

Trypsin-Catalyzed Synthesis of Dipeptide Containing α -Aminoisobutylic Acid Using *p*- and *m*-(Amidinomethyl)phenyl Esters as Acyl Donor

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Two series of inverse substrates, *p*- and *m*-(amidinomethyl)phenyl esters derived from *N*-(*tert*-butoxycarbonyl)amino acid, were prepared as acyl donor components for enzymatic peptide synthesis. They were found to be readily coupled with an acyl acceptor such as *L*-alanine *p*-nitroanilide to produce dipeptide. An α -aminoisobutyric acid containing dipeptide was especially obtained in satisfactory yield. *Streptomyces griseus* trypsin was a more efficient catalyst than the bovine trypsin. The optimum condition for the coupling reaction was studied by changing the organic solvent, pH, and acyl acceptor concentration. It was found that the enzymatic hydrolysis of the resulting product was negligible.

Key words enzymatic peptide synthesis; *p*-(amidinomethyl)phenyl ester; *N*-Boc-aminoisobutylic acid; *m*-(amidinomethyl)phenyl ester; inverse substrate; trypsin

Sterically hindered α -amino acid such as the α -aminoisobutylic acid (Aib, 2-methylalanine) is one of the constituent of naturally occurring antibiotics.^{1–4} Peptides containing Aib are of interest as the model for conformational analysis of the peptide backbone (formation of α - or 3_{10} -helices, or β -turns).^{5,6} However, the introduction of Aib into a peptide is difficult,^{4,7,8} because the reactivity of sterically hindered α,α -disubstituted amino acid is much lower than that of typical α -amino acids. New chemical coupling reagent, 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate, (CIP), for sterically hindered α,α -disubstituted amino acids was developed and chemical synthesis of Alamethicin F-30 was achieved.⁹

Peptide synthesis by protease-catalyzed reverse reaction has been extensively studied with a variety of amino acids and peptide derivatives as coupling.^{10–15} The protease-catalyzed peptide synthesis is superior to the chemical coupling method. The method requires less-side chain protection than the chemical coupling method. A most serious drawback of the enzymatic method, however, is the respective substrate specificity. Thus, the application of proteases for peptide synthesis has not been fully exploited for possible synthesis of a number of biologically important peptides containing D-amino acid or other unusual amino acid.

In a previous paper, we reported that the *p*-amidinophenyl and *p*-guanidinophenyl esters behave as specific substrates for trypsin and trypsin-like enzymes such as thrombin and plasmin, *etc.*^{16–18} In these esters the site-specific groups (charged amidinium and guanidinium) for the enzyme are included in the leaving-group portion instead of being in the acyl moiety. Such a substrate was termed “inverse substrate” by us.¹⁶ The esters provided a novel method for the specific introduction of an acyl group for a wide variety of acyl

groups into a trypsin active site, and such acyl enzymes have proven to be useful for the peptide coupling.^{13,14,19–28} We also reported that α,α -dialkyl amino acid *p*-(guanidinomethyl)phenyl esters were useful substrates for enzymatic peptides synthesis containing such a hindered amino acid.²¹ Recently, we reported that preparation of new two series inverse substrates such as *N*-Boc-amino acid *p*- and *m*-(amidinomethyl)phenyl esters.²⁹ In the present work, we investigated trypsin-catalyzed peptide synthesis using these synthetic substrates as acyl donor components. Comparison was made between two trypsins of different origin (bovine and *Streptomyces griseus* (SG) trypsin) as the catalyst for peptide synthesis as shown in Chart 1.

Trypsin-Catalyzed Peptide Coupling Reaction The trypsin-catalyzed peptide coupling reaction has been studied by using synthetic inverse substrates (**1a–h**, **2a–c**) as acyl donors (Fig. 1). The reaction of *N*-*tert*-butoxycarbonyl-*L*-alanine *p*-(amidinomethyl)phenyl ester (*N*-Boc-*L*-Ala-OpAM) (**1a**) and *L*-alanine *p*-nitroanilide (*L*-Ala-*p*NA) to give *N*-Boc-*L*-Ala-*L*-Ala-*p*NA (**3a**) was examined in dimethyl sulfoxide (DMSO) or *N,N*-dimethylformamide (DMF) as a co-solvent. The reaction was also evaluated under the condition where the pH of the medium was changed and the concentration of the acyl acceptor (*L*-Ala-*p*NA) was changed. The coupling product was obtained in high yield with DMSO rather than with DMF.

Effects of DMSO and DMF concentration on coupling yields are shown in Fig. 2. Coupling yields higher than 50% were observed at the DMSO concentration range of 30–50%, and the best yield (73%) was obtained at 50% DMSO. The DMF showed slightly different behavior from that of DMSO, and the best yield (48%) was obtained at the 20% concentration. Although a high concentration of organic sol-

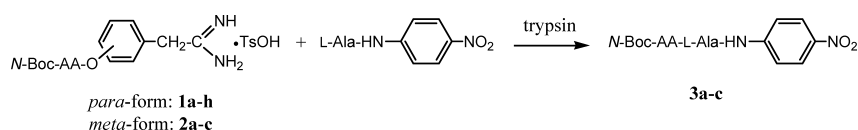


Chart 1

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vent prevents the hydrolysis of the acyl enzyme, it will decrease the enzymatic activity due to the denaturation of trypsin. Consequently, the coupling yield was decreased at a concentration of organic solvents above 60%.

The effect of pH of the buffer component in the medium on the coupling yields was analyzed. DMSO was mixed with

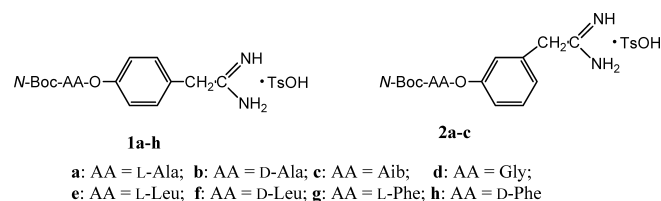


Fig. 1. Structure of Substrates

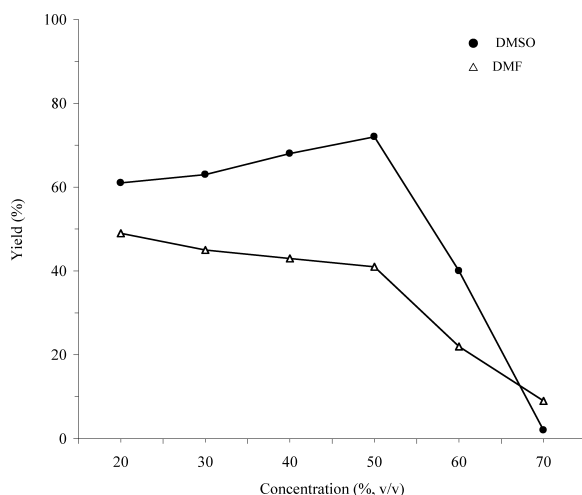


Fig. 2. Effect of Organic Solvent on Bovine Trypsin-Catalyzed Condensation of *N*-Boc-L-Ala-OpAM (**1a**) with L-Ala-*p*NA

Reaction was carried out in 50 mM MOPS buffer (pH 7.0, containing 20 mM CaCl_2). Cosolvents are DMSO (●) and DMF (△) at 25 °C. Product yield was analyzed after a reaction period of 24 h in which the coupling was completed. *N*-Boc-L-Ala-OpAM (**1a**), 1 mM; L-Ala-*p*NA, 20 mM; bovine trypsin, 10 μM .

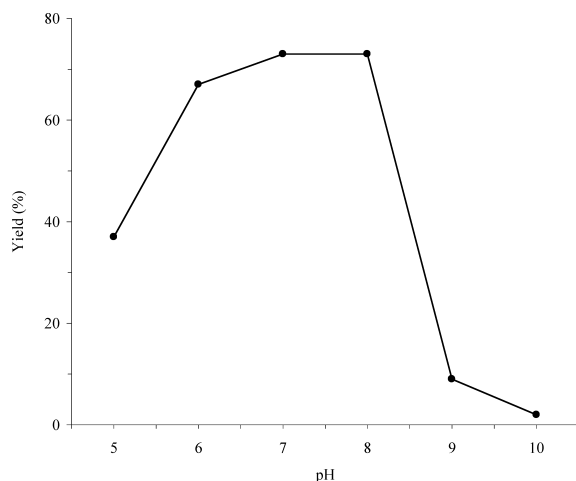


Fig. 3. pH Dependency of Bovine Trypsin-Catalyzed Condensation of *N*-Boc-L-Ala-OpAM (**1a**) with L-Ala-*p*NA

Reaction was carried out in 50% DMSO–50 mM MES buffer (pH 5.0 and 6.0, containing 20 mM CaCl_2), 50 mM MOPS buffer (pH 7.0 and 8.0, containing 20 mM CaCl_2), and 50 mM carbonate buffer (pH 9.0, 10.0) at 25 °C. Product yield was analyzed after a reaction period of 24 h in which the coupling was completed. *N*-Boc-L-Ala-OpAM (**1a**), 1 mM; L-Ala-*p*NA, 20 mM; bovine trypsin, 10 μM .

2-morpholino-1-ethanesulfonate (MES) (50 mM, containing 20 mM CaCl_2), 3-morpholino-1-propanesulfonate (MOPS) (50 mM, containing 20 mM CaCl_2), and carbonate buffers with various pH values, as shown in Fig. 3. The pH values given in Fig. 3 are those of the buffer itself before mixing with organic co-solvent. The pH dependency of the coupling yield for the reaction period of 24 h was determined. The yield was greatly decreased at higher pH than 9. The observed dependency was similar to that of trypsin-catalyzed hydrolysis of the specific ester substrates as reported previously in which the catalytic rate was increased at the higher pH and reached the limit at around pH 9.³¹ The best yield (74%) was obtained around pH8 using MOPS buffer.

The effect of acyl acceptor concentration on the coupling yields in 50% aqueous DMSO is shown in Fig. 4. The dependency can be explained to be due to the saturation of the enzyme binding site with the acyl acceptor. The reaction yield reached maximum (73%) at the concentration around

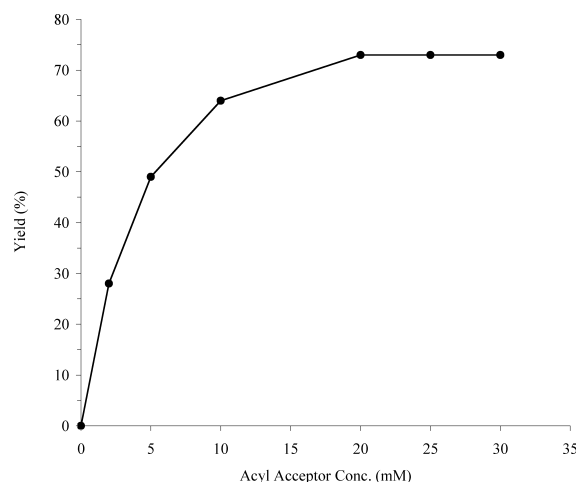


Fig. 4. Effect of Acyl Acceptor Concentration on Bovine Trypsin-Catalyzed Condensation of *N*-Boc-L-Ala-OpAM (**1a**) with L-Ala-*p*NA

Reaction was carried out in 50 mM MOPS buffer containing 50% DMSO at 25 °C. Product yield was analyzed after a reaction period of 24 h in which the coupling was completed. *N*-Boc-L-Ala-OpAM (**1a**), 1 mM; L-Ala-*p*NA, 20 mM; bovine trypsin, 10 μM .

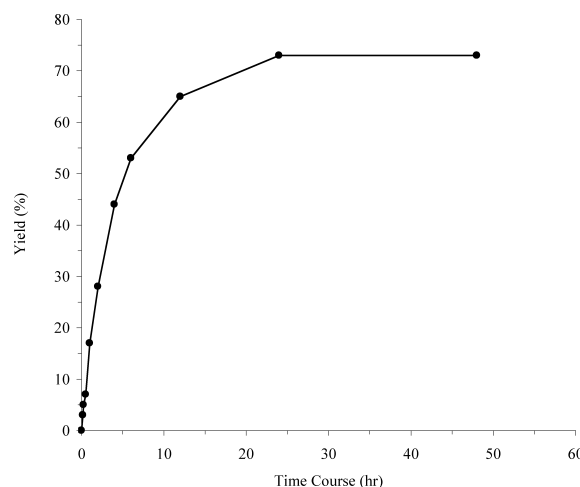


Fig. 5. Time Course Bovine Trypsin-Catalyzed Condensation of *N*-Boc-L-Ala-OpAM (**1a**) with L-Ala-*p*NA

Reaction was carried out in 50 mM MOPS buffer containing 50% DMSO at 25 °C. Product yield was analyzed after a reaction period of 24 h in which the coupling was completed. *N*-Boc-L-Ala-OpAM (**1a**), 1 mM; L-Ala-*p*NA, 20 mM; bovine trypsin, 10 μM .

Table 1. Yields of Bovine Trypsin and SG Trypsin-Catalyzed Peptide Synthesis with Amino Acid Ester as Acyl Donor^{a)}

Entry No.	Enzyme	Acyl donor (No.)	Reaction time (h)	Product (No.)	Yield (%) ^{b)}
1	Bovine trypsin	<i>N</i> -Boc-L-Ala-OpAM (1a)	24	<i>N</i> -Boc-L-Ala-L-Ala-pNA (3a)	73
2	Bovine trypsin	<i>N</i> -Boc-D-Ala-OpAM (1b)	24	<i>N</i> -Boc-D-Ala-L-Ala-pNA (3b)	64
3	Bovine trypsin	<i>N</i> -Boc-Aib-OpAM (1c)	96	<i>N</i> -Boc-Aib-L-Ala-pNA (3c)	83
4	Bovine trypsin	<i>N</i> -Boc-Gly-OpAM (1d)	24	<i>N</i> -Boc-Gly-L-Ala-pNA (3d)	77
5	Bovine trypsin	<i>N</i> -Boc-L-Leu-OpAM (1e)	24	<i>N</i> -Boc-L-Leu-L-Ala-pNA (3e)	79
6	Bovine trypsin	<i>N</i> -Boc-D-Leu-OpAM (1f)	48	<i>N</i> -Boc-D-Leu-L-Ala-pNA (3f)	80
7	Bovine trypsin	<i>N</i> -Boc-L-Phe-OpAM (1g)	48	<i>N</i> -Boc-L-Phe-L-Ala-pNA (3g)	70
8	Bovine trypsin	<i>N</i> -Boc-D-Phe-OpAM (1h)	24	<i>N</i> -Boc-D-Phe-L-Ala-pNA (3h)	63
9	Bovine trypsin	<i>N</i> -Boc-L-Ala-OmAM (2a)	24	<i>N</i> -Boc-L-Ala-L-Ala-pNA (3a)	48
10	Bovine trypsin	<i>N</i> -Boc-D-Ala-OmAM (2b)	24	<i>N</i> -Boc-D-Ala-L-Ala-pNA (3b)	48
11	Bovine trypsin	<i>N</i> -Boc-Aib-OmAM (2c)	48	<i>N</i> -Boc-Aib-L-Ala-pNA (3c)	2
12	SG trypsin	<i>N</i> -Boc-L-Ala-OpAM (1a)	24	<i>N</i> -Boc-L-Ala-L-Ala-pNA (3a)	74
13	SG trypsin	<i>N</i> -Boc-D-Ala-OpAM (1b)	24	<i>N</i> -Boc-D-Ala-L-Ala-pNA (3b)	74
14	SG trypsin	<i>N</i> -Boc-Aib-OpAM (1c)	48	<i>N</i> -Boc-Aib-L-Ala-pNA (3c)	90
15	SG trypsin	<i>N</i> -Boc-Gly-OpAM (1d)	24	<i>N</i> -Boc-Gly-L-Ala-pNA (3d)	76
16	SG trypsin	<i>N</i> -Boc-L-Leu-OpAM (1e)	24	<i>N</i> -Boc-L-Leu-L-Ala-pNA (3e)	78
17	SG trypsin	<i>N</i> -Boc-D-Leu-OpAM (1f)	24	<i>N</i> -Boc-D-Leu-L-Ala-pNA (3f)	90
18	SG trypsin	<i>N</i> -Boc-L-Phe-OpAM (1g)	24	<i>N</i> -Boc-L-Phe-L-Ala-pNA (3g)	58
19	SG trypsin	<i>N</i> -Boc-D-Phe-OpAM (1h)	24	<i>N</i> -Boc-D-Phe-L-Ala-pNA (3h)	59
20	SG trypsin	<i>N</i> -Boc-L-Ala-OmAM (2a)	24	<i>N</i> -Boc-L-Ala-L-Ala-pNA (3a)	64
21	SG trypsin	<i>N</i> -Boc-D-Ala-OmAM (2b)	24	<i>N</i> -Boc-D-Ala-L-Ala-pNA (3b)	64
22	SG trypsin	<i>N</i> -Boc-Aib-OmAM (2c)	48	<i>N</i> -Boc-Aib-L-Ala-pNA (3c)	16

a) Conditions: acyl donor, 1 mM; acyl acceptor (L-Ala-pNA), 20 mM; bovine trypsin and SG trypsin, 10 μ M, respectively; 50% DMSO-MOPS (50 mM, pH 8.0, containing 20 mM CaCl₂); 25 °C. b) The values represent the mean of two runs (each value in within 2% variation).

20 mM of acyl acceptor.

The time courses of the coupling reaction of *N*-Boc-L-Ala-OpAM (**1a**) with L-Ala-pNA is shown in Fig. 5. The D-acyl donors are an efficient substrate for the enzymatic coupling reaction, as well as the L-acyl donors. The coupling yields did not drop even after prolonged reaction period. This result indicated that enzymatic secondary hydrolysis of the resulting peptides is negligible.

Consequently, standard conditions for trypsin-catalyzed peptide coupling reaction used (amidinomethyl)phenyl ester as acyl donor component were selected as described in the methods. The results of bovine trypsin-catalyzed coupling reaction were compared with those of SG trypsin-catalyzed coupling reaction as listed in Table 1. Each product completely coincides with the respective, authentic peptide sample. Coupling reaction of the *p*-(amidinomethyl)phenyl ester (**1a—h**) is much more efficient than that of the *m*-(amidinomethyl)phenyl ester (**2a—c**). In the case of *N*-Boc-Aib-OpAM (**1c**) (Entry 3, 14), Aib containing dipeptide (**3c**) was obtained in high yields with using the both catalysts. The corresponding dipeptides were also obtained using *para*-form substrates in satisfactory yield (Entry 1—8, 12—19).

Experimental

The melting points were measured on a Yanaco micro melting point apparatus. IR spectra were taken on a JASCO VALOR-III FT-IR spectrometer. ¹H- and ¹³C-NMR spectra were recorded on a JEOL JNM-FX-400 FT NMR spectrometer. Chemical shifts are quoted in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) are given in Hz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet; dd, doublet of doublets; m, multiplet. The optical rotations were measured with a JASCO DIP-360 digital polarimeter in a 5 cm cell. The FAB-MS were taken with a JEOL JMS-HX-110 spectrometer. Flash column chromatography was performed using Silica Gel 60N (Kanto Chemical Co., Inc.) as a solid support in the immobile phase. Kieselgel 60 F-254 plates (Merck) were used for thin-layer chromatography

(TLC). Bovine pancreas trypsin (EC 3.4.21.4) was purchased from Worthington Biochemical Corp. (twice crystallized, lot TRL). SG trypsin was prepared following the reported.^{32,33} HPLC grade DMSO and DMF from Kanto Chemical Co., Inc. were used. L-Ala-pNA was purchased from Peptide Institute, Inc. MOPS and MES were purchased from Wako Pure Chemical Industries. Tricine and *p*-methyumbelliferyl *p*'-guanidinobenzoate were purchased from Merck & Co., Inc.

Synthesis of Inverse Substrates All inverse substrates were prepared according to our previous paper.²⁹ The results and some properties of new inverse substrates (**1d—h**) are as follows:

N-(*tert*-Butoxycarbonyl)glycine *p*-(Amidinomethyl)phenyl Ester *p*-Toluenesulfonic Acid Salt (**1d**): mp 177—179 °C (from AcOEt-hexane). IR (KBr) cm⁻¹: 1753. ¹H-NMR (DMSO-*d*₆) δ : 1.39 (9H, s), 2.28 (3H, s), 3.69 (2H, s), 3.95 (2H, d, *J*=5.9 Hz), 7.10—7.13 (4H, m), 7.39—7.49 (5H, m), 8.62 (2H, s), 9.12 (2H, s). ¹³C-NMR (DMSO-*d*₆, 100 MHz) δ : 21.29, 28.64, 37.73, 79.12, 115.98, 122.51, 125.99, 128.69, 130.51, 138.56, 145.47, 150.27, 156.50, 169.33, 169.93. *Anal.* Calcd for C₁₅H₂₁N₃O₄·C₇H₈O₃S·1/3H₂O: C, 54.42; H, 6.16; N, 8.65; S, 6.60. Found: C, 54.59; H, 6.19; N, 8.24; S, 6.60.

N-(*tert*-Butoxycarbonyl)-L-leucine *p*-(Amidinomethyl)phenyl Ester *p*-Toluenesulfonic Acid Salt (**1e**): mp 112—114 °C (from AcOEt-hexane). IR (KBr) cm⁻¹: 1760. ¹H-NMR (DMSO-*d*₆) δ : 0.89—0.94 (6H, m), 1.39 (9H, s), 1.58—1.70 (3H, m), 2.28 (3H, s), 3.68 (2H, s), 4.17 (1H, brs), 7.07—7.12 (4H, m), 7.39—7.49 (5H, m), 8.58 (2H, s), 9.09 (2H, s). ¹³C-NMR (DMSO-*d*₆, 100 MHz) δ : 20.94, 21.42, 22.93, 24.52, 28.29, 37.37, 52.40, 122.05, 125.63, 128.35, 130.14, 138.24, 145.16, 150.05, 155.94, 168.97, 172.18. [α]_D²⁵ -31.1° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₉H₂₉N₃O₄·C₇H₈O₃S: C, 58.30; H, 6.96; N, 7.84; S, 5.99. Found: C, 58.28; H, 6.88; N, 7.68; S, 6.21.

N-(*tert*-Butoxycarbonyl)-D-leucine *p*-(Amidinomethyl)phenyl Ester *p*-Toluenesulfonic Acid Salt (**1f**): mp 177—179 °C (from AcOEt-hexane). IR (KBr) cm⁻¹: 1761. ¹H-NMR (DMSO-*d*₆) δ : 0.90—0.95 (6H, m), 1.41 (9H, s), 1.60—1.75 (3H, m), 2.30 (3H, s), 3.70 (2H, s), 4.17 (1H, brs), 7.08—7.14 (4H, m), 7.43—7.51 (5H, m), 8.30 (2H, s), 9.12 (2H, s). ¹³C-NMR (DMSO-*d*₆, 100 MHz) δ : 19.90, 21.01, 21.41, 22.94, 24.52, 28.30, 36.10, 52.40, 121.42, 125.69, 128.46, 129.90, 134.72, 138.45, 144.96, 149.32, 155.93, 158.05, 168.61, 172.26. [α]_D²⁵ +30.6° (*c*=1.0, MeOH). *Anal.* Calcd for C₁₉H₂₉N₃O₄·C₇H₈O₃S: C, 58.10; H, 6.85; N, 7.85; S, 6.41. Found: C, 58.28; H, 6.88; N, 7.68; S, 6.21.

N-(*tert*-Butoxycarbonyl)-L-phenylalanine *p*-(Amidinomethyl)phenyl Ester *p*-Toluenesulfonic Acid Salt (**1g**): A colourless amorphous powder. IR (KBr)

cm^{-1} : 1760. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.35 (9H, s), 2.28 (3H, s), 3.00–3.10 (2H, m), 3.68 (2H, s), 4.36–4.38 (1H, m), 6.97 (2H, d, $J=8.4$ Hz), 7.11 (2H, d, $J=7.9$ Hz), 7.23–7.35 (5H, m), 7.42 (2H, d, $J=8.4$ Hz), 7.48 (2H, d, $J=7.9$ Hz), 7.58 (1H, d, $J=7.2$ Hz), 8.60 (2H, s), 9.08 (2H, s). $^{13}\text{C-NMR}$ ($\text{DMSO}-d_6$, 100 MHz) δ : 20.93, 28.25, 36.43, 37.36, 55.66, 115.68, 122.01, 125.64, 126.80, 128.33, 128.48, 129.40, 130.13, 131.75, 137.29, 138.20, 145.22, 149.95, 155.74, 168.92, 171.16. $[\alpha]_D^{25} -8.5^\circ$ ($c=1.0$, MeOH). FAB-MS m/z : 570 ($\text{M}^+ + \text{H}$).

N-(*tert*-Butoxycarbonyl)-*D*-phenylalanine *p*-(Amidinomethyl)phenyl Ester *p*-Toluenesulfonic Acid Salt (**1h**): A colourless amorphous powder. IR (KBr) cm^{-1} : 1761. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 1.30 (9H, s), 2.22 (3H, s), 2.94–3.10 (2H, m), 3.62 (2H, s), 4.28–4.33 (1H, m), 6.91 (2H, d, $J=8.4$ Hz), 7.06 (2H, d, $J=37.9$ Hz), 7.27–7.26 (5H, m), 7.36 (2H, d, $J=8.4$ Hz), 7.43 (2H, d, $J=7.9$ Hz), 7.51 (1H, d, $J=7.2$ Hz), 8.54 (2H, s), 9.04 (2H, s). $^{13}\text{C-NMR}$ ($\text{DMSO}-d_6$, 100 MHz) δ : 20.93, 28.26, 36.44, 37.34, 55.66, 115.68, 122.00, 125.64, 126.81, 128.34, 128.48, 129.40, 130.14, 131.77, 137.29, 138.25, 145.15, 149.97, 155.72, 168.97, 171.20. $[\alpha]_D^{25} +7.8^\circ$ ($c=1.0$, MeOH). FAB-MS m/z : 570 ($\text{M}^+ + \text{H}$).

Trypsin-Catalyzed Peptide Coupling Reaction A solution of 50 μl of acyl donor (10 mM solution of inverse substrates in DMSO), 50 μl of acyl acceptor (200 mM solution of L-Ala-*p*NA in DMSO), 240 μl of 50 mM MOPS buffer (containing 20 mM of CaCl_2 , pH 8.0) and 150 μl of DMSO were mixed. To this mixture, 10 μl of trypsin solution (1 mM solution in 1 mM HCl) was added and incubated at 25 $^\circ\text{C}$. The progress of the coupling reaction was monitored by HPLC under the following conditions: Shim-pack CLC-ODS (M) (column i.d. 4.6 $\times$ 250 mm), isocratic elution at 1 ml/min, 0.1% aqueous trifluoroacetic acid/acetonitrile. An aliquot of the reaction mixture was injected and the eluate was monitored at 310 nm (chromophore due to the *p*-nitroanilide moiety). Peak identification was made by correlating the retention time with that of authentic samples which were chemically synthesized.^{34–36} Observed peak areas were used for the estimation of sample concentration.

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References

- Pandey R. C., Meng H., Cook J. C., Jr., Rinehart K. L., Jr., *J. Am. Chem. Soc.*, **99**, 5203–5205 (1977).
- Pandey R. C., Cook J. C., Jr., Rinehart K. L., Jr., *J. Am. Chem. Soc.*, **99**, 5205–5206 (1977).
- Bodo B., Rebuffat S., E. I. Hajji M., Davoust D., *J. Am. Chem. Soc.*, **107**, 6011–6017 (1985).
- Jost M., Greie J.-C., Stemmer N., Wilking S. D., Altendorf K., Sewald N., *Angew. Chem. Int. Ed. Engl.*, **41**, 4267–4269 (2002).
- Marshall G. R., Hodgkin E. E., Langs D. A., Smith G. D., Zabrocki J., Leplawy M. T., *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 487–491 (1990).
- Karle I. L., Balaram P., *Biochemistry*, **29**, 6747–6756 (1990).
- Frerot E., Coste J., Pantaloni A., Dufour M.-N., Jouin P., *Tetrahedron*, **47**, 259–270 (1991).
- Spencer J. R., Antonenko V. V., Delaet N. G. J., Goodman N., *J. Peptide Protein Res.*, **40**, 282–293 (1992).
- Akaji K., Kuriyama N., Kiso T., *Tetrahedron Lett.*, **35**, 3315–3318 (1994).
- Tsuzuki H., Oka T., Morihara K., *J. Biochem. (Tokyo)*, **88**, 669–675 (1980).
- Nakatsuka T., Sasaki T., Kaiser E. T., *J. Am. Chem. Soc.*, **109**, 3808–3810 (1987).
- Wong C.-H., *Science*, **244**, 1145–1152 (1989).
- Schellenberger V., Jakubke H.-D., *Angew. Chem. Int. Ed. Engl.*, **30**, 1434–1449 (1991).
- Bordusa F., *Chem. Rev.*, **102**, 4817–4867 (2002).
- Kumar D., Bhalla T. C., *Appl. Microbiol. Biotechnol.*, **68**, 726–736 (2005).
- Tanizawa K., Kasaba Y., Kanaoka Y., *J. Am. Chem. Soc.*, **99**, 4485–4488 (1977).
- Nakano M., Tanizawa K., Nozawa M., Kanaoka Y., *Chem. Pharm. Bull.*, **28**, 2212–2216 (1980).
- Nozawa M., Tanizawa K., Kanaoka Y., *J. Pharmacobio-Dyn.*, **3**, 213–219 (1980).
- Itoh K., Sekizaki H., Toyota E., Fujiwara N., Tanizawa K., *Bioorg. Chem.*, **24**, 59–68 (1996).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *Chem. Pharm. Bull.*, **44**, 1585–1587 (1996).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *Tetrahedron Lett.*, **38**, 1777–1780 (1997).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *Chem. Pharm. Bull.*, **46**, 846–849 (1998).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *Amino Acids*, **17**, 285–291 (1999).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *Chem. Pharm. Bull.*, **47**, 444–447 (1999).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *Chem. Pharm. Bull.*, **47**, 104–110 (1999).
- Sekizaki H., Murakami M., Itoh K., Toyota E., Tanizawa K., *J. Mol. Catal. B: Enzym.*, **11**, 23–28 (2000).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *Amino Acids*, **21**, 175–184 (2001).
- Sekizaki H., Itoh K., Toyota E., Tanizawa K., *J. Peptide Sci.*, **8**, 521–528 (2002).
- Sekizaki H., Itoh K., Shibuya A., Toyota E., Tanizawa K., *Chem. Pharm. Bull.*, **55**, 1514–1517 (2007).
- West J. B., Wong C.-H., *J. Org. Chem.*, **51**, 2728–2735 (1986).
- Gutfreund H., *Trans. Faraday Soc.*, **51**, 441–446 (1955).
- Yoshida N., Sasaki A., Inoue H., *FEBS Lett.*, **15**, 129–132 (1971).
- Yokosawa H., Hanba Y., Ishii S., *J. Biochem. (Tokyo)*, **79**, 757–763 (1976).
- Bieth J., Weremath C. G., *Biochem. Biophys. Res. Commun.*, **53**, 383–390 (1970).
- Kawai M., Nagai U., *Bull. Chem. Soc. Jpn.*, **55**, 1327–1328 (1982).
- Okada Y., Tsuda Y., Hirata A., Nagamatsu Y., Okamoto U., *Chem. Pharm. Bull.*, **30**, 2212–2216 (1982).