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# RING ENLARGEMENT OF THE β-LACTAM NUCLEUS OF PENICILLINS

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En hommage au Professeur Albert Bruylants qui nous a initiés à la Chimie Organique

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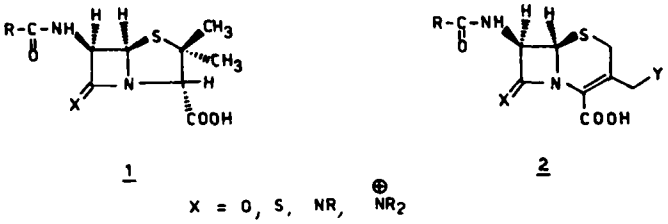
ABSTRACT

Using penicillins as starting materials, a general synthetic route to the bicyclic compounds 7 has been established; they formally result from a ring enlargement of the β-lactam with insertion of one nitrogen atom. The key-step of the procedure is a very mild Lossen rearrangement of hydroxamic acids intermediates upon treatment with N,N-diethylaminopropyne.

## INTRODUCTION

Following Woodward's suggestion[1], the biological activity of penicillins 1 (X=O) and cephalosporins 2 (X=O) has often been discussed in terms of the exceptional lability of their β-lactam ring[2]. It is therefore rather surprising that relatively few studies have been devoted to the replacement of the carbonyl group of the β-lactam by related functions of variable electrophilic character (Scheme 1).



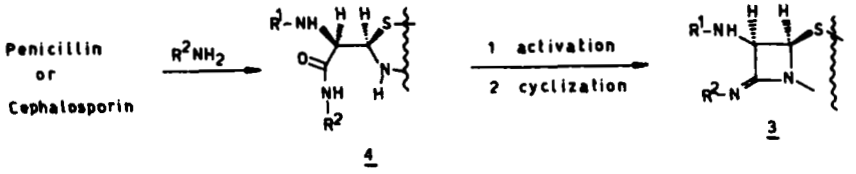
1 2

X = O, S, NR, $\overset{\oplus}{N}R_2$

SCHEME 1

One report[3] describes the synthesis of β-thionolactam analogs of penicillins 1 (X=S) and cephalosporins 2 (X=S). The products were reported to be inactive. More recently, our group has developed a practical method of synthesis of azetidin-2-iminium salts[4]. They can be readily converted into the corresponding azetidin-2-ones, azetidin-2-thiones and azetidin-2-imines[5]. However no derivatives of penicillins or cephalosporins have yet been prepared by this route.

We also considered the possibility of transforming the β-lactam ring of penicillins and cephalosporins into an azetidinimine[6]. As possible route toward such compounds 3 involves the opening of the β-lactam ring with an appropriate amine followed by the intramolecular quenching of an activated form of the resulting amide 4 (Scheme 2).

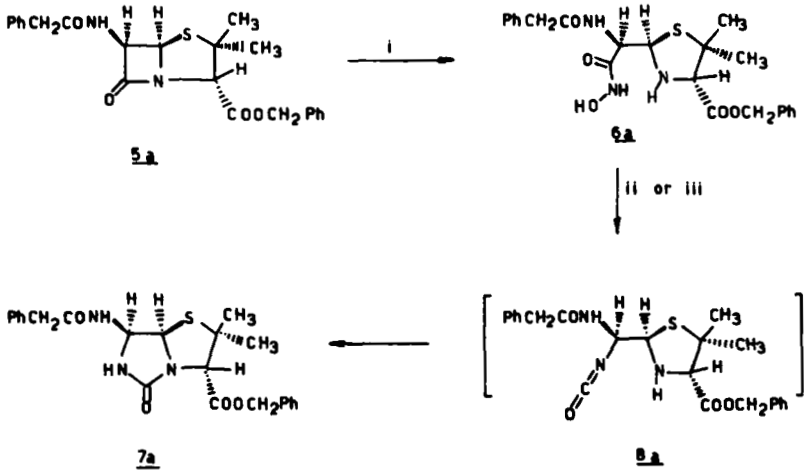



SCHEME 2

During the course of these studies, we discovered that hydroxamic acids (4, $R^2$=OH) derived from penicillins could be converted stereospecifically into new bicyclic derivatives in which the β-lactam ring had been replaced by a five-membered heterocyclic ring.

RESULTS

The readily available[7] Penicillin G benzyl ester 5a was selected for our model experiments. It was converted[8] almost quantitatively into the hydroxamic acid 6a by treatment with hydroxylamine in methanol (Scheme 3). Compound 6a reacted only very slowly with N,N-diisopropylcarbodiimide[9] at room temperature. A product was isolated in rather low yields (25%) which was shown to have the structure 7a[10]. It is characterized by a strong and broad IR absorption at ∿ 1720 $cm^{-1}$. The two protons borne by the carbon atoms of the new five-membered ring give rise, in the $^1H$ NMR spectrum, to an ABX multiplet near 5.70 δ with a vicinal coupling constant of 7 Hz. This confirms the cis configuration of these centers. The proton of the thiazolidine ring shows a singlet at 4.64 δ, as expected for this type of bicyclic structure. No other bicyclic product could be identified in the crude reaction mixture.

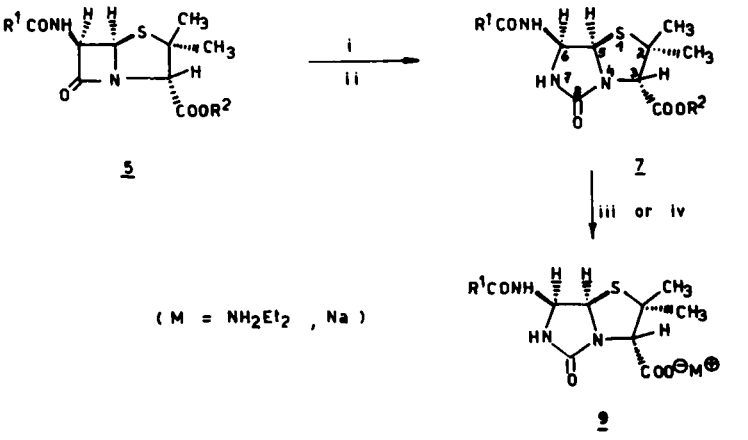



Reagents = (i) $H_2N$-OH, $CH_3OH$, - 30° to 20°C; (ii) $(CH_3)_2CH$-N=C=N-$CH(CH_3)_2$, dioxane-water, 20°C, 15 days (method A) - yield i + ii : 25%; (iii) $CH_3$-C≡C-$N(Et)_2$, $CH_2Cl_2$, - 40° to 20°C (method B) - yield i + iii: 45%.

SCHEME 3

The formation of 7a can be readily explained by a Lossen rearrangement[11] yielding the isocyanate 8a which is trapped by the amine group of the thiazolidine ring. The low rate of reaction probably results from both the weak basicity of the carbodiimide reagent and the weak acidity of the hydroxamic acid 6a. We therefore decided to use a more basic dehydrating agent such as N,N-diethylaminopropyne[12]. The reaction was very fast and took place readily at -40°C in dichloromethane. Furthermore the product 7a was more easily purified and the yield was higher (45%). To our knowledge, this is the first example of a Lossen rearrangement initiated by N,N-diethylaminopropyne; this reagent should be recommended for effecting this rearrangement under very mild conditions and without racemisation.

Attempts to regenerate the free carboxylic acid from the benzyl ester 7a by catalytic hydrogenation[13] (Pd-Carbon, dioxane + 5% AcOH, 20°C, 3 atm), only lead to poor yields : after two runs, the conversion was only ∿ 20% and a large amount of starting material was recovered. We therefore prepared other derivatives of Penicillin G bearing more labile (5b[14] and 5c[15]) or biodegradable (5d[16]) ester groups. They were prepared by conventional methods (see experimental part). We also prepared the biodegradable[17] esters 5e and 5f derived from Penicillin V and Methicillin.




5 7 9

Reagents : (i) $H_2N$-OH, $CH_3OH$, - 30° to 20°C to give 6; (ii) $CH_3$-C≡C-N(Et)$_2$, $CH_2Cl_2$, - 40°C to 20°C; (iii) 7b + 3 equiv. of HCl in $CH_3NO_2$, 30°C, 4h then HN(Et)$_2$; (iv) 7c + HCl - EtOH, reflux, 2h then NaOH.

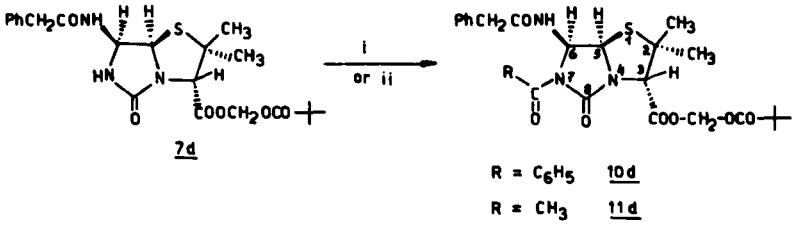
SCHEME 4

Compounds 5b-f smoothly reacted[8] with hydroxylamine to yield almost quantitatively the corresponding penicilloic hydroxamic acids 6, which were used without purification. Upon treatment with N,N-diethylaminopropyne, the bicyclic ureas 7b-f were formed in moderate to good yields (Scheme 4 - Table 1). Structural assignments were further confirmed by an X-ray diffraction analy-

TABLE I : Yields of 7 and 9

| | $R^1$ | $R^2$ | % 7 (from 5) | % 9 | M |
|---|---|---|---|---|---|
| a | $C_6H_5$-$CH_2$ | $C_6H_5$-$CH_2$ | 45(a) | ∿ 20 | H |
| b | $C_6H_5$-$CH_2$ | $CH_3O$-  -$CH_2$ | 36(a) | 55 | H |
| | | | | 30 | $NH_2Et_2$ |
| c | $C_6H_5$-$CH_2$ | $CH_3OCH_2$ | 27(a) | ∿ 90 | Na |
| d | $C_6H_5$-$CH_2$ | $(CH_3)_3C$-$COOCH_2$ | 64(b) | | |
| e | $C_6H_5O$-$CH_2$ | $(CH_3)_3C$-$COOCH_2$ | 68(b) | | |
| f |  | $(CH_3)_3C$-$COOCH_2$ | 48(a) | | |

(a) Yield after column-chromatography
(b) Yield after crystallization from ether




7d

R = $C_6H_5$ 10d

R = $CH_3$ 11d

Reagents : (i) PhCOCl (3 equiv.), $NEt_3$ (3 equiv), $CH_2Cl_2$, 18h, 20°C - Chromatography, 61%; (ii) $CH_3COCl$ (10 equiv), $NEt_3$, (10 equiv), $CH_2Cl_2$, 18h, 20°C - Chromatography, 58%.

SCHEME 5

sis[18] of crystals of 7d.

Treatment of the p-methoxylbenzylester 7b with HCl in nitromethane[19] gave the free acid (∿ 55% crude) which was recrystallized as the diethylammonium salt 9. However, the best precursor of the free carboxylate group was the

methoxymethylester 7c which gave the sodium salt 9 in ∿ 90% yield upon treatment with HCl in refluxing methanol[20], followed by addition of NaOH (Scheme 4 - Table 1).

The X-ray data on 7d[18], suggest that the geometry of the new bicyclic compounds 7 is similar to that of cephalosporins; typical values are the $N_{(4)}$-$C_{(8)}$ bond length (1.40 Å) and the $N_{(4)}$ degree of planarity (expressed as the sum of the bond angles about this atom : 345.1°). These observations prompted us to submit the carboxylate 9 (M = Na) to antibacterial tests. The compound was found to be inactive toward Staphylococcus aureus (ATCC 6538) and Escherichia Coli B (ATCC 11303). This inactivity could result from the rather low electrophilic character of the carbonyl group of the urea function. We therefore decided to prepare, derivatives bearing an electron-withdrawing group on the nitrogen atom $N_{(7)}$ (Scheme 5).

Compound 7d was acylated with benzoyl and acetyl chlorides in the presence of triethylamine to give 10d (60%) and 11d (58%). These derivatives are characterized in the $^1H$ NMR spectra by a strong deshielding of the $H_{(6)}$ protons with respect to 7d. No racemisation was observed during these reactions as shown by the value (7 Hz) of the coupling constant between $H_{(5)}$ and $H_{(6)}$. The structure of 10d was further confirmed by an X-ray diffraction analysis[18].

However, both biodegradable[17] esters 10d and 11d were devoid of biological activity[21].

## EXPERIMENTAL

M.ps (Leitz microscope) are uncorrected. All rotations (Perkin-Elmer 241 MC) were determined in $CH_2Cl_2$ and all IR spectra (Perkin-Elmer 297 and 681, calibration with polystyrene) in $CH_2Cl_2$ as solvent, unless otherwise mentioned. The $^1H$ NMR spectra were recorded ($CDCl_3$ unless otherwise mentioned, with TMS as internal standard) on a Varian T60 spectrometer or, if specified, on XL 100 and XL 200 spectrometers. The $^{13}C$ NMR spectrum was obtained ($CDCl_3$-TMS) with a Varian XL 200 instrument. The Mass spectra were determined with a Varian MAT 311 Spectrometer. Column-Chromatographies were performed with Riedel de Haen alumina (neutral, activity I) and with Merck silica gel 60 (70-230 mesh ASTM). $CH_2Cl_2$, EtOAc and DMF were dried over $P_2O_5$ (reflux) then distilled. $CH_3OH$ and iPropOH were refluxed over CaO and distilled. Diethylether and THF were refluxed on $LiAlH_4$ and distilled. Benzene was dried over Na and distilled.

### Preparation of the Penicillin esters 5

* Penicillin G t.butyloxycarbonylmethyl ester 5d :

To a solution of 18 g (0.05 mol) of Penicillin G sodium salt in 100 ml of dry DMF, 7.5 g (0.055 mol) of chloromethylpivalate were added dropwise. The mixture was stirred at room temperature during 24 hr, and then poured in 500 ml of ice-cold water. The gummy solid obtained was dissolved in $CH_2Cl_2$ and washed with water. After drying ($CaCl_2$), concentration and crystallization from ether, 18g of 5d were isolated (yield : 80%; m.p. 111-112°C; IR : 1780, 1760 (br), 1680 $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.18 (s, 9), 1.43 (s, 6), 3.56 (s, 2), 4.33 (s, 1), ∿ 5.47 (ABq, 2, J=4Hz), ∿ 5.70 (sharp ABq, 2, J=5.5Hz), 7.17 (s, 5) δ].

Chromatography on silica gel (EtOAc - $CH_2Cl_2$ 1:5) furnished the analytically pure sample : Found : C, 58.87; H, 6.35; N, 6.24% - Calcd. for $C_{22}H_{28}O_6N_2S$ (448.46) : C, 58.92; H, 6.29; N, 6.25%.

* Penicillin V t.butyloxycarbonylmethyl ester 5e :

Using the procedure described for 5d, 776 mg (2 mmol) of Penicillin V potassium salt and 360 mg of chloromethylpivalate in 10 ml of DMF, furnished 700 mg of 5e as a gum [yield : 75%, IR : 1785, 1755 (br), 1690 $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.22 (s, 9), 1.53 (s, 3), 1.60 (s, 3), 4.46 (s, 1), 4.53 (s, 2), ∿ 5.62 (ABq, 2, J=5Hz), ∿ 5.78 (sharp ABq, 2, J=6Hz), 6.73-7.50 (m, 5) δ].

* Methicillin t.butyloxycarbonylmethyl ester 5f :

As before, 975 mg (2.4 mmol) of Methicillin sodium salt and 450 mg of chloromethylpivalate in 10 ml of DMF, gave (after 6hr of reaction) 670 mg of 5f [yield : 56%; IR : 1790, 1760 (br), 1683 $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.23 (s, 9), 1.51 (s, 3), 1.63 (s, 3), 3.78 (s, 6), 4.40 (s, 1), ∿ 5.63 (ABq, 2, J=4.5Hz), ∿ 5.81 (ABq, 2, J=4Hz), 6.50 (br d, 2, J=8.5Hz), 7.23 (dxd, 1, J=8.5Hz) δ].

Preparation of the penicilloic hydroxamic acids 6 :

General procedure : a solution of free hydroxylamine was prepared from hydroxylaminehydrochloride (0.01 mol) dissolved in dry methanol (10 ml) and a methanolic solution of NaOH (1N, 10 ml). This solution was added dropwise, with stirring, at - 30°C, to the penicillin esters 5 (0.01 mol) in dry methanol (15 ml). The mixture was allowed to reach 20°C in about 1 hr and to stand at room temperature for a further ∿ 1 hr. After elimination of the solvent to dryness, under vacuum, the crude penicilloic hydroxamic acids 6 (∿ 100%, amorphous solids) were used for cyclization without purification.

* 6a : IR : 3400-3100 (large), 1735, 1660 (br) $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.07 (s, 3), 1.41 (s, 3), 3.50 (s, 1), 3.62 (s, 2), 4.50 (d, 1, J=6Hz), 5.11 (d, 1, J=6Hz), 5.20 (s, 2), 7.29 (s, 5), 7.40 (s, 5) δ.

* 6b : IR : 3400-3100 (large), 1730, 1665 (br), 1610 $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.06 (s, 3), 1.38 (s, 3), 3.50 (s, 1), 3.60 (s, 2), 3.81 (s, 3), 4.51 (d, 1, J=6Hz), ∿ 5.13 (sharp m : ABq, J=10Hz and d, J=6Hz, 2+1), 6.90 (d, 2, J=9Hz), 7.26 (s, 5), 7.33 (d, 2, J=9Hz) δ.

* 6c : IR : 3400-3100 (large), 1735, 1655 (br) $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.20 (s, 3), 1.47 (s, 3), 3.43 and 3.55 (s + br s, 6), 4.53 (br d, 1), 4.93-5.46 (ABq + d, 2+1), 7.23 (s, 5) δ.

* 6d : IR : 3400-3100 (large), 1750, 1662 $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.18 (s, 9+3), 1.41 (s, 3), 3.46 (s, 1), 3.54 (s, 2), 4.40 (d, 1, J=6Hz), 5.00 (d, 1, J=6Hz), 5.55 ($d_{AB}$, 1, J=5Hz), 5.81 ($d_{AB}$, 1, J=5Hz), 7.17 (s, 5) δ.

▪ 6e : IR : 3400-3100 (large), 1750, 1670 $cm^{-1}$; $^1H$ NMR : 1.20 (br s, 9+3), 1.46 (s, 3), 3.68 (s, 1), 4.50 (m, 2+1), 5.13 (m, 1), 5.58 ($d_{AB}$, 1, J=5Hz), 5.85 ($d_{AB}$, 1, J=5Hz), 6.66-7.43 (m, 5) δ.

▪ 6f : IR : 3450-3250 (large), 1758, 1675, 1600 $cm^{-1}$; $^1H$ NMR 1.20 (br s, 9+3), 1.51 (s, 3), 3.76 (br s, 6+1), 4.63 (m, 1), 5.33-5.93 (ABq + m, 2+1), 6.44 (br d, 2, J≃8Hz), 7.16 (dxd, 1, J≃8Hz) δ.

Preparation of the bicyclic ureas 7 :

Method A :

A solution of crude 6a (4.57 g, 0.01 mol) and N,N-diisopropylcarbodiimide (1.9 ml, 0.012 mol) in dioxane (40 ml) and water (20 ml) was stirred for 15 days at room temperature. After concentration under high vacuum, benzene was added (∿ 50 ml) and the precipitated N,N-diisopropylurea was filtered off. Column-chromatography of the filtrate (two runs, alumina, iPropOH-benzene 1:9) gave 1.09 g (25%) of 7a : mp : 169-170°C; $[\alpha]_D$ (± 0.5) : + 40.5° (c = 0.325%); IR : 3435, 3345, 1720 (br), 1670, 1485 $cm^{-1}$; $^1H$ NMR : 1.29 and 1.31 (two s, 6), 3.51 (s, 2), 4.64 (s, 1), 5.10 (s, 2), 5.46-5.88 (mABX, 2), 6.38 (br s, 1), 6.72 (br d, 1, J=7Hz), 7.19 (s, 5), 7.24 (s, 5) δ - after $D_2O$ exchange : 5.65 and 5.81 (two $d_{AB}$, 2, J=7Hz); Mass (EI) : 439 ($M^+$, 0.5%), 3.96 (M-CONH, 1%), 304 (M-G and M-$COOCH_2Ph$, 26%), 250 (M-GCH-NCO, 16%), 168 (26%), 144 (32%), 91 (100%); Anal. Calcd. for $C_{23}H_{25}O_4SN_3$ (439.45) : C, 62.86; H, 5.73; N, 9.56% Found : C, 63.08; H, 5.98; N, 9.66%.

Method B :

▪ bicyclic urea 7a :

To a solution of 18.2 g (0.048 mol) of crude 6a in 100 ml of $CH_2Cl_2$, stirred at -40°C, were added very slowly (∿ 1h 30) 6.63 ml (0.058 mol) of N,N-diethyl-aminopropyne in 20 ml of $CH_2Cl_2$. The solution was allowed to reach r.t. and then concentrated under vacuum. Extraction with ether (3x50 ml) and chromatography of the residue (alumina, iPropOH-benzene 1:9) yielded ∿ 7 g (45%) of 7a, identical with the sample prepared according to the method A.

▪ bicyclic urea 7b :

To a solution of 15g (∿ 0.03 mol) of crude 6b in 150 ml of $CH_2Cl_2$, stirred at 0°C, 4.5 ml (0.032 mol) of ynamine in 10 ml of $CH_2Cl_2$ were added dropwise. The solution was allowed to reach 20° in about 1hr and stirring was continued of 4 hr at r.t. After concentration under vacuum and extraction of the amide with ether (3 x 50 ml), the residue was chromatographied on alumina (iPropOH-benzene 1:9) to give 5 g (36%) of 7b : m.p. : 126-127°C; $[\alpha]_D$ (± 0.5) : +44° (c=0,395%); IR : 3430, 3340, 1727 (br), 1675, 1612, 1510, 1490 $cm^{-1}$; $^1H$ NMR : 1.27 and 1.29 (two s, 6), 3.52 (s, 2), 3.75 (s, 3), 4.64 (s, 1), 5.06 (s, 2), 5.50-5.91 (mABX, 2), 6.21 (br s, 1), 6.66 (br d, 1, J=7Hz), 6.79 (d, 2, J=9Hz), 7.21 (s, 5), 7.23 (d, 2, J=9Hz) δ - after $D_2O$ exchange : 5.62 and 5.81 (two $d_{AB}$, 2, J=7Hz); Mass (EI) : 469 ($M^+$, 1.5%), 349 (M-$CH_2PhOCH_3$, 11%), 334 (M-G, 38%), 280 (M-GCH-NCO, 4%), 175 (18%), 169 (43%), 160 (14%), 135 (26%), 122 (42%), 121 (100%), 91 (> 100%); Anal. Calcd for $C_{24}H_{27}O_5SN_3$ (469.48) : C, 61.40;

H, 5.80; N, 8.95% - Found : C, 61.78; H, 6.10; N, 9.26%.

M bicyclic urea 7c :

7c was prepared, according to the procedure used for 7b, from 10g (∿ 0.024 mol) of crude 6c and 3.5 ml (0.025 mol) of ynamine in 110 ml of $CH_2Cl_2$. Chromatography (alumina, iPropOH-benzene 1:9) yielded 2.78 (27%) of pure 7c as a gum : IR : 3432, 3340, 1725, 1710, 1675, 1490 $cm^{-1}$; $^1H$ NMR 1.34 (s, 3), 1.44 (s, 3), 3.44 (s, 3), 3.54 (s, 2), 4.64 (s, 1), 5.24 (s, 2), 5.51-5.96 (mABX, 2), 6.45 (br s, 1), 6.73 (br d, 1, J=7Hz), 7.21 (s, 5) δ - after $D_2O$ exchange : 5.60 and 5.77 (two $d_{AB}$, 2, J=7Hz); Mass (EI) : 393 ($M^+$, 3%), 348 (M-$CH_2OCH_3$, 4%), 258 (M-G, 100%), 204 (M-GCH-NCO, 80%), 196 (32%), 187 (43%), 169 (72%), 135 (> 100%), 116 (80%), 91 (> 100%).

M bicyclic urea 7d :

To a solution of 19g (∿ 0.039 mol) of crude 6d in 100 ml of $CH_2Cl_2$, stirred at -40°C, 5.6 ml (0.0405 mol) of ynamine were added dropwise. The mixture was allowed to come slowly at room temperature (∿ 2 hr). After a further 1hr at r.t., the solvent was evaporated under vacuum and the residue crystallized in dry ether, at - 18°C (2 days), to furnish 11.5 g (64%) of 7d. One recrystallization in $CHCl_3$-ether gave the analytically pure product : mp : 156°C; $[\alpha]_D$ (± 0.5) : + 46.2° (c=0.485%); I.R. : 3430, 3340, 1745, 1725, 1710, 1677, 1490 $cm^{-1}$; $^1H$ NMR (200 MHz) : 1.18 (s, 9), 1.27 (s, 3), 1.39 (s, 3), 3.57 (s, 2), 4.66 (s, 1), 5.60-5.90 (m, ABX + A'B', 4), ∿ 6.07 (br s, 1), ∿ 6.63 (br d, 1, J ∿ 7Hz), 7.20-7.35 (m, 5) δ - After irradiation of the NH (amide) : 5.67 ($d_{AB}$, J=7.3Hz), 5.71 ($d_{A'B'}$, J=5.5Hz), 5.78 ($d_{AB}$, J=7.3Hz) and 5.81 ($d_{A'B'}$, J=5.5Hz); $^{13}C$ NMR (decoupl., 200 MHz) : 25.30, 26.89; 33.03, 38.79, 43.63, 57.56, 58.37, 69.35, 70.81, 79.63, 127.17, 129.20, 129.57, 133.73, 159.01, 167.38, 171.41, 176.85 ppm; Mass (EI) : 463 ($M^+$, 0.5%), 348 (M-$CH_2OCOC_4H_9$, 1%), 328 (M-G, 61%), 304 (8%), 298 (24%), 274 (M-GCH-NCO, 17%), 244 (20%), 214 (68%), 196 (47%), 169 (M-G-$C_4H_9COOCH_2COOH$, 100%), 135 (36%), 91 (> 100%); Anal. Calcd. for $C_{22}H_{29}O_6N_3S$ (463.4) : C, 57.01; H, 6.31; N, 9.07% - Found : C, 56.96; H, 6.28; N, 9.03%.

M bicyclic urea 7e :

7e was prepared, according to the procedure used for 7d, from 650 mg (1.3 mmol) of crude 6e and 180 µl (1.3 mmol) of ynamine in 10 ml of $CH_2Cl_2$. Crystallization from ether afforded 410 mg (68%) of 7e : m.p. : 115-116°C; IR : 3440, 3360, 1749, 1732, 1690, 1600, 1510, 1495 $cm^{-1}$; $^1H$ NMR : 1.21 (s, 9), 1.48 (s, 6), 4.48 (s, 2), 4.73 (s, 1), 5.63-6.00 (m, ABX + A'B', 4), 6.03 (br s, 1), 6.73-7.40 (m, 5), 7.70 (br d, 1, J≃6Hz) δ - after $D_2O$ exchange : ∿ 5.76 and ∿ 5.82 (two sharp ABq, J=5 and 7Hz, 4); Anal. Calcd. for $C_{22}H_{29}O_7N_3S$ (479.4) : C, 55.11; H, 6.10; N, 8.76% - Found : C, 54.98; H, 6.06; N, 8.75%.

M bicyclic urea 7f :

To a solution of 630 mg (∿ 1.2 mmol) of crude 6f in 6 ml of $CH_2Cl_2$, stirred at - 60°C, were added 180 µl (1.3 mmol) of ynamine (with a syringe through a rubber stopper). The mixture was allowed to come slowly at room temperature and

stirring was continued for 2 hr. Evaporation of the solvent and chromatography (alumina, iPropOH - benzene 8 : 92) yielded 287 mg (48%) of 7f. A second chromatography (alumina, iPropOH-benzene 5 : 95) and precipitation from $CHCl_3$ - pentane gave the analytically pure product : IR : 3445, 3400, 1755-1735 (br), 1680, 1600, 1495, 1480 $cm^{-1}$; $^1H$ NMR : 1.21 (s, 9), 1.50 and 1.53 (two s, 6), 3.76 (s, 6), 4.73 (s, 1), 5.60-6.13 (m, ABX + A'B' + N-H, 5), 6.47 (br d, 2, J≃8Hz), 6.83 (br d, 1, J≃6Hz), 7.20 (dxd, 1, J≃8Hz) δ - after $D_2O$ exchange : ∿ 5.76 and ∿ 5.93 (two sharp ABq, J=5.5 and 7Hz, 4); Anal. Calcd. for $C_{23}H_{31}O_8N_3S$ (509.5) : C, 54.22; H, 6.13; N, 8.25% - Found : C, 54.05; H, 6.17; N, 7.90%.

Preparation of the carboxylates 9 :

* From the ester 7b :

To a solution of 500 mg (∿ 1 mmol) of 7b in 10 ml of $CH_3NO_2$, 3 ml of 1N HCl in $CH_3NO_2$ (3 mmol) were added dropwise at room temperature. After 4 hr at 30°C, the solution was concentrated under vacuum, and the residue, dissolved in $CH_2Cl_2$ (50 ml), was extracted with 5% $NaHCO_3$ aq (4 x 25 ml). The aqueous phase was brought at pH ∿ 2 by addition of conc. HCl, at 0°C, and then extracted with $CHCl_3$ (4 x 50 ml). Drying ($MgSO_4$) and evaporation gave 210 mg (55%) of crude acid 9 (M = H). This material, in 1 ml of $CH_2Cl_2$, was treated with $NHEt_2$ (44 mg, 6 mmol). The diethylammonium salt 9 was precipitated by addition of ether (150 mg, 30%) : m.p. ∿ 130°C (décomp.); IR (KBr) : 3600-3150 (br), 2978, 2940, 2740, 2680, 2625, 2603, 2495, ∿ 1695 (br), 1660, ∿ 1600 (br), 1515 (br), 1420 (br) $cm^{-1}$; $^1H$ NMR : 1.25 (t, 6, J=7Hz), 1.31 (s, 3), 1.48 (s, 3), 2.86 (br q, 4, J=7Hz), 3.55 (s, 2), 4.37 (s, 1), 5.46-6.02 (m ABX, 2), 6.11 (brs, 1), 6.71 (br d, 1, J=7Hz), 7.23 (s, 5), ∿ 8.5 (br m, 2) δ; $^1H$ NMR ($D_2O$) : 1.12 (t, 6, J=7.5Hz), 1.34 (s, 6), 2.92 (q, 4, J=7.5Hz), 3.49 (s, 2), 4.12 (s, 1), 5.66 (br s = sharp ABq, 2, J≃7Hz), 7.23 (s, 5) δ.

* From the ester 7c :

A solution of 570 mg (1.45 mmol) of 7c in 15 ml of Ethanol and 0.4 ml conc. HCl was refluxed for 2 hr. Addition of water (30 ml) and extraction with $CHCl_3$ (3 x 25 ml) furnished, after drying and concentration, 450 mg (∿ 90%) of acid 9 (M=H; IR : 3500-3300 (br), ∿ 1725 (br), 1685, 1495 $cm^{-1}$). The sodium salt was obtained by treatment of 9 (M = H), in a minimum of $CH_2Cl_2$, with aqueous NaOH (1 equiv.) and evaporation to dryness : m.p. ∿ 150°C (decomp.); IR' (KBr): 1695 (br), 1660 (br), 1600, 1510, 1400 (br) $cm^{-1}$; $^1H$ NMR ($D_2O$-100 MHz) : 1.46 (br s, 6), 3.53 (s, 2), 4.23 (s,1), 5.67 (sharp ABq, 2, J=7Hz), 7.17 (s, 5) δ.

Acylation of 7d

* N-Benzoyl derivative 10d :

To a solution of 600 mg (1.3 mmol) of 7d and 700 mg (5 mmol) of benzoylchloride in 13 ml of $CH_2Cl_2$, stirred at 0°C, 505 mg (5 mmol) of $NEt_3$ in 2 ml of $CH_2Cl_2$ were added very slowly. The mixture was left overnight at r.t. The solution was diluted with $CH_2Cl_2$ (15 ml) and washed with water (2 x 20 ml). After drying ($CaCl_2$) and concentration, the residue was extracted with dry ether (2 x 10 ml) and then chromatographied on silica gel (iPropOH - benzene 1:9) to fur-

nish 900 mg of impur 10d. A second chromatography (silica gel, EtOAc-benzene 1:4) gave ∿ pure product : yield : 450 mg (61%); mp : 105-107°C; IR : 3410, 1745 (br), 1690 (br), 1600, 1495 $cm^{-1}$; $^1H$ NMR ($D_2O$ exchange) : 1.18 (s, 9), 1.45 (br s, 6), 3.55 (s, 2), 4.48 (s, 1), 5.66, 5.74 and 5.80 ($d_{AB}$, d and $d_{AB}$, 3, J=5,7 and 5Hz), 6.46 (d, 1, J=7Hz), 7.20 and 7.10-7.56 (s+m, 10) δ; Anal. Calcd. for $C_{29}H_{33}O_7N_3S$ (567.58) : C, 61.36; H, 5.86; N, 7.40% - Found : C, 60.90; H, 6.19; N, 7.97%.

N-acetyl derivative 11d :

To a solution of 450 mg (0.97 mmol) of 7d and 1 ml of acetylchloride (∿ excess 10) in 10 ml of $CH_2Cl_2$, stirred at 5°C, 1.6 ml (11 mmol) of $NEt_3$ in 2 ml of $CH_2Cl_2$ were added very slowly (∿ 2 hr). The mixture was left overnight at r.t. Dilution with $CH_2Cl_2$ (20 ml), washing with water (2x20 ml), drying ($CaCl_2$) and concentration under vacuum gave the crude product. Chromatography (silica gel, iPropOH-benzene 1:9) furnished 570 mg of impur 11d which were chromatographied again (silica gel, EtOAc-benzene 1:4) to yield 287 mg (58%) of 11d. Crystallization from ether (120 mg, 24%) afforded an analytically pure sample : mp : 148°C; IR : 3400, 1742 (br), 1710-1680 (br), 1495 $cm^{-1}$; $^1H$ NMR : 1.20 (s, 9), 1.43 (br s, 6), 2.40 (s, 3), 3.56 (s, 2), 4.50 (s, 1), 5.66, 5.69 and 5.85 (d, $d_{AB}$ and $d_{AB}$, 3, J=7,5 and 5Hz), 6.10 (br d, 1, J=7Hz), 6.26 (dxd, 1, J=7 and 7Hz), 7.23 (s, 5) δ; Anal. calcd. for $C_{24}H_{31}O_7N_3S$ (505.51) : C, 57.02; H, 6.18; N, 8.31% - Found : C, 57.09; H, 6.18; N, 8.35%.

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