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Synthesis of 1,2-Glycosidic Polymers via Ring-Opening Condensation Polymerization of Cyclic Sulfite

環状サルファイトの開環縮合重合による

1,2-グリコシド型ポリマーの合成

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Chapter 1

General Introduction

1.1 Polysaccharides

A carbohydrate is a biological molecule consisting of carbon (C), hydrogen (H), and oxygen (O) atoms, usually with a hydrogen–oxygen atom ratio of 2:1. The term "polysaccharide" means polymeric carbohydrate joined together by glycosidic linkages. Polysaccharides are also called as glycans. They are structurally divided into linear and branched polymers. Polysaccharide exit as storage and structural materials in a living body. Figure 1.1 shows a matrix for the categorization of polysaccharides.

| | Linear | Branched |
|---------------------|------------------|----------------------|
| Storage material | Amylose | Amylopectin, Dextran |
| | Curdlan | Glycogen |
| Structural material | Cellulose | Hemicellulose |
| | Chitin, Chitosan | Arabinoxylan |

Figure 1.1 categories of polysaccharides.

Plants make starch as their storage units, which is found in many plants such as potatoes, rice, wheat, and cereal grains, while animals make glycogen as an energy storage in the liver, muscle tissue, and brain. Glycogen acts as a fuel reserve. When blood sugars become low, the pancreas releases the

hormone *glucagon*, which travels to the liver and triggers the conversion of glycogen to glucose. This process can also be activated by adrenaline as a stress response.

Starch and glycogen consist of glucose as a repeating unit. Starch includes two polymers, amylose and amylopectin. Amylose is an unbranched chain of hundreds of glucose units, while amylopectin is a chain of thousands of units that is branched. The structure of amylopectin is very similar to that of glycogen, although glycogen includes protein at the polymer terminus. Amylose and amylopectin have α -glycosidic linkages without any β -linkage. The anomeric acetal bonds with α -stereochemistry can be easily cleaved, which makes glucose released.

In addition to the storage polysaccharides, structural polysaccharides with other structures are also found in a living body. The polymers give rigidity to cells. They are also comprised of saccharide units with β -anomeric linkages, whose cleavage is more difficult compared with that of α -linkage. Cellulose is a member of the structural polysaccharides, which helps to give plant walls their strength.



Figure 1.2 Polysaccharides with $(1\rightarrow 3)$, $(1\rightarrow 4)$, and $(1\rightarrow 6)$ -glycosidic linkages

Figure 1.2 shows the structures of polysaccharides consisting of glucose as a repeating unit. It is very interesting that the different position of glycosidic linkage for example $(1\rightarrow 6)$ (dextran), $(1\rightarrow 4)$ (amylose, cellulose, chitin, and chitosan), $(1\rightarrow 3)$ (schizophyllan and lentinan) linkages provides different higher-order-structure and unique properties.

Together with the unique structures and activity of polysaccharides, the properties such as atoxicity, hydrophilicity, biocompatibility, multichirality, and multifunctionality confer the additional

importance to polysaccharides as valuable and renewable resources.¹ The chemical modification of polysaccharides is still underestimated regarding the properties of materials.

Wide variety of polymeric carbohydrates are produced by the living organisms.² For long years, the attention has been centered mainly on polysaccharides composed of sugar residues. Noncarbohydrate materials were found in polysaccharide preparations were in fact considered as an impurity. With the improvements in methods for isolation, purification, and characterization of high-molecular-weight natural compounds, it became evident that hybridization between carbohydrates and other constituents occurs widely in nature.

Recently, the research activities have increased in simple and abundant homopolysaccharides like cellulose, chitin, and amylose and less readily obtainable mucopolysaccharides, and polysaccharide moieties of glycoproteins,³ glycopeptides, and lipopolysaccharides. In addition, the interests in polysaccharide biosynthesis⁴ and analytical techniques have markedly increased.

Many natural polysaccharides and oligosaccharides participate in a variety of biochemical reactions *in vivo*. However, it is quite difficult to elucidate the mechanism of the biological activity of the polysaccharide itself, because of the complicated chemical structure of the natural saccharide chain and the existence of impurities.

1.2 Polysaccharides consisting of $(1 \rightarrow 4)$ -glycosidic bonds

1.2.1 Amylose

Amylose is a storage polysaccharide composed of an unbranched chain of D-glucose units, bound to each other through α -(1 \rightarrow 4)-glycosidic bonds having one reducing end (Figure 1.3). Amylose is one of the two components of starch, making up approximately 20-30% of the structure. The other component is amylopectin, which makes up 70–80% of the structure, branched at every 20-35 glucose units via α -(1 \rightarrow 6) linkage.





Figure 1.3 Structures of amylose (A) and amylopectin (B)

Corn starch, copied from free picture

The structural characteristics of amylose and amylopectin lead them to very different uses. For instance, amylopectin is easily digested in the body and is therefore a major source of dietary glucose. It also has several industrial uses because its highly branched nature makes it an excellent thickner and texturant. By contrast, amylose cannot be digested well in human body because its glucose chains tend to repack together even after cooking. Because of its tightly packed structure, amylose is an important form of resistant starch. Amylose is isolated from plant components like corn starch,⁵ cotton leaves,⁶ potato starch, etc. Starch is made from both plant (potato phosphorylase) and animal (muscle phosphorylase) sources, by glycogen phosphorylase.

Amylose forms a left- handed helical structure with a chiral inner cavity.⁷ The helical structure has been reported to be stabilized by a combination of inter-turn hydrogen bonding interactions at the 6positions and a hydrophobic interaction of its main chain,⁸ the introduction of hydrogen-bond functionalities at the 6-positions with respect to the amylose backbone is an important issue in the structural design for advanced functions such as chiral separation,⁹ asymmetric catalysis,¹⁰ and optical anisotropy.¹¹ Several amylose derivatives are known to behave as a host polymer for various compounds such as iodine¹² and linear organic molecules,¹³ accompanied by intermolecular chirality transfer from amylose to achiral guest molecules.¹⁴ Hydrophobic linear polymers also form inclusion complexes with amylose derivatives in water.¹⁵

A lot of functional materials exploiting amylose backbone have been reported to date. The most impressive work is a creation of chiral column reported by Prof. Okamoto and Prof. Yashima.¹⁶ They reported that, the fully protected polymers by carbamates or esters are useful as chiral separation resins to recognize a pair of enantiomers, enabling the optical resolution of racemic compounds



Figure 1.4 Representative structures of chiral stationary phase in chiral column.

1.2.2 Cellulose

Cellulose is a structural polysaccharide consisting of repeated glucose units bonded together by β -(1 \rightarrow 4) linkages (Figure 1.5). Wood is largely cellulose and lignin, while paper and cotton are nearly pure cellulose. It is the most abundant carbohydrate in nature. Humans and many animals lack an enzyme to break the β -linkages, so they do not digest cellulose. Certain animals such as termites can digest cellulose, because the bacteria possessing the enzyme are present in their gut. Cellulose is insoluble in water. It does not change color when mixed with iodine.





Figure 1.5 Structure of cellulose

Cotton, one of cellulose source, copied from free picture

As for the synthesis of cellulose, Prof. Shoda has reported enzymatic synthesis of cellulose utilizing glycosyl fluoride via cellulase-catalyzed polymerization (Scheme 1.1).¹⁷ The polymerization features perfect control of regioselectivity of glycosidation points and stereoselectivity on the anomeric centers.



Scheme 1.1 Enzymatic synthesis of cellulose.

Even though cellulose is the most abundant resource, its application become limited because of its insolubility. However the conversion or depolymerization of cellulose plays a very important role to make it usefull material for biomass conversion processes and equipment to produce fuels, power, heat, and value-added chemicals. Prof. Fukuoka and Prof. Kobayashi reported the catalytic degradation

method of cellulose to glucose and the catalytic transformation method of cellulose to its useful chemicals (Scheme 1.2).¹⁸



Scheme 1.2 Hydrolysis of cellulose.^{18c}

1.2.3 Chitin

Chitin is one of the naturally occurring polymers (Figure 1.6). It is a linear-chain polymer of *N*-acetyl glucosamine and the structural material of cellwall, for instance, fungal cell walls, insect and crustacean exoskeletons (e.g., crabs, lobsters, and shrimps), arthropods, mollusk radulas, and on the scales and other soft tissues of fish and lissamphibians.



Figure 1.6 Structure of chitin.





Shrimp, one of chitin and chitosan source, copied from free picture

It is bio-degradable in the natural environment. Chemically, chitin is closely related to chitosan (a more water-soluble derivative of chitin). Chitosan is the most important derivative of chitin, produced commercially by deacetylation of chitin. These are also closely related to cellulose. Both chitin and chitosan have unbranched chains consisting of glucosamine derivatives.

Natural marine chitin and chitin residues are the exclusive nutrient source for many marine bacteria, which use them efficiently as metabolic fuels. Microbial chitin utilization involves enzymatic breakdown of the biopolymer into short-chain chito-oligomers by secreted chitinases¹⁸ and subsequent efficient internalization of the resultant chitosugars through specialized bacterial outer membrane proteins ("chitoporins").¹⁹ Man-made chitin waste from seafood processing. On the other hand, it is a common starting material for production of commercial purified chitin by processes involving harsh chemical treatment. Both chitin and chitosan has been used in the application of electrochemical biosensors.²⁰ Many reserchers developed the synthetic route for the production of chitin derivatives. Kobayashi *et al.* reported the synthesis of artificial chitin. The method allows the production of the perfectly *N*-acetylated chitins without any damage to *N*-acetyl group (Scheme 1.3).²¹



Scheme 1.3 Synthesis of artificial chitin.

1.2.4 Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β -(1 \rightarrow 4)-D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit) (Figure 1.7). It is made by treating shrimp and other crustacean shells with sodium hydroxide and is more water-soluble. The solubility can be controlled by varying the chain length of the precursor material or the degree of chemical depolymerisation and the extent of deacetylation, which provides useful polymers for a broad range of chemical and physical applications.





Figure 1.7 Structure of chitosan.

One of chitin, chitosan Source, copied from free picture

Chitosan derivatives have practical applications in the form of films, gels, suspensions, microscopic threads, fibers, and also in the fields of biotechnology,²² human²³ and veterinary medicine,²⁴ pharmacy, agriculture,²⁵ food engineering,²⁶ environmental technology,²⁷ and textile and paper industries.²⁸

1.3 Polysaccharides consisting of $(1\rightarrow 3)$ -glycosidic bonds

1.3.1 Schizophyllan

Schizophyllan is an extracellular polysaccharide composed of glucose units derived from the cell wall of the Schizophyllum commune mushroom (Figure 1.8). Schizophyllum species are known collectively as the "split gill" (schizo-) mushrooms. Schizophyllan is a proven biological defense modifier that nutritionally potentiates the immune response. The structure of beta-glucans with the 1,3-glycosidic bonds provides a better molecular "fit", translating into enhanced activation of the immune cascade and a more effective physiological response.



Figure 1.8 Structure of schizophyllans.

The synthesis of 1,3-glycosidic polymer is achievable by cationic ring-opening polymerization of the corresponding anhydro sugar derivatives (Scheme 1.4). The stereoselectivity of ring-opening polymerization of oxetane is controllable by changing of catalyst. The low temperature reaction with Tf₂O takes place through S_N2-type mechanism to give stereoregular glycopyranan at the inversion manner, while the high-temperature reaction affords the other polymer with retention of stereochemistry.



Scheme 1.4 Synthesis of $(1 \rightarrow 3)$ -glucopyranans via ring-opening polymerization.

Since schizophyllan is bound by beta-1,3-glycosidic linkages in the main chain, it forms triple helix with chiral inner cavity, which works as a chiral host polymer for achiral guest molecules.²⁹ For example, it has been reported that oligothiophene guest adopts twisted conformation in the chiral inner cavity (Figure 1.9).



Figure 1.9 Chirality-induced host for achiral guest

1.3.2 Lentinan

Lentinan is also a 1,3-glycosidic polymer (Figure 1.10), which is an intravenous anti-tumor polysaccharide isolated from the fruit body of shiitake mushroom. Lentinan has been approved as an adjuvant for stomach cancer in Japan since 1985. Lentinan is one of the host-mediated anti-cancer drugs which has been shown to affect host defense immune systems.



Figure 1.10 Structure of lentinan



shiitake mushroom, copied from free picture

1.4 Polysaccharides consisting of $(1\rightarrow 6)$ -glycosidic bonds

1.4.1 Dextran

Dextran is a complex branched glucan composed of chains of varying lengths (from 3 to 2000 kilodaltons) (Figure 1.11). The straight chain consists of α -(1 \rightarrow 6)-glycosidic linkages between glucose molecules, while branches begin from α -(1 \rightarrow 3) linkages. Dextran is synthesized from sucrose by certain lactic acid bacteria, the best-known being *Leuconostoc mesenteroides* and *Streptococcus mutans*. It is used medicinally as an antithrombotic to reduce blood viscosity, and as a volume expander in hypovolaemia. Dextran was first discovered by Louis Pasteur as a microbial product in wine.



Figure 1.11 Structure of dextran.

The syntheses of 1,6-glycosidic polymer is also achievable by cationic ring-opening polymerization of the corresponding anhydro sugar derivatives (Scheme 1.5). Dextran with α -(1 \rightarrow 6)-glycosidic linkages is also preparable by the low-temperature ring-opening polymerization.



Scheme 1.5 Synthesis of $(1 \rightarrow 6)$ -glucopyranans via ring-opening polymerization.

1.5 Polysaccharides consisting of $(1 \rightarrow 2)$ -glycosidic bond

1.5.1 (1→2)-β-D-Glucopyranan (nD-β-Glcp-(1-2))

Recently, the author is focussed on the studies of the remaining 1,2-glycosidic polymers. $(1\rightarrow 2)$ - β -Glucopyranan is a natural polysaccharide (Figure 1.12), isolated from *Agrobacterium tumefaciens*. This polymer has glucose units linked together by $(1\rightarrow 2)$ -glycosidic bonds, and has a rigid helical structure having side-chain hydroxyl groups with regulated orientations that may be useful in molecular integration.



Figure 1.12 Structure of $(1\rightarrow 2)$ - β -D-glucopyranan

The soil bacteria can convert nitrogen gas to ammonia after becoming established inside root nodules of legumes. Therefore, legumes such as clover, beans, and soy can sufficiently grow without fertilizer. Although it is strongly expected that this polymer may play a significant role in the interaction between the bacteria and their host plant cell, the interaction mechanism is not clear at the present stage due to a lack of supplied amount of the polymer. The production is affected by external osmolarity, implying the capability to recognize ions.³⁰⁻³²

In spite of such interests, only one pioneering work has been reported as long as I know. The reaction is based on the cationic ring-opening polymerization of highly unstable glycal epoxide, which provides 1,2-glycosidic polymer in middle yield as an anomeric mixture (Scheme 1.6).^{33,34} Therefore, I conceived that there still remains synthetic challenges of 1,2-glucopyranan as follows. First one is the development of practical monomer with improved stability and second is the control of polymerization degree. Third is the introduction of glycosylation reaction-active site to the one polymer terminus, which might be useful for a creation of hybridized materials. Final challenge is the

perfect control of stereochemistry on the anomeric centers.



Scheme 1.6 Synthesis of 1,2-glycosidic polymer from glycal epoxide by cationic ring-opening polymerization.

1.6 Glycosides

Glycosides are compounds containing a sugar unit and a non-sugar residue in the same molecule joined together by glocosidic bond at the anomeric carbon atom. The nonsugar component is called as an aglycone. The sugar component is called as a glycone. Glycosides can be linked by an *O*- (an *Oglycoside*), *N*-(a *glycosylamine*), *S*-(a *thioglycoside*), or *C*-(a *C*-*glycoside*) glycosidic bond. Enzymes can form and break the glycosidic bonds. The representative enzymes are glycoside hydrolases for cleavage and glycosyltransferases for the connection.



Scheme 1.7 Methyl-α-glucoside

Most of glycosides are bioactive compounds. They comprise several important classes of compounds such as hormones, alkaloids, flavonoids, and antibiotics. Several natural glycosides have been used for long time as a folk medicine. It is generally accepted that glycosides are more water soluble than the respective aglycons. Attachment of sugar moiety into the aglycone molecule increases its hydrophilicity and reduces the toxicity. Attachment of polyglycoside to aglycone dramatically increases the solubility in water. The glycone moiety can govern the pharmacokinetic characteristics of glycosides, *e.g.*, circulation, elimination, and the concentrations in the body fluids. Modified hydrophilicity, however, influences mainly the membrane transport. Some compounds enter the cells just because of their "solubility" in the membrane components. Therefore, the solubility of glycoside is very important for the use as a drug when it enters the human body.

Glycosides play important roles in our lives. Many plants store medicinally important chemicals in the form of inactive glycosides. The aglycone contains the biochemically active properties of medical interest. For example, digitalis is a glycoside. The indigestion of the digitalis causes the heart to contract (pump) more forcefully. The glycone and aglycone portions can be chemically separated by hydrolysis in the presence of acid because the acetal bond is labile under acidic condition. There are also numerous enzymes that can form and break the glycosidic bonds.

1.6.1 Monosaccharide- containing glycosides

An example for monosaccharide- containing glycoside is salicin, which is found in the genus *salix* (Figure 1.13). Salicin is converted in the body into salicylic acid, which is closely related to aspirin and has analgesic, antipyretic, and anti-inflammatory effects.



Figure 1.13 Salicin, a glycoside related to aspirin.

Salicin is obtained from different species of *Salix* and *Populus*, but the principal commercial source is *Salix fragilis* (White Willow). Salicin has been used for many years as a remedy in the treatment of fever and rheumatism. It is now used as an analgesic-antipyretic and in case of periodic fever. It is better tolerated in the stomach than sodium salicylate, aspirin, and other antipyretics and antiinflammatory agents, which have largely displaced it in medical practice.

Salicin is hydrolyzed by the enzyme emulsin into saligenin (salicyl alcohol) and glucose. Acid hydrolysis of salicin gives glucose and a phenolic ether, saliretin. Oxidation of saligenin gives salicylic acid, and this accounts for the medicinal value of salicin.

1.6.2 Oligosaccharide containing glycosides

An example for oligosaccharide containing glycoside is glycyrrhizin (or glycyrrhizic acid or glycyrrhizinic acid), which has a disaccharide unit and the aglycone (Figure 1.14). The compound is chief sweet-tasting constituent of *Glycyrrhiza glabra* (liquorice) root, which is known that it is 50 times sweeter than sugar. It is a saponin and has been used as an emulsifier and gel-forming agent in foodstuff and cosmetics. Its aglycone is enoxolone and it has therefore been used as a prodrug for that compound, to prevent liver carcinogenesis in patients with chronic hepatitis C. The other examples for natural oligosaccharide-containing glycosides like cyanidine-3-*O*-sophoroside³⁶ and quercetin-3-*O*-sophoroside³⁶ are reported. These three compounds have $(1\rightarrow 2)$ -glycosidic linkages in the glycone moieties. The effective synthetic method for the introduction of such oligosaccharide to the aglycone has been strongly urged from the view point of the creation of drug.



Figure 1.14 oligosaccharide- containing glycosides.

1.7 Outline of this thesis

This thesis focuses on the development of new synthetic method for 1,2-glycosidic polymers via ring-opening condensation polymerization of a sugar-based cyclic sulfite. In this work, the author discovered the usefulness of the cyclic sulfite a new monomer, for the synthesis of $(1\rightarrow 2)$ -linked polysaccharide. Cationic polymerization of the cyclic sulfite afforded $(1\rightarrow 2)$ -D-glycopyranan along with the elimination of SO₂. The method was also applied to both grafting-onto and grafting-from polymerizations to give the corresponding glycosides. Scheme 1.8 shows the outline of this thesis.

In chapter 2, the author discusses the chemical stability and reactivity of cyclic sulfite as a monomer,

mainly by the comparison with those of glycal epoxide as a conventional monomer. Kinetic studies for the degradations evaluated the higher stability of the cyclic sulfite than the epoxide.

In chapter 3, the author describes the cationic polymerization of cyclic sulfite, where I achieved the synthesis of 1,2-glycosidic polymer bearing pentencyl group at the polymer terminus.

In chapter 4, the author describes the new grafting reaction using cyclic sulfite from glycyrrhetic acid to give glycyrrhetic polyglycosides in one-pot. The reaction enables the rapid preparation of glycyrrhizin derivatives consisting of oligosaccharides with arbitrary polymerization degree.

In chapter 5, this thesis is summarized and concluded, and the future prospect of this work is described.



Scheme 1.8 Outline of this thesis.

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Chapter 2

Synthesis, Kinetic Study and Anionic Ring-Opening Condensation

Polymerization of Cyclic Sulfite
2.1 Introduction

Polysaccharide is a representative class of biopolymers, which consists of sugars with regularlyaligned stereogenic centers as a monomeric unit.¹ The polysaccharide scaffolds are expected to be an indispensable motif for advanced materials such as chiral separation materials,² asymmetric catalysts,³ and optical materials.⁴ To date, the synthetic studies of polysaccharides have been extensively investigated to disclose several fruitful methods for a part of polysaccharides such as cationic ringopening polymerization of anhydro-sugars⁵ and enzymatic polymerizations using non-protected sugars.⁶ Branched and cyclic $(1\rightarrow 2)$ - β -D-glucopyranan (nD- β -Glcp-(1-2)) derivatives are natural polysaccharides isolated from Agrobacterium and Sinorhizobium genera to cause crown gall disease of dicotyledonous plants.^{7,8} The bacterium exhibits nitrogen gas-fixing ability to provide ammonia to the plants. Although it is invoked that nD-β-Glcp-(1-2) derivatives would play an important role to interact between the bacterium and the plant hosts, the interaction mechanism has not been systematically unveiled, urging the chemical synthesis of the structural analogues with a variety of molecular weights and different stereocenters. Meanwhile, the polymer skeleton of $nD-\beta$ -Glcp-(1-2) can be regarded as a helical polyoxyethylene (PEO) possessing hydroxyl groups as the side chain functionalities. Because PEO skeleton recognizes metal cations to change the higher-order structure,⁹ nD-β-Glcp-(1-2) might be a unique asymmetric scaffold available for molecular integration to produce stimuli-responsive optically active materials.

In spite of such interests of $nD-\beta$ -Glcp-(1-2), the synthetic method has not been sufficiently

developed yet; only the synthesis leading to broad polydispersity indices via cationic polymerization of highly unstable glycal epoxide has been reported, where the anomeric carbons of the polymer have ~95% β -configurations.¹⁰ The stereo-selectivity at the anomeric centers depends on three reasons: (i) The polymerization would proceed via an intermediary formation of oxocarbenium cation and (ii) the acetal linkages in the kinetically obtained polymer would undergo further epimerization to give the thermally stable polymer during the acidic condition, which make the stereo-control difficult. In addition, (iii) the S_N2 reaction-based anionic polymerization of the glycal epoxide was infeasible, which seems to provide the stereo-regulated polymer, because the epoxide was highly unstable at the temperature required on the anionic polymerization. Therefore, I planned the development of a new synthetic method of nD- β -Glcp-(1-2) including the following improvements; (i) the use of practical monomer with improved stability, (ii) the controls of polymerization degree and anomeric stereochemistry, and (iii) the introduction of glycosydation-active site to the polymer terminus that might be useful for a creation of the cyclic polymers. Thus I focus on the use of shelf-stable 1,2-Osulfinyl glucopyranose (Scheme 2.1) as a substitute of the unstable epoxy monomer, considering the reports concerning stereospecific introductions of azide,¹¹ diethyloxoglutarate,¹² and cyanide¹³ to the anomeric center of the cyclic sulfite¹⁴.

Schematic illustration for the synthesis of nD- β -Glcp-(1-2) is shown in Scheme 2.1. The thiolate initiator with high nucleophilicity would attack to the anomeric center to give monomeric alkoxide **I** accompanying with a removal of SO₂. Subsequent nucleophilic addition of **I** to the other monomer

would give the corresponding dimer **II** at the stereo-selective manner attributed to an S_N2 reaction. The successive propagation would afford structure-defined nD- β -Glcp-(1-2) skeleton with thioglycoside terminus, which could be easily converted to the corresponding hemi-acetal to give the natural polymer.¹⁵ The thioglycoside could be useful for glycosidation reaction¹⁶ to connect nD- β -Glcp-(1-2) skeleton to aglycons and to graft nD- β -Glcp-(1-2) onto the other polymers (discussed in chapter 4). Moreover, the cyclization reaction under highly diluted condition might also enable the access to cyclic nD- β -Glcp-(1-2) derivative as a natural product.⁸ In this chapter, I describe the synthesis, kinetic study and anionic ring-opening polymerization of the cyclic sulfite.



Scheme 2.1 Schematic illustration for a thiolate-initiated anionic ring-opening polymerization of cyclic sulfite to prepare nD- β -Glcp-(1 \rightarrow 2) framework.

2.2 Results and Discussion

Cyclic sulfite **4** was prepared from tribenzyl-D-glycal **1** according to the literature with a slight modification (Scheme 2.2).¹⁰⁻¹² Glycal **1** was treated with Oxone® to give an epoxide **2** with small amount of impurities¹⁸. The purification of **2** was quite difficult due to the unstability of **2**. Epoxide **2** was exposed to silica gel in ethyl acetate (EtOAc) to give a diol **3** as an anomeric mixture. A formation of cyclic sulfite **4** was performed by a reaction of **3** with freshly prepared thionyl diimidazole in tetrahydrofuran (THF) at room temperature for 30 minutes to give a mixture of two products. From the ¹H NMR spectrum and the literature, it turned out that the products are an *endo/exo*-mixture on the sulfur atom and both compounds are α -anomers.



Scheme 2.2 Synthesis of epoxide 2 and cyclic sulfite 4.

At first I investigated the usefullness of cyclic sulfite over glycal epoxide by a kinetic studies.¹⁹ Through the measurements of ¹H NMR spectra I calculated the thermodynamic parameters for both glycal epoxide and cyclic sulfite discussed below.

Determination of kinetic parameters for the degradation of cyclic sulfite and glycal epoxide. Degradation of glucal cyclic sulfite 4 and epoxide 2 in CDCl₃.

Cyclic sulfite **4** or epoxide **2** (0.01-0.02 mg) was dissolved in CDCl₃ (0.60 mL) in an NMR tube and the solution was heated at defined temperatures (293.15 K, 313.15 K, and 333.15 K). The ¹H NMR spectra were measured after the defined time (Figure 2.4 & 2.5, see page 53). For the cyclic sulfite **4**, the signals corresponding to the anomeric protons at 6.3 and 5.9 ppm progressively decreased and the other peaks changed the shapes along with the degradation of **4**. In the case of epoxide **2**, the anomeric signal at 5.3 ppm decreased faster than that of **4**. These phenomena suggest the chemical stability of **4** is higher than that of **2**. From the integral ratio between the anomeric proton signals and the other aliphatic proton signals, the conversion [**4**]/[**4** $]_0$ was determined.

| 293.15 K | | 313.15 K | | 333.15 K | |
|----------|--------------------------------------|----------|--------------------------------------|----------|------------------|
| Time /h | [4] / [4] ₀ | Time /h | [4] / [4] ₀ | Time /h | [4]/[4] 0 |
| 3 | 0.984 | 3 | 0.987 | 1 | 0.796 |
| 7 | 0.968 | 7 | 0.976 | 4 | 0.756 |
| 21 | 0.967 | 21 | 0.955 | 6 | 0.755 |
| 25 | 0.962 | 25 | 0.871 | 7 | 0.559 |

Table 1. Kinetic analysis for degradation of 4^{a}

 $a[4]_0 = 0.992.$

| Table 2. | Kinetic | analysis | for | degradation of | of 2 . ^{<i>a</i>} |
|----------|---------|----------|-----|----------------|-----------------------------------|
|----------|---------|----------|-----|----------------|-----------------------------------|

| 293.15 K | | 313. | 313.15 K | | 333.15 K | |
|----------|--------------------------------------|---------|--------------------------------------|---------|---------------------------|--|
| Time /h | [2] / [2] ₀ | Time /h | [2] / [2] ₀ | Time /h | [2] / [2] 0 | |
| 1 | 0.860 | 1 | 0.818 | 1 | 0.823 | |
| 2 | 0.828 | 2 | 0.806 | 2 | 0.789 | |
| 3 | 0.810 | 3 | 0.800 | 3 | 0.749 | |
| 4 | 0.814 | 4 | 0.755 | 4 | 0.734 | |
| 5 | 0.803 | 5 | 0.743 | 5 | 0.712 | |
| 6 | 0.800 | 6 | 0.748 | 6 | 0.687 | |
| 7 | 0.804 | 7 | 0.738 | 7 | 0.659 | |
| 25 | 0.789 | 25 | 0.660 | 25 | 0.341 | |
| 27 | 0.788 | 27 | 0.639 | 27 | 0.301 | |
| 29 | 0.773 | 29 | 0.629 | 29 | 0.242 | |
| 71 | 0.747 | 71 | 0.457 | 71 | _b | |
| 101 | 0.746 | 101 | 0.424 | 101 | b | |
| | | | | | | |

^{*a*}[**2**]₀ = 0.864. ^{*b*}Not determined.

Assuming that the degradation reaction obeyed the first-order kinetics, the reaction rate is described with the following equation:

$$-d[4]/dt = -k_{4}[4]$$
(1)

It was converted to equation (2);

$$ln \, [4]/[4]_0 = -k_4 t \tag{2}$$

In a similar way, the conversion $[2]/[2]_0$ was also determined. If the degradation reaction also obeyed the first-order kinetics, the reaction rate would be described with the following equation:

$$-d[\mathbf{2}]/d\mathbf{t} = -k_2[\mathbf{2}] \tag{3}$$

It was also converted to equation (4):

$$ln[\mathbf{2}]/[\mathbf{2}]_0 = -k_2 t \tag{4}$$

Time-course plots of **4** for 293.15 K were drawn to determine the half-life period τ_4 of **4**. The plots were fit by a line and the slope ($-k_4$) was calculated by least squares approximation to determine 5.82 $\times 10^{-8}$ sec⁻¹. The half-life period τ_4 of **4** was estimated from the following equation to be 3307 h:

$$\tau_4 = \ln 2 / k_4 \tag{5}$$

In a similar way, time-course plots of **2** for 293.15 K were drawn to determine the half-life period τ_2 of **2**. The plots were fit by a line and the slope $(-k_2)$ was calculated by least squares approximation to determine $0.30 \times 10^{-6} \text{ sec}^{-1}$. The half-life period τ_2 of **2** was estimated from the following equation to be 497 h:

$$\tau_3 = \ln 2 / k_2 \tag{6}$$

In the same way, k_4 , k_2 , τ_4 , and τ_2 were estimated for different temperatures 313.15 and 333.15 K.

| temp. /K | $k_4 / { m sec}^{-1}$ | $	au_4$ /h |
|----------|-----------------------|------------|
| 293.15 | 5.82×10^{-8} | 3307.4 |
| 313.15 | $0.80 	imes 10^{-6}$ | 224.2 |
| 333.15 | 0.45×10^{-5} | 42.7 |

Table S3. Kinetic parameters for degradation of 4.

Table S4. Kinetic parameters for degradation of 2.

| temp. /K | K_2 /sec ⁻¹ | $	au_2$ /h |
|----------|--------------------------|------------|
| 293.15 | $0.30 	imes 10^{-6}$ | 497.3 |
| 313.15 | 0.29×10^{-5} | 66.1 |
| 333.15 | 1.40×10^{-5} | 13.6 |

Evaluation of activation energy for degradation.

The activation energy for degradation was determined according to the following equation;

$$k = \operatorname{Aexp}\left(-E/\operatorname{RT}\right) \tag{7}$$

It was converted to Arrhenius equation (5);

$$\ln k = -E/RT + \ln A \tag{8}$$

k: dissociation kinetic constant

E: activation energy

R: gas constant (8.31 m² kg s^{$$-2$$} K ^{-1} mol ^{-1})

T: absolute temperature

A: frequency factor of Arrhenius equation

Inverse of temperature was plotted against *ln*k to determine the activation energy E₄. The plots for **4** were fit by a line and the slope $(-E_4/R)$ was calculated by least squares approximation to determine -10.6×10^3 , which give the value of E₄ as 88 kJ/mol. The plots for **2** were fit by a line and the slope $(-E_2/R)$ was calculated by least squares approximation to determine -9.4×10^3 , which give the value of E₄ as 78 kJ/mol.

| Fable 2.5 Analysis of Arrhenius plots for degradation of 4. |
|--|
|--|

| T (K) | 1/T | ln k4 |
|--------|----------|-----------|
| 293.15 | 0.003411 | - 16.6591 |
| 313.15 | 0.003193 | - 14.0386 |
| 333.15 | 0.003002 | - 12.3114 |

Table 2.6 Analysis of Arrhenius plots for degradation of **2**.

| T (K) | 1/T | ln k ₂ |
|--------|----------|-------------------|
| 293.15 | 0.003411 | - 15.0195 |
| 313.15 | 0.003193 | - 12.7508 |
| 333.15 | 0.003002 | - 11.1765 |
| | | |

Evaluation of Gibbs' activation free energy, activation entropy, and activation enthalpy for degradation

Gibbs' activation free energy for degradation was determined according to Eyring equation (9);

$$\Delta G^{\ddagger} = -RT\ln(k \ h/Tk B) \tag{9}$$

 ΔG^{\ddagger} : Gibbs' activation free energy

R: gas constant (8.31 m² kg s⁻² K⁻¹ mol⁻¹)

T: absolute temperature

k: degradation kinetic constant

h: Plank constant ($6.63 \times 10^{-34} \text{ m}^2 \text{kg}^{-3}\text{s}^{-1}$)

*k*_B: Boltzmann constant (1.38 × 10⁻²³ m² kg s⁻²K⁻¹)

 Table S7. Gibbs' activation free energy for degradation of 4.

| temp. /K | ⊿G‡₄ (kJ/mol) |
|----------|---------------|
| 293.15 | -112.300 |
| 313.15 | -113.314 |
| 333.15 | -115.940 |
| | |

| temp. /K | $\Delta G_{2} (kJ/mol)$ |
|----------|-------------------------|
| 293.15 | -108.305 |
| 313.15 | -109.962 |
| 333.15 | -112.798 |
| | |

 Table S8. Gibbs' activation free energy for degradation of 2.

Eyring equation (9) was converted to following equation (10);

$$\ln (k/T) = \ln k_{\rm B} / h + \Delta S^{\ddagger} / R - \Delta H^{\ddagger} / RT$$
(10)

 ΔS^{\ddagger} : activation entropy

ΔH^{\ddagger} : activation enthalpy

Inverse of temperature was plotted against $ln(k_4/T)$ to determine the activation entropy (ΔS^{\ddagger}_4) and enthalpy (ΔH^{\ddagger}_4) for **4**. The plots were fit by a line and the slope ($-\Delta H^{\ddagger}/R$) was calculated by least squares approximation to determine -10.3×10^3 , which gave the value of ΔH^{\ddagger}_4 as 86 kJ/mol, while the intercept [$ln(k_B/h) + \Delta S^{\ddagger}/R$] was also calculated by least squares approximation to determine 13.05, which gave the value of ΔS^{\ddagger}_4 as -31.5 J/K·mol.

In a similar way, the plots for **2** were fit by a line to give the value of slope to be -9.1×10^3 , which gave the value of ΔH^{\ddagger}_2 as 75 kJ/mol. The intercept was 10.4, which gave the value of ΔS^{\ddagger}_2 as -53.7 J/K·mol.

| temp. /K | 1/T | lnk_4 |
|----------|----------|----------|
| 293.15 | 0.003411 | -22.3459 |
| 313.15 | 0.003193 | -19.7853 |
| 333.15 | 0.003002 | -18.1199 |

Table S9. Analysis of Eyring plots for degradation of 4.

Table S10. Analysis of Eyring plots for degradation of 2.

| temp. /K | 1/T | lnk_2 |
|----------|----------|----------|
| 293.15 | 0.003411 | -20.7002 |
| 313.15 | 0.003193 | -18.4975 |
| 333.15 | 0.003002 | -16.9850 |

Table S11. Activation energy, activation entropy, and activation enthalpy for degradation.

| Compound | Activation energy (⊿G‡) / kJ/mol | Activation entropy (⊿S‡) / J/K·mol | Activation enthalpy (Δ H‡) / kJ/mol |
|------------------|-------------------------------------|---------------------------------------|--|
| Cyclic sulfite 4 | 88 | -31 | 86 |
| Epoxide 2 | 78 | -53 | 75 |

Anionic polymerization of cyclic sulfite

Prior to the synthesis of nD- β -Glcp-(1-2), I investigated the synthesis of thioglycoside **5** as a monomeric unit in order to confirm a regio- and stereo-selectivity of nucleophilic addition of thiolate to the cyclic sulfite. Because the sulfite inherently has two reactive carbon centers, I investigated the reactivity using excess amount of potasium thiolate. As a result, the reaction of cyclic sulfite with 200 mol% of TolSK in DMF proceeded selectively to give thioglycoside **5**, along with a formation of hemi-

acetal degraded by humidity.

The structure of **5** was a β -thioglycoside as a single isomer, which was clearly evidenced by a big coupling constant between H-1 and H-2 protons to suggest the structure of 1,2-*trans*-substituents. I also confirmed a generation of alcohol at the 2 position by a removal of SO₂ group, which was determined by the IR spectrum. The structure of **5** clearly indicates a regio-selective addition to the anomeric center bearing weakened C-OSO₂ bond caused by a negative hyperconjugation to the oxygen of pyran, a stereo-selective reaction attributed to an S_N2 reaction, and efficient removal of SO₂ to generate the corresponding alkoxide as a propagation terminus, supporting the applicability of the nucleophilic reaction to polymerization reaction of cyclic sulfite. According to the model reaction condition, I investigated the polymerization of cyclic sulfite using 10 mol% of thiolate initiator (Scheme 2.4). The results are summarized in Table 2.12.



Scheme 2.3 Schematic illustration for synthesis of unit model

The polymerization using 10 mol% of initiator in the presence of drierite in DMF was performed at 100 - 120 °C. The product was purified by reperecipitation by hexane. But polymer is contaminated by polymeric sulfur appeared as a brown gummy solid after the reaction, and found difficult to remove due to its gummy nature. The formation of gummy polymeric sulpfur implying the elimination of SO₂.



Scheme 2.4 Scheme of anionic polymerization

| entry | initiator | additive | temp (°C) | hexane-insoluble | | | hexane-soluble |
|------------|---------------|-------------------|-----------|------------------|-------------|---------------------------|----------------|
| amount (mo | amount (mol%) | | | yield (%) | M_{n}^{a} | $M_{\rm w}/M_{\rm n}^{a}$ | yield (%) |
| 1 | 10 | - | 100 | 11 | 990 | 4.1 | 34 |
| 2 | 10 | MS 4A | 100 | 58 | 510 | 1.5 | 29 |
| 3 | 10 | CaSO ₄ | 100 | 85 | 730 | 2.5 | 19 |
| 4 | 10 | CaSO ₄ | 80 | 63 | 780 | 1.5 | 25 |
| 5 | 10 | CaSO ₄ | 120 | 55 | 890 | 3.2 | 9 |
| 6 | 10 | CaSO ₄ | 140 | 44 | 9000 | 2.0 | 36 |
| 7 | 5 | CaSO ₄ | 120 | 46 | 1200 | 4.0 | 6 |

 Table 2.12 Results of anionic polymerization

^{*a*} Estimated by SEC analyses on the basis of polystyrene standards.

The obtained polymer and unimer was analysed by ¹H NMR, IR, and MALDI–TOF mass spectra and elemental analysis. The ¹H NMR spectrum of polymer clearly supported the absence of characteristic signals of cyclic sulfite at around 6.4 ppm. It was also found that the spectrum of polymer is in a good agreement with that of **5**, implying the formation of $nD-\beta$ -Glcp(1,2) skeleton.



Figure 2.1 ¹H NMR spectra of 4, 5 and polymer (entry-5) (400 MHz, CDCl₃, r.t.).

The IR spectra of cyclic sulfite and polymer is shown below. The IR spectrum of polymer clearly indicates the decrease in the characteristic absorption peak attributed to cyclic sulfite, suggesting that the polymer mainly consisting of polyether linkages. The remained small peak may be corresponds to some other sulfur containing impurities or remained CaSO₄ which I used as a dehydrator for the reaction.



Figure 2.2 IR spectra of cyclic sulfite and polymer (entry-5).

I also measured the MALDI–TOF MS spectrum of polymer. I found that the plural peaks in the spectrum were observed as peaks with even intervals corresponding to the monomeric unit and the observed peak values are in good accordance with those of expected values, strongly supporting that the sulfide-terminated desired polyether was formed.



Figure 2.3 MALDI-TOF MS (matrix: CHCa) of polymer prepared at 120 °C

Despite of such agreements of spectral data with those of theoretical values, the elemental analysis data of the polymer was largely different from those for the proposed structure (calculated for nD- β -Glcp(1-2) is C= 74.98%, H= 6.53%, O= 18.50%, found (polymer entry 5): C= 61.03%, H= 6.23%, S= 4.69%). The observed value was much closer to that of cyclic sulfite (C= 65.31%, H= 5.68%, S= 6.46%). Such results suggested that the products could have a partial polysulfite structure or also be a mixture of nD- β -Glcp(1,2) and some S-atom based compound could be produced. Expected impurity is: SO₂ + 2DMF \rightarrow [(CH₃)₂NCO]₂SO + H₂O \rightarrow 1/8S₈ + X. Prof. Miyamoto reported that a mixing sulfur dioxide in DMF spontaneously generates H₂O and sulfur, although the mechanism has not been clarified.¹⁷

2.3 Conclusion

In conclusion, I invented the usefulness of cyclic sulfite to use it as monomer also I evaluated the higher stability of cyclic sulfite than the glycal epoxide through kinetic studies for the degradation. The anionic ring-opening condensation polymerization of cyclic sulfite seems to lead to a mixture of structures including the proposed structure and some other impurities containing sulfur.

2.4 Experimental

General methods:

Materials. Tetrahydrofuran (THF) (Kanto Chemical Co., Inc.) was freshly distilled in the presence of sodium and benzophenone under nitrogen atromsphere before use. Dichloromethane was distilled over CaH₂ and stored with MS 4Å. MS 3Å as a dehydrator was activated by three times heating for 1 min in a microwave oven following careful heating by a heat gun under vacuum. The other chemicals were used without purification. Cyclic sulfite was prepared according to the literature: A. Benksim, D. Beaupère, A. Wadouachi, *Org. Lett.* **2004**, *6*, 3913-3915..

Measurements. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on JEOL JNM-ESC400 and JNM-ESC600 spectrometers using CDCl₃ as the solvent, calibrated using residual undeuterated solvent and tetramethylsilane as the internal standard. FT-IR spectra were measured using a Thermo Fischer Scientific Nexus 870 spectrometer. Optical rotation was measured with a JASCO P-1030 digital polarimeter. SEC analyses were carried out using a chromatographic system

consisting of a JASCO PU-2080 plus pump with a JASCO UV-1570 (UV detector) equipped with Shodex Asahipak GF-310 HQ (30 x 0.75 (i.d.) cm) (eluent DMF with LiCl (30 mM), flow rate 1.0 mL/min). MALDI–TOF MS spectra were recorded on a Shimadzu Voyager-DE STR-H mass spectrometer (matrix: α – CHCA) at Instrumental Analysis Division, Equipment Management Center Creative Research Institution, Hokkaido University.

Typical procedure for the preparation of cyclic sulfite 4^{4, 11-13}

To a solution of imidazole (5.59 g, 82.0 mmol) in THF (80 mL) was added dropwise SOCl₂ (1.49 mL, 20.5 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 30 min, and filtered. To the resulting *N*,*N*-thionyldiimidazole solution was added a solution of diol (1.54 g, 3.42 mmol) in THF (20 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 30 min, and carefully concentrated in vacuo below 30 °C. The crude was purified by a florisil column chromatography (eluent: hexane : ethyl acetate = 9:1 \rightarrow 3:1) to give the cyclic sulfite **4** (689.1 mg) as an *endo/exo* diastereo-mixture (2:1) on the sulfur atom; [α]p^{25.2} +35.6 (CHCl₃, c. 1.00); ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.37–7.14 (15H, m) 6.37 (0.63H, d, *J* = 4.8 Hz), 6.12 (0.37H, d, *J* = 6.0 Hz), 4.96–4.28 (7H, m), 4.14–3.57 (5H, m) ppm; ¹³C NMR (100 MHz, 298 K, CDCl₃) δ 138.0, 137.9, 137.79, 137.76, 137.4, 137.1, 128.8, 128.61, 128.58, 128.54, 128.49, 128.47, 128.24, 128.21, 128.1, 128.0, 127.94, 127.90, 105.9, 102.4, 83.8, 80.1, 75.8, 75.0, 74.8, 74.6, 74.3, 74.0, 73.7, 73.5, 72.8, 72.6, 71.4, 68.7, 68.6, 67.9 ppm; IR (NaCl) ν 3088, 3063, 3031, 2868, 1773, 1496, 1454, 1365, 1214 (OS(O)O), 1164,

1099, 1028, 989, 893, 819, 773, 740, 699 cm⁻¹; MALDI–TOF MS (matrix: CHC α) calc'd for C₂₇H₂₈NaO₇S⁺ [M+Na⁺] 519.14; found 519.59.

Typical procedure for the preparation of glycal epoxide 2

To a solution of **1** (100 mg, 0.24 mmol) in dichloromethane (1.98 ml) and acetone (0.72 ml) was added a saturated solution of NaHCO₃ at 0 0 C followed by the addition of oxone (885.4 mg, 1.44 mmol) solution in water (2 ml) slowly. The reaction mixture was stirred for 30 minute at 0 0 C and warmed to room temperature, stirred for 5 hours. TLC was checked. TLC showed the absence of starting material. Workup was then carried out by dilution with water and extracted with dichloromethane. The organic layer was separated and concentrated to get the product **2** (102 mg, 98.3%) The product obtained was characterized by ¹H NMR.

The product from the reaction was characterized by ¹H NMR. The analytical details are mentioned below. ¹H NMR (400 MHz, CDCl₃, 293 K) δ 7.50-7.18 (m, 15 H, aromatic H), 4.98 (dd, 1H, $J_{1,2}$ = 2.5 Hz, H-1), 4.88-3.45 (m, 11H), 3.05 (d, 1 H, H-2) ppm.

Typical procedure for the preparation of unit model 5

To a solution of **4** (35 mg, 0.07 mmol) in DMF (7.7 mL) under nitrogen atmosphere potassium salt of 4-methylbenzenethiolate (Initiator) was added (0.011 g, 0.0.070 mmol). The reaction mixture was allowed to stir gently at room temperature for 45 minutes. TLC was checked. TLC showed the absence of starting material and the generation of single product. Workup was then carried out by dilution with water and extracted with diethyl ether. The organic layer was separated and concentrated to get the crude. The crude was purified by column chromatography using silica gel with the solvents hexane and ethyl acetate. The desired fraction was concentrated to get a white solid (20 mg, 51.3%). The product obtained was characterized by ¹H NMR.

The product from the reaction was characterized by ¹H NMR, 2D NMR, IR, ¹³C NMR, MALDI mass, melting range and optical rotation. By the calculation of coupling constant (J= 9.2 Hz) I found the obtained product is β-anomer, The analytical details are mentioned below. Off- white solid. ¹H NMR (400 MHz, CDCl₃, 293 K) δ 7.45 (d, J = 8.2 Hz, 2H), 7.36-7.18 (m, 15 H, Ph), 7.05 (d, J = 8.2 Hz, 2H), 4.91 (d, J = 11.2 Hz, 1H, CH₂Ph), 4.83 (d, J = 11.2 Hz, 1H, CH₂Ph), 4.81 (d, J = 11.5 Hz, 1H, CH₂Ph), 4.61 (d, J = 12.4 Hz, 1H, CH₂Ph), 4.56 (d, J = 11.5 Hz, 1H, CH₂Ph), 4.54 (d, J = 12.4 Hz, 1H, CH₂Ph), 4.40 (d, J = 9.2 Hz, 1H, H-1), 3.78 (dd, J = 11.2, 1.8 Hz, 1H, H-6), 3.73 (dd, J = 11.2, 4.3 Hz, 1H, H-6), 3.59-3.55 (m, 2H, H-4,3), 3.51 (br, 1H, H-5), 3.44 (t, J = 9.2 Hz, 1H, H-2), 2.40 (br, 1H, OH), 2.31 (s,3H, CH₃) ppm. ¹³C NMR (100 MHz, CDCl₃, 293 K) δ 138.47, 138.42, 138.33, 138.06, 133.64, 129.73, 128.49, 128.40, 128.32, 127.98, 127.94, 127.78, 127.62, 127.53, 127.50, 88.06, 85.90, 79.44, 77.33, 75.31, 75.06, 73.41, 72.44, 68.98, 21.14 ppm. [α] ρ^{22} = -31.1250 (c.1.60, CHCl₃)

Typical procedure of anionic polymerization of cyclic sulfite 4

To a solution of **4** in DMF (0.8 M) was added potassium 4-methylbenzenethiolate (0.1 eq) and drierite (100 mg). The suspension was stirred for 24 h at 120 °C. Reaction was monitored by the SEC analysis. After 24 h, reaction mixture was diluted with acetone and then filtered to remove solid additive. Filtration was followed by evaporation to get the crude material as dark yellow syrup. The crude was purified by reprecipitation using chloroform and hexane. Both soluble and insoluble parts were dried under reduced pressure at 35 °C gave yellow syrup which were characterized by ¹H NMR, IR, MALDI-TOF mass, elemental analysis and SEC. ¹H NMR (400 MHz, CDCl₃, 293 K) δ 7.45-7.05 (m), 4.91-4.38 (m), 3.78-3.44 (m), 2.40 (br, 1H, OH), 2.31 (s, CH₃) ppm; IR (NaCl) *v* 3385, 3063, 3030, 2921, 2867, 1496, 1454, 1362, 1210, 1122, 1028, 738, 698 cm⁻¹; Elemental analysis: calc'd for C_{224.24}H_{237.36}O_{41.6}·CHCl₃ based on the polymerization degree (8.12) estimated by the ¹H NMR spectrum (entry 5): C 72.77, H 6.46, Found: C 61.03, H 6.23; S 4.69%.

Figures



Figure 2.4 Spectral change of ¹H NMR spectrum of cyclic sulfite 4 (400 MHz, CDCl₃).



Figure 2.5 Spectral change of ¹H NMR spectrum of epoxide 2 (400 MHz, CDCl₃).



Figure 2.6 First-order kinetic plots for dissociation of cyclic sulfite 4



Figure 2.7 First-order kinetic plots for dissociation of epoxide 2.



Figure 2.8 Arrhenius plots for dissociation of cyclic sulfite 4



Figure 2.9 Arrhenius plots for dissociation of epoxide 2



Figure 2.10 Eyring plots for dissociation of cyclic sulfite 4.



Figure 2.11 Eyring plots for dissociation of epoxide 2



Figure 2.12 ¹H NMR spectrum of cyclic sulfite 4 (400 MHz, CDCl₃, 293 K).



Figure 2.13 ¹³C NMR spectrum of cyclic sulfite 4 (100 MHz, CDCl₃, 293 K).



Figure 2.14 IR spectrum of cyclic sulfite 4 (NaCl).



Figure 2.15 MALDI–TOF MS spectrum of cyclic sulfite 4.



Figure 2.16 ¹H NMR spectrum of 5 (400 MHz, CDCl₃, r.t.).



Figure 2.17 2D NMR spectrum of 5 (400 MHz, CDCl₃, r.t.).



Figure 2.18¹³C NMR spectrum of 5 (400 MHz, CDCl₃, r.t.).



Figure 2.19 ¹³C NMR spectrum of 5.



Figure 2.20 MALDI-TOF MS of 5.

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Chapter 3

Synthesis and Structure of $(1\rightarrow 2)$ -D-Glucopyranan via Cationic Ring-

Opening Condensation Polymerization of Cyclic Sulfite

3.1. Introduction

As I discussed in the chapter 2, the anionic polymerization seems to lead to a mixture of structures including proposed structure and some other impurities containing sulphur. However the polymerization enables selective introductions of stereogenic centers to the anomeric positions with thioglycoside terminus, the product was contaminated with sulfur. I am unable to purify in any method. Therefore, I next focused on the cationic polymerization of the sugar based cyclic sulfite.

It has been reported that the polymerizability of cyclic sulfites under cationic conditions are strongly dependent on the ring size and strain: The cationic polymerization of 7-membered sulfite efficiently gives polysulfite without a lack of sulfite linkage, while that of 6-membered sulfite partially accompanies with elimination of SO₂.¹ Whereas, the both copolymerization^{2,3} with cyclic anhydrides and homopolymerization³ of 5-membered sulfite results in perfect elimination of SO₂ to afford the corresponding polyesters. Therefore, I expected that 5-membered cyclic sulfite I could work as a surrogate of the epoxy monomer to give 1,2-glycosidic polymer through cationic polymerization.



Scheme 3.1 Cationic ring-opening polymerization of 5, 6 and 7- membered cyclic sulfites.

In this work, I selected 4-pentenol as an initiator (R'OH) for the polymerization, because both glycosidation and glycosylation reactions using pentenoyl glycosides have been well established.⁴ 4-Pentenol would attack to the anomeric center in the presence of acid to give monomeric alcohol **II** accompanying with a removal of SO₂. If the glycosydation reaction of the generated secondary alcohol to the other monomer proceeded to furnish **III** at the stereo-selective manner attributed to an S_N2 reaction, the successive propagation would give nD- β -Glcp-(1-2) skeleton with pentenoyl group at the polymer terminus.

3.2 Results and Discussion

Cationic polymerization of cyclic sulfite

Scheme 3.2 shows the schematic illustration for the synthesis of nD- β -Glcp-(1-2) via cationic ringopening condensation polymerization of the cyclic sulfite.


Scheme 3.2 Schematic illustration for a cationic ring-opening condensation polymerization of cyclic sulfite to prepare nD- β -Glcp-(1-2) framework.

I investigated the polymerization of I using 10 mol% of 4-pentenol initiator in the presence of a dehydrator in CH_2Cl_2 (0.6 M). The results are summarized in Table 3.1.



Scheme 3.3 Cationic polymerization of cyclic sulfite

I also investigated the effects of reaction temperature and feed ratio of initiator on the yields and M_n as summarised in the below table. From the results of entries 4–9, I found that the conversions of 4 decrease as the temperature decrease. M_n was moderately controllable by prolonging the reaction time (entries 8 and 9) and changing [M]/[I] (entries 4, 5, 6, 11, and 12).

| entry | initiator (mol%) | acid | dehydrator ^c | temp (°C) | time (h) | yield (%) | $M_{\rm n}{}^d$ (Da) | $M_{ m w}/M_{ m n}^{e}$ | [α] _D ^f |
|-------|---------------------|------------------------------|-------------------------|--------------|-------------|--------------|----------------------|-------------------------|-------------------------------|
| 1 | 10 | CF ₃ COOH | CaSO ₄ | rt | 24 | 0 | _ | _ | _ |
| 2 | 10 | TfOH | CaSO ₄ | rt | 24 | 85 | 4500 | 1.8 | +109 |
| 3 | 10 | TfOH | CaSO ₄ | 0 | 24 | 83 | 4800 | 1.2 | +61 |
| 4 | 10 | TfOH | MS 3Å ^g | rt | 24 | 61 | 1800 | 1.4 | +52 |
| 5 | 5 | $\mathrm{TfOH}^{\mathrm{h}}$ | MS 3Å | rt | 41 | 85 | 4200 | 2.4 | +61 |
| 6 | 10 | TfOH | MS 3Å | -10 | 24 | 73 | 5000 | 1.2 | +55 |
| 7 | 10 | TfOH | MS 3Å | -20 | 41 | 55 | 3000 | 1.3 | +37 |
| 8 | 10 | TfOH | MS 3Å | -40 | 41 | 56 | 2900 | 1.3 | +35 |
| 9 | 10 | TfOH | MS 3Å | -40 | 100 | 42 | 10900 | 1.3 | +56 |
| 10 | 10 | TfOH | MS 3Å | -60 | 41 | 28 | 1900 | 1.2 | +8.7 |
| 11 | 5 | TfOH | MS 3Å | -20 | 41 | 80 | 4300 | 1.4 | +49 |
| 12 | 20 | TfOH | MS 3Å | -20 | 41 | 91 | 1700 | 1.3 | +39 |

 Table 3.1 Results of cationic polymerization.

^{*a*} The reaction was performed by using 4 (0.30–0.40 mmol), initiator (10 mol%), and acid (10 mol%) in the presence of a dehydrator in CH_2Cl_2 (0.6 M). ^{*b*} Monomer conversion ratio was determined by ¹H NMR spectra of the crude mixture. ^{*c*} 50 wt% of dehydrator per 4 was used. ^{*d*} Number-

average molecular weights (M_n) were estimated by the integral ratio in the ¹H NMR spectra. ^{*e*} Polydispersity indices (M_w/M_n) were determined by a size exclusion chromatography (SEC) using DMF as an eluent. ^{*f*} Specific optical rotation ([α]_D) was measured in CHCl₃ at 25–28 °C. ^{*g*} 150 wt% of MS 3Å was used. ^{*h*} 5.0 mol% of TfOH was used.

Polymerization using CF₃COOH (pKa -0.25) resulted in no reaction (entry 1), the use of TfOH (pKa -14) in the presence of CaSO₄ as a dehydrator efficiently facilitated the polymerization at room temperature and 0 °C to afford high yields of polymers (entries 2 and 3). The polymerization reaction was also performed in the presence of acetonitrile in place of dichloromethane at -20 °C, RT and 80 °C. But the result was unfruitful, there was no product formation. The structures of the obtained polymers were evaluated by IR, ¹H NMR, and MALDI–TOF MS spectra.

In the IR spectra, the absence of the characteristic signal of sulfite at around 1200 cm⁻¹ was observed, implying that the polymerization of **4** efficiently eliminates SO₂ to form polyoxyethylene main chain (Figures 3.14, 3.17, 3.20, 3.21, 3.22, 3.23). Elemental analysis of the polymer finally evidenced the absence of sulphur atom in the obtained polymer skeleton. The appearance of olefinic proton signals in the ¹H NMR spectra supports the introduction of pentenoyl group to the polymer terminus. The number average of molecular weights (M_n) were determined on the basis of the integral ratio between the internal olefinic proton of pentenoyl group and the other aliphatic protons in the ¹H NMR spectra. The distributions (M_w/M_n) were estimated by a size exclusion chromatography (SEC)

using *N*,*N*-dimethylformamide (DMF) based on polystyrene standards. Matrix associated laser desorption/ionization time-of-flight mass (MALDI–TOF MS) with the theoretical values corresponding to the polymer, supporting the formation of pentenoyl-group terminated nD-Glcp-(1-2) skeleton. However, I also found the additional minor peaks attributed to the formation of OH-terminated polymers and cyclic polymer in the MS spectra. After considerable efforts, I found that MS 3Å works as a good dehydrator efficiently to suppress the formation of OH-terminated polymers (entries 4 and 5). The MALDI–TOF MS spectrum of the polymer exhibited a simple pattern assignable to the pentenoyl group-terminated polymers excluding the peaks from OH-terminated polymer, strongly suggesting the good dehydrating capability of MS 3Å under the polymerization condition.

I also investigated the effects of reaction temperature and feed ratio of initiator on the yields and M_n (entries 4–11 in Table 3.1). From the results of entries 4–9, I found that the conversions of cyclic sulphite decrease as the temperature decrease. M_n was moderately controllable by prolonging the reaction time (entries 8 and 9) and changing [M]/[I] (entries 4, 5, 6, 11, and 12).

For the purpose to determine the stereochemistry at the anomeric centers of polymer, unit models 5β and 5α were prepared as a reference material (Scheme 3.4). Treatment of cyclic sulphite with 1.2 equivalent of 4-pentenol in the presence of a catalytic amount of TfOH (10 mol%) at room temperature for 13 h in diluted CH₂Cl₂ (0.05 M) gave a 1:1 mixture of 5β and 5α in good yields. The stereochemistry of 5β and 5α were determined by the coupling constants between the 1 and 2 protons in the ¹H NMR spectra.



Scheme 3.4 Synthesis of unit model

To estimate roughly the anomeric stereochemistry of the polymer, I first measured the specific optical rotations ($[\alpha]_D$) of polymers and unit models (Table 1). As a result, it was found that polymer prepared at lower temperature exhibits a smaller positive $[\alpha]_D$ value compared with the polymer prepared at higher temperature. The reported $[\alpha]_D$ s of branched, cyclic nD- β -Glcp-(1-2), and its dimer were -12, -5, and +15, while that of α -kojibiose as a glucose dimer consisting of an α -glycosidic linkage is +162.¹⁰ In addition, the $[\alpha]_D$ s of **5** β and **5** α consisting of β - and α -pentenoly group are -5 and +69, respectively. Therefore, it is expected that the presence of α -glycosidic linkage could lead the $[\alpha]_D$ of polymer to remarkably big positive value. Therefore, the polymers obtained at room temperature (entries 4 and 5) seem to be consisting of much more α -glycosidic linkages than the other polymers, whereas the polymer from entry 10 could mainly include β -glycosidic centers.

I next measured and compared ¹³C NMR spectra of unit models and polymers. Figure 3.1 shows the partial ¹³C NMR spectra of unit models (5β and 5α) and the synthetic polymers in Table 1. In the spectra of unit models (A) and (B), the chemical shifts of anomeric carbon with pentenoly group were 102.9 ppm for **5** β and 98.6 ppm for **5** α . On the other hand, the signals assignable to anomeric carbons in the spectra (C) of polymer obtained at room temperature (entry 5) appeared as multiple peaks, indicating the presence of both α - and β -anomeric centers. The spectra of (D) and (E) from entries 7 and 10 were mainly consisting of two intense signals: The sharp signal at 103 ppm should be a terminal anomeric carbon containing a pentenoyl group, because the chemical shift is similar to that of **5** β (A). The other broad signal at around 104 ppm would be assignable to β -anomeric carbons originating from the glycosidic linkages in the main chain, whose chemical shifts are in good accordance to those of reported natural^{11,12} and synthetic polymers.¹⁰ Therefore, I concluded that (i) the peaks in a range from 90 to 97 ppm in the spectrum (C) could be attributed to the presence of α -anomeric centers in the main chain and (ii) the polymerization at low temperature from -20 °C to -60 °C sufficiently suppresses the formation of α -anomeric center to give nD- β -Glcp-(1-2) skeleton.



Figure 3.1 Partial ¹³C NMR spectra of unit model 5 β (A) and 5 α (B) and synthetic polymers of entry 5 (C), entry 7 (D), and entry 10 (E) (150 MHz, CDCl₃ 293 K).



Scheme 3.5 Plausible reaction mechanism for cationic polymerization of cyclic sulfite.

On the basis of the related mechanism for the ring-opening polymerization of anhydro sugar,¹³ the plausible reaction mechanism for the cationic polymerization of cyclic sulfite is shown in the scheme 3.5. At low temperature, the polymerization would occur in $S_N 2$ inversion manner through the formation of oxonium cation as intermediate, which results in the formation of β -anomeric center.¹³ In the case of high temperature, the reaction would afford the formation of oxocarbenium cation which results in the formation of anomeric mixture.¹³ In addition, the anomerization from β -anomeric compound could be also possible to give the mixture.

To confirm the chemical structure of the polymer, the benzyl groups of the synthesized polymer (entry 7) was deprotected by Birch reduction. By comparision of ¹³C NMR between the reported¹⁰ and the obtained polymers, it was found that the anomeric carbon signals of the synthetic polymer appeared as multiple peaks (Figure 3.2), probably because of the difference in the terminal structure and the chain length to those of the reported polymer. The appearance of pentenoyl group carbons clearly support the formation of pentenoyl-terminated polymer. The anomeric stereochemistry of the polymer was roughly estimated to be $\beta:\alpha = ca. 3:1$.



Scheme 3.6 Debenzylation of polymer prepared by cationic polymerization.



Figure 3.2 ¹³C NMR spectra (125 MHz, D₂O, rt) of reported nD-β-Glcp(1-2) (A) and the obtained polymer (B).

Effects of monomer structure on the cationic ring-opening condensation polymerization



Scheme 3.7 Synthesis of $(1 \rightarrow 2)$ -D-galactopyranan

To clarify the effects of monomer structure on the polymerization, I also examined the polymerization of cyclic sulfite **9** as a D-galactal derivative. The results are shown in the Table 3.2.

| monomer | initiator (mol%) | acid | dehydrator ^c | temp (°C) | time (h) | Conv. (%) | $M_{\rm n}^{d}$ (Da) | $M_{ m w}/M_{ m n}^{e}$ | $[\alpha]_D^f$ |
|---------|---------------------|------|-------------------------|--------------|-------------|--------------|----------------------|-------------------------|----------------|
| 4 | 10 | TfOH | MS 3Å | -20 | 41 | 55 | 3000 | 1.3 | +37 |
| 9 | 10 | TfOH | MS 3Å | -20 | 41 | 99 | 4150 | 3.2 | +75 |

 Table 3.2 Effects of monomer structure on the polymerization.

^{*a*} The reaction was performed by using **9** (0.33 mmol), initiator (10 mol%), and acid (10 mol%) in the presence of a dehydrator in CH₂Cl₂ (0.6 M). ^{*b*} Monomer conversion ratio was determined by ¹H NMR spectra of the crude mixture. ^{*c*} 50 wt% of dehydrator per **I** was used. ^{*d*} Number-average molecular weights (M_n) were estimated by the integral ratio in the ¹H NMR spectra. ^{*e*} Polydispersity indices (M_w/M_n) were determined by a size exclusion chromatography (SEC) using DMF as an eluent. ^{*f*} Specific optical rotation ([α]_D) was measured in CHCl₃ at 25–28 °C.

The polymerization of **9** was performed at the same manner that of **4**, which afforded a quantitative yield of the polymer. The yield is in a good contrast to that from **4**, clearly indicating the higher reactivity of **9**. The reason might be attributed to the stereochemistry of –OBn groups at C3, C4, and C6 positions projecting the β plane, which facilitates the attack of pentenol initiator at C1 position from the α plane. As a result, the resulting polymer would have higher ratio of α -anomeric center than that of β -anomeric center, because of the steric hindrance of the β plane. In this case, the reaction could

form the oxocarbenium cation intermediate. The structures of the obtained polymer were evaluated by IR, ¹H NMR, ¹³C NMR, and MALDI–TOF mass spectra.

In the IR spectrum, the absence of the characteristic signal of sulfite at around 1200 cm⁻¹ was observed, implying that the polymerization of **9** efficiently eliminates SO₂ to form polyoxyethylene main chain (Figure 3.35). The appearance of olefinic proton signals in the ¹H NMR spectrum supports the introduction of pentenoyl group to the polymer terminus. The number average of molecular weight (M_n) was determined on the basis of the integral ratio between the internal olefinic proton of pentenoyl group and the other aliphatic protons in the ¹H NMR spectrum. The distribution (M_w/M_n) was estimated by a size exclusion chromatography (SEC) using *N*,*N*-dimethylformamide (DMF) based on polystyrene standards. In the MALDI–TOF mass spectrum, the observed values were agreed with the theoretical values corresponding to the polymer, supporting the formation of pentenoyl group-terminated polymers.

I also measured the specific optical rotations ($[\alpha]_D$) of the polymer. As a result, it was found that polymer exhibits a higher positive $[\alpha]_D$ value compared with the polymer prepared from 4. Therefore, the polymer obtained from 9 seem to be consisting of much more α -glycosidic linkages than the other polymer from 4.

CD and UV spectra of the polymer prepared at -20 °C and the β -unimer were measured (Figure 3.3). In the UV spectra, the signal of the polymer exhibits hypochromicity (lower intensity) compared with that of unimer, which implies that aromatic groups in the polymer have π -stacked conformation.

In the CD spectrum of the polymer, the positive signal around 205 nm is larger and the negative signal around 194 nm is smaller than those of unimer. Such results suggest that the polymer has a chiral higher-order-structure like a helical structure.



Figure 3.3 CD and UV spectra of β -unimer and polymer (entry 7) at room temperature (cyclohexane,

10⁻⁴ µM).

Application of glycosylation-active terminal group to the synthesis of oligosaccharide-containing glycoside.

The importance of glycosylation-active terminal group of the obtained polymers was demonstrated by using cinnayl alcohol as a glycosyl acceptor. The reaction was performed in the presence of 10 equivalent of cinnamyl alcohol and bis(2,4,6-trimethylpyridine)iodonium hexafluorophosphate as a catalyst at 0 °C.¹⁴ The conversion was estimated by ¹H NMR analysis to be 70%. The observed mass values in the MALDI–TOF MS spectra showed a simple pattern, which agreed with the theoretical values of the structure with cinnamyl alcohol group at the terminus. These results indicate that our synthesized polymer can be grafted onto the materials bearing an alcohol.



Scheme 3.8 Glycosyl activity of terminal pentenoyl group of $(1\rightarrow 2)$ - β -D-glucopyranan.

3.3 Conclusion

In conclusion, I first discovered the usefulness of cyclic sulfite 4 monomer for 1,2-glycosidic polymer as a surrogate of glycal epoxide. I achieved the creation of glycosylation-active polymer with

terminal pentenoyl group via cationic ring-opening condensation polymerization of cyclic sulfite. The anomeric stereocenters of polymer were moderately controllable by changing the reaction temperature. As an application, I demonstrated the glycosylation using the terminal group of the synthetic polymer to cinnamyl alcohol to give the corresponding glycoside.

3.4 Experimental

General methods:

Materials. Tetrahydrofuran (THF) (Kanto Chemical Co., Inc.) was freshly distilled in the presence of sodium and benzophenone under nitrogen atromsphere before use. Dichloromethane was distilled over CaH₂ and stored with MS 4Å. MS 3Å as a dehydrator was activated by three times heating for 1 min in a microwave oven following careful heating by a heat gun under vacuum. The other chemicals were used without purification. Cyclic sulfite was prepared according to the literature: A. Benksim, D. Beaupère, A. Wadouachi, *Org. Lett.* **2004**, *6*, 3913-3915.

Measurements. ¹H NMR (400 and 600 MHz) and ¹³C NMR (100 and 150 MHz) spectra were recorded on JEOL JNM-ESC400 and JNM-ESC600 spectrometers using CDCl₃ as the solvent, calibrated using residual undeuterated solvent and tetramethylsilane as the internal standard. FT-IR spectra were measured using a Thermo Fischer Scientific Nexus 870 spectrometer. Optical rotation was measured with a JASCO P-1030 digital polarimeter. SEC analyses were carried out using a

chromatographic system consisting of a JASCO PU-2080 plus pump with a JASCO UV-1570 (UV detector) equipped with Shodex Asahipak GF-310 HQ (30 x 0.75 (i.d.) cm) (eluent DMF with LiCl (30 mM), flow rate 1.0 mL/min). UV-VIS absorption spectra were measured at room temperature with a JASCO V-570 spectrophotometer. CD spectra were taken on a JASCO J-820 spectrometer. MALDI–TOF MS spectra were recorded on a Shimadzu Voyager-DE STR-H mass spectrometer (matrix: α –CHCA) at Instrumental Analysis Division, Equipment Management Center Creative Research Institution, Hokkaido University.

Typical procedure for the preparation of cyclic sulfite^{7,8,9} (For scheme and spectra, chapter 2)</sup>

To a solution of imidazole (5.59 g, 82.0 mmol) in THF (80 mL) was added dropwise SOCl₂ (1.49 mL, 20.5 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 30 min, and filtered. To the resulting *N*,*N*-thionyldiimidazole solution was added a solution of diol (1.54 g, 3.42 mmol) in THF (20 mL) at 0 °C. The mixture was warmed to room temperature, stirred for 30 min, and carefully concentrated in vacuo below 30 °C. The crude was purified by a florisil column chromatography (eluent: hexane : ethyl acetate = 9:1 \rightarrow 3:1) to give the cyclic sulfite (689.1 mg) as an endo/exo diastereo-mixture (2:1) on the sulfur atom; In case of **D-glucal**; [α]_D^{25.2} +35.6 (CHCl₃, c. 1.00); ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.37–7.14 (15H, m) 6.37 (0.63H, d, *J* = 4.8 Hz), 6.12 (0.37H, d, *J* = 6.0 Hz), 4.96–4.28 (7H, m), 4.14–3.57 (5H, m) ppm; ¹³C NMR (100 MHz, 298 K, CDCl₃) δ 138.0, 137.9, 137.79, 137.76, 137.4, 137.1, 128.8, 128.61, 128.58, 128.54, 128.49, 128.47, 128.24, 128.21,

128.1, 128.0, 127.94, 127.90, 105.9, 102.4, 83.8, 80.1, 75.8, 75.0, 74.8, 74.6, 74.3, 74.0, 73.7, 73.5, 72.8, 72.6, 71.4, 68.7, 68.6, 67.9 ppm; IR (NaCl) υ 3088, 3063, 3031, 2868, 1773, 1496, 1454, 1365, 1214 (OS(O)O), 1164, 1099, 1028, 989, 893, 819, 773, 740, 699 cm⁻¹; MALDI–TOF MS (matrix: α –CHCA) calc'd for C₂₇H₂₈NaO₇S⁺ [M+Na⁺] 519.14; found 519.59. In case of **D-galactal**; ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.37–7.14 (15H, m) 6.37 (0.48H, d, *J* = 4.4 Hz), 6.12 (0.50H, d, *J* = 5.4 Hz), 4.96–4.28 (7H, m), 4.14–3.57 (5H, m) ppm.

Typical procedure for the preparation of unimer

To a solution of cyclic sulfite (157.6 mg, 0.317 mmol) and 4-pentene-1-ol (38.6 μ L, 0.381 mmol) in CH₂Cl₂ (3.0 mL) was added CaSO₄ (100 mg) at room temperature. After stirring for 30 min, trifluoromethanesulfonic acid (2.8 μ L, 0.032 mmol) was added to the mixture and the mixture was stirred for 14 h. The reaction was quenched by the addition of sat. aq. NaHCO₃. The products were extracted with CHCl₃, repeatedly. The combined organic layer was dried over MgSO₄, filtered, and concentrated in vacuo to give a crude material (160.8 mg). The crude was purified by a preparative silica gel column chromatography (eluent: hexane : ethyl acetate = 5:1) to give β-anomeric compound **5**β (65.0 mg, 40%) and α-anomeric compound **5**α (64.3 mg, 39%) as a colorless oil.

5β: [α]_D^{26.7} –5.07 (CHCl₃, c. 1.00); ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.40–7.18 (15H, m), 5.85 (1H, ddd, *J* = 11.2, 7.2, 4.4 Hz), 5.06 (1H, dbrd, *J* = 11.2 Hz), 5.00 (1H, dbrd, *J* = 7.2 Hz), 4.96 (1H, d, *J* = 7.4 Hz), 4.86 (2H, d, *J* = 8.4 Hz), 4.64 (1H, d, *J* = 8.0 Hz), 4.57 (1H, d, *J* = 8.0 Hz), 4.56 (1H, d, J = 8.0 Hz), 4.56

d, J = 7.4 Hz), 4.27 (1H, d, J = 5.2 Hz), 3.97 (1H, dt, J = 6.4, 4.4 Hz), 3.78 (1H, dd, J = 7.2, 1.2 Hz), 3.72 (1H, dd, J = 7.2, 3.2 Hz), 3.65–3.54 (4H, m), 3.51 (1H, brd), 2.43 (1H, brd), 2.17 (2H, q, J = 4.4Hz), 1.77 (2H, sext, J = 4.4 Hz) ppm; ¹³C NMR (100 MHz, 298 K, CDCl₃) δ 138.8, 138.27, 138.26, 138.2, 128.6, 128.53, 128.49, 128.11, 128.07, 127.93, 127.91, 127.84, 127.75, 115.1, 102.9, 84.6, 77.7, 75.3, 75.2, 75.1, 74.9, 73.6, 69.5, 69.0, 30.3, 28.9 ppm; IR (NaCl) υ 3453, 3087, 3064, 3030, 2920, 2868, 1496, 1453, 1361, 1311, 1270 1209, 1114, 1063, 1028, 913, 736, 698 cm⁻¹; MALDI–TOF MS (matrix: α– CHCA) calc'd for C₃₂H₃₈NaO₆⁺ [M+Na⁺] 541.26; found 541.89.

5α: $[\alpha]_{D}^{26.5}$ +69.22 (CHCl₃, c. 1.00); ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.42–7.18 (15H, m), 5.86–5.79 (1H, m), 5.05 (1H, dbrd, J = 11.2 Hz), 5.00 (1H, dbrd, J = 7.2 Hz), 4.98 (1H, d, J = 7.6 Hz), 4.89 (1H, d, J = 10.0 Hz), 4.85 (1H, d, J = 7.2 Hz), 4.65 (1H, d, J = 7.6 Hz), 4.53 (1H, d, J = 10.0 Hz), 4.52 (1H, brd), 3.80–3.65 (7H, m), 3.50 (1H, brd), 2.15 (2H, brd), 1.74 (2H, brd) ppm; ¹³C NMR (100 MHz, 298 K, CDCl₃) δ 138.9, 138.3, 138.09, 138.06, 128.52, 128.51, 128.08, 128.03, 127.93, 127.87, 127.84, 127.77, 127.7, 115.2, 98.6, 83.7, 77.7, 75.5, 75.2, 73.6, 73.2, 70.7, 68.6, 67.7, 30.5, 28.7 ppm; IR (NaCl) ν 3385, 3064, 2921, 2871, 1496, 1453, 1361, 1312, 1276, 1208, 1067, 915, 737, 698 cm⁻¹; MALDI–TOF MS (matrix: CHCα) calc'd for C₃₂H₃₈NaO₆⁺ [M+Na⁺] 541.26; found 541.52.

3.3d Typical procedure for cationic ring-opening condensation polymerization of cyclic sulfite (Table 3.1, entry 4)

To a solution of cyclic sulfite (143.0 mg, 0.288 mmol) and 4-pentene-1-ol (2.9 µL, 0.029 mmol)

in CH₂Cl₂ (0.58 mL) was added a freshly activated MS 3Å (ca. 200 mg) as a dehydrator at room temperature. After stirring for 2.5 h at room temperature, trifluoromethanesulfonic acid (TfOH, 2.9 µL, 0.029 mmol) was added to the mixture. The mixture was stirred for 1 d at the same temperature. The reaction was quenched by the addition of Et₃N (ca. 200 µL) and the following addition of sat. aq. NaHCO₃. The products were extracted with CHCl₃, repeatedly. The combined organic layer was dried over MgSO₄, filtered, and concentrated in vacuo to give a crude material. The crude dissolved in CHCl₃ was reprecipitated into hexane to remove the remaining organic salts as an yellow oil. The filtrate was concentrated in vacuo to give a crude polymer including unreacted monomer (124.1 mg) as a colorless amorphous. The remaining monomer was separated by a gel permeation column chromatography (eluent: CHCl₃) to give the polymer (76.0 mg, 61%) as a colorless oil. The number average molecular weight (M_n) was estimated by integral ratio between the internal olefinic proton at around 5.8 ppm and protons in a range from 5.2 ppm to 3.2 ppm to be M_n 1800 Da. The polydispersity index (M_w/M_n) was estimated by a size exclusion column chromatography analysis (SEC, eluent: DMF) on the basis of polystyrene standards to be M_w/M_n 1.42: $[\alpha]_D^{26.1}$ +52.44 (CHCl₃, c. 1.00); ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.36-6.84 (m) 5.68 (brd), 5.00-3.10 (m), 2.08-1.88 (m), 1.76-1.52 (m) ppm; ¹³C NMR (100 MHz, 298 K, CDCl₃) δ 138.8-137.2 (m), 128.6-127.0 (m), 115.0-92.0 (m), 85.0-68.0 (m), 30.0-28.0 (m) ppm; IR (NaCl) v 3486, 3063, 3030, 2921, 2866, 1496, 1454, 1361, 1209, 1068, 1028, 736, 698 cm⁻¹; Elemental analysis: calc'd for C_{224,24}H_{237,36}O_{41,6}·CHCl₃ based on the polymerization degree (8.12) estimated by the ¹H NMR spectrum (entry 5): C 72.77, H 6.46, Found:

C 72.58, H 6.24; No sulfur atom was detected.

Typical procedure for glycosylation using pentenoyl group of $(1\rightarrow 2)$ - β -D-glucopyranan to cinnamyl alcohol

To a solution of polymer (entry 7, table 3.1) (50.0 mg, 0.016 mmol) and cinnamyl alcohol (21.6 mg, 0.016 mmol) in CH₂Cl₂ (1.5 mL) was added a freshly activated MS 3Å (ca. 75 mg) as a dehydrator at room temperature. After stirring for 1 h at room temperature, I(Coll)₂PF₆ (12.4 mg, 0.024 mmol) was added to the mixture at 0 °C. The mixture was stirred for 19 h at the same temperature (0 °C). The % conversion of cyclic sulfite was estimated on the basis of ¹H NMR of reaction mass in CDCl₃ by integral ratio between olefinic at around 5.8 ppm and protons in a range from 5.2- 3.2 ppm. The reaction was quenched by the addition of Et₃N (ca. 0.1 mL) and the following addition of sat. aq. Na₂S₂O₃. The products were extracted with CHCl₃, repeatedly. The combined organic layer was washed with NaHCO3 and separated organic was dried over MgSO4, filtered, and concentrated in vacuo to give a crude material. The crude material was purified by GPC preparative (eluent: CHCl₃) to give the polymer (38.0 mg, 74.8%) as a colorless oil as a mixture of compound having both α - end pentenoyl and cinnamyl alcohol. The number average molecular weight (M_n) and The polydispersity index (M_w/M_n) was estimated by a size exclusion column chromatography analysis (SEC, eluent: DMF) on the basis of polystyrene standards to be M_n 660 Da and M_w/M_n 2.5 respectively: ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.36-6.84 (m), 6.60 (brd), 6.25 (brd), 5.68 (brd), 5.00-3.10 (m), 2.08-1.20 (m) ppm; ¹³C NMR (100 MHz, 298 K, CDCl₃) δ 138.8-137.2 (m), 128.6-127.0 (m), 105.0-92.0 (m),
85.0-68.0 (m), 30.0-28.0 (m), 22.5 (s), 14.5(s) ppm; IR (NaCl) υ 3476, 3088, 3063, 2920, 2866, 1952,
1879, 1813, 1748, 1605, 1496, 1453, 1361, 1210, 1068, 1028, 751, 736, 699 cm⁻¹.

Figures



Figure 3.4 ¹H NMR spectrum of 5β (400 MHz, CDCl₃, 293 K).



Figure 3.5 ¹³C NMR spectrum of 5β (150 MHz, CDCl₃, 293 K).



Figure 3.6 IR spectrum of 5β (NaCl).



Figure 3.7 ¹H NMR spectrum of 5α (400 MHz, CDCl₃, 293 K).



Figure 3.8 ¹³C NMR spectrum of **5α** (150 MHz, CDCl₃, 293 K).



Figure 3.9 IR spectrum of 5α (NaCl).



Figure 3.10 MALDI–TOF MS spectrum of 5β.



Figure 3.11 MALDI–TOF MS spectrum of 5α.



Figure 3.12 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at

room temperature (Table 3.1, entry 4).



Figure 3.13 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at room temperature (Table 3.1, entry 4).



Figure 3.14 IR spectrum (NaCl) of polymer obtained by the reaction at room temperature (Table 3.1,

entry 4).



Figure 3.15 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of polymer obtained by the reaction using

5 mol% of TfOH at room temperature (Table 3.1, entry 5).



Figure 3.16 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of polymer obtained by the reaction using 5 mol% of TfOH at room temperature (Table 3.1, entry 5).



Figure 3.17 IR spectrum (KBr) of polymer obtained by the reaction using 5 mol% of TfOH at room temperature (Table 3.1, entry 5).



Figure 3.18 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at

-10 °C (Table 3.1, entry 6).



Figure 3.19 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at -10 °C (Table 3.1, entry 6).



Figure 3.20 IR spectrum of polymer obtained by the reaction at -10 °C (Table 3.1, entry 6).



Figure 3.21 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at

-20 °C (Table 3.1, entry 7).



Figure 3.22 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at -20 °C (Table 3.1, entry 7).



Figure 3.23 IR spectrum of polymer obtained by the reaction at -20 °C (Table 3.1, entry 7).



Figure 3.24 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at

-40 °C (Table 3.1, entry 8).



Figure 3.25 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at -40 °C (Table 3.1, entry 8).



Figure 3.26 IR spectrum of polymer obtained by the reaction at -40 °C (Table 3.1, entry 8).



Figure 3.27 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at -60 °C (Table 3.1, entry 10).



Figure 3.28 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of polymer obtained by the reaction at

-60 °C (Table 3.1, entry 10).



Figure 3.29 IR spectrum (NaCl) of polymer obtained by the reaction at -60 °C (Table 3.1, entry 10).



Figure 3.30 MALDI-TOF MS spectrum of polymer obtained by the reaction in the presence of

CaSO₄ at rt (Table 3.1, entry 10).



Figure 3.31 MALDI–TOF MS spectrum of of polymer obtained by the reaction in the presence of MS 3Å at rt (Table 3.1, entry 10).



Figure 3.32 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of D-galactal cyclic sulphite (Table 3.2).



Figure 3.33 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of D-galactal polymer obtained by the reaction at -20 °C (Table 3.2).



Figure 3.34 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of D-galactal polymer obtained by the

reaction at -20 °C (Table 3.2).



Figure 3.35 IR spectrum (NaCl) of D-galactal polymer obtained by the reaction at -20 °C (Table

3.2).



Figure 3.36 MALDI-TOF MS spectrum of D-galactal polymer obtained by the reaction at -20 °C

(Table 3.2).



Figure 3.37 ¹H NMR spectrum (100 MHz, CDCl₃, 293 K) of polymer with cinnamyl alcohol

terminal.


Figure 3.38 ¹³C NMR spectrum (150 MHz, CDCl₃, 293 K) of polymer with cinnamyl alcohol

terminal.



Figure 3.39 IR spectrum (NaCl) of polymer with cinnamyl alcohol terminus.



Figure 3.40 MALDI–TOF MS spectrum of polymer with cinnamyl alcohol terminal.

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Chapter 4

One-Pot Synthesis of Glycyrrhetic Acid Polyglycosides Based on

Grafting-from Method using Cyclic Sulfite.

4.1 Introduction

Natural saponins are a representative member of bioactive natural products.¹ Glycyrrhizin (1) is a triterpenoid saponin consisting of glycyrrhetic acid as an aglycon and $(1\rightarrow 2)$ - β -bis(glucoronic acid) (Figure 4.1), which was isolated from extracts of licorice *Glycyrrhiza glabra* root.² The synthetic studies of the structural analogues have been considerably investigated from the viewpoint of structure-activity relationship, because the compound **1** exhibits unique bioactivities including anti-inflammatory,³ antiulcer,⁴ hypolipidemic,⁵ antiviral against human immunodeficiency virus type 1 (HIV-1) and severe acute respiratory syndrome (SARS)-associated coronavirus,⁶ and interferon-inducing activities.⁷

The previous syntheses were mainly focused on the change of oligosaccharide structure based on the stepwise glycosidations,⁸ which required time-consuming multi-step reactions and purification processes. Therefore, the development of simple preparation method for the derivatives such as glycyrrhetic acid polyglycosides (2) has been strongly urged. Although the powerful polymerization methods to give $(1\rightarrow3)$ -, $(1\rightarrow4)$ -, and $(1\rightarrow6)$ -linked polysaccharides have been constructed to date,⁹ that for $(1\rightarrow2)$ -linked polysaccharides has not been sufficiently developed yet. On the other hand, it is known that glycal epoxide is a potentially useful glycosyl donor for the synthesis of $(1\rightarrow2)$ -linked oligosaccharide-containing glycosides (Figure 2). Danishefsky *et al.* demonstrated the simple stepwise glycosidation of saccharide via ring-opening nucleophilic addition of alcohol to the epoxide.¹⁰ Several processes concerning protection-deprotection can be shortened by using epoxide because the nucleophilic addition to epoxide accompanies with the generation of a free secondary alcohol. However, the applications of epoxide to graft polymerization techniques were difficult due to the high unstability of epoxide.



Figure 4.1 Structures of glycyrrhizin (1) and glycyrrhetic acid polyglycosides (2).

In chapters 2 and 3, I discovered that shelf-stable cyclic sulfite 4 works as a practical monomer for the preparation of $(1\rightarrow 2)$ -linked polysaccharide skeletons. The cationic ring-opening condensation

polymerization of **4** resulted in a perfect elimination of SO₂ linkages to afford the corresponding $(1\rightarrow 2)$ -linked glucopyranan.^{11,12}

Herein, I describe the synthesis of glycyrrhetic acid polyglycosides (2) as the application of our previous work to a grafting polymerization. The cationic ring-opening condensation polymerization of 4 was performed in the presence of glycyrrhetic acid 5 as an initiator and trifluoromethane sulfonic acid (TfOH) as a catalyst.¹³ This method enables not only the one-pot synthesis of 2 but also the control of polymerization degree of $(1\rightarrow 2)$ -linked oligosaccharide skeleton.

4.2 Results and Discussion

Cationic ring-opening condensation polymerization of 4 initiated by glycyrrhetic acid (5)

Cyclic sulfite **4** was prepared according to the literature¹⁴. I investigated the grafting reaction of **4** from glycyrrhetic acid (**5**) to give the benzyl-protected skeleton of **2**, as shown in Scheme 4.1.



Scheme 4.1 Cationic ring-opening condensation polymerization of 4 initiated by 5.

Initiator (5) was prepared from commercially available glycyrrhetic acid via benzyl protection of carboxylic acid. The grafting polymerization of **4** from **5** were performed in CH₂Cl₂ in the presence of TfOH and molecular sieves 3Å (MS 3Å) at room temperature. The results are summarized in Table 4.1. The conversion of **4**, the polymerization degree, and the number average molecular weight (M_n) were determined by the ¹H NMR analysis of crude mixture. The conversion of **5** and the polydispersity index (M_w/M_n) were estimated by a size exclusion chromatography (SEC) using THF on the basis of polystyrene standards. At first, I used the stoichiometric amount of both **5** and TfOH to that of **4**.

| Entry | Initiator (5) | TfOH | Time | Conv. of | Conv. of | Polymerization | $M_{\rm n}{}^c$ | $M_{\rm w}/M_{\rm n}^{f}$ |
|----------------|---------------|------|------|-------------------------|------------------|---------------------|-----------------|---------------------------|
| | mol% | mol% | h | 4 % ^d | 5 % ^e | Degree ^d | | |
| 1 | 100 | 100 | 1 | 100 | 45.8 | 2.9 | 1,800 | 1.1 |
| 2 | 150 | 100 | 1 | 100 | 30.8 | 2.5 | 1,700 | 1.1 |
| 3 | 50 | 50 | 2 | 100 | 63.7 | 4.7 | 2,600 | 1.6 |
| 4 | 20 | 20 | 94 | 76.1 | 98.5 | 4.6 | 2,600 | 1.1 |
| 5 | 10 | 10 | 94 | 46.8 | 100 | 4.0 | 2,300 | 1.1 |
| 6 ^b | 10 | 10 | 94 | 71.1 | 100 | 8.0 | 4,000 | 1.1 |
| 7 | 5 | 5 | 137 | 14.6 | 100 | 3.0 | 1900 | 1.1 |
| 8° | 5 | 5 | 137 | 45.2 | 100 | 8.9 | 4400 | 1.1 |

Table 4.1 Effects of the feed ratio on the conversions and M_n^a

^{*a*}The reaction was performed by using cyclic sulfite **4** (0.18 mmol) in the presence of TfOH, glycyrrhetic acid, and MS 3Å (50 wt%) in CH₂Cl₂ (0.6 M per **4**) at room temperature. ^{*b*}MS 3Å (25 wt%) was used. ^{*c*}MS 3Å (12.5 wt%) was used. ^{*d*}Determined by ¹H NMR analysis of the crude mixture. ^{*e*}Determined by size exclusion chromatography (SEC) analysis of the crude mixture. ^{*f*}Determined by SEC on the basis of polystyrene standards.

Contrary to our expectation that the reaction would mainly give the monosaccharide-containing glycoside, the conversion of **5** was middle (45.8%) and that of **4** was quantitative (entry 1, Table 4.1). The average polymerization degree was 2.9, indicating that M_n of **Poly-6** is 1,800 Da. The M_w/M_n of **Poly-6** was a relatively narrow value (1.1). Such results indicate that the bulky alcohol of (**5**) as a

neopentyl alcohol would slowly initiate the glycosylation reaction of **4** along with the elimination of SO₂ to generate a free alcohol at the 2 position of the sugar. The resultant secondary alcohol could exhibit a higher reactivity than the neopentyl alcohol of **5**, which would facilitate further nucleophilic addition to the other cyclic sulfite **4**, leading to the ring-opening polycondensation of **4** initiated by **5**. It is noted that the polymer with a narrow M_w/M_n was obtained, even when the excess amount of **5** was used (entry 2, Table 4.1).

I next investigated the effects of feed ratio of initiator (5) on the conversions and M_n (entries 3–8, Table 4.1). With the decrease of the feed ratio of 5 against 4, the conversion of 4 decreased whereas that of 5 increased (entries 3–5, Table 4.1), which afforded the similar M_n values. The decreased amount of TfOH prolonged the reaction time, probably because the reaction rate would be strongly dependent on the concentration of acid. On the other hand, when the amount of MS 3Å was decreased to 25 wt% (entry 6, Table 4.1), the conversion of 4 was dramatically improved, resulting in the formation of the polymer with higher M_n . In the cases of entries 7 and 8 with 5 mol% of TfOH, the reduced amount of MS 3Å (12.5 wt%) also enabled the high conversion of 4 to give the highermolecular-weight polymer. Such results suggest that M_n of **Poly-6** was moderately controllable by the change of feed ratio. The use of MS 3Å as a dehydrator might reduce the effective concentration of acid. In the case of entry 7 (Table 4.1) with 5 mol% of TfOH, the reaction hardly proceeded.

The crude material after a typical workup was precipitated into MeOH to remove the remaining 4 and 5. The structure of the polymer as a MeOH-insoluble part was evaluated by IR, ¹H NMR, and

MALDI-TOF mass spectra. In the IR spectrum, the absence of the characteristic signal of sulfite at around 1200 cm⁻¹ was observed, suggesting that the polymerization of **4** efficiently eliminates SO₂ to form $(1\rightarrow2)$ -linked oligosaccharide skeletons. The presence of enone absorption band at around 1660 cm⁻¹ was also confirmed. In the ¹H NMR spectrum, the signals attributed to glycyrrhetic acid and oligosaccharides appeared along with the disappearance of the protons of **4**, supporting the efficient conversion of **4**. The chemical shift of olefinic proton signal of the polymer almost agreed with that of **5**, also supporting the retention of the enone moiety after the polymerization. It has been reported that the Ag- or Hg-catalyzed glycosylation to glycyrrhetic acid skeleton gave the corresponding enol glycoside,^{8b} highlighting the present reaction condition is a useful method to suppress the side reaction.

I also measured the ¹³C NMR spectrum of **Poly-6** to clarify the anomeric stereochemistry (Figure S14). The β/α -stereo-ratio on the anomeric centers was roughly estimated to be 14.5:1 by the integral ratio between the carbon signals at 110–100 ppm for β carbon and those at 100–90 ppm for α carbon, considering the reported chemical shifts of natural product and synthetic derivatives.2,8 The results indicate that the polymerization would mainly undergo at SN2-like inversion manner to give the corresponding β -anomers as a kinetic product, because no specific participating group is included in the skeleton of **4**. The use of a catalytic amount of TfOH with MS 3Å might also suppress the anomerization reaction to afford the thermodynamically preferred α -anomers.

Matrix associated laser desorption/ionization time-of-flight (MALDI–TOF) mass spectrum of **Poly-6** exhibited a simple pattern with an even interval agreed with the theoretical values corresponding to the Benzyl-protected glycyrrhetic acid-terminated polymers (Figure 4.2), which finally evidenced that the polymerization of **4** accompanied with the perfect elimination of SO₂ to give **Poly-6** skeleton. The additional minor peaks attributed to the polymers initiated by a trace of H₂O were barely detectable in the spectrum, indicating the important role of MS 3Å as a dehydrator.



Figure 4.2 MALDI-TOF mass apectrum of Poly-6

4.3 Conclusion

In conclusion, I demonstrated a new grafting reaction of cyclic sulfite 4 from glycyrrhetic acid 5 to give glycyrrhetic acid polyglycosides in one-pot via cationic ring-opening condensation polymerization of 4.

The method enables the rapid preparation of glycyrrhizin derivatives consisting oligosaccharides with arbitrary polymerization degree. Because other natural glycosides bearing $(1\rightarrow 2)$ -linked oligosaccharides such as cyaniding-3-*O*-sophoroside¹⁵ and quercetin-3-*O*-sophoroside¹⁶ have been reported, this method would be also applicable to the preparations of their derivatives. The present results may provide new insights into not only natural product syntheses but also the creation of high performance polymers based on a grafting reaction.

4.4 Experimental

General methods:

Materials. Molecular sieves 3Å (MS 3Å) as a dehydrator was activated by careful heating by a heat gun under vacuum. Dry dichloromethane (TCI) and trifluoromethanesulfonic acid (Kanto chemicals) were used as obtained for polymerization. The other chemicals were used without purification. Cyclic sulfite **4** was prepared according to the literature: Benksim, A.; Beaupère, D.; Wadouachi, A. *Org. Lett.* **2004**, *6*, 3913–3915.

Measurements. ¹HNMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on JEOL JNM-

ESC400 and Bruker AVANCE II 400 spectrometers using CDCl₃ as the solvent, calibrated using residual undeuterated solvent and tetramethylsilane as the internal standard. FT-IR spectra were measured using a Perkin Elmer spectrum 100 spectrometer. Optical rotation was measured with an ATAGO AP-300 automatic polarimeter. SEC analyses were carried out using a chromatographic system consisting of a Shimadzu LC-20AT pump with a Shimadzu SPD-20A (UV detector) equipped with two consecutive linear polystyrene gel columns (Tosoh TSKgel GMH_{HR}-H and TSKgel G3000H_{HR}) at room temperature according to polystyrene standards using THF as an eluent (flow rate: 1.0 mL/min). MALDI–TOF MS spectra were recorded on a Shimadzu AXIMA-CFR plus mass spectrometer (matrix: CHC α).

Typical procedure for the preparation of initiator (5)

To a solution of glycyrrhetic acid (1.00 g, 2.12 mmol) in a mixture of acetone–DMF (10:1, 11 mL) was added K₂CO₃ (587 mg, 4.25 mmol) and benzyl bromide (303 µL, 2.55 mmol) at room temperature. After stirring for 40.5 h, water was added to the mixture to give white precipitates. The precipitates were collected by filtration, washed with water repeatedly, and dried in vacuo to give **5** (1.08 g, 90.8%) as a white solid: m.p. 118.0–120.0 °C; $[\alpha]_D^{25}$ +153.2 (CHCl₃, c. 1.00); ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.40–7.33 (m, 5H), 5.55 (s, 1H), 5.19 (d, *J* = 12.4 Hz, 1H), 5.09 (d, *J* = 12.4 Hz, 1H), 3.22 (brd, 1H), 2.79 (brd, 1H), 2.31 (s, 1H), 2.04–1.91 (m, 4H), 1.80 (brd, 1H), 1.67–1.54 (m, 7H), 1.48–1.25 (m, 7H), 1.16–1.11 (m, 9H), 1.00–0.94 (m, 5H), 0.80 (s, 3H), 0.73–0.67 (s, 4H) ppm; ¹³C

NMR (100 MHz, 298 K, CDCl₃) δ 200.1, 176.2, 169.2, 136.1, 128.6, 128.5, 128.3, 128.2, 78.7, 66.2, 61.8, 54.9, 53.7, 48.2, 45.3, 44.0, 43.1, 41.0, 39.1, 37.6, 37.0, 32.7, 31.8, 31.1, 28.4, 28.3, 28.1, 27.3, 26.44, 26.37, 23.3, 18.6, 17.5, 16.4, 15.6 ppm; IR (neat) *υ* 3360, 2934, 2868, 1727, 1660, 1620, 1456, 1387, 1358, 1313, 1279, 1258, 1209, 1146, 1084, 1042, 995, 981, 955, 913, 878, 749, 645 cm⁻¹; MALDI–TOF MS (matrix: CHCα) calc'd for C₃₇H₅₂NaO₄⁺ [M+Na⁺] 583.37; found 583.85.

Typical procedure for grafting polymerization of 4 from 5 (run 4 in Table 4.1)

To a solution of cyclic sulfite 4 (90.3 mg, 0.18 mmol) and initiator (5) (20.4 mg, 0.04 mmol) in CH₂Cl₂ (0.30 mL) was added a freshly activated MS 3Å (ca. 45 mg) as a dehydrator at room temperature. After stirring for 1 h at room temperature, TfOH (3.2 μ L, 0.04 mmol) was added to the mixture. The mixture was stirred for 94 h at the same temperature. The reaction was quenched by the addition of sat. aq. NaHCO₃. The products were extracted with CHCl₃, repeatedly. The combined organic layer was dried over MgSO₄, filtered, and concentrated in vacuo to give a crude material. The conversion of 4 was estimated by the ¹H NMR measurement of the crude material to be 76.1%. The number average molecular weight (M_n) was estimated by integral ratio between the olefinic proton at around 5.55 ppm and protons in a range from 4.2 ppm to 3.0 ppm to be M_n 2,600 Da. The conversion of 5 was estimated by the size exclusion column chromatography (SEC, eluent: THF) of the crude solution (conc. 1.0 mg/mL) to be 98.5 % from the peak area of 5 according to the molar absorbance of 5 at 254 nm. The crude sample (60 mg) was purified for the analytical purpose through reprecipitation using

MeOH (30 mL) and CHCl₃ (0.1 mL) to get 7.0 mg of the polymer as a MeOH-insoluble part and 50 mg as a MeOH-soluble part. The polydispersity index (M_w/M_n) was also estimated by SEC analysis (eluent: THF) on the basis of polystyrene standards to be M_w/M_n 1.1: [α]_D²⁵ +63.3 (CHCl₃, c. 1.00); ¹H NMR (400 MHz, 298 K, CDCl₃) δ 7.40–6.98 (m), 5.68 (m), 5.55 (m), 5.29–2.72 (m), 2.27–0.73 (m) ppm; IR (neat) υ 2919, 2851, 1727, 1659, 1497, 1454, 1361, 1311, 1260, 1211, 1050, 1026, 912, 800, 733 cm⁻¹.





Figure 4.3 ¹H NMR spectrum of 5 (400 MHz, CDCl₃, 298 K).



Figure 4.4 ¹³C NMR spectrum of **5** (100 MHz, CDCl₃, 298 K).



Figure 4.5 IR spectrum of 5 (neat).



Figure 4.6 MALDI–TOF mass spectrum of (5) (matrix: CHCα).



Figure 4.7 ¹H NMR spectrum of Poly-6 (400 MHz, CDCl₃, 298 K).



Figure S14. ¹³C NMR spectrum of Poly-6 (100 MHz, CDCl₃, 298 K).



Figure 4.8 IR spectrum of Poly-6 (neat).



Figure 4.9 MALDI–TOF mass spectrum of Poly-6 (matrix: CHCα).

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Chapter 5

General Conclusion

In this thesis, the author has described the new synthetic method of 1,2-glycosidic polymers via ring-opening condensation polymerization of a sugar- based cyclic sulfite. Cationic polymerization of the cyclic sulfite afforded $(1\rightarrow 2)$ -D-glycopyranan along with the elimination of SO₂. The method was also applied to both grafting-onto and grafting-from polymerizations to give the corresponding glycosides. The results of this study are summarised as follows. In chapter 1, the author described the general backgrounds of polysaccharides and glycosides. Through the historical survey, the importance on the synthetic studies of 1,2-glycosidic polymers was particularly emphasized.

In chapter 2, the author discussed the chemical stability and reactivity of cyclic sulfite as a monomer, mainly by comparision with those of glycal epoxide as a conventional monomer. Kinetic studies for the degradations evaluated the higher stability of the cyclic sulfite than the epoxide. At first, the anionic ring-opening condensation polymerization of cyclic sulfite was investigated according to the model studies of nucleophilic substitution on the anomeric center. However, it was found that the anionic polymerization gave a mixture of the polymer and some other impurities containing sulfur.

In chapter 3, the author achieved the synthesis of glycosylation-active polymer bearing pentenoyl group at the polymer terminus. The anomeric stereochemistry of the polymer was moderately controllable by changing the reaction temperature. As an application, the author demonstrated the glycosylation reaction using the terminal pentenoyl group. The reaction could provide a new pathway for the creation of polysaccharide- containing graft copolymers and the cyclic polymer.

In chapter 4, I demonstrated a new grafting reaction of the cyclic sulfite from glycyrrhetic acid to

give glycyrrhetic acid polyglycosides in one-pot. The reaction enables the rapid preparation of glycyrrhizin derivatives consisting oligosaccharides with arbitrary polymerization degree.

As stated above, this thesis demonstrates the usefulness of the sugar-based cyclic sulfite as a new monomer for the first time. The author hopes that this study will be a cornerstone for the preparation of 1,2-glycosidic polymers. The present results may provide new insights into not only natural product syntheses but also the creation of high performance polymers based on a grafting reaction.