ACS Macro Letters
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Direct Poly(β-alanine) Synthesis via Polycondensation in Ionic Liquids

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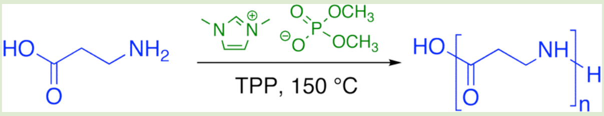
Supporting Information

ABSTRACT: Poly(β-alanine) was successfully synthesized by an alternative method, which is the direct polyamidation of β-alanine in ionic liquids with triphenylphosphite as a condensing agent. It was found that 1,3-dimethylimidazolium dimethylphosphate was the most suitable reaction medium, in which a number-average degree of polymerization up to 49.5 was obtained. It was shown that the method is also applicable to the direct synthesis of polypeptides, for example, poly(L-valine) and poly(L-isoleucine).



Naturally occurring β-amino acid β-alanine is found as a component of vitamin B5 and several proteins.[1] The oligomers and polymers of β-alanine derivatives are inherently protein mimetic[2] and can adopt secondary structures.[3] They have been attracting a lot of attention as biomimetic materials with potential biomedical applications.[3,4]

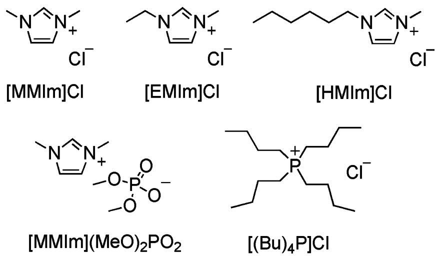
Poly(β-amino acid)s can be prepared by the ring-opening polymerization (ROP) of β-propiolactam and its derivatives in the presence of strong bases.[5] However, due to the acidity of active hydrogens, side reactions take place during the polymerization of the unsubstituted β-propiolactam, leading to chain branching. This method has therefore been mainly applied to substituted derivatives, with the recent exceptions of phosphazene-catalyzed[6] and of enzyme-catalyzed[7] polymerizations.

The synthesis of poly(β-alanine) by the base-catalyzed Michael polyaddition of acrylamide has been extensively investigated. It is usually carried out at 80−120 °C in aprotic solvents, like dimethylformamide (DMF), in the presence of a strong base catalyst, such as sodium *tert*-butoxide.[8,9] The reaction proceeds mainly by the addition of a primary amide anion to acrylamide double bond but also involves secondary amide anion addition, resulting in the formation of branched polymers.[10]

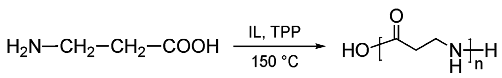
The polycondensation of β-alanine could be a straightforward method of poly(β-alanine) synthesis. However, this polymer is insoluble in most organic solvents and undergoes degradation reactions before melting and, therefore, cannot be prepared by conventional polycondensation. During recent years, ionic liquids (ILs) have drawn wide attention, as they are nonvolatile, easily recyclable reaction media and have high thermal and chemical stabilities.[11] Many chain polymerizations and polycondensations have been investigated in ILs.[12,13] Aromatic polyamides were synthesized in ILs from diamines and diacid chlorides at low temperatures (0−60 °C)[13] or from diamines and diacids with addition of triphenyl phosphite (TPP) at higher temperatures.[14]

On the basis of these investigations, we report here which is, to our best knowledge, the first example of β-alanine direct polycondensation. The reaction was carried out in various ILs (Chart 1) in the presence of a stoichiometric amount of condensing agent, triphenyl phosphite (TPP; Scheme 1). To get insight into the polymer structure, the resulting polymers were carefully examined by NMR spectroscopy and matrix-assisted desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

Chart 1. Ionic Liquids Used in the Current Study



Scheme 1. Synthesis of Poly(β-alanine) in Ionic Liquid in the Presence of TPP



Received: May 25, 2012
Accepted: July 31, 2012
Published: August 7, 2012

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As expected for polyamides, the polymers were insoluble in common organic solvents.[8] They were not soluble in polyamide solvents either, such as chloroform trifluoroacetic anhydride mixtures, but were soluble in pure formic acid.

The Fourier transform infrared (FTIR) spectra of the resulting products reflect the formation of a polyamide, as two intense bands at 3291 cm^{-1} (s) and 1629 cm^{-1} (s) can be observed, which correspond to typical N—H and C═O amide stretching vibrations.

The MALDI-TOF mass spectra (Figure 1) of the yielded products show a distribution of peaks with a mass increment of $m/z = 71.07$, which corresponds exactly to one monomer unit. Taking the peak at $m/z = 1674.70$ for instance, it corresponds to $H-[NH-CH_2-CH_2-CO]_{23}-OH,Na^+$, that is, the macromolecule with 23 β-alanine repeating units cationized by Na^+. It proves that poly(β-alanine) was produced by direct polymerization in ILs.

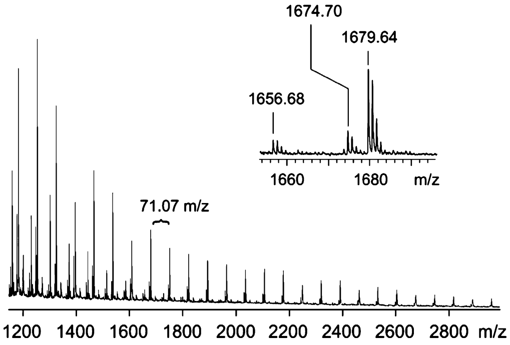
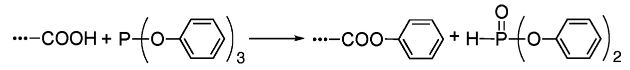


Figure 1. Polymerization of β-alanine in [HMIm]Cl at 150 °C for 18 h (β-alanine/IL = 1:2 wt/wt, β-alanine/TPP = 1:1 mol/mol): MALDI-TOF mass spectrum of resulting poly(β-alanine).

Carboxylic acids react with TPP during the formation of polyamide, forming phenyl ester and diphenyl phosphite (DPP; Scheme 2).[15] Phenyl ester was formed in the current study as well, since a series of peaks corresponding to $H-[NH-CH_2-CH_2-CO]_n-OC_6H_5,Na^+$ is detected in the MALDI-TOF mass spectrum, with an example at 1679.64 m/z ($n = 22$) (Figure 1). As expected for a polycondensation process,[16] macrocyclic poly(β-alanine) species were also formed during the polymerization. For example, the peak at 1656.68 m/z corresponds to the cyclic poly(β-alanine) molecule with 23 repeating units. Therefore, the MALDI-TOF MS study confirms that poly(β-alanine) was synthesized in the ILs and shows that both carboxylic acid- and phenyl ester-terminated polymers were formed during the reaction, together with cyclic species.

Scheme 2. Formation of Phenyl Ester and DPP during β-Alanine Polymerization



The ^1H NMR spectra of the polymers (Figure 2) present classical polyamide patterns with broad peaks at 2.31 and 3.25 ppm, assigned to the methylene groups of repeating units (H^2 and H^3, respectively). The end groups can be detected as well: There is a resonance at 3.31 ppm, coupled with another resonance at 2.28 ppm that overlaps with H^2 in the zone of $-CH_2-CO-NH-$ resonances and can be assigned to the methylene H^{14} in β position to amine end groups (Supporting Information, Figure S2, 2D $^1H-^1H$ COSY-45 NMR spectrum). The peak at 3.31 ppm corresponds, therefore, to the methylene H^{15} in the α position to amine end groups. Two triplets of low intensity are present at 2.52 and 2.61 ppm, which can be assigned to the methylene connected to either the carboxylic acid or the phenyl ester chain ends detected in the MALDI-TOF mass spectrum. The resonance at 2.52 ppm is assigned to the $-CH_2-COOH$ methylene (H^6), with the help of the ^1H NMR spectrum of *N,N′*-(*Z*-octadec-9-endioyl)bis(β-alanine) (**1**), a model compound which presents a $-CONH-CH_2-CH_2-COOH$ methylene resonance at 2.53 ppm in formic acid solution. The quadruplet at 3.19 ppm, coupled with H^6 in the 2D $^1H-^1H$ COSY-45 NMR spectrum, is then easily assigned to the methylene (H^7) in the β position to carboxylic acid end groups. The remaining triplet at 2.61 ppm is consequently assigned to the methylene H^{10} of phenyl ester chain ends. The three peaks present in the aromatic zone between 6.7 and 7.1 ppm (H^{17} (6.76 ppm), H^{19} (6.93 ppm), and H^{18} (7.06 ppm)) reflect the presence of the corresponding phenyl groups. It is worth mentioning that their integrations correspond exactly to the required values for phenyl ester end-groups, as expected from H^{10} integration. The quadruplet at 3.37 ppm, coupled with H^{10} is finally assigned to H^{11}. These assignments were confirmed by the 2D HSQC and HMBC NMR spectra. This NMR study demonstrates, therefore, that poly(β-alanine) with the expected structure was obtained and that the carboxylic acid end groups were partially modified to the corresponding phenyl ester.

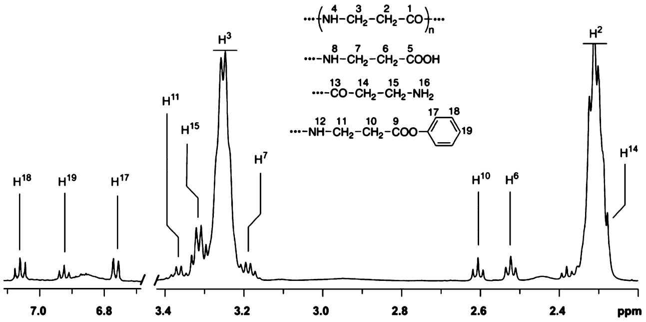


Figure 2. Polymerization of β-alanine in $[(Bu)_4P]Cl$ at 150 °C for 2 h (β-alanine/IL = 1:2 wt/wt, β-alanine/TPP = 1:1 mol/mol): ^1H NMR spectrum of resulting poly(β-alanine) (500 MHz, formic acid with external acetone-d_6, ref δ (acetone-d_6) = 2.05 ppm).

The solubility of β-alanine in different ILs was tested at 150 °C. The monomer showed limited solubility in the ILs with the Cl^- anion ([MMIm]Cl, [EMIm]Cl, and [HMIm]Cl) but was soluble in $[MMIm](MeO)_2PO_2$, showing that its solubility strongly depends on IL anion structure. The results of β-alanine polymerization in different ILs are presented in Table 1.

The number-average degree of polymerization (DP_n) was determined from the ratio of the integrations of repeating units and end-group resonances. When the reaction was conducted without TPP, no polymer was formed (Table 1, entries 1 and 2), while in the presence of TPP poly(β-alanine) was obtained in all of the ionic liquids. When the reaction was carried out during 2 h, no obvious difference was observed between [EMIm]Cl and [HMIm]Cl, as the DP_n of poly(β-alanine) was respectively 12.2 (entry 6) and 13.5 (entry 8). On the other hand, when the reaction time was prolonged to 18 h, DP_n increased to 31.3 in[EMIm]Cl (entry 7) but remained around 20 in [HMIm]Cl (entry 9). The polymerization in [MMIm]Cl

Table 1. Polymerization of β-Alanine at 150 °C, with β-Alanine/IL (w/w) = 1:2 and β-Alanine/TPP (mol/mol) = 1:1. Yields and Number-Average Degrees of Polymerization (DP_n) Obtained by ^1H NMR

entry	ionic liquid	time (h)	DP_n	yield[b] (%)
1[a]	[HMIm]Cl	18		
2[a]	[MMIm]$(MeO)_2PO_2$	18		
3	[MMIm]Cl	2	28.4	67
4	[MMIm]Cl	8	29.5	87
5	[MMIm]Cl	18	27.8	94
6	[EMIm]Cl	2	12.2	68
7	[EMIm]Cl	18	31.3	82
8	[HMIm]Cl	2	13.5	65
9	[HMIm]Cl	18	20.0	77
10	$[(Bu)_4P]Cl$	2	17.2	65
11	$[(Bu)_4P]Cl$	8	19.2	67
12	$[(Bu)_4P]Cl$	18	19.9	71
13	[MMIm]$(MeO)_2PO_2$	2	24.5	66
14	[MMIm]$(MeO)_2PO_2$	8	35.5	75
15	[MMIm]$(MeO)_2PO_2$	18	49.5	83

[a] Reaction carried out without TPP. No polymer was formed. [b] Yield of resulting product after purification.

was more efficient at the beginning of the reaction than those in both [EMIm]Cl and [HMIm]Cl, as the DP_n was around 28 after 2 h (Entry 3) but remained almost constant when it was heated for a longer time (Entries 4 and 5). Increasing the alkyl chain length of imidazolium ILs with Cl^- counterion does not favor the reaction. Poly(β-alanine) was obtained in $[(Bu)_4P]Cl$ as well, and the DP_n remained around 20 after 8 and 18 h (entries 11 and 12). Comparing to other ILs, the polymerization was much more efficient in [MMIm]$(MeO)_2PO_2$, as a DP_n of 24.5 was obtained after 2 h (entry 13) and went up to 49.5 when the reaction was carried out for 18 h (entry 15).

This direct synthesis approach in ionic liquids was also employed to prepare several polypeptides from their corresponding α-amino acids. For example, poly(L-isoleucine) and poly(L-valine) were successfully obtained from L-isoleucine and L-valine, when the reactions were carried out in a very common ionic liquid [EMIm]Cl.

The MALDI-TOF MS study confirms that poly(L-isoleucine) was obtained in the IL, as the mass spectrum of the product (Figure 3) shows a distribution of peaks with a mass increment of m/z = 113.07, which corresponds exactly to one L-isoleucine monomer unit. It was also found that macromolecules with various end-groups are present. Taking the peak at m/z = 1850.34 for example, it corresponds to $H-[NH-CH(iso\text{-}C_4H_9)\text{-}CO]_{16}-OH,Na^+$, that is, the macromolecule with 16 L-isoleucine repeating units cationized by Na^+. Another species $H-[NH-CH(iso\text{-}C_4H_9)\text{-}CO]_{16}\text{-}ONa,Na^+$, corresponds to the peak at m/z = 1872.32. Macrocyclic poly(L-isoleucine) species were also formed during the polymerization. For example, the peak at 1832.33 m/z corresponds to the cyclic poly(L-isoleucine) molecule with 16 repeating units. Furthermore, phenyl ester was formed as well, as a series of peaks corresponding to $H-[NH-CH(iso\text{-}C_4H_9)-CO]_n-OC_6H_5,Na^+$ is detected, with an example at 1813.29 m/z (n = 15). In a word, as previously mentioned in the study of poly(β-alanine), carboxylic acid- and phenyl ester-terminated polymers, as well as cyclic species, were formed during the polymerization of L-isoleucine.

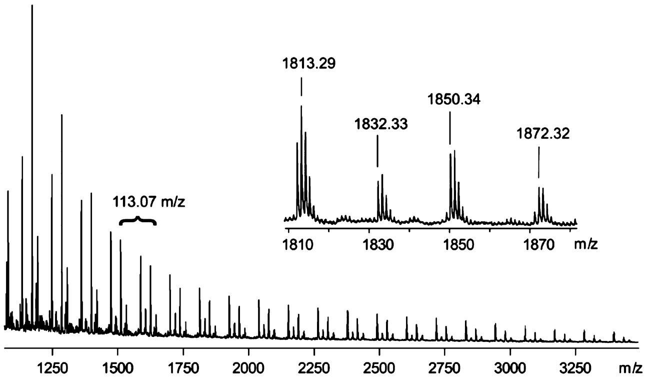


Figure 3. Polymerization of L-isoleucine in [EMIm]Cl at 150 °C for 18 h (L-isoleucine/IL = 1:2 wt/wt, L-isoleucine/TPP = 1:1 mol/mol): MALDI-TOF mass spectrum of resulting poly(L-isoleucine).

In conclusion, unsubstituted poly(β-alanine) was synthesized for the first time, using a straightforward solution polymerization of β-alanine in ionic liquids in the presence of TPP. Polymers with a number-average degree of polymerization up to 49.5 were obtained. As generally observed in conventional polyamidation, small amounts of macrocyclic species were also formed in the ionic liquids. It was also found that part of the carboxylic acid end groups was modified to its corresponding phenyl ester, which might be involved in the reaction mechanism. This is the first example of direct polycondensation of β-alanine. It was also found that this straightforward method can be applied to the synthesis of polypeptides from natural α-amino acids, such as L-isoleucine and L-valine.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental and characterization details; IR, 2D NMR, and MALDI-TOF MS spectra of poly(β-alanine); MALDI-TOF MS spectrum of poly(L-valine). 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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