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Research paper

Efficient synthesis and activity of beneficial intestinal flora of two lactulose-derived oligosaccharides

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ABSTRACT

Lactulose is considered as a prebiotic because it promotes the intestinal proliferation of *Lactobacillus acidophilus* which is added to various milk products. Moreover, lactulose is used in pharmaceuticals as a gentle laxative and to treat hyperammonemia. This study was aimed at the total synthesis of two Lactulose-derived oligosaccharides: one is 3-O-β-D-galactopyranosyl-D-fructose, D-fructose and β-D-galactose bounded together with β-1,3-glycosidic bound, the other is 1-O-β-D-galactopyranosyl-D-fructose, D-fructose and β-D-galactose bounded together with β-1,1-glycosidic bound, which were accomplished in seven steps from D-fructose and β-D-galactose and every step of yield above 75%. This synthetic route provided a practical and effective synthetic strategy for galactooligosaccharides, starting from commercially available monosaccharides. Then we evaluated on their prebiotic properties in the search for potential agents of regulating and improving the intestinal flora of human. The result showed that the prebiotic properties of Lactulose-derived oligosaccharides was much better than Lactulose. Among them, 3-O-β-D-galactopyranosyl-D-fructose displayed the most potent activity of proliferation of *L. acidophilus*.

1. Introduction

Lactulose (4-O-β-D-galactopyranosyl-D-fructose), a synthetic disaccharide composed of two sugar molecules D-fructose and β-D-galactose bounded together with β-1,4-glycosidic bound. Lactulose is 1.5 times sweeter than Lactose and can be crystallized from alcohol solution. The β-glycosidic linkage of the lactulose is not hydrolyzed by mammalian digestive enzymes and ingested lactulose passes the stomach and small intestine without degradation. It is characteristically utilized by all the species of *Lactobacillus acidophilus*, which resides in the human intestine tract [1]. In the colon, large number of bacteria metabolizes lactulose and consumes it as their own food. In doing so, these bacteria produce lactic, acetic, and formic acid as well as carbon dioxide gas. These acids biochemically draw fluid into the bowel which softens the stool, hence the lactulose can be used as a laxative [2]. Lactulose has prebiotic property, because it stimulates the growth of health-promoting bacteria in the gastrointestinal tract, such as *lactobacilli* and *bifidobacteria* and at the same time inhibits pathogenic bacteria such as *Salmonella* [3]. In addition, lactulose is used in the pharmaceutical field for treating constipation, hepatic encephalopathy and complications of liver disease, and it maintains blood glucose and insulin levels [4].

Lactulose synthetic methods usually can be divided into chemical and enzymetic. The chemical methods are essentially based on the isomerization of lactose by using many catalysts, such as sodium hydroxide, potassium hydroxide, sodium carbonate, magnesium oxide [5–7]. Montgomery and Hudson synthesized lactulose with calcium hydroxide for the first time [8]. These processes generally produce a high level of lactulose degradation, which leads to the formation of a considerable percentage of difficult to separate, colored by-products which lower the lactulose yield. Another group of processes uses complexing reagents such as aluminates and borates, which facilitate the reaction with a minimum of secondary reactions and result in a high yield of lactulose

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by eliminating lactulose from the reaction equilibrium mixture in the form of a complex [9,10]. However, they are unsatisfactory from the industrial aspect because of the difficulty of eliminating the aluminate and borate. Also in these processes a large excess of borate and aluminate is necessary for optimal yield. The chemical methods have several drawbacks since reaction is poorly specific, side reactions occurring so that low product yields are obtained, colored by-products are formed and intense purification is required [11,12]. For these reasons any study to develop a feasible process to produce lactulose is very important.

The enzymatic transgalactosylation reaction from lactose by β-galactosidases obtaining complex mixtures of oligosaccharides with different glycosidic linkages and degree of polymerization depending on the source of the enzymes and experimental conditions [13–16]. In addition, these products contain glucose, galactose and lactose, without prebiotic properties, which increase the calorific value of the product. Until recently, lactulose have been obtained only from lactose, but currently there is a great interest in obtaining new prebiotic carbohydrates with improved properties, addressed to reach the distal regions of the colon unaltered to promote the growth of specific bacteria. Due to the prebiotic properties that lactulose show, Martínez-Villaluenga, C. and Cardelle-Cobas, A. carried out enzymatic and chemical approaches for the synthesis of new lactulose-derived oligosaccharides, which may be also bioactive compounds and whose beneficial properties should be investigated [17,18]. Makras et al. reported Galactooligosaccharides synthesized by β-galactosidases from lactobacilli and bifidobacteria contain mainly β-(1–1) or β-(1–3) linked di- and trisaccharides, which may have prebiotic effects specifically targeting those strains better than β-(1–4) linkages [19].

In this work we report the chemical synthesis of two Lactulose-derived oligosaccharides: 3-O-β-D-galactopyranosyl-D-fructose and 1-O-β-D-galactopyranosyl-D-fructose from commercially available D-fructose and β-D-galactose (Fig. 1). It is valuable to develop an efficient way to prepare this kind of oligosaccharides to promote further studies, such as thorough pharmacological research and further structure-activity relationship investigation. The preliminary activity of regulating and improving the intestinal flora of these synthetic oligosaccharides was then investigated.

2. Chemistry

The synthetic procedures and reaction conditions are shown in Schemes 1 and 2. Reaction of β-D-galactose (**4**) in acetic anhydride with sodium acetate gave compound **5**. Reaction of compound **5** in diethyl ether with benzylamine provided compound **6**, which, after protection with trichloroacetimidate, gave compound **7**. Reaction of D-fructose (**1**) in acetone with sulfuric acid gave compound **2,3**. Compound **7** reacted with compound 2,3 respectively in the presence of trimethylsily trifluoromethanesulfonate (TMSOTf) to provide compound **8,11**, which were deprotected in methanol with sodium methylate to give compound **9,12**. Then these two compounds after deprotection with acetic acid conditions to give compound **10,13**. The structures of all the synthetic compounds were fully characterized by spectroscopic data (NMR, MS).

3. Results and discussion

For many methods, including the indican enzyme synthesis and chemical synthesis, enzymatic synthesis is of advantages, such as mild reaction, good region, and stereoselectivety. However, it requires glycosyltransferase and glycosidase-catalysed, and the enzyme is expensive. The enzyme on the substrate specificity is strong. Relatively, the chemical synthesis is indispensable. The core of the formation of glycosidic bond is an anomeric acetal synthesis, because the dehydration directly generating glycosidic bond is a violent reaction, so the usual method is to use a leaving groups at anomeric position (donor), which reacts with an alcohol (accepter) to give, in the presence of a promoter, obtained the desired glycoside.

Knorr-Koenigs reaction is a classic glycosylation, using a bromide as donor and heavy metals as promoters, and the problem of this reaction is that the promoting agents are expensive and environment unfriendly. The Schmidt method in 1980, so-called imidate method, is the most valuable glycosidic bond synthesis method, which has advantage of good stability, easy operation, high yield, good selectivity, etc. In the reaction, we chose trichloroacetimidate as β-D-galactose 1-OH activation group, Trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promoter, Acetyl groups have been used as hydroxyl protection. As shown in Schemes 1 and 2, were readily prepared in good yields from the corresponding 1-OH sugars. The reaction of glycosylation achieved in the conditions described above, gave the compound 3-O-β-D-Galactopyranosyl-D-fructose in 86% yield and 1-O-β-D-Galactopyranosyl-D-fructose in 85% yield. The stereochemistry of the newly introduced linkage was determined to be β on the basis of the Glc H-1, H-2 coupling constant ($J_{1,2} = 7.6$ Hz) and Gal H-1, H-2 Coupling constant ($J_{1,2} = 8.4$ Hz) [20]. *L. acidophilus* was grown in simulated intestinal fluid demonstrated that 3-O-β-D-galactopyranosyl-D-fructose is the best carbon source for promoting the growth of *L. acidophilus*, which proliferation activity is better than lactulose.

4. Conclusion

Lactulose chemical structures show a great variability, this affects prebiotic properties. Nevertheless, Studies including synthetic lactulose are scarce, and little information is available about the specific preferences of bacteria with respect to lactulose linkage or composition to compare with. In this work the influence of factors have been investigated both qualitatively and quantitatively. Intestinal flora (*L. acidophilus)* were shown to be able to utilize lactulose and different lactulose-derived as carbon sources. It was observed that glycosidic linkage affected the individual strains growth. Our data showed a general preference of the strains

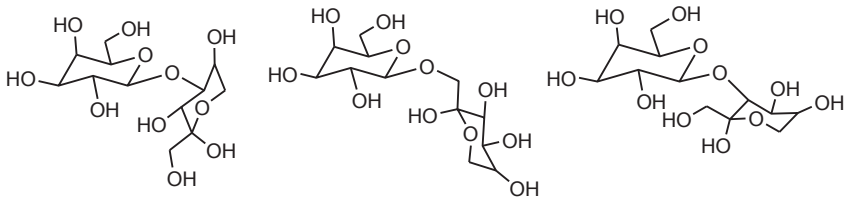
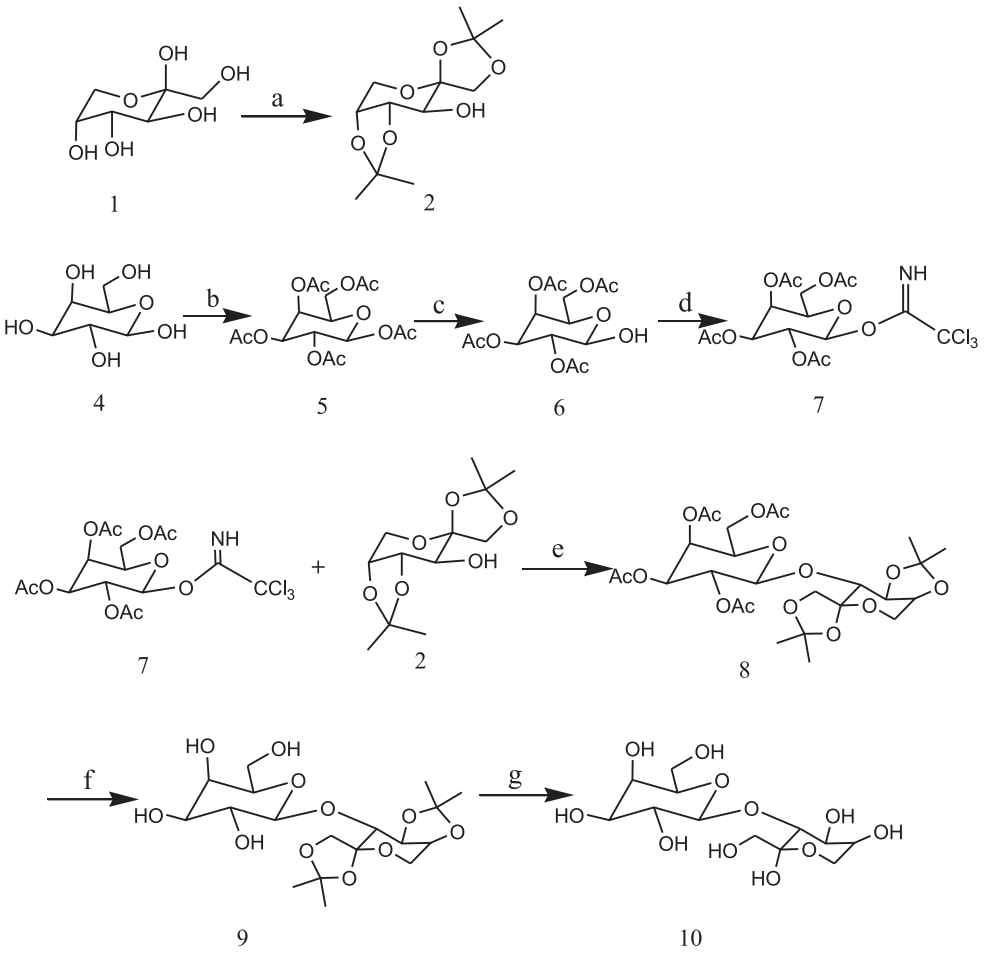


Fig. 1. Structures of compounds Lactulose (4-O-β-D-galactopyranosyl-D-fructose), Compound 13 (1-O-β-D-galactopyranosyl-D-fructose) and Compound 10 (3-O-β-D-galactopyranosyl-D-fructose).



Scheme 1. The synthetic route of Lactulose-derived oligosaccharides(**Compound 10**). Reagents and conditions: (a) H_2SO_4, acetone, r.t, 2 h, 88%; (b) CH_3COONa, $(CH_3CO)_2O$, 120 °C, 1.5 h, 96%; (c) benzylamine, diethyl ether, 0 °C, 2.5 h, 86%; (d) trichloroacetonitrile, DBU, CH_2Cl_2, 0 °C, 4 h, 88%; (e) TMSOTf, CH_2Cl_2, -20 °C, 2 h, 75%; (f) CH_3ONa, CH_3OH, r.t, 2 h, 85%; (g) CH_3COOH, 60 °C, 8 h, 86%.

towards β-galactosyl residues having β(1–3) and β(1–1) linkages over those of β(1–4). Besides, results indicate that oligosaccharides derived from lactulose emerge as candidates to have prebiotic properties, although further research on their functionality and safety is required.

5. The activity of promoting Lactobacillus acidophilus proliferation

A drug's solubility and dissolution behavior within the gastrointestinal tract is a key property for successful administration by the oral route and one of the key factors in the biopharmaceutics classification system. A compound's pKa for example can induce extreme effects due to the variation of pH in the intestine about 6.8, This can greatly affect solubility of for example weakly basic compounds, which could rapidly dissolve in the intestine [21]. This property can be determined by investigating drug solubility in human intestinal fluid but this is difficult to obtain and highly variable, which has led to the development of multiple simulated intestinal fluid recipes. In this work stock solution of the various components of the simulated intestinal media were freshly prepared: 3.4 g KH_2PO_4 dissolved in 250 mL distilled water, with 0.4 g/100 mL NaOH solution to adjust pH value to 6.8, then diluted to 500 mL, and added to trypsin in the solution, made its mass concentration of 1 g/100 mL, blending dissolves adequately, used aperture 0.20 microns of microporous membrane filter, made simulated intestinal fluid.

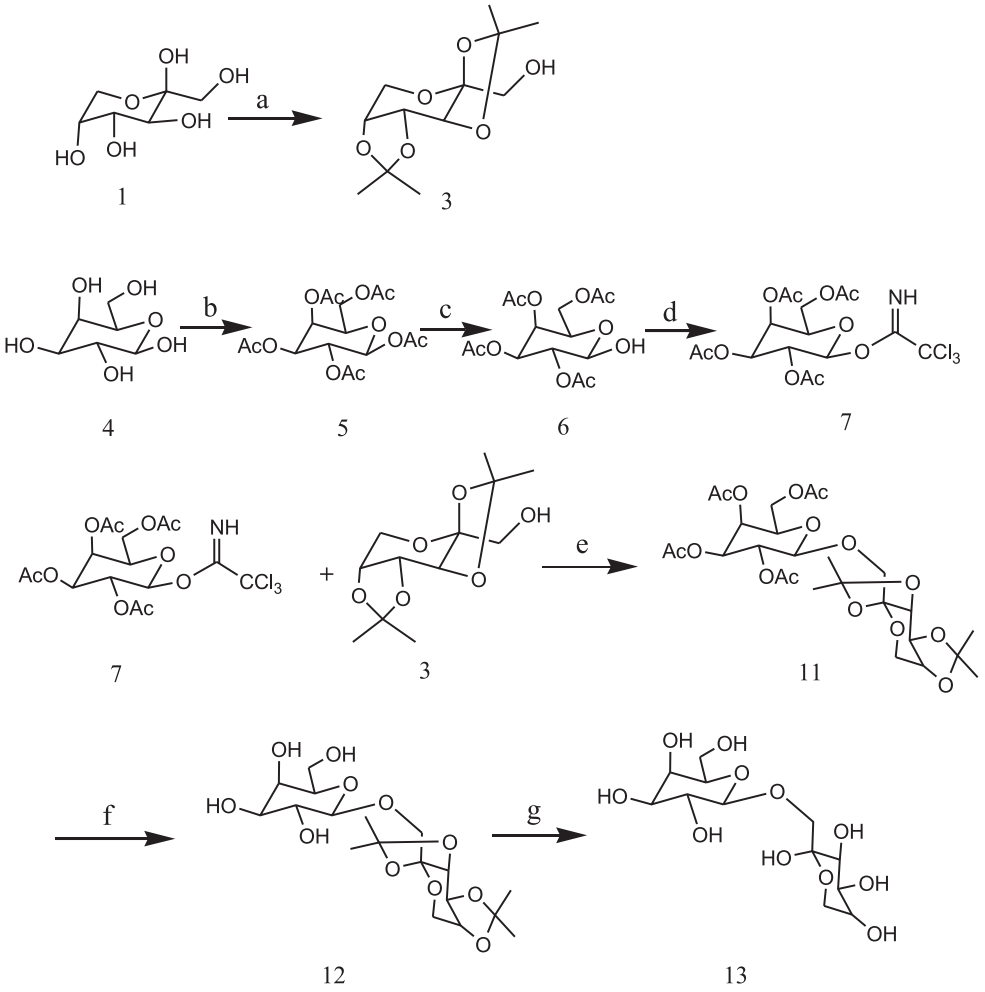
L. acidophilus was provided by the Research Center of Food Biotechnology, Tianjin University of Science and Technology. The Prefabricated MRS(Man-Rogosa-Sharpe) medium contained 10 g proteose peptone, 10 g beef extract, 5 g sodium acetate, 0.1 g magnesium sulfate, 0.05 g manganese sulfate and 2 g dipotassium phosphate per liter at pH = 6.5. For long-time storage, glycerol-stocks (33% v/v) of a stationary phase culture were prepared and maintained at −80 °C, MRS broth medium was used for activation of strains.

1% inoculum of *L. acidophilus* was grown in simulated intestinal fluid with added to different cabon sources (4-O-β-D-galactopyranosyl-D-fructose, 3-O-β-D-galactopyranosyl-D-fructose and 1-O-β-D-galactopyranosyl-D-fructose) and incubated together at 36 °C in a shaker. The cultures were halted after 1 h, 2 h, 3 h and their optical densities were measured at 600 nm (OD_{600}), the results are illustrated in (Fig. 2).

6. Experimental section

6.1. Chemistry

All chemicals (reagent grade) used were purchased from Sigma–Aldrich (U.S.A) and Aladdin-Reagent Co.,Ltd (China). TLC (thin



Scheme 2. The synthetic route of Lactulose-derived oligosaccharides(**Compound 13**). Reagents and conditions as same as Scheme 1.

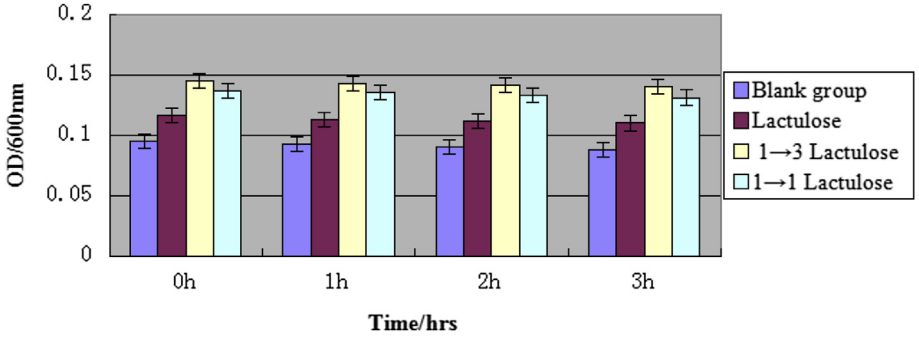


Fig. 2. Comparison of *Lactobacillus acidophilus* using different carbon source in simulated intestinal fluid growth of OD_{600}.

layer chromatography) was run on the silica gel coated aluminum sheets (Silica Gel 60 GF254, E, Merck, Germany). ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AVANCE instrument (400 MHz) with tetramethylsilane as internal standard, and chemical shifts values were recorded. ESI (Electrospray Ionization) mass spectra were obtained on an LCQ-Advantayc-MAX (LAM10188, Finnigan, Co., Ltd, USA). Specific rotation were obtained on an Automatic Polarimeter (WZZ-2B, Shanghai precision scientific instrument, Co.,Ltd, China). Flash column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Group Co., Qingdao, china).

6.1.1. The acetyl-β-D-galactopyranose ***5***

Sodium acetate (1.0 g, 12 mmol) was added in acetic anhydride 20 mL was heated to 120 °C for 30 min, then β-D-galactopyranose (2.0 g, 11 mmol) was added dropwise in the solution under stirring. After 1 h of the reaction at 120 °C, the mixture was cooled and poured into cold water, then washed with saturated $NaHCO_3$ solution to pH = 7. The precipitate was separated and the mixture was extracted with dichloromethane. The combined organic phases were washed with saturated brine and water, successively. Then, the organic layer was dried with sodium sulfate and concentrated. The residue was purified by silica gel column chromatography

(petroleum ether-ethyl acetate 3:1, Rf = 0.35) to obtain compound **5** (4.2 g, 96%).

*6.1.2. 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranose **6***

To a mixture of compound 5 (4 g, 10.3 mmol) and 4 Å molecular sieves (1.8 g) in diethyl ether 20 mL, then benzylamine 40 mL was added dropwise in the solution under stirring. The reaction mixture was continuously stirred for 2.5 h at 0 °C, the solvent was removed by rotary evaporator. The residue was dissolved in dichloromethane, washed by 1 M HCl solution to pH = 7, The organic phases were washed with saturated brine. Then, the organic layer was dried with sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether-ethyl acetate 2:1, Rf = 0.5) to give compound **6** (3.1 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 2.051, 2.067, 2.097, 2.106 (s, 12H), 4.440 (m, J = 8.2 Hz, 1H), 5.176 (d, J = 3.6 Hz, 1H), 5.414 (s, 1H), 5.433 (s, 1H), 5.441 (s, 1H), 5.474 (s, 1H), 5.802 (s, 1H), 7.324 (m, 1H).

*6.1.3. 2,3,4,6-Tetra-O-acetyl- β-D-galactopyranosyl trichloroacetimidate **7***

To a mixture of compound 6 (2.65 g, 7.6 mmol) and 4 Å molecular sieves (1.2 g) in anhydrous dichloromethane 10 mL, after that DBU (0.23 g) was added in the solution under stirring for 10 min at 0 °C. Then trichloroacetonitrile (2.7 mL) was added dropwise in the solution for 3.5 h at room temperature. The mixture was poured into cold water, then washed with saturated brine. Then, the organic layer was dried with sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether-ethyl acetate 1:1, Rf = 0.55) to give compound **7** (3.25 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 2.017, 2.021, 2.030, 2.169 (s, 12H), 4.109 (m, J = 8.2 Hz, 1H), 4.176 (m, 1H), 4.443 (m, J = 13.6 Hz, 1H), 5.385 (m, 2H), 5.449 (s, 1H), 6.661 (s, 1H), 8.667 (s, 1H).

*6.1.4. 1,2:4,5-di-O-(1-methyl) ethylidene-D-fructose **2***

Sulfuric acid (0.3 mL) was added in acetone (60 mL) under stirring for 10 min, then D-fructopyranose (3.2 g) was added, and the mixture was stirred at room temperature. After 2 h, TLC showed no remaining material, and 1 mol/L sodium hydroxide solution (8.5 mL) was added dropwise in the solution at 0 °C. The precipitate was separated and the mixture was concentrated in vacuo, then dissolved in the dichloromethane 100 mL, and washed with water and saturated brine, successively. The organic layer was dried with sodium sulfate and concentrated in vacuo. Obtain light yellow solid and recrystallization with petroleum ether, after filtration and concentrated in vacuo to give the white solid **2**(3.9 g, 88%, Rf = 0.45). $[\alpha]_D^{20}$ − 98 (c = 0.01, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 1.357, 1.404, 1.488, 1.554 (s, 12H); 3.682 (m, 2H); 3.942 (d, J = 2.0 Hz, 1H); 3.910 (d, J = 1.6 Hz, 1H); 4.238 (d, J = 1.2 Hz, 1H); 4.257 (s, 1H); 1.625 (d, 1H); 1.918 (s, 1H).

*6.1.5. 2,3:4,5-di-O-(1-methyl) ethylidene-D-fructose **3***

sulfuric acid (2.0 mL)was added in acetone (46 mL) under stirring for 10 min, then D-fructose (4.7 g) was added, and the mixture was stirred at room temperature. After 2 h, TLC showed no remaining material, and 1 mol/L sodium hydroxide solution (7.5 mL) was added dropwise in the solution at 0 °C. The precipitate was separated and the mixture was concentrated in vacuo, then dissolved in the dichloromethane 100 mL, and washed with water and saturated brine, successively. The organic layer was dried with sodium sulfate and concentrated in vacuo. Obtain light yellow solid and recrystallization with petroleum ether, after filtration and concentrated in vacuo to give the white solid **3** (5.6 g, 83%, Rf = 0.45). $[\alpha]_D^{20}$ − 49 (c = 0.01, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 1.372, 1.444, 1.516, 1.536 (s, 12H); 3.674 (d, J = 7.2 Hz, 1H); 4.112 (m, J = 10.8 Hz, 2H); 4.218 (m, J = 6.2 Hz, 1H); 4.140 (s, 1H); 4.123 (m, J = 10.2 Hz, 2H); 1.984 (s,1H).

*6.1.6. 1,2:4,5-di-O-(1-methyl)ethylidene-3-O-(2,3,4,6-tetra-O-acetyl-β-D-Galactopyranosyl)-D-f-ructose **8***

To a mixture of compound 7(108.0 mg, 0.22 mmol), compound 2 (29 mg, 0.11 mmol) and 4 Å molecular sieves (300 mg) in anhydrous dichloromethane 5 mL. It was stirred for 0.5 h at 0 °C, and then TMSOTf (4.0 μL, 0.022 mmol) was added under Ar protection and the mixture was stirred at −20 °C for 2 h, TLC showed no remaining material, then neutralized with Et_3N. The reaction mixture was then diluted with dichloromethane, and filtered to remove the molecular sieves, which was washed with water and saturated brine, dried with sodium sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether-ethyl acetate 3:2) to give compound **8** (65 mg, 75%, Rf = 0.55). $[\alpha]_D^{20}$ − 28 (c = 0.01, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 1.259, 1.350, 1.458, 1.526 (s, 12H); 1.977 (s, 3H); 2.035 (m, J = 8.4 Hz, 8H); 2.170 (s, 3H); 3.711 (m, 2H); 3.933 (d, J = 9.4 Hz, 3H); 4.152 (m, J = 8.6 Hz, 4H); 4.240 (d, J = 7.2 Hz, 1H); 4.426 (s, 1H); 4.590 (d, J = 5.6 Hz, 2H); 5.014 (dd, J = 12.6 Hz, 1H); 5.226 (m, J = 10.4 Hz, 1H); 5.382 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$, δppm): 20.56, 20.64, 20.72, 20.81, 24.02, 25.45, 25.83, 26.59, 61.07, 61.32, 67.15, 68.86, 70.12, 70.83, 70.98, 71.20, 76.72, 77.04, 77.36, 100.74, 101.88, 108.69, 108.98, 169.31, 170.16, 170.33, 170.42. HR-ESI-MS *m/z:* 590.5711, calculated for $C_{26}H_{38}O_{15}$ $[M + NH_4]^+$ 608.5211.

*6.1.7. 2,3:4,5-di-O-(1-methyl)ethylidene-1-O-(2,3,4,6-tetra-O-acetyl-β-D-Galactopyranosyl)-D-f-ructose **11***

The procedure is the same as that for compound 8, silica gel column chromatography (petroleum ether-ethyl acetate 3:2) to give compound **11** (65 mg, 75%, Rf = 0.55). $[\alpha]_D^{20}$ − 32 (c = 0.01, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 1.344, 1.351, 1.458, 1.526 (s, 12H); 1.977, 2.026, 2.036, 2.171 (s, 12H); 3.711 (m, J = 11.4 Hz, 2H); 3.932 (d, J = 10.4 Hz, 3H); 4.153 (d, J = 7.4 Hz, 2H); 4.241 (d, J = 8.0 Hz, 1H), 4.425 (d, J = 8.4 Hz, 1H); 4.612 (d, J = 8.0 Hz, 2H); 5.015 (dd, J = 14.2 Hz, 1H); 5.231 (m, J = 10.4 Hz, 1H); 5.380 (d, J = 3.2 Hz, 1H). ^{13}C NMR (400 MHz, CDCl3, δppm): 24.01, 25.42, 25.81, 26.56, 61.05, 61.30, 67.14, 68.85, 69.60, 69.78, 70.10, 70.81, 70.95, 71.17, 76.72, 77.04, 77.36, 100.72, 101.86, 108.65, 108.94, 169.26, 170.10, 170.28, 170.36. HR-ESI-MS *m/z:* 590.5782, calculated for $C_{26}H_{38}O_{15}$ $[M + NH_4]^+$ 608.6204.

*6.1.8. 1,2:4,5-di-O-(1-methyl)ethylidene-3-O-(β-D-Galactopyranosyl)-D-fructose **9***

To a mixture of compound 8(100 mg, 0.17 mmol) and sodium methylate (10.8 mg) in 10 mL of methanol was stirred at room temperature for 2 h. The solvent was removed by rotary evaporator. The residue was dissolved in dichloromethane, washed by water and saturated brine, successively. The organic layer was dried with sodium sulfate and concentrated in vacuo. The residue was purified by silica gel column chromatography (dichloromethane-methanol = 10:1, Rf = 0.45) to give compound **9** as white powder (60 mg, 85%). $[\alpha]_D^{20}$ − 65 (c = 0.01, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 1.422, 1.446, 1.472, 1.523 (s, 12H); 3.460 (s, 1H); 3.561 (d, J = 4.8 Hz, 1H); 3.781 (m, 5H); 3.932 (m, 3H); 4.117 (s, 1H); 4.247 (d, J = 8.2 Hz, 1H); 4.349 (m, J = 10.8 Hz, 2H); 4.491 (s, 1H); 4.616 (d, J = 10.2 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$, δppm): 23.92, 25.40, 25.74, 26.12, 26.47, 27.97, 29.69, 70.16, 70.81, 71.26, 71.41, 76.73, 77.05, 77.37, 102.21, 103.56, 108.77, 109.02. HR-ESI-MS *m/z:*422.4162, calculated for $C_{18}H_{30}O_{11}$ $[M + NH_4]^+$ 440.4056.

6.1.9. 2,3:4,5-di-O-(1-methyl)ethylidene-1-O-(β-D-Galactopyranosyl)-D-fructose ***12***

The procedure is the same as that for compound 9, silica gel column chromatography dichloromethane-methanol = 10:1, Rf = 0.45) to give compound **12** as white powder (65 mg, 75%, Rf = 0.55). $[\alpha]_D^{20}$ − 68 (c = 0.01, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 1.357, 1.423, 1.496, 1.543 (s, 12H); 3.663 (m, J = 14.8 Hz, 1H); 3.747 (m, 2H); 3.769 (s, 1H); 3.778 (s, 1H); 3.807 (s, 1H); 3.834 (s, 1H); 3.897 (d, J = 1.6 Hz, 1H); 3.933 (m, 2H); 4.009 (m, 1H); 4.131 (m, 1H); 4.265 (d, J = 8.6 Hz, 1H), 4.311 (d, J = 8.2 Hz, 1H); 4.622 (s, 1H); 4.646 (d, J = 7.8 Hz, 1H). ^{13}C NMR (400 MHz, CDCl3, δppm): 23.90, 25.43, 25.69, 26.46, 61.06, 62.74, 69.25, 70.00, 70.35, 70.87, 71.53, 71.77, 73.36, 75.23, 76.70, 77.02, 77.34, 102.23, 103.67, 108.61, 109.30. HR-ESI-MS *m/z:*422.4284, calculated for $C_{18}H_{30}O_{11}$ $[M + NH_4]^+$ 440.5384.

6.1.10. 3-O-β-D-Galactopyranosyl-D-fructose ***10***

To a mixture of compound 9 (100 mg, 0.17 mmol) in the solution of acetic acid (15 mL) was heated to 60 °C for overnight. TLC (dichloromethane-methanol = 2:1) showed no remaining material, the solvent was removed by rotary evaporator. The residue was dissolved in water, washed by dichloromethane. The aqueous phase was concentrated in vacuo to obtain compound **10** (62 mg, 86%). $[\alpha]_D^{20}$ − 44 (c = 0.01, H_2O). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 3.374 (m, J = 12.4 Hz, 1H); 3.498 (m, J = 10.6 Hz, 2H); 3.581 (s, 2H); 3.724 (m, J = 10.4 Hz, 5H); 3.837 (s, 1H); 3.909 (s, 1H); 3.978 (m, J = 11.0 Hz, 1H); 4.154 (m, J = 9.2 Hz, 1H); 4.364 (m, J = 8.7 Hz, 1H). ^{13}C NMR (400 MHz, CDCl3, δppm): 60.23, 61.49, 62.42, 69.32, 71.14, 72.65, 74.11, 75.49, 81.43, 92.23, 97.83, 103.52. HR-ESI-MS *m/z:*342.1097, calculated for $C_{12}H_{22}O_{11}$ $[M+2NH_4]^+$ 377.0762.

6.1.11. 1-O-β-D-Galactopyranosyl-D-fructose ***13***

The procedure is the same as that for compound 10, $[\alpha]_D^{20}$ − 42 (c = 0.01, H_2O). ^{1}H NMR (400 MHz, $CDCl_3$, δppm): 3.497 (m, J = 10.2 Hz, 1H); 3.681 (m, 3H); 3.746 (m, 3H); 3.756 (d, 2H); 3.854 (m, 1H); 4.105 (m, J = 9.4 Hz, 2H); 4.370 (m, J = 8.5 Hz, 1H). ^{13}C NMR (400 MHz, CDCl3, δppm): 61.06, 63.43, 67.77, 68.64, 68.75, 68.86, 70.71, 71.56, 72.51, 75.15, 97.58, 103.34. HR-ESI-MS *m/z:*342.1097, calculated for $C_{12}H_{22}O_{11}$ $[M+2NH_4]^+$ 377.0762.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.03.007.

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