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DESIGN, SYNTHESIS AND BIOLOGICAL STUDIES OF SUGAR-BASED ESTER SURFACTANTS

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Coordinatore: Ch.mo Prof. Alessandro Bogliolo

Supervisore: Ch.mo Prof. Simone Lucarini

Co-Supervisore: Ch.mo Prof. Andrea Duranti

Dottorando: Michele Verboni

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List of Abbreviations

δ	Chemical shift
Ac	Acetyl
ACN	Acetonitrile
AmB	Amphotericin B
APC	Antigen presenting cell
Ar	Aryl
AS	Anionic surfactants
ASGPR	Asialoglycoprotein receptors
Bn	Benzyl
B.p.	Boiling point
[Bmim][TfO]	1-*n*-butyl-3-methylimidazolium trifluoromethane sulfonate
[Bmim][Tf_2N]	1-Butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide
CAPE	Caffeic acid phenethyl ester
Cbz	Carbobenzyloxy
CMC	Critical Micellar Concentration
COX	Cyclooxigenase
CS	Cationic surfactants
DBU	1,8-Diazabicyclo [5.4.0] undec-7-ene
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DCF	2′,7′-dichlorofluorescein
DIAD	Diisopropyl azodicarboxylate
DIPEA	*N*,*N*′-Diisoproylethylamine
DLS	Dynamic Light Scattering
DMAP	4-Dimethylaminopyridine
DMEs	Dimannopyranose ester
DMF	*N*,*N*′-Dimethylformamide

DMSO	Dimethyl sulfoxide
Dppf	1,1′-Bis(diphenylphosphino) ferrocene
DTA/DSC	Differential Thermal/scanning analyses
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EDG	Electron-donating group
EtOAc	Ethyl acetate
EWG	Electron-withdrawing group
FD-4	Fluorescein isothiocyanate-labelled dextran 4 kDa
FITC-OVA	Fluorescein Isothiocyanate Ovalbumin
HaCaT	Immortalized human keratinocyte
HLB	Hydrophilic Lipophilic Balance
HMBC	Heteronuclear Multiple Bond Correlation
HPPA	3-(4-hydroxyphenyl) propionic acid
ICAM	Intercellular Adhesion Molecule
IL	Ionic liquid
iNOS	Inducible Nitric Oxide Synthase
LA	Lewis Acid
LBE	Lactose-based ester
LDH	Lactate dehydrogenase
LGs	Leaving group
logP	Octanol–water portion coefficient
LOX	Lipoxigenase
LR	Lectin receptor
LPS	Lipopolysaccharides
LTA	Lactose Tetra Acetal
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
2M2B	2-Methyl-2-butanol

MBE	Mannose-based Ester
MR	Mannose receptor
MS	Molecular Sieves
MS (ESI)	Electrospray ionization mass
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide
MW	Molecular Weight
NIS	Non-ionic surfactants
NO	Nitric Oxide
NP	Nanoparticle
NSAID	Non-Steroid Anti-Inflammatory Drug
P_{app}	Permeability apparent coefficient
p-TSA	*para*-toluenesulfonic acid
PA	Palmitic acid
PE	Permeability enhancer
PEG	Polyethylene glycol
PG	Protecting group
PKC	Protein Kinase C
py	Pyridine
RhL	Rhamnolipid
SBE	Sucrose-based ester
SBFAE	Sugar-based fatty acid ester
SCAED	Sugar cinnamic acid ester derivative
SDS	Sodium dodecyl sulphate
SDBS	Sodium dodecyl benzene sulfonate
SEM	Scanning Electron Microscope
SRB	Sulforhodamine B
t-BuOH	*tert*-butanol

TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TCA	Trichloroacetimidate
TEA	Triethylamine
TEER	Trans Epithelial Electrical Resistance
TEM	Trasmission Electron Microscope
TFA	Trifluoroacetic acid
TGA	Thermogravimetry analysis
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TME	Trimannopyranose ester
TMSOTf	Trimethylsilyl triflate
TPSA	Topological Polar Surface Area,
TJ	Tight junction
TNF-α	Tumor necrosis factor alfa
UA	Undecylenic acid
ZS	Zwitterionic surfactants

1. General Introduction

1.1. Surfactants

Surfactants are amphiphilic molecules constituted by a hydrophilic moiety and a lipophilic chain. Due to this characteristic, surfactant molecules orient at the interface of systems formed by two non-miscible phases (air-liquid, liquid-liquid and solid-liquid). Hence, surfactants low the interface tension avoiding aggregation and coalescence. Thus, they are utilized as wetting agents, stabilizers of emulsions and foams, detergents, and disinfectants [1]. The disposition of the surfactants on the surface of water depends on the concentration. When the surface is saturated by surfactant molecules, spherical structures called micelles are formed in which the hydrophilic heads are placed externally in contact with water and the lipophilic chains are inside of the micelles. This supramolecular aggregation is favoured when the CMC is reached. The CMC value is specific to each surfactant and depends on its structural characteristics (e.g., type of hydrophilic head, length, and ramifications of the lipophilic tail), on external factors such as temperature and on the presence of co-solvents and other substances (e.g., electrolytes) in solution [2]. The HLB is a parameter that measures the degree of hydrophilicity or lipophilicity of a surfactant. It is commonly calculated with the Griffin method [3]. The value ranges from 0 (completely hydrophobic) to 20 (completely hydrophilic) and determinates the application of surfactants (Table 1).

Table 1. HLB values and correlated surfactant applications.

HLB	Application
2-3	Antifoaming agents
3-6	w/o emulsifier
7-9	Wetting agents
8-16	o/w emulsifier
13-15	Detergents
15-18	Solubilizing agents

Surfactants are classified into four groups based on the ionic properties of the hydrophilic portions: anionic, cationic, zwitterionic and non-ionic surfactants (Table

2) [4]. Anionic surfactants (AS) are characterized by a negative charge, usually represented by carboxylates, sulphates, or sulfonate. Their use as detergents is declined because they are more aggressive and irritating than other classes of surfactants. However, AS are relevant in the pharmaceutical field because of their bacteriostatic action against Gram-positive bacteria. Cationic surfactants (CS) have positively charged heads, typically constituted by quaternary ammonium salts. CS are employed for topic use, especially for cleaning wounds since they have broad-spectrum bactericidal activity against Gram-positive and a thinner spectrum against Gram-negative. Zwitterionic surfactants (ZS) have the hydrophilic portion containing groups that can be charged positively and/or negatively in relation to the pH of the solution. Some examples are phospholipids, phosphatidylcholine, and sphingomyelins. ZS have a good detergent, emulsifier, and bactericidal activity. Moreover, they are widely used in cosmetic and personal care products due to their non-irritating action on the skin. Finally, non-ionic surfactants (NIS) are molecules that have no charge on the hydrophilic polar head. They are insensitive to pH variations and to the presence of electrolytes. NIS are excellent detergents; emulsifying agents and they are well tolerated by the skin. Additionally, NIS are subclassed into lipophilic (e.g., ethoxylated alcohols and sorbitan esters or Span®), and hydrophilic (e.g., PEG and polysorbates or Tween®).

Table 2. Structure and classification of surfactants.

Classes	Structures	Examples
Anionic		SDS, Sodium stearate, SDBS
Cationic		Laurylamine hydrochloride, Trimethyl dodecylammonium chloride Cetyl trimethylammonium bromide
Zwitterionic		Dodecyl betaine, Lauramidopropyl betaine Cocoamido-2-hydroxypropyl sulfobetaine
Non-ionic		Span®, Tween®, Glycolipids

Lipophilic non-ionic surfactants are mainly used as antifoaming agents, wetting agents and water-in-oil emulsifiers, while the hydrophilic ones are mainly used as oil-in-water solubilizing and emulsifying agents. Finally, surfactants could be also divided into

petrochemical or bio-based depending on the origin production. Due to their properties and favourable characteristics biosurfactants will be described.

1.2. Biosurfactants

Research on biosurfactants has exponential increased in last ten years [5]. Biosurfactants of microbial origin represent a great alternative to their chemical counterpart [6]. In fact, they are characterized by low toxicity, both high biodegradability and biocompatibility and present other advantages such as low CMC, greater thermostability and tolerance in severe extreme conditions [7]. Biosurfactants are naturally produced directly by microbial metabolic processes through various microorganisms such as bacteria, fungi, and yeast. Moreover, they are obtainable at low costs from natural renewable cheap resources (e.g., sugars, fatty acids, amino acids, peptides, polyalcohol) [8]. Biosurfactants are widely employed in different sectors (pharmaceuticals, industrial, environmental, agriculture) thanks to their numerous properties. The biosurfactants can be classified into high molecular weight biosurfactants and low molecular weight biosurfactants [9]. The first class is represented by polymeric surfactants, while the second contains lipopeptides, lipoproteins, lipopeptides, phospholipids, and glycolipids.

1.3. Sugar-based fatty acid esters

Sugar-based fatty acid esters (SBFAEs) are non-ionic glycolipids, largely demanded in pharmaceutical, food, and cosmetic industries [10, 11]. They are constituted by a sugar polar moiety (mono- or oligosaccharide) linked *via* an ester bond to a fatty acid chain (Figure 1). SBFAEs are non-toxic, tasteless, odourless, not irritant, and biodegradable amphiphilic compounds [12, 13]. They display notable surface properties and stabilizing emulsions and foams [14, 15]. Additionally, SBFAEs can be applied in the biodegradation of spill oil [16]. Therefore, they represent a suitable alternative to petrochemical surfactants. Some sucrose fatty acid esters have already been commercialized in various countries, where they are mainly used as emulsifiers and food preservatives [17]. Moreover, SBFAEs exhibit excellent antimicrobial activity [18, 19], antitumoral activity [20] and recently have shown drug permeability

enhancing effects [21]. These glycolipids have also the advantages to be produced by microbial fermentation, chemical or enzymatic reactions using renewable resources.

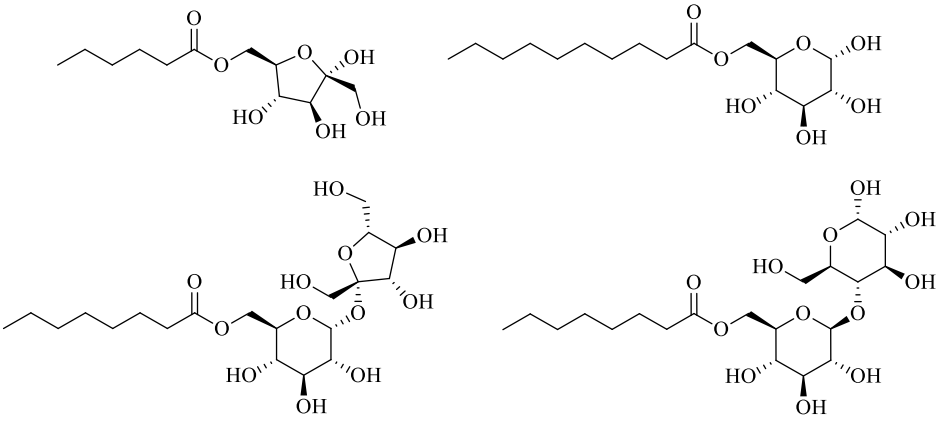


Figure 1. Example of SBFAEs.

In fact, SBFAEs are mainly synthesized by simple reaction starting from carbohydrates (mono- or oligosaccharides) with fatty acid (Figure 2). Clearly, due to the numerous hydroxyl groups present in the carbohydrate moiety, the main challenge is represented by the regioselective esterification of the appropriate hydroxyl group of the carbohydrate.

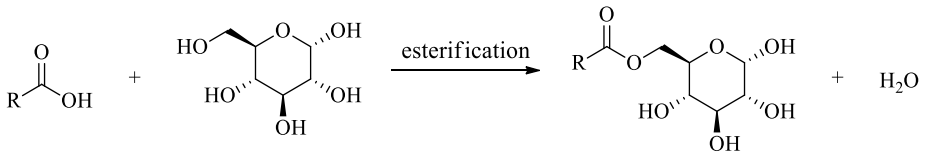


Figure 2. Schematic esterification of glucose esters.

Chemical glycolipid synthesis is mainly applied in industry for large scale production of SBFAEs at low cost and high yields [22]. Nevertheless, chemical esterification reactions have several drawbacks such as the use of high-boiling solvents (e.g., pyridine, DMF), high temperature and toxic acylating agents (such as acyl chlorides) [23]. Moreover, chemical syntheses are not regioselective, leading to the formation of a mixture of different di-, tri- and polyacylated sugar esters with a lower emulsifier and antibacterial properties than the most desirable monoesters [22]. On the other hand, enzymatic glycolipid reactions often consent the regioselective esterification of sugar. They are usually conducted under mild temperatures with eco-friendly solvents (e.g., acetone, ethanol, *tert*-butanol) with low water activity. Enzymes are versatile specific, and reusable, catalysts [24]. Moreover, enzymatic reactions use carboxylic

acids or vinyl esters as acyl donors in place of acyl chlorides [25]. They require easy purification steps leading to safe SBFAEs for food, cosmetic and pharmaceutical applications. The limits of enzymatic reactions of SBFAEs could be represented by the high cost of enzymes, long reaction time and modest yields. In this introduction are presented chemical synthetic procedure (**1.4**), the enzymatic synthesis and the parameter to optimize (**1.5**), followed by biological activities (**1.6**) of SBFAEs.

1.4. Chemical synthetic procedures

Chemical synthesis is very versatile for each type of sugar substrate. However, SBFAEs production in a single-step, cheap and regioselective is very difficult because of the different hydroxyl groups on the carbohydrate moieties. Hence, the regioselective esterification results more difficult for the disaccharides (e.g., sucrose, maltose, trehalose, lactose) than the monosaccharides (e.g., glucose, mannose, galactose, fructose). For example, the esterification of sucrose (eight hydroxyl groups) occurs specially at the primary hydroxyl groups, in this order: 6-OH (glucose unit) > 6′-OH (fructose unit) > 1′-OH (fructose unit) [26]. However, the mono-esterification remains very difficult to achieve due to the small difference in reactivity of hydroxyl groups. In fact, the large-scale synthesis of SBFAEs lead to a mixture of monoesters, di-esters, and polyesters derivatives. Moreover, the current industrial procedures often required high temperatures, presenting many complications, such as (i) the elimination of hazardous high-boiling solvents (e.g., DMSO b.p. 189 °C) from the reaction mixture, which could compromise the thermal stability of SBFAEs, (ii) the presence of products having residual toxicities (iii) high manufacturing cost [27]. Regarding lab-scale syntheses of SBFAEs, free fatty acids, fatty acid esters or acyl chlorides are commonly employed as acyl donors (Figure 3). Alfindee and co-workers synthesized a series of monosaccharides monoesters (glucose, mannose and *N*-acetylglucosamine) using different fatty acyl chlorides (C2-C16), with DMAP in pyridine. By using the same equivalent of acyl chlorides (1.5 eq.), it was observed a major regioselectivity for position 6 of glucose rather than mannose and *N*-acetylglucosamine. In fact, the mannose esters were obtained in a mixture 4:1 of 6-*O*-acylmannose and 2-*O*-acylmannose, while *N*-acetylglucosamine esters were produced in a mixture of 6-*O*-acyl-*N*-acetylglucosamine and 3,6-diacyl-*N*-acetylglucosamine. However, a higher

formation of 6-*O*-acyl-*N*-acetylglucosamine was observed instead of diester product by using a lower amount of acyl chlorides (0.8 eq.) [28].

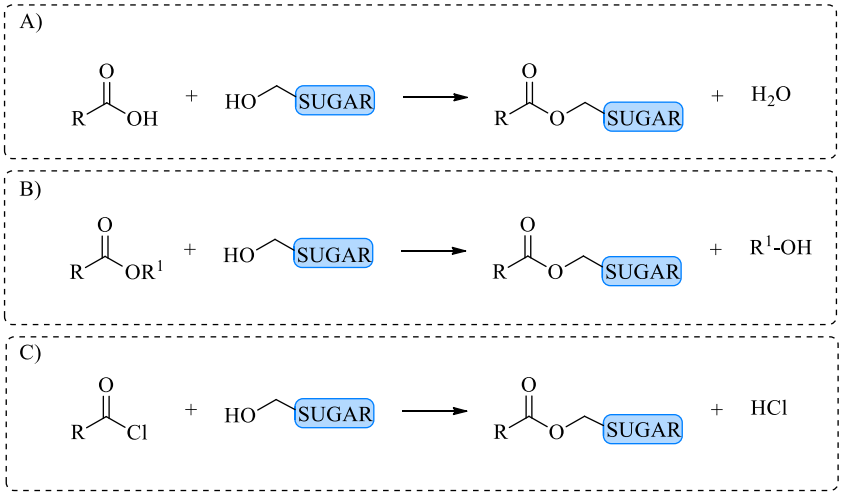


Figure 3. General esterification procedures of SBFAEs. A) esterification; B) transesterification; C) *via* acyl chlorides esterification.

Jumina and co-workers also reported the use of acyl chloride for the syntheses of myristoyl monoesters derivatives of glucose, fructose, and galactose with a 1:3 sugar/myristoyl chloride ratio in pyridine at 95 °C for 40 minutes [29]. Recently, this acyl chloride was employed to produce methyl β-D-galactopyranoside ester [30]. This latter derivative was obtained in high yield using an equimolar ratio of sugar and myristoyl chloride, DMAP, TEA in DMF at 0 °C. Regarding sucrose fatty acid esters, the Mitsunobu reaction [31] was widely employed for their synthesis. Molinier and co-workers applied this procedure for the formation of a mixture of 6 and 6′-*O*-monoesters and 6,6′-*O*-diester of sucrose using different fatty acids with PPh_3, DIAD in DMF. Interestingly, it was highlighted the formation of 6-*O*-acyl-3′,6′-anhydrosucrose besides mono and diesters by intramolecular mechanism [32]. Recently, the Mitsunobu reaction was also employed for the synthesis of β-glucose-1-*O*-esters in regioselective and stereospecific manner starting from pure α-glucose [33]. Solvent-free transesterification are also employed for the synthesis of sucrose esters. This reaction foresees the sucrose dissolution in the esters (reagent and product). Remarkably, its solubility was subject to a high increase in some methyl fatty acid esters with the addition of low amounts of sodium stearate and/or some surfactants [34]. Uronium-based coupling agent was employed for the synthesis of trehalose fatty acid esters. The reaction of trehalose with different fatty acids (1 eq.) catalysed by

TBTU (1 eq.) in pyridine at room temperature provided the formation of the 6-*O*-monoester in good yield, together with a small quantity of the 6,6′-*O*-diester. On the other hand, an increase in the fatty acid equivalents (2 eq.) gave mainly the diester compounds rather than monoester [35]. Protecting groups can be also required for obtained monoesters in a regioselective way. Some lactose-3-*O*-esters derivatives were synthesized by initial esterification of protected lactose as benzyl 2,6-di-*O*-benzyl-4-*O*-(2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranose with typical DCC coupling procedure (Steglich reaction) [36] in the presence of appropriate fatty acids, followed by the remotion of the 3',4'-*O*-isopropylidene group, and the successive hydrogenolysis of the benzyl groups [37]. Recently, solid catalysts have been reported for SBFAEs synthesis [38, 39]. Aluminosilicate zeolites were used as Lewis acid catalysts for the esterification of lactose-6′-*O*-laurate starting from pure lactose and lauric acid [38]. Aluminosilicate minerals are very cheap and highly accessible from waste materials. Moreover, they act as molecular sieves favouring the formation of ester products. The best result was achieved at 1:2 molar ratio lactose/lauric acid, using *tert*-butanol in 10 days. On the other hand, a weakly basic resin (Diaion WA20) was used in the transesterification between sucrose and methyl oleate [39]. This resin showed a very low decomposition of reactants and products, respect to stronger basic resins (Diaion PA306S) [40]. In fact, the yield of the product was considerably enhanced compared to the strongly basic resin with a molar ratio between sucrose and methyl oleate of 12:1. These catalysts represent a valid alternative to enzymes, and they could be employed in the large-scale synthesis of SBFAEs.

1.5. Enzymatic synthesis

Enzymatic synthesis is a greener alternative to chemical synthesis of SBFAEs. In fact, enzymes lead to biocompatible carbohydrate esters requiring easy purification steps. In addition, they give regioselective, enantioselective, diastereoselective, and chemoselective products which are difficult to obtain utilizing classical coupling agents. The enzymes commonly used for esterification or transesterification of sugar compounds are proteases and lipases. These latter are the most employed for sugar esterification because they are biodegradable and versatile enzymes characterized by

stability at different conditions (pH, temperature, and solvents) [24]. Immobilized lipases are more stable in various reaction conditions and can be recovered and reused for other reaction cycles without significant loss of activity [41]. Generally, lipases require only a small amount of water as the reaction medium to maintain their catalytic capability. However, an inadequate quantity of water is deleterious since it favours the inverse hydrolysis reaction, with a consequent reduction of ester yield (Figure 4).

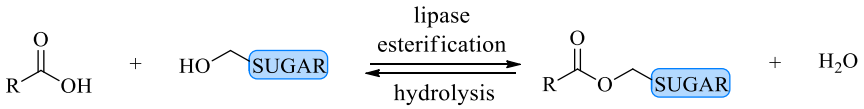


Figure 4. Lipase esterification of sugar and hydrolysis of sugar esters.

Furthermore, hydrolysis of the SBFAE can also occur when anhydrous organic solvents are utilized. Hence, the addition of molecular sieves in the reaction medium is often reported [42]. The lipase-catalysed transesterification seems to follow a "bi-bi ping-pong" mechanism with competitive substrate inhibition by the sugar [43] (Figure 5).

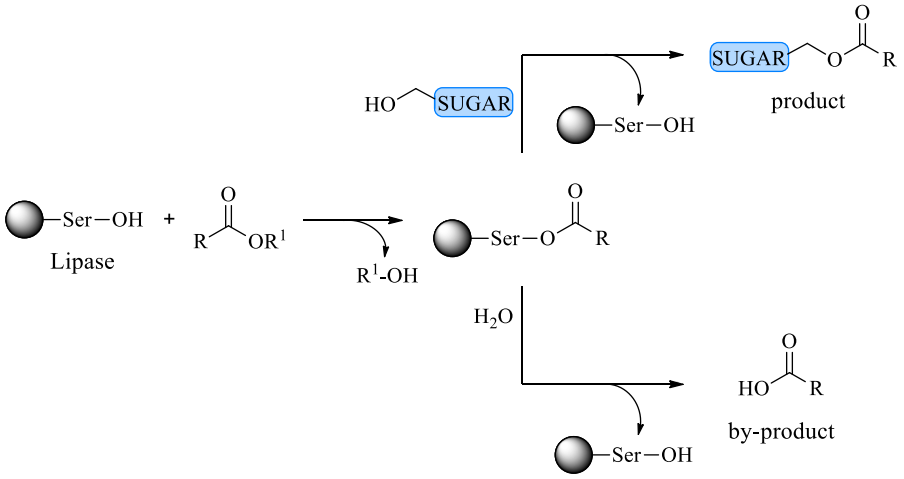


Figure 5. Mechanism of lipase-catalyzed transesterification reactions.

Lipases that catalysed SBFAEs synthesis in nonaqueous environment have a microbial origin [44]. The commercial immobilized lipases mostly used for the regioselective esterification of sugars derives from *Candida antarctica*, *Candida rugosa*, *Rhizomucor miehei*, *Thermomyces lanuginous*, *Mucor miehei*, *Pseudomonas cepacia* and *Pseudomonas sp.* (Table 3). They often catalysed the esterification of the primary hydroxyl groups of different carbohydrates. *Candida antarctica* lipase was widely employed for the synthesis of monosaccharide monoesters due to its versatility,

stability, and low cost. This enzyme consents the regioselective acylation in position 6 of glucose and other monosaccharide [45].

Table 3. Commercial lipases used in the enzymatic reaction and their immobilized form.

Lipase	**Immobilized form**
Candida antarctica	Novozyme 435®
Thermomyces lanuginosus	Lipozyme® TL IM, Lipolase 100L
Candida rugosa	NER-CRL
Rhizomucor miehei	Lipozyme® IM
Mucor miehei	Lipozyme®
Pseudomonas cepacia	Amano Lipase PS-C I
Pseudomonas sp.	Lipase PS

Regarding the disaccharides, the presence of various hydroxyl groups enhances the challenges of a regioselective mono-esterification. However, several lipases were found to catalyse primarily the 6 positions of disaccharides such as sucrose and lactose (Figure 6) [27, 46]. On the other hand, proteases catalysed the regioselective esterification of the 1′-hydroxyl position of sucrose.

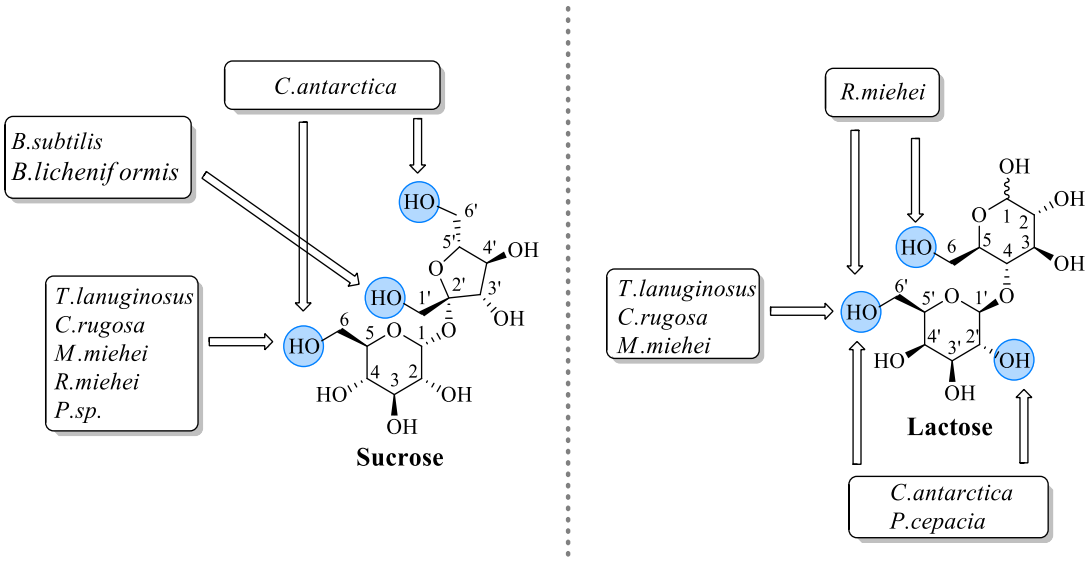


Figure 6. Esterification sites of sucrose and lactose by lipases and proteases

However, the regioselectivity as well as the conversion into the desired SBFAE is not correlated only to the type of the enzyme. Below are discussed the main parameter that influence the enzymatic synthesis of SBFAEs evaluated by considering the conversion of the acyl donor (as generally reported in the literature) or the yield of the sugar ester.

1.5.1. Effect of solvent and water amount

The choice of the reaction solvent is a crucial point for the optimization of enzymatic reactions. In fact, the solvent can influence the SBFAE yield acting on the solubility of the reagents and on the activity of the lipase. Non-polar solvents, such as n-hexane and toluene lead to a poor dissolution of the sugar causing low yields, but the conformation of the lipase is preserved. On the other hand, polar solvents such as DMSO, dioxane and pyridine, induce a reduction in the lipase activity despite the sugar being very soluble in them [45]. Hence, a great solvent system has to offer a good dissolution of sugar and acyl donor, protect the enzyme activity and preventing the hydrolysis of the SBFAE. Acetone, ACN, tertiary alcohols (e.g., *t*-BuOH, 2M2B) are widely employed as green solvents in lipase-catalysed systems. As mentioned above, a correct level of hydration of the reaction is fundamental in lipase- catalysed reactions. In fact, an initial small water amount is necessary to preserve the lipase conformation and its effect [47]. However, the water formed as a by-product in the esterification reactions promote the hydrolysis of the product. For this reason, molecular sieves or sodium sulphate are commonly used as adsorbent agents [42]. Arcens and co-workers tested several organic solvents on the synthesis of glucose-6-*O*-palmitate C16 catalysed by *Candida antartica* [45]. It was observed that the pre-treatment (drying or addition of MS 3 Å) of the best solvents (acetone, THF, dioxane, ACN and *t*-BuOH) is essential for achieving the higher conversions degree (80-100%) at 45 °C in 72 h, which although is not obtainable. Particularly, dry ACN gave a complete conversion. Therefore, the influence of residual water amount in the SBFAE formation was confirmed. Enayati and co-workers evaluated the trend of the conversion rate of lauric acid to lactose-6-*O*-laurate C12 with free and immobilized CALB (*Candida.antarctica* lipase B) using different solvents (hexanes, acetone, *t*-BuOH, EtOAc, THF and ACN) [48]. The highest conversions of lauric acid were 93% and 77% for immobilized and free lipase respectively, both using hexane, with an equimolar ratio of lactose and lauric acid at 50 °C in 12 days. The free lipase was highly influenced by the type of solvent than the immobilized ones. In fact, immobilized lipases showed a better conversion associated to a less solvent dependency. Moreover, immobilization leads to a simpler purification of the lactose ester and an easier recovery of the lipase, which can be reused for other reaction cycles. Dual-solvent systems with different polarities

can provide the dissolution of the sugar (e.g., pyridine, DMSO) and the good stability of the enzyme (e.g., THF, *t*-BuOH), justifying their large use in lipase-catalysed reaction. For example, Zhu and co-workers reported the synthesis of a series of sucrose monoesters with long fatty acid chains catalysed by Lipozyme TLIM (*Thermomyces lanuginosus*) in anhydrous *t*-BuOH/pyridine (1:1 v/v) [15]. The same lipase was also employed for the synthesis of lactose fatty acid monoesters in THF/pyridine (1:1 v/v) [14]. A recent study evaluated the effect of DMSO percentage in the esterification of glucose with palmitic acid catalysed by *Candida antartica* lipase B [49]. The maximum conversion was achieved using DMSO/2M2B (4:1 v/v), at 55 °C in 24 h. A small percentage of DMSO led to both a lower conversion and lipase activity, while a high percentage of DMSO favoured the synthesis of diester [49]. On the contrary, an amount from 10% to 20% of DMSO was found to drastically improve the conversion of vinyl laurate into sucrose monolaurate products instead of their diester derivatives in the transesterification catalyzed by Lipolase 100L (*Thermomyces. lanuginosus*) [50]. Ionic liquids (ILs) are considered a green alternative to the classic organic solvents for the enzymatic synthesis of SBFAEs due to their near-zero vapor pressure, thermal stability, and recyclability [51]. The benefits of ILs consist in maintain a high activity of lipase and enhancing both sugar and acyl donor solubilities [51]. Shin and collaborators showed a higher solubility of glucose and vinyl laurate in ionic liquid mixtures than other organic solvents, causing a conversion two times superior [52]. The reaction was performed using Novozym 435 (*Candida antartica*) and [Bmim][TfO]:[Bmim][Tf_2N] (1:1 v/v). Shao and collaborators reported a yield of 66% of sucrose-6-*O*-laurate (C12) in [3CIM(EO)][NTf_2]/2M2B (1.5:1 v/v) from sucrose and vinyl laurate catalysed by Lipozyme TLIM (*Thermomyces lanoginosus*) [18]. In this case IL/organic solvent mixture was necessary due to the low solubility of sucrose in ILs pure system. Galactose was also found to be not very soluble in ILs [53]. Abdulmalek and co-wokers applied high polar DMSO as co-solvent of imidazolium-base-IL to increase dissolution and conversion of the galactose into the galactose-6-*O*-oleate (C18:1). The esterification reaction catalyzed by Lipozyme RM IM. DMSO:IL 1:20 (v/v) converted 77 % of the oleic acid in 2 h. Lower percentages of DMSO as co-solvent were not able to solubilize galactose, while higher percentages probably could lead to denaturation of the Lipozyme. Thanks to its non-toxic and non-flammable

nature, supercritical CO_2 was also employed as a solvent in various lipase-catalysed reactions. For example, immobilized *Candida antarctica* lipase B was used in supercritical CO_2 system to afford sucrose laurate (C12) with a 74 % of lauric acid conversion at 60 °C in 24 h. [54]. Notably, it was highlighted the importance of molecular sieves which raised the conversion of 27%.

1.5.2. Effect of temperature

The enzyme stability, the reaction time and the solubility of substrates are dependent on the temperature. Each lipase usually works in mild conditions and presents an optimal effect at a determinate temperature range. For example, *Candida Antarctica* lipase, which is one of the most used in enzymatic reactions shows to be active from 40 °C to 80 °C. Higher temperature causes the thermal denaturation of enzymes and consequently the loss of their activity. However, an increase of temperature can be useful to enhance the kinetic of the reaction and to improve the solubility of sugar in the selected solvent, causing major yield of monoester. On the other hand, an enhanced monoester solubility at high temperatures could reduce its yield. Therefore, monoesters with low solubility in the reaction medium are necessary to try avoiding the diesters formation or its hydrolysis. Various studies evaluated the effect of temperature on the lipase efficiency in SBFAEs esterification. Regarding *Candida Antarctica* it was studied the effect of the temperature levels in the synthesis of glucose-6-*O*-palmitate (C16) starting by vinyl palmitate in ACN [45]. The kinetic of the reaction proved that the formation of glucose-6-*O*-palmitate (C16) considerably improved from 20 °C to 60 °C. Moreover, conversion rates were higher at higher temperatures. A 94 % of vinyl palmitate conversion was detected at 60 °C in 20 h, lowering to 86 % at 70 °C. No increase in glucose-6-*O*-palmitate (C16) production was noted after 20 h, probably due to lipase thermal denaturation. Neta and co-workers studied the esterification of fructose with oleic acid C18:1 using *C. antarctica* at different temperature levels were (from 46.6 to 63.4 °C). The best temperature was 57.2 °C with 88 % conversion of oleic acid in almost 37.8 h [55]. In a study of lactose caprate (C10) synthesis catalysed by *Candida rugosa* an increase of capryl acid conversions of 20 % was observed when the temperature goes from 45 °C to 55 °C [56]. This increment of conversion was probably due to an increase of the contact between molecules in the reaction mixture. Nevertheless, at 60 °C was not observed

an evident increment of the conversion. Notably, the free lipase application led to a decrement of conversion with the same conditions. Thus, proved that immobilization raises the enzyme's thermal stability [41]. Moreover, immobilization on supports soluble in the reaction medium boost the availability of the enzyme active sites, causing an improvement of the catalytic process. The same lipase was also employed in the solvent free synthesis of fructose oleate (C18:1) from free oleic acid and fructose [57]. This reaction was conducted in a fluidized bed reactor giving 80% of conversion at 60 °C in 48 h. Importantly, a drastic reduction of the conversion was observed in 72 h, which was caused by the hydrolysis of the product. Moreover, it was highlighted that the formation of monoester is favoured by a high saccharide/acyl donor molar ratio.

1.5.3. Effect of the sugar/acyl donor molar ratio

The correction of the molar ratio between sugar and acyl donor is also essential for improving the formation of SBFAEs. Generally, an excess of acyl donors is desirable. However, a large excess of these latter can increase the formation of diester instead of the monoester and can deactivate the enzyme. Instead, an excess of sugar can lead to a reduced lipase activity due to a decreased solubility in the reaction medium. Ren and co-workers studied the influence of the molar ratio in the esterification of glucose with three different fatty acid (caproic C6, lauric C12 and palmitic acid C16) catalysed by *Candida antartica* lipase B. It was observed that 1:1 and 3:1 molar ratio caused poor solubility of glucose in the reaction medium, in which sugar crystals could be observed throughout the process, causing low conversion rate of fatty acids into the product. On the contrary, it was highlighted the higher conversion of fatty acids in 1:3 molar ratio due to a major amount of fatty acid in the reaction medium than the amount of water created during the esterification reaction. Therefore, the esterification reaction is favoured more than the reverse hydrolysis reaction [49]. Zaidan and co-workers evaluated the effect of the molar ratio in the synthesis of lactose caprate (C10) esters catalysed by *Candida rugosa* lipase. It was observed that the conversion of capric acid increased from 30 % to 70 % by reducing the sugar concentration until 2:1. Therefore, an excess of sugar occasionally promotes the conversion of fatty acid. Probably, this is due to its lypo-protectant effect that reduces the water activity in the reaction medium favouring the esterification. On the other hand, slightly reduced conversion

rates were obtained with increases in capric acid molar ratio to 1:1, 1:2, and 1:3 because of acid inhibition of lipase [56]. The determination of the correct molar ratio is also fundamental for the formation of desired monoester instead of diesters. Ferrer and co-workers analysed the effect of molar ratio in the transesterification reaction catalyzed by Lipolase 100L (*T. lanuginosus*) of sucrose with vinyl laurate conduced in 2M2B/DMSO (4:1) at 40 °C in 24 h. A 1:10 molar ratio gave 50% conversion to sucrose monolaurate C12 and <5% to diesters. A reduction of the amount of vinyl laurate led to a 20% reduction of conversion to monoester, while its increment gave the same conversion of monoester but enhancing the undesired diester formation [50].

1.5.4. Effect of the type of sugar and acyl donor

The reactivity of the hydroxyl groups of the sugar and the nature and length of the acyl donor are important parameters that influenced their behaviours in different solvents and their affinity to the enzyme. As regard the sugar moiety, *Candida antarctica* lipase was the most versatile enzyme for different monosaccharides and disaccharides. The esterification of three different sugar, i.e., fructose, sucrose, and lactose with oleic acid catalyzed by Novozyme 435 conducted in ethanol at 40 °C, for 72 h was explored [55]. The best oleic acid conversion was achieved for lactose (84 %), subsequently fructose (74 %) and sucrose (56 %). Zaiden and co-workers reported the esterification of two monosaccharides (ribose and mannose) and two disaccharides (lactose and trehalose) with capric acid catalysed by free and immobilized *Candida rugosa* lipase in acetone [56]. The major conversion rate of capric acid was observed in the order : lactose > ribose > mannose > trehalose. Monosaccharides are inclined to be more highly soluble in acetone than disaccharides. However, the higher ester conversions for lactose could be clarified by its two primary hydroxyl groups at the C6 and C6′, which increases its reactivity with the fatty acid. As regard the acyl donors, several studies reported a decreased ester yield correlated to an increase of chain length. However, this trend is not always observed [14, 15]. Ren and co-workers explored the influence of three fatty acids (palmitic C16, lauric C12 and caproic C6), in the esterification of glucose esters catalysed by *Candida antarctica* lipase B [49]. The conversions trend for each fatty acid was observed in the order: glucose caproate C6 (113%) > glucose palmitate C16 (97%) > glucose laurate C12 (77 %). The higher conversions of caproic acid C6 can

be explained by its high polarity and its solubility in the dual solvent system (DMSO/2M2B 4:1 v/v). In addition, caproic acid, constituted by a shorter chain, possesses a very low stearic hindrance facilitating its access to the lipase active sites. However, traces of diester compound could be formed using more reactive caproic acid.

1.5.5. Effect of the type and amount of lipase

As mentioned above lipases represented the best choice in the enzymatic catalysis of SBFAEs. Different lipases were used in the esterification of monosaccharides (e.g., glucose, mannose, galactose, fructose, ribose, xylose) and oligosaccharides (e.g., sucrose, lactose, maltose, trehalose). Arcens and co-workers evaluated the activity of different immobilized lipases from *Candida antarctica* (A and B), *Thermomyces lanuginosus*, *Rhizomucor miehei*, *Pseudomonas cepacia*, and *Fusarium solani pisi* in the synthesis of glucose-6-*O*-palmitate. Independently of the reaction condition, *Candida antarctica* lipase B revealed the best conversion of vinyl palmitate (100 % in ACN) followed by *Thermomyces lanuginosus* lipase (36 % in ACN). On the other hand, low conversions were obtained with *R. miehei*, *P. cepacia*, and *F. solani pisi* (0-18 %) The *Candida antarctica* lipase A did not show any effect in all solvents tested, because of the geometry of the lipase active site [45]. Moreover, the influence of the immobilization type of *Candida Antarctica* lipase B on the supporting acrylic beads, both by adsorption and by covalent linkage, was studied. Based on the conversion results, the adsorbed lipase was more efficient than the covalently linked ones in all solvents. Depending on the type of lipase-support interaction, the active site of the enzyme may be obstructed/exposed, which may affect its accessibility to the sugar. Ferrer and co-workers screened different lipases for the synthesis of sucrose laurate C12 performed starting from sucrose and vinyl laurate (1:10) in 2M2B/DMSO (4:1 v/v) at 40 °C in the presence of molecular sieves [50]. The best results in terms of vinyl laurate conversion were 51%, 45% and 39% obtained with Lipolase 100L (*Thermomyces lanuginosus*), Novozyme 435 (*Candida antarctica*) and Lipase PS (*Pseudomonas sp.*) respectively. However, Novozyme 435 furnished two major monoesters in a nearly equimolar ratio. Walsh and co-workers evaluated the impact of different lipases on the synthesis of sucrose and lactose laurate in 2M2B at 55 °C [58]. It was observed good yield of lactose laurate with *Pseudomonas cepacia* (57%) and

Mucor miehei (52%). On the contrary, these lipases furnished low yields of sucrose laurate (34% and 7%), highlighting their major affinity for lactose than sucrose. In another study, immobilized and free lipases were examined for the synthesis of galactose oleates (C18:1) in a dual solvent system of IL/DMSO [53]. Lipozyme RM IM showed the best conversion of oleic acid, (77 %) followed by Novozym 435 (73 %) at the same conditions. On the other hand, free lipases (*Candida rugosa* and *Geobacillus zalihae*) exhibited lower conversion. These difference in activities between free and immobilized lipases is due to the better stability of these latter in the polar two-solvent system and to their rigid conformation. The optimum amounts of lipase were also tested, observing a decrease in the conversion of galactose when the Lipozyme RM IM amount was increased from 2% to 5% (w/w). This trend could be explained by a reduction of the mass transfer due to enhanced viscosity of the reaction media [53]. Another study from the same author highlighted this relation between the lipase amount and the conversion [59]. It was observed a slight conversion reduction of caproic acid to xylose caproate C6, by an increasing amount of Novozyme 435 from the optimal 16 % to 24 % (w/v) in DMSO/acetone 1:10. Even in this case the overloaded lipase amount restricts medium fluidity, and its overcrowding limits the interaction of the substrates with the lipase active site.

1.5.6. Summary

Enzymes can offer environmental-friendly and more regioselective esterification or transesterification than chemical methods. The most utilized enzymes are lipases, which require mild conditions with easy purification steps. Many physicochemical and biochemical parameters are important to consider in lipase-catalyzed reactions (Table 4). Hence their optimizations are fundamental to obtain the desired products with good conversions and regioselectively. The drawbacks are represented by the substrate specificity of lipases (e.g., aromatic acid and substituted sugar are not tolerated) and their high costs. Therefore, the translation of enzymes in industrial processes for large-scale production of sugar-based monoesters is still challenging [28].

Table 4. Physicochemical and biochemical parameters involved in reported lipase-catalysed reaction. a = sugar/acyl donor molar ratio; b = acyl donor conversion, as reported in indicated articles; c = isolated yield of sugar ester.

Lipase	**Sugar**	**Acyl donor**	**Solvents v/v**	**R[a]**	**T °C**	**t h**	**%[b]**	**Ref.**
C. antarctica	Glucose	Vinyl palmitate	ACN	1:1	45	72	100	[45]
		Caproic acid Lauric acid Palmitic acid	DMSO/ 2M2B (4:1)	1:3	55	24	113 77 97	[49]
		Vinyl laurate	IL	1:2	40	12	55	[52]
	Fructose Sucrose Lactose	Oleic acid	EtOH	1.2:1 1:1 1:1	57 40 40	38 72 72	88 56 84	[55]
	Xylose	Caproic acid	acetone/ DMSO (10:1)	1:4	60	24	64	[59]
	Sucrose	Lauric acid	SC CO_2	1:1	60	24	74	[54]
	Lactose	Lauric acid	Hexane	1:1	50	288	93	[48]
T. lanuginosus	Sucrose	Vinyl laurate	2M2B/ DMSO (4:1)	1:10	40	24	51	[50]
			IL/2M2B (1.5:1)	1:4	60	72	66[c]	[18]
		Vinyl esters (C12-C22)	*t*- BuOH/py. (1:1)	1:4	40	8	67-80[c]	[15]
	Lactose	Vinyl esters (C6-C18)	dry THF/py. (1:1)	1:3	55	48	58-78[c]	[14]
C. rugosa	Fructose	Oleic acid	Solvent free	1:4	60	48	80	[57]
	Ribose Mannose Trehalose Lactose	Capric acid	Acetone	1:1	55	48	63 62 56 67	[56]
R. mucor	Galactose	Oleic acid	DMSO/IL 20:1	1:2	60	2	77	[53]
P.cepacia	Lactose Sucrose	Vinyl laurate	2M2B	1:3	55	336	57 34	[58]

1.6. Biological activities

1.6.1. Antimicrobial activity

Food pathogens caused significant problems on human health, mainly via digestive diseases [60]. The classical dangerous bacteria are represented by *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhimurium*, while an ordinary harmful fungus is *Candida albicans* [61, 62]. Some antimicrobial agents used in the food industry are toxic and not biodegradable. Therefore, SBFAEs receive great attention due to their antimicrobial activity linked to their green characteristics and safe biocompatible and biodegradable profile. The antibacterial and antifungal activity of SBFAEs have been widely examined and MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) values have been achieved in different conditions for several bacteria and fungi. Generally, SBFAEs possess a major inhibition effect versus Gram-positive than Gram-negative bacteria [18]. Based on the dose, they can be both bacteriostatic [63] and bactericidal [64]. The antimicrobial activity is correlated to the nature of the carbohydrate moiety, the type, the length, and the number of fatty acid chains [65]. Zhao and co-workers studied the antibacterial activities of different SBFAEs versus five bacteria (*Staphylococcus aureus, Bacillus Cereus, Bacillus Subtilis, Escherichia coli* and *Salmonella typhimurium)*. Their results indicated a major effect against Gram-positive bacteria than Gram-negative. The antibacterial activities of sucrose fatty acid esters decreased by an increase of the chain length of fatty acids [sucrose caprate (C10) > sucrose laurate (C12) > sucrose myristate (C14)]. Moreover, no antibacterial activity was displayed for sucrose palmitate C16 and sucrose monostearate C18 against all the tested bacteria [19]. Karlova and co-workers also reported a rapid decrease in antibacterial activity by the increase of the chain length of fructose monoesters for *Bacillus Cereus* and *Escherichia coli* [66]. The same trend was observed for lactose monoester derivatives. In fact, lactose stearate C18 was found to be inactive as well as lactose with a very short side chain (lactose caproate, C6) against Gram-positive bacteria [14]. Surprisingly, in a recent work, reported by Zhu and co-workers, was found an inverse trend: for *Staphylococcus aureus* and *Listeria monocytogenes* the MIC diminished as the length of the acyl chain

of sucrose esters increased [15]. Additionally, good activities against *Escherichia coli* were revealed by using two long-chain unsaturated lactose esters [67]. On the other hand, the antibacterial activities of caprates with different carbohydrate heads indicated that disaccharide monoesters exhibited better antibacterial activity than monosaccharide monoesters for *Bacillus Cereus, Bacillus Subtilis, Staphylococcus aureus* and *Escherichia coli* (sucrose C10 > maltose C10 > fructose C10 ≥ glucose C10) [19]. Jumina and co-workers reported three monosaccharide myristates (glucose C14, fructose C14 and galactose C14) with moderate antibacterial activity against the Gram-positive bacteria and low antibacterial activity against the Gram-negative bacteria. Interestingly, the pyranoses C14 ester exhibited higher antibacterial activity than the furanose C14 derivative [29]. Remarkably, some trehalose fatty acid esters displayed an inhibition solely of Gram-negative bacteria due to the disruption of the cell membrane [68]. Several SBFAEs have demonstrated to have a good antifungal activity versus many fungi species [28, 29, 69]. Mannose myristate C14 was found to have a broad-spectrum activity against a series of fungi (*Aspergillus flavus, Fusarium graminearum, Candida albicans, Cryptococcus neoformans, Rhodotorula pilimanae*) [28]. A small library of sucrose unsaturated esters with short chains inhibited different *Aspergillus, Penicillum* and *Trichoderma* fungi species [69]. Fructose C14 was more active than pyranoses (glucose C14 and galactose C14) against *C.Albicans* [29]. Nobmann and co-workers proved a limit of esterification degree for a good antimicrobial activity: one for monosaccharide, two for disaccharide [70]. In fact, a major number of acyl chains (e.g., triesters) lead to a SBFAEs poor water solubility and to a less activity [70–72**]**. The activity of SBFAEs (with α-glucose, β-glucose, α-mannose, and α-galactose), against *Listeria monocytogenes*, was also compared with respective polyalcohol, proving a superior or comparable efficacy of these SBFAEs respect to market available molecules used for this purpose (monoglycerides of capric C10 and lauric acids C12) [70]. Moreover, in this study it was observed that pentaerythritol esterified with the same chain and an equal number of free hydroxyl groups of monosaccharide esters, are less active than the SBFAEs, highlighting the importance of the carbohydrate moiety for the antimicrobial activity [73]. The mechanism of the inhibition effect of these SBFAEs is still studied. Probably sugar esters could recognize the cell membrane perturbing its permeability, which causes a

spill of essential metabolites [74]. A recent plausible mechanism of the antibacterial activity of sucrose monolaurate (C12) against Gram-positive bacteria was proposed by Shao and co-workers [18]. Initially, sucrose monolaurate interrupts the integrity of the cell membrane of the bacteria. This leads to the collapse of the cytoplasm and the subsequent dispersion of intracellular enzymes, the release of K^+ from the cytosol, the disruption of proteins localization and, consequently the deactivation of the bacteria. Concerning the antifungal mechanism, Matin and co-workers observed an interruption of ergosterol production by enzyme CYP51A1, due to its hydrophobic interactions with mannopyranoside ester derivatives, which causes a damage to the membrane with a subsequent death of the pathogen [75]. In conclusion, SBFAEs can potentially be applied as non-toxic food preservatives in the food industry.

1.6.2. Permeability enhancer activity

Currently, the research of non-invasive routes of drug administration is still fundamental to enhance the possibilities of patient compliances [76]. The transport of low permeation macromolecular and peptides across the mucosal surface is a great problem for present drug delivery system [77]. Generally, permeability enhancers (PEs) represent a suitable solution by improving the permeability across the paracellular route by opening tight junctions (TJs) and/or by transcellular perturbation of the membrane [78]. Nevertheless, some PEs exhibited high toxicity profiles due to non-transient effect [79]. Several SBFAEs were studied as potential PEs, particularly, alkylmaltosides, sucrose esters, rhamnolipids and lactose esters. [21, 80, 81]. A plausible mechanism of alteration of membrane integrity was showed by Mayer and co-workers [80]. They proposed that PEs such as sucrose laurate (C12) or sucrose stearate (C18) insert into the plasma membrane causing a destabilisation and removal of phospholipids and other components into micellar structures, which lead to the modification of barrier permeability (Figure 7). About alkylmaltose, maltose laurate (C12) and maltose myristate (C14) are in advanced clinical trials to improve the nasal absorption of a parathyroid hormone analogue peptide drug [82]. Maltose C12 and C14 were also tested at low concentrations on Caco-2 monolayer (intestinal cell lines) causing a decrease in TEER (Trans Epithelial Electrical Resistance), which were recovered to the initial value in a short time. On the other hand, at high concentrations

there was only a partial recovery of TEER values, indicating damage to the monolayer cells.

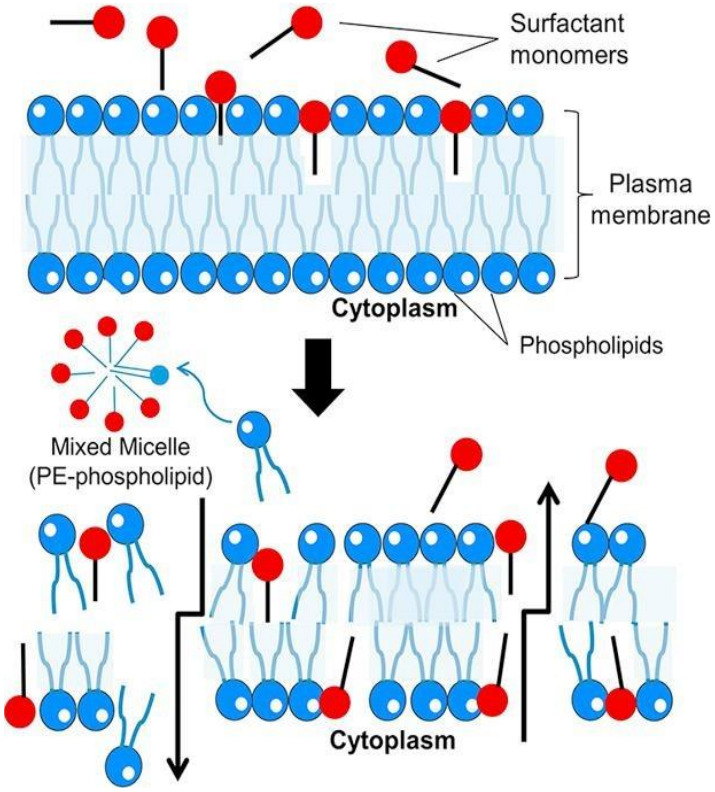


Figure 7. Proposed mechanism of alteration of membrane integrity by surfactant-based PEs. Reproduced with permission from Ref. [80]. Copyright Clearance Center's RightsLink®.

Moreover, maltose laurate and maltose myristate increased the P_{app} (Permeability apparent coefficient) of [^{14}C]-mannitol and fluorescein isothiocyanate-labelled dextran 4 kDa (FD-4) across Caco-2 monolayers and colonic mucosae. Regarding the mechanism, a combination of paracellular and transcellular transport was assessed. In addition, *in vivo* cytotoxicity results established that they may help the delivery of peptides across the colon [83]. Sucrose laurate (C12), sucrose myristate (C14) and sucrose palmitate (C16) were found to be able to increase the absorption of drugs such as atenolol, rhodamine, and vinblastine through Caco-2 monolayers [84]. Furthermore, *in vivo* studies using the same sucrose esters revealed their safety profile for oral administration. In fact, they are readily hydrolysed into sucrose and non-toxic fatty acids by intestinal enzymes, bacterial lipases or by chemical hydrolysis on the digestive system [85]. The possible mechanism of sucrose esters is similar to those alkylmaltosides previously reported [84]. Interestingly, in this case, the activity of P-glycoprotein (the most common of the efflux transporter proteins), does not appear to

be affected. Moreover, sucrose esters in low concentrations exhibits a fluidizing effect of the plasma membrane of epithelial cells, suggesting an improved transcellular transport of drugs [84]. Rhamnolipids (RhLs) were investigated as possible PEs agents for oral and respiratory administration of macromolecules. RhLs are produced by the fermentation process of *P.aeruginosa* as a mixture of mono- and di- RhLs linked to different types of 3-(hydroxylalkanoyloxy) alkanoic acids with several chain length (8C-16C). Perinelli and co-workers established a dose-dependent effect of RhLs on TEER and FD-4 or FD-10 permeability across Calu-3 (pulmonary cell lines) and Caco-2 monolayers at safe concentrations. The mechanism seems to be involved in a reversible TJs opening (paracellular route) [81]. Additionally, similar studies have been carried out for lactose esters [67, 11]. Lactose palmitoleate C16:1 and lactose nervonate C24:1 showed an increase of permeability of FITC-OVA 45 kDa (Fluorescein Isothiocyanate Ovalbumin) across Caco-2 monolayers at low non-cytotoxic dose by transcellular pathway [67]. On the other hand, lactose oleate C18:1 was found to enhance the paracellular route of FD-4 on Caco-2 by the reversible opening of TJs, but the implication of the transcellular route is possible [11]. Recently, McCartney and collaborators showed an enhanced permeability of [^{14}C]-mannitol across rat intestinal mucosae of three carbohydrate laurate (sucrose C12, lactose C12 and trehalose C12) *via* TJs modulation [21]. Overall, SBFAEs can be considered as potential green PEs to be inserted in oral or pulmonary pharmaceutical formulations.

1.6.3. Anticancer activity

Carbohydrate transporters are overexpressed on cancer cells [86]. Therefore, SBFAEs, could be plausible non-toxic candidates to act as anticancer agents. Notably, SBFAEs showed an inhibition effect of enzymes involved in lower glycolysis pathway in bacteria [73]. Nishikawa and co-workers studied the effect of maltose and sucrose fatty acid esters to Ehrlich ascites carcinoma in mice. Monoesters were more effective than the highly esterified analogues. Additionally, monoesters fatty acids with long chains were more effective than the shorter ones [87]. Ferrer and co-workers showed a cytotoxic effect of maltotriose palmitate derivatives versus Hep-G2 and HeLa [20] activities. Recently Fang and co-workers discovered that a sucrose pentyl-isovalerate ester (isolated from *Ainsliaea yunnanensis Franch*), produced a cytotoxic effect against the A549 (lung adenocarcinoma) cell line. Interestingly, this derivative can

interrupt the G_0/G_1 phase, and it induces cell apoptosis by a diminution in mitochondrial membrane potential and an amount of ROS level [88]. Sucrose fatty acid esters have also been used in drug delivery systems. For example, different mixtures of sucrose fatty acid esters (C8-C18) were used in the nanoencapsulation of caffeic acid phenethyl ester (CAPE), enhancing its capacity to treat colon and breast cancers. Moreover, possible synergic effects cannot be excluded [89]. Recently, glucose esters of eicosapentaenoic acid and γ-linolenic acid were observed to induce apoptosis in SKOV-3 ovarian cancer cells [90].

1.6.4. Insecticidal activity

The control of yellow fever mosquitoes (*Aedes aegypti*) is a serious public health problem [91]. Glucose esters of eicosapentaenoic acid and γ-linolenic acid exhibited insecticidal activity against *Aedes aegypti larvae*. [90]. Indeed, some sucrose esters were found to possess also insecticidal activity. Particularly, sucrose caprylate (C8) was found to be higher toxic than other analogues with similar acyl fatty acid chains versus a broad range of arthropod species [92]. Probably, two mechanism-of-action might be involved in SBFAEs insecticidal activity: asphyxia caused by mechanical obstructions of body openings and dryness of insect cuticles.

1.6.5 Anti-inflammatory activity

The research for safer drug alternatives represents an important step for contrasting the drawbacks of common anti-inflammatory therapies. Several plants of the Solanaceae family (*Physalis peruviana and Physalis philadelphic*a) or Leguminosae family (*Astragalus membranaceus*) are used in traditional medicine to treat inflammatory pathologies. The purification of the extracts of these plants with isolation of single components allowed the evaluate of the possible anti-inflammatory activity of sucrose esters. The sucrose esters derivatives extracted from *P. philadelphica* (polyesters with decanoic acid and isobutyric acid) showed an inhibitory activity towards the COX-1 and COX-2 enzymes, which convert arachidonic acid into prostaglandins, in a manner comparable to some NSAIDs such as aspirin, ibuprofen and naproxen [93]. On the other hand, sucrose ester derivatives obtained from *P. peruviana* (triesters with isobutyric or 2-methylbutanoic acid) caused down-regulation of iNOS and COX-2 enzymes, with reduced nitric oxide and prostaglandin E2 production. Their action,

however, does not appear to influence TNF-α release from macrophages [94]. A step forward in this context is represented by the sucrose monoesters obtained from *Astragalus membranaceus*, particularly, sucrose-6-*O*-palmitate C16:1, sucrose-6'-*O*-linoleate C18:2 and sucrose-6'-*O*-linolenoate C18:3. These derivatives were able to decrease the transcriptional activity of TNF-α-induced NF-kB, with reduced expression of iNOS, ICAM-1, proinflammatory cytokines, COX-2, LOX and other signaling molecules. Thus, resulting in a reduction of inflammation on LPS-stimulated murine macrophages [95]. Furthermore, this mechanism of action was also observed with free linoleic and linolenic fatty acids [96]. Hence, this would justify the better activity of 6'-*O*-sucrose linolenoate (C18:3) than other derivatives, including 6'-*O*-sucrose linoleate (C18:2) and the saturated analogues extracted from *Astragalus*. Recently, trehalose fatty acid esters showed as LOX inhibition [67]. In detail, the inhibitory effect is amplified with the increase of the acyl fatty acid chain length and diesters were more active than monoesters, suggesting an important contribution of the acyl chain in the inhibition of 15-LOX. Moreover, trehalose fatty acid esters were found to be more potent than the analogues sucrose fatty acid esters, probably due to the high affinity of trehalose with proteins. In fact, trehalose can attenuate the 5-LOX and COX pathways [97]. These results encourage the study of new SBFAEs as anti-inflammatory agents.

1.6.6. Site specific drug delivery

Lectin receptors (LRs) are widely expressed in the surface of numerous cells and can recognize different carbohydrates such as mannose, galactose, fructose, fucose and lactose [98]. Due to this interaction, sugars are used as ligand in the site-specific targeting of several drugs. Notably, sugars can be conjugated to the drug carriers (e.g., nanoparticles, liposome) leading to the glycosylated ones. Therefore, these sugar surface decorated carriers are recognized and endocytosed by LRs of targeted cell, followed by the drug is released in the cytoplasm (Figure 8). This approach could be led to a possible increase of the desired therapeutic effect and reduction of the toxicity. LRs are largely expressed by hepatocytes, where ASGPR of parenchymal cells recognize galactose [99], while ASGPR of non-parenchymal cells (e.g., endothelial cells and Kupffer cells) recognize mannose [100].

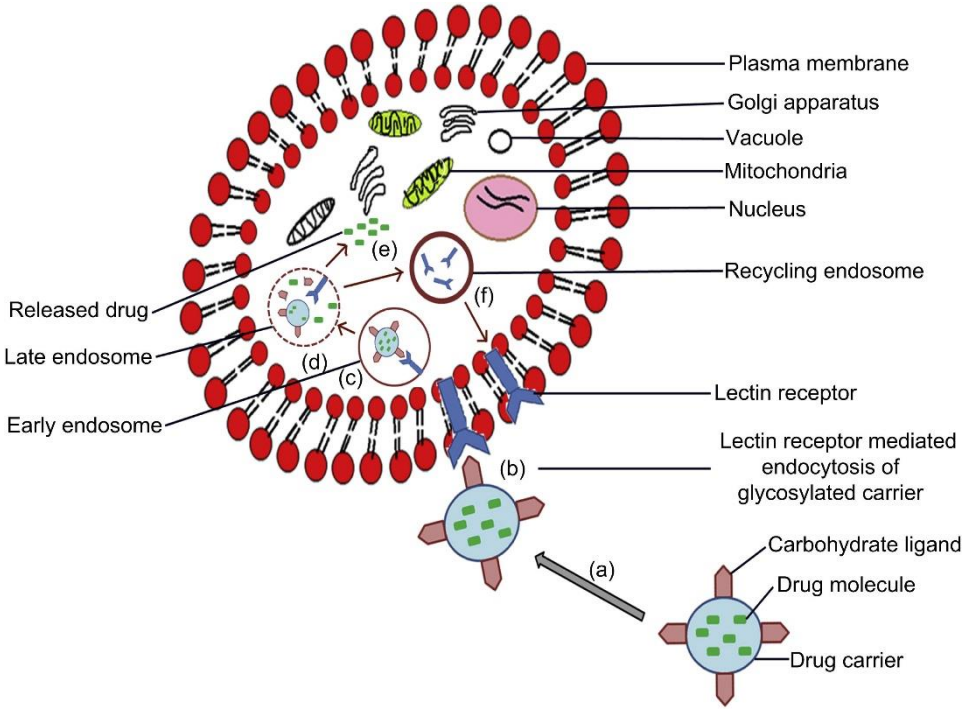


Figure 8. Scheme for LRs-mediated delivery of glycosylated carriers (a) Recognition of glycosylated carrier by lectin receptors; (b) lectin receptor-mediated endocytosis; (c) early endosome, (d) endosome rupture (e) release of drug into cytoplasm; (f) recycling of LRs. Reproduced with permission from Ref. [98]. Copyright Clearance Center's RightsLink®.

Moreover, mannose receptors (MRs), are also expressed by alveolar, splenic, peritoneal macrophages and in high density by dendritic cells. Hence, the mannosylated carries could be employed in several pharmaceutical fields (e.g., anti-inflammatory, anticancer, antimicrobial, antiviral) with the aim to improve the delivery of the drugs into the specific tissue target. Furthermore, these approaches may prove potentially useful to contrast chronic infections such as tuberculosis, HIV and leishmaniasis [98], which are currently treated with combinations of drugs with limited efficacy and several drawbacks. Moreover, Schafer and co-workers showed a good efficacy of mannose-conjugated glycolipids, constituted by trimannose polar heads linked to a saturated lipid chain, against dengue virus by reducing the entry of the viral genome into the cytoplasm of host cells [101]. Thus, they could represent a great alternative to antiviral drugs or vaccines, which have low efficacy. Nanoparticles (NPs) can delivery mRNA avoiding its enzymatic degradation by encapsulation [102]. Goswami and co-workers observed that mannose conjugates surface decorated nanoparticles can improve the potency of self-amplifying RNA replicon vaccines by improved uptake by antigen presenting cells (APCs). Dimannose-NPs were found to

produce an augmented effect than mannose-NPs and conventional NPs. On the other hand, no further enhancement was observed with the application of longer mannans NPs (trimannose and tetramannose). Moreover, the reduction of PEG molecules in these mannosylated-NPs lead to a high improve of their immunogenicity, probably due to increased mannose exposure. Moreover, it is highlighted the potential use of self-amplifying RNA vaccines delivered by mannose nanoparticles for intradermal and intramuscular delivery [103]. Recently, some studies have emphasised the advantages to use the mannose-NPs instead of amphotericin (AmB) and commercially available AmBisome® (liposomial AmB) in the treatment of visceral leishmaniasis [104, 105]. Vaghela and co-workers showed that mannose-NPs containing bovine serum albumin AmB were higher recognized by infected macrophages than non-mannosylated ones. In addition, the AmB-mannose-NPs demonstrated a relative bioavailability across intestinal membrane, which was more superior to AmB [104]. Eventually, mannose-anchored thiomer-based AmB nanocarriers showed to improve bioavailability and increase oral administration by controlled release of AmB. Furthermore, this nanocarrier formulation was biocompatibility with red blood cells as compared to AmB and AmBisome® formulation. Therefore, this mannose-anchored thiomer-based nanocarriers could improve treatment of visceral leishmaniasis thanks to the enhanced intestinal permeation of AmB due to mannose-targeted macrophage internalization [105].

2. Research Proposal

Sugar-based esters represent a class of non-ionic amphiphilic surfactants characterized by a carbohydrate polar head linked to a hydrophobic chain. In the last years, they have attracted great attention in different fields due to their physicochemical properties, brought about by a desirable biodegradability and a safe biocompatibility profile. These compounds exhibit variable biological properties and applications such as antimicrobial activity and permeability enhancer effect. Even though numerous research regarding sugar-based esters have been reported, the synthesis of less studied sugar esters such as lactose-based esters or sugar-based-esters bearing polyunsaturated chains or aryl(alkyl) lipophilic moieties is still poorly investigated. Moreover, there is still few investigations regarding their further possible applications, like wound care or in the targeted treatment of infectious diseases.

Therefore, the purpose of this thesis is the synthesis of several sugar-based monoester surfactants with different sugar heads and various hydrophobic portions using both enzymatic and chemical esterification reaction. The chapters division is based on the polar head group: lactose, sucrose, and mannose. Finally, in the last chapter is presented a novel synthetic approach in order to obtain protected mono- and disaccharide monoesters.

To start with, an emerging class represented by 6′-*O*-lactose-based esters with different fatty acid chains and aryl(alkyl) moieties were designed and synthesized with the aim to investigate the following properties: (i) antimicrobial activity, (ii) antibiofilm inhibition, (iii) anti-inflammatory activity, (iv) antioxidant activity. Then, the *in vivo* wound healing promotion of 6′-*O*-lactose-based polyunsaturated fatty acid esters was studied.

Secondly, to extend our studies on sugar esters, the optimization of the 6-*O*-sucrose-based monoesters synthesis was presented. These sucrose esters were produced with different hydrophobic tails in order to analyse their influence on antibacterial and antifungal activities and the best product was also selected for anti-inflammatory studies. Then, a novel study focused on the permeability enhancer activity of 6-*O*-

sucrose-based aryl(alkyl) esters was conducted using 6′-*O*-lactose esters analogues as a comparison.

Another research was focused on designing multistep syntheses of new mannose-type esters through glycosylation and esterification steps. These compounds were synthesized with the aim to develop a drug delivery method based on mannose-decorated liposomes to target infected macrophages by leishmania pathogen, improving drug bioavailability and diminishing drug toxicity.

Finally, for the first time an innovative palladium-catalysed oxidative alkoxycarbonylation of variously substituted styrenes was applied for the synthesis of different sugar-based *trans*-cinnamic esters.

3. Synthesis of 6′-*O*-Lactose-Based Esters as Possible Antibiofilm Agents and Their Application in Wound Care

3.1. Introduction

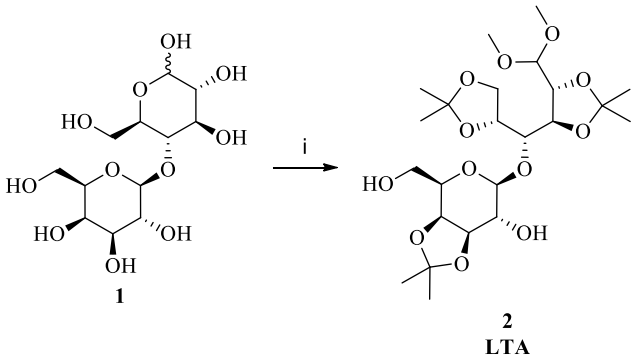
Food pathogens are responsible of significant economic losses in food industry and cause different human diseases [106]. Indeed, microbials manifest their toxicity in human beings principally by the production of mycotoxins enterotoxins in contaminated food, which could be thermostable and survive using the common food conservation method [107]. Biofilm formation is a survival strategy of bacteria defence in a large range of adverse conditions. Biofilms are sessile groups of attached bacterial cells to each other, which are inserted in their matrix which is constituted by extracellular polymeric substances. Four phases are involved in the biofilm formation: (i) planktonic, (ii) reversible and irreversible attachment, (iii) maturation, and (iv) dispersion [108]. This system is fundamental in the protection of bacteria against the action of most commercial disinfectant products [109]. The classic disinfection practices in the food industry are often high-priced and dangerous for human health and the environment. In addition, some of them can remove biofilms but do not kill bacterial cells, which can re-attach to new surfaces and generate a new biofilm [110]. Other practices, such as the use of maleic acid as a classic food preservative like, are demonstrated to be unsafe and toxic [111]. Therefore, the research of new molecules to contrast this problem is still necessary. In this context, SBFAEs could represent a suitable alternative to the commonly preservatives to eradicate biofilms [18]. SBFAEs are non-toxic, non-irritant, highly biodegradable amphiphilic compounds and they possess many other activities such as anti-inflammatory [93]. Among all SBFAEs, glucose and sucrose fatty acid esters are widely studied and employed in food, cosmetic and pharmaceutical sectors, while lactose esters have received less consideration, but some of their promising properties and activities are recently emerged [45]. For this reason, in this chapter, the synthesis of a small library of 6′-*O*-lactose esters (LBEs) constituted by different saturated (C8, C10, C12, C14 and C16) or unsaturated (C11:1, C18:2 and C18:3) fatty acids and aryl(alkyl) (phenylacetic, biphenylacetic, *p*-phenylbenzoic and terphenylacetic) acids has been reported using both chemical and enzymatic esterification. Then, the biological tests are divided in

two sections. In the first section LBEs with saturated fatty acids and aryl(alkyl) chains were tested to determine their MIC values *versus* different Gram-positive, Gram-negative bacteria, and fungi. Subsequently, the two best LBEs candidates were also evaluated as antibiofilm agents and their cytocompatibility was assessed. In the second section, since sugar esters bearing unsaturated chains have shown anti-inflammatory activity [95], the synthesized 6′-*O*-lactose (poly)unsaturated esters were also studied for their *in vitro* cytocompatibility, anti-inflammatory and antioxidant activity, in addition to antimicrobial one. Lastly, 6′-*O*-lactose polyunsaturated esters were also tested *in vivo* alone and in combinations with sucrose C12 (commercially available) and chitosan to evaluate their wound healing effect.

3.2. Results and discussion

3.2.1. Synthesis of 6′-*O*-lactose-based esters

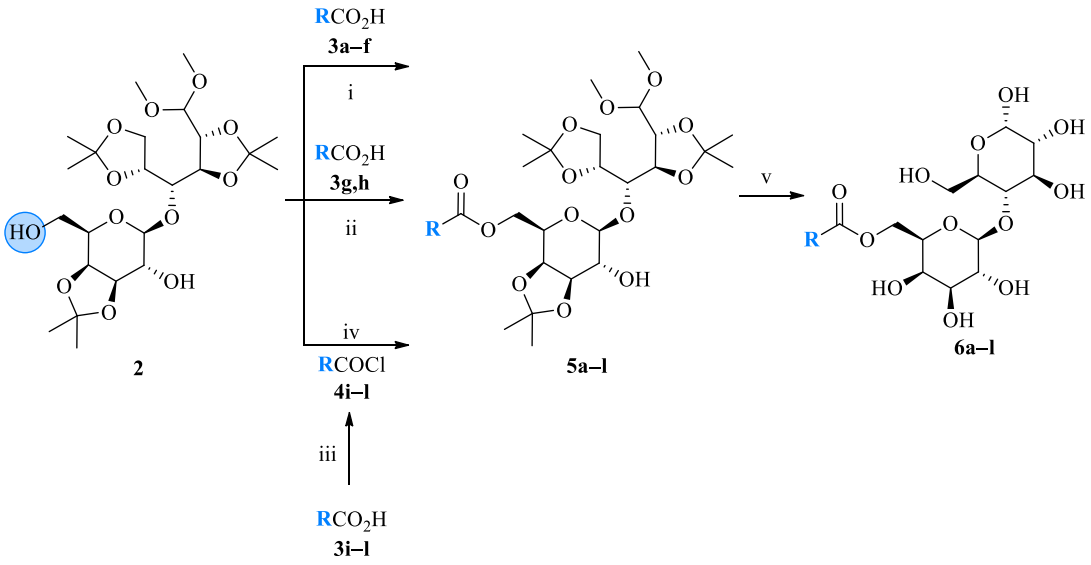
The syntheses of 6′-*O*-lactose esters (LBEs) were conducted using a two-step procedure starting from 4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose (lactose tetra acetal, LTA, **2**), which was previously obtained protecting the lactose hydroxyl groups (**1**) with 2,2-dimethoxypropane and *para*-toluenesulfonic acid (*p*-TSA) at reflux (Scheme 1) [112].



Scheme 1. Synthetic procedure for the protection of lactose to LTA **2**. Reagents and conditions. (i): 2,2-dimethoxypropane, *p*-TSA, reflux, 4 h.

To start with, an esterification reaction (chemical or enzymatic) of LTA (**2**) with fatty acids and aryl(alkyl) acids was investigated (Scheme 2). Due to the different reactivity and stability of acyl donor substrates, three distinct esterification strategies were adopted for the esterification of LTA with saturated/monounsaturated (**3a–f**), and

polyunsaturated (**3g,h**) fatty acids or aryl(alkyl) acids (**3i–l**). The second step was a commonly acidic deprotection of the acetals adducts (**5a–l**), which provided the desired 6′-*O*-lactose esters (**6a–l**) (Scheme 2).



R

Saturated and monounsaturated fatty acid chains

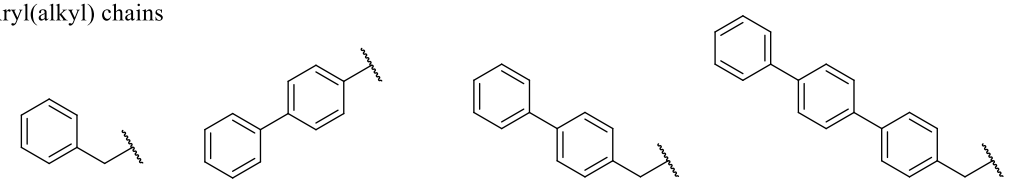
-$(CH_2)_6CH_3$	-$(CH_2)_8CH_3$	-$(CH_2)_8CH=CH_2$
a: caprylate (C8)	**b**: caprate (C10)	**c**: undecylenate (C11:1)
5a (55%)	**5b** (53%)	**5c** (79%)
6a (46%)	**6b** (75%)	**6c** (37%)
-$(CH_2)_{10}CH_3$	-$(CH_2)_{12}CH_3$	-$(CH_2)_{14}CH_3$
d: laurate (C12)	**e**: myristate (C14)	**f**: palmitate (C16)
5d (50%)	**5e** (44%)	**5f** (34%)
6d (44%)	**6e** (65%)	**6f** (80%)

Polyunsaturated fatty acid chains

-$(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$
g: linoleate (C18:2)
5g (19%)
6g (70%)

-$(CH_2)_7CH=CHCH_2CH=CHCH_2CH=CHCH_2CH_3$
h: linolenate (C18:3)
5h (25%)
6h (66%)

Aryl(alkyl) chains



i: phenylacetate	**j**: *p*-phenylbenzoate	**k**: biphenylacetate	**l**: terphenylacetate
5i (83%)	**5j** (8%)	**5k** (44%)	**5l** (17%)
6i (66%)	**6j** (52%)	**6k** (72%)	**6l** (58%)

Scheme 2.Two-step syntheses of 6′-*O*-lactose esters. Reagents, conditions and yields. (i): Lipozyme®, toluene, 75 °C, 12 h; (ii): EDCI·HCl, DMAP, dry TEA, dry DCM, 0 °C, then rt, 72 h, (iii): [$(COCl)_2$], DMF cat., rt, 2h; (iv): DIPEA, dry DCM, rt, 16 h; (v): HBF_4·Et_2O cat., H_2O cat., dry MeCN, 0-30 °C, 3-5 h. Ester isolated yields are reported in brackets.

An enzymatic catalyzed esterification by Lipozyme® (immobilized lipase from *Mucor miehei*) was selected for the synthesis of 6′-*O*-LTA saturated (C8–C16) or

monounsaturated (C11:1) fatty acid esters (**5a–f**). In detail, the LTA and the appropriate fatty acid reacted in equimolar ratio in the presence of 20% w/w of Lipozyme® (refer to LTA) in toluene at 75 °C for 12 h furnishing the respective 6′-*O*-LTA esters with good yields ranging from 34-79%. No correlation between product yields and the length of the fatty acid chain was noted, in agreement with what already reported for similar processes [14, 15]. Moreover, the presence of the terminal double bond of undecylenic acid (C11:1) did not affected the yield. In fact, 6′-*O*-LTA-undecylenate was obtained with 79% yield. Notably, lipases can be recovered with simple purification steps and reused for other catalytic cycles [24]. In this case, Lipozyme® was recovered by simple filtration of the crude reaction under vacuum, washed with ethyl acetate and dried. Its catalytic efficiency was evaluated after each enzymatic reaction (Figure 9). Gratifyingly, the enzyme can be reused at least three times without significant loss in the activity, potentially reducing processes-associated costs.

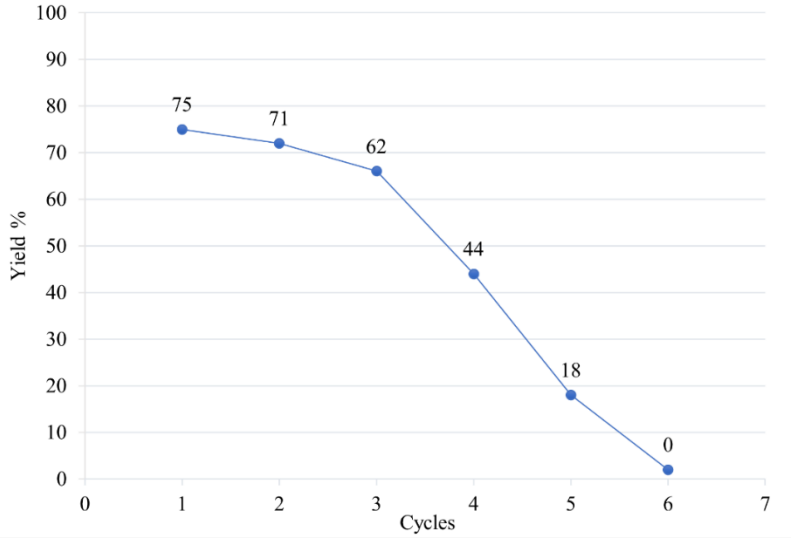


Figure 9. Trend of catalytic efficiency of Lipozyme®. The study was conducted using capric acid (C10) as acyl donor.

Lipozyme® gave regioselective esterification only at the primary hydroxyl group 6′-OH, which was unambiguously assessed by HMBC correlation. It was observed that the two methylene protons H6′ correlate with the carbonyl carbon of the ester group, thus confirming the site of esterification (Figure 10). The acidic deprotection was conducted with catalytic amounts of tetrafluoroboric acid diethyl ether complex ($HBF_4 \cdot Et_2O$) and water in dry ACN in 1:5:500 v/v respectively at 30 °C for 3 h, leading

to the cleavage of acetal groups of 6′-*O*-LTA adducts. The formed precipitate was filtered and recrystallized affording LBEs **6a–f** as α anomers. The α anomer was assigned through ^{1}H-NMR-analysis, considering the coupling between the proton H1 and the proton H2 (Figure 11). In fact, the observed a *J* value of 4.0 Hz indicates the disposition equatorial-axial of H1 and H2 confirming the formation of α anomer.

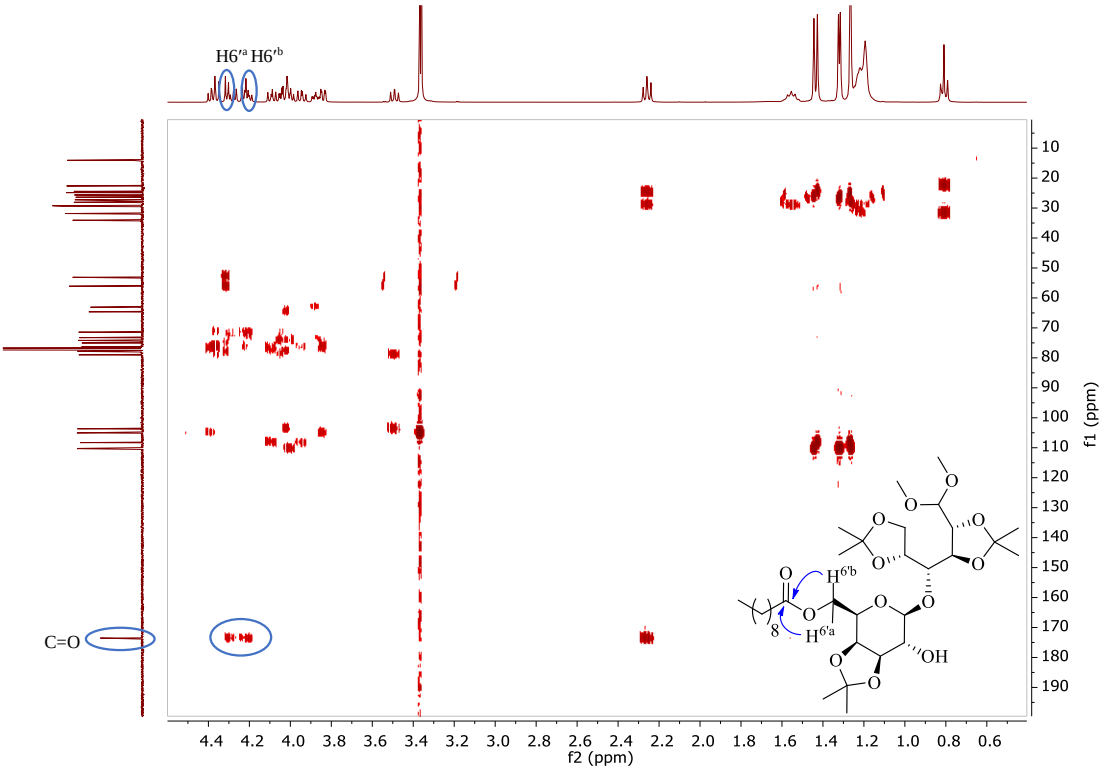


Figure 10. HMBC correlation for 6′-*O*-LTA-caprate. The key three bond correlation between the C=O carbon and the H6′ protons has been evidenced.

Regarding the synthesis of 6′-*O*-LTA-polyunsaturated esters (**5g** and **5h**), by using the already mentioned Lypozyme® method, which needs a high temperature (75 °C) for a relatively long time (12 h) or by employing acyl chlorides of linoleic acid (**3g**) and linolenic acid (**3h**), under strong acid conditions, several double bonds isomerization were observed. Therefore, a modified Steglich esterification [36] was found to be the most effective procedure to obtain **5g** and **5h** in pure form. The reaction was performed in LTA/fatty acid 1:1.2 molar ratio, using EDCI·HCl instead of DCC as coupling agent in the presence of dry triethylamine and catalytic amount of DMAP in dry DCM at room temperature for 16 h. Extremely pure LTA adducts were obtained with this approach, although in low yields, and no double bond isomerization was observed. The subsequent deprotection was conducted at 0 °C and furnished the 6′-*O*-lactose

linoleate C18:2 (**6g**) and 6′-*O*-lactose linolenate C18:3 (**6h**), utilizing the already described procedure for obtaining **6a–f**. These two lactose polyunsaturated esters were synthesized for the first time.

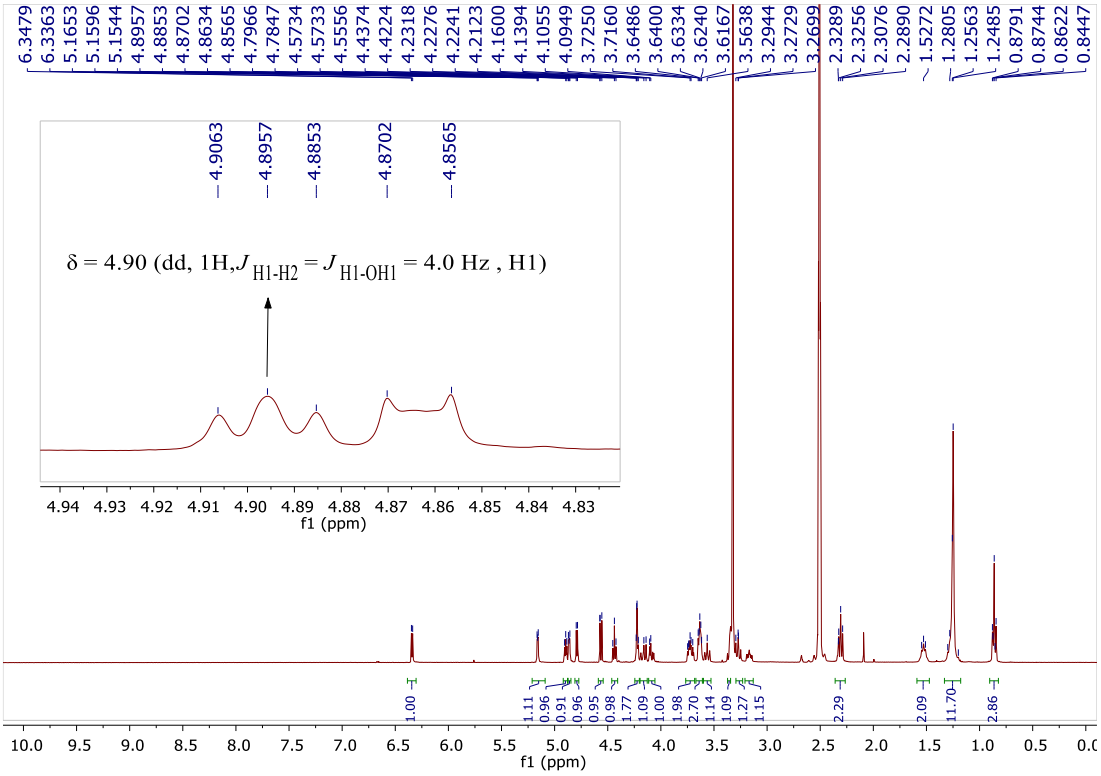
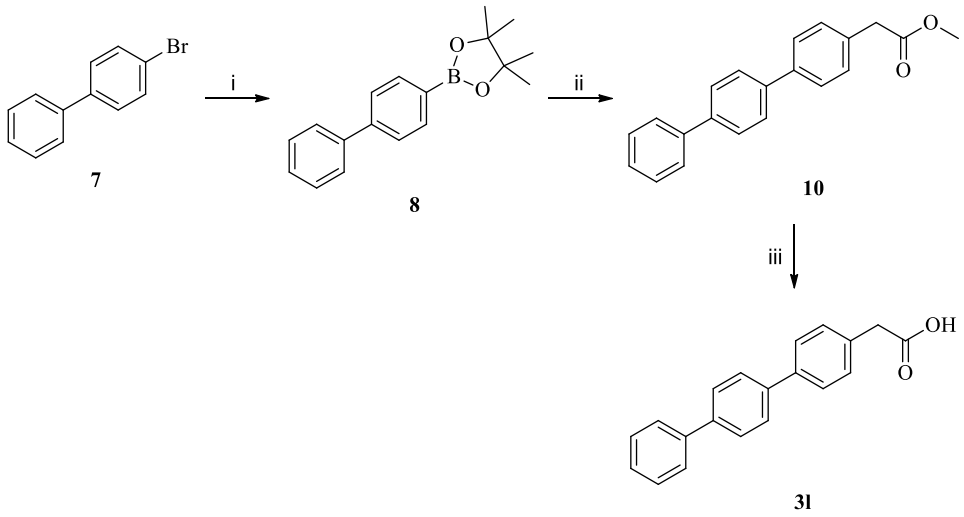


Figure 11. ^{1}H NMR of lactose caprate **6b** with the region from 5.0 ppm to 4.8 ppm zoomed.

Lastly, 6′-*O*-LTA-aryl(alkyl) esters (**5i–l**) were synthesized using a traditional esterification procedure *via* acyl chloride formation, because the Lipozyme® and other lipases tested (i.e., Novozyme 435® from *Candida antarctica*, Acylase I from *Aspergillus melleus* and Lipase from *Porcine pancreas*) did not tolerate aromatic acid or alkyl aromatic acid with less of three methylene units [114]. About the aryl(alkyl) fatty acid (**3i–l**), three of them are commercially available (**3i–k**), while the terphenylacetic acid (**3l**) was synthesized with a three-step procedure starting from *p*-phenylbromobenzene (**7**) which through borylation with B_2pin_2 gave the correspondent dioxoborilane derivative **8** (Scheme 3). The latter was coupled with methyl 2-(4-bromophenyl)acetate (**9**) via Pd(II) catalysed Suzuki reaction, leading to the methyl 2-[(1,1′:4′,1′′-terphenyl)-4-yl]acetate **10**, which was subsequently subjected to basic hydrolysis with LiOH, furnishing the desired terphenylacetic acid **3l** [115] (Scheme 3). Regarding the formation of acyl chlorides (**4i–l**) (Scheme 2), the

appropriate aryl(alkyl) acids (**3i–l**) were treated with oxalyl chloride [$(COCl)_2$] in presence of a catalytic amount of DMF. The successive reaction with LTA (**2**), in an equimolar ratio, utilizing DIPEA in dry DCM for 72 h, led to the respective 6′-*O*-LTA-aryl(alkyl) ester (**5i–l**).



Scheme 3. Synthesis of terphenylacetic acid. Reagents and conditions: (i) B_2pin_2, $Pd(dppf)Cl_2$·DCM, dppf, KOAc, dry dioxane, 80 °C, 16 h; ii) methyl 2-(4-bromophenyl)acetate (**9**), $Pd(II)(dba)_3$, PCy_3, K_3PO_4/H_2O, dioxane:H_2O 2:1, 75 °C, 16 h; iii) LiOH, MeOH:H_2O 3:1, 60 °C, 5 h.

Importantly, this procedure was found to be regioselective for position 6′, and no 2′,6′-diester or 2’-*O*-LTA-monoester traces were spotted in the crude medium. Despite the regioselectivity of the processes, low yields were obtained with *p*-phenylbenzoic acid and terphenylacetic acid, confirming that the steric hindrance of acyl donor plays an important role. As a matter of fact, only the 6’-*O*-LTA phenylacetate ester **5i** was achieved in high yield. The final LBEs (**6i–l**) were gained by filtration, trituration and lyophilization after deprotection (at 30 °C and 0 °C for **6i**, **6k** and **6j**, **6l** respectively).

3.2.2. Physicochemical properties of 6′-*O*-lactose-based esters

For LBEs derivatives **6a–l**, some physicochemical properties, such as hydrophilic–lipophilic balance (HLB), octanol–water portion coefficient (logP) (Table 5) and topological polar surface area (TPSA) were calculated. HLB calculated values of LBEs ranging from 9.7 to 12.9, therefore they could be classified as hydrophilic surfactants (HLB > 8) acting as oil-in-water emulsifiers. Regarding logP, both positive and negative values were assessed (from −2.5 to 2.8). In general, the aryl(alkyl) derivatives

(**6i–l**) display lower logP, probably indicates their higher solubility in water, a part for the terphenylacetate ester (**6l**). Due to the different conformations in solution, lactose (alkyl) aromatic esters (**6i–l**) could show peculiar and interesting properties compared to the others (**6a–g**).

Table 5. Calculated physicochemical properties of LBEs surfactants. [a] HLB calculated by Griffin's method for non-ionic surfactants [3]. HLB = 20 × (MW hydrophilic portion/MW). [b] Calculated octanol-water portion coefficient LogP (by OSIRIS Property Explorer) [116].

6	Lactose ester	MW	HLB[a]	logP[b]
a	Caprylate C8	468.5	12.7	−1.3
b	Caprate C10	496.6	12.0	−0.3
c	Undecylenate C11:1	508.6	11.7	−0.1
d	Laurate C12	524.6	11.4	0.6
e	Myristate C14	552.7	10.8	1.5
f	Palmitate C16	580.7	10.3	2.4
g	Linoleate C18:2	604.7	9.9	2.8
h	Linoleneate C18:3	602.7	9.9	2.5
i	Phenylacetate	460.4	12.9	−2.5
j	*p*-Phenylbenzoate	522.5	11.4	−0.9
k	Biphenylacetate	536.5	11.1	−0.9
l	Terphenylacetate	612.6	9.7	0.8

The topological polar surface area (TPSA), was calculated as 195.6 $Å^2$ for all LBEs, suggesting that this value is not altered by the different physicochemical characteristics of the analysed compounds.

3.2.3. Biological tests for 6′-*O*-lactose saturated fatty acid esters and 6′-*O*-lactose aryl(alkyl) esters.

3.2.3.1. Antimicrobial activity

The capacity of LBEs to act as antimicrobial agents was evaluated against Gram-positive (*E. faecalis*, *L. monocytogenes*, *S. aureus*) and Gram-negative (*E. coli* , *K. pneumoniae*, *P. aeruginosa*, *S. enteritidis*) bacteria and *C. albicans* and was compared to gentamicin and parabens (Table 6).

Table 6. MIC values (μg/mL) of the tested lactose esters against selected microorganisms. [a] Fluconazole was used as control for *C. albicans* ATCC 10231.

Lactose esters	*E. faecalis* ATCC 29212	*L.monocytogenes* ATCC 7644	*S. aureus* ATCC 43387	*E. coli* O157:H7 ATCC 35150	*K. pneumoniae* ATCC 13883	*P. aeruginosa ATCC 9027*	*S.enteritidis* ATCC 13076	*C. albicans* ATCC 10231
6a	256	256	256	256	256	>256	256	256
6b	128	256	256	256	256	256	256	128
6d	>256	>256	>256	>256	>256	>256	>256	256
6e	256	>256	>256	>256	>256	>256	>256	256
6f	>256	>256	>256	>256	>256	>256	>256	256
6i	256	256	>256	256	256	>256	256	256
6j	256	256	256	256	256	256	256	256
6k	256	256	256	256	256	256	256	256
6l	256	256	256	256	256	256	256	256
Gentamicin	64	8	16	16	8	16	4	1 [a]
Parabens	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024

In general, the results obtained with lactose fatty acid esters (**6a,b,d–f**), revealed an inhibition of microbial growth at MICs > 256 μg/mL. Among all LBEs, lactose caprylate **6b** was the most effective, with a MIC of 128 μg/mL versus *E. faecalis* and *C. albicans* , and 256 μg/mL for the other bacteria. It was noted a general reduction of inhibition activities with increasing length of the chain, as also reported in literature [14]. On the other hand, lactose aryl(alkyl) esters (**6i–l**), displayed MICs of 256 μg/mL for all the tested pathogens (only for **6i** against the two bacteria strains *S. aureus* and *P. aeruginosa* the MICs were not established). Moreover, they showed comparable MIC values to the corresponding saturated medium-length chain esters (C8–C12). Notably, the antimicrobial activities of LBEs with aromatic moiety is reported for the first time. Regarding the internal control, gentamicin inhibited the growth of *S. enteritidis* with the lowest MIC value of 4 μg/mL, while the highest MIC (64 μg/mL) was observed for *E. faecalis* and the growth of *C. albicans* was inhibited by fluconazole at MIC of 1 μg/mL. Importantly, all tested LBEs of this first group resulted more active than the parabens mixture, which showed MIC values >1024 μg/mL for all the examined bacteria and fungi.

3.2.3.2. Antibiofilm inhibition and cytotoxicity assay

Biofilm formation processes operated by some microbes represent a great problem for the epidemic infectious disease due to their major resistance to antibiotics and some physical treatments [109]. One possibility to contrast the biofilm growth is to inhibit its formation phase with the SBFAEs herein studied. However, the vulnerability of biofilms to the action of these molecules can change in their different phases. Based on the LBEs antimicrobial activity previously assessed, lactose caprate **6b** and lactose biphenylacetate **6k** were selected to study the inhibition effect on biofilm formation of four bacteria strains (*E. coli*, *L. monocytogenes*, *S. aureus* and *S. enteritidis*) after 24 h, 48 h and 5 days of their incubation at MIC and 2X MIC concentrations. The percentage values of inhibition of the biofilm formation generated by the two LBEs have been evaluated, and 30%-50% was considered a good percentage of antibiofilm activity (Table 7). Lactose caprate **6b** inhibited the biofilms formation only after five days, but it was not active versus *S. enteritidis*. On the contrary, lactose biphenylacetate

6k exhibited promising results, being active just after 24 h against the biofilm formation of most of the bacteria. The best result was obtained against *E. coli* after 5 days (MIC = 85.2%, 2X MIC = 92.0%), but consistent antibiofilm properties were achieved just after 24 h (MIC = 40.1%, 2X MIC = 47.0%) and 48 h (MIC = 61%, 2X MIC 66%). Excellent results were also obtained against *L. monocytogenes*, but only after 5 days (MIC = 76.1%, 2X MIC = 78.8%).

Table 7**.** Percentage values of biofilm formation inhibition after 24 h, 48 h and 5 days of incubation with lactose caprate **6b** and lactose biphenylacetate **6k** at their relative MIC and 2× MIC values. In bold are indicated the percentage values > 30% herein considered as index of good antibiofilm activity.

	24 h		**48 h**		**5 days**	
	MIC	2× MIC	MIC	2× MIC	MIC	2× MIC
	6b					
E. coli O157:H7 ATCC 35150	7.5%	11.7%	9.7%	26.8%	**35.5%**	**43.6%**
L. monocytogenes ATCC 7644	9.6%	16.7%	13.5%	27.8%	15.4%	**48.9%**
S. aureus ATCC 43387	6.7%	18.1%	21.1%	24.7%	**41.4%**	**55.3%**
S. enteritidis ATCC 13076	3.6%	12.8%	3.6%	14.8%	11.0%	21.5%
	6k					
E. coli O157:H7 ATCC 35150	**40.1%**	**47.0%**	**61.3%**	**65.7%**	**85.2%**	**92.0%**
L. monocytogenes ATCC 7644	19.4%	27.1%	27.0%	**34.7%**	**76.1%**	**78.8%**
S. aureus ATCC 43387	15.4%	**39.0%**	22.5%	**40.3%**	**33.0%**	**53.9%**
S. enteritidis ATCC 13076	20.6%	**38.4%**	**45.4%**	**52.2%**	**48.1%**	**53.3%**

Overall, this data indicates that the lactose biphenylacetate restricts the initial formation of the biofilm and persists to interfere with its subsequent maturation. The cytotoxicity of this two LBEs was assessed by MTT assay on Caco-2 cells (Figure 12). Notably, the cytotoxic effect depends on numerous factors such as the capacity of the hydrocarbon chain to arrange into the lipid bilayer [117]. After 24 h, both LBEs did not exhibit toxicity to Caco-2 cells at lower tested concentrations (0.01–0.1 mM), while a slight reduction in cell viability was detected for **6b** at 0.5 mM (248 μg/mL, 73.0%). On the contrary, highest

concentrations (1 and 2 mM) were associated with a decrease in cell viability for both LBEs. Importantly, it can be observed that at MICs values, no significant toxicity was noticed for LBEs on this cell line. This cytotoxicity study represents a preliminary examination of the LBEs toxicological profile, which should be proved by additional studies.

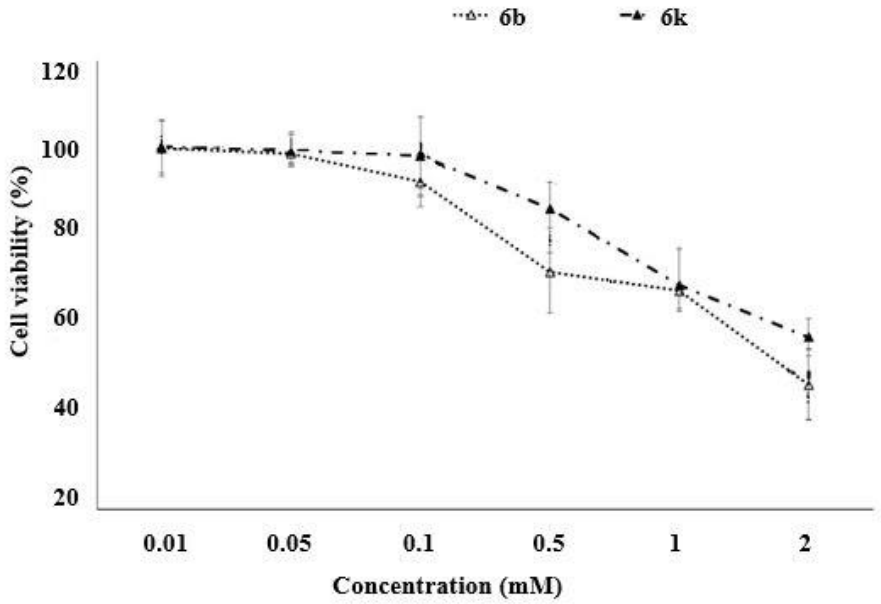


Figure 12. MTT assay of LBEs on Caco-2 cell lines.

3.2.4. Biological tests for 6′-*O*-lactose (poly)unsaturated esters

3.2.4.1. Antimicrobial activity

The antibacterial activity of the lactose undecylenate (**6c**), lactose linoleate (**6g**) and lactose linolenate (**6h**) was evaluated against *S. aureus* and *P. aeruginosa* strains (Table 8). Unfortunately, lactose undecylenate was not able to inhibit the growth of the tested bacteria strains (MIC > 1024 μg/mL). Similar results were obtained with lactose linoleate and lactose linoleneate, which showed none or neglectable inhibitory effect against *S. aureus* and *P. aeruginosa* strains, with MIC values of 1024 or > 1024 μg/mL. Conversely, other monounsaturated lactose esters reported by our research group, such as lactose palmitoleate (C16:1) and lactose nervonate (C24:1), were able to inhibit the growth of Gram-positive and Gram-negative bacteria, showing MIC values between 64 and 128 μg/mL [67]. On the other hand, LBEs **6c**, **6g** and **6h**

displayed interesting antifungal activity versus *C. albicans* and filamentus fungi (Table 9). In this study, the undecylenic acid (UA) was used as internal control due to its reported antifungal activities [118, 119]. In fact, it was reported that UA caused the deformation of cell surface and inhibited the adhesion capacity, mitochondrial activity, and cell proliferation of *C. albicans*. Regarding the mechanism of action of UA, it was assumed that the fungal cell membrane represents one of its targets, indicating that enzymes or metabolites linked with lipid homeostasis may be altered by fatty acids. Additionally, these explanations were confirmed by the antimicrobial activity of different SBFAEs [120, 121].

Table 8. MIC value (μg/mL) of lactose undecylenate (**6c**), lactose linoleate (**6g**) and lactose linolenate (**6h**) assessed against *S. aureus* and *P. aeruginosa* strains.

	6c	**6g**	**6h**
S. aureus HCS026	>1024	1024	1024
S. aureus 2/5	>1024	1024	1024
S. aureus 28/10	>1024	1024	1024
S. aureus 18/9	>1024	1024	1024
S. aureus MRSA HCS002	>1024	1024	>1024
S. aureus ATCC 43300	>1024	>1024	>1024
S. aureus ATCC 43387	>1024	1024	1024
P. aeruginosa C86	>1024	1024	>1024
P. aeruginosa ATCC 27583	>1024	1024	>1024

Concerning the obtained results, lactose undecylenate (**6c**) showed to be active only versus *T. rubrum* F2 and *C. albicans* while its correspondent UA was active against all fungi with MIC values ranging from 128 to 512 μg/mL. Better results were assessed with lactose linoleate (**6g**) and lactose linolenate (**6h**). In detail, both lactose polyunsaturated esters were able to inhibit the growth of *T. mentagrophytes* F6 and *E. floccosum* F12 with a same MIC value (256 for *T. mentagrophytes* F6 and 128 μg/mL *E. floccosum* F12), while against *C. albicans* lactose linoleate **6g** resulted more active than **6h** with a MIC value of 256 μg/mL. However, the best inhibition activity versus

C. albicans (128 μg/mL) observed can be ascribed to the lactose oleate C18:1 (previously synthetized by our research group [11]), probably indicating that a progressive decrease of the number of the unsaturation in the same side hydrophobic chain (C18) lead to an improved inhibitory effect.

Table 9. MIC (μg/mL) value of antifungal activities of lactose undecylenate (**6c**), lactose linoleate (**6g**) and lactose linolenate (**6h**) assessed against filamentous fungi and *C. albicans strains*. Undecylenic acid (UA) was used as internal control.

	UA	**6c**	**6g**	**6h**
T. mentagrophytes F6	512	> 1024	256	256
T. rubrum F2	256	512	>512	>512
T. violaceum F11	512	> 1024	>512	>512
E. floccosum F12	256	> 1024	**128**	**128**
C. albicans ATCC 10231	128	512	256	>512

3.2.4.2. Anti-inflammatory properties

Nitric oxide (NO) is a mediator and regulator of the inflammatory response. In fact, the exposure of inflammatory cells to microbial agents, such as bacterial lipopolysaccharide (LPS), induces the expression of inducible nitric oxide synthase (iNOS), which in turn increases NO production [122]. Here, the anti-inflammatory activity of lactose unsaturated fatty acid esters has been evaluated by Griess assay. The application of LPS to RAW 264.7 cells (murine macrophages) led to NO production and release in the culture medium, while NO release could not be detected in untreated control cells (CTR). When cells were pre-treated for 2 h with these LBEs, a dose-dependent reduction in LPS-induced NO release was detected (Figure 13). The major inhibitions of NO release were achieved at 50 μM for all tested LBEs, following this order: lactose linoleate **6g** (–60%) > lactose linolenate **6h** (–50%) > lactose undecylenate **6c** (–40%). However, the assessment of cell viability by SRB test displayed that NO reduction by lactose linoleate (**6g**) was partly owing to an affiliated decrease of cell viability at just 25 μM, as well as for lactose undecylenate at 50 μM (**6c**) (Figure 14). Consequently, lactose linolenate **6h** was the best compound to reduce NO release by LPS-activated macrophages without remarkable cytotoxic effects,

representing a potential anti-inflammatory surfactant versus microbial infections. Eventually, it is worth mentioning that this was the first study regarding the anti-inflammatory properties of 6′-*O*-lactose-based fatty acid esters surfactants.

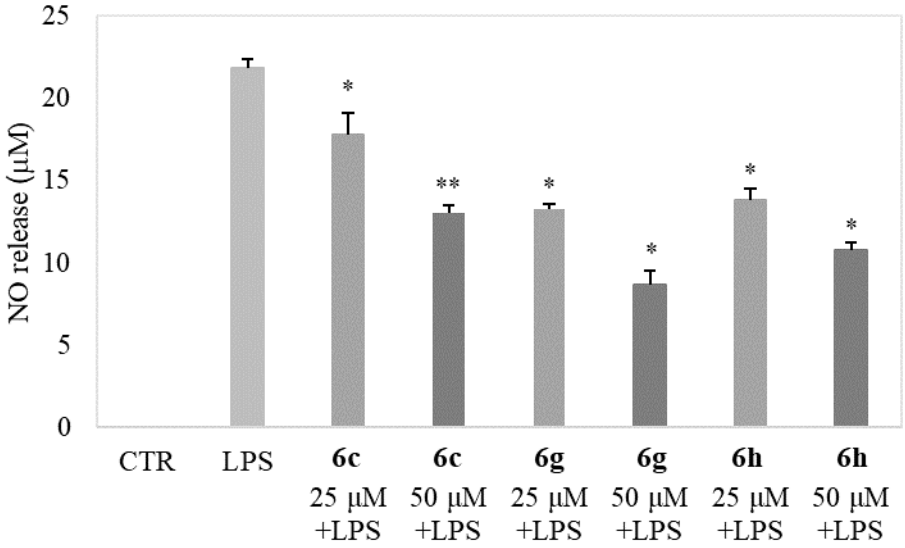


Figure 13. Assessment of the anti-inflammatory properties of LBEs **6c**, **6g** and **6h** versus LPS-induced NO production in RAW 264.7 cells by the reagent Griess. * $p < 0.05$ vs. LPS, ** $p < 0.01$ vs. LPS.

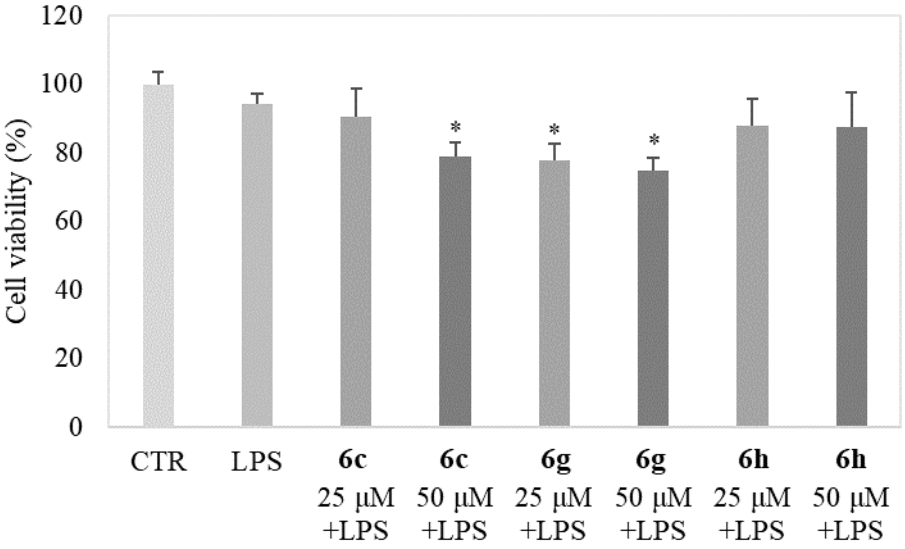


Figure 14. Evaluation of cell viability after LBEs **6c**, **6g** and **6h** applications to RAW 264.7 cell lines stimulated with LPS (SRB test). * $p < 0.05$ vs. LPS, ** $p < 0.01$ vs. LPS.

3.2.4.3. Antioxidant properties

The application of H_2O_2 to cultured human keratinocyte (HaCaT) cell lines led to an important increase of 2′,7′-dichlorofluorescein (DCF) fluorescence emission, representing augmented intracellular oxidation levels as compared to untreated cells (Figure 15).

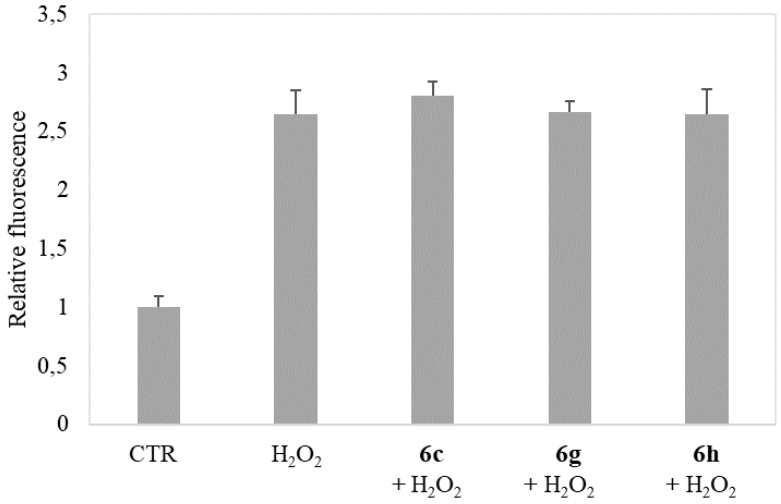


Figure 15. Assessment of the antioxidant ability of LBEs **6c**, **6g** and **6h** versus H_2O_2-induced oxidation in HaCaT cell lines by the probe DCFH-DA.

When cells were pre-treated for 2 h with **6c**, **6g**, and **6h** at 100 μM, no appreciable reduction of H_2O_2-induced oxidation was detected, suggesting that these surfactants did not display antioxidant protective ability against H_2O_2.

3.2.4.4. Cytotoxicity assay

Potential cytotoxicity of lactose (poly)unsaturated esters administration has been analyzed in HaCaT cell lines by SRB assays (Figure 16). Lactose linoleate (**6g**) and lactose linolenate (**6h**) showed a significant reduction of cell viability only at the maximum tested dose of 200 μM, while lactose undecylenate (**6c**) was not cytotoxic in the range of concentrations tested.

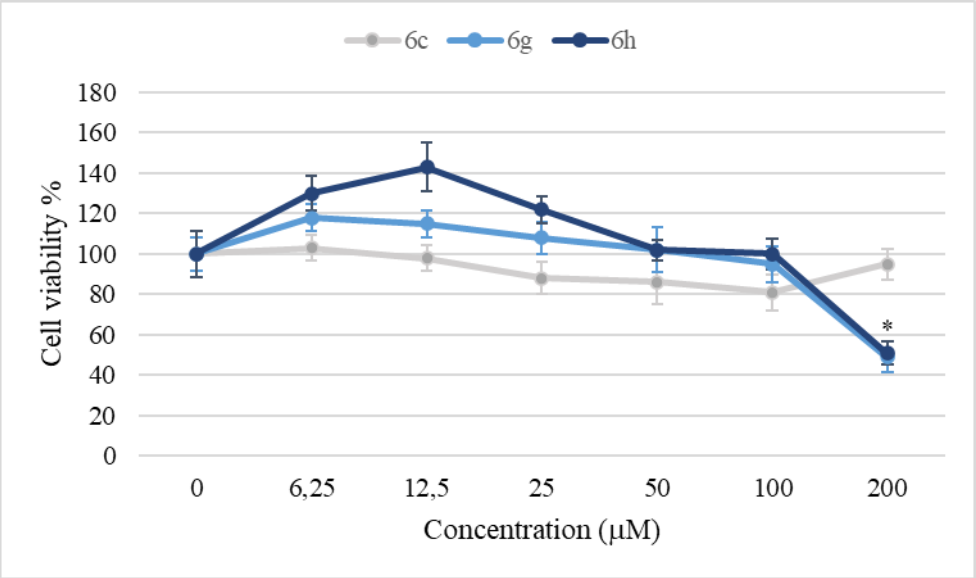


Figure 16. HaCaT cell viability after LBEs **6c**, **6g** and **6h** administration evaluated by SRB assay. * $p < 0.05$ vs. control.

3.2.5. *In vivo* wound healing studies

Wound healing is a complex bioprocess that comprises successively connected hemostasis, inflammation, re-epithelialization, and tissue maturation steps, which critically influence health-care providence [123, 124]. Overstated and unrestrained oxidative damage and the consequent pathological uncontrolled inflammation are the primary causes responsible for delayed and uncoordinated wound healing [125]. Wound medication including chitosan have been widely used due to their biodegradability, stability, and multimodal advantageous activities for wound care [126, 127]. In fact, chitosan-based wound sprays, gels, patches and bandages are commercially available as safe and easily biopolymer wound medications [127]. Probably, these biomaterials could open new perspective in skin wound care. In this chapter, chitosan (C), and sucrose laurate (SL), lactose linoleate (**6g**) and lactose linolenate (**6h**) were selected to evaluate their capacity to contribute at *in vivo* wound healing processes in male Wistar albino rats. The two mentioned LBEs were chosen due to their better antimicrobial and anti-inflammatory activities compared to the lactose undecylenate **6c**. Formulation of single compounds or binary and triple combinations of these sugar esters and chitosan have been designed and probed for this purpose and compared to Healosol® (a commercial spray product containing phenytoin). Moreover, the mechanism of wound healing associated with these

biomaterials was investigated by profiling the expression of the key factors of Wnt/β-catenin signaling pathway, because of the crucial role of this signaling pathway at molecular level in wound healing [128]. Briefly, Wnt signaling is started by the bond of Wnt ligands, such as Wnt-1, with their receptors, causing a cytoplasmic and nuclear growth of β-catenin which eventually initiates target genes (e.g., c-myc) that acts in many cellular processes [129]. Additionally, β-catenin was involved in epidermal cell proliferation, differentiation, and migration, accelerating the wound healing [130]. Wnt-1 activates the β-catenin-dependent Wnt pathway, leading to an increased production of β-catenin, which was found to regulate wound repair and regeneration of the skin [131], while the Wnt-2 ligand was associated with skin fibrosis [132]. Conversely, its downregulation was reported to block fibrosis in human keloid fibroblast [133]. This study represents the first evaluation of *in vivo* wound healing processes involved in the interaction with the key factors of Wnt/β-catenin signaling pathway applying lactose polyunsaturated fatty acid esters and their relative combination with chitosan (C) and sucrose laurate (SL). The wound area and closure in different groups were assessed at 3, 5, 7 and 15 days after damage. The healing course was faster when the tested wound care solutions of lactose linoleate **6g**, lactose linolenate **6h** and chitosan, both in single and combination formulation, were utilized in the wound. On the contrary, a little wound closure was also observed on day 15 post treatment. Remarkably, sucrose laurate solution and its combinations exhibited better healing responses with almost complete closure of wounds at the end of the test. In relation to the onset of healing response results, solutions of single components; sucrose laurate (SL), lactose linoleate (**6g**) and lactose linolenate (**6h**) showed a significant quick onset of healing response at 3 days after injury (Figure 17A). They were shown a considerably faster healing response than those obtained with chitosan (C) and Healosol® as well as the positive control untreated wounds. Nevertheless, in comparison to solutions of single molecules, their multiple combinations were found to be more effective in increasing the rate of wound healing. Particularly, the time for 50% reduction of wound area was between 3 and 5 days and 5 and 7 days after wound generation for solutions of tested single forms and Healosol® were observed respectively (Figure 17A).

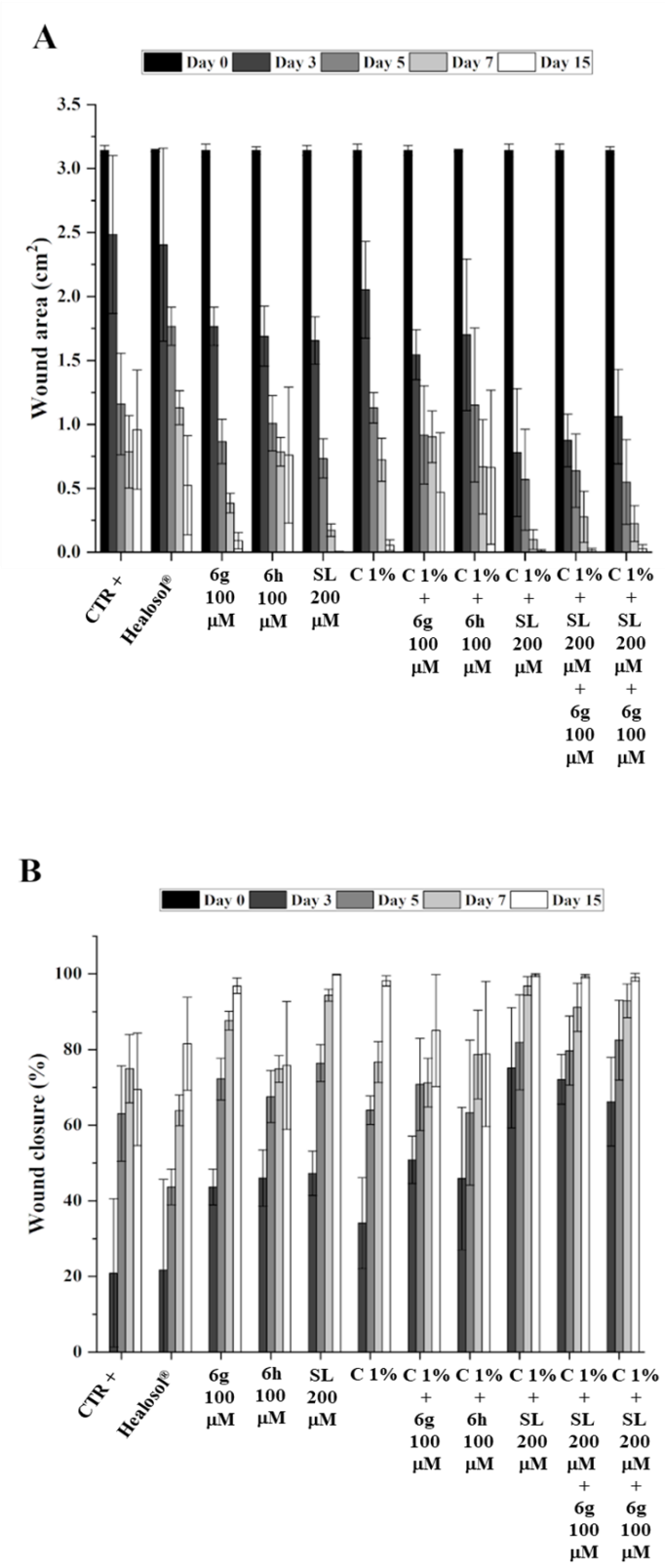


Figure 17. Effects of sugar derivatives and their combinations on wound area and on percent of wound closure as a function of time in different treatment groups. Data are presented as mean ± SEM (n = 6). (A) Wound area, (B) Wound closure.

Relatively, more than 50% reduction in the wound area was achieved with rats treated with all multiple combinations at 3 days after injury, not including chitosan and lactose linolenate combination, which showed 50% wound contraction between 3 and 5 days (Figure 17A). Unfortunately, the wound closure in treated groups with chitosan, lactose linoleate (**6g**), lactose linolenate (**6h**) solutions, and their combinations was lower than with sucrose laurate treated groups. The lowest efficacy and partly healed tissue were observed with the rats treated with lactose linolenate (**6h**), binary mixture of lactose linolenate (**6h**) and chitosan (C), and Healosol® displaying only 78.8%, 84.8%, and 83.3% wound closure at the end of the test respectively (Figure 17B). In contrast the treated group with sucrose laurate (SL) solution and its combinations showed a significant restorative effect (> 90% wound closure in 7 days) and wound recovery (15 days after injury). In summary, sucrose laurate and its combinations exhibited the best results in wound care in terms of increased wound rate.

Successively, the effect of these sugar esters was investigated on the expression of key factors (Wnt-1, Wnt-2 c-myc and β-catenin) in Wnt/β-catenin signaling pathway. Sucrose laurate (SL) and lactose linoleate **6g** both in single and combined formulations showed increased gene expressions in treated groups. On the other hand, lactose linolenate **6h** led to a considerable increase in c-myc gene expression with no significant rise in Wnt-1 and β-catenin gene expression in treated groups as compared to the untreated wound group (positive control). Notably, no significant increase was assessed with chitosan and Healosol® in Wnt-1, c-myc and β-catenin genes expression as compared to untreated wound group (positive control). Indeed, chitosan (C) and Healosol® showed a considerable decrease in Wnt-1 and in β-catenin gene expression respectively, as compared to the unwounded group (negative control).

Therefore, these results highlighted the greater beneficial effects of the SBFAEs tested mainly for sucrose laurate and lactose linoleate. Remarkably, sucrose laurate (SL) in single and combined formulations showed the highest increase in Wnt-1, c-myc, and β-catenin genes expression. However, this surfactant led to a substantial reduction in Wnt-2 gene expression in single or combined formulations as compared to the untreated wound group (positive control) (Figure 18).

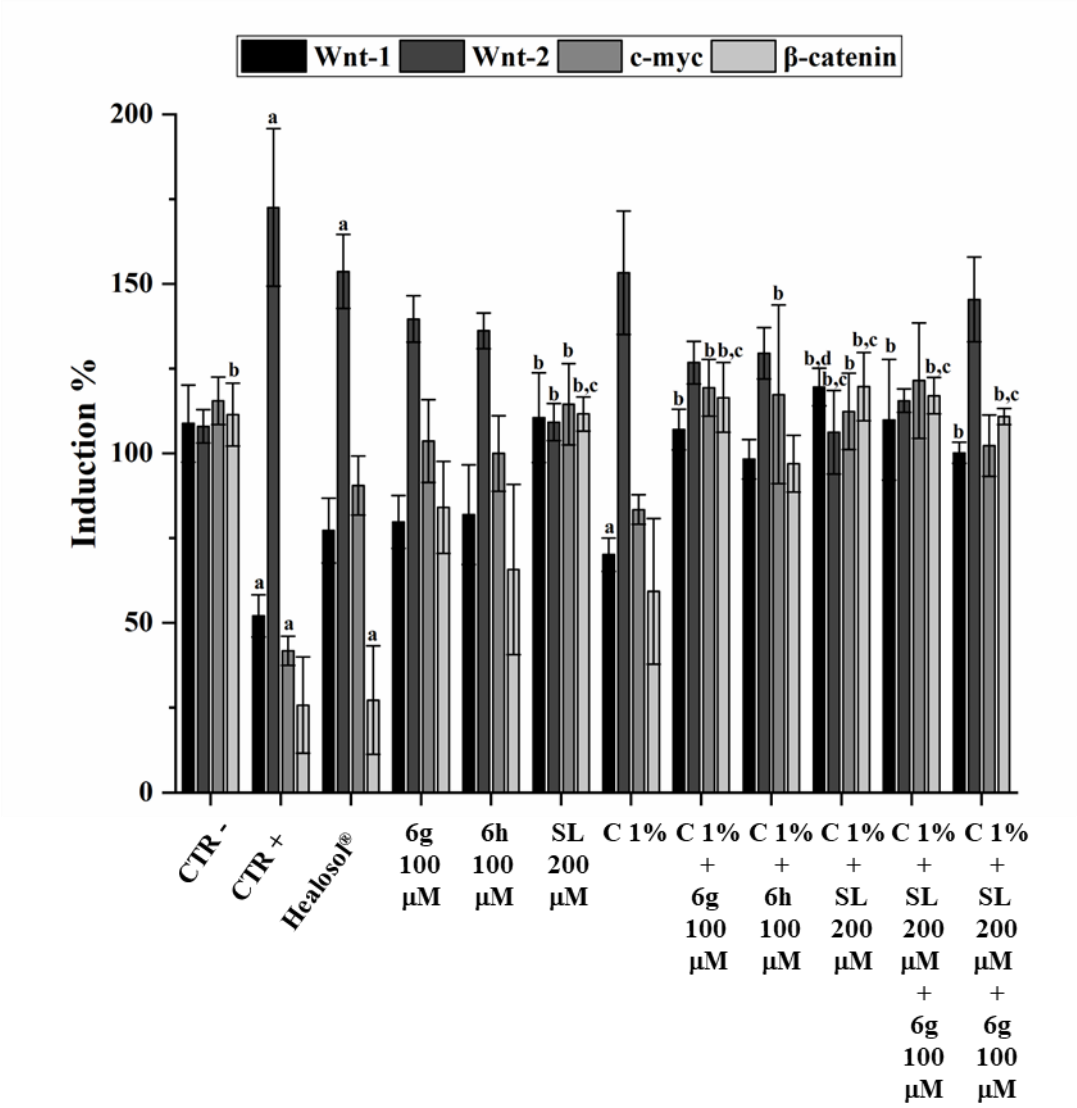


Figure 18. Effects of sugar derivatives and their combinations on Wnt/β-catenin signaling expression genes. Data are presented as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's test as post-hoc test. a) Significantly different from Control group at p <0.05; b) Significantly different from Positive control group at p <0.05; c) Significantly different from Healosol® group at p <0.05; d) Significant different from Chitosan group at p <0.05.

3.3. Conclusion

A small library of 6′-*O*-lactose esters was obtained using three different esterification pathways starting from previously synthesized LTA. For LTA-aliphatic saturated/mono-unsaturated fatty acid esters an enzymatic esterification catalysed by Lipozyme® lipase was adopted. Regarding LTA-polyunsaturated fatty acid esters, a modified Steglich esterification was found to be the most effective procedure. Finally, the LTA-aryl(alkyl)esters were obtained by a conventional esterification method by

acyl chloride formation. The common final step for all three methods is an acidic deprotection, which leads to the desired 6′-*O*-lactose esters. The antimicrobial activities of 6′-*O*-lactose saturated fatty acid and aryl(alkyl) esters were assessed with a set of different microorganisms, achieving MICs values ranging from 128 to 256 μg/mL. Then, the antibiofilm activity of the most relevant LBEs, i.e., lactose caprate **6b** and lactose biphenylacetate **6k**, was evaluated at different times of development of representative bacteria strains. **6k** was found to increase percentages of inhibition of biofilm formation for all bacteria strains. Particularly, the best result was achieved against *E. coli* with 92.0% of biofilm formation inhibition. Importantly, at MICs values, no toxicity for the selected LBEs was observed on the Caco-2 using the MTT assay. From these results, the LBEs investigated, particularly **6k**, can be proposed as possible antibacterial agents for food and pharmaceutical applications. Regarding, 6′-*O*-lactose unsaturated fatty acid esters, they showed good antifungal activities, with the best results displayed by lactose linoleate **6g** and lactose linolenate **6h** versus *E. floccusum* F12 with MIC value of 128 μg/mL. Even though these LBEs did not show appreciable antioxidant activities, they were found to reduce the release of NO in RAW 264.7 cells induced by LPS. Finally, **6g** and **6h** were tested *in vivo* to evaluate their wound healing effect both alone or as mixtures with sucrose laurate and chitosan. Formulation of lactose linoleate in combination with sucrose laurate and chitosan showed a wound closure above 90% in male Wistar albino rats associated with the repair of the Wnt/β-catenin signaling, which controls stem cells to induce epidermal cell proliferation accelerating wound healing. It is worth underlining that this novel combination employed in wound dressing could represent a new frontier in skin wound care.

3.4. Materials and methods

3.4.1. Chemicals

Chitosan chloride (Chitoceuticals® Chitosan HCl code 54040) was purchased from Heppe Medical Chitosan GmbH (Halle, Germany), linolenic and linoleic acids from Fluorochem (Hadfield, UK), caprylic, capric, lauric, myristic and palmitic acids, triethylamine (TEA) anhydrous from TCI (Zwijndrecht, Belgium), lactose monohydrate and 4-(dimethylamino)pyridine (DMAP) from Carlo Erba (Milan, Italy).

Sucrose monolaurate, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl), Lipozyme® (immobilized from *Mucor miehei*), *p*-TSA·H_2O, 2,2-dimethoxypropane, tetrafluoroboric acid diethyl ether complex ($HBF_4 \cdot Et_2O$), *p*-bromophenyl benzene, 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2'-bis-1,3,2-dioxaborolane. [bis(pinacolate)diboron (B_2pin_2), [1,1'-bis(diphenylphosphino)ferrocene] dichloropalladium (II) [Pd(dppf)Cl_2], 1,1′-bis(diphenylphosphino) ferrocene (dppf), tris(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba)_3$], tricyclohexylphosphine [$P(Cy)_3$], undecylenic, phenylacetic, biphenylacetic, *p*-phenylbenzoic acids, and all organic solvents were purchased from Sigma-Aldrich (Milan, Italy). Healosol® spray (phenytoin) was obtained from the Egyptian company for advanced pharmaceuticals (Egypt). Prior to use, acetonitrile and dichloromethane were dried with molecular sieves with an effective pore diameter of 4 Å. The structures of compounds were unambiguously assessed by MS (ESI), ^{1}H NMR, ^{13}C NMR. MS (ESI) spectra were recorded with a Waters Micromass ZQ spectrometer in a negative or positive mode using a nebulizing nitrogen gas at 400 L/min and a temperature of 250 °C, cone flow 40mL/min, capillary 3.5 kV and cone voltage 60 V; only molecular ions $[M - H]^-$ or $[M + NH_4]^+$, $[M + Na]^+$ and $[M + HCOO]^-$ are given. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 400 and 101, respectively, spectrometer and analysed using the TopSpin 1.3 software package. Chemical shifts were measured by using the central peak of the solvent. Column chromatography purifications were performed under "flash" conditions using Merck 230–400 mesh silica gel. TLC was carried out on Merck silica gel 60 F254 plates, which were visualized by exposure to ultraviolet light and to an aqueous solution of ceric ammonium molybdate.

3.4.2. General procedure for the synthesis of 4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-O-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose (lactose tetra acetal, LTA, 2)

p-TSA·H_2O (0.056 g, 0.324 mmol) was added to a suspension of lactose monohydrate (8.170 g, 22.2 mmol) in 2,2-dimethoxypropane (58.42 mL, 477.4 mmol). The mixture was heated up to reflux and stirred at reflux temperature for 2 h. The mixture was cooled at rt, Amberlyst IR-400 was added, and the mixture stirred for further 10 minutes. Then it was filtered to remove the resin and the filtrate was concentred under

vacuo. Purification of the residue by column chromatography (cyclohexane/EtOAc 6:4) gave the product **2** as white solid [112].

Yield: 59% (6.652 g). ^{1}H NMR. (400 MHz, $CDCl_3$): δ = 1.27 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 3.45 (s, 3H, OCH_3), 3.46 (s, 3H, OCH_3), 3.47 (m, 1H, H2′), 3.48 (m, 1H, H3′), 3.74 (m, 1H, H4), 3.86 (m, 1H, H3), 3.65 (m, 1H, H6′a), 3.92 (m, 1H, H6′b), 3.94 (m, 1H, H4′), 4.02 (m, 1H, H5), 3.98 (m, 1H, H6a), 4.16 (m, 1H, H6b), 4.28 (m, 1H, H-5′), 4.33 (d, 1H, H1), 4.39 (d, 1H, $J_{H1'\text{-}H2'}$ = 8.3 Hz, H1′), 4.56 (dd, 1 H, $J_{H1\text{-}H2}$ = 6.7 Hz, $J_{H2\text{-}H3}$ = 8.0 Hz, H2) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.8, 25.5, 26.1 (2C), 27.0, 28.0, 54.3, 57.4, 62.3, 64.4, 73.4, 74.0, 74.5, 75.2, 75.7, 77.4, 78.1, 79.3, 103.3, 107.0, 108.2, 109.8, 110.4 ppm.

3.4.3. General procedure for the synthesis of 6′-*O*-lactose tetra acetal fatty acid esters (5a–f)

Lipozyme® (0.078 g) was added to a solution of lactose tetra acetal **2** (0.79 mmol) and the appropriate fatty acid (**3a–f**) (0.79 mmol) and (0.402 g, 0.79 mmol) in toluene (0.5 mL, 1.58 M) at room temperature [112]. The mixture was stirred at 75 °C for 12 h, cooled and diluted with acetone, then the lipase was filtered, and the filtrate was concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 8:2) gave **5a–f** as pale-yellow oils.

6′-*O*-capryl-4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose, LTA caprylate (**5a**) [112]

Yield = 55% (0.275 g). MS (ESI): 652 $[M + NH_4]^+$, 679 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.92 (t, 3H, *J* = 6.7 Hz, CH_3), 1.31 (s, 6H, 2 CH_3), 1.33–1.37 (m, 18H), 1.39 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.61–1.67 (m, 2H, CH_2CH_2COOR), 2.40 (t, 2H, *J* = 7.0 Hz, CH_2COOR), 3.45–3.47 (m, 6H, OCH_3), 3.47 (dd, 1H, $J_{H2'\text{-}H3'}$ = 7.1 Hz, $J_{H2'\text{-}H1'}$ = 8.0 Hz, H2′), 3.91 (dd, 1H, $J_{H4\text{-}H3}$ = 1.0 Hz, $J_{H4\text{-}H5}$ = 5.0 Hz, H4), 4.04 (ddd, 1H, $J_{H5'\text{-}H6a'}$ = 1.0 Hz, $J_{H5'\text{-}H4'}$ = 2.0 Hz, $J_{H5'\text{-}H6b'}$ = 7.0 Hz, H5′), 4.05 (dd, 1H, $J_{H6b\text{-}H5}$ = 6.0 Hz, $J_{H6b\text{-}H6a}$ = 8.5 Hz, H6b), 4.08 (dd, 1H, $J_{H3'\text{-}H4'}$ = 5.5 Hz, $J_{H3'\text{-}H2'}$ = 7.0 Hz, H3′), 4.15 (dd, 1H, $J_{H3\text{-}H4}$ = 1.0 Hz, $J_{H3\text{-}H2}$ = 7.5 Hz, H3),

4.17 (dd, 1H, J_{H6a-H5} = 6.0 Hz, $J_{H6a-H6b}$ = 8.5 Hz, H6a), 4.22 (dd, 1H, $J_{H4'-H5'}$ = 2.0 Hz, $J_{H4'-H3'}$ = 5.5 Hz, H4′), 4.27 (dd, 1H, $J_{H6b'-H5'}$ = 7.0 Hz, $J_{H6b'-H6a'}$ = 11.5 Hz, H6′b), 4.30 (dd, 1H, $J_{H6a'-H5'}$ = 1.0 Hz, $J_{H6a'-H6b'}$ = 11.5 Hz, H6′a), 4.30 (ddd, J_{H5-H4} = 5.0 Hz, $J_{H5-H6a} \cong J_{H5-H6b}$ = 6.0 Hz, H5), 4.41 (d, 1H, J_{H1-H2} = 6.0 Hz, H1), 4.51 (d, 1H, $J_{H1'-H2'}$ = 8.0 Hz, H1′), 4.51 (dd, 1H, J_{H2-H1} = 6.0 Hz, J_{H2-H3} = 7.5 Hz, H2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.0, 22.3, 24.2, 24.6, 25.1, 25.5, 25.6, 26.2, 27.0, 28.8, 29.0, 29.1, 29.2, 29.3, 29.3, 29.4, 31.7, 33.5, 53.0, 55.1, 63.1, 65.5, 70.8, 73.3, 73.6, 75.4, 76.4, 76.8, 77.6, 79.4, 103.1, 105.7, 108.5, 109.7, 109.9, 173.8 ppm.

6′-*O*-caproyl-4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl- D-glucopyranose, LTA caprate (**5b**) [112]

Yield: 53% (0.275 g). MS (ESI): 680 $[M + NH_4]^+$, 707 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.92 (t, 3H, *J* = 6.7 Hz, CH_3), 1.32 (s, 6H, 2 CH_3), 1.33–1.37 [m, 12H, $(CH_2)_6$], 1.39 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.61–1.67 (m, 2H, CH_2CH_2COOR), 2.40 (t, 2H, *J* = 7.0 Hz, CH_2COOR), 3.46 (s, 6H, 2 OCH_3), 3.47 (dd, 1H, $J_{H2'–H3'}$ = 7.0 Hz, $J_{H2'–H1'}$ = 8.0 Hz, H2′), 3.91 (dd, 1H, $J_{H4–H3}$ = 1.2 Hz, $J_{H4–H5}$ = 5.0 Hz, H4), 4.04 (ddd, 1H, $J_{H5'–H6'a}$ = 1.5 Hz, $J_{H5'–H4'}$ = 2.1 Hz, $J_{H5'–H6'b}$ = 6.8 Hz, H5′), 4.05 (dd, 1H, $J_{H6b–H5}$ = 6.0 Hz, $J_{H6b–H6a}$ = 8.7 Hz, H6b), 4.08 (dd, 1H, $J_{H3'–H4'}$ = 5.5 Hz, $J_{H3'-H2'}$ = 7.0 Hz, H3′), 4.14 (dd, 1H, $J_{H3–H4}$ = 1.2 Hz, $J_{H3–H2}$ = 7.5 Hz, H3), 4.17 (dd, 1H, $J_{H6a–H5}$ = 6.0 Hz, $J_{H6a–H6b}$ = 8.7 Hz, H6a), 4.22 (dd, 1H, $J_{H4'–H5'}$ = 2.1 Hz, $J_{H4'–H3'}$ = 5.5 Hz, H4′), 4.27 (dd, 1H, $J_{H6'b–H5'}$ = 6.8 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6′b), 4.30 (dd, 1H, $J_{H6'a–H5'}$ = 1.5 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.31 (ddd, 1H, $J_{H5–H4}$ = 5.0 Hz, $J_{H5–H6a} \cong J_{H5–H6b}$ = 6.0 Hz, H5), 4.41 (d, 1H, J_{H1-H2} = 6.2 Hz, H1), 4.51 (d, 1H, $J_{H1'-H2'}$ = 8.0 Hz, H1′), 4.51 (dd, 1H, J_{H2-H1} = 6.2 Hz, J_{H2-H3} = 7.5 Hz, H2) ppm. 13C NMR (100 MHz, CD_3OD) δ: 13.0, 22.3, 24.2, 24.6, 25.1, 25.5, 25.6, 26.2, 27.0, 28.8, 29.00, 29.02, 29.2, 31.6, 33.5, 53.0, 55.1, 63.0, 65.5, 70.8, 73.3, 73.5, 75.4, 76.4, 76.8, 77.6, 79.4, 103.1, 105.7, 108.5, 109.7, 109.9, 173.8 ppm.

6′-*O*-Undec-10-enoyl-4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose, LTA undecylenate (**5c**)

Yield: 79% (0.401 g). MS (ESI): 692 $[M + NH_4]^+$, 719 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26–1.31 [m, 8H, $(CH_2)_4$], 1.32–1.35 (m, 8H, 2 CH_3, $CH_2CH_2CH=CH_2$) 1.38 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.58–1.64 (m, 2H, CH_2CH_2COOR), 2.00–2.06 (m, 2H, $CH_2CH=CH_2$), 2.32 (t, 2H, J = 7.5 Hz, CH_2COOR), 3.42 (s, 3H, OCH_3), 3.43 (s, 3H, OCH_3), 3.56 (dd, 1H, $J_{H2'-H3'}$ = 7.0 Hz, $J_{H2'-H1'}$ = 8.0 Hz, H2′), 3.90 (dd, 1H, J_{H3-H4} = 1.5 Hz, J_{H3-H2} = 7.5 Hz, H3), 3.94 (ddd, 1H, J_1 = 2.0 Hz, J_2 = 5.0 Hz, J_3 = 7.0 Hz), 4.01 (dd, 1H, J_1 = 7.0 Hz, J_2 = 9.0 Hz), 4.04–4.10 (m, 2H), 4.11 (dd, 1H, J_1 = 2.0 Hz, J_2 = 5.5 Hz), 4.16 (dd, 1H, J_1 = 6.5 Hz, J_2 = 9.0 Hz), 4.25–4.30 (m, 2H, H5, H6b), 4.35 (dd, 1H, $J_{H6'a-H5'}$ = 5.0 Hz, $J_{H6'a-H6'b}$ = 11.5 Hz, H6′a), 4.37 (d, 1H, J_{H1-H2} = 6.0 Hz, H1), 4.42 (d, 1H, $J_{H1'-H2'}$ = 8.0 Hz, H1'), 4.45 (dd, 1H, J_{H2-H1} = 6.0 Hz, J_{H2-H3} = 7.5 Hz, H2), 4.92 (dddd, 1H, $J_{gem} \cong J_1$ = 1.5 Hz, J_2 = 4.0 Hz, J_{cis} = 10.0 Hz, HCH=CH), 4.98 (dddd, 1H, $J_{gem} \cong J_1 \cong J_2$ = 1.5 Hz, J_{trans} = 17.0 Hz, HCH=CH), 5.80 (dddd, 1H, $J_1 \cong J_2$ = 7.0 Hz, J_{cis} = 10.0 Hz, J_{trans} = 17.0 Hz, HCH=CH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.6, 25.0, 25.8, 26.4, 26.5, 27.4, 28.2, 29.0, 29.17, 29.28, 29.34, 29.4, 33.9, 34.2, 53.3, 56.2, 63.2, 64.8, 71.5, 73.4, 74.3, 75.2, 76.5, 78.0, 78.1, 79.1, 103.8, 105.2, 108.4, 110.4, 110.5, 114.3, 139.2, 173.6 ppm.

6′-*O*-lauroyl-4′-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose, LTA laurate (**5d**) [112]

Yield: 50% (0.274 g). MS (ESI): 708 $[M + NH_4]^+$, 735 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.92 (t, 3H, J = 6.7 Hz, CH_3), 1.32 (s, 6H, 2 CH_3), 1.33–1.37 [m, 16H, $(CH_2)_8$], 1.39 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.62–1.67 (m, 2H, CH_2CH_2COOR), 2.40 (t, 2H, J = 7.0 Hz, CH_2COOR), 3.46 (s, 6H, 2 OCH_3), 3.47 (dd, 1H, $J_{H2'–H3'}$ = 7.0 Hz, $J_{H2'–H1'}$ = 8.0 Hz, H2′), 3.91 (dd, 1H, $J_{H4–H3}$ = 1.2 Hz, $J_{H4–H5}$ = 5.0 Hz, H4), 4.05 (ddd, 1H, $J_{H5'H6a'}$ = 1.2 Hz, $J_{H5'-H4'}$ = 2.1 Hz, $J_{H5'–H6'b}$= 6.8 Hz, H5′), 4.05 (dd, 1H, J_{H6b-H5} = 6.0 Hz, $J_{H6b-H6a}$ = 8.7 Hz, H6b), 4.08 (dd, 1H, $J_{H3'–H4'}$ = 5.5 Hz, $J_{H3'-H2'}$ = 7.0 Hz, H3′), 4.14 (dd, 1H, J_{H3-H4} = 1.2 Hz, J_{H3-H2} = 7.5 Hz, H3), 4.17 (dd, 1H, J_{H6-H5} = 6.0 Hz, $J_{H6a–H6b}$ = 8.7 Hz, H6a), 4.22 (dd, 1H, $J_{H4'–H5'}$ = 2.1 Hz, $J_{H4'–H3'}$ = 5.5 Hz, H4′), 4.27 (dd, 1H, $J_{H6'b–H5'}$ = 6.8 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6'b), 4.30 (dd, 1H, $J_{H6'a–H5'}$ = 1.2 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.31 (ddd, 1H,

$J_{H5–H4}$ = 5.0 Hz, $J_{H5–H6a} \cong J_{H5–H6b}$ = 6.0 Hz, H5), 4.41 (d, 1H, J_{H1-H2} = 6.2 Hz, H1), 4.51 (d, 1H, $J_{H1'-H2'}$ = 8.0 Hz, H1′), 4.51 (dd, 1H, J_{H2-H1} = 6.2 Hz, J_{H2-H3} = 7.5 Hz, H2) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ: 13.0, 22.3, 24.2, 24.6, 25.1, 25.5, 25.7, 26.2, 27.0, 28.8, 29.0, 29.1, 29.2, 29.3, 31.7, 33.5, 53.0, 55.1, 63.1, 65.5, 70.8, 73.3, 73.5, 75.4, 76.4, 76.8, 77.6, 79.4, 103.1, 105.7, 108.5, 109.7, 109.9, 173.8 ppm.

6′-*O*-Myristoyl-4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*- methyl-D-glucopyranose, LTA myristate (**5e**) [112]

Yield: 44% (0.248 g). MS (ESI): 736 $[M + NH_4]^+$, 763 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD) δ: 0.92 (t, 3H, *J* = 6.7 Hz, CH_3), 1.30–1.33 [m, 20H, $(CH_2)_{10}$], 1.35 (s, 6H, 2 CH_3), 1.39 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.61–1.67 (m, 2H, CH_2CH_2COOR), 2.40 (t, 2H, *J* = 7.0 Hz, CH_2COOR), 3.46 (s, 6H, 2 OCH_3), 3.47 (dd, 1H, $J_{H2'-H3'}$ = 7.1 Hz, $J_{H2'-H1'}$ = 8.0 Hz, H2′), 3.91 (dd, 1H, J_{H4-H3} = 1.2 Hz, J_{H4-H5} = 5.0 Hz, H4), 4.04 (ddd, 1H, $J_{H5'-H6'a}$ = 1.0 Hz, $J_{H5'-H4'}$ = 2.2 Hz, $J_{H5'–H6'b}$ = 6.8 Hz, H5′), 4.05 (dd, 1H, J_{H6b-H5} = 6.0 Hz, $J_{H6b-H6a}$ = 8.7 Hz, H6b), 4.08 (dd, 1H, $J_{H3'–H4'}$ = 5.6 Hz, $J_{H3'-H2'}$ = 7.1 Hz, H3′), 4.15 (dd, 1H, J_{H3-H4} = 1.2 Hz, J_{H3-H2} = 7.5 Hz, H3), 4.17 (dd, 1H, J_{H6-H5} = 6.0 Hz, $J_{H6a–H6b}$ = 8.7 Hz, H6a), 4.22 (dd, 1H, $J_{H4'–H5'}$ = 2.2 Hz, $J_{H4'–H3'}$ = 5.6 Hz, H4′), 4.27(dd, 1H, $J_{H6'b–H5'}$ = 6.8 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6′b), 4.30 (dd, 1H, $J_{H6'a–H5'}$ = 1.0 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.31 (ddd, 1H, $J_{H5–H4}$ = 5.0 Hz, $J_{H5–H6a} \cong J_{H5–H6b}$ = 6.0 Hz, H5), 4.41 (d, 1H, J_{H1-H2} = 6.2 Hz, H1), 4.51 (d, 1H, $J_{H1'-H2'}$ = 8.0 Hz, H1′), 4.51 (dd, 1H, J_{H2-H1} = 6.2 Hz, J_{H2-H3} = 7.5 Hz, H2) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ: 13.0, 22.3, 24.2, 24.6, 25.1, 25.5, 25.6, 26.2, 27.0, 28.8, 29.0, 29.1, 29.2, 29.31, 29.34, 29.4, 31.7, 33.5, 53.0, 55.1, 63.1, 65.5, 70.8, 73.3, 73.6, 75.4, 76.4, 76.8, 77.6, 79.4, 103.1, 105.7, 108.5, 109.7, 109.9, 173.8 ppm.

6′-*O*-Palmitoyl-4-*O*-(3′,4′-O-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose, LTA palmitate (**5f**) [112]

Yield: 34% (0.200 g). MS (ESI): 764 $[M + NH_4]^+$, 791 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD) δ: 0.92 (t, 3H, *J* = 6.7 Hz, CH_3), 1.30–1.33 [m, 24H, $(CH_2)_{12}$], 1.35 (s, 6H, 2 CH_3), 1.39 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.60–1.67 (m, 2H, CH_2CH_2COOR), 2.40 (t, 2H, *J* = 7.4 Hz, CH2COOR), 3.46 (s, 6H,

2 OCH_3), 3.47 (dd, 1H, $J_{H2'\text{-}H3'}$ = 7.1 Hz, $J_{H2'\text{-}H1'}$ = 8.0 Hz, H2′), 3.91 (dd, 1H, $J_{H4\text{-}H3}$ = 1.2 Hz, $J_{H4\text{-}H5}$ = 5.0 Hz, H4), 4.04 (ddd, 1H, $J_{H5'H6a'}$ = 1.5 Hz, $J_{H5'\text{-}H4'}$ = 2.2 Hz, $J_{H5'–H6b'}$ = 6.8 Hz, H5′), 4.05 (dd, 1H, $J_{H6b\text{-}H5}$ = 6.0 Hz, $J_{H6b\text{-}H6a}$ = 8.7 Hz, H6b), 4.08 (dd, 1H, $J_{H3'–H4'}$ = 5.5 Hz, $J_{H3'\text{-}H2'}$ = 7.1 Hz, H3′), 4.14 (dd, 1H, $J_{H3\text{-}H4}$ = 1.2 Hz, $J_{H3\text{-}H2}$ = 7.5 Hz, H3), 4.17 (dd, 1H, $J_{H6\text{-}H5}$ = 6.0 Hz, $J_{H6a–H6b}$ = 8.7 Hz, H6a), 4.22 (dd, 1H, $J_{H4'–H5'}$ = 2.2 Hz, $J_{H4'–H3'}$ = 5.5 Hz, H4′), 4.27 (dd, 1H, $J_{H6'b–H5'}$ = 6.8 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6′b), 4.30 (dd, 1H, $J_{H6'a–H5'}$ = 1.5 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.31 (ddd, 1H, $J_{H5–H4}$ = 5.0 Hz, $J_{H5–H6a} \cong J_{H5–H6b}$ = 6.0 Hz, H5), 4.41 (d, 1H, $J_{H1\text{-}H2}$ = 6.2 Hz, H1), 4.51 (d, 1H, $J_{H1'\text{-}H2'}$ = 8.0 Hz, H1′), 4.51 (dd, 1H, $J_{H2\text{-}H1}$ = 6.2 Hz, $J_{H2\text{-}H3}$ = 7.5 Hz, H2) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ: 13.0, 22.3, 24.2, 24.6, 25.1, 25.7, 26.2, 27.0, 28.8, 29.1, 29.2, 29.4, 31.7, 33.5, 53.0, 55.1, 63.1, 65.5, 70.8, 73.3, 73.6, 75.4, 76.4, 76.9, 77.6, 79.4, 103.1, 105.7, 108.5, 109.7, 109.9, 173.8 ppm.

3.4.4. General procedure for the synthesis of 6′-*O*-lactose tetra acetal polyunsaturated fatty acid esters (5g,h)

DMAP (0.048 g, 0.4 mmol) and linoleic acid (**3g**) or linolenic acid (**3h**) (2 mmol) were added to a solution of lactose tetra acetal (LTA, **2**) (1.219 g, 2.4 mmol) in dry DCM (20.0 mL, 0.1 M) at room temperature under N_2 atmosphere. The mixture was cooled at 0 °C and added of dry TEA (0.334 mL, 2.4 mmol) and EDCI·HCl (0.460 g, 2.4 mmol), then stirred at 0 °C for 10 min and at room temperature for 3 days, diluted with DCM, and washed with saturated aqueous NH_4Cl and $NaHCO_3$ solutions. The organic phase was dried on anhydrous Na_2SO_4, filtered, and concentrated. Purification of the residue by column chromatography (cyclohexane/EtOAc 7:3) gave **5g** and **5h** as a pale-yellow oil.

Octadec-9*Z*-12*Z*-dienoyl-4-*O*-(3′,4′-*O*-isopropilydene-β-D-galactopyranosyl) 2,3:5,6-di-*O* isopropilydene-1,1-di-*O*-methyl-D-glucopyranose, LTA linoleate (**5g**)

Yield: 19% (0.292 g). MS (ESI): 789 $[M + NH_4]^+$, 794 $[M + Na]^+$.^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 3H, *J* = 7.0 Hz, CH_3), 1.23–1.40 [m, 26H, 4 CH_3, $(CH_2)_7$], 1.48 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.58–1.67 (m, 2H, CH_2CH_2COOR), 2.02–2.10 (m, 4H, $CH_2CH{=}CHCH_2CH{=}CHCH_2$), 2.33–2.50 (m, 2H, CH_2CH_2COOR), 2.78 (dd, $J_1 = J_2$= 8.0 Hz, $CH_2CH{=}CHCH_2CH{=}CHCH_2$), 3.49 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.64–

3.71 (dd, 1H, $J_{H6a'\text{-}H5'}$ = 1.5 Hz, $J_{H6a'\text{-}H6b'}$ = 11.5 Hz, H6′a), 3.82–3.88 (m, 2H, H3′, H5′), 3.91–3.95 (m, 2H, H4′, H6a), 3.96 (dd, 1H, $J_{H6b'\text{-}H5'}$ = 9.0 Hz, $J_{H6b'\text{-}H6a'}$ = 11.5 Hz, H6b′) 4.07 (dd, 1H, $J_{H6b\text{-}H5}$ = 2.0 Hz, $J_{H6b\text{-}H6a}$ = 5.5 Hz, H6b), 4.07–4.09 (m, 1H, H4), 4.14 (dd, 1H, $J_{H3\text{-}H4}$ = 5.0 Hz, $J_{H3\text{-}H2}$ = 8.0 Hz, H3), 4.30 (ddd, 1H, $J_{H5\text{-}H6b}$=2.0 Hz, $J_{H5\text{-}H4} \cong J_{H5\text{-}H6a}$ = 7.0 Hz,, H5), 4.38 (d, 1H, $J_{H1'\text{-}H2'}$ = 7.0 Hz, H1′), 4.63 (dd, 1H, $J_{H2'\text{-}H1'}$ = 7.0Hz, $J_{H2'\text{-}H3'}$ = 7.5 Hz, H2′), 4.64 (d, 1H, $J_{H1\text{-}H2}$ = 9.0 Hz, H1), 5.06 (dd, 1H, $J_{H2\text{-}H3}$ = 8.0 Hz, $J_{H2\text{-}H1}$ = 9.0 Hz, H2), 5.29–5.43 (m, 4H, C*H*=C*H*CH$_2$C*H*=C*H*) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1, 22.6, 24.6, 24.7, 25.6, 26.2, 26.4, 27.1, 27.2, 27.3, 27.8, 29.0, 29.18, 29.25, 29.3, 29.6, 31.5, 33.8, 53.7, 57.6, 62.3, 64.6, 72.6, 73.8, 73.9, 74.2, 74.9, 77.7, 78.2, 78.3, 100.7, 107.1, 108.0, 110.4, 110.8, 127.9, 128.0, 130.1, 130.2, 172.4 ppm.

Octadec-9*Z*-12*Z*-15*Z*-trienoyl-4-*O*-(3′,4′-*O*-isopropilydene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropilydene-1,1-di-*O*-methyl-D-glucopyranose, LTA linolenate (**5h**)

Yield: 25% (0.372 g). MS (ESI): 786 $[M + NH_4]^+$, 791 $[M + Na]^+$.^{1}H NMR (DMSO-d_6): δ = 0.93 (t, 3H, *J* = 7.5 Hz, CH_3), 1.23–1.32 [m, 20H, 4 CH_3, $(-CH_2-)_4$], 1.33 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.50–1.55 (m, 2H, *CH*$_2$CH$_2$COOR), 2.00–2.09 (m, 4H, *CH*$_2$CH=CH$_2$CH=CHCH$_2$CH=CH*CH*$_2$), 2.30–2.37 (m, 2H, CH$_2$COOR), 2.71–2.80 (m, 4H, CH=CH*CH*$_2$CH=CH*CH*$_2$CH=CH), 3.24 (ddd, 1H, $J_{H2'\text{-}OH2'}$ = 4.5 Hz, $J_{H2'\text{-}H3'} = J_{H2'\text{-}H1'}$ = 7.5 Hz, H2′), 3.32 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 3.63 (d, 1H, *J* = 7.0 Hz), 3.94 (dd, 1H, J_1= 5.5 Hz, J_2 = 8.5 Hz), 3.97–4.01 (m, 2H), 4.05–4.08 (m, 1H), 4.08–4.17 (m, 5H), 4.34 (d, 1H, $J_{H1\text{-}H2}$ = 6.5 Hz, H1), 4.38 (dd, 1H, $J_{H2\text{-}H1} = J_{H2\text{-}H3}$ = 6.5 Hz, H2), 4.41 (d, 1H, $J_{H1'\text{-}H2'}$ = 7.5 Hz, H1′), 5.25–5.38 (m, 6H, C*H*=C*H*CH$_2$*CH=CH*CH$_2$*CH=CH*), 5.39 (d, 1H, $J_{OH2'\text{-}H2'}$ = 4.5 Hz, OH2′) ppm. ^{13} C NMR (DMSO-d_6): δ = 14.5, 21.2, 24.8, 25.6, 25.7, 26.0, 26.7, 27.1 (3C), 27.8, 28.4, 28.9, 28.9, 29.0, 29.4, 33.7, 53.8, 55.7, 63.4, 66.2, 70.3, 72.8, 73.5, 75.3, 76.3, 77.4, 77.5, 79.7, 103.5, 105.6, 108.5, 109.3, 109.6, 127.4, 128.0, 128.4, 128.4, 130.3, 131.9, 173.3 ppm.

3.4.5. General procedure for the synthesis of 6′-*O*-lactose tetra acetal aryl(alkyl) esters (5i–l)

Oxalyl chloride (1.08 mL, 12.80 mmol) and catalytic dry DMF (two drops) were added to the appropriate acid (**3i–l**) (0.8 mmol). The mixture was stirred at room temperature

for 2 h, diluted with DCM, and concentrated to give the desired acyl chloride (**4i–l**) as pale yellow oils, which was used without furhter purifications. The opportune **4i–l** in dry DCM (2.4 mL) was then added dropwise at 0 °C to a solution of DIPEA (0.382 g, 0.51 mL, 2.96 mmol) and LTA (**2**) (0.300 g, 0.59 mmol) in dry DCM (2.4 mL, 0.25 M). The mixture was stirred at 0 °C for 1 h and at room temperature for 16 h, then extracted with DCM. The combined organic layers were washed with $NaHCO_3$ saturated solution, dried on anhydorus Na_2SO_4, filtered, and concentrated. Purification of the residue by column chromatography (cyclohexane/ethyl acetate 8:2) gave **5i–l** as pale yellow oils.

6′-*O*-(2-Phenylethanoyl)-4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl) 2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-d-glucopyranose, LTA phenylacetate (**5i**)

Yield = 83% (0.306 g). MS (ESI): 644 $[M + NH_4]^+$, 649 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.22 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.43 (s, 6H, 2 CH_3), 3.360 (s, 3H, OCH_3), 3.364 (s, 3H, OCH3), 3.48 (dd, 1H, $J_{H2'\text{-}H3'}$ = 7.0 Hz, $J_{H2'\text{-}H1'}$ = 8.0 Hz, H2′), 3.59 (s, 2H, CH_2), 3.84 (dd, 1H, $J_{H3\text{-}H4}$ = 2.0 Hz, $J_{H3\text{-}H2}$ = 7.6 Hz, H3), 3.86 (ddd, 1H, $J_{H5\text{-}H6a}$ = 2.0 Hz, $J_{H5\text{-}H6b} \cong J_{H5\text{-}H4}$ = 6.0 Hz, H5), 3.92–3.95 (m, 2H), 3.97 (dd, 1H, $J_{H3'\text{-}H4'}$ = 5.6 Hz, $J_{H2'\text{-}H3'}$ = 7.0 Hz, H3′), 4.00 (dd, 1H, $J_1 \cong J_2$ = 1.5 Hz), 4.09 (dd, 1H, J_1 = 6.4 Hz, J_2 = 8.8 Hz), 4.22 (ddd, 1H, $J_{H5'\text{-}H6'a}$ = 2.4 Hz, $J_{H5'\text{-}H4'} \cong J_{H5'\text{-}H6'b}$ = 6.5 Hz, H5′), 4.23–4.26 (m, 1H), 4.27 (dd, 1H, $J_{H4\text{-}H3}$ = 2.0 Hz, $J_{H4\text{-}H5}$ = 6.0 Hz, H4), 4.30 (d, 1H, $J_{H1\text{-}H2}$ = 6.0 Hz, H1), 4.34 (d, 1H, $J_{H1'\text{-}H2'}$ = 8.0 Hz, H1′), 4.39 (dd, 1H, $J_{H2\text{-}H1}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 7.6 Hz, H2), 7.19–7.26 (m, 5H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.4, 25.7, 26.2, 26.4, 27.3, 28.1, 41.1, 53.5, 56.2, 63.4, 64.6, 71.2, 73.1, 74.1, 75.2, 76.4, 77.8, 78.0, 78.9, 103.7, 105.3, 108.3, 110.3, 110.3, 127.2, 128.6, 129.2, 133.8, 171.3 ppm.

6′-*O*-[2-(4-Phenyl)benzoyl]-4-*O*-(3′,4′-*O*-isopropylidene- β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene- 1,1-di-*O*-methyl- D -glucopyranose, LTA *p*-phenylbenzoate (**5j**)

Yield = 8% (0.032 g). MS (ESI): 706 $[M + NH_4]^+$, 711 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.26 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 3.27 (s, 3H, OCH_3), 3.28 (s, 3H, OCH_3), 3.54

(dd, 1H, $J_{H2'\text{-}H3'}$ = 7.0 Hz, $J_{H2'\text{-}H1'}$ = 8.0 Hz, H2′), 3.84 (dd, 1H, $J_{H3\text{-}H4}$ = 2.0 Hz, $J_{H3\text{-}H2}$ = 7.6 Hz, H3), 3.94 (dd, 1H, *J*1 = 6.8 Hz, *J*2 = 8.8 Hz), 4.02–4.08 (m, 3H), 4.10 (dd, 1H, J_1 = 6.8 Hz, J_2 = 8.8 Hz), 4.15 (dd, 1H, $J_{H4\text{-}H3}$ = 2.0 Hz, $J_{H4\text{-}H5}$ = 6.0 Hz, H4), 4.19–4.23 (m, 1H), 4.22 (d, 1H, $J_{H1\text{-}H2}$ = 5.6 Hz, H1), 4.41 (d, 1H, $J_{H1'\text{-}H2'}$ = 8.0 Hz, H1′), 4.41 (dd, 1H, $J_{H2\text{-}H1}$ = 5.6 Hz, $J_{H2\text{-}H3}$ = 7.6 Hz, H2), 4.49 (dd, 1H, $J_{H6b'\text{-}H5'}$ = 7.2 Hz, $J_{H6b'\text{-}H6a'}$ = 11.6 Hz, H6b′), 4.56 (dd, 1H, $J_{H6a'\text{-}H5'}$ = 4.8 Hz, $J_{H6a'\text{-}H6b'}$ = 11.6 Hz, H6a′), 7.30–7.33 (m, 1H, ArH), 7.37–7.41 (m, 2H, ArH), 7.53–7.55 (m, 2H, ArH), 7.59 (d, 2H, *J* = 8.5 Hz, ArH), 8.05 (d, 2H, *J* = 8.5 Hz, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 24.5, 24.9, 25.6, 25.7, 26.2, 26.3, 26.4, 27.2, 28.1, 33.9, 49.2, 53.3, 56.4, 64.0, 64.7, 71.6, 73.4, 74.3, 75.2, 76.5, 76.7, 77.2, 77.8, 77.9, 79.0, 103.8, 105.1, 108.3, 110.2, 110.4, 126.8, 127.1, 127.3, 128.2, 128.5, 129.0, 130.3, 139.9, 145.9, 166.2 ppm.

6′-*O*-[2-(4-Phenyl)phenylethanoyl]-4-*O*-(3′,4′-*O*-isopropylidene-β-D galactopyranosyl)-2,3:5,6-di-O-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose, LTA biphenylacetate (**5k**)

Yield = 44% (0.182 g). MS (ESI): 701 $[M - H]^-$, 720 $[M + NH_4]^+$, 725 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.16 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.42 (s, 6H, 2 CH_3), 3.30 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 3.46 (dd, 1H, $J_{H2'\text{-}H3'}$ = 7.0 Hz, $J_{H2'\text{-}H1'}$ = 8.0 Hz, H2′), 3.82–3.87 (m, 3H), 3.91–3.95 (m, 2H), 3.97 (dd, 1H, $J_1 \cong J_2$ = 1.5 Hz), 4.09 (dd, 1H, J_1 = 6.4 Hz, J_2 = 8.8 Hz), 4.22 (ddd, 1H, $J_{H5'\text{-}H6'a}$ = 2.4 Hz, $J_{H5'\text{-}H4'} \cong J_{H5'\text{-}H6'b}$ = 6.8 Hz, H5′), 4.23–4.26 (m, 1H), 4.27 (dd, 1H, $J_{H4\text{-}H3}$ = 2.0 Hz, $J_{H4\text{-}H5}$ = 6.0 Hz, H4), 4.30 (d, 1H, $J_{H1\text{-}H2}$ = 6.0 Hz, H1), 4.34 (d, 1H, $J_{H1'\text{-}H2'}$ = 8.0 Hz, H1′), 4.38 (dd, 1H, $J_{H2\text{-}H1}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 7.6 Hz, H2), 4.99 (s, 2H, CH_2), 7.15–7.27 (m, 9H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 26.0, 26.5, 27.0, 27.1, 27.8, 28.4, 53.9, 55.7, 56.1, 63.8, 66.1, 69.9, 72.7, 73.2, 75.3, 76.3, 77.4, 77.5, 79.5, 103.7, 105.5, 108.5, 109.2, 109.6, 127.6, 128.7, 128.9, 129.0, 139.2, 139.4, 172.2 ppm.

6′-*O*-[2-(4,4′-Biphenyl)phenylethanoyl]-4-*O*-(3′,4′-*O*-isopropylidene- β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose, LTA terphenylacetate (**5l**)

Yield = 17% (0.078 g). MS (ESI): 777 $[M - H]^-$, 796 $[M + NH_4]^+$, 801 $[M + Na]^+$. ^{1}H

NMR (400 MHz, $CDCl_3$): δ = 1.22 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 3.37 (s, 3H, OCH3), 3.38 (s, 3H, OCH3), 3.47–3.51 (m, 1H, H2′), 3.65 (s, 2H, CH_2), 3.84 (dd, 1H, $J_{H3\text{-}H4}$ = 2.0 Hz, $J_{H3\text{-}H2}$ = 7.6 Hz, H3), 3.89 (ddd, 1H, $J_{H5\text{-}H6a}$ = 2.0 Hz, $J_{H5\text{-}H6b} \cong J_{H5\text{-}H4}$ = 6.0 Hz, H5), 3.94 (dd, 1H, $J_{H4'\text{-}H5'}$ = 6.8 Hz, $J_{H3'\text{-}H4'}$ = 8.5 Hz, H4′), 3.96–3.99 (m, 3H), 4.10 (dd, 1H, $J_{H3'\text{-}H2'}$ = 6.4 Hz, $J_{H3'\text{-}H4'}$ = 8.5 Hz, H3′), 4.22 (ddd, 1H, $J_{H5'\text{-}H6'a}$ = 2.0 Hz, $J_{H5'\text{-}H4'} \cong J_{H5'\text{-}H6'b}$ = 6.5 Hz, H5′), 4.25–4.33 (m, 2H), 4.31 (d, 1H, $J_{H1\text{-}H2}$ = 6.4 Hz, H1), 4.36 (d, 1H, $J_{H1'\text{-}H2'}$ = 8.0 Hz, H1′), 4.41 (dd, 1H, $J_{H2\text{-}H1}$ = 6.4 Hz, $J_{H2\text{-}H3}$ = 7.6 Hz, H2), 7.27–7.32 (m, 3H, ArH), 7.36–7.41 (m, 2H, ArH), 7.54 (d, 2H, *J* = 8.5 Hz, ArH), 7.57 (d, 2H, *J* = 8.5 Hz, ArH), 7.57–7.59 (m, 4H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 24.4, 25.7, 26.3, 26.4, 27.3, 28.1, 40.7, 53.5, 56.3, 63.6, 64.6, 71.2, 73.1, 74.2, 75.3, 76.5, 77.9, 78.0, 79.0, 103.7, 105.4, 108.3, 110.3, 110.3, 127.0, 127.3, 127.4, 127.5, 128.8, 129.7, 132.9, 139.6, 139.7, 140.2, 140.6, 171.3 ppm.

3.4.6. General procedure for the synthesis of 6′-*O*-lactose esters (6a–l)

Compounds **5a–l** (0.25 mmol) were dissolved in $HBF_4.Et_2O/H_2O$/dry MeCN (2.1 mL, 1:5:500 v/v) and the mixture was stirred at 30 °C for 3 h (except for **5c**,**5g**,**5h** and **5j** at 0 °C for 5 h). The white solids precipitated were then filtered, washed with MeCN and dried. Purification by recrystallization for **5a–f** from methanol or by trituration with petroleum ether for **5g–l** gave the desired final lactose esters **6a–f** as white solids.

6′-*O*-capryloyl-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose caprylate (**6a**) [112]

Yield = 46% (0.053 g). MS (ESI): 467 $[M - H]^-$, 486 $[M + NH_4]^+$, 491 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.87 (t, 3H, *J* = 6.5 Hz, CH_3), 1.20–1.34 [m, 8H, $(CH_2)_4$], 1.52–1.55 (m, 2H, CH_2CH_2COOR), 2.31 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.17 (ddd, 1H, $J_{H2\text{-}H1}$ = 4.0 Hz, $J_{H2\text{-}OH2}$ = 7.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.28 (dd, 1H, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.0 Hz, H4), 3.32–3.38 (m, 2H, H2′, H3′), 3.57 (dd, 1H, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.0 Hz, H3), 3.63–3.65 (m, 3H, H6a, H6b, H4′), 3.69–3.75 (m, 2H, H5, H5′), 4.09 (dd, 1H, $J_{H6a'\text{-}H5'}$ = 4.0 Hz, $J_{H6a'\text{-}H6b'}$ = 11.5 Hz, H6a′), 4.17 (dd, 1H, $J_{H6b'\text{-}H5'}$ = 8.5 Hz, $J_{H6b'\text{-}H6a'}$ = 11.5 Hz, H6b′), 4.20–4.24 (m, 2H, H1′, OH3), 4.43 (dd, 1H, $J_{OH6\text{-}H6a} \cong J_{OH6\text{-}H6b}$ = 6.0 Hz, OH6), 4.56 (d, 1H, $J_{OH2\text{-}H2}$ = 7.0 Hz, OH2), 4.79 (d, 1H, $J_{OH4'\text{-}H4'}$ = 5.0 Hz,

OH4′), 4.86 (brs, 1H, OH), 4.90 (dd, 1H, $J_{H1\text{-}OH1}$ = 4.5 Hz, $J_{H1\text{-}H2}$ = 4.0 Hz, H1), 5.15 (brs, 1H, OH), 6.34 (d, 1H, $J_{OH1\text{-}H1}$ = 4.5 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.4, 22.5, 24.8, 28.8, 28.9, 31.6, 33.8, 60.9, 63.8, 68.7, 70.2, 70.8, 71.7, 72.7, 72.9, 73.3, 81.5, 92.5, 104.0, 173.4 ppm.

6′-*O*-Caproyl-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose caprate (**6b**) [112]

Yield: 75% (0.093 g). MS (ESI): 495 $[M - H]^-$, 514 $[M + NH_4]^+$, 519 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.20–1.32 [m, 12H, $(CH2)_6$], 1.48–1.57 (m, 2H, CH_2CH_2COOR), 2.31 (t, 2H, J = 7.3 Hz, CH_2COOR), 3.17 (ddd, 1H, $J_{H2–H1}$ = 4.0 Hz, $J_{H2–OH2}$ = 7.0 Hz, $J_{H2–H3}$ = 9.5 Hz, H2), 3.27 (dd, 1H, $J_{H4–H3} \cong J_{H4–H5}$ = 9.5 Hz, H4), 3.33–3.37 (m, 2H, H2′, H3′), 3.57 (dd, 1H, $J_{H3–H2} \cong J_{H3–H4}$ = 9.5 Hz, H3), 3.60–3.67 (m, 3H, H6a, H6b, H4′), 3.68–3.76 (m, 2H, H5, H5′), 4.09 (dd, 1H, $J_{H6'b–H5'}$ = 4.5 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6′b), 4.17 (dd, 1H, $J_{H6'a–H5}$ = 8.5 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.20–4.25 (m, 2H, H1′, OH3), 4.43 (dd, 1H, $J_{OH6–H6a} \cong J_{OH6–H6b}$ = 6.0 Hz, OH6), 4.55 (d, 1H, $J_{OH2–H2}$ = 7.0 Hz, OH2), 4.78 (d, 1H, $J_{OH4'–H4'}$ = 5.0 Hz, OH4′), 4.86 (d, 1H, J = 3.0 Hz, OH), 4.90 (dd, 1H, $J_{H1–OH1} \cong J_{H1–H2}$ = 4.0 Hz, H1), 5.15 (d, 1H, J = 3.0 Hz, OH), 6.33 (d, 1H, $J_{OH1–H1}$ = 4.0 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.4, 22.6, 24.8, 28.9, 29.1, 29.2, 29.3, 31.7, 33.8, 60.9, 63.8, 68.7, 70.2, 70.8, 71.7, 72.7, 72.9, 73.3, 81.6, 92.5, 104.0, 173.4 ppm.

6′-*O*-undec-10-enoyl-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose undecy lenate (**6c**)

Yield: 37% (0.110 g). MS(ESI): 507 $[M - H]^-$, 526 $[M + NH_4]^+$, 531 $[M + Na]^+$, 553 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.21–1.28 [m, 8H, $(CH_2)_4$], 1.32–1.35 (m, 2H, $CH_2CH_2CH{=}CH_2$), 1.47–1.56 (m, 2H, CH_2CH_2COOR), 1.97–2.04 (m, 2H, $CH_2CH{=}CH_2$), 2.30 (t, 2H, J = 7.5 Hz, CH_2COOR), 3.17 (ddd, 1H, $J_{H2–H1}$ = 4.0 Hz, $J_{H2–OH2}$ = 7.0 Hz, $J_{H2–H3}$ = 9.5 Hz, H2), 3.27 (dd, 1H, $J_{H4–H3} \cong J_{H4–H5}$ = 9.5 Hz, H4), 3.32–3.38 (m, 2H, H2′, H3′), 3.56 (dd, 1H, $J_{H3–H2} \cong J_{H3–H4}$ = 9.5 Hz, H3), 3.61–3.66 (m, 3H, H6a, H6b, H4′), 3.68–3.75 (m, 2H, H5, H5′), 4.08 (dd, 1H, $J_{H6a'–H5}$ = 4.0 Hz, $J_{H6a'–H6b'}$ = 11.5 Hz, H6a′), 4.16 (dd, 1H, $J_{H6b'–H5'}$ = 8.0 Hz, $J_{H6b'–H6a'}$ = 11.5 Hz, H6b′), 4.20–4.26 (m, 2H, H1′, OH3), 4.47 (dd, 1H, $J_{OH6–H6a} \cong J_{OH6–H6b}$ = 6.0 Hz, OH6), 4.60

(d, 1H, $J_{OH2–H2}$ = 7.0 Hz, OH2), 4.82 (d, 1H, $J_{OH4'–H4'}$ = 6.5 Hz, OH4′), 4.87 (d, 1H, $J_{OH2'–H2'}$ = 5.0 Hz, OH2′), 4.89 (dd, 1H, $J_{H1–OH1}$ = 4.5 Hz, $J_{H1–H2}$ = 4.0 Hz, H1), 4.93 (1H, dddd, J_{cis} = 10.0 Hz, J_2 = 4.0 Hz, $J_{gem} \cong J_1$ = 1.5 Hz, HCH=CH), 4.99 (1H, dddd, J_{trans} = 17.0 Hz, $J_{gem} \cong J_1 \cong J_2$ = 1.5 Hz, HCH=CH), 5.79 (dddd, 1H, $J_1 \cong J_2$ = 7.0 Hz, J_{cis} = 10.0 Hz, J_{trans} = 17.0 Hz, HCH=CH), 6.37 (d, 1H, $J_{OH1–H1}$ = 4.5 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 24.8, 28.7, 28.9 (2C), 29.13, 29.147, 33.6, 33.8, 60.9, 63.8, 68.7, 70.2, 70.7, 71.7, 72.6, 72.9, 73.3, 81.5, 92.5, 104.0, 115.1, 139.3, 173.4 ppm.

6′-*O*-Lauryol-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose laurate (**6d**) [112]

Yield: 44% (0.058 g). MS (ESI): 523 $[M – H]^–$, 542 $[M + NH_4]^+$, 547 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.19–1.30 [m, 16H, $(CH2)_8$], 1.48–1.57 (m, 2H, CH_2CH_2COOR), 2.31 (t, 2H, J = 7.3 Hz, CH_2COOR), 3.17 (ddd, 1H, $J_{H2–H1}$ = 4.0 Hz, $J_{H2–OH2}$ = 7.0 Hz, $J_{H2–H3}$ = 9.5 Hz, H2), 3.27 (dd, 1H, $J_{H4–H3} \cong J_{H4–H5}$ = 9.5 Hz, H4), 3.33–3.38 (m, 2H, H2′, H3′), 3.56 (dd, 1H, $J_{H3–H2} \cong J_{H3–H4}$ = 9.5 Hz, H3), 3.60–3.67 (m, 3H, H6a, H6b, H4′), 3.68–3.76 (m, 2H, H5, H5′), 4.09 (dd, 1H, $J_{H6'b–H5'}$ = 4.5 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6′b), 4.17 (dd, 1H, $J_{H6'a–H5}$ = 8.5 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.20–4.25 (m, 2H, H1′, OH3), 4.43 (dd, 1H, $J_{OH6–H6a} \cong J_{OH6–H6b}$ = 6.0 Hz, OH6), 4.56 (d, 1H, $J_{OH2–H2}$ = 7.0 Hz, OH2), 4.79 (d, 1H, $J_{OH4'–H4'}$ = 5.0 Hz, OH4′), 4.86 (d, 1H, J = 5.0 Hz, OH), 4.90 (dd, 1H, $J_{H1–OH1} \cong J_{H1–H2}$ = 4.0 Hz, H1), 5.16 (d, 1H, J = 4.0 Hz, OH), 6.34 (d, 1H, $J_{OH1–H1}$ = 4.0 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ: 14.4, 22.6, 24.8, 28.9, 29.2, 29.4, 29.46, 29.48, 31.8, 33.8, 60.9, 63.8, 68.7, 70.2, 70.7, 71.7, 72.7, 72.9, 73.3, 81.6, 92.5, 104.0, 173.4 ppm.

6′-*O*-Myristoyl-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose myristate (**6e**) [112]

Yield: 65% (0.090 g). MS (ESI): 551 $[M – H]^–$, 570 $[M + NH_4]^+$, 575 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.17–1.32 [m, 20H, $(CH_2)_{10}$], 1.48–1.57 (m, 2H, CH_2CH_2COOR), 2.30 (t, 2H, J = 7.4 Hz, CH_2COOR), 3.16 (ddd, 1H, $J_{H2–H1}$ = 4.0 Hz, $J_{H2–OH2}$ = 7.0 Hz, $J_{H2–H3}$ = 9.5 Hz, H2), 3.27 (dd, 1H, $J_{H4–H3} \cong J_{H4–H5}$ = 9.5 Hz, H4), 3.31–3.37 (m, 2H, H2′, H3′), 3.56 (dd, 1H, $J_{H3–H2} \cong J_{H3–}$

$_{H4}$ = 9.5 Hz, H3), 3.60–3.66 (m, 3H, H6a, H6b, H4′), 3.67–3.76 (m, 2H, H5, H5′), 4.08 (dd, 1H, $J_{H6'b–H5'}$ = 4.5 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6′b), 4.16 (dd, 1H, $J_{H6'a–H5}$ = 8.5 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.20–4.25 (m, 2H, H1′, OH3), 4.47 (dd, 1H, $J_{OH6–H6a} \cong J_{OH6–H6b}$ = 6.0 Hz, OH6), 4.60 (d, 1H, $J_{OH2–H2}$ = 7.0 Hz, OH2), 4.82 (d, 1H, $J_{OH4'–H4'}$ = 5.0 Hz, OH4′), 4.89 (d, 1H, J = 4.0 Hz, OH), 4.90 (dd, 1H, $J_{H1–OH1} \cong J_{H1–H2}$ = 4.0 Hz, H1), 5.19 (d, 1H, J = 4.0 Hz, OH), 6.37 (d, 1H, $J_{OH1–H1}$ = 4.0 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.4, 22.6, 24.8, 29.0, 29.2, 29.2, 29.4, 29.5, 29.5, 29.5, 31.8, 33.8, 60.9, 63.8, 68.7, 70.2, 70.7, 71.7, 72.7, 72.9, 73.3, 81.6, 92.5, 104.0, 173.4 ppm.

6′-*O*-Palmitoyl-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose palmitate (**6f**) [112]

Yield: 80% (0.116 g). MS (ESI): 579 $[M – H]^–$, 598 $[M + NH_4]^+$, 603 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 3H, J = 6.6 Hz, CH_3), 1.18–1.32 [m, 24H, $(CH_2)_{12}$], 1.47–1.58 (m, 2H, CH_2CH_2COOR), 2.31 (t, 2H, J = 7.4 Hz, CH_2COOR), 3.18 (ddd, 1H, $J_{H2–H1}$ = 4.0 Hz, $J_{H2–OH2}$ = 7.0 Hz, $J_{H2–H3}$ = 9.5 Hz, H2), 3.27 (dd, 1H, $J_{H4–H3} \cong J_{H4–H5}$ = 9.5 Hz, H4), 3.32–3.38 (m, 2H, H2′, H3′), 3.57 (dd, 1H, $J_{H3–H2} \cong J_{H3–H4}$ = 9.5 Hz, H3), 3.61–3.67 (m, 3H, H6a, H6b, H4′), 3.68–3.76 (m, 2H, H5, H5′), 4.09 (dd, 1H, $J_{H6'b–H5'}$ = 4.5 Hz, $J_{H6'b–H6'a}$ = 11.5 Hz, H6′b), 4.17 (dd, 1H, $J_{H6'a–H5}$ = 8.5 Hz, $J_{H6'a–H6'b}$ = 11.5 Hz, H6′a), 4.20–4.28 (m, 2H, H1′, OH3), 4.39 (dd, 1H, $J_{OH6–H6a} \cong J_{OH6–H6b}$ = 6.0 Hz, OH6), 4.51 (d, 1H, $J_{OH2–H2}$ = 7.0 Hz, OH2), 4.75 (d, 1H, $J_{OH4'–H4'}$ = 5.0 Hz, OH4′), 4.82 (br s, 1H, OH), 4.90 (dd, 1H, $J_{H1–OH1} \cong J_{H1–H2}$ = 4.0 Hz, H1), 5.12 (br s, 1H, OH), 6.31 (d, 1H, $J_{OH1–H1}$ = 4.0 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.4, 22.5, 24.8, 29.0, 29.1, 29.2, 29.4, 29.5, 29.5, 31.7, 33.8, 61.0, 63.7, 68.7, 70.2, 70.8, 71.7, 72.7, 72.9, 73.3, 81.6, 92.5, 104.0, 173.4 ppm.

Octadec-9*Z*-12*Z*-dienoyl-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose linoleate (**6g**)

Yield: 70% (0.160 g). MS (ESI): 603 $[M – H]^–$, 622 $[M + NH_4]^+$, 627 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 3H, J = 7.0 Hz, CH_3), 1.22–1.30 [m, 14H, $(CH_2)_7$], 1.49–1.55 (m, 2H, CH_2CH_2COOR), 1.98–2.05 (m, 4H, $CH_2CH{=}CHCH_2CH{=}CHCH_2$), 2.31 (t, 2H, J = 7.5 Hz, CH_2COOR), 2.74 (dd, 2H, $J_1 \cong J_2$ = 6.0 Hz, $CH{=}CHCH_2CH{=}CH$), 3.16 (dd, 1H, J_{H2-H1} = 3.5 Hz, J_{H2-H3} = 9.0 Hz,

H2), 3.27 (dd, 1H, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.0 Hz, H4), 3.30–3.36 (m, 2H, H2′, H3′), 3.56 (dd, 1H, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.0 Hz, H3), 3.61–3.65 (m, 3H, H6a, H6b, H4′), 3.68–3.76 (m, 2H, H5, H5′), 4.08 (dd, 1H, $J_{H6a'\text{-}H5'}$ = 4.0 Hz, $J_{H6a'\text{-}H6b'}$ =11.5 Hz, H6a′), 4.16 (dd, 1H, $J_{H6b'\text{-}H5'}$ = 8.5 Hz, $J_{H6b'\text{-}H6a'}$ = 11.5 Hz, H6b′), 4.22 (m, 2H, H1′, OH3), 4.49 (brs, 1H, OH6), 4.81 (brs, 1H, OH4′), 4.87-4.93 (m, 1H, H1), 5.18 (brs, 1H, OH), 5.27–5.39 (m, 4H, C*H*=C*H*CH$_2$C*H*=C*H*), 6.36 (brs, 1H, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.4, 22.4, 24.8, 25.7, 27.06, 27.09, 28.9, 29.0, 29.1, 29.2, 29.5, 31.3, 33.7, 60.9, 63.7, 68.7, 70.2, 70.8, 71.7, 72.7, 72.9, 73.3, 81.5, 92.5, 104.0, 128.2, 128.2, 130.2 (2C), 173.3 ppm.

Octadec-9*Z*-12*Z*-15*Z*-trienoyl-4-*O*-(β-D-galactopyranoyl)-D-glucopyranose, lactose linolenate (**6h**)

Yield = 66% (0.151 g). MS (ESI): 601 $[M - H]^-$, 620 $[M + NH_4]^+$, 625 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.93 (t, 3H, *J* = 7.5 Hz, CH_3), 1.22–1.32 [m, 8H,$(CH_2)_4$], 1.49–1.55 (m, 2H, C*H*$_2$CH$_2$COOR), 1.98–2.08 (m, 4H, C*H*$_2$CH=CHCH$_2$CH=CHCH$_2$CH=CHC*H*$_2$), 2.31 (t, 2H, *J*= 7.5 Hz, CH$_2$COOR), 2.71–2.81 (m, 4H, CH=CHC*H*$_2$CH=CHC*H*$_2$CH=CH), 3.16 (dd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.27 (dd, 1H, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.33-3.43 (m, 2H, H2′, H3′), 3.56 (dd, 1H, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.5 Hz, H3), 3.61–3.65 (m, 3H, H6a, H6b, H4′), 3.68-3.75 (m, 2H, H5, H5′), 4.08 (dd, 1H, $J_{H6a'\text{-}H5}$ = 4.5 Hz, $J_{H6a'\text{-}H6b'}$ =11.5 Hz, H6a′), 4.16 (dd, 1H, $J_{H6b'\text{-}H5'}$ = 8.5 Hz, $J_{H6b'\text{-}H6a'}$ = 11.5 Hz, H6b′), 4.21–4.22 (m, 1H, H1′), 4.90(d,1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.24–5.41 (m, 6H, C*H*=C*H*CH$_2$C*H*=C*H*CH$_2$C*H*=C*H*), 6.37 (brs, 1H, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.6, 20.5, 24.8, 25.6, 25.7, 27.1, 28.9, 29.0, 29.1, 29.5, 33.7, 60.9, 63.8, 68.7, 70.2, 70.7, 71.7, 72.6, 72.8, 73.2, 81.5, 92.5, 104.0, 127.4, 128.0, 128.4, 128.4, 130.4, 132.0, 173.3 ppm.

6′-*O*-(2-Phenylethanoyl)-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose phenylacetate (**6i**)

Yield = 66% (0.076 g). MS (ESI): 459 $[M - H]^-$, 478 $[M + NH_4]^+$, 483 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.21–3.24 (m, 1H, H2), 3.30–3.41 (m, 3H, H4, H2′, H3′), 3.59–3.68 (m, 4H, H3, H6a, H6b, H4′), 3.70 (s, 2H, CH_2), 3.72–3.77 (m, 2H, H5, H5′), 4.11 (dd, 1H, $J_{H6'b\text{-}H5'}$ = 4.0 Hz, $J_{H6'b\text{-}H6'a}$ = 11.2 Hz, H6′b), 4.24 (dd, 1H, $J_{H6'a\text{-}}$

$_{H5'}$ = 2.0 Hz, $J_{H6'a-H6'b}$ = 11.2 Hz, H6′a), 4.25–4.27 (m, 1H, H1′), 4.34 (brs, 1H, OH), 4.47 (dd, 1H, $J_{OH6-H6a} \cong J_{OH6-H6b}$ = 6.0 Hz, OH6), 4.64 (brs, 1H, OH), 4.80 (brs, 1H, OH), 4.86 (brs, 1H, OH), 4.92 (dd, 1H, $J_{H1-OH1} \cong J_{H1-H2}$ = 4.0 Hz, H1), 5.16 (brs, 1H, OH), 6.36 (d, 1H, J_{OH1-H1} = 4.0 Hz, OH1), 7.25–7.33 (m, 5H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 31.2, 60.9, 64.3, 68.7, 70.2, 70.8, 71.7, 72.7, 72.8, 73.3, 81.7, 92.5, 104.1, 127.2, 128.8, 130.0, 134.7, 171.7 ppm.

6′-*O*-[2-(4-Phenyl)benzoyl]-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose *p*-phenylbenzoate (**6j**)

Yield = 52%. MS (ESI): 521 $[M - H]^-$, 540 $[M + NH_4]^+$, 545 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.22 (dd, 1H, J_1 = 3.6 Hz, J_2 = 9.6 Hz), 3.33–3.38 (m, 2H), 3.38–3.41 (m, 2H), 3.60–3.70 (m, 3H), 3.70–3.76 (m, 2H), 3.92 (dd, 1H, J_1 = 4.0 Hz, J_2 = 8.5 Hz), 4.26–4.36 (m, 3H), 4.48–4.56 (m, 2H), 4.91 (d, 1H, J = 4.0 Hz), 7.42–7.46 (m, 1H, ArH), 7.50–7.54 (m, 2H, ArH), 7.74–7.77 (m, 2H, ArH), 7.82–7.85 (m, 2H, ArH), 8.14–8.18 (m, 2H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 60.8, 64.8, 68.9, 70.2, 70.8, 71.7, 72.8, 73.1, 73.3, 81.3, 92.6, 104.0, 127.3, 127.5, 128.8, 128.9, 129.6, 130.7, 139.4, 145.2 166.1 ppm.

6′-*O*-[2-(4-Phenyl)phenylethanoyl]-4-*O*-(β-D-galactopyranosyl)-D-glucopyranose, lactose biphenylacetate (**6k**)

Yield = 72% (0.096 g). MS (ESI): 535 $[M - H]^-$, 554 $[M + NH_4]^+$, 559 $[M + Na]^+$. ^{1} H NMR (400 MHz, DMSO-d_6): δ = 3.04 (dd, 1H, $J_1 \cong J_2$ = 8.0 Hz), 3.23 (dd, 1H, J_1 = 3.6 Hz, J_2 = 9.6 Hz), 3.28–3.38 (m, 6H), 3.58–3.66 (m, 4H), 3.70–3.76 (m, 3H), 4.17–4.28 (m, 4H), 4.37 (d, 1H, J = 8.0 Hz), 4.94 (d, 1H, J = 3.6 Hz), 5.23 (s, 2H, CH_2), 7.25–7.38 (m, 9H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 56.2, 60.9, 64.7, 68.7, 70.2, 70.3, 70.7, 71.7, 72.7, 73.2, 75.2, 81.8, 92.6, 103.9, 127.5, 127.5, 128.9, 129.0, 129.1, 139.3, 139.4, 172.4 ppm.

6′-*O*-[2-(4,4′-Biphenyl)phenylethanoyl]-4-*O*-(β-D-galactopyranosyl)-D glucopyranose, lactose terphenylacetate (**6l**)

Yield = 58%. MS (ESI): 459 $[M - H]^-$, 478 $[M + NH_4]^+$, 483 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.25 (dd, 1H, J_1 = 3.2 Hz, J_2 = 9.6 Hz), 3.30–3.42 (m, 4H), 3.51

(brs, 1H), 3.61–3.68 (m, 4H), 3.72–3.80 (m, 4H), 4.13 (dd, 1H, J_1 = 3.2 Hz, J_2 = 11.6 Hz), 4.27–4.39 (m, 4H), 4.93 (d, 1H, J = 3.2 Hz), 7.39–7.51 (m, 5H, ArH), 7.68–7.72 (m, 8H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 60.9, 64.4, 68.7, 70.2, 70.8, 71.8, 72.7, 72.9, 73.3, 81.8, 92.6, 104.2, 126.96, 127.02, 127.6, 127.7, 128.0, 129.5, 130.7, 134.1, 138.5, 139.3, 139.5, 140.1, 171.6 ppm.

3.4.7. Synthesis of terphenylacetic acid (3l)

2-([1,1′-biphenyl] -4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**8**) [134]

A mixture of *p*-phenylbromobenzene (**7**) (0.534 g, 2.29 mmol), B_2pin_2 (1.635 g, 6.44 mmol), Pd(dppf)Cl_2·DCM (0.170 g, 0.21 mmol), dppf (0.083 g, 0.15 mmol) and KOAc (1.320 g, 13.43 mmol) in dry dioxane (11.6 mL) was stirred at 80 °C for 20 h, then filtered on Celite®, and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The purification of the residue by column cromatography (cyclohexane/EtOAc 98:2) gave **8** as a white solid.

Yield = 96%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (s, 12H, 6 CH_3), 7.26–7.31 (m, 1H, ArH), 7.35–7.39 (m, 2H, ArH), 7.53–7.56 (m, 4H, ArH), 7.81–7.83 (m, 2H, ArH).

Methyl 2-[(1,1′:4′,1″-trephenyl)-4-yl] acetate (**10**) [134]

A K_3PO_4 1,27 M aqueous solution (4 mL) was added to solution of **8** (0.999 g, 3.57 mmol), methyl 2-(4-bromophenyl) acetate (**9**) (0.682 g, 2.98 mmol), $Pd_2(dba)_3$ (0.164 g, 0.18 mmol), PCy_3 (0.114 g, 0.41 mmol) in a mixture 2:1 dioxane/H_2O (8 mL). The solution was stirred at 80 °C for 20 h, then filtered on Celite®, and extracted with EtOAc. The combined organic layers were washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The purification by column cromatography (cyclohexane/EtOAc 98:2 then petroleum ether/Et_2O 95:5) gave **10** as a white solid.

Yield = 47%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.62 (s, 2H, CH_2), 3.65 (s, 3H, CH_3), 7.29–7.31 (m, 3H, ArH), 7.34–7.41 (m, 2H, ArH), 7.53–7.58 (m, 4H, ArH), 7.59–7.61 (m, 4H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 40.8, 52.1, 127.0, 127.2, 127.3, 127.4, 127.5, 128.8, 129.7, 133.1, 139.6, 139.7, 140.2, 140.7, 172.0 ppm.

Terphenylacetic acid (**3l**) [135]

A solution of **10** (0.420 g, 1.39 mmol) and LiOH (0.292 g, 6.95 mmol) in a mixture 3:1 MeOH/H_2O (9.3 mL) was stirred at 60 °C for 5 h, then acidified with HCl 2N to pH = 2, and extracted with EtOAc. The combined organic layers were washed with H_2O, dried on Na_2SO_4, and concentrated. The purification by recrystallization from EtOAc gave **3l** as a white solid.

Yield = 79%. MS (ESI): 287 $[M - H]^-$, 289 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-d_6): δ = 3.64 (s, 2H, CH_2), 7.37–7.40 (m, 3H, ArH), 7.47–7.51 (m, 2H, ArH), 7.67–7.69 (m, 2H, ArH), 7.72–7.74 (m, 2H, ArH), 7.75–7.78 (m, 4H, ArH), 12.36 (brs, 1H, COOH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 40.8, 126.9, 127.0, 127.52, 127.56, 127.7, 128.0, 129.5, 130.5, 134.9, 138.4, 139.4, 139.5, 140.1, 173.11 ppm.

4. Synthesis of 6-*O*-Sucrose Esters by Mitsunobu Reaction as Potential Antifungal Agents and Drug Permeability Enhancer Studies

4.1. Introduction

Fungal invasive infections are a significant risk to human health and can be correlated with at least 1.5-2 million deaths worldwide [136, 137]. Fungal infections are commonly present in patients having an impaired immune system exposed to anticancer treatments or organ transplants. Currently, most of the antifungals present in the market, (especially, those administrated by systemic and oral routes), possess several drawbacks, such as toxicity, narrow spectrum of activity, low safety, unfavorable pharmacokinetic properties, and drug resistant strains [138]. Therefore, the research of new antifungals agents is a compelling necessity. In this context, sucrose fatty acid esters could represent a suitable solution thanks to their favorable properties [27]. Notably, sucrose fatty acid ester derivatives have the advantages to be synthesized by inexpensive and renewable materials [27]. Their antibacterial [15, 19], antifungal [27] and insecticidal [27, 92] activities have been largely reported in the literature. In addition, they displayed also permeability enhancing properties [21, 84, 85]. For all these reasons, sucrose fatty acid esters are very useful amphiphilic molecules in the agriculture, food, nutraceutical, cosmetic, dental, and pharmaceutical industries. As an extension of our previous studies focused on synthesis, biological activities and applications of 6′-*O*-lactose ester derivatives, a small series of 6-*O*-sucrose-based esters (SBEs) has been created. Moreover, SBEs glycolipids are probably more soluble than the corresponding lactose esters, favoring their solubility in water medium for biological screening. Despite numerous studies reporting the synthesis and biological properties of sucrose esters, there is still a lack of information as regard sucrose with unsaturated fatty acid chains or aryl(alkyl) moiety as lipophilic portion. For this purpose, in this chapter, several 6-*O*-sucrose-based esters were synthesized in a single step through an appropriately revised Mitsunobu reaction [32] starting from sucrose and different fatty acids (C8, C11:1, C16:1, C18:1, C18:2, C18:3, C24:1), and aryl(alkyl) acids (benzoic, phenylacetic, *p*-phenylbenzoic, biphenylacetic

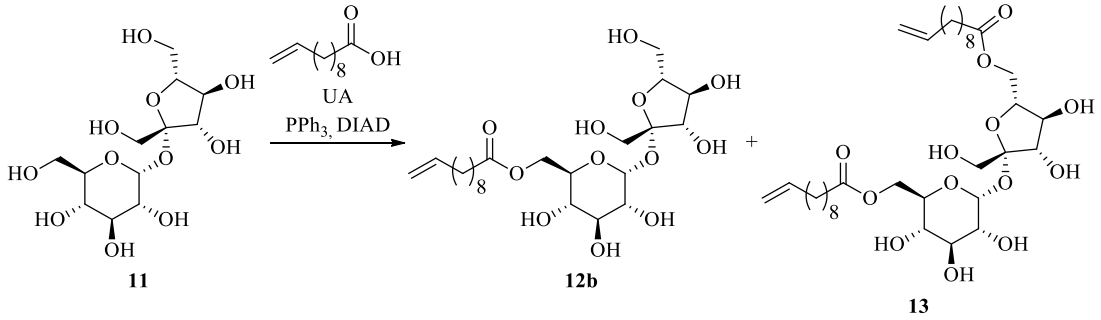
and terphenylacetic). Subsequently, these compounds were tested to determine their minimum inhibitory concentration (MIC) values against different Gram-positive and Gram-negative bacteria and fungi. Successively, the anti-inflammatory effect and biocompatibility properties of the best SBE were evaluated. In addition, sucrose aryl(alkyl) esters (phenylacetate, *p*-phenylbenzoate and biphenylacetate) have been evaluated for their permeability enhancer abilities. In detail, the chemical characterization and biocompatibility properties of these SBEs were assessed in comparison with lactose esters analogues, followed by their related evaluation as permeability enhancer surfactants through Transepithelial Electrical Resistance (TEER) and permeability study.

4.2. Results and discussion

4.2.1. Synthesis of 6-*O*-sucrose-based esters

6-*O*-sucrose-based monoesters could be synthesized by enzymatic and chemical procedures [15, 22, 23, 27]. In this chapter, several synthetic approaches were screened using unprotected sucrose **11** and undecylenic acid (**UA**) or its acyl analogues (i.e., vinyl undecylenate and undecylenoyl chloride) as starting materials (Scheme 4). The enzymatic esterification and transesterification reaction with commercially available enzymes i.e., Lipozyme®, from *Mucor miehei*, Novozyme 435® from *Candida antarctica*, Acylase I from *Aspergillus melleus* and Lipase from *Porcine pancreas*, gave insufficient results in terms of yield and regioselectivity. Regarding the chemical methodologies, both the Steglich and the acylation (*via* acyl chloride) reactions furnished a mixture of diesters and triesters of sucrose. On the other hand, following a procedure reported by Grindley et al. for the direct synthesis of trehalose monoesters and diesters with uronium-based coupling agent 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) [35], both 6-*O*-sucrose undecylenate and 6,6′-*O*-sucrose di-undecylenate were obtained. However, also in this case low yield of desired monoester derivatives was assessed. Finally, the Mitsunobu reaction type reported by Molinier et al. [32] was found to be the most appropriate method to achieve 6-*O*-sucrose undecylenate **12b** in satisfactory yield (34%), together with other side products including the 6,6′-*O*-sucrose-di-undecylenate **13** [32] (Scheme 4 and

Table 10). In order to improve the yield of the desired monoester **12b**, the reaction has been optimized has reported in Table 10.



Scheme 4. Mitsunobu-type reaction for the formation of sucrose 6-*O*-undecylenate **12b**. Diester **13** was obtained as major side-product.

Table 10. Screening of the Mitsunobu-type reaction conditions using sucrose **11** and undecylenic acid (**UA**). a = isolated yield.

Entry	eq. UA	eq. PPh_3:DIAD	T (°C)	t (h)	Dry solvent	Yield[a] (%) 12b	Yield[a] (%) 13
1	2.5	2.7:2.7	20	24	DMF	34	38
2	1	2.5:2.5	20	24	DMF	19	trace
3	**1.5**	**2.5:2.5**	**20**	**24**	**DMF**	**39**	**15**
4	1.5	1.5:1.5	20	24	DMF	26	trace
5	1	1:1	20	24	DMF	trace	-
6	1.5	3:3	20	24	DMF	39	16
7	1.5	2.5:2.5	60	24	DMF	trace	41
8	1.5	2.5:2.5	20	6	DMF	12	2
9	1.5	2.5:2.5	20	12	DMF	18	9
10	1.5	2.5:2.5	20	24	THF	trace	–
11	1.5	2.5:2.5	20	24	dioxane	trace	–

Initially, the yield of diester **13** was higher than monoester **12b** (38% and 34% respectively), as shown in entry 1 of Table 10. Hence, with the aim to reduce the percentage of diester formation, the equivalents of undecylenic acid **UA** were decreased. In fact, with 1 equivalent of **UA**, the presence of the diester was not detected, however a concomitant reduction of the yield of **12b** was assessed (entry 2). On the other hand, using 1.5 equivalent of the undecylenic acid the best result in terms of yield of the desired monoester **12b** (39%, entry 3) was obtained, despite 15% yield

of **13**. We proceeded our investigation by lowering the amount of DIAD and PPh_3, with the aim to avoid the formation of 6-*O*-undecylenyl-3′,6′-anhydrosucrose (side product observed in low yield) [32], (entries 4 and 5). Unfortunately, this objective was not achieved. On the contrary, enhancing the amount of DIAD and PPh_3 did not lead to an amount of **12b** formation (entry 6). An increase of the temperature caused a high formation of the diester **13** with 41% yield accompanied with traces of monoester (entry 7). Also, by reducing the reaction time unsatisfactory yields of **12b** were achieved (entries 8 and 9). Lastly, other polar aprotic solvents, such as THF and dioxane were tested but even in this case only traces of the product were obtained (entries 10 and 11), probably due to the very low solubility of sucrose in these solvents. The regioselective formation of product **12b** was confirmed by NMR analysis. From ^{1}H-^{1}H COSY spectrum registered in DMSO-d_6 it is possible to distinguish between the three different sugar's CH_2 signals (Figure 19).

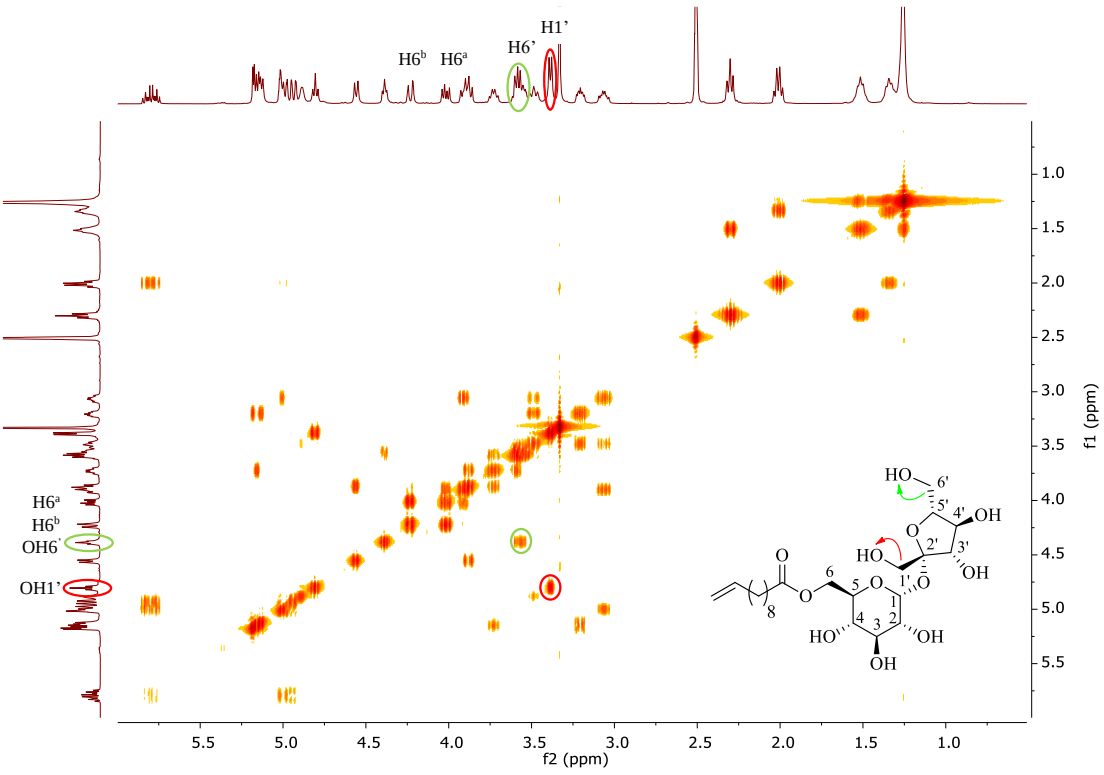


Figure 19. ^{1}H-^{1}H COSY correlations for 6-*O*-sucrose-undecylenate (C11:1). H1′-OH1′ and H6′-OH6′ couplings are highlighted with red circle and green circle respectively.

In particular, the correlation between the hydroxyl groups OH1′ and OH6′ with H1′ and H6′ respectively can be detected, while the coupling with the hydroxyl group in position 6 and H6 is no more observed and the $H6^a$ and $H6^b$ signals were transformed

from *ddd* (sucrose) to *dd* (sucrose 6-*O*-undecylenate). In addition, the structure was also confirmed by HMBC correlation. Indeed, cross peaks between the carbon of the ester carbonyl group and the diasterotopic protons $H6^a$ and $H6^b$ are present, further proving the site of esterification at hydroxyl group in position 6 (Figure 20).

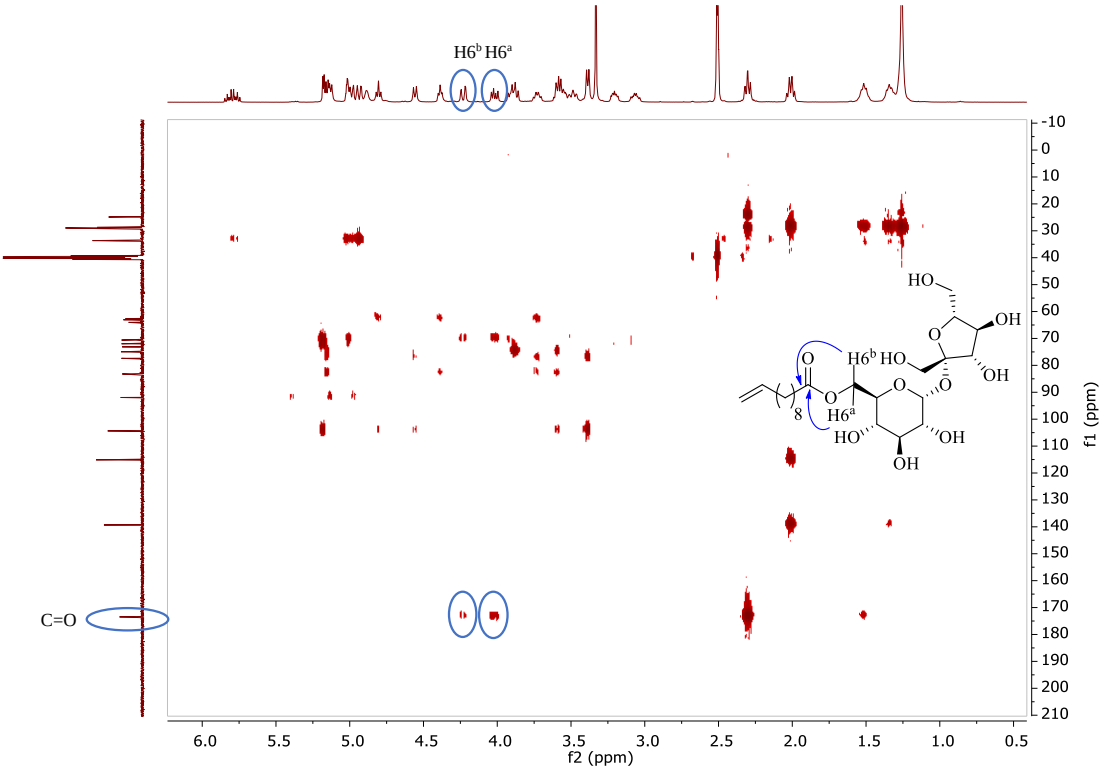
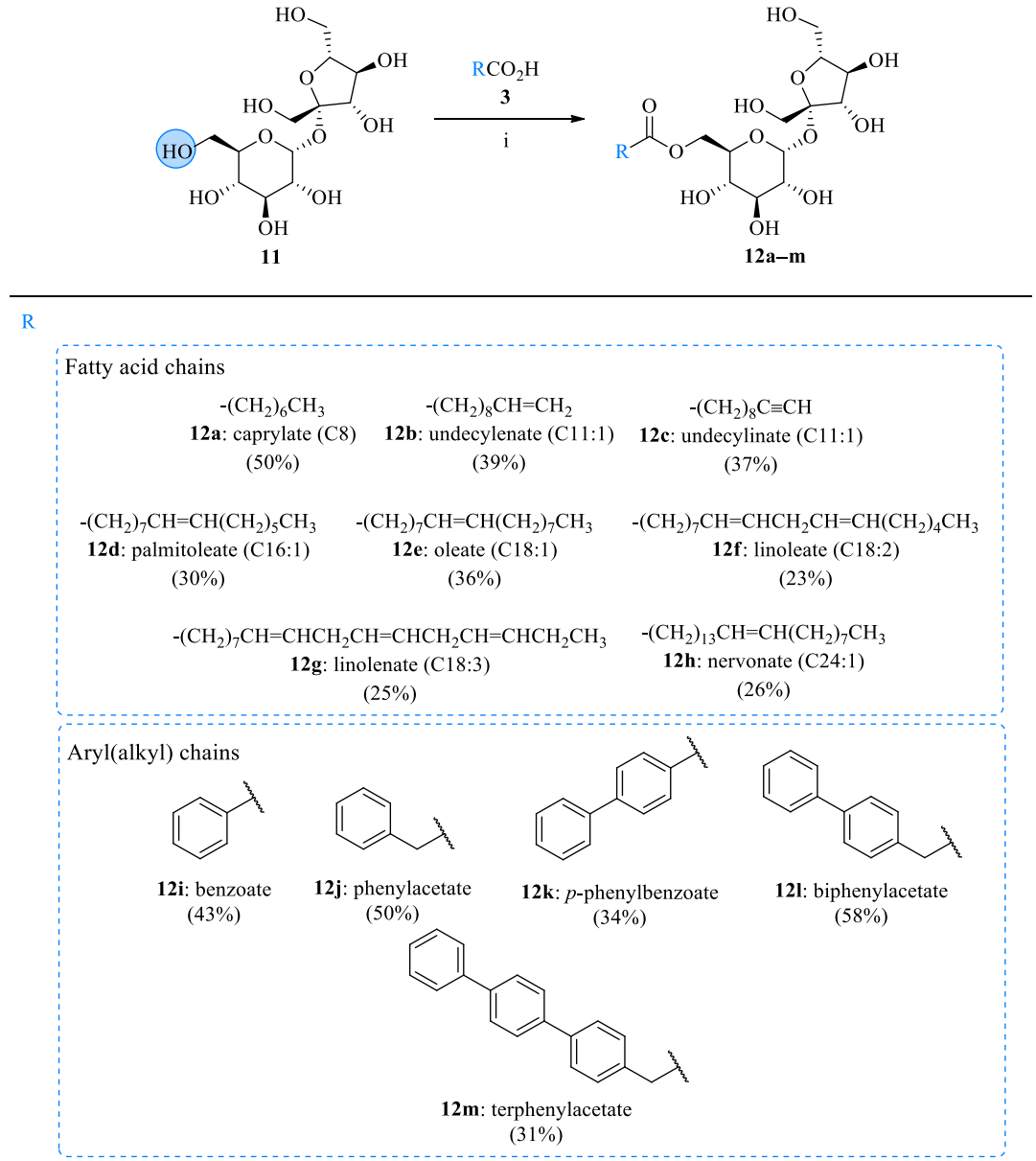


Figure 20. Key HMBC correlation for 6-*O*-sucrose-undecylenate (C11:1).

Moreover, a further confirmation was given by the multiplicity of $H6^a$ and $H6^b$ signals by ^{1}H NMR, using DMSO-d_6 as solvent. In fact, since the coupling with the hydroxyl group in position 6 is no more possible, the $H6^a$ and $H6^b$ signals were transformed from *ddd* (sucrose) to *dd* (sucrose 6-*O*-undecylenate). Considering these results, this Mitsunobu type reaction, optimized with the conditions of entry 3 Table 10, was selected to synthesize a series of 6-*O*-sucrose esters from sucrose and commercially available carboxylic acid (except terphenylacetic acid, which was synthesized as reported in Chapter 3). This methodology was found to be versatile for all the acid substrates, leading to the relative 6-*O*-sucrose esters in low to moderate yields (Scheme 5). As regard sucrose fatty acid esters, the best result was achieved for sucrose caprylate **12a** with 50% yield, while sucrose biphenylacetate **12l** gave the highest yield within the sucrose aryl(alkyl) ester series with a 58% of yield.



Scheme 5. Syntheses of 6-*O*-sucrose esters through Mitsunobu reaction. Reagents, conditions, and yields: (i)PPh_3, DIAD, dry DMF, rt, 24–30 h. Ester isolated yields are reported in brackets.

Despite the modest yields, this procedure has some various advantages such as the use of renewable reagents, the avoidance of protection and deprotection steps, and it is highly regioselective for the primary position of the sucrose. Therefore, here we have demonstrated the versatility of this developed Mitsunobu type reaction, which could be successfully applied for the synthesis of different types of 6-*O*-sucrose monoesters.

4.2.2. Physicochemical properties of 6-*O*-sucrose esters

The most important physicochemical constants in the landscape of surfactants were calculated for 6-*O*-sucrose esters (Table 11) likewise already done for 6′-*O*-lactose-based esters (see Chapter 3). The calculated TPSA was 196 $Å^2$ for all sucrose esters and do not represent a discriminating parameter due to the same polar head for all these SBEs. The synthesized SBEs presented variable ranges of HLB and logP values, which can be possibly correlated with the biological activity.

Table 11. Calculated physicochemical properties of SBEs surfactants. [a]HLB calculated by Griffin's method for non-ionic surfactants [**3**]. HLB = 20 × (MW hydrophilic portion/MW). [b] Calculated octanol-water portion coefficient LogP (by OSIRIS Property Explorer) [116].

12	Sucrose ester	MW	HLB[a]	logP[b]
a	Caprylate C8	468.5	12.7	-1.4
b	Undecylenate C11:1	508.6	11.7	-0.2
c	Undecylinate C11:1	506.6	11.8	-0.9
d	Palmitoleate C16:1	578.7	10.3	2.0
e	Oleate C18:1	606.7	9.8	2.9
f	Linoleate C18:2	604.7	9.9	2.6
g	Linolenate C18:3	602.7	9.9	2.4
h	Nervonate C24:1	690.9	8.6	5.9
i	Benzoate	446.4	13.4	-2.7
j	Phenylacetate	460.4	12.9	-2.1
k	*p*-Phenylbenzoate	522.5	11.4	-0.3
l	Biphenylacetate	536.5	11.1	-0.4
m	Terphenylacetate	612.6	9.7	0.6

All the SBEs (except for the sucrose nervonate, **12h**) possess HLB values major than 9.5 (ranging from 9.7 to 13.4) and therefore can be classified as hydrophilic surfactants (i.e., are able to act as oil-in-water emulsifiers). Moreover, the synthesized SBEs are enclosed in a widespread calculated logP ranging from a negative value -2.7 (sucrose benzoate, **12i**) to a positive 2.9 (sucrose oleate, **12e**). Sucrose nervonate **12h** showed a logP value out of the range (5.9) probably because of its very long hydrophobic chain.

4.2.3. Antibacterial activity

The antibacterial activity of the SBEs **12a–m** was evaluated against both Gram-positive (*E. faecalis* ATCC 29212, *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 43387 and *S. aureus* ATCC 43300) and Gram-negative (*E. coli* O157:H7 ATCC 35150, *K. pneumoniae*, ATCC 13833, *P. aeruginosa*, ATCC 9027 *S. enteritidis* ATCC 13076) bacteria (Table 12). In general, it was observed a moderate antibacterial activity for the tested SBEs against Gram-positive bacteria, while no activity was assessed versus Gram-negative ones (MIC > 1024 µg/mL). Several SBEs displayed a MIC value of 1024 µg/mL versus *E. faecalis* ATCC 29212, while sucrose linoleate **12f** and sucrose linolenate **12g** showed MIC values of 256 µg/mL and 512 µg/mL, respectively. Moreover, sucrose palmitoleate **12d**, sucrose oleate **12e** and sucrose linoleate **12f** resulted active against the other Gram-positive bacteria with a MIC value of 1024 µg/mL. Sucrose nervonate **12h**, sucrose benzoate **12i** and sucrose *p*-phenylacetate **12j** showed efficacy only against *E. faecalis* ATCC 29212 with a MIC 1024 µg/mL, while sucrose *p*-phenylbenzoate **12k**, sucrose undecylinate **12c** and linolenate **12g** were also active against *L. monocytogenes* ATCC 7644 (MIC = 1024 µg/mL). Sucrose caprylate **12a**, sucrose undecylenate **12b**, sucrose biphenylacetate **12l**, and sucrose terphenylacetate **12m** did not show antibacterial activity against all the selected microorganisms. The antibacterial mechanism of sucrose fatty acid esters is probably correlated to their permeability enhancing effect on the cell membrane, which leads to the subsequent release of proteins and other important components [18, 19]. The MIC values highlighted that Gram-negative bacteria were more resistant to the tested SBEs compared to Gram-positive bacteria, probably due to their outer membrane, which limits the diffusion of SBEs. This low efficacy of tested SBEs versus Gram-negative agreed to data reported for other sucrose fatty acid esters. Unfortunately, due to the higher MIC values of these SBEs, it was impossible to correlate the activity with their physicochemical properties and hydrophobic chain length.

Table 12. MIC values (μg/mL) of 6-*O*-sucrose-based esters against selected bacteria

Sucrose ester	*E. faecalis* ATCC 29212	*L.monocytogenes* ATCC 7644	*S. aureus* ATCC 43387	*S.aureus* ATCC 43300	*E. coli* O157:H7 ATCC 35150	*K. pneumoniae* ATCC 13883	*P. aeruginosa* ATCC 9027	*S.enteritidis* ATCC 13076
12a	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
12b	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
12c	**1024**	**1024**	>1024	>1024	>1024	>1024	>1024	>1024
12d	**1024**	**1024**	**1024**	**1024**	>1024	>1024	>1024	>1024
12e	**1024**	**1024**	**1024**	**1024**	>1024	>1024	>1024	>1024
12f	**256**	**1024**	**1024**	**1024**	>1024	>1024	>1024	>1024
12g	**512**	**1024**	>1024	>1024	>1024	>1024	>1024	>1024
12h	**1024**	>1024	>1024	>1024	>1024	>1024	>1024	>1024
12i	**1024**	>1024	>1024	>1024	>1024	>1024	>1024	>1024
12j	**1024**	>1024	>1024	>1024	>1024	>1024	>1024	>1024
12k	**1024**	**1024**	>1024	>1024	>1024	>1024	>1024	>1024
12l	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
12m	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024

4.2.4. Antifungal activity

The antifungal activity of the tested SBEs is shown in Table 13. In contrast to the antibacterial activity, they exhibited good antifungal activity. Remarkably, sucrose undecylenate **12b**, palmitoleate **12d**, oleate **12e** and linoleate **12f** were active versus all the selected fungi with MIC value ranging from 16 to 1024 µg/mL. Among all SBEs, the best results were obtained with sucrose palmitoleate **12d** and sucrose oleate **12e** with MIC value of 16 µg/mL against *Candida albicans* ATCC 10231 and *Aspergillus fumigatus* IDRAH01 respectively. Moreover, **12d** showed good inhibitory activity versus *Fusarium spp* with a MIC of 32 µg/mL as well as sucrose linoleate **12f** against *A. fumigatus* IDRAH01. Sucrose undecylenate **12b** showed MIC value of 512 µg/mL against *Aspergillus niger* ATCC 9642 and *Fusarium* spp., while sucrose linolenate **12g** showed MIC values of 128 µg/mL and 512 µg/mL for *Fusarium* spp. and *C. albicans* ATCC 10231, respectively. On the other hand, sucrose aryl(alkyl) ester derivatives, resulted active against *Fusarium* spp. and *C. albicans* ATCC 10231 with MIC values ranging from 256 to 1024 µg/mL, except for sucrose terphenylacetate **12m** which resulted inactive versus all selected fungi. Hence, SBEs bearing (poly)unsaturated fatty acid chains resulted the best antifungal compounds tested. In detail, **12d** was one of the most active SBEs against all the four fungi strains (MIC from 16 to 512 µg/mL), while sucrose undecylenate **12b** showed low activity (MIC from 512 to 1024 µg/mL), indicating that the reduction of antifungal activity can be correlated with the decrease of the fatty chain length. A further decrease of activity was detected by the substitution of the terminal alkene of **12b** with the alkyne (**12c**) (MIC 1024 µg/mL or higher). As general trend, an increase in the chain length led to less active SBEs. In fact, **12d** showed lower MIC values than the SBEs with longer fatty acid chain (**12e-h**). The only SBE with saturated fatty acid chain (**12a**) is less effective than **12d** as well as the aryl(alkyl) esters (**12i-m**). Regarding the SBEs with alkyl aromatic moiety, only sucrose benzoate (**12i**) and sucrose phenylacetate (**12j**) showed antifungal activity against *Fusarium* spp. (MIC 512 and 256 µg/mL, respectively) and *C. albicans* (MIC 256 and 1024 µg/mL, respectively) strains. In summary, the small library of SBEs synthesized was explored for antibacterial and antifungal activities. The compound with the best activities was sucrose palmitoleate **12d**, hence, it was selected for further assessment of its properties.

Table 13. MIC values (µg/mL) of 6-*O*-sucrose esters against selected fungi.

Sucrose ester	*A. fumigatus* IDRAH01	*A. niger* ATCC 9642	*Fusarium spp*.	*C. albicans* ATCC 10231
12a	1024	1024	>1024	1024
12b	1024	512	512	1024
12c	>1024	>1024	1024	1024
12d	**64**	512	**32**	**16**
12e	**16**	1024	**128**	1024
12f	**32**	1024	**128**	1024
12g	1024	>1024	**128**	**512**
12h	1024	>1024	1024	>1024
12i	>1024	>1024	512	**256**
12j	1024	>1024	**256**	1024
12k	>1024	>1024	1024	1024
12l	>1024	>1024	1024	1024
12m	>1024	>1024	>1024	>1024

4.2.5. Cytotoxicity assay

The assessment of sucrose palmitoleate C16:1 cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay showed a significant decrease of HaCaT cell viability with concentration up to 256 µg/mL (Figure 21). The IC_{50} calculated (230 µg/mL) classified **12d** as a weakly cytotoxic compound [139]. Notably, the cell growth is dependent on both hydrocarbon chain length and sucrose ester concentration as previously highlighted in cytotoxicity studies of 6′-*O*-lactose fatty acid ester derivatives [21, 67, 113]. However, the cytotoxic concentration of **12d** in HaCaT cells was higher than its MIC showed against most of the selected fungi strains. Indeed, **12d** is considered biocompatible and safe antifungal agents at least up to 128 µg/mL.

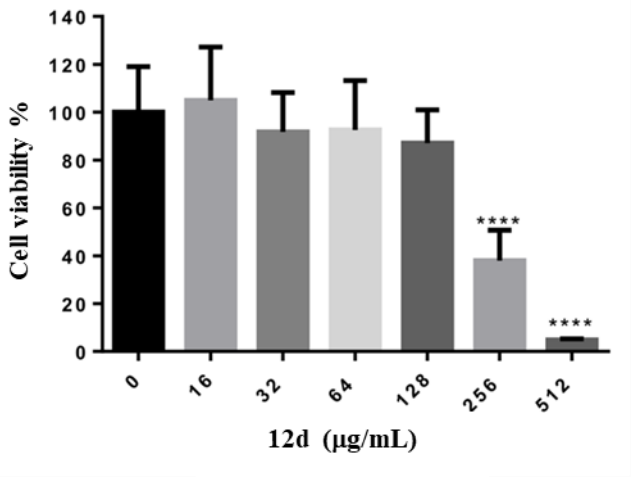


Figure 21. Cell viability evaluation after **12d** administration to HaCaT cells for 24 h. Data are expressed as the mean ± SD (n = 3). ****$p < 0.0001$ vs untreated control cells (Tukey's post hoc test).

4.2.6. Anti-inflammatory activity

The stimulation of RAW 264.7 cells by LPS (CTR+) led to a major extracellular release NO as compared to untreated control cells (CTR–). When LPS-exposed cells were co-incubated with **12d**, a dose-dependent reduction of NO release was detected (Figure 22).

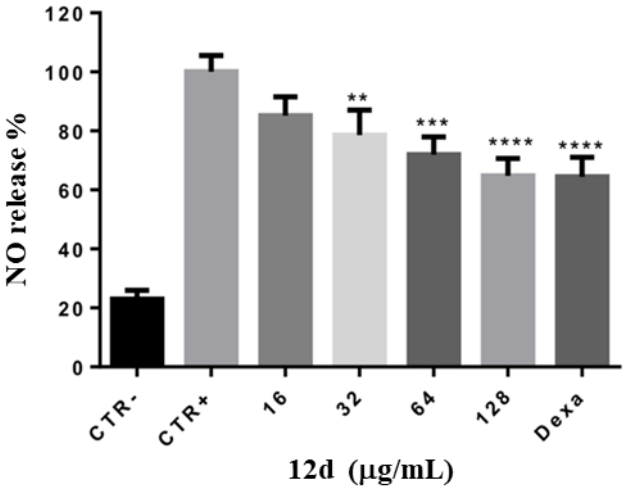


Figure 22. Extracellular NO release after RAW 264.7 stimulation by LPS for 24 h in the presence of sucrose palmitoleate **12d** (16-128 µg/mL). CTR-: negative control (untreated cells); CTR+: positive control (LPS); Dexa: dexamethasone 2 µg/mL. Data are expressed as the mean ± SD (n = 3). **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ vs CTR+ (Tukey's post hoc test).

The same anti-inflammatory activity observed using dexamethasone 2 µg/mL was achieved with a concentration of **12d** of 128 µg/mL. This indicated **12d** as possible anti-inflammatory surfactant. The anti-inflammatory effect of sucrose fatty acid esters

was also demonstrated through the inhibition of NF-κB activation [95]. In addition, also lactose polyunsaturated esters have displayed a reduction of NO production in LPS-stimulated macrophages as showed in Chapter 3. The anti-inflammatory activity of **12d** was also related to their single components i.e., sucrose and palmitoleic acid (C16:1) (Figure 23). Sucrose palmitoleate and C16:1 displayed a comparable activity linked to a significant decrease of the LPS-induced NO release. On the other hand, no anti-inflammatory effect was observed for sucrose. Therefore, the anti-inflammatory activity of **12d** is probably due to the palmitoleate chain. The anti-inflammatory activity of palmitoleic acid was previously assessed by the inhibition of the inflammasome pathway [140], supporting the observed results.

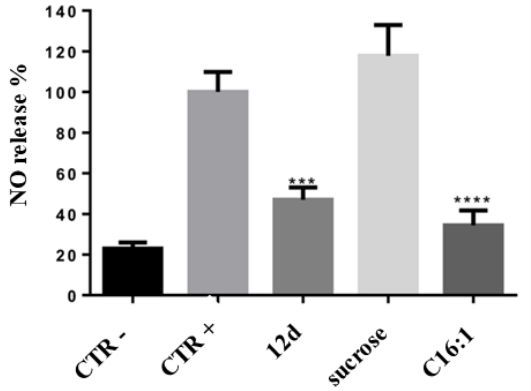


Figure 23. Extracellular NO release after RAW 264.7 stimulation by LPS for 24 h in the presence of sucrose palmitoleate **12d**, sucrose and palmitoleic acid C16:1 (128 µg/mL). CTR-: negative control (untreated cells); CTR+: positive control (LPS). Data are expressed as the mean ± SD (n = 3). ***$p < 0.001$, ****$p < 0.0001$ vs CTR+ (Tukey's post hoc test).

Nevertheless, the employment of palmitoleic acid could be limited because of its low solubility and its irritant behavior. Therefore, sucrose palmitoleate could represent a safe potential prodrug of palmitoleic acid with improved physicochemical and pharmacokinetic properties. Moreover, no cytotoxic effects were observed after **12d** and dexamethasone administration to RAW 264.7 cells compared to untreated control cells (Figure 24), indicating the safe profile of sucrose palmitoleate at concentrations ≤ 128 µg/mL, as also highlighted with cytotoxicity assay on HaCaT cells.

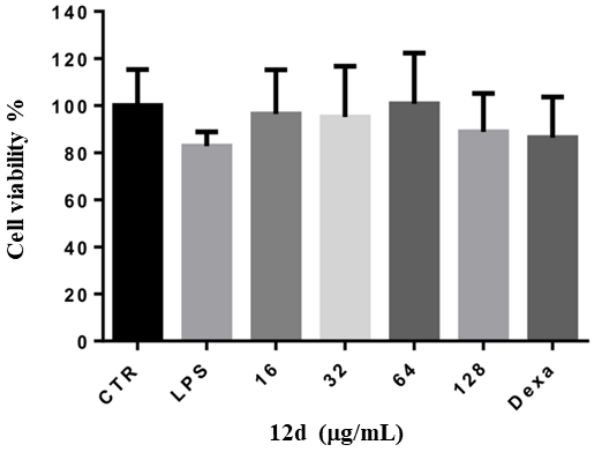


Figure 24. Cell viability evaluation after LPS (1 µg/mL), sucrose palmitoleate **12d** (16-128 µg/mL), and dexamethasone (2 µg/mL) administration to RAW 264.7 cells for 24 h. Data are expressed as the mean ± SD (n = 3).

4.2.7. Permeability enhancer studies

Sugar esters could represent promising absorption enhancers with a safe toxicological profile, which might improve the mucosal absorption of macromolecules [21, 66, 81]. Concerning the sucrose fatty acid esters several studies were reported on their permeability enhancer ability and application [21, 84, 85]. However, in this context, no studies are present in the literature for sucrose esters with aryl(alkyl) chains. Therefore, here the evaluation of three 6-*O*-sucrose aryl(alkyl) esters **12j–l** to act as absorption enhancer was carried out in comparison to that of the corresponding 6′-*O*-lactose aryl(alkyl) ester analogues **6i–k** previously tested in chapter 3 (Figure 25).

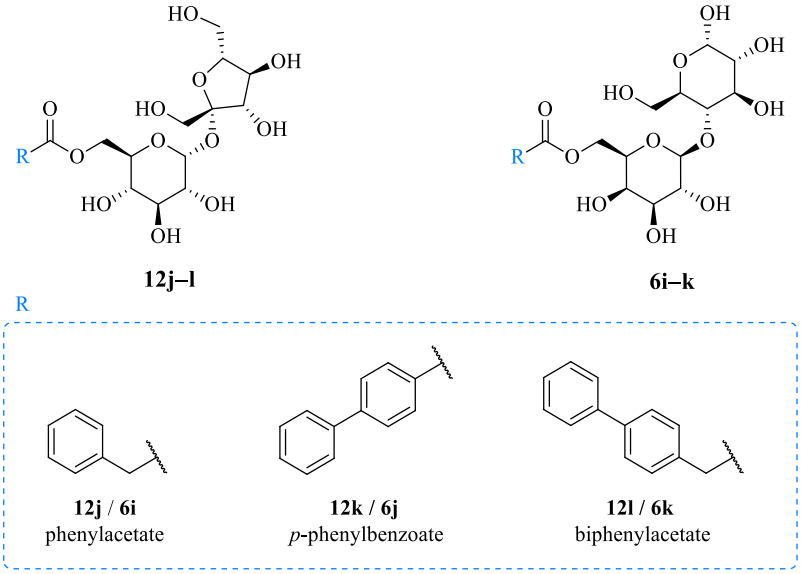


Figure 25. Chemical structure of selected SBEs **12j–l** and their LBEs analogues **6i–k**.

Firstly, the thermogravimetry analysis (TGA), the differential thermal/scanning analyses (DTA/DSC) and CMC measurement were conducted. Then, their biocompatibility was evaluated on Calu-3 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assay. Successively, TEER measurements were performed to evaluate their permeability enhancing activity. Finally, sugar esters with significant TEER decreases were selected for macromolecule permeability assay, conducted using fluorescein isothiocyanate (FITC)-dextran.

4.2.7.1. TGA and DTA/DSC measurements

All TGA showed two thermal events associated with the weight loss of sugar esters (Figure 26). In the temperature range of 50-125 °C, was observed the first loss of weight (≤ 5% of the initial mass), which is correlated to adsorbed water desorption.

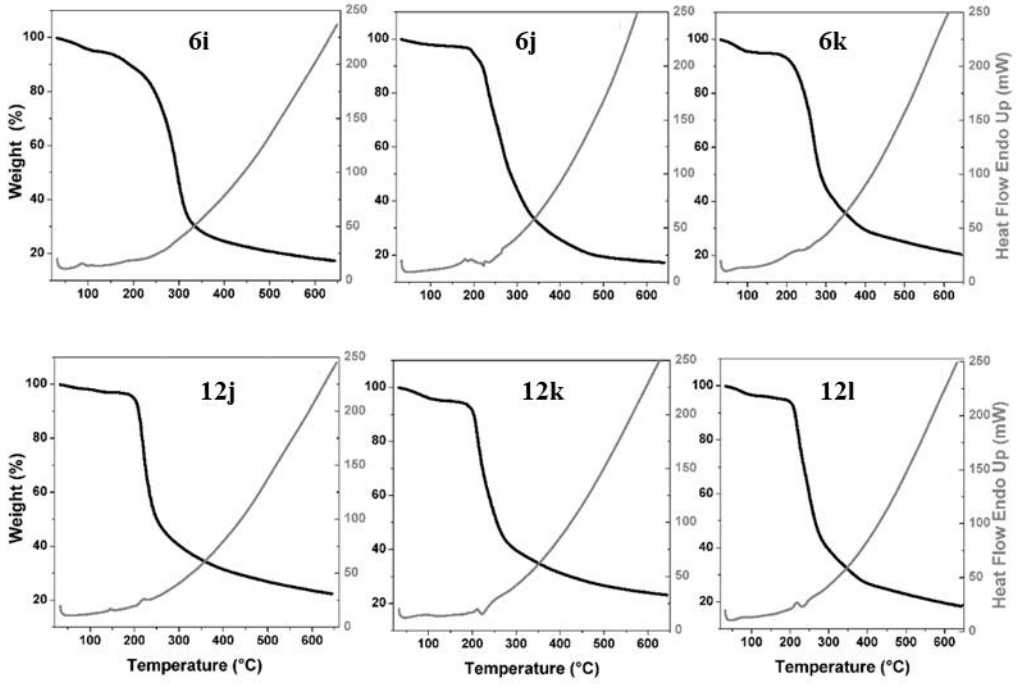


Figure 26. TGA and DTA of sucrose-based esters and lactose-based esters.

The second loss (~ 65-70% of the initial mass) is due to the thermal degradation of sugar esters and was assessed in the temperature range of 200-400 °C. No significant endothermic transitions were observed by DTA/DSC profiles at temperatures lower than the degradation ones, indicating the probably concomitant melting and

degradation of the sugar esters. In general, both 6-*O*-sucrose esters **12j-l** and 6′-*O*-lactose esters **6i-k** show comparable thermal properties.

4.2.7.2. CMC measurements

The deviation of pyrene fluorescence emission (I, III peaks ratio) was exploited to calculate CMC values (Figure 27). Pyrene is a fluorescence probe, largely applied to study the aggregation state of surfactant in aqueous solution because its emission properties are dependent by the polarity of the solutions. Particularly, a reduction in the ratio between I and III peaks indicated an increase of the hydrophobicity in the environments of pyrene due to the formation of micelles or supramolecular aggregates [141]. All plots display a sigmoidal decrease of the I and III pyrene peaks over concentration, implying a self-assembling of surfactants in water. The CMC value is mainly determined by the HLB of surfactants [142]. Particularly, the hydrophobicity of the lipophilic chain of surfactants has a major impact on the CMC value than the variation of the polar head. The sigmoidal profiles are shifted toward lower concentration from **12j** to **12l** and from **6i** to **6k**. This trend is correlated to the different hydrophobicity of the sugar esters related to the presence of a phenyl (**12j** and **6i**) or a biphenyl substituent (**12k**, **12l**, **6j**, **6k**), which changed the CMC values (Table 14).

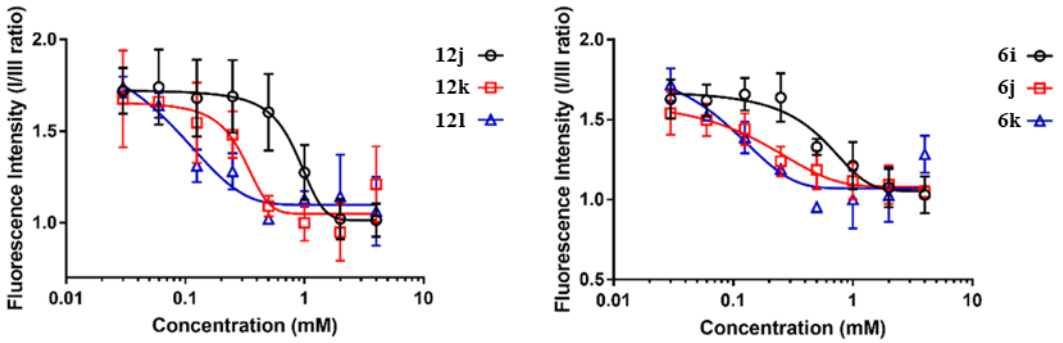


Figure 27. Fluorescence intensity (peak I, III) *vs* concentration plots 6-*O*-sucrose aryl(alkyl) esters **12j-l** and their 6′-*O*-lactose aryl(alkyl) ester analogues **6i-k**.

The higher CMC values were observed for the two sugar esters with phenylacetate moiety (**12j, 6i**). Regarding the sugar linked to a biphenyl moiety, they showed a significant decrease in the CMC, due to the increased hydrophobic character. Moreover, the insertion of a methylene group (**12l**, **6k**) leads to a further diminution in the CMC. On the other hand, no notable difference was observed between sucrose and

lactose esters with the same aryl(alkyl) group, highlighting the less dependency of CMC by the sugar type, as demonstrated also for other sugar surfactants [143]. These self-assembling properties were also confirmed by DLS from counts analysis, which correlated the alteration of the scattering intensities to the detector (kCps) with the concentration of the tested SBEs and LBEs (Figure 28).

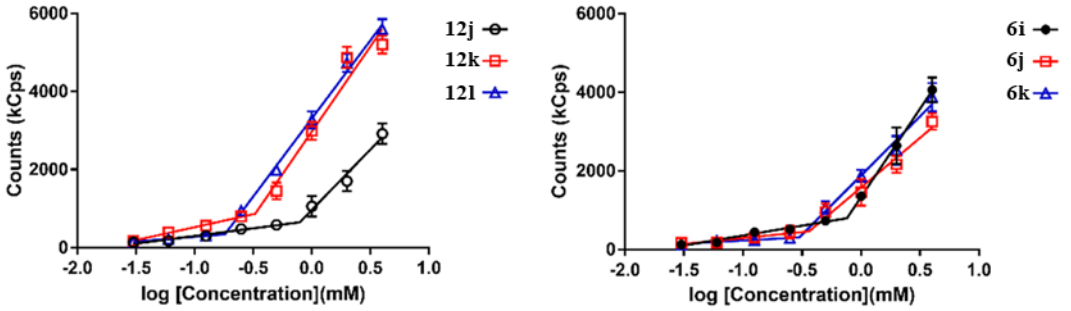


Figure 28. Counts (kCps) *vs* concentration plots from DLS measurements for 6-*O*-sucrose aryl(alkyl) esters **12j–l** and their 6′-*O*-lactose aryl(alkyl) ester analogues **6i–k**.

The CMC is represented by the inflection point, from which a rapid increase in the measured counts of the sugar ester solutions is exhibited due to the onset of micelles formation. [11]. Instead, in the unimeric form, the hydrodynamic sizes of the sugar esters are not big enough and the scattering properties of solutions are not strikingly different from those of the medium. The CMC measurements are reported in Table 10.

Table 14. CMC values calculated for sucrose aryl(alkyl) esters (**12j-l**) and lactose aryl(alkyl) esters (**6i-k**) from fluorescence and DLS measurements.

Entry	Fluorescence spectroscopy CMC (mM)	Dynamic light scattering CMC (mM)
12j	0.861±0.076	0.791±0.045
12k	0.305±0.091	0.329±0.023
12l	0.095±0.015	0.181±0.026
6i	0.753±0.084	0.729±0.075
6j	0.270±0.015	0.344±0.034
6k	0.110±0.017	0.259±0.063

4.2.7.3. Cytotoxicity assays

The cytotoxicity of the sucrose and lactose aryl(alkyl) esters was evaluated on Calu-3 cells. MTT colorimetric assay was applied to measure cellular metabolic activity as a value of cell viability and proliferation. The tested surfactants did not exhibit variation of the cell viability at the concentrations tested apart for the sucrose *p*-phenyl benzoate **12k**, which presented a reduction in viability to ~70% at the highest tested concentration of 4.5 mM, and lactose phenylacetate **6i**, which lead to a reduction to 70% (Figure 29). In addition, the cytotoxicity of the sugar esters was also evaluated by LDH release assay, which is correlated to damages to the plasma membrane of cells. The data obtained corroborated the high biocompatibility of these sugar esters at the tested concentrations (Figure 30).

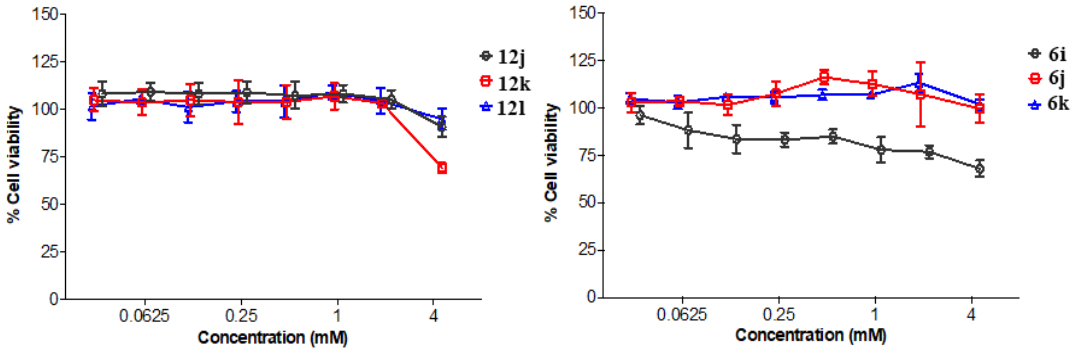


Figure 29. MTT cell viability study of 6-*O*-sucrose aryl(alkyl) esters **12j–l** and their 6′-*O*-lactose aryl(alkyl) ester analogues **6i–k** on Calu-3 cells.

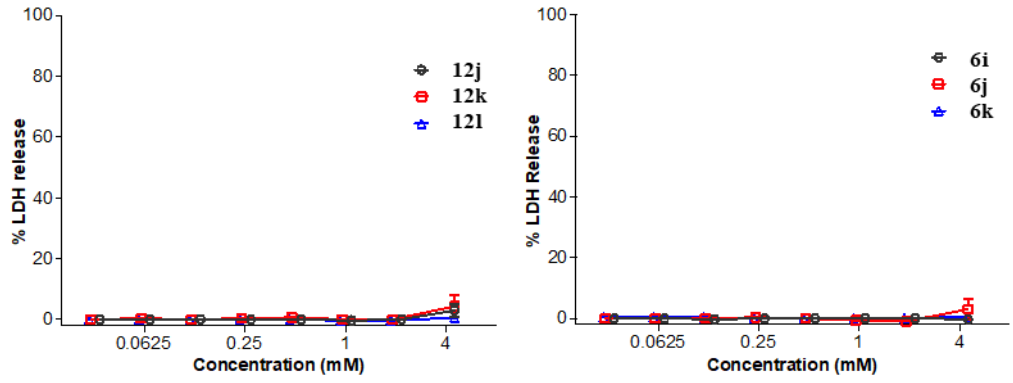


Figure 30. LDH release study of 6-*O*-sucrose aryl(alkyl) esters **12j–l** and their 6′-*O*-lactose aryl(alkyl) ester analogues **6i–k** on Calu-3 cells.

4.2.7.4. TEER experiments

TEER studies were conducted in Calu-3 cells to estimate the capacity of sucrose and lactose esters to act as PEs through transmucosal perturbation by the transient opening of the TJs (Figure 31). TEER measurements were evaluated in parallel to the cytotoxic profile of the tested sugar esters to confirm that the TEER variations are not linked to the permeant damage of the membrane. In fact, a transient modulation of TJs opening generally affect in a reversible effect on the TEER, while a permanent modification of the membrane integrity is highlighted by an irreversible effect on the TEER.

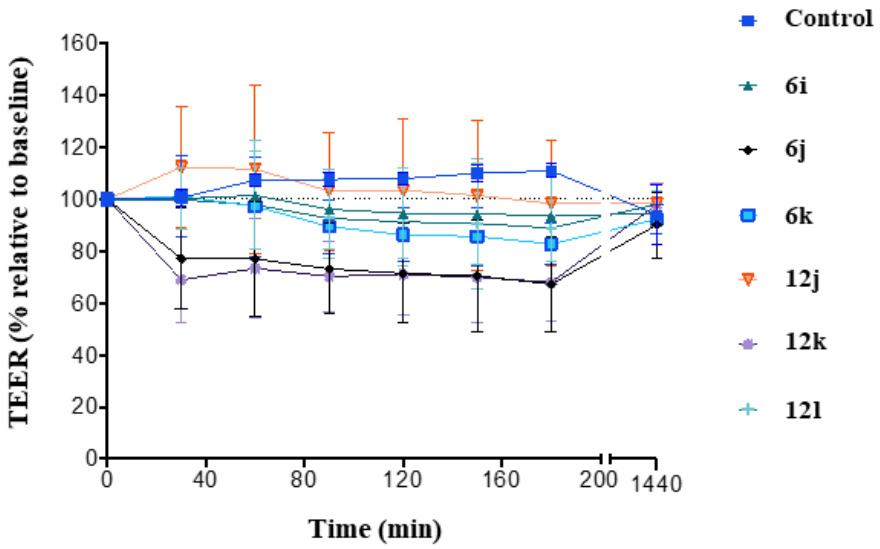


Figure 31. TEER study of 6-*O*-sucrose aryl(alkyl) esters **12j–l** and their 6′-*O*-lactose aryl(alkyl) ester analogues **6i–k** at a concentration of 4 mM on Calu-3 monolayers.

However, the reversible effect on TEER could be also correlated to other mechanisms. The concentration selected for each sugar-based surfactants in these TEER studies was 4 mM, because no cytotoxic effect was observed at this concentration by the MTT and LDH assays above reported. Sucrose *p*-phenylbenzoate **12k** and lactose *p*-phenyl benzoate **6j** showed a major decrease in TEER, while the other two lactose esters (**6i,k**), and sucrose biphenylacetate **12l** displayed only a small decrement in the TEER. Finally, sucrose phenylacetate **12j** only poorly altered the TEER, implying a reduced effect in TJs opening (or other membrane perturbation mechanisms). The ability of **12k** and **6j** to lower considerably the TEER suggested that these two sugar esters have a good balance between safety and efficacy. Remarkably, TEER reversed to the initial value after 24 h with all the tested surfactants, suggesting a transient effect on the

membrane permeability, possibly by TJs opening. Sucrose *p*-phenylbenzoate **12k** and lactose *p*-phenylbenzoate **6j** were selected for further permeability studies conducted to evaluate their potentiality as PEs, as reported in the next chapter 4.2.6.5.

4.2.7.5. Permeability study

The apparent permeability coefficient (P_{app}) values of FITC-Dextran in the absence (control) and in the presence of sucrose *p*-phenyl benzoate **12k** and lactose *p*-phenyl benzoate **6j** at a concentration of 4 mM was measured across Calu-3 cell layers (Figure 32). In agreement with TEER measurements, both sugar *p*-phenyl benzoate showed an increase in FITC-Dextran permeation. The best result was achieved with **12k**, which remarked a more promising permeation enhancing effect, while **6j** displayed a mild permeation effect but with a better safety profile. Further study of **6j** and **12k** on other mucosal epithelial cells will be necessary.

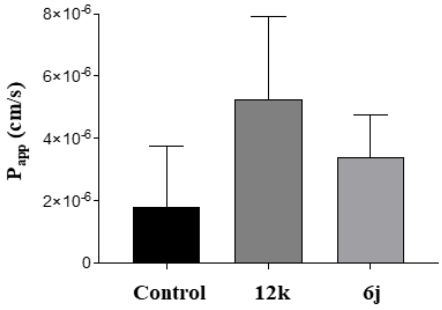


Figure 32. The P_{app} coefficient of FITC-Dextran in the presence of . Sucrose *p*-phenyl benzoate **12k** and lactose *p*-phenyl benzoate **6j** at a concentration of 4 mM across Calu-3 monolayers.

4.3 Conclusion

A series of 6-*O*-sucrose esters was synthesized by applying a modified Mitsunobu type reaction, which was found to be very versatile for several acid substrates. The best conditions were assessed by a reduction of equivalent of fatty acid and successively applied for substrate scope. Unprotected sucrose was coupled with several fatty acids and aryl(alkyl) acids with the aim to obtain compounds with different physicochemical properties. Successively, the biological activities of the obtained surfactants were evaluated. The antimicrobial tests highlighted a better antifungal activity respect to the antibacterial one. The best result was achieved with 6-*O*-sucrose palmitoleate **12d** which showed MIC values of 16, 32, 64 μg/mL against *C. albicans*, *Fusarium* spp. and

A. fumigatus strains, respectively. Therefore, sucrose palmitoleate was chosen for further biological studies. This sucrose ester surfactants showed a safe profile on HaCaT cells and RAW 264.7 cells at least up to 128 μg/mL. Moreover, it exhibited considerable anti-inflammatory activity in LPS-activated macrophages, indicating this surfactant as possible agent for controlling the inflammatory response. **12j–l** were also studied as adsorption enhancer and compared to their corresponding 6′-*O*-lactose-based esters. In general, these surfactants showed insignificant cytotoxicity *in vitro* on Calu-3 cells at their effective concentrations. 6-*O*-Sucrose *p*-phenylbenzoate **12k** and its relative lactose ester **6j** presented the largest significant decrease in the TEER measurement. Their permeability enhancing effect was also confirmed through an increasing of the P_{app} by permeability study. In particular, the best result was assessed for 6-*O*-sucrose *p*-phenylbenzoate. Based on these studies, sucrose palmitoleate **12d** could be proposed as potential alternative antifungal and anti-inflammatory agent in food and other industries. On the other hand, due to its optimal cytocompatibility, sucrose *p*-phenylbenzoate **12k** could be employed as PEs in pharmaceutical applications.

4.4. Materials and methods

4.4.1. Chemicals

Caprylic and undecynoic acids were purchased from TCI (Zwijndrecht, Belgium). Undecylenic, palmitoleic, oleic, linoleic, linolenic and nervonic acids and sucrose were purchased from Fluorochem (Hadfield, UK), diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (PPh_3) from Alfa Aesar (Ward Hill, Massachusetts, USA), benzoic, phenylacetic, phenylbenzoic, and biphenylacetic acids and all solvent from Sigma-Aldrich (Milan, Italy). The structures of 6-*O*-sucrose esters were assessed by MS, ^{1}H NMR, ^{13}C NMR, and IR. ESI-MS spectra were recorded with a Waters Micromass ZQ spectrometer in a negative or positive mode using a nebulizing nitrogen gas at 400 L/min and a temperature of 250 °C, cone flow 40 mL/min, capillary 3.5 kV and cone voltage 60 V; only molecular ions $[M - H]^-$ or $[M+ NH_4]^+$ or $[M + Na]^+$ are given. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 400 or 101, respectively, spectrometer and analyzed using the TopSpin 1.3 software package. Chemical shifts were measured by using the central peak of the solvent. Column

chromatography purifications were performed under “flash” conditions using Merck 230–400 mesh silica gel. TLC was carried out on Merck silica gel 60 F254 plates, which were visualized by exposure to ultraviolet light and by exposure to an aqueous solution of ceric ammonium molybdate.

4.4.2. General procedure for the synthesis of 6-*O*-sucrose esters (12a–m)

Sucrose (**11**) (0.342 g, 1 mmol) was dissolved in dry DMF (7.9 mL) at 70 °C and stirred under N_2 atmosphere. The mixture was cooled to room temperature and then PPh_3 (0.656 g, 2.5 mmol), the appropriate carboxylic acid (**3a–m**) (1.5 mmol), and DMF (2.1 mL) were added. After complete dissolution, the mixture was cooled to 0 °C and DIAD (0.493 mL, 2.5 mmol) introduced. The mixture was stirred at room temperature for 24-30 h and concentrated. Purification of the residue by column chromatography (DCM/acetone/MeOH/H_2O 7.8:1:1:0.2) gave **12a–m** as solids.

6-*O*-Capryl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose caprylate (**12a**) [144]

Yield: 50% (0.234 g). MS (ESI): 467 $[M - H]^-$, 486 $[M + NH_4]^+$, 491 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 0.86 (t, 3H, J = 7.0 Hz, CH_3) 1.22–1.30 [m, 8H, $(CH_2)_4$], 1.48–1.55 (m, 2H, CH_2CH$_2$COOR), 2.29–2.33 (m, 2H, CH_2COOR), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 6.0 Hz, $J_{H4\text{-}H5}$ = 9.0 Hz, $J_{H4\text{-}H3}$ = 9.5 Hz, H4), 3.21 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.38–3.41 (m, 2H, H1′a, H1′b), 3.49 (ddd, 1H, $J_{H3\text{-}OH3}$ = 5.0 Hz, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.5 Hz, H3), 3.53–3.60 (m, 3H, H5′, H6′a, H6′b), 3.70–3.76 (m, 1H, H4′), 3.88 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz, H3′), 3.90 (ddd, 1H, $J_{H5\text{-}H6b}$ = 1.5 Hz, $J_{H5\text{-}H6a}$ = 6.0 Hz, $J_{H5\text{-}H4}$ = 9.0 Hz, H5), 4.02 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.23 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.38 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 6.0 Hz, OH6′), 4.55 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.80 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.5 Hz, OH1′), 4.88 (d, 1H, $J_{OH3\text{-}H3}$ = 5.0 Hz, OH3), 5.00 (d, 1H, $J_{OH4\text{-}H4}$ = 6.0 Hz, OH4), 5.12 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.15 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1) ppm. ^{13}C NMR (DMSO-d_6): δ = 14.4, 22.5, 24.9, 28.8, 28.9, 31.6, 33.8, 62.6, 63.1, 64.0, 70.4, 70.6, 72.0, 73.1, 75.0, 77.3, 83.2, 91.9, 104.3, 173.5 ppm.

6-*O*-Undec-10-enoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose undec-10-enoate (**12b**)

Yield: 39% (0.198 g). MS (ESI): 507 $[M - H]^-$, 526 $[M + NH_4]^+$, 531 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 1.24–1.27 [m, 8H, $(CH_2)_4$], 1.33–1.35 (m, 2H, CH_2CH$_2$CH=CH$_2$), 1.49–1.53 (m, 2H, CH_2CH$_2$COOR), 1.98–2.04 (m, 2H, CH_2CH=CH$_2$), 2.28–2.32 (m, 2H, CH_2COOR), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 5.0 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.20 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.37–3.40 (m, 2H, H1′a, H1′b), 3.48 (m, 1H, H3), 3.53–3.62 (m, 3H, H5′, H6′a, H6′b), 3.70–3.76 (m, 1H, H4′), 3.88 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$= 8.0 Hz, H3′), 3.91 (ddd, 1H, $J_{H5\text{-}H6b}$ = 1.5 Hz, $J_{H5\text{-}H6a}$ = 6.0 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 4.01 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.23 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.39 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.0 Hz, OH6′), 4.56 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.81 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.0 Hz, OH1′), 4.89 (brs, 1H, OH3), 4.94 (dddd, 1H, $J_{gem} \cong J_1$ = 1.5 Hz, J_2 = 4.0 Hz, J_{cis} = 10.0 Hz, HC*H*=CHCH$_2$), 5.00 (dddd, 1H, $J_{gem} \cong J_1 \cong J_2$ = 1.5 Hz, J_{trans} = 17.0 Hz, *H*CH=CHCH$_2$), 5.01 (d, 1H, $J_{OH4\text{-}H4}$ = 5.0 Hz, OH4), 5.13 (d,1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.16 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.80 (dddd, 1H, $J_1 \cong J_2$ = 7.0 Hz, J_{cis} = 10.0 Hz, J_{trans} = 17.0 Hz, CH$_2$=C*H*CH$_2$) ppm. ^{13}C NMR (DMSO-d_6): δ = 24.9, 28.7, 28.9, 29.1, 29.1, 29.2, 33.6, 33.8, 62.6, 63.1, 64.0, 70.5, 70.6, 72.0, 73.1, 75.0, 77.4, 83.2, 91.9, 104.4, 115.1, 139.3, 173.5 ppm.

6-*O*-Undec-10-inoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose undec-10-inoate (**12c**)

Yield: 37% (0.187 g). MS (ESI): 505 $[M - H]^-$, 524 $[M + NH_4]^+$, 529 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 1.23–1.27 [m, 6H, $(CH_2)_3$], 1.30–1.37 (m, 2H, CH_2CH$_2$CH$_2$CCH), 1.40–1.47 (m, 2H, CH_2CH$_2$CCH), 1.49–1.55 (m, 2H, CH_2CH$_2$COOR), 2.14 (td, 2H, J_1 = 2.5 Hz, J_2 = 7.0 Hz, CH_2CCH), 2.28–2.33 (m, 2H, CH_2COOR), 2.72 (t, 1H, J = 2.5 Hz, C*CH*), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 5.0 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.21 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.36–3.41 (m, 2H, H1′a, H1′b), 3.49 (ddd, 1H, $J_{H3\text{-}OH3}$ = 5.0 Hz, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.5 Hz, H3), 3.53–3.62 (m, 3H, H5′, H6′a, H6′b), 3.70–3.76 (m, 1H, H4′), 3.88 (dd,

1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz, H3′), 3.90 (ddd, 1H, $J_{H5\text{-}H6b}$ = 1.5 Hz, $J_{H5\text{-}H6a}$ = 6.0 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 4.02 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.23 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.38 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.0 Hz, OH6′), 4.56 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.80 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.0 Hz, OH1′), 4.88 (d, 1H, $J_{OH3\text{-}H3}$ = 5.0 Hz, OH3), 5.00 (d, 1H, $J_{OH4\text{-}H4}$ = 5.0 Hz, OH4), 5.13 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.16 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1) ppm. ^{13}C NMR (DMSO-d_6): δ = 18.1, 24.9, 28.4, 28.5, 28.8, 28.9, 29.1, 33.8, 62.7, 63.0, 64.0, 70.5, 70.6, 71.5, 72.0, 73.1, 75.0, 77.4, 83.2, 85.0, 91.9, 104.4, 173.5 ppm.

6-*O*-Hexadec-9-enoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose palmitoleate (**12d**) [32]

Yield: 30% (0.173 g). MS (ESI): 577 $[M - H]^-$, 596 $[M + NH_4]^+$, 601 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 0.86 (t, 3H, J = 7.0 Hz, CH_3), 1.26–1.30 [m, 16H, $(CH_2)_8$], 1.50–1.53 (m 2H, CH_2CH$_2$COOR), 1.97–2.00 (m, 4H, CH_2CH=CHCH_2), 2.28–2.32 (m, 2H, CH_2COOR), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 5.0 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.19 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 4.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.38–3.39 (m, 2H, H1′a, H1′b), 3.49 (m, 1H, H3), 3.54–3.62 (m, 3H, H5′, H6′a, H6′b), 3.71–3.76 (m, 1H, H4′), 3.88 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz, H3′), 3.91 (ddd, 1H, $J_{H5\text{-}H6b}$ = 1.5 Hz, $J_{H5\text{-}H6a}$ = 6.0 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 4.03 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.23 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.38 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.0 Hz, OH6′), 4.55 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.80 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.0 Hz, OH1′), 4.88 (brs, 1H, OH3), 5.00 (d, 1H, $J_{OH4\text{-}H4}$ = 5.0 Hz, OH4), 5.12 (d, 1H, $J_{OH2\text{-}H2}$ = 4.0 Hz, OH2), 5.14 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.31 (ddd, 1H, $J_1 \cong J_2$ = 6.0 Hz, J_3 = 11.0 Hz, CH=CH), 5.34 (ddd, 1H, $J_1 \cong J_2$ = 6.0 Hz, J_3 = 11.0 Hz, CH=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 14.4, 22.5, 24.8, 27.1 (2C), 28.7, 29.0, 29.1, 29.6, 31.6, 33.8, 62.6, 63.1, 64.0, 70.4, 70.6, 72.0, 73.1, 75.0, 77.4, 83.2, 91.9, 104.4, 130.1 (2C), 173.5 ppm.

6-*O*-Octadec-9-enoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose oleate (**12e**) [145]

Yield: 36% (0.218 g). MS (ESI): 605 $[M - H]^-$, 624 $[M + NH_4]^+$, 629 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 0.86 (t, 3H, J = 6.5 Hz, CH_3) 1.22–1.33 [m, 20H, $(CH_2)_{10}$], 1.47–1.55 (m, 2H, CH_2CH$_2$COOR), 1.95–2.01 (m, 4H, CH_2CH=CHCH_2), 2.28–2.32 (m, 2H, CH_2COOR), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 6.0 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.20 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.39–3.40 (m, 2H, H1′a, H1′b), 3.48 (ddd, 1H, $J_{H3\text{-}OH3}$ = 5.0 Hz, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.5 Hz, H3), 3.52–3.62 (m, 3H, H5′, H6′a, H6′b), 3.70–3.76 (m, 1H, H4′), 3.87 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$= 8.0 Hz, H3′), 3.91 (ddd, 1H, $J_{H5\text{-}H6b}$ = 1.5 Hz, $J_{H5\text{-}H6a}$ = 6.0 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 4.02 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.23 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.39 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.0 Hz, OH6′), 4.56 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.81 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.0 Hz, OH1′), 4.88 (d, 1H, $J_{OH3\text{-}H3}$ = 5.0 Hz, OH3), 5.00 (d, 1H, $J_{OH4\text{-}H4}$ = 6.0 Hz, OH4), 5.13 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.16 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.31 (ddd, 1H, $J_1 \cong J_2$ = 6.0 Hz, J_3 = 11.0 Hz, CH=CH), 5.35 (ddd, 1H, $J_1 \cong J_2$ = 6.0 Hz, J_3 = 11.0 Hz, CH=CH) ppm. ^{13}C NMR (DMSO-d_6): δ = 14.4, 22.5, 24.9, 27.0, 27.1, 28.95, 28.97. 29.03, 29.06, 29.12, 29.3, 29.6, 31.7, 33.7, 62.6, 63.0, 64.0, 70.4, 70.6, 72.0, 73.1, 75.0, 77.4, 83.2, 92.0, 104.4, 130.1(2C), 173.5 ppm.

6-*O*-Octadec-9,12-enoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose linoleate (**12f**) [95]

Yield: 23% (0.138 g). MS (ESI): 603 $[M - H]^-$, 622 $[M + NH_4]^+$, 627 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 0.86 (t, 3H, J = 6.5 Hz, CH_3), 1.22–1.34 [m, 14H, $(CH_2)_7$], 1.49–1.54 (m 2H, CH_2CH$_2$COOR), 2.00–2.05 (m, 4H, CH_2CH=CHCH$_2$CH=CHCH_2), 2.28–2.32 (m, 2H, CH_2COOR), 2.74 (m, 2H, CH=CHCH_2CH=CH), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 5.5 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.0 Hz, H4), 3.20 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.0 Hz, H2), 3.39–3.40 (m, 2H, H1′a, H1′b), 3.48 (m, 1H, H3), 3.53–3.61 (m, 3H, H5′, H6′a, H6′b), 3.71–3.77 (m, 1H, H4′), 3.87 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz ,H3′), 3.90–3.93 (m, 1H, H5), 4.02 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ =

11.5 Hz, H6a), 4.22 (dd, 1H, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.38 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.0 Hz, OH6′), 4.56 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.81 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.0 Hz, OH1′), 4.89 (d, 1H, $J_{OH3\text{-}H3}$ = 4.5 Hz, OH3), 5.00 (d, 1H, $J_{OH4\text{-}H4}$ = 5.5 Hz, OH4), 5.12 (d,1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.14 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.5 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.27–5.38 (m, 4H, C*H*=C*H*CH$_2$C*H*=C*H*) ppm. ^{13}C NMR (DMSO-d_6): δ = 14.4, 22.4, 24.9, 25.7, 27.0, 27.1, 28.95, 29.0, 29.1, 29.2, 29.5, 31.3, 33.8, 62.6, 63.0, 64.0, 70.4, 70.6, 72.0, 73.0, 75.0, 77.4, 83.2, 91.9, 104.4, 128.2(2C), 130.2(2C), 173.5 ppm.

6-*O*-octadec-9,12,15-enoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose linolenate (**12g**)

Yield: 25% (0.150 g). MS (ESI): 601 $[M - H]^-$, 620 $[M + NH_4]^+$, 625 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 0.93 (t, 3H, J = 7.5 Hz, CH_3) 1.19–1.26 [m, 8H, $(CH_2)_4$], 1.49–1.54 (m 2H, C*H*$_2$CH$_2$COOR), 2.02–2.08 (m, 4H, C*H*$_2$CH=CHCH$_2$CH=CHCH$_2$CH=CHC*H*$_2$), 2.28–2.32 (m, 2H, C*H*$_2$COOR), 2.73–2.79 (m, 4H, CH=CHC*H*$_2$CH=CHC*H*$_2$CH=CH), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 6.0 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.21 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.38–3.39 (m, 2H, H1′a, H1′b), 3.49 (ddd, 1H, $J_{H3\text{-}OH3}$ = 5.0 Hz, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.5 Hz, H3), 3.54–3.61 (m, 3H, H5′, H6′a, H6′b), 3.70–3.76 (m, 1H, H4′), 3.86 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz ,H3′), 3.90–3.93 (m, 1H, H5), 4.02 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.23 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.38 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.0 Hz, OH6′), 4.56 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.80 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.5 Hz, OH1′), 4.89 (d, 1H, $J_{OH3\text{-}H3}$ = 5.0 Hz, OH3), 5.00 (d, 1H, $J_{OH4\text{-}H4}$ = 6.00 Hz, OH4), 5.13 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.15 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.25–5.40 (m, 6H, C*H*=C*H*CH$_2$C*H*=C*H*CH$_2$C*H*=C*H*) ppm. ^{13}C NMR (DMSO-d_6): δ = 14.6, 20.5, 24.8, 25.6, 25.7, 27.1, 28.9, 29.0, 29.1, 29.5, 33.8, 62.6, 63.0, 64.0, 70.4, 70.6, 72.0, 73.1, 75.0, 77.4, 83.2, 92.0, 104.4, 127.4, 128.0, 128.3, 128.4, 130.4, 132.0, 173.5 ppm.

6-*O*-tetracos-15-enoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose nervonate (**12h**)

Yield: 26% (0.179 g). MS (ESI): 689 $[M - H]^-$, 708 $[M + NH_4]^+$, 713 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 0.86 (t, 3H, J = 7.0 Hz, CH_3), 1.24–1.29 [m, 32H, $(CH_2)_{16}$], 1.49–1.53 (m 2H, CH_2CH$_2$COOR), 1.96–2.00 (m, 4H, CH_2CH=CHCH_2), 2.30 (t, 2H, J = 7.5 Hz, CH_2COOR), 3.06 (ddd, 1H, $J_{H4\text{-}OH4}$ = 5.5 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.21 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.38–3.40 (m, 2H, H1′a, H1′b), 3.49 (dd, 1H, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.5 Hz, H3), 3.54–3.62 (m, 3H, H5′, H6′a, H6′b), 3.71–3.76 (m, 1H, H4′), 3.88 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz ,H3′), 3.89–3.93 (m, 1H, H5), 4.02 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.23 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.37 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.5 Hz, OH6′), 4.56 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.80 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.5 Hz, OH1′), 4.89 (d, 1H, $J_{OH3\text{-}H3}$ = 3.5 Hz, OH3), 5.00 (d, 1H, $J_{OH4\text{-}H4}$ = 5.5 Hz, OH4), 5.13 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.15 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.18 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.31 (ddd, 1H, $J_1 \cong J_2$ = 6.0 Hz, J_3 = 11.0 Hz, CH=CH), 5.34 (ddd, 1H, $J_1 \cong J_2$ = 6.0 Hz, J_3 = 11.0 Hz CH=CH) ppm ^{13}C NMR (DMSO-d_6): δ = 14.3, 22.6, 24.9, 27.0, 29.0, 29.1, 29.29, 29.31, 29.4, 29.5, 31.75, 33.8, 62.7, 63.0, 64.0, 70.5, 70.6, 72.0, 73.1, 75.0, 77.4, 83.2, 92.0, 104.4, 130.1, 173.5 ppm.

6-*O*-benzoyl-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose benzoate (**12i**) [146]

Yield: 43% (0.192 g). MS (ESI): 445 $[M - H]^-$, 428 $[M + NH_4]^+$, 433 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 3.26 (ddd, 1H, $J_{H4\text{-}OH4}$ = 6.0 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.28 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.41–3.43 (m, 2H, H1′a, H1′b), 3.50–3.61 (m, 4H, H3, H5′, H6′a, H6′b), 3.79 (ddd, 1H, $J_{H4'\text{-}OH4}$ = 6.00 Hz, $J_{H4'\text{-}H3'} \cong J_{H4'\text{-}H5'}$ = 8.00 Hz, H4′), 3.91 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz, H3′), 4.04–4.11 (m, 1H, H5), 4.34 (dd, 1H, $J_{H6a\text{-}H5}$ = 5.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.37 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.5 Hz, OH6′), 4.45 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.65 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.83 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.0 Hz, OH1′), 4.92 (d, 1H, $J_{OH3\text{-}H3}$ = 5.0 Hz, OH3), 5.14 (d, 1H, $J_{OH4\text{-}H4}$ =

6.0 Hz, OH4), 5.15 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.19 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 5.23 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 7.51–7.57 (m, 2H, ArH), 7.67 [dddd, 1H, $J_1 \cong J_2$ = 1.0 Hz, $J_3 \cong J_4$ = 8.5 Hz, ArH(*p)*], 7.97–8.01 (m, 2H, ArH) ppm. ^{13}C NMR (DMSO-d_6): δ = 62.6, 63.0, 64.8, 70.5, 72.0, 73.1, 75.0, 77.4, 83.1, 92.2, 104.5, 129.2, 129.7, 130.1, 133.7, 173.5 ppm.

6-*O*-(2-Phenylethanoyl)-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose phenylacetate (**12j**) [147]

Yield: 50% (0.230 g). MS (ESI): 459 $[M - H]^-$, 478 $[M + NH_4]^+$, 483 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 3.07 (ddd, 1H, $J_{H4\text{-}OH4}$ = 6.0 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.21 (ddd, 1H, $J_{H2\text{-}H1}$ = 3.5 Hz, $J_{H2\text{-}OH2}$ = 6.0 Hz, $J_{H2\text{-}H3}$ = 9.5 Hz, H2), 3.39–3.43 (m, 2H, H1′a, H1′b), 3.50 (ddd, 1H, $J_{H3\text{-}OH3}$ = 5.0 Hz, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.5 Hz, H3), 3.58–3.64 (m, 3H, H5′, H6′a, H6′b), 3.67 (d, *J* = 16.0 Hz, 1H, *H*CHAr), 3.72 (d, *J* = 16.0 Hz, 1H, HC*H*Ar), 3.76–3.82 (m, 1H, H4′), 3.90 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz ,H3′), 3.95 (m, 1H, H5), 4.05 (dd, 1H, $J_{H6a\text{-}H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.28 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.43 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.5 Hz, OH6′), 4.60 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.83 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.5 Hz, OH1′), 4.90 (d, 1H, $J_{OH3\text{-}H3}$ = 5.0 Hz, OH3), 5.03 (d, 1H, $J_{OH4\text{-}H4}$ = 6.0 Hz, OH4), 5.13 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.19 (d, 1H, $J_{H1\text{-}H2}$ = 3.5 Hz, H1), 5.20 (d, 1H, $J_{OH4'\text{-}H4'}$ = 6.0 Hz, OH4′), 7.24–7.35 (m, 5H, ArH) ppm. ^{13}C NMR (DMSO-d_6): δ = 21.2, 62.7, 63.1, 64.6, 70.6, 72.0, 73.1, 75.0, 77.4, 83.2, 92.0, 104.4, 127.2, 128.7, 129.9, 134.8, 171.7 ppm.

6-*O*-[2-(4-Phenyl)benzoyl]-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose *p*-phenylbenzoate (**12k**)

Yield: 34% (0.177 g). MS (ESI): 521 $[M - H]^-$, 540 $[M + NH_4]^+$, 545 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 3.25–3.32 (m, 2H, H4, H2), 3.40–3.44 (m, 2H, H1′a, H1′b), 3.48 (ddd, 1H, $J_{H3\text{-}OH3}$ = 5.0 Hz, $J_{H3\text{-}H2} \cong J_{H3\text{-}H4}$ = 9.0 Hz, H3), 3.52–3.62 (m, 3H, H5′, H6′a, H6′b), 3.78–3.83 (m, 1H, H4′), 3.92 (dd, 1H, $J_{H3'\text{-}OH3'} \cong J_{H3'\text{-}H4'}$ = 8.0 Hz, H3′), 4.09 (ddd, 1H, $J_{H5\text{-}H6b}$ = 1.5 Hz, $J_{H5\text{-}H6a}$ = 5.0 Hz, $J_{H5\text{-}H4}$ = 9.0 Hz, H5), 4.36 (dd, 1H, $J_{H6a\text{-}H5}$ = 5.0 Hz, $J_{H6a\text{-}H6b}$ = 12.0 Hz, H6a), 4.41 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 6.0 Hz, OH6′), 4.47 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 12.0 Hz, H6b), 4.68 (d, 1H, $J_{OH3'\text{-}H3'}$

= 8.0 Hz, OH3′), 4.85 (dd, 1H, $J_{OH1'-H1'a} \cong J_{OH1'-H1'b}$ = 6.5 Hz, OH1′), 4.96 (d, 1H, J_{OH3-H3} = 5.0 Hz, OH3), 5.17 (d, 1H, J_{OH4-H4} = 5.0 Hz, OH4), 5.18 (d,1H, J_{OH2-H2} = 6.0 Hz, OH2), 5.21 (d, 1H, $J_{OH4'-H4'}$ = 5.5 Hz, OH4′), 5.24 (d, 1H, J_{H1-H2} = 3.5 Hz, H1), 7.42–7.46 (m, 1H, ArH), 7.50–7.54 (m, 2H, ArH), 7.74–7.76 (m, 2H, ArH), 7.82–7.85 (m, 2H, ArH), 8.05–8.08 (m, 2H, ArH) ppm. ^{13}C NMR (DMSO-d_6): δ = 62.5, 63.0, 64.8, 70.5, 70.6, 72.0, 73.1, 74.9, 77.3, 83.1, 92.2, 104.5, 127.4, 127.5, 128.9, 129.0, 129.6, 130.4, 139.4, 145.1, 166.1 ppm.

6-*O*-[2-(4-Phenyl)phenylethanoyl]-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose *p*-biphenyl acetate (**12j**)

Yield: 58% (0.306 g). MS (ESI): 527 $[M - H]^-$, 536 $[M + NH_4]^+$, 541 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ = 3.03 (ddd, 1H, J_{H4-OH4} = 6.0 Hz, $J_{H4-H3} \cong J_{H4-H5}$ = 9.5 Hz, H4), 3.13 (ddd, 1H, J_{H2-H1} = 3.5 Hz, J_{H2-OH2} = 6.0 Hz, J_{H2-H3} = 9.5 Hz, H2), 3.37–3.41 (m, 2H, H1′a, H1′b), 3.48 (ddd, 1H, J_{H3-OH3} = 5.0 Hz, $J_{H3-H2} \cong J_{H3-H4}$ = 9.5 Hz, H3), 3.56–3.63 (m, 3H, H5′, H6′a, H6′b), 3.76–3.81 (m, 1H, H4′), 3.88–3.96 (m, 2H, H3′, H5), 4.14 (dd, 1H, J_{H6a-H5} = 5.0 Hz, $J_{H6a-H6b}$ = 11.5 Hz, H6a), 4.33 (dd, 1H, J_{H6b-H5} = 1.0 Hz, $J_{H6b-H6a}$ = 11.5 Hz, H6b), 4.42 (dd, 1H, $J_{OH6'-H6'a} \cong J_{OH6'-H6'b}$ = 5.5 Hz, OH6′), 4.62 (d, 1H, $J_{OH3'-H3'}$ = 8.0 Hz, OH3′), 4.82 (dd, 1H, $J_{OH1'-H1'a} \cong J_{OH1'-H1'b}$ = 6.0 Hz, OH1′), 4.89 (d, 1H, J_{OH3-H3} = 5.0 Hz, OH3), 5.02 (d, 1H, J_{OH4-H4} = 6.0 Hz, OH4), 5.13 (d, 1H, J_{OH2-H2} = 6.0 Hz, OH2), 5.17 (d, 1H, J_{H1-H2} = 3.5 Hz, H1) 5.20 (d, 1H, $J_{OH4'-H4'}$ = 5.5 Hz, OH4′), 7.24–7.28 (m, 2H, ArH), 7.29–7.37 (m, 7H, ArH) ppm. ^{13}C NMR (DMSO-d_6): δ = 56.1, 62.5, 63.0, 64.6, 70.3, 70.4, 71.9, 73.1, 75.0, 77.4, 83.2, 92.0, 104.5, 127.4, 127.5, 128.9, 130.0, 139.48, 139.52, 172.4 ppm.

6-*O*-[2-(4,4′-Biphenyl)phenylethanoyl]-1-*O*-(β-D-fructofuranosyl)-α-D-glucopyranose, sucrose terphenylacetate (**12m**)

Yield: 31% (0.190 g). MS (ESI): 611 $[M - H]^-$, 630 $[M + NH_4]^+$, 635 $[M + Na]^+$.^{1}H NMR (DMSO-d_6): δ = 3.08 (ddd, 1H, J_{H4-OH4} = 5.0 Hz, $J_{H4-H3} \cong J_{H4-H5}$ = 9.5 Hz, H4), 3.23 (ddd, 1H, J_{H2-H1} = 4.0 Hz, J_{H2-OH2} = 6.0 Hz, J_{H2-H3} = 9.5 Hz, H2), 3.38–3.43 (m, 2H, H1′a, H1′b), 3.46–3.54 (m, 1H, H3), 3.57–3.66 (m, 3H, H5′, H6′a, H6′b), 3.73 (d, *J* = 16.0 Hz, 1H, *H*CHAr), 3.77 (d, *J* = 16.0 Hz, 1H, HC*H*Ar), 3.79–3.82 (m, 1H, H4′), 3.90 (dd, 1H, $J_{H3'-OH3'} \cong J_{H3'-H4'}$ = 8.0 Hz , H3′), 3.97 (m, 1H, H5), 4.08 (dd, 1H, J_{H6a-}

$_{H5}$ = 6.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.31 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 11.5 Hz, H6b), 4.46 (dd, 1H, $J_{OH6'\text{-}H6'a} \cong J_{OH6'\text{-}H6'b}$ = 5.5 Hz, OH6′), 4.62 (d, 1H, $J_{OH3'\text{-}H3'}$ = 8.0 Hz, OH3′), 4.83 (dd, 1H, $J_{OH1'\text{-}H1'a} \cong J_{OH1'\text{-}H1'b}$ = 6.0 Hz, OH1′), 4.84 (brs, 1H, OH3), 5.06 (d, 1H, $J_{OH4\text{-}H4}$ = 5.0 Hz, OH4), 5.15 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz, OH2), 5.21 (m, 2H, H1, OH4′), 7.35–7.42 (m, 3H, ArH), 7.45–7.51 (m, 2H, ArH), 7.66–7.78 (m, 8H, ArH) ppm. ^{13}C NMR (DMSO-d_6): δ = 62.7, 63.1, 64.7, 70.5, 70.6, 72.0, 73.1, 75.0, 77.3, 83.2, 91.9, 104.4, 126.9, 127.0, 127.5, 127.55, 127.65, 128.0, 129.4, 130.6, 134.2, 138.5, 139.3, 139.6, 140.1, 171.7 ppm.

5. Synthesis of Mannopyranose Ester Derivatives for Innovative Liposomal Anti-Leishmanial Therapy

5.1. Introduction

Leishmaniasis is a neglected parasitic disease considered endemic over 90 countries, mainly belonging to North-East Africa, East Asia and Central America [148]. It is estimated that affects 12 million people with 350 million people at risk of infection worldwide [149, 150]. This disease is caused by *leishmania* parasites, obligate intracellular protozoa, which are transmitted by the bite of infected female sandfly, called phlebotomine. This sandfly injects promastigotes into the skin of the host, which are recognized and phagocyted by macrophages, where they are transformed into flagellar amastigotes [148]. The type of infection varies by the strain of *leishmania,* and the type of immune response of the host [151]. Leishmaniasis occurs mainly in three forms: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL) [149]. VL, is the most serious form, proving 20000-40000 cases each year [150]. Leishmania parasites can elude the defense mechanisms of the host and treatments with conventional drugs do not guarantee the complete eradication of intracellular pathogens [152]. Therefore, leishmaniasis is considered a global public health problem. In fact, no vaccine is still available to prevent this disease and the current commercial drugs (e.g., Pentamidine, Amphotericin B, Miltefosine, Paromomycin) possess several drawbacks such as the lack of safety and efficacy, high costs, high toxicity, difficulty in administration, long treatment duration and drug resistance [152, 153]. Hence, the research focused on the development of new efficacious therapeutic antileishmanial strategies, with low toxicity and reasonable cost, is still fundamental. Nowadays, many drawbacks of conventional formulations can be overcome using nanocarriers systems [154]. Additionally, *leishmania* parasites mainly infect macrophages, leading to M1 (pro-inflammatory) or M2 (anti-inflammatory, permissive to infection) activation of macrophages. Particularly, M2-like macrophages overexpress mannose receptor (MRC1/CD206). Therefore, trying to increase drug bioavailability and to target M2-like macrophages nanosized carriers targeting CD206 have been exploited [154]. Mannose was conjugated on different types of nanoparticles showing increased

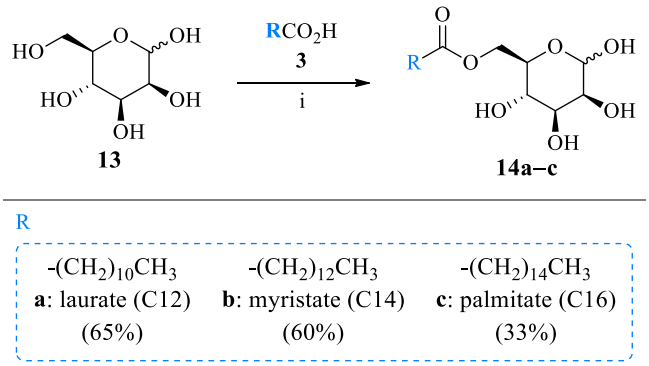
selective uptake by infected macrophages [104, 105]. Among nanoparticles, also liposomes have been explored for the leishmaniasis treatment (e.g., AmBisome) [155]. Liposomal formulations with mannose-based active targeting have been studied showing improved properties compared to non-targeted ones [156]. Nevertheless, in all cases, the preparation method involved chemical reactions to effectively conjugate the mannose molecule to the liposomal surface increasing the overall complexity. To the best of our knowledge no mannose-decorated liposomes have been produced by simply mixing appropriate excipients containing sugar moieties. This research project has the aim to produce mannose esters decorated liposome, through mixing methodology (thin layer evaporation and microfluidic), able to deliver antileishmanial drug (e.g., Miltefosine) or other synthetic molecules, such as bisindole derivatives [157] to the infected macrophages, trying to reduce drug amounts and consequent toxicity, at the same time increasing bioavailability. For this purpose, a small library of mono, di- and tri-mannopyranose monoester derivatives was obtained. Firstly, the 6-*O*-mannose-based esters (MBEs) were synthesized through an enzymatic esterification in a single step using three aliphatic fatty acids (C12, C14 and C16). The same fatty acids substrates were employed for the synthesis of 6′-*O*-α1,2-dimannopyranose (DMEs) and 6′′-*O*-α-1,2 trimannopyranose (TMEs) esters. Hence, two similar multistep synthetic approaches starting from mannose were designed. The key steps are represented by the *O*-glycosylation and the esterification reactions. Once achieved the opportune protected glycosyl acceptor and donors from different pathways, the conditions of the glycosylation reactions were performed. Then, different esterification reactions were screened using palmitic acid (C16) as reagent. The best ones were selected, optimized, and successively applied for the coupling with the other fatty acids. The final products were chemically characterized, and they will be employed to produce mannose esters surface decorated liposomes for delivery of antileishmanial drug.

5.2. Results and discussion

5.2.1. Synthesis of 6-*O*-mannose-based esters

MBEs **14a–c** were synthesized using a reported lipase-catalysed reaction (Scheme 6) [158]. In detail, mannose **13** was coupled with saturated fatty acids (lauric, myristic

and palmitic) in 1:3 molar ratio in the presence of Novozyme 435® and MS 4Å, in dry acetone at room temperature for four days (Scheme 6). The final mannose esters were obtained as a mixture of anomers (α/β ratio 1:0.1). Good yields were achieved for mannose laurate **14a** and mannose myristate **14b**, while a considerable reduction was observed with mannose palmitate **14c**. Hence, it was assessed a correlation between the reduction of yield with the increase of the chain length. The advantages of this reaction were constituted by the regioselective esterification in 6 position and the easy step of purification. Moreover, Novozyme 435® have a reasonable price compared to the other lipases.

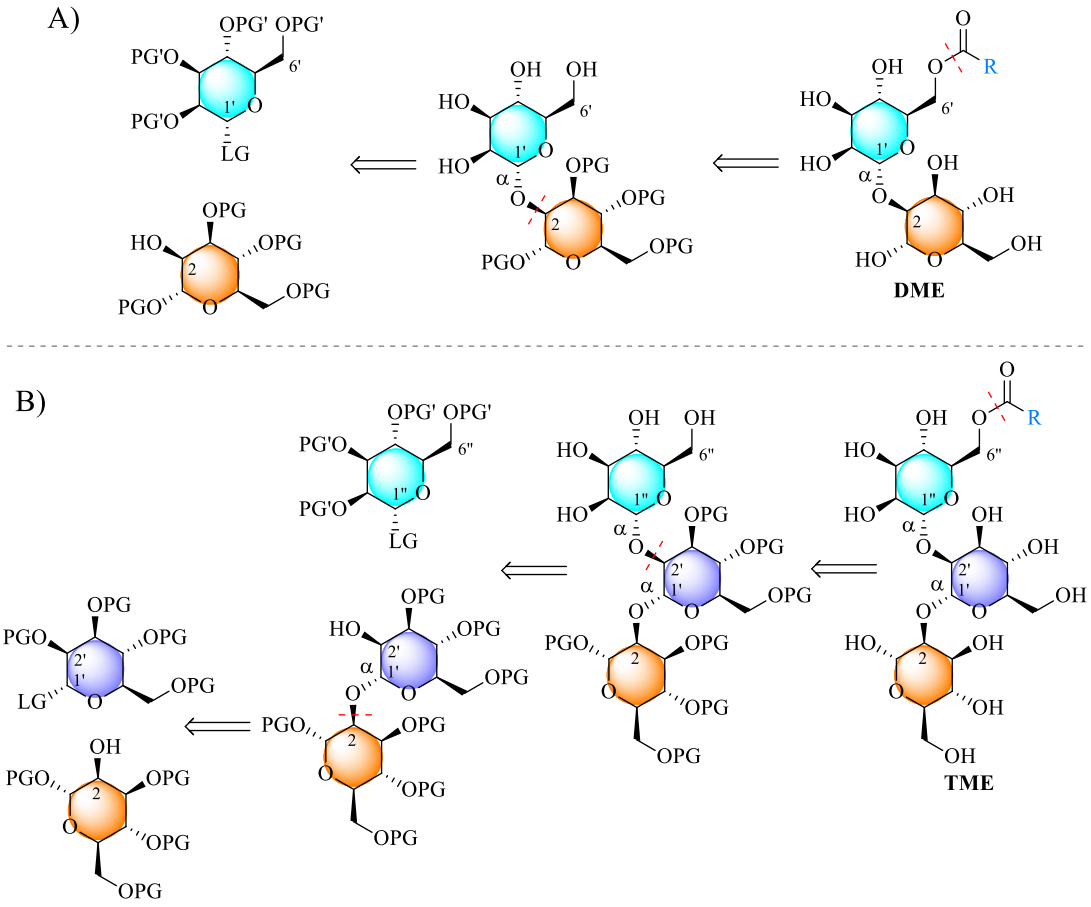


Scheme 6. Enzymatic synthesis of 6-*O*-mannose esters. Reagents, conditions and yields: (i) Novozyme 435®, MS 4Å, dry acetone, rt, 96 h.

5.2.2. Design of the multistep synthesis of DMEs and TMEs

Once synthesized the 6-*O*-mannopyranose esters, multistep syntheses of 6′-*O*-α1,2-dimannopyranose (DMEs) and 6′′-*O*-α-1,2-trimannopyranose (TMEs) esters were designed. These two polar heads were selected because they have recently shown high affinity to the MRs in lipidic nanoparticles [103]. The retrosynthetic analysis of DMEs and TMEs highlighted the importance of two steps (Scheme 7): the *O*-glycosylation and the esterification reaction. Regarding the first one, the installation of protecting groups (PGs) on the glycosyl donors and glycosyl acceptors as well as the leaving groups (LGs) on glycosyl donor and the choice of promoter agent are essential parameter to reach a stereo-controlled *O*-glycosylation in good yields [159, 160]. The mechanism of glycosylation reaction involves the activation of the LG in the anomeric position of glycosyl donor by the promoter (generally a Lewis acid), followed by its elimination giving the resultant oxocarbenium ion. Then, electrophile glycosyl donor

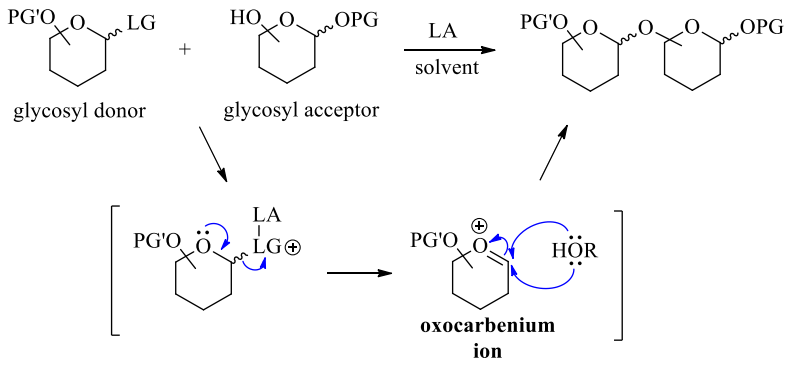
undergoes the nucleophilic attack of glycosyl acceptor, which afforded the glycosidic linkage (Scheme 8). The formation of α or β glycosidic linker is dependent by several parameters, such as the steric hindrance, the solvent effect and neighbor-group participation [160]. The choice of PGs also assumed relevance in the esterification reaction. In fact, the installation of orthogonal groups on the different carbohydrate units could be decisive to obtain a regioselective esterification in the primary 6′ position, avoiding the formation of undesired diester derivatives.



Scheme 7. Retrosynthetic mechanism of 6′-*O*-α-1,2-dimannopyranose (DMEs, A) and 6′′-*O*-α-1,2-trimannopyranose (TMEs, B). PG = protecting group. LG = leaving group.

Therefore, for the synthesis of DMEs will be require a protected glycosyl donor with a LG in position 1 and a glycosyl acceptor with free OH group in position 2 and different protecting groups respect to those of glycosyl donor. After glycosylation reaction and orthogonal deprotection, the dimannopyranose derivative will be esterified in position 6′ with the acyl donor to give the desired DMEs (Scheme 7A). The same synthetic approach will be utilized for the synthesis of TMEs. In this case

will be necessary a further protected glycosyl donor with LG in position 1, an additional *O*-glycosylation reaction and deprotection step (Scheme 7B).

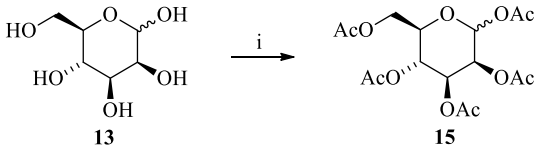


Scheme 8. General mechanism of glycosylation reaction. PG = protecting group, LG = leaving group, LA = Lewis acid.

Clearly, the multistep synthesis of DMEs and TMEs started from the formation of the appropriate glycosyl donor and glycosyl acceptor, which will be discussed below.

5.2.3. Synthesis of the glycosyl donors and glycosyl acceptor

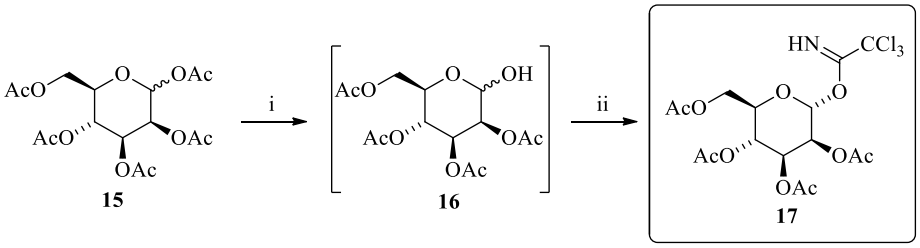
All the desired mannosyl monomers were produced starting from mannose pentaacetate (**15**), which was previously obtained through the per-*O*-acetylation of D-mannose (**13**) with acetic anhydride in quantitative yield (Scheme 9) [161].



Scheme 9. Synthesis of mannose pentaacetate (**15**). Reagents and conditions: (i) Ac_2O, pyridine, rt, 12 h.

Tetra-*O*-acetyl-mannopyranosyl-1-trichloroacetimidate **17** was selected as glycosyl donor both for DMEs and TMEs pathways due to its advantages, such as easy purification step, the relatively high stability and the potential stereochemical control of the glycosylation reaction, which led to the formation of only α-anomer. This monomer was synthetized by two step procedure (Scheme 10) [162]. An initial selective mono-hydrolysis of mannose pentaacetate (**15**) in position 1 with hydrazine acetate led to the intermediate **16**, which was successively treated with trichloroacetonitrile (CCl_3CN) and catalytic amount of 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) to form the trichloroacetamidate activating group (TCA) in the position

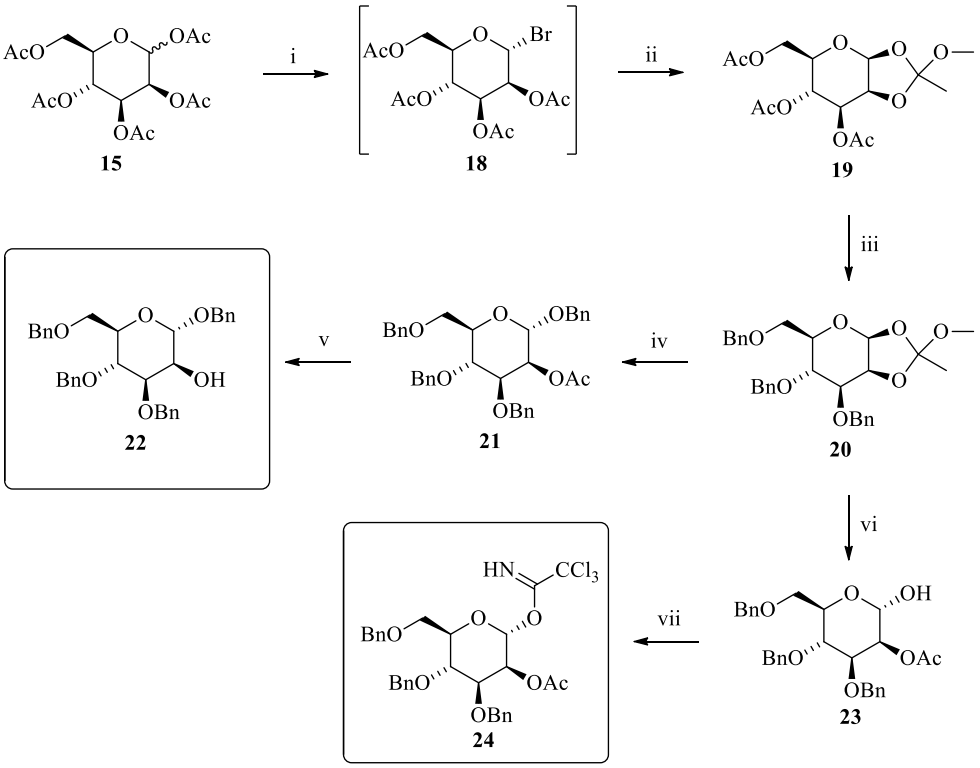
1. The glycosyl donor **17** was obtained with an overall yield of 82%. To be noted that DBU promoted thermodynamic control of the reaction, which led only to the more stable α-anomer.



Scheme 10. Synthesis of glycosyl donor **17**. Reagents and conditions: (i) N_2H_4, AcOH; dry DMF, rt, 1 h. (ii) CCl_3CN, DBU cat., dry DCM, rt, 12 h.

Regarding the glycosyl acceptor, the 1,3,4,6-tetra-*O*-benzyl-2-hydroxy-mannopyranose **22** was selected for the *O*-glycosylation reactions of both DMEs and TMEs pathways. The benzyl group was chosen as PG because of its orthogonal deprotection in comparison with acetyl groups of glycosyl donor **17**. Five steps were required for the synthesis of this glycosyl acceptor (Scheme 11). Firstly, mannose pentaacetate **15** was treated with HBr, AcOH 30% v/v in dry DCM to replace the acetyl group in position 1 with bromine. The resulted 2,3,4,6-tetra-*O*-acetyl-1-bromo-mannopyranose intermediate (**18**) was not isolated, but immediately used due to its low stability. Successive intramolecular substitution mediated by methanol in presence of 2,6-lutidine gave the 3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-1,2-orthoester **19**, which was subsequently deacetylated and benzylated in one step procedure with a large excess of KOH (15 eq.) and benzyl bromide (17 eq.) to afford the 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-1,2-orthoester **20** [162] with an overall yield of 67%. Then, its acidic hydrolysis catalysed by an equimolar amount of boron trifluoride–ether complex ($BF_3{\cdot}OEt_2$) was conducted in the presence of high excess of benzyl alcohol and MS 4Å to give 2-*O*-acetyl-1,3,4,6-tetra-*O*-benzyl-mannopyranose **21** [163]. The final deacetylation of **21** with sodium methoxide (NaOMe) in MeOH furnished the desired 1,3,4,6-tetra-*O*-benzyl-2-hydroxy-mannopyranose **22**. The overall yield of glycosyl acceptor **22** was 55%. Then, the 3,4,6-tri-*O*-benzyl-1,2-orthoester mannopyranose (**20**) was also employed for the synthesis of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-mannopyranosyl-1-TCA **24**. This mannose derivative was selected for the *O*-glycosylation reaction of the TMEs pathway because of its double action. In

fact, it can act as glycosyl donor by the activation of TCA group and successively as glycosyl acceptor through the selective remotion of acetyl group position 2. Regarding its synthesis, the orthoester **20** was subjected to an acidic hydrolysis with acetic acid (90% v/v), which act both as solvent and reagent, giving the compound **23** [164], which was subsequently transformed into the desired TCA derivative (**24**) using CCl_3CN and catalytic amount of DBU [168].



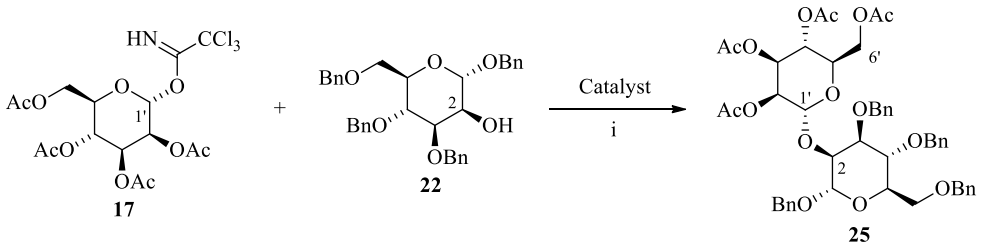
Scheme 11. Synthesis of glycosyl acceptor **22** and donor **24**. Reagents and conditions: (i): HBr, AcOH 30% v/v, dry DCM, rt., 2 h. (ii): 2,6-lutidine, dry DCM/MeOH (1:1 v/v), rt, 12 h. (iii): BnBr, KOH, THF, reflux, 12 h. (iv): $BF_3 \cdot OEt_2$, BnOH, MS 4Å, dry DCM, rt, 2 h. (v): NaOMe, MeOH, rt, 2 h, Amberlyst 15, 10 min. (vi): acetic acid (90% v/v), rt, 5 h. (vii) CCl_3CN, DBU cat., dry DCM, rt, 30 min.

The overall yield of this glycosyl donor **24**, starting from mannose pentaacetate **15**, was 58%. To summarize, the mannose derivatives **17** and **22** were efficiently synthetized for the DMEs and TMEs synthetic pathways. Moreover, the synthesis of mannosyl donor **24** was also assessed for its employment in TMEs pathway.

5.2.4. Multistep synthesis of DMEs

5.2.4.1. Optimization of the O-glycosylation reaction

Generally, the *O*-glycosylation reaction represents the essential step in the synthesis of complex oligomannosides [160, 166]. In this case, for the formation of α-1,2-glycosydic link between glycosyl donor **17** and glycosyl acceptor **22**, different conditions were explored using boron trifluoride–ether complex ($BF_3{\cdot}OEt_2$) or trimethylsilyl triflate (TMSOTf) as promoter Lewis acids in the presence of MS 4Å using dry DCM as non-polar solvent (Scheme 12, Table 15). These two catalysts were utilized because they have been revealed to work well in the formation of *O*-glycosidic links [166]. Moreover, they are safe and represent a suitable alternative to the high toxic mercury compounds. As shown in Table 15, it was observed an increment of product yield from 48% to 62% with 1.5 eq. (entry 2) of glycosyl donor **17** instead of 1.0 eq. (entry 1) using $BF_3{\cdot}OEt_2$ (0.06 eq.) at –20 °C in 3 hours.



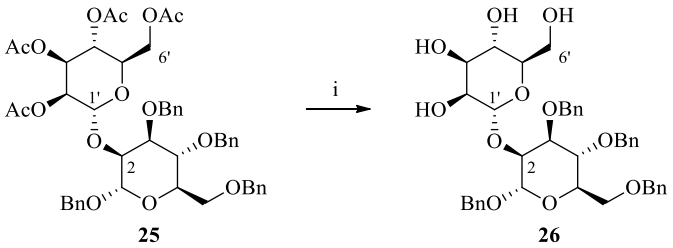
Scheme 12. General scheme of *O*-glycosylation reaction between glycosyl donor **17** and acceptor **22**. Reagents and conditions: (i) Catalyst, MS 4Å, dry DCM.

Table 15. Optimization of the *O*-glycosylation reaction between **17** and **22**.

Entry	Catalyst	Catalyst (eq.)	17 (eq.)	T (°C)	t (h)	Yield 25 (%)
1	$BF_3{\cdot}OEt_2$	0.06	1.0	–20	3	48
2	$BF_3{\cdot}OEt_2$	0.06	1.5	–20	3	62
3	TMSOTf	0.06	1.0	–20	1	77
4	**TMSOTf**	**0.06**	**1.5**	**–20**	**1**	**87**
5	TMSOTf	0.06	1.5	0	1	66

However, the better yields were obtained replacing $BF_3{\cdot}OEt_2$ with TMSOTf, which is more suitable for the glycosyl donor activation with the TCA as leaving group. Indeed, it was achieved a 77% of yield with equimolar ratio of the reagents at –20 °C in 1 h (entry 3). The best result was obtained by increase the equivalent of glycosyl donor to

1.5 eq. at –20 °C in 1 h (entry 4) while an increasing of the temperature to 0 °C led to a reduction to 66% of yield (entry 5). The best reaction conditions (entry 4) furnished only the α-anomer form in 87% of yield. The stereoselectivity of glycosidic link formation was led by the 2-*O*-acyl vicinal group on the donor (anchimeric assistance). The anomeric form of the product **25** was unambiguously assessed by the coupling constant of anomeric proton $J_{H1'\text{-}H2'}$ = 2.0 Hz, which corresponding to desired α-anomer, according to Karplus equation [167]. Therefore, the optimized *O*-glycosylation reaction (entry 4) was selected for the gram-scale production of tetra-*O*-acetyl-tetra-*O*-benzyl-dimannopyranose **25**, which was subsequently deacetylated by basic hydrolysis using NaOMe, giving tetra-*O*-benzyl-dimannopyranose **26** in quantitative yield. This orthogonal deprotection allows to obtain the free hydroxyl groups in only one monomer unit, which was essential for the successive esterification step.

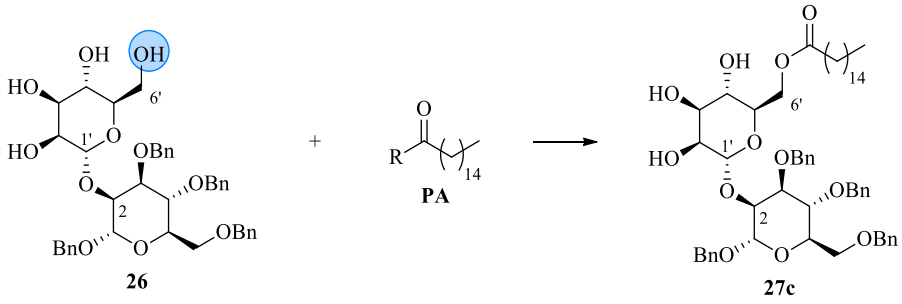


Scheme 13. Deacetylation of protected dimannopyranose **25**. Reagents and conditions: (i) NaOMe, DCM/MeOH (1:1 v/v), rt, 2 h, then DOWEX(H^+), 15 min.

5.2.4.2. Screening of the esterification reactions

The regioselective esterification of the primary 6′ position represents the main challenge for this multistep synthesis. In this case, the mannose derivative **26** is formed by one mannose unit presenting free hydroxyl groups, and one mannose unit with benzyl groups. This allows to enhance the probability of regioselective monoester formation in the 6′ position, which is the only primary free hydroxyl position. Nowadays, no esterification reactions have been reported in literature for this 1,2-α-dimannopyranose. Hence, several esterification reactions were tested to achieve only the 6′-*O*-monoester product. Both enzymatic (Novozyme 435® and Lipozyme®) and chemical reactions (Mitsunobu, Steglich, *via* acyl chlorides) were screened using palmitic acid (**PA**) or its derivatives as acyl donors (Scheme 14, Table 16). The best

result in term of yield (67%) and regioselectivity was obtained using a Lipozyme®-catalysed reaction in dry toluene at 75 °C in 24 h (entry 1). The reaction conditions were similar to that adopted for the synthesis of lactose saturated fatty acid esters as shown in chapter 3. This enzymatic esterification allowed to the regioselective formation of the ester bond in position 6′ as highlighted by heteronuclear correlation between the H6′^{a} and H6′^{b} with the carbon of the carbonyl group as showed by HMBC spectra (Figure 33).



Scheme 14. General esterification reaction for the formation of tetra-*O*-benzyl-6′′-*O*-palmityl-dimannopyranose (**27c**) using palmitic acid (**PA**).

Table 16. Screening of the esterification procedure for the synthesis of tetra-*O*-benzyl-6′′-*O*-palmityl-dimannopyranose (**27c**)

Entry	Reaction conditions	R	Molar ratio 26/PA	Yield 27c (%)
1	**Lipozyme®, dry toluene 75 °C, 24 h**	**OH**	**1:2**	**67**
2	Novozyme 435®, dry acetone, MS 4Å, rt, 4 d	OH	1:3	22
3	Dry TEA, dry DCM, rt, 6 h	Cl	1:1.1	48
4	PPh_3, DIAD, dry DMF, rt, 24 h	OH	1:1.5	23
5	TBTU, DIPEA, dry DMF, rt, 24 h	OH	1.1:1	20
6	EDCI· HCl, DMAP, TEA, dry DCM, rt, 72 h	OH	1.35:1	38

Then, the Novozyme 435®-catalysed reaction [158], previously used for the synthesis of MBEs, furnished only 22% in a regioselective manner (entry 2). On the other hand, the chemical reactions gave less yield compared to Lipozyme®-catalysed reaction. The best yield for the chemical reactions was obtained with *via* acyl chloride procedure (entry 3), which led to a moderate yield (48%) of monoester product at room temperature in 6 h. Additionally it was observed also a lower quantity of diester product as well as with the Mitsunobu reaction (entry 4). This latter was selected by

the precedent work on the synthesis of 6-*O*-sucrose ester (see chapter 4) and gave only 23% of yield using PPh_3 and DIAD at room temperature for 24 h. The lowest yield (20%) was observed in entry 5 with the application of a similar procedure of Paul et al. [35] which required TBTU as uronium-based coupling agent in the presence of DIPEA. Lastly, a modified Steglich reaction (see Chapter 3) led to a regioselective formation of desired monoester in 38 % of yield but in very long reaction time (72 h) (entry 6).

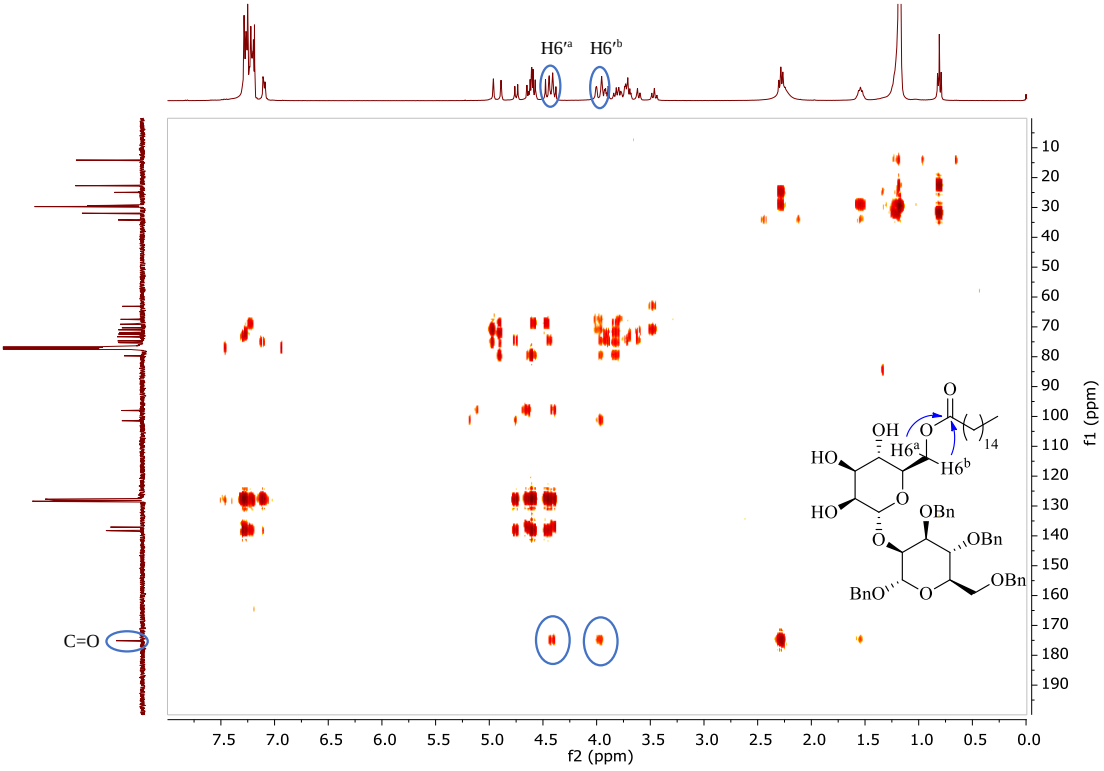
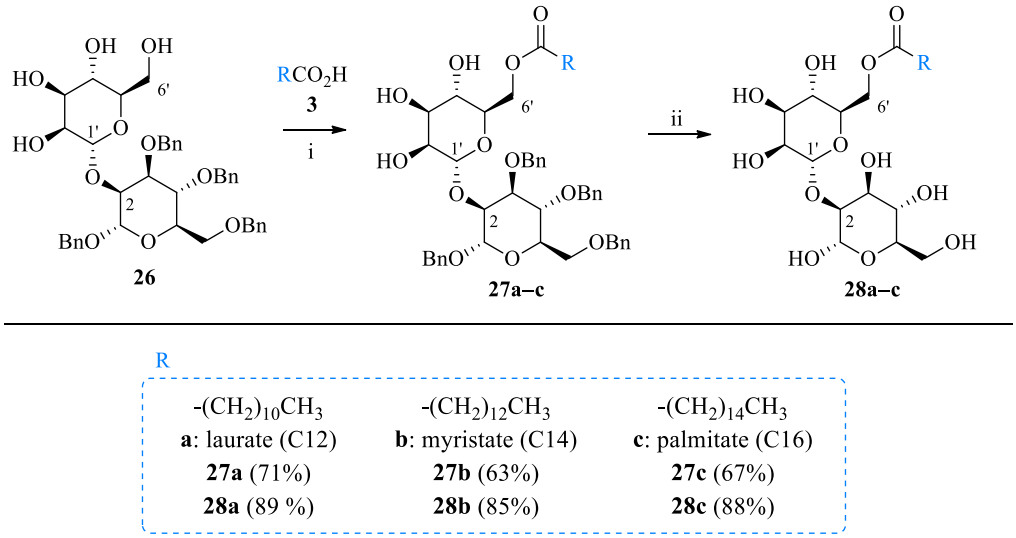


Figure 33. Key HMBC correlation for tetra-*O*-benzyl-6′′-*O*-palmitoyl-dimannopyranose ester **27c**.

Therefore, the Lipozyme®-catalyzed reaction was selected to synthesize tetra-*O*-benzyl-6′′-*O*-acyl-dimannopyranose esters with different fatty acid chains **27a–c**. As depicted in Scheme 15, protected sugar **26** was coupled with lauric acid, myristic acid and palmitic acid in a single step using the conditions reported in entry 1, giving the corresponding protected dimannopyranose esters **27a–c** in good yields (**27a** = 71%, **27b** = 63%, **27c** = 67%). Finally, the final step was represented by the removal of the benzyl groups to give the final DMEs **28a–c**. In detail, tetra-benzylated dimannopyranose esters **27a–c** were deprotected by hydrogenolysis reaction

conducted with Pd/C 10% [166], under hydrogen atmosphere (3.5 atm.) over night, to led the final DMEs **28a–c** in high yields as white solids.

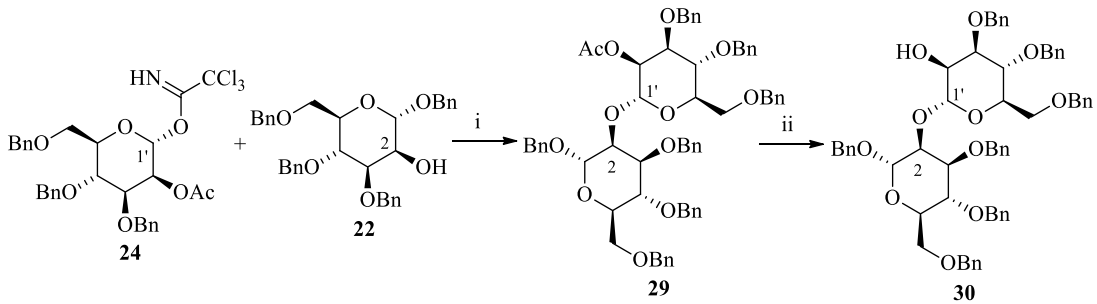


Scheme 15. Lipase-catalysed esterification for the formation of Tetra-*O*-benzyl-6′-*O*-acyl dimannopyranose esters (**27a–c**) and their deprotection into the final DMEs (**28a–c**). Reagents, conditions and yields: (i) Lipozyme®, dry toluene, 75 °C, 24 h. (ii) H_2; Pd/C (10%); dry MeOH; o.n.

5.2.5. Multistep synthesis of TMEs

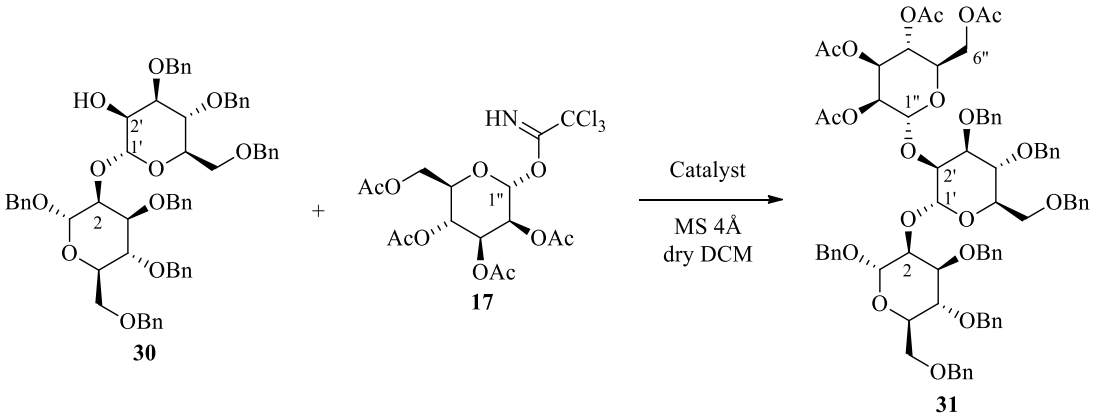
5.2.5.1. O-Glycosylation reactions and deprotection steps

The *O*-glycosylation reaction for the formation of the 2′-*O*-acetyl-hepta-*O*-benzyl-dimmanopyranose **29** was performed using the best conditions assessed for the glycosylation reported above, with an increase of TMSOTf equivalent (Scheme 16). Specifically, the glycosyl donor **24** was activated by catalytic TMSOTf (0.12 eq.) and reacted with glycosyl acceptor **22** in the presence of MS 4Å in dry DCM leading to selective formation of compound **29** with a 78% of yield in 1h at –20 °C.



Scheme 16. Synthesis of hepta-*O*-Benzyl-dimannopyranose **30**. Reagents and conditions: (i) TMSOTf cat., MS 4Å, dry DCM, –20 °C, 1 h. (ii) NaOMe, DCM/MeOH (1:1 v/v), rt, 3 h.

Successively, the basic deprotection of the acetyl group in position 2′ transformed compound **29** into hepta-*O*-benzyl-2′-*O*-hydroxy-dimmanopyranose (Scheme 16), which acted as glycosyl acceptor **30** for the subsequent formation of α-1′′,2′ glycosyl bond with tetra-*O*-acetyl-mannopyranosyl-1-TCA **17** (Scheme 17). For this second glycosylation step were tested different reaction conditions using TMSOTf and $BF_3{\cdot}OEt_2$ as catalyst (Table 17) in the presence of MS 4Å in dry DCM, with the aim to achieve high yield of protected trimannopyranose derivative **31**.



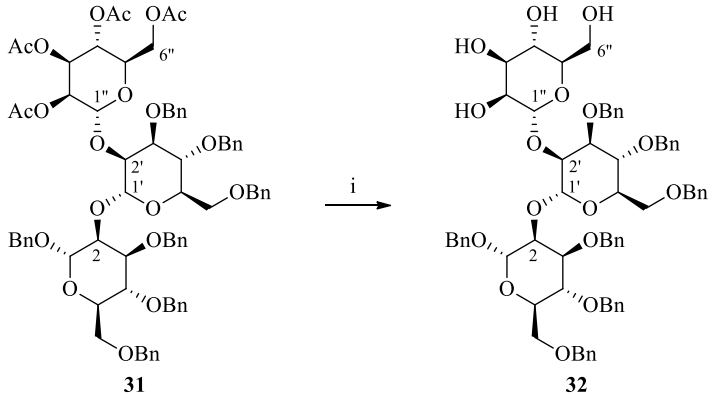
Scheme 17. General scheme of *O*-glycosylation between glycosyl acceptor **30** and donor **17**.

Table 17. Optimization of the *O*-glycosylation reaction between **30** and **17**.

Entry	Catalyst	Catalyst (eq.)	17 (eq.)	T (°C)	t (h)	Yield 31 (%)
1	TMSOTf	0.06	1.5	-20	1	61
2	$BF_3{\cdot}OEt_2$	0.06	1.5	-20	3	38
3	TMSOTf	0.06	1.5	0	1	41
4	TMSOTf	0.12	1.5	-20	1	77
5	**TMSOTf**	**0.12**	**2.0**	**-20**	**1**	**92**

In entry 1 was reported the selected conditions utilized in the DMEs pathways, which furnished a product yield of 61%. Also in this case, it was confirmed a significant decrease of the yield (38%) using $BF_3{\cdot}OEt_2$ (entry 2) instead of TMSOTf, as well as the increase of the temperature from –20 °C to 0 °C which led to a decrease of yield (entry 3). Then, trying to improve the result of entry 1, a double amount of TMSOTf (0.12 eq) provided an evident improvement of the yield (entry 4). The optimal conditions were assessed using 2 eq. of **17** instead of 1.5 eq. in the presence of catalytic TMSOTf (0.12 eq) at – 20 °C in 1 h. These conditions led to the tetra-*O*-acetyl-hepta-

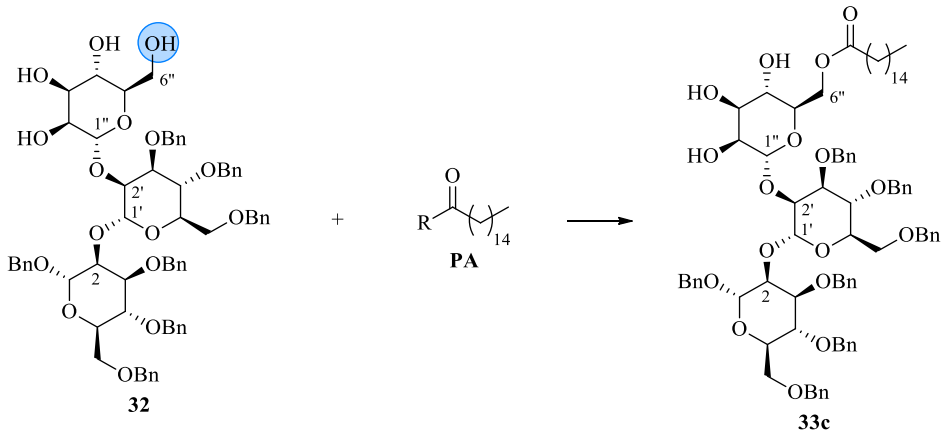
O-benzyl-trimannopyranose derivative **31** with a 92% of yield. The successive basic hydrolysis of **31** permitted the orthogonal deprotection of only acetylated mannose unit which led to hepta-*O*-benzyl-dimannopyranose **32** (Scheme 18).



Scheme 18. Synthesis of hepta-*O*-Benzyl-dimannopyranose **32** by deacetylation of **31**. Reagents and conditions: (i) NaOMe, DCM/MeOH (1:1 v/v), rt, 3 h.

5.2.5.2. Screening of the esterification reactions

As showed for the dimannopyranose esterification, a screening of several enzymatic and chemical reaction was conducted to obtain the hepta-*O*-benzyl-6′′-*O*-palmityl-trimannopyranose ester **33c** starting by sugar **32** and palmitic acid **PA** (Scheme 19). In Table 18 are reported the same reaction conditions adopted in the DMEs pathway. Unfortunately, both lipase-catalyzed esterification gave poor yields (entry1,2). In fact, it was confirmed a low yield (21%) with Novozyme 435® (entry 2), as showed for dimannopyranose. Unfortunately, a drastically reduction of the yield was detected using Lipozyme® compared to dimannopyranose esterification. This low yield is probably due to the steric hindrance of the sugar **32**, which bearing seven benzyl groups, makes difficult its interaction with the active site of the lipase. Focalizing on the chemical methods, the best result was achieved using the conditions in entry 3. In detail, protected sugar **32** reacted with palmitoyl chloride in a molar ratio of 1:1.1 in the presence of 2.2 equivalent of dry TEA in dry DCM to give the desired esterified in position 6′′ with a yield of 56 % in 6 h at room temperature.

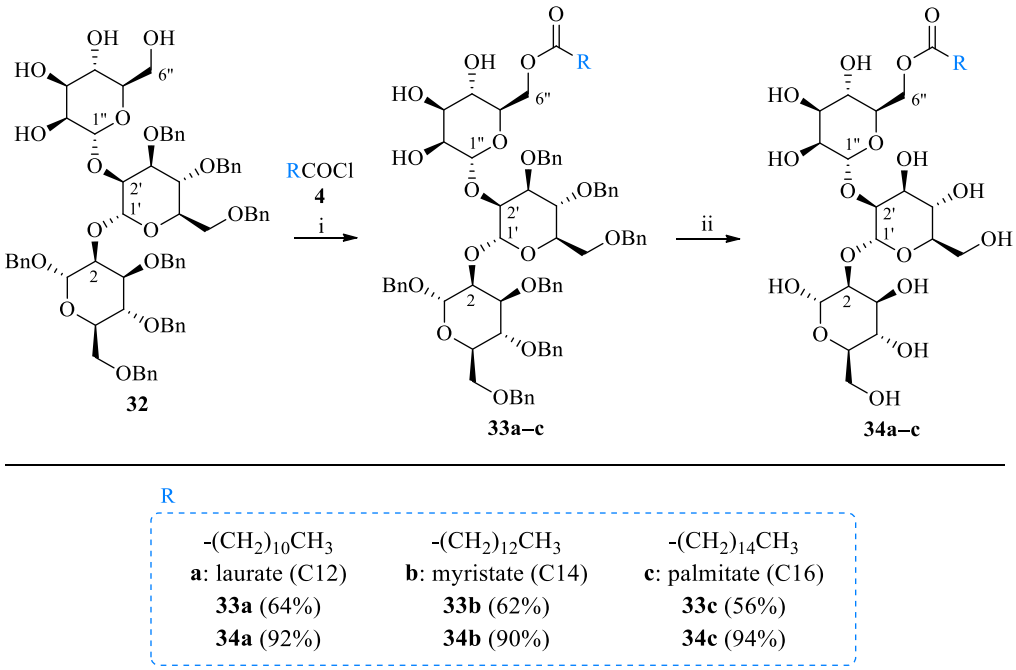


Scheme 19. General esterification reaction for the formation of hepta-*O*-Benzyl-6′′-*O*-palmityl-trimannopyranose ester **33c** using palmitic acid **PA**.

Table 18. Screening of the esterification procedure for the synthesis of hepta-*O*-Benzyl-6′′-*O*-palmityl-trimannopyranose ester **33c**

Entry	**Reaction conditions**	**R**	**Molar ratio 32/PA**	**Yield 33c (%)**
1	Lipozyme®, dry toluene 75 °C, 24 h	OH	1:2	27
2	Novozyme 435®, dry acetone, MS 4Å, rt, 4 d	OH	1:3	21
3	**Dry TEA, dry DCM, rt, 6 h**	**Cl**	**1:1.1**	**56**
4	PPh_3, DIAD, dry DMF, rt, 24 h	OH	1:1.5	21
5	TBTU, DIPEA, dry DMF, rt, 24 h	OH	1.1:1	18
6	EDCI· HCl, DMAP, TEA, dry DCM, rt, 72 h	OH	1.35:1	15

Despite the difficulty to achieve the regioselective monoester by chemical methods, only trace of diester was detected in this case. Regarding the other screened chemical procedure (entry 4–6), unsatisfactory yields were obtained (from 15 % to 21%). Indeed, the esterification using acyl chloride was selected for the synthesis of the hepta-*O*-benzyl-6′′-*O*-acyl-trimannopyranose esters **33a–c** (Scheme 20). These protected trimannopyranose esters **33a–c** were isolated in good yields. To be noted a slightly decrease of yield correlated to an increase of the chain length (**33a** = 64%, **33b** = 62%, **34c** = 56%). The subsequent debenzylation of **33a–c** operated by the same conditions for the DMEs pathway [166] gave the desired TMEs **34a–c** in high yields as white solids.



Scheme 20. Esterification *via* acyl chloride for the formation of hepta-*O*-Benzyl-6′′-*O*-acyl trimannopyranose **33a–c**. and their deprotection to the final TMEs **34a–c**. Reagents, conditions, and yields: (i) TEA, dry DCM.; rt, 6 h, (ii) H_2, Pd/C (10%), dry MeOH; o.n.

5.3. Conclusion

Three little series of mannose-type fatty acid monoesters were efficiently achieved with the aim to apply them as recognizing molecule in liposomal nanocarrier for the targeted treatment of leishmaniasis. The 6-*O*-mannopyranose esters (MBEs) were easily obtained by Novozyme 435®-catalysed reaction in good yield. Then, a multistep syntheses of new 6′-*O*-α-1,2-dimmanopyranose esters 6′′-*O*-α-1,2-trimmanopyranose esters were designed. Initially, the protected glycosyl donors and glycosyl acceptor were synthetized by three different synthetic pathways. Then, the *O*-glycosylation for the formation of α-1,2-glycosyl bond between the protected mannose units were optimized. The best yields for *O*-glycosylation reactions were achieved by catalytic amounts of TMSOTf. After the deacetylation step of the resulted compounds, benzylated α-1,2-dimannospyranose and α-1,2-trimannopyranose were used as reagents in the screening of the different esterification reactions using palmitic acid as acyl donor. The best result in term of regioselectivity and yield was achieved by a Lipozyme®-catalysed reaction for benzylated 6′-*O*-dimannopyranose ester, while a chemical procedure using acyl chloride was selected for the synthesis of benzylated

6′′-*O*-α-1,2-trimannopyranose. The subsequent hydrogenolysis gave the desired DMEs and TMEs, which were chemically characterized. In future perspective, these mannose-type fatty acid monoesters will be exposed in the surface of liposomes for the targeting of infected macrophages by leishmania pathogens. Liposomes will be produced using a mixture of synthetic lipids (i.e., DOPC, DOPG, DSPC, and cholesterol) and the synthesized mannose-type esters. First, the conventional thin layer evaporation method will be used to investigate the best lipidic mixture necessary to obtain nanovesicles with the desired characteristics. Then, microfluidic devices, allowing precise control over nanovesicle characteristics, will be employed to produce liposomes with the best lipidic mixture. The new mannose-decorated-liposomes will be first loaded with traditional anti-leishmania drugs (miltefosine) and successively with bisindole derivatives synthesized from our research group, which showed excellent antileishmanial activity. Then, toxicity and efficacy of encapsulated molecules will be tested in *L. infantum* promastigotes, host cell lines and *in vitro* infection models. Liposome uptake by infected macrophages will be monitored by TEM/confocal microscopy analysis. This innovative liposome system could represent an excellent solution to contrast the toxicity of commercial antileishmanial drugs and to improve their bioavailability. These results could be useful to design new targeted therapeutic strategies and to improve the relation between research and scientific results transferability to public health management of infectious diseases.

5.4. Materials and Methods

5.4.1.Chemicals

D-Mannose palmitic acid and hydrogen bromide (30% v/v) were purchased from TCI (Zwijndrecht, Belgium), myristic acid, hydrazine acetate, 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), trimethylsilyl trifluoromethanesulfonate (TMSOTf) from Fluorochem (Hadfield, UK). Diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (PPh_3) from Alfa Aesar (Ward Hill, Massachusetts, USA), lauric acid, acetic anhydride, 2,6-lutidine, potassium hydroxide (KOH), benzylic alcohol, boron trifluoride–ether complex ($BF_3{\cdot}OEt_2$), sodium methoxide (NaOMe), Novozyme 435®, Lipozyme®, palladium on carbon (Pd/C) and all organic solvent were purchased from Sigma-Aldrich (Milan, Italy). Prior to use, methanol and

dichloromethane were dried with molecular sieves with an effective pore diameter of 4 Å. The structures of compounds were assessed by ESI-MS, ^{1}H NMR, ^{13}C NMR, and IR. ESI-MS spectra were recorded with a Waters Micromass ZQ spectrometer in a negative or positive mode using a nebulizing nitrogen gas at 400 L/min and a temperature of 250 °C, cone flow 40 mL/min, capillary 3.5 kV and cone voltage 60 V; molecular ions $[M - H]^-$ or $[M + NH_4]^+$ or $[M + Na]^+$ are given. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 400 or 101, respectively, spectrometer and analyzed using the TopSpin 1.3 software package. Chemical shifts were measured by using the central peak of the solvent. Column chromatography purifications were performed under "flash" conditions using Merck 230–400 mesh silica gel. TLC was carried out on Merck silica gel 60 F254 plates, which were visualized by exposure to ultraviolet light and by exposure to an aqueous solution of ceric ammonium molybdate and potassium permanganate.

5.4.2. General procedure for the synthesis of 6-*O*-mannose-based esters (14a–c)

Novozyme 435® (0.400 g) and MS 4Å (0.800 g) were added to a solution of D-mannose (**13**) (0.252 g, 1.4 mmol) and the appropriate fatty acid (**3a–c**) (4.2 mmol, 3 eq.) in dry acetone (8.48 mL, 0.165 M) [158]. The mixture was stirred at room temperature for 96 h, filtered, and the filtrate was concentrated. Purification of the residue by flash chromatography (cyclohexane/EtOAc 2:8) gave **14a–c** as white solids.

6-O-Lauroyl-D-mannopyranose (mannose laurate) (**14a**) [168]

Yield = 65% (0.329 g). α/β ratio = 1:0.1. MS (ESI): 361 $[M - H]^-$, 380 $[M + NH_4]^+$, 385 $[M + Na]^+$. Data for α anomer: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 3H, J = 6.5 Hz, CH_3), 1.24–1.28 [m, 16H, $(CH_2)_8$], 1.48–1.55 (m, 2H, CH_2CH_2COOR), 2.27 (t, 2H, J = 7.5 Hz CH_2COOR), 3.37 (ddd, 1H, J_{H4-OH4} = 5.5 Hz, $J_{H4-H3} \cong J_{H4-H5}$ = 9.5 Hz, H4), 3.49–3.56 (m, 2H, H2, H3), 3.71 (ddd, 1H, J_{H5-H6b} = 2.0 Hz, J_{H5-H6a} = 7.0 Hz, J_{H5-H4} = 9.5 Hz, H5), 3.99 (dd, 1H, J_{H6a-H5} = 7.0 Hz, $J_{H6a-H6b}$ = 11.5 Hz, H6a), 4.29 (dd, 1H, J_{H6b-H5} = 2.0 Hz, $J_{Hba-H6a}$ = 11.5 Hz, H6b), 4.53 (d, 1H, J_{OH2-H2} = 6.0 Hz OH2), 4.59 (d, 1H, J_{OH3-H3} = 4.0 Hz, OH3), 4.86 (dd, 1H, $J_{H1-H2} \cong J_{H1-OH1}$ = 4.5 Hz, H1), 4.87 (d, 1H, J_{OH4-H4} = 5.5 Hz, OH4), 6.35 (d, 1H, J_{OH1-H1} = 4.5 Hz, OH1) ppm. ^{13}C NMR

(101 MHz, DMSO-d_6): δ = 14.4, 22.6, 24.9, 28.9, 29.2, 29.2, 29.4, 29.5, 31.8, 33.9, 64.7 (C6), 67.6 (C5), 70.8 (C4), 70.9 (C3), 71.8 (C2), 94.5 (C1), 173.5 (CO) ppm.

6-O-Myristoyl-D-mannopyranose (mannose myristate) (**14b**) [168]

Yield = 60% (0.328 g). α/β ratio = 1:0.1. MS (ESI): 389 $[M - H]^-$, 408 $[M + NH_4]^+$, 413 $[M + Na]^+$. Data for α anomer: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.87 (t, 3H, J = 6.5 Hz, CH_3), 1.24–1.28 [m, 20H, $(CH_2)_{10}$], 1.47–1.54 (m, 2H, CH_2CH_2COOR), 2.27 (t, 2H, J = 7.5 Hz, CH_2COOR), 3.37 (ddd, 1H, $J_{H4\text{-}OH4}$ = 5.5 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.50–3.56 (m, 2H, H2, H3), 3.71 (ddd, 1H, $J_{H5\text{-}H6b}$ = 2.0 Hz, $J_{H5\text{-}H6a}$ = 7.0 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 3.99 (dd, 1H, $J_{H6a\text{-}H5}$ = 7.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.29 (dd, 1H, $J_{H6b\text{-}H5}$ = 2.0 Hz, $J_{Hba\text{-}H6a}$ = 11.5 Hz, H6b), 4.53 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz OH2), 4.59 (d, 1H, $J_{OH3\text{-}H3}$ = 4.0 Hz, OH3), 4.86 (dd, 1H, $J_{H1\text{-}H2} \cong J_{H1\text{-}OH1}$ = 4.5 Hz, H1), 4.87 (d, 1H, $J_{OH4\text{-}H4}$ = 5.5 Hz, OH4), 6.35 (d, 1H, $J_{OH1\text{-}H1}$ = 4.5 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.4, 22.6, 24.9, 29.0, 29.1, 29.2, 29.4, 29.5, 31.8, 33.9, 64.7 (C6), 67.6 (C5), 70.8 (C4), 70.9 (C3), 71.8 (C2), 94.5 (C1), 173.4 (CO) ppm.

6-O-Palmitoyl-D-mannopyranose (mannose palmitate) (**14c**) [28]

Yield = 33% (0.193 g). α/β ratio = 1:0.1. MS (ESI): 417 $[M - H]^-$, 436 $[M + NH_4]^+$, 441 $[M + Na]^+$. Data for α anomer: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 3H, J = 6.5 Hz, CH_3), 1.24–1.30 [m, 24H, $(CH_2)_{12}$], 1.48–1.53 (m, 2H, CH_2CH_2COOR), 2.27 (t, 2H, J = 7.5 Hz, CH_2COOR), 3.37 (ddd, 1H, $J_{H4\text{-}OH4}$ = 5.5 Hz, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 3.50–3.56 (m, 2H, H2, H3), 3.71 (ddd, 1H, $J_{H5\text{-}H6b}$ = 2.0 Hz, $J_{H5\text{-}H6a}$ = 7.0 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 3.99 (dd, 1H, $J_{H6a\text{-}H5}$ = 7.0 Hz, $J_{H6a\text{-}H6b}$ = 11.5 Hz, H6a), 4.29 (dd, 1H, $J_{H6b\text{-}H5}$ = 2.0 Hz, $J_{Hba\text{-}H6a}$ = 11.5 Hz, H6b), 4.53 (d, 1H, $J_{OH2\text{-}H2}$ = 6.0 Hz OH2), 4.59 (d, 1H, $J_{OH3\text{-}H3}$ = 4.0 Hz, OH3), 4.85–4.86 (m, 2H, OH4, H1), 6.35 (d, 1H, $J_{OH1\text{-}H1}$ = 4.5 Hz, OH1) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 14.4, 22.6, 24.9, 29.0, 29.1, 29.2, 29.4, 29.5, 29.5, 31.8, 33.9, 64.7 (C6), 67.6 (C5), 70.8 (C4), 70.9 (C3), 71.8 (C2), 72.0 (C2), 94.5 (C1), 173.4 (CO) ppm.

5.4.3. Synthesis of 1,2,3,4,6-penta-*O*-Acetyl-D-mannopyranose, mannose pentaacetate (15)

Acetic anhydride (52.50 mL, 556.0 mmol, 10 eq.) was added dropwise to a solution of D-mannose **13** (10 g, 55.6 mmol) in pyridine (49.20 mL, 1.13 M) at 0 °C, under nitrogen atmosphere. The mixture was stirred at rt for 12 h, then poured into ice water and extracted with ethyl acetate (x3). The organic phase was washed with saturated $NaHCO_3$, twice with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure to obtain the product **15** as colourless oil [161].

Yield = 100% (21.68 g). MS (ESI): 413 $[M + Na]^+$. Data for α anomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.96 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 3.95-4.13 (m, 2H, H5, H6b), 4.24 (dd, 1H, J_{H6a-H5} = 5.0 Hz, $J_{H6a-H6b}$ = 12.5 Hz, H6a), 5.22 (dd, 1H, $J_{H2-H1} \cong J_{H2-H3}$ = 2.0 Hz, H2), 5.26-5.37 (m, 2H, H3, H4), 6.04 (d, 1H, J_{H1-H2} = 2.0 Hz, H1) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 20.7, 20.7, 20.7, 20.8, 20.9, 62.1, 65.5, 68.3, 68.8, 70.6, 90.6, 168.1, 169.6, 168.8, 170.0, 170.7 ppm.

5.4.4. Synthesis of 2,3,4,6-tetra-*O*-Acetyl-α-D-mannopyranosyl-1-trichloroacetimidate (17)

Hydrazine acetate (1.868 g, 20.31 mmol, 1.1 eq.). was added to a solution of D-mannose pentaacetate **15** (7.199 g, 18.46 mmol) in dry DMF (59.60 mL, 0.31 M) at rt. The reaction mixture was stirred at rt for one hour and then DCM was added. The organic phase was washed with cold saturated $NaHCO_3$ (x3), dried over Na_2SO_4, filtered and concentrated. The product **16** was re-dissolved in DCM (148.8 mL) and CCl_3CN (18.50 mL; 180.5 mmol; 10 eq.), DBU (0.690 mL; 4.62 mmol; 0.25 eq.) were added and the reaction mixture stirred at rt overnight. Solvents were removed in vacuo and the residue purified by flash column chromatography (cyclohexane/EtOAc 8:2) to yield the product **17** as yellow solid [162].

Yield = 82% (8.177 g). MS (ESI): 514 $[M + Na]^+$.^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.00 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 4.16-4.21 (m, 2H, H5, H6b), 4.27 (dd, 1H, J_{H6a-H5} = 4.5 Hz, $J_{H6a-H6b}$ = 12 Hz, H6a), 5.39-5.42 (m, 2H, H3, H4), 5.47 (dd, 1H, J_{H2-H1} = 2.0 Hz, J_{H2-H3} = 3.0 Hz, H2),

6.28 (d, 1H, $J_{H1\text{-}H2}$ = 2.0 Hz, H1), 8.79 (s, 1H, NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 20.6, 20.7, 20.8, 62.0, 65.3, 67.8, 68.8, 71.2, 90.5, 94.5, 159.7, 169.2, 169.7, 169.8, 170.6 ppm.

5.4.5. Synthesis of 3,4,6-tri-*O*-Acetyl-β-D-mannose-1,2-(methyl orthoacetate) (19)

HBr 30% v/v (39.30 mL) was added dropwise to a solution of mannose pentaacetate **15** (14.46 g; 37.08 mmol) in dry DCM (296.70 mL; 0.125 M) at 0 °C. The reaction mixture was stirred at rt for 2 h. The solution was poured into ice water and the aqueous phase was extracted with DCM (x2). The organic phases were combined and washed once with saturated $NaHCO_3$ and once with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to yield the mannose bromide **18** which was used without further purification. **18** was dissolved in a mixture of dry MeOH and dry DCM (1:1 v/v, 30 mL, 1.15 M) and 2,6-lutidine (3 mL) was added dropwise. The reaction mixture was stirred at rt for 12 h. The solution was diluted with DCM and the organic phase was washed with saturated $NaHCO_3$ (x2) and brine (x1), dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (cyclohexane/EtOAc 8:2) to give the product **19** as white solid [162].

Yield = 89% (11.946 g). MS (ESI): 385 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.72 (s, 1H, CH_3), 2.03 (s, 1H, $COCH_3$), 2.05 (s, 1H, $COCH_3$), 2.09 (s, 1H, $COCH_3$), 3.26 (s, 1H, OCH_3), 3.66 (ddd, 1H, $J_{H5\text{-}H6b}$ = 2.5 Hz, $J_{H5\text{-}H6a}$ = 5.0 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 4.12 (dd, 1H, $J_{H6b\text{-}H5}$ = 2.5 Hz, $J_{H6b\text{-}H6a}$ = 12.0 Hz, H6b), 4.22 (dd, 1H, $J_{H6a\text{-}H5}$ = 5.0 Hz, $J_{H6a\text{-}H6b}$ = 12.0 Hz, H6a), 4.59 (dd, 1H, $J_{H2\text{-}H1}$ = 2.5 Hz, $J_{H2\text{-}H3}$ = 4.0 Hz, H2), 5.13 (dd, 1H, $J_{H3\text{-}H2}$ = 4.0 Hz, $J_{H3\text{-}H4}$ = 10.0 Hz, H3), 5.27 (dd, 1H, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 10.0 Hz, H4), 5.48 (d, 1H, $J_{H1\text{-}H2}$ = 2.5 Hz, H1) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 20.6, 20.7, 20.7, 24.3, 49.9, 62.2, 65.4, 70.6, 71.2, 76.5, 97.3, 124.5, 169.4, 170.3, 170.6 ppm.

5.4.6. Synthesis of 3,4,6-tri-*O*-Benzyl-β-D-mannose-1,2-(methyl orthoacetate) (20)

Benzyl bromide (68.31 mL; 558.8 mmol; 17 eq.) was added to a solution of 3,4,6-tri-*O*-acetyl-D-mannose-1,2-(methyl orthoacetate) **19** (11.900 g, 32.87 mmol) in dry THF

(117.39 mL, 0.28 M) and the reaction heated to 80 °C. After 15 minutes, the reaction mixture was cooled and powdered KOH (27.60 g, 493.0 mmol, 15 eq.) was added and heating overnight at 80 °C. Then, water was added, and the reaction mixture was diluted with DCM. The organic phase was separated, washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (cyclohexane/EtOAc 8:2) to yield the product **20** as white solid [162].

Yield = 75% (12.474 g). MS (ESI): 529 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.75 (s, 3H, CH_3), 3.30 (s, 1H, OCH_3), 3.43 (ddd, 1H, $J_{H5\text{-}H6b}$ = 2.5 Hz, $J_{H5\text{-}H6a}$ = 4.5 Hz, $J_{H5\text{-}H4}$ = 9.5 Hz, H5), 3.68-3.78 (m, 3H, H3, H6a, H6b), 3.93 (dd, 1H, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.5 Hz, H4), 4.41 (dd, 1H, $J_{H2\text{-}H1}$ = 2.5 Hz, $J_{H2\text{-}H3}$ = 4.0 Hz, H2), 4.54-4.63 (m, 3H, CH_2Ar; CH*H*Ar), 4.79 (d, 2H, *J* = 1.5 Hz, CH_2Ar), 4.91 (d, 1H, *J* = 11.0 Hz, CH*H*Ar), 5.36 (d, 1H, $J_{H1\text{-}H2}$ = 2.5 Hz, H1), 7.23-7.42 (m, 15H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 24.4, 49.7, 68.9, 72.3, 73.3, 74.1, 74.1, 75.2, 77.1, 79.0, 97.5, 123.9, 127.5, 127.7, 128.0, 128.0, 128.3, 128.4, 128.5, 137.8, 138.2 ppm.

5.4.7. Synthesis of 1,3,4,6-tetra-*O*-Benzyl-α-D-mannopyranose (22)

2-O-Acetyl-1,3,4,6-tetra-O-benzyl-α-D-mannopyranose (**21**)

Benzyl alcohol (6.27 mL, 60.0 mmol, 5 eq.) were added to a solution of 3,4,6-tri-*O*-benzyl-β-D-mannose-1,2-(methyl orthoacetate) **20** (6.072 g; 12.0 mmol) in dry DCM (92.30 mL, 0.13 M) were added. The mixture was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (1.494 mL; 12.0 mmol; 1 eq.) was added dropwise. Then, the reaction mixture was allowed to warm up to rt and kept under nitrogen atmosphere for 2 h. After completion of the reaction, the mixture was washed with saturated $NaHCO_3$, brine, and extracted with DCM. The organic phase was dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (cyclohexane/EtOAc 9.5:0.5) to give compound **21** as colourless oil [163].

Yield = 82% (5.726 g). MS (ESI): 605 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.14 (s, 3H, $COCH_3$), 3.70 (dd, 1H, $J_{H6b\text{-}H5}$ = 1.5 Hz, $J_{H6b\text{-}H6a}$ = 10.5 Hz, H6b), 3.81 (dd, 1H, $J_{H6a\text{-}H5}$ = 4.0 Hz, $J_{H6a\text{-}H6b}$ = 10.5 Hz, H6a), 3.85 (m, 1H, H5), 3.91 (dd, 1H, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 9.0 Hz, H4), 4.03 (dd, 1H, $J_{H3\text{-}H2}$ = 3.5 Hz, $J_{H3\text{-}H4}$ = 9.0 Hz, H3), 4.45-

4.88 (m, 8H, 4 x CH_2Ar), 4.94 (d, 1H, J_{H1-H2} = 1.5 Hz, H1), 5.42 (dd, 1H, J_{H2-H1} = 1.5 Hz, J_{H2-H3} = 3.5 Hz, H2), 7.14-7.16 (m, 2H, ArH), 7.26-7.38 (m, 18H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.3, 68.9, 69.0, 69.5, 71.7, 72.0, 73.6, 74.5, 75.4, 78.5, 97.3, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.5, 128.6, 137.0, 138.1, 138.4, 138.5, 170.6 ppm.

1,3,4,6-tetra-O-Benzyl-α-D-mannopyranose (**22**)

21 (5.820 g, 10.0 mmol) was dissolved in MeOH (25.0 mL, 0.4 M) and NaOMe (0.560 g, 10.0 mmol) was added. The reaction mixture was stirred at rt for 2 h under nitrogen atmosphere. After the completion of the reaction, the mixture was further stirred with Amberlyst 15 for 10 minutes. Then, the mixture was filtered to eliminate the resin and concentrated under reduced pressure. The residue was purified by flash column chromatography (cyclohexane/EtOAc 8.5:1.5) to obtain the product **22** as colourless oil [163].

Yield = 97% (5.238 g). MS (ESI): 558 $[M + NH_4]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.70 (dd, 1H, J_{H6b-H5} = 1.0 Hz, $J_{H6b-H6a}$ = 10.5 Hz, H6b), 3.77 (dd, 1H, J_{H6a-H5} = 4.0 Hz, $J_{H6a-H6b}$ = 10.5 Hz, H6a), 3.81-3.91 (m, 2H, H4, H5), 3.92-3.95 (m, 1H, H3), 4.08 (dd, 1H, J_{H2-H1} = 1.5 Hz, J_{H2-H3} = 3.0 Hz, H2), 4.46-4.85 (m, 8H, 4 x CH_2Ar), 5.00 (d, 1H, J_{H1-H2} = 1.5 Hz, H1), 7.16-7.18 (m, 2H, ArH), 7.25-7.38 (m, 18H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 68.6, 69.1, 69.2, 71.4, 72.2, 73.6, 74.5, 75.3, 80.4, 98.6, 127.7, 127.8, 128.0, 128.0, 128.0, 128.1, 128.2, 128.46, 128.49, 128.5, 128.6, 137.3, 138.0, 138.4 ppm

5.4.8. Synthesis of 2-*O*-acetyl-3,4,6-tri-*O*-Benzyl-α-D-mannopyranosyl 1-trichloroacetimidate (24)

2-O-acetyl-3,4,6-tri-O-benzyl-mannopyranose (**23**)

A solution of **20** (6.072 g, 12.00 mmol) in acetic acid 90% (54.54 mL, 0.22 M) was stirred at rt for 5 h. Then the mixture was poured into ice water and extracted with DCM (x3). The organic phases were combined, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash column

chromatography (cyclohexane/EtOAc 7:3) to obtain the compound **23** as colourless oil [164].

Yield = 88% (5.195 g). MS (ESI): 515 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.15 (s, 3H, $COCH_3$), 3.42 (d, 1H, J_{OH1-H1} = 3.5 Hz, OH1), 3.69-3.71 (m, 2H, H6a, H6b), 3.75 (dd. 1H, $J_{H4-H3} \cong J_{H4-H5}$ = 9.0 Hz, H4), 4.04 (dd, 1H, J_{H3-H2} = 3.0 Hz, J_{H3-H4} = 9.0 Hz, H3), 4.06-4.09 (m, 1H, H5), 4.45-4.55 (m, 3H, 3CH*H*Ar),4.61 (d, 1H, *J* = 12.0 Hz, CH_2Ar), 4.71 (d, 1H, *J* = 11.0 Hz, CH_2Ar), 4.86 (d, 1H, *J* = 11.0 Hz, CH_2Ar), 5.22 (dd, 1H, J_{H1-H2} = 2.0 Hz, J_{H1-OH} = 3.5 Hz, H1), 5.37 (dd, 1H, J_{H2-H1} = 2.0 Hz, J_{H2-H3} = 3.0 Hz, H2), 7.13-7.18 (m, 2H, ArH), 7.26-7.36 (m, 13H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.2, 69.0, 69.2, 69.3, 71.2, 71.8, 73.5, 74.6, 75.3, 77.6, 92.6, 127.3, 127.5, 127.6, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 137.4, 137.6, 137.9, 138.1, 138.3, 170.5 ppm.

2-O-acetyl-3,4,6-tri-O-benzyl-mannopyranosyl-trichloroacetimidate (**24**)

CCl_3CN (5.38 mL, 51.83 mmol, 5 eq.) and DBU (0.16 mL, 1.07 mmol, 0.1 eq.) were added to a solution of **23** (5.100 g; 10.36 mmol) in dry DCM (86.333 mL, 0.12 M) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for one hour at 0 °C, then solvents were removed in vacuo and the residue purified by flash column chromatography (cyclohexane/EtOAc 8:2) to yield the product **24** as colourless oil [165].

Yield = 99% (6.512 g). MS (ESI): 658 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.20 (s, 3H, $COCH_3$), 3.72 (dd, 1H, J_{H6a-H5} = 1.5 Hz, $J_{H6a-H6b}$ = 11.0 Hz, H6a), 3.85 (dd, 1H, J_{H6b-H5} = 3.5 Hz, $J_{H6b-H6a}$ = 11.0 Hz, H6b), 3.98-4.06 (m, 3H, H3, H4, H5), 4.49-4.55 (m, 4H, $2CH_2Ar$), 4.61 (d, 1H, *J* = 11.0 Hz, CHAr),4.68 (d, 1H, *J* = 12.0 Hz, CHAr), 4.73 (d, 1H, *J* = 11.0 Hz, CHAr), 4.86 (d, 1H, *J* = 11.0 Hz, CHAr), 5.50 (dd, 1H, $J_{H2-H1} \cong J_{H2-H3}$ = 2.0 Hz, H2), 6.30 (dd, 1H, J_{H1-H2} = 2.0 Hz, H1), 7.17-7.20 (m, 2H, ArH), 7.26-7.36 (m, 13H, ArH), 8.69 (s, 1H, NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 20.9, 67.3, 68.3, 72.3, 73.4, 73.8, 74.5, 75.3, 76.7, 77.0, 77.2, 77.3, 77.6, 92.4, 98.4, 127.3, 127.5, 127.6, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 137.4, 137.6, 137.8, 137.9, 138.1, 159.9, 170.0. ppm.

5.4.9. Synthesis of 2-*O*-[(2′,3′,4′,6′-tetra-*O*-acetyl)mannopyranosyl]-1,3,4,6-tetra-*O*-benzylmannopyranose (25)

MS 4Å was added to a mixture of **17** (2.725 g, 5.55 mmol, 1.5 eq.) and **22** (1.961 g, 3.70 mmol, 1 eq.) in dry DCM (61.70 mL, 0.06 M). The reaction mixture was cooled at –20 °C, TMSOTf (0.043 mL; 0.222 mmol; 0.06 eq.) was added and the mixture was stirred for one hour. After that, the mixture was neutralized with TEA, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography (cyclohexane/EtOAc 9:1) to give **25** as colourless oil.

Yield = 87% (2.801 g). MS (ESI): 888 $[M + NH_4]^+$, 893 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.97 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.08 (s, 3H, $CO\mathit{CH_3}$), 3.67-3-74 (m, 2H, H6a, H6b), 3.81 (ddd, 1H, $J_{H5\text{-}H6a}$ = 2.5 Hz, $J_{H5\text{-}H6b}$ = 4.0 Hz, $J_{H5\text{-}H4}$ = 8.5 Hz, H5), 3.88 (dd, 1H, $J_{H4\text{-}H3} \cong J_{H4\text{-}H5}$ = 8.5 Hz, H4), 3.92-3.97 (m, 3H, H6′b, H2, H3), 4.11 (ddd, $J_{H5'\text{-}H6'b}$ = 2.0 Hz, $J_{H5'\text{-}H6'a}$ = 5.0 Hz, $J_{H5'\text{-}H4'}$ = 10.0 Hz, H5′), 4.19 (dd, 1H, $J_{H6'a\text{-}H5'}$ = 5.0 Hz, $J_{H6'a\text{-}H6'b}$ = 12.0 Hz, H6′a), 4.47 (t, 2H, *J* = 11.0 Hz, 2 x CH*H*Ar), 4.55-4.65 (m, 3H, 3 x CH*H*Ar), 4.70 (dd, 2H, J_1 = 1.5 Hz, J_2 = 11.5 Hz, 2 x CH*H*Ar), 4.80 (d, 1H, *J* = 11.0 Hz, CH*H*Ar), 4.94-4.97 (m, 2H, H1′, H1), 5.23 (dd, 1H, $J_{H4'\text{-}H3'} \cong J_{H4'\text{-}H5'}$ = 10.0 Hz, H4′), 5.38 (dd, 1H, $J_{H3'\text{-}H2'}$ = 3.5 Hz, $J_{H3'\text{-}H4'}$ = 10.0 Hz, H3′), 5.44 (dd, 1H, $J_{H2'\text{-}H1'}$ = 2.0 Hz, $J_{H2'\text{-}H3'}$ = 3.5 Hz, H2′), 7.11-7.14 (m, 2H, ArH), 7.22-7.38 (m, 18H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 20.7, 20.7, 20.7, 20.9, 62.4, 66.1, 68.8, 69.1, 69.1, 69.1, 69.4, 72.0, 72.5, 73.2, 74.8, 75.3, 76.2, 79.6, 97.8, 99.3, 127.40, 127.46, 127.5, 127.6, 127.7, 128.1, 128.3, 128.4, 128.5, 137.0, 138.20, 138.22, 138.3, 169.6, 169.7, 169.8, 170.6 ppm.

5.4.10. Synthesis of 2-*O*-(Mannopyranosyl)-1,3,4,6-tetra-*O*-benzylmannopyranose (26)

NaOMe was added (0.256 g, 4.74 mmol, 1.5 eq.) to a solution of **25** (2.750 g, 3.16 mmol; 1 eq.) in dry MeOH/dry DCM 1:1v/v (43.89 mL, 0.072 M) and the reaction mixture was stirred for one hour at rt. After the completion of the reaction, the dowex $[H^+]$ resin was added, and the mixture was further stirred for 15 minutes. Then the mixture was filtered and concentrated under reduced pressure. The residue was

purified by flash column chromatography (DCM/MeOH 9.5:0.5) to yield the product **26** as white solid.

Yield = 97% (2.151 g). MS (ESI): 720 $[M + NH_4]^+$, 725 $[M + Na]^+$, 747 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.58-3.65 (m, 3H), 3.70 (dd, 1H, J_1 =3.5 Hz, J_2 = 11.0 Hz), 3.75-3.83 (m, 2H), 3.91-4.02 (m, 5H), 4.09 (s, 1H, H2), 4.40 (dd, 2H, J_1 = 3.0 Hz, J_2 =12.0 Hz, 2 x CH*H*Ar), 4.50 (d, 1H, *J* = 12.0 Hz, CH*H*Ar), 4.56-4.66 (m, 4H, 4 x CH*H*Ar), 4.67-4.73 (m, 4H, 4 x OH), 4.76 (d, 1H, *J* = 10.5 Hz, CH*H*Ar), 4.91 (s, 1H, H1′), 5.08 (s, 1H, H1), 7.08-7.12 (m, 2H, ArH), 7.19-7.33 (m, 18H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 60.9, 66.2, 69.0, 69.1, 71.1, 71.5, 71.9, 72.3, 72.8, 73.3, 74.9, 75.1, 75.2, 79.6, 98.0, 101.8, 127.6, 127.6, 127.8, 127.8, 127.8, 127.9, 128.0, 128.3, 128.4, 128.4, 128.5, 137.1, 138.2, 138.2, 138.3 ppm.

5.4.11. General procedure for synthesis of 6′-*O*-Acyl-2-*O*-(mannopyranosyl)-1,3,4,6-tetra-*O*-benzyl-mannopyranose (27a–c)

Lipozyme® (0.160 g; 40% w/w) was added to a solution of 2-*O*-(mannopyranosyl)-1,3,4,6-tetra-*O*-benzylmannopyranoside **26** (0.400 g, 0.57 mmol, 1 eq.) and the appropriate fatty acid (1.14 mmol, 2 eq.) in dry toluene (4.60 mL, 0.125 M). The reaction mixture was stirred at 75 °C for 24 h, then it was filtered, and concentrated under reduced pressure. Purification by flash column chromatography (DCM/MeOH 9.5:0.5) furnished the correspondent **27a–c** as colourless oils.

6′-O-Lauroyl-2-O-(mannopyranosyl)-1,3,4,6-tetra-O-benzyl-mannopyranose (**27a**)

Yield = 71% (0.360 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, 3H, *J* = 6.5 Hz, CH_3), 1.22-1.33 [m, 16H, $(CH_2)_8$], 1.57-1.65 (m, 2H, CH_2CH_2COOR), 2.35 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.53 (dd, 1H, $J_{H4'\text{-}H3'} \cong J_{H4'\text{-}H5'} = 9.5$ Hz, H4′), 3.67-3.70 (m, 1H), 3.75-3.80 (m, 3H), 3.83-3.91 (m, 2H), 3.96-3.99 (m, 2H), 4.02 (dd, 1H, $J_{H2'\text{-}H1'} \cong J_{H2'\text{-}H3'}$ = 2.0 Hz, H2′), 4.07 (dd, 1H, $J_{H2\text{-}H1}$ = 1.5 Hz, $J_{H2\text{-}H3}$ = 3.5 Hz, H2), 4.45-4.55 (m, 4H, 3 x CH*H*Ar, H6′a), 4.63-4.72 (m, 4H, 4 x CH*H*Ar), 4.82 (d, 1H, *J* = 10.5 Hz, CH*H*Ar), 4.96 (d, 1H, $J_{H1'\text{-}H2'}$ = 2.0 Hz, H1′), 5.02 (d, 1H, $J_{H1\text{-}H2}$ = 1.5 Hz, H1), 7.17-7.19 (m, 2H, ArH), 7.26-7.36 (m, 18H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 14.1, 22.7, 24.9, 29.1, 29.3, 29.3, 29.5, 29.6, 31.9, 34.2, 63.2, 67.6, 69.0, 69.1, 70.3, 71.0, 71.1,

72.0, 72.4, 73.3, 74.7, 75.20, 75.25, 79.7, 98.1, 101.4, 127.6, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.4, 128.5, 137.1, 138.2, 138.3, 138.3, 175.0 ppm.

6′-O-Myristoyl-2-O-(mannopyranosyl)-1,3,4,6-tetra-O-benzyl-mannopyranose (**27b**)

Yield = 63% (0.330 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, 3H, *J* = 6.5 Hz, CH_3), 1.22-1.33 [m, 20H, $(CH_2)_{10}$], 1.58-1.65 (m, 2H, *CH*$_2$CH$_2$COOR), 1.80-2.22 (m, 4H, 4 x OH), 2.36 (t, 2H, *J* = 7.5 Hz, CH$_2$COOR), 3.53 (dd, 1H, $J_{H4'\text{-}H3'}$ $J_{H4'\text{-}H5'}$ = 9.5 Hz, H4′), 3.66-3.70 (m, 1H), 3.75-3.81 (m, 3H), 3.83-3.91 (m, 2H), 3.97-4.01 (m, 2H), 4.01 (dd, 1H, $J_{H2'\text{-}H1'} \cong J_{H2'\text{-}H3'}$ = 2.0 Hz, H2′), 4.07 (dd, 1H, $J_{H2\text{-}H1}$ = 1.5 Hz, $J_{H2\text{-}H3}$ = 3.5 Hz, H2), 4.45-4.55 (m, 4H, 3 x CH*H*Ar, H6′a), 4.63-4.72 (m, 4H, 4 x CH*H*Ar), 4.82 (d, 1H, *J* = 10.5 Hz, CH*H*Ar), 4.96 (d, 1H, $J_{H1'\text{-}H2'}$ = 2.0 Hz, H1′), 5.02 (d, 1H, $J_{H1\text{-}H2}$ = 1.5 Hz, H1), 7.16-7.19 (m, 2H, ArH), 7.26-7.36 (m, 18H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 14.1, 22.7, 24.9, 29.1, 29.3, 29.4, 29.5, 29.61, 29.64, 29.7, 31.9, 63.1, 67.5, 69.0, 69.1, 70.3, 71.0, 71.1, 72.0, 72.4, 73.3, 74.7, 75.2, 75.3, 79.7, 98.0, 101.4, 127.6, 127.65, 127.7, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.4, 128.5, 137.1, 138.2, 138.3, 138.3, 175.0 ppm.

6′-O-Palmitoyl-2-O-(mannopyranosyl)-1,3,4,6-tetra-O-benzyl-mannopyranose (**27c**)

Yield = 67% (0.360 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, 3H, *J* = 6.5 Hz, CH_3), 1.22-1.32 [m, 24H, $(CH_2)_{12}$], 1.58-1.65 (m, 2H, *CH*$_2$CH$_2$COOR), 2.26-2.40 (m, 4H, 4 x OH), 2.36 (t, 2H, *J* = 7.5 Hz, CH$_2$COOR), 3.53 (dd, 1H, $J_{H4'\text{-}H3'} \cong J_{H4'\text{-}H5'}$ = 9.5 Hz, H4′), 3.67-3.70 (m, 1H), 3.76-3.81 (m, 3H), 3.84-3.91 (m, 2H), 3.98-4.01 (m, 2H), 4.03 (dd, 1H, $J_{H2'\text{-}H1'} \cong J_{H2'\text{-}H3'}$ = 1.5 Hz, H2′), 4.07 (dd, 1H, $J_{H2\text{-}H1} \cong J_{H2\text{-}H3}$ = 1.5 Hz, H2), 4.45-4.55 (m, 4H, 3 x CH*H*Ar, H6′a), 4.64-4.72 (m, 4H, 4 x CH*H*Ar), 4.82 (d, 1H, *J* = 10.5 Hz, CH*H*Ar), 4.96 (d, 1H, $J_{H1'\text{-}H2'}$ = 1.5 Hz, H1′), 5.03 (s, 1H, H1), 7.16-7.18 (m, 2H, ArH), 7.26-7.36 (m, 18H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 14.1, 22.7, 25.0, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.7, 31.9, 34.2, 63.1, 67.5, 69.0, 69.1, 70.3, 71.0, 71.0, 72.0, 72.3, 73.3, 74.7, 75.2, 75.3, 79.7, 98.0, 101.4, 127.6, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.4, 128.4, 128.4, 128.5, 137.1, 138.2, 138.2, 138.3, 175.1 ppm.

5.4.12. General procedure for the synthesis of 6′-*O*-acyl-α-1′,2-dimannopyranose esters (28a–c)

A solution of appropriate **27a–c** (0.34 mmol) with Pd/C 10% in dry MeOH (17 mL; 0.02 M) was stirred at room temperature under atmospheric hydrogen (3.5 atm.) overnight. The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. Purification by flash column chromatography (DCM/MeOH 8.5:1.5) furnished the final products as white solids [166].

6′-O-Lauroyl-α-1′,2-dimannopyranose (dimannose laurate) (**28a**)

Yield = 89% (0.159 g). MS (ESI): 569 $[M + HCOO]^-$, 542 $[M + NH_4]^+$, 547 $[M + Na]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.90 (t, 3H, *J* = 6.5 Hz, CH_3), 1.26-1.36 [m, 16H, $(CH_2)_8$], 1.59-1.66 (m, 2H, CH_2CH_2COOR), 2.38 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.57-3.64 (m, 2H), 3.68-3.74 (m, 3H), 3.79-3.85 (m, 2H), 3.88-3.91 (m, 2H), 3.98 (dd, 1H, $J_{H2'-H1'}$ = 1.5 Hz, $J_{H2'-H3'}$ = 3.0 Hz, H2′), 4.18 (dd, 1H, $J_{H6'b-H5'}$ = 7.0 Hz, $J_{H6'b-H6'a}$ = 11.5 Hz, H6′b), 4.41 (dd, 1H, $J_{H6'a-H5'}$ = 2.0 Hz, $J_{H6'a-H6'b}$ = 11.5 Hz, H6′a), 4.95 (d, 1H, $J_{H1'-H2'}$ = 1.5 Hz, H1′), 5.30 (d, 1H, J_{H1-H2} = 1.5 Hz, H1) ppm. ^{13}C NMR (101 MHz, CD_3OD): δ = 13.0, 22.3, 24.6, 28.8, 29.0, 29.1, 29.2, 29.3, 31.7, 33.6, 61.7, 63.7, 67.4, 67.7, 70.3, 70.4, 70.9, 71.0, 72.6, 79.8, 93.0, 102.7, 174.4 ppm.

6′-O-Myristoyl-α-1′,2-dimannopyranoside (dimannose myristate) (**28b**)

Yield = 85% (0.154 g). MS (ESI): 597 $[M + HCOO]^-$, 570 $[M + NH_4]^+$, 575 $[M + Na]^+$. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.90 (t, 3H, *J* = 6.5 Hz, CH_3), 1.25-1.35 [m, 20H, $(CH_2)_{10}$], 1.59-1.65 (m, 2H, CH_2CH_2COOR), 2.38 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.57-3.64 (m, 2H), 3.67-3.74 (m, 3H), 3.79-3.85 (m, 2H), 3.88-3.92 (m, 2H), 3.99 (dd, 1H, $J_{H2'-H1'}$ = 1.5 Hz, $J_{H2'-H3'}$ = 3.0 Hz, H2′), 4.18 (dd, 1H, $J_{H6'b-H5}$ = 7.0 Hz, $J_{H6'b-H6'a}$ = 11.5 Hz, H6′b), 4.41 (dd, 1H, $J_{H6'a-H5'}$ = 2.0 Hz, $J_{H6'a-H6'b}$ = 11.5 Hz, H6′a), 4.95 (d, 1H, $J_{H1'-H2'}$ = 1.5 Hz, H1′), 5.30 (d, 1H, J_{H1-H2} = 1.5 Hz, H1) ppm. ^{13}C NMR (101 MHz, CD_3OD): δ = 13.1, 22.3, 24.6, 28.8, 29.1, 29.3, 29.4, 29.4, 29.4, 31.7, 33.6, 61.7, 63.8, 67.5, 67.7, 70.3, 70.5, 70.9, 71.0, 72.6, 79.8, 93.0, 102.7, 174.4 ppm.

6′-O-Palmitoyl-α-1′,2-dimannopyranoside (dimannose palmitate) (**28c**)

Yield = 88% (0.163 g). MS (ESI): 598 $[M + NH_4]^+$, 603 $[M + Na]^+$ 625 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.90 (t, 3H, *J* = 6.5 Hz, CH_3), 1.27-1.35 [m, 24H, $(CH_2)_{12}$], 1.59-1.65 (m, 2H, *CH*$_2$$CH_2COOR$), 2.38 (t, 2H, *J* = 7.5 Hz, *CH*$_2$COOR), 3.57-3.64 (m, 2H), 3.68-3.73 (m, 3H), 3.79-3.82 (m, 2H, H2), 3.88-3.91 (m, 2H), 3.99 (dd, 1H, $J_{H2'\text{-}H1'}$ = 1.5 Hz, $J_{H2'\text{-}H3'}$ = 3.0 Hz, H2′), 4.18 (dd, 1H, $J_{H6'b\text{-}H5'}$ = 7.0 Hz, $J_{H6'b\text{-}H6'a}$ = 11.5 Hz, H6′b), 4.41 (dd, 1H, $J_{H6'a\text{-}H5'}$ = 2.0 Hz, $J_{H6'a\text{-}H6'b}$ = 11.5 Hz, H6′a), 4.95 (d, 1H, $J_{H1'\text{-}H2'}$ = 1.5 Hz, H1′), 5.30 (d, 1H, $J_{H1\text{-}H2}$ = 1.5 Hz, H1) ppm. ^{13}C NMR (101 MHz, CD_3OD): δ = 13.0, 22.3, 24.6, 28.8, 29.1, 29.3, 29.4, 29.4, 31.7, 33.6, 61.7, 63.7, 67.5, 67.7, 70.3, 70.4, 70.9, 71.0, 71.6, 79.8, 92.9, 102.7, 174.4 ppm.

5.4.13. Synthesis of 2-*O*-[(2′-*O*-Acetyl-3′,4′,6′-tri-*O*-benzyl)mannopyranosyl] 1,3,4,6-tetra-*O*-benzylmannopyranose (29)

MS 4Å were added to a mixture of **22** (3.277 g, 6.07 mmol) and **24** (5.782 g, 7.28 mmol, 1.5 eq.) in dry DCM (101.2 mL, 0.06 M). The reaction mixture was cooled at –20 °C, TMSOTf (0.141 mL, 0.73 mmol, 0.12 eq.) was added dropwise and the mixture was stirred for one hour. After that, the mixture was neutralized with TEA, filtered, and concentrated. The residue was purified by flash column chromatography (cyclohexane/EtOAc 9:1) to obtain the product **29** as colorless oil.

Yield = 78% (4.801 g). MS (ESI): 1032 $[M + NH_4]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.10 (s, 3H, $COCH_3$), 3.54 (dd, 1H, $J_{H6a\text{-}H5}$ = 1.5 Hz, $J_{H6a\text{-}H6b}$ = 10.5 Hz, H6a), 3.69-3.90 (m, 7H), 3.95-3.99 (m, 2H), 4.04 (dd, 1H, $J_{H2\text{-}H1}$ = 2.0 Hz, $J_{H2\text{-}H3}$ = 3.0 Hz, H2), 4.36-4.44 (m, 4H, 4x CHAr), 4.54-4.58 (m, 2H, 2x CHAr),4.63-4.69 (m, 6H, 6x CHAr), 4.84 (d, 1H, *J* = 11.0 Hz), 4.86 (d, 1H, *J* = 11.0 Hz), 4.97 (d, 1H, $J_{H1\text{-}H2}$ = 2.0 Hz, H1), 5.07 (d, 1H, $J_{H1'\text{-}H2'}$= 1.5 Hz, H1′), 5.54 (dd, 1H, $J_{H2'\text{-}H1'}$= 1.5 Hz, $J_{H2'\text{-}H3'}$ = 3.0 Hz, H2′), 7.14-7.36 (m, 35H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.1, 68.7, 68.9, 69.0, 69.2, 71.8, 71.9, 72.1, 72.1, 73.3, 73.4, 74.3, 74.7, 74.9, 75.1, 75.2, 78.2, 79.7, 98.0, 99.6, 127.4, 127.5, 127.5, 127.6, 127.6, 127.7, 127.8, 127.8, 127.9, 128.1, 128.2, 128.3, 128.3, 128.3, 128.4, 137.3, 138.0, 138.2, 138.4, 138.4, 138.5, 138.5, 170.1 ppm.

5.4.14. Synthesis of 2-*O*-[(3′,4′,6′-tri-*O*-Benzyl-2′-hydroxy)mannopyranosyl]-1,3,4,6-tetra-*O*-benzylmannopyranose (30)

NaOMe (0.758 g; 14.04 mmol; 3 eq.) was added to a solution of **29** (4.750 g; 4.68 mmol; 1 eq.) in dry MeOH/DCM (1/1 v/v, 62.40 mL; 0.075 M) and the reaction mixture was stirred for 3 h at rt. After the completion of the reaction, the Amberlyst resin was added, and the mixture was further stirred for 15 minutes. Then the mixture was filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (cyclohexane/EtOAc 8.5:1.5) to yield the product **30** as colorless oil.

Yield = 96% (4.367 g). MS (ESI): 995 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.39 (br s, 1H, OH), 3.57 (dd, 1H, $J_{H6a\text{-}H5}$ = 1.5 Hz, $J_{H6a\text{-}H6b}$ = 10.5 Hz, H6a), 3.67-3.71 (m, 2H), 3.79-3.86 (m, 6H), 3.97 (dd, 1H, J_1 = 3.0 Hz, J_2 = 9.0 Hz), 4.07 (dd, 1H, $J_1 \cong J_2$ = 2.0 Hz), 4.13 (dd, 1H, $J_1 \cong J_2$ = 2.0 Hz), 4.35 (d, 1H, J = 12.0 Hz), 4.45-4.58 (m, 6H), 4.60-4.62 (m, 2H), 4.64-4.72 (m, 4H), 4.79-4.85 (m, 2H), 5.00 (d, 1H, J = 1.5 Hz), 5.14 (d, 1H, J = 1.0 Hz), 7.16-7.36 (m, 35H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 68.5, 68.7, 69.0, 69.2, 71.4, 72.0, 72.2, 72.4, 73.3, 73.4, 74.2, 74.8, 75.0, 75.1, 79.7, 79.9, 91.8, 98.1, 101.0, 127.4, 127.5, 127.6, 127.7, 127.7, 127.7, 127.8, 127.8, 127.9, 128.0, 128.0, 128.3, 128.3, 128.4, 128.5, 137.2, 137.9, 137.9, 138.2, 138.3, 138.3, 138.5, 163.8 ppm.

5.4.15. Synthesis of 2-*O*-{[2′-*O*-(2′,3′,4′,6′-tri-*O*-Acetyl)mannopyranosyl]-3′,4′,6′-tetra-*O*-benzylmannopyranosyl}-1,3,4,6-tetra-*O*-benzylmannopyranose (31)

MS 4Å were added to a solution of **30** (4.300 g, 4.42 mmol) and **17** (4.340 g, 8.84 mmol, 2 eq.) in dry DCM (73.67 mL, 0.06 M). The reaction mixture was cooled at –20 °C, and TMSOTf (0.101 mL, 0.53 mmol, 0.12 eq.) was added dropwise and the mixture was stirred for an hour. After that, the mixture was neutralized with TEA, filtered and concentrated. The residue was purified by flash column chromatography (cyclohexane/EtOAc 8:2) to give the product **31** as colourless oil.

Yield = 92% (5.294 g). MS (ESI): 1320 $[M + NH_4]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.97 (s, 3H, COCH_3), 1.99 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 2.12 (s, 3H,

$COCH_3$), 3.60 (m, 1H), 3.65-3.70 (m, 2H), 3.79-3.81 (m, 2H), 3.88-3.90 (m, 2H), 3.92-3.97 (m, 2H), 4.01 (dd, 1H, $J_1 \cong J_2$ = 2.0 Hz), 4.03 (dd., 1H, $J_1 \cong J_2$ = 2.0 Hz), 4.08-4.15 (m, 2H), 4.30 (d, 1H, J = 12.0 Hz), 4.48-4.59 (m, 6H), 4.61-4.65 (m, 3H), 4.70 (d, 1H, J = 12.0 Hz), 4.81 (d, 1H, J = 3.0 Hz) , 4.83 (d, 1H, J = 3.5 Hz), 4.90 (d, 1H, J = 1.5 Hz), 5.01 (d, 1H, J = 2.0 Hz), 5.18 (d, 1H, J = 2.0 Hz), 5.25 (br. t, J = 10.0 Hz), 5.39 (dd, 1H, J_1 = 3.5 Hz, J_2 = 9.5 Hz), 5.43 (dd, J_1 = 2.0 Hz, J_2 = 3.5 Hz) 7.16-7.37 (m, 35H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 20.7, 20.7, 20.9, 62.2, 66.1, 68.9, 69.1, 69.2, 69.5, 72.1, 72.2, 72.4, 72.5, 73.1, 73.4, 74.8, 75.0, 75.1, 75.6, 79.2, 79.6, 98.2, 99.2, 100.5, 127.4, 127.4, 127.5, 127.6, 127.7, 127.7, 127.8, 127.9, 128.0, 128.3, 128.3, 128.3, 128.4, 128.4, 137.3, 138.3, 138.4, 138.4, 138.5, 169.7, 169.7, 169.8, 170.5 ppm.

5.4.16. Synthesis of 2-*O*-[3′,4′,6′-tri-*O*-Benzyl-2′-*O*-(mannopyranosyl)mannopyranosyl]-1,3,4,6-tetra-*O*-benzylmannopyranose (32)

NaOMe was added (0.437 g, 8.11 mmol, 2 eq.) to a solution of **31** (5.280 g, 4.06 mmol) in dry MeOH/DCM (1:1 v/v, 40.60 mL; 0.1 M) and the reaction mixture was stirred for 3 h at rt. After the completion of the reaction, the Amberlyst resin was added, and the mixture was further stirred for 15 minutes. Then the mixture was filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (cyclohexane/EtOAc 3:7) to give the product **33** as white solid.

Yield = 85% (3.916 g). MS (ESI): 1157 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.27 (br s, 1H, OH), 3.53-3.73 (m, 9H), 3.75-3.86 (m, 7H), 3.89 (dd, 1H, J_1 = 3.0 Hz, J_2 = 9.0 Hz), 3.94 (dd, 1H, J_1 = 3.0 Hz, J_2 = 8.5 Hz), 4.00-4.03 (m, 2H), 4.07 (d, 1H, J = 2.0 Hz), 4.32 (d, 1H, J = 12.0 Hz), 4.43-4.47 (m, 2H), 4.50-4.64 (m, 8H), 4.68 (d, 1H, J = 12.0 Hz), 4.78 (d, 1H, J_1 = 11.0 Hz) , 4.82 (d, 1H, J_1 = 11.0 Hz), 4.97 (d, 1H, J = 1.5 Hz), 5.02 (br d, 1H), 5.14 (d, 1H, J = 1.5 Hz), 7.13-7.35 (m, 35H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 61.1, 66.7, 69.1, 69.3, 71.0, 71.6, 72.1, 72.1, 72.4, 72.4, 72.8, 73.3, 73.3, 74.9, 75.0, 75.1, 75.5, 79.4, 98.2, 100.8, 101.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.8, 127.8, 127.8, 127.9, 128.0, 128.3, 128.3, 128.3, 128.4, 128.4, 128.5, 137.4, 138.2, 138.2, 138.4, 138.4, 138.5 ppm.

5.4.17. General procedure for the synthesis of 6′′-*O*-acyl-2-*O*-[3′,4′,6′-tri-*O*-benzyl-2′-*O*-(mannopyranosyl)mannopyranosyl]-1,3,4,6-tetra-*O*-benzylmannopyranose (33a–c)

In a two-necked round bottom flask, under nitrogen atmosphere, TEA was added (0.27 mL, 1.96 mmol, 2.2 eq.) was added to a solution of **33** (1 g, 0.88 mmol, 1 eq.) in dry DCM (8.80 mL, 0.01 M). The reaction flask was cooled to 0 °C and the appropriate acyl chloride **3** (0.97 mmol, 1.1 eq.) was added dropwise. The reaction mixture was stirred for 12 h at rt. After the competition of the reaction, the mixture was concentrated. Purification by flash column chromatography (DCM/MeOH 9.9:0.1) gave the products (**34a–c**) as white solids.

6′′-O-lauroyl-2-O-[3′,4′,6′-tri-O-benzyl-2′-O-(mannopyranosyl)mannopyranosyl]-1,3,4,6-tetra-O-benzylmannopyranose (**33a**)

Yield = 64% (0.741 g). MS (ESI): 1339 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, 3H, *J* = 6.5 Hz, CH_3), 1.20-1.32 [m, 16H, $(CH_2)_8$], 1.54-1.61 (m, 2H, CH_2CH_2COOR), 2.33 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.50 (t app., 1H, *J* = 9.5 Hz), 3.55 (d, 1H, *J* = 10.5 Hz), 3.67-3.92 (m, 10H), 3.96 (m, 1H), 4.05 (br t., 1H), 4.07 (br.t, 1H), 4.34-4.39 (m, 2H), 4.44-4.70 (m, 10H), 4.80 (d, 1H, *J* = 10.5 Hz), 4.83 (d, 1H, *J* = 11.0 Hz), 4.97 (br d, 2H), 5.20 (br d, 1H), 7.17-7.35 (m, 35H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 14.1, 22.7, 24.9, 29.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 34.1, 62.8, 67.3, 69.1, 69.2, 70.3, 71.0, 71.1, 72.1, 72.2, 72.4, 72.6, 73.2, 73.4, 74.7, 74.9, 75.0, 75.1, 79.8, 98.2, 100.5, 127.4, 127.5, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 127.9, 128.3, 128.4, 128.5, 128.5, 137.3, 138.2, 138.3, 138.4, 138.5, 175.2 ppm.

6′′-O-myristoyl-2-O-[3′,4′,6′-tri-O-benzyl-2′-O-(mannopyranosyl)mannopyranosyl]-1,3,4,6-tetra-O-benzylmannopyranose (**33b**)

Yield = 62% (0.744 g). MS (ESI): 1367 $[M + Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, 3H, *J* = 6.5 Hz, CH_3), 1.21-1.33 [m, 20H, $(CH_2)_{10}$], 1.55-1.62 (m, 2H, CH_2CH_2COOR), 2.33 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.49 (t app., 1H, *J* = 9.5 Hz), 3.56 (d, 1H, *J* = 10.5 Hz), 3.67-3.91 (m, 10H), 3.96 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.5 Hz), 4.03-4.08 (m, 2H), 4.34-4.39 (m, 2H), 4.45-4.70 (m, 10H), 4.80 (d, 1H, *J* = 10.5 Hz),

4.83 (d, 1H, *J* = 11.0 Hz), 4.97 (s, 2H), 5.21 (br d., 1H) 7.17-7.35 (m, 35H, ArH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 14.1, 22.7, 24.9, 29.1, 29.3, 29.3, 29.4, 29.6, 29.6, 29.7, 31.9, 34.1, 62.8, 67.3, 69.1, 69.2, 69.3, 70.3, 71.0, 71.05, 72.1, 72.2, 72.4, 72.6, 73.2, 73.4, 74.7, 74.93, 74.97, 75.02, 75.1, 75.5, 79.1, 79.8, 98.2, 100.5, 101.0, 127.4, 127.5, 127.6, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 128.3, 128.3, 128.4, 128.5, 137.3, 138.2, 138.3, 138.4, 138.5, 175.2 ppm.

6′′-O-palmitoyl-2-O-[3′,4′,6′-tri-O-benzyl-2′-O-(mannopyranosyl)mannopyranosyl]-1,3,4,6-tetra-O-benzylmannopyranose **(33c)**

Yield = 56% (0.676 g). MS (ESI): 1395 $[M + Na]^+$ ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, 3H, *J* = 6.5 Hz, CH_3), 1.21-1.33 [m, 24H, $(CH_2)_{12}$], 1.55-1.62 (m, 2H, CH_2CH_2COOR), 2.33 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.49 (t app., 1H, *J* = 9.5 Hz), 3.56 (d, 1H, *J* = 10.5 Hz), 3.67-3.91 (m, 10H), 3.96 (dd, 1H, J_1 = 2.5 Hz, J_2 = 8.5 Hz), 4.03-4.08 (m, 2H), 4.34-4.39 (m, 2H), 4.45-4.70 (m, 10H), 4.80 (d, 1H, *J* = 10.5 Hz), 4.83 (d, 1H, *J* = 11.0 Hz), 4.97 (s, 2H), 5.20 (d., 1H, *J* = 2.0 Hz) 7.17-7.35 (m, 35H, ArH) ppm. . ^{13}C NMR (101 MHz, $CDCl_3$): δ = 14.1, 22.7, 24.9, 29.1, 29.2, 29.3, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 34.2, 62.9, 67.4, 69.1, 69.2, 69.2, 70.3, 71.0, 71.0, 72.1, 72.2, 72.4, 72.6, 73.2, 73.4, 74.7, 75.0, 75.0, 75.1, 75.5, 79.1, 79.8, 98.2, 100.5, 101.0, 127.4, 127.5, 127.6, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 128.3, 128.3, 128.4, 128.5, 137.3, 138.1, 138.2, 138.2, 138.5, 175.1 ppm.

5.4.18. General procedure synthesis of 6′′-*O*-Acyl-α-1-2-trimannopyranose esters (34a–c)

A solution of appropriate **33a–c** (0.34 mmol) and Pd/C 10% in MeOH (17 mL; 0.02 M) was stirred at rt under atmospheric hydrogen (3.5 atm) overnight. The reaction mixture was filtered through Celite, and the organic phase concentrated under reduced pressure. Trituration by petroleum ether (DCM/MeOH 8.5:1.5) gave the final products **34a–c** as white solids.

6′′-O-Lauroyl-α-1,2-trimannopyranose (trimannose laurate) (**34a**)

Yield = 96% (0.223 g). MS (ESI): 704 $[M + NH_4]^+$, 709 $[M + Na]^+$, 731 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD):δ = 0.90 (t, 3H, *J* = 6.5 Hz, CH_3), 1.26-1.36 [m, 16H,

$(CH_2)_8$], 1.58-1.65 (m, 2H, *CH*$_2$CH$_2$COOR), 2.41 (t, 2H, *J* = 7.5 Hz, *CH*$_2$COOR), 3.58-3.74 (m, 8H), 3.80-3.93 (m, 6H), 3.98 (dd, 1H, J_1 = 2.0 Hz, J_2 = 3.0 Hz), 4.02 (dd, 1H, J_1 = 2.0 Hz, J_2 = 3.0 Hz), 4.16 (dd, 1H, $J_{H6''b\text{-}H5''}$ = 6.5 Hz, $J_{H6''b\text{-}H6''a}$ = 11.5 Hz, H6′′b), 4.43 (dd, 1H, $J_{H6''a\text{-}H5''}$ = 1.5 Hz, $J_{H6''a\text{-}H6''b}$ = 11.5 Hz, H6′′a), 4.95 (d, 1H, *J* = 1.0 Hz), 5.23 (d, 1H, *J* = 1.5 Hz), 5.35 (d, 1H, *J* = 1.5 Hz) ppm. ^{13}C NMR (101 MHz, CD_3OD) δ 11.5, 20.8, 23.1, 27.3, 27.6, 27.6, 27.8, 27.9, 30.2, 32.2, 60.1, 60.2, 62.4, 65.9, 66.1, 66.4, 68.8, 68.9, 69.3, 69.4, 71.0, 72.0, 77.9, 79.2, 91.3, 99.7, 101.2, 173.1 ppm.

6′′-O-Myristoyl-α-1,2-trimannopyranose (trimannose myristate) (**34b**)

Yield = 90% (0.219 g). MS (ESI): 732 $[M + NH_4]^+$, 737 $[M + Na]^+$, 759 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.90 (t, 3H, *J* = 6.5 Hz, CH_3), 1.26-1.36 [m, 20H, $(CH_2)_{10}$], 1.58-1.64 (m, 2H, *CH*$_2$CH$_2$COOR), 2.41 (t, 2H, *J* = 7.5 Hz, *CH*$_2$COOR), 3.58-3.72 (m, 8H), 3.80-3.93 (m, 6H), 3.98 (dd, 1H, J_1 = 2.0 Hz, J_2 = 3.0 Hz), 4.02 (dd, 1H, J_1 = 2.0 Hz, J_2 = 3.0 Hz), 4.16 (dd, 1H, $J_{H6''b\text{-}H5''}$ = 6.5 Hz, $J_{H6''b\text{-}H6''a}$ = 11.5 Hz, H6′′b), 4.43 (dd, 1H, $J_{H6''a\text{-}H5''}$ = 1.5 Hz, $J_{H6''a\text{-}H6''b}$ = 11.5 Hz, H6′′a), 4.95 (d, 1H, *J* = 1.0 Hz), 5.23 (d, 1H, *J* = 1.5 Hz), 5.35 (d, 1H, *J* = 1.5 Hz) ppm. ^{13}C NMR (101 MHz, MeOD) δ 13.0, 22.3, 24.6, 28.8, 29.1, 29.1, 29.3, 29.4, 29.4, 31.7, 33.7, 61.6, 61.7, 63.9, 67.4, 67.6, 67.9, 70.3, 70.5, 70.9, 71.0, 72.5, 73.5, 79.4, 80.7, 92.8, 101.2, 102.8, 174.6 ppm.

6′′-O-Palmitoyl-a-1,2-trimannopyranose (trimannose palmitate) (**34c**)

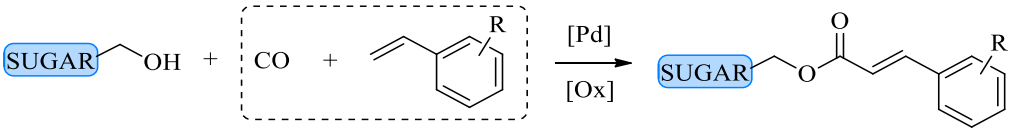
Yield = 94% (0.237 g). MS (ESI): 760 $[M + NH_4]^+$, 765 $[M + Na]^+$, 787 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.90 (t, 3H, *J* = 6.5 Hz, CH_3), 1.26-1.36 [m, 24H, $(CH_2)_{12}$], 1.58-1.65 (m, 2H, *CH*$_2$CH$_2$COOR), 2.41 (t, 2H, *J* = 7.5 Hz, CH_2COOR), 3.58-3.74 (m, 8H), 3.81-3.90 (m, 6H), 3.98 (dd, 1H, J_1 = 2.0 Hz, J_2 = 3.0 Hz), 4.02 (dd, 1H, J_1 = 2.0 Hz, J_2 = 3.0 Hz), 4.16 (dd, 1H, $J_{H6''b\text{-}H5''}$ = 6.5 Hz, $J_{H6''b\text{-}H6''a}$ = 11.5 Hz, H6′′b), 4.43 (dd, 1H, $J_{H6''a\text{-}H5''}$ = 1.5 Hz, $J_{H6''a\text{-}H6''b}$ = 11.5 Hz, H6′′a), 4.94 (d, 1H, *J* = 1.0 Hz), 5.23 (s, 1H), 5.35 (s, 1H) ppm. ^{13}C NMR (101 MHz, CD_3OD) δ 13.0, 22.3, 24.7, 28.8, 29.1, 29.1, 29.3, 29.4, 29.4, 31.7, 33.7, 61.6, 61.7, 63.9, 67.4, 67.6, 67.9, 70.3, 70.5, 70.9, 71.0, 72.5, 73.5, 79.4, 80.7, 92.8, 101.2, 102.7, 174.6.

6. Regioselective and Stereoselective Synthesis of Sugar Cinnamic Acid Monoesters by Oxidative Alkoxycarbonylation of Olefins

6.1. Introduction

Nowadays, sugar cinnamic acid ester derivatives (SCAEDs) have been reported to possess a plethora of biological activities. They are a class of natural products constituted by one or more phenylacrylic groups linked through an ester bond to a carbohydrate moiety. The aromatic portion can contain several substituents such as hydroxyl and methoxy groups, while the sugar portion can be from monosaccharide to hepta-saccharide form. More than 300 SCAEDs have been isolated from several plants [169]. Among them, Verbascoside represents one of the most SCAEDs present in plants, and it is widely applied to treat cancer and other diseases. In fact, it showed an interesting inhibition activity of PKC in the rat brain (IC_{50} = 25 μM), which plays an important role in the cell cycle control and tumorigenesis [170]. Other SCAEDs such as Tenuifolioses A and B demonstrated to have a neuroprotective effect versus glutamate and serum deficiency at 10 uM concentration [171]. Whereupon, some smilasides substituted in the feruloyl group were found to exhibit stronger antioxidant activity by DPPH radical scavenging assays, which was comparable to that of α-tocopherol [172]. Furthermore, some phenylpropanoid glycosides isolated from *Paulownia tomentosa* stems showed significant antibacterial activity versus *S. aureus* and *S. faecium* [173]. Despite these remarkable biological activities, there are still few studies concerning the SCAEDs synthesis. In this context, the enzymatic esterification strategies have been shown several limitations such as low conversion and/or yields and long reaction times. Buzatu et al. reported the chemoenzymatic synthesis of different sugars acetals coupled with 3-(4-hydroxyphenyl) propionic acid (HPPA) catalysed by Novozyme 435® [114]. It was emerged low conversions of HPPA into the sucrose acetals monoesters mixture (27%) and inulin monoesters mixture (24%) in 96 h, while the esterified lactose acetal monoester was not detected. These results were probably due to the bulky phenyl group used in the esterification reaction. In fact, the low affinity of aromatic substrates for lipases was also established by Compton et al.

that reported the formation of ferulic acid octyl ester with Novozyme 435® at 60 °C in *t*-BuOH, in 13% of yield after 300 h [174]. A possible strategy for SCAEDs synthesis could be represented by metal-catalysed carbonylation reactions, which utilized carbon monoxide as cheap and easily accessible C1 source to convert alkenes and alkynes into carbonylated compounds (Scheme 21) [175, 176]. Among them, palladium-catalysed oxidative alkoxycarbonylation of styrenes is probably one of the most attractive ways for the synthesis of cinnamate esters [177, 178]. Here, different palladium catalysts, bearing various ligands, have been utilized in the presence of a suitable oxidizing agent, for driving the selectivity towards the desired carbonylated product. Moreover, from the point of view of the sugar, CO/olefinic system can be considered as the ideal acylating reagent for its esterification.



Scheme 21. Pd-catalyzed oxidative carbonylation of sugar with styrenes

The main issues related to carbonylation reactions are probably related with the usually drastic reaction conditions, such as high temperature and/or high CO pressure. In addition, high boiling point solvents, whose remotion from the reaction results hard, are often employed. On the other hand, an accurate choice of the solvent system is essential for the optimal proceeding of the reaction. [179] As an example, Lei et al. reported the oxidative carbonylative esterification of olefins for the synthesis of α,β-unsaturated esters, using, 3 mol% of $PdCl_2$, molecular oxygen as terminal oxidant, in a solvent mixture of toluene/DMSO 10:1 at 80 °C [180]. More recently, Della Ca′ and co-workers investigated the oxidative alkoxycarbonylation of α-olefins using heterogeneous palladium source such as Pd/C, Pd/Al_2O_3 and $Pd/CaCO_3$ in conjunction with CuI. The reaction, which utilized ACN as solvent, required 2 atm of CO and 35 atm of air (O_2 as terminal oxidant) and proceeded at 120°C [181].

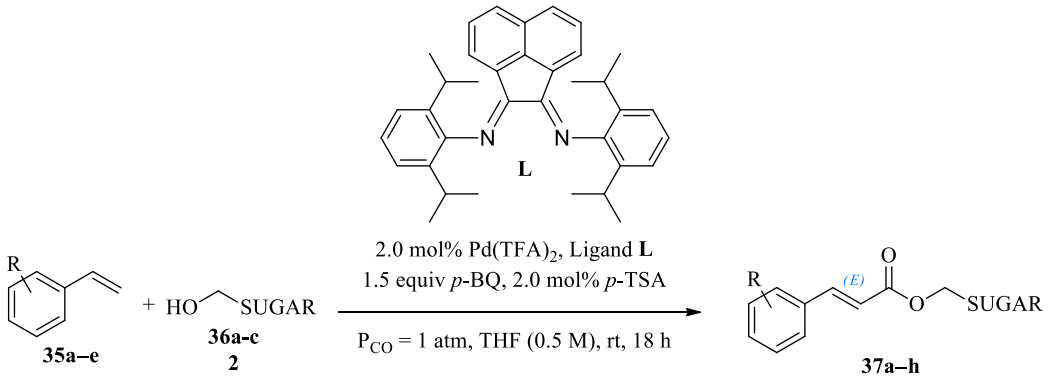
In this chapter is reported an efficient palladium-catalysed oxidative alkoxycarbonylation of styrenes utilizing carbohydrate derivates for the stereoselective synthesis of sugar-based *trans*-cinnamic esters. Remarkably, mild reaction conditions have been used (P_{CO} = 1 atm, 20°C). In particular, monosaccharide

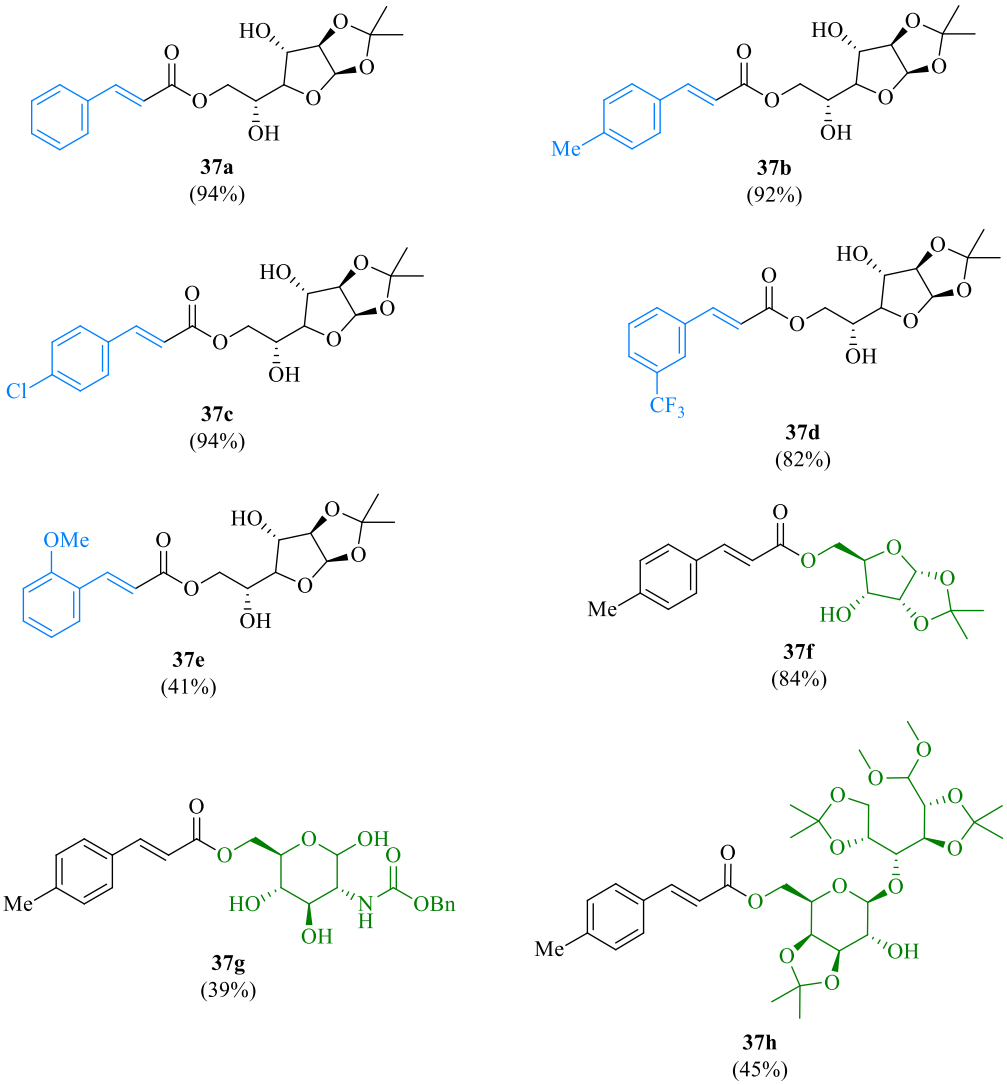
acetal derivatives, *N*-Cbz-glucosamine and LTA have been regioselectively esterified at the primary hydroxyl group, with moderate to excellent yields. To the best of our knowledge this represents the first example of an oxidative alkoxycarbonylation reaction applied to carbohydrates.

6.2. Results and discussion

The envisioned synthesis sugar-based *trans*-cinnamic esters has been realized by modifying the conditions of a previously reported oxidative carbonylation reaction [182–184]. In particular, the catalyst is formed in situ by mixing $Pd(TFA)_2$ and the aryl α-diimine ligand **L**, displaying a 1,4-diazabutadiene scaffold merged with a naphthalene backbone [185]. In the presence of equimolar amount of the sugar and styrene derivatives, the reaction proceeds in THF using *p*-benzoquinone (1.5 eq.) as the oxidant and 2.0 mol% of *p*-TSA under 1 atm of CO and at rt (Scheme 22). Due to the low solubility of sugar in the reaction medium, protected sugar, commercially available or previously synthesized, were utilized. To start with, the 1,2-*O*-isopropyliden-α-D-glucofuranose **36a** was coupled with carbon monoxide and different ortho, meta and para substituted styrenes **35a–e**. In detail, despite the presence of three different hydroxyl groups in the sugar moiety, the esterification occurs in regioselective manner only at the primary OH6 position. Regardless the presence of an EWG or EDG on the aromatic ring, excellent yields have been achieved for compounds **37a–c** with styrene (94%), *p*-methylstyrene (92%) and *p*-chlorostyrene (94%) respectively. With *m*-trifluoromethylstyrene **37d** 82% of isolated yield was obtained while slightly less satisfactory results were assessed with the *o*-methoxystyrene **37e** (41%) probably due to steric hindrance reason. Successively, the 1,2-*O*-isopropyliden-α-D-ribose **36b**, *N*-benzyloxycarbonyl-D-glucosamine **36c** and LTA **2** were coupled using *p*-methylstyrene. The result obtained with the ribose derivative **36b** is comparable to those obtained with the glucofuranose **36a**, achieving the cinnamic ester **37f** with 84% isolated yield. Interesting results was observed with the D-glucosamine derivative. Indeed, despite the presence of four hydroxyl group, the carbonylation still regioselectively proceeds at the OH6 and the modest yield (39% isolated yield of **37g**) observed can be ascribed to the very low solubility of this sugar in THF which led to a poor conversion (sugar conversion 45%). Lastly, the reaction

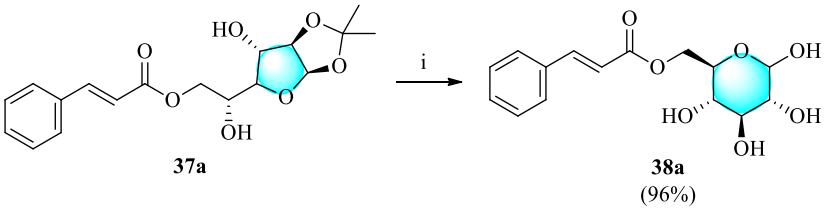
was extended to the disaccharide LTA **2** (45% isolated yield of **37h**). This developed alkoxycarbonylation reaction proceeds with a completed stereoselectivity, since only the *E*-isomer was formed.





Scheme 22. Scope of Pd-catalyzed oxidative alkoxycarbonylation with sugar and styrene derivatives.

Noteworthy, this new methodology led to higher yields of SCAEDs respect to those previously reported in literature [114, 174]. To summarize, this alkoxycarbonylation reaction was found to be more suitable for the stereoselective synthesis of sugar-based *trans*-cinnamic esters. Successively, 6-*O*-cinnamyl-1,2-*O*-isopropyliden-α-D-glucofuranose **37a** was deprotected by acidic hydrolysis conducted using TFA in DCM to give the correspondent glucose cinnamic acid ester **38a** in 96% of yield in α/β mixture (Scheme 23). This reaction converted the furanose form into the pyranose form as reported by Redmann [186]. In fact, ^{1}H-NMR analysis highlighted the change of multiplicity of H5 proton which change from *dddd* to *ddd* indicating the formation of pyranose form.



37a **38a** (96%)

Scheme 23. Synthesis of 6-*O*-cinnamyl-D-glucopyranose **38a** through acidic hydrolysis. Reagents, conditions, and yield: (i) TFA (90% v/v), DCM, rt, 1h.

The other sugar cinnamic esters derivatives will be successively deprotected. Both protected and deprotected sugar-based *trans*-cinnamic esters will be tested as possible antimicrobial, anti-inflammatory and antioxidant agents and for other biological activities.

6.3. Conclusion

In conclusion a new approach for the synthesis of sugar aromatic esters has been developed. Through this Pd-catalysed oxidative alkoxycarbonylation different sugar-based *trans*-cinnamic monoesters were successfully synthesized in moderate to excellent yields employing partially protected carbohydrates, variously substituted styrenes and carbon monoxide. The reaction is completely regioselective, indeed the esterification take place only on the primary hydroxyl group of the sugar, and stereoselective, since only the *E*-isomer of the cinnamic ester was observed. Successive sugar deprotection reaction of 6-*O*-cinnamoyl-1,2-*O*-isopropyliden-α-D-glucofuranose did not affect the double bond and the ester functionalities and the

respective product can be isolated in high yield. In future perspective, this Pd-catalyzed oxidative alkoxycarbonylation will be applied to other sugar derivative and olefin. These sugar-based trans cinnamic esters and their relative deprotected form will be biologically evaluated as regard their antimicrobial, anti-inflammatory, antioxidant and other activities.

6.4. Materials and Methods

6.4.1. Chemicals

Carbon monoxide (Cp grade 99.99%) was supplied by Air Liquide. (Caution: carbon monoxide is a toxic gas with potentially lethal action, therefore adequate precautions must be observed). The *p*-benzoquinone was purchased by Alfa Aesar and was filtered off a plug of silica gel washing with DCM, obtaining a yellow solid after drying the solution under vacuum. Olefins were purchased from Merck Sigma-Aldrich and were filtered off a plug of neutral Al_2O_3 and used without further purification. Anhydrous THF was distilled from sodium-benzophenone. $Pd(TFA)_2$ was purchased by Fluorochem. All other chemicals were purchased from Merck Sigma-Aldrich and used without further purification. The ligand **L** was synthesized according to literature procedures [185]. LTA (lactose tetra acetal) and *N*-Cbz-D-glucosamine were previously synthetized using reported procedures [112, 187]. All reactions were carried out under nitrogen atmosphere with dry solvents under anhydrous conditions, by using Schlenk technique. All solid reagents were weighed in an analytical balance without excluding moisture and air. The structures of compounds were assessed by MS (ESI), ^{1}H NMR, ^{13}C NMR, and IR. MS (ESI) spectra were recorded with a Waters Micromass ZQ spectrometer in a negative or positive mode using a nebulizing nitrogen gas at 400 L/min and a temperature of 250 °C, cone flow 40 mL/min, capillary 3.5 kV and cone voltage 60 V; molecular ions $[M + Na]^+$ and $[M + HCOO]^-$ are given. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 400 or 101, respectively, spectrometer and analyzed using the TopSpin 1.3 software package. Chemical shifts were measured by using the central peak of the solvent. Column chromatography purifications were performed under "flash" conditions using Merck 230–400 mesh silica gel. TLC was carried out on Merck silica gel 60 F254, which were visualized by

exposure to ultraviolet light and by exposure to an aqueous solution of ceric ammonium molybdate.

6.4.2. General procedure for the sugar carbonylation reaction (37a–h)

In a nitrogen flushed dried Schlenk tube, equipped with a magnetic stirring bar the $Pd(TFA)_2$ (3.3 mg, 0.01 mmol) and THF (1.0 mL) were added in sequence. After the mixture turned in a red/brown color (10 min), the ligand **L** (5.5 mg, 0.011 mmol) was added. The mixture was left under stirring for 10 min, turning in a dark orange color. Then, *p*-benzoquinone (81.2 mg, 0.75 mmol), *p*-TSA·H_2O (1.9 mg, 0.01 mmol), the cinnamic acid derivative **35** (0.5 mmol) and the appropriate sugar **36** (0.5 mmol) were added in sequence. The reaction was vigorously stirred at rt under CO (1 atm), for 18 h. Then, the CO was removed, and the crude was dried under reduced pressure and the product was obtained after column chromatography on silica gel.

6-*O*-Cinnamoyl-1,2-*O*-isopropyliden-α-D-glucofuranose (**37a**)

Yield = 94% (0.164 g). MS (ESI): 373 $[M + Na]^+$, 395 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.24 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 3.92-4.02 (m, 2H, H4, H5) 4.06-4.09 (m, 2H, H6a, H3), 4.36 (dd, 1H, J_{H6b-H5} = 2.0 Hz, $J_{H6b-H6a}$ = 11.0 Hz, H6b), 4.42 (d, 1H, J = 3.5 Hz, H2), 5.10 (d, 1H, J_{OH5-H5} = 6.0 Hz, OH5), 5.26 (d, 1H, J_{OH3-H3} = 5.0 Hz, OH3), 5.82 (d, 1H, J_{H1-H2} = 3.5 Hz, H1), 6.64 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 7.43-7.44 (m, 2H, ArH), 7.69 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, CH=C*H*), 7.69-7.73 (m, 2H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 26.6, 27.2, 65.8, 67.4, 73.4, 80.8, 85.1, 105.0, 111.1, 118.7, 128.7, 129.4, 130.9, 134.5, 144.9, 166.7 ppm.

6-*O*-(*p*-Methyl)cinnamoyl-1,2-*O*-isopropyliden-α-D-glucofuranose (**37b**)

Yield = 92% (0.167 g). MS (ESI): 387 $[M + Na]^+$, 409 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.24 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.91-4.00 (m, 2H, H4, H5), 4.04-4.09 (m, 2H, H3, H6b), 4.34 (dd, 1H, J_1 = 2.0 Hz, J_2 = 11.0 Hz, H6b), 4.41 (d, 1H, J = 3.5 Hz, H2), 5.09 (d, 1H, J_{OH5-H5} = 6.0 Hz, OH5), 5.26 (d, 1H, J_{OH3-H3} = 5.0 Hz, OH3), 5.82 (d, 1H, J_{H1-H2} = 3.5 Hz, H1), 6.57 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 7.25 (d, 2H, J = 8.0 Hz, ArH), 7.60 (d, 2H, J = 8.0 Hz, ArH), 7.65 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, CH=C*H*) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ =

21.5, 26.6, 27.2, 65.8, 67.3, 73.4, 80.8, 85.1, 105.0, 111.10, 117.6, 128.7, 130.0, 131.8, 140.9, 144.9, 166.8 ppm.

6-*O*-(*p*-Chloro)cinnamoyl-1,2-*O*-isopropyliden-α-D-glucofuranose (**37c**)

Yield = 94% (0.180 g). MS (ESI): 407 $[M + Na]^+$, 429 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.24 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 3.91-4.00 (m, 2H, H4, H5), 4.06-4.11 (m, 2H, H3, H6b), 4.35 (dd, 1H, J_1 = 2.0 Hz, J_2 = 11.0 Hz, H6b), 4.41 (d, 1H, J = 3.5 Hz, H2), 5.09 (d, 1H, J_{OH5-H5} = 6.0 Hz, OH5), 5.26 (d, 1H, J_{OH3-H3} = 5.0 Hz, OH3), 5.82 (d, 1H, J_{H1-H2} = 3.5 Hz, H1), 6.67 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 7.48-7.52 (m, 2H, ArH), 7.68 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, CH=C*H*), 7.74-7.78 (m, 2H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 26.6, 27.1, 65.8, 67.4, 73.4, 80.7, 85.1, 105.0, 111.1, 119.4, 129.5, 130.5, 133.4, 135.4, 143.5, 166.5 ppm.

6-*O*-(*m*-Trifluoromethyl)cinnamoyl-1,2-*O*-isopropyliden-α-D-glucofuranose (**37d**)

Yield = 82% (0.171 g). MS (ESI): 441 $[M + Na]^+$, 463 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.24 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 3.91-4.00 (m, 3H, H4, H5), 4.05-4.13 (m, 2H, H3, H6b), 4.36 (dd, 1H, J_1 = 2.0 Hz, J_2 = 11.0 Hz, H6b), 4.42 (d, 1H, J = 3.5 Hz, H2), 5.09 (d, 1H, J_{OH5-H5} = 6.0 Hz, OH5), 5.27 (d, 1H, J_{OH3-H3} = 5.0 Hz, OH3), 5.82 (d, 1H, J_{H1-H2} = 3.5 Hz, H1), 6.84 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 7.67 (t, 1H, J = 8.0 Hz, ArH), 7.75-7.82 (m, 1H, ArH), 7.79 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, CH=C*H*), 8.06 (d, 1H, J = 8.0 Hz, ArH), 8.10 (s, 1H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 26.6, 27.1, 65.8, 67.5, 73.4, 80.8, 85.1, 105.0, 111.0, 120.9, 124.4 (q, J_{C-F} = 273.5 Hz), 125.5 (q, J_{C-F} = 3.2 Hz), 127.1 (q, J_{C-F} = 3.7 Hz), 130.3 (q, J_{C-F} = 32.1 Hz), 130.5, 132.3, 135.7, 143.1, 166.4 ppm.

6-*O*-(*o*-Methoxy)cinnamoyl-1,2-*O*-isopropyliden-α-D-glucofuranose (**37e**)

Yield = 41% (0.078 g). MS (ESI): 403 $[M + Na]^+$, 425 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.24 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 3.91-4.00 (m, 2H, H4, H5), 4.04-4.10 (m, 2H, H3, H6b), 4.36 (dd, 1H, J_1 = 2.0 Hz, J_2 = 11.0 Hz, H6b), 4.41 (d, 1H, J = 3.5 Hz, H2), 5.09 (d, 1H, J_{OH5-H5} = 6.0 Hz, OH5), 5.25 (d, 1H, J_{OH3-H3} = 5.0 Hz, OH3), 5.82 (d, 1H, J_{H1-H2} = 3.5 Hz, H1), 6.62 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 7.00 (m, 1H, ArH), 7.11 (m, 1H, ArH), 7.43 (m, 1H, ArH), 7.70

(m, 1H, ArH), 7.90 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, CH=C*H*) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 26.7, 27.2, 56.1, 65.8, 67.3, 73.4, 80.8, 85.1, 105.0, 111.1, 112.2, 118.8, 121.2, 122.7, 129.2, 132.5, 139.7, 158.3, 166.5 ppm.

5-*O*-(*p*-Methyl)cinnamoyl-1,2-*O*-isopropyliden-α-D-ribose (**37f**)

Yield = 84% (0.140 g). MS (ESI): 357 $[M + Na]^+$, 379 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.28 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.34 (s, 3H, $ArCH_3$), 3.79 (ddd, 1H, J_{H3-H2} = 4.5 Hz, J_{H3-OH3} = 7.0 Hz, J_{H4-H3} = 9.0 Hz, H3), 3.97 (ddd, 1H, J_{H4-H5b} = 2.0 Hz, J_{H4-H5a} = 6.0 Hz, J_{H4-H3} = 9.0 Hz, H4), 4.12 (dd, 1H, J_{H5a-H4} = 6.0 Hz, $J_{H5a-H5b}$ = 12.0 Hz, H5a), 4.44 (dd, 1H, J_{H5b-H4} = 2.0 Hz, $J_{H5b-H5a}$ = 12.0 Hz, H5b), 4.48-4.54 (m, 1H, H2), 5.29 (d, 1H, J_{OH3-H3} = 7.0 Hz, OH3), 5.71 (d, 1H, J_{H1-H2} = 3.5 Hz, H1), 6.62 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 7.24 (d, 2H, *J* = 8.0 Hz, ArH) 7.61-7.67 (m, 3H, ArH, CH=C*H*), ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 21.5, 26.8, 27.0, 63.8, 71.7, 77.4, 79.3, 103.9, 111.9, 117.1, 128.9, 130.0, 131.7, 141.0, 145.4, 166.7 ppm.

6-*O*-(*p*-Methyl)cinnamoyl-*N*-benzyloxycarbonylglucosamine (**37g**)

Yield = 39% (0.089 g). MS (ESI): 480 $[M + Na]^+$, 502 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.34 (s, 3H, $ArCH_3$), 3.20 (ddd, 1H, J_{H4-OH4} = 5.5 Hz, $J_{H4-H3} \cong J_{H4-H5}$ = 9.5 Hz, H4), 3.36-3.40 (m, 1H, H2), 3.53 (ddd, 1H, J_{H3-OH3} = 5.5 Hz, J_{H3-H2} = 8.5 Hz, J_{H3-H4} = 9.5 Hz, H3), 3.87 (ddd, 1H, J_{H5-H6b} = 1.0 Hz, J_{H5-H6a} = 6.0 Hz, J_{H5-H4} = 9.5 Hz, H5), 4.21 (ddd, 1H, J_{H6a-H5} = 6.0 Hz, $J_{H6a-H6b}$ = 11.5 Hz, H6a), 4.40 (ddd, 1H, J_{H6b-H5} = 1.0 Hz, $J_{H6b-H6a}$ = 11.5 Hz, H6b), 4.80 (d, 1H, J_{OH3-H3} = 5.5 Hz, OH3), 4.97 (dd, 1H, $J_{H1-H2} \cong J_{H1-OH1}$ = 4.0 Hz, H1), 5.00-5.05 (m, 2H, CH_2Ar), 5.22 (d, 1H, J_{OH4-H4} = 5.5 Hz, OH4), 6.57 (d, 1H, J_{OH1-H1} = 4.0 Hz, OH1), 6.60 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 6.99 (d, 1H, J_{NH-H2} = 8.5 Hz, NH), 7.24 (d, 1H, *J* = 8.0 Hz, ArH), 7.30-7-34 (m, 1H, ArH), 7.35-7-39 (m, 4H, ArH), 7.60-7.66 (m, 3H, ArH, CH=C*H*) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ = 21.5, 56.7, 64.4, 65.7, 69.8, 70.6, 71.5, 91.3, 116.1, 117.3, 128.21, 128.24, 128.8, 128.9, 130.0, 121.7, 137.6, 141.0, 145.1, 150.2, 156.6, 166.8 ppm.

6′-*O*-(*p*-Methyl)cinnamoyl-4-*O*-(3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-2,3:5,6-di-*O*-isopropylidene-1,1-di-*O*-methyl-D-glucopyranose, LTA *p*-methylcinnamate (**37h**)

Yield = 45% (0.147 g). MS (ESI): 675 $[M + Na]^+$, 697 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 2.38 (s, 3H, $ArCH_3$), 3.40 (s, 3H, OCH_3), 3.41 (s, 3H, OCH_3), 3.59 (dd, 1H, $J_{H2'\text{-}H3'}$ = 7.0 Hz, $J_{H2'\text{-}H3'}$ = 8.0 Hz, H2′), 3.90 (dd, 1H, $J_{H3\text{-}H4}$ = 1.5 Hz, $J_{H3\text{-}H2}$ = 7.5 Hz, H3), 4.00-4.06 (m, 2H), 4.08-4,18 (m, 4H), 4.29 (ddd, 1H, *J* = 2.5 Hz, *J* = 7.5 Hz), 4.37-4.52 (m, 5H), 6.42 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, C*H*=CH), 7.19 (d, 2H, *J* = 8.0 Hz, ArH), 7.44 (d, 2H, *J* = 8.0 Hz, ArH), 7.69 (d, 1H, $J_{CH=CH}$ = 16.0 Hz, CH=C*H*) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 21.5, 24.5, 25.7, 26.3, 26.4, 27.2, 28.1, 53.3, 56.2, 63.5, 64.7, 71.6, 73.4, 74.3, 75.1, 76.5, 77.9, 79.0, 103.8, 105.0, 108.3, 110.2, 110.2, 110.4, 116.1, 116.5, 128.1, 129.6, 131.6, 140.9, 145.4, 166.9 ppm.

6.4.3. Synthesis of the 6-*O*-Cinnamoyl-D-glucopyranose (38a)

TFA (90% v/v, 2.041 mL) was added dropwise to a stirred suspension of 6-*O*-cinnamoyl-1,2-*O*-isopropyliden-α-D-glucofuranose (100 mg, 0.29 mmol) in DCM (2.041 mL, 0.14 M) at 0 °C and the reaction was stirred at rt for 1 h. Then the solvent and TFA were removed by co-evaporation in vacuo with toluene and the residue was purified by flash column chromatography (DCM/MeOH 9:1) to give the 6-*O*-cinnamoyl-D-glucopyranose as light brown solid.

Yield = 96% (0.085 g). Rapporto α/β = 1:0.9. MS (ESI): 309 $[M - H]^-$, 311 $[M + H]^+$, 328 $[M + NH_4]^+$, 333 $[M + Na]^+$, 355 $[M + HCOO]^-$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.94 (ddd, 0.8H, $J_{H2\beta\text{-}OH2\beta}$ = 5.0 Hz, $J_{H2\beta\text{-}H1\beta} \cong J_{H2\beta\text{-}H3\beta}$, 7.5 Hz, $H2^{\beta}$), 3.08-3.20 (m, 2H +1.6H, $H4^{\alpha}$, $H2^{\alpha}$, $H3^{\beta}$, $H4^{\beta}$), 3.38–3.42 (m, 0.8H, $H5^{\beta}$)3.46 (ddd, 1H, $J_{H3\alpha\text{-}OH3\alpha}$ = 5.0 Hz, $J_{H3\alpha\text{-}H2\alpha} \cong J_{H3\alpha\text{-}H4\alpha}$ = 9.0 Hz, $H3^{\alpha}$), 3.86 (ddd, 1H, $J_{H5\alpha\text{-}H6b\alpha}$ = 1.5 Hz, $J_{H5\alpha\text{-}H6a\alpha}$ = 6.5 Hz, $J_{H5\alpha\text{-}H4\alpha}$ = 9.5 Hz, $H5^{\alpha}$), 4.16 (dd, 0.8H, $J_{H6a\beta\text{-}H5\beta}$ = 6.5 Hz, $J_{H6a\beta\text{-}H6b\alpha\beta}$ = 11.5 Hz, $H6a^{\beta}$), 4.19 (dd, 1H, $J_{H6a\alpha\text{-}H5\alpha}$ = 6.5 Hz, $J_{H6a\alpha\text{-}H6b\alpha}$ = 11.5 Hz, $H6a^{\alpha}$), 4.34 (dd, 0.8H, $J_{H1\beta\text{-}OH1\beta}$ = 6.5 Hz, $J_{H1\beta\text{-}H2\beta}$ = 7.5 Hz, $H1^{\beta}$), 4.40 (dd, 1H, $J_{H6b\alpha\text{-}H5\alpha}$ = 1.5 Hz, $J_{H6b\alpha\text{-}H6a\alpha}$ = 11.5 Hz, $H6b^{\alpha}$), 4.44 (dd, 1H, $J_{H6b\beta\text{-}H5\beta}$ = 1.5 Hz, $J_{H6b\beta\text{-}H6a\beta}$ = 11.5

Hz, H6b$^{\beta}$), 4.55 (d, 1H, $J_{OH2\alpha\text{-}H2\alpha}$ = 6.5 Hz, OH2$^{\alpha}$) 4.77 (d, 1H, $J_{OH3\alpha\text{-}H3\alpha}$ = 5.0 Hz, OH3$^{\alpha}$), 4.91-4.94 (m, 1H +0.9H, H1$^{\alpha}$, OH2$^{\beta}$), 4.97 (d, 1H, $J_{OH3\beta\text{-}H3\beta}$ = 4.5 Hz, OH3$^{\beta}$), 5.09 (d, 1H, $J_{OH4\alpha\text{-}H4\alpha}$ = 5.5 Hz, OH4$^{\alpha}$), 5.15 (d, 1H, $J_{OH4\beta\text{-}H4\beta}$ = 5.0 Hz, OH4$^{\beta}$), 6.37 (d, 1H, $J_{OH1\alpha\text{-}H1\alpha}$ = 4.5 Hz, OH1$^{\alpha}$), 6.64 (d, 1H, $J_{CH\alpha=CH\alpha}$ = 16.0 Hz, CH$^{\alpha}$=C*H*$^{\alpha}$), 6.68 (d, 0.9H, $J_{CH\beta=CH\beta}$ = 16.0 Hz, CH$^{\beta}$=C*H*$^{\beta}$), 6.69 (d, 1H, $J_{OH1\beta\text{-}H1\beta}$ = 6.5 Hz, OH1$^{\beta}$), 7.41-7.46 (m, 3H +2.7H, ArH$^{\alpha}$, ArH$^{\beta}$), 7.65 (d, 1H, $J_{CH\alpha=CH\alpha}$ = 16.0 Hz, C*H*$^{\alpha}$=CH$^{\alpha}$), 7.67 (d, 0.9H, $J_{CH\beta=CH\beta}$ = 16.0 Hz, C*H*$^{\beta}$=CH$^{\beta}$), 7.72–7.76 (m, 2H +1.8H, ArH$^{\alpha}$, ArH$^{\beta}$) ppm. ^{13}C NMR (101 MHz, DMSO-*d*$_6$): δ = 64.21(1C), 64.24(0.9C), 69.2(0.9C), 70.2(1C), 70.6(0.9C), 72.2(0.9C), 72.9(0.9C), 73.5(1C), 74.7(1C), 76.4(1C), 92.3(0.9C), 96.9(1C), 117.9(1C), 118.0(0.9C), 128.38(0.9C), 128.41(1C), 128.9(1.9C), 130.48(0.9C), 130.51,(1C), 134.0(1.9C), 144.6(0.9C), 144.7(1C), 166.25(0.9C), 166.26(1C).

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