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Biocatalytic and chemical investigations in the synthesis of sucrose analogues

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Abstract—Herein, we report about the synthesis of sucrose analogues, obtained by two different approaches: a chemical and an enzymatic. The one step synthesis of the sucrose analogues with the exo-fructosyltransferase (EC 2.4.1.162) from *Bacillus subtilis* NCIMB 11871, which transfers the fructosyl residue of the substrate sucrose to the monosaccharide acceptors galactose, mannose, xylose and fucose, has been developed. Effects in the fructosylation by variation of the positions of the hydroxyl-groups in glycopyranoside acceptors have been studied in respect to their acceptor properties. In contrast, the chemical equivalent nonenzymatic organic synthesis of galacto-sucrose and manno-sucrose has been achieved including six synthetic steps.

1. Introduction

Oligosaccharides such as galacto-oligosaccharides, xylo-oligosaccharides and lactosucrose have been produced in industrial scale[1] and developed as bulking sugar substitutes that have beneficial health effects.[2] For example, the sucrose analogue sucralose has been examined for its usefulness as noncariogenic sweeting agent. It is 600 times sweeter than sucrose and inhibits certain oral bacterial species including *mutans streptococci* (MS).[3] More recently, these compounds have been demonstrated to exhibit immunomodulatory effects on systemic immune response. Thus, the life sciences industry has an increasing demand in oligosaccharides, because these biomolecules have potential application as therapeutics.[4] Some studies have concluded that fucose and mannose appeared to be the most effective of the essential sugars when it came to slowing the growth of cancer cells.[5] Fucose studies are also showing, that it plays a significant role in many diseases, including cancer and its spread and neuron transmission in the brain.[6]

However, the degree of molecular diversity that can be generated from glycosidic linkage assembly is enormous and the synthesis of specific glycosidic linkages is difficult, as carbohydrates are highly functionalized with hydroxyl groups of similar reactivity.[7] To obtain relatively simple oligosaccharides, a wide range of selective protecting-group strategies has to be planed in synthetic routes.[8] In nature, there are hundreds of different enzymes involved in the synthesis of oligosaccharides. We are recently interested in the synthesis of oligosaccharides by enzymes called non Leloir-glycosyltransferases, which utilize the substrate sucrose.[9] The binding energy of substrates, preserved in sucrose analogues, is used in further/subsequent synthesis, as synthons. In our studies, we present the chemical and enzymatic synthesis of the galactose, xylose, mannose and fucose analogues of sucrose.

2. Results and discussion

2.1. Synthetic approach

The chemistry of sucrose is limited due to the eight hydroxyl groups of similar reactivity. Thus, regioselective protection is difficult.[10] For the synthesis, we started a synthetic classical approach and a parallel enzymatic route. Chemical synthesis of sucrose analogues has been studied by Lichtentaler et al.[11] According to their previous work, we got access to the sucrose analogue β-D-fructofuranosyl-α-D-mannopyranoside, which was obtained in 26% overall yield, respectively.

Inspired by this work, a new route for the synthesis of β-D-fructofuranosyl-α-D-galactopyranoside (Gal-Fru) **6** was investigated (Scheme 1). Thus, isopropylidenation of commercially and cheap available sucrose **1** using 2,2′-dimethoxypropane (DMP) afforded 4,6-mono-*O*-isopropylidenesucrose **2** in

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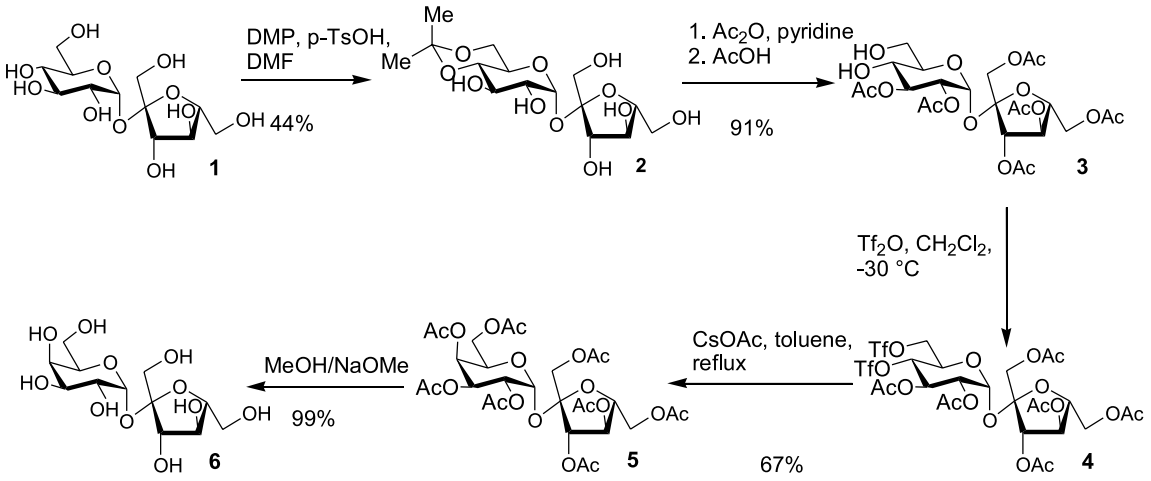


Scheme 1.

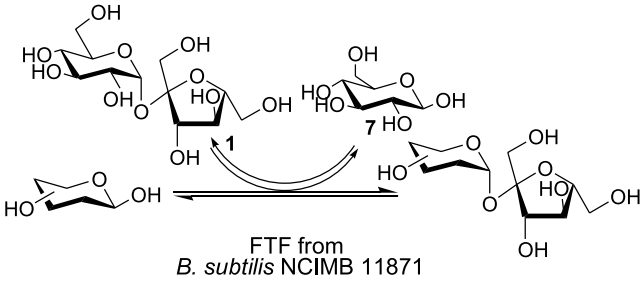
44% yield.[12] Peracetylation, followed by deacetylation using acetic acid, gave 1,3,4,6-tetra-*O*-acetyl-β-D-fructofuranosyl 2,3-di-*O*-acetyl-α-D-glucopyranoside **3** in excellent yield. The free diol was converted in the corresponding ditriflate **4**, which was highly unstable. Thus, refluxing **4** in toluene with caesium acetate gave 1′,2,3,3′,4,4′,6,6′-octa-*O*-acetyl-β-D-fructofuranosyl-α-D-galactopyranoside **5**, which upon deactetylation afforded Gal-Fru **6** in 66% overall yield.

2.2. Enzymatic synthesis

The sucrose analogue synthesis is a time-consuming process, due to the protective group manipulations and the isolation of the intermediates, which decreases overall efficiency. Recently, Römer et al. reported on the synthesis of the sucrose analogue β-D-fructofuranosyl-α-D-xylopyranoside **12** from the donor substrate UDP-α-D-xylose and D-fructose as acceptor by a recombinant sucrose synthase (SuSy1) from potato, respectively.[13] In contrast, in our studies we used an enzyme for a transfructosylation process, which does not require sugar nucleotides, as do all glycosyltransferases of the Leloir pathway, with respect for industrial purposes.

The FTF produced by *Bacillus subtilis* NCIMB 11871[14,15] was tested for its ability to synthesize sucrose analogues by fructosyltransfer from sucrose in the presence of glycopyranosides as in acceptors (Scheme 2). In the presence of D-galactose **8** (400 g/L) and sucrose **1** (400 g/L) the FTF formed the disaccharide Gal-Fru **6**. Optimization of the media and temperature revealed, that the yield of the desired Gal-Fru **6** was maximized at 54%, because an equilibrium is formed,[9] which relies on two transfer reactions: the transfer of the fructosyl residue from sucrose **1** to the acceptor D-galactose **8**, and the reverse reaction the transfer of the fructosyl residue from Gal-Fru **6** to the D-glucose **7**. We also observed the hydrolysis of Gal-Fru **6**. Consequently, the acceptor spectrum for the transfructosylation reaction was expanded. In contrast to D-galactose **8** the acceptor D-mannose **9** demonstrated to be a weak acceptor. The reason should be addressed to its axial position of the hydroxyl group at C-2. Only a maximum yield of 25 g/L manno-sucrose **10** was observed even by variation of the reaction conditions. In addition, the formation of xylosyl-sucrose **12** using D-xylose **11** as acceptor was observed in maximum concentrations of 226 g/L, respectively. The results indicate that the hydroxyl groups of D-glycopyranosides in position 4 and 6 are not crucial for the transfructosylation, in contrast to the position 2. Very recently, Kalovidouris et al. demonstrated that Fuc-α-(1–2)-Gal carbohydrates are capable of modulating neuronal outgrowth and morphology.[16]

This observation prompted us to investigate the acceptor properties of L-fucose **13**. Surprisingly in our studies, the L-fucose **13** was also fructosylated by the enzyme in a concentration of 54 g/L^{-1} (Fig. 1). Because the



Scheme 2.

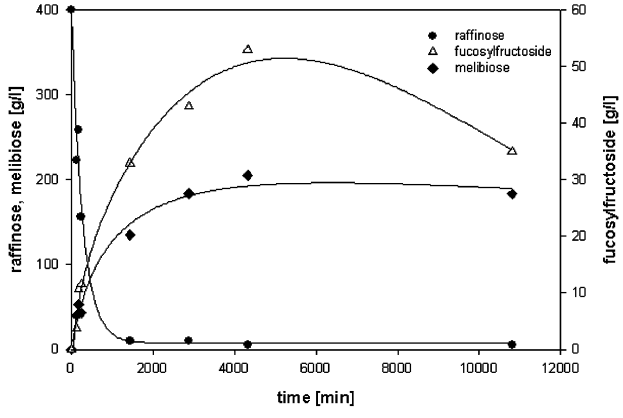


Figure 1.