An Efficient Chemoenzymatic Synthesis of the Bactericide Lapyrium Chloride

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An efficient route for large-scale preparation of lapyrium chloride, a broad-spectrum antimicrobial surfactant, was developed from chloroacetic acid in four steps, three of them enzymatic. Due to the chemoselective behavior of the biocatalysts, lapyrium chloride was obtained in a high degree of purity and yield, from mild reaction conditions and following a low environmental impact methodology.

Introduction

1-[(2-Dodecanoyloxyethylcarbamoyl)methyl]pyridinium chloride (**1**) is well known in the chemical industry as lapyrium chloride. It is used in a 5–10% water solution as an antiseptic and disinfectant (Figure 1). It is nontoxic,[1] and does not burn or stain. It can be used on healthy skin or open wounds showing broad-spectrum antimicrobial activity. As an antiseptic it is used for diaper dermatitis, skin infections, burns and skin surgical asepsis. As a disinfectant it is useful for bottles, diapers and baby clothes asepsis, ceramic wash and ambient cleaning. Because of its surfactant activity it is also used as an ingredient in personal care products such as hair and skin conditioners,[2] toothpastes,[3] antiplaque mouth rinse solutions,[4] etc. Moreover, it is used in domestic waste-water treatment[5] and in corrosion inhibitor formulations.[6]

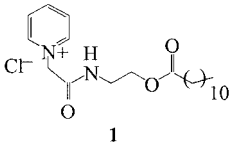

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Figure 1. Lapyrium chloride.

Considering its structure, lapyrium chloride could be prepared using the synthetic procedures shown in Scheme 1.

Among the three possibilities, only route 2 was performed as it was reported in two patents.[7,8] Both of the processes suffer from some important disadvantages. In the Epstein process[7] the formation of intermediate ester **7** is accompanied by the release of irritating, corrosive fumes of hydrogen halide gas. The formation of lapyrium chloride by heating intermediate **7** in pyridine causes the lapyrium chloride to be contaminated with pyridine hydrochloride which coprecipitates and must subsequently be removed by repeated slurrying in benzene. Although Gordon et al.[8] reported a new approach that could avoid the fumes of hydrogen chloride and the use of benzene, the processes also used toxic and air sensitive reagents and drastic reaction conditions. Due to the lack of selectivity of chemical reactions, it was necessary to isolate and purify the intermediates from a complex mixture. Then, following any of the two processes, it was difficult to obtain the product in a high degree of purity and yield.

Since lapyrium chloride is a commercially useful bactericide, it is highly desirable to have an efficient and clean method for its preparation. This is why we have decided to apply an enzymatic approach.

The use of enzymes in the synthesis of pharmaceuticals has been increasingly developed over the last few years.[9–12] Biotransformation is an area of great interest for the preparation of therapeutic agents which are difficult to obtain by conventional chemical methods.[13] It is recognised that enzymes are capable of accepting a wide array of substrates, and catalyze enantio-, chemo- and regioselective reactions. As a result, biocatalysts allow us to carry out different chemical transformations without the need for tedious protection and deprotection steps, especially in compounds with several functional groups, such as hydroxyl and amino groups in ethanolamine in the case of lapyrium chloride synthesis. Over the last few years, biocatalysis in nonaqueous media has been widely used for aminolysis and ammonolysis of esters[14,15] and have been shown to be of great utility for the resolution of amines[16] and in the preparation of chiral amides, carbonates and carbamates.[17]

Encouraged by our success in lipase-catalyzed esterification reactions[18,19] and in the preparation of an intermediate in the synthesis of Alfuzosin by a one-pot aminolysis of esters,[20] we envisaged the application of this methodology to the synthesis of lapyrium chloride. In contrast with the traditional methods, in this synthetic procedure, the biocat-

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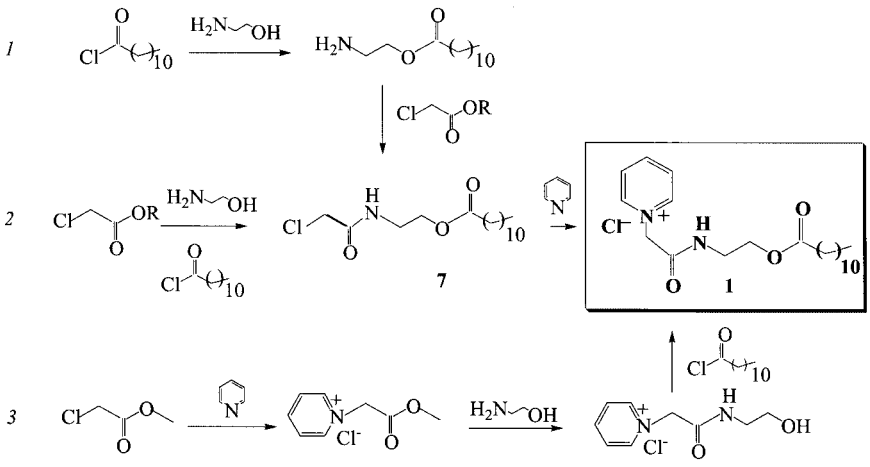
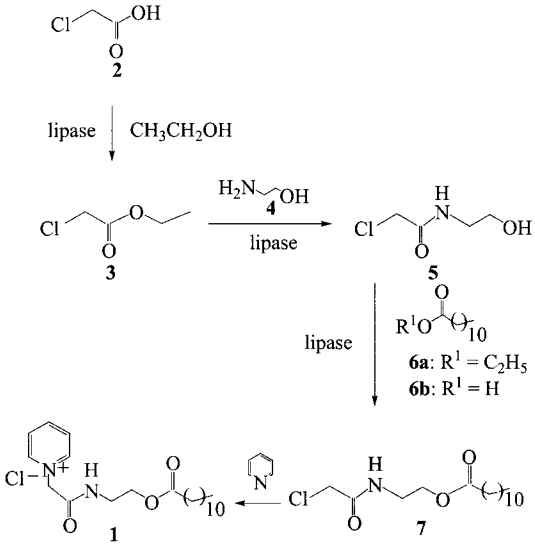


Scheme 1. Possible synthetic procedures for lapyrium chloride **1**.

alysts showed high activity and chemoselectivity allowing the product to be obtained in high purity and yield.

Among the several possibilities of coupling from the retrosynthetic analysis, we decided to carry out the synthetic procedure described in Scheme 2.



Scheme 2. Chemoenzymatic synthesis of lapyrium chloride **1**.

Results and Discussion

The chemoenzymatic synthesis of lapyrium chloride was performed as depicted in Scheme 2.

It involves three consecutive enzymatic steps: esterification of chloroacetic acid (**2**), aminolysis of ethyl chloroacetate (**3**), and esterification or transesterification of the chloroacetamide (**5**).

Esterification of Chloroacetic Acid (2)

Ethyl chloroacetate (**3**) was obtained through esterification of chloroacetic acid (**2**) with ethanol, catalyzed by lipases from several sources: the yeasts *Candida rugosa* lipase (CRL) and *Candida antarctica* lipases A and B (CAL A and CAL B), lipozyme from the fungus *Rhizomucor miehei* (LIP), and lipase from *Pseudomonas* sp. (PS-C). The results of the lipase-catalyzed esterification of (**2**) are summarized in Table 1 (column 4). Among the six commercial lipases tested in the esterification of (**2**), the lipase from *Candida antarctica* B (CAL B) gave the most satisfactory results at both temperatures. Without enzymes, chloroacetic acid did not react at all.

Table 1. Lipase-catalyzed preparation of **3**, **5** and **7**.

Entry	Enzyme	Temperature (°C)	Products Yield (%)[a]			
			3[b]	**5**[c]	**7**[d]	**7**[e]
1	CAL B	25	97	42	70	95
2	LIP	25	45	41	75	95
3	CAL A	25	12	20	46	43
4	PSL	25	43	22	29	26
5	PPL	25	16	18	37	32
6	CRL	25	39	21	43	46
7	CAL B	55	99	98	70	95
8	LIP	55	44	96	75	95
9	CAL A	55	23	43	54	57
10	PSL	55	45	41	35	37
11	PPL	55	22	40	45	40
12	CRL	55	45	44	50	47

[a] Determined by GC analysis. [b] E/S: 0.5; time: 36 h. [c] E/S: 1; ethanolamine/**3**: 1; solvent: acetone; time: 48 h. [d] Ethyl laurate as acylating agent. E/S: 3; ethyl laurate/**5**: 2.3; solvent: acetone; time: 24 h molecular sieves as additive. [e] Lauric acid as acylating agent, E/S: 3; lauric acid/**5**: 1; solvent: acetone; time: 24 h, molecular sieves as additive.

To optimize the reaction conditions we performed several experiments by changing the reaction parameters such as temperature (25 °C and 55 °C) and enzyme-substrate ratio (E/S). As can be seen in Table 1, an increase in temperature improved the results in the case of CAL A and PPL, but both maintained the low reactivity towards the esterification reaction.

Regarding the influence of the enzyme:substrate ratio, we performed experiments varying the ratio from 0.1 to 5

(Table 2, column 3), and observed that an E/S = 0.5 gave the best results. We chose the following standard conditions for the biocatalytic esterification: CAL B as biocatalyst, excess of ethanol, room temperature (97% to 25 °C and 99% to 55 °C) and E/S ratio of 0.5.

Table 2. Effect of enzyme/substrate ratio on lipase-catalyzed preparation of **3**, **5** and **7**.

Entry	E/S	Products Yield (%)[a]		
		3[b]	**5**[c]	**7**[d]
1	0.1	40	32	28
2	0.25	78	60	47
3	0.5	95	85	69
4	1	97	96	79
5	3	96	98	95
6	5	98	98	95

[a] Determined by GC analysis. [b] CAL B; 25 °C; time: 36 h. [c] LIP; ethanolamine/**3**: 1; 55 °C; solvent: acetone; time: 48 h. [d] LIP; lauric acid as acylating agent; lauric acid/**5**: 1; 25 °C; solvent: acetone; time: 24 h; molecular sieves as additive.

The preparation of ethyl chloroacetate has been previously reported according to known chemical procedures, by refluxing the acid with an excess of absolute ethanol with sulfuric acid as the catalyst.

The product was obtained in 75% yield.[21] The enzymatic approach we have applied gave the product in almost quantitative yield. The procedure is simple, can be carried out under mild reaction conditions and only uses ethanol as a solvent and esterifying agent.

Aminolysis of Ethyl Chloroacetate (3)

Different lipases were screened for the enzymatic aminolysis reaction of ethyl chloroacetate with ethanolamine (**4**). The results are summarized in Table 1, column 5. Lipases showed variable activity. Working at 55 °C, CAL B and LIP gave the most satisfactory results using an E/**3** ratio of 0.5 and 1, respectively (Table 2).

The product was isolated and identified by spectroscopic methods as *N*-(hydroxyethyl)chloroacetamide (**5**). It is remarkable that the enzymes act in a chemoselective manner, exclusively producing the amide (**5**). The isomeric aminoester was not detected. The reaction was carried out at different ester and ethanolamine ratios and the best yield was obtained at an amine/ester ratio equal to 1 and at 2 M ester concentration.

We examined the aminolysis with different organic solvents. As ethanolamine is only soluble in polar solvents, nonpolar solvents such as hexane and toluene were not effective. Using LIP as the biocatalyst, THF and dioxane gave the product (**5**) in about 89–90% yield, and the yield was 96% in acetone and 98% in acetonitrile. The reaction without solvent afforded (**5**) in 89% yield. The high yield obtained using ethanol as the solvent, is quite remarkable (90%). Even in high concentrations of ethanol the aminolysis reaction prevailed over transesterification, showing the excellent chemoselectivity displayed by the enzymes LIP and CAL B.

Esterification or Transesterification of Chloroacetamide (5)

The third enzymatic step involves the transformation of chloroacetamide (**5**) into 2-(2-chloroacetylamino)ethyl dodecanoate (**7**).

This step was studied by two alternative enzymatic reactions: transesterification and esterification.

The transesterification was carried out by treating (**5**) with ethyl laurate and the esterification with lauric acid. The results are presented in Table 3.

Table 3. Solvent effect in lipase-catalyzed preparation of **5** and **7**.

Entry	Enzyme	Solvent	Products Yield (%)[a]		
			5[b]	**7**[c]	**7**[d]
1	CAL B	no solvent[c]	87	–	–
2	CAL B	ethanol	90	–	–
3	CAL B	acetonitrile	98	76	96
4	CAL B	acetone	98	73	94
5	CAL B	THF	85	60	50
6	CAL B	dioxane	87	77	80
7	LIP	no solvent[c]	89	–	–
8	LIP	ethanol	90	–	–
9	LIP	acetonitrile	98	74	96
10	LIP	acetone	96	75	95
11	LIP	THF	88	50	45
12	LIP	dioxane	89	72	75

[a] Determined by GC analysis. [b] E/S: 1; ethanolamine/**3**: 1; 55 °C; time: 36 h. [c] Ethyl laurate as acylating agent. E/S: 3; ethyl laurate/**5**: 2.3; 25 °C; time: 48 h; molecular sieves as additive. [d] Lauric acid as acylating agent, E/S: 3; lauric acid/**5**: 1; 25 °C; time: 24 h; molecular sieves as additive.

The esterification reaction afforded the highest yields with both the enzymes CAL B and LIP. Moreover, the ratio lauric acid/**5** = 1 in the esterification is better than in the transesterification, which is ethyl laurate/**5** = 2.3. To obtain **7** in high purity, the excess of ethyl laurate or lauric acid must be eliminated at the end of the reaction. While the separation of ethyl laurate is difficult, working with lauric acid, the product **7** can be obtained in high purity by washing the crude residue with hexane. An additional advantage of lauric acid is that it is more economic and less toxic than ethyl laurate.

Although the highest performance in the enzymatic esterification was achieved using acetonitrile as solvent we chose acetone because it is less toxic and more economic than acetonitrile and the difference in yield (1 or 2 points) does not justify its use. Considering the performance displayed by both lipases and their cost, lipozyme was the enzyme of choice used for the second and third enzymatic steps, in terms of the economy of the procedure.

Taking into account our previous work,[18] we tried a one-pot procedure for the preparation of **7**. The second and third enzymatic steps corresponding to the formation of **5** and **7,** respectively, were performed successively in the same pot. In this case, chloroacetamide (**5**) was prepared using

acetone as solvent (entry 4, Table 2); and then lauric acid, more LIP, more solvent and molecular sieves were added. The global yield when applying both enzymatic steps together was only 62%. It could be observed that it is more convenient to isolate **5** and then carry out the enzymatic esterification with lauric acid, because a higher yield ($96 \times 95 = 91\%$) can be achieved.

It could be supposed that the presence of some ethanolamine in excess from the first step, could prevent, in the second step, the formation of **7** in high yield.

Moreover, we observed that a considerable amount of product **5** remained adsorbed to the enzyme surface at the end of the reaction. The yield of **5** was remarkably increased when the enzyme was washed several times with acetone. This washing was not carried out in the one-pot procedure, and could be a reason for the decreased yield.

We carried out a preparative scale reaction **3** → **5** → **7** in the same manner as described above with 20 g of LIP. Even though the yield in **7** showed a slight decrease from 91% to 86%, the result confirmed the feasibility of the procedure for large-scale production of lapyrium chloride.

Finally, lapyrium chloride (**1**) was obtained by treating (**7**) with pyridine overnight at room temperature. The product, which precipitates after addition of hexane, furnishes an 87% yield.

Conclusions

This work describes the application of lipases to the preparation of lapyrium chloride, an antimicrobial surfactant. The product is obtained from chloroacetic acid in four steps, three of them enzymatic. The enzymatic steps consisted of two esterifications (first and third step) and an aminolysis reaction. Due to the chemoselective behavior of the biocatalysts, a mixture of intermediates is avoided and lapyrium chloride can be obtained in high purity and yield.

Moreover, because no pyridine hydrochloride is contained in the product, produced by the enzymatic procedure, a 1% aqueous dispersion of **1** has a pH 5.0 to 6.0 at 25 °C, while a similar preparation of the previous chemical approach has a pH of 2.5 to 3.8.

From the results obtained in the screening of the lipases and the study of the reaction conditions we can conclude that the best outcome was obtained when using the following conditions: CAL B as biocatalyst in the esterification reaction **2** → **3** at room temperature. LIP in the aminolysis to obtain **5** (55 °C) and in the esterification to obtain **7** (room temperature). Acetone as the solvent in the aminolysis and the second esterification.

Some of the advantages of the enzymatic methodology such as mild reaction conditions and low environmental impact, make the biocatalysis an appropriate means for preparing high purity lapyrium chloride. This is an essential requisite for a product designed for human consumption.

Experimental Section

General Remarks: All solvents and reagents were reagent grade and used without purification. Lipase from *Candida rugosa* (CRL) (905 U/mg solid), and type II crude from porcine pancreas (PPL) (190 U/mg protein) were purchased from Sigma Chemical Co.; *Candida antarctica* lipase A, (CAL A): Chirazyme L-5, c.-f. lyo (400 U/g) was purchased from Roche Diagnostics GmbH; *Candida antarctica* lipase B (CAL B): Novozyme 435 (7400 PLU/g) and Lipozyme RM 1 M (LIP) (7800 U/g) were generous gifts of Novozymes Latinoamerica Ltda. and Novozymes A/S; *Pseudomonas* lipase: Lipase PS Amano (PSL) (33200 U/g) was purchased from Amano Pharmaceutical Co. All enzymes were used "straight from the bottle".

Enzymatic reactions were carried out with an Innova 4000 digital incubator shaker, New Brunswick Scientific Co., at 25 °C and 55 °C and 200 rpm. Melting points were measured with a Fisher–Johns apparatus and are uncorrected. Enzymatic reactions were followed by TLC on Merck Silica gel 60F-254 aluminium sheets (0.2 mm thickness). For column chromatography Merck Silica gel 60 (60–230 mesh) was used. GC analyses were performed with a Hewlett–Packard 5890 gas chromatograph, using ULTRA-2 column (25 m x 0.33 mm x 0.20 μm). The following temperature program was employed: 120 °C (1 min)/10 °C/min/280 °C (5 min). FT-IR spectra were obtained on a Shimadzu FTIR-8300 spectrophotometer.^{1}H NMR and ^{13}C NMR spectra were recorded with a 200 MHz Bruker AC-200 spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane (TMS) set at 0 δ, and coupling constants are given in Hz. Solvents are indicated. EI-MS were obtained at 70 eV with a TRIO-2 VG Masslab Shimadzu QP-5000 and a Finnigan TSQ70 instrument mass spectrometers, in *m*/*z* (%). GC-MS analyses were performed with a Shimadzu GCMS-QP5050A gas chromatograph-mass spectrometer, using ULTRA-2 column (25 m × 0.33 mm × 0.20 μm). The following temperature program was employed 120 °C (1 min)/10 °C/min/280 °C (5 min).

Lipase-Catalysed Preparation of Ethyl Chloroacetate (3): CAL B lipase (150 mg) was added to a solution of chloroacetic acid (284 mg, 3 mmol) in ethanol (5 mL). The suspension was shaken (200 rpm) at room temperature and the progress of the reaction monitored by GLC. When the acid was converted into the ethyl ester (36 h), the enzyme was filtered off. After evaporation of the solvent an oily residue identified as **3** was obtained, 357 mg (97%). FT-IR (film): $\tilde{\nu}_{max}$ = 2986, 1759 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 4.29 (q, J = 7.1, 2 H, CH_2CH_3), 4.10 (s, 2 H, CH_2–Cl), 1.30 (t, J = 7.1 Hz, 3 H, CH_3CH_2–) ppm. ^{13}C NMR ($CDCl_3$, 200 MHz): δ = 169.5 (COO), 62.6 (CH_2–O), 41.2 (CH_2–Cl), 15.3 (CH_3CH_2) ppm. EIMS: *m*/*z* (%) = 107 $[M]^+$ (1), 79 (9), 77 (27), 51 (4), 49 (13), 29 (100). Tests with other lipases were performed under the same experimental conditions. Yields are reported in Table 1.

2-Chloro-*N*-(2-hydroxyethyl)acetamide (5): LIP (300 mg) was added to a solution of ethyl chloroacetate (366 mg, 3 mmol) and ethanolamine (183 mg, 3 mmol) in acetone (5 mL). The suspension was shaken (200 rpm) at 55 °C and the progress of the reaction monitored by GLC. Once **3** had been converted into the amide **5** (48 h), the enzyme was filtered off, and washed with acetone (3 × 5 mL). Evaporation of the solvent resulted in 537 mg (98%) of an oil identified as **5**. FT-IR (film): $\tilde{\nu}_{max}$ = 3300, 1641, 1545 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 4.07 (s, 2 H, CH_2–Cl), 3.62 (t, J = 5.5 Hz, 2 H, CH_2–O), 3.35 (t, J = 5.5 Hz, 2 H, CH_2–NH) ppm. ^{13}C NMR ($CDCl_3$, 200 MHz): δ = 162.5 (CONH), 65.4 (CH_2–O), 58.9 (CH_2–Cl), 49.3 (CH_2–NH) ppm. EIMS: *m*/*z* (%) = 140 $[M^+ + 2]$ (34), 138 (M^+) (100), 120 (42), 106 (18), 94 (20), 72 (20), 60 (10). Tests with other lipases were performed under the same experimental conditions and are reported in Table 1.

2-(2-Chloroacetylamino)ethyl Dodecanoate (7). Ethyl Laurate as Acylating Agent: LIP (1.5 g) and 200 mg of molecular sieves were

added to a solution of **5** (480 mg, 3.5 mmol) and ethyl laurate (1.8 g, 8 mmol) in acetone (10 mL). The suspension was shaken (200 rpm) at room temperature and the progress of the reaction monitored by GLC. When 97% of conversion was achieved (24 h), the enzyme was filtered off and the solvent evaporated. The residue was washed with hexane and 840 mg (75%) of a solid identified as **7** was obtained. M.p. 67–70 °C. FT-IR (KBr): $\tilde{\nu}_{max}$ = 2900, 2877, 1732, 1625 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ = 4.19 (t, J = 5.9 Hz, 2 H, CH_2–O), 4.07 (s, 2 H, CH_2–Cl), 3.59 (t, J = 5.8 Hz, 2 H, CH_2–NH), 2.32 (t, J = 7.0 Hz, 2 H, CH_2COO), 1.61 (q, J = 7.1 Hz, 2 H, CH_2CH_2), 1.35–1.21 (m, 16 H, $8\times CH_2CH_2$), 0.89 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 200 MHz): δ = 173.9 (COO), 166.3 (CONH), 62.4 (CH_2–O), 42.4 (CH_2–Cl), 39.1 (CH_2–NH), 34.1 (CH_2COO), 33.9, 31.8, 29.5, 29.4, 29.2, 29.0, 24.7, 22.6 ($8\times CH_2$), 14.0 (CH_3) ppm. EIMS: m/z (%) = 319 $[M]^+$ (4), 200 (2), 183 (23), 119 (98), 84 (54), 55 (51), 43 (100). Tests with other lipases were performed under the same experimental conditions and are reported in Table 1.

Lauric Acid as Acylating Agent: As described with ethyl laurate but using 720 mg (3.5 mmol) of lauric acid. Yield: 1.07 g (95%) of **7**.

One-Pot Procedure: LIP (300 mg) was added to a solution of ethyl chloroacetate (366 mg, 3 mmol) and ethanolamine (183 mg, 3 mmol) in of acetone (5 mL). The suspension was shaken (200 rpm) at 55 °C and the progress of the reaction monitored by GLC. Once **3** was converted into the amide **5** (48 h), lauric acid (3.5 mmol), LIP (1.2 g), acetone (5 mL) and molecular sieves (200 mg) were added. After standing for 24 h, the enzyme was filtered off, the solvent was evaporated, and the crude residue purified by flash chromatography on silicia gel and identified by ^1H NMR spectroscopy as **7**. Yield: 695 mg (62%).

1-[(2-Dodecanoyloxyethylcarbamoyl)methyl]pyridinium Chloride (1): Pyridine (4 mL) was added dropwise to **7** (440 mg), in an ice-bath, with vigorous stirring. The reaction mixture was allowed to stand overnight at room temperature. After addition of hexane (35 mL), a precipitate identified as **1** (477 mg, 87%) appeared. It was separated by filtration and washed twice with hexane to eliminate the excess of pyridine and lauric acid. M.p. 141–144 °C. FT-IR (KBr): $\tilde{\nu}_{max}$ = 3360, 2988, 2830, 1738, 1622 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ = 9.37 (d, J = 5.5 Hz, 1 H, CH=N), 8.45 (d, J = 7.9 Hz, 2 H, CH=CH), 8.05 (dd, J = 6.6 Hz, 2 H, $2\times$CH=CH), 5.99 (s, 2 H, CH_2–Cl), 4.18 (t, J = 5.8 Hz, 2 H, CH_2–O), 3.50 (t, J = 5.8 Hz, 2 H, CH_2–NH), 2.34 (t, J = 7.4 Hz, 2 H, CH_2COO), 1.58(c J = 7.24 Hz, 2 H, CH_2CH_2), 1.34–1.22(m, 16 H, $8\times CH_2CH_2$), 0.88 (t J = 7.04 Hz, 3 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 200 MHz): δ = 173.8 (COO), 164.3 (CONH), 146.0 (CH=CH), 145.1 (CH=N), 127.6 (CH=CH), 62.3 (CH_2–O), 61.7 (CH_2–N), 39.0 (CH_2–NH), 34.2 (CH_2COO), 31.8, 29.6, 29.3, 29.1, 24.8, 22.6 (CH_2), 14.1 (CH_3) ppm. EIMS: m/z (%) = 363 $[M]^+$ (4), 183 (14), 138 (28), 119 (55), 98 (19), 84 (21), 79 (100), 57 (33), 52 (91).

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