Revolutionary Phosgene-Free Synthesis of α-Amino Acid *N*-Carboxyanhydrides Using Diphenyl Carbonate Based on Activation of α-Amino Acids by Converting into Imidazolium Salts

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INTRODUCTION

N-Carboxylic anhydrides of α-amino acids (NCAs) are highly useful monomers, because their living anionic ring-opening polymerizations permit precise construction of various polymer architectures having well-defined polypeptide sequences.[1–10] Currently, NCAs are synthesized conveniently by treatment of α-amino acids with phosgene, phosgene dimer, or phosgene trimer.[11–14] These carbonyl sources are highly reactive, but at the same time, they are lethally toxic so that their uses are limited to only small-scale operations. Another method established eariler than the phosgene method is "Leuch's method," which is based on the cyclization of *N*-alkoxycarbonyl amino acid chloride.[15] This method allows a straightforward access to NCA; however, its application to industrial production of NCA would be difficult, because of the requirement of the use of corrosive halogenating reagents (such as PBr_3, PCl_5, and $SOCl_2$) and the formation of carcinogenic alkyl halides as a result of the cyclization.

For industrial production of NCAs, development of new methods with using safer reagents without formation is strongly desired. As a phosgene-alternative, we have focused our attention on diphenyl carbonate (DPC): DPC is a much less toxic and inexpensive chemical, which can be produced by a completely phosgene-free process from ethylene oxide, phenol, and carbon dioxide.[16–18] We examined the reactivity of α-amino acids with DPC; however, despite surveying various conditions, they did not react with DPC at all. On the other hand, amino acids were successfully converted to NCAs by treatment with analogous but much more electrophilic carbonates such as bis(2,4-dinitrophenyl)carbonate.[19,20] These results implied that the protonated amino group of α-amino acids in a zwitter-ionic form was not enough nucleophilic to react with less electrophilic DPC. Another barrier was the insoluble nature of α-amino acids, which was so serious to be overcome by weak electrostatic interaction between α-amino acids and DPC. To accomplish this challenging task, that is, use of DPC as a safer and less-expensive phosgene-alternative for NCA synthesis, we designed a new route that consisted of three steps (Scheme 1): The first step is derivation of α-amino acids in a zwitter ionic form into their imidazolium salts **1**, which were reported by Ohno and co-workers.[21,22] We considered that this derivation can be regarded as a facile method to liberate the amino group from the protonation, and at the same time, as a convenient method to transform α-amino acids into "soluble" derivatives. The second step is a reaction of the free amino group of **1** with DPC to obtain the corresponding urethane derivative **2**, and the third step is the cyclization of **2** to give NCA **3**.

RESULTS AND DISCUSSION

The imidazolium salts of various α-amino acids were readily prepared according to the reported method by Ohno and co-workers.[21,22] All of the obtained imidazolium salts herein were soluble in chloroform, dichloromethane, 2-butanone, acetonitrile, and not soluble in tetrahydrofuran. *N*-Carbamoylation of the obtained imidazolium salts **1** with DPC was successfully conducted in acetonitrile, which can dissolve L-phenylalanine imidazolium salt **1a**, to give the corresponding **2a** within 10 min without any side reactions. ^{1}H- and ^{13}C-NMR of **2a** are shown in Figure S1 (Supporting Information). To confirm that this process was free from racemization, **2a** was treated with trimethylsilyl chloride in dry methanol to obtain the corresponding methyl ester **4a** in Figure 1(a),[23] and its optical purity was studied by high-performance liquid chromatography (HPLC) equipped with a column of chiral stationary phase. The resulting chromatogram, which is shown in Figure 1(b) along with that for the

Additional Supporting Information may be found in the online version of this article. Correspondence to: T. Endo (E-mail: tendo@mol-eng.fuk.kindai.ac.jp)

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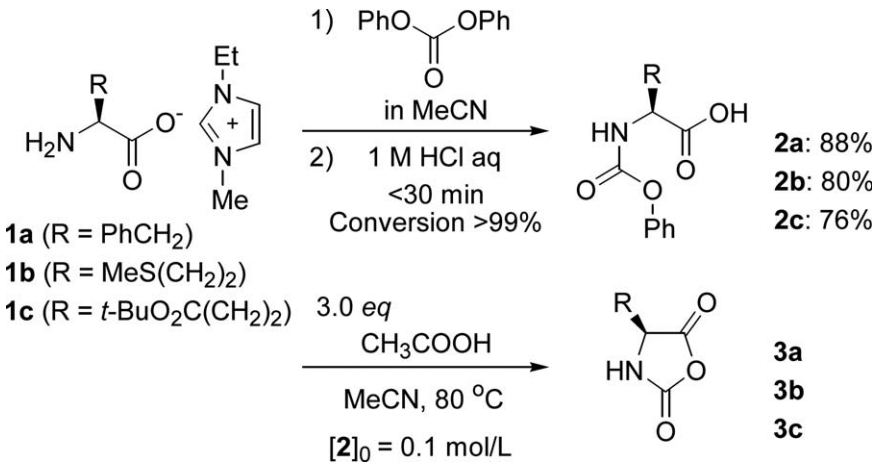

SCHEME 1 Synthesis of urethane derivatives **2** by reaction of imidazolium salts of α-amino acids **1** with diphenylcarbonate and their cyclization reactions.

authentic racemic mixture of **2a** synthesized from DL-phenylalanine, clearly confirmed that racemization of the chiral center during the *N*-carbamoylation step was negligible. In a similar way, the other imidazolium salts **1b** and **1c** were also successfully converted to the corresponding urethanes **2b**–**2c**. The reactions completed within 30 min, and the urethanes were isolated in approximately 80% yield.

The last highlight of this work was the selective cyclization of the urethane derivatives **2** into NCA. First, **2a** was heated in acetonitrile at 80 °C to find whether it underwent the cyclization very slowly. As a result, the corresponding NCA **3a** was obtained in 9% yield at 40 h (Fig. 2). Encouraged by this result, we performed the reaction at higher temperature for prolonged reaction time; however, it was interfered by serious oligomerization of the formed NCA. Addition of basic compounds such as triethylamine and sodium bicarbonate for the purpose of enhancing the nucleophilicity of the carboxyl group was also not suitable, because they induced further serious oligomerization of the formed NCA. Eventually, we examined addition of acidic compounds and discovered that acetic acid (AA) was effective additive to accelerate the cyclization reaction of **2a** into the corresponding NCA **3a**. In Figure 2, the time-dependence of the yield of **3a**, which was measured by ^{1}H-NMR, is shown. A series of the corresponding spectra are shown in Figure 3. As Figure 2 shows, the addition of a 3.0 equivalent amount of AA resulted in complete transformation of **2a** into the

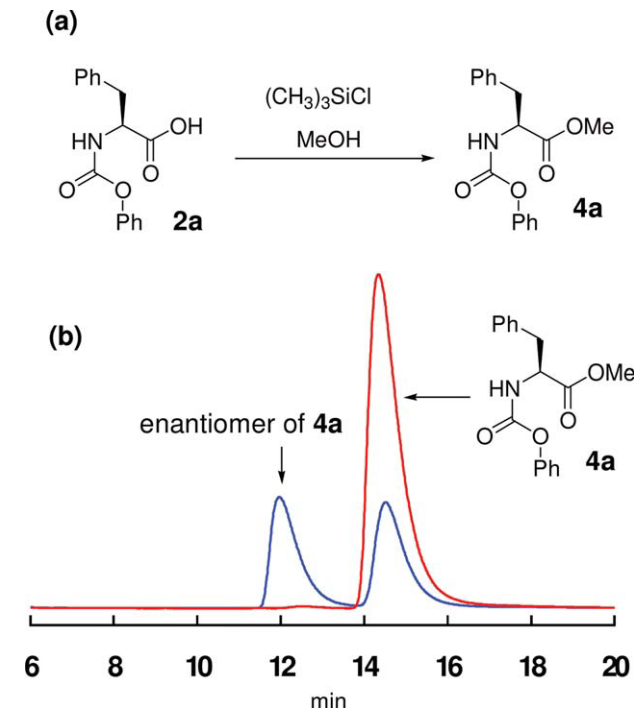

FIGURE 1 (a) Derivation of urethane **2a** into the corresponding methyl ester **4a**. (b) HPLC chromatogram of L-phenylalanine-derived methyl ester **4a** and that of DL-phenylalanine-derived methyl ester rac-**4a** (column: Daicel Chiralpak OD-H, eluent: hexane/2-propanol = 5/2 (v/v)).

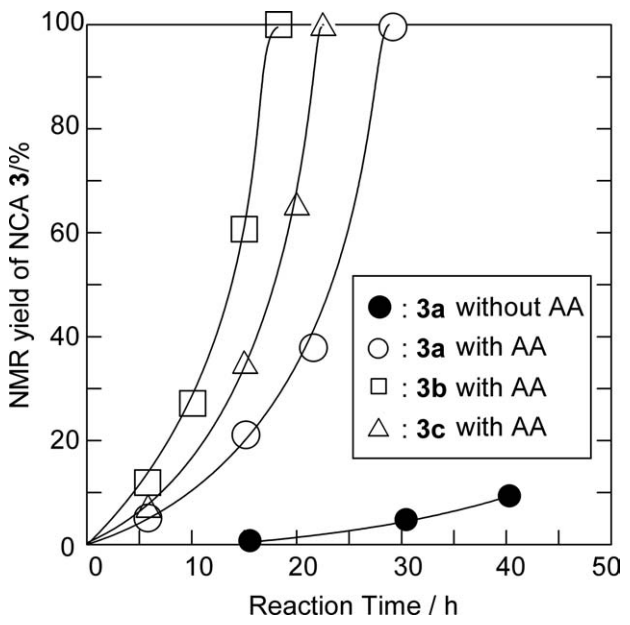

FIGURE 2 Time-conversion relationships for the cyclization of the carbamate **2a** into NCA **3a** at 60 °C.

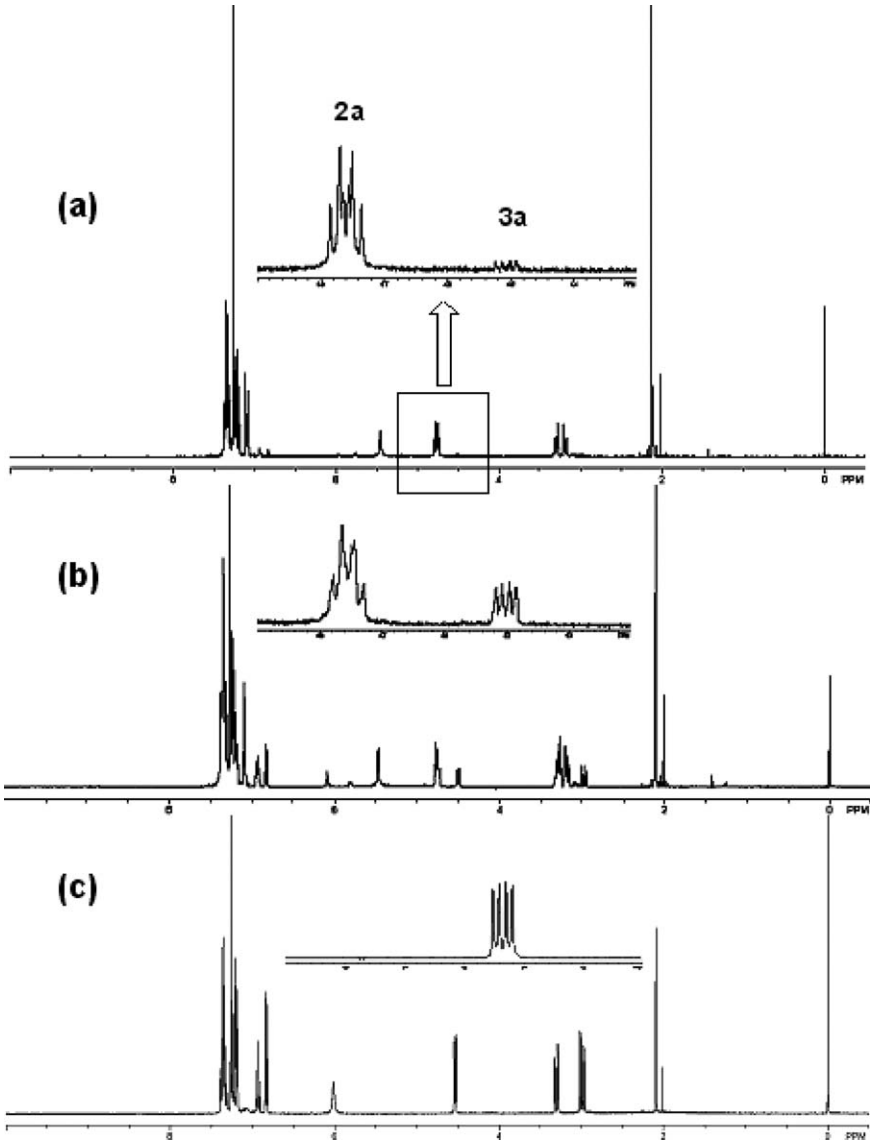

FIGURE 3 ^{1}H-NMR spectra of the reaction mixture (a) after 6 h, (b) after 15 h, and (c) after 29 h in the intramolecular cyclization of **2a** into NCA **3a**.

corresponding NCA **3a** within 30 h. This acceleration effect by AA was quite unexpected, and its mechanism is not clear, but it may involve enhancement of electrophilicity of the urethane moiety by the added carboxylic acid. In a similar way, the cyclization reactions of L-methionine-derived urethane **2b** and γ-*t*-butyl-L-glutamate-derived urethane **2c** were performed. As shown in Figure 2, **2b** and **2c** were smoothly converted to the corresponding NCAs **3b** and **3c**, respectively.

The cyclization of urethanes **2** was accompanied by formation of an equimolar amount of phenol, and, thus, NCAs **3** were obtained as a mixture with phenol. We found that phenol was efficiently removed by adsorption on activated charcoal to permit facile isolation of NCA: The cyclization of **2a** was performed in acetonitrile with using AA as a promoter as was described above. To the resulting reaction mixture containing NCA **3a** and phenol, 1 *M* hydrochloric acid (1/10 volume of the solution) was added. By addition of hydrochloric acid, hydrophilicity of the medium was increased to enhance hydrophobic interaction between phenol and charcoal. Then, activated charcoal (1250 wt % to the theoretical weight of phenol released by the cyclization reaction) was added to the mixture. The mixture was filtered to obtain a solution of NCA **3a** free from phenol, from which the volatiles (acetonitrile and AA) were removed under reduced pressure to obtain NCA **3a** in 78% yield. Its ^{1}H-NMR spectrum is shown in Figure S2 (Supporting Information). NCA **3a** was stable even in the presence of water, if the medium was enough acidified. The melting point of the obtained phenylalanine NCA was 89.1–90.4 °C, which was in good agreement with the reported one (88–90 °C)[24] to confirm the high purity of the obtained NCA.

Summary

In summary, we developed a new "phosgene-free" synthetic route from α-amino acids to NCAs with using DPC, a much less toxic and more easily available carbonyl source than phosgene. All the steps are free from toxic and corrosive

reagents, free from special caution to moisture, and free from toxic by-products. This "safety" ensured in the present phosgene-free synthesis will open up a new vista of the future industrial production of NCAs and its contribution to the prospect of polypeptide-related science and technology.

EXPERIMENTAL

Materials and Measurements

L-Phenylalanine, L-methionine, 1-ethyl-3-methylimidazolium bromide, AA, D,L-phenylalanine, and activated charcoal were purchased from Wako Pure Chemical Industries (Wako). γ-*t*-Butyl-L-glutamic acid was purchased from Watanabe Chemical. Amberlite IRA 400 CL (anion exchange resin) was purchased from Aldrich. Diphenylcarbonate was purchased from Tokyo Chemical Industry Co. These reagents were used as received. Acetonitrile was purchased from Wako Pure Chemical Industries and dried over calcium hydride and then distilled before use. ^{1}H-NMR (400 MHz; $\delta_{\text{tetramethylsilane}} = 0.00$ ppm, $\delta_{\text{chloroform}} = 7.26$ ppm) and ^{13}C-NMR (100.6 MHz; $\delta_{\text{chloroform}} = 77.00$ ppm) were recorded on a Varian NMR spectrometer model Unity INOVA. HPLC analysis was performed on JASCO Intelligent LC1500 with detection at 258 nm at 20 °C, with using a chiral column (Daicel Chiralpak OD-H). The flow rate was 1.0 mL/min. Optical rotation was measured with JASCO DIP-1000 at 21.6 °C, using a quartz cell of 100 mm length.

Synthesis of Amino Acid Imidazolium Salt 1

Typical procedure is as follows: 1-Ethyl-3-methylimidazolium bromide (4.77 g, 25.0 mmol) was dissolved in water (25 mL) and was passed through a column of Amberlite IRA 400 CL (50 cm^3) using 250 mL of water as an eluent. The eluted solution of 1-ethyl-3-methylimidazolium hydroxide (25.0 mmol) was added dropwise to a solution of L-phenylalanine (4.96 g, 30.0 mmol) in water at 0 °C, and the resulting solution was stirred at 0 °C. After 12 h, the solution was concentrated under reduced pressure with heating at 40–50 °C. To the resulting residue, acetonitrile (400 mL) and methanol (50 mL) were added, and the resulting mixture was stirred vigorously at 0 °C. By this process, unconsumed L-phenylalanine precipitated out of the solution. The mixture was filtered, and the filtrate was concentrated under reduced pressure and then dried *in vacuo* for 5 h at 60 °C to obtain L-phenylalanine imidazolium salt **1a** (5.78 g, 21.2 mmol, 84%).

L-Methionine imidazolium salt **1b** (4.99 g, 19.3 mmol, 77%) was obtained in a manner similar to that of L-phenylalanine imidazolium salt **1a**. Similarly, from γ-*t*-butyl-L-glutamic acid (2.03 g, 12.0 mmol) and 1-ethyl-3-methylimidazolium bromide (1.91 g, 10.0 mmol), γ-*t*-butyl-L-glutamic acid imidazolium salt **1c** (2.97 g, 9.47 mmol, 95%) was obtained.

Synthesis of Urethane Derivative 2

Typical procedure is as follows: To a solution of diphenylcarbonate (427 mg, 2.00 mmol) in acetonitrile (5 mL), a solution of **1a** (550 mg, 2.0 mmol) in acetonitrile (15 mL) was added dropwise at room temperature, and the reaction mixture was stirred at room temperature. After 15 min, the reaction mixture was poured into 1 *M* hydrochloric acid. The mixture was concentrated under reduced pressure, and the resulting residue was transferred into a separating funnel with diluting with water (25 mL) and ethyl acetate (50 mL). The ethyl acetate layer was separated, and the aqueous layer was extracted with 50 mL of ethyl acetate twice. The combined organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was charged on a silica gel column (4 cm × 15 cm) and eluted with ethyl acetate/*n*-hexane = 1/3 to remove phenol first; then, **2a** was eluted with ethyl acetate. The ethyl acetate solution was concentrated under reduced pressure to obtained **2a** (502 mg, 1.76 mmol, 88%) as a white solid: $[\alpha_D^{20}] + 91.2$ (in chloroform, $c = 1.0$); ^{1}H-NMR ($CDCl_3$, δ in ppm): 3.00–3.35 (m, 2H), 4.76 (m, 1H), 5.45 (d, $J = 8.2$ Hz, 1H), 7.09 (d, $J = 7.7$ Hz, 2H), 7.24 (m, 3H), 7.34 (m, 5H); ^{13}C-NMR ($CDCl_3$, δ in ppm) 37.60, 54.66, 121.5, 125.6, 127.4, 128.8, 129.30, 129.33, 135.3, 150.7, 154.2, 176.2.

From L-methionine imidazolium salt **1b** (519 mg, 2.00 mmol), the corresponding urethane **2b** (439 mg, 1.62 mmol, 82%) was obtained as a white solid: ^{1}H-NMR ($CDCl_3$, δ in ppm): 2.02–2.34 (m, 5H), 2.65 (m, 2H), 4.58 (m, 1H), 5.45 (d, $J =$ 8.3 Hz, 1H), 7.10–7.42 (m, 5H).

From γ-*t*-butyl-L-glutamic acid imidazolium salt **1c** (627 mg, 2.00 mmol), the corresponding urethane **2c** (470.1 mg, 2.00 mmol, 76%) was obtained as a white solid: ^{1}H-NMR ($CDCl_3$, δ in ppm): 1.45 (s, 9H), 1.98–2.32 (m, 2H), 2.45 (m, 2H), 4.45 (m, 1H), 5.89 (d, $J = 8.3$ Hz, 1H), 7.15–7.42 (m, 5H).

Derivation of Urethane 2a into the Corresponding Methyl Ester 4a

To a solution of the alanine-derived urethane **2a** (285 mg, 1.00 mmol) in dry methanol (10 mL), trimethylsilyl chloride (0.297 mL, 2.33 mmol) was added at room temperature, and the resulting solution was stirred at room temperature. After 18 h, the solution was concentrated under reduced pressure to obtain the corresponding methyl ester **4a** (294 mg, 98%) as a colorless oil: $[\alpha_D^{25}] + 83.4$ (in chloroform, $c = 1.0$); ^{1}H-NMR ($CDCl_3$, δ in ppm) 3.00–3.35 (m, 2H), 3.77 (s, 3H), 4.76 (m, 1H), 5.45 (d, $J = 8.2$ Hz, 2H), 7.09 (d, $J = 7.7$ Hz, 2H), 7.24 (m, 3H), 7.34 (m, 5H). Optical purity of the resulting methyl ester **4a** was 99% e.e., which was determined by HPLC analysis with chiral column (Daicel Chiralpak OD-H). A mixture of hexane and 2-propanol (5/2 in v/v) was used as the eluent.

Intramolecular Cyclization of 2 into NCA 3 and Its Isolation

Typical procedure is as follows: In an oven-dried glassware, **2a** (570 mg, 2.00 mmol) and AA (360 mg, 6.00 mmol) **2a** were dissolved in acetonitrile (20 mL), and the resulting solution was stirred at 80 °C under nitrogen. During the reaction, several portions of the reaction mixture were taken out at prescribed times, concentrated under reduced pressure, and dissolved in $CDCl_3$ for ^{1}H-NMR analysis. The characteristic multiplet signal for the methine proton of NCA was observed at 4.4 ppm, and this signal was used for calculating NMR yields of NCA. The reaction mixture was cooled to room temperature, and then 1 M HCl solution (2.0 mL)

and activated charcoal (2.4 g) were added to the reaction mixture. After stirring for 1 h at room temperature, the mixture was filtered, and the filtrate was concentrated under reduced pressure to obtain NCA **3a** (302 mg, 1.56 mmol, 78%) as a white solid: m.p. = 89.1–90.4 °C.

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