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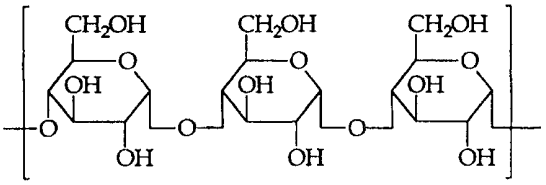

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(57) Abstract

The present invention relates to a process for preparing α-(1,4) linked oligosaccharide, and more particularly, to a process for preparing oligosaccharide expressed by Formula (1) by reacting amylose, easily available from the natural product, with enzyme under a specific condition. Since the oligosaccharide prepared from the present invention is α-(1,4) linked and has a suitable sugar distribution, the same is particularly useful as source material for preparing optically pure (*S*)-3-hydroxy-γ-butyrolactone.

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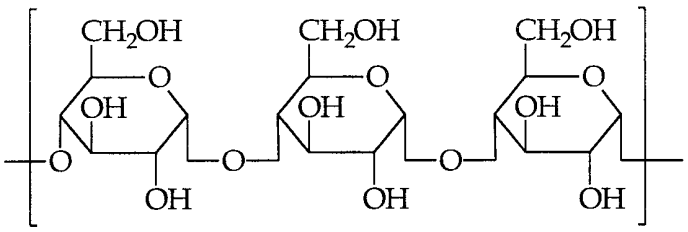
PROCESS FOR PREPARING ALPA-(1,4) LINKED OLIGOSACCHARIDE

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a process for preparing α-(1,4) linked oligosaccharide, and more particularly, to a process for preparing oligosaccharide expressed by the following Formula 1 by reacting amylose, easily available from the natural product, with enzyme under a specific condition.



(1)

Since the oligosaccharide prepared from the present invention has α-(1,4) linkage units and a specific sugar distribution, the same is particularly useful as source material for preparing optically pure (*S*)-3-hydroxy-γ-butyrolactone, useful intermediate for chiral compounds.

Description of the Related Arts

(*S*)-3,4-Dihydroxybutyric acid derivatives and (*S*)-3-hydroxy-γ-butyrolactone are used as synthetic intermediates for preparing various chiral compounds. For example, it is well known that they act as key intermediates for preparing neuromeidator (*R*)-GABOB [*Tetrahedron*, **46**, 4277(1990)], treatment for hyperlipemia (Atorvastatin; HMG-CoA reductase inhibitor) [*Tetrahedron Lett.*, **33**, 2279(1992)], (*S*)-oxiracetam which is improvement agent in brain metabolism [International patent publication

WO93/06,826], L-carnitine which is health supplement agent [International patent publication WO99/05,092], (*S*)-3-hydroxytetrahydrofuran [*J. Am. Chem. Soc.*, **117**, 1181(1995); International patent publication WO94/05,639] which is an essential intermediate of AIDS drug (Agenerase; HIV protease inhibitor), (*S*)-mono-betalactam [Japanese patent publication 64-13,069(1989)], ester of (*S*)-3-hydroxy-4-bromobutyric acid [Japanese patent publication 4-149,151(1992); Japanese patent publication 6-172,256(1994)], potentiating intermediate of satiety agent [*Bull. Chem. Soc. Jpn.*, **61**, 2025(1988)] and neuroleptic drug [USP 4,138,484] and useful intermediates in synthetic efforts towards natural products [*J. Org. Chem.*, **50**, 1144 (1985); *Can. J. Chem.*, **65**, 195 (1987), *Tetrahedron Lett.*, 507 (1992)]. Optical purity is the most important factor in preparing these chiral compounds.

The conventional technologies for preparing (*S*)-3,4-dihydroxybutyric acid derivatives and (*S*)-3-hydroxy-γ-butyrolactone, which are useful for preparing the said chiral compounds, are explained in detail hereunder.

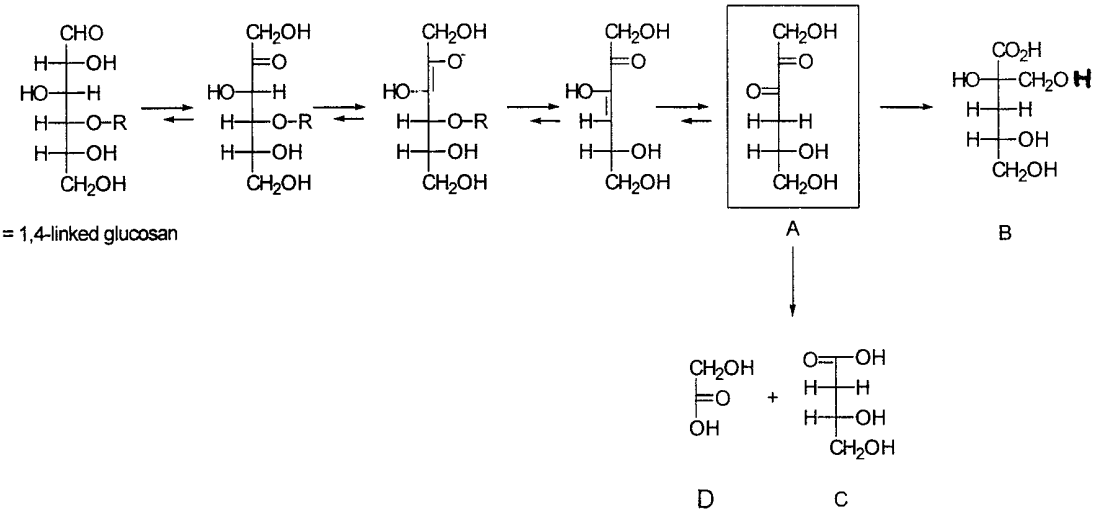
Methods of preparing (*S*)-3-hydroxybutyric acid derivatives from the enzymatic or catalytic reduction of β-ketoester were known [*J. Am. Chem. Soc.*, **105**, 5925~5926(1983); *Teterahedron Lett.*, **31**, 267~270(1990); European patent publication 452,143A2]. These methods have difficulty in that the prochiral center should be reduced to one-side to generate chiral center and expensive metal catalyst should be used.

A technology preparing ester of (*S*)-3,4-dihydroxybutyric acid and (*S*)-3-hydroxy-γ-butyrolactone by selective reduction of (L)-malic acid ester was known [*Chem. Lett.*, 1389~1392(1984); USP 5,808,107]. This technology has disadvantage in that reduction should be performed selectively to only one of the two ester functional groups.

Many methods of preparing (*S*)-3,4-dihydroxybutyric acid derivatives and (*S*)-3-hydroxy-γ-butyrolactone from carbohydrate have been reported.

A technology preparing isosaccharinic acid (B) or (*S*)-3,4-dihydroxybutyric acid (C) is reported [*J. Chem. Soc.*, 1924~1931(1960)] by alkaline degradation of carbohydrate containing glucose substituent in the 4-position, such as 4-*O*-methyl-(D)-glucose, maltose, amylose and cellulose, elimination of C-4 substituent as leaving group, forming dicarbonyl compound (A; 4-deoxy-2,3-hexodiulose), and reacting the formed dicarbonyl compound with base as shown in Scheme 1. However, the yield of (*S*)-3,4-dihydroxybutyric acid is low.

Scheme 1



Also, it has been reported that (*S*)-3,4-dihydroxybutyric acid (C) and glycolic acid (D) were obtained as major products by forming dicarbonyl compound (A) from alkaline degradation of carbohydrate containing glucose substituent in the 4-position, and separating the formed dicarbonyl compound (A) and reacting it with hydrogen peroxide [*J. Chem. Soc.*, 1932~1938(1960)]. This method has a serious problem that the product exists as small amount of isomers due to tautomerization and a mixture of cyclic compounds and hydrates derived from dicarbonyl compound (A). So, the dicarbonyl compound (A) cannot be separated in good yields from the reaction mixture. Another problem is that the prepared (*S*)-3,4-dihydroxybutyric acid is degraded

to formic acid and glycolic acid due to the overoxidation.

A similar technology for preparing (*S*)-3,4-dihydroxybutyric acid from carbohydrate either using base only or using oxygen in base was known. It proposed that the dicarbonyl compound (A) was a synthetic intermediate for (*S*)-3,4-dihydroxybutyric acid as shown in the Scheme 1. But the yield was reported to be as low as about 30% [*J. Res. Natl. Bur. Stand.*, **32**, 45(1944); *J. Am. Chem. Soc.*, 2245~2247(1953); *J. Am. Chem. Soc.*, 1431~1435(1955); *Carbohyd. Res.*, **11**, 17~25(1969); *J. Chromatography*, **549**, 113~125(1991)]. In these methods, (*S*)-3,4-dihydroxybutyric acid is produced with various kinds of mixtures including glycolic acid (D), isosaccharinic acid (B), formic acid, ketone, diketone and glyceric acid. Since the yield of (*S*)-3,4-dihydroxybutyric acid is very low, these methods are also considered as not suitable for industrial use.

A method for preparing (*S*)-3,4-dihydroxybutyric acid from disaccharide (lactose) using base and oxidant has been reported [International patent publication WO98/04543]. In this work, (*S*)-3,4-dihydroxybutyric acid was cyclized to (*S*)-3-hydroxy-γ-butyrolactone under the reaction condition and purified by protection of the two hydroxy groups to acetonide ester compound, methyl (*S*)-3,4-*O*-isopropylidene-3,4-dihydroxybutanoate, which was recyclized to (*S*)-3-hydroxy-γ-butyrolactone under acidic media.

Preparing methods of (*S*)-3,4-dihydroxybutyric acid including the process of alkaline oxidation of carbohydrate containing glucose substituent in the 4-position have been known [USP 5,292,939, 5,319,110 & 5,374,773(1994)]. In these methods, dicarbonyl compound (A) intermediate is formed at first, oxidized to (*S*)-3,4-dihydroxybutyric acid (C) and glycolic acid (D). However, optical purity, the most important physical property of chiral compounds, is not mentioned at all. Also, purification of target compound is very difficult, considering the reaction mechanism. In the case of disaccharides such as maltose or lactose, only one sugar unit in the disaccharide

forms (*S*)-3,4-dihydroxybutyric acid and the other sugar unit functions as leaving group, so that the target product and leaving group coexist as 1:1 mixture. Accordingly, it is very difficult to separate and purify (*S*)-3,4-dihydroxybutyric acid or (*S*)-3-hydroxy-γ-butyrolactone from the reaction mixture. The maximum mass conversion obtainable is 28.3 wt%. In other words, 28.3g of (*S*)-3-hydroxy-γ-butyrolactone can be obtained from 100g of disaccaride. For polysaccharides, such as maltodextrin, starch and cellulose, mentioned in the above patents, the (1,4) and/or (1,6) glucose units are linked complexly like nets. The problem is that the step-by-step oxidation proceeding from the reducing end units comprising (1,4) linkage terminates at (1,6) linkage unit. Therefore, no more target product is formed. Also, the polysaccharides are degraded by overoxidation of reducing end units to complex acid mixtures containing formic acid, oxalic acid, glycolic acid and erythronic acid [*J. Am. Chem. Soc.*, **81**, 3136(1959); *Starch* **41** Nr. 8, S. 303~309(1989); *Synthesis*, 597~613(1997)].

There was an attempt to improve the yield of (*S*)-3,4-dihydroxybutyric acid or (*S*)-3-hydroxy-γ-butyrolactone for polysaccharide by degradation of higher-molecular sugars to relatively lower-molecular sugars through acid or base hydrolysis. Though the reactivity by this method is increased to a degree, (1,4) linkage and (1,6) linkage are not hydrolyzed selectively to afford random distribution. Accordingly, there is a fundamental problem in preparing (*S*)-3,4-dihydroxybutyric acid derivatives in high yield [*Encyclopedia of Chemical Technology*, 3rd ed. 492~507].

Regarding the preparation of (*S*)-3-hydroxy-γ-butyrolactone using (1,4)-linked polysaccharide, the step-by-step oxidation proceeds continuously from the reducing end units to non-reducing end units to afford (*S*)-3,4-dihydroxybutyric acid until the last chain unit (leaving group) remains. Namely, if (1,4)-linked polysaccharide is used as a source material for preparing

(*S*)-3-hydroxy-γ-butyrolactone, the theoretical mass conversion yield obtainable is 63 wt%, about two times more compared with the method using disaccharide. In other words, 63g of (*S*)-3-hydroxy-γ-butyrolactone can be obtained from 100g of (1,4)-linked polysaccharide. Also, since the small amount of leaving group is produced in the reaction mixture compared with disaccharide, the target product is easily purified. Therefore, the use of (1,4)-linked polysaccharide promises the enhanced productivity.

However, regarding conventional polysaccharides, the target product and by-products (acids such as formic acid, oxalic acid, glycolic acid and erythronic acid) are formed competitively in the step-by-step oxidation due to the compact structure having random (1,4) linkage and (1,6) linkage. Thus, selective degradation technique of polysaccharide to a suitable sugar distribution range having (1,4) linkage is required.

On the other hand, there have been many reports of transforming higher-molecular sugars to lower-molecular sugars using biological enzymatic treatment process for industrial use.

The reported technologies include preparing glucose, maltose and ethanol through enzymatic treatment of starch [USP 3,791,865(1974); USP 3,922,200(1975); USP 4,855,232(1989): Japanese patent publication 4-158,795(1992); *Methods Carbohydr. Chem.*, **10**, 231~239(1994); *Methods Carbohydr. Chem.*, **10**, 245~248(1994)], and preparing maltodextrin with adequate dextrose equivalent (DE) [USP 3,986,890(1976); USP 4,447,532(1984); USP 4,612,284(1986); USP 5,506,353(1996)]. In these references, through the degradation or transformation of high molecular polysaccarides, they are converted to adequate materials for medicines, food additives, and diagnostic reagents.

But, the method for preparing (1,4)-linked oligosaccharides suitable for the mass production of (*S*)-3-hydroxy-γ-butyrolactone by biological treatment of higher molecular polysaccharides with enzymes is not known at present.

SUMMARY OF THE INVENTION

The inventors of the present invention made intensive efforts to develop a method for preparing optically pure (*S*)-3-hydroxy-γ-butyrolactone from commercially available amylose with ease. We found that α-(1,4)-linked oligosaccharide from amylose with enzymatic reactions followed by oxidation, esterification, and cyclization under specific conditions, formation of by-products from oxidation reaction is minimized due to the structural specificity of oligosaccharide. Furthermore, oxidation reaction can be performed continuously in the same reactor without additional separation and purification of the prepared oligosaccharide.

Accordingly, an object of this invention is to provide a method for preparing oligosaccharide as source material for preaparing optically pure (*S*)-3-hydroxy-γ-butyrolactone in high yield without additional purification of intermediates.

Brief Description of the Drawings

Fig. 1a represents the optical purity analysis results by gas chromatography (GC) of racemic 3-hydroxy-γ-butyrolactone.

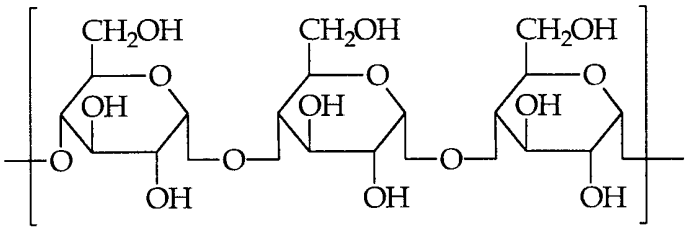
Fig. 1b represents the optical purity analysis results by gas chromatography (GC) of 3-hydroxy-γ-butyrolactone prepared from disaccharide of the conventional method.

Fig. 1c represents the optical purity analysis results by gas chromatography (GC) of 3-hydroxy-γ-butyrolactone prepared from oligosaccharide of the present invention.

Detailed Description of the Invention

The present invention is characterized by an enzymatic reaction of

amylose to α-(1,4)-linked oligosaccharide expressed by the Formula 1 under the condition of pH 4.0~8.0 and 40~120℃.



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The detailed description of the present invention is given hereunder.

The present invention relates to a process for transforming amylose to oligosaccharide with the optimal sugar distribution for preparing the target product using specific enzyme. Namely, the present invention facilitates the subsequent oxidation by solving the problem caused by the specific structure of amylose, i.e. double helix structure due to the intramolecular hydrogen bond. The fundamental inventive concept of the present invention is degradation of amylose to oligosaccharide with the optimal sugar using specific enzyme for mass production of (*S*)-3-hydroxy-γ-butyrolactone.

Preparing process of the present invention is advantageous in that the double helix structure due to the very strong intramolecular hydrogen bond is degraded with α-amylase to obtain the oligosaccharide with suitable sugar distribution from the said amylose. In this kind of enzymatic reaction, if α-amylase is reacted for a long time, amylose is excessively degraded, so the intended oligosaccharide is not obtainable. Accordingly, in this invention, a technology of inactivating the transformed oligosaccharide was introduced.

The enzymatic reaction of the present invention is performed in water or buffer solution of pH 4.0~8.0 at 40~120℃. α-Amylase is used in the range of 0.001~10 wt% of amylose, and enzymatic reaction of α-amylase is performed for 30 min ~ 4 hr, and then remaining α-amylase is inactivated.

Inactivation is performed under acidic (pH 2.0~4.5) and high temperature (60~150℃) conditions and maintained for 10 min ~ 4 hr. Reducing end units and molecular weight distribution of the prepared oligosaccharide are analyzed from reducing end units and dextrose equivalent analysis by an optical analyzer, HPLC analysis, and gel permeation chromatography (GPC) analysis.

The oligosaccharide is obtained from the selective enzymatic reaction and has distribution mostly between 3~50 glucose units, and preferably 5~50 glucose units. Since the prepared oligosccaride has suitable sugar distribution for the preparation of (*S*)-3-hydroxy-γ-butyrolactone, the yield of (*S*)-3-hydroxy-γ-butyrolactone is very high through continuous sequential reactions with minimizing the by-products (e.g., acid mixtures of formic acid, oxalic acid, glycolic acid and erythronic acid). Furthermore, the obtained (*S*)-3-hydroxy-γ-butyrolactone was identified to be optically very pure (>99.9%ee).

The detailed explanation of the preparation process of this invention is as follow. It comprises; 1) a step preparing oligosaccharide with characteristic α-(1,4) linkage expressed in Formula 1 by degradation of amylose using biological treatment of specific enzymes, 2) a step preparing ester of (*S*)-3,4-dihydroxybutyric acid through oxidation, and 3) a step preparing optically pure (*S*)-3-hydroxy-γ-butyrolactone in high yield through cyclization of the prepared ester compound. Especially, the preparation process of this invention is characterized by preparing (*S*)-3-hydroxy-γ-butyrolactone in the same reactor without additional purification of the intermediates (oligosaccharide and ester of (*S*)-3,4-dihydroxybutyric acid).

Oxidation of oligosaccharide is performed by adding base and oxidant dropwise for 6~36 hr under the condition of 30~65℃. Hydrogen peroxide, alkali metal peroxides, akaline earth metal peroxides and alkyl hydroperoxides

are used for the oxidants, and hydrogen peroxide or *t*-butylhydroperoxide is the most preferable. The oxidant is used in the range of 1~3 equivalents per molar glucose unit of amylose. The base is selected from the group consisting of alkali metal hydroxide or alkaline earth metal hydroxide, and sodium hydroxide or potassium hydroxide is preferable. The base is used in the range of 2~4 equivalents per molar glucose unit of amylose.

The preparation yields of (*S*)-3-hydroxy-γ-butyrolactone depending on the source material are compared as follows [Refer to Experimental example 1.]. If maltose (disaccharide) or lactose (disaccharide) obtained from cheese by-product is used as source material, the theoretical mass conversion yield of (*S*)-3-hydroxy-γ-butyrolactone is not more than 28.3 wt% of the source material weight used. On the other hand, if amylose among polysaccharides with more than 50 glucose units is used, the theoretical mass conversion yield of (*S*)-3-hydroxy-γ-butyrolactone is similar to that of prepared oligosaccaride of the present invention. But, the double helix structure due to very strong intramolecular hydrogen bond limits the step-by-step oxidation, so the yield becomes very low. However, by using oligosaccharide of the present invention as source material, the yield of (*S*)-3-hydroxy-γ-butyrolactone is very high as 57.2 wt% of the source material weight used.

In order to synthesize (*S*)-3-hydroxy-γ-butyrolactone from the prepared (*S*)-3,4-dihydroxybutyric acid, esterification and cyclization is performed sequentially.

Esterification of the present invention is performed in the presence of acid catalyst using alcohol as both solvent and reagent in the range of 30~80℃. Inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid and nitric acid, and organic acids such as fluoroalkylsulfonic acid, aralkylsulfonic acid, hydrate of aralkylsulfonic acid and trifluoroacetic acid are used as acid catalyst. Linear or branched alcohol with 1~5 carbon atoms is used for the

alcohol.

Cyclization of the present invention is performed at the temperature range of 30~80℃ for 2~5hr in the presence of acid catalyst to afford the target compound, (*S*)-3-hydroxy-γ-butyrolactone. Inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid and nitric acid, and organic acids such as fluoroalkylsulfonic acid, aralkylsulfonic acid, hydrate of aralkylsulfonic acid and trifluoroacetic acid are used as acid catalyst

As explained above, the present invention is excellent in that the low reactivity of amylose to oxidation is overcome by transforming amylose to oligosaccharide with the application of specific enzymes. Furthermore, by-product formation is minimized and optically pure (*S*)-3-hydroxy-γ-butyrolactone can be prepared in high yield with very simple purification process.

The following examples are intended to be illustrative of the present invention and should not be construed as limiting the scope of this invention defined by the appended claims.

Example 1: Preparation of methyl (*S*)-3,4-dihydroxybutanoate

10 L of water and 5 kg of dried amylose were put into a 50 L reactor. After heating the reactor to 55℃, 12 g of α-amylase (BAN; EC 3.2.1.1 from *Bacillus licheniformis*, Novo Nordisk) was added. After heating this reaction solution to 75℃, the same was stirred for 2 hr at the same temperature. 5 mL of 0.1N HCl solution was added to adjust the pH of the reaction solution to 3.0~3.5, and then the same was stirred for 1 hr at 90℃ to inactivate the remaining α-amylase. After slowly cooling the reaction mixture to 60℃, 40% NaOH (8.64 kg) solution and 30% H_2O_2 (5.25 kg) solution were added dropwise for 24 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The prepared sodium salt of

(*S*)-3,4-dihydroxybutyric acid was identified using NMR analysis.

^{1}H-NMR (D_2O, ppm) δ 2.27 (dd, 1H), 2.39 (dd, 1H), 3.41 (dd, 1H), 3.51 (dd, 1H), 3.8~3.9 (m, 1H)

The reaction solution was concentrated, and 10L of methanol was added. Sulfuric acid was added to adjust the pH to 4~5, and then the same was stirred for 3 hr at 50℃. Sodium carbonate was added to neutralize the solution, and the same was filtered to remove the by-product, and then methanol was concentrated to obtain methyl (*S*)-3,4-dihydroxybutanoate. The formation of methyl (*S*)-3,4-dihydroxybutanoate (conversion ratio: 92%) was identified through NMR analysis by comparison with internal standard.

^{1}H-NMR ($CDCl_3$, ppm) δ 2.5 (dd, 2H), 3.5 (dd, 1H), 3.6 (dd, 1H), 3.7 (s, 3H), 4.1 (m, 1H)

Example 2: Preparation of (*S*)-3-hydroxy-γ-butyrolactone

10L of water and 5kg of dried amylose were put into a 50L reactor. After heating the reactor to 55℃, 12g of α-amylase (Teramyl; EC 3.2.1.1 from *Bacillus amyloliquefaciens*, Novo Nordisk) was added. After heating this reaction solution to 85℃, the same was stirred for 2 hr at the same temperature. 5mL of 0.1N HCl solution was added to adjust the pH of the reaction solution to 3.0~3.5, and then the same was stirred for 1 hr at 90℃ to inactivate the remaining α-amylase. After slowly cooling the reaction to 60℃, 40% NaOH (8.64kg) solution and 30% H_2O_2 (5.25kg) solution were added dropwise for 24 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The prepared (*S*)-3,4-dihydroxybutyric acid sodium salt was identified using NMR analysis.

^{1}H-NMR (D_2O, ppm) δ 2.27 (dd, 1H), 2.39 (dd, 1H), 3.41 (dd, 1H), 3.51 (dd, 1H), 3.8~3.9 (m, 1H)

The reaction solution was concentrated, and 10L of methanol was added. In this solution, methanesulfonic acid was added to adjust the pH to 4~5, and then the same was stirred for 3 hr at 50℃. After cooling, sodium carbonate was added to neutralize the solution, and the same was filtered to remove the by-product, and then methanol was concentrated to obtain methyl (*S*)-3,4-dihydroxybutanoate. Formation of methyl (*S*)-3,4-dihydroxybutanoate (conversion ratio: 93%) was identified using NMR analysis comparing with the internal standard.

^{1}H-NMR ($CDCl_3$, ppm) δ 2.5 (dd, 2H), 3.5 (dd, 1H), 3.6 (dd, 1H), 3.7 (s, 3H), 4.1 (m, 1H)

The prepared methyl (*S*)-3,4-dihydroxybutanoate was cyclized at 65℃ under reduced pressure by adding 0.5 wt% of concentrated HCl without any separation. The resultant solution was dissolved with ethyl acetate and the same was neutralized with sodium carbonate. After filtrating and concentrating the same, (*S*)-3-hydroxy-γ-butyrolactone (2.86kg, 57.2 wt% of the amylose weight used) was obtained.

^{1}H-NMR ($CDCl_3$, ppm) δ 2.28 (dd, 1H), 2.74 (dd, 1H), 4.13 (dd, 1H), 4.32 (dd, 1H), 4.4~4.5 (m, 1H)

Comparative example 1: Preparation of (*S*)-3-hydroxy-γ-butyrolactone from starch

20L of water and 5kg of dried starch were put into a 50L reactor, and the temperature was raised to 70℃. 40% NaOH (8.64kg) solution and 30% H_2O_2 (5.25kg) solution were added dropwise for 48 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The same was esterified and cyclized as in Example 2 to obtain (*S*)-3-hydroxy-γ-butyrolactone (1.1kg, 22.0 wt% of starch weight used).

Comparative example 2: Preparation of (*S*)-3-hydroxy-γ-butyrolactone from starch

10L of 0.5N HCl solution and 5kg of dried starch were put into a 50L reactor, and the starch was hydrolyzed for 20 min at 100℃. After cooling the solution to 20℃, the same was neutralized with 100mL of 40% NaOH solution and the temperature was raised to 70℃. 40% NaOH (8.64kg) solution and 30% H_2O_2 (5.25kg) solution were added dropwise for 48 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The same was esterified and cyclized as in Example 2 to obtain (*S*)-3-hydroxy-γ-butyrolactone (1.22kg, 24.4 wt% of starch weight used).

Comparative example 3: Preparation of (*S*)-3-hydroxy-γ-butyrolactone from amylose

20L of water and 5kg of dried amylose were put into a 50L reactor, and the temperature was raised to 70℃. 40% NaOH (8.64kg) solution and 30% H_2O_2 (5.25kg) solution were added dropwise for 48 hr to the reaction solution and the same was stirred for 1 hr at the same temperature. The same was esterified and cyclized as in the Example 2 to obtain (*S*)-3-hydroxy-γ-butyrolactone (1.35kg, 27.0 wt% of amylose weight used).

Experimental example 1: Comparison of (*S*)-3-hydroxy-γ-butyrolactone yield depending on the source material

For the reaction solutions containing each of the carbohydrates shown in Table 1, oxidation, esterification and cyclization were performed as in the Example 2 to obtain (*S*)-3-hydroxy-γ-butyrolactone. The yields of (*S*)-3-hydroxy-γ-butyrolactone are shown in Table 1.

Table 1

Source material (5kg)		Product (wt% compared with source material weight)
Oligosaccharide of the present invention (Example 2)		2.86kg (57.2 wt%)
Polysaccharide	Amylopectin	1.01kg (20.2 wt%)
	Amylose	1.35kg (27.0 wt%)
Disaccharide (maltose)[a]		1. 19kg (23.7 wt%)
[a] Examples 1 & 2 of USP 5,292,939, 5,319,110 & 5,374,773		

Table 1 shows that for disaccharide the relative mass conversion yield is low as 23.7 wt%. On the other hand, if amylose is transformed to oligosaccharide with specific enzyme treatment, the relative mass conversion yield is enhanced to 57.2 wt%, almost two times compared with disaccharide. If amylose is not treated with enzymes, the relative mass conversion yield is relatively low as 27.0 wt%.

Experimental example 2: Optical purity analysis of (*S*)-3-hydroxy-γ-butyrolactone

(*S*)-3-Acetoxy-γ-butyrolactone was synthesized by the following method in order to analyze optical purity of (*S*)-3-hydroxy-γ-butyrolactone prepared from the present invention and the conventional preparing method.

102mg (1mmol) of (*S*)-3-hydroxy-γ-butyrolactone prepared from each method was dissolved in 3mL of methylene chloride, and 0.4mL (5mmol) of pyridine and 0.47mL (5mmol) of acetic anhydride were added to the same.

After 3 hr, the reaction was quenched with 1N HCl. (*S*)-3-Acetoxy-γ-butyrolactone was extracted with the methylene chloride. After work up, the same was purified with silica gel column chromatography. The obtained (*S*)-3-acetoxy-γ-butyrolactone was dissolved in methylene chloride, and 0.5㎕ was taken with syringe for GC analysis. The result is shown in the following Table 2 and Figs. 1a~1c.

Table 2

Source Material	Optical Purity
Disaccharide (maltose) [a)]	94%ee
Oligosaccharide of the present invention (Example 2)	99.9%ee
[a)]Examples 1 & 2 of USP 5,292,939, 5,319,110 & 5,374,773	

To improve the medicinal efficiency and minimize the side effect, more than 99.5%ee of high optical purity is required for chiral compounds. Table 2 and Figs. 1a~1c show that the optical purity of (*S*)-3-hydroxy-γ-butyrolactone prepared from oligosaccharide of the present invention is very high as 99.9%ee. So, the same is very useful for the intermediates of other chiral compounds. The results are illustrated in Figure 1a, 1b and 1c, respectivly.

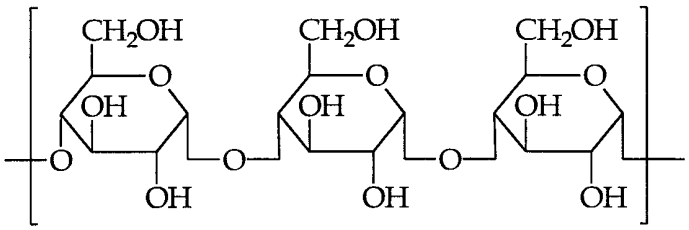
The preparing method of the present invention gives optically pure (*S*)-3-hydroxy-γ-butyrolactone, which is very useful for industrial uses because the by-product formation is minimized and the purification process is very simple. It comprises alkaline oxidation of α-(1,4)-linked oligosaccharide from the enzymatic reaction of amylose in a specific condition followed by esterifying and cyclizing to afford the target product. The present invention has overcome the disadvantage of using expensive metal catalyst for selective asymmetric reduction, and enables easy preparation from inexpensive natural product having optically pure chiral center, thereby the industrial

utility as chiral intermediates of various medicine can be maximized. Furthermore, the relative mass conversion yield is almost double compared with disaccharides.

CLAIMS

What is claimed is:

1. A process for preparing α-(1,4) linked oligosaccharide expressed by the Formula 1 by α–amylase enzymatic reaction of amylose under the condition of pH 4.0~8.0 and 40~120℃.



(1)

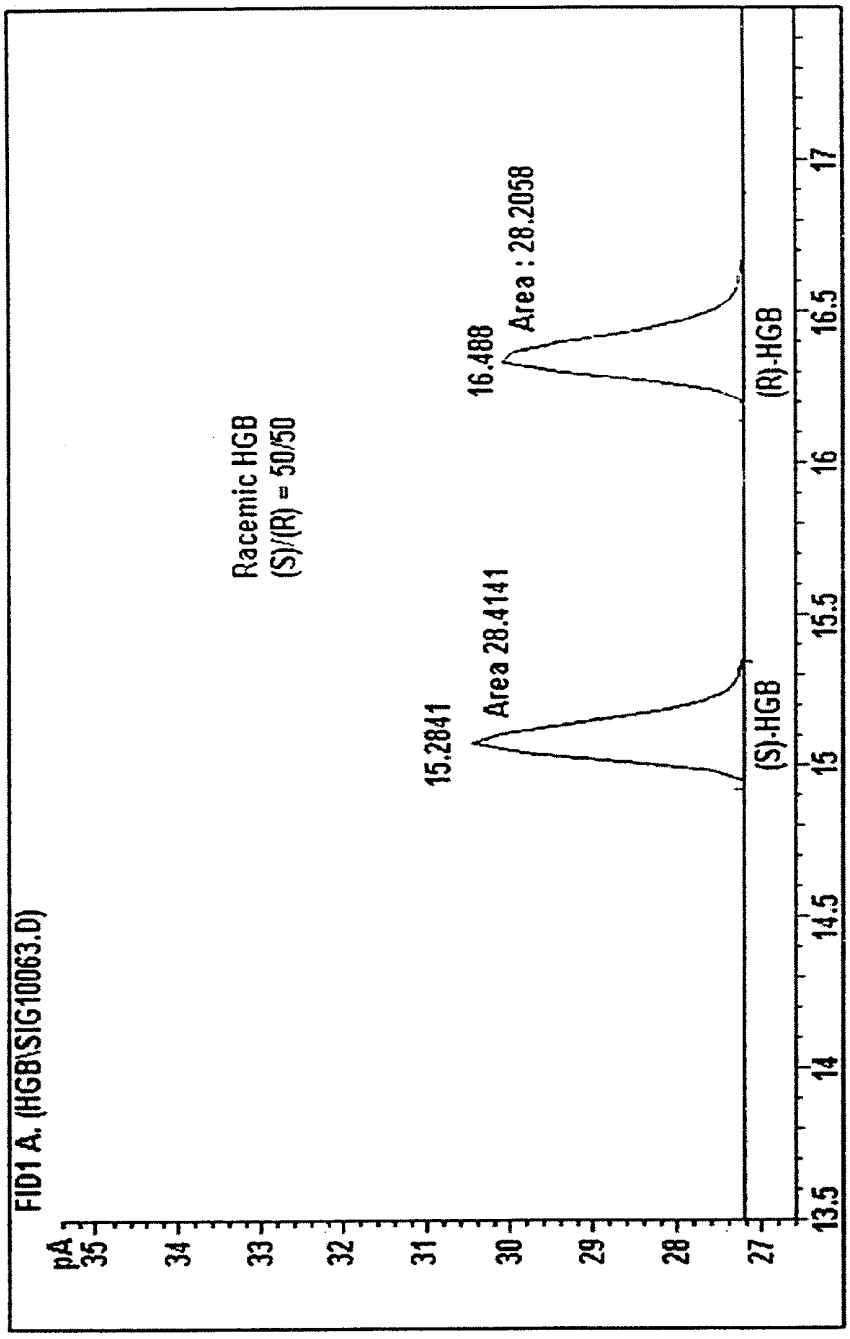
2. The process according to claim 1, wherein the said enzymatic reaction is performed in water or in pH 4~8 buffer solution.

3. The process according to claim 1, wherein the said α-amylase is used in the range of 0.001~10 wt% of amylose.

4. The process according to claim 1, wherein the said oligosaccharide has the range of 3~50 glucose units.

5. The process according to claim 1, wherein the remaining α-amylase after the α-amylase enzymatic reaction is inactivated under the condition of pH 2.0~4.5 and temperature of 60~150℃.

Fig. 1a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pA
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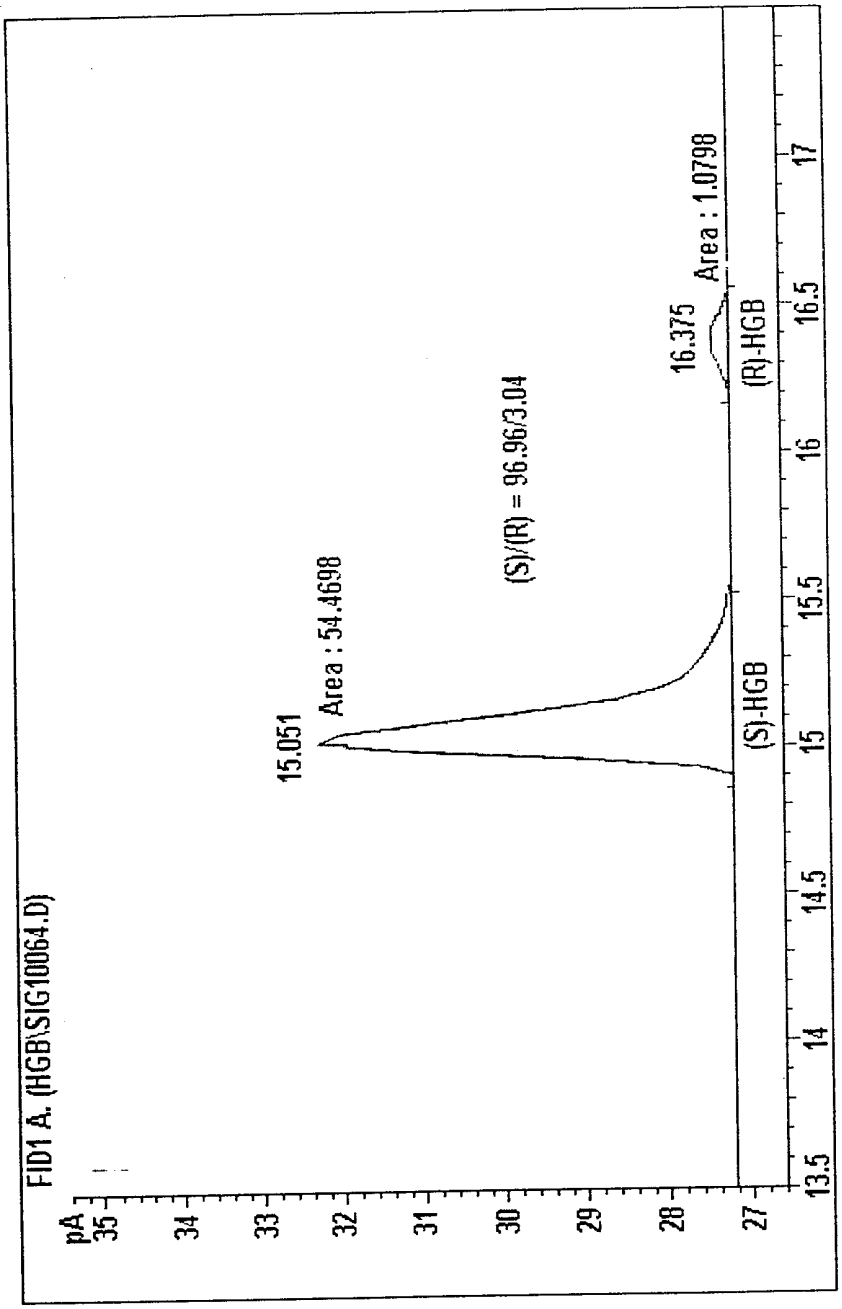
34
33
32
31
30
29
28
27
13.5
14
14.5
15
15.5
16
16.5
17
Racemic HGB
(S)/(R) = 50/50
15.2841
Area 28.4141
(S)-HGB
16.488
Area : 28.2058
(R)-HGB

Fig. 1b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32
31
30
29
28
27
13.5
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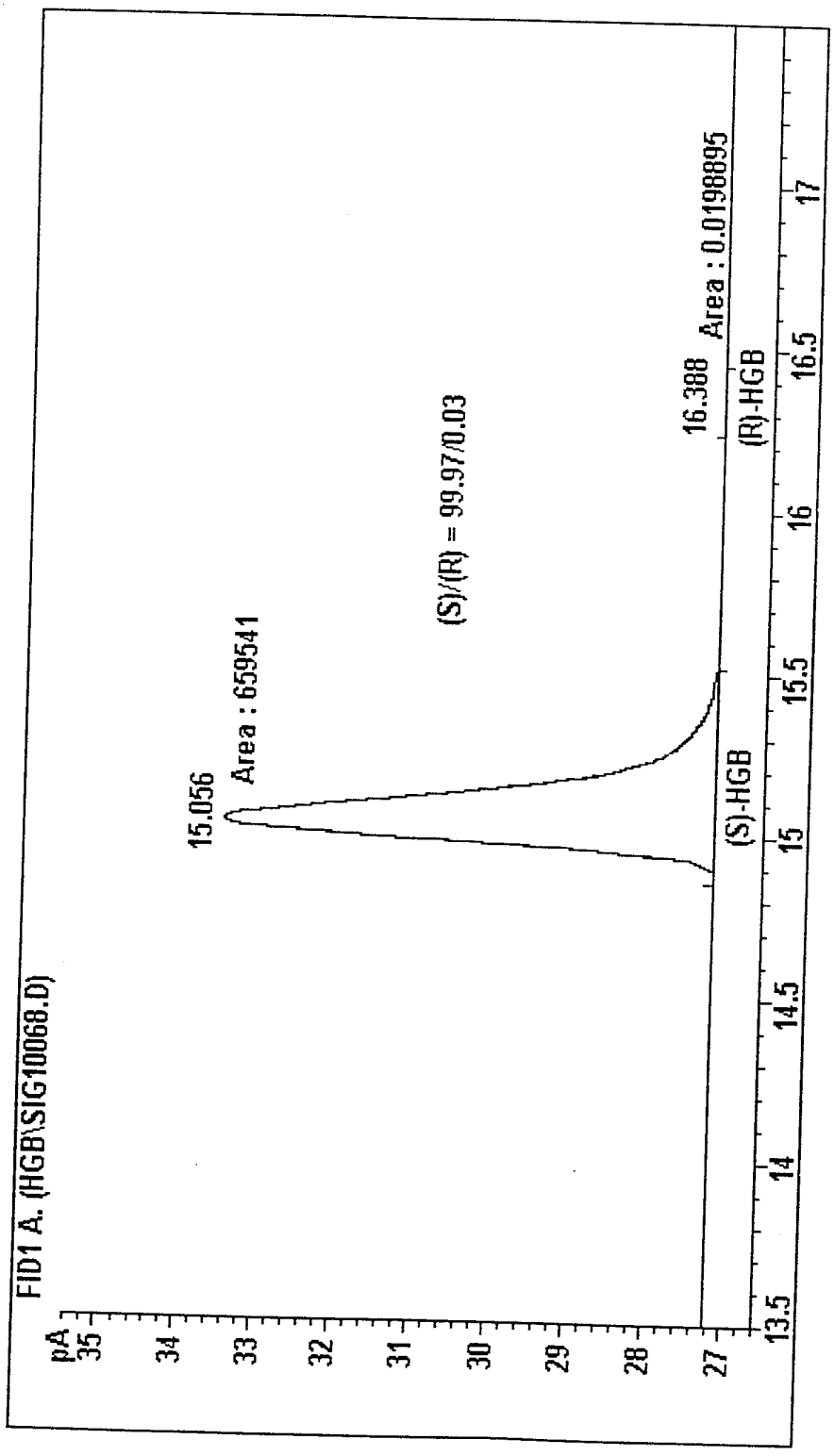
14.5
15
15.5
16
16.5
17
15.051
Area : 54.4698
(S)/(R) = 96.96/3.04
16.375
Area : 1.0798
(S)-HGB
(R)-HGB

Fig. 1c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32
31
30
29
28
27
13.5
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14.5
15
15.5
16
16.5
17
15.056
Area : 659541
(S)/(R) = 99.97/0.03
16.388
Area : 0.0198895
(S)-HGB
(R)-HGB

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 99/00397

A. CLASSIFICATION OF SUBJECT MATTER

IPC[7]: C 12 P 19/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: C 12 P 19/14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	US 5108913 A (RAUSCHER et al.) 28 April 1992 (28.04.92) abstract.	1
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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 November 1999 (08.11.99)	06 December 1999 (06.12.99)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/200	Authorized officer Wolf Telephone No. 1/53424/436

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

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International application No.
PCT/KR 99/00397

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International application No.
PCT/KR 99/00397

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