Cumulated advantages of enzymatic and carbene chemistry for the non-organometallic synthesis of (co)polyesters†

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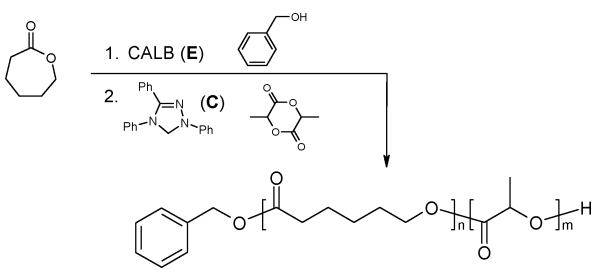
Enzymatic and carbene catalysed ring opening polymerisation can be combined in a one-pot reaction for the metal-free synthesis of degradable block copolymers.

Aliphatic polyesters such as polylactide (PLA) and polycaprolactone (PCL) have an increasing presence among biodegradable polymers in various medical applications.[1] In many cases, random or block copolymers are synthesised so as to advantageously modify the material properties. Commonly PCL and PLA and their (block) copolymers are produced by organometallic, *e.g.*, tin(II) 2-ethylhexanoate, catalysed ring-opening polymerisation (ROP) of the corresponding cyclic esters.[2] However, biomaterials are among the most sensitive materials with respect to product safety and purity. While tin(II) 2-ethylhexanoate is approved for biomedical grade polymers, there is increasing pressure on its use for medical-grade polymers due to some possible toxicity issues. The development of organometallic-free alternatives is thus of great interest in the biomedical and polymer communities.

Two promising metal-free alternatives are the enzymatic and carbene catalyzed ROP, respectively. It has been recently demonstrated that 1,3,4-triphenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-ylidene carbene catalyzes ROP of lactide (LA) in the presence of alcohol initiators.[3] These polymerisations proceed with first-order kinetics and exhibit a linear correlation between molecular weight and conversion and can yield polymers with low polydispersity indices (PDI) around 1.1. The exceptional control observed in this system is attributed to the reversible formation of a dormant alkoxyl triazoline, which keeps both the free carbene and the alcohol chain ends at low concentrations, thereby minimizing the rate of transesterification of the polymer chains. Unfortunately, the cited-carbene catalyzed polymerisation of caprolactone (CL) has not been successful yet. Lipases like immobilised *Candida Antarctica* lipase B (CALB), on the other hand, have proven high catalytic activity in the ROP of lactones of all ring sizes.[4] In contrary to carbenes, the CALB catalyzed ROP of L-lactides has so far been unsuccessful. While the reversible inhibition of the CALB by the lactide has been suggested[5] also the fact that ROP of the monomer produces a chiral secondary alcohol end-group largely prevents further propagation.

While both metal-free ROP's offer certain advantages, they seem to be highly complementary in the classes of monomers they can polymerise. CALB does polymerise lactones but not lactides. Carbenes, on the other hand, are highly active catalysts for the polymerisation of lactides but not for lactones. This prompted us to investigate whether the chemoenzymatic combination of both catalysts is possible in order to overcome the individual limitations and develop a completely metal-free ROP to biomedically relevant degradable aliphatic polyesters (Scheme 1). While chemoenzymatic polymerisations using CALB and controlled polymerisations have been reported, those either involve an organometallic metal catalyst or produce non-degradable block copolymers.[6]



Scheme 1

Table 1 Results of LA and CL polymerisations initiated from benzyl alcohol using enzymatic (**E**) and/or carbene (**C**) catalysis. Polymerisations performed for a monomer concentration of 4 M for 6 hours at 90 °C at a total monomer-to-initiator ration of 50 : 1

Entry	Cat.	Monomer	M_n/g mol^{-1} [a]	PDI[a]	DP_{CL}[b]	DP_{LA}[b]
1	E	CL	10 400	1.8	30	—
2	E	LA	—[c]	—	—	—
3	C	CL	—[c]	—	—	—
4	C	LA	7700	1.1	—	26
5	E	CL, LA	—[c]	—	—	—
6	C	CL, LA	—[c]	—	—	—
7	E, C	CL	—[c]	—	—	—
8	E, C	LA	7200	1.3	—	27

[a] Determined by SEC in THF with polystyrene standards. [b] Determined by ^{1}H NMR analysis: $DP_{CL} = [I_{4.1}/I_{3.65}]$; $DP_{LA} = [I_{5.1}/(2 \times I_{4.35})]$. [c] No polymerisation took place.

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The combined enzymatic–carbene polymerisation was first addressed in a number of control experiments. All reactions were performed under identical reaction conditions of concentration, polymerisation time, temperature and monomer-to-initiator ratio (Table 1). In the first experiment, CL was polymerised by the immobilised CALB enzyme (**E**) from benzyl alcohol at 90 °C for 6 hours yielding PCL with a molecular weight of 10 400 g mol^{-1} (Table 1, entry 1). The PDI of 1.8 is in the typical range for enzymatic ROP due to the inevitable transesterification reactions. As anticipated, when CL was replaced by LA no polymerisation reaction was observed (Table 1, entry 2), which is in agreement with the state of the art. The same experiments were then reinitiated replacing the lipase by the 1,3,4-triphenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-ylidene carbene catalyst (**C**) keeping all other conditions unchanged (Scheme 1). As expected, LA was polymerised to a high conversion (~80%) and a molecular weight of 7700 g mol^{-1} with a perfect end-group fidelity and a low PDI (1.1) according to the controlled character of this polymerisation (Table 1, entry 4). On the other hand, the CL polymerisation did not yield any polymer from the carbene catalyst under the same conditions (Table 1, entry 3). As suspected, the CL polymerisation from **C** did not proceed due to the sterically encumbered structure of the triazole-based carbene catalyst confirming the inactivity of the imidazolin-2-ylidene towards CL polymerisation. These reactions show the distinct activities of the two catalysts regarding both monomers. The next experiments were designed to find out the apparent complementarities and apply those towards the synthesis of CL/LA block copolymers.

In order to avoid intermediate work-up procedures, it was the aim to conduct both polymerisations consecutively in one pot, which requires a detailed understanding of the mutual interactions of both techniques. Copolymerisation experiments were thus performed to test the compatibility of both techniques with respect to monomers and catalysts. CALB was first used to initiate the CL/LA copolymerisation from benzyl alcohol. After 6 hours of reaction, no polymerisation was observed (Table 1, entry 5). This result is in agreement with that reported by Gross *et al.* who suggested that LA acts as a reversible inhibitor of CALB during its copolymerisation with CL.[5] Moreover, the secondary hydroxy end-group of a ring-opened lactide is a sterically hindered nucleophile thus leading to a highly reduced reaction kinetic.

Surprisingly, the carbene catalyzed polymerisation of LA did not occur in the presence of CL (Table 1, entry 6). To our knowledge, this phenomenon has not been reported yet and the latent mechanism is still under investigation. The reactions corresponding to entries 7 and 8 in Table 1 were designed to investigate the catalyst compatibility. Therefore, the two catalysts were mixed with one of the monomers and a polymerisation initiated. In these experiments the mixture of CL, lipase and carbene did not yield any polymer (Table 1, entry 7). This result suggests that the enzymatic ROP of CL is inhibited in the presence of the carbene. The exact mechanism of inhibition, *e.g.*, blocking of the active site of the enzyme, or its reversibility, is yet unknown. In contrast, no inhibition was observed when the carbene catalysed-polymerisation of LA was performed in the presence of the enzyme (Table 1, entry 8), which confirms that CALB has no influence on the catalytic activity of the carbene. In summary, poor compatibility for the system was observed for the enzymatic ROP in the presence of CL and carbene, while for the carbene-catalysed ROP a high acceptance for Novozym 435 and LA was shown when polymerisations were performed in toluene at 90 °C.

The results of the compatibility tests presented above have to be considered when designing a "one-pot" reaction. With the mutual inhibition described, difficulties were expected in making the copolymer by simply mixing two monomers and two catalysts in "one-pot" at elevated temperature. A successful "one-pot" block copolymer synthesis must therefore be divided into two steps in which each polymerisation step is triggered individually. At temperatures well below 90 °C the triazolinium carbene forms a stable adduct with primary alcohols such as the benzyl alcohol used in our synthesis.[3] We rationalised that this could bind the carbene so as to prevent inhibition of the enzyme. While enzymatic polymerisation is still possible at low temperatures (water initiated),[7] the carbene catalysed ROP of LA should be prevented and only initiated when the temperature is raised above 90 °C. We therefore attempted to vary the reaction temperature so as to allow first the enzymatic polymerisation of CL at lower temperatures (60 °C and 30 °C, respectively) from a mixture containing all reaction components and subsequently initiate the carbene-catalyzed polymerisation of LA by increasing the temperature (Table 2, entries 1 and 2). In both cases only the formation of PLA homopolymer was observed, which suggests the inhibition of the enzyme due to blocking of the active site possibly by LA.[5] Then, and as expected, by increasing the temperature to 90 °C, the carbene is thermally generated and promotes the LA polymerisation. Even though the control over the LA ROP appears somehow less efficient leading to higher PDI in this reaction, the polymerisation occurs effectively.

Due to the incompatibility of the enzymatic reaction with both, the LA and the carbene, the synthesis of block copolymers must be achieved by sequential addition of reactants (Table 2, entry 3). Since carbene polymerisation of LA is more controlled than enzymatic ROP, carbene macroinitiation will result in a higher block copolymer yield. Thus, the enzymatic reaction has to be performed first. This was realised by first polymerising CL in the presence of CALB at 60 °C for 6 h. During this time a quantitative CL conversion was achieved. Subsequently, the carbene, together with the LA, was added to the reaction flask. Further polymerisation at 90 °C for 6 h yielded the expected block copolymer (Table 2, entry 3). An advantage of this

Table 2 Results of the chemoenzymatic "one-pot" reaction of LA and CL using enzymatic (**E**) and/or carbene (**C**) catalysts

	Conditions					
Entry	Step 1	Step 2	M_n/ g mol^{-1} [a]	PDI[a]	DP_{CL}[b]	DP_{LA}[b]
1	C, E, CL, LA (60 °C, 2 h)	(90 °C, 6 h)	5500	1.5	—	25
2	C, E, CL, LA (30 °C, 24 h)	(60 °C, 24 h)	5000	1.4	—	21
3	E, CL (60 °C, 6 h)	C, LA (90 °C, 6 h)	10 500	1.5	29	22

[a] Determined by SEC in THF with polystyrene standards. [b] Determined by ^{1}H-NMR analysis: $DP_{CL} = [I_{4.1}/(I_{3.65} + 2I_{4.35})]$; $DP_{LA} = [I_{5.1}/(I_{3.65} + 2I_{4.35})]$.

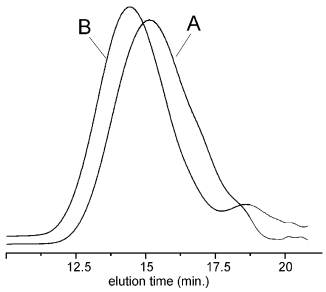

Fig. 1 GPC traces of ''one-pot'' carbene–enzyme catalyzed block copolymer synthesis (Table 2, entry 3): (A) PCL obtained after step 1 and (B) P(CL-*b*-LA).

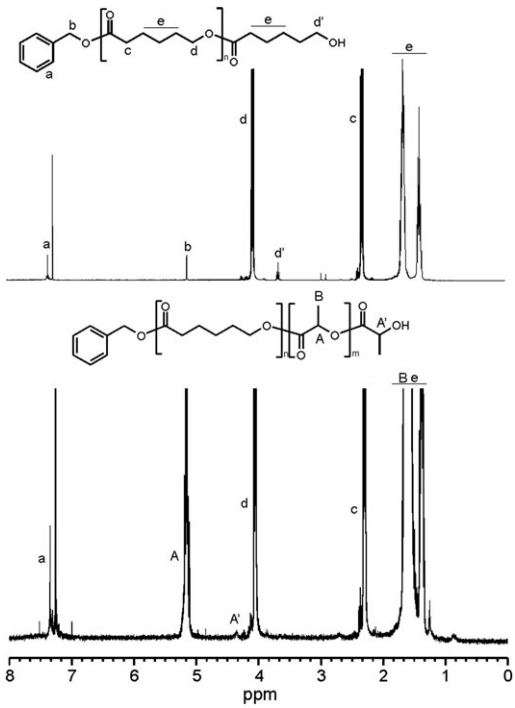

Fig. 2 ^{1}H NMR spectra of ''one-pot'' carbene–enzyme catalysed block copolymer synthesis (Table 2, entry 3): (top) PCL obtained after step 1 and (bottom) P(CL-*b*-LA).

procedure is that deactivation of the enzyme prior to the second reaction step is unnecessary because the carbene itself acts as an internal inhibitor for the enzyme. This prevents enzyme-catalyzed transesterification reactions, which could lead to a randomisation of the block copolymer.

After the enzymatic polymerisation of CL a polymer with a molecular weight of 5300 g mol^{-1} was obtained. GPC analysis revealed a clear molecular weight shift to 10 500 g mol^{-1} after addition of the carbene and LA comonomer (Fig. 1). This provides first evidence that the macroinitiation took place. Further proof could be found in the comparison of the ^{1}H NMR spectra of the block copolymer and of a sample taken before the addition of carbene/LA. In particular the disappearance of the caproic ω-hydroxymethylene end-group initially present at 3.65 ppm (Fig. 2, d′) in the final product confirms that the hydroxyl end-groups of PCL were completely converted by initiating the polymerisation of LA.

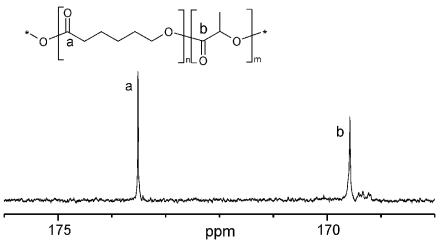

Fig. 3 ^{13}C NMR spectrum of the carbonyl region of P(CL-*b*-LA) obtained from one-pot enzyme–carbene ROP.

Furthermore, in the spectrum of the block copolymer a signal at 4.35 ppm could be detected and was assigned to the ω-hydroxy end-group of the PLA block (Fig. 2, A′). Evidence for the block structure was obtained from ^{13}C NMR analysis. Fig. 3 shows the significant region of the relevant carbonyl signals. Only two peaks at 169.5 ppm and 173.5 ppm, respectively, are observed. This confirms that only two species of ester bonds are present in the polymer, namely LA-LA and CL-CL.[8] Random copolymers would have shown a plethora of additional peaks in between the predicted carbon signals due to the formation of different triads based on caproyl and lactoyl units (LA-LA-CL, CL-LA-CL, *etc*...).

Finally, this approach was extended to the synthesis of block copolymers with various degrees of polymerisation of CL and LA (CL/LA of 15/35, 25/25 and 35/15). In agreement with the achievable control over the molecular parameters, DP values of 13/38, 26/25 and 37/13, were obtained as calculated from ^{1}H NMR spectra (see ESI†).

In conclusion, the first example of an organometallic free combination of carbene and enzymatic ROP has been shown. A ''one-pot'' two-step reaction allowed the synthesis of defined block copolymers from LA and CL.

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