Controlled Synthesis of Glycopolymer-type Macromonomers and their Use for the Preparation of Carbohydrate-Decorated Polymer Particles

DISSERTATION

Doctoral Thesis of Materials Chemistry Program, International Graduate Program for Interdisciplinary Study in Science and Technology, Graduate School of Science and Technology.

by

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Acknowledgments

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Abstract

Macromonomers containing one or more polymerizable groups in the polymer chains have been recognized as useful building blocks of various polymer assembly architectures. Among them, the use of amphiphilic macromonomers for the dispersion or emulsion copolymerization with hydrophobic monomers in polar media can produce polymer particles of a wide range of sizes. The resultant polymer particles are categorized as hairy or core-shell particles and hence afford stable aqueous dispersions, where the dispersion stability is attributed to the steric stabilization attained by the hydrophilic polymer chains fixed on the particle surfaces. It is worth noting that the surface functionality of the particles can be widely designed by varying the structure of the macromonomers because the polymer chain moieties are covalently attached to the particle surfaces. In particular, polymer particles decorated with carbohydrates on their surfaces have been of great interest in the biomedical field, because they have potential ability for providing useful materials for diagnosis based on their specific interactions with biomolecules such as proteins and viruses etc. In order to produce carbohydrate-decorated polymer particles, the author designed novel amphiphilic glycopolymer-type macromonomers, which are synthesized by controlled polymerizations, thereby possess well-defined architecture consisting of pendant carbohydrate moieties and a terminal vinyl group. Moreover, chain lengths of the macromonomers and composition ratios of the block copolymer-type versions can be precisely controlled.

This thesis deals with the following research topics: (i) The synthetic strategies for yielding various glycopolymer-type macromonomers were developed. Firstly, a combination of living cationic polymerization of alkyne-

substituted vinyl ether (VEEP) and copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) allowed the poly(vinyl ether) (polyVE)-based glycopolymer-type macromonomers, where the precursor macromonomer having pendant alkynes and a methacryloyl group at α–terminus was synthesized by living cationic polymerization of VEEP, followed by CuAAC click reaction with maltosyl azide. The resultant glycopolymer-type macromonomer possesses both pendant maltose residues and a terminal polymerizable group at α–end. (ii) The synthetic methodology in (i) was then applied for the synthesis of amphiphilic glycopolymer-type macromonomers composed of a hydrophobic poly(alkylVE) segment and a maltose-substituted hydrophilic polyVE segment. Through the dispersion copolymerization with styrene in polar media, this amphiphilic macromonomer afforded core-shell polymer particles decorated with maltose residues on their surfaces. (iii) An alternative approach for the synthesis of glycopolymer-type macromonomers was developed based on the living radical copolymerization by RAFT (reversible addition-fragmentation chain transfer) process of maltose-containing and other acrylamides and subsequent functional conversion of the terminal function. The precursor maltose-carrying polyacrylamide was successfully synthesized by RAFT copolymerization, and its terminal trithiocarbonate function was reduced and then subjected to thiol-ene reaction with a diacrylate compound to give the target macromonomer. The obtained macromonomers possess both pendant maltose residues and an acryloyl group at ω–end, and were found to be capable of forming maltose-decorated core-shell polymer particles by dispersion copolymerization with styrene in polar media. (iv) Lectin binding assay using concanavalin A (Con A) revealed that the prepared maltose-decorated polymer particles specifically interact with Con A.

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Chapter I

Overview

I.1 General introduction

Development of carbohydrate-based functional materials has been attracting considerable interest since their specific interactions with biomolecules such as proteins and viruses etc. are crucial to the potential applications in biomedical and related fields. Among carbohydrate-based polymeric materials, synthetic polymers having pendant carbohydrate residues, which are referred to as "glycopolymers", have been attracting much attention and a large number of studies on them were so-far reported[1]. These glycopolymers have been synthesized either by conventional radical polymerization or by controlled ionic and radical polymerizations of various glycomonomers. An alternative approach for the glycopolymer synthesis is post-functionalization of the precursor polymers with carbohydrate derivatives as exemplified in copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) between an alkyne-substituted polymer and a glycosyl azide[2]. In this study, the author focused on the controlled synthesis of glycopolymers having a polymerizable group at α- or ω-terminus, which compounds correspond to glycopolymer-type macromonomers. Although a large variety of glycopolymers with well-defined structure have been hitherto synthesized by controlled polymerizations[3], there have been only a few examples of glycopolymer-type macromonomers[4]. Here the author designed amphiphilic glycopolymer-type macromonomers consisting of a hydrophobic segment and a carbohydrate-carrying hydrophilic segment. It is well known that amphiphilic macromonomers can afford polymer particles through the

dispersion copolymerization with a low-molecular-weight hydrophobic monomer in polar media, where the formed amphiphilic grafted polymers in the early stage of the copolymerization combined each other to form polymer particles (Scheme 1.4). It is notable that, in the dispersion state, the hydrophilic polymer chains derived from the macromonomers segregated from the hydrophobic particles and located on the particle surfaces, resulting in the formation of stable dispersion. This suggests that the amphiphilic macromonomers act as the steric stabilizer in this dispersion system. This fact prompted the author to design amphiphilic glycopolymer-type macromonomers and then utilize them in the dispersion copolymerization for the preparation of polymer particles decorated with glycopolymer chains on their surfaces. Glycopolymers have been known to exhibit multivalent effect in their recognition with biomolecules, where a carbohydrate-carrying glycopolymer specifically interact with the corresponding lectin in 10^2-10^3 times higher extent compared to the carbohydrate itself. On the other hand, polymer particles are useful functional materials having large specific surface area. It is therefore expected that the glycopolymer-decorated polymer particles exhibit remarkable surface functions by the synergy effect of the two factors mentioned above. Moreover, the surface functionality of the carbohydrate-decorated particles could be widely designed by varying the structure of the glycopolymer-type macromonomers.

In attempt to create various glycopolymer-type macromonomers, the author utilized two different controlled polymerization systems. In addition, CuAAC click reaction and thiol-ene reactions were also applied. Firstly, a combination of living cationic polymerization of alkyne-substituted vinyl ether (VEEP) and CuAAC click reaction allowed the poly(vinyl ether) (polyVE)-based glycopolymer-type macromonomers, where the precursor macromonomer having pendant alkynes and a methacryloyl group at α-

terminus was synthesized by living cationic polymerization of VEEP (Scheme 1.1), followed by CuAAC click reaction with maltosyl azide. The resultant glycopolymer-type macromonomer possesses both pendant maltose residues and a terminal polymerizable group at α-end. This synthetic methodology was then applied for the synthesis of amphiphilic glycopolymer-type macromonomers composed of a hydrophobic poly(alkylVE) segment and a maltose-substituted hydrophilic polyVE segment. An alternative approach for the synthesis of glycopolymer-type macromonomers was developed based on the living radical copolymerization by RAFT (reversible addition-fragmentation chain transfer) process of maltose-containing and other acrylamides (Scheme 1.2) and subsequent functional conversion of the terminal function. The precursor maltose-carrying polyacrylamide was synthesized by the trithiocarbonate agent-mediated RAFT copolymerization, and its terminal trithiocarbonate function was reduced and then subjected to thiol-ene reaction with a diacrylate compound to give the target macromonomer. The obtained macromonomers possess both pendant maltose residues and an acryloyl group at ω-end. Moreover, the author demonstrated the preparation of carbohydrate-decorated polymer particles with employing the two types of glycopolymer-type macromonomers mentioned above.

It should also be emphasized that the synthetic procedures in this study for the preparation of glycopolymer-type macromonomers present several advantages. The first benefit is that the glycopolymer-type macromonomers act not only as steric stabilizer in dispersion copolymerization but also as functionalization agent on the particle surfaces, yielding stable aqueous dispersions of carbohydrate-decorated polymer particles. The second is that the present methodologies are versatile and atom-economical because all the synthetic protocols are free from protecting / deprotecting processes of the multiple hydroxyls of the carbohydrates and pendant alkynes. Most of the

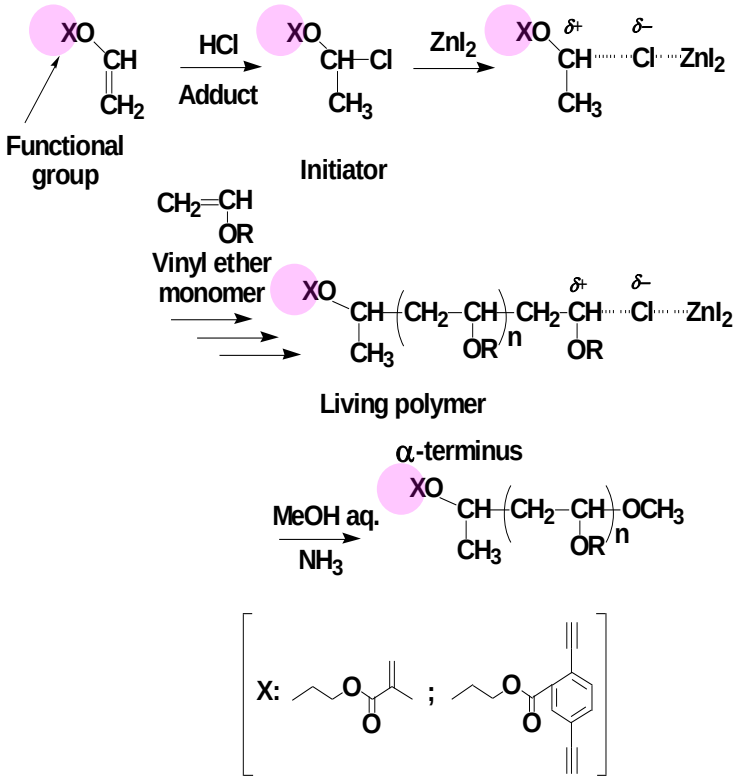
precursor polymers employed to date for CuAAC click reaction require protecting / deprotecting processes of the alkyne proton[2,5]. The last is the versatility of the methodology for preparation of carbohydrate-decorated polymer particles, namely, these synthetic methodologies are applicable for all of the reducing sugars in principle.

I.2 Background of research

I.2.1 Synthesis of end-functionalized poly(vinyl ether)s by living cationic polymerization

There have been a lot of studies about the living cationic polymerization of functionalized vinyl ethers (VEs). The mechanism of living cationic polymerization is shown in Scheme 1.1. This polymerization procedure has many advantages, for example, the obtained polymers possess controlled molecular weights and narrow molecular weight distributions as well as a large variety of pendant functional groups[6]. In this study, the author focused on the usefulness in the control of the functionality at α-terminus of the obtained polymers. With employing a functionalized initiator, polymers having a functional group at α-terminus can be quantitatively obtained (Scheme 1.1), making it possible to synthesize a macromonomer by living cationic polymerization of VEs with using a methacryloyl group-functionalized initiator. Indeed, many kinds of functional groups have been incorporated in the polyVE pendants by using the corresponding pendant-functionalized VEs, but living cationic polymerization of alkynyl group-substituted VEs has not yet been reported to date. Here the author designed a new bifunctional VE with an alkynyl group in the pendant and investigated on its living cationic polymerization. It is noteworthy that the present VE possessing an unprotected alkynyl pendant can be directly subjected to living cationic polymerization. Most of the alkyne-substituted polymers so far reported required protecting / deprotecting processes before

and after the polymerization. Thus, the author designed a polyVE-based macromonomer having both a methacryloyl function at α-end and unprotected alkynes in the pendants.

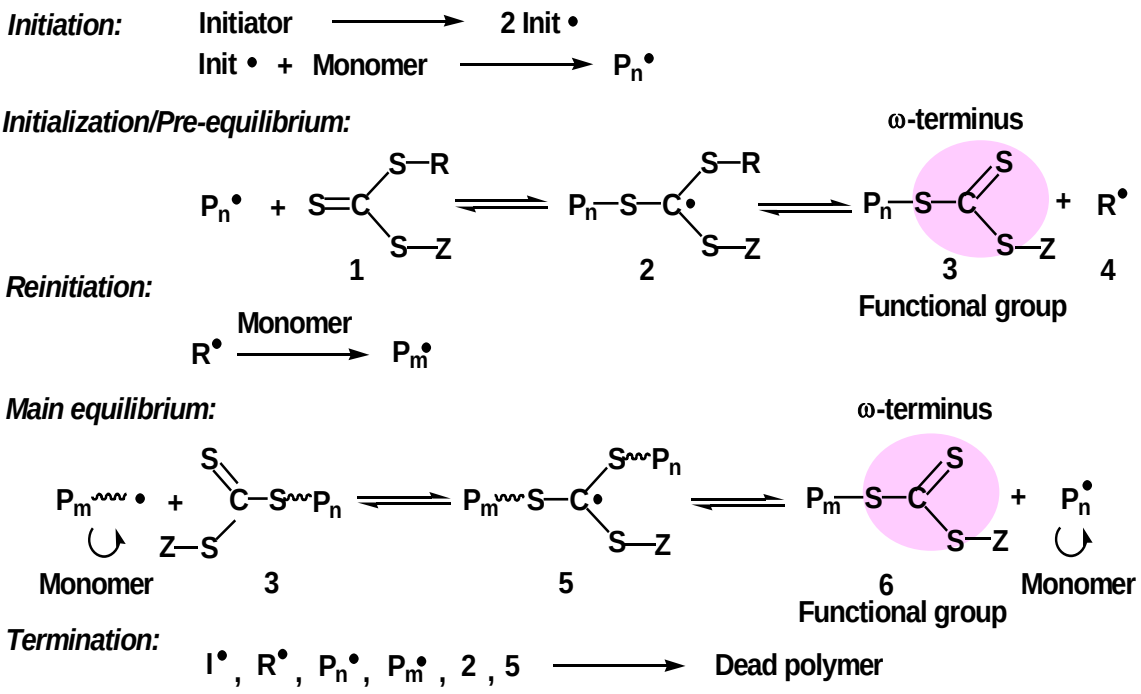


Scheme 1.1: Synthesis of end-functionalized polyVEs by living cationic polymerization.

I.2.2 Living radical polymerization by reversible addition-fragmentation chain transfer polymerization (RAFT) process

Living radical polymerization by RAFT process (hereafter referred to as RAFT polymerization) is a versatile method to synthesize a wide variety of tailored polymers. As shown in Scheme 1.2, RAFT polymerization is basically operated on the principle of degenerative chain transfer and its deactivation-activation equilibrium[7]. For the RAFT polymerization, many kinds of chain transfer agent (CTA) have been utilized. Among them, dithioester and trithiocarbonate compounds are most commonly used depending on the type of vinyl monomers. One of the characteristic features

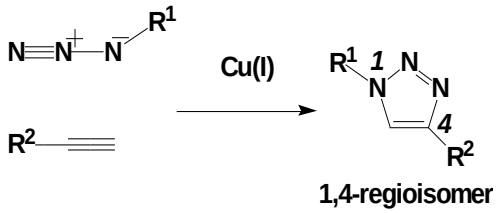
of RAFT polymerization is that the resulting polymer possesses a functionalized ω-terminus. For example, the use of dithioester compound as the CTA shown in Scheme 1.2 yields a polymer with a dithioester moiety at ω-terminus. In this study, the author employed trithiocarbonate compound as the CTA for the RAFT polymerization of acrylamide derivatives, hence the resultant polyacrylamide has a trithiocarbonate-functionalized ω-end. The terminal functions such as dithioester and trithiocarbonate can be quantitatively converted to thiol under reducing conditions, and then subjected to further post reaction such as thio-ene reaction[8] and Au-S bond formation[9] etc. The author demonstrated the RAFT copolymerization of acrylamide derivatives and subsequent post reactions composed of reduction of the ω-end trithiocarbonate group and thiol-ene reaction with an excess amount of diacrylate compounds, resulting in the successful synthesis of a polyacrylamide-based macromonomer having an acryloyl group at ω-end.



Scheme 1.2: Mechanism of living radical polymerization by RAFT process.

I.2.3 CuAAC click reaction with maltosyl azide for forming glycomonomers and glycopolymers

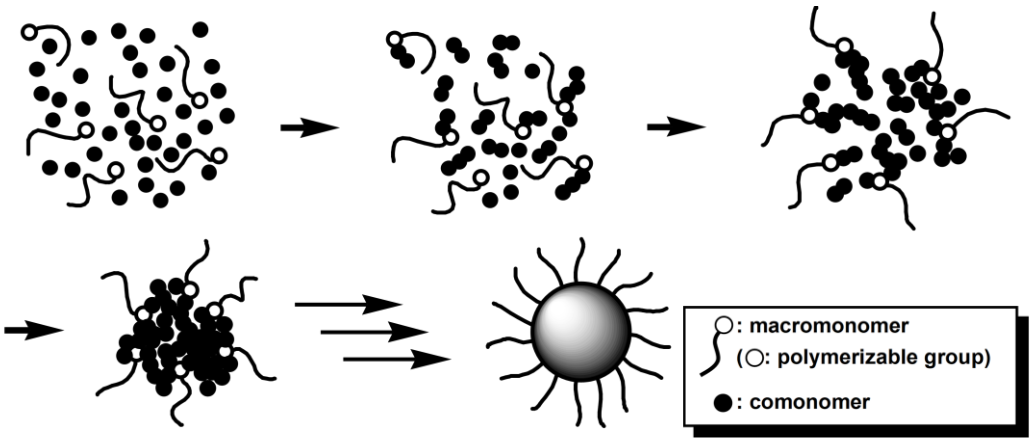
In this research, CuAAC click reaction is one of the most important reactions for the synthesis of glycopolymer-type macromonomers and a glycomonomer. It has widely been recognized that Cu catalyst-mediated click reactions between an alkyne and an azide compounds exclusively yields 1,4-regioisomer of triazole derivatives (Scheme 1.3) whereas 1,5-regioisomer generates by the reactions using Ru-based catalyst or acetylide anions. CuAAC click reactions efficiently proceed with high tolerance of functional groups and solvent variations under a moderate reaction temperature[10]. In this study, CuAAC click chemistry was utilized to synthesize carbohydrate-substituted polyVE-based macromonomers from their precursor alkyne-substituted versions, and also to prepare carbohydrate-carrying acrylamide monomer as a glycomonomer. Here, the author emphasizes again that the combined methodologies of living cationic polymerization or RAFT-based living radical polymerization and CuAAC click reactions are free from any protecting / deprotecting of the hydroxyl and the alkyne functions throughout the synthetic processes.



Scheme 1.3: Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC).

I.2.4 Dispersion polymerization for the preparation of hairy or core-shell polymer particles

A great variety of polymer particles have been prepared to date. They have various shapes and morphologies including spheres, truncated spheres, hemispheres, hairy or core-shell spheres, hollow spheres, spheres with multiple surface dimples, and so on[11]. In addition, many methodologies for preparing polymer particles have been developed[12]. Among them, in this study, the author deals with hairy or core-shell polymer particles prepared by the dispersion copolymerization of amphiphilic or hydrophilic macromonomers and hydrophobic low-molecular-weight vinyl monomers in polar media. The formation process of the hairy or core-shell polymer particles by dispersion copolymerization was shown in Scheme 1.4, where the nuclei of polymer particles are formed by the formation and aggregation of the grafted copolymers in the initial phase and the small particles having hydrophilic polymer chains on their surfaces grow up to form a stable aqueous dispersion in the middle and late phase of the polymerization. The most characteristic feature of this methodology is that the surfaces of the obtained polymer particles are covered with numerous hydrophilic polymer chains derived from the macromonomer employed[13], indicating the surface functionality of the core-shell polymer particles can be widely designed by the structure of the amphiphilic macromonomers. That is to say, carbohydrate-decorated polymer particles can be readily prepared with employing glycopolymer-type macromonomers for the dispersion copolymerization.



Scheme 1.4: Mechanism of dispersion copolymerization by using amphiphilic or hydrophilic macromonomers.

I.3 Aim of this study

The objectives of the present study are synthesizing of glycopolymer-type macromonomers via two different approaches based on living cationic polymerization (LCP) and RAFT polymerization through the protecting free-group processes and their applications to the preparing carbohydrate-decorated core-shell-type polymer particles. The details were divided into three major themes.

The first theme is to design LCP of a functionalized VE monomer (VEEP) having an unprotected alkynyl pendant and to synthesize macromonomer-type polyVE (MA-PVEEP) by LCP. Furthermore, glycopolymer-type macromonomer [MA-P(VE-Mal)] with both pendant maltose residues and a methacryloyl group at α-terminus was synthesized by CuAAC click reaction between the precursor alkyne-functionalized macromonomer and maltosyl azide.

In the second theme, the synthetic methodology described in the first part was applied to the synthesis of amphiphilic block copolymer-type macromonomers with pendant maltose residues. The precursor

macromonomer was synthesized by sequential block LCP of IBVE and VEEP monomers in this order with using a methacryloyl-functionalized initiator. Similar to the first part, CuAAC click reaction of the alkyne-substituted precursor block copolymer with maltosyl azide yielded the target amphiphilic block copolymer-type macromonomers with pendant maltose moieties. In this part, maltose-decorated polymer particles were prepared through the dispersion copolymerization of the segmented glycopolymer-type macromonomers with styrene (St) in polar media and the specific interaction of the resultant polymer particles with a lectin was also examined.

The third theme concerns with the synthetic methodology for designing glycopolymer-type macromonomers with a terminal polymerizable group at ω-end. By RAFT polymerization, a maltose-appended polyacrylamide copolymer having a trithiocarbonate terminal function was firstly synthesized and then subjected to the extended RAFT polymerization of poly(*N*-*tert*-butylacrylamide) with the precursor polyacrylamide as macro CTA to afford the segmented polyacrylamide copolymer. Its terminal function conversion under a reducing condition and subsequent thiol-ene reaction with a diacrylate compound afforded an amphiphilic block copolymer composed of maltose-carrying polyacrylamide backbone and a terminal acryloyl group. Maltose-decorated polymer particles were prepared by the dispersion copolymerization of the polyacrylamide-based macromonomers with St and were also found to specifically recognize a corresponding lectin.

I.4 Structure of thesis

This thesis consists of four chapters. In chapter I, the author describes the objectives of this study and shows the related fundamental principles in polymer and organic chemistry, which are indispensable for doing this research. Chapters II and III were devoted to the controlled synthesis of polyVE-based glycopolymer-type macromonomers by LCP and CuAAC click reaction and their use for the preparation of carbohydrate-decorated polymer particles. In chapter IV, a new synthetic approach to the formation of polyacrylamide-based glycopolymer-type macromonomer was demonstrated based on a combination of RAFT polymerization and thiol-ene reaction, and carbohydrate-decorated polymer particles were obtained from the macromonomer. In chapters III and IV, surface functionality of the carbohydrate-decorated polymer particles was demonstrated by lectin binding assay experiments.

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Chapter II

Living cationic polymerization of a vinyl ether with an unprotected pendant alkynyl group and their use for the synthesis of glycopolymer-type macromonomers via CuAAC with maltosyl azides

II.1 Introduction

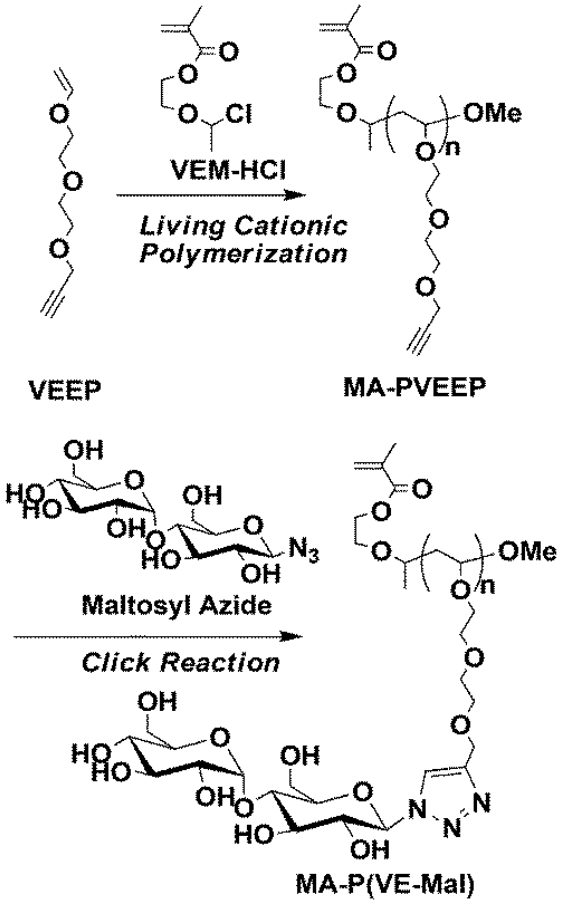
Glycopolymers[1] have recently been becoming an important research topic due to their potential abilities as functional materials in a wide variety of areas such as biotechnology[2], biosensing[3], drug and gene delivery[4-5], nanomedicine[6], and so on. To synthesize well-defined glycopolymers having various architectures[7-10] such as linear block glycopolymers consisting of multiple segments and branched glycopolymers such as glycodendrimers[11-12] and star-shaped glycopolymers[13-14], scientists have utilized controlled polymerization of carbohydrate-containing monomers or post-functionalization of the precursor reactive polymers using carbohydrate derivatives as exemplified in copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC)[15-16]. A large number of papers have reported the controlled synthesis of glycopolymers[7] where various types of controlled polymerization such as living radical polymerization[10, 17-18], living ionic polymerization[19-21], ring-opening metathesis polymerization[14] have been applied. An alternative approach to the synthesis of well-defined glycopolymers is a CuAAC-based procedure in which alkyne-carrying precursor polymers are decorated with azide-functionalized carbohydrates by click reaction. Among the glycopolymer syntheses mentioned above, living radical polymerization of carbohydrate-bearing monomers by RAFT process and CuAAC-based post-

functionalization are tolerant to multiple hydroxyl functions of carbohydrates, meaning these approaches can be carried out by protecting group-free procedures.

In this chapter, the author demonstrates the synthesis of well-defined glycopolymer by a combination of living cationic polymerization and CuAAC. It has been well recognized that tailor-made poly(vinyl ether)s (polyVEs) having a large variety of pendant functional groups can be synthesized by living cationic polymerization of functionalized vinyl ethers (VEs)[22]. However, it has not yet been reported about living cationic polymerization of VEs having pendant alkynyl groups. The author newly designed an alkyne-functionalized VE (3-[2-(2-vinyloxyethoxy)-ethoxy]-propyne, VEEP). To be emphasized is that the synthesis of alkyne-substituted polyVEEP in this study can be directly synthesized by living cationic polymerization of VEEP without any protecting / deprotecting processes. To the best of author's knowledge, only a few reports have described the direct polymerization of monomers having unprotected alkynyl group(s)[23-25]. For example, ring-opening cationic polymerization of an alkyne-substituted oxazoline derivative has been reported[23].

Here, the author proposes a synthetic strategy for designing novel glycopolymers with unique architecture, that is, macromonomer-type glycopolymer possessing both a terminal polymerizable group at α-end and pendant carbohydrate moieties (Scheme 2.1). By employing an initiator bearing a methacryloyl group for the living cationic polymerization of VEEP, α-terminus of polyVEEP can be quantitatively modified with a methacryloyl group. This precursor macromonomer is then subjected to CuAAC-based click reaction with glycosyl azides to form a macromonomer-type glycopolymer. This chapter concerns the synthesis of maltose-substituted polyVE-based macromonomer by a combination of living cationic polymerization of VEEP and CuAAC using maltosyl azide. Maltosyl azide

and analogous glycosyl azides can be synthesized using Shoda's activation of unprotected sugars[26] where unprotected mono saccharides and oligosaccharides are directly converted in aqueous solutions with 2-chloro-1,3-dimethylimidazolinium chloride (DMC). The synthetic procedure for the macromonomer-type glycopolymers in the present study offers several advantages. The first is the well-defined macromonomer structure of the resultant glycopolymer, that is, one glycopolymer molecule possesses one polymerizable group at the α-end. The second is the simple and atom-economical synthetic procedure of the precursor polymers with pendant alkynyl groups for CuAAC, which can be directly obtained by a protecting-free process using an alkyne-containing monomer. Most of the precursor polymers for CuAAC require protecting / deprotecting processes of the alkyne proton[8, 27-30]. The last is the versatility, thus, the designed macromonomer-type glycopolymers can be decorated with a wide variety of carbohydrates through protecting group-free pathways. In addition, the resultant reactive glycopolymers would be capable of fabricating branched glycopolymers by post-(co)polymerization of the terminal methacryloyl group and preparation of core-shell polymer particles covered with glycopolymers on their surfaces through emulsion or dispersion polymerization in polar media[31-32].



Scheme 2.1: Synthesis of a maltose-substituted macromonomer MA-P(VE-Mal) by a combination of living cationic polymerization and CuAAC-based click chemistry.

II. 2 Experimental sections

II.2.1 Materials and measurements

II.2.1.1 Materials

3-Bromo-1-propyne (Wako, 95.0%), 2-(vinyloxy-ethoxy)-ethanol (DEGV; Maruzen Petrochemical, 99.8%), potassium hydroxide (Wako, 85.0%), $^{n}Bu_4NI$ (Tokyo Chemical Industry Co., Ltd., 98.0%), ZnI_2 (Aldrich, 99.99+%), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA; TCI, 97.0%), copper (II) sulfate pentahydrate (Wako, 99.5%), L-ascorbic acid sodium salt (Wako, 98.0%), D(+)-maltose monohydrate (Wako, 98.0%), sodium azide (Wako, 98.0%), 2-chloro-1,3-dimethylimidazolinium chloride (DMC; Wako, 97.0%), *N*,*N*-diisopropylethylamine (DIPEA; TCI, 98.0%) were used as received. Synthesis of 2-(vinyloxy) ethyl methacrylate (VEM) and VEM-HCl adduct were carried out according to the procedure reported in the literature[33].

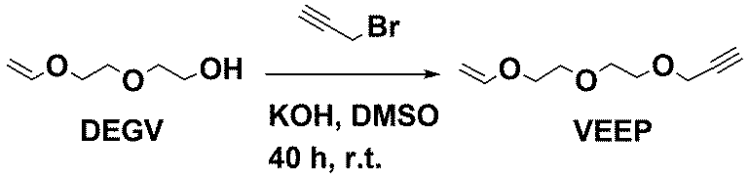
II.2.1.2 Measurements

^{1}H and ^{13}C nuclear magnetic resonances (NMR) spectra were recorded at 25 °C on a Bruker model AC-500 spectrometer, operating at 500 and 125 MHz, respectively, where chemical shifts (δ in ppm) were determined with respect to non-deuterated solvent residues as internal standards. Preparative size exclusion chromatography (SEC) was performed at 25 °C using 21.5 mm x 300 mm polystyrene gel columns (TOSOH TSKgel G2000H, G2500H, and G3000H) on a TOSOH model CCPE equipped with RI-8022 RI detector. Analytical SEC was performed in THF at 40 °C, using 8.0 mm x 300 mm polystyrene gel columns (Shodex KF-804 x 2) on a TOSOH model DP-8020 equipped with a UV-8000 variable-wavelength UV-vis detector and a RI-8022 RI detector. The number-average molecular weight (M_n) and polydispersity ratio (M_w/M_n) were calculated from the chromatographs with

respect to 15 polystyrene standards (Scientific Polymer Products, Inc.; M_n = 580−670000 g mol^{-1}, M_w/M_n = 1.01−1.07). Analytical SEC was performed in 0.2 mol L^{-1} $NaNO_3$ aqueous solution at 40 °C, using 7.8 mm x 300 mm gel columns (TOSOH TSKgel α−3000 x 3) on a JASCO model PU2089 equipped with a UV−2075 variable-wavelength UV−vis detector and an RI−2031 RI detector. The number-average molecular weight (M_n) and polydispersity ratio (M_w/M_n) were calculated from the chromatographs with respect to poly(ethylene glycol)s standards (Scientific Polymer Products, Inc.; M_n = 590−11900 g mol^{-1}, M_w/M_n = 1.05−1.11).

II.2.2 Experimental sections

II.2.2.1 Synthesis of 3-[2-(2-vinyloxyethoxy)-ethoxy]-propyne (VEEP)



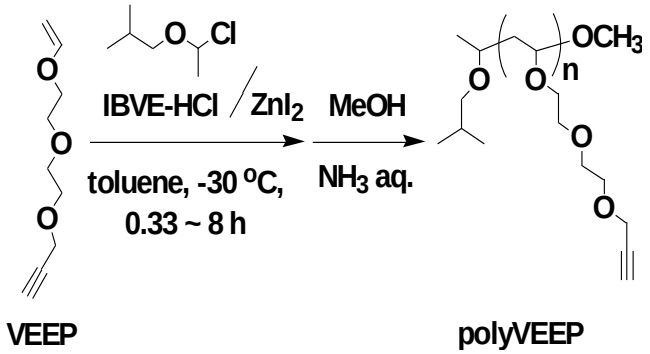
Scheme 2.2: Synthesis of alkyne-substituted monomer VEEP.

The reaction of DEGV (10 mL, 0.078 mol) with propargyl bromide (5.91 mL, 0.078 mol) was carried out in dimethyl sulfoxide (DMSO) (60 mL) in a 500 mL, three-necked, round-bottomed flask equipped with a paddle stirrer and a reflux condenser. KOH (5.38 g, 0.078 mol) was then added at room temperature (Scheme 2.2). After a period of 40 h, the mixture was obtained as a yellow heterogeneous mixture, then extracted with 10% NaCl aq. (100 mL), diethyl ether (300 mL) and dried overnight with Na_2SO_4. The extract was filtered and concentrated by evaporating off the ether and the unreacted chemicals under reduced pressure. By column chromatography using mixed solvents of ethyl acetate and hexane (R_f = 0.33) (1/9, v/v), monomer VEEP was obtained as a colourless oil (9.18 g, yield: 69% based on

DEGV). Prior to polymerization, the monomer was distilled over CaH_2 (72-73 °C/1.8 Torr).

^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.50 (dd, J_1 = 14.3 Hz, J_2 = 6.9 Hz, 1H, CH_2=C*H*-), 4.21 (d, J = 2.4 Hz, 2H; -CH_2-), 4.18 (dd, J_1 = 14.4 Hz, J_2 = 2.2 Hz, 1H, C*H*$_2$=CH-), 4.01 (dd, J_1 = 6.9 Hz, J_2 = 2.2 Hz, 1H, C*H*$_2$=CH-), 3.86 (t, J = 3.2 Hz, 2H; -CH_2-), 3.75 (t, J = 3.2 Hz, 2H; -CH_2-), 3.71 (m, 4H; -CH_2-), 2.43 (t, J = 2.4 Hz, 1H; CH≡). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 151.8, 86.8, 80.2, 77.0, 69.4, 68.7, 68.4, 67.2, 57.4.

II.2.2.2 Living cationic polymerization of VEEP



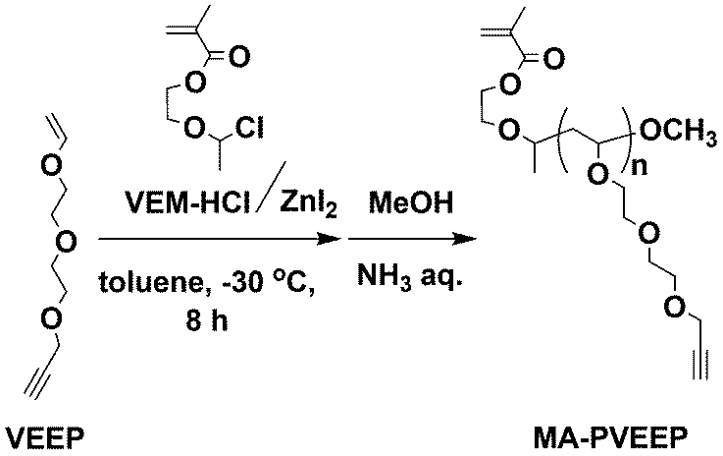
Scheme 2.3: Synthesis of poly(VEEP) via living cationic polymerization.

Polymerization of VEEP was carried out under dry nitrogen atmosphere in a baked glass tube equipped with a three-way stopcock. A typical example for the polymerization procedure is given (Scheme 2.3). VEEP monomer (800 mmol L^{-1}, 3.6 mL in toluene) and a prechilled solution of IBVE-HCl as an initiator (27 mmol L^{-1}, 0.4 mL in toluene) were added in this order at -30 °C. To the solution was added ZnI_2 solution as an activator (2.7 mmol L^{-1}, 0.4 mL in diethyl ether), then added 0.1 mL aliquot of toluene to prepare the reaction mixture of 4.5 mL. After the reaction time of 0.33, 0.50, 0.75, 1, 1.5, 2, 4, 6, 7, and 8 h, each polymerization was quenched with an excess of amount of chilled ammoniacal methanol (MeOH/NH_3 aq.). The conversion of VEEP monomer was determined by ^{1}H NMR analysis. The solution was

diluted with toluene and then washed with 20% aqueous sodium thiosulfate solution and evaporated under reduced pressure, then vacuum-dried to yield the target polymer [poly(VEEP)]. M_n and M_w/M_n of the obtained polymers were determined by analytical SEC in THF. The polymer structure was also analysed by ^{1}H NMR measurement.

^{1}H NMR (500 MHz, $CDCl_3$, δ): 4.19 (2H, -CH_2-), 3.67-3.59 (8H, -CH_2-), 2.50 (1H, CH≡), 1.85-1.54 (3H x n, (-CH_2-CH-)$_n$), 0.91 (6H, CH_3-).

II.2.2.3 Synthesis of precursor macromonomer MA-PVEEP



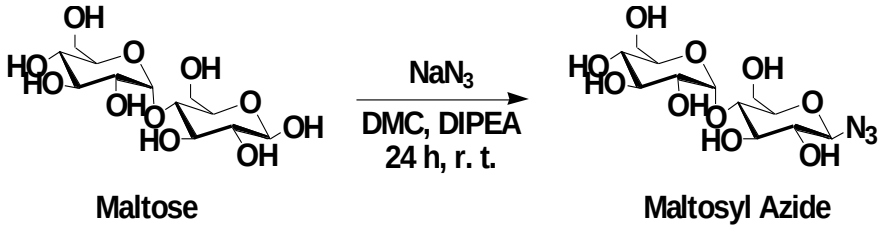
Scheme 2.4: Synthesis of precursor macromonomer MA-PVEEP.

Synthesis of the precursor macromonomer (MA-PVEEP) having both a terminal methacryloyl group and pendant alkynyl functions was carried out by living cationic polymerization of VEEP (1200 mmol L^{-1}, 2.7 mL in toluene) at -30 °C with the HCl adduct of 2-(vinyloxy) ethyl methacrylate (VEM-HCl, 40 mmol L^{-1}, 0.40 mL in toluene) and ZnI_2 (4.0 mmol L^{-1}, 0.40 mL in diethyl ether) in a similar way mentioned above (Scheme 2.4). After a period of 8 h, the polymerization was quenched with an excess of amount of chilled ammoniacal methanol (MeOH/NH_3 aq.). The reaction mixture obtained was diluted with toluene and washed with 20% aqueous sodium thiosulfate solution and water to remove the salts, evaporated to dryness

under reduced pressure, then vacuum dried to give the precursor macromonomer (MA-PVEEP). The product was purified by preparative SEC in $CHCl_3$ and used for further experiments. The M_n and M_w/M_n of MA-PVEEP were estimated by analytical SEC in THF. Furthermore, the structural analysis of the product was performed by ^1H NMR spectroscopy in $CDCl_3$. The conversion reached nearly 90% within 8 h based on ^1H NMR spectrum.

^1H NMR (500 MHz, $CDCl_3$, δ): 6.15 and 5.59 (2H, CH_2=), 4.21 (2H, -CH_2-), 3.69-3.64 (8H, -CH_2-), 2.50 (1H, CH≡), 1.94 (3H, CH_3-), 1.84-1.55 (3H x n, (-CH_2-CH-)$_n$). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 166.54, 137.12, 125.77, 80.28, 76.94, 73.48, 70.09, 69.57, 68.57, 67.48, 57.57, 39.87, 18.01.

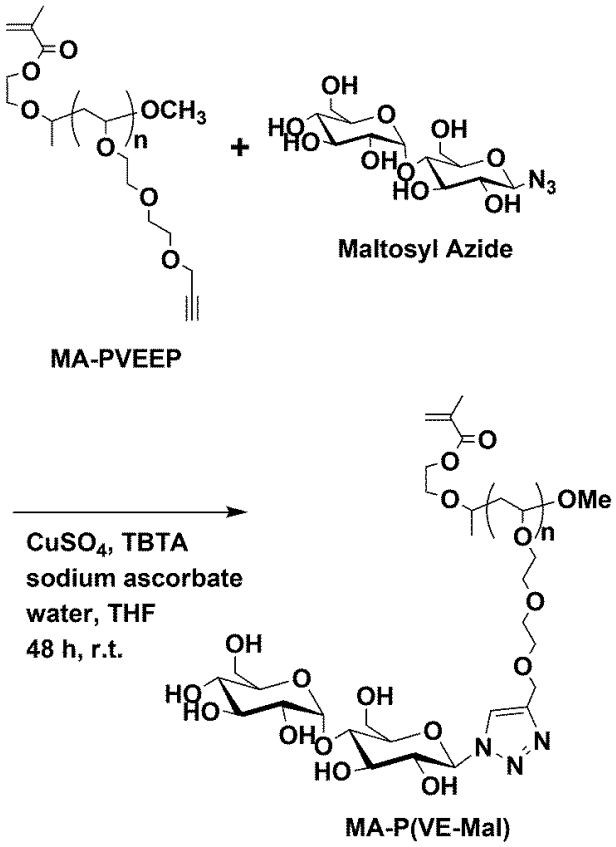
II.2.2.4 Synthesis of maltosyl azide



Scheme 2.5: Synthesis of maltosyl azide (Mal-N_3).

A typical procedure is described as follows[26]. 2-chloro-1,3-dimethyl-imidazolinium chloride (DMC; 1.54 g, 0.009 mol) was added to a mixture of D (+)-maltose monohydrate (1.03 g, 0.003 mol), *N*,*N*-diisopropylethylamine (DIPEA; 4.71 mL, 0.027 mol), and NaN_3 (1.95 g, 0.03 mol) in distillated water (11 mL) at room temperature (Scheme 2.5). After stirring for 24 h, the mixture was filtered and washed by ethanol, then was extracted by CH_2Cl_2 (200 mL). By ion-exchange column chromatography of amberlite quaternary ammonium with distilled water, then the product was obtained (2.78 g, yield: 94%).

II.2.2.5 Click reaction of pendant alkynes of MA-PVEEP with maltosyl azide



Scheme 2.6: Click reaction between MA-PVEEP and maltosyl azide.

Precursor macromonomer MA-PVEEP (100 mg, 27 μmol) (DP_n = 21) and maltosyl azide (630 mg, 1.7 mmol) were suspended in a 1:1 mixture of THF and water (15 mL) (Scheme 2.6). Sodium ascorbate (68 mg, 0.34 mmol) was added, followed by the addition of copper (II) sulfate pentahydrate (42 mg, 0.17 mmol) and TBTA (91 mg, 0.17 mmol). The heterogeneous mixture was stirred vigorously for 48 h under nitrogen atmosphere at room temperature, at which point it became transparent and thin-layer chromatography (TLC) analysis indicated complete consumption of the reactants. The reaction mixture was extracted with toluene and evaporated to dryness under reduced pressure. The water solution of reaction mixture was poured into a

large amount of methanol to precipitate the polymers. The resultant polymer was collected by centrifugation and dried under reduced pressure. For further purification, the crude product was purified by dialysis membrane with a molecular weight cut-off (MWCO) of 2000 in distilled water for more than 1 week and then stirring with metal scavenger (SiliaMets® Imidazole, 200 mg) for 20 h. After removing of metal scavenger by filtration, the filtrate was concentrated *in vacuo* and freeze-dried to give off-white colour solid (153 mg, yield: 49%).

^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.25 (1H, =CH-N), 6.01 and 5.99 (2H, CH_2=), 5.90-3.10 (12H, Mal), 4.50 (2H, -CH_2-), 1.89 (3H, CH_3-), 1.90-1.10 (3H x n, (-CH_2-CH-)$_n$). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 143.92, 123.22, 101.39, 87.63, 79.56, 78.42, 77.06, 74.0, 73.71, 72.89, 72.07, 70.51, 70.33, 70.13, 69.58, 63.87, 61.24, 60.74, 40.45, 18.46.

II.3 Results and discussion

II.3.1 Synthesis of VEEP

An asymmetric bifunctional monomer having both vinyl and alkynyl moieties (VEEP) was synthesized by the reaction of DEGV and propargyl bromide in DMSO in the presence of KOH as a catalyst (Scheme 2.2). After purification by column chromatography and distillation, the monomer was obtained as a colourless oil in decent yield (69%). As seen in Figure 2.1(a), the ^{1}H NMR spectrum in $CDCl_3$ of VEEP show signals at 6.50 ppm (peak g) and 2.42 ppm (peak a) ascribable to vinyl and alkynyl protons, respectively. Peak intensity ratio of these signals is unity, indicating the monomer equivalently contains a vinyl and an alkynyl group. The bifunctional structure was also confirmed by ^{13}C NMR spectrum.

II.3.2 Living cationic polymerization of VEEP

As shown in Scheme 2.3, the author polymerized VEEP in toluene at -30 °C using the CH_3-CH(O*i*Bu)-Cl (IBVE-HCl) / zinc iodide (ZnI_2) initiating system, which is one of the typical initiating systems so far reported[31-34] for living cationic polymerization of VEs ($[VEEP]_0$ / $[IBVE\text{-}HCl]_0$ / $[ZnI_2]_0$ = 30 / 1 / 0.1, $[VEEP]_0$ = 800 mmol L^{-1}). The resulting polymer poly(VEEP) was analyzed by RI-detected size exclusion chromatography (SEC), ^{1}H NMR and ^{13}C NMR. By ^{1}H NMR, all the key signals arising from VEEP repeating units (2.50, 3.59-3.67 and 4.19 ppm) and the *iso*-butyl group at α-end (0.91 ppm) are consistent with those of the expected structure for poly(VEEP). And the number-average degree of polymerization (DP_n) of poly(VEEP) can be determined by the peak intensities of the terminal alkyl group proton peaks of the IBVE moiety and pendant alkynyl protons in the poly(VEEP) segment [Figure 2.1(b), peaks j' and a']. Figure 2.2(b) shows the time-conversion curve obtained for the polymerization of

VEEP, showing that the polymerization proceeded smoothly without an induction phase then reached over 90% conversion within 8 h. The SEC curves obtained for the polymers shifted to higher molecular weight regions as the reaction time increased (Figure 2.2(a)). In Figure 2.2(c), the values of number-average molecular weight (M_n) and polydispersity index (M_w/M_n) for the polymers were plotted against monomer conversions. A linear increase in M_n against conversion as well as maintaining of narrow molecular weight distribution ($M_w/M_n < 1.24$) of the polymers indicate that the polymerization of VEEP proceeded in a controlled manner. Furthermore, the DP_ns of poly(VEEP) determined by ^{1}H NMR were in good agreement with the theoretical values, which are evaluated based on the feed molar ratio of $[VEEP]_0/[IBVE\text{-}HCl]_0$ and monomer conversions. These results clearly indicate that quantitative initiation and precisely controlled cationic polymerization of VEEP were accomplished using the IBVE-HCl/ZnI_2 initiating system.

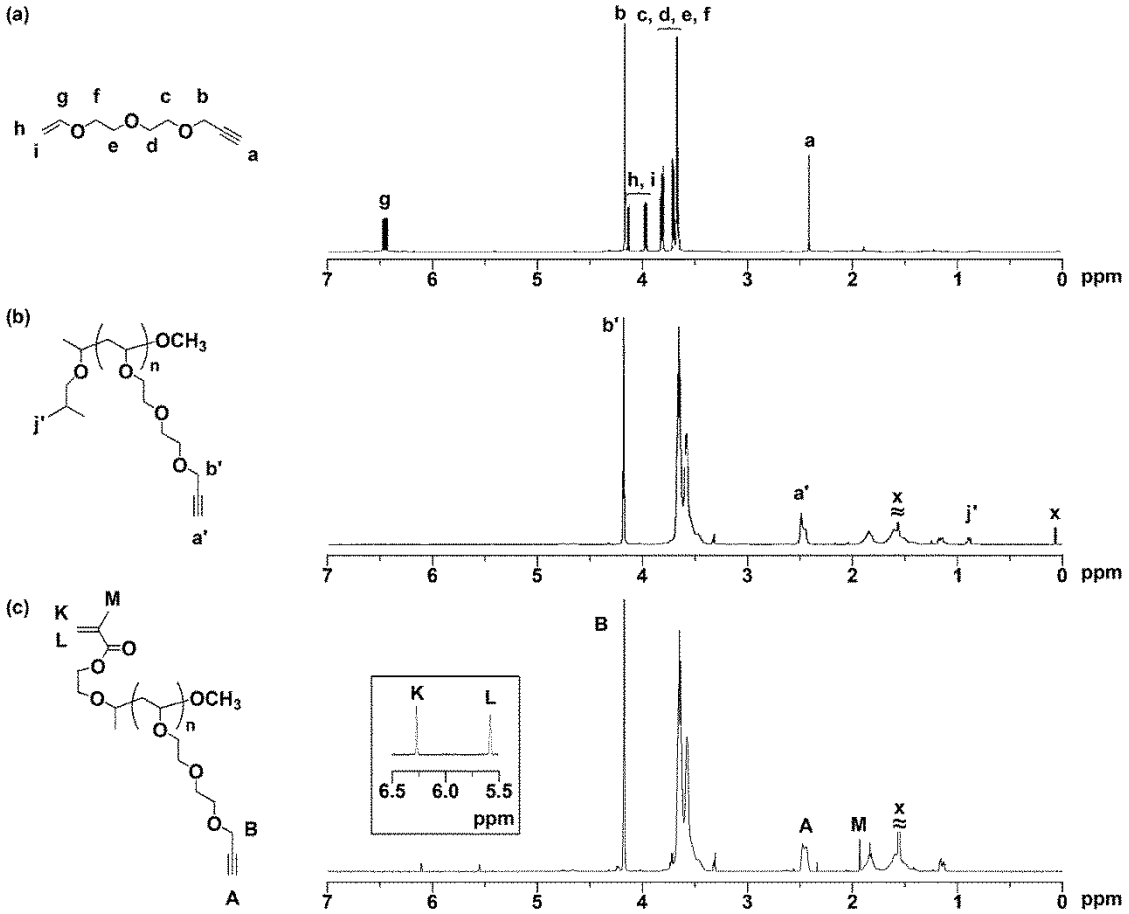


Figure 2.1: ^{1}H NMR spectra of (a) VEEP, (b) poly(VEEP), and (c) MA-PVEEP in $CDCl_3$ (x: remaining solvents).

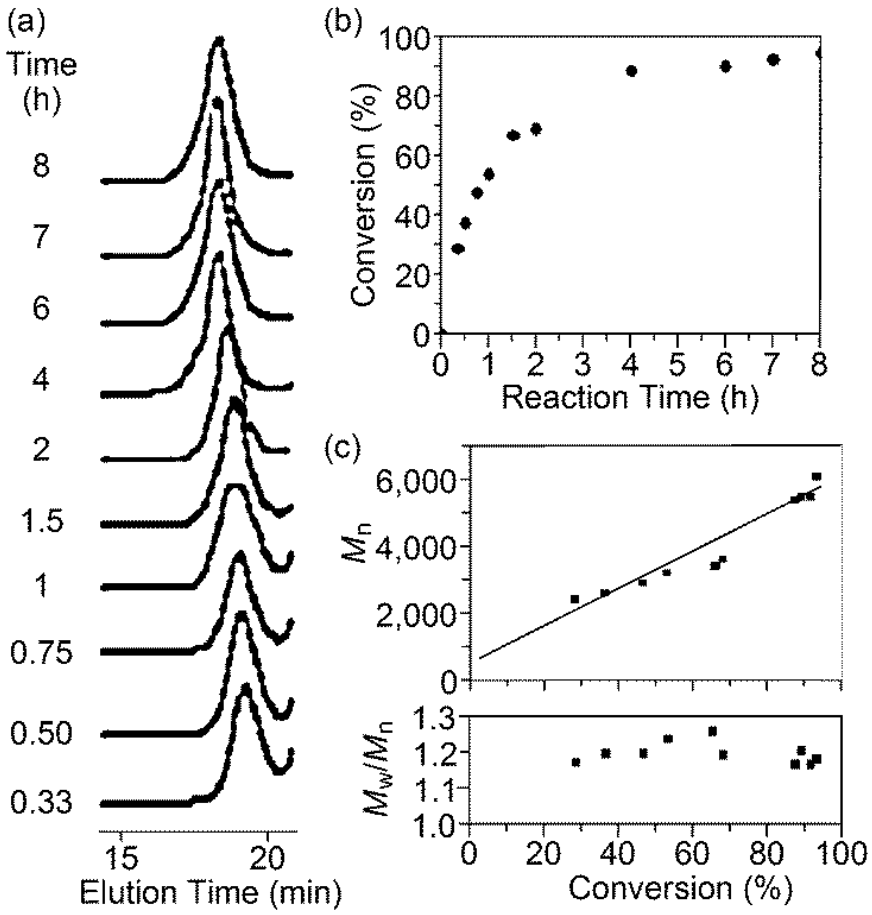


Figure 2.2: (a) SEC curves of poly(VEEP) using THF as an eluent, (b) Time-conversion curve for polymerization of VEEP, (c) M_n and M_w/M_n values of poly(VEEP) plotted against monomer conversion.

II.3.3 Synthesis of precursor macromonomer MA-PVEEP

The macromonomer (MA-PVEEP) possessing a methacryloyl group at α-end and pendant alkynyl functions was synthesized by living cationic polymerization of VEEP using the HCl adduct of 2-(vinyloxy)ethyl methacrylate (VEM-HCl) as an initiator in conjunction with ZnI_2 in toluene at -30 °C ($[VEEP]_0/[VEM\text{-}HCl]_0/[ZnI_2]_0$ = 30/1/0.1, $[VEEP]_0$ = 1,200 mmol L^{-1}) (Scheme 2.4). After quenching the polymerization, the product was purified by preparative SEC to remove the unreacted VEEP. The product was soluble in common organic solvents. The SEC data of the isolated

macromonomer depict a unimodal MWD curve without any shoulders, as well as a narrow molecular weight distribution in Figure 2.3(a) (M_w/M_n = 1.24), indicating that neither the terminal methacryloyl moiety nor the pendant alkynyl groups induced any side reactions throughout the living cationic polymerization.

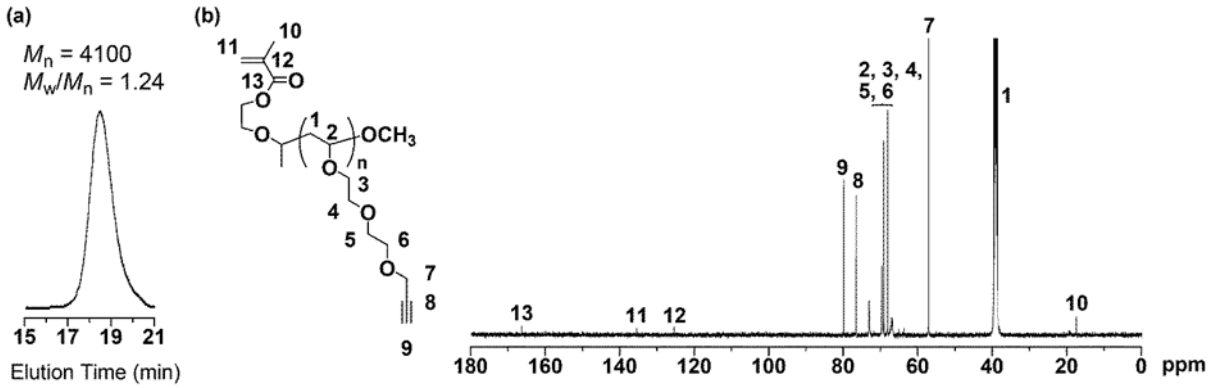


Figure 2.3: (a) SEC curve of MA-PVEEP using THF as the eluent. (b) ^{13}C NMR spectrum of MA-PVEEP macromonomer in DMSO-d_6.

The quantitative formation of the target macromonomer was also confirmed by ^1H NMR measurement in $CDCl_3$ in Figure 2.1(c). All the key signals arising from the methacryloyl group at α-end (5.59 and 6.15 ppm) and VEEP repeating units (2.50, 3.69-3.64, and 4.21 ppm) are consistent with those of the expected structure for MA-PVEEP. The DP_n of MA-PVEEP was determined to be 21 based on the peak intensity ratio of the alkynyl protons (peak A) and terminal vinyl protons (peak K). These results prove that the macromonomer MA-PVEEP having both a terminal methacryloyl group and pendant alkynyl moieties was precisely synthesized by living cationic polymerization. Furthermore, ^{13}C NMR spectrum in Figure 2.3(b) clearly shows the carbon peaks assignable to the pendant alkynyl groups (peak 8, 9) and the terminal methacryloyl group (peak 10-13), respectively.

II.3.4 Synthesis of maltosyl azide

The maltosyl azide was obtained as white color product, giving rise to the obtained Mal-N_3 in 94% yield based on weight balance. The ^{1}H NMR spectrum of Mal-N_3 show signals at 5.01 and 5.66 ppm, ascribable to two anomeric protons at the anomeric carbon (C_1) of the first and second glucose unit, respectively. It means that the azide group had been introduced in stereo-selective manner, affording Mal-N_3 with a *β*-configuration. The author achieved a direct synthesis of *β*-maltosyl azide without protection of hydroxyl groups and proceeds smoothly in aqueous media (Figure 2.4).

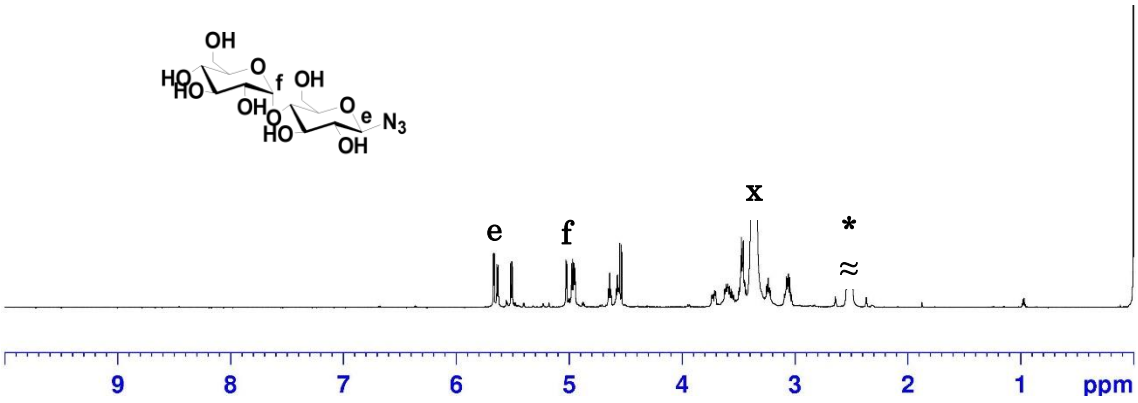


Figure 2.4: ^{1}H NMR spectrum of Mal-N_3 in DMSO-d_6 (* and x: remaining solvents).

II.3.5 Click reaction of the pendant alkynyl groups of MA-PVEEP with maltosyl azide

To demonstrate the potential ability of MA-PVEEP as an alkyne-substituted precursor polymer for CuAAC, MA-PVEEP was subjected to click reaction with maltosyl azide. The click reaction was performed under a typical CuAAC condition and the product was purified by dialysis. In this study, a small excess amount of maltosyl azide was reacted with the

pendant alkynyl moieties ([maltosyl azide]$_0$ / [pendant alkynyl moiety]$_0$ = 3.0) (Scheme 2.6). Reprecipitation of the reaction mixtures from water into MeOH was conducted to remove the Cu catalyst and sodium ascorbate, followed by centrifugation. After removal of the unreacted maltosyl azide and trace of the Cu catalyst by dialysis and metal scavenger, the SEC trace in Figure 2.5(a) indicates the successful isolation of the maltose-substituted macromonomer-type glycopolymer.

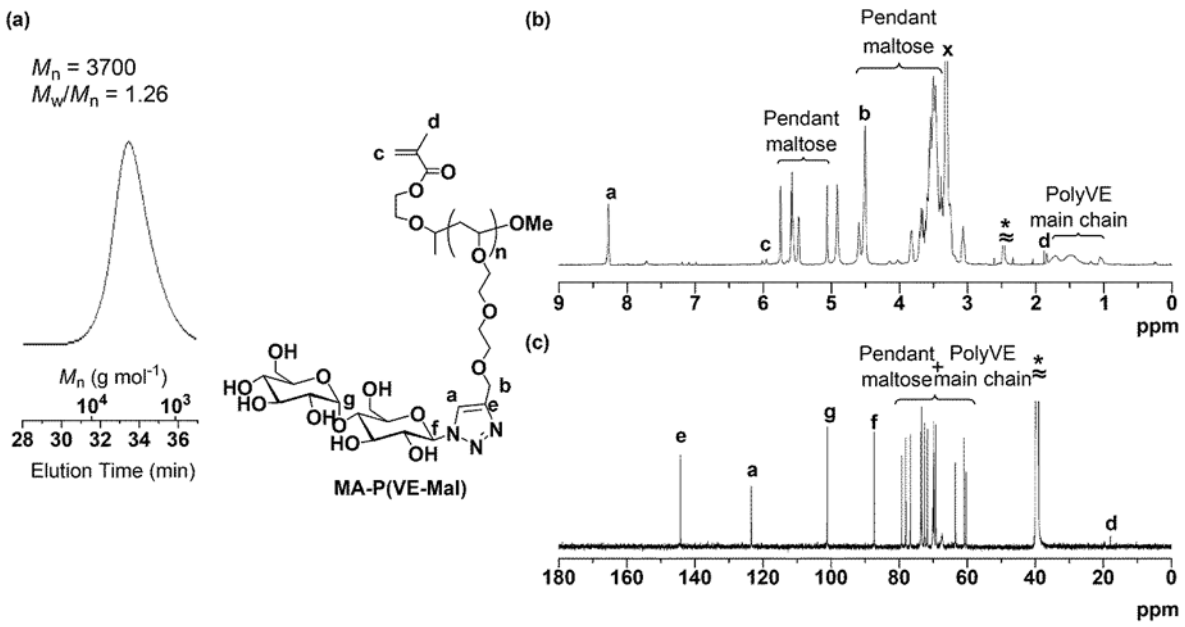


Figure 2.5: (a) RI detected SEC curve of MA-P(VE-Mal) (eluent: 0.2 M $NaNO_3$ aq.). (b) ^{1}H NMR and (c) ^{13}C NMR spectra of the click reaction product MA-P(VE-Mal) in DMSO-d_6 (* and x: remaining solvents).

The isolated polymer was subjected to ^{1}H NMR analysis. As shown in Figure 2.5(b), multiple signals at 5.9-3.1 ppm and broadened signals at 1.9-1.1 ppm are observed, which are assignable to the pendant maltose moieties and the polyVE main chain moiety, respectively. In addition, strong key signals appeared at 8.25 ppm and 4.5 ppm. These are ascribable to the linkage 1,4-substituted 1,2,3-triazole proton (peak a), and methylene protons adjacent to the triazole moiety (peak b), respectively. And ^{1}H NMR spectrum also shows a signal arising from a vinyl proton of the terminal

methacryloyl group at around 6.0 ppm (peak c). And no signal of the alkynyl protons are observed at 3.3-3.4 ppm in DMSO-d_6 because these alkynyl protons are overlapped by maltose protons. Based on the peak intensity ratio of the triazole proton to the methylene protons adjacent to the residual alkynyl group (4.2 ppm) after click reaction, 92% of the pendant alkynes were converted to the triazole units, indicating the click reaction of the pendant alkynes of MA-PVEEP and maltosyl azide nearly quantitatively proceeded. Furthermore, by employing the peak intensity ratio of the linkage triazole proton (peak a) and terminal vinyl proton (peak c), DP_n of MA-P(VE-Mal) was determined to be 20, which is in good agreement with the theoretical value based on the DP_n of the precursor polymer (MA-PVEEP) and the pendant alkynes' conversion (0.92). In Figure 2.5(c), ^{13}C NMR spectrum shows characteristic signals of triazole carbons at 143.92 ppm and 123.22 ppm and other signals assignable to the target maltose-substituted macromonomer-type glycopolymer [MA-P(VE-Mal)].

II.4 Conclusions

It could be concluded that the author succeeded in the selective and living cationic polymerization of asymmetric bifunctional monomer (VEEP), which is structurally characterized by the terminal VE and alkynyl functions in both ends. The procedure was applied to the synthesis of a macromonomer (MA-PVEEP) having a terminal methacryloyl moiety and pendant alkynyl groups. The resultant polymer was proved to be useful as the precursor for CuAAC-based modification with maltosyl azide, afforded the novel macromonomer-type glycopolymer [MA-P(VE-Mal)] with a terminal polymerizable group and pendant maltose residues. Furthermore, the terminal polymerizable group on the macromonomer-type glycopolymer also implies the potential polymerizability of MA-P(VE-Mal) in radical polymerization, which will be discussed in the next chapter.

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Chapter III

Controlled synthesis of poly(vinyl ether)-based amphiphilic glycopolymer-type macromonomers and their use for the preparation of carbohydrate-decorated polymer particles

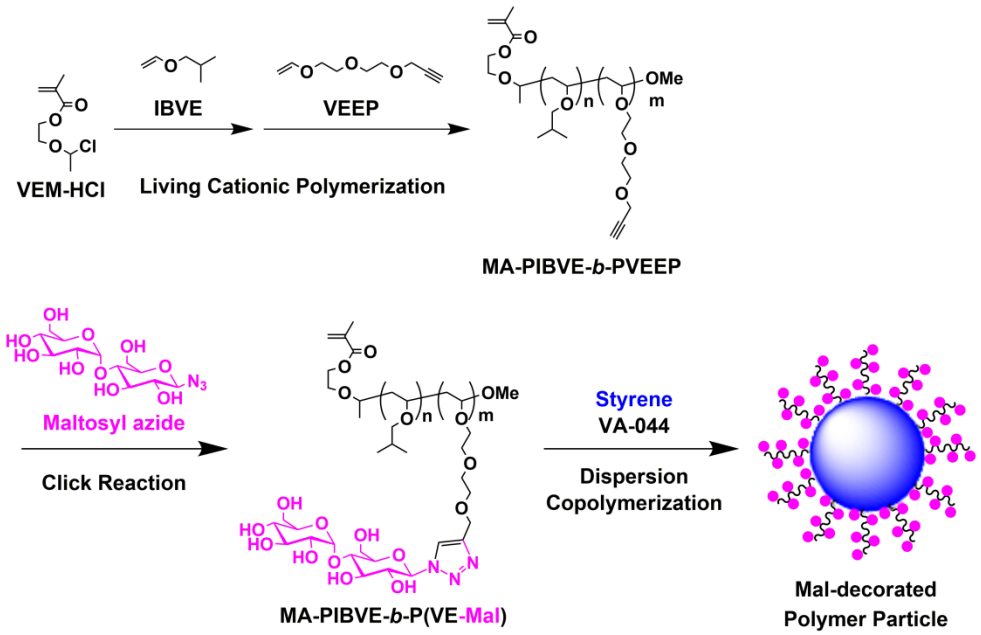
III.1 Introduction

Surface-functionalized polymer particles have attracted increased attention because of their scientific and technical importance[1] as versatile materials with extremely large specific surface areas. In particular, polymer particles decorated with carbohydrates on their surfaces have been of great interest in the biomedical field, because they have the potential ability for providing useful materials for diagnosis based on their specific interactions with biomolecules such as proteins and viruses etc.[2]. One effective method for producing surface-functionalized particles is the use of amphiphilic macromonomers for the dispersion or emulsion copolymerization with hydrophobic monomers in polar media[3]. The resultant polymer particles afford stable aqueous dispersions, where the dispersion stability is attributed to the steric stabilization attained by the hydrophilic polymer chains fixed on the particle surfaces. It is worth noting that the surface functionality of the particles can be widely designed by varying the structure of the macromonomers because they are covalently attached to the particle surfaces. The author and his coworkers have already reported that poly(vinyl ether) (polyVE)-based hydrophilic macromonomers bearing functional side chains can produce monodispersed polymer particles, where the surfaces of the polymer particles can be modified with a variety of functions originating from the pendant functions of the macromonomers

employed[4]. In contrast, there is still little information available on the use of glycopolymer-type macromonomers for the formation of core-shell polymer particles[5]; therefore, synthesis of such macromonomers having glycopolymer backbones, and studies on their potential ability as steric stabilizers (*glycostabilizers*) for forming polymer particles are strongly required. The synthesis of well-defined glycopolymers have been achieved by controlled polymerization of carbohydrate-containing monomers, or the post-functionalization of the precursor reactive polymers using carbohydrate-based agents as exemplified in copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC)[6]. A large number of papers have reported the synthesis of well-defined glycopolymers[7] by various controlled polymerizations such as living radical polymerization[8], living ionic polymerization[9], and ring-opening metathesis polymerization[10]. An alternative approach to the synthesis of well-defined glycopolymers is a CuAAC-based procedure, in which alkyne-carrying precursor polymers are precisely synthesized and then decorated with azide-functionalized carbohydrates by click reaction.

In chapter III, for the preparation of carbohydrate-decorated polymer particles, the author proposes a synthetic strategy for designing novel glycostabilizers that correspond to the glycopolymer-type macromonomers that possess both a terminal polymerizable group and a carbohydrate-substituted amphiphilic block copolymer backbone (Scheme 3.1). The living cationic sequential block copolymerization of isobutyl vinyl ether (IBVE) and alkyne-functionalized VE (VEEP) using the initiator bearing a methacryloyl group (VEM-HCl) produced block copolymer-type precursor macromonomers (MA-PIBVE-*b*-PVEEP) bearing a methacryloyl group at α-terminus. This precursor macromonomer was then subjected to the CuAAC click reaction with maltosyl azide to form the target amphiphilic glycopolymer-type macromonomer [MA-PIBVE-*b*-P(VE-Mal)]. The maltose-decorated (Mal-

decorated) polymer particles were then prepared by the dispersion copolymerization of the obtained macromonomer and styrene, and their lectin-binding properties were also investigated.



Scheme 3.1: Synthesis of glycopolymer-type amphiphilic macromonomers [MA-PIBVE-*b*-P(VE-Mal)] and their application to the preparation of maltose-decorated (Mal-decorated) polymer particles by dispersion polymerization.

III. 2 Experimental sections

III.2.1 Materials and measurements

III.2.1.1 Materials

D(+)-maltose monohydrate, sodium azide, 2-chloro-1, 3-dimethylimidazolinium chloride (DMC), copper (II) sulfate pentahydrate, L-ascorbic acid sodium salt, 2,2'-azobis-[2-(2-imidazolin2-yl)-propane] dihydrochlroride (VA-044), styrene, concanavalin A (Con A) from Canavalia ensiformis, and bovine serum albumin (BSA) were purchased from

FUJIFILM Wako Pure Chemical Corporation. *N*,*N*-diisopropyl ethylamine (DIPEA) and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) were purchased from Tokyo Chemical Industry Co., Ltd. Isobutyl vinyl ether (IBVE) was purchased from Sigma-Aldrich. Cellulose dialysis tubes size 20 with molecular weight cut-off MWCO = 14 kDa and pre-wetted dialysis tubing with MWCO = 1 kDa and 2 kDa (nominal flat width: 18 mm, diameter 11.5 mm, volume: 1.1 mL cm^{-1}, length: 10 m) (Spectrum) was performed in a 1 L beaker by changing the distilled water four or five times over a period of 24 h. Anthrone reagent was prepared by adding 200 mg anthrone into 100 mL ice-cold 95% H_2SO_4. All other reagents were commercially available and used without further purification.

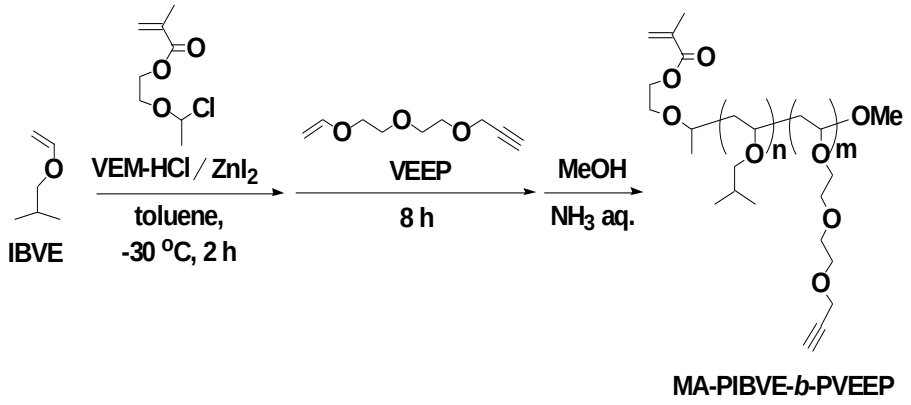
III.2.1.2 Measurements

^1H and ^{13}C NMR spectra were recorded at 25 °C on a Bruker model AC-500 spectrometer, operating at 500 and 125 MHz, respectively, where chemical shifts (δ in ppm) were determined with respect to non-deuterated solvent residues as internal standards. Preparative size exclusion chromatography (SEC) was performed at 25 °C by using 21.5 mm x 300 mm polystyrene gel columns (TOSOH TSKgel G2000H, G2500H and G3000H) on a TOSOH model CCPE equipped with RI-8022 RI detector. Analytical SEC was performed in tetrahydrofuran (THF) at 40 °C, using 8.0 mm x 300 mm polystyrene gel columns (Shodex KF-804 x 2) on a TOSOH model DP-8020 equipped with a UV-8000 variable-wavelength UV−vis detector and a RI-8022 RI detector. The number-average molecular weight (M_n) and polydispersity ratio (M_w/M_n) were calculated from the chromatographs with respect to 15 polystyrene standards (Scientific Polymer Products, Inc.; M_n = 580−670000 g mol^{-1}, M_w/M_n = 1.01−1.07). Analytical SEC was performed in 0.2 mol L^{-1} $NaNO_3$ aqueous solution at 40 °C, using 7.8 mm x 300 mm gel columns (TOSOH TSKgel α−3000 x 3) on a JASCO model PU2089 equipped

with a UV-2075 variable-wavelength UV-vis detector and an RI−2031 RI detector. The number-average molecular weight (M_n) and polydispersity ratio (M_w/M_n) were calculated from the chromatographs with respect to poly(ethylene glycol)s standards (Scientific Polymer Products; M_n = 590−11900 g mole^{-1}, M_w/M_n = 1.05−1.11). Scanning electron microscopy (SEM) images were observed using a JEOL JSM−7600F. UV−vis spectra were recorded on a SHIMADZU Type UV−2550 spectrometer.

III.2.2 Experimental sections

III.2.2.1 Synthesis of MA-PIBVE-*b*-PVEEP



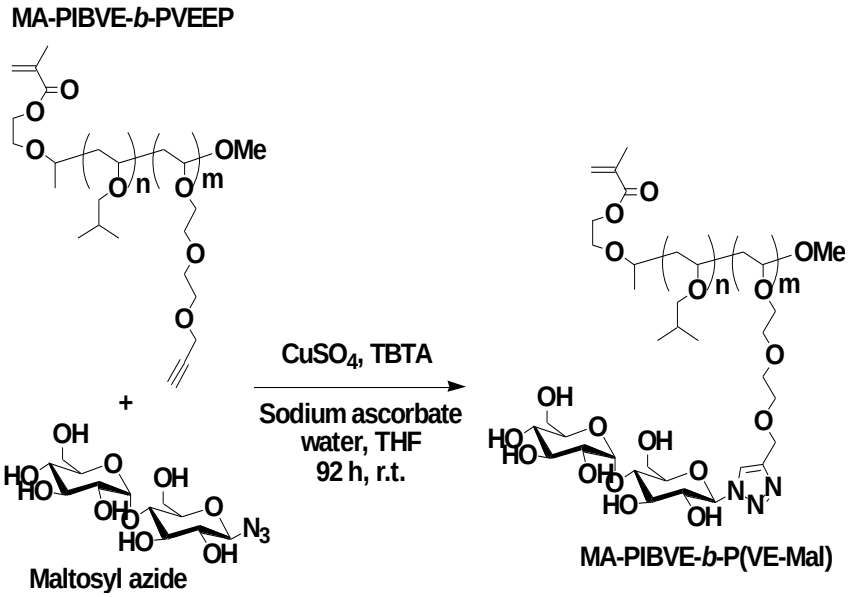
Scheme 3.2: Synthesis of precursor macromonomer MA-PIBVE-*b*-PVEEP via living cationic polymerization.

Synthesis of the precursor block macromonomer (MA-PIBVE-*b*-PVEEP) having both a terminal methacryloyl group and pendant alkynyl functions was carried out by the living cationic polymerization of IBVE (600 mmol L^{-1}, 1.35 mL in toluene) and VEEP (600 mmol L^{-1}, 1.35 mL in toluene) in this order with a prechilled solution of VEM-HCl (20 mmol L^{-1}, 0.45 mL in toluene) as an initiator and ZnI_2 solution (2.0 mmol L^{-1}, 0.45 mL in toluene) as an activator at -30 °C under dry nitrogen atmosphere in a baked glass tube equipped with a three-way stopcock (Scheme 3.2). Toluene (0.9 mL) was added to the solution mixture to make up to 4.5 mL in total volume. After a period of 10 h, the polymerization was quenched with an excess of

amount of chilled ammonia solution in methanol (MeOH/NH_3 aq.). The obtained reaction mixture was diluted with toluene and washed with 20% aqueous sodium thiosulfate solution and water to remove the salts, evaporated to dryness under reduced pressure, then vacuum-dried to yield the precursor macromonomer (MA-PIBVE-*b*-PVEEP) (Products **P1** and **P2**). The M_n and M_w/M_n of MA-PIBVE-*b*-PVEEP were estimated by analytical SEC in THF. Furthermore, the structural analysis of the target product was performed by ^{1}H NMR spectroscopy in $CDCl_3$.

^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.12 and 5.55 (2H, CH_2=), 4.19 (2H, -CH_2-), 3.67-3.50 (8H, -CH_2- of VEEP), 3.33-3.10 (3H, -CH_2-CH= of IBVE), 2.50 (1H, CH≡), 1.94 (3H, CH_3-), 1.84-1.54 (3H x (n + m), (-CH_2-CH-)$_n$-*b*-(-CH_2-CH-)$_m$), 0.90 (6H, CH_3- of IBVE). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 80.68, 79.57, 77.36, 73.86, 70.48, 70.25, 69.96, 68.95, 67.53, 57.96, 28.78, 19.44.

III.2.2.2 Synthesis of MA-PIBVE-*b*-P(VE-Mal)



Scheme 3.3: Synthesis of MA-PIBVE-*b*-P(VE-Mal) via CuAAC.

Precursor macromonomers (MA-PIBVE-*b*-PVEEP) and maltosyl azide (Mal-N_3) were suspended in a 1:1 mixture of THF and water (20 mL)

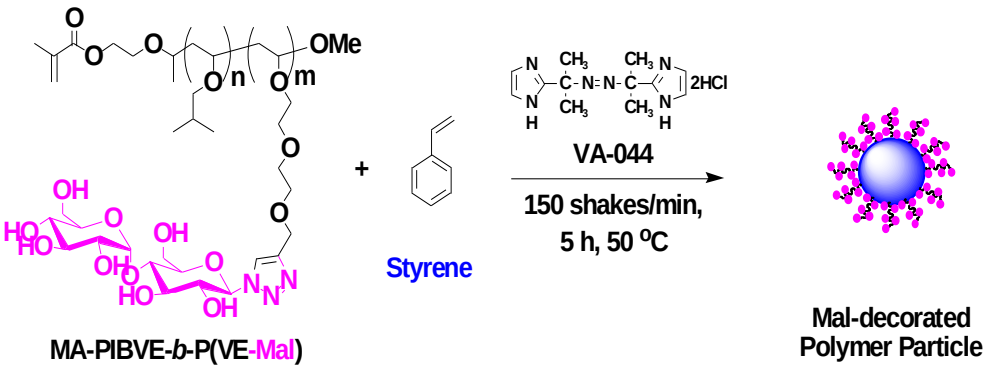
(Scheme 3.3). Sodium ascorbate (20 mol%) was added, followed by the addition of copper (II) sulfate pentahydrate (10 mol%) and TBTA (10 mol%) (Table 3.1). The heterogeneous mixture was stirred vigorously for 92 h under nitrogen atmosphere at room temperature, at which point it became transparent, and TLC analysis indicated complete consumption of the reactants. The reaction mixture was extracted with toluene and evaporated to dryness under reduced pressure. The water solution of reaction mixture was poured into a large amount of methanol to precipitate the polymers. The resultant polymer was collected by centrifugation and dried under reduced pressure. For further purification, the crude product was purified by dialysis membrane with a molecular weight cut-off (MWCO) of 2000 in distilled water for more than two weeks and, then recovered by freeze-drying. The products (**P3** and **P4**) were off-white colour solid.

^{1}H NMR (500 MHz, DMSO-d_6, δ): 8.30 (1H, =CH-N), 5.90-3.10 (12H, Mal), 4.50 (2H, -CH_2-), 1.90-1.10 (3H x (n + m), (-CH_2-CH-)$_n$-*b*-(-CH_2-CH-)$_m$), 0.83 (6H, CH_3- of IBVE). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 143.81, 123.09, 100.08, 87.06, 78.97, 77.84, 76.49, 73.42, 73.13, 72.32, 71.49, 69.75, 69.55, 69.01, 63.29, 60.67, 60.15, 59.31, 27.88, 19.05.

Table 3.1: Reaction conditions of synthesizing MA-PIBVE-*b*-P(VE-Mal).

MA-PIBVE-*b*-PVEEP (mg; mmol)	Mal-N_3 (mg; mmol)	AsANa (mg; mmol)	$CuSO_4$ (mg; mmol)	TBTA (mg; mmol)	Product
P1 (257; 0.1) DP_n of VEEP = 10	37; 1.0	40; 0.2	20; 0.1	53; 0.1	**P3**
P2 (244; 0.1) DP_n of VEEP = 30	1,050; 2.90	113; 0.57	72; 0.29	152; 0.29	**P4**

III.2.2.3 Preparation of Mal-decorated polymer particles



Scheme 3.4: Preparation of Mal-decorated polymer particles by dispersion copolymerization.

The obtained block macromonomers MA-PIBVE-*b*-P(VE-Mal), styrene, and VA-044 were dissolved in mixture solvent (10 mL, EtOH/H_2O = 4/1, v/v) in a glass tube (Table 3.2). The solution was degassed by several freeze-thaw cycles, then the glass tube was sealed off and was shaken (150 shakes per minute) at 50 °C for 5 h. The products were purified by dialysis (MWCO 14,000) against deionized water and freeze-dried to give polymer particles (**P3-Particle** and **P4-Particle**). The morphologies of the resultant polymer particles were examined by SEM analysis (Scheme 3.4).

Table 3.2: Conditions of synthesizing polymer particles.

MA-PIBVE-*b*-P(VE-Mal) (mg; μmol)	VA-044 (mg; μmol)	Styrene (mg; μmol)	Product
P3 (41; 5.0)	16.2; 50	518; 5,000	**P3-Particle**
P4 (94; 5.0)	16.2; 50	518; 5,000	**P4-Particle**

III.2.2.4 Lectin binding assay and estimation of maltose density

a. Lectin binding assay

The protein (Con A or BSA; 0.1 mL, 300 μM) was added to the polymer particle suspension (0.1 g L^{-1}) in the buffer solution (4.0 mL, 0.1 M Tris-HCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 10 mM NaCl, pH 7.5). The transmittance of the supernatant was monitored at 550 nm after 2 h.

b. Determination of maltose density on particle surface

Polymer particles (10 mg) were heated at 80 °C under acid condition (2.5 M HCl aq., 5 mL) for 3 h. The resultant solution was neutralized by sodium carbonate. The supernatant was collected, and anthrone solution was added (200 mg anthrone and 100 mL H_2SO_4). The mixture was heated at 80 °C for 10 min and then cooled rapidly. The absorbance at 630 nm was measured by UV-Vis spectrometer. The standard curve was made by using glucose solution.

III.3 Results and discussion

III.3.1 Synthesis of MA-PIBVE-*b*-PVEEP

The precursor block macromonomer (MA-PIBVE-*b*-PVEEP) possessing a methacryloyl group at *α*-terminus, and pendant alkynyl functions, was synthesized by living cationic sequential block copolymerization of IBVE and VEEP using the VEM-HCl adduct / ZnI_2 initiating system[4]. ($[IBVE]_0$ / $[VEEP]_0$ / $[VEM\text{-}HCl]_0$ / $[ZnI_2]_0$ = 30 / 30 / 1 / 0.1, $[IBVE]_0$ and $[VEEP]_0$ = 600 mmol L^{-1}). After quenching the polymerization, the product was purified by preparative SEC to remove the unreacted IBVE and VEEP. The product was soluble in common organic solvents. Table 3.3 shows the results of SEC and ^1H NMR analysis for the isolated block copolymers. As shown in Figure 3.1(a), the analytical SEC data of the isolated block macromonomer depicts a unimodal MWD curve without any shoulders, as well as narrow molecular weight distribution (M_w/M_n < 1.63) compared to those obtained by conventional cationic polymerization. These results indicate that neither the methacryloyl moiety at the *α*-end, nor the pendant alkynes, inhibit living cationic polymerization. The formation of the target block macromonomer was confirmed by ^1H NMR measurement in $CDCl_3$ (Figure 3.2(a)). All the key signals arising from the methacryloyl group at the *α*-terminus (5.55 and 6.12 ppm) and IBVE repeating units (0.90, 3.10-3.30 ppm) and VEEP repeating units (2.50, 3.50-3.67 and 4.19 ppm) were consistent with those of the expected structure for the MA-PIBVE-*b*-PVEEP. The number-average degree of polymerization (DP_n) of MA-PIBVE-*b*-PVEEP was determined by the peak intensity ratio of the isobutyl protons (peak c) and the alkynyl protons (peak a), based on the terminal vinyl protons (peak e) for IBVE and VEEP segments, respectively. Furthermore, the ^{13}C NMR spectrum in Figure 3.1(b) clearly showed the carbon peaks that were assignable to the pendant alkynyl groups (79.57 and 80.68 ppm) and dimethyl groups of IBVE (28.78 and 19.44 ppm), respectively. However, the vinyl and carbonyl

carbons of the terminal methacryloyl moiety were difficult to observe probably due to their much lower concentrations compared to the pendant alkynyl and methyl carbons. In addition, as for the carbonyl carbon, no appearance in the spectra might also be caused by its inherent lower intensity than other carbons, in ^{13}C NMR spectroscopy. These results indicate that block macromonomer MA-PIBVE-*b*-PVEEP, having both a terminal methacryloyl group and an alkynyl groups-substituted block copolymer backbone, was precisely synthesized by living cationic polymerization.

Table 3.3: Synthesis of MA-PIBVE-*b*-P(VE-Mal).

Run	Precursor Macromonomer					CuAAC click reaction Product				
		M_n[b]	M_w/M_n[a]	DP_n of PIBVE[b]	DP_n of PVEEP[b]		M_n[b]	M_w/M_n[c]	Yield (%)[d]	Degree of substitution (%)[b]
1	**P1**	4,400	1.63	25	10	**P3**	8,100	1.43	20	Quant.
2	**P2**	7,800	1.60	25	30	**P4**	18,800	2.13	15	Quant.

a) Estimated by polystyrene-calibrated SEC. b) Determined by ^1H NMR spectrum. c) Estimated by poly(ethylene glycol)-calibrated SEC. d) Isolated yield.

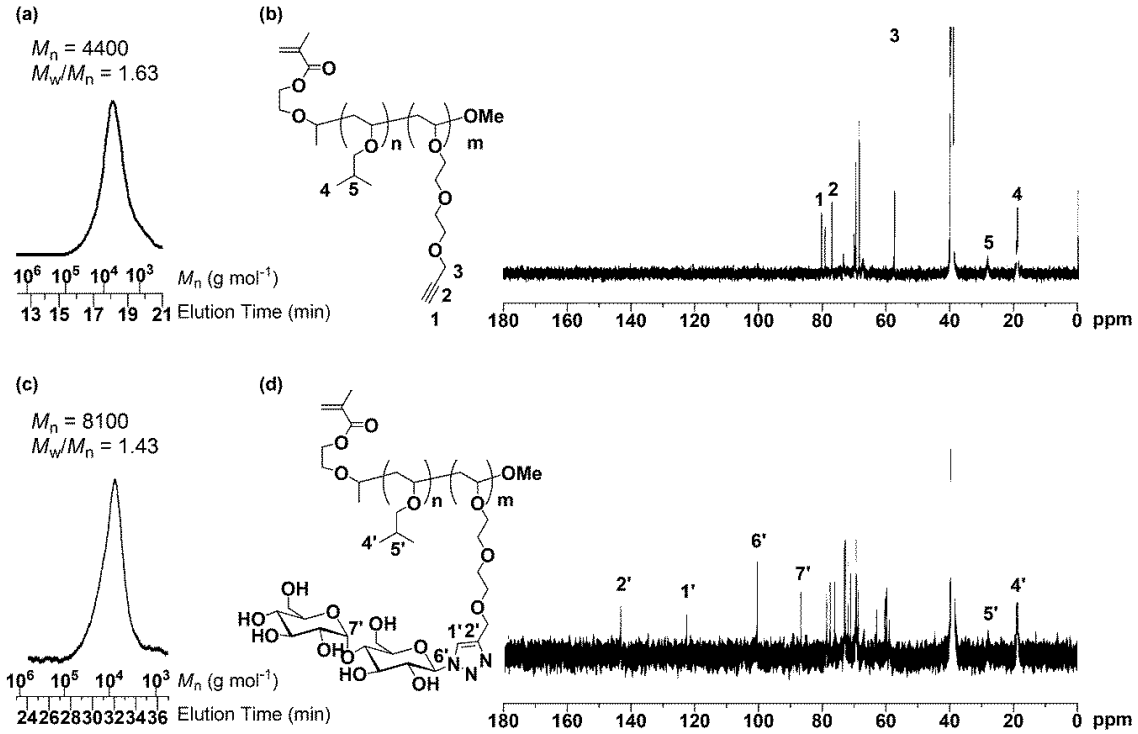


Figure 3.1: (a) SEC curve of MA-PIBVE$_{25}$-*b*-PVEEP$_{10}$ using THF as the eluent. (b) ^{13}C NMR spectrum of MA-PIBVE$_{25}$-*b*-PVEEP$_{10}$ macromonomer in DMSO-*d*$_6$. (c) SEC curve of MA-PIBVE$_{25}$-*b*-P(VE-Mal)$_{10}$ using 0.2 mol L^{-1} $NaNO_3$ aq. as the eluent. (d) ^{13}C NMR spectrum of MA-PIBVE$_{25}$-*b*-P(VE-Mal)$_{10}$ macromonomer in DMSO-*d*$_6$.

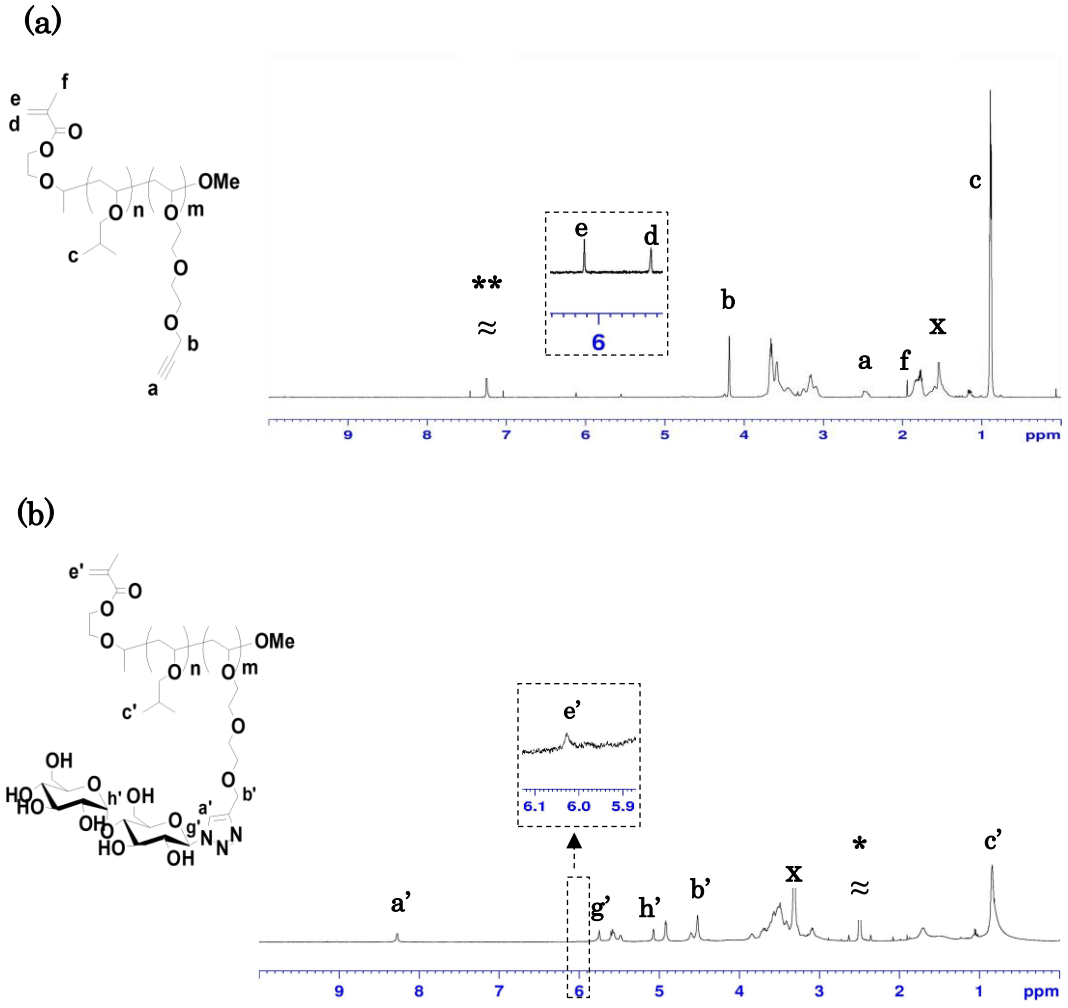


Figure 3.2: ^{1}H NMR spectra of (a) MA-PIBVE$_{25}$-*b*-PVEEP$_{10}$ in $CDCl_3$ and (b) MA-PIBVE$_{25}$-*b*-P(VE-Mal)$_{10}$ in DMSO-d_6 (*; **; and x: remaining solvents).

III.3.2 Synthesis of MA-PIBVE-*b*-P(VE-Mal)

The author investigated the conversion of the pendant alkynyl groups of MA-PIBVE-*b*-PVEEP to carbohydrate residues by CuAAC click reaction, which was carried out in THF/H_2O (1/1, v/v) at 30 °C under dry nitrogen atmosphere for 92 h in the presence of $CuSO_4$ and sodium ascorbate as a catalyst and a reductant, respectively (Table 3.3). In this study, a small excess amount of Mal-N_3 was reacted with the pendant alkynyl moieties ([VEEP*]$_0$ / [Mal-N_3]$_0$ / [$CuSO_4$]$_0$ / [sodium ascorbate]$_0$ / [TBTA] = 1 / 1.01 / 0.1 / 0.2 / 0.1) ([VEEP*] shows the calculated molar concentration of the

VEEP repeating units). After the reaction mixture was washed with toluene to remove the TBTA, the product was purified by reprecipitation and dialysis to remove the unreacted Mal-N_3 and traces of the copper catalyst. The isolated yields were relatively low, probably due to loss in the purification processes, because the author thoroughly purified the product by combining reprecipitation and dialysis over a couple of weeks, because the high purity of the macromonomer was indispensable for the success of the post-reactions. Consequently, the SEC trace in Figure 3.1(c) indicates the successful purification of the maltose-substituted glycopolymer-type macromonomer. The occurrence of CuAAC click reaction with Mal-N_3 led to the drastic change in solubility characteristics of the macromonomers. As a result, the obtained maltose-appended macromonomers (**P3** and **P4**) were readily soluble in a mixture of EtOH and water, while the precursor macromonomers (MA-PIBVE-*b*-PVEEP, **P1** and **P2**) were sparingly soluble in EtOH and insoluble in water. The isolated polymer was subjected to ^{1}H NMR analysis. As shown in Figure 3.2(b), multiple signals at 5.90-3.10 ppm, a sharp signal at 0.83 ppm, and broadened signals at 1.90-1.10 ppm were observed, which were assignable to the pendant maltose moieties, the dimethyl group of the IBVE segment, and the polyVE main chain moiety, respectively. In addition, strong key signals appeared at 8.30 ppm and 4.50 ppm. These are ascribable to the linkage triazole proton (peak a') and methylene protons adjacent to the triazole moiety (peak b'), respectively. Based on the peak intensity ratio of triazole proton to the methylene protons adjacent to the residual alkynyl group (4.20 ppm) after click reaction, more than 95% of the pendant alkynes were converted to the triazole units, indicating that the click reaction of the pendant alkynes of MA-PIBVE-*b*-PVEEP and maltosyl azide nearly quantitatively proceeded. The proton concentrations of the terminal methacryloyl moiety is quite low, however, the ^{1}H NMR spectrum showed a signal arising from a vinyl proton of the terminal methacryloyl group at around 6.0 ppm (peak e') (Figure 3.2(b),

inset). These results confirm that glycopolymer-type amphiphilic macromonomers MA-PIBVE-*b*-P(VE-Mal) having both a terminal methacryloyl group and pendant maltose moieties was precisely synthesized by a combination of living cationic polymerization and CuAAC click reaction.

III.3.3 Preparation of Mal-decorated polymer particles

To prepare Mal-decorated polymer particles, the obtained glycopolymer-type amphiphilic macromonomers [MA-PIBVE-*b*-P(VE-Mal)] were subjected to dispersion copolymerization with styrene. In this study, two kinds of MA-PIBVE-*b*-P(VE-Mal) having different DP_n values of P(VE-Mal) segment (**P3**; DP_n = 10 and **P4**; DP_n = 30, respectively) were synthesized and employed. Dispersion polymerization was carried out in EtOH/water (4/1, v/v) with VA-044 as the initiator at 50 °C, and over 150 shakes per minute for 5 h. After dialysis of the reaction mixture for removal of the unreacted amphiphilic macromonomer, followed by lyophilization, the polymerization product was obtained as a white powder (Table 3.4). SEM of an air-dried sample from the suspensions in EtOH indicated the presence of submicron-sized uniform and spherical polymer particles (Figure 3.3). It should be noted that the size of the polymer particles was dependent on the DP_n of the P(VE-Mal) segment of the macromonomer: the higher the DP_n of the P(VE-Mal) segment, the larger the particle diameter. This result indicates that the size of the polymer particles can be controlled by varying the DP_n of the glycopolymer-type macromonomer. The glycopolymer-based amphiphilic macromonomer with a hydrophilic P(VE-Mal) segment, and hydrophobic PIBVE segment thus effectively worked as a surfactant, allowing the preparation of styrene-based core-shell polymer particles by dispersion polymerization in aqueous solvent. Furthermore, stable dispersions of the Mal-decorated polymer particles can be obtained, not only in alcoholic solvents such as a mixture EtOH and water but also in buffer solutions.

Table 3.4: Preparation of Mal-decorated polymer particles.

Particle	Yield (%)[a)]	D_n (nm)[b)]	PDI[b)]
P3-Particle	55	460 ± 80	1.19
P4-Particle	55	550 ± 110	1.24

a) Isolated yield. b) Determined by SEM analysis (Average of 100 particles).

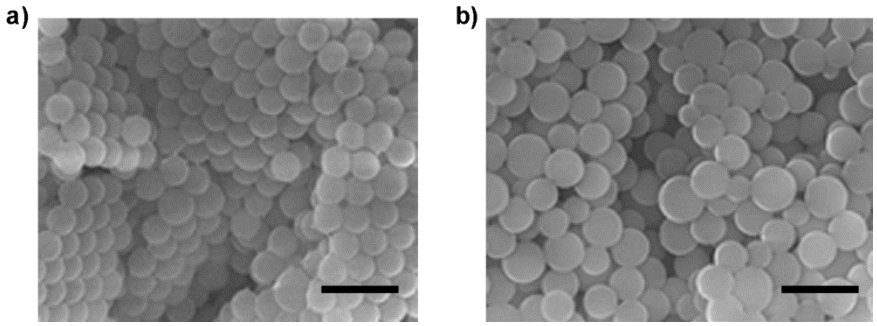


Figure 3.3: SEM images of the Mal-decorated polymer particles obtained by dispersion copolymerization employing (a) P3 and (b) P4 of MA-PIBVE-*b*-P(VE-Mal). Bars: 1 μm.

III.3.4 Lectin binding assay and estimation of maltose density

It has been reported that lectin Con A specifically interacts with the α-glucoside residue of a maltose moiety. Thus, the author investigated the interaction of the Mal-decorated particles with Con A. Some differences in the results of lectin binding assay were observed dependent of the DP_n of the P(VE-Mal) segment of the macromonomer employed. As for **P3**, no significant differences in transmittance were observed for three aqueous suspensions (control, with Con A, and with BSA) (Figure 3.4(a)), indicating Mal-decorated polymer particles prepared from the glycopolymer-type macromonomer **P3** with a shorter P(VE-Mal) segment did not strongly interact with Con A. In sharp contrast, the transmittance of the aqueous suspension of the polymer particle prepared from **P4** with a longer P(VE-Mal) segment increased remarkably upon addition of Con A, this was due to the precipitation of the lectin-particle conjugates (Figure 3.4(b)). Compared to the result of the addition of Con A, polymer particles prepared with **P4** showed no increase in transmittance upon addition of BSA as a control protein. These results support that the particles prepared using **P4** more strongly bound to Con A than those prepared with **P3**. The strength of the interaction of the polymer particles with Con A is presumably dependent on the carbohydrate density on the particle surfaces. Then, the density of maltose moieties on the particle surfaces was quantitatively evaluated by measuring the amount of the liberated glucose moieties that are released from the polymer particle surfaces by hydrolysis under acidic conditions. This assay showed that the surface densities of maltose moieties on the particles prepared using **P4** and **P3** was 3.30 and 1.83 μg cm^{-2}, respectively (Table 3.5 (a) and (b)). These results suggest that the higher density of the maltose residues on the particle surfaces brought about the higher extent in the interaction with Con A (glycocluster effect)[11]. Here, it should be emphasized that the saccharide density of the polymer particle surfaces can

be regulated by the hydrophilic / hydrophobic balance and the DP_n of the block copolymer-type glycostabilizer.

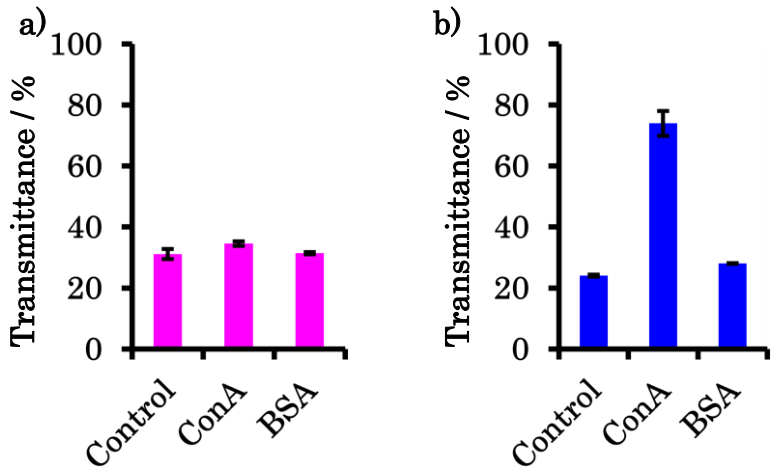


Figure 3.4: Transmittance of Mal-decorated polymer particle suspensions prepared using (a) P3 and (b) P4 after adding lectins for 2 h. Control: polymer particle suspension before the addition of Con A and BSA.

Table 3. Determination of maltose amounts on the Mal-decorated polymer particle surfaces.

Particle	Absorb. at 630 nm [a)]	Conc. of free glucose (μg mL^{-1})	Amount of free glucose (mg per 8.1 mg of particle)	Amount of maltose (mg per 8.1 mg of particle)	D_n (nm) [b)]	Surface area of the polymer particle (cm^2 x10^{-10})	Surface density of maltose moieties on the particles (μg cm^{-2})
P3-Particle	0.328	23.8	0.298	0.595	460	16.6	1.83
P4-Particle	0.419	35.6	0.447	0.894	550	24.1	3.30

a) Estimated by polystyrene-calibrated SEC. b) Determined by SEM analysis (Average of 100 particles).

III.4 Conclusions

The author has succeeded in the synthesis of new glycopolymer-type macromonomers [MA-PIBVE-*b*-P(VE-Mal)], consisting of amphiphilic block copolymer backbone bearing maltose moieties and a vinyl group at the *α*-terminus, by a combination of living cationic sequential block copolymerization of IBVE and VEEP, and a CuAAC click reaction with maltosyl azide without any protecting / deprotecting processes. The author has also demonstrated the preparation of nearly monodispersed Mal-decorated polymer particles by dispersion copolymerization with styrene, using the glycopolymer-type macromonomers as steric stabilizers. The author further confirmed the successful formation of the Mal-decorated polymer particles and their capability of specific binding to Con A by the lectin binding assay employing BSA as a control protein. Glycopolymer-type macromonomers with controlled architecture are expected to make a notable contribution in the development of carbohydrate-based functional polymeric materials.

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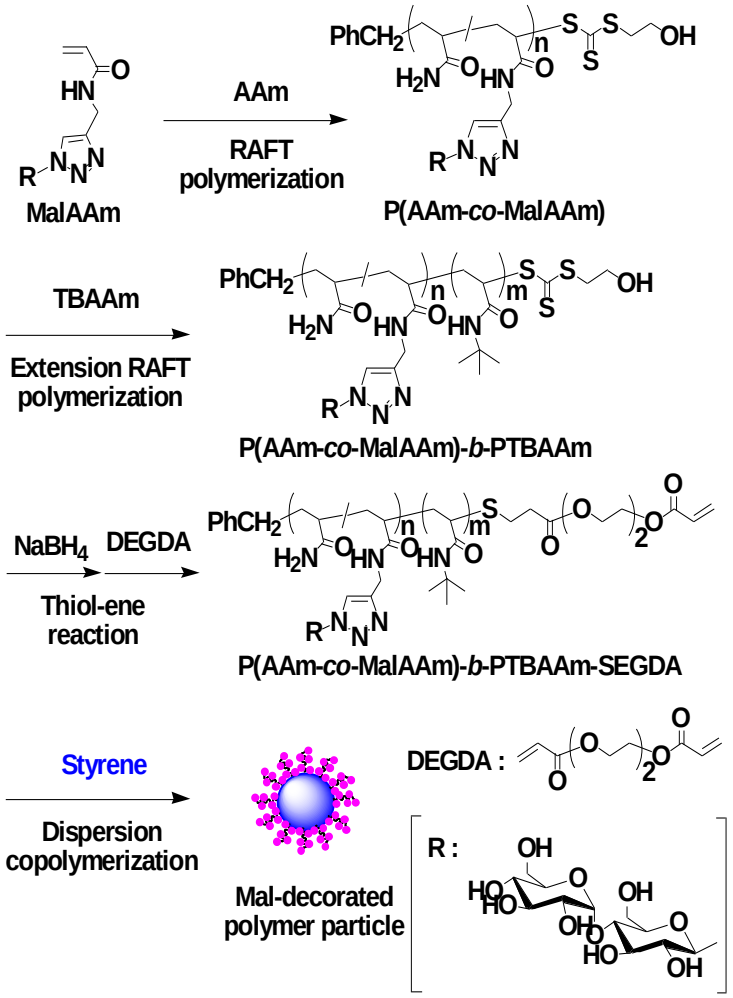
Chapter IV

Controlled synthesis of polyacrylamide-based amphiphilic glycopolymer-type macromonomers and their use for the preparation of carbohydrate-decorated polymer particles

IV.1 Introduction

Carbohydrates immobilized on particle surfaces are significant interest because their interactions with proteins such as lectins, and with viruses, are important for many recognition applications[1]. Spherical polymer particles covered with a hydrophilic shell are typically generated by dispersion polymerization using a polymeric surfactant as a steric stabilizer. Amphiphilic macromonomers with a polymerizable group at the ω-terminus are utilized as steric stabilizers and fixed on the surfaces of the polymer particles during dispersion polymerization[2]. In the author's group, core-shell particles were previously prepared using a well-defined poly(vinyl ether)-based macromonomer as a steric stabilizer[3]. In contrast, there is little information available on the use of glycopolymer-type stabilizers (*glycostabilizers*) for the formation of core-shell polymer particles[4] and thus synthesis of macromonomer-type glycostabilizers is required. The author's group recently developed a protecting-group-free synthetic protocol for glycopolymers with a vinyl group from free saccharides via direct anomeric azidation using 2-chloro-1,3-dimethylimidazolinium chloride (DMC); called "Shoda activation"[5], followed by a copper-catalyzed azide-alkyne cycloaddition reaction[6] and RAFT polymerization[7]. This simple synthetic method for the glycopolymers was used to synthesize block copolymers composed of polyacrylamide (PAAm) bearing saccharide moieties and

poly(*N*-isopropyl acrylamide) via consecutive RAFT polymerizations[8]. An amphiphilic block copolymer bearing saccharides would have two functions: (a) as a steric stabilizer for particle formation, even at low concentration, and (b) as a moiety for binding lectins to the polymer particles. In this study, the author synthesized novel amphiphilic glycopolymer-type macromonomers with a vinyl group at the ω-terminus. The macromonomers, composed of PAAm bearing saccharide moieties and PTBAAm, were synthesized by consecutive RAFT polymerizations and subsequent thiol-ene reaction. Carbohydrate-decorated polymer particles were then prepared by dispersion copolymerization with styrene using the glycopolymer-type macromonomers, and their lectin-binding properties were evaluated (Scheme 4.1).



Scheme 4.1: Synthesis of Mal-decorated polymer particles.

IV.2 Experimental sections

IV.2.1 Materials and measurements

IV.2.1.1 Materials

Propargylamine, acryloyl chloride, acrylamide (AAm), 2,2'-azobis-[2-(2-imidazolin-2-yl)-propane] dihydrochlroride (VA-044), triethylamine (Et_3N), AIBN and diethylene glycol diacrylate (DEGDA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). AAm was used after recrystallization from chloroform/methanol = 10/3. *N-tert*-Butylacryamide (TBAAm) and sodium borohydride ($NaBH_4$) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). TBAAm was used after recrystallization from ethanol. 2-(Benzylsulfanylthiocarbonylsulfanyl)-ethanol (BTSE) was synthesized using 2-mercaptoethanol, carbon disulfide, and benzyl bromide[9]. Styrene was purchased from Wako Pure Chemical Industries (Osaka, Japan) and used after distillation over CaH_2 under reduced pressure. Concanavalin A (Con A) from *Canavalia Ensiformis* and bovine serum albumin (BSA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were commercially available and used without further purification.

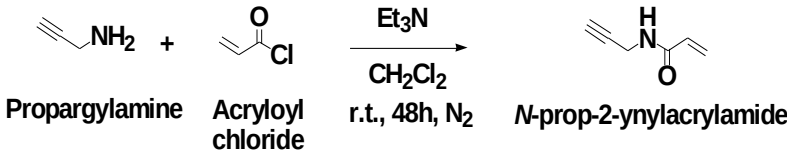
IV.2.1.2 Measurements

^1H and ^{13}C NMR spectra were recorded at 25 °C on a Bruker model AC-500 spectrometer, operating at 500 MHz. GPC measurements were conducted using a system consisting of a JASCO PU2089 pump, a CO-2065 column oven, an RI-2031 refractive index detector using three TOSOH TSKgel α-3000 columns (7.8 mm x 300 mm) and 0.2 M $NaNO_3$ aqueous solution was used as an eluent at a flow rate of 0.8 mL min^{-1} at 40 °C. Poly(ethylene glycol) samples were used as standards. UV-Vis absorption spectra were recorded using a JASCO UV-2550 spectrometer. Scanning

electron microscope (SEM) images were observed using a JEOL JSM-7600F. DLS measurements were conducted in water using an Otsuka Electronics ELSZ-1000 with 667 nm at room temperature.

IV.2.2 Experimental sections

IV.2.2.1 Synthesis of *N*-prop-2-ynylacrylamide

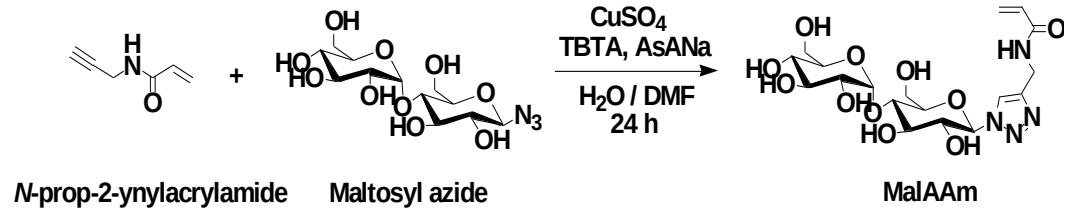


Scheme 4.2: Synthesis of *N*-prop-2-ynylacrylamide.

According to the procedure[10], acryloyl chloride (4.89 g, 54 mmol) was tardily added into a solution of propargylamine (2.98 g, 54 mmol) and Et_3N (10.92 g, 108 mmol) in 70 mL of CH_2Cl_2, at room temperature (Scheme 4.2). The reaction was equipped with a paddle stirrer. After 48 h, the mixture was obtained as an orange heterogeneous mixture, evaporated off CH_2Cl_2 and Et_3N, then extracted with 0.01 N HCl solution (100 mL) and $CHCl_3$ (100 mL). The pure *N*-prop-2-ynylacrylamide was purified by column chromatography using mixed solvents (hexane/ethyl acetate = 6.5/3.5, v/v; R_f = 0.26) as a white solid (3.76 g, yield: 67%).

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 6.36 (d, 1 H, CH_2=), 6.15 (dd, 1 H, -CH=), 5.73 (br, 1 H, -NH-), 5.71 (d, 1 H, CH_2=), 4.18 (dd, 2 H, -CH_2-), 2.28 (t, 1 H, CH≡). ^{13}C NMR (500 Hz, in $CDCl_3$); δ (ppm) 165.5, 130.52, 127.83, 79.71, 72.31, 29.77.

IV.2.2.2 Synthesis of MalAAm

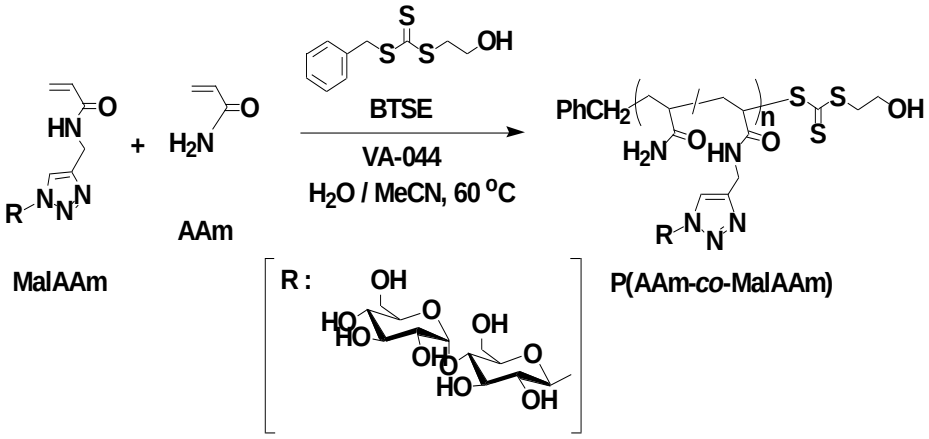


Scheme 4.3: Synthesis of MalAAm.

According to the procedure[11], the *N*-prop-2-ynylacrylamide (67 mg, 0.65 mmol) and Mal-N_3 (236 mg, 0.65 mmol) were added in 10 ml a mixture (DMF : H_2O = 1 : 1). Then, AsANa (25.6 mg, 0.13 mmol) was added, followed by $CuSO_4.5H_2O$ (16.1 mg, 0.065 mmol) and TBTA (34.1 mg, 0.065 mmol) (Scheme 4.3). The heterogeneous mixture was vigorously stirred for 24 h under N_2 gas at room temperature, at which point it cleared and TLC analysis indicated complete consumption of the reactants. After concentration of the reaction mixture under reduced pressure, the reaction mixture was extracted with toluene and purified by silica gel column chromatography (H_2O/CH_3CN = 1/6, v/v), and then stirring with metal scavenger (SiliaMetS® Imidazole, 81 mg, 5 equiv. for Cu) overnight at room temperature. The obtained MalAAm monomer was a white solid (148 mg, yield: 49%).

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.64 (1H, s, -NH-), 8.12 (1H, s, N-CH=), 6.28 (1H, dd, -CH=), 6.12 and 5.61 (2H, d, CH_2=), 5.76 (1H, d), 5.06 (1H, d), 4.95 (2H, t, CH_2-), 4.60 (1H, s), 4.54 (1H, d), 4.39 (2H, q, -CH_2-NH). ^{13}C NMR (500 Hz, DMSO-d_6, δ ppm): 164.45, 144.52, 131.42, 125.40, 121.97, 100.85, 87.05, 79.05, 77.90, 76.60, 73.48, 73.16, 72.35, 71.48, 69.79, 60.68, 60.19, 34.02.

IV.2.2.3 Synthesis of P(AAm-*co*-MalAAm)



Scheme 4.4: Synthesis of P(AAm-*co*-MalAAm).

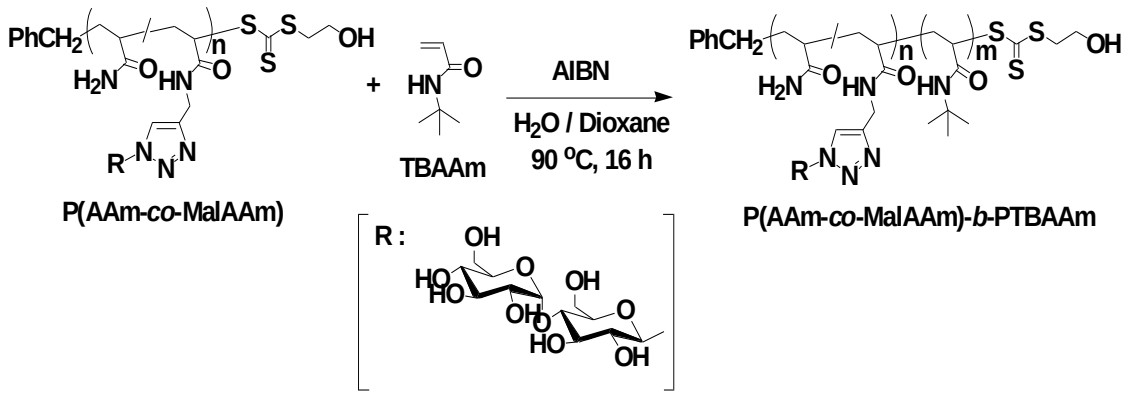
MalAAm, AAm, VA-044, and BTSE were dissolved in mixture solvent (1.0 mL, H_2O/CH_3CN = 4/3, v/v) in a small round-bottomed flask (Table 4.1). The solution was degassed by several freeze-thaw cycles, then the glass tube was sealed under vacuum and heated at 60 °C (Scheme 4.4). The products were purified by dialysis (MWCO 2,000) against deionized water and freeze-dried to give P(AAm-*co*-MalAAm).

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.13 (1H, =CH-N), 7.40-7.25 (5H, Ph), 5.75 (1H, H-1 of Mal) 5.46 (1H, H-1' of Mal), 4.72-4.47 (2H, C*H*$_2$-NH), 4.03-3.44 (12H, Mal), 2.21-1.61 (3H × n, $(-CH_2-CH-)_n$).

Table 4.1: Reaction conditions of synthesizing P(AAm-*co*-MalAAm).

Run	AAm (mg; mmol)	MalAAm (mg; mmol)	BTSE (mg; µmol)	VA-044 (mg; µmol)	[AAm]:[MalAAm] : [VA-044]:[BTSE]	Reaction time (h)	Product
1	26.5; 0.372	177; 0.372	1.9; 7.44	2.5; 7.44	50 : 50 : 1 : 1	6	**P5**
2	53.0; 0.736	350; 0.736	3.6; 14.72	4.8; 14.72	50 : 50 : 1 : 1	24	**P6**

IV.2.2.4 Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm



Scheme 4.5: Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm.

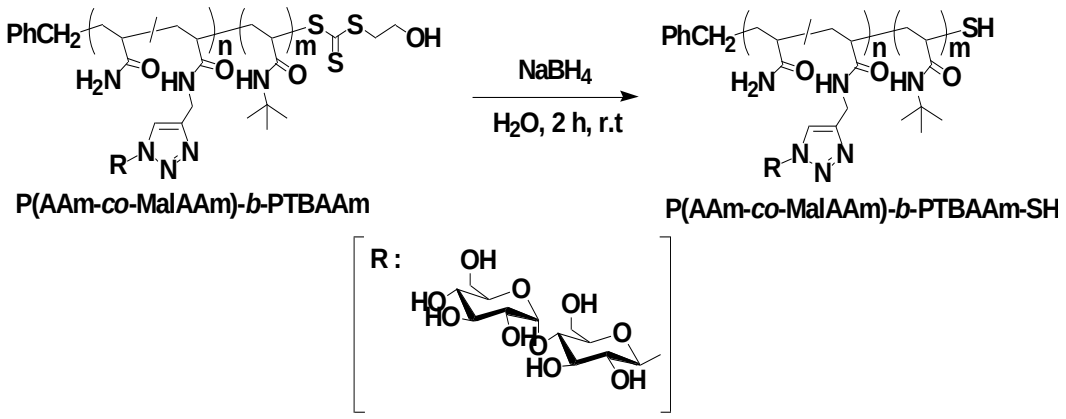
P(AAm-*co*-MalAAm), TBAAm, and AIBN were dissolved in mixture solvent (10 mL, H_2O/1,4-dioxane = 1/1, v/v) in a small round-bottomed flask (Scheme 4.5). The solution was degassed by several freeze-thaw cycles, then the glass tube was sealed under vacuum and heated at 70 °C under nitrogen for 16 h (Table 4.2). The products were purified by extraction with $CHCl_3$, precipitation in hexane, and then dialysis in deionized water (MWCO 2,000) and freeze-dried to give P(AAm-*co*-MalAAm)-*b*-PTBAAm.

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.13 (1H, =CH-N), 7.40-7.25 (5H, Ph), 5.56 (1H, H-1 of Mal), 5.09 (1H, H-1' of Mal), 3.95 (2H, C*H*$_2$-NH), 3.80-3.31 (12H, Mal), 2.21-1.31 (3H × (n+m), (-CH_2-CH-)$_n$-*b*-(-CH_2-CH-)$_m$), 1.20 (9H, CH_3 of TBAAm).

Table 4.2: Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm.

Run	MacroCTA (mg, μmol)	TBAAm (mg, μmol)	[MacroCTA]/ [AIBN]	[TBAAm]/ [MacroCTA]	Product
1	**P5** (70; 6.39)	16.3; 128	3.3	20	**P7**
2	**P6** (100; 7.29)	37.2; 292	3.3	40	**P8**

IV.2.2.5 Synthesis of thiol-terminated P(AAm-*co*-MalAAm)-*b*-PTBAAm



Scheme 4.6: Synthesis of thiol-terminated P(AAm-*co*-MalAAm)-*b*-PTBAAm.

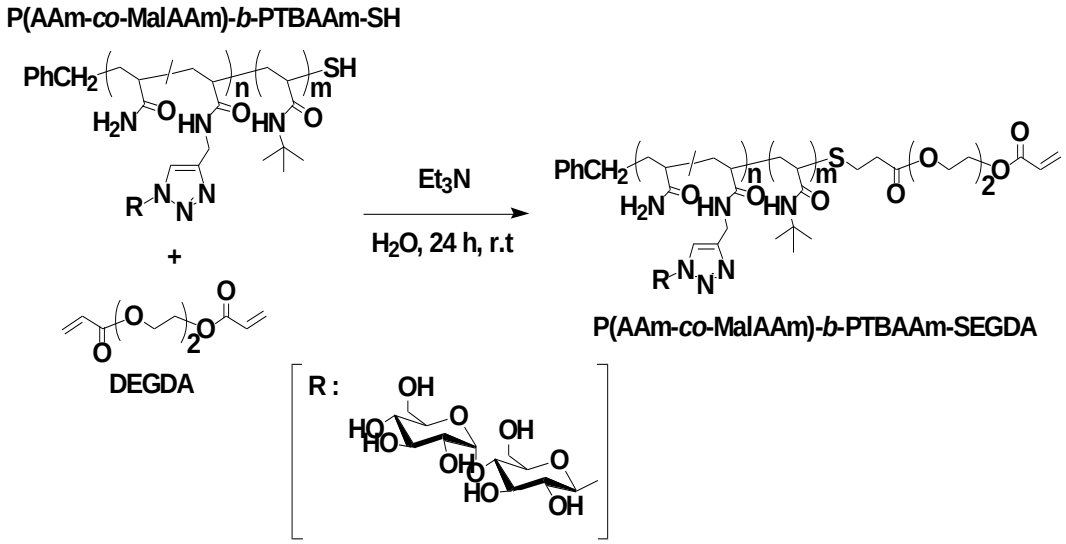
A mixture of block copolymer (**P7** and **P8**) and $NaBH_4$ in 20 mL of water was stirred at room temperature for 2 h (Scheme 4.6). The products were purified by dialysis (MWCO 2,000) against deionized water and freeze-dried to give thiol-terminated P(AAm-*co*-MalAAm)-*b*-PTBAAm, **P7-SH** and **P8-SH** (Table 4.3).

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.05 (1H, =CH-N), 7.24-7.11 (5H, Ph), 5.56 (1H, H-1 of Mal), 5.15 (1H, H-1' of Mal), 3.85 (2H, C$\underline{H_2}$-NH), 3.71-3.11 (12H, Mal), 2.21-1.21 (3H × (n+m), (-CH_2-CH-)$_n$-*b*-(-CH_2-CH-)$_m$), 1.20 (9H, CH_3 of TBAAm).

Table 4.3: Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm-SH.

Run	P(AAm-*co*-MalAAm)-*b*-PTBAAm (mg; μmol)	$NaBH_4$ (mg; μmol)	Product
1	**P7** (62.8; 10.9)	4.2; 109	**P7-SH**
2	**P8** (100; 6.19)	2.34; 61.9	**P8-SH**

IV.2.2.6 Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm-SEGDA

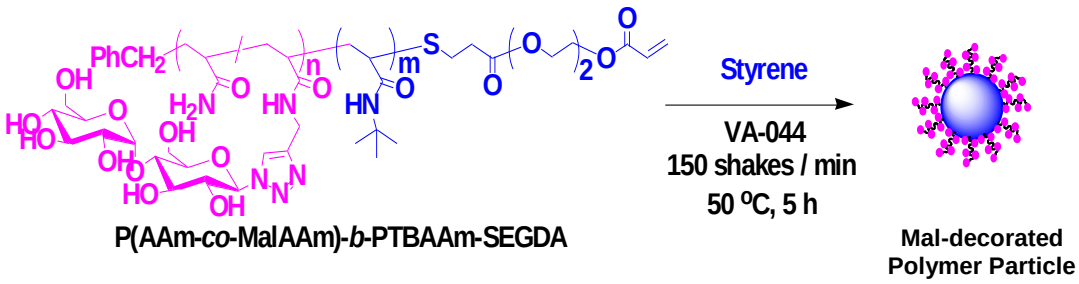


Scheme 4.7: Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm-SEGDA.

A mixture of thiol-terminated block copolymer, DEGDA, and Et_3N in water (10.0 mL) was stirred at room temperature for 24 h (Scheme 4.7). The products were purified by precipitation in MeOH and dialysis (MWCO 2,000) against deionized water and freeze-dried to give **P9** and **P10** (Table 4.6).

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 8.32 (1H, =CH-N), 7.23-7.12 (5H, Ph), 6.33 and 5.9 (3H, vinyl), 5.56 (1H, H-1 of Mal), 5.12 (1H, H-1' of Mal), 3.86 (2H, CH_2-NH), 3.70-3.09 (12H, Mal), 2.21-1.21 (3H × (n+m), (-CH_2-CH-)$_n$-*b*-(-CH_2-CH-)$_m$), 1.20 (9H, CH_3 of TBAAm).

IV.2.2.7 Preparation of Mal-decorated polymer particles



Scheme 4.8: Synthesis of Mal-decorated polymer particles.

P(AAm-*co*-MalAAm)-*b*-PTBAAm-SEGDA, styrene, and VA-044 were dissolved in mixture solvent (10 mL, EtOH/H_2O = 4/1, v/v) in a glass tube (Table 4.4). The solution was degassed by several freeze-thaw cycles, then the glass tube was sealed off and was shaken (150 shakes per minute) at 50 °C for 5h (Scheme 4.8). The products were purified by dialysis (MWCO 14,000) against deionized water and freeze-dried to give polymer particles.

Table 4.4: Conditions of synthesizing polymer particles.

Run	Macromonomer (mg; µmol)	Styrene (mg; µmol)	VA-044 (mg; µmol)	Product
1	**P9** (31.1; 2.5)	518; 5000	16.2; 50	**P9-Particle**
2	**P10** (40.3; 2.5)	518; 5000	16.2; 50	**P10-Particle**

IV.2.2.8 Lectin binding assay and estimation of maltose density on polymer particles

a. Lectin binding assay

The protein (Con A or BSA; 0.2 mL, 300 μM) was added to the obtained polymer particle suspension (0.2 g L^{-1}) in the buffer solution (4.0 mL, 0.1 M Tris-HCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 10 mM NaCl, pH 7.5). The transmittance of the supernatant was monitored at 550 nm.

b. Determination of maltose density on polymer particles

Polymer particles (10 mg) were heated at 80 °C under acid condition (2.5 M HCl aq., 5 mL) for 3 h. The resultant solution was neutralized by sodium carbonate. The supernatant was collected and added anthrone solution (200 mg anthrone and 100 mL H_2SO_4). The mixture was heated at 80 °C for 10 min and then cooled rapidly. The absorbance at 630 nm was measured by UV-Vis spectrometer. The standard curve was made by using free glucose.

IV.3 Results and discussion

IV.3.1 Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm

The synthetic procedure for amphiphilic glycopolymer-type macromonomers bearing maltose moieties is shown in scheme 4.5. Mal-bearing PAAms (P(AAm-*co*-MalAAm), **P7** and **P8**) were synthesized by RAFT polymerization using the Mal-carring acrylamide derivative (MalAAm) and acrylamide (AAm) as monomers, 2-(benzylsulfanylthio-carbonylsulfanyl)ethanol (BTSE) as a chain transfer agent (CTA), and 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) as an initiator. Chain extension reaction by RAFT polymerization was conducted in aqueous 1,4-dioxane using **P5** and **P6** as macro-CTAs and TBAAm as a monomer. The chain extension reaction at different molar ratios of macro-CTA and TBAAm monomer (1/20 and 1/40) provided block copolymers (P(AAm-*co*-MalAAm)-*b*-PTBAAm, **P7** and **P8**) (Table 4.5). The molecular weights of the product block copolymers increased (**P5**→**P7**; 11,000→12,400, **P6**→**P8**; 13,400→16,200) and the polymer dispersity (M_w/M_n) remained low (< 1.33). The chain extension reactions resulted in a clear increase in the methyl proton signals attributed to the TBAAm segment at 1.2 ppm in ^{1}H NMR spectra (Figure 4.1(a) and (b)). Each copolymer exhibited different degrees of polymerization (DP_n) of the TBAAm unit (**P7**; 11, **P8**; 19). Furthermore, GPC curves shows that the amphiphilic copolymer (**P7**) possesses narrow molecular weight distribution after extension RAFT polymerization (Figure 4.2(a) and (b)). The results demonstrate that hydrophobic monomer TBAAm was successfully inserted into the amphiphilic copolymers [P(AAm-*co*-MalAAm)-*b*-PTBAAm] under extension RAFT polymerization.

Table 4.5: Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm.

Feeding molar ratio of P(AAm-*co*-MalAAm)/ TBAAm	Product	Conv. (%)[a]	Yield (%)[b]	M_n[a]	M_w/M_n[c]	DP_n of AAm/MalAAm/ TBAAm in product[a]
1 (**P5**)[d]/20	**P7**	55	55	12,400	1.33	49/15/11
1 (**P6**)[e]/40	**P8**	48	48	16,200	1.24	41/22/19

a) Determined by ^{1}H NMR. b) Isolated yield. c) Determined by GPC.

d) $M_{n(NMR)}$ = 11,000, M_w/M_n = 1.30, DP_n of AAm/MalAAm = 49/15.

e) $M_{n(NMR)}$ = 13,400, M_w/M_n = 1.41, DP_n of AAm/MalAAm = 41/22.

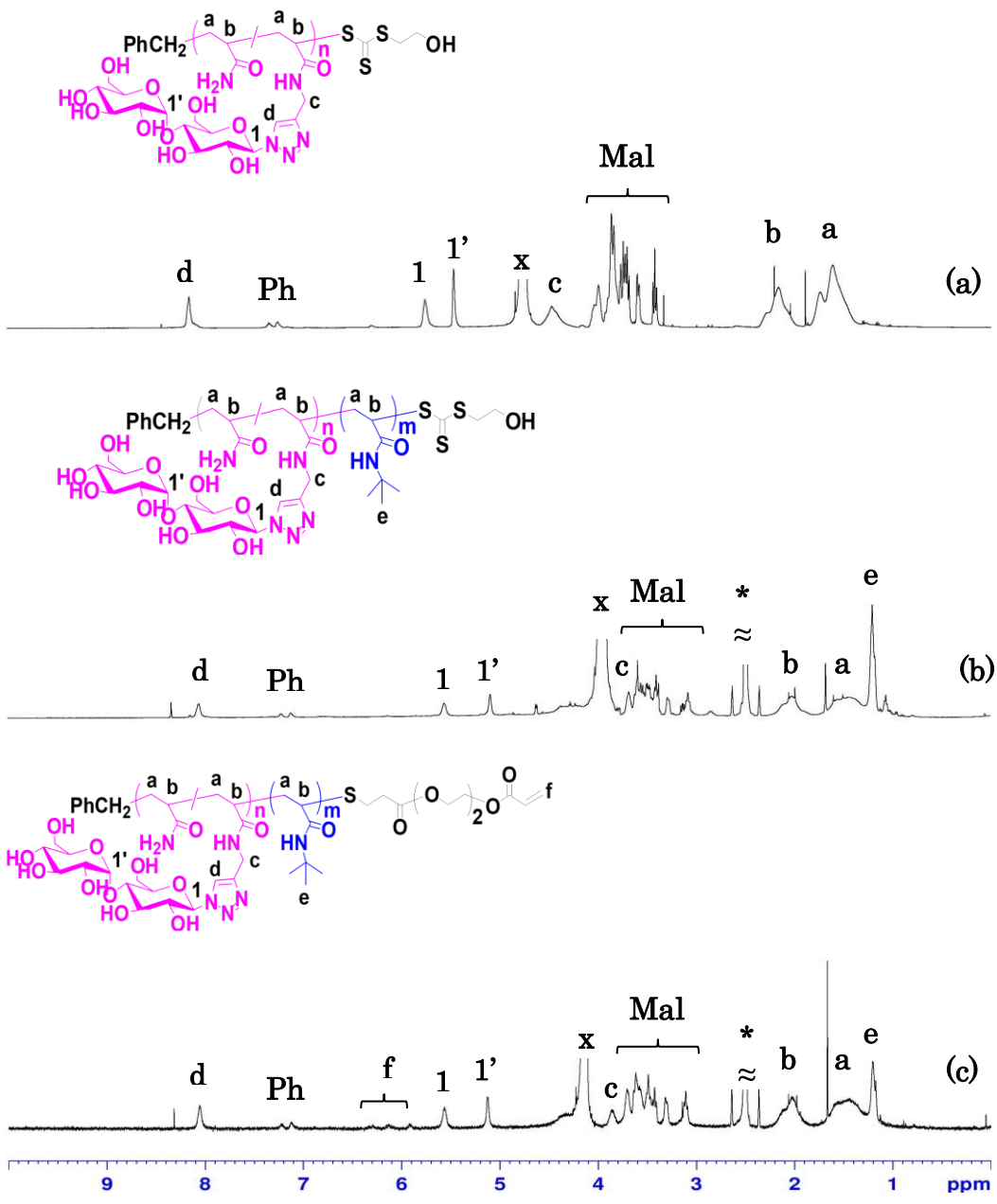


Figure 4.1: ^{1}H NMR spectra of (a) P5 in D_2O, (b) P7 and (c) P9 in DMSO-*d_6* (* and x: remaining solvents).

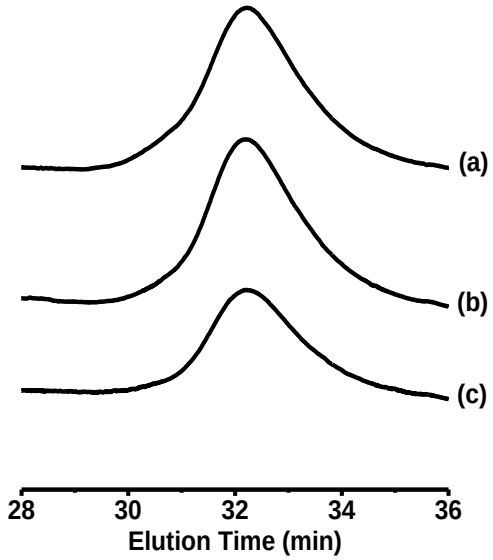


Figure 4.2: GPC analysis of (a) P5, (b) P7, and (c) P9 in 0.2 M $NaNO_3$ aq.

IV.3.2 Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm-SEGDA

The author obtained glycopolymer-type macromonomers by reducing the trithiocarbonate (TC) group to a thiol at the terminus of the copolymer, followed by thiol-ene reaction with diethylene glycol diacrylate (DEGDA). Block copolymers **P7** and **P8** were reacted with $NaBH_4$ in water. After the reduction of TC terminal group to a thiol, thiol-ene reaction[12] between the thiol-terminated block copolymers and DEGDA was then conducted using an excess of DEGDA in the presence of triethylamine (Et_3N) to obtain the amphiphilic glycopolymer-type macromonomers P(AAm-*co*-MalAAm)-*b*-PTBAAm-SEGDA (**P9** and **P10**). The degree of substitution was quantitative (Table 4.6). Vinyl protons were observed at 6.20 and 5.90 ppm by ^1H NMR (Figure 4.1(c)). Furthermore, GPC analysis shows that the amphiphilic copolymer (**P9**) possesses narrow molecular weight distribution after thiol-

ene reaction (Figure 4.2(c)), indicating that the desired glycopolymer-type macromonomers **P9** and **P10** were successfully synthesized via consecutive RAFT polymerizations and subsequent thiol-ene reaction.

Table 4.6: Synthesis of P(AAm-*co*-MalAAm)-*b*-PTBAAm-SEGDA.

Thiol-terminated block copolymer (mg; μmol)	DEGDA (mg; μmol)	Et_3N (mg; μmol)	Product	Yield (%)[a]	Degree of substitution (%)[b]	M_n[b]	M_w/M_n[c]
P7-SH (48.8; 8.69)	190; 869	1.8; 17.4	**P9**	86	quant.	12,400	1.33
P8-SH (84.7; 5.34)	58; 267	1.1; 10.7	**P10**	81	quant.	16,100	1.24

a) Isolated yield. b) Determined by ^1H NMR. c) Determined by GPC.

IV.3.3 Preparation of Mal-decorated polymer particles

Mal-decorated polymer particles were prepared using **P9** and **P10** as macromonomers by dispersion polymerization with styrene in ethanol/water (4/1, v/v). The obtained polymer particles were spherical and submicron in diameter (Table 4.7). SEM images of the products show uniform spherical shapes with approximately 400 nm in diameter, while DLS analysis of those shows around 500 nm in diameter (Figure 4.3 and 4.4). This is because the SEM particle samples were dried in a vacuum condition, but the DLS particle samples were measured the whole of 3 ml of particle suspension solution. The dispersion stability of polymer particles in water is

satisfactory. There is no participate at least for 24 h, and the transmittance of dispersion remained low (Figures 4.6 and 4.7). The amphiphilic glycopolymers act as a surfactant, with a hydrophilic P(AAm-*co*-MalAAm) segment and hydrophobic PTBAAm segment, allowing the preparation of styrene-based polymer particles by dispersion polymerization in aqueous solvent.

Table 4.7: Preparation of Mal-decorated polymer particles.

Run	Macro monomer	Yield (%)[a)]	D_n[b)] (nm)	D_n[c)] (nm)	PDI[b)]
1	**P9**	58	410 ± 30	540	1.02
2	**P10**	49	390 ± 20	520	1.04

a) Isolated yield. b) Determined by SEM analysis (Average of 100 particles). c) Determined by DLS analysis.

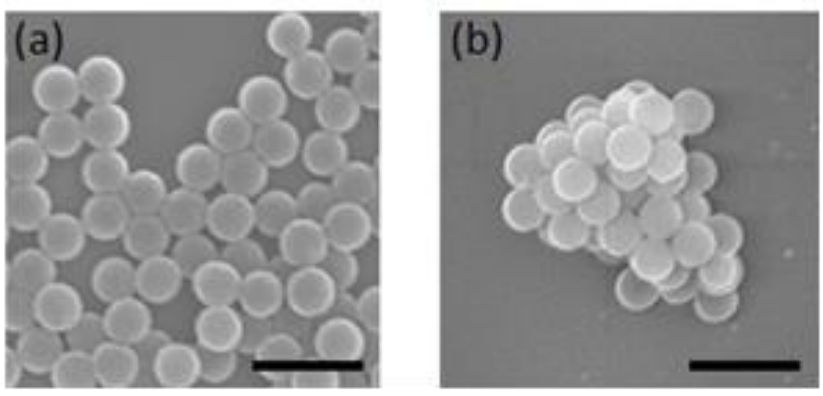


Figure 4.3: SEM images of polymer particles prepared using (a) P9 and (b) P10. Bars: 1 μm.

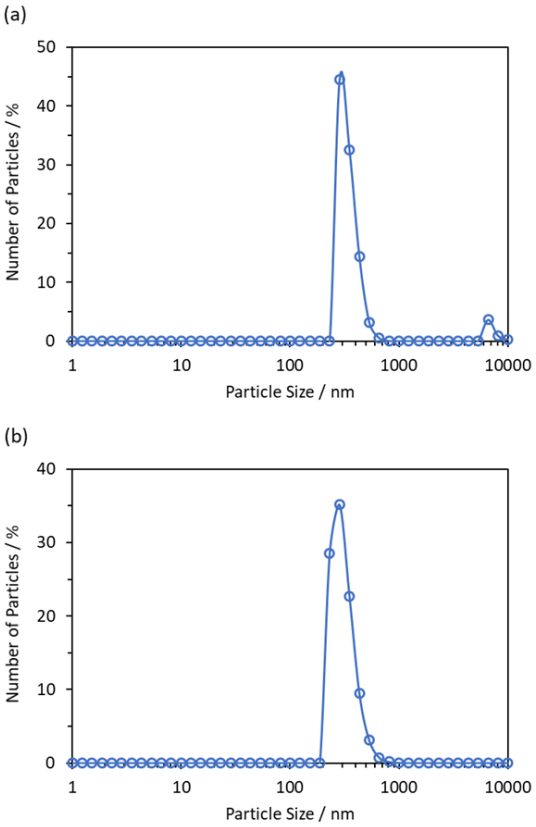


Figure 4.4: DLS measurements of polymer particles prepared using (a) P9 and (b) P10.

IV.3.4 Lectin binding assay and estimation of maltose density on polymer particles

The synthesized polymer particles presumably display Mal moieties on the surface and thus the author investigated the interaction of these particles with the lectin Con A. The transmittance of the polymer particle aqueous suspension increased remarkably following the addition of Con A due to precipitation of the lectin-particle conjugates (Figure 4.5 and 4.6). In contrast, no increase in transmittance was observed when bovine serum albumin (BSA) was added as a control protein to the particle suspension. Particles prepared using **P10** bound Con A more strongly than those prepared using **P9**. The surface density of Mal moieties on the particles was

evaluated by quantitating the amount of free glucose hydrolyzed and released from the polymer particle surfaces in an acidic condition. This assay showed that the surface density of Mal moieties on the particles prepared using **P9** and **P10** was 6.49 and 8.33 μg cm^{-2}, respectively. These results suggest that the longer hydrophobic segment in **P10** is more favorable to the incorporation of Mal moieties onto the polymer particle surfaces, hence amplified the interaction with Con A (glycocluster effect)[13]. Thus, the saccharide density of the polymer particle surfaces can be regulated by the hydrophilic/hydrophobic balance of the block copolymer-type glycostabilizer.

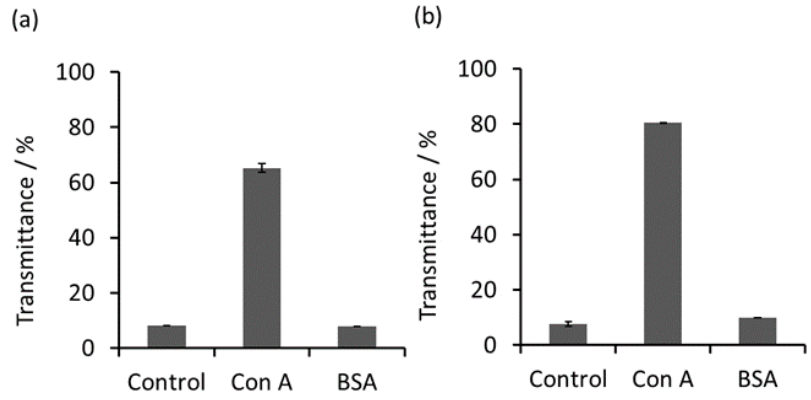


Figure 4.5: Transmittance of polymer particle suspensions prepared using (a) P9 and (b) P10 after the addition of proteins for 11 h. Control: polymer particle suspension before the addition of Con A or BSA.

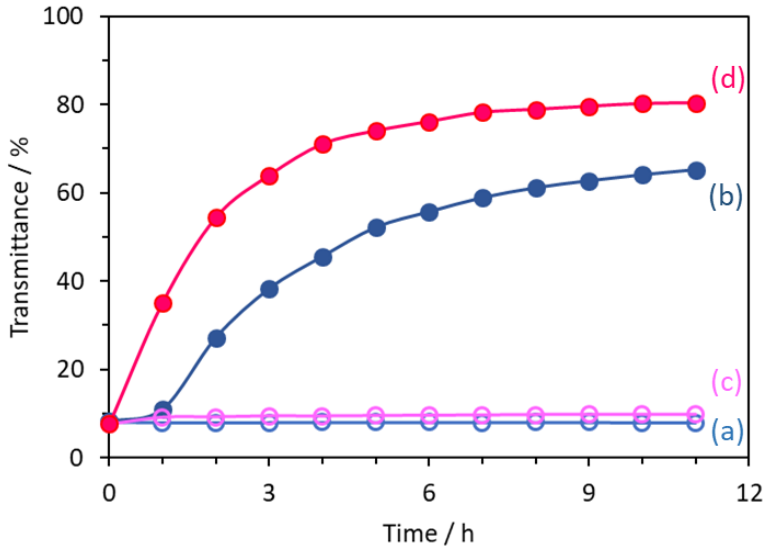


Figure 4.6: The transmittance of polymer particle suspension. (a) Polymer particles prepared using P9 + BSA, (b) Polymer particles prepared using P9 + Con A, (c) Polymer particles prepared using P10 + BSA, (d) Polymer particles prepared using P10 + Con A.

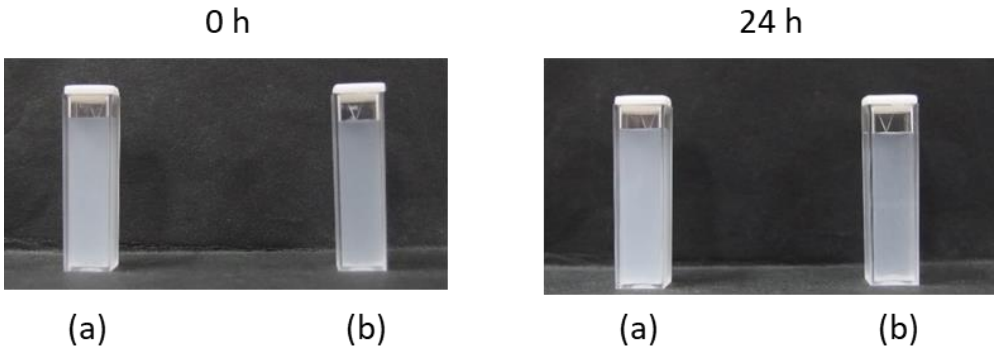


Figure 4.7: Photos of polymer particle suspension in water prepared using (a) P9 and (b) P10.

Table 4.8: Analysis of maltose density on the polymer particle surface.

Particle	Absorbance at 630 nm	Conc. of free glucose ($\mu g\ mL^{-1}$)	Amount of free glucose (mg per 10 mg of polymer particle)	Amount of maltose (mg per 10 mg of polymer particle)
P9-Particle	0.33	24.0	1.20	2.40
P10-Particle	0.39	32.0	1.60	3.20

Table 4.9: Estimation of maltose density on polymer particles.

Particle	D_n (nm)[a)]	Volume ($cm^3 \times 10^{-15}$)	Area of the polymer particle ($cm^2 \times 10^{-10}$)	Styrene (molecule per particle $\times 10^6$)	Surface density of maltose moieties ($\mu g\ cm^{-2}$)
P9-Particle	410	35.0	12.9	205	6.49
P10-Particle	390	31.3	12.0	181	8.33

IV.4 Conclusions

In conclusion, the novel glycopolymer-type macromonomers P(AAm-*co*-MalAAm)-*b*-PTBAAm-SEGDAs (**P9** and **P10**), consisting of amphiphilic block copolymers bearing saccharides and a vinyl group at the terminus, were successfully synthesized and used to prepare carbohydrate-decorated polymer particles via consecutive RAFT polymerizations, terminal conversion, and thiol-ene reaction. Dispersion polymerization with styrene using the glycopolymer-type macromonomers as steric stabilizers produced carbohydrate-decorated polymer particles. These polymer particles were uniformly spherical and capable of binding the target lectin due to the multivalency of the saccharide moieties on the particle surfaces. Glycopolymer-type macromonomers will contribute to the development of various carbohydrate-based polymer materials, including the preparation of lectin-binding particles and surface modified particles.

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List of Publications

Chapter II

Living cationic polymerization of a vinyl ether with an unprotected pendant alkynyl group and their use for the protecting group-free synthesis of macromonomer-type glycopolymers via CuAAC with maltosyl azides

Nguyen Minh Tan, Ryota Mori, Tomonari Tanaka, Jin Motoyanagi, and Masahiko Minoda

Journal of Polymer Science Part A: Polymer Chemistry, **2019**, 57, 681-688.

Chapter III

Protecting Group-Free Synthesis of Glycopolymer-type Amphiphilic Macromonomers and their Use for the Preparation of Carbohydrate-Decorated Polymer Particles

Jin Motoyanagi, Minh Tan Nguyen, Tomonari Tanaka, and Masahiko Minoda

Biomolecules, **2019**, 9, 72.

Chapter IV

Amphiphilic Glycopolymer-type Macromonomers for the Preparation of Carbohydrate-decorated Polymer Particles

Tomonari Tanaka, Minh Tan Nguyen, and Masahiko Minoda

Chemistry Letters, **2018**, 47, 1519-1521.

Other Publications

1/ Synthesis of well-defined poly(vinyl ether)-based macromonomers having pendant glycerols via living cationic polymerization and their application to the preparation of core–shell polymer particles

Jin Motoyanagi, Nguyen Minh Tan, and Masahiko Minoda

Polymer International, **2014**, 63, 459-464.