acceptor **5** and glycosyl donor **9** by deprotection of Y- and X-groups, respectively, for further glycosylation.

Then, β-glycosylation is performed between glycosyl donor **2** and acceptor **5** (Fig. 1-1), and glycosyl donor **9** and acceptor **3** (Fig. 1-2), to afford cellotriose derivative **6**, respectively. Compound **6** is again converted into glycosyl acceptor **7** (Fig. 1-1) and glycosyl donor **10** (Fig. 1-2) that reacted with compound **2** or **3**, respectively, to give cellotetraose derivative **8**. After repeating above two reactions, a series of cello-oligosaccharides (whose D.P.= 1, 2, 3, 4, ···) could be theoretically obtained.

In the convergent method (Fig. 1-3), β-glycosylation conducted between glycosyl acceptor **5** and glycosyl donor **9** gives cellotetraose derivative **8** having the same protective system as compound **4**. Further, β-glycosylation between glycosyl donor and acceptor derived from compound **8** produces cellooctaose derivative. Thus, in this method, a series of cello-oligosaccharides by doubling molecular chain length (whose D.P.= 1, 2, 4, 8, ···) with one-stage glycosylation could be theoretically prepared.

In the present synthetic design, selection of the three kinds of protective groups and β-glycosylation method are very important.

1 — 2 Selection of protective groups

A possible combination of X-, Y- and R-protective groups is shown in Fig. 1-4. In this combination, benzyl and acetyl groups could be considered as candidates for the R-group because these groups are quite widely used as a protective group in the carbohydrate chemistry. In comparison between them, acetyl group was avoided here because of its possibility of intramolecular migration which makes it uncertain regiospecificity, and instability under basic conditions.

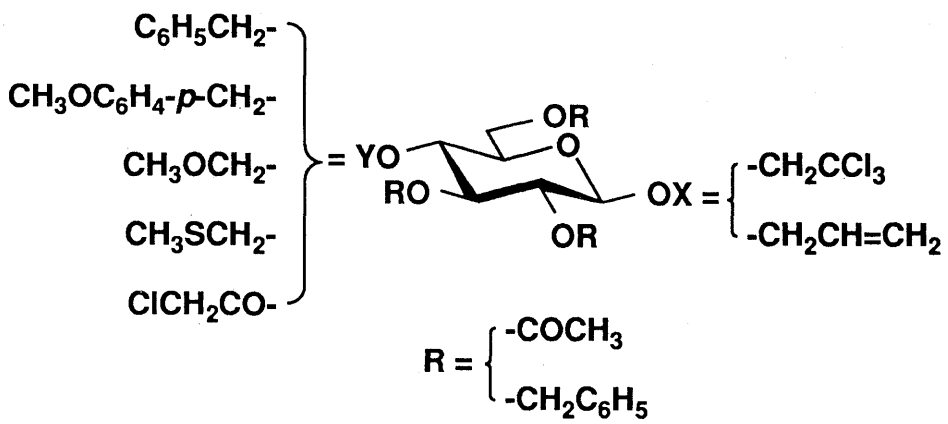


Fig. 1-4. Possible combination of X-, Y- and R-protective groups.

Thus, benzyl group was selected as the R-group, persistent protective group. The merits of the benzyl group as the persistent protective group are as follows:

(1) The benzyl group has high stability under both acidic and basic conditions. This makes it possible to utilize a wide range of reaction conditions for the introduction or removal of the temporary protective groups[22]. (2) There is no possibility of migration with the benzyl group, which makes sure the regiospecific control. (3) Benzyl derivatives of glucose are usually more reactive than acetyl derivatives, especially, in the case of glycosylation reaction, probably because of the electron donating effect of the benzene ring. (4) The UV absorption of the benzyl group makes it possible to easily monitor the reaction products by thin layer chromatography (TLC) under UV-light (254 nm). (5) And benzyl glucose derivatives have good solubility in organic solvents and could be easily crystallized. However, β-glucosidic bond formation resulting from the neighboring group participation is not expected.

Next, 2,2,2-trichloroethyl group was compared with allyl group as a possible X-group (Fig. 1-4). 2,2,2-Trichloroethyl group was found to be introduced by Magnusson method[45] and selectively removed by zinc powder in acetic acid. However, it was found that 2,2,2-trichloroethyl group is unstable under basic reaction conditions such as benzylation reaction to convert into 2,2-dichlorovinyl group[48]. In addition, 2,2-dichlorovinyl group is known to be difficult to be removed selectively. Thus, 2,2,2-trichloroethyl group was eliminated from the candidates for X-group.

Allyl group can be regiospecifically introduced into anomeric hydroxyl group by Köigs-Knorr glycosylation method[42]. The allyl group can be selectively removed by the method consisting of isomerization of the double bond by potassium *t*-butoxide/DMSO and subsequent acid

hydrolysis of the resulted enol ether by hydrochloric acid in acetone[19]. It could be also isomerized under relatively mild conditions (e.g., tristriphenylphosphinerhodium (I) chloride, under neutral conditions[18]). The allyl group had been used for the protection of the anomeric hydroxyl group[5,7,8,20,34]. Thus, it was concluded that the allyl group is suitable as X-group when benzyl group is used as R-group.

When benzyl and allyl groups were used as R- and X-groups, respectively, *p*-methoxybenzyl group is thought to be the most suitable Y-group among the candidates shown in Fig. 1-4. The reasons are:

(1) *p*-Methoxybenzyl group can be introduced to O-4 position by the regioselective reductive cleavage of 4,6-*O*-*p*-methoxybenzylidene derivative of glucose with lithium aluminum hydride and aluminum chloride[32] or with sodium cyanoborohydride and trimethylsilyl chloride[31]. (2) The *p*-methoxybenzyl group are removed oxidatively by the use of cerium (IV) ammonium nitrate (CAN)[31] or of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[49] without any influence on benzyl persistent protective group.

Hence, allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (**11**) was selected as a starting material **1**.

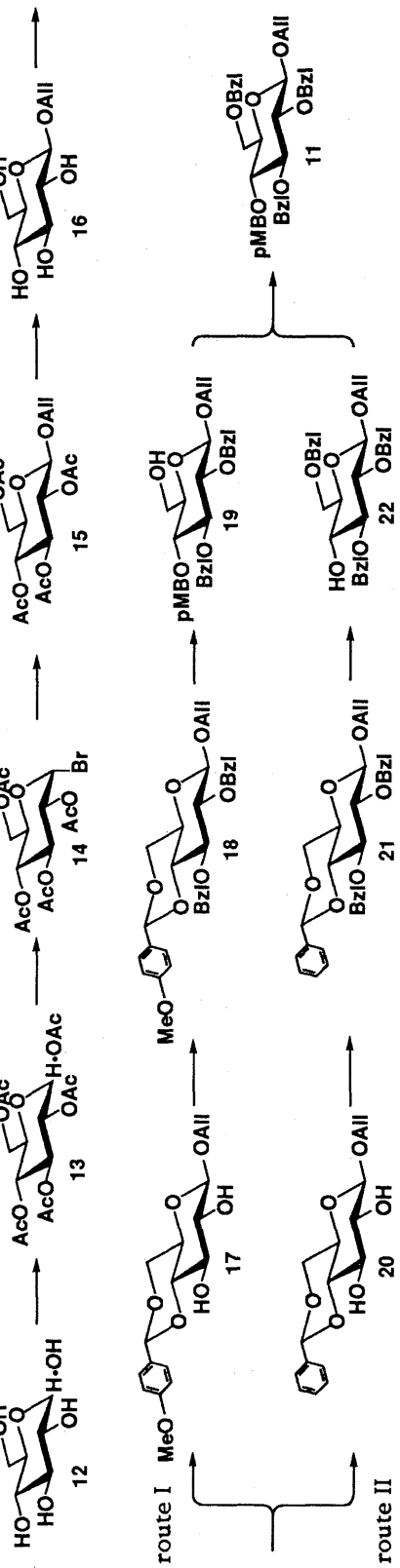
1 — 3 Synthesis of allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside

A synthetic route for the starting material, allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (**11**), is shown in Fig. 1-5.

As the first step of the synthesis, introduction of allyl group into the O-1 position of D-glucose was conducted: the target compound, allyl β-D-glucopyranoside (**16**), was synthesized by a modified method reported by Lee and Lee[44].

As the next step, introduction of the *p*-methoxybenzyl group into the C-4 hydroxyl group of compound **16** should be achieved. The two hydroxyl group at C-4 and C-6 can be distinguished from those at C-2 and C-3 by the different reactivity for benzaldehyde derivatives. In order to distinguish the C-4 hydroxyl group from the C-6 hydroxyl group, regioselective reductive ring-opening reactions will be available. On the other hand, the selective benzylation of benzyl 2,3-di-*O*-benzyl-α-D-glucopyranoside has been reported to give the 6-*O* benzyl ether in a 80 % yield[9], but a synthetic route for the compound **11** *via* this selective benzylation is thought to require more reaction steps and give a lower overall yield than that of proposed synthetic route *via* the reductive ring-opening reaction.

Recently, Garegg and Hultberg[16,17] and Johansson and Samuelsson[31] have been studied on reductive ring-opening reaction of the 4,6-*O*-benzylidene acetal. They concluded that the direction of the reductive opening depends on the relative size of acid used and on the difference in nucleophlicity between the C-4 and the C-6 acetal oxgens. Specifically, the reductive cleav-



12→13: Ac_2O / NaOAc, 80°C, over night, quantitative yield. **13→14**: 30 % - HBr in AcOH, CH_2Cl_2, 0°C→r.t., 4 h, quantitative yield.
14→15: $Hg(CN)_2$ / CH_2=$CHCH_2OH$, Ms4Å, 50°C, 1 h, 93 %. **15→16**: NaOMe, MeOH, 0°C→r.t., 1.5 h, 95 %.
16→17: MeO-C_6H_4-*p*-$CH(OMe)_2$ / *p*-TsOH, dioxane, 30°C, 30 mmHg, 20 min, 87%. **17→18**: $C_6H_5CH_2Br$ / NaH / $(n\text{-}Bu)_4NI$, THF, reflux, 10 h, 95 %. **18→19**: $LiAlH_4$ / $AlCl_3$, CH_2Cl_2-Et_2O, -15°C, 2 h, 96 %. **19→11**: $C_6H_5CH_2Br$ / NaH / $(n\text{-}Bu)_4NI$, THF, reflux, 4 h, 87 %.
16→20: C_6H_5-$CH(OMe)_2$ / *p*-TsOH, dioxane, 30°C, 30 mmHg, 30 min, 75%. **20→21**: $C_6H_5CH_2Br$ / NaH / $(n\text{-}Bu)_4NI$, THF, reflux, 7 h, 81 %.
21→22: $NaCNBH_3$ / HCl-Et_2O, THF, Ms4Å, r.t., 30 min, 72 %. **22→11**: *p*-MeO-$C_6H_4CH_2Cl$ / NaH / $(n\text{-}Bu)_4NI$, THF, reflux, 16 h, 90 %.

Fig. 1-5. Two synthetic routes for glucose derivative **11**.

age of the 4,6-benzylidene acetal of a glucose derivative using lithium aluminum hydride and aluminum chloride (a Lewis acid with relatively large size) preferentially afforded the 4-*O* benzyl derivative. The results from the coordination of the Lewis acid with the C-6 acetal oxygen, due to steric hindrance toward the O-4 position exerted by the 3-*O* and 2-*O* benzyl group. On the other hand, in the reductive cleavage of the same acetal with hydrogen chloride (a molecule much smaller than aluminum chloride) and sodium cyanoborohydride, the protonation preferentially occurred at the C-4 acetal oxygen, which has a higher electron density than that of the C-6 acetal oxygen, to give primarily the 6-*O* benzyl derivative.

Thus, two synthetic routes shown in Fig. 1-5 are examined to obtain starting material **11**.

Compound **16** was treated with *p*-methoxybenzaldehyde dimethylacetal and a catalytic amount of *p*-toluenesulfonic acid (0.05 eq.) to produce compound **17** in 92% yield. The structure of compound **17** was confirmed by ^{1}H-NMR spectral data. The characteristic signal of an acetal proton appeared at δ 5.50 (1H, s) and equatorial proton at C-6 in the rigid acetal ring was deshielded (δ 4.34, dd, J = 10.3, 4.8) and the axial proton at C-6 appeared in a higher field (δ 3.78, t, J = 10.3). The benzylation of compound **17** was made with benzyl bromide/sodium hydride/tetra-*n*-butyl ammonium iodide/THF system. The reaction was found to proceed quantitatively according to TLC, but yield of crystal was low. This low yield may be caused by the removal of the *p*-methoxybenzylidene group from compound **18** by hydrobromic acid generated from excess benzyl bromide during the solvent evaporation. To prevent the formation of hydrobromic acid, excess benzyl bromide was decomposed by addition of methanol to the reaction mixture, and then the reaction mixture was diluted with ethyl acetate, washed with brine, drying over Na_2SO_4, and evaporated *in vacuo* to afford a neutral oil. Thus, compound **18** was obtained in 95% yield after crystallization from ethanol.

Compound **18** was then converted to compound **19** in 96% yield by a modified method reported by Joniak *et al*[32]. using the lithium aluminum hydride/aluminum chloride reaction system. The structure of compound **19** was confirmed by ^{1}H-NMR spectral data. The characteristic signal of the acetal proton appeared at δ 5.50 ppm (present in the spectra of compounds **17** and **18**) had disappeared. Down field shifts of the signals of two protons at C-6 were observed in the ^{1}H-NMR spectrum of the acetate of compound **19**: namely, from δ 3.86 ppm (dd, J = 10.5, 2.5) and δ 3.74 ppm (t, J = 10.5) to δ 4.24 ppm (dd, J = 12.0, 7.5) and δ 4.31 ppm (d, J = 12.0). Thus, the presence of a free hydroxyl group at C-6 in compound **19** was confirmed.

When this reductive ring-opening reaction, using the lithium aluminum hydride/aluminum chloride system, was applied directly to compound **17**, selectivity of the reductive ring-opening was low: both 6-*O* and 4-*O* *p*-methoxybenzyl derivatives were obtained in about 60% and 30% yields, respectively. These results suggest that the 3-*O* and 2-*O* benzyl groups provide

steric hindrance which is indispensable to obtain high selectivity for the production of the desired cleavage product (**19**). Compound **19** was benzylated using the method described above for the preparation of compound **18** to produce compound **11** in 96% yield. The overall yield from compound **16** to compound **11** using synthetic route I was 81%.

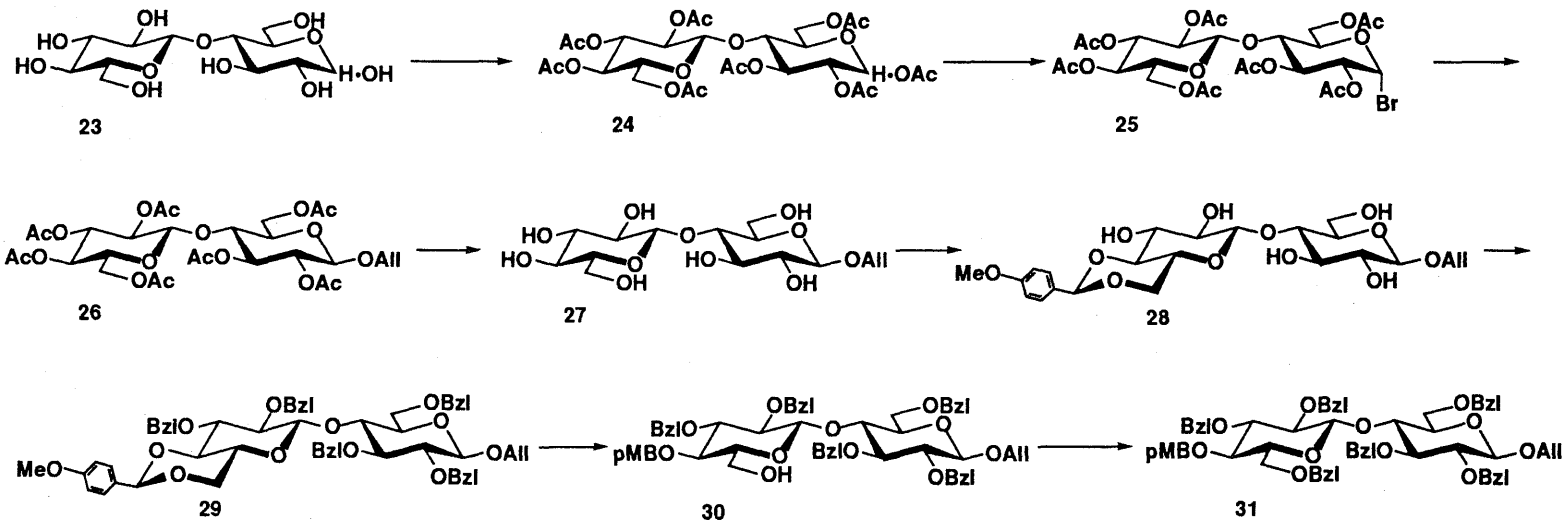
The synthesis of compound **11** was also attempted using the alternative route II. Compound **16** was converted to compound **21** *via* compound **20** by the reported method[58]. The acetal ring of compound **21** was opened selectively with sodium cyanoborohydride/hydrogen chloride in diethyl ether/THF to produce compound **22** in 72% yield. The resulting compound **22** was converted to compound **11** by *p*-methoxybenzylation in 90% yield (overall yield from **16**: 39 %).

Both synthetic route I and II were found to be useful as a synthetic route for compound **11**. Route I seems to be more favorable route because of the higher overall yield from compound **16** to compound **11** without using poisonous reagents such as sodium cyanoborohydride. However, compound **22** may be served as a useful intermediate for the preparation of the benzylated allyl glucose derivatives containing various protective groups (tetrahydropyranyl, methoxymethyl, methylthiomethyl group, and so forth) introduced into the C-4 hydroxyl group as a Y-group.

1 — 4 Synthesis of allyl 2,3,6,2',3',6'-hexa-*O*-benzyl-4'-*O*-(*p*-methoxybenzyl)-β-D-cellobioside from D-cellobiose

Using an analogous synthetic route to the route I shown in Fig. 1-5, allyl 2,3,6,2',3',6'-hexa-*O*-benzyl-4'-*O*-(*p*-methoxybenzyl)-β-D-cellobioside (**31**) was synthesized from D-cellobiose **23** (Fig. 1-6).

Allyl β-D-cellobioside (**23**) was synthesized by deacetylation of allyl 2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-D-cellobioside (**26**) which was prepared by glycosylation of 2,3,6,2',3',4',6'-hepta-*O*-acetyl-α-D-cellobiosyl bromide (**25**) with allyl alcohol in the presence of mercury (II) cyanide. Compound **27** was treated with *p*-methoxybenzaldehyde dimethylacetal and a catalytic amount of *p*-toluenesulfonic acid (0.05 eq.) to produce allyl 4',6'-*O*-(*p*-methoxybenzylidene)-β-D-cellobioside (**28**). Allyl 2,3,6,2',3'-penta-*O*-benzyl-4',6'-*O*-(*p*-methoxybenzylidene)-β-D-cellobioside (**29**), prepared by benzylation of compound **28** with benzyl bromide in sodium hydride/tetra-*n*-butyl ammonium iodide/THF system, was converted into allyl 2,3,6,2',3'-penta-*O*-benzyl-4'-*O*-(*p*-methoxybenzyl)-β-D-cellobioside (**30**) by a modified method as described above using the lithium aluminum hydride/aluminum chloride reaction system. The expected compound **31** was obtained by the benzylation of 6'-OH group in compound **30** by the same reaction system as described above. Overall yield of compound **31** from **23** was 60 %.



23→**24**: Ac_2O / NaOAc, 50°C, over night, quantitative yield. **24**→**25**: 30 % - HBr in AcOH, CH_2Cl_2, 0°C→r.t., 2.5 h, quantitative yield.
25→**26**: $Hg(CN)_2$ / CH_2=$CHCH_2OH$, Molecular Sieve 4Å, 50°C, 1.5 h, 93 %. **26**→**27**: NaOMe, MeOH, 0°C→r.t., 1.5 h, 94 %.
27→**28**: *p*-MeO-C_6H_4-$CH(OMe)_2$ / *p*-TsOH, dioxane, 20-30°C, 30 mmHg, 20 min, 83%. **28**→**29**: $C_6H_5CH_2Br$ / NaH / $(n\text{-}Bu)_4NI$, THF, reflux, 12 h, 91 %.
29→**30**: $LiAlH_4$ / $AlCl_3$, CH_2Cl_2-Et_2O, -15°C, 1 h, 98 %. **30**→**31**: $C_6H_5CH_2Br$ / NaH / $(n\text{-}Bu)_4NI$, THF, reflux, 4.5 h, 92 %.

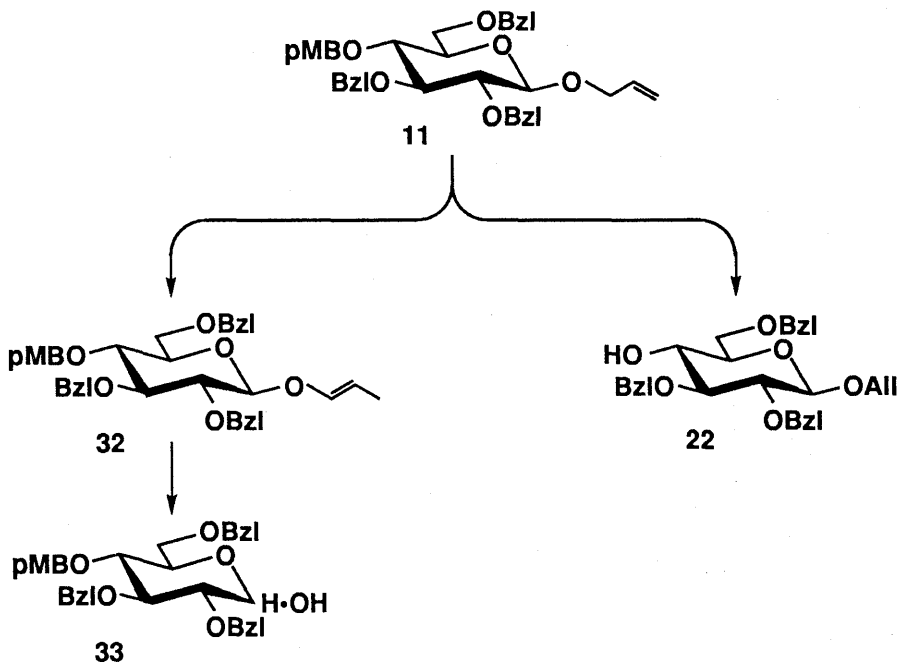
Fig. 1-6. Synthetic route for cellobiose derivative **31**.

1 — 5 Preparation of glycosyl donor and glycosyl acceptor

As shown in Fig. 1-7, the starting material **11** was converted into both glycosyl donor **33** and acceptor **22** by removal of the allyl and the *p*-methoxybenzyl groups, respectively, without any influence on the remaining protective groups.

The glycosyl donor **33** could be prepared from the starting compound **11** by removal of the allyl group. Following three methods were examined for the removal of allyl group, i.e., oxidation with selenium (Ⅳ) dioxide[34], treatment with palladium on activated carbon (Pd/C)[5] and a two-step method[19] consists of isomerization of the double bond to produce enol ether and following acid hydrolysis of the resulted enol ether.

With selenium (Ⅳ) dioxide in acetic acid/dioxane, the allyl group was removed to produce compound **33** in 62% yield. Using Pd/C in the presence of *p*-toluene sulphonic acid, the allyl group could not be satisfactorily removed probably because of the acid-labile *p*-methoxybenzyl group. Finally, the allyl group was removed successfully by the two-step method. Potassium *t*-butoxide in DMSO was used to isomerize the double bond to produce compound **32** in 92% yield. Subsequent acid hydrolysis of the enol ether **32** produced the desired product **33** in 87 % yield (overall yield: 80%). The two-step method was found to be predominant.



11→22: CAN / CH_3CN - H_2O (9 : 1, v/ v), r.t., 15 min., 74 %.
11→32: KO-*t*-Bu, DMSO, 110°C, 12 h, 91 %.
32→33: 3.65 % - HCl, acetone, reflux, 2 h, 71 %.

Fig. 1-7. Preparation of glycosyl acceptor **22** and glycosyl donor **33**.

The glycosyl acceptor **22** was prepared from the compound **11** by the removal of the *p*-methoxybenzyl group. Four methods for the removal of the *p*-methoxybenzyl group were examined, i.e., acid hydrolysis, nitric acid oxidation, DDQ oxidation[49] and CAN oxidation [31].

When the compound **11** was subjected to acid hydrolysis using *p*-toluenesulfonic acid, the *p*-methoxybenzyl group was not removed without partial cleavage of the allyl group. The *p*-methoxybenzyl group was not removed selectively by nitric acid, that is, hydrolysis of both the allyl group and *p*-methoxybenzyl group was observed. The DDQ oxidation gave a low yield (about 60%) because of side reactions. Finally, the *p*-methoxybenzyl group was removed effectively using CAN to produce compound **22** in a 74% yield.

1 — 6 Summary

For the synthesis of cello-oligosaccharide, regiospecific and stereospecific glycosylation reaction should be conducted. In this chapter, basic design for the starting material was discussed at first. Glucose derivative with three kinds of protective groups, X-, Y- and R-groups, allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (**11**), was selected as a starting material. Then, synthetic route for the starting material was investigated. As the result, it was found that the synthetic route including regioselective reductive ring-opening reactions of the *p*-methoxybenzylidene with lithium aluminum hydride/aluminum chloride to give compound **19** is more favorable than the other route because of higher overall yield.

The synthesized starting material **11** was successfully converted into the glycosyl donor **33** and glycosyl acceptor **22**. That is, the glycosyl donor **33** was prepared from compound **11** by the selective removal of allyl group using two-step reactions in 80% yield. On the other hand, the glycosyl acceptor **22** was prepared by the selective removal of *p*-methoxybenzyl group using CAN from the starting material **11** in 74% yield.

In addition, a cellobiose derivative, the compound **31** which has the same protective group system as the starting material **11** was also synthesized successfully from D-cellobiose.

CHAPTER 2 Synthesis of cello-oligosaccharide derivatives

In chapter 1, selection of the starting material for cello-oligosaccharide synthesis and the preparation method of the glycosyl donor **33** and the acceptor **22** from the compound **11**, allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside, were described. Next step is to achieve the stereospecific glycosylation of glycosyl acceptor **22** with donor **33**.

In this chapter, selection of a highly stereoselective glycosylation method and reactivity of glycosyl acceptor **22** with donor **33** on the selected glycosylation are described.

2 − 1 Selection of glycosylation method

Since the first glycosylation reaction was reported by Michael in 1879[47], a number of synthetic methods for *O*-glycoside have been proposed and many reviews have been published[4,30,43,45,62], i.e., alcholysis of acid-catalyzed reducing sugars[11,12,13], synthesis from 1-halo sugars[42], from 1-*O*-acyl sugars[27,29] and so on. However, no universal glycosylation method applicable to the preparation of all kinds of glycosides has been established so far. Thus, the most suitable method should be selected for the preparation of individual glycoside.

Although β-glycoside is successfully prepared by utilizing neighboring group participation of the 2-*O*-acyl group[50], there are only a few methods applied to β-D-glycosylation of benzyl derivative, such as compound **33**, without neighboring group participation.

In the case of benzyl derivatives, it is essential to select a glycosylation method that proceeds *via* a complete SN2 mechanism with Walden inversion at the C-1 position to produce the β-glycoside in high yield.

It has been reported[25] that the fluoride method using α-D-glycosyl fluoride as a glycosyl donor gave the β-D-glycoside in acetonitrile with high stereoselectivity. This method could be applied to a substrate having benzyloxy group at C-2 position, however, it cannot be applied to compound **33**, with an acid-sensitive *p*-methoxybenzyl group because the preparation of the glycosyl fluoride must be carried out in the presence of a strong acid (hydrogen fluoride).

Recently, a highly stereoselective β-glycosylation method, so-called imidate method using trichloroacetimidoyl group as a leaving group introduced into C-1 position of glycosyl donor was developed by Schmidt[53,54]. This method proceeds *via* a complete SN2 mechanism under the selected reaction conditions to afford β-glycoside in high yield from α-imidate, and therefore is applicable to the glycosyl donor without effect of the neighboring group participation exerted by 2-*O* acyl group. Thus, the imidate method is thought to be the most promising method for the glycosylation of glycosyl acceptor **22** with donor **33**.

The imidate method has the following advantages:

(1) Glycosylation can be conducted under mild reaction conditions, i.e., at -78℃ for about 30 min, in the presence of catalytic amounts of BF_3-Et_2O (0.1 eq.). (2) Only β-glycoside is obtained from α-imidate *via* SN2 reaction mechanism. (3) By the selection of catalysts and protective groups, comparable reactivities to Köigs-Knorr's method can be expected. (4) Schmidt and Michel prepared cellotetraose derivative protected with the benzyl group in a high yield by this method[54].

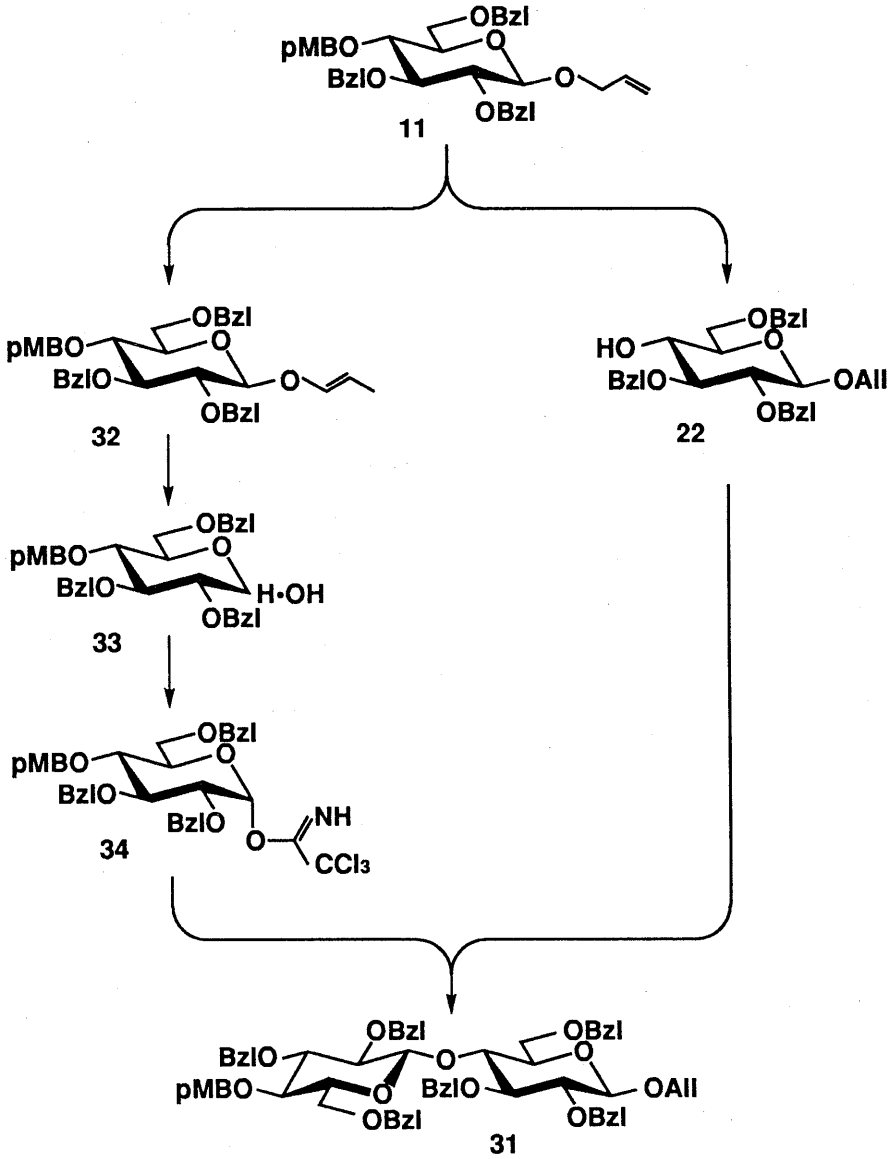
2 — 2 Imidoylation reaction

With the imidate method, the β-glucopyranoside is obtained exclusively when the α-imidate reacted with a glucosyl acceptor in the presence of an acid catalyst in the manner of an SN2 type reaction. Thus, to obtain only the β-glycoside it is indispensable to synthesize the α-imidate in high yield.

The imidoylation reaction gives an equilibrium mixture consisting of α- and β-anomers during the reaction process. Generally, β-imidate is produced under kinetically controlled reaction conditions, but α-anomer under thermodynamically controlled conditions[55], that is, the β-anomer is formed preferentially in early stage of the imidoylation reaction, but the α-anomer gradually becomes the dominant product after longer reaction times. It is also reported that the equilibrium is strongly affected by base[57].

In this study, sodium hydride (NaH)[56] and 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU)[59] were examined as a base for the imidoylation of compound **33**. In the case of NaH, rate of the reaction was accelerated by using a large amount of NaH, but numerous side reactions occurred, and the reaction mixture turned dark. In addition, removal of the by-products from the reaction mixture is difficult because of instability of the imidate. However, in the case of DBU, the reaction mixture did not color. Moreover, the anomerization from the β-imidate to the α-imidate proceeded more rapidly with DBU than with NaH. Thus, DBU was found to be a more suitable base for the present imidoylation.

Thus, compound **33** was treated with 2.0 molar equivalent of trichloroacetonitrile (TCA) in the presence of 0.2 molar equivalent of DBU in dichloromethane at room temperature. After 1 h, conversion to the α-imidate went essentially to completion which was detected on an analytical silica-gel TLC-plate. However, the α-imidate was found to be extremely unstable even on a preparative TLC plate (silica-gel) for the purification, and all purification procedures should be carried out as rapidly as possible. So, the reaction mixture was passed through an alumina column to remove DBU and by-products. The solvent was evaporated as rapidly as possible, and the residue was triturated with *n*-hexane to afford an expected pure α-imidate derivative, trichloroacetimidoyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-α-D-glucopyranoside (**34**), as a colorless powder, in 89% yield. The α-imidate thus obtained is stable for at



11→22: CAN / CH_3CN - H_2O (9 : 1, v/ v), r.t., 15 min., 74 %.
11→32: KO-*t*-Bu, DMSO, 110°C, 12 h, 91 %.
32→33: 3.65 % - HCl, acetone, reflux, 2 h, 71 %.
33→34: TCA / DBU, CH_2Cl_2, r.t., 1.2 h, 89 %.
22+34→31: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 10 min, 84 %.

Fig. 2-1. Synthetic route for cellobiose derivative **31** from **11**.

least one month when it is stored below -20℃ under nitrogen atmosphere.

The structure of compound **34** was confirmed by ^{1}H-NMR spectral data. The imino proton appeared at δ 8.60 ppm (1H, s) and H-1 at δ 6.53 ppm (1H, d, J = 3.5). The chemical shift and coupling constant of H-1 supports the expected α-configuration.

2 — 3 Synthesis of cellobiose derivative

The glycosylation of glycosyl acceptor **22** with imidate **34** was accomplished by the reported method[53,54] (Fig. 2-1). Imidate **34** and glycosyl acceptor **22** were dissolved in anhydrous dichloromethane containing molecular sieve 4 Å. Then the solution was cooled below -70℃ and a catalytic amount of BF_3-Et_2O (0.1 eq.) was added. Under these reaction conditions three kinds of protective groups (benzyl, allyl and *p*-methoxybenzyl groups) were stable. The reaction was followed by a silica-gel TLC (ethyl acetate - *n*-hexane, 1 : 4 v/v). When the imidate **34** is designated as a standard compound (R_S = 1.00) for calculation of R_S value, the R_S value of the glycosyl acceptor **22** is 0.67. As soon as the reaction started, a single new spot appeared at an R_S value of 0.82. The new spot turned purple-red when a silica-gel TLC plate was soaked in a 5% solution of phosphomolybdic acid in ethanol and then heated. This characteristic purple-red was derived from the *p*-methoxybenzyl group[35,36]. Intensity of the new spot gradually increased whereas those of the starting materials decreased. Thus, the product (new spot) may correspond to the expected product formed by a combination of both starting materials. After compound **34** had been consumed, the reaction mixture was worked up and purified by TLC to obtain colorless crystals. All data for the product, such as R_f value, m.p., $[\alpha]_D^{20}$, elemental analysis, ^{1}H- and ^{13}C-NMR were perfectly identical with those of the authentic sample which was synthesized from D-cellobiose by a method similar to that used for the synthesis of compound **31** from D-glucose as described in section 1-3.

Particularly, the ^{1}H- and ^{13}C-NMR data were useful for the identification of the product. In the ^{1}H-NMR spectrum of compound **31** shown in Fig. 2-5, the individual peaks corresponding to benzylic- and pyranosyl ring-protons are fairly difficult to assign because of their overlap, but the signals derived from the allyl (appearing at δ 5.95, 5.31 and 5.19 ppm) and *p*-methoxybenzyl group (appearing at δ 7.05, 6.78 and 3.75 ppm) are assigned clearly. The existence of these characteristic signals provides the structural proof: glycosylation occurred between glycosyl donor having a *p*-methoxybenzyl group and glycosyl acceptor having allyl group. In addition, degree of polymerization of the product can be calculated from the ratio of aromatic protons derived from the benzyl and *p*-methoxybenzyl groups.

Configuration of the anomeric position of this compound newly formed by the glycosylation can be determined by ^{13}C-NMR (Fig. 2-6 (dimer)), but not by ^{1}H-NMR. That is, the peaks appeared at δ 102.7 and 102.3 ppm in ^{13}C-NMR spectrum conclusively indicated the β-

configuration: no signal at around δ 97 ppm for α-configuration[2,3]. The peaks corresponding to the allyl and *p*-methoxybenzyl groups appeared at δ 134.1, 116.9 and 70.0 ppm, and at δ 159.2, 130.5, 129.4, 113.7, 77.8 and 55.1 ppm, respectively. This information from ^{1}H- and ^{13}C-NMR spectral data is extremely important for the identification of the present cello-oligosaccharide derivative (with higher degree of polymerization) which will be discussed later.

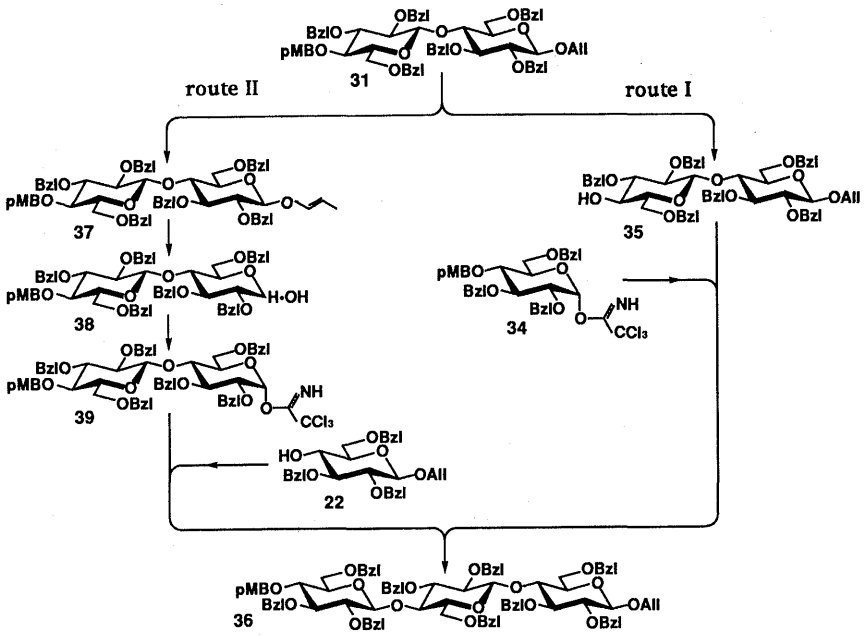
Thus, it was confirmed clearly that the imidate method is an excellent method for the β-glycosylation of 1,4-substituted glucose derivatives having the benzyl group as a persistent protective group.

2 — 4 Synthesis of cellotriose derivative

The synthesized cellobiose derivative **31**, having the same protective group system as that of starting glucose derivative **11**, may be converted to cello-oligosaccharide derivative with a higher D.P. by the same method as that of synthesis of cellobiose derivative **31**. In this section, synthesis of cellotriose derivative starting from synthesized cellobiose derivative is described.

In the synthesis of cellotriose derivative **36** from cellobiose derivative **31**, two synthetic routes as shown in Fig. 2-2 were examined. In the route I, monomer imidate **34** was used as a glycosyl donor for the glycosylation with dimer glycosyl acceptor **35**, and in the route II, dimer imidate **39** was used as a glycosyl donor for the glycosylation with monomer glycosyl acceptor **22**.

First, possibility in the route I was examined because the imidate **39** seems to be unstable and its purification may be difficult. Cellobiose derivative **31** was converted to a dimer acceptor **35** in 89.5% yield by the oxidative removal of the *p*-methoxybenzyl group with CAN. Compound **35** was glycosylated using α-imidate **34** in the presence of a catalytic amount of BF_3-Et_2O (0.1 eq.). The reaction was followed by silica-gel TLC (ethyl acetate - *n*-hexane, 1 : 4, v/v). When imidate **34** was designed as a standard compound for calculation of R_S value (R_S = 1.00), R_S value of the compound **35** and newly appeared product were 0.61 and 0.78, respectively. After all of the compound **34** was consumed, the reaction mixture was worked up and purified by TLC to afford the product (R_S = 0.78) as a colorless oil in 79.1% yield. ^{1}H-NMR spectrum of the product is shown in Fig 2-5 (trimer). Both characteristic signals assigned to allyl (appeared at δ 5.99, 5.33 and 5.15 ppm) and *p*-methoxybenzyl group (appeared at δ 7.05, 6.82 and 3.79 ppm) are clearly appeared. The existence of these characteristic signals provides the structural proof: two starting materials, compound **35** and imidate **34**, are linked each other to afford the product. Because three kinds of protective groups (allyl, *p*-methoxybenzyl and benzyl groups) are stable under the present glycosylation conditions, there is no possibility of glycosidic bond formation at any other position except for C-4'.



31→35: CAN / CH_3CN - H_2O (9 : 1, v/ v), r.t., 15 min., 74 %. **34+35→36**: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 20 min, 79 %.
31→37: KO-*t*-Bu, DMSO, 110°C, 2 h, 85 %. **32→33**: 3.65 % - HCl, acetone, reflux, 2 h, 71 %.
38→39: TCA / DBU, CH_2Cl_2, r.t., 2.5 h, 52 %. **39+22→36**: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 20 min, 50 %.

Fig. 2-2. Two synthetic routes for cellotriose derivative **36**.

The molecular weight of cellotriose derivative **36** was determined to be 1470 by vapor pressure osmometry (VPO), which corresponds to D.P. 2.99. Degree of polymerization of the product was also calculated from ^{1}H-NMR data shown in Fig. 2-5, i.e., from a comparison of the peak areas of the aromatic protons appearing at δ 6.82 ppm (doublet peaks assigned to two aromatic protons of *p*-methoxybenzyl group; 2H) and at δ 7.08 - 7.94 ppm (multiplet peaks assigned to the other aromatic protons including two aromatic protons of *p*-methoxybenzyl group; 47H). Thus, D.P. of the product calculated from NMR data was found to be 3.03. Consequently, the D.P. of the synthesized cello-oligosaccharide derivatives were found to be conveniently determined by ^{1}H-NMR analysis.

The configuration of the newly formed anomeric center by glycosylation was determined by the ^{13}C-NMR spectrum shown in Fig. 2-6 (trimer). The peaks of anomeric centers appearing at δ 102.7, 102.5 and 102.4 ppm indicate the β-configuration (the peak at around δ 97 ppm indicating α-configuration does not appear). The allyl and *p*-methoxybenzyl groups gave the characteristic peaks at δ 134.3, 117.1 and 70.1, and at δ 159.3, 130.2, 129.5, 113.7, 77.5 and 55.3, respectively. These spectral data confirm that the product is surely the expected compound, cellotriose derivative **36**. The overall yield of cellotriose derivative **36** from cellobiose

derivative **31** by route I was 70.8% based on cellobiose derivative **31**.

Next, the possibility by the synthetic route II was examined. In this case, it is indispensable to obtain an α-imidate, compound **39**, as a pure state, which is probably unstable. The allyl group of cellobiose derivative **31** was removed by the two-step method consisting of isomerization of the double bond and acid hydrolysis of the resulted enol ether to give compound **38** in 80.0% yield. Trichloroacetimidoylation of compound **38** was carried out firstly under the same conditions as those of imidoylation of compound **33** (Table 2-1, reaction No.1), but the reaction did not go to completion and unexpected β-imidate still remained. An expected dimer α-imidate **39** was unstable as same as the case of monomer α-imidate **34**: purification of dimer α-imidate **39** by preparative TLC (silica-gel) was also impossible. Then, the imidoylation-reaction conditions of cellobiose derivative **38** was further examined for rapid completion of the reaction. The reaction was traced by the TLC. In the early stage of the reaction, about same amount of α-imidate (ethyl acetate - *n*-hexane, 2 : 3, v/v; R_f = 0.71) and β-imidate (R_f = 0.67) were observed. After the reaction reached to equilibrium (2 h), α / β ratio and amount of the unreacted compound **38** were checked by TLC as shown in Table 2-1. It was found that the α / β ratio after equilibrium depends on the amount of TCA used. Consequently, the reaction No.3 using 0.2 molar equivalent of DBU and 5.0 molar equivalent of TCA were found to be optimum conditions.

Table 2-1. Imidoylation reaction conditions of cellobiose derivative **38**.

No.	DBU[a] (mol. eq.)	TCA[b] (mol. eq.)	α / β [c]	unreacted compound **38** (%)[c]
1	0.2	2.0	8/2	10
2	0.2	4.0	9/1	0
3	0.2	5.0	10/1	0
4	0.2	8.0	7/3	10

a) 1,8-diazabicyclo [5.4.0] undec-7-ene.
b) trichloroacetonitrile.
c) detected by TLC.

The reaction mixture was passed through an alumina column to remove DBU and by-products (see p.20). The solvent was evaporated as rapidly as possible because α-imidate **39** was very unstable. The residue was crystallized from a mixture of ethanol and *n*-hexane (1 : 1, v/v) to afford a colorless crystal of α-imidate **39** in 52% yield. It was observed that α-imidate **39** was partially converted to ethyl β-cellobioside derivative during longer period of crystalliza-

tion (over 24 h) from ethanol. The structure of compound **39** was confirmed by ^{1}H-NMR spectral data: the imino proton appeared at δ 8.66 ppm and H-1 at δ 6.43 ppm (d, J = 3.4) whose coupling constant indicates the expected α-configuration.

Imidate **39** was reacted with the glycosyl acceptor **22** in the presence of a catalytic amount of BF_3-Et_2O (0.1 eq.). The purified product was obtained as a colorless oil in 50.4% yield. All data for the product were perfectly identical with those of the authentic compound **36** obtained by route I. The overall yield of cellotriose derivative **36** from cellobiose derivative **31** by route II was 28.7%.

Route I, giving higher overall yield, is superior to route II, but imidate **39** may be a useful compound for the synthesis of cellotetraose derivative **41** from compound **35** by the convergent synthetic method.

2 — 5 Synthesis of a series of cello-oligosaccharide derivatives up to cellooctamer by linear synthetic method

Since the cellotriose derivative, compound **36**, was successfully synthesized in high yield, it is expected that the synthesis of a series of cello-oligosaccharide derivatives with higher D. P. can be achieved by the same strategy (linear synthetic method) as that of route I shown in Fig. 2-2.

Then, synthesis of a series of cello-oligosaccharide derivatives with higher D.P. by linear synthetic method was examined as follow: *p*-Methoxybenzyl group of cellotriose derivative **36** was removed with CAN to give cellotriose glycosyl acceptor **40**, which was glycosylated with monomer imidate **34** to afford the expected cellotetraose derivative **41** in 78.8% as planned. Furthermore, after four times repeating a set of two reactions, the removal of *p*-methoxybenzyl group and the β-glycosylation reaction with α-imidate **34**, a series of cello-oligosaccharide derivatives up to octamer were synthesized, as shown in Figs. 2-3 and 2-4. Reaction time and yield of each glycosylation reaction were summarized in Table 2-2. Although the yield of each glycosylation reaction is fairly good, glycosylation of the oligomers with higher D.P. requires longer reaction time.

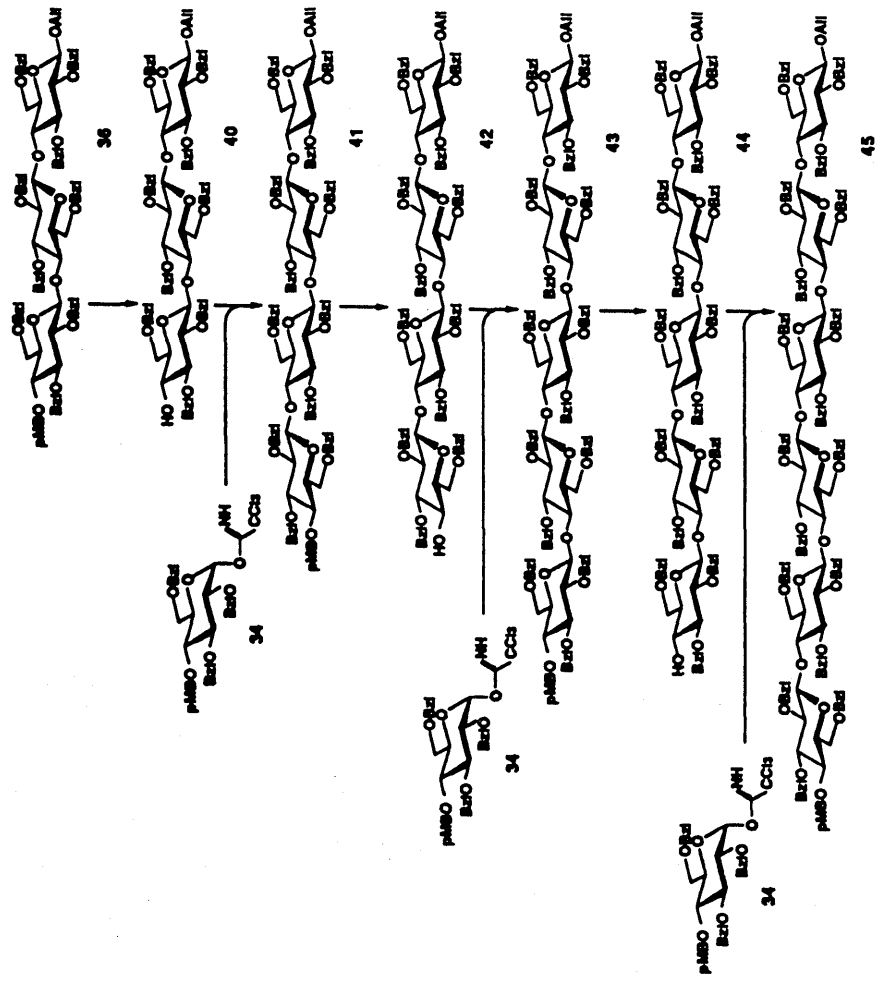
The structures of synthesized cello-oligosaccharide derivatives were confirmed by ^{1}H- and ^{13}C-NMR, and TLC analyses. Figure 2-5 shows the ^{1}H-NMR spectra of cello-oligosaccharide derivative series from monomer to octamer. Peaks derived from allyl and *p*-methoxybenzyl group are clearly assigned. Peak areas of the protons of allyl and *p*-methoxybenzyl groups linked to the terminal units of oligomers decrease with increasing D.P., but the chemical shifts of these protons scarcely change by increase of D.P. Degree of polymerization of the synthesized oligomers estimated by the peak area of ^{1}H-NMR spectrum indicates that each synthesized oligomer has the expected D.P. value (Table 2-2.).

^{13}C-NMR spectra of cello-oligosaccharide derivative series are shown in Fig. 2-6. As mentioned in section 2-3, ^{13}C-NMR is especially useful for the determination of the configuration of the synthesized glycosidic center, namely, peaks appearing at around δ 102 ppm and no anomeric peak at around δ 97 in these spectra reveal that all synthesized glycosidic centers are exactly β-configurations and that the synthesized oligomers are only expected cello-oligosaccharides. The characteristic peaks ascribed to the terminal group, allyl and *p*-methoxybenzyl groups are also easily assigned because the heights of these peaks gradually decrease with increase of D.P., but the chemical shifts of these peaks are not influenced by increasing D.P.

Table 2-2. Reaction conditions of glycosylation and estimated D.P.

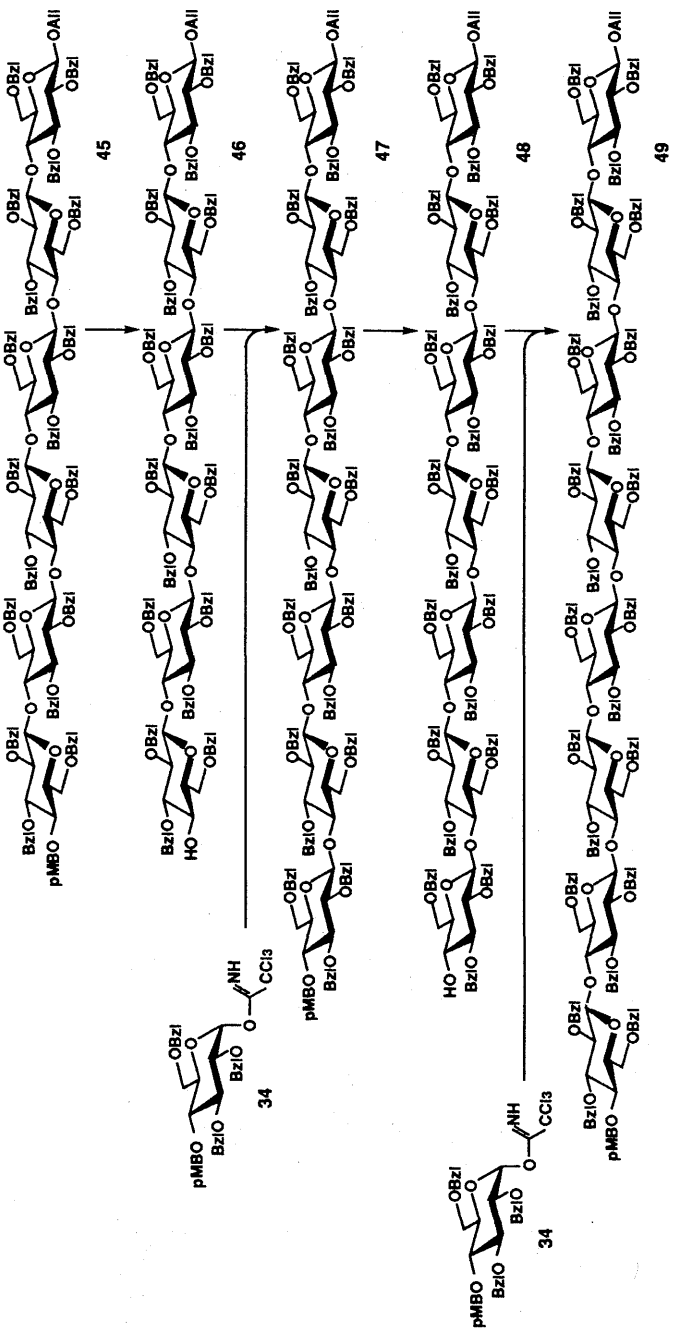
product	reaction time (min)	yield (%)	D.P.*
timer **(36)**	20	79.1	3.03
tetramer **(41)**	25	78.7	3.80
pentamer **(43)**	30	71.8	4.81
hexamer **(45)**	30	76.4	5.72
heptamer **(47)**	45	62.5	6.70
octamer **(49)**	90	73.3	8.09

* estimated from the peak area of NMR spectrum.



36→40: CAN, CH_3CN - H_2O (9 : 1, v/v), r.t., 30 min, 87 %. **40+34→41**: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 25 min, 79 %.
41→42: CAN, CH_3CN - H_2O (9 : 1, v/v), r.t., 15 min, 55 %. **42+34→43**: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 30 min, 72 %.
43→44: CAN, CH_3CN - H_2O (19 : 1, v/v), r.t., 50 min, 93 %. **44+34→45**: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 30 min, 76 %.

Fig. 2-3. Linear synthetic route for a series of cello-oligosaccharide derivatives from cellotriose derivative **39** to cellohexaose **45**.



45→46: CAN, CH_3CN - H_2O (19 : 1, v/v), r.t., 90 min, 39 %. **46+34→47**: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 45 min, 63 %.
47→48: CAN, CH_3CN - H_2O (19 : 1, v/v), r.t., 90 min, 38 %. **48+34→49**: BF_3-Et_2O, Ms4Å, CH_2Cl_2, -70°C, 90 min, 73 %.

Fig. 2-4. Linear synthetic route for a series of cello-oligosaccharide derivatives from cellohexaose derivative **45** up to cellooctaose derivative **49**.

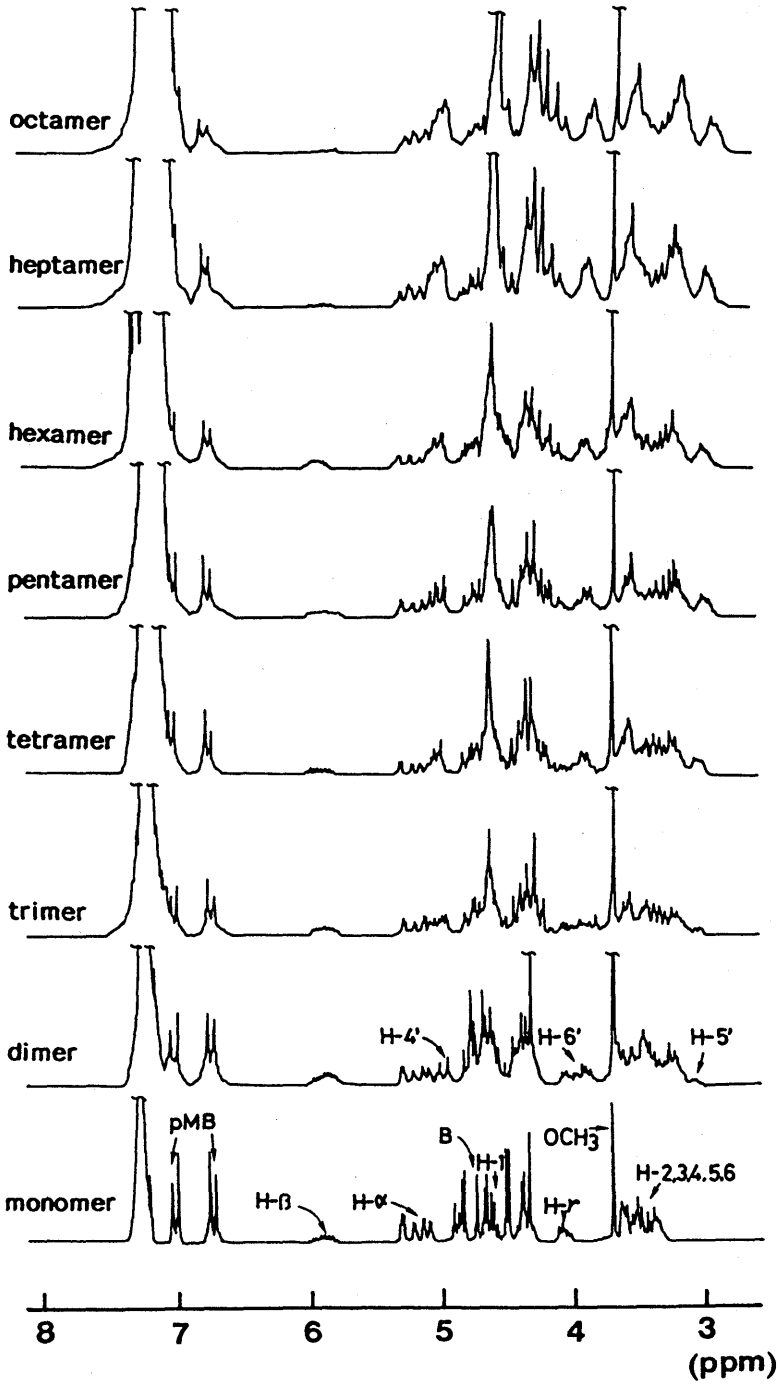


Fig. 2-5. ^{1}H-NMR spectra of cello-oligosaccharide derivatives.

Legends : B=-C*H*$_2$-C$_6$H$_5$, H-α =-CH$_2$-C*H*$_2$

H-β =-CH$_2$-C*H*=CH$_2$, H-γ =-C*H*$_2$-CH=CH$_2$

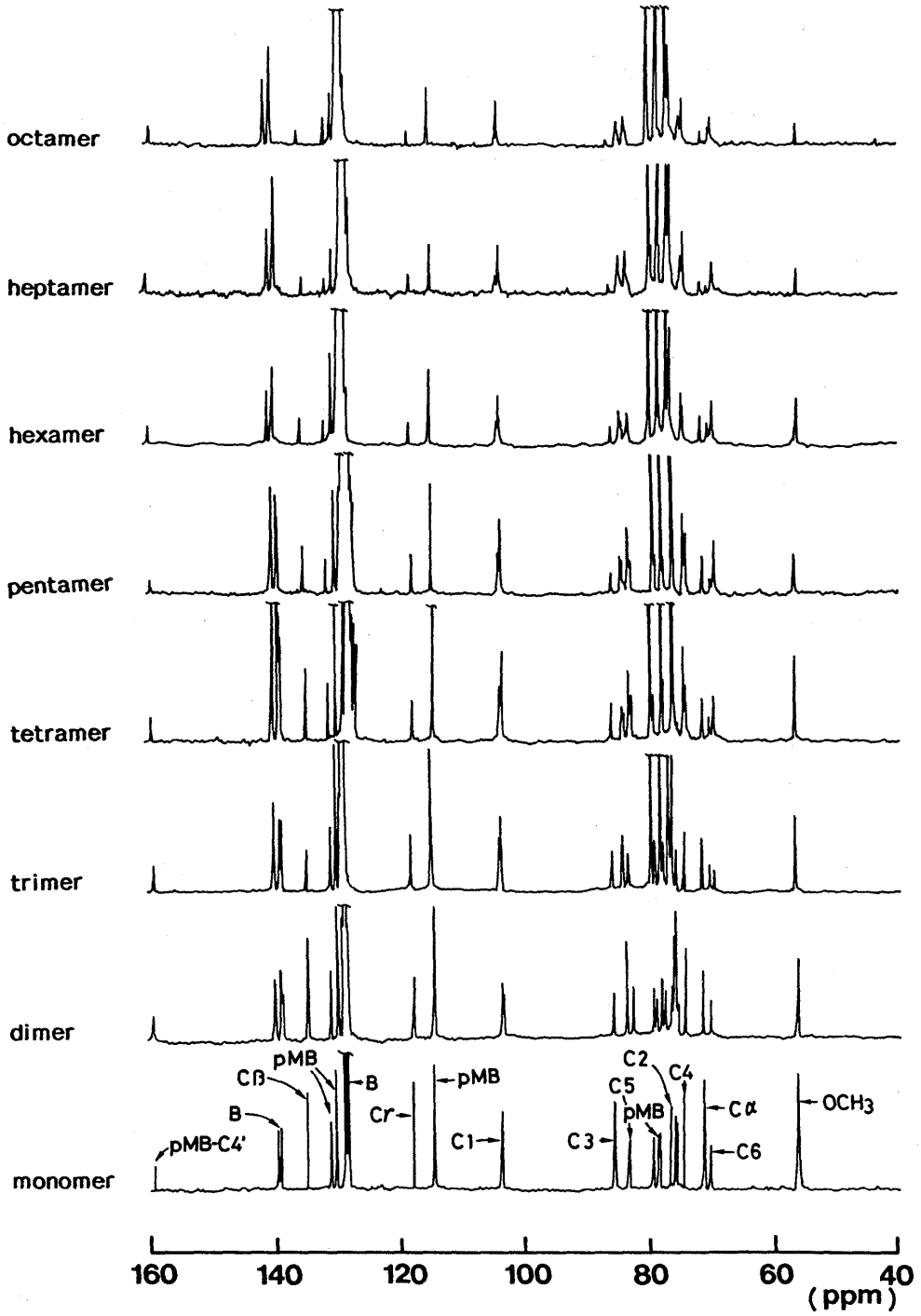


Fig. 2-6. ^{13}C-NMR spectra of cello-oligosaccharide derivatives.

Legends : B=-CH_2-C_6H_5, C α =-CH_2-CH=CH_2

C β =-CH_2-CH=CH_2, C γ =-CH_2-CH=CH_2.

2 — 6 Synthesis of cellotetraose and cellooctaose derivatives by convergent synthetic method

The convergent synthetic route is shown in Fig. 2-7. The glycosylation of dimer glycosyl acceptor **35** with dimer imidate **39** was accomplished by a modified method of that described in section 1-5. Glycosyl acceptor **35** and a catalytic amount of BF_3-Et_2O (0.1 eq.) were dissolved in anhydrous dichloromethane containing molecular sieve 4 Å, and the mixture was cooled below -70℃. To the mixture, the solution of imidate **39** (1.0 molar equivalent for the glycosyl acceptor **35**) in dichloromethane was added. The reaction was monitored by TLC, in which imidate **39** and glycosyl acceptor **35** appeared at R_f = 0.40 and 0.29, respectively (ethyl acetate - *n*-hexane, 1 : 4, v/v). A new spot (R_f = 0.37) appeared. After 20 min of the reaction time, all imidate **39** added was consumed, but unreacted glycosyl acceptor **35** was still remained. Hence, imidate **39** was added in stepwise until glycosyl acceptor **35** was consumed. The reaction mixture was worked up to give colorless oil. Purification was done with preparative TLC to afford cellotetraose derivative **41** as a colorless syrup in 77% yield based on glycosyl acceptor **35**. The signals patterns of ^1H- and ^{13}C-NMR spectra and R_f-value of the product were perfectly identical with those of the authentic sample **41** synthesized by the linear synthetic method.

As cellotetraose derivative **41** has the same protective system as that of compound **31**, it may be converted to cellooctaose derivative **49** by the same convergent method. Compound **41** was converted into the glycosyl donor **51** and glycosyl acceptor **42**. Then, compound **51** was converted into tetramer imidate **52**, but purification of imidate **52** was found to be difficult: the starting material **51** still remained even under the optimum reaction conditions, No.2 or 3, shown in Table 2-1 and the imidate **52** was too unstable and decomposed during purification procedure. Thus, after the reaction mixture was passed through aluminum column (dichloromethane - *n*-hexane, 1 : 1, v/v) to remove DBU, it was submitted to the convergent synthesis with compound **42** without further purification. The TLC analysis of the glycosylation reaction mixture indicated that only trace amount of the expected compound **49** (R_f = 0.35) was produced.

From these results, the convergent synthesis of cellooctaose derivative may be accomplished by isolation of the pure α-imidoyl-cellotetraose derivative. For this purpose, imidoylation-reaction conditions of the compound **51** should be examined, otherwise alternative protective system to stabilize imidate derivative, such as acyl group have to be used.

2 — 7 Summary

In this chapter, synthesis of cello-oligosaccharide derivatives up to octamer was described. For the glycosylation of compound **22**, the imidate method was selected (section 2-1), and

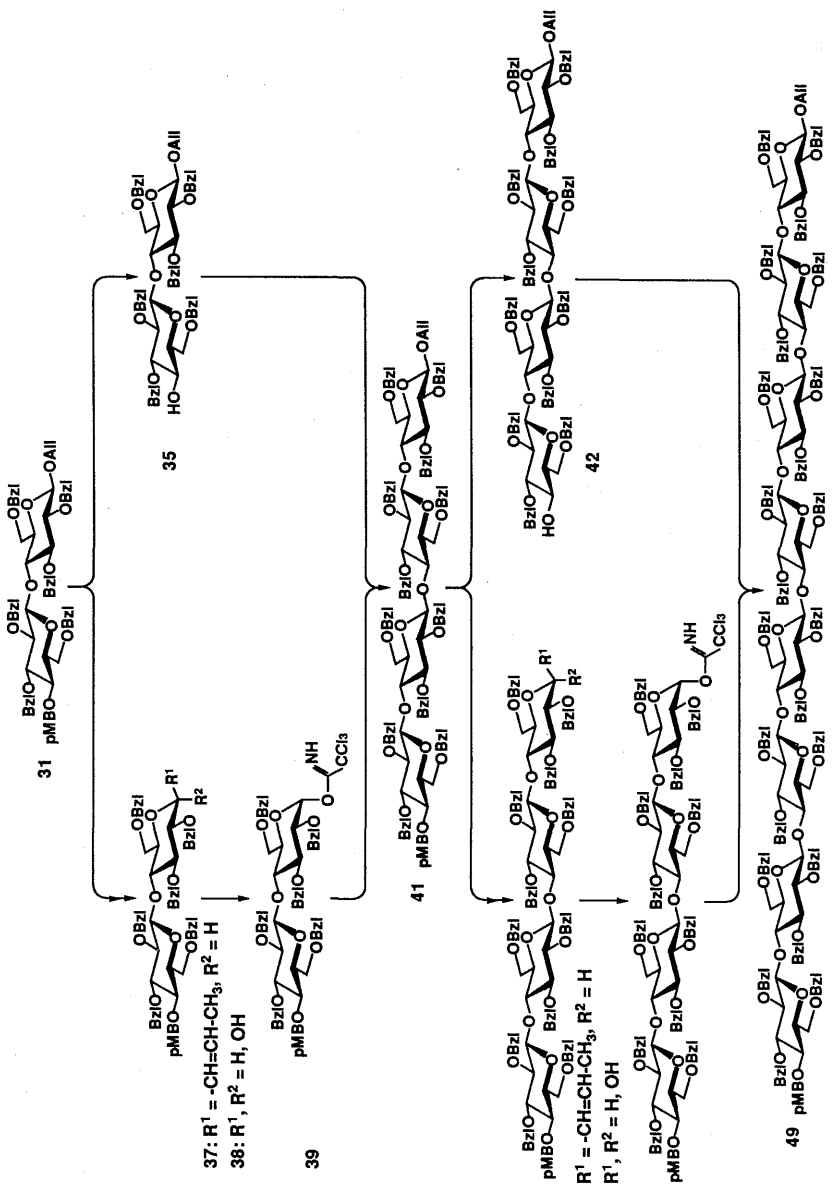


Fig. 2-7. Convergent synthetic route for cellotetraose derivative **41** and cellooctaose derivative **49**.

a leaving group, trichloroacetimidoyl group, was selectively introduced into C-1 position in the presence of a catalytic amount of DBU (0.2 eq.) to afford α-imidate **34** (section 2-2). α-Imidate **34** was obtained as a colorless powder in 89% yield.

Then, by the glycosylation of glycosyl acceptor **22** with α-imidate **34**, the cellobiose derivative **31** was synthesized in 84% yield (section 2-3). The information from ^{1}H- and ^{13}C-NMR spectral data was found to be useful for identification of the chemical structure.

Synthesized cellobiose derivative **31** has the same protective system as starting compound **11**. In section 2-4, synthesis of cellotriose derivative **36** from cellobiose derivative **31** was investigated by two routes, I and II (Fig. 2-2). In route I, dimer acceptor **35** was glycosylated with monomer α-imidate **34** to afford cellotriose derivative **36** in 79.1% yield (overall yield from cellobiose derivative **31** was 70.8%). In route II, monomer glycosyl acceptor **22** was glycosylated with dimer α-imidate **39** in 50.4% yield (overall yield from **31** was 28.7%). Route I, giving higher overall yield is superior to route II. Dimer α-imidate **39** may be useful for the synthesis of cellotetraose derivative by convergent synthetic method.

Furthermore, repeating a set of two reactions, removal of *p*-methoxybenzyl group and β-glycosylation reaction of α-imidate **34** (linear synthetic method), a series of cello-oligosaccharide derivatives up to octamer was synthesized (section 2-5). Glycosylation of the oligomer with the higher D.P. took longer reaction time, but the yield of each glycosylation reaction was fairly good (Table 2-2).

Next, the convergent synthetic method (Fig. 2-7) was investigated (section 2-6). Although cellotetraose derivative **41** was synthesized by the glycosylation of dimer glycosyl acceptor **35** with dimer α-imidate **39** in 77% yield (based on compound **35**), only trace amount of cellooctaose derivative **49** was produced by the glycosylation of tetramer glycosyl acceptor **42** with tetramer α-imidate **52**. This is due to the preparation of tetramer α-imidate **52**.

CHAPTER 3 Preparation of cello-oligosaccharides

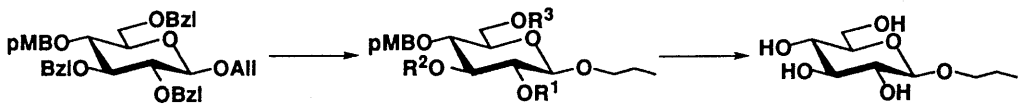
Final stage of the synthesis is removal of three kinds of protective groups from cello-oligosaccharide derivatives. In this chapter, reactivities and removal of three kinds of protective groups and identification of the obtained products are discussed.

3 — 1 Reactivities of protective groups

Among three kinds of protective groups of synthesized cello-oligosaccharide derivatives, two protective groups, allyl and *p*-methoxybenzyl group, can be removed by the method described in section 1-5. Hence, the removal method for benzyl group is discussed in this section.

Benzyl group can be readily removed by a neutral hydrogenolysis in the presence of catalyst[46]. However, some catalyst such as Raney nickel causes reduction of hemiacetal linkage to convert aldoses into their corresponding alditols[33], which should be avoided in this case. Fortunately, the cleavage of benzyl group by hydrogenation with a Pd/C catalyst proceeds without converting into a corresponding glycitol[1]. Therefor, the Pd/C catalyst was selected for our purpose.

As a model experiment, the catalytic hydrogenation of glucose derivative **11**, which has the same protective group system as the synthesized oligosaccharide derivatives, was conducted in a mixed solvent of ethanol and acetic acid (10 : 1, v/v) at 50℃ in the presence of 5% Pd/C and monitored by TLC. After 15 min reaction, spot of the starting compound **11** disappeared, and eight spots appeared on a TLC plate under UV-light (254 nm). When the TLC plate was soaked in 5% solution of phosphomolybdic acid in ethanol and subsequently heated, these eight spots turned to purple-red which indicates the presence of a *p*-methoxybenzyl group[35,36]. Structure of these eight compounds were determined by ^{1}H-NMR analysis after isolation by preparative TLC.



53: $R^1 = R^2 = R^3 = Bzl$ **57**: $R^1 = Bzl, R^2 = R^3 = H$

54: $R^1 = Bzl, R^2 = H, R^3 = Bzl$ **58**: $R^1 = H, R^2 = Bzl, R^3 = H$

55: $R^1 = H, R^2 = R^3 = Bzl$ **59**: $R^1 = R^2 = H, R^3 = Bzl$

56: $R^1 = R^2 = Bzl, R^3 = H$ **60**: $R^1 = R^2 = R^3 = H$

11→(53,54,55,56,57,58,59,60)→61: H_2, Pd/C, EtOH - AcOH (10 : 1, v/v), 50 °C, 4 h, quantitative yield.

Fig. 3-1. Hydrogenation of compound **11**.

Table 3-1 shows the ^{1}H-NMR chemical shifts of these eight compounds and their acetates, together with the R_f-values developed with two solvent systems, A [methanol and dichloromethane (5 : 95, v/v)] and B [ethyl acetate and *n*-hexane (1 : 2, v/v)]. ^{1}H-NMR spectra of all these

Table 3-1. R_f-value and chemical shifts of ^{1}H-NMR spectra of compounds **53-60**.

Compounds	R_f-Values		Chemical shifts (ppm)			Chemical shifts of acetate (ppm)		
	Solvent A[a]	Solvent B[b]	*H*-2	*H*-3	*H*-6	*H*-2	*H*-3	*H*-6
53	0.99	0.81	3.43	3.54	3.65 3.73	—	—	—
54	0.72	0.66	3.26	3.48	3.66 3.75	3.32	5.18	3.67 3.72
55	0.61	0.56	3.54	3.57	3.67 3.73	4.98	3.63	3.67 3.74
56	0.46	0.47	3.41	3.53	3.82 3.92	3.43	3.51	4.22 4.32
57	0.31	0.27	3.23	3.46	(3.6)[c] (3.7)[c]	3.32	5.22	4.21 4.32
58	0.29	0.18	3.51	(3.5)[d]	(3.6)[c] (3.7)[c]	4.98	3.67	4.21 4.35
59	0.17	0.11	3.42	3.50	3.69 3.76	4.93	5.21	(3.5)[c] (3.8)[c]
60	0.09	0.07	3.36	3.48	3.80 3.90	4.89	5.24	4.20 4.35

a) Methanol - dichloromethane (5 : 95, v/v).

b) Ethyl acetate - *n*-hexane (1 : 2, v/v).

c) These peaks were too broad to be assigned.

d) This signal was present too near to be distingusighed from signal of *H*-4.

eight compounds gave the characteristic signals for *p*-methoxybenzyl group at δ 3.80 ppm (3H, s, -OC*H*$_3$), δ 6.90 ppm (2H, d, J = 8.8, aromatic-*H*α) and δ 7.29 ppm (2H, d, J = 8.8, aromatic-*H*β) and for propyl group at δ 0.90 ppm (3H, t, J = 7.0, -CH$_2$-CH$_2$-C*H*$_3$), δ 1.59 ppm (2H, dt, J = 7.0, 7.0, -CH$_2$-C*H*$_2$-CH$_3$) and δ 3.44 and 3.84 ppm (each 1H, dt, J = 9.2, 7.0, -C*H*$_2$-CH$_2$-CH$_3$).

The positions of the free hydroxyl groups were estimated by acetylation, which caused a down-field shift of the signal of the methine proton attached to the free hydroxyl group. *H*-2, *H*-3 and *H*-6 signals were shifted by acetylation about 1.5, 1.7 and 0.5 ppm to lower field, respectively.

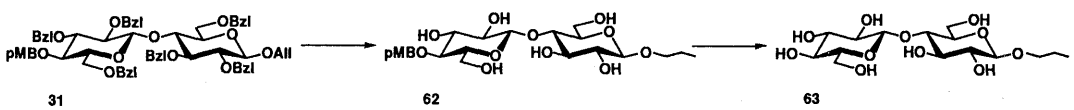
As a result, these eight spots were determined to be a series of propyl 4-*O*-*p*-methoxybenzyl glucose derivatives **53**-**60** shown in Fig. 3-1. Compound **53** is tribenzyl derivative, compounds **54**, **55** and **56** are dibenzyl derivatives, compounds **57**, **58** and **59** are monobenzyl derivatives, and compound **60** has no benzyl group.

In addition to the above, the R$_f$-values of compounds **53**-**60** suggested a polar nature of the 2-OH, 3-OH and 6-OH groups. Generally, R$_f$-values on a normal phase TLC plate (silica gel) increase with decrease of polarities of compounds. Among the dibenzyl derivatives (compounds **54**-**56**), the R$_f$-values of the compounds decreased in the order of **54**>**55**>**56**, suggesting that the polarity increased in the order of 3-OH<2-OH<6-OH. Among the monobenzyl derivatives (compounds **57**-**59**), the polar nature of 2-OH, 3-OH and 6-OH also were suggested in the above order, except for compound **59**. Compound **59**, in which the 6-OH group having a greater polarity than the 2-OH and 3-OH groups was protected by a benzyl group, was presumed to have a lesser polarity than compounds **57** and **58**, in which the 6-OH group is free. Contrary to this presumption, R$_f$-values showed that compound **60** has a greater polar nature than compound **58** and **59**. The vicinal-diol system such as in compound **60** is thought to have a special adsorption behavior on a silica gel plate different from the isolated-diol system.

After 2 h, the product gave only two spots corresponding to compounds **60** and **61**. Finally, compound **61** was isolated in almost quantitative yield after 4 h.

The above results on hydrogenation can be summarized as follows:

1) The allyl group was reduced to a propyl group within 15 min prior to debenzylation. 2) The benzyl group on 6-*O* was removed faster than the others on 2-*O* and 3-*O*, probably



31→(**62**)→**63**: H_2, Pd/C, EtOH - AcOH (10 : 1, v/v), 50°C, 6 h, 99 %.

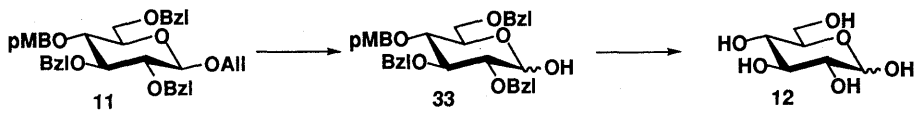
Fig. 3-2. Hydrogenation of compound **31**.

because of their different steric hindrances. 3) The *p*-methoxybenzyl group exhibited less reactivity than did the benzyl group because of an electron-donating effect of the methoxyl group.

As shown in Fig. 3-2, catalytic hydrogenation of cellobiose derivative **31** proceeded in similar manner to that of glucose derivative **11**. Propyl β-D-cellobioside (**63**) was obtained in a quantitative yield *via* propyl 4'-*O*-(*p*-methoxybenzyl)-β-D-cellobioside (**62**) after 6 h. The glycosidic bond was stable under the hydrogenation conditions[38].

Although the reaction conditions of catalytic hydrogenation in a mixed solvent of ethanol and acetic acid at 50℃ in the presence of 5% Pd/C was exhibited to remove benzyl and *p*-methoxybenzyl groups successively without any influence on glycosidic bonds, hydrogenated allyl group (propyl group) was not removed. There is no method to remove the propyl group without any influence on glycosidic bonds. This means that allyl group should be removed before hydrogenolysis.

Conversion of compounds **11** and **31** into glucose and cellobiose based on the above results are shown in Figs. 3-3 and 3-4, respectively. Compound **11** and **31** were at first converted to 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-D-glucopyranose (**33**)[35] and 2,3,6,2',3',6'-hexa-*O*-benzyl-4'-*O*-(*p*-methoxybenzyl)-D-cellobiose (**38**)[37], respectively. Then, both compounds **33** and **38** were converted to **12** and **23**, respectively, in almost quantitative yields under the reaction conditions of catalytic hydrogenation in a mixed solvent of ethanol and acetic acid at 50 ℃ in the presence of 5% Pd/C. NMR spectra of these acetate derivatives were identical to the authentic samples.

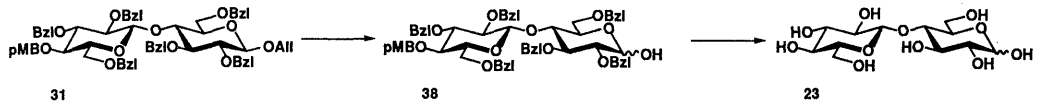


11→33: KO-*t*-Bu, DMSO, 110°C, 12 h; 3.65 % - HCl, acetone, reflux, 2 h, total 65 %.
33→12: H_2, Pd/C, EtOH - AcOH (10 : 1, v/v), 50°C, 3 h, quantitative yield.

Fig. 3-3. Deprotection of compound **11**.

The present hydrogenation was found to be strongly effected by the solvents used. Deprotection of compounds **33** and **38** was completed in 48 h in ethanol and in 2 h in acetic acid. Deprotection in a mixed solvent of formic acid and methanol (1 : 9, v/v)[52] also gave expected products in 16 h in over 90% yields.

Thus, three kinds of protective groups could be removed in quantitative yields without any influences on glycosidic bonds: the removal of the allyl group by the two-step method followed by the hydrogenolysis of the benzyl and *p*-methoxybenzyl groups with the Pd/C catalyst. This method will be applied to prepare cello-oligosaccharide with higher D.P.



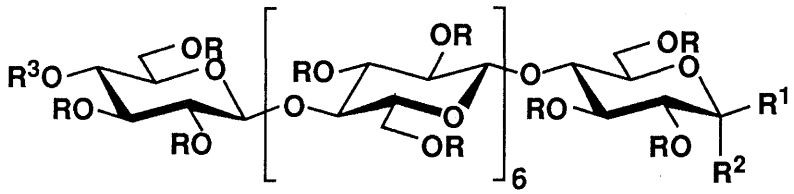
31→**38**: KO-*t*-Bu, DMSO, 110°C, 2 h; 3.65 % - HCl, acetone, reflux, 2 h, total 60 %.
38→23: H_2, Pd/C, EtOH - AcOH (10 : 1, v/v), 50°C, 4 h, quantitative yield.

Fig. 3-4. Deprotection of compound **31**.

3 — 2 Removal of protective groups from cellooctaose derivative

Removal of the three kinds of protective groups of synthesized cellooctaose derivative **49**, prepared by the linear synthetic method, was tried by the modified method described in section 3-1 (Fig. 3-5).

First, allyl group of compound **49** was removed by the method described in section 1-5 to obtain 1-OH free cellooctaose derivative **64**. Hydrogenolysis of compound **64** and subsequent acetylation gave the expected cellooctaose acetate **69**, with less than 10% yield. Then, to improve the yield, a modified method was examined. That is, compound **64** was acetylated in order to protect an anomeric hydroxyl group to afford compound **65**. *p*-Methoxybenzyl group of compound **65** was removed with CAN to afford compound **66**, and the hydroxyl group newly generated was acetylated to give compound **67**. Removal of benzyl groups of compound **67** by a hydrogenolysis with 10% Pd/C catalyst in a mixture of ethanol and acetic acid (10 : 1, v/v) to obtain compound **68** and subsequent acetylation with acetic anhydride and pyridine produced cellooctaose acetate **69** as a syrup in 63% yield. Crystallization of compound **69** from 95% ethanol[6,65,66] was failed as shown in Fig. 3-6 (A). However, it was found that crystallization from methanol/dichloromethane solvent system to give colorless leaflet-like crystals was successful (m.p. 268-270℃, Figs. 3-6 (B) and (C))[39].



49: R= Bzl, R^1= OAll, R^2= H, R^3= pMB
64: R= Bzl, R^1, R^2= H•OH, R^3= pMB
65: R= Bzl, R^1, R^2= H•OAc, R^3= pMB
66: R= Bzl, R^1, R^2= H•OAc, R^3= H
67: R= Bzl, R^1, R^2= H•OAc, R^3= Ac
68: R= H, R^1, R^2= H•OAc, R^3= Ac
69: R= Ac, R^1, R^2= H•OAc, R^3= Ac
70: R= Ac, R^1= H, R^2= OAc, R^3= Ac

Fig. 3-5. Synthetic route for α-D-cellooctaose hexacosaacetate **70**.

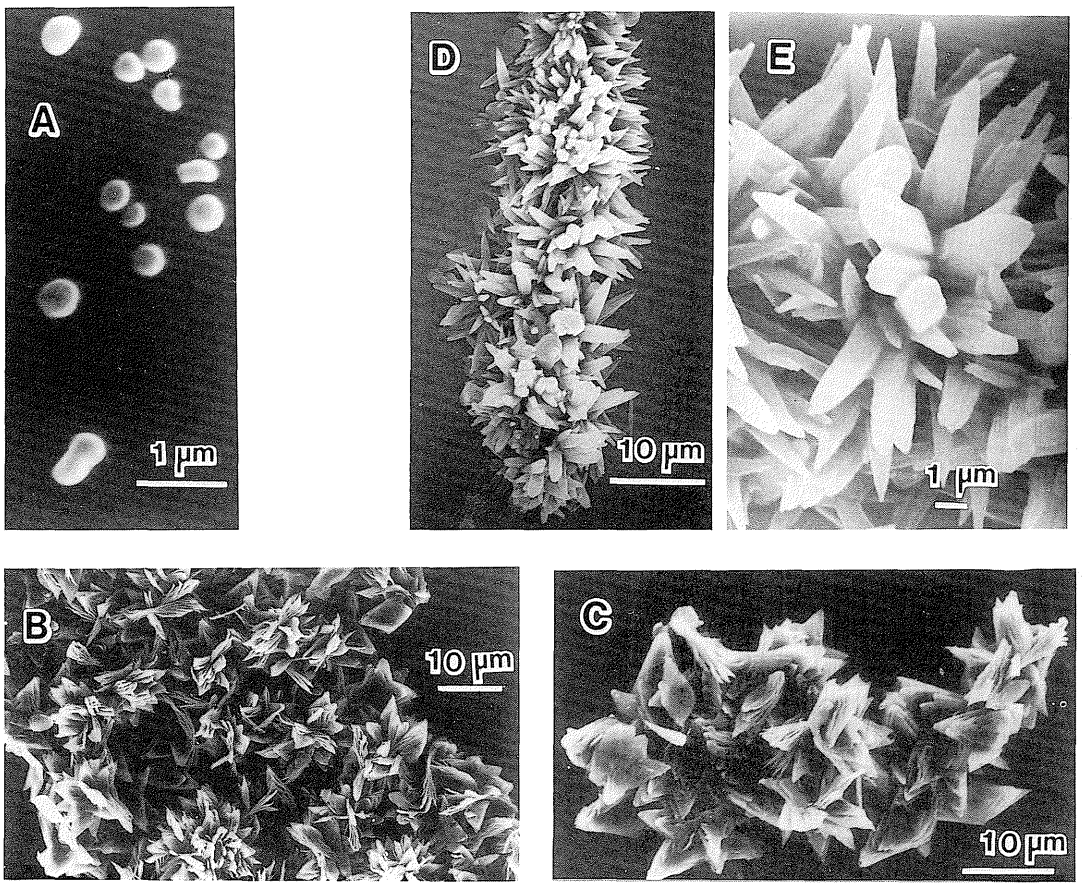


Fig. 3-6. Scaning electron micrographs of cellooctaose acetates.
Legends: A: precipitate from 95% ethanol.
B, C: crystals of compound **69**.
D, E: crystals of compound **70**.

The fast atom bombardment mass spectrometry (FABMS) of compound **69** employing 3-nitrobenzyl alcohol as a sample matrix gave a spectrum shown in Fig. 3-7. The spectrum gave the molecular ion peak at m/z 2349 which was assigned as $(M-OAc)^+$ ion peak caused by loss of the anomeric acetate occurred in ionization. No other peaks appeared at higher field than m/z 2350. The ion peaks at m/z 2060, 1772, 1483, 1195, 907, 619 and 331 were afforded with subsequent fragmentation at each glycosidic linkage. These loss of m/z 288 or 289 (value of 289 was afforded with the effect of ^{13}C isotope) are corresponding to one glucose residue. Any small peaks would be derived from contaminations, which should appear in the mass spectrum of cello-oligosaccharide acetates prepared by acetolysis of cellulose[6], were not observed.

For identification with the authentic sample reported[6], pure α-acetate **70** was prepared from compound **69** by treating a mixture of acetic anhydride and acetic acid in the presence of zinc (II) chloride. The product was crystallized from methanol and dichloromethane (1 : 1, v/v) to afford colorless crystals (crystal A), and further crystallization from the mother liquid gave colorless crystals (crystal B, m.p. 264-269℃).

^1H-NMR spectra of crystals A and B are shown in Fig. 3-8. The signals were assigned by the comparison with the chemical shifts of cellobiose acetate and also with the values in the literature[6]: acetyl protons at δ 1.95-2.18 ppm, H-5 at δ 3.50-3.62 ppm, H-4 at δ 3.70-3.79 ppm, H-6a at δ 3.97-4.14 ppm, H-6b and H-1 of internal and non reducing end residue at δ 4.31-4.80 ppm, H-2 and H-3 at δ 4.78-5.15 ppm and H-3 of reducing end at δ 5.41 ppm.

Furthermore, in the spectrum of crystal A (Fig. 3-8 (a)), the signal appeared at δ 6.24 ppm (d, J = 3.77 Hz) and 5.65 ppm (d, J = 8.11 Hz) are assigned as reducing end H-1 of α- and β-anomers, respectively: the ratio of α/β is 8.6/1. On the other hand, in the spectrum of crystal B (Fig. 3-8 (b)), the signals derived from β-anomer is not appeared: crystal B consists of only α-anomer.

The ^{13}C-NMR of crystal B (compound **70**) is shown in Fig. 3-9. Signals derived from the methyl carbons (appearing at δ 20.50-20.88 ppm), the carbonyl carbons of acetyl groups (appearing at δ 169.28-170.20 ppm) and the ring carbons (appearing at δ 62.11, 67.92, 69.48, 70.83, 71.70, 72.18 and 72.93 ppm) were well identical with those reported[6]. Anomeric carbon of reducing end residue gave the signal corresponding to α-anomer appeared at δ 89.03 ppm. Other anomeric carbons, existed on internal and non reducing end residues gave signals at δ 100.49 and 100.81 ppm, respectively.

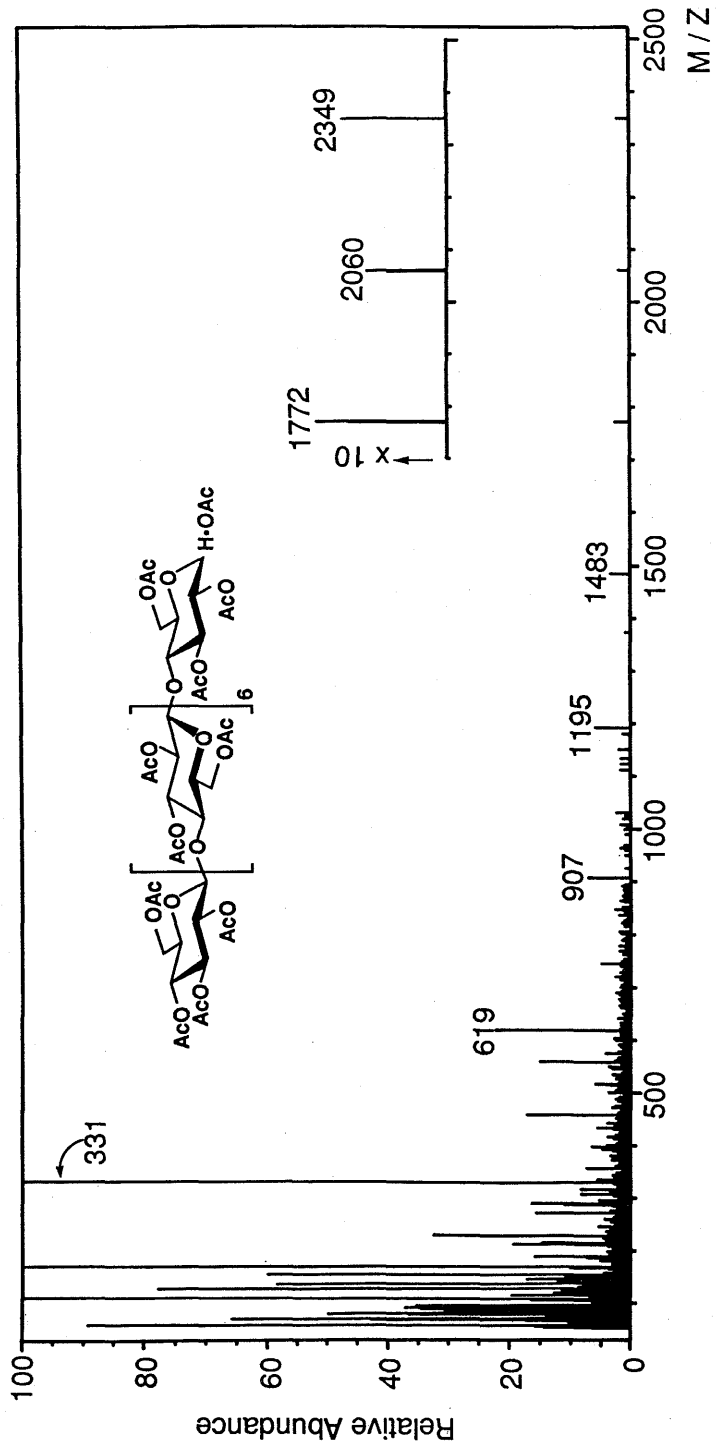


Fig. 3-7. Mass spectrum of compound **69**.

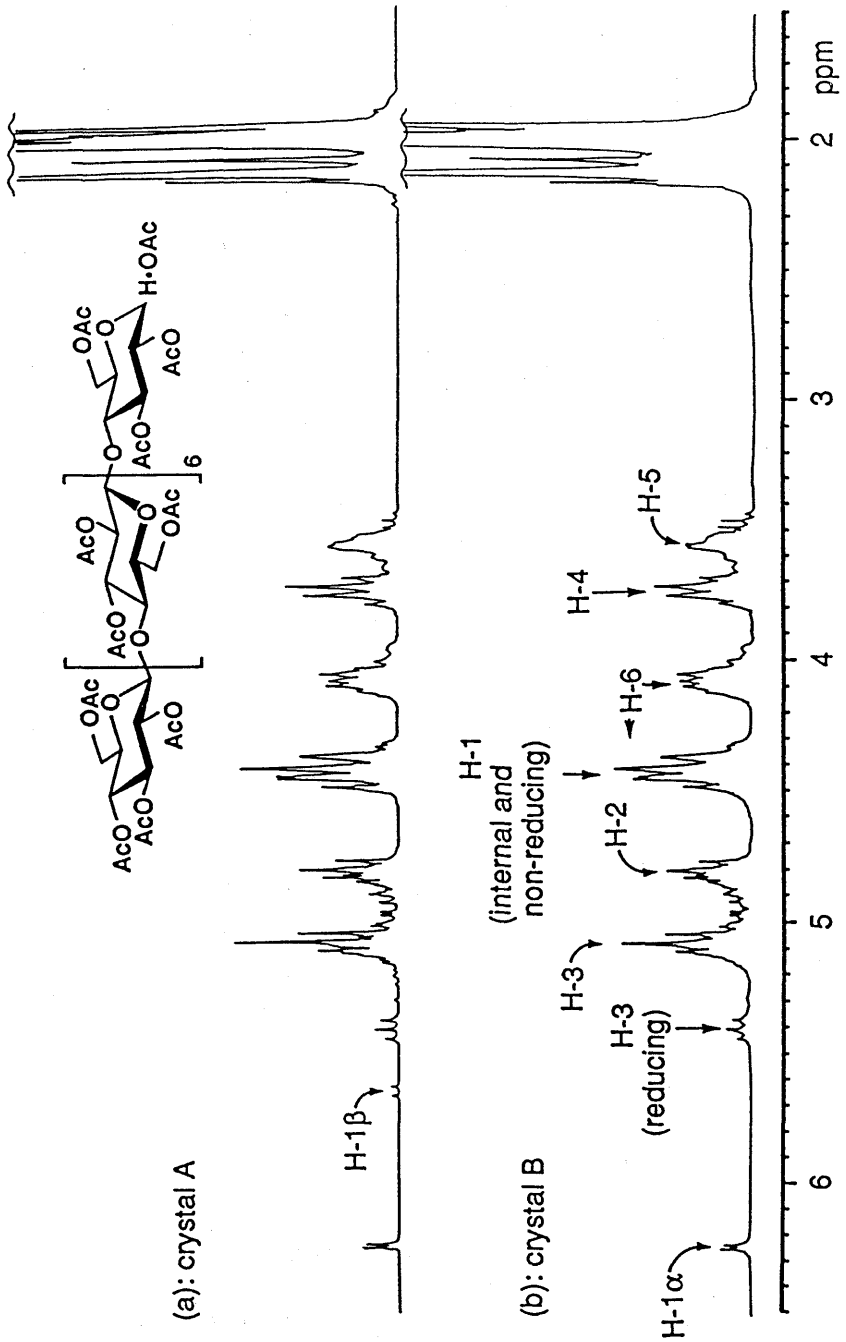


Fig. 3-8. ^{1}H-NMR spectra of synthesized cellooctaose acetates.

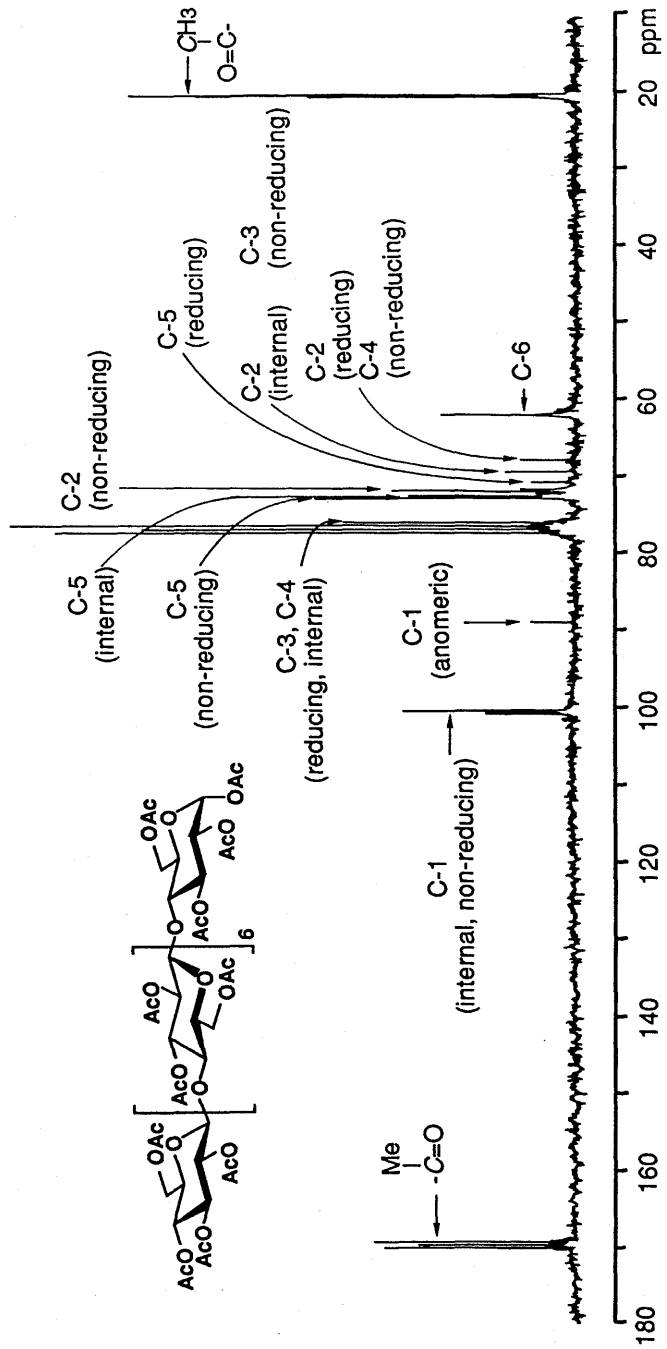


Fig. 3-9. ^{13}C-NMR spectrum of compound **70**.

3 — 3 Summary

At a final step for the synthesis of cello-oligosaccharides with free hydroxyl groups, three kinds of protective groups must be removed.

In this chapter, the reaction conditions for removal of three kinds of protective groups, allyl, *p*-methoxybenzyl and benzyl groups from D-glucose derivative **11** and D-cellobiose derivative **31** were established firstly.

By the established method, three kinds of protective groups of cellooctaose derivative **49**, prepared by the linear synthetic method, was removed, and the following acetylation afforded D-cellooctaose hexacosaacetate **69**.

Crystallization of compound **69** from methanol-dichloromethane afforded a colorless crystal consisting of α/β-D-cellooctaose hexacosaacetate (crystal A). Crystallization from the mother liquid gave another colorless leaflet-like crystals of **70**, α-D-cellooctaose hexacosaacetate.

CONCLUSION

For the synthesis of cello-oligosaccharides, two problems, regiospecific 1,4-bond formation and stereospecific β-linkage formation, have to be solved. The former will be solved by the use of a starting material which has special protective groups introduced by regiospecific control, the latter by the stereospecific β-glycosylation reaction. In this thesis, the investigation of the synthetic method for cello-oligosaccharides is described.

In chapter 1, the basic synthetic design was proposed at first. In the synthetic design, substituted glucose derivative **1** having X-, Y- and R-groups is used as a starting material (Figs. 1-1, 1-2 and 1-3). Then, allyl, *p*-methoxybenzyl and benzyl group were selected for X-, Y- and R-group, respectively. According to the synthetic design, the synthesis of the starting material, allyl 2,3,6-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-β-D-glucopyranoside (**11**), from D-glucose (**12**) was investigated. Here, two possible synthetic routes, using *p*-methoxybenzylidene (route I in Fig. 1-5) and benzylidene (route II in Fig. 1-5), were examined. As a result, the synthetic route for compound **11** by route I was established giving a higher overall yield.

In chapter 2, synthesis of cello-oligosaccharide derivatives up to octamer by linear synthetic method was described. The preparation method of glycosyl donor and acceptor from the starting material **11** was investigated at first. From compound **11**, selective removal of *p*-methoxybenzyl group with CAN and allyl group by two-step method afforded glycosyl donor **33** and glycosyl acceptor **22**, respectively. Glycosylation reaction to achieve stereospecific β-linkage formation was selected and imidate method was found to be the best for the present glycosylation. Thus, compound **33** was converted into α-trichloroacetimidoyl derivative **34** by TCA/DBU, which was reacted with compound **22** to afford cellobiose derivative **31** (Fig. 2-1).

For synthesizing cellotriose derivative **36**, two linear synthetic routes, the route using monomer imidate **34** as the glycosyl donor and the alternative route using dimer imidate **39**, were examined (Fig. 2-2). As a result, the former method was found to be suitable because of affording a higher overall yield. Then, by this strategy of linear synthetic method, i.e., repeating a set of two reactions consisting of the removal of *p*-methoxybenzyl group from synthesized oligomer and the β-glycosylation with monomer α-imidate **34**, a series of cello-oligosaccharide derivatives up to octamer was synthesized (Fig. 2-3 and 2-4). Glycosylation of the oligomer having higher D.P. took longer reaction time, but the yield of each glycosylation reaction was fairly good (Table 2-2).

Then, convergent synthetic method was investigated (Fig. 2-7). Although cellotetraose derivative **41** was synthesized, but only trace amount of cellooctaose derivative **49** was obtained.

This is responsible for preparation of the tetramer α-imidate **52**.

In chapter 3, reactivity and removal method of three kinds of the protective groups in cello-oligosaccharide derivatives synthesized were investigated. First, reaction conditions for cleavage of three kinds of protective groups were examined using D-glucose derivative **11** and D-cellobiose derivative **31** as model cases. According to the deprotection method established by the model experiments, three kinds of the protective groups of synthesized cellooctaose derivative **49** were removed and the product was finally converted by acetylation into cellooctaose hexacosaacetate **69**. By crystallizing compound **69** from a mixture of methanol and dichloromethane, colorless leaflet-like crystal of α/β-anomers was formed. From the mother liquid, crystals of α-anomer **70** appeared. Comparison of compound **70** with that obtained by acetolysis of cellulose indicated that the first chemical synthesis of cellooctaose hexacosaacetate was achieved.

EXPERIMENTAL

GENERAL

All melting points (m.p.) were measured by a hot-plate method with Yanagimoto Micro Apparatus and the values were not corrected. ^{1}H- and ^{13}C-NMR spectra were taken with a Varian XL-270FT-NMR (200 MHz), a JEOL FX-90 FT-NMR (90 MHz) and a JEOL JNM GX-270 FT-NMR (270 MHz) spectrometers with tetramethylsilane (TMS) as an internal standard in chloroform-*d*. Chemical shifts and coupling constants are given in δ-values (ppm and Hz, respectively). Optical rotations were measured using a Jasco Dip-4 digital polarimeter. The vapor pressure osmometry (VPO) was performed in chloroform with Knauer Vapor Pressure Osmometer. TLC was done on silica gel plates (Kieselgel 60 F_{245}, Merck). The standard work-up procedures consisted of extraction with ethyl acetate, washing with brine, drying over Na_2SO_4, and evaporation *in vacuo*. Unless otherwise indicated, the acetylation was done with acetic anhydride and pyridine by stirring the mixture overnight at room temperature.

EXPERIMENTAL (chapter 1)

Allyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (15)

To a mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[26] (**14**, 33.0 g, 80 mmol) and mercury (II) cyanide (22.0 g, 88 mmol) in anhydrous allyl alcohol (720 ml, distilled from CaO), powdered molecular sieve 4 Å (50 g) was added. The suspension was stirred at 50℃ for 1 h. The solution from the reaction mixture was evaporated to dryness *in vacuo*, and the residue was dissolved in ethyl acetate and neutralized with a $NaHCO_3$ solution. The resulting mixture was worked up and the product (**15**) was crystallized from 95% ethanol (29.0 g, 93.0%); m.p. 86℃ (reported[44] 86℃ for β-anomer, 97℃ for α-anomer), $[\alpha]_D^{20}$ -21.6° (c 3.47, chloroform). *Anal.* Calcd. for $C_{17}H_{24}O_{10}$: C, 52.57; H, 6.23. Found: C, 52.69; H, 6.25.

Allyl β-D-glucopyranoside (16)

To a suspension of compound **15** (19.0 g, 50 mmol) in methanol (50 ml), 28% sodium methoxide in methanol (10.08 ml, 50 mmol) was added dropwise at 0℃ over 1 h period. This solution was stirred for an additional 30 min at room temperature. The solution was decationized by mixing it with Amberlite IR-120 B (H^+) (12 g) and stirring for 30 min to complete the neutralization. The resin was filtered off, and the filtrate was evaporated *in vacuo* to give crystals which were recrystallized from 1-propanol/diethyl ether (1 : 1, v/v) to

give compound **16** (10.5 g, 95.0%), $[\alpha]_D^{20}$ -36.8° (c 3.37, water). *Anal.* Calcd. for $C_9H_{16}O_6$: C, 49.09; H, 7.27. Found: C, 48.60; H, 7.31.

Allyl 4,6-O-(p-methoxybenzylidene)-β-D-glucopyranoside (17)

A solution of compound **16** (11.0 g, 50 mmol), *p*-methoxybenzaldehyde dimethyl acetal (11 ml, 60 mmol, prepared by the method of Johansson and Samuelsson[31]), and *p*-toluenesulfonic acid (430 mg, 2.5 mmol) in dioxane (85 ml) was agitated under reduced pressure (30 mmHg) at 20-30℃ for 20 min. To this reaction mixture, $NaHCO_3$ (1 g) was added. The solvent was distilled at 60℃ *in vacuo* until about 10 ml remained, and the solution was poured onto a stirred mixture of ice (50 g) and diethyl ether (50 ml). After 30 min the resulting crystals were collected, washed successively with *n*-hexane and with water, then dried at 100℃ for 12 h to afford compound **17** (14.7 g, 87.3%); m.p. 153℃, $[\alpha]_D^{20}$ -54.8° (c 3.27, chloroform); ^{1}H-NMR (chloroform-*d*) δ: 3.43 (1H, dt, J = 4.9, 10.3, *H*-5), 3.54, 3.55, 3.83 (3H, three t, J = 10.5, *H*-2, *H*-3, *H*-4), 3.78 (1H, t, J = 10.3, *H*ax-6), 3.82 (3H, s, -OC*H*$_3$), 4.16, 4.38 (2H, two broad dd, J = 12.5 and 6.5 or 5.6, -C*H*$_2$-CH=CH$_2$), 4.34 (1H, dd, J = 10.3, 4.8, *H*eq-6), 4.46 (1H, d, J = 7.0, *H*-1), 5.26 (1H, broad dd, J = 10.5, 1.5, -CH$_2$-CH=C*H*cis), 5.35 (1H, broad dd, J = 17.0, 1.5, -CH-CH=C*H*trans), 5.50 (1H, s, acetal *H*), 5.96 (1H, dddd, J = 17.0, 10.7, 6.5, 5.6, -CH$_2$-C*H*=CH$_2$), 6.90 (2H, d, J = 8.5, aromatic *H*), and 7.43 (2H, d, J = 8.5, aromatic *H*). *Anal.* Calcd. for $C_{17}H_{22}O_7$: C, 60.34; H, 6.55. Found: C, 60.45; H, 6.61.

Allyl 2,3-di-O-benzyl-4,6-O-(p-methoxybenzylidene)-β-D-glucopyranoside (18)

To a solution of compound **17** (13.5 g, 40 mmol) in THF (320 ml), 60% NaH dispersed in mineral oil (6.9 g, 240 mmol) and tetra-*n*-butyl ammonium iodide (1.4 g, 4 mmol) were added and then the solution was refluxed for 30 min. After addition of benzyl bromide (14.4 ml, 120 mmol), the solution was refluxed for an additional 10 h. The excess benzyl bromide was decomposed by the addition of methanol (7.2 ml, 180 mmol) under reflux for 30 min. The reaction mixture was worked up and the product was crystallized from ethanol (**18**, 19.7 g, 95.0%); m.p. 124℃, $[\alpha]_D^{20}$ -31.5° (c 2.07, chloroform); ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **17** except for new signals at 4.78, 4.92, and 4.80, 4.93 (4H, four d, J = 11.0, O-2- and O-3-C*H*$_2$-C$_6$H$_5$) and 7.21-7.50 (10H, m, aromatic *H*). *Anal.* Calcd. for $C_{31}H_{34}O_7$: C, 71.79; H, 6.61. Found: C, 71.74; H, 6.65.

Allyl 2,3-di-O-benzyl-4-O-(p-methoxybenzyl)-β-D-glucopyranoside (19)

To a solution of compound **18** (5.18 g, 10 mmol) in anhydrous dichloromethane/diethyl ether (140 ml, 1 : 1, v/v), $LiAlH_4$ (1.9 g, 50 mmol) was added. A solution of $AlCl_3$ (6.8 g,

50 mmol, dried over P_2O_5 *in vacuo*) in anhydrous diethyl ether (36 ml) was added dropwise at -15℃ over 2 h period. The reaction mixture was treated carefully with water (2 ml) in THF (10 ml) to decompose the excess reagents and then diluted with ethyl acetate (72 ml). The resulting filtrate was evaporated *in vacuo* to give a colorless syrup, and the product was crystallized from ethanol (**19**, 5.02 g, 96.1%); m.p. 79℃, $[\alpha]_D^{20}$ -3.56° (c 3.37, chloroform); ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **18** except for the losses of the signals for the acetal proton and the appearance at 4.57, 4.79 (2H, two d, J = 10.0, -C*H*$_2$-C$_6$H$_4$-OCH$_3$) and an upfield signal at 3.86 (1H, dd, J = 10.5, 2.5, *H*eq-6). *Anal.* Calcd. for $C_{31}H_{36}O_7$: C, 71.25; H, 6.97. Found: C, 71.70; H, 7.00.

Allyl 2,3,6-tri-O-benzyl-4-O-(p-methoxybenzyl)-β-D-glucopyranoside (11)

To a solution of compound **19** (5.2 g, 10 mmol) in THF (80 ml), 60% NaH dispersed in mineral oil (1.2 g, 30 mmol) and tetra-*n*-butyl ammonium iodide (369 mg, 1 mmol) were added, and the solution was refluxed for 4 h. The excess benzyl bromide was decomposed by the addition of methanol (2.7 ml, 22.5 mmol) under reflux for 30 min. The reaction mixture was worked up to give a crystalline product which was recrystallized from ethanol (**11**, 5.3 g, 86.9%); m.p. 113℃, $[\alpha]_D^{20}$ +0.03° (c 3.37, chloroform); ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **19** except for a signal at 7.28-7.54 (new 15H, m, aromatic *H*), and new signals at 4.54, and 4.63 (2H, two d, J = 12.5, O-6-C*H*$_2$-). *Anal.* Calcd. for $C_{38}H_{42}O_7$: C, 74.73; H, 6.93. Found: C, 74.98; H, 6.95.

Allyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (22) from compound 21

To a solution of compound **21** (122 mg, 0.25 mmol) in anhydrous THF (4 ml) containing molecular sieve 4 Å, $NaBH_3CN$ (157.2 mg, 2.5 mmol) was added. To the resulting suspension, diethyl ether (0.6 ml) saturated with hydrogen chloride was added dropwise over 30 min period. The reaction mixture was worked up to give a colorless syrup (204.8 mg). This syrup was purified by TLC with ethyl acetate/*n*-hexane (1 : 4, v/v). The expected compound (**22**) was obtained as a colorless oil (88.2 mg, 72.0%); all analytical data, such as $[\alpha]_D^{20}$, ^{1}H-NMR, and elementary analysis were the same as those obtained for compound **22**, described below. An unexpected compound (C-6 hydroxyl, C-4 benzyl) also was obtained (4.0 mg, 3.3%).

EXPERIMENTAL (chapter 2)

Allyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (22) from compound 11

To a solution of compound **11** (122 mg, 0.2 mmol) in CH_3CN/water (1.8 ml, 9 : 1, v/v),

CAN (329 mg, 0.6 mmol) was added. This solution was stirred for 15 min at room temperature, then diluted with ethyl acetate. After neutralization with a saturated $NaHCO_3$ solution, the reaction mixture was worked up to give a yellow syrup (131 mg). This syrup was purified by TLC with ethyl acetate/*n*-hexane (1 : 4, v/v). The expected compound (**22**) was obtained as a colorless oil (87.3 mg, 74.0%) which was triturated in *n*-hexane (5 ml) at -90℃ to give a colorless powder (70.0 mg, 59.2%); $[\alpha]_D^{20}$ -26.40° (c 3.37, chloroform); ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **11** except for the loss of the signals assigned to the *p*-methoxybenzyl group. *Anal.* Calcd. for $C_{30}H_{34}O_6$: C, 73.47; H, 6.94. Found: C, 73.11; H, 7.00.

1'-Propenyl 2,3,6-tri-O-benzyl-4-O-(p-methoxybenzyl)-β-D-glucopyranoside (32)

To a solution of compound **11** (6.1 g, 12 mmol) in DMSO (50 ml), potassium *t*-butoxide (1.34 g, 12 mmol) was added. The resulting suspension was stirred for 12 h at 110℃, diluted with ethyl acetate (100 ml), and washed with brine until the washings became neutral. The ethyl acetate layer was evaporated to give a crude crystalline product (5.3 g) which was recrystallized from ethanol (**32**, 5.2 g, 91.2%); m.p. 100℃, $[\alpha]_D^{20}$ -6.96° (c 3.45, chloroform); ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **11** except for the losses of signals assigned to the allyl group and the appearance of signals for the 1'-propenyl group at 1.68 (3H, dd, J = 7.0, 2.0, =C-C*H*$_3$), 4.61 (1H, m, -O-C=C*H*), and 6.28 (1H, dq, J = 6.0, 2.0. -O-C*H*=C). *Anal.* Calcd. for $C_{38}H_{42}O_7$: C, 74.75; H, 6.93. Found: C, 74.64; H, 6.92.

2,3,6-Tri-O-benzyl-4-O-(p-methoxybenzyl)-β-D-glucopyranose (33)

To a solution of compound **32** (5.49 g, 9 mmol) dissolved in acetone (20 ml) at 50℃, 3.65% HCl solution (8 ml) was added. After refluxing for 2 h, $NaHCO_3$ (0.5 g) was added. The suspension was worked up to give a crystalline product which was recrystallized from ethanol (**33**, 3.65 g, 71.2%). The overall yield of compound **33** from compound **11** was 64.9%; m.p. 137℃, $[\alpha]_D^{20}$+11.65° (c 3.45, chloroform); ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **32** except for the losses of the signals assigned to the 1'-propenyl group. *Anal.* Calcd. for $C_{35}H_{38}O_7$: C, 73.68; H, 6.67. Found: C, 73.77; H, 6.76.

Trichloroacetimidoyl 2,3,6-tri-O-benzyl-4-O-(p-methoxybenzyl)-α-D-glucopyranoside (34)

To a solution of compound **33** (855 mg, 1.5 mmol) in anhydrous dichloromethane (15 ml), DBU (41.2 μl, 0.3 mmol) was added. The resulting solution was stirred at room temperature for 30 min; then TCA (300 μl, 3.0 mmol) was added. This solution was stirred for an additional 40 min; then the solvent was distilled of below 25℃ *in vacuo* until about 2 ml

remained. To the resulting solution, *n*-hexane (4 ml) was added; purification was made by a column chromatography (Alumina Woelm B, Akt. I, 24 mm×10 mm) using dichloromethane /*n*-hexane (1 : 2, v/v) as an eluant. The solvent was evaporated *in vacuo* to give a colorless syrup (1.22 g) that was triturated with *n*-hexane to afford a colorless solid (953.4 mg, 89.1%); m.p. 118℃, $[\alpha]_D^{20}$+49.85° (c 3.43, chloroform); ^{1}H-NMR (chloroform-*d*) δ: 3.80 (3H, s, -OC*H*$_3$), 4.47, 4.60, 4.66, 4.76, 4.83, 4.97, 4.45, 4,77 (8H, eight d, J = 12.0, 11.5, 11.0, and 10.5, benzyl *H*), 6.53 (1H, d, J = 3.5, *H*-1), 6.80, 7.03 (4H, two d, J = 8.5, -C$_6$H$_4$-), 8.60 (1H, s, =N*H*), 3.46-4.10 (6H, broad, *H*-2, *H*-3, *H*-4, *H*-5, and *H*-6). *Anal.* Calcd. for $C_{37}H_{38}Cl_3NO_7$: C, 62.15; H, 5.36; N, 1.96. Found: C, 62.16; H, 5.48; N, 2.00.

Allyl 2,3,6,2',3',6'-hexa-O-benzyl-4'-O-(p-methoxybenzyl)-β-D-cellobioside (31)

Compound **22** (25 mg, 0.05 mmol) and compound **34** (71 mg, 0.1 mmol), well dried over P_2O_5 in a vacuum desiccator, were dissolved in anhydrous dichloromethane (1 ml; freshly distilled from P_2O_5) containing molecular sieve 4 Å (50 mg), and then cooled below -70℃. To the resulting suspension, 0.1 ml of a solution of BF_3-Et_2O (12.3 μl, 0.1 mmol) in anhydrous dichloromethane (1 ml) was added (the actual amount of BF_3-Et_2O was 1.23 μl, 0.01 mmol) and stirred for 10 min. The reaction mixture was extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. The solution was evaporated *in vacuo* to give a colorless syrup (105.8 mg). This syrup was purified using TLC with ethyl acetate/*n*-hexane (1 : 4, v/v) to give a colorless oil. The product crystallized spontaneously. Recrystallization was carried out from ethanol to obtain 43.6 mg (83.7%) of the title compound; m.p. 110.5℃, $[\alpha]_D^{20}$+14.29° (c 3.50, chloroform); ^{1}H-NMR (chloroform-*d*) δ: 5.95 (1H, broad ddd, J = 16.5, 10.5, 5.0, -C-C*H*=C), 5.31 (1H, broad dd, J = 16.5, 1.5, -C-C=C*H*trans), 5.19 (1H, broad dd, J = 10.5, 1.5, -C-C=C*H*cis), 7.05, 6.78 (two 2H, two d, J = 8.0, -C$_6$*H*$_4$-), 3.75 (3H, s, -OC*H*$_3$), 7.10-7.26 (30H, aromatic *H*), 4.02-5.08 (20H), and 3.18-4.00 (10H); ^{13}C-NMR (chloroform-*d*) δ: 159.2 (CH$_3$O-*C*), 134.1 (-CH$_2$-*C*H=CH$_2$), 116.9 (-CH$_2$-CH=*C*H$_2$), 102.6, 102.2 (two anomeric *C*), 70.0 (-*C*H$_2$-CH=CH$_2$), and 55.1 (*C*H$_3$O-).

Allyl 2,3,6,2',3',6'-hexa-O-benzyl-β-D-cellobioside (35)

To a solution of compound **31** (1.04 g, 1 mmol) in CH$_3$CN/water (2.0 ml, 9 : 1, v/v), CAN (1.64 g, 3 mmol) was added. This solution was stirred for 15 min at room temperature, then diluted with ethyl acetate. After neutralization with a saturated $NaHCO_3$ solution, the reaction mixture was worked up to give a yellow syrup (902 mg). This syrup was purified by TLC with ethyl acetate/*n*-hexane (1 : 4, v/v). The expected compound (**22**) was obtained

as a colorless oil (682 mg, 74.0%).

Allyl 2,3,6,2',3',6',2",3",6"-nona-O-benzyl-4"-O-(p-methoxybenzyl)-β-D-cellotrioside (36) from 34 and 35

Compound **35** (274.9 mg, 0.3 mmol) and compound **34** (425.2 mg, 0.6 mmol), well dried over P_2O_5 in a vacuum desiccator, were dissolved in anhydrous dichloromethane (2 ml; freshly distilled from P_2O_5) containing molecular sieve 4 Å (50 mg), and then cooled below -70℃. To the resulting suspension, BF_3-Et_2O (3.69 μl, 0.03 mmol) was added and stirred for 20 min. The reaction mixture was extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. The solution was evaporated *in vacuo* to give a colorless syrup (570.0 mg). This syrup was purified using TLC with ethyl acetate/*n*-hexane (1 : 4, v/v) to give a colorless oil (347.4 mg, 79.1%); ^{1}H-NMR (chloroform-*d*) δ: 3.20-4.20 (26H), 3.79 (3H, s, -OC*H*$_3$), 4.28-5.02 (21H), 5.21 (1H, broad dd, J = 10.5, 1.5, -C-C=C*H*cis), 5.33 (1H, broad dd, J = 16.5, 1.5, -C-C=C*H*trans), 5.99 (1H, broad ddd, J = 16.5, 10.5, 5.0, -C-C*H*=C), 7.05, 6.78 (two 2H, two d, J = 8.0, -C$_6$*H*$_4$-), and 7.08-7.94 (47H, aromatic *H*); ^{13}C-NMR (chloroform-*d*) δ: 159.3 (CH_3O-*C*), 134.3 (-CH_2-*C*H=CH_2), 117.1 (-CH_2-CH=*C*H$_2$), 102.6 (three anomeric *C*), 70.3 (-*C*H$_2$-CH=CH_2), and 55.3 (*C*H$_3$O-).

1'-Propenyl 2,3,6,2',3',6'-hexa-O-benzyl-4'-O-(p-methoxybenzyl)-β-D-cellobioside (37)

To a solution of compound **31** (2.08 g, 2.0 mmol) in DMSO (50 ml), potassium *t*-butoxide (1.14 g, 10 mmol) was added. The resulting suspension was stirred for 2 h at 110℃, diluted with ethyl acetate (100 ml), and washed with brine until the washings became neutral. The ethyl acetate layer was evaporated to give a crude crystalline product (2.0 g) which was recrystallized from ethanol (**37**, 1.77 g, 85.0%); ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **31** except for the losses of signals assigned to the allyl group and the appearance of signals for the 1'-propenyl group at 1.68 (3H, dd, J = 7.0, 2.0, =C-C*H*$_3$), 4.61 (1H, m, -O-C=C*H*), and 6.28 (1H, dq, J = 6.0, 2.0. -O-C*H*=C).

2,3,6,2',3',6'-Hexa-O-benzyl-4'-O-(p-methoxybenzyl)-β-D-cellobiose (38)

To a solution of compound **37** (1.77 g, 1.7 mmol) dissolved in acetone (20 ml) at 50℃, 3.65% HCl solution (8 ml) was added. After refluxing for 2 h, $NaHCO_3$ (0.5 g) was added. The suspension was worked up to give a crystalline product which was recrystallized from ethanol (**38**, 1.60 g, 94.1%). The overall yield of compound **38** from compound **31** was 80.0 %; ^{1}H-NMR (chloroform-*d*) δ: similar to that of compound **37** except for the losses of the signals assigned to the 1'-propenyl group.

Trichloroacetimidoyl 2,3,6,2',3',6'-hexa-O-benzyl-4'-O-(p-methoxybenzyl)-α-D-cellobioside (39)

To a solution of compound **38** (100.2 mg, 0.1 mmol) in anhydrous dichloromethane (2 ml), DBU (3 μl, 0.02 mmol) was added. The resulting solution was stirred at room temperature for 30 min, then TCA (50 μl, 0.5 mmol) was added. the reaction mixture was stirred for an additional 2 h, and *n*-hexane (2 ml) was added to the reaction mixture. Purification was made by a column chromatography (Alumina Woelm B, Akt I, 6 mm×25 mm) using dichloromethane and *n*-hexane (10 ml, 1 : 1, v/v) as an eluant. The solvent was evaporated *in vacuo* to give a colorless syrup (63.0 mg, 55.0%) that was crystallized from ethanol (59.6 mg, 52%); m. p. 109.5-110.0℃; ^{1}H-NMR (chloroform-*d*) δ: 3.79 (3H, s, -OC*H*$_3$), 6.43 (1H, d, *J* = 3.4, *H*-1), 6.89, 7.70 (two 2H, two d, *J* = 9.4, -C$_6$*H*$_4$-), 8.66 (1H, s, -N*H*).

Allyl 2,3,6,2',3',6',2'',3'',6''-nona-O-benzyl-4''-O-(p-methoxybenzyl)-β-D-cellotrioside (36) from 39 and 22

Compound **39** (105.3 mg, 0.09 mmol) and compound **22** (108.5 mg, 0.18 mmol), well dried over P_2O_5 in a vacuum desiccator, were dissolved in anhydrous dichloromethane (2.5 ml; freshly distilled from P_2O_5) containing molecular sieve 4 Å (50 mg), and then cooled below -70 ℃. To the resulting suspension, BF_3-Et_2O (1.24 μl, 9 μmol) was added and stirred for 20 min. The reaction mixture was extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. The solution was evaporated *in vacuo* to give a colorless syrup (191.4 mg). This syrup was purified using TLC with ethyl acetate/*n*-hexane (1 : 4, v/v) to give a colorless oil (**36**, 66.86 mg, 50.4%); all analytical data, such as R_f-value, ^{1}H-NMR and ^{13}C-NMR were the same as those obtained for compound **36** from compound **34** and **35**.

Perbenzylated allyl 4^n-OH-β-D cello-oligosaccharides 40, 42, 44, 46 and 48

Compounds **40**, **42**, **44**, **46** and **48** were obtained by the selective removal of *p*-methoxybenzyl group according to the similar procedure as described for the synthesis of allyl 2,3,6,2',3',6'-hexa-*O*-benzyl-β-D-cellobioside (**22**) from compound **11**. Amount of starting material and CAN, solvent, reaction time, yield, and R_f-value (ethyl acetate - *n*-hexane, 1 : 4, v/v, ×3) of each reaction are described below. The structure of the synthetic compounds was supported by the ^{1}H-NMR spectra; similar to that of the starting material except for the loss of the signals assigned to the *p*-methoxybenzyl group.

Compound 40 (trimer)

A starting material (**36**, 180.0 mg, 0.12 mmol); CAN (197.3 mg, 0.36 mmol); in CH_3CN-water (2 ml, 9 : 1, v/v); reaction time 30 min; yield 141.4 mg (87.0%); R_f-value 0.596.

Compound 42 (tetramer)

A starting material (**41**, 171.5 mg, 0.09 mmol); CAN (147.9 mg, 0.27 mmol); in CH_3CN-water (4 ml, 9 : 1, v/v); reaction time 15 min; yield 79.0 mg (54.6%); R_f-value 0.523.

Compound 44 (pentamer)

A starting material (**43**, 46.76 mg, 0.02 mmol); CAN (32.88 mg, 0.06 mmol); in CH_3CN-water (2 ml, 19 : 1, v/v); reaction time 50 min; yield 41.6 mg (93.0%); R_f-value 0.436.

Compound 46 (hexamer)

A starting material (**45**, 19.39 mg, 7 μmol); CAN (15.30 mg, 28 μmol); in CH_3CN-water (2 ml, 19 : 1, v/v); reaction time 90 min; yield 7.3 mg (39.3%); R_f-value 0.401.

Compound 48 (heptamer)

A starting material (**47**, 12.80 mg, 4 μmol); CAN (10.96 mg, 20 μmol); in CH_3CN-water (1.5 ml, 19 : 1, v/v); reaction time 90 min; yield 4.7 mg (38.1%); R_f-value 0.362.

Perbenzylated allyl 4$^{n'}$-(p-methoxybenzyl)-β-D-cello-oligosaccharides 41, 43, 45, 47 and 49

Compounds **41**, **43**, **45**, **47** and **49** were synthesized by the glycosylation of α-imidate **34** with compounds **40**, **42**, **44**, **46** and **48**, respectively, according to the similar procedure as described for the synthesis of compound **36** from **34** and **35**. Amount of starting material, α-imidate **34** and BF_3-Et_2O, yield, R_f-value (ethyl acetate - *n*-hexane, 1 : 2, v/v), $[\alpha]_D^{20}$ of each compound are described below. The structure of the synthetic compounds was supported by the ^{1}H-NMR (Fig. 2-5) and ^{13}C-NMR (Fig. 2-6) spectra.

Compound 41 (tetramer)

A starting material (**40**, 135 mg, 0.1 mmol); α-imidate **34** (142.7 mg, 0.2 mmol); BF_3-Et_2O (2.46 μl, 0.02 mmol); reaction time 25 min; yield 150.0 mg (78.7%); R_f-value 0.476; $[\alpha]_D^{20}$ 13.6 (c 4.79, chloroform).

Compound 43 (pentamer)

A starting material (**42**, 71.4 mg, 0.04 mmol); α-imidate **34** (57.1 mg, 0.08 mmol); BF_3-Et_2O (0.98 μl, 8 μmol); reaction time 30 min; yield 67.2 mg (71.8%); R_f-value 0.388; $[\alpha]_D^{20}$ 11.5 (c 3.81, chloroform).

Compound 45 (hexamer)

A starting material (**44**, 39.9 mg, 18 μmol); α-imidate **34** (25.7 mg, 36 μmol); BF_3-Et_2O

(0.44 μl, 6 μmol); reaction time 30 min; yield 38.08 mg (76.4%); R_f-value 0.377; $[\alpha]_D^{20}$ 10.4 (c 3.86, chloroform).

Compound 47 (heptamer)

A starting material (**46**, 18.5 mg, 7 μmol); α-imidate **34** (7.9 mg, 14 μmol); BF_3-Et_2O (0.17 μl, 1.4 μmol); reaction time 45 min; yield 14.0 mg (62.5%); R_f-value 0.309; $[\alpha]_D^{20}$ 9.9 (c 2.72, chloroform).

Compound 49 (octamer)

A starting material (**48**, 4.6 mg, 1.5 μmol); α-imidate **34** (0.038 mg, 0.3 μmol); BF_3-Et_2O (0.17 μl, 1.4 μmol); reaction time 90 min; yield 4.0 mg (73.3%); R_f-value 0.283; $[\alpha]_D^{20}$ 8.8 (c 1.36, chloroform).

Compound 41 (tetramer) from compound 39 and 35

As for this experiment, dichloromethane (CH_2Cl_2) fleshly distilled from P_2O_5 was used.

Compound **35** (11 mg, 0.012 mmol), well dried over P_2O_5 in a vacuum deciccator, was dissolved in CH_2Cl_2 (1 ml) containing molecular sieve 4 Å, and then cooled below -70℃. To the suspension, 0.05 ml of a solution of BF_3-Et_2O (12.3 μl, 0.1 mmol) in CH_2Cl_2 (1 ml) was added (the actual amount of BF_3-Et_2O was 0.6 μl, 5 μmol). To the resulting mixture, 1 ml of a solution of imidate **39** in CH_2Cl_2 (55.38 mg, 0.048 mmol, 2 ml) [solution A] was added (the actual amount of imidate **39** was 27.69 mg, 0.024 mmol), and the reaction mixture was stirred for 20 min. Then, to the reaction mixture, another portion of the solution A (0.5 ml: the actual amount of imidate **39** was 13.85 mg, 0.012 mmol) was added then the reaction mixture was stirred for additional 20 min. Then the last portion of solution A (0.5 ml) was added and stirred for more than 20 min. The reaction products were extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. The solvent was evaporated *in vacuo* to give a colorless syrup (59.5 mg). This syrup was purified using TLC with ethyl acetate and *n*-hexane (1 : 4, v/v) to give a colorless syrup (**41**, 17.5 mg, 77% from compound **35**, 38 % from compound **39**); ^{1}H-NMR (chloroform-*d*) δ: 3.00-3.75 (24H), 3.78 (3H, s, -OC*H*$_3$), 3.09-5.00 (32H), 5.18 (1H, broad dd, J = 10.1, 1.8, -C-C=C*H*cis), 5.32 (1H, broad dd, J = 17.9, 1.8, -C-C=C*H*trance), 5.69 (1H, broad ddd, J = 17.9, 10.1, 6.1, -C-C*H*=C), 6.80, 7.06 (two 2H, two d, J = 8.5, -C$_6$*H*$_4$-), 7.08-7.40 (60H, aromatic *H*): ^{13}C-NMR (chloroform-*d*) δ: 55.2 (*C*H$_3$O-), 70.2 (-*C*-C=C), 102.5-102.7 (anomeric-*C*), 117.0 (-C-C=*C*), 134.2 (-C-*C*=C), 159.2 (MeO-*C*).

EXPERIMENTAL (chapter 3)

General procedure for hydrogenolysis

To a solution of the compound in a mixed solvent of ethanol and acetic acid (10 : 1, v/v; solvent A), 5% Pd/C (twice the weight of the compound) was added. The suspension was stirred under a hydrogen atmosphere for 3-6 h at 50℃. The catalyst was filtered off and washed with ethanol. The filtrate and washings were combined which was concentrated *in vacuo*. The residue was analytically pure.

Propyl β-D-glucopyranoside (61)

Compound **11** (30 mg, 0.5 mmol) was dissolved in solvent A (30 ml) and hydrogenated for 4 h to give compound **61** (111 mg, 100%); R_f-value 0.49 (*n*-propanol-ethyl acetate, 2 : 3, v/v).

Acetate of compound **61** was recrystallized from ethanol to give colorless crystals in a quantitative yield; m.p. 89-90℃; ^{1}H-NMR (chloroform-*d*) δ: 0.90 (3H, t, J = 7.0, -CH_2-CH_2-CH_3), 1.59 (2H, tq, J = 7.0, 7.0, -CH_2-CH_2-CH_3), 2.00, 2.03, 2.05, 2.09 (four 3H, s, CH_3-CO-), 3.44 (1H, dt, J = 9.2, 7.0, one of -CH_2-CH_2-CH_3), 3.70 (1H, ddd, J = 9.2, 4.9, 3.2, *H*-5), 3.84 (1H, dt, J = 9.2, 7.0, one of -CH_2-CH_2-CH_3), 4.11 (1H, dd, J = 12.3, 3.2, one of *H*-6), 4.27 (1H, dd, J = 12.3, 4.9, one of *H*-6), 4.50 (1H, d, J = 7.4, *H*-1), 5.00 (1H, dd, J = 7.4, 9.2, *H*-2), 5.09 (1H, t, J = 9.2, *H*-4), 5.21 (1H, t, J = 9.2, *H*-3).

Propyl β-D-cellobioside (63)

Compound **31** (1.04 g, 1 mmol) was dissolved in solvent A (100 ml) and hydrogenated for 6 h to give compound **63** (381 mg, 99%); R_f-value 0.43 (*n*-propanol-ethyl acetate, 2 : 3, v/v).

Acetate of compound **63** was recrystallized from ethanol to give colorless crystals in a quantitative yield; m.p. 203℃; ^{1}H-NMR (chloroform-*d*) δ: 0.90 (3H, t, J = 6.8, -CH_2-CH_2-CH_3), 1.59 (2H, tq, J = 6.8, 6.8, -CH_2-CH_2-CH_3), 1.99, 2.00, 2.01, 2.02, 2.10, 2.12 (21H, seven CH_3-CO-), 3.42 (1H, dt, J = 9.4, 6.8, one of -CH_2-CH_2-CH_3), 3.58 (1H, ddd, J = 2.2, 5.1, 9.6, *H*-5), 3.66 (1H, ddd, J = 2.2, 4.6, 9.6, *H*-5'), 3.78 (1H, t, J = 9.6, *H*-4), 3.80 (1H, dt, J = 9.4, 6.8, one of -CH_2-CH_2-CH_3), 4.06 (1H, dd, J = 2.2, 12.3, one of *H*-6'), 4.10 (1H, dd, J = 5.1, 12.0, one of *H*-6), 4.37 (1H, dd, J = 4.6, 12.3, one of *H*-6'), 4.45 (1H, d, J = 8.1, *H*-1'), 4.52 (1H, d, J = 8.1, *H*-1), 4.53 (1H, dd, J = 2.2, 12.0, one of *H*-6), 4.90 (1H, dd, J = 8.1, 9.6, *H*-2'), 4.93 (1H,

dd, J = 8.1, 9.6, H-2), 5.06 (1H, t, J = 9.6, H-4'), 5.15 (1H, t, J = 9.6, H-3'), 5.19 (1H, t, J = 9.6, H-3).

D-Glucose (12) from compound 33

2,3,6-Tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-D-glucopyranose (**33**, 285 mg, 0.5 mmol) was dissolved in solvent A (10 ml) and hydrogenated for 3 h to give colorless syrup (D-glucose, 90 mg, 100%); R_f-value 0.58 (*n*-propanol-ethyl acetate-water, 6 : 1 : 3, v/v/v).

Acetate of compound **12** was recrystallized from ethanol to give colorless crystals in a quantitative yield, whose α/β ratio was 1/6.8 by NMR analysis; m.p. 116-123℃ (112-113 ℃ for α-anomer and 132℃ for β-anomer[69]).

D-Cellobiose (23) from compound 38

2,3,6,2',3',6'-Hexa-*O*-benzyl-4'-*O*-(*p*-methoxybenzyl)-D-cellobiose (**38**, 501 mg, 0.5 mmol) was dissolved in solvent A (100 ml) and hydrogenated for 4 h to give a colorless syrup (D-cellobiose, 171 mg, 100%); R_f-value 0.54 (*n*-propanol-ethyl acetate-water, 6 : 1 : 3, v/v/v).

Acetate of compound **23** was recrystallized from ethanol to give colorless crystals in a quantitative yield. The α/β ratio was estimated to be 1/1.4 by NMR analysis; m.p. 201-203℃ (229.5℃ for α-anomer[67], 202.0-202.5℃ for β-anomer[68]).

Conversion of compound 49 into α-D-cellooctaose hexacosaacetate (70)

To a solution of compound **49** (377.4 mg, 0.104 mmol) in DMSO (2 ml), potassium *t*-butoxide (22.4 mg, 0.2 mmol) was added. The suspension was stirred for 8 h at 110℃, diluted with ethyl acetate (10 ml) and washed with brine until the washings became neutral. The ethyl acetate layer was dried over Na_2SO_4 and evaporated to give a yellow oil (380 mg).

The resulted oil was dissolved into acetone (2 ml) and to the solution 3.65% HCl solution (0.8 ml) was added. After refluxing for 2.5 h, $NaHCO_3$ was added to neutralize. The suspension was diluted with ethyl acetate (20 ml), washed with water and brine, dried over Na_2SO_4 and evaporated to give a yellow oil (**64**, 135.1 mg, 37% from compound **49**).

The resulted oil was dissolved into acetic anhydride (1 ml) and pyridine (1 ml). The solution was stirred overnight at room temperature, and evaporated to give a yellow oil (**65**, 101.9 mg, 73%).

To a solution of the resulted oil in CH_3CN-water (9 : 1, v/v, 2 ml), CAN (138 mg, 0.252 mmol) was added. The solution was stirred for 4 h at room temperature, then diluted with ethyl acetate (25 ml), neutralized with $NaHCO_3$ solution, washed with water and brine, dried over Na_2SO_4 and the ethyl acetate layer was evaporated to give a yellow oil (84.7 mg). This oil was purified by TLC with ethyl acetate-*n*-hexane (1 : 4, v/v) to give a colorless syrup

(**66**, 55.8 mg, 57%) which was treated with acetic anhydride (1 ml) and pyridine (1 ml) to give a colorless oil (**67**, 39.8 mg, 68%).

The resulted oil was dissolved into a mixed solvent of ethanol-acetic acid (10 : 1, v/v, 10 ml), and to the solution, 10% Pd/C (100 mg) was added. The suspension was stirred under hydrogen atmosphere for 4 h at 50℃. The catalyst was filtered off and washed with ethanol. The filtrate and washings were combined which was concentrated *in vacuo* to give colorless oil (**68**, 9.9 mg, 63%).

The resulted oil was treated with acetic anhydride (1 ml) and pyridine (1 ml) to give a colorless oil which was crystallized from ethanol to give crude crystal of compound **69** (12.6 mg, 71%, overall yield from compound **49** was 4.8%).

The crude crystal of **69** (12.6 mg, 0.005 mmol) was treated with acetic anhydride (2 ml) and $ZnCl_2$ (0.3 mg) at 80℃ for 7 h, then the reaction mixture was diluted with ethyl acetate (10 ml), neutralized with $NaHCO_3$ solution, washed with water and brine, dried over Na_2SO_4 and the ethyl acetate layer was evaporated to give a yellow oil (12.3 mg) which crystallized from methanol-dichloromethane (1 : 1, v/v, 1 ml) to give first crystal (7.8 mg); ^{1}H-NMR (chloroform-*d*) δ: 1.95-2.18 (-C(=O)-CH_3), 3.50-3.62 (*H*-5), 3.70-3.79 (*H*-4), 3.97-4.14 (*H*-6), 4.13-4.80 (*H*-6, internal and non reducing end residue *H*-1), 4.78-5.15 (*H*-2, *H*-3), 5.41 (reducing end residue *H*-3), 5.65 (anomeric *H*-1β) and 6.24 (anomeric *H*-1α, α/β ratio is 8.6/1), and second crystal (**70**, 3.3 mg); m.p. 264.2-269.1℃ (m.p. 258-262 ℃[10]); ^{1}H-NMR (chloroform-*d*) δ: similar to that of the first crystal except for the loss of signal derived from *H*-1β; ^{13}C-NMR (chloroform-*d*) δ: 20.50-20.88 (-C(=O)-CH_3), 62.11 (*C*-6), 67.92 (reducing end residue *C*-2, non reducing end residue *C*-4), 69.48 (internal residue *C*-2, non reducing end residue *C*-3), 70.83 (reducing end residue *C*-5), 71.70 (non reducing end residue *C*-2), 72.18 (non reducing end residue *C*-5), 72.93 (internal residue *C*-5), 76.10 (reducing end and internal residues *C*-3, *C*-4), 89.03 (anomeric *C*-1), 100.49, 100.81 (internal and non reducing end residues *C*-1), 169.28 - 170.20 (-*C*(=O)-CH_3).

Mass spectrum

Mass spectrum of compound **69** was obtained on a JEOL JMS-DX303 HF mass spectrometer and JMA-DA500 mass data system equipped with a FAB gun operated at 2 keV. The instrument was scanned from 2500 to 50 amu in 15 s with an interscan delay time of 1 s at a resolution of ca. 3000. The spectrometer was calibrated with cesium iodide. Samples were prepared by wetting the FABMS probe tip with 3-nitrobenzyl alcohol. The tip of a plumger from a 10 μl Hamilton syringe was wetted with 3-nitrobenzyl alcohol and used to transfer 10-20 μl of the compound **69** to the FABMS probe. About 5 s was required for the signal strength to reach maximum after switching on the ion beam; 3-8 scans were averaged to obtain the spectra.

SEM observation

Samples were suspended in water. One drop of the suspension was mounted on a glass plate (about 8×8 mm) attached on SEM specimen stub, air-dried, and then coated with platinum in a high vacuum evaporation unit. The specimen was examined at 15 kV with 15 mm of working distance in a HITACHI X-650 scanning electron microscope.

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要　　旨

純有機化学的手法によりD-グルコースから一連のセロオリゴ糖を合成する方法について検討した。

まず、D-グルコースの１，４位にそれぞれ一時的保護基を、２，３，６位に永続的保護基を持つ誘導体を出発物質として用いる合成デザインを策定し、使用可能な保護基を検討したのち、出発物質としてアリル２，３，６-トリ-*O*-ベンジル-4-*O*-(*p*-メトキシベンジル)-β-D-グルコピラノシドを選択し、その合成経路を確立した（D-グルコースから８段階、総収率60％）。次いで、この出発物質からアリル基と*p*-メトキシベンジル基を脱離させて、それぞれグリコシルドナーとグリコシルアクセプターを調製し、これらのイミデート法によるグリコシル化反応によりセロビオース誘導体（出発物質と同様の保護基の組み合わせを持つ）を合成した（収率84％）。上記の操作、すなわち保護基の脱離とグリコシル化、を繰り返す直線型合成法により８量体までの一連のセロオリゴ糖誘導体を合成した（各収率とも約70％）。最後に、保護基の脱離を行い、セロオクタオースアセテートの合成に成功した。なお、本合成法は（1→2）β-、（1→3）β-、（1→6）β-、グリカン由来のオリゴ糖の調製にも適用され得るものである。