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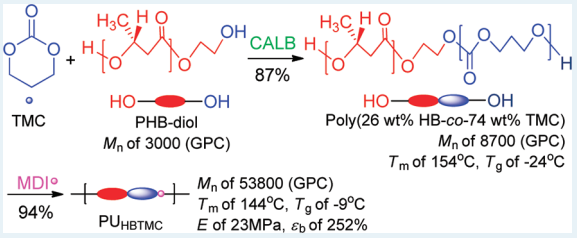
Enzymatic Ring-Opening Polymerization of Trimethylene Carbonate with Macrodiol: Synthesis of Block Poly(ester-*co*-carbonate) for Biomaterial Preparation

Shiyao Dai, Liang Xue, and Zhi Li*

Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576

Supporting Information

ABSTRACT: Novozym 435-catalyzed ring-opening polymerization (ROP) of trimethylene carbonate (TMC) with telechelic hydroxylated poly[(*R*)-3-hydroxybutyrate] [PHB-diol; M_n = 3000 g/mol (GPC)] as initiator gave di-block poly(HB-*co*-TMC) with different weight percents of the blocks and M_n of 4400−8700 g/mol (GPC) in 54−89% yield, being the first enzymatic preparation of block poly(ester-*co*-carbonate). The generality of the novel enzymatic method was demonstrated by the enzymatic ROP of TMC with poly(ε-caprolactone)-diol [PCL-diol; M_n of 4200 g/mol (GPC)] to give A−B−A tri-*block*-poly(TMC-*co*-CL-*co*-TMC) with different weight percents of the blocks and M_n of 7700−10600 g/mol (GPC) in 54−67% yield. The prepared block poly(ester-*co*-carbonate) with two terminal hydroxyl groups was proven to be a useful starting material for the further preparation of thermoplastic block copolymers. Polymerization of di-block poly(24 wt % HB-*co*-76 wt % TMC) with methylene diphenyl-4,4′-diisocyanate (MDI) afforded the corresponding polyurethane with M_n of 53 800 g/mol (GPC) in 94% yield. The polymer showed excellent thermoplastic properties (T_m of 144 and 152 °C, T_g of −9 °C, ε_b of 252%, σ_{max} of 6.37 MPa, and E of 23 MPa), being potentially useful for soft tissue engineering.



KEYWORDS: enzyme catalysis, ring-opening polymerization, block poly(ester-*co*-carbonate), biomaterials, poly[(*R*)-3-hydroxybutyrate], trimethylene carbonate

1. INTRODUCTION

Enzymatic polymerization is highly selective and non-toxic, thus receiving increasing attention for the synthesis of biomaterials with novel structures, desired properties, and nontoxicity. A well-known enzymatic polymerization is the lipase-catalyzed ring-opening polymerization (ROP), which has become a useful tool for the synthesis of polyesters and polycarbonates.[1−4] In such an enzyme-catalyzed ROP, a lactone or cyclic carbonate is opened by a lipase to give an acyl-enzyme intermediate, which is then reacted with the OH group of an initiator to give an ester or carbonate containing a terminal OH group; further reaction of this molecule with the acyl-enzyme intermediate leads to the elongation of the chain. Many enzymes, such as Porcine pancreatic lipase, *Pseudomonas* sp. lipase, and *Candida antarctica* lipase B (CALB), have been reported as good catalysts for ROP.[3] Enzymatic ROP of trimethylene carbonate (TMC) with water and lactone are known to give the corresponding poly(trimethylene carbonate) (PTMC)[5−9] and random poly(ester-*co*-carbonate),[10−12] respectively. PTMC[13−15] is a biodegradable and biocompatible material, with a Young's modulus (E) of 3−7 MPa, elongation at break (ε_b) of 1000%, and glass transition temperature (T_g) of −25 °C.[16−18] The soft and elastic properties of PTMC make it an attractive soft segment for the preparation of block *co*-polymers with designed thermoplastic properties. Therefore, a synthetic method for such a type of block *co*-polymers is highly desirable. We are interested in developing a novel and general enzymatic method to prepare block poly(ester-*co*-carbonate) containing PTMC soft blocks via enzymatic ROP of TMC with a low molecular weight polyester macrodiol to achieve desired thermal and mechanical properties for biomedical application.

PHB is a well-known biodegradable and biocompatible microbial polyester, produced in large amounts[19] and applied in several environmental and biomedical fields.[20−25] However, PHB is a hard and brittle material[26−29] and thus cannot be directly used as a thermoplastic material. Nevertheless, PHB can be used as a hard segment, together with an appropriate soft segment, to prepare block *co*-polymers with improved thermoplastic properties. For instance, block *co*-polyesterurethanes containing PHB hard block and poly(ε-caprolactone) (PCL)[30] or microbial poly[(*R*)-3-hydroxyoctanoate] (PHO)[31] soft block are useful thermoplastic materials for manufacturing implants and medical devices. Since PTMC is less stiff and more elastic than PCL (E = 216 MPa, ε_b = 746%, T_m = 60 °C, and T_g = −65 °C)[32] and PHO (soft, sticky material; T_m = 61 °C, T_g = −30 °C),[33−37] we want to explore and evaluate the incorporation of PTMC block with PHB block to achieve the desired thermoplastic and mechanical properties for soft tissue engineering.[38−40] The enzymatic

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ROP is an ideal tool for the controlled synthesis and setting of PTMC and PHB blocks to prepare the desired polymers, with the possibility of easily adjusting the polymer properties.

Here, we report the enzymatic syntheses of di-block poly(HB-*co*-TMC) via ROP of TMC with telechelic PHB-diol[41,42] as a novel and useful method for modifying PHB, the enzymatic syntheses of A−B−A tri-block poly(TMC-*co*-CL-*co*-TMC) via ROP of TMC with PCL-diol to demonstrate the generality of the novel method for preparing the block poly(ester-*co*-carbonate) containing PTMC soft block, and the further polymerization of di-block poly(HB-*co*-TMC) with methylene diphenyl 4,4′-diisocyanate (MDI) to prepare the corresponding polyurethane with good physical and mechanical properties as thermoplastic soft biomaterials.

2. EXPERIMENTAL SECTION

Materials. Novozym 435 (immobilized *Candida antarctica* lipase B, 10 000 PLU/g) was purchased from Novozymes, Denmark. Trimethylene carbonate (TMC) (99%) was purchased from Boehringer Ingelheim. ε-Caprolactone(99%), 1,4-dioxane (99.8%), toluene (99.8%), methylene diphenyl 4,4′-diisocyanate (MDI) (98%), and *N*,*N*-dimethylformamide (DMF, 99%) were purchased from Aldrich. Ethylene glycol (99%) was obtained from Merck. Chloroform (HPLC, 99.9%) and methanol (HPLC, 99.9%) were obtained from Tedia. Telechelic hydroxylated poly-[(*R*)-3-hydroxybutrate] (PHB-diol, M_n of 3000 g/mol, GPC) was prepared according to the published procedures.[41,42] Novozym 435 and PHB-diol were dried in a vacuum oven at 40 °C for 12 h, 1,4-dioxane and toluene were dried by refluxing over sodium/benzophenone under argon, and DMF was dried with CaH_2 for 24 h and freshly distilled before use.

Enzymatic Ring-Opening Polymerization of TMC with PHB-Diol To Synthesize Di-*block*-poly[HB (26 wt %)-*co*-TMC (74 wt %)]. To a predried mixture of PHB-diol [66 mg; M_n = 2200 (NMR) and 3000 (GPC) g/mol], TMC (305 mg), and Novozym 435 (72 mg) in a Schlenk tube containing a magnetic stirring bar was added under argon atmosphere freshly distilled toluene (1.2 mL). The polymerization was performed at 50 °C for 8 h, and the reaction was terminated by the addition of chloroform (6 mL). Novozym 435 was then removed by filtration using filter paper, and the filtrate was subjected to evaporation under reduced pressure to remove toluene and chloroform. The residue was dissolved in chloroform (2 mL) and treated with methanol (18 mL), and the product was precipitated at 4 °C for 30 min. After filtration, the precipitates were dried under vacuum in a rotary evaporator and then in a vacuum oven at 40 °C for 24 h. This gave 323 mg (87% yield) of poly(HB-*co*-TMC) with a M_n of 8700 g/mol (GPC), a weight ratio of PHB/PTMC of 26/74, T_m of 154 °C, and T_g of −24 °C.

Enzymatic Ring-Opening Polymerization of TMC with PCL-Diol To Synthesize Tri-*block*-poly[TMC-*co*-CL-*co*-TMC] Containing 29 wt % PCL and 71 wt % PTMC. A mixture of Novozym 435 (400 mg), freshly distilled ε-caprolactone (20 g), and ethylene glycol (1.0 mL) in a dry Schlenk tube were stirred under argon atmosphere at 70 °C for 8 h. After reaction, the mixture was treated with chloroform (8 mL) and stirred at room temperature for 30 min. The Novozym 435 was filtered out, and chloroform was removed by evaporation under reduced pressure. The product was dissolved in chloroform/methanol (20 mL/180 mL) and precipitated at 4 °C for 30 min. The final product was collected by filtration and then dried in a vacuum oven at 40 °C for 24 h, which gave 16.86 g (84% yield) of PCL-diol with M_n = 4200 g/mol (GPC), T_m = 46 °C, and T_g = −58 °C.

To a mixture of Novozym 435 (97 mg), PCL-diol prepared above (62 mg), and TMC (402 mg) in a Schlenk tube was added freshly distilled toluene (1.6 mL) under argon atmosphere, and the reaction was performed at 50 °C for 8 h. Workup with the same procedure as that for the synthesis of poly(HB-*co*-TMC) gave 296 mg (64% yield) of poly(TMC-*co*-CL-*co*-TMC) with M_n = 10 600 g/mol (GPC), a weight ratio of PCL/PTMC of 29/71, and T_g = −42 °C.

Synthesis of *block*-*co*-poly(ester-carbonate-urethane) PU_{HBTMC} by Polymerization of Poly[Hb(26 wt %)-*co*-TMC(74 wt %)] with MDI. To a solution of poly[HB(26 wt %)-*co*-TMC(74 wt %)] (1997 mg) in dry DMF (10 mL) in a Schlenk tube containing a magnetic stirring bar was added a solution of MDI (104 mg) in DMF (5 mL) dropwise under argon atmosphere. The mixture was stirred at 85 °C for 12 h. After cooling, methanol (60 mL) was added, and the mixture was magnetically stirred for 30 min at room temperature. The product was precipitated, collected by filtration, washed with a mixture of DMF/methanol (15 mL/60 mL), and dried in a vacuum oven at 50 °C for 24 h. A 1982 mg yield of PU_{HBTMC} (94% yield) was obtained, with M_n = 53800 g/mol (GPC), T_m = 144 °C, T_g of −9 °C, E = 23 MPa, and ε_b = 252%.

Synthesis of Block *co*-poly(ester-carbonate-urethane) PU_{CLTMC} by Polymerization of Poly[TMC-*co*-CL-*co*-TMC] Containing 38 wt % PCL and 62 wt % PTMC with MDI. Similar to the procedure for the preparation of PU_{HBTMC}, poly[TMC-(62 wt %)-*co*-CL(38 wt %)] [M_n = 7600 g/mol (GPC); 2011 mg] and MDI (64 mg) in DMF (15 mL) were reacted at 85 °C for 12 h, followed by the workup and precipitation. 1892 mg yield of PU_{CLTMC} (91% yield) was obtained, with M_n = 41500 g/mol (GPC), E = 19 MPa, and ε_b = 62.35%.

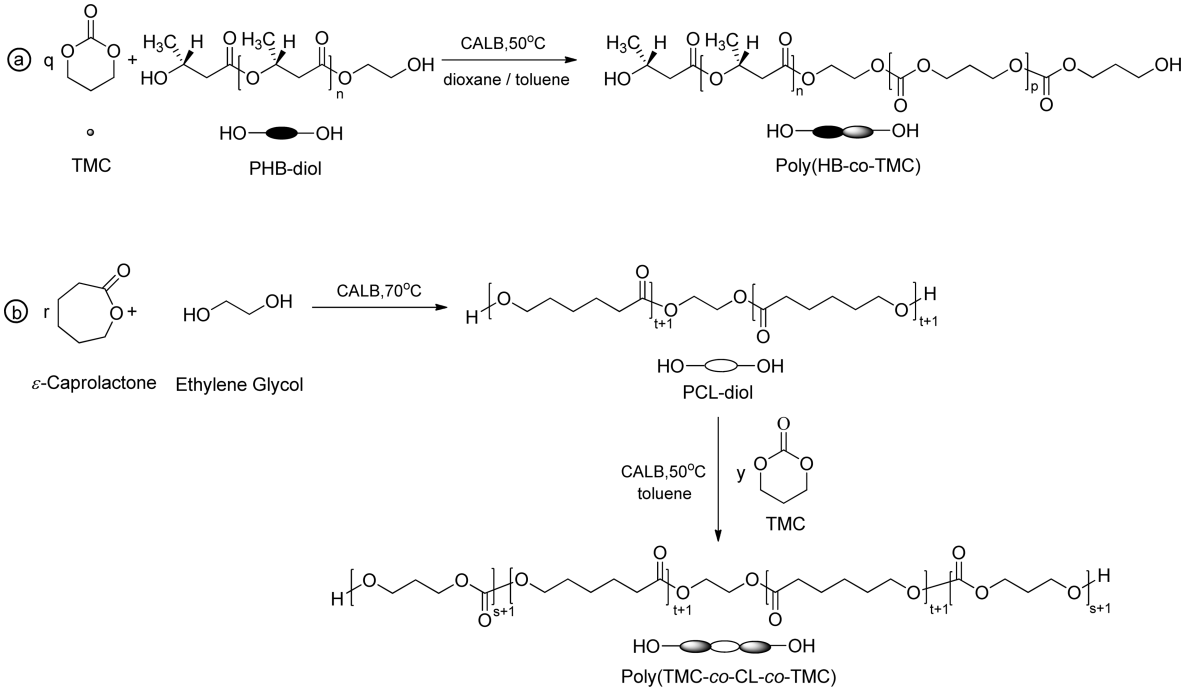
Gel Permeation Chromatography (GPC). Molecular weight analysis (M_n and polydispersity index M_w/M_n) was performed by using a Waters instrument, with a Waters 510 pump; Waters 410 refractive index detector; and Waters HR4E, HR5E, and HR6 columns placed in series. THF was used as the eluent for the measurement of PHB-diol and poly(HB-*co*-TMC)s at a flow rate of 1.0 mL/min and at 30 °C. DMF was used as the eluent for the analysis of polyurethanes at a flow rate of 1.0 mL/min and at 35 °C. The sample concentration was ∼0.1% (w/v), and the injection volume was 100 μL. Polystyrene standards with molecular weights of 1310, 2970, 13 900, 30 200, 197 000, and 696 000 g/mol were used to generate a calibration curve.

Nuclear Magnetic Resonance. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded with a Bruker AMX500 NMR instrument in DMSO-d_6 at 333 K. Chemical shifts were referred to TMS at 0 ppm.

Differential Scanning Calorimetry. The thermal properties of the polymers were measured on a Mettler Toledo DSC 822 system. Nitrogen was used as the purge gas with a flow rate of 20 mL/min. Samples of 10 mg were prepared in aluminum foils, where the aluminum weights of the sample and reference were closely matched. The samples were heated from room temperature to 180 °C, cooled to −100 °C, and heated again to 180 °C, all at a rate of 20 °C/min. T_m and T_g of the samples were obtained from the second heating curves.

Fourier Transform Infrared Spectrophotometer. IR spectra of the polymers were analyzed with a Shimadzu FTIR-8400 system using DMF as solvent.

Scheme 1



Tension Test. Tension tests of polymer films were carried out on an Instron 5569 double column microforce tester with a 10 N load cell at a crosshead speed of 5 mm/min at room temperature. The films were prepared as follows: 1.0 g of polymer was dissolved in 10 mL of DMF, the solution was cast onto a PTFE plate, and the temperature was kept at 65 °C to remove DMF; the obtained film was further dried in a vacuum oven at 65 °C for 48 h; and finally, dog bone-shaped specimens [50 mm (length) × 3.25 mm (width) × 0.2 mm (thickness)] were prepared for tension tests.

3. RESULTS AND DISCUSSION

Enzymatic Ring-Opening Polymerization of TMC with PHB-diol To Prepare Di-*block*-poly(HB-*co*-TMC). *Ring-Opening Polymerization.* To incorporate PTMC into PHB, Novozym 435 [immobilized *Candida Antarctica* lipase B (CALB)]-catalyzed ROP of TMC with telechelic hydroxylated poly-[(*R*)-3-hydroxybutyrate] (PHB-diol) as initiator was explored (Scheme 1a). Novozym 435 was chosen as the catalyst for the ROP because of its well-known high catalytic activity and stability at high temperature, good solvent resistance, and good recyclability for the reduction of catalyst cost. PHB-diol (M_n = 3000 g/mol, GPC) was prepared by transesterification of PHB with ethylene glycol according to the published procedures.[41,42] 1,4-Dioxane and toluene with boiling point higher than 100 °C and good solubility for PHB-diol were examined as the solvent for the ROP. To avoid water-initiated ROP of TMC, both 1,4-dioxane and toluene were dried and freshly distilled before use, and the reactions were carried out at anhydrous conditions under argon atmosphere. The reaction temperatures examined were from room temperature to 70 °C, and different ratios of TMC/enzyme, TMC/PHB-diol, and TMC/solvent were explored to obtain the best polymerization conditions as well as polymers with different ratios of PHB/PTMC blocks. Initial tests showed that the highest molecular weight of the product was achieved at 8 h; thus, a series of reactions were performed for 8 h. After the reaction, the reaction mixtures were treated with chloroform, the enzyme was removed through filtration, the solvent was removed, and the product was precipitated in chloroform/methanol (1:9). After drying under high vacuum at 50 °C for 24 h, the corresponding block copolymers poly(HB-*co*-TMC)s were obtained in 57−89% yield. The M_n of the polymers was determined to be 4400−8700 g/mol by GPC in THF with polystyrene standards without correction. The reaction conditions, yields, and the molecular weights of the polymers are summarized in Table 1.

In entries 1−3 (Table 1), ratios of TMC/enzyme from 2:1 to 4:1 were studied for polymerization in dioxane at 50 °C at fixed ratios of TMC/PHB-diol and TMC/solvent. The molecular weight and yield of the resulting product increased with the increase in the catalyst amount: M_n of 4700, 5000, and 5500 g/mol were obtained at ratios of TMC/enzyme of 4:1, 3:1, and 2:1, respectively, after 8 h of reaction. To avoid the use of too much catalyst, the ratio of TMC/enzyme of 4:1 was used for further study of the polymerization. For entries 4 and 3, the reduction of ratio of TMC/PHB-diol from 100:1 to 75:1 resulted in a slight decrease in the polymer molecule weight.

Toluene proved to be a much better solvent than dioxane for the polymerization. As shown in entries 3 and 5, the M_n of the polymers increased from 4700 to 8700 g/mol by simple replacement of dioxane with toluene. Here again, the reduction of the ratio of TMC/PHB-diol from 100:1 to 75:1 (entry 5 vs 6) or from 100:1 to 50:1 (entry 7 vs 8) resulted in the decrease in M_n of the polymer. This effect can be used to synthesize polymers with different molecule weights and thus different ratio of PHB/PTMC. Further decrease of catalyst amount from a ratios of TMC/enzyme of 4:1 to 6:1 gave rise to a significant decrease in the polymer M_n (entry 5 vs 7). The temperature effects were

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Table 1. Novozym 435-Catalyzed Ring-Opening Polymerization of TMC with PHB-Diol or PCL-Diol As Initiator for 8 h

entry	monomer	initiator	M_n[a] (g/mol)	M[b]: I[c]	M:E[d]	M:S[e]	solvent	temp (°C)	M_n[f] (g/mol)	M_w/M_n	yield (%)	code
1	TMC	PHB-diol	2200	100:1	2:1	1:4	D[g]	50	5500	1.36	64	
2	TMC	PHB-diol	2200	100:1	3:1	1:4	D	50	5000	1.28	58	
3	TMC	PHB-diol	2200	100:1	4:1	1:4	D	50	4700	1.26	57	
4	TMC	PHB-diol	2200	75:1	4:1	1:4	D	50	4500	1.30	71	A
5	TMC	PHB-diol	2200	100:1	4:1	1:4	T[h]	50	8700	1.58	87	B
6	TMC	PHB-diol	2200	75:1	4:1	1:4	T	50	7400	1.59	89	
7	TMC	PHB-diol	2200	100:1	6:1	1:4	T	50	6600	1.57	84	
8	TMC	PHB-diol	2200	50:1	6:1	1:4	T	50	5400	1.65	85	C
9	TMC	PHB-diol	2200	100:1	4:1	1:4	T	40	7000	1.56	84	
10	TMC	PHB-diol	2200	100:1	4:1	1:4	T	60	8500	1.69	82	
11	TMC	PHB-diol	2200	100:1	4:1	1:4	T	70	7800	1.43	81	
12	TMC	PHB-diol	2200	100:1	4:1	1:4	T	r.t.	7100	1.45	84	D
13	TMC	PCL-diol	3000	100:1	4:1	1:4	T	50	9200	1.61	67	
14	TMC	PCL-diol	3000	200:1	4:1	1:4	T	50	10600	1.64	64	E
15	TMC	PCL-diol	3000	100:1	2:1	1:4	T	70	7700	1.58	55	F
16	TMC	PCL-diol	3000	200:1	4:1	1:4	T	70	8100	1.62	54	

[a] Calculated from ^{1}H NMR. [b] M: monomer (TMC). [c] I: initiator, PHB-diol or PCL-diol. [d] E: enzyme, Novozym 435. [e] S: solvent. [f] Measured by GPC in THF at 30 °C using polystyrene standard without correction. [g] D: dioxane. [h] T: toluene.

examined from room temperature to 70 °C in entries 5 and 9−12. The polymer molecular weight increased from room temperature to 50 °C, reached the maximum at 50−60 °C, and decreased from 60 to 70 °C. Thus, the best reaction temperature is 50 °C for the enzymatic ROP of TMC.

Structure Analysis. From the ^{1}H NMR spectrum of PHB-diol (M_n = 3000 g/mol, GPC) shown in Figure 1(i), the number of monomer repeating units, *n*, in PHB-diol was established as 24 on the basis of the signal intensities of proton *m*/*u*. The M_n of PHB-diol was thus deduced as 2200 g/mol. In the ^{1}H NMR spectrum of poly(HB-*co*-TMC) (sample B) shown in Figure 1(ii), signals of protons c, f, and h of PHB-diol disappeared, and a new signal of proton e was observed. This indicates that the terminal primary OH group of PHB-diol was reacted with TMC to form the polymer. On the other hand, the ratio of signal intensity of proton *m*/*u* remained unchanged, and the signal of proton d was clearly observed. This suggests that the terminal secondary OH group of PHB-diol did not react with TMC and remained as the terminal group of the di-block polymer. Therefore, the ring-opening polymerization of TMC was initiated selectively with the primary OH terminal group of PHB-diol, which is similar to the ring-opening polymerization of ε-caprolactone with PHB-diol.[41] The unchanged ratio of proton *m*/*u* suggests also no degradation or transesterification of the PHB block during the reaction. The signals of protons of aa, bb, cc, and dd of PTMC block were easily assigned. On the basis of the signal ratio of the a-proton and aa-proton of 7.8/38 (= *n*/2*p*), the number of TMC monomer repeating units, *p*, was deduced to be 58. Therefore, the molecular weight (M_n) of the block copolymer was established as 8200 g/mol. This value is very close to the M_n of 8700 g/mol determined by GPC. On the basis of the M_n and structure established by NMR, the ratio of PHB and PTMC in the polymer can be deduced as 26/74 (w/w).

The analysis of the ^{13}C NMR spectrum of poly(HB-*co*-TMC) (sample B) (Figure S1 of the Supporting Information) further confirmed that the polymer is a block copolymer rather than a random copolymer: there were only two signals at 154 and 169 ppm in the range of 150−170 ppm, which were assigned to the carbonyl groups in PHB block and PTMC block, respectively. If random copolymer were produced, there would be additional signals for the new type of carbonyl groups between 154 and 169 ppm. The di-block structure of poly(HB-*co*-TMC) (sample B) with a primary and a secondary terminal OH group was further evidenced in the IR spectrum (Figure S2 of the Supporting Information): there were two absorption peaks at 3555 and 3332 cm^{-1}, indicating the existence of two different types of OH groups. This is similar to the IR spectrum of PHB-diol, which showed two different absorptions at 3559 and 3332 cm^{-1} for the primary and the secondary terminal OH groups, respectively. If the block-copolymer were to have the structure of PTMC−PHB−PTMC, it would have the same type of terminal OH group at both ends and thus would give only one absorption peak in the IR spectrum.

The structures of other poly(HB-*co*-TMC) samples (A, C, and D) were also confirmed as di-block copolymers by NMR analysis. Similarly, the M_n and the block ratio of PHB/PTMC of each sample were established. As shown in Table 2, the M_n established from NMR analysis is nearly the same as the M_n obtained from the GPC analysis for each sample. By controlling the ROP conditions, di-block poly(HB-*co*-TMC) at different block ratios of PHB/PTMC of 26:74, 32:68, 41:59, and 46:54 (w/w) were successfully prepared.

Physical Properties. The melting temperature (T_m) and glass transition temperature (T_g) of poly(HB-*co*-TMC)s, PHB-diol, and PTMC were measured by DSC (Figure S3 of the Supporting Information). The data are summarized in Table 2. The di-block poly(HB-*co*-TMC)s with M_n of 4500−8700 g/mol (samples A−D) showed the T_m of PHB block at 149−154 °C, which is slightly higher than the T_m of PHB-diol (143 °C). The T_g of the di-block poly(HB-*co*-TMC)s were between −20 and −24 °C, which was obviously from PTMC block. The T_g of PHB block was not detectable.

Enzymatic Ring-Opening Polymerization of TMC with PCL-Diol To Prepare A−B−A Tri-block Poly(TMC-*co*-PCL-*co*-TMC). *Ring-Opening Polymerization.* To examine the generality of the enzymatic ROP of TMC with a polymer macrodiol to

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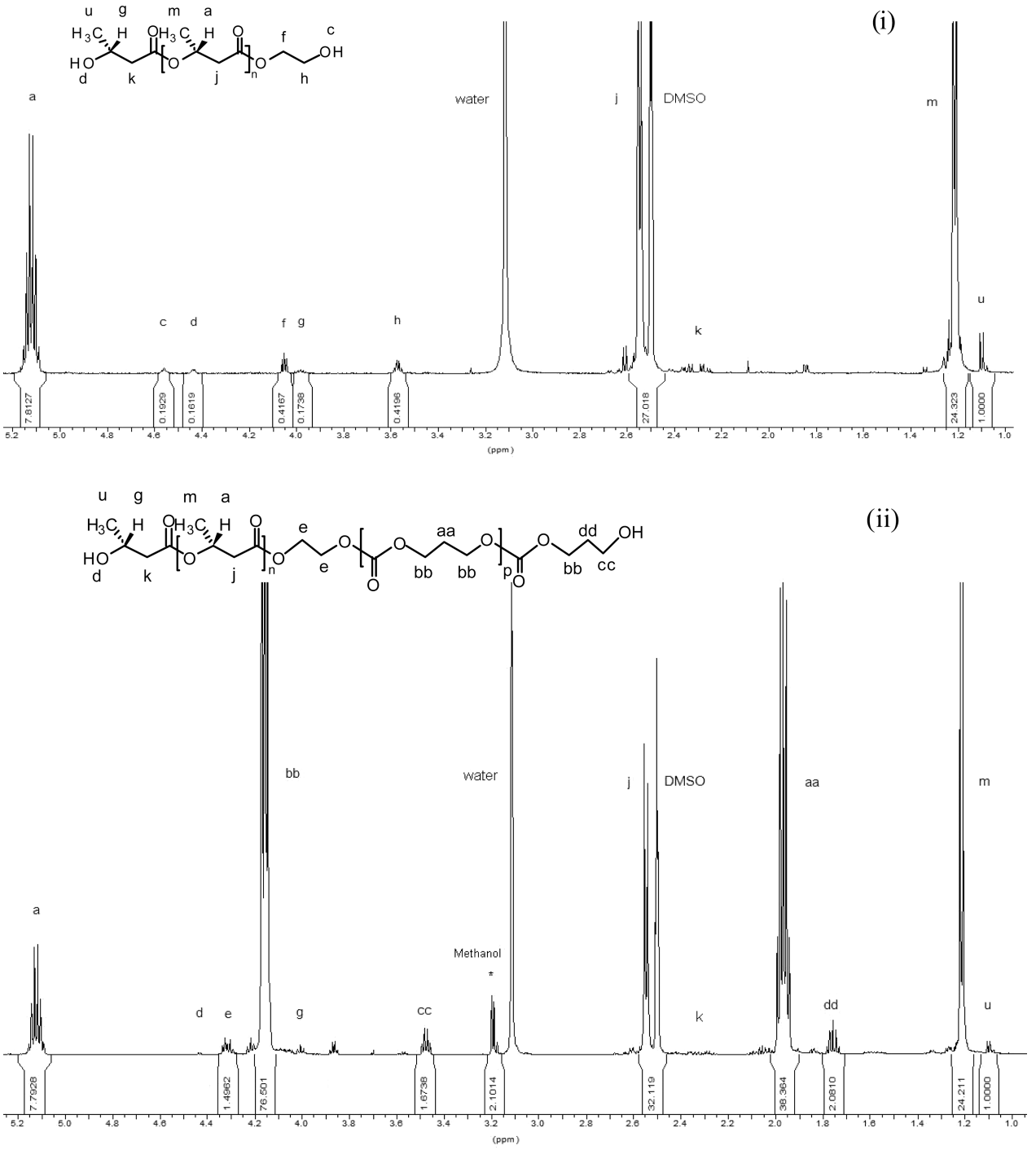

Figure 1. ^{1}H NMR spectra of (i) PHB-diol and (ii) poly[HB(26 wt %)-*co*-TMC (74 wt %)] (sample B, Table 1) in DMSO-d_6 at 333 K.

prepare block copolymers and explore the possibility of combining PTMC and PCL blocks for soft material synthesis, ROP of TMC with PCL-diol was carried out to prepare block copolymer poly(CL-*co*-TMC)s. At first, PCL-diol with a M_n of 3000 (^{1}H NMR) was prepared in 84% yield by Novozym 435-catalyzed ROP of ε-caprolactone with ethylene glycol as initiator at 70 °C without using any solvent (Scheme 1b). PCL-diol was then used as the initiator for enzymatic ROP of TMC in toluene at different molar ratios of TMC/PCL-diol for 8 h at 50 and 70 °C (Table 1, entries 13−16). The products were isolated in 54−67% yield with an M_n (GPC) of 7700−10 600 g/mol by using the same procedure for the preparation of poly(HB-*co*-TMC)s. As shown in entries 13 vs 14 and 15 vs 16 of Table 1, the increase in the ratio of TMC/PCL-diol increased the M_n of the final polymers at both temperatures. This phenomenon is similar to that observed in the ROP of TMC with PHB-diol. From entries 14 and 16 of Table 1, it can be seen that a reaction temperature of 50 °C is better than 70 °C for producing higher molecular weight polymers. All effects of different parameters on the molecular weigh of the product in the enzymatic ROP of TMC with PCL-diol are similar to those of the ROP of TMC with PHB-diol described above.

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Table 2. Physical Properties of Poly(Hb-*co*-TMC)s and Poly(TMC-*co*-CL-*co*-TMC) Compared with PHB-diol, PCL-diol, and PTMC

polymer	sample code	M_n[a] (GPC) (g/mol)	M_n (NMR) (g/mol)	CL/TMC[b] (wt %)	HB/TMC[b] (wt %)	T_m[c] (°C)	T_g[c] (°C)
PHB-diol		3000	2200			143	−5
PCL-diol[d]		4200	3000			46	−58
PTMC[e]		3400	2000				−27
poly(HB-*co*-TMC)	A	4500	4800		46/54	151	−20
poly(HB-*co*-TMC)	B	8700	8200		26/74	154	−24
poly(HB-*co*-TMC)	C	5400	5400		41/59	153	−21
poly(HB-*co*-TMC)	D	7100	6900		32/68	149	−21
poly(TMC-*co*-CL-*co*-TMC)	E	10600	10500	29/71			−42
poly(TMC-*co*-CL-*co*-TMC)	F	7700	7700	40/60			−48
poly(TMC-*co*-CL-*co*-TMC)[f]	G	7600	7600	38/62			−48

[a] Measured by GPC in THF at 30 °C using polystyrene standard without correction. [b] Calculated on the basis of NMR analysis. [c] Obtained from DSC. [d] Prepared by enzyme-catalyzed ROP of ε-caprolactone with ethylene glycol as initiator. [e] Prepared by enzyme-catalyzed ROP of TMC with water as initiator. [f] Scale-up experiment of F.

Structure Analysis. The ^{1}H NMR spectrum of PCL-diol prepared above by enzymatic ROP of ε-caprolactone with ethylene glycol is shown in Figure 2(i). No signals of protons from (−OCH_2−CH_2OH) were detected, and the signal of proton e was clearly observed. This suggests that both OH groups of the ethylene glycol were reacted. The protons from the PCL backbone (z, w, x, y, v) as well as the protons from the terminals (zz, ww, yy) adsorbed at the expected areas. The signal intensity of proton zz is nearly the same as that of proton e, which confirmed the polymer structure shown in Figure 2(i). On the basis of the signal ratio of proton z/zz of 12/1, the CL repeating *t* from the structure was deduced as 12. The M_n of PCL-diol was then established as 3000 g/mol.

The ^{1}H NMR spectrum of the copolymer poly(CL−TMC) (sample E, Table 1) prepared from the PCL-diol is shown in Figure 2(ii). The protons aa and bb of PTMC backbone and z, y, x, w, and v absorbed in the expected areas. Moreover, the signals for protons zz, ww, yy from the PCL-diol ending group disappeared, and new signals for protons cc and dd of the ending groups of PTMC block were observed. This confirmed the structure of the polymer as A−B−A tri-block poly(TMC-*co*-CL-*co*-TMC). This structure is the logical result for the ROP of TMC with PCL-diol that contains two equal terminal OH groups. On the basis of the signal ratio of protons aa/v of 2.77/1, the PTMC repeating unit s in the polymer structure was deduced as 36. The M_n of poly(TMC-*co*-CL-*co*-TMC) was thus established as 10 500 g/mol, which is nearly the same as the value obtained by GPC analysis. The ratio of PCL and PTMC was thus established as 29/71 (w/w). In the ^{13}C NMR spectrum of the same polymer (Figure S4, Supporting Information), there were only two signals, at 154 and 172 ppm, which were assigned to the carbonyl groups of TMC and PCL blocks, respectively. This excluded the formation of random copolymer and thus confirmed the block copolymer structure. The tri-block structure of poly(TMC-*co*-CL-*co*-TMC) with two primary terminal OH groups was further confirmed in the IR spectrum: there was only one OH absorption peak at 3450 cm^{-1}, similar to the IR spectrum of PCL-diol.

Although a tri-block poly(ether-carbonate) consisting of PTMC and poly(PEG-*co*-cyclic acetal) (PECA) was reported by the enzymatic ROP of TMC with PECA glycol containing stable and non-degradable ether functions,[43] it is the first example of the synthesis of tri-block poly(ester-*co*-carbonate) containing both biodegradable ester and carbonate functions by ROP with polyester-diols (PCL-diol).

Polymer samples F and G were also analyzed by NMR: they are the tri-block poly(TMC-*co*-CL-*co*-TMC); their M_n determined from NMR analysis are nearly the same as the M_n obtained from GPC analysis; the block ratio of PCL/PTMC is 40:60 and 38:62 (w/w) for samples F and G, respectively.

Physical Properties. The thermal properties of poly(TMC-*co*-CL-*co*-TMC)s were analyzed by DSC (Figure S3 of the Supporting Information), and the data are summarized in Table 2. Poly(TMC-*co*-CL-*co*-TMC)s with a M_n of 10 500 (sample E) and 7700 g/mol (sample F or G) did not show any T_m, possibly due to the low percentage of PCL in the polymers, but they gave a T_g of −42 and −48 °C, respectively. These values are higher than the T_g of PCL-diol (−58 °C) and lower than that of PTMC (−27 °C). Sample E containing 71 wt % PTMC has a higher T_g value than sample F or G containing 60−62 wt % PTMC, possibly due to the incorporation of more PTMC into the polymers.

Preparation and Characterization of Block *co*-Poly(ester-carbonate-urethane)s by Polymerization of the Block Poly(ester-*co*-carbonate)s with MDI. *Polymer Synthesis.* The above enzymatically prepared di-block poly(HB-*co*-TMC)s and tri-block poly(TMC-*co*-CL-*co*-TMC)s have two terminal hydroxyl groups and relatively low molecular weight, thus, being useful segments for the preparation of block copolymers with high molecular weight. Such a possibility was explored by the preparation of block *co*-poly(ester-carbonate-urethane)s by the polyaddition of MDI with the poly(ester-*co*-carbonate) or polyester macrodiols (Scheme 2). The polymerizations were performed in DMF at 85 °C for 12 h without using any catalyst. After reaction, the mixture was treated with methanol and precipitated twice in DMF/methanol (1:4) at 4 °C. The polymers were collected by filtration and then dried in a vacuum oven at 50 °C for 24 h. The PUs were obtained in 88−94% yield with a M_n of 34 600−53 800 g/mol (GPC). The polymerizations and results are summarized in Table 3.

Polymer Properties. The thermal properties of block *co*-poly(ester-carbonate-urethane)s (samples H−J) were measured by DSC and are summarized in Table 4. PU_{HBTMC} (samples H and I) had a T_m of 144−152 °C, similar to those of the corresponding starting materials poly(HB-*co*-TMC)s (samples A

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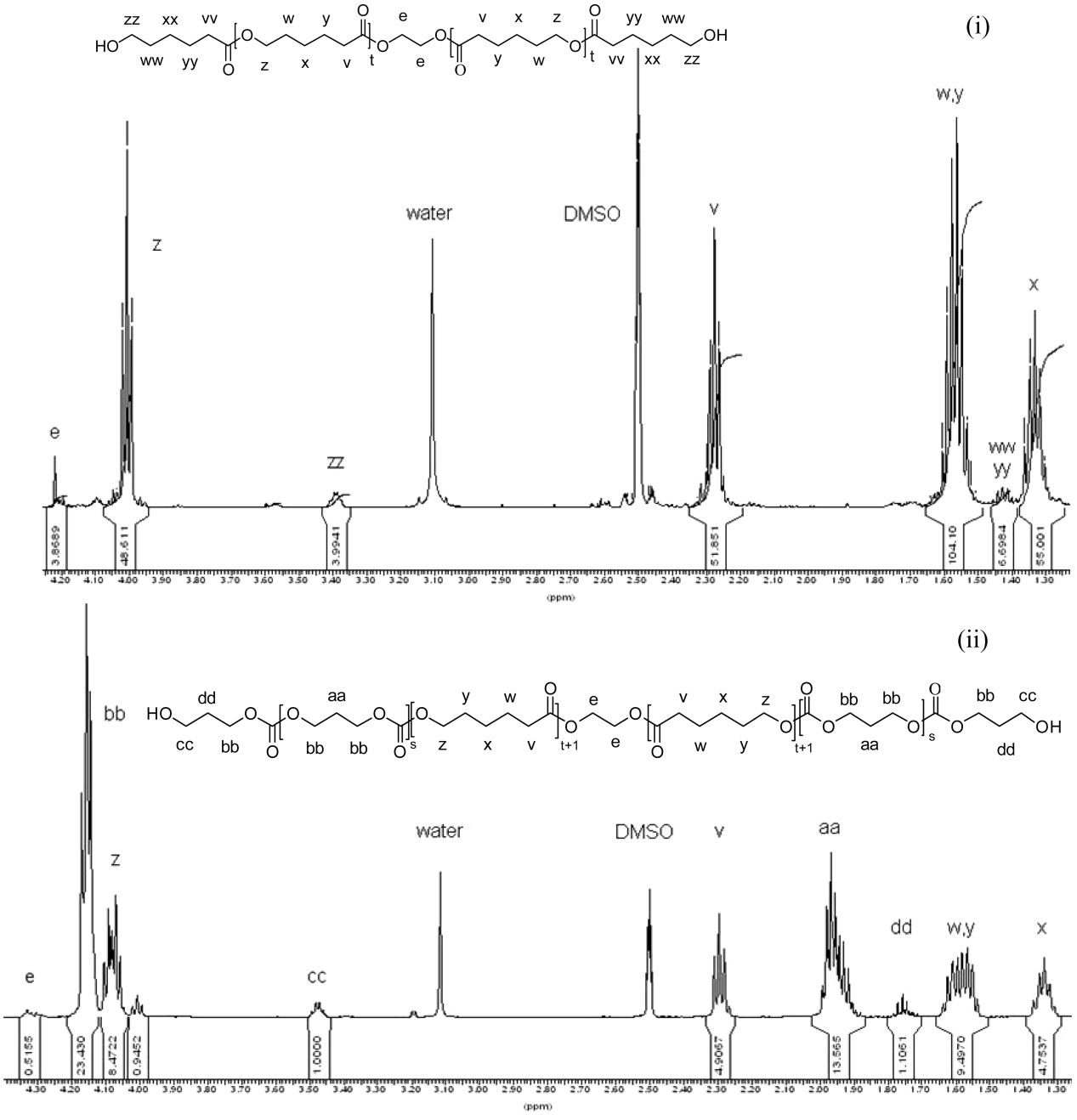

Figure 2. [1]H NMR spectra of (i) PCL-diol and (ii) poly[TMC-*co*-CL-*co*-TMC] containing 29 wt % PCL and 71 wt % PTMC (sample E in Table 1) in DMSO-d_6 at 333 K.

and B) (Table 2). On the other hand, PU_{HBTMC} (samples H and I) showed a T_g of −5 to −9 °C which is higher than the observed values of the corresponding starting materials poly(HB-*co*-TMC)s (samples A and B). Possibly, the T_g observed for PU_{HBTMC} is from the PHB block, whereas the T_g observed for poly(HB-*co*-TMC)s is from the PTMC block.

The mechanical properties of block *co*-poly(ester carbonate urethane)s (samples H−J) were measured using polymer films with a microforce tester at room temperature. As listed in Table 4, PU_{HBTMC} with different weight percentages of PHB and PTMC showed significantly different mechanical properties. PU_{HBTMC} (sample H) containing 46 wt % PHB and 54 wt % PTMC gave rather poor plastic properties, with ε_b = 4.76% and σ_{max} = 6.62 MPa, and was quite stiff, with E = 285 MPa. On the other hand, PU_{HBTMC} (sample I) containing 24 wt % PHB and 76 wt % PTMC showed excellent plastic properties with ε_b = 252%, σ_{max} = 6.37 MPa and was quite soft with E = 23 MPa (Figure 3). Obviously, the incorporation of more PTMC soft block significantly increased the plastic properties and softness of the PHB-based block copolymers. Thus, we have established a novel method to engineer PHB-based soft block copolymers by incorporating PTMC at a desired weight percentage. The mechanical data for PU_{HBTMC} (sample I) are close to that for several soft tissues.[38] Therefore, such a biodegradable, biocompatible, and thermoplastic block copolymer is potentially useful biomaterial for soft tissue engineering.

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Scheme 2

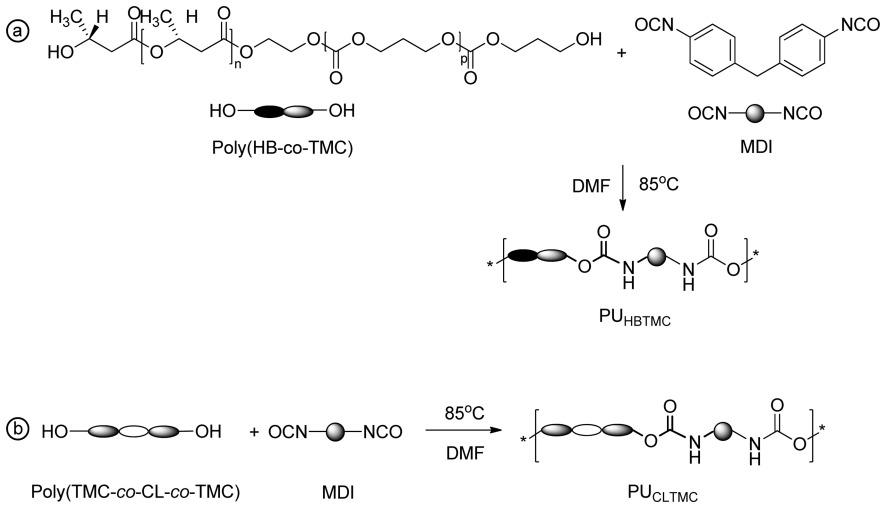

Table 3. Polymerization of MDI with Poly(HB-*co*-TMC)s and Poly(TMC-*co*-CL-*co*-TMC), respectively, in DMF at 85 °C and for 12 h

entry	block-*co*-polymer-diol	sample code	diols/MDI (mol/mol)	product	sample code	M_n (GPC)[a] (g/mol)	yield (%)
1	poly(HB-*co*-TMC)	A	1:1	PU$_{HBTMC}$	H	41 800	88
2	poly(HB-*co*-TMC)	B	1:1	PU$_{HBTMC}$	I	53 800	94
3	poly(TMC-*co*-CL-*co*-TMC)	G	1:1	PU$_{CLTMC}$	J	41 500	91

[a] Determined by GPC in DMF at 35 °C using polystyrene standards without corrections.

Table 4. Physical and Mechanical Properties of PUs Containing Poly(HB-*co*-TMC) or Poly(HB-*co*-CL-*co*-TMC) blocks

entry	PU	sample code	M_n (GPC)[a] (g/mol)	HB/CL/TMC[b] (wt/wt/wt)	T_m (°C)	T_g (°C)	E[c] (MPa)	σ_y[d] (MPa)	ε_y[e] (%)	σ_b[f] (MPa)	ε_b[g] (%)	σ_{max}[h] (MPa)
1	PU$_{HBTMC}$	H	41 800	46: 0: 54	149	−5	285	3.31	1.44	6.61	4.76	6.62
2	PU$_{HBTMC}$	I	53 800	24: 0: 76	144, 152	−9	23	1.17	4.84	5.59	252	6.37
3	PU$_{CLTMC}$	J	41 500	0: 38: 62	ND[i]	ND	19	1.23	5.49	2.64	62.4	4.27

[a] Measured by GPC in DMF at 35 °C using polystyrene standard without correction. [b] Measured by NMR. [c] E: Young's modulus. [d] σ_y: tensile stress at yield. [e] ε_y: elongation at yield (offset 0.1%). [f] σ_b: tensile stress at break. [g] ε_b: elongation at break. [h] σ_{max}: maximum tensile stress. [i] ND: not detected.

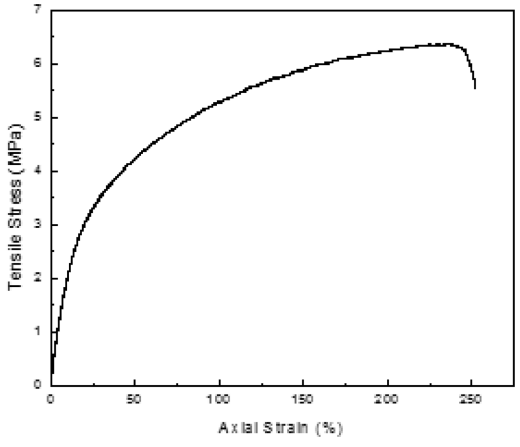

Figure 3. Stress−strain curves of PU$_{HBTMC}$ containing poly[HB-(26 wt %)-*co*-TMC(74 wt %)] (sample I, Table 3).

PU$_{CLTMC}$ (sample J) with a ratio of PCL/PTMC of 38:62 (w/w) gave an ε_b = 62.4%, σ_{max} = 4.27 MPa, and E = 19 MPa, being and is also a soft material with potential application in soft tissue engineering. A further increase in the plastic properties of PU$_{CLTMC}$ might be achieved via incorporation of a higher percentage of PTMC.

4. CONCLUSIONS

Enzymatic ring-opening polymerization of TMC with PHB-diol gave di-block poly(HB-*co*-TMC)s with controlled weight percentage of the blocks and controlled molecular weight, being the first example of enzymatic preparation of block poly(ester-*co*-carbonate). The syntheses are high-yielding, selective, green, and novel. The di-block structures of poly(HB-*co*-TMC)s were confirmed by NMR analyses. Poly(HB-*co*-TMC)s with two terminal hydroxyl groups were proven to be useful starting materials for the further preparation of thermoplastic block copolymers. Polymerization of di-block poly(HB-*co*-TMC)s

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with MDI afforded the corresponding block *co*-poly(ester carbonate urethane)s in high yield. The polymers showed good thermal properties, with a T_m of 140−144 °C and a T_g of −5 to −9 °C, and their mechanical and plastic properties were tunable by adjusting the weight percentage of PHB and PTMC blocks. Block *co*-polyurethane containing di-block poly(24 wt % HB-*co*-76 wt % TMC) segments demonstrated excellent plastic properties, with ε_b = 252% and σ_{max} = 6.37 MPa, and good "softness", with E = 23 MPa, being potentially useful biodegradable thermoplastic material for soft tissue engineering.

The generality of the novel enzymatic method for the preparation of block (polyester-*co*-carbonate) was demonstrated. Enzymatic ring-opening polymerization of TMC with PCL-diol afforded A−B−A *tri*-block poly(TMC-*co*-CL-*co*-TMC) with controlled weight percentage of the blocks and controlled molecular weight in good yields. NMR analysis confirmed the tri-block structure containing two reactive hydroxy ending groups. The tri-block polymer was shown to be a good soft segment for the preparation of soft materials. Polymerization of poly(TMC-*co*-CL-*co*-TMC) containing 38 wt % PCL and 62 wt % PTMC with MDI gave the corresponding block *co*-polyurethane in high yield, with ε_b = 62.4%, σ_{max} = 4.27 MPa, and E = 19 MPa.

■ ASSOCIATED CONTENT

Supporting Information. ^{13}C NMR spectra of samples B and E; IR spectra of PHB-diol, sample B, PCL-diol, and sample E; DSC spectra of PHB-diol, samples A, B, C, and D, PTMC, samples H, and I. This information is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +65-6516 8416. Fax: +65-6779 1936. E-mail: chelz@nus.edu.sg.

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