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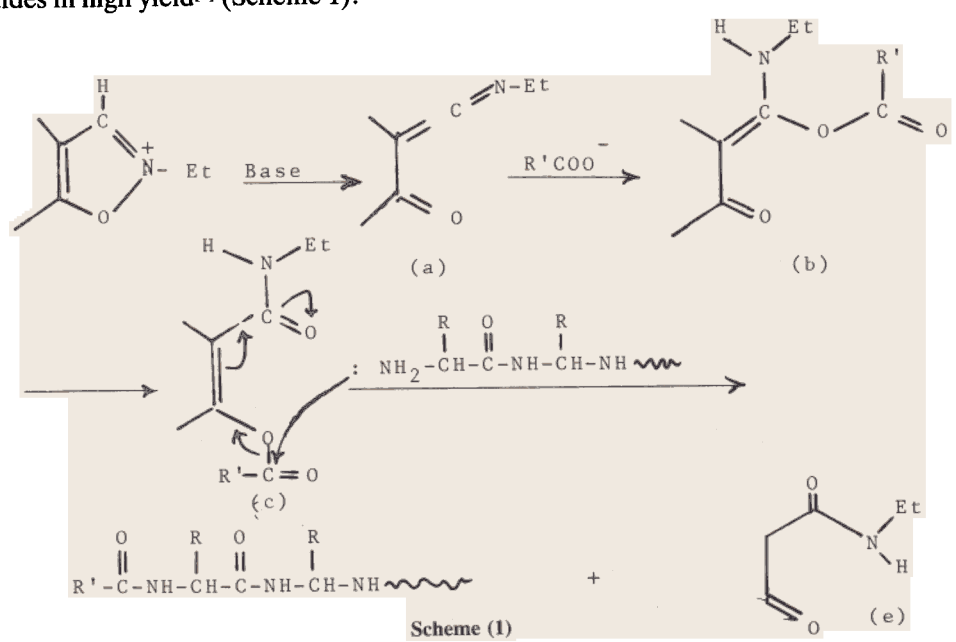
Synthesis of Some Peptides

SYED A. RAHMAN, REDA A. KABLI AND ABDULLAH B. EL-TAHIR
Department of Chemistry, Faculty of Science,
King Abdulaziz University, Jeddah, Saudi Arabia.

ABSTRACT. A practical synthesis of some dipeptides and tripeptides by the reaction of amino acids derivatives with activated enol esters derived from the reaction of N-ethylnaphth (1, 2-d) isoxazolium cation with carboxylic acids has been reported.

Introduction

Woodward[1] showed that amino carboxylic acids react rapidly and smoothly with N-ethylisoxazolium salt under very mild conditions to yield activated enol esters. The latter undergoes a facile reaction with amino groups to give the corresponding peptides in high yield[2] (Scheme 1).



Scheme (1)

The initial step in the coupling process was postulated[1] to proceed *via* ketoketenimine formed by the ring opening of the ylide. The existence of ketoketenimine (a) was subsequently confirmed by its isolation[3,4]. Attack by a carboxylate ion proceeds across the reactive cumulative system to give the intermediate (b) which suffers an internal transacylation to the active ester (c). The use of N-ethylnaphth (1, 2-d) isoxazolium salt has been reported[5,6] to yield active esters of N-protected amino acids in high yield and with considerable ease through the same pathway.

Results and Discussion

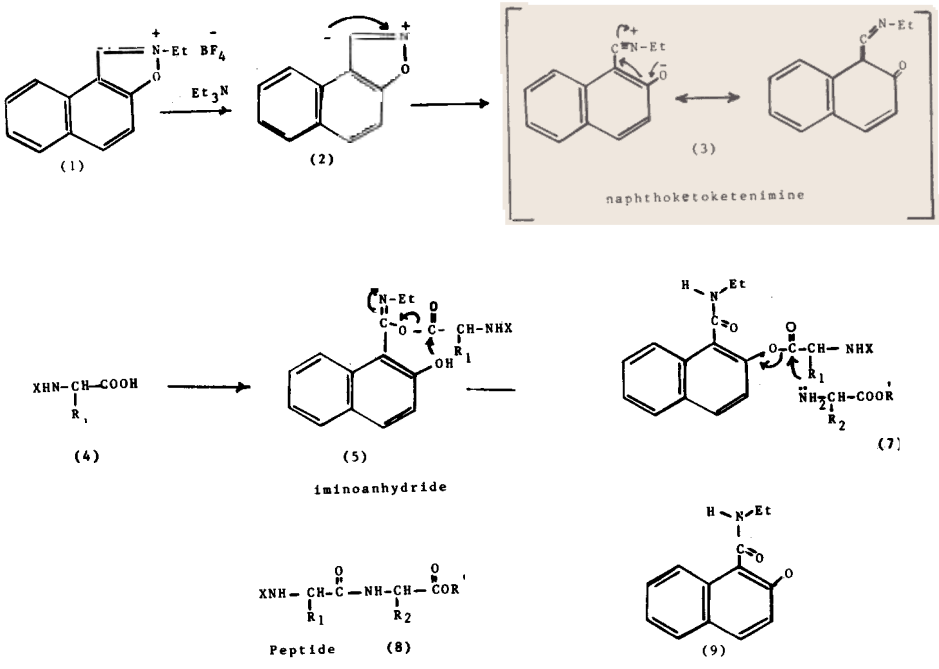
The synthesis of the di- and tripeptides was done by adopting the general procedure described in the literature[7]. Thus, the carboxy groups of a number of nitrogen blocked amino acids (Compd. 4) has been activated, almost quantitatively at room temperature in a few minutes, in solution of acetonitrile or nitromethane by reacting them with equimolar equivalent of N-ethylnaphth (1, 2-d) isoxazolium cation (Compd. 1) and triethylamine. Treatment of the resulting activated esters (Compd. 6) with an equivalent amount of the amino acid ester hydrochloride or the peptide ester hydrochloride (Compd. 7) in the presence of triethylamine afforded the di- and tripeptides (Compd. 8) respectively. The reaction proceeds smoothly and the resulting protected peptides (Table 1) have different solubility properties from those of the byproduct (Compd. 9), which simplifies the problem of separation and purification of the resulting peptides. The structure of the peptides is shown in table 1, and were confirmed by studying their spectra.

Table (1)

Peptide	Yield	and	m.p. (C)
Phth. Gly | Gly. OEt	86%		193-194°C
Z. L-Phe | Gly. OEt	90%		109-110°C
Z. L-Phe | L-Leu. OMe	90%		106-107°C
Z. Gly | Gly. Gly. OEt	67%		167-168°C

The peptide bond was produced at the point indicated by vertical stroke in the accompanying table.

The formation of the peptides (Scheme 2) from N-ethylnaphth (1, 2-d) isoxazolium ion could be rationalized on the basis of the known mechanism. Thus, the initial step between the naphth (1, 2-d) isoxazolium cation and the carboxylate ion of the N-protected amino acids leads to the intermediate naphthoketoketenimine (Compd. 3). Attack by the carboxylate ion proceeds across the reactive cumulative system producing an iminoanhydride (Compd. 5) that transacylates to the active ester (Compd. 6), namely 2-O-acyl-l-N′-ethylnaphthamide. A subsequent attack of the amino-acid derivative (Compd. 7) on the carbonyl ester group afforded the peptide and amide (Compd. 9) as a byproduct.



Scheme (2)

Reaction of N-ethylnaphth (1, 2-d) isoxazolium cation with N-protected amino acid to yield an active ester[6] and subsequent acylation of an amino acid ester or peptide ester to form a peptide[8].

Experimental

General Methods

Melting points were determined with Gallen kamp melting point apparatus and are uncorrected. Infrared spectra were recorded, for potassium bromide disc, with a Perkin-Elmer Spectrophotometer. Nuclear magnetic resonance spectra were recorded with EM-390 (90 MHz) NMR Spectrometer for solutions in deuteriochloroform using tetramethylsilane as standard. Elemental analysis were performed on Perkin-Elmer 240B Elemental Analyser.

Ethyl-N-phthalylglycylglycine

A solution of 2-O-N-phthalylglycine-l-N′-ethylnaphthamide (0.402 g; 1×10^{-3} mole) in 5 ml dichloromethane containing triethylamine (0.15 ml; 1×10^{-3} mole) was added to a solution of glycine ethyl ester hydrochloride (0.14 g; 1×10^{-3} mole) in 10 ml of the same solvent. The reaction mixture was stirred at room temperature over-

night. It was concentrated to give a white crystalline solid. The product was filtered off and recrystallized from acetonitrile to give the title compound (0.28 g, 86.2%), m.p. 193°C-194°C.

(**found** C, 58; H, 4.9; N, 9.5; $C_{14}H_{14}N_2O_5$; **Calc:** C, 57.9; H, 4.8; N, 9.6%).

Infrared spectrum (KBr) : 3420 (N-H stretch); 1740 (C = 0); 1660 and 1560 cm^{-1} (–CONH).

Nmr spectrum (chloroform-d) : showed signals at δ1.3 (3-proton triplet, methyl protons of $-\overset{O}{\overset{\|}{C}}-O-CH_2-\underline{CH_3}$); δ4.3 (2-proton quartet, methylene protons of $-\overset{O}{\overset{\|}{C}}-O-\underline{CH_2}-CH_3$); δ4.1 (2-proton doublet, methylene protons of $-CONH-\underline{CH_2}-COOCH_2-CH_3$); δ6.5 (1-proton broad singlet; N-H proton); δ4.5 (2-proton singlet, methylene protons of N-phthalylglycine moitey); and δ7.8 (4-proton multiplet, aromatic protons).

Carbobenzoxy-L-phenylalanine glycine ethyl ester

A solution of carbobenzoxy-L-phenylalanine (300 mg; 1×10^{-1} mole) in acetonitrile was treated with triethylamine (101 mg; 1×10^{-3} mole), followed by N-ethyl-naphth (1, 2-d) isoxazolium fluoborate (300 mg; 1×10^{-3} mole). The reaction mixture was stirred for 60 min. at 0°C. Glycine ethyl ester hydrochloride (140 mg; 1×10^{-3} mole) and triethylamine (101 mg; 1×10^{-3} were then added, and the mixture was left overnight at room temperature. The solvent was then evaporated under reduced pressure and the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was dried and concentrated to give the title compound. It was recrystallized from ethyl acetate-petroleum ether (0.350 g, 90%) m.p. 109°C-110°C.

(**found:** C, 65.5; H, 6.1; N, 7.2; $C_{21}H_{24}N_2O_5$; **Calc:** C, 65.6; H, 6.3; N, 7.3%).

Infrared spectrum (KBr) : 3400 (N-H stretch); 1735 (C = O of ester group); and 1650 cm^{-1} (–CONH-peptide linkage).

Nmr spectrum (chloroform-d) : showed signals at δ1.3 (3-proton triplet, methyl protons of $-\overset{O}{\overset{\|}{C}}-O-CH_2-\underline{CH_3}$); δ4.25 (2-proton quartet, methylene protons of $-\overset{O}{\overset{\|}{C}}-O-\underline{CH_2}-CH_3$); δ4.1 (2-proton doublet, methylene protons of $-NH-CH_2-COOC_2H_3$); δ4.5 (1-proton multiplet, methine proton of phe); δ2.3 (2-proton doublet, benzyl protons of phe); δ6.5 (2-proton broad absorption, N-H proton); and δ7.9 (10-proton multiplet, aromatic protons of phe and Z group).

Carbobenzoxy-L-phenylalanyl-L-leucine methyl ester

A solution of carbobenzoxy-L-phenylalanine (1.5 g; 5×10^{-3} mole) in acetonitrile (10 ml), containing triethylamine (0.5 g; 5×10^{-3} mole) was added to solution of N-ethylnapth (1, 2-d) isoxazolium fluoroborate (1.5 g; 5×10^{-3} mole) in 15 ml of the same solvent. The reaction mixture was stirred at 0°C for 60 min. and then treated with L-leucine methyl ester hydrochloride (0.9 g; 5×10^{-3} mole) and triethylamine (0.5 g; 5×10^{-3} mole). The mixture was left overnight at room temperature. The solvent was then evaporated to dryness and the residue so obtained was partitioned between ethyl acetate and water. The organic layer was dried and concentrated to give the crude product which was recrystallized from ethyl acetate-petroleum ether; yield 1.9 g; 90% m.p. 106°C-107°C.

(**found:** C, 67.7; H, 6.9; N, 6.7; $C_{24}H_{30}N_2O_5$; **Calc:** C, 67.6; H, 7.1; N, 6.6%).

Infrared spectrum (KBr) : 3350 (N-H stretch; 1735 (C = O of ester group); 1650 (–CONH-peptide linkage); and 1380 and 1370 cm^{-1} dimethyl of L-leu.

Nmr spectrum (chloroform-d) : showed signals at δ4.1 (3-proton singlet, methyl protons of leu ester); δ1.2 (6-proton doublet, isopropyl group of leu); δ1.8 (1-proton multiplet, methine proton of leu); δ1.4 (2-proton triplet, methylene protons of leu); δ4.3 (2-proton multiplet, –NH–CH–C(=O)–); δ2.3 (2-proton doublet, benzyl proton of phe); δ6.5 (2-proton broad absorption, N-H proton of –CONH–); and δ7.8 (10-proton multiplet, aromatic protons).

Carbobenzoxy triglycine ethyl ester

A solution of carbobenzoxyglycine (413 mg; 2×10^{-3} mole) in nitromethane (10 ml) containing triethylamine (202 mg; 2×10^{-3} mole) was added to a solution of N-ethylnaphth (1, 2-d) isoxazolium fluoroborate (570 mg; 2×10^{-3} mole) in 10 ml of the same solvent. The reaction mixture was stirred for 1 h at room temperature and then a solution of glycylglycine ethyl ester hydrochloride (393 mg; 2×10^{-3} mole) was added. The reaction mixture was left overnight at room temperature, and the solvent was then evaporated. The residue was dissolved in hot water which on cooling gave the pure peptide, which was filtered, washed with water and dried. Yield 475 mg; 67% m.p. 167°C-168°C.

(**found:** C, 54.5, H, 5.8; N, 11.8; $C_{16}H_{21}N_3O_6$; **Calc:** C, 54.7; H, 6.0; N, 12.0%).

Infrared spectrum (KBr) : 3400 (N-H stretch; 1735 (C = O of ester); 1640 cm^{-1} (broad-CONH- band, peptide linkage).

Nmr spectrum (chloroform-d) : showed signals at δ1.3 and δ4.25 group); (5-proton triplet and quartet $-OCH_2-CH_3$ protons of ester group); δ4.5 (6-proton doublet, methylene protons of $-CONH-CH_2-$); δ6.5 (broad absorption, N-H protons); and δ7.8 (5-proton multiplet, aromatic protons).

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تحضـــير بعض الببتيـــــدات

سيد عبد الرحمن ، رضا علي كابلي و عبد الله بابكر الطاهر
قسم الكيمياء ، كلية العلوم ، جامعة الملك عبد العزيز ، جـــدة ، المملكة العربية السعودية

في هذا البحث تم تحضير بعض الببتيدات الثنائية والثلاثية عن طريق تفاعل مشتقات الأحماض الأمينية مع إسترات الإينول المنشّطة المشتقة من تفاعل كاتيون ن - إيثيل نافث (١ ، ٢ - د) أيزوكسازوليم مع الأحماض الكربوكسيلية .