

Synthesis of α -Hydroxy- ω -Aminotelechelic Polypeptide from α -Amino Acid *N*-Carboxyanhydrides Catalyzed by Alkali-metal Borohydrides*

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Abstract It is reported that alkali-metal borohydrides (MBH₄, M = Li, Na and K) are efficient catalysts for ring opening polymerization (ROP) of α -amino acid *N*-carboxyanhydrides (NCAs). Polypeptides are prepared in quantitative yields with relatively narrow molecular weight distributions (MWDs = 1.1~1.5) which depend on the reaction temperature. End groups of the produced polypeptide are studied in detail by MALDI-ToF MS, ¹H-NMR, ¹³C-NMR, ¹H-¹H COSY and ¹H-¹³C HMQC analyses. The results indicate that α -hydroxy- ω -aminotelechelic polypeptides are formed which are suitable for post-polymerization functionalization.

Keywords: Polypeptide; Alkali-metal borohydride; α -Amino acid *N*-carboxyanhydride; End group functionalization.

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INTRODUCTION

Synthetic polypeptides have attracted attention recently for their special ability to self-assemble into highly-ordered hierarchical structures and excellent biocompatibility^[1, 2]. Their most typical preparation method is the ring opening polymerization (ROP) of α -amino acid *N*-carboxyanhydrides (NCA)^[3]. Over the past decades, many initiators and catalysts have been developed for their synthesis including traditional primary amine, tertiary amine, sodium methoxide^[4–6], zero-valent nickel complexes bipyNi(COD) (bipy = 2,2'-bipyridyl, COD = 1,5-cyclooctadiene)^[7, 8] and hexamethyldisilazane (HMDS)^[9, 10]. Some improvements for a better controllability by the primary amine have been reported, including using high vacuum techniques^[11], lowering reaction temperature^[12–14] and treating primary amine with hydrochloride^[15]. In polymerization mechanistic aspects, great progress has benefited from clear end group-identification by MALDI-ToF^[16–18], NALDI-ToF^[19] and nonaqueous capillary electrophoresis^[20] analytical methods. DFT calculation by our group also provides insights into the elemental reactions behind the overall polymerization phenomena^[21].

The nature of polymer end groups is not only essential to polymerization mechanisms but also important for

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chain-end modification and chain extension. Generally, the prepared polypeptide contains an amino group at one end and an initiator residue or carboxylic group at the another one^[16, 18]. Because of the sensitivity of NCA, the initiator residue is generally an alkyl or alkoxy group which is inert for further modifications. In an effort to produce end group-functionalized polypeptides, primary amines containing allyl^[10] and porphyrin-groups^[22] are used as initiators. However, these polymerizations suffer from chain termination reactions resulting bi-modal GPC trace and unpredictable molecular weights (MWs). A better method was reported by Cheng using derivatives of hexamethyldisilazane (HMDS)^[10], by which allyl, propargyl, mPEG-functionalized polypeptides were synthesized with controllable MWs and narrow molecular weight distributions (MWDs < 1.3). However, it is not possible to use any HMDS derivative containing a hydroxyl group as initiator.

Sodium borohydride (NaBH₄) was used to polymerize γ -benzyl-L-glutamate *N*-carboxyanhydride (BLG NCA) decades ago^[23] with very limited characterization of the polypeptide structure due to the limited characterization methods at that time. This commercially-available compound, however, hasn't been examined in NCA polymerization thereafter. Here, we report alkali metal borohydrides (MBH₄, M = Li, Na and K) as catalysts for the ROP of NCAs producing α -hydroxy- ω -aminotelechelic polypeptides. Compared to the alkyl and alkoxy end groups, the hydroxyl end group allows a new modification site for drug conjugation. It also allows chain extension such as the use of polypeptides as macroinitiators for the ROP of lactones, lactides and cyclic carbonates. The nontoxic alkali metal ion makes the easily accessible MBH₄ a promising catalyst for the synthesis of polypeptide intended for biological and medical applications.

EXPERIMENTAL

Materials

γ -Benzyl-L-glutamate (99.0%, Shanghai Hanhong Chemical, China), L-alanine (98.5%, Sinopharm Chemical Reagent, China), sodium borohydride (96.0%, Sinopharm Chemical Reagent, China), lithium borohydride (95.0%, Acros) and potassium borohydride (98.0%, Alfa-Aesar) were used as received. Tetrahydrofuran (THF) and hexane were refluxed over potassium/benzophenone ketyl prior to use. Ethyl acetate was stirred over CaH₂ and distilled. *N,N*-Dimethylformamide (DMF) was purified by drying over 0.4 nm molecular sieves followed by vacuum distillation.

Synthesis of γ -benzyl-L-glutamate *N*-carboxyanhydride (BLG NCA) and L-alanine *N*-carboxyanhydride (ALA NCA)

In a typical procedure, γ -benzyl-L-glutamate (10.1 g, 42.2 mmol) was charged into a 250 mL round bottom Schlenk flask under argon followed by 100 mL anhydrous THF. Triphosgene (4.60 g, 15.5 mmol), dissolved in 20 mL anhydrous THF, was added dropwise into the BLG solution over a period of half an hour. The reaction was left to proceed for a further 2 h under argon atmosphere until the solution became clear. The solution was concentrated and poured into anhydrous hexane. The crude white precipitate was purified by recrystallization four times using a mixture of ethyl acetate and hexane resulting in BLG NCA in crystalline form (10.37 g, 83%). ALA NCA (53%) was synthesized following the same procedures.

Polymerization of BLG NCA

All polymerizations were carried out in Schlenk tubes under argon. In a typical polymerization, a mixture of BLG NCA (0.355 g, 1.35 mmol) in 2.7 mL DMF and 0.18 mL NaBH₄ (0.0555 mol/L in DMF) was sealed in a tube and placed in a 40 °C thermostated oil bath for 24 h. The polymer was isolated by precipitation from diethyl ether and dried in vacuum (yield = 99.2%).

Polymerization of ALA NCA

ALA NCA (0.3396 g, 2.95 mmol) was dissolved in 3 mL THF. 0.10 mL NaBH₄ (0.9062 mol/L in DMF) was then added by a syringe. The tube was sealed and placed at room temperature for 3 days. The produced polymer was isolated by precipitation from diethyl ether, washed with THF and dried in vacuum (yield > 99%).

Characterizations

NMR spectra were recorded on a Bruker Avance DMX 500 spectrometer (^1H : 500 MHz, ^{13}C : 125 MHz) using CF_3COOD as solvent and trifluoroacetic acid (CF_3COOH) as internal reference. MWs and MWDs were determined by gel permeation chromatography/multiangle laser light scattering (GPC/MALLS) which consisted of a Waters 1515 isocratic high performance liquid chromatograph pump, a Wyatt DAWN DSP multiangle laser light scattering detector, a Wyatt Optilab DSP interferometric refractometer and two columns of Styragel (HT3 and HT4). DMF containing 0.1 mol/L LiBr was used as the eluent with a flow rate of 1.0 mL/min at 60 °C. The MALDI-ToF mass spectra were measured on an Applied Biosystems Voyager System 4350 equipped with a nitrogen laser ($\lambda = 337$ nm). All mass spectra were recorded in the reflection mode with an acceleration voltage of 20 kV. The irradiation targets were prepared from hexafluoroisopropyl alcohol solutions with 2,5-dihydroxybenzoic acid as matrix and potassium trifluoroacetate as dopant.

RESULTS AND DISCUSSION

MBH_4 ($\text{M} = \text{Li}, \text{Na}$ and K) are efficient catalysts for ROP of BLG NCA as shown in Table 1. Generally, the polymerizations were finished within 24 h as confirmed by FTIR and the products were isolated in high yields (> 90%). The MWs of polypeptides depend on monomer to catalyst feed ratios but deviate from the theoretical values. The polypeptide MWDs are relatively narrow in the range of 1.1 and 1.6 depending on reaction temperatures. At lower reaction temperatures, PBLGs with relatively narrow MWDs can be prepared (Table 1).

Table 1. Polymerizations of BLG NCA catalyzed by MBH_4 ($\text{M} = \text{Li}, \text{Na}$ and K)^a

Run	MBH_4	Temp. (°C)	$[\text{NCA}]/[\text{MBH}_4]$	Yield (%)	M_n^b (10^4Da)	MWD ^b
1	LiBH_4	40	33	89.0	3.47	1.16
2	LiBH_4	40	67	95.2	4.31	1.35
3	LiBH_4	40	125	96.7	4.75	1.52
4	NaBH_4	40	33	97.1	3.23	1.32
5	NaBH_4	40	67	98.2	4.35	1.37
6	NaBH_4	40	125	99.2	5.60	1.54
7	KBH_4	40	32	90.6	2.56	1.29
8	KBH_4	40	67	94.7	3.23	1.52
9	KBH_4	40	125	95.7	5.27	1.41
10	LiBH_4	15	125	96.4	4.03	1.36
11	KBH_4	15	125	95.4	5.63	1.18

^a Reaction conditions: [BLG NCA] = 0.5 mol/L, 24 h in DMF;

^b Determined by GPC/MALLS in DMF containing 0.1 mol/L LiBr at 60 °C

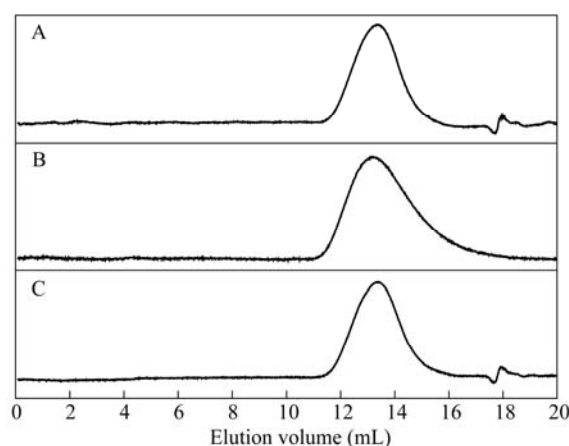


Fig. 1 GPC traces of PBLGs catalyzed by LiBH_4 (run 3 in Table 1) (A), NaBH_4 (run 6 in Table 1) (B) and KBH_4 (run 9 in Table 1) (C)

Different alkali metal borohydride compounds (MBH_4 , $\text{M} = \text{Li}$, Na and K) do not show obvious differences. As shown in Fig. 1, GPC traces of PBLG exhibit a symmetrical mono-modal shape implying a single active species is responsible for chain growth.

Unlike PBLG, PALA is not soluble in aprotic solvents and precipitates during polymerization resulting in low MW polypeptides useful for NMR and mass spectrum analysis^[24]. The MALDI-ToF MS of PALA synthesized by NaBH_4 at room temperature is shown in Fig. 2. One population of polymer chains is resolved corresponding to the polypeptides with hydroxyl- and amino-end groups (series A). Another set of peaks (B) with lower intensity is the sodium cationized PALA chains. The adjacent peaks have an interval of 71 Da corresponding to an ALA repeating unit. PALAs prepared by LiBH_4 and KBH_4 generally have the same end group structures as shown in Fig. S1 and Fig. S2 (Supporting Information).

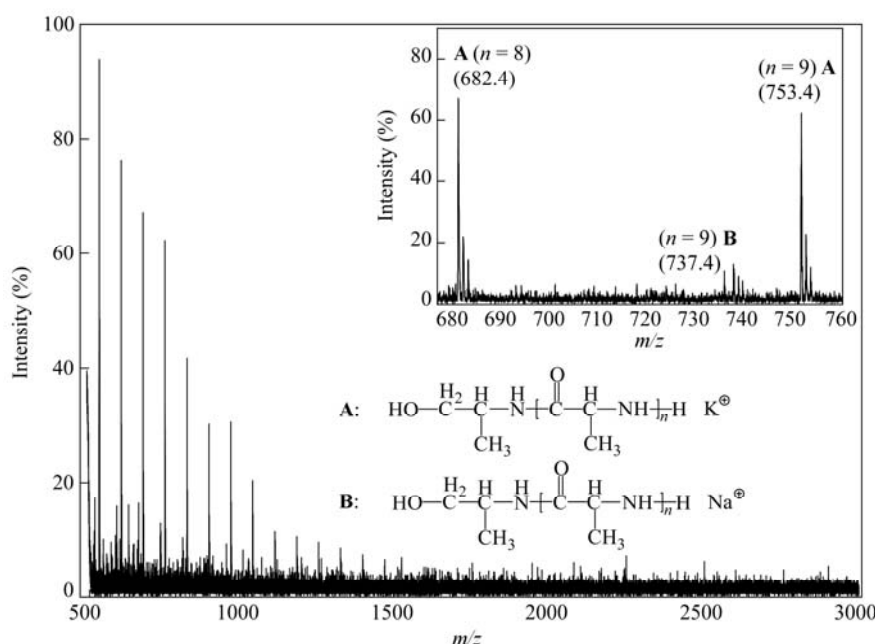


Fig. 2 MALDI-ToF mass spectrum (K^+ doping) of PALA catalyzed by NaBH_4 at 40°C in THF with $[\text{ALA NCA}]/[\text{BH}_4] = 30/1$

Evidence for end groups of PALA prepared by NaBH_4 is also provided by fully-assigned ^1H -NMR, ^{13}C -NMR, ^1H - ^1H COSY and ^1H - ^{13}C HMQC spectra as illustrated in Figs. 3–6. As shown in Fig. 3, in comparison with α -carboxylic- ω -amino-PALA which is prepared in the presence of water (Figs. 3B and 3C), a signal (H_f) around $\delta = 1.45$ is seen in NaBH_4 -catalyzed PALA which is ascribed to the methyl-proton of the last unit next to the hydroxyl end group. Besides, signals belong to the methylene (H_{g1} and H_{g2}) and methenyl (H_c) of the hydroxyl-contained unit are also found while these signals are absent in water-catalyzed one. Because the methylene group is linked to a chiral carbon (H_c), its signal splits into two peaks residing at $\delta = 4.50$ (H_{g1}) and $\delta = 4.66$ (H_{g2}) with a geminal coupling constant of 80 Hz. Although the signals of H_c and H_{g2} overlap with the main peak of H_b , they can be distinguished by ^1H - ^1H COSY (Fig. 5) and ^1H - ^{13}C HMQC spectra (Fig. 6). In Fig. 5, the coupling signal H_c - H_{g1} can be resolved indicating the existence of H_c . In ^1H - ^{13}C HMQC spectrum (Fig. 6) H_{g2} - C_g and H_{g1} - C_g coupling signals are found. The amino end-signals (H_a and H_d) in Fig. 3A are ascribed by comparison with water-catalyzed α -carboxylic- ω -amino-PALA (Figs. 3B and 3C), which is further confirmed through ^1H - ^1H COSY (Fig. 5) and ^1H - ^{13}C HMQC spectra (Fig. 6) by the coupling signals of H_a - H_d , H_a - C_a and H_d - C_d . It is in accordance with ^1H -NMR spectrum of PALA methylate reported by Kricheldorf^[25]. It is noteworthy that the methylene protons linked to a hydroxyl group (HOCH_2-) which usually locate at $\delta = 3\sim 4$ in

CDCl_3 solvent appear at $\delta = 4.50$ ($\text{H}_{\text{g}1}$) and 4.66 ($\text{H}_{\text{g}2}$) in Fig. 5. This down-field shift is caused by the solvent CF_3COOD since the same end group (HOCH_2-) signal in a poly(ϵ -caprolactone) chain originally located at $\delta = 3.65$ in CDCl_3 moves to $\delta = 4.40$ in a mixed solvent $\text{CDCl}_3/\text{CF}_3\text{COOH}$ ($V:V = 3:1$).

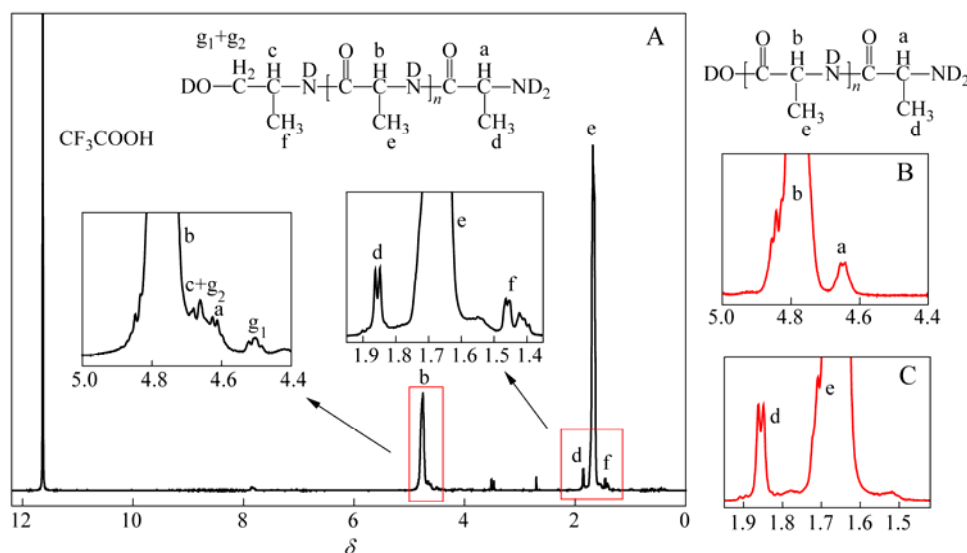


Fig. 3 ^1H -NMR spectra of PALA catalyzed by NaBH_4 (A) and H_2O (B and C) at 25°C in THF with $[\text{ALA NCA}] / ([\text{BH}_4] \text{ or } [\text{H}_2\text{O}]) = 30/1$

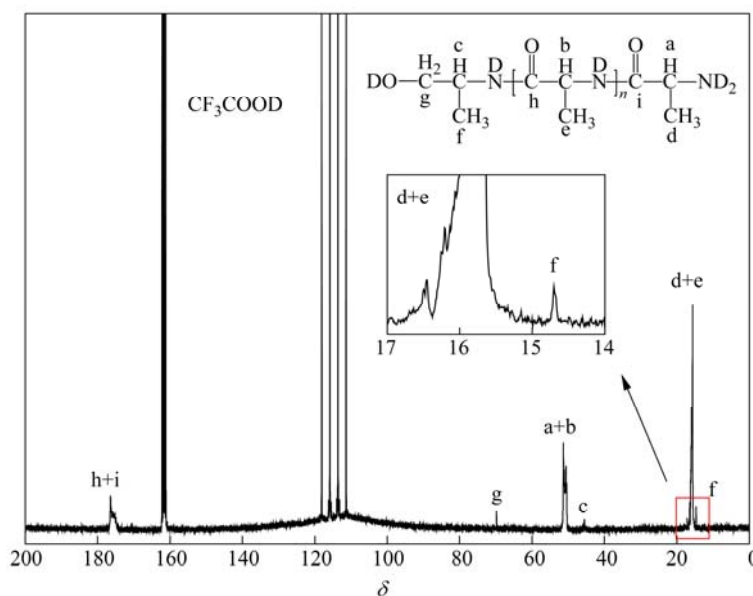


Fig. 4 ^{13}C -NMR spectrum of PALA catalyzed by NaBH_4 at 25°C in THF with $[\text{ALA NCA}] / [\text{BH}_4] = 30/1$

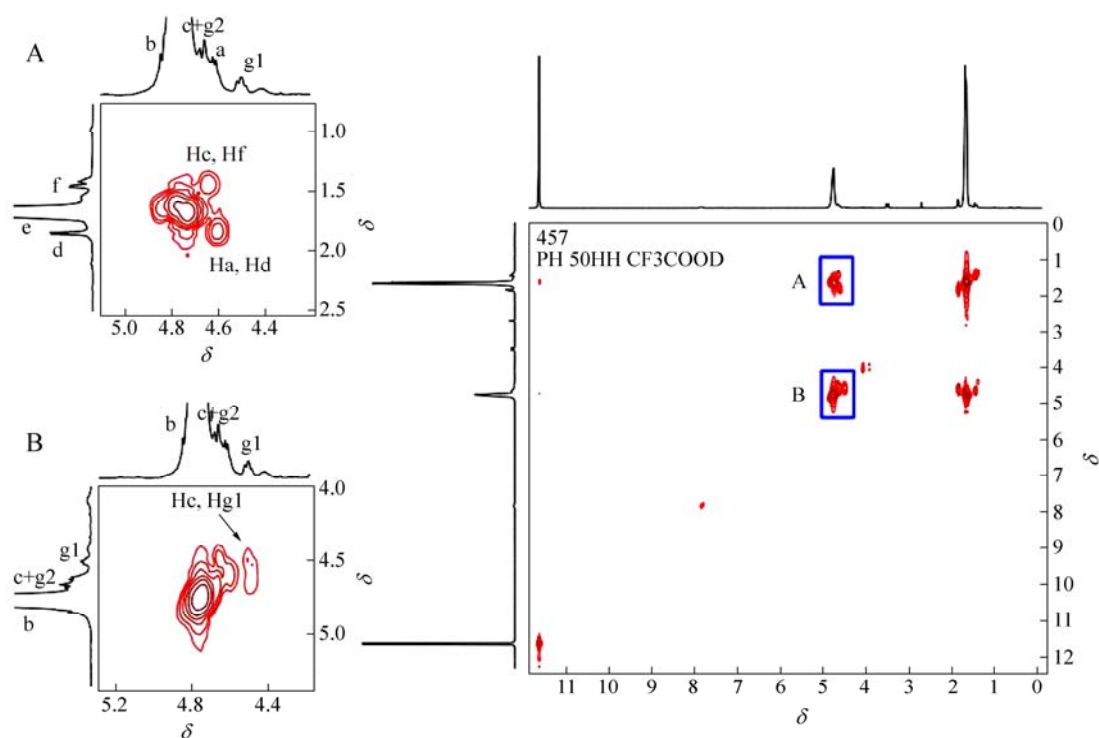


Fig. 5 ^1H - ^1H COSY spectrum of PALA catalyzed by NaBH_4 at 25°C in THF with $[\text{ALA NCA}]/[\text{BH}_4] = 30/1$

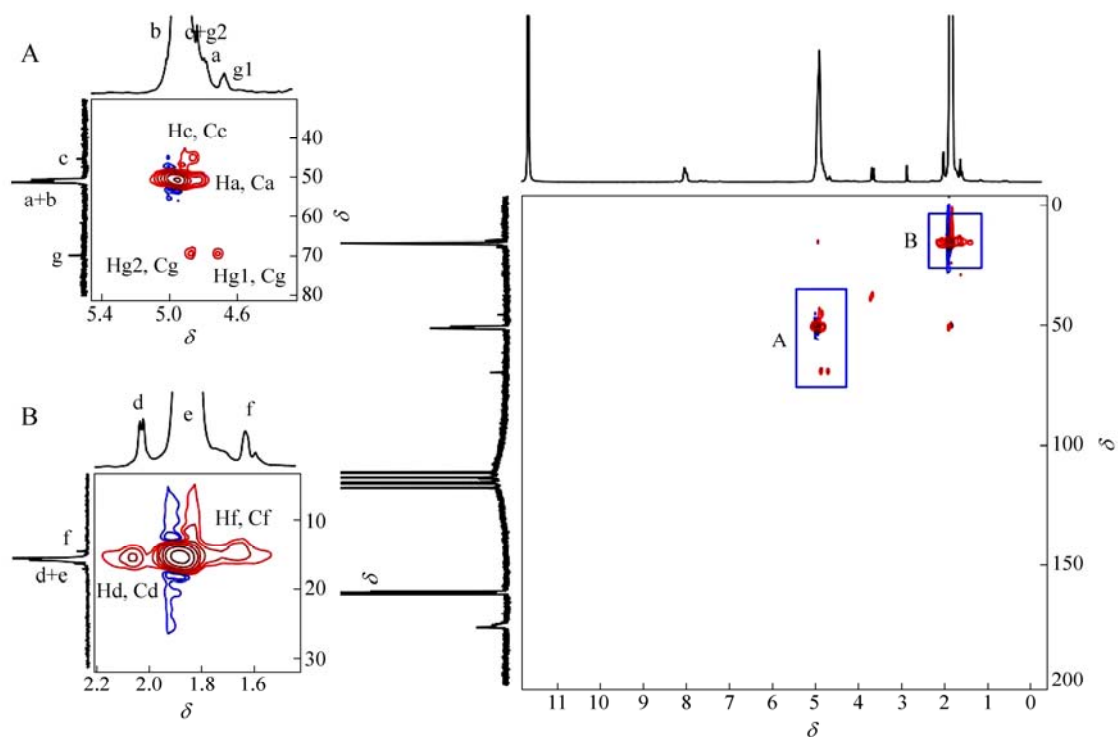
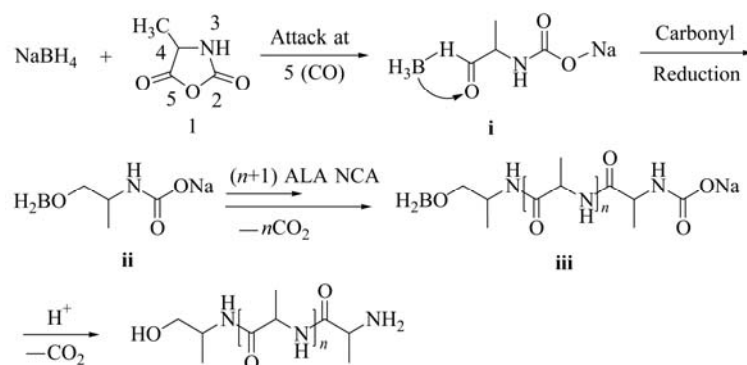


Fig. 6 ^1H - ^{13}C HMQC spectrum of PALA catalyzed by NaBH_4 at 25°C in THF with $[\text{ALA NCA}]/[\text{BH}_4] = 30/1$

Based on our previous report on rare earth metallic borohydride compounds catalyzed NCA polymerization^[24, 26], a possible mechanism to form into α -hydroxyl- ω -amino polyalanine is proposed in Scheme 1. NaBH₄ attacks 5-CO of ALA NCA, generating the compound **i** which forms into **ii** by an intramolecular reduction of the carbonyl group by the adjacent BH₃ group. The intermediate **ii** initiates further NCA polymerization on the other chain end and forms into α -hydroxyl- ω -amino polyalanine after termination.



Scheme 1 Possible polymerization mechanism of ALA NCA catalyzed by NaBH₄

Very recently, we used rare earth tris(borohydride) complexes as catalysts for NCA polymerizations which give a mixture of α -hydroxyl- ω -aminotelechelic polypeptide and α -carboxylic- ω -aminotelechelic one at room temperature or above^[24, 26]. When we tried to use common reducing agents including LiAlH₄, H₂/Pd-C and NaBH₄ to reduce the corresponding carboxylic end groups, none of them was successful in preparing α -hydroxyl- ω -aminotelechelic polypeptide with high purity.

CONCLUSIONS

In this work, we demonstrate that MBH₄ (M = Li, Na and K) are feasible and unique catalysts for the synthesis of α -hydroxyl- ω -aminotelechelic polypeptides with relatively narrow MWDs in high yields (> 90%) within 24 h at room temperature. This polypeptide is promising for further chain extension and biological applications.

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