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Chemoenzymatic Synthesis of New Aromatic Esters of Mono- and Oligosaccharides

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Abstract: An efficient and convenient chemoenzymatic route for the synthesis of novel phenolic mono-, di- and oligosaccharide esters is described. Acetal derivatives of glucose, sucrose, lactose and inulin were obtained by chemical synthesis. The fully characterized pure sugar acetals were subjected to enzymatic esterification with 3-(4-hydroxyphenyl) propionic acid (HPPA) in the presence of Novozyme 435 lipase as a biocatalyst. The aromatic esters of alkyl glycosides and glucose acetal were obtained with good esterification yields, characterized by mass spectrometry (MALDI-TOF MS), infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (^{1}H NMR, ^{13}C NMR). The synthesis of aromatic esters of disaccharide acetals was successful only for the enzymatic esterification of sucrose acetal. The new chemoenzymatic route allowed the synthesis of novel aromatic esters of inulin as the inulin monoacetal monoester and diester and the inulin diacetal monoester with a polymerization degree of two, as well as the inulin monoacetal monoester with a degree of polymerization of three, were obtained by enzymatic acylation of inulin acetals with HPPA. These compounds could represent a new class of sugar ester surfactants with enhanced bioactivity, antioxidative and antimicrobial properties and with potential application in drug delivery systems.

Keywords: sugar ester; lipases; aromatic oligosaccharides

1. Introduction

Synthetic strategies for sugar esters are needed because the isolation, profiling, characterization and quantification of naturally occurring acylated sugars, important metabolites of plants, are difficult and laborious processes that require complex protocols [1]. As chemical pathways are not environmentally friendly and require harsh reaction conditions that can lead to multiple secondary products, the use of biocatalysts could be an alternative route [2,3]. Due to their unique properties, enzymes offer effective and sustainable possibilities for utilization in the carbohydrate field. The synthetic potential of lipases has been extensively reported since, compared to most enzymes, they exhibit higher stability in aqueous and nonaqueous solvents, high enantio- and stereoselectivity and require mild reaction conditions. Depending on the reaction medium, lipases can perform hydrolysis, aminolysis, esterification and transesterification reactions, accepting a wide range of substrates including aliphatic and aromatic carboxylic acids, alcohols, sugars and sugar derivatives [4–8]. The use of immobilized lipases is

undeniably advantageous as they exhibit improved chemical, mechanical and thermal stability without affecting their enantioselectivity/stereoselectivity and reusability potential [9–11].

The first biocatalytic approach towards the esterification of sugars involved well-known medium- and long-chain fatty acids [12], but the huge potential of the enzymatic synthesis allowed the widening of the research field to many other important bioconjugates, such as aromatic esters [13]. Esters of sugars and sugar derivatives have been reported to possess great potential as surfactants [2,14] and for drug delivery formulations due to their anti-inflammatory, antiviral, antidepressant, cytotoxic, immunopotentiating, as well as antioxidant activity if holding a phenolic moiety [15,16]. The biological activity and importance of naturally occurring aromatic sugar esters are uncontested. The important pharmaceutical properties of oligosaccharide esters with phenylpropanoid groups (coumaroyl, feruloyl, caffeoyl, sinapoyl, 3,4,5-trimethoxycinnamoyl and cinnamoyl, *p*-methoxybenzoyl and *p*-hydroxybenzoyl) isolated from medicinal plants and extensively used in traditional Chinese medicine are well documented [15,17]. Good surfactant and food preservative properties of oleic acid esters of fructose, sucrose and lactose have been reported for the stabilization of the emulsion of fresh coconut milk [18]. The antimicrobial activities of sugar fatty acid esters produced by lipase-catalyzed synthesis and of chemically synthesized aromatic lactose esters have also been tested. The highest inhibition for *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enteritidis* was exhibited by mannose C10 ester and lactose biphenylacetate [19]. The antioxidant properties of sugar cane juice phenolic extracts of cinnamic, caffeic and sinapic acid were demonstrated by inhibition of spontaneous peroxidation of rat brain homogenates, suggesting the potential of such compounds for oxidative stress therapy [20]. Various structures derived from rhamnolipids and 3-(4-hydroxyphenyl) propionic acid (HPPA) were prepared by chemoenzymatic routes using glycosylation or enzymatic esterification and metathesis steps, combining the antioxidant properties of phenolic compounds and surfactant properties of sugar fatty acid esters [21]. The glycosylation of phenolic OH groups can be accomplished using glycosidases, but it is a challenging reaction. However, the progress in this field is amazing, as demonstrated by the recent synthesis of glycosyl esters of various phenolic acids, using rutin as glycosyl donor and a rutinosidase from *Aspergillus niger* as a biocatalyst [22]. Based on the unique combination of properties, the esters of sugars with phenolic acids could attract high scientific and practical interest. Apart from the well-known radical scavenging properties, phenolic acids are found ubiquitously and well documented for other health-protective effects such as antimicrobial, anti-inflammatory, antimutagenic, etc. [23].

Although the efficiency and versatility of lipases has been demonstrated for a wide range of esterification reactions, including aromatic substrates [24], the literature data on the lipase-mediated synthesis of phenolic esters of monosaccharides and oligosaccharide derivatives are very scarce. The great challenge of the lipase-catalyzed esterification of sugars with phenolic acids is the solubility of sugars in highly polar organic solvents in which lipases can be denaturated or inactivated [25,26], mainly because polar solvents can penetrate the active site of enzyme, leading to protein unfolding [27]. The possibility of tuning the lipase catalytic activity or organic solvent stability by protein engineering represents a very promising approach [28,29] but is usually targeting a specific application and the mutant enzymes are commercially unavailable. Therefore, sugar acetals and alkyl derivatives are mostly used to overcome the solubility impediment. Acetal transfer using 2,2-dimethoxypropane, 2,3-dimethoxypropene or 2-methoxypropene in the presence of an acid catalyst is frequently used to introduce an isopropylidene group [30,31]. *O*-Isopropylidenation of carbohydrates was also successfully achieved with acetone, using a macroporous acidic cation exchange resin as a heterogeneous catalyst [32].

The abundancy, biodegradability and biocompatibility of inulin, a polymer of (2–>1)-*β*-D-fructofuranan with a terminal α-D-glucopyranosyl group, G(F)n, where n = 2–20 fructose units, a natural soluble storage molecule of the chicory roots, Jerusalem artichoke, dahlias and artichokes, gives several advantages for food and medical purposes. Inulin propionate esters showed an appetite-suppressing effect by suppressing energy intake and stimulating the release of satiety hormones, thus preventing

weight gain [33]. The conjugation of inulin with some antibiotic drugs proved to be favorable for the delivery of the drug in the treatment of tuberculosis [34]. Polyesters of inulin with various carboxylic acids, obtained by chemical synthesis, demonstrated excellent properties for paint compositions as a binder [35]. The chemical synthesis of inulin esters with ferulic, cinnamic, vanillic acid with a maximum degree of substitution of 1.1 was carried out by acylation with 4-acetoxybenzenecarboxylic acids chlorides in a water-organic environment, followed by the removal of the protective acetyl group [36]. However, enzymatic synthesis of polyesters of inulin with aromatic acids has not been reported yet.

In this work, phenolic sugar esters were synthesized from sugar acetals and HPPA using an enzymatic approach. The experiments covered a wide range of sugars and sugar derivatives, including alkyl glycosides, monosaccharides and oligosaccharides. The selection of Novozyme 435 as a biocatalyst was based on a previous screening of several native and immobilized lipases, whereby Novozyme 435 exhibited the highest yield of products [8]. Sugar acetals such as 1,2-*O*-isopropylidene-D-glucofuranose, 3,4-*O*-isopropylidene-sucrose, 2,1′:4,6-di-*O*-isopropylidene-sucrose, 2,3:5,6:4,6′-tri-*O*-isopropylidene dimethyl lactose, 2,3:5,6:3′,4′-tri-*O*-isopropylidene dimethyl lactose and mono-, di- and tri-isopropylidene inulin derivatives of DP = 2–5 were synthesized by chemical acetalation. The determination of the enzymatic reaction yields and product distribution was performed using liquid chromatography, whereas the structures of aromatic sugar esters and intermediates were assessed using advanced physicochemical techniques: mass spectrometry, infrared spectroscopy and nuclear magnetic resonance spectroscopy. To the best of our knowledge, this is the first report on the chemoenzymatic synthesis of aromatic inulin esters.

2. Materials and Methods

2.1. Materials

Sucrose, lactose monohydrate, *n*-decosan, butanone, methyl *tert*-butyl ether (Across Organics), Orafti inulin (DP = 2–9, BENEO GmbH, Mannheim, Germany), acetone, ethyl acetate, anhydrous sodium carbonate and sulfuric acid 96% (Chimopar, București, Romania) were used as-purchased from the vendors. *Candida antarctica B* (Novozyme 435) and *Thermomyces lanuginosus* lipases were purchased from Novozymes A/S (Bagsværd, Denmark). 3-(4-Hydroxyphenyl) propionic acid (HPPA, 97%), *tert*-butanol (≥99%), molecular sieves (4A, 4–8 mesh), methyl-α-D-glucopyranoside, octyl-β-D-glucopyranoside, *N*,*N*-dimethylformamide, α-D-glucose, 1-octanol, 3-(4-hydroxyphenyl) propionic acid methyl ester (HPPME), ferulic acid, caffeic acid, *p*-coumaric, gallic acid, vanillic acid, ethyl ferrulate, propyl gallate, protocatechuic acid ethyl ester, *Pseudomonas fluorescence* lipase were from Sigma-Aldrich. *Candida rugosa*, *A. niger*, *Rhizopus oryzae*, pig pancreas and wheat germ lipases and dimethylsulfoxide (DMSO) were from Fluka. Acetonitrile (99.5%), hexane, *tert*-amyl alcohol, methanol, sodium hydroxide, chloroform, cyclohexane, 2,2-dimethoxypropane, and toluene-*p*-sulphonic acid monohydrate 99% were analytical reagents bought from Merck.

2.2. Enzymatic Esterification/Transesterification of 1-octanol with Aromatic Substrates

Acylations were performed in 2 mL glass vials, charged with a mixture of 1-octanol (0.05 m/mol), aromatic compound (HPPA, HPPME, ferulic acid, caffeic acid, *p*-coumaric, gallic acid, vanillic acid, ethyl ferulate, propyl gallate or protocatechuic acid ethyl ester) (0.15 m/mol), *n*-decosan as internal standard (1 mg), *tert*-butanol (1 mL) and native lipase from *C. antarctica B*, *C. rugosa*, *T. lanuginosus*, *A. niger, R. oryzae*, pig pancreas or wheat germ (10 mg). The vial containing the reaction mixture was placed at 40 °C and stirred at 250 rpm in a Memmert Modell 500 incubator (Memmert GmbH, Schwabach, Germany) with a controlled temperature using a rotator (Stuart SB2) for parallel reactions. An enzyme-free reaction (blank) was performed under the same conditions, and all reactions were carried out in duplicate. Samples were taken at different time intervals. The enzyme was removed by centrifugation (10 min, 15,300× *g*), if solid, or inactivated by boiling (10 min), if liquid. Based on the gas

chromatography analysis, the amount of synthesized ester, the ester yield, the specific transesterification activity and the total transesterification activity were calculated by the internal standard method at different reaction times (a calibration curve was set for HPPME). The specific activity was calculated using the protein content determined by the Bradford method.

2.3. Enzymatic Transesterification of Sugars Using HPPME

HPPME was added in molar ratios of 1:1, 2:1, 3:1 and 5:1 to different amounts of sugars (α-D-glucose, sucrose, lactose and inulin). The reactions were performed in various reaction media: 10% *tert*-butanol/DMSO; 10–30% water/*t*-amyl alcohol; *t*-amyl alcohol; 5–60% DMSO/*t*-butanol; phosphate buffer (pH = 7); 10% phosphate buffer/methyl *tert*-butyl ether; 10% phosphate buffer/butanone; 10%, 20%, 30% water/*tert*-amyl alcohol; buffer/hexane/*tert*-butanol mixture. The reactions were started by the addition of lipases from *P. fluorescens*, *C. antarctica B* and *T. lanuginosus* (2, 5, 8, and 10 units of activity, calculated based on the reaction between 1-octanol and the corresponding phenolic compound). The reactions were performed in a Radley parallel carousel of reactions at 50 °C. At the end of the reaction, the solid enzyme was removed by centrifugation (10 min, 15,300× *g*), and the liquid enzyme was inactivated by boiling for 10 min. The sugars were previously dried for 24 h in a vacuum oven at 50 °C, and the solvents were kept on a molecular sieve. The water content of sugars and solvents was determined by Karl Fischer titration to be less than 1%. In parallel, under the same conditions but without the enzyme, a control reaction (blank) was performed. The reactions were performed in duplicate. The reaction products were identified based on mass spectra, using the UPLC MS Waters Alliance 2695 HPLC equipment. The conversion of the phenolic compound was assessed by chromatographic analysis (Waters liquid chromatograph), using a calibration curve for HPPME.

2.4. Chemical Synthesis of Sugar Acetals

2.4.1. Synthesis of 1,2-*O*-isopropylidene-D-glucofuranose

The synthesis of 1,2-*O*-isopropylidene-D-glucofuranose was performed according to the procedure previously described by Schmidt [37]. Five grams of anhydrous α-D-glucose was stirred vigorously with 100 mL acetone in an ice bath, and 4 mL of H_2SO_4 (96%) was added in 0.5 mL amounts within 10–15 min while maintaining the temperature at 5–10 °C. After the addition of sulfuric acid, vigorous stirring was continued for 5 h, allowing the temperature to rise gradually to 20–25 °C. The solution was cooled again in an ice bath, and 50% (*w*/*v*) of NaOH solution was slowly added to obtain neutrality. A small amount of $NaHCO_3$ was added to maintain the solution near neutrality. After standing overnight, the salts were removed by filtration, and the acetone solution was concentrated under reduced pressure to a thick syrup that solidifies on standing. The mixture was dissolved in chloroform, and the solution was extracted with water. Based on TLC analysis, the chloroform solution contained the di-*O*-isopropylidene derivative, and the water phase contained the mono-*O*-isopropylidene derivative. The mono-*O*-isopropylidene derivative was crystallized from ethyl acetate (yield 23%). The di-*O*-isopropylidene derivative was recrystallized from cyclohexane (56% yield). Ten milliliters of water was added to the di-*O*-isopropylidene derivative, and the aqueous mixture was adjusted to pH 2 with 1 N HCl and heated 3 h at 40 °C with constant stirring. The hydrolysate was neutralized to pH 8 with NaOH, filtered and the filtrate was concentrated at reduced pressure to obtain the 1,2-*O*-isopropylidene-D-glucofuranose as a white solid.

2.4.2. Acetalation of Sucrose, Lactose and Inulin

A solution of sugar (3 m/mol) in *N*,*N*-dimethylformamide (40 mL) was mixed with 2,2-dimethoxypropane (4 mL) and *p*-toluene sulphonic acid (30 mg). Acetalation was carried out 4 h at 25 °C for sucrose and 48 h at 70 °C for inulin. For the acetalation of lactose, a suspension of 9.5 mmol lactose monohydrate and 25 mg *p*-toluene sulphonic acid was added to 25 mL 2,2-dimethoxypropane and refluxed for 24 h. The mixture was neutralized with sodium carbonate, filtered, concentrated by

evaporation and subjected to silica gel column chromatography. The elution used for column chromatography separation for the sucrose derivatives (mono-*O*-isopropylidene sucrose and di-*O*-isopropylidene sucrose) was performed using a 1:9 ratio of methanol/ethyl acetate. A white-yellow syrup was obtained after solvent evaporation (η = 64%); the di-isopropylidene derivative of sucrose was purified by column chromatography using a 19:1 ratio of ethyl acetate/methanol, and a white solid was obtained. For the elution of the lactose mixture, a solvent system with an ethyl acetate/hexane ratio of 6:4 was used, and two tri-isopropylidene derivatives were obtained, namely 2,3:5,6:3′, 4′-tri-*O*-isopropylidene dimethyl lactose (η = 68%) and 2,3:5,6:4′,6′-tri-*O*-isopropylidene dimethyl lactose (9% yield), in the form of white amorphous solids. A 4:1 ethyl acetate/methanol ratio was used as a solvent to separate the inulin *O*-isopropylidene derivatives from unreacted inulin (52% yield).

2.5. Enzymatic Reactions

2.5.1. Enzymatic Esterification of Alkyl Glycosides and Sugar Acetals by HPPA

Novozyme 435 (0.05 g) was added to the reaction mixture, containing 0.05 m/mol of sugar derivative (methyl-α-D-glucopyranoside, octyl-β-D-glucopyranoside, 1,2-*O*-isopropylidene-D-glucofuranose, *O*-isopropylidene derivatives of sucrose, lactose and inulin), 0.15 m/mol of HPPA, 0.03 g of 4 Å molecular sieves and 1 mL *tert*-butanol. The reactions were performed in 4 mL glass vials using an incubator (ILW 115 STD; Pol-Eko-Aparatura, Poland) equipped with an orbital shaker (MIR-S100; Sanyo, Japan) at 300 rpm and 60 °C. Samples were taken at fixed time intervals up to 72 h. The activity of Novozyme 435 for this type of aromatic substrate was previously determined as 25 U/g in the transesterification reaction of methyl-3-(4-hydroxyphenyl)-propionate with 1-octanol and in the *tert*-butanol reaction medium at 40 °C [8]. The progress of the esterification reaction was monitored by HPLC. All experiments were performed in duplicate, and the sampling was also done in duplicate. Results are given as the mean value of the four measurements. The conversions were calculated based on the consumption of the phenolic acid, and ester yields were calculated based on calibration curves of the pure compounds, isolated and purified in our laboratory. Identification of the products was accomplished by matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS) based on the similarity between the *m*/*z* values identified in the spectra and the calculated values of the appropriate esters.

2.5.2. Separation and Purification of Aromatic Sugar Esters

At the end of the reaction, the enzyme and molecular sieves were removed by filtration, *tert*-butanol was removed in vacuo and the resulting syrup was applied onto a silica gel column. The elution was carried out using ethyl acetate to isolate the aromatic ester of methyl-α-D-glucopyranoside and a 5:3 ratio of ethyl acetate/hexane for the aromatic esters of octyl-β-D-glucopyranoside and 1,2-*O*-isopropylidene-D-glucofuranose. The isolated products were obtained as white solids. The following compounds were purified using this procedure: 6-*O*-[3-(4-hydroxyphenyl)propionyl]methyl-α-D-glucoside (64.7% yield), 6-*O*-[3-(4-hydroxyphenyl) propionyl]octyl-β-D-glucoside (58% yield), and 6-*O*-[3-(4-hydroxyphenyl)propionyl]-1,2-*O*-isopropylidene-D-glucofuranose (53% yield). The purified products were used as standards for the HPLC analysis.

2.5.3. HPLC Analysis

The reaction mixtures were analyzed by HPLC (Jasco HPLC system, equipped with a PU-2089 Plus quaternary pump and a UV-2070 Plus detector) equipped with a reverse-phase Synergy 4u Hydro-RP 80A, 250 mm × 4.6 mm column (Phenomenex, Torrance, CA, USA). The compounds were eluted using an acetonitrile/water mixture gradient from 2% to 98% (17.5 min), followed by isocratic elution with 98:2 acetonitrile/water (4.5 min) at a flow rate of 0.6 mL/min, and UV detection was carried out at 276 nm.

2.5.4. UHPLC Analysis

UHPLC MS analyses were performed with a UPLC system—MS Waters Alliance 2695 HPLC, equipped with a UV detector (Waters 2996), MS detector (Waters ZQ-4000) and Waters BEH C18 column (reverse phase). The MS component was a Thermo LTQ-XL electrospray ionization–ion trap MS (ESI/MS) system. Measurements were performed in the negative ion mode. The carrier gas was helium, and the auxiliary gas was nitrogen. The capillary temperature was set at 250 °C and the spray potential at 4.7 kV. The analysis of the samples was initially performed on the entire domain m/z = 300–2000, and the MS2 analysis of the most intense ion was performed using a normalized collision energy of 35. For data acquisition and processing, the Xcalibur software package (Thermo, Waltham, MA, USA) was used.

2.5.5. Gas Chromatography (GC) Analysis

Gas chromatography analysis was performed on a Trace GC Ultra gas chromatograph (Thermo Scientific) equipped with a flame ionization detector, autosampler and Restek Rxi-5 ms column (30 m × 0.25 mm × 0.25 μm). Analysis conditions for samples were as follows: injector temperature: 275 °C; detector temperature: 300 °C; carrier gas: helium with a flow rate of 1 mL/min; temperature program: 100 (2 min)–300 °C (3 min) with a heating speed of 10 °C/min; *n*-decosan as the internal standard.

2.5.6. Thin-Layer Chromatography

The reactions were monitored over time using the thin-layer chromatography technique and alumina silica gel plates (Merck, Kenilworth, NJ, USA, DC-Autofolien Kiesegel 60 F254) with dimensions 20 × 20 cm and 0.2 mm thick. The eluents were selected according to the polarity of the substrates and reaction products. Visualization of the spots was performed by immersing the plate in an ethanolic solution of phosphomolybdic acid of a 5% concentration, followed by heating.

2.6. Structural Analysis

MALDI-TOF MS spectra were recorded on an Ultraflex Workstation, using FlexControl and FlexAnalysis software packages for acquisition and processing of the data (Bruker Daltonics, Bremen, Germany). The instrument was calibrated using a mixture of maltodextrins with known molecular masses (Avebe, Veendam, The Netherlands). One microliter of the reaction mixture was mixed with 9 μL of matrix solution (10 mg/mL 2,5-dihydroxy benzoic acid solubilized in acetonitrile: water, 1:1). Two microliters of this mixture was subsequently transferred to a target plate and dried under a stream of dry air. Measurements were performed in the positive mode. Ions were accelerated with a 25 kV voltage after a delayed extraction time of 200 ns. Detection was performed in the reflector mode. The lowest laser intensity needed to obtain a good quality spectrum was applied. NMR spectra were recorded on a Bruker Avance III spectrometer operating at 400.17 MHz (^{1}H) and 100.62 MHz (^{13}C). DMSO-d_6 (99.9% D, Aldrich, St. Louis, MO, USA) was used as a solvent. Attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectra were recorded on a Jasco FT/IR-430 spectrophotometer.

2.6.1. 6-*O*-[3-(4-hydroxyphenyl)propionyl] methyl-α-D-glucoside (**1**)

FTIR (cm^{-1}): 3348 (ν(O-H)), 2924 (ν(C-H)), 1715 (ν(C=O) ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.15 (br s, 1H, H-1′), 7.00 (d, 2H, J = 8.5 Hz, H-3′), 6.66 (d, 2H, J = 8.5 Hz, H-2′), 5.2–3.3 (multiple couplings, 10H, H-1 + H-2 + H-3 + H-4 + H-5 + H-6 + OH of carbohydrate moiety [5.12, d, 1H, 4.87, br s, 1H, 4.77, br d, 1H, 4.54, d, 1H, 4.30, d, 1H, 4.02, dd, 1H, 3.53, t, 1H, 3.46, br s, 1H, 3.40, t, 1H, 3.08, m, 1H]), 3.24 (s, 3H, CH3 of methoxy group), 2.74 (t, 2H, J = 7.5 Hz, H-4′), 2.54 (t, 2H, J = 7.5 Hz, H-5′); ^{13}C NMR (100 MHz, DMSO-d_6, δ) and proton-decoupled DEPT-135 (negative signals due to CH_2, positive signals due to CH and CH_3, $CDCl_3$, δ): 172.31 (C-7′, C=O), 155.66 (C-1′), 130.68 (C-4′),

129.17 (C-3′), 115.20 (C-2′), 99.75 (C-1), 73.34 + 71.91 + 70.52 + 69.62 (C-2 + C-3 + C-4 + C-5), 63.80 (C-6), 54.42 (C-a), 35.64 (C-6′), 29.55 (C-5′).

2.6.2. 6-*O*-[3-(4-hydroxyphenyl)propionyl] octyl-β-D-glucoside (**2**)

FTIR (cm^{-1}): 3479 (ν(O-H)), 2921 (ν(C-H)), 1719 (ν(C=O) ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.13 (br s, 1H, H-1′), 6.99 (d, 2H, J = 8.5 Hz, H-3′), 6.65 (d, 2H, J = 8.5 Hz, H-2′), 5.12 (d, 1H, J = 5.3 Hz, anomeric H), 5.00 + 4.99 (d + s, 2H, H-a + H-b), 4.30 (dd, 1H, J1 = 11.8 Hz, J2 = 2.0 Hz, H-6,7), 4.13 (d, 1H, J = 7.8 Hz, H-1), 4.02 (dd, 1H, J1 = 11.8 Hz, J2 = 7.0 Hz, H-6,7), 3.66 (dt, 1H, J1 = 9.8 Hz, J2 = 6.8 Hz, H-8″a,b), 3.42 (dt, 1H, J1 = 9.8 Hz, J2 = 6.8 Hz, H-8″a,b), 3.32 (m, 1H, H-5), 3.15 (m, 1H, H-3), 3.06 (m, 1H, H-4), 2.96 (m, 1H, H-2), 2.74 (t, 2H, J = 7.5 Hz, H-4′), 2.54 (t, 2H, J = 7.5 Hz, H-5′), 1.50 (m, 2H, H-7″), 1.22 (br m, 10H, H-6″, H-5″, H-4″ H-3″, H-2″), 0.84 (t, 3H, J = 7.0 Hz, H-1″); ^{13}C NMR (100 MHz, DMSO-d_6, δ) and proton-decoupled DEPT-135 (negative signals due to CH2, positive signals due to CH and CH_3, $CDCl_3$, δ): 172.11 (C-7′, C=O), 155.58 (C-1′), 130.48 + 128.98 + 115.04 (C-4′ + C-3′ + C-2′), 102.85 + 76.47 + 73.55 + 73.30 + 70.17 (C-1 + C-3 + C-5 + C-2 + C-4), 68.68 (C-8″), 63.73 (C-6), 35.60 + 29.53 (C-6′ + C-5′), 31.21 + 29.29 + 28.78 + 28.66 + 25.46 + 22.04 (C-7″ + C-6″ + C-5″ + C-4″ + C-3″ + C-2″), 13.89 (C-1″).

2.6.3. 6-*O*-[3-(4-hydroxyphenyl)propionyl]-1,2-*O*-isopropylidene-D-glucofuranose (**3**)

FTIR (cm^{-1}): 3400 (ν(O-H)), 2800–3000 (ν(-CH2-)), 1707 (ν(C=O) ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.14 (br s, 1H, H-1′), 7.00 (d, 2H, J = 8.5 Hz, H-3′), 6.65 (d, 2H, J = 8.5 Hz, H-2′), 5.80 (d, 1H, J = 3.5 Hz, H-1), 5.21 (br s, 1H, OH of carbohydrate moiety), 5.00 (br s, 1H, OH of carbohydrate moiety), 4.39 (d, 1H, J = 3.5 Hz, H-2), 4.21 (d, 1H, J = 9.5 Hz, H-6,7), 4.05 (br s, 1H, H-3), 3.95–3.88 (multiple couplings, 3H, H-4 + H-5 + H-6,7), 2.75 (t, 2H, J =7.5 Hz, H-4′), 2.55 (t, 2H, J = 7.5 Hz, H-5′), 1.37 + 1.23 (2x s, 2x 3H, 2x CH3 of acetal, H-8,9); ^{13}C NMR (100 MHz, DMSO-d_6, δ) and proton-decoupled DEPT-135 (negative signals due to CH_2, positive signals due to CH and CH3, CDCl3, δ): 172.24 (C-7′, C=O), 155.52 (C-1′), 130.58 (C-4′), 129.02 + 115.07 (C-3′ + C-2′), 104.47 + 84.61 + 80.20 + 72.88 + 65.18 (C-1 + C-2 + C-4 + C-3 + C-5), 66.65 (C-6), 35.67 + 29.45 (C-6′ + C-5′), 26.65 + 26.09 (C-8 + C-9).

2.6.4. 2,1:4,6-di-*O*-isopropylidene sucrose (**4**)

1. H-NMR (400 MHz, DMSO-d_6, δ): 1.21 + 1.27 + 1.37 + 1.49 (12 H, 3s, 8-CH_3, 9-CH_3, 8′-CH_3 and 9′-CH_3); 3.2–4.3 (13 H, m, sucrose protons); 6.1 (1 H, d, 1-H). ^{13}C-NMR: (δ ppm)19.3 + 29.5 + 24.5 + 25.4 (C-8 + C-9 + C-8′ + C-9′) methyl groups from acetal; 62.7 + 64.1 + 70.1 (C-1′ + C-6′ + C-6) methylene from sucrose 66.9 + 74.2 + 74.3 + 74.5 + 74.8 + 80.1 +84.1 + 91.9 (C-4 + C-3′ + C-4′ + C-2 + C-3 + C-5 + C-5′ + C-1) CH from sucrose; 99.9 + 102.1 (C-7 + C-7′) from acetal; 104.1 (C-2′).

2.6.5. 2,3: 5,6: 3′, 4′-tri-*O*-isopropylidene dimethyl lactose (**5**)

1. H-NMR (400 MHz, DMSO-d_6, δ): 1.32 (s, 3H, H-11); 1.33 (s, 3H, H-12); 1.39 (s, 6H, H1-13 and H-14); 2.00 (s, 6H, H-16 and H-17); 3.46 + 3.48 (2s, 3H, 2 x OMe); 3.62–4,58 (14H, m, lactose protons). ^{13}C-RMN (δ ppm) 107.6 + 76.39 + 78.8 + 76.78 + 78.1 + 104.6 + 74.8 + 80.8 + 74.56 + 75.34 (C-1 + C-2 + C-3 +C-4 + C-5 + C-1′ + C-2′ + C-3′ + C-4′ + C-5′) of CH from lactose; 62.58 + 65.9 (C-6 + C-6′) of -CH_2 from lactose; 25.3 + 26.5 + 26.7 + 26.8 + 27.5 + 28.5 (C-11 + C-12 + C-13 + C-14 + C-15 + C-16 + C-17) of -CH_3 from lactose 54.5 + 57.01 (C-7 + C-8) of -OCH_3; 108.8+ 110.06 + 110.11 (C-9 + C-10 + C-15) CMe2 from acetals.

3. Results and Discussion

3.1. Specificity of Native Lipases for Aromatic Acids and Esters

It is well known that substrate specificity of lipases is largely dependent on several factors including the lipase source. To develop efficient lipase-catalyzed processes, in-depth knowledge of their substrate specificity is required [38]. Therefore, prior to the enzymatic esterification of sugar

derivatives, several aromatic substrates and lipases were screened in reaction conditions resulting from preliminary experiments (data not shown).

Nine native lipases from different sources (*P. fluorescens*, *P. cepacia*, *C. antarctica* B, *C. rugosa*, *T. lanuginosus*, *A. niger*, *R. oryzae*, wheat germ lipase and pig pancreatic lipase) were tested as biocatalysts for esterification and transesterification reactions of 1-octanol with 10 aromatic acids and esters (chemical structures presented in Figure S1, Supplementary Materials) as acylating agents. The reactions were carried out in *tert*-butanol at 40 °C for 48 h, and the analyses were performed by GC chromatography.

Among the tested substrates, only methyl 3-(4-hydroxyphenyl) propionate (HPPME) and 3-(4-hydroxyphenyl) propionic acid (HPPA) resulted in the formation of the corresponding esters. Compton et al. reported the synthesis of ferulic acid octyl ester with Novozyme 435 at 60 °C in *tert*-butanol, although the yield was very low, only about 13% after 300 h of reaction [39], which makes it economically an inefficient process.

The activity of the most efficient three lipases for HPPME and HPPA esterification is shown in Figure 1. The results were expressed as specific activities (μmol/h mg protein).

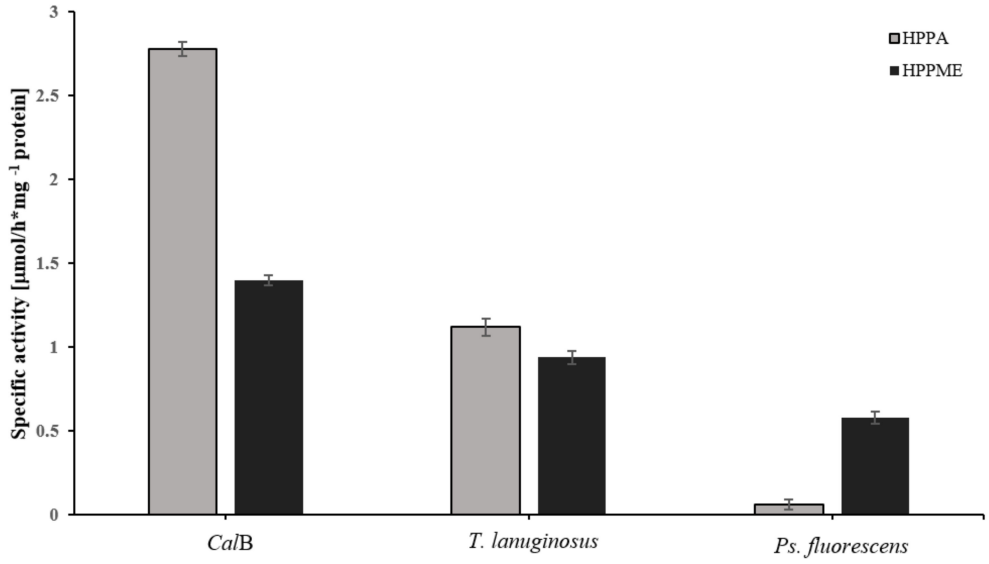


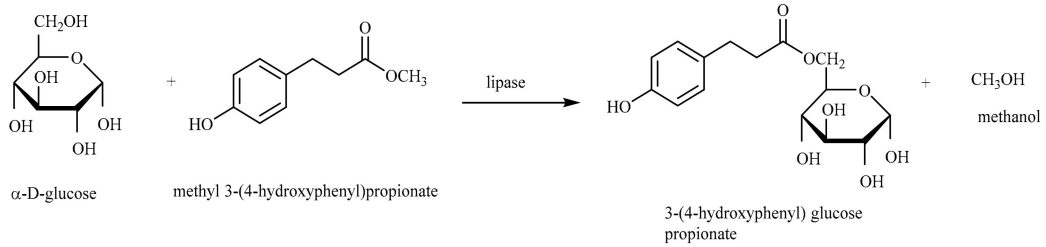
Figure 1. The esterification activities of *C. antarctica* B (*Cal*B), *T. lanuginosus* and *P. fluorescens* lipases in the esterification of 3-(4-hydroxyphenyl) propionic acid (HPPA) and transesterification of methyl 3-(4-hydroxyphenyl) propionate (HPPME) with 1-octanol after a 3 h reaction time.

Among the selected lipases (Figure 1), the most effective was from *C. antarctica* B, followed by *T. lanuginosus* lipase and the lipase from *P. fluorescens*. Regarding the substrates, the best results were obtained in the case of HPPA. Consequently, *C. antarctica* B lipase and HPPA as an acyl donor were selected for the subsequent enzymatic experiments. The screening results also revealed that the methoxy substituent and the double bond in the side chain conjugated to the aromatic ring probably affected the formation of the acyl-enzyme intermediate in the lipase active site. Enzymatic activity may be influenced in this case by electronic or steric effects. The reactivity of the carboxylic group may be affected by aromatic ring substituents with an electron donor effect, thus limiting the nucleophilic attack of alcohol. It seems that the simultaneous presence of a side chain double bond conjugated to the aromatic ring and a hydroxyl group in the *para* position has an inhibitory effect on the activity of lipases. This phenomenon is due more to an electronic effect than to a steric effect, although the steric effect cannot be ruled out. The presence of several hydroxyl groups on the benzene ring also inhibits the esterification of the acid group if the latter is conjugated to the ring, either by direct ring binding or by a double bond. The absence of conjugation and a double bond in the side chain favors

the esterification reaction. Inductive electronic effects can also alter the reactivity of the carboxylic group [40].

3.2. Specificity of Native Lipases for Aromatic Esters of Carbohydrates

The specificity of the selected native lipases for carbohydrates was determined in transesterification reactions of carbohydrates having a different chain length and monosaccharide sequence with HPPME. The native lipases from *C. antarctica* B, *T. lanuginosus* and *P. fluorescens* were selected based on the higher efficiency in the acylation reactions with HPPA and HPPME, as shown above. Kennedy et al. [4] indicated that the direct esterification of sugar catalyzed by lipases is not efficient; therefore, in our study, only HPPME was used for the transesterification of four selected sugars (α-D-glucose (Scheme 1), sucrose, lactose and inulin). The reactions were performed in 10% (*v*/*v*) DMSO/*t*-butanol, and the formation of the reaction products was evidenced by UHPLC-MS.



Scheme 1. Transesterification reaction of α-D-glucose with methyl 3- (4-hydroxyphenyl) propionate (HPPME), catalyzed by lipases.

After a 72 h reaction, time the mass spectra indicated the formation of reaction products for all tested sugars. A typical example is shown in Figure 2, where inulin was used as a sugar substrate. It can be observed that, using *P. fluorescens* lipase, monoesters of inulin oligomers with polymerization degrees of two and three (*m*/*z*: 490 and 652) were formed as the main reaction products, whereas with *T. lanuginosus* lipase, the oligosaccharide monoesters with polymerization degrees between two and five (*m*/*z*: 490, 652, 814 and 976) were detected. For glucose, sucrose and inulin the results were slightly evident under the given conditions compared to lactose (Table S1, Supplementary Materials).

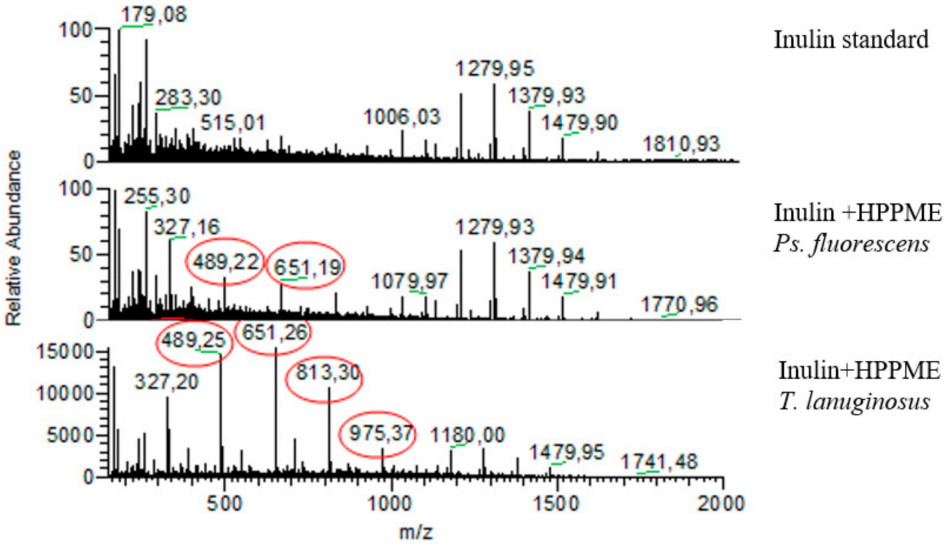


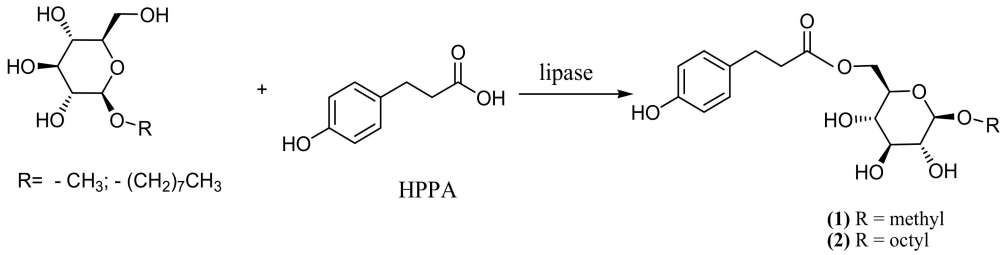
Figure 2. UHPLC MS spectra for inulin transesterification reactions with HPPME in 10% (*v*/*v*) DMSO/*tert*-butanol, catalyzed by lipases, at a 72 h reaction time. Red circles indicate the reaction products identified.

Even though the formation of reaction products was demonstrated, the quantitative analysis results revealed very low conversion yields, although different reaction conditions were studied (different reaction media, different sugar concentrations, different biocatalyst concentrations and different molar ratios, data not shown). As shown in Table S1, the conversion yields obtained after 72 h did not exceed 6%, most probably due to the very low solubility of the oligosaccharides in the organic solvents used, as we previously reported [41].

Therefore, we targeted increasing the solubility of the carbohydrate substrates in the reaction medium by introducing easily removable hydrophobic groups. Chemical derivatization of sugars with hydrophobic protecting groups and subsequent enzymatic esterification of intermediates, followed by removal of the protecting groups in acid medium, is a chemoenzymatic method described in the literature to obtain aliphatic esters of sugars [42–44] and may be an alternative also for the synthesis of aromatic esters of sugars. Two different approaches have been used, using (a) alkyl glycosides and (b) glycoside acetals as substrates. The reaction temperature was set at 60 °C to achieve a reasonable yield without affecting the stability of sugars and to achieve complete solubility of the substrates.

3.3. Enzymatic Synthesis and Characterization of Aromatic Esters of Alkyl Glycosides

Two alkyl glucosides, α-methyl-glucose and β-octyl glucose (Scheme 2) were subjected to the esterification reaction with HPPA in the presence of Novozyme 435 lipase, and aromatic esters with yields higher than 90% were obtained after 120 h of reaction. Samples were collected at different time intervals. As an example, after 72 h, the substrate conversions were 63% for α-methyl glucose and 77% for β-octyl glucose. The solubility of alkyl glycosides in the reaction medium (*t*-butanol) is higher compared to glucose due to the presence of hydrophobic alkyl groups in the molecule, so their accessibility in the catalytic active site of the lipase is favored.



Scheme 2. Reaction scheme for enzymatic synthesis of 6-*O*-[3-(4-hydroxyphenyl)propionyl] methyl-α-D-glucoside (1), and 6-*O*-[3-(4-hydroxyphenyl)propionyl] octyl-β-D-glucoside (2).

The reaction products were purified by column chromatography and their structures were demonstrated by mass spectrometry (MALDI-TOF MS) FTIR and NMR spectroscopy. In the MALDI-TOF MS spectra of the reaction mixture (Figure S2, Supplementary Materials), the formation of 6-*O*-[3-(4-hydroxyphenyl)propionyl] methyl-α-D-glucoside was demonstrated by the presence of its sodium adduct at *m*/*z* 364.86. The formation of 6-*O*-[3-(4-hydroxyphenyl)propionyl] octyl-β-D-glucoside was confirmed by the presence of its sodium adduct at *m*/*z* 463.01 (Figure S3, Supplementary Materials). The characteristic bands from 1715 and 1719 cm^{-1} from the FTIR spectra (Figures S4 and S5, Supplementary Materials) confirm the presence of the ester bond together with the characteristic bands of alkylglucoside at 2924 cm^{-1}, 2921 cm^{-1} and phenolic OH groups at 3300–3400 cm^{-1}, respectively.

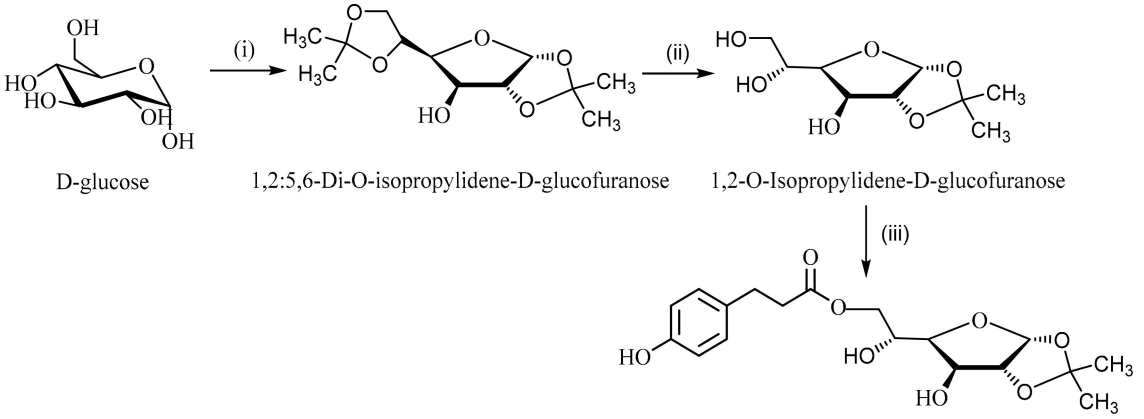
The α-methylglucose monosubstitution at the OH-6 group was confirmed by the ^{1}H-NMR, ^{13}C-NMR, 135 DEPT spectra, where six signals for the alkylglucoside, seven signals for the acyl residue containing the aromatic ring and one signal for the group methoxy were present (Figures S6–S8, Supplementary Materials). Based on ^{1}H, ^{13}C 135DEPT NMR spectra (Figure S9–S11, Supplementary Materials), the structure of β-octyl-glucose 6-*O*-3-(4-hydroxyphenyl)-propionate was confirmed. For example, the ^{13}C-NMR spectra show 14 signals for alkylglucoside and 7 signals for the remaining

acyl containing the aromatic nucleus. The complete signal assignment is shown in the materials and methods section.

Based on the NMR spectra, it was demonstrated that the reactions occurred with high regioselectivity, exclusively at the primary hydroxyl groups of the tested alkylglucosides. These results are in concordance with previously reported results, where the regioselective esterification of sugar derivatives was demonstrated [4].

3.4. Chemoenzymatic Synthesis and Characterization of 6-O-[3-(4-hydroxyphenyl)propionyl]-1,2-O-isopropylidene-D-glucofuranose

Instead of using alkyd glycosides, the enzymatic synthesis of aromatic esters of D-glucose was evaluated using 1,2-*O*-isopropylidene-D-glucofuranose as a substrate. The substrate was chemically synthesized and characterized in detail by different spectroscopic techniques. The chemoenzymatic synthesis strategy (Scheme 3) starts from α-D-glucose and involves diacetalation in acid catalysis (conc. H_2SO_4) to obtain the di-isopropylidene derivative of glucose. The next step, hydrolysis in acid medium followed by neutralization, led to the selective deprotection of the hydroxyl groups at Positions 5 and 6, obtaining the monoisopropylidene derivative of glucose with a 56% yield. The primary hydroxyl group at Position 6 was thus available for esterification with HPPA in *tert*-butanol catalyzed by Novozyme 435 to give the aromatic ester of the glucose monoacetal. The conversion values determined by HPLC were 92% after 120 h, comparable with the values obtained for the alkyl glycoside substrates.



Scheme 3. Reaction scheme for chemoenzymatic synthesis of 6-*O*-[3-(4-hydroxyphenyl) propionyl]-1,2-*O*-isopropylidene-D-glucofuranose (**3**). (i) Acetone, H_2SO_4, 0 °C–25 °C, 5 h; (ii) HCl 1N, 3 h, 40 °C, NaOH 50%; (iii) HPPA, 60 °C, 120 h, Novozyme 435.

The ^{13}C-NMR, ^{1}H-NMR and 135 DEPT spectra confirmed the structure of 1,2-*O*-isopropylidene glucofuranose (Figures S12–S14, Supplementary Materials). The FTIR spectra revealed a considerable decrease of the intensity of the band corresponding to the hydroxyl groups (at about 3400 cm^{-1}) following their isopropylidenation in Positions 1,2 and 5,6, simultaneously with the intensification of the characteristic signals of the etheric bond at 1377 cm^{-1} (Figure S15, Supplementary Materials).

The FTIR spectra of the product (3) confirms the structure of the aromatic ester of monoacetonglucose (Figure S16, Supplementary Materials). The significant bands correspond to the stretching vibrations of the hydroxyl groups in the glucose molecule and HPPA (at approx. 3400 cm^{-1}), the methylene groups (between 2800 and 3000 cm^{-1}) and the ester carbonyl group (1707 cm^{-1}).

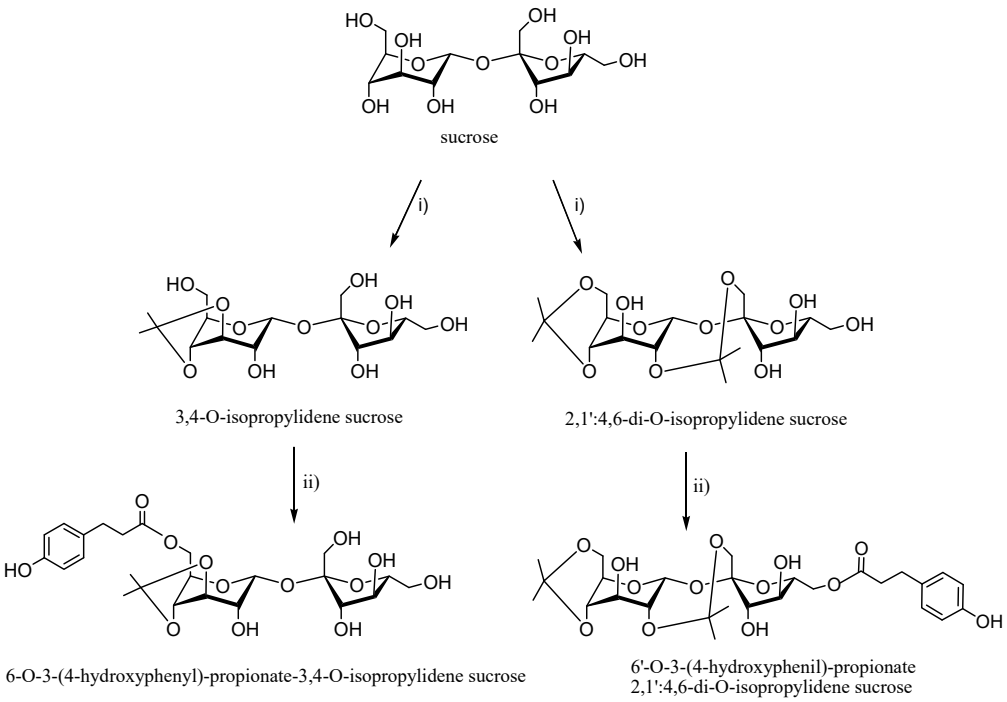
The formation of the monoester of glucose acetal (3) was also demonstrated by the presence of a peak at *m*/*z* 390.91 in the MALDI-TOF MS spectra (Figure S17, Supplementary Materials). Based on the ^{1}H-NMR, ^{13}C-NMR and 135-DEPT (Figures S18–S20, Supplementary Materials), the structure was confirmed. In the ^{13}C-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl]-

1,2-*O*-isopropylidene-ɒ-glucofuranose, seven signals for the acyl residue containing the aromatic ring, six signals for the carbohydrate and two signals for the isopropylidene group are present. The complete signal assignments are presented in the materials and method section.

3.5. Chemoenzymatic Synthesis and Characterization of Aromatic Esters of Disaccharides

Two disaccharides were investigated in this study: sucrose and lactose. Sucrose was derivatized by isopropylidination in acid catalysis (*p*-toluenesulfonic acid) with 2,2-dimethoxypropane, and a mixture of its monoisopropylidene and di-isopropylidene derivatives (2,1′:4,6-di-*O*-izopropylidensucrose) was obtained. The total conversion of sucrose in this step was 64%. In the second step, the mixture of isopropylidene derivatives of sucrose was subjected to enzymatic esterification with HPPA in the presence of Novozyme 435 in *tert*-butanol to yield the aromatic monoester of mono-*O*-isopropylidene sucrose, together with 2,1′-6,1-*O*-3- (4-hydroxyphenyl)-propionate: 4,6-di-*O*-isopropylidiene sucrose. The di-isopropylidene derivative of sucrose was purified by column chromatography and characterized by FTIR and NMR spectroscopy. Based on the NMR spectra, the diacetalation of sucrose occurred at the hydroxyl groups at Positions 2, 1′ and 4, 6. The ^{13}C-NMR spectrum of the di-isopropylidene derivative of sucrose shows 12 signals for sucrose and 6 signals for the two isopropylidene residues (details in the materials section and Figures S12–S14, Supplementary Materials).

The total conversion of the enzymatic esterification step, determined by HPLC based on HPPA consumption, was 27% after 96 h. The structure of the monoisopropylidene derivative of sucrose could not be confirmed by NMR spectroscopy, as the column chromatography purification of this compound was not successful. Given the structure of sucrose, acetalization could occur in either Positions 3,4 or 4,6 and the enzymatic esterification most likely took place at the primary hydroxyl group at Positions 6 or 6′. A proposed reaction scheme is shown in Scheme 4.



Scheme 4. Proposed reaction scheme for the chemoenzymatic synthesis of HPPA esters of sucrose acetals (i) 2,2-dimethoxypropane, DMF, *p*-toluene sulphonic acid, 4 h, 25 °C; (ii) HPPA, 60 °C, 120 h, Novozyme 435.

MALDI-TOF MS was used for the identification of reaction intermediates and end products. In the MALDI-TOF MS spectrum (Figure 3), the *m*/*z* values 404.90 and 444.95 represent the Na^+ adducts of

the sucrose of mono- and diacetal, respectively. The formation of the aromatic monoesters of mono- and diacetal was confirmed by the peaks *m*/*z* 553.03 and 593.08 (also as Na^+ adducts), respectively.

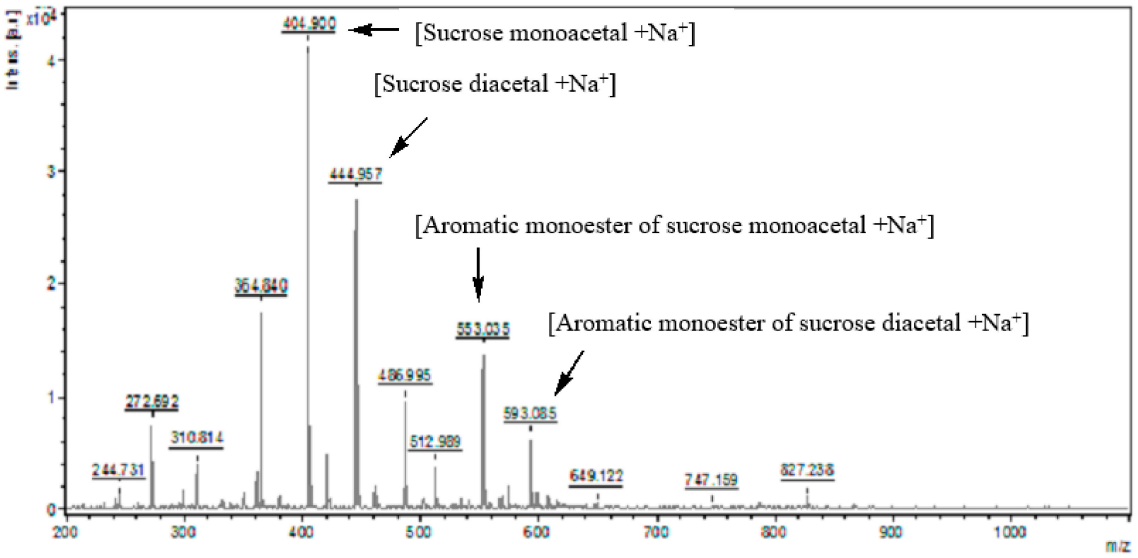


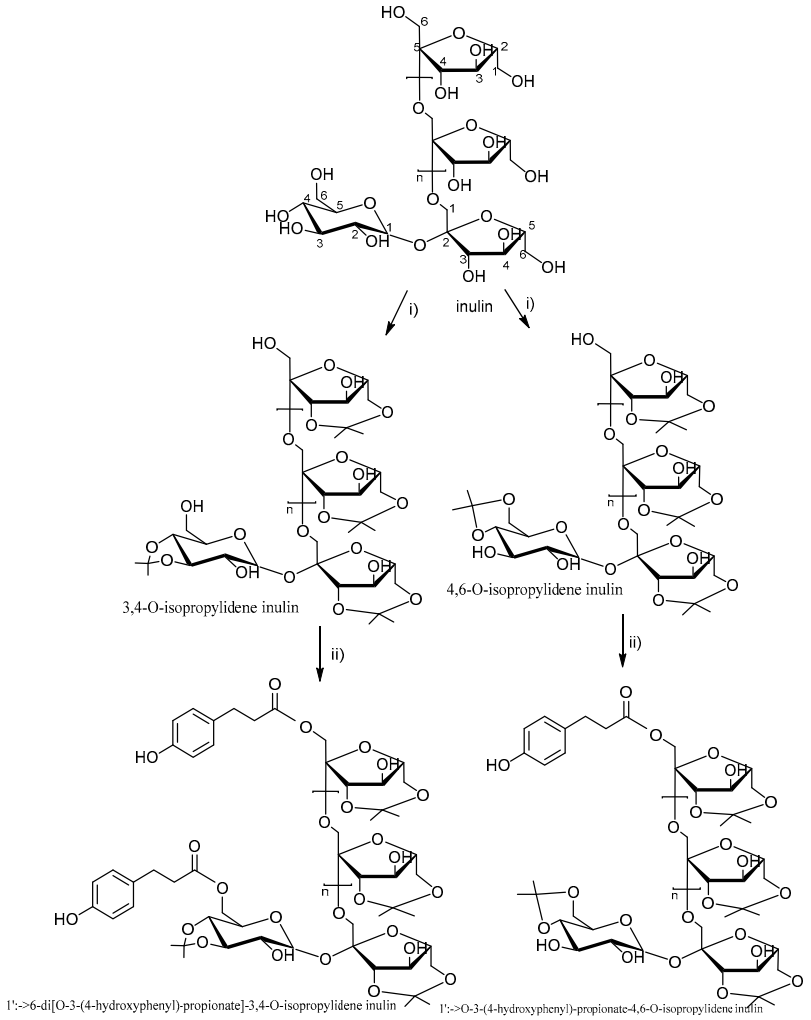
Figure 3. MALDI-TOF MS spectra of the reaction mixture from acylation of monoisopropylidensucrose and 2,1′,4,6-di-*O*-izopropiliden sucrose with HPPA in the presence of Novozyme 435, after 72 h.

A similar chemical approach was used for lactose. First, the derivatization by isopropylidenation was accomplished in acidic medium (*p*-toluenesulfonic acid) with 2,2-dimethoxypropane at reflux temperature to obtain a mixture of two tri-isopropylidene derivatives, namely 2,3: 5,6: 4′,6′-tri-*O*-isopropylidene dimethyl lactose and 2,3: 5,6: 3′, 4′-tri-*O*-isopropylidene dimethyl lactose (Scheme S1, Supplementary Materials). The resulted compounds were separated and purified by column chromatography, and the 2,3: 5,6: 3′,4′-tri-*O*-isopropylidene dimethyl lactose was characterized by NMR spectroscopy (assignment presented in the materials and methods section and spectra in Figures S25–S28, Supplementary Materials). Finally, the 2,3: 5,6: 3′,4′-tri-*O*-isopropylidene dimethyl lactose compound was subjected to enzymatic esterification with HPPA in *tert*-butanol at 60 °C in the presence of Novozyme 435. The enzymatic esterification reaction was monitored by thin-layer chromatography, HPLC and MALDI-TOF MS. The presence of the peak at *m*/*z* 531.07 confirmed the presence of the lactose triacetal (Na^+ adduct) in the reaction mixture (Figure S28, Supplementary Materials). However, the absence of other specific peaks means that the enzymatic esterification of the primary hydroxyl group of the tri-*O*-isopropylidene derivative did not occur. This can be due to the steric hindrance by the presence of the acetal group at Positions 3′, 4′ and the presence of the bulky phenyl group in the acylation reagent.

3.6. Synthesis and Characterization of Aromatic Esters of Inulin

The envisaged reaction pathway started from inulin (mixture of oligosaccharides with DP = 2–7, the most abundant being those with DP = 3, 4 and 5) and involved its isopropylidation in acid catalysis (*p*-toluenesulfonic acid) with 2,2-dimethoxypropane to give a mixture of isopropylidene derivatives of inulin. The crude mixture was further subjected to enzymatic esterification with HPPA in *tert*-butanol in the presence of Novozyme 435. The acetalization reaction of the inulin was monitored over time by thin-layer chromatography, and the enzymatic esterification reaction of the inulin acetals was monitored by HPLC. The total conversion of the acetalization stage of inulin was 52%, whereas the enzymatic esterification yield was 24% after 96 h of reaction, determined by HPLC analysis based on HPPA consumption. The chemical structures of inulin and inulin derivatives are shown for DP = 4 (as an example) in Scheme 5, considering the possibilities of inulin acetalization and esterification of

inulin acetals. To the best of our knowledge, these inulin derivatives were not previously described in the literature.



Scheme 5. Reaction scheme for chemoenzymatic synthesis of inulin-based aromatic esters (DP = 4). (i) 2,2-dimethoxypropane, DMF, *p*-toluene sulphonic acid, 70 °C 48 h; (ii) HPPA, 60 °C, 120 h, Novozyme 435.

The formation of mono-, di- and tri-isopropylidene derivatives of inulin at DP = 2–5, as well as of the mono- and aromatic diester of inulin monoacetal at polymerization degree two and the aromatic monoester of inulin monoacetal at polymerization degree three were demonstrated by MALDI-TOF MS spectrometry (Figures 4 and 5).

In the MALDI-TOF MS spectrum of the acetalization reaction mixture (Figure 4), the *m*/*z* values corresponding to the sodium adducts of inulin acetals, at different degrees of polymerization, were identified. For example, the peak from *m*/*z* 405 corresponds to the monoacetal sodium adduct with polymerization degree two, the peak from *m*/*z* 607 corresponds to the diacetal sodium adduct with DP 3 and the peak from *m*/*z* 809 correspond to the sodium adduct of the triacetal with DP 4.

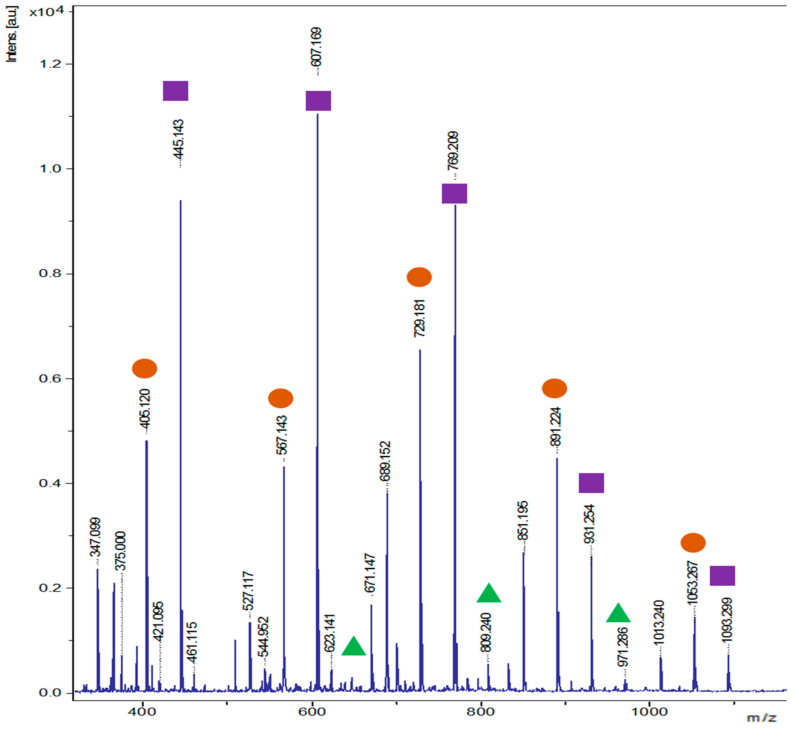


Figure 4. MALDI-TOF MS spectrum of the acetalation product of inulin. The identified acetals (sodium adducts) are marked with orange circles (monoacetals), purple rectangles (diacetals) (purple) and green triangles (triacetals).

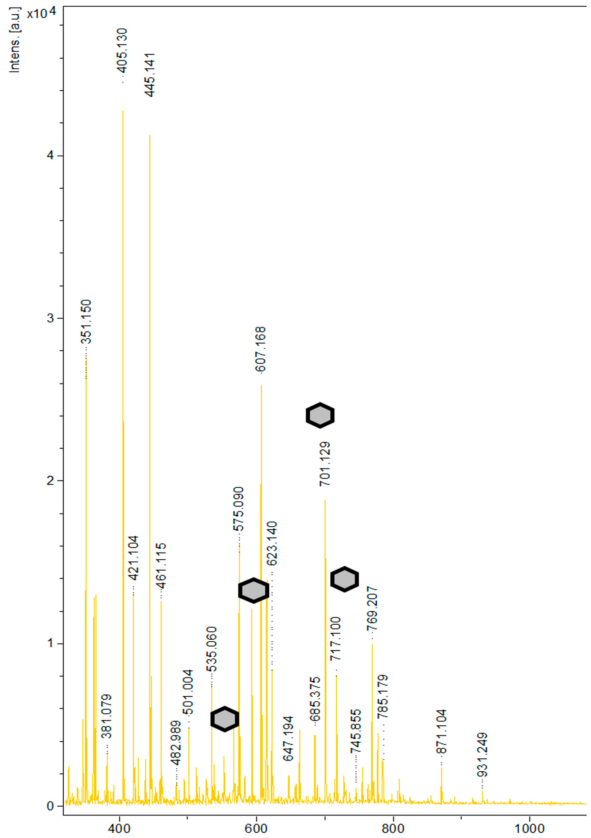


Figure 5. MALDI-TOF MS spectrum of the reaction mixture from the acylation of inulin acetals with HPPA, catalyzed by Novozyme 435, after 72 h of reaction. Identified acetals (sodium adducts) of aromatic esters formed with isopropylidene derivatives of inulin are marked with hexagons.

In the MALDI-TOF MS spectrum of the reaction mixture from the enzymatic acylation of inulin acetals with HPPA (Figure 5), the *m*/*z* values corresponding to the sodium adducts of aromatic esters formed with isopropylidene derivatives of inulin were identified. At polymerization degree two, the sodium adduct of monoester and aromatic diester with monoacetal were identified (*m*/*z* = 553 and *m*/*z* = 701), aromatic monoester with diacetal (with *m*/*z* = 593), whereas at degree of polymerization three, the sodium adduct of the aromatic monoester with the monoacetal was identified (*m*/*z* = 717). It is for the first time that the enzymatic synthesis of phenylcarboxylic esters of short-chain inulin oligosaccharides using lipase as a catalyst has been demonstrated. The inulin esters of phenylcarboxylic acids represent a new class of biobased bioactive compounds, with strong antioxidative and antimicrobial properties imparted by the phenyl moieties and potential application in the food and pharmaceutical industry.

4. Conclusions

The chemoenzymatic synthesis of five new aromatic sugar esters was demonstrated. The presence of alkyl and isopropylidene groups in the structure of sugars led to an increase of their solubility in the reaction medium and higher accessibility to the lipase active site. Higher esterification yields were achieved when glucose monoesters were used as substrates. The derivatization of sugars with isopropylidene groups took place with yields exceeding 50%, and the primary hydroxyl groups (from Position 6 in the case of glucose and Position 6′ in the case of lactose and sucrose) remained unprotected for enzymatic esterification. Based on NMR spectroscopy analysis, acylation with the aromatic acid occurred only at the primary hydroxyl groups. When sucrose and inulin were used as substrates, the synthesis of acetals and aromatic esters was demonstrated based on MALDI-TOF MS analysis. The formation of aromatic esters of inulin mono- and diacetals at a degree of polymerization of two and the aromatic ester of inulin monoacetal at a degree of polymerization of three demonstrates the feasibility of this chemoenzymatic system.

In the perspective of green synthesis pathways for novel oligomeric sugar esters, this work is a feasible proof of concept for the regioselective esterification of sugars and could open the way to explore this route for several other substrates.

Supplementary Materials: The following are available online at http://www.mdpi.com/2227-9717/8/12/1638/s1, Figure S1. Chemical structures of aromatic compounds tested as substrates for lipases; Figure S2. MALDI-TOF MS spectrum of the reaction mixture from α-methyl-glucose acylation with HPPA catalyzed by Novozyme 435, after 72 h, containing the 6-*O*-[3-(4-hydroxyphenyl)propionyl] methyl-α-D-glucoside product; Figure S3. MALDI-TOF MS spectrum of the reaction mixture from α-methyl-glucose acylation with HPPA catalyzed by Novozyme 435, after 72 h., containing 6-*O*-[3-(4-hydroxyphenyl) propionyl] octyl-β-D-glucoside (2), after 72 h; Figure S4. FTIR spectra of α-methyl-glucose (a); HPPA (b) and 6-*O*-[3-(4-hydroxyphenyl) propionyl] methyl-α-D-glucoside (c). Insert is a zoomed-in version of the spectrum at wavenumber 2000–600 cm^{-1}; Figure S5. FTIR spectra of β-octyl-glucose (a); HPPA (b) and 6-*O*-[3-(4-hydroxyphenyl)propionyl] octyl-β-D-glucoside (c). Insert is a zoomed-in version of the spectrum at wavenumber 2000–600 cm^{-1}; Figure S6. 1H-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl) propionyl] methyl-α-D-glucoside (collected in DMSO-d_6); Figure S7. ^{13}C-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl) propionyl] methyl-α-D-glucoside (collected in DMSO-d_6); Figure S8. 135DEPT-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl] methyl-α-D-glucoside (collected in DMSO-d_6); Figure S9. ^{1}H-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl] octyl-β-D-glucoside (2); Figure S10. ^{13}C-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl] octyl-β-D-glucoside (2); Figure S11. 135DEPT-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl] octyl-β-D-glucoside (2); Figure S12. ^{1}H-NMR spectrum of 1,2-*O*-isopropylidene-glucofuranose; Figure S13. ^{13}C-NMR spectrum of 1,2-*O*-isopropylidene-glucofuranose; Figure S14. 135DEPT-NMR spectrum of 1,2-*O*-isopropylidene-glucofuranose; Figure S15. FTIR spectra of glucose (a); 1,2-*O*-isopropylideneglucose (b) 1,2:5,6-di-*O*-isopropylideneglucose (c). Insert is a zoomed-in version of the spectrum at wavenumber 2000–600 cm^{-1}; Figure S16. FTIR spectra of D-glucose (a), isopropylidene-D-glucose (b), 6-*O*-[3-(4-hydroxyphenyl)propionyl]-1,2-*O*-isopropylidene-D-glucofuranose (3). Insert is a zoomed-in version of the spectrum at wavenumber 2000–600 cm^{-1}. Figure S17. MALDI-TOF MS spectrum of the reaction mixture after acylation of 1,2-*O*-isopropylidene sucrose with HPPA, catalyzed by Novozyme 435 after 72 h. Figure S18. ^{13}C-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl]-1,2-*O*-isopropylidene-D-glucofuranose. Figure S19. ^{1}H-NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl]-1,2-*O*-isopropylidene-D-glucofuranose. Figure S20. 135DEPT NMR spectrum of 6-*O*-[3-(4-hydroxyphenyl)propionyl]-1,2-*O*-isopropylidene-D-glucofuranose. Figure S21. FTIR spectra of (a) sucrose; (b) 2,1′:4,6-di-*O*-isopropylidene sucrose. Figure S22. ^{13}C-NMR spectrum of 2,1′:4,6-di-*O*-isopropylidene sucrose. Figure S23. 1H-NMR spectrum of 2,1′:4,6-di-*O*-isopropylidene sucrose.

Figure S24. 135 DEPT NMR spectrum of 2,1′:4,6-di-*O*-isopropylidene sucrose. Figure S25. 13C-NMR spectrum of 2,3:5,6:3′,4′-tri-*O*-isopropylidene dimethyl lactose. Figure S26. ^{1}H-NMR spectrum of 2,3:5,6:3′,4′-tri-*O*-isopropylidene dimethyl lactose. Figure S27. 135DEPT NMR spectrum of 2,3:5,6:3′,4′-tri-*O*-isopropylidene dimethyl lactose. Figure S28. MALDI-TOF MS spectrum of the reaction mixture after esterification of 2,3:5,6:3′, 4′-tri-*O*-isopropylidene dimethyl lactose with HPPA, in tert-butanol at 60 °C in the presence of Novozyme 435. Scheme S1. Reaction scheme of 2,3: 5,6: 4′, 6′-tri-*O*-isopropylidene dimethyl lactose and 2,3: 5,6: 3′, 4′-tri-*O*-isopropylidene dimethyl lactose synthesis by acetalization of lactose with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid, at reflux, 24 h. Table S1. Transesterification reaction conversions of α-D-glucose, sucrose, lactose, inulin with methyl 3-(4-hydroxyphenyl) propionate (HPPME) catalyzed by native lipases after 72 h of reaction.

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