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N-Methylation of Peptides on Selected Positions during the Elongation of the Peptide Chain in Solution Phase

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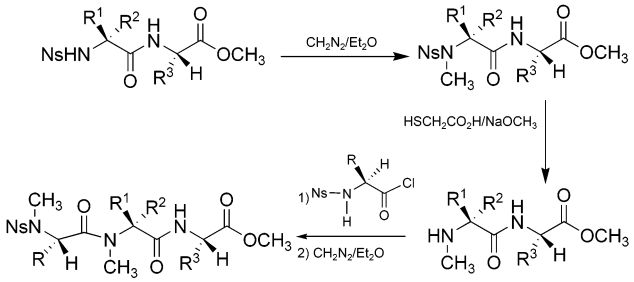

An efficient and general solution-phase method for the site-specific *N*-methylation of peptides has been developed. This novel procedure involves synthesis of *N*-nosyl protected peptides and their subsequent *N*-methylation with diazomethane. Its efficiency was proved by the successful synthesis of various hindered oligopeptides containing *N*-methyl amino acid residues with excellent yield and purity. The method is particularly attractive in that the adopted conditions do not cause any detectable racemization of the peptide stereocenters and the process does not require chromatographic purification of the methylated products. A further advantage is the compatibility of this methodology with Fmoc solution-phase peptide synthesis.

Introduction

Peptide analogues that possess an increased biological activity and bioavailability with respect to the corresponding naturally occurring peptides can be obtained by modifying appropriately the peptide backbone.[1]

N-Methylation of a peptide bond provides a bulky steric modification that can change the conformation of the peptide. It can also block potential intramolecular hydrogen bonding sites and proteolytic enzyme cleavage sites. These properties, together with the enhanced hydrophobicity, can increase the bioavailability and the therapeutic potential of the peptide.[2] *N*-Methyl peptides also represent important systems for structure−activity studies.

Several naturally occurring peptides that exhibit interesting biological activities such as cyclosporines,[3] dolastatins,[4] and didemnins[5] are characterized by the presence in their chain of *N*-methylated peptide bonds.

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N-Methylated peptides are generally prepared by incorporation of previously synthesized protected *N*-methylated amino acids[6] into the peptide chain in solution or solid phase.[7] The difficulty of these syntheses is often due to the sterically hindered N-methyl amino acids which resist coupling.[8] Therefore, preparation of *N*-methylated peptides can be challenging because racemization[9] and diketopiperazine formation[10] are common side reactions.

Recently we introduced a new and efficient solution method for preparing *N*-methylated amino acid methyl esters by methylation of *N*-*p*-nitrobenzensulfonyl-α-amino acid methyl esters with diazomethane.[6a]

Direct methylation of the terminal amino function of *N*-nosyl peptides and the subsequent elongation of the methylated peptide chains could represent an interesting goal in the peptide synthesis. The successful application of the direct *N*-methylation to peptides *N*-nosyl protected could be used afterward to carry out *N*-methylation of peptide bond in key positions of peptide chain and to study the effects of *N*-methylation at every residue of a biologically active peptide.

N-Methylation of *N*-nosyl-peptides could circumvent the problem of racemization since it avoids the use of protected *N*-methyl amino acids as activated components. In fact, protected *N*-methyl-amino acids activated on the carboxyl function are more subjected to racemization,[7a] proceeding through α-deprotonation or enolization of the oxazolonium ion, than the corresponding amino acids not methylated on the protected amino function.

Furthermore, we are interested in the use of highly reactive aminoacyl chlorides protected at the nitrogen atom by the nosyl group as reagents for peptide coupling. Nosyl protected amino acids chlorides should allow practical synthesis of hindered peptides containing *N*-methyl amino acid residues using solution-phase methodology. In addition, amino acid chlorides protected on the amino function with arenesulfonyl group can avoid the risk of oxazolone formation.[11]

Only a few examples of direct N-methylation of peptides are reported, the majority of which on the solid phase.[12] Some of these procedures are characterized by nonselective permethylation of peptides,[12f] or modest yields[12d] or incompatibility with standard Fmoc peptide synthesis procedures.[12e]

In this work we describe a new, straightforward and general method for site-specific *N*-methylation of peptides during the elongation of peptide chain in solution phase. It involves the synthesis of *N*-nosyl-protected peptides and their subsequent *N*-methylation.[6a] The latter process requires simply an ethereal solution of diazomethane,[6a] that acting as a base, generates in situ the methyl diazonium ion[13] responsible for the *N*-methylation. The use of bases in the methylation reaction can be crucial, since weak bases can provide poor yield of methylated peptide while strong bases can cause methylation of the amide backbone. The choice of diazomethane as methylating reagent could be significant to achieve specific *N*-methylation of the amino terminal function.

The proposed methodology is developed for solution peptide synthesis and consequently it is applied to short peptide sequences considering the poor solubility of larger peptides in most of organic solvents.

Results and Discussion

To explore the direct methylation of oligopeptides with diazomethane, the synthesis of *N*-nosyl-protected dipeptides was undertaken. The preparation of dipeptides **5**−**9** required the synthesis of *N*-nosyl-α-amino acids and their subsequent activation.

The starting *N*-nosyl-α-amino acids **2a**−**g** were prepared from *p*-nitrobenzensulfonyl chloride (Ns-Cl) and the corresponding α-amino acids **1a**−**g** dissolved in an aqueous solution of NaOH 1N[11a,14] (Scheme 1, Table 1). The reaction of **2a**−**g** with thionyl chloride gave the corresponding *N*-nosyl amino acids chlorides **3a**−**g** in quantitative yields (Scheme 2).

The synthesis of *N*-nosyl-dipeptides **5**−**9** was then accomplished by coupling of *N*-nosyl amino acids chlorides **3a**−**g** with α-amino acid methyl esters **4a,b** (Scheme 2). The proceeding of the coupling reaction was initially verified by treating *N*-nosyl-l-phenylalanine chloride (**3c**),

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SCHEME 1

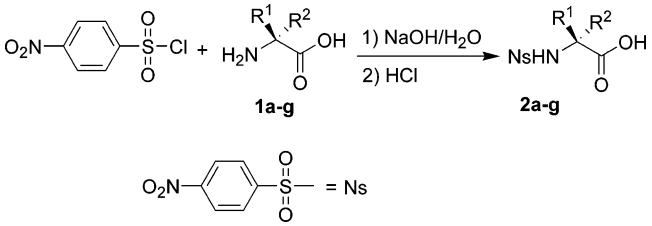


TABLE 1. Results of the Syntheses of *N*-Nosyl Amino Acids 2a−g

compound	R^1	R^2	yield (%)[a]
2a	**$CH_3CH_2(CH_3)CH$**	**H**	**84**
2b	**$(CH_3)_2CH$**	**H**	**83**
2c	**$C_6H_5CH_2$**	**H**	**95**
2d	**H**	**$C_6H_5CH_2$**	**93**
2e	**CH_3**	**H**	**84**
2f	**H**	**CH_3**	**82**
2g	$(CH_3)_2CHCH_2$	**H**	**84**

[a] Isolated yield.

TABLE 2. Results of the Syntheses of *N*-Nosyl Dipeptides Methyl Esters 5−9

compound	R^1	R^2	R^3	yield (%)[a]
5	**$C_6H_5CH_2$**	**H**	**CH_3**	**94**
6	**H**	**$C_6H_5CH_2$**	**CH_3**	**85**
7	**$CH_3CH_2(CH_3)CH$**	**H**	**CH_3**	**94**
8	**H**	**CH_3**	**$(CH_3)_2CH$**	**74**
9	$(CH_3)_2CHCH_2$	**H**	CH_3	**76**

[a] Isolated yield.

dissolved in ethanol free chloroform, with an aqueous basic solution of l-alanine methyl ester hydrochloride **4a** at room temperature (Scheme 2). The reaction was complete within 1h and afforded the dipeptide *N*-nosyl-l-phenylalanyl-l-alanine (**5**) in 94% yield (Scheme 2, Table 2).

To study the stereochemical aspects of coupling reaction, the dipeptide *N*-nosyl-d-phenylalanyl-l-alanine (**6**) diastereomer of **5** was synthesized for comparison under the same conditions using *N*-nosyl-d-phenylalanine chloride (**3d**). GC/MS analysis of the single crude products **5** and **6** showed the presence of only one diastereomer in both samples.

GC/MS analysis performed on an appropriately prepared mixture of the two diastereomers **5** and **6** showed instead the presence of two peaks corresponding to the two diastereomers. Hence *N*-nosyldipeptide methyl ester **5** was obtained without measurable epimerization. Also, the comparison of ^{1}H NMR spectrum of the mixture to those obtained from the single crude products **5** and **6** excluded the formation of epimerized products.

N-Nosyldipeptide methyl esters **7**−**9** were also obtained as previously described in high yields and purity (Scheme 2, Table 2).

Methylation reaction was performed, at room temperature, by treating *N*-nosyldipeptides **5**−**9** with an ethereal solution of diazomethane (0.66 N) in the molar ratio 1:8 (Scheme 3). The reaction was completed within 30 min and the corresponding *N*-nosyl-*N*-methyl-dipeptides **5a**−**9a** were recovered with quantitative yields and high purity after evaporation of the solvent under reduced pressure (Scheme 3).

The methylation reaction is chemospecific, as determined by analyzing the products **5a**−**9a** by NMR and GC/MS methods; in fact, only products monomethylated on the sulfonamide nitrogen atom were identified, no traces of other methylated products were observed. Mass spectra of the obtained *N*-methylated dipeptides allowed the unequivocal assignment of the methylation site in each dipeptide. The *N*-nosyl-*N*-methylated residue was easily identified by a considerable *N*-nosyl-*N*-methylimmonium ion peak. Analysis of the other mass fragments and of the ^{1}H NMR spectra confirmed the location of the *N*-methyl group in the peptide chain.

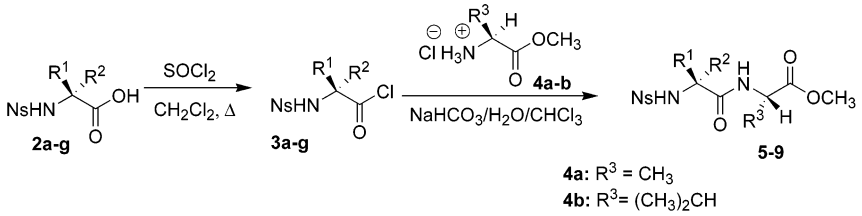
The application of the adopted *N*-methylation conditions to two diastereomeric dipeptides **5** and **6** allowed us to exclude the formation of epimeric methylated products. The diastereomeric *N*-methyl-dipeptides **5a** and **6a** were readily resolved by GC/MS. Furthermore careful examination of ^{1}H NMR spectra of both *N*-methylated products **5a** and **6a** showed the presence of signals corresponding to only one diastereomer. In particular, comparison of the peaks of *N*-methyl and N−H amide groups of both products **5a** and **6a** was employed to analyze the presence in each spectrum of the other diastereomer.

The comparison signals were found with chemical shifts that differs in the two diastereomers as showed by ^{1}H NMR spectrum of a mixture of **5a** and **6a** (Figure 1).

In light of the excellent results obtained with *N*-nosyldipeptides, the methylation reaction with diazomethane was then investigated also with tripeptide systems.

The tripeptide N-nosyl-l-Ile-d-Ala-l-Val-OMe (**11**), prepared by coupling of *N*-nosyl-l-isoleucine chloride (**3a**) with the dipeptide d-alanyl-l-valine-OMe (**10**), was readily *N*-methylated according to the described procedure (Scheme 4). The resulting *N*-nosyl-protected tripeptide **11a** methylated on the nitrogen atom of the *N*-terminal residue was isolated in quantitative yield as the sole reaction product. ^{1}H NMR and mass spectra of tripeptide **11a** confirmed the specific *N*-methylation of the sulfonamide group.

SCHEME 2



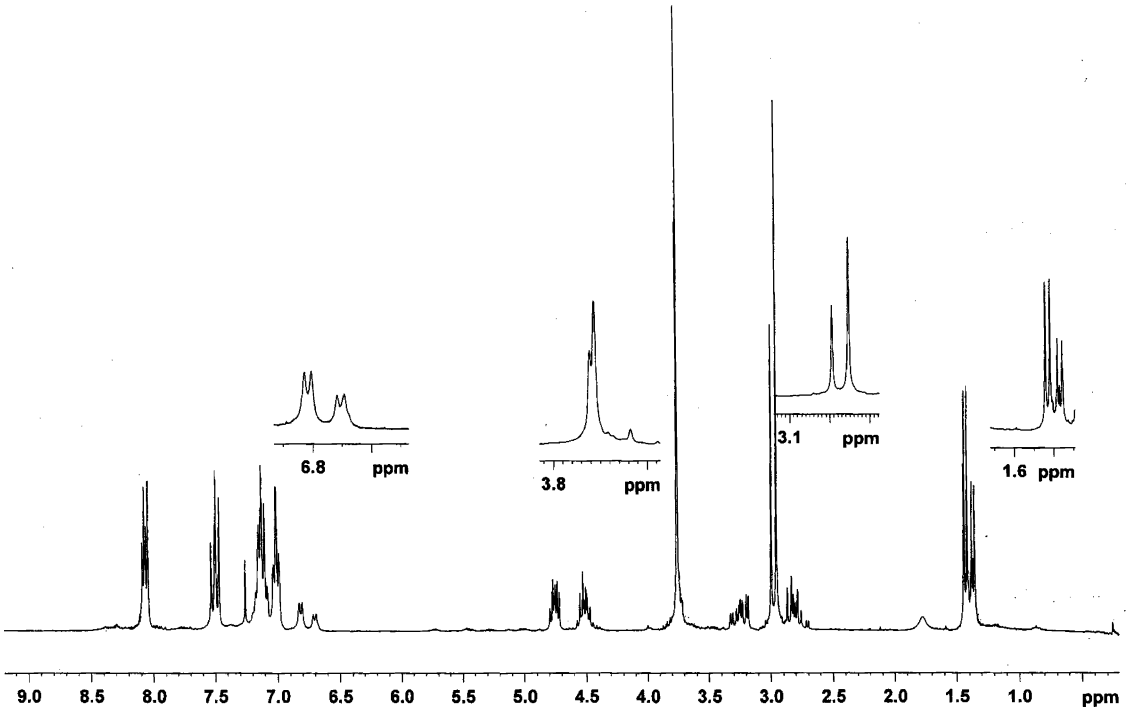
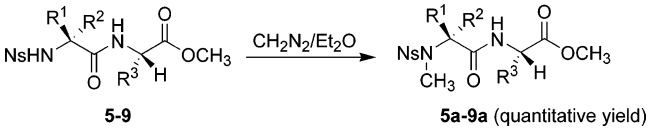
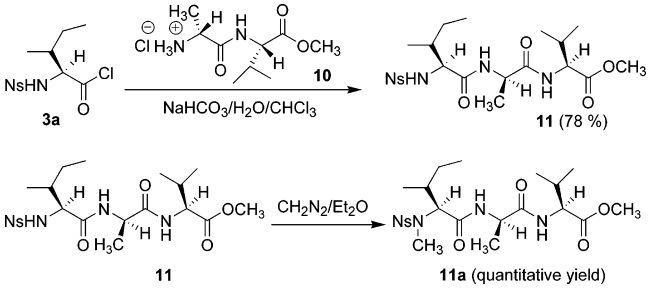


FIGURE 1. ^{1}H NMR spectrum of a mixture of *N*-nosyl-(Me)-l-Phe-l-Ala-OMe (**5a**) and *N*-nosyl-(Me)-d-Phe-l-Ala-OMe (**6a**).

SCHEME 3



SCHEME 4



SCHEME 5

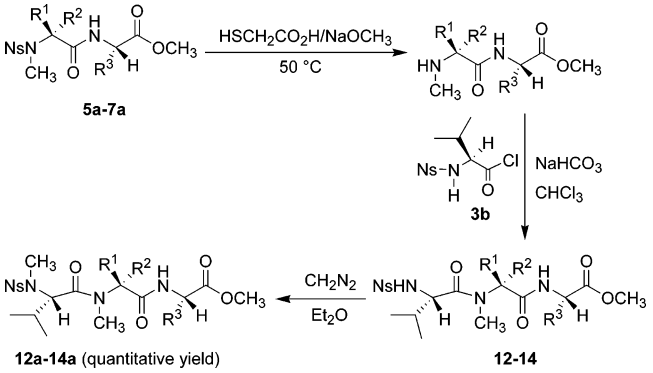


TABLE 3. Results of the Syntheses of Tripeptides Methyl Esters 12−14

compound	R^1	R^2	R^3	yield (%)[a]
12	$C_6H_5CH_2$	**H**	**CH_3**	**86**
13	**H**	$C_6H_5CH_2$	**CH_3**	**88**
14	$CH_3CH_2(CH_3)CH$	**H**	**CH_3**	**72**

[a] Isolated yield.

To demonstrate that our procedure could be used to methylate each amino acid in a peptide chain, polymethylated peptides were prepared by sequential *N*-methylation of the *N*-terminal amino acid residue during the elongation of the peptide.

N-Methylated dipeptides **5a** and **7a** were easily deprotected on the terminal amino function by the reagent system mercaptoacetic acid/sodium methoxide[6a] and then coupled with *N*-nosyl-l-valine chloride (**3b**) (Scheme 5, Table 3). The resulting monomethylated tripeptides **12** and **14** were recovered in high yields.

The tripeptide **13** epimer of **12** was also prepared starting from the methylated dipeptide **6a** diastereomer of **5a** to test the epimerization. There was no cross-contamination of epimers according to ^{1}H NMR spectra of both tripeptides.

The methylated *N*-nosyltripeptides **12**−**14** were further methylated with diazomethane to provide the corresponding dimethylated tripeptides *N*-nosyl-(Me)-l-Val-(Me)-l-Phe-l-Ala-OMe (**12a**), *N*-nosyl-(Me)-l-Val-(Me)-d-Phe-l-Ala-OMe (**13a**), and *N*-nosyl-(Me)-l-Val-(Me)-l-Ile-l-Ala-OMe (**14a**) (Scheme 5).

Finally, to complete our study, it was also investigated the compatibility of standard Fmoc solution peptide synthesis techniques (Fmoc-SPS) with the use of *p*-nitrobenzensulfonyl protecting group required when *N*-methylation is desired.

Therefore in an additional experiment *N*-nosyl-*N*-methyldipeptides **6a**, **8a**, and **9a**, after removal of the sulfonamide group, were coupled with *N*-Fmoc-amino acid chlorides **15a,b** (Scheme 6, Table 4) to give the

SCHEME 6

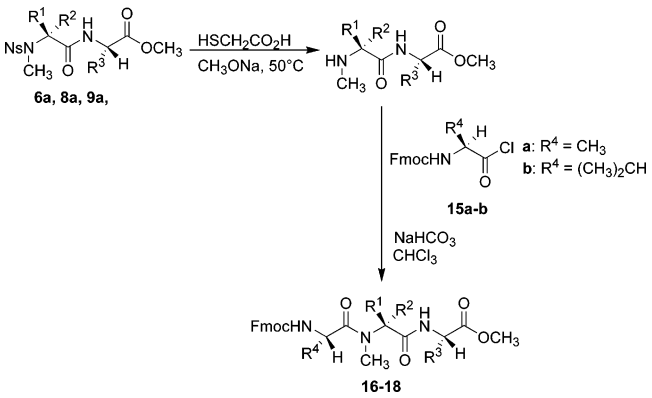
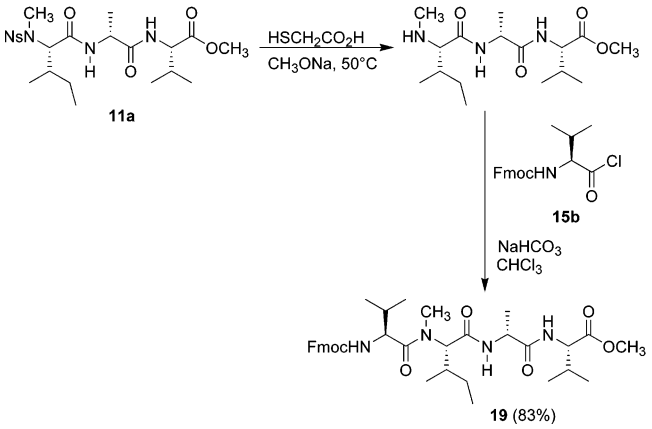


TABLE 4. Results of the Syntheses of *N*-Fmoc *N*-Methylated Tripeptides Methyl Esters 16−18

compound	R^1	R^2	R^3	R^4	yield (%)[a]
16	**H**	$C_6H_5CH_2$	**CH_3**	**CH_3**	**92**
17	**H**	**CH_3**	**$(CH_3)_2CH$**	**$(CH_3)_2CH$**	**94**
18	$(CH_3)_2$-CHCH$_2$	**H**	**CH_3**	**$(CH_3)_2CH$**	**93**

[a] Isolated yield.

SCHEME 7



corresponding *N*-Fmoc-tripeptides **16**−**18** methylated on the nitrogen atom of the second amino acid unit. Removal of the Fmoc protecting group has been successfully tested for these systems.[16]

Also, the *N*-nosyl-*N*-methyltripeptide **11a** was subjected to the above-described two-step sequence composed of terminal amine deprotection, followed by coupling with *N*-Fmoc-valine chloride (**15b**) to afford the corresponding methylated tetrapeptide *N*-Fmoc protected **19** (Scheme 7).

The synthesis of methylated *N*-nosyl- or *N*-Fmoc-protected structures occurs in a simple way and in high yields. Furthermore, the high reactivity of the amino acid chloride, formed cleanly using $SOCl_2$ procedure, is more than sufficient to overcome the steric hindrance by the *N*-methyl substituent of the deprotected methylated dipeptides in the coupling step. Therefore products derived from incomplete peptide coupling or competitive cyclization reactions were not detected.

Conclusions

In conclusion, a novel and efficient solution-phase method for the site-specific *N*-methylation of peptides was developed.

The method described allows the specific methylation of the sulfonamide nitrogen atom of *N*-nosyl peptides and, after removal of the nosyl group, to elongate easily the *N*-methylated peptide chain in order to obtain stereochemically pure peptides *N*-methylated on specific amide bonds.

The highly efficient coupling, the chemospecific and quantitative *N*-methylation reaction and the rapid and complete deprotection step allowed us to obtain *N*-methylated peptides with high yields and excellent purity without time-consuming chromatographic workup.

In addition, the elongation of peptide chain carried out successfully using standard Fmoc SPS procedures to the position where *N*-methylation was desired, demonstrated that this methodology combines very well with solution peptide synthesis based on Fmoc-chemistry.

The adopted methodology represents a useful tool to label with a methyl group the nitrogen atom of every amino acid residue of biologically active peptide sequences for structure−activity studies.

The application on the solid phase of this new procedure for the *N*-methylation of amino acids and peptides is under investigation.

Experimental Section

Synthesis of *N*-Methyl-*N*-nosyldipeptides 5a−9a and *N*-Methyl-*N*-nosyltripeptide 11a. General Procedure A. A 0.66 M solution of diazomethane in diethyl ether (8 mmol) was added cautiously dropwise to a magnetically stirred solution of the *N*-nosyldipeptides methyl esters **5**−**9** (1 mmol) or *N*-nosyltripeptide methyl ester **11** in dry dichloromethane (10 mL). The resulting mixture was maintained at room temperature and under N_2 for 30 min. TLC analysis (chloroform/methanol, 98:2 v/v) showed complete conversion of the precursor. Evaporation of the solvent under reduced pressure afforded the *N*-methyl-*N*-nosyldipeptides methyl esters **5a**−**9a** or *N*-methyl-*N*-nosyltripeptide methyl ester **11a** in quantitative yields.

N-Methyl-*N*-nosyl-L-phenylalanyl-L-alanine Methyl Ester (5a). The product was prepared by general procedure A using 0.059 g (0.135 mmol) of **5** in dry dichloromethane and 1.64 mL (1.08 mmol) of 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding *N*-methyl dipeptide **5a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, 2 H, J = 8.9 Hz), 7.48 (d, 2 H, J = 8.9 Hz), 6.98−7.20 (m, 5 H), 6.83 (d, 1 H, J = 7.0 Hz), 4.75 (dd, 1 H, J = 10.3, 5.3 Hz), 4.52 (m, 1 H), 3.75 (s, 3 H), 3.21 (dd, 1 H, J = 14.5, 5.2 Hz), 2.95 (s, 3 H), 2.82 (dd, 1 H, J = 14.6, 10.3 Hz), 1.42 (d, 3 H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 168.5, 149.7, 144.5, 136.7, 129.0, 128.8, 128.00, 127.00, 124.2, 61.6, 52.7, 48.4, 34.0, 30.0, 18.1. MS (EI) *m/z* (rel intensity %) 390 (M^+ − $COOCH_3$, 1), 358 (1), 319 (100), 303 (2), 263 (21), 233 (21), 186 (4), 132 (45), 122 (18), 91 (36), 76 (9), 59 (5). Anal. Calcd. For $C_{20}H_{23}N_3O_7S$: C, 53.44; H, 5.16; N, 9.35; S, 7.13. Found: C, 53.46; H, 5.15; N, 9.34; S, 7.11.

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N-Methyl-*N*-nosyl-L-isoleucyl-D-alanyl-L-valine Methyl Ester (11a). The product was prepared by general procedure A using 0.50 g (1.01 mmol) of **11** in dry dichloromethane and 12 mL (8.01 mmol) of 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding *N*-methyl tripeptide **11a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, 2 H, J = 8.9 Hz), 7.95 (d, 2 H, J = 8.9 Hz), 6.82 (d, 1 H, J = 6.5 Hz), 6.68 (d, 1 H, J = 8.1 Hz), 4.55 (dd, 1 H, J = 8.6, 4.9 Hz), 4.35 (m, 1 H), 4.06 (d, 1 H, J = 6.7 Hz), 3.75 (s, 3 H), 2.93 (s, 3 H), 2.17 (m, 1 H), 1.95 (m, 1 H), 1.47−1.21 (m, 2 H), 1.34 (d, 3 H, J = 6.9 Hz), 0.96−0.82 (m, 12 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 171.5, 168.4, 149.9, 144.6, 128.5, 124.3, 64.0, 57.7, 52.4, 48.4, 33.0, 31.3, 30.2, 24.8, 19.3, 19.1, 18.9, 17.7, 10.5. MS (EI) *m/z* (rel intensity %) 514 ($M^{+\bullet}$, 1), 384 (1), 356 (2), 328 (33), 285 (100), 270 (7), 255 (12), 229 (40), 186 (9), 122 (6). Anal. Calcd. For $C_{23}H_{36}N_4O_8S$: C, 52.26; H, 6.86; N, 10.60; S, 6.07. Found: C, 52.24; H, 6.88; N, 10.63; S, 6.04.

Synthesis of *N*-Nosyltripeptides 12−14, *N*-Fmoc-tripeptides 16−18, and *N*-Fmoc-tetrapeptide 19. General Procedure B. Mercaptoacetic acid (3 mmol) was added to a solution of **5a**−**9a** or **11a** (1 mmol) in dry acetonitrile (10 mL) under N_2 at 50 °C. Solid 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 ∼1 h monitoring the conversion of **5a**−**9a** or **11a** by TLC (diethyl ether/petroleum ether, 60:40 v/v). Aqueous HCl 1 N 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 *N*-deprotected products, were then treated with a solution of *N*-nosyl-L-valine chloride **3b** (1 mmol) or *N*-Fmoc amino acid chlorides **15a**−**b** 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 with Na_2SO_4 and the solvents evaporated under vacuum to afford the corresponding *N*-Nosyl-tripeptides **12**−**14** in 72−88% yields, the *N*-Fmoc-tripeptides **16**−**18** in 92−94%, and the *N*-Fmoc-tetrapeptide **19** in 83% yield.

N-Nosyl-L-valyl-*N*-methyl-L-phenylalanyl-L-alanine Methyl Ester (12). The product was prepared by general procedure B using 0.15 g (0.33 mmol) of **5a** in dry acetonitrile (10 mL), 0.069 mL (1.00 mmol) of mercaptoacetic acid, and 0.14 g (2.67 mmol) of sodium methoxide in methanol (2 mL). The reaction was stirred at 50 °C for 50 min. The afforded unmasked dipeptide in an aqueous 9% solution of $NaHCO_3$ was treated with 0.11 g (0.33 mmol) of **3b** in dry methylene chloride. The reaction was stirred at RT for 1 h. The subsequent work up afforded 0.156 g of the title compound **12** (0.28 mmol, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37−8.24 (m, 2 H), 8.07−7.95 (m, 2 H), 7.31−7.10 (m, 5 H), 7.10 (d, 1 H, J = 8.1 Hz), 6.48 (d, 1 H, J = 7.8 Hz), 5.16 (dd, 1 H, J = 8.7, 6.6 Hz), 4.41 (m, 1 H), 3.77 (m, 1 H), 3.66 (s, 3 H), 3.21 (dd, 1 H, J = 13.9, 8.7 Hz), 2.96 (s, 3 H), 2.50 ((dd, 1 H, J = 13.9, 6.6 Hz), 2.03 (m, 1 H), 1.23 (d, 3 H, J = 7.2 Hz), 1.03 (d, 3 H, J = 6.7 Hz), 0.87 (d, 3 H, J = 6.7 Hz).^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 171.4, 168.5, 149.8, 146.1, 136.1, 129.7, 128.6, 128.7, 128.6, 124.1, 58.5, 57.8, 52.2, 47.8, 33.7, 31.1, 30.7, 19.5, 17.1, 16.6. FAB^+ MS *m/z* 549 $(M + H)^+$. Anal. Calcd. For $C_{25}H_{32}N_4O_8S$: C, 54.73; H, 5.88; N, 10.21; S, 5.84. Found: C, 54.70; H, 5.89; N, 10.23; S, 5.85.

Synthesis of N-Fmoc-L-alanyl-*N*-methyl-D-phenylalanyl-L-alanine Methyl Ester (16). The product was prepared by general procedure B using 0.20 g (0.45 mmol) of **6a** in dry acetonitrile (10 mL), 0.09 mL (1.34 mmol) of mercaptoacetic acid, and 0.19 g (3.56 mmol) of sodium methoxide in methanol (5 mL). The reaction was stirred at 50 °C for 40 min. The afforded unmasked dipeptide in an aqueous 9% solution of $NaHCO_3$ was treated with 0.14 g (0.45 mmol) of **15a** in dry methylene chloride. The reaction was stirred at RT for 45 min. The subsequent work up afforded 0.22 g of the title compound **16** (0.39 mmol, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81−7.15 (m, 13 H), 6.80 (d, 1 H, J = 7.4 Hz), 5.66 (dd, 1 H, J = 11.2, 5.6 Hz), 5.60 (d, 1 H, J = 6.8 Hz), 4.69−4.43 (m, 2 H), 4.36−4.17 (m, 3 H), 3.76 (s, 3 H), 3.46 (dd, 1 H, J = 14.9, 5.6 Hz), 2.99 (s, 3 H), 2.95 (dd, 1 H, J = 14.9, 11.2 Hz), 1.42 (d, 3 H, J = 7.2 Hz), 1.35 (d, 3 H, J = 7.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.1, 172.5, 171.1, 156.4, 143.0, 141.4, 129.2, 128.9, 128.2, 127.7, 127.4, 127.1, 125.1, 120.0, 58.3, 56.5, 51.7, 51.0, 49.9, 38.9, 35.1, 31.7, 18.9, 18.2. FAB^+ MS *m/z* 558 $(M+H)^+$. Anal. Calcd. For $C_{32}H_{35}N_3O_6$: C, 68.92; H, 6.33; N, 7.54. Found: C, 68.94; H, 6.31; N, 7.52.

Synthesis of *N*-Fmoc-L-valyl-*N*-methyl-l-isoleucyl-D-alanyl-L-valine Methyl Ester (19). The product was prepared by general procedure B using 0.069 g (0.13 mmol) of **11a** in dry acetonitrile (10 mL), 0.028 mL (0.40 mmol) of mercaptoacetic acid, and 0.058 g (1.07 mmol) of sodium methoxide in methanol (2 mL). The reaction was stirred at 50 °C for 1 h. The afforded unmasked dipeptide in an aqueous 9% solution of $NaHCO_3$ was treated with 0.048 g (0.13 mmol) of **15b** in dry methylene chloride. The reaction was stirred at RT for 1h. The subsequent work up afforded 0.073 g of the title compound **19** (0.11 mmol, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85−7.26 (m, 8 H), 6.94 (d, 1 H, J = 8.5 Hz), 6.80 (d, 1 H, J = 8.2 Hz), 5.78 (d, 1 H, J = 8.7 Hz), 4.55−4.18 (m, 6 H), 3.71 (s, 3 H), 3.11 (s, 3 H), 1.98−2.28 (m, 3 H), 1.42−1.31 (m, 2 H), 1.36 (d, 3 H, J = 7.1 Hz), 1.04 (d, 3 H, J = 6.9 Hz), 0.96−0.82 (m, 15 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4, 172.1, 171.4, 170.1, 156.5, 143.8, 141.3, 128.1, 127.8, 127.3, 125.2, 67.0, 57.0, 56.0, 52.0, 48.6, 47.2, 46.4, 31.4, 31.3, 31.0, 30.8, 24.4, 19.5, 18.9, 17.7, 17.4, 17.3, 15.7, 10.5. MS *m/z* (%) 673.0778 [$(M + Na)^+$, 100] 689.0432 [$(M+K)^+$, 54]. Anal. Calcd. For $C_{36}H_{50}N_4O_7$: C, 66.44; H, 7.74; N, 8.61. Found: C, 66.44; H, 7.74; N, 8.61.

Synthesis of Dimethylated *N*-Nosyltripeptides 12a−14a. General Procedure A.

N-Methyl-*N*-nosyl-L-valyl-*N*-methyl-L-phenylalanyl-L-alanine Methyl Ester (12a). The product was prepared by general procedure A using 0.069 g (0.13 mmol) of **12** in dry dichloromethane and 1.57 mL (1.04 mmol) of 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding dimethylated tripeptide **12a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40−8.32 (m, 2 H), 8.06−7.88 (m, 2 H), 7.40−7.10 (m, 5 H), 6.47 (d, 1 H, J = 8.1 Hz), 5.38 (m, 1 H), 4.52 (m, 1 H), 4.48 (m, 1 H), 3.68 (s, 3 H), 3.38 (dd, 1 H, J = 14.3, 7.6 Hz), 3.19 (s, 3 H), 2.96 (dd, 1 H, J = 14.1, 5.2 Hz), 2.87 (s, 3 H), 2.30 (m, 1 H), 1.34 (d, 3 H, J = 7.2 Hz), 0.95 (d, 3 H, J = 6.7 Hz), 0.77? (d, 3 H, J = 6.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 171.3, 169.0, 149.1, 145.3, 136.6, 128.9, 128.5, 127.7, 126.7, 123.5, 60.4, 57.2, 52.3, 48.0, 33.7, 31.6, 29.8, 28.3, 19.5, 18.7, 17.9. FAB^+ MS *m/z* 563 $(M + H)^+$. Anal. Calcd. For $C_{26}H_{34}N_4O_8S$: C, 55.50; H, 6.09; N, 9.96; S, 5.70. Found: C, 55.52; H, 6.07; N, 9.96; S, 5.71.

Supporting Information Available: Experimental details for the synthesis of compounds **2a**−**g**, **5**−**9**, **11**, **6a**−**9a**, **13**−**14**, **17**−**18,** and **13a**−**14a**. GC/MS analyses and ^{1}H NMR spectra of dipeptides **5a** and **6a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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