Synthesis of Novel Poly(ϵ-caprolactone)s Functionalized with a Thioester End-Group via a Living Ring Opening Polymerization and Their Application in Chemoselective Ligation with Compounds Containing a Cysteine Terminal

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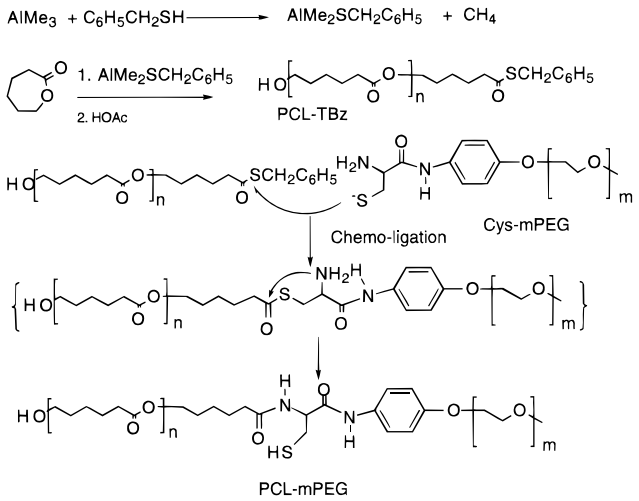
In this paper, we report a facile method for the introduction of a thioester end-group to poly(ϵ-caprolactone) (PCL) via living polymerization and results on the reactivity of this functional group in chemoselective ligation processes. The aliphatic polyesters, such as PCL, polylactide (PLA), and polyglycolide (PGA), and their copolymers are of great interest for applications in biological and biomedical areas due to their desirable properties of biodegradability, biocompatibility, and permeability.[1] Their incorporation into other biocompatible polymeric materials or bioactive species (peptides, enzymes, etc.) may lead to novel biomaterials. In fact, novel drug delivery systems[2] and potential prodrugs[3] have been developed, and in this respect, their functionalized derivatives are truly valuable intermediates, as in the case of poly(ethylene glycol) (PEG) chemistry.[4]

Ring opening polymerization (ROP) of lactones and related compounds has been the major polymerization approach to the syntheses of these polyesters. The synthesis of well-defined aliphatic polyesters with precisely controlled end-group functionalities has been pursued extensively.[5] Living polymerization provides a particularly powerful tool,[6] either through functionalized initiators or by selective transformation of living polymer chain ends. For example, dialkylaluminum alkoxides (R_2AlOR') initiate polymerization of lactones in a living fashion, leading to the formation of polyesters with a hydroxy functional end and an alkyl carboxylate (−COOR′) end-group from the initiator. However, the ester end-groups in these aliphatic polyesters are difficult to further manipulate without affecting the polymer backbones. To utilize this terminal functionality in further chemical reactions, more reactive functional groups are needed. A thioester group is one of such functionalities, which is a moderately activated carboxy derivative.

Our interest in introducing thioester functional group into PCL is motivated by the following considerations. First, thioesters have many synthetic applications,[7] and recent applications of the chemoselective ligation between thioester and *N*-cysteine in peptide synthesis make it a particularly interesting functional group.[8] Novel biomaterials such as PCL−peptide conjugates may be synthesized through this approach. Second, dimethylaluminum benzylthiolate, which is known to be very effective in the preparation of thioesters from lactones or esters,[9] is an analogue of R_2AlOR' and soluble in common organic solvents such as toluene. Thus, it is reasonably expected that the ROP of ϵ-CL (caprolactone = CL) with R_2AlSR' will also show a living character since after the initiation, the propagation center is the same as that when AlR_2OR' is used as the initiator. As a result, synthesis of well-defined PCL and its copolymers with thioester ends could be readily achieved. Last but not least, further modifications of these heterobifunctional PCLs (i.e., one thioester end and one hydroxy end) will make them useful candidates in a variety of applications.

Scheme 1. Synthesis of Diblock Copolymers via Chemoselective Ligation



The initiator**,** dimethylaluminum benzylthiolate, was prepared by reaction of benzyl mercaptan with trimethylaluminum as described by Corey et al. (Scheme 1).[9] Polymerizations of ϵ-CL under different reaction conditions have been systematically conducted. It was found that the molecular weight distributions (MWDs) of polyesters are quite narrow (1.09−1.34). A linear relationship between the number-average molecular weight (M_n) at total monomer conversion and the monomer-to-initiator ratio (M/I) exists, implying the living character of the polymerization process. The living nature of the polymerization was further confirmed from the polymerization resumption experiment. In that experiment, more ϵ-CL monomer was added after the polymerization of the first addition had gone to completion. Figure 1a shows that the molecular weight increased for the final polymer (peak B, M_n = 20400, MWD = 1.21), relative to the first peak (peak A, M_n = 7800, MWD = 1.13), and no residual peak was detected. Since we are more concerned with the polymer chain ends, ^{1}H NMR studies on this system were carried out in detail. As a typical example, shown in Figure 2, the ^{1}H NMR spectrum of PCL-20 (the number 20 indicates the designed

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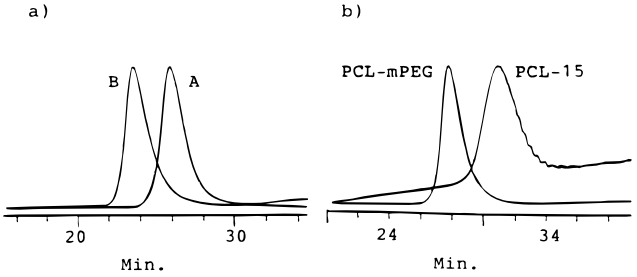

Figure 1. (a) GPC profiles of polymerization resumption experiment: (peak A) PCL after first addition of ϵ-CL, $M/I = 60$; (peak B) PCL after second addition of ϵ-CL, $M/I = 150$. (b) GPC profiles of PCL-15 and its ligation product PCL-mPEG.

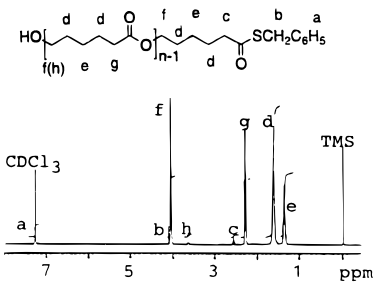

Figure 2. ^{1}H NMR spectrum of PCL-20 in $CDCl_3$.

M/*I*) gave an intensity ratio (1.02:1.00) between H_c ($-CH_2-$ from ϵ-CL at the thioester chain end) and H_h ($-CH_2-$ from ϵ-CL at the hydroxy end) close to one, which is in agreement with our expectation that the polymer chain should be capped with one thioester and one hydroxy end, respectively. Moreover, further study on the initiator in $CDCl_3$ has shown that during the initiation step, all of the initiators were reactive, and only acyl−oxygen cleavage was involved. All of these results allow us to conclude that this initiator is effective not only for the ROP of ϵ-CL but for the quantitative introduction of the thioester end-group as well. On the other hand, these results also suggest that this initiator might be suitable for the synthesis of pure block copolymers such as PCL-*b*-PLA. Consequently, the synthesis of PCL-*b*-PLA with dimethylaluminum benzylthiolate was carried out. Both GPC and ^{13}C NMR characterization of the resulting polymer support our prediction, and PCL-*b*-PLA with a narrow molecular weight distribution (MWD = 1.20) was obtained.

The reactivity of the thioester end-group is essential for the purpose of the synthesis of novel PCL conjugates via the chemoselective ligation approach. To test the applicability, we first chose L-cysteine ethyl ester hydrochloride as a small model compound to react with the benzylthioester-functionalized PCL (PCL-TBz). The reaction went smoothly under appropriate reaction conditions (e.g., THF/H_2O, 4:1 v/v, 18-crown-6, and pH ca. 8). The ^{1}H NMR spectra of the resulting products showed complete disappearance of the 2.58 ppm triplet ($-CH_2-$ from ϵ-CL at the PCL thioester end); instead, a new signal characteristic of the formation of an amide bond ($-NH-$) appeared at 6.47 ppm. To exclude the interference caused by hydrolysis of the thioester end or the ester backbone of PCL, PCL-TBz was also treated under the same conditions without adding L-cysteine ethyl ester hydrochloride as a control. According to the ^{1}H NMR and GPC results, there were no detectable changes from the PCL-TBz samples before and after the treatment. Therefore, the thioester at PCL-TBz chain end is indeed reactive toward *N*-cysteine group.

Furthermore, *N*-Cys-terminated poly(ethylene glycol) methyl ether (Cys-mPEG) has been synthesized for the studies of chemoselective ligation. The reasons for choosing this system are manifold: PEG is water soluble and the coupling conditions developed should resemble those of polypeptides, the results from these reactions can thus be readily generalized; its coupling product may be interesting for drug delivery systems. Detailed studies on the chemoselective ligation using PCL-TBz and Cys-mPEG have been carried out by systematically changing solvents, reagents, and reaction temperature as well (Scheme 1). The preliminary results from our experiments are very encouraging. High coupling efficiencies (>80%) with regard to the benzyl thioester were observed under several different reaction conditions. For example, under room temperature, using CH_3CN/THF as mixing solvent and $KHCO_3/K_2CO_3$/18-Crown-6 as reagents, the coupling went to completion within 18 h. The GPC profile and ^{1}H NMR spectrum of the ligation product are shown in Figure 1b and Figure 3, respectively. The signal appeared at 6.63 ppm unambiguously indicates the formation of a new amide linkage. Although the complete disappearance of the 2.58 ppm triplet, which is due to the loss of thioester end, does not ensure a quantitative coupling, the results from its GPC profile (i.e., no residual peak and no MWD broadening) excluded the presence of any detectable side reactions under these conditions. Combined with the observation that there was virtually no reaction using ethanolamine instead of Cys-mPEG under the same reaction conditions, the coupling reaction is believed to proceed in a similar fashion as that in peptide synthesis (Scheme 1).

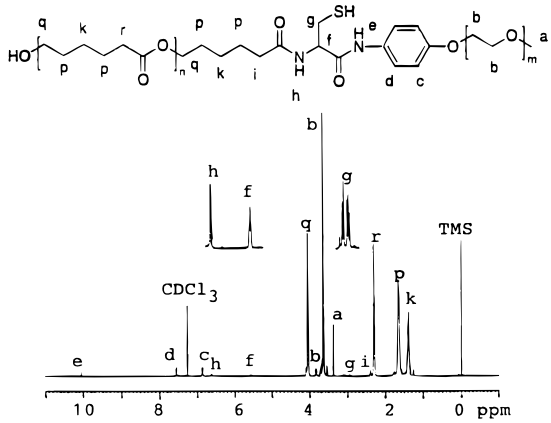

Figure 3. ^{1}H NMR spectrum of PCL-mPEG in $CDCl_3$.

In conclusion, a facile method for the introduction of a thioester end-group in the ROP of ϵ-CL using dimethylaluminum benzylthiolate as an initiator has been developed. The living character shown in the polymerization process has enabled us to synthesize PCL-*b*-PLA block copolymer in a controlled way. More importantly, the chemoselective ligation approach has been demonstrated to be applicable to thioester functionalized PCL. This may provide a new approach for the design and synthesis of novel PCL conjugates such as PCL−peptide conjugates. Further studies in this regard are currently underway in our laboratory.

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Supporting Information Available: Experimental details (8 pages). See any current masthead page for ordering information and Web access instructions.

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