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One-pot synthesis of glycyrrhetic acid polyglycosides based on grafting-from method using cyclic sulfite

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Abstract

Glycyrrhetic acid polyglycosides were synthesized in one-pot via cationic ring-opening condensation polymerization of cyclic sulfite (4) initiated by glycyrrhetic acid as an aglycon. Sulfite 4 worked as a practical monomer for the preparation of (1→2)-linked polysaccharide skeletons. The chemical stability of 4 was evaluated by the comparison of thermodynamic parameters with those of conventional epoxide (2). The grafting reaction of sulfite 4 from glycyrrhetic acid (5) was performed in the presence of TIOH and MS 3A in CH₂Cl₂ at room temperature. The polymerization degree was moderately controllable by the change of feed ratio of initiator.

Introduction

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

The previous syntheses were mainly focused on the change of oligosaccharide structure based on the stepwise glycosidations,8 which required time-consuming multi-step reactions and purification processes. Therefore, the development of simple preparation method for the derivatives such as glycyrrhetic acid polyglycosides (2) has been strongly urged. Although the powerful polymerization methods to give (1→3)-(1→4), and (1→6)-linked polysaccharides have been constructed to date,9 that for (1→2)-linked polysaccharides has not been sufficiently developed yet.10 On the other hand, it is known that glycal epoxide 3 is a potentially useful glycosyl donor for the synthesis of (1→2)-linked oligosaccharide-containing glycosides (Fig. 2). Danishefsky et al. demonstrated the simple stepwise glycosidation via ring-opening nucleophilic addition of alcohol to the epoxide 3.11 Several processes concerning protection–deprotection can be shortened using 3 because the nucleophilic addition to 3 accompanies with the generation of a free secondary alcohol. However, the applications of 3 to graft polymerization techniques were difficult due to the high unstability of 3.

We recently discovered that shelf-stable cyclic sulfite (4) works as a practical monomer for the preparation of (1→2)-linked polysaccharide skeletons. The cationic ring-opening condensation polymerization of 4 resulted in a perfect elimination of SO₂ linkages to afford the corresponding (1→2)-linked glucopyranan.12 The results indicate that cyclic sulfite 4 can be regarded as a stable surrogate of 3.13

Herein, we describe the synthesis of glycyrrhetic acid polyglycosides (2) as the application of our previous work to a grafting polymerization. The cationic ring-opening condensation polymerization of 4 was performed in the presence of glycyrrhetic acid (5) as an initiator and trifluoromethane sulfonic acid (TIOH) as a catalyst.14 This method enables not only the one-pot synthesis of 2 but also the control of polymerization degree of (1→2)-linked oligosaccharide skeleton. The analyses of chemical stability of 3 and 4 were also investigated.
CDCl₃ was kept at arbitrary temperatures (20, 40, and 60 °C) on the sulfur atom,¹²,¹⁶ which was used for the next studies with analyzation by ¹H NMR measurements.¹⁷,¹⁸ Assuming that the degradation reactions of the literature. Sulfite analyses of chemical stability between cyclic sulfite and glycal epoxide of chemical stability between the feed ratio of 4, as shown in Scheme 1. Initiator 5 was prepared from commercially available glycyrrhetic acid via benzyl protection of carboxylic acid. The grafting polymerization of 4 from 5 were performed in CH₂Cl₂ in the presence of TFOH and molecular sieves 3Å (MS 3A) at room temperature. The results are summarized in Table 1. The conversion of 4, the polymerization degree, and the number average molecular weight (Mₙ) were determined by the ¹H NMR analyses of crude mixture. The conversion of 5 and the polydispersity index (Mₘ/Mₙ) were estimated by a size exclusion chromatography (SEC) using THF on the basis of polystyrene standards. At first, we used the stoichiometric amount of both 5 and TFOH to that of 4. Contrary to our expectation that the reaction would mainly give the monosaccharide-containing glycoside, the conversion of 5 was middle (45.8%) and that of 4 was quantitative (entry 1, Table 1). The average polymerization degree was 2.9, indicating that Mₙ of Poly-6 is 1,800 Da. The Mₘ/Mₙ of Poly-6 was a relatively narrow value (1.1). Such results indicate that the bulky alcohol of 5 as a neopentyl alcohol would slowly initiate the glycosylation reaction of 4 along with the elimination of SO₂ to generate a free alcohol at the 2 position of the sugar. The resultant secondary alcohol could exhibit a higher reactivity than the neopentyl alcohol of 5, which would facilitate further nucleophilic addition to the other cyclic sulfite 4, leading to the ring-opening polycondensation of 4 initiated by 5. It is noted that the polymer with a narrow Mₘ/Mₙ was obtained, even when the excess amount of 5 was used (entry 2).

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

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

Having shelf-stable 4 in hand, we next investigated the grafting reaction of 4 from glycyrrhetic acid (5) to give the benzyl-protected skeleton of 2, as shown in Scheme 1. Initiator 5 was prepared from commercially available glycyrrhetic acid via benzyl protection of carboxylic acid. The grafting polymerization of 4 from 5 were performed in CH₂Cl₂ in the presence of TFOH and molecular sieves 3Å (MS 3A) at room temperature. The results are summarized in Table 1. The conversion of 4, the polymerization degree, and the number average molecular weight (Mₙ) were determined by the ¹H NMR analyses of crude mixture. The conversion of 5 and the polydispersity index (Mₘ/Mₙ) were estimated by a size exclusion chromatography (SEC) using THF on the basis of polystyrene standards. At first, we used the stoichiometric amount of both 5 and TFOH to that of 4. Contrary to our expectation that the reaction would mainly give the monosaccharide-containing glycoside, the conversion of 5 was middle (45.8%) and that of 4 was quantitative (entry 1, Table 1). The average polymerization degree was 2.9, indicating that Mₙ of Poly-6 is 1,800 Da. The Mₘ/Mₙ of Poly-6 was a relatively narrow value (1.1). Such results indicate that the bulky alcohol of 5 as a neopentyl alcohol would slowly initiate the glycosylation reaction of 4 along with the elimination of SO₂ to generate a free alcohol at the 2 position of the sugar. The resultant secondary alcohol could exhibit a higher reactivity than the neopentyl alcohol of 5, which would facilitate further nucleophilic addition to the other cyclic sulfite 4, leading to the ring-opening polycondensation of 4 initiated by 5. It is noted that the polymer with a narrow Mₘ/Mₙ was obtained, even when the excess amount of 5 was used (entry 2).

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### Analyses of chemical stability of cyclic sulfite and glycal epoxide

Epoxide ³¹⁵ and cyclic sulfite ⁴¹⁶ were prepared according to the literature. Sulfite 4 was the α-anomer and an endo/exo-mixture on the sulfur atom,⁹²,¹⁶ which was used for the next studies without separation. While 4 was sufficiently stable for purification on a florisil column chromatography, epoxide 3 decomposed easily under the same purification condition. To evaluate the difference of chemical stability between 3 and 4, each solution of 3 and 4 in CDCl₃ was kept at arbitrary temperatures (20, 40, and 60 °C) and analyzed by ¹H NMR measurements.¹⁷,¹⁸ Assuming that the degradation reactions obey first-order kinetics, the rate constants were determined from the time vs. ln[(3 or 4)³(3 or 4)₀] plots using the integral ratios from the ¹H NMR spectra. As a result, it was found that 4 survived for longer time than 3 at all temperatures.

![ Structures of glycyrrhizin (1) and glycyrrhetic acid polyglycosides (2). ]

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

The half-lives (τ) of 3 and 4 were also calculated by the equation:

\[ \tau = \ln(2)/k \]

The activation energy (ΔE) was obtained by the Arrhenius plots and the activation enthalpy (ΔH⁰) and activation entropy (ΔS⁰) were obtained by the Eyring plots.¹⁷ The kinetic and thermodynamic parameters for the degradations are summarized in Table 1. The degradation half-lives of 4 were approximately 3–6 times greater than those of 3, leading to the higher ΔE for the degradation of 4 than that of 3. It also turned out that the enthalpy term (ΔH⁰) was dominant rather than the entropy term (TΔS⁰).

### Table 1

| Thermodynamic parameters for the degradation reactions of 3 and 4⁴¹⁶ | 20 °C | 40 °C | 60 °C |
|---------------------------------------------------------------|------|------|------|
| Epoxide 3           | 497.3 | 66.1 | 13.6 |
| Cyclic sulfite 4    | 3307.4 | 224.2 | 42.7 |
| Activation energy (ΔE) kJ/mol |
| Activation enthalpy (ΔH⁰) kJ/mol |
| Activation entropy (ΔS⁰) J/(mol K) |

*a* Determined by ¹H NMR analyses of 3 and 4 in CDCl₃ (0.02–0.03 mol/L). The details of experiments and calculations are given in Supporting information.¹⁶
The crude material after a typical workup was precipitated into MeOH to remove the remaining 4 and 5. The structure of the polymer as a MeOH-insoluble part was evaluated by IR, $^1$H NMR, and MALDI-TOF mass spectra. In the IR spectrum, the absence of the characteristic signal of sulfite at around 1200 cm$^{-1}$ was observed, suggesting that the polymerization of 4 efficiently eliminates SO$_2$ to form (1→2)-linked oligosaccharide skeletons. The presence of enone absorption band at around 1660 cm$^{-1}$ also supported the retention of the enone moiety after the polymerization. It has been reported that the Ag- or Hg-catalyzed glycosylation to glycyrrhetic acid skeleton gave the corresponding enol glycoside, highlighting the efficient conversion of 4 to give glycyrrhetic acid polyglycosides in one-pot via cationic ring-opening condensation polymerization of 4. The method enables the rapid preparation of glycyrrhizin derivatives consisting oligosaccharides with arbitrary polymerization degree. Because other natural glycides bearing (1→2)-linked oligosaccharides such as cyaniding-3-O-sophoroside$^{19}$ and quercetin-3-O-sophoroside$^{20}$ have been reported, this method would be also applicable to the preparations of their derivatives.

Matrix associated laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrum of Poly-6 exhibited a simple pattern with an even interval agreed with the theoretical values corresponding to the Bn-protected glycyrrhetic acid-terminated polymers (Fig. 3), which finally evidenced that the polymerization of 4 accompanied with the perfect elimination of SO$_2$ to give Poly-6 skeleton. The additional minor peaks attributed to the polymers initiated by a trace of H$_2$O were barely detectable in the spectrum, indicating the important role of MS 3A as a dehydrator.

In conclusion, we demonstrated a new grafting reaction of cyclic sulfite 4 from glycyrrhetic acid 5 to give glycyrrhetic acid polyglycosides in one-pot via cationic ring-opening condensation polymerization of 4. The method enables the rapid preparation of glycyrrhizin derivatives consisting oligosaccharides with arbitrary polymerization degree. Because other natural glycides bearing (1→2)-linked oligosaccharides such as cyaniding-3-O-sophoroside and quercetin-3-O-sophoroside have been reported, this method would be also applicable to the preparations of their derivatives. The present results may provide new insights into not only natural product syntheses but also the creation of high performance polymers based on a grafting reaction. Further investigations on the control of stereochemistry on the anomeric centers and the evaluation of structure–activity relationship are currently in progress.
Acknowledgments

This work was financially supported by JSPS KAKENHI Grant Numbers JP24685023 and JP15H00718.

Supplementary data

Supplementary data (kinetic studies of 3 and 4, synthetic details, 1H NMR, 13C NMR, IR, and MALDI-TOF mass spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.07.001.

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