SHORT COMMUNICATION

DOI: 10.1002/ejoc.200((will be filled in by the editorial staff))

Synthesis of novel non-proteinogenic amino acids: *N*-ethyl-α,β-dehydroamino acid methyl esters

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

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

Two routes for the synthesis of *N*-(4-nitrobenzenesulfonyl), *N*-ethyl-α,β-dehydroamino acid derivatives from serine, threonine and phenylserine derivatives are presented. One route consists of dehydration of *N*-(4-nitrobenzenesulfonyl)-β-hydroxyamino acid methyl esters with *tert*-butylpyrocarbonate catalyzed by dimethylaminopyridine, followed by alkylation of the *N*-(4-nitrobenzenesulfonyl)-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 a α,β-dehydro moiety.

[a] Chemistry Centre, University of Minho, Gualtar, 4710-057 Braga, Portugal.
Fax: +351 253604382
E-mail: monteiro@quimica.uminho.pt

Introduction

Non-proteinogenic 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 non-proteinogenic 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*-methyl-leucine is found in cyclosporines.[3] A substitution of *N*-methyl-leucine of cyclosporine A by various *N*-ethyl amino 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 co-workers 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, making these groups unique for the preparation of *N*-alkyl peptides.[8] *N*-Nitrobenzenesulfonyl 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-nitrobenzenesulfonyl (Nosyl) protected amino acids using triethyloxonium tetrafluoroborate (Et_3OBF_4) as alkylating agent to give *N*-ethyl amino 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.

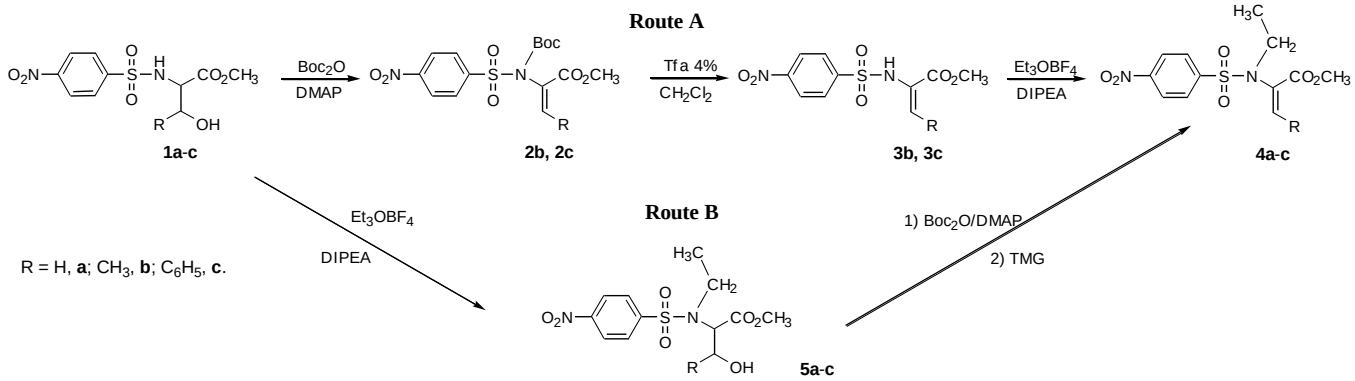
Dehydroamino acids can be found in several yeasts and bacteria, in which they contribute with a catalytic role in the active sites of some enzymes, as well as in a variety of peptide antibiotics of bacterial origin that include the lantibiotics (nisin, epidermin, subtilin, gallidermin).[10] Since they 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. In our laboratories we developed an efficient method for the synthesis of *N,N*-diacyl-α,β-dehydroamino acid derivatives by using two equivalents of *tert*-butylpyrocarbonate (Boc_2O) and 4-dimethylaminopyridine (DMAP) as catalyst in dry acetonitrile.[12] In order to allow the synthesis of *N*-acyl-α,β-dehydroamino acid derivatives, a modification of this method was subsequently reported.[13] Thus, by reacting β-hydroxyamino acid derivatives with one equivalent of *tert*-butylpyrocarbonate 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 non-proteinogenic 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 equivalents 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 protecting and subsequent deprotecting, 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).



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

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 one equivalent of *tert*-butylpyrocarbonate using DMAP as catalyst, followed by treatment with TMG.[13] However, this method led to complex mixtures resulting from *tert*-butylcarbonylation of the hydroxyl group and also of the sulfonamide function. Thus, the alternative reaction with two equivalents of *tert*-butylpyrocarbonate was carried out.[12] In the case of reaction with compounds **1b** and **1c** the corresponding *N*-(4-nitrobenzenesulfonyl), *N*-(*tert*-butyloxycarbonyl)-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*-(4-nitrobenzenesulfonyl), *N*-ethyl-α,β-dehydroamino acids.*

Compound	Yield/%	Compound	Yield/%	Compound	Yield/%
2a	--	**3a**	--	**4a**	--
2b	81	**3b**	84	**4b**	70
2c	99	**3c**	83	**4c**	78

* Route A (Scheme 1).

However, in the case of reaction with coupound **1a** the major product obtained was the methyl ester of *N*-(*tert*-butyloxycarbonyl), β-(4-nitrophenylsulfinyl)-α,β-dehydroserine.[14] The introduction of the *tert*-butyloxycarbonyl group makes necessary a deprotection step prior to alkylation, so 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 subject to ethylation using the conditions proposed by Liguori et al.[9b] [2.5 equivalents of triethyloxonium tetrafluoroborate, 3.5 equivalents of *N,N*-diisopropylethylamine (DIPEA) in dry dichloromethane] to give the corresponding *N*-Nosyl, *N*-ethyl-α,β-dehydroamino acid derivatives in good yields (compounds **4b** and **4c**, Scheme 1, Table 1).

In order to avoid the *tert*-butyloxycarbonyl 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 reacted directly without side chain protection with 1 equivalent of triethyloxonium tetrafluoroborate. Fortunately, the reaction was regioselective giving in high yield (92-94%) the corresponding *N*-Nosyl, *N*-ethyl-β-hydroxyamino acid derivative (compounds **5a-c**, Scheme 1, Table 2).

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

Compound	Yield/%	Compound	Yield/%
5a	92	**4a**	73
5b	94	**4b**	--
5c	92	**4c**	62

*Route B (Scheme 1).

These could now be dehydrated by reaction with one equivalent of *tert*-butylpyrocarbonate followed by treatment with TMG. In the case of reaction with compounds **5a** and **5c**, good yields in the corresponding *N*-Nosyl, *N*-ethyl-α,β-dehydroamino acid derivative were obtained. Attempts in dehydration of compound **5b** resulted in long reaction times and complex mixtures which did not allow isolation of the wanted 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

Melting points (ºC) were determined in a Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus at 300 and 75.4 MHz, respectively or on a Bruker Avance II^{+} at 400 and 100.6 MHz, respectively. ^{1}H-^{1}H spin-spin decoupling, DEPT θ 45º,

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 on 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 boiling range 40-60 ºC. When solvent gradient was used, the increase of polarity was made from neat petroleum ether to mixtures of diethyl ether/petroleum ether, increasing 10% of diethyl ether each time until the isolation of the product. Solvents were used without purification except for acetonitrile and dichloromethane which were dried using standard procedures.

Synthesis of the methyl esters of *N*-(4-nitrobenzenesulfonyl)-β-hydroxyamino acids

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

Synthesis of Nosyl-L-Thr-OMe (compound 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 eq. of triethylamine and 1 eq. of 4-nitrobenzenesulfonyl chloride with vigorous stirring and cooling in an ice bath. The reaction mixture was stirred at room temperature for 4 hours. The solvent was then evaporated at reduced pressure. The extract was partitioned between 100 cm^3 of ethyl acetate and 30 cm^3 of $KHSO_4$ (1 mol dm^{-3}), and washed with $KHSO_4$ (1 mol dm^{-3}) and brine (2 times, 30 cm^3 each). After drying over $MgSO_4$ 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, 3H, γCH_3), 3.58 (s, 3H, CH_3OMe), 3.93 (dd, *J* = 2.4 Hz, *J* = 6.8 Hz, 1H, βCH), 4.26-4.33 (m, 1H, αCH), 5.61 (d, *J* = 9.9 Hz, 1H, NH), 7.66 (d, *J* = 8.4 Hz, 2H, ArH), 8.05 (d, *J* = 8.7 Hz, 2H, 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. Found C, 41.51; H, 4.43; N, 8.80; S, 10.07; $C_{11}H_{14}N_2O_7S$ (318.30) requires C, 41.36; H, 4.48; N, 8.72; S, 10.11.

Synthesis of Nosyl-D,L-Phe(β-OH)-OMe (compound 1c): The same procedure as described for the preparations of **1b** was followed 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, 3H, CH_3OMe), 4.14 (dd, *J* = 5.6 Hz, *J* = 5.2 Hz, 1H, βCH), 5.08 (s, 1H, αCH), 5.81 (s, 1H, NH), 7.09-7.16 (m, 3H, ArH Phe), 7.23-7.26 (m, 2H, ArH Phe), 7.72 (d, *J* = 9.0 Hz, 2H, ArH Nosyl), 8.18 (d, *J* = 9.0 Hz, 2H, 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. Found C, 50.05; H, 4.23; N, 7.22; S, 8.50; $C_{16}H_{16}N_2O_7S$ (380.37) requires C, 50.52; H, 4.24; N, 7.36; S, 8.43.

Synthesis of the methyl esters of *N*-(4-nitrobenzenesulfonyl), *N*-(*tert*-butyloxycarbonyl)-α,β-dehydroamino acids

Synthesis of Nosyl-ΔAbu(*N*-Boc)-OMe (compound 2b): Nosyl-L-Thr-OMe (**1b**) (0.955 g, 3 mmol) was dissolved in dry acetonitrile (0.5 mol dm^{-3}), followed by addition 0.1 eq. of DMAP and 2.2 eq. of *tert*-butylpyrocarbonate. The reaction mixture was stirred for 18 hours. 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 x 30 cm^3) and then was dried over $MgSO_4$. Removal of the solvent afford **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, 9H, CH_3 Boc), 2.05 (d, *J* = 7.2 Hz, 3H, γCH_3), 3.80 (s, 3H, CH_3OMe), 7.35 (q, *J* = 7.2 Hz, 1H, βCH), 8.37 (s, 4H, ArH) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 14.92 (γCH_3), 27.73 [C(*C*H_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 (compound 2c): The same procedure as described for the preparations of **2b** was followed 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, 9H, CH_3 Boc), 3.87 (s, 3H, CH_3OMe), 7.42-7.45 (m, 3H, ArH Phe), 7.65-7.67 (m, 2H, ArH Phe), 7.93 (s, 1H, βCH), 8.23 (d, *J* = 9.2 Hz, 2H, ArH Nosyl), 8.30 (d, *J* = 9.2 Hz, 2H, ArH Nosyl) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 27.64 [C(*C*H_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. Found C, 54.22; H, 4.88; N, 6.00; S, 6.62; $C_{21}H_{22}N_2O_8S$ (462.48) requires C, 54.54; H, 4.79; N, 6.06; S, 6.93.

Synthesis of the methyl esters of *N*-(4-nitrobenzenesulfonyl)-α,β-dehydroamino acids

Synthesis of Nosyl-ΔAbu-OMe (compound 3b): Nosyl-ΔAbu(*N*-Boc)-OMe (**2b**) (0.961 g, 2.4 mmol) was dissolved in dichloromethane (25 cm^3) followed by addition of TFA (1 cm^3). The reaction mixture was stirred for 18 hours. Then dichloromethane (75 cm^3) was added and the organic phase was washed with $KHSO_4$ (1 mol dm^{-3}) and brine (3 x 30 cm^3) and was dried over $MgSO_4$. Removal of the solvent afford **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, 3H, γCH_3), 3.49 (s, 3H, CH_3OMe), 6.18 (s, 1H, NH), 7.09 (q, *J* = 7.2 Hz, 1H, βCH), 8.01 (d, *J* = 9.2 Hz, 2H, ArH), 8.33 (d, *J* = 9.2 Hz, 2H, 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. Found C, 43.46; H, 4.08; N, 9.21; S, 10.60; $C_{11}H_{12}N_2OS$ (300.29) requires C, 44.00; H, 4.03; N, 9.33; S, 10.68. HRMS (ESI): calcd. for $C_{11}H_{12}N_2NaO_6S$ 323.0314; found 323.0308.

Synthesis of Nosyl-ΔPhe-OMe (compound 3c): The same procedure as described for the preparations of **3b** was followed 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, 3H, CH_3OMe), 6.33 (s, 1H, NH), 7.35-7.37 (m, 3H, ArH), 7.60 (s, 1H, βCH), 7.44-7.78 (m, 2H, ArH), 7.96 (d, *J* = 8.7 Hz, 2H, ArH Nosyl), 8.25 (d, *J* = 8.7 Hz, 2H, 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*-(4-nitrobenzenesulfonyl), *N*-ethyl-α,β-dehydroamino acids by alkylation of the methyl esters of *N*-(4-nitrobenzenesulfonyl)-α,β-dehydroamino acids

Synthesis of Nosyl-ΔAbu(*N*-Et)-OMe (compound 4b): Nosyl-ΔAbu-OMe (**3b**) (0.150 g, 0.5 mmol) was dissolved in dry dichloromethane (0.05 mol dm^{-3}) followed by addition 3.5 eq. of *N*,*N*-diisopropylethylamine and 2.2 eq. triethyloxonium tetrafluoroborate under inert atmosphere. 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 x 15 cm^3 each) and was dried over $MgSO_4$. Removal of the solvent afford **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, 3H, CH_2CH_3), 2.05 (d, *J* = 7.2 Hz, 3H, γCH_3), 3.18 (br. s, 1H, CH_2CH_3), 3.55 (s, 3H, CH_3OMe), 3.73 (br. s, 1H, CH_2CH_3), 7.39 (q, *J* = 7.2 Hz, 1H, βCH), 8.01 (d, *J* = 9.2 Hz, 2H, ArH), 8.34 (d, *J* = 9.2 Hz, 2H, 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. Found C, 47.03; H, 4.99; N, 8.36; S, 9.52;

$C_{13}H_{16}N_2O_6S$ (328.24) requires C, 47.55; H, 4.91; N, 8.53; S, 9.77. HRMS (ESI): calcd. for $C_{13}H_{16}N_2NaO_6S$ 351.0627; found 351.0621.

Synthesis of Nosyl-ΔPhe(*N*-Et)-OMe (compound 4c): The same procedure as described for the preparations of **4b** was followed 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, 3H, CH_2CH_3), 3.46 (br. s, 1H, CH_2CH_3), 3.64 (s, 3H, CH_3 OMe), 3.77 (br. s, 1H, CH_2CH_3), 7.43-7.45 (m, 3H, ArH Phe), 7.88-7.90 (m, 3H, ArH Phe + βCH), 8.03 (d, *J* = 9.2 Hz, 2H, ArH Nosyl), 8.34 (d, *J* = 9.2 Hz, 2H, 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. Found C, 55.28; H, 4.70; N, 7.11; S, 7.93; $C_{18}H_{18}N_2O_6S$ (390.41) requires C, 55.38; H, 4.65; N, 7.18; S, 8.21.

Synthesis of the methyl esters of *N*-(4-nitrobenzenesulfonyl), *N*-ethyl-β-hydroxyamino acids

Synthesis of Nosyl-L-Ser(*N*-Et)-OMe (compound 5a): Nosyl-L-Ser-OMe (**1a**) (0.304 g, 1 mmol) was dissolved in dry dichloromethane (0.05 mol dm^{-3}) and 3.5 eq. of *N*,*N*-diisopropylethylamine and 1.0 eq. of triethyloxonium tetrafluoroborate added under inert atmosphere. 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 x 25 cm^3) and was dried over $MgSO_4$. 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, 3H, CH_3), 3.17-3.26 (m, 1H, NCH_2CH_3), 3.39-3.48 (m, 1H, NCH_2CH_3), 3.63 (s, 3H, CH_3 OMe), 3.92-3.97 (m, 1H, βCH_2), 4.11-4.16 (m, 1H, βCH_2), 4.66 (t, *J* = 6.0 Hz, 1H, αCH), 8.07 (d, *J* = 9.0 Hz, 2H, ArH), 8.36 (d, *J* = 9.0 Hz, 2H, 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 (compound 5b): The same procedure as described for the preparations of **5a** was followed substituting Nosyl-L-Thr-OMe (**1b**) (0.318 g, 1 mmol) for **1a** to give **5b** (0.324 g, 94%) as brown oil. M.p. 91.5-93.0 ºC (from diethyl ether/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32 (t, *J* = 7.2 Hz, 3H, CH_3), 1.36 (d, *J* = 6.4 Hz, 3H, γCH_3), 3.26-3.46 (m, 1H, CH_2), 3.41-3.48 (m, 1H, CH_2), 3.54 (s, 3H, CH_3 OMe), 4.39-4.45 (m, 1H, βCH), 4.47 (d, *J* = 5.2 Hz, 1H, αCH), 8.05 (d, *J* = 8.8 Hz, 2H, ArH), 8.36 (d, *J* = 8.8 Hz, 2H, 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 (compound 5c): The same procedure as described for the preparations of **5a** was followed 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. M.p. 164.0-166.0 °C (from ethyl acetate/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (t, *J* = 6.8 Hz, 3H, CH_3), 3.47 (s, 3H, CH_3 OMe), 3.47-3.58 (m, 2H, CH_2), 4.81 (d, *J* = 7.2 Hz, 1H, αCH), 5.23 (d, *J* = 7.2 Hz, 1H, βCH), 7.34-7.37 (m, 5H, ArH), 7.86 (d, *J* = 8.4 Hz, 2H, Nosyl ArH), 8.24 (d, *J* = 8.4 Hz, 2H, 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*-(4-nitrobenzenesulfonyl), *N*-ethyl-α,β-dehydroamino acids by dehydration of the methyl esters of *N*-(4-nitrobenzenesulfonyl), *N*-ethyl-β-hydroxyamino acids

Synthesis of Nosyl-ΔAla(*N*-Et)-OMe (compound 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 0.1 eq. of DMAP followed by 1.1 eq. of *tert*-butylpyrocarbonate added. The reaction mixture was stirred for 30 minutes and then TMG added. After stirring for 15 more minutes, 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 x 30 cm^3) and then was dried over $MgSO_4$. Removal of the solvent afforded compound **4a** (0.115 g, 73%) as a yellow oil. M.p. 69.0-70.0 ºC (from ethyl acetate/petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.16 (t, *J* = 7.2 Hz, 3H, CH_3), 3.50 (q, *J* = 7.2 Hz, 2H, NCH_2CH_3), 3.68 (s, 3H, CH_3 OMe), 5.96 (s, 1H, βCH_2), 6.54 (s, 1H, βCH_2), 8.01 (d, *J* = 9.2 Hz, 2H, ArH), 8.35 (d, *J* = 9.2 Hz, 2H, 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 (compound 4c): The same procedure as described for the preparations of **4a** was followed 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

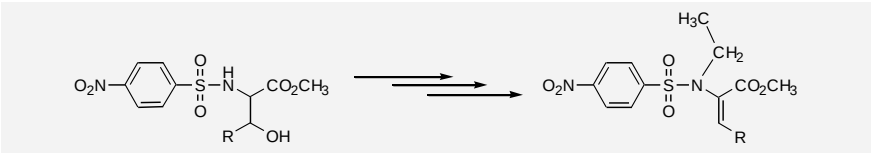
Foundation for Science and Technology (FCT) – Portugal and Fundo Europeu de Desenvolvimento Regional (FEDER) for financial support to Chemistry Centre of University of Minho. The NMR spectrometer Bruker Avance II$^+$ 400 is part of the National NMR Network and was purchased in the framework of the National Programme for Scientific Re-equipment, contract REDE/1517/RMN/2005, with funds from 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 cited 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: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

Layout 2:



Luís S. Monteiro*, Joanna Kołomańska, Ana C. Suarez ... Page No. – Page No.

Synthesis of novel non-proteinogenic amino acids: *N*-ethyl-α,β-dehydroamino acid methyl esters

Two routes for the synthesis of *N*-(4-nitrobenzenesulfonyl), *N*-ethyl-α,β-dehydroamino acid derivatives from serine, threonine and phenylserine derivatives are presented. In both a sequential alkylation-dehydration procedure is carried but in alternative sequence. This methodology allowed the synthesis for the first time of new non-natural amino acids which incorporate both the *N*-ethyl and α,β-dehydro moieties.

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