Synthesis of γ-Lactam Analogues of Carbapenems with Substituted-thio Groups at the C-3 Position

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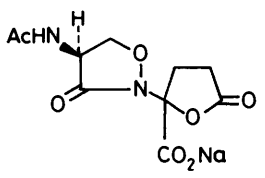
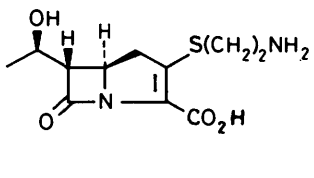
γ-Lactam analogues of carbapenems {(7*S*)-7-acylamino-2-carboxy-3-(substituted-thio)-1-azabicyclo-[3.3.0]oct-2-en-8-ones} (**2**) were synthesized starting from L-aspartic acid. Condensation of the oxo ester (**4**) with 2,4-dimethoxybenzylamine followed by cyclization gave preferentially the *E*-γ-lactam (**6**), which was transformed into the 3,5-*cis*-5-carboxymethyl-γ-lactam *cis*-(**9**) *via* stereoselective catalytic reduction. The major product *cis*-(**9**) and its *trans*-isomer were converted into compound (**2**) *via* a carbene insertion reaction. The antibacterial activity of the *trans*-acetamidoethylthio derivative (**23**) slightly exceeded that of the corresponding *cis*-derivative (**17**).

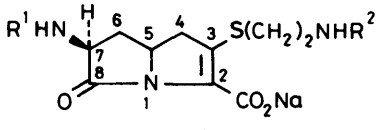
A screening programme for new inhibitors of cell wall synthesis produced by bacteria using β-lactam hypersensitive mutants led to the discovery of a new type of antibiotic, lactivicin (**1**) {2-[(4*S*)-4-acetamido-3-oxoisoxazolidin-2-yl]-5-oxotetrahydrofuran-2-carboxylic acid sodium salt} from culture filtrates of *Empedobacter lactamgenus* YK-258 and *Lysobacter albus* YK-422.[1a,b] Although lacking a β-lactam ring, compound (**1**) had various biological properties commonly associated with β-lactam antibiotics.[1a] Extensive studies on the chemical modification of (**1**) have been carried out using both semi- and total-synthetic approaches.[1c–e] The structure-activity relationships[1d] also suggest that (**1**) and its derivatives show antibacterial activity *via* a mechanism similar to that of traditional β-lactams, in which the C–N bond of the cycloserine ring activated by the lactone moiety may act as the activite site in binding to target enzymes. Based on these findings, we hypothesized that γ-lactam derivatives in which the C–N bond is activated by suitable functionalities might also have antibacterial activity. The C–N bond of bicyclic β-lactam antibiotics is activated by electronic and conformational effects of the adjacent fused ring: in particular, the reactivity of the C–N bond of carbapenems (*e.g.*, thienamycin) is known to be highly enhanced,[2] which is reflected in the excellent antibacterial activity. Thus, as part of our research programme on the chemical modification of (**1**), we prepared the γ-lactam analogues of carbapenems (**2**), which possess acylamino groups at the C-7 position and cysteamine moieties at the C-3 position. During the course of our studies three groups reported the syntheses of γ-lactam analogues of carbapenems[3b] and related nuclei,[3a,c–f] based on molecular modelling studies. We describe here a new approach to the γ-lactam analogues of carbapenems (**2**) starting from L-aspartic acid as a chiral source.



Lactivicin (**1**)



Thienamycin



(**2**)

Table 1. ^{1}H N.m.r. chemical shifts of vinyl γ-lactams[a]

(**6**)	(**7a**)		(**7b**)	
vinyl-H	vinyl-H	N–H	vinyl-H	N–H
5.40	5.36	8.69	4.68	9.97
(5.22)[b]	(5.36	9.4)[c]	(5.00	9.9)[d]

[a] In $CDCl_3$. [b] δ for (**8a**). [c] δ for (**8b**). [d] δ for (**8c**).

The α-benzyl ester of benzyloxycarbonyl (Cbz)-L-aspartic acid (**3**)[4] was transformed into the oxo ester (**4**) (76.7%) by successive treatment with *N*,*N*′-carbonyldi-imidazole (CDI) and the magnesium salt of monobenzyl malonate.[5] Condensation of (**4**) with 2,4-dimethoxybenzylamine (DMB-NH_2) and subsequent cyclization gave selectively the *E*-γ-lactam (**6**) (52.1%), the olefin geometry of which was assigned by n.m.r. (*vide infra*). Removal of the DMB group was successfully effected by treating compound (**6**) with ceric ammonium nitrate (CAN) in aqueous solution at 5 °C for 1.5 h to afford a 5:2 mixture of the *E*-γ-lactam (**7b**) and its *Z*-isomer (**7a**) (68.8%). Increasing the reaction time merely led to an increase in the yield of (**7a**), which may have been due to isomerization of (**7b**) under the reaction conditions.

Assignment of olefin geometry in (**6**), (**7b**), and (**7a**) rests on n.m.r. evidence and correlation with the data reported for similar compounds (**8**),[6] which are summarized in Table 1. The amide protons of the *Z*-isomers, which can form an intramolecular hydrogen-bond, are more deshielded and hence have lower chemical shifts than those of the *E*-isomers. Selective formation of the *E*-isomer (**6**) from the enamine (**5**) is consistent with mechanistic considerations: the initial stage of the reaction would undoubtedly be the formation of the *Z*-enamine (**5a**), which is stabilized by hydrogen-bonding of the N–H with the carbonyl group. Cyclization might occur *via* isomerization into the *E*-isomer (**5b**), the nitrogen atom of which is more reactive than that of (**5a**). The isomerization of (**6**) into its *Z*-isomer is prohibited due to the steric interaction which occurs in the *Z*-isomer between the N-substituent and the CO_2Bn group.[6]

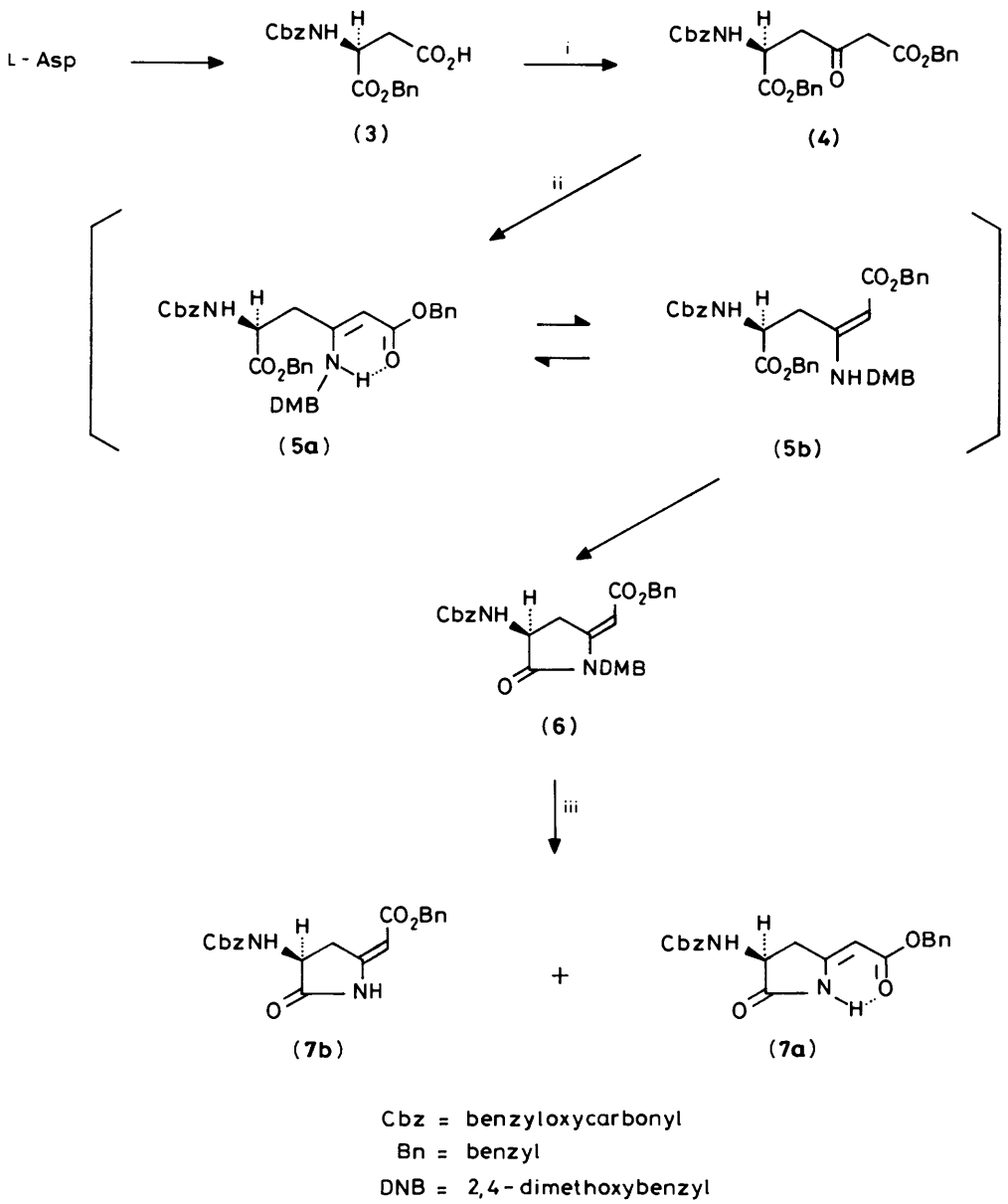
Catalytic reduction of compound (**7b**) using 10% palladium–charcoal (Pd–C) in a two-phase system and subsequent treatment with Cbz chloride gave a 10:1 diastereoisomeric mixture of the *cis* and *trans* isomers (**9**), from which *cis*-(**9**) was easily isolated after a single crystallization procedure. Catalytic reduction of (**7a**) and the mixture of (**7a**) and (**7b**) also afforded the same ratio (10:1) of the mixture. The ratio was determined by h.p.l.c. analysis of the *p*-bromobenzyl ester (**10**) derived from (**9**) (see Table 2 for n.m.r. data).

The stereochemistry of the major isomer *cis*-(**9**) was con-

Table 2. ^1H and ^{13}C N.m.r. chemical shifts of γ-lactams (**10**)

		cis-(**10**)				*trans*-(**10**)			
		δ_H [a]			δ_C [b]	δ_H [a]			δ_C [b]
3-H		4.21			52.08	4.27			50.57
	α-H	1.61	$J_{3,4}$ 9.24			2.21	$J_{3,4}$ 9.41		
			$J_{4,5}$ 8.25				$J_{4,5}$ 8.25		
C-4				J_{gem} 12.5	35.88			J_{gem} 13.5	34.52
	β-H	2.84	$J_{3,4}$ 6.77			2.39	$J_{3,4}$ 8.41		
			$J_{4,5}$ 4.79				$J_{4,5}$ 0.00		
5-H		3.90			47.35	4.01			47.67
		2.49	$J_{5,6}$ 8.42			2.57	$J_{5,6}$ 8.58		
6-H				J_{gem} 16.8	40.54			J_{gem} 17.2	40.39
		2.67	$J_{5,6}$ 1.65			2.61	$J_{5,6}$ 5.04		

[a] 270 MHz, $CDCl_3$. [b] 67.5 MHz, $CDCl_3$.

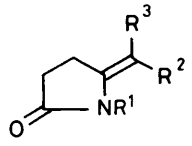


Scheme 1. *Reagents and conditions:* i, CDI, $Mg(O_2CCH_2CO_2Bn)_2$/THF; ii, $DMBNH_2$–toluene, heat; iii, CAN/acetone–MeOH

Table 3. ^1H and ^{13}C N.m.r. chemical shifts of bicyclic compounds (**14b**) and (**16**)

		(**14b**) (*cis*)				(**16**) (*trans*)			
		δ_H [a]			δ_C [b]	δ_H [a]			δ_C [b]
	α-H	2.78	$J_{4,5}$ 10.6			2.73	$J_{4,5}$ 11.9		
C-4				J_{gem} 17.1	37.32			J_{gem} 16.5	31.72
	β-H	3.27	$J_{4,5}$ 9.4			3.17	$J_{4,5}$ 8.6		
5-H		4.40			57.39	4.63			60.34
	α-H	1.78	$J_{5,6}$ 9.2			2.30	$J_{5,6}$ 5.8		
			$J_{6,7}$ 11.9				$J_{6,7}$ 7.8		
C-6				J_{gem} 11.9	31.56			J_{gem} 13.9	33.25
	β-H	3.01	$J_{5,6}$ 6.3			2.38	$J_{5,6}$ 6.8		
			$J_{6,7}$ 8.9				$J_{6,7}$ 3.3		
7-H		4.56			54.73	4.33			56.08

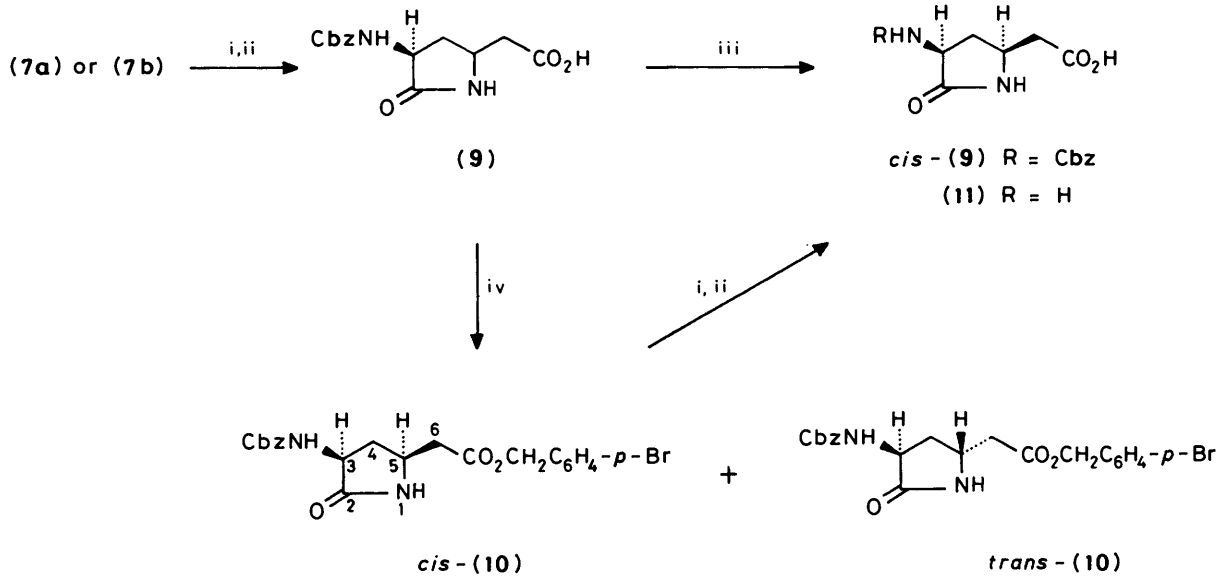
[a] 400 MHz, $CDCl_3$ + D_2O. [b] 100 MHz, $CDCl_3$ + [$^2H_6$]DMSO + D_2O.



(8) a; R^1 = Pr, R^2 = CO_2Et, R^3 = H
b; R^1 = R^3 = H, R^2 = CO_2Et
c; R^1 = R^2 = H, R^3 = CO_2Et

the magnesium salt of the mono-*p*-nitrobenzyl ester of malonic acid. The reaction of the esters (**13a,b**) with toluene-*p*-sulphonyl azide in the presence of triethylamine followed by a carbene insertion reaction assisted by rhodium acetate afforded the bicyclic compounds, which were converted into the desired products (**14a,b**) by successive treatment with diphenylphosphoryl chloride and the thiol compounds in the presence of a base.

The 5,7-*trans*-bicyclic compound (**16**) was prepared from *trans*-(**10**) *via* (**15**) in a similar manner to that described for the

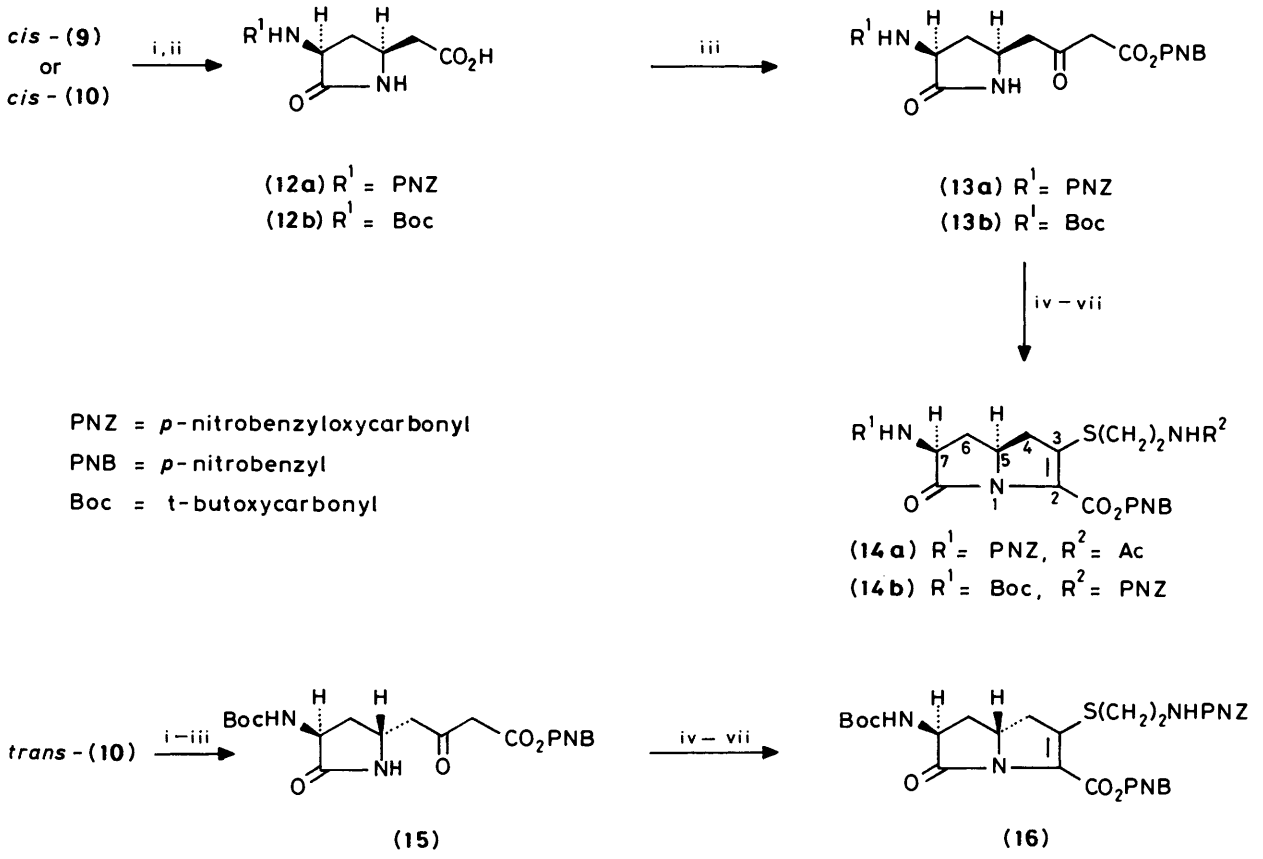


Scheme 2. *Reagents and conditions:* i, H_2, 10% Pd–C/EtOAc–H_2O; ii, Cbz–Cl, $NaHCO_3/H_2O$–THF; iii, crystallization; iv, *p*-bromobenzyl bromide, K_2CO_3–DMF

firmed to be 3*S*,5*S* by *X*-ray crystallographic analysis of the amino acid (**11**) derived from *cis*-(**9**). The selective reduction of (**7**) is explained by preferential approach of the catalyst to the double bond from the less hindered α-side of the molecule to form *cis*-(**9**) as the major product.

The acids (**12a,b**) were prepared from *cis*-(**9**) or *cis*-(**10**) by hydrogenolysis followed by protection of the amino group. Further conversion of (**12a,b**) into the 5,7-*cis*-bicyclic compounds (**14a,b**) was achieved by the sequence of reactions[7] shown in Scheme 3. Thus, compounds (**12a,b**) were transformed into the oxo esters (**13a,b**) by successive treatment with CDI and synthesis of the corresponding 5,7-*cis*-bicyclic isomer (**14b**). N.m.r. data for (**14b**) and (**16**) are given in Table 3.

Deprotection of compound (**14a**) by hydrogenolysis and subsequent acylation with the 1-hydroxybenzotriazole (HOBT) ester of D-2-(4-ethyl-2,3-dioxopiperazin-1-ylcarbonylamino)-2-phenylacetic acid (EPPA)[8] afforded the γ-lactam analogue (**17**). The compound having a 2-aminothiazol-4-yl-(*Z*)-2-methoxyiminoacetyl side chain (**18**) was prepared from (**14a**) *via* hydrogenolysis, acylation with 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl chloride hydrochloride,[9] and removal of the chloroacetyl group with sodium *N*-methyldi-



Scheme 3. *Reagents and conditions:* i, H_2, 10% Pd–C/EtOAc–H_2O; ii, PNZ–Cl, $NaHCO_3$ or $(Boc)_2O$, Et_3N/H_2O–THF; iii, CDI, $Mg(O_2CCH_2CO_2PNB)_2$/THF; iv, toluene-*p*-sulphonyl azide, Et_3N/MeCN; v, cat. $Rh_2(OAc)_3$/benzene–THF; vi, diphenylphosphoryl chloride, Pr^i_2NEt/MeCN; vii, $HS(CH_2)_2NHR^2$, Pr^i_2NEt/MeCN

thiocarbamate. Deprotection of compound (**14b**) with trifluoroacetic acid (TFA) and subsequent acylation with the HOBT ester of EPPA gave the ester (**19**), which was then converted into the lactam analogue (**20**) by hydrogenolysis. Oxidation of (**20**) with *m*-chloroperbenzoic acid (MCPBA) in aqueous solution gave the sulphone (**21**). Conversion of the 5,7-*trans*-isomer (**16**) into compound (**23**) was achieved in a similar manner to that described for the preparation of (**20**) and subsequent acetylation.

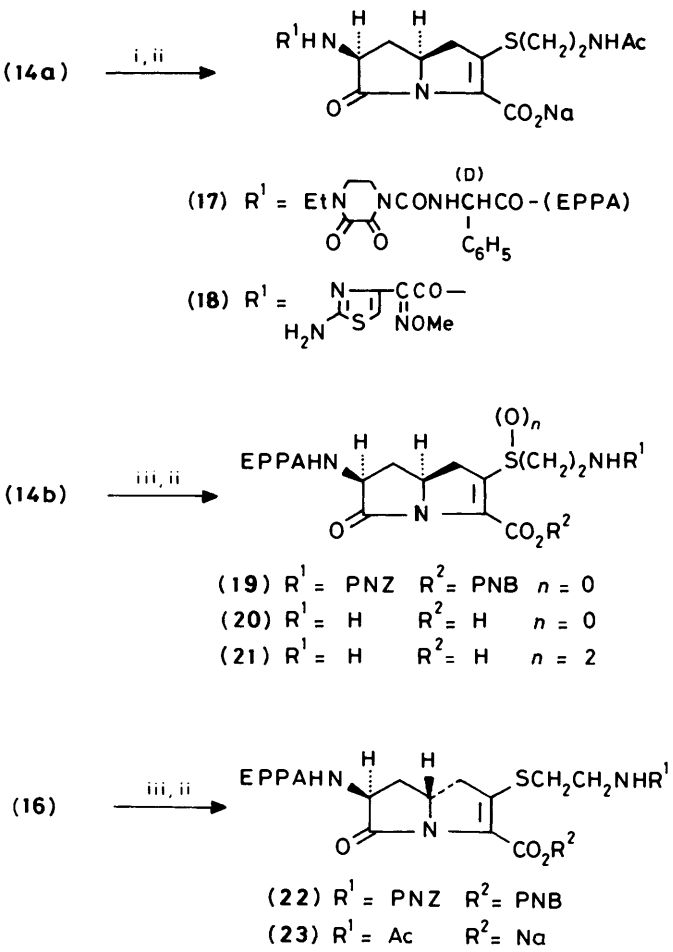
The γ-lactam analogues (**17**), (**18**), (**20**), (**21**), and (**23**) showed slight, but appreciable *in vitro* antibacterial activity against the gram negative organisms tested. The sulphone derivative (**21**) exhibited more potent antibacterial activity than the corresponding sulphide derivative (**20**) [MIC (minimum inhibitory concentration): μg ml^{-1}, 10^6 colony forming units ml^{-1}: *e.g.*, *Escherichia coli* PG-8S: (**20**), 100; (**21**), 50;*Proteus mirabillis* ATCC 21100: (**20**), >100; (**21**), 100]; the electron withdrawing sulphone group might play an important role in activating the C–N bond by delocalization of the lone pair of electrons on the bridgehead nitrogen. Interestingly, the 5,7-*trans*-isomer (**23**) was slightly more active than its corresponding 5,7-*cis*-isomer (**17**) [MIC: *e.g.*, *E. coli* PG-12: (**23**), 25; (**17**), 100; *P. mirabillis* ATCC 21100: (**23**), 6.25; (**17**), 25; *Klebsiella pneumoniae* IFO 3317: (**23**), 25; (**17**), 100]. This result clearly contrasts with the hitherto observed significantly diminished antibacterial activity of 5-epipenicillins,[10] 6-epicephalosporins,[10] and 5-epicarbapenem[11] compared with that of their corresponding isomers.

Comparison of the conformation of these analogues with that of the known β-lactam antibiotics by molecular modelling studies is in progress in order to interpret the unique structure-activity relationship.

Experimental

M.p.s were determined using a Yanagimoto m.p. apparatus and are uncorrected. I.r. spectra were measured with a Hitachi 215 spectrophotometer, and mass spectra with JEOL TMS-DX303 and Hitachi M-80A mass spectrometers. ^1H N.m.r. spectra were taken on a Varian EM-390 (90 MHz), a JEOL JNM-GX270FT (270 MHz), and a JEOL JNM-GX400FT (400 MHz) spectrometer, and ^{13}C n.m.r. spectra were measured with a JEOL JNM-GX270FT (67.5 MHz) and a JEOL JNM-GX400FT (100 MHz) spectrometer with tetramethylsilane as an internal standard. U.v. spectra were taken with a Hitachi 557 spectrophotometer. The optical rotations were recorded with a JASCO DPI-181 digital polarimeter. H.p.l.c. data were obtained using a Hitachi 655A equipment, and the analytical conditions were as follows: YMC-GEL CN; hexane–Pr^iOH (9:1, v/v). Extracted solutions were dried over magnesium sulphate unless otherwise stated.

Dibenzyl [*5S*]-*5-Benzyloxycarbonylamino*-3-*oxohexane*-1,6-*dioate* (**4**).—*N,N'*-Carbonyldi-imidazole (CDI) (5.34 g, 32.9 mmol) was added to a solution of the α-benzyl ester of benzyloxycarbonyl (Cbz)-L-aspartic acid[3] (**3**) (10.7 g, 29.2 mmol) in dry THF (130 ml) at room temperature. When the mixture had been stirred for 2 h at room temperature, the magnesium salt of the mono-benzyl ester of malonic acid (6.45 g, 15.7 mmol) was added. The mixture was stirred for 16 h at room temperature, concentrated under reduced pressure, and the concentrate dissolved in ethyl acetate. The solution was washed successively with aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, dried, and evaporated to dryness. Chromatography of the residue on



Scheme 4. *Reagents and conditions:* i, H_2, 10% Pd–C/EtOAc–phosphate buffer pH 7; ii, acylation; iii, TFA–CH_2Cl_2, $NaHCO_3$

silica gel with hexane–ethyl acetate as eluant (2:1 ⟶ 1:1, v/v) gave the *oxo ester* (**4**) (11.2 g, 76.7%) as pale yellow crystals, m.p. 82—86 °C (Found: C, 68.5; H, 5.6; N, 2.9. $C_{28}H_{27}NO_7$ requires C, 68.7; H, 5.6; N, 2.9%); $\nu_{max.}$(KBr) 3 300, 1 745, 1 720, 1 695, 1 535, and 1 290 cm^{-1}; δ_H(90 MHz; $CDCl_3$) 3.18 (2 H, t, *J* 5 Hz, CH_2CO), 3.43 (2 H, s, $COCH_2CO$), 4.28—4.78 (1 H, m, 5-H), 5.10 (2 H, s, OCH_2Ph), 5.12 (4 H, s, OCH_2Ph × 2), 5.70 (1 H, d, *J* 9 Hz, CONH), and 7.30 (15 H, s, Ph × 3); $[\alpha]_D^{23}$ +13.5° (*c* 0.585, $CHCl_3$).

(3S)-3-*Benzyloxycarbonylamino*-5-(E)-*benzyloxycarbonylmethylidene*-1-(2,4-*dimethoxybenzyl*)*pyrrolidin*-2-*one* (**6**).—A mixture of the oxo ester (**4**) (11.0 g, 22.5 mmol) and 2,4-dimethoxybenzylamine (3.76 g, 22.5 mmol), and toluene (50 ml) was refluxed for 30 min. The solvent was evaporated off and the residue was heated at 150—160 °C under reduced pressure (20 mmHg) for 1 h. The resulting reddish brown oil was chromatographed on silica gel. Elution with hexane–ethyl acetate (1:1, v/v) gave the *pyrrolidinone* (**6**) (3.2 g, 52.1%) as pale yellow crystals, m.p. 153—154 °C (Found: C, 68.1; H, 5.65; N, 5.1. $C_{30}H_{30}N_2O_7$ requires C, 67.9; H, 5.7; N, 5.3%); $\nu_{max.}$(KBr) 3 340, 1 740, 1 695, 1 610, 1 145, and 1 130 cm^{-1}; δ_H(90 MHz; $CDCl_3$) 2.80—3.21 (1 H, m, CH_2C=), 3.72 and 3.80 (3 H × 2, s, OCH_3 × 2), 3.96—4.43 (1 H, m, CH_2C=), 4.65 (2 H, s, NCH_2Ar), 5.07 and 5.10 (2 H × 2, s, OCH_2Ph × 2), 5.40 (1 H, s, =CHCO), 5.46 (1 H, d, *J* 8 Hz, CONH), 6.30—6.48 and 6.92—7.14 (3 H, m, Ar), and 7.30 (10 H, s, Ph × 2); $[\alpha]_D^{23}$ −88.5° (*c* 0.39, $CHCl_3$).

(3S)-3-*Benzyloxycarbonylamino*-5-(E)-*benzyloxycarbonylmethylidenepyrrolidin*-2-*one* (**7b**) *and its Z-Isomer* (**7a**).—A solution of ceric ammonium nitrate (CAN) (4.58 g, 8.48 mmol) in water (50 ml) was added dropwise to a solution of (**6**) (1.5 g, 2.48 mmol) in acetone (150 ml) and MeOH (50 ml) at 5 °C. The mixture was stirred for 30 min at 5 °C and additional CAN (4.58 g) was added to the mixture. The reaction mixture was stirred for 1 h at 5 °C and concentrated under reduced pressure. The concentrate was extracted with ethyl acetate and the extract was washed successively with aqueous sodium hydrogen carbonate and brine, and then dried. The solution was evaporated to dryness and the residue was chromatographed on silica gel. Gradient elution with hexane–ethyl acetate (2:1 ⟶ 2:3, v/v) gave the *Z-isomer* (**7a**) (200 mg, 18.6%) and the *E-isomer* (**7b**) (540 mg, 50.2%). Compound (**7b**), m.p. 165—168 °C (Found: C, 66.3; H, 5.2; N, 7.3. $C_{21}H_{20}N_2O_5$ requires C. 66.3; H, 5.3; N, 7.4%); $\nu_{max.}$(KBr) 1 740, 1 700, 1 690, 1 540, 1 265, and 1 135 cm^{-1}; δ_H(90 MHz; $CDCl_3$) 3.04 (1 H, ddd, *J* 1, 7, and 9 Hz, 4α-H), 3.77 (1 H, dd, *J* 10 and 19 Hz, 4β-H), 4.03—4.38 (1 H, m, 3-H), 5.09 and 5.11 (2 H × 2, s, OCH_2Ph × 2), 5.36 (1 H, br, =CHCO), 5.64 (1 H, d, *J* 7 Hz, CONH), 7.25 and 7.28 (5 H × 2, s, Ph × 2), and 8.60—8.88 (1 H, br, NH); $[\alpha]_D^{25.5}$ −133.0° (*c* 0.33, ethyl acetate).

Compound (**7a**), m.p. 105—107 °C (Found: C, 66.4; H, 5.4; N, 7.3); $\nu_{max.}$(KBr) 1 760, 1 685, 1 645, 1 530, 1 265, and 1 200 cm^{-1}; δ_H(90 MHz; $CDCl_3$) 2.80 (1 H, ddd, *J* 1, 7, and 16 Hz, 4α-H), 3.26 (1 H, dd, *J* 9 and 16 Hz, 4β-H), 4.10—4.43 (1 H, m, 3-H), 4.68 (1 H, br, CHCO), 5.10 and 5.14 (2 H × 2, s, OCH_2Ph × 2), 5.55 (1 H, d, *J* 6 Hz, CONH), 7.30 and 7.32 (5 H × 2, s, Ph × 2), and 9.85—10.10 (1 H, br, NH); $[\alpha]_D^{25.5}$ −45.5° (*c* 0.415, ethyl acetate).

(3S,5S)-3-*Benzyloxycarbonylamino*-5-*carboxymethylpyrrolidin*-2-*one* cis-(**9**).—A mixture of the *E*-isomer (**7b**) and the *Z*-isomer (**7a**) (*ca.* 5:2) (1.8 g, 4.73 mmol) was dissolved in ethyl acetate (10 ml), and a suspension of 10% palladium–charcoal (1.8 g) in water (10 ml) was added to the solution. The mixture was vigorously stirred under hydrogen at room temperature for 8 h. The catalyst was filtered off, washed with water, and the combined filtrate and washings were washed twice with ethyl acetate. To the aqueous solution was added sodium hydrogen carbonate (1.19 g, 14.2 mmol). Then, a solution of Cbz chloride (969 mg, 5.68 mmol) in THF (10 ml) was added dropwise to the mixture at room temperature. The mixture was stirred for 18 h at room temperature, washed with hexane, acidified with aqueous potassium hydrogen sulphate, and extracted three times with ethyl acetate. The extract was dried and concentrated under reduced pressure. The concentrate was treated with ethyl acetate to give the cis-*pyrrolidinone* (**9**) (1.0 g, 72.5%) as colourless crystals. Recrystallization from ethyl acetate provided an analytical sample, m.p. 169—171 °C (Found: C, 57.5; H, 5.5; N, 9.4. $C_{14}H_{16}N_2O_5$ requires C, 57.5; H, 5.5; N, 9.6%); $\nu_{max.}$(KBr) 3 380, 1 705, 1 690, 1 660, 1 525, and 1 260 cm^{-1}; δ_H(90 MHz; [$^2H_6$]DMSO) 1.30—1.80 and 1.90—2.60 (1 H × 2, m, 4-H × 2), 2.28—2.70 (2 H, m, CH_2CO), 3.52—3.94 (1 H, m, 5-H), 3.94—4.35 (1 H, m, 3-H), 5.02 (2 H, s, OCH_2Ph), 7.33 (5 H, s, Ph), and 7.75 (1 H, s, NH); $[\alpha]_D^{23}$ −23.0° (*c* 0.33, EtOH).

(3S,5S)-3-*Benzyloxycarbonylamino*-5-(4-*bromobenzyloxycarbonyl*)*methylpyrrolidin*-2-*one* cis-(**10**) *and Its* (3S,5R)-*Isomer* trans-(**10**).—A mixture of the crude pyrrolidinone (**9**), prepared from (**7b**) (190 mg, 0.5 mmol) by the above method, *p*-bromobenzyl bromide (125 mg, 0.5 mmol), potassium carbonate (138 mg, 1 mmol), and DMF (5 ml) was stirred for 18 h at room temperature. The mixture was diluted with ethyl acetate, washed successively with water and brine, and then dried. Evaporation of the solvent gave the diastereoisomeric mixture of *cis*-(**10**) and *trans*-(**10**) as a pale yellow oil; the ratio was

determined to be 10:1 by h.p.l.c. analysis. A part of the mixture was subjected to chromatography on silica gel. Gradient elution with hexane–ethyl acetate (1:1 ⟶ 1:3, v/v) gave cis-*pyrrolidinone* (**10**) and trans-*pyrrolidinone* (**10**), respectively. Compound *cis*-(**10**), m.p. 159—160 °C (Found: C, 54.7; H, 4.6; N, 6.0. $C_{21}H_{21}N_2O_5Br$ requires C, 54.7; H, 4.6; N, 6.1%); $\nu_{max.}$(KBr) 3 300, 1 720, 1 680, 1 530, 1 290, 1 260, 1 190, and 1 080 cm^{-1}; $[\alpha]_D^{23}$ +5.7° (*c* 0.21, $CHCl_3$). Compound *trans*-(**10**), m.p. 123—124 °C (Found: C, 54.5; H, 4.5; N, 6.0); $\nu_{max.}$(KBr) 3 310, 1 720, 1 690, 1 540, 1 290, 1 250, and 1 080 cm^{-1}; $[\alpha]_D^{23}$ −22.7° (*c* 0.22, $CHCl_3$). ^1H N.m.r. and ^{13}C n.m.r. data are summarized in Table 2.

(3S,5S)-3-*Amino*-5-*carboxymethylpyrrolidin*-2-*one* (**11**).—A mixture of *cis*-(**9**) (100 mg, 0.342 mmol), 10% palladium–charcoal (100 mg), MeOH (5 ml), and water (5 ml) was vigorously stirred for 2 h under hydrogen at room temperature. The catalyst was filtered off and washed with water. The combined filtrate and washings were evaporated under reduced pressure to give the *pyrrolidinone* (**11**) as colourless crystals, m.p. 245—248 °C (Found: C, 44.6; H, 6.4; N, 17.2. $C_6H_{10}N_2O_3 \cdot \frac{1}{5}H_2O$ requires C, 44.6; H, 6.5; N, 17.3%); $[\alpha]_D^{24}$ −23.4° (*c* 0.145, H_2O); $\nu_{max.}$(KBr) 3 280, 1 715, 1 575, 1 550, and 1 390 cm^{-1}. Recrystallization from EtOH–water gave a sample for *X*-ray crystallographic analysis[12] as colourless needles.

(3S,5S)-5-*Carboxymethyl*-3-(4-*nitrobenzyloxycarbonylamino*)*pyrrolidin*-2-*one* (**12a**).—A mixture of the *cis*-(**9**) (1.5 g, 5.13 mmol), 10% palladium–charcoal (1.5 g), and ethyl acetate (20 ml) and water (20 ml) was vigorously stirred for 2 h under hydrogen at room temperature. The catalyst was filtered off, washed with water, and the combined filtrate and washings were washed with ethyl acetate. To the aqueous solution were added sodium hydrogen carbonate (862 mg, 10.3 mmol) and a solution of the *p*-nitrobenzyl chloroformate (1.22 g, 5.65 mmol) in THF (30 ml) at room temperature. After being stirred for 18 h at room temperature, the mixture was washed twice with ether, acidified with aqueous potassium hydrogen sulphate, and extracted three times with a 3:1 mixture of ethyl acetate and THF. The combined extracts were dried and concentrated under reduced pressure. The resulting crystals were collected by filtration and washed with ether to give the *pyrrolidinone* (**12a**) (1.56 g, 90.2%) as colourless crystals, m.p. 200—203 °C (Found: C, 49.8; H, 4.5; N, 12.35. $C_{14}H_{15}N_3O_7$ requires C, 49.85; H, 4.5 N, 12.5%); $\nu_{max.}$(KBr) 3 380, 1 705, 1 690, 1 660, 1 510, 1 350, and 1 260 cm^{-1}; δ_H(90 MHz; [$^2H_6$]DMSO) 1.31—1.78 and 2.00—2.60 (1 H × 2, m, 4-H × 2), 2.20—2.70 (2 H, m, CH_2CO), 3.55—3.92 (1 H, m, 5-H), 3.92—4.39 (1 H, m, 3-H), 5.20 (2 H, s, OCH_2Ar), 7.80 (1 H, s, NH), and 7.64 and 8.25 (2 H × 2, d, *J* 9 Hz, Ar × 2); $[\alpha]_D^{23}$ −31.0° (*c* 0.2, EtOH).

(3S,5S)-5-*Carboxymethyl*-3-(*t-butoxycarbonylamino*)*pyrrolidin*-2-*one* (**12b**).—Treatment of *cis*-(**10**) (300 mg, 0.650 mmol) with di-t-butyl dicarbonate (0.3 ml) in a similar manner to that described for the preparation of (**12a**) gave the *pyrrolidinone* (**12b**) (121 mg, 72%) as colourless crystals, m.p. 184—186 °C (Found: C, 51.05; H, 7.05; N, 10.8. $C_{11}H_{18}N_2O_5$ requires C, 51.2; H, 7.0; N, 10.85%); $\nu_{max.}$(KBr) 3 400, 2 970, 1 730, 1 710, 1 675, 1 510, and 1 165 cm^{-1}; δ_H(90 MHz; [$^2H_6$]DMSO) 1.35 (9 H, s, CH_3), 1.20—1.76 and 2.01—2.50 (1 H × 2, m, 4-H × 2), 2.27—2.63 (2 H, m, CH_2CO), 3.48—3.85 (1 H, m, 5-H), 3.70—4.23 (1 H, m, 3-H), 6.86 (1 H, d, *J* 9 Hz, CONH), and 7.70 (1 H, s, NH); $[\alpha]_D^{23}$ −11.4° (*c* 0.21, EtOH).

(3S,5S)-3-(4-*Nitrobenzyloxycarbonylamino*)-5-[3-(4-*nitrobenzyloxycarbonyl*)-2-*oxopropyl*]*pyrrolidin*-2-*one* (**13a**).—CDI (836 mg, 5.16 mmol) was added to a suspension of the pyrrolidinone (**12a**) (1.45 g, 4.30 mmol) in dry THF (50 ml) at room temperature. When the mixture had been stirred for 2 h at room temperature, the magnesium salt of mono-*p*-nitrobenzyl ester of malonic acid (1.08 g, 2.15 mmol) was added. The mixture was stirred for 18 h at room temperature, diluted with ethyl acetate, washed successively with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, and then dried. Concentration of the extract gave the oxo ester (**13a**) (2.02 g, 100%) as an amorphous solid; $\nu_{max.}$(KBr) 3 380, 1 705br, 1 515, and 1 345 cm^{-1}; δ_H(90 MHz: $CDCl_3$) 1.50—2.80 (2 H, m, 4-H), 2.65—2.97 (2 H, m, CH_2CO), 3.56 (2 H, s, $COCH_2CO$), 3.55—4.40 (2 H, m, 3-H and 5-H), 5.16 and 5.24 (2 H × 2, s, OCH_2Ar × 2), 5.70 (1 H, d, *J* 6 Hz, CONH), 6.60 (1 H, s, NH), and 7.35—7.60 and 8.10—8.32 (4 H × 2, m, Ar × 2).

(3S,5S)-5-[3-(4-*Nitrobenzyloxycarbonyl*)-2-*oxopropyl*]-3-(*t-butoxycarbonylamino*)*pyrrolidin*-2-*one* (**13b**).—Treatment of compound (**12b**) (481 mg, 1.86 mmol) in a similar manner to that described for the preparation of (**13a**) gave the pyrrolidinone (**13b**) (408 mg, 50.2%) as a colourless amorphous solid; $\nu_{max.}$(KBr) 3 330, 1 750, 1 715, 1 680, 1 520, 1 350, and 1 165 cm^{-1}; δ_H(90 MHz; $CDCl_3$) 1.45 (9 H, s, CH_3), 2.63 (1 H, dd, *J* 9 and 18 Hz, CH_2CO), 2.98 (1 H, dd, *J* 4 and 18 Hz, CH_2CO), 3.54 (2 H, s, $COCH_2CO$), 3.67—4.30 (2 H, m, 3-H and 5-H), 5.28 (2 H, s, OCH_2Ar), and 7.52—8.25 (2 H × 2, d, *J* 9 Hz, Ar × 2).

(5S,7S)-3-(2-*Acetamidoethylthio*)-2-(4-*nitrobenzyloxycarbonyl*)-7-(4-*nitrobenzyloxycarbonylamino*)-1-*azabicyclo*[3.3.0]*oct*-2-*en*-8-*one* (**14a**).—Triethylamine (435 mg, 4.30 mmol) was added dropwise to a solution of the oxo ester (**13a**) (2.01 g, 4.30 mmol) and toluene-*p*-sulphonyl azide (848 mg, 4.30 mmol) in acetonitrile (30 ml) at 5 °C. The mixture was stirred for 1 h at 5 °C and diluted with ethyl acetate. The solution was washed successively with aqueous potassium hydrogen sulphate and brine, and then dried. The extract was evaporated to dryness and the residue dissolved in a mixture of dry THF (40 ml) and dry benzene (80 ml). The solution was heated at 80—90 °C in the presence of a catalytic amount of $Rh_2(OAc)_4$ (63 mg) under argon for 2 h; the solvent was evaporated off under reduced pressure, and the residue was dissolved in acetonitrile (50 ml). To the solution was added successively di-isopropylethylamine (611 mg, 4.73 mmol) and diphenyl chlorophosphate (1.27 g, 4.73 mmol) at 5 °C. The mixture was stirred for 30 min at 5 °C, a solution of *N*-acetylcysteamine (564 mg, 4.30 mmol) and di-isopropylethylamine (611 mg, 4.73 mmol) in acetonitrile (10 ml) was added at 5 °C, and the mixture was allowed to stand in a refrigerator for 18 h. After being diluted with ethyl acetate, the mixture was washed successively with water, aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate and brine, and then dried. The solvent was evaporated off and the residue was chromatographed on silica gel. Gradient elution with hexane–ethyl acetate (1:2, v/v) ⟶ ethyl acetate–MeOH (7:3, v/v) gave the bicyclic compound (**14a**) (1.55 g, 58.7%) as a pale yellow powder. Recrystallization from acetone–hexane provided an analytical sample as pale yellow crystals, m.p. 177—180 °C; *m/z* 613 (M^+); δ_H(400 MHz; $CDCl_3$) 1.80—1.88 (1 H, m, 6-H), 1.98 (3 H, s, CH_3), 2.76—2.83 (1 H, m, 4-H), 2.81—2.90 and 2.99—3.05 (1 H × 2, m, CH_2S × 2), 3.05—3.10 (1 H, m, 6-H), 3.30—3.37 and 3.44—3.48 (1 H × 2, m, CH_2N × 2), 3.36—3.42 (1 H, m, 4-H), 4.43—4.46 (1 H, m, 5-H), 4.60—4.65 (1 H, m, 7-H), 5.22 (2 H, s, OCH_2Ar), 5.27 and 5.47 (1 H, d, *J* 13.4 Hz, OCH_2Ar × 2), 7.52 and 7.65 (2 H × 2, d, *J* 8.8 Hz, Ar × 2), and 8.21 and 8.22 (2 H × 2, d, *J* 8.8 Hz, Ar × 2); $\nu_{max.}$(KBr) 3 400, 1 720, 1 520, and 1 345 cm^{-1}; $[\alpha]_D^{24}$ −15.2° (*c* 0.105, $CHCl_3$).

(5S,7S)-2-(4-*Nitrobenzyloxycarbonyl*)-3-[2-(4-*nitrobenzyloxycarbonylamino*)*ethylthio*]-7-(*t-butoxycarbonylamino*)-1-*azabicyclo*[3.3.0]*oct*-2-*en*-8-*one* (**14b**).—Treatment of compound

(**13b**) (408 mg, 0.937 mmol) in a similar manner to that described for the preparation of (**14a**) gave the *bicyclic compound* (**14b**) (350 mg, 55.6%) as a pale yellow powder. Recrystallization from ethyl acetate provided an analytical sample as pale yellow crystals, m.p. 178—180 °C (Found: C, 53.2; H, 4.9; N, 10.3. $C_{30}H_{33}N_5O_{11}S$ requires C, 53.6; H, 4.95; N, 10.4%); $\nu_{max.}$(KBr) 3 400, 1 720, 1 690, 1 520, 1 345, 1 260, and 1 160 cm^{-1}; $[\alpha]_D^{24}$ −6.7° (*c*, 0.075, $CHCl_3$). ^1H N.m.r. and ^{13}C n.m.r. data are summarized in Table 3.

(3S,5R)-5-[3-(4-*Nitrobenzyloxycarbonyl*)-2-*oxopropyl*]-3-(*t-butoxycarbonylamino*)*pyrrolidin-2-one* (**15**).—Treatment of *trans*-(**10**) (300 mg, 0.650 mmol) in a similar manner to that described for the preparation of the corresponding *cis*-isomer (**13b**) gave the pyrrolidinone (**15**) (181 mg, 64.0%) as colourless crystals; this crude product was used in the next reaction without further purification, m.p. 174—175 °C; $\nu_{max.}$(KBr) 3 320, 1 720, 1 680, 1 520, 1 350, and 1 160 cm^{-1}; δ_H(90 MHz; $CDCl_3$) 1.44 (9 H, s, CH_3), 2.00—2.53 (2 H, m, 4-H), 2.80 (2 H, d, *J* 7 Hz, CH_2CO), 3.50 (2 H, s, $COCH_2CO$), 3.80—4.37 (2 H, m, 3-H and 5-H), 5.00—5.20 (1 H, br, CONH), 5.25 (2 H, s, OCH_2Ar), 6.13—6.35 (1 H, br, NH), and 7.52 and 8.25 (2 H × 2, d, *J* 9 Hz, Ar × 2); $[\alpha]_D^{24}$ −14.8° (*c* 0.135, $CHCl_3$).

(5R,7S)-2-(4-*Nitrobenzyloxycarbonyl*)-3-[2-(4-*nitrobenzyloxycarbonylamino*)*ethylthio*]-7-(*t-butoxycarbonylamino*)-1-*azabicyclo*[3.3.0]*oct-2-en-8-one* (**16**).—Treatment of compound (**15**) (180 mg, 0.413 mmol) in a similar manner to that described for the preparation of (**14b**) gave the *bicyclic compound* (**16**) (185 mg, 66.5%) as pale yellow crystals, m.p. 165—167 °C (Found: C, 53.25; H, 4.8; N, 10.0. $C_{30}H_{33}N_5O_{11}S$ requires C, 53.6; H, 4.95; N, 10.4%); $\nu_{max.}$(KBr) 3 350, 1 720—1 680br, 1 510, 1 345, and 1 160 cm^{-1}; $[\alpha]_D^{24}$ +11.4° (*c* 0.07, $CHCl_3$), ^1H N.m.r. and ^{13}C n.m.r. data are summarized in Table 3.

Sodium (5S,7S)-3-(2-*Acetamidoethylthio*)-7-[(2R)-2-(4-*ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-*phenylacetamido*]-8-*oxo*-1-*azabicyclo*[3.3.0]*oct*-2-*ene*-2-*carboxylate* (**17**).—A mixture of compound (**14a**) (200 mg, 0.326 mmol), 10% palladium–charcoal (200 mg), and ethyl acetate (10 ml) and phosphate buffer (pH 7, 10 ml) was stirred for 2 h under hydrogen. The catalyst was filtered off and washed with water, and the combined filtrate and washings were washed with ethyl acetate. To the aqueous solution was added a mixture of THF (10 ml) and 1-hydroxybenzotriazole (HOBt) ester of D-2-(4-ethyl-2,3-dioxo-1-piperazin-1-ylcarbonylamino)-2-phenylacetic acid (EPPA),[7] which was prepared from HOBt (50 mg, 0.489 mmol), the acid (156 mg, 0.489 mmol), and dicyclohexylcarbodi-imide (DCC) (135 mg, 0.652 mmol). The mixture was stirred for 2 h at room temperature, washed with ethyl acetate, and the aqueous solution was concentrated under reduced pressure. The residue was subjected to chromatography on Amberlite XAD-II (150 ml). Gradient elution with water–EtOH (1:0 ⟶ 4:1, v/v) and lyophilization of the eluate gave *compound* (**17**) (66 mg, 32.5%) as a white powder (Found: C, 47.3; H, 5.3; N, 12.6. $C_{27}H_{31}N_6O_8SNa\cdot\frac{7}{2}H_2O$ requires C, 47.3; H, 5.6; N, 12.3%); $\nu_{max.}$(KBr) 3 400, 1 710, 1 675, 1 510, 1 390, and 1 365 cm^{-1}; δ_H(90 MHz, D_2O) 1.35 (3 H, t, *J* 7 Hz, CH_3), 2.63—3.75 (12 H, m, CH_2N, CH_2S, and 4-H), 3.51—3.88 (1 H, m, 5-H), 3.95—4.20 (1 H, m, 7-H), 5.58 [1 H, s, COCH(N)Ph], and 7.59 (5 H, s, Ph); $[\alpha]_D^{23}$ −39.5° (*c* 0.21, H_2O).

Sodium (5S,7S)-3-(2-*Acetamidoethylthio*)-7-[2-(2-*aminothiazol*-4-*yl*)-(*Z*)-2-*methoxyiminoacetamido*]-8-*oxo*-1-*azabicyclo*[3.3.0]*oct*-2-*ene*-2-*carboxylate* (**18**).—Treatment of compound (**14a**) (200 mg, 0.326 mmol) with 2-(2-chloroacetamidothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl chloride hydrochloride[8] (130 mg, 0.391 mmol) in a similar manner to that described for the preparation of (**17**) gave *compound* (**18**) (65 mg, 39.5%) as a white powder (Found: C, 39.0; H, 4.7; N, 14.7. $C_{18}H_{21}N_6O_6S_2Na\cdot 3H_2O$ requires C, 38.7; H, 4.9; N, 15.05%); $\nu_{max.}$(KBr) 3 300br, 1 690, 1 650, 1 595, 1 530, 1 415, and 1 390 cm^{-1}; δ_H(90 MHz; D_2O) 2.08 (3 H, s, CH_3), 2.68—3.28 (4 H, m, CH_2S and 4-H), 3.30—3.60 (2 H, m, CH_2N), 4.06 (3 H, s, CH_3), 5.00—5.35 (2 H, m, 5-H and 7-H), and 7.25 (1 H, s, thiazole); $[\alpha]_D^{23}$ −28.3° (*c* 0.205, H_2O).

(5S,7S)-7-[(2R)-2-(4-*Ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-*phenylacetamido*]-2-(4-*nitrobenzyloxycarbonyl*)-3-[2-(4-*nitrobenzyloxycarbonylamino*)*ethylthio*]-1-*azabicyclo*[3.3.0]*oct*-2-*en*-8-*one* (**19**).—Trifluoroacetic acid (6 ml) was added to a solution of the bicyclic compound (**14b**) (300 mg, 0.447 mmol) in CH_2Cl_2 (6 ml) at 5 °C. The mixture was stirred for 2 h at 5 °C. The solution was evaporated to dryness and the residue was dissolved in aqueous sodium hydrogen carbonate, and was extracted twice with CH_2Cl_2. The combined extracts were dried over sodium sulphate and concentrated under reduced pressure. To the concentrate was added a mixture of THF (5 ml) and the HOBt ester of EPPA, which was prepared from HOBt (66 mg, 0.491 mmol), the acid (157 mg, 0.491 mmol), and DCC (111 mg, 0.536 mmol). The mixture was stirred for 4 h at room temperature, diluted with ethyl acetate, washed successively with water and brine, and then dried; it was then evaporated to dryness, and the residue was subjected to chromatography on silica gel. Gradient elution with $CHCl_3$–ethyl acetate (3:1, v/v) ⟶ $CHCl_3$–ethyl acetate–MeOH (5:5:1, v/v) gave compound (**19**) (400 mg, 98.0%) as a yellow powder; this crude product was used in the next reaction without further purification, m.p. 130—141 °C; $\nu_{max.}$(KBr) 3 320, 1 720, 1 680, 1 625, 1 515, and 1 350 cm^{-1}; δ_H(400 MHz; $CDCl_3$) 1.21 (3 H, t, *J* 7.2 Hz, CH_3), 1.53—1.64 (1 H, m, 6-H), 2.65—3.66 (9 H, m, 4-H, 6-H, CH_2N and CH_2S), 3.55 (2 H, q, *J* 7.2 Hz, CH_2N), 3.93—4.17 (2 H, m, CH_2N), 4.36—4.47 (1 H, m, 5-H), 4.74—4.83 (2 H, m, 7-H), 5.18 (2 H, s, OCH_2Ar), 5.24 and 5.45 (1 H × 2, d, *J* 13.6 Hz, OCH_2Ar × 2), 5.46 [1 H, d, *J* 6.4 Hz, COCH(N)Ph], 7.31—7.64 (9 H, m, Ar), 8.17—8.25 (4 H, m, Ar), and 9.93 (1 H, d, *J* 6.4 Hz, CONH); $[\alpha]_D^{24}$ −22.7° (*c* 0.075, $CHCl_3$).

(5S,7S)-3-(2-*Aminoethylthio*)-2-*carboxy*-7-[(2R)-2-(4-*ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-*phenylacetamido*]-1-*azabicyclo*[3.3.0]*oct*-2-*en*-8-*one* (**20**).—A mixture of the ester (**19**) (150 mg, 0.172 mmol) and 10% palladium–charcoal (150 mg), ethyl acetate (10 ml), and phosphate buffer (pH 7) (10 ml) was stirred for 2 h at room temperature under hydrogen. The catalyst was filtered off, washed with water, and the combined filtrate and washings were adjusted at pH 4 with 1M hydrochloric acid. The resulting solution was washed with ether and concentrated under reduced pressure. The concentrate was subjected to chromatography on Amberlite XAD-II. Gradient elution with water–EtOH (1:0 ⟶ 1:1, v/v), and lyophilization of the eluate gave the compound (**20**) (32 mg, 33.3%) as a colourless powder; secondary ion mass spectral analysis (s.i.m.s.) *m/z* 559 (*M* + 1) and 581 (*M* + Na); $\nu_{max.}$(KBr) 3 400, 1 705, 1 670, 1 500, 1 390, and 1 180 cm^{-1}; δ_H(90 MHz; D_2O) 1.26 (3 H, t, *J* 7 Hz, CH_3), 1.70—2.50 (2 H, m, 6-H), 2.55—3.10 (2 H, m, 4-H), 2.98—3.45 (2 H, m, CH_2S), 3.42—3.90 (4 H, m, CH_2N), 3.90—4.30 (2 H, m, CH_2N), 4.20—5.00 (2 H, m, 5-H and 7-H), 5.60 [1 H, s, COCH(N)Ph], and 7.58 (5 H, s, Ar); $[\alpha]_D^{26}$ −40.0° (*c* 0.07, H_2O); $\lambda_{max.}$(H_2O) 283 nm (ε 9 540).

(5S,7S)-3-(2-*Aminoethylsulphonyl*)-2-*carboxy*-7-[(2R)-2-(4-*ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-*phenylacetamido*-1-*azabicyclo*[3.3.0]*oct*-2-*en*-8-*one* (**21**).—*m*-Chloroperbenzoic acid (107 mg, 0.619 mmol) and sodium hydrogen carbonate (52 mg, 0.619 mmol) were added to an aqueous solution

of the crude bicyclic compound (**20**), which was prepared from the ester (**19**) (180 mg, 0.206 mmol). The mixture was stirred for 3 h at room temperature, and adjusted to pH 4 with 1M hydrochloric acid. The solution was concentrated under reduced pressure, and the concentrate was subjected to chromatography on Amberlite XAD-II. Gradient elution with water–EtOH (1:0 ⟶ 1:1, v/v) and lyophilization of the eluate gave the compound (**21**) (28 mg, 23%) as a colourless powder; s.i.m.s. *m/z* 591 (*M* + 1); $\nu_{max.}$(KBr) 3 400, 1 715, 1 670, and 1 510 cm^{-1}; δ_H(90 MHz; D_2O) 1.25 (3 H, t, *J* 7 Hz, CH_3), 2.42—3.15 (2 H, m, 4-H), 3.43—3.90 (6 H, m, CH_2N and CH_2SO_2), 3.90—4.25 (2 H, m, CH_2N), 5.58 [1 H, s, COCH(N)Ph], and 7.58 (5 H, s, Ph); $[\alpha]_D^{26}$ −50.0 (*c* 0.06, H_2O); $\lambda_{max.}$(H_2O) 271 nm (ε 11 800).

(5R,7S)-7-[(2R)-2-(4-*Ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-*phenylacetamido*]-2-(4-*nitrobenzyloxycarbonyl*)-3-[2-(4-*nitrobenzyloxycarbonylamino*)*ethylthio*]-1-*azabicyclo*[3.3.0]*oct*-2-*en*-8-*one* (**22**). Treatment of the bicyclic compound (**16**) (180 mg, 0.268 mmol) in a similar manner to that described for the preparation of the corresponding *cis*-isomer (**19**) gave compound (**22**) (185 mg, 79.1%) as a pale yellow powder; this crude product was used in the next reaction without further purification, m.p. 135—138 °C; $\nu_{max.}$(KBr) 3 400, 1 715—1 670br, 1 510, and 1 340 cm^{-1}; δ_H(400 MHz; $CDCl_3$) 1.08 (3 H, t, *J* 7.2 Hz, CH_3), 2.85—2.97 (3 H, m, 6-H and CH_2S), 3.12—3.40 (7 H, m, 6-H, 4-H and CH_2N), 3.52—3.59 (2 H, m, CH_2N), 3.87, 3.95 (2 H, m, CH_2N), 4.47—4.63 (2 H, m, 5-H and 7-H), 5.17 (2 H, s, OCH_2Ar), 5.31 and 5.46 (1 H × 2, d, *J* 13.9 Hz, OCH_2Ar × 2), 5.49 [1 H, d, *J* 7.6 Hz, COCH(N)Ph], 7.28—7.77 (9 H, m, Ar), and 8.13—8.25 (4 H, m, Ar).

Sodium (5R,7S)-3-(2-*Acetamidoethylthio*)-7-[(2R)-2-(4-*ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-*phenylacetamido*]-8-*oxo*-1-*azabicyclo*[3.3.0]*oct*-2-*ene*-2-*carboxylate* (**23**).—A mixture of the ester (**22**) (140 mg, 0.160 mmol), 10% palladium–charcoal (140 mg), ethyl acetate (10 ml), and phosphate buffer (pH 7, 10 ml) was stirred for 2 h at room temperature under hydrogen. The catalyst was filtered off and washed with water. To the combined filtrate and washings was added a solution of acetic anhydride (0.1 ml) in dry THF (20 ml) at room temperature. The mixture was stirred for 2 h at room temperature and washed with ethyl acetate; the aqueous solution was concentrated under reduced pressure, and the residue subjected to chromatography on Amberlite XAD-II. Gradient elution with water–EtOH (1:0 ⟶ 1:1, v/v) and lyophilization of the eluate gave compound (**23**) (28 mg, 28.0%) as a colourless powder; s.i.m.s. *m/z* 623 (*M* + 1); $\nu_{max.}$(KBr) 3 400, 1 710, 1 670, 1 590, 1 390, 1 365, and 1 180 cm^{-1}; δ_H(90 MHz; D_2O) 1.25 (3 H, t, *J* 7 Hz, CH_3), 2.05 (3 H, s, CH_3), 2.62—3.20 (2 H, m, 4-H), 3.33—3.88 (4 H, m, CH_2N), 3.96—4.25 (2 H, m, CH_2N), 5.33 [1 H, s, COCH(N)Ph], and 7.58 (5 H, s, Ph); $[\alpha]_D^{24}$ −38.4° (*c* 0.185, H_2O); $\lambda_{max.}$(H_2O) 274 nm (ε 11 070).

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