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Oligosaccharide synthesis by dextransucrase: new unconventional acceptors

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Dedicated to Professor Dr. Dr. h.c. Frieder W. Lichtenthaler on the occasion of his 70th birthday

Abstract

The acceptor reactions of dextransucrase offer the potential for a targeted synthesis of a wide range of di-, tri- and higher oligosaccharides by the transfer of a glucosyl group from sucrose to the acceptor. We here report on results which show that the synthetic potential of this enzyme is not restricted to 'normal' saccharides. Additionally functionalized saccharides, such as alditols, aldosuloses, sugar acids, alkyl saccharides, and glycals, and rather unconventional saccharides, such as fructose dianhydride, may also act as acceptors. Some of these acceptors even turned out to be relatively efficient: α-D-glucopyranosyl-(1→5)-D-arabinonic acid, α-D-glucopyranosyl-(1→4)-D-glucitol, α-D-glucopyranosyl-(1→6)-D-glucitol, α-D-glucopyranosyl-(1→6)-D-mannitol, α-D-fructofuranosyl-β-D-fructofuranosyl-(1,2′:2,3′)-dianhydride, 1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol ('D-glucal'), and may therefore be of interest for future applications of the dextransucrase acceptor reaction.

Keywords: Oligosaccharide synthesis; Glycosylation; Dextransucrase; Acceptor reactions

1. Introduction

Dextransucrase (EC 2.4.1.5) is a bacterial enzyme produced extracellularly by various bacterial species of *Leuconostoc*, *Lactobacillus*, and *Streptococcus* which catalyzes two kinds of reaction: (1) the primary reaction (substrate reaction), the synthesis of dextran from sucrose; and (2) the secondary reaction (acceptor reaction), the transfer of D-glucose from sucrose to carbohydrate acceptors that are added. The mechanisms for these reactions were studied first by Ebert and co-workers and later extensively by Robyt and co-workers.[1–8] They proposed that dextran is synthesized by a two-site insertion mechanism in which the D-glucosyl group is added to the reducing-end of a covalently linked, growing dextran chain. The chain, or the covalently bound glucosyl residue, is released from the active site by acceptor reactions.[9,10] Nevertheless, at the molecular level, the catalytic action of dextransucrase is still not understood in detail, since all attempts to crystallize this enzyme have failed thus far. However the amino acid sequences of some dextransucrases have recently been reported and the probable location of the catalytic sites identified by sequence comparison and site-directed mutagenesis.[11,12]

The acceptor reaction has been of special interest to us in that it has many possible technical applications for the synthesis of new oligosaccharide derivatives. Many different mono-,[2,10,13–17] di-,[10,15,18–21] tri-,[5,10,13,22] and oligo-[23,24] saccharides may act as acceptors for dextransucrase. With the exception of a few acceptors, the acceptor product itself can serve as an acceptor, leading to the formation of an homologous series of isomalto-oligosaccharides attached to the acceptor, which is located at the reducing end of the product. The efficiency and extent of the acceptor reaction varies, depending on the particular acceptor.[10,17,20,23–25] So far,

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the glucodisaccharides maltose and isomaltose were found to be the best acceptors, whereas several other saccharides do not seem to be acceptors at all.[10,13,15,17,20,21,26,27] For many acceptors, D-glucose is transfered to the 6-hydroxyl group to give an α-(1→6) glycosidic linkage, but all of the other possible α-glycosidic linkages also have been observed to be formed, e.g., (1→1),[27–29] (1→2),[10,15,30–35] (1→3),[17,35] (1→4),[17] and (1→5),[22,26] depending on the particular structure of the acceptor.

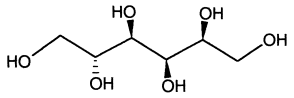
To date, there has not been a detailed explanation as to why there are these differences in bond formation specifity as well as in acceptor efficiency, and only few systematic investigations concerning the relationships between the molecular structure of a given saccharide and its acceptor properties have been carried out (methyl α-D-glucopyranoside analogues,[17] maltooligosaccharides[23,24] and gluco-disaccharides[20]). Moreover, little is known about the acceptor properties of derivatized saccharides, although numerous saccharide derivatives have been tested as inhibitors for dextransucrase, for example, fluoro saccharides,[36–42] chloro saccharides,[43,44] amino saccharides,[45–47] and thio saccharides.[39,40,42,47] Our interest was in studying the potential of other saccharides and their derivatives as acceptors to give new products, and to demonstrate further synthetic potential of the dextransucrase glucosylation reaction. The focus here is on such saccharides as alditols, aldosuloses, aldonic acids, fructose dianhydrides, and glycals. They may be building blocks for further synthesis carrying the potential for enhanced solubility, hydrophilicity, and reduced toxicity.

On the basis of our kinetic model for dextransucrase,[27,48–50] a kinetic definition for the strength of the acceptor was developed. This method is in contrast to the method based on the final yield of product used by Robyt et al.[10,17] It has the advantage of being independent of both the initial saccharide concentration and of the eventual acceptor efficiency of the products formed. The strength of the acceptors was determined by carrying out at least one kinetic experiment, using iterative computation of the kinetic parameters.

2. Results and discussion

In the dextransucrase acceptor reaction, sucrose was used as the substrate, where the glucosyl group is transfered, by tranglycosylation, to the acceptor and fructose is released as a product (see graphical abstract). Dextran is formed in major or minor amounts, depending on the strength of the acceptor and the reaction conditions. The following acceptors were tested:

alditols, D-glucitol (**1**)[†] and D-mannitol (**2**), the disaccharide derivatives α-D-glucopyranosyl-(1→4)-D-glucitol (**3**, 'maltitol'), α-D-glucopyranosyl-(1→6)-D-glucitol (**4**), and α-D-glucopyranosyl-(1→6)-D-mannitol (**5**);[‡]



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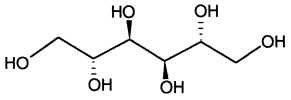

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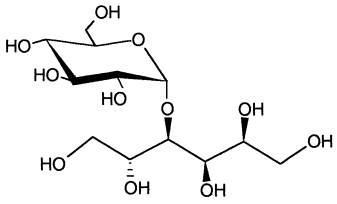

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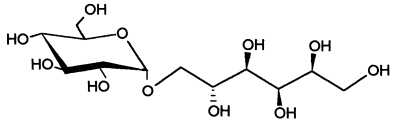

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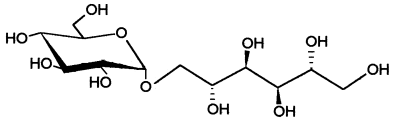
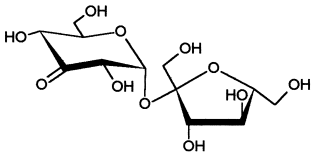


5

the aldosuloses derived from sucrose and its (regio-) isomers, by microbial oxidation of the 3-position of the glucopyranosyl group, α-D-*arabino*-hexos-3-ulopyranosyl-β-D-fructofuranoside (**6**, '3-keto-sucrose'), α-D-*arabino*-hexos-3-ulopyranosyl-(1→5)-D-fructose (**7**), α-D-*arabino*-hexos-3-ulopyranosyl-(1→6)-D-fructose (**8**), and the oxidation product of **5**, α-D-*arabino*-hexos-3-ulopyranosyl-(1→6)-D-mannitol (**9**); the sugar acid,

[†] Earlier, Ebert and co-workers[61,62] reported from their observation, that the two hexitols glucitol and mannitol cause a decrease in the average molecular weight of the dextran formed during dextransucrase catalysis. They therefore assumed both these hexitols to be dextransucrase acceptors, although they never succeeded in detecting the corresponding low molecular weight acceptor products.

[‡] Evidence for acceptor properties of this saccharide was first obtained by D. Prinz.[21]



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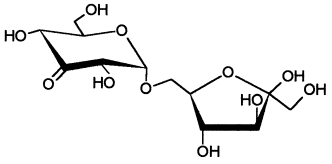
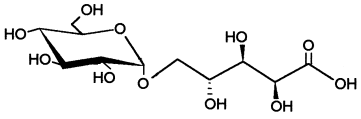


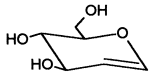
8

the oxidation product from α-D-glucopyranosyl-(1 → 6)-D-fructose ('isomaltulose'), α-D-glucopyranosyl-(1 → 5)-D-arabinonic acid (**10**, evidence for acceptor properties of this saccharide was first obtained by D. Prinz[21]);



10

the glycals 1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol ('D-glucal', **11**), ethyl 2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside ('ethyl pseudoglucal', **12**), 2,6-anhydro-1-deoxy-D-*arabino*-hex-1-enitol (**13**);



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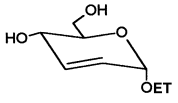

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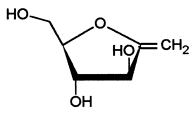
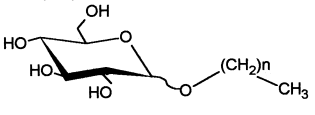


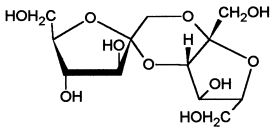
13

the *n*-alkyl glucosides *n*-octyl β-D-glucopyranoside (**14**) and *n*-decyl [α-D-glucopyranosyl-(1 → 4)-] α-D-glucopyranoside (**15**);



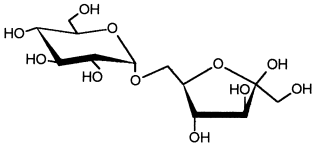
14, 15

the fructose dianhydride α-D-fructofuranosyl-β-D-fructofuranosyl-(1,2′:2,3′)-dianhydride (**16**); and



16

the sucrose regioisomers α-D-glucopyranosyl-(1 → 4)-D-fructose ('maltulose', **17**), α-D-glucopyranosyl-(1 → 6)-D-fructose ('isomaltulose', **18**), and α-D-glucopyranosyl-(1 → 1)-D-fructose ('trehalulose', **19**).



18

Primary results from the tests of acceptor properties.—The chromatograms obtained by analysis with either the HPAEC system (Fig. 1) or the aminopropyl silica gel column and the cation exchanger column in the silver form clearly shows that all alditols, two aldosuloses, **8** and **9**, α-D-glucopyranosyl-(1 → 5)-D-arabinonic acid (**10**), one glycal, **11**, the fructose dianhydride **16**, and the sucrose analogue α-D-glucopyranosyl-(1 → 4)-D-fructose (**17**), gave rise to a homologous series of acceptor products. Furthermore, it was especially remarkable that we were able to detect two different primary acceptor products both for the alditol, D-glucitol, and the sucrose regioisomer **17**. The two other sucrose regioisomers (**18** and **19**) were already known to act as acceptors.[21] Remarkably, in case of the acceptor reaction of **17**, one of the two primary products was completely consumed during the course of the reaction.

For the two glycals, **12** and **13**, and the two remaining aldosuloses, **6** and **7**, we did not succeed in detecting any acceptor product using any of our chromatographic systems. It must be assumed, therefore, that these saccharide derivatives are not acceptors in the dextransucrase reaction. In the case of the two glycals, the lack of verifiable acceptor reactions may be explained by conformational changes in the pyranoside rings, caused by the presence of a double bond. Moreover, the hydroxyl groups, which are missing in these molecules, usually play a key role in the acceptor reaction of gluco- and fructo-pyranosyl saccharides. In a systematic investigation, Robyt et al.[17] have shown that the 2-, 3- and 4-hydroxyl groups play an important role for the acceptor properties of glucopyranose analogues. In the case of the two aldosuloses, we indeed did not expect to observe any acceptor products, as the unmodified disaccharides, sucrose and its isomer, α-D-

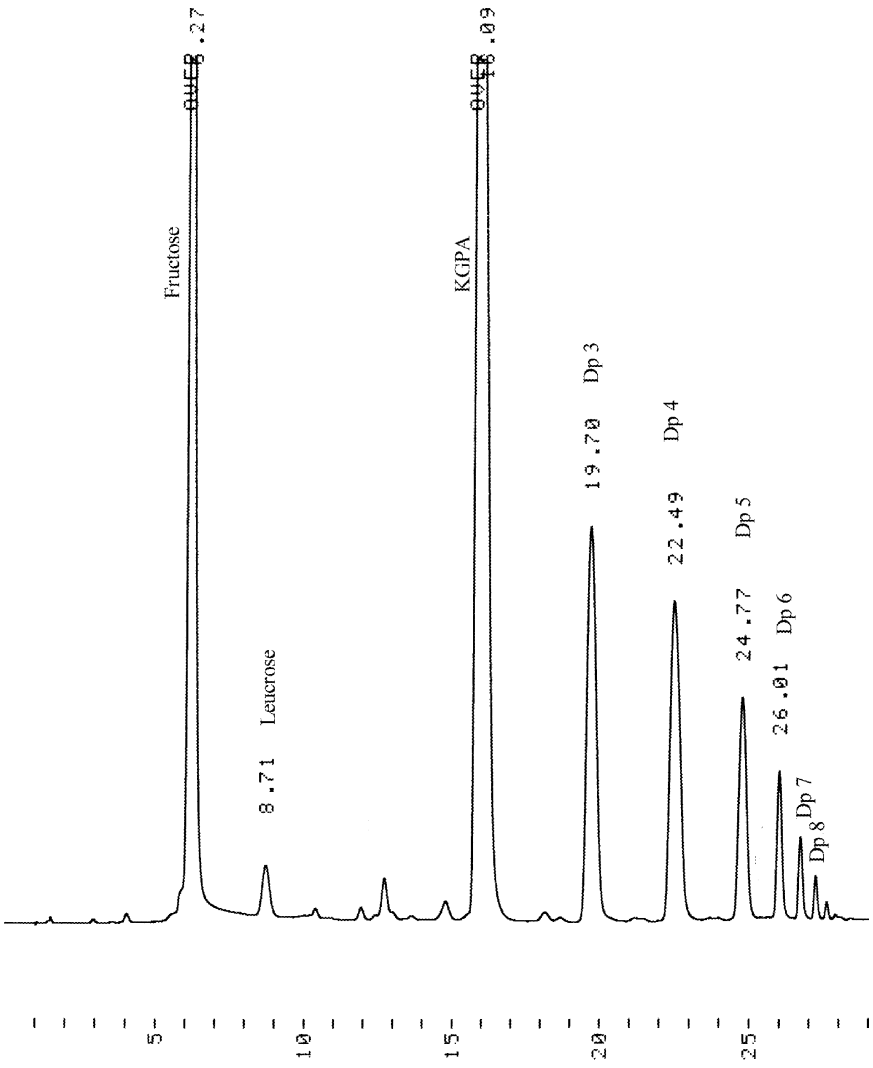


Fig. 1. HPAEC chromatogram from a sample of an acceptor reaction digest of α-D-glucopyranosyl-(1→5)-D-arabinonic acid (**10**).

glucopyranosyl-(1→5)-D-fructose ('leucrose'), do not act as acceptors for dextransucrase.

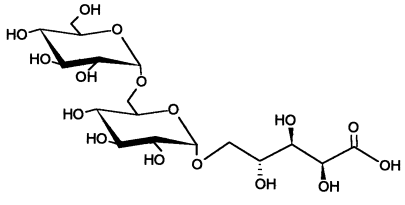
For *n*-alkyl glycosides, we were not able to discover any new products in the case of *n*-octyl β-D-glucopyranoside (**14**), whereas the chromatograms from the reactions with *n*-decyl [α-D-glucopyranosyl-(1→4)-] α-D-glucopyranoside (**15**) revealed the formation of a small amount of a new product: A new signal appeared in the region of the chromatograms where the corresponding primary acceptor product was expected.

Structure of the primary products from D-glucitol and D-mannitol.—As already mentioned, there were two different primary acceptor products detected in case of D-glucitol, whereas mannitol only gave a single product. Assuming that both of these alditols are capable of reacting by nucleophilic attack of either the 1- or 6-hydroxyl group, this observation may be explained as follows: Due to the symmetry of the mannitol molecule only a single primary product is possible, α-D-glucopyranosyl-(1→6)-D-mannitol (**5**). The D-glucitol molecule, on the other hand, does not have such symmetry and two different products should result, α-D-glucopyranosyl-(1→6)-D-glucitol (**4**), from reaction of the 6-hydroxyl group and α-D-glucopranosyl-(1→6)-D-gulitol from that of the 1-hydroxyl group. A comparison of the retention times on HPAEC under various analytical conditions showed that the primary acceptor product of mannitol was indeed **5**, as well as the higher homologues of the mannitol acceptor product series, which showed the same retention times as those of the acceptor-product series of **5**. Likewise, one of the two primary acceptor products of D-glucitol was identified as **4**. On basis of these findings, it therefore stands to reason that the hexitols are able to react as acceptors with both their 1- and 6-hydroxyl groups. It must be pointed out however that the acceptor reaction of the hexitols is much slower, by one order of magnitude, than that of the first (and subsequent two) acceptor products, notably the disaccharide alcohols **4** and **5** (see later, Fig. 3). No di-D-glucopyranosyl product was

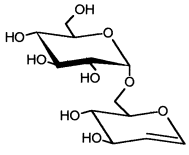
found. This is reasonable, since the first acceptor products—the D-glucopyranosyl-glucitol, or -mannitol, respectively, are further glucosylated at 6-OH; this reaction is much more rapid, as compared to the glucosylation of the sugar alcohols **1** and **2**.

NMR characterization of selected acceptor products.—In order to gain more information about the molecular structure of the new acceptor products, the primary acceptor products of α-D-glucopyranosyl-(1→5)-D-arabinonic acid (**10**) and 1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol ('D-glucal', **11**), as well as the secondary acceptor products of the aldosulose **8** (the isolated secondary acceptor product of **8** still contained some primary acceptor product as contamination) and the fructose dianhydride **16** were isolated from the reaction mixtures by preparative chromatography and then analyzed by ^{1}H and ^{13}C NMR spectroscopy.

The resulting ^{1}H NMR spectra of the primary products **10a** and **11a** from **10** and **11** both showed, when compared to the original spectra of **10** and **11**, a new doublet at δ 5.01 and 5.00 ppm with a coupling constant of 3.7 Hz, which according to Bradbury et al.[55] is characteristic for the anomeric proton of an α-(1→6)-glucosidic linkage. Moreover, the ^{13}C NMR spectra also displayed the corresponding characteristic signals.[35,56–58] In the case of the **11**, the signal for C-6 of the glucosyl moiety carrying the double bond appeared at 66.4 ppm and the signal of the C-1′ of the transfered glucosyl residue at 99.0 ppm. For the acceptor product of **10**, analogous signals for C-6′ and C-1″ were found at 66.5 and 98.7 ppm. It can thus be concluded that both **10** and **11** react as 'normal' acceptors, namely by nucleophilic attack of their 6-hydroxyl group.

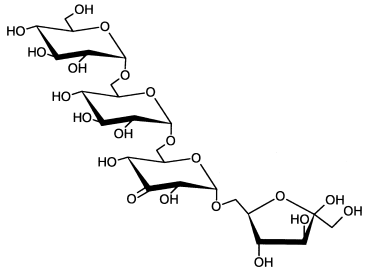


10a

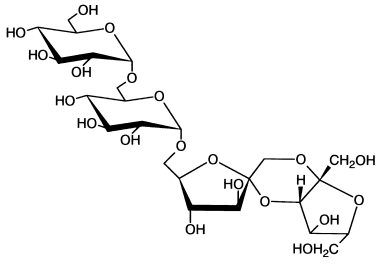


11a

Similar results were obtained from the NMR investigations of the secondary acceptor products **8b** and **16b** obtained from **8** and **16**. In the ^{1}H NMR spectrum of the acceptor product **8b**, the presence of two more doublets centered at 5.01 and 5.07 ppm in addition to the doublets at 5.41 ppm resulting from the anomeric proton of the α-(1→6)-glycosidic bond between the fructofuranosyl and the modified glucopyranosyl residue and the expected ^{13}C NMR signals at 98.8, 98.6, and 66.4 ppm (two peaks) confirmed the existence of two α-(1→6)-glucosidic linkages between the glucosyl residues. By analogy, the secondary product **16b** gave, when compared to the spectrum of of the fructose dianhydride **16** itself, two additional characteristic doublets in the ^{1}H NMR spectrum. These doublets were centered at 4.99 and 5.01 ppm, both showing a coupling constant of ^{3}J 3.7 Hz. The corresponding ^{13}C NMR signals occured at 99.2, 98.6 ppm (C-1 of the two transferred glucopyranosyl residues) and 67.6 and 66.3 ppm, indicating that C-6 of the α-fructofuranosyl moiety and C-6 of the first transfered glucopyranosyl residue act as nucleophiles; comparison with the NMR data of **16** itself revealed that the molecule must have reacted by nucleophilic attack of the 6-hydroxyl group of the α-fructofuranosyl moiety.



8b



16b

Further experimental evidence for the molecular structure of the acceptor products.—Additional evidence concerning the structure of the various acceptor products was obtained by HPAEC chomatography: Samples from dextransucrase acceptor reaction digests of glucose, maltose and α-D-glucopyranosyl-(1→6)-D-fructose ('isomaltulose', **18**) and D-glucitol (**1**) [as well as α-D-glucopyranosyl-(1→6)-D-glucitol, **4**] D-mannitol (**2**) [as well as α-D-glucopyranosyl-(1→6)-D-mannitol, **5**], α-D-glucopyranosyl-(1→4)-D-glucitol (**3**), α-D-glucopyranosyl-(1→5)-D-arabinonic acid (**10**), **11** ('D-glucal'), the fructose dianhydride **16**, the sucrose analogues α-D-glucopyranosyl-(1→4)-D-fructose ('maltulose', **17**) and

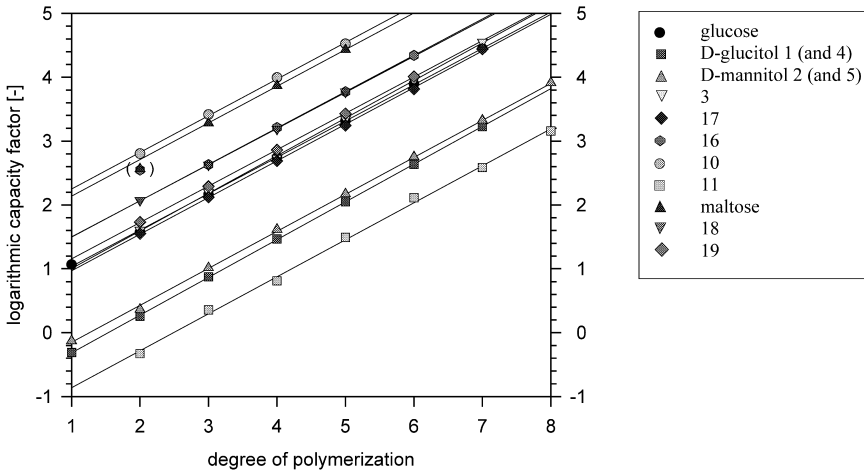


Fig. 2. Logarithmic capacity factors obtained from the HPAEC system under isocratic elution conditions (98% of eluent A and 2% of eluent B) for various dextransucrase acceptors and their respective acceptor products as a function of the degree of polymerization; correlation of numbers with saccharides: D-glucitol (**1**), D-mannitol (**2**), α-D-glucopyranosyl-(1→4)-D-glucitol ('maltitol', **3**), α-D-glucopyranosyl-(1→6)-D-glucitol (**4**), α-D-glucopyranosyl-(1→6)-D-mannitol (**5**), α-D-glucopyranosyl-(1→5)-D-arabinonic acid (**10**), 1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol ('D-glucal', **11**), α-D-fructofuranosyl-β-D-fructofuranosyl-(1,2′:2,3′)-dianhydride (**16**), α-D-glucopyranosyl-(1→4)-D-fructose ('maltulose', **17**), α-D-glucopyranosyl-(1→6)-D-fructose (isomaltulose, **18**), α-D-glucopyranosyl-(1→1)-D-fructose ('trehalulose', **19**).

α-D-glucopyranosyl-(1→1)-D-fructose ('trehalulose', **19**) were analyzed under specific isocratic elution conditions (98% of eluent A and 2% of eluent B). Then the logarithmic capacity factors[§] of the various acceptors and their respective acceptor products were calculated from the observed retention times and plotted versus the degree of polymerization. As may be seen from Fig. 2, straight lines were obtained for each of the homologous series of acceptor products and—what is especially remarkable—the slopes of all these lines were identical (see also Table 1).

It is well known that glucose, maltose and **18** ('isomaltulose') all give a series of 'normal' acceptor products with isomalto oligosaccharide groups attached via α glycosidic bonds to the 6-hydroxyl group of the non-reducing glucosyl moiety.[5,10,15] This observation suggests that the observed slope is characteristic for a series of oligosaccharides with α-(1→6)-linked glucopyranosyl residues under the given analytical conditions. We also found a completely different elution behavior in the case of the maltooligosaccharide series, where all D-glucopyranosyl residues are α-(1→4)-linked. In other words, these findings can be interpreted as another indication that all acceptors, which were taken into consideration in this investigation, react in the 'normal' way.

As mentioned in the first paragraph of this paper, the HPAEC chromatograms obtained from the sucrose analogue α-D-glucopyranosyl-(1→4)-D-fructose ('maltulose', **17**) acceptor reaction digest showed that **17** gives rise to two different primary acceptor products. According to our findings from the logaritmic plot of capacity factors (preceding paragraph), one of these two acceptor products should be a 'normal' one, that is **17** should have reacted by nucleophilic attack of the 6-hydroxyl group of its glucopyranosyl residue. The second primary acceptor product initially is formed during the course of the reaction and later disappears completely from the reaction mixture. It may be assumed that **17** reacts also as an acceptor in its β-fructo-

Table 1
Results from the logarithmic plot of the acceptor products capacity factors versus their degree of polymerization

Acceptor	Slope[a]	Intercept
D-Glucose (and isomaltose)	0.575	0.437
D-Glucitol **1** (and **4**)	0.591	−0.907
D-Mannitol **2** (and **5**)	0.579	−0.730
3 ('Maltitol')	0.417	0.589
17 ('Maltulose')	0.574	0.396
16 (Fructose dianhydride)	0.569	0.927
α-D-Glucopyranosyl-(1→5)-D-arabinonic acid (**10**)	0.574	1.677
11 (D-'Glucal')	0.579	−1.441
Maltose	0.574	1.565
18 ('Isomaltulose')	0.565	0.936
19 ('Trehalulose')	0.586	0.570

[a] Average slope of lines: $m = 0.576$ (± 0.002) (standard error: 0.4%).

[§] The capacity factor k_i for any substance i was determined according to the equation $k_i = (t_{r,i} - t_0)/t_0$ ($t_{r,i}$: retention time of substance i under the given analytical conditions; t_0: dead time of the analytical system).

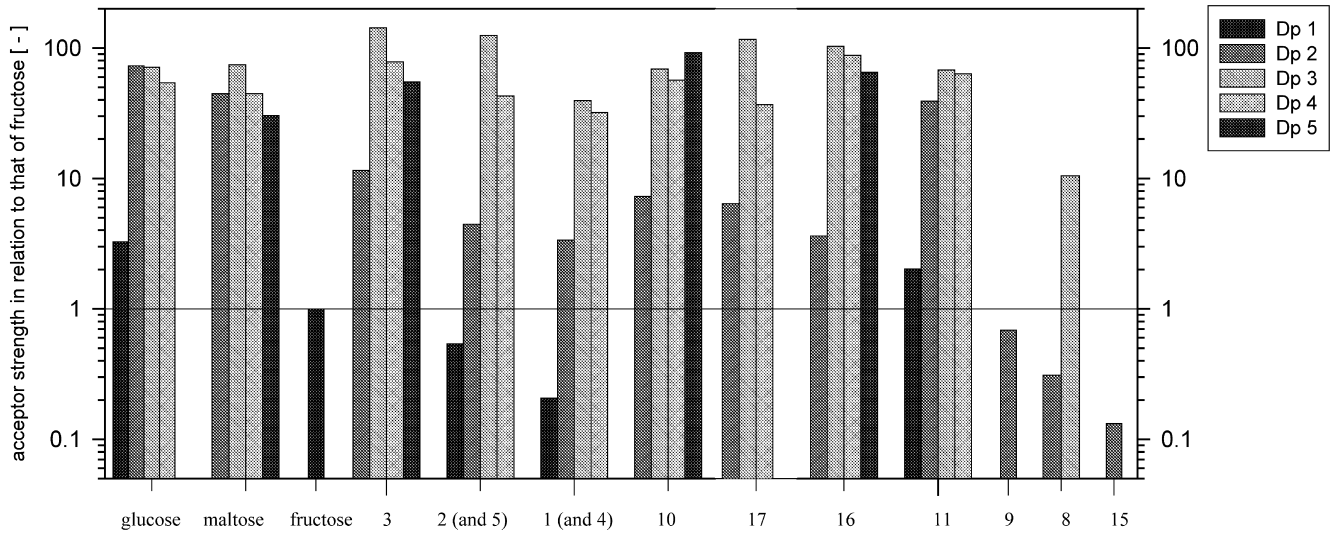


Fig. 3. Acceptor strength of various mono- and di-saccharides and their acceptor products in relation to the acceptor efficiency of D-fructose (logarithmic scale; DP: degree of polymerization); for correlation of numbers with saccharides see Fig. 2; further: α-D-*arabino*-hexos-3-ulopyranosyl-(1→6)-D-fructose (**8**), α-D-*arabino*-hexos-3-ulopyranosyl-(1→6)-D-mannitol (**9**), *n*-decyl [α-D-glucopyranosyl-(1→4)-] α-D-glucopyranoside (**15**).

furanoside form via the 2-hydroxyl group of the fructosyl moiety to give α-D-glucopyranosyl-(1→4)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside as the second primary acceptor-product. This follows from the structural analogy to β-D-galactopyranosyl-(1→4)-D-fructose ('lactulose', **20**) which is known to give an acceptor product bound similarly,[33] which in turn is a substrate for dextransucrase, giving dextran and **20** as reaction products.[34] This would explain the consumption of one of the acceptor products of **17** during the course of the reaction.

Determination of the kinetic parameters and acceptor efficiencies.—For a given acceptor, the value of the kinetic parameter describing the rate of the acceptor reaction step itself can be viewed as a general criterion for its acceptor strength. In this work, we decided to determine the kinetic parameters for the acceptor reaction of not only each of the new acceptors themselves (namely the various saccharide derivatives, which were initially added to the reaction mixtures), but also of their acceptor products. Therefore, the kinetic model for the dextransucrase catalysis, which we have presented in several earlier publications,[27,49,50] had to be extended: new parameters were introduced for the acceptor reactions of all members of the homologous series of acceptor products. Moreover, potential inhibition caused by the acceptor products were also taken into consideration in the extended model.

The kinetic parameters for the acceptor reaction for each of the acceptors were then determined by iterative parameter estimation, taking all data for sucrose, fructose, α-D-glucopyranosyl-(1→5)-D-fructose (**21**, 'leucrose'), acceptor and acceptor product (only the first four members of the acceptor product series were considered) concentrations into account. (For a more detailed descripition of the calculation prodedure see Refs. 50 and 59.) Fig. 3 presents the main results obtained from these calculations, namely the acceptor reaction parameters estimated for each of the new acceptors and also those of the respective acceptor products in relation to that of fructose. Moreover, for better comparison, Fig. 3 also shows our previous modeling results for the glucose and the maltose acceptor.[50,60]

As may be seen from Fig. 3, maltitol (**3**) is the best acceptor among the saccharide derivatatives studied in our investigations. Nevertheless, its acceptor efficiency is considerably less than that of α-D-glucopyranosyl-(1→6)-D-glucose (**22**, 'isomaltose') and maltose (**23**), which were the strongest acceptors known so far.[10,23–25] Compound **3** is followed by the sugar acid α-D-glucopyranosyl-(1→5)-D-arabinonic acid (**10**), and then by α-D-glucopyranosyl-(1→6)-D-mannitol (**5**), the fructose dianhydride **16**, α-D-glucopyranosyl-(1→6)-D-glucitol (**4**) and **11** ('D-glucal'). The remaining new acceptors, the aldosuloses **8** and **9**, mannitol, D-glucitol and the *n*-alkyl glycoside **15** must be considered as weak acceptors, since their acceptor strength is below that of D-fructose.

In accordance with the results obtained by J. F. Robyt et al.,[17] the data given in Fig. 3 generally illustrate, that the unchanged glucopyranosyl group at the non-reducing-end of a saccharide is the best structural component suited for dextransucrase acceptors. Among all monosaccharides examined here, D-glucose was found to be the best acceptor, followed by **11** ('D-glucal'), which is the only other monosaccharide here with an analogous glucopyranosyl molecular structure.

Moreover, the same tendency was also observed in case of the disaccharides. The acceptor efficiency of the disaccharides **20** ('isomaltose') and maltose **23**, which both have two unchanged glucopyranosyl units, was considerably higher than that of the disaccharides having only one unmodified glucopyranosyl unit, namely **3** ('maltitol'), **10**, **17** ('maltulose'), and the disaccharide alditols **4** and **5**. The acceptor strength of the two aldosuloses derived from disaccharides, **8** and **9** with both glucopyranosyl units modified were found to be only somewhere in the order of that of the monosaccharides.

On the whole, the most striking evidence for the good suitability of the glucopyranosyl (and the isomaltosyl) unit was obtained from our findings that the subsequent higher homologue acceptor products—especially the trisaccharides—are altogether very good acceptors for dextransucrase. These modeling results do not mean, however, that all saccharides containing an unaffected glucopyranosyl residue necessarily have to be relatively good acceptors for dextransucrase. Instead, it must be kept in mind, that every attached group may have a severe influence on the given saccharide's acceptor properties. In this work, for instance, the presence of long *n*-alkyl chains turned out to be definitely disadvantegous for the acceptor properties of saccharide derivatatives.

Another example, demonstrating the importance of attached groups and their structural arrangement is provided from the series of sucrose analogues. Among these, both α-D-glucopyranosyl-(1→6)-D-fructose ('isomaltulose', **18**) and α-D-glucopyranosyl-(1→1)-D-fructose ('trehalulose', **19**) have, according to the results of Prinz,[21] to be classified as strong acceptors. In contrast, α-D-glucopyranosyl-(1→5)-D-fructose ('leucrose') and sucrose itself do not seem to be able to act as acceptors at all.[10] The two remaining members of the sucrose analogue series, α-D-glucopyranosyl-(1→3)-D-fructose ('turanose')[10] and α-D-glucopyranosyl-(1→4)-D-fructose ('maltulose', **17**), finally, may be looked at as moderately efficient acceptors. In order to explain the differences observed, a closer look was taken at the molecular structure of the various sucrose analogs. Thereby, a correlation between the acceptor efficiency and the flexibility of the attached fructosyl residue concerning structural arrangement became obvious. In the case of the two strong dextransucrase acceptors, **18** and **19**, the fructosyl group is bound very flexibly via a hydroxymethylene group. The remaining sucrose analogues do not have such a hydroxymethylene-group linking the two monosaccharide groups. Nevertheless, in the case of **17** ('maltulose') and α-D-glucopyranosyl-(1→3)-D-fructose ('turanose', **24**), the fructosyl group is still somewhat flexible, since in these molecules it may undergo structural change forming either the α- or β-furanoside or the α- or β-pyranoside form. In α-D-glucopyranosyl-(1→5)-D-fructose ('leucrose', **21**), however, the fructosyl group is fixed strictly in the α- or β-pyranoside form, and in the sucrose molecule to the β-furanoside form.

Summarizing our investigations, the acceptor reactions of dextransucrase offer the potential for a targeted synthesis of a wide range of di-, tri- and higher oligosaccharides by the transfer of glucose to the acceptor. The synthetic potential of this enzyme is not restricted to 'normal' saccharides: Also additionally functionalized saccharides, such as alditols (**1**–**4**), aldosuloses (**8**, **9**), a sugar acid (**10**), D-glucal (**11**), and rather unconventional saccharides, such as the fructose dianhydride **16**, may act as acceptors. Some of these acceptors even turned out to be relatively efficient (e.g., **10**, **3**–**5**, **16**, **11**) and may therefore be of interest for future applications of the dextransucrase acceptor reaction.

3. Experimental

Enzyme.—All experimental investigations were carried out with the dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F, since the enzyme from this species shows a high specifity in bond formation. The enzyme was a gift from the Pfeiffer & Langen company, Cologne, Gemany. For determination of the dextransucrase activity, the maltose test (see below)[51] was used.

Substrates and acceptors.—The sugar alcohols α-D-glucopyranosyl-(1→6)-D-glucitol (**4**) and α-D-glucopyranosyl-(1→6)-D-mannitol (**5**) were a gift from the 'Südzucker AG', Mannheim, Germany, and α-D-glucopyranosyl-(1→4)-D-fructose ('maltulose', **17**) was a gift from Professor F. W. Lichtenthaler (Technical University, Darmstadt, Germany).

All of the aldusuloses (**6**–**9**) were synthesized by microbial oxidation with *Agrobacterium tumefaciens*.[52] α-D-Glucopyranosyl-(1→5)-D-arabinonic acid (**10**) and α-D-fructofuranosyl-β-D-fructofuranosyl-(1,2′:2,3′) dianhydride (**16**) were gifts from 'Südzucker AG' and 'Nordzucker AG' (Braunschweig, Germany), respectively.

The two glycals **11** and **12** were both chemically synthesized from 3,4,6-tri-*O*-acetyl-D-glucal according to the literature.[53] The glycal **13** was prepared from D-fructose by a four-step synthesis similar to that described by Lichtenthaler et al.[54] The remaining saccharide derivatatives were commercially available chemicals.

Experiments for determination of the kinetic parameters and potential acceptor properties.—With all saccharides mentioned, at least one kinetic experiment was carried out. Standard reaction conditions used were the following: initial sucrose concentration: 526 mM; initial

concentration of potential acceptor: 920 mM (in the case of **5** and the alkyl glycosides, **14** and **15**, different initial concentrations had to be chosen because of the lower water solubility of these saccharides at 25 °C); dextransucrase activity: 0.80 U/mL; pH 5.4 (maintained by a 25 mM calcium acetate buffer); 25 °C. 1 Unit is defined by the formation of 1 μmol of fructose per min, as measured in the 'maltose test' with 0.146 M sucrose as the substrate and 0.73 M maltose as an acceptor, other conditions as before.[51]

The reactions were allowed to proceed for at least 24 h, within this time samples were repeatedly taken from the reaction mixtures. For determination of the saccharide concentrations and for detection of potential new acceptor products, these samples were then diluted, filtered on a membrane, and analyzed by various appropriate HPLC systems.

HPLC.—Analysis of the samples from assays with sugar alcohols as well as **17**, **10**, **16**, and glycals were mainly performed with the highly sensitive HPAEC system from the Dionex corporation (column: 'CarboPak PA 1', 10 μm, 4 × 250 cm; eluents: A) 0.1 M NaOH and B) 1.0 M NaOAc in 0.1 M NaOH; gradient: in 20 min from 100% A to 97% A, in 5 min from 97% A to 85% A, 3 min at 85% A; temperature: 30 °C; flow rate: 1.0 mL/min). Aldusuloses decompose rapidly under strongly basic conditions and thus the HPAEC system was not suitable for these samples. Therefore, other HPLC systems were used: an aminopropyl silica gel column for determination of the mono-, di- and trisaccharides (column: 'LiChroSpher 100 NH2', 5 μm, 4 × 250 mm, from Merck, Darmstadt, Germany; eluent: 80:20 (v/v) or 70:30 (v/v) acetonitrile–water; flow rate: 0.8 mL/min; temperature: 22 °C); and a strong cation exchanger column in the silver form for detection of higher oligosaccharides (column: 'Aminex HPX 65 A', 7.8 × 300 mm from BioRad Laboratories, Richmond, USA; eluent: distilled water; flow rate: 0.6 mL/min; temperature: 70 °C). The analytical determination of the *n*-alkyl glycosides was performed with an octadecyl silica gel column (column: 'LiChroSpher 100 RP-18e', 5 μm, 4 × 250 mm, from Merck; eluent: acetonitrile/water in the range 40:60 (v/v) to 65:35 (v/v); flow rate: 0.8 mL/min, temperature: 22 °C).

Preparative chromatography.—For preparative chromatography of the reaction mixtures obtained with **10**, **11**, and **8** the octadecyl silica gel 'Labomatic Labochrom Gel RP-18 Silica HD-Sil-18-30-10' (20–40 μm) (Krannich, Göttingen, Germany) was used as the stationary phase. Column dimensions were 1–1.3 m × 1 cm; eluent: distilled water; flow rate: in the range 0.16–0.27 mL/min; temperature: 2 °C.

The reaction mixture obtained from **10** was separated by weak anion exchange chromatography on a preparative aminopropyl silica gel HPLC column ('LiChroSorb NH2', 7 μm, 40 × 250 mm, from Merck); eluent: 10 mM NaOAc buffer with a pH of 4.7; flow rate: 7.0 mL/min; ambient temperature. Removal of the acetate from the product fractions was performed by preparative chromatography on a Sephadex G-10 column (Pharmacia, Uppsala, Sweden).

NMR spectroscopy.—^{1}H and ^{13}C NMR spectroscopy was performed with a Bruker AM-400 spectrometer at 400 and 100 MHz, respectively. D_2O was used as the solvent.

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