β-Nitro-α-amino acids as latent α,β-dehydro-α-amino acid residues in solid-phase peptide synthesis

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Dedicated to Prof. Rod Rickards on the occasion of his 70th birthday, with thanks for the good humor and generous advice and support

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Abstract

β-Nitro-α-amino acids, that are readily accessible through either the reaction of bromoglycine derivatives with alkyl nitronates or the three-component coupling of amines, nitroalkanes and glyoxalate in aqueous base, are easily converted to the corresponding *N*-*t*-Boc-amino acids. These undergo solid-phase Merrifield peptide synthesis, with elimination of nitrous acid, either during or subsequent to cleavage of the peptide from the resin, converting the nitro amino acids to dehydro amino acid residues. The method has been applied successfully with two β,β-disubstituted nitro amino acids and *N*-methyl-β-nitronorvaline, but failed with β-nitroalanine.

Keywords: Amino acids, peptides, solid-phase synthesis, dehydro amino acids

Introduction

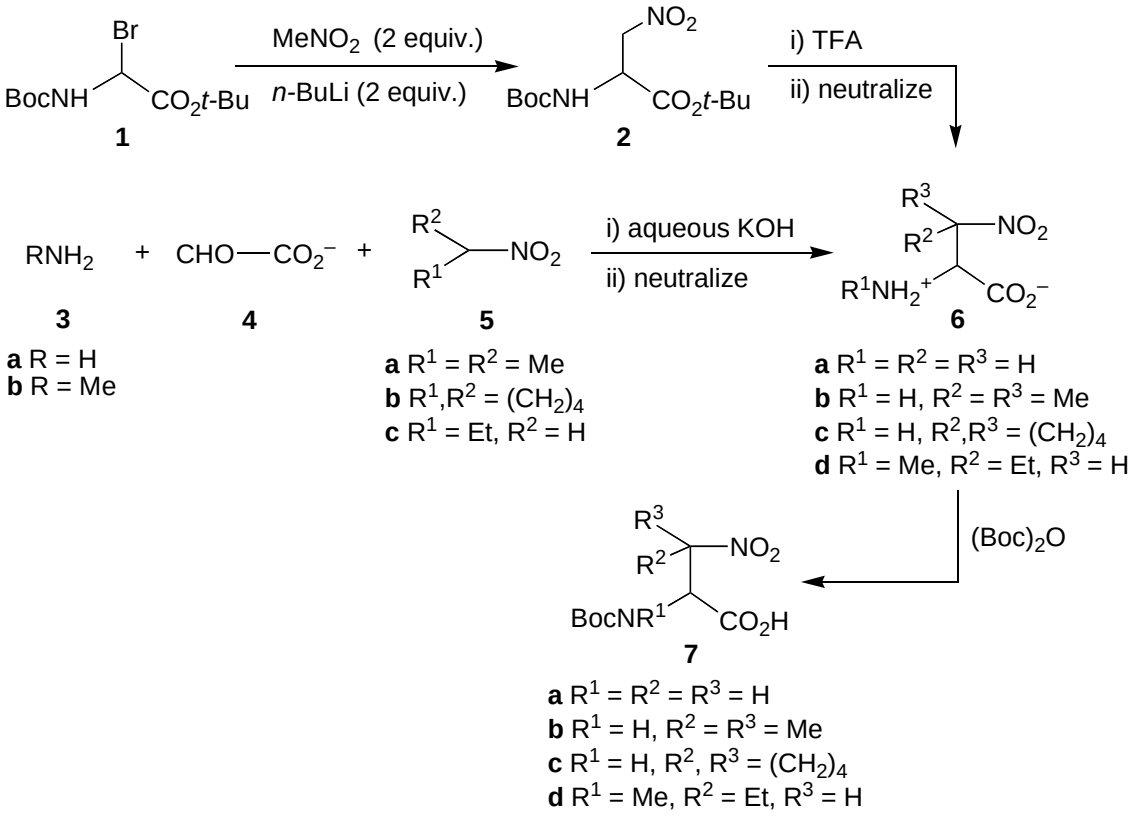
Peptides containing α,β-dehydro-α-amino acid residues are of interest as synthetic targets, principally because of the physiological activity of many naturally occurring compounds of this type.[1] Free dehydro amino acids are generally unsuitable for direct peptide synthesis because they are unstable and, being enamines, are only poor nucleophiles.[2] Instead, the most common approach is to incorporate suitably protected β-hydroxy amino acids in their place,[3] although other β-functionalized amino acids can also be used.[4] After peptide synthesis, the β-substituted amino acids are elaborated to unmask dehydro amino acid residues.

This method is somewhat limited by the lack of ready access to β-hydroxy amino acids other than serine and threonine, except through synthesis,[5] and the need for selective functional group protection and deprotection strategies, particularly in cases where the target peptide contains more than one dehydro amino acid or both dehydro and hydroxy amino acids. Therefore, we are

developing a complementary approach involving the incorporation of β-nitro-α-amino acids in peptides as latent α,β-dehydro-α-amino acid residues. β-Nitro amino acids with a wide variety of side chains are easily prepared through reaction of α-bromoglycine derivatives with alkyl nitronates[6,7] or, more directly, through the three-component coupling of amines, nitroalkanes and glyoxylate in aqueous base.[8] Previously we have shown that the amino acid *tert*-butyl esters can be incorporated as the *C*-terminal residues in *N*- and *C*-protected dipeptides, using solution-phase methods.[9] We now report on the utility of the nitro amino acids in solid-phase peptide synthesis, and the isolation and characterization of the free peptides.

Results and Discussion

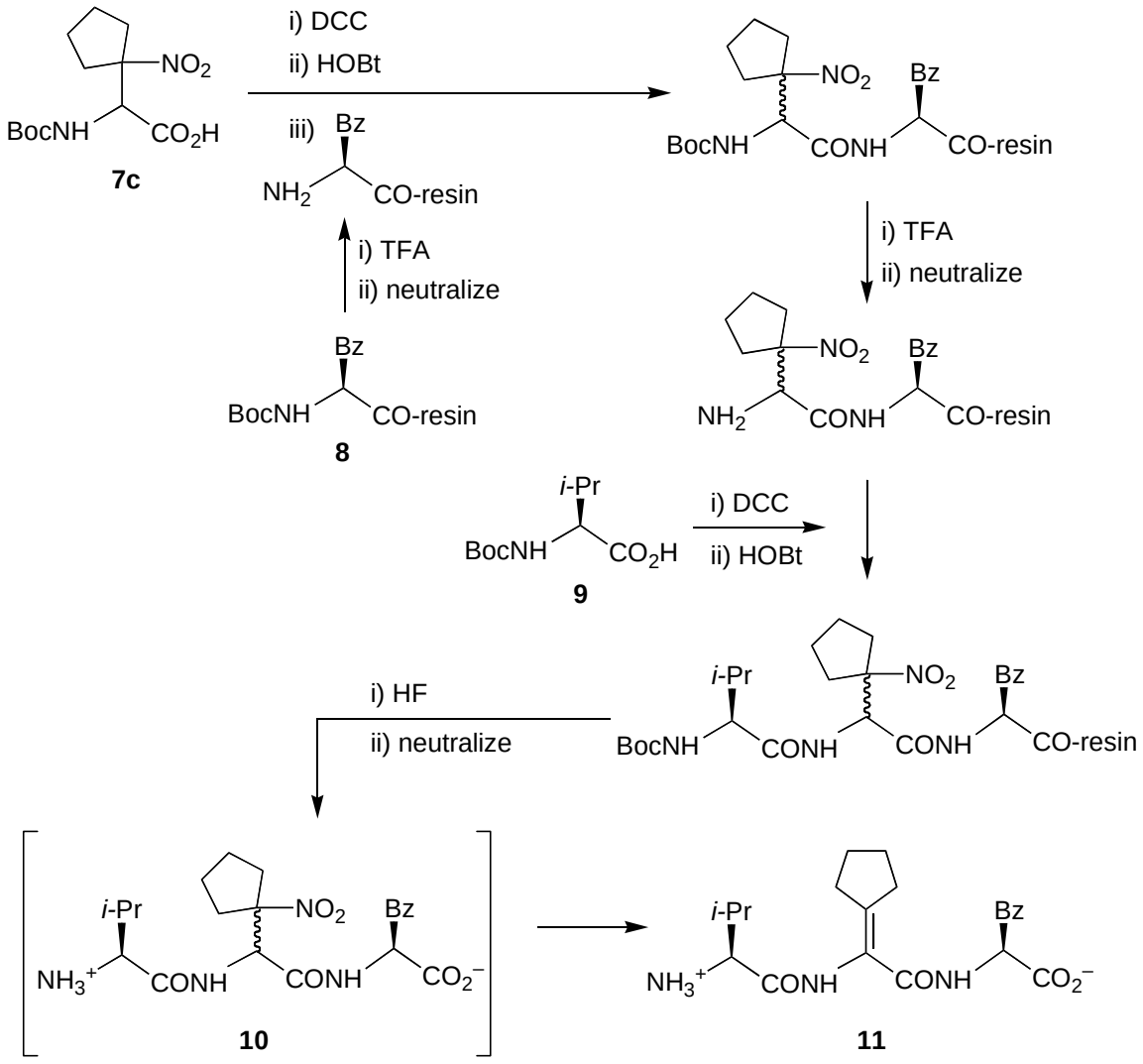
The β-nitro-α-amino acids used in this study were prepared as shown in Scheme 1. β-Nitroalanine **6a** was obtained by treatment of the bromoglycine derivative **1** with methyl nitronate, followed by removal of the protecting groups from the alanine derivative **2** through reaction with trifluoroacetic acid.[7]



Scheme 1

β-Nitrovaline **6b** and β-nitrocyclopentylglycine **6c** were prepared in three-component coupling reactions of ammonia **3a** and glyoxylate **4**, with 2-nitrovaline **5a** and nitrocyclopentane **5b**, respectively, in water under basic conditions.[8] An analogous reaction of methylamine **3b**, glyoxylate **4** and 1-nitropropane **5c** afforded a 6:1 mixture of the diastereomers of *N*-methyl-β-nitronorvaline **6d**. Each of the nitro amino acids **6a–d** is assumed to be racemic but this is unimportant for the synthesis of dehydro amino acid derivatives since any stereochemical information is lost in the interconversion. For peptide synthesis the free amino acids **6a–d** were *N*-protected by treatment with di-*tert*-butyl dicarbonate to give **7a–d**, as a 1:1 mixture of diastereomers in the case of **7d**.

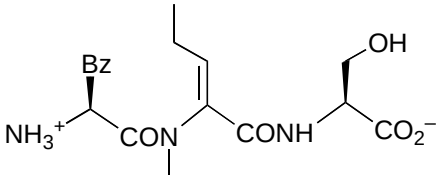
Peptide synthesis was carried out under standard conditions on Merrifield polystyrene resin.[10] The procedure is illustrated in Scheme 2 for the tripeptide **11**.



Scheme 2

Resin substituted with *N*-*t*-Boc-(*S*)-phenylalanine **8** was treated with trifluoroacetic acid to remove the *N*-protecting group. The resin was then washed and neutralized before being treated with the nitrocyclopentylglycine **7c** that had been activated by pre-treatment with dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The cycle of deprotection with trifluoroacetic acid and coupling with an activated amino acid was repeated, this time using *N*-*t*-Boc-(*S*)-valine **9**. After washing and drying, the resin was treated with hydrogen fluoride/anisole, then the hydrogen fluoride was removed by distillation at 0 °C. The residual solution was separated from the resin and treated with diethyl ether to give a white precipitate, which was subjected to preparative HPLC, to give the tripeptide **11**.

In a similar manner, the nitronorvaline derivative **7d** and *N*-*t*-Boc-(*S*)-phenylalanine were sequentially coupled to resin substituted with *N*-*t*-Boc-*O*-Bz-(*S*)-serine. In this case treatment with hydrogen fluoride cleaved the product from the resin as well as removing the benzyl protecting group, to give the tripeptide **12**. The assignment of *Z*-stereochemistry to the dehydronorvaline residue in the tripeptide **12** is made on the basis of the tendency of dehydro amino acid derivatives to favor this configuration.[11] When the same resin was treated with the valine derivative **7b** and then *N*-*t*-Boc-(*S*)-phenylalanine, only a trace of the tripeptide **14** was obtained. Instead the diastereomers of the β-nitrovaline derivative **13** were the major components isolated. These were easily converted to the dehydrovaline derivative **14** on treatment with base. When the serine-substituted resin was treated with the nitroalanine **7a** and then *N*-*t*-Boc-(*S*)-phenylalanine, neither the tripeptide **15** nor the corresponding β-nitroalanine-containing peptide was obtained.



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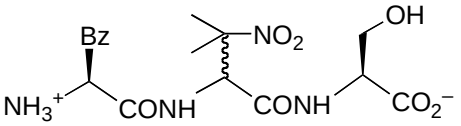

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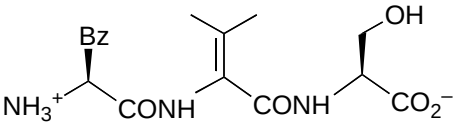

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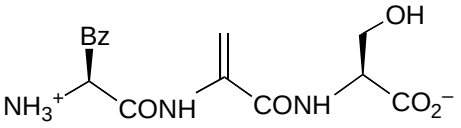

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These experiments show that β-nitro-α-amino acids can be used in solid-phase peptide synthesis. Presumably elimination of nitrous acid then occurs under the conditions of cleavage from the resin in the cases of the peptides **11** and **12**, the putative precursor of the former being the nitrocyclopentylglycine derivative **10**. The method works equally well with the *N*-unsubstituted and *N*-methyl amino acid derivatives **7c** and **7d**, respectively. Elimination of

nitrous acid from the nitrovaline derivative **13** is less facile and separate treatment with base is therefore required for the efficient production of the tripeptide **14**. The decreased reactivity in this system can be attributed to the increased steric constraints associated with bringing the substituents at the α- and β-positions into coplanarity, during the change in hybridization.[6] By analogy, loss of nitrous acid from β-nitroalanine derivatives is more facile[6] and this may be occurring during the use of the amino acid **7a** in peptide synthesis, leading to decomposition of reactive dehydroalanine derivatives and the failure to produce the peptide **15**. However, since dehydroalanine derivatives are readily prepared from serine, they are already accessible by methods that are truly complementary to the approach presented here.

Experimental Section

General Procedures. Melting points (mp) were determined on a Kofler hot-stage melting point apparatus under a Reichert microscope and are uncorrected. Microanalyses were performed by the Research School of Chemistry Microanalytical Service at the Australian National University, on a Carlo–Erba 1106 autoanalyser. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 300 spectrometer. Proton (^{1}H) NMR spectra were recorded at 300 MHz and carbon (^{13}C) NMR spectra were recorded at 75.5 MHz. Infrared (IR) spectra were recorded as KBr disks on a Perkin–Elmer 1800 infrared spectrophotometer. Electron impact (EI) mass spectra were obtained using a VG Autospec double focussing trisector mass spectrometer. Electrospray (ESI) mass spectra were obtained using a VG Quatro II triple quadrupole mass spectrometer operating in the positive ion mode.
β-Nitroalanine **6a**,[7] β-nitrovaline **6b**,[8] β-nitrocyclopentylglycine **6c**[8] and *N*-methyl-β-nitronorvaline **6d**[8d] were prepared as reported previously, the latter as a 6:1 mixture of diastereomers. *N*-*t*-Boc-(*S*)-Phenylalanine and *N*-*t*-Boc-(*S*)-valine **9**, and Merrifield polystyrene resins substituted with *N*-*t*-Boc-(*S*)-phenylalanine **8** and *N*-*t*-Boc-*O*-Bz-(*S*)-serine were available from Auspep Pty. Ltd. Methanol was dried using sodium. HPLC was performed using a Superspher® 250-4, LiChroCART, 100 RP-18 column eluting with acetonitrile/water (90/10, v/v) containing 0.1% trifluoroacetic acid.

***N*-*t*-Boc-β-nitroalanine (7a).** A mixture of β-nitroalanine **6a** (0.47 g, 3.5 mmol), di-*t*-butyl dicarbonate (1.51 g, 6.9 mmol) and triethylamine (0.56 mL, 4.0 mmol) in dry methanol (5.6 mL) was heated at reflux under a nitrogen atmosphere for 0.5 h, before it was cooled and concentrated under reduced pressure. The residue was partitioned between aqueous hydrochloric acid (0.5 mol dm^{-3}, 20 mL) and ethyl acetate (20 mL). The aqueous phase was extracted with ethyl acetate (3 × 20 mL) and the combined organic fractions were extracted with aqueous potassium hydroxide (0.5 mol dm^{-3}, 3 × 40 mL). The combined basic extracts were acidified with aqueous hydrochloric acid (2.0 mol dm^{-3}) and the resultant precipitate was collected by filtration, to give the title compound **7a** as a colorless solid (0.40 g, 49%); mp 112–115 °C; ^{1}H NMR (CD_3OD) δ

4.88 (2H, m), 4.76 (1H, t, *J* = 5.5 Hz), 1.44 (9H, s); ^{13}C NMR (CD_3OD) δ 171.7, 157.6, 81.1, 76.4, 52.8, 28.6; IR ν_{max} 3403, 2989, 1758, 1675, 1558, 1517, 1396, 1374, 1202, 1159, 1083, 846 cm^{-1}; MS (EI) *m/z* (%) 189 (35) $[M–CO_2H]^{+\bullet}$, 179 (15), 135 (15), 89 (15), 57 (100); Anal. Calcd. for $C_8H_{14}N_2O_6$: C, 41.03; H, 6.02; N, 11.96. Found: C, 40.67; H, 6.03; N, 11.97%.

***N*-*t*-Boc-β-nitrovaline (7b).** Treatment of β-nitrovaline **6b** (0.3 g, 1.85 mmol) with di-*t*-butyl dicarbonate (0.81 g, 3.7 mmol), as described above for the synthesis of *N*-*t*-Boc-β-nitroalanine **7a**, afforded the title compound **7b** as a colorless solid (0.24 g, 49%); mp 154–156 °C; ^{1}H NMR (DMSO-d_6) δ 7.54 (1H, br d, *J* = 10 Hz), 4.92 (1H, d, *J* = 10 Hz), 1.53 (3H, s), 1.47 (3H, s), 1.39 (9H, s); ^{13}C NMR (CD_3OD) δ 171.6, 158.1, 89.9, 81.2, 60.4, 28.6, 25.1, 22.7; IR ν_{max} 3321, 2978, 2600, 1719, 1653, 1539, 1404, 1370, 1349, 1267, 1158, 1053, 850 cm^{-1}; MS (EI) *m/z* (%) 217 (7) $[M–CO_2H]^{+\bullet}$, 160 (20), 142 (15), 116 (25), 89 (30), 70 (30), 57 (100); Anal. Calcd. for $C_{10}H_{18}N_2O_6$: C, 45.90; H, 6.92; N, 10.68. Found: C, 45.49; H, 6.69; N, 10.72%.

***N*-*t*-Boc-β-nitrocyclopentylglycine (7c).** Treatment of β-nitrocyclopentylglycine **6c** (0.8 g, 4.3 mmol) with di-*t*-butyl dicarbonate (1.88 g, 8.6 mmol), as described above for the synthesis of *N*-*t*-Boc-β-nitroalanine **7a**, afforded the title compound **7c** as a colorless solid (0.90 g, 73%); mp 149–150.5 °C; ^{1}H NMR (CD_3OD) δ 4.97 (1H, s), 2.42 (2H, m), 2.20 (1H, m), 2.01 (1H, m), 1.77 (4H, m), 1.46 (9H, s); ^{13}C NMR (CD_3OD) δ 172.1, 158.3, 101.0, 81.2, 59.6, 38.4, 36.0, 28.6, 26.1, 25.5; IR ν_{max} 3304, 3102, 2981, 2615, 1741, 1648, 1546, 1407, 1367, 1259, 1159, 1055, 1028, 853, 776, 681 cm^{-1}; MS (EI) *m/z* (%) 243 (15) $[M–CO_2H]^{+\bullet}$, 186 (20), 142 (35), 125 (55), 115 (30), 97 (40), 57 (100); Anal. Calcd. for $C_{12}H_{20}N_2O_6$: C, 49.99; H, 6.99; N, 9.72. Found: C, 49.71; H, 7.05; N, 9.54%.

***N*-*t*-Boc-*N*-methyl-β-nitronorvaline (7d).** Treatment of *N*-methyl-β-nitronorvaline **6d** (3.27 g, 18.6 mmol, 6:1 mixture of diastereomers) with di-*t*-butyl dicarbonate (8.14 g, 37.1 mmol), as described above for the synthesis of *N*-*t*-Boc-β-nitroalanine **7a**, afforded a 1:1 mixture of diastereomers of the title compound **7d**, as a colorless solid after recrystallization from ethyl acetate/petroleum ether (1.85 g, 38%); mp 123.5–125 °C; ^{1}H NMR (CD_3OD) δ 5.02 (1H, m), 4.90 and 4.69 (total 1H, d, *J* = 10 Hz, and d, *J* = 10 Hz), 2.96 and 2.91 (total 3H, s and s), 1.87 (2H, m), 1.46 and 1.44 (total 9H, s and s), 0.94 (3H, q, *J* = 7.5 Hz); ^{13}C NMR (CD_3OD) δ 171.9, 157.5, 156.4, 89.1, 88.2, 83.1, 82.3, 63.3, 62.1, 36.5, 35.5, 28.4, 25.3, 10.1, 9.9; IR ν_{max} 2984, 1745, 1638, 1556, 1414, 1370, 1320, 1244, 1211, 1165, 856 cm^{-1}; MS (EI) *m/z* (%) 231 (2) $[M–CO_2H]^{+\bullet}$, 203 (4), 185 (3), 174 (10), 131 (35), 129 (35), 85 (35), 57 (100); Anal. Calcd. for $C_{11}H_{20}N_2O_6$: C, 47.82; H, 7.30; N, 10.14. Found: C, 47.77; H, 7.06; N, 10.16%.

(*S*)-Valyl-α,β-dehydrocyclopentylglycyl-(*S*)-phenylalanine (11). *N*-*t*-Boc-(*S*)-phenylalanine-substituted Merrifield polystyrene resin **8** (1.0 g, 1.0 mmol) was shaken with trifluoroacetic acid (20 mL) at room temperature for 0.5 h. The resin was then washed repeatedly with dichloromethane and diisopropylethylamine in dichloromethane (5%, v/v), before it was treated with a solution that had been prepared from *N*-*t*-Boc-β-nitrocyclopentylglycine **7c** (0.85 g, 2.95 mmol), dicyclohexylcarbo-diimide (0.61 g, 2.95 mmol), 1-hydroxybenzotriazole (0.40 g, 2.95 mmol) and dichloromethane (5 mL). The mixture was shaken at room temperature for 4 h, before the resin was separated and washed with dichloromethane. The cycle of treatment with

trifluoroacetic acid and coupling with an amino acid was then repeated, using *N*-*t*-Boc-(*S*)-valine **9** (0.64 g, 2.95 mmol) instead of *N*-*t*-Boc-β-nitrocyclopentylglycine **7c**. The resin was then washed with *N*,*N*-dimethylformamide, dichloromethane, methanol and diethyl ether, and dried overnight, before it was treated with hydrogen fluoride-anisole (9:1, v/v) at 0 °C for 1 h. The hydrogen fluoride was removed by distillation at 0 °C, then diethyl ether (20 mL) was added and the mixture was filtered. Acetonitrile in water (30%, v/v) was added to the solid and the resultant solution was separated from the resin before being concentrated to dryness. The residue was dissolved in water and a sample of the solution (ca. 10%) was applied to an HPLC column, eluting with a gradient of acetonitrile in water (0–20%, 40 min) to give the title compound **11** as a colorless solid (16 mg); ^{1}H NMR (D_2O) δ 7.19 (5H, m), 4.59 (1H, m), 3.78 (1H, d, *J* = 5.0 Hz), 2.99 (2H, m), 2.05 (5H, m), 1.51 (4H, m), 0.92 (3H, d, *J* = 7.0 Hz),), 0.86 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (D_2O) δ 176.1, 169.4, 167.7, 137.5, 130.3, 129.7, 128.1, 119.4, 101.4, 59.4, 55.4, 37.6, 33.6, 33.1, 31.0, 27.7, 25.9, 18.9, 17.5; MS (ESI, +ve) *m/z* (%) 387 (100) [$M^{+\bullet}$].

(*S*)-Phenylalanyl-*N*-methyl-(*Z*)-α,β-dehydronorvalyl-(*S*)-serine (12). Treatment of *N*-*t*-Boc-*O*-Bz-(*S*)-serine-substituted Merrifield polystyrene resin (1.5 g, 1.0 mmol) with *N*-*t*-Boc-*N*-methyl-β-nitronorvaline **7d** (2.0 g, 7.2 mmol) and *N*-*t*-Boc-(*S*)-phenylalanine (1.9 g, 7.2 mmol), as described above for the synthesis of the tripeptide **11**, afforded the title compound **12** as a colorless solid (8.5 mg); ^{1}H NMR (D_2O) δ 7.20 (5H, m), 5.76 (1H, m), 5.31 (2H, m), 4.11 (1H, m), 3.69 (2H, m), 3.56 (1H, s), 3.04 (2H, m), 2.52 (3H, s), 1.65 (3H, m); MS (ESI, +ve) *m/z* (%) 387 (15) [$(M+Na)^{+\bullet}$], 363 (100) [$(M+H)^{+\bullet}$].

(*S*)-Phenylalanyl-(*RS*)-β-nitrovalyl-(*S*)-serine (13). Treatment of *N*-*t*-Boc-*O*-Bz-(*S*)-serine-substituted Merrifield polystyrene resin (0.7 g, 0.7 mmol) with *N*-*t*-Boc-β-nitrovaline **7b** (0.30 g, 1.14 mmol) and *N*-*t*-Boc-(*S*)-phenylalanine (0.32 g, 1.2 mmol), as described above for the synthesis of the tripeptide **11**, afforded a 2:1 mixture of the diastereomers of the title compound **13** as a colorless solid (5 mg); ^{1}H NMR (D_2O) major diastereomer δ 7.20 (5H, m), 5.07 (1H, s), 4.26 (2H, m), 3.93 (1H, dd, *J* = 5.0 and 12.0 Hz), 3.82 (1H, dd, *J* = 5.0 and 12.0 Hz), 3.12 (2H, m), 1.61 (3H, s), 1.55 (3H, s); the minor diastereomer showed separate resonances at δ 5.10 (1H, s), 1.57 (3H, s); ^{13}C NMR (D_2O) major diastereomer δ 174.2, 169.9, 168.0, 134.4, 130.2, 130.1, 128.9, 90.2, 61.8, 59.0, 56.5, 55.0, 37.8, 24.5, 21.9; the minor diastereomer showed separate resonances at δ 59.1, 22.3; MS (ESI, +ve) *m/z* (%) 396 (100) [$(M+H)^{+\bullet}$]. (*S*)-Phenylalanyl-α,β-dehydrovalyl-(*S*)-serine **14** (1 mg) was obtained as a minor by-product and characterized as described below.

(*S*)-Phenylalanyl-α,β-dehydrovalyl-(*S*)-serine (14). A 2:1 mixture of the diastereomers of (*S*)-phenylalanyl-(*RS*)-β-nitrovalyl-(*S*)-serine **13** (3.5 mg, 10 μmol) was dissolved in aqueous piperidine (1 mol dm^{-3}, 0.1 mL) and the mixture was stirred at room temperature for 17 h, before it was concentrated under reduced pressure. The residue was dissolved in water and the solution was applied to an HPLC column, eluting with a gradient of acetonitrile in water (0–20%, 40 min) to give the title compound **14** as a colorless solid (3.5 mg, quantitative); ^{1}H NMR (D_2O) δ 7.23 (5H, m), 4.20 (2H, m), 3.73 (2H, m), 3.10 (2H, m), 1.81 (3H, s), 1.30 (3H, s); MS (ESI, +ve) *m/z* (%) 349 (100) [$M^{+\bullet}$].

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