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ENZYMATIC SYNTHESIS OF GLYCAMIDE SURFACTANTS BY AMIDIFICATION REACTION

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Abstract : The condensation of a secondary amine (N-methyl-glucamine) with oleic acid was selected as a reaction model to study the production of glycamide surfactants by enzymatic amidification. Reactions catalyzed by immobilized lipase from *Candida antarctica* were carried out in 2-methyl-2-butanol. The acido-basic conditions (through the N-methyl-glucamine / oleic acid ratio) control the chemoselectivity of the reaction allowing the synthesis of either amide, ester or amide-ester. At 90°C and with a N-methyl-glucamine / acid ratio of 1, a 100 % conversion yield with 97 % of amide synthesis was obtained in less than 50 hours. The process was applied to the preparation of a range of amides using various amines and acyl donors. This process is the first that describes successful amide bond synthesis from a hydroxylated secondary amine and fatty acid or fatty acid ester.

INTRODUCTION

Biosurfactants that contain amide bonds constitute a new class of amphiphilic molecules, greatly appreciated by surfactant manufactures. The amide bond is chemically and physically very stable in alkaline media. In this class, glycamides (N-acyl-N-alkyl-glycamines) are surfactants produced from natural fats and oils. These nonionic and fully biodegradable surfactants cause less damage to the environment than petroleum chemical surfactants. They find applications in detergents, food, cosmetics and pharmaceuticals thanks to their surface active properties[1].

The chemical synthesis of these molecules has been developed but it is not very specific and requires very fastidious steps of hydroxyl group protection / deprotection. The high temperatures of the chemical reactions also prevent the use of fragile molecules and may cause coloration of final products. In addition, the coproduction of salts, and the use of toxic solvents (DMF, methanol) that must be eliminated at the end of the reaction increase the cost of the processes.

An interesting alternative is the use of biocatalysts. The enzymatic approach presents several advantages : there are no by-products and no need for protection / deprotection of the reagent, and the enzymes are regio, stereo and chemoselective. Nevertheless, for biocatalysis to be competitive, surfactants must be produced at low cost, preferably from cheap, renewable materials and at high yields. Enzymatic syntheses of amide bonds have been envisaged between a fatty acid and hydrophilic amine (such as a sugar

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amine). These reactions must be carried out in organic media. The first strategy that can be employed consists of using proteases. But it seems difficult to use these enzymes beyond their usual scope of application, because they are very specific for certain amino acids and are too sensitive to organic solvents[2]. As early as 1984, Inada *et al.* and Zaks and Klibanov demonstrated that lipases could serve as catalysts for amide bond synthesis reactions, in organic solvents containing a minimal amount of water[3]. Since, lipases have been used for the synthesis of peptides[4], fatty amides[5] and N-acyl-amino acids[6] and the acylation of aminopropanol[7]. However, the yields are too low for development on an industrial scale.

The present article reports the chemoselective acylation of N-alkyl-glycamines catalyzed by the immobilized lipase from *Candida antarctica* (Novozym®) in organic media for synthesis of glycamide surfactants. A convenient process that provides high reaction yields for the production of oleoyl-N-methyl-glucamide (**3a**) is described. This method has been extended to the synthesis of a range of glycamides by reverse hydrolysis or transacylation.

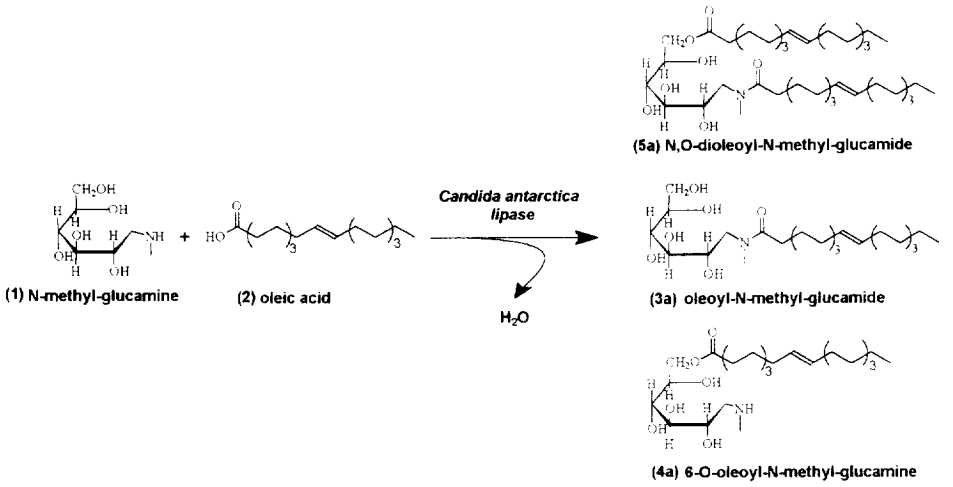
RESULTS AND DISCUSSION

Amidification of N-methyl-glucamine with oleic acid.

The synthesis by reverse hydrolysis of oleoyl-N-methyl-glucamide **3a** from N-methyl-glucamine **1** and oleic acid **2** was chosen as the reaction model. The two substrates **1, 2** of the reaction are molecules of different polarities and solubilities. Fatty acids are soluble in hydrophobic solvents, while N-methyl-glucamine is poorly soluble in such solvents. We used 2-methyl-2-butanol (polar protic solvent) for oleoyl-N-methyl-glucamide synthesis because it is a nontoxic solvent and this alcohol is not a substrate for lipase. Moreover, it partially dissolves N-methyl-glucamine (6 g/l at 55°C). The *Candida antarctica* lipase (Novozym®) was selected as a catalyst because this immobilized enzyme is commercially available, is stable in organic media and can be easily recovered. N-methyl-glucamine contains several hydroxyl functions that can react with oleic acid to produce unwanted by-products (N-methyl-glucamine mono-ester **4a** and N-methyl-glucamine amide-ester **5a**) (Scheme 1). It was thus necessary to find suitable operating conditions allowing the selective N-acylation of N-methyl-glucamine.

The first reaction was run under standard conditions, i.e. at 55°C and atmospheric pressure. From HPLC analysis (Figure 1), the decrease of oleic acid concentration was seen to be concomitant with the synthesis of two products **3a** and **4a**. Products **3a** and **4a** were easily distinguished by their respective UV absorbance. Product **3a** absorbed much more than **4a,** suggesting that it contained an amide bond. The isolation, purification and characterization of products **3a** and **4a** further confirmed that **3a** corresponded to oleoyl-N-methyl-glucamide and **4a** to the ester of N-methyl-glucamine.

Figure 2, shows the time course of the reaction up to 130 hours. At that point, thermodynamic equilibrium was reached ; 40 % of the oleic acid had been consumed, to give 80 % amide and 20 % ester.



Scheme 1

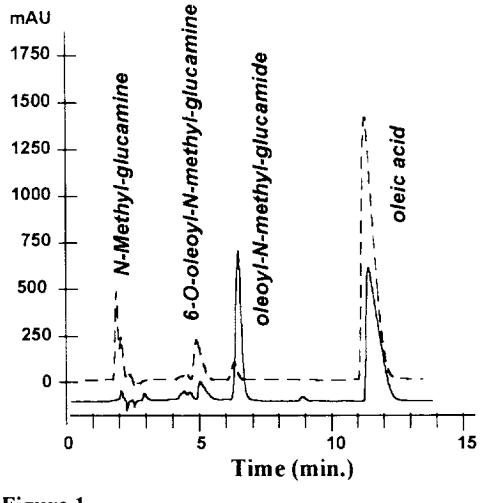


Figure 1.
HPLC Analysis of the products of N-methyl-glucamine acylation by oleic acid.
(N-methyl-glucamine was eluted at 2 minutes, followed by the mono-ester (at 5 minutes), the amide (at 6.3 minutes) and oleic acid (at 11.45 minutes).
Column C18 (250 x 4 mm), methanol/water/TFA (90/10/0.3, v/v/v), 1 ml/min., 40°C.
UV detection at 210 nm (—), Refractometer(- - -)

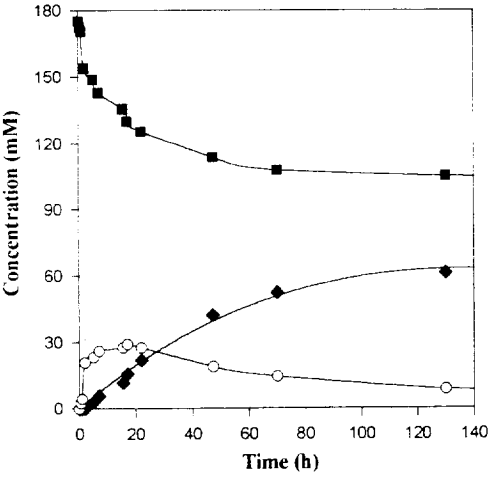


Figure 2.
Condensation of **1** (175 mM) with **2** (175 mM), in 2-methyl-2-butanol at 55°C under 1000 mBar with 10 g/l of Novozym®.
◆ oleoyl-N-methyl-glucamide (3a), O 6-O-oleoyl-N-methyl-glucamine (4a), ■ oleic acid (2).

Lipozyme® (lipase from *Rhizomucor miehei*) has also been tested in same conditions. The results obtained are not as good as the results with *Candida antarctica* lipase (Novozym®). With Proteases N-amano®, Proleather® (proteases from *Bacillus*) and α-chymotrypsine (protease from Bovine Pancreas), using the

standard reaction procedure, no reaction was observed, probably because they hardly hydrolyze the amide bond.

Reaction selectivity with Acid / N-methyl-glucamine ratio.

Several experiments were carried out using various acid / N-methyl-glucamine ratios. Table 1 reports the variations of selectivity and N-methyl-glucamine conversion versus the acid / N-methyl-glucamine ratio. An increase in the conversion of N-methyl-glucamine is observed with the increase of acid / N-methyl-glucamine ratio, but the corresponding selectivity [defined as the molar ratio amide / (amide + monoester + amide-ester)] for N-acylation dropped dramatically. The selectivity of the reaction, seems to be dependent on the acido-basic conditions of the reaction. In fact, when the acid / N-methyl-glucamine ratio is higher than 1, the medium is acid. In these conditions, the amine group is protonated and consequently cannot react with the acyl-enzyme. This enhances N-methyl-glucamine esterification. In contrast, when the ratio is lower than 1, the medium is more basic and the N-methyl-glucamine amidification becomes preponderant because the amine group is more reactive. A molar acid/amine ratio of 1 represents the best compromise between acylation yield and chemoselectivity.

Table 1. Chemoselective acylation of N-methyl-glucamine versus molar acid/amine ratio.

Molar ratio acid/amine	Acid conversion (%)	Amine conversion (%)	Amidification (%)
2/1	30	60	40
1/1	42	42	80
1/2	25	12.5	95

(Reactions were carried out at 55°C under atmospheric pressure, in 10 ml of 2-methyl-2-butanol, with 10 g/l of Novozym®)

Optimization of oleoyl-N-methyl-glucamide synthesis.

To shift the equilibrium toward synthesis, the coproduced water had to be eliminated. A temperature of 55°C did not provide optimum conditions for eliminating coproduced water efficiently during reverse hydrolysis. To efficiently remove water, higher temperatures compatible with the enzyme activity had to be used. Several reactions were thus attempted at 90 °C and under various pressures (Figure 3a et 3b).

At 90°C and 1000 mBar pressure, the enzyme activity increased and water generated during the reaction was partially removed. The equilibrium of the reaction was shifted towards synthesis. After 50 hours of reaction (Figure 3a), 100 % of oleic acid had been transformed and amide yield reached 97 %. A new product with 3 % yield, was synthesized. It was identified as an amide-ester (**5a**).It was probably formed from the ester **4a** produced during the first hours of the reaction and which had completely disappeared at the end of the reaction.

To improve the system performance at 90°C, the reaction was run under reduced pressure (500 mBar). The water was then removed more rapidly than under atmospheric pressure. Acid conversion reached 100 % in only 20 hours. However, amide-ester production was greater (10 %)(Figure 3b), and was then reduced by using higher amine concentrations in the reaction mixture (Table 2). But in these conditions,

excess amine still remained at the end of the reaction. Consequently, optimum conditions for selective amide production correspond to reaction at 90°C under atmospheric pressure, using a fatty acid / amine ratio of 1. These high yields are comparable to the values obtained by Ducret *et al.* for sugar ester formation by *Candida antarctica* lipase[8].

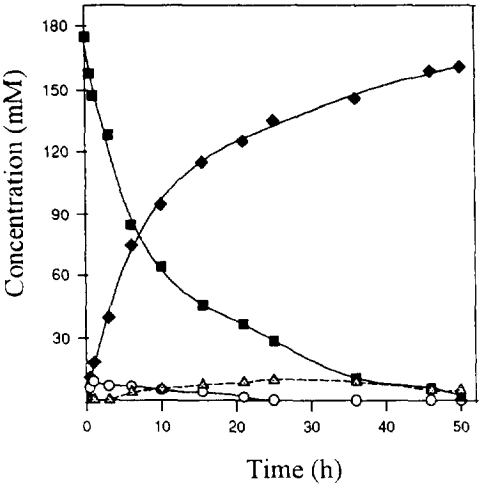


Figure 3 a. Condensation of N-methyl-glucamine (175 mM) with oleic acid (175 mM). The reaction was run in 2-methyl-2-butanol at 90°C under 1000 mBar with 10 g/l of Novozym®.
◆ oleoyl-N-methyl-glucamide, O 6-O-oleoyl-N-methyl-glucamine, ▲ N,O-dioleoyl-N-methyl-glucamide, ■ oleic acid.

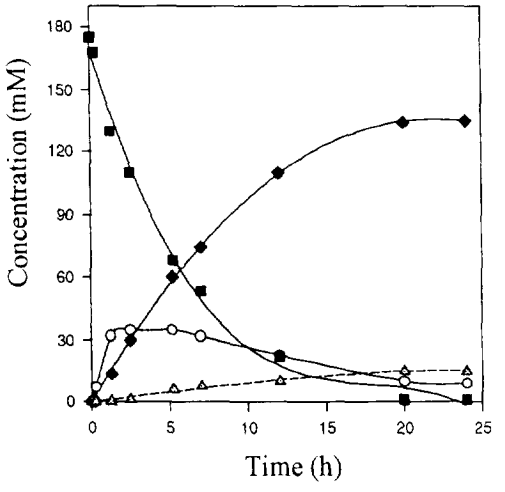


Figure 3 b. Condensation of N-methyl-glucamine (175 mM) with oleic acid (175 mM). The reaction was run in 2-methyl-2-butanol at 90°C under 500 mBar with 10 g/l of Novozym®.
◆ oleoyl-N-methyl-glucamide, O 6-O-oleoyl-N-methyl-glucamine, ▲ N,O-dioleoyl-N-methyl-glucamide, ■ oleic acid

Table 2. Effect of acid/N-methyl-glucamine molar ratio on amide-ester formation.

Oleic acid/N-methyl-glucamine ratio (mM/mM)	Amide-ester fraction **5a** (%)	Ester fraction **4a** (%)	Amide fraction **3a** (%)	Selectivity for amidification (%)	Total amine conversion (%)
175/90	30	20	50	50	100
175/175	10	5	75	83	90
175/350	3	1	92	93	48

(Reactions were carried out in 2-methyl-2-butanol at 90°C under 500 mBar with 10 g/l of Novozym®)
*Selectivity = **3a** / (**3a** + **4a** + **5a**)*

Synthesis of amide bond surfactants by transacylation reaction.

The synthesis of glucamides by transacylation using ester from natural sources and triglyceride was then tested. The reactions were completed in shorter times than for reverse hydrolysis (Table 3). With methyl oleate as the substrate, the kinetics of the transformation were shorter than with oleic acid as the acyl donor (Figure 4). During the first hours, both amide (**3a**) and ester (**4a**) of N-methyl-glucamine were synthesized, and after 3 hours the ester was consumed to give rise to amide-ester (**5a**). With Diester® (Colza fatty acid methyl ester), comparable results were obtained yielding more than 89 % of amide. The efficiency of the synthesis with Diester® means that the future development of the process on a large scale and at low cost will be possible.

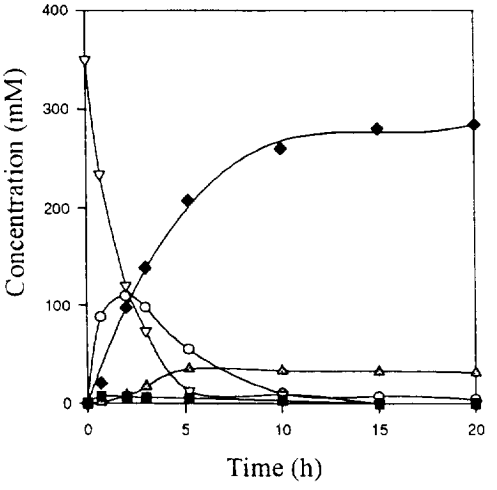


Figure 4. Condensation of N-methyl-glucamine (350 mM) with methyl oleate (350 mM). The reaction was run in 2-methyl-2-butanol at 90°C under 500 mBar with 10 g/l of Novozym®.
◆ oleoyl-N-methyl-glucamide, ○ 6-O-oleoyl-N-methyl-glucamine, ▲ N,O-dioleoyl-N-methyl-glucamide, ■ oleic acid, ∇ methyl oleate.

Table 3. Synthesis of glucamides by transacylation reaction.

Acyl donor	amine donor	[A] (mM)	[B] (mM)	Acid conversion (%)	Amine conversion (%)	Selectivity for amide (%)	Reaction time (hours)
ethyl oleate	N-methyl-glucamine	350	350	100	90	89	20
methyl oleate	N-methyl-glucamine	350	350	100	90	89	15
Diester®	N-methyl-glucamine	350	350	100	90	89	15
ethyl laurate	N-methyl-glucamine	350	350	100	90	89	20
trilaurine	N-methyl-glucamine	140	420	100	91	83	20

(Reactions were carried out in 2-methyl-2-butanol at 90°C under 500 mBar with 10 g/l of Novozym®).
[A] acyl donor concentration, [B] amine concentration
Selectivity = amide / (amide + monoesters + amide-esters)

Extension of the process.

Various amines were screened with respect to their ability to be used as substrates for amide bond surfactant synthesis.The experiments were all run at 90°C. For all the N-alkyl-glycamines tested (N-methyl-glucamine, N-methyl-galactamine, N-octyl-glucamine), the reactions were complete yielding more than 80 % amide (Table 4). Different acyl donors were also used : decanoic acid, dimethyl adipate and ethyl lactate. Again, we observed a complete reaction with an excellent selectivity for amidification. The results are presented in Table 4 .

Table 4. Extension of the amide synthesis.

Acyl donor	amine donor	[A] (mM)	[B] (mM)	Acid conversion (%)	Amide yield (%)	Reaction time (hours)
dimethyladipate[1]	N-methyl-glucamine	350	350	100	71	60
ethyl lactate	N-methyl-glucamine	350	350	100	90	100
decanoic acid	N-methyl-glucamine	175	175	100	83	25
oleic acid	N-methyl-galactamine	175	175	100	85	25
ethyl laurate	N-octyl-glucamine	72	72	100	84	90

All reactions were carried out at 90°C at 500 mBar with 10 g/l of of Novozym® except reaction **1** which used 25 g/l of Novozym®.
[A] acyl donor concentration, [B] amine concentration
Selectivity = amide / (amide + monoesters + amide-esters)

CONCLUSION

In conclusion, an efficient method has been developed for the lipase-driver synthesis of stable amide-bond surfactants such as oleoyl-N-methyl-glucamide. To our knowledge, this process is the first that describes amide bond synthesis from hydroxylated secondary amines and fatty acids, triglycerides or fatty acid esters by reverse hydrolysis or transacylation with yields reaching 97 %. A low cost, natural and renewable raw material (Diester®) was also demonstrated to be a very good substrate for the reaction. This is essential to envisage further industrial development. Finally, it was established that the process can be extended to various sources of amine or acyl donors.

EXPERIMENTAL PROCEDURES

Biological and Chemical Material

Novozym® SP 435 (lipase from *Candida antarctica* immobilized on an acrylic resin), Lipozyme® (lipase from *Rhizomucor miehei* immobilized on an anionic macroporous resin, Duolite 568N), were from Novo Industries (Denmark). Proteases N-amano® and Proleather® (proteases from *Bacillus*) were from

Amano Industries (Japan). α-chymotrypsine (protease from Bovine Pancreas) was from Sigma Chemical Co.(USA).

The solvents, all analytical grade, trilaurine, trioleine, dimethyl-adipate and ethyl lactate were from Fluka. N-methyl-glucamine (Sigma Chemical Co., USA.), N-methyl-galactamine (Aldrich) and N-octyl-glucamine (Aldrich) were more than 99 % pure. Except Diester® (Colza fatty acid methyl esters) which was from Sidobre Sinova, all the acyl donors were more than 99 % pure. All the fatty acids methyl or ethyl esters and fatty acids were supplied by Sigma Chemical Co. (USA).

General procedure for the enzymatic reaction

Amine, acyl agent and Novozym were mixed in 2-methyl-2-butanol. The reactions were carried out in 25 ml flasks mechanically stirred on a rotary evaporator (Büchi) which was used as a reactor under atmospheric or reduced pressure and at controlled temperature. In standard conditions, 1.75 mmoles (494.4 mg) of oleic acid was reacted with 1.75 mmoles (341.6 mg) of N-methyl-glucamine and 100 mg of Novozym® in 10 ml of 2-methyl-2-butanol. The reaction was run at 55°C under atmospheric pressure for 120 hours. These standard conditions were used except when otherwise stated in the text.

HPLC Analysis

Analyses were performed with an HPLC system from Hewlett Packard (processor, pump, UV detector and injector model 1050, differential refractometer (RI) model 1047A), equipped with an Ultrasep C18 (250 x 4 mm, 6 µ) reverse phase column from ICS, France. 25 µl of the proper dilution of the reaction mixture were injected. For reactions with long-chain fatty acids (more than 12 carbon atoms), a mixture of methanol/water/TFA, 90/10/0.3 (v/v/v) was used as eluent at 40°C and a flow rate of 1 ml/min. For reactions with short chain fatty acids (less than 12 carbon atoms), a mixture of methanol/water/TFA, 80/20/0.3 (v/v/v) was used as eluent at 40°C at a flow rate of 1 ml/min. With polyacid and lactic acid reaction, a mixture of methanol/water/TFA, 20/80/0.3 (v/v/v) was used as eluent a 40°C at a flow rate of 1 ml/min. Products were detected using a UV detector at 210 nm and a differential refractometer. The samples were quantitated by means of calibration curves with pure reagent.

Purification of reaction products

At the end of the reaction, the biocatalyst was removed by filtration and the solvent evaporated under reduced pressure. The remaining oil was separated into amide (N-acyl), monoesters of N-alkyl-glycamine (O-acyl) and amide-esters of N-alkyl-glycamine (N,O-diacyl), by chromatography using a silica gel (60 H, Merck) column (30 cm x 20 mm). The concentrated oil sample was diluted in a minimum volume of chloroform/methanol (9/1, v/v) and was deposited at the top of the column previously equilibrated with chloroform/methanol (9/1, v/v). The column was eluted with chloroform/methanol mixtures from 9/1 to 7/3 (v/v) for elution. All the fractions obtained were analysed by HPLC before structural analysis.

Structural analysis

Carbon 13 Nuclear Magnetic Resonance (^{13}C NMR) spectra were recorded using an AC 250 MHz spectrometer from Brüker, with an internal reference of tetramethylsilane. Infra-Red (IR) spectra were recorded using a Perkin Elmer IRFT 1760-x spectrometer for KBr pellets. Mass spectra were obtained by chemical ionisation (DCI/NH3), using a NERMAG R10-10 spectrometer.

1-deoxy-1-[methyl(1-oxo-9-octadecenyl)amino]-D-glucitol, (oleoyl-N-methyl-glucamide)(3a):

IR : ν (OH) = 3500 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} and ν (CO-N) = 1620 cm^{-1}.

^{13}C NMR/$CDCl_3$ (δ in ppm) : 175 (CO-N), 130 (2 CH=CH), 22.7-35.8 (14 CH2), 14 (CH3), 70-73 (4 CH-OH), 63.7 (CH2OH), 51(CH2N), 37.5 (CH3N). Anal. Calcd for $C_{25}H_{49}NO_6$: C, 65.42 ; H, 10.66. Found : C, 65.53 ; H, 10.74. Mass (DCI/NH3) : 461 ($M + H^+$), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3+ H^+).

1-deoxy-1-methylamino-6-(1-oxo-9-octadecenyl)-D-glucitol, (6-O-oleoyl-N-methyl-glucamine)(4a):

IR : ν (OH) = 3500 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} and ν (CO-O) = 1735 cm^{-1}.

^{13}C NMR/$CDCl_3$ (δ in ppm) : 178 (CO-O), 130 (2 CH=CH), 22.7-35.8 (14 CH2), 14 (CH3), 70-73 (4 CH-OH), 65.7 (CH2O), 49(CH2NH), 37.5 (CH3N). Anal. Calcd for $C_{25}H_{49}NO_6$: C, 65.42 ; H, 10.66. Found : C, 65.45 ; H, 10.84. Mass (DCI/NH3) : 461 ($M + H^+$), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3+ H^+).

1-deoxy-1-[methyl(1-oxo-9-octadecenyl)amino]-6-(1-oxo-9-octadecenyl)-D-glucitol, (N-O-dioleoyl-N-methyl-glucamide)(5a):

IR : ν (OH) = 3500 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} , ν (CO-O) = 1730 cm^{-1} and ν (CO-N) = 1620 cm^{-1}.

^{13}C NMR/$CDCl_3$ (δ in ppm) : 175 (CO-N), 176 (CO-O), 130-129.7 (4 CH=CH), 22-36 (28 CH2), 14 (2 CH3), 70-73 (4 CH-OH), 65.7 (CH2O), 51(CH2N), 37.5 (CH3N). Anal. Calcd for $C_{43}H_{81}NO_7$: C, 71.25 ; H, 11.18. Found : C, 71.33 ; H, 11.05. Mass (DCI/NH3) : 725 ($M - H^+$), 460 (M - 265 + H^+), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3+ H^+).

1-deoxy-1-[methyl(1-oxododecyl)amino]-D-glucitol, (lauroyl-N-methyl-glucamide)(3b):

IR : ν (OH) = 3500 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} and (CO-N) = 1620 cm^{-1}.

^{13}C NMR/D_2O (δ in ppm) : 177.8 (CO-N), 25.2-36.5 (10 CH2), 16.3 (CH3), 72.2-74.5 (4 CH-OH), 65.4 (CH2OH), 54.3 (CH2N), 39.6 (CH3N). Anal. Calcd for $C_{19}H_{39}NO_6$: C, 60.39 ; H, 10.33. Found : C, 61.02 ; H, 10.12. Mass (DCI/NH3) : 378 ($M + H^+$), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3+ H^+).

1-deoxy-1-[methyl(1-oxodecyl)amino]-D-glucitol, (decanoyl-N-methyl-glucamide)(3c):

IR : ν (OH) = 3500 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} and (CO-N) = 1620 cm^{-1}.

^{13}C NMR/$CDCl_3$ (δ in ppm) : 175.4 (CO-N), 22.7-34.2 (8 CH2), 14.3 (CH3), 70.2-72.5 (4 CH-OH), 63.4 (CH2OH), 51.3 (CH2N), 37.4 (CH3N). Anal. Calcd for $C_{17}H_{35}NO_6$: C, 58.37 ; H, 10.01. Found : C, 58.43 ; H, 10.20. Mass (DCI/NH3): 349 ($M + H^+$), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3 + H^+).

1-deoxy-1-[methyl(1-oxo-9-octadecenyl)amino]-D-galactitol, (oleoyl-N-methyl-galactamide)(3d):

IR : ν (OH) = 3500 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} and (CO-N) = 1620 cm^{-1}.

^{13}C NMR/$CDCl_3$ (δ in ppm) : 175 (CO-N), 130 (2 CH=CH), 22.7-35.8 (14 CH2), 14 (CH3), 70-73 (4 CH-OH), 63.7 (CH2OH), 51(CH2N), 37.5 (CH3N). Anal. Calcd for $C_{25}H_{49}NO_6$: C, 65.42 ; H, 10.66. Found : C, 65.49 ; H, 10.55. Mass (DCI/NH3) : 461 ($M + H^+$), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3+ H^+).

1-deoxy-1-[octyl(1-oxododecyl)amino]-D-glucitol, (lauroyl-N-octyl-glucamide)(3e):

IR : ν (OH) = 3500 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} and (CO-N) = 1620 cm^{-1}.

^{13}C NMR/$CDCl_3$ (δ in ppm) : 175 (CO-N), 25.2-34.15 (10 CH2), 14.05 (CH3), 72.2-74.5 (4 CH-OH), 63.9 (CH2OH), 49.7 (CH2N), 50 (C7H15-CH2-N), 25.2-34.15 (6 CH2), 14 (CH3). Anal. Calcd for $C_{26}H_{53}NO_6$: C, 65.58 ; H, 11.14. Found : C, 65.45 ; H, 10.98. Mass (DCI/NH3) : 476 ($M + H^+$), 294 (CH_2OH-$(CHOH)_4$-CH_2-NH-C_8H_{17} + H^+).

1-deoxy-1-[methyl(1-oxo-6-oxomethoxy-adipoyl)amino]-D-glucitol (3f):

IR : ν (OH) = 3400 cm^{-1}, ν (CH) = 2800-2900 cm^{-1}. (CO-N) = 1620 cm^{-1} and ν (CO-OCH3) = 1735 cm^{-1}.

^{13}C NMR/D_2O (δ in ppm) : 179 (CO-N), 179.6 (CO-O), 54.6 (OCH3), 34.5-36.7 (αCH2), 26.1-28 (βCH2), 70-74 (4 CH-OH), 65.2 (CH2OH), 54 (CH2N), 39.5 (CH3N). Anal. Calcd for $C_{14}H_{27}NO_8$: C, 49.79 ; H, 8.01. Found : C, 49.65 ; H, 7.98. Mass (DCI/NH3) : 338 ($M + H^+$), 324 (M - CH3 + $2H^+$), 355 ($M + NH4^+$), 306 (M - CH3 - H2O + $2H^+$), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3+ H^+).

1-deoxy-1-[methyl(2-methyl-2-hydroxy-1-oxopropyl)amino]-D-glucitol (3g):

IR : ν (OH) = 3350 cm^{-1}, ν (CH) = 2800-2900 cm^{-1} and (CO-N) = 1630 cm^{-1}.

^{13}C NMR/D_2O (δ in ppm) : 178.9 (CO-N), 66.9 (CH-OH), 10.4 (CH3), 72.6-74.5 (4 CH-OH), 66.5 (CH2-OH), 54.2 (CH2N), 38.7 (CH3N). Anal. Calcd for $C_{10}H_{21}NO_7$: C, 44.89 ; H, 7.85. Found : C, 44.81 ; H, 7.75. Mass (DCI/NH3) : 268 ($M + H^+$), 196 (CH_2OH-$(CHOH)_4$-CH_2-NH-CH_3+ H^+).

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