A New Synthetic Method for Dipeptides Containing α,β-Didehydroamino Acids Utilizing an α-Tosylglycine Residue

Yohsuke Shiraishi, Hiroshi Yamauchi, Takashi Takamura, and Hideki Kinoshita*

Department of Chemistry, Faculty of Science, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192

Received May 10, 2004; E-mail: kinosita@cacheibm.s.kanazawa-u.ac.jp

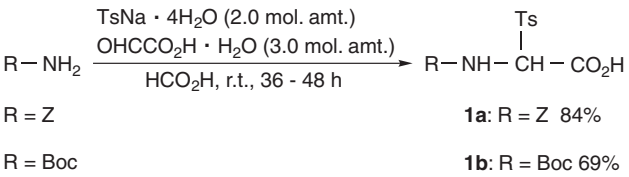
The reaction of peptides containing an *N*-Boc- or *N*-Z-α-tosylglycine residue at the *N*-terminus with a variety of excess nitro compounds under basic conditions to give the nitro compound-adducts in good yields, followed by the elimination of nitrous acid from the adducts to afford the corresponding dipeptide containing α,β-didehydroamino acids with (*Z*)-configuration predominantly. Treatment of the same starting materials with aldehydes in the presence of base and tributylphosphine also gave a dipeptide containing α,β-didehydroamino acids with high (*Z*)-selectivity in satisfactory yields. The present method can be successfully applied to the synthesis of a protected leucine enkephalin analog.

α,β-Didehydroamino acid residues are common components of naturally occurring peptides that remarkably affect the physiological activity of peptides,[1] as well as their conformations,[2] and their resistance to enzyme-catalyzed degradation.[3] Consequently, there are many reports related to methods for the syntheses of peptides containing α,β-didehydroamino acid residues.[4]

In previous papers, we already reported that *N*-*t*-butoxycarbonyl (Boc)- or *N*-benzyloxycarbonyl (Z)-α-tosylglycine ester was reacted with either a variety of nitroalkanes in the presence of base[5a] or various kinds of aldehydes in the presence of base and tributyl phosphine[5b] to afford the corresponding α,β-didehydroamino acid derivatives with high (*Z*)-selectivity in good yields. The latter procedure was successfully applied to the syntheses of optically active 4-hydroxyprolines.[5b]

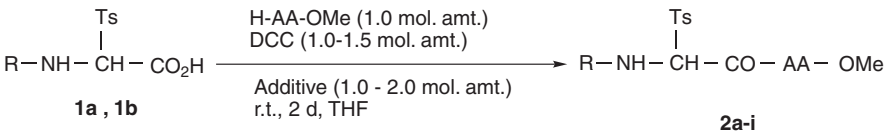
In the light of our results mentioned above, we now wish to report a convenient method for the preparation of dipeptides **17**–**31** containing an α,β-didehydroamino acid residue starting from dipeptides containing α-tosylglycine residue at *N*-terminus **2**. Z- and Boc-α-tosylglycines, **1a** and **1b**, were prepared by the reaction of sodium sulfinate, glyoxylic acid, and Z- or Boc-carbamate in 99% or 50% formic acid in 84% and 69% yields, respectively (Scheme 1).

First, dipeptide **2a** was obtained as a mixture of diastereomers in a 71% yield by the coupling reaction of methyl L-leucinate and *N*-Z-α-tosylglycine (**1a**) using the dicyclohexylcarbodiimide (DCC)–*N*-hydroxysuccinimide (HONSu) method. Dipeptides **2b**–**d** were obtained in moderate yields, respectively (Entries 2–4 in Table 1). Similarly, *N*-Boc protected dipeptides



Scheme 1. Preparation of Z- and Boc-α-tosylglycine.

Table 1. Preparation of Dipeptides **2a**–**i** Containing Tosylglycine Residue



Entry	R	AA	Additive	Product/%		Ratio of diastereomer[a)]
1	Z	Leu	HONSu	**2a**	71	56/44
2	Z	Ala	HOBt	**2b**	51	55/45
3	Z	Gly	HOBt	**2c**	65	
4	Z	Val	HOBt	**2d**	56	56/44
5	Boc	Leu	HOBt	**2e**	78	67/33
6	Boc	Ala	HOBt	**2f**	74	54/46
7	Boc	Gly	HOBt	**2g**	76	
8	Boc	Val	HONSu	**2h**	45	53/47
9	Boc	Ile	HOBt	**2i**	62	68/32

a) Determined by NMR spectrum.

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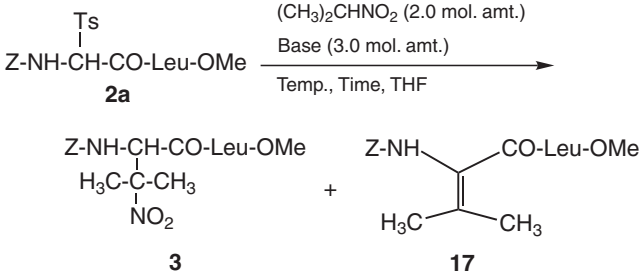
2e–i were also prepared from *N*-Boc-α-tosylglycine (**1b**) with the corresponding amino acid esters in moderate yields (Entries 5–9 in Table 1).

As preliminary experiments, compound **2a** was treated with 2 molar amounts of 2-nitropropane in the presence of 3 molar amounts of potassium *t*-butoxide (KO^{t}Bu) in tetrahydrofuran (THF) at 0 °C for 2 h. Contrary to our previous studies,[5a] the reaction did not give the expected dipeptide **17** containing an α,β-didehydroamino acid residue, but the nitro compound-adduct **3** in 50% yield, as shown in Table 2 (Entry 1).[6] Although 3 molar amounts of sodium hydride were used instead of KO^{t}Bu, only **3** was obtained in a 44% yield (Entry 2 in Table 2). The usage of 3 molar amounts of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base at 50 °C for 6 h afforded both **3** and the desired product **17** in 18% and 42% yields, respectively (Entry 3 in Table 2).

Table 2. Reaction of Dipeptide **2a** with 2-Nitropropane

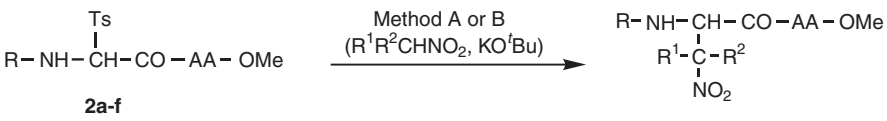


Entry	Base	Temp.	Time/h	**3**/%	**17**/%
1	KO^{t}Bu	0 °C	2	50	0
2	NaH	r.t.	2	44	0
3	DBU	r.t. → 50 °C	6	18	42

After isolation of the nitro compound-adduct **3**, the conversion of **3** into **17** was examined under various conditions. Eventually, the slow addition of **2a** into a mixture of 3 molar amounts of KO^{t}Bu and 6 molar amounts of 2-nitropropane at −40 °C in THF succeeded in forming **3** in quantitative yield, as shown in Table 3 (**Method A**, Entry 1). In the same way, *N*-protected α-tosylglycine derivatives **2a–g** were successfully converted into the corresponding nitro compound-adducts **4–14** in high yields. The results are listed in Table 3. Moreover, in order to reduce the quantity of nitro compound used for the preparation of nitro compound-adduct, various reaction conditions were examined. The reaction of **2a** with 2 molar amounts of 2-nitropropane in the presence of 2 molar amounts of KO^{t}Bu in THF at −40 °C for 1 h proceeded to afford the desired compound **3** in 61% yield (**Method B**, Entry 2 in Table 3). Similarly, compounds **4–6** and **13–16** were furnished in good yields according to method B (Table 3).

Next, the elimination of the nitrous acid from the nitrocompound-adduct to form a dipeptide containing an α,β-didehydroamino acid residue was performed. Compound **16** was readily converted into methyl *N*-Boc-dehydrovalyl-L-isoleuci-

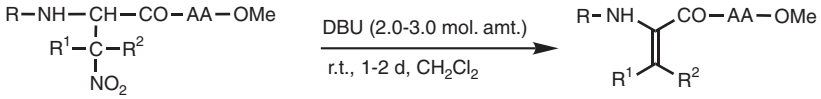
Table 3. Reaction of a Variety of Dipeptides with Nitro Compounds



Entry	Substrate	R	AA	Method[a)]	R^1	R^2	Product	Yield/%	ds[b)]
1	**2a**	Z	Leu	A	Me	Me	**3**	quant.	50/50
2	**2a**	Z	Leu	B	Me	Me	**3**	61	—[c)]
3	**2a**	Z	Leu	A	Me	H	**4**	90	—[d)]
4	**2a**	Z	Leu	B	Me	H	**4**	82	—[d)]
5	**2b**	Z	Ala	A	Me	Me	**5**	83	53/47
6	**2b**	Z	Ala	B	Me	Me	**5**	83	—[c)]
7	**2c**	Z	Gly	A	Me	Me	**6**	80	
8	**2c**	Z	Gly	B	Me	Me	**6**	89	
9	**2d**	Z	Val	A	Me	Me	**7**	99	51/49
10	**2d**	Z	Val	A	Me	H	**8**	95	—[d)]
11	**2e**	Boc	Leu	A	Me	Me	**9**	95	55/45
12	**2e**	Boc	Leu	A	Me	H	**10**	95	55/45
13	**2e**	Boc	Leu	A	Ph	H	**11**	71	—[d)]
14	**2e**	Boc	Leu	A	Et	Me	**12**	94	—[d)]
15	**2f**	Boc	Ala	A	Me	Me	**13**	95	55/45
16	**2f**	Boc	Ala	B	Me	Me	**13**	99	—[c)]
17	**2g**	Boc	Gly	A	Me	Me	**14**	96	
18	**2g**	Boc	Gly	B	Me	Me	**14**	89	
19	**2h**	Boc	Ala	B	Me	Me	**15**	83	—[c)]
20	**2i**	Boc	Ile	B	Me	Me	**16**	94	53/47

a) Method A: the reaction was performed utilizing 6 equimolar amounts of nitro compound and 3 equimolar amounts KO^{t}Bu in THF at −40 °C to room temperature for 1–3 h. Method B: the reaction was performed utilizing 2 equimolar amounts of nitro compound and KO^{t}Bu in THF at −40 °C for 1 h. b) Means the ratio of diastereomers determined by NMR spectrum. c) Not determined. d) Consisted of four diastereomers.

Table 4. Preparation of Dipeptides Containing Dehydroamino Acid Residue



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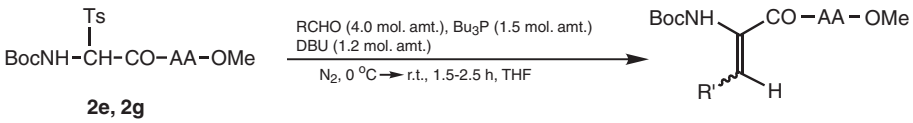
Entry	Substrate	R	R^1	R^2	AA	Product	Yield/%	E/Z[a]
1	**3**	Z	Me	Me	Leu	**17**	70	—
2	**4**	Z	Me	H	Leu	**18**	48	0/100
3	**5**	Z	Me	Me	Ala	**19**	53	—
4	**6**	Z	Me	Me	Gly	**20**	47	—
5	**9**	Boc	Me	Me	Leu	**21**	84	—
6	**10**	Boc	Me	H	Leu	**22**	66	0/100
7	**11**	Boc	Ph	H	Leu	**23**	34	0/100
8[b]	**11**	Boc	Ph	H	Leu	**23**	62	0/100
9	**12**	Boc	Et	Me	Leu	**24**	55	14/86
10	**13**	Boc	Me	Me	Ala	**25**	67	—
11	**14**	Boc	Me	Me	Gly	**26**	81	—
12	**15**	Boc	Me	Me	Val	**27**	70	—
13	**16**	Boc	Me	Me	Ile	**28**	91	—

a) Determined by NOE measurement. b) 7.0 equimolar amounts of 1,4-diazabicyclo[2.2.2]octane (DABCO) were used instead of DBU.

Table 5. Reaction of Dipeptides Containing Tosylglycine Residue with Aldehydes



2e, 2g

Entry	Substrate	AA	RCHO	R′	Product	Total yield/%	E/Z
1	**2e**	Leu	MeCHO	Me	(*Z*)-**22** (*E*)-**22′**	89	5/95[a]
2	**2e**	Leu	PhCHO	Ph	**23**	64	0/100[a]
3	**2e**	Leu	EtCHO	Et	(*Z*)-**29** (*E*)-**29′**	97	10/90[a]
4	**2g**	Gly	MeCHO	Me	(*Z*)-**30** (*E*)-**30′**	82	6/94[b]
5	**2g**	Gly	EtCHO	Et	(*Z*)-**31** (*E*)-**31′**	quant.	6/94[a]

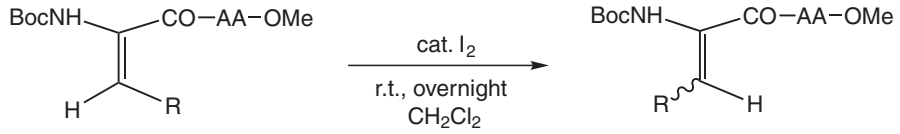
a) Determined by NOE measurement. b) The major product was assigned to be (*Z*)-isomer by comparison with the authentic sample.

nate (**28**) in 91% yield utilizing 2 molar amounts of DBU in dichloromethane at room temperature for 2 days under nitrogen. Although the yield in Entry 7 was not so high, it was improved by using 7 molar amounts of 1,4-diazabicyclo[2.2.2]octane (DABCO) instead of DBU (Entry 8 in Table 4). In the cases of Entries **2** and **6–8** (Table 4), the products **18**, **22**, and **23** were obtained with only (*Z*)-configuration. The elimination reaction of the nitrous acid proceeded smoothly with high (*Z*)-selectivity to afford the products **17–28** in good yields (Table 4). The stereochemistry was assigned by NOE measurement.

We furthermore examined the direct conversion of a dipeptide containing an α-tosylglycine residue to a dipeptide containing an α,β-didehydroamino acid residue. Namely, compounds **2e** and **2g** were treated with 4 equimolar amounts of aldehyde in the presence of 1.5 molar amounts of tributylphosphine (Bu_3P) and 1.2 molar amounts of DBU. Accordingly, the reaction proceeded through a Wittig-type reaction[5b] to afford dipeptides; the (*Z*)-isomer of **22**, **23**, and **29–31** as major products in good yields accompanied by a small amount of (*E*)-isomer, except for Entry 2 (Table 5). The results are summarized in Table 5. The stereochemistry of the major product **30** in Entry 4 was assigned to be (*Z*)-configuration by comparison with the authentic sample prepared by the coupling reaction of (*Z*)-2-(*t*-butoxycarbonylamino)-2-butenoic acid and methyl glycinate.

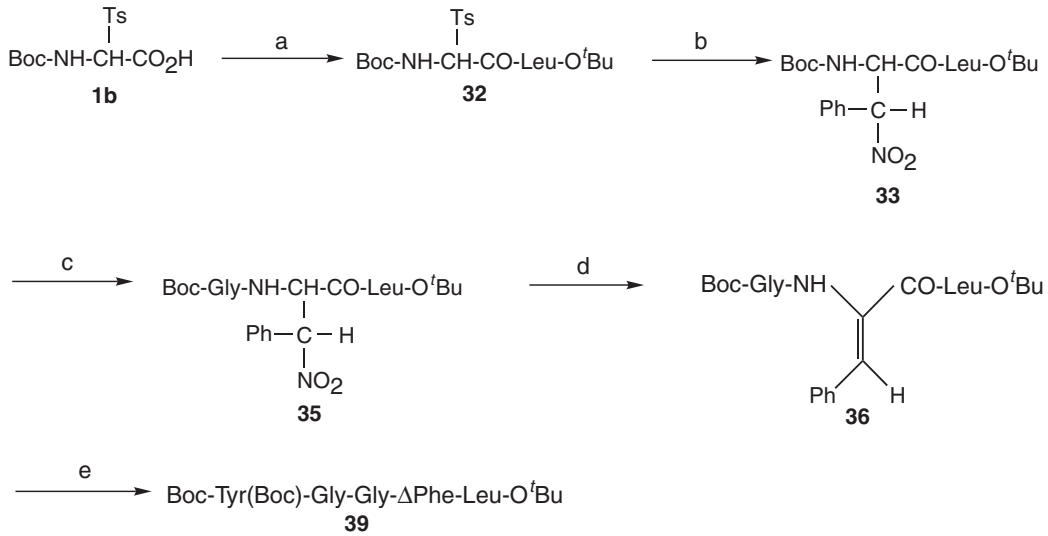
Moreover, it was found that treatment of isolable (*E*)-isomer **22′** with a catalytic amount of iodine in dichloromethane at room temperature resulted in the isomerization to the corresponding (*Z*)-isomer in an 83% yield (Entry 1 in Table 6). In a similar way, **29′**, **30′**, and **31′** were also isomerized to their (*Z*)-isomers in good yields (Table 6).

Table 6. Isomerization of (*E*)- to (*Z*)-isomer by Iodine



22', 29', 30', 31'

Entry	Substrate	R	AA	Yield/%	*E*/*Z*
1	**22′**	Me	Leu	83	5/95
2	**29′**	Et	Leu	90	20/80
3	**30′**	Me	Gly	83	5/95
4	**31′**	Et	Gly	80	2/98



Scheme 2. Synthesis of protected ΔPhe[4]-leucine enkephalin analog. a) H-Leu-O^{t}Bu (1.0 mol. amt.), DCC (1 mol. amt.), HOBt (1 mol. amt.) in THF, r.t., 23 h. **32** 77%. b) $PhCH_2NO_2$ (2 mol. amt.), KO^{t}Bu (2 mol. amt.) in THF, -40 °C $\rightarrow$ r.t., **33** 70%. c) 1) CH_3COCl (17 mol. amt.), MeOH (17 mol. amt.) in AcOEt, r.t., 8 h. HCl salt **34** quant. 2) Boc-Gly-OH (1.1 mol. amt.), IBCF (1.0 mol. amt.), NMM (1.1 mol. amt.) in THF, -15 °C, 8 min; **34** (1 mol. amt.), NMM (1.1 mol. amt.) in THF/DMF (2/1), -15 °C to r.t., 5 h. **35** 76%. d) DABCO (7 mol. amt.) in CH_2Cl_2, r.t., 8 h. **36** 81%. e) 1) CH_3COCl (17 mol. amt.), MeOH (17 mol. amt.) in AcOEt, r.t., 8 h. HCl salt **37** quant. 2) Boc-Tyr(Boc)-Gly-OH (**38**) (1 mol. amt.), IBCF (1.1 mol. amt.), NMM (1.1 mol. amt.) in THF, -15 °C, 15 min; **37** (1 mol. amt.), NMM (1.1 mol. amt.) in THF/DMF (2/1), -15 °C to r.t., overnight. **39** 72%. DCC = dicyclohexylcarbodiimide, HOBt = 1-hydroxybenzotriazole, IBCF = isobutyl chloroformate, NMM = 4-methylmorpholine, DABCO = 1,4-diazabicyclo[2.2.2]octane.

As nitro compound-adducts (**3**–**6** and **9**–**16**) could be easily converted to the corresponding dipeptide containing an α,β-didehydroamino acid residue under basic conditions in moderate to good yields (Table 4), the present method was applied to the synthesis of protected analog **39** of the opioid peptide, leucine-enkephalin, according to Scheme 2.[7] Initially, *N*-Boc-α-tosylglycine (**1b**) was coupled with *t*-butyl L-leucinate using the DCC–HOBt method to afford the dipeptide **32** in 77% yield, which was treated with (nitromethyl)benzene in the presence of 2 molar amounts of KO^{t}Bu to give *t*-butyl *N*-Boc-(β-nitro)-phenylalanyl-L-leucinate **33** in 70% yield. Next, selective deprotection of the *N*-Boc protecting group in compound **33** was examined under various acidic conditions. Eventually, it was found that *N*-deprotection of **33** with hydrogen chloride in MeOH[4o] gave the hydrogen chloride salt **34** in quantitative yield. Compound **34** was mediated with *N*-Boc-glycine by means of the mixed anhydride method using isobutyl chlorodormate to afford the tripeptide **35** in 76% yield, which was treated with 2 molar amounts of DBU in dichloromethane at room temperature for 2 days under nitrogen to afford the desired Boc-Gly-ΔPhe-Leu-O^{t}Bu (**36**) with (*Z*)-configuration in only 34% yield. The conditions used for the preparation of compound **23** (Entry 8 in Table 4) were then applied. Namely, the treatment of **35** with 7 molar amounts of DABCO in dichloromethane at room temperature for 8 h afforded **36** in 81% yield. The stereochemistry of the α,β-dehydrophenylalanine residue of **36** were determined by NOE measurement. Finally, after *N*-deprotection of **36** with hydrogen chloride under the same reaction conditions as described for compound **33** the resulting amine hydrogen chloride salt **37** was condensed with *N*,*O*-diBoc-L-tyrosylglycine (**38**) using the mixed anhydride method to give the desired protected leucine-enkephalin analog

(**39**) in 72% yield.

As mentioned above, the α-tosylglycine residue proved to be a very useful precursor for the α,β-didehydroamino acid residue. The utility of the present method was demonstrated by the synthesis of the protected leucine-enkephalin analog.

Experimental

All of the melting points were determined with a micro melting apparatus (Yanagimoto Seisakusho) and were uncorrected. The ^{1}H NMR, IR, and MS spectra were recorded on JEOL JNM-LA 400FT (400 MHz) and LA 300FT (300 MHz) NMR spectrometers, a JASCO FT/IR-230 infrared spectrometer, and a JEOL SX-102A mass spectrometer, respectively. The chemical shifts of the NMR spectra are reported in δ relative to TMS as an internal standard. All of the solvents were distilled and stored over a drying agent. Thin-layer chromatography (TLC) and flash-column chromatography were performed using Merck's silica-gel 60 PF_{254} (Art. 7749) and Cica-Merck's silica-gel 60 (No. 9385-5B), respectively.

***N*-Z-α-Tosylglycine (1a).** A solution of benzyl carbamate (1.51 g, 17 mmol), glyoxylic acid monohydrate (1.84 g, 20 mmol), and sodium *p*-toluenesulfinate tetrahydrate (7.50 g, 30 mmol) in 99% formic acid (9 mL) was allowed to stir for 48 h at room temperature under air. The solution was then poured into a large amount of water. The crystalline product was collected, washed with water, and dried to afford the desired product **1a** in 84% yield (3.17 g). Mp 81.0–82.0 °C (ethyl acetate–hexane); IR (KBr) 3289, 2372, 2338, 1687, 1655, 1552, 1436, 1300, 1258, 1110, 1062, 976, 757, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (s, 3H), 5.01 (s, 2H), 5.58 (d, J = 9.7 Hz, 1H), 6.26 (d, J = 9.9 Hz, 1H), 7.25–7.36 (m, 7H), 7.80 (d, J = 8.3 Hz, 2H). One proton of the carboxylic acid was not assigned. **1a** was converted to the corresponding methyl ester by means of diazomethane for elementary analysis. Mp 127.0–127.5 °C (ethyl acetate–hexane). Found: C, 57.34; H, 5.05; N, 3.60%. Calcd for $C_{18}H_{19}NO_6S$: C, 57.28; H, 5.07; N, 3.71%.

***N*-Boc-α-Tosylglycine (1b).** A solution of *t*-butyl carbamate (3.98 g, 34 mmol), glyoxylic acid monohydrate (6.26 g, 68 mmol), and *p*-toluenesulfinate tetrahydrate (25.50 g, 102 mmol) in 50% aqueous formic acid (15 mL) was allowed to stir for 36 h at room temperature under air. The solution was then poured into 800 mL of water. The crystalline product was collected, washed with water, and dried to afford the desired product **1b** in 69% yield (7.73 g). Mp 142.0–144.0 °C (ethyl acetate–hexane); IR (KBr) 3354, 2960, 1727, 1698, 1595, 1517, 1467, 1429, 1366, 1325, 1227, 1162, 1146, 1082, 1056, 923, 854, 815, 778, 659 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 9H), 2.44 (s, 3H), 3.64 (br, 1H), 5.66 (d, J = 9.8 Hz, 1H), 5.81 (d, J = 9.8 Hz, 1H), 7.36 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 8.1 Hz, 2H). One proton of the carboxylic acid was not assigned. Found: C, 51.09; H, 5.86; N, 4.09%. Calcd for $C_{14}H_{19}NO_6S$: C, 51.05; H, 5.81; N, 4.25%.

A General Procedure for the Preparation of Dipeptides Containing a Tosylglycine Residue (2a–i). To a suspension of methyl leucinate hydrochloride (927 mg, 5.10 mmol) in dry THF (15 mL) was added a solution of triethylamine (526 mg, 5.20 mmol) in dry THF (2 mL) at 0 °C under nitrogen. After stirring for 2 h at room temperature, **1a** (151 g, 4.00 mmol), DCC (825 mg, 4.00 mmol), and *N*-hydroxysuccinimide (460 mg, 4.00 mmol) were added. The mixture was stirred for 2 d at room temperature under air. After filtration, the filtrate was concentrated in vacuo to give a residue, which was redissolved into ethyl acetate. The insoluble substances were filtered off, and the filtrate was successively washed with 10% citric acid, 10% $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford methyl *N*-Z-α-tosylglycyl-L-leucinate (**2a**) as an inseparable mixture of diastereomers in 71% yield (1.39 g). Mp 105.0–106.0 °C (ethyl acetate–hexane); IR (KBr) 3284, 3033, 2957, 2871, 1737, 1717, 1669, 1628, 1597, 1523, 1456, 1328, 1232, 1181, 1147, 1083, 1058, 816, 771, 741, 698, 670 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (major product = M) δ 0.97 (d, J = 6.1 Hz, 6H), 1.64–1.73 (m, 2H), 1.83–1.94 (m, 1H), 2.40 (s, 3H), 3.74 (s, 3H), 4.69 (dd, J = 7.6, 7.6 Hz, 1H), 4.86 (d, J = 12.2 Hz, 1H), 4.94–4.97 (m, 1H), 5.65 (d, J = 9.3 Hz, 1H), 6.20 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 7.8 Hz, 1H), 7.18–7.38 (m, 7H), 7.74 (d, J = 8.8 Hz, 2H). (Minor product = m) δ 0.90–0.94 (m, 6H), 1.64–1.73 (m, 2H), 1.83–1.94 (m, 1H), 2.40 (s, 3H), 3.79 (s, 3H), 4.60–4.68 (m, 1H), 4.96 (d, J = 12.4 Hz, 1H), 5.04 (d, J = 12.2 Hz, 1H), 5.68 (d, J = 10.5 Hz, 1H), 6.15 (d, J = 8.8 Hz, 1H), 7.18–7.38 (m, 7H), 7.41 (d, J = 7.3 Hz, 1H), 7.76 (d, J = 8.5 Hz, 2H). Found: C, 58.97; H, 6.22; N, 5.85%. Calcd for $C_{24}H_{30}N_2O_7S$: C, 58.76; H, 6.16; N, 5.71%.

The physical and spectral data of compounds **2b**–**2i** are shown in the following. The products, except for **2c** and **2g**, were obtained as an inseparable mixture of diastereomers.

Methyl *N*-Z-α-Tosylglycyl-L-alaninate (2b): Mp 150.5–151.0 °C (ethyl acetate); IR (KBr) 3343, 3278, 3033, 2953, 1738, 1663, 1597, 1524, 1454, 1380, 1356, 1324, 1305, 1265, 1227, 1180, 1146, 1083, 1056, 982, 912, 833, 818, 772, 741, 698, 669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (M) δ 1.47 (d, J = 7.3 Hz, 3H), 2.41 (s, 3H), 3.81 (s, 3H), 4.54–4.68 (m, 1H), 4.93–5.03 (m, 2H), 5.62 (d, J = 8.5 Hz, 1H), 7.22–7.35 (m, 8H), 7.76 (d, J = 8.3 Hz, 2H). (m) δ 1.51 (d, J = 7.3 Hz, 3H), 2.41 (s, 3H), 3.76 (s, 3H), 4.54–4.68 (m, 1H), 4.89–5.00 (m, 2H), 5.62 (d, J = 8.5 Hz, 1H), 6.16 (d, J = 8.9 Hz, 1H), 7.22–7.35 (m, 7H), 7.52 (d, J = 6.1 Hz, 1H), 7.73 (d, J = 8.5 Hz, 2H). Found: C, 54.07; H, 6.82; N, 6.31%. Calcd for $C_{20}H_{30}N_2O_7S$: C, 54.28; H, 6.83; N, 6.33%.

Methyl *N*-Z-α-Tosylglycylglycinate (2c): Mp 153.0–154.0 °C (ethyl acetate); IR (KBr) 3312, 3283, 3033, 2947, 2853, 1739, 1704, 1674, 1658, 1597, 1523, 1439, 1386, 1323, 1268, 1233, 1181, 1135, 1107, 1083, 1051, 983, 873, 742, 699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H), 3.79 (s, 3H), 4.09 (dd, J = 5.7, 18.1 Hz, 1H), 4.20 (dd, J = 5.7, 18.1 Hz, 1H), 4.93 (d, J = 12.2 Hz, 1H), 4.99 (d, J = 12.4 Hz, 1H), 5.65 (d, J = 8.8 Hz, 1H), 6.14 (d, J = 8.8 Hz, 1H), 7.23–7.35 (m, 7H), 7.78 (d, J = 8.3 Hz, 2H). Found: C, 55.25; H, 5.12; N, 6.46%. Calcd for $C_{20}H_{22}N_2O_7S$: C, 55.29; H, 5.10; N, 6.45%.

Methyl *N*-Z-α-Tosylglycyl-L-valinate (2d): Mp 117.0–118.0 °C (ethyl acetate–diethyl ether–hexane); IR (KBr) 3338, 3270, 3065, 2962, 1745, 1725, 1672, 1598, 1534, 1455, 1438, 1375, 1327, 1265, 1234, 1182, 1147, 1084, 1020, 840, 815, 737, 714, 697, 669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (M) δ 0.96 (dd, J = 2.1, 7.0 Hz, 6H), 2.18–2.34 (m, 1H), 2.41 (s, 3H), 3.80 (s, 3H), 4.65 (dd, J = 4.6, 8.8 Hz, 1H), 4.95 (d, J = 11.2 Hz, 1H), 5.03 (d, J = 12.2 Hz, 1H), 5.65 (d, J = 9.0 Hz, 1H), 6.17 (d, J = 8.8 Hz, 1H), 7.13 (d, J = 8.8 Hz, 1H), 7.16–7.38 (m, 7H), 7.75 (d, J = 8.3 Hz, 2H). (m) δ 1.04 (dd, J = 2.1, 7.0 Hz, 6H), 2.18–2.34 (m, 1H), 2.41 (s, 3H), 3.76 (s, 3H), 4.53 (dd, J = 4.9, 8.1 Hz, 1H), 4.85 (d, J = 12.4 Hz, 1H), 4.93 (d, J = 12.2 Hz, 1H), 5.63 (d, J = 9.0 Hz, 1H), 6.17 (d, J = 9.0 Hz, 1H), 7.16–7.38 (m, 7H), 7.40 (d, J = 8.1 Hz, 1H), 7.75 (d, J = 8.3 Hz, 2H). Found: C, 57.75; H, 5.90; N, 5.85%. Calcd for $C_{23}H_{28}N_2O_7S$: C, 57.97; H, 5.92; N, 5.88%.

Methyl *N*-Boc-α-Tosylglycyl-L-leucinate (2e): Mp 84.0–86.0 °C (diethyl ether–hexane); IR (KBr) 3309, 2957, 2871, 1729, 1703, 1681, 1655, 1597, 1556, 1521, 1453, 1393, 1367, 1287, 1256, 1147, 1084, 1056, 1019, 977, 867, 817, 732, 708, 670 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 0.99 (d, $J = 6.3$ Hz, 6H), 1.21 (s, 9H), 1.64–1.78 (m, 2H), 1.82–1.92 (m, 1H), 2.42 (s, 3H), 3.76 (s, 3H), 4.71 (dt, $J = 7.7$, 7.7 Hz, 1H), 5.49 (d, $J = 8.8$ Hz, 1H), 5.92 (d, $J = 8.5$ Hz, 1H), 6.97 (d, $J = 8.1$ Hz, 1H), 7.34 (d, $J = 7.3$ Hz, 2H), 7.80 (d, $J = 7.8$ Hz, 2H). (m) δ 0.95 (d, $J = 5.9$ Hz, 3H), 0.96 (d, $J = 5.9$ Hz, 3H), 1.26 (s, 9H), 1.64–1.78 (m, 2H), 1.82–1.92 (m, 1H), 2.42 (s, 3H), 3.80 (s, 3H), 4.62–4.68 (m, 1H), 5.49 (d, $J = 8.8$ Hz, 1H), 5.87 (d, $J = 9.0$ Hz, 1H), 7.24 (d, $J = 7.8$ Hz, 1H), 7.34 (d, $J = 7.8$ Hz, 2H), 7.82 (d, $J = 7.8$ Hz, 2H). Found: C, 55.02; H, 7.26; N, 6.19%. Calcd for $C_{21}H_{32}N_2O_7S$: C, 55.25; H, 7.06; N, 6.14%.

Methyl *N*-Boc-α-Tosylglycyl-L-alaninate (2f): Mp 144.5 °C (ethyl acetate); IR (KBr) 3420, 3331, 3278, 2979, 2953, 1742, 1684, 1650, 1597, 1518, 1455, 1366, 1345, 1322, 1308, 1291, 1254, 1224, 1152, 1085, 1050, 1021, 975, 866, 831, 768, 715, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 1.23 (s, 9H), 1.53 (d, $J = 7.1$ Hz, 3H), 2.43 (s, 3H), 3.78 (s, 3H), 4.67 (dq, $J = 7.1$, 7.3 Hz, 1H), 5.52 (d, $J = 8.8$ Hz, 1H), 5.94 (d, $J = 8.8$ Hz, 1H), 7.16 (d, $J = 7.3$ Hz, 1H), 7.35 (d, $J = 7.6$ Hz, 2H), 7.80 (d, $J = 7.6$ Hz, 2H). (m) δ 1.26 (s, 9H), 1.50 (d, $J = 7.1$ Hz, 3H), 2.43 (s, 3H), 3.81 (s, 3H), 4.64 (dq, $J = 7.1$, 7.3 Hz), 5.50 (d, $J = 9.0$ Hz, 1H), 5.92 (d, $J = 9.0$ Hz, 1H), 7.35 (d, $J = 8.3$ Hz, 2H), 7.46 (d, $J = 6.6$ Hz, 1H), 7.82 (d, $J = 8.3$ Hz, 2H). Found: C, 51.91; H, 6.31; N, 6.63%. Calcd for $C_{18}H_{26}N_2O_7S$: C, 52.16; H, 6.32; N, 6.76%.

Methyl *N*-Boc-α-Tosylglycylglycinate (2g): Mp 158.0–159.5 °C (ethyl acetate); IR (KBr) 3356, 3306, 2977, 1743, 1720, 1683, 1599, 1545, 1517, 1442, 1404, 1375, 1338, 1315, 1300, 1256, 1227, 1209, 1162, 1133, 1081, 1040, 974, 934, 869, 841, 813, 767, 706, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (s, 9H), 2.43 (s, 3H), 3.80 (s, 3H), 4.10 (dd, $J = 5.9$, 18.3 Hz, 1H), 4.26 (dd, $J = 4.9$, 18.3 Hz, 1H), 5.56 (d, $J = 9.0$ Hz, 1H), 5.92 (d, $J = 9.0$ Hz, 1H), 7.26–7.30 (br, 1H), 7.35 (d, $J = 8.3$ Hz, 2H), 7.81 (d, $J = 8.3$ Hz, 2H). Found: C, 50.75; H, 6.01; N, 6.95%. Calcd for $C_{17}H_{24}N_2O_7S$: C, 50.99; H, 6.04; N, 7.00%.

Methyl *N*-Boc-α-Tosylglycyl-L-valinate (2h): Mp 135.0–136.0 °C (ethyl acetate–diethyl ether–hexane); IR (KBr) 3341, 3069, 2965, 2882, 1737, 1720, 1679, 1597, 1551, 1513, 1440, 1366, 1334, 1320, 1307, 1255, 1223, 1166, 1140, 1081, 1058, 1021, 983, 908, 864, 845, 819, 774, 729, 708 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 1.03 (d, $J = 7.0$ Hz, 3H), 1.08 (d, $J = 7.0$ Hz, 3H), 1.20 (s, 9H), 2.20–2.36 (m, 1H), 3.78 (s, 3H), 4.67 (dd, $J = 4.8$, 8.8 Hz, 1H), 5.50 (d, $J = 8.8$ Hz, 1H), 5.93 (d, $J = 8.8$ Hz, 1H), 7.05 (d, $J = 8.8$ Hz, 1H), 7.34 (d, $J = 8.6$ Hz, 2H), 7.81 (d, $J = 8.6$ Hz, 2H). (m) δ 0.98 (d, $J = 7.0$ Hz, 6H), 1.26 (s, 9H), 2.20–2.36 (m, 1H), 3.81 (s, 3H), 4.56 (dd, $J = 5.0$, 8.1 Hz, 1H), 5.50 (d, $J = 8.8$ Hz, 1H), 5.87 (d, $J = 9.0$ Hz, 1H), 7.29–7.31 (br, 1H), 7.34 (d, $J = 8.6$ Hz, 2H), 7.81 (d, $J = 8.6$ Hz, 2H). Found: C, 54.07; H, 6.82; N, 6.31%. Calcd for $C_{20}H_{30}N_2O_7S$: C, 54.28; H, 6.83; N, 6.33%.

Methyl *N*-Boc-α-Tosylglycyl-L-isoleucinate (2i): A solid; IR (KBr) 3343, 2971, 2879, 1739, 1680, 1597, 1495, 1458, 1439, 1368, 1327, 1305, 1253, 1211, 1164, 1146, 1083, 1057, 1020, 980, 900, 864, 814, 768, 733, 707, 660 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 0.96 (t, $J = 7.6$ Hz, 3H), 1.05 (d, $J = 6.8$ Hz, 3H), 1.20 (s, 9H), 1.30–1.37 (m, 2H), 1.46–1.56 (m, 1H), 2.42 (s, 3H), 3.77 (s, 3H), 4. 71 (dd, $J = 4.6$, 8.8 Hz, 1H), 5.52 (d, $J = 8.8$ Hz, 1H), 5.94 (d, $J = 8.8$ Hz, 1H), 7.11 (d, $J = 8.8$ Hz, 1H), 7.34 (d, $J = 8.3$ Hz, 2H), 7.81 (d, $J = 8.3$ Hz, 2H). (m) δ 0.95 (t, $J = 7.3$. Hz, 3H), 1.05 (d, $J = 6.8$ Hz, 3H), 1.20 (s, 9H), 1.30–1.37 (m, 2H), 1.46–1.56 (m, 1H), 2.42 (s, 3H), 3.80 (s, 3H), 4.61 (dd, $J = 4.6$, 7.8 Hz, 1H), 5.51 (d, $J = 8.8$ Hz, 1H), 5.91 (d, $J = 9.3$ Hz, 1H), 7.35 (d, $J = 8.3$ Hz, 1H), 7.37–7.40 (br, 1H), 7.81 (d, $J = 8.3$ Hz, 2H). EI-MS m/z 456 (M^+, 0.12%).

A General Procedure for the Preparation of Dipeptides Containing a β-Nitro-α-amino Acid Residue 3–16. Method A: To a solution of KO^{t}Bu (270 mg, 2.4 mmol) in dry THF (1 mL) was added a solution of 2-nitropropane (428 mg, 4.8 mmol) in dry THF (2 mL) at −40 °C under nitrogen. After 5 min, a solution of **2a** (392 mg, 0.80 mmol) in dry THF (2 mL) was added over 60 min. The reaction mixture was gradually warmed to room temperature, and then quenched with a saturated aqueous solution of NH_4Cl. The THF was removed in vacuo to give a residue, which was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. The combined extracts were washed with brine and dried over anhydrous $MgSO_4$. Removal of the solvent gave methyl *N*-Z-β-nitrovalyl-L-leucinate **3** as an inseparable mixture of diastereomers in quantitative yield (335 mg). Mp 92.0–93.0 °C (ethyl acetate–hexane); IR (KBr) 3291, 3068, 2960, 1713, 1692, 1652, 1551, 1455, 1390, 1360, 1288, 1248, 1153, 1052, 748, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 0.89 (d, $J = 6.1$ Hz, 3H), 0.91 (d, $J = 6.1$ Hz, 3H), 1.50–1.67 (m, 3H), 1.57 (s, 3H), 1.71 (s, 3H), 3.70 (s, 3H), 4.49–4.56 (m, 1H), 4.96 (d, $J = 8.3$ Hz, 1H), 5.11–5.17 (m, 2H), 5.97–6.01 (m, 1H), 6.66 (d, $J = 7.8$ Hz, 1H), 7.32–7.36 (m, 5H). (m) δ 0.89–0.92 (m, 6H), 1.50–1.67 (m, 3H), 1.60 (s, 3H), 1.71 (s, 3H), 3.72 (s, 3H), 4.49–4.56 (m, 1H), 4.98 (d, $J = 8.8$ Hz, 1H), 5.11–5.17 (m, 2H), 5.97–6.01 (m, 1H), 6.68–6.74 (br, 1H), 7.32–7.36 (m, 5H). Found: C, 56.57; H, 6.90; N, 9.92%. Calcd for $C_{20}H_{29}N_3O_7$: C, 56.73; H, 6.90; N, 9.92%.

Method B: To a suspension of KO^{t}Bu (185 mg, 1.65 mmol) in dry THF (6 mL) was added a solution of 2-nitropropane (1.453 g, 1.63 mmol) in THF (2 mL) at −40 °C under nitrogen. After 15 min, a solution of **2a** (402 mg, 0.82 mmol) in THF (4 mL) was added dropwise over 30 min at −40 °C. The mixture was stirred for 1 h and then quenched with a saturated aqueous solution of NH_4Cl. The THF was removed in vacuo to give a residue, which was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. The combined extracts were washed with brine and dried over anhydrous $MgSO_4$. Removal of the solvent and recrystallization from AcOEt–hexane gave **3** as an inseparable mixture of diastereomers in 61% yield (211 mg).

Methyl 2-(Benzyloxycarbonylamino)-3-nitrobutanoyl-L-leucinate (4): Mp 85.0–86.0 °C (ethyl acetate–hexane); IR (KBr) 3287, 3076, 2959, 1748, 1713, 1693, 1658, 1555, 1455, 1389, 1361, 1285, 1244, 1153, 1056, 1030, 873, 739, 698 cm^{-1}. Compound **4** was found to be a mixture of four diastereomers based on the NMR spectrum. Found: C, 55.94; H, 6.62; N, 10.16%. Calcd for $C_{19}H_{27}N_3O_7$: C, 55.74; H, 6.65; N, 10.26%.

Methyl *N*-Z-β-Nitrovalyl-L-alaninate (5): Mp 110.0–112.0 °C (ethyl acetate–hexane); IR (KBr) 3328, 3269, 3070, 3002, 2953, 1754, 1736, 1715, 1665, 1540, 1457, 1400, 1383, 1345, 1324, 1289, 1262, 1213, 1152, 1061, 1016, 912, 859, 797, 757, 741, 728, 698, 677 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 1.37 (d, $J = 7.1$ Hz, 3H), 1.61 (s, 9H), 1.73 (s, 3H), 3.73 (s, 3H), 4.42–4.58 (m, 1H), 4.93 (d, $J = 9.8$ Hz, 1H), 5.10–5.19 (m, 2H), 5.97 (d, $J = 7.8$ Hz, 1H), 6.76–6.90 (br, 1H), 7.30–7.40 (m, 5H). (m) δ 1.37 (d, $J = 7.1$ Hz, 3H), 1.57 (s, 3H), 1.72 (s, 3H), 4.42–4.58 (m, 1H), 4.97 (d, $J = 9.5$ Hz, 1H), 5.10–5.19

(m, 2H), 5.99 (d, $J = 8.8$ Hz, 1H), 6.76–6.90 (br, 1H), 7.30–7.40 (m, 5H). Found: C, 53.43; H, 6.13; N, 11.09%. Calcd for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.08; N, 11.02%.

Methyl *N*-Z-β-Nitrovalylglycinate (6): Mp 140.5–141.5 °C (ethyl acetate); IR (KBr) 3303, 3067, 2954, 2851, 1745, 1717, 1665, 1540, 1499, 1455, 1439, 1393, 1376, 1345, 1323, 1259, 1223, 1137, 1105, 1057, 1012, 981, 902, 856, 794, 778, 742, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H), 1.73 (s, 3H), 3.73 (s, 3H), 3.89 (dd, $J = 5.6$, 18.3 Hz, 1H), 4.07 (dd, $J = 5.6$, 18.3 Hz, 1H), 5.01 (d, $J = 9.8$ Hz, 1H), 5.10 (d, $J = 12.2$ Hz, 1H), 5.15 (d, $J = 12.0$ Hz, 1H), 6.03 (d, $J = 9.8$ Hz, 1H), 6.94 (br, 1H), 7.31–7.38 (m, 5H). Found: C, 52.54; H, 5.83; N, 11.51%. Calcd for $C_{16}H_{21}N_3O_7$: C, 52.31; H, 5.76; N, 11.44%.

Methyl *N*-Z-β-Nitrovalyl-L-alaninate (7): Mp 110.0–112.0 °C (ethyl acetate–hexane); IR (KBr) 3328, 3269, 3070, 2953, 1754, 1736, 1715, 1665, 1540, 1457, 1400, 1383, 1345, 1324, 1289, 1262, 1213, 1152, 1061, 1016, 912, 859, 797, 757, 741, 728, 698, 677 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 1.37 (d, $J = 7.1$ Hz, 3H), 1.61 (s, 9H), 1.73 (s, 3H), 3.73 (s, 3H), 4.42–4.58 (m, 1H), 4.93 (d, $J = 9.8$ Hz, 1H), 5.10–5.19 (m, 2H), 5.97 (d, $J = 8.8$ Hz, 1H), 7.30–7.40 (m, 5H). (m) δ 1.37 (d, $J = 7.1$ Hz, 3H), 1.57 (s, 3H), 1.72 (s, 3H), 3.73 (s, 3H), 4.42–4.58 (m, 1H), 4.97 (d, $J = 9.5$ Hz, 1H), 5.10–5.19 (m, 2H), 5.99 (d, $J = 8.8$ Hz, 1H), 6.76–6.90 (br, 1H), 7.30–7.40 (m, 5H). Found: C, 53.43; H, 6.13; N, 11.09%. Calcd for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.08; N, 11.02%.

Methyl 2-(Benzyloxycarbonylamino)-3-nitrobutanoyl-L-alaninate (8): Mp 131.5–134.0 °C (ethyl acetate–hexane); IR (KBr) 3287, 3068, 3035, 2966, 2934, 2878, 2851, 2786, 1957, 1744, 1715, 1664, 1539, 1456, 1437, 1376, 1346, 1258, 1146, 1115, 1102, 1087, 1059, 1014, 911, 857, 787, 754, 739, 699, 686 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ (d, $J = 6.8$ Hz, 3H), 0.89 (d, $J = 6.8$ Hz, 3H), 1.60 (s, 3H), 1.72 (s, 3H), 2.10–2.20 (m, 1H), 3.72 (s, 3H), 4.43 (dd, $J = 4.9$, 8.3 Hz, 1H), 4.96 (d, $J = 9.8$ Hz, 1H), 5.15 (s, 2H), 6.04 (d, $J = 9.8$ Hz, 1H), 6.73–6.75 (br, 1H), 7.32–7.38 (m, 5H). (m) δ 0.86 (d, $J = 6.8$ Hz, 3H), 0.89 (d, $J = 6.8$ Hz, 3H), 1.58 (s, 3H), 1.72 (s, 3H), 2.10–2.20 (m, 1H), 3.72 (s, 3H), 4.46 (dd, $J = 4.7$, 8.4 Hz, 1H), 5.00 (d, $J = 8.8$ Hz, 1H), 5.15 (s, 2H), 5.98 (d, $J = 8.8$ Hz, 1H), 6.64–6.66 (br, 1H), 7.32–7.38 (m, 5H). Found: C, 55.59; H, 6.74; N, 10.28%. Calcd for $C_{19}H_{27}N_3O_7$: C, 55.74; H, 6.65; N, 10.26%.

Methyl *N*-Boc-β-Nitrovalyl-L-leucinate (9): Mp 141.0–144.0 °C (ethyl acetate–hexane); IR (KBr) 3280, 3105, 2957, 2874, 1743, 1692, 1655, 1546, 1457, 1435, 1394, 1369, 1345, 1332, 1272, 1163, 1099, 1060, 1020, 984, 879, 854, 803, 754, 715 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 0.93 (d, $J = 5.3$ Hz, 6H), 1.43–1.68 (m, 3H), 1.47 (s, 9H), 1.59 (s, 3H), 1.74 (s, 3H), 3.72 (s, 3H), 4.40–4.60 (m, 1H), 4.81 (d, $J = 9.7$ Hz, 1H), 5.66–5.76 (br, 1H), 6.55 (d, $J = 7.9$ Hz, 1H). (m) δ 0.91 (d, $J = 5.5$ Hz, 6H), 1.43–1.68 (m, 3H), 1.46 (d, $J = 5.5$ Hz, 6H), 1.62 (s, 3H), 1.74 (s, 3H), 3.72 (s, 3H), 4.40–4.60 (m, 1H), 4.80 (d, $J = 7.9$ Hz, 1H), 5.66–5.76 (br, 1H), 6.55 (d, $J = 7.9$ Hz, 1H). Found: C, 52.39; H, 8.04; N, 10.58%. Calcd for $C_{17}H_{31}N_3O_7$: C, 52.43; H, 8.02; N, 10.79%.

Methyl 2-(*t*-Butoxycarbonylamino)-3-nitrobutanoyl-L-leucinate (10): Mp 161.0–164.0 °C (ethyl acetate–hexane); IR (KBr) 3342, 3278, 3088, 2976, 1755, 1687, 1655, 1554, 1520, 1455, 1392, 1369, 1305, 1253, 1204, 1160, 1112, 1055, 874, 755 cm^{-1}. Compound **10** was found to be a mixture of four diastereomers based on the NMR spectrum. Found: C 51.12; H, 7.83; N, 11.18%. Calcd for $C_{16}H_{29}N_3O_7$: C, 51.19; H, 7.79; N, 11.19%.

Methyl *N*-Boc-(β-nitro)phenylalanyl-L-leucinate (11): Mp 169.0–170.0 °C (ethyl acetate–hexane); IR (KBr) 3302, 3089, 2959, 2871, 1726, 1693, 1657, 1557, 1525, 1458, 1438, 1369, 1329, 1289, 1249, 1208, 1168, 1050, 1024, 863, 801, 750, 719 cm^{-1}. Compound **11** was found to be a mixture of four diastereomers based on the NMR spectrum. Found: C 57.53; H, 7.17; N, 9.60%. Calcd for $C_{21}H_{31}N_3O_7$: C, 57.65; H, 7.14; N, 9.60%.

Methyl *N*-Boc-β-Nitroisoleucyl-L-leucinate (12): Mp 143.5–145.0 °C (ethyl acetate); IR (KBr) 3326, 2979, 2883, 1750, 1688, 1655, 1548, 1461, 1380, 1369, 1350, 1323, 1275, 1254, 1212, 1156, 1061, 1012, 985, 881, 857, 769, 669 cm^{-1}. Compound **12** was found to be a mixture of four diastereomers based on the NMR spectrum. Found: C, 53.57; H, 8.23; N, 10.41%. Calcd for $C_{18}H_{33}N_3O_7$: C, 53.58; H, 8.24; N, 10.42%.

Methyl *N*-Boc-β-Nitrovalyl-L-alaninate (13): Mp 152.5–154.0 °C (ethyl acetate); IR (KBr) 3279, 3089, 2982, 2955, 1753, 1696, 1665, 1546, 1456, 1381, 1369, 1347, 1328, 1281, 1254, 1218, 1162, 1052, 1013, 877, 856, 835, 806, 751, 669 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 1.39 (d, $J = 7.1$ Hz, 3H), 1.47 (s, 9H), 1.62 (s, 3H), 1.74 (s, 3H), 3.74 (s, 3H), 4.46–4.58 (m, 1H), 4.80 (d, $J = 9.8$ Hz, 1H), 5.69 (d, $J = 9.3$ Hz, 1H), 6.71 (d, $J = 5.9$ Hz, 1H). (m) δ 1.40 (d, $J = 7.3$ Hz, 3H), 1.47 (s, 9H), 1.59 (s, 3H), 1.75 (s, 3H), 3.75 (s, 3H), 4.46–4.58 (m, 1H), 4.84 (d, $J = 10.0$ Hz, 1H), 5.69 (d, $J = 9.3$ Hz, 1H), 6.70–6.82 (m, 1H). Found: C, 48.26; H, 7.31; N, 12.00%. Calcd for $C_{14}H_{25}N_3O_7$: C, 48.41; H, 7.25; N, 12.10%.

Methyl *N*-Boc-β-Nitrovalylglycinate (14): Mp 173.0–180.0 °C (ethyl acetate); IR (KBr) 3281, 3096, 3005, 2984, 1762, 1697, 1668, 1549, 1456, 1438, 1405, 1394, 1381, 1370, 1350, 1322, 1282, 1258, 1209, 1179, 1100, 1059, 1038, 1015, 982, 931, 877, 855, 791, 706, 662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.62 (s, 3H), 1.77 (s, 3H), 3.75 (s, 3H), 3.97 (dd, $J = 5.6$, 18.3 Hz, 1H), 4.05 (dd, $J = 5.6$, 18.3 Hz, 1H), 4.83 (d, $J = 9.8$ Hz, 1H), 5.68 (d, $J = 9.8$ Hz, 1H), 6.62 (br, 1H). Found: C, 46.75; H, 6.98; N, 12.56%. Calcd for $C_{13}H_{23}N_3O_7$: C, 46.84; H, 6.95; 12.61%.

Methyl *N*-Boc-β-Nitrovalyl-L-valinate (15): Mp 143.0 °C (benzene–hexane); IR (KBr) 3326, 2971, 2935, 2879, 1749, 1691, 1653, 1549, 1457, 1392, 1379, 1369, 1351, 1327, 1278, 1255, 1216, 1157, 1062, 1012, 981, 953, 881, 858, 792, 769, 752, 662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 0.89 (d, $J = 7.1$ Hz, 3H), 0.93 (d, $J = 7.1$ Hz, 3H), 1.47 (s, 9H), 1.61 (s, 3H), 1.75 (s, 3H), 2.13–2.23 (m, 1H), 4.44 (dd, $J = 4.6$, 8.8 Hz, 1H), 4.77 (d, $J = 9.5$ Hz, 1H), 5.78 (d, $J = 9.5$ Hz, 1H), 6.56–6.58 (br, 1H). (m) δ 0.87 (d, $J = 7.1$ Hz, 3H), 0.89 (d, $J = 7.1$ Hz, 3H), 1.47 (s, 9H), 1.59 (s, 3H), 1.74 (s, 3H), 2.13–2.20 (m, 1H), 3.74 (s, 3H), 4.46 (dd, $J = 4.6$, 10.0 Hz, 1H), 4.85 (d, $J = 9.5$ Hz, 1H), 5.69 (d, $J = 9.5$ Hz, 1H), 6.53–6.56 (br, 1H). Found: C, 51.00; H, 7.81; N, 11.07%. Calcd for $C_{16}H_{29}N_3O_7$: C, 51.19; H, 7.79; N, 11.19%.

Methyl *N*-Boc-β-Nitrovalyl-L-isoleucinate (16): Mp 123.0–123.5 °C (benzene–hexane); IR (KBr) 3326, 2979, 2883, 1750, 1688, 1655, 1548, 1461, 1380, 1369, 1350, 1323, 1275, 1254, 1212, 1156, 1061, 1012, 881, 857, 769 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 0.89 (t, $J = 9.3$ Hz, 3H), 0.90 (d, $J = 11.0$ Hz, 3H), 1.11–1.21 (m, 1H), 1.35–1.42 (m, 1H), 1.47 (s, 9H), 1.61 (s, 3H), 1.74 (s, 3H), 1.82–1.96 (m, 1H), 3.73 (s, 3H), 4.48 (m, 1H), 4.80 (d, $J = 9.8$ Hz, 1H), 5.77 (d, $J = 9.3$ Hz, 1H), 6.63–6.66 (m, 1H). (m) δ 0.90 (d, $J = 11.0$ Hz, 3H), 0.90 (t, $J = 9.3$ Hz, 3H), 1.11–1.21 (m, 1H), 1.35–1.42 (m, 1H), 1.47 (s, 9H), 1.59 (s, 3H), 1.74 (s, 3H), 1.82–1.96 (m, 1H), 3.73 (s, 3H), 4.41 (dd, $J = 4.6$, 8.5 Hz, 1H), 4.85 (d, $J = 9.3$ Hz, 1H), 5.70 (d, $J =$

9.3 Hz, 1H), 6.60–6.63 (m, 1H). Found: C, 52.18; H, 8.01; N, 10.59%. Calcd for $C_{17}H_{31}N_3O_7$: C, 52.43; H, 8.02; N, 10.79%.

A General Procedure for the Preparation of Dipeptide Containing an α,β-Didehydroamino Acid Residue (17–28). To a solution of compound **16** (164 mg, 0.42 mmol) in dry CH_2Cl_2 (4 mL) was added a solution of DBU (128 mg, 0.42 mmol) in CH_2Cl_2 (2 mL) at 0 °C under nitrogen. After stirring for 24 h at room temperature, the solvent was removed in vacuo to afford a residue, which was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined extracts were washed with 10% citric acid, 10% $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford a residue, which was subjected to preparative TLC (SiO_2, hexane:ethyl acetate = 2/1, v/v) to afford methyl *N*-Boc-α,β-didehydrovalyl-L-isoleucinate (**28**) in 91% yield (131 mg). Mp 78.0–79.0 °C (hexane); $[\alpha]_D^{25} = -7.1°$ (0.27, MeOH); IR (KBr) 3319, 3060, 2973, 1745, 1691, 1670, 1629, 1549, 1458, 1368, 1348, 1254, 1201, 1168, 1055, 856 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, $J = 7.3$ Hz, 3H), 0.93 (d, $J = 6.8$ Hz, 3H), 1.14–1.30 (m, 1H), 1.40–1.50 (m, 1H), 1.46 (s, 9H), 1.79 (s, 3H), 1.87–1.97 (m, 1H), 2.04 (s, 3H), 3.73 (s, 3H), 4.61 (dd, $J = 4.9$, 8.5 Hz, 1H), 5.95 (s, 1H), 6.85 (br, 1H). Found; C, 59.39; H, 8.87; N, 7.93%. Calcd for $C_{17}H_{30}N_2O_5$: C, 59.63; H, 8.83; N, 8.18%.

The physical and spectral data of compounds **17**–**27** are shown in the following.

Methyl *N*-Z-α,β-Didehydrovalyl-L-leucinate (17): Mp 128.0–129.0 °C (ethyl acetate–hexane); $[\alpha]_D^{25} = -24.4°$ (0.59, MeOH); IR (KBr) 3284, 3048, 2956, 2869, 1756, 1703, 1626, 1559, 1509, 1250, 1229, 1202, 1157, 1060, 738 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87–0.96 (br, 6H), 1.51–1.72 (m, 3H), 1.78 (s, 3H), 2.06 (s, 3H), 4.60–4.67 (br, 1H), 5.15 (s, 2H), 5.95 (s, 1H), 6.45–6.55 (br, 1H), 7.35–7.52 (m, 5H). Found: C, 63.59; H, 7.53; N, 7.36%. Calcd for $C_{20}H_{28}N_2O_5$: C, 63.81; H, 7.50; N, 7.44%.

Methyl (*Z*)-2-Benzyloxycarbonylamino-2-butenoyl-L-leucinate (18): Mp 77.5–78.0 °C (ethyl acetate); $[\alpha]_D^{25} = -27.8°$ (0.50, MeOH); IR (KBr) 3244, 3042, 2956, 2870, 1752, 1698, 1671, 1630, 1551, 1507, 1252, 1232, 1202, 1158, 1099, 740, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90–0.92 (m, 2H), 1.51–1.66 (m, 2H), 1.74 (d, $J = 7.0$ Hz, 3H), 3.71 (s, 3H), 4.62–4.70 (m, 1H), 5.14 (s, 2H), 6.53 (q, $J = 7.0$ Hz, 1H), 6.57 (d, $J = 8.3$ Hz, 1H), 7.31–7.38 (br, 5H). Found: C, 61.25; H, 7.21; N, 7.32%. Calcd for $C_{19}H_{26}N_2O_5 \cdot 1/2H_2O$: C, 61.42; H, 7.33; N, 7.54%.

Methyl *N*-Z-α,β-Didehydrovalyl-L-alaninate (19): Mp 83.0–84.0 °C (benzene–hexane); $[\alpha]_D^{25} = -7.8°$ (0.83, MeOH); IR (KBr) 3276, 2987, 2951, 1745, 1687, 1631, 1543, 1507, 1257, 1219, 1161, 1042, 860, 755, 732, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.32–1.50 (br, 3H), 1.77 (s, 3H), 2.05 (s, 3H), 3.72 (s, 3H), 4.50–4.70 (br, 1H), 5.14 (s, 2H), 6.22 (s, 1H), 6.60–6.80 (br, 1H), 7.28–7.40 (br, 5H). Found: C, 61.02; H, 6.64; N, 8.22%. Calcd for $C_{17}H_{22}N_2O_5$: C, 61.07; H, 6.63; N, 8.38%.

Methyl *N*-Z-α,β-Didehydrovalylglycinate (20): Mp 107.0–108.0 °C (ethyl acetate–hexane); IR (KBr) 3298, 2947, 1764, 1707, 1663, 1499, 1446, 1388, 1356, 1329, 1208, 1186, 1118, 1011, 961, 928, 915, 787, 765, 752, 718, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.78 (s, 3H), 2.07 (s, 3H), 3.74 (s, 3H), 4.03–4.17 (br, 2H), 5.13 (s, 2H), 6.17 (s, 1H), 6.50–6.78 (br, 1H), 7.35–7.40 (m, 5H). Found: C, 59.93; H, 6.31; N, 8.60%. Calcd for $C_{16}H_{20}N_2O_5$: C, 59.99; H, 6.29; N, 8.74%.

Methyl *N*-Boc-α,β-Didehydrovalyl-L-leucinate (21): Mp 133.0 °C (ethyl acetate–hexane); $[\alpha]_D^{25} = -37.5°$ (0.36, MeOH); IR (KBr) 3279, 2960, 2868, 1757, 1699, 1624, 1541, 1492, 1458, 1370, 1333, 1251, 1232, 1201, 1162, 1053, 1031, 986, 897, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90–0.92 (m, 6H), 1.46 (s, 9H), 1.54–1.73 (m, 3H), 1.79 (s, 3H), 2.05 (s, 3H), 3.73 (s, 3H), 4.63 (dt, $J = 4.8$, 8.8 Hz, 1H), 5.68 (br, 1H), 6.54–6.65 (br, 1H). Found: C, 59.42; H, 8.91; N, 8.04%. Calcd for $C_{17}H_{30}N_2O_5$: C, 59.63; H, 8.83; N, 8.18%.

Methyl (*Z*)-2-(*t*-Butoxycarbonylamino)-2-butenoyl-L-leucinate (22): Mp 110.0–112.0 °C (ethyl acetate) $[\alpha]_D^{25} = -33.7°$ (1.00, MeOH) IR (KBr) 3334, 3220, 3049, 2964, 2868, 1759, 1688, 1670, 1626, 1548, 1488, 1453, 1371, 1344, 1304, 1252, 1233, 1201, 1159, 1096, 1053, 1031, 989, 905, 859, 785, 711, 666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, $J = 6.3$ Hz, 3H), 0.95 (d, $J = 6.1$ Hz, 3H), 1.46 (s, 9H), 1.57–1.71 (m, 3H), 1.76 (d, $J = 7.1$ Hz, 3H), 3.73 (s, 3H), 4.67 (m, 1H), 6.25 (s, 1H), 6.53 (q, $J = 7.1$ Hz, 1H), 6.69 (d, $J = 8.3$ Hz, 1H). HRMS (FAB) ($M^+ + 1$): Found: m/z 329.2068. Calcd for $C_{16}H_{29}N_2O_5$: 329.2076. When the carbamate NH proton was irradiated, 3.3%, 2.5%, and 2.2% of the NOE were observed for the ^{t}Bu protons of the Boc group, the methyl protons at the double bond, and the amide NH proton, respectively.

Methyl (*Z*)-*N*-Boc-α,β-Didehydrophenylalanyl-L-leucinate (23): Mp 104.0–105.0 °C (ethyl acetate–hexane); $[\alpha]_D^{25} = -43.0°$ (1.00, MeOH) [Lit. mp 99–101.0 °C, $[\alpha]_D^{23} = -41.8°$ (1.00, MeOH)];[41] IR (KBr) 3230, 3056, 2957, 2871, 1743, 1680, 1658, 1629, 1550, 1504, 1489, 1446, 1391, 1366, 1342, 1304, 1250, 1201, 1164, 1078, 1061, 1029, 962, 938, 882, 841, 783, 754, 689 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.59 (d, $J = 5.7$ Hz, 3H), 0.97 (d, $J = 5.7$ Hz, 3H), 1.40 (s, 9H), 1.58–1.79 (m, 3H), 3.73 (s, 3H), 4.70–4.78 (m, 1H), 6.29 (s, 1H), 6.90 (d, $J = 8.1$ Hz, 1H), 7.21 (s, 1H), 7.27–7.46 (m, 5H). Found: C, 64.38; H, 7.79; N, 7.04%. Calcd for $C_{21}H_{30}N_2O_5$: C, 64.60; H, 7.74; N, 7.17%. When the carbamate NH proton was irradiated, 2.4% and 8.9% of the NOE were observed for the amide NH proton and the ortho protons of the phenyl group, respectively.

Methyl (*E*,*Z*)-*N*-Boc-α,β-Didehydroisoleucyl-L-leucinate (24): A mixture of (*Z*)- and (*E*)-isomers. Mp 108.0–108.5 °C (ethyl acetate); IR (KBr) 3349, 3058, 2938, 2740, 2344, 1758, 1690, 1637, 1491, 1386, 1332, 1252, 1201, 1093, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (*Z*)-isomer δ 0.93 (d, $J = 5.9$ Hz, 3H), 0.94 (d, $J = 5.9$ Hz, 3H), 1.03 (t, $J = 7.6$ Hz, 3H), 1.45 (s, 9H), 1.55–1.73 (m, 3H), 2.01 (s, 3H), 2.15 (q, $J = 7.6$ Hz, 2H), 3.72 (s, 3H), 4.62–4.68 (m, 1H), 5.89 (s, 1H), 7.30 (br, 1H). (*E*)-isomer δ 0.93 (d, $J = 5.9$ Hz, 3H), 0.94 (d, $J = 5.9$ Hz, 3H), 1.08 (t, $J = 7.6$ Hz, 3H), 1.45 (s, 9H), 1.55–1.73 (m, 3H), 1.76 (s, 3H), 2.15 (q, $J = 7.6$ Hz, 2H), 3.72 (s, 3H), 4.62–4.68 (m, 1H), 7.30 (br, 1H). Found: C, 60.45; H, 9.06; N, 7.78%. Calcd for $C_{18}H_{32}N_2O_5$: C, 60.65; H, 9.05; N, 7.86%.

Methyl *N*-Boc-α,β-Didehydrovalyl-L-alaninate (25): Mp 118.5–119.0 °C (ethyl acetate–hexane); $[\alpha]_D^{25} = -14.4°$ (0.73, MeOH); IR (KBr) 3282, 3060, 2982, 1753, 1688, 1665, 1635, 1542, 1457, 1367, 1339, 1278, 1252, 1210, 1164, 1055, 1029, 689 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (d, $J = 7.1$ Hz, 3H), 1.46 (s, 9H), 1.79 (s, 3H), 2.05 (s, 3H), 3.75 (s, 3H), 4.63 (dq, $J = 7.1$, 7.3 Hz, 1H), 5.83 (s, 1H), 6.75 (br, 1H). Found: C, 56.04; H, 8.13; N, 9.13%. Calcd for $C_{14}H_{24}N_2O_5$: C, 55.99; H, 8.05; N, 9.33%.

Methyl *N*-Boc-α,β-Didehydrovalylglycinate (26): Mp 164.0–165.0 °C (ethyl acetate–hexane); IR (KBr) 3335, 3189, 2985, 1761, 1691, 1659, 1634, 1540, 1456, 1435, 1397, 1365,

1333, 1288, 1258, 1202, 1175, 1135, 1085, 1058, 1034, 1013, 981, 900, 831, 779, 658 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.80 (s, 3H), 2.07 (s, 3H), 3.76 (s, 3H), 4.11 (d, $J = 5.7$ Hz, 2H), 5.74 (br, 1H), 6.70 (br, 1H). Found: C, 54.39; H, 7.76; N, 9.79%. Calcd for $C_{13}H_{22}N_2O_5$: C, 54.53; H, 7.74; N, 9.78%.

Methyl *N*-Boc-α,β-Didehydrovalyl-L-valinate (27): Mp 120.5–121.0 °C (hexane); $[\alpha]_D^{25} = -9.7°$ (0.48, MeOH); IR (KBr) 3299, 2976, 2364, 1750, 1686, 1638, 1543, 1510, 1365, 1273, 1252, 1174, 1057, 900 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (d, $J = 6.8$ Hz, 3H), 0.97 (d, $J = 6.8$ Hz, 3H), 1.46 (s, 9H), 1.80 (s, 3H), 2.05 (s, 3H), 2.16–2.24 (m, 1H), 3.73 (dd, $J = 4.8$, 8.7 Hz, 1H), 5.95 (s, 1H), 5.76 (br, 1H), 6.77 (br, 1H). Found; C, 58.39; H, 8.65; N, 8.32%. Calcd for $C_{16}H_{28}N_2O_5$: C, 58.52; H, 8.59; N, 8.53%.

Reaction of *N*-Boc-α-Tosylglycylamino Acid Methyl Ester with Aldehydes. To a solution of Bu_3P (124 mg, 0.61 mmol) and DBU (73 mg, 0.48 mmol) in dry THF (3 mL) was added 0.12 mL of propanal (95 mg, 1.6 mmol) at 0 °C under nitrogen. A solution of **2e** (187 mg, 0.41 mmol) in dry THF (1 mL) was added to the solution over 10 min at 0 °C under nitrogen. The mixture was allowed to stir for 5.5 h at room temperature. After removal of the solvent, a residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residual oil was subjected to preparative TLC (SiO_2, hexane:ethyl acetate = 4/1, v/v) to afford (*Z*)-isomer **29** (132 mg, 88%) and (*E*)-isomer **29′** (13 mg, 9%) in 97% yields, respectively. (*Z*)-isomer **29**: Mp 136.5–137.5 °C (ethyl acetate); $[\alpha]_D^{25} = -34.4°$ (1.00, MeOH); IR (KBr) 3350, 3224, 3053, 2965, 2870, 1759, 1690, 1667, 1631, 1549, 1485, 1368, 1324, 1296, 1249, 1230, 1159, 1096, 1053, 1020, 981, 903, 857, 825, 790, 752, 669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, $J = 5.9$ Hz, 3H), 0.95 (d, $J = 5.6$ Hz, 3H), 1.06 (t, $J = 7.6$ Hz, 3H), 1.46 (s, 9H), 1.54–1.74 (m, 2H), 2.17 (dq, $J = 7.3$, 7.6 Hz, 2H), 3.73 (s, 3H), 4.69 (dt, $J = 4.6$, 8.5 Hz, 1H), 5.95 (s, 1H), 6.40–6.43 (m, 1H), 6.57 (d, $J = 8.1$ Hz, 1H). Found: C, 59.34; H, 8.86; N, 8.13%. Calcd for $C_{17}H_{30}N_2O_5$: C, 59.36; H, 8.83; N, 8.18%. When the carbamate NH proton of the major product **29** was irradiated, 2.2% and 4.4% of the NOE were observed for the amide NH proton and methylene protons of the ethyl group, respectively.

(*E*)-isomer **29′**: A solid; $[\alpha]_D^{25} = -45.5°$ (0.40, MeOH); IR (KBr) 3255, 3058, 3009, 2964, 2935, 2870, 2365, 1760, 1693, 1655, 1636, 1545, 1503, 1457, 1384, 1369, 1329, 1251, 1232, 1197, 1158, 1050, 1022, 985 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (d, $J = 5.6$ Hz, 3H), 0.96 (d, $J = 5.9$ Hz, 3H), 1.10 (t, $J =$ 7.6 Hz, 3H), 1.46 (s, 9H), 1.57–1.71 (m, 3H), 2.44 (dq, $J = 7.3$, 7.6 Hz, 2H), 3.75 (s, 3H), 4.70 (dt, $J = 4.9$, 8.3 Hz, 1H), 6.16 (br, 1H), 6.32–6.46 (m, 2H). HRMS (FAB) ($M^+ + 1$): Found: m/z 343.2247. Calcd for $C_{17}H_{31}N_2O_5$: 343.2233.

The physical and spectral data of compounds **22′**, **30**, **30′**, **31**, and **31′** are shown in the following.

Methyl (*E*)-2-(*t*-Butoxycarbonylamino)-2-butenoyl-L-leucinate (22′): A solid; $[\alpha]_D^{25} = -15.8°$ (0.18, MeOH); IR (KBr) 3336, 3225, 3060, 3014, 2965, 2927, 2869, 2367, 2346, 1761, 1693, 1663, 1631, 1555, 1504, 1455, 1386, 1371, 1252, 1199, 1158, 1130, 1048, 1024, 987 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (d, $J = 6.3$ Hz, 3H), 0.97 (d, $J = 6.1$ Hz, 3H), 1.45 (s, 9H), 1.55–1.85 (m, 3H), 2.01 (d, $J = 7.6$ Hz, 3H), 3.75 (s, 3H), 4.71 (dt, $J = 5.1$, 8.5 Hz, 2H), 6.28 (br, 1H), 6.38 (q, $J = 7.6$ Hz, 1H). HRMS (FAB) ($M^+ + 1$): Found: m/z 329.2077. Calcd for $C_{16}H_{29}N_2O_5$: 329.2076.

Methyl (*Z*)-2-(*t*-Butoxycarbonylamino)-2-butenoylglycinate (30): Mp 116.0–118.0 °C (ethyl acetate); IR (KBr) 3324, 3207, 2973, 1762, 1691, 1674, 1631, 1536, 1498, 1456, 1436, 1405, 1367, 1298, 1273, 1254, 1208, 1173, 1097, 1050, 1010, 986 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.77 (d, $J = 7.1$ Hz, 3H), 3.75 (s, 3H), 4.10 (d, $J = 5.4$ Hz, 2H), 5.70–5.85 (br, 1H), 6.52 (q, $J = 7.1$ Hz, 1H), 6.50–6.60 (br, 1H). Found: C, 52.86; H, 7.51; N, 10.21%. Calcd for $C_{12}H_{20}N_2O_5$: C, 52.93; H, 7.40; N, 10.29%. In order to confirm the stereochemistry of the major product **30**, an authentic sample was prepared as follows: (*Z*)-2-(*t*-butoxycarbonylamino)butenoic acid and methyl glycinate were condensed by the DCC–HOBt method (57%). The NMR spectrum of the product was consistent with that of **30**.

Methyl (*E*)-2-(*t*-Butoxycarbonylamino)-2-butenoylglycinate (30′): A solid: IR (KBr) 3326, 3195, 2928, 2850, 1756, 1687, 1662, 1640, 1552, 1519, 1433, 1397, 1364, 1284, 1258, 1205, 1165, 1122, 1050, 1033, 1017, 982 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.02 (d, $J = 7.5$ Hz, 3H), 3.79 (s, 3H), 4.16 (d, $J = 6.3$ Hz, 2H), 6.20–6.30 (br, 1H), 6.30–6.40 (br, 1H), 6.45–6.60 (m, 1H). HRMS (FAB) ($M^+ + 1$); Found: m/z 273.1434. Calcd for $C_{12}H_{21}N_2O_5$: 273.1450.

Methyl (*Z*)-2-(*t*-Butoxycarbonylamino)-2-pentenoylglycinate (31): A solid: IR (KBr) 3265, 3060, 3006, 2978, 2937, 2875, 1763, 1748, 1704, 1683, 1666, 1639, 1513, 1460, 1434, 1414, 1392, 1366, 1319, 1279, 1254, 1213, 1173, 1124, 1107, 1059, 1039, 1020, 984 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (t, $J = 7.6$ Hz, 3H), 1.47 (s, 9H), 2.18 (dq, $J = 7.3$, 7.6 Hz, 2H), 3.77 (s, 3H), 4.12 (d, $J = 5.1$ Hz, 2H), 5.80 (br, 1H), 6.41 (br, 1H), 6.62 (br, 1H). HRMS (FAB) ($M^+ + 1$): Found: m/z 287.1614. Calcd for $C_{13}H_{23}N_2O_5$: 287.1607. When the methylene protons of the ethyl group were irradiated, 9.7%, 6.7%, and 2.7% of the NOE were observed for the methyl protons of the ethyl group, the olefinic proton, and the carbamate NH proton, respectively.

Methyl (*E*)-2-(*t*-Butoxycarbonylamino)-2-pentenoylglycinate (31′): A solid: IR (film) 3323, 2929, 2850, 1749, 1732, 1716, 1697, 1684, 1521, 1506, 1489, 1457, 1369, 1211, 1160, 1144, 1069, 1047, 1021, 984 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (t, $J = 7.6$ Hz, 3H), 1.46 (s, 9H), 2.45 (dq, $J = 7.3$, 7.6 Hz, 2H), 3.78 (s, 3H), 4.14 (d, $J = 5.1$ Hz, 2H), 6.12 (br, 1H), 6.37 (br, 1H), 6.52 (br, 1H). HRMS (FAB) ($M^+ + 1$): Found: m/z 287.1600. Calcd for $C_{13}H_{23}N_2O_5$: 287.1607.

Isomerization of the (*E*)- to (*Z*)-Isomer by Iodine. To a solution of **22′** (105 mg, 0.32 mmol) in dry CH_2Cl_2 (2 mL) was added a solution of a catalytic amount of iodine in dry CH_2Cl_2 (2 mL) at room temperature under a nitrogen. The solution was allowed to stand overnight with stirring, and was then concentrated in vacuo to give a residue, which was redissolved into AcOEt. The solution was washed with $NaHSO_3$, $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo to afford a residue, which was subjected to preparative TLC (SiO_2, hexane:AcOEt = 2:1, v/v); (*Z*)-isomer 83 mg, (*E*)-isomer 5 mg.

Compounds **29′**, **30′**, and **31′** were isomerized to their (*Z*)-isomer in the same way. The results are summarized in Table 6.

Synthesis of Protected Leucine-Enkephalin Analog (39). *t*-Butyl *N*-Boc-α-Tosylglycyl-L-leucinate (32): To a suspension of *t*-butyl L-leucinate hydrochloride (985 mg, 44 mmol) in dry THF (2 mL) was added dropwise a solution of 4-methylmorpholine (546 mg, 5.4 mmol) in dry THF (2 mL) at 0 °C under air. After stirring for 1 h at room temperature, **16** (1.472 g, 4.5 mmol), DCC (927 mg, 4.5 mmol), and HOBt (608 mg, 4.5 mmol) were added. After the mixture was stirred for 23 h at room temperature,

the insoluble substances were filtered off and washed with AcOEt. The filtrate was concentrated in vacuo to afford a residue, which was dissolved into AcOEt. The AcOEt solution was successively washed with 10% citric acid, 10% $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give the desired product as a mixture of diastereomers, which was recrystallized from ethyl acetate–hexane (1.726 g, 77%). Mp 143.0–146.0 °C (ethyl acetate–hexane); $[\alpha]_D^{25} = -29.1°$ (1.00, MeOH); IR (KBr) 3421, 3377, 2967, 2871, 1719, 1698, 1596, 1549, 1499, 1394, 1367, 1317, 1289, 1255, 1227, 1148, 1084, 1054, 950, 871, 848, 818, 737, 708, 666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) (M) δ 0.99 (d, $J = 6.6$ Hz, 6H), 1.21 (s, 9H), 1.48 (s, 9H), 1.64–1.71 (m, 2H), 1.80–1.92 (m, 1H), 2.42 (s, 3H), 4.49–4.63 (m, 1H), 5.46 (d, $J = 3.5$ Hz, 1H), 5.91 (d, $J = 8.8$ Hz, 1H), 7.18 (d, $J = 7.5$ Hz, 1H), 7.35 (d, $J = 3.5$ Hz, 2H), 7.79 (d, $J = 5.3$ Hz, 2H). (m) δ 0.95 (d, $J = 4.6$ Hz, 6H), 1.26 (s, 9H), 1.52 (s, 9H), 1.64–1.71 (m, 2H), 1.80–1.92 (m, 1H), 2.42 (s, 3H), 4.49–4.62 (m, 1H), 5.43 (d, $J = 3.5$ Hz, 1H), 5.85 (d, $J = 9.4$ Hz, 1H), 6.92 (d, $J = 7.5$ Hz, 1H), 7.32 (d, $J = 3.7$ Hz, 2H), 7.82 (d, $J = 5.5$ Hz, 2H). Found: C, 57.68; H, 7.68; N, 5.55%. Calcd for $C_{24}H_{38}N_2O_7S$: C, 57.81; H, 7.68; N, 5.62%.

***t*-Butyl *N*-Boc-(β-Nitro)phenylalanyl-L-leucinate (33):** To a suspension of KO^tBu (169 mg, 1.5 mmol) in dry THF (1.5 mL) was added dropwise a solution of (nitromethyl)benzene[5a] (384 mg, 2.8 mmol) in dry THF (2 mL) at −40 °C under nitrogen. After 10 min, a solution of **32** (249 mg, 0.5 mmol) in dry THF (3 mL) was added dropwise over 40 min. The reaction mixture was gradually warmed to room temperature and then quenched with a saturated aqueous solution of NH_4Cl. The THF was removed in vacuo to afford a residue, which was partitioned between AcOEt and water. The aqueous solution was extracted with AcOEt. The combined extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to preparative TLC (SiO_2, hexane:ethyl acetate = 10/1, v/v) to give **33** as a mixture of diastereomers in 70% yield (168 mg). Mp 131.0–136.0 °C (ethyl acetate–hexane); IR (KBr) 3317, 2979, 2871, 2352, 1726, 1696, 1658, 1556, 1529, 1457, 1393, 1369, 1327, 1283, 1249, 1168, 1049, 1023, 947, 849, 803, 743, 717, 695 cm^{-1}. The product was found to be a mixture of four diastereomers based on the NMR spectrum. Found: C, 59.92; H, 7.83; N, 8.71%. Calcd for $C_{24}H_{37}N_3O_7$: C, 60.11; H, 7.78; N, 8.76%.

***t*-Butyl *N*-Boc-Glycyl-(β-nitro)phenylalanyl-L-leucinate (35):** A mixed solution of AcOEt (3 mL), abs. methanol (122 mg = 0.15 mL, 3.8 mmol), and acetyl chloride (299 mg = 0.27 mL, 3.8 mmol) was stirred for 30 min at room temperature under nitrogen. The solid **33** (108 mg, 0.22 mmol) was added to the resulting solution at 0 °C, and the solution was then stirred for 8 h at room temperature to afford the hydrogen chloride salt **34** in quantitative yield, which was used in the subsequant coupling reaction without further purification.

To a solution of *N*-Boc-Gly-OH (40 mg, 0.23 mmol) in dry THF (0.5 mL) was added a solution of isobutyl chloroformate (30 mg, 0.22 mmol) in dry THF (1.5 mL) at −15 °C under nitrogen, followed by the addition of a solution of NMM (24 mg, 0.23 mmol) in dry THF (1.5 mL). After 3 min, a solution of **34** and NMM (24 mg, 0.23 mmol) in dry DMF (1.5 mL) was added to the mixed-anhydride solution prepared above. The mixture was kept at −15 °C for 30 min and then stirred for 2 h at room temperature. After the insoluble substances were filtered off and washed with AcOEt, the filtrate was concentrated under reduced pressure to afford a residue, which was partitioned between AcOEt and water. The aqueous layer was extracted with AcOEt and the combined extracts were successively washed with 10% citric acid, 10% $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to preparative TLC (SiO_2, hexane:ethyl acetate = 2/1, v/v) to give **35** as a mixture of diastereomers in 76% yield (82 mg). Mp 114.0–116.0 °C (ethyl acetate–hexane); IR (KBr) 3308, 3068, 2961, 2872, 1732, 1699, 1657, 1556, 1471, 1456, 1368, 1280, 1247, 1152, 1047, 947, 847, 718, 694 cm^{-1}. Found: C, 57.53; H, 7.17; N, 9.55%. Calcd for $C_{24}H_{37}N_3O_7$: C, 57.65; H, 7.14; N, 9.60%.

***t*-Butyl *N*-Boc-Glycyl-α,β-didehydrophenylalanyl-L-leucinate (36):** To a solution of **35** (29 mg, 0.06 mmol) in CH_2Cl_2 (0.5 mL) was added a solid of DABCO (7 mg, 0.06 mmol) at 0 °C under air. After stirring for 5 h at room temperature, an additional DABCO (42 mg, 0.36 mmol) was added. The solution was stirred for 18 h and quenched with a phosphate buffer solution (pH = 7). The solvent was removed in vacuo to afford a residue, which was partitioned between AcOEt and water. The aqueous layer was extracted with AcOEt, and the combined extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to preparative TLC (SiO_2, chloroform:methanol = 15/1, v/v) to afford **36** in 81% yield (22 mg). Mp 114.0–116.0 °C; $[\alpha]_D^{25} = -8.9°$ (0.42, MeOH); IR (KBr) 3365, 3290, 3056, 2976, 2932, 2871, 1725, 1687, 1657, 1623, 1523, 1457, 1391, 1367, 1274, 1253, 1153, 1050, 1030, 969, 946, 849, 764, 689 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, $J = 6.6$ Hz, 6H), 1.42 (s, 9H), 1.46 (s, 9H), 1.62 (dd, $J = 7.1$, 7.1 Hz, 2H), 1.75 (tq, $J = 6.6$, 6.6 Hz, 1H), 3.86 (d, $J = 5.1$ Hz, 2H), 4.53 (dt, $J = 7.3$, 7.3 Hz, 1H), 5.48 (t, $J = 5.1$ Hz, 1H), 7.06 (d, $J = 6.8$ Hz, 1H), 7.20 (s, 1H), 7.28–7.42 (m, 5H), 8.10 (s, 1H). EI-MS m/z 447 (M^+: 18.4%). When the amide NH proton of the α,β-didehydrophenylalanine residue was irradiated, 3.2%, 2.1%, 1.4%, and 1.8% of the NOE were observed for the ortho-protons of the phenyl group, the NH and the methylene protons of the glycine residue, and the NH proton of the leucine residue.

***N*,*O*-(DiBoc)-L-Tyrosylglycine (38):** A mixed solution of Boc-Tyr(Boc)-OH[10] (2.830 g, 7.43 mmol), HONSu (941 mg, 8.17 mmol), and DCC (1.533 g, 7.43 mmol) in dry THF (40 mL) was stirred for 4 h at room temperature under air. After filtration, the filtrate was treated with an aqueous solution (3 mL) of H-Gly-OH (837 mg, 11.5 mmol) and $NaHCO_3$ (937 mg, 11.15 mmol) at room temperature overnight. The THF was removed in vacuo to give a residue, which was diluted with a small amount of water and extracted with AcOEt. The aqueous layer was acidified with 10% citric acid. After salting out, the liberated oil was extracted with AcOEt. The organic solution was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to afford the desired product, which was recrystallized from ethyl acetate (2.51 g, 77%). Mp 147.0–148.5 °C; $[\alpha]_D^{25} = -1.2°$ (1.0, MeOH); IR (KBr) 3310, 2935, 2981, 2602, 2345, 1760, 1655, 1532, 1510, 1455, 1394, 1369, 1335, 1280, 1257, 1225, 1152, 1105, 1049, 1019, 897, 846, 834, 782, 671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 9H), 1.55 (s, 9H), 3.04 (br, 2H), 3.98 (br, 2H), 4.50 (br, 1H), 5.22 (br, 1H), 6.65 (br, 1H), 7.08 (d, $J = 8.5$ Hz, 2H), 7.21 (d, $J = 8.5$ Hz, 2H). One proton of the carboxylic acid was not assigned. Found: C, 57.32; H, 6.97; N, 6.34%. Calcd for $C_{21}H_{30}N_2O_8$: C, 57.52; H, 6.90; N, 6.39%.

***t*-Butyl *N*,*O*-(DiBoc)-L-Tyrosylglycylglycyl-α,β-didehydrophenylalanyl-L-leucinate (39):** To 1.5 mL of AcOEt was added methanol (60 mg = 0.076 mL, 1.87 mmol) followed by the addition of acetyl chloride (147 mg = 0.134 mL, 1.87 mmol) at room

temperature under nitrogen. The solution was stirred for 80 min at room temperature, and a solution of **36** (54 mg, 0.11 mmol) in ethyl acetate (2 mL) was then added at 0 °C. The solution was gradually warmed to room temperature and then stirred for 20 h. The solvent was removed under reduced pressure to afford the hydrogen chloride salt **37** in quantitative yield (47 mg), which was then used in the subsequent reaction. To a solution of **38** (44 mg, 0.10 mmol) in dry THF (2 mL) was added a solution of NMM (11 mg, 0.11 mmol) in dry THF (1 mL) followed by the addition of IBCF (15 mg, 0.11 mmol) in dry THF (1 mL) at −15 °C under nitrogen. After 30 min, a solution of the hydrogen chloride salt prepared above and NMM (12 mg, 0.12 mmol) in dry DMF (1 mL) was added while the solution was kept −15 °C for 15 min, and then gradually warmed to room temperature. After stirring overnight, the THF was removed in vacuo to afford a residue, which was partitioned between ether and water. The aqueous layer was extracted with ether. The combined extracts were successively washed with 10% citric acid, 10% $NaHCO_3$, and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residual oil was subjected to preparative TLC (SiO_2, chloroform:ethyl acetate:ethanol = 40:8:1, v/v). Yield, 64 mg (a solid, 72%); $[\alpha]_D^{25} = -1.4^\circ$ (0.41, MeOH); IR (film) 3314, 3059, 2960, 2933, 2872, 2354, 1759, 1667, 1510, 1392, 1369, 1275, 1256, 1223, 1150, 1048, 1019, 970, 896, 846, 758, 693 cm^{-1}; selected ^1H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 9H), 1.45 (s, 9H), 1.55 (s, 9H); HRMS (FAB) ($M^+ + 1$): Found: m/z 810.4253. Calcd for $C_{42}H_{60}N_5O_{11}$: 810.4289. Amino acid analysis showed the presence of tyrosine, glycine, and leucine in the ratio of 0.61:2.00:1.10.

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