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Incorporation of primary amines into a poly(1,5-dioxepan-2-one) *via* lipase-catalyzed ring-opening polymerization

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Abstract

A simple chemo-enzymatic strategy for the synthesis of functionalized poly(1,5-dioxepane-2-one)esters has been developed. The protocol involves first the reaction of a primary amine with 1,5-dioxepane-2-one (**2**) to give an amide carrying a terminal primary hydroxyl group, followed by an enzymatic ring-opening polymerization catalyzed by Novozym 435. The proposed method is easy to handle and is suitable for the incorporation of different amines, as it has been shown with the model benzyl amine (**3**), the functionalized amines tyramine (**4**) and propargylamine (**5**), and the bioactive compound *N*-deacetylthiocolchicine (**6**).

Keywords: Ring-opening polymerization; *Candida antarctica* lipase; Novozym 435; 1,5-dioxepan-2-one; Polyesters

1. Introduction

The exploitation of enzymes for the synthesis and the modification of polymers to produce new materials with improved properties is an expanding area of research [1–3]. On this respect, the so-called enzyme-catalyzed ring-opening polymerization (eROP) has proved to be an efficient and mild methodology for the preparation of end-functionalized polyesters with low PDI values. Several examples have been reported in the literature so far [4], most of them based on the peculiar performances of Novozym 435, an industrial formulation of the immobilized lipase B from *Candida antarctica* [1,2].

In the context of our ongoing research activity in applied biocatalysis [5], we became interested in this methodology as a tool to obtain the "macromolecularization" of natural products [6]. Accordingly, in a recent paper, we have described a simple chemo-enzymatic strategy for the incorporation of bioactive and suitable functionalized molecules into a polyester chain [7]. The protocol involves first the reaction of a primary amine with ε-caprolactone (**1**, ε-CL) to give an amide carrying a terminal primary hydroxyl group, followed by the enzymatic growth of the polymeric chain triggered by Novozym 435 (Scheme 1, $X = CH_2$). The method has proved to be easy to handle and suitable for the incorporation of different amines into a polyester chain.

Here we report an extension of our previous research, describing the results obtained with a different cyclic lactone, the 1,5-dioxepan-2-one (DXO, **2**) and oxygenated analogue of ε-CL. We propose that, all together, these results show new opportunities towards the synthesis of end-functionalized polyesters that, in turn, can be used for the preparation of suitable block copolymers [8].

2. Experimental

2.1. Materials and methods

N-Deacetylthiocolchicine (**6**) was prepared from thiocolchicine (gift from Indena S.p.A., Milano, Italy) following a standard procedure [9]. Tetrahydro-4*H*-pyran-4-one was from Dayang Chemicals Co., China. *m*-Chloroperbenzoic acid, benzylamine (**3**), tyramine (**4**) and propargylamine (**5**) were from Aldrich. Novozym 435 was a gift from Novozymes Inc. Benzylamine (**3**) and 1,5-dioxepane-2-one (DXO, **2**) were distilled (Benzylamine (**3**), $T = 185\,^{\circ}C$ at $p = 1$ atm; Dioxepanone (DXO,

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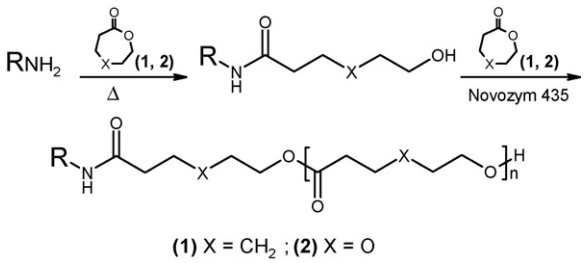

Scheme 1. Chemoenzymatic ring-opening polymerization of ε-caprolactone (**1**) or 1,5-dioxepan-2-one (**2**).

2), $T = 85\,^{\circ}C$ at $p = 4$ 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 deionizated water). Preparative TLC was performed on silica plates (Merck 60, 230–400 mesh).

2.1.1. NMR spettroscopy

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.

2.1.2. 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 mm × 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 of flow rate, about 3 mg/mL 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.

2.2. Baeyer–Villiger oxidation of tetrahydro-4H-pyran-4-one

Tetrahydro-4*H*-pyran-4-one (22.3 g, 223 mmol) and *m*-chloroperbenzoic acid (70%, 67.8 g, 275 mmol) were mixed in CH_2Cl_2 (250 mL). The suspension was heated under reflux for 3 h. The reaction mixture was cooled in an ice bath, and the solids were filtered over Celite and washed with cold CH_2Cl_2 (2× 50 mL). The organic solution was washed with 10% $Na_2S_2O_3$ solution (2× 200 mL), saturated Na_2CO_3 solution (2× 200 mL), and saturated NaCl solution (1× 200 mL). The organic layer was dried with Na_2SO_4, filtered, and evaporated in vacuo. The resulting liquid was distilled in vacuum to afford the pure lactone DXO (**2**) as a solid in 70% yields.

2: white solid; ^{1}H NMR δ (ppm, $CDCl_3$): 4.25 (2H, t, $J = 3.2$ Hz, CH_2-6); 3.85 (2H, t, $J = 3.2$ Hz, CH_2-5); 3.80 (2H, t, $J = 4.9$ Hz, CH_2-3); 2.85 (2H, t, $J = 4.9$ Hz, CH_2-2).

2.3. Synthesis of 3-(2-hydroxyethoxy)-propionamides (*3a–6a*)

The primary amines (**3**–**6**, 0.1 mmol) and 1,5-dioxepane-2-one (**2**, 4 mmol) were added to a round bottom flask. The flask was immersed in an oil bath at 60 °C and the reaction was monitored by TLC. After consumption of the amine (typically 2 h), the products were isolated by preparative TLC.

3a: white oil; $R_f = 0.27$ (eluent AcOEt : MeOH 10 : 0.5); ^{1}H-NMR δ (ppm, $CDCl_3$): 7.30 (5H, m, aromatic protons); 4.49 (2H, d, $J = 5.7$ Hz, CH_2-Ph); 3.80 (2H, m, CH_2-b); 3.70 (2H, m, CH_2-c); 3.60 (2H, t, $J = 4.8$ Hz, CH_2-d); 2.52 (2H, t, $J = 5.6$ Hz, CH_2-a).

4a: white oil; $R_f = 0.23$ (eluent AcOEt: MeOH 10 : 0.5); ^{1}H-NMR δ (ppm, $CDCl_3$): 7.01 (2H, d, $J = 8.4$ Hz, ArH); 6.80 (2H, d, $J = 8.4$ Hz, ArH); 4.14 (2H, $J = 6.9$ Hz, CH_2–N); 3.75 (2H, t, $J = 5.6$ Hz, CH_2-b); 3.65 (2H, m CH_2-c); 3.59 (2H, t, $J = 4.8$ Hz, CH_2-d); 3.48 (2H, t, $J = 6.9$ Hz, CH_2–Ar); 2.60 (2H, t, $J = 5.9$ Hz, CH_2-a).

5a: white oil; $R_f = 0.40$ (eluent AcOEt : MeOH 95 : 5); ^{1}H-NMR δ (ppm, $CDCl_3$): 4.05 (2H, dd, $J_1 = 2.4$ Hz, $J_2 = 5.2$ Hz, CH_2–N); 3.75 (4H, m, CH_2-b, CH_2-c); 3.61 (2H, t, $J = 4.4$ Hz, CH_2-d); 2.50 (2H, t, $J = 5.6$ Hz, CH_2-a); 2.25 (1H, t, $J = 2.8$ Hz, C–H).

6a: yellow oil; $R_f = 0.50$ (eluent AcOEt : MeOH 10: 0.7); ^{1}H-NMR δ (ppm, $CDCl_3$): 7.45 (1H, s, H-8); 7.35 (1H, d, $J = 10.4$ Hz, H-11); 7.10 (1H, d, $J = 10.5$ Hz, H-12); 6.55 (1H, s, H-4); 4.70 (1H, m, H-7); 3.95 (3H, s, OCH_3); 3.90 (3H, s, OCH_3); 3.80–3.60 (6H, m, CH_2-b, CH_2-c, CH_2-d); 3.65 (3H, s, OCH_3); 2.70–2.20 (6H, m, CH_2-5, CH_2-6, CH_2-a); 2.52 (3H, s, SCH_3).

2.4. Polymerization of 1,5-dioxepane-2-one in the presence of primary amines as an initiator

In a typical polymerization reaction, DXO (melted in an oil bath at 35 °C), and the amine in a suitable ratio (see Table 1) were added with a syringe into a two necks round-bottom flask. The flask was heated at 60 °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 DXO)

Table 1
Incorporation of amines into poly DXO obtained by chemo-enzymatic ROP[a]

Amine	Yield[b] (%)	Inc.[c] (%)	DP[d]	M_n[e]	M_w[e]	PDI[e]
3	64	50	25	6450	9,650	1.50
4	85	90	35	6200	11,700	1.89
5	86	65	30	3900	6,100	1.56
6	90	80	32	8000	11,600	1.45

[a] Reaction conditions: monomer (DXO) to amine molar feed ratio, 40:1; Temperature, 60 °C.
[b] Estimated by the ratio between the mass of the isolated polymer and the mass of the starting monomer DXO.
[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).

and the molecular sieves (150 mg) were added, and the reaction was left overnight at 60 °C. After cooling to 40 °C, a small volume of $CHCl_3$ was added, the enzyme and the molecular sieves were filtrated. The solution was poured in cold methanol, and the amorphous polymer was recovered by centrifugation (isolated yields usually in the order of 80%, w/w added monomer). As reported in Table 1, different polymers carrying different amines were synthesized.

3b: colourless amorphous solid; ^{1}H-NMR δ (ppm, $CDCl_3$): polymer chain: 4.25 (t, $J = 4.9$ Hz, CH_2-d); 3.78 (t, $J = 6.4$ Hz, CH_2-b); 3.67 (t, $J = 4.9$ Hz, CH_2-c); 3.60 (t, $J = 4.9$ Hz, CH_2-e); 2.65 (t, $J = 6.4$ Hz, CH_2-a). Benzyl amine moiety: 7.30 (m, aromatic proton); 4.48 (d, $J = 7$ Hz, CH_2-Ph). SEC (THF) $M_n = 3900$, $M_w = 6100$, $d = 1.56$.

4b: colourless amorphous solid; ^{1}H-NMR δ (ppm, $CDCl_3$): polymer chain: 4.25 (t, $J = 4.4$ Hz, CH_2-d); 3.78 (t, $J = 6.4$ Hz, CH_2-b); 3.69 (t, $J = 4.8$ Hz, CH_2-c); 3.60 (2H, t, CH_2-e); 2.65 (t, $J = 6.4$ Hz, CH_2-a). Tyramine moiety: 7.05 (d, $J = 8.4$ Hz, H-3); 6.79 (d, $J = 8.2$ Hz, H-4); 4.12 (t, $J = 6.9$ Hz, CH_2–N); 3.50 (t, $J = 6.7$ Hz, CH_2–Ar). SEC (THF) $M_n = 6450$, $M_w = 9650$, $d = 1.50$.

5b: colourless amorphous solid; ^{1}H-NMR δ (ppm, $CDCl_3$): polymer chain: 4.24 (t, $J = 4.8$ Hz, CH_2-d); 3.77 (t, $J = 6.4$ Hz, CH_2-b); 3.68 (t, $J = 4.8$ Hz, CH_2-c); 3.60 (t, CH_2-e); 2.64 (t, $J = 6.4$ Hz, CH_2-a). Propargylamine moiety: 3.85 (dd, $J_1 = 2.5$ Hz, $J_2 = 5.1$ Hz, CH_2–N); 2.25 (t, $J = 2.5$ Hz, C–H). SEC (THF) $M_n = 8000$, $M_w = 11600$, $d = 1.45$.

6b: yellow amorphous solid; ^{1}H-NMR δ (ppm, $CDCl_3$): polymer chain: 4.25 (t, $J = 4.8$ Hz, CH_2-d); 3.77 (t, $J = 6.4$ Hz, CH_2-b); 3.67 (t, $J = 4.8$ Hz, CH_2-c); 3.59 (t, CH_2-e); 2.63 (t, $J = 6.4$ Hz, CH_2-a). Thiocolchicine moyety (selected data): 7.25 (d, $J = 10.6$ Hz, H-11); 7.24 (s, H-8); 7.05 (d, $J = 10.3$ Hz, H-12); 6.55 (s, H-4); 4.65 (m, H-7); 3.95 (s, OCH_3); 3.90 (s, OCH_3); 2.45 (s, SCH_3). SEC (THF) $M_n = 6200$, $M_w = 11700$, $d = 1.89$.

3. Results and discussion

Poly(1,5-dioxepane-2-one) (poly-DXO) is an amorphous polyester-polyether which has been proposed as a fast degrading component in block and random copolymers [10]. The synthesis of homo- and copolymers of DXO have been deeply investigated by Albertsson and coworkers, initially exploiting organometallic catalysis [11]. The need to remove all traces of metallic residues (a requirement for the proposed pharmaceutical and biomedical applications of these macromolecules) have made attractive the use of alternative biocatalyzed approaches, extensively explored by the same research group, and specifically the Novozym 435-catalyzed ring-opening polymerization [12]. With this literature information in hands, we thought the DXO could be an ideal monomeric substrate for an extension of our chemo-enzymatic approach to the synthesis of end-functionalized polyesters [7]. Four different primary amines were used as a substrate: the model benzyl amine (**3**), the functionalized tyramine (**4**) and propargylamine (**5**), and the bioactive compound *N*-deacetylthiocolchicine (**6**) (Fig. 1).

According to Scheme 1 (X = O), the intermediate hydroxyamides **3a**–**6a** were prepared by heating the corresponding

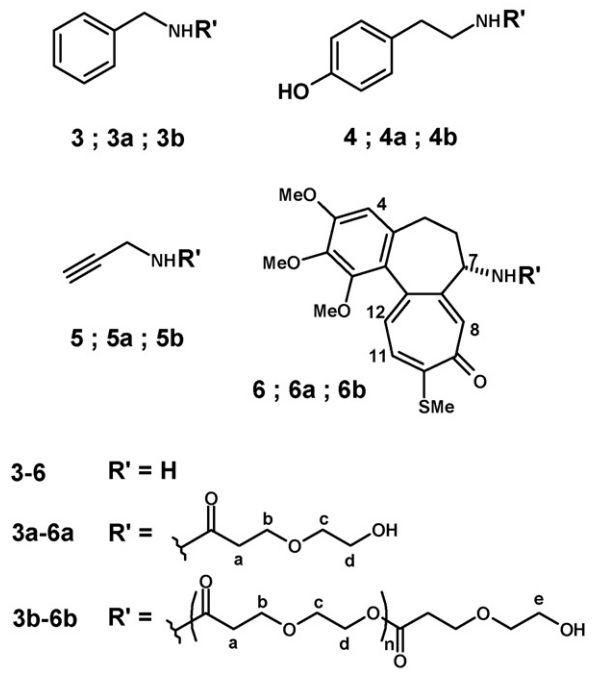

Fig. 1. Compounds: **3**–**6**, **3a**–**6a**, **3b**–**6b**.

amines **3**–**6** in the presence of an excess-typically 40 equiv. of DXO (2 h, 60 °C). In the complete chemo-enzymatic processes, compounds **3a**–**6a** were not isolated and, after 2 h of preliminary heating, the enzymatic ring opening polymerization was started by adding Novozym 435 to the reaction mixture. The reactions were left overnight at 60 °C, and the polyesters **3b**–**6b** were isolated and characterized. In comparison to ε-caprolactone which gave polyesters that were as semicrystalline solids [7], 1,5-dioexepane-2-one furnished amorphous materials.

As an example, Fig. 2 shows the ^{1}H-NMR spectrum of the polyesters mixture **6b**. The typical intense signals due the four CH_2 moieties (a–d) of the polymeric chains are clearly visible. In addition, other small peaks due to the presence of the aminic compound are recognizable: the aromatic protons (7.2–6.8 ppm), the sharp singlets due to two of the three OMe groups (at 3.95 and 3.90 ppm) and to the SMe (2.45 ppm), and the multiplet at 4.65 ppm due to H-7 that is downfield shifted (in comparison to signal resonating at 3.80 ppm in the starting compound **6**) and thus clearly demonstrates the incorporation of the thiocolchicine moiety in the polymer.

Additionally, the NMR spectrum allowed the determination of two other important parameters: the percentage of incorporation of the amine in the polyester and the average degree of polymerization (DP). The percentage of incorporation could be evaluated by comparing the integral value of one of the signals due to the amine "initiator" (one of the aromatic protons, or the multiplet at 4.65 ppm due to H-7, or one of the singlets at 3.95 and 3.90 ppm, due to the OMe moieties) with the one due to the terminal CH_2OH of the polymeric chain (resonating as a triplet at 3.60 ppm). The ratio between the very intense signals at 4.25 (a triplet due to the "internal" –CH_2OCO– esterified moieties) and at 3.60 ppm (the above described triplet due to the terminal

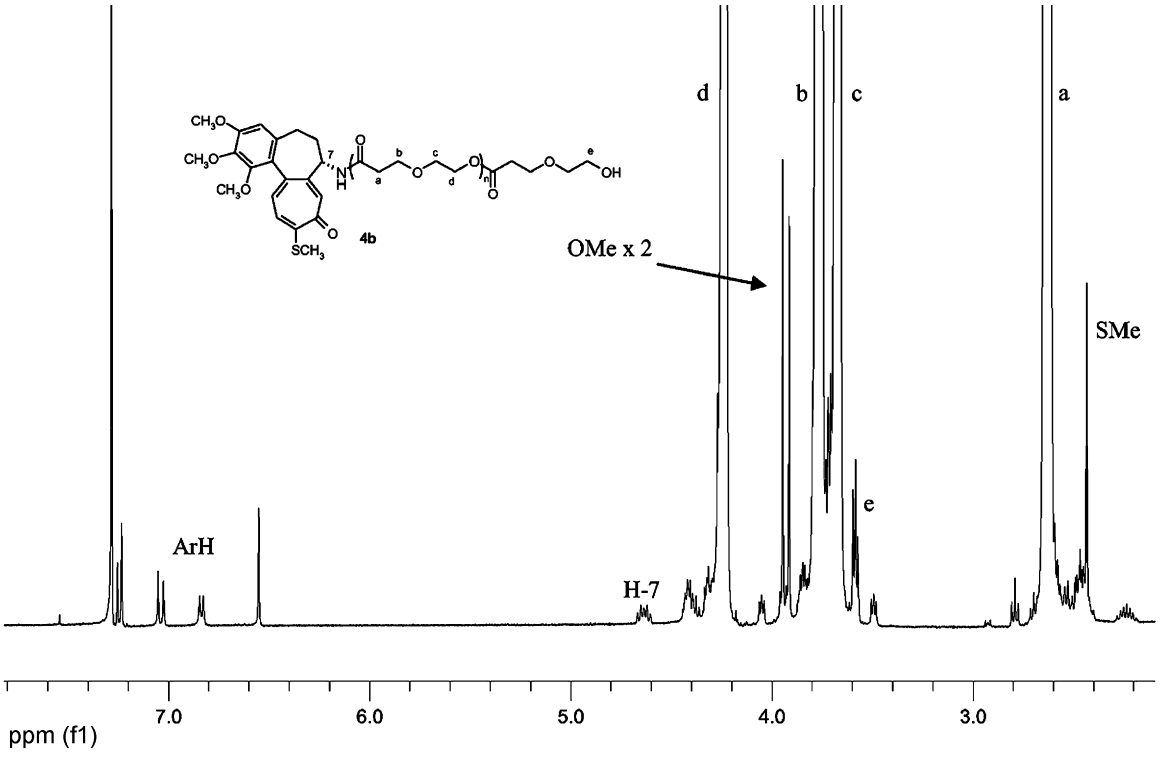

Fig. 2. ^{1}H NMR spectrum of the polyester **6b**.

CH_2OH) allowed also estimating a degree of polymerization (DP) value of 35.

Finally, the number average molecular weight (M_n) and the weight average molecular weight (M_w) of **6b** were determined by size exclusion chromatography, which allowed also calculating a polydispersity value (PDI) of 1.89.

Some of the relevant data of the polymeric products **3b**–**6b** are reported in Table 1.

4. Conclusions

We have shown that functionalized primary amines can be incorporated into a poly(1,5-dioxepane-2-one) polyester chain by a combination of chemical and enzymatic processes, which involve first the reaction of a primary amine with DXO to form the corresponding ω-hydroxy-amides, followed by the enzymatic ROP catalyzed by Novozym 435.

The reported data confirm the versatility of the suggested protocol for the preparation of terminal-functionalized polymers. Specifically, the functionalized polyesters **4b** and **5b** can be utilized for the preparation of different polymers architecture, exploiting either the radical polymerization of phenols [13] or the so-called "click chemistry" [14].

References

[1] S. Kobayashi, H. Uyama, S. Kimura, Chem. Rev. 101 (2001) 3793–3818.
[2] R.A. Gross, A. Kumar, B. Karla, Chem. Rev. 101 (2001) 2097–2124.
[3] D.-Y. Kim, X. Wu, J.S. Dordick, ACS Symp. Ser. 840 (2002) 34–49.
[4] R.K. Srivastava, A.C. Albertsson, Macromolecules 39 (2006) 46–54, and references therein.
[5] (a) S. Riva, J. Mol. Catal. B Enzym. 19–20 (2002) 43–54;
(b) G. Carrea, S. Riva, Angew Chem. Int. Ed. 39 (2000) 2226–2254.
[6] (a) A. Cordova, T. Iversen, K. Hult, Macromolecules 31 (1998) 1040–1045;
(b) K.S. Bisht, F. Deng, R.A. Gross, D.L. Kaplan, G. Swift, J. Am. Chem. Soc. 120 (1998) 1363–1367.
[7] M. Marzorati, K. Hult, S. Riva, B. Danieli, Adv. Synth. Catal. 349 (2007) 1963–1968.
[8] M. De Geus, L. Schormans, A.R.A. Palmans, C.E. Koning, A. Heise, J. Polym. Sci. A: Polym. Chem. 44 (2006) 4290–4297, and references therein.
[9] P. Kerekes, P.N. Sharma, A. Brossi, C.F. Cignell, F.R. Quinn, J. Med. Chem. 28 (1985) 1204–1208.
[10] (a) N. Andranova, R.K. Srivastava, A.C. Albertsson, Polymer 46 (2005) 6746–6755;
(b) U. Edlund, A.C. Albertsson, J. Polym. Sci. A: Polym. Chem. 37 (1999) 1877–1884.
[11] See, for instance:
(a) H. Von Schenck, M. Ryner, A.C. Albertsson, M. Svensson, Macromolecules 35 (2002) 1556–1562;
(b) M. Ryner, A. Valdre, A.C. Albertsson, J. Polym. Sci. A: Polym. Chem. 40 (2002) 2049–2054;
(c) A.C. Albertsson, M. Gruvegard, Polymer 36 (1995) 1009–1016;
(d) T. Mathisen, K. Masus, A.C. Albertsson, Macromolecules 22 (1989) 3842–3846.
[12] (a) R.K. Srivastava, K. Kumar, I.K. Varma, A.C. Albertsson, Eur. Polym. J. 43 (2007) 808–817;
(b) R.K. Srivastava, A.C. Albertsson, J. Polym. Sci. A: Polym. Chem. 43 (2005) 4206–4216.
[13] T. Fukuoka, H. Uyama, S. Kobayashi, Macromolecules 37 (2004) 8481–8484.
[14] (a) R. Riva, S. Schmeits, C. Jerome, R. Jerome, P. Lecomte, Macromolecules 40 (2007) 796–803;
(b) J.F. Lutz, Angew. Chem. Int. Ed. 46 (2007) 1018–1025.