

Synthesis of N_ε-protected-L-lysine and γ-benzyl-L-glutamate N-carboxyanhydrides (NCA) by carbamoylation and nitrosation

W. Vayaboury, O. Giani, H. Collet, A. Commeyras, and F. Schué

Université de Montpellier II, UMR 5073 CNRS, Montpellier, France

Received March 18, 2004

Accepted July 20, 2004

Published online September 22, 2004; © Springer-Verlag 2004

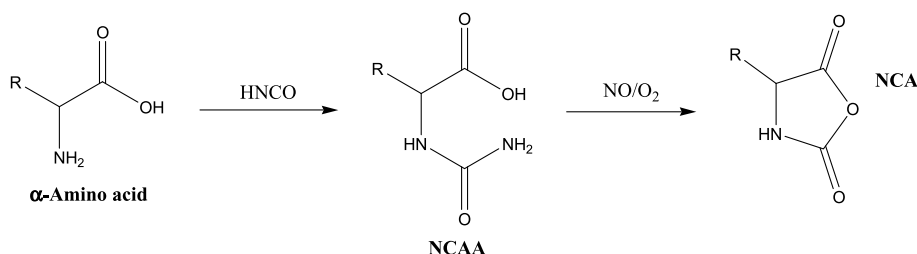
Summary. This paper reports on an original process to synthesize N-carboxyanhydrides, which consists of nitrosating N-carbamoylamino acids with a NO/O₂ gas mixture in acetonitrile. The synthesis of several N-carbamoylamino acids of L-lysine was described using potassium cyanate in water. The latter were then nitrosated to yield the corresponding NCA with more or less efficiency. Indeed, the NCA carrying an acid-sensitive protecting group led to a partial deprotection to give the L-lysine NCA salt. The NCA of N_ε-trifluoroacetyl-L-lysine, N_ε-benzyloxycarbonyl-L-lysine and γ-benzyl-L-glutamate were successfully synthesized with satisfactory yields. Their polymerizability was compared to that of the N_ε-trifluoroacetyl-L-lysine NCA initiated by n-hexylamine in N,N-dimethylformamide. It also showed that this new process of NCA synthesis could be applied to the synthesis of polypeptides and more generally to the protein chemistry.

Keywords: N-Carboxyanhydride – Carbamoylation – Nitrosation – Poly(N_ε-trifluoroacetyl-L-lysine) – Poly(L-lysine)

Introduction

Polypeptides are very important biological macromolecules and have recently become very attractive as high-performance materials (Byrom, 1991; Vincent 1990). Their excellent physical properties, biocompatibility and biodegradability make them well suited for biomedical applications such as sutures, artificial tissues, implants and drug delivery (Urry, 1988; Capello and Crissman, 1990; Waite, 1990; Viney et al., 1992). Among all the polypeptides, poly(L-lysine) (PLL) is one of the most important for these applications, in particular because of its hydrosolubility and functionalized side chain. As an illustration that the topic is of hot interest, the majors of chemical industry have taken more than 30 patents for the last 5 years, concerning applications of PLL (Goto et al., 2003; Lueke et al., 2003; Motegi, 2003).

Excluding stepwise protein syntheses (i.e. Merrifield-type reactions) (Greenstein and Winitz, 1961), which can produce only small amounts of high molecular weight materials, the best technique for chemical PLL synthesis involves polymerization of α-amino acid N-carboxyanhydrides (NCA) (Kricheldorf, 1987). The principal drawback to the PLL and more generally to polypeptides is the high cost of NCA monomers. Since the discovery by Leuchs (1906) of NCA, two new routes, one by Curtius et al. (1930) and the other by Fuchs (1922) and Farthing and Reynolds (1950) were proposed and optimized (Mobashery and Johnston, 1985; Daly and Poché, 1988; Wilder and Mobashery, 1992) to obtain these products. However, these syntheses present the disadvantage of handling hazardous species such as phosgene. Until now, they lead to several by-products that reveal harmful to the polymerization processes. Recently, we described a new and efficient route to NCA without racemization and formation of secondary organic products compared to the other ways. It uses mild conditions, works quantitatively and liberates HNO₂, HNO₃, H₂O and N₂ as by-products (Collet et al., 1996). This process consists of nitrosating N-carbamoylamino acids (NCAA) with a NO/O₂ gas mixture (Scheme 1). Though other organic synthetic methods exist, such as transcarbamoylation from urea degradation (Gonko et al., 1978), hydantoin hydrolysis by strong bases (Rousset et al., 1980) or enzymatic systems (Yamashiro et al., 1988; Keil et al., 1995), a simpler and cheaper way to prepare NCAA by reacting the free α-amino acid with aqueous mineral cyanate is here used (Taillades et al., 2001).



Scheme 1. Synthesis of NCA by carbamoylation and nitrosation

In this paper, we describe the synthesis of a few NCA by this method and evaluate their polymerizability in organic medium with a primary amine.

Materials and methods

Melting points were measured using a Buchi 520 apparatus. IR spectra were recorded on a Bruker IFS 25 instrument in the transmittance mode. Wavelengths are given in cm^{-1} . ^1H NMR spectra were recorded on a Bruker Avance DPX 200 (200 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 (400 MHz) spectrometer. All chemical shifts were reported in parts per million (ppm) downfield of tetramethylsilane and coupling constants (J values) are given in Hz. The following abbreviations s, d, t and m are used and respectively designated singlet, doublet, triplet and multiplet. MS spectra were recorded on a Jeol JMS-DX 300 spectrometer using a FAB technique. The optical activities were taken on a Perkin Elmer Modèle 341 polarimeter. The pH regulations were performed using a Metrohm Titrino SM 702 autotitrator.

SEC-MALLS analysis of the (PLL) resulting from the deprotection of the PTL was performed using a Waters HPLC 515 pump (Saint-Quentin en Yvelines, France), with a flow rate of 0.5 mL/min using 1 M acetate buffer (pH 4.65) as mobile phase. The eluent was filtrated through 0.45 and 0.1 μm hydrophilic membranes (Milex SV, Millipore, Molsheim, France). The samples were injected through a 100 μL loop and eluted on a Waters Ultrahydrogel linear column of 30 cm \times 7.8 mm. The temperature of the column and of the detectors was set at 35°C. The polymer samples were prepared at 5 g/L in the eluent and were filtered through a 0.45 μm hydrophilic membrane before injection. A MALLS detector (Dawn DSP, Wyatt Technology Co, Santa Barbara, CA) coupled with a refractive index detector (Optilab, Wyatt Technology Co, Santa Barbara, CA) as concentration detector was used to obtain on line determination of the absolute molar mass for each elution fraction of ~ 0.01 mL.

Deionized water was further purified with a Milli-Q system from Millipore (Molsheim, France). Molecular sieve (4 Å) was from Roth (Lauterbourg, France). Methanol (MeOH), ethanol (EtOH), isopropanol (iPrOH), acetonitrile (MeCN) and N,N -dimethylformamide (DMF) were purchased from Carlo Erba (Val de Reuil, France). Potassium cyanate (97%), deuterated trifluoroacetic acid (99.5%) and L-lysine monohydrate (99%) were purchased from Acros (Noisy-le-Grand, France). Hexane, anhydrous Na_2SO_4 , sodium acetate trihydrate, piperidine (98%) and hydrochloric acid (36%) were from Prolabo (Paris, France). n -Hexylamine (99%) was from Avocado (La Tour du Pin, France). γ -benzyl-L-glutamate (97%) was from Aldrich (Saint-Quentin Fallavier, France). Sulfonic acid resin AG 50 W-X4 was from Bio-Rad (Chassieu, France). Ethyl acetate (AcOEt) was from Baeckeroot (Jacou, France). Deuterated dimethylsulfoxide (DMSO-d_6 , 99.8%) and D_2O (99.9%) were from Euriso-Top (Gif-Sur-Yvette, France). N_ϵ -trifluoroacetyl-L-lysine, N_ϵ -benzyloxycarbonyl-L-lysine and N_ϵ -formyl-L-lysine were from Degussa (Vénissieux, France). NO, O_2 and N_2 were from Air Liquide (Vedène, France). All solvents were used as received

except MeCN, AcOEt, hexane and DMF which were dried on molecular sieve before use. All reagents were used as received except potassium cyanate, which was washed with MeOH, then dried in vacuo prior to use.

N_α -Carbamoyl- N_ϵ -trifluoroacetyl-L-lysine (1)

This product was synthesized according to the procedure described by Taillades et al. (2001). Anal.: calculated for $\text{C}_9\text{H}_{14}\text{O}_4\text{N}_3\text{F}_3$ (C 37.90, H 4.95, N 14.73, O 22.44), found (C 36.99, H 4.90, N 14.79, O 23.87).

N_α -Carbamoyl- N_ϵ -benzyloxycarbonyl-L-lysine (2)

This product was synthesized according to the procedure described by Schlögl and Fabitschowitz (1953). $[\alpha]_D^{20} = +6$ ($c = 0.35$ g/100 mL, EtOH); IR (KBr): 3439, 3360 and 3281 (NH), 1686 ($\text{C}=\text{O}_{\text{acid}}$), 1637 ($\text{C}=\text{O}_{\text{urea}}$), 1528 (NH), 735 ($\text{C}=\text{C}$); ^1H NMR (DMSO-d_6) δ : 1.34 (m, 4H, $\text{H}_\gamma + \text{H}_\delta$), 1.58 (m, 2H, H_β), 2.98 (td, 2H, $J_{\text{NH}} = J_\delta = 6.3$ Hz, H_ϵ), 4.03 (m, 1H, H_α), 5.01 (s, 2H, CH_2O), 5.59 (s, 2H, NH_2), 6.22 (d, 1H, $J_\alpha = 8.2$ Hz, NH), 7.27 (t, 1H, NH), 7.35 (m, 5H, H_{Ar}), 12.49 (s, 1H, CO_2H); ^{13}C NMR (DMSO-d_6) δ : 23.4 (C_γ), 30 (C_δ), 32.6 (C_β), 40 (C_ϵ), 53 (C_α), 65.9 (CH_2O), 128.6 ($\text{C}_\text{o} + \text{C}_\text{p}$), 129.2 (C_m), 138.1 (C_{Ar}), 156.9 (CO_2), 159.2 (CO_α), 175.6 (CO_2H); MS: $m/z = 647$ ($2\text{M} + \text{H}^+$), $m/z = 324$ ($\text{M} + \text{H}^+$); Mp: 174–5°C.

N -Carbamoyl- γ -benzyl-L-glutamate (3)

To a suspension of γ -benzyl-L-glutamate (1 g, 4.2 mmol) in 100 mL water at 50°C was added potassium cyanate (0.51 g, 6.3 mmol). The pH was then adjusted to 6 using the autotitrator to control continuous 4M HCl addition. After stirring 10h, the reaction mixture was cooled by means of an ice bath and acidified by adding 4M HCl until reaching pH 2. After filtration, the crude product was recrystallized from EtOH/ H_2O (96/4 v/v) mixture and dried in vacuo. Yield: 0.76 g (65%); IR (KBr): 3447 and 3313 (NH), 1723 ($\text{C}=\text{O}_{\text{ester}}$), 1685 ($\text{C}=\text{O}_{\text{acid}}$), 1631 ($\text{C}=\text{O}_{\text{urea}}$), 1547 (NH); ^1H NMR (DMSO-d_6) δ : 1.79 (m, 1H, H_β); 1.98 (m, 1H, H_β); 2.41 (m, 2H, H_γ); 4.11 (m, 1H, H_α); 5.09 (s, 2H, CH_2O); 5.63 (s, 2H, NH_2); 6.29 (d, 1H, $J_\alpha = 8.2$ Hz, NH); 7.37 (m, 5H, H_{Ar}); 12.49 (s, 1H, CO_2H); ^{13}C NMR (DMSO-d_6) δ : 28.2 (C_β), 30.8 (C_γ), 52.4 (C_α), 66.4 (CH_2O), 128.7 (C_o), 128.8 (C_p), 129.3 (C_m), 137 (C_{Ar}), 159.2 (CO_α), 173 (CO_γ), 175.1 (CO_2H); MS: $m/z = 281$ ($\text{M} + \text{H}^+$); Anal.: calculated for $\text{C}_{13}\text{H}_{16}\text{O}_5\text{N}_2$ (C 55.71, H 5.75, N 9.99), found (C 55.49, H 5.78, N 10.08); Mp: 181°C.

N_α -Carbamoyl- N_ϵ -formyl-L-lysine (4)

To a warm (50°C), stirred solution of N_ϵ -formyl-L-lysine (8.7 g, 50 mmol) in 100 mL water was added potassium cyanate (4.87 g, 60 mmol). The pH was then regulated to 7.5 using AG 50W-X4 cation exchange resin (H^+ form) addition. After 10h stirring at 50°C, the mixture was filtered through a column containing AG 50W-X4 cation exchange resin (H^+ form; further elution with water). After water evaporation in vacuo and freeze-drying, pure material was obtained as a white solid. Yield: 9.98 g

(92%); $[\alpha]_D^{20} = +18$ ($c = 0.5$ g/100 mL, EtOH); IR (KBr): 3339 (NH), 1711 (C=O_{acid}), 1659 (C=O_{urea}), 1659 (C=O_{formyl}), 1547 (NH); ¹H NMR (DMSO-*d*₆) δ : 1.34 (m, 4H, H _{γ} + H _{δ}); 1.56 (m, 2H, H _{β}); 3.06 (td, 2H, J_{NH} = J _{δ} = 6.2 Hz, H _{ϵ}); 4.03 (m, 1H, H _{α}); 5.60 (s, 2H, NH₂); 6.22 (d, 1H, J _{α} = 8.2 Hz, NH); 7.97 (m, 2H, H(CO)NH); 12.52 (s, 1H, CO₂H); ¹³C NMR (DMSO-*d*₆) δ : 23.4 (C _{γ}), 29.5 (C _{δ}), 32.6 (C _{β}), 37.8 (C _{ϵ}), 53.1 (C _{α}), 159.3 ((CO) _{α}), 161.8 ((CO)H), 175.6 (CO₂H); MS: $m/z = 218$ (M + H⁺); Anal.: calculated for C₈H₁₅O₄N₃ (C 44.23, H 6.96, N 19.34), found (C 44.18, H 6.97, N 19.29); Mp: 154–5°C.

N,N'-Dicarbamoyl-L-lysine (**5**)

Same procedure as **4**, using L-Lysine monohydrate (12.3 g, 75 mmol) in 150 mL water and potassium cyanate (13.97 g, 172.5 mmol), reaction at 50°C, at pH 7.5 for 24 h. The crude product was recrystallized from MeOH/iPrOH (9/1 v/v) mixture to yield pure material. Yield: 12.7 g (73%); $[\alpha]_D^{20} = +6$ ($c = 0.5$ g/100 mL, EtOH); IR (KBr): 3338 (NH), 1711 (C=O_{acid}), 1658 (C=O_{urea}), 1548 (NH); ¹H NMR (DMSO-*d*₆) δ : 1.31 (m, 4H, H _{γ} + H _{δ}), 1.56 (m, 2H, H _{β}), 2.90 (td, 2H, J_{NH} = J _{δ} = 6.1 Hz, H _{ϵ}), 4.03 (m, 1H, H _{α}), 5.38 (s, 2H, NH₂⁺), 5.59 (s, 2H, NH₂⁺), 5.91 (t, 1H, NH), 6.22 (d, 1H, J _{α} = 8.1 Hz, NH), 12.49 (s, 1H, CO₂H). ¹³C NMR (DMSO-*d*₆) δ : 23.5 (C _{γ}), 30.6 (C _{δ}), 32.7 (C _{β}), 40 (C _{ϵ}), 53.2 (C _{α}), 159.3 ((CO)N _{α}), 159.7 ((CO)N _{ϵ}), 175.8 (CO₂H); MS: $m/z = 465$ (2M + H⁺), $m/z = 233$ (M + H⁺), $m/z = 216$ (M – NH₃⁺); Anal.: calculated for C₈H₁₆O₄N₄ (C 41.37, H 6.94, N 24.12), found (C 41.57, H 6.71, N 23.55); Mp: 139°C.

N_ε-Trifluoroacetyl-L-lysine NCA (**6**)

In a 2L-reactor, a suspension of **1** (2 g, 7 mmol) in 80 mL MeCN was degassed with nitrogen during 10 minutes. Then, NO (472 mL, 21 mmol) and O₂ (118 mL, 5.2 mmol) was added to the reaction mixture which was stirred at room temperature. The reaction was stopped 10 minutes after obtaining a homogeneous solution by a fast degasification with nitrogen during 10 minutes. The crude mixture was then dried over anhydrous Na₂SO₄ and the solvent was evaporated to dryness in vacuo at a temperature lower than 40°C to yield pure material. However, a purification of the NCA monomer was achieved before polymerization reaction. The NCA was dissolved in 150 mL AcOEt, then washed with an aqueous solution of NaHCO₃ (0.5% w/w) at 0°C until neutralization of the aqueous phase. The organic phase was dried over anhydrous Na₂SO₄ and then concentrated to 10 mL in vacuo at a temperature lower than 40°C. The addition of a minimum of hexane disturbed the solution which was centrifuged, and the supernatant was then recovered (this operation was repeated until the appearance of white crystals). The product was finally recrystallized twice in an AcOEt/hexane mixture and dried in vacuo. Yield: 1.54 g (82% before purification) and 0.92 g (49% before polymerization); IR (KBr): 1858 (C=O_{anhydride}), 1791 (C=O_{urethane}), 1726 (C=O_{amide}); ¹H NMR (DMSO-*d*₆) δ : 1.35 (m, 2H, H _{γ}), 1.50 (m, 2H, H _{δ}), 1.71 (m, 2H, H _{β}), 3.18 (td, 2H, J_{NH} = J _{δ} = 6.4 Hz, H _{ϵ}), 4.44 (t, 1H, J _{β} = 6.2 Hz, H _{α}), 9.11 (s, 1H, NH), 9.43 (t, 1H, CF₃(CO)NH); ¹³C NMR (DMSO-*d*₆) δ : 22.3 (C _{γ}), 28.7 (C _{δ}), 31.4 (C _{β}), 39.7 (C _{ϵ}), 57.8 (s, C _{α}), 116.8 (q, J_{CF} = 288.2 Hz, CF₃), 152.8 ((CO)N _{α}), 156.8 (q, J_{CF} = 37.5 Hz, (CO)N _{ϵ}), 172.5 ((CO) _{α}); MS: $m/z = 559$ (2M + Na⁺); $m/z = 537$ (2M + H⁺); $m/z = 307$ (M + K⁺); $m/z = 291$ (M + Na⁺); $m/z = 269$ (M + H⁺); $m/z = 267$ (M – H⁺); $m/z = 69$ (CF₃[–]); HR-MS: calculated for C₉H₁₂O₄N₂F₃ (M + H⁺) 269.0749, found 269.0775.

N_ε-Benzyloxycarbonyl-L-lysine NCA (**7**)

Same procedure as **6**, using **2** (2 g, 6.2 mmol) in 80 mL MeCN, NO (416 mL, 18.6 mmol) and O₂ (104 mL, 4.6 mmol). Yield: 1.23 g (65%); ¹H NMR (DMSO-*d*₆) δ : 1.29 (m, 4H, H _{γ} + H _{δ}), 1.65 (m, 2H, H _{β}), 3.71 (t, 2H, J _{δ} = 6.8 Hz, H _{ϵ}), 4.39 (t, 1H, J _{β} = 6.4 Hz, H _{α}), 5.51 (s, 2H, CH₂O); 7.44 (m, 6H, H_{Ar} + NH _{ϵ}); 9.1 (s, 1H, NH _{α}); ¹³C NMR (DMSO-*d*₆) δ : 22.6

(C _{γ}), 26.7 (C _{δ}), 31.3 (C _{β}), 41.3 (C _{ϵ}), 57.7 (C _{α}), 70.1 (CH₂O), 129.2 (C_O + C_p), 129.4 (C_m), 135.7 (C_{Ar}), 152.8 (N _{α} (CO)), 154 (N _{ϵ} (CO)), 172.4 ((CO) _{α}); MS: $m/z = 307$ (M + H⁺), $m/z = 91$ (C₇H₇⁺).

γ -Benzyl-L-glutamate NCA (**8**)

Same procedure as **6**, using **3** (2 g, 7.1 mmol) in 80 mL MeCN, NO (640 mL, 28.4 mmol) and O₂ (160 mL, 7.1 mmol). Yield: 1.47 g (79%); ¹H NMR (DMSO-*d*₆) δ : 2 (m, 2H, H _{β}), 2.54 (t, 2H, J _{β} = 7.6 Hz, H _{γ}), 4.49 (t, 1H, J _{β} = 6.7 Hz, H _{α}), 5.12 (s, 2H, CH₂O), 7.38 (m, 5H, H_{Ar}), 9.14 (s, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ : 27.3 (C _{β}), 29.9 (C _{γ}), 57.1 (C _{α}), 66.6 (CH₂O), 128.8 (C_O), 128.9 (C_p), 129.3 (C_m), 136.9 (C_{Ar}), 152.7 ((CO)N _{α}), 172.2 ((CO) _{γ}), 172.6 ((CO) _{α}); MS: $m/z = 527$ (2M + H⁺), $m/z = 286$ (M + Na⁺), $m/z = 264$ (M + H⁺); HR-MS: calculated for C₁₃H₁₄O₅N (M + H⁺) 264.0872, found 264.0891.

N_ε-Formyl-L-lysine NCA (**9**)

Same procedure as **6**, using **4** (2 g, 9.2 mmol) in 80 mL MeCN, NO (619 mL, 27.6 mmol) and O₂ (155 mL, 6.9 mmol). Yield: product non isolated; ¹H NMR (DMSO-*d*₆) δ : 1.37 (m, 4H, H _{γ} + H _{δ}), 1.68 (m, 2H, H _{β}), 3.07 (m, 2H, H _{ϵ}), 4.43 (m, 1H, H _{α}), 7.99 (s, 2H, H(CO)NH), 9.08 (s, 1H, NH).

Salt of L-lysine NCA (**10**)

Same procedure as **6**, using **5** (2 g, 8.6 mmol) in 80 mL MeCN, NO (772 mL, 34.4 mmol) and O₂ (193 mL, 8.6 mmol). Yield: product non isolated; ¹H NMR (DMSO-*d*₆) δ : 1.48 (m, 4H, H _{γ} + H _{δ}), 1.75 (m, 2H, H _{β}), 2.8 (m, 2H, H _{ϵ}), 4.48 (t, 1H, J _{β} = 5.5 Hz, H _{α}), 7.68 (s, 3H, NH₃), 9.1 (s, 1H, NH); ¹³C NMR (DMSO-*d*₆) δ : 22 (C _{γ}), 27.3 (C _{δ}), 31.1 (C _{β}), 39.4 (C _{ϵ}), 57.2 (C _{α}), 152.8 (N(CO)), 172.5 (CO).

Poly(*N_ε*-trifluoroacetyl-L-lysine) (PTLL) (**11**)

11 was synthesized by ring opening polymerization of **6** in DMF (Sela et al., 1963). DMF was distilled on a 4 Å molecular sieve in vacuo before the polymerization. In a Schlenk flask fitted with a stir bar and a silicon septum, **6** (2.144 g, 8 mmol) was dissolved under nitrogen in 50 mL of distilled DMF. Then, *n*-hexylamine (5.3 μ L, 40 μ mol) corresponding to a molar ratio of monomer to initiator (M/I) of 200 was added and the reaction mixture was stirred at room temperature. After 48 h, the reaction mixture was poured into a 25-fold excess of water. The precipitated polypeptides were isolated by filtration and subsequently dried in vacuo at 50°C. Yield: 1.50 g (84%); IR (KBr): 1633 (C=O_{peptide}), 1687 (C=O_{amide}), 1546 (NH), 1219, 1180 and 1156 (CF₃); ¹H NMR (TFA-*d*) δ : 1.52 (m, 2H_n, (H _{γ})_n), 1.72 (m, 2H_n, (H _{δ})_n), 1.92 (m, 2H_n, (H _{β})_n), 3.48 (m, 2H_n, (H _{ϵ})_n), 4.63 (t, 1H_n, J _{β} = 6.0 Hz, (H _{α})_n); ¹³C NMR (DMSO-*d*₆) δ : 23.8 (C _{γ}), 28.8 (C _{δ}), 30.3 (C _{β}), 40.5 (C _{ϵ}), 58 (C _{α}), 116.8 (q, J_{CF} = 288.1 Hz, CF₃), 157 (q, J_{CF} = 36 Hz, (CO) _{ϵ}), 176.1 ((CO) _{α}).

Poly(L-lysine) (**12**)

11 (1 g, 4.5 mmol) was deprotected and **12** was purified according to the procedure described by Sela et al. (1963). Yield: 0.95 g (88%); ¹H NMR (D₂O) δ : 1.39 (m, 2H_n, (H _{γ})_n), 1.66 (m, 4H_n, (H _{β})_n + (H _{δ})_n), 3.48 (t, 2H_n, J _{δ} = 7.5 Hz, (H _{ϵ})_n), 4.25 (t, 1H_n, J _{β} = 6.9 Hz, (H _{α})_n); ¹³C NMR (D₂O) δ : 22.5 (C _{γ}), 26.7 (C _{δ}), 31 (C _{β}), 39.6 (C _{ϵ}), 53.7 (C _{α}), 174 (CO); SEC-MALLS analysis: M_n = 24000 g/mol and M_w/M_n = 1.38.

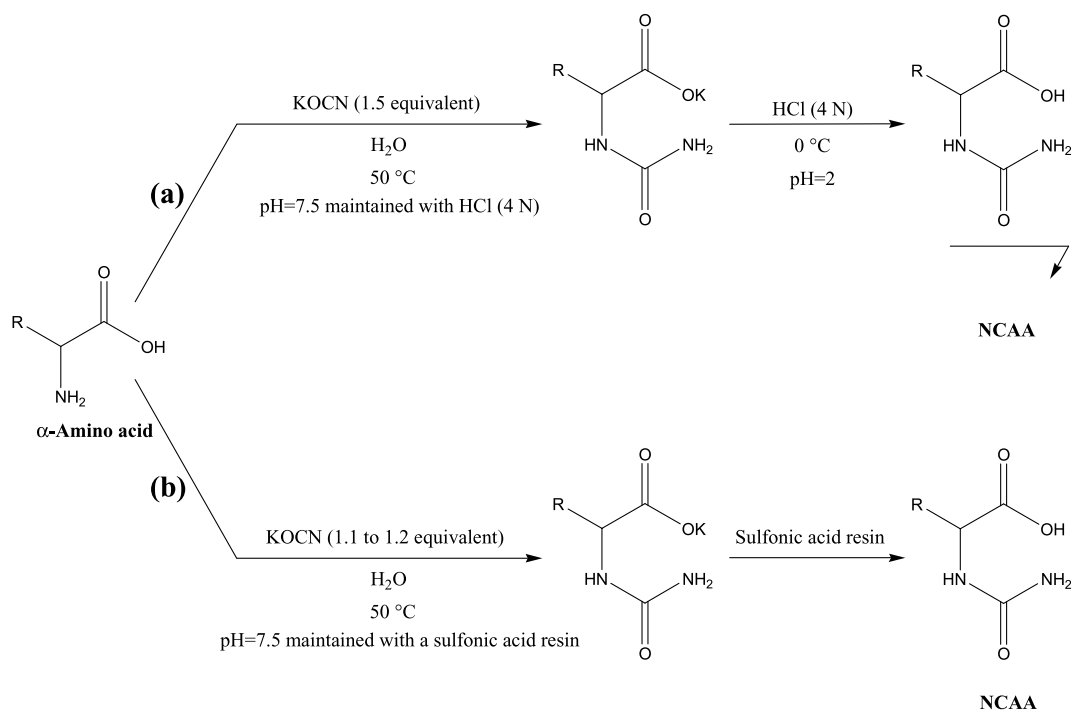
Results and discussion

In a previous study of the N-carbamoylation reaction, we defined the optimal conditions for the synthesis and the

extraction of NCAA (Taillades et al., 2001). Indeed, according to computer simulations evaluated at several pH and temperatures, the optimum conditions for the synthesis of NCAA, minimizing the urea formation (which can become critical in the purification of very water soluble NCAA), are obtained by reacting 1.2–1.5 equivalent of mineral cyanate, with pH regulated to 8.5–9, at a moderate temperature and by stopping the reaction after having reached 99% of α -amino acid conversion. In these conditions, a complete reaction requires approximately 100 hours. However, this time can be decreased to 10–24 hours by using a less basic pH (between 7.5–8) at 50°C with good yields. For the product extraction, the reaction medium is then acidified to pH 2 in order to hydrolyze the cyanate excess. Therefore, to limit the NCAA cyclization to hydantoin and to precipitate the NCAA, the acidification is performed at low temperatures (typically at 0°C). Depending on the NCAA, these reaction conditions can vary to facilitate the synthesis and isolation of the final product in particular as regards pH regulation, acidification of the medium and quantity of mineral cyanate. Indeed, two general processes can be considered: that of NCAA with non-polar or not very polar side chain (insoluble in water) and that of NCAA with polar side chain (water soluble).

In the first one, the strategy consists in using a cyanate excess (1.5 equivalent) for the synthesis and acidifying after the synthesis by a solution of HCl to precipitate the final product. By this way (Scheme 2, route a), NCAA of the N_ϵ -trifluoroacetyl-L-lysine **1** was obtained with a satisfactory yield from 50% after recrystallization. Because of a very weak hydrosolubility, the N_ϵ -benzyl-oxycarbonyl-L-lysine carbamoylation was carried out under reflux conditions and without pH control (yield from 64% after recrystallization) closed to the experimental conditions described by Schlögl and Fabitschowitz (1953). The cyanation of the γ -benzyl-L-glutamate is performed with pH regulated to 6 since the side chain is sensitive to basic hydrolysis (yield from 65% after recrystallization).

The formation of hydrophilic NCAA must be carried out using a different approach because the isolation of the products by precipitation after acidification of the medium is not appropriate since polar NCAA are very water soluble. In order to facilitate the isolation of the final product, the pH of the solution is maintained by cation exchange resins and a light cyanate excess (1.1 to 1.2 equivalent) is used. Then, the pure NCAA is obtained by a simple filtration of the crude reaction mixture through a cation exchange column. With this process (Scheme 2, route b), two NCAA with polar side chain such as the N,N' -



Scheme 2. Carbamoylation of α -amino acid: with non polar side chain (a) and polar side chain (b)

dicarbamoyl-L-lysine **5** and the N $_{\alpha}$ -carbamoyl-N $_{\epsilon}$ -formyl-L-lysine **4** were synthesized in acceptable yields respectively from 75% after recrystallization and from 92% without any purification.

After the carbamoylation reaction of α -amino acid, the nitrosation of NCA synthesized was investigated. This reaction consists of using a NO/O $_2$ gas mixture containing nitrogen oxides (in particular N $_2$ O $_3$ and N $_2$ O $_4$) which generate the nitrosyl cation NO $^+$, recognized as the reactive entity. The mechanism of this NCA synthesis will be discussed in a forthcoming paper. The nitrosation reaction is carried out in organic medium (MeCN) or in solid phase (NCAA)/gas (NO/O $_2$). Our choice for MeCN is to prevent hydrolysis of the NCA by in situ water formed during the nitrosation. Indeed, MeCN which dissolves the NCA, ensures a better dispersion of the final product in the organic medium and limits the direct contact between the NCA and the in situ formed water. In experiments, the reaction is generally performed with the suspension of the NCAA in MeCN in the presence of an excess of NO/O $_2$ gas mixture (3 or 4 moles of NO per mole of NCAA with a proportion from 20 to 25% of O $_2$ compared to NO). The nitrosation is carried out at room temperature and is completed in 10 minutes after formation of a homogenous solution. The reaction work-up consists of drying the organic phase and after filtration, of evaporating the solvent under reduced pressure at a temperature lower than 40°C. Through this procedure, the NCA of N $_{\epsilon}$ -trifluoroacetyl-L-lysine **6**, of the N $_{\epsilon}$ -benzyloxycarbonyl-L-lysine **7** and of γ -benzyl-L-glutamate **8** were prepared and isolated (Table 1). However, the nitrosation of NCAA with very sensitive groups to the acid medium like the N $_{\alpha}$ -carbamoyl-N $_{\epsilon}$ -formyl-L-lysine and the N,N'-dicarbamoyl-L-lysine led to a partial deprotection. Indeed, the N $_{\epsilon}$ -formyl-L-lysine NCA **9** synthesis is accompanied by that of the L-lysine salt NCA (probably nitrite and/or nitrate salt) which results from the deprotection of the formyl

group and this whatever the nitrosation conditions (2, 3 or 4 moles of NO and 20 or 25% of O $_2$). All our attempts to isolate these two NCA failed. In a similar way, the N,N'-dicarbamoyl-L-lysine nitrosation leads to the carbamoyl group deprotection of the side chain to form the L-lysine salt NCA **10** which could not be isolated. Consequently, the compounds **9** and **10** cannot be used in organic synthesis as well as in polymerization because they are not pure or because they are or contain acid derivatives (nitrite and/or nitrate salt of L-lysine NCA).

In order to evaluate the polymerizability of NCA synthesized by this process, a *n*-hexylamine initiated polymerization of N $_{\epsilon}$ -trifluoroacetyl-L-lysine NCA **6** was performed in DMF for a M/I molar ratio of 200. Before the polymerization reaction, the monomer was purified from traces of mineral impurities (nitrite and nitrate salts, HNO $_2$ and HNO $_3$) by washing NCA with aqueous bicarbonate at 0°C according to an unconventional method described by Poché et al. (1999) and from possible traces of organic impurities by two successive recrystallizations in an AcOEt/hexane mixture. Indeed, the amine initiated polymerization of NCA are highly sensitive to the presence of acid compounds since the basicity and nucleophilicity of the initiator can be affected by the protonation of the amine group. After precipitation of the reaction crude, a yield from 84% is obtained for the polymerization. This yield does not reflect an incomplete polymerization reaction but results from the presence of PTLL oligomers (Cottet et al., 2003; Simo et al., 2004; Vayaboury et al., 2004) which are soluble in the precipitation medium (water). Figure 1 shows the SEC-MALLS analysis of the PLL obtained for this polymerization after deprotection of the trifluoroacetyl group of the PTLL (Sela et al., 1963). Taking into account the counterion condensation according to the Manning theory, the average molar mass of a monomer for the PLL in acetate buffer is $0.59 \times 129 + 0.51 \times 188 = 159$ g/mol (Cottet et al., 2003). Thus, a theoretical number-average molar mass of 32000 g/mol is expected for an M/I molar ratio of 200. The determination of absolute molar mass indicates a number-average molar mass of 24000 g/mol which is lower than the theoretical value. This discrepancy may be explained by two side reactions which clearly demonstrated in two papers (Cottet et al., 2003; Simo et al., 2004). The quite large polydispersity index (1.38) is certainly due to these termination reactions because there is not chain scission during the deprotection which has been proved by comparing the polydispersity index before and after deprotection (Collet et al., 2003). This attempt of polymerization thus confirms the polymerizability of NCA (synthesized by carbamoylation and

Table 1. Nitrosation of N-carbamoylamino acids with NO/O $_2$ gas mixture in MeCN

Reagent	Proportion of NO/O $_2$ ^a	Product	Yield before purification (%)
1	3/25	6	82
2	3/25	7	65
3	4/25	8	79
4	3/25	9 + 10	–
5	4/25	10	–

^a number of NO moles per mole of reagent/number of O $_2$ moles expressed as a percentage of the number of NO moles

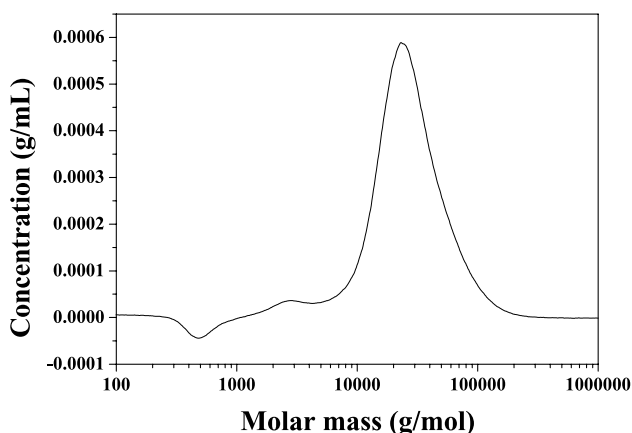


Fig. 1. SEC-MALLS analysis of poly(L-lysine) **12** obtained for a M/I molar ratio of 200. Eluent: 1 M acetate buffer pH = 4.65. M_n = 24000 g/mol. Polydispersity index = 1.38

nitrosation) which is also supported by the ^1H NMR and IR analyses of PTLL.

Conclusion

In this work, an original method to synthesize NCA has been applied in particular to N_ϵ -protected-L-lysine. Carbamoylation and nitrosation reactions of these α -amino acids have been described and led to the synthesis of the NCA most interesting for ring opening polymerization. Their polymerizability has been studied using a primary amine initiated polymerization of N_ϵ -trifluoroacetyl-L-lysine NCA in DMF. A good aptitude to prepare polypeptides has been observed in spite of the presence of side reactions. The possibility to obtain living polymerization upon decreasing the reaction temperature has been just reported in a rapid communication (Vayaboury et al., 2004) and will be discussed in more detail in a future full paper.

Acknowledgements

We are grateful to the CNRS and the French Ministries of Education and Research for their financial support and Degussa-Hüls AG (Germany) for the amino acids. The authors thank also Dr. André Deratani from the Institut Européen des Membranes (IEM, Université de Montpellier II, France) for the SEC-MALLS experiments and Céline Bauzac for her help in revising the English expression of this article.

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- Authors' address:** Dr. O. Giani, Laboratoire Organisation Moléculaire, Évolution et Matériaux Fluorés, UMR 5073 CNRS, Université de Montpellier II, case courrier 17, Place Eugène Bataillon, 34095 Montpellier cedex 5, France,
Fax: (+33) 4-6754-4757, E-mail: ogiani@univ-montp2.fr