DOI: 10.1002/adsc.200600642

Incorporation of Primary Amines into a Polyester Chain by a Combination of Chemical and Lipase-Catalyzed ε-Caprolactone Ring-Opening Processes

Mattia Marzorati,[a,b] Karl Hult,[c] Sergio Riva,[b,*] and Bruno Danieli[a]

[a] Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, Via Venezian 21, 20133 Milano, Italy
[b] Istituto di Chimica del Riconoscimento Molecolare, C.N.R., Via Mario Bianco 9, 20131 Milano, Italy
Fax: (+39)-02-2890-1239; e-mail: sergio.riva@icrm.cnr.it
[c] Department of Biochemistry, Royal Institute of Technology, Stockholm, 10691 Sweden

Received: December 19, 2006; Revised: June 1, 2007

Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

Abstract: A simple chemoenzymatic strategy for the incorporation of bioactive and suitably functionalized molecules into a polyester chain has been developed. The protocol involves first the reaction of a primary amine with ε-caprolactone to give an amide carrying a terminal primary hydroxy group, followed by the enzymatic growth of the polymeric chain triggered by Novozym 435. This method is versatile and easy to handle and is suitable for the incorporation of different amines into polyesters, as has been shown with the model compounds benzylamine and tryptamine, the bioactive compound *N*-deacetylthiocolchicine and the functionalized propargylamine and tyramine .

Keywords: amines; *Candida antarctica* lipase; ε-caprolactone; enzyme catalysis; Novozym 435; ring-opening polymerization

Introduction

Enzyme-catalyzed polymerizations of suitable reactive monomers is an expanding area of applied biocatalysis.[1–3] Specifically, enzyme-catalyzed ring-opening polymerization (eROP) has proved to be an efficient tool for the preparation of terminally functionalized and block polyesters with low PDI values.[4] Several examples have been reported in the literature so far, most of them exploiting the peculiar performances of the immobilized lipase B from *Candida antarctica* (Novozym 435).[1,2]

In the context of our ongoing research activity finalized to the biocatalyzed modification of natural products,[5] we became interested in a series of papers describing the regioselective lipase-catalyzed ε-caprolactone (ε-CL) eROP initiated by simple alkyl glucopyranosides.[6,7] We were attracted by this approach as a tool to obtain the "macromolecularization" of natural products, with the opportunity to confer either a hydrophobic or a hydrophilic character to these compounds, depending on the lactone used in the eROP process (i.e., ε-caprolactone or 1,5-dioxepan-2-one).

Thiocolchicoside (**1**, 3-*O*-β-D-glucopyranosyl-3-*O*-demethylthiocolchicine) is a synthetic derivative of the naturally occuring compound colchicoside.[8,9] It has been previously chosen by us as a model target molecule for successful biotransformations,[5,10,11] and, specifically, we have shown that the sugar moiety of **1** could be regioselectively acylated at its primary OH with short chain aliphatic acids by proteases and lipases (and specifically, among the latter enzymes, by Novozym 435)[10,11] in organic solvents.

As will be described in the following, the glucoside **1** could be incorporated into the polymeric product, but the results were not fully satisfactory. However, in spite of these initial data, we thought that the thiocolchicinoid moiety could be included into a growing polymer by exploiting the reactivity of functional groups different from the OHs at the sugar moiety, namely the C-7-NH_2 of *N*-deacetylthiocolchicine (**2**), an easily accessible derivative of **1**.

The general outcome of these experiments was the development of a combination of simple chemical and enzymatic processes for the incorporation of suitably functionalized molecules into a polyester chain. These results offer new opportunities to the preparation of end-functionalized polyesters that, in turn, can be used for the preparation of suitable block copolymers.[12]

Results and Discussion

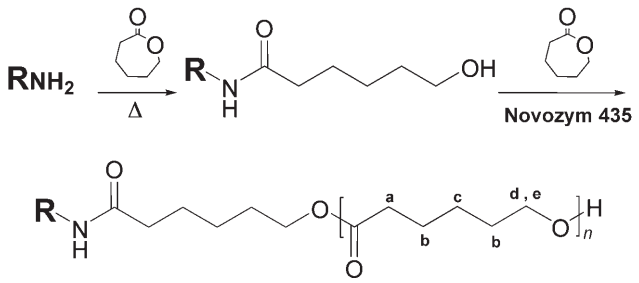
Following standard protocols,[6,7] the Novozym 435-catalyzed ε-CL-ROP initiated by the model glucoside thiocolchicoside (**1**) was performed, furnishing a yellow polymeric mixture **1a**. The ^{1}H NMR analysis of the isolated product showed that **1** was indeed incorporated into the macromolecular product, but the percentage of thiocolchicoside-initiated polyesters **1a** in the obtained polymeric mixture was unsatisfactory, due to the presence of significant amounts of water-initiated poly ε-CL.[13] Our attempts to decrease the water competition resulted in a slightly increase of the percentage of **1a** in the polyester product mixture, percentage that, however, did never exceeded 50% (see the NMR spectra in the Supporting Information). Apparently, despite our attempts, we were not able to completely eliminate water from the reaction mixture, and water proved to be a much better (and natural) nucleophile for the intermediate acyl-enzyme than the primary sugar OH of the bulky thiocolchicoside.

Pursuing our goal to introduce the pharmacologically valuable thiocolchicine moiety into a polyester chain, we turned our attention to the cognate derivative *N*-deacetylthiocolchicine (**2**), with the hope that the primary amino group at C-7 of this molecule could be a better initiator than the sugar hydroxy groups of **1**.

Despite the fact that it is well-known that lipase can also catalyze the formation of amide bonds,[14] to the best of our knowledge and to our surprise, the use of amines as initiators in an enzymatic-catalyzed ε-CL ROP has been only described once in the literature, by Gross and co-workers.[15] The goal of their study was not synthetic but mechanicistic, and was finalized to the comparison of the performances of an amine (butylamine) and of the corresponding alcohol (butanol) in order to get information on the effect of the initiator on the product structure, propagation kinetics and mechanism of the enzymatic polymerization.

After some experimentation (for details see Supporting Information), we came to the conclusion that **2** was also a poor substrate for *Candida antarctica* lipase, probably due to steric reasons. However, the nucleophilicity of the C-7 amino group allowed **2** to be acylated in the presence of a suitable reactive ester, even in the absence of the enzyme. We reasoned that the cyclic ε-CL, due to its ring strain,[16] could be assimilated to an activated acyl donor when suffering the attack of a primary amino group, like that present in **2**. In this way a new compound (**2a**) carrying a terminal, more accessible primary OH could be formed *in situ*. This adduct, in turn, could act as a more efficient initiatior for the lipase-catalyzed ε-CL-ROP (Scheme 1).

Confirming this hypothesis, compound **2** and also two other model primary amines – benzylamine (**3**) and triptamine (**4**) – as well as two functionalized amines – propargylamine (**5**) and tyramine (**6**) – were efficiently incorporated into the polyesters obtained by the biocatalyzed ε-CL-ROP. On the contrary, secondary amines (like, for instance, *N*-benzyl-*N*-methylamine, **7**) could not be efficiently incorporated using the same protocol.



Scheme 1. Chemoenzymatic ε-caprolactone ring-opening polymerization.

According to Scheme 1, the intermediate hydroxy amides **2a–6a** were easily prepared (by heating the corresponding amines **2–6** in the presence of an excess – typically 40 equivs. – of ε-CL at 70 °C for 2 h), and isolated to be characterized. In the complete chemoenzymatic processes, compounds **2a–6a** were not isolated and the enzymatic ROPs were started by adding, after 2 h, a suitable amount of Novozym 435 to the reaction mixture. The reactions were left overnight at 60 °C, and the monomer conversion was evaluated by ^{1}H NMR analysis of the crude reaction mixtures. The polyesters **2b–6b** were isolated following usual work-up protocols and characterized. Some of the relevant data are reported in Table 1.

Table 1. Incorporation of amines into poly ε-CL obtained by chemoenzymatic ROP.[a]

Amine	Conversion[b] [%]	Incorporation[c] [%]	DP[d]	M_n[e]	M_w[e]	PDI[e]
2	>95	>95	40	6,850	12,250	1.79
3	>95	85	37	8,050	14,500	1.80
4	>95	85	37	7,400	13,500	1.82
5	>95	90	38	8,150	14,600	1.79
6	>95	85	38	8,100	14,500	1.79

[a] *Reaction conditions:* monomer ε-caprolactone (M) to amine (A) molar feed ratio, 40:1; temperature, 60 °C.
[b] Monomer conversion determined by ^{1}H NMR spectroscopy of the crude reaction mixture.
[c] Percentage of amino incorporation into the polyester determined by ^{1}H NMR spectroscopy of the precipitated polymer.
[d] Determined by ^{1}H NMR spectroscopy of the precipitated polymer.
[e] Obtained by SEC analysis in comparison with polystyrene standards (detector, RI).

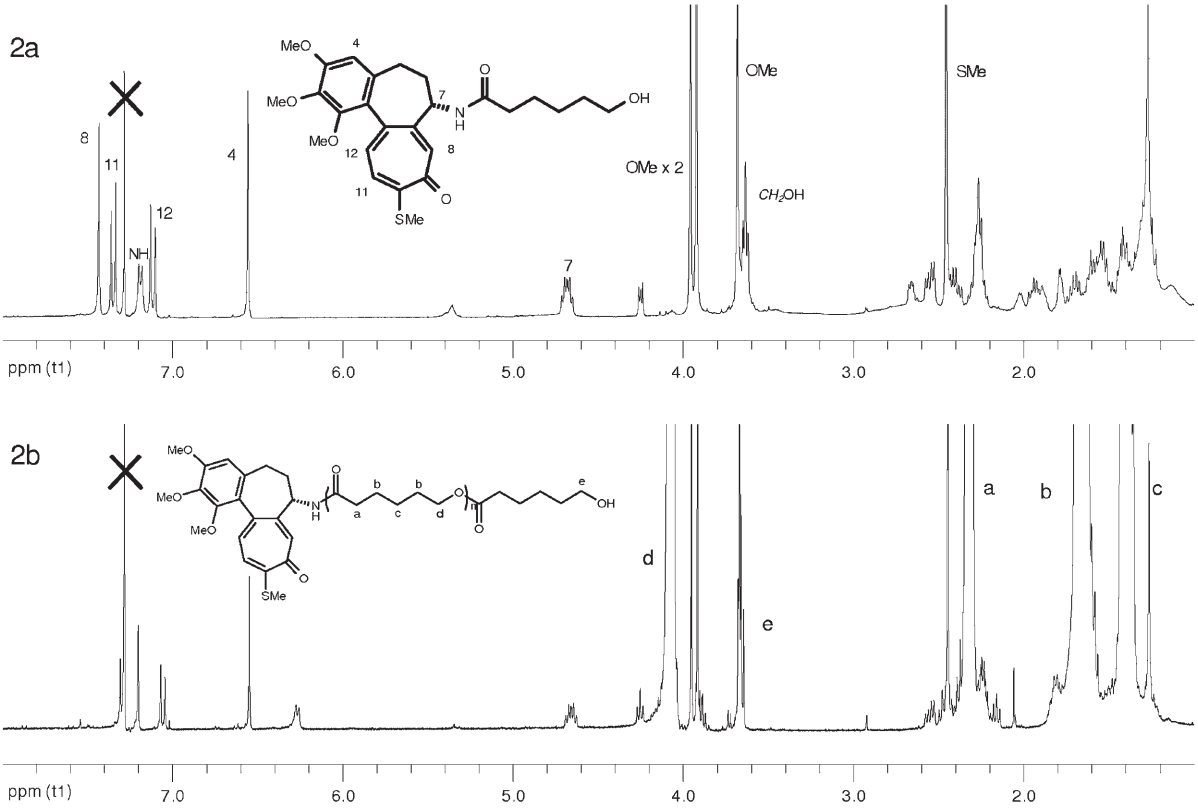


Figure 1. . ^{1}H NMR spectra of compound **2a** and of the polyester **2b**.

As an example, Figure 1 shows the ^{1}H NMR spectra of the adduct **2a** and of the corresponding polyesters mixture **2b**. The incorporation of a 6-hydroxyhexanoyl moiety in **2a** was clearly demonstrated by the triplet at 3.64 (corresponding to the terminal CH_2OH of the 6-hydroxyhexanoyl moiety partially overlapping the OCH_3 singlet due to the C-1 methoxy moiety). Additionally, the ratio between the integrated signals at 3.68–3.64 (a singlet due to a OCH_3 moiety of **2** plus the above described triplet) and at 4.68 ppm (a dt due to H-7 of **2**) was approximately 5, thus confirming the *ca.* 1:1 ratio between the starting amine **2** and the added aliphatic chain. A similar ratio between the integration values of these signals in the polyester **2b** confirmed the complete incorporation of the amine into the polyester product. The ratio between the very intense signals at 4.08 (a triplet due to the "internal" $-CH_2OCO-$ esterified moieties) and at 3.68–3.64 ppm (the above described triplet due to the terminal CH_2OH and the singlet due to a OCH_3 moiety) allowed us also to estimate a degree of polymerization (DP) value of 40. The number average molecular weight (M_n) and the weight average molecular weight (M_w) were determined by size exclusion chromatography, which allowed us also to calculate a polydispersity value (PDI) of 1.79. Additional information was obtained by the high resolution ESI-MS of the polymer, which showed a Gaussian distribution of peaks separated by 114.14 Da (corresponding to one ε-CL unit). Besides that, the evaluation of the exact mass of some single peaks confirmed the incorporation of **2** into the polyester. For instance, the peak at 1764.94223 Da corresponded to the Na^+ adduct of **2b** with $n=12$ (error 0.7 ppm), the one at 1879.00924 Da corresponded to the Na^+ adduct of **2b** with $n=13$ (error 0.1 ppm), etc.

Conclusions

We have shown that functionalized primary amines can be incorporated into a polyester chain by a combination of chemical and enzymatic processes, which involves first the reaction of an amine with ε-CL to form the corresponding ω-hydroxy amides, followed by the enzymatic growth of the polymeric chain triggered by Novozym 435.

The suggested protocol is versatile, suitable for the incorporation of different primary amines and easy to handle (i.e., it does not require special apparatus, like dry boxes, to keep the water content under control), therefore it might allow one to further extend the available array of terminal-functionalized polymers. [4] Specifically, the functionalized polyesters **5b** and **6b**

can be utilized for the preparation of different polymer architectures, exploiting either the so-called "click chemistry"[17] or the radical polymerization of phenols.[18]

To our surprise, the well-known higher chemical reactivity of amines, that, in contrast to alcohols or thiols, make this functional group able to easily open an ε-CL molecule,[19–21] has never been synthetically exploited before for biocatalyzed polymerizations.

Experimental Section

Materials and Methods

Thiocolchicoside (**1**; Figure 2) and thiocolchicine were a gift from Indena S.p.A., Milano, Italy. *N*-Deacetylthiocolchicine (**2**; Figure 2) was simply prepared from thiocolchicine following a standard procedure.[22] Novozym 435 was a gift from Novozymes Inc. The other substrates and reagents were from Fluka. Tryptamine (**4**) was crystallized from petroleum ether before use, while benzylamine (**3**) and ε-caprolactone were distilled (**3**, $T=185\,°C$ at $p=1$ atm; ε-caprolactone, $T=98\,°C$ at $p=5$ mm Hg). TLC analysis were performed on silica plates (Merck 60 F_{254}) and treated with the molybdate reagent [$(NH_4)_6MoO_{24}\cdot 4H_2O$, 42 g; $Ce(SO_4)_2$, 2 g; H_2SO_4 concentrated 62 mL; made up to 1 L with deionized water]. Preparative TLC were performed on silica plates (Merck 60, 230–400 mesh).

NMR Spectroscopy: The percentage of monomer (ε-caprolactone) conversion, the percentage of amine incorporation and the degree of polymerization were determined by ^{1}H NMR spectroscopy with a Bruker AC400 (400 MHz) using $CDCl_3$ as the solvent. Specifically, the monomer conversion was evalated by comparing the area of the triplet due to the CH_2OCO of ε-caprolactone (resonating at 4.25 ppm) with that of the triplet due to the polymeric CH_2OCO (resonating at 4.07 ppm).

Mass Spectroscopy: High resolution electrospray mass spectra (HR-ESI-MS) were acquired with an FT-ICR (Fourier Transfer Ion Cyclotron Resonance) APEX™ II model (Bruker Daltonics) equipped with a 4.7 Tesla cryo-magnet (Magnex). Samples were dissolved in CH_3CN and injected into the instrument equipped with its own ESI source. Spectra were recorded in the HR mode with resolutions ranging from 20,000 to 30,000.

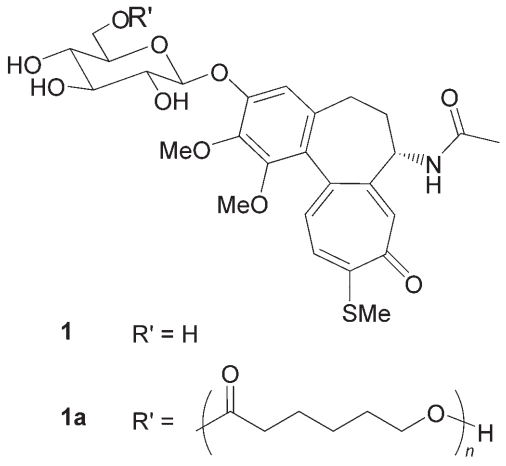


Figure 2. Compounds **1–1a.**

SEC: The molecular weight distribution (MWD) of polymers were determined by a modular Alliance 2690 size exclusion chromatography (SEC) system from Waters equipped with a 2414 differential refractometer as on-line concentration detector. The columns set was composed of two PLGel Mixed C columns (300 × 7.8 mm, 5 μm of particle size) from Polymer Laboratories. The experimental conditions consisted of tetrahydrofuran as mobile phase stabilized with 0.05% of BHT, 35 °C of temperature, 0.8 mL min^{-1} of flow rate, about 3 mg mL^{-1} of sample concentration and 100 μL of injection volume. Narrow MWD polystyrene standards were used for the calibration of the SEC system. Empower chromatographic software from Waters was used to process the data.

Polymerization of ε-CL in the Presence of Thiocolchicoside (1) as an Initiator

Thiocolchicoside (**1**, 282 mg), the enzymatic preparation Novozym 435 (77 mg) and molecular sieves (150 mg) were added to a two-necked, round-bottom flask and dried overnight in a dessicator over P_2O_5. Freshly distilled ε-caprolactone (2.2 mL) was added and the mixture was left at 60 °C overnight. The crude reaction mixture was dissolved in 4 mL of dioxane, the solid enzyme and the molecular sieves were filtered. Addition of cold water to the solution caused the precipitation of a yellow solid. After vacuum-drying, direct NMR analysis of the crude sample showed that it was a mixture of thiocolchicoside-initiated polyester **1a** and water-initiated polyester.

The ^{1}H NMR (ppm, $CDCl_3$) spectrum is reported in the Supplementary Materials Section. δ (polymer chain) = 4.07 (t, $J=6.8$ Hz, CH_2-d); 3.65 (m, terminal CH_2OH, CH_2-e), 2.32 (t, $J=7.2$ Hz, CH_2-a), 1.67 (m, CH_2-b), 1.41 (m, H-c); δ (thiocolchicoside moiety, selected data) = 7.25 and 7.05 (1H each, AB system, $J=10.5$ Hz, H-11 and H-12), 7.22 (1H, s, H-8), 6.8 (1H, s, H-4), 4.82 (1H, d, $J=7.4$ Hz, H-1′), 4.60 (1H, dt, $J_1=11.3$ Hz, $J_2=6.7$ Hz, H-7), 4.50 (1H, dd, $J_1=12.0$ Hz, $J_2=5.1$ Hz, H-6′$_a$), 4.42 (1H, dd, $J_1=12.0$ Hz, $J_2=2.2$ Hz, H-6′$_b$), 3.99 (3H, s, OCH_3), 3.65 (m, OCH_3, H-2′, H-3′, H-4′), 2.45 (3H, s, SCH_3); ESI-MS (selected data, Da): $m/z=1042.44874$ (**1a** + Na^+, with $n=5$; calculated for $[C_{27}H_{33}NO_{10}S(C_6H_{10}O_2)_5Na]^+$: 1042.44406), 1058.42216 (**1a** + K^+, with $n=5$; calculated $[C_{27}H_{33}NO_{10}S(C_6H_{10}O_2)_5K]^+$: 1058.41799), 1067.61738 (water-initiated poly ε-CL + Na^+, with $n=9$; calculated for $[H_2O(C_6H_{10}O_2)_9+Na]^+$: 1067.61738), 1083.58988 (water-initiated poly ε-CL + K^+, with $n=9$; calculated for $[H_2O(C_6H_{10}O_2)_9+K]^+$: 1083.58644).

The integrated NMR signals indicated that the ratio between thiocolchicoside-initiated polyester **1a** and water-initiated polyester was less than 1:1. This value was obtained by comparing the integration value of the signal at 4.82 ppm due to H-1′ (or of the signal at 4.60 due to H-7) with the integration value of the multiplet at 3.65 due to the overlapping of the signals of one OCH_3, of the sugar protons H-2′, H-3′, H-5′ and of the terminal oxymethylene of the polymeric chains.

Synthesis of 6-Hydroxyhexanoyl Amides (2a–6a; Figure 3)

The primary amines (**2**–**6**, 0.1 mmol) and ε-CL (4 mmol) were added to a round-bottom flask. The flask was immersed in an oil bath at 70 °C and the reaction was monitored by TLC. After consumption of the amine (typically 2 h), the products were isolated by preparative TLC.

2a: yellow solid; $R_f = 0.39$ (eluent AcOEt : MeOH 10:1); ^{1}H NMR (ppm, $CDCl_3$, see Figure 1, selected data): δ = 7.43 (1 H, s, H-8), 7.35 (1 H, d, $J = 10.4$ Hz, H-11), 7.19 (1 H, br d, $J = 7.1$ Hz, NH), 7.15 (1 H, d, $J = 10$ Hz, H-12), 6.56 (1 H, s, H-4), 4.68 (1 H, dt, $J_1 = 11.4$ Hz, $J_2 = 6.8$ Hz, H-7), 3.96 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 3.68 (3 H, s, OCH_3), 3.64 (2 H, t, $J = 6.1$ Hz, CH_2OH, CH_2-a), 2.45 (3 H, s, SCH_3); ESI-MS (Da): $m/z = 510.19240$ (**2a** + Na^+, calculated for $[C_{26}H_{33}NO_6S + Na]^+$: 510.19208), 997.39418 (2 × **2a** + Na^+, calculated for $[C_{52}H_{66}N_2O_{12}S_2 + Na]^+$: 997.39494).

3a: white solid; $R_f = 0.27$ (eluent AcOEt); ^{1}H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ = 7.36–7,27 (5 H, m, ArH), 4.44 (2 H, d, $J = 5.7$ Hz, CH_2-1), 3.64 (2 H, t, $J = 6.4$ Hz, CH_2-e), 2.24 (2 H, t, $J = 7.4$ Hz, CH_2-a), 1.70 (2 H, q, $J = 6.4$ Hz, CH_2-b or CH_2-d) and 1.59 (2 H, q, $J = 6.4$ Hz, CH_2-b or CH_2-d), 1.42 (2 H, m, CH_2-c); ESI-MS (Da): $m/z = 244.13052$ (**3a** + Na^+, calculated for $[C_{13}H_{19}NO_2 + Na]^+$: 244.13080), 465.27315 (2 × **3a** + Na^+, calculated for $[C_{26}H_{38}N_2O_4 + Na]^+$: 465.27238).

4a: white solid; $R_f = 0.12$ (eluent AcOEt); H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ (aromatic protons) = 7.62 (1 H, d, $J = 8.0$ Hz), 7.40 (1 H, d, $J = 8.0$ Hz), 7.22 (1 H, t, $J = 8.1$ Hz), 7.15 (1 H, t, $J = 8.0$ Hz) 7.06 (1 H, s); δ (aliphatic protons) = 3.65 (4 H, m, CH_2-1 and CH_2-e), 3.00 (2 H, t, $J = 6.8$ Hz, CH_2-2), 2.14 (2 H, t, $J = 6.8$ Hz, CH_2-a), 1.67 (2 H, q, $J = 7.2$ Hz, CH_2-b or CH_2-d), 1.56 (2 H, q, $J = 7.2$ Hz, CH_2-b or CH_2-d), 1.37 (2 H, m, CH_2-c); ESI-MS (Da): $m/z = 297.15735$ (**4a** + Na^+, calculated for $[C_{16}H_{22}N_2O_2 + Na]^+$: 297.15735), 571.32609 (2 × **4a** + Na^+, calculated for $[C_{32}H_{44}N_4O_4 + Na]^+$: 571.32548).

5a: white solid; $R_f = 0.50$ (eluent AcOEt-MeOH 10–0.5); H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ = 4.07 (2 H, dd, $J = 2.5$ Hz, $J = 5.2$ Hz, CH_2-2), 3.67 (2 H, t, $J = 6.4$ Hz, CH_2-e), 2.25 (1 H, t, $J = 2,6$ Hz, CH-1), 2.24 (2 H, t, $J = 7.3$ Hz, CH_2-a), 1.70 (2 H, q, $J = 7,6$ Hz, CH_2-b or CH_2-d) and 1.61 (2 H, q, $J = 7.1$ Hz, CH_2-b or CH_2-d), 1.43 (2 H, m, $J = 7.1$ Hz, CH_2-c).

6a: white solid; $R_f = 0.10$ (eluent AcOEt); H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ = 7.07 (2 H, d, $J = 8.2$ Hz) and 6.81 (2 H, d, $J = 8.2$ Hz), aromatic protons, 3.66 (2 H, t, $J = 6.5$ Hz, CH_2-e), 3.51 (2 H, quart, $J = 8.2$ Hz, CH_2-NH), 2.77 (2 H, t, $J = 6.7$ Hz, CH_2-Ph), 2.16 (2 H, t, $J = 7.2$ Hz, CH_2-a), 1.59 (4 H, m, CH_2-b and CH_2-d), 1.40 (2 H, m, CH_2-c).

Polymerization of ε-CL in the Presence of Primary Amines as an Initiator

In a typical polymerization reaction, the lactone, and the amine in a 40:1 ratio (see Table 1) were added with a syringe into a two-necked, round-bottom flask. The flask was heated at 70 °C for 2 h with continuous stirring and under vacuum (generated by a water pump). The reaction mixture was flushed with N_2, then the enzyme (3 % w/w ε-CL) and the molecular sieves (150 mg) were added, and the reaction

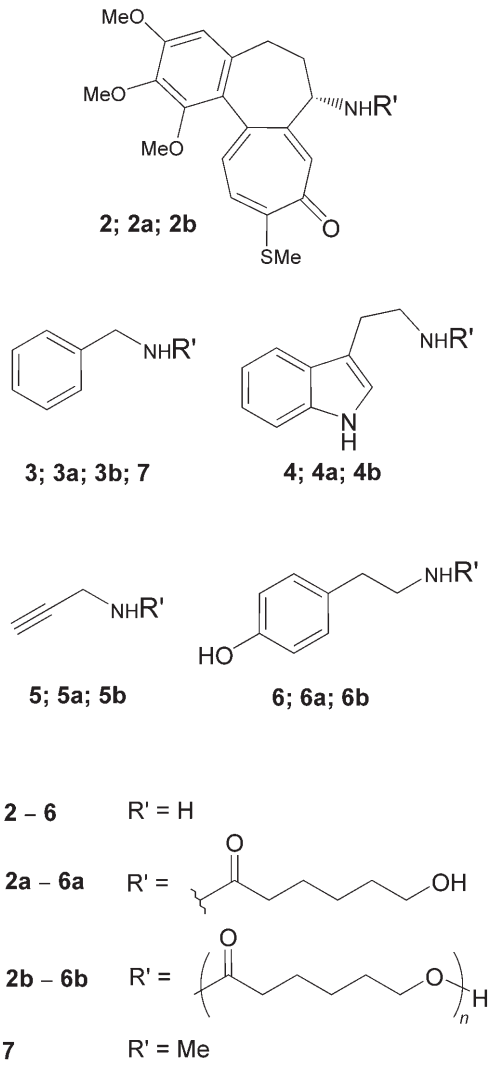


Figure 3. Compounds **2–6**, **2a–6a**, **2b–6b**.

was left overnight at 60 °C (see Table 1). After cooling to 40 °C, a small volume of $CHCl_3$ was added, the enzyme and the molecular sieves were filtered, and a portion of the crude reaction mixture was analyzed by ^{1}H NMR to determine the percentage of monomer (ε-CL) conversion (Table 1). The remaining polymer was precipitated by adding cold hexane, filtered and dried under vacuum (isolated yields usually in the order of 80 % w/w added monomer). As reported in Table 1, different polymers carrying different amines were synthesized, showing different degrees of polymerization.

2b: yellow solid; H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ (polymer chain) = 4.08 (t, $J = 6.8$ Hz, CH_2-d), 3.67 (t , $J = 6.8$ Hz, CH_2OH, CH_2-e), 2.33 (t, $J = 7.5$ Hz, CH_2-a), 1.68 (m, CH_2-b); 1.40 (m, CH_2-c); δ (thiocolchicine moiety, selected data) = 7.40 (s, H-8), 7.35 (d, $J = 10.0$ Hz, H-11), 7.05 (1 H, d, $J = 10.1$ Hz, H-12), 6.55 (1 H, s, H-4), 4.66 (1 H, dt, $J_1 = 11.3$ Hz, $J_2 = 6.7$ Hz, H-7), 3.96 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 3.67 (3 H, s, OCH_3), 2.45 (3 H, s, SCH_3); ESI-MS (selected data, Da): $m/z = 1650.87072$ (**2b** + Na^+, with $n = 11$; calculated for $[C_{20}H_{22}NO_4S(C_6H_{10}O_2)_{11} + Na]^+$: 1650,87288),

1764.94223 (**2b** + Na^+, with $n=12$; calculated for $[C_{20}H_{22}NO_4S(C_6H_{10}O_2)_{12}+Na]^+$: 1764.94095), 1879.00924 (**2b** + Na^+, with $n=13$; calculated for $[C_{20}H_{22}NO_4S(C_6H_{10}O_2)_{13}+Na]^+$: 1879.00903), SEC (THF): $M_n=6{,}850$, $M_w=12{,}250$, $d=1.79$.

3b (M/I 40/1): white solid; H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ (polymer chain) = 4.07 (t, $J=6.7$ Hz, CH_2-d), 3.66 (t, $J=6.7$ Hz, CH_2OH, CH_2-e), 2.32 (t, $J=7.2$ Hz, CH_2-a), 2.22 (t, $J=7.1$ Hz, CH_2-f), 1.66 (m, CH_2-b), 1.40 (m, CH_2-c); δ (benzylamine moiety) = 7.38–7.26 (m, ArH), 4.43 (d, $J=5.6$ Hz, $PhCH_2$); ESI-MS (selected data, Da): $m/z=1156.67576$ (**3b** + Na^+, with $n=9$; calculated for $[C_7H_9N(C_6H_{10}O_2)_9Na]^+$: 1156.67544), 1384.81374 (**3b** + Na^+, with $n=11$; calculated for $[C_7H_9N(C_6H_{10}O_2)_{11}Na]^+$: 1384.81160), 1612.95520 (**3b** + Na^+, with $n=13$; calculated for $[C_7H_9N(C_6H_{10}O_2)_{13}+Na]^+$: 1612.94775), SEC (THF): $M_n=8{,}050$, $M_w=14{,}500$, $d=1.80$.

4b: white solid; H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ (polymer chain) = 4.07 (t, $J=6.8$ Hz, CH_2-d), 3.66 (m, CH_2OH, CH_2-e), 2.31 (t, $J=7.6$ Hz, CH_2-a), 2.16 (t, $J=7.2$ Hz, CH_2-f), 1.67 (m, CH_2-b), 1.41 (m, CH_2-c); δ (tryptamine moiety) = 7.61 (d, $J=8$ Hz), 7.40 (d, $J=8.0$ Hz), 7.23 (t, $J=8.0$ Hz), 7.15 (t, $J=8.0$ Hz), 7.06 (s), 3.65 (m, CH_2NH), 2.99 (t, $J=6.8$ Hz, $ArCH_2$); ESI-MS (selected data, Da): $m/z=1209.70755$ (**4b** + Na^+, with $n=9$; calculated for $[C_{10}H_{12}N(C_6H_{10}O_2)_9+Na]^+$: 1209.70199), 1665.97807 (**4b** + Na^+, with $n=13$; calculated for $[C_{10}H_{12}N(C_6H_{10}O_2)_{13}+Na]^+$: 1665.97430); SEC (THF): $M_n=7{,}400$, $M_w=13{,}500$, $d=1.82$.

5b: white solid; H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ (polymer chain) = 4.07 (t, $J=6.7$ Hz, CH_2-d), 3.65 (t, $J=6.5$ Hz, CH_2OH, CH_2-e), 2.33 (t, $J=7.3$ Hz, CH_2-a), 2.21 (t, $J=7.1$ Hz, CH_2-f), 1.66 (m, CH_2-b), 1.39 (m, CH_2-c); δ (propargyl amine moiety) = 4.08 (m, CH_2-2), 2.23 (m, CH_2-1): ESI-MS (selected data, Da): $m/z=762.44001$ (**5b** + Na^+, with $n=6$; calculated for $[C_3H_5N(C_6H_{10}O_2)_6Na]^+$: 762.43990), 876.50966 (**5b** + Na^+, with $n=7$; calculated for $[C_3H_5N(C_6H_{10}O_2)_7Na]^+$: 876.50798), 990.57637 (**5b** + Na^+, with $n=8$; calculated for $[C_3H_5N(C_6H_{10}O_2)_8Na]^+$: 990.57606); SEC (THF): $M_n=8{,}150$, $M_w=14{,}600$, $d=1.79$.

6b: white solid; H NMR (ppm, $CDCl_3$, the spectrum is depicted in the Supporting Information): δ (polymer chain) = 4.08 (t, $J=6.8$ Hz, CH_2-d), 3.65 (t, $J=6.5$ Hz, CH_2OH, CH_2-e), 2.32 (t, $J=7.2$ Hz, CH_2-a), 2.20 (t, $J=7.1$ Hz, CH_2-f), 1.66 (m, CH_2-b), 1.38 (m, CH_2-c); δ (tyramine amine moiety) = 7.06 and 6.79 (d each, ArH), 3.50 (quart, $J=6.9$ Hz, CH_2-NH), 2.76 (t, $J=7.2$ Hz, CH_2-Ph); ESI-MS (selected data, Da): $m/z=844.48251$ (**6b** + Na^+, with $n=6$; calculated for $[C_8H_{11}NO(C_6H_{10}O_2)_6Na]^+$: 844.48176), 958.55094 (**6b** + Na^+, with $n=7$; calculated for $[C_8H_{11}NO(C_6H_{10}O_2)_7Na]^+$: 958.55094), 1072.61929 (**5b** + Na^+, with $n=8$; calculated for $[C_8H_{11}NO(C_6H_{10}O_2)_8Na]^+$: 1072.61792); SEC (THF): $M_n=8{,}100$, $M_w=14{,}500$, $d=1.79$.

Supporting Information Available

^{1}H NMR spectra of compounds **1a**, **3a**, **3b**, **4a**, **4b**, **5a**, **5b**, **6a** and **6b**.

Acknowledgements

We thank Dr. Raniero Mendichi (ISMAC-CNR, Milano) for his analytical support (SEC analysis).

References

[1] S. Kobayashi, H. Uyama, S. Kimura, *Chem. Rev.* **2001**, *101*, 3793–3818.
[2] R. A. Gross, A. Kumar, B. Karla, *Chem. Rev.* **2001**, *101*, 2097–2124.
[3] D.-Y. Kim, X. Wu, J. S. Dordick, *ACS Symp. Ser.* **2002**, *840*, 34–49.
[4] R. K. Srivastava, A. C. Albertsson, *Macromolecules* **2006**, *39*, 46–54.
[5] S. Riva, *J. Mol. Catal. B: Enzymatic* **2002**, *19–20*, 43–54.
[6] A. Cordova, T. Iversen, K. Hult, *Macromolecules* **1998**, *31*, 1040–1045.
[7] K. S. Bisht, F. Deng, R. A. Gross, D. L. Kaplan, G. Swift, *J. Am. Chem. Soc.* **1998**, *120*, 1363–1367.
[8] J. I. Clark, T. N. Margulis, *Life Sciences* **1980**, *26*, 833–836.
[9] M. H. Zweig, C. F. Chignell, *Biochemical Pharmacology* **1973**, *22*, 2141–2150.
[10] B. Danieli, P. De Bellis, G. Carrea, S. Riva, *Gazz. Chim. It.* **1991**, *121*, 123–126.
[11] B. Danieli, M. Luisetti, G. Sampognaro, G. Carrea, S. Riva, *J. Mol. Catal. B: Enzymatic* **1997**, *3*, 193–201.
[12] M. De Geus, L. Schormans, A. R. A. Palmans, C. E. Koning, A. Heise, *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4290–4297.
[13] H. Uyama, S. Suda, S. Kobayashi, *Acta Polym.* **1998**, *49*, 700–703.
[14] S. Tawaki, A. M. Klibanov, *J. Am. Chem. Soc.* **1992**, *114*, 1882–1884.
[15] L. A. Henderson, Y. Y. Svirkin, R. A. Gross, D. L. Kaplan, G. Swift, *Macromolecules* **1996**, *29*, 7759–7766.
[16] L. Van der Mee, F. Helmich, R. de Bruijn, J. A. J. M. Vekemans, A. R. A. Palmans, E. W. Meijer, *Macromolecules* **2006**, *39*, 5021–5027.
[17] a) R. Riva, S. Schmeits, C. Jerome, R. Jerome, P. Lecomte, *Macromolecules* **2007**, *40*, 796–803; b) J. F. Lutz, *Angew. Chem. Int. Ed.* **2007**, *46*, 1018–1025.
[18] T. Fukuoka, H. Uyama, S. Kobayashi, *Macromolecules* **2004**, *37*, 8481–8484.
[19] M. Schappacher, A. Soum, S. M. Guillaume, *Biomacromolecules* **2006**, *7*, 1373–1379.
[20] T. J. Deming, *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 275–278.
[21] A. Kowalski, J. Libiszowski, T. Biela, M. Cypryc, A. Duda, S. Penczek, *Macromolecules* **2005**, *38*, 8170–8175.
[22] P. Kerekes, P. N. Sharma, A. Brossi, C. F. Cignell, F. R. Quinn, *J. Med. Chem.* **1985**, *28*, 1204–1208.

