DOI: 10.1002/ejoc.201001302

Synthesis of Novel Nonproteinogenic Amino Acids: *N*-Ethyl-α,β-dehydroamino Acid Methyl Esters

Luís S. Monteiro,*[a] Joanna Kołomańska,[a] and Ana C. Suarez[a]

Keywords: Amino acids / Elimination / α,β-Dehydroamino acids / Alkylation / *N*-Ethyl-α,β-dehydroamino acids

Two routes for the synthesis of *N*-ethyl-*N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino acid derivatives from serine, threonine and phenylserine derivatives are presented. One route consists of dehydration of *N*-(4-nitrophenylsulfonyl)-β-hydroxyamino acid esters with di-*tert*-butyl dicarbonate catalyzed by 4-(dimethylamino)pyridine, followed by alkylation of the *N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino acid methyl esters obtained with triethyloxonium tetrafluoroborate. The second strategy applied the same procedures but in inverse order: alkylation followed by dehydration. These methods made it possible to obtain for the first time, new non-natural amino acids, which incorporate both an *N*-ethyl and an α,β-dehydro moiety.

Introduction

Nonproteinogenic amino acids are an important class of organic compounds that can have intrinsic biological activity or can be found in peptides with antiviral, antitumor, anti-inflammatory or immunosuppressive activities. This type of compounds is also important in drug development, in the elucidation of biochemical pathways and in conformational studies. Incorporation of non-natural amino acids in peptide chains can change the properties of these compounds, for example increasing enzymatic stability and bioavailability. Among nonproteinogenic amino acids are the *N*-alkylamino acids and dehydroamino acids, which can be found in many biologically important peptides.[1]

N-Alkylation of the peptide bond causes changes in the volume and conformation of peptides. *N*-Alkylation results in reduced flexibility, increase of permeability for the membrane (increased lipophilicity) and prevention of cleavage by proteolytic enzymes.[2] Several *N*-alkylated peptides show antibiotic, anticancer or antiviral activity. For example *N*-methylleucine is found in cyclosporines.[3] A replacement of *N*-methylleucine of cyclosporine A by various *N*-ethylamino acids was performed, and the corresponding *N*-ethylated derivatives resulted in analogues exhibiting immunosuppressive and anti-HIV activity.[4] Many methods of synthesis of *N*-alkylamino acids have been developed, most of them are *N*-methylations.[2] However, only a few methods for the synthesis of *N*-ethylated amino acids and their derivatives are available in the literature.[5] Papaioannou and coworkers described a Mitsunobu-type *N*-ethylation of tosylamino esters with excess ethanol.[6] Difficulties found in the chemical detosylation step could be overcome by reductive electrochemical cleavage of the protecting group.[7] The stronger electron-withdrawing effect of nitroarylsulfonamides further enhances the acidity of the α-amide hydrogen atom, making these groups unique for the preparation of *N*-alkyl peptides.[8] *N*-(Nitrophenylsulfonyl)amino acid chlorides have also been used to couple to extremely hindered amines on a solid support giving better results than analogous Fmoc-amino acid chlorides.[8] Recently, Liguori et al. proposed the ethylation of several 4-nitrophenylsulfonyl (nosyl) protected amino acids using triethyloxonium tetrafluoroborate (Et_3OBF_4) as alkylating agent to give *N*-ethylamino acid derivatives in high yields.[9b] These authors demonstrated the compatibility of the procedure with standard Fmoc chemistry.[9] Thus, removal of the nosyl group was accomplished by an aromatic nucleophilic substitution (mercaptoacetic acid/sodium methoxide), and the amino function was reprotected with the Fmoc group by treatment with Fmoc chloride.

Dehydroamino acids can be found in several yeasts and bacteria, in which they play a catalytic role in the active sites of some enzymes, as well as in a variety of peptide antibiotics of bacterial origin that include the lantibiotics (nisin, epidermin, subtilin, gallidermin).[10] Since they affect both chemical reactivity and conformation, dehydroamino acids have been introduced into peptides for structure–function relationship studies and have also been used as linkers in solid-phase peptide synthesis.[11] The chemical synthesis of α,β-dehydroamino acids and their derivatives has been attempted through several methods. Those that follow the biosynthetic routes involving elimination reactions of β-hydroxyamino acids, β-mercaptoamino acids and *N*-hydroxyamino acids are the most important ones. In our laboratories we developed an efficient method for the synthesis of *N*,*N*-diacyl-α,β-dehydroamino acid derivatives by

[a] Chemistry Centre, University of Minho,
Gualtar, 4710-057 Braga, Portugal
Fax: +351-253-604382
E-mail: monteiro@quimica.uminho.pt

using 2 equiv. of di-*tert*-butyl dicarbonate (Boc_2O) and 4-(dimethylamino)pyridine (DMAP) as catalyst in dry acetonitrile.[12] For the synthesis of *N*-acyl-α,β-dehydroamino acid derivatives, a modification of this method was subsequently reported.[13] Thus, by treating β-hydroxyamino acid derivatives with 1 equiv. of di-*tert*-butyl dicarbonate and DMAP, followed by treatment with *N*,*N*,*N*′,*N*′-tetramethylguanidine (TMG), *N*-monoprotected α,β-dehydroamino acid derivatives could be obtained in high yields.

In this paper we report the use of a combination of the alkylation procedure reported by Liguori et al.[9b] and our dehydration methodologies[12,13] to obtain new nonproteinogenic amino acids, namely, *N*-ethyl-α,β-dehydroamino acids.

Results and Discussion

The methodology proposed by Liguori et al. for *N*-ethylation of *N*-nosyl-protected amino acid derivatives using 2.5 equiv. of the alkylating agent triethyloxonium tetrafluoroborate, requires the use of side-chain protection in the case of side-chain-functionalized amino acids.[9b] To avoid the need for side-chain protection and subsequent deprotection, our initial approach for the synthesis of *N*-ethyl-α,β-dehydroamino acid derivatives was a two-step procedure in which the first step was dehydration followed by the alkylation reaction (Route A, Scheme 1).

Thus, the amine function of the β-hydroxyamino acids serine, threonine and phenylserine was protected with the nosyl group by reaction of their methyl esters with 4-nitrobenzenesulfonyl chloride (compounds **1a**–**c**, Scheme 1).

Dehydration was initially attempted by reaction with 1 equiv. of di-*tert*-butyl dicarbonate by using DMAP as catalyst, followed by treatment with TMG.[13] However, this method led to complex mixtures resulting from *tert*-butoxycarbonylation of the hydroxy group and also of the sulfonamide function. Thus, the alternative reaction with 2 equiv. of di-*tert*-butyl dicarbonate was carried out.[12] In the case of reaction with compounds **1b** and **1c** the corresponding *N*-(*tert*-butoxycarbonyl)-*N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino acid derivatives were obtained (compounds **2b** and **2c**, Scheme 1, Table 1).

Table 1. Yields obtained in the synthesis of the methyl esters of *N*-ethyl-*N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino acids.[a]

Compound	Yield [%]	Compound	Yield [%]	Compound	Yield [%]
2a	–	**3a**	–	**4a**	–
2b	81	**3b**	84	**4b**	70
2c	99	**3c**	83	**4c**	78

[a] Route A (Scheme 1).

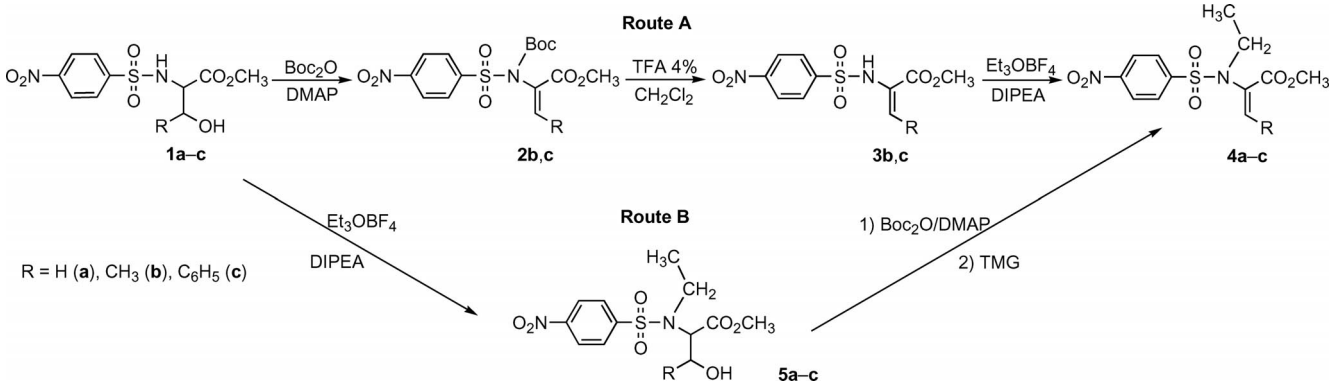
However, in the case of the reaction with coupound **1a** the major product obtained was the methyl ester of *N*-(*tert*-butoxycarbonyl)-β-(4-nitrophenylsulfinyl)-α,β-dehydroserine.[14] The introduction of the *tert*-butoxycarbonyl group makes necessary a deprotection step prior to alkylation; thus, compounds **2b** and **2c** were treated with a 4% solution of trifluoroacetic acid (TFA) in dichloromethane to give compounds **3b** and **3c** in high yields. These *N*-nosyl-α,β-dehydroamino acid derivatives were subjected to ethylation by using the conditions proposed by Liguori et al.[9b] [2.5 equiv. of triethyloxonium tetrafluoroborate, 3.5 equiv. of *N*,*N*-diisopropylethylamine (DIPEA) in dry dichloromethane] to give the corresponding *N*-ethyl-*N*-nosyl-α,β-dehydroamino acid derivatives in good yields (compounds **4b** and **4c**, Scheme 1, Table 1).

In order to avoid the *tert*-butoxycarbonyl group removal step required by Route A, an alternative strategy, in which alkylation occurs prior to dehydration, was attempted (Route B, Scheme 1). Thus, compounds **1a**–**c** were treated directly without side-chain protection with 1 equiv. of triethyloxonium tetrafluoroborate. Fortunately, the reaction was regioselective giving in high yield (92–94%) the corresponding *N*-ethyl-*N*-nosyl-β-hydroxyamino acid derivative (compounds **5a**–**c**, Scheme 1, Table 2).

Table 2. Yields obtained in the synthesis of the methyl esters of *N*-ethyl-*N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino acids.[a]

Compound	Yield [%]	Compound	Yield [%]
5a	92	**4a**	73
5b	94	**4b**	–
5c	92	**4c**	62

[a] Route B (Scheme 1).



Scheme 1. Synthesis of *N*-ethyl-α,β-dehydroamino acid derivatives from *N*-(4-nitrophenylsulfonyl)-β-hydroxyamino acid methyl esters.

These could now be dehydrated by reaction with 1 equiv. of di-*tert*-butyl dicarbonate followed by treatment with TMG. In the case of the reaction with compounds **5a** and **5c**, good yields of the corresponding *N*-ethyl-*N*-nosyl-α,β-dehydroamino acid derivatives were obtained. Attempts in dehydration of compound **5b** resulted in long reaction times and complex mixtures, which did not allow isolation of the desired product.

Conclusions

Two routes to obtain *N*-ethyl-α,β-dehydroamino acid derivatives involving alkylation and dehydration of *N*-nosyl-β-hydroxyamino acid derivatives are proposed. The route, in which alkylation occurs prior to dehydration, results in one step less, giving the *N*-ethyl derivatives of dehydroalanine and dehydrophenylalanine in overall yields of 67% and 57%, respectively. The corresponding dehydroaminobutyric acid and dehydrophenylalanine derivatives could also be obtained by the alternative route (dehydration prior to alkylation) in overall yields of 48% and 64%, respectively.

Thus, it was possible to obtain for the first time new non-natural amino acids, which incorporate both the *N*-ethyl and α,β-dehydro moieties. These can be interesting precursors of new peptides with potential pharmacological activity.

Experimental Section

General: Melting points [°C] were determined with a Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity Plus at 300 and 75.4 MHz, respectively, or with a Bruker Avance II$^+$ at 400 and 100.6 MHz, respectively. ^{1}H-^{1}H spin-spin decoupling, DEPT θ 45°, HMQC and HMBC were used to attribute some signals. Chemical shifts are given in ppm and coupling constants in Hz. MS and HRMS data were recorded by the mass spectrometry service of the University of Vigo, Spain; elemental analysis was performed with a LECO CHNS 932 elemental analyzer. The reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed on Macherey–Nagel silica gel 230–400 mesh. Petroleum ether refers to the fraction with boiling range 40–60 °C. When a solvent gradient was used, the increase of polarity was made from neat petroleum ether to mixtures of diethyl ether/petroleum ether by increasing the percentage of diethyl ether by 10% each time until the isolation of the product. Solvents were used without purification except for acetonitrile and dichloromethane, which were dried by using standard procedures.

Synthesis of the Methyl Esters of *N*-(4-Nitrophenylsulfonyl)-β-hydroxyamino Acids

Synthesis of Nosyl-L-Ser-OMe (1a): The synthesis of this compound was described elsewhere.[14]

Synthesis of Nosyl-L-Thr-OMe (1b): HCl·H-L-Thr-OMe (0.848 g, 5 mmol) was dissolved in dichloromethane (0.1 mol dm^{-3}) followed by addition of 2.2 equiv. of triethylamine and 1 equiv. of 4-nitrobenzenesulfonyl chloride with vigorous stirring and cooling in an ice bath. The reaction mixture was stirred at room temperature for 4 h. The solvent was then evaporated at reduced pressure. The residue was partitioned between ethyl acetate (100 cm^3) and $KHSO_4$ (1 mol dm^{-3}) (30 cm^3), and washed with $KHSO_4$ (1 mol dm^{-3}) and brine (2 times, 30 cm^3 each). After drying with $MgSO_4$, the extract was taken to dryness at reduced pressure to give **1b** (1.309 g, 82%) as a white solid. M.p. 120.0–122.0 °C (from ethyl acetate/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33 (d, J = 6.6 Hz, 3 H, γCH_3), 3.58 (s, 3 H, CH_3 OMe), 3.93 (dd, J = 2.4, J = 6.8 Hz, 1 H, βCH), 4.26–4.33 (m, 1 H, αCH), 5.61 (d, J = 9.9 Hz, 1 H, NH), 7.66 (d, J = 8.4 Hz, 2 H, ArH), 8.05 (d, J = 8.7 Hz, 2 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 20.03 (γCH_3), 52.89 (OCH_3), 60.96 (βCH), 68.27 (αCH), 124.21 (CH), 128.42 (CH), 145.79 (C), 150.09 (C), 170.45 (C=O) ppm. $C_{11}H_{14}N_2O_7S$ (318.30): calcd. C 41.36, H 4.48, N 8.72, S 10.11; found C 41.51, H 4.43, N 8.80, S 10.07.

Synthesis of Nosyl-D,L-Phe(β-OH)-OMe (1c): The same procedure as described for the preparation of **1b** was applied, substituting HCl·H-D,L-Phe(βOH)-OMe (1.158 g, 5 mmol) for HCl·H-L-Thr-OMe to give **1c** (1.693 g, 89%) as a yellow oil. M.p. 166.0–168.0 °C (from ethyl acetate/petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.44 (s, 3 H, CH_3 OMe), 4.14 (dd, J = 5.6, J = 5.2 Hz, 1 H, βCH), 5.08 (s, 1 H, αCH), 5.81 (s, 1 H, NH), 7.09–7.16 (m, 3 H, ArH Phe), 7.23–7.26 (m, 2 H, ArH Phe), 7.72 (d, J = 9.0 Hz, 2 H, ArH nosyl), 8.18 (d, J = 9.0 Hz, 2 H, ArH nosyl) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 51.94 (OCH_3), 62.72 (βCH), 72.45 (αCH), 123.94 (CH), 126.27 (CH), 127.08 (CH), 127.60 (CH), 127.66 (CH), 140.88 (C), 146.78 (C), 148.97 (C), 169.97 (C=O) ppm. $C_{16}H_{16}N_2O_7S$ (380.37): calcd. C 50.52, H 4.24, N 7.36, S 8.43; found C 50.05, H 4.23, N 7.22, S 8.50.

Synthesis of the Methyl Esters of *N*-(*tert*-Butoxycarbonyl)-*N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino Acids

Synthesis of Nosyl-ΔAbu(*N*-Boc)-OMe (2b): Nosyl-L-Thr-OMe (**1b**) (0.955 g, 3 mmol) was dissolved in dry acetonitrile (0.5 mol dm^{-3}), followed by the addition DMAP (0.1 equiv.) and di-*tert*-butyl dicarbonate (2.2 equiv.). The reaction mixture was stirred for 18 h. The solvent was evaporated at reduced pressure. Diethyl ether (100 cm^3) was added to the extract. The organic phase was washed with $KHSO_4$ (1 mol dm^{-3}), $NaHCO_3$ (1 mol dm^{-3}) and brine (3×30 cm^3) and then dried with $MgSO_4$. Removal of the solvent afforded **2b** (0.973 g, 81%) as a brown oil that solidified on standing. M.p. 95.0–96.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.33 (s, 9 H, CH_3 Boc), 2.05 (d, J = 7.2 Hz, 3 H, γCH_3), 3.80 (s, 3 H, CH_3 OMe), 7.35 (q, J = 7.2 Hz, 1 H, βCH), 8.37 (s, 4 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 14.92 (γCH_3), 27.73 [C(CH_3)$_3$], 52.52 (OCH_3), 85.44 [C(CH_3)$_3$], 123.46 (CH), 127.12 (C) 130.81 (CH), 144.66 (C), 145.03 (CH), 150.51 (C), 163.91 (C=O), 171.14 (C=O) ppm. HRMS (ESI): calcd. for $C_{16}H_{20}N_2NaO_8S$ 423.0838; found 423.0833.

Synthesis of Nosyl-ΔPhe(*N*-Boc)-OMe (2c): The same procedure as described for the preparation of **2b** was applied, substituting nosyl-D,L-Phe(β-OH)-OMe (**1c**) (0.761 g, 2 mmol) for **1b** to give **2c** (0.916 g, 99%) as a yellow oil. M.p. 135.0–136.0 °C (from ethyl acetate/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (s, 9 H, CH_3 Boc), 3.87 (s, 3 H, CH_3 OMe), 7.42–7.45 (m, 3 H, ArH Phe), 7.65–7.67 (m, 2 H, ArH Phe), 7.93 (s, 1 H, βCH), 8.23 (d, J = 9.2 Hz, 2 H, ArH nosyl), 8.30 (d, J = 9.2 Hz, 2 H, ArH nosyl) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 27.64 [C(CH_3)$_3$], 52.81 (OCH_3), 85.49 [C(CH_3)$_3$], 123.26 (C), 123.36 (CH), 129.04 (CH), 130.14 (CH), 131.06 (CH), 131.17 (CH), 132.09 (C), 142.79 (βCH), 144.28 (C), 149.50 (C), 150.50 (C=O), 164.96 (C=O) ppm. $C_{21}H_{22}N_2O_8S$ (462.48): calcd. C 54.54, H 4.79, N 6.06, S 6.93; found C 54.22, H 4.88, N 6.00, S 6.62.

Synthesis of the Methyl Esters of *N*-(4-Nitrophenylsulfonyl)-α,β-dehydroamino Acids

Synthesis of Nosyl-ΔAbu-OMe (3b): Nosyl-ΔAbu(*N*-Boc)-OMe (**2b**) (0.961 g, 2.4 mmol) was dissolved in dichloromethane (25 cm^3) followed by the addition of TFA (1 cm^3). The reaction mixture was stirred for 18 h. Then dichloromethane (75 cm^3) was added, and the organic phase was washed with $KHSO_4$ (1 mol dm^{-3}) and brine (3×30 cm^3) and dried with $MgSO_4$. Removal of the solvent afforded **3b** (0.605 g, 84%) as a white solid. M.p. 116.0–118.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.10 (d, J = 7.2 Hz, 3 H, γCH_3), 3.49 (s, 3 H, CH_3 OMe), 6.18 (s, 1 H, NH), 7.09 (q, J = 7.2 Hz, 1 H, βCH), 8.01 (d, J = 9.2 Hz, 2 H, ArH), 8.33 (d, J = 9.2 Hz, 2 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 15.24 (γCH_3), 52.56 (OCH_3), 123.98 (CH), 124.83 (C), 128.83 (CH), 141.92 (βCH), 144.83 (C), 150.23 (C), 164.11 (C=O) ppm. $C_{11}H_{12}N_2O_6S$ (300.29): calcd. C 44.00, H 4.03, N 9.33, S 10.68; found C 43.46, H 4.08, N 9.21, S 10.60. HRMS (ESI): calcd. for $C_{11}H_{12}N_2NaO_6S$ 323.0314; found 323.0308.

Synthesis of Nosyl-ΔPhe-OMe (3c): The same procedure as described for the preparation of **3b** was applied, substituting nosyl-ΔPhe(*N*-Boc)-OMe (**2c**) (0.462, 1 mmol) for **2b** to give **3c** (0.299 g, 83%) as a yellow oil. M.p. 149.0–150.0 °C (from ethyl acetate/petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.64 (s, 3 H, CH_3 OMe), 6.33 (s, 1 H, NH), 7.35–7.37 (m, 3 H, ArH), 7.60 (s, 1 H, βCH), 7.44–7.78 (m, 2 H, ArH), 7.96 (d, J = 8.7 Hz, 2 H, ArH nosyl), 8.25 (d, J = 8.7 Hz, 2 H, ArH nosyl) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 52.92 (OCH_3), 122.08 (C), 123.90 (CH), 128.65 (CH), 128.82 (CH), 130.69 (CH), 130.82 (CH), 132.32 (C), 138.46 (βCH), 145.14 (C), 150.17 (C), 165.19 (C=O) ppm. HRMS (ESI): calcd. for $C_{16}H_{14}N_2NaO_6S$ 385.0470; found 385.0467.

Synthesis of the Methyl Esters of *N*-Ethyl-*N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino Acids by Alkylation of the Methyl Esters of *N*-(4-Nitrophenylsulfonyl)-α,β-dehydroamino Acids

Synthesis of Nosyl-ΔAbu(*N*-Et)-OMe (4b): Nosyl-ΔAbu-OMe (**3b**) (0.150 g, 0.5 mmol) was dissolved in dry dichloromethane (0.05 mol dm^{-3}) followed by the addition of *N*,*N*-diisopropylethylamine (3.5 equiv.) and triethyloxonium tetrafluoroborate (2.2 equiv.) under an inert gas. The reaction mixture was stirred at room temperature for 30 min. Then dichloromethane (20 cm^3) was added. The organic phase was washed with $KHSO_4$ (1 mol dm^{-3}), $NaHCO_3$ (1 mol dm^{-3}) and brine (3×15 cm^3 each) and dried with $MgSO_4$. Removal of the solvent afforded **4b** (0.114 g, 70%) as a yellow oil that solidified on standing. M.p. 90.0–91.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.14 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 2.05 (d, J = 7.2 Hz, 3 H, γCH_3), 3.18 (br. s, 1 H, CH_2CH_3), 3.55 (s, 3 H, CH_3 OMe), 3.73 (br. s, 1 H, CH_2CH_3), 7.39 (q, J = 7.2 Hz, 1 H, βCH), 8.01 (d, J = 9.2 Hz, 2 H, ArH), 8.34 (d, J = 9.2 Hz, 2 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 13.97 (CH_3), 15.44 (γCH_3), 44.49 (CH_2), 52.06 (OCH_3), 123.82 (CH), 128.03 (C), 128.93 (CH), 145.44 (C), 147.55 (βCH), 149.93 (C), 163.79 (C=O) ppm. $C_{13}H_{16}N_2O_6S$ (328.24): calcd. C 47.55, H 4.91, N 8.53, S 9.77; found C 47.03, H 4.99, N 8.36, S 9.52. HRMS (ESI): calcd. for $C_{13}H_{16}N_2NaO_6S$ 351.0627; found 351.0621.

Synthesis of Nosyl-ΔPhe(*N*-Et)-OMe (4c): The same procedure as described for the preparation of **4b** was applied, substituting nosyl-ΔPhe-OMe (**3c**) (0.050 g, 0.138 mmol) for **3b** to give **4c** (0.042 g, 78%) as a yellow oil. M.p. 96.0–98.0 °C (from diethyl ether/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.08 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 3.46 (br. s, 1 H, CH_2CH_3), 3.64 (s, 3 H, CH_3 OMe), 3.77 (br. s, 1 H, CH_2CH_3), 7.43–7.45 (m, 3 H, ArH Phe), 7.88–7.90 (m, 3 H, ArH Phe, βCH), 8.03 (d, J = 9.2 Hz, 2 H, ArH nosyl), 8.34 (d, J = 9.2 Hz, 2 H, ArH nosyl) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 13.32 (CH_3), 44.70 (CH_2), 52.37 (OCH_3), 123.74 (CH), 124.50 (C), 128.84 (CH), 129.31 (CH), 131.18 (CH), 131.31 (CH), 132.14 (C), 144.35 (CH), 145.11 (C), 149.98 (C), 165.19 (C=O) ppm. $C_{18}H_{18}N_2O_6S$ (390.41): calcd. C 55.38, H 4.65, N 7.18, S 8.21; found C 55.28, H 4.70, N 7.11, S 7.93.

Synthesis of the Methyl Esters of *N*-Ethyl-*N*-(4-nitrophenylsulfonyl)-β-hydroxyamino Acids

Synthesis of Nosyl-L-Ser(*N*-Et)-OMe (5a): Nosyl-L-Ser-OMe (**1a**) (0.304 g, 1 mmol) was dissolved in dry dichloromethane (0.05 mol dm^{-3}), and *N*,*N*-diisopropylethylamine (3.5 equiv.) and triethyloxonium tetrafluoroborate (1.0 equiv.) were added under an inert gas. The reaction mixture was stirred at room temperature for 30 min. Then dichloromethane (80 cm^3) was added. The organic phase was washed with $KHSO_4$ (1 mol dm^{-3}), $NaHCO_3$ (1 mol dm^{-3}) and brine (3×25 cm^3) and dried with $MgSO_4$. Removal of the solvent afforded **5a** (0.307 g, 92%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26 (t, J = 7.2 Hz, 3 H, CH_3), 3.17–3.26 (m, 1 H, NCH_2CH_3), 3.39–3.48 (m, 1 H, NCH_2CH_3), 3.63 (s, 3 H, CH_3 OMe), 3.92–3.97 (m, 1 H, βCH_2), 4.11–4.16 (m, 1 H, βCH_2), 4.66 (t, J = 6.0 Hz, 1 H, αCH), 8.07 (d, J = 9.0 Hz, 2 H, ArH), 8.36 (d, J = 9.0 Hz, 2 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 15.83 (CH_3), 42.48 (NCH_2CH_3), 52.56 (OCH_3), 61.59 (βCH_2), 61.73 (αCH), 124.04 (CH), 128.74 (CH), 145.70 (C), 150.01 (C), 169.66 (C=O) ppm. HRMS (ESI): calcd. for $C_{12}H_{16}N_2NaO_7S$ 355.0576; found 355.0570.

Synthesis of Nosyl-L-Thr(*N*-Et)-OMe (5b): The same procedure as described for the preparation of **5a** was applied, substituting nosyl-L-Thr-OMe (**1b**) (0.318 g, 1 mmol) for **1a** to give **5b** (0.324 g, 94%) as brown oil, which was crystallized from diethyl ether/petroleum ether. M.p. 91.5–93.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32 (t, J = 7.2 Hz, 3 H, CH_3), 1.36 (d, J = 6.4 Hz, 3 H, γCH_3), 3.26–3.46 (m, 1 H, CH_2), 3.41–3.48 (m, 1 H, CH_2), 3.54 (s, 3 H, CH_3 OMe), 4.39–4.45 (m, 1 H, βCH), 4.47 (d, J = 5.2 Hz, 1 H, αCH), 8.05 (d, J = 8.8 Hz, 2 H, ArH), 8.36 (d, J = 8.8 Hz, 2 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 15.76 (CH_3), 19.87 (γCH_3), 43.20 (CH_2), 52.31 (OCH_3), 65.42 (αCH), 66.91 (βCH), 123.89 (CH), 128.87 (CH), 145.36 (C), 149.96 (C), 169.76 (C=O) ppm. HRMS (ESI): calcd. for $C_{13}H_{18}N_2NaO_7S$ 369.0732; found 369.0728.

Synthesis of Nosyl-D,L-Phe(β-OH)(*N*-Et)-OMe (5c): The same procedure as described for the preparation of **5a** was applied, substituting nosyl-D,L-Phe(β-OH)-OMe (**1c**) (0.380 g, 1 mmol) for **1a** to give **5c** (0.376 g, 92%) as a yellow oil, which was crystallized from ethyl acetate/petroleum ether. M.p. 164.0–166.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (t, J = 6.8 Hz, 3 H, CH_3), 3.47 (s, 3 H, CH_3 OMe), 3.47–3.58 (m, 2 H, CH_2), 4.81 (d, J = 7.2 Hz, 1 H, αCH), 5.23 (d, J = 7.2 Hz, 1 H, βCH), 7.34–7.37 (m, 5 H, ArH), 7.86 (d, J = 8.4 Hz, 2 H, nosyl ArH), 8.24 (d, J = 8.4 Hz, 2 H, nosyl ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 14.14 (CH_3), 42.01 (CH_2), 52.22 (OCH_3), 65.32 (αCH), 72.22 (βCH), 123.83 (CH), 126.72 (CH), 128.49 (CH), 128.61 (CH), 128.77 (CH), 139.15 (C), 145.79 (C), 149.81 (C), 169.40 (C=O) ppm. HRMS (ESI): calcd. for $C_{18}H_{20}N_2NaO_7S$ 431.0889; found 431.0887.

Synthesis of the Methyl Esters of *N*-Ethyl-*N*-(4-nitrophenylsulfonyl)-α,β-dehydroamino Acids by Dehydration of the Methyl Esters of *N*-Ethyl-*N*-(4-nitrophenylsulfonyl)-β-hydroxyamino Acids

Synthesis of Nosyl-ΔAla(*N*-Et)-OMe (4a): Nosyl-L-Ser(*N*-Et)-OMe (**5a**) (0.166 g, 0.5 mmol) was dissolved in dry acetonitrile (0.1 mol dm^{-3}), and DMAP (0.1 equiv.) followed by di-*tert*-butyl dicarbonate (1.1 equiv.) were added. The reaction mixture was stirred for 30 min and then TMG added. After stirring for additional 15 min, the solvent was evaporated at reduced pressure. Diethyl

ether (100 cm^3) was added to the extract. The organic phase was washed with $KHSO_4$ (1 $mol\,dm^{-3}$), $NaHCO_3$ (1 $mol\,dm^{-3}$) and brine (3 × 30 cm^3) and then dried with $MgSO_4$. Removal of the solvent afforded compound **4a** (0.115 g, 73%) as a yellow oil. M.p. 69.0–70.0 °C (from ethyl acetate/petroleum ether). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.16 (t, J = 7.2 Hz, 3 H, CH_3), 3.50 (q, J = 7.2 Hz, 2 H, NCH_2CH_3), 3.68 (s, 3 H, CH_3 OMe), 5.96 (s, 1 H, βCH_2), 6.54 (s, 1 H, βCH_2), 8.01 (d, J = 9.2 Hz, 2 H, ArH), 8.35 (d, J = 9.2 Hz, 2 H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 13.99 (CH_3), 44.30 (NCH_2CH_3), 52.58 (OCH_3), 124.04 (CH), 128.84 (CH), 130.48 (βCH_2), 134.43 (αC), 145.00 (C), 150.06 (C), 163.63 (C=O) ppm. HRMS (ESI): calcd. for $C_{12}H_{14}N_2NaO_6S$ 337.0470; found 337.0465.

Synthesis of Nosyl-ΔPhe(*N*-Et)-OMe (4c): The same procedure as described for the preparation of **4a** was applied, substituting nosyl-D,L-Phe(β-OH)(*N*-Et)-OMe (**5c**) (0.380 g, 0.93 mmol) for **5a** to give **4c** (0.226 g, 62%).

Acknowledgments

The Foundation for Science and Technology (FCT) – Portugal and the Fundo Europeu de Desenvolvimento Regional (FEDER) are acknowledged for financial support to the Chemistry Centre of the University of Minho. The NMR spectrometer Bruker Avance II$^+$ 400 is part of the National NMR Network and was purchased in the framework of the National Programme for Scientific Re-equipment (contract REDE/1517/RMN/2005) with funds from the Programa Operacional Ciência e Inovação (POCI 2010), FEDER and FCT.

[1] T. Goodman, L. Moroder in *Synthesis of Peptides and Peptidomimetics*, Houben-Weyl, Thieme, Stuttgart, Germany, **2003**, vol. E22c, pp. 215–271.
[2] L. Aurelio, R. T. C. Brownlee, A. B. Hughes, *Chem. Rev.* **2004**, *104*, 5823–5846, and references therein.
[3] R. M. Wenger, *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 77–85.
[4] R. T. Shuman, E. L. Smithwick, R. C. A. Frederickson, P. D. Gesellchen, *Peptides: Proceedings of the 7th American Peptide Symposium* (Eds.: D. Rich, E. Gross), Pierce Chemical Co., Rockford, IL, USA, **1981**, pp. 617–618.
[5] T. Ruckle, B. Dubray, F. Hubler, M. J. Mutter, *Pept. Sci.* **1999**, *5*, 56–58.
[6] D. Papaioannou, C. Athanassopoulos, V. Magafa, N. Karamanos, G. Stavropoulos, A. Napoli, G. Sindona, D. W. Aksnes, G. W. Francis, *Acta Chem. Scand.* **1994**, *48*, 324–333.
[7] C. Athanassopoulos, H. L. S. Maia, L. S. Monteiro, D. Papaioannou *Peptides 1996: Proceedings of the 24th European Peptide Symposium* (Eds.: R. Ramage, R. Epton), Mayflower Scientific Ltd., Kingswinford, U.K., **1998**, pp. 219–220.
[8] F. Albericio, *Biopolymers* **2000**, *55*, 123–139.
[9] a) M. L. Di Gioia, A. Leggio, A. Liguori, F. Perri, C. Siciliano, M. C. Viscomi, *Amino Acids* **2010**, *38*, 133–143; b) E. L. Belsito, R. De Marco, M. L. Di Gioia, A. Liguori, F. Perri, M. C. Viscomi, *Eur. J. Org. Chem.* **2010**, 4245–4252.
[10] a) D. E. Palmer, C. Pattaroni, K. Nunami, R. K. Chadha, M. Goodman, T. Wakamiyia, K. Fukase, S. Horimoto, M. Kitazawa, H. Fujita, A. Kubo, T. Shiba, *J. Am. Chem. Soc.* **1992**, *114*, 5634–5642; b) G. Jung, *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1051–1068; c) C. Chatterjee, M. Paul, L. Xie, W. A. Van Der Donk, *Chem. Rev.* **2005**, *105*, 633–684.
[11] a) U. Schmidt, J. Hausler, E. Olher, H. Poisel, *Fortschr. Chem. Org. Naturst.* **1977**, *37*, 251–300; b) K. Noda, Y. Shimohigashi, N. Izumiya, *The Peptides, Analysis, Synthesis Biology*; Academic Press: New York, **1983**, Vol. 5, pp. 286; c) U. Schmidt, A. Lieberknecht, J. Wild, *Synthesis* **1988**, 159–172; d) S. Nomoto, A. Sano, T. Shiba, *Tetrahedron Lett.* **1979**, *20*, 521–522; e) R. H. Mazur, D. R. Pilipauskas, *Peptides 1982* (Ed.: W. de Gruyter), Berlin, **1983**, pp. 319; f) C. Bonauer, T. Walenzyk, B. König, *Synthesis* **2006**, *1*, 1–20.
[12] P. M. T. Ferreira, H. L. S. Maia, L. S. Monteiro, J. Sacramento, *J. Chem. Soc. Perkin Trans. 1* **1999**, 3697–3703.
[13] P. M. T. Ferreira, L. S. Monteiro, G. Pereira, L. Silva, L. Ribeiro, J. Sacramento, *Eur. J. Org. Chem.* **2007**, 5934–5949.
[14] P. M. T. Ferreira, H. L. S. Maia, L. S. Monteiro, *Eur. J. Org. Chem.* **2003**, *14*, 2635–2644.

Received: September 17, 2010
Published Online: October 28, 2010

