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Convenient and simple homologation of N^{α}-urethane protected α-amino acids to their β-homologues with concomitant *o*-nitrophenylesters formation

Kupanna Ananda, Hosahudya N Gopi & Vommina V Suresh Babu*
Department of Studies in Chemistry, Central College, Bangalore University, Bangalore 560 001

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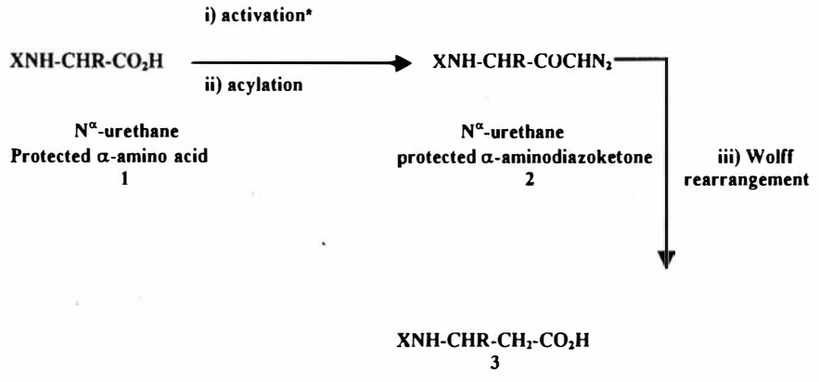
The Wolff rearrangement of α-aminodiazoketones derived from N^{α}-urethane protected α-amino acids in presence of *o*-nitrophenol catalyzed by silver acetate at low temperature is described. The potential utility of the well-known ketene intermediate is exploited for the synthesis of crystalline, optically pure β-amino acid active esters. Thus the homologation of Boc-/Z-/Fmoc-α-amino acids to β-amino acids with concomitant formation of the corresponding *o*-nitrophenyl esters has been achieved by this method. All the β-amino acid active esters have been obtained in good yield and purity and fully characterized by IR and NMR spectra.

The synthesis and characterization of peptides consisting of β-amino acids, the so called β-peptides is a field of research that has been receiving more and more interest in recent years[1,2]. The β-peptides, similar to their α-counterparts, have been found to have stable secondary structures[3-9]. They are often characterized by lower rates of metabolic degradation[6].

The Arndt-Eistert method for the synthesis of β-amino acids via Wolff rearrangement of diazoketones derived from N^{α}-protected α-amino acids has been utilized since 1940s[10-12]. This protocol was reinvestigated thoroughly with respect to possible epimerization of the chiral center and to the potential utility of the ketene intermediate. The acylation of diazomethanes, in general, was achieved by the mixed anhydride method using isobutyloxycarbonyl chloride or ethylchloro formate not only for the Boc-/Z-α-amino acids but also for the Fmoc-α-amino acids as well[13,14]. Recently the utility of Fmoc amino acid chlorides[15,16], pentafluorophenyl esters[17,18] and DCC[19] has been described for this purpose. The Wolff rearrangement of α-diazoketones can be accomplished thermally, photochemically or by metal (Ag^{+}) ion catalysis. This homologation process is based on the fact that the Wolff rearrangement of diazoketones containing a chiral center next to the carbonyl group occurred with retention of configuration[12-20]. Silver oxide catalysis usually involves a heterogeneous reaction at high temperature. It has been replaced by the Newman-Beal method consisting of a homogeneous, Ag^{+} catalyzed decomposition in presence of excess of a tertiary base, proceeding usually at low temperatures[20,21]. The presence of a base, therefore, has often been assumed to be inevitable for the progress of the reaction. For simple derivatives, thus, the homologation of α-amino acids to β-amino acids according to Arndt-Eistert approach giving entiomerically pure, crystalline N^{β}-protected β-amino acids in only two steps nicely complements the synthetic repertoire. This method is further attractive as the reactive intermediate arising from the Wolff rearrangement of a diazoketone can be trapped by a substituted phenol. By applying this strategy, we now made β-amino acid derivatives with concomitant formation of *o*-nitrophenyl esters.

Formation of the peptide bond under mild conditions requires a derivative of the participating carboxyl group with enhanced reactivity[22-24]. The activation of the carboxyl group in the form of 'active ester' is known to result the coupling reaction unambiguously[25-27]. The advantages of the use of active esters include having isolated stable crystalline intermediates (commercially) available on shelf, selectively react with the amine component in coupling and free from side reactions including racemization. Active esters are most commenly prepared by dicyclohexylcarbodiimide coupling between a protected acid and the ester moiety[24].

The Boc- and Z-α-aminodiazoketones required for the present studies were made by the mixed anhydride method using isobutyloxycarbonyl chloride (IBC-Cl) in presence of triethylamine at -5^{0}C and then subsequent upon acylation with diazomethane. In the case of Fmoc derivatives, Fmoc-amino acid chlorides were



i) activation via anhydride or active ester of DCC or acid chloride (if x=Fmoc) method;
ii) CH_2N_2, THF; iii) Ag+, triethylamine, 70°C, 3-4 hr.

Scheme I—Homologation of α-amino acid to β-amino acid by the Arndt-Eistert method

used as acylating agents (**Scheme I**). All the compounds (**2a-j**) made were isolated and fully characterized. They were found to have the same physical constants as reported earlier.

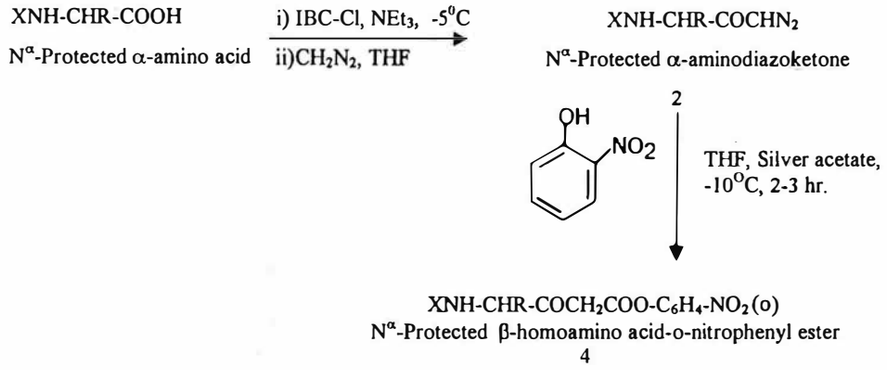
Herein, we report the synthesis of *o*-nitrophenyl esters of N^{β}-protected β-amino acids using the sequential Arndt-Eistert homologation with concomitant active ester formation (**Scheme II**). Until now, there are only few reports on the trapping of a ketene intermediate leading to the homologation and then concomitant formation of the peptide bond due to the insertion of the amino functionality[28-31].

The diazoketones (**2a-j**) were decomposed in THF as a solvent, in the presence of catalytic amount of silver acetate added as homogeneous solution in triethylamine. The rearrangement was accomplished in the presence of an equimolar quantity of *o*-nitrophenol. It was found that during this step, the insertion of *o*-nitrophenolic moiety takes place efficiently leading to the formation of the corresponding *o*-nitrophenyl ester (**Scheme III**). The course of the reaction was followed by TLC. It took about 2 hr for the completion of the reaction. The IR spectra of the β-amino acid *o*-nitrophenyl esters made has a strong peak at about 1771 cm^{-1} corresponding to the CO group vibrational stretching frequency, which is a characteristic peak for ONp esters. The CO group stretching of the corresponding α -amino acid esters was also observed in the same region in IR. The simple aqueous work-up of the reaction mixture gave N-protected β-homoamino acid *o*-nitrophenyl esters. All the compounds (**4a-j**) made were obtained in good yield and purity. The results are summarized in **Table I**. The physical properties of Fmoc-β-HLeu-ONp made by this strategy were found to have the similar properties to that obtained separately by using Fmoc-β-HLeu, *o*-nitrophenol and dicyclohexylcarbodiimide (DCC). The optical rotation of the compound was also found to be same.

Thus, the ketene intermediate arising from the Wolff rearrangement of α-aminodiazoketones has been trapped by *o*-nitrophenol. It is a simple method for the homologation of α-amino acid to β-homoamino acid with concomitant active ester formation. The resulting Fmoc-/Boc-/Z-β-homoamino acid *o*-nitrophenyl esters will be useful as building blocks for the synthesis of β-peptides by both solution and solid phase methods.

Experimental Section

The melting points were determined by using Leitz-Wetzlar melting point apparatus and are uncorrected. Optical rotations were measured with an automatic AA-10 polarimeter (Optical Activity, U.K.). IR spectra were recorded on a Nicolet model Impact 400D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution) and ^1H NMR spectra on a ACF 200 MH_z spectrometer using Me_4Si as an internal standard. Elemental analyses were done using Perkin Elmer Analyser and the samples were dried for 24 hr under vacuum before analysis. TLC was carried on precoated silica gel plates using solvent systems i) chlo-



	X	R		X	R
a)	Z	CH_2Ph	g)	Fmoc	CH_3
b)	Z	$CH_2CH(CH_3)_2$	h)	Fmoc	
c)	Z	$CH_2CH_2CH_3$			
d)	Z	Ph	i)	Fmoc	$CH_2C_6H_5OCH_2C_6H_5$
e)	Boc	CH_2Ph	j)	Fmoc	CH_2Ph
f)	Boc	$CH_2OCH_2C_6H_5$	k)	Fmoc	

Scheme II—Synthesis of *o*-nitrophenyl esters of N^{α}-protected β-homoamino acid

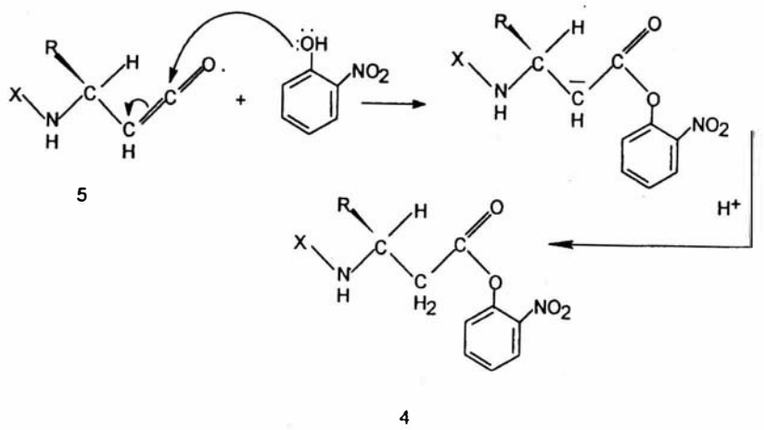
roform : methanol : acetic acid (40 : 2 : 1, v/v/v) and ii) ethyl acetate : hexane (35 : 65, v/v) and R_f values are designated as R_fA and R_fB respectively. The diazomethane solution in dry THF was prepared from N-methyl-N-nitroso-toluene-p-sulphonamide using reported procedures[32]. The N^{α}-protected α-aminodiazoketones were synthesized according to the procedures reported earlier.

Synthesis of Fmoc-/Boc-/Z-β-homoamino acid *o*-nitrophenyl esters : General procedure. The Fmoc-/Boc-/Z-α-aminodiazoketone (1 mmole) and *o*-nitrophenol (1.2 mmole) were dissolved in dry THF (10 mL). The reaction mixture was cooled to -15^0C and then silver acetate (0.12 mmole) and triethylamine (1 eq) were added. The reaction mixture was stirred for about 2 hr. The progress of the reaction was monitored by TLC and IR. The reaction mixture was filtered and the solvent evaporated *in vacuo*. The residue was dissolved in ethyl acetate and washed with 0.1N NaOH (10x3 mL), water (10x3 mL), 0.5 N HCl (10x3 mL), and brine, and dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* and the resulting residue was crystallised using absolute ethanol.

Z-β-HPhe-ONp (4a). Z-Phe-DAM (**2a**, 0.323g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17g, 1.2 mmole) in dry THF (10 mL), yield 0.35g. Anal. Found : C, 65.98; H, 5.01; N, 6.41. Calcd for $C_{24}H_{22}N_2O_6$ (434.41) : C, 66.35; H, 5.09; N, 6.45%. ^{1}H NMR ($CDCl_3$): δ 2.5 (2H, d, α-CH_2), 4.2 (1H, m, CHNH), 5.01 (2H, s, $CH_2C_6H_5$), 5.46 (1H, br, NH), 7.25 (14H, s, aryl).

Z-β-HLeu-ONp (4b). Z-Leu-DAM (**2b**, 0.277 g, 1 mmole) was stirred in the presence of silver acetate (5 mg 0.12 mmole), Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.32g. Anal. Found : C, 62.79; H, 5.98 ; N, 6.87. Calcd for $C_{21}H_{24}N_2O_6$ (400.39) : C, 62.99 ; H, 6.03 ; N, 6.99%. ^{1}H NMR ($CDCl_3$) : δ 0.8 [6H, d, $CH(CH_3)_2$], 1.1 [2H, m, $CH_2CH(CH_3)_2$], 2.30 [2H, m, α-CH_2), 3.8 (1H, m, NHCH), 5.0 (2H, s, $CH_2C_6H_5$), 5.6 (1H, br, NH), 7.25 (14H, m, aryl).

Z-β-D-HNva-ONp (4c). Z-D-Nva-DAM (**2c**, 0.275 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10



Scheme III—The trapping of ketene intermediate by *o*-nitrophenol

Table I—*o*-Nitrophenyl esters of N^{α}-Fmoc-/Boc-/Z-β-homoamino acids

Sl No.	Name of the N^{α}-protected α-aminodiazoketone	Name of the product (4)	Yield (%)	m.p. (^{0}C)	TLC R_f value	$[\alpha]^{25}_D$ (c=1, $CHCl_3$)	IR ν_{max} in cm^{-1}
1	Z-PHe-DAM	Z-β-HPhe-ONp	76	115-118	0.88	-21.24	3314,1771,1688
2	Z-Leu-DAM	Z-β-HLeu-ONp	75	Gum	0.85	-18.14	3320,1770,1700
3	Z-D-Nva-DAM	Z-β-D-HNva-ONp	78	85-88	0.85	+11.16	3316,1772,1698
4	Z-D-Phg-DAM	Z-β-D-HPhg-ONp	79	88-90	0.81	+10.19	3322,1776,1690
5	Boc-Phe-DAM	Boc-β-HPhe-ONp	74	118-120	0.80	-20.14	3318,1772,1698
6	Boc-Ser(OBzl)DAM	Boc-β-HSer(OBzl)-ONp	79	78-80	0.82	+2.6	3310,1771,1698
7	Fmoc-Ala-DAM	Fmoc-β-HAla-ONp	68	130-132	0.81	-11.04	3341,1774,1692
8	Fmoc-Pro-DAM	Fmoc-β-HPro-ONp	67	110-112	0.79	-24.12	1771, 1688
9	Fmoc-Tyr(OBu^t)-DAM	Fmoc-β- HTyr(OBu^t)-ONp	64	106-108	0.83	-8.32	3334, 1772,1700
10	Fmoc-Phe-DAM	Fmoc-β-HPhe-ONp	68	144-146	0.84	-12.26	3336,1771,1698
11	Fmoc-Nip-DAM	Fmoc-δ-HNip-ONp	69	92-94	0.85	-	1774, 1698

*Abbreviations used for amino acids are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature published in 'Pure and Applied Chemistry', 40, 1974, 314. Additional ones are used as Phg, Phenylglycine; Nva-α-amino-valeric acid; Nip, nipecotic acid (3-piperidine carboxylic acid)

mL), yield 0.3 g. Anal. Found : C, 62.19; H, 5.81; N, 7.14. Calcd for $C_{20}H_{24}N_2O_6$ (386.37) : C, 62.16; H, 5.73; N, 7.25%. ^{1}H NMR ($CDCl_3$) : δ 1.2-1.5 [7H, m, $(CH_2)_2CH_3$], 2.45 (2H, d, α-CH_2), 3.8 (1H, d, CHNH), 5.6 (1H, br, NH), 7.2 (9H, m, aryl).

Z-β-D-HPhg-ONp (4d). Z-D-Phg-DAM (**2d**, 0.309 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.33g. Anal. Found: C, 65.48; H, 4.81; N, 6.69. Calcd for $C_{23}H_{20}N_2O_6$ (420.39) : C, 65.70; H, 4.79; N, 6.66%. ^{1}H NMR ($CDCl_3$) : δ 2.5 (2H, d, α-CH_2), 4.2 (1H, m, CHNH), 5.01 (2H, s, $CH_2C_6H_5$), 5.46 (1H, br, NH), 7.25 (14H, m, aryl).

Boc-β-HPhe-ONp (4e). Boc-Phe-DAM (**2e**, 0.289 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole) Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.32 g. Anal. Found : C, 62.91; H, 6.09; N,6.87. Calcd for $C_{21}H_{24}N_2O_6$ (400.39) : C, 62.99; H, 6.03; N, 6.99%. ^{1}H NMR ($CDCl_3$) : δ 1.38 [9H, s, $C(CH_3)_3$], 2.38 (2H, d, α-CH_2) 2.72 (2H, d, $CH_2C_6H_5$), 4.01 (1H, m, CHNH), 5.0 (1H, m, NH), 7.26 (9H, m, aryl).

Boc-β-HSer(OBzl)-ONp (4f). Boc-Ser (OBzl)–DAM (**2f**, 0.32 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2

mmole) in dry THF (10 mL), yield 0.34 g. Anal. Found : C,61.41 ; H, 6.11; N, 6.47. Calcd for $C_{22}H_{26}N_2O_7$ (430.42) : C, 61.38; H, 6.08; N, 6.50 %. ^{1}H NMR ($CDCl_3$) : δ 1.38 (9H, s, Boc), 2.50 – 2.62 (2H, m, α-CH_2), 4.26 – 4.43 (3H, m, $CHCH_2O$), 5.08 (2H, s, $C_6H_5CH_2$), 5.6 (1H, br, NH), 7.26 (10H, m, aryl).

Fmoc-β-HAla-ONp (4g). Fmoc-Ala-DAM (**2g**, 0.348 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.36 g. Anal. Found: C, 67.34; H, 4.99; N, 6.31. Calcd for $C_{25}H_{24}N_2O_6$ (446.42): C, 67.25; H, 4.96; N, 6.27%. ^{1}H NMR ($CDCl_3$) : δ 1.1 (3H, d, $CHCH_3$), 2.4 (2H, d, α-CH_2), 4.01 (1H, m, CHNH), 4.3 (3H, br, CH_2CO, CH Fmoc); 4.59 (2H, d, CH_2O), 5.2 (1H, br, NH), 7.2-7.8 (8H, m, aryl).

Fmoc-β-HPr*o*-ONp (4h). Fmoc-Pr*o*-DAM (**2h**, 0.361 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1 mL 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.32 g. Anal. Found : C, 68.58 ; H, 5.08; N, 5.84. Calcd for $C_{27}H_{24}N_2O_6$ (472.45) : C, 68.63; H, 5.11; N, 5.93%. ^{1}H NMR ($CDCl_3$) : δ 2.0 (4H, br, CH_2CH_2CH), 2,72 (2H, m, α-CH_2), 3.47 (2H, t, CH_2N), 3.80 (1H, m, $CHCH_2COOH$), 4.10 (1H, m, CHFmoc), 4.46 (2H, d, CH_2O), 7.2-7.8 (12H, m, aryl).

Fmoc-β-HTyr(OBu^t)-ONp (4i). Fmoc–Tyr(OBu^t)-DAM (**2i**, 0,48 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N(0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.34 g. Anal. Found : C, 69.26; H, 4.61; N, 5.26. Calcd for $C_{31}H_{26}N_2O_7$ (538.51): C, 69.13; H, 4.86; N, 5.20%. ^{1}H NMR ($CDCl_3$): δ 1.41 (9H, s, Boc), 2.52 (2H, m, γCH2), 2.74 (2H, m, α-CH_2), 3.61 (1H, m, β-CH), 4.10 (1H, m, CH Fmoc), 4.45 (2H, d, CH_2O), 5.52 (1H, br, NH), 7.2-7.8 (16H, m, aryl).

Fmoc-β-HPhe-ONp (4j). Fmoc-Phe-DAM (**2j**, 0.411 g, 1mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1 mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.53 g. Anal. Found: C, 70.89; H, 5.09; N, 5.43. Calcd for $C_{31}H_{26}N_2O_6$ (522.51) : C, 71.25; H, 5.01; N, 5.36%. ^{1}H NMR ($CDCl_3$) : δ 2.40 (2H, m, $CH_2C_6H_5$), 2.70 (2H, m, α-CH_2), 3.60 (1H, m, $CHCH_2C_6H_5$), 4.1 (1H, m, CHFmoc), 4.41 (2H, d, CH_2O), 5.45 (1H, br, NH), 7.2-7.8 (17H, m, aryl).

Fmoc-δ-HNip-ONp (4k) (*o*-nitrophenyl ester of N-Fmoc-3 piperidinylacetic acid). Fmoc-Nip-DAM (**2k**, 0.375 g, 1 mmole) was stirred in the presence of silver acetate (5 mg, 0.12 mmole), Et_3N (0.1mL, 1 mmole) and *o*-nitrophenol (0.17 g, 1.2 mmole) in dry THF (10 mL), yield 0.28 g. Anal. Found : C, 68.52; H, 5.48; N, 5.92. Calcd for $C_{27}H_{26}N_2O_6$ (474.47) : C, 68.14; H, 5.51; N, 5.90%. ^{1}H NMR ($CDCl_3$) : δ 1.32-2.4 (4H, m, CH_2CHCH_2), 2.78 (2H, d, α-2H), 3.1-3.5 (5H, m, CH_2NCH_2 CH_2CHCH_2), 4.0 (1H, br, CH Fmoc), 4.25 (2H, d, CH_2O), 7.28 (13H, m, aryl).

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