Regioselective Synthesis of Galactosylated Tri- and Tetrasaccharides by Use of \( \beta \)-Galactosidase from \textit{Bacillus circulans}.

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

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Abstract: \( \beta \)-Galactosidase from \textit{Bacillus circulans} (EC 3.2.1.23) catalyzes the transfer of galactose units to various glucose and galactose derivatives, forming \( \beta \rightarrow 4 \) linkages. The synthesis of several biologically relevant tri- and tetrasaccharides \((\beta-D\text{-Galp}\rightarrow 4)\beta-D\text{-Galp}\rightarrow 4)\alpha-D\text{-GlcP}\) (2), \( \beta-D\text{-Galp}\rightarrow 4)\alpha-D\text{-GlcP}\rightarrow 6)\beta-D\text{-Fru}\) (6), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 4)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (8), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (10). \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (12a), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (14a), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (14b). The kinetic approach or transglycosylation.

Key words: transglycosylations, \( \beta \)-galactosidase, \textit{Bacillus circulans}, enzymes, tri- and tetrasaccharides

Oligosaccharides are involved in many biological processes such as cell recognition and communication, growth regulation, and antibody interactions.1 Another more recently upcoming interest is the formation of health-food additives.2 Balances of intestinal bacterial flora are related to human health and a predominance of \textit{Bifidobacterium} is considered to be very important. It is known that diseases and ageing causes significant decrease or disappearance of \textit{Bifidobacterium} in intestines. In this context, some reports stated that the presence of certain oligosaccharides, such as galacto-, fructo-, isomalt-, and xylooligosaccharides, or galactooligosaccharides in diets of humans and farm animals strengthen their health by favouring the growth of Bifidobacteria and positively affecting the immunological status of intestinal cells.3-5

Among these oligosaccharides \( \beta-(1\rightarrow 4) \) galactosylated structures play an important role. Broad application in the health-food sector, therefore, depends on the availability of larger quantities of some basic di-, tri-, and higher galactose-containing oligosaccharide structures.

Syntheses of such oligosaccharides by chemical methods have been developed in the last decade. However, these multistep approaches involving protection and deprotection procedures are of limited suitability for large scale productions. To overcome these difficulties, synthetic strategies using enzymes as catalysts have been introduced.6-13 Approaches using glycosyltransferases are promising, but at present, the transferases and their cofactors as well as nucleoside-sugar donors are not available in large quantities and they are very expensive.

More practical approaches take advantage of the transglycosylation activity of glycoside hydrolases (glycosidases).14-17 Glycosidases are stereospecific catalysts which are readily available in larger quantities and which allow the use of less complex glycosyl donor substrates, such as simple sugars or sugar glycosides. Glycosidases usually cleave glycosidic bonds, but can also be used in a reverse mode for the formation of glycosidic bonds by either kinetic or thermodynamic approaches. Success in reversing enzyme action has been achieved especially by derivatising the substrate with superior leaving groups, thus altering the kinetics of the binding of enzyme and substrate (in the kinetic approach or transglycosylation).

\( \beta \)-Galactosidase from \textit{Bacillus circulans}14-24 catalyses the transfer of galactose from lactose or Gal\( \beta \)O\( \beta \)NP predominantly to the OH-4 position of Glc, Gal, Gal\( \beta \)NAc, and Gal\( \beta \)NAc.

We report here the application of lactose (1), isomalt (3), isomaltulose (5), sucrose (7), 1-kestose (9), isomelezitose (11), and raffinose (13) as glycosyl acceptors in the enzymatic synthesis of the following tri- and tetrasaccharides using \( \beta \)-galactosidase from \textit{Bacillus circulans}:

\( \beta-D\text{-Galp}\rightarrow 1)\beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (2), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (4), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (6), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (8), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (10), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (12a), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (12b), \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (14a), and \( \beta-D\text{-Galp}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\alpha-D\text{-GlcP}\rightarrow 1)\beta-D\text{-Fru}\) (14b).
Furthermore, the glycosyl donor properties of four β-galactosides were compared: \( p \)-nitrophenyl \( \beta \)-D-galactopyranoside (16), lactose (1), lactulose (17), and lactitol (18). Employing methyl \( \beta \)-D-glucopyranoside (15) as a model acceptor the formation of two possible regioisomers \( 1 \rightarrow 4 \) and \( 1 \rightarrow 6 \) was examined.

### Enzymatic Glycosylation of Various Acceptors with β-Galactosidase from *Bacillus circulans* Employing Lactose as Donor Substrate

In order to synthesise novel potential food additives, various di- and trisaccharides were chosen such as lactose (1), isomalt (3), isomaltulose (5), sucrose (7), 1-kestose (9), isomelezitose (11), and raffinose (13) as glycosyl acceptors, which are known food constituents or intermediates in food-processing pathways. For the whole synthetic sequence special care has to be taken that no potentially toxic compounds are involved. Therefore, the generally used \( p \)-nitrophenyl \( \beta \)-D-galactopyranoside does not meet the demand of food grade requirements as it involves the liberation of \( p \)-nitrophenol. Lactose (1) as natural substrate of \( \beta \)-galactosidases, therefore, represents an ideal alternative donor substrate. The results summarised in Table 1, show that the structure of the acceptor has a pronounced influence on the preferred galactosylation site.

Lactose (1), isomalt (3), isomaltulose (5), sucrose (7), and 1-kestose (9) could be regioselectively transglycosylated at the C-4 hydroxyl group at the terminal non-reducing sugar unit (Scheme 1). In contrast, transglycosylation of isomelezitose (11) produced the positional isomers \( 12a/b \) in a 1:1 ratio and raffinose (13) yielded \( 14a/b \) in a 4:3 ratio.

### Table 1 Results of the Enzymatic Galactosylations Using Different Acceptors

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Product(s)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lactose (1)</td>
<td>2</td>
<td>15*</td>
</tr>
<tr>
<td>isomaltitol (3)</td>
<td>4</td>
<td>59</td>
</tr>
<tr>
<td>isomaltulose (5)</td>
<td>6</td>
<td>59</td>
</tr>
<tr>
<td>sucrose (7)</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>kestose (9)</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>isomelezitose (11)</td>
<td>12 a/b</td>
<td>21</td>
</tr>
<tr>
<td>raffinose (13)</td>
<td>14 a/b</td>
<td>38</td>
</tr>
</tbody>
</table>

\* Donor/acceptor ratio = 1:6.

### Scheme 1 Enzymatic galactosylation of lactose (1), isomalt (3), and isomaltulose (5) with lactose (1) as donor using \( \beta \)-galactosidase from *Bacillus circulans*
(Scheme 2). Regarding these results a general trend can be postulated that neither furanosyl units nor open glycitol structures offer possible galactosylation sites for *Bacillus circulans* β-galactosidase. On the other hand, if terminal pyranosyl units with non-obstructed C-4 positions were present in the oligosaccharides, as in the case of isomelezitose (11) and raffinose (13), competitive galactosylation took place (Scheme 3). As shown in Table 1 yields were in a range of 15–59%. In the case of lactose, donor and acceptor were identical, and therefore, the yield was given based on donor-acceptor ratio of 1:6.

### Enzymatic Glycosylation of GlcαOMe as Model Acceptor with β-Galactosidase from *Bacillus circulans* Employing Different Donor Substrates

The donor substrate properties of the following β-galactosides were compared: *p*-nitrophenyl β-D-galactopyranoside (16), lactose (1), lactulose (17), and lactitol (18). Methyl α-D-glucopyranoside (15) was chosen as a simple model acceptor substrate. The enzymatic glycosylations were carried out according to the general procedure C and the results are summarised in Table 2. The best transfer yield (57%) was obtained using *p*-nitrophenyl β-D-galactopyranoside (16), but the use of lactose (1) led to the formation of 19a/b in remarkable yield (38%) (Scheme 1). The application of lactulose (17) and lactitol (18) resulted in the disaccharides 19a/b in much smaller yields (15% and 11%, respectively). The existence of hydrophobic regions in the active site of the donor substrate is indicated by the fact that most glycosidases have a considerably higher affinity for glycosides with hydrophobic aglycons than for the corresponding oligosaccharides. Thus, the interaction of the donor and the enzyme is significantly influenced by the nature of the donor aglycon. The structure of the donor has not only an influence on the yield, but plays a vital role in the product formation. When lactulose (17) and lactitol (18) were used as donors, the regioselectivity was significantly reduced compared to the reactions with *p*-nitrophenyl β-D-galactopyranoside (16) and lactose (1). Lactulose (17) and lactitol (18) produced a 1:1 ratio of the 1→4-linked 19a and 1→6-linked 19b regioisomers (Table 2). By employing lactitol (18) as donor two side products were also observed in traces: the monogalactosylated lactitol, β-D-Gal-(1→4)-β-D-Gal-(1→4)-Glc-ol, {MALDI-TOF: *m/z* = [M + Na]*+, 528.95, [M + K]* 544.89}, and the digalactosylated lactitol, β-D-Gal-(1→4)-β-D-Gal-(1→4)-β-D-Gal-(1→4)-Glc-ol, {MALDI-TOF: *m/z* = [M + Na]*+, 690.92, [M + K]* 704.84}.

### Table 2 Results of the Enzymatic Galactosylations Using Different Donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Ratio of 19a and b</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>p</em>NP-β-D-Galp (16)</td>
<td>10:1</td>
<td>57</td>
</tr>
<tr>
<td>lactose (1)</td>
<td>10:1</td>
<td>38</td>
</tr>
<tr>
<td>lactitol (17)</td>
<td>1:1</td>
<td>15</td>
</tr>
<tr>
<td>lactitol (18)</td>
<td>1:1</td>
<td>11</td>
</tr>
</tbody>
</table>

The reactions were monitored by TLC analysis using silica gel plates (Kieselgel 60 F254, Merck). Compounds were visualised by spraying with 20% H2SO4 in EtOH, followed by charring at 150 °C and/or UV irradiation. Column chromatography was performed on Biogel P2 (BioRad-Pharmacia) with H2O as eluent. Optical rotations were determined at r.t. with a Perkin-Elmer 241 and 341 polarimeters. NMR spectra were recorded with a Bruker AMX 400 spectrometer. Chemical shifts are given in ppm (δ). Mass spectra were recorded with a Bruker MALDI-TOF mass spectrometer (with N2 laser operating at 337 nm and 5 μL of 2,5-dihydroxybenzoic acid as matrix). β-Galactosidase from *Bacillus circulans* (EC 3.2.1.23) was purchased from ‘Biolacta’ Daiwa Kasei Co., Ltd., Osaka, Japan, and *p*-nitrophenyl β-D-galactopyranoside and lactose from Sigma, Germany.

### Enzymatic Glycosylations; General Procedures

**Procedure A**: Lactose (1) as donor (1 mmol) and lactose (1), sucrose (7), or isomelezitose (11) as acceptors (6 mmol) suspended in aq NaOAc buffer (2 mL, 50 mM, pH 5.0) were incubated with β-galactosidase from *Bacillus circulans* (2 U/mmol acceptor) at 25 °C for 3 h. The reaction was terminated by heating to 90 °C for 10 min. The separation of the products from starting material and desalination was achieved by column chromatography (Biogel P2). The structures of the products were confirmed by 1H and 13C NMR spectroscopy and MALDI-TOF mass spectrometry. The 13C NMR data are collected in Tables 3–5.

**Procedure B**: Lactose (1) as donor (1 mmol) and isomaltose (3), isomaltulose (5), 1-kestose (9), or raffinose (13) as acceptors (10 mmol, 10 equiv) suspended in aq NaOAc buffer (5.9 mL, 50 mM, pH 5.0) were incubated with β-galactosidase from *Bacillus circulans* (1.2 U/mmol acceptor) at 55 °C for 2 h. The reaction was terminated by
heating to 90 °C for 10 min. The separation of the products from starting material and desalination was achieved by column chromatography (Biogel P2).

Procedure C: Methyl α-D-glucopyranoside (15) as acceptor (10 mmol) and p-nitrophenyl β-D-galactopyranoside (16), lactose (1), lactulose (17), or lactitol (18) as donors (1 mmol) suspended in aq NaOAc buffer (5.9 mL, 50 mM, pH 5.0) were incubated with β-galactosidase from *Bacillus circulans* (1.2 U/mmol acceptor) at 55 °C for 2 h. The reaction was terminated by heating to 90 °C for 10 min. The separation of the products from starting material and desalination was achieved by column chromatography (Biogel P2).

β-D-Galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-α/β-D-glucopyranose [β-D-Galp-(1→4)-β-D-Galp-(1→4)-αβ-D-Glcp, 2]

Prepared from lactose (1; 504.5 mg, 1.4 mmol) according to General Procedure A; yield: 14.6 mg (15% based on donor-acceptor ratio 1:6); white amorphous solid; [α]_D20 +40 (c = 1.2, MeOH). The ratio of α/β-anomer is 1:2.

Scheme 3  Enzymatic galactosylation of isomelezitose (11), and raffinose (13) with lactose (1) as donor using β-galactosidase from *Bacillus circulans*
Scheme 4 Enzymatic galactosylation of methyl β-D-glucopyranoside (15), with the following donors using β-galactosidase from Bacillus circulans: p-nitrophenyl β-D-galactopyranoside (16), lactose (1), lactulose (17), and lactitol (18).

Table 3 13C NMR Chemical Data for Glycosyl Acceptors 1, 3, 5, 7, 9, 11, and 13 in D2O

<table>
<thead>
<tr>
<th>Compound Residue</th>
<th>Chemical Shifts, δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-1</td>
</tr>
<tr>
<td>1 Galp-(1→4)</td>
<td>103.27</td>
</tr>
<tr>
<td>α-Glc</td>
<td>92.17</td>
</tr>
<tr>
<td>β-Glc</td>
<td>96.11</td>
</tr>
<tr>
<td>3 Glcp-(1→6)</td>
<td>98.55/98.51</td>
</tr>
<tr>
<td>Glcp-ol</td>
<td>63.63</td>
</tr>
<tr>
<td>Manp-ol</td>
<td>62.79</td>
</tr>
<tr>
<td>5 Glcp-(1→6)</td>
<td>98.63</td>
</tr>
<tr>
<td>β-Fru</td>
<td>63.02</td>
</tr>
<tr>
<td>7 Glcp-(1→2)</td>
<td>92.52</td>
</tr>
<tr>
<td>Fru</td>
<td>61.68</td>
</tr>
<tr>
<td>9 Glcp-(1→2)</td>
<td>92.83</td>
</tr>
<tr>
<td>Fru/(1→2)</td>
<td>60.76</td>
</tr>
<tr>
<td>Fru</td>
<td>61.24</td>
</tr>
<tr>
<td>11 Glcp-(1→6)</td>
<td>98.61</td>
</tr>
<tr>
<td>Glcp-(1→2)</td>
<td>92.20</td>
</tr>
<tr>
<td>Fru</td>
<td>61.88</td>
</tr>
<tr>
<td>13 Galp-(1→6)</td>
<td>98.82</td>
</tr>
<tr>
<td>Glcp-(1→2)</td>
<td>92.45</td>
</tr>
<tr>
<td>Fru</td>
<td>61.75</td>
</tr>
</tbody>
</table>

* Not distinguishable from other signals.
\[ \text{Table 4} \quad ^{13}C \text{ NMR Chemical Data for Compounds 2, 4, 6, 8, and 10 in D}_2\text{O} \]

<table>
<thead>
<tr>
<th>Compound Residue</th>
<th>Chemical Shifts, ( \delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-1</td>
</tr>
<tr>
<td>2 Galp-(1→4)</td>
<td>104.57</td>
</tr>
<tr>
<td></td>
<td>103.24</td>
</tr>
<tr>
<td>( \alpha )-GlcP</td>
<td>92.17</td>
</tr>
<tr>
<td>( \beta )-GlcP</td>
<td>96.12</td>
</tr>
</tbody>
</table>

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{19}H_{32}O_{16}, 506.53; \text{ found: } 527.17 [M + Na]^+. \]

\[ \beta\text{-d-Galactopyranosyl-(1→4)-d-glucopyranosyl-(1→6)-d-glucopyranosyl-mannitol} \quad \beta\text{-d-Galp-(1→4)-d-Glc-(1→6)-d-Glcol/Mannol, 4) \]

Prepared from isomalt monohydrate (3; 360.3 mg, 1 mmol) and lactose (1; 36.0 mg, 0.1 mmol according to General Procedure B; yield: 29.8 mg (59%); white amorphous solid; [\( \alpha \])_D^{20} +61 (c = 0.8, H_2O).

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{19}H_{32}O_{16}, 506.53; \text{ found: } 527.17 [M + Na]^+. \]

\[ \beta\text{-d-Galactopyranosyl-(1→4)-d-glucopyranosyl-(1→6)-d-fructofuranose} \quad \beta\text{-d-Galp-(1→4)-d-Glc-(1→6)-d-Fru, 6) \]

Prepared from isomaltulose (5; 342.3 mg, 1 mmol) and lactose (1; 36.0 mg, 0.1 mol) according to General Procedure B; yield: 29.7 mg (59%); white amorphous solid; [\( \alpha \])_D^{20} +74 (c = 0.5, H_2O).

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{20}H_{34}O_{16}, 545.63 [M + K]^+. \]

\[ \beta\text{-d-Galactopyranosyl-(1→4)-d-glucopyranosyl-(1→6)-d-fructofuranoside} \quad \beta\text{-d-Galp-(1→4)-d-Glc-(1→6)-d-Fru, 8) \]

Prepared from sucrose (7; 432.4 mg, 1.2 mmol) and lactose (1; 72.0 mg, 0.2 mol) according to General Procedure A; yield: 16.9 mg (17%); white amorphous solid; [\( \alpha \])_D^{20} +51 (c = 0.7, MeOH).

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{22}H_{42}O_{21}, 666.65; \text{ found: } 689.32 [M + Na]^+. \]

\[ \beta\text{-d-Galactopyranosyl-(1→4)-d-glucopyranosyl-(1→6)-d-fructofuranoside} \quad \beta\text{-d-Galp-(1→4)-d-Glc-(1→6)-d-Fru, 10) \]

Prepared from isomaltulose dihydrate (9; 900.0 mg, 0.6 mmol) and lactose (1; 21.6 mg, 0.06 mol) according to General Procedure B; yield: 12.6 mg (32%); white amorphous solid; [\( \alpha \])_D^{20} +26 (c = 0.4, H_2O).

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{24}H_{42}O_{21}, 666.65; \text{ found: } 689.32 [M + Na]^+. \]

\[ \beta\text{-d-Galactopyranosyl-(1→4)-d-glucopyranosyl-(1→6)-d-fructofuranoside} \quad \beta\text{-d-Galp-(1→4)-d-Glc-(1→6)-d-Fru, 12) \]

Prepared from isomaltulose dihydrate (11; 648.5 mg, 1.2 mmol) and lactose (1; 72.0 mg, 0.2 mmol) according to General Procedure A; yield: 27.4 mg (21%); white amorphous solid; [\( \alpha \])_D^{20} +96 (c = 1.1, H_2O). The ratio of 12a/12b was 1:1.

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{24}H_{44}O_{22}, 705.30 [M + Na]^+. \]

\[ ^1H \text{ NMR (400 MHz, D}_2\text{O}): \delta = 5.54 \text{ (d, 1 H, } J_{1,2} = 4.1 \text{ Hz, H-1)}, \]
\[ 4.30 \text{ (d, 1 H, } J_{3,2} = 7.6 \text{ Hz, H-1}), \]
\[ 4.04 \text{ (d, 1 H, } J_{5,4} = 7.6 \text{ Hz, H-3}), \]
\[ 3.87 \text{ (dd, 1 H, } J_{3,4} = 8.7 \text{ Hz, H-4}). \]

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{24}H_{44}O_{22}, 705.30 [M + Na]^+. \]

\[ ^1H \text{ NMR (400 MHz, D}_2\text{O)}: \delta = 5.24 \text{ (d, 1 H, } J_{1,2} = 4.1 \text{ Hz, H-1)}, \]
\[ 4.30 \text{ (d, 1 H, } J_{3,2} = 7.6 \text{ Hz, H-1}), \]
\[ 4.04 \text{ (d, 1 H, } J_{5,4} = 7.6 \text{ Hz, H-3}), \]
\[ 3.87 \text{ (dd, 1 H, } J_{3,4} = 8.7 \text{ Hz, H-4}). \]

\[ \text{MALDI-TOF}: m/z \text{ calcd for } C_{24}H_{44}O_{22}, 705.30 [M + Na]^+. \]
α-D-Galactopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside (α-D-Galp-(1→6)-[β-D-Galp-(1→4)]-α-D-Glcp-(1→2)-β-D-Fru, 14a) and β-D-Galactopyranosyl-(1→4)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→4)-α-D-Galp-(1→6)-α-D-Glcp-(1→2)-β-D-Fru, 14b) Prepared from raffinose pentahydrate (13; 594.5 mg, 1 mmol) and lactose (1; 360.0 mg, 0.1 mmol) according to General Procedure B; yield: 25.3 mg (38%); white amorphous solid; $[\alpha]_{D}^{20} +42$ ($c = 0.7$, H$_2$O). The ratio of 14a/14b was 4:3.

$^1$H NMR (400 MHz, D$_2$O): $\delta = 5.38$ (d, 1 H, $J_{1,2} = 3.6$ Hz, H-1x), 4.24 (d, 1 H, $J_{1,2} = 4.1$ Hz, H-1a), 5.22 (d, 1 H, $J_{1,2} = 4.1$ Hz, H-1a), 4.80 (d, 1 H, $J_{1,2} = 6.1$ Hz, H-1''), 4.39 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1''), 4.02 (d, 1 H, $J_{1,2} = 8.7$ Hz, H-3').

MALDI-TOF: $m/z$ calcd for C$_{36}$H$_{58}$O$_{16}$, 666.65; found, 689.45 [M + Na$^+$], 705.43 [M + K$^+$].

Methyl β-D-Galactopyranosyl-(1→4)-α-D-glucopyranoside (β-D-Galp-(1→4)-α-D-Glcp-OMe, 19a) and Methyl β-D-Galactopyranosyl-(1→6)-α-D-glucopyranoside (β-D-Galp-(1→6)-α-D-Glcp-OMe, 19b) Prepared from methyl α-D-glucopyranoside (15; 388.4 mg, 2 mmol) and the following donors: a) pNP-β-D-Gal (16; 60.3 mg, 0.2 mmol, b) lactose (1; 72.0 mg, 0.2 mmol), c) lactulose (17; 68.2 mg, 0.2 mmol), and d) lactitol (18; 68.2 mg, 0.2 mmol) according to General Procedure B; yield: a) 40.9 mg (57%), b) 26.8 mg (38%), c) 10.5 mg (15%), and d) 7.5 mg (11%); white amorphous solid.

19a

$[\alpha]_{D}^{20} +109$ ($c = 0.4$, H$_2$O).

$^1$H NMR (400 MHz, D$_2$O): $\delta = 4.65$ (d, 1 H, $J_{1,2} = 4.1$ Hz, H-1), 4.28 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1''), 3.26 (s, 3 H, OCH$_3$).

$^1$C NMR (100.67 MHz, D$_2$O): $\delta = 55.47$ (OCH$_3$); other signals in Table 5.

MALDI-TOF: $m/z$ calcd for C$_{20}$H$_{32}$O$_{12}$, 356.32; found, 379.21 [M + Na$^+$], 395.18 [M + K$^+$].

19b

$[\alpha]_{D}^{20} +39$ ($c = 0.4$, H$_2$O).

$^1$H NMR (400 MHz, D$_2$O): $\delta = 4.61$ (d, 1 H, $J_{1,2} = 4.1$ Hz, H-1), 4.24 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1''), 4.00 (dd, 1 H, $J_{1,2} = 2.0$ Hz, $J_{1,2} = 11.7$ Hz, H-6a), 3.24 (s, 3 H, OCH$_3$).

$^1$C NMR (100.67 MHz, D$_2$O): $\delta = 55.63$ (OCH$_3$); other signals in Table 5.

MALDI-TOF: $m/z$ calcd for C$_{20}$H$_{32}$O$_{12}$, 356.32; found, 379.21 [M + Na$^+$], 395.18 [M + K$^+$].

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