

Electronic Supplementary Information for Chemical Communications

**Chemoenzymatic synthesis of polypeptides
containing the unnatural amino acid 2-
aminoisobutyric acid**

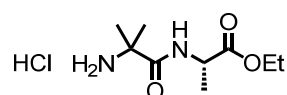
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Experimental

Materials. Papain was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used as received. The activity was approximately 0.5 U g^{-1} , where one unit is defined to hydrolyze 1 mmol of *N*-benzoyl-DL-arginine *p*-nitroanilide per minute at pH 7.5 and 25 °C. Diethyl ether and triethylamine were dried over 4Å molecular sieves and stored under nitrogen. The Aib-containing dipeptide AibAla-OEt and tripeptide, AlaAibAla-OEt, were synthesized via a modified conventional solution method according to the reported procedure.¹ All other reagents were used as received without any purification unless otherwise noted.

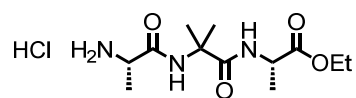
Synthesis of AibAla-OEt HCl salt.



To a flask equipped with an addition funnel and a stir bar were added α -(Bocamino)isobutyric acid (10.16 g, 50 mmol), L-alanine ethyl ester hydrochloride (7.68 g, 50 mmol), 1-hydroxybenzotriazole (HOBt) monohydrate (6.76 g, 50 mmol), triethylamine (7.0 mL, 50 mmol) and chloroform (50 mL) at -10 °C under nitrogen. A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (water-soluble carbodiimide, WSCI) hydrochloride (9.59 g, 50 mmol) in chloroform (50 mL) was added dropwise over

30 min, and the resulting mixture was stirred at $-10\text{ }^{\circ}\text{C}$ for 30 min and then at $25\text{ }^{\circ}\text{C}$ for 24 h. The mixture was washed sequentially with water, 5% NaHCO_3 aq., and brine. The organic layer was dried with Na_2SO_4 and concentrated using a rotary evaporator. The product was dried in vacuo to give Boc-AibAla-OEt in a yield of 13.9 g (92%). The obtained Boc-AibAla-OEt was then subjected to deprotection of the Boc group. To the solution of Boc-AibAla-OEt (7.56 g, 25 mmol) in dichloromethane (15 mL) was slowly added trifluoroacetic acid (9.6 mL, 0.125 mol) at $0\text{ }^{\circ}\text{C}$ under nitrogen. The mixture was stirred at $0\text{ }^{\circ}\text{C}$ for 10 min and then at $25\text{ }^{\circ}\text{C}$ for 24 h. After the solvent was removed under reduced pressure, the crude product was dissolved in dioxane/HCl (4 M, 10 mL). The solution was poured into diethyl ether. The precipitate was filtered, washed with diethyl ether, and dried in vacuo to afford AibAla-OEt as a white hygroscopic solid. The yield was 5.47 g (92%).

Synthesis of AlaAibAla-OEt HCl salt.



To a flask equipped with an addition funnel and a stir bar were added *N*-Boc-L-alanine (1.59 g, 8.4 mmol), AibAla-OEt hydrochloride (2.0 g, 8.4 mmol), HOBt monohydrate (1.28 g, 8.4 mmol), triethylamine (1.2 mL, 8.4 mmol) and chloroform (10 mL) at $-10\text{ }^{\circ}\text{C}$ under nitrogen. A solution of WSCI hydrochloride (1.61 g, 8.4 mmol) in chloroform (10

mL) was added dropwise over 30 min, and the resulting mixture was stirred at $-10\text{ }^{\circ}\text{C}$ for 30 min and then at $25\text{ }^{\circ}\text{C}$ for 24 h. The mixture was washed sequentially with water, 5% NaHCO_3 aq., and brine. The organic layer was dried with Na_2SO_4 and concentrated using a rotary evaporator. The product was dried in vacuo to give Boc-AlaAibAla-OEt in a yield of 2.45 g (78%). The obtained Boc-AlaAibAla-OEt was subsequently subjected to deprotection of the Boc group. To the solution of Boc-AlaAibAla-OEt (2.45 g, 6.6 mmol) in dichloromethane (6.0 mL) was slowly added trifluoroacetic acid (9.6 mL, 0.125 mol) at $0\text{ }^{\circ}\text{C}$ under nitrogen. The mixture was stirred at $0\text{ }^{\circ}\text{C}$ for 10 min and then at $25\text{ }^{\circ}\text{C}$ for 24 h. After the solvent was removed under reduced pressure, the crude product was dissolved in dioxane/HCl (4 M, 3 mL). The solution was poured into diethyl ether. The precipitate was filtered, washed with diethyl ether, and dried in vacuo to afford AlaAibAla-OEt as a white hygroscopic solid. The yield was 1.98 g (97%).

General procedure of chemoenzymatic polymerization using papain. Poly(L-alanine) (polyAla) was synthesized by papain-catalyzed polymerization of L-alanine ethyl ester hydrochloride according to a previously reported procedure.² The general procedure for the chemoenzymatic polymerization of Aib-containing monomers was as follows (Run 6 in Table 1): to a glass tube equipped with a stir bar were added AlaAibAla-OEt HCl salt (0.155 g, 0.5 mmol) and phosphate buffer (1 mL, 1 M, pH = 8.0), and the mixture

was stirred at 40 °C until all of the substrates were completely dissolved. To this solution was added a solution of papain (0.100 g) in phosphate buffer (0.5 mL) in one portion. The final concentrations of AlaAibAla-OEt and papain were 0.25 M and 50 mg/mL, respectively. The mixture was stirred at 800 rpm and 40 °C for 6 h. After the mixture cooled to room temperature, the precipitate was collected by centrifugation at 7000 rpm for 10 min at 4 °C. The crude product was washed twice with deionized water and lyophilized to provide the oligopeptide as a white solid. The yield was 0.037 g (33%).

Time course experiment on chemoenzymatic polymerization of AlaAibAla-OEt. To a glass tube equipped with a stir bar were added AlaAibAla-OEt HCl salt (0.155 g, 0.5 mmol) and phosphate buffer (1 mL, 1 M, pH = 8.0), and the mixture was stirred at 40 °C until all the substrates were completely dissolved. To this solution was added a solution of papain (0.100 g) in phosphate buffer (0.5 mL) in one portion. After the reaction was started at 40 °C, an aliquot (10 μ L) of the final solution was taken at 0, 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, and 120 min, and dissolved in dimethylsulfoxide- d_6 (DMSO- d_6 , 0.5 mL). These samples were subjected to ^1H nuclear magnetic resonance (NMR) measurement, and the signal intensities of α -methyl protons in Ala and Aib residues and of methyl group in ethanol (leaving group) in ^1H NMR spectra was compared to determine the conversion.

Analyses. The infrared (IR) spectra of the bulk samples were recorded by using an IRPrestige-21 Fourier transform infrared spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with a MIRacle A single-reflection attenuated total reflection (ATR) unit using a Ge prism. The ^1H and ^{13}C NMR spectra were recorded using a JNM-EX270 (JEOL, Tokyo, Japan) at 25 °C and at frequencies of 270 and 67.5 MHz, respectively. DMSO- d_6 with trifluoroacetic acid- d (TFA- d) (5/1 in volume) was used as the solvent for the polypeptides; tetramethylsilane was used as the internal standard. A Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI TOF) mass spectrometric analysis was conducted using an ultrafleXtreme MALDI-TOF spectrophotometer (Bruker Daltonics, Billerica, MA, USA) operated in reflection mode at an accelerating voltage of 15 kV. The sample was dissolved in water/acetonitrile (0.8 mg/mL,) containing 0.1% TFA, mixed with a solution of α -cyano-4-hydroxycinnamic acid (CHCA) in water/acetonitrile (10 mg/mL), and deposited onto an MTP 384 ground steel BC target plate. A circular dichroism (CD) spectroscopic analysis was conducted using a Jasco J-820 CD spectropolarimeter (JASCO, Tokyo, Japan). Measurements were conducted on polypeptide solutions (100 μM) in 2,2,2-trifluoroethanol (TFE) using a quartz cuvette with a 0.1 cm path length. Each spectrum represents the average of 10 scans from 190 to 290 nm with a resolution of 1 nm, obtained at 200 nm min^{-1} with a bandwidth of 1 nm.

References

1. E. Galoppini and M. A. Fox, *J. Am. Chem. Soc.*, 1996, **118**, 2299.
2. P. J. Baker and K. Numata, *Biomacromolecules*, 2012, **13**, 947.

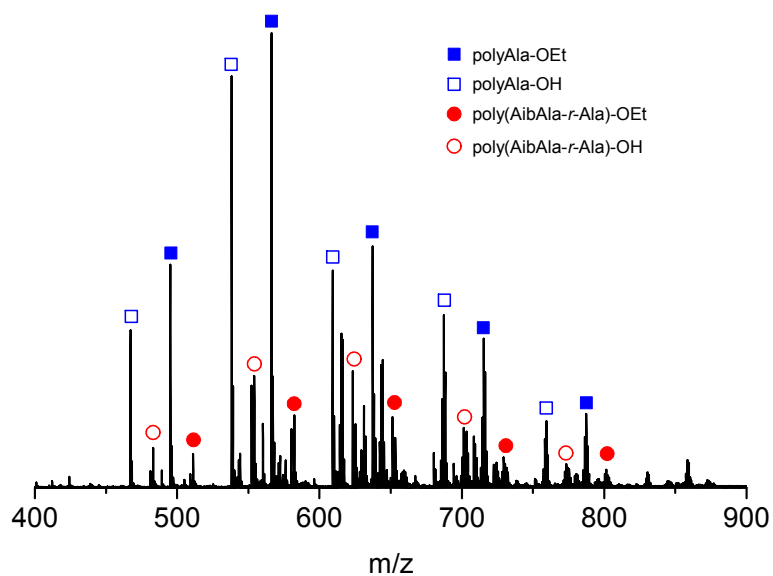


Fig. S1 MALDI-TOF mass spectrum of poly(AibAla-*r*-Ala) (Run 4, Table 1). Filled and open symbols indicate the series of peaks for polypeptides with ethyl ester and carboxylic acid (hydrolyzed) terminal groups, respectively.

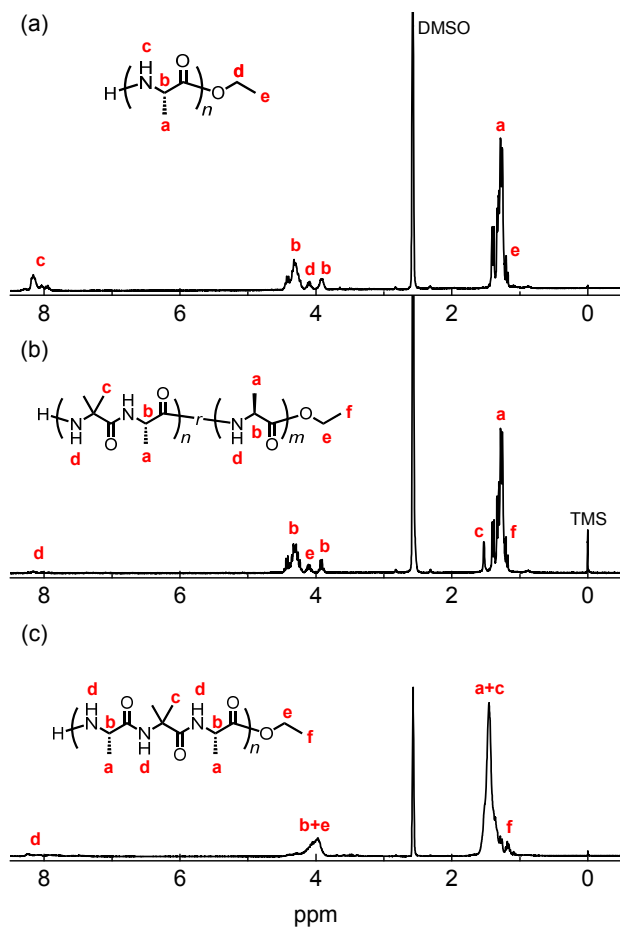


Fig. S2 ^1H NMR spectra of (a) poly(L-alanine) (polyAla), (b) poly(AibAla-*r*-Ala) (Run 4, Table 1), and (c) poly(AlaAibAla) (Run 6, Table 1) in DMSO- d_6 /TFA- d (5/1 in volume).

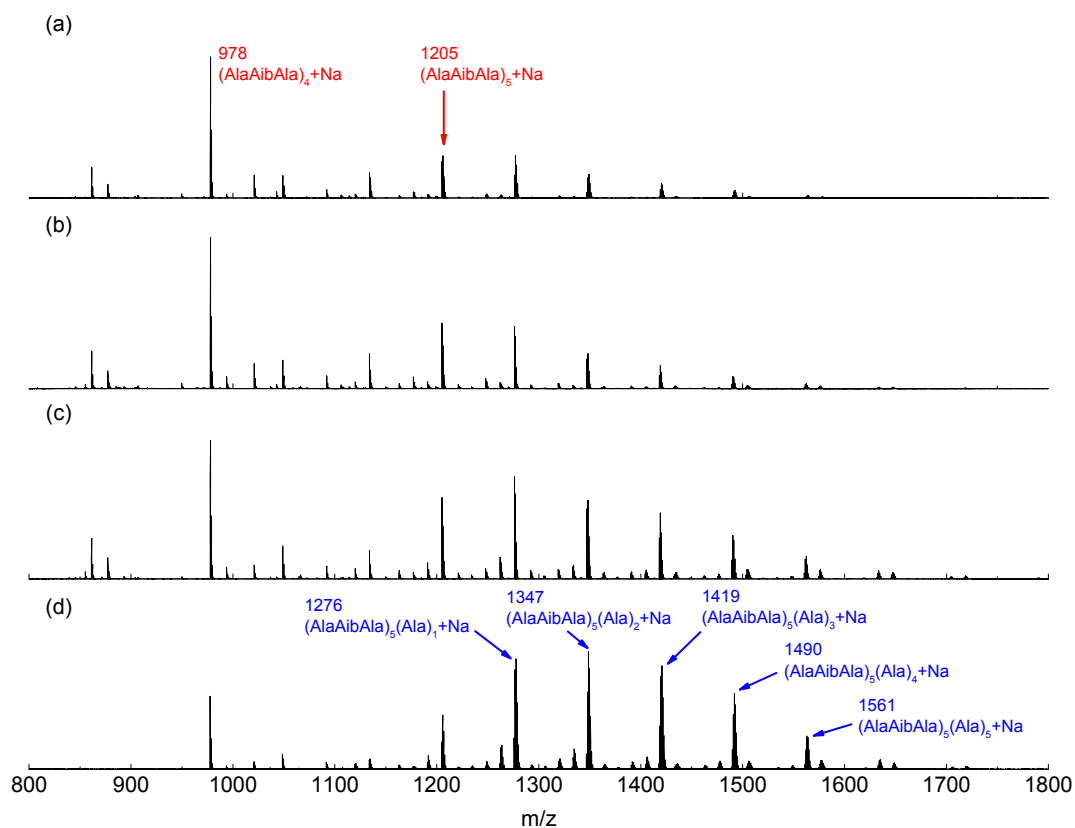


Fig. S3 MALDI-TOF mass spectra of the poly(AlaAibAla)s obtained by chemoenzymatic polymerization using papain at monomer feed concentrations of (a) 0.17 M (Run 5, Table 1), (b) 0.25 M (Run 6, Table 1), (c) 0.33 M (Run 7, Table 1), and (d) 0.5 M (Run 8, Table 1).

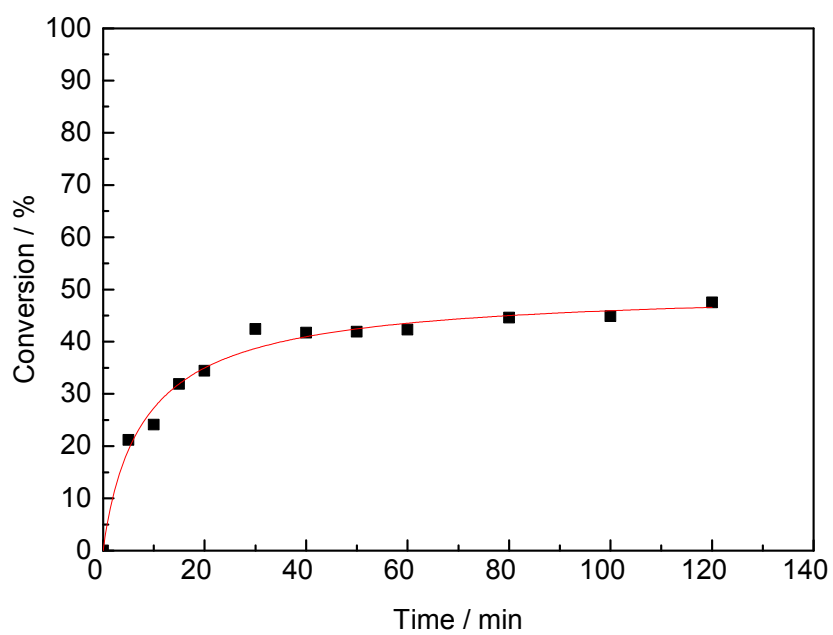


Fig. S4 Time course experiment on the enzymatic polymerization of AlaAibAla-OEt (0.25 M) using papain at 40 °C.