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

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SUPPORTING INFORMATION

General Experimental Methods

Solvents were purified and dried by standard procedures and distilled prior to use. Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz respectively and using $CDCl_3$ or [d_6]DMSO as solvents. Chemical shifts are reported in units of parts per million and all coupling constants are reported in Hertz.

GC/MS analyses were performed using an HP-5MS (30 m × 0.25 mm, PhMesiloxane 5 %) capillary column 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. The MALDI mass spectrum was acquired on a 4700 proteomics analyzer mass spectrometer equipped with 200 Hz, Nd: YAG laser at 355-nm wavelength. The MS spectrum was acquired in reflectron mode (20 KeV accelerating voltage), with 400ns delayed extraction, averaging 2000 laser shots. α-cyano-4-hydroxycinnamic acid (HCCA) was used as matrix. 0.45 μl of a premixed solution of HCCA and sample (800:1) dissolved in MeOH/$H_2$0 (1:1) were spotted on the matrix target, dried at room temperature and analysed into the mass spectrometer.

The progress of all reactions was monitored by thin-layer chromatography using silica gel 60-F_{254} precoated glass plates. 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 using a classical procedure.[13,15] The concentration of the diazomethane solution (0.66 M) was obtained by a back-titration performed with a standard benzoic acid solution.[13,15] Caution: diazomethane is highly

toxic. Hence, this reagent must be handled carefully. Diethyl ether solutions of diazomethane are stable for long periods if stored on KOH pellets at –20°C.

Synthesis of *N*-Nosyl amino acids 2a-g. General Procedure C. The α-amino acids **1a-g** (1 mmol) were dissolved in a 1 N NaOH solution and cooled to 0° C. *p*-Nitrobenzenesulfonylchloride (1.5-2 mmol) was added slowly and the homogenous reaction mixture, maintained at a pH value of 9, was stirred for 2-3 h, monitoring the conversion of **1a-g** by TLC (chloroform/methanol 90:10 v/v). The reaction mixture was extracted with ethyl acetate (3 × 10 mL). The aqueous phase was acidified with a 1 N HCl solution (pH= 2) and extracted with ethyl acetate. The organic layer was washed with water and brine and then dried with Na_2SO_4. The solvent was evaporated to afford the corresponding *N*-Nosyl amino acids **2a-g** as pale yellow solids in 82-95 % overall yields.

***N*-Nosyl-L-Isoleucine (2a).** The product was prepared by General Procedure C using 0.50 g (3.81 mmol) L-Isoleucine (**1a**) in 1 N NaOH solution (4 ml) and 1.27 g (5.73 mmol) *p*-nitrobenzensolfonyl chloride. The reaction was stirred at RT for 3 h. The subsequent work up afforded 1.01 g of the title compound (3.19 mmol, 84 %) as a pale yellow solid: mp 128-130°C; IR (KBr) 3120, 2965, 1721, 1665, 1538, 1361, 1065, 860,747 cm^{-1}. ^1H NMR (300 MHz, [d_6]DMSO) δ 12.75 (s br, 1 H), 8.50 (d, 1 H, *J*= 9.3 Hz), 8.40 (d, 2 H, *J*= 8.7 Hz), 8.01 (d, 2 H, *J*= 8.7 Hz), 3.64 (dd, 1 H, *J*= 9.1, 6.3 Hz), 1.75 (m, 1 H), 1.35 (m, 1 H), 1.15 (m, 1 H), 0.91-0.73 (m, 6 H). Anal. Calcd. For $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86; S, 10.14. Found: C, 45.58; H, 5.08; N, 8.89; S, 10.12.

***N*-Nosyl-L-Valine (2b).** The product was prepared by General Procedure C using 0.50 g (4.27 mmol) L-Valine (**1b**) in 1 N NaOH solution (4 ml) and 1.42 g (6.41 mmol) *p*-nitrobenzensolfonyl chloride. The reaction was stirred at RT for 2 h. The subsequent work up afforded 1.07 g of the title compound (3.54 mmol, 83 %) as a pale yellow solid: mp 185-187 °C; IR (KBr) 3120, 2965, 1721, 1665, 1538, 1361, 1065, 860, 747 cm^{-1}. ^1H NMR (300 MHz, [d_6]DMSO) δ 12.70 (s br, 1 H), 8.49 (d, 1 H, *J*= 9.3 Hz), 8.38 (d, 2 H, *J*=8.1 Hz), 8.03 (d, 2 H, *J*=8.1 Hz), 3.60 (dd, 1 H, *J*=8.4, 6.0 Hz),

2.10-1.89 (m, 1 H), 0.83 (d, 3 H, *J*=6.7 Hz), 0.78 (d, 3 H, *J*=6.7 Hz). Anal. Calcd. For $C_{11}H_{14}N_2O_6S$: C, 43.70; H, 4.67; N, 9.27; S, 10.61. Found: C, 43.68; H, 4.69; N, 9.30; S, 10.61.

***N*-Nosyl-L-Phenylalanine (2c).** The product was prepared by General Procedure C using 0.50 g (3.03 mmol) L-Phenylalanine (**1c**) in 1 N NaOH solution (5 ml) and 1.01 g (4.51 mmol) *p*-nitrobenzensolfonyl chloride. The reaction was stirred at RT for 3 h. The subsequent work up afforded 1.01 g of the title compound (2.88 mmol, 95 %) as a pale yellow solid: mp 163-165 °C; IR (KBr) 3110, 2970, 2960, 1760, 1665, 1532, 1361, 1064, 850, 747 cm^{-1}. ^1H NMR (300 MHz, [d_6]DMSO) δ 12.75 (s br, 1 H), 8.76 (d, 1 H, *J*=9.0 Hz), 8.13-8.26 (m, 2 H), 7.70-7.60 (m, 2 H), 7.05-7.25 (m, 5 H), 3.95 (m, 1 H), 2.97 (dd, 1 H, *J*=13.7, 10.1 Hz), 2.69 (dd, 1 H, *J*=13.7, 4.1 Hz). Anal. Calcd. For $C_{15}H_{14}N_2O_6S$: C, 51.42; H, 4.03; N, 8.00; S, 9.15. Found: C, 51.46; H, 4.02; N, 7.97; S, 9.13.

***N*-Nosyl-D-Phenylalanine (2d).** The product was prepared by General Procedure C using 0.63 g (3.81 mmol) D-Phenylalanine (**1d**) in 1 N NaOH solution (5 ml) and 1.70 g (7.67 mmol) *p*-nitrobenzensolfonyl chloride. The reaction was stirred at RT for 3 h. The subsequent work up afforded 1.24 g of the title compound (3.54 mmol, 93 %) as a pale yellow solid: mp 163-165 °C; IR 3110, 2970, 2960, 1760, 1665, 1532, 1361, 1064, 850, 747 cm^{-1}. ^1H NMR (300 MHz, [d_6]DMSO) δ 12.75 (s br, 1 H), 8.76 (d, 1 H, *J*=9.0 Hz), 8.26-8.13 (m, 2 H), 7.60-7.70 (m, 2 H), 7.25-7.05 (m, 5 H), 3.95 (m, 1 H), 2.97 (dd, 1 H, *J*=13.7, 10.1 Hz), 2.69 (dd, 1 H, *J*=13.7, 4.1 Hz). Anal. Calcd. For $C_{15}H_{14}N_2O_6S$: C, 51.42; H, 4.03; N, 8.00; S, 9.15. Found: C, 51.46; H, 4.02; N, 7.97; S, 9.13.

***N*-Nosyl-L-Alanine (2e).** The product was prepared by General Procedure A using 1.00 g (11.23 mmol) L-Alanine (**1e**) in 1 N NaOH solution (10 ml) and 3.73 g (16.84 mmol) *p*-nitrobenzensolfonyl chloride. The reaction was stirred at RT for 3 h. The subsequent work up afforded 2.58 g of the title compound (9.41 mmol, 84 %) as a pale yellow solid: mp 140-142 °C; IR 3110, 2972, 2962, 1720, 1665, 1532, 1361, 1064, 870, 747 cm^{-1}. ^1H NMR (300 MHz, [d_6]DMSO) δ 12,70 (s br, 1 H), 8.60 (d, 1 H, *J*=8,1 Hz), 8.40-8.20 (m, 2H), 8.10-7.80 (m, 2H), 3.91-3.70 (m, 1

H), 1.10 (d, 3 H, *J*=7,1 Hz). Anal. Calcd. For $C_9H_{10}N_2O_6S$: C, 39.42; H, 3.68; N, 10.21; S, 11.69. Found: C, 39.46; H, 3.69; N, 10.19; S, 11.66.

***N*-Nosyl-D-Alanine (2f).** The product was prepared by General Procedure C using 1.00 g (11.23 mmol) D-Alanine (**1f**) in 1 N NaOH solution (10 ml) and 3.73 g (16.84 mmol) *p*-nitrobenzensolfonyl chloride. The reaction was stirred at RT for 3 h. The subsequent work up afforded 2.54 g of the title compound (9.27 mmol, 82%) as a pale yellow solid: mp 140-142 °C; IR (KBr) 3110, 2972, 2962, 1720, 1665, 1532, 1361, 1064, 870, 747 cm^{-1}. ^1H NMR (300 MHz, [d_6]DMSO) δ 12.72 (s br, 1 H), 8.60 (d, 1 H, *J*=8.1 Hz), 8.42-8.37 (m, 2 H), 8.07-8.01 (m, 2 H), 3.92-3.81 (m, 1 H), 1.20 (d, 3 H, *J*=7.2 Hz). Anal. Calcd. For $C_9H_{10}N_2O_6S$: C, 39.42; H, 3.68; N, 10.21; S, 11.69. Found: C, 39.46; H, 3.69; N, 10.19; S, 11.66.

***N*-Nosyl-L-Leucine (2g).** The product was prepared by General Procedure C using 1.00 g (7.63 mmol) L-Leucine (**1g**) in 1 N NaOH solution (10 ml) and 2.53 g (11.42 mmol) *p*-nitrobenzensolfonyl chloride. The reaction was stirred at RT for 3 h. The subsequent work up afforded 2.03 g of the title compound (6.42 mmol, 84 %) as a pale yellow solid: mp 134-136°C; IR (KBr) 3120, 2965, 1721, 1665, 1538, 1361, 1065, 860, 747 cm^{-1}. ^1H NMR (300 MHz, [d_6]DMSO) δ 12.69 (s br, 1 H), 8.58 (d, 1 H, *J*=9 Hz), 8.40-8.32 (m, 2 H), 8.10-7.95 (m, 2 H), 3.80-3.70 (m, 1 H), 1.62-1.52 (m, 1 H), 1.45-1.38 (m, 2 H), 0.81(d, 3H, *J*=6.6 Hz), 0.72 (d, 3 H, *J*=6.5 Hz). Anal. Calcd. For $C_{12}H_{16}N_2O_6S$: C, 45.56; H, 5.10; N, 8.86; S, 10.14. Found: C, 45.59; H, 5.07; N, 8.82; S, 10.16.

Synthesis of dipeptides 5-9 and tripeptide 11 . General Procedure D. Tionyl chloride (12 mmol) was added to a magnetically stirred dry dichloromethane solution of the *N*-Nosyl amino acids **2a-g** (1 mmol). The resulting reaction mixture was maintained under N_2 and refluxed for 30 minutes. TLC analysis (chloroform/methanol, 90:10 v/v) showed complete conversion of the precursor. Evaporation of the solvent under reduced pressure afforded the *N*-Nosyl amino acid chlorides **3a-g** as yellowish solids in quantitative yields. The *N*-Nosyl amino acid chlorides **3a-g** (1 mmol) were immediately dissolved in ethanol-free chloroform and treated with a 9% $NaHCO_3$ solution of the

amino acid methyl ester hydrochlorides **4a-b** (1 mmol) or D-Alanyl-L-Valine methyl ester hydrochloride (**10**) (1 mmol). The reaction was stirred at room temperature for ~ 1 h and then the organic layer was separated. The aqueous phase was extracted with three additional portions of dichloromethane (3 ×10 mL). The combined organic extracts were washed with a 1N HCl solution and brine and then dried with Na_2SO_4. The solvent was evaporated under vacuum to afford the corresponding *N*-Nosyl dipeptides **5-9** in 74-94% overall yields and the N-Nosyl tripeptide **11** in 78% yield.

***N*-Nosyl-L-Phenylalanyl-L-Alanine methyl ester (5).** The product was prepared by General Procedure D using 0.70 g (1.90 mmol) *N*-Nosyl-L-Phenylalanine chloride (**3c**) and 0.26 g (1.90 mmol) L-Alanine methyl ester hydrochloride (**4a**). The reaction was stirred at RT for 1 h. The subsequent work up afforded 0.78 g of the title compound (1.79 mmol, 94%): mp 169-171°C; IR (KBr) 3351, 3119, 2976, 1729, 1645, 1536, 1352, 1042, 856, 800, 742 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8,15 (d, 2 H, *J*=6.9), 7.75 (d, 2 H, *J*=6.9), 7.20-7.01 (m, 5 H), 6.75 (d, 1 H, *J*=7.0 Hz), 6.01 (d, 1 H, *J*=8.2 Hz), 4.48 (m, 1 H), 4.03 (m, 1 H), 3.73 (s, 3 H), 3.07 (dd, 1 H, *J*=14.0, 5.4 Hz), 2.90 (dd, 1 H, *J*=14.0 *J*=8.6 Hz), 1.31 (d, 3 H, *J*=7.15 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 169.6, 149.9, 145.1, 135.3, 129.2, 128.9, 128.2, 127.4, 124.2, 58.4, 52.7, 48.4, 38.9, 18.2. MS (EI) m/z (rel intensity %) 376 (M^+ - $COOCH_3^{\cdot}$, 5), 344 (9), 305 (100), 233 (49), 186 (29), 156 (31), 122 (43), 92 (38), 91 (68), 76 (15), 65 (14); Anal. Calcd. For $C_{19}H_{21}N_3O_7S$: C, 52.41; H, 4.86; N, 9.65; S, 7.36. Found: C, 52.43; H, 4.85; N, 9.67; S, 7.35.

***N*-Nosyl-D-Phenylalanyl-L-Alanine methyl ester (6).** The product was prepared by General Procedure D using 1.50 g (4.07 mmol) *N*-Nosyl-D-Phenylalanine chloride (**3d**) and 0.57 g (4.08 mmol) L-Alanine methyl ester hydrochloride (**4a**). The reaction was stirred at RT for 40 min. The subsequent work up afforded 1.52 g of the title compound (3.49 mmol, 85%): mp 168-171°C; IR 3352, 3119, 2976, 1729, 1645, 1536, 1352, 1042, 856, 800, 742 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, 2 H, *J* = 8.6 Hz), 7.78 (d, 2 H, *J* = 8.6 Hz), 7.20-6.95 (m, 5 H), 6.62 (d, 1 H, *J* = 7.5 Hz), 6.02 (d, 1 H, *J* = 8.2 Hz), 4.40 (m, 1 H), 3.98 (m, 1 H), 3.72 (s, 3 H), 3.05 (dd, 1 H, *J* = 13.9, 6.4

Hz), 2.92 (dd, 1 H, *J* = 13.9, 8.4 Hz), 1.28 (d, 3 H, *J* =7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0, 169.4, 149.9, 144.8, 135.4, 129.2, 128.9, 128.2, 127.4, 124.2, 67.1, 58.5, 52.7, 48.1, 39.3. MS (EI) m/z (rel intensity %) 376 (M^+ - $COOCH_3^{\cdot}$, 5), 344 (9), 305 (100), 233 (49), 186 (29), 156 (31), 122 (43), 92 (38), 91 (68), 76 (15), 65 (14); Anal. Calcd. For $C_{19}H_{21}N_3O_7S$: C, 52.41; H, 4.86; N, 9.65; S, 7.36. Found: C, 52.43; H, 4.85; N, 9.67; S, 7.35.

***N*-Nosyl-L-Isoleucyl-L-Alanine methyl ester (7).** The product was prepared by General Procedure D using 0.35 g (1.04 mmol) *N*-Nosyl-L-Isoleucine chloride (**3a**). and 0.14 g (1.04 mmol) L-Alanine methyl ester hydrochloride (**4a**). The reaction was stirred at RT for 45 min. The subsequent work up afforded 0.39 g of the title compound (0.98 mmol, 94%): mp 168-171°C; IR (KBr) 3347, 3119, 2968, 1732, 1645, 1536, 1348, 1040, 857, 803, 742 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (d, 2 H, *J* = 8.6 Hz), 8.03 (d, 2 H, *J* = 8.6 Hz), 6.20 (d, 1 H, *J* = 7.2 Hz), 5.68 (d, 1 H, *J* = 9.1 Hz), 4.35 (m, 1 H), 3.71 (s, 3 H), 3.62 (dd, 1 H, *J* = 9.1, 5.7 Hz), 2.52-1.40 (m, 3 H), 1.38 (d, 3 H, *J* = 7.1 Hz), 0.90-0.85 (m, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 169.3, 150.1, 145.8, 128.6, 124.2, 61.5, 52.7, 48.1, 38.5, 24.3, 18.1, 15.4, 11.2. MS (EI) m/z (rel intensity %) 342 (M^+ - $COOCH_3^{\cdot}$, 2), 314 (1), 271 (100), 255 (2), 241 (10), 229 (3), 215 (70), 186 (28), 122 (25), 92 (11). Anal. Calcd. For $C_{16}H_{23}N_3O_7S$: C, 47.87; H, 5.77; N, 10.47; S, 7.99. Found: C, 47.85; H, 5.79; N, 10.50; S, 7.98.

***N*-Nosyl-D-Alanyl-L-Valine methyl ester (8).** The product was prepared by General Procedure D using 1.83 g (6.26 mmol) *N*-Nosyl-D-Alanine chloride (**3f**). and 1.05 g (6.26 mmol) L-Valine methyl ester hydrochloride (**4b**). The reaction was stirred at RT for 1 h. The subsequent work up afforded 1.79 g of the title compound (4.63 mmol, 74%): mp 124-126°C; IR (KBr) 3350, 3119, 2976, 1730, 1645, 1536, 1352, 1042, 856, 742 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (d, 2 H, *J* = 9.2 Hz), 8.04 (d, 2 H, *J* = 8.8 Hz), 6.42 (d, 1 H, *J*=8.8 Hz), 5.85 (d, 1 H, *J* = 6.7 Hz), 4.33 (dd, 1 H, *J*=8.8, 4.7 Hz), 3.96 (m, 1 H), 3.73 (s, 3 H), 2.13 (m, 1 H), 1.37 (d, 3 H, *J* = 7.0 Hz), 0.87 (d, 3 H, *J* = 6.8 Hz), 0.83 (d, 3 H, *J* = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 170.8, 150.1, 145.4, 128.5, 124.4, 56.9, 52.6, 52.5, 31.3, 20.3, 18.9, 17.5. MS (EI) m/z (rel intensity %) 328 (M^+ - $COOCH_3^{\cdot}$, 13), 274 (7), 229 (100), 213 (4), 186 (34), 158 (9), 130 (25), 122 (36), 72 (30). Anal.

Calcd. For $C_{15}H_{21}N_3O_7S$: C, 46.50; H, 5.46; N, 10.85; S, 8.28. Found: C, 46.47; H, 5.47; N, 10.87; S, 8.29.

***N*-Nosyl-L-Leucyl-L-Alanine methyl ester (9).** The product was prepared by General Procedure D using 2.08 g (6.23 mmol) *N*-Nosyl-L-Leucine chloride (**3g**). and 0.87 g (6.23 mmol) L-Alanine methyl ester hydrochloride (**4a**). The reaction was stirred at RT for 50 min. The subsequent work up afforded 1.90 g of the title compound (4.73 mmol, 76%): mp 153-155°C; IR (KBr) 3351, 3118, 2976, 1729, 1645, 1536, 1352, 1042, 856, 742 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, 2 H, *J* = 8.7 Hz), 8.08 (d, 2 H, *J* = 8.7 Hz), 6.46 (d, 1 H, *J* = 7.2 Hz), 5.96 (d, 1 H, *J* = 8.9 Hz), 4.36 (m, 1 H), 3.85 (m, 1 H), 3.75 (s, 3 H), 1.73 (m, 1 H), 1.54-1.47 (m, 2 H), 1.19 (d, 3 H, *J* = 7.1 Hz), 0.91 (d, 3 H, *J* = 6.5 Hz), 0.85 (d, 3 H, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9, 170.6, 150.1, 145.7, 128.6, 124.3, 55.5, 52.8, 48.1, 42.5, 24.3, 22.9, 21.3, 18.2. MS (EI) m/z (rel intensity %) 342 (M^+ - $COOCH_3^{\cdot}$, 2), 271 (100), 255 (2), 229 (18), 215 (43), 186 (36), 159 (19), 122 (29). Anal. Calcd. For $C_{16}H_{23}N_3O_7S$: C, 47.87; H, 5.77; N, 10.47; S, 7.99. Found: C, 47.90; H, 5.75; N, 10.46; S, 7.98.

***N*-Nosyl-L-Isoleucyl-D-Alanyl-L-Valine methyl ester (11).** The product was prepared by General Procedure D using 0.96 g (2.86 mmol) *N*-Nosyl-L-Isoleucine chloride (**3a**). and 0.68 g (2.86 mmol) D-Alanyl-L-Valine methyl ester hydrochloride (**10**). The reaction was stirred at RT for 1 h. The subsequent work up afforded 1.12 g of the title compound (2.24 mmol, 78%): mp 208-212 °C; IR (KBr) 3378, 1637, 1545, 1361, 1352, 858, 800, 741 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, 2 H, *J* = 8.6 Hz), 8.05 (d, 2 H, *J* = 8.6 Hz), 7.51 (d, 1 H, *J* = 8.4 Hz), 7.06 (d, 1 H, *J* = 7.8 Hz), 6.74 (d, 1 H, *J* = 9.0 Hz), 4.69-4.55 (m, 2 H), 3.86 (s, 3 H), 3.70 (m, 1 H), 2.18 (m, 1 H), 1.57-1.78 (m, 3 H), 1.27 (d, 3 H, *J* = 6.8 Hz), 0.94-0.82 (m, 12 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.5, 171.6, 169.8, 149.7, 146.3, 127.8, 124.1, 61.6, 56.8, 52.8, 48.4, 38.4, 31.7, 24.9, 19.1, 18.8, 17.6, 15.1, 11.1. MS (EI) m/z (rel intensity %) 500 ($M^{+\cdot}$, 1), 430 (18), 383 (9), 342 (16), 327 (16), 314 (9), 297 (8), 271 (100), 253 (23), 186 (25), 157 (13), 122 (21), 76 (14). Anal. Calcd. For $C_{22}H_{34}N_4O_8S$: C, 51.35; H, 6.66; N, 10.89; S, 6.23. Found: C, 51.37; H, 6.64; N, 10.90; S, 6.23.

Synthesis of *N*-methyl-*N*-nosyl-dipeptides 6a-9a.

***N*-Methyl *N*-Nosyl-D-Phenylalanyl-L-Alanine methyl ester (6a).** The product was prepared by General Procedure A using 0.072 g (0.165 mmol) **6** in dry dichloromethane and 2 mL (1.32 mmol) 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding *N*-methyl dipeptide **6a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, 2 H, *J* = 8.9 Hz), 7.51 (d, 2 H, *J* = 8.6 Hz), 7.20-6.98 (m, 5 H), 6.70 (d, 1 H, *J* = 7.3 Hz), 4.78 (dd, 1 H, *J* = 9.7, 5.9 Hz), 4.49 (m, 1 H), 3.75 (s, 3 H), 3.29 (dd, 1 H, *J* = 14.4, 5.9 Hz), 2.98 (s, 3 H), 2.80 (dd, 1 H, *J* = 14.4, 9.7 Hz), 1.36 (d, 3 H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9, 168.6, 149.7, 144.3, 136.9, 129.1, 128.8, 128.1, 127.00, 124.2, 61.8, 52.6, 48.2, 34.3, 30.2, 17.8. MS (EI) m/z (rel intensity %) 390 (M^+ - $COOCH_3^{\cdot}$, 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.

***N*-Methyl-*N*-Nosyl-L-Isoleucyl-L-Alanine methyl ester (7a).** The product was prepared by General Procedure A using 0.2 g (0.49 mmol) **7** in dry dichloromethane and 6.03 mL (3.98 mmol) 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding *N*-methyl dipeptide **7a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, 2 H, *J* = 8.6 Hz), 7.76 (d, 2 H, *J* = 8.6 Hz), 6.28 (d, 1 H, *J* = 7.3 Hz), 4.35 (m, 1 H), 4.23 (m, 1 H), 3.71 (s, 3 H), 2. 96 (s, 3 H), 2.52-1.42 (m, 3 H), 1.38 (d, 3 H, *J* = 7.1 Hz), 0.90-0.85 (m, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 170.1, 151.6, 145.8, 128.2, 124.4, 61.5, 52.5, 48.2, 36.5, 32.3, 24.7, 18.2, 14.9, 10.7. MS (EI) m/z (rel intensity %) 356 (M^+ - $COOCH_3^{\cdot}$, 1), 285 (100), 269 (2), 229 (54), 186 (11), 122 (10), 86 (9). Anal. Calcd. For $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; S, 7.72. Found: C, 49.13; H, 6.08; N, 10.12; S, 7.70.

***N*-Methyl-*N*-Nosyl-D-Alanyl-L-Valine methyl ester (8a).** The product was prepared by General Procedure A using 0.2 g (0.52 mmol) **8** in dry dichloromethane and 6.3 mL (4.13 mmol) 0.66 M

diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding *N*-methyl dipeptide **8a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (d, 2 H, *J* = 9.0 Hz), 8.03 (d, 2 H, *J* = 9.0 Hz), 6.78 (d, 1 H, *J* = 8.8 Hz), 4.64 (q, 1 H, *J* = 7.2 Hz), 4.46 (dd, 1 H, *J* = 8.8, 4.7 Hz), 3.76 (s, 3 H), 2.91 (s, 3 H), 2.22 (m, 1 H), 1.14 (d, 3 H, *J* = 7.2 Hz), 0.97 (d, 3 H, *J* = 6.8 Hz), 0.92 (d, 3 H, *J* = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0, 171.1, 151.6, 145.4, 128.2, 124.5, 56.7, 53.0, 52.7, 31.7, 30.7, 18.9, 18.5, 14.4. MS (EI) m/z (rel intensity %) 342 (M^+ - $COOCH_3^{\cdot}$, 1), 243 (100), 215 (17), 186 (8), 130 (4), 122 (21), 76 (8). Anal. Calcd. For $C_{16}H_{23}N_3O_7S$: C, 47.87; H, 5.77; N, 10.47; S, 7.99. Found: C, 47.89; H, 5.75; N, 10.48; S, 7.99.

***N*-Methyl-*N*-Nosyl-L-Leucyl-L-Alanine methyl ester (9a).** The product was prepared by General Procedure A using 0.15 g (0.37 mmol) **9** in dry dichloromethane and 4.53 mL (2.99 mmol) 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding *N*-methyl dipeptide **9a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, 2 H, *J* = 9.2 Hz), 8.02 (d, 2 H, *J* = 8.7 Hz), 6.48 (d, 1 H, *J* = 7.0 Hz), 4.51-4.40 (m, 2 H), 3.76 (s, 3 H), 2.90 (s, 3 H), 1.72 (m, 1 H), 1.52-1.30 (m , 2 H), 1.36 (d, 3 H, *J* = 7.2 Hz), 0.88 (d, 6 H, *J* = 6.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9, 169.1, 150.1, 144.9, 128.4, 124.3, 57.8, 52.6, 48.1, 37.3, 30.1, 24.6, 22.7, 21.8, 18.1. MS (EI) m/z (rel intensity %) 356 (M^+ - $COOCH_3^{\cdot}$, 1), 285 (100), 269 (2), 243 (14), 229 (23), 186 (15), 122 (11), 76 (6). Anal. Calcd. For $C_{17}H_{25}N_3O_7S$: C, 49.15; H, 6.07; N, 10.11; S, 7.72. Found: C, 49.17; H, 6.05; N, 10.10; S, 7.73.

Synthesis of *N*-Nosyl-tripeptides 13-14 and *N*-Fmoc-tripeptides 17-18

***N*-Nosyl-L-Valyl-*N*-methyl-D-Phenylalanyl-L-Alanine methyl ester (13).** The product was prepared by General Procedure B using 0.15 g (0.33 mmol) **6a** in dry acetonitrile (10 mL), 0.069 mL (1.00 mmol) mercaptoacetic acid and 0.14 g (2.67 mmol) sodium methoxide in methanol (2 mL). The reaction was stirred at 50 °C for 45 min. The afforded unmasked dipeptide in an aqueous

9% solution of $NaHCO_3$ was treated with 0.11 g (0.33 mmol) **3b** in dry methylene chloride. The reaction was stirred at RT for 50 min. The subsequent work up afforded 0.16 g of the title compound **13** (0.29 mmol, 88%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35-8.26 (m, 2 H), 8.05-7.97 (m, 2 H), 7.35-7.11 (m, 5 H), 7.08 (d, 1 H, *J* = 8.1 Hz), 6.44 (d, 1 H, *J* = 7.8 Hz), 5.18 (dd, 1 H, *J* = 8.7, 6.6 Hz), 4.38 (m, 1 H), 3.77 (m, 1 H), 3.68 (s, 3 H), 3.25 (dd, 1 H, *J* = 13.9, 8.7 Hz), 2.98 (s, 3 H), 2.53 ((dd, 1 H, *J* = 13.9, 6.6 Hz), 2.03 (m, 1 H), 1.25 (d, 3 H, *J* = 7.2 Hz), 1.01 (d, 3 H, *J* = 6.7 Hz), 0.85 (d, 3 H, *J* = 6.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 171.4, 168.6, 149.9, 146.3, 136.2, 129.9, 128.8, 128.7, 128.3, 124.1, 58.6, 57.6, 52.3, 47.9, 33.9, 31.1, 30.6, 19.6, 17.2, 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.

***N*-Nosyl-L-Valyl-*N*-methyl-L-Isoleucyl-L-Alanine methyl ester (14).** The product was prepared by General Procedure B using 0.20 g (0.48 mmol) **6a** in dry acetonitrile (10 mL), 0.1 mL (1.45 mmol) mercaptoacetic acid and 0.21 g (3.85 mmol) sodium methoxide in methanol (5 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 0.15 g (0.48 mmol) **3b** in dry methylene chloride. The reaction was stirred at RT for 45 min. The subsequent work up afforded 0.17 g of the title compound **14** (0.33 mmol, 72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, 2 H, *J* = 9.0 Hz), 7.92 (d, 2 H, *J* = 9.0 Hz), 7.08 (d, 1 H, *J* = 8.1 Hz), 6.51 (d, 1 H, *J* = 7.8 Hz), 4.62 (d, 1 H, *J* = 10.4 Hz), 4.50 (m, 1 H), 4.01 (m, 1 H), 3.65 (s, 3 H), 2.95 (s, 3 H), 2.19-2.05 (m, 2 H), 1.48-1.27 (m, 2 H), 1.28 (d, 3 H, *J* = 7.2 Hz), 0.90 (d, 3 H, *J* = 6.4 Hz), 0.82 (d, 3 H, *J* = 6.6 Hz), 0.81-0.72 (m, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 171.2, 169.4, 150.6, 145.8, 128.3, 124.1, 59.5, 57.5, 51.9, 48.2, 34.4, 31.9, 30.6, 25.0, 17.8, 17.7, 17.2, 15.0, 10.6. FAB^+ MS *m/z* 515 $(M+H)^+$. Anal. Calcd. For $C_{22}H_{34}N_4O_8S$: C, 51.35; H, 6.66; N, 10.89; S, 6.23. Found: C, 51.37; H, 6.64; N, 10.90; S, 6.23.

Synthesis of *N*-Fmoc-L-Valyl-*N*-Methyl-D-Alanyl-L-Valine methyl ester (17). The product was prepared by General Procedure B using 0.13 g (0.32 mmol) **8a** in dry acetonitrile (10 mL), 0.067

mL (0.97 mmol) mercaptoacetic acid and 0.14 g (2.59 mmol) sodium methoxide in methanol (2 mL). The reaction was stirred at 50 °C for 1h. The afforded unmasked dipeptide in an aqueous 9% solution of $NaHCO_3$ was treated with 0.12 g (0.32 mmol) **15b** in dry methylene chloride. The reaction was stirred at RT for 50 min. The subsequent work up afforded 0.16 g of the title compound **17** (0.30 mmol, 94%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82-7.22 (m, 8 H), 6.87 (d, 1 H, *J* = 8.5 Hz), 5.68 (d, 1 H, *J* = 8.2 Hz), 5.35 (q, 1 H, *J* = 7.1 Hz), 4.48-4.16 (m, 5 H), 3.61 (s, 3 H), 3.06 (s, 3 H), 2.18-2.02 (m, 2 H), 1.37 (d, 3 H, *J* = 7.1 Hz), 1.05-0.97 (m, 6 H), 0.90 (d, 3 H, *J* = 6.9 Hz), 0.84 (d, 3 H, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.9, 174.0, 170.9, 156.4, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 67.1, 58.8, 57.6, 56.7, 52.3, 52.0, 47.1, 31.1, 30.4, 19.2, 19.0, 18.3, 17.8, 17.5. FAB$^+$ MS *m/z* 538 $(M+H)^+$. Anal. Calcd. For $C_{30}H_{39}N_3O_6$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.04; H, 7.30; N, 7.81.

Synthesis of *N*-Fmoc-L-Valyl-*N*-Methyl-L-Leucyl-L-Alanine methyl ester (18). The product was prepared by General Procedure B using 0.16 g (0.38 mmol) **9a** in dry acetonitrile (10 mL), 0.08 mL (1.16 mmol) mercaptoacetic acid and 0.17 g (3.08 mmol) sodium methoxide in methanol (3 mL). The reaction was stirred at 50 °C for 55 min. The afforded unmasked dipeptide in an aqueous 9% solution of $NaHCO_3$ was treated with 0.13 g (0.38 mmol) **15b** in dry methylene chloride. The reaction was stirred at RT for 40 min. The subsequent work up afforded 0.195 g of the title compound **18** (0.35 mmol, 93%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81-7.20 (m, 8 H), 6.73 (d, 1 H, *J* = 7.4 Hz), 5.83 (d, 1 H, C *J* = 8.9 Hz), 5.17 (m, 1 H), 4.57-4.48 (m, 2 H), 4.38-4.19 (m, 3 H), 3.72 (s, 3 H), 3.04 (s, 3 H), 2.04 (m, 1 H), 1.76-1.48 (m, 3 H), 1.35 (d, 3 H, *J* = 7.2 Hz), 0.98-0.89 (m, 9 H), 0.85 (d, 3 H, *J* = 6.6 Hz).^{13}C NMR (75 MHz, $CDCl_3$): δ 173.2, 173.1, 170.0, 156.5, 143.8, 141.3, 127.7, 127.1, 125.1, 120.0, 67.1, 56.0, 54.6, 52.5, 47.9, 47.2, 36.2, 31.1, 30.9, 24.8, 22.9, 22.1, 19.5, 18.2, 17.5. FAB$^+$ MS *m/z* 552 $(M+H)^+$. Anal. Calcd. For $C_{31}H_{41}N_3O_6$: C, 67.49; H, 7.49; N, 7.62. Found: C, 67.50; H, 7.50; N, 7.60.

Synthesis of dimethylated *N*-Nosyl-tripeptides 13a-14a.

***N*-Methyl-*N*-Nosyl-L-Valyl-*N*-methyl-D-Phenylalanyl-L-Alanine methyl ester (13a).** The product was prepared by General Procedure A using 0.069 g (0.13 mmol) **13** in dry dichloromethane and 1.57 mL (1.04 mmol) 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding dimethylated tripeptide **13a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42-8.30 (m, 2 H), 8.08-7.90 (m, 2 H), 7.38-7.12 (m, 5 H), 6.43 (d, 1 H, *J* = 8.1 Hz), 5.35 (m, 1 H), 4.55 (m, 1 H), 4.48 (m, 1 H), 3.71 (s, 3 H), 3.38 (dd, 1 H, *J* = 14.3, 7.6 Hz), 3.21 (s, 3 H), 2.98 (dd, 1 H, *J* = 14.1, 5.2 Hz), 2.89 (s, 3 H), 2.30 (m, 1 H), 1.32 (d, 3 H, *J* = 7.2 Hz), 0.92 (d, 3 H, *J* = 6.7 Hz), 0.75 (d, 3 H, *J* = 6.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 171.4, 169.0, 149.1, 145.3, 136.7, 129.0, 128.5, 127.8, 126.8, 123.5, 60.4, 57.2, 52.3, 48.0, 33.8, 31.5, 29.9, 28.4, 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.

***N*-Methyl-*N*-Nosyl-L-Valyl-*N*-methyl-L-Isoleucyl-L-Alanine methyl ester (14a).** The product was prepared by General Procedure A using 0.13 g (0.25 mmol) **14** in dry dichloromethane and 3.06 mL (2.02 mmol) 0.66 M diazomethane in diethyl ether. The reaction was stirred for 30 min. Evaporation of the solvent afforded the corresponding dimethylated tripeptide **14a** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, 2 H, *J* = 9.0 Hz), 7.92 (d, 2 H, *J* = 9.0 Hz), 6.51 (d, 1 H, *J* = 7.8 Hz), 4.62 (d, 1 H, *J* = 10.4 Hz), 4.50 (m, 1 H), 4.01 (m, 1 H), 3.65 (s, 3 H), 3.20 (s, 3 H), 2.95 (s, 3 H), 2.19-2.05 (m, 2 H), 1.48-1.27 (m, 2 H), 1.28 (d, 3 H, *J* = 7.2 Hz), 0.90 (d, 3 H, *J* = 6.4 Hz), 0.82 (d, 3 H, *J* = 6.6 Hz), 0.81-0.72 (m, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0, 171.6, 169.3, 149.9, 145.1, 128.5, 124.4, 60.5, 55.0, 52.4, 47.9, 31.7, 31.6, 29.8, 28.2, 24.8, 19.6, 18.6, 17.8, 15.5, 10.3. FAB$^+$ MS *m/z* 529 $(M+H)^+$. Anal. Calcd. For $C_{23}H_{36}N_4O_8S$: C, 52.26; H, 6.86; N, 10.60; S, 6.07. Found: C, 52.29; H, 6.85; N, 10.58; S, 6.05.

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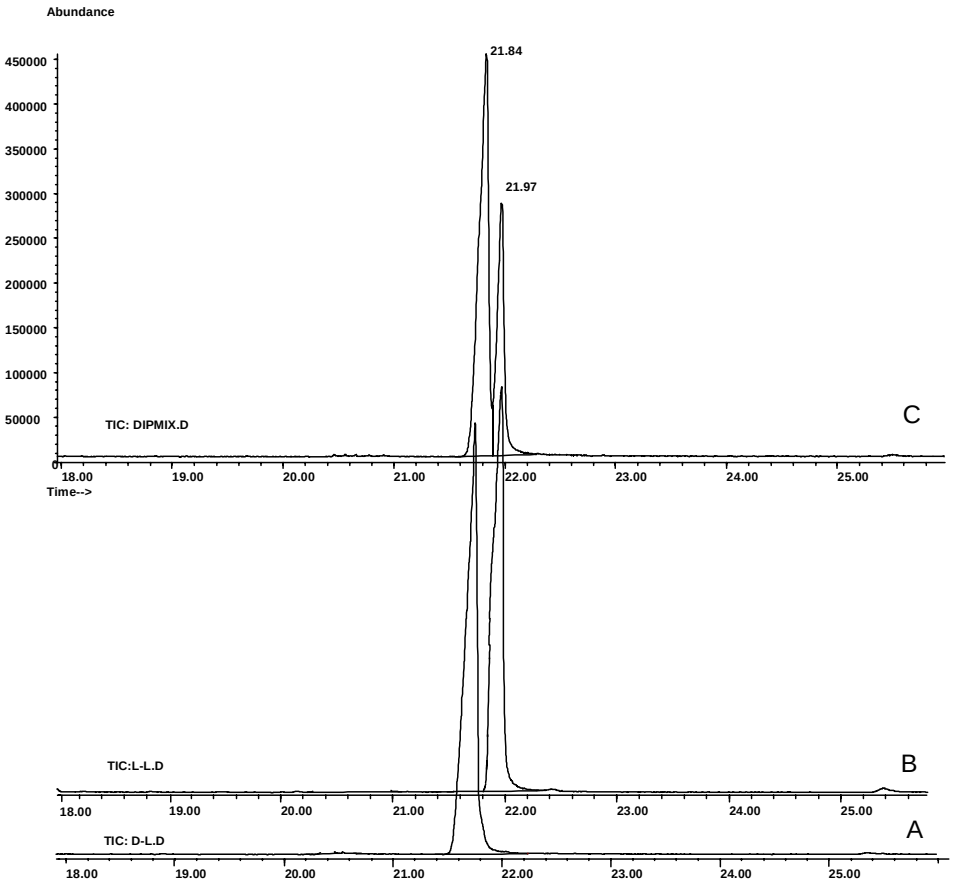

Figure 2. GC/MS analyses of *N*-Nosyl-*N*-methylated dipeptides. (A) *N*-Nosyl-D-(Me)-Phe-L-Ala-OMe **6a** (rt: 21.84 min.); (B) *N*-Nosyl-L-(Me)-Phe-L-Ala-OMe **5a** (rt: 21.97 min.); (C) A mixture of **5a** and **6a**.

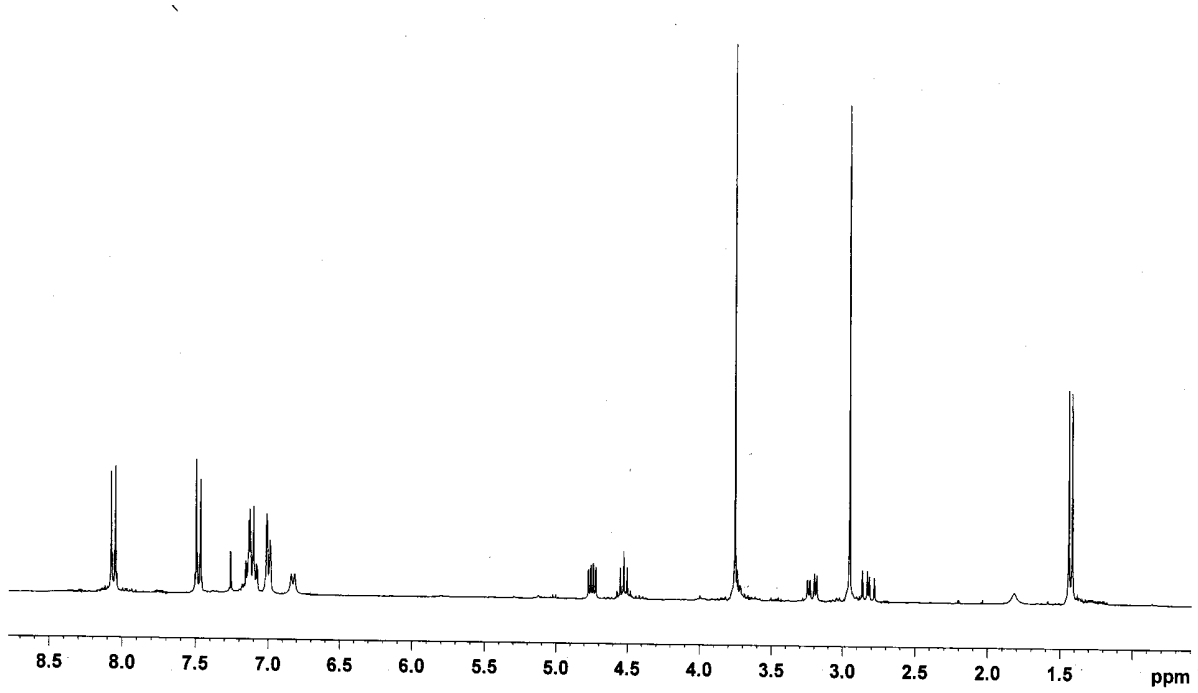

Figure 3. ^{1}H NMR spectrum of *N*-Nosyl-(Me)-L-Phe-L-Ala-OMe (**5a**)

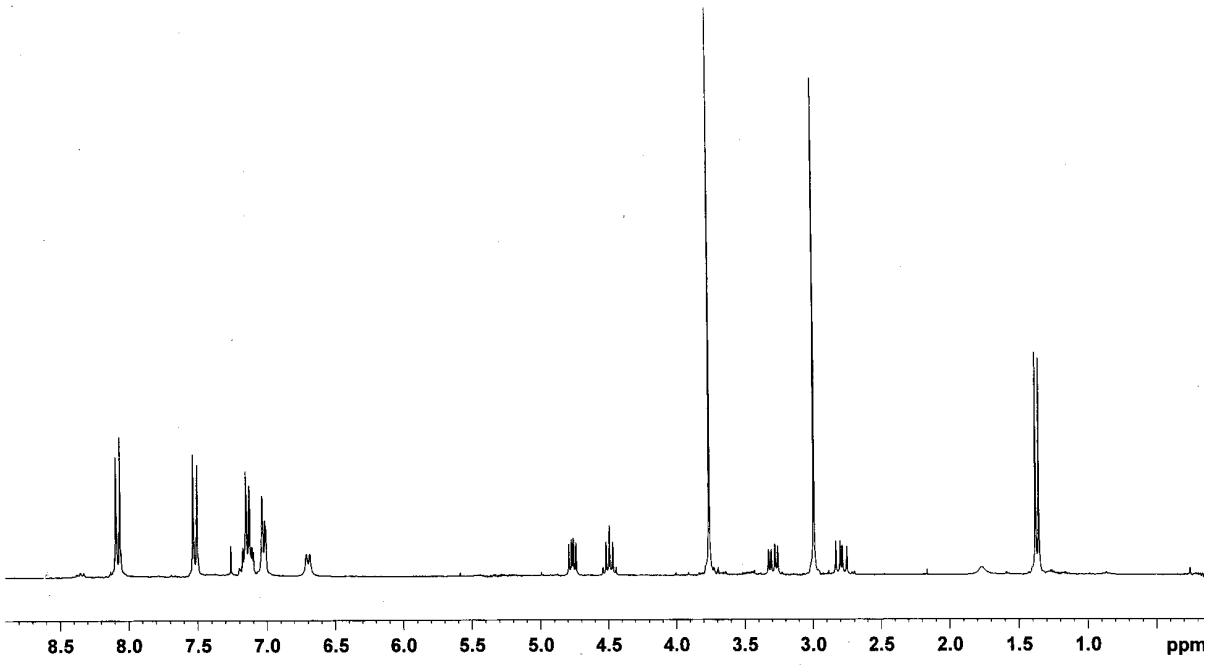

Figure 4. ^{1}H NMR spectrum of *N*-Nosyl-(Me)-D-Phe-L-Ala-OMe (**6a**)

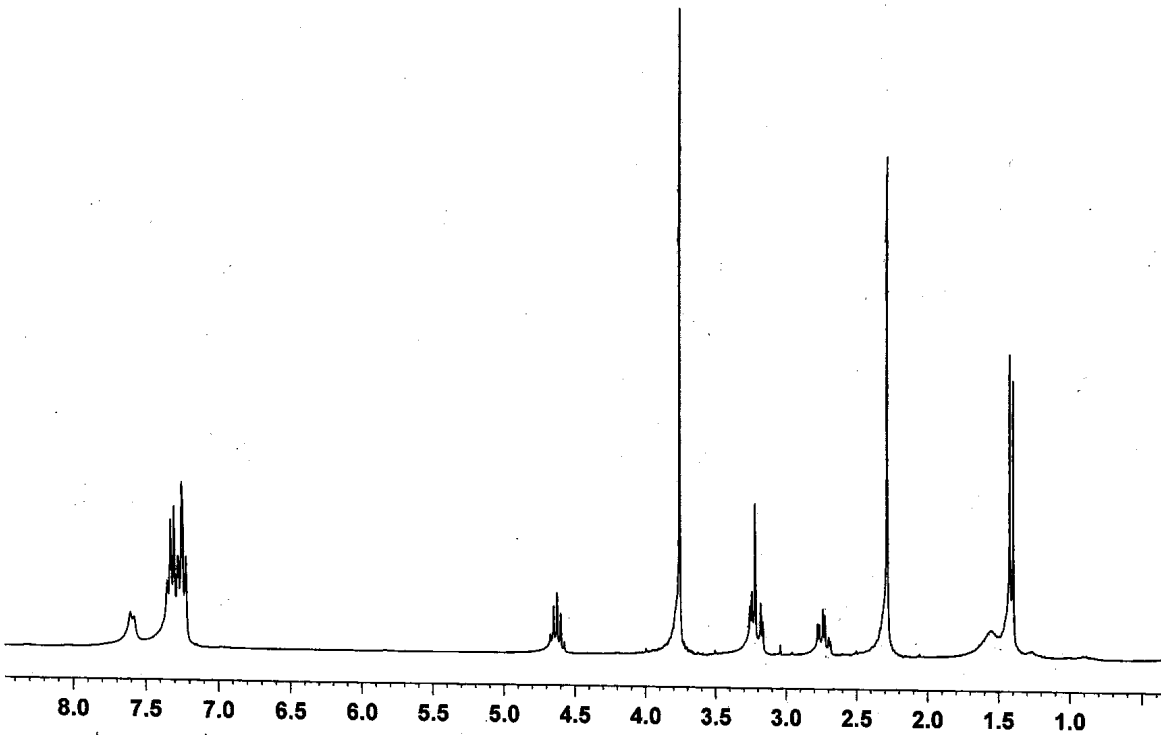

Figure 5. ^{1}H NMR spectrum of the crude product obtained by deprotection of dipeptide **6a**