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A Simple Procedure for the Regioselective Synthesis of Fatty Acid Esters of Maltose, Leucrose, Maltotriose and *n*-Dodecyl Maltosides

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Abstract—The enzymatic acylation of several di- and trisaccharides with acyl donors ranging from 8 to 18 carbon atoms was carried out by transesterification with the corresponding vinyl esters. The reaction was performed in 2-methyl-2-butanol/dimethylsulfoxide mixtures using the lipase from *Humicola lanuginosa* (immobilized on Celite). Maltose, maltotriose and *n*-dodecyl maltosides were specifically acylated in the primary hydroxyl of the non-reducing-end glucose moiety; the acylation of leucrose occurred preferentially in the primary hydroxyl of the glucose ring. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Carbohydrate fatty acid esters are non-ionic surfactants that can be synthesized from renewable sources. The main properties of these compounds compared with other petroleum-derived surfactants are their biodegradability and non-toxicity. In addition, their hydrophilic-lipophilic balance (HLB) may be modulated by varying the fatty acid, the sugar moiety and the degree of substitution.

Although less studied than sucrose esters (widely used in food, detergent and personal-care industries), fatty acid esters of maltose have potential applications in cosmetics[1] and medicine as antitumoral agents.[2] Most of the procedures described for the synthesis of maltose fatty acid esters are based on the use of acyl chlorides at high temperatures, and give rise to complex mixtures of products, ranging from mono- to higher esters.

Selective monoacylation of maltose has been achieved chemically using exceptionally low temperatures (−20°C) in the presence of basic organic catalysts[1] or via a Mitsunobu reaction,[3] yielding in both cases the monoester at the primary hydroxyl 6′-OH (corresponding to the non-reducing glucose moiety). A process for the acylation of the anomeric hydroxyl of maltose (1-OH) based on the use of sterically-hindered acyl donors has been also described.[4] The main drawbacks associated with these processes are the need to synthesize special electrophiles, the extreme conditions required and the low yields obtained.

The ability of enzymes in organic solvents to work in the reverse direction to that in water has resulted in a great number of enzyme-catalyzed synthetic reactions including regioselective transformations of mono and disaccharides. The regioselective monoacylation of maltose in one-step has only been reported with butyryl donors using the protease subtilisin[5] or the lipase from *Candida antarctica*.[6] The enzymatic acylation using ethyl laurate has been described in refluxing *tert*-butanol (82°C) but with low yield.[6] A chemo-enzymatic process based on the formation of maltose acetal followed by enzymatic esterification with fatty acids in toluene was also reported.[7] In all cases, selectivity towards 6′-OH was found.

The difficulties to develop a lipase-catalyzed reaction for the acylation of maltose with long fatty acids derive from the fact that lipases are readily inactivated by the polar solvents required to dissolve disaccharides (dimethylsulfoxide, dimethylformamide, dimethylacetamide, etc.). Similar limitations have been found for the acylation of other di- and trisaccharides as well as alkyl-maltosides.[8–11]

In this work, continuing our contribution to the study of the acylation of carbohydrates,[12–14] we have developed a lipase-catalyzed process suitable for the acylation of maltose, leucrose, maltotriose and *n*-dodecyl maltosides. The key

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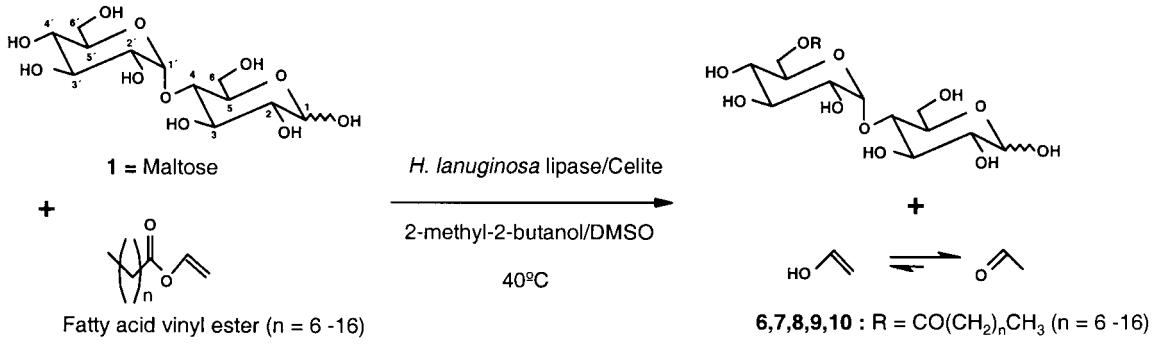


Scheme 1.

point is the use of a medium constituted by two miscible solvents (2-methyl-2-butanol/dimethylsulfoxide) that facilitates the solubilization of the saccharide and avoids the inactivation of lipases. This strategy was recently developed in our laboratory to enhance the reactivity of sucrose.[14]

Results and Discussion

The transesterification of maltose (**1**) with vinyl laurate was assayed in 2-methyl-2-butanol at 40°C in the presence of the lipase from *Humicola lanuginosa* immobilised on Celite. Vinyl laurate was chosen as acyl donor since the equilibrium can be shifted towards the ester formation.[15] The reactions were conducted in the presence of molecular sieves, in order to dry the reaction medium and thus avoid the formation of fatty acid (by lipase-catalyzed hydrolysis of the vinyl ester).

Using 0.12 M maltose and 0.3 M acyl donor as starting concentrations, a significant conversion (38%, determined by HPLC) to monoester was obtained in 24 h. As well as the monoester, higher esters (6% conversion in 24 h) were also detected. The monoester was isolated as described in the Experimental, and the position of acylation determined by ^{1}H NMR and correlation bidimensional analysis ^{1}H–^{1}H (COSY, TOCSY) and ^{1}H–^{13}C NMR (HMQC, HMBC), demonstrating that 6′OH was acylated (Scheme 1). It is well established that the 6′-position of maltose is more reactive than the 6- since the latter is more sterically hindered.[16,17] Probably for this reason, there exists a good correlation between enzymatic and chemical selectivity. This is not the case with other disaccharides; for example, the chemical acylation of sucrose normally gives a mixture of the 6- and 6′-monoesters, whereas the protease-catalyzed transesterification with activated esters leads to the 1′-monoester.[18]

In order to increase the rate of the process, we tested the acylation of maltose with vinyl laurate in *tert*-amyl alcohol containing 5–20% DMSO (Fig. 1). As we observed in a previous work with sucrose,[14] the addition of DMSO caused a notable acceleration of the reaction, resulting in a higher conversion to 6′-*O*-lauroylmaltose (72% in 24 h, using 5% DMSO). However, the formation of diesters was also appreciable (13% in 24 h). When the reaction was carried out in a medium containing 20% DMSO, only a moderate acceleration was observed (47% conversion in 24 h) but, interestingly, the presence of diesters was almost negligible (<1%).

Different immobilized lipases (commercial or prepared in the laboratory) were screened in a medium containing 20% DMSO (the hydrolytic activity of the lipases were first measured, and the same number of enzyme units were used to compare the experiments). Table 1 shows that *H. lanuginosa* lipase on Celite gave the best results. Our

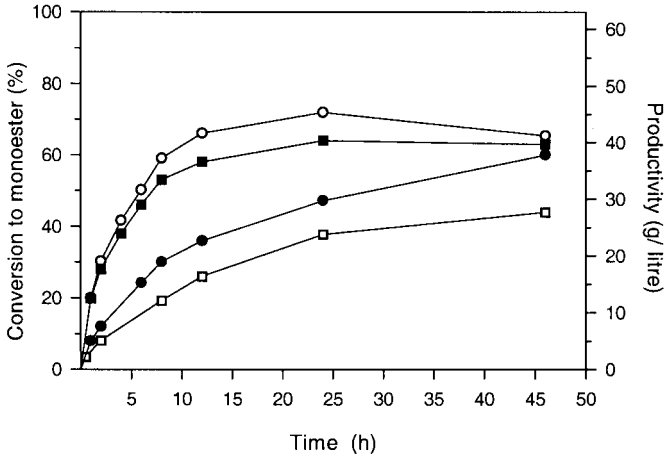


Figure 1. Kinetics of formation of 6′-*O*-lauroylmaltose varying the percentage of DMSO in *tert*-amyl alcohol: 0% (□), 5% (○), 10% (■), 20% (●). Conditions: 0.12 M maltose, 0.3 M vinyl laurate, 25 mg/mL biocatalyst, 25 mg/mL molecular sieves (3 Å), 40°C. Conversion and productivity determined by HPLC.

Table 1. Acylation of maltose with vinyl laurate catalysed by different lipases in *tert*-amyl alcohol/DMSO (4:1 v/v). Conditions: 0.12 M maltose, 0.3 M acyl donor, 2.3 μkat/mL lipase, 40°C. The same weight of molecular sieves (3 Å) as biocatalyst was used

Lipase	Hydrolytic activity (μkat/g)[a]	Support	Conversion (%)[b]
Humicola lanuginosa (Lipolase 100L)	90	Celite	47
Candida antarctica (Novozyme 435)	20	Acrylic macroporous resin	39
Pseudomonas sp. (Lipase PS)	25	Toyonite-200-P	25
Humicola lanuginosa (Lipolase 100T)	32	Acrylic macroporous resin	20
Penicillium chrysogenum	10	Celite	14

[a] Enzymatic activity determined in the hydrolysis of tributyrin. 1 μkat (the amount of enzyme that liberates 1 μmol of fatty acid per second) is equal to 60 U (enzyme units).
[b] Conversion in 24 h determined by HPLC.

previous experience in the acylation of sucrose with trichloroethyl esters demonstrated the substantial improvement of the transesterification rate by immobilisation of the enzyme on Celite.[12,13] With all the enzymes assayed, regioselectivity at the 6′-OH was observed.

The length of the fatty acid donor was varied between 8 and 18 carbons. Table 2 summarises the conversions and yields obtained using different vinyl esters. Except for vinyl stearate, the longer the carbon chain, the higher the conversion.

The effect of the activation of the acyl donor was also studied. Using ethyl laurate, the conversion is one order of magnitude lower than that obtained with the vinyl ester. However, in a medium containing 5% DMSO, the conversion in 48 h was nearly 25%, using 0.06 M maltose and 0.3 M ethyl laurate as starting concentrations.

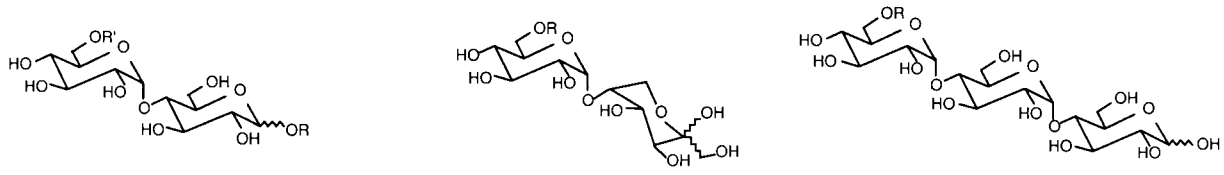
The α- and β-dodecyl glycosides (**2** and **3**, respectively) of maltose were also used as substrates. The reactions were performed in *tert*-amyl alcohol due to the higher solubility of the maltosides compared with maltose. In fact, the acylation using the lipase from *H. lanuginosa*/Celite proceeded extraordinarily fast: quantitative conversion of dodecyl maltosides to monolaurate was achieved in only 1 h using a 10-fold molar excess of acylating agent. The monoester was isolated, and ^{1}H NMR analysis showed that with both α- and β-D-maltosides the product was the 6′-*O*-monoester (Scheme 2). This selectivity is similar to that described in the acylation of the same substrates with vinyl acetate in *tert*-amyl alcohol using the lipase from *C. antarctica*.[10]

Table 3 summarises the yields obtained with the different acyl donors. In all cases, the conversion to 6′-monoesters of maltosides was higher than 85% in 3 h, without the formation of higher esters. Leucrose (**4**), an isomer of sucrose with α(1→5)-glucosyl-fructose link, and the trisaccharide maltotriose (**5**) were also tested under similar conditions. The acylation of leucrose (**4**) gave rise to three monoesters, although 6-*O*-lauroyl-leucrose (Scheme 2) represented 92% of the total. In addition, 6,1′-di-*O*-lauroyl-leucrose was formed (8% conversion in 8 h). As in the case of maltose, when studying the acylation of maltotriose (**5**), the longer the fatty acid, the higher the conversion. The hydroxyl 6″-OH in the non-reducing end was selectively acylated (Scheme 2). With this trisaccharide, the yield of monoesters could be optimised by increasing the biocatalyst concentration to 100 mg/mL. Although the use of 5% DMSO gives rise to a notable acceleration of the process (about 3.5-fold compared with 20% DMSO), the presence of 10% diesters makes these conditions less convenient to get the monoester.

Table 2. Acylation of maltose in mixtures *tert*-amyl alcohol/DMSO catalyzed by *H. lanuginosa* lipase/Celite. Conditions: 0.12 M maltose, 0.3 M acyl donor, 40°C. The same weight of molecular sieves (3 Å) as biocatalyst was used

Acyl donor	Percentage of DMSO	Amount of biocatalyst (mg/mL)	Conversion (%)[a]		Yield (%)[b]
			Monoester	Diester	
Vinyl laurate	0	25	38	6	25
Vinyl laurate	0	100	50	15	38
Vinyl laurate	5	25	72	13	64
Vinyl laurate[c]	5	50	78	22	65
Vinyl laurate	10	25	64	10	60
Vinyl laurate	20	25	47	<1	45
Vinyl laurate	20	100	75	<1	70
Vinyl laurate[c]	20	100	84	<1	80
Ethyl laurate	20	100	8.4	<1	–
Vinyl caprylate	20	100	70	<1	60
Vinyl myristate	20	100	77	<1	72
Vinyl palmitate	20	100	82	<1	76
Vinyl stearate	20	100	65	<1	55

[a] Conversion in 24 h determined by HPLC.
[b] Referred to the weight of isolated monoester.
[c] Using 0.06 M maltose, 0.3 M acyl donor and 10 h reaction.



2, 3 : R = $(CH_2)_{11}CH_3$; R' = H (*n*-dodecyl maltoside) **4 :** R = H (leucrose) **5 :** R = H (maltotriose)

11, 12, 13, 14, 15 : R = $(CH_2)_{11}CH_3$; R' = $CO(CH_2)_nCH_3$ (n = 10-16) **16 :** R = $CO(CH_2)_{10}CH_3$ **17, 18, 19, 20 :** R = $CO(CH_2)_nCH_3$ (n = 10-16)

Scheme 2.

Table 3. Acylation of di- and trisaccharides with vinyl fatty acid esters in *tert*-amyl alcohol or in mixtures *tert*-amyl alcohol/DMSO catalysed by *H. lanuginosa* lipase/Celite. Conditions: 0.03 M *n*-dodecyl maltosides or 0.06 M leucrose and maltotriose, 0.3 M acyl donor, 50 mg/mL biocatalyst, 50 mg/mL molecular sieves (3 Å), 40°C

Carbohydrate	Percentage of DMSO	Acyl donor	Reaction time (h)	Conversion (%)[a]		Yield (%)[b]
				Monoester	Diester	
2	0	Vinyl laurate	1	98	<1	90
3	0	Vinyl laurate	3	95	<1	86
3	0	Vinyl myristate	3	95	<1	84
3	0	Vinyl palmitate	3	92	<1	78
3	0	Vinyl stearate	3	85	<1	77
4	20	Vinyl laurate	8	82[c]	8	70
5	5	Vinyl laurate	24	88	10	74
5	20	Vinyl laurate	24	25	<1	21
5	20	Vinyl myristate	24	32	<1	26
5	20	Vinyl palmitate	24	33	<1	28
5	20	Vinyl stearate	24	38	<1	27

[a] Determined by HPLC.
[b] Referred to the weight of isolated monoester.
[c] Referred to the total amount of monoesters (92% of 6-ester and 8% of two unidentified isomers).

A study was carried out to compare the reactivity of different disaccharides in these two-solvent mixtures under strictly the same conditions. Similar reactivity was found for sucrose, maltose and leucrose (Fig. 2). However, the reaction is notably low using lactose. The relative reactivity determined in these experiments is similar to those reported in the acylation with ethyl butanoate in *tert*-butyl alcohol;[6] the low conversion obtained with lactose seems to be related to its high crystal energy, thus making its solubility very low.

In summary, the results described above are very promising when compared with similar processes reported in the literature. To our knowledge, this is the first report of a method to acylate enzymatically maltose, leucrose, maltotriose and alkyl maltosides with long fatty acids in one-step, under

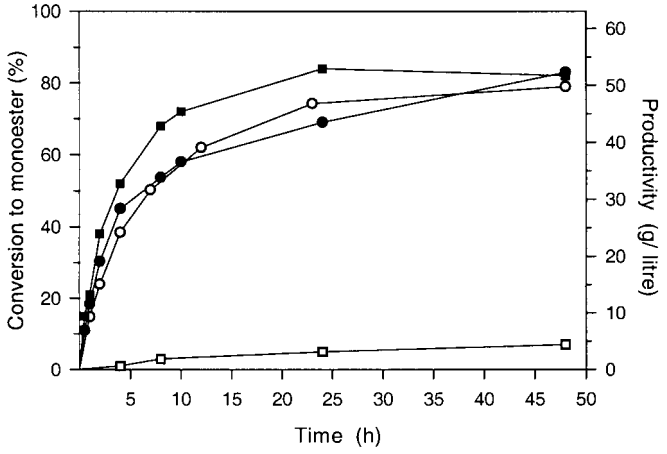


Figure 2. Kinetics of the acylation of various disaccharides with vinyl laurate in *tert*-amyl alcohol/DMSO 4:1 (v/v). Conditions: 0.12 M disaccharide, 0.3 M vinyl laurate, 100 mg/mL biocatalyst, 100 mg/mL molecular sieves (3 Å), 40°C. The following disaccharides were tested: sucrose (●), maltose (○), leucrose (■) and lactose (□). Conversion and productivity determined by HPLC.

mild reaction conditions. In addition, this method offers the advantage that the monoacylated products can be easily isolated from the reaction mixtures by simple precipitation (followed by crystallisation). The monoesters obtained in this way present a purity higher than 98% (determined by HPLC), thus avoiding column chromatography. In conclusion, the reaction we propose is quite simple and, in our opinion, may be of interest for both enzymologists and organic chemists.

Experimental

General methods

Dimethyl sulfoxide was supplied by Merck. 2-Methyl-2-butanol was from Sigma. Solvents were dried over 3 Å molecular sieves for 24 h. All reactions were monitored by thin layer chromatography (TLC) on silica gel 60 plates (Merck) using chloroform/methanol 4:1 as eluent. Spots were detected by immersion of plates into a solution of orcinol/ferric chloride (Bial's reagent) diluted with ethanol (4 volumes), drying and heating at 120°C for 5 min. The progress of the reactions was followed by reverse-phase high-performance liquid chromatography (HPLC) using a system equipped with a Spectra-Physics pump, a Sugelabor Nucleosil 100-C18 column (250×4.6 mm), and a refraction-index detector (Spectra-Physics). Integration was carried out using the Varian Star 4.0 software. For esters of octanoic, lauric and myristic acids, and for *n*-dodecyl maltoside esters, methanol:water 85:15 (v/v) was used as mobile phase, flow rate 1.5 mL/min; for esters of palmitic and stearic acids, methanol:water 95:5 (v/v) was used at 1.1 mL/min. In all cases, the temperature of the column was kept constant at 40°C. Purification on column chromatography was made with Merck Silica gel 60 (0.06–0.2 mm, 70–230 mesh).

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA (300 MHz) spectrometer at 30°C for solutions in CD_3OD. 2D NMR spectra were recorded on a Varian UNITY spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). Chemical shifts are referred to the methanol multiplet, centered at 3.30 ppm for ^{1}H NMR and 49.0 ppm for ^{13}C NMR. The ^{1}H NMR spectra of the corresponding octanoyl, miristoyl, palmitoyl and stearoyl derivatives showed chemical shifts and coupling constants within ±0.005 and ±0.5 Hz, respectively, of those found for the lauroyl compounds. Melting point analyses were carried out using a Gallenkamp apparatus and are uncorrected. Optical density measurements were performed in a Perkin–Elmer 241C polarimeter. High resolution mass spectral analyses were obtained on a VG AutoSpec spectrometer (FAB was performed with Cs as the fast atom using a *m*-nitrobenzylic alcohol matrix and polyethylene glycol as internal calibration standard). Infrared spectra were recorded using a Nicolet 5ZDX FTIR spectrophotometer.

Lipolase 100L and 100T (lipases from *Humicola lanuginosa*) and Novozyme SP-435 (lipase from *Candida antarctica*) were kindly donated by Novo Nordisk. Lipase from *Pseudomonas* sp. (lipase PS) immobilised on Toyonite-200-P was a gift from Amano. All starting carbohydrates were from Sigma. Lipase activity using tributyrin was tested titrimetrically in a pHstat (Radiometer) using 1 M NaOH at 25°C. The reaction mixture (10 mL) contained 200 μL tributyrin (68 mM), 0.1 M NaCl, 0.1 M $CaCl_2$, 1 mM Tris–HCl (pH 7.0) and 3% acetonitrile (v/v).

For immobilisation of lipase from *H. lanuginosa* onto Celite, the pH of commercial Lipolase 100L solution (100 mL) was adjusted to 7.0. The support (8 g) was added and the suspension stirred for 30 min at 4°C. Then, 200 mL of cold acetone (0°C) were slowly added with stirring. The immobilised enzyme was filtered, washed with acetone, dried in vacuo, and stored at 0°C. Celite (diatomaceous earth, 30–80 mesh) was purchased from BDH.

General method for the synthesis of maltose esters

Maltose monohydrate (**1**) (1.08 g, 3 mmol) was dissolved in 5 mL of dimethylsulfoxide. Then, 2-methyl-2-butanol was slowly added to a final volume of 25 mL. The biocatalyst (*H. lanuginosa* lipase immobilised on Celite, 2.5 g) and 3 Å molecular sieves (2.5 g) were then added and the suspension maintained 30 min at 40°C with magnetic stirring. Then, the proper fatty acid vinyl ester (7.5 mmol, dried overnight with molecular sieves) was added. When the conversion of maltose to monoester reached the maximum value (determined by HPLC), the mixture was cooled, filtered and washed with 2-methyl-2-butanol to get a final concentration of 5% DMSO (v/v). The maltose monoesters were precipitated by addition of 2.5–10 volumes of *n*-heptane. The mixture was then filtered, and the solid (white powder) was recrystallised in acetone or ethyl acetate and dried in vacuo. The residual vinyl ester was recovered from the liquid phase by evaporating the *tert*-amyl alcohol and the *n*-heptane, followed by extraction with 2 volumes of hexane or petroleum ether.

6′-*O*-Octanoylmaltose (6). By following the general procedure outlined above, adding vinyl octanoate (1.45 mL, 7.5 mmol). The reaction was maintained for 24 h. Quenching of the reaction mixture as outlined above yielded a white solid (0.84 g, 60%) with a purity by HPLC higher than 98% of 6′-*O*-octanoylmaltose. The solid was submitted to column chromatography using a gradient chloroform: methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **6** (0.80 mg, 57%) as a white solid crystalline powder. Mp 130°C; $[\alpha]_D^{25}=+83.8$ (*c* 5 in methanol); ν_{max}/cm^{-1} (KBr disks): 3430 br (O–H), 1743 (C=O); HRMS (FAB): calcd for $C_{20}H_{36}O_{12}Na$ ($M+Na^+$) 491.212852, found 491.212095.

6′-*O*-Lauroylmaltose (7). By following the general procedure outlined above, adding vinyl laurate (1.95 mL, 7.5 mmol). The reaction was maintained for 24 h. Quenching of the reaction mixture as outlined above yielded a white solid (1.02 g, 70%) with a purity by HPLC higher than 98% of 6′-*O*-lauroylmaltose. The solid was submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **7** (991 mg, 68%) as a white solid crystalline powder. Mp 195°C; $[\alpha]_D^{25}=+81.0$ (*c* 5 in methanol); ν_{max}/cm^{-1} (KBr disks): 3467 br (O–H), 1749 (C=O); ^{1}H NMR (δ, ppm): the spectrum shows that it is a mixture of

α- and β-anomers: 5.12 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′α), 5.11 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′β), 5.10 (d, 1H, $J_{1,2}$=3.8 Hz, H-1α), 4.48 (d, 1H, $J_{1,2}$=3.8 Hz, H-1β), 4.38 (dd, 2H, $J_{5',6'a}$=2.2 Hz; H-6′aα+H-6′aβ), 4.16 (dd, 2H, $J_{5',6'b}$= 5.0 Hz; H-6′bα), 4.15 (dd, 2H, $J_{5',6'b}$=5.0 Hz; H-6′bβ), 3.89 (dd, 2H, $J_{5,\ 6b}$=2.2 Hz; $J_{6a,\ 6b}$=12.2 Hz; H-6aβ), 3.85 (m, 1H, H-5′α), 3.84 (m, 1H, H-5′β), 3.84 (m, 1H, H-5α), 3.82 (m, 2H, H-6aα+H-6bα), 3.60+3.59 (m, 3H, H-3′α+H-3β+H-3′β), 3.46 (dd, 1H, $J_{3,4}$=10.1 Hz; $J_{4,5}$=9.0 Hz, H-4β+1H, H-4α), 3.43 (dd, 2H, $J_{2',3'}$=9.7 Hz, H-2′β+H-2′α), 3.41 (dd, 1H, H-2α), 3.38 (m, 1H, H-5β), 3.25 (m, 1H, H-4′α), 3.24 (dd, 1H, $J_{4',5'}$=10.0 Hz; H-4′α), 3.16 (dd, 1H, $J_{2,3}$=9.4 Hz, H-2β), 2.36 (m, 2H, J=7.5 Hz, –CH_2–CO–), 1.61 (m, 2H, –CH_2–CH_2–CO–), 1.29 (m, 16H, –CH_2– chain), 0.89 (t, 3H, J=6.7 Hz, CH_3–); ^{13}C NMR (δ, ppm): 175.5 (C=O), 103.1 (C-1′), 98.2 (C-1β), 93.8 (C-1α), 82.6 (C-4α), 82.2 (C-4β), 77.9 (C-3β), 76.8 (C-5β), 75.8 (C-2β), 75.1+75.0 (C-3′α+3′β), 74.7 (C-3α), 74.3+74.1 (C-2′α+2′β), 73.5 (C-2α), 72.3 (C-5′), 71.8 (C-5α), 71.7 (C-4′), 65.0 (C6′), 62.4 (C-6α+β), 34.9 (–CH_2–CO–), 33.0, 30.7, 30.6, 30.4, 30.2 (–CH_2–lauroyl backbone), 26.0 (–CH_2–CH_2–CO–), 23.7 (–CH_2–CH_3), 14.4 (CH_3–); HRMS (FAB): calcd for $C_{24}H_{44}O_{12}Na$ (M+Na^+) 547.273047, found 547.274671.

6′-*O*-Myristoylmaltose (8). By following the general procedure outlined above, adding vinyl myristate (2.2 mL, 7.5 mmol). The reaction was maintained for 24 h. Quenching of the reaction mixture as outlined above yielded a white solid (1.2 g, 72%) with a purity by HPLC higher than 98% of 6′-*O*-myristoylmaltose. The solid was submitted to column chromatography using a gradient chloroform: methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **8** (1.14 g, 69%) as a white solid crystalline powder. Mp 208°C with decomp.; $[\alpha]_D^{25}$= +70.0 (*c* 5 in methanol); ν_{max}/cm^{-1} (KBr disks): 3431 br (O–H), 1742 (C=O); HRMS (FAB): calcd for $C_{26}H_{48}O_{12}Na$ (M+Na^+) 575.304347, found 575.305522.

6′-*O*-Palmitoylmaltose (9). By following the general procedure outlined above, adding vinyl palmitate (2.38 mL, 7.5 mmol). The reaction was maintained for 24 h. Quenching of the reaction mixture as outlined above yielded a white solid (1.32 g, 76%) with a purity by HPLC higher than 98% of 6′-*O*-palmitoylmaltose. The solid was submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **9** (1.2 g, 72%) as a white solid crystalline powder. Mp 209°C with decomp.; ν_{max}/cm^{-1} (KBr disks): 3466 br (O–H), 1747 (C=O); HRMS (FAB): calcd for $C_{28}H_{52}O_{12}Na$ (M+Na^+) 603.335647, found 603.335904.

6′-*O*-Stearoylmaltose (10). By following the general procedure outlined above, adding vinyl stearate (2.35 g, 7.5 mmol). The reaction was maintained for 24 h. Quenching of the reaction mixture as outlined above yielded a white solid (1.0 g, 55%) with a purity by HPLC higher than 98% of 6′-*O*-stearoylmaltose. The solid was submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **10** (0.94 g, 52%) as a white solid crystalline powder. Mp 210°C with decomp.; $[\alpha]_D^{25}$= +62.6 (*c* 5 in methanol),; ν_{max}/cm^{-1} (KBr disks): 3459 br (O–H), 1747 (C=O); HRMS (FAB): calcd for $C_{30}H_{56}O_{12}Na$ (M+Na^+) 631.366948, found 631.365948.

General method for the synthesis of *n*-dodecylmaltoside esters

n-Dodecyl α-D-maltoside (**2**) or *n*-dodecyl β-D-maltoside (**3**) (153 mg, 0.3 mmol) were dissolved in 10 mL of 2-methyl-2-butanol. The biocatalyst (0.5 g) and 3 Å molecular sieves (0.5 g) were then added and the suspension maintained 30 min at 40°C with magnetic stirring. Then, fatty acid vinyl ester (3 mmol, dried overnight with 3 Å molecular sieves) was added. For the purification of *n*-dodecyl α- and β-D-maltoside fatty acid esters, the reaction mixture was cooled, filtered and washed with *tert*-amyl alcohol or acetone. The alcohol was evaporated under reduced pressure, and the residue was redissolved with *n*-heptane. The monoester was precipitated selectively by cooling at 4°C after filtration of the unreacted maltoside. The mixture was filtered, and the solid washed with *n*-heptane and dried in vacuo. The residual vinyl ester was recovered from the liquid phase by evaporating the *n*-heptane.

6′-*O*-Lauroyl α-D-dodecylmaltoside (11). By following the general procedure outlined above, adding vinyl laurate (0.78 mL, 3 mmol). The reaction was maintained for 1 h. Quenching of the reaction mixture as outlined above yielded a white solid (187 mg, 90%) with a purity by HPLC higher than 98% of compound **11**. The solid was submitted to column chromatography using a gradient chloroform: methanol 15:1 to 8:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **11** (184 mg, 88%) as a white solid crystalline powder. Mp 125°C; $[\alpha]_D^{25}$=+83.5 (*c* 5 in acetone); ν_{max}/cm^{-1} (KBr disks): 3440 br (O–H), 1741 (C=O); ^{1}H NMR (δ, ppm): 5.09 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′), 4.76 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 4.40 (dd, 1H, $J_{5',6'a}$=2.0 Hz, $J_{6'a,6'b}$=11.9 Hz, H-6′a), 4.14 (dd, 1H, $J_{5',6'b}$=6.6 Hz, $J_{6'a,6'b}$=11.9 Hz, H-6′b), 3.89 (m, 1H, H-5′), 3.83 (dd, H-3), 3.82 (m, 2H, H-6a+H-6b), 3.71+3.44 (m, 2H, –CH_2–O–), 3.62 (dd, 1H, $J_{2',3'}$= 9.0 Hz; $J_{3',4'}$=9.6 Hz, H-3′), 3.46 (1H, H-4), 3.44 (dd, 1H, $J_{1',2'}$=3.8 Hz, $J_{2',3'}$=9.0 Hz, H-2′), 3.43 (dd, 1H, $J_{1,2}$= 3.8 Hz, $J_{2,3}$=9.7 Hz, H-2), 3.62 (m, 1H, H-5), 3.23 (dd, 1H, $J_{3',4'}$=9.6 Hz; $J_{4',5'}$=10.0 Hz, H-4′), 2.36 (t, 2H, J= 7.4 Hz, –CH_2–CO–), 1.61 (m, 2H, –CH_2–CH_2–CO–), 1.29 (m, H, –CH_2– chain), 0.89 (t, 6H, J=6.9 Hz, CH_3–); ^{13}C NMR (δ, ppm): 175.5 (C=O), 103.2 (C-1′), 100.0 (C-1), 82.6 (C-4), 75.1 (C-3′), 74.9 (C-5′), 74.3 (C-2′), 73.2 (C-2), 72.4 (C-3+C-5), 71.7 (C-4′), 69.4 (–CH_2–O–, dodecyl), 65.1 (C-6′), 62.3 (C-6), 35.0 (–CH_2–CO–), 33.1 (–CH_2–CH_2–O–), 30.8, 30.6, 30.5, 30.4, 30.2, 27.3, 26.0, and 23.7 (–CH_2–lauroyl+dodecyl-backbones); HRMS (FAB): calcd for $C_{36}H_{68}O_{12}Na$ (M+Na^+) 715.460848, found 715.461840.

6′-*O*-Lauroyl β-D-dodecylmaltoside (12). By following the general procedure outlined above, adding vinyl laurate (0.78 mL, 3 mmol). The reaction was maintained for 3 h. Quenching of the reaction mixture as outlined above yielded a white solid (179 mg, 86%) with a purity by HPLC higher

than 98% of compound **12**. The solid was submitted to column chromatography using a gradient chloroform: methanol 15:1 to 8:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **12** (175 mg, 84%) as a white solid crystalline powder. Mp 121°C; $[\alpha]_D^{25}$=+27.7 (*c* 5 in acetone); ν_{max}/cm^{-1} (KBr disks): 3421 br (O–H), 1739 (C=O); ^{1}H NMR (δ, ppm): 5.11 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′), 4.38 (dd, 1H, $J_{5',6'a}$=2.0 Hz, $J_{6'a,6'b}$=11.8 Hz, H-6′a), 4.26 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 4.14 (dd, 1H, $J_{5',6'b}$=6.6 Hz, $J_{6'a,6'b}$=11.8 Hz, H-6′b), 3.91 (dd, 1H, $J_{5,6a}$=1.9 Hz, $J_{6a,6b}$=12.0 Hz, H-6a), 3.88+3.53 (m, 2H, –CH_2–O–), 3.86 (m, 1H, H-5′), 3.78 (dd, 1H, $J_{5,\ 6b}$= 5.0 Hz, $J_{6a,6b}$=12.0 Hz, H-6b), 3.60 (t, 1H, $J_{2',3'}$=9.8 Hz; $J_{3',4'}$=8.7 Hz, H-3′), 3.60 (t, 1H, $J_{2,3}$=9.3 Hz; $J_{3,\ 4}$= 9.0 Hz, H-3), 3.47 (dd, 1H, H-H), 3.43 (dd, 1H, $J_{1',2'}$=3.8 Hz, $J_{2',3'}$=9.8 Hz, H-2′), 3.35 (m, 1H, H-5), 3.24 (dd, 1H, $J_{3',4'}$=8.7 Hz; $J_{4',5'}$=10.1 Hz, H-4′), 3.22 (dd, 1H, $J_{1,2}$=3.8 Hz, $J_{2,3}$=9.3 Hz, H-2), 2.36 (m, 2H, *J*=7.5 Hz, –CH_2–CO–), 1.61 (m, 2H, –CH_2–CH_2–CO–), 1.29 (m, H, –CH_2– chain), 0.89 (t, 6H, *J*=7.0 Hz, CH_3–); ^{13}C NMR (δ, ppm): 175.5 (C=O), 104.3 (C-1), 103.0 (C-1′), 82.0 (C-4), 77.8 (C-3), 76.7 (C-5), 75.0 (C-3′), 74.7 (C-2), 74.1 (C-2′), 72.4 (C-5′), 71.7 (C-4′), 71.0 (–CH_2–O–), 65.0 (C-6′), 62.4 (C-6), 34.9 (–CH_2–CO–), 33.1, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 27.1, 26.0, and 23.7 (–CH_2–lauroyl+‴ dodecyl backbones), 14.4 (CH_3–lauroyl+dodecyl moieties); HRMS (FAB): calcd for $C_{36}H_{68}O_{12}Na$ (M+Na^+) 715.460848, found 715.461047.

6′-*O*-Myristoyl β-D-dodecylmaltoside (13). By following the general procedure outlined above, adding vinyl myristate (0.88 mL, 3 mmol). The reaction was maintained for 3 h. Quenching of the reaction mixture as outlined above yielded a white solid (182 mg, 84%) with a purity by HPLC higher than 98% of compound **13**. The solid was submitted to column chromatography using a gradient chloroform: methanol 25:1 to 15:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **13** (178 mg, 82%) as a white solid crystalline powder. Mp 130°C; $[\alpha]_D^{25}$=+24.0 (*c* 5 in acetone); ν_{max}/cm^{-1} (KBr disks): 3425 br (O–H), 1739 (C=O); HRMS (FAB): calcd for $C_{38}H_{72}O_{12}Na$ (M+Na^+) 743.492148, found 743.492923.

6′-*O*-Palmitoyl β-D-dodecylmaltoside (14). By following the general procedure outlined above, adding vinyl palmitate (0.95 mL, 3 mmol). The reaction was maintained for 3 h. Quenching of the reaction mixture as outlined above yielded a white solid (175 mg, 78%) with a purity by HPLC higher than 98% of compound **14**. The solid was submitted to column chromatography using a gradient chloroform: methanol 35:1 to 25:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **14** (168 mg, 75%) as a white solid crystalline powder. Mp 120°C; $[\alpha]_D^{25}$= +25.2 (*c* 5 in acetone); ν_{max}/cm^{-1} (KBr disks): 3420 br (O–H), 1739 (C=O); HRMS (FAB): calcd for $C_{40}H_{76}O_{12}Na$ (M+Na^+) 771.523448, found 771.525069.

6′-*O*-Stearoyl β-D-dodecylmaltoside (15). By following the general procedure outlined above, adding vinyl stearate (0.94 g, 3 mmol). The reaction was maintained for 3 h. Quenching of the reaction mixture as outlined above yielded a white solid (233 mg, 77%) with a purity by HPLC higher than 98% of compound **15**. The solid was submitted to column chromatography using a gradient chloroform: methanol 45:1 to 35:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **15** (221 mg, 73%) as a white solid crystalline powder. Mp 115°C; $[\alpha]_D^{25}$=+23.6 (*c* 5 in acetone); ν_{max}/cm^{-1} (KBr disks): 3431 br (O–H), 1739 (C=O); HRMS (FAB): calcd for $C_{42}H_{80}O_{12}Na$ (M+Na^+) 799.554749, found 799.553055.

6-*O*-Lauroyl-leucrose (16). Leucrose (514 mg, 1.5 mmol) was dissolved in 5 mL of dimethylsulfoxide. Then, 2-methyl-2-butanol was slowly added to a final volume of 25 mL. The biocatalyst (*H. lanuginosa* lipase immobilised on Celite, 1.25 g) and 3 Å molecular sieves (1.25 g) were then added and the suspension maintained 30 min at 40°C with magnetic stirring. Then, vinyl laurate (7.5 mmol, dried overnight with molecular sieves) was added. After 8 h, the mixture was cooled, filtered and washed with *tert*-amyl alcohol to get a final concentration of 5% DMSO (v/v). The leucrose monoesters were partially precipitated by addition of 2.5–10 volumes of *n*-heptane. The mixture was then filtered, and the solid (white powder) was recrystallised in acetone, filtered and dried in vacuo. The residual vinyl ester was recovered from the liquid phase by evaporating the *tert*-amyl alcohol and *n*-heptane, and further extraction with 2 volumes of hexane or petroleum ether. The remaining dimethylsulfoxide (containing the rest of reaction compounds) was treated with water (10 mL). The monoesters were extracted with cyclohexane:butanol 1:1 (v/v) (1×40 mL). The solvent was evaporated, the residue was mixed with the solid obtained by precipitation, and the mixture submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded 6-*O*-lauroyl-leucrose (551 mg, 70%) as a white solid crystalline powder. Mp 183°C; ν_{max}/cm^{-1} (KBr disks): 3431 br (O–H), 1722 (C=O); ^{1}H NMR (δ, ppm): 4.93 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 4.44 (dd, 1H, $J_{5,6a}$=1.6 Hz, $J_{6a,6b}$=12.5 Hz, H-6a), 4.12 (m, 1H, H-6b), 4.08 (m, 1H, $J_{5,6a}$=1.6 Hz, $J_{5,\ 6b}$= 6.2 Hz, H-5), 3.95 (b.s., 2H, H-6′a+H-6′b), 3.82 (b.s., 3H, H-3′+H-4′+H-5′), 3.67 (t, 1H, $J_{3,4}$=8.9 Hz, H-3), 3.65 (d, 1H, $J_{1'a,\ 1'b}$=12.2 Hz, H-1′a), 3.48 (d, 1H, H-1′b), 3.37 (dd, 1H, $J_{2,3}$=9.8 Hz, H-2), 3.26 (dd, 1H, $J_{4,5}$=9.8 Hz, H-4), 2.33 (t, 2H, *J*=7.4 Hz, –CH_2–CO–), 1.60 (m, 2H, –CH_2–CH_2–CO–), 1.29 (m, 16H, –CH_2– chain), 0.89 (t, 3H, *J*=7.1 Hz, CH_3–); ^{13}C NMR (δ, ppm): 175.7 (C=O), 102.8 (C-1), 99.1 (C-2′), 81.9 (C-5′), 75.0 (C-3), 74.1 (C-2), 72.1 (C-4′), 72.0 (C-4), 71.4 (C-5), 70.1 (C-3′), 65.9 (C-1′), 65.0 (C-6), 63.4 (C-6′), 35.1 (–CH_2–CO–), 33.0, 30.7, 30.6, 30.4, 30.2, 26.0, and 23.7 (–CH_2–lauroyl backbone), 14.4 (CH_3–lauroyl); HRMS (FAB): calcd for $C_{24}H_{44}O_{12}Na$ (M+Na^+) 547.273047, found 547.271354. After purification, 6,1′-di-*O*-lauroyl-leucrose is obtained as a secondary product (6.3 mg, 6%). ^{1}H NMR (δ, ppm): 4.91 (d, 1H, $J_{1,2}$= 3.9 Hz, H-1), 4.41 (dd, 1H, $J_{5,6a}$=1.5 Hz, $J_{6a,6b}$=11.5 Hz, H-6a), ca. 4.12 (m, 1H, H-5′), ca. 4.11 (dd, 1H, H-6b), *ca.*4.10 (m, 1H, H-5+2H, H-6′a+H-6′b), 4.02 (dd, 1H, $J_{3',4'}$=10.4 Hz, $J_{4',5'}$=2.7 Hz, H-4′), 3.93 (d, 1H, H-3′), 3.84 (d, 1H, $J_{1'a,\ 1'b}$=12.2 Hz, H-1′a), 3.81 (d, 1H, H-1′b), 3.66 (dd, 1H, $J_{2,3}$=9.8 Hz; $J_{3,4}$=8.9 Hz, H-3), 3.37 (dd, 1H, H-2), 3.27 (dd, 1H, $J_{4,5}$=9.4 Hz, H-4), 2.32 (t, 2H, *J*=6.6 Hz, –CH_2–CO–), 2.31 (t, 2H, *J*=6.6 Hz, –CH_2–CO–), 1.59 (m, 4H, 2×–CH_2–CH_2–CO–), 1.28 (m, 32H, –CH_2– backbone), 0.89 (t, 6H, *J*=6.6 Hz, 2×–CH_3).

General method for the synthesis of maltotriose esters

Maltotriose (303 mg, 0.6 mmol) was dissolved in 2 mL of dimethylsulfoxide. Then, 2-methyl-2-butanol was slowly added to a final volume of 10 mL. The biocatalyst (*H. lanuginosa* lipase immobilised on Celite, 0.5 g) and 3 Å molecular sieves (0.5 g) were then added and the suspension maintained 30 min at 40°C with magnetic stirring. Then, fatty acid vinyl ester (3 mmol, dried overnight with molecular sieves) was added. After 24 h, the mixture was cooled, filtered and washed with *tert*-amyl alcohol to get a final concentration of 5% DMSO (v/v). The maltotriose monoesters were precipitated by addition of 2.5–10 volumes of *n*-heptane. The mixture was filtered, and the solid (white powder) was recrystallised in acetone and dried in vacuo. The residual vinyl ester was recovered from the liquid phase by evaporating the *tert*-amyl alcohol and *n*-heptane, followed by extraction with 2 volumes of hexane or petroleum ether.

6″-*O*-Lauroylmaltotriose (17). By following the general procedure outlined above, adding vinyl laurate (0.78 mL, 3 mmol). Quenching of the reaction mixture as outlined above yielded a white solid (87 mg, 21%) with a purity by HPLC higher than 98% of 6″-*O*-lauroylmaltotriose. The solid was submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **17** (84 mg, 20%) as a white solid crystalline powder. Mp 185°C; ν_{max}/cm^{-1} (KBr disks): 3407 br (O–H), 1724 (C═O); ^{1}H NMR (δ, ppm): the spectrum shows that it is a mixture of α- and β-anomers: 5.15 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′α), 5.12 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′β), 5.11 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′), 5.09 (d, 1H, $J_{1,2}$=3.8 Hz, H-1α), 4.48 (d, 1H, $J_{1,2}$=3.8 Hz, H-1β), 4.38 (dd, 2H, $J_{5',6'a}$=2.1Hz; $J_{6'a,6'b}$=11.9 Hz; H-6′a), 4.16 (dd, 2H, $J_{5',6'b}$=6.4 Hz; H-6′b), 3.91 (m, 1H, H-3α), 3.90 (m, 1H, H-6aβ), 3.88 (dd, 1H, H-6′a), 3.86 (m, 1H, H-5′), 3.85 (m, 1H, H-5′α), 3.84 (m, 1H, H-5′β), 3.84 (m, 1H, H-5α), 3.82 (m, 2H, H-6aα+H-6bα), 3.78 (m, 2H, H-6a+H-6b), 3.76 (m, 3H, H-6bβ+H-3′+H-6′b), 3.60+3.59 (m, 3H, H-3′α+H-3β +H-3′), 3.52 (dd, 1H. $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.9 Hz, H-4α), 3.48 (dd, 1H, H-2′), 3.44 (m, 1H, H-4′), 3.43 (dd, 2H, $J_{2',3'}$=9.7 Hz, H-2′), 3.40 (dd, 1H, $J_{2,3}$=9.7 Hz, H-2α), 3.38 (m, 2H, H-4β+H-5β), 3.25 (m, 1H, $J_{4',5'}$=9.9 Hz, H-4′), 3.25 (dd, 1H, $J_{4',5'}$=9.9 Hz; H-4′α), 3.16 (dd, 1H, $J_{2,3}$=9.4 Hz, H-2β), 2.36 (m, 2H, J=7.5 Hz, –C*H*$_2$–CO–), 1.61 (m, 2H, –C*H*$_2$–CH$_2$–CO–), 1.29 (m, 16H, –CH$_2$–chain), 0.89 (t, 3H, J=6.7 Hz, CH$_3$–); ^{13}C NMR (δ, ppm): 175.5 (C═O), 103.4 (C-1′), 102.7 (C-1′), 98.2 (C-1β), 93.9 (C-1α), 82.1 (C-4α), 81.9 (C-4β), 81.5 (C-4′), 77.9 (C-3β), 76.7 (C-5β), 75.9 (C-2β), 75.0 (C-3′α+3′β+C-3′), 74.6 (C-3α), 74.3+74.0 (C-2′+2′), 73.4 (C-2α), 72.3 (C-5′ +C-5′), 71.8 (C-5α), 71.7 (C-5+C-4′), 64.9 (C6′), 62.5 (C-6α+β), 62.2 (C-6′), 34.9 (–*C*H$_2$–CO–), 33.1, 30.8, 30.7, 30.4, 30.2 (–*C*H$_2$-backbone), 26.0 (–*C*H$_2$–CH$_2$–CO–), 23.7 (–*C*H$_2$–CH$_3$), 14.4 (CH$_3$–); HRMS (FAB): calcd for $C_{30}H_{54}O_{17}Na$ (M+Na$^+$) 709.325871, found 709.325662.

6″-*O*-Myristoylmaltotriose (18). By following the general procedure outlined above, adding vinyl myristate (0.88 mL, 3 mmol). Quenching of the reaction mixture as outlined above yielded a white solid (111 mg, 26%) with a purity by HPLC higher than 98% of 6″-*O*-myristoylmaltotriose. The solid was submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **18** (105 mg, 24%) as a white solid crystalline powder. Mp 208°C with decomp.; $[\alpha]_D^{25}$=+88.1 (*c* 5 in methanol); ν_{max}/cm^{-1} (KBr disks): 3406 br (O–H), 1739 (C═O); HRMS (FAB): calcd for $C_{32}H_{58}O_{17}Na$ (M+Na$^+$) 737.357171, found 737.359364.

6″-*O*-Palmitoylmaltotriose (19). By following the general procedure outlined above, adding vinyl palmitate (0.96 mL, 3 mmol). Quenching of the reaction mixture as outlined above yielded a white solid (125 mg, 28%) with a purity by HPLC higher than 98% of 6″-*O*-palmitoylmaltotriose. The solid was submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **19** (116 mg, 26%) as a white solid crystalline powder. Mp 202°C with decomp.; $[\alpha]_D^{25}$=+73.9 (*c* 5 in methanol); ν_{max}/cm^{-1} (KBr disks): 3419 br (O–H), 1739 (C═O); HRMS (FAB): calcd for $C_{34}H_{62}O_{17}Na$ (M+Na$^+$) 765.388471, found 765.387054.

6″-*O*-Stearoylmaltotriose (20). By following the general procedure outlined above, adding vinyl stearate (932 mg, 3 mmol). Quenching of the reaction mixture as outlined above yielded a white solid (125 mg, 27%) with a purity by HPLC higher than 98% of 6″-*O*-stearoylmaltotriose. The solid was submitted to column chromatography using a gradient chloroform:methanol 10:1 to 4:1 v/v. Concentration of the pure fractions in vacuo afforded monoester **20** (111 mg, 24%) as a white solid crystalline powder. Mp 212°C with decomp.; $[\alpha]_D^{25}$=+75.6 (*c* 5 in methanol); ν_{max}/cm^{-1} (KBr disks): 3430 br (O–H), 1740 (C═O); HRMS (FAB): calcd for $C_{36}H_{66}O_{17}Na$ (M+Na$^+$) 793.419771, found 793.417560.

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