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New Non-Proteogenic Aminoacids Bearing an Enol Aryl-Ether Moiety.

M. DAUMAS, L. VO-QUANG*, F. Le GOFFIC

Laboratoire de Bioorganique et Biotechnologies
URA CNRS 1386, ENSCP
11, rue P. & M. Curie ; 75231 PARIS Cedex 05 ; FRANCE

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Abstract: Aminoacids bearing an enol aryl-ether moiety have been synthesized by a new method allowing a great versatility in the introduction of N-protective groups and enol ether functions. This method involves a Wittig-Horner condensation affording alpha,beta-dehydro homoserine ether derivatives, followed by a regio and stereoselective isomerization into the desired E enol ether. Clean deprotection was achieved providing new 2-amino-4-aryloxybut-3(E)-enoic acids 3.

Beta,gamma-dehydro aminoacids have received much attention in recent literature especially as they are efficient inhibitors of Pyridoxal Phosphate (PLP) dependent enzymes[1]. Aminoacids bearing an enol ether moiety are a particular class of beta,gamma-dehydro aminoacids which exhibit specific properties [2-16].

2(S)-Amino-4-Methoxybut-3(E)-enoic acid (L-trans AMB, **1a**) was demonstrated to be the first naturally occurring enzyme-activated irreversible inhibitor (k_{cat} inhibitor) of the PLP linked enzyme aspartate amino-transferase[13]. The naturally occurring compounds (**1a**, **1b**, **1c**) possess strong potentialities either as plant growth regulators[7-9] or as antibacterial agents[2,3,10-12]. Furthermore, the unnatural derivative 2(S)-Amino-4-Methoxybut-3(Z)-enoic acid (L-cis AMB) was shown to exhibit antitumoral properties *in vitro*[14].

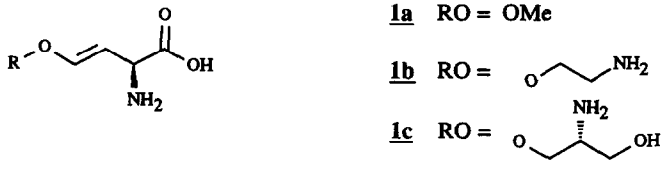


Figure 1

Most of the biological properties of known beta,gamma-enol ethers amino acids are thought to rely on their ability to inhibit PLP-dependant enzymes involved in the metabolism of methionine[4,9,15,16]. Rhizobitoxine (**1c**) is a close analogue of cystathionine, the natural substrate of beta-cystathionase, an enzyme of methionine biosynthesis in plants and bacteria. Its strong inhibitory effect is thought to rely on this analogy but the presence of the enol ether function seems mandatory to ensure an irreversible effect[15].

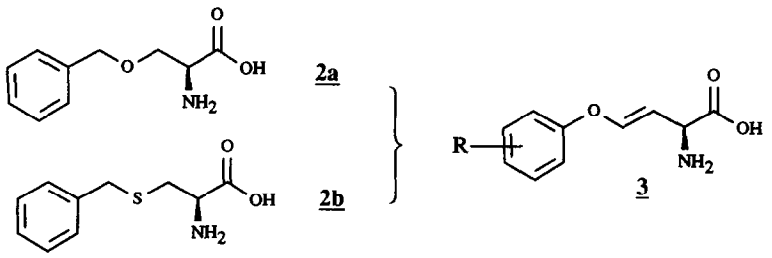


Figure 2

This simplistic structure activity correlation[12-16] led us to synthesize analogs of other α-aminoacids enol-ethers. The amino acids bearing an enol aryl-ether side chain function (**3**) were chosen as synthetic targets since these compounds are close, rigidified analogues of O-benzylserine (**2a**) or S-benzylcysteine (**2b**), two substrates of beta-cystathionase[12,16]. Moreover, the presence of the enol ether moiety was expected to lead to the aforementioned irreversible inhibition of the target enzyme.

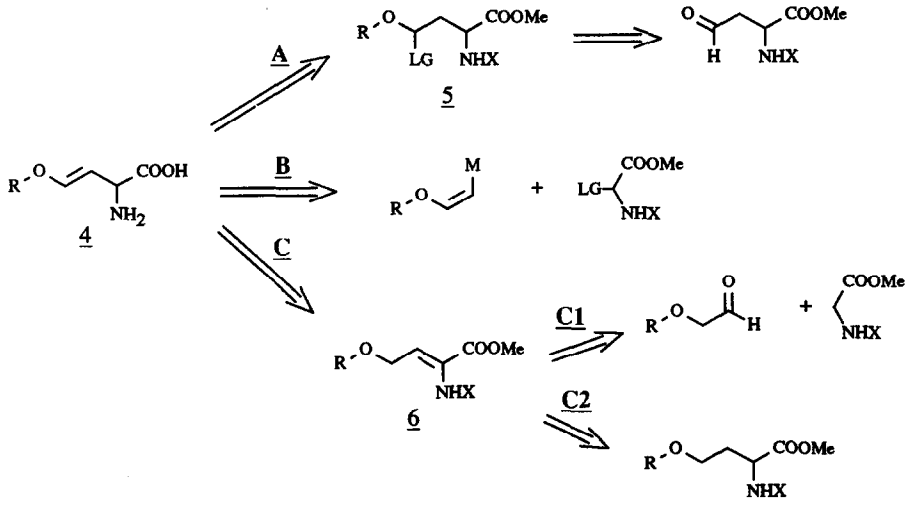
The synthesis of these enol ethers encounters specific difficulties :

* Generation of an enol ether moiety in the presence of potentially reactive aminoacid substituents ;
* Deprotection of the latter groups without degrading the enol ether moiety ;
* Stereocontrol of the double bond geometry and of the chiral centre as well.

The retrosynthetic scheme (Scheme 1) revealed three major routes used in previously published syntheses to overcome these difficulties[17-21].

Route A is the historical way, developed by Keith and co-workers[17] for the first synthesis of Rhizobitoxine (1c). The key step of this synthesis is an elimination reaction on a beta-aspartic semi aldehyde derivative (5) to afford the desired enol ether[18a]. Recent modifications of experimental conditions ensure high yield and "trans" stereoselectivity in the case of methoxyvinylglycine (1a)[18b]. This route seems nevertheless restricted to the synthesis of simple enol ethers, owing to the rather poor yield reported in the case of Rhizobitoxine[17].

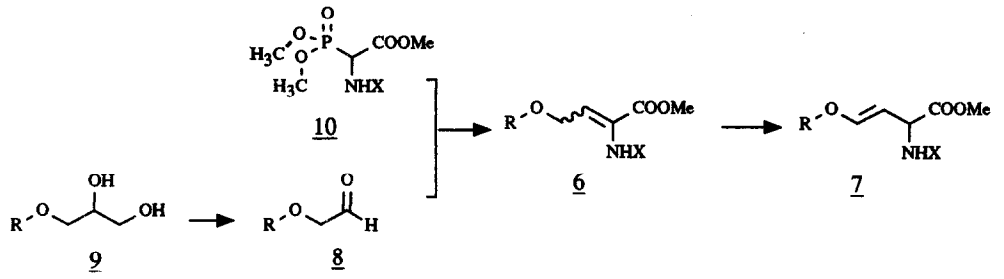
Route B, although synthetically attractive owing to its simplicity and "Cis" stereoselectivity also seems limited by the poor yield reported in the coupling step[19].



Scheme 1

Route C involves an isomerization step to generate the enol ether from an alpha,beta-unsaturated aminoacid (6)[20,21]. In this case, stereocontrol of the chiral centre would be difficult, but this route is however attractive owing to the facility in introducing different enol ether residues. Moreover, the introduction of various amino-protecting groups is possible[28,29] which is suitable to ensure complete study in the deprotection steps. Finally, stereospecific isomerization of allyl ethers into enol ethers has already been reported[22-24].

According to these statements, we recently described in a preliminary paper a new route of type C for the synthesis of racemic enol alkyl-ethers aminoacids[25]. The full sequence employed is outlined in Scheme 2.



Scheme 2

The main step of the sequence is a Wittig-Horner condensation affording the alpha,beta-unsaturated aminoacid derivative (6) followed by a regio and stereospecific isomerization into the corresponding E-enol ether (7).

We now wish to report this work in detail. In particular, the successful application of this optimized scheme to the synthesis of the new non-natural aminoacid derivatives : 2-amino-4-aryloxybut-3(E)-enoic acids (3).

Alpha-aryloxy acetaldehydes (8, R= aryl) were obtained from alpha-aryloxyglycerol (9, R= aryl) according to previously reported procedures[26]. Wet silica gel supported sodium metaperiodate[27] was the oxidant of choice for small scale experiments. Crude aldehydes (8) were used directly in the following step.

N-protected phosphonates (10) are readily available compounds according to the early work of Schmidt *et al.*[28,29]. A specific sequence was developed to obtain the N-formyl derivative (10, X= CHO) in multigram quantities[31]. The Wittig-Horner olefination proceeded without particular problems using slight modifications of a previously published procedure[28]. The results of this two-step sequence are summarized in Table 1.

N°	R	X	Yield [1] ; (% Z)	mp	MS (DCI/NH3) [2]
6a	Me	Ac	47 % (90)	oil	205, 187, 156
6b	iBu	CHO	56 % (87)	oil	233, 216, 183
6c	C6H5	Ac	55 % (98)	72-73°C	267, 250, 156
6d	C6H5	CHO	66 % (98)	95-96°C	253, 236, 142
6e	4-ClC6H4	Ac	81 % (90)	136°C	301, 284, 156
6f	4-FC6H4	CHO	63 % (90)	95°C	271, 254, 142
6g	4-NO2C6H4	Ac	64 % (90)	190°C	312, 295, 156
6h	4-NO2C6H4	CHO	58 % (90)	205°C (dec)	280 [3]

[1] Yields refer to the two-step sequence : oxidative cleavage of glycerol monoethers and Wittig-Horner olefination. The ratio of isomers was determined by ^{1}H NMR.

[2] MS : Desorption and chemical ionization with NH_3. $(M+NH_4)^+$,$(M+H)^+$, $(M+H-ROH)^+$ respectively.

[3] MS : electronic impact desorption and ionization.

Table 1 : Methyl 4-alkoxy or 4-aryloxy-2-N-acylaminobut-2-enoates 6.

The isomerization step was performed with slight modifications of the procedure developed by Schöllkopf *et al.*[21]. Using two equivalents of lithium di-isopropylamide in tetrahydrofuran at low (-78°C) temperature and quenching the reaction mixture with ammonium chloride in methanol afforded the desired E-enol ether (7) in fair yields. Results of this isomerization step are presented in Table 2. Our results are in good agreement with those previously reported[21,25] : no trace of Z-enol ether could be detected in any reaction attempted to date.

4-Nitrophenoxy derivatives proved particularly difficult to isomerize. Under optimized conditions, compounds (**6g**) and (**6h**) led only to low yields of enol ethers and extensive recovery of starting material. Modifications of reaction conditions employing lower temperatures or increased amounts of base led to recovery or degradation of starting material respectively. This should be related to the reported incompatibility between nitro-aryl moiety and lithium or magnesium based organometallics[30].

N°	R	X	Yield [1]	mp	MS (DCI/NH3) [2]
7a	Me	Ac	51 %	oil	205, 187
7b	iBu	CHO	34 % [3]	oil	233, 216
7c	C6H5	Ac	34 % [3]	72-73°C	267, 250
7d	C6H5	CHO	50 %	88-89°C	253, 236
7e	4-Cl-C6H4	Ac	68 %	132°C	301, 284
7f	4-F-C6H4	CHO	79 %	92 °C	271, 254
7g	4-NO2C6H4	Ac	18 %	120°C (dec)	312, 295
7h	4-NO2C6H4	CHO	20 %	80-85°C	280 [4]

[1]Yields of E isomer, Z isomer has never been detected.

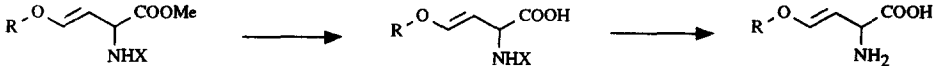
[2]MS : Desorption and chemical ionization with NH_3. $(M+NH_4)^+$ and $(M+H)^+$ respectively.

[3]Reaction conditions not optimized.

[4]MS : electronic impact desorption and ionization.

Table 2 : Methyl 4-alkoxy or 4-aryloxy-2N-acylaminobut-3E-enoates 7.

The deprotection of the enol ethers (7) took place according to Scheme 3. Hydrolysis of the methyl ester moiety occurred easily using lithium hydroxide in methanol[18,20].



7 11 3

Scheme 3

As expected, deprotection of the amine function proved to be the most sensitive step of the sequence. The formyl group appeared to be the most suitable protecting group in this case since it is easily removed using hydrazine hydrate under mild conditions (40°C, 2 hours) ; yields are higher than those obtained with the N-acetyl group (see Table 3) and N-formyl derivatives (7) and (11) are generally stable crystalline materials.

N°	R	Yield [1]	mp	MS (DCI/NH3) [3]
3a	Me	36 % [2]	235°C (dec)	149, 132
3b	iBu	63 %	228°C (dec)	191, 174
3c	C6H5	71 %	250°C (dec)	211, 194
3d	4- ClC6H4	36 %	255°C (dec)	245, 228
3e	4- FC6H4	92 %	265°C (dec)	229, 212

[1] Yields refer to both steps of the deprotection procedure.

[2] Lower yield from N-acetyl derivative 7.

[3] MS : chemical desorption and ionization with NH_3. $(M+NH_4)^+$ and $(M+H)^+$ respectively.

Table 3 : 4-alkoxy or 4-aryloxy-2-aminobut-3(E)-enoic acids 3.

4-Nitrophenoxy derivatives (7e-f) and (11e-f) exhibited particularly high reactivity towards lithium hydroxide in methanol and hydrazine hydrate respectively. 4-Nitrophenol was isolated as a side product in the hydrolysis step and extensive degradation occurred during hydrazinolysis.

In conclusion, our work led us to synthesize a new class of non natural aminoacids : 4-aryloxy-2-aminobut-3(E)-enoic acids (3). The N-formyl protecting group proved to be the most suitable choice ensuring crystalline intermediates and a high yielding deprotection step. These new compounds did, however, show limited antibacterial properties in controlled media, this probably being due to a poor uptake within the cell as they possess high affinity for beta-cystathionase. Biochemical and biological results will be published elsewhere.

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Experimental Section :

All reagents and solvents were of commercial purity grade and were used without further purification. Tetrahydrofuran was dried by passing through a pad of neutral alumina and storing over 4 Å molecular sieves. Solvents were purchased from Prolabo S. A.. Reagents were from either Fluka A. G., Aldrich Chem. Corp. or Janssen Chimica. Column chromatography was performed at ambient pressure, on silica gel (Merck, grade 60, 200-400 Mesh).

^{1}H NMR spectra were recorded on a Brucker 250 MHz spectrometer using either $CDCl_3$, acetone D6 or D_2O as solvents and tetramethylsilane (TMS) as internal reference.

Mass spectra were recorded on a quadrupole Nermag R10-10 spectrometer, at the Laboratoire de spectrometrie de masse de l'ENSCP.

Preparation of 1-aryloxypropan-2,3-diols 9 : General Procedure.

To a solution of the appropriate phenol (50 mmoles), in acetonitrile, (150 ml) was added potassium carbonate (250 mmoles, 5 eq.) and the mixture was heated at reflux for 1 hour. 3-chloropropan-1,2-diol (5.53 g, 50 mmoles) was then added dropwise to the refluxing mixture. After refluxing overnight, the mixture was cooled to room temperature diluted with dichloromethane (100 ml), filtered on a sintered glass before the solvents were removed under reduced pressure. The resulting oil was taken up in dichloromethane (150 ml) and the solution was washed with HCl solution (0.5 N, 50 ml) and with brine (50 ml). Drying over magnesium sulfate, filtration and evaporation of the solvent afforded 9 as an oil which crystallized in light petroleum (bp 40-60 °C).

1-phenoxypropan-2,3-diol : Yield 63%, mp 62°C (Lit.[26] mp 61-62°C)
1-(4-chlorophenoxy)propan-2,3-diol :Yield 76%, mp 80°C (Lit.[32] mp 80°C)
1-(4-fluorophenoxy)propan-2,3-diol :Yield 65%, mp 54°C (Lit.[33] mp 53-54°C)
1-(4-nitrophenoxy)propan-2,3-diol :Yield 28%, mp 64°C, MS (DCI/NH_3): 214 $(M+H)^+$, 231 $(M+NH_4)^+$.

Preparation of 1-aryloxyethanals 8 : General Procedure.

To a vigorously stirred suspension of chromatographic grade silica gel (20 g) in dichloromethane (120 ml) in a 250 ml Erlenmeyer flask, was added dropwise an aqueous solution of sodium metaperiodate (0.65 M, 20 ml, 13 mmoles) until a flaky suspension was formed. Diol 9 (10 mmoles), in dichloromethane (15 ml), was then added and the reaction was monitored by TLC until disappearance of the starting material. The suspension was then filtered on a sintered glass and the silica gel was carefully washed with dichloromethane (50 ml, twice). Evaporation of the solvent afforded aldehyde 8 as an oil which was used in the next step without further purification.

Preparation of methyl 4-aryloxy-2N-acylaminobut-2-enoates 6 : General Procedure.

In a flame dried 50 ml reactor, fitted with thermometre, magnetic stirring bar and argon inlet and outlet, was placed potassium tert-butoxide (0.45 g, 4 mmoles). The reactor was cooled to -70°C (Acetone/dry ice bath) and dichloromethane (10 ml) was then added. To this suspension was slowly added a solution of N-acylaminophosphonate 10 (4 mmoles) in dichloromethane (10 ml). The mixture was kept at -70°C until all potassium tert-butoxide went into solution and a yellow colour developed. Freshly prepared aldehyde 8 (4 mmoles) in dichloromethane (10 ml) was then added and the mixture was kept at -70°C for two hours. The clear mixture was allowed to reach room temperature and stirred overnight until a viscous precipitate formed.

The solvent was removed *in vacuo* and the residue was taken up in ethyl acetate (100 ml). The solution was washed with water (40 ml) and with a 5% ammonium chloride aqueous solution (40 ml). The organic phase was then dried over magnesium sulfate, filtrated and concentrated in vacuo to afford alpha,beta-dehydroaminoacid derivatives **6** as oils which were purified by column chromatography on a case by case basis.

^{1}H NMR data refer to the major isomer isolated. Yields, melting points and mass spectrometry data are given in Table 1.

Methyl 4-methoxy-2-N-acetylaminobut-2(Z)-enoate (6a) : (using dichloromethane/ethanol gradient, 0-10%). *See Lit. Ref.* [18].

Methyl 4-(2-methylpropoxy)-2-N-formylaminobut-2(Z)-enoate (6b) : (1,1,1-Trichloroethane 100: ethanol 2). ^{1}NMR ($CDCl_3$), δ (ppm) : 8.23, s, 1H, CHO ; 7.49, br s, 1H, NH ; 6.71, t, 1H, 5.5Hz, =CH ; 4.17, d, 2H, 5.5Hz, OCH_2- ; 3.80, s, 3H, OMe ; 3.21, d, 2H, 6.6Hz, OCH_2- ; 1.85, m, 1H, >CH- ; 0.89, d, 6H, 6.6Hz, CH_3.

Methyl 4-phenoxy-2-N-acetylaminobut-2(Z)-enoate (6c) : (Ethyl acetate 1:heptane 2). ^{1}NMR ($CDCl_3$), δ (ppm): 7.32-7.24, m, 3H, arom+NH ; 7.0-6.85, m, 3H, arom ; 6.79, t, 1H, 5.6Hz, =CH ; 4.67, d, 2H, 5.6Hz, OCH_2- ; 3.81, s, 3H, OMe ; 2.18, s, 3H, CH_3.

Methyl 4-phenoxy-2-N-formylaminobut-2(Z)-enoate (6d) : (1,1,1-Trichloroethane 100: ethanol 8). ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.32, br s, 1H, CHO ; 7.42, br s, 1H, NH ; 7.35-7.21, m, 2H, $arom_o$; 6.97, *pt*, 1H, 7.5Hz, arom ; 6.89, *pd*, 2H, 8.4Hz, arom ; 6.95, t, 1H, 5.5Hz, =CH ; 4.70, d, 2H, 5.5Hz, OCH_2- ; 3.83, s, 3H, OMe.

Methyl 4-(4-chlorophenoxy)-2-N-acetylaminobut-2(Z)-enoate (6e) : (1,1,1-Trichloroethane). ^{1}H NMR ($CDCl_3$) : 7.29, br s, 1H, NH, 7.27-7.15, m, 2H, $arom_o$; 6.88-6.75, m, 2H, $arom_m$; 6.74, t, 1H, 5.5Hz, =CH ; 4.63, d, 2H, 5.5Hz, OCH_2- ; 3.82, s, 3H, OMe ; 2.18, s, 3H, CH_3.

Methyl 4-(4-fluorophenoxy)-2-N-formylaminobut-2(Z)-enoate (6f) : (1,1,1-Trichloroethane 1:heptane 2). ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.31, br s, 1H, CHO ; 7.39, br s, 1H, NH ; 7.05-6.75, m, 5H, arom + =CH ; 4.65, d, 2H, 5.5Hz, OCH_2- ; 3.84, s, 3H, OMe.

Methyl 4-(4-nitrophenoxy)-2-N-acetylaminobut-2(Z)-enoate (6g) : Crystallized from dichloromethane/ether mixture. ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.22-8.15, m, 2H, arom ; 7.38, br s, 1H, NH, ; 6.98-6.91, m, 2H, arom ; 6.72, t, 1H, 5.5Hz, =CH ; 4.76, d, 2H, 5.5Hz, OCH_2- ; 3.83, s, 3H, OMe ; 2.21, s, 3H, CH_3.

Methyl 4-(4-nitrophenoxy)-2-N-formylaminobut-2(Z)-enoate (6h) : First crop crystallized from dichloromethane/ether mixture, second crop by chromatography of mother liquors (1,1,1-Trichloroethane). ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.34, br s, 1H, CHO ; 8.20, d, 2H, 9.3Hz, $arom_m$; 7.51, br s, 1H, NH ; 6.96, d, 2H, 9.3Hz, $arom_o$; 6.78, t, 1H, 5.4Hz, =CH ; 4.73, d, 2H, 5.4Hz, OCH_2- ; 3.82, s, 3H, OMe.

Preparation of methyl 4-aryloxy-2-N-acylaminobut-3E-enoates **7** : General procedure.

In a flame dried 200 ml reactor fitted with thermometre, magnetic stirring bar and argon inlet and outlet, were placed diisopropylamine (1.0 g, 10 mmoles) and dried tetrahydrofuran (60 ml). The reactor was cooled to -70°C (acetone/dry ice bath) under a stream of argon, and n-butyllithium in hexane (2.5 M solution, 4.1 ml, 10.2 mmoles) was then added. The mixture was stirred at -70°C for 15 minutes and a solution of methyl 4-aryloxy-2-N-acylaminobut-2-enoate **6** (5 mmoles) in tetrahydrofuran (25 ml) was then added *over a period of at least 20 minutes*. Care should be taken to keep the temperature below -65°C during the course of the addition. The reaction mixture was stirred at -70°C for 30 minutes and a solution of ammonium chloride in a methanol/water mixture (10 ml, 1:1 mixture, 5 % solution) was rapidly added, in one portion, without cooling. The solution was allowed to reach room temperature, and ether (50 ml) was then added. The organic phase was separated and washed successively with ammonium chloride solution (5 % solution 50 ml) and

saturated aqueous sodium bicarbonate (50 ml). The organic phase was dried over magnesium sulfate and the solvent was evaporated. The crude E-enol ether 7 was then chromatographed on silica gel using the eluents reported below.

4-(4-nitrophenoxy) derivatives should be recrystallized prior to chromatography otherwise crystalization of remaining starting material occurs during elution.

Yields, melting points and mass spectrometry data are given in Table 2.

Methyl 4-methoxy-2-N-acetylaminobut-3(E)-enoate (7a) : (1,1,1-trichloroethane 20:ethanol 1). *See Lit. Ref.* [18]

Methyl 4-(2-methylpropoxy)-2-N-formylaminobut-3(E)-enoate (7b) : (Diethyl ether 4:pentane 1: triethylamine 0.1%). ^{1}NMR ($CDCl_3$), δ (ppm) : 8.19, s, 1H, CHO ; 6.64, d, 1H, 12.5Hz, =CH-O ; 6.26, br s, 1H, NH ; 5.03, m, 1H, CH_α ; 4.73, dd, 1H, 12.5 & 8.6Hz, =CH ; 3.77, s, 3H, OMe ; 3.45, d, 2H, 6.6Hz, OCH_2- ; 1.93, m, 1H, >CH- ; 0.93, d, 6H, 6.7Hz, CH_3.

Methyl 4-phenoxy-2-N-acetylaminobut-3(E)-enoate (7c) : (Ethyl acetate 1:heptane 2). ^{1}NMR ($CDCl_3$), δ (ppm) : 7.35-7.25, m, 2H, $arom_o$; 7.09, *pt*, 1H, 15Hz, $arom_p$; 7.05-6.85, m, 2H, $arom_m$; 6.83, d, 1H, 12.0Hz, =CH-O ; 6.51, br d, 1H, 7.0Hz, NH ; 5.28, dd, 1H, 12.0 & 8.5Hz, CH= ; 5.10, *pt*, 1H, 7.9Hz, CH_α ; 3.78, s, 3H, OMe ; 2.04, s, 3H, CH_3.

Methyl 4-phenoxy-2-N-formylaminobut-3(E)-enoate (7d) : (Using diethylether/pentane gradient : 50-25%). ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.23, br s, 1H, CHO ; 7.37-7.27, m, 2H, $arom_o$; 7.03-6.65, m, 3H, $arom_{m+p}$; 6.85, d, 1H, 11.5Hz, =CH-O ; 6.42, br s, 1H, NH ; 5.28, dd, 1H, 11.5 & 8.5Hz, CH= ; 5.21-5.13, m, 1H, CH_α ; 3.80, s, 3H, OMe.

Methyl 4-(4-chlorophenoxy)-2-N-acetylaminobut-3(E)-enoate (7e) : (1,1,1-trichloroethane 100:ethanol 2). ^{1}H NMR ($CDCl_3$), δ (ppm) : 7.28, d, 2H, 9.3Hz, $arom_o$; 6.93, d, 2H, 9.3Hz, $arom_m$; 6.77, d, 1H, 11.5Hz, =CH-O ; 6.18, br d, 1H, 6Hz, NH ; 5.28, dd, 1H, 12.0 & 8.5Hz, OCH= ; 5.10-5.03, m, 1H, CH_α ; 3.79, s, 3H, OMe ; 2.05, s, 3H, CH_3.

Methyl 4-(4-fluorophenoxy)-2-N-formylaminobut-3(E)-enoate (7f) : (1,1,1-trichloroethane 20:ethanol 1). ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.25, br s, 1H, CHO ; 7.10-6.90, m, 4H, arom ; 6.79, d, 1H, 11.8Hz, =CH-O ; 6.35, br s, 1H, NH ; 5.30-5.10, m, 2H, CH= & CH_α ; 3.81, s, 3H, OMe.

Methyl 4-(4-nitrophenoxy)-2-N-acetylaminobut-3(E)-enoate (7g) : Crystallized from dichloromethane/ether mixture prior to chromatography (dichloromethane/ethyl acetate 1%). ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.25-8.15, m, 2H, arom ; 7.10-7.00, m, 2H, arom ; 6.85, d, 1H, 11.9Hz, =CH-O ; 6.40, br d, 1H, 6.8Hz, NH ; 5.46, dd, 1H, 11.9 & 8.4Hz, CH= ; 5.13, dd, 1H, 7.2 & 8.1Hz, CH_α ; 3.83, s, 3H, OMe ; 2.20, s, 3H, CH_3.

Methyl 4-(4-nitrophenoxy)-2-N-formylaminobut-3(E)-enoate (7h) : Crystallized from trichloro-1,1,1-ethane/ethanol mixture prior to chromatography (1,1,1-trichloroethane 10:ethanol 1). ^{1}H NMR ($CDCl_3$), δ (ppm) : 8.27, br s, 1H, CHO ; 8.23, d, 2H, 9.3Hz, $arom_m$; 7.08, d, 2H, 9.3Hz, $arom_o$; 6.89, d, 1H, 11.9Hz, =CH-O ; 6.55, br d, 1H, 5.3Hz, NH ; 5.49, dd, 1H, 11.9 & 8.3Hz, CH= ; 5.22, dd, 1H, 7.3 & 8.2Hz, CH_α ; 3.82, s, 3H, OMe.

Hydrolysis of methyl ester 7 : General Procedure.

The fully protected enol aryl-ether 7 (3 mmoles) was dissolved in methanol (40 ml) and a freshly prepared solution of lithium hydroxide (0.4 N solution, 10 ml, 1.3 eq.) was then added. The reaction was monitored by TLC and was generally complete within 2 hours. The solvent was evaporated and the crude product was taken up in hydrochloric acid (0.3 N solution, 60 ml) and ethyl acetate (50 ml). The aqueous layer was extracted with ethyl acetate (50 ml). The combined organic phases were washed with brine and dried over magnesium sulfate. Filtration and evaporation of the solvent afforded acids **11** as white powders which were directely used in the next step.

4-methoxy-2-N-acetylaminobut-3(E)-enoic acid (11a) : Yield 65%. ^{1}H NMR (acetone D6) : *see Lit. Ref.*[18].

4-(2-methylpropoxy)-2-N-formylaminobut-3(E)-enoic acid (11b): Yield 93%. *Compound not isolated, directly used in the next step.*

4-phenoxy-2-N-formylaminobut-3(E)-enoic acid (11c) : Yield 90% ; mp 160°C (dec) ; MS : 239 $(M+NH_4)^+$, 222 $(M+H)^+$; ^{1}H NMR (acetone D6), δ (ppm) : 7.97, br s, 1H, CHO ; 7.52, br d, 1H, 7Hz, NH ; 7.12-7.04, m, 2H, $arom_o$; 6.90-6.80, m, 1H, $arom_p$; 6.78-6.73, m, 2H, $arom_m$; 6.60, d, 1H, 12.1Hz, O-CH= ; 5.16, dd, 1H, 8.0 & 12.1Hz, =CH- ; 4.91-4.81, m, 1H, CH_α.

4-(4-chlorophenoxy)-2-N-acetylaminobut-3(E)-enoic acid (11d) : Yield 75% ; mp 140°C (dec) ; MS : 270 $(M+NH_4)^+$, 239 $(M+H)^+$; ^{1}H NMR (acetone D6), δ (ppm) : 7.60, br d, 1H, 7.5Hz, NH ; 7.41-7.30, m, 2H, $arom_o$; 7.11-7.05, m, 2H, $arom_m$; 6.99, d, 1H, 12.5Hz, O-CH= ; 5.46, dd, 1H, 7.5 & 12.5Hz, =CH- ; 5.03, *pt*, 1H, 7.5Hz, CH_α ; 1.95, s, 3H, CH_3.

4-(4-fluorophenoxy)-2-N-formylaminobut-3(E)-enoic acid (11e) : Yield 94% ; mp 179°C (dec) ; MS : 257 $(M+NH_4)^+$, 240 $(M+H)^+$; ^{1}H NMR (acetone D6), δ (ppm) : 8.19, br s, 1H, CHO ; 7.52, br s, 1H, NH ; 7.20-7.0, m, 4H, arom ; 6.97, d, 1H, 12.1Hz, O-CH= ; 5.40, dd, 1H, 8.3 & 12.1Hz, =CH- ; 5.09, *pt*, 1H, 8.5Hz, CH_α.

4-(4-nitrophenoxy)-2-N-formylaminobut-3(E)-enoic acid (11f) : Yield 18% ; mp 177°C (dec) ; MS : 284 $(M+NH_4)^+$, 267 $(M+H)^+$; ^{1}H NMR (acetone D6), δ (ppm) : 8.30, m, 2H, arom ; 8.23, s, 1H, CHO ; 7.85-7.75, m, 1H, NH ; 7.30, m, 2H, arom ; 7.19, d, 1H, 12.0Hz, O-CH= ; 5.70, dd, 1H, 8.3 & 12.0Hz, =CH- ; 5.24-5.16, m, 1H, CH_α.

Hydrazinolysis of N-formyl aminoacids 11 : General Procedure.

4-Aryloxy-2-N-formylaminobut-3E-enoic acid **11** from the previous step (1.5 mmoles) was dissolved in hydrazine hydrate (85 % solution, 8 ml) and the mixture was heated at 40°C for 2 hours. Hydrazine was then evaporated. The crude product was taken up in methanol (30 ml) and the solvent was evaporated. This procedure was repeated twice to ensure removal of traces of hydrazine. The amino acid **3** was then precipitated from acetone.

This procedure has been optimized for N-formyl derivatives. Acetyl group was removed using a similar procedure, but hydrazinolysis was led at 70°C for 2 hours to ensure complete removal of the protecting group.

Yields, melting points and mass spectrometry data of aminoacids **3** are provided in table 3. Yields refer to both steps of the deprotection procedure.

4-methoxy-2-aminobut-3(E)enoic acid (3a) : Yield : 36% ; ^{1}H NMR (D_2O) : *see lit. ref.*[18].

4-(2-methylpropoxy)-2-aminobut-3(E)-enoic acid (3b) : Yield : 63% ; ^{1}H NMR (D_2O), δ (ppm) : 6.29, d, 1H, 12.5Hz, OCH= ; 4.70-4.40 (m, 1H, =CH- ; 3.48, d, 1H, 8.7Hz, CH_α ; 3.24, d, 2H, 6.7Hz, CH_2O ; 1.80-1.65, m, 1H, -CH< ; 0.70, d, 6H, 6.7Hz, $(CH_3)_2$.

4-phenoxy-2-aminobut-3(E)-enoic acid (3c) : Yield : 71% ; ^{1}H NMR (D_2O), δ (ppm) : 7.30-7.20, m, 2H, $arom_o$; 7.0-6.92, m, 3H, $arom_{m+p}$; 6.67, d, 1H, 12.1Hz, OCH= ; 5.38, dd, 1H, 12.1 & 8.7Hz, =CH- ; 3.67, d, 1H, 8.7Hz, CH_α.

4-(4-chlorophenoxy)-2-aminobut-3(E)-enoic acid (3d) : Yield : 36% ; ^{1}H NMR (D_2O), δ (ppm) : 7.24, *pd*, 2H, 8.8Hz, $arom_o$; 6.92, *pd*, 2H, 9.0Hz $arom_m$; 6.60, d, 1H, 12.2Hz, OCH= ; 5.17, dd, 1H, 12.1 & 8.5Hz, =CH- ; 3.67, d, 1H, 8.5Hz, CH_α.

4-(4-fluorophenoxy)-2-aminobut-3(E)-enoic acid (3e) : Yield : 71% ; ^{1}H NMR (D_2O), δ (ppm) : 6.95-6.65, m, 4H, arom ; 6.54, d, 1H, 12.2Hz, OCH= ; 5.17, dd, 1H, 12.2 & 8.5Hz, =CH- ; 3.63, d, 1H, 8.5Hz, CH_α.

References :

1- a)Schmidt U., Lieberknecht A., Wild J. *Synthesis* **1988**, 159-172.
b)Angst C. *Pure and Appl. Chem.* **1987**, 59, 373-380.
2- Pruess D.L., Scannel J.P., Kellet H.A., Ax H.A., Janecek J., Williams T.H., Stempel A., Berger J.J. *J. Antibiot.* **1974**, 27, 229-233.
3- Scannel J.P., Pruess D.L., Demny T.H., Sello L.H., Williams T.H., Stempel A. *J. Antibiot.* **1972**, 25, 122-127.
4- Sufrin J.R., Lombardini J.B., Keith D.D. *Biochem. Biophys. Research Commun.* **1982**, 106, 251-255.
5- Dashman T. *Life Sciences* **1980**, 27, 1415-1422.
6- Cornell N.W., Zuurendonk P.F., Kerich M.J., Straight C.B. *Biochem. J.* **1984**, 220, 707-716.
7- Owens L.D., Wright D.A. *Plant Physiol.* **1965**, 40, 927.
8- Mattoo A.K., Anderson J.D., Chalutz E., Lieberman M. *Plant Physiol.EI* **1979**, 64, 289-292.
9- Even-Chen Z., Mattoo A.K., Goren R. *Plant Physiol.* **1982**, 65, 385-388.
10-Owens L.D., Guggenheim S., Hilton J.L. *Biochim. Biophys. Acta* **1968**, 158, 219-225.
11-Sahm U., Knobloch G., Wagner F. J. *J. Antibiot.* **1973**, 26, 389.
12-Bouthier de la Tour C. *Thèse de doctorat de l'université P. et M. Curie* **1987**, Paris.
13-Rando R.R., Relya N., Cheng L.J. *J. Biol. Chem.* **1976**, 251, 3306-3312.
14-Kramer D.L., Sufrin J.R., Porter C.W. *Biochem. Biophys. Acta* **1988**, 249, 581.
15-Giovanelli J., Owens L.D., Mudd H.S. *Biochem. Biophys. Acta* **1971**, 227, 671-684 ; *Plant Physiol.* **1972**, 51, 492-503.
16-Martel A., Bouthier de la Tour C., Le Goffic F. in *"Biochemistry of vitamin B6"*, Birkhaüser Verlag, Basel **1987**, 341-344.
17-Keith D.D., Tortora J.A., Ineichen K., Leimgruber W. *Tetrahedron* **1975**, 31, 2633-2636.
18-a)Keith D.D., Tortora J.A.,Yang R.J. *J. Org. Chem.* **1978**, 43, 3711-3713.
b)Alks V., Sufrin J. R. *Tetrahedron Lett.* **1990**, 31, 5257-5260.
19-Alks V., Sufrin J.R. *Synth. Commun.* **1989**, 19, 1479-1486.
20-Keith D.D., Yang R., Tortora J.A., Weigele M. *J. Org. Chem.* **1978**, 43, 3713-3716.
21-Hoppe I., Schöllkopf U. *Synthesis* **1982**, 129-131.
22-Hine J., Mahone L.G., Liotta C. L. *J. Org. Chem.* **1967**, 32, 2600-2609.
23-Rhoads S.J., Chattopadhyay J.K., Walli E.E. *J. Org. Chem.* **1970**, 35, 3358-3361.
24-Rhoads S.J., Walli E.E. *J. Org. Chem.* **1970**, 35, 3352-3357.
25-Daumas M., Vo-Quang L., Vo-Quang Y., Le Goffic F. *Tetrahedron Lett.* **1989**, 30, 5121-5124.
26-Hatch L.F., Nesbitt S.S. *J. Am. Chem. Soc.* **1945**, 67, 39-41.
27-Daumas M., Vo-Quang L., Vo-Quang Y., Le Goffic F. *Synthesis* **1989**, 64-66.
28-Schmidt U., Wild J., Lieberknecht A. *Synthesis* **1984**, 53-60.
29-Schmidt U., Lieberknecht A., Schanbacher U., Beuttler T., Wild J. *Angew. Chem.* **1982**, 94, 797 ; *Angew. Chem. Int. Ed. Engl.* **1982**, 21, 776.
30-Thompson W.J., Gaudino J. *J. Org. Chem.* **1984**, 49, 5237-5243.
31-Daumas M., Vo-Quang L., Le Goffic F. *Synth. Commun.* **1991**, 20, 3394-3401.
32-Bradley W., Forrest J., Stephenson O.J. *J. Chem. Soc.* 1951, 1589-1593.
33-Lindenstruth A.F., Fellman J.H., Van der Werf C.A. *J. Am. Chem. Soc.* **1950**, 72, 1886-1887.