Synthesis of glycero amino acid-based surfactants. Part 2.[1] Lipase-catalysed synthesis of 1-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) and 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) derivatives

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An enzymatic procedure for the synthesis of mono- and dilauroylated amino acid glyceride conjugates is presented. The reaction consisted of the lipase-catalysed esterification of one or two hydroxy groups of the amino acid glyceryl ester derivatives by lauric acid. Solvent-free media with continuous removal of the water produced at atmospheric pressure were the reaction systems of choice. It is found that the mono- and dilauroylation yields and enzymatic activity depended crucially on the reaction temperature, the molar ratio of lauric acid to amino acid glyceryl ester, and concentration of phosphate salts using *Candida antarctica* (Novozym® 435) and *Rhizomucor miehei* (Lipozyme® IM) lipases as catalysts. These reaction variables are identified and studied systematically using Boc–Arg–OGl as a model substrate and modulated accordingly for the target amino acid-based compounds. As in conventional glycerides, spontaneous intramolecular acyl-migration reactions are observed for the lauroyl and aminoacyl moieties of the glycerol backbone. This reaction led to a number of regioisomers, which are identified and quantified by mono- and bidimensional NMR techniques. The method developed allowed us to prepare glycero conjugates of arginine, aspartic acid, glutamic acid, asparagine, glutamine and tyrosine in isolated yields of the regioisomeric mixtures ranging from 22 to 69%.

Introduction

Mono- and diacylglycerol amino acid conjugates constitute a novel class of speciality bio-based surfactants, which can be considered analogues of partial glycerides and phospholipids. They consist of one or two aliphatic chains and one polar head, *i.e.* the amino acid, linked together through a glycerol moiety. The resulting structures resemble the acid esters of monoglycerides such as lactic, citric, tartaric and succinic glyceride esters used as food emulsifiers.[2] The physicochemical and biological properties of amino acid glyceride conjugates have not been extensively explored yet. On the basis of our preliminary observations, these novel compounds combine the advantages of both partial glycerides and lipo-amino acids.[3] For instance, we have observed that they possess antimicrobial activity, like long-chain N^{α}-acyl amino acid derivatives,[4] and form lamellar phases and vesicles, characteristic of partial glycerides and phospholipids. Moreover, the possibility of introducing different ionic groups (*i.e.*, by selecting the appropriate amino acid) increases the swelling properties by promoting the electric repulsion between charged-group bilayers.[2,5] Furthermore, mono- and diacylglyceride amino acid conjugates may lead to a number of lipid analogues of potential therapeutic interest and as adjuvants for drug and gene delivery (*i.e.*, transfection).[6] For all these reasons, they constitute a promising family of compounds with a great potential interest in pharmaceutical and food formulations.

The enzymatic preparation of mono- and diacylglyceride amino acid conjugates is a current topic of interest in our laboratory. The strategy devised for their synthesis consists of two steps. The first involves the enzymatic preparation of the amino acid glyceryl ester derivatives [*rac*-1-*O*-(N^{α}-acetyl-L-aminoacyl)glycerol]. In the second step the enzymatic acylation of the hydroxy groups of the amino acid glyceryl ester by fatty acids is performed. We have recently reported[1] the selective enzymatic preparation of a number of amino acid glyceryl ester derivatives: Ac–Arg–OGl·HCl, Ac–Asp–OGl, Ac–Glu–OGl, Ac–Asn–OGl, Ac–Gln–OGl and Ac–Tyr–OGl as well as Boc–Arg–OGl·HCl, where –OGl stands for glyceryl ester. We here present a study of the lipase-catalysed mono- and diacylation of the above amino acid glyceryl ester derivatives. An enzymatic acylation methodology has been reported by Valivety *et al.*[7] However, the procedure described afforded exclusively the monoacylated hydrophobic and basic amino acid glyceride conjugates. The goal of the present work was to prepare the 1-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) (monolauroylated) **3**, **5**, **7**, **9**, **11**, **13** and 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) (dilauroylated) **4**, **6**, **8**, **10**, **12**, **14** derivatives (Scheme 1) by means of an efficient enzymatic methodology. Hence, the reaction variables were first investigated systematically on a model reaction for obtaining fundamental information on the effect of reaction variables on both enzymatic activity and product yield. The results obtained were instrumental in finding the best conditions for the preparation of the target compounds. The methodology developed provides a general strategy for the synthesis of amino acid and peptide glyceride conjugates in a simple and clean manner. This is especially appropriate if the products are intended to be used in food, pharmaceutical and personal-care formulations.

Results and discussion

The enzymatic synthesis of 1-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-protected-L-amino acid) (monolauroylated product) and

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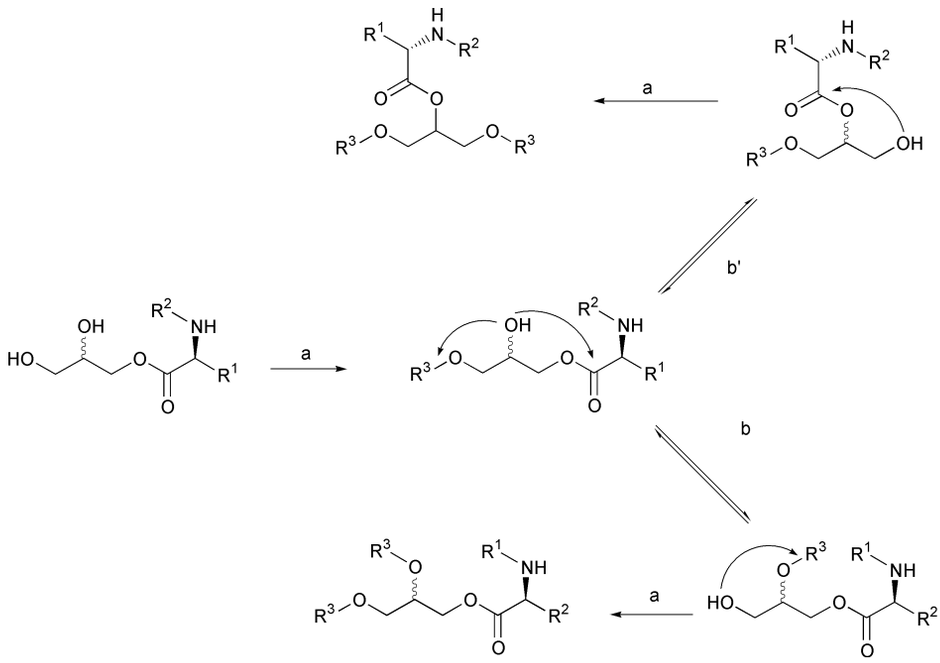
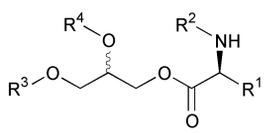


Scheme 1 Reaction pathway for the synthesis of monolauroylated and dilauroylated products: a, lipase-catalysed esterification; b, spontaneous intramolecular acyl-migration reaction of the lauroyl moiety; b′, acyl migration of the aminoacyl moiety. R^1, R^2 and R^3 as defined in structures **1**–**14**.



1 R^1 = -$CH_2CH_2CH_2NHC(NH_2)_2^+Cl^-$, R^2 = Bu^tOCO-,
R^3 = $CH_3(CH_2)_{10}CO$-, R^4 = H

2 R^1 = -$CH_2CH_2CH_2NHC(NH_2)_2^+Cl^-$, R^2 = Bu^tOCO-,
R^3 = R^4 = $CH_3(CH_2)_{10}CO$-

3 R^1 = -$CH_2CH_2CH_2NHC(NH_2)_2^+Cl^-$, R^2 = CH_3CO-,
R^3 = $CH_3(CH_2)_{10}CO$-, R^4 = H

4 R^1 = -$CH_2CH_2CH_2NHC(NH_2)_2^+Cl^-$, R^2 = CH_3CO-,
R^3 = R^4 = $CH_3(CH_2)_{10}CO$-

5 R^1 = -CH_2COOH, R^2 = CH_3CO-, R^3 = $CH_3(CH_2)_{10}CO$-, R^4 = H

6 R^1 = -CH_2COOH, R^2 = CH_3CO-, R^3 = R^4 = $CH_3(CH_2)_{10}CO$-

7 R^1 = -CH_2CH_2COOH, R^2 = CH_3CO-, R^3 = $CH_3(CH_2)_{10}CO$-, R^4 = H

8 R^1 = -CH_2CH_2COOH, R^2 = CH_3CO-, R^3 = R^4 = $CH_3(CH_2)_{10}CO$-

9 R^1 = -CH_2CONH_2, R^2 = CH_3CO-, R^3 = $CH_3(CH_2)_{10}CO$-, R^4 = H

10 R^1 = -CH_2CONH_2, R^2 = CH_3CO-, R^3 = R^4 = $CH_3(CH_2)_{10}CO$-

11 R^1 = -$CH_2CH_2CONH_2$, R^2 = CH_3CO-, R^3 = $CH_3(CH_2)_{10}CO$-, R^4 = H

12 R^1 = -$CH_2CH_2CONH_2$, R^2 = CH_3CO-, R^3 = R^4 = $CH_3(CH_2)_{10}CO$-

13 R^1 = -$CH_2C_6H_4OH$, R^2 = CH_3CO-, R^3 = $CH_3(CH_2)_{10}CO$-, R^4 = H

14 R^1 = -$CH_2C_6H_4OH$, R^2 = CH_3CO-, R^3 = R^4 = $CH_3(CH_2)_{10}CO$-

1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-protected-L-amino acid) (dilauroylated product) derivatives consists of the lipase-catalysed esterification of one or two hydroxy groups, respectively, of the N^{α}-protected amino acid glyceryl esters with lauric acid. To this end, both 1,3-regioselective lipases for the 1(3)-lauroylated products and non-selective lipases for the 1,2-dilauroylated products were considered. However, mono- and dilauroylation may be carried out using selective lipases by taking advantage of the spontaneous intramolecular acyl-migration reaction that occurs in partial glycerides.[8–10] Thus, the 1(3)-lauroylated product may undergo intramolecular 1(3)⟶2 acyl migration and the resulting 1,2(2,3)-isomer subsequently acylated at the free primary hydroxy group by the lipase. Accordingly, the yield of dilauroylated product will depend on both the enzymatic esterification of the 2,1(3)-isomer and the rate of the intramolecular acyl-migration reaction. Both processes are influenced by the reaction conditions[9,10] such as solvent, support for enzyme immobilization, buffer salts and on the amino acid glyceryl ester derivative.

There are two possible strategies for lipase-catalysed acylations, namely esterification and transesterification. We selected the direct esterification, using simple lauric acid as acyl donor, for two main reasons. First, Millqvist-Fureby *et al.*[11] found that, in lipase-catalysed glyceride synthesis in solvent-free systems, simple capric acid (*i.e.*, direct esterification) was the most efficient acyl donor compared with tricaprin and ethyl caprate. Second, fatty acid esters have to be prepared previously, increasing the number of synthetic steps.

The enzymatic acylations were carried out in solvent-free media, considering both the environmental standpoint and the recent trends in biocatalysed reactions.[12] We chose the melted substrate approach to achieve intimate contact of the solid substrate mixture.[13,14] The amino acid glyceryl esters were normally high-density oils or vitreous solids with undefined melting point. Thermal analysis of the reaction mixtures by differential scanning calorimetry (DSC) revealed that in all instances they melted around 44 °C, which corresponds to the melting point of lauric acid. Hence, the reactions were performed at temperatures above 44 °C and therefore the melted lauric acid was acting as reagent and solvent. According to the literature data,[10,11,13] the following variables were selected for the optimization of product yields: temperature, the molar ratio of lauric acid to amino acid glyceryl ester, presence of solid buffer salts, and biocatalyst type and configuration.

Reaction progress

For experimental convenience, we explored first the reaction between Boc–Arg–OGl and lauric acid to give compounds **1** and **2**. Among the lipases commercially and readily available

which can catalyse the reaction, *Candida antarctica* lipase B, Novozym® 435 (CAL-B), was the first choice. The model reaction was carried out at 45 °C in open reactors to continuously remove the water produced and shift efficiently the thermodynamic equilibrium in favour of the synthesis. When the reactions were carried out in closed reactors, the substrate conversion was only 30%. Reduced pressure was also considered for removing the water produced. However, similar results to those with open reactors at atmospheric pressure were obtained.[14] Fig. 1 shows the time progress of the reaction for Boc–Arg–OGl, the monolauroylated product **1** and dilauroylated product **2**. As can be seen, monolauroylation was the major reaction during 24 h. Prolonged incubation times resulted in an increase of **2** up to 25% in 72 h. Considering that CAL-B is a 1,3-selective lipase, the diacylated product may have been formed in two steps. First, product **1** isomerized by spontaneous acyl-migration reaction of the lauroyl moiety to position 2, giving 2-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-Boc-L-arginine). Second, enzymatic acylation of the 2,3-regioisomer at the primary C1 hydroxy group occurred to give the diacylated product **2**. Obviously, the yield of **2** will depend on the rate of the spontaneous intramolecular acyl-migration, which in turn depends on the reaction variables and on the amino acid at position C3.

The mono- and dilauroylated product yields given in this study correspond to the maximum yields obtained for each set of reaction conditions, unless otherwise stated. In all instances, they were calculated from the time-progress curves of amino acid glyceryl ester consumption and product formation. While in all cases the monolauroylated product showed a clear maximum (see Fig. 1 as an example) between 8 and 24 h, the yield of dilauroylation reached a plateau between 48–72 hours of incubation. Hence, the yields of dilauroylated product were always given at 48–72 hours of reaction. In all cases, the products were identified using LC/MS analysis.

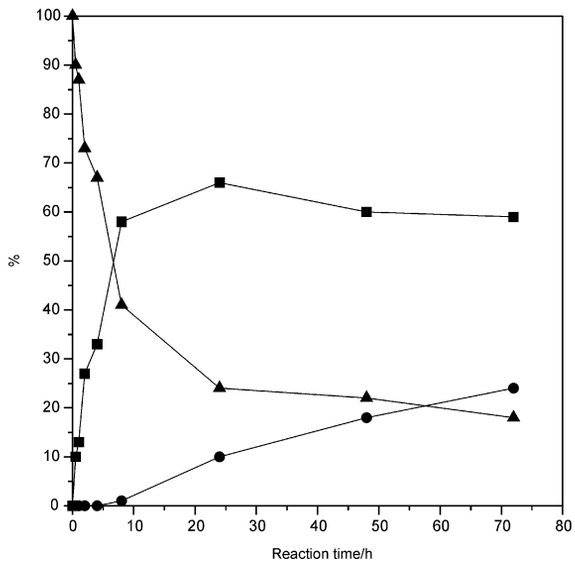


Fig. 1 Lipase-catalysed esterification of lauric acid with *rac*-1-*O*-(N^{α}-Boc-L-arginyl)glycerol (Boc–Arg–OGl). Reaction time-course of monolauroylated (**1**; ■), dilauroylated (**2**; ●) products and substrate glyceryl ester (▲). The reaction was carried out in an open-flask reactor. Boc–Arg–OGl·HCl (96.0 mg, 0.25 mmol) and lauric acid (0.10 g, 0.50 mmol) were mixed with 200 mM aqueous phosphate, pH 7, buffer (500 µl) and the mixture was freeze-dried during 48 h. At the end of this period the solid contained 56 mg of phosphate salts per mmol of Boc–Arg–OGl·HCl. To this solid mixture was added water (4 µl) and the reactor was placed at 45 °C. After 5 min, CAL-B (50 mg) was added.

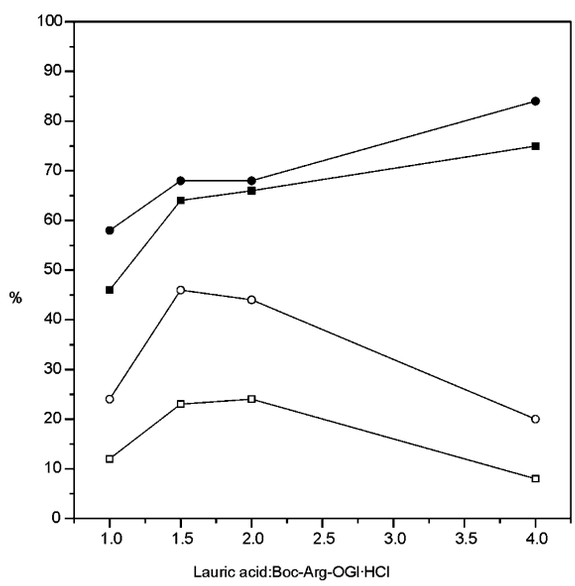


Fig. 2 Lipase-catalysed esterification of lauric acid with *rac*-1-*O*-(N^{α}-Boc-L-arginyl)glycerol. Influence of the ratio of lauric acid to Boc–Arg–OGl and the temperature on the maximum yields of monolauroylated (**1**; filled symbols) and dilauroylated (**2**; empty symbols) products. Reactions were carried out under the conditions described in the caption to Fig. 1 at 45 °C (□, ■) and at 55 °C (○, ●).

The reaction temperature and the ratio of lauric acid to Boc–Arg–OGl

In melted-substrate reaction systems both the temperature and substrate molar ratio are of paramount importance for the reaction performance. Both variables influence the catalytic properties of the enzyme, the reaction rate, the equilibrium position of the reaction and the rate of intramolecular acyl-migration. The maximum yields of mono- **1** and dilauroylated **2** were measured as a function of the molar ratio of lauric acid to Boc–Arg–OGl at 45 and 55 °C. As Fig. 2 shows, the yield of **2** rose approximately two-fold with the temperature while the yield of **1** remained practically unchanged (1.1 to 1.2-fold). This may be explained by an increase in both the catalytic and acyl-migration rates with temperature.[9,15] Unexpectedly, the maximum yield of monolauroylated product **1** increased with the ratio of lauric acid to amino acid glyceryl ester. Moreover, the yield of dilauroylated product **2** reached a maximum between 1.5 and 2 molar ratio and tended to a minimum for the highest lauric acid content assayed. If CAL-B were considered a 1,3-specific lipase, the results would be suggesting that at high lauric acid concentration the rate of intramolecular acyl migration might be inhibited. Alternatively, if the specificity of CAL-B depended on both the reactants and reaction conditions, 1,3-specificity might increase with the amount of lauric acid.

Effect of buffer salts

It has been demonstrated that the presence of suspended solid buffer salts in the reaction mixture may alter the rate of acyl migration of partial glycerides dissolved in organic solvents.[9,16] Moreover, buffer salts can also control the enzyme's ionization state[17] and the thermodynamic water activity (*i.e.*, the enzyme hydration level)[18] which are critical parameters for the catalytic activity of the enzyme. In open reactors, with continuous water evaporation, it is important to keep an optimum enzyme hydration level to avoid inactivation.[19] Although the precise mechanism of water removal is not yet clearly understood, there is evidence[14] that the water generated during the reaction tends to leave the surface of the immobilized enzyme preparation, migrate to the bulk of the reaction mixture, and evaporate. The extent of this evaporation will depend upon the water activity of the reaction medium that is, in turn, controlled by the different components of the mixture such as the buffer salts.[20]

Table 1 Lipase-catalysed synthesis of 1-*O*-lauroyl- **1** and 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-Boc-L-arginine) hydrochloride **2**. Effect of buffer phosphate salts on the maximum product yields, substrate conversion, initial reaction rate and product distribution[a]

	Monolauroylation				Dilauroylation				
Phosphate salts[b] (mg mmol^{-1})	**1** Yield (%)	Reaction time (*t*/h)	Conversion (%)	me[c] (%)	**2** Yield (%)	Reaction time (*t*/h)	Conversion (%)	me[c] (%)	v_o (μmol h^{-1} mg^{-1})
0	47	72	50	88	3	72	50	88	0.4
56	68	8	78	74	44	72	89	0.1	1.5
112	65	8	76	71	44	72	88	0	1.1

[a] Reactions were carried out in open reactors. Boc–Arg–OGl·HCl (96.0 mg, 0.25 mmol) and lauric acid (0.10 g, 0.50 mmol) were mixed with plain water (500 μl) or aqueous phosphate, pH 7, buffer, 200 mM (56 mg mmol^{-1}) or 400 mM (112 mg mmol^{-1}) (500 μl) and the mixture was freeze-dried during 48 h. To the solid obtained was added water (4 μl) and the reactors were placed at 55 °C. After 10 min, CAL-B (50 mg) was added. [b] Referred to mmol of Boc–Arg–OGl·HCl. [c] Monolauroylation excess (me): (%monolauroylated − %dilauroylated/%monolauroylated + %dilauroylated)

Table 2 Influence of biocatalyst type and configuration on the maximum yields of **1** and **2** and substrate conversion[a]

	Monolauroylation			Dilauroylation		
Biocatalyst[b]	**1** Yield (%)	Reaction time (*t*/h)	Conversion (%)	**2** Yield (%)	Reaction time (*t*/h)	Conversion (%)
CAL-BL	42	24	46	6	72	44
CAL-B	84	24	91	20	72	93
CAL-BL-C545	51	48	52	3	72	54
RML	77	8	84	47	72	97
CRL	12	72	12	0	72	12
CRL-EP100	15	72	15	0	72	15
CRL-C545	16	72	16	0	72	16

[a] Conditions described in footnote *a* of Table 1. In all instances, 50 mg of enzyme preparation was added. [b] CAL-BL: Novozym® 545 L; an aliquot of this industrial preparation (74 μl) was freeze-dried and the residue (50 mg) added to the reaction. CAL-B: Novozym® 435. CAL-BL–C545: Novozym® 545 L adsorbed onto Celite 545® (1 g of liquid preparation g^{-1} of support). RML: Lipozyme® IM. CRL: lipase from *Candida rugosa* used without immobilization as powder straight from the bottle. CRL-EP100: lipase from *Candida rugosa* adsorbed onto EP100 (1 g of pure protein g^{-1} of support). CRL-C545: lipase from *Candida rugosa* adsorbed onto Celite 545® (50 mg of pure protein g^{-1} of support).

The effect of the buffer salts on the enzymatic activity and yield was investigated in the model reaction. Before any enzymatic reaction, the Boc–Arg–OGl and lauric acid were mixed with plain water or aqueous phosphate buffer and then this mixture was freeze-dried (see Experimental section). As shown in Table 1 the presence of buffer phosphate salts had a positive effect on the yield and reaction rate. The results indicated that buffer salts may either prevent excessive enzyme dehydration or promote an adequate enzyme's ionization state. Thermogravimetric analysis of the lyophilysed salts showed that they contained 6% w/w of water, which cannot be removed by simple evaporation at the reaction temperature. However, as we demonstrate below, both the ionization and hydration effects depended on the substrates used as well, therefore in some instances the presence of salts had opposite effects on the reaction performance. Concerning the product distribution, the monolauroylation excess (me) at the maximum yield of **1** and **2** decreased with the use of buffer salts because of an enhancement of the acyl-migration rate.

Biocatalyst type and configuration

The synthetic applicability of a particular biocatalyst is usually limited by its own substrate specificity. With the aim of extending the procedure to other amino acid derivatives, we tested other lipases, either 1,3-selective or nonselective, for the acylation of Boc–Arg–OGl. Furthermore, free and adsorbed lipases onto polypropylene powder (EP100) and silica (Celite 545®) were also assayed owing to the influence of the biocatalyst configuration on the reaction performance. The results of the experiments done with different free and immobilized lipases are shown in Table 2. Lipases from *Pseudomonas cepacia* (lipase PS® nonselective) and from *Aspergillus niger* (lipase A® moderately 1,3-selective) both adsorbed onto EP100 and Celite-545 failed as a catalyst for this reaction (results not shown) while the nonselective *Candida rugosa* lipase (CRL) displayed very low catalytic activity. The commercial immobilized preparations of 1,3-selective lipases from *Candida antarctica*, Novozym® 435 (CAL-B) and *Rhizomucor miehei*, Lipozyme® IM (RML) gave the best results and were used for the rest of this study. Lipases normally show little preference for saturated fatty acids[21] in a range of chains lengths between C_2 and C_{18}. Hence, the differences observed in reactivity may be exclusively related to the selectivity towards the amino acid glyceryl ester. It is worth mentioning that supported *Candida antarctica* lipase gave better results than that in free form, due to either a stabilization of biocatalyst or mass-transfer effects.[22] As seen in Table 2, RML exhibited less selectivity for the monolauroylation than did CAL-B. As observed by Millqvist-Fureby *et al.*,[9] the apparent lack of specificity of the RML preparation may be due to an increase in the acyl-migration rate catalysed by the weak anion-exchange resin support used.[23]

Synthesis of 1(2)-*O*-lauroyl-*rac*-glycero-3(2)-*O*-(N^{α}-acetyl-L-amino acid) and 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) derivatives

Enzymatic synthesis conditions. The scope of the proposed synthetic methodology was extended to the synthesis of the other target compounds. For each derivative, an optimization of the reaction yield was conducted on an analytical scale considering the previous results with the model reaction. The standard conditions were: temperature 55 °C, ratio of lauric acid to amino acid glyceryl ester 4 : 1, 56 mg of phosphate pH 7 buffer salts per mmol of amino acid glyceryl ester, and both CAL-B and RML as catalysts. These reaction variables were conveniently varied for achieving adequate reaction yields of both mono- and dilauroylated products. The optimal reaction conditions found and the corresponding reaction and isolated yields obtained are summarized in Table 3. HPLC analysis of the reaction samples revealed that the monolauroylated products gave broad peaks, whereas for the dilauroylated products

Table 3 Lipase-catalysed synthesis of 1(2)-*O*-lauroyl-*rac*-glycero-3(2)-*O*-(N^{α}-acetyl-L-amino acid) and 1,2(1,3)-di-*O*-lauroyl-*rac*-glycero-3(2)-(N^{α}-acetyl-L-amino acid) derivatives[a]

Product	Lauric acid : Ac–AA–OGl ratio	Phosphate salts mg mmol^{-1}	Temperature (θ/°C)	Reaction time (t/h)	Substrate conversion (%)	Reaction yield[b] (%)	Biocatalyst	Isolated yield[b] (g) (% of theor.)
3	4 : 1	560	65	18	75	62	CAL-B	2.0 (21)
4	8 : 1	560	65	96	97	74	RML	1.7 (22)
5	4 : 1	0	55	24	83	72	RML	0.9 (50)
6	4 : 1	280	55	72	90	58	RML	0.5 (22)
7	4 : 1	0	55	7	91	75	CAL-B	0.8 (40)
8	4 : 1	56	55	96	96	60	CAL-B	0.6 (32)
9	4 : 1	0	65	4	82	70	CAL-B	0.8 (22)
10	8 : 1	0	65	48	97	76	RML	1.3 (41)
11	4 : 1	0	65	2	80	77	CAL-B	1.1 (32)
12	8 : 1	0	65	24	86	63	RML	0.9 (25)[c]
13	4 : 1	56	55	6	91	82	RML	0.8 (46)
14	4 : 1	56	55	72	98	72	CAL-B	1.3 (69)

[a] Reaction conditions as described in the Experimental section. [b] Mixture of regioisomers. [c] Values corresponding to pure 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-glutamine) derivatives. The regioisomer 1,3-di-*O*-lauroyl-*rac*-glycero-2-*O*-(N^{α}-acetyl-L-glutamine) was obtained in 17% yield (0.6 g).

two signals were distinguished although not baseline separated (less than 0.2 min of retention time). Analysis of the samples by liquid–liquid mass spectrometry (LC/MS) demonstrated that this behaviour was caused by the different isomers of the mono- and dilauroylated products. The formation, nature and structure of these isomers are discussed below. Hence, the reaction yield, based on HPLC analysis, and the overall yields given in Table 3 correspond to mixtures of isomers of each individual amino acid glyceride conjugate.

It is clear that CAL-B and RML lipases were versatile catalysts for this reaction accepting all the target amino acid glyceryl ester derivatives for the esterification reaction. In many instances, the reaction performance of both lipases was similar. Overall, the use of RML favoured the yield of dilauroylated product due to the effect of the immobilization support on the rate of the intramolecular acyl migration.[24] Expectedly, we observed that the best reaction conditions varied largely with each amino acid glyceryl ester. For instance, the N^{α} protecting group of the arginine glyceryl ester derivative had a strong effect on both reaction performance and conditions. Using CAL-B, no reaction was observed in the absence of buffer salts with Ac–Arg–OGl compared with 50% conversion obtained with Boc–Arg–OGl (see Table 1). On the other hand, RML gave 55% conversion during the esterification of Ac–Arg–OGl in the absence of salts. The reactions needed 560 mg mmol^{-1} of buffer salts and a temperature of 65 °C to give sufficient substrate conversion for the preparation of the mono- **3** and dilauroylated **4** conjugates in gram quantities. Under these conditions, both CAL-B and RML gave similar yields of **3** (62%), while RML was much better for the dilauroylation reaction at a ratio of lauric acid to Ac–Arg–OGl of 8 : 1. As stated above, this was probably due to the effect of the support on the acyl-migration rate.[9]

Different behaviour was observed during the synthesis of aspartic acid derivatives **5**, **6**, and glutamic acid derivatives **7**, **8**. In this case, the glyceryl ester substrate conversion was not affected by the presence of buffer salts using either CAL-B or RML as catalyst. This suggest that, in this case, neither the ionization state of the enzyme nor its hydration level was affected by the buffer salts. At the same time, phosphate buffer salts had a negative influence on the yield of monolauroylated products **5** and **7**; between 43 and 58% maximum yield with buffer (280 mg mmol^{-1} of glyceryl ester substrate) as compared with 66 and 72% without salts, respectively. The same trend was also observed in the monolauroylation excess (me): from 73–88% without to 20–63% with salts, suggesting an enhancement of the rate of acyl-migration reaction by the buffer salts. Furthermore, at pH 7 (*i.e.*, in the presence of buffer salts) the $^{\beta}$COOH and $^{\gamma}$COOH of aspartic acid and glutamic acid, respectively, were in the form of sodium carboxylates, contributing to acceleration of the rate of acyl migration.

From the above observations, it is clear that the presence of buffer salts favoured the synthesis of the dilauroylated aspartic acid derivative **6**, the best yield (58%) being achieved in the presence of 280 mg mmol^{-1}. However, the synthesis of the dilauroylated derivative of glutamic acid, compound **8**, behaved somewhat differently. Surprisingly, the yield of **8** decreased with use of the buffer salt when using CAL-B (*i.e.*, from 66% without salts to 54% at 280 mg mmol^{-1}), while it was not affected by this variable using RML (52–57%). The time–progress curves revealed that the production of **8** was initially higher in the presence of salts than that in water and then levelled off after 24 h. This suggests a possible inhibition of the enzymatic dilauroylation reaction, which increased with both the presence of salts and reaction time. Hence, in this case the best yields were achieved with CAL-B either without or at low (56 mg mmol^{-1}) buffer salts content (Table 3).

The buffer salts had a negative influence on substrate conversion in the synthesis of both asparagine derivatives **9**, **10** and glutamine derivatives **11**, **12**; 46–64% substrate conversion with salts (280 mg mmol^{-1}) compared with 78–87% in systems without buffer salts, in both cases at 55 °C and a molar ratio of lauric acid to amino acid glyceryl ester of 4 : 1. The results indicated that the water balance induced by the buffer salts may not favour the equilibrium position towards the esterification products. However, although the conversions without buffer salts were acceptable, the reactions showed poor selectivity-resulting in yields ranging from 43 to 57% for the monolauroylated (**9**, **11**) and from 31 to 58% for the dilauroylated derivatives **10**, **12**. The reaction performance was further enhanced by raising either the temperature to 65 °C or the molar ratio of lauric acid to amino acid glyceryl ester to 8 : 1, or both. By increasing the temperature, the monolauroylation yield and substrate conversion increased upon using CAL-B (Table 3). The effect of raising both variables was rather the same regardless of the lipase used: improvement of both dilauroylated yields and substrate conversion (Table 3).

The reaction between tyrosine glyceryl ester and lauric acid for the production of **13** and **14**, under the standard conditions (*i.e.*, 55 °C, buffer salts 56 mg mmol^{-1}, molar ratio of lauric to tyrosine glyceryl ester of 4 : 1), afforded good reaction conversions as well as mono- **13** and dilauroylated **14** yields (Table 3). To our surprise, in this case the monolauroylation was clearly favoured using RML: 82% *versus* 62% with CAL-B. Moreover, as in the synthesis of dilauroylated glutamic acid and asparagine conjugates, CAL-B gave slightly higher dilauroylated

yields (72%) than did RML (66%). These results probably reflect the fact that the effect of the immobilization support of the RML lipase depended also on the substrates involved in the biotransformation.

Finally, the maximum initial reaction rate (v_o^m) was calculated and plotted (Fig. 3) for each starting amino acid glyceryl

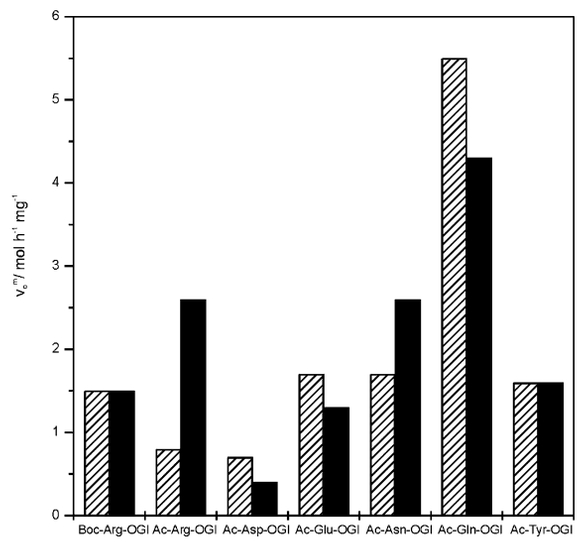


Fig. 3 Lipase-catalysed esterification of lauric acid with *rac*-1-*O*-(N^{α}-acetyl-L-aminoacyl)glycerol. Maximal initial reaction rates (v_o^m) for the different amino acid glyceryl ester derivatives at the best esterification reaction conditions using Novozym® 435 (line shading) and Lipozyme® IM (black) as catalysts.

ester. It is important to have in mind that the v_o^m-value shown in Fig. 3 corresponded to the maximum found for each substrate, so the reaction conditions are not necessarily the same in all instances. However, the data can give an idea of the reactivity of both lipases towards the amino acid glyceryl ester substrates. One can see the overwhelming preference of the lipases assayed for glutamine glyceryl ester, while the lowest reactivity was found towards the aspartic acid derivative regardless of the lipase used. It is also noteworthy that using CAL-B the v_o^m increased 2-fold upon changing the N^{α} protecting group of the arginine glyceryl ester from Ac to Boc, whereas the reverse situation was found for RML. The reactivity of both lipases towards glutamic acid, asparagine and tyrosine derivatives was rather close, especially for CAL-B.

Purification and structural characterization. Purification of the amino acid glyceride conjugates was achieved by preparative HPLC on a C4 reversed-phase column eluted with gradients of acetonitrile in aq. trifluoroacetic acid. This was a convenient method for the arginine, aspartic acid, glutamic acid, asparagine **9**, and tyrosine glyceride conjugates. Purification with flash chromatography on silica was employed successfully for the asparagine **10** and glutamine conjugates. Both methodologies provided similar recovery yields. Reversed-phase HPLC on a C4 column was better suited for ionic derivatives while silica was preferred for neutral products. Both methodologies provided highly pure products but, although the purification was not optimized, the recoveries of these particular compounds were usually low due to their inherent tendency to adsorb onto solid surfaces.

As stated above, the broad peaks observed by HPLC analysis were due to the different isomers of the products. It is noteworthy that in the case of the dilauroylated glutamine derivative the two isomers were separated and purified by flash chromatography on silica. The structures of both products were ascertained unequivocally by mono- and bidimensional NMR spectroscopic techniques. We found that the peak at $k' = 1.8$

Table 4 Lipase-catalysed synthesis of dilauroyl amino acid glyceride conjugates. Percentage of 1,3-di-*O*-lauroyl-glycero-2-*O*-(N^{α}-protected-L-amino acid) **A** (Fig. 4) in the dilauroylated products[a]

Amino acid	1,3-Dilauroylated regioisomer **A** (%)
Arginine	30
Aspartic acid	24
Glutamic acid	61
Asparagine	54
Glutamine	40[b]
Tyrosine	44

[a] The percentage of regioisomer was measured by ^{13}C NMR. [b] Calculated from the weight of pure isomer.

corresponded to the title compound **12**, 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-glutamine), and that at $k' = 2.0$ to the 1,3-di-*O*-lauroyl-glycero-2-*O*-(N^{α}-acetyl-L-glutamine) [Fig. 4 **A**

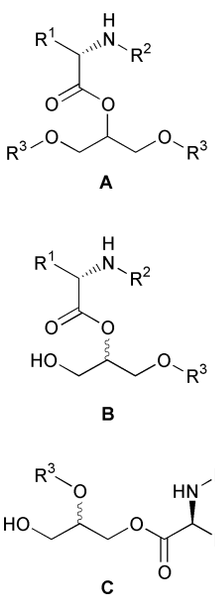


Fig. 4 Regioisomers of the mono- and dilauroylated products: 1,3-di-*O*-lauroyl-glycero-2-*O*-(N^{α}-protected-L-amino acid) **A**, 1(3)-*O*-lauroyl-*rac*-glycero-2-*O*-(N^{α}-protected-L-amino acid) **B**, 2-*O*-lauroyl-*rac*-glycero-1(3)-*O*-(N^{α}-protected-L-amino acid) **C**. R^1, R^2 and R^3 as defined in structures **1**–**14**.

with $R^1 = CH_2CH_2CONH_2$, $R^2 = CH_3CO$, $R^3 = CH_3(CH_2)_{10}CO$] regioisomers. This finding was also used to quantify the proportions of both regioisomers in the dilauroylated products by ^{13}C NMR (Table 4); the ester substitution of the secondary hydroxy group led to a signal at δ_C 69.56 as opposed to one at δ_C 70.67 for the C2 carbon of the glycerol backbone for the 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) (the title compounds) and 1,3-di-*O*-lauroyl-glycero-2-*O*-(N^{α}-acetyl-L-amino acid) (Fig. 4 **A**), respectively. The latter regioisomer could be formed if, during the reaction, the aminoacyl moiety can also migrate spontaneously to position C2 of the glycerol skeleton and if the resulting 1(3)-lauroylated product is subsequently esterified enzymatically (Scheme 1). The different proportions of regioisomer observed for each derivative (Table 4) indicated that this process depended on the amino acid residue.

Analysis of the monolauroylated products by ^1H NMR spectroscopy showed the presence of a major product, namely 1-*O*-lauroyl-*rac*-glycero-3-*O*-(N^{α}-acetyl-L-amino acid) (the title compounds) and a minor one corresponding to the acylation of the secondary hydroxy group of the glycerol moiety. This acylation was unequivocally assigned by comparing the chemical shift of the proton at C2 with that of the 2-*O*-(N^{α}-Ac-L-aspart-1-yl)glycerol, obtained in our previous work,[1] and the

Table 5 Lipase-catalysed synthesis of monolauroyl amino acid glyceride conjugates. Percentage of 1,2(2,3)-regioisomer (Fig. 4 **B** or **C**) in the monolauroylated products

Amino acid	N^{α}-Protection	1,2(2,3)-Regioisomer[a] (**B** or **C**) (%)
Arginine	Boc	13
Arginine	Ac	21
Aspartic acid	Ac	20
Glutamic acid	Ac	18
Asparagine	Ac	20
Glutamine	Ac	16
Tyrosine	Ac	63

[a] The 2-regioisomer could be either 1(3)-*O*-lauroyl-*rac*-glycero-2-*O*-(N^{α}-acetyl-L-amino acid) **B** or 2-*O*-lauroyl-*rac*-glycero-1(3)-*O*-(N^{α}-acetyl-L-amino acid) **C** and was measured by ^{1}H NMR.

dilauroylated glutamine regioisomers discussed above. However, it was not possible to distinguish whether the amino acid [*i.e.*, 1(3)-*O*-lauroyl-*rac*-glycero-2-*O*-(N^{α}-acetyl-L-amino acid) Fig. 4 **B**] or lauric acid [*i.e.*, 2-*O*-lauroyl-*rac*-glycero-1(3)-*O*-(N^{α}-acetyl-L-amino acid)] Fig. 4 **C**) was acylating this position because the chemical shift of the proton at C2 ($\delta \approx 5.2$) of the glycerol backbone was identical for both regioisomers. Moreover, no differentiation was observed in the ^{13}C NMR signals corresponding to the chemical shifts of C1, C2 and C3 of the glycerol moiety. The percentages of the 1,2(2,3)-regioisomer in the monolauroylated derivatives were calculated by ^{1}H NMR spectroscopy and are summarized in Table 5. Assuming that these values correspond to the equilibrium values, the percentage of the 1,2(2,3)-regioisomer (13–20%) was lower than in conventional glycerides (40%)[9] with the exception of the tyrosine derivative. The driving force of isomerization (*i.e.*, intramolecular acyl migration) is believed to be steric, so that linear structures, which are more thermodynamically stable, are favoured.[15,25] Moreover, differences in the positions of equilibrium concentrations may be explained by van der Waals, hydrophobic and/or steric interactions between the adjacent molecules acylating the glycerol.[25] Branched and hydrophilic amino acids may favour the equilibrium towards the 1,3-regioisomer, even more than in conventional diglycerides. On the other hand, the hydrophobic attractions between the tyrosine residue and the lauroyl chain may be the major effect causing the shift, in this case, of the regioisomeric equilibrium to favour the 1,2(2,3)-regioisomer (63%). Furthermore the relatively low percentage of 1,2(2,3)-regioisomer observed with the Boc derivative is noteworthy and may be attributed to steric hindrance of the bulky Boc group.

As ascertained by ^{13}C NMR spectroscopy the monolauroylated and dilauroylated products, except the 1,3-di-*O*-lauroyl-glycero-2-*O*-(N^{α}-acetyl-L-amino acid), were mixtures (approximately 50 : 50) of diastereoisomers. In the dilauroylated products, they were distinguishable mainly by the C3 [CH_2O(aminoacyl)–CHO(lauroyl)-CH_2O(lauroyl)] carbon atom, while the monolauroylated products could be distinguished either by the C1, C2 or C3 carbon atoms of the glycerol backbone. It seems likely that the lipases used could not distinguish between the two diastereoisomers of the starting *rac*-1-*O*-(N^{α}-acetyl-L-aminoacyl)glycerol derivatives and those generated during the monolauroylation reaction.

In light of these observations, we conclude that the reaction pathway leading to the formation of the amino acid glyceride conjugates by lipases in solvent-free systems involves esterification and acyl-migration steps of both the aminoacyl and the lauroyl moieties (Scheme 1). The percentage of regioisomers in the monolauroylated products depended on the isomerization equilibrium for each compound and, in turn, on the amino acid residue. The percentage of 1,3-dilauroylated regioisomer depended on the relative tendency of either the lauroyl or aminoacyl moiety to migrate. The results, shown in Table 4, suggest that when the amino acid was asparagine, glutamine or tyrosine the migration of the lauroyl and the amino acid residues took place at a similar rate. For arginine and aspartic acid the lauroyl moiety was more prone to migrate than the amino acid residue, whereas for glutamic the opposite situation is true. In summary, by changing the reaction variables it is possible to drive the esterification reaction of *rac*-1-*O*-(N^{α}-acetyl-L-aminoacyl)-glycerol derivatives towards either mono- or dilauroylation while the proportions of regioisomers in the final products depended mainly on the amino acid residue.

Experimental

Materials

Candida antarctica lipase, Novozym® 435 (CAL-B) (EC 3.1.1.3) (7000 PLU g-1, PLU: propyl laurate units), *Candida antarctica* liquid preparation, Novozym® 545 (CAL-BL) and *Rhizomucor miehei* lipase, Lipozyme® IM (RML) were a generous gift from Novo Nordisk A/S (Bagsvaerd, Denmark). *Aspergillus niger* lipase (lipase A®, 136 000 U g^{-1} Amano's method) and *Pseudomonas cepacia* lipase (lipase PS®, 32 100 U g^{-1} Amano's method) were generously donated by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). *Candida rugosa* lipase (CRL) [240 U mg^{-1}, one unit (U) corresponds to 1 μmol of fatty acid liberated in the assay conditions described by Sánchez *et al.*[26]] was donated by the Dept. of Chemical Engineering (UAB) (Bellaterra, Spain). *rac*-1-*O*-(N^{α}-*tert*-Butoxycarbonyl-L-argininyl) glycerol hydrochloride (Boc–Arg–OGl·HCl), *rac*-1-*O*-(N^{α}-acetyl-L-argininyl)glycerol hydrochloride (Ac–Arg–OGl·HCl), *rac*-1-*O*-(N^{α}-acetyl-L-aspart-1-yl)glycerol (Ac-Asp-OGl), *rac*-1-*O*-(N^{α}-acetyl-L-glutam-1-yl)glycerol (Ac-GLu-OGl), *rac*-1-*O*-(N^{α}-acetyl-L-asparaginyl)glycerol (Ac–Asn–OGl), *rac*-1-*O*-(N^{α}-acetyl-L-glutaminyl)glycerol (Ac–Gln–OGl) and *rac*-1-*O*-(N^{α}-acetyl-L-tyrosinyl)glycerol (Ac–Tyr–OGl) were synthesized in our lab.[1] Celite® [type 545, particle size 26 μm; mean pore diameter 17 000 nm; specific surface area (BET method) 2.19 m^2 g^{-1}] was obtained from Fluka. Polypropylene EP100 [particle size 200–400 μm; mean pore diameter 900 nm; specific surface area (BET method) 78 m^2 g^{-1}] was a generous gift from Akzo (Obernburg, Germany). Solvents and other chemicals used in this work were of analytical grade.

Instrumentation

Mass spectrometry and HPLC-mass spectrometry (LC/MS) analyses were performed at the Serveis Departamentals d'Assistencia a la Investigació of the University of Lleida. LC-Electrospray-MS analyses were recorded on a Waters 2690 (Milford, MA, USA) solvent-management system interfaced with a Waters/Micromass ZMD single-quadrupole mass spectrometry and with a Waters 996 diode-array detector. Compounds were eluted with CH_3CN gradients in aq. trifluoroacetic acid (0.1%) under the conditions described below. High-resolution mass spectrometry (HR–FAB–MS) analyses were performed at the Unidad de Espectrometría de Masas of the University of Córdoba. Nuclear magnetic resonance (NMR) analyses were carried out at the Instituto de Investigaciones Químicas y Ambientales-CSIC and at the Serveis Cientificotècnics (Unitat de Ressonància Magnètica Nuclear) of the University of Barcelona. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were observed with a Unity-300 spectrometer from Varian (Palo Alto, California, USA) for d_6-DMSO and CD_3OD solutions. Homonuclear ^{1}H–^{1}H (COSY and TOCSY-70 experiments), one-bond ^{1}H–^{13}C (HSQC) and two- and three-bond ^{1}H–^{13}C connectivities (HMBC experiments) were measured on a Varian Inova 500 500 MHz spectrometer. Specific rotations were measured with a Perkin Elmer model 341 (Überlingen, Germany) polarimeter. $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}.

Methods

Enzyme adsorption. The general procedure for the deposition of the enzymes onto solid support materials was the following. An enzyme solution in the proper buffer (1 ml) was mixed thoroughly with the support (1 g). The mixture was then evaporated under vacuum overnight. For lipase A®, lipase PS®, 100 mg of enzyme g^{-1} of Celite® were dissolved in 50 mM Tris·HCl, pH 7.8, buffer. Adsorption of lipases onto polypropylene powder EP100 was carried out following the methodology described by Gitlesen *et al.*[27] using 20 mM phosphate buffer, pH 7.0.

HPLC analysis. The amount of reactants and products in the enzymatic reactions was measured by HPLC analysis. HPLC analyses were performed on a Merck-Hitachi (Darmstadt, Germany) Lichrograph system using a Lichrocart 250–4 HPLC cartridge, 250 × 4 mm, filled with Lichrosphere® 100 CN (Propylcyano), 5 μm or Lichrosphere® 100, RP-18, 5 μm (Merck). Samples (5–10 mg) were withdrawn from the reaction medium at different times: immediately before addition of the enzyme and at 0.5, 1, 2, 3, 4, 8, 24, 48 and 72 h. The samples were dissolved with a solution of acetonitrile–water–acetic acid 16 : 3 : 1 (2 ml) to stop any further enzymatic reaction, centrifuged to separate the solid particles, and the supernatant was analysed directly by HPLC on the 100 CN (Propylcyano) column. This provided the qualitative and quantitative analysis of the lauric acid, mono- and dilauroylated products. After that, an aliquot of the supernatant obtained previously (500 μl) was diluted with pure water (500 μl) and the solid formed was centrifuged. The supernatant was evaporated under vacuum to dryness, partly dissolved in water (500 μl), and the residue was centrifuged again. The supernatant was analysed directly by HPLC on the C18 column, allowing the quantification of the amino acid glyceryl ester derivative. Quantitative analysis was performed from peak areas by means of the external standard method. Preparative HPLC runs were performed on a Waters (Milford, MA, USA) Prep LC 4000 pumping system and a Waters PrepPack® 1000 module fitted with a PrepPack® (Waters) column (47 × 300 mm) filled with Deltapack C4, 300 Å, 15 μm stationary phase.

Enzymatic reactions. Reactions on an analytical scale were carried out in 5 ml open flasks placed on a hot-plate thermostatted at 45, 50, 55 or 65 °C depending on the experiment. The reactants were prepared as follows. The amino acid glyceryl ester derivative (0.25 mmol) was dissolved in water (500 μl) or aq. phosphate (200 mM, 400 mM or 1 M), pH 7, buffer (500 μl or 1000 μl depending on the mg of phosphate salts per mmol of amino acid glyceryl ester derivative used). To this solution was added lauric acid (0.25, 0.38, 0.5, 1 or 2 mmol depending on the experiment). After being stirred vigorously with vortex and ultrasonication, the mixture was freeze-dried during 48 h. Then water (4 μl, 0.22 mmol) was added to the resulting solid and the reactors were placed on a hot-plate at the corresponding temperature. After 10 min, the reactions were started by the addition of the immobilized or free enzyme (50 mg).

HPLC reaction monitoring. *Synthesis of **1** and **2**.* Solvent system: solvent [A]: 0.1% (v/v) trifluoroacetic acid (TFA) in H_2O, solvent [B]: 0.085% (v/v) TFA in H_2O–CH_3CN 1 : 4, gradient elution from 40 to 100% B in 30 min; retention factor (k') for each product was: lauric acid (5.0), **1** (7.0) and **2** (10.9).

*Synthesis of **3** and **4**.* Solvent system solvent [C]: aq. triethylamine–phosphoric acid buffer, pH 6.0 (TEAP pH 6.0) in H_2O, solvent [D]: TEAP, pH 6.0–CH_3CN 1 : 4, gradient elution from 35 to 100% [D] in 26 min, k'-values for each product were: lauric acid (2.6), **3** (6.2) and **4** (11.6). For the LC/MS analysis of **3** and **4** the same gradient elution was used but the solvent system was [A] and [B]. Under these conditions, no separation between lauric acid and **3** was obtained.

*Synthesis of **5**–**12**.* Gradient elution from 35 to 100% [B] in 26 min.

*Synthesis of **13** and **14**.* Gradient elution from 40 to 100% [B] in 26 min; k'-values for each product were: lauric acid (6.0), **5** (5.1) and **6** (9.1), **7** (5.3), **8** (9.1), **9** (4.1), **10** (8.6), **11** (4.3), **12** (8.6), **13** (5.2) and **14** (10.0). HPLC analysis of the corresponding *rac*-3-*O*-(N^{α}-acetyl-L-aminoacyl)glycerol was performed under the conditions described in the previous paper.[1] In all cases the flow rate was 1 ml min^{-1} and UV detection at 254 or 215 nm.

Enzymatic reactions at the preparative level. Reactions were carried out in 250 ml open flasks. Scale-up of the reactions was made on the basis of approximately 1–2 g of pure product, considering the reaction and purification yields obtained at analytical level. The procedure was the same as at the analytical level. The *rac*-1-*O*-(N^{α}-acetyl-L-aminoacyl)glycerol (2.9–18.3 mmol) was dissolved in water or aqueous phosphate, pH 7, buffer at the adequate molarity depending on the required mg of phosphate salts per mmol of amino acid glyceryl ester. To this solution was added lauric acid (11.6–73.2 mmol) and after stirring the mixture was freeze-dried for 48 hours. To the solid obtained was added water [4% w/w with respect to the *rac*-1-*O*-(N^{α}-acetyl-L-aminoacyl)glycerol] and the reactor was placed at the corresponding temperature. The reactions were initiated by the addition of the enzyme (0.6–3.7 g) and monitored by HPLC. At the end of the reaction the mixture was diluted with a solution of CH_3CN–water–acetic acid 16 : 3 : 1 [2 ml per mmol of initial *rac*-1-*O*-(N^{α}-acetyl-L-aminoacyl)glycerol] to stop any further enzymatic reaction and to dissolve the solid particles of product and substrates. This process was conducted at the reaction temperature. The resulting mixture was filtered through Celite®-545 (100 ml of bed volume) and then the filter cake was washed with CH_3CN–water–acetic acid 16 : 3 : 1 (4 × 100 ml). The filtrated was cooled to −20 °C overnight and during this time a precipitate appeared, which was filtered off. The filtrates always contained the monolauroylated products with the presence of some dilauroylated compounds, amino acid glyceryl ester, and minor quantities of unchanged lauric acid. The amount of dilauroylated product depended on both the selectivity of the reaction and the product's solubility properties. Filtrates were concentrated under vacuum and during the process more lauric acid precipitated out. An exception was the monolauroylated glutamic acid derivative **7** for which the product and lauric acid precipitated out together during this operation. In this case, after filtration the residue was re-dissolved in CH_3CN; the solution was cooled to separate selectively the lauric acid as a solid. The dilauroylated products and the unchanged lauric acid were mainly located in the residues with the exception of the arginine (**4**) and glutamic acid (**8**) derivatives which were in the filtrate and the asparagine **10** which was 50% distributed in the filtrate and residue. In this latter case, the filtrate was concentrated until both **10** and lauric acid precipitated out again and the residues were pooled. Filtrates and residues containing the crude materials were dried under vacuum and purified by preparative HPLC on reversed-phase C4 or flash chromatography on silica.

Purification procedures. *Reversed-phase chromatography on C4*. This methodology was used for compounds **1**, **3**–**9**, **13** and **14**. An aliquot of the residue containing crude product (2 g) was loaded onto the preparative PrepPack® (Waters) column (47 × 300 mm), filled with Deltapack C4, 300 Å 15 μm cartridge, and eluted using an aq. TFA solution (0.1% v/v) and gradient elution from 30 to 56% CH_3CN in the aq. TFA in 30 min for the monolauroylated products and from 54 to 100% CH_3CN in the aq. TFA in 30 min for the dilauroylated compounds. The flow rate was 100 ml min^{-1} and the products were detected at 215 nm.

Purification of asparagine (**10**) and glutamine (**11**, **12**) derivatives was accomplished by flash chromatography on silica (bed volume 150 ml). The crude (2 g) was loaded onto the silica (60 A CC Chromagel 35–70 microns, SDS, Peypin, France) and washed with methylene dichloride (1 l) (**11**, **12**) or diethyl ether (1 l) (**10**) to eliminate the lauric acid. Then the products were eluted isocratically with a solution of CH_2Cl_2–acetone 1 : 1 (35 × 25 ml) for **11** and **12**, or $CHCl_3$ (10 × 25 ml) for **10**.

In all cases, the operation was repeated until the whole crude was consumed. Analysis of the fractions was accomplished by HPLC using the conditions previously described. Pure fractions were pooled and lyophilysed from CH_3CN–water mixtures.

Purity of the products as mixtures of regioisomers was assessed by HPLC. The relative amounts of each regioisomer were measured by NMR spectroscopy. Physical and spectroscopy data for each compound are given below.

1-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-Boc-L-arginine) hydrochloride ***1***. Purity of the regioisomeric mixture was 92.3% by HPLC. NMR: δ_H(300 MHz; d_4-MeOH) 5.18 (0.13 H, m, C*H*OCO-(aminoacyl or lauroyl) regioisomers type **B** or **C**), 4.19–3.99 (5H, m, NHC*H*CO + COOCH_2CHOH + CHOHCH_2OCO(CH$_2$)$_{10}$CH$_3$), 3.68 (1H, m, C*H*OH), 3.20 (2H, t, NHCH_2CH$_2$), 2.36 (2H, t, CH$_2$OCOCH_2(CH$_2$)$_9$CH$_3$), 1.68–1.59 (6H, m, CHCH_2CH_2CH$_2$ + CH$_2$OCOCH$_2$CH_2(CH$_2$)$_8$CH$_3$), 1.44 (9 H, s, 3 × CH_3), 1.29 (16H, s, CH_2), 0.90 (3H, t, CH_3CH$_2$); δ_C(75 MHz; d_4-MeOD) 175.26 (H$_2$CO*C*O-(CH$_2$)$_{10}$CH$_3$), 173.64 (CH*C*OCH$_2$), 158.63 (*C*=N guanidine function), 157.32 ((CH$_3$)*C*CONH), 80.79 ((CH$_3$)*C*CONH), 68.32 (*C*HOH), 66.64 (O*C*H$_2$CHOH), 65.92 (*C*H$_2$OCO-(CH$_2$)$_{10}$CH$_3$), 54.58 (NH*C*HCO), 41.86 (*C*H$_2$NH), 34.87–25.98 (*C*H$_2$), 28.69 ((*C*H$_3$)$_2$C), 26.31 (CH*C*H$_2$CH$_2$CH$_2$), 23.73 (CHCH$_2$*C*H$_2$CH$_2$), 14.45 (*C*H$_3$CH$_2$); *m*/*z* (ES) 348.8 ([M + H]$^+$. $C_{14}H_{29}N_4O_6$ requires *m*/*z*, 349.2).

1-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-arginine) hydrochloride ***3***. Purity of the regioisomeric mixture was 94.1% by HPLC. NMR: δ_H(300 MHz; d_4-MeOH) 5.18 (0.21 H, m, C*H*O-CO-(aminoacyl or lauroyl) regioisomers type **B** or **C**), 4.45 (1H, m, NHC*H*CO), 4.20–4.03 (4H, m, COOCH_2CHOH + CHOHCH_2OCO(CH$_2$)$_{10}$CH$_3$), 3.72 (1H, m, C*H*OH), 3.24 (2H, t, NHCH_2CH$_2$), 2.36 (2H, t, CH$_2$OCOCH_2(CH$_2$)$_9$CH$_3$), 2.00 (3H, s, CH_3CO), 1.80–1.53 (6H, m, CH_2), 1.29 (16H, s, CH_2), 0.90 (3H, t, CH_3CH$_2$); δ_C(75 MHz; d_4-MeOD) 175.27 (H$_2$CO*C*O-(CH$_2$)$_{10}$CH$_3$), 173.53 (CH*C*OCH$_2$), 172.99 (CH$_3$*C*O), 158.61 (*C*=N guanidine function), 68.29 (*C*HOH), 66.81 (O*C*H$_2$-CHOH), 65.86 (*C*H$_2$OCO(CH$_2$)$_{10}$CH$_3$), 53.38 (NH*C*HCO), 41.85 (*C*H$_2$NH), 35.57–23.74 (*C*H$_2$), 22.33 (*C*H$_3$CO), 14.45 (*C*H$_3$CH$_2$); *m*/*z* (ES) 473.2 ([M + H]$^+$. $C_{23}H_{45}N_4O_6$ requires *m*/*z*, 473.3); $[\alpha]_D^{20}$ −9.16 (*c* 1 in MeOH) (Found: C, 51.6; H, 9.2; N, 10.7. $C_{23}H_{45}ClN_4O_6$·1.5H$_2$O requires C, 51.6; H, 9.0; N, 10.5%).

1,2-Di-O-lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-arginine) hydrochloride ***4***. Purity of the regioisomeric mixture was 97.3% by HPLC. NMR: δ_H(300 MHz; d_4-MeOH) 5.29 (1 H, m, COCH$_2$C*H*OCO), 4.44–4.10 (5 H, m, NHC*H*COOCH_2 + *H*$_2$COCO(CH$_2$)$_{10}$CH$_3$), 3.21 (2 H, t, NHCH_2CH$_2$), 2.33 (4 H, m, CHOCOCH_2(CH$_2$)$_9$CH$_3$ + H$_2$COCOCH_2(CH$_2$)$_9$CH$_3$), 2.0 (3 H, s, CH_3CO), 1.66 (6 H, m, CH_2 + CHOCOCH$_2$CH_2-(CH$_2$)$_8$CH$_3$ + H$_2$COCOCH$_2$CH_2(CH$_2$)$_8$CH$_3$), 1.29 (32 H, s, CH_2), 0.90 (6 H, t, 2 × CH_3CH$_2$); δ_C(75 MHz; d_4-MeOD) 174.84 (H$_2$CO*C*O(CH$_2$)$_{10}$CH$_3$ regioisomer type **A**), 174.79 (O*C*O-(CH$_2$)$_{10}$CH$_3$), 172.99 (CH*C*OCH$_2$), 173.47 (CHO*C*OCH(CH$_2$-CH$_2$NHC(NH$_2$)$_2$)(NHCOCH$_3$) regioisomer type **A**), 172.43 (CH$_3$*C*O), 158.72 (*C*=N guanidine group), 71.82 (*C*HOCO-CH(CH$_2$CH$_2$NHC(NH$_2$)$_2$)(NHCOCH$_3$) regioisomer type **A**), 70.47 (*C*HOCOCH_2(CH$_2$)$_9$CH$_3$), 64.16 (*C*H$_2$OCO(CH$_2$)$_{10}$CH$_3$ regioisomer type **A**), 64.07 (O*C*H$_2$CHOH), 63.00 (*C*H$_2$OCO-(CH$_2$)$_{10}$CH$_3$), 53.26 (NH*C*HCO), 41.87 (*C*H$_2$NH), 35.02 (*C*H$_2$), 22.33 (*C*H$_3$CO), 14.47 (*C*H$_3$CH$_2$); *m*/*z* (ES) 655.6 ([M + H]$^+$. $C_{35}H_{67}N_4O_7$ requires *m*/*z*, 655.5); $[\alpha]_D^{20}$, −7.95 (*c* 1 in MeOH) (Found: C, 59.3; H, 10.2; N, 7.7. Calc. for $C_{35}H_{67}ClN_4O_7$·H$_2$O: C, 59.3; H, 9.8; N, 7.9%).

1-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-α-aspartic acid) ***5***. Purity of the regioisomeric mixture was 98.8% by HPLC. NMR: δ_H(300 MHz; d_6-acetone) 12.22 (1H, s, β-COO*H*), 8.12 (1H, d, CON*H*CH), 4.76 (1H, m, NHC*H*CO), 5.05 (0.20 H, m, C*H*OCO-(aminoacyl or lauroyl) regioisomers type **B** or **C**), 4.18–3.99 (4H, m, CHCOOCH_2 + CHOHCH_2OCO-(CH$_2$)$_{10}$CH$_3$), 3.65 (1H, m, COOCH$_2$C*H*OH), 2.85 (2H, m, CHOHCH_2COO(CH$_2$)$_{10}$CH$_3$), 2.31–2.02 (2 H, m, CHCH_2-COOH), 2.01 (3H, s, CH_3CO), 1.59 (2H, t, H$_2$COCOCH$_2$-CH_2(CH$_2$)$_8$CH$_3$), 1.27 (16H, s, CH_2), 0.86 (3H, t, CH_3CH$_2$); δ_C(75 MHz; d_6-acetone) 172.62 (β-*C*OOH), 172.01 (CH$_2$O*C*-OCH$_2$), 170.84 (NHCH*C*OO), 169.96 (CH$_3$*C*ONH), 66.02 (*C*HOH), 65.07 (O*C*H$_2$CHOH), 64.62 (*C*H$_2$OCO), 48.42 (NH*C*HCO), 35.89 (CH*C*H$_2$COOH), 33.29–21.89 (*C*H$_2$), 22.17 (*C*H$_3$CO), 13.73 (*C*H$_3$CH$_2$); *m*/*z* (ES) 432.2 ([M + H]$^+$. $C_{21}H_{37}NO_8$ requires *M*, 431.3); $[\alpha]_D^{20}$ +2.37 (*c* 1 in acetone) (Found: C, 57.3; H, 8.7; N, 3.2. Calc. for $C_{21}H_{37}NO_8$·0.5H$_2$O: C, 57.2; H, 8.6; N, 3.2%).

1,2-Di-O-lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-α-aspartic acid) ***6***. Purity of the regioisomeric mixture was 98.7% by HPLC. NMR: δ_H(300 MHz; d_6-acetone) 12.22 (1H, s, β-COO*H*), 7.42 (1H, d, CON*H*CH), 5.25 (1H, m, COCH$_2$-C*H*OCO), 4.78 (1H, m, NHC*H*CO), 4.30–4.13 (4H, m, COOCH_2CHOCO + CH_2OCO(CH$_2$)$_{10}$CH$_3$), 2.87 (2H, m, CHCH_2COOH), 2.31 (4H, m, CHOCOCH_2(CH$_2$)$_9$CH$_3$ + H$_2$COCOCH_2(CH$_2$)$_9$CH$_3$), 1.94 (3H, s, CH_3CO), 1.58 (4H, t, CHOCOCH$_2$CH_2(CH$_2$)$_8$CH$_3$ + H$_2$COCOCH$_2$CH_2(CH$_2$)$_8$CH$_3$), 1.23 (32 H, s, CH_2), 0.84 (6H, t, 2 × CH_3CH$_2$); δ_C(75 MHz; d_6-acetone) 173.37 (β-*C*OOH), 173.16 (O*C*OCH_2(CH$_2$)$_9$CH$_3$ regioisomer type **A**), 172.33 (O*C*O(CH$_2$)$_{10}$CH$_3$), 170.82 (O*C*O-CH(CH$_2$COOH)(NHCOCH$_3$)), 170.16 (O*C*OCH(CH$_2$COOH)-(NHCOCH$_3$) regioisomer type **A**), 168.62 (CH$_3$*C*O), 70.54 (*C*HOCOCH(CH$_2$COOH)(NHCOCH$_3$) regioisomer type **A**), 69.70 (*C*HOCOCH_2(CH$_2$)$_9$CH$_3$), 63.20 (NHCHCOO*C*H$_2$), 62.73 (*C*H$_2$CO(CH$_2$)$_{10}$CH$_3$ regioisomer type **A**), 62.51 (*C*H$_2$-COCH_2(CH$_2$)$_9$CH$_3$), 49.54 (NH*C*HCO), 36.87 (*C*H$_2$COOH), 34.56–23.25 (*C*H$_2$), 22.61 (*C*H$_3$CO), 14.27 (*C*H$_3$CH$_2$); *m*/*z* (ES) 614.6 ([M + H]$^+$. $C_{33}H_{59}NO_9$ requires *M*, 613.4); $[\alpha]_D^{20}$ +2.93 (*c* 1 in acetone) (Found: C, 64.6; H, 10.0; N, 2.4. Calc. for $C_{33}H_{59}NO_9$: C, 64.6; H, 9.7; N, 2.3%).

1-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-α-glutamic acid) ***7***. Purity of the regioisomeric mixture was 99.9% by HPLC. NMR: δ_H(300 MHz; d_6-DMSO) 12.05 (1 H, s, γ-COO*H*), 8.15 (1 H, d, CON*H*CH), 4.94 (0.18 H, m, C*H*OCO-(aminoacyl or lauroyl) regioisomers regioisomer type **B** or **C**), 4.23 (1 H, m, NHC*H*CO), 4.04 (4 H, m, NHCHCOOCH_2 + CH_2OCO), 3.87 (1 H, m, C*H*OH), 2.28 (4 H, m, CH$_2$CH_2COOH + CH$_2$OCO-CH_2(CH$_2$)$_9$CH$_3$), 1.99–1.74 (5 H, m, CH_3CO + CHCH_2CH$_2$-COOH), 1.51 (2 H, t, CH$_2$OCOCH$_2$CH_2(CH$_2$)$_8$CH$_3$), 1.23 (16 H, s, CH_2), 0.84 (3 H, t, CH_3CH$_2$); δ_C(75 MHz; d_6-DMSO) 173.36 (γ-*C*OOH), 172.58 (CHOHCH$_2$O*C*OCH_2(CH$_2$)$_9$CH$_3$), 171.54 (NHCH*C*OO), 169.32 (CH$_3$*C*ONH), 66.02 (*C*HOH), 65.21 (O*C*H$_2$CHOH), 64.58 (*C*H$_2$OCOCH_2(CH$_2$)$_9$CH$_3$), 51.23 (NH*C*HCO), 33.28–22.03 (*C*H$_2$), 29.78 (CH$_2$*C*H$_2$COOH), 26.09 (*C*H$_2$CH$_2$COOH), 21.89 (*C*H$_3$CO), 13.72 (*C*H$_3$CH$_2$); *m*/*z* (ES) 446.4 ([M + H]$^+$. $C_{22}H_{39}NO_8$ requires *M*, 445.3); $[\alpha]_D^{20}$ −9.0 (*c* 1 in acetone). High-resolution MS *m*/*z* (FAB+) 446.2764 [M + H]$^+$. $C_{22}H_{40}NO_8$ requires *m*/*z*, 446.2754.

1,2-Di-O-lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-α-glutamic acid) ***8***. Purity of the regioisomeric mixture was 98.0% by HPLC. NMR: δ_H(300 MHz; d_6-DMSO) 12.10 (1 H, s, γ-COO*H*), 8.20 (1H, d, CON*H*CH), 5.18 (1 H, m, COCH$_2$-C*H*OCO), 4.25–4.10 (5 H, m, C*H*COOCH_2CHOCO + *H*$_2$CCO(CH$_2$)$_{10}$CH$_3$), 2.26 (6 H, m, CH$_2$CH_2COOH + HCO-COCH_2(CH$_2$)$_9$CH$_3$ + H$_2$COCOCH_2(CH$_2$)$_9$CH$_3$), 1.85 (5 H, m, CH_2CH$_2$COOH + CH_3CO), 1.50 (4H, t, HCOCOCH$_2$CH_2-(CH$_2$)$_8$CH$_3$ + H$_2$COCOCH$_2$CH_2(CH$_2$)$_8$CH$_3$), 1.23 (32 H, s, CH_2), 0.84 (6 H, t, 2 × CH_3CH$_2$); δ_C(75 MHz; d_6-DMSO) 172.33 (γ-*C*OOH), 172.29 (CH$_2$O*C*O(CH$_2$)$_{10}$CH$_3$ regioisomer type **A**), 171.98 (O*C*O(CH$_2$)$_{10}$CH$_3$), 171.14 (O*C*OCH(CH$_2$-

$CH_2COOH)(NHCOCH_3)$ regioisomer type **A**), 171.56 (CH-$OCOCH(CH_2CH_2COOH)(NHCOCH_3)$), 169.24 ($CH_3CO$), 69.29 ($CHOCOCH(CH_2CH_2COOH)(NHCOCH_3)$ regioisomer type **A**), 68.48 ($CHOCO(CH_2)_{10}CH_3$), 62.19 (NHCHCOO-CH_2), 61.74 ($CH_2OCO(CH_2)_{10}CH_3$ regioisomer type **A**), 61.51 ($CH_2CO(CH_2)_{10}CH_3$), 51.38 (NH$CHCO$), 33.30–21.88 (CH_2), 29.84 (CH_2COOH), 26.02 (CH_2CH_2COOH), 21.88 (CH_3CO), 13.70 (CH_3CH_2); *m/z* (ES) 628.6 ($[M + H]^+$. $C_{34}H_{61}NO_9$ requires *M*, 627.4); $[\alpha]_D^{20}$ −7.42 (*c* 1 in acetone) (Found: C, 63.2; H, 9.8; N, 2.9. Calc. for $C_{34}H_{61}NO_9 \cdot 0.5CH_3CN \cdot H_2O$: C, 63.0; H, 9.7; N, 3.2%).

1-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-asparagine) ***9***. Purity of the regioisomeric mixture was 98.8% by HPLC. NMR: δ_H(300 MHz; d_6-acetone) 7.48 (1 H, d, CONHCH), 7.09 (1 H, s, $CONH_2$), 6.51 (1 H, s, $CONH_2$), 5.18 (0.20 H, m, CHOCO-(aminoacyl or lauroyl) regioisomers type **B** or **C**), 4.71 (1 H, m, NHCHCO), 4.27–4.01 (5 H, m, $COOCH_2CH$-OH + $H_2COCO(CH_2)_{10}CH_3$), 2.88–2.67 (2 H, m, $CHCH_2$-$CONH_2$), 2.30 (2 H, t, $H_2COCOCH_2(CH_2)_9CH_3$), 1.94 (3 H, s, CH_3CO), 1.58 (2 H, t, $H_2COCOCH_2CH_2(CH_2)_8CH_3$), 1.27 (16 H, s, CH_2), 0.86 (3 H, t, CH_3CH_2); δ_C(75 MHz; d_6-acetone) 173.62 ($CHCH_2CONH_2$), 172.83 ($CH_2OCOCH_2CH_2(CH_2)_8$-CH_3), 171.86 ($CHCOOCH_2$), 170.28 (CH_3CO), 67.99 ($CHOH$), 66.59 ($CHCOOCH_2CHOH$), 65.38 ($CH_2OCOCH_2CH_2(CH_2)_8$-CH_3), 49.94 (NHCHCO), 37.70 (CH_2CONH_2), 34.40–23.26 (CH_2), 22.65 (CH_3CO), 14.31 (CH_3CH_2); *m/z* (ES) 431.3 ($[M + H]^+$. $C_{21}H_{38}N_2O_7$ requires *M*, 430.3); $[\alpha]_D^{20}$ +1.19 (*c* 1 in acetone). High-resolution MS *m/z* (FAB+) 431.2752 $[M + H]^+$. $C_{21}H_{39}N_2O_7$ requires *m/z*, 431.2757.

1,2-Di-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-asparagine) ***10***. Purity of the regioisomeric mixture was 99.8% by HPLC. NMR: δ_H(300 MHz; d_6-acetone) 7.34 (1H, d, CONHCH), 6.92 (1H, s, $CONH_2$), 6.35 (1H, s, $CONH_2$), 5.23 (1H, m, $COCH_2$-$CHOCO$), 4.71 (1H, m, NHCHCO), 4.37–4.14 (4H, m, $CHCOOCH_2$ + $H_2COCO(CH_2)_{10}CH_3$), 2.85–2.64 (2H, m, CH-CH_2CONH_2), 2.29 (4H, m, $CHOCOCH_2(CH_2)_9CH_3$ + H_2CO-$COCH_2(CH_2)_9CH_3$), 1.92 (3H, s, CH_3CO), 1.58 (4H, t, $CHOCOCH_2CH_2(CH_2)_8CH_3$ + $H_2COCOCH_2CH_2(CH_2)_8CH_3$), 1.28 (32H, s, CH_2), 0.87 (6H, t, 2 × CH_3CH_2); δ_C(75 MHz; d_6-acetone) 173.45 ($CHCH_2CONH_2$), 173.18 ($OCO(CH_2)_{10}CH_3$ regioisomer type **A**), 171.77 ($OCO(CH_2)_{10}CH_3$), 171.75 (NHCH$COOCH_2$), 171.44 ($CHOCOCH(CH_2CONH_2)$(NH-$COCH_3$) regioisomer type **A**), 169.90 (CH_3CO), 70.89 ($CHOCOCH(CH_2CONH_2)(NHCOCH_3)$ regioisomer type **A**), 69.74 ($CHOCO(CH_2)_{10}CH_3$), 63.49 ($CHCOOCH_2$), 62.78 ($CH_2OCO(CH_2)_{10}CH_3$ regioisomer type **A**), 62.48 (H_2COCO-$(CH_2)_{10}CH_3$), 49.83 (NHCHCO), 37.44 (CH_2CONH_2), 34.54–23.29 (CH_2), 22.66 (CH_3CO), 14.32 (CH_3CH_2); *m/z* (ES) 613.0 ($[M + H]^+$. $C_{33}H_{60}N_2O_8$ requires *M*, 612.4); $[\alpha]_D^{20}$ +11.84 (*c* 1 in $CHCl_3$) (Found: C, 63.5; H, 9.75; N, 4.8. Calc. for $C_{33}H_{60}N_2O_8 \cdot 0.5H_2O$: C, 63.7; H, 9.8; N, 4.5%).

1-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-glutamine) ***11***. Purity of the regioisomeric mixture was 99.2% by HPLC. NMR: δ_H(300 MHz; d_6-acetone) 7.67 (1 H, d, CONHCH), 6.93 (1 H, s, $CONH_2$), 6.35 (1 H, s, $CONH_2$), 5.08 (0.16 H, m, CHOCO-(aminoacyl or lauroyl) regioisomers type **B** or **C**), 4.58 (1 H, m, NHCHCO) 4.38 (1 H, m, $COOCH_2CHOH$), 4.42–4.01 (4 H, m, $COOCH_2CHOH$ + $H_2COCO(CH_2)_{10}CH_3$), 2.28 (4 H, m, $CH_2CH_2CONH_2$ + $H_2COCOCH_2(CH_2)_9CH_3$), 1.95 (5 H, m, CH_3CO + $CHCH_2CH_2$), 1.58 (2 H, t, H_2COCO-$CH_2CH_2(CH_2)_8CH_3$), 1.27 (16 H, s, CH_2), 0.87 (3 H, t, CH_3CH_2); δ_C(75 MHz; d_6-acetone) 174.83 (γ-$CONH_2$), 173.64 ($CH_2OCO(CH_2)_{10}CH_3$), 172.60 (NHCH$COOCH_2$), 170.52 (CH_3CONH), 67.89 ($CHOH$), 66.54 (OCH_2CHOH), 65.49 ($CH_2OCO(CH_2)_{10}CH_3$), 53.13 (NHCHCO), 34.41 (CH_2-$CONH_2$), 32.57–23.26 (CH_2), 27.85 ($CH_2CH_2CONH_2$), 22.58 (CH_3CO), 14.30 (CH_3CH_2); *m/z* (ES) 445.3 ($[M + H]^+$. C_{22}-$H_{40}N_2O_7$ requires *M*, 444.3); $[\alpha]_D^{20}$ −7.38 (*c* 1 in acetone). High-resolution MS *m/z* (FAB+) 445.2908 $[M + H]^+$. $C_{22}H_{41}N_2O_7$ requires *m/z*, 445.2914.

1,2-Di-O-lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-glutamine) ***12***. Purity of 1,2-di-*O*-lauroyl-*rac*-glycero-3-*O*-(*N*$^{\alpha}$-acetyl-L-glutamine) was 99.7% by HPLC and 90% by NMR spectroscopy. NMR: δ_H(300 MHz; d_6-acetone) 7.55 (1H, d, CONHCH), 6.82 (1H, s, $CONH_2$), 6.21 (1H, s, $CONH_2$), 5.27 (1H, m, $CHOCO(CH_2)_{10}CH_3$), 4.40–4.14 (5H, m, NHCHCOOCH_2 + $H_2COCO(CH_2)_{10}CH_3$), 2.31 (6H, m, $CH_2CH_2CONH_2$ + $CHOCOCH_2(CH_2)_9CH_3$ + $H_2COCOCH_2(CH_2)_9CH_3$), 2.05–1.89 (5H, m, $CHCH_2CH_2CONH_2$ + CH_3CO), 1.58 (4H, t, $CHOCOCH_2CH_2(CH_2)_8CH_3$ + $H_2COCOCH_2CH_2(CH_2)_8CH_3$), 1.27 (32H, s, CH_2), 0.86 (6H, t, 2 × CH_3CH_2); δ_C(75 MHz; d_6-acetone) 174.18 (γ-$CONH_2$), 172.99 ($OCO(CH_2)_{10}CH_3$), 172.38 (NHCHO$COCH_2$), 169.98 (CH_3CO), 69.56 ($CHOCO(CH_2)_{10}$-CH_3), 63.24 (NHCHCOOCH_2), 62.42 ($H_2COCO(CH_2)_{10}CH_3$), 53.08 (NHCHCO), 34.53 (CH_2CONH_2), 34.38–23.29 (CH_2), 27.76 ($CH_2CH_2CONH_2$), 22.57 (CH_3CO), 14.32 (CH_3CH_2). Other minor signals corresponding to ≈10% of the regioisomer type **A**: 173.25 ($CH_2OCO(CH_2)_{10}CH_3$), 172.32 ($HCOCOCH$-$(CH_2CH_2CONH_2)(NHCOCH_3)$), 70.53 ($CHOCOCH(CH_2$-$CH_2CONH_2)(NHCOCH_3)$), 62.58 ($CH_2OCO(CH_2)_{10}CH_3$); *m/z* (ES) 627.0 ($[M + H]^+$. $C_{34}H_{62}N_2O_8$ requires *M*, 626.4); $[\alpha]_D^{20}$ −9.55 (*c* 1 in acetone) (Found: C, 64.4; H, 10.0; N, 4.7. Calc. for $C_{34}H_{62}N_2O_8 \cdot 0.5\ H_2O$: C, 64.2; H, 9.9; N, 4.4%).

Purity of the 1,3-di-*O*-lauroyl-glycero-2-*O*-(*N*$^{\alpha}$-acetyl-L-glutamine) (regioisomer type **A**) was 98.6% by HPLC and >90% by NMR spectroscopy (no signals of the 1,2-di-*O*-lauroyl regioisomer were detected). NMR: δ_H(300 MHz; d_6-acetone) 7.56 (1H, d, CONHCH), 6.83 (1H, s, $CONH_2$), 6.21 (1H, s, $CONH_2$), 5.28 (1H, m, $CHOCOCH(CH_2CH_2CONH_2)(NH$-$COCH_3)$), 4.41–4.08 (5H, m, NHCHCO + 2 × H_2COCO-$(CH_2)_{10}CH_3$), 2.30 (6H, m, $CH_2CH_2CONH_2$ + 2 × H_2COCO-$CH_2(CH_2)_9CH_3$), 2.05–1.89 (5H, m, $CHCH_2CH_2CONH_2$ + CH_3CO), 1.58 (4H, t, 2 × $H_2COCOCH_2CH_2(CH_2)_8CH_3$), 1.27 (32H, s, CH_2), 0.86 (6H, t, 2 × CH_3CH_2); δ_C(75 MHz; d_6-acetone) 174.20 (γ-$CONH_2$), 173.39 ($CH_2OCO(CH_2)_{10}CH_3$), 172.19 (NHCHO$COCH_2$), 170.09 (CH_3CO), 70.67 ($CHOCO$-$(CH_2)_{10}CH_3$), 62.50 ($CH_2OCO(CH_2)_{10}CH_3$), 53.18 (NH-CHCO), 34.29 (CH_2CONH_2), 32.60–23.28 (CH_2), 27.78 ($CH_2CH_2CONH_2$), 22.57 (CH_3CO), 14.32 (CH_3CH_2); *m/z* (ES) 627.0 ($[M + H]^+$. $C_{34}H_{62}N_2O_8$ requires *M*, 626.4); $[\alpha]_D^{20}$ −5.70 (*c* 1 in acetone) (Found: C, 64.2; H, 10.0; N, 4.7. Calc. for $C_{34}H_{62}N_2O_8 \cdot 0.5H_2O$: C, 64.2; H, 9.9; N, 4.4%).

1-O-Lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-tyrosine) ***13***. Purity of the regioisomeric mixture was 98.0% by HPLC. NMR: δ_H(300 MHz; d_6-DMSO) 9.12 (1H, s, C=C(O*H*)C), 8.12 (1 H, d, CONHCH), 5.33 (0.63 H, m, CHOCO-(aminoacyl or lauroyl) regioisomers type **B** or **C**), 6.97 (2 H, d, *H*C=C(CH_2)C=C*H*), 6.65 (2 H, d, *H*C=C(OH)C=C*H*), 4.38 (1H, m, NHCHCO), 3.96 (4 H, m, $CHCOOCH_2$ + $H_2COCO(CH_2)_{10}CH_3$), 3.83 (1 H, m, $COOCH_2CHOHCH_2OCO$), 2.88–2.71 (2 H, m, $CHCH_2$), 2.28 (2 H, t, $H_2COCOCH_2(CH_2)_9CH_3$), 1.78 (3H, s, CH_3CO), 1.51 (2 H, t, $H_2COCOCH_2CH_2(CH_2)_8CH_3$), 1.23 (18 H, s, CH_2), 0.84 (3 H, t, CH_3CH_2); δ_C(75 MHz; d_6-DMSO) 172.58 ($CH_2OCO(CH_2)_{10}CH_3$), 171.43 (NHCH$COOCH_2$), 169.13 (CH_3CO), 155.86 (C=*C*OH), 129.72 (CH_2C=C), 127.11 (CH_2C=*C*), 114.90 (HOC=*C*H), 66.00 ($CHOH$), 65.07 (NHCH-$COOCH_2$), 64.49 ($CH_2CO(CH_2)_{10}CH_3$), 53.80 (NHCHCO), 35.88–21.89 (CH_2), 22.06 (CH_3CO), 13.3 (CH_3CH_2); *m/z* (ES) 480.5 ($[M + H]^+$. $C_{26}H_{41}NO_7$ requires *M*, 479.3); $[\alpha]_D^{20}$ +6.51 (*c* 1 in acetone) (Found: C, 64.1; H, 8.8; N, 3.2. Calc. for $C_{26}H_{41}NO_7 \cdot 0.5H_2O$: C, 63.9; H, 8.6; N, 2.9%).

1,2-Di-O-lauroyl-rac-glycero-3-O-(N$^{\alpha}$-acetyl-L-tyrosine) ***14***. Purity of the regioisomeric mixture was 99.3.0% by HPLC. NMR: δ_H(300 MHz; d_6-DMSO) 9.12 (1 H, s, C=C(O*H*)C), 8.14 (1 H, d, CONHCH), 6.98 (2 H, m, *H*C=C(CH_2)C=C*H*), 6.63 (2 H, m, *H*C=C(OH)C=C*H*), 5.14 (1 H, m, $COCH_2$-$CHOCO$), 4.35 (1 H, m, NHCHCO), 4.22–4.09 (4 H, m, $COOCH_2CHOCO$ + $CH_2OCO(CH_2)_{10}CH_3$), 2.86–2.75 (2 H, m, $CHCH_2$), 2.26 (4 H, m, $CHOCOCH_2(CH_2)_9CH_3$ + H_2CO-$COCH_2(CH_2)_9CH_3$), 1.77 (3 H, s, CH_3CO), 1.50 (4 H, t,

$CHOCOCH_2CH_2(CH_2)_8CH_3$ + $H_2COCOCH_2CH_2(CH_2)_8CH_3$), 1.23 (32 H, s, CH_2), 0.84 (6 H, t, 2 × CH_3CH_2); δ_C(75 MHz; d_4-MeOD) 174.82 ($CH_2OCO(CH_2)_{10}CH_3$ regioisomer type **A**), 174.37 ($OCO(CH_2)_{10}CH_3$), 173.09 ($NHCHCOOCH_2$), 173.04 ($HCOCOCH(CH_2C_6H_5)(NHCOCH_3)$ regioisomer type **A**), 172.45 (CH_3CO), 157.49 (C=COH), 131.14 (CH_2C=C), 128.57 (CH_2C=C), 116.29 (C=C(OH)C), 71.61 ($CHOCOCH(CH_2$-$C_6H_5)(NHCOCH_3)$ regioisomer type **A**), 70.43 ($CHOCO$-$(CH_2)_{10}CH_3$), 64.02 ($NHCHCOOCH_2$), 63.23 (CH_2OCO-$(CH_2)_{10}CH_3$ regioisomer type **A**), 63.09 ($H_2COCO(CH_2)_{10}CH_3$), 55.63 ($NHCHCO$), 37.65–23.70 (CH_2), 22.25 (CH_3CO), 14.41 (CH_3CH_2); *m*/*z* (ES) 662.5 ($[M + H]^+$. $C_{38}H_{63}NO_8$ requires *M*, 661.5); $[\alpha]_D^{20}$ +16.64 (*c* 1 in $CHCl_3$) (Found: C, 65.2; H, 9.6; N, 3.9. Calc. for $C_{38}H_{63}NO_8 \cdot CH_3CN \cdot 2H_2O$: C, 65.0; H, 9.5; N, 3.8%).

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