Tetrahedron Vol 45, No 14, pp 4537 to 4550, 1989
Printed in Great Britain.

0040-4020/89 $3 00+.00

A γ-LACTAM ANALOGUE OF THE PENEMS POSSESSING ANTIBACTERIAL ACTIVITY

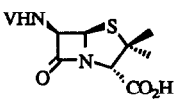
Jack E. Baldwin,* Richard T. Freeman, Christopher Lowe,
Christopher J. Schofield, and Eun Lee.+

The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY
+ The Department of Chemistry, Seoul National University, Seoul 151, Korea.

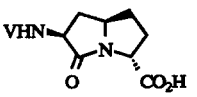
(*Received in UK* 17 *April* 1989)

Abstract: The synthesis of a γ-lactam analogue of penems, from aspartic acid semi-aldehyde, which possessed antibacterial activity is described.[1,2]

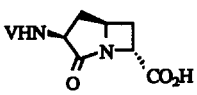
The antibacterial activity of the β-lactam antibiotics [e.g. penicillin V (1)] involves the acylation of transpeptidases involved in cell wall biosynthesis.[3] The minimum structural requirement for activity appears to be a suitably activated β-lactam. The possibility of similarly biologically active compounds devoid of a β-lactam ring has been explored by ourselves and others. We have prepared several γ-lactams, for example (2)[4] and (3)[5] and the literature contains several related efforts.[6] However, with the exception of the azete (4)[7] none were shown to possess biological activity, either as antibacterials or β-lactamase inhibitors.



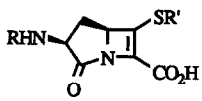
1



2



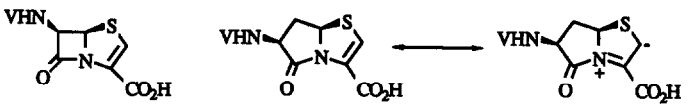
3



4

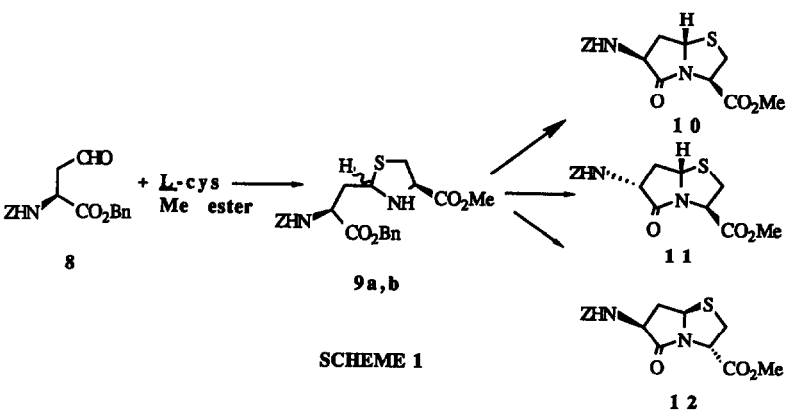
V = $PhOCH_2CO$

We reasoned that the absence of antibacterial activity observed for (2) and (3) possibly derived from the diminished reactivity of the γ-lactams compared to that of the β-lactam ring systems.[8] Hence a γ-lactam analogue (6) of the 6β-acylamino penems (5)[9], might show increased reactivity and biological activity due to delocalisation of the lactam-N lone pair through the olefinic double bond, as in (6) → (7). In this connection it is of interest that the 6β-acylamino-penems (5) have been considered too reactive for practical use as antibiotics.[10]



5 6 7

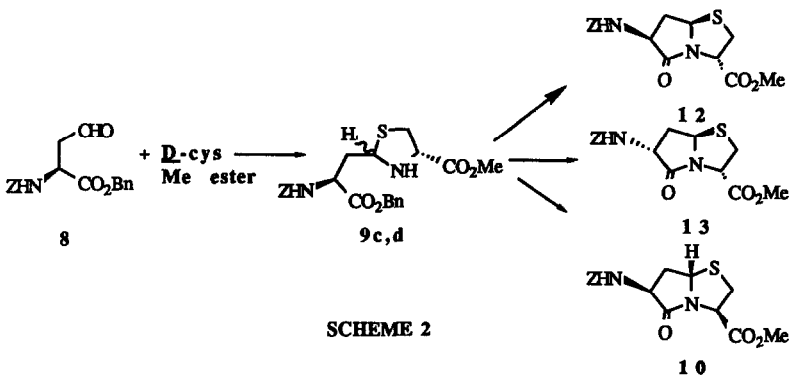
We chose cysteine and aspartic acid semi-aldehyde[11] as readily available chiral precursors. Initially we examined the reaction of (8) with L-cysteine methyl ester. Thus condensation of (8) with L-cysteine methyl ester in pyridine in the presence of activated molecular sieves (20°C, 5 hours) gave a ca 1:1 mixture of the diastereomeric thiazolidines (9a, 9b). Equilibration of separated (9a) and (9b) was observed on silica gel and upon standing in chloroform solution. Reflux of a mixture of (9a) and (9b) in pyridine effected ring closure to give the bicyclic lactam (10) (45%) {$[\alpha]_D^{20}$ = -208° ($CHCl_3$, c =1.48)} and a minor product assigned as probably a mixture of the enantiomers (11) and (12) {$[\alpha]_D^{20}$ = -117° ($CHCl_3$, c=0.11)} (Scheme 1). These assignments were made on the basis of nuclear Overhauser experiments and further experimentation.



8 9a,b 10 11 12

SCHEME 1

The C-5 stereochemistry of (10) was the opposite to that desired, consequently the condensation was repeated, without isolation of the intermediate thiazolidines, using D-cysteine methyl ester. ^{1}H n.m.r. analysis indicated two bicyclic products the major of which was assigned as the desired diastereomer (12) (38%) {$[\alpha]_D^{20}$ = + 175° ($CHCl_3$, c = 0.6)} and a minor which was assigned as a mixture of the enantiomers (10) and (13) {$[\alpha]_D^{20}$ = + 168° ($CHCl_3$, c = 1.13)} (Scheme 2).

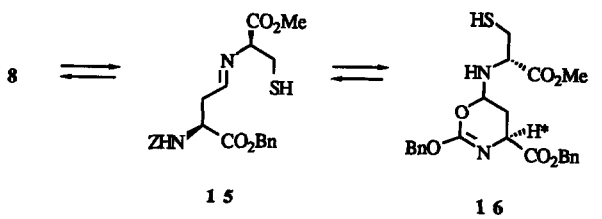


8 9c,d

SCHEME 2

The final ring closures to afford the bicyclic γ-lactams (10-13) were shown to be irreversible by refluxing pure samples [by ^{1}H n.m.r. (500 MHz)] of (10) and (12) in pyridine (>16 hours). No isomerisation was observed by tlc or ^{1}H n.m.r. and the optical integrity of the starting materials was maintained. A similar test on the aldehyde (8) at 20°C also indicated no racemisation.

A plausible rationale for the observed partial racemisation of the minor products was provided by consideration of the mechanism of the ring closures. For the L- cysteine case, formation of the imine (15) allows partial epimerisation at the cysteine C-2 position to occur in pyridine at elevated temperatures. Similarly epimerisation at C-7 of the thiazolidines can be rationalised by reversible cyclisation of the O-benzyloxycarbonyl moiety onto the imine (15) to give (16) which renders the C-7 position H* exchangeable (Scheme 3).

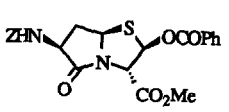


15 16

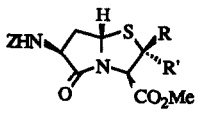
SCHEME 3

We have previously reported methodology for stereospecific functionalisation α- to sulphur in a synthesis of penicillin G.[12] Thus reflux of (12) in benzene with benzoyl peroxide (cat. cupric acetoacetate) provided as a single product the trans-benzoate (17) (59%). Similarly, reaction of (10) with benzoyl peroxide gave a 3:1 mixture of the diastereomeric benzoates (18) and (19).

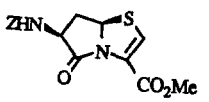
Syn-elimination of benzoic acid from (17) was achieved either by neat thermolysis or by reflux in N,N'-dimethylaniline to give the olefin (20) (40%). Similar treatment of the trans-benzoate (18) gave a ca 4:1 mixture of olefins (20) and (21) respectively. The minor cis-benzoate (19) also gave (20) and (21) but with a substantially altered ratio (1:1). It is possible that in addition to a simple E2 type elimination an alternative process occurs in which at high temperature an initial displacement of the sulphur occurs to give a thiolate (22), thereby explaining the observed loss of stereochemistry at C-5 in the transformations [(19) → (21) + (22) and (18) → (21) + (22)] (Scheme 4).



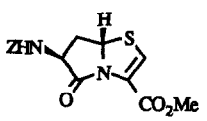
17



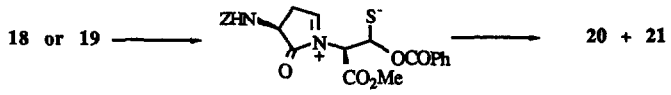
18, R = H, R' = OCOPh
19, R = OCOPh, R' = H



20

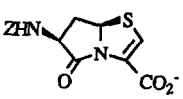


21

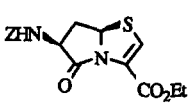


22

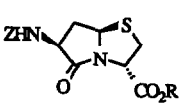
SCHEME 4



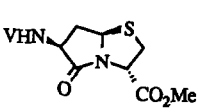
23



24

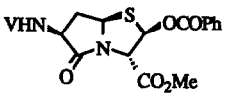


38, R = H
39, R = $CH_2C_6H_4NO_2$

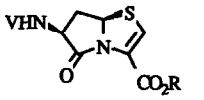


25

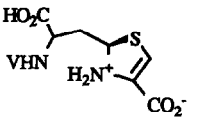
The analogue (20) was saponified (LiOH/THF/H_2O) then dissolved in pH 7.6 50mM KH_2PO_4 - K_2HPO_4-KCl buffer to give a solution of (23). The carboxylate salt (23) could be re-esterified (DMF, ethyl iodide) to the corresponding ethyl ester (24) [74% from (20)], thereby proving the existence of the free carboxylate (23).



27

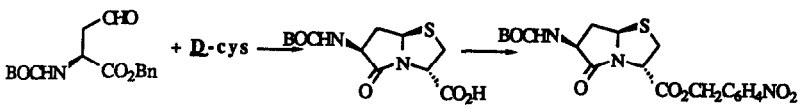


26, R = Me
28, R = Bn
29, R = K



30

The phenoxyacetamido ('V') side chain was introduced by deprotection (45% HBr/AcOH) of (12), followed by reacylation to give (25). The olefinic linkage was introduced as before to yield (26) via the benzoate (27). Hydrolysis of (26) gave the desired γ-lactam analogue (6) as the carboxylate which could be re-esterified to give (28).

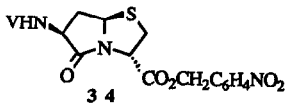


31 32 33

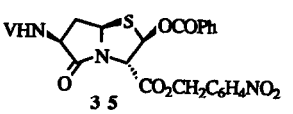
SCHEME 5

The final deprotection step proceeded in low yield, presumably due to hydrolysis of the bicyclic lactam to give (30). We therefore developed a modified synthesis of (6) using a p-nitrobenzyl protecting group for the carboxylic acid (Scheme 5).

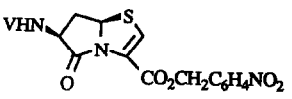
Condensation of aldehyde (31) with free D-cysteine gave the crude bicyclic lactam (32) which was esterified to the bicyclic ester (33). Functionalisation as before followed by deprotection [H_2/Pd/C/tetrahydrofuran /H_2O/$NaHCO_3$ (1 equiv.)] gave (6) cleanly. The analogue (6) showed weak, but real biological activity against Staphylococcus aureus. In contrast the saturated analogue (38) exhibited no biological activity.



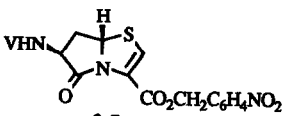
34



35



36



37

Acknowledgements We thank the SERC, for a studentship (to C.L.) and the British Council, for support (to R.T.F.)

Experimental

Standard chemical procedures as previously reported were employed.[5]

Melting points (m.p.) were determined on a Buchi 510 capillary apparatus and are uncorrected.

Infra red spectra were recorded as nujol mulls or in chloroform solution on Perkin-Elmer 681 and Perkin-Elmer 1750 Fourier Transform spectrometers. Absorption maxima were recorded in wavenumbers. The following abbreviations were used: s, strong; m, medium; w, weak; and b, broad.

Low resolution mass spectra (m/e) in the electron impact (E.I.) or chemical ionisation (C.I.) modes were recorded on a V.G. Micromass 16F spectrometer. Samples requiring desorption chemical ionisation (D.C.I.), in beam electron impact (I.B.E.I.) techniques and accurate mass measurements (E.I. or I.B.E.I.) were recorded on V.G. Micromass 30F or ZAB 1F spectrometers. The m/e values are quoted with the relative abundance (base ion = 100%) and assignment in parentheses. Only molecular ions (M^+), fragments of molecular ions and major peaks were reported.

Proton magnetic resonance spectra (^{1}H NMR) were recorded at 300 MHz, 500 MHz and 250 MHz on Bruker WH300, Bruker AM500 and Bruker AM250 spectrometers respectively. For ^{1}H NMR spectra recorded in $CDCl_3$ and acetone-d_6, chemical shifts (δH) were reported in parts per million (ppm) downfield of internal or external tetramethylsilane at 0.00 ppm. For ^{1}H NMR spectra recorded in D_2O, chemical shifts were reported in ppm downfield of sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP) at 0.00 ppm. The following abbreviations were used: s, singlet, d, doublet; t, triplet; q, quartet; m, multiplet and b, broad. Coupling constants were recorded in Hertz to the nearest 0.5 Hz.

Carbon magnetic resonance spectra (^{13}C NMR) were recorded at 62.5 MHz and 125 MHz on Bruker AM250 and Bruker AM500 spectrometers respectively. Chemical shifts (δ_C) are quoted in ppm and are referenced to $CDCl_3$ unless otherwise stated.

Relative assignments of stereochemistry of all products were made using nuclear Overhauser experiments - only selected data are reported and all values are approximate. α and β refer to the stereochemistry as drawn in the diagrams.

Thin layer chromatography (t.l.c.) was performed using Merck aluminium foil backed plates, pre-coated with Kieselgel 60 GF_{254} (No. 5554). Plates were developed by spraying with either 3% w/v dodeca-phosphomolybdic acid in ethanol or 10% w/v ammonium molybdate in 2N sulphuric acid; followed by warming to 60°C. Preparative layer chromatography (PLC) was performed using 200 x 200 x 1 mm layers of Merck Kieselgel 60 GF_{254} (No. 7730). Flash column chromatography was carried out using Merck Kieselgel 60 GF_{254}, 230-400 mesh.

Test for stability of the aldehyde (8)

The aldehyde (8)[11] (200 mg, 0.59 mmol) was stirred in pyridine (20 ml) at 20°C over ca 3 h and was re-isolated by chromatography to give [flash silica (20 g), diethyl ether] (8) (175 mg, 88%), identical by ^{1}H NMR (300 MHz) and optical rotation, ($[\alpha]_D^{25}$ + 6.8°, c = 0.5) to the starting material, ($[\alpha]_D^{25}$ + 6.3°, c = 0.5).

D-Cysteine methyl ester hydrochloride

D-Cysteine hydrochloride monohydrate (2.00 g, 11.4 mmol) was dissolved in dry distilled methanol (100 ml), and HCl gas was bubbled through the solution for ca 2 h, whilst stirring at room temperature (20°C). Stirring was then continued for a further 20 h. The solvent was evaporated in vacuo to yield an oil, which, upon tituration with diethyl ether, yielded the title compound (2.15 g, 99%) as white crystals; m.p. 140-141°C, (Lit.[14], 140-141°C); $[\alpha]_D^{25}$ + 2.3° (CH_3OH, c=10.3), (Lit.[14], $[\alpha]_D^{20}$ + 2.1° (CH_3OH, c=10); ν_{max} (nujol) 1740 s, 1580 m, 1513 s, 1378 s, 1335 m, 1248 m, 1220 m, 1077 m; δ_H (300 MHz, D_2O) 3.00 (2H, ca dd, J 6, 5 Hz, 3-H), 3.72 (3H, s, OCH_3), 4.27 (1H, ca t, J 6 Hz, 2-H); δ_C (62.5 MHz, CD_3OD) 25.1 (t, CH_2S), 53.9 (q, OCH_3), 55.8 (d, $CHCO_2$); m/e (NH_3, D.C.I.) 136 (MH^+, 100%); [Found C, 27.98%; H, 5.95%; N, 8.15%. $C_4H_{10}NO_2SCl$ requires C, 27.99%; H, 5.83%; N, 8.16%].

(2R,4S and 2S,4S)-[(2S)-2-(Benzyloxycarbonylaminopropanoic acid benzyl ester)]-3-aza-4-(methoxycarbonyl)tetrahydrothiophene (9c and 9d) - From D-cysteine

D-Cysteine methyl ester hydrochloride monohydrate (54.0 mg, 0.29 mmol) and the aldehyde (8) (96.4 mg, 0.28 mmol) were dissolved in pyridine (2.5 ml) and stirred under nitrogen at room temperature (20°C) for 18 h. The excess pyridine was evaporated *in vacuo* (0.1 mmHg) and the residue partitioned between diethyl ether (20 ml) and water (20 ml). The organic layer was washed with brine (20 ml), dried (sodium sulphate) and evaporated *in vacuo* to yield the thiazolidines (9c and 9d) (116 mg, 90%) as an oil; t.l.c. [diethyl ether] showed two spots, R_f 0.6 which equilibrated on silica, corresponding to an epimeric mixture of the two thiazolidines (9c,d); δ_H (300 MHz, $CDCl_3$) 2.83-2.88 (2H, m, CH_2S), 3.26-3.28 (2H, m, CH_2S), 3.76 (3H, s, OCH_3), 5.10 (2H, s, $PhCH_2$), 5.11 (2H, s, $PhCH_2$), 5.71 (1H, d, *J* 7 Hz, NH), 6.22 (1H, d, *J* 7 Hz, CONH), 7.29-7.36 (10H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; m/e (NH_3, C.I.) 459 (MH^+, 100%), 351 (65), 108 (22), 91 (48).

(2R,4R and 2S,4R)-[(2S)-2-(Benzyloxycarbonylaminopropanoic acid benzyl ester)]-3-aza-4-(methoxycarbonyl)tetrahydrothiophene (9a and 9b) - From L-cysteine.

Prepared by a procedure analogous to that used for the preparation of 9c and 9d. Rapid chromatographic separation on silica gel could separate the epimers to a reasonable extent, but they equilibrated rapidly in $CDCl_3$ at 20°C. Selected ^1H NMR data: δ_H (300 MHz, $CDCl_3$) one epimer showed peaks at 2.86 (1H, dd, *J* 9, 11 Hz, CH_2S) and 3.29 (1H, dd, *J* 7, 11 Hz, CH_2S) and the other epimer showed peaks at 2.93 (1H, dd, *J* 9, 11 Hz, CH_2S) and 3.25 (1H, dd, *J* 7, 11 Hz, CH_2S).

(2S,5R,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid methyl ester (12) - From D-cysteine

D-Cysteine methyl ester hydrochloride monohydrate (80.0 mg, 0.42 mmol) and the aldehyde (8) (146 mg, 0.43 mmol) were dissolved in pyridine (3.5 ml) and stirred for *ca* 3 h, at 20°C, then refluxed for 15 h under an inert atmosphere. The excess pyridine was evaporated *in vacuo* (0.1 mmHg) and the residue partitioned between diethyl ether (30 ml) and water (30 ml). The organic layer was washed with brine (30 ml), dried (sodium sulphate) and evaporated *in vacuo* to yield an oil. The crude product was purified by chromatography [(flash silica, 10 g), diethyl ether] to yield (12) (55.5 mg, 38%) as an oil; $[\alpha]_D^{\circ}$ + 175.3° ($CHCl_3$, c=0.6); t.l.c. [diethyl ether] R_f 0.35; ν_{max} ($CHCl_3$) 3026 m, 3013 m, 2956 m, 1715 bs (CO), 1512 s, 1455 m, 1438 m, 1407 s, 1341 m, 1279 m, 1223 s, 1171 m, 1132 m, 865 m; δ_H (250 MHz, $CDCl_3$) 2.03-2.12 (1H, m, 6-H), 3.19-3.27 (1H, m, 6-H), 3.36-3.42 (2H, m, 3-H), 3.78 (3H, s, OCH_3), 4.63-4.74 (1H, m, 7-H), 5.11-5.20 (4H, m, $PhCH_2$, 2-H, 5-H), 5.37 (1H, bd, *J* 5 Hz, NH), 7.32-7.40 (5H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; [Selected n.O.e. data: irradiation at 5.11-5.20 (5-H) showed enhancements at 2.03-2.12 (6 -αH) = 5% and 3.36-3.42 (3-αH) = 3%. Irradiation at 2.03 - 2.12 (6 -αH) showed enhancements at 3.19-3.27 (6 - βH) = 30% and 4.63-4.74 (7 - H) = 8%]; δ_C (62.5 MHz, $CDCl_3$) 35.0 (t, 3-C), 38.5 (t, 6-C), 52.9 (q, OCH_3), 54.6, 57.7, 61.7 (3 x d, 2-C, 5-C, 7-C), 67.1 (t, $PhCH_2$), 128.1, 128.2, 128.5 (3 x d, ArC), 136.0 (s, phenyl-*ipso*-C), 155.9 (s, CO), 169.4 (s, CO), 171.1 (s, CO); m/e (I.B.E.I.), 350 (M^+, 10%), 259 (M^+-$CH_2C_6H_5$, 22), 156 (20), 91 (100), 86 (25); [Found C, 54.71%; H, 5.38%; N, 8.04%. $C_{16}H_{18}N_2O_5S$ requires C, 54.86%; H, 5.18%; N, 8.00%].

(2S,5R,7R)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid methyl ester (13) and (2R,5S,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo [3.3.0] octane-2-carboxylic acid methyl ester (10) From D-cysteine

Prepared by a procedure analogous to that used for the preparation of (12). Yield [143 mg, 5% from (8) (3.00 g, 8.79 mmol)]; $[\alpha]_D^{\circ}$ + 168° ($CHCl_3$, c=1.13); t.l.c. [diethyl ether] R_f 0.5; δ_H (300 MHz, $CDCl_3$) 2.40-2.50 (1H, m, 6-H), 2.69-2.78 (1H, m, 6-H), 3.36-3.54 (2H, m, 3-H), 3.78 (3H, s, OCH_3), 4.42-4.50 (1H, m, 7-H), 5.06 (1H, dd, *J* 9, 5 Hz, 2-H), 5.12 (2H, s, $PhCH_2$), 5.18 (1H, *ca* d, *J* 7 Hz, 5-H), 5.26 (1H, bd, *J* 5 Hz, NH), 7.30-7.39 (5H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; [Selected n.O.e. data: irradiation at 5.18 (5-H) showed enhancements at 2.40-2.50 (6 -βH) = 8%. Irradiation at 2.40-2.50 (6 -αH) showed enhancements at 5.18

(5-H) and 2.67-2.78 (6 - βH) = 15%. Irradiation at 2.67 - 2.78 (6 - αH) showed enhancement at 2.40 - 2.50 (6 - βH) = >15% and 4.42-4.50 (7 - H) = 20%]; m/e (NH_3, D.C.I.) 368 (MNH_4^+, 98%), 351 (MH^+, 50%), 260 (100), 243 (27), 108 (10).

(2R,5S,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid methyl ester (10) From L-cysteine

Prepared by a procedure analogous to that used for the preparation of (12), using the aldehyde (8) (49.0 mg, 0.14 mmol) and L-cysteine methyl ester hydrochloride (26.0 mg, 0.15 mmol). Yield [22 mg, 45% from (8)]; $[\alpha]_D^{25}$ - 208° ($CHCl_3$, c=1.48) ν_{max} ($CHCl_3$) 2956 m, 1715 bs (CO), 1510 s, 1439 m, 1400 m, 1300 s, 1222 s, 1060 m; t.l.c. [diethyl ether] R_f 0.5; δ_H (300 MHz, $CDCl_3$) 2.41-2.48 (1H, m, 6-H), 2.71-2.74 (1H, m, 6-H), 3.37-3.53 (2H, m, 3-H), 3.79 (3H, s, OCH_3), 4.44-4.48 (1H, m, 7-H), 5.07 (1H, dd, J 8.5, 4.5 Hz, 2-H), 5.14 (1H, s, $PhCH_2$), 5.19 (1H, d, J 7 Hz, 5-H), 5.27 (1H, bd, J 5 Hz, NH), 7.32-7.39 (5H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13]; δ_C (62.5 MHz, $CDCl_3$) 29.9 (t, 3-C), 36.9 (t, 6-C), 52.8 (q, OCH_3), 52.1, 58.4, 63.8 (3 x d, 2-C, 5-C, 7-C), 67.1 (t, $PhCH_2$), 128.05, 128.10, 128.43 (3 x d, Ar), 136.0 (s, phenyl-ipso-C), 155.9 (s,CO), 170.0 (s,CO), 174.7 (s,CO); m/e (NH_3, D.C.I.) 368 (MNH_4^+, 50%), 351 (MH^+, 92%), 260 (100), 243 (48) 217 (22), 108 (18), 91 (12); [Found C, 54.66%; H, 5.21%; N, 7.87%. $C_{16}H_{18}N_2O_5S$ requires C,54.86%; H, 5.18%; N, 8.00%].

(2S,5R,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid methyl ester (12) and (2R,5S,7R)-1Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo [3.3.0] octane-2-carboxylic acid methyl ester (11) - From L- cysteine

Prepared by a procedure analogous to that used for the preparation of (12). Yield [2.0 mg, 4% from (8) (49.0 mg, 0.14 mmol)]; $[\alpha]_D^{25}$ - 117° ($CHCl_3$, c=0.11); t.l.c. [diethyl ether] R_f 0.35; δ_H(300 MHz, $CDCl_3$) 2.04-2.11 (1H, m, 6-H), 3.20-3.27 (1H, m, 6-H), 3.36-3.41 (2H, m, 3-H), 3.79 (3H, s, OCH_3), 4.63-4.73 (1H, m, 7-H), 5.12-5.19 (4H, m, $PhCH_2$, 2-H, 5-H), 5.38 (1H, bd, J 5 Hz, NH), 7.33-7.42 (5H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated.[13]

(2S,5R,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid(38)

The saturated bicyclic lactam (12) (25.0 mg, 7.14 x 10^{-2} mmol) was dissolved in tetrahydrofuran: water (3:1) (1.0 ml), and cooled to ca 4°C. Lithium hydroxide monohydrate (7.0 mg, 0.17 mmol) was then added, and the reaction mixture stirred for 6 h at 5-10°C. The tetrahydrofuran was evaporated in vacuo, the residue dissolved in water and acidified (to ca pH 2) by dropwise addition of 2N hydrochloric acid. The solution was extracted into ethyl acetate (2 x 10 ml), the combined organic extracts were dried (sodium sulphate) and evaporated in vacuo to yield (38) (20.6 mg, 86%) as an oil; (38) showed no antibacterial activity against E.coli or S.aureus at a concentration of 100 mg ml^{-1}. ν_{max} 3432 bw (OH), 1718 bs (CO), 1507 m, 1420 m, 1282 m, 1225 m; δ_H (500 MHz acetone-d_6) 2.12-2.18 ($CHCl_3$) (1H, m, 6-H), 3.03-3.09 (1H, m, 6-H), 3.32-3.41 (2H, m, 3-H), 4.71-4.74 (1H, m, 7-H), 5.09-5.15 (4H, m, $PhCH_2$, 2-H, 5-H), 6.71 (1H, bd, J 8 Hz, NH), 7.28-7.36 (5H, m, ArH); m/e (I.B.E.I.) 336 (M^+, 8%), 245 ($M^+-CH_2C_6H_5$, 30), 91 (100).

(2S,5R,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid p-nitrobenzyl ester (39)

The free acid (38) (13 mg, 3.9 x 10^{-2} mmol) was dissolved in dry, distilled dimethylformamide (0.5 ml). Sodium hydrogen carbonate (3.3 mg, 3.9 x 10^{-2} mmol) was then added, followed by p-nitrobenzyl bromide (9.2 mg, 4.3 x 10^{-2} mmol) and a catalytic amount of sodium iodide (<1 mg). This mixture was stirred for ca 16 h at room temperature (20°C), quenched with water (5 ml) and extracted into ethyl acetate (3 x 5 ml). The combined organic layers were washed with brine (10 ml), dried (sodium sulphate) and evaporated in vacuo to yield crude (39). This residue was purified by PLC [diethyl ether: acetic acid (99:1)] R_f 0.4 to yield (39) (12 mg, 67%); ν_{max} ($CHCl_3$) 3026 m, 1721 bs (CO), 1609 m, 1526 s, 1456 m, 1350 m; δ_H (500 MHz, $CDCl_3$) 2.07-2.13 (1H, m, 6-H), 3.19-3.23 (1H, m, 6-H), 3.47-3.50 (2H, m, 3-H), 4.65-4.70 (1H, m, 7-H), 5.11-5.13 (3H, m

$PhCH_2$, 5-H), 5.24-5.26 (1H, dd, J 6, 5 Hz, 2-H), 5.29 (2H, s, $CH_2C_6H_4NO_2$), 5.34 (1H, bs, NH), 7.32-7.39 (5H, m, C_6H_5), 7.51 [2H, d, J 8.5 Hz, $C_6H_4NO_2$ (o-H)], 8.25 [2H, d, J 8.5 Hz, $C_6H_4NO_2$ (m-H)]. A Jeneer experiment was consistent with the connectivity as indicated[13] ; m/e (I.B.E.I.) 471 (M^+, 3%), 380 ($M^+-CH_2C_6H_5$, 15), 91 (100).

(2R,3S,5S,7S)-1-Aza-3-benzoyloxy-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0] octane-2-carboxylic acid methyl ester (18) and (2R,3R,5S,7S)-1-Aza-3-benzoyloxy-7-benzyloxycarbonyl-amino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid methyl ester (19)

(10) (36 mg, 0.10 mmol) was dissolved in dry benzene (10 ml) and benzoyl peroxide (68 mg, 2.81 mmol) was added. The mixture was refluxed for 1.5 h, evaporated to dryness and the residue purified by chromatography [(flash silica 25 g), diethyl ether] to yield (18) (14 mg, 30%) and 19 (4 mg, 9%) as colourles oils. For (18): t.l.c. (diethyl ether) R_f 0.45; δ_H (300 MHz, $CDCl_3$) 2.52-2.64 (1H, m, 6-H), 2.71-2.84 (1H, m, 6-H), 3.85 (3H, s, OCH_3), 4.40-4.49 (1H, m, 7-H), 5.14 (2H, ca s, CH_2Ph), 5.36 (1H, br s, NH), 5.47 (1H, d, J 7 Hz, 5-H), 5.63 (1H, s, 2-H), 6.73 (1H, s, 3-H), 7.28-7.39 (5H, m, ArH) 7.45 (2H, t, J 7.5 Hz, ArH), 7.59 (1H, t, J 7.5 Hz, ArH), 7.93 (2H, d, J 7 Hz, ArH); m/e (NH_3, D.C.I.) 488 (MNH_4^+, 7%), 471 (MH^+, 2), 366 (MNH_4^+ - $PhCO_2H$, 76), 349 (MH^+ - $PhCO_2H$, 100). For (19): t.l.c. [diethyl ether] R_f 0.5; δ_H (300 MHz, $CDCl_3$) 2.52-2.59 (1H, m, 6-H), 2.77-2.84 (1H, m, 6-H), 3.71 (3H, s, OCH_3), 4.45-4.48 (1H, m, 7-H), 5.15 (2H, s, OCH_2Ph), 5.27 (1H, d, J 7 Hz, 2-H), 5.31 (1H, br s, NH) 5.64 (1H, d, J 6 Hz, 5-H), 7.02 (1H, d, J 7 Hz, 3-H), 7.37 (5H, m, ArH), 7.45 (2H, t, J 7.5 Hz, ArH), 7.61 (1H, t, J 7.5 Hz, ArH), 7.96 (2H, d, J 7 Hz, ArH); m/e (NH_3, D.C.I.) 488 (MNH_4^+, 5%), 471 (MH^+, 3%), 366 (MNH_4^+ -$PhCO_2H$, 48), 349 (MH^+ - $PhCO_2H$, 100).

(2S,3R,5R,7S)-1-Aza-3-benzoyloxy-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0] octane-2-carboxylic acid methyl ester (17)

The bicyclic compound (12) (505 mg, 1.07 mmol) was dissolved in dry benzene (20 ml). Benzoyl peroxide (1.50 g, 6.20 mmol) and cupric acetoacetate (45.0 mg, 0.17 mmol) were added to a solution, which was then stirred at 55°C for 2 h. The benzene was evaporated in vacuo and the residue purified by chromatography [(flash silica, 200 g), ethyl acetate: 40-60 petroleum ether (50:50)] to yield (17) (286 mg, 57%) as an oil; t.l.c. [diethyl ether] R_f 0.5; ν_{max} ($CHCl_3$) 1758 m, 1726 s, 1507 m, 1453 m, 1400 m, 1263 m, 1225 s, 1090 m, 1068 m, 1026 m, 909 m; δ_H (500 MHz, $CDCl_3$) 2.12-2.17 (1H, m, 6-C), 3.31-3.34 (1H, m, 6-H), 3.84 (3H, s, OCH_3), 4.73-4.78 (1H, m, 7-H), 5.12 (2H, s, $PhCH_2$), 5.30 (1H, ca dd, J 7, 6.5 Hz), 5.39 (1H, d, NH), 5.66 (1H, s, 3-H), 6.74 (1H, s, 2-H), 7.30-7.62 (8H, m, ArH), 7.95 [2H, d, J 8 Hz, OPh (o-H)]; δ_C (125 MHz, $CDCl_3$) 41.0 (t, 6-C), 53.4 (q, OCH_3), 53.9, 61.9, 64.7 (3 x d, 2-C, 5-C, 7-C), 67.2 (t, $PhCH_2$), 83.2 (d, 3-C), 128.1, 128.3, 128.4, 128.6, 128.7, 129.8 (6 x d, Ar), 133.8 (s, phenyl-ipso-C), 135.9 (s, phenyl-ipso-C), 156.0 (s, CO), 164.9 (s, CO), 166.3 (s, CO), 172.5 (s, CO); m/e (NH_3, D.C.I.) 488 (MNH_4^+, 3%), 471 (MH^+, 1%), 366 (MNH_4^+ - $C_6H_5CO_2H$, 42), 349 (MH^+ -$C_6H_5CO_2H$, 100), 108 (20).

Preparation of (20) and (5S,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0] oct-2-ene-2-carboxylic acid methyl ester (21) from (18).

(18) (50 mg, 0.11 mmol) was dissolved in N,N'-dimethylaniline (10 ml) and the solution refluxed for 25 minutes. Work up as for the preparation of (20), followed by chromatography [(flash silica), diethyl ether: ethyl acetate (3:1)] gave (20) (16 mg, 42%) and (21) (3.5 mg, 9%) as colourless oils. For (21): t.l.c. [diethyl ether: ethyl acetate (3:1)] R_f 0.55; δ_H (300 MHz, $CDCl_3$) 2.55-2.61 (1H, m, 6-H), 3.00-3.03 (1H, m, 6-H), 3.85 (3H, s, OCH_3), 4.61-4.64 (1H, m, 7-H), 5.14 (2H, m, $PhCH_2O$), 5.34 (1H, br s, NH), 5.98-6.10 (1H, m, 5-H), 7.33 (1H, s, 3-H), 7.29-7.42 (9H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; m/e (I.B.E.I.) 348 (M^+, 7%), 234 (23), 91 (100).

(5R,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]oct-2-ene-2-carboxylic acid methyl ester (20)

(17) (220 mg, 0.47 mmol) was dissolved in N,N'-dimethylaniline (30 ml) and refluxed at 194°C under nitrogen for 45 min. The reaction mixture was partitioned between ethyl

acetate (100 ml) and 2N hydrochloric acid (100 ml), the organic layer washed with 2N hydrochloric acid (2 x 100 ml) and sodium bicarbonate solution (100 ml), dried (sodium sulphate) and evaporated *in vacuo* to yield an oil. The crude product was purified by chromatography [(flash silica, 40 g), diethyl ether: ethyl acetate (3:1)] to yield the olefin (20) (60.5 mg, 37%) as an oil; $[\alpha]_D^{\circ}$ + 84.3° ($CHCl_3$, c=0.195); t.l.c. [diethyl ether: ethyl acetate (3:1)] R_f 0.5 ν_{max} ($CHCl_3$) 1739 s (lactam CO), 1721 s (ester CO), 1671 m (urethane CO), 1510 m, 1440 m, 1375 m, 1273 m, 1222 s, 1081 m; δ_H (500 MHz, $CDCl_3$) 2.42-2.47 (1H, m, 6-H), 3.34-3.40 (1H, m, 6-H), 3.84 (3H, s, OCH_3), 4.64-4.69 (1H, m, 7-H), 5.15 (2H, s, $PhCH_2$), 5.51 (1H, bs, NH), 5.91-5.95 (1H, *ca* dd, *J* 8, 6.5 Hz, 5-H), 7.21 (1H, s, 3-H), 7.34-7.40 (5H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; δ_C (125 MHz, $CDCl_3$) 37.9 (t, 6-C) 52.3 (q, OCH_3), 54.0 (d, 7-C), 67.2 (t, $PhCH_2$), 68.1 (d, 5-C), 126.3 (d, 3-C), 130.8 (s, 2-C), 128.1, 128.4 , 128.6 (3 x d, Ar), 136.0 (s, phenyl-*ipso*-C), 156.0 (s, CO), 158.5 (s, CO), 172.2 (s, CO); m/e (I.B.E.I.) 348 (M^+, 8%), 224 (26), 91 (100).

(5R,7S)-1-Aza-7-benzyloxycarbonylamino-8-oxo-4-thiabicyclo[3.0]oct-2-ene-carboxylic acid ethyl ester (24)

Aqueous lithium hydroxide monohydrate solution (0.29 M, 0.1 ml, 2.9 x 10^{-2} mmol) was added to a solution of the olefin (20) (9.0 mg, 2.6 x 10^{-2} mmol) in tetrahydrofuran (0.3 ml) and stirred at 0°C for 30 minutes. The solvent was evaporated *in vacuo*, the residue dried thoroughly on a high vacuum line (*ca* 1 mmHg) and then redissolved in dry, distilled dimethylformamide (0.3 ml). Ethyl iodide (3.0 µl, 3.8 x 10^{-2} mmol) was added and the reaction mixture stirred at 20°C for 20 h. The reaction was quenched with water (5 ml) and extracted with ethyl acetate (3 x 10 ml). The combined organic layers were washed with brine (10 ml), dried (sodium sulphate) and evaporated *in vacuo* to yield an oil which was purified by PLC [diethyl ether: acetic acid (99:1)] R_f 0.5 to yield (24) (5.6 mg, 60%) as an oil; ν_{max} ($CHCl_3$) 1727 bs (CO), 1603 m, 1514 m; δ_H (500 MHz, $CDCl_3$) 1.31 (3H, t, *J* 7 Hz, OCH_2CH_3), 2.41-2.45 (1H, m, 6-H), 3.34-3.39 (1H, m, 6-H), 4.27-4.32 (2H, q, *J* 7 Hz, OCH_2CH_3), 4,63-4.68 (1H, m, 7-H), 5.13 (2H, s, $PhCH_2$), 5.45 (1H, bs, NH), 5.91 (1H, *ca* dd, *J*, 7 6 Hz, 5-H), 7.18 (1H, s, 3-H), 7.18 (1H, s, 3-H), 7.34-7.38 (5H, m, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ;m/e (I.B.E.I.) 362 (M^+, 6%), 91 (100).

(2S,5R,7S)-1-Aza-7-phenoxymethylamido-8-oxo-4-thiabicyclo[3.0]-2-carboxylic acid methyl ester (25)

The bicyclic lactam (12) (394 mg, 1.12 mmol) was dissolved in dry dichloromethane (4.0 ml) and cooled to 0°C in an ice-water bath. A solution of hydrogen bromide in acetic acid (45% w/v, *ca* 2 ml) was added and a calcium chloride drying tube attached to the reation vessel to exclude atmospheric moisture. The reaction mixture was allowed to warm to 20°C and stirred until evolution of carbon dioxide had ceased (*ca* 0.5 h) and for a further 0.5 h. The solvent was then evaporated *in vacuo* (<2 mmHg) and the residue redissolved in dry dichloromethane (4.0 ml). Dry triethylamine (235 µl, 1.68 mmol) was added to the solution at 0°C, after which phenoxyacetyl chloride (170 µl, 1.23 mmol) and triethylamine (160 µl, 1.15 mmol) were added simultaneously to the solution (over *ca* 15 min) and the reaction mixture stirred for a further 4 h at room temperature. Dichloromethane (10 ml) was then added and the resultant solution was washed with 1N hydrochloric acid (10 ml) and aqueous sodium hydrogen carbonate solution (5% w/v 10 ml), dried (sodium sulphate) and evaporated *in vacuo* to yield the crude product which was purified by chromatography [(flash silica, 400 g), diethyl ether:ethyl acetate (1:1)] to yield (25) (260 mg, 66%) as an oil; t.l.c. [diethyl ether:ethyl acetate (1:1)] R_f 0.5; ν_{max} ($CHCl_3$) 1756 m, 1718 s, 1685 s, 1600 m, 1522 m, 1496 m, 1442 m, 1405 m; δ_H (500 MHz, $CDCl_3$) 2.03-2.10 (1H, m, 6-H), 3.24-3.29 (1H, m, 6-H), 3.37-3.43 (2H, m, 3-H), 3.79 (3H, s, OCH_3), 4.53, 4.54 (2H, ABq, *J* 16 Hz, $PhOCH_2$), 4.90-4.96 (1H, m, 7-H), 5.16-5.21 (2H, m, 2-H, 5-H), 6.94 [2H, d, *J* 8 Hz, OPh (*o*-H)], 7.04 [1H, t, *J* 7.5 Hz, OPh (*p*-H)], 7.16 (1H, d, *J* 6 Hz, NH), 7.33 [2H, dd, *J* 8.5, 7.5 Hz, OPh (*m*-H]. A Jeneer experiment was consistent with the connectivity as indicated[13] ; δ_C (125 MHz, $CDCl_3$) 35.0 (t, 3-C), 38.0 (t, 6-C), 52.8 (q, OCH_3), 52.9, 57.8, 61.8 (3 x d, 2-C, 5-C, 7-C), 67.3 (t, $PhOCH_2$) 114.8 [d, OPh (*o*-C)], 122.2 [d, OPh (*p*-C)], 129.7 (d, OPh (*m*-C)], 157.1 (s, phenyl-*ipso*-C), 168.6 (s,

CO), 169.4 (s, CO), 170.9 (s, CO); m/e (I.B.E.I.) 350 (M^+, 100%), 257 ($M^+ - OC_6H_5$, 18), 215 ($M^+ - C_6H_5OCH_2CO$, 27), 199 (70), 107 (43), 86 (50), 77 (92), 59 (25).

(2S,3R,5R,7S)-1-Aza-3-benzoyloxy-7-phenoxymethylamido-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid methyl ester (27)

The bicyclic lactam (25) (750 mg, 2.14 mmol) was dissolved in dry benzene (2.0 ml). To this solution was added dry benzoyl peroxide (1.84 g, 7.60 mmol) and cupric acetoacetate (50.0 mg, 0.19 mmol), and the mixture stirred under nitrogen at 70°C for 2 h. The solvent was evaporated in vacuo and the residue purified by chromatography [(flash silica, 200 g), diethyl ether] to yield (27) (344 mg, 34%) as an oil; ν_{max} ($CHCl_3$), 1729 s, 1695 s, 1603 m, 1496m, 1452 m, 1375 m; δ_H (500 MHz, $CDCl_3$) 2.11-2.17 (1H, m, 6-H), 3.38-3.43 (1H, m, 6-H), 4.52, 4.53 (2H, ABq, J 15 Hz, $PhOCH_2$), 5.01-5.06 (1H, m, 7-H), 5.35 (1H, dd, J 7, 6 Hz, 5-H), 5.69 (1H, s, 2-H), 6.75 (1H, s, 3-H), 6.87 [2H, d, J 9 Hz, OPh (o-H)], 7.03 [1H, t, J 7.5 Hz, OPh (p-H)], 7.22 (1H, d, J 6 Hz, NH), 7.27-7.64 (8H, m, PhO, PhCOO [m-H, p-H]), 7.96 (2H, d, J 8.5 Hz, PhCOO [o-H]; m/e (NH_3, D.C.I.) 471 (MH^+, 2%) 366 ($MNH_4^+ - PhCO_2H$, 30), 349 ($MH^+ - PhCO_2H$).

(5R,7S)-1-Aza-7-phenoxymethylamido-8-oxo-4-thiabicyclo[3.3.0]oct-2-ene-2-carboxylic acid methyl ester (26)

The benzoate (27) (280 mg, 0.60 mmol) was dissolved in N,N'-dimethylaniline (50 ml) and refluxed under a nitrogen atmosphere for 45 minutes, then allowed to cool to 20°C. The reaction mixture was dissolved in ethyl acetate (100 ml) and washed with 2N hydrochloric acid (4 x 100 ml) to remove all traces of N,N'-dimethylaniline. The organic layer was then washed with aqueous sodium hydrogen carbonate (solution 100 ml), dried (sodium sulphate) and evaporated in vacuo to yield the crude product. This was purified by chromatography [(flash silica, 20 g), diethyl ether: ethyl acetate (1:1)] to yield (26) (76 mg, 37%) as an oil; t.l.c. [diethyl ether: ethyl acetate (1:1)] R_f 0.5; ν_{max} ($CHCl_3$) 1740 s, 1719 s, 1685 s, 1601 s, 1522 m, 1496 m, 1441 m, 1383 m, 1325 m; δ_H (500 MHz, $CDCl_3$) 2.40-2.47 (1H, m, 6-H), 3.44-3.49 (1H, m, 6-H), 3.84 (3H, s, OCH_3), 4.55 (2H, s, $PhOCH_2$), 4.86-4.91 (1H, m, 7-H), 5.97 (1H, dd, J 7.5, 6 Hz, 5-H), 6.94 [2H, ca d, J 8 Hz, OPh (o-H)], 7.04 [1H, ca t, J 7.5 Hz, OPh (p-H)], 7.22 (1H, s, 3-H), 7.33 [2H, ca dd, J 8.5, 7.5 Hz, OPh (m-H)]. A Jeneer experiment was consistent with the connectivity as indicated [13]; [Selected n.O.e. data: irradiation at 2.40-2.47 (6-βH) showed enhancement at 3.44-3.69 (6-αH) = 26%. Irradiation at 3.44-3.69 (6-αH) showed enhancements at 2.40-2.47 (6-βH) = 28%, 4.86-4.91 (7-H) = 10% and 5.97 (5-H) = 18%. Irradiation at 4.86-4.91 (7-H) showed enhancements at 2.40-2.47 (6-βH) < 2%, 3.44-3.69 (6-αH) = 6%, and 5.97 (5-H) = 9%]; δ_C (125 MHz, $CDCl_3$) 37.6 (t, 6-C), 52.2 (q, OCH_3), 52.3 (d, 7-C), 67.3 (t, $PhOCH_2$), 68.3 (d, 5-C), 114.8 [d, OPh (o-C)], 122.3 [d, OPh (p-C)], 126.3 (s, 2-C), 127.1 (d, 3-C), 129.8 [d, OPh (m-C)], 157.1 (s, phenyl-ipso-C), 158.5 (s, CO), 168.9 (s, CO), 172.4 (s, CO); m/e (I.B.E.I) 348 (M^+, 24%), 44 (100), 107 (50), 77 (90).

(5R,7S)-1-Aza-7-phenoxymethylamido-8-oxo-4-thiabicyclo[3.3.0]oct-2-ene-2-carboxylic acid benzyl ester (28)

To a stirred solution of the olefin (26) (14.3 mg, 4.11 x 10^{-2} mmol) in tetrahydrofuran (500 µl) was added an aqueous solution of lithium hydroxide (0.24 M, 180 µl, 4.20 x 10^{-2} mmol). The pale yellow solution was stirred at 0°C for ca 30 minutes. The solvent was evaporated in vacuo to yield the crude carboxylate which was thoroughly dried on a high vacuum line. This was dissolved in dry, distilled dimethylformamide (450 µl); benzyl bromide (99% w/w) (12 µl, 0.10 mmol) and sodium iodide (ca 1 mg) were then added, and the solution stirred for ca 20 h under nitrogen at 20°C. The reaction was quenched with water (10 ml) and extracted into ethyl acetate (3 x 10 ml). The combined organic layers were washed with brine (20 ml), dried (sodium sulphate) and evaporated in vacuo to yield the crude product which was purified by PLC (diethyl ether) to yield (28) [6.3 mg, 36% from (26)] as an oil; ν_{max} ($CHCl_3$) 1740 s, 1711 s, 1685 s, 1598 m, 1522 m, 1497 s, 1442 m, 1363 m, 1242 m; δ_H (500 MHz, $CDCl_3$) 2.40-2.47 (1H, m, 6-H), 3.43-3.48 (1H, m, 6-H), 4.55 (2H, s, $PhOCH_2$), 4.86-4.91 (1H, m, 7-H), 5.28 (2H, s, $PhCH_2$), 5.94-5.97 (1H, dd, J 7.5, 6 Hz, 5-H), 6.96 [2H, ca d, J 8 Hz, OPh (o-H)], 7.04 (1H, ca t, J 7 Hz, OPh (p-H)], 7.21 (1H, ca d, J 8 Hz, NH), 7.25 (1H, s, 3-H), 7.28-7.42 (7H, m, Ar-H); m/e (I.B.E.I.) 424

(M^+, 10%), 333 ($M^+ - CH_2C_6H_5$, 30), 305 (22), 107 (23), 91 (100), 77 (28); m/e (NH_3, D.C.I.) 442 (MNH_4^+, 35%), 425 (MH^+, 100), 108 (40).

(2S,5R,7S)-1-Aza-7-t-butyloxycarbonylamino-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid p-nitrobenzyl ester (33)

Aldehyde (31) (2.78 g, 9.07 mmol) and D-cysteine hydrochloride monohydrate (1.59 g, 9.07 mmol) were dissolved in pyridine (30 ml) and refluxed for 16 h. The reaction mixture was then evaporated to dryness *in vacuo* and the residue partitioned between ethyl acetate (100 ml) and 5% aqueous sodium bicarbonate (100 ml); the aqueous phase was separated, acidified and re-extracted with ethyl acetate (2 x 100 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated *in vacuo* to give crude (32) (2.00 g, 6.62 mmol). The crude acid (32) was dissolved in dry dimethylformamide (50 ml), to which potassium bicarbonate (729 mg, 7.28 mmol), potassium iodide (*ca* 20 mg), sodium sulphate (*ca* 20 mg), and p-nitrobenzylbromide (1.57 g, 7.28 mmol), were added. The reaction mixture was stirred for 72 h, after which the solvent was removed *in vacuo* and the residue dissolved in ethyl acetate (100 ml), washed [5% aqueous sodium bicarbonate solution (100 ml) and brine (100 ml)], dried (sodium sulphate) and evaporated *in vacuo* to give (33) (2.89 g, 73%); $[\alpha]_D^{20}$ + 142.3° ($CHCl_3$, c = 1.5) t.l.c. [(diethyl ether)] R_f 0.45; ν_{max} ($CHCl_3$) 3430 m, 2980 s, 2880 m, 1750 m, 1710 s, 1610 m, 1525 s, 1350 s, 1160 s; δ_H (200 MHz, $CDCl_3$) 1.46 [9H, s, $(CH_3)_3C$], 1.95-2.10 (1H, m, 6-H), 3.10-3.26 (1H, m, 6-H), 3.35 (2H, *ca* d, J 6 Hz, 3-H), 4.59-4.70 (1H, m, 7-H), 5.10 (1H, t, J 7 Hz, 2-H), 5.23 (1H, t, J 5 Hz, 5-H), 5.30 (2H, s, CH_2Ar), 7.52, 8.26 (4H, A_2B_2, J 8.5 Hz, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; [Selected n.O.e. data: irradiation at 1.95-2.10 (6-βH) showed enhancement at 3.10-3.26 (6-αH) = 24%. Irradiation at 3.10-3.26 (6-αH) showed enhancements at 1.95-2.10 (6-βH) = 29%, 4.59-4.70 (7-H) = 13%, and 5.23 (5-H) = 19%. Irradiation at 4.59-4.70 (7-H) showed enhancements at 3.10-3.26 (6-αH) = 7% and 5.23 (5-H) = 11%]; δ_C (62.5 MHz, $CDCl_3$) 28.1 [q, $(CH_3)_3C$] 34.9 (t, 3-C), 38.8 (t, 6-C), 54.2 (d, 7-C), 57.8 (d, 2-C), 61.7 (d, 5-C), 66.1 (t, CH_2Ar), 80.1 [s, $C(CH_3)_3$], 124.1 (d, ArC), 128.6 (d, ArC), 142.2 (s, ipso-ArC), 148.2 (s, ipso-ArC), 155.6 (s, CO), 168.9 (s, CO), 171.9 (s, CO); m/e (NH_3, D.C.I.) 455 (MNH_4^+, 17%), 48 (MH^+, 22), 399 (100). [Found C, 52.13; H, 5.40; N, 9.39%. $C_{20}H_{21}N_3SO_7$ requires C, 52.17; 3H, 5.26; N, 9.61%].

(2S,5R,7S)-1-Aza-7-phenoxymethylamido-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid p-nitrobenzyl ester (34)

(33) (2.89 g, 6.62 mmol) was dissolved in formic acid (25 ml) and stirred at 20°C for 3 h, after which the reaction mixture was evaporated to dryness. The residue was partitioned between ethyl acetate (100 ml) and 2N hydrochloric acid (3 x 100 ml). The aqueous extracts were combined, basified (to pH 10, with 2N sodium hydroxide) and extracted with ethyl acetate (3 x 50 ml). The organic extracts were dried (sodium sulphate) and evaporated *in vacuo* to give the crude amine as an oil. T.l.c. [(diethyl ether)] R_f 0.1; ν_{max} ($CHCl_3$) 3690 m, 3010 s, 1755 m, 1712 s, 1605 m, 1525 s, 1350 s, 1205 s, 718 s; δ_H (200 MHz, $CDCl_3$) 1.79 (2H, br s, NH_2), 1.80-1.91 (1H, m, 6-H), 2.91-3.05 (1H, m, 6-H), 3.35 (2H, d, J 5 Hz, 3-H), 3.80-3.90 (1H, m, 7-H), 5.05 (1H, *ca* t, J 6.5 Hz, 2-H), 5.17 (1H, t, J 5.5 Hz, 5-H), 5.25 (2H, s, CH_2Ar), 7.48, 8.19 (4H, A_2B_2, J 9 Hz, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; δ_C (62.5 MHz, $CDCl_3$) 35.1 (t, 3-C), 39.3 (t, 6-C), 55.2, 57.5, 61.7 (3 x d, 7-C, 2-C, 5-C), 65.9 (t, CH_2Ar), 124.0, 128.5 (2 x d, ArC), 142.3 (s, ipso-ArC), 147.9 (s, ipso-ArC), 169.2 (s, CO), 175.6 (s, CO); m/e (NH_3, D.C.I.) 355 (MNH_4^+, 7%), 338 (MH^+, 100).
The crude amine (1.49 g, 4.43 mmol) was dissolved in dichloromethane (30 ml), the solution cooled to 0°C and triethylamine (0.92 ml, 6.64 mmol) was added. Then further triethylamine (0.68 ml, 4.87 mmol) and phenoxyacetyl chloride (0.67 ml, 4.87 mmol) were added simultaneously over 15 minutes. The reaction mixture was allowed to warm to 20°C and stirred for 16 h. The solvent was removed *in vacuo*, the residue dissolved in ethyl acetate (100 ml) and washed [5% sodium bicarbonate solution (100 ml), 2N hydrochloric acid (100 ml) and brine (100 ml)], dried (magnesium sulphate) and evaporated *in vacuo*. The residue was chromatographed [(flash silica) ethyl acetate: diethyl ether (1:1)] to give

(34) (1.92 g, 92%); t.l.c. [diethyl ether: ethyl acetate (2:1)] R_f 0.6; ν_{max} ($CHCl_3$) 3690 m, 3410 w, 3010 s, 1755 s, 1720 s, 1685 s, 1525 s, 1350 s; δ_H (200 MHz, $CDCl_3$) 2.07-2.19 (1H, m, 6-H), 3.11-3.24 (1H, m, 6-H), 3.39 (2H, d, J 5 Hz, 3-H), 4.49 (2H, s, CH_2OPh), 4.90 -4.97 (1H, m, 7-H), 5.13 (1H, t, J 7 Hz, 5-H), 5.23-5.27 (3H, m, 2-H, CH_2Ar), 6.90 (2H, d, J 8 Hz, ArH), 7.00 (1H, t, J 8 Hz, ArH), 7.25-7.34 (3H, m, NH, ArH), 7.50, 8.21 (4H, A_2B_2, J 8 Hz, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13] ; (3H, m, NH, ArH) δ_C (62.5 MHz,$CDCl_3$) 34.9 (t, 3-C), 37.8 (t, 6-C), 52.7, 57.9, 61.7 (3 x d, 7-C, 2-C, 5-C), 66.1 (t, CH_2Ar), 67.1 (t, CH_2OPh), 114.8, 122.3, 124.0, 128.6, 129.9 (5 x d, ArC), 142.2 (s, ipso-ArC), 148.0 (s, ipso-ArC), 157.2 (s, ipso-ArC), 168.9 (s, CO), 171.4 (s, CO), 171.4 (s, CO); m/e (NH_3, D.C.I.) 489 (MNH_4^+, 5%), 472 (MH^+, 75), 357 (M^+- 134, 100).

(2S,3R,5R,7S)-1-Aza-3-benzyloxy-7-phenoxymethylamido-8-oxo-4-thiabicyclo[3.3.0]octane-2-carboxylic acid p-nitrobenzyl ester (35)

To (34) (1.28 g, 2.72 mmol) in benzene (40 ml) was added dry benzoyl peroxide (3.79 g, 16.3 mmol) and cupric acetoacetate and the solution stirred at 55°C for 1 h. The benzene was removed in vacuo and the residue purified by chromatography [flash silica (diethyl ether)] to give (35) (360 mg, 23%); t.l.c. [(diethyl ether)] R_f 0.3; ν_{max} ($CHCl_3$) 3420 w, 3020 m, 1770 m, 1755 s, 1740 s, 1730 s, 1685 s, 1605 s, 1525 s, 1495 s, 1350 s, δ_H (200 MHz, $CDCl_3$) 2.06-2.22 (1H, m, 6-H), 3.33-3.50 (1H, m, 6-H), 4.50 (2H, s, CH_2OPh), 4.95-5.08 (1H, m, 7-H), 5.28-5.48 (3H, m, 5-H, CH_2Ar), 5.75 (1H, s, 2-H), 6.75 (1H, s, 3-H), 6.87 (2H, d, J 9 Hz, ArH), 7.03 (1H, t, J 7.5 Hz, ArH), 7.16 (1H, d, J 6 Hz, NH), 7.25-7.42 (5H, m, ArH), 7.56, 8.26 (4H, A_2B_2 J 9 Hz, ArH), 7.91-7.95 (2H, m, J ArH); δ_C (62.5 MHz, $CDCl_3$) 40.3 (t, 6-C), 51.8 (d, 7-C), 62.1, 65.0 (2 x d, 5-C, 2-C), 67.2, 66.6 (2 x t, 2 x CH_2Ar), 83.0 (d, 3-C), 114.9, 122.5, 124.2, 128.3, 129.9, 134.1 (5 x d, 5 x ArC), 141.6 (s, ipso-ArC), 148.0 (s, ipso-ArC), 157.2 (s, ipso-ArC), 158.2 (s, ipso-ArC), 165.1 (s, CO), 165.8 (s, CO), 168.3 (s, CO), 172.9 (s, CO); m/e (NH_3, D.C.I.) 591 (MH^+, 1%), 487 (MNH_4^+ - $PhCO_2H$,40), 470 (MH^+ - $PhCO_2H$, 50).

(5R,7S)- and (5S,7S)1-Aza-7-phenoxymethylamido-8-oxo-4-thiabicyclo[3.3.0]oct-2-ene-2-carboxylic acid -nitrobenzyl ester (36) and (37)

A solution of 35 (360 mg, 0.61 mmol) in N,N'-dimethylaniline (30 ml) was refluxed for 45 minutes. The reaction mixture was cooled, diluted with ethyl acetate (100 ml), washed [2N hydrochloric acid (5 x 100 ml), 5% aqueous sodium carbonate (3 x 100 ml) and brine (100 ml)], dried (magnesium sulphate) and evaporated to dryness. The residue was chromatographed [flash silica, (diethyl ether)] to give (36) and (37) as a colourless oil. For (36): (136 mg, 47%) $[\alpha]_D^{\circ}$ + 75.4 t.l.c. (diethyl ether) R_f 0.30; ν_{max} ($CHCl_3$) 3690 m, 3625 m, 3040 s, 3020 s, 1720 s, 1690 s, 1604 m, 1525 s, 1500 s, 1482 m, 1325 s, 1220 s, 1050 s; δ_H (200 MHz, $CDCl_3$) 2.31 - 2.55 (1H, m, 6-H), 3.34-3.53 (1H, m, 6-H), 4.56 (2H, s, CH_2OPh), 4.86-4.99 (1H, m, 7-H), 5.30, 5.38 (2H, ABq, J 14 Hz, CH_2Ar), 5.95 (1H, t, J 7 Hz, 5-H), 6.92 (2H, d, J 8 Hz, ArH), 7.03 (1H, t, J 7 Hz, ArH), 7.28 (1H, s, 3-H), 7.33 (2H, t, J 9 Hz, ArH), 7.43 (1 H, d, J 7 Hz, NH), 7.56, 8.21 (4H, A_2B_2, J 9 Hz, ArH). A Jeneer experiment was consistent with the connectivity as indicated 13; [Selected n.O.e. data: irradiation at 3.34-3.53 (6-αH) showed enhancements at 2.31-2.55 (6-βH) = 21%; 4.86-4.99 (7-H) = 9%, and 5.95 (5-H) = 12%]. δ_C (62.5 MHz, $CDCl_3$) 37.3 (t, 6-C), 52.0 (d, 7-C), 65.7, 67.1 (2 x t, 2 x CH_2Ar), 68.4 (d, 5-C), 122.5, 123.9, 125.9, 128.7, 130.0 (5 x d, ArC), 132.7 (d, 3-C). 142.8, 147.9, 157.3 (3 x s, 3 x ipso-ArC), 158.0, 169.1, 172.9 (3 x s, 3 x CO); m/e (NH_3, D.C.I.) 470 (MH^+, 7%), 122 (MH^+ - 348, 100). For (37): (<5%); t.l.c. (diethyl ether) R_f 0.35; δ_H (200 MHz, $CDCl_3$), 2.50-2.66 (1H, m, 6-H), 3.05-3.18 (1H, m, 6-H), 4.55 (2H, s, CH_2OPh), 4.83-4.95 (1H, m, 7-H), 5.33, 5.43 (2H, ABq, J 13 Hz, CH_2CO_2), 6.35 (1H, ca d, J 5 Hz, 5-H), 6.93 (2H, d, J 7 Hz, ArH), 7.05 (1H, t, J 7 Hz, ArH), 7.23 (1H, d, J 7 Hz, NH), 7.27 (1H, s, 3-H), 7.38 (2H, t, J 7 Hz, ArH), 7.59, 8.23 (4H, A_2B_2, J 9 Hz, ArH); m/e (NH_3, D.C.I.) 470 (MH^+, 10%), 122 (MH^+ - 348, 100).

Preparation of (6) from (36)

To a solution of (36) (43 mg, 0.09 mmol) in tetrahydrofuran/water (3 ml, 3:5) was added potassium bicarbonate (9.1 mg, 0.09 mmol) and 10% palladium on charcoal (ca 400 mg).

The reaction mixture was hydrogenated at atmospheric pressure for 45 minutes, after which it was filtered (Celite) and evaporated to dryness in vacuo. The residue was partitioned between ethyl acetate (3 x 20 ml) and water (20 ml). The aqueous layer was evaporated in vacuo to yield (6) (13mg, 44%) δ_H (500 MHz, D_2O, TSP referenced) 2.35-2.41 (1H, m, 6-H), 2.87-2.93 (1H, m, 6-H), 4.50 (2H, s, CH_2OPh), 4.78 (1H, ca t, J 9 Hz, 7-H), 5.83 (1H, t, J 7 Hz, 5-H), 6.79 (1H, s, 3-H), 6.84 (2H, d, J 7 Hz, ArH), 6.90 (1H, t, J 7 Hz, ArH), 7.20 (2H, t, J 7 Hz, ArH). A Jeneer experiment was consistent with the connectivity as indicated[13]; [Selected n.O.e. data: irradiation at 2.87-2.93 (6-αH) showed enhancements at 2.35-2.41 (6-βH) = 32%, 4.78 (7-H) = 19%, and 5.83 (5-H) = 12%]; δ_C (125 MHz, D_2O) 28.0 (t, 6-C), 4.67 (d, 7-C), 60.5 (t, CH_2Ar), 61.9 (d, 5-C), 108.7, 116.0, 123.8 (3 x t, ArC), 119.8 (s, 3-C). 131.9, 132.4 (2 x s, ipso-ArC, 2-C), 150.9, 165.4, 167.20 (3 x s, 3 x CO); m/e (fast atom bombardment) 373 MH^+). (6) Showed ca 5% of the biological activity of penicillin G against S. aureus NC No. 6071.

1 This work has been published in a preliminary form: J.E. Baldwin, C. Lowe, C.J. Schofield, and E. Lee, Tetrahedron Lett., 1986, 3461.

2 After completion of this work we were informed by Dr. L.D. Hatfield (Eli Lilly & Co., Indianapolis, U.S.A.) that they had prepared examples of similar substances, see: D.B. Boyd, T.K. Elzey, L.D. Hatfield, M.D. Kinnick, and John M. Morin Jr., Tetrahedron Lett., 1986, 3453; D. Boyd, B.J. Foster, L.D. Hatfield, W.J. Hornback, N.D. Jones, J.E. Munroe, and J.K. Swartzendruber, ibid., 1986, 3457; L.N. Jungheim, S.K. Sigmund, and J.W. Fisher, ibid., 1987, 285; L.N. Jungheim, S.K. Sigmund, N. Jones, and J.K. Swartzendruber, ibid., 1987, 289. See also S. Hashiguchi, H. Natsugari, and M. Ochiai, J.Chem.Soc., Perkin Trans. 1, 1988, 2345.

3 E.F. Gale, E. Cundliffe, P.E. Reynolds, N.H. Richmond, and M.J. Waring, "The Molecular Basis of Antibiotic Action", John Wiley and Sons, Ltd., London, 1981, pp 79-136.

4 J.E. Baldwin, M.F. Chan, G. Gallacher, P. Monk, and K. Prout, J.Chem.Soc.,Chem. Commun., 1983, 250; J.E. Baldwin, M.F. Chan, G. Gallacher, P. Monk, and K. Prout, Tetrahedron, 1984, 40, 4513.

5 J.E. Baldwin, R.M. Adlington, R.H. Jones, C.J. Schofield, C. Zaracostas, C.W. Greengrass, J.Chem.Soc.Chem.Commun., 1985, 194; idem, Tetrahedron, 1986, 42, 4879.

6 V. Vigneaud, and F.H. Carpenter, in "The Chemistry of Penicillin", Eds., H.T. Clarke, J.R. Johnson, and R. Robinson, Princeton University Press, Ithaca, New York, 1949, p. 1004; H. Wasserman, F.M. Precopio, and T.C. Liu, J.Am.Chem.Soc., 1952, 74, 4093; E.M. Gorden and J. Pluscec, Tetrahedron Lett., 1983, 3419.

7 United States Pat. 4,428, 960 (1984); Chem Abstr., 1984, 100, (23), 191655.

8 There is evidence for a correlation of the reactivity of the β-lactam ring of certain cephalosporins and their antibacterial activity. See e.g. D.B. Boyd, J.Med.Chem., 1973, 16, 1195. For alternative viewpoints see M.I. Page, Acc.Chem.Res., 1984, 17, 144 and N.C. Cohen, J.Med.Chem., 1983, 26, 259.

9 H.R. Pfaendler, J. Gosteli, and R.B. Woodward, J.Am.Chem.Soc., 1980, 102, 2039; I. Ernest, J. Gosteli, C.W. Greengrass, W. Holick, D.E. Jackman, H.R. Pfaendler, and R.B. Woodward, J.Am.Chem.Soc., 1978, 100, 8214; M. Lang, K. Prasad, W. Holik, J. Gosteli, and R.B. Woodward, J.Am.Chem.Soc., 1979, 101, 6296; I. Ernest, J. Gosteli, and R.B. Woodward, J.Am.Chem.Soc., 1979, 101, 6301.

10 I. Ernest in "Chemistry and Biology of β-Lactam Antibiotics", Eds., R.B. Morin and M. Gorman, Academic Press, 1982, 1, pp 357-358.

11 D.D. Keith, J.A. Tortora, K. Ineichen, and W. Leimgruber, Tetrahedron, 1975, 31, 2633

12 J.E. Baldwin, M.A. Christie, S.B. Haber, and L.I. Kruse, J.Am.Chem.Soc., 1976, 98, 3405; J.E. Baldwin, A. Au, M. Christie, S.B. Haber and D. Hesson, J.Am.Chem.Soc., 1975, 97, 5957.

13 A.Bax, "Two Dimensional Nuclear Magnetic Resonance in Liquid", Reidel, London, 1982.

14 J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", John Wiley, New York, 1961, p. 1901.