SYNTHESIS OF BICYCLIC PYRIDINE TRIPEPTIDES

Abd El-Hamid ATTIA, Mohamed H. ABO-GHALIA and Osama I. ABD EL-SALAM

National Research Centre,
Dokki, Cairo, Egypt

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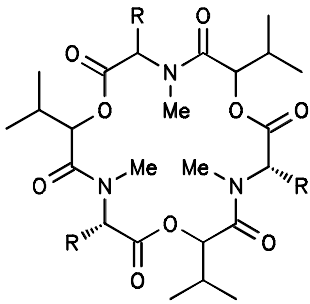
Dinicotinic acid reacted with L-phenylalanine affording *N*-dinicotinoyl-bis-L-phenylalanine (*VII*). The same product was also obtained by mild alkaline hydrolysis of the corresponding ester *VI*. Coupling of *VII* with L-ornithine or L-ornithine methyl ester gave rise to the formation of the desired chiral bicyclic tripeptides *IIIa* and *IIIb* as enniatin analogues.

Cyclic peptides are interesting biological compounds of diverse activities[1]. The cyclic peptide antibiotic enniatin[2] (*I*) is merely an example. Recently, Talma et al.[3], starting from 3,5-pyridine dicarboxylic acid (dinicotinic acid) and a variety of L-amino acids, reported the synthesis of chiral bridged bis-coupled amino acid dihydropyridines *II*. These compounds, which have an architectural resemblance to enniatin (*I*), were also valuable for their chemical activity comparable to that of enzyme cofactors. Thus, they were sufficiently active to carry out enantioselective reductions of keto groups to corresponding secondary alcohols.

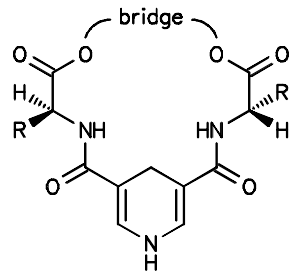
The present study deals with the synthesis of similar chiral cyclic tripeptide systems, where L-ornithine performs the final ring closure rather than a dihalogeno compound used by Talma[3]. The lateral substituents present positions at which other moieties could be easily linked. Nicotinoylphenylalanine derivatives are reported to possess biological activities such as antimicrobial[4] or glucose and cholesterol level lowering[5].

Synthesis of *VII* was initially attempted by DCCI/HOSu method[6–9] (Scheme 1). The in situ prepared *N*-hydroxysuccinimide active ester of dinicotinic acid *IV* was coupled with L-phenylalanine in alkaline aqueous medium in a poor yield (16%). It was then found more convenient to get *VII* by the mild alkaline hydrolysis of its corresponding ethyl ester *VI*, which was successfully obtained by a low temperature coupling of a freshly prepared 3,5-pyridinedicarbonyl chloride (*V*) with the amino acid ethyl ester in presence of triethylamine (TEA). Cyclization of *VII* by the potentiated DCCI/1-hydroxybenzotriazole (HOBt) method[10–13] afforded a pure bis-coupled product *IIIa* in 42% yield. Characterization of the obtained compound *IIIa* was based on the qualitative chromatographic analysis (TLC) of its hydrolyzed products where dinicotinic acid, phenylalanine and ornithine were detected. The structure of *IIIa* was confirmed in light

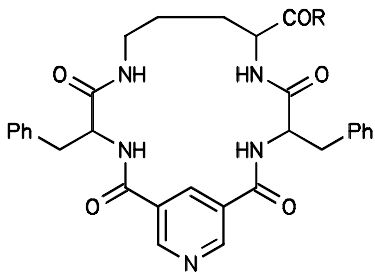
of its spectroscopic features. Its mass spectrum is devoid of the molecular ion peak [M^+], which may easily split off the carboxylic group. The ion [M – COOH]$^+$ (*m*/*z* 512, 2%) was detected which on further fragmentation revealed ion at *m*/*z* 459 (10%) after cleavage of the bridged alkyl group. Other ion peaks were found to be in agreement with the assigned structure (Scheme 2). The ^{1}H NMR spectrum proved the bridged methylene protons in the range 1.1 – 2.9 ppm and the methine proton at δ 3.1.



I



II

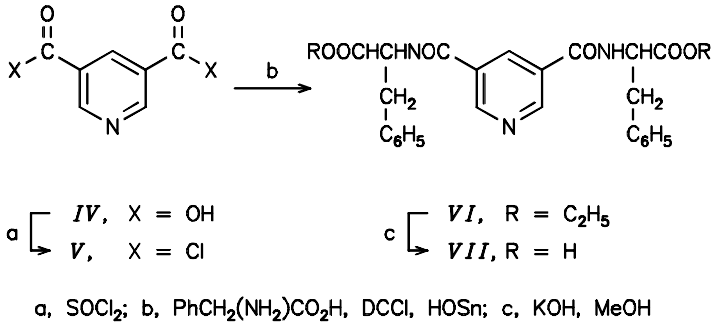


III

a, R = OH

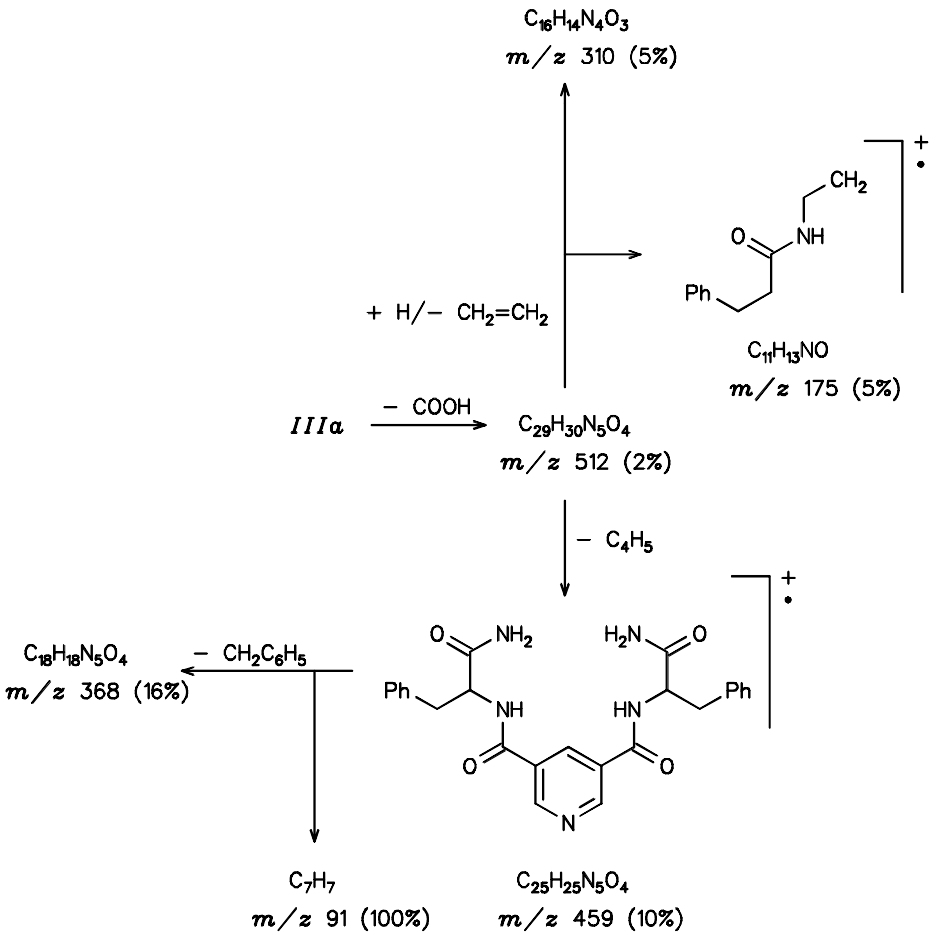
b, R = OCH_3

A trial to esterify *IIIa* by classical Curtius method[14] was unsuccessful. Typical hydrolyzed products were obtained as identified by TLC qualitative analysis. This may be attributed to ring instability under the used reaction conditions. However, coupling L-ornithine methyl ester with *VII* by the DCCI/HOBt method afforded the desired bis-coupled ester *IIIb* which was further purified by preparative TLC (silica gel). The IR



a, $SOCl_2$; b, $PhCH_2(NH_2)CO_2H$, DCCl, HOSn; c, KOH, MeOH

SCHEME 1



SCHEME 2

spectrum showed bands at 3 040 cm^{-1} and 2 950 cm^{-1} [ν(CH), aliphatic and aromatic], at 1 660, 1 550, 1 290 cm^{-1} (amide I, II and III bands) and at 1 780 cm^{-1} [ν(C=O, ester)]. Its ^1H NMR spectrum exhibited the same signals found in that of *IIIa* with the exception of the downfield difference of 1.1 ppm for methyl ester protons. In addition, ^{13}C NMR spectrum of *IIIb* gave signals corresponding to the bridgehead methylene carbons in the range 39.57 – 33.88 ppm and the methine carbon at δ 56.68.

Results for the biological properties of the obtained products will be published separately.

EXPERIMENTAL

All melting points are uncorrected. The infrared spectra (in KBr, given in cm^{-1}) were recorded on Unicam SP-1000 spectrophotometer. The ^1H NMR spectra (given in δ ppm) were recorded on EM-390 Varian spectrophotometer in $(CD_3)_2SO$ using tetramethylsilane as an internal standard.

The mass spectrum (ionization by electron impact, EI) was performed by the organic chemistry laboratory, Bonn University, Germany. Acceleration voltage: 2 kV, electron energy: 70 eV (100 μA) and the ion source temperature: 150 °C. The spectra were recorded during heating of the sample (150 °C).

The reactions were followed up and the purity of the synthesized compounds was checked by thin-layer chromatography (Merck, aluminium sheets, silica gel 60, F_{254}) in the proper solvent systems BuOH–pyridine–AcOH–H_2O 120 : 40 : 12 : 48 (S1); acetone–AcOH–H_2O 18 : 1 : 2 (S2); S2–light petroleum 2 : 1 (S3), visualizing the spots in ultraviolet light and/or ninhydrin.

3,5-Pyridinedicarbonyl Dichloride (*V*)

A mixture of dinicotinic acid (*IV*) (1.67 g, 10 mmol) and thionyl chloride (5 ml) in dry dioxane (15 ml) was refluxed for 1 h. Excess solvent was distilled off under reduced pressure and the crude mass was triturated with dry benzene (3 × 15 ml). The acid chloride was obtained as a dark brown oil which was immediately used.

N^{α}-Dinicotinoyl-bis-L-phenylalanine Ethyl Ester (*VI*)

Triethylamine (5.7 ml; 40 mmol) was added in portions to a well stirred cold solution (–5 °C) of 3,5-pyridinedicarbonyl dichloride (*V*) (2 g; 10 mmol) and L-phenylalanine ethyl ester hydrochloride (4 g; 20 mmol) in 450 ml mixture benzene–methylene chloride 4 : 5. The reaction mixture was stirred for 3 h at the same temperature with simultaneous adjustment of pH ≈ 8. Triethylamine hydrochloride was filtered off, and washed with water (3 × 10 ml), 1 M sodium bicarbonate (3 × 10 ml) and 1 M HCl (3 × 10 ml); then dried over anhydrous sodium sulfate. The solvent was distilled off in vacuo and the obtained oil was solidified and recrystallized from ethanol–hexane to afford white crystals of *VI* (3.45 g; 67% yield); m.p. 122 – 123 °C; R_F = 0.52 (S1); $[\alpha]_D^{30}$ = –77.3 (*c* = 0.05, absolute ethanol); IR spectrum (KBr): 3 313 (NH), 1 733 (C=O, ester), 1 647 (amide I), 1 527 (amide II) and 1 293 (amide III). For $C_{29}H_{31}N_3O_6$ (517.6) calculated: 67.29% C, 6.04% H, 8.12% N; found: 67.40% C, 5.99% H, 8.03% N.

N^{α}-Dinicotinoyl-bis-L-phenylalanine (*VII*)

Method A: Dry HCl gas was bubbled in a suspension of 3,5-pyridinedicarboxylic acid (*IV*) (0.84 g; 5 mmol) in glacial acetic acid for 30 min. The solvent was distilled off and the crude mass was left in dessicator over sodium hydroxide pellets under reduced pressure. To a cold solution (–5 °C) of the

obtained acid hydrochloride and HOSu (1.27 g; 11 mmol) in 50 ml tetrahydrofurane, DCCI (2.27 g; 11 mmol) was added in portions. A solution of L-Phe (1.65 g; 10 mmol) in 1 M $NaHCO_3$ (11 ml) was added and the reaction mixture was stirred for 3 h at 0 °C with adjustment of its pH ≈ 8 and left overnight at room temperature. Dicyclohexylurea was filtered off and THF was distilled off. The cold (≈ 0 °C) water solution was acidified to pH ≈ 3 with 1 M HCl. The obtained gelatinous product was extracted with ether and the ethereal solution was dried over anhydrous sodium sulfate. Solvent was distilled off under reduced pressure and the obtained product was identified as *VII*, white crystals when crystallized from aqueous ethanol (0.37 g; 16% yield); m.p. 223 – 224 °C; R_F = 0.49 (S2); $[\alpha]_D^{30}$ = −70 (*c* 0.05, absolute ethanol); IR spectrum (KBr): 3 313 (NH), 2 500 (OH), 1 705 (C=O, acid), 1 674 (amide I), 1 573 (amide II) and 1 293 (amide III). ^{1}H NMR spectrum ($(CD_3)_2SO$): 3.2 d, 4 H (2 CH_2); 4.6 m, 2 H (2 CH); 6.7 s, 2 H (2 COOH); 7 – 7.4 m, 10 H (2 C_6H_5); 8.4 s, 1 H (pyr-H-4); 8.8 – 9.1 d, 4 H (pyr-H-2,6 + 2 NH). For $C_{25}H_{23}N_3O_6$ (461.5) calculated: 65.07% C, 5.02% H, 9.11% N; found: 65.00% C, 5.14% H, 9.14% N.

Method B: A solution of 1 M KOH (5 ml, 5 mmol) was added dropwise to a stirred cold solution (−5 °C) of *VI* (1.04 g; 2 mmol) in methyl alcohol (15 ml). Stirring was maintained at the same temperature for 3 h. Methyl alcohol was distilled off under reduced pressure and water was added to a volume of 25 ml. The cold aqueous solution (−5 °C) was acidified with 1 M HCl to pH ≈ 3, while stirring. The obtained gelatinous product was then extracted with ether and the ethereal extract was dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure and the resulted residue was found to be *VII* by TLC when compared with authentic sample, which was recrystallized from aqueous ethanol to give white crystals of *VII* (0.49 g; 53% yield); m.p. 223 – 224 °C.

Cyclo[N^{α}-dinicotinoyl-bis-L-phenylalanyl-L-ornithine] (*IIIa*)

To a well stirred solution of *VII* (115 mg; 0.25 mmol) and HOBt (67.5 mg; 0.5 mmol) in dimethylformamide (3 ml), L-ornithine (51 mg; 25 mmol) was added with equivalent amount of triethylamine in dimethylformamide (2 ml), at 0 °C. The reaction temperature was kept at about −5 °C. Then, DCCI (103 mg; 0.5 mmol) was added portionwise to the reaction mixture over 20 min. After the addition was completed, the mixture was stirred for 5 h at −5 °C, the formed dicyclohexylurea was filtered off and the reaction mixture was washed with acetonitrile (2 × 5 ml). The combined filtrate was kept in refrigerator overnight and the newly formed dicyclohexylurea was filtered off. Solvent was removed and the obtained residue was triturated with water, 1 M HCl and water again; on treatment with ether then solidified. The obtained solid was filtered off, washed thoroughly with ether and recrystallized from ethanol–ether to give 63 mg (42% yield) of *IIIa* as a yellowish white powder; m.p. 154 °C (decomp.); R_F = 0.61 (S2); $[\alpha]_D^{30}$ = −93° (*c* 0.05 absolute ethanol). IR spectrum: 3 230 (NH), 2 500 (OH), 1 725 (C=O), 1 660 (amide I), 1 530 (amide II), and 1 275 (amide III). ^{1}H NMR spectrum: 1.1 m, 2 H (CH_2); 2.9 m, 8 H (4 CH_2); 3.1 d, 3 H (3 CH); 7.1 – 7.4 m, 10 H (2 C_6H_5); 8 – 8.5 m, 5 H (pyr-H-4 + 4 NH); 9 d, 3 H (pyr-H-2,6 + COOH). Mass spectrum, *m/z*: 512 (M^+ − COOH), 484 (2), 471 (3), 459 (10), 310 (5), 175 (5), 91 (100).

Cyclo[N^{α}-dinicotinoyl-bis-L-phenylalanyl-L-ornithine Methyl Ester] (*IIIb*)

L-Ornithine methyl ester (37 mg; 0.25 mmol) was added to a well stirred mixture of *VII* (115 mg; 0.25 mmol) and HOBt (67.5 mg; 0.5 mmol) in dimethylformamide. The reaction temperature was kept at −5 °C. Then, DCCI (103 mg; 0.5 mmol) was added in portions to the reaction mixture over 20 min, and stirring was maintained for 5 h at same temperature. The reaction mixture was diluted with 25 ml acetonitrile and kept overnight in refrigerator. The formed dicyclohexylurea was filtered off and washed with ethyl acetate (2 × 5 ml). The obtained residue after solvent distillation was dissolved in 25 ml ethyl acetate, washed with water, 1 M $NaHCO_3$, water, 1 M HCl, water and dried over

anhydrous magnesium sulfate. The solvent was distilled off and the obtained oily product was purified by preparative thin-layer chromatography (silica gel plates). A product of R_F = 0.5 (S3) was separated in methanol to give 54 mg (36%) brownish white crystals identified as *IIIb*; m.p. 172 °C; $[\alpha]_D^{30}$ = 115 (*c* 0.05, absolute ethanol); IR spectrum: 3 300 (NH), 1 780 (C=O, ester), 1 660 (amide I), 1 550 (amide II) and 1 290 (amide III). ^{1}H NMR spectrum: 1.1 t, 2 H (CH_2); 2.8 m, 4 H (2 CH_2); 3.1 m, 4 H (2 CH_2); 3.6 s, 3 H (CH_3); 4.5 m, 2 H (CH_2); 7 – 7.4 m, 10 H (2 C_6H_5); 8.2 s, 4 H (4 NH); 8.45 s, 1 H (pyr-H-4); 9 s, 2 H (pyr-H-2,6). ^{13}C NMR spectrum: 174.36 d, 1 C (carboxy); 166.26 t, 4 C (C-2, 5, 12, 15); 152.41 d, 2 C (C-16, 20); 140.21 t, 2 C (C-17, 19); 136.12 d, 1 C (C-1); 130.96 s, 6 C (*o*, *p*-Ph); 129.94 s, 4 C (*m*-Ph); 128.12 s, 2 C (*i*-Ph); 56.68 m, 3 C (C-4, 7, 13); 53.57 s, 1 C (CH_3); 46.95 s, 2 C (CH_2-benzyl); 39.57 m, 1 C (C-10); 38.82 m, 1 C (C-8); 33.88 m, 1 C (C-9).

Esterification of *IIIa*

Dry HCl gas was bubbled in 10 ml cold methanolic solution of *IIIa* (55 mg; 0.1 mmol) over a period of 30 min. The resulted clear solution was then left at room temperature overnight. Solvent was distilled off under reduced pressure and the obtained residue was dissolved in water (10 ml) and neutralized with 1 M $NaHCO_3$. By TLC qualitative analysis, the expected product *IIIb* was not identified, but only decomposition products were formed [dinicotinic acid (R_F = 0.43), L-phenylalanine (R_F = 0.05), L-ornithine (R_F = 0.1); (S1)].

Alkaline Hydrolysis of *IIIb*

1 M KOH was added to a cold (–5 °C) stirred methanolic solution of *IIIb*. Stirring was maintained at the same temperature for 3 h. Methyl alcohol was distilled off under reduced pressure and the water solution was acidified with 1 M citric acid to pH 3. The solid formed was filtered off and identified as *IIIa* on comparison with authentic sample by TLC.

Acidic Hydrolysis of *IIIa* and *IIIb*

An acidic solution of *IIIa* or *IIIb* (10 mg; 5 ml 6 M HCl) was heated on a steam bath for 5 h. TLC qualitative analysis of the neutralized reaction mixture indicated the presence of dinicotinic acid, L-phenylalanine and L-ornithine on comparison with authentic samples.

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