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Synthesis of branched cyclomaltooligosaccharide carboxylic acids (cyclodextrin carboxylic acids) by microbial oxidation☆

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Abstract

Novel branched cyclomaltooligosaccharide carboxylic acid (cyclodextrin carboxylic acid) derivatives were synthesized by microbial oxidation using *Pseudogluconobacter saccharoketogenes* to oxidize five types of branched cyclodextrins, including maltosyl β-cyclodextrin (maltosyl-β-CyD). For each novel cyclodextrin carboxylic acid derivative synthesized, the hydroxymethyl group of the terminal glucose residue in the branched part of the molecule was regiospecifically oxidized to a carboxyl group to give the corresponding uronic acid. In addition, the physicochemical properties of cyclomaltoheptaosyl-(6→1)-α-D-glucopyranosyl-(4→1)-α-D-glucopyranosiduronic acid (GUG-β-CyD) (**1**) and its sodium salt were studied more extensively, as these compounds are most likely to have a practical application. © 2001 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Pseudogluconobacter saccharoketogenes*; Microbial oxidation; Branched cyclodextrin carboxylic acids; Branched cyclomaltooligosaccharide carboxylic acids; Novel cyclodextrin derivatives; GUG-β-CyD

1. Introduction

The potential medical and food uses of natural cyclomaltooligosaccharides (cyclodextrins, CyDs) and their synthetic derivatives have been extensively studied in order to improve certain properties of the drugs, such as solubility, stability, and/or bioavailablity.[2,3] Though many CyDs have been synthesized, there are very few examples currently in medical use because of the difficulty of producing practical yields of pure CyDs on an industrial scale.

In order to develop novel uses of cyclodextrin derivatives, we have investigated the synthesis of branched cyclodextrin carboxylic acids in which the terminal glucose residue in the branched part of CyD is regiospecifically oxidized to a carboxyl group using alcohol dehydrogenase. Although several bacterial alcohol dehydrogenases are known (e.g., from the genera *Acetobacter*,[4] *Gluconobacter*,[5] *Pseudomonus*[6]), they are specific to lower alkyl alcohols and monosaccharides only. Furthermore, while catalytic oxidation of primary

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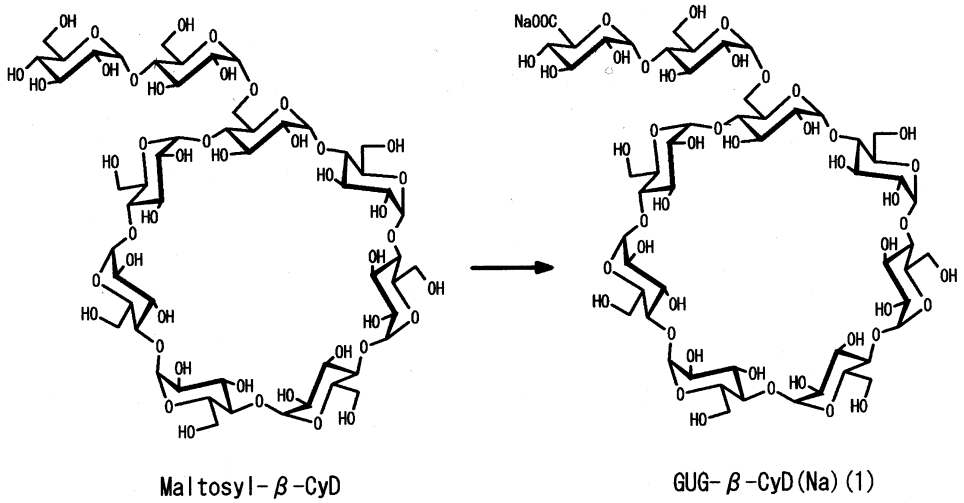


Scheme 1. Synthesis of GUG-β-CyD (Na) (**1**) by microbial oxidation using *Pseudogluconobacter saccharoketogenes*.

alcohols using the nitroxyl radical, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), has been used in organic synthesis,[7–9] the regiospecificity of this reaction is limited.

In the course of our study of the substrate specificity of *Pseudogluconobacter saccharoketogenes (P. saccharoketogenes)*, we found a remarkably broad specificity for oxidative substrates including oligosaccharides and saccharide derivatives.[1,10,11] Among these compounds, maltosyl β-CyD, a branched cyclodextrin, is a good substrate for cellular oxidation by *P. saccharoketogenes*, giving cyclomaltoheptaosyl-(6→1)-α-D-glucopyranosyl-(4→1)-α-D-glucopyranosiduronic acid (GUG-β-CyD) (**1**).

In this paper, we describe a convenient method for the synthesis of **1** (GUG-β-CyD) using the alcohol dehydrogenase activity of *P. saccharoketogenes*. We also describe synthesis of five novel branched cyclodextrin carboxylic acid derivatives. Of these compounds, **1** is most likely to have a practical application (Scheme 1).

2. Results and discussion

Microbial oxidation of branched CyDs by P. saccharoketogenes.—Resting cells of *P. saccharoketogenes* Rh 47[12–17] were incubated with branched CyDs (glucosyl-α-CyD, glucosyl-β-CyD, maltosyl-α-CyD, maltosyl-β-CyD, and maltosyl-γ-CyD) and β-CyD at 32 °C for 22 hours in water adjusted to pH 6.3 with 1 N NaOH. Samples of the reaction mixture were removed, for analysis by HPLC. The time course of microbial oxidation of maltosyl-β-CyD is shown in Fig. 1. After 22 h of incubation, maltosyl-β-CyD was completely oxidized. Similar oxidation experiments were carried out with the other branched CyD substrates, yielding branched cyclodextrin carboxylic acid derivatives **2**, **3**, **4**, and **5** for glucosyl-α-CyD, glucosyl-β-CyD, maltosyl-α-CyD and maltosyl-γ-CyD, respectively. However, β-CyD was not oxidized at all.

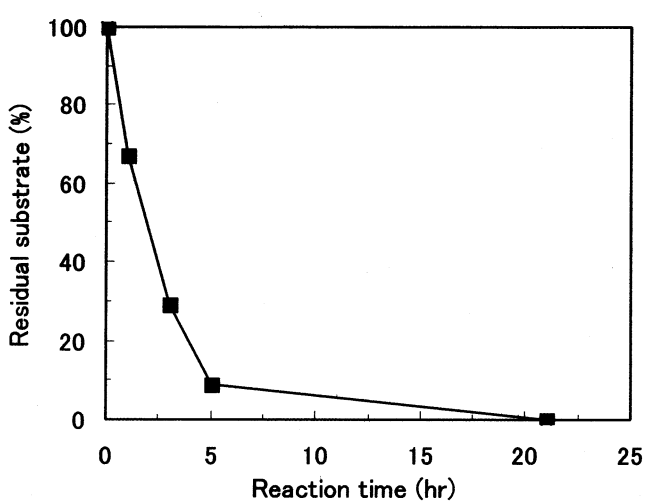
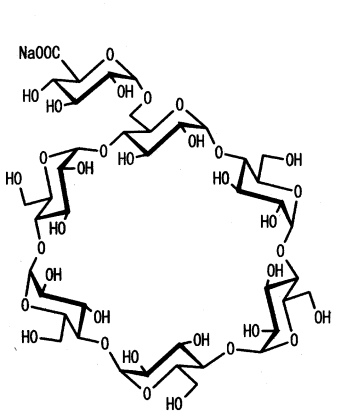
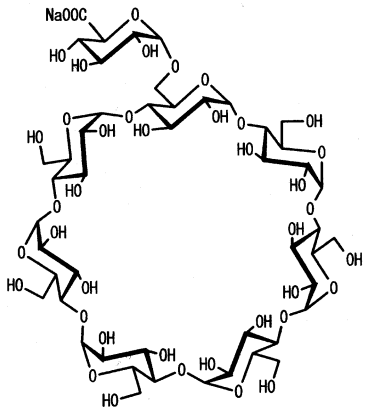


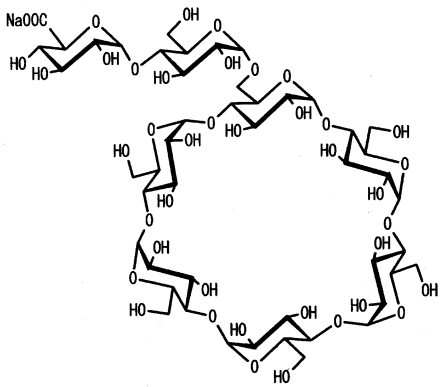
Fig. 1. Time course of microbial oxidation of G_2-β-CyD.



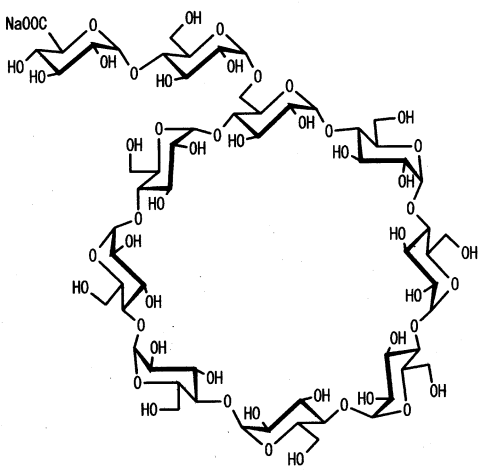
GU-α-CyD(Na) (2)



GU-β-CyD(Na) (3)



GUG-α-CyD(Na) (4)



GUG-γ-CyD(Na) (5)

*Structural characterization of GUG-β-CyD (**1**) and related compounds.*—The low-resolution FAB mass spectrum of **1** (Fig. 2) showed negatively charged molecular ions $[M-H]^-$ at *m/z* 1493 and $[M-Na]^-$ at *m/z* 1471 and fragment ions at *m/z* 1295 and 1133, respectively.

The high-resolution FAB mass spectrum of **1** revealed the presence of negatively charged ions $[M-H]^-$ at *m/z* 1493.4249 for $C_{54}H_{86}NaO_{46}$ and also $[M-Na]^-$ at 1471.4472 for $C_{54}H_{87}O_{46}$, respectively. Therefore, the molecular formula of **1** was determined to be $C_{54}H_{87}NaO_{46}$. Furthermore, fragment ions of **1** were observed at *m/z* 1295.4138 for $C_{48}H_{79}O_{40}$, resulting from the loss of a glucuronyl residue (a*), and also at *m/z* 1133.3585 for $C_{42}H_{69}O_{35}$, resulting from the loss of a glucuronyl glucosyl residue (b*) (Fig. 3).

These results suggest that the glucuronyl residue is on the terminal side-chain glucose of **1**. This conclusion was confirmed by the ^{13}C NMR spectrum of **1** (Fig. 4) where the peak at 177.84 ppm is assigned to the carbonyl carbon of a glucuronyl residue. Furthermore, the peaks at 100.01, 101.58, 103.25, 103.31 and 103.37 ppm are assigned to the C-1 resonance of the glucose residues of cyclodextrin ring and side-chain residues.

From these spectral data the structure of **1** was confirmed to be sodium cyclomaltoheptosyl-(6→1)-β-D-glucopyranosyl-(4→1)-α-D-glucopyranosiduronate. The physicochemical properties of **1** are shown in Table 1. Other branched cyclodextrin carboxylic acids were

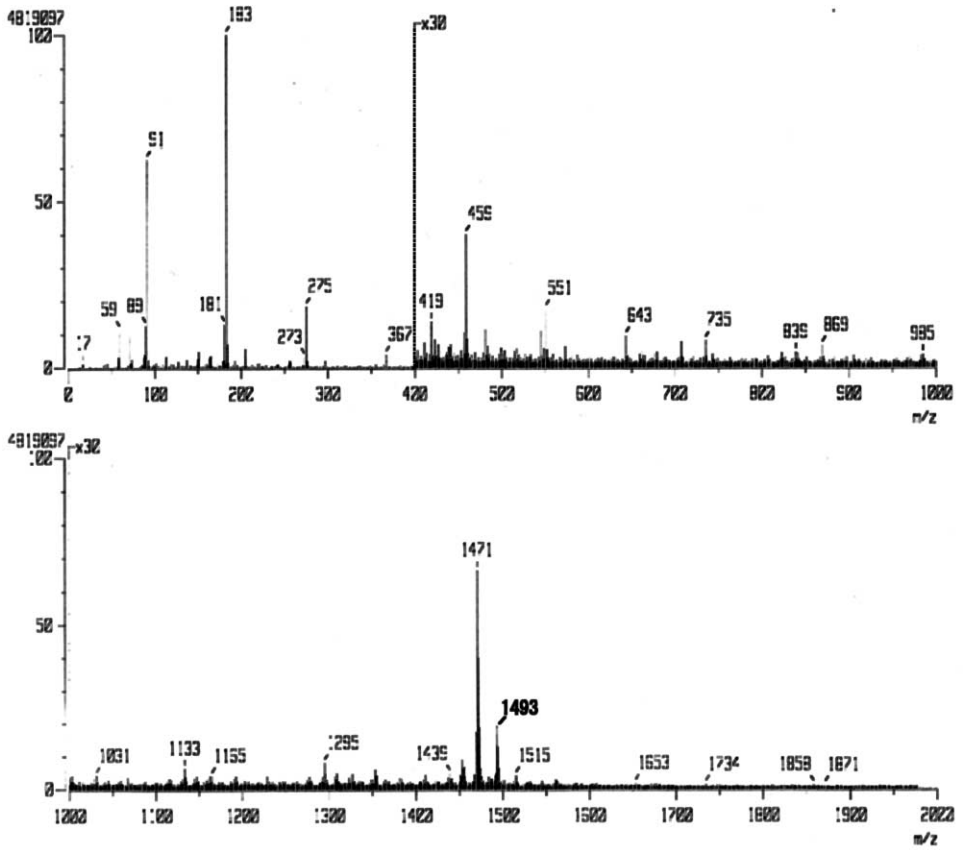


Fig. 2. FAB-MS of GUG-β-CyD (Na) (**1**).

characterized in a similar manner: (**2**), GU-α-CyD (Na); (**3**), GU-β-CyD (Na); (**4**), GUG-α-CyD(Na); and (**5**), GUG-γ-CyD (Na), and

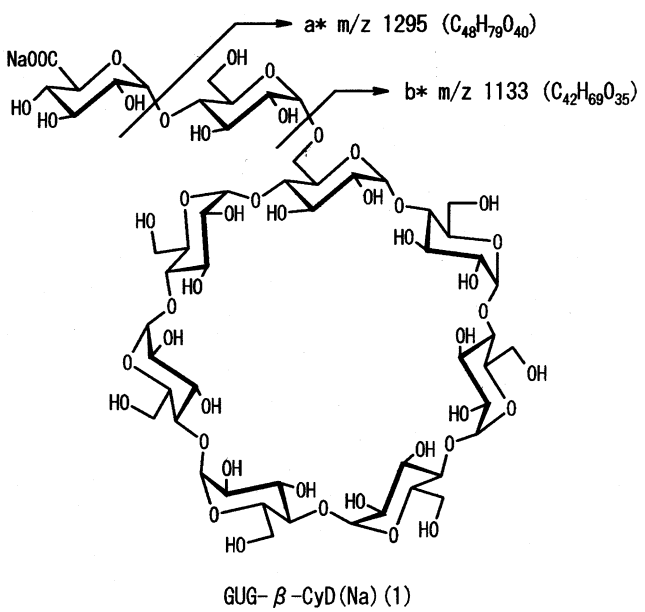


Fig. 3. Mass fragmentation of GUG-β-CyD (Na) (**1**).

their structures were determined by comparison of each spectrum with the spectrum of **1**. The ^{13}C NMR spectra of the branched cyclodextrin carboxylic acids are shown in Table 2.

*Enzymatic degradation of **1** by α-glucoamylase.*—The glucuronyl residue was assigned to the terminal side-chain glucose of **1** by analysis of digestion products of α-glucoamylase. Either **1** or maltosyl-β-CyD was incubated with α-glucoamylase at 37 °C for 30 min and

Table 1
Physicochemical properties of GUG-β-CyD (Na) (**1**)

Appearance	White amorphous powder
Formula	$C_{54}H_{87}NaO_{46}$
Molecular weight	1495.25
Elemental analysis	Anal. Calc. for $C_{54}H_{87}NaO_{46}\cdot 4.5H_2O$: C, 41.15; H, 6.14. Found: C, 41.29; H, 6.27%
$[\alpha]_D^{20}$	156.2° (*c* 1.04, water)
HRFABMS	[M − H] = 1493.4249

Table 2
^{13}C NMR chemical shifts of branched cyclodextrin carboxylic acids

		δ ppm (67.8 MHz, D_2O)
GU-α-CyD (Na)	(**2**)	63.27, 63.35, 63.39, 69.83, 73.52, 74.12, 73.37, 74.58, 74.70, 74.76, 74.86, 74.97, 75.69, 76.17, 76.27, 84.08, 84.16, 84.21, 84.27, 101.70, 104.23, 104.26, 104.39, 104.43, 179.61.
GU-β-CyD (Na)	(**3**)	61.56, 66.90, 68.33, 68.29, 71.88, 72.54, 72.87, 73.10, 73.30, 73.38, 74.04, 74.36, 82.40, 82.45, 82.58, 82.68, 82.71, 100.30, 103.14, 103.19, 103.30, 178.07.
GUG-α-CyD (Na)	(**4**)	61.01, 67.94, 71.07, 71.61, 71.76, 72.35, 72.43, 72.53, 73.52, 73.92, 74.24, 78.49, 81.73, 81.84, 82.13, 99.28, 100.70, 102.44, 177.11.
GUG-β-CyD (Na)	(**1**)	61.69, 61.89, 68.54, 71.84, 72.16, 72.47, 73.14, 73.21, 73.30, 73.49, 73.57, 74.23, 74.33, 74.50, 74.71, 79.62, 82.52, 82.57, 82.74, 83.02, 100.01, 101.58, 103.25, 103.31, 103.37, 177.84.
GUG-γ-CyD (Na)	(**5**)	62.83, 63.11, 69.73, 81.06, 83.07, 83.20, 83.33, 83.38, 83.72, 101.30, 102.94, 104.34, 104.47, 179.12.

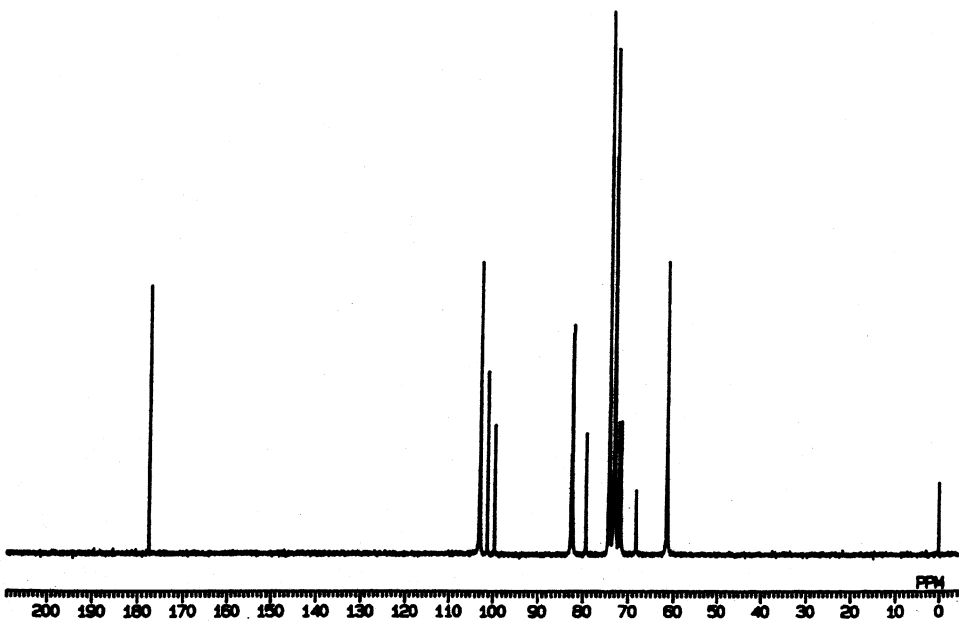


Fig. 4. ^{13}C NMR spectrum of GUG-β-CyD (Na) (**1**).

analyzed by HPLC on an NH2P-50 column. From the HPLC data, the time course of remaining (%) residue of **1** and maltosyl-β-CyD was plotted as shown in Fig. 5. We found that **1** was resistant to α-glucoamylase hydrolysis, whereas maltosyl-β-CyD was almost completely digested in 10 min. This result suggests that the carboxyl group is attached at C-6 of the terminal glucose residue in the branched part of **1**.

Specificity of the alcohol dehydrogenation reaction of P. saccharoketogenes.—Regiospecific synthesis of **1** was achieved via oxidation of the hydroxymethyl group of the terminal glucose residue in the branched part of maltosyl-β-CyD, using *P. saccharoketogenes*. The other branched CyDs, glucosyl-α-CyD, glucosyl-β-CyD, maltosyl-α-CyD, maltosyl-β-CyD and maltosyl-γ-CyD were similarly oxidized, producing **2**, **3**, **4**, and **5**, respectively, in practical yields. However, β-CyD, which does not have a branched glucose residue in the CyD ring, was not oxidized at all.

These data indicate that cyclodextrin substrates must have at least one glucose residue in the branched part of the molecule in order to be oxidized by *P. saccharoketogenes*. The bulkiness of the CyD ring may have some steric effects on the reaction intermediates generated by *P. saccharoketogenes* so that the

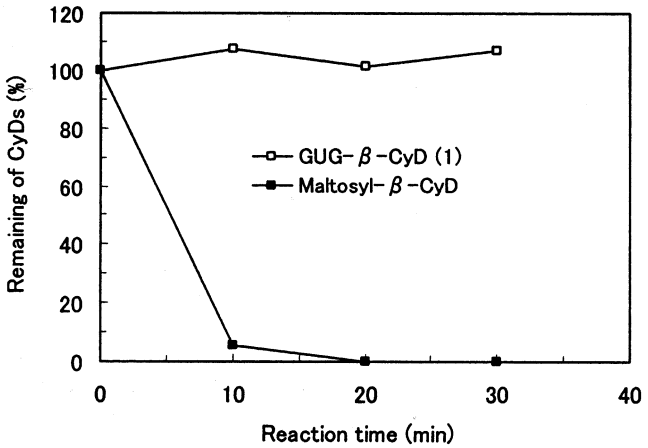


Fig. 5. Time course of the degradation of (**1**) and Maltosyl-CyD by Glucoamylase.

hydroxymethyl group of C-6 of glucose in the branched CyDs provides a side-chain donor, which is not present in β-CyD.

3. Experimental

Materials.—Glucosyl-α-CyD, glucosyl-β-CyD, maltosyl-α-CyD, maltosyl-β-CyD and maltosyl-γ-CyD were supplied by the Ensuiko Sugar Refining Co. Ltd., (Yokohama, Japan). β-CyD and glycoamylase (*Rhizopus* sp.) were purchased from Wako Pure Chemical Industries Ltd., (Tokyo, Japan).

General methods.—Melting points were measured with a micro melting point apparatus (Yanagimoto, Kyoto, Japan) and are uncorrected. Optical rotations were determined with a DIP-360 digital polarimeter (JASCO, Tokyo, Japan). The pH measurements were made on an F-22 pH meter (Horiba, Kyoto, Japan). Lyophilization was carried out with a Virtis freeze-dryer, Freeze Mobile-12 (Tokyo Rika, Tokyo, Japan). High-performance liquid chromatography (HPLC) analyses were performed using a Shimadzu LC-6A system HPLC pump, a model LC-6 A injector, SIL-6A, and a refractive index (RI) detector, RID-6A (all from Shimadzu, Kyoto, Japan). The columns used were Asahipak NH2P-50 (250 × 4.6 mm i.d.; Showa Denko, Tokyo, Japan).

Low- and high-resolution fast-atom bombardment mass spectrometry (FABMS) was carried out with a JMS-700T mass spectrometer (JEOL, Tokyo, Japan) using xenon atoms having a kinetic energy equivalent to 6 kV at an emission current 5–10 mA. The mass marker was calibrated with perfluoroalkylphosphazine (Ultramark 1621). Branched cyclodextrin carboxylic acids were analyzed in the negative-ion mode with water as a solvent and glycerol as a matrix. ^{13}C NMR spectra (125.65 MHz) were recorded at 50 °C on 3% solutions in D_2O with an A-500 spectrometer (JEOL, Tokyo, Japan). Chemical shifts are expressed in ppm downfield from the signal of sodium trimethylsilylpropionate (0.0 ppm). Moisture was measured by Karl Fischer analysis.

Microorganisms and culture conditions

Preparation of resting cells of P. saccharoketogenes *Rh 47.* The seed medium was prepared as follows. 10 g of lactose, 30 g of corn steep liquor (CSL), 10 g of yeast extract and 3 g of $(NH_4)_2SO_4$ were dissolved in 900 mL of water, and the pH was adjusted to 7.0 with 30% NaOH. After pH adjustment, 20 g of $CaCO_3$ were added, water was added to a final volume of 1000 mL, and the broth was dispensed into a flask and autoclaved. One loopful of cells was transferred from a slant culture to a 1000 mL flask containing 200 mL of the seed medium described above and cultured on a rotary shaker (200 rpm) at 30 °C for two days.

A jar fermenter was charged with 2 L of a solution containing 60 g of CSL, 15 g of yeast extract, 9 g of $(NH_4)_2SO_4$, 3 g of $FeSO_4 \cdot 7H_2O$, 3 mg of Vitamin B_1 and 0.15 g of actcol (Takeda Chemical Industries Ltd., Japan), and the pH was adjusted to 7.0 with 30% NaOH. After autoclaving, 600 mL of 10% glucose, 100 mL of 0.3% $LaCl_3 \cdot 7H_2O$ and 300 mL of seed culture were added, and cultivation was carried out at 30 °C. After approximately 20 h of incubation, 2 L of culture was centrifuged, and the resulting cell pellet was suspended in 500 mL of sterile water.

Preparation of ***1*** *(Na salt) by cellular reaction of* P. saccharoketogenes *Rh 47.*—A 5 L jar fermentor was charged with a solution of 200 g of maltosyl-β-CyD in 1.5 L of sterilized water, followed by addition of 0.5 L of *P. saccharoketogenes* Rh 47 cell suspension, equivalent to a wet weight of 43.5 g of cells.

Cultures were aerated at a rate of 1.6 L min^{-1} and cultured at 32 °C with stirring at 800 rpm for 22 h. During the experiment, the pH was continuously monitored and maintained at pH 6.3 with N NaOH. The oxidation reaction was monitored by a time-course plot of (%) remaining maltosyl-β-CyD as determined by HPLC. Samples (500 μL) of the reaction mixture were taken, centrifuged (15,000 rpm, 5 min), and filtered through a 0.45-μm Millipore filter. The filtrate was diluted ten-fold and analyzed by HPLC under the following parameters and settings: Column: NH_2P-50 (Asahipak); Mobile phase: 48:52 MeCN–water supplemented with 0.005 M paired ion chromatography (PIC) reagent A (Waters); Flow rate: 0.8 mL min^{-1}; Detection: RI. The results of the time-course plot of % remaining maltosyl-β-CyD, as determined by HPLC, are shown in Fig. 1.

Purification of ***1*** *(Na salt).*—Two liters of the above reaction mixture were centrifuged at 800 rpm to remove the cells, and the supernatant (2.22 L) was passed through a 0.45 μm Millipore filter. The filtrate (2.16 L) was loaded onto an IR-120B (H) column (1 L) and washed with water (2 L), and the combined washing fraction was loaded onto an HP-20 chromatography column (1 L). The column was washed (8 L) and eluted (12 L) with water. This eluate was concentrated to 500 mL and adjusted to pH 7.45 with 134 mL of N NaOH and lyophilized to provide 194 g (92%) of **1** as a white powder (Na salt).

Characterization of ***1*** *(Na salt).*—White amorphous powder; $[\alpha]_D^{20}$ +156.2° (*c* 1.04, water). Purity: 99% (HPLC). Anal. Calc. for $C_{54}H_{87}NaO_{46}\cdot 4H_2O$: C, 41.15; H, 6.14. Found: C, 41.29; H, 6.27. Moisture 4.3%. MW: $C_{54}H_{87}NaO_{46}$; 1495.25; HRFABMS $[M-H]^-$ 1493.4249 for $C_{54}H_{86}NaO_{46}$; $[M-Na]^-$, 1471.4472 for $C_{54}H_{87}O_{46}$. The ^{13}C NMR spectrum of **1** (Na salt) is shown in Fig. 4. The signal for 177.84 was assigned to the carbonyl carbon of the glucuronyl residue, and the signals for 100.01, 101.58, 103.25, 103.31, and 103.37 ppm were assigned as C-1 resonances of the glucose residues of cyclodextrin ring and side-chain residues. The chemical shifts of GUG-β-CyD (Na salt) are provided in Table 2.

Enzymatic degradation of ***1*** *by α-glucoamylase.*—To an aliquot of **1** (Na salt) and maltosyl-β-CyD solution (10 mM) in water in separate experiments was added α-glucoamylase from *Rhizopus* sp. at a final concentration of 2 units mL^{-1}. The reaction mixture was incubated in a water bath at 37 °C. Then, 500-μL samples of the reaction mixture were taken, heated at 100 °C for 15 min to inactivate the enzyme, centrifuged (15,000 rpm, 5 min), and filtered through a Millipore filter (cut-off molecular weight 10,000). The filtrate was diluted ten-fold and analyzed by HPLC under the following parameters and settings: Column: NH2P-50 (Asahipak); Mobile phase: 48:52 MeCN–water, supplemented with 0.005 M PIC reagent A; Flow rate: 0.8 mL min^{-1}; Detection: RI. The result of enzymatic degradation of **1** (Na salt) by α-glucoamylase is shown in Fig. 5.

Preparation and characterization of ***1*** *(free-OH).*—A solution of **1** (Na salt) (10 g) in water (20 mL) was loaded onto an IR-120B (H) column (400 mL) and washed with water (1 L). This eluate was concentrated to 6 mL to provide 8.5 g of colorless plates of **1** (free-OH), mp 216–220 °C (dec.), $[\alpha]_D^{20}$ +138.3° (*c* 1.20, water). Purity: 99% (HPLC). ^{13}C NMR spectrum of GUG-β-CyD (free-OH); 62.03, 62.26, 68.85, 71.97, 72.49, 72.87, 72.95, 73.21, 73.48, 73.57, 73.67, 73.83, 73.96, 74.12, 74.15, 74.17, 74.39, 74.65, 74.67, 74.87, 75.04, 79.59, 82.89, 83.13, 83.31, 100.37, 101.72, 103.54, 103.65, 103.75, 103.89, 175.06.

Preparation of some branched cyclodextrin-carboxylic acids.—The branched cyclodextrins, glucosyl-α-CyD, glucosyl-β-CyD, maltosyl-α-CyD and maltosyl-γ-CyD were subjected to microbial oxidation as described above for **1**, yielding **2** (Na), **3** (Na), **4** (Na) and **5** (Na), respectively.

Data for **2** (Na): white amorphous powder; $[\alpha]_D^{20}$ +125.4° (*c* 0.96, water). ^{13}C NMR (67.8 MHz, D_2O); δ 63.27, 63.35, 63.39, 69.83, 73.52, 74.12, 74.37, 74.58, 74.70, 74.76, 74.86, 74.97, 75.69, 76.17, 76.27, 84.08, 84.16, 84.21, 84.27, 101.70, 104.23, 104.26, 104.39, 104.43, 179.61.

Data for **3** (Na): white amorphous powder; $[\alpha]_D^{20}$ +139.0° (*c* 0.92, water). ^{13}C NMR (67.8 MHz, D_2O): δ 61.56, 66.90, 68.33, 68.39,

71.88, 72.54, 72.87, 73.10, 73.30, 73.38, 74.04, 74.36, 82.40, 82.45, 82.58, 82.68, 82.71, 100.30, 103.14, 103.19, 103.30, 178.07.

Data for **4** (Na): white amorphous powder; $[\alpha]_D^{20}$ +137.3° (*c* 0.68, water). ^{13}C NMR (67.8 MHz, D_2O): δ 61.01, 67.94, 71.07, 71.61, 72.35, 72.43, 72.53, 73.52, 73.92, 74.24, 78.49, 81.73, 81.84, 82.13, 99.28, 100.70, 102.44, 177.11.

Data for **5** (Na): white amorphous powder; Anal. Calc. for $C_{60}H_{97}NaO_{51}\cdot 11.5H_2O$: C, 38.65; H, 6.49. Found: C, 38.60, H, 6.20%. $[\alpha]_D^{20}$ +155.9° (*c* 1.04, water). ^{13}C NMR (67.8 MHz, D_2O): δ 62.83, 63.11, 69.73, 81.06, 83.07, 83.20, 83.33, 83.38, 83.72, 101.30, 102.94, 104.34, 104.47, 179.12. Table 2 shows ^{13}C NMR chemical shifts of the branched cyclodextrin carboxylic acids: **2** (Na), **3** (Na), **4** (Na) and **5** (Na).

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References

[1] (a) Ishiguro, T.; Oka, M.; Yamaguchi, T.; Nogami, I. *Japan Kokai Tokkyo Koho*, (1995) 76594. (b) Ishiguro, T.; Oka, M.; Yamaguchi, T.; Nogami, I. Eur. Pat. 599646, 1994. (c) Kurasawa, T.; Fuse, T.; Nakamichi, M.; Yasumura, Y.; Ishiguro, T. *Ann. Meeting Jpn. Pharm. Soc.*, Tokyo, March 1994.

[2] Szejtli, J. In *Cyclodextrin Technology*; Davies, J. E. D., Ed.; Kluwer Academic: Dordrecht, 1988.

[3] Irie, T.; Uekama, K. *J. Pharm. Sci.* **1997**, *86*, 147–163.

[4] Adachi, O.; Miyagawa, E.; Shinagawa, E.; Matsushita, K.; Ameyama, M. *Agric. Biol. Chem.* **1978**, *42*, 2331–2340.

[5] (a) Adachi, O.; Miyagawa, E.; Shinagawa, E.; Matsushita, K.; Ameyama, M. *Agric. Biol. Chem.* **1978**, *42*, 2045–2056. (b) Adachi, O.; Miyagawa, E.; Shinagawa, E.; Matsushita, K.; Ameyama, M. *Agric. Biol. Chem.* **1990**, *54*, 3123–3129.

[6] (a) Shinagawa, E.; Matsushita, K. Adachi, O. Ameyama, M. *Agric. Biol. Chem.* **1982**, *46*, 135–141. (b) Sugisawa, T.; Hoshino, T.; Nomura, S.; Fujita, A. *Agric. Biol. Chem.* **1991**, *55*, 363–370. (c) Matsushita, K.; Ohno, Y.; Shinagawa, E.; Adachi, O.; Ameyama, M. *Agric. Biol. Chem.* **1980**, *44*, 1505–1512.

[7] Anelli, P. L.; Biffi, C.; Montanari, F.; Ouichi, S. *J. Org. Chem.* **1987**, *52*, 2559–2562.

[8] Yamaguchi, M.; Tanaka, T.; Endo, T. *J. Org. Chem.* **1990**, *55*, 1490–1492.

[9] Jiang, B.; Drouet, E.; Milas, M.; Rinaudo, M. *Carbohydr. Res.* **2000**, *327*, 455–461.

[10] Ishiguro, T.; Oka, M.; Kumagai, M. *Proc. 4th Pacific Rim Biotechnol. Conf.*, Melbourne, February 1995, pp. 285–286.

[11] (a) Nogami, I.; Shirafuji, H.; Oka, M.; Yamaguchi, T. Jpn. Pat. Applic. S-62-228288, 1987. (b) Nogami, I.; Yamaguchi, T.; Oka, M.; Shirafuji, H. Jpn. Pat. Applic. S-64-85088, 1989. (c) Shirafuji, H.; Yamaguchi, T.; Nogami, I. Jap. Pat. Applic. S-62-228287, 1987.

[12] Taylor, P. M.; Whelan, W. J. *Arch. Biochem. Biophys.* **1966**, *113*, 500–502.

[13] French, D.; Abdullah, M. *Biochem. J.* **1966**, *100*, 6P–7.

[14] Hizukuri, S.; Kuwano, S.; Abe, J.; Koizumi, K.; Utamura, T. *Biotech. Appl. Biochem.* **1989**, *11*, 60–73.

[15] Hizukuri, S.; Abe, J.; Koizumi, K.; Okada, Y.; Kubota, Y.; Sakai, S.; Mandai, T. *Carbohydr. Res.* **1989**, *185*, 191–198.

[16] Shiraishi, T.; Fujimoto, D.; Sakano, Y. *Agric. Biol. Chem.* **1989**, *53*, 3093–3095.

[17] Shiraishi, T.; Kusano, S.; Tsumuraya, Y.; Sakano, Y. *Agric. Biol. Chem.* **1989**, *53*, 2181–2188.