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Carbohydrate Research 338 (2003) 1961–1967
CARBOHYDRATE RESEARCH
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Alternansucrase acceptor reactions with methyl hexopyranosides

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Received 28 April 2003; accepted 17 June 2003

Dedicated to the memory of Professor Edward J. Hehre

Abstract

Alternansucrase (EC 2.4.1.140, sucrose: (1→6), (1→3)-α-D-glucan 6(3)-α-D-glucosyltransferase) is a D-glucansucrase that synthesizes an alternating α-(1→3), (1→6)-linked D-glucan from sucrose. It also synthesizes oligosaccharides via D-glucopyranosyl transfer to various acceptor sugars. We have studied the acceptor products arising from methyl glycosides as model compounds in order to better understand the specificity of alternansucrase acceptor reactions. The initial product arising from methyl β-D-glucopyranoside was methyl β-isomaltoside, which was subsequently glucosylated to yield methyl β-isomaltotrioside and methyl α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-β-D-glucopyranoside. These products are analogous to those previously described from methyl α-D-glucopyranoside. The major initial acceptor product from methyl α-D-mannopyranoside was methyl α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside, but several minor products were also isolated and characterized, including a 3,6-di-*O*-substituted mannopyranoside. Methyl α-D-galactopyranoside yielded two initial products, methyl α-D-glucopyranosyl-(1→3)-α-D-galactopyranoside and methyl α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside, in a 2.5:1 molar ratio. Methyl D-allopyranosides were glucosylated primarily at position 6, yielding methyl α-D-glucopyranosyl-(1→6)-D-allopyranosides. The latter subsequently gave rise to methyl α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-D-allopyranosides. In general, the methyl α-D-hexopyranosides were better acceptors than the corresponding β-glycosides.

Keywords: Alternansucrase; Glucansucrase; Glycopyranoside acceptors; Oligosaccharide

1. Introduction

Glucansucrases are D-glucosyltransferases that synthesize α-D-glucans from sucrose. They are of interest not only for their ability to synthesize unique polysaccharides, but also for their ability to synthesize oligosaccharides via an acceptor reaction. Alternansucrase (EC 2.4.1.140), which synthesizes an alternating α-(1→3), (1→6)-linked D-glucan[1] is especially interesting in this regard. Not only do its acceptor products differ in structure from those synthesized by *Leuconostoc mesenteroides* NRRL B-512F dextransucrase,[2,3] but they are also produced somewhat more efficiently.[3] These oligosaccharide acceptor products may be useful as prebiotics.[4]

In this study, we describe the structures of alternansucrase acceptor products arising from various methyl hexopyranosides and compare them with those produced by *L. mesenteroides* NRRL B-512F dextransucrase.[5] Methyl glycosides have the advantage of occurring in a single anomeric form and are easily purified using ion-exchange chromatography.

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[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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doi:10.1016/S0008-6215(03)00324-0

2. Experimental

2.1. Materials

Methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl α-D-galactopyranoside, and methyl β-D-mannopyranoside were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methyl β-D-galactopyranoside and methyl α-D-mannopyranoside were purchased from Pfanstiehl Corp. (Waukegan, IL, USA). Methyl β-D-allopyranoside was synthesized from D-allose and purified by crystallization from EtOH.[6] Methyl α-D-allopyranoside was isolated from the mother liquor of the same reaction product mixture by ion-exchange chromatography and high performance liquid chromatography (HPLC) (details below). Crystalline food-grade sucrose was purchased from a local grocery store. All other chemicals were reagent grade.

Table 1
^{13}C NMR peak assignments

	C-1	C-2	C-3	C-4	C-5	C-6	OMe
Disaccharides							
α-D-Glc*p*-(1→6)-α-D-Man*p*-OMe							
Man	101.1	69.8	70.8	66.3	70.8	65.4	54.8
Glc	97.8	71.4	73.0	69.5	71.7	60.4	
α-D-Glc*p*-(1→3)-α-D-Man*p*-OMe							
Man	100.7	69.7	78.5	66.0	72.6	60.8	54.7
Glc	100.5	71.7	72.7	69.5	72.2	60.4	
α-D-Glc*p*-(1→2)-α-D-Man*p*-OMe							
Man	99.3	79.6	70.4	67.0	72.5	60.9	54.8
Glc	100.9	71.8	72.7	69.7	72.2	60.7	
α-D-Glc*p*-(1→4)-α-D-Gal*p*-OMe							
Gal	99.5	68.3	69.1	78.6	71.2	60.2	55.2
Glc	100.2	71.8	72.7	69.4	72.0	60.5	
α-D-Glc*p*-(1→3)-α-D-Gal*p*-OMe							
Gal	99.5	66.6	74.4	65.5	70.6	61.2	55.1
Glc	94.9	71.3	72.9	69.5	71.8	60.4	
α-D-Glc*p*-(1→6)-β-D-Glc*p*-OMe							
Glc	103.3	75.9	74.1	73.0	71.4	65.3	57.2
Glc′	97.8	69.4	73.0	69.2	71.7	60.4	
α-D-Glc*p*-(1→6)-β-D-All*p*-OMe							
All	101.2	70.2	71.0	66.4	71.7	65.6	57.1
Glc	97.7	71.5	73.1	69.4	71.8	60.4	
α-D-Glc*p*-(1→6)-α-D-All*p*-OMe							
All	99.4	65.9	71.1	65.4	67.2	65.5	55.6
Glc	97.7	71.4	73.0	69.4	71.7	60.4	
Trisaccharides							
α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Man*p*-OMe							
Man	101.1	69.8	70.6	66.3	70.8	65.5	54.8
Glc	97.8	71.3	73.3	69.4	70.1	65.4	
Glc′	97.6	71.4	73.0	69.4	71.8	60.4	
α-D-Glc*p*-(1→6)-[α-D-Glc*p*-(1→3)]-α-D-Man*p*-OMe							
Man	101.1	69.6	79.6	65.1	70.8	65.0	54.9
Glc (1→3)	100.8	71.8	72.8	69.5	72.3	60.4	
Glc (1→6)	97.7	71.5	73.0	69.4	71.8	60.3	
α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-OMe							
Glc	103.3	76.0	74.1	73.0	71.4	65.4	57.2
Glc′	97.7	70.2	73.3	69.4	71.3	65.4	
Glc″	97.8	69.4	73.05	69.3	71.76	60.4	
α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-OMe							
Glc	103.4	76.0	74.1	73.1	71.6	65.2	57.2
Glc′	97.9	70.0	79.4	69.3	72.8	60.2	
Glc″	99.0	71.6	72.8	69.9	71.7	60.2	
α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-All*p*-OMe							
All	99.4	65.9	71.1	65.4	67.2	65.6	55.6
Glc	97.7	71.4	73.3	70.2	71.4	65.4	
Glc′	97.7	69.4	73.0	69.4	71.8	60.6	
α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-β-D-All*p*-OMe							
All	101.2	70.2	71.0	66.4	71.8	65.6	57.1
Glc	97.7	71.4	73.3	69.4	71.4	65.4	
Glc′	97.7	69.4	73.0	69.3	71.8	60.4	

2.2. Acceptor reaction conditions

Acceptor reactions were carried out essentially as previously described,[2,4] using alternansucrase from *L. mesenteroides* NRRL B-21297. Dialyzed and concentrated cell-free culture fluid was used as alternansucrase without further purification, as this strain produces only alternansucrase with no detectable levels of dextransucrase.[7] Variations in the concentrations of sucrose and acceptor affect the distribution of acceptor products, but do not influence their structures.[8]

2.3. Analytical and preparative methods

Non-reducing acceptor products were isolated from reaction mixtures by ion-exchange chromatography as previously described,[9,10] followed by purification using aqueous gel-permeation chromatography over Bio-Gel P-2. When necessary, final purification of products was carried out by HPLC using a Synergi 10-μm RP 80 Å reversed-phase preparative column (21.2 × 250 mm) (Phenomenex Corp., Torrance, CA). Elution was accomplished with 1% MeOH in water, at a flow rate of 3–4 mL/min with refractive index detection. Thin-layer chromatography (TLC) was performed on Whatman K5 silica gel plates, using multiple ascents in 4:1 MeCN–water (v/v) as previously described.[2,11] Structures were determined by methylation/GC–MS and NMR spectroscopy.[10] ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR spectra were recorded for each sample on a Bruker 400 MHz instrument in D_2O at 27 °C. Peak assignments for NMR spectra (Table 1) were made based on the 2D NMR experiments, and the glycosidic linkages were confirmed by HMBC long-range coupling experiments.

2.4. Comparison of relative acceptor strengths

Relative quantitative effects of acceptors were measured by determining their ability to diminish the yield of alternan from sucrose.[12] Reaction mixtures contained 45 μL of 10% (w/v) sucrose, 45 μL of 10% (w/v) methyl glycoside, and 10 μL of alternansucrase solution (2.5 U/mL). Reactions were monitored by TLC and were judged to be complete when all of the sucrose had been consumed. Upon completion of the reaction, each was mixed with 0.2 mL of EtOH, and the alternan thus precipitated was pelleted by centrifugation. The alternan was redissolved in 0.3 mL water, reprecipitated with 0.6 mL EtOH, and redissolved in 1 mL water. These aqueous solutions were analyzed for total carbohydrate content.[13]

3. Results

Results of the quantitative comparisons of relative acceptor strengths appear in Fig. 1.

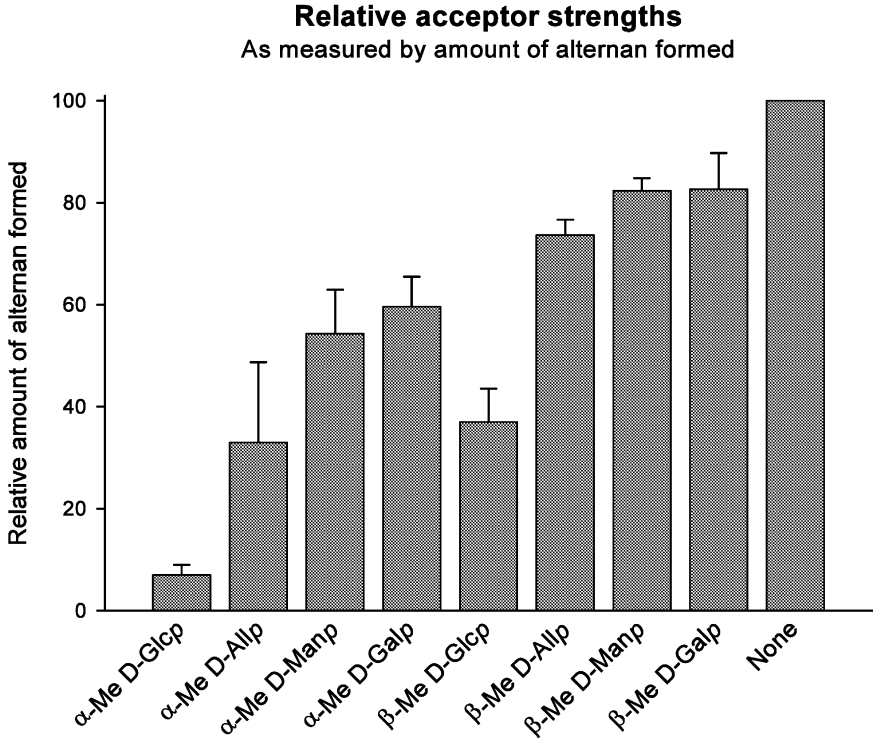

Fig. 1. Relative acceptor strengths of methyl hexopyranosides, as measured by ability to reduce alternan formation. Better acceptors give rise to less alternan.

As the data show, methyl α-D-glucopyranoside was the best acceptor in this group, with an approximately 90% diversion of D-glucosyl units away from alternan formation and into oligosaccharides. Methyl α-D-allopyranoside and methyl β-D-glucopyranoside were approximately equal in their ability to serve as acceptors, as were methyl α-D-mannopyranoside and methyl α-D-galactopyranoside. Methyl β-D-galactopyranoside and methyl β-D-mannopyranoside were the poorest acceptors, and their products were not studied.

Methyl β-D-glucopyranoside gave rise to a series of products that migrated on TLC identically to those arising from methyl α-D-glucopyranoside.[2] The initial product was shown by ^{13}C and ^{1}H NMR to be methyl β-isomaltoside. Two trisaccharides were also isolated from the reaction mixture. The slower-migrating one (on TLC) was identified by NMR as methyl β-isomaltotrioside, and the other, faster-migrating product was shown by NMR and methylation/GC–MS to be methyl α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-β-D-glucopyranoside. These products (Fig. 2) are analogous to the products arising from glucose and methyl α-D-glucopyranoside.[2] Traces of higher-DP products were also observed, but were not isolated.

Two major products were isolated from the reaction with methyl α-D-allopyranoside. The first of these was methyl α-D-glucopyranosyl-(1→6)-α-D-allopyranoside. This disaccharide was subsequently glucosylated at position 6′ to give rise to the trisaccharide methyl α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-allopyranoside. Higher DP products were observed but not isolated. Methyl β-D-allopyranoside gave rise to the corresponding α-(1→6)-linked products, but in lower yields.

A mixture of reaction products arose when methyl α-D-galactopyranoside was added as an acceptor. Two disaccharide products were isolated. Results of methylation/GC–MS analysis showed the major disaccharide to be methyl α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside and the minor disaccharide to be methyl α-D-glucopyranosyl-(1→3)-α-D-galactopyranoside (Fig. 3). The NMR spectra of both are consistent with the data presented by Baumann and co-workers.[14] The molar ratio of major/minor disaccharide was 2.5:1, as

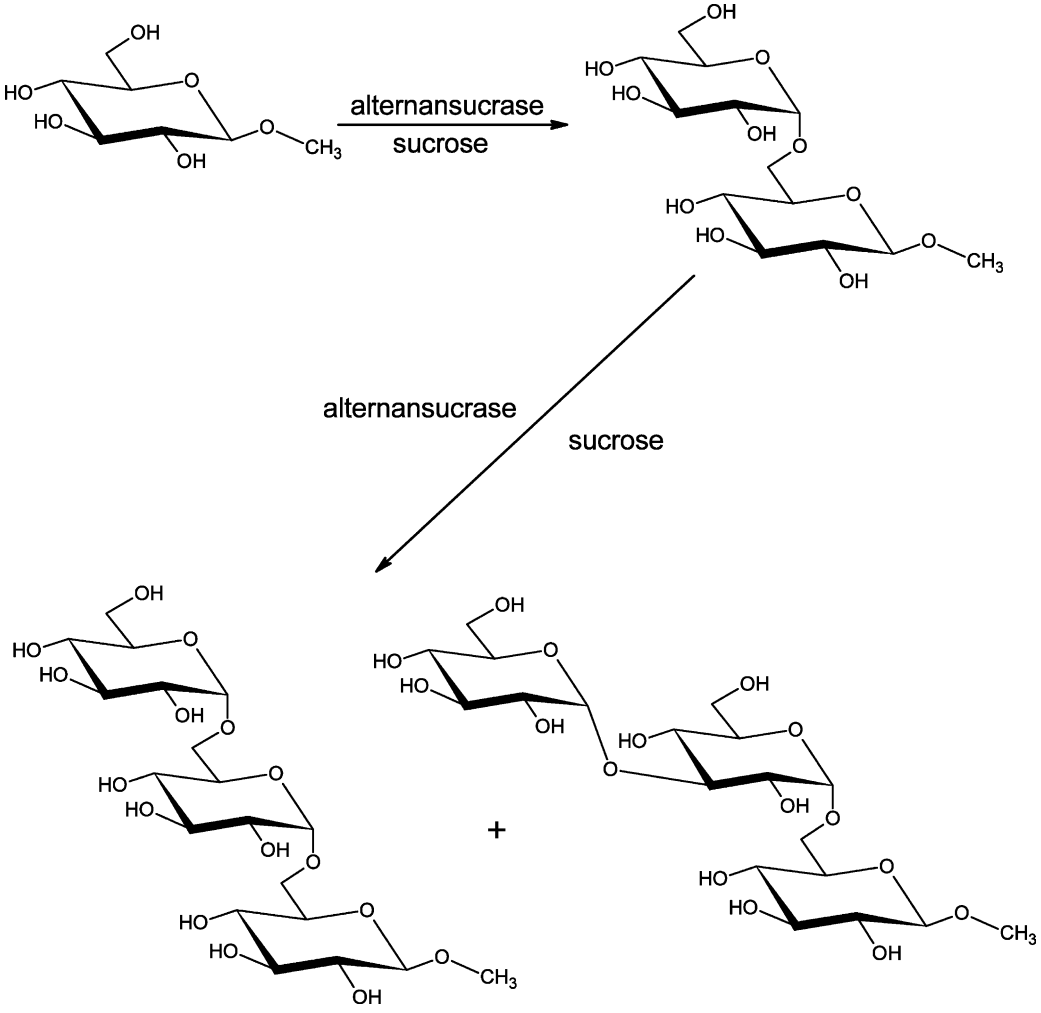

Fig. 2. Acceptor reaction products from methyl β-D-glucopyranoside.

determined by NMR spectroscopy and methylation/GC–MS analysis of the mixture prior to separation. Fu and co-workers[5] found that *L. mesenteroides* NRRL B-512F dextransucrase synthesized only methyl α-D-glucopyranosyl-(1→4)-α-D-galactopyranoside, which has also been described recently by Liotta and co-workers.[15] This compound is of interest due to its structural similarity to a portion of the repeating segment of the O-specific antigen polysaccharide of *Salmonella* spp.[16] Small amounts of higher DP products were observed but not isolated in pure form. Methylation analysis of the mixture suggested that it contained 6-*O*-α-D-glucosylated derivatives of both disaccharides.

Several products were isolated by ion-exchange and size-exclusion chromatography from methyl α-D-mannopyranoside reaction mixtures. The major disaccharide product was shown by NMR spectroscopy and methylation/GC–MS to be methyl α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside. This is the same product isolated from the acceptor reaction with dextransucrase.[5] The α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside structure also occurs as part of the cell wall glucomannan in certain fungi.[17] The major trisaccharide product was methyl α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside, which arises from 6-*O*-α-D-glucosylation of the major disaccharide product. Two minor disaccharide products, methyl α-D-glucopyranosyl-(1→2)-α-D-mannopyranoside and methyl α-D-glucopyranosyl-(1→3)-α-D-mannopyranoside, were also formed but were not separately isolated. The most unusual product isolated was identified as methyl α-D-glucopyranosyl-(1→6)-[α-D-glucopyranosyl-(1→3)]-α-D-mannopyranoside. Two conceivable pathways exist for the formation of this di-*O*-substituted mannoside, as shown in Fig. 4. The tetrasaccharide methyl α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside was also isolated and identified by methylation/GC–MS analysis.

4. Discussion

Some conclusions may be drawn from these results. In general, the methyl α-D-hexopyranosides are better acceptors than their corresponding β-linked isomers. Within a series (α or β), the order of acceptor strengths is the same, i.e., D-glucopyranoside > D-allopyranoside > D-mannopyranoside ≥ D-galactopyranoside. At least in the cases of D-glucopyranoside and D-allopyranoside, the products arising from the methyl α-D-hexopyranosides are structurally analogous to those arising from the corresponding β-linked isomers. Also for these products, the hydroxyls above the plane of the ring (those *cis* to the anomeric oxygen in methyl β-D-glycopyranosides or *trans* to anomeric oxygen in methyl α-D-glycopyranosides) are acceptors,

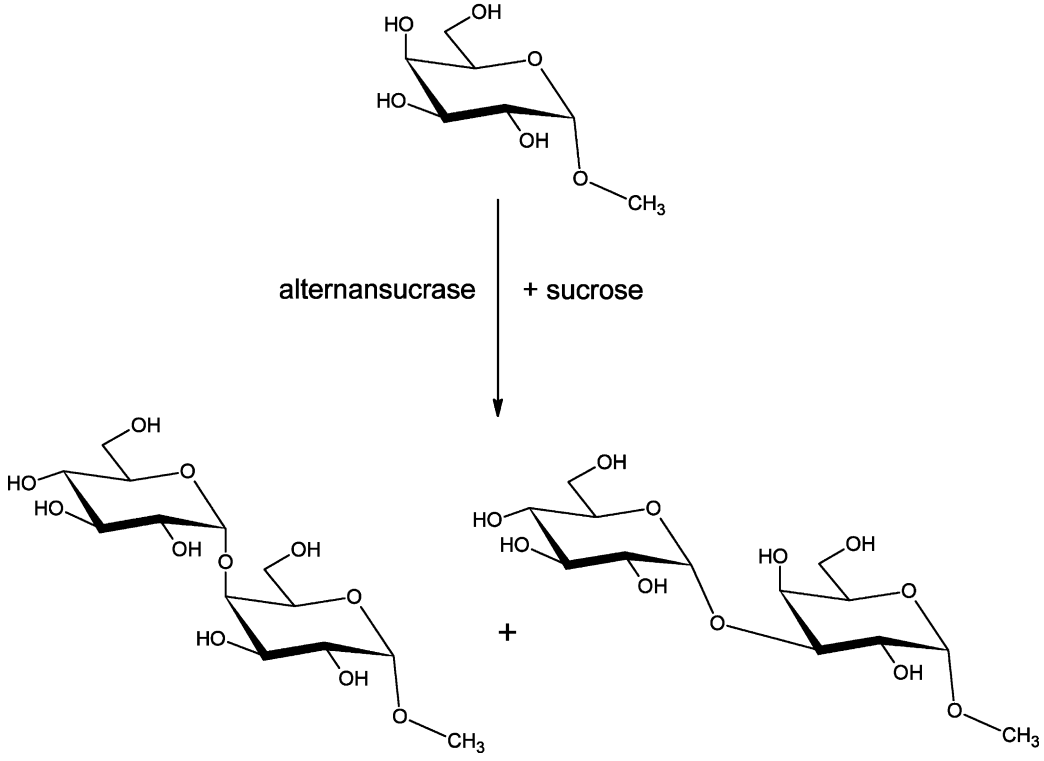

Fig. 3. Initial acceptor reaction products from methyl α-D-galactopyranoside.

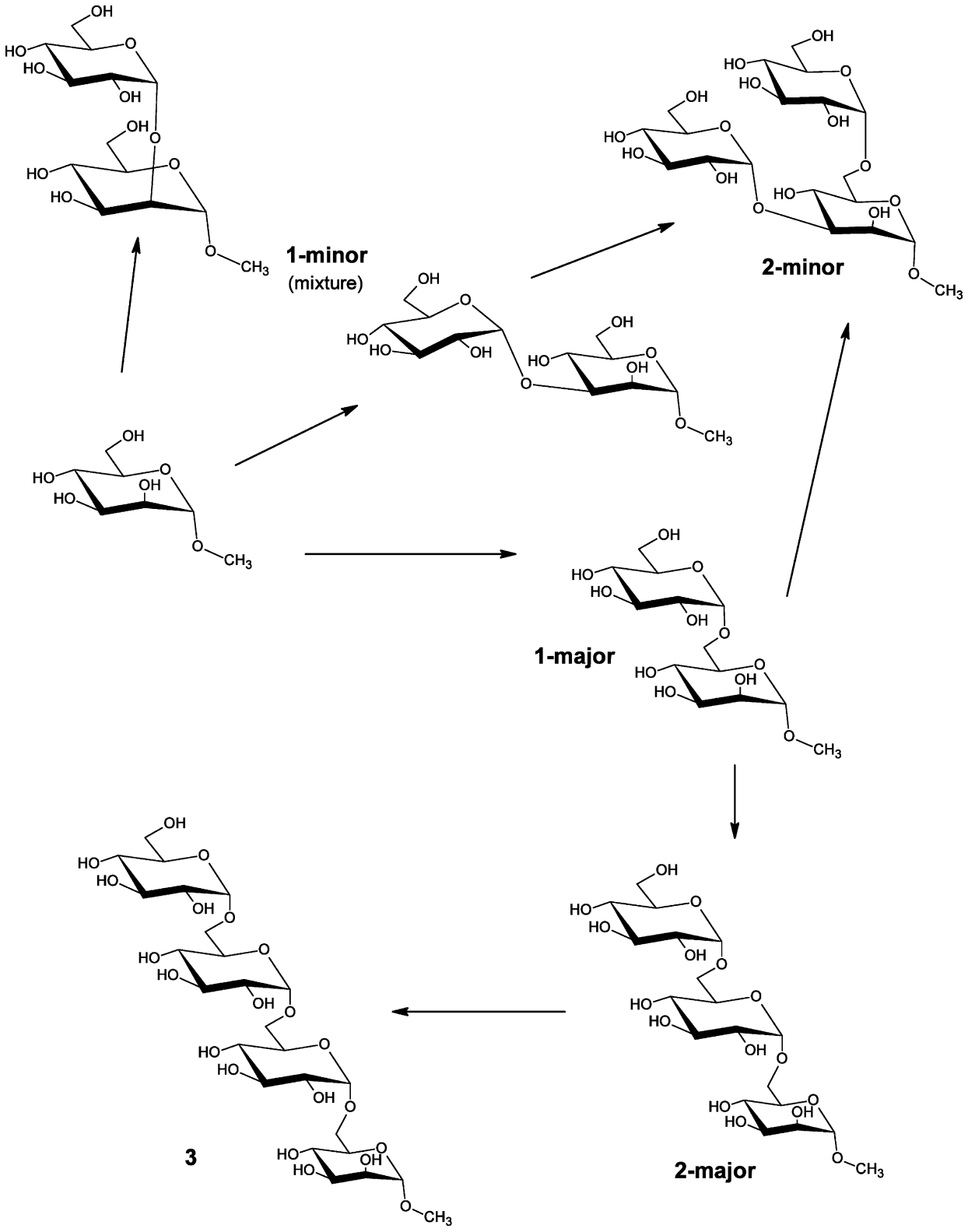

Fig. 4. Acceptor reaction products from methyl α-D-mannopyranoside.

while all hydroxyls below the ring are not. Interestingly, the one exception to this observation is the lack of the methyl α-D-glucopyranosyl-(1→6)-α-D-galactopyranoside as an acceptor product.

With the exception of leucrose, alternansucrase acceptor products previously described in the literature have all been derived from D-glucose-containing acceptors.[2,3,8,18] The compounds described here provide

additional examples showing that the regioselectivity of alternansucrase acceptor reactions differs from that of *L. mesenteroides* NRRL B-512F dextransucrase, particularly in the fact that alternansucrase produces a variety of linkages, whereas dextransucrases typically produces only a single linkage type for any given acceptor.[5] They have also been useful to us as model compounds in our studies of prebiotic oligosaccharides produced by alternansucrase, especially those derived from α-D-galactopyranosides such as raffinose and melibiose.[4]

Our published report[4] and many others, too numerous to cite, on the prebiotic activity of various oligosaccharides make it clear that the structure–function relationship of prebiotic carbohydrates is not understood. Knowledge of the carbohydrate structures is but a single first step toward that goal.

Acknowledgements

The authors are grateful to James Nicholson for performing methylation analyses and to Dr David Weisleder for obtaining the NMR spectra.

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