Pergamon

Tetrahedron, Vol. 53, No. 12, pp. 4457-4468, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00118-X

Products of Ozonolysis of L-1,4-Cyclohexadienylalanine. Intramolecular Cyclization and Cyclization with Hydroxylamine. The Synthesis of Two Isomers of L-Isoxazolylalanine.

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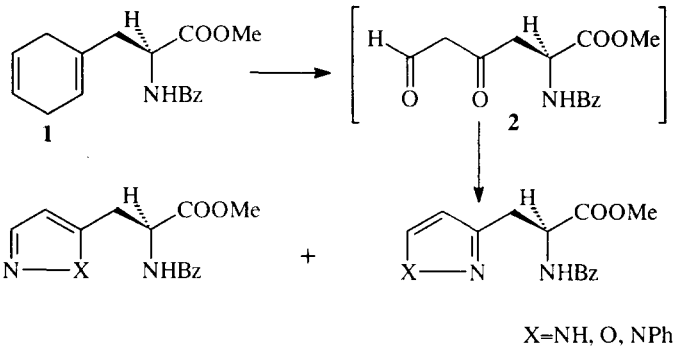
Abstract: Protected L-phenylalanine was reduced to L-1,4-cyclohexadienylalanine followed by ozonolysis of the unsaturated ring. Intramolecular cyclization of the ozonolysis product occured upon treatment with acid. The cyclization of the product of ozonolysis with hydroxylamine was studied as well. The formation of both 5- and 3-isoxazolylalanine is demonstrated. Mechanism of formation of these products and structure of intermediates are discussed. © 1997 Elsevier Science Ltd.

INTRODUCTION

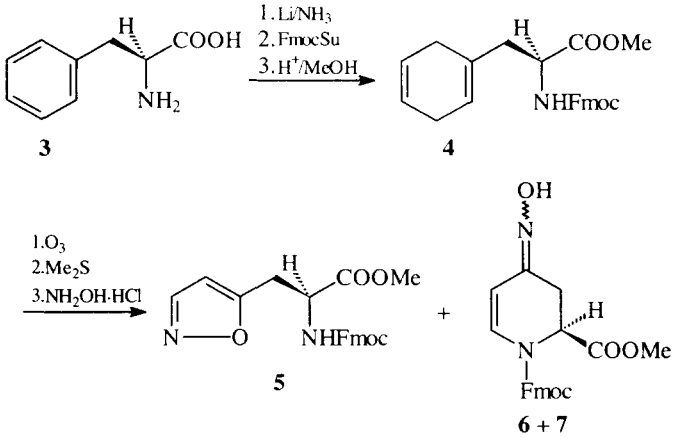
Amino acids are enjoying unprecedented renaissance in virtually all disciplines. Many nonproteinogenic α-amino acids have been found to have biological and pharmacological activities[1-5] and are also incorporated in semisynthetic penicillins[6], cephalosporins and biologically active peptides[7-8]. An approach to the transformation of L-phenylalanine into other optically active amino acids with the retention of optical activity, by a combination of Birch reduction of the aromatic ring, followed by ozonolysis of the resulting L-(1,4-cyclohexadienyl)alanine was shown recently[9]. The ozonolysis of D-(1,4-cyclohexadienyl)glycine was studied as well, leading to racemic products[10] . The methyl ester of the N-benzoyl derivative (**1**) was treated with ozone resulting in cleavage of both double bonds. However, the expected synthon (2) was neither isolated nor observed in the reaction mixture[9].



Scheme 1

RESULTS AND DISCUSSION

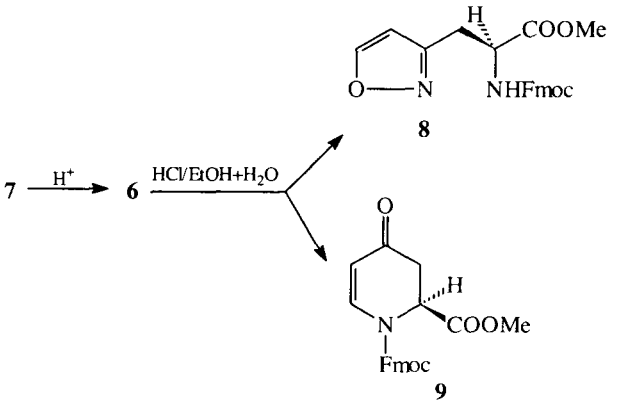
The present work uncovers several intermediates in this procedure and provides a route to the preparation of both 3- and 5-isoxazolylalanines. Thus, using the N-fluorenylmethyloxycarbonyl derivative (**4**), the resulting products can readily be deprotected[11] or used in synthesis of new pseudopeptides, new semisynthetic antibiotics, *etc.*



Scheme 2

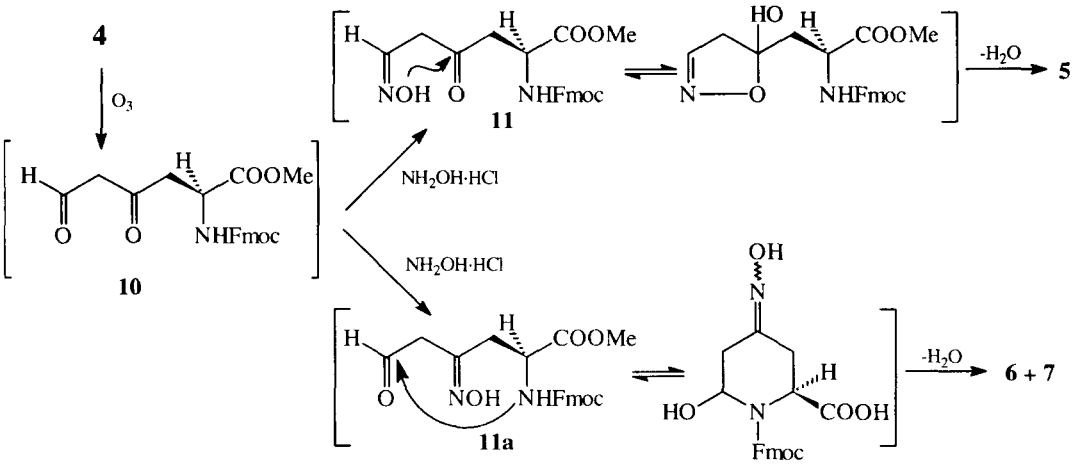
Heating the mixture resulting from the process of the ozonolysis of L-N-Fmoc(1,4-cyclohexadienyl)alanine methyl ester (**4**) with hydroxylamine hydrochloride yielded three major products. These were identified as L-N-Fmoc(isoxazol-5-yl)alanine methyl ester (**5**, 15%) and the two geometric

isomers of the pyridone oxime derivative (**6** and **7**, 26 and 21%, respectively) which could be separated by column chromatography. One of the isomers (**6**) had its vinylic ^1H absorption at 6.00 ppm while the other isomer (**7**) at 5.57 ppm. A minor product was methyl L-N-Fmoc-2-amino-4-oxopentanoate which is formed by an exhaustive ozonolysis, as described earlier[9] in the ozonolysis of the N-benzoyl derivative (**1**). Upon further heating of the cyclic oxime (**6**) in HCl for several hrs L-N-Fmoc(isoxazol-3-yl)alanine methyl ester (**8**) was obtained together with the hydrolysis product (**9**). Treatment of the second isomer (**7**) in HCl gave products **8** and **9** in the same proportions. The isomerization of the latter (**7**) into its isomer (**6**) by HCl, before its conversion to **8** and **9** could be detected (Scheme 3).



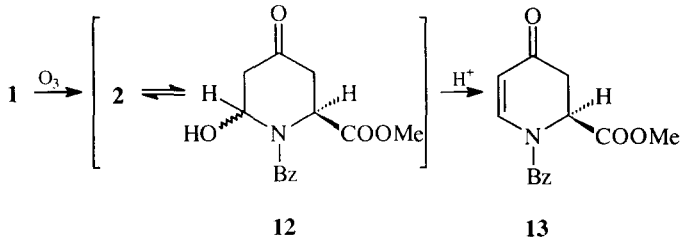
Scheme 3

It is suggested, therefore, that when hydroxylamine traps the postulated intermediate **10** by condensation at the aldehydic terminus forming oxime **11** (Scheme 4), it cyclizes to yield the isoxazol-5-ylalanine derivative (**5**). Competing reactions are both the condensation at the keto group to form an oxime (**11a**) and the cyclization of the unreacted aldehyde group with the nitrogen at the α-position. They can lead to the formation of both pyridone oximes **6** and **7**. Evidently, the protecting group does not prevent this cyclization. The reopening of the pyridone ring in the oxime takes place with HCl in methanol and water, allowing the irreversible cyclization to the isoxazole ring to produce **8**. A competing hydrolysis of the oxime group leads to the formation of **9**. The ratio of these products is 1:1 with overall yield 80%. Upon using anhydrous methanolic HCl no reaction took place and **6** was recovered unchanged. As expected, heating the mixture of the ozonolysis of **4** with methanolic HCl, without treatment with NH_2OH, the formation of **9** was observed. The procedure described above was the only way for the seperation between **5** and **8**, because in a one pot reaction in which heating in HCl was carried out before the separation of **5**, the two isomers came out from the column together.



Scheme 4

Treatment of the recently described[9] ozonolysis mixture of N-benzoyl derivative (**1**) with HCl led to the formation of the pyridone derivative **13** (Scheme 5) in 55% yield. The HCl is probably needed for the acid catalyzed dehydration of the saturated ring in intermediate **12**. A similar reaction using DL-cyclohexadienylalanine gave the same cyclic product, however, in a crystalline form and improved yield (72%). The racemic mixture of **13** separates as cubic crystals for which single crystal X-ray analysis could be carried out (Figure 1). The optically active L-isomer is isolated as a colorless oil, however it shows identical spectral data.



Scheme 5

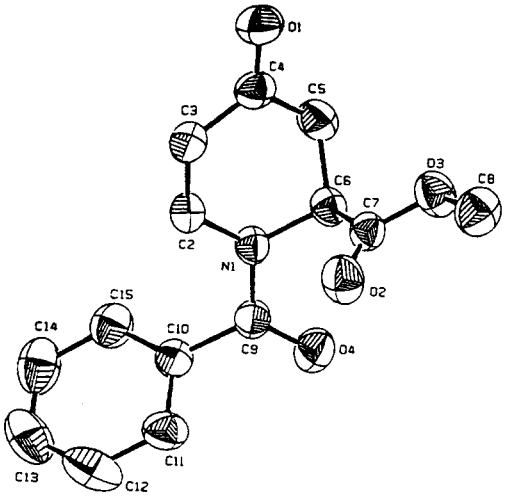
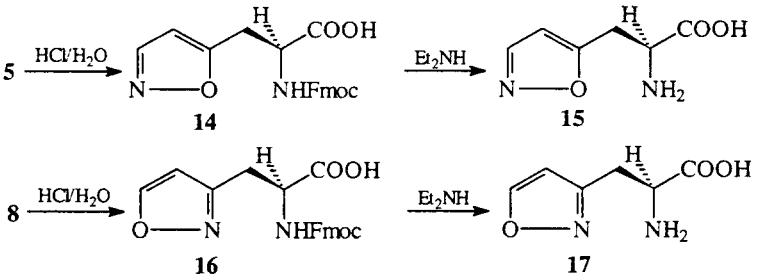


Figure 1: Structure of N-Benzoyl-6-carbomethoxy-1,4,5,6-tetrahydro-4-oxopyridine (13) by X-Ray Single Crystal Analysis

Consequently, it is assumed that the initial product is the piperidine derivative (**12**) which is in equilibrium with the open chain intermediate (**2**) (Scheme 5). Thus, in the presence of nucleophiles the open chain (2) is the reactive species and under acidic catalysis the dehydration of **12** occurs to yield **13** as the major product.

Heating the isoxazolylalanine derivatives **5** and **8** in aqueous HCl gave the corresponding N-protected amino acids **14** and **16** respectively with good yields (87-91%). Removal of the protecting Fmoc group was achieved by treatment with diethylamine[11], thus concluding the synthesis of the two isomers of isoxazolylalanine, i.e. L-isoxazol-5-ylalanine (15) and L-isoxazol-3-ylalanine (**17**).



Scheme 6

ACKNOWLEDGMENT

We wish to thank Dr. Shmuel Cohen for his dedication in carrying out the X-ray diffraction analysis.

EXPERIMENTAL SECTION

General Methods. Melting points are uncorrected. Chromatographic separation was carried out with silica gel (230-400 mesh) on a 450x10 mm column.

X-Ray Crystal Structure Analysis data were measured on an RNRAF-NONITS CAD-4 Computer-Controlled Difractometer. CuK_α (λ=1.54178 Å) radiation with graphite crystal monochromator in the incident beam was used. The unit cell dimensions were obtained by a least-squares fit of 24 centered reflections in the range of $25 \leq \Theta \leq 30°$.

Intensity data were collected using the ω–2θ technique to a maximum 2θ of 120°. The scan width, $\Delta\omega$, for each reflection was $0.80+0.15\tan\theta$. An aperture with a height of 4 mm and a variable width, calculated as $(2.0+0.5\tan\theta)$ mm, was located 173 mm from the crystal. Reflections were measured with a scan of 8.24°/min. The rate of the final scan was calculated from the preliminary scan results so that the ratio I/σ (I) would be at least 40 but the maximum scan time would not exceed 60 seconds. If in the preliminary scan I/σ (I)<2, this measurement was used as the datum. Scan rates varied from 1.27 to 8.24°/min. Of the 96 steps in the scan, the first and the last 16 steps were considered to be background. During data collection the intensities of three standard reflections were monitored after every hour of X-ray exposure. No decay was observed. In addition, three orientation standards were checked after 100 reflections to check the effects of crystal movement. If the standard deviation of the h, k, and l values of any orientation reflection exceeded 0.08, a new orientation matrix was calculated on the basis of the recentering of the 24 reference reflections.

Intensities were corrected for Lorentz and polarization effects All non-hydrogen atoms were found by using the results of the SHELXS-86 direct method analysis[12]. After several cycles of refinements[13] the positions of hydrogen atoms were calculated and added to the refinement process. Refinement proceeded to convergence by minimizing the function $\Sigma\omega(|F_o| - |F_c|)^2$. A final difference Fourier synthesis map showed several peaks less than 0.2 e/Å^{-3} scattered about the unit cell without a significant feature. The discrepancy indices, $\Sigma\omega(|F_o| - |F_c|)/\Sigma|F_o|$ and $R_\omega=[\Sigma\omega(|F_o| - |F_c|)^2/\Sigma(|F_o|^2]^{1/2}$ are presented with other pertinent crystallographic data in Table 1

Table 1. Crystallographic data[a]:

formula	$C_{15}H_{17}N_3O_3$
space group	I2/a
a, Å	18.319(3)
b, Å	11.971(2)
c, Å	144.117(2)
β ,deg	106.52(1)
v, Å^3	2968.0(6)
z	8
ρ_{calcd}, g cm^{-3}	1.29
$\mu(MoK_\alpha)$, cm^{-1}	0.86
no. of unique reflections	2750
no. of reflections with $I \geq 2\sigma_I$	2040
R	0.043
R_ω	0.055

[a]Standard calibrations, data formats and positional parameters, structure factors and U values were submitted to the Editor.

L-N-9-Fluorenylmethyloxycarbonyl(1,4-cyclohexadienyl)alanine: A solution of 6.0 g of L-phenylalanine in 300 mL of liquid NH_3 on aceton-dry ice bath was slowly diluted with 100 mL of t-butanol, during 1 h. Lithium (3.0 g) was added in small portions until the blue color persisted. Most of the NH_3 evaporated by stirring overnight at room temperature and the remaining solvent was removed under reduced pressure. The white residue was dissolved in water (100 mL) and acidified to pH 7 by HCl. FmocOSu (14 g) in acetonitrile (200 mL) and triethylamine (11.2 mL) were added and the mixture was stirred at room temperature for 6 h. Water (250 mL) was added and the solution was washed with petroleum ether (3x100 mL) then acidified with 1 N HCl (60 mL) and extracted with ethyl acetate (3x100 mL). The organic layer was dried over $MgSO_4$ and the product crystallized upon concentration under reduced pressure. Yield 13.1 g (92%); m.p. 115-6°C (chloroform-diisopropyl ether); $[\alpha]_D^{25}$ -11° (c = 1, methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.37-2.63 (6 H, m, β-CH_2 and cyclohexadienyl CH_2), 4.21 (1 H, t, J = 7.02 Hz, Fmoc), 4.37 (2 H, dt, J_1 = 7.02 Hz, J_2 = 1.16 Hz, Fmoc), 4.44 (1 H, dd, J_1 = 7.72 Hz, J_2 = 5.11 Hz, α-CH), 5.37 (1 H, d, J = 7.72 Hz, NH), 5.52 (1 H, s, vinyl), 5.66 (1 H, s, vinyl), 7.28 (2 H, dt, J_1 = 7.33 Hz, J_2 = 1.19 Hz, Fmoc), 7.37 (2 H, t, J = 7.33 Hz, Fmoc), 7.57 (2 H, d, J = 7.33 Hz, Fmoc), 7.73 (2 H, d, J = 7.33 Hz, Fmoc), 9.33 (1 H, s, COOH). Anal. Calcd. for $C_{24}H_{23}NO_2$: C, 80.64; H 6.49; N 3.92. Found: C, 80.68; H 6.62; N 3.61.

Methyl L-9-Fluorenylmethyloxycarbonyl(1,4-cyclohexadienyl)alaninate (4): A solution of 3.0 g of N-9-fluorenylmethyloxycarbonyl(1,4-cyclohexadienyl)alanine and 1.3 g of camphorsulfonic acid in methanol (50 mL) was heated under reflux for 3 h. Methanol was evaporated, the residue was dissolved in water, neutralized by $NaHCO_3$ and extracted with 3 portions (50 mL) of ethyl acetate. The product crystallized upon concentration under reduced pressure. Yield: 2.9 g (86 %); m.p. 91-2°C (chloroform-diisopropyl ether); $[\alpha]_D^{25}$ +5° (c = 1, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.48-2.66 (6 H, m, β-CH_2 and cyclohexadienyl CH_2), 3.68 (1 H, s, COOMe), 4.21 (1 H, t, J = 7.21 Hz, Fmoc), 4.38 (2 H, dt, J_1 = 7.21 Hz, J_2 = 1.16 Hz, Fmoc), 4.46 (1 H, dd, J_1 = 8.01 Hz, J_2 = 4.98, Hz, α-CH), 5.38 (1 H, d, J = 8.01 Hz, NH), 7.29 (2 H, t, J = 7.39 Hz, Fmoc), 7.38 (2 H, t, J = 7.44 Hz, Fmoc), 7.57 (2 H, d, J = 7.39 Hz, Fmoc), 7.74 (2 H, d, J = 7.44 Hz, Fmoc). Anal. Calcd. for $C_{25}H_{25}NO_4$: C, 74.42; H 6.25; N 3.47. Found: C, 74.26; H 66.23; N 3.19.

Ozonolysis of methyl L-N-fluorenylmethyloxycarbonyl(1,4-cyclohexa- dienyl)alaninate and cyclization with hydroxylamine hydrochloride **cyclization:** Methyl L-N-9-fluorenylmethyloxycarbonyl(1,4-cyclohexa-dienyl)alaninate (**4**, 1.0 g) in 10 mL dichloromethane was added to a saturated solution of ozone in dichloromethane (25 mL) on aceton-dry ice bath, buffered with 0.2 g of $NaHCO_3$. More ozone was added until blue color persisted. The mixture was washed with nitrogen, dimethyl sulfide (5 mL) was added and allowed to warm to room temperature overnight. The solution was filtered and the solvent was removed under reduced pressure. The residue dissolved in MeOH (25 mL), hydroxylamine hydrochloride (0.5 g) was added and the mixture refluxed for 4 h, then cooled, diluted with ice-water and neutralized with sodium bicarbonate. The crude product was extracted with ethyl acetate and chromatographed with a solvent gradient: ethyl acetate-petroleum ether 1:20 - 1:4. The product eluted first was methyl N-9-fluorenylmethyloxycarbonyl(isoxazol-5-yl)-L-alaninate (**5**): 0.15 g; m.p. 146-7°C; $[\alpha]_D^{25}$ +33° (c = 1, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.39 (2 H, dd, J_1 = 5.18 Hz, J_2 = 16.42 Hz, β-CH_2), 3.80 (3 H, s, ester), 4.25 (1 H, t, J = 6.59 Hz, Fmoc), 4.43 (2 H, dt, J_1 = 6.59 Hz, J_2 = 1.12 Hz, Fmoc), 4.76 (1 H, dd, J_1 = 7.16 Hz, J_2 = 5.18 Hz, α-CH), 5.48 (1 H, d, J = 7.16 Hz, NH), 6.04 (1 H, d, J ~ 1 Hz, isoxazolyl), 7.32 (2 H, t, J = 7.31 Hz, Fmoc), 7.41 (2 H, t, J = 7.43 Hz, Fmoc), 7.58 (2 H, d, J = 7.31 Hz, Fmoc), 7.77 (2 H, d, J = 7.43 Hz, Fmoc), 8.17 (1 H, d, J ~ 1 Hz, isoxazolyl). Anal. Calcd. for $C_{22}H_{20}N_2O_5$: C, 67.34; H 5.14; N 7.14. Found: C, 67.13; H 5.02; N 6.81.

Pyridone oxime (**7**, 0.25 g, oil). The ^{1}H NMR in DMSO-d_6 (300 MHz) indicates it to be a 1:1 mixture of nitrogen invertomers (in $CDCl_3$ the ratio is 3:2): 2.56 (2 H, m, CH_2), 3.59 (1.5 H, s, COOMe), 3.64 (1.5 H, s, COOMe), 4.23 0.5 H, t, J = 5.11 Hz, Fmoc), 4.29-4.59 (2.5 H, m, Fmoc), 4.88 (0.5 H, d, J = 6.88 Hz, (α-CH), 5.09 (0.5 H, d, J = 6.91, α-CH), 5.46 (0.5 H, d, J = 8.35 Hz, vinyl), 5.49 (0.5 H d, J = 8.50 Hz, vinyl), 6.97 (0.5 H, d, J = 8.35 Hz, vinyl), 7.07 (0.5 H, d, J = 8.50 Hz, vinyl), 7.31-7.36 (2 H, m, Fmoc), 7.41-7.44 (2 H, m, Fmoc), 7.61 (2 H, m, Fmoc), 7.91 (2 H, m, Fmoc), 10.96 (0.5 H, s, NOH), 10.98 (0.5 H, s, NOH). This product is not stable under conditions of crystallization, it is partially converted to **6**.

The third isolated product (**6**, 0.2 g, oil) is a 3: 2 mixture of two nitrogen invertomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ: major: 2.69-2.98 (2 H, m, CH_2), 3.66 (3 H, s, COOMe), 4.25-4.56 (3 H, m, Fmoc), 5.07 (1 H, d, J = 5.18, α-CH), 6.00 (1 H, 2 d, J = 8.51 Hz, vinyl), 7.13-7.27 (4 H, m, Fmoc+vinyl), 7.52 (2 H, m, Fmoc), 7.70 (2 H, 2d, J = 7.12 Hz, Fmoc), 9.20 (1 H, bs, NOH). Minor: 2.69-2.98 (2 H, m, CH2), 3.58 (3 H, s, COOMe), 4.16 (1 H, t, J = 5.16 Hz, Fmoc), 4.25-4.56 (2 H, m, Fmoc), 4.66 (1 H, d, J = 5.25 Hz, (α-CH), 6.00 (1 H, 2 d, J = 8.51 Hz, vinyl), 7.13-7.27 (4 H, m, Fmoc+vinyl), 7.52 (2 H, m, Fmoc), 7.70 (2 H, 2d, J = 7.12 Hz, Fmoc), 9.20 (1 H, bs, NOH). Anal. Calcd. for $C_{22}H_{20}N_2O_5$: C, 67.34; H 5.14; N 7.14. Found: C, 66.97; H 4.85; N 6.95.

One pot reaction in which heating in HCl was carried out before the column separation, both **5** and **8** came out from the column together. Another isolated product was identified as methyl L-N-Fmoc-2-amino-4-oxopentanoate (**4**): yield 0.085 g; m.p. 111-2°C; $[\alpha]_D^{25}$ +21° (c = 1, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.19 (3 H, s, Me), 3.12 (2 H, dd, J_1 = 4.25 Hz, J_2 = 18.42 Hz, CH_2), 3.75 (3 H, s, COOMe), 4.25, (1 H, t, J = 6.96 Hz, Fmoc), 4.38 (2 H, bt, J = 6.96 Hz, Fmoc), 4.56 (1 H, dd, J_1 = 4.25 Hz, J_2 = 8.05 Hz, α-CH), 5.80 (1 H, d, J = 8.05 Hz, NH), 7.31 (2 H, t, J = 7.21 Hz, Fmoc), 7.39 (2 H, t, J = 7.28 Hz, Fmoc), 7.60 (2 H, d, J = 7.21 Hz, Fmoc), 7.77 (2 H, d, J = 7.28 Hz, Fmoc). Anal. Calcd. for $C_{21}H_{21}NO_5$ requires C, 68.65; H 5.76; N 3.81. Found: C, 68.33; H 5.61; N 3.42.

Heating of pyridone oxime derivative (6) in HCl (MeOH-H_2O 10:3): Pyridone derivative (**6**, 0.1 g) was heated under reflux in the mixture of MeOH (10 mL) and 38 % HCl (3 mL) for 36 h. The solvent removed under reduced pressure and the product was chromatographed with a solvent gradient: ethyl acetate-petroleum ether 1:5 - 1:3. Two products were isolated. One was methyl N-9-fluorenylmethyloxycarbonyl(isoxazol-3-yl)-L-alaninate (**8**): 0.04 g (40 %); m.p. 142-3°C; $[\alpha]_D^{25}$ +18° (c = 1, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.39 (2 H,d, J = 5.31 Hz, β-CH_2), 3.77 (3 H, s, COOMe), 4.22 (1 H, t, J = 6.90 Hz, Fmoc), 4.39 (2 H, dt, J_1 = 6.90 Hz, J_2 = 1.12 Hz, Fmoc), 4.76 (1 H, dd, J_1 = 8.06 Hz, J_2 = 5.31 Hz, α-CH), 5.65 (1 H, d, J = 8.06, NH), 6.18 (1 H, d, J ~ 1 Hz, isoxazolyl), 7.31 (2 H, t, J = 7.41 Hz, Fmoc), 7.40 (2 H, t, J = 7.31 Hz, Fmoc), 7.58 (2 H, d, J = 7.41 Hz, Fmoc), 7.76 (2 H, d, J = 7.31 Hz, Fmoc), 8.35 (1 H, d, J ~ 1 Hz, isoxazolyl). Anal. Calcd. for $C_{22}H_{20}N_2O_5$ requires C, 67.34; H 5.14; N 7.14. Found: C, 67.22; H 5.09; N 7.03.

The second eluated product was pyridone derivative **9** which is a 1:1 mixture of two nitrogen invertomers: 0.38 g (40 %, oil); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.94 (1 H, m, CH_2), 3.64 (1.5 H, s, COOMe), 3.75 (1.5 H, s, COOMe), 4.26 (0.5 H, bt, Fmoc), 4.56 (2 H, bd, Fmoc), 4.78 (0.5 H, d, J = 4.01 Hz, α-CH), 5.24, (0.5 H, d, J = 5.72 Hz, α-CH), 5.36 (1 H, 2 bd, vinyl), 7.34 (2 H, t, J = 7.07 Hz, Fmoc), 7.43 (2 H, t, J = 7.43 Hz, Fmoc), 7.51-7.57 (2 H, 2 bd, Fmoc), 7.78 (2.5 H, 2d, J = 7.43 Hz, Fmoc + vinyl), 7.92 (0.5 H, d, J = 7.51 Hz, vinyl). Anal. Calcd. for $C_{22}H_{19}NO_5$: C, 70.02; H 5.07; N 3.71. Found: C, 70.32; H 5.22; N 3.55.

L-N-benzoyl-6-carbomethoxy-1,4,5,6-tetrahydro-4-oxopyridine (13): Methyl L-N-benzoyl(1,4-cyclohexadienyl)alaninate (**1**, 0.5 g) was subjected to ozonolysis and reduction as reported recently1. The resulted reaction mixture was refluxed in 3N HCl/MeOH for 40 min, then cooled, diluted with ice-water and neutralized with sodium bicarbonate. The crude product was extracted with ethyl acetate and chromatographed with a solvent gradient: ethyl acetate-petroleum ether 1:10-1:3. Yield: 0.25 g (55 %, oil); $[\alpha]_D^{25}$ -75° (c = 1, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.98 (2 H,m, CH_2), 3.71 (3 H, s, COOMe), 5.24 (1 H, d, *J* = 8.37 Hz, vinyl), 5.42 (1 H, d, *J* = 5.72 Hz, α-CH), 7.41-7.55 (5 H, m, benzoyl), 7.42 (1 H, d, *J* = 8.37 Hz, vinyl). Anal. Calcd. for $C_{14}H_{13}NO_4$: C, 64.86; H 5.05; N 5.40. Found: C, 64.68; H 4.92; N 5.21. Methyl L-2-benzoylamino-4-oxopentanate[9] (**4**, 0.12 g) was also isolated.

DL-N-Benzoyl(1,4-cyclohexadienyl)alanine: Was prepared by the procedure which was described for the preparation of L-N-benzoyl(1,4-cyclohexadienyl)alanine[9]. Yield 72%; m.p. 183-4°C (ethanol). Spectral and analytical data are identical to those for the L-isomer[9].

Methyl DL-N-benzoyl(1,4cyclohexadienyl)alaninate: Was prepared by the procedure which was described for the preparation of methyl L-N-benzoyl(1,4-cyclohexadienyl)alanine[9]. Yield 72%; semisolid. Spectral and analytical data are identical to those for the L-isomer[9].

DL-N-benzoyl-6-carbomethoxy-1,4,5,6-tetrahydro-4-oxopyridine: Was obtained by the same procedure as for the L-form above from methyl DL-N-benzoyl(1,4-cyclohexadienyl)alaninate. Yield 40 %; m.p. 135-6°C. Spectral and analytical data are identical to those for the L-isomer. Molecular structure by single crystal X-ray analysis is shown in Fig.1.

N-9-Fluorenylmethyloxycarbonyl(isoxazol-5-yl)-L-alanine (14): Methyl N-9-fluorenylmethyloxycarbonyl-(isoxazol-5-yl)-L-alaninate (**5**, 0.25 g) was dissolved in 1:1 mixture of 2 N HCl-dioxane (15 mL) and refluxed for 6 h. The product crystallized upon concentration under reduced pressure. Yield 0.21 g (87 %); m.p. 159-60°C (chloroform); $[\alpha]_D^{25}$ -14° (c = 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.41 (2 H, dd, J_1 = 5.12 Hz, J_2 = 12.79 Hz, β-CH_2), 4.20 (1 H, t, *J* = 6.64 Hz, Fmoc), 4.42 (2 H, bd, *J* = 6.64 Hz, Fmoc), 4.74 (1 H, dd, J_1 = 7.78 Hz, *J*2 = 5.12 Hz, α-CH), 5.62 (1 H, d, *J* = 7.78 Hz, NH), 6.08 (1 H, d, *J* ~ 1 Hz, isoxazolyl), 7.30 (2 H, t, *J* = 7.41 Hz, Fmoc), 7.39 (2 H, t, *J* = 7.37 Hz, Fmoc), 7.56 (2 H, d, *J* = 7.41 Hz, Fmoc), 7.75 (2 H, d, *J* = 7.37 Hz, Fmoc), 8.17 (1 H, d, *J* ~ 1 Hz, isoxazolyl). Anal. Calcd. for $C_{21}H_{18}N_2O_4$: C, 69.60; H 5.01; N 7.73. Found: C, 69.45; H 4.91; N 7.52.

Isoxazol-5-yl-L-alanine (15): N-9-Fluorenylmethyloxy-carbonyl(isoxazol-5-yl)-L-alanine (**14**, 0.05 g) was dissolved in ethanol (10 mL) and diethylamine (1 mL) was added. After leaving overnight at room temperature the solvent was evaporated under reduced pressure. The crude product was redissolved in water

(10 mL) and washed by ethyl acetate (3x10 mL). The product crystallized upon concentration. Yield 0.02 g (93 %); m.p. 165-6°C; $[\alpha]_D^{25}$ -16° (c = 1, H_2O); ^1H NMR (300 MHz, D_2O) δ: 3.53 (2 H, m, β-CH_2), 4.18 (1 H, t, J = 5.89 Hz, α-CH), 6.46 (1 H, d, J ~ 1 Hz, isoxazolyl), 8.46 (1 H, d, J ~ 1 Hz, isoxazolyl). Anal. Calcd. for $C_6H_8N_2O_3{\cdot}C_4H_{11}N$: C, 52.39; H 8.35; N 18.33. Found: C,52.95; H 8.77; N 18.09.

N-9-Fluorenylmethyloxycarbonyl(isoxazol-3-yl)-L-alanine (16): Methyl N-fluorenylmethyloxycarbonyl-(isoxazol-3-yl)-L-alaninate (**8**, 0.25 g) was dissolved in 1:1 mixture of 2 N HCl-dioxane (15 mL) and refluxed for 6 h. The product crystallized upon concentration under reduced pressure. Yield 0.22 g (91 %); m.p. 127-8°C (chloroform); $[\alpha]_D^{25}$ +16° (c = 1, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ: 3.33 (2 H, bd, J = 5.32 Hz, β-CH_2), 4.22 (1 H, t, J = 6.96 Hz, Fmoc), 4.40 (2 H, bd, J = 6.59 Hz, Fmoc), 4.77 (1 H, bd, J_1 = 7.59 Hz, J_2 = 5.32 Hz, α-CH), 5.74 (1 H, d, J = 7.59 Hz, NH), 6.23 (1 H, d, J ~ 1 Hz, isoxazolyl), 7.31 (2 H, t, J = 7.38 Hz, Fmoc), 7.40 (2 H, t, J = 7.49 Hz, Fmoc), 7.57 (2 H, d, J = 7.38 Hz, Fmoc), 7.76 (2 H, d, J = 7.49 Hz, Fmoc), 8.35 (1 H, d, J ~ 1 Hz, isoxazolyl). Anal. Calcd. for $C_{21}H_{18}N_2O_4$: C, 69.60; H 5.01; N 7.73. Found: C, 69.33; H 4.88; N 7.43.

Isoxazol-3-yl-L-alanine (17): N-9-Fluorenylmethyloxy-carbonyl(isoxazol-3-yl)-L-alanine (**16**, 0.05 g) was dissolved in ethanol (10 mL) and diethylamine (1 mL) was added. After leaving overnight at room temperature the solvent was evaporated under reduced pressure. The crude product was dissolved in water (10 mL) and washed with ethyl acetate (3x10 mL). The product crystallized upon concentration. Yield 0.02 g (93 %); m.p. 165-6°C; $[\alpha]_D^{25}$ -5° (c = 1, H_2O); ^1H NMR (300 MHz, D_2O) δ: 3.42 (2 H, dd, J_1 = 4.75 Hz, J_2 = 11.26 Hz, β-CH_2), 4.18 (1 H, d, J = 4.75 Hz, α-CH), 6.56 (1 H, d, J = 1.62 Hz, isoxazolyl), 8.67 (1 H, d, J = 1.62 Hz, isoxazolyl). Found: C, 52.18; H 8.67; N 18.19. Anal. Calcd. for $C_6H_8N_2O_3{\cdot}C_4H_{11}N$: C, 52.39; H 8.35; N 18.33.

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(*Received in UK* 16 *December* 1996; *revised* 29 *January* 1997; *accepted* 30 *January* 1997)