

OXAZIRIDINES AND NITRONES AS POTENTIAL INHIBITORS OF BACTERIAL D,D-PEPTIDASES

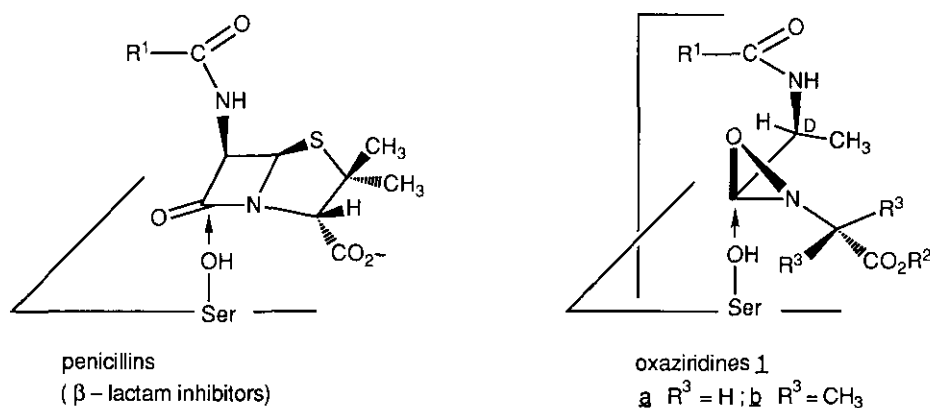
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Abstract—Novel functionalized oxaziridines and nitrones have been prepared and examined as potential irreversible inhibitors of bacterial PBPs. N-(β -Hydroxycarbonyl)ethyloxaziridines were found to undergo a fast isomerisation in protic solvent to yield the corresponding nitrones.

INTRODUCTION

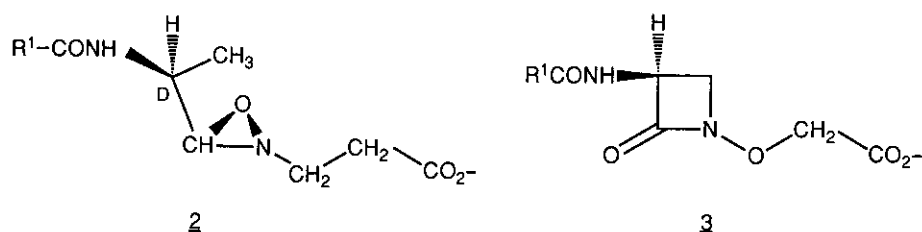
The most important clinical problems encountered in antibiotherapy using β -lactam derivatives¹ arise from the widespread emergence of resistant pathogenic bacteria producing β -lactamases.² Therefore, intensive efforts have been made to discover new active β -lactams that will overcome the bacterial defense mechanism.³ Concurrently, the design and synthesis of non- β -lactam analogs have received increasing attention.^{4,5} We have ourselves examined several classes of heterocycles^{6,7} susceptible to interact with the penicillins binding proteins (PBPs)^{3,2} in the same manner as the β -lactams. Oxaziridines⁸ have been considered as potential irreversible alkylating inhibitors of the bacterial serine D,D-transpeptidases.



Scheme 1

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Taking account of stereoelectronic factors for nucleophilic ring opening and topological requirements for molecular recognition, we selected structures **1** as first synthetic targets⁸ (Scheme 1). Oxaziridines **1a** were found to be unstable, giving rise to a rapid β -elimination.^{9,10} On the other hand, oxaziridines **1b**, bearing no hydrogen atom α to the carbonyl group, could be isolated as esters ($R^2 \neq H$) and one derivative ($R^1 = PhCH_2$, $R^2 = CH_2OCOC_4H_9$, $R^3 = CH_3$) showed a weak bacteriostatic activity in vitro.⁸ Unfortunately, attempts to prepare the free acids **1b** ($R^2 = H$) failed probably as a result of intramolecular cleavage of the three-membered ring by the carboxyl group. We therefore decided to synthesize the higher homologs **2** (Scheme 2) which can be considered as topological analogs of oxamazins **3**,^{11,12} a novel class of β -lactam antibiotics. These compounds having no acidic hydrogen were expected to be more stable.

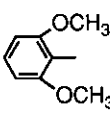


Scheme 2

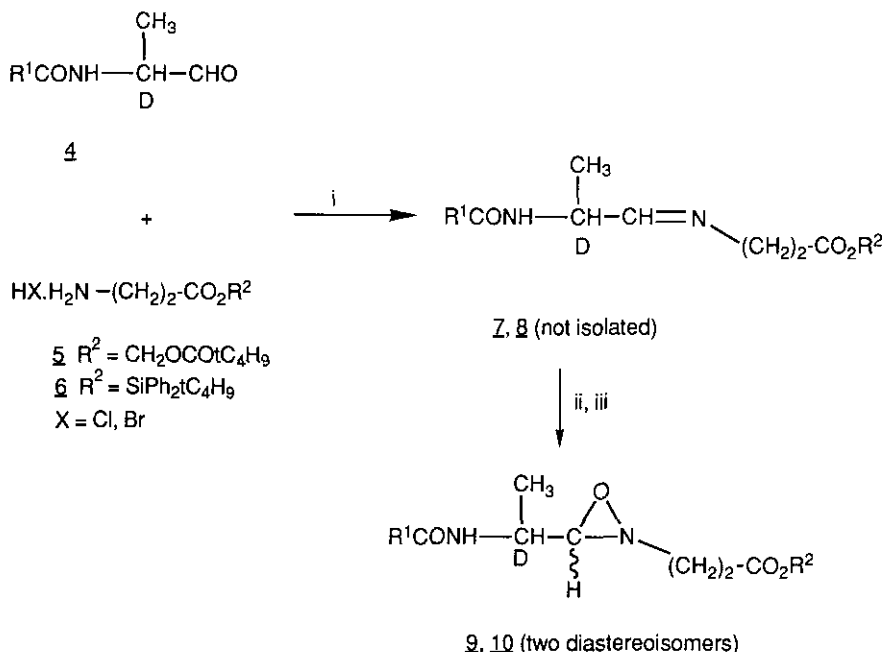
RESULTS

Oxaziridines **9-10** (Table I) were readily prepared by a convergent synthetic plan involving the oxidation of Schiff bases **7-8** derived from D- α -alaninals **4** and β -alaninates **5-6** (Scheme 3). Aldehydes **4** obtained by reduction of the appropriate D-alanine derivatives have been previously described.^{8,13} They readily reacted with pivaloyloxymethyl- or t-butylidiphenylsilyl- β -alaninates **5** and **6** to yield respectively the (E)-imines **7** and **8**. These crude intermediates were directly oxidized with m-chloroperbenzoic acid at room temperature; the stable oxaziridines **9** and **10** were obtained as mixtures of two diastereoisomers, resulting from unselective formation of a new asymmetric center in the oxidation process.¹⁴ The proton on the oxaziridine ring gives rise to two doublets near δ 4.00 and the carbon atom shows two typical lines near 82 ppm, in the nmr spectra.

Table I : Preparation of oxaziridines (Scheme 3)

R^1	R^2	Compd	Yield (from 4)
$PhCH_2$	$CH_2OCOC_4H_9$	9a	40%
$PhOCH_2$	$CH_2OCOC_4H_9$	9b	47%
	$CH_2OCOC_4H_9$	9c	52%
$PhCH_2$	$SiPh_2tC_4H_9$	10a	60%
$PhOCH_2$	$SiPh_2tC_4H_9$	10b	54%

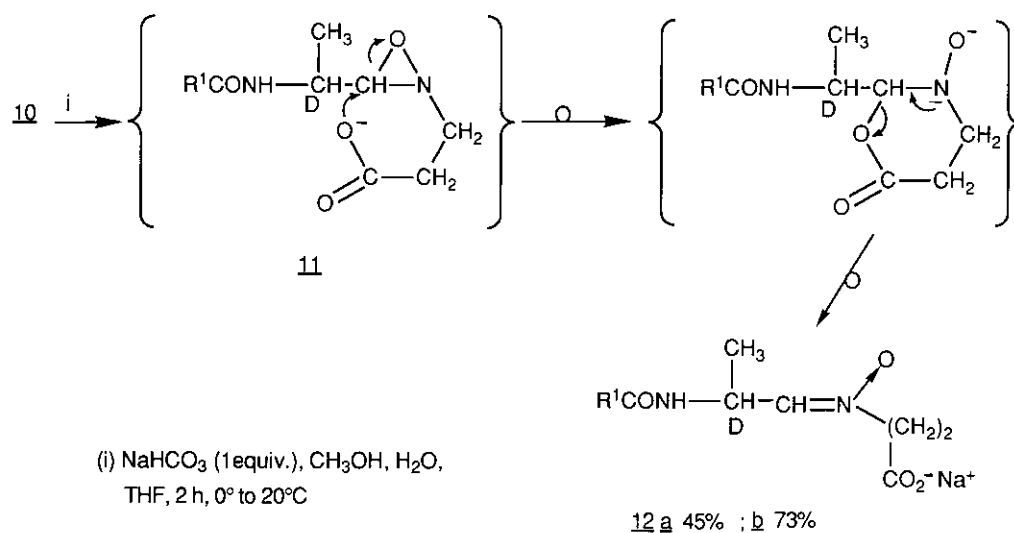
The biodegradable¹⁵ esters **9a-c** were submitted to biological evaluation towards representative gram-positive and gram-negative bacterial strains. No activity was found for concentrations up to 200 μ M.¹⁶



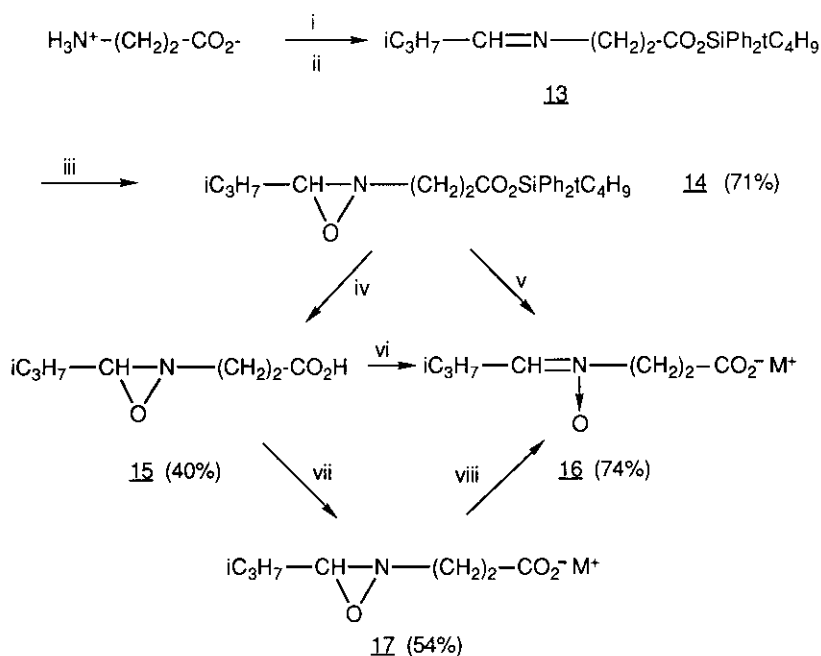
- (i) $(\text{C}_2\text{H}_5)_3\text{N}$ (1equiv.), 3Å molecular sieves, CH_2Cl_2 , 20°C, 3 h;
(ii) MCPBA (1equiv.), CH_2Cl_2 , 20°C, 2 h; (iii) chromatography on SiO_2

Scheme 3

Mild acidic hydrolysis (HOAc , H_2O -THF, 0° to 20°C) of the silyl ester **10** gave untractable mixtures. Smooth basic hydrolysis (NaHCO_3 , CH_3OH - H_2O , 0° to 20°C) also resulted in the cleavage of oxaziridine ring. Nitrones **12** were the only isolated products in moderate to good yields (Scheme 4). These new compounds are well characterized in ^1H nmr by a doublet near δ 7.10-7.20 assigned to the azomethinic proton. The carbon atom of the $\text{C}=\text{N}$ double bond gives a typical line near 145 ppm in ^{13}C nmr. The chemical unstability of the deprotected oxaziridines **11** probably results from an anchimeric assistance of the carboxylate group to nucleophilic cleavage of the oxaziridine C-O bond (Scheme 4). Indeed, oxaziridine rearrangements^{17,18} into nitrones normally occur under more drastic conditions, for instance in the presence of strong Lewis acid catalyst^{19,20} or at much higher temperature.^{19,21} Since the presence of an acylamino side chain in **10-11** could also contribute to the cleavage of the oxaziridine ring, we investigated the behaviour of the model compound **15**. The precursor **14** of the free acid was prepared in 71% overall yield from the oxidation of the imine **13** derived from β -alanine and isobutyraldehyde (Scheme 5). Treatment of **14** with an excess of acetic acid in aqueous THF solution smoothly cleaved the silyl ester²² to give the stable oxaziridine **15** which was purified by chromatography on silica gel. The corresponding carboxylate **17** ($\text{M}^+=\text{Na}^+$) obtained by addition of aqueous sodium hydrogenocarbonate immediately yielded the corresponding nitrone **16**. However, the oxaziridine ring of **17** was found to be stable in non-protic solvent: addition of potassium 2-ethylhexanoate to **15** in dry ethyl acetate led to the precipitation of the potassium salt **17**. The stability of this material was further examined by ^1H nmr spectroscopy in CD_3OD : rearrangement into nitrone **16** ($\text{M}^+=\text{K}^+$) occurred within 20 minutes at 30°C.



Scheme 4

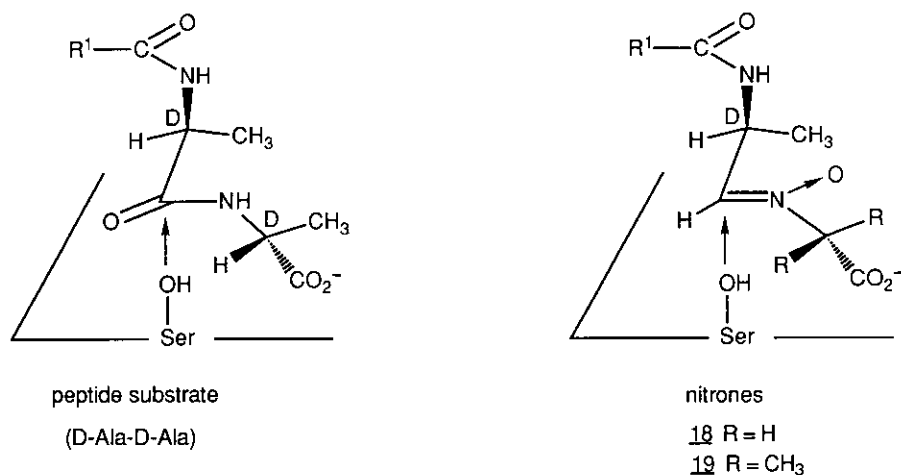


(i) $\text{ClSiPh}_2\text{tC}_4\text{H}_9$, CHCl_3 , CH_3CN , Δ ; (ii) $(\text{C}_2\text{H}_5)_3\text{N}$, $3\text{\AA}$ molecular sieves, $\text{iC}_3\text{H}_7\text{-CHO}$, CH_2Cl_2 ; (iii) MCPBA, CH_2Cl_2 , 20°C ; (iv) $\text{HOAc-THF-H}_2\text{O}$ (2:1:1), 20°C , 24 h; (v) NaHCO_3 (1equiv.), CH_3OH , THF , H_2O , 2 h, 20°C ; (vi) NaHCO_3 (1equiv.), Acetone, H_2O ; (vii) K^+ 2-ethylhexanoate, $\text{C}_2\text{H}_5\text{OAc}$, 20°C ; (viii) CD_3OD , 30°C .

Scheme 5

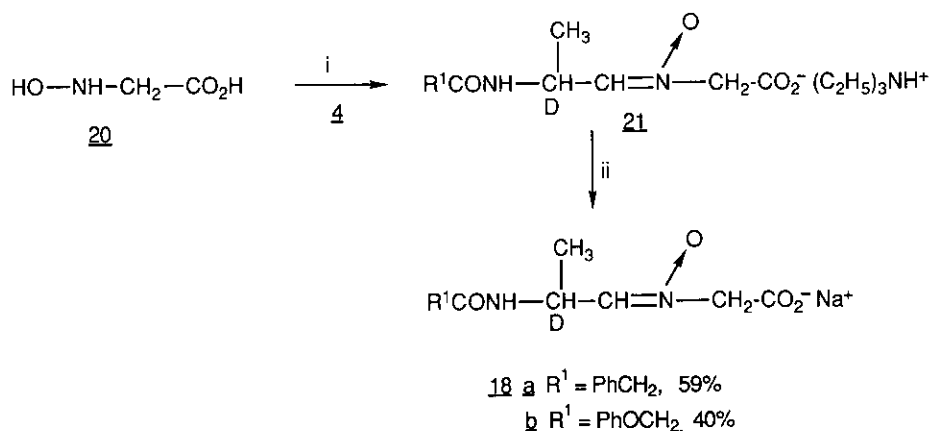
Thus oxaziridines **2** bearing a β -(hydroxycarbonyl)ethyl chain on the nitrogen atom are expected to be converted into the corresponding nitrones at physiological pH. On the other hand, oxaziridines **9,10,14** and **15** were found to be stable under usual chromatographic conditions. No rearrangement into corresponding amides was observed.²³

The nitrones themselves^{24,25} were examined as potential D,D-peptidases inhibitors. Compounds **18-19** can be regarded as modified peptide substrates which should be activated towards nucleophilic attack²⁶ (Scheme 6).



Scheme 6

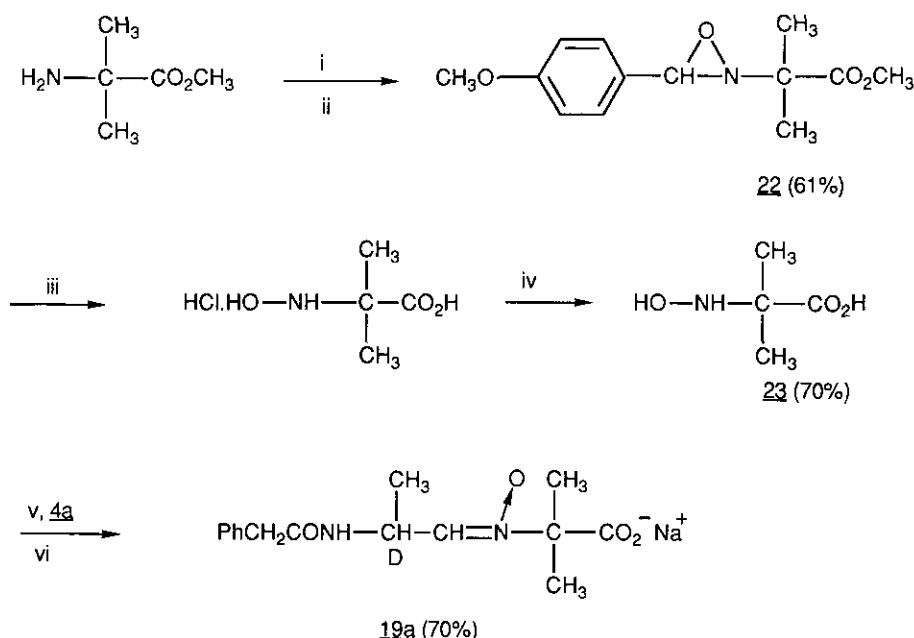
Nitrones **18** were conventionally prepared²⁴ by coupling the known hydroxylamine derivative **20**²⁷ with aldehydes **4** in the presence of triethylamine (Scheme 7). The triethylammonium carboxylate intermediates **21** were washed with aqueous NaHCO₃ to yield the sodium salts **18**.



(i) (C₂H₅)₃N(1equiv.), 3Å molecular sieves, CH₂Cl₂, 40°C; (ii) NaHCO₃, H₂O, 20°C

Scheme 7

Nitrone **19a** was prepared according to Scheme 8. The Schiff base derived from methyl α -aminoisobutyrate and p-methoxybenzaldehyde was oxidized to oxaziridine **22**. Strong acidic hydrolysis²⁸ of **22** yielded the hydroxylamine derivative **23** which was coupled with aldehyde **4a** to give the nitrone **19a**.



(i) $\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CHO}$, benzene, Δ ; (ii) MCPBA, CH_2Cl_2 ; (iii) 12N HCl, 100°C ; (iv) $(\text{CH}_3)_3\text{SiCl}$ (excess), $(\text{C}_2\text{H}_5)_3\text{N}$ (excess), CHCl_3 , Δ then $\text{C}_2\text{H}_5\text{OH}$; (v) $(\text{C}_2\text{H}_5)_3\text{N}$ (1equiv.), CH_2Cl_2 , 3Å molecular sieves, 40°C ; (vi) NaHCO_3 , $\text{C}_2\text{H}_5\text{OAc}-\text{H}_2\text{O}$.

Scheme 8

Nitrones **12a**, **12b**, **18a**, **18b** and **19a** were found devoid of antibiotic properties at $100\ \mu\text{M}$ concentration.¹⁶ They were also tested as potential inhibitors of isolated D,D-peptidases²⁹ and β -lactamases.³⁰ No activity could be detected at final concentrations up to $100\ \mu\text{M}$. Thus, nitrones structurally related to the β -lactam antibiotics family (penicillins and oxamazins) appear totally inactive in vitro. As an interesting consequence, the weak bacteriostatic activity previously observed^{6,8} for oxaziridine **1b** could be well assigned to the presence of the reactive small ring, since its potentially rearrangement product **19a** under physiological conditions lacks of activity.³¹

EXPERIMENTAL

Melting points were taken with a Leitz microscope and are uncorrected. Rotations ($\pm 0.1^\circ$) were determined on a Perkin-Elmer 241 MC polarimeter in CH_2Cl_2 at 20°C . Ir spectra were taken with a Perkin-Elmer 297 instrument in CH_2Cl_2 (unless otherwise mentioned) and calibrated with polystyrene. ^1H Nmr spectra were recorded on Varian T60, Varian XL-200 or Varian Gemini 200 spectrometers in CDCl_3 (unless otherwise mentioned) with TMS as internal standard. ^{13}C Nmr spectra were recorded on Varian FT-80A or Varian XL-200 spectrometers. Mass spectra were obtained on a Varian MAT 44 instrument (CI-IB, chemical ionisation with isobutene). Microanalyses were performed at the University of Vienna (Austria). Column-chromatographies were performed with Merck silica gel 60 (70-230 mesh ASTM). DMF, CH_2Cl_2 , CHCl_3 and

C_2H_5OAc were dried over P_2O_5 at reflux, then distilled. Ether was dried over $LiAlH_4$ and benzene was distilled over Na. $(C_2H_5)_3N$ was stored on KOH pellets.

Pivaloyloxymethyl β -alaninate **5** :

N-(*t*-Butyloxycarbonyl)- β -alanine : β -Alanine (8.9 g, 0.1 mol) in 1:1 acetone-water solution (120 ml) was stirred with $(C_2H_5)_3N$ (21 ml, 0.15 mol) and BOC-ON (27 g, 0.11 mol) for 5 h at 20°C. After addition of water (170 ml), the solution was washed with ether (2 x 100 ml), then acidified to pH 4 with 2N HCl and extracted with ether (2 x 100 ml). Drying on $MgSO_4$ and concentration of the organic layers gave the N-protected β -alanine: yield 17.4 g (92 %); 1H nmr (60 MHz) δ 1.46 (s, 9H), 2.53 (t, 2H, $J=6$ Hz), 3.37 (txd, 2H, $J=6$ and 6 Hz), 5.10 (br d, 1H, NH), 8.06 (m, 1H, COOH).

Pivaloyloxymethyl-*N*-(*t*-butyloxycarbonyl)- β -alaninate : BOC- β -alanine (17.4 g, 0.092 mol) in DMF solution (125 ml) was treated with chloromethyl pivalate (13.25 ml, 0.092 mol) in the presence of solid $KHCO_3$ (10.7 g, 0.11 mol). After stirring for 24 h at 20°C, the solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (CH_2Cl_2 - C_2H_5OAc 4:1) to furnish the PIV ester : yield 11.5 g (42%); 1H nmr (60 MHz) δ 1.22 (s, 9H), 1.43 (s, 9H), 2.40 (t, 2H, $J=6$ Hz), 3.38 (txd, 2H, $J=6$ and 6 Hz), 4.97 (m, 1H, NH), 5.67 (s, 2H).

BOC deprotection : N-BOC- β -alanine PIV ester (11.5 g, 0.038 mol) was dissolved in 0.5N HBr- CH_2Cl_2 solution (150 ml) and stirred for 5 h at 20°C. Evaporation, addition of dry ether and filtration gave the hydrobromide of β -alanine PIV ester **5** : yield 9.4 g (88%); 1H nmr (60 MHz) δ 1.22 (s, 9H), 3.1 (br t, 2H), 3.47 (m, 2H), 5.80 (s, 2H), 7.90 (m, 3H, NH_3^+).

α -(*N*-Hydroxy)-aminoisobutyric acid **23** :

Oxaziridine **22** : *p*-Methoxybenzaldehyde (1.034 ml, 8.51 mmol) and methyl α -aminoisobutyrate (0.969 g, 8.51 mmol) in benzene solution (35 ml) were refluxed for 3 days. The benzene was distilled and replaced by CH_2Cl_2 (30 ml). MCPBA (1.75 g, 85% purity, 8.52 mmol) in CH_2Cl_2 solution (20 ml) was added dropwise. After 4 h of stirring at 20°C, the solvent was evaporated. Addition of *n*-hexane, filtration of MCBA, concentration and chromatography on silica gel (hexane-ether 7:3) gave the oxaziridine **22** : yield 1.32 g (61 %); ir (film) 1737, 1614, 1518, 1460, 1440, 1393 cm^{-1} ; 1H nmr (60 MHz) δ 1.40 (s, 6H, $C(CH_3)_2$), 3.70 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.83 (s, 1H, H oxaziridine), 6.83 (d, 2H, $J=8.5$ Hz, Ph), 7.33 (d, 2H, $J=8.5$ Hz, Ph).

Hydrolysis : Oxaziridine **22** (6 g, 24 mmol) was heated at 100°C for 3 h in 12N HCl (114 ml). After washing with CH_2Cl_2 (3x50 ml), the aqueous phase was concentrated under vacuum. The residue was dissolved in acetone and dried over $MgSO_4$: yield 2.65 g of hydrochloride **23**, 76.8%.

Free hydroxylamine : A mixture of hydrochloride **23** (0.534 g, 3.43 mmol), trimethylchlorosilane (1.73 ml, 3 equiv.) and $(C_2H_5)_3N$ (1.91 ml, 4 equiv.) in $CHCl_3$ solution (25 ml) was refluxed for 3 h (complete dissolution). After concentration, $HCl.(C_2H_5)_3N$ was precipitated by addition of dry ether. Filtration, evaporation and addition of C_2H_5OH (8 ml) led the acid **23** crystallize smoothly : yield 0.287 g (70%); mp 171-172°C; ms (CI-IB) 120 (100%, $M^+ + 1$), 101 (15%, $M^+ - H_2O$), 74 (75%, $M^+ - CO_2H$).

Oxaziridines **9** :

*R*¹ = benzyl, **9a** : Aldehyde **4a** (0.5 g, 2.61 mmol) in CH_2Cl_2 solution (20 ml) was treated at room temperature with HBr- β -alaninate **5** (0.741 g, 2.61 mmol) in the presence of $(C_2H_5)_3N$ (0.367 ml, 2.61 mmol) and 3Å molecular sieves. After 3 h, the mixture was filtered and concentrated under vacuum. Addition of ether, filtration of $HBr.(C_2H_5)_3N$ and evaporation yielded crude imine **7a**. This material in CH_2Cl_2 solution (15 ml) was oxidized by dropwise addition of MCPBA (0.531 mg, 85 % purity, 2.60 mmol) in CH_2Cl_2 (10 ml). The

mixture was stirred for 2 h at 20°C. The solvent was removed under vacuum and the residue was chromatographed twice on silica gel (hexane - ether - C₂H₅OAc 5:5:2) to give pure oxaziridine **9a** as an oil : yield 0.202 g (20%); [α]_D -10.1° (c=0.81); ir 1750, 1670 cm⁻¹; ¹H nmr (200 MHz) δ (one diastereoisomer) 1.11 (d, 3H, J = 6.9 Hz, CH-CH₃), 1.23 (s, 9H, tC₄H₉), 2.56-2.86 (m, 3H, N-CH-CH₂-COO), 3.10-3.26 (m, 1H, N-CH-CH₂), 3.54 (s, 2H, PhCH₂), 3.96 (d, 1H, J=1.7 Hz, H oxaziridine), ~ 4.35 (qxdxd, 1H, J=6.9, 7.0, 1.7 Hz, CH-CH₃), 5.73 (br d, 1H, J = 7.0 Hz, NH), 5.77 (sharp ABq, J=12 Hz, 2H, COOCH₂O), 7.20-7.43 (m, 5H, Ph); ¹³C nmr (50 MHz) ppm (one diastereoisomer) 15.2 (CH-CH₃), 27.6 (C(CH₃)₃), 32.8 (N-CH₂-CH₂), 39.2 (C(CH₃)₃), 44.0 (PhCH₂), 44.4 (CH-CH₃), 56.8 (N-CH₂-CH₂), 80.0 (COOCH₂O), 82.8 (C oxaziridine), 126.8, 128.8, 130.0 and 135.2 (Ph), 170.0 (CO amide), 171.6 (CO ester), 177.6 (CO ester); Anal. Calcd for C₂₀H₂₈O₆N₂ : C, 61.22; H, 7.14; O, 24.48; N, 7.14 : Found : C, 60.65; H, 7.15; O, 25.13; N, 7.02.

*R*¹=Phenoxymethyl, **9b**: Oxaziridine **9b** was prepared according to the procedure described for **9a**, from 0.525 g (2.53 mmol) of aldehyde **4b**, 0.721 g (2.53 mmol) of HBr. β -alaninate **5**, 0.35 ml of (C₂H₅)₃N and then 0.514 g of MCPBA (80% purity). Column chromatography on silica gel (hexane-ether-C₂H₅OAc-CH₂Cl₂, 5:5:2:2) gave pure **9b** as an oil : yield 0.49 g (47%); [α]_D -0.85° (c=1.3); ir 3420, 1751, 1680, 1600, 1590, 1522, 1491 cm⁻¹; ¹H nmr (200 MHz) δ (two diastereoisomers 60 : 40) 1.20 (s, 9H, tC₄H₉), 1.21 and 1.22 (two d, 3H, J=7 Hz, CH-CH₃), 2.58-2.90 (m, 3H, N-CH-CH₂), 3.10-3.32 (m, 1H, N-CH-CH₂), 4.01 (d, 1H, J=2.2 Hz, H oxaziridine), 4.42 (m, 1H, CH-CH₃), 4.46 and 4.47 (two s, 2H, PhOCH₂), 5.74 and 5.74 (s and sharp ABq, J= 12 Hz, 2H, COOCH₂O), 6.60 and 6.72 (two br d, 1H, NH, J=8 Hz), 6.90-7.09 (m, 3H, Ph), 7.24-7.38 (m, 2H, Ph); ¹³C nmr (50 MHz) ppm (two diastereoisomers) 15.15 and 15.29 (CH-CH₃), 26.78 (C(CH₃)₃), 32.51 (N-CH₂-CH₂), 38.69 (C(CH₃)₃), 43.91 and 43.99 (CH-CH₃), 55.95 and 56.11 (N-CH₂-CH₂), 67.33 (PhOCH₂), 79.52 and 79.56 (COOCH₂O), 81.94 and 82.41 (C oxaziridine), 114.77, 122.05, 129.65 and 157.18 (OPh), 168.17 (CO amide), 169.97 and 170.05 (CO ester), 177.05 (CO ester); Anal. Calcd for C₂₀H₂₈O₇N₂ : C, 58.82; H, 6.86; O, 27.45; N, 6.86 : Found : C, 58.51; H, 6.97; O, 27.49; N, 6.72.

*R*¹ = 2,6-dimethoxyphenyl, **9c**: Oxaziridine **9c** was prepared according to the procedure described for **9a**, from 0.840 g (3.54 mmol) of aldehyde **4c**, 1.005 g (3.54 mmol) of HBr. β -alaninate **5**, 0.439 ml of (C₂H₅)₃N and then 0.732 g of MCPBA (80 % purity, 3.54 mmol). Column chromatography on silica gel (ether-hexane-C₂H₅OAc-CH₂Cl₂ 10:2:2:2) gave pure **9c** as an oil : yield 0.806 g (52%); [α]_D + 0.586° (c=0.87); ir 3420, 1755, 1670, 1600, 1509, 1472 cm⁻¹; ¹H nmr (200 MHz) δ (two diastereoisomers 55:45) ~ 1.20 (two s + d, J=6 Hz, 9H + 3/2 H, tC₄H₉ + CH-CH₃), 1.46 (d, 3/2 H, J=6 Hz, CH-CH₃), 2.60-2.90 (m, 3H, N-CH-CH₂), 3.25 and 3.57 (two m, 1H, N-CH-CH₂), 3.84 (two s + d, J=2 Hz, 6H + 1/2 H, (OCH₃)₂ + H oxaziridine), 4.18 (d, 1/2 H, J=2 Hz, H oxaziridine), 4.60 and 4.72 (two m, 1H, CH-CH₃), 5.75 (two sharp ABq, J=10 Hz, 2H, COOCH₂O), 5.90 and 6.42 (two br d, 1H, J=7 Hz, NH amide), 6.56 and 6.60 (two d, J=8 Hz, 2H, aryl), 7.30 (m, 1H, aryl); ¹³C nmr (50 MHz) ppm (two diastereoisomers) 14.56 and 18.03 (CH-CH₃), 27.05 (C(CH₃)₃), 32.93 and 34.05 (N-CH₂-CH₂), 35.36 (C(CH₃)₃), 45.09 and 49.38 (CH-CH₃), 56.27 and 56.38 (N-CH₂-CH₂), 79.92 and 80.05 (COOCH₂O), 82.47 (C-oxaziridine), 104.43 (aryl), 128.55, 130.14, 130.50, 131.33, 131.57 and 133.48 (aryl), 157.87 and 158.00 (aryl), 166.32 (CO amide), 171.3 (CO ester), 173.19 (CO ester); Anal. Calcd for C₂₁H₃₀O₈N₂ : C, 57.53; H, 6.84; N, 6.39 : Found : C, 58.00; H, 7.17; N, 6.21.

Oxaziridines **10** :

*R*¹= benzyl, **10a**: A mixture of β -alanine (0.235 g, 2.67 mmol) and t-butylchlorodiphenylsilane (0.706 ml, 2.7 mmol) in CHCl₃ (6 ml) and CH₃CN (1 ml) was refluxed for 2 h (complete dissolution : **6**). (C₂H₅)₃N (0.371 ml, 2.67 mmol), then aldehyde **4a** (0.508 g, 2.67 mmol) and 3Å molecular sieves were added at room temperature. After 3 h of stirring, the solution was filtered and concentrated under vacuum. Addition of dry ether, filtration of HCl. (C₂H₅)₃N and evaporation yielded crude imine **8a**. This material, in CH₂Cl₂ solution (10 ml), was treated

with MCPBA (0.559 g, 85% purity, 2.67 mmol) in CH_2Cl_2 (10 ml, dropwise addition at 20°C). After 1.5 h of stirring, the solvent was removed under vacuum and the residue was chromatographed twice on silica gel (hexane-ether 7:3 and hexane-ether- $\text{C}_2\text{H}_5\text{OAc}$ 5:5:3; $R_f=0.45$) to furnish **10a** as a gum : yield 0.586 g (60%); ir 1750, 1660 cm^{-1} ; ^1H nmr (200 MHz) δ (one diastereoisomer) 1.03 (d, 3H, $J=6.8$ Hz, $\text{CH}-\text{CH}_3$), 1.12 (s, 9H, tC_4H_9), 2.65-2.90 (m, 3H, $\text{N}-\text{CH}-\text{CH}_2$), 3.00-3.14 (m, 1H, $\text{N}-\text{CH}-\text{CH}_2$), 3.45 (s, 2H, PhCH_2), 3.91 (d, 1H, $J=2$ Hz, H oxaziridine), 4.28 (qxdxd, 1H, $J=6.8$, 2 and 8 Hz, $\text{CH}-\text{CH}_3$), 5.72 (d, 1H, $J=8$ Hz, NH), 7.15-7.80 (m, 15H, Ph).

R1=phenoxymethyl, **10b** : Oxaziridine **10b** was obtained according to the procedure described for **10a**, from 0.332 g (3.73 mmol) of β -alanine, 0.969 ml (3.8 mmol) of *t*-butylchlorodiphenylsilane, 0.519 ml of $(\text{C}_2\text{H}_5)_3\text{N}$, 0.772 g (3.73 mmol) of aldehyde **4b** and 0.659 g of MCPBA (85% purity). Two successive chromatographies on silica gel (hexane-ether 5:3 and hexane-ether- $\text{C}_2\text{H}_5\text{OAc}$ 5:5:3; $R_f=0.61$) gave **10b** as a gum : yield 1.075 g (54%); ir 1745, 1680 cm^{-1} ; ^1H nmr (60 MHz) δ 1.10 (s, 9H, tC_4H_9), 1.16 (d, 3H, $J=7$ Hz, $\text{CH}-\text{CH}_3$), 2.66-3.00 (m, 3H, $\text{N}-\text{CH}-\text{CH}_2$), 3.03-3.33 (m, 1H, $\text{N}-\text{CH}-\text{CH}_2$), 3.96 (d, 1H, $J=2$ Hz, H oxaziridine), 4.23 (qxdxd, $J=7$, 8 and 2 Hz, 1H, $\text{CH}-\text{CH}_3$), 4.40 (s, 2H, PhOCH_2), 6.56 (br d, 1H, $J=8$ Hz, NH), 6.70-7.73 (m, 15H, Ph).

Oxaziridine **14** :

Crude imine **13** was prepared according to the procedure described for **10a**, from 0.534 g (6 mmol) of β -alanine, 1.592 ml (6 mmol) of *t*-butylchlorodiphenylsilane, 0.834 ml (6 mmol) of $(\text{C}_2\text{H}_5)_3\text{N}$ and 0.555 ml (6 mmol) of isobutyraldehyde : yield 2.27g (98%); ir (film) 1730, 1670, 1590 (w), 1465, 1430, 1364 cm^{-1} ; ^1H nmr (60 MHz) δ 1.01 (d, 6H, $J=7$ Hz, $\text{CH}(\text{CH}_3)_2$), 1.08 (s, 9H, tC_4H_9), 2.26 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.73 (t, 2H, $J=6$ Hz, $\text{N}-\text{CH}_2-\text{CH}_2$), 3.63 (t, 2H, $J=6$ Hz, $\text{N}-\text{CH}_2-\text{CH}_2$), 7.26 and 7.30-7.80 (d, $J=6$ Hz and m, 1H + 10H, $\text{CH}=\text{N}$ and Ph).

Oxidation : MCPBA (1.255 g, 82% purity, 5.9 mmol) in CHCl_3 (30 ml) solution was added dropwise at 20°C to a CHCl_3 solution (50 ml) of the imine **13** (2.27 g, 5.9 mmol) and the mixture was stirred for 2 h. Evaporation, addition of hexane and filtration of MCPBA gave crude oxaziridine **14** which was chromatographed on silica gel (hexane-ether- $\text{C}_2\text{H}_5\text{OAc}$ 5:5:1): yield 1.682 g (71%); ^1H nmr (60 MHz) δ 0.93 (d, 6H, $J=6$ Hz, $\text{CH}(\text{CH}_3)_2$), 1.11 (s, 9H, tC_4H_9), 1.74 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.73-3.00 (m, 3H, $\text{N}-\text{CH}-\text{CH}_2$), 3.00-3.32 (m, 1H, $\text{N}-\text{CH}-\text{CH}_2$), 3.56 (d, 1H, $J=5$ Hz, H oxaziridine), 7.17-7.80 (m, 10H, Ph).

Oxaziridine **15** :

Silyl ester **14** (0.36 g, 0.835 mmol) in 1:1 THF- H_2O solution (3 ml) was treated at 20°C with acetic acid (3 ml). After 24 h, the solvents were evaporated under reduced pressure and the residue was chromatographed on silica gel (hexane-ether- $\text{C}_2\text{H}_5\text{OAc}$ 5:5:3) to furnish the free acid **15** : yield 60 mg (40 %); ^1H nmr (60 MHz) δ 1.00 (d, 6H, $J=7$ Hz, $\text{CH}(\text{CH}_3)_2$), 1.63 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.60-3.33 (m, 4H, $\text{N}-\text{CH}_2-\text{CH}_2$), 3.60 (d, 1H, $J=6$ Hz, H oxaziridine), 10.40 (br s, 1H, COOH).

Nitrone **16** :

Oxaziridine **15** (0.317 g, 0.8 mmol) in THF solution (4 ml) was treated at 0°C with cold methanol (9 ml) and aqueous NaHCO_3 (67 mg, 0.8 mmol in 3 ml of H_2O). After 2 h of stirring at 20°C , the organic solvents were evaporated and the aqueous layer was extracted twice with CH_2Cl_2 . Concentration of the aqueous phase under high vacuum gave a white powder : yield 0.107 g (74 %); ir (KBr) 1580, 1410, 1310 (w), 1180, 1117 cm^{-1} ; ^1H nmr (60 MHz, D_2O) δ 1.16 (d, 6H, $J=7$ Hz, $\text{CH}(\text{CH}_3)_2$), 2.76 (t, 2H, $J=7$ Hz, $\text{N}-\text{CH}_2-\text{CH}_2$), 3.13 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 4.06 (t, 2H, $J=7$ Hz, $\text{N}-\text{CH}_2-\text{CH}_2$), 7.23 (d, 1H, $J=8$ Hz, $\text{CH}=\text{N}\rightarrow\text{O}$); ^{13}C nmr (20 MHz, CD_3OD) ppm 19.2 ($\text{CH}(\text{CH}_3)_2$), 27.5 ($\text{CH}(\text{CH}_3)_2$), 36.2 ($\text{N}-\text{CH}_2-\text{CH}_2$), 62.8 ($\text{N}-\text{CH}_2-\text{CH}_2$), 154.5 ($\text{CH}=\text{N}\rightarrow\text{O}$), 179.3 ($\text{C}=\text{O}$).

Nitrones 12:

*R*¹=benzyl; 12a: Oxaziridine 10a (0.328 g, 0.64 mmol) in THF solution (3 ml) was treated at 0°C with CH₃OH (7 ml) and aqueous NaHCO₃ (53 mg, 0.64 mmol in 3 ml of H₂O). The mixture was allowed to reach slowly 20°C. After evaporation of the organic solvents under vacuum, the aqueous layer was extracted with CH₂Cl₂ (removal of HOSi(Ph)₂C₄H₉). Lyophilization gave a powder which was washed with acetone and then ethanol: yield 88 mg (45%); ir (KBr) 3330, 1650, 1585, 1530, 1420 (br), 1175 (N→O) cm⁻¹; ¹H nmr (60 MHz, D₂O + CD₃OD) δ 1.50 (d, 3H, J=7 Hz, CH-CH₃), 2.80 (t, 2H, J=7 Hz, N-CH₂-CH₂), 3.66 (s, 2H, PhCH₂), 4.06 (t, 2H, J=7 Hz, N-CH₂-CH₂), 4.83 (qxd, 1H, J=7 and 6 Hz, CH-CH₃), 7.11 (d, 1H, J=6 Hz, CH=N), 7.26 (s, 5H, Ph); ¹³C nmr (20 MHz, D₂O + CD₃OD) ppm 16.00 (CH-CH₃), 36.10 (N-CH₂-CH₂), 43.33 (PhCH₂), 44.37 (CH-CH₃), 62.71 (N-CH₂-CH₂), 128.49, 130.09, 130.29 and 136.10 (Ph), 148.11 (CH=N→O), 175.21 (CO amide), 179.07 (COO⁻).

*R*¹=phenoxymethyl; 12b: Nitrone 12b was obtained according to the procedure described for 12a, from 0.296 g (0.55 mmol) of oxaziridine 10b and 46 mg of NaHCO₃: yield 0.127 mg (73%); ¹H nmr (60 MHz, D₂O) δ 1.46 (d, 3H, J=7 Hz, CH-CH₃), 2.83 (t, 2H, J=7 Hz, N-CH₂-CH₂), 4.10 (t, 2H, J=7 Hz, N-CH₂-CH₂), 4.56 (s, 2H, PhOCH₂), 4.96 (qxd, J=7 and 6 Hz, 1H, CH-CH₃), 6.96 and 6.83-7.53 (d, J=6 Hz, and m, 1H+5H, CH=N→O and Ph); ¹³C nmr (20 MHz, DMSO-d₆) ppm 16.84 (CH-CH₃), 43.05 (CH-CH₃), 49.62 (N-CH₂-CH₂), 62.81 (N-CH₂-CH₂), 67.47 (PhOCH₂), 115.71, 122.68 and 130.72 (Ph), 143.23 (CH=N→O), 158.32 (OPh), 169.55 (CO amide), 176.26 (COO⁻).

Nitrones 18:

*R*¹=benzyl; 18a: Hydroxylamine 20 (0.4 g, 4.39 mmol) in CH₂Cl₂ (30 ml) was heated at 35°C in the presence of (C₂H₅)₃N (0.611 ml, 4.39 mmol) up to dissolution. Aldehyde 4a (0.839 g, 1 equiv.) in CH₂Cl₂ solution (5 ml) was added dropwise and then molecular sieves (3Å, 500 mg) were introduced in the flask. The mixture was refluxed for 5 h. Filtration and concentration under vacuum gave crude 21a. This material in C₂H₅OAc solution (15 ml) was treated with aqueous NaHCO₃ (0.35 g, 4.16 mmol, in 12 ml of H₂O). After 30 min of stirring at 20°C, the aqueous phase was lyophilized and the solid residue was washed with C₂H₅OH: yield 0.741 g (59%) of sodium salt 18a; ir (KBr) 3515, 3250, 1640, 1610, 1550, 1403, 1364, 1308, 1265 (N→O), 1188 (N→O), cm⁻¹; ¹H nmr (200 MHz, D₂O) δ 1.38 (d, 3H, J=7.1 Hz, CH-CH₃), 3.61 (s, 2H, PhCH₂), 4.36 (ABd, 1H, J=15.5 Hz, N-CH-COO), 4.45 (ABd, 1H, J=15.5 Hz, N-CH-COO), 4.82 (m, 1H, CH-CH₃), 7.16 (d, 1H, J=6.5 Hz, CH=N→O), 7.28-7.45 (m, 5H, Ph); ¹³C nmr (20 MHz, D₂O) ppm 15.53 (CH-CH₃), 42.83 (CH-CH₃), 44.04 (PhCH₂), 68.38 (N-CH₂-COO), 128.07, 129.69, 129.88 and 135.63 (Ph), 148.92 (CH=N→O), 172.06 (CO amide), 174.96 (COO⁻).

*R*¹=phenoxymethyl; 18b: The nitrone 18b was obtained according to the procedure described for 18a, from 0.2 g (2.2 mmol) of hydroxylamine 20, 0.305 ml (2.2 mmol) of (C₂H₅)₃N, 0.455 g (1 equiv.) of aldehyde 4b and then 0.166 g (0.9 equiv.) of NaHCO₃: yield 0.263 g (40%) of sodium salts 18b; ir (KBr) 1660, 1615, 1600, 1585, 1240 (N→O), 1180 (N→O) cm⁻¹; ¹H nmr (200 MHz, D₂O) δ 1.39 (d, 3H, J=7 Hz, CH-CH₃), 4.38 (ABd, 1H, J=15.3 Hz, N-CH-COO), 4.48 (ABd, 1H, J=15.3 Hz, N-CH-COO), 4.64 (s, 2H, PhO-CH₂), 4.98 (dxd, 1H, J=7 and 6.3 Hz, CH-CH₃), 6.98-7.14 (m, 3H, Ph), 7.19 (d, 1H, J=6.3 Hz, CH=N→O), 7.32-7.46 (m, 2H, Ph); ¹³C nmr (50 MHz, D₂O) ppm 15.23 (CH-CH₃), 42.94 (CH-CH₃), 66.77 (PhOCH₂), 67.88 (N-CH₂-COO), 114.88, 122.33 and 130.05 (Ph), 147.46 (CH=N→O), 157.22 (OPh), 171.14 (CO amide), 171.39 (COO⁻); ms (CI-IB) 260 (M⁺-CO₂+1).

Nitron 19:

The nitron 19a was prepared according to the procedure described from 21a, 0.132 g (1.11 mmol) of

hydroxylamine **23**, 0.154 ml (1.11 mmol) of $(C_2H_5)_3N$ and 0.212 g (1 equiv.) of aldehyde **4a**: yield 0.432 g (100 %) of triethylammonium salt of **19**; ir 3420, 1665, 1620, 1500 cm^{-1} ; 1H nmr (60 MHz) δ 1.20 (t, 9H, $J=7$ Hz, $N(CH_2-CH_3)_3$), 1.33 (d, 3H, $J=6.5$ Hz, $CH-CH_3$), 1.57 (br s, 6H, $C(CH_3)_2$), 2.93 (q, 6H, $J=7$ Hz, $N(CH_2-CH_3)_3$), 3.50 (s, 2H, $PhCH_2$), 4.80 (qxdxd, 1H, $J=6.5$, 6 and 8 Hz, $CH-CH_3$), 7.07 (d, 1H, $J=6$ Hz, $CH=N\rightarrow O$), 7.26 (s, 5H, Ph), 7.87 (br d, 1H, $J=8$ Hz, NH); ^{13}C nmr (50 MHz), ppm 8.66 ($N(CH_2-CH_3)_3$), 15.99 ($CH-CH_3$), 24.84 ($C(CH_3)_2$), 43.24 ($CH-CH_3$), 43.51 ($PhCH_2$), 44.99 ($N(CH_2-CH_3)_3$), 77.31 ($C(CH_3)_2$), 126.71, 128.46, 129.04 and 134.06 (Ph), 135.12 ($CH=N\rightarrow O$), 170.27 (CO amide), 175.40 (COOH). The sodium salt **19a** was obtained following the procedure described for **18a**; 1H nmr (60 MHz, D_2O) δ 1.49 (d, 3H, $J=7$ Hz, $CH-CH_3$), 1.66 (br s, 6H, $C(CH_3)_2$), 3.73 (s, 2H, $PhCH_2$), 5.00 (qxd, 1H, $J=7$ and 6.5 Hz, $CH-CH_3$), 7.33 (d, 1H, $J=6.5$ Hz, $CH=N\rightarrow O$), 7.46 (s, 5H, Ph).

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