Fully enzymatic esterification/transesterification sequence for the preparation of symmetrical and unsymmetrical trehalose diacyl conjugates

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Abstract:

Monoacyl and diacyl trehalose were synthesized in two steps from trehalose and carboxylic acids. The carboxylic acids were converted first into the corresponding 2,2,2-trifluoroethyl esters through a biocatalyzed esterification. The acyl donor was then transferred to the disaccharide through biocatalyzed transesterification. Thanks to microwave reaction conditions the transesterification proceeded selectively to the monoacyl trehalose or to the diacyl counterparts depending on the sole amount of acyl donor. These reaction conditions applied also for the preparation of unsymmetrical diacyl trehalose in a fully enzymatic sequence.

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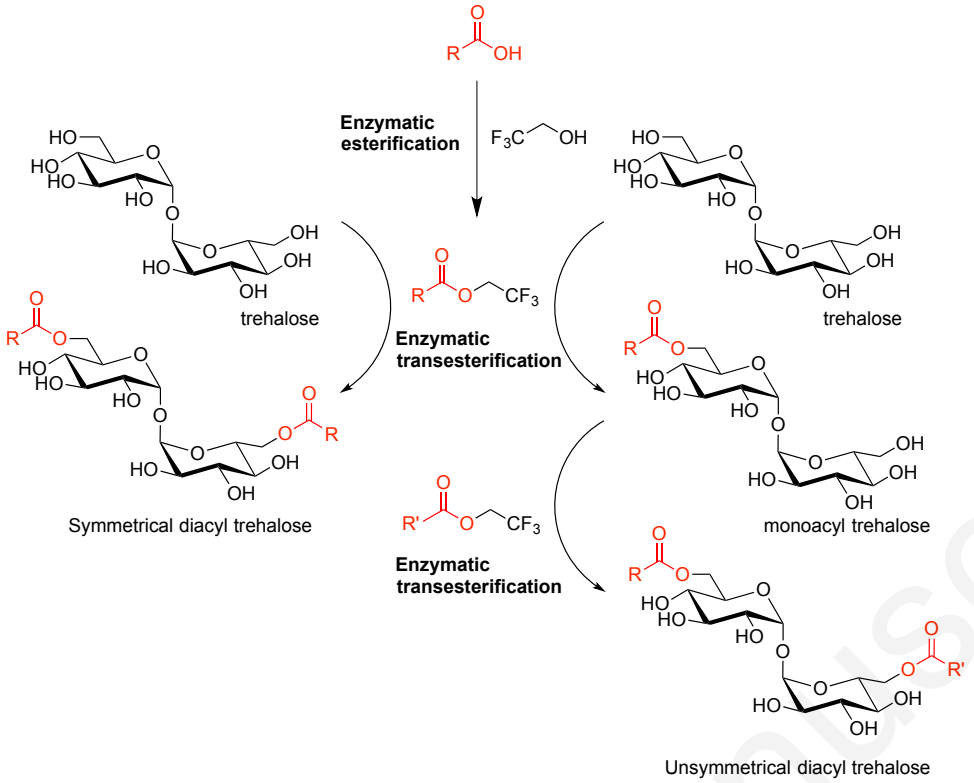
1. Introduction

Biocatalysis is nowadays part of the organic chemist toolbox for the design of synthetic schemes. It offers several advantages such as mild reaction conditions, selectivity, reduction of protection/deprotection steps and limited uses of toxic reagents or solvents. Indeed, the development of efficient biocatalysis processes dramatically enhances organic synthetic schemes. Among enzymatic processes, lipase mediated esterification is one of the most studied with the aim of reducing environmental impact of such chemical transformation that classically required acyl chlorides or toxic coupling agents.[1] Lipases are used for numerous purposes such as kinetic resolution of asymmetric compounds,[2-6] hydrolysis[7] or alcoholysis[8, 9] of triglycerides, modification of saccharides[10-18] and polysaccharides[19]. The last two examples are of importance as they lead to various types of biologically active glycolipids. Indeed lipases are usually selective for the primary alcohol of hexosides which is useful for a selective protection with an acyl group[15-17] or for the selective functionalization of the C6 position with various acyl chains[10-12]. However, enzymatic esterifications often lead to moderate yields due to the equilibrium with the hydrolysis pathway. To circumvent this drawback, one would prefer transesterification of vinyl or alkyl esters in low water content media. This transesterification is much more efficient displacing the equilibrium towards the formation of the desired ester, the release of aldehydes or less nucleophilic alcohols being the driving force of the reaction. Although the conversion yield is higher, this strategy definitely introduces a chemical step for the preparation of the required starting vinyl or alkyl esters.

We describe hereafter a fully enzymatic esterification/transesterification sequence applied to the preparation of glycolipids. It includes the preparation of the acylating agent and its reaction on an alcohol function both with the same enzymatic strategy. We developed this strategy on trehalose which is an important disaccharide involved for instance in the cell wall of mycobacteria.[20, 21] Indeed, this sugar has two primary hydroxyl groups and the corresponding

mono and diesters are active compounds against this microorganism.[21-24] Besides, trehalose lipids have gained increased interest as biosurfactants for their potential applications in bioremediation technologies and oil/petroleum industry, and in biomedical/healthcare industry as antimicrobial and therapeutic agents.[25] Until now the preparation of such glycolipids requires multi-step synthesis[26-29] and lipase mediated esterifications are not efficient enough, conducing only to monoester derivatives[30] or to diesters compounds but in a long reaction time[31]. The synthetic scheme described here, includes two consecutive lipase-mediated steps starting from fatty acid and trehalose. A 2,2,2-trifluoroethyl ester would be prepared by an enzymatic esterification and this ester would be involved in an enzymatic transesterification step leading to the final glycolipid (Scheme 1). Trifluoroethyl esters have several advantages in terms of reactivity (good leaving group) and of access from trifluoroethanol. The challenge is i) to develop an efficient enzymatic access to 2,2,2-trifluoroethyl esters, and ii) to control the transesterification process towards the selective preparation of diacyl and/or monoacyl trehalose opening the access to unsymmetrical derivatives.



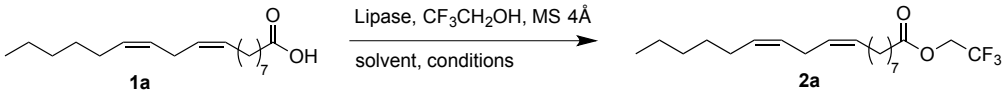
Scheme 1. Fully enzymatic strategy towards the selective esterification of trehalose.

2. Results and discussion

Lipase mediated transesterifications are dramatically improved when the starting ester is derived from vinyl or 2,2,2-trifluoroethyl moieties for instance. Among them we have chosen to work on 2,2,2-trifluoroethyl esters due to the availability 2,2,2-trifluoroethanol and their common use as acyl donor in enzymatic[13, 32-34] and non-enzymatic[35] transesterification reactions. These esters are classically prepared by reaction of 2,2,2-trifluoroethanol on activated carboxylic acid derivative such as acyl chloride or anhydride, or using Steglich esterification conditions (DCC/DMAP).[13, 36-39] No biocatalyzed methodology has been described so far. The development of a lipase mediated access to 2,2,2-trifluoroethyl esters would therefore result in a fully enzymatic two-step esterification process. We first optimized reaction conditions on linoleic acid by screening the type of lipase, solvent, type of activation (thermal or microwave) and amount of 2,2,2-trifluoroethanol (Table 1). To limit as much as possible the hydrolysis of

the newly formed ester, all reactions were carried out in the presence of molecular sieves (MS 4 Å) that trapped the released water. The results of this optimization are gathered in Table 1. Four lipases were selected: immobilized *Candida antartica* Lipases A and B (CAL-A (Imb) and CAL-B (Imb)), *Candida rugosa* Lipase (CRL) and porcine pancreas lipase (PPL). Under thermal activation (Δ, 46 °C), among these four lipases, CAL-B afforded the best yields after 12h of reaction in *t*-BuOH (74%) and in the presence of 5 equivalents of CF_3CH_2OH (Entries 1-4). Thus, we continued the study with CAL-B (Imb). Changing the solvent to toluene led to a very poor yield (<5%, entry 5) while THF and *n*-hexane provided lower yields (respectively 65% and 52%, entries 6-7). Indeed, *t*-BuOH represents a good compromise in terms of hydrophilicity and hydrophobicity. The use of 10 equivalents of CF_3CH_2OH instead of 5 gave a similar yield (72%), however reduction to 2 equivalents found the limit of the reaction and led to a poorer yield of 35% (Entries 8-9). Microwave activation is known to accelerate lipase mediated hydrolysis, esterification or transesterification. [2, 4, 7, 40-45] We thus switched from thermal to microwave activation (180 W) but keeping the same reaction temperature (46 °C). We were pleased to observe complete reactions after 1h instead of 12 h and in addition to increased yields (Entries 10-12). Indeed, whatever the solvent CAL-B (Imb) mediated esterifications were more efficient (Entries 10-12). As for the thermal activation, a maximum yield was reached in *t*-BuOH (82%, entry 12). It is noteworthy that similar yields were obtained under thermal or microwave conditions, the later impacted more the reaction time than the reaction yield. Again, attempts to reduce to 2 equivalents of CF_3CH_2OH gave a lower yield (42%, entry 13), so that 5 equivalents were optimal for this reaction.

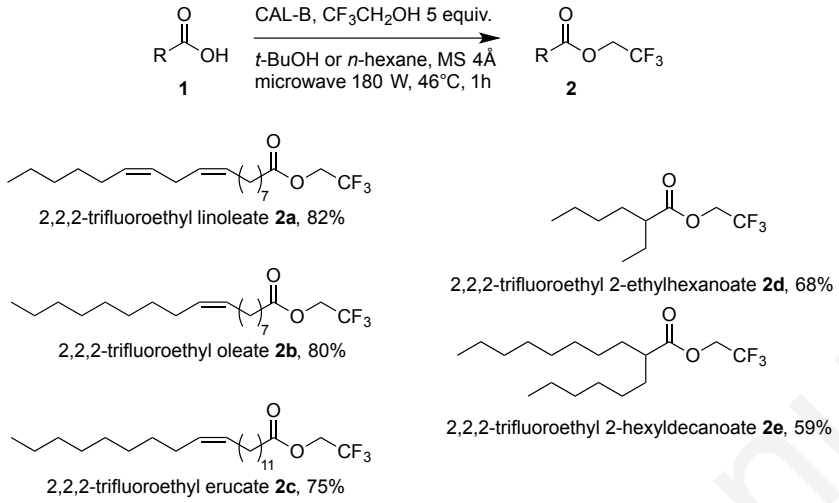
Table 1. Optimization of the enzymatic esterification of linoleic acid into 2,2,2-trifluoroethyl linoleate



Entry	Lipase	CF_3CH_2OH (equiv.)	Solvent	Conditions	Yield (%)
1	CAL-A	5	*t*-BuOH	12 h, Δ, 46 °C	10
2	PPL	5	*t*-BuOH	12 h, Δ, 46 °C	20
3	LCR	5	*t*-BuOH	12 h, Δ, 46 °C	10
4	CAL-B	5	*t*-BuOH	12 h, Δ, 46 °C	74
5	CAL-B	5	toluene	12 h, Δ, 46 °C	<5
6	CAL-B	5	THF	12 h, Δ, 46 °C	65
7	CAL-B	5	*n*-hexane	12 h, Δ, 46 °C	52
8	CAL-B	10	*t*-BuOH	12 h, Δ, 46 °C	72
9	CAL-B	2	*t*-BuOH	12 h, Δ, 46 °C	35
10	CAL-B	5	THF	1 h, Mw 180 W, 46 °C	73
11	CAL-B	5	*n*-hexane	1 h, Mw 180 W, 46 °C	59
12	CAL-B	5	*t*-BuOH	1 h, Mw 180 W, 46 °C	82
13	CAL-B	2	*t*-BuOH	1 h, Mw 180 W, 46 °C	42

Therefore, optimized conditions were settled as follow: CAL-B (Imb) lipase in the presence of 5 equivalents of CF_3CH_2OH, molecular sieve (4 Å) in *t*-BuOH and under a microwave activation of 1 h (180 W, 46 °C) (Scheme 2). These conditions were applied to other linear fatty acids **1** (oleic and erucic acids) with similar yields (**2a-c**, 75-82%). Surprisingly, the esterifications of branched fatty acids **1d-e** were performed in a *n*-hexane/*t*-BuOH : 1:1 mixture

in order to reach good conversions (**2d-e**, 59-68%). Concerning these two branched carboxylic acids we suppose that they do not fit perfectly within the catalytic site of the enzyme explaining the slightly lower yields.



Scheme 2. Enzymatic esterification of fatty acids into 2,2,2-trifluoroethyl esters.

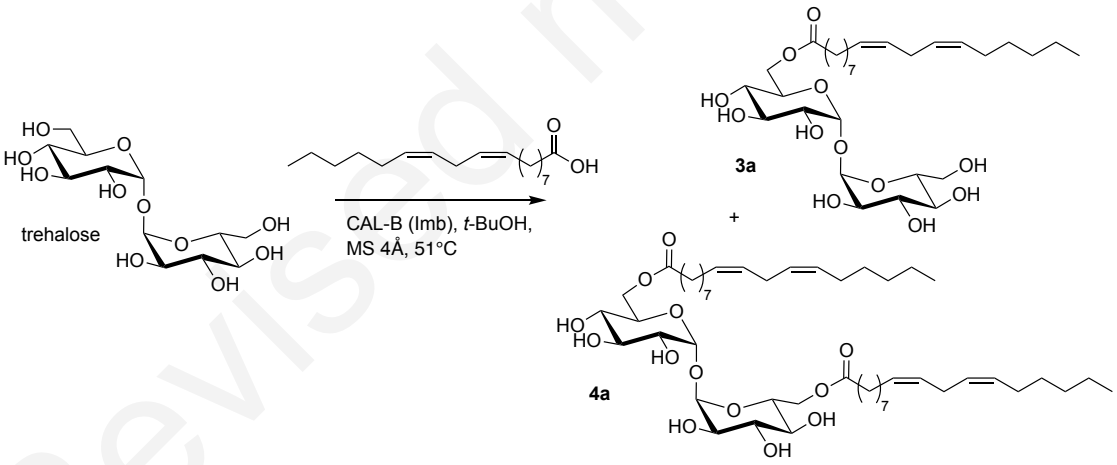
Having the activated esters in hands, we further studied the biocatalyzed monoacylation and diacylation of trehalose. This sugar is a α,α-1,1-disacharide of D-glucose. Its structure reveals two primary hydroxyl functions that are the less sterically hindered and the more nucleophilic sites.[46] Depending on the number of equivalent of fatty ester or on the reaction conditions one can expect mono (**3**) and diacylation (**4**) of trehalose (Table 2).

Based on the esterification procedure described above, reactions were carried out with the same lipase (CAL-B (Imb)) in *t*-BuOH, in the presence of activated molecular sieves and at 51 °C under thermal and microwave activation with linoleic acid or the corresponding 2,2,2-trifluoroethyl linoleate as acylating agent. As previously observed,[30, 31] the direct acylation of trehalose with 1.1 or 4 equivalents (Table 2, Entries 1-2) of carboxylic acid **1a** (R = H) were inefficient or required long reaction time. Almost no diacyl trehalose **4a** was formed even in

the presence of 4 equiv. of acid **1a**, only the monolinoleate **3a** was isolated in 42% yield. Moreover, with 1.1 equivalents, only traces of monoacyl derivative **3a** were obtained.

We therefore envisaged an *in situ* activation of the fatty acid by addition of CF_3CH_2OH into the reaction media following our optimized protocol. Interestingly, 4 equivalents of linoleic acid **1a** in the presence of 5 equivalents of CF_3CH_2OH gave selectively after 2 h of reaction the monoacyl derivative **3a** in 65% yield and only traces of diacyl compound **4a** (Entry 3). It is noteworthy that this *in situ* procedure gave both better yield and selectivity compared to the reaction carried out without CF_3CH_2OH (Mono 42%, Di 15% entry 2). It revealed that the acylation of trehalose proceeded at least partly through a transesterification of the *in situ* formed 2,2,2-trifluoroethyl ester. Therefore, this transesterification is responsible for the high selectivity towards a monoacylation. Thus, we envisaged the stepwise esterification.

Table 2. Enzymatic esterification of linoleic acid and trehalose

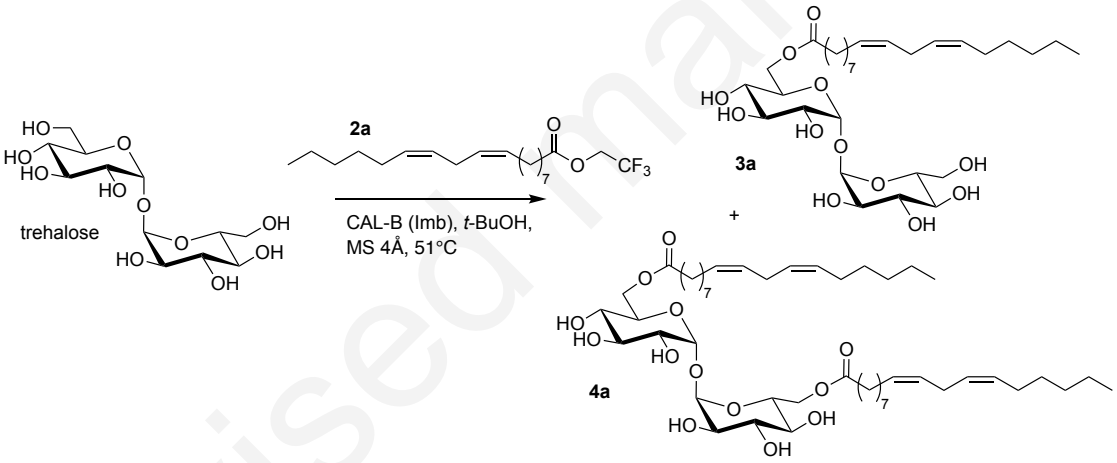


Entry	Equiv.	Conditions	Yield (%)		
			Mono **3a**	Di **4a**	trehalose
1	1.1	Microwave, 2 h	traces	-	90
2	4	Microwave, 2 h	42	15	35
3[a]	4	Microwave, 2 h	65	traces	10

a) The reaction was carried out in the presence of 5 equiv. of CF_3CH_2OH

In the presence of 1.1 equiv. of 2,2,2-trifluoroethyl linoleate **2a**, our reaction conditions led to the monoacyl trehalose **3a** in 73% yield after 1h under microwave activation (Entry 4). It confirmed that the transesterification of trehalose occurred selectively towards the formation of the sole monoacylated trehalose **3a**. In the presence of 4 equiv. of 2,2,2-trifluoroethyl linoleate **2a**, the transesterification proceeded smoothly towards the diacyl derivative both under thermal (Entry 5) and microwave (Entry 6) activations. Again the reaction was much more efficient under microwave (180 W) leading to the diacyl trehalose **4a** in 85% yield (Entry 6).

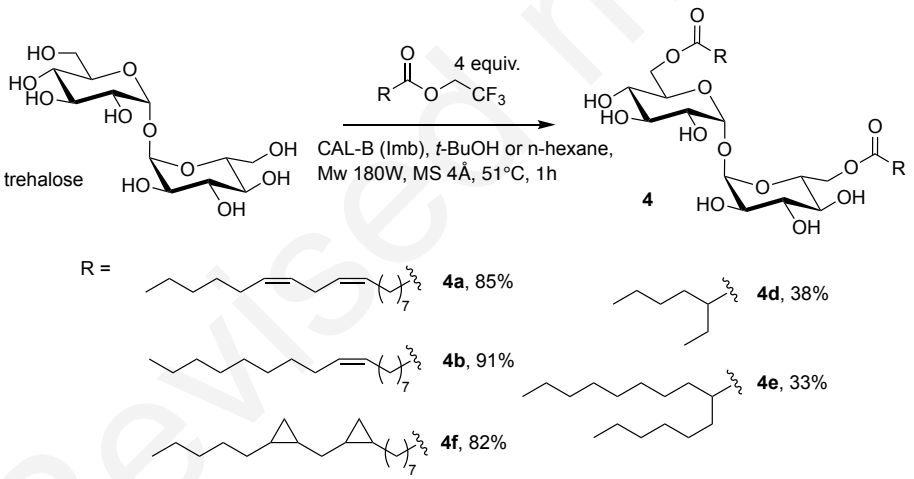
Table 3. Enzymatic transesterification of linoleic acid 2,2,2-trifluoroethyl ester by trehalose



Entry	Equiv.	Conditions	Yield (%)		
			Mono **3a**	Di **4a**	trehalose
1	1.1	Microwave, 1 h	73	traces	<5
2	4	Thermal, 18h	15	62	20
3	4	Microwave, 1 h	<5	85	<5
4[a]	4	Microwave, 1h	0	0	100

a) The reaction was carried without enzyme

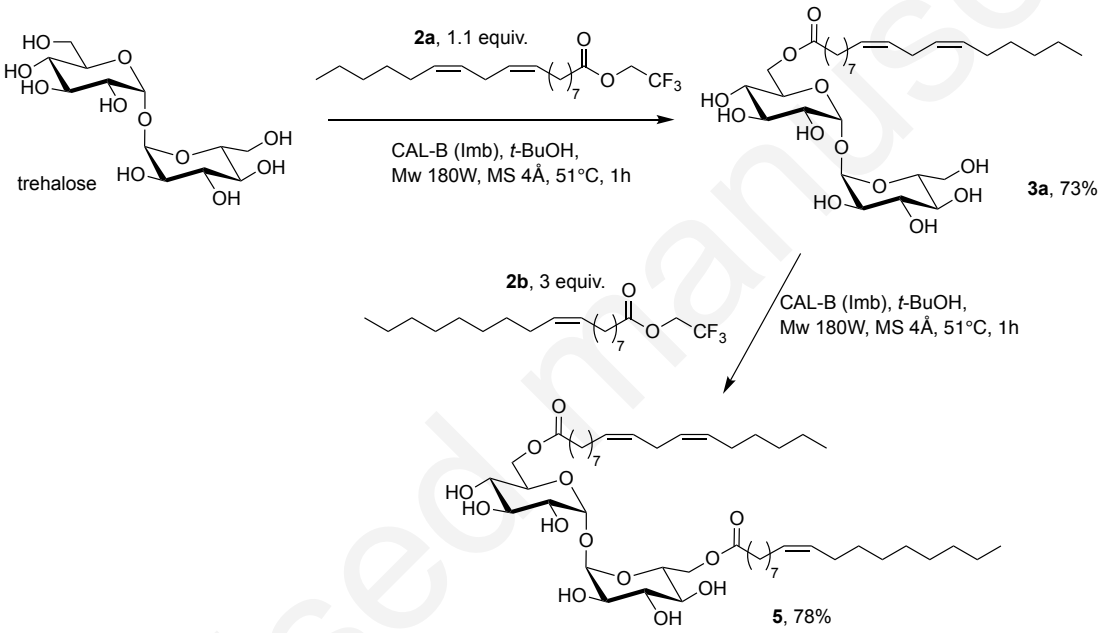
Therefore, transesterification conditions were established for the selective mono- or diacylation of trehalose in the case of linoleate derivatives. The diacylation conditions were successfully applied to other fatty acid 2,2,2-trifluoroethyl esters (**2**) such as oleic (**2b**) or dicyclopropaneted stearic (**2f**) leading to the corresponding diacyl trehalose **4** in good yields (**4a-b,f**, 82-91%) (Scheme 3). The branched esters (**2d-e**) provided the diacyl trehalose (**4d-e**) in lower yields ranging from 33 to 38%. As for the esterification step described above, the lipase (CAL B) would have a reduced activity with such branched esters due to unfavorable steric hindrance within the active site affording lower yields compared to linear fatty chains. Interestingly, ester **2f** bearing 2 cyclopronane moieties is a good model of mycolic acid conjugates. This ester was prepared from the linoleate **2a** after adaptation of a known procedure.[47, 48]



Scheme 3. Enzymatic diacylation of trehalose

Since the reaction conditions developed here can yield selectively mono trehalose conjugates we subsequently evaluated the ability of this approach for the synthesis of unsymmetrical diester derivatives (Scheme 4). This type of trehalose glycolipids, such as maradolipid, are

found in several organisms and require multistep synthesis or the use of coupling agents (TBTU).[29, 49] As a proof of concept, we developed the synthesis of the unsymmetrical diacyl trehalose **5** following our fully enzymatic sequence without any protection/deprotection steps. As mentioned here before (Table 2, entry 4), the reaction of a first 2,2,2-trifluoroethyl linoleate (1.1 equiv.) gave the monoacyl trehalose **3a** (Scheme 4, 73%). Starting from this isolated monolinoleyl trehalose **3a**, the second acylation was performed with 3 equivalents of 2,2,2-trifluoroethyloleate **2b** leading to the desired unsymmetrical diacyl trehalose **5** in 78% yield.



Scheme 4. Access to unsymmetrical diacyl trehalose by stepwise enzymatic acylations.

Finally, to gather more information on these enzymatic esterification/transesterification reactions, we performed additional kinetic and residual activity experiments. After 2h of reaction (microwave or thermal activation), the immobilized CALB was involved in the hydrolysis of a *p*-nitrophenyl linoleate. The colorimetric follow up of this hydrolysis clearly demonstrated that the enzyme is still active after exposure to the reaction conditions used in this work (See supporting information). We also determined the kinetic parameters of the

esterification of linoleic acid with 2,2,2-trifluoroethanol. The impact of the quantity of both substrates on the initial rate of the reaction was evaluated using the Michaelis-Menten model (See supporting information). First the initial reaction rates (V0) increased with increasing concentration of either the acid or the alcohol. It demonstrated that no inhibition occurred by both substrates of the lipase or by the ester formed. The double reciprocal plot or Lineweaver-Burk plot 1/V0 versus the reciprocal of linoleic acid concentration at fixed trifluoroethanol concentration showed a set of parallel lines characteristic of a Ping-pong bi-bi mechanism. Such mechanism has been generally found with soluble or immobilized lipase. The resulting calculation for Km of both substrates gave the value of 0.7 $mol.L^{-1}$ for the linoleic acid and 0.9 $mol.L^{-1}$ for the trifluoroethanol. It means that both substrates bind to the enzyme with the same affinity. This low affinity is linked to the nature of the lipase, and its interfacial mechanism. They are conforming to the ones found in the literature.[50-52]

3. Conclusion

The fully enzymatic strategy developed herein allowed the straightforward and efficient synthesis of trehalose glycolipid derivatives which represent important class of biologically active molecules. The final diacyl trehalose were obtained by a lipase mediated transesterification with 2,2,2-trifluoroethyl fatty esters. The choice for this later was particularly appropriate as it made possible the development of an enzymatic approach to the corresponding 2,2,2-trifluoroethyl fatty esters so that a unique biocatalyst was used for a two-step esterification/transesterification reaction. Thus, this work renews the access to symmetrical and also unsymmetrical diacyl trehalose compounds in an environmentally friendlier manner avoiding any classical esterification step.

4. Experimental

4.1 General methods

All reactions were carried out in oven-dried glassware. Optical rotations were measured at 20 °C on a Perkin-Elmer 341 polarimeter. NMR spectra were recorded on a Bruker Avance III 400 spectrometer operating at 400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C. Chemical shifts are given in δ units (ppm) and referenced to CD_3OD or $CDCl_3$. Coupling constants J were calculated in Hertz (Hz). MS spectra were recorded on a Waters Micromass Q-TOF equipped with a Z-spray ion source.

4.2. General procedure for preparation of 2,2,2-trifluoroethyl fatty esters. Carboxylic acid (1 equiv., typical quantity 100 mg), 2,2,2-trifluoroethanol (5 equiv.) and lipase (Immobilized CAL B, 93 mg per mmol of carboxylic acid, 3000 U/g) were placed in *t*-BuOH in the presence of activated molecular sieves (4Å) and under nitrogen atmosphere. The reaction mixture was placed in a microwave oven using a temperature control mode (180 W, 46 °C) for 1h. The supported lipase was removed by filtration and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (Cyclohexane/EtOAc) to afford the required compound.

2,2,2-trifluoroethyl linoleate **2a**

The general procedure for the preparation of 2,2,2-trifluoroethyl esters has been applied to the synthesis compound **2a**. The titled compound was isolated as a colorless oil in 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.45- 5.27 (4H, m), 4.46 (2H, q, J_{H-F} = 8.5 Hz), 2.84-2.68 (2H, m), 2.41 (2H, t, J = 7.5 Hz), 2.10-1.94 (4H, m), 1.66 (2H, m), 1.42-1.21 (14H, m), 0.88 (3H, t, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.28, 130.38, 130.14, 128.23, 128.03, 123.17 (q, J_{C-F} = 277.1 Hz), 60.27 (q, J_{C-F} = 36.6 Hz), 33.79, 31.68, 29.71, 29.50, 29.23, 29.20, 29.08, 27.36, 27.32, 25.78, 24.83, 22.73, 14.22; HRMS *m/z* calcd for $C_{20}H_{33}O_2F_3Na$

$[M+Na]^+$: 385.2325, found: 385.2329.

2,2,2-trifluoroethyl oleate **2b**

The general procedure for the preparation of 2,2,2-trifluoroethyl esters has been applied to the synthesis compound **2b**. The titled compound was isolated as a yellowish oil in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.44-5.28 (2H, m), 4.45 (2H, q, J_{H-F} = 8.5 Hz), 2.40 (2H, t, J = 7.5 Hz), 2.10-1.92 (4H, m), 1.71-1.59 (2H, m), 1.41-1.18 (20H, m), 0.87 (3H, t, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.21, 130.16, 129.81, 123.16 (q, J_{C-F} = 277.1 Hz), 60.24 (q, J_{C-F} = 36.5 Hz), 33.75, 32.07, 29.92, 29.80, 29.68, 29.48, 29.23, 29.19, 29.09, 27.36, 27.35, 27.28, 24.84, 22.83, 14.21; HRMS *m/z* calcd for $C_{20}H_{35}O_2F_3Na$ $[M+Na]^+$: 387.2481, found: 387.2480.

2,2,2-trifluoroethyl erucate **2c**

The general procedure for the preparation of 2,2,2-trifluoroethyl esters has been applied to the synthesis compound **2c**. The titled compound was isolated as a colorless oil in 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.44-5.30 (2 H, m), 4.45 (2 H, q, J_{H-F} = 8.5 Hz), 2.40 (2 H, t, J= 7.5 Hz), 2.06-1.91 (4 H, m), 1.72-1.59 (2 H, m), 1.39-1.15 (28 H, m), 0.87 (3 H, t, J= 6.8 Hz);^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.16, 130.52, 130.48, 123.19 (q, J_{C-F} = 277.1 Hz), 60.22 (q, J_{C-F} = 36.6 Hz), 33.75, 32.78, 32.11, 32.07, 29.84, 29.81, 29.75, 29.71, 29.59, 29.56, 29.52, 29.36, 29.32, 29.14, 27.08, 24.86, 22.86, 14.19; HRMS *m/z* calcd for $C_{24}H_{43}O_2F_3Na$ $[M+Na]^+$: 443.3107, found: 443.3109.

2,2,2-trifluoroethyl 2-ethylhexanoate **2d**

The general procedure for the preparation of 2,2,2-trifluoroethyl esters has been applied to the synthesis compound **2d**. The titled compound was isolated as a yellowish oil in 68% yield. ^{1}H

NMR (400 MHz, $CDCl_3$) δ (ppm) 4.47 (2H, q, J_{H-F} = 8.5 Hz), 2.42-2.33 (1H, m), 1.71-1.38 (4H, m), 1.37-1.17 (4H, m), 0.89 (3 H, t, J = 7.5 Hz), 0.87 (3 H, t, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.85, 123.20 (q, J_{C-F} = 278.1 Hz), 59.98 (q, J_{C-F} = 36.4 Hz), 47.09, 31.68, 29.56, 27.06, 25.44, 22.67, 13.94, 11.71; HRMS *m/z* calcd for $C_{10}H_{17}O_2F_3Na$ $[M+Na]^+$: 249.1073, found: 249.1075.

2,2,2-trifluoroethyl 2-hexyldecanoate **2e**

The general procedure for the preparation of 2,2,2-trifluoroethyl esters has been applied to the synthesis compound **2e**. The titled compound was isolated as a colorless oil in 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.46 (2H, q, J_{H-F} = 8.5 Hz), 2.51-2.36 (1H, m), 1.69-1.54 (2H, m), 1.55-1.38 (2H, m), 1.35-1.15 (20H, m), 0.89 (6 H, t, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.99, 123.22 (q, J_{C-F} = 277.2 Hz), 59.98 (q, J_{C-F} = 36.5 Hz), 45.56, 32.45, 32.01, 31.79, 29.62, 29.55, 29.38, 29.29, 27.43, 27.39, 27.07, 22.81, 22.71, 14.18, 14.12; HRMS *m/z* calcd for $C_{18}H_{33}O_2F_3Na$ $[M+Na]^+$: 361.2325, found: 361.2329.

2,2,2-trifluoroethyl 9,12-dicyclopropylstearate **2f**

Under nitrogen atmosphere a solution of **2a** (0.20 g, 0.55 mmol) in dry CH_2Cl_2 (2 mL) was cooled down to -23 ^{o}C, and diethyl zinc (1.0 M solution in hexane, 3.3 mL, 3.3 mmol) was added. The reaction mixture was stirred at the same temperature for 5 min before the dropwise addition of diiodomethane (0.5 mL, 5.5 mmol). After 12 h at -23 ^{o}C to 0 ^{o}C, the reaction mixture was treated with aqueous NH_4Cl and extracted with Et_2O. The combined organic phases were washed with saturated aqueous $NaHCO_3$ and brine and were dried over $MgSO_4$. The solvent were removed under reduced pressure and the residue was purified by flash chromatography on silica gel (Cyclohexane/EtOAc) to afford ester **2f** (0.2 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.45 (2H, q, J_{H-F} = 8.5 Hz), 2.40 (2H, t, J = 7.5 Hz), 1.71-1.61 (2H, m), 1.54-1.22 (18H,

m), 1.21-0.98 (2H, m), 0.89 (3H, t, J = 7.1 Hz), 0.86-0.53 (6H, m), (-0.22)-(-0.34) (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.22, 123.17 (q, $J_{C\text{-}F}$ = 277.1 Hz), 60.23 (q, $J_{C\text{-}F}$ = 36.6 Hz), 33.77, 32.06, 30.26, 30.05, 29.55, 29.38, 29.14, 29.05, 28.88, 28.19, 28.04, 24.85, 22.88, 16.20, 16.07, 16.02, 15.81, 15.75, 14.22, 11.16, 10.98; HRMS *m/z* calcd for $C_{22}H_{37}O_2F_3Na$ $[M+Na]^+$: 413.2638, found: 413.2642.

4.3. General procedure for the acylation of trehalose.

General procedure. Trehalose (1 equiv., typical quantity 30 mg), acyl donor and lipase (immobilized CAL B, 126 mg per mmol of trehalose, 3000 U/g) were placed in *t*-BuOH under nitrogen atmosphere and in the presence of activated molecular sieves (4 Å, 10% w/w). The reaction mixture was placed in a microwave oven under temperature control (180 W, 51 ^{o}C) for 1 h. The supported lipase was removed by filtration and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH) to afford required compounds.

6-linoleoyl trehalose **3a**

The general procedure has been applied to the synthesis of monoester **3a**. The titled compound was isolated as acolorless oil in 73% yield. $[\alpha]^{20}_D$ +7.3 (*c* 1.0; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD : 1:1) δ (ppm) 5.40-5.23 (4H, m), 5.09 (2H, dd, J = 12.0, 3.7 Hz), 4.33 (1H, dd, J =12.0, 2.2 Hz), 4.23 (1H, dd, J = 12.0, 4.9 Hz), 3.99 (1H, ddd, J =10.2, 4.8, 2.2 Hz), 3.87-3.74 (4H, m), 3.72-3.61 (2H, m), 3.50 (2H, td, J = 10.2, 3.7 Hz), 3.42-3.27 (4H, m), 2.74 (2H, t, J = 6.3 Hz), 2.33 (2H, t, J = 7.5 Hz), 2.03 (4H, dt, J = 8.5, 6.4 Hz), 1.59 (2H, q, J = 7.3 Hz), 1.39-1.21 (14H, m), 0.87 (3H, t, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD : 1:1) δ (ppm) 175.17, 130.61, 130.48, 128.62, 128.51, 94.54, 94.41, 73.94, 73.72, 73.11, 72.47, 72.40,

71.31, 71.09, 70.71, 63.85, 62.24, 34.66, 32.12, 30.21, 29.94, 29.80, 29.72, 27.74, 26.16, 25.47, 23.13, 14.31; HRMS *m/z* calcd for $C_{30}H_{52}O_{12}Na$ $[M+Na]^+$: 627.3351, found: 627.3350.

6,6'-dilinoleoyl trehalose **4a**

The general procedure has been applied to the synthesis of diester compound **4a**. The titled compound was isolated as a white solid in 85% yield. mp: 121-123 °C; $[\alpha]^{20}_D$ +5.9 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.31-5.13 (8H, m), 4.97 (2H, d, *J* = 3.7 Hz), 4.24-4.10 (4H, m), 4.00 (7H, s), 3.85 (2H, ddd, *J* = 10.1, 4.7, 2.5 Hz), 3.75 (2H, t, *J* = 9.4 Hz), 3.42 (2H, dd, *J* = 9.8, 3.7 Hz), 3.31-3.19 (2H, m), 2.68-2.60 (3H, m), 2.21 (4H, t, *J* = 7.6 Hz), 1.92 (7H, d, *J* = 6.9 Hz), 1.48 (4H, m), 1.29-1.08 (29H, m), 0.76 (6H, t, *J* = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD: 1:1) δ (ppm) 174.65, 130.27, 130.08, 128.14, 127.98, 93.60, 73.14, 71.71, 70.36, 70.14, 63.27, 34.19, 31.60, 29.71, 29.42, 29.31, 29.24, 29.22, 27.28, 27.27, 25.70, 24.92, 22.65, 14.06; HRMS *m/z* calcd for $C_{48}H_{82}O_{13}Na$ $[M+Na]^+$: 889.5648, found: 889.5646.

6,6'-dioleoyl trehalose **4b**

The general procedure has been applied to the synthesis of diester **4b**. The titled compound was isolated as a yellowish solid in 91% yield. mp: 153-154 °C; $[\alpha]^{20}_D$ +6.5 (*c* 0.8; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD : 1:1) δ (ppm) 5.40-5.24 (4H, m), 5.07 (2H, d, *J* = 3.8 Hz), 4.32 (2H, dd, *J* = 12.0, 2.3 Hz), 4.23 (2H, dd, *J* = 12.0, 5.1 Hz), 3.96 (2H, ddd, *J* = 10.2, 5.1, 2.3 Hz), 3.75 (2H, dd, *J* = 9.7, 8.9 Hz), 3.49 (2H, dd, *J* = 9.7, 3.8 Hz), 3.38-3.33 (4H, m), 2.32 (4H, t, *J* = 7.5 Hz), 2.08-1.89 (7H, m), 1.60 (4H, m), 1.39-1.16 (39H, m), 0.86 (6H, t, *J* = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD : 1:1) δ (ppm) 175.15, 130.48, 130.26, 94.33, 73.96, 72.36, 71.08, 70.71, 63.88, 34.68, 33.13, 32.48, 30.31, 30.27, 30.25, 30.21, 30.18, 30.07, 30.05, 29.92, 29.89, 29.84, 29.83, 29.76, 29.70, 29.67, 27.72, 27.69, 25.46, 23.21, 23.19, 14.34, 14.32; HRMS *m/z* calcd for $C_{48}H_{86}O_{13}Na$ $[M+Na]^+$: 893.5961, found: 893.5969.

6,6'-diethylhexanoyl trehalose **4d**

The general procedure has been applied to the synthesis of monoester **4d** (n-hexane/t-BuOH was used instead of t-BuOH alone). The titled compound was isolated as a white solid in 38% yield. mp: 181-183 °C; $[\alpha]^{20}_{D}$ +4.7 (*c* 1.0; MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 5.05 (2H, dd, *J* = 3.7, 1.5 Hz), 4.41 (2H, dd, *J* = 11.8, 2.1 Hz), 4.21 (2H, dd, *J* = 11.9, 5.2 Hz), 4.01 (2H, ddd, *J* = 10.1, 5.2, 2.1 Hz), 3.79 (2H, dd, *J* = 9.8, 8.8 Hz), 3.46 (2H, ddd, *J* = 9.8, 3.8, 1.4 Hz), 3.40-3.32 (2H, m), 2.30 (2H, tt, *J* = 8.9, 5.3 Hz), 1.70-1.42 (8H, m), 1.42-1.22 (14H, m), 0.95-0.84 (12H, m); ^{13}C NMR (100 MHz, CD_3OD): δ_C 177.95, 95.08, 74.58, 73.17, 71.99, 71.94, 71.53, 64.13, 64.06, 54.80, 32.99, 32.96, 32.14, 30.75, 30.70, 30.08, 26.63, 26.59, 23.69, 23.67, 14.35, 14.33, 12.21, 12.18; HRMS *m/z* calcd for $C_{28}H_{50}O_{13}Na$ $[M+Na]^+$: 617.3144, found: 617.3155.

6,6'-dihexyldecanoyl trehalose **4e**

The general procedure has been applied to the synthesis of monoester **4e** (n-hexane/t-BuOH was used instead of t-BuOH alone). The titled compound was isolated as a white solid in 33% yield. mp: 164-166 °C; $[\alpha]^{20}_{D}$ +5.4 (*c* 1.0; MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 5.06 (2H, d, *J* = 3.7 Hz), 4.37 (2H, dd, *J* = 11.8, 2.1 Hz), 4.21 (2H, dd, *J* = 11.8, 5.3 Hz), 4.02 (2H, ddd, *J* = 10.1, 5.3, 2.2 Hz), 3.80 (2H, dd, *J* = 9.7, 8.8 Hz), 3.46 (2H, dd, *J* = 9.7, 3.8 Hz), 3.39-3.31 (1H, m), 2.36 (2H, tt, *J* = 9.0, 5.2 Hz), 1.65-1.55 (4H, m), 1.53-1.41 (5H, m), 1.29 (45H, m), 0.94-0.86 (12H, m); ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 178.08, 95.01, 74.59, 73.21, 72.02, 71.42, 64.23, 47.14, 33.65, 33.06, 32.89, 32.86, 30.70, 30.69, 30.60, 30.58, 30.45, 30.38, 30.37, 28.54, 28.52, 28.49, 28.47, 23.77, 23.70, 14.50, 14.48; HRMS *m/z* calcd for $C_{44}H_{82}O_{13}Na$ $[M+Na]^+$: 841.5648, found: 841.5658.

6,6'-di-(dicyclopropyl)-stearoyl trehalose **4f**

The general procedure has been applied to the synthesis of diester **4f**. The titled compound was isolated as a colorless oil in 82% yield. $[\alpha]^{20}_{D}$ +4.6 (*c* 1.0; MeOH); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$: 1:1) δ (ppm) 4.91 (2H, d, *J* = 3.5 Hz), 3.77 (2H, dt, *J* = 7.7, 2.3 Hz), 3.61-3.53 (2H, m), 3.47 (3H, d, *J* = 3.3 Hz), 3.37-3.29 (2H, m), 3.24-3.12 (8H, m), 2.16 (4H, dd, *J* = 8.7, 6.3 Hz), 1.42 (4H, s), 1.28-1.02 (38H, m), 0.76-0.63 (8H, m), 0.59 (4H, m), 0.49 (4H, s), 0.41 (4H, m), (-0.39)-(-0.59) (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$: 1:1) δ (ppm) 174.94, 93.94, 77.89, 73.70, 72.00, 70.68, 70.36, 63.55, 34.45, 32.20, 30.45, 30.18, 29.77, 29.64, 29.46, 29.19, 29.03, 28.35, 28.20, 25.18, 22.99, 16.34, 16.21, 16.17, 15.94, 15.90, 14.23, 11.24, 11.07, 11.06; HRMS *m/z* calcd for $C_{52}H_{90}O_{13}Na$ $[M+Na]^+$: 945.6274, found: 945.6283.

6-linoleoyl-6'-oleoyl trehalose **5**

The general procedure has been applied to the synthesis of diester **5** from **3a**. The titled compound was isolated as a yellowish oil in 78% yield. $[\alpha]^{20}_{D}$ +6.7 (*c* 1.0; MeOH); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$: 1:1) δ (ppm) 5.07-4.91 (6H, m), 4.73 (2H, d, *J* = 3.7 Hz), 3.99 (2H, dd, *J* = 12.0, 2.3 Hz), 3.90 (2H, dd, *J* = 12.0, 5.1 Hz), 3.64 (2H, ddd, *J* = 10.2, 5.1, 2.2 Hz), 3.43 (2H, dd, *J* = 9.7, 8.9 Hz), 3.16 (2H, dd, *J* = 9.7, 3.8 Hz), 3.05-2.94 (4H, m), 2.47-2.34 (2H, m), 1.99 (4H, t, *J* = 7.6 Hz), 1.75-1.64 (8H, m), 1.26 (4H, m), 1.06-0.89 (38H, m), 0.58-0.48 (6H, m); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$: 1:1) δ (ppm) 175.15, 130.61, 130.48, 130.47, 130.27, 128.62, 128.50, 110.72, 94.37, 73.96, 72.39, 71.11, 70.74, 63.90, 34.69, 32.51, 32.12, 30.33, 30.29, 30.20, 30.09, 29.94, 29.91, 29.86, 29.78, 29.70, 27.74, 26.16, 25.47, 23.23, 23.13, 14.35, 14.32; HRMS *m/z* calcd for $C_{48}H_{84}O_{13}Na$ $[M+Na]^+$: 891.5804, found: 891.5815.

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References

1 J. Otera and J. Nishikido, *Esterification: Methods, Reactions, and Applications, 2nd Edition*, Wiley-VCH Verlag GmbH & Co. KGaA, 2010.
2 D. F. Izquierdo, J. M. Bernal, M. I. Burguete, E. Garcia-Verdugo, P. Lozano and S. V. Luis, *RSC Adv.*, 2013, **3**, 13123-13126.
3 K. Engstroem, M. Shakeri and J.-E. Baeckvall, *Eur. J. Org. Chem.*, 2011, 1827-1830.
4 R. O. M. A. de Souza, O. A. C. Antunes, W. Kroutil and C. O. Kappe, *J. Org. Chem.*, 2009, **74**, 6157-6162.
5 M.-J. Kim, H. M. Kim, D. Kim, Y. Ahn and J. Park, *Green Chem.*, 2004, **6**, 471-474.
6 M. Bertau and G. E. Jeromin, in *Biocatalysis in organic synthesis*, eds. K. Faber, W.-D. Fessner and N. J. Turner, Georg Thieme Verlag KG, Stuttgart, 2015, vol. 1, pp. 129-187.
7 C.-C. Chen, P. M. Reddy, C. S. Devi, P.-C. Chang and Y.-P. Ho, *Enzyme Microb. Technol.*, 2016, **82**, 164-172.
8 D.-H. Yu, L. Tian, D.-X. Ma, H. Wu, Z. Wang, L. Wang and X.-X. Fang, *Green Chem.*, 2010, **12**, 844-850.
9 T. Tan, J. Lu, K. Nie, L. Deng and F. Wang, *Biotechnol. Adv.*, 2010, **28**, 628-634.
10 M. B. Abdul Rahman, M. Arumugam, N. S. K. Khairuddin, E. Abdulmalek, M. Basri and A. B. Salleh, *Asian J. Chem.*, 2012, **24**, 5058-5062.
11 Y.-G. Shi, J.-R. Li and Y.-H. Chu, *J. Chem. Technol. Biotechnol.*, 2011, **86**, 1457-1468.
12 T. Kobayashi, *Biotechnol. Lett.*, 2011, **33**, 1911-1919.
13 P. Magrone, F. Cavallo, W. Panzeri, D. Passarella and S. Riva, *Org. Biomol. Chem.*, 2010, **8**, 5583-5590.
14 L. Lay, L. Panza, S. Riva, M. Khitri and S. Tirendi, *Carbohydrate Res.*, 1996, **291**, 197-204.
15 S. Martinez-Montero, S. Fernandez, Y. S. Sanghvi, V. Gotor and M. Ferrero, *Eur. J. Org. Chem.*, 2012, 5483-5490.
16 L.-H. Du and X.-P. Luo, *RSC Adv.*, 2012, **2**, 2663-2665.
17 P. Galletti, F. Moretti, C. Samori and E. Tagliavini, *Green Chem.*, 2007, **9**, 987-991.
18 T. Rodriguez-Perez, I. Lavandera, S. Fernandez, Y. S. Sanghvi, M. Ferrero and V. Gotor, *Eur. J. Org. Chem.*, 2007, 2769-2778.
19 S. Adak and R. Banerjee, *Carbohydr. Polym.*, 2016, **150**, 359-368.
20 V. M. F. Lima, V. L. D. Bonato, K. M. Lima, S. A. Dos Santos, R. R. Dos Santos, E. D. C. Goncalves, L. H. Faccioli, I. T. Brandao, J. M. Rodrigues-Junior and C. L. Silva, *Infect. Immun.*, 2001, **69**, 5305-5312.
21 J. Indrigo, R. L. Hunter, Jr. and J. K. Actor, *Microbiology (Reading, U. K.)*, 2002, **148**, 1991-1998.

22 K. Sakamoto, M. J. Kim, E. R. Rhoades, R. E. Allavena, S. Ehrt, H. C. Wainwright, D. G. Russell and K. H. Rohde, *Infect. Immun.*, 2013, **81**, 764-776.
23 J. Guiard, A. Collmann, M. Gilleron, L. Mori, G. De Libero, J. Prandi and G. Puzo, *Angew. Chem. Int. Ed.*, 2008, **47**, 9734-9738.
24 B. L. Stocker and M. S. M. Timmer, *Carbohydr. Res.*, 2014, **389**, 3-11.
25 A. Franzetti, I. Gandolfi, G. Bestetti, T. J. P. Smyth and I. M. Banat, *Eur. J. Lipid Sci. Technol.*, 2010, **112**, 617-627.
26 S. G. Taher, J. a. R. Al Dulayymi, H. G. Tima, H. M. Ali, M. Romano and M. S. Baird, *Tetrahedron*, 2016, **72**, 3863-3876.
27 S. G. Taher, M. Muzael, J. R. Al Dulayymi and M. S. Baird, *Chem. Phys. Lipids*, 2015, **189**, 28-38.
28 B. L. Stocker, A. A. Khan, S. H. Chee, F. Kamena and M. S. M. Timmer, *ChemBioChem*, 2014, **15**, 382-388.
29 N. K. Paul, J.-d. A. K. Twibanire and T. B. Grindley, *J. Org. Chem.*, 2013, **78**, 363-369.
30 S.-W. Hsieh, M.-R. Lee, C.-W. Tsai, L.-Y. Lai, T. Yeh, C.-W. Hsieh, T.-J. Yang and S.-W. Chang, *Food Bioprod. Process.*, 2015, **95**, 163-172.
31 R. Csuk, A. Schultheiß, S. Sommerwerk and R. Kluge, *Tetrahedron Lett.*, 2013, **54**, 2274-2276.
32 P. You, J. Qiu, E. Su and D. Wei, *Eur. J. Org. Chem.*, 2013, 557-565.
33 T. Miyazawa and W. Iguchi, *Biotechnol. Lett.*, 2013, **35**, 1639-1643.
34 S. Kim, Y. K. Choi, J. Hong, J. Park and M.-J. Kim, *Tetrahedron Lett.*, 2013, **54**, 1185-1188.
35 L. Lemiègre, R. L. Stevens, J.-C. Combret and J. Maddaluno, *Org. Biomol. Chem.*, 2005, **3**, 1308-1318.
36 W. Lu, K. Xie, Z. Chen, Y. Pan and C. Zheng, *J. Fluorine Chem.*, 2014, **161**, 110-119.
37 T. Angelini, S. Bonollo, D. Lanari, F. Pizzo and L. Vaccaro, *Org. Lett.*, 2012, **14**, 4610-4613.
38 M. S. Azam, S. L. Fenwick and J. M. Gibbs-Davis, *Langmuir*, 2011, **27**, 741-750.
39 C. M. Monteiro, N. M. T. Lourenco and C. A. M. Afonso, *Tetrahedron: Asymmetry*, 2010, **21**, 952-956.
40 F. Yang, Z. Wang, H. Wang, C. Wang and L. Wang, *RSC Adv.*, 2015, **5**, 57122-57126.
41 S. D. Shinde and G. D. Yadav, *Appl. Biochem. Biotechnol.*, 2015, **175**, 2035-2049.
42 I. C. R. Costa, F. K. Sutili, G. V. V. da Silva, S. G. F. Leite, L. S. M. Miranda and R. O. M. A. de Souza, *J. Mol. Catal. B: Enzym.*, 2014, **102**, 127-131.
43 N. E. Leadbeater, L. M. Stencel and E. C. Wood, *Org. Biomol. Chem.*, 2007, **5**, 1052-1055.
44 M. Happe, P. Grand, S. Farquet, S. Aeby, J.-C. Heritier, F. Corthay, E. Mabillard, R. Marti, E. Vanoli, A.-F. Grogg, S. Nussbaum, A. Roduit, F. Tieche, S. Salem, C. Constantin, E. Schmitt, S. Zahno, C. Ellert, A. Habib, J. Wyss and F. Fischer, *Green Chem.*, 2012, **14**, 2337-2345.
45 B. Major, I. Kelemen-Horvath, Z. Csanadi, K. Belafi-Bako and L. Gubicza, *Green Chem.*, 2009, **11**, 614-616.
46 C. M. Pedersen, J. Olsen, A. B. Brka and M. Bols, *Chem. Eur. J.*, 2011, **17**, 7080-7086.
47 T. Morikawa, H. Sasaki, R. Hanai, A. Shibuya and T. Taguchi, *J. Org. Chem.*, 1994, **59**, 97-103.
48 J. Furukawa, N. Kawabata and J. Nishimura, *Tetrahedron*, 1968, **24**, 53-58.
49 V. A. Sarpe and S. S. Kulkarni, *J. Org. Chem.*, 2011, **76**, 6866-6870.
50 S. D. Shinde and G. D. Yadav, *Process Biochem.*, 2015, **50**, 230-236.

51 A. Chowdhury and D. Mitra, *Biotechnol. Progress*, 2015, **31**, 1494-1499.
52 A. Zaidi, J. L. Gainer, G. Carta, A. Mrani, T. Kadiri, Y. Belarbi and A. Mir, *J. Biotechnol.*, 2002, **93**, 209-216.