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Note

Chemoenzymatic synthesis of 6$^{\omega}$-modified maltooligosaccharides from cyclodextrin derivatives

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Abstract—Regioselectively substituted maltooligosaccharides were prepared by enzymatic transformation of modified cyclodextrins by using simultaneously two different enzymes: cyclodextrin glucanotransferase (CGTase) and amyloglucosidase. Oligosaccharides were obtained in very good yields and their structures were identified by 1D and 2D NMR spectroscopy. These results provided new information about the specificity of the catalytic sites of CGTase and amyloglucosidase. They also offered new ways for the synthesis of regioselectively modified maltooligosaccharides.

Keywords: Carbohydrate; Enzyme; CGTase; Amyloglucosidase; Modified maltooligosaccharides; Cyclodextrins

Substrate analogues have been used to identify the nature of amino acids involved in the active site of enzymes. Studies on the coupling reaction of CGTase (EC 2.4.1.19, GH family 13) between modified α-cyclodextrin and glucosyl residues allowed some information about the specificity of the donor subsites.[1–4] Specificity of the acceptor part of the active site has been shown by using native cyclodextrin and various acceptors.[5–7] Acceptor subsites admit a wide range of modified glucosyl residues unlike donor subsites, which accommodate only few modifications. These studies also demonstrated that only glucopyranose rings in $^4C_1$ conformation fit between −2 and +2 subsites of the active site of CGTase.

Amyloglucosidase (EC 3.2.1.3, GH family 15) is a starch degrading enzyme, which catalyses the splitting of glucosyl residues from the nonreducing end of oligosaccharides and liberates β-D-glucose residues. Its substrate specificity has been investigated using various maltose derivatives.[8–11]

In this paper, we report the synthesis in good yield of modified maltooligosaccharides by coupling reactions of mono-modified α- and β-cyclodextrins **1**–**6** on various acceptors followed by amyloglucosidase action (Scheme 1). It is worthy to note that maltooligosaccharides modified at their nonreducing end were also obtained by action of Taka-amylase or porcine pancreatic α-amylases on substituted cyclodextrins.[12–14]

All compounds were characterized by NMR and FAB mass spectroscopy.

The coupling reaction of **1** with D-glucopyranose or methyl β-D-glucopyranoside as acceptors gave, respectively, the tetrasaccharides **9** and **10** after 12 h. Compound **9** was obtained in almost quantitative yield, suggesting a preferential binding of cyclodextrin bearing one carboxylic acid group in the −2 subsite of the active site of CGTase (Fig. 1). After 48 h of incubation time, the reaction with glucose led to the trisaccharide **8**. The −1 subsite of CGTase cannot accommodate a glucuronic residue,[15] so this enzyme is not responsible for the conversion of compound **9** into **8**. It is also known that for short incubation times, a maltose substituted

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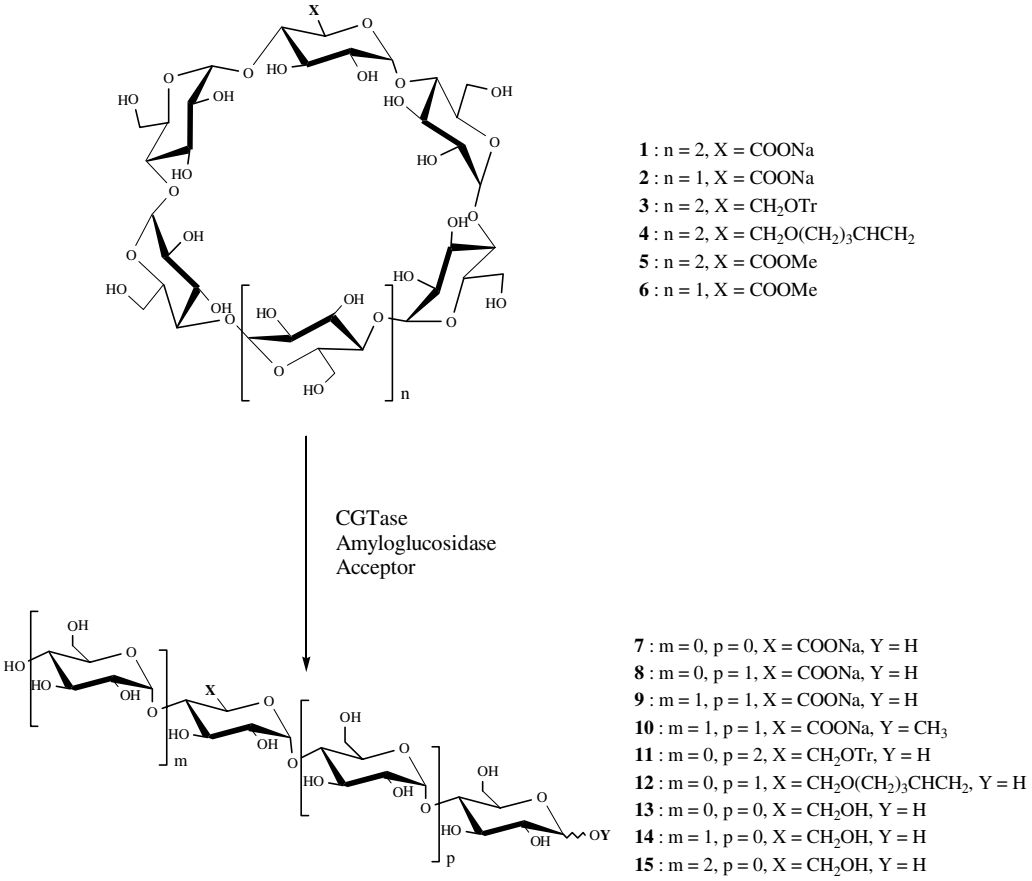

1 : n = 2, X = COONa
2 : n = 1, X = COONa
3 : n = 2, X = CH_2OTr
4 : n = 2, X = $CH_2O(CH_2)_3CHCH_2$
5 : n = 2, X = COOMe
6 : n = 1, X = COOMe

7 : m = 0, p = 0, X = COONa, Y = H
8 : m = 0, p = 1, X = COONa, Y = H
9 : m = 1, p = 1, X = COONa, Y = H
10 : m = 1, p = 1, X = COONa, Y = CH_3
11 : m = 0, p = 2, X = CH_2OTr, Y = H
12 : m = 0, p = 1, X = $CH_2O(CH_2)_3CHCH_2$, Y = H
13 : m = 0, p = 0, X = CH_2OH, Y = H
14 : m = 1, p = 0, X = CH_2OH, Y = H
15 : m = 2, p = 0, X = CH_2OH, Y = H

Scheme 1. Synthesis of modified maltooligosaccharides.

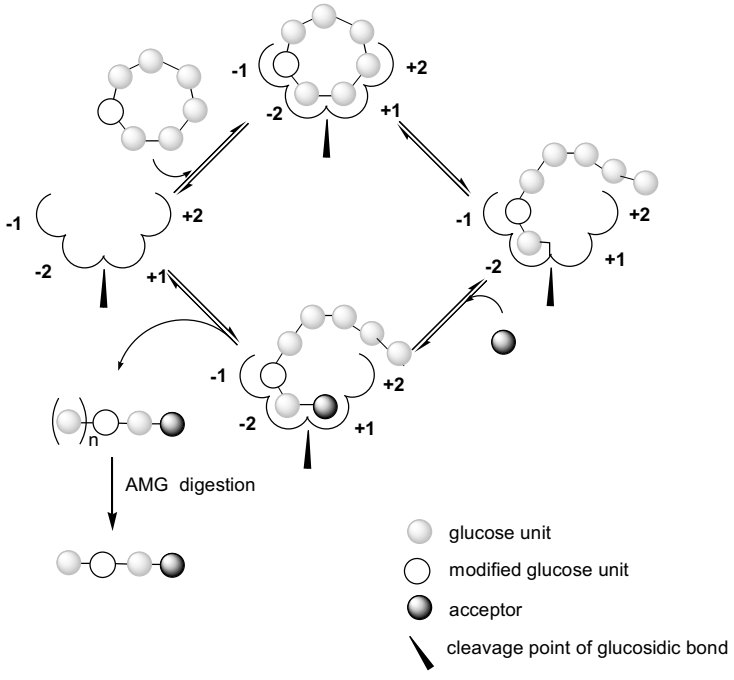

Figure 1. Schematic representation of enzymatic coupling of compound **1** by CGTase and amyloglucosidase.

at C-6[I] by a charged group (amino or carboxy) cannot be hydrolyzed by amyloglucosidase.[10] To the best of our knowledge, no experiment has been performed when the incubation time has been increased. Consequently, we can rationally assume that, for a long incubation time, amyloglucosidase can cleave glycosidic bond between a non substituted glucosyl residue and a glucuronic one (Fig. 2). This fact was confirmed by incubation for 48 h of compound **9** in the presence of amyloglucosidase alone, which afforded exclusively compound **8**.

In the same way, the reaction of **2** with D-glucopyranose as acceptor gave the major compound **8**, after an incubation time of 48 h. In this case, the formation of **9** was not observed after 12 h. This phenomena could be explained by the fact that we used a 'β-CGTase', which reacts faster on β-cyclodextrin derivatives than on α-cyclodextrin ones and, as already shown for longer reaction time, amyloglucosidase can cleave glycosidic bond between a glucosyl residue and a glucuronic one. If the rates of formation of compound **9** and its cleavage by amyloglucosidase are similar, the formation of the intermediate compound **9** should not be observed.

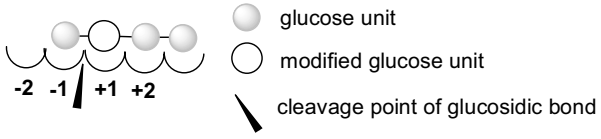

Figure 2. Schematic representation of enzymatic hydrolysis of tetrasaccharide **9** by amyloglucosidase.

The reaction of substituted cyclodextrin **3** with D-glucopyranose as acceptor provided the tetrasaccharide **11**, whereas substituted cyclodextrin **4** gave the trisaccharide **12**. These two experiments demonstrated that the −2 subsite of CGTase can accommodate a glucosyl unit substituted at C-6 by a pentenyl group but not by a trityl one. They also showed that amyloglucosidase can admit in the +1 subsite of the active site a glucosyl residue which bears a hydrophobic group as well as a bulky group. The reaction of methyl ester derivatives (**5** and **6**) led exclusively to the formation of disaccharide **7**. As shown previously, a hydrolytic cleavage of the expected trisaccharide by CGTase can explain the formation of the disaccharide obtained but the hydrolysis of methyl ester group remains unclear.

The structures of the different maltooligosaccharides synthesized were established by NMR spectroscopy. The complete assignment of proton and carbon resonances of oligosaccharides were done by using a combination of one- and two-dimensional ^{1}H–^{1}H homonuclear and ^{1}H–^{13}C heteronuclear experiments such as COSY, TOCSY and HMQC (Tables 1–6). Anomeric protons are the most downfield shifted resonances in the proton NMR spectra of oligosaccharides. They

Table 1. Assignments of signals in the ^{1}H NMR spectra (in ppm) and coupling constants (in hertz, given in parentheses) of disaccharides (**7**) and (**13**)

Disaccharides	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
Maltose (**13**)							
α-D-Glc*p*-(1→4)	5.34 (3.7)	3.51 (9.9)	3.67 (9.2)	3.34 (9.3)	3.63 (1.6; 6.2)	3.68 (12.3)	3.82
α-D-Glc*p*	5.15 (3.7)	3.49 (9.2)	3.92 (9.1)	3.57 (9.2)	3.64 (1.6; 6.2)	3.68 (12.3)	3.82
β-D-Glc*p*	4.58 (8.1)	3.20 (8.1)	3.70 (8.5)	3.59 (8.4)	3.52	3.76	3.88
Compound (**7**)							
	$5.32\ (3.8)_{\alpha}$		$3.62\ (8.9)_{\alpha}$		3.92_{α}		
α-D-GlcA*p*-(1→4)		3.55 (9.9)		3.42 (10.1)		—	—
	$5.31\ (3.8)_{\beta}$		$3.64\ (8.9)_{\beta}$		3.91_{β}		
α-D-Glc*p*	5.15 (3.8)	3.50 (9.9)	3.89 (8.7)	3.57 (10.5)	3.88 (2.4; 4.7)	3.71 (12.0)	3.76
β-D-Glc*p*	4.58 (8.0)	3.21 (9.5)	3.69 (8.7)	3.56 (11.3)	3.54 (1.9; 4.8)	3.68 (12.1)	3.82

Table 2. Assignments of signals in the ^{13}C NMR spectra (in ppm) of disaccharides (**7**) and (**13**)

Disaccharides	C-1	C-2	C-3	C-4	C-5	C-6	COONa
Maltose (**13**)							
		72.2_{α}					
α-D-Glc*p*-(1→4)	100.1		73.3	69.8	73.0	61.0	—
		72.1_{β}					
α-D-Glc*p*	92.2	71.7	73.5	77.9	70.4	61.0	—
β-D-Glc*p*	96.2	74.4	76.5	77.6	75.0	61.2	—
Compound (**7**)							
		72.0_{α}					
α-D-GlcA*p*-(1→4)	100.1		73.3	72.2	73.1	—	176.8
		71.9_{β}					
α-D-Glc*p*	92.2	71.6	73.5	77.8	70.4	60.9	—
β-D-Glc*p*	96.1	74.3	76.5	77.7	75.0	61.1	—

Table 3. Assignments of signals in the ^{1}H NMR spectra (in ppm) and coupling constants (in hertz, given in parentheses) of trisaccharides (**8**), (**12**) and (**14**)

Trisaccharides	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
Maltriose (**14**)							
α-D-Glc*p*-(1→4)[a]	5.32 (3.7)	3.51 (10.1)	3.66 (9.6)	3.35 (9.6)	3.61	3.75	3.79
α-D-Glcp-(1→4)[b]	5.33 (3.7)	3.58 (11.9)	3.90 (9.3)	3.58 (9.8)	3.71	3.75	3.79
α-D-Glc*p*	5.15 (4.0)	3.49 (10.4)	3.90 (9.3)	3.58 (9.8)	3.71	3.75	3.79
β-D-Glc*p*	4.58 (8.1)	3.20 (9.2)	3.70 (10.1)	3.58 (9.8)	3.52 (2.0)	3.70 (12.1)	3.83
Compound (**8**)							
α-D-GlcA*p*-(1→4)[a]	5.27 (3.8)	3.50 (9.9)	3.58 (8.9)	3.35 (10.1)	3.86	—	—
α-D-Glc*p*-(1→4)[b]	5.26 (3.8)	3.49 (9.8)	3.80 (9.2)	3.47 (9.8)	3.72	3.76	3.81
α-D-Glc*p*	5.11 (3.8)	3.46 (9.9)	3.85 (8.7)	3.54 (10.7)	3.84	3.67	3.72
β-D-Glc*p*	4.53 (8.0)	3.16 (9.5)	3.64 (8.7)	3.51 (11.3)	3.49	3.63	3.78
Compound (**12**)							
α-D-Glc*p*-(1→4)[a]	5.29 (3.9)	3.49	3.59	3.33	3.67–3.87 5.01 (2.2; 17.2; 1.6)	3.67–3.87	3.67–3.87
C6-(O6)	3.52	1.61	2.04	5.83 (6.6)	 4.94 (10.2; 1.2)	—	—
α-D-Glc*p*-(1→4)[b]	5.31 (3.9)	3.55	3.89	3.51	3.67–3.87	3.67–3.87	3.67–3.87
α-D-Glc*p*	5.14 (3.9)	3.48	3.89	3.56	3.67–3.87	3.67–3.87	3.67–3.87
β-D-Glc*p*	4.56 (8.0)	3.19	3.69	3.51	3.67–3.87	3.67–3.87	3.67–3.87

[a] Terminal nonreducing unit.
[b] Penultimate unit.

Table 4. Assignments of signals in the ^{13}C NMR spectra (in ppm) of trisaccharides (**8**), (**12**) and (**14**)

Trisaccharides	C-1	C-2	C-3	C-4	C-5	C-6	COONa
Maltriose (**14**)							
α-D-Glc*p*-(1→4)[a]	100.3	72.2	73.4	69.9	73.1	61.0	—
	100.0_{α}	72.0_{α}					
α-D-Glc*p*-(1→4)[b]			73.7	77.7	71.7	61.0	—
	99.9_{β}	71.9_{β}					
α-D-Glc*p*	92.3	71.7	73.5	78.0	70.5	61.0	—
β-D-Glc*p*	96.2	74.5	76.5	77.8	75.0	61.2	—
Compound (**8**)							
α-D-GlcA*p*-(1→4)[a]	100.3	71.6–72.2	73.6	72.2	73.1	—	176.7
α-D-Glc*p*-(1→4)[b]	99.8	72.0	73.3	77.4	71.6	60.8	—
α-D-Glc*p*	92.3	71.8	73.5	77.6	70.3	60.1	—
β-D-Glc*p*	96.2	74.4	76.5	77.7	74.9	60.9	—
Compound (**12**)							
α-D-Glc*p*-(1→4)[a]	100.3	71.8–72.0	73.2	70.3	72.0	69.5	—
C6-(O6)	71.3	28.2	29.9	139.3	115.1	—	—
	99.9_{α}						
α-D-Glc*p*-(1→4)[b]		74.9	69.5–75.0	77.5	71.5–71.8	61.0	—
	99.8_{β}						
α-D-Glc*p*	92.3	71.5–71.8	73.7	77.2	69.5–75.0	61.0	—
β-D-Glc*p*	96.1	74.3	76.5	77.3	69.5–75.0	61.0	—

[a] Terminal nonreducing unit.
[b] Penultimate unit.

are used as starting points for the determination of coupling network of a corresponding sugar. After complete assignment of proton and carbon NMR spectra for each glucosyl residue, the type of glycosidic linkage was determined by using an HMBC experiment. This experiment showed two- and three-bond ^{1}H–^{13}C couplings of a corresponding sugar unit but also across the glycosidic linkage between two adjacent residues. The NMR spectra of maltose (**13**), maltotriose (**14**) and maltotetraose (**15**) were used as standards. Some of these data have already been published.[16–18] In the ^{13}C spectra of maltooligosaccharides modified on their terminal nonreducing end, signals of C-4 and C-6 of modified units were shifted downfield relative to the underivatized glucose residues, excepted for compound **12** where they were shifted upfield. Maltooligosaccharides modified on the penultimate glucose unit starting from their nonreducing end exhibited a C-4 signal shifted upfield. Sur-

Table 5. Assignments of signals in the ^{1}H NMR spectra (in ppm) and coupling constants (in hertz, given in parentheses) of tetrasaccharides (**9**), (**10**) and (**11**)

Tetrasaccharides	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	OCH_3	OTr
Compound (**9**)									
α-D-Glc*p*-(1→4)[a]	5.30 (3.9)	3.34 (9.8)	3.52 (9.7)	3.23 (9.8)	3.38–3.76	3.38–3.76	3.38–3.76	—	—
α-D-GlcA*p*-(1→4)[b]	5.16 (3.9)	3.50 (9.8)	3.75 (9.4)	3.59 (9.8)	3.91	—	—	—	—
α-D-Glc*p*-(1→4)[c]	5.22 (3.8)	3.45 (9.8)	3.76 (9.2)	3.43 (9.8)	3.68	3.38–3.76	3.38–3.76	—	—
α-D-Glc*p*	5.05 (3.8)	3.39 (9.8)	3.80 (9.2)	3.48 (9.2)	3.38–3.76	3.38–3.76	3.38–3.76	—	—
β-D-Glc*p*	4.48 (8.0)	3.10 (9.1)	3.59 (9.4)	3.48 (9.2)	3.38–3.76	3.38–3.76	3.38–3.76	—	—
Compound (**10**)									
α-D-Glc*p*-(1→4)[a]	5.30 (3.9)	3.33 (9.8)	3.52 (9.7)	3.22 (9.6)	3.38–3.76	3.38–3.76	3.38–3.76	—	—
α-D-GlcA*p*-(1→4)[b]	5.16 (4.0)	3.48 (9.8)	3.75 (9.4)	3.59 (9.7)	3.90	—	—	—	—
α-D-Glc*p*-(1→4)[c]	5.21 (3.7)	3.44 (9.8)	3.76 (9.2)	3.45 (9.8)	3.38–3.76	3.38–3.76	3.38–3.76	—	—
β-D-Glc*p*	4.22 (8.0)	3.12 (9.3)	3.59 (9.4)	3.44 (9.2)	3.38–3.76	3.38–3.76	3.38–3.76	3.39	—
Compound (**11**)									
α-D-Glc*p*-(1→4)[a]	5.08 (3.6)	3.38 (9.8)	3.41 (9.7)	3.35-3.98	3.35–3.98	3.27	2.84	—	7.25–7.44
α-D-Glc*p*-(1→4)[b]	5.33 (3.6)	3.49 (9.8)	3.63 (9.4)	3.35–3.98	3.35–3.98	3.35–3.98	3.35–3.98	—	—
α-D-Glc*p*-(1→4)[c]	5.36 (3.6)	3.59 (9.8)	3.90 (9.2)	3.35–3.98	3.35–3.98	3.35–3.98	3.35–3.98	—	—
α-D-Glc*p*	5.15 (3.8)	3.50 (9.8)	3.90 (9.2)	3.72	3.35–3.98	3.35–3.98	3.35–3.98	—	—
β-D-Glc*p*	4.57 (8.0)	3.20 (9.3)	3.69 (9.4)	3.58 (9.4)	3.51	3.69	3.88	—	—

[a] Terminal nonreducing unit.
[b] Penultimate unit.
[c] Antepenultimate unit.

Table 6. Assignments of signals in the ^{13}C NMR spectra (in ppm) of tetrasaccharides (**9**), (**10**), (**11**) and (**15**)

Tetrasaccharides	C-1	C-2	C-3	C-4	C-5	C-6	COONa	OCH_3
Maltotetraose (**15**)								
α-D-Glc*p*-(1→4)[a]	100.2	72.2	73.4	69.9	73.1	61.0	—	—
α-D-Glc*p*-(1→4)[b]	100.0	72.0	73.6	77.7	71.7	61.0	—	—
α-D-Glc*p*-(1→4)[c]	100.0	71.9	73.6	77.8	71.7	61.0	—	—
α-D-Glc*p*	92.3	71.7	73.5	78.0	70.4	61.2	—	—
β-D-Glc*p*	96.2	74.4	76.5	77.8	75.0	61.0	—	—
Compound (**9**)								
α-D-Glc*p*-(1→4)[a]	98.8	72.0	73.1	69.6	71.6–71.8	60.4-60.7	—	—
α-D-GlcA*p*-(1→4)[b]	100.5	72.1	74.0	76.7	73.6	—	175.9	—
α-D-Glc*p*-(1→4)[c]	99.8	74.9	73.5	78.4	71.5	60.4–60.7	—	—
α-D-Glc*p*	92.2	71.7	73.5	77.4	70.3	60.8	—	—
β-D-Glc*p*	96.1	74.3	76.5	77.3	75.0	61.0	—	—
Compound (**10**)								
α-D-Glc*p*-(1→4)[a]	98.7	71.5–72.0	71.5–72.0	69.5	71.5–72.0	60.7	—	—
α-D-GlcA*p*-(1→4)[b]	100.5	71.5–72.0	73.5	76.6	73.2	—	175.9	—
α-D-Glc*p*-(1→4)[c]	99.7	74.8	73.5	78.3	71.5	60.3	—	—
β-D-Glc*p*	103.4	74.0	76.6	77.2	74.9	60.9	—	57.5
Compound (**11**)								
α-D-Glc*p*-(1→4)[a]	100.6	72.1	73.0	68.8	72.8	59.7	—	—
α-D-Glc*p*-(1→4)[b]	99.2	72.1	73.8	79.2	71.8	61.3	—	—
α-D-Glc*p*-(1→4)[c]	100.1	71.8	73.7	76.8	71.8	61.3	—	—
α-D-Glc*p*	92.3	70.4	73.6	77.5	70.7	60.9	—	—
β-D-Glc*p*	96.2	74.3	76.5	76.5	75.0	61.0	—	—

[a] Terminal nonreducing unit.
[b] Penultimate unit.
[c] Antepenultimate unit.

prisingly, no effect was observed for C-5 carbon of modified residues. In the ^{1}H spectra, only signals of H-4, H-5 and H-6 of modified units were shifted either upfield or downfield depending on their substitution. The chemical structures of all maltooligosaccharides obtained were confirmed by FABMS spectrometry.

In this work, we have decided to reinvestigate the substrate specificity of the active site of CGTase and amyloglucosidase using modified cyclodextrins as donors and glucose or methyl glucoside as acceptors. From our results, the methyl ester at C-6^{II} of the maltosyl unit can accommodate in the +1 subsite of amyloglucosidase

and in the −2 subsite of CGTase, but negative interactions occur when a carboxylate derivative was used, as stated by previous workers.[10] This study allows the preparation of new maltooligosaccharides in good overall yields.

1. Experimental

1.1. Materials

CGTase from *Bacillus* sp. was a gift from Wacker Chimie S.A. (France) and amyloglucosidase G2 from *Aspergillus niger* was a gift from Dr. B. Svensson from Carlsberg Research Laboratory (Copenhagen, Denmark). D-(1-^{13}C)-glucopyranose was provided by CEA. D-Glucopyranose and methyl β-D-glucopyranoside are commercially available. 5-Carboxy-cyclomaltoheptaose (**1**), 5-carboxy-cyclomaltohexaose (**2**) and 6-*O*-trityl-cyclomaltoheptaose (**3**) were prepared as previously described.[19–21] 6-*O*-Pentenyl-cyclomaltoheptaose (**4**) was supplied by Chiralsep S.A. (France). All other chemicals were of commercial analytical grade unless otherwise noted and were used as received.

1.2. General methods

NMR experiments were recorded on a Bruker Avance 400 (^{1}H frequency of 400.132 MHz and ^{13}C frequency of 100.613 MHz), using the Bruker standard pulse sequences. Samples were studied as solutions in D_2O (5 mg in 2 mL of solvent) at 298 K in 5 mm od tubes. HOD signal (δ = 4.65) was used as an internal standard. ^{1}H spectra were recorded using 90° pulses, 4006 Hz spectral width, 65,536 data points, 8.179 s acq. time and 64 scans were accumulated. ^{13}C NMR experiments were obtained on the same spectrometer and 10,000 scans were accumulated (internal acetone ^{13}C (CH_3) δ = 31.5 relative to Me_4Si).

Low resolution fast atom bombardment mass spectra (FABMS) in the positive mode were recorded on a R 1010C quadripolar mass spectrometer (model 2000, Nermag, Reuil-Malmaison, France), equipped with a M Scan Wallis-type gun (8 kV, 20 mA). The samples were dissolved in a glycerol matrix and submitted to Xe (9 kV) bombardment.

Thin layer chromatography (TLC) was performed on silica gel F_{254} (Merck) with detection by UV light and/or charring with 1:15:15 sulfuric acid–MeOH–water solution.

1.3. Enzymatic hydrolysis

Carboxylic acids (**1**–**2**) were converted into their methyl esters (**5**–**6**) as follows: Carboxylic acids (20 mg) were dissolved in anhydrous methanol (10 mL). 2,2-Dimethoxypropane (1 mL) and one drop of concentrated chlorohydric acid were added. The solution was stirred at room temperature for 24 h. Solvent was removed under reduced pressure. The residue was dissolved in water, mixed-bed resin (Amberlite Duolite MB-6113) was added and after stirring for 30 min, the resin was filtered off, the solvent removed at 45 °C in vacuum and compounds were freeze dried. Compound **5**: FABMS (positive mode): m/z = 1001 $(M+H)^+$. Compound **6**: FABMS (positive mode): m/z = 1186 $(M+Na)^+$.

To a solution of modified cyclodextrin ((**1**–**6**), 10 mg) in water (1 mL) were added cyclodextrin glucanotransferase (17 μL, 11 U), amyloglucosidase (1 mg, 2 U) and glucose or its methyl derivative (10 mg). The mixture was incubated at 40 °C to give maltooligosaccharides (**7**–**12**). Enzymes were inactivated by boiling for 10 min. Ionic maltooligosaccharides were purified by HPLC on a TKHW40F column with distilled water as eluent (**7**–**10**). Other maltooligosaccharides were purified on Sep-Pack C18 eluted first with water to eliminate D-glucopyranoside and then, with a gradient water–MeOH to give a pure product (**11** and **12**). All compounds were finally freeze dried. ^{1}H and ^{13}C NMR assignments are shown in Tables 1–6.

1.3.1. (Sodium α-D-glucopyranosyluronate)-(1→4)-D-glucopyranose 7. (3.3 mg, 96%)—Donor: compound (**5**) or (**6**). Acceptor: D-(1-^{13}C)-glucopyranoside. Incubation time: 48 h. FABMS (positive mode): m/z = 401 $(M+Na)^+$.

1.3.2. (Sodium α-D-glucopyranosyluronate)-(1→4)-α-D-glucopyranosyl-(1→4)-D-(1-^{13}C)-glucopyranose 8. (2.6 mg, 56%)—Donor: compound (**1**) or (**2**). Acceptor: D-(1-^{13}C)-glucopyranoside. Incubation time: 48 h. FABMS (positive mode): m/z = 564 $(M+Na)^+$.

1.3.3. α-D-Glucopyranosyl-(1→4)-(sodium α-D-glucopyranosyluronate)-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose 9. (5.9 mg, 98%)—Donor: compound (**1**). Acceptor: D-glucopyranoside. Incubation time: 12 h. FABMS (positive mode): m/z = 725 $(M+Na)^+$.

1.3.4. Methyl (α-D-glucopyranosyl-(1→4)-(sodium α-D-glucopyranosyluronate)-(1→4)-α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside 10. (5.2 mg, 85%)—Donor: compound (**1**). Acceptor: methyl β-D-glucopyranoside. Incubation time: 12 h. FABMS (positive mode): m/z = 739 $(M+Na)^+$.

1.3.5. (6-*O*-Trityl-α-D-glucopyranosyl)-(1→4)-α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose 11. (3.5 mg, 53%)—Donor: compound (**3**). Acceptor: D-glucopyranose. Incubation time: 18 h. FABMS (positive mode): m/z = 932 $(M+Na)^+$.

1.3.6. (6-*O*-Pentenyl-α-D-glucopyranosyl)-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose 12. (2.6 mg, 57%)—Donor: compound (**4**). Acceptor: D-glucopyranose. Incubation time: 18 h. FABMS (positive mode): $m/z = 572$ $(M+Na)^+$.

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