Synthesis and characterization of isomaltulose-derived oligosaccharides produced by transglucosylation reaction of Leuconostoc mesenteroides dextransucrase

Montserrat Barea-Alvarez[1], Maria Teresa Benito[2], Agustín Olano[1], Maria Luisa Jimeno[2], F. Javier Moreno*[1]

[1]Dpto. Bioactividad y Análisis de Alimentos. Instituto de Investigación en Ciencias de la Alimentación, CIAL (CSIC-UAM), CEI (UAM+CSIC), c/Nicolás Cabrera 9, 28049 Madrid, Spain.

[2]Centro Química Orgánica "Lora-Tamayo" (CSIC), c/Juan de la Cierva 3, 28006 Madrid, Spain.

* Corresponding author: Tel.: +34 91 0017948; E-mail address: javier.moreno@csic.es

Abstract

This article reports the efficient enzymatic synthesis of a homologous series of isomaltulose-derived oligosaccharides with degrees of polymerization ranging from 3 to 9 through the transglucosylation reaction using a dextransucrase from *Leuconostoc mesenteroides* B-512F. The total oligosaccharide yield obtained under optimal conditions was of 41-42% (in weight respect to the initial amount of isomaltulose) after 24-48 hours of reaction. Nuclear magnetic resonance (NMR) structural characterization indicated that dextransucrase specifically transferred glucose moieties of sucrose to the C-6 of the nonreducing glucose residue of isomaltulose. Likewise, the monitoring of the progress of the content of each individual oligosaccharide indicated that oligosaccharide acceptor products of low molecular weight acted in turn as acceptors for further transglucosylation to yield oligosaccharides of higher degree of polymerization. The produced isomaltulose-derived oligosaccharides can be considered as isomalto-oligosaccharides (IMOs) since they are linked by only α-(1→6) bonds. In addition, having isomaltulose as core structure, these IMO-like structures could possess appealing bioactive properties which could find potential applications as functional food ingredients.

Keywords: isomaltulose, bioactive oligosaccharides, transglycosylation, dextransucrase, *Leuconostoc mesenteroides*

Introduction

Isomaltulose is a reducing disaccharide (6-*O*-α-D-glucopyranosyl-D-fructose) occurring naturally in honey or sugar cane juice and derived products[1]. It is considered as a multifunctional carbohydrate ingredient with beneficial properties, such as non-cariogenicity or reduction of post-prandial glycemic responses among others, which may be particularly favorable for both diabetics and pre-diabetics[2,3]. These properties make isomaltulose suitable for sucrose replacer in a number of foods and beverages by combining the functional benefits mentioned above with its organoleptic and technological properties such as improved texture, good stability under acidic conditions or thermal processing, as well as mild and sweet taste[3,4].

Isomaltulose is approved for the use in food and beverages in the United States, European Union, Japan and elsewhere. In addition, regulatory authorities such as EFSA or FDA have ruled this ingredient as non-cariogenic or with the capacity to induce lower post-prandial glycemic and insulinemic responses[5,6]. These facts explain that there is a renewed interest in isomaltulose commercialization, which is normally marketed under the trade name "Palatinose®". Commercial isomaltulose is produced enzymatically from food-grade sucrose by rearrangement of the glycosidic linkage from a 1,2-fructoside to a 1,6-fructoside followed by crystallization[1].

Isomaltulose can be considered as an isomaltooligosaccharide (IMO)[7]. IMOs involve a heterogeneous group of glucosyl saccharides with the predominant α-(1→6) linkage and this type of oligosaccharides are of interest in the fields of foods, pharmaceuticals, and cosmetics due to their unique properties, including prebiotic character, cholesterol regulation, immunomodulatory activity or prevention and

resistance to various diseases[8]. In this context, the development of simple and convenient methods for the efficient synthesis of oligosaccharides derived from isomaltulose could offer opportunities to develop new derivatives with improved or additional functionalities, as well as to further expand the use of isomaltulose as a high-value added ingredient.

Of particular interest are the so-called acceptor reactions of microbial glucansucrases, including dextransucrases (EC 2.4.1.5) and alternansucrases (2.4.1.140). This type of enzymes polymerize the glucosyl moiety of sucrose to form dextran, an α-(1→6) linked glucan with optionally α-(1→2), α-(1→3), or α-(1→4) branch linkages depending on the origin of the enzyme[9]. When, in addition to sucrose, other carbohydrates (mainly mono- or disaccharides) are present, D-glucosyl units are transferred from sucrose to the carbohydrate through the acceptor reaction diverting the dextran formation to yield α-D-glucopyranosyl acceptor products[8,10-14]. Whilst maltose has proven to be an excellent acceptor for a variety of bacterial glucansucrases to yield glucooligosaccharides[15-19], isomaltulose has been hardly used to act as acceptor in this type of reaction. Concretely, Demuth et al[12] and Côté et al.[14,17] showed isomaltulose to be a moderate acceptor for glucansucrases, and only the corresponding trisaccharide acceptor product could be structurally characterized as isomaltotriulose following transglucosylation reaction catalyzed by an alternansucrase from *L. mesenteroides* NRRL B-21297[17]. However, the rest of acceptor products were neither structurally characterized nor quantified. Finally, isomaltotriulose was also detected in trace amounts in broths of dextran-producing cultures of *Streptococcus bovis* containing sucrose as substrate[20].

In this work the efficient enzymatic synthesis of a homologous series of isomaltulose-derived oligosaccharides catalyzed by a dextransucrase from *Leuconostoc mesenteroides* B-512F, as well as their comprehensive NMR structural characterization are reported.

Materials and methods

Chemicals, reagents, standards and enzymes

Fructose, glucose, sucrose, maltotriose, maltotetraose, maltopentaose and maltohexaose were purchased from Sigma-Aldrich (Steinheim, Germany), whereas isomaltulose was bought from Carbosynth (Compton, UK). Acetonitrile (HPLC grade) was obtained from Lab-scan (Gliwice, Poland) and deuterium oxide from Merck Millipore (Darmstadt, Germany). Ultrapure water quality (18.2 MΩ cm) with 1-5 ppb total organic carbon (TOC) and <0.001 EU mL^{-1} pyrogen levels was produced in-house using a laboratory water purification Milli-Q Synthesis A10 system from Millipore (Billerica, MA). Purified dextransucrase (E.C. 2.4.1.5) from *Leuconostoc mesenteroides* B-512F was purchased from CRITT Bio-Industries (Toulouse, France). Specific activity was 0.4 U mg^{-1}, where 1 unit is the amount of enzyme required to transfer 1 μmol of glucose per minute, with 100 g of sucrose per liter as the substrate in 20 mM sodium acetate buffer (pH 5.2) with 10 mg L^{-1} of $CaCl_2$ at a working temperature of 30 °C. All other chemicals were of analytical grade and commercially available.

Enzymatic synthesis of isomaltulose-derived oligosaccharides

Oligosaccharide synthesis was carried out by transglucosylation reaction catalyzed by dextransucrase from *Leuconostoc mesenteroides* B-512F (0.8 U mL^{-1}) at 30 ºC in 20 mM sodium acetate buffer with 0.34 mM $CaCl_2$ (pH 5.2) in the presence of 250 g L^{-1} sucrose (donor) and 250 g L^{-1} isomaltulose (acceptor). The reaction was allowed to proceed up to 48 h, and aliquots were repeatedly taking from the reaction mixture at suitable time intervals (1, 3, 5, 8, 24 and 48 h). The enzyme was inactivated by heating at 100 °C for 5 min, and inactivated samples were then 40-fold diluted with acetonitrile:water (50:50, v:v), filtered using a 0.45 μm syringe filter (Symta, Madrid, Spain), and analyzed by liquid chromatography with refractive index detector (LC-RID). All enzymatic syntheses were carried out in triplicate.

Chromatographic Determination of Carbohydrates by Liquid Chromatography with Refractive Index Detector (LC-RID).

The progress of the synthesis process of isomaltulose-derived oligosaccharides was monitored by liquid chromatography with refractive index detector (LC-RID) on an Agilent Technologies 1220 Infinity LC System – 1260 RID (Boeblingen, Germany). The separation of carbohydrates was carried out with an analytical Zorbax® NH_2 column (150 × 4.6 mm, 5 μm particle size) and an analytical Zorbax® NH_2 guard column (12.5 × 4.6mm, 5 μm particle size) (Agilent, Boeblingen, Germany) using isocratic elution with acetonitrile:water at 75:25 or 70:30 (v/v) as the mobile phase to quantify mono- and disaccharides or oligosaccharides, respectively, and at a flow rate of 1.0 mL min^{-1} for 60 min. Injection volume was 50 μL (750 μg of total carbohydrates). Data acquisition and processing were performed using Agilent ChemStation software (Agilent Technologies, Boeblingen, Germany).

Carbohydrates in the reaction mixtures were identified by comparing their retention times with those of standard sugars. Quantitative analysis was performed by the external standard method, using calibration curves in the range 0.1 - 5 mg mL^{-1} for fructose, glucose, sucrose, isomaltulose, maltotriose, maltotetraose, maltopentaose and maltohexaose. Individual maltodextrins were used to quantify the isomaltulose-derived oligosaccharides with the same degree of polymerization (DP). Maltohexaose was also used for the quantification of oligosaccharides with DP above 6. All analyses were carried out in triplicate. Determination coefficients obtained from these calibration curves, which were linear over the range studied, were high (R^2 > 0.999). Reproducibility of the method was estimated on the basis of the intra-day and inter-day precision, calculated as the relative standard deviation (RSD) of concentrations of oligosaccharide standards obtained in $n \geq 6$ independent measurements, obtaining RSD values below 10% in all cases.

Purification and structural characterization of the isomaltulose-derived oligosaccharides by Nuclear Magnetic Resonance

Isomaltulose-derived oligosaccharides from DP 3 to 7 were isolated and purified by LC-RID from sucrose:isomaltulose mixtures after 24 hours of transglucosylation reaction and using a semi-preparative column Kromasil® (100-NH_2) column (250 × 10 mm, 5 μm particle size) (Akzo Nobel, Brewster, NY). Thus, 500 μL of reaction mixtures (7.5 mg of total carbohydrates) was repeatedly eluted with acetonitrile:water (70:30, v:v) as the mobile phase at a flow rate of 5 mL min^{-1}, and fractions corresponding to the main synthesised oligosaccharide were manually collected, pooled,

evaporated in a rotatory evaporator R-200 (Büchi, Switzerland) below 25 ºC and freeze-dried for its subsequent characterization.

Structure elucidation of the purified oligosaccharides was accomplished by Nuclear Magnetic Resonance spectroscopy (NMR). NMR spectra were recorded at 298 K, using D_2O as solvent, on a Varian SYSTEM 500 NMR spectrometer (^1H 500 MHz, ^{13}C 125 MHz) equipped with a 5-mm HCN cold probe. Chemical shifts of ^1H (δ_H) and ^{13}C (δ_C) in ppm were determined relative to an external standards of sodium [2, 2, 3, 3-$^2H_4$]-3-(trimethylsilyl)-propanoate in D_2O (δ_H 0.00 ppm) and 1, 4-dioxane (δ_C 67.40 ppm) in D_2O, respectively. One-dimensional (1D) NMR experiments (^1H and ^{13}C) were performed using standard Varian pulse sequences. Two-dimensional (2D) [^1H, ^1H] NMR experiments (gradient correlation spectroscopy [gCOSY] and total correlation spectroscopy [TOCSY]) were carried out with the following parameters: a delay time of 1 s, a spectral width of 1,675.6 Hz in both dimensions, 4,096 complex points in t2 and 4 transients for each of 128 time increments, and linear prediction to 256. The data were zero-filled to 4,096 × 4,096 real points. 2D [^1H–^{13}C] NMR experiments (gradient heteronuclear single-quantum coherence [gHSQC] and gradient heteronuclear multiple-bond correlation [gHMBC]) used the same ^1H spectral window, a ^{13}C spectral window of 30,165 Hz, 1 s of relaxation delay, 1,024 data points, and 128 time increments, with a linear prediction to 256. The data were zero-filled to 4,096 × 4,096 real points. Typical numbers of transients per increment were 4 and 16, respectively.

Results

The optimal conditions, i.e. pH, temperature, enzyme charge and concentration ratio of donor:acceptor, for the transglucosylation activity of dextransucrase in acceptor

178 reactions using disaccharides as lactose or lactulose were previously established by
179 Diez-Municio et al.[21,22]. Based on these reaction conditions, the enzymatic synthesis
180 was monitored by LC-RID as a function of time. **Figure 1**, which depicts a typical
181 chromatogram obtained after 48 hours of transglucosylation reaction of isomaltulose,
182 shows a series of oligosaccharide acceptor products with degrees of polymerization
183 (DP) ranging from 3 to 9 resolved in 60 min using an isocratic elution with
184 acetonitrile:water 70:30 (v:v). Sucrose and isomaltulose could not be well resolved
185 under these chromatographic conditions and a mobile phase composition with a weaker
186 elution strength (i.e., acetonitrile:water 75:25, v:v) was used to quantify mono- and
187 disaccharides (chromatograms not shown).

188 As it is indicated in **Table 1**, sucrose was readily hydrolyzed throughout the
189 enzymatic reaction and only 3.5% remained after 48 hours whilst fructose was
190 progressively released as the sucrose hydrolysis advanced. Concomitantly, only traces
191 of glucose could be detected whereas isomaltulose content substantially decreased,
192 which was indicative of the efficient transfer of glucose moieties as well as of the
193 capacity of isomaltulose to act as acceptor. Concerning oligosaccharide acceptor
194 products, the isomaltulose-derived trisaccharide was the predominant acceptor product
195 during the first 8 hours of reaction, whereas at 24 and 48 hours of reaction the most
196 abundant oligosaccharides were the tetrasaccharide and the pentasaccharide acceptor
197 products, respectively (**Table 1**). Overall, the maximum formation of isomaltulose-
198 derived oligosaccharides was obtained after 24-48 hours of reaction by producing
199 101.6-104 g L^{-1} which is equivalent to a yield of 41-42% (in weight respect to the initial
200 amount of isomaltulose).

201 **Figure 2** shows the evolution of each individual oligosaccharide acceptor
202 product expressed as percent of the total carbohydrate composition (including fructose,

sucrose and isomaltulose). The maximum relative percentage of the trisaccharide reached a plateau from the fifth hour of reaction and this was kept until the end of the reaction, whilst the tetrasaccharide reached a plateau at 24 hours and the pentasaccharide exhibited a moderate increase between 24 and 48 hours of reaction. In contrast, hexa-, hepta-, octa- and nonasaccharide were initially detected at 3, 5, 8 and 24 hours of reaction, respectively, and did not reach a maximum formation at 48 hours of reaction. This behavior clearly indicated that oligosaccharide acceptor products of low molecular weight could in turn act as an acceptor for further transglucosylation, thus yielding a homologous series of oligosaccharide products with an elongated chain.

In order to comprehensively characterize the synthetized oligosaccharides as well as to confirm the enzyme mechanism in the synthesis of oligosaccharides, the resulting carbohydrate mixture obtained after 24 hours of reaction was repeatedly analyzed using a semipreparative LC column to purify the oligosaccharides with DP ranging from 3 to 7. These isolated oligosaccharides (structures DP3, DP4, DP5, DP6 and DP7 respectively, **Figure 3**) were then fully characterized by 1D (^{1}H and ^{13}C) and 2D [^{1}H, ^{1}H] and [^{1}H, ^{13}C] NMR experiments (gCOSY, TOCSY, multiplicity-edited gHSQC and gHMBC). ^{1}H and ^{13}C NMR chemical shifts observed are summarized in **Table 2**.

These compounds showed duplicate NMR signals in 1D ^{1}H and ^{13}C NMR spectra, indicating the presence of two quite different populated isomers at room temperature, probably due to an anomeric mixture of β and α fructose anomers. Structural determinations were performed on the major isomer for all structures.

1D ^{13}C spectrum of DP3 showed a major set of signals corresponding to 18 carbons including three anomeric carbons (δ102.31, δ98.95 and δ98.40), indicative of

the presence of a trisaccharide with three hexose sugars in its structure. A multiplicity-edited gHSQC spectrum was used to link the carbon signals to the corresponding proton resonances. So, the anomeric carbon at δ98.95 correlated with an alpha anomeric proton at δ4.98 (J(H1,H2)=3.8 Hz) and the anomeric carbon at δ98.40 correlated with an alpha anomeric proton at δ4.95 (J(H1,H2)=3.8 Hz). The anomeric carbon at δ102.31 was a quaternary carbon. In addition, four methylene carbons at δ68.61, δ66.12, δ63.22 and δ61.05 were identified. Careful analysis of the 2D gCOSY and TOCSY spectra revealed the ^{1}H signals of two units of glucopyranose and a unit of fructofuranose (**Figure 3**). To assign the configuration of each anomeric center, the values of the vicinal coupling constants for the glucopyranose anomeric protons, and the ^{13}C chemical shift values of the fructofuranose anomeric carbons were used[23]. These results were consistent with the structure of a trisaccharide with the presence of two α-D-glucopyranose,residues (G_1 and G_2) and as the reducing terminal unit F_1 a fructose residue in the form of a furanosyl ring with β and α forms in a 3:1 ratio. The position of glycosidic linkages was analyzed as follows: gHMBC experiment showed correlations between the anomeric proton of G_1 (4.98 ppm) and C-6 of the terminal fructose F_1 (68.61ppm), between the anomeric carbon of G_1 (98.95 ppm) and the methylene H6 protons of the terminal fructose F_1 (3.88, 3.70 ppm), between the anomeric proton of G_2 (4.95 ppm) and C-6 of G_1 (66.12 ppm), and between the anomeric carbon of G_2 (98.40 ppm) and the methylene H6 protons of G_1 (3.97, 3.76 ppm). Finally, the major isomer of compound DP3 was identified as α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside and whose common name is isomaltotriulose.

The 1D ^{1}H NMR spectrum of DP_4 showed three resonances in the anomeric region, and besides 1D ^{13}C NMR spectrum showed a major set of signals corresponding to 24 carbons including four anomeric carbons (δ102.31, δ98.94, δ98.35 and δ98.32),

indicative of the presence of a tetrasaccharide. Careful analysis of the 2D gCOSY and TOCSY spectra revealed the ^{1}H signals of three units of glucopyranose and a unit of fructofuranose. To assign the configuration of each glucopyranose, the values of ^{3}J(H1,H2) coupling constants (3.8, 3.8 and 3.8 Hz) were used. Following the same procedure used for the trisaccharide, using multidimensional solution NMR and 13C chemical shift comparison (**Figure 4**), the major isomer of compound DP4 was identified as α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside.

1D ^{1}H NMR spectra of DP5, DP6 and DP7 showed one, two and three additional doublets in the anomeric region, respectively. Likewise the corresponding 1D ^{13}C NMR spectra showed five (δ102.32, δ98.95, δ98.35, δ98.34 and δ98.24), six (δ102.31, δ98.94, δ98.35, δ98.34, δ98.29 and δ98.27) and seven anomeric carbons (δ102.31, δ98.94, δ98.35, δ98.34, δ98.29, δ98.28 and δ98.27), consistent with the presence of a penta- hexa- and a heptasaccharide, respectively. Following the same procedure, as for DP3, compound DP5 was identified as α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside, compound DP6 α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside and compound DP7 as α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside.

Discussion

This work describes the efficient synthesis of a homologous series of 6-*O*-α-glucosylated isomaltulose with DP ranging from 3 to 9 by transferring the glucosyl moiety from sucrose to the C-6 of the non-reducing end of isomaltulose. To the best of our knowledge, this article contains the first structural NMR data of isomaltulose-derived oligosaccharides from DP 4 to 7. Côté et al.[17] previously published the NMR shifts for the isomaltulose trisaccharide product derived from a transglucosylation reaction catalyzed by an alternansucrase from *L. mesenteroides* NRRL B-21297. Also, Seo et al.[24] identified isomaltulose and the corresponding 6-O-α-glucosylated isomaltuloses of DP 3, 4 and 5 which were produced in minor amounts when dextransucrase was used to hydrolyze concentrated sucrose solutions, although in that case, the authors used a combination of enzymatic approaches and MALDI-TOF MS analysis.

The formation of α-(1→6) glucosidic bonds is in good agreement with the reaction mechanism described for the dextransucrase from *L. mesenteroides* NRRL B-512F which catalyzes the formation of the polysaccharide dextran with a 95% of α-(1→6)-linked glucan with 5% α–(1→3) branch linkages. In addition, the bond formed during the acceptor reaction is normally an α-(1→6) glucosidic linkage[10,25,26]. As an example, this was the case for maltose which gave rise to a series of seven homologous acceptor products having isomaltodextrin residues attached to the C-6 of the non-reducing glucosyl residue of maltose[16]. However, some exceptions have been described for this enzyme as it was the case of the main production of 2-α-glucosyl-cellobiose, 2-α-glucosyl-lactose or lactulosucrose through the formation of an α-(1→2) glucosidic linkage to the reducing end of cellobiose, lactose or lactulose, respectively[21,22,27].

Elegant works led by Robyt and co-authors determined that at high sucrose concentrations (≥ 200 mM) in the presence of equimolar amounts of suitable acceptors, dextransucrase trends to catalyze the synthesis of negligible amounts of dextran but does catalyze large amounts of acceptor products[28-30]. Our data confirmed this observation and revealed the suitability of isomaltulose as acceptor to yield around 42% of oligosaccharides after 24 h of reaction using equimolar amounts of sucrose and isomaltulose (**Table 1**). Likewise, the progress of the content of the acceptor products throughout the enzymatic reaction (**Figure 2**) as well as their characterized structures indicated the ability of the acceptor products to also serve as acceptors to give oligosaccharides of higher DP, thus, yielding a homologous series of isomaltulose-derived oligosaccharides of decreasing amounts as DP increase (**Table 1**).

The produced isomaltulose-derived oligosaccharides can be considered as IMO in the strict sense since they are linked by only α-(1→6) bonds. In addition, bearing in mind that isomaltulose is the core structure, these oligosaccharides could possess a series of bioactive properties based on isomaltulose functionality. In this sense, Seo et al.[24] synthetized a series of oligosaccharides from high concentrations of sucrose (2.5-4 M) using a dextransucrase prepared from a mutant *Leuconostoc mesenteroides* strain. Among these oligosaccharides, low levels (between 2.7 and 3.7%) of 6-O-α-glucosylated isomaltuloses of DP 3, 4 and 5 were present. These authors reported that the mixture of oligosaccharides effectively inhibited the formation of insoluble glucan by *Streptoccocus sobrinus* which play an important role in the aetiology of dental caries, suggesting their potential use as anti-cariogenic sucrose substitute. Interestingly, Kashimura et al.[31] demonstrated that isomaltulose and its hydrogenated disaccharide products which were also linked through an α-(1→6) glucosyl bond, i.e. α-O-D-glucopyranosyl-1,6-D-sorbitol and α-O-D-glucopyranosyl-1,6-D-mannitol,

competitively inhibited rat small intestinal α-glucosidases (sucrase, maltase and glucoamylase) and reduced the rate of hydrolysis of sucrose and other α-glucosylsaccharides such as maltose, dextrin or soluble starch. Considering that maltitol, a disaccharide alcohol with an α-(1→4) glucosyl linkage, did not exert inhibitory effects, these authors suggested that the inhibitory action of isomaltulose and its derivatives could be related to the α-(1→6) glucosyl linkage. Given that the rates of hydrolysis of isomaltulose[3] and its hydrogenated derivatives are low in the small intestine[32], they could contribute to reduce glycemic and insulinemic responses following their ingestion. In fact, there are solid scientific evidences that point out isomaltulose as an efficient low-glycemic sweetener with capacity to reduce the speed of digestion and absorption resulting in lower post-prandial blood glucose[33-40]. In this context, and given that the isomaltulose-derived oligosaccharides described in the current work possess an α-1,6 glucosyl linkage as unique structural bond, it will be of interest to investigate their role on glycemic control.

Considering their inhibitory action on small intestinal α-glucosidases and reduced absorption, IMO-like structures might have the ability to reach the large intestine, at least, partially intact[8]. Nevertheless, IMOs do not conform strictly to the non-digestibility criterion as they could be hydrolyzed by the membrane-bound isomaltase and maltase-glucoamylase enzymatic complexes in the small intestine, despite not all IMO species seem to be digested at the same rate[41]. In this context, composition and dosage in the diet should be key parameters for the prebiotic potential of IMOs[8] and, consequently, additional studies on the effect of isomaltulose-derived oligosaccharides on the modulation of both the composition and activity of the gut microbiota should be warranted. In this sense, some *in vivo* studies carried out in humans have provided evidences on the prebiotic effect after IMO administration in

adult and elderly individuals[42,43]. Furthermore, Yen et al.[44] also observed an improvement in bowel movement in chronically constipated elderly subjects, as well as a beneficial effect on blood cholesterol levels after long-term supplementation (8 weeks) of IMOs.

To conclude, this article reports the efficient enzymatic synthesis of a homologous series (DP 3 to 9) of 6-O-α-glucosylated oligosaccharides derived from the transglucosylation reaction of isomaltulose catalyzed by a *Leuconostoc mesenteroides* B-512F dextransucrase (EC 2.4.1.5). According to the oligosaccharide structures elucidated using a comprehensive NMR approach, these isomaltulose-derived oligosaccharides are consecutively elongated by the addition of glucose moieties linked by α-(1→6) glycosidic bonds at the non-reducing glucose residue while they keep the fructose unit at the reducing end. By having isomaltulose as core structure and the α-(1→6) linkage as unique structural feature, these IMO-like structures could possess appealing bioactive properties, which might warrant their potential use as functional ingredients. The reported enzymatic synthesis offers a good yield of isomaltulose-derived oligosaccharides, as well as it provides a relatively non-complex carbohydrate mixture which should make easier their purification for further investigation of their functional properties.

Acknowledgements

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Table 1. Carbohydrate composition (g L^{-1}) determined by LC-RID and produced upon the transglucosylation reaction mixture using 250 g L^{-1} sucrose (donor) and 250 g L^{-1} isomaltulose (acceptor) as starting substrates.

				Isomaltulose-derived oligosaccharides							
Time (hours)	Fructose	Sucrose	Isomaltulose	DP 3	DP 4	DP 5	DP 6	DP 7	DP 8	DP 9	Total oligosaccharide
0	0.0	247.4±1.3[a]	261.2±11.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	30.7±4.4	158.6±6.6	198.8±11.0	12.3±1.5	5.6±0.2	1.4±0.1	0.0	0.0	0.0	0.0	19.3
3	81.3±0.3	156.6±9.6	176.2±13.4	18.2±0.9	13.3±0.9	7.1±0.9	4.3±0.7	0.0	0.0	0.0	42.9
5	110.7±14.0	125.8±1.5	165.1±24.1	21.8±1.6	19.6±1.1	13.9±1.0	7.9±0.2	6.0±0.5	0.0	0.0	69.2
8	134.2±8.6	98.6±13.5	154.8±14.6	20.7±0.5	19.2±0.0	14.8±0.2	8.3±0.2	5.1±0.3	3.7±0.3	0.0	71.8
24	216.3±4.1	23.8±1.4	119.6±16.9	20.8±0.6	24.1±0.2	23.9±1.7	14.4±0.7	9.4±0.6	5.8±0.3	3.2±0.1	101.6
48	210.4±10.2	8.9±0.4	94.4±10.2	18.8±0.6	22.1±0.8	24.0±0.7	15.3±0.1	11.4±0.2	7.3±0.3	5.1±0.1	104.0

[a] Standard deviation (*n* =3).

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for isomaltulose-derived oligosaccharides from **DP3 to DP7**[a].

Structure	Position	G_{n+2}		G_2-G_{n+1}		G_1		F_1	
		δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
DP3 (n=0) α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside	1	4.95 (3.8)	98.40			4.98 (3.8)	98.95	3.57 3.54	63.22
	2	3.57	72.06			3.55	71.93	-	102.31
	3	3.73	73.64			3.71	73.92	4.19	75.83
	4	3.43	70.09			3.51	70.05	4.11	75.15
	5	3.73	72.42			3.89	70.96	3.97	79.43
	6	3.85 3.77	61.05			3.97 3.76	66.12	3.88 3.70	68.61
DP4 (n=1) α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside	1	4.97 (3.8)	98.32	4.97 (3.8)	98.35	4.99 (3.8)	98.94	3.59 3.54	63.22
	2	3.57	72.08	3.57	71.96	3.57	71.93	-	102.31
	3	3.73	73.68	3.73	73.94	3.73	73.92	4.20	75.83
	4	3.43	70.10	3.52	70.08	3.52	70.08	4.12	75.15
	5	3.73	72.42	3.91	70.84	3.91	70.90	3.98	79.43
	6	3.85 3.77	61.05	3.99 3.77	66.19	3.99 3.77	66.11	3.88 3.71	68.61
DP5 (n=2) α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside	1	4.97 (3.8)	98.28	4.97 (3.8)	98.34, 98.35	4.99 (3.8)	98.95	3.58 3.53	63.23
	2	3.57	72.08	3.57	71.99, 71.97	3.57	71.93	-	102.32
	3	3.73	73.68	3.73	73.96, 73.95	3.73	73.95	4.20	75.82
	4	3.43	70.15	3.52	70.09, 70.09	3.52	70.09	4.12	75.14
	5	3.73	72.42	3.91	70.85, 70.78	3.91	70.89	3.98	79.44
	6	3.85 3.77	61.05	3.99 3.77	66.18, 66.18	3.99 3.77	66.10	3.89 3.72	68.61
DP6 (n=3) α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside	1	4.97 (3.8)	98.29	4.97 (3.8)	98.35, 98.34, 98.27	4.99 (3.8)	98.94	3.58 3.55	63.23
	2	3.57	72.07	3.57	71.99, 71.99, 71.96	3.57	71.93	-	102.31
	3	3.73	73.67	3.73	73.98, 73.95, 73.95	3.73	73.95	4.20	75.83
	4	3.43	70.13	3.52	70.13, 70.08, 70.08	3.52	70.08	4.12	75.14
	5	3.73	72.42	3.91	70.84, 70.77, 70.76	3.91	70.89	3.98	79.43
	6	3.85 3.77	61.05	3.99 3.77	66.17, 66.17, 66.13	3.99 3.77	66.10	3.89 3.71	68.60
DP7 (n=4) α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→6)-β-D-fructofuranoside	1	4.97 (3.8)	98.29	4.97 (3.8)	98.35, 98.34, 98.28, 98.27	4.99 (3.8)	98.94	3.58 3.55	63.22
	2	3.57	72.07	3.57	71.99, 71.99, 71.99, 71.96	3.57	71.93	-	102.31
	3	3.73	73.67	3.73	73.98, 73.98, 73.95, 73.95	3.73	73.95	4.20	75.83
	4	3.43	70.13	3.52	70.13, 70.08, 70.08, 70.08	3.52	70.08	4.12	75.14
	5	3.73	72.42	3.91	70.84, 70.78, 70.77, 70.77	3.91	70.89	3.98	79.43
	6	3.85 3.77	61.05	3.99 3.77	66.17, 66.17, 66.13, 66.13	3.99 3.77	66.10	3.89 3.71	68.66

[a] Chemical shift (δ, ppm) and coupling constants (J in Hz, in parentheses).

Figure captions

Figure 1. LC-RID profile of transglucosylation reaction with 250 g isomaltulose L^{-1} and 250 g sucrose L^{-1} as initial substrates and catalyzed by dextransucrase from *Leuconostoc mesenteroides* B-512F (0.8 U mL^{-1}) at 30 °C, in 20 mM sodium acetate buffer at pH 5.2, for 48 hours. DP = Degree of polymerization.

Figure 2. Isomaltulose-derived oligosaccharides synthesis during the time course of the transglucosylation reaction performed at 30 °C at an initial concentration of 250 g isomaltulose L^{-1} and 250 g sucrose L^{-1}, 0.8 U mL^{-1} of enzyme in 20 mM sodium acetate buffer at pH 5.2. DP = Degree of polymerization.

Figure 3. Structures of the isomaltulose-derived oligosaccharides from DP3 to DP7.

Figure 4. One-dimensional NMR spectra of isomaltulose-derived oligosaccharides from DP3 to DP7 at 500 MHz in D_2O. Data represent ^{1}H (left) and ^{13}C (right) anomeric region spectra.

Figure 1. Barea et al.

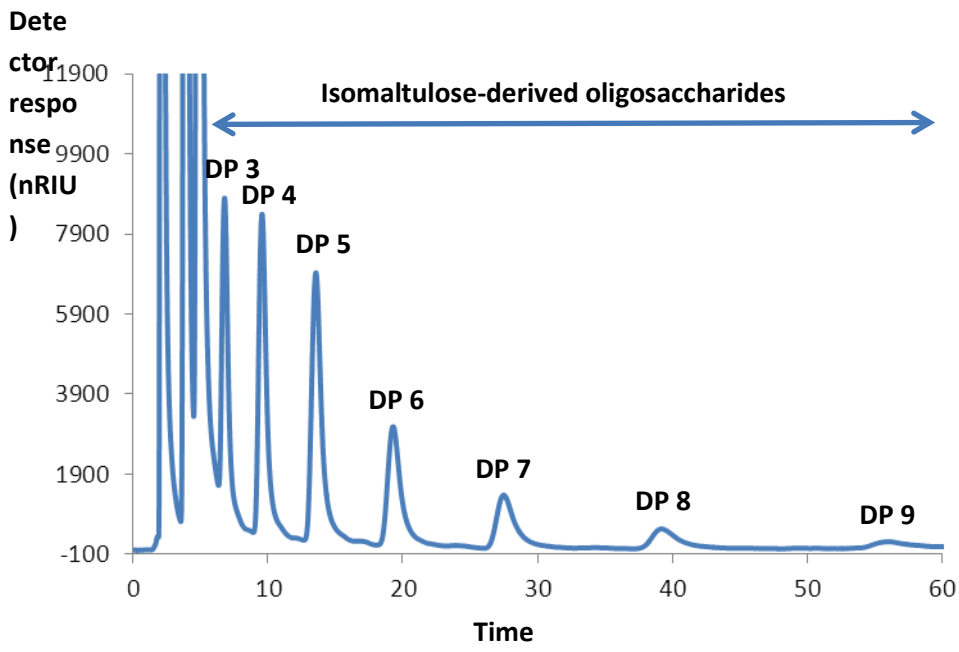


Figure 2. Barea et al.

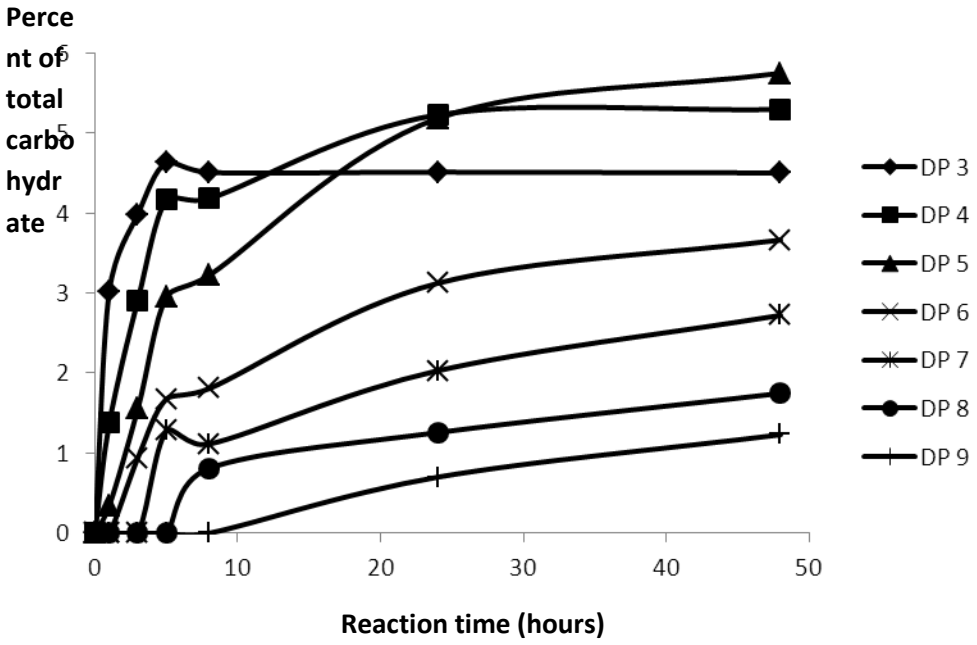
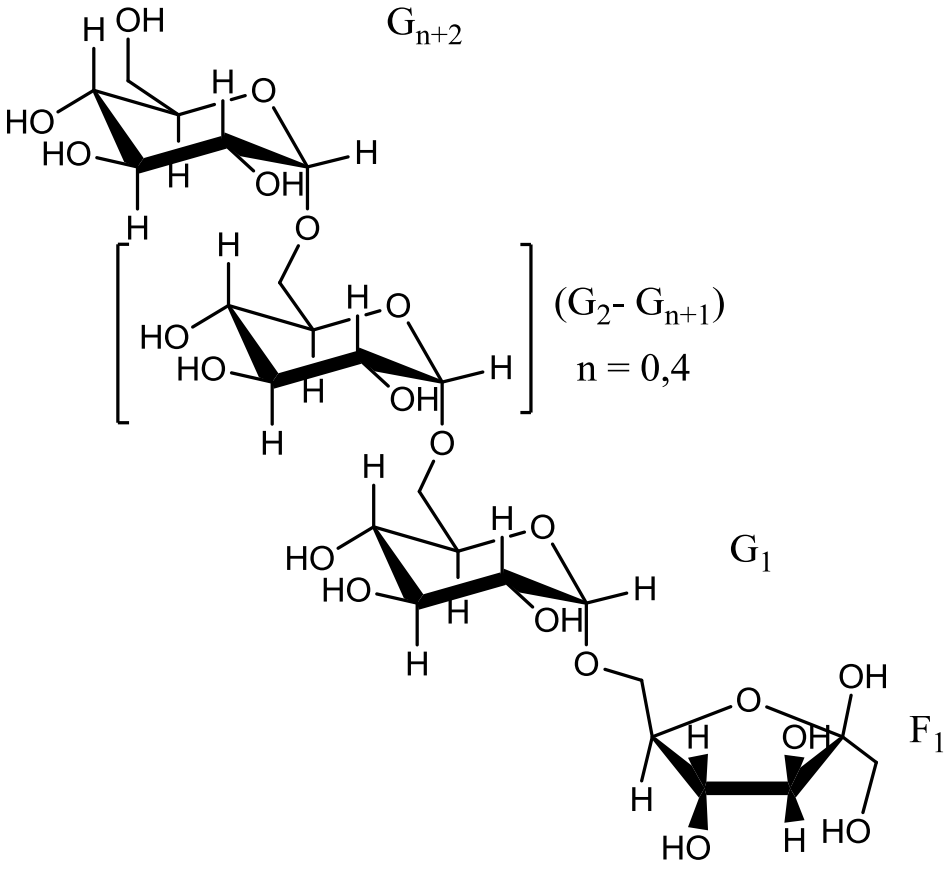
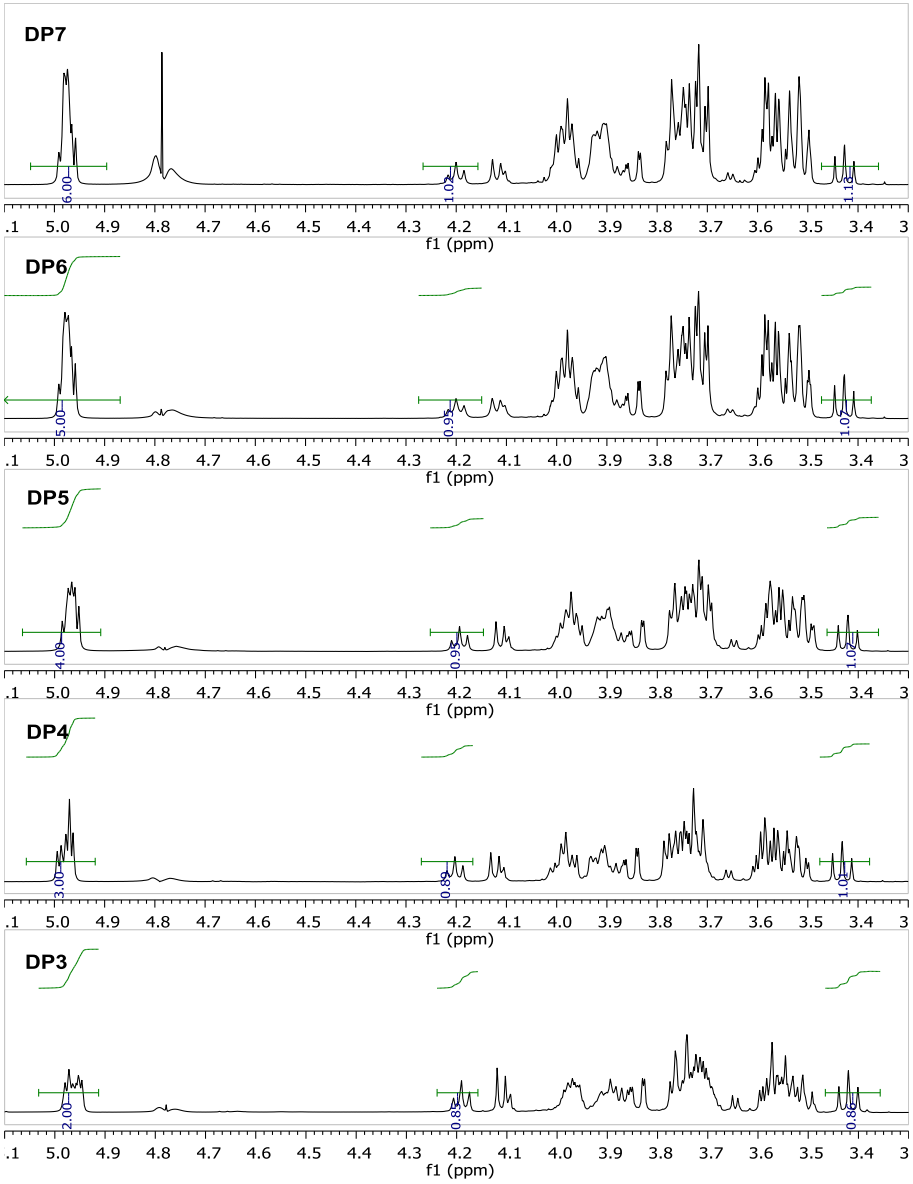


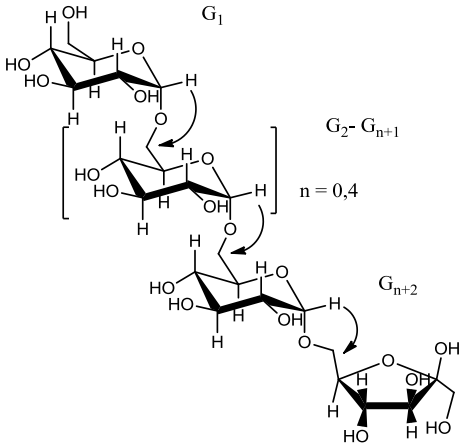
Figure 3. Barea et al.



DP3 n=0
DP4 n=1
DP5 n=2
DP6 n=3
DP7 n=4

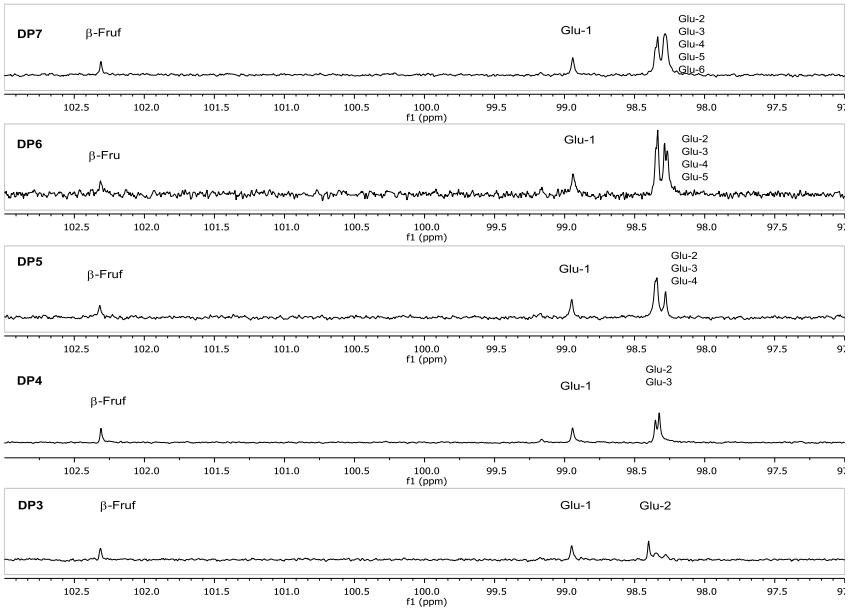
Figure 4. Barea et al.



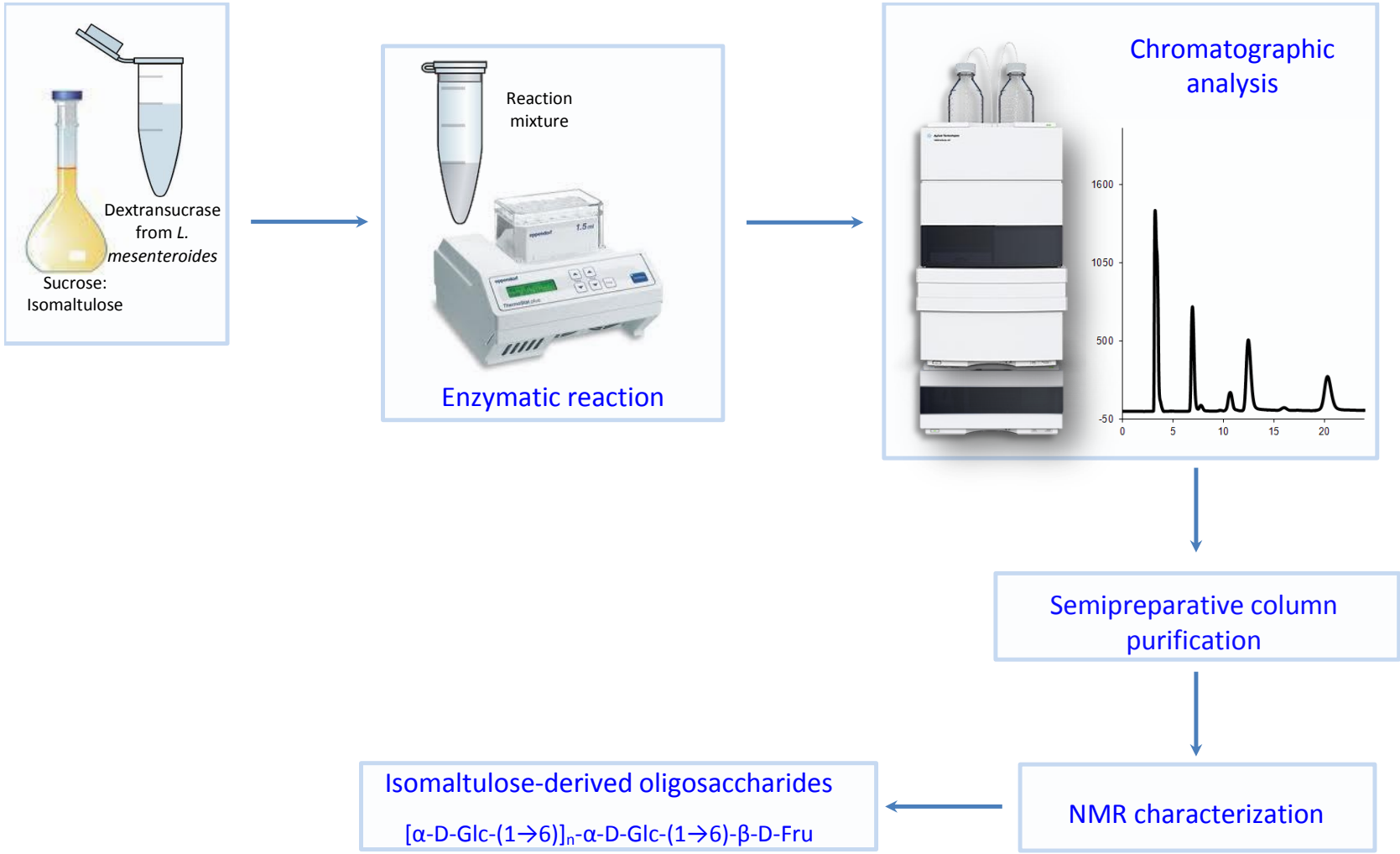


DP3 n=0
DP4 n=1
DP5 n=2
DP6 n=3
DP7 n=4

Observed gHMBC correlations(arrows)



TOC GRAPHIC


Dextransucrase from *L. mesenteroides*
Sucrose: Isomaltulose
Reaction mixture
Enzymatic reaction
Chromatographic analysis
1600
1050
500
-50
0
5
10
15
20
Semipreparative column purification
NMR characterization
Isomaltulose-derived oligosaccharides
[α-D-Glc-(1→6)]$_n$-α-D-Glc-(1→6)-β-D-Fru