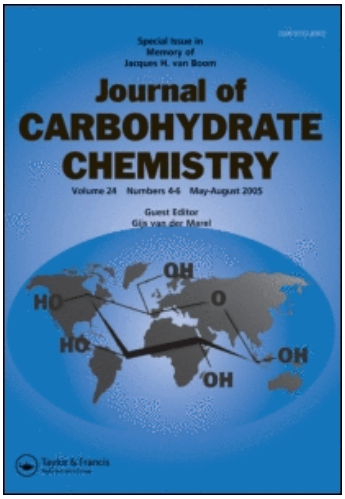

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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Masayasu Takada; Koichi Ogawa; Takeomi Murata; Taichi Usui

To cite this Article Takada, Masayasu , Ogawa, Koichi , Murata, Takeomi and Usui, Taichi(1999) 'Inhibition of Human α-Amylases By Synthetic 6³-*O*-Benzyl-and 6³-*O*-β-D-Galactosyl Maltotrionolactones', Journal of Carbohydrate Chemistry, 18: 2, 149 — 163

To link to this Article: DOI: 10.1080/07328309908543988

URL: http://dx.doi.org/10.1080/07328309908543988

J. CARBOHYDRATE CHEMISTRY, 18(2), 149-163 (1999)

INHIBITION OF HUMAN α-AMYLASES BY SYNTHETIC 6^3-*O*-BENZYL- AND 6^3-*O*-β-D-GALACTOSYL MALTOTRIONOLACTONES

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Received May 25, 1998 - Final Form November 5, 1998

ABSTRACT

Maltooligosaccharides were transformed into both endo-modified oligosaccharidonolactones as substrate analogue inhibitors for human α-amylases. *p*-Nitrophenyl 6^5-*O*-benzyl-α-maltopentaoside (**1**) and *p*-nitrophenyl 6^5-*O*-β-D-galactosyl-α-maltopentaoside (**11**), which have been synthesized as substrates for human α-amylases in serum, were selectively hydrolyzed by specific α-amylases to afford 6^3-*O*-benzyl maltotriose (**8**) and 6^3-*O*-β-D-galactosyl maltotriose (**10**), respectively. Both the modified oligomers were chemically oxidized to 6^3-*O*-benzyl maltotrionolactone (**6**) and 6^3-*O*-β-D-galactosyl maltotrionolactone (**7**), respectively. Compound **6**, which is a strong competitive inhibitor, had K_i values of 2.8 and 9.6 × 10^{-6} M for human salivary α-amylase (HSA) and human pancreatic α-amylase (HPA), respectively. On the other hand, the inhibition activity of **7** was about ten times less than **6**. These results indicated that the 6-*O*-substituted hydrophobic benzyl group enhances the binding with enzymes, whereas the corresponding hydrophilic 6-*O*-β-D-galactosyl substituted product weakens binding.

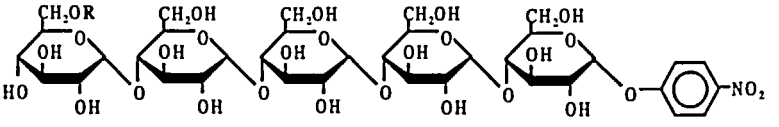
INTRODUCTION

The *O*-benzyl-maltopentaoside **1** and *p*-nitrophenyl 4^5-*O*-β-D-galactosyl-α-maltopentaoside (**2**), have been accepted as suitable substrates for α-amylase assay in diagnosis for pancreatic disease.[1-5] These facts prompted us to design substrate analogue inhibitors for human α-amylases. We have recently reported the chemo-enzymatic transformation of maltooligosaccharides into both end-modified oligosaccharidonolactones, 4^2-*O*-β-D-galactosyl maltobionolactone (**3**) and 4^3-*O*-β-D-galactosyl maltotrionolactone (**4**), as substrate analogue inhibitors for mammalian α-amylases.[6] In this case, the terminal 4-*O*-subsituted galactosyl residue of the oligosaccharidono-lactones does not act as a barrier on the binding with the enzymes and exhibits tolerance to digestion by α-glucosidase and glucoamylase. In the same regard, 6-*O*-benzyl- (**6**) and -galactosyl derivatives (**7**) of maltotrionolactone (**5**) modified at the terminal glucosyl group were of interest to elucidate a relationship between the structure and the inhibition activity. Systematic trends in inhibition studies on the structural modification of substrates would be helpful in revealing the requirements for binding and catalytic specificity.

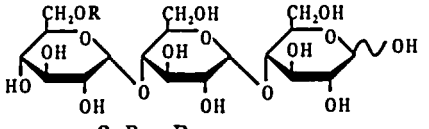
The present paper describes a preparative synthetic method involving the chemo-enzymatic transformation of maltooligosaccharide into 6-*O*-benzyl maltooligosaccharidonolactone (**6**) and 6-*O*-β-D-galactosyl maltooligosaccharidonolactone (**7**) as substrate analogue inhibitors for human α-amylases, with particular focus on the influence of 6-*O*-substituent groups.

RESULTS AND DISCUSSION

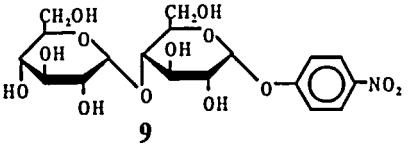
Preparation of 6 and 7. *p*-Nitrophenyl 6^5-*O*-benzyl-α-maltopentaoside **1**, which is a useful substrate for human α-amylases assay in serum, was used as a starting substance for the synthesis of **6**.[9] Thus, **1** was hydrolyzed predominantly to **8** and *p*-nitrophenyl α-maltoside (**9**) by HSA. The resulting product **9** in the reaction mixture was further treated with α-glucosidase and glucoamylase. The chromatographic



1 R = Bn (Benzyl)
11 R = β-D-Gal
12 R = H



8 R = Bn
10 R = β-D-Gal



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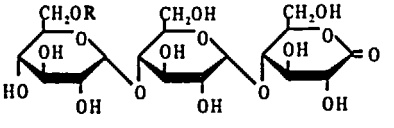
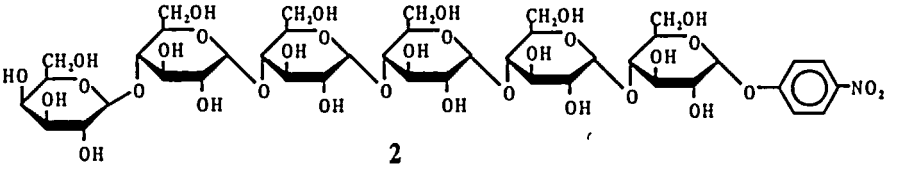


5 R = H
6 R = Bn
7 R = β-D-Gal



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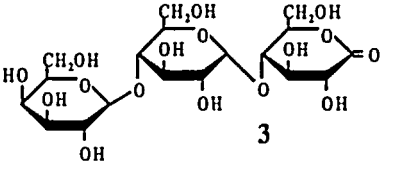

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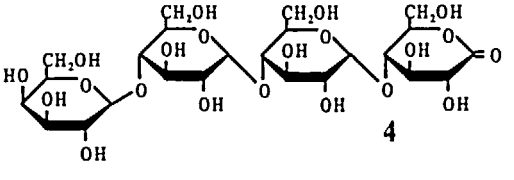


4

separation of the desired compound **8** from **9** was carried out using a Toyopearl HW-40S column. Benzyl maltotriose **8** was obtained in a yield of 26.9 % (w/w) based on **1** added. Galactosyl maltotriose **10** was prepared from **11**, which was synthesized using a *Bacillus circulans* β-D-galactosidase mediated-transglycosylation from lactose to *p*-nitrophenyl α-maltopentaoside (**12**).[8] *p*-Nitrophenyl 6^5-*O*-β-D-galactosyl-α-maltopentaoside **11** was selectively hydrolyzed with an amylase of *Thermo-monospora viridis* (TVA) to give **10** and **9**. The separation of former product from latter was done by successive chromatographies on Duolite S876 and charcoal-Celite columns. Compound **10** was obtained in a yield of 47.6 % (w/w) based on **11** added. Compounds **8** and **10** were then oxidized with potassium hypoiodate in water at 40 °C and the resulting potassium oligosaccharidosyl aldonates were neutralized by passing through an Amberlite IR120B (H^+) column to give **6** and **7** in yields of 85.0 and 66.2 % (w/w) based on **8** and **10**, respectively.

Characterization of 6 and 7. The structures of **6** and **7** were elucidated from their ^1H and ^{13}C NMR spectra. NMR signal assignments were made using ^1H-detected heteronuclear signal quantum coherence spectroscopy. The lactone contents of **6** and **7** in D_2O at 30 °C were determined by a ^1H NMR method. In aqueous solution, a lactone is known to be readily hydrolyzed to its aldonic acid form. In the present study, oxidized product **6** exists in both forms, i. e., the lactone and aldonic acid (6^3-*O*-benzyl maltotrionic acid) forms, even immediately after dissolution. The ^1H NMR spectra of **6** recorded immediately after dissolution and after 24 h were correlated for the glycosidic protons as shown in Figure 1., because the glycosidic proton signals at lower field were the more clearly differentiated. On comparison of the chemical shifts of **6** with those of the corresponding **8**, peaks δ 5.34 (B) and 5.33 (C) ppm were attributed to glycosidic protons involved in H-1' and H-1" due to the lactone form, and peaks δ 5.35 (A) and 5.13 (D) ppm involved in H-1' and H-1" due to the aldonic acid one, respectively. There were significant variations in their intensities with time. Immediately after dissolution in D_2O, the relative intensity of peaks of A + B + C to peak D (1.0) was about 3.3. As a result, the proportion of the lactone and aldonic acid form was calculated to be 70 : 30. After 24 h, the peaks B and C decreased, whereas the peaks A and D increased in relative intensity, corresponding to a change in the proportion of lactone and aldonic acid forms to 29 : 71. These results indicate that the lactone form of **6** was gradually hydrolyzed to

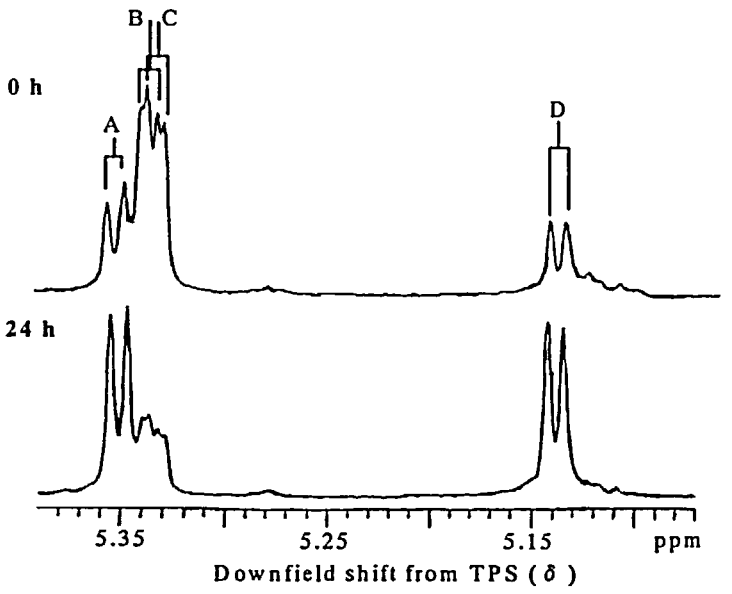


Figure 1. 500 MHz ^{1}H NMR spectra of **6** in D_2O. Compound **6** was analyzed in D_2O (2.5 % w/v) at 30 °C. The proportion of the lactone and aldonic acid forms of **6** was determined from the relative intensities of peaks A and D to peaks B and C.

its open-chain form. The ^{13}C NMR spectra of **6** immediately after dissolution (A) and after 24 h (B) also provide useful information on their composition as shown in Figure 2. All of the different carbon lines were resolved by comparison with earlier data [6,9] and their chemical shifts are recorded in Table 2. By comparison of the relative intensities of spectra A and B, peaks at δ 176.23, 83.65, 78.53, 73.97 and 63.22 ppm, whose signals are attributed to C-1, C-2, C-4, C-3 and C-6 involved in the lactone residue, were clearly observed to decrease with time. The structure assignment of **7** was also based upon NMR. From ^{1}H NMR spectral data, the proportion of the lactone to aldonic acid forms in D_2O was calculated to be 70 : 30, immediately after dissolution, while its proportion changed to 33 : 67 after 24 h. We have recently reported that HPLC was also useful for determining the ratios of the ring and open-chain forms of oxidized compounds in an aqueous solution.[6] The separation patterns of the lactone (L) and aldonic acid (A) forms of **7** in D_2O with time at 30 °C were investigated using a Shodex DC-613 column (data not shown). Thus, peak L was greater than peak A in the initial stage, but this relation was

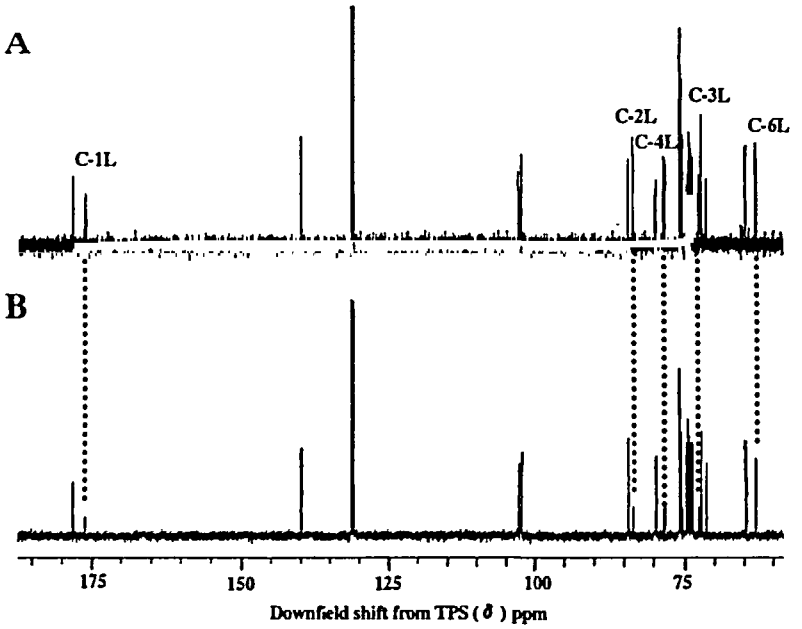


Figure 2. 125 MHz ^{13}C NMR spectra of **6** in D_2O. Compound **6** was analyzed in D_2O (2.5 % w/v) at 30 °C. (A) Immediately after dissolution, (B) After 24 h.

reversed after 24 h. A sequence of structural changes similar to these were also observed using the NMR analysis mentioned above, although the analytical conditions were different. However, the ratio of the lactone to aldonic acid forms of **6** could not be determined by HPLC, because the peaks due to lactone form overlapped the H_2O peak.

Inhibition Analysis. Prior to α-amylase inhibition study, the ratio of the lactone to aldonic acid forms of **7** with time was determined by HPLC. When the inhibition reaction was followed for 5 min, the extent of the conversion corresponded to less than 5 % of the initial lactone content. Consequently, the inhibition study was based on the assumption that the lactone form of **7** is not converted into the aldonic acid one in the initial stage of reaction (5 min). This was also applied to the case of **6**.

Kinetic studies have been done on the effect of inhibition on the hydrolysis of **2**. The mode of inhibition of **6** and **7** for human α-amylases was shown to be competitive by Dixon plots as shown in Figure 3. Furthermore, the K_i values of **6** and **7** for the two enzymes were determined as in Table 1. Compound **6** was shown to be a strong

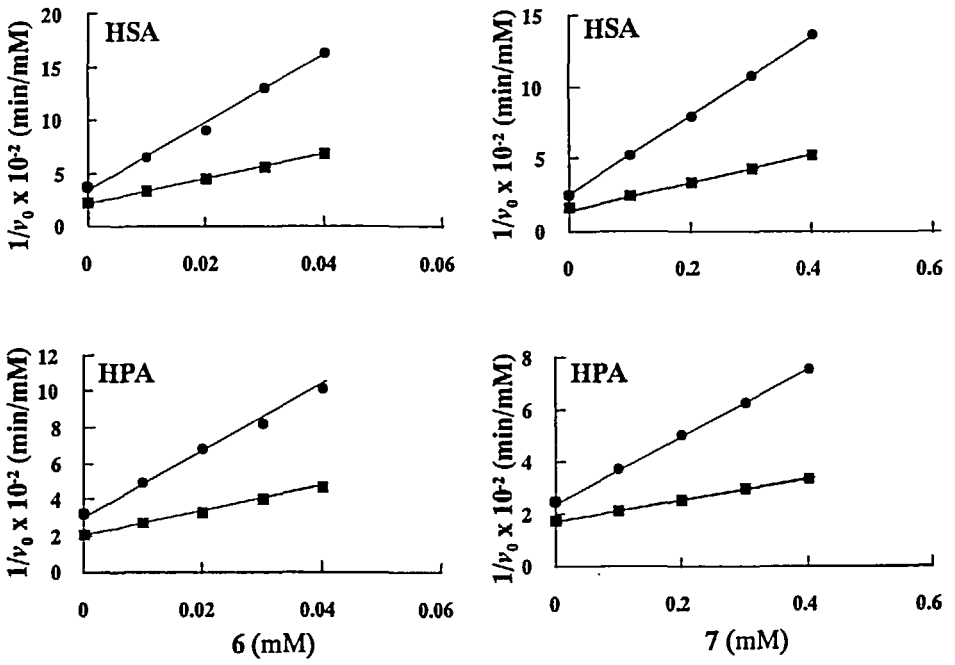


Figure 3. Dixon plots of inhibition of α-amylases by **6** and **7**. The inhibition assay is described in the **EXPERIMENTAL** section. Substrate concentration; ●: 0.1 mM and ■: 0.25 mM.

Table 1. K_i (M) values of mammalian α-amylases

Lactone	HSA	HPA
5*	5.0×10^{-6}	1.7×10^{-5}
3*	4.9×10^{-6}	1.8×10^{-5}
4*	3.4×10^{-6}	1.1×10^{-5}
6	2.8×10^{-6}	9.6×10^{-6}
7	4.5×10^{-5}	8.0×10^{-5}

* See ref. 6

competitive inhibitor with K_i values of 2.8 and 9.6 × 10^{-6} M for HSA and HPA, respectively. These values were about twice that of **5** which served as a control sample, despite the introduction of the hydrophobic benzyl group at the terminal position. In contrast, the K_i values of **7** were five to nine times less than those of **5**. These results

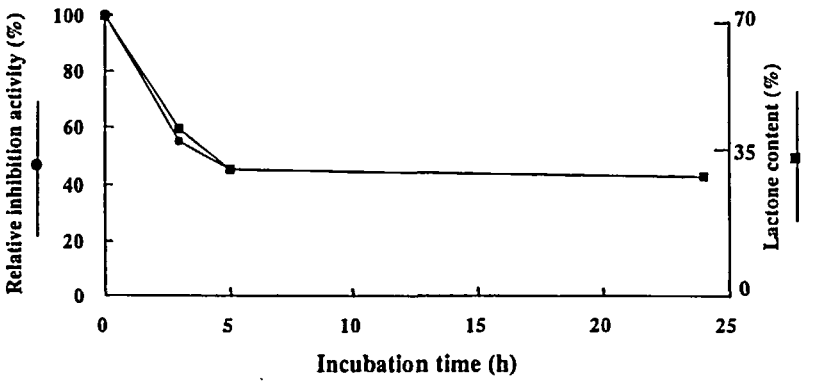


Figure 4. Time course relationship between the lactone content of **6** and its inhibition activity toward HSA. The remaining lactone contents of **6** were analyzed by ^{1}H NMR. The inhibition activity was conveniently determined as the amount of **6** required to give 50 % inhibition (IC_{50}) toward HSA by the Dixon plot method. The IC_{50} of **6** immediately after dissolution was arbitrarily set at 100 %.●; lactone content, ■; relative inhibition activity (%).

indicated that 6-*O* substituted benzyl group enhances the binding with the enzymes, while the corresponding hydrophilic galactosyl group weakens the binding. In this process, the two synthetic inhibitors **6** and **7** were not hydrolyzed by human α-amylases during the entire course of reaction. We have recently reported that **3** and **4** are strong inhibitors for HSA, HPA and PPA (Porcine pancreatic α-amylase), and exist in the active lactone and inactive aldonic acid forms.[6] There was a distinct correlation between the lactone content and the inhibition activity with time. This was also the case for **6**. The time course of the lactone content was compared with that of the relative inhibition activity as shown in Figure 4. It shows a good correlation between the lactone content and inhibition activity with time. The inhibition activity showed a remarkable decrease (40 % of the value) within 5 h, but only a gentle decrease during the subsequent reaction. Its profile well corresponds to that of the lactone content with respect to time. It confirms that only the lactone form takes part in the occurrence of the enzyme inhibition. A similar result was also observed with **7**. The active sites of HSA and HPA have been shown to have five binding sites for glucose residues of the substrates.[15] Taking into account the earlier data and present inhibition kinetic results, we reasonably concluded that **6** is bound to subsites geometrically complementary to three glucose residues, 1, 2,

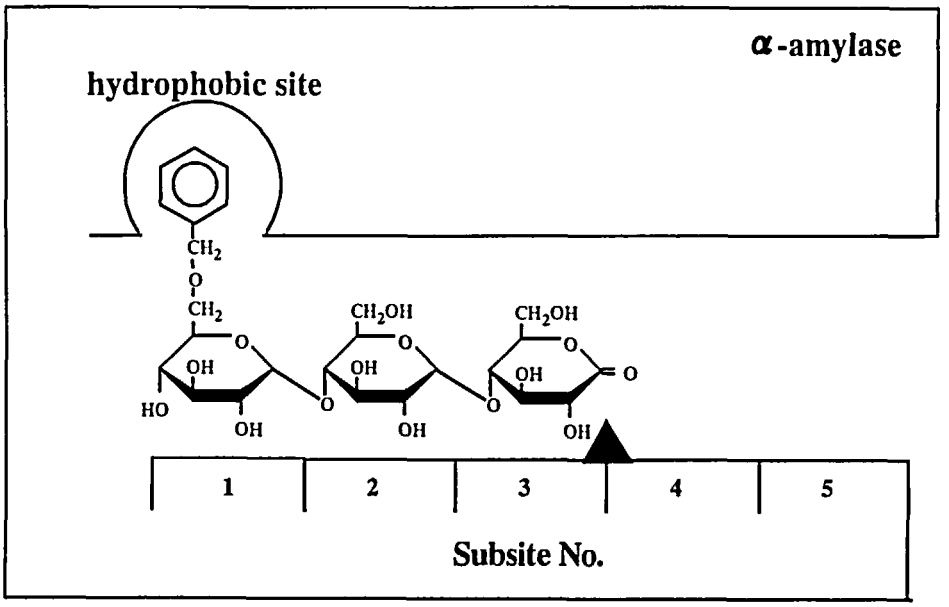


Figure 5. Binding models of **6** on the subsites of α-amylases. The arrow head indicated the catalytic site and each box numbered in order represents a subsite.

and 3 as shown in Figure 5. Such binding is expected to result in competitive inhibition as to the substrates. Thus, the lactone form of **6** is favorably located in subsite 3 and the 6-*O*-benzyl group serves as hydrophobic binding group in Figure 5. Such binding is expected to act as a transition state analogue of the substrate and induce strong inhibition. Some researchers have reported *p*-nitrophenyl α-maltooligosaccharides with a benzyl group or pryridyl amino group at C-6 of the non-reducing D-glucosyl residue are suitable as substrates for human α-amylases and cause a decrease in K_m values and an increase in the relative rate of hydrolysis.[13, 14] This added strong support for the suggestion that hydrophobic site is present near the subsite 1 located at terminal of the five binding sites. On the contrary, the enzyme does not much accept **7** as inhibitor in which hydrophobic benzyl group replaces the hydrophilic galactosyl group. The decrease of inhibition of **7** is explained on the basis of a mutual repulsion of the hydrophilic galactosyl group with a hydrophobic site located in the neighborhood of the subsites mentioned above, because the K_i values for **7** were 4.7 ~ 16 and 4.0 ~ 13 times greater than those for **6** and **4**, respectively. As a result, the inhibition of **7** and **4** was shown to be remarkably

influenced by the position of the substituted galactosyl group. Such studies of enzyme inhibition permit us to map the active sites of enzymes.

In conclusion, the chemo-enzymatic process for obtaining the 6-*O*-substituted derivatives of maltooligosaccharidonolactones **6** and **7** is simple and the yield is sufficiently high to make the method practical. Both endo-modified oligosaccharidonolactones as transition-state analogue inhibitors were useful tools for elucidating the substrate specificity of HSA and HPA.

EXPERIMENTAL

General Procedure. HPLC analysis for sugars was done using a Shodex DC-613 column (6.0 × 150 mm) or Asahipak GS-220HQ column (7.5 × 300 mm) in a Hitachi 6000-series liquid chromatography equipped with a Hitachi L-4000 ultraviolet detector (220 nm) and L3350 RI monitor. Elution of the former column was effected with 30:70 (v/v) H_2O-acetonitrile at room temperature and that of the latter with distilled water at 65 °C. The flow rate was 0.9 mL/min and 0.6 mL/min, respectively. Analysis for oxidized sugars was also done under the following condition: Shodex DC-613 column (6.0 × 150 mm); mobile phase, 30:70 (v/v) H_2O-acetonitrile containing 0.1 M $NaNO_3$; flow rate, 0.8 mL/min; temperature, 30 °C; and detection, RI. Before injection, 0.2 mL of the reaction digest containing about 2 mg of each lactone was mixed thoroughly with 0.2 mL of 0.2 M $NaNO_3$ solution, and then the mixture was injected into the apparatus immediately. NMR spectra were measured on JEOL JNM-EX 270 and JNM-EX 500 spectrometers in D_2O using sodium 4,4-dimethyl-4-silapropanoate (TPS) as an internal standard. FABMS analyses were carried out in the positive- and negative-ion mode using a JEOL DX -303 HF mass spectrometer, coupled to a JEOL DA-500 data system operating at the full accelerating potential (3KV). The molecular weight of the sample was estimated from the *m/z* value of the quasi-molecular-ion $[M+1]^+$ peak. Specific rotations were determined with a Digital Automatic Polarimeter PM-101 apparatus (Union Giken Corp., LTD).

Materials. Crude β-D-galactosidase (5,420 IU/g, *Bacillus circulans*) and yeast α-glucosidase (14 U/mg) were obtained from Daiwa Kasei (Japan) and Oriental yeast

Co., LTD (Japan), respectively. A saccharogenic α-amylase of TVA (2,000 U/mL) was purified according to the method of Muramatsu *et al.*[7] Cyclomaltodextrin glucanotransferase (10,000 U/mL, BMA) of *B. macerans*, glucoamylase (44 U/mg, GA Amano II, *Rhizopus sp.*), and β-amylase (20,000 U/mL, Biozyme L, *Asp. Niger*) were obtained from Amano Seiyaku (Japan). A series of *p*-nitrophenyl α-maltooligosaccharides were purchased from Calbiochem-Boehring (USA). Alpha cyclodextrin (α-CD) was a product of Nihon Shokuhin Kako (Japan). Compounds **1** and **11** were prepared according to our method.[8,9] Human salivary α-amylase (HSA) from Sigma Chemical (USA) was purified as described by Mayo and Carlson.[10] Human pancreatic α-amylase (HPA), which was purified by the method of Matsuura *et al.*,[11] was a generous gift from Dr. Watazu of Kokusai Shiyaku (Japan). Other chemicals were of the highest quality available commercially.

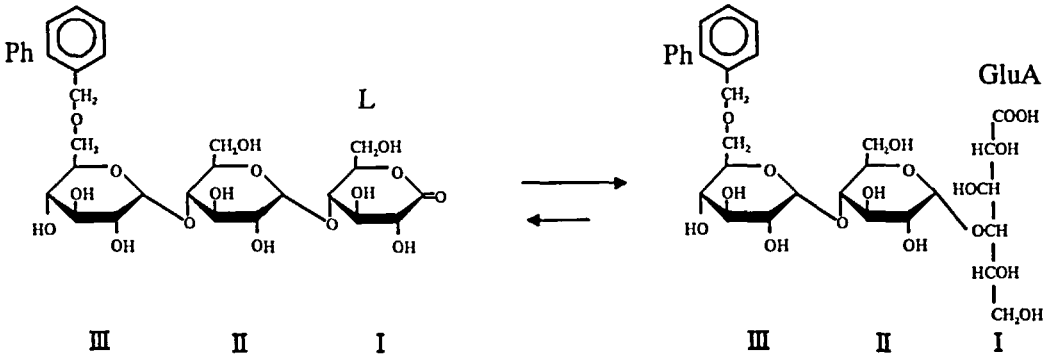
Enzyme Assay. Beta-D-galactosidase activity was determined by the following method; The reaction mixture (1.0 mL) consisting of 2 mM *o*-nitrophenyl β-D-galactoside, 20 mM Na-phosphate buffer (pH 6.0) and the enzyme solution (0.1 mL) was incubated at 40 °C for 10 min. The reaction was terminated by adding 0.1 M Na_2CO_3 solution (5 mL) and then *o*-nitrophenol liberated was determined at 420 nm. One unit of the enzyme activity was defined as the amount of enzyme that formed 1 μmol of *o*-nitrophenol per minute under this condition. TVA and glucoamylase activities were determined according to our method.[6]

Assay of Inhibition Activity. The inhibition of α-amylase activities by some oligosaccharidonolactones was determined under the following condition. The reaction mixture (2.0 mL) containing 0.25 mM or 0.10 mM **2**, 100 mM HEPES buffer (pH 7.3) containing 60 mM sodium chloride, 1.5 mM potassium chloride, 0.60 % (w/v) α-CD and the coupling enzymes (30 U/mL glucoamylase and 15 U/mL α-glucosidase) was incubated at 37 °C for 5 min preliminary. The reaction was done with about 0.08 U of α-amylase in the presence of 0 ~ 40 μM of **6** and 0 ~ 0.5 mM of **7**, respectively. The reaction was monitored by the increase in absorbance at 405 nm. The inhibition constant (K_i) for various oxidized sugars was calculated from the Dixon and Webb plots.[12] The K_m (mM) values with **2** were 0.10 mM for HSA and 0.12 mM for HPA, respectively.[8]

(a) Preparation of 8. Compound **1** (3.0 g), dissolved in 100 mL of 20 mM K-phosphate buffer (pH 6.9), was incubated with 30 U of HSA at 37 °C for 12 h. After the reaction was terminated by heating, insoluble materials were removed by centrifugation. The pH of the supernatant was adjusted to 6.0 with 4.0 % HCl. The solution was incubated with α-glucosidase (20 U) and glucoamylase (30 U) at 37 °C for 12 h. After termination of the reaction by heating, the resulting insoluble materials were removed by centrifugation. The supernatant was loaded onto a Toyopearl HW-40S column (φ2.6 × 90 cm). The eluate (10 mL fractions) showed two main peaks (F-I: tube 27 ~ 34 and F-II: tube 40 ~ 43). The fractions corresponding to F-II were concentrated and lyophilized to afford **8** (807 mg): $[\alpha]_D^{25}$ +130.41° → +127.80° (*c* 1, H_2O); FAB-MS *m/z* 595 $[M+H]^+$; ^1H NMR (D_2O) δ 5.22 (d, 1H, J = 3.96 Hz, H-1α), 4.66 (d, 1H, J = 7.5 Hz, H-1β), 5.38 (d, 1H, J = 3.63 Hz, H-1'), 5.37 (d, 1H, J = 3.30 Hz, H-1''); ^{13}C NMR (D_2O) δ 140.16 (C-Ph), 131.59 (*m*-Ph), 131.25 (*o*-Ph), 131.16 (*p*-Ph), 102.59 (C''-1), 102.42 (C'1-α), 102.35 (C'1β), 98.65 (C-1β), 94.77 (C-1α), 80.07 (C-4α), 79.84 (C-4β, C'-4), 79.03 (C-3β), 77.43 (C-5β), 76.85 (C-2β), 76.15 (C3), 76.10 (C''-3), 74.55 (C'-5), 74.48 (C''-2), 74.16 (C-5α), 74.09 (C'-2), 74.36 (C-3α), 72.83 (C-2α), 72.43 (C''-4, CH_2Ph), 71.59 (C''-6), 63.58 (C-6β), 63.64 (C'-6, C-6).

(b) Preparation of 6. Compound **8** was oxidized according to our method.[6] 6^3-*O*-benzyl maltotriose **8** (400 mg) was dissolved in water (8.4 mL) and added to an iodine (548 mg) solution in methanol (10.52 mL) at 40 °C. At this temperature, a 4 % w/v potassium hydroxide solution in methanol was added dropwise with magnetic stirring for 50 min until the color of iodine disappeared. The solution was concentrated to a syrup and crystallized from a mixture of ethanol and water (5:1 v/v). The resulting potassium lactonate was then converted into the free acid by passing the aqueous solution through a column of Amberlite IR-120B (H^+) (φ3.5 × 10 cm). The acidic eluate was collected and concentrated to a small volume (3 mL). The solution was loaded onto the same Toyopearl HW-40S column for removal of the iodine. The eluate (10 mL fractions) showed one main peak (F-I: tube 28 ~ 32). The fractions corresponding to F-I were concentrated and reconcentrated several times from aqueous solution. The concentrate was dissolved in a small amount of water and lyophilized to afford **6** (340 mg): $[\alpha]_D^{25}$

Table 2. ^{13}C chemical shifts of **6**

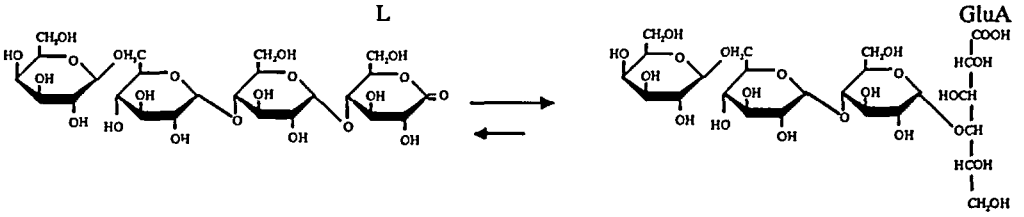


		C-1	C-2	C-3	C-4	C-5	C-6
I	L	176.23	83.65	73.97	78.53	72.84	63.22
	GluA	178.38	74.99	74.70	84.44	74.24	64.84
II	L	102.48	74.08	76.09	79.70	74.14	63.22
	GluA	103.03	74.08	76.09	79.70	74.14	63.22
III		102.56	74.49	76.01	72.37	75.70	71.51
		m-Ph	*o*-Ph	*p*-Ph	C-Ph	CH_2Ph	
Ph		131.59	131.25	131.16	140.16	72.43	

+108.24° → +108.56° (*c* 1, H_2O); FAB-MS *m/z* 593 $[M+H]^+$ and *m/z* 611 $[M+H]^+$ attributed to lactone and its aldonic acid forms, respectively; ^1H NMR (D_2O) δ 5.34 (d, 1H, J = 3.66 Hz, H-1'), δ 5.33 (d, 1H, J = 3.97 Hz, H-1"): anomeric protons attributed to lactone form. δ 5.13 (d, 1H, J = 3.97 Hz, H-1'), 5.35 (d, 1H, J = 3.97 Hz, H-1"): anomeric protons attributed to aldonic acid form. The ^{13}C NMR data are shown in Table 2.

Preparation of 10. Compound **11** (600 mg) dissolved in 50 mL of 20 mM Na-phosphate buffer (pH 7.0) was incubated with TVA (60 U) at 40 °C for 12 h. After the reaction was stopped by heating, the pH of the solution was adjusted to 6.0 and further treated with glucoamylase (60 U) and α-glucosidase (60 U) at 37 °C for 20 h. After termination of the reaction by heating, the resulting insoluble materials were removed. The supernatant was loaded onto a Duolite S876 column (6.0 × 30 cm). The non-

Table 3. ^{13}C chemical shifts of **7**



		C-1	C-2	C-3	C-4	C-5	C-6
I	L	176.35	83.74	73.80	76.21	72.92	63.36
	GluA	178.58	74.82	74.57	84.56	74.32	64.92
II		103.11	73.80	75.08	79.96	74.14	63.36
III		102.71	74.32	76.03	72.06	75.69	71.32
IV		106.23	73.69	75.60	71.57	78.06	63.92

adsorbed fraction was loaded onto a charcoal-Celite column (1:1 by weight, 6.0 × 30 cm). The column was first washed with H_2O (3L) and then eluted with 14.0 % ethanol (1L). The eluted fraction was concentrated and lyophilized to afford 286 mg of a white powder: $[\alpha]_D^{25}$ +108.25° → +106.01° (*c* 1, H_2O); FAB-MS *m/z* 667 $[M+H]^+$; ^1H NMR (D_2O) δ 5.23 (d, 1H, J = 3.96 Hz, H-1α), 4.66 (d, 1H, J = 7.91 Hz, H-1β), 5.40 (d, 1H, J = 3.62 Hz, H-1'), 5.39 (d, 1H, J = 3.30 Hz, H-1''), 4.44 (d, 1H, J = 7.59 Hz, H-1'''); ^{13}C NMR (D_2O) δ 106.25 (C'''-1), 102.78 (C''-1), 102.35 (C'-1), 98.65 (C-1β), 94.77(C-1α), 80.14 (C-4α), 79.89 (C'-4), 79.03 (C-3β), 79.89 (C-4β), 78.04 (C'''-5), 77.43 (C-5β), 76.85 (C-2β), 76.17 (C'-3), 76.05 (C''-3), 75.67 (C''-5), 75.58 (C'''-3), 74.55 (C'-5), 74.43 (C''-2), 74.34 (C-3α), 74.14 (C-5α), 74.02 (C'-2), 73.67 (C'''-2), 72.83 (C-2α), 72.06 (C''-4), 71.57 (C'''-4), 71.37 (C''-6), 63.90 (C'''-6), 63.58 (C-6β), 63.47 (C-6α, C'-6).

(b) Preparation of 7. Compound **10** (207 mg) was oxidized to **7** (137 mg) in the same manner as **8**: $[\alpha]_D^{25}$ +98.91° → +97.56° (*c* 1, H_2O); FAB-MS *m/z* 665 $[M+H]^+$

and *m/z* 683 $[M+H]^+$ attributed to lactone and its aldonic acid forms, respectively; ^1H NMR (D_2O) δ 5.42 (d, 1H, J = 4.29 Hz, H-1'), 5.40 (d, 1H, J = 3.95 Hz, H-1''), 4.45 (d, 1H, J = 7.59 Hz, H-1'''): anomeric protons attributed to lactone form. δ 5.20 (d, 1H, J = 3.63 Hz, H-1'): anomeric protons attributed to aldonic acid form. The ^{13}C NMR data are shown in Table 3.

ACKNOWLEDGMENT

This work was supported by Leading Research Utilizing Potential of Regional Science & Technology from Science and Technology Agency, Japan.

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