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

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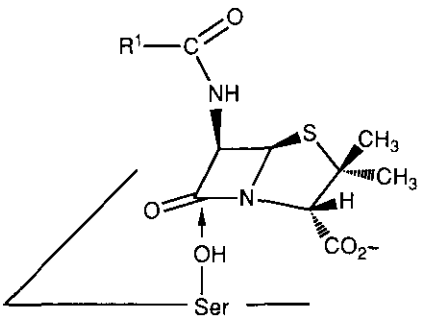
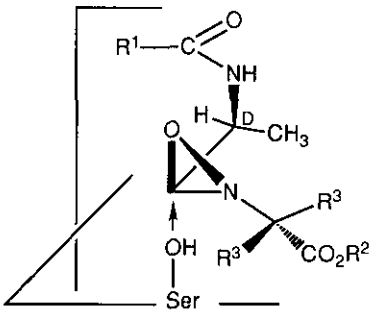
Abstract – Novel functionalized oxaziridines and nitrones have been prepared and examined as potential irreversible inhibitors of bacterial PBPs. N-(β–Hydroxycarbonyl)ethyloxaziridines were found to undergo a fast isomerisation in protic solvent to yield the corresponding nitrones.

INTRODUCTION

The most important clinical problems encountered in antibiotherapy using β-lactam derivatives[1] 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)[32] in the same manner as the β-lactams. Oxaziridines[8] have been considered as potential irreversible alkylating inhibitors of the bacterial serine D,D-transpeptidases.



penicillins
(β – lactam inhibitors)



oxaziridines 1
a $R^3 = H$; b $R^3 = CH_3$

Scheme 1

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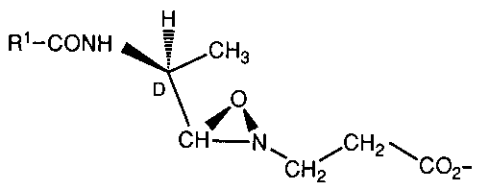
Taking account of stereoelectronic factors for nucleophilic ring opening and topological requirements for molecular regognition, we selected structures 1 as first synthetic targets[8] (Scheme 1). Oxaziridines 1a were found to be unstable, giving rise to a rapid β-elimination.[9,10] On the other hand, oxaziridines 1b, bearing no hydrogen atom α to the carbonyl group, could be isolated as esters ($R^2 \neq H$) and one derivative ($R^1=PhCH_2$, $R^2=CH_2OCOtC_4H_9$, $R^3=CH_3$) 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.



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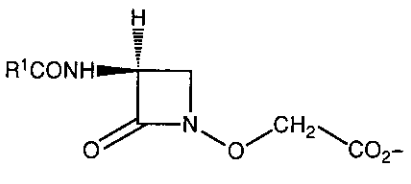


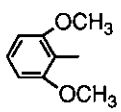
3

Scheme 2

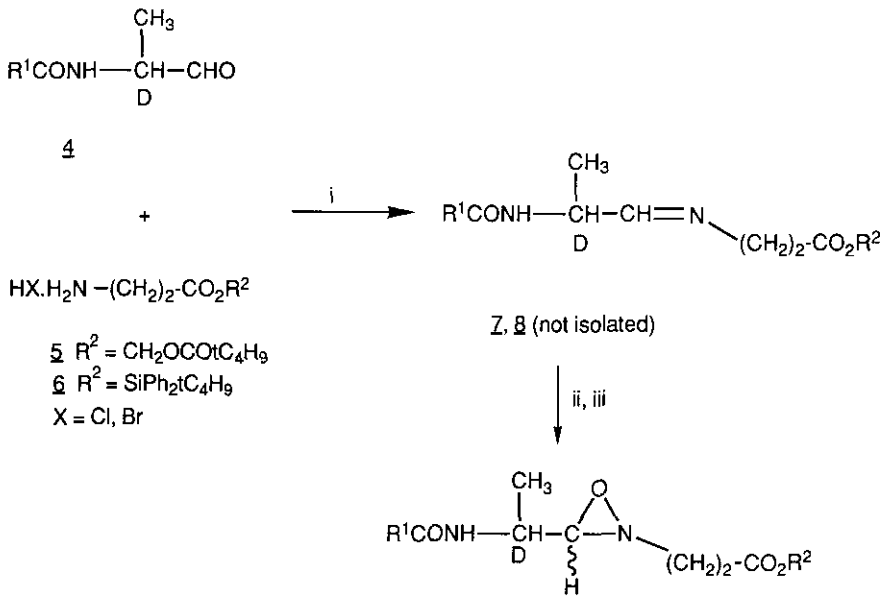
RESULTS

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-butyldiphenylsilyl-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 rize 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)
$PhCH_2$	$CH_2OCOtC_4H_9$	9a	40%
$PhOCH_2$	$CH_2OCOtC_4H_9$	9b	47%
	$CH_2OCOtC_4H_9$	9c	52%
$PhCH_2$	$SiPh_2tC_4H_9$	10a	60%
$PhOCH_2$	$SiPh_2tC_4H_9$	10b	54%

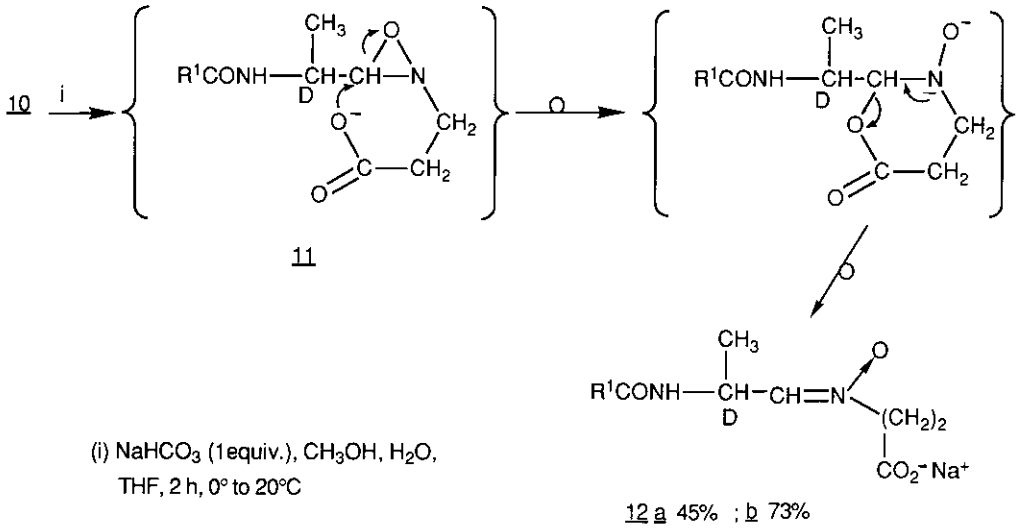
The biodegradable[15] esters 9a-c were submitted to biological evaluation towards representative gram-positive and gram-negative bacterial strains. No activity was found for concentrations up to 200 μ M.[16]



(i) $(C_2H_5)_3N$ (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

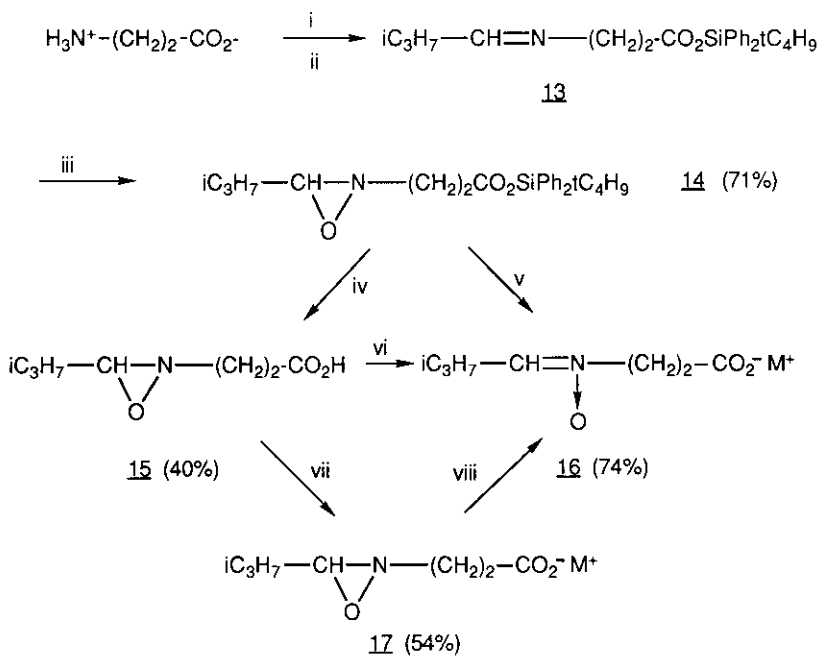
Mild acidic hydrolysis (HOAc, H_2O-THF, 0° to 20°C) of the silyl ester 10 gave untractable mixtures. Smooth basic hydrolysis ($NaHCO_3$, CH_3OH-H_2O, 0° to 20°C) also resulted in the cleavage of oxaziridine ring. Nitrones 12 were the only isolated products in moderate to good yields (Scheme 4). These 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 C=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 ester[22] to give the stable oxaziridine 15 which was purified by chromatography on silica gel. The corresponding carboxylate 17 (M+=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 (M+=K+) occurred within 20 minutes at 30°C.



(i) $NaHCO_3$ (1equiv.), CH_3OH, H_2O, THF, 2 h, 0° to 20°C

12 a 45% ; b 73%

Scheme 4

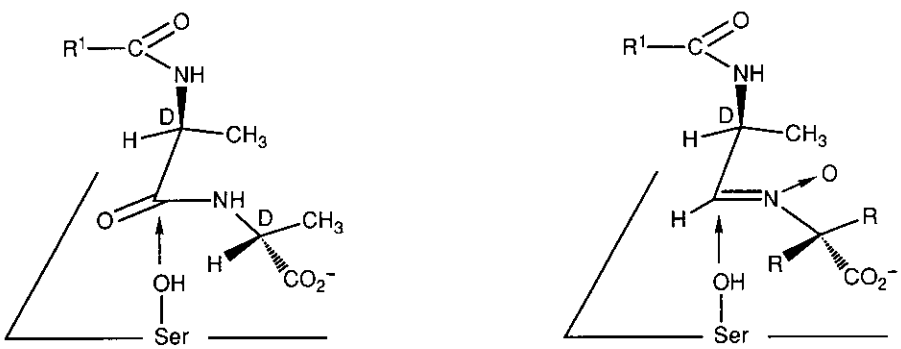


(i) $ClSiPh_2tC_4H_9$, $CHCl_3$, CH_3CN, Δ; (ii) $(C_2H_5)_3N$, 3Å molecular sieves, iC_3H_7-CHO, CH_2Cl_2; (iii) MCPBA, CH_2Cl_2, 20°C; (iv) HOAc-THF-H_2O (2:1:1), 20°C, 24 h; (v) $NaHCO_3$ (1equiv.), CH_3OH, THF, H_2O, 2 h, 20°C; (vi) $NaHCO_3$ (1equiv.), Acetone, H_2O; (vii) K^+ 2-ethyl-hexanoate, C_2H_5OAc, 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, oxaridinines 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 attack[26] (Scheme 6).

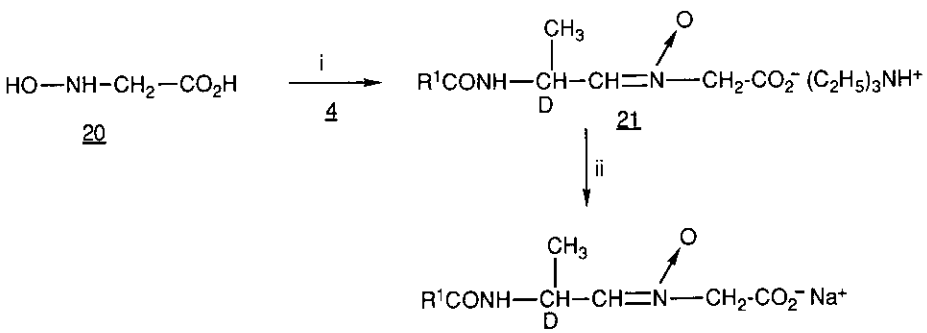


peptide substrate
(D-Ala-D-Ala)

nitrones
18 R = H
19 R = CH_3

Scheme 6

Nitrones 18 were conventionally prepared[24] 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 $NaHCO_3$ to yield the sodium salts 18.

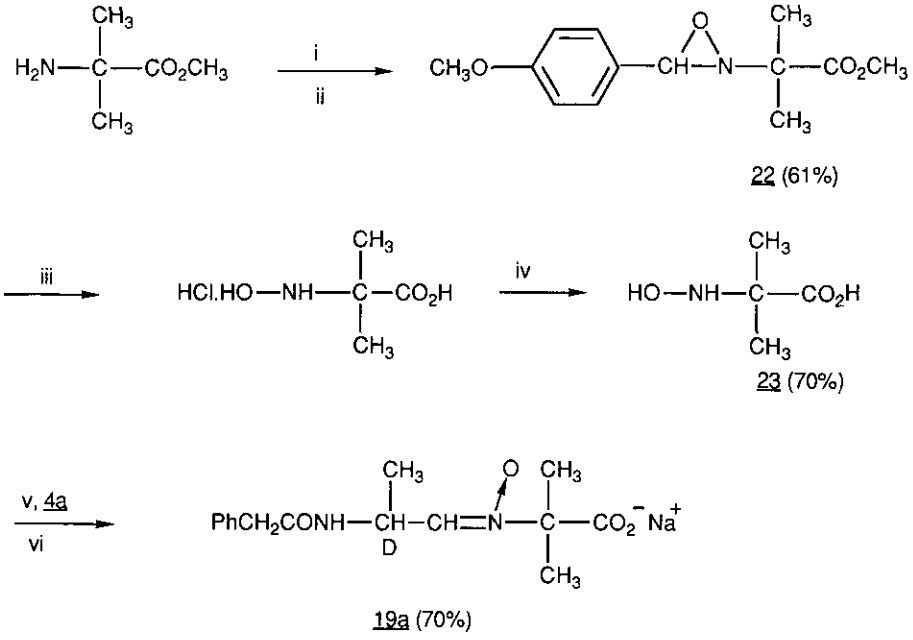


18 a $R^1 = PhCH_2$, 59%
b $R^1 = PhOCH_2$, 40%

(i) $(C_2H_5)_3N$(1equiv.), 3Å molecular sieves, CH_2Cl_2, 40°C; (ii) $NaHCO_3$, H_2O, 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 hydrolysis[28] of 22 yielded the hydroxylamine derivative 23 which was coupled with aldehyde 4a to give the nitrone 19a.



(i) CH_3O-C_6H_4-CHO, benzene, Δ; (ii) MCPBA, CH_2Cl_2; (iii) 12N HCl, 100°C; (iv) $(CH_3)_3SiCl$ (excess), $(C_2H_5)_3N$ (excess), $CHCl_3$, Δ then C_2H_5OH; (v) $(C_2H_5)_3N$ (1equiv.), CH_2Cl_2, 3Å molecular sieves , 40°C; (vi) $NaHCO_3$, C_2H_5OAc-H_2O.

Scheme 8

Nitrones 12a, 12b, 18a, 18b and 19a were found devoid of antibiotic properties at 100 μM concentration.[16] They were also tested as potential inhibitors of isolated D,D-peptidases[29] and β–lactamases.[30] No activity could be detected a final concentrations up to 100 μM. 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 (± 0.1°) 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 storred 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 - C_2H_5OAc 5:5:2) to give pure oxaziridine 9a as an oil : yield 0.202 g (20%); $[\alpha]_D$ -10.1° (c=0.81); ir 1750, 1670 cm^{-1}; ^1H nmr (200 MHz) δ (one diasteroisomer) 1.11 (d, 3H, J = 6.9 Hz, CH-C<u>H</u>$_3$), 1.23 (s, 9H, tC_4H_9), 2.56-2.86 (m, 3H, N-C<u>H</u>-C<u>H</u>$_2$-COO), 3.10-3.26 (m, 1H, N-C<u>H</u>-CH_2), 3.54 (s, 2H, $PhCH_2$), 3.96 (d, 1H, J=1.7 Hz, H oxaziridine), ~ 4.35 (qxdxd, 1H, J=6.9, 7.0, 1.7 Hz, C<u>H</u>-CH_3), 5.73 (br d, 1H, J = 7.0 Hz, NH), 5.77 (sharp ABq, J=12 Hz, 2H, $COOCH_2O$), 7.20-7.43 (m, 5H, Ph); ^{13}C nmr (50 mHz) ppm (one diastereoisomer) 15.2 (CH-<u>C</u>H$_3$), 27.6 (C(<u>C</u>H$_3$)$_3$), 32.8 (N-<u>C</u>H$_2$-CH$_2$), 39.2 (<u>C</u>(CH$_3$)$_3$), 44.0 (Ph<u>C</u>H$_2$), 44.4 (<u>C</u>H-CH$_3$), 56.8 (N-CH$_2$-<u>C</u>H$_2$), 80.0 (COO<u>C</u>H$_2$O), 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 $C_{20}H_{28}O_6N_2$: 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 $(C_2H_5)_3N$ and then 0.514 g of MCPBA (80% purity). Column chromatography on silica gel (hexane-ether-C_2H_5OAc-CH_2Cl_2, 5:5:2:2) gave pure 9b as an oil : yield 0.49 g (47%); $[\alpha]_D$ -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, tC_4H_9), 1.21 and 1.22 (two d, 3H, J=7 Hz, CH-C<u>H</u>$_3$), 2.58-2.90 (m, 3H, N-C<u>H</u>-C<u>H</u>$_2$), 3.10-3.32 (m, 1H, N-C<u>H</u>-CH$_2$), 4.01 (d, 1H, J=2.2 Hz, H oxaziridine), 4.42 (m, 1H, C<u>H</u>-CH$_3$), 4.46 and 4.47 (two s, 2H, PhOC<u>H</u>$_2$), 5.74 and 5.74 (s and sharp ABq, J= 12 Hz, 2H, COO C<u>H</u>$_2$O), 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); ^{13}C nmr (50 MHz) ppm (two diasteroisomers) 15.15 and 15.29 (CH-<u>C</u>H$_3$), 26.78 (C(<u>C</u>H$_3$)$_3$), 32.51 (N-<u>C</u>H$_2$-CH$_2$), 38.69 (<u>C</u>(CH$_3$)$_3$), 43.91 and 43.99 (<u>C</u>H-CH$_3$), 55.95 and 56.11 (N-CH$_2$-<u>C</u>H$_2$), 67.33 (PhO<u>C</u>H$_2$), 79.52 and 79.56 (COO-<u>C</u>H$_2$-O), 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 $C_{20}H_{28}O_7N_2$: 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 $(C_2H_5)_3N$ and then 0.732 g of MCPBA (80 % purity, 3.54 mmol). Column chromatography on silica gel (ether-hexane-C_2H_5OAc-CH_2Cl_2 10:2:2:2) gave pure 9c as an oil : yield 0.806 g (52%); $[\alpha]_D$ + 0.586° (c=0.87); ir 3420, 1755, 1670, 1600, 1509, 1472 cm^{-1}; ^1H nmr (200 MHz) δ (two diasteroisomers 55:45) ~ 1.20 (two s + d, J=6 Hz, 9H + 3/2 H, tC_4H_9 + CH-C<u>H</u>$_3$), 1.46 (d, 3/2 H, J=6 Hz, CH-C<u>H</u>$_3$), 2.60-2.90 (m, 3H, N-C<u>H</u>-C<u>H</u>$_2$), 3.25 and 3.57 (two m, 1H, N-C<u>H</u>-CH$_2$), 3.84 (two s + d, J=2 Hz, 6H + 1/2 H, $(OCH_3)_2$ + H oxaziridine), 4.18 (d, 1/2 H, J=2 Hz, H oxaziridine), 4.60 and 4.72 (two m, 1H, C<u>H</u>-CH$_3$), 5.75 (two sharp ABq, J=10 Hz, 2H, COOC<u>H</u>$_2$O), 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); ^{13}C nmr (50 MHz) ppm (two diastereoisomers) 14.56 and 18.03 (CH-<u>C</u>H$_3$), 27.05 (C(<u>C</u>H$_3$)$_3$), 32.93 and 34.05 (N-<u>C</u>H$_2$.CH$_2$), 35.36 (<u>C</u>(CH$_3$)$_3$), 45.09 and 49.38 (<u>C</u>H-CH$_3$), 56.27 and 56.38 (N-CH$_2$-<u>C</u>H$_2$), 79.92 and 80.05 (COO<u>C</u>H$_2$O), 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 $C_{21}H_{30}O_8N_2$: 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 $CHCl_3$ (6 ml) and CH_3CN (1 ml) was refluxed for 2 h (complete dissolution : 6). $(C_2H_5)_3N$ (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. $(C_2H_5)_3N$ and evaporation yielded crude imine 8a. This material, in CH_2Cl_2 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-C_2H_5OAc 5:5:3; Rf=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, CH-C$\underline{H}_3$), 1.12 (s, 9H, tC_4H_9), 2.65-2.90 (m, 3H, N-C$\underline{H}$-C$\underline{H}_2$), 3.00-3.14 (m, 1H, N-C$\underline{H}$-CH_2), 3.45 (s, 2H, PhC$\underline{H}_2$), 3.91 (d, 1H, J=2 Hz, H oxaziridine), 4.28 (qxdxd, 1H, J=6.8, 2 and 8 Hz, C$\underline{H}$-CH_3), 5.72 (d, 1H, J=8 Hz, NH), 7.15-7.80 (m, 15H, Ph).

R^1=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 $(C_2H_5)_3N$, 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-C_2H_5OAc 5:5:3; Rf=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, CH,-C$\underline{H}_3$), 2.66-3.00 (m, 3H, N-C$\underline{H}$-C$\underline{H}_2$), 3.03-3.33 (m, 1H, N-C$\underline{H}$-CH_2), 3.96 (d, 1H, J=2 Hz, H oxaziridine), 4.23 (qxdxd, J=7, 8 and 2 Hz, 1H, C$\underline{H}$-CH_3), 4.40 (s, 2H, PhOC$\underline{H}_2$), 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 $(C_2H_5)_3N$ 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, CH(C$\underline{H}_3$)$_2$), 1.08 (s, 9H, tC_4H_9), 2.26 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.73 (t, 2H, J=6 Hz, N-CH_2-C$\underline{H}_2$), 3.63 (t, 2H, J=6 Hz, N-C$\underline{H}_2$-CH_2), 7.26 and 7.30-7.80 (d, J=6 Hz and m, 1H + 10H, C$\underline{H}$=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 MCBA gave crude oxaziridine 14 which was chromatographed on silica gel (hexane-ether-C_2H_5OAc 5:5:1): yield 1.682 g (71%); ^1H nmr (60 MHz) δ 0.93 (d, 6H, J=6 Hz, CH(C$\underline{H}_3$)$_2$), 1.11 (s, 9H, tC_4H_9), 1.74 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.73-3.00 (m, 3H, N-C$\underline{H}$-C$\underline{H}_2$), 3.00-3.32 (m, 1H, N-C$\underline{H}$-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-C_2H_5OAc 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, CH(C$\underline{H}_3$)$_2$), 1.63 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.60-3.33 (m, 4H, N-C$\underline{H}_2$-C$\underline{H}_2$), 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, CH(C$\underline{H}_3$)$_2$), 2.76 (t, 2H, J=7 Hz, N-CH_2-C$\underline{H}_2$), 3.13 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 4.06 (t, 2H, J=7 Hz, N-CH_2-C$\underline{H}_2$), 7.23 (d, 1H, J=8 Hz, C$\underline{H}$=N→O); ^{13}C nmr (20 MHz, CD_3OD) ppm 19.2 (CH(C$\underline{H}_3$)$_2$), 27.5 (C$\underline{H}$(CH$_3$)$_2$), 36.2 (N-C$\underline{H}_2$-CH_2), 62.8 (N-CH_2-C$\underline{H}_2$), 154.5 (C$\underline{H}$=N→O), 179.3 (C$\underline{O}$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 CH_3OH (7 ml) and aqueous $NaHCO_3$ (53 mg, 0.64 mmol in 3 ml of H_2O). The mixture was allowed to reach slowly 20°C. After evaporation of the organic solvents under vacuum, the aqueous layer was extracted with CH_2Cl_2 (removal of $HOSi(Ph)_2tC_4H_9$). 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, D_2O + CD_3OD) δ 1.50 (d, 3H, J=7 Hz, CH-C$\underline{H}_3$), 2.80 (t, 2H, J=7 Hz, N-CH_2-C$\underline{H}_2$), 3.66 (s, 2H, PhC$\underline{H}_2$), 4.06 (t, 2H, J=7 Hz, N-C$\underline{H}_2$-CH_2), 4.83 (qxd, 1H, J=7 and 6 Hz, C$\underline{H}$-CH_3), 7.11 (d, 1H, J=6 Hz, C$\underline{H}$=N), 7.26 (s, 5H, Ph); ^{13}C nmr (20 MHz, D_2O + CD_3OD) ppm 16.00 (CH-$\underline{C}H_3$), 36.10 (N-$\underline{C}H_2$-CH_2), 43.33 (Ph$\underline{C}H_2$), 44.37 ($\underline{C}$H-CH_3), 62.71 (N-CH_2-$\underline{C}H_2$), 128.49, 130.09, 130.29 and 136.10 (Ph), 148.11 ($\underline{C}$H=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 $NaHCO_3$: yield 0.127 mg (73%); ^1H nmr (60 MHz, D_2O) δ 1.46 (d, 3H, J=7 Hz, CH-C$\underline{H}_3$), 2.83 (t, 2H, J=7 Hz, N-CH_2-C$\underline{H}_2$), 4.10 (t, 2H, J=7 Hz, N-C$\underline{H}_2$-CH_2), 4.56 (s, 2H, PhOC$\underline{H}_2$), 4.96 (qxd , J=7 and 6 Hz, 1H, C$\underline{H}$-CH_3), 6.96 and 6.83-7.53 (d, J=6 Hz, and m, 1H+5H, C$\underline{H}$=N→O and Ph); ^{13}C nmr (20 MHz, DMSO-d_6) ppm 16.84 (CH-$\underline{C}H_3$), 43.05 ($\underline{C}$H-CH_3), 49.62 (N-$\underline{C}H_2$-CH_2), 62.81 (N-CH_2-$\underline{C}H_2$), 67.47 (PhO$\underline{C}H_2$), 115.71, 122.68 and 130.72 (Ph), 143.23 ($\underline{C}$H=N→O), 158.32 (OPh), 169.55 (CO amide), 176.26 (COO^-).

Nitrones 18 :

R^1=benzyl; 18a : Hydroxylamine 20[27] (0.4 g, 4.39 mmol) in CH_2Cl_2 (30 ml) was heated at 35°C in the presence of $(C_2H_5)_3N$ (0.611 ml, 4.39 mmol) up to dissolution. Aldehyde 4a (0.839 g, 1 equiv.) in CH_2Cl_2 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 C_2H_5OAc solution (15 ml) was treated with aqueous $NaHCO_3$ (0.35 g, 4.16 mmol, in 12 ml of H_2O). After 30 min of stirring at 20°C, the aqueous phase was lyophilized and the solid residue was washed with C_2H_5OH : 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, D_2O) δ 1.38 (d, 3H, J=7.1 Hz, CH-C$\underline{H}_3$), 3.61 (s, 2H, PhC$\underline{H}_2$), 4.36 (ABd, 1H, J=15.5 Hz, N-C$\underline{H}$-COO), 4.45 (ABd, 1H, J=15.5 Hz, N-C$\underline{H}$-COO), 4.82 (m, 1H, C$\underline{H}$-CH_3), 7.16 (d, 1H, J=6.5 Hz, C$\underline{H}$ = N→O), 7.28-7.45 (m, 5H, Ph); ^{13}C nmr (20 MHz, D_2O) ppm 15.53 (CH-$\underline{C}H_3$), 42.83 ($\underline{C}$H-CH_3), 44.04 (Ph$\underline{C}H_2$), 68.38 (N-$\underline{C}H_2$-COO), 128.07, 129.69, 129.88 and 135.63 (Ph), 148.92 ($\underline{C}$H=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 $(C_2H_5)_3N$, 0.455 g (1 equiv.) of aldehyde 4b and then 0.166 g (0.9 equiv.) of $NaHCO_3$: 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, D_2O) δ 1.39 (d, 3H, J=7 Hz, CH-C$\underline{H}_3$), 4.38 (ABd, 1H, J=15.3 Hz, N-C$\underline{H}$-COO), 4.48 (ABd, 1H, J=15.3 Hz, N-C$\underline{H}$-COO), 4.64 (s, 2H, PhO-C$\underline{H}_2$), 4.98 (dxd, 1H, J=7 and 6.3 Hz, C$\underline{H}$-CH_3), 6.98-7.14 (m, 3H, Ph), 7.19 (d, 1H, J=6.3 Hz, C$\underline{H}$=N→O), 7.32-7.46 (m, 2H, Ph); ^{13}C nmr (50 MHz, D_2O) ppm 15.23 (CH-$\underline{C}H_3$), 42.94 ($\underline{C}$H-CH_3), 66.77 (PhO$\underline{C}H_2$), 67.88 (N-$\underline{C}H_2$-COO), 114.88, 122.33 and 130.05 (Ph), 147.46 ($\underline{C}$H=N→O), 157.22 (OPh), 171.14 (CO amide), 171.39 (COO^-); ms (CI-IB) 260 (M^+ -CO_2+1).

Nitrone 19 :

The nitrone 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, $N(CH_2\text{-}CH_3)_3$), 1.33 (d, 3H, J=6.5 Hz, $CH\text{-}CH_3$), 1.57 (br s, 6H, $C(CH_3)_2$), 2.93 (q, 6H, J=7 Hz, $N(CH_2\text{-}CH_3)_3$), 3.50 (s, 2H, $PhCH_2$), 4.80 (qxdxd, 1H, J=6.5, 6 and 8 Hz, $CH\text{-}CH_3$), 7.07 (d, 1H, J=6 Hz, CH=N→O), 7.26 (s, 5H, Ph), 7.87 (br d, 1H, J=8 Hz, NH); ^{13}C nmr (50 MHz), ppm 8.66 ($N(CH_2\text{-}CH_3)_3$), 15.99 ($CH\text{-}CH_3$), 24.84 ($C(CH_3)_2$), 43.24 ($CH\text{-}CH_3$), 43.51 ($PhCH_2$), 44.99 ($N(CH_2\text{-}CH_3)_3$), 77.31 ($C(CH_3)_2$), 126.71, 128.46, 129.04 and 134.06 (Ph), 135.12 (CH=N→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, $CH\text{-}CH_3$), 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, $CH\text{-}CH_3$), 7.33 (d, 1H, J=6.5 Hz, CH=N→O), 7.46 (s, 5H, Ph).

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