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Polymers from Amino acids: Development of Dual Ester-Urethane Melt Condensation Approach and Mechanistic Aspects

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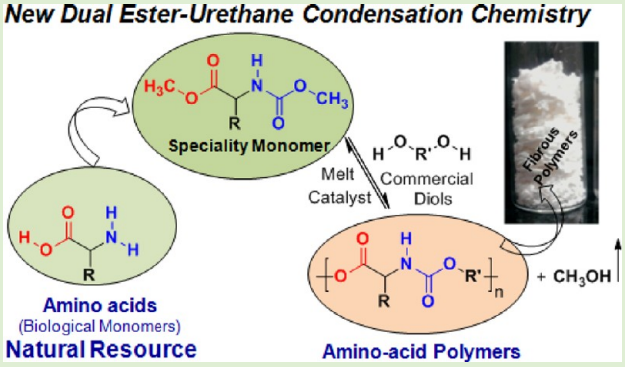
Supporting Information

ABSTRACT: A new dual ester-urethane melt condensation methodology for biological monomers−amino acids was developed to synthesize new classes of thermoplastic polymers under eco-friendly and solvent-free polymerization approach. Naturally abundant L-amino acids were converted into dual functional ester-urethane monomers by tailor-made synthetic approach. Direct polycondensation of these amino acid monomers with commercial diols under melt condition produced high molecular weight poly(ester-urethane)s. The occurrence of the dual ester-urethane process and the structure of the new poly(ester-urethane)s were confirmed by ^{1}H and ^{13}C NMR. The new dual ester-urethane condensation approach was demonstrated for variety of amino acids: glycine, β-alanine, L-alanine, L-leucine, L-valine, and L-phenylalanine. MALDI-TOF-MS end group analysis confirmed that the amino acid monomers were thermally stable under the melt polymerization condition. The mechanism of melt process and the kinetics of the polycondensation were studied by model reactions and it was found that the amino acid monomer was very special in the sense that their ester and urethane functionality could be selectively reacted by polymerization temperature or catalyst. The new polymers were self-organized as β-sheet in aqueous or organic solvents and their thermal properties such as glass transition temperature and crystallinity could be readily varied using different L-amino acid monomers or diols in the feed. Thus, the current investigation opens up new platform of research activates for making thermally stable and renewable engineering thermoplastics from natural resource amino acids.



INTRODUCTION

Amino acids are biological monomers and their macromolecular peptide sequence and chain length played a crucial role on the size, function, and secondary structure of proteins.[1,2] Synthetic polymers based on amino acids have been of great interest in chemistry−biology interface due to their potential application in therapeutics, cosmetics, and biodegradable and biocompatible engineering thermoplastics.[3,4] Peptide linkages (amide bonds) were routinely made by the self-condensation of amino acid using water removal agents such as DCC; however, this route was not capable of making higher molecular weight polymers having more than 8−10 repeating units.[5] In an indirect approach, amino acids were converted into dicarboxylic acid derivatives and polymerized with diols or diamines to produce poly(ester-amide) and their random copolymers.[6−8] Ring-opening polymerization of amino acids via *N*-carboxyanhydride (NCA) intermediate was another important approach to make linear, block, and star-shaped polypeptides.[9] Better control over the NCA intermediates was achieved by Deming and co-workers via Ni(COD)bpy transition metal catalyzed polymerization process.[10] However, the interference of functional groups in multifunctional amino acids (like serine) with the catalyst restricted this approach to a limited range of amino acids.[11] Other than the NCA route, so far no effort has been put to utilize amino acids as monomers for making high molecular weight polymers for commercial application. Amino acids are naturally available bioresources, and therefore, developing new synthetic polymerization strategies using them would open up a new direction of research activities toward amino acid polymeric materials for applications in biomedical components and thermoplastics.

The present work is emphasized to develop new eco-friendly melt polycondensation approach for L-amino acid monomers. Solvent-free melt polycondensation process is one of the most widely employed synthetic approach for producing commercial engineering thermoplastics such as polyesters, polycarbonates and polyamides.[12] In this process, raw materials were melted and subjected to condensation reaction to produce high viscous resins which could be directly processes into desired objects. In 1946, Whinfield developed first melt polymerization route transesterification in which a diester monomer was polycondensed with diols to produce polyesters (see Scheme 1).[12]

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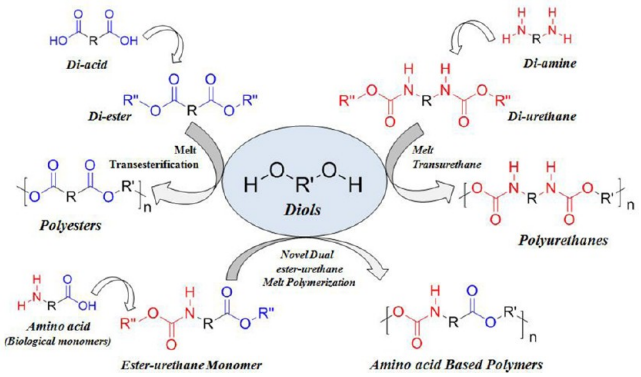
Scheme 1. Development of New Dual Ester-Urethane Melt Polycondensation for Amino Acids



This route is adopted even today for manufacturing a few million tons of polyesters every year. Ramakrishnan and co-workers had reported melt transetherification approach for linear and hyperbranched polyethers based on fully protected bis-benzyl ethers.[13] From our research group, Deepa et al. had reported eco-friendly melt transurethane process (see Scheme 1) for polyurethane and the approach was aimed to replace the toxic and hazardous isocyanate chemical pathways.[14] In this process, commercially available diamines were converted into diurethane monomers which were polymerized with diols to make polyurethanes (see Scheme 1). It is reasonable to consider amino acids as half an acid and half an amine; thus, development of solvent-free melt condensation approach by combining the melt transurethane process developed in our laboratory with the whinfield one (transesterification) would open up a new avenue of research opportunities for amino acid based condensation polymers.

Herein, we developed a new *dual ester-urethane* melt polycondensation approach for amino acid monomers as shown in scheme-1. In this new process, amino acids were readily converted into dual ester-urethane monomers and polycondensed with diols under melt conditions to produce high molecular weight polymers. The new synthetic process was tested for more than half-dozen of amino acids and diols. The mechanistic aspects of the new process were studied by NMR and MALDI-TOF-MS, and control reactions were carried out to understand the kinetics of the polymerization. The role of the catalyst, polymerization temperature, and repeating unit structure on the molecular weight of the polymers and their thermal properties were also investigated. The newly synthesized amino acid polymers were found to self-organize as either β-sheet or polyproline type-II secondary structures. The present synthetic polymer approach, dual ester-urethane process, is expected to pave the way for new research platforms for amino acids, which has huge potential in thermoplastic and biomedical applications.

EXPERIMENTAL SECTION

Materials. Glycine, L-alanine, β-alanine, L-valine, L-leucine, L-phenylalanine, 1,4-cyclohexanedimethanol (CHDM), 1,12-dodecandiol (DD), diethyleneglycol (DEG), triethyleneglycol (TREG), tetraethylene glycol (TEG), and titanium tetrabutoxide ($Ti(OBu)_4$) were purchased from Aldrich chemicals and used without further purification. Methylchloroformate, thionyl chloride, and other solvents were purchased locally and purified prior to use.

General Procedures. ^1H and ^{13}C NMR were recorded using 400 MHz JEOL NMR spectrophotometer. All NMR spectra were recorded in $CDCl_3$ containing TMS as internal Standard. High resolution mass spectra were obtained from Micro Mass ESI-TOF MS spectrometer. MALDI-TOF MS of the polymers was determined using Applied Biosystems 4800 PLUS Analyzer. The polymer samples were dissolved in tetrahydrofuran (THF) at 10 mg/mL and dihydroxybenzoic acid (DHB) was used as matrix. The matrix solution was prepared by dissolving 30 mg in 1.0 mL of THF. A 1−2 μL aliquot of the polymer/matrix mixture was used of the MALDI-TOF MS analysis. FT-IR spectra of all compounds were recorded using Bruker alphaT Fourier transform infrared spectrophotometer. Gel permeation chromatographic (GPC) analysis of the polymer samples were performed using Viscotek VE 1122 pump, Viscotek VE 3580 RI detector and Viscotek VE 3210 UV/vis detector in tetrahydrofuran (THF) or dimethylformaamide (DMF) using polystyrene as standards. Thermal analysis of the polymers was performed using TA Q20 Differential Scanning Calorimeter. The instrument was calibrated with indium standards. All the polymers were heated to melt before recording their thermograms to remove their previous thermal history. Polymers were heated and cooled at 10 °C/min under nitrogen atmosphere and their thermograms were recorded. Thermal stability of the polymers was determined using Perkin Elmer thermal analyzer STA 6000 model at a heating rate of 10 °C/min in nitrogen atmosphere. Circular dichroism (CD) analysis of the polymer samples were done using JASCO J-815 CD spectrometer at 25 °C in THF, methanol, and water, depending up on their solubility.

Synthesis of Methyl Esters of Amino Acids. Typical procedure for carboxylic methyl esters of amino acids was described for L-phenylalanine. To a suspension of L-phenylalanine (8.67 g, 0.052 mol) in methanol (85 mL), thionylchloride (11.4 mL, 18.74 g, 0.157 mol) was added dropwise at 5 °C under a nitrogen atmosphere. The reaction was refluxed for 12 h by stirring under nitrogen. The solvent and excess thionylchloride were removed by distillation. The residue solid was washed with dry diethylether (120 mL) and dried to get product as white solid. Yield = 10.4 g (93%). ^1H NMR (400 MHz, D_2O) δ ppm: 7.41−7.24 (m, 5H, Ar**H**), 4.39 (m, 1H, C**H**CH_2Ar), 3.79 (s, 3H, $COOCH_3$), 3.33−3.18 (d, 2H, CH_2Ar). FT-IR (cm^{-1}): 3926, 2852, 2618, 1742, 1581, 1491, 1443, 1232, 1145, and 1056. HR-MS (ESI^+): m/z [M + H^+] Calcd for $C_{10}H_{13}NO_2$ [M^+], 180.1025; found, 180.1025.

The methyl ester of other amino acids, glycine, β-alanine, L-valine, L-leucine, and L-alanine, was prepared as described above and the details are provided in the Supporting Information.

Synthesis Ester-Urethane Monomers of Amino Acids. Typical procedure is described for L-phenylalanine monomer. Hydrochloride salt of the above methyl ester of L-phenylalanine (7.10 g, 0.032 mol) was stirred in sodium bicarbonate solution (25 wt %, 65 mL) + dichloromethane (45 mL) at 5 °C under a nitrogen atmosphere. To this ice cold solution, methyl chloroformate (5.1 mL, 0.066 mol) was added dropwise and the reaction was continued for 12 h at 25 °C. The reaction mixture was extracted with dichloromethane and the organic layer was dried over anhydrous Na_2SO_4. The liquid was further purified by passing through silica gel column using ethyl acetate and pet ether (1:4 v/v) as eluent. Yield = 6.0 g (89%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.32−7.24 (m, 3H, Ar**H**), 7.12 (d, 2H, Ar**H**), 5.12 (b, 1H, -N**H**), 4.64 (q, 1H, C**H**CH_2Ar), 3.73 (s, 3H, $COOCH_3$), 3.67 (s, 3H, $NHCOOCH_3$), 3.11 (d, 2H, CH_2Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 172.95, 156.24, 135.69, 129.15 (2C), 128.52 (2C), 127.06, 54.69, 52.24 (2C), 38.13. FT-IR (cm^{-1}): 3338, 2954, 1705, 1518, 1444, 1355, 1253, 1210, and 1057. HR-MS (ESI^+): m/z [M + Na^+] Calcd for $C_{12}H_{15}NO_4$ [M^+], 260.0898; found, 260.0899.

Dual ester-urethane monomers of other amino acids, glycine, β-alanine, L-valine, L-leucine, and L-alanine, were prepared as described above and details are provided in the Supporting Information.

Dual Ester-Urethane Melt Polycondensation Process. Typical dual ester-urethane melt polymerization procedure is explained for L-phenylalanine monomer with 1,12-dodecanediol. Equimolar amounts of amino acid monomer, L-phenylalanine monomer (0.76 g, 3.0 mmol) and 1,12-dodecanediol (0.65 g, 3.0 mmol) were taken in a test tube-shaped polymerization vessel and melted by placing the tube in oil bath at 100 °C (see reaction vessel in the Supporting Information, SF-

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1). The polycondensation apparatus was made oxygen and moisture free by purging with nitrogen and subsequent evacuation by vacuum under constant stirring. Titanium tetrabutoxide (11.0 mg, 0.03 mmol, 1.0 mol % equivalent to monomer) was added as catalyst and the melt polycondensation was carried out at 150 °C for 4 h with constant stirring under nitrogen purge. During this stage, the methanol was removed along with the purge gas and the polymerization mixture became viscous. The viscous melt was further subjected to high vacuum (0.01 mm of Hg) at 150 °C for 2 h under stirring. At the end of the polycondensation, the polymer, poly(ester-urethane), was obtained as a white mass (weight = 0.98 g (82%). It was purified by dissolving in tetrahydrofuran, filtered, and precipitated into methanol to obtain fibrous product. Yield = 0.72 g (59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.31−7.12 (m, 5H, Ar**H**), 5.13 (b, 1H, N**H**), 4.62 (m, 1H, C**H**), 4.10−4.04 (m, 4H, COOC**H**$_2$, NHCOOC**H**$_2$), 3.10 (t, 2H, C**H**$_2$Ar), 1.56−1.25 (m, 20H, C**H**$_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 171.74, 155.93, 135.85, 129.25, 128.46, 126.98, 65.56, 65.29, 54.65, 38.35, 29.50, 29.16, 28.89, 28.38, and 25.76. FT-IR (cm^{-1}): 3348, 2924, 2853, 1717, 1505, 1458, 1397, 1346, 1249, 1196, and 1057. Molecular weights are given in Table 1.

Table 1. Monomers, Molecular Weights, % Conversion, and Thermal Properties of Polymers

monomer	diol[a]	M_n[b] (g/mol)	M_w[b] (g/mol)	x_n[c]	P[d]	T_D[e] (°C)	T_g[f] (°C)
glycine	DEG	4600	6700	24	96	240	8.6
	TREG	4900	8800	21	95	240	−2.9
	TEG	5200	8600	18	94	250	−13.3
	CHDM	9300	10400	40	97	280	58.3
	DD	5500	14100	19	95	275	14.8
L-alanine	TEG	4800	7300	16	93	250	−26.6
	CHDM	3400	7200	14	92	280	6.7
	DD	8600	14200	28	96	250	6.0
β-alanine	TEG	6100	10900	20	95	250	−24.3
	CHDM	8600	15300	18	97	260	24.1
	DD	5600	11000	35	94	270	13.7
L-valine	TEG	4400	6700	14	93	255	−29.6
	CHDM	5600	11000	20	95	260	43.5
	DD	8400	11000	25	96	270	-
L-leucine	TEG	5900	9800	18	94	250	−15.7
	CHDM	5900	11900	20	95	260	50.4
	DD	13300	17000	39	97	300	−13.1
L-phenyl-alanine	TEG	7000	14000	19	95	265	−0.1
	CHDM	4800	8300	15	93	300	−8.3
	DD	17900	32700	47	98	300	64.0

[a]DEG, TREG, and TEG refer di-, tri-, and tetra-ethylene glycols, respectively. CHDM and DD refer 1,4-cyclohexanedimethanol and 1,2-dodecane diol, respectively. [b]Number (M_n) and weight average (M_w) molecular weights were determined by GPC using THF as solvent. [c]The degree of polymerization (x_n) was calculated using the formula $M_n = nM_o$, where M_o is repeating unit mass. [d]The percent conversion (P) was calculated using Carothers eq $x_n = 1/(1 - P)$. [e]The decomposition temperature (T_D) of the sample was determined for 10% weight loss using TGA under nitrogen. [f]The glass transition temperature (T_g) was determined by DSC at 10°/min.

All other amino acid-based polymers with various diols: 1,4-cyclohexanedimethanol (CHDM), 1,12-dodecandiol, diethylene glycol (Di-EG), triethylene glycol (Tri-EG), and tetraethylene glycol (Tetra-EG) are provided in the Supporting Information.

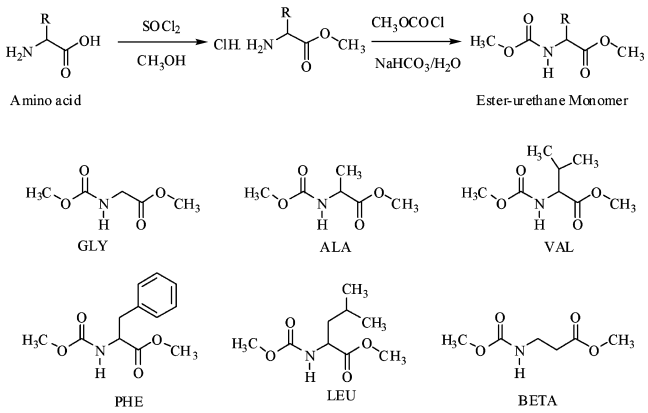
Model Reactions for Kinetic Studies. Typical reaction is described for benzyl alcohol (**Bz**) with the glycine monomer. Benzyl alcohol (2.21 g, 20.0 mol) and glycine monomer (1.50 g, 10.0 mol) were taken in a test-tube shaped polymerization apparatus and melted by placing in an oil bath at 100 °C with constant stirring. After degassing, as described for the polymerization, $Ti(OBu)_4$ (0.035 g, 1 mol %) was added and the condensation was carried out at 120 °C under a nitrogen purge for 3 h. Subsequently, the temperature was increased to 150 °C under a nitrogen purge for 3 h. Further, controlled vacuum (1 mm of Hg) was applied at 150 °C for 2 h. During the nitrogen and vacuum stage, various aliquots were taken every half an hour interval. At the end of the condensation reaction, the product was obtained as a slight yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.36 (m, 10H, Ar**H**), 5.18 (b, 1H, -N**H**), 5.09 (s, 2H, PhC**H**$_2$COO), 5.03 (s, 2H, PhC**H**$_2$NHCOO), 3.91 (d, 2H, C**H**$_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.19, 156.56, 136.44, 135.39, 128.91 (2C), 128.79 (2C), 128.66 (2C), 128.47, 128.37, 67.45, 67.38, and 43.08. FT-IR (cm^{-1}): 3359, 2923, 1712, 1524, 1361, 1161, and 1052. MALDI-TOF MS: m/z [M + Na$^+$] Calcd for $C_{17}H_{17}NO_4$ [M$^+$], 322.1055; found, 322.0435.

The other model reactions, (i) stepwise in the absence of catalyst and (ii) direct at 150 °C in the presence of catalyst, are provided in the Supporting Information.

RESULTS AND DISCUSSION

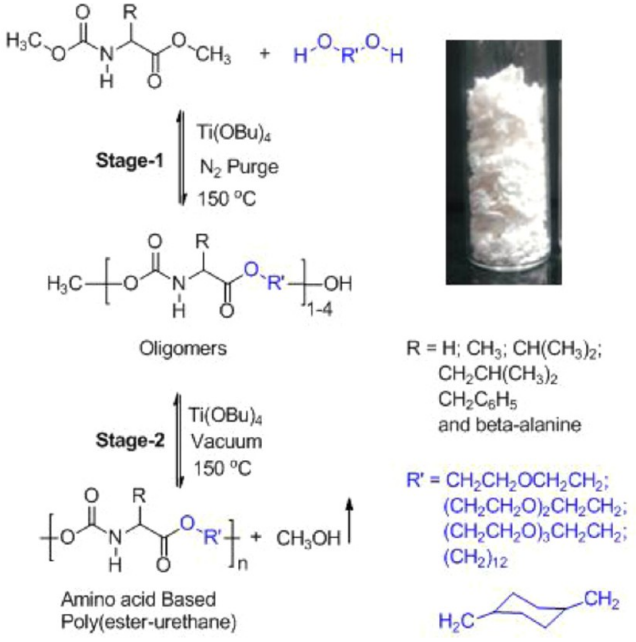
Monomer Design and Dual Ester-Urethane Melt Polycondensation. Ester-urethane monomers were synthesized starting from naturally available L-amino acids, as shown in Scheme 2. Amino acids were converted into carboxylic acid

Scheme 2. Synthesis of Dual Ester-Urethane Monomer from Amino Acids



chlorides and subsequently reacted with methanol to yield amino acid methyl ester hydrochloride salts. The amine salt was converted into its free amine by washing with aqueous $NaHCO_3$, then reacted with methyl chloroformate to obtain dual ester-urethane monomer. Monomers of glycine, L-alanine, L-valine, L-phenylalanine, L-leucine, and β-alanine were synthesized as described above and their structures were characterized by NMR, FT-IR, and mass spectroscopic analysis (see Supporting Information). The monomers were synthesized in an average yield of 75−84%. A dual ester-urethane melt condensation reaction was carried out in one pot and two steps, as shown in Scheme 3. Equimolar amounts of amino acid monomer and diols were polycondensed using $Ti(OBu)_4$ as catalyst (1 mol %) under nitrogen purge and high vacuum (0.01 mm of Hg). During this process, the viscosity of the melt increased very rapidly and the stirring stopped at the end of the polycondensation. The white polymer mass was purified using the precipitation technique and dried in a vacuum oven (0.1 mm of Hg) prior to further analysis. In the current process, the diol reacted with both methyl ester and methyl urethane units in the amino acid monomers; as a result, the new class of polymers are named as poly(ester-urethane)s. The new polycondensation process was tested for various diols of di-,

Scheme 3. Synthesis of Polymers via Dual Ester-Urethane Melt Polycondenstaion[a]



[a]The sample in the vial showed the fibrous polymer obtained using 1,12-dodecane diol with glycine monomer.

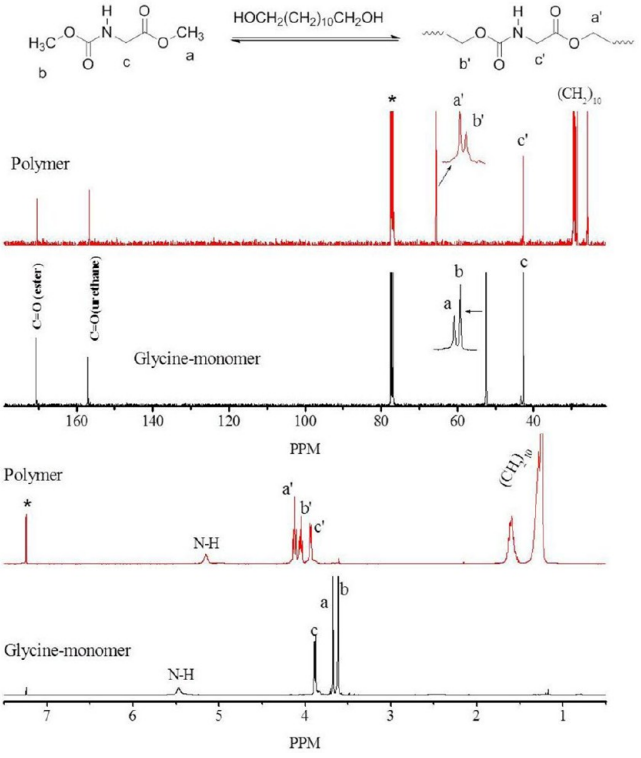


Figure 1. ^{1}H and ^{13}C NMR spectra of glycine monomer and its 1,12-dodecane diol polymer.

tri-, and tetraethylene glycols, 1,12-dodecanol (linear aliphatic diols), and 1,4-cyclohexanedimethanol (cycloaliphatic diols) and also various amino acid monomers (see Scheme 2). Both diols and amino acid monomers were varied in the feed to produce wide range of polymer structures. The structures of poly(ester-urethane)s are provided in the Supporting Information (SF-2).

NMR Spectroscopy and Molecular Weights. The occurrence of a dual ester-urethane process and the structure of the new poly(ester-urethane)s were confirmed by NMR spectroscopy. NMR spectra of glycine monomer and its corresponding polymer with 1,12-dodecane diol are shown in Figure 1. The different types of the protons and carbon atoms in the monomer and polymer structure were assigned by alphabets.

In the ^{1}H NMR spectrum of the monomer, the methyl ester protons in the ester (a) and the urethane (b) appeared very closely as a singlet at 3.67 and 3.62 ppm, respectively. The N−H proton and the NH-C**H**$_2$-COO (c) from the amino acid unit appeared at 5.47 and 3.88 ppm, respectively. Upon polymerization, two new triplets appeared at 4.12 ppm and 4.05 ppm corresponding to $-CH_2CH_2OOC$ (a′) and $-CH_2CH_2OOCNH$ (b′) protons in the ester and urethane linkages. All other aliphatic protons in the dodecane diol unit appeared below 2.00 ppm. During the polycondensation process, the nucleophilic attack of −OH groups in the diols at the carbonyl of ester and urethane linkages lead to the removal of low boiling alcohol (as methanol in the present case). The fast and efficient removal of methanol from the equilibrium drove the polycondensation to produce higher molecular weight chains. The comparison of NMR spectra revealed that $-OCH_3$ protons and carbon in the monomer had completely vanished in the polymer spectra (at 3.70−3.64 ppm), which confirmed the occurrence of the dual ester-urethane process as well as formation of high molecular weight polymers. ^{13}C NMR spectra of the monomer and polymers also confirmed the occurrence of the polycondensation. A similar NMR spectral analysis was done for all amino acid monomers in polycondensation with various diols and the details are provided in the Supporting Information (SF-3−5). Similarly FT-IR spectra of the polymers were recorded to confirm the formation of ester and urethane linkages (see Supporting Information, SF-6). The polymers showed peaks at 3317 and 1526 cm^{-1} with respect to N−H and C−N stretching frequencies, respectively. The peaks for ester and urethane linkages appeared at 1739 and 1687 cm^{-1}, respectively,[14] which confirmed the formation of expected poly(ester-urethane) structure.

The molecular weight of the newly synthesized amino acid poly(ester-urethane)s were determined by gel permeation chromatography (GPC) in THF. The GPC chromatograms are provided in the Supporting Information (SF-7) and their molecular weights are summarized in Table 1. Almost all the polymers showed monomodal distribution, indicating the formation of uniform molecular weight chains by the melt route (see SF-7). The molecular weight of the polymers were obtained in the range of M_n = 5.0−19.2 × 10^3 and M_w = 10.1−32.6 × 10^3 g/mol with the average polydispersity of 2.1. The number average degrees of polymerization (x_n) of the polymers were determined from the equation, $M_n = (M_o) \times (x_n)$, where M_o is the repeating units mass.[15] Based on the Carrothors equation,[15] $x_n = 1/(1 - P)$ for the step condensation process, where "P" is the percent conversion (or extent of the reaction), both x_n and p were obtained as 20−40 units and 96−98% of occurrence of reaction, respectively (see table 1). These values are very good for newly developed laboratory scale (1.0−2.0 g) melt polycondenstaion process. The molecular weights of the polymer samples were also determined in dimethylformaamide

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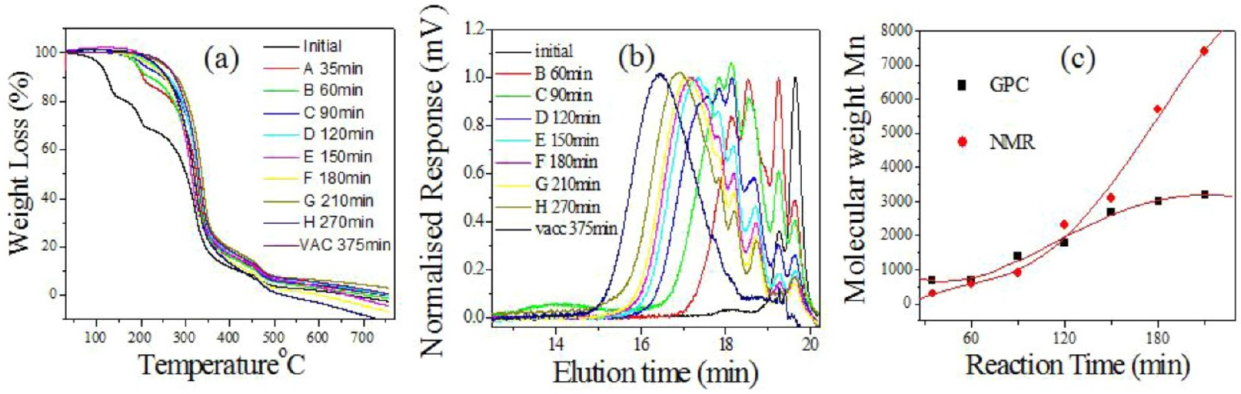

Figure 2. TGA profiles (a) and GPC chromatograms (b) of glycine monomer and 1,12-dodecane diol polymerization aliquots. The M_n determined by NMR and GPC technique were plotted for aliquots at various reaction times (c).

(DMF) GPC columns and their molecular weights are given in Table ST1 in the Supporting Information. The molecular weights determined by DMF columns were much higher up to M_w = 62000, which again confirmed that the new dual ester-urethane methodology developed in the present investigation very robust in producing high molecular weight polymers based on amino acid monomers. Further, the comparison of molecular weights in Table 1 indicated that oligoethylene diols produced relatively low molecular weight polymer chains compared to that of the linear aliphatic (1,12-dodecanol) and cycloaliphatic diols (CHDM). Among the amino acid monomers, phenylalanine produced very high molecular polymers with fibrous products compared to that of other counterparts.

Glycine monomer with 1,12-dodecane diol polymerization was carried out and aliquots were collected at various intervals to check the thermal stability and chain growing ability of the newly developed dual ester-urethane process. The TGA profiles (see Figure 2a) of the aliquots showed continuous enhancement of the thermal stability of the polymers with formation of higher molecular weight chains from 180 to 280 °C. The GPC chromatograms (see Figure 2b) of the aliquots also showed enhancement in the molecular weight and the polymer samples shifted from multimodel distribution to monomodel distribution with respect to the formation of high molecular weight chain lengths. The number average molecular weights (M_n) of the aliquots were determined by comparing the intensities of repeating unit peaks with end groups in the ^{1}H NMR spectra (see SF-8). From the expression, $M_n = x_n M_o$, where M_o is the repeating unit mass, the number average molecular weight (M_n) of polymer chains in the aliquots were estimated based on NMR. The molecular weight (M_n) obtained from GPC and NMR techniques was plotted against the reaction time and shown in Figure 2c. The molecular weight of the polymers linearly increased with time up to M_n = 3000 amu and showed sudden increase at larger reaction time (especially in the NMR data). The increase in the molecular weight at higher conversion was attributed to the rapid increase in the melt viscosity of the polymerization during the condensation process. The M_n based on NMR was much higher than that obtained from GPC in the higher molecular weight range. This suggested that the polystyrene standard used for the calibration of the GPC column (especially in THF solvent) could be underestimating the actual molecular weights of the polymers at higher conversion. Therefore, the molecular weight of the synthesized polymers could be much higher than that summarized in Table 1. Thus, the above studies confirmed that the newly developed dual ester-urethane process is thermally stable and is a very efficient synthetic methodology for amino acid polymers under solvent-free melt conditions.

End Group Analysis by MALDI-TOF-MS. The MALDI-TOF MS technique is a very powerful tool for studying the polymerization reaction via end group analysis. In typical A−A- and B−B-type melt polycondensation reactions, four different types of end groups are possible: (i) chain with AA ends, (ii) chain with BB ends, (iii) chain with AB ends, and (iv) macrocycles having the mass of repeating units (see scheme in SF-9).[16] To trace the functional groups in the chain ends, aliquots were collected at various intervals in the polymerization of glycine monomer and 1,12-dodecanediol and they were subjected to MALDI-TOF MS analysis. MALDI-TOF MS spectra of the aliquots corresponding to a nitrogen purge stage (1 h and end of the N_2 purge stage) and a vacuum stage are shown in Figure 3. Mass values corresponding to the polymer chain with end groups: A-$(P)_n$-A, B-$(P)_n$-B and A-$(P)_n$-B and cyclic $(P)_n$ were theoretically calculated for n = 1−12 and the

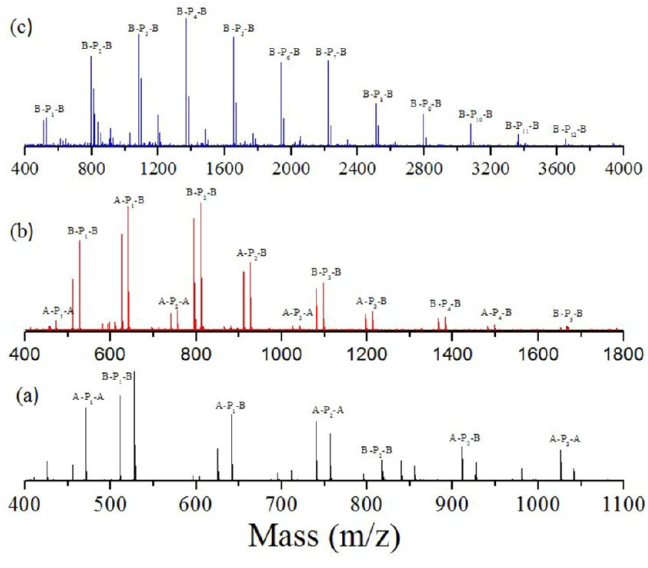

Figure 3. MALDI-TOF mass spectra of glycine monomer and 1,12-dodecane diol polymer at 1 h (a), end of nitrogen purge at 4 h (b) and at the vacuum stage at 6 h (c).

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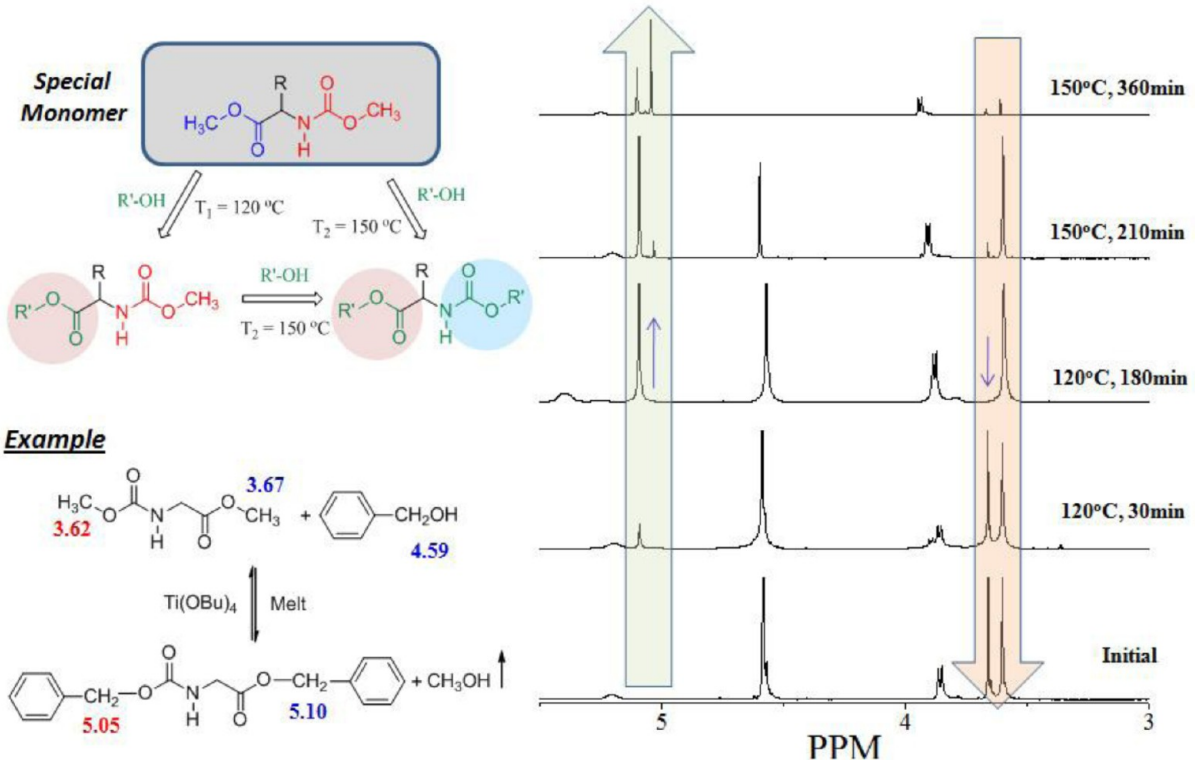

Figure 4. Model reaction of benzyl alcohol with glycine monomer at 120 and 150 °C. ^{1}H NMR spectra of the aliquots are given for various time intervals.

values are given in Table SF9. After a 1 h reaction time (see Figure 3a), the samples showed mass peaks in the range of m/z = 300−1100 amu with respect to the formation of oligomer species. The peaks were assigned (Na^+ and K^+ ions) to the chain-AA, chain-BB, and chain-AB end groups (for values, see Table SF-9). At the end of the nitrogen purge stage (in Figure 3b), the chain length increased up to five repeating units. Subsequent condensation under vacuum increased the molecular weight of the polymers and chain length with repeating units up to 12 were clearly visible in Figure 3c.

Few observations from the MALDI-TOF MS analysis were (i) the ester and urethane functional groups of the amino acid monomers were stable under high temperature melt polymerization process, (ii) the molecular weight of the polymers increased with increase in the reaction time, (iii) all three possible chain ends like AA, BB, and AB were involved in the condensation route and (iv) there is no macrocycle formation in the dual ester-urethane polycondensation process. The absence of macrocycle formation in the present melt condensation approach is a very crucial point. This is because polycondensation chemistry for other naturally occurring monomers like lactic acid[17] or NCA mediated route[9a] for amino acids were known to form macrocycles which hampered their high molecular weight formation. Interestingly, the amino acid monomers underwent linear chain formation rather than cyclization in the dual ester-urethane process which is very crucial for high molecular weight formation. Similarly, MALDI-TOF MS analysis was done for the polymerization reaction of 1, 12-dodecane diol with alanine (see SF-10). Thus, it could be concluded that the amino acid functional groups (urethane and ester groups) were thermally stable in the newly developed dual ester-urethane melt polymerization and produced high molecular weight polymers.

Temperature-Dependent Reactivity and Kinetics. To study the mechanism and the role of the melt temperature on the dual ester-urethane process; model reactions were carried as shown in Figure 4. Typically, temperature and catalyst are the two important parameters that drove the polycondensation synthetic process. The thermogravimetric analysis of the amino acid monomers showed that the ester and urethane linkages were thermally stable only up to 150−180 °C depending on their structures (see SF-11). Therefore, the melt reaction was performed at 80, 100, 120, and 150 °C and it was found that both ester and urethane units in the amino acid monomer were only active above 120 °C. Based on these finding, two temperatures were chosen for the model reaction at 120 and 150 °C. Benzylalcohol was selected as suitable alcohol for the condensation because it provided possibility for identification and quantification of ester and urethane products in the dual ester-urethane reaction by ^{1}H NMR. Glycine monomer was condensed with twice the amount of benzyl alcohol and the following approaches were adopted: (i) stepwise increase of the temperature at 120 and 150 °C in the presence of catalyst, (ii) stepwise increase of the temperature at 120 and 150 °C in the absence of catalyst and (iii) direct condensation at 150 °C in the presence of catalyst. Aliquots were taken at regular interval and they were subjected to ^{1}H NMR analysis to quantify the unreacted monomers and products. ^{1}H NMR spectra of the aliquots for the model reaction (i) are shown in Figure 4 [for model reactions (ii) and (iii), see SF-12 and SF-13].

In the initial mixture, peaks corresponding to the protons $C_6H_5CH_2OH$, gly-CH_2COOCH_3 (ester), and gly-HNCOO-CH_3(urethane) appeared at 4.59, 3.67, and 3.62 ppm, respectively (the chemical shift region above 5.5 not shown for simplicity). At 120 °C, only the carboxylic ester of the amino acid monomer underwent reaction with benzyl alcohol;

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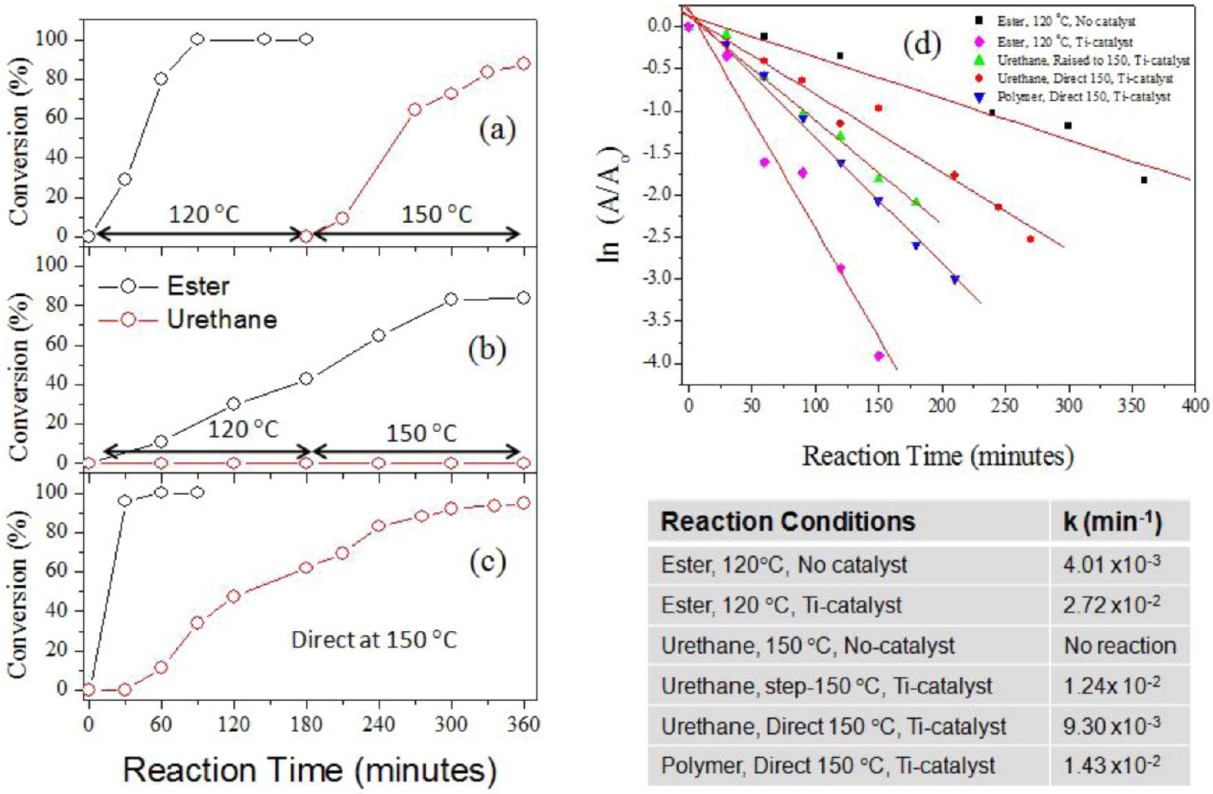

Reaction Conditions	k (min^{-1})
Ester, 120°C, No catalyst	4.01 x10^{-3}
Ester, 120 °C, Ti-catalyst	2.72 x10^{-2}
Urethane, 150 °C, No-catalyst	No reaction
Urethane, step-150 °C, Ti-catalyst	1.24x 10^{-2}
Urethane, Direct 150 °C, Ti-catalyst	9.30 x10^{-3}
Polymer, Direct 150 °C, Ti-catalyst	1.43 x10^{-2}

Figure 5. Plots of percent conversion vs reaction time in the presence of Ti-catalyst (a) and in the absence of catalyst (b) at stepwise 120−150 °C reaction, and direct condensation at 150 °C in the presence of Ti-catalyst (c). First order kinetic plots vs reaction time (d) and the rate constant (k) were determined from the slopes of linear fitting, and the values are summarized in the table.

as a result, the protons corresponding to ester part (at 3.67 ppm) disappeared and new peak with respect to protons in the product PhCH$_2$OOCCH$_2$-gly appeared at 5.10 ppm. During this ester-exchange process, the urethane part in the amino acid monomer was completely inert. Upon subsequent increase in the reaction temperature to 150 °C, the urethane functionality in the monomer became active and underwent reaction with benzyl alcohol to produce new urethane linkage PhC**H**$_2$OOCNH-gly. The disappearance of protons at 3.62 ppm of gly-HNCOOC**H**$_3$ and appearance of new peak at 5.05 ppm confirmed the formation of PhC**H**$_2$OOCNHCH$_2$-gly. Thus, it is clearly evident that the ester and urethane reactivity could be easily varied by adjusting the appropriate condensation temperature in the present dual ester-urethane process. The extent of the ester or urethane exchange reaction at particular temperature (either at 120° and 150 °C) over a period of reaction time was determined by comparing relative peak intensities in the gly monomer at 3.67 or 3.62 ppm. The extent of the reaction (or percent conversion) monomer was plotted against the reaction time in Figure 5. In the presence of catalyst (see Figure 5a), at 120 °C, the ester-exchange reaction occurred more than 98% within 90 min. Upon increasing the temperature of the same vessel to 150 °C (180 min was corresponding to the initial time for the reaction at 150 °C), the urethane unit became active and gave 98% conversion at the end of reaction. In the absence of catalyst, the ester part of the amino acid monomer underwent slow exchange with benzyl alcohol to produce 80% of product at the end of the reaction (see Figure 5b). However, the urethane unit was completely inert in the absence of catalyst throughout the reaction. Direct polycondensation at 150 °C (see Figure 5c), in the presence of catalyst, both ester and urethane exchange reaction occurred simultaneously. The ester exchange was very rapid (95% within 30 min) compared to the urethane exchange reaction which occurred over a period of 360 min.

These percent conversion data indirectly reflected on the composition and the amount of unreacted monomer at any given time. Therefore, these data could be directly utilized for determination of kinetic rate constant for the condensation process. The data were fitted in the first order ester exchange reaction using the logarithmic equation $\ln\{[A]/[A_o]\} = -kt$, where $[A]$ is the concentration of unreacted monomer at time "t", $[A_o]$ is the initial concentration of the monomer, and "k" is the rate constant. The plots of $\ln\{[A]/[A_o]\}$ versus "t" for all model reactions were given in Figure 5d. The rate constant for the model reaction were obtained from the slope of the plots and the values are summarized in the table in Figure 5. At 120 °C, the rate constant for ester-exchange reaction was obtained as 2.7×10^{-2}min^{-1}, which was in accordance with the literature values.[18] In the absence of the Ti-catalyst, the ester-exchange process was one order magnitude lower. The rate constants for the urethane-exchange reaction were obtained as of $0.9-1.2 \times 10^{-2}$ min^{-1}, which was almost half compared to its ester counterpart. The comparison of the rate constant values revealed that the ester exchange process was almost twice as fast as the urethane-exchange in the dual ester-urethane process of amino acid based monomers. The rate constant for the polymerization reaction was also determined using the aliquots data of glycine monomer with 1,12-dodecanol at 150 °C (see Figure 2b). Based on the procedure explained for model reactions, the rate constant for the polycondensation was obtained as 1.4×10^{-2} min^{-1} (see plot in Figure 5d). The rate

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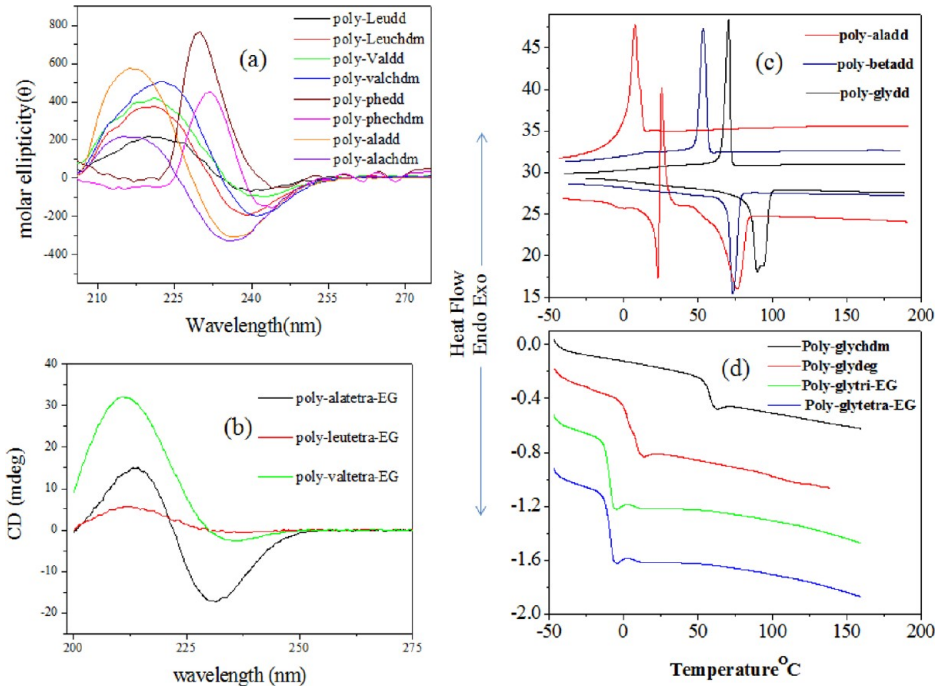

Figure 6. CD spectra of polymers in tetrahydrofuran (a) and in water (b) at 25 °C. DSC thermograms of the semicrystalline (c) and amorphous (d) polymers at 10°/min heating/cooling rates.

constants for the polymerization were almost comparable to that of the model reaction, hence, it may be concluded that both model reaction and polycondensation followed similar kinetics in the dual ester-urethane process. The above model reactions revealed that the amino acid monomers were very special for the condensation approach. Unlike the ester units, the urethane linkage is very selective to the temperature of the reaction (at 150 °C) and also need Ti-catalyst for the exchange reaction to occur. Hence, using appropriate reaction conditions, one could easily fine-tune the dual ester-exchange process for making bis- or triurethane small molecules as well as block copolymers based on naturally available amino acid starting materials. Currently, the dual ester-urethane synthetic methodology is continued in these directions.

Secondary Structures and Thermal Properties of Amino Acid Polymers. The newly synthesized polymers were derived from repeating units of L-amino acid monomers, and therefore, circular dichroism (CD) spectroscopic analysis was carried out for the polymers to investigate their ability to form secondary structures. Generally, CD measurements are based on the difference in the absorbance between right and left circularly polarized light of optically active molecules. In the present case, the ester-urethane chemical linkage of the chains absorbed in the far-UV region with three electronic transitions: (i) $n-\pi^*$ transition at ~220 nm polarized along the carbonyl bond, (ii) $\pi-\pi^*$ transition at 185−200 nm polarized in the direction of the C−N bond, and (iii) $\pi-\pi^*$ transition at 140 nm polarized approximately perpendicular to the C−N bond direction.[19] Typically, peptides showed a positive band at 192 nm and two negative bands at 208 and 222 nm with respect to α-helical conformation and the β-sheet structure exhibited a positive CD band at 195−198 nm and a negative CD band at 218 nm.[20a] Random coil structures are expected to show single negative CD band at 195 nm.[20a]

Among the amino acid monomers chosen for the present investigation, the monomers L-alanine, L-valine, L-leucine, and L-phenylalanine are optically active, and therefore, their polymers could be expected to have tendency to form self-assembled secondary structures like those observed in peptides and proteins. Polymers belonging to these amino acid monomers were subjected to CD studies in tetrahydrofuran, water, and methanol depending on their solubility. CD spectra of representative polymers in THF and water are shown in Figure 6 (for methanol, SF-14). In THF, all the polymers (except phenylalanine), showed one positive CD band at 210−215 nm for $\pi-\pi^*$ and a negative CD band around 230 nm for $n-\pi^*$ transition corresponding to the secondary structure in β-sheet conformation.[20a] The water-soluble polymers also showed CD bands, as observed in THF solvent, which confirmed that the β-sheet secondary structure was retained even in the aqueous medium. Phenylalanine-based polymers showed positive CD band at 230 nm, which was attributed to the aromatic side chains, and their spectral features resembled the polyproline type II helical coil conformation.[20b] The above results confirmed that the newly synthesized amino acid poly(ester-urethanes) are very efficient in forming secondary structures similar to that of polypeptides. Hence, these new polymers could be very useful for applications in biological systems.

TGA analysis of the polymers revealed that these newly synthesized polymers were thermally stable up to 280−300 °C (see SF-15).The thermal properties of the newly synthesized polymers were analyzed by DSC and few representative thermograms are shown in Figure 6c (see SF-16). Most of the poly(ester-urethanes) were sluggish to crystallize and showed only glass transition temperatures (T_g; see Table 1). Interestingly, polymers of 1,12-DD with glycine, alanine, and β-alanine showed clear melting and crystallization peaks under the heating/cooling cycles (see Figure 6c). Their transition temperatures and enthalpy values are given in Table ST-2 in the Supporting Information. The crystalline nature was attributed to the less steric hindrance provided by these amino acid units

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in the polymer backbone. Glycine-based polymer with PEGs showed a decrease in T_g with an increase in the length of the ethylene oxide units (see Figure 6d). The polymers synthesized from phenylalanine were found to have maximum T_g values, which is attributed to the high rigidity contributed by the amino acid repeating units. The thermal analysis suggested that by choosing appropriate amino acid based monomers and diols, the thermal properties of the poly(ester-urethane)s could be easily fine-tuned for various thermoplastic applications.

CONCLUSION

A dual ester-urethane condensation approach was successfully developed for biological monomers-amino acids to make new classes of thermoplastic polymers under solvent free polymerization methodology. L-aminoacids were converted into their corresponding ester-urethane monomers by simple tailor-made approach. These monomers were condensed with various commercial diols under melt polycondensation process in one-pot in two stages. In this process, oligomers of 1−4 repeating units were initially obtained under nitrogen purge which upon further polycondensation under reduced vacuum produced high molecular weight polymers. The occurrence of the melt dual ester-urethane process and the structure of the new poly(ester-urethane)s were confirmed by ^{1}H and ^{13}C NMR spectroscopies. The new dual ester-urethane condensation approach was demonstrated for a variety of amino acids: glycine, β-alanine, L-alanine, L-leucine, L-phenylalanine, and L-valine, along with commercial diols di-, tri-, and tetraethylene glycols, 1,12-dodecane diol (linear aliphatic diols), and 1,4-cyclohexanedimethanol (cycloaliphatic diols). The molecular weights of the polymers were obtained in the range of moderate to high with polydispersity ∼2.1. The percentage conversion of the new process was found to be more than 97-98% with respect to degree of polymerization, x_n = 30−40 units. The end group analysis by MALDI-TOF MS revealed that the amino acid functional groups (urethane and ester groups) were very much thermally stable in the dual ester-urethane melt polymerization process and produced high molecular weight polymers. The mechanism of melt dual ester-urethane process and the kinetics of the polycondensation were studied by model reactions using benzyl alcohol. It was found that the amino acid monomer was very special in the sense that their ester and urethane reaction selectivity could be tuned either by the catalyst or the polymerization temperature. The presence of Ti-catalyst and 150 °C are essential for the urethane-exchange reaction, whereas the ester-exchange could occur at much lower temperatures at 120 °C. The rate constant values revealed that the ester exchange process was almost twice as fast as the urethane-exchange in the amino acid based monomers. The thermal properties of the newly synthesized polymers obtained using 1,12-dodecane diol along with glycine, alanine, and β-alanine were found to be semicrystalline solid and showed melting and crystallization peaks. CD analysis of the synthesized polymers confirmed that these new poly(ester-urethane)s were efficient structures to produce self-organized β-sheets like polypeptides in water or organic solvents. In a nut shell, the new dual ester-urethane process is very robust in producing a thermally stable, high molecular weight β-sheet structure forming poly(ester-urethanes) from largely abundant naturally occurring biological monomers-amino acids. Further, the temperature-selective reactivity of the current process could be exploited for making tailor-made block copolymers and oligomers in amino acid chemistry. Thus, the new melt condensation approach will open up new platform of research activates based on amino acids in polymer synthesis literature.

ASSOCIATED CONTENT

Supporting Information

NMR data, FTIR spectra, HR-MS data, GPC chromatograms, MALDI-TOF mass spectra, TGA profile, circular dichorism (CD), and DSC profile, photographs of the reaction vessel, and structure of the polymers are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Deming, T. J. *Adv. Mater.* **1997**, *9*, 299−311.
(2) Yu, M.; Nowak, A. P.; Deming, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 12210−12211.
(3) Katz, J. S.; Zhong, S.; Ricart, B. G.; Pochan, D. J.; Hammer, D. A.; Burdick, J. A. *J. Am. Chem. Soc.* **2010**, *132*, 3654−3655.
(4) Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. *Chem. Rev.* **1999**, *99*, 3181−3198.
(5) Wadhvani, P.; Afonin, S.; Leronimo, M.; Buerck, J.; Ulrich, A. S. *J. Org. Chem.* **2006**, *71*, 55−61.
(6) Sun, H.; Meng, F.; Dias, A. A.; Hendriks, M.; Feijen, J.; Zhong, Z. *Biomacromolecules* **2011**, *12*, 1937−1955.
(7) (a) Guo, K.; Chu, C. C. *Biomacromolecules* **2007**, *8*, 2851−2861. (b) Guo, K.; Chu, C. C. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 1595−1606. (c) Deng, M..; Wu, J.; Reinhart-King, C. A.; Chu, C. C. *Biomacromolecules* **2009**, *10*, 3037−3047. (d) De Wit, M. A.; Wang, Z.; Atkins, K. M.; Mequanint, K.; Gillies, E. R. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 6376−6392.
(8) (a) Paredes, N.; Rodriguez-Galan, A.; Puiggali, J. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 1271−1282. (b) Asin, L.; Armelin, E.; Montane, J.; Rodriguez-Galan, A.; Puiggali, J. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *39*, 4283−4293.
(9) (a) Kricheldorf, H. R. *Angew. Chem., Int. Ed.* **2006**, *45*, 5752−5784. (b) Zhong, B.; Fischer, K.; Schmidt, M. *Macromol. Chem. Phys.* **2005**, *206*, 157−162. (c) Inoue, K.; Sakai, H.; Ochi, S.; Itaya, T.; Tanigaki, T. *J. Am. Chem. Soc.* **1994**, *116*, 10783−10784.
(10) Deming, T. J. *J. Am. Chem. Soc.* **1998**, *120*, 4240−4241.
(11) Deming, T. J. *Nature* **1997**, *390*, 386−389.
(12) Whinfield, J. R. *Nature* **1946**, *158*, 930.
(13) (a) Jayakannan, M.; Ramakrishnan, S. *Chem. Commun.* **2000**, *19*, 1967−1968. (b) Jayakannan, M.; Ramakrishnan, S. *Macromol. Chem. Phys.* **2000**, *201*, 759−767. (c) Jayakannan, M.; Ramakrishnan, S. *Macromol. Rapid Commun.* **2001**, *22*, 1463−1473. (d) Behera, G. C.; Ramakrishnan, S. *Macromolecules* **2004**, *37*, 9814−9820. (e) Roy, R. K.; Ramakrishnan, S. *Macromolecules* **2011**, *44*, 8398−8406.
(14) (a) Deepa, P.; Jayakannan, M. U.S. Patent 2007/0117950 A1, 2007. (b) Deepa, P.; Jayakannan, M. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 2445−2458. (c) Deepa, P.; Jayakannan, M. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2351−2366.
(15) Odian, G. *Principles of Polymerization*, 3rd ed.; John Wiley and Sons, Inc.: New York, 1991; Chapter 2, p 53.
(16) Jayakannan, M.; van Dongen, J. L. J.; Janssen, R. A. J. *Macromolecules* **2001**, *34*, 5386−5393.

(17) Chmura, A. J.; Chuck, C. J.; Davidson, M. G.; Jones, M. D.; Lunn, M. D.; Bull, S. D.; Mahon, M. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 2280−2283.
(18) (a) Collins, S.; Kenwright, A. M.; Pawson, C.; Peace, S. K.; Richards, R. W.; MacDonald, W. A.; Mills, P. *Macromolecules* **2000**, *33*, 2974−2980. (b) Yang, H.; He, J.; Liang, B. *J. Polym. Sci., Part B: Polym. Chem.* **2001**, *39*, 2607−2614.
(19) Violette, A.; Averlant-Petit, M. C.; Semetey, V.; Hemmerlin, C.; Casimir, R.; Graff, R.; Marraud, M.; Braiand, J. P.; Rongnan, D.; Guichard, G. *J. Am. Chem. Soc.* **2005**, *127*, 2156−2164.
(20) (a) Greenfield, N.; Davidson, B.; Fasman, G. D. *Biochemistry* **1967**, *6*, 1630−1637. (b) Shi, Z.; Olson, C. A.; Rose, G. D.; Baldwin, R. L.; Kallenbach, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9190−9195.