A Facile Synthesis of *N*-Carboxyanhydrides and Poly(α-amino acid) Using Di-*tert*-butyltricarbonate

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The chemistry of amino acids and peptides is important in the structural elucidation and synthesis of oligo- and polypeptides with useful biological function.[1] We can now obtain optically pure amino acids for low prices due to recent remarkable improvements in the technology in fermentation and organic synthesis. Consequently, amino acids and some oligopeptides are now widely used for drug, food, chiral sources in organic synthesis, and polymers from chemical and biological methods. Because synthetic polypeptides with high molecular weight can be models for the general study of physical, chemical, and biological properties of proteins, a wide variety of methods for polypeptides synthesis have been proposed.[2−7] Chemical synthetic methods for polypeptides can be divided into two methods. The first one is sequential condensation including solid-phase synthesis that afford polypeptides with highly regulated sequences.[4] These polypeptides have been widely applied in the field of biological and medicinal areas, although this method requires demanding processes and is not suitable for mass production. The second one is polycondensation[5] of activated amino acid derivatives and ring-opening polymerization[6,7] of α-amino acid anhydrides (NCAs) that affords polypeptides comprising linkage of one kind of amino acids, which can easily produce high molecular weight polypeptides. These polypeptides are regarded as important materials in biological and industrial areas. Ring-opening polymerization of NCAs is advantageous over the polycondensation because ring-opening polymerization, which is a chain polymerization, is capable of providing polypeptides with precise topology (e.g., block and graft polymers) and controlled architecture. The starting materials NCAs have been generally prepared from amino acids with phosgene or triphosgene, which are highly toxic.[8] The reaction generates hydrogen chloride that makes preparation of pure NCAs without contamination of amino acid hydrochloride salts as byproducts difficult. To solve these troubles, we selected di-*tert*-butyltricarbonate (DBTC)[8,9] instead of phosgene (see Supporting Information). DBTC has been already reported to enable facile syntheses of multiisocyanate from primary amines[10] and α,ω-isocyanato alcohol from amino alcohol.[11] We have also reported a one-pot synthesis of an L-tyrosine-based polyurethane by using the DBTC method.[12] Herein, we describe (1) a facile synthesis of NCAs from α-amino acids and DBTC and (2) a one-pot synthesis of poly(amino acid) from an amino acid and DBTC as a dehydrating agent.

Table 1. Synthesis of NCA with Di-*tert*-butyltricarbonate[a]

run	α-amino acid	yield (%)
1	γ-benzyl-L-glutamate	84
2	L-alanine	41
3	L-leucine	33
4	L-phenylalanine	25

[a] Conditions: α-amino acid (1.0 mmol) and di-*tert*-butyltricarbonate (1.0 mmol) in THF (20 mL) at 60 °C for 4 h.

We first tried to prepare γ-benzyl-L-glutamate−*N*-carboxyanhydride (BLG−NCA) from BLG and DBTC (Scheme 1; Table 1, run 1). BLG was added to a solution of DBTC (1.1 equiv) in dry tetrahydrofuran (THF) (0.1 M) at 60 °C. Although the starting reaction mixture was heterogeneous, the mixture changed to a homogeneous solution after 2 h. Strong IR aborption assignable to the isocyanate was observed at 2252.5 cm^{-1} in $CHCl_3$ solution of the mixture after 2 h. After 4 h, this absorption completely disappeared, and a new IR aborption assignable to the carbonyl moieties in *N*-carboxyanhydride was observed at 1851.3 and 1781.9 cm^{-1}. The resulting mixture was concentrated by a rotary evaporator, and residual mass was washed with dry *n*-hexane, followed by recrystallization from a mixed solvent (THF and *n*-hexane) to obtain a colorless solid, BLG−NCA, in high yield. In a similar fashion, NCAs could be prepared from several α-amino acids such as L-alanine, L-leucine, and L-phenylalanine (Table 1, runs 2−4).[13]

Although BLG gave the corresponding NCA efficiently, other amino acids gave in lower yields. Because the reaction remained heterogeneous after 4 h, the lower yield may originate from the poor solubility of the amino acids. The stoichiometry and the intermediates leading to NCA formation are illustrated in Scheme 1 (path A). Nucleophilic attack of an amine moiety in an amino acid to a carbonyl moiety in DBTC forms an isocyanato acid intermediate that is transformed to the corresponding NCA via intramolecular cyclization. As a consequence, this reaction affords NCAs in a simple procedure where the byproducts are 2 equiv of CO_2 and *tert*-butyl alcohol, which are inert to NCAs and easily separated.

When this reaction was carried out under more concentrated solutions of α-amino acids, some intermolecular reactions should predominantly take place to afford poly(amino acid)s (Scheme 1, path B). BLG as a monomer was selected as a typical example. Polymerization of BLG was carried out with DBTC as a dehydrating agent in several solvents (1.0 M) for 24 h to afford poly(BLG). The polymerization proceeded heterogeneously, which is in contrast to the case of the NCA synthesis. After 24 h, insoluble BLG was removed by filtration, and the polymers were isolated by precipitation with methanol. Dichloromethane and 1,4-dioxane were suitable solvents to obtain polymers with high molecular weight ($M_n > 10^4$) in good yields (Table 2, runs 2−4). The ^1H NMR,^{13}C NMR, and IR spectra of the obtained poly(BLG) were in identical to the authentic spectra of poly(BLG) (see Supporting Information).[14]

In this polymerization system, the following two mechanisms are possible: (1) intermolecular polycon-

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Scheme 1

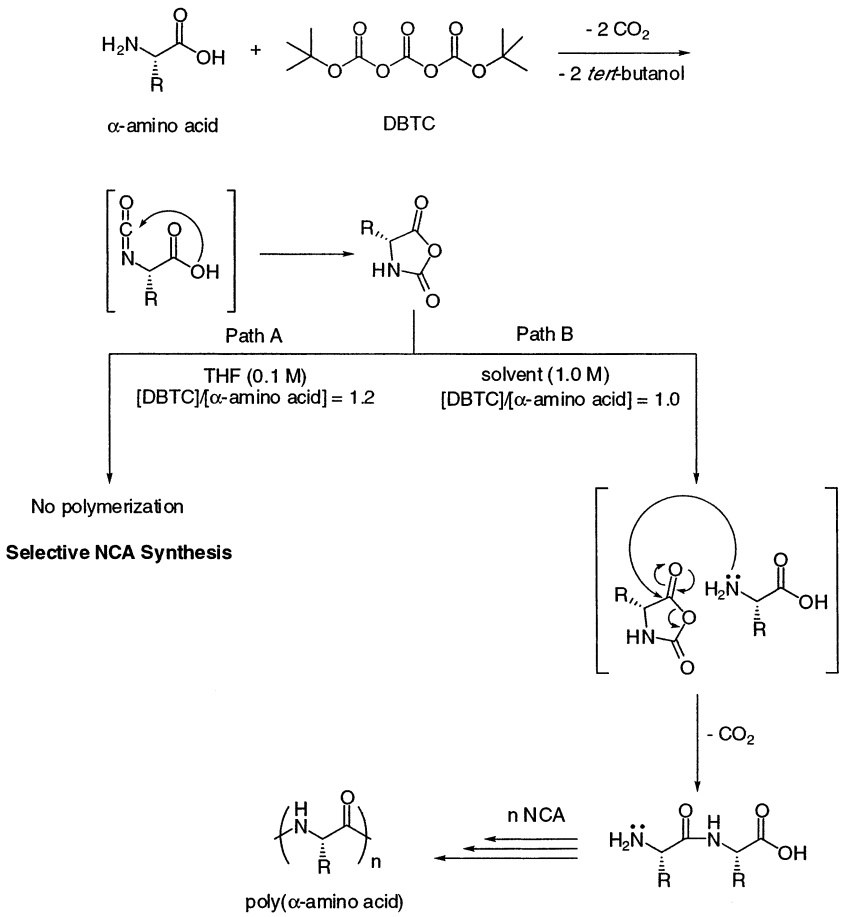

Table 2. Polymerization of BLG with DBTC in Several Solvents[a]

run	solvent	temp (°C)	yield (%)[b]	M_n[c]	M_w/M_n[c]
1	THF	60	21	4 400	3.87
2	1,4-dioxane	60	71	17 400	2.11
3	1,4-dioxane	30	72	25 600	3.09
4	CH_2Cl_2	30	79	22 800	2.83

[a] Conditions: γ-benzyl-L-glutamate (1.0 mmol), di-*tert*-butyltricarbonate (1.0 mmol), solvent (1.0 M), time 24 h. [b] Methanol-insoluble part. [c] Estimated by SEC (polystyrene standard, eluent; DMF containing 5.0 mM lithium bromide and 5.0 mM phosphoric acid).

densation of the isocyanato acid as an intermediate and (2) ring-opening polymerization of the NCA with unreacted BLG as an initiator. The polymerization behavior was followed by IR spectrometric analysis of the reaction mixture during the polymerization. Although IR aborption assignable to the isocyanate moiety could not be observed after 30 min, that assignable to the carbonyl moieties in *N*-carboxyanhydride was observed (see Supporting Information). These data suggest that intramolecular reaction of isocyanato acids to give NCAs is faster than the intermolecular polycondensation. Namely, the polymerization system proceeded through ring-opeing polymerization of BLG−NCA initiated by unreacted BLG (Scheme 1, path B).

In conclusion, we have demonstrated a facile synthesis of α-amino acid−*N*-carboxyanhydrides (NCAs) and convenient direct polymerization of amino acid to obtain a poly(amino acid) with high molecular weight ($>10^4$). Because this system would be suitable to synthesize NCAs and poly(amino acid)s from amino acids having acid-sensitive protecting groups, we are directing our attention to this challenge.

Supporting Information Available: Synthetic method of di-*tert*-butyltricarbonate, the IR spectra of the mixture during the polymerization, and the ^1H, ^{13}C NMR, and IR spectra of poly(BLG) obtained in Table 2 (run 2). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hey, D. H., John, D. I., Eds.; *Amino Acids, Peptides and Related Compounds*; Butterworth: London, 1973.
(2) Li, C. H., Ed.; *Hormonal Peptides*; Academic Press: New York, 1973; Vol. 2, p 64.
(3) Katchalshi, E., Sela, M., Eds.; *Advance in Protein Chemistry*; Academic Press: New York, 1958; p 438.
(4) Chan, W. C., White, P. D., Eds.; *Fmoc Solid Phase Peptides Aynthesis: A Practical Approach*; Oxford University Press: Oxford, 2000; p 277.
(5) Iwakura, Y.; Uno, K.; Oya, M. *J. Polym. Sci., Part A*: *Polym. Chem.* **1967**, *6*, 216.
(6) (a) Berger, A.; Katchalski, E. *J. Am. Chem. Soc.* **1951**, *73*, 4084. (b) Perly, B.; Chachaty, C.; Tsutsumi, A. *J. Am. Chem. Soc.* **1980**, *102*, 1521. (c) Saudek, V.; Stejskal, J.; Schmidt, P.; Zimmermann, K.; Skarda, V.; Kratochvil, P.; Drbnik, J. *Biopolymers* **1987**, *26*, 705.
(7) (a) Deming, T. J. *Nature (London)* **1997**, *390*, 386. (b) Deming, T. J. *J. Am. Chem. Soc.* **1997**, *119*, 2759. (c) Deming, T. J. *J. Am. Chem. Soc.* **1998**, *120*, 4240. (d) Deming, T. J. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 3011.
(8) Daly, W. H.; Poche, D. *Tetrahedron Lett.* **1988**, *29*, 5859.
(9) (a) Dean, C. S.; Tarbell, D. S.; Fehlner, J. R. *J. Org. Chem.* **1970**, *35*, 3393. (b) Yamamoto, Y.; Tarbell, D. S.; Fehlner, J. R.; Pope, B. M. *J. Org. Chem.* **1973**, *38*, 2521.
(10) Peerlings, H. W.; Meijer, E. W. *Tetrahedron Lett.* **1999**, *40*, 1021.

(11) Versteegen, R. M.; Sijbesma, R. P.; Meijer, E. W. *Angew. Chem., Int. Ed.* **1999**, *38*, 2917.

(12) (a) Kudo, H.; Nagai, A.; Ishikawa, J.; Endo, T. *Macromolecules* **2001**, *34*, 5355. (b) Nagai, A.; Ishikawa, J.; Kudo, H.; Endo, T. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 1143.

(13) γ-Benzyl-L-glutamate−NCA: yield = 84%; mp = 93−94 °C (lit.[8] 96−97 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.04−2.20 (m, 2H, −CH_2−), 2.51−2.56 (m, 2H, −CH_2−), 4.32 (t, J = 6.35 Hz, 1H, ⟩CH−), 5.07 (s, 2H, −CH_2−Ph), 6.45 (s, 1H, −NH−), 7.27−7.34 ppm (m, 5H, −C$_6H_5$). ^{13}C NMR (270 MHz, $CDCl_3$): δ = 26.80, 29.81, 56.93, 67.1, 128.47, 128.70, 128.80, 135.24, 151.81, 169.49, 172.60 ppm. IR (KBr): 3332.4, 3263.0, 1859.0, 1781.9, 1257.4, 1195.7, 933.4 cm^{-1}. L-Alanine−NCA: yield = 41%; mp = 93−94 °C (lit.[8] 91−92 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.50−1.52 (m, 3H, −CH_3), 4.35 (q, J = 6.75 Hz, 1H, ⟩CH−), 6.39 (s, 1H, −NH−) ppm. ^{13}C NMR (270 MHz, $CDCl_3$): δ = 17.5, 53.28, 152.39, 170.20 ppm. IR (KBr): 3340.1, 1859.0, 1781.9, 1365.4, 1288.2, 1141.7, 933.4 cm^{-1}. L-Leucine−NCA: yield = 33%; mp = 80−81 °C (lit.[8] 78−79 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.94−1.05 (m, 6H, −(CH_3)$_2$), 1.66−1.78 (m, 1H, −CH⟨), 1.80−1.86 (m, 2H, −CH_2−), 4.33−4.38 (m, 1H, ⟩CH−), 7.05 (s, 1H, −NH−) ppm. ^{13}C NMR (270 MHz, $CDCl_3$): δ = 21.35, 22.57, 24.89, 40.69, 56.12, 153.19, 170.1 ppm. IR (KBr): 3301.5, 2962.1, 1805.0, 1758.7, 1373.1, 1292.9, 941.1 cm$^{-1.}$ L-Phenylalanine−NCA: yield = 25%; mp = 90−91 °C (lit.[8] 91−92 °C). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 2.98−3.22 (m, 2H, −CH_2−), 4.45−4.47 (m, 1H, ⟩CH−), 5.95 (s, 1H, −NH−), 7.20−7.31 ppm (m, 5H, −C$_6$H$_5$). ^{13}C NMR (270 MHz, $CDCl_3$): δ = 37.80, 58.75, 128.14, 129.23, 129.39, 134.0, 151.4, 168.75 ppm. IR (KBr): 3293.8, 1843.6, 1781.9, 1365.4, 1295.9, 1095.4, 933.4 cm^{-1}.

(14) Block, H. *Poly(g-benzyl-L-glutamate) and Other Glutami Acid Containing Polymers*; Gordon and Breach: New York, 1983.

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