Chemo-Enzymatic Synthesis of Amino Acid-Based Surfactants

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ABSTRACT: The application of lipases to the synthesis of amino acid-based surfactants was investigated. Low yields (2–9%) were obtained in the acylation of free amino acids, such as L-serine and L-lysine, as well as their ethyl esters and amides with fatty acids, owing in part to low miscibility of the reactants. When the *N*-carbobenzyloxy (Cbz)-L-amino acids were used in an effort to improve miscibility of the amino acid derivatives with the acyl donor, a dramatic improvement was observed for *N*-Cbz-L-serine (92% yield) but not for N_α-Cbz- or N_ζ-Cbz-L-lysine (7 and 2% yield, respectively). As an alternative, an efficient synthesis of N_ζ-acyl-L-lysines was developed, based on the regiospecific chemical acylation of copper(II) lysinate. In pursuit of a general route to amino acid–fatty acid surfactants, the utility of a polyol linker was investigated. Thus, the glycerol ester of N_α,N_ζ-di-Cbz-L-lysine was prepared and evaluated as a substrate for acylation. As expected, this and other glycer-1-yl esters of *N*-protected amino acids were excellent substrates for lipase-catalyzed acylation. Their reaction with myristic acid in the presence of Novozyme resulted in the regioselective acylation of the primary hydroxyl group of the glycerol moiety to afford the corresponding 1-*O*-(*N*-Cbz-L-aminoacyl)-3-*O*-myristoylglycerols with conversions of 50–90%. These were readily deprotected to give a range of 1-*O*-(aminoacyl)-3-*O*-myristoylglycerols with overall yields of 27–71%.

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Surfactants constitute an important class of bulk and speciality chemicals with applications in many sectors of industry. Until recently, the synthesis of surfactants has been considered solely within the capabilities of organic chemistry. However, the rapid advance of biotechnology has led to a surge of interest in "biosurfactants," i.e., surface-active agents produced *via* mediation of biological catalysts in the form of microorganisms (1–4) or isolated enzymes (5). The main advantages associated with the use of biocatalysts are mild reaction conditions and high enzymatic specificity, which often eliminates the need for regioselective protection of multifunctional starting materials. This point has been clearly demonstrated by a number of recent reports dealing with the application of enzymes to synthesis and/or modification of sugar fatty acid esters, phospholipids, and alkyl glycosides (5). This paper is aimed at exploring the usefulness of enzymes in the preparation of yet another interesting group of surface-active agents, amino acid-based surfactants.

Recently, amino acid esters and amides have been a subject of intensive investigations owing to their excellent emulsifying properties, biocompatibility, and strong antimicrobial activity (6–14). These features have made them attractive for applications in cosmetics and personal-care products, food, and pharmaceutical formulations. Additionally, there are good reasons for considering amino acid-based surfactants as potential bulk detergents for industrial and household cleaning usages. Indeed, their production would rely on the use of renewable and inexpensive raw materials (e.g., amino and fatty acids) with expected rapid and complete biodegradability in the environment. Furthermore, the structural diversity of amino acids should enable manufacturers to tailor the functional properties of these surfactants to suit particular applications.

The wide range of functionalities displayed by amino acids should be a great asset where any "fine-tuning" of the surfactant's performance is concerned. However, this characteristic is also a potential drawback from a synthetic viewpoint owing to the requirement for temporary protection of any reactive functional groups. It is somewhat surprising therefore to find that relatively few attempts to circumvent some of the synthetic difficulties, by utilizing the selectivity of enzymes, have been reported (15–17). In this communication, we report the results of our initial investigations, aimed at the development of a facile chemo-enzymatic approach to the preparation of amino acid-based surfactants. In particular, the usefulness of lipases as catalysts for acylation of glycerol esters of amino acid was investigated in some detail.

EXPERIMENTAL PROCEDURES

Materials. *N*-Carbobenzyloxy (Cbz)-glycine, *N*-Cbz-L-tyrosine, *N*-Cbz-L-phenylalanine, *N*-Cbz-L-aspartic acid, *N*-Cbz-L-serine, and N_α,N_ζ-di-Cbz-L-lysine were obtained from Sigma Chemical Company (Poole, Dorset, England). Novozyme (im-

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mobilized *Candida antarctica* lipase) and Lipozyme (immobilized *Mucor miehei* lipase) were purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). L-Lysine, capryloyl chloride, caproyl chloride, lauroyl chloride, myristoyl chloride, palmitoyl chloride, stearoyl chloride, myristic acid, glycerol, formic acid, palladium on alumina catalyst (10% w/w), boron trifluoride etherate, 2,2-dimethyl-1,3-dioxolane-4-methanol (solketal), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid, d_6-dimethylsulfoxide (d_6-DMSO), EDTA disodium salt, and basic alumina (Brockmann grade 1) were acquired from Aldrich Chemical Company (Poole, Dorset, England). All solvents employed were of the highest available purity and were dried over 3A molecular sieves before use.

Analytical methods. Reactions were followed by reverse-phase high-performance liquid chromatography (HPLC), with a Gilson 305/306 system (Anachem Luton, United Kingdom), connected with a Gilson 231 Autosampler, an Applied Chromatography Systems (ACS; Cheshire, United Kingdom) 750/12 ultraviolet detector or an ACS 750/14 light-scattering detector, and a Hewlett-Packard Chemstation 35900 (Winnersh, United Kingdom) for data acquisition and processing. Analyses were carried out on a 0.46 × 15 cm Hichrom RPB/5-μm column (Reading, United Kingdom), maintained at 45°C and operated at a flow rate of 1 mL · min^{-1}. Methanol and 4:1 water/methanol, both containing 0.05% vol/vol phosphoric acid, were employed as mobile phases. Fast atom bombardment mass spectroscopy (FAB-MS) was carried out on a Kratos MS9/50TC spectrometer (Manchester, United Kingdom) with xenon at 5–7 kV and a source potential of 5.33 kV. Glycerol was used as the matrix, and spectra were recorded at 0.1 mm in positive or negative ionization mode, with polyglycerol ions as reference. Optical rotations were measured on a Thorn NPL-type 243 polarimeter (Nottingham, United Kingdom) at 589.3 nm, 25°C, in a 0.5-cm cell, with methanol or 1:1 methanol/trifluoroacetic acid as the solvent. Melting points were determined with a Stuart Scientific SMP-1 apparatus (Red Hill, United Kingdom) using sealed capillaries and are uncorrected. All compounds were also fully characterized by ^1H, ^{13}C, DEPT, ^1H-^1H correlation spectroscopy (COSY), and ^1H-^{13}C COSY nuclear magnetic resonance (NMR) experiments. These spectra were recorded on a Jeol JNM EX 270 Fourier transform spectrometer (Tokyo, Japan) at 35°C, with d_6-DMSO as solvent. Proton spectra were recorded at 270.0 MHz and carbon-13 spectra at 67.8 MHz with broad-band proton decoupling, both by using a DMSO field lock. ^1H-^1H COSY spectra were recorded in symmetrized mode.

General procedure for acylation of free amino acids and their derivatives. The acylation of L-serine, L-lysine, and their amide and methyl/ethyl ester derivatives was conducted as follows. The amino acid substrate (1 mmol) was dispersed in fatty acid or fatty acid methyl ester (2 or 4 mmol) at 50, 60, or 70°C, with or without the addition of solvent (5 or 10 mL where employed). Immobilized lipase (100–500 mg) was then added, and the mixture was stirred vigorously at 50, 60 or 70°C by means of a heating mantle/stirrer. Samples for HPLC analysis were withdrawn at the appropriate time intervals, quenched with methanol containing 5% vol/vol acetic acid, and analyzed by HPLC.

Preparation of N_ζ-acyl-L-lysines. Aqueous bis(L-lysinato)copper(II) complex was prepared by adding aqueous potassium L-lysinate (30 mL of 3.3 M solution, 100 mmol) to ice-cold aqueous copper(II) sulfate (40 mL of 1.3 M solution, 52 mmol) with vigorous stirring and cooling in an ice bath. Solid potassium bicarbonate (15 g, 150 mmol) was then added with stirring, followed by dropwise addition of the acid chloride (125 mmol in 10 mL of acetone) over a period of 1.5–2 h. The mixture was left stirring in the ice bath for a further 2–3 h, then allowed to warm to room temperature; stirring was continued for another 3–5 h, to give a viscous solution or suspension of the bis(N_ζ-acyl-L-lysinato)copper(II) complex. The mixture was then heated to 50°C, an aqueous suspension of EDTA disodium salt (500 mL of 0.25 M, 125 mmol) was added with vigorous stirring, and the pH of the mixture was adjusted to between 4.0 and 6.0 with 2.0 M aqueous hydrochloric acid. The precipitate formed was filtered off and washed successively with 2 × 50 mL water, 2 × 50 mL of 1:1 water/ethanol, 1 × 50 mL ethanol, 1 × 50 mL of 1:1 ethanol/ether, and finally 4 × 50 mL ether, to give the required N_ζ-acyl-L-lysine as a white solid (51–64% yield). The analytical data are presented below for a representative member of this class of compounds, N_ζ-myristoyl-L-lysine (1):

N_ζ-Myristoyl-L-lysine (**1**). M.p. > 230°C (decomp.); $[\alpha]_{D,25}$ + 10.07° (*c* 1.38, 1:1 methanol/trifluoroacetic acid); FAB-MS: (M + H) calcd. for $C_{20}H_{41}N_2O_3$ 357.3117, (M + H) obsvd. 357.3070.

Preparation of 1-O-(N-Cbz-L-aminoacyl)glycerols. 1-*O*-(*N*-Cbz-glycyl)-, 1-*O*-(*N*-Cbz-L-phenylalanyl)-, 1-*O*-(*N*-Cbz-L-tyrosyl)-, 1-*O*-(*N*-Cbz-L-seryl)-, and 1-*O*-(N_α,N_ζ-di-Cbz-L-lysyl)-glycerol were synthesized from the corresponding *N*-Cbz-L-amino acids by esterification with glycerol and boron trifluorode etherate as catalyst (18). *N*-Cbz-L-amino acid (50 mmol) was dissolved in a mixture of dimethylformamide (50 mL) and glycerol (500 mL). The solution was heated to 60°C, boron trifluoride etherate (25 mL) was added over a period of 1 h to the stirred mixture, and the reaction was allowed to proceed for 20 h. Brine (300 mL) and 0.6 M sodium bicarbonate (400 mL) was then added, and the reaction mixture was extracted with 3 × 500-mL portions of ethyl acetate. The organic layers were pooled and washed successively with 4 × 50 mL of aqueous 0.1 M sodium bicarbonate containing 0.2 M sodium chloride, 4 × 50 mL of aqueous 0.1 M citric acid containing 0.2 M sodium chloride, and finally with 4 × 50 mL of 0.4 M sodium chloride. Drying over anhydrous magnesium sulfate, followed by rotary evaporation, gave the pure product in 80–90% yield.

*1-*O-(N-*Cbz-glycyl)glycerol* (**2a**). M.p. 68–70°C; $[\alpha]_{D,25}$ 0° (*c* 1.10, methanol); FAB-MS: (M + H) calcd. for $C_{13}H_{17}NO_6$ 284.1137, (M + H) obsvd. 284.1104.

*1-*O-(N-*Cbz-L-phenylalanyl)glycerol* (**3a**). M.p. 43–44°C; $[\alpha]_{D,25}$–9.43° (*c* 1.96, methanol); FAB-MS: (M + H) calcd. for $C_{20}H_{24}NO_6$ 374.1606, (M + H) obsvd. 374.1588.

*1-*O-(N-*Cbz-L-tyrosyl)glycerol* (**4a**). M.p. 70–72°C; $[\alpha]_{D,25}$–5.00° (*c* 1.57, methanol); FAB-MS: (M + H) calcd. for $C_{20}H_{24}NO_7$ 390.1555, (M + H) obsvd. 390.1519.

1-O-*(*N-*Cbz-*L*-seryl)glycerol (***5a***)*. M.p. Oil; $[\alpha]_{D,25}$–6.56° (*c* 1.72, methanol); FAB-MS: (M + H) calcd. for $C_{14}H_{20}NO_7$ 314.1242, (M + H) obsvd. 314.1260.

1-O-*(*$N_\alpha N_\zeta$*-Di-Cbz-*L*-lysyl)glycerol (***6a***)*. M.p. 47–48°C; $[\alpha]_{D,25}$ –8.35° (*c* 2.10, methanol); FAB-MS: (M + H) calcd. for $C_{25}H_{33}N_2O_8$ 489.2242, (M + H) obsvd. 489.2238.

*Preparation of α,γ-diglycer-1-yl-*N-*Cbz-*L*-aspartate (***7a***)*. The direct esterification of *N*-Cbz-L-aspartic acid with glycerol was unsuitable for the preparation of α,γ-di-glycer-1-yl-*N*-Cbz-L-aspartate, because of its decomposition to a mixture of monoesters during the extraction procedures. Therefore, the following route, based on the disolketal ester, was employed. *N*-Cbz-L-aspartic acid (20 g, 75 mmol) and 4-(*N,N*-dimethylamino)pyridine (DMAP, 0.4 g, 3 mmol) were dissolved in a mixture of solketal (38 mL, 300 mmol) and 400 mL of acetonitrile. DCC (34 g, 165 mmol), dissolved in acetonitrile (50 mL), was then added over a period of 0.5 h to the stirred solution, and the reaction was allowed to proceed over a period of 24 h. Glacial acetic acid (10 mL) was then added, and the mixture was stirred for a further 0.5 h, after which time it was filtered and rotary-evaporated to a syrup. This was dissolved in 500 mL ethyl acetate and washed successively with 4 × 50 mL of aqueous 0.05 M potassium bicarbonate containing 0.3 M sodium chloride, 4 × 50 mL of aqueous 0.05 M citric acid containing 0.3 M sodium chloride, and finally 4 × 50 mL of 0.4 M sodium chloride (0.05 M). The organic layer was dried over anhydrous magnesium sulfate, then rotary-evaporated to give the pure product as a syrup. Further purification by flash column chromatography on silica gel (3 × 50 cm column containing 400 g of silica gel 40/60) with 7:1 petroleum ether/ethanol as eluent gave the pure α,γ-di(2,3-isopropylideneglycer-1-yl)-*N*-Cbz-L-aspartate as a colorless syrup (25.9 g, 70% yield). The disolketal ester was deprotected by dissolving it in 200 mL of 4:1 acetonitrile/1 M aqueous hydrochloric acid, followed by stirring at room temperature for 6 h. The solution was then concentrated by rotary evaporation at 30°C, followed by freeze-drying, to give α,γ-diglycer-1-yl-*N*-Cbz-L-aspartate as a colorless viscous liquid (21.4 g, 98% deprotection yield, 69% overall yield). M.p. oil; $[\alpha]_{D,25}$ –6.80° (*c* 1.04, methanol); FAB-MS: (M + H) calcd. for $C_{18}H_{26}NO_{10}$ 416.1561, (M + H) obsvd. 416.1556.

Solvent-free enzymatic esterification of 1-O-*(*N-*Cbz-*L*-aminoacyl)glycerols and α,γ-diglycer-1-yl-*N-*Cbz-*L*-aspartate.* The 1-*O*-(*N*-Cbz-L-aminoacyl)glycerols and α,γ-diglycer-1-yl-*N*-Cbz-L-aspartate were enzymatically esterified as follows. The glycer-1-yl ester (20 mmol) was thoroughly mixed with pentan-3-one (20% w/w), myristic acid (40 mmol, twofold excess except for α,γ-diglycer-1-yl-*N*-Cbz-L-aspartate, where a threefold excess of the fatty acid was employed), and Novozyme (2% w/w of substrate mixture) in a widemouthed reaction flask, which was then transferred to a heating mantle–stirrer maintained at 70°C. After 25–60 h, over 95% of the glycer-1-yl ester substrate had reacted, and the conversion to the 3-*O*-myristoyl ester had reached 50–90%. The reaction mixture was then extracted with methanol, the immobilized enzyme was filtered off, and the extract was rotary-evaporated to dryness at 30°C to give the crude product as a semisolid. This product was then dissolved in ether, and applied to a basic alumina column (3 × 50 cm with 400 g of alumina, Brockmann grade II), and purified by eluting with ether [for 1-*O*-(*N*-Cbz-L-tyrosyl)-, 1-*O*-(*N*-Cbz-L-phenylalanyl)-, and 1-*O*-(*N*-Cbz-L-seryl)-3-*O*-myristoylglycerol]. For 1-*O*-(N_α,N_ζ-di-Cbz-L-lysyl)-3-*O*-myristoylglycerol, 1-*O*-(*N*-Cbz-glycyl)-3-*O*-myristoylglycerol, and α,γ-di(3-*O*-myristoylglycer-1-yl)-*N*-Cbz-L-aspartate, the product was eluted with pure ethanol.

1-O-*(*N-*Cbz-glycyl)-3*-O-*myristoylglycerol (***2b***)*. M.p. 81–82°C; $[\alpha]_{D,25}$ 0° (*c* 1.01, methanol); FAB-MS: (M + H) calcd. for $C_{27}H_{42}NO_7$ 492.2963, (M + H) obsvd. 492.2990.

1-O-*(*N-*Cbz-*L*-phenylalanyl)-3*-O-*myristoylglycerol (***3b***)*. M.p. Oil; $[\alpha]_{D,25}$ –6.73° (*c* 1.17, methanol); FAB-MS: (M + H) calcd. for $C_{34}H_{50}NO_7$ 584.3590, (M + H) obsvd. 584.3617.

1-O-(N-*Cbz-*L*-tyrosyl)-3*-O-*myristoylglycerol* (**4b**). M.p. oil; $[\alpha]_{D,25}$ –3.80° (*c* 1.19, methanol); FAB-MS: (M + H) calcd. for $C_{34}H_{50}NO_8$ 600.3539, (M + H) obsvd. 600.3527.

1-O-*(*N-*Cbz-*L*-seryl)-3*-O-*myristoylglycerol (***5b***)*. M.p. oil; $[\alpha]_{D,25}$ –4.17° (*c* 1.60, methanol); FAB-MS: (M + H) calcd. for $C_{28}H_{46}NO_8$ 524.3235, (M + H) obsvd. 524.3246.

1-O-*(*$N_\alpha N_\zeta$*-Di-Cbz-*L*-lysyl)-3*-O-*myristoylglycerol (***6b***)*. M.p. 28–29°C; $[\alpha]_{D,25}$ –6.20° (*c* 2.19, methanol); FAB-MS: (M + H) calcd. for $C_{39}H_{59}N_2O_9$ 699.4226, (M + H) obsvd. 699.4245.

Di-(3-O-*myristoylglycer-1-yl)*-N-*Cbz-*L*-aspartate (***7b***)*. M.p. oil; $[\alpha]_{D,25}$ +1.84° (*c* 2.23, methanol); FAB-MS: (M + H) calcd. for $C_{46}H_{77}NO_{12}$ 836.5524, (M + H) obsvd. 836.5546.

Deprotection of 1-O-*(*N-*Cbz-*L*-aminoacyl)-3*-O-*myristoylglycerols and α,γ-di-(3*-O-*myristoylglycer-1-yl)*-N-*Cbz-*L*-aspartate.* The *N*-Cbz-derivatives were deprotected by transfer-hydrogenation with formic acid as donor. 1-*O*-(*N*-Cbz-L-aminoacyl)-3-*O*-acyl-glycerol or α,γ-di-(3-*O*-myristoylglycer-1-yl)-*N*-Cbz-L-aspartate (300 mg) was dissolved in 4% vol/vol formic acid in methanol (10 mL). Palladium on alumina catalyst [300 mg, except for 1-*O*-(N_α,N_ζ-di-Cbz-L-lysyl)-3-*O*-myristoylglycerol, where 600 mg was employed) was then added, and the mixture was stirred at room temperature until deprotection was complete (typically less than 0.5 h). The reaction mixture was then filtered through a bed of Celite, and the filtrate was concentrated by rotary evaporation at 30°C. The syrup obtained was then diluted with water (10 mL) and freeze-dried, and the solid obtained was purified by crystallization from petroleum ether, to give pure 1-*O*-L-aminoacyl-3-*O*-myristoylglycerol or α,γ-di(3-*O*-myristoylglycer-1-yl)-L-aspartate in almost quantitative yield as a white solid.

1-O-*Glycyl-3*-O-*myristoylglycerol (formate salt,* **2c***)*. M.p. 60–61°C; $[\alpha]_{D,25}$ 0° (*c* 0.85, methanol); FAB-MS: (M + H) calcd. for $C_{19}H_{37}NO_5$ 360.2747, (M + H) obsvd. 360.2734.

1-O-*L*-Phenylalanyl-3*-O-*myristoylglycerol (formate salt,* **3c***)*. M.p. 63–64°C; $[\alpha]_{D,25}$ +5.50° (*c* 1.03, methanol); FAB-MS: (M + H) calcd. for $C_{26}H_{43}NO_5$ 450.3219, (M + H) obsvd. 450.3225.

*1-*O*-L-Tyrosyl-3-*O*-myristoylglycerol (formate salt,* **4c***).* M.p. 69–71°C; $[\alpha]_{D,25}$ +4.67° (*c* 0.96, methanol); FAB-MS: (M + H) calcd. for $C_{26}H_{43}NO_6$ 466.3163, (M + H) obsvd. 466.3140.

*1-*O*-L-Seryl-3-*O*-myristoylglycerol (formate salt,* **5c***).* M.p. 40–41°C; $[\alpha]_{D,25}$ +3.50° (*c* 0.43, methanol); FAB-MS: (M + H) calcd. for $C_{20}H_{40}NO_6$ 390.2852, (M + H) obsvd. 390.2860.

*1-*O*-L-Lysyl-3-*O*-myristoylglycerol (diformate salt,* **6c***).* M.p. 58–59°C; $[\alpha]_{D,25}$ +3.96° (*c* 0.37, 1:1 methanol/trifluoroacetic acid); FAB-MS: (M + H) calcd. for $C_{23}H_{46}N_2O_5$ 431.3485, (M + H) obsvd. 431.3479.

*Di-(3-*O*-myristoylglycer-1-yl)-L-aspartate (formate salt,* **7c***).* M.p. 39–41°C; $[\alpha]_{D,25}$ –0.68° (*c* 1.49, methanol); FAB-MS: (M + H) calcd. for $C_{38}H_{71}NO_{10}$ 702.5156, (M + H) obsvd. 702.5171.

*Preparation of 3-*O*-myristoyl-*N*-Cbz-L-serine (***8a***). N-*Cbz-L-Serine (4.78 g, 20 mmol) was mixed with myristic acid (9.14 g, 40 mmol), pentan-3-one (5 mL) and Novozyme (0.5 g), and the mixture was incubated with stirring in an open flask at 70°C. After 60 h, when the esterification level had reached 93%, the reaction mixture was dissolved in ether (100 mL) and filtered. Rotary evaporation, followed by recrystallization from petroleum ether, afforded the title product as a white solid (7.2 g, 80% yield). M.p. 49–50°C; $[\alpha]_{D,25}$ +3.47° (*c* 1.08, methanol); FAB-MS (M + H) calcd. for $C_{25}H_{40}NO_6$ 450.2858, (M + H) obsvd. 450.2846.

*3-*O*-Myristoyl-L-serine (formate salt,* **8b***).* 3-*O*-Myristoyl-*N*-Cbz-L-serine was deprotected by transfer hydrogenation with formic acid as described above, to give 3-*O*-myristoyl-L-serine formate salt in quantitative yield. M.p. 43–44°C; $[\alpha]_{D,25}$ –0.90° (*c* 0.55, methanol); FAB-MS: (M + H) calcd. for $C_{17}H_{33}NO_4$ 316.2488, (M + H) obsvd. 316.2476.

N_ζ-Myristoyl-L-lysine (**1**). ^{1}H NMR, δ: 0.82 (*t*, 3H, *J* = 6.6 Hz, Myr-H_{14}), 1.22 (*m*, 22H, Myr-$H_{4–13}$), 1.50 (*m*, 6H, Myr-H_3 + Lys-H_γ + Lys-H_δ), 1.82 (*m*, 2H, Lys-H_β), 2.20 (*t*, 2H, *J* = 7.9 Hz, Myr-H_2), 3.20 (*m*, 2H, Lys-H_ζ), 3.80 (*m*, 1H, Lys-H_α), 8.20 (*m*-broad, 3H, Lys_α-N*H* + Lys_ζ-N*H*); ^{13}C NMR, δ: 13.2 (Myr-C_{14}), 21.6/21.9 (Myr-C_{13} + Lys-C_β), 25.5 (Myr-C_3), 28.0 (Lys-C_γ), 28.5–29.0 (Myr-$C_{4–11}$), 29.5 (Lys-C_δ), 31.3 (Myr-C_{12}), 35.0 (Myr-C_2), 38.6 (Lys-C_ζ), 52.3 (Lys-C_α), 171.0/175.0 (carbonyl).

*1-*O*-(*N*-Cbz-glycyl)glycerol* (**2a**). ^{1}H NMR, δ: 3.45 (*m*, 2H, Glc-H_3), 3.75 (*m*, 1H, Glc-H_2), 3.85 (*d* 2H, $J_{\alpha,NH}$ = 6.3 Hz, Gly-H_α), 4.10 (*m*, 2H, Glc-H_1), 5.15 (*s*, 2H, Cbz-CH_2), 7.33–7.45 (*m*, 5H, Cbz-Arom-*H*), 7.60 (*t*, 1H, $J_{\alpha,NH}$ = 6.1 Hz, Gly-N*H*); ^{13}C NMR, δ: 42.1 (Gly-C_α), 62.5 (Glc-C_3), 65.5 (Cbz-*C*H$_2$), 66.1 (Glc-C_1), 69.2 (Glc-C_2), 127.0/128.0/128.5/137.0 (Cbz-Arom-*C*), 156.0/170.0 (carbonyl).

*1-*O*-(*N*-Cbz-L-phenylalanyl)glycerol* (**3a**). ^{1}H NMR, δ: 2.95 (*m*, 1H, Phe-$H_{\beta1}$), 3.25 (*m*, 1H, Phe-$H_{\beta2}$), 3.50 (*d*, 2H, *J* = 5.0 Hz, Glc-H_3), 3.80 (*m*, 1H, Glc-H_2), 4.15 (*m*, 2H, Glc-H_1), 4.45 (*m*, 1H, Phe-H_α), 5.10 (*s*, 2H, Cbz-CH_2), 7.30–7.44 (*m*, 10H, Phe-Arom-*H* + Cbz-Arom-*H*), 7.82 (*d*, 1H, $J_{\alpha,NH}$ = 8.3 Hz, Phe-N*H*); ^{13}C NMR, δ: 36.6 (Phe-C_β), 55.6 (Phe-C_α), 62.6 (Glc-C_3), 65.4 (Cbz-*C*H$_2$), 66.3 (Glc-C_1), 69.3 (Glc-C_2), 126.5/129.0/136.9/137.5 (Phe-Arom-*C* + Cbz-Arom-*C*), 156.0/171.7 (carbonyl).

*1-*O*-(*N*-Cbz-L-tyrosyl)glycerol* (**4a**). ^{1}H NMR, δ: 2.90 (*m*, 1H, Tyr-$H_{\beta1}$), 3.15 (*m*, 1H, Tyr-$H_{\beta2}$), 3.50 (*d*, 2H, *J* = 5.3 Hz, Glc-H_3), 3.78 (*t*, 1H, *J* = 5.1 Hz, Glc-H_2), 4.15 (*m*, 2H, Glc-H_1), 4.40 (*m*, 1H, Tyr-H_α), 5.10 (*s*, 2H, Cbz-CH_2), 6.80 (*d*, 2H, $J_{o,m}$ = 8.6 Hz, Tyr-Arom-H_o), 7.14 (*d*, 2H, $J_{o,m}$ = 8.6 Hz, Tyr-Arom-H_m), 7.33–7.44 (*m*, 5H, Cbz-Arom-*H*), 7.82 (*d*, 1H, $J_{\alpha,NH}$ = 8.3 Hz, Tyr-N*H*); ^{13}C NMR, δ: 36.6 (Tyr-C_β), 55.9 (Tyr-C_α), 62.6 (Glc-C_3), 65.4 (Cbz-*C*H$_2$), 66.2 (Glc-C_1), 69.3 (Glc-C_2), 115.1/127.2/127.7/128.1/130.0/136.9 (Tyr-Arom-*C* + Cbz-Arom-*C*), 156.0/171.6 (carbonyl).

*1-*O*-(*N*-Cbz-L-seryl)glycerol* (**5a**). ^{1}H NMR, δ: 3.50 (*m*, 2H, Glc-H_3), 3.60 (*m*, 1H, Glc-H_2), 3.80 (*m*, 2H, Ser-H_β), 4.20 (*t*, 1H, $J_{\alpha,NH}$ = 4.9 Hz, Ser-H_α), 4.70 (*m*, 2H, Glc-H_1), 5.16 (*s*, 2H, Cbz-CH_2), 7.33–7.42 (*m*, 5H, Cbz-Arom-*H*); ^{13}C NMR, δ: 57.0 (Ser-C_α), 61.6 (Glc-C_3), 62.8 (Ser-C_β), 65.9 (Cbz-*C*H$_2$), 69.5 (Glc-C_2), 72.4 (Glc-C_1), 127.0/127.8/128.5/137.0 (Cbz-Arom-*C*), 156.0/170.0 (carbonyl).

*1-*O*-($N_\alpha N_\zeta$-Di-Cbz-L-lysyl)glycerol* (**6a**). ^{1}H-NMR, δ: 1.24 (*m*, 2H, Lys-H_β), 1.78 (*m*, 2H, Lys-H_δ), 2.50 (*m*, 2H, Lys-H_γ), 3.10 (*t*, 2H, $J_{\delta,\zeta}$ = 6.0 Hz, Lys-H_ζ), 3.50 (*m*, 4H, Glc-H_1 + Glc-H_3), 3.80 (*m*, 1H, Glc-H_2), 4.20 (*m*, 1H, Lys-H_α), 5.13 (*s*, 2H, Cbz_ζ-CH_2), 5.16 (Cbz_α-CH_2), 7.30 (*m*, 1H, Lys-NH_ζ), 7.35–7.45 (*m*, 10H, Cbz_α-Arom-*H* + Cbz_ζ-Arom-*H*), 7.70 (*d*, 1H, $J_{\alpha,NH}$ = 8.0 Hz, Lys-NH_α); ^{13}C-NMR, δ: 22.8 (Lys-C_β), 29.0 (Lys-C_δ), 30.6 (Lys-C_γ), 40.4 (Lys-C_ζ), 55.1 (Lys-C_α), 63.3 (Glc-C_3), 65.2 (Cbz_ζ-*C*H$_2$), 65.5 (Cbz_α-*C*H$_2$), 69.4 (Glc-C_1), 72.4 (Glc-C_2), 127.0/127.3/128.0/136.9/137.3/138.7 (Cbz_α-Arom-*C* + Cbz_ζ-Arom-*C*), 156.3/172.5 (carbonyl).

*α,γ-Diglycer-1-yl-*N*-Cbz-L-aspartate* (**7a**). ^{1}H NMR, δ: 2.90 (*d*, 2H, $J_{\alpha,\beta}$ = 7.7 Hz, Asp-H_β), 3.40 (*m*, 4H, Glc_α-H_3 + Glc_γ-H_3), 3.75 (*m*, 2H, Glc_α-H_2 + Glc_γ-H_2), 4.10 (*m*, 4H, Glc_α-H_1 + Glc_γ-H_1), 4.50 (*m*, 1H, Asp-H_α), 5.10 (*s*, 2H, Cbz-CH_2), 7.32–7.42 (*m*, 5H, Cbz-Arom-*H*); ^{13}C NMR, δ: 36.4 (Asp-C_β), 51.0 (Asp-C_α), 63.0 (Glc_γ-C_1), 63.5 (Glc_α-C_1), 66.2 (Cbz-*C*H$_2$), 66.9 (Glc_α-C_3 + Glc_γ-C_3), 69.7 (Glc_α-C_2 + Glc_γ-C_2), 128.1/128.4/128.9/137.2 (Cbz-Arom-*C*), 156.4/170.5/171.4 (carbonyl).

*1-*O*-(*N*-Cbz-glycyl)-3-*O*-myristoylglycerol* (**2b**). ^{1}H NMR, δ: 0.85 (*t*, 3H, *J* = 6.4 Hz, Myr-H_{14}), 1.24 (*m*, 20H, Myr-$H_{4–13}$), 1.52 (*m*, 2H, Myr-H_3), 2.30 (*t*, 2H, *J* = 7.3 Hz, Myr-H_2), 3.85 (*d*, 2H, $J_{\alpha,NH}$ = 6.0 Hz, Gly-H_α), 4.00 (*m*, 1H, Glc-H_2), 4.10 (*m*, 4H, Glc-H_1 + Glc-H_3), 5.10 (*s*, 2H, Cbz-CH_2), 7.31–7.42 (*m*, 5H, Cbz-Arom-*H*), 7.60 (*t*, 1H, $J_{\alpha,NH}$ = 6.0 Hz, Gly-N*H*); ^{13}C NMR, δ: 14.1 (Myr-C_{14}), 22.2 (Myr-C_{13}), 24.6 (Myr-C_3), 28.4–29.2 (Myr-$C_{4–11}$), 31.5 (Myr-C_{12}), 33.6 (Myr-C_2), 42.9 (Gly-C_α), 64.9 (Glc-C_3), 65.7 (Glc-C_1 + Cbz-*C*H$_2$), 69.2 (Glc-C_2), 127.8/128.2/128.5/137.9 (Cbz-Arom-*C*), 156.7/170.2/172.9 (carbonyl).

*1-*O*-(*N*-Cbz-L-phenylalanyl)-3-*O*-myristoylglycerol* (**3b**). ^{1}H NMR, δ: 0.94 (*t*, 3H, *J* = 6.6 Hz, Myr-H_{14}), 1.30 (*m*, 20H, Myr-$H_{4–13}$), 1.60 (*m*, 2H, Myr-H_3), 2.33 (*t*, 2H, *J* = 7.3 Hz, Myr-H_2), 3.05 (*m*, 1H, Phe-$H_{\beta1}$), 3.20 (*m*, 1H, Phe-$H_{\beta2}$), 4.00 (*m*, 1H, Glc-H_2), 4.10–4.20 (*m*, 4H, Glc-H_1 + Glc-H_3), 4.40 (*m*, 1H, Phe-H_α), 5.06 (*s*, 2H, Cbz-CH_2), 7.30–7.40 (*m*, 10H, Phe-Arom-*H* + Cbz-Arom-*H*), 7.82 (*d*, 1H, $J_{\alpha,NH}$ = 8.0 Hz, Phe-N*H*); ^{13}C NMR, δ: 13.7 (Myr-C_{14}), 22.0 (Myr-C_{13}), 24.4 (Myr-C_3), 28.5–29.0 (Myr-$C_{4–11}$), 31.3 (Myr-C_{12}), 33.4 (Myr-

C_2), 36.6 (Phe-C_β), 55.4 (Phe-C_α), 64.6 (Glc-C_3), 65.1 (Glc-C_1), 65.5 (Cbz-CH$_2$), 66.1 (Glc-C_2), 126.3/128.9/136.7/137.3 (Phe-Arom-C + Cbz-Arom-C), 155.8/171.8/172.1 (carbonyl).

*1-*O*-(*N*-Cbz-L-tyrosyl)-3-*O*-myristoylglycerol* (**4b**). ^{1}H NMR, δ: 0.94 (*t*, 3H, J = 6.3 Hz, Myr-H_{14}), 1.32 (*m*, 20H, Myr-$H_{4–13}$), 1.60 (*m*, 2H, Myr-H_3), 2.30 (*m*, 2H, Myr-H_2), 2.90 (*m*, 1H, Tyr-$H_{\beta 1}$), 3.05 (*m*, 1H, Tyr-$H_{\beta 2}$), 4.00 (*m*, 1H, Glc-H_2), 4.15 (*m*, 4H, Glc-H_1 + Glc-H_3), 4.35 (*m*, 1H, Tyr-H_α), 5.10 (*s*, 2H, Cbz-CH_2), 6.75 (*d*, 2H, $J_{o,m}$ = 8.3 Hz, Tyr-Arom-H_o), 7.10 (*d*, 2H, $J_{o,m}$ = 8.3 Hz, Tyr-Arom-H_m), 7.30–7.40 (*m*, 5H, Cbz-Arom-H), 7.82 (*d*, 1H, $J_{\alpha,NH}$ = 8.6 Hz, Tyr-NH); ^{13}C NMR, δ: 13.8 (Myr-C_{14}), 22.0 (Myr-C_{13}), 24.3 (Myr-C_3), 28.4–29.3 (Myr-$C_{4–11}$), 31.2 (Myr-C_{12}), 33.3 (Myr-C_2), 35.8 (Tyr-C_β), 55.8 (Tyr-C_α), 64.6 (Glc-C_3), 65.0 (Glc-C_1), 65.3 (Cbz-CH$_2$), 66.1 (Glc-C_2), 115.0/127.3/127.4/127.6/128.2/129.8/136.8 (Tyr-Arom-C + Cbz-Arom-C), 156.0/171.6/172.6 (carbonyl).

*1-*O*-(*N*-Cbz-L-seryl)-3-*O*-myristoylglycerol* (**5b**). ^{1}H NMR, δ: 0.95 (*t*, 3H, J = 7.0 Hz, Myr-H_{14}), 1.33 (*m*, 20H, Myr-$H_{4–13}$), 1.62 (*m*, 2H, Myr-H_3), 2.36 (*t*, 2H, J = 5.0 Hz, Myr-H_2), 3.82 (*m*, 2H, Ser-H_β), 4.00 (*m*, 1H, Glc-H_2), 4.10 (*m*, 4H, Glc-H_1 + Glc-H_3), 4.15(*m*, 1H, Ser-H_α), 5.16 (*s*, 2H, Cbz-CH_2), 7.32–7.41 (*m*, 5H, Cbz-Arom-H); ^{13}C NMR, δ: 13.7 (Myr-C_{14}), 22.2 (Myr-C_{13}), 24.5 (Myr-C_3), 28.6–29.1 (Myr-$C_{4–11}$), 31.4 (Myr-C_{12}), 33.5 (Myr-C_2), 56.8 (Ser-C_α), 61.4 (Ser-C_β), 64.9 (Glc-C_3), 65.5 (Glc-C_1), 65.7 (Cbz-CH$_2$), 66.2 (Glc-C_2), 127.3/127.5/128.2/136.9 (Cbz-Arom-C), 156.6/170.0/172.6 (carbonyl).

*1-*O*-(*N$_\alpha$,N$_\zeta$*-Di-Cbz-L-lysyl)-3-*O*-myristoylglycerol* (**6b**). ^{1}H NMR, δ: 0.94 (*t*, 3H, J = 6.5 Hz, Myr-H_{14}), 1.33 (*m*, 24H, Myr-$H_{4–13}$ + Lys-H_β + Lys-H_δ), 1.60 (*m*, 4H, Myr-H_3 + Lys-H_γ), 2.40 (*m*, 2H, Myr-H_2), 3.10 (*m*, 2H, Lys-H_ζ), 4.00 (*m*, 1H, Glc-H_2), 4.10 (*m*, 5H, Lys-H_α + Glc-H_1 + Glc-H_3), 5.10 (*s*, 2H, Cbz$_\zeta$-CH_2), 5.15 (Cbz$_\alpha$-CH_2), 7.25 (*m*, 1H, Lys-NH_ζ), 7.30–7.40 (*m*, 10H, Cbz$_\alpha$-Arom-H + Cbz$_\zeta$-Arom-H), 7.70 (*d*, 1H, $J_{\alpha,NH}$ = 7.3 Hz, Lys-NH_α); ^{13}C NMR, δ: 13.7 (Myr-C_{14}), 22.2 (Myr-C_{13}), 22.7 (Lys-C_β), 24.4 (Myr-C_3), 28.5–29.0 (Lys-C_δ + Myr-$C_{4–11}$), 30.4 (Lys-C_γ), 31.4 (Myr-C_{12}), 33.4 (Myr-C_2), 40.1 (Lys-C_ζ), 54.0 (Lys-C_α), 65.1 (Glc-C_3), 65.5 (Glc-C_1), 66.2 (Glc-C_2), 64.6 (Cbz$_\zeta$-CH$_2$), 65.3 (Cbz$_\alpha$-CH$_2$), 127.2/127.3/127.5/127.6/128.0/136.0/137.0 (Cbz$_\zeta$-Arom-C + Cbz$_\zeta$-Arom-C), 156.0/156.1/172.0/172.4/174.0 (carbonyl).

*Di-(3-*O*-myristoylglycer-1-yl)-*N*-Cbz-L-aspartate* (**7b**). ^{1}H NMR, δ: 0.94 (*t*, 6H, J = 6.6 Hz, Myr$_\alpha$-H_{14} + Myr$_\gamma$-H_{14}), 1.32 (*m*, 40H, Myr$_\alpha$-$H_{4–13}$ + Myr$_\gamma$-$H_{4–13}$), 1.60 (*m*, 4H, Myr$_\alpha$-H_3 + Myr$_\gamma$-H_3), 2.33 (*t*, 4H, J = 7.2 Hz, Myr$_\alpha$-H_2 + Myr$_\gamma$-H_2), 2.90 (*m*, 2H, Asp-H_β), 3.50 (2*d*, 4H, $J_{2,3}$ = 5.2 Hz, Glc$_\alpha$-H_3 + Glc$_\gamma$-H_3), 3.60 (*m*, 2H, Glc$_\alpha$-H_2 + Glc$_\gamma$-H_2), 4.00 (*m*, 4H, Glc$_\alpha$-H_1 + Glc$_\gamma$-H_1), 4.50 (*m*, 1H, Asp-H_a), 5.10 (*s*, 2H, Cbz-CH_2), 7.32–7.41 (*m*, 5H, Cbz-Arom-H), 7.82 (*d*, 1H, J = 7.5 Hz, Asp-NH); ^{13}C NMR, δ: 13.8 (Myr$_\alpha$-C_{14} + Myr$_\gamma$-C_{14}), 22.1 (Myr$_\alpha$-C_{13} + Myr$_\gamma$-C_{13}), 24.4 (Myr$_\alpha$-C_3 + Myr$_\gamma$-C_3), 28.5–29.0 (Myr$_\alpha$-$C_{4–11}$ + Myr$_\gamma$-$C_{4–11}$), 31.4 (Myr$_\alpha$-C_{12} + Myr$_\gamma$-C_{12}), 33.5 (Myr$_\alpha$-C_2 + Myr$_\gamma$-C_2), 36.0 (Asp-C_β), 50.4 (Asp-C_α), 62.7 (Glc$_\alpha$-C_1 + Glc$_\gamma$-C_1), 65.4 (Cbz-CH$_2$), 65.6 (Glc$_\alpha$-C_3 + Glc$_\gamma$-C_3), 69.8 (Glc$_\alpha$-C_2 + Glc$_\gamma$-C_2), 127.6/127.7/128.3 (Cbz-Arom-C), 156.0/172.0/173.0/174.0 (carbonyl).

*1-*O*-Glycyl-3-*O*-myristoylglycerol (formate salt,* **2c***).* ^{1}H NMR, δ: 0.94 (*t*, 3H, J = 6.3 Hz, Myr-H_{14}), 1.33 (*m*, 20H, Myr-$H_{4–13}$), 1.61 (*m*, 2H, Myr-H_3), 2.37 (*t*, 2H, J = 7.3 Hz, Myr-H_2), 3.60 (*m*, 2H, Gly-H_α), 3.86 (*m*, 1H, Glc-H_2), 4.05 (*m*, 4H, Glc-H_1 + Glc-H_3); ^{13}C NMR, δ: 13.7 (Myr-C_{14}), 21.9 (Myr-C_{13}), 24.3 (Myr-C_3), 28.6–28.9 (Myr-$C_{4–11}$), 31.4 (Myr-C_{12}), 33.4 (Myr-C_2), 44.2 (Gly-C_α), 62.6 (Glc-C_3) 65.3 (Glc-C_1), 69.2 (Glc-C_2), 166.0/173.0 (carbonyl).

*1-*O*-L-Phenylalanyl-3-*O*-myristoylglycerol (formate salt,* **3c***).* ^{1}H NMR, δ: 0.94 (*t*, 3H, J = 6.6 Hz, Myr-H_{14}), 1.32 (*m*, 20H, Myr-$H_{4–13}$), 1.60 (*m*, 2H, Myr-H_3), 2.35 (*t*, 2H, J = 7.4 Hz, Myr-H_2), 2.85 (*dd*, 1H, $J_{\beta 1,\beta 2}$ = 14.0 Hz, $J_{\alpha,\beta 1}$ = 7.3 Hz, Phe-$H_{\beta 1}$), 3.00 (*dd*, 1H, $J_{\beta 1,\beta 2}$ = 14.0 Hz, $J_{\alpha,\beta 2}$ = 6.2 Hz, Phe-$H_{\beta 2}$), 3.70 (*m*, 1H, Phe-H_α), 3.90 (*m*, 1H, Glc-H_2), 3.96 (*m*, 4H, Glc-H_1 + Glc-H_3), 7.30–7.40 (*m*, 5H, Phe-Arom-H); ^{13}C NMR, δ: 13.8 (Myr-C_{14}), 21.9 (Myr-C_{13}), 24.3 (Myr-C_3), 28.3–28.6 (Myr-$C_{4–11}$), 31.2 (Myr-C_{12}), 33.3 (Myr-C_2), 40.5 (Phe-C_β), 55.6 (Phe-C_α), 64.5 (Glc-C_3), 64.9 (Glc-C_1), 66.0 (Glc-C_2), 126.1/127.9/129.1/137.8 (Phe-Arom-C), 172.6/174.6 (carbonyl).

*1-*O*-L-Tyrosyl-3-*O*-myristoylglycerol (formate salt,* **4c***).* ^{1}H NMR, δ: 0.94 (*t*, 3H, J = 7.1 Hz, Myr-H_{14}), 1.32 (*m*, 20H, Myr-$H_{4–13}$), 1.60 (*m*, 2H, Myr-H_3), 2.38 (*m*, 2H, Myr-H_2), 2.80 (*m*, 2H, Tyr-H_β), 3.65 (*m*, 1H, Tyr-H_α), 3.90 (*m*, 1H, Glc-H_2), 4.10 (*m*, 4H, Glc-H_1 + Glc-H_3), 6.73 (*d*, 2H, $J_{o,m}$ = 8.6 Hz, Tyr-Arom-H_o), 7.05 (*d*, 2H, $J_{o,m}$ = 8.6 Hz, Tyr-Arom-H_m); ^{13}C NMR, δ: 13.8 (Myr-C_{14}), 21.9 (Myr-C_{13}), 24.3 (Myr-C_3), 28.4–29.2 (Myr-$C_{4–11}$), 31.2 (Myr-C_{12}), 33.3 (Myr-C_2), 40.5 (Tyr-C_β), 55.6 (Tyr-C_α), 64.5 (Glc-C_3), 64.9 (Glc-C_1), 66.0 (Glc-C_2), 126.1/127.9/129.1/137.8 (Tyr-Arom-C), 172.6/174.6 (carbonyl).

*1-*O*-L-Seryl-3-*O*-myristoylglycerol (formate salt,* **5c***).* ^{1}H NMR, δ: 1.00 (*t*, 3H, J = 6.6 Hz, Myr-H_{14}), 1.40 (*m*, 20H, Myr-$H_{4–13}$), 1.65 (*m*, 2H, Myr-H_3), 2.33 (*m*, 2H, Myr-H_2), 3.80 (*m*, 2H, Ser-H_β), 3.95 (*m*, 1H, Glc-H_2), 4.10 (*m*, 5H, Glc-H_1 + Glc-H_3 + Ser-H_α); ^{13}C NMR, δ: 13.8 (Myr-C_{14}), 22.0 (Myr-C_{13}), 24.3 (Myr-C_3), 28.8–29.0 (Myr-$C_{4–11}$), 31.2 (Myr-C_{12}), 33.3 (Myr-C_2), 56.1 (Ser-C_α), 62.6 (Ser-C_β), 65.0 (Glc-C_3), 65.4 (Glc-C_1), 69.2 (Glc-C_2), 172.8/174.0 (carbonyl).

*1-*O*-L-Lysyl-3-*O*-myristoylglycerol (diformate salt,* **6c***).* ^{1}H NMR, δ: 0.95 (*t*, 3H, J = 6.6 Hz, Myr-H_{14}), 1.33 (*m*, 24H, Myr-$H_{4–13}$ + Lys-H_β + Lys-H_δ), 1.60 (*m*, 4H, Myr-H_3 + Lys-H_γ), 2.40 (*m*, 2H, Myr-H_2), 2.80 (*t*, 2H, $J_{\delta,\zeta}$ = 7.3 Hz, Lys-H_ζ), 3.60 (*m*, 1H, Lys-H_α), 4.00 (*m*, 1H, Glc-H_2), 4.10 (*m*, 4H, Glc-H_1 + Glc-H_3); ^{13}C NMR, δ: 13.8 (Myr-C_{14}), 21.9 (Myr-C_{13}), 22.0 (Lys-C_β), 24.3 (Myr-C_3), 27.0 (Lys-C_δ), 28.8–29.0 (Myr-$C_{4–11}$ +Lys-C_γ), 31.2 (Myr-C_{12}), 33.5 (Myr-C_2), 38.5 (Lys-C_ζ), 53.6 (Lys-C_α), 64.6 (Glc-C_3), 65.0 (Glc-C_1), 66.0 (Glc-C_2), 172.6/174.0 (carbonyl).

*Di-(3-*O*-myristoylglycer-1-yl)-L-aspartate (formate salt,* **7c***).* ^{1}H NMR, δ: 0.93 (*t*, 6H, J = 6.6 Hz, Myr$_\alpha$-H_{14} + Myr$_\gamma$-H_{14}), 1.32 (*m*, 40H, Myr$_\alpha$-$H_{4–13}$ + Myr$_\gamma$-$H_{4–13}$), 1.60 (*m*, 4H, Myr$_\alpha$-H_3 + Myr$_\gamma$-H_3), 2.25 (*m*, 4H, Myr$_\alpha$-H_2 + Myr$_\gamma$-H_2), 2.90 (*m*, 2H, Asp-H_β), 3.45 (*m*, 4H, Glc$_\alpha$-H_3 + Glc$_\gamma$-H_3), 3.75 (*m*, 2H, Glc$_\alpha$-H_2 + Glc$_\gamma$-H_2), 3.80 (*m*, 1H, Asp-H_α), 4.00 (*m*, 4H, Glc$_\alpha$-H_1 + Glc$_\gamma$-H_1); ^{13}C NMR, δ: 13.8 (Myr$_\alpha$-C_{14} + Myr$_\gamma$-C_{14}), 22.0 (Myr$_\alpha$-C_{13} + Myr$_\gamma$-C_{13}), 24.4 (Myr$_\alpha$-C_3 +

Myr$_\gamma$-C_3), 28.5–28.9 (Myr$_\alpha$-C_{4-11} + Myr$_\gamma$-C_{4-11}), 31.2 (Myr$_\alpha$-C_{12} + Myr$_\gamma$-C_{12}), 33.4 (Myr$_\alpha$-C_2 + Myr$_\gamma$-C_2), 39.0 (Asp-C_β), 51.0 (Asp-C_α), 62.6 (Glc$_\alpha$-C_1 + Glc$_\gamma$-C_1), 65.4 (Glc$_\alpha$-C_3 + Glc$_\gamma$-C_3), 69.2 (Glc$_\alpha$-C_2 + Glc$_\gamma$-C_2), 172.8/174.0 (carbonyl).

3-O-Myristoyl-N-Cbz-L-serine (**8a**). ^{1}H NMR, δ: 0.94 (*t*, 3H, J = 6.6 Hz, Myr-H_{14}), 1.33 (*m*, 20H, Myr-H_{4-13}), 1.60 (*m*, 2H, Myr-H_3), 2.33 (*t*, 2H, J = 7.0 Hz, Myr-H_2), 4.25 (*m*, 1H, Ser-$H_{\beta 1}$), 4.45 (*m*, 2H, Ser-H_α + Ser-$H_{\beta 2}$), 5.15 (*s*, 2H, Cbz-CH_2), 7.40–7.50 (*m*, 5H, Cbz-Arom-H), 7.63 (*d*, 1H, J = 8.2 Hz, Ser-NH); ^{13}C NMR, δ: 13.6 (Myr-C_{14}), 22.0 (Myr-C_{13}), 24.3 (Myr-C_3), 28.2–29.0 (Myr-C_{4-11}), 31.3 (Myr-C_{12}), 33.3 (Myr-C_2), 54.0 (Ser-C_α), 62.9 (Ser-C_β), 65.6 (Cbz-CH$_2$), 127.2/127.6/128.2/136.9 (Cbz-Arom-C), 156.0/172.5/174.2 (carbonyl).

3-O-Myristoyl-L-serine (formate salt, **8b**). ^{1}H NMR, δ: 0.94 (*t*, 3H, J = 6.6 Hz, Myr-H_{14}), 1.32 (*m*, 20H, Myr-H_{4-13}), 1.60 (*m*, 2H, Myr-H_3), 2.24 (*t*, 2H, J = 7.3 Hz, Myr-H_2), 4.10 (*m*, 1H, Ser-$H_{\beta 1}$), 4.50 (*m*, 2H, Ser-H_α + Ser-$H_{\beta 2}$); ^{13}C NMR, δ: 13.8 (Myr-C_{14}), 22.0 (Myr-C_{13}), 24.5 (Myr-C_3), 28.5–29.0 (Myr-C_{4-11}), 31.2 (Myr-C_{12}), 33.7 (Myr-C_2), 55.0 (Ser-C_α), 62.0 (Ser-C_β), 174.0 (carbonyl).

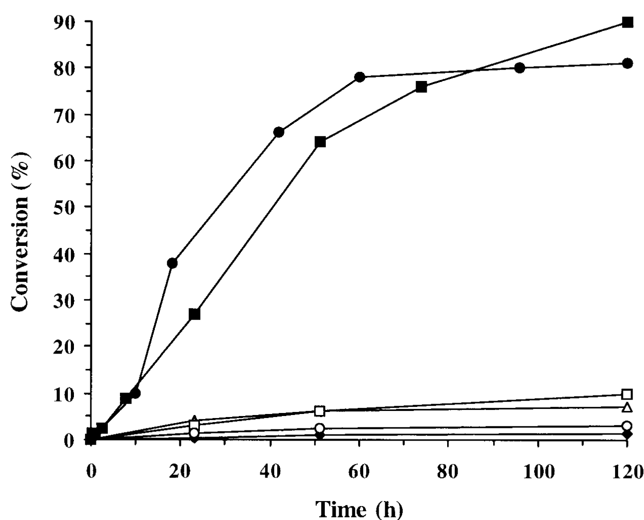

FIG. 1. Lipase-catalyzed acylation of L-serine and L-lysine derivatives with myristic acid. Substrates: ■, *N*-Cbz-L-serine; ●, 1-*O*-(N_α,N_ζ-di-Cbz-L-lysyl)glycerol; □, N_α-Cbz-L-lysine methyl ester; △, N_α-Cbz-L-lysine; ○, N_ζ-Cbz-L-lysine methyl ester; ◆, N_α-Cbz-L-lysine.

RESULTS AND DISCUSSION

Initially, we investigated the feasibility of lipase-catalyzed acylation of L-lysine and L-serine with a range of long-chain fatty acids and their methyl esters as acyl donors. Special attention was focused on the synthesis of myristoyl derivatives because *N*-myristoyl-L-amino acids have been shown to exhibit optimal surface-active and antimicrobial properties (14). The reactions were carried out with a range of immobilized lipases, including Lipozyme and Novozyme, with up to 3 mol equivalents of the acylating agent in the absence of solvent at 60–80°C. However, the reaction rates were poor, with only traces of product (2–4%) being detected after several days of incubation. The yields remained below 10% even when the corresponding amino acid amides or ethyl or methyl esters were used as substrates. No significant improvement was observed upon the addition of organic solvents nor on replacement of the fatty acid with triglycerides, such as triolein. The low conversions obtained in these experiments were initially attributed to low solubility of the amino acid derivatives in the oil phase.

In an attempt to improve the miscibility of the reactants, the *N*-Cbz-L-amino acids were examined as alternative substrates. We found that *N*-Cbz-L-serine was an excellent substrate for lipases and provided the required *O*-acyl-*N*-Cbz-L-serine in over 90% yield (Fig. 1). In contrast, the corresponding lysine derivatives were poor substrates, regardless of their solubility in the oil phase. Thus, N_α-Cbz-L-lysine (and N_ζ-Cbz-L-lysine) was acylated only to a minor extent (Fig. 1), despite its structural similarity to the serine compound. Even use of N_α- and N_ζ-Cbz-L-lysine methyl esters failed to significantly improve the reactions (Fig. 1), in spite of their much higher solubility in the fatty acid phase. These results indicated that substrate miscibility was not the sole factor responsible for the disappointing performance of lipases in these reactions. Indeed, it may well be that lipases are inherently poor amidation catalysts where *N*-acylation of amino acids is concerned, perhaps reflecting the lack of suitable structural provisions for the binding of these substrates as acyl acceptors (19–22).

In pursuit of an alternative route to N_ζ-acyl-L-lysines, we examined the chemical acylation of copper(II) lysinate, in which the α-amino (but not the ζ-amino group) is coordinated to Cu(II). Chemical amidation of this chelate complex with fatty acid chlorides proceeded readily and regiospecifically in aqueous media, thereby providing access to N_ζ-acyl-L-lysines in good yields. For example, the capryloyl, caproyl, lauroyl, and myristoyl derivatives were synthesized with preparative yields of 69, 64, 51, and 63%, respectively, by using this approach (see the Experimental Procedures section). Although this approach proved to be more facile for regiospecific N_ζ-amidation of lysine than use of enzymes, it showed little promise where the side-chain modification of other multifunctional amino acids, such as serine, threonine, and arginine, was concerned.

As alcohols are known to be good substrates for lipases, we reasoned that use of a polyol, such as glycerol, as a linker between amino and fatty acids (Fig. 2) may prove to be a superior strategy for the enzymatic synthesis of a range of amino acid-based surfactants. As well as providing a facile method for attaching lipid functionalities to amino acids, such glycerol-linked compounds should possess interesting physico-chemical properties that are intermediate to those displayed by long-chain amino acid esters and monoglycerides, and should also retain the good biocompatibilities of the constituents. To investigate the feasibility of this approach, we prepared the glycer-1-yl ester of N_α,N_ζ-di-Cbz-L-

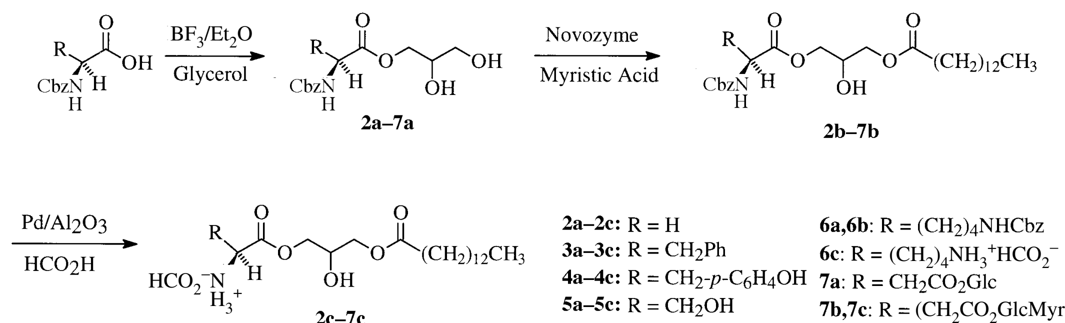

FIG. 2. Chemo-enzymatic approach to the synthesis of 1-*O*-(L-aminoacyl)-3-*O*-myristoylglycerols.

lysine (Fig. 2, **6a**) and studied its enzymatic esterification. As expected, **6a** was a good substrate for Novozyme and Lipozyme catalysts, and a highly regioselective acylation of the primary hydroxyl of the glycerol moiety was observed (Fig. 1). Thus, a conversion of 80% was attained in a matter of hours when the reaction was performed in a 1:2 (mole ratio) mixture of **6a** and myristic acid in the presence of a small amount of pentan-3-one (20% w/w, added at the beginning of the reaction, then allowed to evaporate as acylation proceeded), which was included to improve miscibility of the substrates.

Encouraged by this result, we proceeded to investigate whether this methodology was equally applicable to the synthesis of other glycerol-linked amino acid–fatty acid esters (Fig. 2). This was found to be the case, and conversions of 50–90% were attained for Novozyme-catalyzed acylations with myristic acid. The substrates were used at 0.85–1.09 M, and under these conditions, 0.31–0.61 g of product was formed per gram of reaction mixture. After purification, the 1-*O*-(*N*-Cbz-L-aminoacyl)-3-*O*-myristoylglycerols were obtained with preparative yields of 35–80% from the corresponding *N*-Cbz-L-derivatives of glycine, phenylalanine, tyrosine, serine, and aspartic acid (Table 1). The effects of varying the substrate ratio and the amount of immobilized enzyme added on the synthesis of a representative 1,3-di-*O*-acylglycerol, 1-*O*-(*N*-Cbz-L-phenylalanyl)-3-*O*-myristoylglycerol (**3b**), are shown in Figures 3A and B, respectively. Subsequent *N*-deprotection by transfer hydrogenation gave the required 1-*O*-(L-aminoacyl)-3-*O*-myristoylglycerols in overall yields of 27–71%.

The developed methodology provides easy access to a range of cationic and zwitterionic surfactants, which can be used as obtained, or alternatively, can serve as efficient building blocks for further transformations, such as the synthesis

TABLE 1
Novozyme-Catalyzed Synthesis of 1-*O*-(*N*-Cbz-L-aminoacyl)-3-*O*-Myristoylglycerols

Product	Time (h)	Conversion (%)[a]	Product concentration (gg^{-1})[b]	Yield (%)[c]	FAB-MS (M + H, calcd.)	FAB-MS (M + H, obsvd.)
2b	25	50	0.31	35	492.2963	492.2990
3b	25	91	0.54	80	584.3590	584.3617
4b	40	90	0.61	68	600.3539	600.3527
5b	25	78	0.50	55	524.3235	524.3246
6b	25	80	0.58	56	699.4226	699.4245
7b	35	60	0.51	40	836.5524	836.5546
8a	60	93	0.55	80	450.2858	450.2846

[a]Percentage of product formed, as determined by high-performance liquid chromatography analysis upon completion of the reaction.
[b]Calculated on the basis of the mass of the reaction mixture excluding biocatalyst, gram per gram.
[c]Yield after purification. In all cases 20% w/w of pentan-3-one was added at the beginning of the reaction, then allowed to evaporate as acylation progressed. A 1:2 molar ratio of glycer-1-yl ester/myristic acid was employed in all cases, except in the preparation of (**7b**) where a 1:3 ratio was used. Abbreviation: FAB-MS, fast atom bombardment mass spectroscopy; **2b**: 1-*O*-(*N*-Cbz-glycyl)-3-*O*-myristoylglycerol; **3b**: 1-*O*-(*N*-Cbz-L-phenylalanyl)-3-*O*-myristoylglycerol; **4b**: 1-*O*-(*N*-Cbz-L-tyrosyl)-3-*O*-myristoylglycerol; **5b**: 1-*O*-(*N*-Cbz-L-seryl)-3-*O*-myristoylglycerol; **6b**: 1-*O*-(N_α,N_ζ-di-Cbz-L-lysyl)-3-*O*-myristoyl glycerol; **7b**: di-(3-*O*-myristoylglycer-l-yl)-*N*-Cbz-L-aspartate; **8a**: 3-*O*-myristoyl-*N*-Cbz-L-serine.

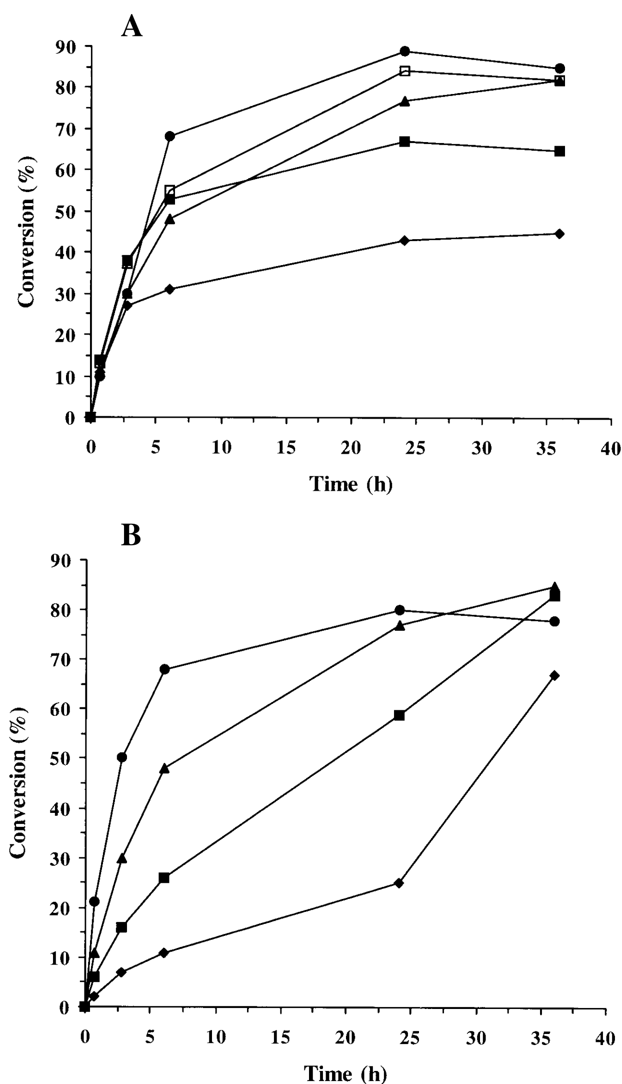

FIG. 3. Synthesis of 1-*O*-(*N*-Cbz-L-phenylalanyl)-3-*O*-myristoylglycerol. A: Effect of substrate/fatty acid ratio on Novozyme-mediated synthesis. 1-*O*-(*N*-Cbz-L-phenylalanyl)glycerol/myristic acid molar ratio: ●, 1:2; □, 1:1.5; ▲, 1:1; ■, 1:0.75; ◆, 1:0.5. B: Effect of Novozyme concentration on synthesis. Amount of Novozyme added (% w/w): ●, 20%; ▲, 10%; ■, 5%; ◆, 2%.

of peptide–lipid conjugates. The properties of these products and their usefulness for the preparation of lipidated dipeptides are currently under investigation in our laboratory.

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