SUPPORTING INFORMATION

Chemoselective Enzymatic Acylation of Glycine as a Green Route to *N*-Acyl aminoacid Surfactants

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EXPERIMENTAL PROCEDURES

General methods

Reagents and solvents

Lauroyl chloride (Tokio Chemical industry Co., Ltd.; 98 %); benzylamine (Sigma-Aldrich; 99 %); glycine ethyl ester hydrochloride (Fluka; 99 %); glycine methyl ester hydrochloride (Fluka; 99 %); L-alanine methyl ester hydrochloride (Fluka; 99 %); L-glutamic acid dimethyl ester hydrochloride (Thermo Fisher Scientific; 98 %); ethyl laurate (Tokio Chemical industry Co., Ltd.; 99 %); methyl laurate (Tokio Chemical industry Co., Ltd.; 98 %); methyl palmitate (Fluka; 97 %); methyl oleate (Tokio Chemical industry Co., Ltd.; 60 %); triethylamine (Apollo Scientific; 100 %); potassium bicarbonate (Carlo Erba; 99,7 %); sodium bicarbonate (Carlo Erba; 99,7 %); sodium carbonate (Carlo Erba; 99,8 %); sodium hydroxide (VWR Chemicals; 98,6 %); tris(hydroxymethyl)aminomethane (Sigma-Aldrich; 99,8 %).

Isopropanol (Sigma-Aldrich; 99,5 %); trifluoroethanol (Tokio Chemical industry Co., Ltd.; 99 %); cyrene (Circa FC5; 99,3 %); tetrahydrofuran (Sigma-Aldrich; 99,9 %); heptane (Sigma-Aldrich; 99 %); tert-butyl alcohol (Merck; 99,5 %); acetonitrile (VWR Chemicals; 100 %); diisopropyl ether (Sigma-Aldrich; 99 %); tetramethyl tetrahydrofuran (**prepared in house**); methanol (Sigma-Aldrich; 100 %); ethanol (VWR Chemicals; 99,7 %).

Enzymes

Novozym 435 (Novozymes A/S; product code LC200232); Lipase from porcine pancreas (Sigma; product code L3126); Lipase AS Amano (Amano Enzyme Inc.; product code LAI1051510S); Lipase M Amano (Amano Enzyme Inc.; product code LMO1251205K); Lipase AYS Amano (Amano Enzyme Inc.; product code LAYH0252411S); Lipase from candida cylindracea (Sigma; product code L1754); Lipozyme RM IM (Novozymes A/S; product code LUX00213); Lipase from Aspergillus niger (Fluka; product code 62301); Lipase from Penicillium roqueforti (Fluka; product code 62308); Papain (Sigma; product code P3375); Lipase A Amano 6 (Amano Pharmaceutical Co., Ltd.; product code LT02515); Lipozyme TL IM (Novozymes A/S; product code LA 330045); Lipase PS Amano SD (Amano Enzyme Inc.; product code LPO1250704SD); Lipase AK Amano (Amano Enzyme Inc.; product code LAKO0752107).

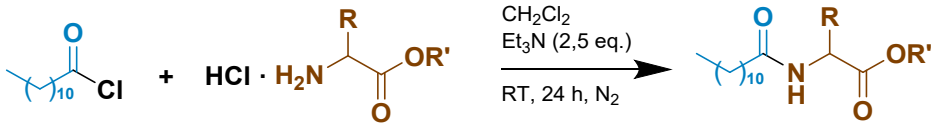
Routine analysis

TLC analysis was performed using glass supported silica gel plates (Macherey-Nagel 60 0.25 mm); basic potassium permanganate (1.5 g $KMnO_4$, 10 g K_2CO_3, 1 mL NaOH 10% in 200 mL water) was used for stains developing.

Column chromatography was carried out using “flash” methodology using silica gel 60 Å (230-400 mesh).

Regardless of the reaction and dryness of the solvent, all reactions were carried out in an N_2 atmosphere.

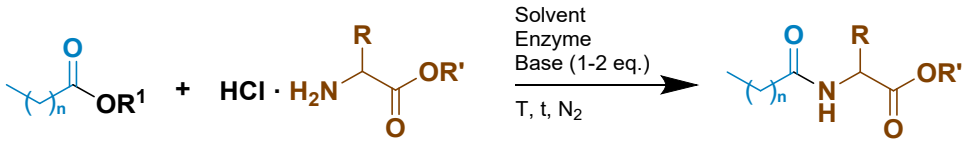
General procedure 1 (GP-1) for the synthesis of N-lauroyl aminoacid ester – reference compound for GC-MS calibration and quantitative NMR measurements



Aminoacid ester hydrochloride (2 mmol, 1 eq.) was dissolved in anhydrous dichloromethane in inert atmosphere (N_2) at 0 °C. When the mixture became homogeneous, lauroyl chloride (2.2 mmol, 1.1 eq.) was added, followed by triethylamine (5 mmol, 2.5 eq.). The reaction was carried out at room temperature in 24 h. Workup exists in the addition of aqueous K_2CO_3 solution (70 mg mL^{-1}) and stirring for 30 min; extraction with EtOAc; washing with NH_4Cl; drying with Na_2SO_4 and filtration through a cotton. Finally, EtOAc was removed using a rotary evaporator and a vacuum pump.

Scope of aminoacid esters: glycine ethyl ester, L-alanine methyl ester, L-glutamic acid dimethyl ester, benzylamine (amine).

General procedure 2 (GP-2) for the enzymatic synthesis of *N*-acyl aminoacid esters



Amino acid ester hydrochloride (1.75 mmol, 1 eq.) was weighed with fatty acid ester (1.75 mmol, 1 eq.), base (1.75-3.5 mmol, 1-2 eq.) and enzyme (137-275 mg / 1 mmol fatty acid ester) in a round-bottom flask with a magnetic stirrer. After addition of anhydrous solvent (1 mL / 0.25-1 mmol) the mixture was stirred in 3-24 hours at 40-60 °C. Workup included the addition of EtOAc and mixing in 30 min; filtration through celite; washing with NH_4Cl and drying with Na_2SO_4. Finally, EtOAc was removed using a rotary evaporator and a vacuum pump.

Scope of fatty acid esters (FAE): ethyl laurate, methyl laurate, methyl palmitate, methyl oleate.

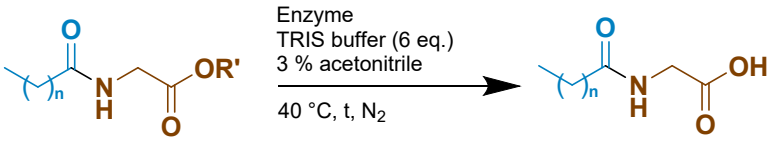
Scope of aminoacid esters (AAE) hydrochlorides: glycine ethyl ester, glycine methyl ester, L-alanine methyl ester, L-glutamic acid dimethyl ester, benzylamine (amine).

Scope of solvents: isopropanol, trifluoroethanol (TFE), cyrene, tetrahydrofuran (THF), tetramethyl tetrahydrofuran (TM-THF), heptane, diisopropyl ether (DIPE), tert-butyl alcohol (*t*BuOH), acetonitrile (MeCN).

Scope of enzymes: Novozym 435, Lipase from porcine pancreas, Amano Lipase AS, Amano Lipase M, Amano Lipase AYS, Lipase from candida cylindracea, Lipozyme RM IM, Lipase from Aspergillus niger, Lipase from Penicillium roqueforti, Papain, Amano Lipase A 6, Lipozyme TL IM, Amano Lipase PS SD, Amano Lipase AK.

Scope of bases: Et_3N, $KHCO_3$, $NaHCO_3$, Na_2CO_3.

General procedure 3 (GP-3) for enzymatic hydrolysis of N-acyl amino acid ester to N-acyl amino acid



The mixture of *N*-acyl aminoacid ester (0.15 mmol, 1 eq.), enzyme (1 mg / 3 µmols) was stirred in tris(hydroxymethyl)aminomethane (TRIS) buffer (pH=7, 0.25 mol L^{-1}, 6 eq.) with the addition of acetonitrile as the co-solvent (3 % by volume to TRIS buffer) in 24-72 h at 40 °C. Workup included the addition of EtOAc; filtration through celite; extraction with saturated solution of NaCl and 1M HCl (pH < 2) and drying with Na_2SO_4. Finally, EtOAc was removed using a rotary evaporator and a vacuum pump.

Scope of *N*-acyl aminoacid esters: *N*-lauroyl glycine ethyl ester, *N*-palmitoyl glycine methyl ester.

Scope of enzymes: Novozym 435, Amano Lipase AK.

See Table 1 of paper or Table of S.I.

General procedure 4 (GP-4) for chemical hydrolysis of *N*-acyl aminoacid ester to *N*-acyl aminoacid

N-acyl aminoacid ester (0.15 mmol) was suspended in a 1:1 solution of alcohol and 1M NaOH (2 eq.). The mixture was stirred for 72 h at RT. Work up included the addition of 1M HCl (pH < 2), saturated solution of NaCl and EtOAc; drying the organic phase with Na2SO4. Finally, EtOAc was removed using a rotary evaporator and a vacuum pump.

Scope of *N*-acyl aminoacid esters: *N*-lauroyl glycine ethyl ester

COMPREHENSIVE TABLE OF RESULTS

Entry	Electrophile	Nucleophile	Solvent	Enzyme	Base	Time, h	Results, %		
							Electrophile	Product	By-product
1	Ethyl laurate	$BnNH_2$	Isopropanol	Novozym 435	-	3	100	-	-
2	Ethyl laurate	$BnNH_2$	TFE	Novozym 435	-	3	100	-	-
3	Ethyl laurate	$BnNH_2$	Cyrene	Novozym 435	-	3	100	-	-
4	Ethyl laurate	$BnNH_2$	THF	Novozym 435	-	3	69	31	-
5	Ethyl laurate	$BnNH_2$	THF + 3% H_2O	Novozym 435	-	3	100	-	-
6	Ethyl laurate	$BnNH_2$	Heptane	Novozym 435	-	3	53	47	-
7	Ethyl laurate	$BnNH_2$	Heptane + 3% H_2O	Novozym 435	-	3	92	8	-
8	Ethyl laurate	$BnNH_2$	t-BuOH	Novozym 435	-	3	52	48	-
9	Ethyl laurate	$BnNH_2$	t-BuOH + m.s.	Novozym 435	-	3	25	75	-
10	Ethyl laurate	$BnNH_2$	MeCN	Novozym 435	-	3	16	84	-
11	Ethyl laurate	$BnNH_2$	DIPE	Novozym 435	-	3	12	88	-
12	Ethyl laurate	$BnNH_2$	DIPE + 3% H_2O	Novozym 435	-	3	92	8	-
13	Ethyl laurate	$BnNH_2$	TM-THF	Novozym 435	-	3	5	95	-
14	Ethyl laurate	Gly-OEt	TM-THF	Novozym 435	Et_3N, 1 eq.	3	100	-	-
15	Ethyl laurate	Gly-OEt	DIPE	Novozym 435	Et_3N, 1 eq.	3	100	-	-
16	Ethyl laurate	Gly-OEt	MeCN	Novozym 435	Et_3N, 1 eq.	3	68	32	
17	Ethyl laurate	Gly-OEt	t-BuOH + m.s.	Novozym 435	Et_3N, 1 eq.	3	65	22	13
18	Ethyl laurate	Gly-OEt	MeCN	Lipase from porcine pancreas	Et_3N, 1 eq.	18	100	-	-
19	Ethyl laurate	Gly-OEt	MeCN	Amano Lipase AS	Et_3N, 1 eq.	18	100	-	-
20	Ethyl laurate	Gly-OEt	MeCN	Amano Lipase M	Et_3N, 1 eq.	18	100	-	-
21	Ethyl laurate	Gly-OEt	MeCN	Amano Lipase AYS	Et_3N, 1 eq.	18	100	-	-
22	Ethyl laurate	Gly-OEt	MeCN	Lipase from candida cylindracea	Et_3N, 1 eq.	18	100	-	-

23	Ethyl laurate	Gly-OEt	MeCN	Lipozyme RM IM	Et_3N, 1 eq.	18	100	-	-
24	Ethyl laurate	Gly-OEt	MeCN	Lipase from Aspergillus niger	Et_3N, 1 eq.	18	100	-	-
25	Ethyl laurate	Gly-OEt	MeCN	Lipase from Penicillium roqueforti	Et_3N, 1 eq.	18	100	-	-
26	Ethyl laurate	Gly-OEt	MeCN	Papain	Et_3N, 1 eq.	18	100	-	-
27	Ethyl laurate	Gly-OEt	MeCN	Amano Lipase A 6	Et_3N, 1 eq.	18	100	-	-
28	Ethyl laurate	Gly-OEt	MeCN	Lipozyme TL IM	Et_3N, 1 eq.	18	96	4	-
29	Ethyl laurate	Gly-OEt	MeCN	Amano Lipase PS SD	Et_3N, 1 eq.	18	95	5	-
30	Ethyl laurate	Gly-OEt	MeCN	Amano Lipase AK	Et_3N, 1 eq.	18	88	12	-
31	Methyl laurate	Gly-OEt	MeCN	Amano Lipase AK	Et_3N, 1 eq.	18	83	16	1
32	Methyl laurate	Gly-OMe	MeCN	Amano Lipase AK	Et_3N, 1 eq.	18	70	29	1
33	Methyl palmitate	Gly-OEt	MeCN	Amano Lipase AK	Et_3N, 1 eq.	18	72	27	1
34	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	Et_3N, 1 eq.	18	49	50	1
35	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	Et_3N, 1 eq.	18, 60 °C	59	40	1
36	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	$KHCO_3$, 1 eq.	18	21	74	5
37	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	$KHCO_3$, 2 eq.	18	19	77	4
38	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	Et_3N, 1 eq.	18	17	81	2
39	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	$KHCO_3$, 1.1 eq.	18	10	88	2
40	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	$KHCO_3$, 1.5 eq.	18	4	92	4
41	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	$KHCO_3$, 2 eq.	18	3	93	4
42	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	$NaHCO_3$, 2 eq.	18	10	86	4
43	Methyl palmitate	Gly-OMe	MeCN	Amano Lipase AK	Na_2CO_3, 2 eq.	18	14	82	4
44	Methyl palmitate	Gly-OMe	t-BuOH	Amano Lipase AK	$KHCO_3$, 2 eq.	18	-	97	3
45	Methyl laurate	Gly-OMe	MeCN	Amano Lipase AK	$KHCO_3$, 2 eq.	24	32	64	4

46	Methyl laurate	Gly-OMe	t-BuOH	Amano Lipase AK	$KHCO_3$, 2 eq.	24	5	84	11
47	Methyl oleate	Gly-OMe	MeCN	Amano Lipase AK	$KHCO_3$, 2 eq.	24	29	66	5
48	Methyl oleate	Gly-OMe	t-BuOH	Amano Lipase AK	$KHCO_3$, 2 eq.	24	6	88	6
49	Methyl palmitate	Ala-OMe	t-BuOH	Amano Lipase AK	$KHCO_3$, 2 eq.	24	100	-	-
50	Methyl palmitate	Ala-OMe	t-BuOH	Novozym 435	$KHCO_3$, 2 eq.	24	100	-	-
51	Methyl palmitate	Glu-$(OMe)_2$	t-BuOH	Amano Lipase AK	$KHCO_3$, 2 eq.	24	100	-	-
52	Methyl palmitate	Glu-$(OMe)_2$	t-BuOH	Novozym 435	$KHCO_3$, 2 eq.	24	100	-	-
53	Coconut oil	Gly-OMe	t-BuOH +3 % MeOH	Amano Lipase AK	$KHCO_3$, 2 eq.	24	53	45	2
54	Coconut oil	Gly-OMe	t-BuOH +3 % MeOH	Amano Lipase AK	$KHCO_3$, 2 eq.	72	23	73	4
55	*N*-lauroyl glycine ethyl ester	H_2O	TRIS buffer, 6 eq. + 3 % MeCN (pH=7)	Novozym 435	-	24	-	100	-
56	*N*-lauroyl glycine ethyl ester	H_2O	TRIS buffer, 6 eq. + 3 % MeCN (pH=7)	Amano Lipase AK	-	72	74	26	
57	*N*-palmitoyl glycine methyl ester	H_2O	TRIS buffer, 6 eq. + 3 % MeCN (pH=7)	Novozym 435	-	24	-	100	-
58	*N*-lauroyl glycine ethyl ester	^-OH	1M NaOH : EtOH 1 : 1	-	NaOH, 2 eq.	72, RT	0,5	99,5	-
Conditions for entries 1-37: 275 mg of enzyme / 1 mmol of fatty acid ester; 1 mL solvent / 0.25 mmol of all reactants Conditions for entries 38-54: 137 mg of enzyme / 1 mmol of fatty acid ester; 1 mL solvent / 1 mmol of all reactants Conditions for entries 55-58: 1 mg enzyme / 1 mg of *N*-acyl aminoacid ester									

ANALYTICAL METHODS

GC-MS measurement

GC-MS measurements were performed with the use of Shimadzu GC−MS-QP2010 SE spectrometer with the HI-5 MS column (0.25 mm, 0.25 mm, 30 m). The following method was used for GC-MS analysis: starting temperature 150 °C, starting time 3 min, temperature rate 25 °C min^{-1}, final temperature 300 °C, column flow 0,96 mL min^{-1}, split ratio 1:10, volume injected 1 µL.

For GC-MS calibration, two curves were created: the area ratio of various concentrations of 1. initial ethyl laurate and 2. the product of GP-1 (15, 30, 50, 80, 100 mg L^{-1}) to fixed concentration of internal standard (50 mg L^{-1}). Methyl palmitate was used as the internal standard due to high structural similarity with ethyl laurate. These two curves can be described by the equation ***y = kx + m***, where ***y*** is the ratio of the peak areas of internal standard to 1. ethyl laurate or 2. product, and ***x*** is the concentration (mg L^{-1}) of ethyl laurate or the product.

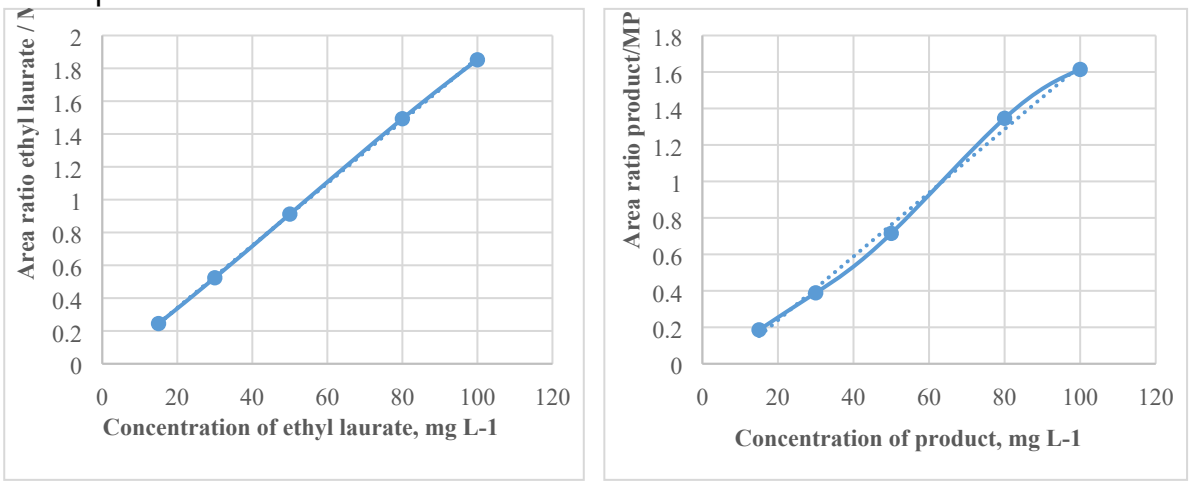


Figure 1. Example of calibration curves for calculating the conversion

The reaction crude after workup (GP-2) was dissolved in the mixture of EtOAc and dichloromethane. Approximate concentration of obtained mixture is 100 mg L^{-1} (starting fatty acid ester + N-acyl amino acid ester).

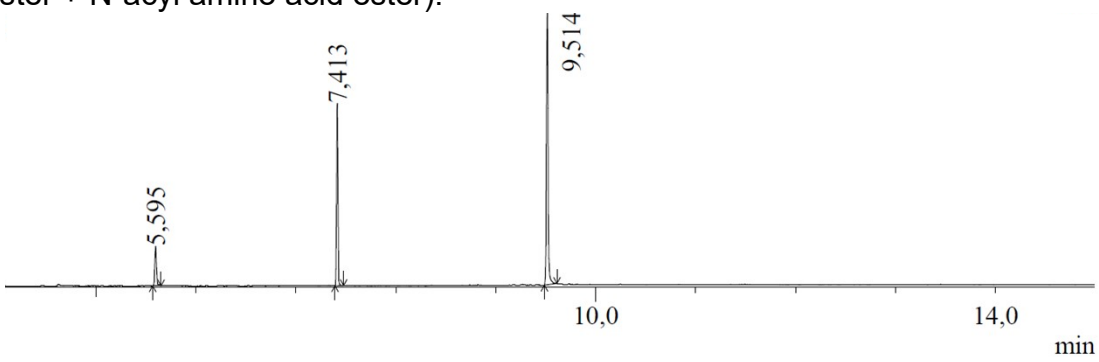


Figure 2. Example of GC-MS spectra: reaction between ethyl laurate and benzylamine in MeCN (entry 10)

The efficiency of the reaction is assessed by the amount of fatty acid esters that have converted into the product. Analysis of the residual amount of amino acid ester is not possible because it is extracted into the aqueous phase during workup.

The ratios of the areas under the peaks in the GC-MS spectra of the starting material (5.6 min) and the product (9.5 min) to the internal standard (7.4 min) are used to calculate the residual concentration of ethyl laurate and the concentration of the synthesized product (mg L^{-1}) according to the following equation: ***C = (area ratio – m) / k***, where ***m*** and ***k*** are taken from the calibration curves; ***area ratio*** = *area (ethyl laurate or product) / area (internal standard)* from the measurement results (Table 1; column "Area").

Table 1. Peak report on the GC-MS spectra of Figure 2

Peak	**R. Time, min**	**Area**	**Area, %**	**Height, %**
1 (ethyl laurate)	5,595	706405	8,88	7,98
2 (internal standard)	7,413	2679158	33,69	36,82
3 (product)	9,514	4565946	57,42	55,19

When the concentration is known (mg L^{-1}), the molar concentration (µmol L^{-1}) was calculated as: $C_M = C / M$, where ***M*** is the molar weight of compound.

When the molar concentration is known, conversion of ethyl laurate to the product was calculated as: $Conversion = 100\ \% \cdot C_M\ (product) / SUM\ C_M$, where $SUM\ C_M$ is the sum of the molar concentrations of ethyl laurate and product.

Table 2. Example of calculation

Substrate	**Area ratio**		**C, mg L^{-1}**	**C_M, µmol L^{-1}**	**Conversion, %**
Ethyl laurate	0.264		16	70.1	83,6
Internal standard		1.7			
Product			103.4	357.2	

The method was used to quantitatively measure the conversion of GP-2 reactions (entry 1–17); when the presence of by-product was detected, GC-MS spectroscopy was replaced by NMR spectroscopy.

^{1}H NMR measurement

NMR spectra were recorded at 400 MHz with the use of JEOL 400 and analyzed with MestReNova (Mestrelab Research® v. 14.2) as the software. NMR measurements were carried out at RT in chloroform-D for the crude after enzymatic synthesis (entry 18-54); in DMSO D_6 – after enzymatic hydrolysis (entry 55-58). Chemical shifts of NMR signals are given in δ = ppm (part per million); their multiplicity is abbreviated as s-singlet; d-doublet; t-triplet; q-quartet; m-multiplet; b-broad signal.

As with GC-MS amalysis, the efficiency of the reaction was assessed by the amount of fatty acid esters that have converted into the product and by-product. Analysis of the residual amount of amino acid ester is not possible because it is extracted into the aqueous phase during workup.

Since the reaction crude of GP-2 was not purified by flesh column chromatography, it consists of the mixture of starting fatty acid ester, product and by-product. Therefore, to determine the position of the NMR signals, the spectra of the individual compounds (product and by-product) were recorded and compared at the beginning; NMR spectra of fatty acid esters are known in literature.

Typical ^{1}H NMR spectra of GP-2 consist of a singlet of tetramethylsilane (TMS) as a reference (δ=0.00); a broad peak of water (δ=1.65) and a shape peak of chloroform D (δ=7.27). The remaining peaks correspond to the crude of the reaction:

1. Protons on terminal carbon of an aliphatic chain: δ=0.86-0.89 (t, 3H, CH_3) of starting FAE, product and by-product (eclipsed).
2. Protons on aliphatic chain carbons: δ=1.25-1.35 (b, 2H, CH_2) of starting FAE, product and by-product (eclipsed).
3. Protons on carbon of aliphatic chain, directly adjacent to carbonyl group: δ=2.22-2.25 (t, 2H, CH_2) of product and by-product (eclipsed); δ=2.28-2.31 (t, 2H, CH_2) of starting FAE.
4. If a methyl ester of amino acid and FAE are used, protons on methyl carbon of an ester group: δ=3.65 (s, 3H, CH_3) of by-product; δ=3.67 (s, 3H, CH_3) of starting FAE; δ=3.77 (s, 3H, CH_3) of product.
   If an ethyl ester of amino acid and FAE are used, protons on methyl carbon of an ester group: δ=1.27-1.31 (t, 3H, CH_3) for the starting FAE, product and by-product (eclipsed); protons of methylene carbon directly adjacent to ester oxygen: δ=4.20-4.25 (q, 2H, CH_2) of product; δ=4.19-4.25 (q, 2H, CH_2) of by-product; δ=4.1-4.15 (q, 2H, CH_2) of starting FAE.
5. Protons on methylene carbon directly adjacent to nitrogen: δ=3.98-4.00 (d, 2H, CH_2) of by-product and δ=4.05-4.06 (d, 2H, CH_2) of product and by-product (eclipsed).
6. Protons on nitrogen of an amide bond: δ=5.95 (b, 1H, NH) of product; δ=6.28 (b, 1H, NH) and δ=6.64 (b, 1H, NH) of by-product.

The integrated signals of protons on carbon of aliphatic chain, directly adjacent to carbonyl group were used to calculate the conversion of fatty acid esters to the mixture of product and by-product. Example (Figure 3): $\mathbf{I_1}$ (δ=2.28-2.31) is 1; $\mathbf{I_2}$ (δ=2.22-2.25) is 9.16. Therefore, $\boldsymbol{Conversion = 100\ \% \cdot I_2 / (I_1+I_2)}$ = 90 %.

The integrated signals of protons on methylene carbon directly adjacent to nitrogen were used to calculate the amount of by-product. Example (Figure 3): $\mathbf{I_3}$ (δ=3.97-3.99) is 0.17; $\mathbf{I_4}$ (δ=4.04-4.06) is 9.15. Therefore, $\boldsymbol{By\text{-}product\ quantity = I_3 / I_4 \cdot Conversion}$ = 2 %.

Result of calculation: 10 % of starting FAE; 88 % of product; 2 % of by-product.

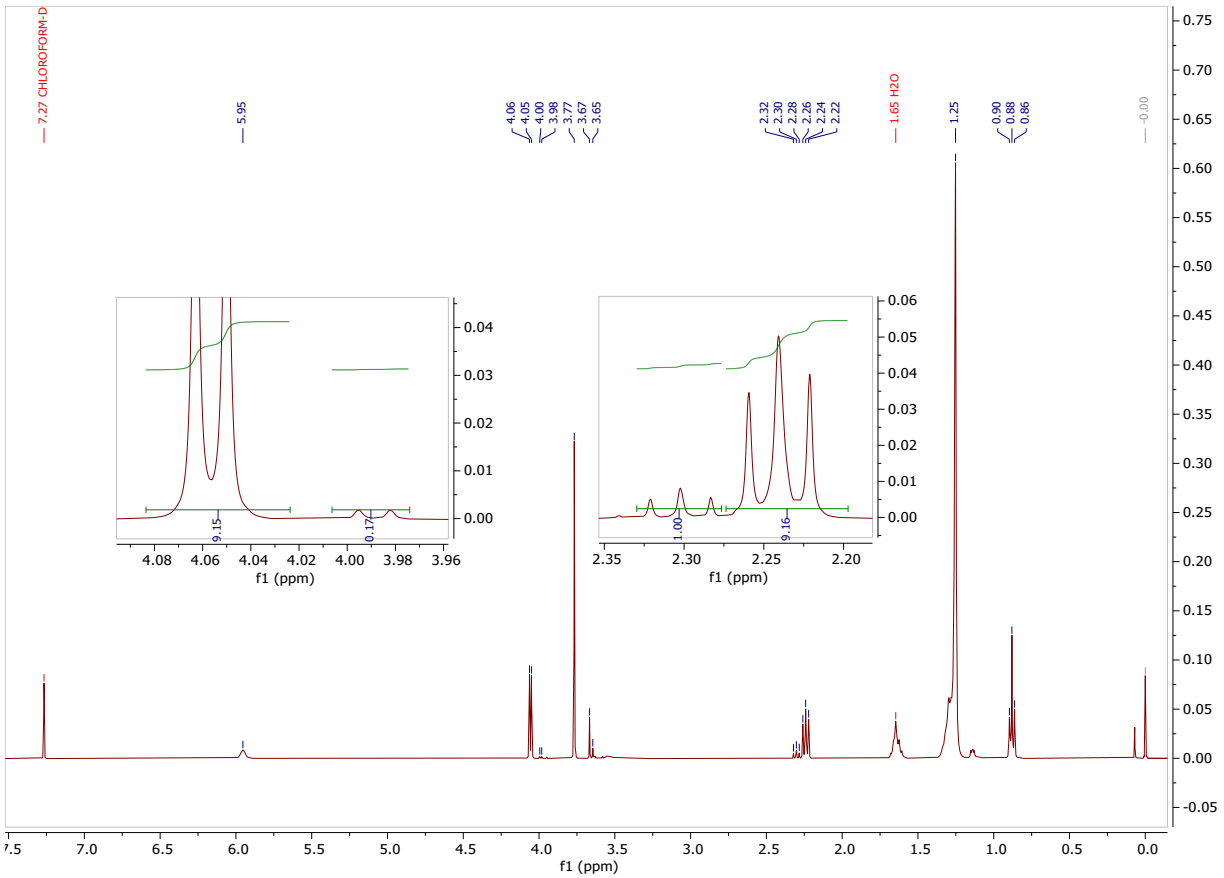


Figure 3. Example of NMR spectra: reaction between methyl palmitate and glycine methyl ester in MeCN with Lipase AK Amano (entry 39)

Typical ^{1}H NMR spectra of GP-3 consist of a singlet of TMS as a reference (δ=0.00); a broad peak of water (δ=1.65) and a broad peak of DMSO D_6 (δ=2.5). The remaining peaks correspond to the crude of the reaction:

1. Protons on terminal carbon of an aliphatic chain: δ=0.84-0.87 (t, 3H, CH_3) of starting *N*-acyl aminoacid ester and *N*-acyl aminoacid (eclipsed).
2. Protons on aliphatic chain carbons: δ=1.2-1.3 (b, 2H, CH_2) of starting *N*-acyl aminoacid ester and *N*-acyl aminoacid (eclipsed).
3. Protons on carbon of aliphatic chain, directly adjacent to carbonyl group: δ=2.08-2.12 (t, 2H, CH_2) of starting *N*-acyl aminoacid ester and *N*-acyl aminoacid (eclipsed).
4. If the starting material is *N*-acyl aminoacid methyl ester, protons on methyl carbon of an ester group: δ=3.62 (s, 3H, CH_3).
   If the starting material is *N*-acyl aminoacid ethyl ester, protons on methyl carbon of an ester group: δ=1.16-1.20 (t, 3H, CH_3); protons of methylene carbon directly adjacent to ester oxygen: δ=4.05-4.10 (q, 2H, CH_2).
5. Protons on methylene carbon directly adjacent to nitrogen: δ=3.77-3.78 (d, 2H, CH_2) of starting N-acyl amino acid ester and δ=3.70-3.72 (d, 2H, CH_2) of *N*-acyl aminoacid.
6. Protons on nitrogen of an amide bond: δ=8.18-8.21 (t, 1H, NH) of starting *N*-acyl aminoacid ester and δ=8.07-8.10 (t, 1H, NH) of *N*-acyl aminoacid.

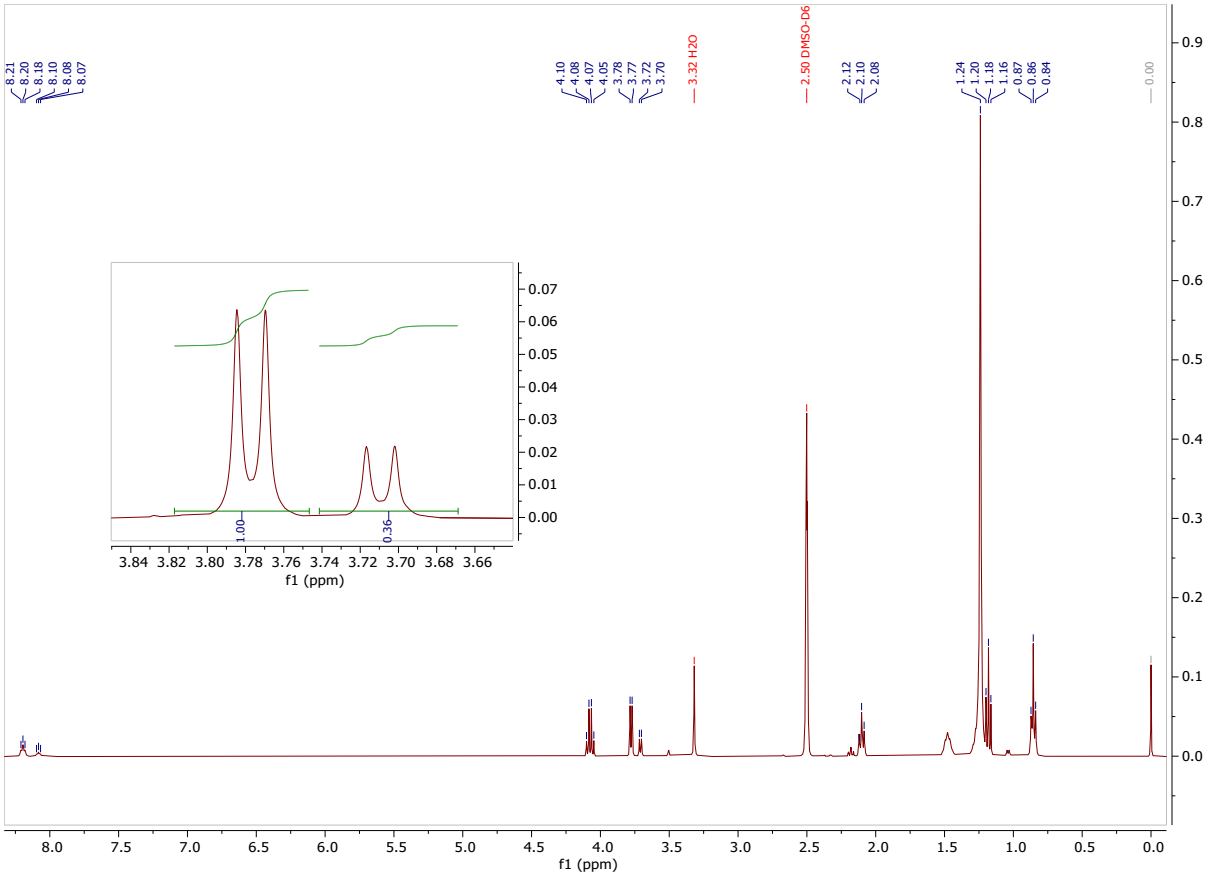


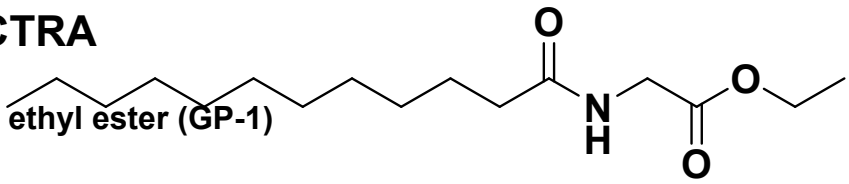
Figure 4. Example of NMR spectra: enzymatic hydrolysis of N-lauroyl glycine ethyl ester to N-lauroyl glycine with Lipase AK Amano (entry 56)

Hydrolysis efficiency was estimated as the conversion of *N*-acyl aminoacid esters to *N*-acyl aminoacids; the integrated signals of protons on methylene carbon directly adjacent to nitrogen were used to calculate it. Example (Figure 4): $\mathbf{I_1}$ (δ=3.77-3.78) is 1; $\mathbf{I_2}$ (δ=3.70-3.72) is 0.36. Therefore, $\boldsymbol{Conversion = 100\ \% \cdot I_2 / (I_1+I_2)}$ = 26 %.

Result of calculation: 74 % of starting *N*-acyl aminoacid ester; 26 % of *N*-acyl aminoacid.

NMR SPECTRA

N-acyl glycine ethyl ester (GP-1)



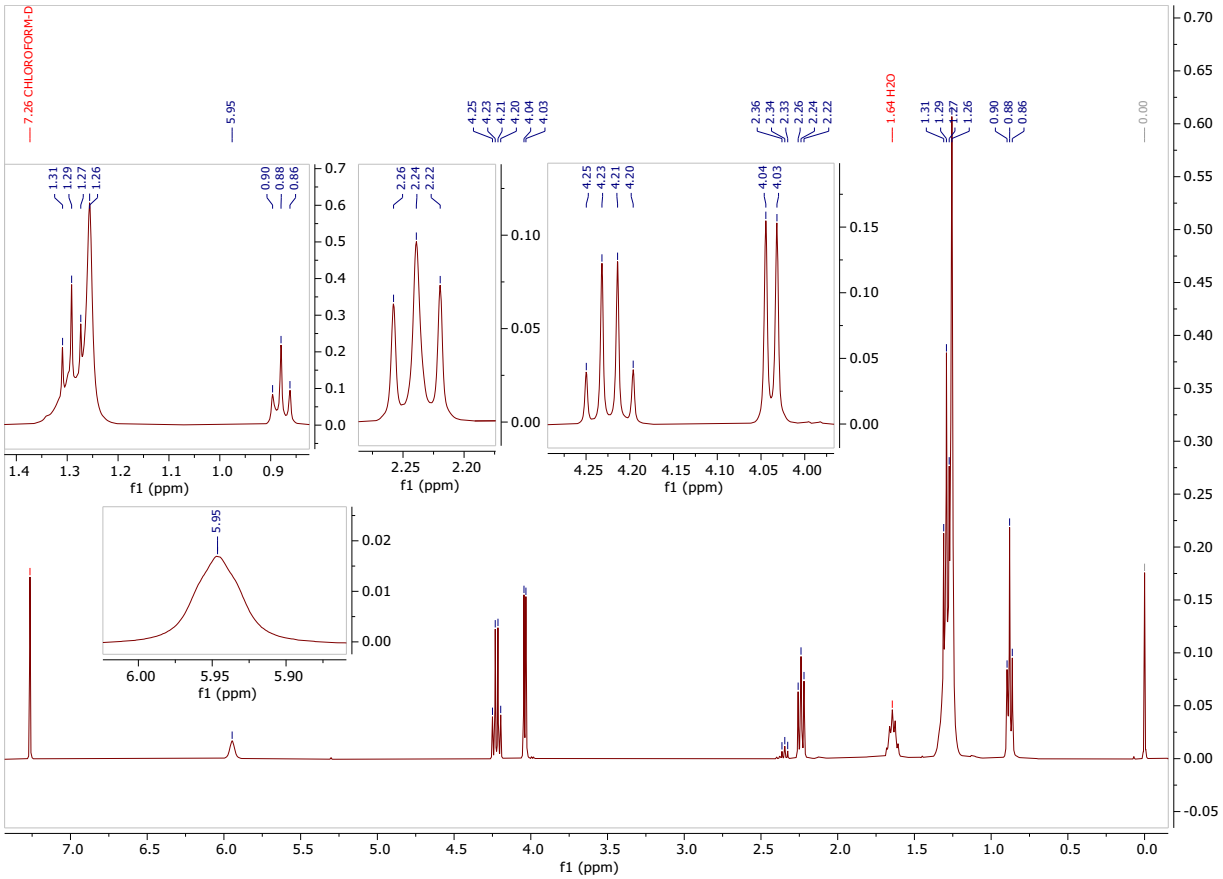
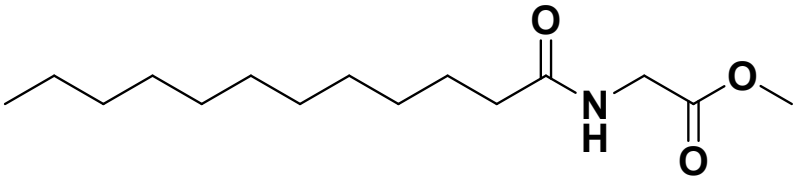


Figure 5. NMR spectra of N-lauroyl glycine ethyl ester

^{1}H NMR (400 MHz; $CDCl_3$, RT): δ=0.88 (t, *J*= 6.9, 3H, C*H*$_3$), 1.22-1.35 (m, 16H, C*H*$_2$), 1.29 (t, *J*= 7.2, 3H, O-CH$_2$-*CH*$_3$), 1.64 (quint., *J*= 7.4, 2H, C*H*$_2$CH$_2$C=O)(in part superimposed with H_2O peak), 2.24 (t, *J*= 7.6, 2H, CH$_2$C*H*$_2$C=O), 4.04 (d, *J*= 5.0, 2H, NHC*H*$_2$), 4.22 (q, *J*= 7.2, 2H, O-*CH*$_2$-CH$_3$), 5.95 (s, 1H, NH).

Since the GP-1 involved the use of 1.1 eq. of FAE and they were hydrolysed during workup, NMR spectra contain little presence of fatty acid (< 0.1 eq.): δ=2.34 (t, *J*= 7.5, 2H, C*H*$_2$C=O). Other proton signals are eclipsed with the signals of *N*-lauroyl glycine ethyl ester.

N-lauroyl glycine methyl ester (GP-2; entry 44)



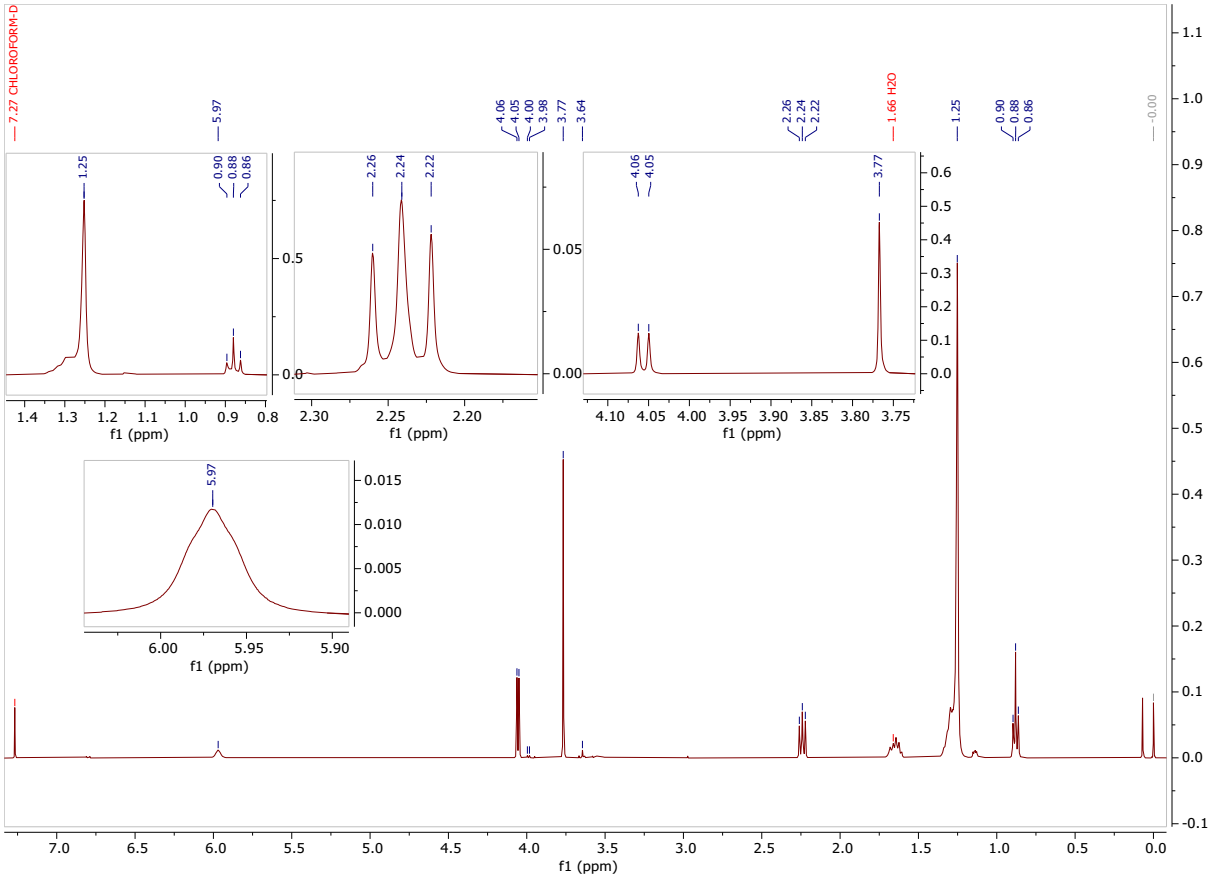


Figure 6. NMR spectra of N-lauroyl glycine methyl ester

^{1}H NMR (400 MHz; $CDCl_3$, RT): δ= 0.88 (t, *J*= 7.0, 3H, C*H*$_3$), 1.22-1.35 (m, 16H, C*H*$_2$), 1.64 (quint., *J*= 7.4, 2H, C*H*$_2$CH$_2$C=O)(in part superimposed with H_2O peak), 2.24 (t, *J*= 7.6, 2H, CH$_2$C*H*$_2$C=O), 3.77 (s, 3H, OC*H*$_3$), 4.06 (d, *J*= 5.1, 2H, NHC*H*$_2$), 5.97 (s, 1H, NH).

Since a small amount of by-product is present in the GP-2 reactions, its signals are present in the NMR spectra: δ=3.64 (s, 3H, O-*CH*$_3$), 3.99 (d, *J*= 5.4, 2H, CH$_2$). Other proton signals are eclipsed with the signals of *N*-lauroyl glycine methyl ester.

By-product of enzymatic synthesis – N-lauroyl glycylglycine ethyl ester (isolated)

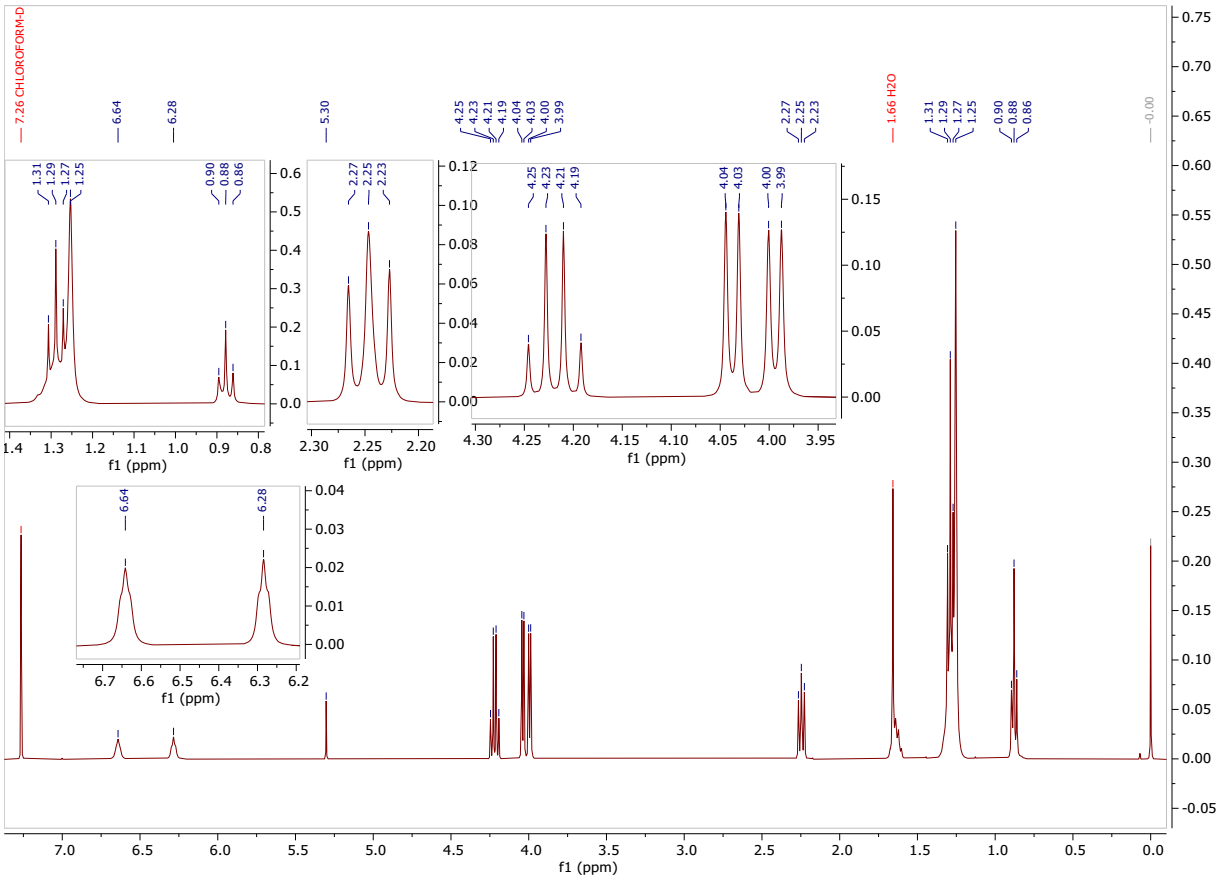


Figure 7. NMR spectra of N-lauroyl glycylglycine ethyl ester

^{1}H NMR (400 MHz; $CDCl_3$, RT): δ=0.88 (t, *J*= 6.9, 3H, C*H*$_3$), 1.22-1.35 (m, 16H, C*H*$_2$), 1.29 (t, *J*= 7.0, 3H, O-CH$_2$-*CH*$_3$), 1.64 (quint., *J*= 7.4, 2H, C*H*$_2$CH$_2$C=O)(in part superimposed with H_2O peak), 2.25 (t, *J*= 7.6, 2H, CH$_2$C*H*$_2$C=O), 3.99 (d, *J*= 5.1, 2H, NHC*H*$_2$), 4.04 (d, *J*= 5.3, 2H, NHC*H*$_2$), 4.22 (q, *J*= 7.2, 2H, O-*CH*$_2$-CH$_3$), 6.28 (s, 1H, NH), 6.64 (s, 1H, NH).

N-lauroyl glycine (GP-3; entry 55)

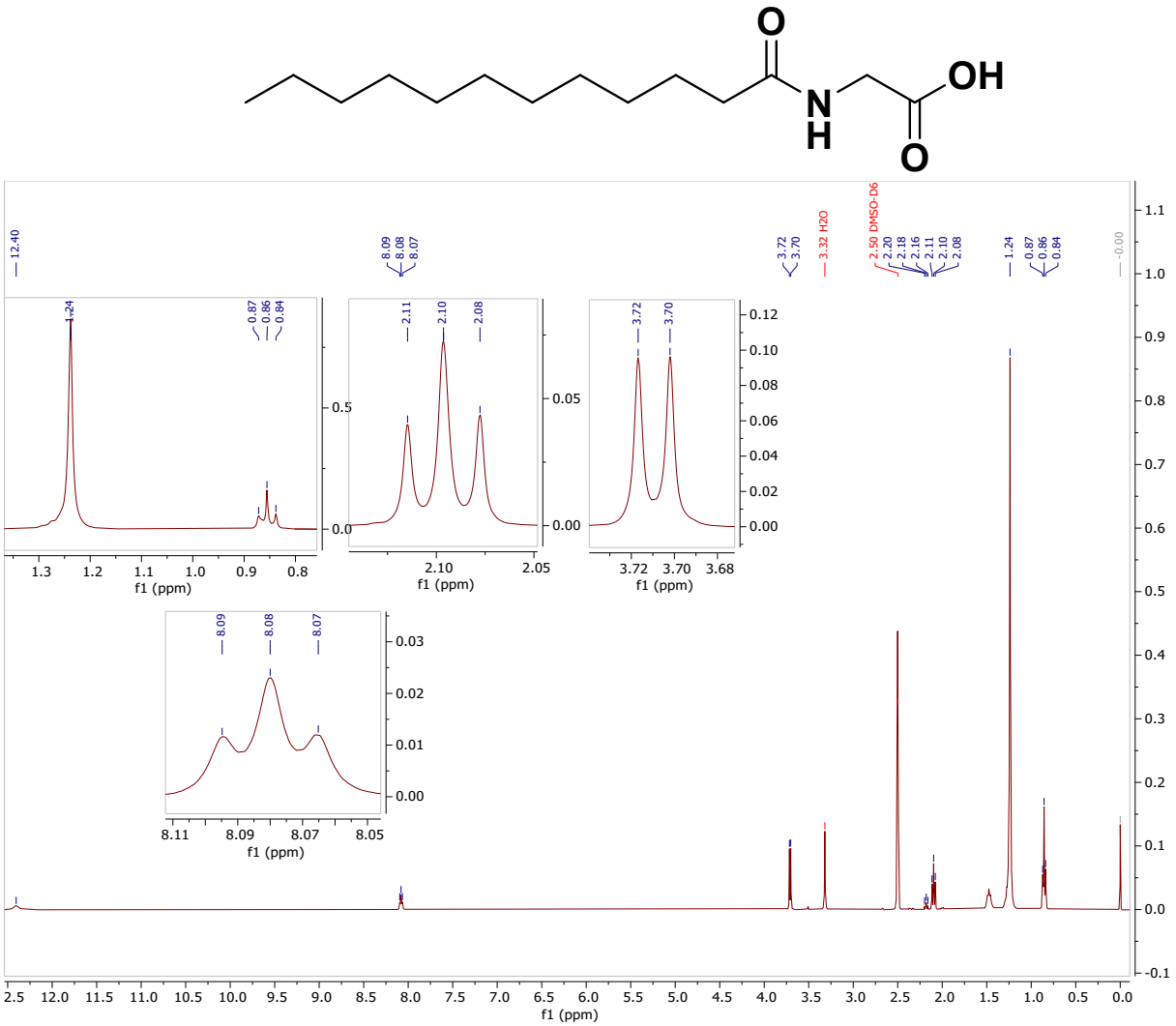


Figure 8. NMR spectra of N-lauroyl glycine

^{1}H NMR (400 MHz; d_6-DMSO; RT): δ= 0.86 (t, *J*= 6.8, 3H, C*H*$_3$), 1.20-1.33 (m, 16H, C*H*$_2$), 1.48 (quint., *J*= 6.8, 2H, C*H*$_2$CH$_2$C=O), 2.10 (t, *J*= 7.4, 2H, CH$_2$C*H*$_2$C=O), 3.71 (d, *J*= 5.9, 2H, NHC*H*$_2$), 8.08 (t, *J*= 5.8, 1H, NH), 12.40 (broad s, 1H, CO$_2$*H*).

Since the product of GP-1 was used for GP-3, the NMR spectra also contain little presence of fatty acid (< 0.1 equiv.): δ= 2.18 (t, *J*= 7.3, 2H, CH$_2$). Other proton signals are eclipsed with the signals of *N*-lauroyl glycine.

The central DMSO peak at δ=2.506 was used as a reference.