Supporting Information

Innovative mechanochemical synthesis of lipopeptide surfactants through direct amidation of fatty esters: application to silk-derived amphiphilic lipopeptides

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‖These authors made an equal contribution to this work.

Table S1. Calculation of Green Metrics for lipopeptides of silk sericin with C14 fatty acid chain.

	Conventional acylation reaction		CDI pre-activation by mechanochemistry		Amidation reaction by mechanochemistry	
$m_{peptides}$ (mg)	708.7		500		500	
$M_{w\ peptides}$ (g/mol)[a]	219		162		157	
$m_{fatty\ chain}$ (mg)	fatty acid chloride	202.3	fatty acid	152.7	fatty acid ester	166
$M_{w\ fatty\ chain}$ (g/mol)		246.8		228.4		242.4
m_{water} (mg)	20000		-		-	
$M_{w\ water}$ (g/mol)	18		-		-	
m_{CDI} (mg)	-		108.5		-	
$M_{w\ CDI}$ (g/mol)	-		162.1		-	
$m_{t\text{-}BuOK}$ (mg)	-		-		115	
$M_{w\ t\text{-}BuOK}$ (g/mol)	-		-		112.2	
$m_{lipopeptide}$ (mg)[b]	373.5		332.7		326.3	
$M_{w\ lipopeptide}$ (g/mol)	428		327.4		367.4	
AR (%)	41 ± 3		48 ± 1		49 ± 1	
E-factor	54.9		1.2		1.6	
PMI	56.0		2.3		2.6	
AE (%)	78.4		68.9		66.6	

[a] estimated considering the amino acid composition of sericin and the degree of hydrolysis
[b] calculated taking into account the acylation rate (AR) of each lipopeptide mixture

General procedures for synthesis of lipoaminoacids and lipopeptides

***N*-acyl-glycine** (example for Entry 3, Table 1): 0.971g (0.01 mol, 1 equivalent) of glycine previously pretreated with NaOH (1:1 molar ratio) and freeze dried were introduced in the jar with 2.143g (0.01 mol, 1 equivalent) of methyl laurate and 1.683g (0.015 mol, 1.5 equivalents) of potassium tert-butoxide. The reaction mixture was ground during 90 min at 650 rpm. At the end of the reaction, the reaction medium was a white, pasty material; however, the alcohols (MeOH and t-BuOH) rapidly evaporated at room temperature, and the mixture became solid. The final product was collected using a plastic spatula.

On the ^{1}H NMR and ^{13}C NMR raw spectrum of N-acyl-glycine preparation appears peaks of the N-acyl-glycine as well as the unreacted glycine and fatty alkyl chain. They are described bellow and a comparison of the spectra of the mixture and the pure glycinate and pure fatty carboxylate is presented in the Fig 1. of the manuscript:

^{1}H NMR (400 MHz, D_2O): δ(ppm) 8.33 (s, NH) 3.61 (s, CH_2 of N-acyl-glycine) 3.06 (s, CH_2 of unreacted glycine), 2.17 (t, αCH_2 of N-acyl-glycine), 2.04 (t, αCH_2 of residual fatty chain), 1.48 (m, $_{\beta}CH_2$ of N-acyl-glycine), 1.45 (t, $_{\beta}CH_2$ of residual fatty chain), 1.17 (s, CH_2 of fatty chains), 0.75 (t, CH_3 of fatty chain).

^{13}C NMR (100 MHz, D_2O): δ(ppm) 183.34 (COO- residual fatty chain noted with ⁎), 176.80 (COO- of N-acyl-glycine), 176.16 (COO- residual glycine noted with ◦), 173.63 (CONH), 43.26 (αCH_2 of

N-acyl-glycine), 37.79 (αCH_2), 35.90 (βCH_2), 31.71 (CH_2), 29.17 (CH_2), 25.57 (CH_2), 22.61 (CH_2), 13.57 (CH_3).

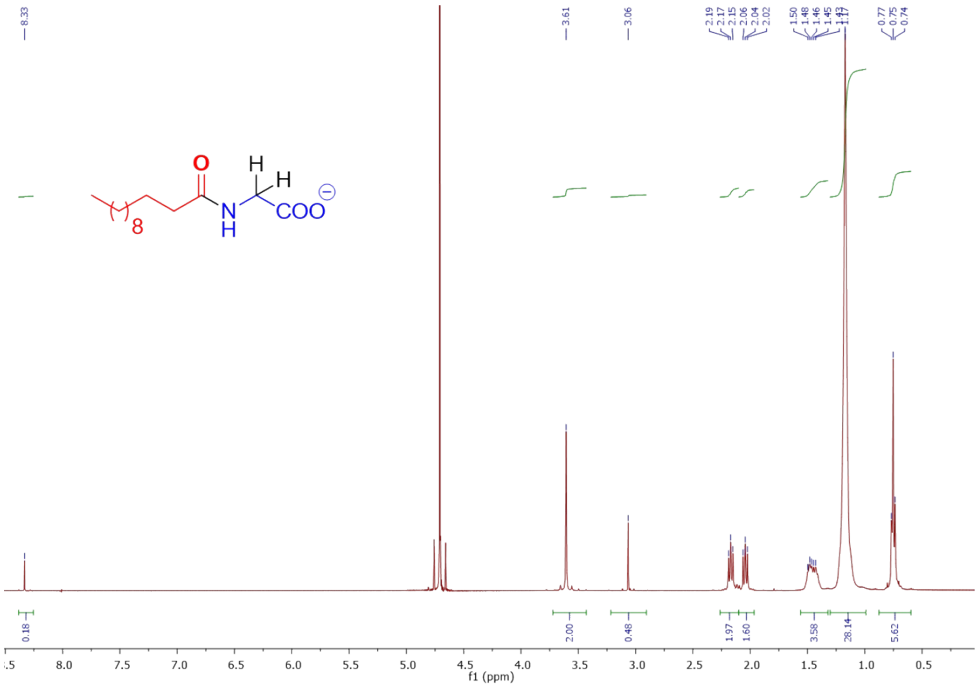


Figure S1. ^{1}H NMR raw spectrum of N-acyl-glycine preparation

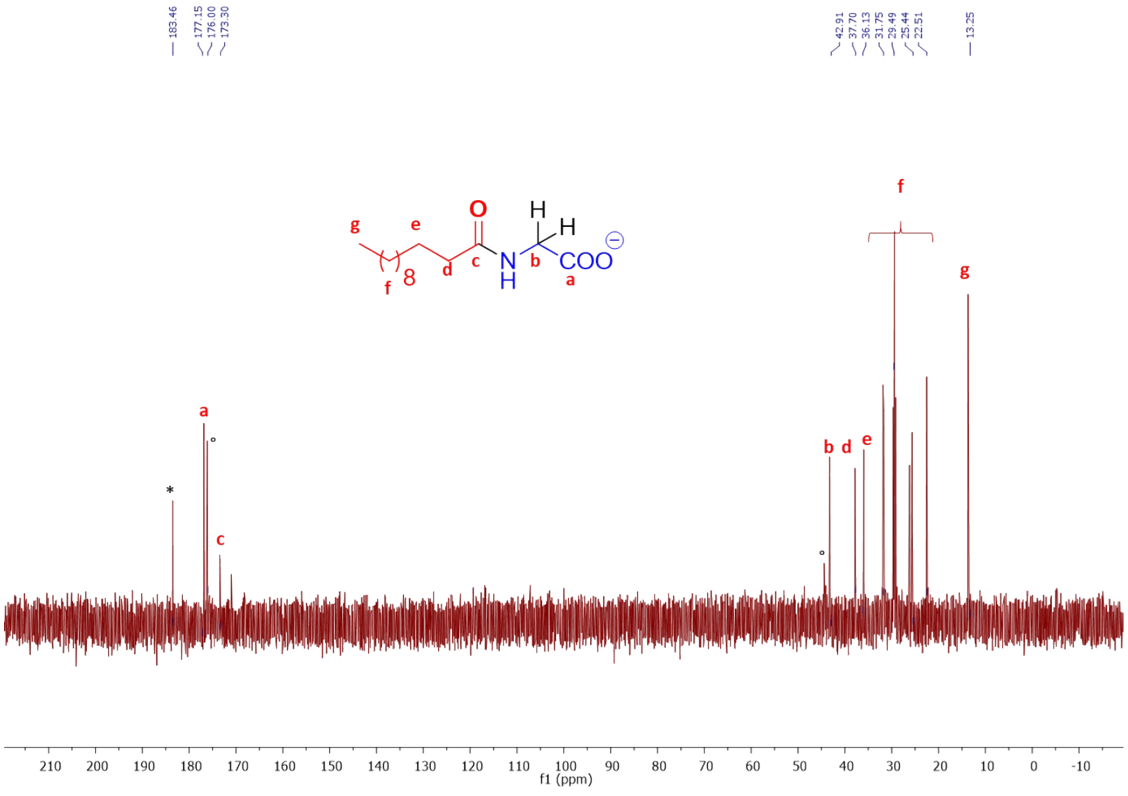


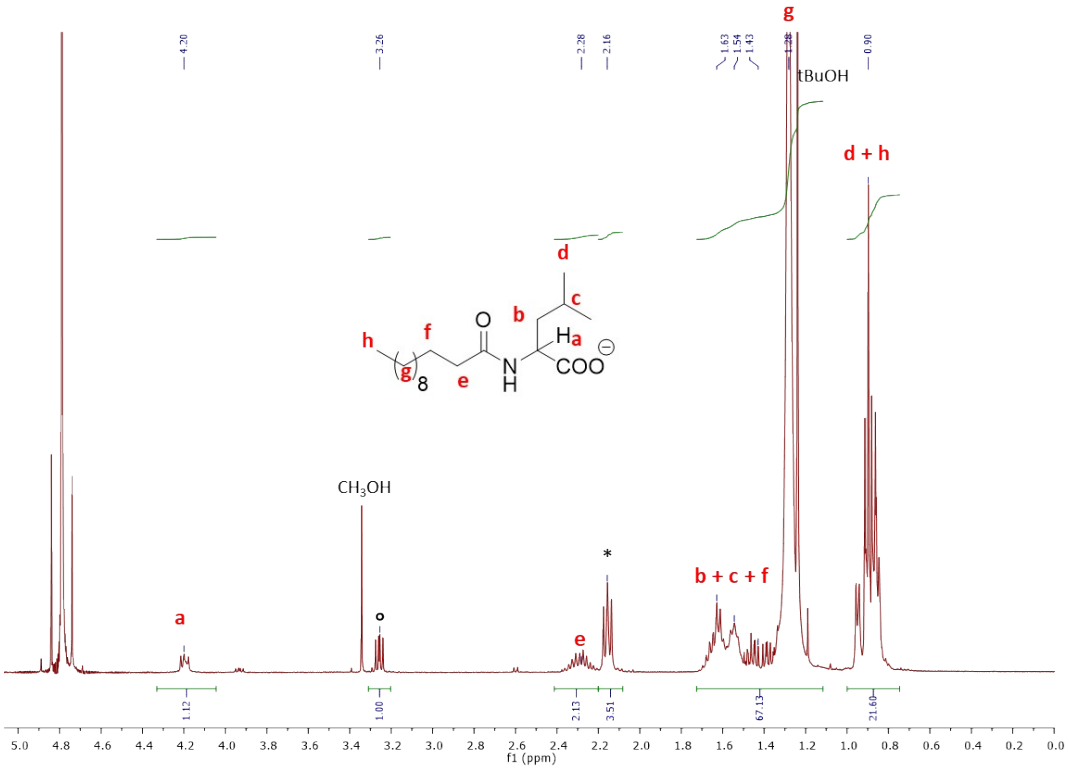
Figure S2. ^{13}C NMR raw spectrum of N-acyl-glycine preparation

***N*-acyl-leucine** (example for Entry 1, Table 2): 1.532g (0.01 mol, 1 equivalent) of leucine previously pretreated with NaOH (1:1 molar ratio) and freezy dried were introduced in the jar with 2.143g (0.01 mol, 1 equivalent) of methyl laurate and 1.683g (0.015 mol, 1.5 equivalents) of potassium tert-butoxide. The reaction mixture was ground during 90 min at 650 rpm. At the end of the reaction, the reaction medium was a white, pasty material; however, the alcohols (MeOH and t-BuOH) rapidly evaporated at room temperature, and the mixture became solid. The final product was collected using a plastic spatula.

On the ^{1}H NMR and ^{13}C NMR raw spectrum of N-acyl-leucine preparation several peaks of the N-acyl-leucine as well as the unreacted leucine and fatty alkyl chain are observed and described below. In addition, Figure S4 presents the spectra of the raw N-acylated Leucine compared to fatty carboxylate and leucinate spectra.

^{1}H NMR (400 MHz, D_2O): δ(ppm) 8.27 (s, NH) 4.20 (s, CH of N-acyl-leucine) 3.26 (s, CH of unreacted leucine noted °), 2.28 (m, αCH_2 of N-acyl-leucine), 2.16 (t, αCH_2 of residual fatty chain noted *), 1.63, 1.54 and 1.43 (several multiplets corresponding to $_{\beta}CH_2$ of N-acyl-leucine, $_{\beta}CH_2$ of residual fatty chain, CH_2 and CH of Leucine side chain noted b+c+f), 1.28 (s, CH_2 of fatty chains), 0.90 (t, CH_3 of fatty chain + multiplet signal from the -CH_3 of Leucine side chain noted d+h). Finally, the peak at 1.24 ppm and 3.26 ppm are ascribed to remaining traces of tBuOH and MeOH in the medium.

^{13}C NMR (100 MHz, D_2O): δ(ppm) 183.4 (COO- residual fatty chain), 181.2 (COO- residual glycine), 180.5 (COOH of N-acyl-glycine), 175.6 (CONH), 54.1 (αCH of N-acyl-leucine), 53.4 (CH, Leucine side chain), 37.8 (αCH_2), 31.8 (βCH_2), form 30 to 21 (fatty chain CH2 + CH2 leucine side chain), 13.6-13.7 (CH_3 of the fatty chain + CH_3 of leucine side chain).



∘ residual non acylated Leucine

∗ residual fatty acid

Figure S3. [1]H NMR raw spectrum of N-acyl-leucine preparation

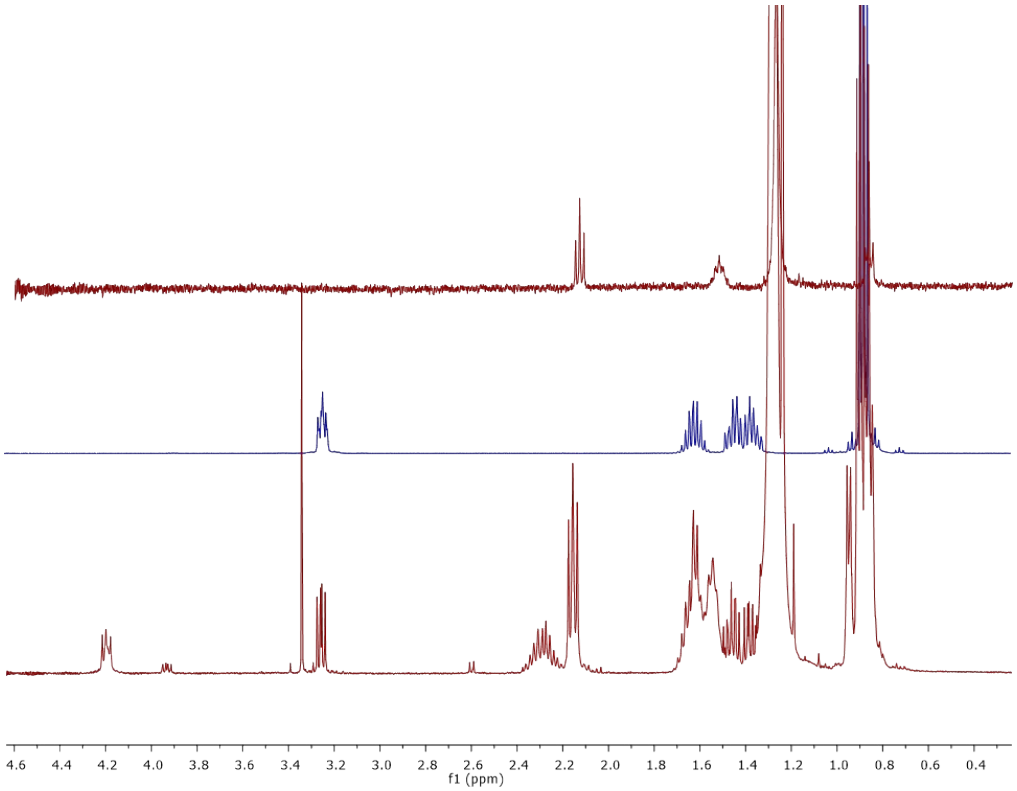


Figure S4. Comparison of [1]H NMR spectra of (from top to bottom) fatty carboxylate, Leucinate and raw N-acyl-leucine preparation

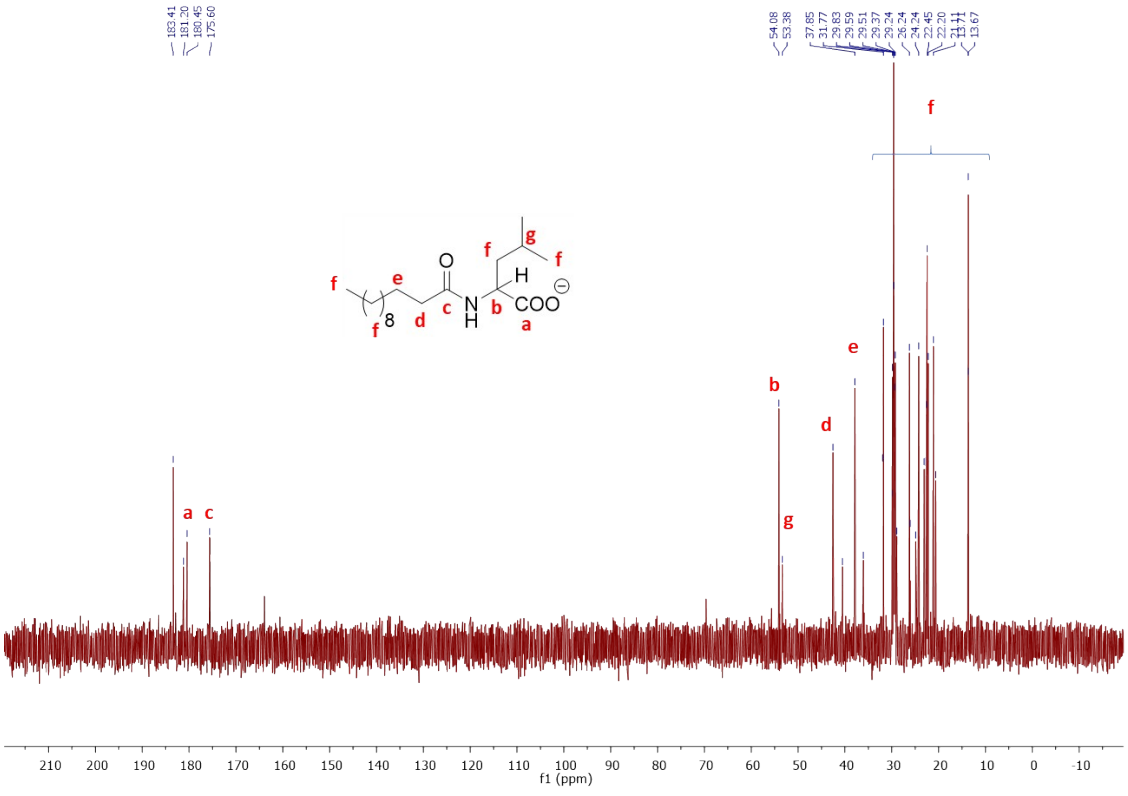


Figure S5. [13]C NMR (100 MHz, D_2O) raw spectrum of N-acyl-leucine preparation:

***N*-acyl-serine** (example for Entry 4, Table 2): 1.051g (0.01 mol, 1 equivalent) of serine were introduced in the jar with 2.143g (0.01 mol, 1 equivalent) of methyl laurate and 2.806g (0.025 mol, 2.5 equivalents) of potassium tert-butoxide. The reaction mixture was ground during 90 min at 650 rpm. At the end of the reaction, the reaction medium was a white, pasty material; however, the alcohols (MeOH and t-BuOH) rapidly evaporated at room temperature, and the mixture became solid. The final product was collected using a plastic spatula.

On the ^{1}H NMR and ^{13}C NMR raw spectrum of N-acyl-serine preparation peaks of the N-acyl-serine as well as the unreacted serine and fatty alkyl chain are observed and described below. In addition, Figure S7 presents the spectra of the raw N-acylated serine compared to fatty carboxylate and serinate spectra.

^{1}H NMR (400 MHz, D_2O): δ(ppm) 8.40 (s, NH) 4.20 (t, CH of N-acyl-serine noted a), 3.78 (m, CH_2 of N-acyl-serine side chain noted b), 3.68 (m, CH_2 of serine side chain noted ◦) 3.32 (t, CH of unreacted serine noted ◦), 2.27 (m, αCH_2 of N-acyl-serine), 2.12 (t, αCH_2 of residual fatty chain noted *), 1.54 and 1.50 (2 multiplets corresponding to $_{\beta}CH_2$ of N-acyl-serine and $_{\beta}CH_2$ of residual fatty chain noted d), 1.25 (s, CH_2 of fatty chains), 0.83 (t, CH_3 of fatty chain).

^{13}C NMR (100 MHz, D_2O): δ(ppm) 183.5 (COO- residual fatty chain), 179.6 (COOH residual serine), 178.0 (COOH of N-acyl-serine), 176.0 (CONH), 64.1 (CH_2OH of N-acyl-serine), 62.1 (CH_2OH of residual serine), 57.4 (αCH of N-acyl-serine), 37.9 (αCH_2 fatty chain), 35.7 (βCH_2 fatty chain), form 32 to 21 (fatty chain CH_2), 13.5 (CH_3 of the fatty chain).

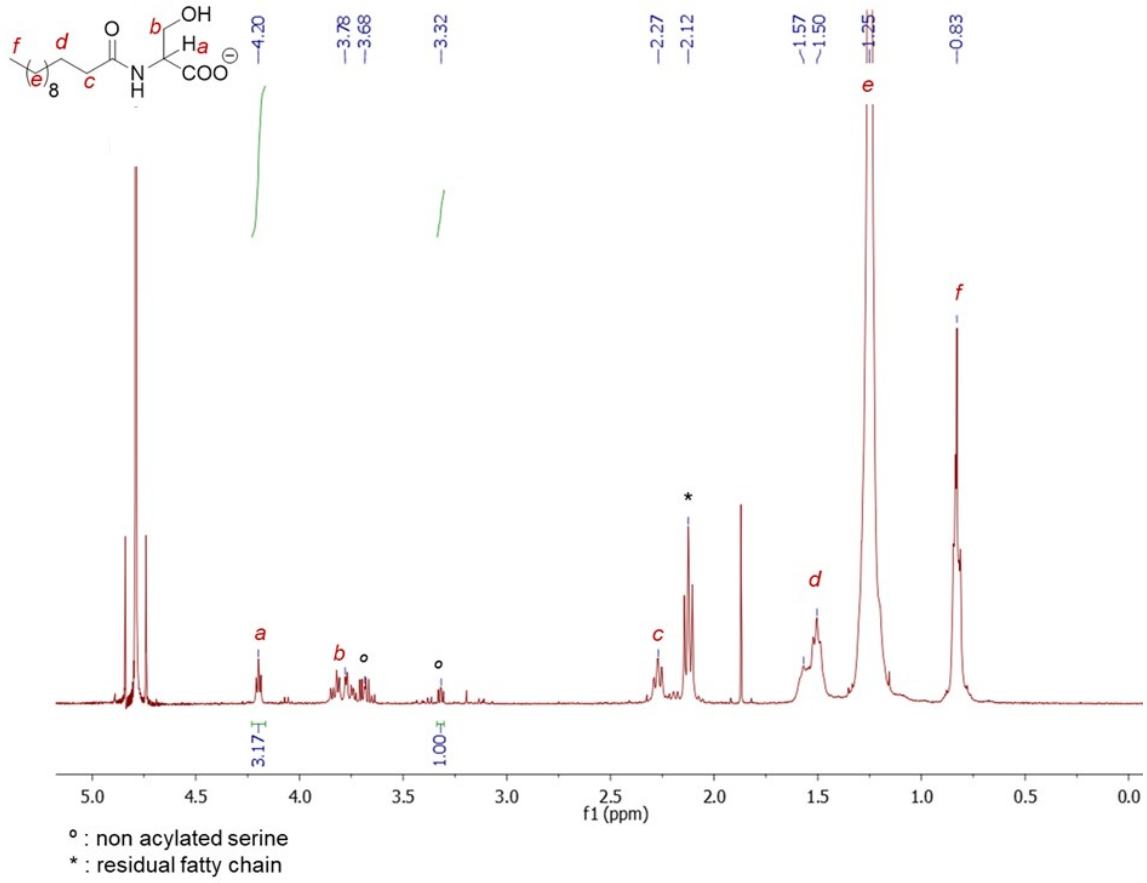


Figure S6. ^{1}H NMR raw spectrum of *N*-acyl-serine preparation

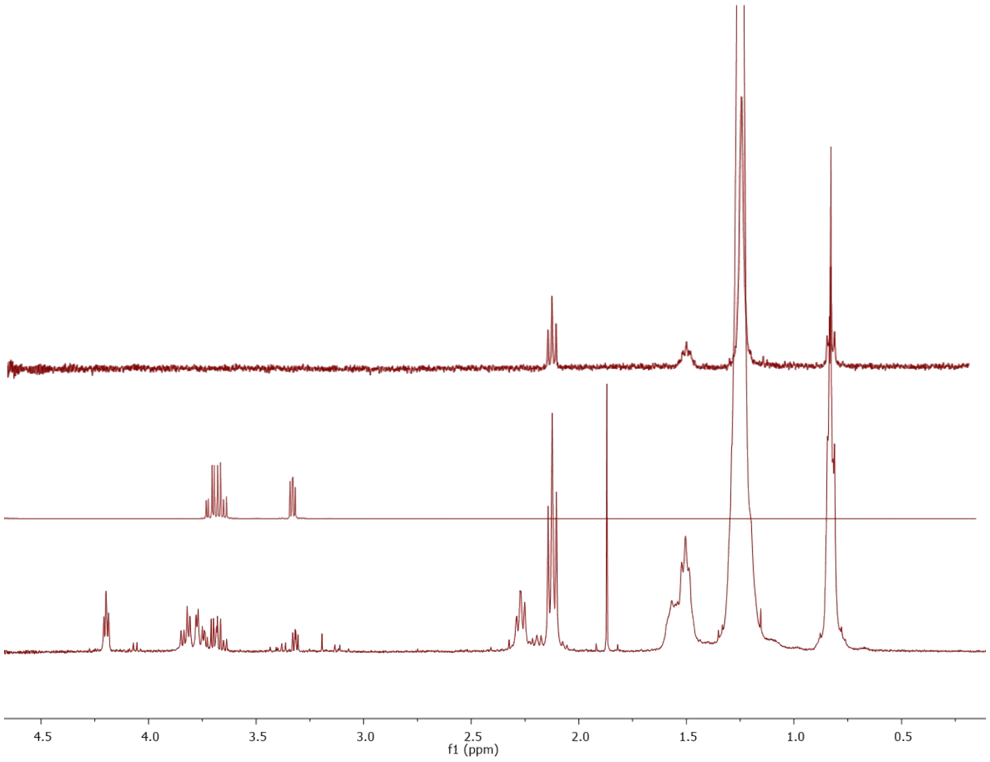


Figure S7. Comparison of ^{1}H NMR spectra of (from top to bottom) fatty carboxylate, Serinate and raw N-acyl-serine preparation

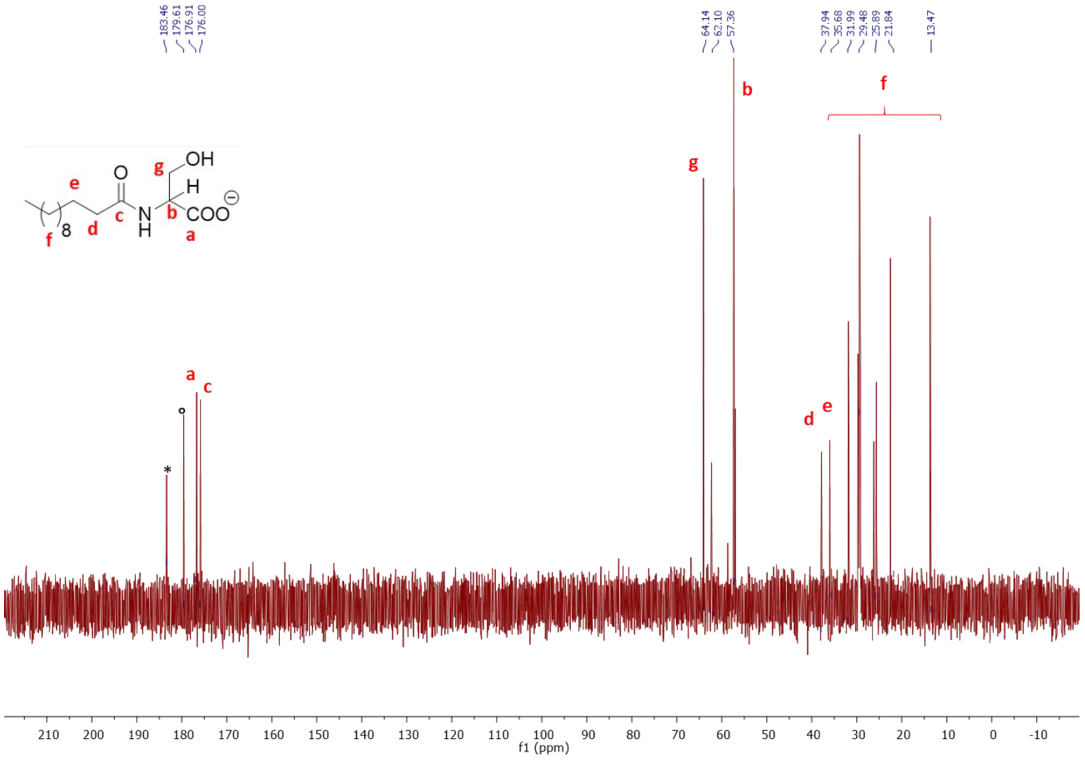


Figure S8. ^{13}C NMR raw spectrum of N-acyl-serine preparation

LP-C12 sericin lipopeptides: 0.5g (0.00068 mol, 1 equivalent) of sericin peptides (0.00137 mol NH_2/g) pretreated with NaOH (1:1 molar ratio) after enzymatic hydrolysis and freezy dried were introduced in the jar with 0.146 g (0.00068 mol, 1 equivalent) of methyl laurate with 0.115 g (0.001025 mol, 1.5 equivalent) of potassium tert-butoxide. The reaction mixture was ground during 90 min at 650 rpm. At the end of the reaction, the lipopeptide mixture was a pale yellow, pasty material; however, the alcohols (MeOH and t-BuOH) rapidly evaporated at room temperature, and the mixture became solid. The final product was collected using a plastic spatula.