COMPARATIVE BIOLOGICAL PROPERTIES OF SOME SYNTHETIC OLIVANIC ACID ANALOGUES

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A series of olivanic acid/thienamycin analogues have been prepared by total synthesis. Particular attention was given to the effect of the side-chain substituents on the chemical, β-lactamase and metabolic stability of the final products. All of the compounds possessed a broad and high level of *in vitro* antibacterial activity against Gram-positive and Gram-negative organisms including β-lactamase-producing strains. Two derivatives (**8c**) and (**8j**) were selected for further evaluation on the basis of *in vitro* activity, ease of synthesis and stability parameters. The improved metabolic stability of the selected analogues, relative to the naturally-occurring olivanic acid, MM 13902, could be demonstrated in terms of better activity, higher blood levels and improved urinary recovery in *in vivo* studies in mice.

Over the last two decades the penicillins and cephalosporins have provided the medicinal chemist with a wide variety of compounds differing in chemical structure and biological properties. The discovery of clavulanic acid in 1976[1] provided the first example of a naturally-occurring β-lactam containing a novel bicyclic ring system since the discovery of the penicillins and cephalosporins to exhibit useful biological properties. The isolation of thienamycin[2], the olivanic acids[3], PS-5[4], carpetimycins[5] and asparenomycins[6] between 1976 and 1981 provided a further family of β-lactam antibiotics with potent antibacterial properties and a common chemical relationship in the presence of the 7-oxo-1-azabicyclo-[3.2.0]hept-2-ene(carbapenem) ring system.

This new series of β-lactam antibiotics has representatives which possess high *in vitro* activity against a wide range of Gram-positive and Gram-negative bacteria including β-lactamase-producing strains and anaerobes. It also includes several members that are potent β-lactamase inhibitors. However, to date, the major drawbacks of the series have been chemical instability and extensive metabolism *in vivo*. The poor metabolic stability both we (unpublished results) and others[7,8] have found to be essentially due to degradation of the compound by renal dehydropeptidase, an enzyme present in the kidney. Following the isolation of the olivanic acids[3] in our laboratories, a chemical programme was set up to synthesise analogues and to determine the features required in these antibiotics to optimise the properties of high antibacterial activity coupled with improved metabolic stability. Key factors in this respect with regard to the olivanic acid series were the nature of the C-3 and C-8 side-chain substituents. Our synthetic methodology[9] gave ready access to compounds having the *trans* stereochemistry about the β-lactam ring and possessing the 1-hydroxyethyl substituent in the C-6 position. We chose in the first instance to synthesise some basic analogues and to examine the effect on biological properties of altering the stereochemistry at the asymmetric centre in the C-6 side-chain. Later attention was turned to modification of the C-3 substituent and it was suggested that the presence of an unsaturated side-chain conjugated

through sulphur could lead to compounds with improved general stability. We, therefore chose to prepare a range of suitable analogues and to test this hypothesis.

Chemistry

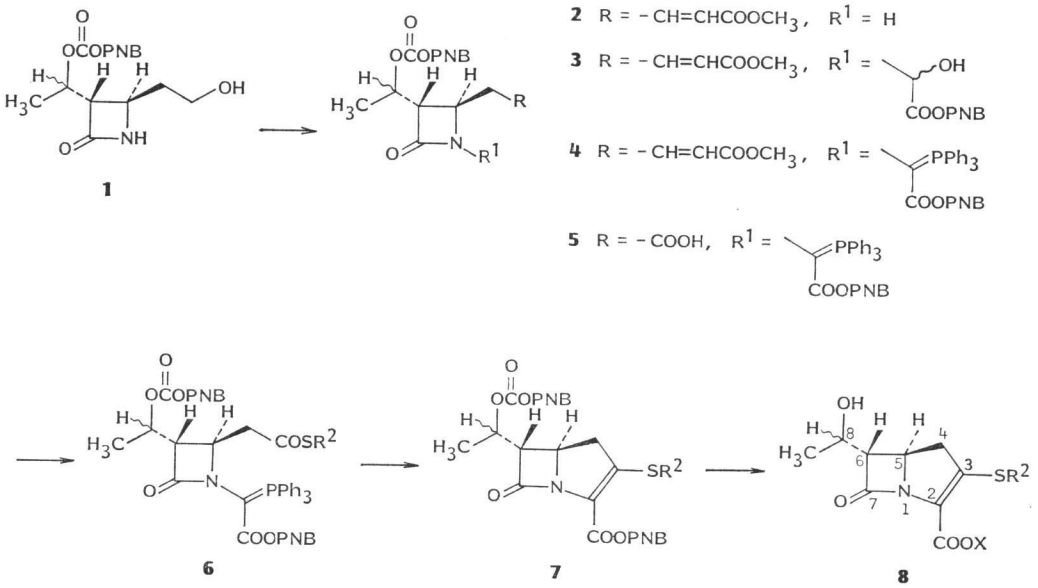
The preparation of the olivanic acid analogues described in this study is outlined in Scheme 1. Synthesis of the intermediate azetidinone **1** has been previously described[9,10]. The compound may conveniently be used as a mixture of diastereoisomers and the resulting products separated at a later stage in the synthetic route. Alternatively the azetidinone **1** may be used as a pure single diastereoisomer and carried through the reaction sequence as depicted. The assignment of stereochemical configuration at C-8 was based on correlation of NMR data[9,10] as was the assignment of *E* or *Z* configuration of the C-3 side-chains[11].

Oxidation of the alcohol **1** using pyridinium chlorochromate in methylene chloride and trapping of the intermediate aldehyde using a stabilised Wittig reagent, carbomethoxymethylenetriphenylphosphorane, gave **2** (60%). Conversion of **2** to **3** was carried out by condensation of **2** with *p*-nitrobenzylglyoxylate to provide **3** in 48% yield. This compound was then elaborated in the usual way to the phosphorane **4** by treatment with thionyl chloride followed by 2,6-lutidine and triphenylphosphine (80%). Ozonolysis of **4** and oxidation using *m*-chloroperbenzoic acid led to the phosphorane-acid **5** (90%) which provided the building-block for the synthesis of the thioesters **6**.

Two methods were used for the preparation of the thioester-phosphoranes **6**. The phosphorane-acid **5** was either converted to the acid chloride and treated with the appropriate silver thiolate[11] or reacted as the mixed phosphonic anhydride with the corresponding lithium thiolate to provide the thioester-phosphoranes **6** in yields ranging from 44 to 73% (Table 1). The more unusual side-chain thiolates were prepared as outlined in Scheme 2 utilising standard amino-acid chemistry. For example *t*-butyloxycarbonylglycine may be converted to the *m*-nitrophenylester **10** (n=1, 88%), deprotected to **11**

Scheme 1.

All compounds are racemic. Only the enantiomer corresponding to the stereochemistry of the naturally-occurring materials is depicted.



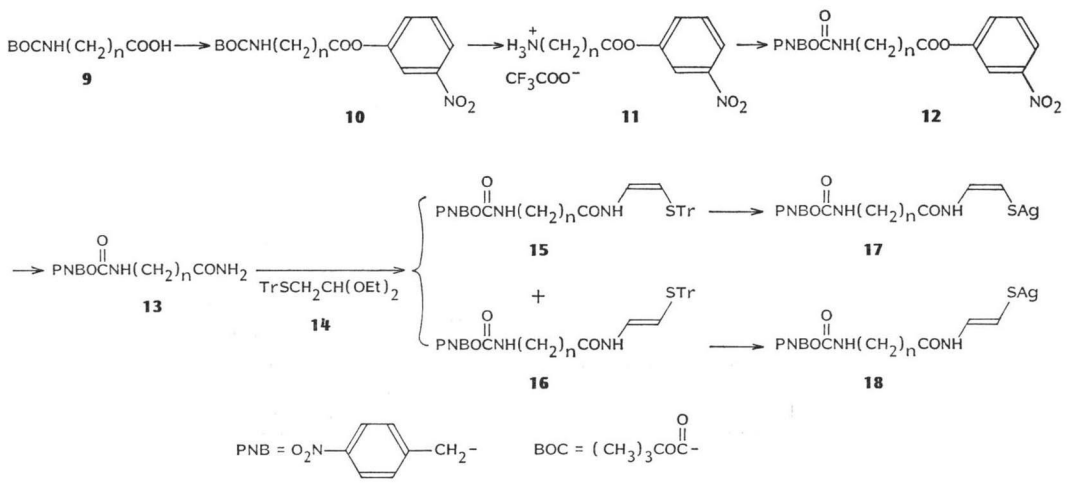
1 **6** **7** **8**

Table 1. Methods and yields of olivanic acid analogues **8** from phosphorane-acid **5**.

No.	R^2	C-8	Preparation of thioester **6**			Cyclisation to **7**				Yield (%) of hydrogenolysis to **8**
			Method	Yield (%)	mp (°C) (solvent)	Solvent	Time (hours)	Yield (%)	mp (°C) (solvent)	
a		8*S*	Ag	55	110~112 (Ether)	T	16	15	Triturated solid	47
b		8*R*							Triturated solid	38
c		8*R*	Ag	52	Viscous oil	X	3.5	19	Viscous oil	55
d		8*R*	Ag	44	Viscous oil	X	3.5	18	Viscous oil	34
e		8*R*			Isomerisation				Viscous oil	20
f		8*R*			Isomerisation				Viscous oil	31
g		8*R*	Ag	50	Viscous oil	X	3	13	Viscous oil	33
h		8*R*	Ag	55	Viscous oil	X	3	10	Viscous oil	30
i		8*S*	Li	60	Viscous oil	T	3	73	Viscous oil	64
j		8*R*							172~174 (EtOAc)	58
k		8*R*	Li	62	Viscous oil	T	3	62	157~158 (EtOAc)	75
l		8*R*	Li	73	Viscous oil	T	3	76	165~167 (EtOAc)	71

Ag=Acid chloride/silver salt method. Li=Mixed phosphonic anhydride/lithium salt method.
T =Toluene, X=xylene.
* Protected as the *p*-nitrobenzyloxycarbonyl derivative.

Scheme 2.



(n=1) and acylated using *p*-nitrobenzylchloroformate to provide **12** (n=1, 88% overall). Treatment of **12** (n=1) with gaseous ammonia in methylene chloride[12] provided **13** (n=1, 70%) which was condensed with tritylthioethyldiethylacetal **14** leading to a mixture of ethenyl sulphides **15** (n=1, 11%) and **16** (n=1, 10%) which were separable by chromatography. Cleavage of the sulphur protecting group using silver nitrate in methanol led to the yellow silver salts **17** (n=1) and **18** (n=1) in high yield which were then coupled to the phosphorane-acid **5** as described above. The thioester-phosphoranes **6** were generally obtained as viscous oils after chromatography and purity assayed by IR and TLC. Where oils were obtained, the yields of thioester-phosphorane **6** (Table 1) related, not to finally purified and characterised products, but to material obtained after chromatography. These materials were cyclised directly to the bicyclic β-lactams **7**.

Cyclisation of the thioester-phosphoranes was carried out by heating the compound in refluxing toluene or xylene under argon for varying periods depending upon the example (Table 1). Yields were invariably low for the ethenylthio examples (**7a**~**h**) ranging from 10 to 19% although substantial amounts of unreacted thioester-phosphorane generally remained. Lengths of reaction times depended upon the balance of degradation against formation of product and were often determined as a result of several experiments. In the case of the pyrimidine examples (**6i**~**l**) cyclisation was particularly facile and high yields (>70%) of product generally resulted.

Removal of protecting groups was performed by hydrogenolysis of the bicyclic azetidinones (**7a**~**e**) in aqueous dioxan over 10% Pd/C followed by the addition of one equivalent of sodium bicarbonate to provide the sodium salts of the acids (**8a**~**e**, X=Na, Table 1). The zwitterions (**8f**~**l**, X=H) were obtained by hydrogenolysis of the protected azetidinone in the presence of 0.5 M phosphate buffer.

In some cases separation of the cyclised product was difficult owing to the similarity in polarity between the starting thioester-phosphorane and the cyclised product and in these instances an alternative approach was adopted (Scheme 3). Treatment of the protected azetidinone **19** with mercuric chloride in aqueous acetonitrile[13] affords an equilibrium mixture of *E* and *Z* isomers **19** and **20** which are separable by chromatography. In a similar way compounds (**7e** and **7f**) were prepared and deprotected to provide examples (**8e** and **8f**).

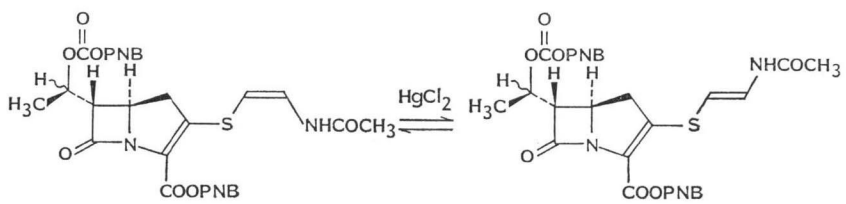
The purity of the products **8** was determined by assay using the characteristic olivanic acid/thienamycin chromophore at λ_{max} 295 to 310 nm and compounds **8** corrected for purity. All MIC values quoted (Table 2) for compounds **8** were carried out on essentially pure materials.

Biological Materials and Methods

Antibiotics

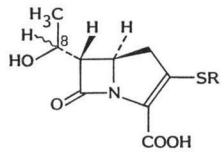
Amoxicillin free acid was a Beecham Laboratory reference standard (Beecham Pharm, Worthing,

Scheme 3.



19 **20**

Table 2. Comparative antibacterial activities of some synthetic olivanic acid/thienamycin analogues **8**.



No.	R	C-8	MIC (μg/ml)									
			Escherichia coli Amp^S	*E. coli* R-TEM Amp^R	*Klebsiella aerogenes* Cep^S	*Proteus mirabilis* Amp^S	*Enterobacter cloacae*	*Serratia marcescens*	*Pseudomonas aeruginosa*	*Staphylococcus aureus* Amp^S	*S. aureus* Amp^R	*Streptococcus faecalis*
a	 (±MM 22383)	8*S*	25	25	25	>50	25	25	>50	12.5	12.5	>50
b	 (±*N*-Ac-dehydrothienamycin)	8*R*	0.8	0.8	0.8	3.1	1.6	1.6	50	0.4	0.8	6.2
c		8*R*	0.8	0.8	0.4	3.1	12.5	3.1	>50	0.2	0.2	3.1
d		8*R*	0.8	0.8	0.2	3.1	3.1	3.1	>100	0.4	0.4	3.1
e		8*R*	0.4	0.4	0.2	3.1	1.6	1.6	>50	0.4	0.4	6.2
f		8*R*	0.4	0.4	0.8	3.1	1.6	1.6	50	≤0.1	0.2	1.6
g		8*R*	0.2	0.4	0.8	1.6	1.6	0.8	50	0.1	0.4	0.8
h		8*R*	0.8	0.8	≤0.1	6.2	3.1	0.8	100	≤0.1	≤0.1	0.8
i		8*S*	6.2	3.1	1.6	12.5	6.2	12.5	100	0.2	0.8	50
j		8*R*	0.4	0.4	0.2	0.8	0.8	0.8	100	≤0.1	≤0.1	3.1
k		8*R*	1.6	0.8	0.2	0.8	0.4	3.1	100	0.4	0.2	3.1
l		8*R*	12.5	5.0	1.2	5.0	12.5	25.0	>50	0.2	0.5	5.0

England) and cefazolin (Kefzol, Eli Lilly & Co. Ltd.) was a commercial preparation. MM 13902 was prepared by fermentation of *Streptomyces olivaceus* ATCC 31365 as described previously[8].

Minimum Inhibitory Concentrations (MIC)

Compounds (**8a**~**l**) were tested in aqueous solution and the concentrations assayed by the ultraviolet absorption of the characteristic olivanic acid/thienamycin chromophore at λ_{max} 295 to 310 nm.

The compounds were serially diluted in 0.05 ml volumes of Nutrient Broth (Oxoid) using microtitre equipment (Dynatech). The microtitre trays were inoculated with a multipoint inoculator which deli-

vered 0.001 ml of a 1/10 dilution of an overnight broth culture, an inoculum equivalent to 10^8 cfu/ml. MIC values were determined after incubation at 37°C for 18 hours as the lowest concentration of antibiotic preventing visible growth.

Mouse Protection Tests

Mice (MFI-Olac strain, Oxford Laboratory Animal Colony, Oxford, England, weight range 18~22 g) were injected by the intraperitoneal route with 0.5 ml of a suspension of an overnight broth culture of the test organism in hog gastric mucin (American Laboratories Inc., Omaha, Nebr.) standardised to give an infective inoculum of 100~1,000 median lethal doses. The antibiotics were administered at 1 hour after infection (0.2 ml/20 g) as solutions in phosphate-buffered saline to groups of 10 mice at each dose level. The numbers of animals surviving 4 days after infection were recorded and the dose of compound required to produce 50% protection of infected animals was calculated.[14]

Plasma Levels and Urinary Recoveries in Mice

Plasma levels of the antibiotics were determined following subcutaneous administration of the compounds at either 20 mg/kg or 50 mg/kg to groups of five mice. The mice were killed by dislocation of the neck at intervals during the 1-hour period after administration of the antibiotics. Samples of blood were collected from the axillary region, and 0.3 ml volumes were added to 0.3 ml volumes of heparin (100 U/ml; Weddal Pharmaceuticals Ltd., Wrexham, Clwyd.). The blood was then centrifuged at 12,000×*g* for 1 minute, following which the plasma was assayed for antibiotic content as described below. To determine urinary recovery, six groups of five mice were dosed orally with 1 ml water and given 50 mg/kg of test compound by subcutaneous injection. Urine was collected over 0~1 hour, 1~2 hours and 2~4 hours periods. Antibiotic concentrations in plasma and urine samples were determined by microbiological assay using *Bacillus subtilis* ATCC 6633 against standards prepared either in normal mouse plasma or in 0.5 M phosphate buffer.

Preparation of Crude Kidney Enzyme

Crude preparations of both mouse and human enzymes were prepared using essentially the same methods. Samples of kidney tissue were used fresh or after storage at −20°C. No loss of activity for either tissue or crude enzyme preparation was detected during this type of storage.

Kidney tissue was sliced into small pieces and suspended in 0.05 M 3-(*N*-morpholino)propane sulphonic acid (MOPS) buffer at pH 7.0. All further steps in the enzyme preparation were carried out on ice. The suspension was homogenised for 3 periods of 30 seconds, with 30-second intervals using a IKA Ultra-Turrax TP 18/2 homogeniser (Janke & Kunkel, IKA Products, Belmont, Surrey, England). The homogenate was further treated by ultrasonication (MSE 100 Watt Ultrasonic Disintegrator from MSE Scientific Instruments, Crawley, Sussex, England) for a total of 90 seconds in 3 equal periods with 30-second intervals between treatments. The resulting sonicate was centrifuged at 2,200×*g* for 15 minutes, the supernatant was used as the crude enzyme preparation and stored at −20°C.

Stability Assay

The concentration of kidney enzyme used in the stability test was adjusted to give a standard rate of degradation of MM 13902. In all stability tests a control reaction with MM 13902 was included.

Reaction mixtures containing 0.2 ml of test compound at 200 μg/ml and 0.2 ml kidney enzyme preparation (or MOPS buffer) were prepared and incubated at 37°C. Control reactions contained 0.2 ml MM 13902 (200 μg/ml) in place of the test compound. Samples (20 μl) were removed from each reaction mixture at time 0 and at suitable intervals and assayed for residual test compound by high performance liquid chromatography (HPLC). The HPLC was carried out using a Waters 6000A pump, U6K injector and a reverse phase C_{18} μBondapak column (Waters Associates Ltd., Northwich, England). A guard column packed with co-pell ODS (Whatman Chemicals Separation Division, Maidstone, Kent, England) was included before the main column to prolong its life. Compounds were detected by their UV absorption at 300 nm using a Cecil 212 UV spectrophotometer (Cecil Instruments, Cambridge, England) with an 8 μl flow cell and 10 mm path length. The column eluant was based on 0.05 M ammonium dihydrogen phosphate buffer (pH 4.7), with the addition of various concentrations of acetonitrile to give a suitable retention time for each compound.

The stability of MM 13902 was arbitrarily assigned the value 1.0 and the stability of the test compound was expressed as the ratio:

$$\frac{\text{Initial rate of inactivation of test compound } (\mu g/ml/minute)}{\text{Initial rate of inactivation of MM 13902 } (\mu g/ml/minute)}$$

Thus, compounds having a stability figure of >1.0 are proportionally more stable than MM 13902 and <1.0 are less stable than MM 13902.

Results and Discussion

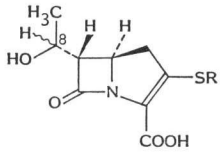
The comparative antibacterial activities of the synthetic olivanic acid/thienamycin analogues prepared above are shown in Table 2. Most of the compounds tested were highly active against a wide range of penicillin-sensitive and penicillin-resistant Gram-positive and Gram-negative organisms. The importance of the stereochemistry at the C-8 epimeric centre was determined at an early stage in the study. A comparison of the activity of compound **8a** (±MM 22383) with **8b** (±*N*-acetyldehydrothienamycin) confirmed previous findings[15] in that the biological activity was markedly affected by the stereochemistry of the hydroxyethyl group at C-8, the 8*R* example **8b** being on average some ten-fold more active than the 8*S* isomer **8a**. In the 2-thiopyrimidine series the same was also true where compound **8j** was found to be some ten-fold more active overall than **8i**.

Simple modification to the C-3 side-chain substituent also had an effect, albeit less pronounced, on overall activity. For example, comparison of the (*Z*)-isomer **8c** with the (*E*)-isomer **8b** showed that **8c** had greater activity than **8b** against Gram-positive organisms but at the expense of potency against some Gram-negative organisms such as *Enterobacter cloacae*. Modification of the C-3 side-chain amide substituent made little difference to activity, examples **8d**, **8g** and **8h** being very similar to **8c** and **8e**, and **8f** very similar to **8b**. Compound **8h** however, was the most active compound overall against Gram-positive organisms. The introduction of a primary amino function as in compounds (**8f**~**h**) in order to increase the hydrophilicity of the molecule and to encompass activity against *Pseudomonas* sp. was unrewarding, the improvements in activity being marginal.

In the 2-thiopyrimidine series introduction of hydrophobic substituents into the pyrimidine ring reduced activity cf. **8l**, **8k** and **8j**. Overall **8j** was similar in activity to most of the ethenylthio analogues and more active than racemic *N*-acetyldehydrothienamycin. The majority of the compounds were highly stable to β-lactamases and equally active against both ampicillin sensitive and resistant bacteria. Most examples particularly those with the 8*R* side-chain stereochemistry were relatively inactive as β-lactamase inhibitors in comparison with MM 13902 and other naturally-occurring olivanic acids (C. READING, Personal communication).

All of the compounds in this study were examined for their stability to mouse and human kidney tissue homogenates (Table 3) and their rates of degradation were measured relative to the standard compound MM 13902. Those compounds bearing the 8*R* side-chain stereochemistry were 2 to >10 times more stable than MM 13902 in the presence of mouse kidney homogenate and either similar to or up to 3 times more stable against the human kidney preparation. Compounds **8c**, **8h** and **8j** gave the best overall tissue stability values. Their rates of degradation by 50% mouse and human plasma were also measured, but the variation in these rates was considered insignificant in relation to those of the kidney homogenates. Based on activity *in vitro*, stability data and ease of preparation, two compounds **8c** and **8j** were selected for further evaluation *in vivo*.

Table 3. Comparative tissue stabilities of some synthetic olivanic acid/thienamycin analogues.



No.	R	C-8	Stability relative to standard MM 13902 in	
			2% Mouse kidney homogenate	10% Human kidney homogenate
a	 (±MM 22383)	8*S*	2.3	1.0
b	 (±*N*-Ac-dehydrothienamycin)	8*R*	4.0	1.8
c		8*R*	10.0	3.5
d		8*R*	9.7	1.9
e		8*R*	4.9	1.6
f		8*R*	2.6	0.7
g		8*R*	7.5	2.2
h		8*R*	>10	2.7
i		8*S*	2.4	1.2
j		8*R*	5.6	2.6
k		8*R*	9.0	1.9
l		8*R*	7.9	1.7

Blood levels and activity against experimental infections in the mouse are shown for these two derivatives in comparison to MM 13902 (Table 4). It can be seen that both compounds produced higher and more prolonged plasma levels than MM 13902 in the mouse, **8c** being 2.5 times higher and **8j** some 8 times higher than MM 13902. This in turn led to the compounds being very much more effective than MM 13902 against experimental infections caused by *Staphylococcus aureus* Smith or by ampicillin-sensitive (E8) and ampicillin-resistant (E96) strains of *Escherichia coli*. In addition the urinary recoveries of **8c** and **8j** in the mouse were much higher than that of MM 13902 reflecting their improved stability to the mouse kidney enzyme preparation.

Table 4. *In vivo* activity and pharmacokinetic data of compounds **8c** and **8j** in the mouse.

Compound	PD_{50} value (mg/kg)[a]			Mean plasma concentration (μg/ml) at minute after 50 mg/kg subcutaneous dose							Elimination half-life ($t_{\frac{1}{2}}$, minutes)	0~4 hours urinary recovery (%)
	S. aureus Smith	*E. coli* 8	*E. coli* E96	5	10	15	20	30	45	60		
8c	0.4	3.8	2.8	8.3[b]	5.8	—	2.1	<1.2			8.0	20
8j	0.7	2.8	4.0	56.2	52.4	35.1	9.4	4.5	1.7	0.2	6.7	7.9
MM 13902	60	>100	>100	9.2	6.0	2.4	1.0	<0.3	—	—	4.5	1.0
Cefazolin	NT	NT	8.6			NT					NT	NT
Amoxycillin	0.02	2.9	>100			NT					NT	NT

[a] Dosed subcutaneously 1 hour after infection.
[b] 20 mg/kg dosed subcutaneously.
NT: Not tested.

In terms of overall stability most of the analogues prepared were found to be at least as stable as analogues containing a saturated C-3 substituent (unpublished results). It was difficult to assess whether unsaturation in the C-3 side-chain alone led to increased stability since changes in other structural features, *e.g.* the stereochemistry of the side-chain double bond, also tended to have a marked effect on stability properties. Both derivatives selected for evaluation were chemically stable as judged by their stability as solids or in aqueous solution at room temperature compared to other members of the series.

Experimental

UV spectra were recorded on a Pye-Unicam SP 8000 or a Perkin-Elmer 554 spectrophotometer. Unless stated otherwise IR spectra were recorded for solutions in chloroform on a Perkin-Elmer 197 or 457 machine. ^{1}H NMR spectra were recorded at 60 MHz on a Varian EM 360, at 80 MHz on a Varian CFT 20, at 90 MHz on a Perkin-Elmer R32, and at 250 MHz on a Bruker WM 250 instrument, for solutions in $CDCl_3$ with tetramethylsilane as internal standard unless otherwise stated. The purity of all compounds was tested by TLC on Merck precoated silica gel 60 F_{254} plates. Preparative chromatography was carried out on columns of Merck silica gel 60 (finer than 230 mesh or 230~400 mesh ASTM) with eluants as stated using the slightly increased pressure provided by a Medcalf Hy-flo pump. Solutions were dried with magnesium sulphate and solvents were removed by evaporation under reduced pressure using a rotary evaporator. Mps were determined with a Kofler hot-stage apparatus and are uncorrected. All compounds prepared are racemic.

3(*R*,*S*),4(*S*,*R*)-4-(3-Methoxycarbonyl-2-propen-1-yl)-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)azetidin-2-one (**2**)

3(*R*,*S*),4(*S*,*R*)-4-(2-Hydroxyethyl)-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)azetidin-2-one (**1**, 1.2 g) was dissolved in methylene chloride (50 ml) and pyridinium chlorochromate (1.2 g) was added. The reaction was stirred at room temperature for 2 hours. A further quantity of pyridinium chlorochromate (0.5 g) was added and stirring continued for 1.5 hours. The solution was filtered and carbomethoxymethylenetriphenylphosphorane (1.75 g) was added. The mixture was stirred at room temperature for 1 hour and for 30 minutes at 50°C. The solution was applied directly to a silica H column and eluted with ethyl acetate - petroleum ether (60~80°C) to afford **2** as an oil (0.85 g, 60%). IR ν_{max} ($CHCl_3$) 3400, 1760, 1720 (sh), 1600, 1525, 1350 cm^{-1}. ^{1}H NMR δ ppm ($CDCl_3$) 1.43 (3H, d, J=7 Hz, CH_3), 2.54 (2H, t, J=7 Hz, CH_2), 2.82 to 3.25 (1H, m, C3-H for two isomers), 3.68 (4H, s, CO_2CH_3 covering C4-H), 5.11 (1H, m, CH_3CH), 5.26 (2H, s, CH_2Ar), 5.90 (1H, d, J=16 Hz, CH=$CHCO_2CH_3$), 6.57 (1H, s, NH [exchangeable]), 6.86 and 6.94 (1H, 2×d, J=16 Hz, CH=$CHCO_2CH_3$ for two isomers), 7.56 (2H, d, J=9 Hz, Ar), 8.21 (2H, d, J=9 Hz, Ar).

3(*R,S*),4(*S,R*)-1-(1-Hydroxy-1-*p*-nitrobenzyloxycarbonylmethyl)-4-(3-methoxycarbonyl-2-propen-1-yl)-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)azetidin-2-one (**3**)

p-Nitrobenzylglyoxylate hydrate (0.75 g) was refluxed in benzene (20 ml) for 1 hour with removal of water (Dean-Stark). The azetidinone **2** (0.79 g) was added and the mixture refluxed for 9 hours. The solvent was evaporated and the residue dissolved in ethyl acetate and chromatographed. Elution with 80% ethyl acetate - petroleum ether (60~80°C) gave the product **3** as an oil (0.55 g, 48%). IR ν_{max} ($CHCl_3$) 3550, 1750, 1725 (sh), 1610, 1525, 1355, 1265 cm^{-1}. ^1H NMR δ ppm ($CDCl_3$) 1.25 (3H, 2×d, J=7 Hz, CH_3–CH's [4 isomers]), 1.55 (1H, br. s, OH [exchangeable]), 2.25 to 2.70 (2H, m, CH_2), 2.80 to 3.20 (1H, m, C3-H), 3.60 and 3.56 (3H, 2×s, isomeric CO_2CH_3's), 3.50 to 4.00 (2H, m, C*H*–OH and C4-H), 4.75 to 5.20 (1H, m, C*H*–CH_3), 5.12 and 5.23 (4H, 2×s, CH_2-Ar's), 5.76 (1H, d, J=16 Hz, C*H*=$CHCO_2CH_3$), 6.45 to 6.95 (1H, m, CH=C*H*CO_2CH_3), 7.40 (4H, d, J=8 Hz, Ar), 8.10 (4H, d, J=8 Hz, Ar).

3(*R,S*),4(*S,R*)-4-(3-Methoxycarbonyl-2-propen-1-yl)-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)-1-(1-*p*-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidenemethyl)azetidin-2-one (**4**)

A stirred solution of the carbinol **3** (0.45 g) in tetrahydrofuran (15 ml) was treated with 2,6-lutidine (0.16 g) followed by thionyl chloride (0.18 g) in tetrahydrofuran (3 ml) at −20°C. Stirring was continued at −20°C for 20 minutes. The solution was filtered and azeotroped twice with toluene. Dioxan (15 ml) was added followed by 2,6-lutidine (0.16 g) and triphenylphosphine (0.393 g). The reaction was stirred at room temperature overnight, the solvent removed *in vacuo* and the residue chromatographed to provide the phosphorane **4** as an oil (0.5 g, 80%). IR ν_{max} ($CHCl_3$) 1745, 1720 (sh), 1620, 1605, 1520, 1435, 1340, 1255 cm^{-1}.

3(*R,S*),4(*S,R*)-4-Carboxymethyl-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)-1-(1-*p*-nitrobenzyloxy-carbonyl-1-triphenylphosphoranylidenemethyl)azetidin-2-one (**5**)

The phosphorane **4** (0.42 g) was dissolved in methylene chloride (12 ml) and trifluoroacetic acid (0.4 ml) was added at 0°C. The solution was stirred at 0°C for ten minutes and cooled to −60°C. Ozone was passed into the solution until it went slightly blue and excess ozone was expelled with argon. *m*-Chloroperbenzoic acid (0.09 g) was added, the mixture allowed to warm to room temperature and stirred overnight. The solvent was evaporated and the residue azeotroped four times with ethyl acetate - toluene. Chromatography of the product using ethyl acetate - petroleum ether (60~80°C) gave the product **5** (0.36 g, 90%) as a separable mixture of isomers. For both isomers IR ν_{max} ($CHCl_3$) 3000 (br), 1730, 1600, 1520, 1430, 1365, 1340, 1245 cm^{-1}.

3(*R,S*),4(*S,R*)-4-(*E*)-Acetamidoethenylthiocarbonylmethyl-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)-1-(1-*p*-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidenemethyl)azetidin-2-one (**6a** and **6b**)

The phosphorane-acid **5** (mixture of isomers, 0.4 g) was dissolved in dry acetonitrile (5 ml) containing *N,N*-dimethylformamide (3 drops). Thionyl chloride (0.06 g) in acetonitrile (1 ml) was added followed by finely-ground (*E*)-2-acetamidoethenyl silver thiolate[11] (0.16 g, 1.25 equiv). The reaction was stirred at room temperature for 1 hour and filtered. The solvent was evaporated and the residue chromatographed using ethyl acetate - petroleum ether (60~80°C) as eluant. The product (a mixture of **6a** and **6b**) was eluted off the column using ethyl acetate and obtained as a colourless gum which solidified by trituration with ether mp 110~112°C (Found: C 59.3, H 4.3, N 6.0%. Calcd. $C_{46}H_{41}N_4O_{12}SP \cdot 1\frac{1}{2}H_2O$: C 59.3, H 4.6, N 6.0%) (0.25 g, 55%). IR ν_{max} ($CHCl_3$) 3410, 1735, 1690, 1620, 1600, 1510, 1430, 1365, 1340 cm^{-1}.

p-Nitrobenzyl 5(*R,S*),6(*S,R*)-3-(*E*)-2-Acetamidoethenylthio-6-(1-*p*-nitrobenzyloxycarbonyloxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**7a** and **7b**)

The thioester phosphorane **6** (mixture of diastereoisomers, 0.15 g) was dissolved in dry toluene and refluxed vigorously under argon for 16 hours. The solvent was evaporated and the residue chromatographed using ethyl acetate - petroleum ether (60~80°C) as eluant. The first eluted compound was recovered starting thioester-phosphorane (0.12 g). The product **7a** was then collected as a light yellow oil which solidified by trituration with ether. (0.011 g, 11%, 53% based on recovered phosphorane). UV λ_{max} (EtOH) 324, 264 nm. IR ν_{max} ($CHCl_3$) 3420, 1780, 1745, 1700, 1620, 1610, 1525, 1350, 1340,

1260 cm^{-1}. ^1H NMR δ ppm ($CDCl_3$) 1.48 (3H, d, J=7.5 Hz, CH_3CH), 2.07 (3H, s, $COCH_3$), 3.04 (2H, dd, J=5, 8.5 Hz, C4-CH_2), 3.47 (1H, dd, J=3.0, 4.5 Hz, C6-H), 4.08 (1H, m, C5-H), 5.00 to 5.60 (5H, m including s at 5.25 and ABq at 5.20 and 5.49, J=13.5 Hz for CH_2Ar, $-OCOCH_2Ar$, and CH_3CH), 5.85 (1H, d, J=14 Hz, S–CH=), 7.18 (1H, dd, J=11, 14 Hz, NH–CH=), 7.55 and 8.20 (4H, ABq, J=8.5 Hz, Ar), 7.63 and 8.20 (4H, ABq, J=8.5 Hz, Ar), 7.50 (1H, obscured by Ar signals, NH).

The recovered phosphorane (0.10 g) was dissolved in dry toluene (125 ml) and refluxed under argon for 48 hours. The solvent was evaporated and the residue chromatographed to yield the starting phosphorane (0.065 g) containing a slightly more polar product the concentration of which had increased following recyclisation. The product **7b** (0.011 g) was collected as a light yellow oil which was rechromatographed twice using chloroform - ethanol as eluant to afford the other diastereoisomer **7b** initially as a gum, but which solidified by trituration with ether (0.005 g). UV λ_{max} (EtOH) 325, 264 nm. IR ν_{max} ($CHCl_3$) 3400, 1780, 1750, 1700, 1625, 1605, 1525, 1350 cm^{-1}. ^1H NMR δ ppm ($CDCl_3$) 1.45 (3H, d, J=7.5 Hz, CH_3CH), 2.08 (3H, s, $COCH_3$), 3.01 (2H, dd, J=4, 8.5 Hz, C4-CH_2), 3.32 (1H, dd, J=2.5, 8 Hz, C6-H), 4.10 (1H, m, C5-H), 5.00 to 5.60 (5H, m, including s at 5.24 and ABq at 5.20 and 5.50, J=13.5 Hz for CH_2Ar, $-OCOCH_2Ar$ and CH_3CH), 5.84 (1H, d, J=14 Hz, S–CH=), 7.20 (1H, dd, J=11, 14 Hz, NH–CH=), 7.52 and 8.22 or 8.24 (4H, ABq, J=8.5 Hz, Ar), 7.62 and 8.22 or 8.24 (4H, ABq, J=8.5 Hz, Ar), 7.50 (1H, obscured by Ar signals, NH).

Sodium 5(*R*,*S*),6(*S*,*R*)-3-(*E*)-2-Acetamidoethenylthio-6-[1(*S*,*R*)hydroxyethyl]-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (**8a**)

The bicyclic azetidinone **7a** (0.028 g) was dissolved in 30% aqueous dioxan (3 ml) containing 5% Pd/C (0.05 g) which had previously been hydrogenated for 20 minutes. The solution was hydrogenated at ambient temperature and pressure for 2 hours. The solution was neutralised with $NaHCO_3$ (0.0038 g) in water (3.8 ml) and filtered through Kieselguhr. The organic solvent was evaporated and the aqueous phase extracted with ethyl acetate (3×5 ml). Examination of the aqueous phase by UV showed it to contain the product **8a**. The aqueous phase was reduced in volume to approximately 2 ml and chromatographed on HP20 ion exchange resin using water - ethanol to elute the product. The solution containing the product was freeze-dried to give a white fluffy solid (0.007 g, 47%). UV λ_{max} (H_2O) 308, 225 nm. IR ν_{max} (KBr) 3400, 1755 cm^{-1}. ^1H NMR δ ppm (D_2O) 1.10 (3H, d, J=6 Hz, CH_3), 1.86 (3H, s, $COCH_3$), 2.85 and 2.98 (2H, 2×dd, J=18, 10 Hz, C4-CH_2), 3.23 (1H, dd, J=4.5, 2.5 Hz, C6-H), 3.95 (2H, m, CHOH and C5-H), 5.82 (1H, d, J=14 Hz, –SCH=), 6.94 (1H, d, J=14 Hz, =CH–NH).

Sodium 5(*R*,*S*),6(*S*,*R*)-3-(*E*)-2-Acetamidoethenylthio-6-[1(*R*,*S*)-hydroxyethyl]-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (**8b**)

The bicyclic azetidinone **7b** (0.035 g) was hydrogenated as described for the previous example to yield (0.005 g, 30%) of product **8b**. UV λ_{max} (H_2O) 308, 228 nm. IR ν_{max} (KBr) 3400, 1755, 1640, 1610, 1510 cm^{-1}.

Isomerisation of (*Z*)-Diastereoisomer **8c** to (*E*)-Diastereoisomer **8b**

The (*Z*)-diastereoisomer **8c** (0.08 g) was dissolved in 25% aqueous acetonitrile (10 ml) and treated with mercuric chloride (0.04 g) at room temperature. After ten minutes ethyl acetate (25 ml) was added and the solution washed with dil. sodium bicarbonate solution (3×10 ml). The organic phase was dried ($MgSO_4$) and evaporated. Chromatography using ethyl acetate - petroleum ether (60~80°C) gave recovered (*Z*)-diastereoisomer **8c** (0.042 g, 50%) and (*E*)-diastereoisomer **8b** (0.032 g, 40%).

3-(*Z*) and (*E*)-2-Glycinamidoethenylthio Side-chains

t-Butoxycarbonylglycine-*m*-nitrophenyl Ester **10** (n=1): *t*-Butoxycarbonylglycine (8.7 g) was dissolved in methylene chloride (25 ml), *m*-nitrophenol (7.5 g) added at 0°C followed by DCCI (11.9 g) and the mixture stirred at room temperature overnight. The solution was filtered and washed with 20% citric acid (25 ml) and dilute sodium bicarbonate solution (3×20 ml). The organic phase was dried ($MgSO_4$) and the solvent evaporated to give the product **10** (n=1) from ethyl acetate - petroleum ether (60~80°C), (13 g, 88%), mp 77~78°C (Found: C 53.0, H 5.5, N 9.5%. Calcd. $C_{13}H_{16}N_2O_6$: C 52.7, H 5.4, N 9.5%). IR ν_{max} ($CHCl_3$) 3300, 1790, 1710, 1680, 1530, 1470 cm^{-1}.

Glycine *m*-Nitrophenyl Ester **11** (n=1): The ester **10** (n=1, 20 g) was dissolved in trifluoroacetic acid (10 ml) and stirred at room temperature for 30 minutes. Ethyl acetate (100 ml) was added and the product **11** (n=1) was collected, washed well with ethyl acetate and dried (19 g, 95%), mp 170~171°C (Found: C 38.8, H 2.8, N 8.8%. Calcd. $C_{10}H_9N_2O_6F_3$: C 38.8, H 2.9, N 9.0%). IR ν_{max} ($CHCl_3$) 3130, 3100, 2645, 1780, 1670, 1525 and 1350 cm^{-1}.

p-Nitrobenzyloxycarbonylglycine-*m*-nitrophenylester **12** (n=1): The salt **11** (n=1, 19 g) was dissolved in methylene chloride (25 ml) and treated with triethylamine (2.2 g). The mixture was cooled to −20°C and *p*-nitrobenzylchloroformate (4.5 g) was added. The mixture was stirred at −20°C for 30 minutes and allowed to warm to 0°C. The solution was washed with 20% citric acid (25 ml) and saturated sodium bicarbonate solution (3×25 ml) and dried. Evaporation of the solvent and chromatography of the residue gave the product **12** (n=1) as a colourless solid from ethyl acetate (14 g, 93%). mp 115~116°C (Found: C 51.8, H 3.8, N 11.2%. Calcd. $C_{16}H_{13}N_3O_8$: C 51.2, H 3.5, N 11.2%). IR ν_{max} (Nujol) 3100, 1775, 1525, 1460, 1375, 1345 cm^{-1}.

p-Nitrobenzyloxycarbonylglycinamide **13** (n=1): The ester **12** (n=1, 7.5 g) was dissolved in methylene chloride (125 ml) and ammonia was bubbled into the solution for 30 minutes at room temperature. The reaction was left at room temperature for a further hour. The precipitated amide **13** (n=1) was filtered off and collected as a white solid, mp 192~193°C (3.5 g, 70%) (Found: C 47.0, H 4.2, N 16.1%. Calcd. $C_{10}H_{11}N_3O_5$: C 47.4, H 4.3, N 16.6%). IR ν_{max} ($CHCl_3$) 3450, 3300, 3150, 1705, 1675, 1605, 1545, 1510, 1460, 1345 cm^{-1}. ^1H NMR δ ppm [$(CD_3)_2SO$] 3.57 (2H, d, J=6.5 Hz, CH_2), 5.17 (2H, s, CH_2Ar), 6.98 and 7.39 (3H, br signals [exchangeable] NH and $CONH_2$), 7.61 and 8.20 (4H, ABq, J=9 Hz, Ar).

(*Z*)- and (*E*)-2-*p*-Nitrobenzyloxycarbonylglycinamido-1-triphenylmethylthioethene (**15** and **16**, n=1): The amide **13** (n=1, 2.5 g) was dissolved in *N*,*N*-dimethylformamide (30 ml) and treated with tritylthioethyldiethylacetal **14** (3.9 g) followed by toluene-*p*-sulphonic acid monohydrate (1.95 g). The reaction was stirred at 90°C for 2 hours. The solvent was evaporated and the residue chromatographed eluting with ethyl acetate - petroleum ether (60~80°C) to yield a mixture of isomers **15** (n=1) and **16** (n=1) complete separation of which was accomplished by repeat chromatography. Thus the (*Z*)-isomer **15** (n=1) was isolated as a colourless solid from ethyl acetate - petroleum ether (60~80°C), mp 79~80°C (0.6 g, 11%) (Found: C 65.4, H 5.0, N 6.9%. Calcd. $C_{31}H_{27}N_3O_5S\cdot H_2O$: C 65.1, H 5.1, N 7.3%). IR ν_{max} ($CHCl_3$) 3430, 3380, 1730, 1700, 1625, 1525, 1475 and 1350 cm^{-1}. ^1H NMR δ ppm [$(CD_3)_2SO$] 3.78 (2H, d, J=6.5 Hz, CH_2), 4.80 (1H, d, J=8 Hz, CH–S), 5.16 (2H, s, CH_2Ar), 6.72 (1H, dd, J=11, 8 Hz, CH–N), 7.25 (15H, s, Tr), 7.56 and 8.18 (4H, ABq, J=8.5 Hz, Ar), 9.41 (1H, d, J=11 Hz, NH).

The (*E*)-isomer **16** (n=1) crystallised from ethyl acetate as a colourless solid, mp 170~171°C (0.5 g, 10%) (Found: C 66.4, H 5.3, N 7.3%. Calcd. $C_{31}H_{27}N_3O_5S\cdot\frac{1}{2}H_2O$: C 66.2, H 5.0, N 7.5%). IR ν_{max} (Nujol) 3450, 3330, 3280, 1720, 1675, 1610, 1510, 1460 cm^{-1}. ^1H NMR δ ppm [$(CD_3)_2SO$] 3.58 (2H, d, J=6.5 Hz, CH_2), 5.18 (2H, s, CH_2Ar), 5.38 (1H, d, J=16 Hz, =CH–S), 6.89 (1H, m, collapses to d, J=16 Hz, on D_2O exchange, CH=N*H*–), 7.26 (15H, s, Tr), 7.59 and 8.22 (4H, ABq, J=8.5 Hz, Ar), 10.07 (1H, d, J=9.5 Hz, NH). One NH obscured by aromatic protons.

Silver (*Z*)-2-*p*-Nitrobenzyloxycarbonylglycinamido-1-ethenylthiolate **17** (n=1): The sulphide **15** (n=1, 0.55 g) was dissolved in methanol (25 ml) and finely-ground silver nitrate (0.17 g) in methanol (5 ml) was added with pyridine (0.08 g). The mixture was stirred at room temperature for 3 hours and the yellow silver salt **17** (n=1) collected by centrifugation. The collected salt was washed twice with methanol and twice with ether and dried (0.37 g, 90%). IR ν_{max} (Nujol) 3300, 1700, 1630, 1605, 1520, 1465, 1350 cm^{-1}.

Silver (*E*)-2-*p*-Nitrobenzyloxycarbonylglycinamido-1-ethenylthiolate **18** (n=1): The sulphide **16** (n=1, 0.55 g) was dissolved in refluxing methanol (50 ml) and finely-ground silver nitrate (0.17 g) in methanol (5 ml) was added together with pyridine (0.08 g). The mixture was stirred at room temperature for 1 hour and centrifuged. The residue was washed twice with methanol and twice with ether and dried (0.33 g, 97%). IR ν_{max} (Nujol) 3300, 1700, 1665, 1625, 1520, 1460 cm^{-1}.

3-(*Z*)-2-*β*-Alaninamidoethenylthio Side-chain

The side-chain **17** (n=2) was prepared in an analogous way to that described for the 3-(*Z*)-2-glycin-

amidoethenylthio side-chain **17** (n=1) above.

t-Butoxycarbonyl-β-alanine-*m*-nitrophenyl Ester **10** (n=2): The ester **10** (n=2) was obtained as described for **10** (n=1) in 71% yield from ethyl acetate - petroleum ether (60~80°C) as a colourless solid. mp 81°C (Found: C 54.0, H 5.8, N 8.9%. Calcd. $C_{14}H_{18}N_2O_6$: C 54.2, H 5.8, N 9.0%). IR ν_{max} ($CHCl_3$) 3400, 1760, 1675, 1520, 1460 cm^{-1}.

β-Alanine-*m*-nitrophenyl Ester **11** (n=2): The ester **11** (n=2) was obtained as detailed for **11**(n=1) by precipitation with ether in 94% yield, mp 147~150°C (Found: C 41.0, H 3.7, N 8.5%. Calcd. $C_{11}H_{11}N_2O_6F_3$: C 40.7, H 3.4, N 8.6%). IR ν_{max} ($CHCl_3$) 3100, 1770, 1670, 1525, 1465, 1335 cm^{-1}.

p-Nitrobenzyloxycarbonyl-β-alanine-*m*-nitrophenyl Ester **12** (n=2): The ester **12** (n=2) obtained as described for **12** (n=1), crystallised from ethyl acetate - petroleum ether (60~80°C), mp 87°C (50%) (Found: C 52.4, H 3.9, N 10.7%. Calcd. $C_{17}H_{15}N_3O_8$: C 52.4, H 3.9, N 10.8%). IR ν_{max} ($CHCl_3$) 3300, 1755, 1700, 1610, 1525, 1460, 1355 cm^{-1}.

p-Nitrobenzyloxycarbonyl-β-alaninamide **13** (n=2): The amide **13** (n=2) was obtained as detailed for **13** (n=1) by precipitation from methylene chloride as a white solid, mp 158~159°C (75%) (Found: C 49.3, H 4.9, N 15.6%. Calcd. $C_{11}H_{13}N_3O_5$: C 49.4, H 4.9, N 15.7%). IR ν_{max} ($CHCl_3$) 3400, 3350, 3200, 1685, 1645, 1610, 1520, 1455, 1345 cm^{-1}. ^1H NMR δ ppm [$(CD_3)_2SO$] 2.26 (2H, t, J=7 Hz, CH_2CO), 3.25 (2H, dt, J=7 Hz, CH_2NH), 5.18 (2H, s, CH_2), 6.81 and 7.32 (3H, br signals [exchangeable] NH and $CONH_2$), 7.62 and 8.26 (4H, ABq, J=8.5 Hz, Ar).

(*Z*)- and (*E*)-2-*p*-Nitrobenzyloxycarbonyl-β-alaninamido-1-triphenylmethylthioethene (**15** and **16**, n=2): (**15** and **16**, n=2) were obtained as described for (**15** and **16**, n=1). The (*Z*)- isomer **15** (n=2) crystallised from ethyl acetate - petroleum ether (60~80°C) as a light yellow solid, mp 146~147°C (13%) (Found: C 67.5, H 5.2, N 7.4%). Calcd. $C_{32}H_{29}N_3O_5S$: C 67.7, H 5.1, N 7.4%). IR ν_{max} ($CHCl_3$) 3210, 3070, 1705, 1660, 1635, 1515, 1460 cm^{-1}. ^1H NMR δ ppm [$(CD_3)_2SO$] 2.51 (2H, t, J=6.5 Hz, CH_2CO), 3.24 (2H, dt, J=7 Hz, CH_2NH), 4.77 (1H, d, J=8 Hz, CH–S), 5.17 (2H, s, CH_2Ar), 6.76 (1H, dd, J=10.5, 8 Hz, CH–N), 7.27 (15H, s, Tr), 7.59 and 8.22 (4H, ABq, J=9 Hz, Ar), 9.47 (1H, d, J=11 Hz, NH).

The (*E*)-isomer crystallised from ethyl acetate - petroleum ether (60~80°C) as a light yellow solid, mp 108~110°C (16%) (Found: C 67.5, H 5.2, N 7.3%. Calcd. $C_{32}H_{29}N_3O_5S$: C 67.7, H 5.1, N 7.4%). IR ν_{max} ($CHCl_3$) 3250, 3060, 1695, 1650, 1615, 1510, 1455 cm^{-1}. ^1H NMR δ ppm [$(CD_3)_2SO$] 2.30 (2H, t, J=7 Hz, CH_2CO), 3.20 (2H, dt, J=7 Hz, CH_2NH), 5.14 (2H, s, CH_2Ar), 5.29 (1H, d, J=14 Hz, =CH–S), 6.88, (1H, dd, J=14, 10 Hz, =CH–NH), 7.28 (15H, s, Tr), 7.58 and 8.20 (4H, ABq, J=9 Hz, Ar), 10.10 (1H, d, J=10 Hz, NH).

Silver (*Z*)-2-*p*-Nitrobenzyloxycarbonyl-β-alaninamido-1-ethenylthiolate **17** (n=2): The sulphide **15** (n=2) gave the yellow silver salt **17** (n=2) in 91% yield as described for **15** (n=1). IR ν_{max} (Nujol) 3300, 1680, 1620, 1510, 1455, 1345 cm^{-1}.

3(*R*,*S*),4(*S*,*R*)-4-Pyrimidin-2-ylthiocarbonylmethyl-3-(1-*p*-nitrobenzyloxycarbonyloxyethyl)-1-(1-*p*-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidenemethyl)azetidin-2-one (**6i** and **6j**)

The phosphorane-acid **5** (mixture of isomers, 0.4 g) was dissolved in dry tetrahydrofuran (15 ml) and treated with triethylamine (0.06 g) and diethyl chlorophosphate (0.1 g). The solution was stirred at room temperature under an argon atmosphere for 2 hours and treated with freshly-prepared lithium-2-pyrimidinyl thiolate (0.07 g), and stirred for a further hour at room temperature. The reaction mixture was filtered, evaporated and the residue chromatographed using ethyl acetate as eluant, to afford the product as a pale yellow foam (0.27 g, 60%), IR ν_{max} ($CHCl_3$) 1750, 1525, 1350 cm^{-1}.

p-Nitrobenzyl 5(*R*,*S*),6(*S*,*R*)-3-(Pyrimidin-2-ylthio)-6-(1-*p*-nitrobenzyloxycarbonyloxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**7i** and **7j**)

The thioesters (**6i** and **6j**, 0.06 g) were dissolved in dry toluene (60 ml) and heated under reflux for 3 hours under an argon atmosphere in an apparatus fitted with a Dean and Stark trap. Evaporation of solvent followed by careful chromatography of the residue eluting with petroleum ether (60~80°C) grading to ethyl acetate afforded the two diastereoisomers (**7i** and **7j**).

The faster-running component **7j** (8*R* diastereoisomer) was obtained as a yellow oil (0.016 g, 54%) which crystallised from ethyl acetate, mp 172~174°C (Found: C 53.8, H 3.8, N 11.0%. Calcd. $C_{28}H_{23}$-

$N_5O_{10}S$: C 53.9, H 3.7, N 11.2%). UV λ_{max} (EtOH) 320 nm. IR ν_{max} ($CHCl_3$) 1780, 1740, 1520, 1350 cm^{-1}. ^1H NMR δ ppm ($CDCl_3$) 1.49 (3H, d, J=6.5 Hz, CH_3), 3.25 (1H, dd, J=10, 18 Hz, C4-H), 3.45 (1H, dd, J=3, 7.5 Hz, C6-H), 3.82 (1H, dd, J=10, 18 Hz, C4-H), 4.31 (1H, ddd, J=10, 10, 3 Hz, C5-H), 5.22 (1H, dq, J=7.5, 6.5 Hz, C8-H), 5.26 (2H, s, CH_2Ar), 5.31 (1H, d, J=14 Hz, OCH_AH_BAr), 5.52 (1H, d, J=14 Hz, OCH_AH_BAr), 7.12 (1H, t, J=5 Hz, pyrimidine-H), 7.54 and 8.24 or 8.21 (4H, ABq, J=8.5 Hz, Ar), 7.66 and 8.24 or 8.21 (4H, ABq, J=8.5 Hz, Ar), 8.58 (2H, d, J=5 Hz, pyrimidine-H).

The slower-running component, obtained as a yellow oil (0.005 g, 17%) was identified as **7i** (8*S* diastereoisomer). UV λ_{max} (EtOH) 320 nm. IR ν_{max} ($CHCl_3$) 1780, 1740, 1520, 1350 cm^{-1}. ^1H NMR δ ppm ($CDCl_3$) 1.49 (3H, d, J=6.5 Hz, CH_3), 3.22 (1H, dd, J=9, 18 Hz, C4-H), 3.56 (1H, dd, J=3, 4 Hz, C6-H), 3.84 (1H, dd, J=9, 18 Hz, C4-H), 4.25 (1H, ddd, J=9, 9, 3 Hz, C5-H), 5.15~5.25 (1H, m, C8-H), 5.26 (2H, s, CH_2Ar), 5.31 (1H, d, J=14 Hz, OCH_AH_BAr), 5.52 (1H, d, J=14 Hz, OCH_AH_BAr), 7.12 (1H t, J=5 Hz, pyrimidine-H), 7.54 and 8.24 or 8.21 (4H, ABq, J=8.5 Hz, Ar), 7.66 and 8.24 or 8.21 (4H, ABq, J=8.5 Hz, Ar), 8.58 (2H, d, J=5 Hz, pyrimidine-H).

5(*R*,*S*),6(*S*,*R*)-3-(Pyrimidin-2-ylthio)-6[1(*R*,*S*)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8j**)

The *p*-nitrobenzyl protected intermediate **7j** (0.014 g) was dissolved in dioxan containing water (1.5 ml), phosphate buffer (1/20 M, 1.5 ml) and 10% Pd/C catalyst (0.025 g) and hydrogenated at ambient temperature and pressure for 2 hours. The solution was filtered through Kieselguhr, washed with water and the dioxan evaporated. The aqueous solution was washed with ether (3×5 ml). Examination of the aqueous phase by UV showed it to contain the product. This was freeze-dried to provide **8j** as a yellow solid (0.004 g, 58%). UV λ_{max} (H_2O) 295, 245 nm. IR ν_{max} (KBr) 1765, 1600, 1380 cm^{-1}. ^1H NMR δ ppm (D_2O) 1.05 (3H, d, J=6.5 Hz, CH_3), 2.81 (1H, dd, J=9, 18 Hz, C4-H), 3.13 (1H, dd, J=9, 18 Hz, C4-H), 3.23 (1H, dd, J=3, 6 Hz, C6-H), 4.04 (1H, dq, J=6, 6.5 Hz, C8-H), 4.08 (1H, ddd, J=3, 9, 9 Hz, C5-H), 7.13 (1H, t, J=5 Hz, pyrimidine-H), 8.42 (2H, d, J=5 Hz, pyrimidine-H).

5(*R*,*S*),6(*S*,*R*)-3-(Pyrimidin-2-ylthio)-6-[1(*S*,*R*)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8i**)

The *p*-nitrobenzyl protected intermediate **7i** (0.012 g) was deprotected as described for the preparation of **8j**, to yield the zwitterion **8i** (0.004 g, 65%) as a yellow solid after freeze-drying. UV λ_{max} (H_2O) 295, 244 nm. IR ν_{max} (KBr) 1760, 1600, 1380 cm^{-1}.

Compounds (**8c**~**h**), **8k** and **8l** were prepared in an analogous way to the examples described above.

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