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"One-Pot" Methylation of *N*-Nosyl-α-amino Acid Methyl Esters with Diazomethane and Their Coupling To Prepare *N*-Methyl Dipeptides

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N-Nosyl-α-amino acid methyl esters are methylated quantitatively with diazomethane. After proper deprotection of the amino function by treatment with the reagent system mercaptoacetic acid/sodium methoxide, the obtained *N*-methyl amino acid methyl esters are coupled with *N*-Fmoc amino acid chlorides to afford the corresponding dipeptides. The obtained products do not show any detectable extent of racemization by ^{1}H NMR and HPLC.

Introduction

N-Methylation of amino acids essentially changes the chemical and physical properties and the biological activity of peptide systems. In fact, the steric hindrance of the methylated amino group modulates the intensity of their activity and modifies their selectivity of action. *N*-Methylation of amino acids eliminates the hydrogen on the nitrogen atom of the peptide bond. Thus, the hydrogen-bonding pattern of peptides containing these amino acids is different from that of the unmethylated peptides and results in restriction of flexibility and consequent modification of the biological activity.[1]

Peptides containing *N*-methyl amino acids exhibit antibiotic,[2] anticancer, and antiviral activity.[3]

Various methods have been developed for the synthesis of optically active *N*-methyl amino acids.[4] However, all known synthetic approaches are characterized by harsh reaction conditions and by a lack of generality of the procedure due to low reactivity or racemization.[5,6]

Results and Discussion

In this work we report the results of our study, dealing with the development of a straightforward methylation procedure that can successfully be employed in the preparation of *N*-methyl amino acid methyl esters and their peptide derivatives. Our initial attempts involved the direct *N*-alkylation of amino acid methyl esters, performed with a large excess of diazomethane in the presence of aluminum trichloride, which provided mixtures of products whose composition depended on the reaction times. Diazomethane was prepared in diethyl ether starting from *N*-methyl-*N*-nitrosourea.[7] Due to the toxicity of diazomethane, reactions were performed under safe conditions. The starting amino acid methyl esters and the corresponding *N*-methyl and *N*,*N*-dimethyl derivatives were detected as the main components of the reaction mixtures. Acylation of the crude reaction products, performed with acetic anhydride, enabled separation of the *N*-acetyl amino acid methyl esters and the *N*-acetyl-*N*-methyl amino acid methyl esters from the *N*,*N*-dimethylated product that did not undergo the acetylation. In a typical experiment, the model compound leucine methyl ester hydrochloride (**1e**) was treated with diazomethane in the presence of 2 equiv of aluminum trichloride. The subsequent derivatization of the reaction products, with acetic anhydride, allowed for the recovery of a mixture of *N*-acetyl-*N*-methyl-leucine methyl ester (40% yield), *N*,*N*-dimethyl-leucine methyl ester (18%), and *N*-acetyl-leucine methyl ester (36%).

In an attempt to increase the acidity of the proton on the α amino group, various protecting groups were introduced on the amino function. *N*-Methylation of the *N*-acetyl amino acid methyl esters carried out with diazomethane proceeded at markedly reduced reaction rates. The *N*-acetyl-*N*-methyl amino acid methyl ester

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SCHEME 1

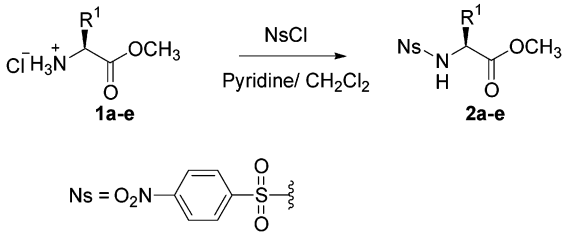


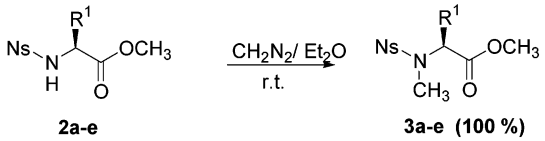
TABLE 1

compd	R^1	yield (%)
2a	CH_3	81
2b	CH_2Ph	97
2c	$CH(CH_3)_2$	84
2d	$CH_2CH(CH_3)_2$	82
2e	$CH(CH_3)CH_2CH_3$	87

was recovered in a very low yield and the starting material remained almost totally unchanged. In fact, reaction of the *N*-acetyl phenylalanine methyl ester with diazomethane in methanol afforded, after 12 h, the corresponding *N*-acetyl-*N*-methyl phenylalanine methyl ester only in 20% total yield. The acidity of the amide proton is too low to generate the methyl diazonium ion by interaction with diazomethane.[8] As reported,[9] the pK_a value of the methyldiazonium ion, the conjugated acid of diazomethane, is 8; hence, it was necessary to increase the acidity of the amide proton by introducing a more efficient protecting group that could assist the proton abstraction from the α-nitrogen atom of the amino acid methyl esters. For this reason, the *p*-nitrobenzenesulfonyl group (nosyl) was selected to protect and activate the amino function of the amino acid derivatives; this strong electron-withdrawing masking group enhances the reactivity of the N−H function toward diazomethane allowing the easy formation of the species responsible for methylation. Literature[10] reports note that alkylation of *N*-nosyl amino acid methyl esters is best performed with very reactive electrophiles, such as allylic or benzylic substrates, due to the low nucleophilicity of the α-nitrogen atom. Less electrophilic alkyl reactants need higher temperatures; however, these conditions can cause thermal decomposition and structural rearrangement of the expected products. To study the direct methylation with diazomethane, the α-amino functions of amino acid methyl esters **1a**−**e** were protected with the nosyl group (Scheme 1).

Treatment of **1a**−**e** with nosyl chloride in the presence of pyridine in dry methylene chloride gave the corresponding *N*-nosyl amino acid methyl esters **2a**−**e** in 81−97% yields of isolated product (Table 1). Lipophilic amino acids were chosen as model systems in order to study more accurately the methylation reaction and to evaluate better the effect of the reagents used on possible and competitive racemization processes. The obtained protected compounds were pure enough to be used in the subsequent methylation step, without need of further purification. Nosylation did not involve racemization of

SCHEME 2



a: R^1=-CH_3
b:R^1= -CH_2Ph
c:R^1= -$CH(CH_3)_2$
d:R^1= -$CH_2CH(CH_3)_2$
e:R^1= -$CH(CH_3)CH_2CH_3$

the chiral substrates as shown by ^{1}H NMR, for example **2e**. ^{1}H NMR analysis, performed on the crude product from the nosylation of isoleucine methyl ester, clearly showed the presence of signals corresponding to only one diastereoisomer; no traces of the other possible diastereoisomer of **2e** were detected. TLC and GC/MS analysis also revealed **2e** as the single reaction product. *N*-Nosyl amino acid methyl esters **2a**−**e** obtained were then allowed to react overnight with diazomethane in dry diethyl ether at room temperature. The *N*-nosyl-*N*-methyl amino acid methyl esters **3a**−**e** (Scheme 2) were obtained in quantitative yields.

^{1}H NMR spectra of **3a**−**e** differed from those of the corresponding unmethylated substrates **2a**−**e** with the presence of a new singlet at 3.43 ppm, due to the methyl group on the α-amino function, and the absence of the N−H resonance at 5.60 ppm. Diazomethane is a highly efficient reagent for the methylation of the α-amino function of *N*-nosyl amino acid methyl esters. These methylation reactions are very clean and no racemization of the final products was observed. The coupling of *N*-methyl-*N*-nosyl amino acid methyl esters **3a**−**e** with activated amino acids to produce dipeptides required the unblocking of the amino function. Removal of the nosyl group was first tried with thiophenol as the nucleophile in the presence of potassium carbonate, at room temperature. Unfortunately, no conversion of the starting material was observed. Although removal of the nosyl group may be carried out with either thiophenol or *β*-mercaptoethanol, the use of mercaptoacetic acid is more convenient because the thioether formed as coproduct can be easily removed by just washing the reaction mixture with a saturated aqueous solution of $NaHCO_3$.[12,13] Moreover mercaptoacetic acid, being less volatile as a salt in basic medium, has less odor than some other mercapto deprotection reagents. Removal of the nosyl group was initially performed by subjecting **3a**−**e** to a S_NAr reaction with the reagent system mercaptoacetic acid/1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), in dry DMF at room temperature,[11] but the reaction rates were impratically low and after 24 h the starting substrates were recovered mostly unchanged. The unblocking procedure was improved by raising the temperature to 50 °C with 3 equiv of mercaptoacetic acid in the presence of 8 equiv of sodium methoxide. The reaction was performed in acetonitrile by adding variable amounts of methanol to facilitate the

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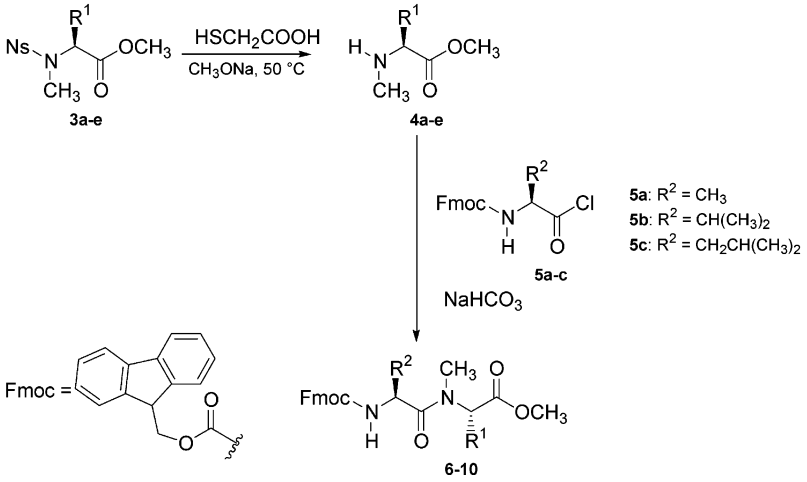
SCHEME 3



sodium methoxide solubilization. It is worth noting that deprotection went to completion in only 10 min. *N*-Methyl-*N*-nosyl-isoleucine methyl ester (**3e**) was chosen as a model system to verify, under the selected experimental conditions, the stereochemical features of the deprotection reaction, and to evaluate possible racemization. The deprotection reaction of *N*-methyl-*N*-nosyl isoleucine methyl ester (**3e**) afforded the corresponding *N*-methyl isoleucine methyl ester (**4e**) in 86% yield. The recovered product **4e** was characterized by ^{1}H NMR to check the optical purity. The ^{1}H NMR spectrum of the crude reaction product (**4e**), compared to the ^{1}H NMR spectrum of an authentic sample of *N*-methyl-D-*allo*-isoleucine methyl ester (**4e′**) obtained under the same deblocking conditions, did not reveal any signals corresponding to **4e′**. *N*-Methyl amino acid methyl esters obtained were converted into dipeptide systems. *N*-Methyl-*N*-nosyl amino acid methyl esters **3a**−**e** were thus unblocked with mercaptoacetic acid and sodium methoxide at 50 °C, as previously described, and the unmasked products **4a**−**e** were treated with *N*-Fmoc amino acid chlorides[14] **5a**−**c** in a methylene chloride solution containing aqueous $NaHCO_3$ (Scheme 3).

The crude reaction mixture was fractionated by short-column flash chromatography, to afford the corresponding dipeptides **6**−**10** in 69−98% yields (Table 2). ^{1}H NMR of the obtained dipeptides showed the presence of only one of the possible diastereoisomers of compounds **6**−**10**.

N-Methyl-L-alanine methyl ester (**4a**) and *N*-methyl-D-alanine methyl ester (**4a′**) prepared under the same reaction conditions as those for **4a** were chosen as model compounds to evaluate the enantiomeric purity of the *N*-methyl amino acid methyl esters **4a**−**e**. **4a** and **4a′** were converted respectively into dipeptide **6** and its diastereoisomer *N*-Fmoc-L-Leu-D-(Me)-Ala-OMe (**11**) by coupling with *N*-Fmoc-L-leucine chloride. The crude reaction products **6** and **11** and a suitably prepared mixture of the two diastereoisomers were subsequently analyzed by High Performance Liquid Chromatography (HPLC). The two diastereoisomers of the mixture were readily resolved by HPLC. Chromatograms of both crude dipeptides **6** and **11** showed the presence of only one diastereoisomer, hence *N*-methyl amino acid methyl esters **4a** and **4a′** are enantiomerically pure.

TABLE 2.

compd	R^1	R^2	yield (%)
6	CH_3	$CH_2CH(CH_3)_2$	69
7	CH_2Ph	$CH(CH_3)_2$	78
8	$CH(CH_3)_2$	CH_3	72
9	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	84
10	$CH(CH_3)CH_2CH_3$	CH_3	98

In an additional experiment *N*-methyl-L-alanine methyl ester (**4a**) and *N*-methyl-D-alanine methyl ester (**4a′**) were coupled with *N*-nosyl-L-leucine chloride to obtain respectively the dipeptide *N*-nosyl-L-Leu-L-(Me)-Ala-OMe (**12**) and its diastereoisomer *N*-nosyl-L-Leu-D-(Me)-Ala-OMe (**13**). GC/MS analysis was performed on the crude products **12** and **13** with the purpose of investigating if racemization took place during the methylation of *N*-nosyl amino acid methyl esters and the subsequent unblocking process of the amino function.

The recorded chromatograms, compared to that obtained analyzing an appropriately prepared mixture of the two diastereoisomers **12** and **13** (Figure 1), showed the presence of only one diastereoisomeric *N*-nosyl protected dipeptide in both crude products. GC/MS analysis of the *N*-nosyl dipeptides **12** and **13** in combination with the HPLC data for the *N*-Fmoc dipeptides **6** and **11** exclude any detectable racemization throughout the entire synthetic procedure.

Conclusions

N-Methylated amino acid methyl esters are readily prepared starting from *N*-nosyl-protected amino acid methyl esters. The methylation reaction is performed by treatment of nosyl derivatives with diazomethane. Finally, the *N*-nosyl group is readily removed with mercaptoacetic acid in the presence of sodium methoxide, to give the desired *N*-methyl analogues that are efficiently coupled to *N*-Fmoc amino acids chlorides to afford the corresponding dipeptides in excellent total yields and with retention of the configuration of the carbon atom of the precursors as confirmed by GC/MS, HPLC, and ^{1}H NMR spectroscopy.

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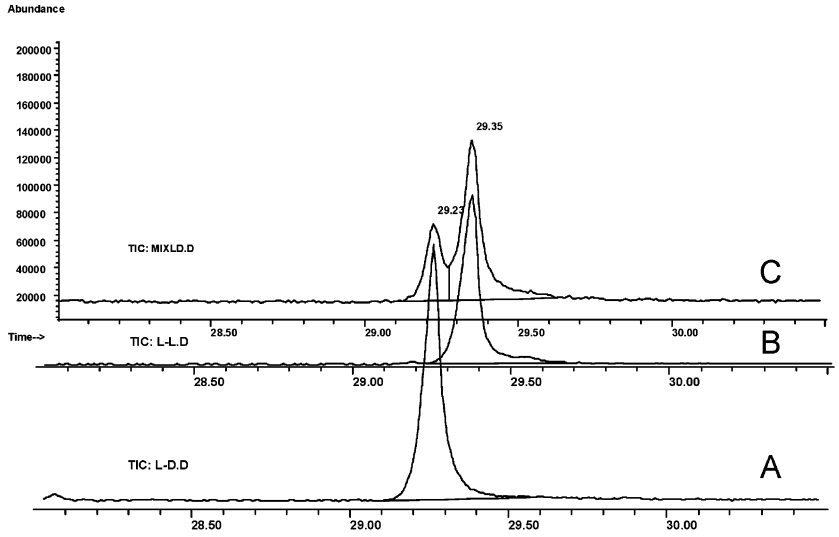

FIGURE 1. GC/MS analyses of *N*-nosyl dipeptides: (A) *N*-nosyl-L-Leu-D-(Me)-Ala-OMe (**13**) (rt; 29.22 min); (B) *N*-nosyl-L-Leu-L-(Me)-Ala-OMe (**12**) (rt: 29.36 min); and (C) a mixture of **12** and **13**.

Experimental Section

Solvents were purified and dried by standard procedures and distilled prior to use. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz with $CDCl_3$ as solvent. HPLC analyses were performed with a Lichrosorb SI-60 column (250 × 4 mm i.d. and 5 μm particle size) and a mixture of hexane/ethyl acetate/methanol (40:6:1) as the mobile phase (isocratic at a flow of 1 mL/min and UV detector at 260 nm).

GC/MS analyses were carried out on a 30 m HP-35MS capillary column with a 0.25 mm internal diameter and a 0.25 μm film thickness. The mass detector was operated in the electron impact ionization mode (EIMS) with an electron energy of 70 eV. Mass spectra were recorded on a Vacuum Generators ZAB-2F spectrometer, using 3-nitrobenzyl alcohol as matrix, by fast atom bombardment (FAB^+ MS), with a neutral Xenon beam operating at 8 keV and a total current of 10 μA.

Reaction mixtures were monitored by TLC, using silica gel 60-F_{254} precoated glass plates. Short column flash chromatography (SCFC) was performed on Kieselgel 60 H without gypsum. When required, the reactions were carried out under an inert atmosphere (N_2). The diethyl ether solution of diazomethane was prepared from *N*-methyl-*N*-nitrosourea following a classical procedure.[7] The concentration of the diazomethane solution (0.66 M) was obtained by a back-titration performed with a standard benzoic acid solution. **Caution:** Diazomethane is highly toxic. Hence, this reagent must be handled carefully.[7] Diethyl ether solutions of diazomethane are stable for long periods if stored on KOH pellets at −20 °C. All reactions were carried out under an inert atmosphere (N_2). Exact masses were determined by ESI-TOF MS analysis performed on a Q-STAR Pulsar-i-instrument.

Synthesis of *N*-Methyl-*N*-nosyl Amino Acid Methyl Esters 3a−e. General Procedure. A 0.66 M solution of diazomethane[7,12] in diethyl ether (8 mmol) was added cautiously dropwise to a stirred solution of *N*-nosyl-amino acid methyl esters **2a**−**e** (1 mmol) in dry diethyl ether (10 mL). The resulting mixture was maintained under an inert atmosphere (N_2) and stirred at room temperature overnight. TLC analysis (chloroform/methanol, 80:20 v/v) showed complete conversion of the precursor. Evaporation of the solvent under reduced pressure afforded the *N*-methyl-*N*-nosyl amino acid methyl esters **3a**−**e** as yellowish solids in quantitative yields. **3a**−**e** were used in the next step without further purification because the TLC analysis of the crude reaction mixture revealed the presence of only one product.

3a: pale yellow solid, mp 74−76 °C. IR (KBr) ν 3110, 2962, 1730, 1531, 1345, 1069, 850, 745 cm^{-1}. ^{1}H NMR δ 1.35 (d, J = 6.8 Hz, 3 H), 2.81 (s, 3 H), 3.47 (s, 3 H), 4.76 (q, J = 7.8 Hz, 1 H), 7.88−7.98 (m, 2 H), 8.24−8.38 ppm (m, 2 H). FAB^+ MS *m*/*z* (%) 303 (60) [(M + H)$^+$], 319 (65), 243 (100), 227 (25), 186 (32). Anal. Calcd for $C_{11}H_{14}N_2O_6S$: C, 43.70; H, 4.67; N, 9.27; O, 31.75; S, 10.61. Found: C, 43.71; H, 4.66; N, 9.29; S, 10.60.

3a′: pale yellow solid, mp 78−80 °C. IR (KBr) ν 3111, 2966, 1734, 1527, 1350, 1072, 851, 745 cm^{-1}. ^{1}H NMR δ 1.35 (d, J = 6.8 Hz, 3 H), 2.78 (s, 3 H), 3.47 (s, 3 H), 4,75 (q, J = 7.8 Hz, 1 H), 7.88−7.96 (m, 2 H), 8.24−8.34 ppm (m, 2 H). FAB^+ MS: *m*/*z* (%) 303 (58) [(M + H)$^+$], 319 (69), 243 (100), 227 (20), 186 (36). Anal. Calcd for $C_{11}H_{14}N_2O_6S$: C, 43.70; H, 4.67; N, 9.27; O, 31.75; S, 10.61. Found: C, 43.71; H, 4.66; N, 9.29; S, 10.60.

3b: pale yellow solid, mp 107−110 °C. IR (KBr) ν 3110, 2960, 1763, 1522, 1347, 1160, 850, 748 cm^{-1}. ^{1}H NMR δ 2.89 (s, 3 H), 3.27−3.36 (m, 2 H), 3.64 (s, 3 H), 4.98 (dd, J_1 = 5.8 Hz, J_2 = 10.7 Hz, 1 H), 7.09−7.27 (m, 5 H), 7.57−7.65 (m, 2 H), 8.11−8.20 ppm (m, 2 H). FAB^+ MS *m*/*z* (%) 379 (40) [(M + H)$^+$], 319 (65), 288 (100), 257 (35), 186 (58). Anal. Calcd for $C_{17}H_{18}N_2O_6S$: C, 53.96; H, 4.79; N, 7.40; O, 25.37; S, 8.47. Found: C, 53.98; H, 4.77; N, 7.38; S, 8.48.

3c: pale yellow solid, mp 53−56 °C. IR (KBr) ν 3104, 2972, 1734, 1530, 1351, 1086, 856, 747 cm^{-1}. ^{1}H NMR δ 0.96 (d, J = 6.8 Hz, 3 H), 1.02 (d, J = 6.8 Hz, 3 H), 2.13 (m, 1 H), 2.92 (s, 3 H), 3.46 (s, 3 H), 4.18 (d, J = 10.7 Hz, 1 H), 7.92−8.05 (m, 2 H), 8.29−8.42 ppm (m, 2 H). FAB^+ MS *m*/*z* (%) 331 (63) [(M + H)$^+$], 287 (27), 271 (100), 255 (15). Anal. Calcd for $C_{13}H_{18}N_2O_6S$: C, 47.26; H, 5.49; N, 8.48; O, 29.05; S, 9.71. Found: C, 47.23; H, 5.50; N, 8.49; S, 9.73.

3d: pale yellow solid, mp 89−91 °C. IR (KBr) ν 3107, 2957, 1746, 1530, 1345, 1155, 855, 741 cm^{-1}. ^{1}H NMR δ 0.92−1.01 (m, 6 H), 1.61−1.72 (m, 3 H), 2.85 (s, 3 H), 3.47 (s, 3 H), 4.71 (m, 1 H), 7.90−7.99 (m, 2 H), 8.27−8.39 ppm (m, 2 H). FAB^+ MS *m*/*z* (%) 345 (15) [(M + H)$^+$], 286 (30), 285 (100), 269 (18), 186 (16). Anal. Calcd for $C_{14}H_{20}N_2O_6S$: C, 48.83; H, 5.85; N, 8.13; O, 27.87; S, 9.31. Found: C, 48.84; H, 5.84; N, 8.16; S, 9.29.

3e:. pale yellow solid, mp 95−97 °C. IR (KBr) ν 3116, 2974, 1732, 1538, 1352, 1086, 857, 743 cm^{-1}. ^{1}H NMR δ 0.87−0.98 (m, 6 H), 1.10−1.25 (m, 1 H), 1.54−1.65 (m, 1 H), 1.86−1.99 (m, 1 H), 2.92 (s, 3 H), 3.43 (s, 3 H), 4.26 (d, J = 10.7 Hz, 1 H), 7.95−8.03 (m, 2 H), 8.31−8.38 ppm (m, 2 H). FAB^+ MS *m*/*z* (%) 345 (71) [(M + H)$^+$], 286 (39), 285 (100), 271 (54), 269 (49), 229 (25), 186 (24). Anal. Calcd for $C_{14}H_{20}N_2O_6S$: C, 48.83; H, 5.85; N, 8.13; O, 27.87; S, 9.31. Found: C, 48,81; H, 5.86; N, 8.14; S, 9.32.

Deprotection of *N*-Methyl-*N*-Nosyl-isoleucine Methyl Ester (3e) and *N*-Methyl-*N*-nosyl-D-*allo*-isoleucine Methyl Ester (3e′). Mercaptoacetic acid (3 mmol) was added to a solution of **3e** or **3e′** (1 mmol) in dry acetonitrile (10 mL) under N_2 at 50 °C. Sodium methoxide (8 mmol) was then added to the solution with methanol (5 mL). The resulting mixture was stirred for 10 min monitoring the conversion of **3e** or **3e′** by TLC (diethyl ether/petroleum ether 60:40 (v/v)). Aqueous 1 N HCl was then added and the acidified solution (pH 2) was extracted with ethyl acetate (3 × 10 mL). The aqueous phase was basified with saturated aqueous $NaHCO_3$ (pH 8) and then extracted with methylene chloride (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to afford **4e** or **4e′** as a colorless oil in 86% or 84% yield, respectively.

4e: 86% yield, colorless oil. ^{1}H NMR δ 0.85−0.93 (m, 6H), 1.21 (m, 1H), 1.53 (m, 1H), 1.73 (m, 1H), 2.01 (s, 1H), 2.39 (s, 3H), 3.09 (d, J = 5.8 Hz, 1H), 3.75 ppm (s, 3H). ESI-TOF MS calcd for $(C_8H_{17}NO_2 + H)^+$ 159.1259, found 159.1272.

4e′: 84% yield, colorless oil. ^{1}H NMR δ 0.85−0.93 (m, 6H), 1.21 (m, 1H), 1.53 (m, 1H), 1.73 (m, 1H), 2.01 (s, 1H), 2.37 (s, 3H), 3.05 (d, J = 5.85 Hz, 1H), 3.75 ppm (s, 3H). ESI-TOF MS calcd for $(C_8H_{17}NO_2 + H)^+$ 159.1259, found 159.1276.

Synthesis of Dipeptides 6−11. General Procedure. Mercaptoacetic acid (3 mmol) was added to a solution of **3a**−**e** (1 mmol) in dry acetonitrile (10 mL) under N_2 at 50 °C. Sodium methoxide (8 mmol) was then added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The resulting mixture was stirred for 5−10 min monitoring the conversion of **3a**−**e** by TLC (diethyl ether/petroleum ether 60:40 (v/v)). Aqueous 1 N HCl was then added and the acidified solution (pH 2) was extracted with ethyl acetate (3 × 10 mL). The aqueous phase was basified with saturated aqueous $NaHCO_3$ (pH 8). The basic liquors, containing the deprotected products **4a**−**e**, were then treated with a solution of *N*-Fmoc amino acid chlorides **5a**−**c** (0.5 mmol) in dry methylene chloride (10 mL). The reaction mixture was stirred at room temperature for ∼1 h, and the organic layer was separated. The aqueous phase was extracted with three additional portions of methylene chloride (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to give a crude reaction product. The subsequent chromatographic purification (diethyl ether/petroleum ether 15:85 (v/v)) afforded dipeptides **6**−**10** in 69−98% overall yields.

6: 69% yield, colorless oil. IR (KBr) ν 3308, 3068, 2962, 1746, 1698, 1650, 1538, 1256, 1045, 760, 733 cm^{-1}. ^{1}H NMR δ 1.07 (d, J = 6.8 Hz, 3 H), 1.15 (d, J = 6.3 Hz, 3 H), 1.53 (d, J = 7.3 Hz, 3 H), 1.58−1.69 (m, 2 H), 1.87 (m, 1 H), 3.12 (s, 3 H), 3.80 (s, 3 H), 4.31 (m, 1 H), 4.42−4.51 (m, 2 H), 4.88 (m, 1 H), 5.38 (q, J = 7.3 Hz, 1 H), 5.83 (d, J = 9.2 Hz, 1 H), 7.31−7.88 ppm (m, 8 H). FAB$^+$ MS m/z (%) 453 (47) [(M + H)$^+$], 421 (12), 239 (28), 191 (50), 179 (100), 178 (80), 165 (59). Anal. Calcd for $C_{26}H_{32}N_2O_5$: C, 69.01; H, 7.13; N, 6.19; O, 17.68. Found: C, 69.02; H, 7.12; N, 6.21.

7: 78% yield, colorless oil. IR (KBr) ν 3313, 3065, 2959, 1740, 1692, 1644, 1498, 1223, 1029, 759, 740 cm^{-1}. ^{1}H NMR δ 0.93 (d, J = 6.8 Hz, 3 H), 0.98 (d, J = 6.8 Hz, 3 H), 1.92−2.06 (m, 1 H), 2.95 (s, 3 H), 3.35−3.43 (dd, J_1 = 14.6 Hz, J_2 = 4.9 Hz, 1 H), 3.46−3.51 (dd, J_1 = 14.6 Hz, J_2 = 4.9 Hz, 1 H), 3.71 (s, 3 H), 4.13−4.45 (m, 4 H), 5.36 (dd, J_1 = 4.9 Hz, J_2 = 5.8 Hz, 1 H), 5.45(d, J = 8.8 Hz, 1 H), 7.02−7.80 ppm (m, 8 H). FAB$^+$ MS m/z (%) 515 (60) [(M + H)$^+$], 484 (36), 333 (74), 319 (30), 294 (28), 221 (25), 195 (100), 179 (80), 178 (79), 165 (60). Anal. Calcd for $C_{31}H_{34}N_2O_5$: C, 72.35; H, 6.66; N, 5.44; O, 15.55. Found: C, 72.36; H, 6.67; N, 5.43.

8: 72% yield, colorless oil. IR (KBr) ν 3320, 3070, 2970, 1746, 1691, 1640, 1504, 1454, 1246, 1066, 762, 742 cm^{-1}. ^{1}H NMR δ 0.85 (d, J = 6.8 Hz, 3 H), 1.03 (d, J = 6.8 Hz, 3 H), 1.37 (d, J = 6.8 Hz, 3 H), 2.22 (m, 1 H), 3.04 (s, 3 H), 3.68 (s, 3 H), 4.20 (m, 1 H), 4.32−4.38 (m, 2 H), 4.70 (m, 1 H), 4.86 (d, J = 9.8 Hz, 1 H), 5.86 (d, J = 7.8 Hz, 1 H), 7.23−7.80 ppm (m, 8 H). FAB$^+$ MS m/z (%) 439 (87) [(M + H)$^+$], 407 (35), 243 (50), 191 (81), 179 (100), 178 (82), 165 (87). Anal. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39; O, 18.24. Found: C, 68.45; H, 6.91; N, 6.41.

9: 84% yield, colorless oil. IR (KBr) ν 3302, 3070, 2961, 1748, 1681, 1651, 1535, 1258, 1030, 760, 740 cm^{-1}. ^{1}H NMR δ 0.85−0.91 (m, 6 H), 0.94 (d, J = 6.8 Hz, 3 H), 1.02 (d, J = 6.8 Hz, 3 H), 1.45 (m, 1 H), 1.65−1.75 (m, 2 H), 2.05 (m, 1 H), 2.98 (s, 3 H), 3.65 (s, 3 H), 4.18 (m, 1 H), 4.29−4.36 (m, 2 H), 4.54 (dd, J_1 = 8.8 Hz, J_2 = 5.8 Hz, 1 H), 5.33 (dd, J_1 = 9.8 Hz, J_2 = 5.8 Hz, 1 H), 5.62 (d, J = 8.8 Hz, 1 H), 7.21−7.76 ppm (m, 8 H). FAB$^+$ MS m/z (%) 481 (55) [(M + H)$^+$], 449 (55), 293 (14), 284 (18), 193 (18), 190 (100), 188 (87), 179 (80), 178 (78), 165 (60). Anal. Calcd for $C_{28}H_{36}N_2O_5$: C, 69.98; H, 7.55; N, 5.83; O, 16.65. Found: C, 69.99; H, 7.54; N, 5.82.

10: 98% yield, colorless oil. IR (KBr) ν 3305, 3068, 2962, 1746, 1685, 1643, 1555, 1454, 1250, 1054, 764, 735 cm^{-1}. ^{1}H NMR δ 0.73−0.82 (m, 3 H), 0.87 (d, J = 6.8 Hz, 3 H), 0.92−1.04 (m, 2 H), 1.27 (d, J = 6.8 Hz, 3 H), 1.91 (m, 1 H), 2.97 (s, 3 H), 3.62 (s, 3 H), 4.13 (m, 1 H), 4.25−4.33 (m, 2 H), 4.62 (m, 1 H), 4.92 (d, J = 9.8 Hz, 1 H), 5.78 (d, J = 7.8 Hz, 1 H), 7.16−7.69 ppm (m, 8 H). FAB$^+$ MS m/z (%) 453 (67) [(M + H)$^+$], 421 (65), 266 (28), 257 (47), 195 (81), 179 (100), 178 (80), 165 (57). Anal. Calcd for $C_{26}H_{32}N_2O_5$: C, 69.01; H, 7.13; N, 6.19; O, 17.68. Found: C, 69.02; H, 7.12; N, 6.18.

11: 75% yield, colorless oil. IR (KBr) ν 3312, 3066, 2959, 1752, 1696, 1642, 1540, 1260, 1046, 761, 739 cm^{-1}. ^{1}H NMR δ 1.08 (d, J = 6.8 Hz, 3 H), 1.12 (d, J = 6.3 Hz, 3 H), 1.56 (d, J = 7.3 Hz, 3 H), 1.55−1.66 (m, 2 H), 1.84 (m, 1 H), 3.05 (s, 3 H), 3.75 (s, 3 H), 4.30 (m, 1 H), 4.39−4.50 (m, 2 H), 4.92 (m, 1 H), 5.35 (q, J = 7.3 Hz, 1 H), 5.92 (d, J = 9.2 Hz, 1 H), 7.29−7.87 ppm (m, 8 H). FAB$^+$ MS m/z (%) 453 (35) [(M + H)$^+$], 421 (10), 239 (21), 191 (42), 179 (100), 178 (85), 165 (53). Anal. Calcd for $C_{26}H_{32}N_2O_5$: C, 69.01; H, 7.13; N, 6.19; O, 17.68. Found: C, 69.02; H, 7.12; N, 6.21.

Synthesis of Dipeptides 12 and 13. General Procedure. Mercaptoacetic acid (3 mmol) was added to a solution of **3a** or **3a′** (1 mmol) in dry acetonitrile (10 mL) under N_2 at 50 °C. Sodium methoxide (8 mmol) was then added to the solution with a variable amount of methanol to facilitate the sodium methoxide solubilization. The resulting mixture was stirred for 5−10 min, monitoring the conversion of **3a** or **3a′** by TLC (diethyl ether/petroleum ether 60:40 (v/v)). Aqueous 1 N HCl was then added and the acidified solution (pH 2) was extracted with ethyl acetate (3 × 10 mL). The aqueous phase was basified with saturated aqueous $NaHCO_3$ (pH 8). The basic liquors, containing the deprotected products **4a** or **4a′**, were then treated with a solution of *N*-nosyl-leucine chloride (0.5 mmol) in dry methylene chloride (10 mL). The reaction mixture was stirred at room temperature for ∼1 h, and the organic layer was separated. The aqueous phase was extracted with three additional portions of methylene chloride (3 × 10 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under vacuum to give a crude reaction product. The subsequent chromatographic purification (diethyl ether/petroleum ether 15:85 (v/v)) afforded dipeptides **12** (83% yield) or **13** (85% yield).

12: 83% yield, pale yellow solid. IR (KBr) ν 3293, 3104, 2962, 1740, 1640, 1534, 1401, 1354, 1261, 1170, 802, 740 cm^{-1}. ^{1}H NMR δ 0.99 (d, J = 6.7 Hz, 3 H), 1.03 (d, J = 6.5 Hz, 3 H), 1.17 (d, J = 7.3 Hz, 3 H), 1.45−1.59 (m, 2 H), 1.98 (m, 1 H), 2.84 (s, 3 H), 3.69 (s, 3 H), 4.23 (m, 1 H), 4.84 (q, J = 7.3 Hz, 1 H), 5.77 (d, J = 8.2 Hz, 1 H), 8.00−8.05 (m, 2 H), 8.32−8.38 ppm (m, 2 H). GC/MS m/z (%) 415 (2) [(M)$^+$], 400 (3), 356 (4), 271 (100), 229 (22), 215 (40), 186 (35), 146 (15), 122 (31). Anal. Calcd for $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; O, 26.96; S, 7.72. Found: C, 49.13; H, 6.08; N, 10.12; S, 7.71.

13: 85% yield, pale yellow solid. IR (KBr) ν 3298, 3106, 2960, 1742, 1645, 1537, 1407, 1350, 1258, 1172, 805, 743 cm^{-1}. ^{1}H NMR δ 0.95 (d, J = 6.7 Hz, 3 H), 1.02 (d, J = 6.5 Hz, 3 H), 1.26 (d, J = 7.3 Hz), 1.41−1.54 (m, 2 H), 2.00 (m, 1 H), 2.86 (s,

3 H), 3.62 (s, 3 H), 4.27 (m, 1 H), 4.78 (q, J = 7.3 Hz, 1 H), 5.71 (d, J = 7.5 Hz, 1 H), 7.96−8.01 (m, 2 H), 8.29−8.33 ppm (m, 2 H). GC/MS m/z (%) 415 (1) [$(M)^+$], 400 (3), 356 (5), 271 (100), 229 (25), 215 (37), 186 (31), 146 (12), 122 (24). Anal. Calcd for $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; O, 26.96; S, 7.72. Found: C, 49.12; H, 6.08; N, 10.12; S, 7.70.

Supporting Information Available: Compound characterization data for **2a**−**e** and HPLC analyses of **6** and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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