

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF C-2 ALKENYLTHIO-CARBAPENEM DERIVATIVES[†]

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A number of C-2 aminoalkenylthio-carbapenem derivatives possessing both the (5*R*,6*R*,8*S*)- and the (5*R*,6*S*,8*R*)-stereochemistries have been prepared from the olivanic acids MM 22382 and MM 22383, respectively. Certain members of this new class of compounds displayed potent, broad spectrum antibacterial activity as well as improved stability to human kidney homogenate.

Since the isolation of the olivanic acids^{1,2)} and thienamycin³⁾ from soil microorganisms, a wide range of structurally related natural products have been reported.⁴⁾ In general these streptomycete metabolites, collectively known as the carbapenem antibiotics, exhibit a high degree of activity against a broad range of Gram-positive and Gram-negative bacteria.

Although most carbapenems display remarkable stability towards bacterial β -lactamase, they are extensively metabolised by the renal dipeptidase enzyme, dehydropeptidase I (DHP-I).⁵⁾ These compounds give only low urinary recoveries and, in some instances, are nephrotoxic.^{5,6)}

The metabolic instability of imipenem (*N*-formimidoylthienamycin; MK 0787)⁷⁾ has been overcome by co-administration with cilastatin (MK 0791),⁶⁾ which is a potent inhibitor of the β -lactam destroying renal dipeptidase enzyme.

Our aim was to prepare by chemical modification of the olivanic acids a broad spectrum antibacterially active carbapenem derivative which showed good stability to the DHP-I enzyme. Our preceding paper⁸⁾ described the preparation of a number of (5*R*,6*R*,8*S*)- and (5*R*,6*S*,8*R*)- C-2 carboxyethenylthio-carbapenem derivatives from MM 22382 and MM 22383, after inversion of configuration at C-8 in the latter case. Whilst they were extremely active against a broad range of Gram-positive and Gram-negative bacteria, they were only weakly active against *Pseudomonas* sp. The low level of antipseudomonal activity may be attributed to poor penetration of the bacterial cell, which in turn may be due to the bulky nature of the C-2 side chain. We therefore turned our attention to the preparation of carbapenem derivatives with smaller side chains which retained both the (*Z*)-ethenylthio-substituent and the hydrophilic amino-group. These features we felt were desirable for both tissue stability and antipseudomonal activity.

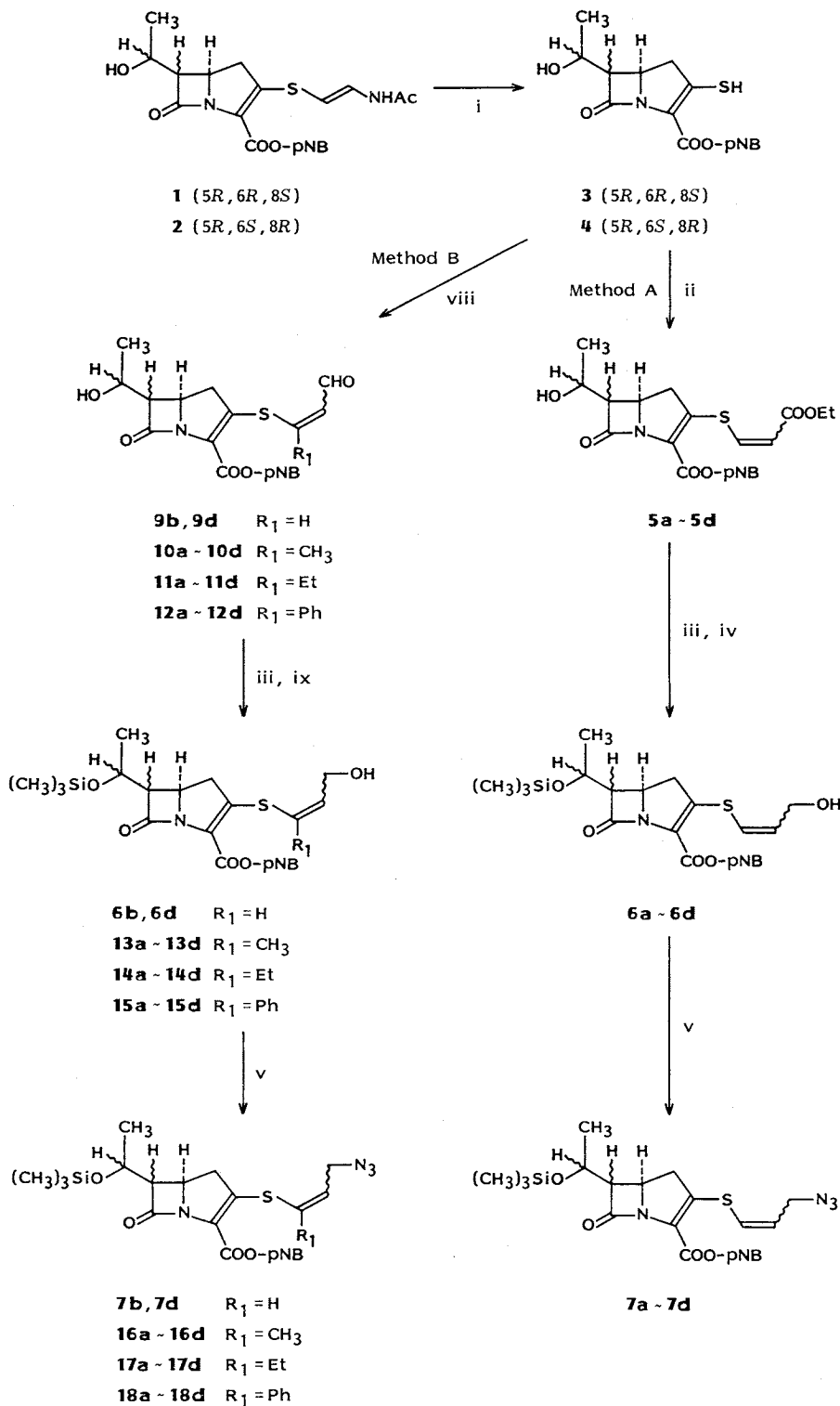
The present paper describes the preparation and some pertinent *in vitro* biological properties of a series of C-2 (*Z*)- and (*E*)-aminoalkenylthio-carbapenem derivatives.

Chemistry

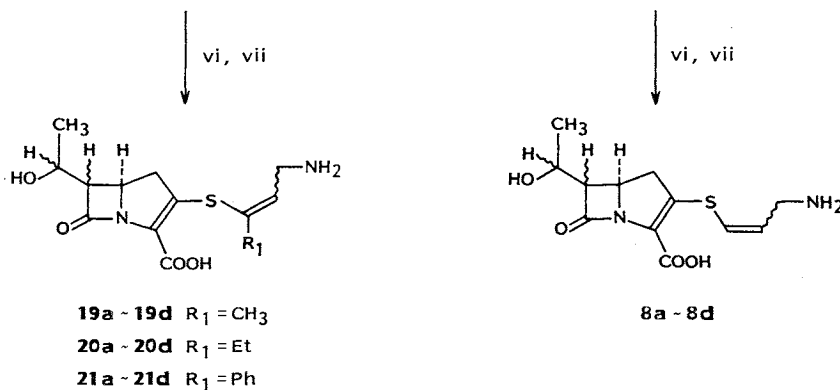
The aminopropenyl derivatives **8a** and **8c** were prepared from the *p*-nitrobenzyl esters of MM 22382 (**1**) and *N*-acetyldehydrothienamycin (**2**), respectively, by the procedure outlined in Scheme 1 (Method A). Compound **2**, which was the starting material for the synthesis of all the (5*R*,6*S*,8*R*)-derivatives, was prepared from MM 22383 by the previously reported C-8 inversion procedure.⁹⁾

[†] Numbering based on 'trivial' carbapenem nomenclature. For nomenclature of β -lactam antibiotics, see ref 15.

Scheme 1.



Scheme 1. (Continued)



pNB: *p*-Nitrobenzyl.

(a) (5*R*,6*R*,8*S*)-; (*Z*)-isomer, (b) (5*R*,6*R*,8*S*)-; (*E*)-isomer, (c) (5*R*,6*S*,8*R*)-; (*Z*)-isomer, (d) (5*R*,6*S*,8*R*)-; (*E*)-isomer.

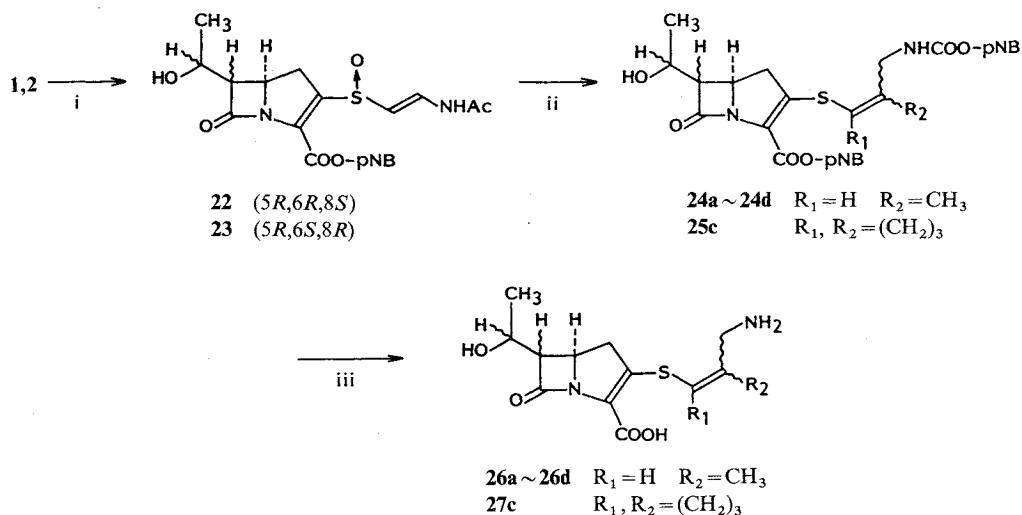
Reagents: i) *N*-Bromoacetamide, aq 1,4-dioxan, room temperature, 5 minutes; ii) $\text{HC}\equiv\text{CCOOEt}$, K_2CO_3 , DMF, room temperature, 15 minutes; iii) $(\text{CH}_3)_3\text{SiCl}$, pyridine, room temperature, 30 minutes; iv) $^i\text{Bu}_2\text{AlH}$, THF, 0°C , 1.5 hours; v) PPh_3 , HN_3 , $^i\text{PrOOCN}=\text{NCOO}^i\text{Pr}$, THF, 0°C then room temperature, 2 hours; vi) H_2 , 5% Pd/C, aq 1,4-dioxan, 0.05 M pH 7.0 potassium phosphate buffer, room temperature, 1.5 hours; vii) pH 2.5, 0.5 minute, then pH 7.0; viii) $\text{R}_1\text{C}\equiv\text{CCHO}$, K_2CO_3 , DMF, room temperature, 20 minutes; ix) NaBH_4 , 20% aq THF, 40°C , 15 minutes.

Addition of the C-2 thiol (**3**),¹⁰ to ethyl propiolate afforded as the major product the (*Z*)-ethoxycarbonylethenylthio-derivative (**5a**) in 31% yield. The configurations of the geometrical isomers were unambiguously assigned by ^1H NMR on the basis of the coupling constants for the vinylic protons; $J_{\text{CH}=\text{CH}}$ for the *cis*-isomer (**5a**) is typically 10 Hz whilst $J_{\text{CH}=\text{CH}}$ for *trans*-compounds is in the order of 13~16 Hz. TMS protection of the C-8 hydroxyl group in **5a**, followed by diisobutylaluminium hydride (DIBAH) reduction provided the allylic alcohol (**6a**) in low yield (23%). Elaboration of **6a** by the procedure outlined in Scheme 1, Method A gave the amino acid (**8a**). In an attempt to overcome the low yielding DIBAH reduction, the thiol (**3**) was reacted with 3-trimethylsilylpropargaldehyde. The sole product was the (*E*)-isomer of the α,β -unsaturated aldehyde (**9b**) ($J_{\text{CH}=\text{CH}}$ 16 Hz) in 24% yield. Silylation, followed by sodium borohydride reduction provided the allylic alcohol (**6b**) (52%), which was progressed to the (*E*)-amino acid (**8b**) (Scheme 1).

A similar series of reactions commencing with the *p*-nitrobenzyl ester of *N*-acetyldehydrothienamycin afforded the (5*R*,6*S*,8*R*)-amino acids (**8c** and **8d**).

The 3-amino-1-methylprop-1-en-1-ylthio-carbapenem derivatives (**19a**~**19d**) were prepared by the procedure outlined in Scheme 1 (Method B). Addition of thiol (**3**) to but-2-yn-1-al gave an isomeric mixture of α,β -unsaturated aldehydes (**10a** and **10b**) (ratio 5:1) in 66% yield. The major product was assumed to be the (*Z*)-isomer (**10a**) based on a comparison of calculated and observed ^1H NMR chemical shifts for the vinylic proton in the two isomers.¹¹ The geometrical isomers could be separated by careful silica gel column chromatography and progression of each isomer individually gave amino acids (**19a** and **19b**). Similarly, reaction of thiol (**4**) with but-2-yn-1-al provided, after further elaboration, amino acids (**19c** and **19d**).

Scheme 2.

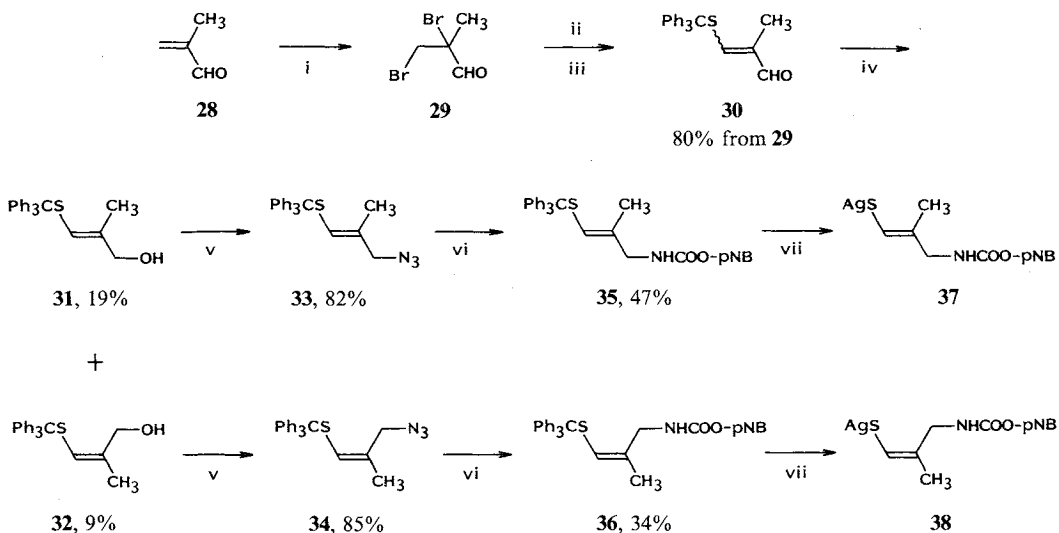


pNB: *p*-Nitrobenzyl.

(a) (5*R*,6*R*,8*S*)-; (*Z*)-isomer, (b) (5*R*,6*R*,8*S*)-; (*E*)-isomer, (c) (5*R*,6*S*,8*R*)-; (*Z*)-isomer, (d) (5*R*,6*S*,8*R*)-; (*E*)-isomer.

Reagents: i) *m*-Chloroperbenzoic acid, CH_2Cl_2 ; ii) $AgSC(R_1)=C(R_2)CH_2NHCOOpNB$, CH_3CN , NaI (10 equiv), $5^\circ C$, 20 minutes; iii) see vi) in Scheme 1.

Scheme 3.

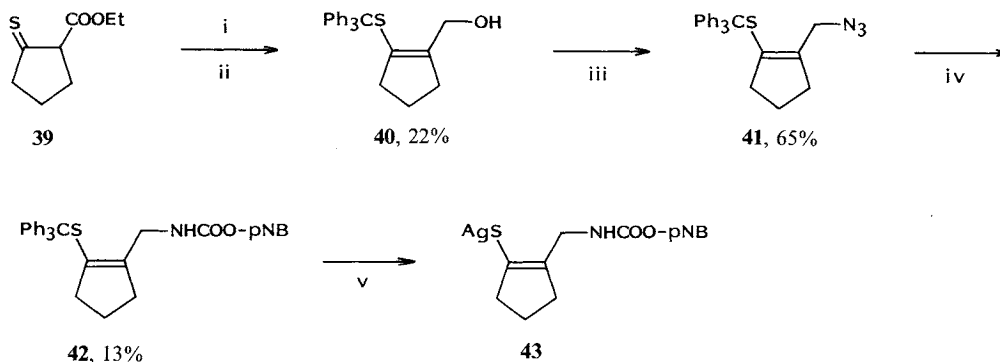


pNB: *p*-Nitrobenzyl.

Reagents: i) Br_2 , CCl_4 , room temperature; ii) DBU (1 equiv), DMF, room temperature, 4 hours; iii) Ph_3CSNa , DMF, room temperature, 1.5 hours; iv) $NaBH_4$, aq THF, $-40^\circ C$, 1 hour; v) diethylazodicarboxylate, PPh_3 , HN_3 , room temperature, 15 minutes; vi) (a) PPh_3 , $70^\circ C$, 1 hour, (b) $ClCOOpNB$, toluene, $5^\circ C$, 30 minutes, (c) $NaHCO_3$ (excess); vii) $AgNO_3$, pyridine, CH_3OH , room temperature, 3 hours.

Abbreviation: DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene.

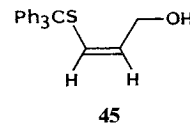
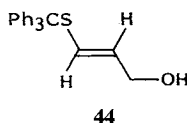
Scheme 4.



pNB: *p*-Nitrobenzyl.

Reagents: i) (a) NaH, THF, room temperature, 1 hour; (b) Ph_3CCl , THF, 2 hours; ii) diisobutylaluminium hydride, THF, room temperature, 1 hour; iii, iv) and v) as in (v), (vi) and (vii) of Scheme 3.

Utilisation of the sulfoxide displacement procedure reported by Sanraku-Ocean Co., Ltd.¹²⁾ provided an entry to the previously inaccessible 2-substituted and 1,2-disubstituted aminoalkenylthio-carbapenem derivatives (Scheme 2). Thus, reaction of sulfoxides (22 and 23) with silver salts (37 and 38), and subsequent hydrogenolysis furnished the 3-amino-2-methylprop-1-en-1-ylthio-carbapenem derivatives (26a~26d). Yields for the sulfoxidation/sulfoxide displacement reaction sequence ranged from 21 to 48% whilst yields for the hydrogenolyses ranged from 13 to 45%. The silver salts (37 and 38) were prepared from methacrolein (28) by the procedure outlined in Scheme 3. Bromination of 28, followed by dehydrobromination and reaction with NaSCPh_3 gave the isomeric mixture of aldehydes (30), which upon sodium borohydride reduction provided the separable alcohols 31 and 32.

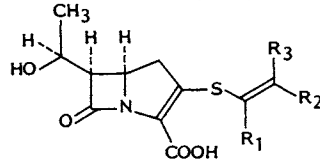


The geometries of the double bonds in products 31 and 32 were assigned by virtue of a comparison of their ^1H NMR chemical shifts with those of allylic alcohols (44 and 45), whose geometries were unambiguously assigned on the basis of their $J_{\text{CH}=\text{CH}}$ coupling constants. Additional confirmation of these assignments was provided by a comparison of the calculated and observed ^1H NMR chemical shifts for the vinylic protons in esters (24a~24d).¹¹⁾ Elaboration of alcohols (31 and 32) provided silver salts 37 and 38.

Reaction of sulfoxide (23) with silver salt (43), prepared from ethyl 2-thionocyclopentanecarboxylate (39)¹³⁾ (Scheme 4), provided the 2-aminomethylcyclopent-1-enylthio-derivative (27c) after hydrogenolysis (Scheme 2).

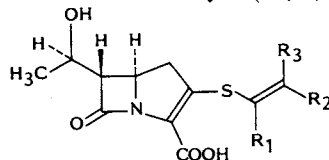
There was evidence that the silver salts 37, 38, and 43 degraded upon isolation. They were therefore freshly prepared from the respective tritylthio-precursors 35, 36, and 42, (2 molar equivalents) and used immediately in the sulfoxide displacement reaction.

The amidines (Table 1, entries 47 and 48) were prepared by the procedure outlined in the preceding paper.⁸⁾

Table 1. *In vitro* antibacterial activity of (5*R*,6*R*,8*S*) derivatives.

Compound	R ₁	R ₂	R ₃	Antibacterial activity (μg/ml)																Human kidney stability (% left after 1 hour)
				<i>E.cl.</i> N1	<i>E.c.</i> JT39 (R ⁺)	<i>E.c.</i> JT39 (R ⁺)	<i>K.p.</i> A	<i>P.m.</i> 977	<i>P.v.</i> W091	<i>P.a.</i> A	<i>P.a.</i> Fr11	<i>P.a.</i> NCTC 10662	<i>P.a.</i> T447B	<i>S.m.</i> US20	<i>S.a.</i> Ox	<i>S.a.</i> Ru (R ⁺)	<i>S.a.</i> 1517	<i>S.f.</i> I	<i>S.p.</i> CN10	
8a	H	H	CH ₂ NH ₂	0.4	≤0.1	0.2	0.2	0.8	0.4	6.2	3.1	6.2	6.2	0.4	≤0.1	≤0.1	1.6	≤0.1	—	40
8b	H	CH ₂ NH ₂	H	0.4	0.2	≤0.1	0.2	0.8	3.1	12.5	3.1	6.2	6.2	0.2	≤0.1	≤0.1	3.1	≤0.1	≤0.1	7
19a	CH ₃	H	CH ₂ NH ₂	1.6	≤0.1	≤0.1	0.2	3.1	6.2	25	—	—	—	1.6	≤0.1	≤0.1	6.2	≤0.1	≤0.1	51
19b	CH ₃	CH ₂ NH ₂	H	≤0.1	≤0.1	0.8	0.8	0.8	1.6	25	12.5	12.5	—	0.8	≤0.1	≤0.1	1.6	0.2	≤0.1	13
21a	Ph	H	CH ₂ NH ₂	6.2	3.1	1.6	1.6	3.1	6.2	100	—	100	—	6.2	0.2	0.4	12.5	0.4	≤0.1	63
26a	H	CH ₃	CH ₂ NH ₂	1.6	0.2	6.2	0.8	6.2	12.5	25	25	25	—	0.8	<0.1	<0.1	12.5	<0.1	<0.1	7
26b	H	CH ₂ NH ₂	CH ₃	—	0.2	1.6	0.8	3.1	6.2	25	25	25	—	1.6	<0.1	0.2	6.2	0.2	<0.1	0
46	H	H	CH ₂ OH	0.8	0.2	3.1	0.2	0.4	1.6	100	—	—	—	3.1	0.2	0.2	6.2	0.4	—	—
47	H	H		0.4	≤0.1	0.4	0.8	3.1	6.2	6.2	—	—	—	0.4	≤0.1	≤0.1	0.8	≤0.1	—	20
48	H	H		0.8	0.2	0.4	0.4	1.6	3.1	12.5	—	—	—	0.8	≤0.1	0.1	0.8	≤0.1	≤0.1	42
MK 0787	—	—	—	—	<0.1	0.4	0.4	6.2	1.6	3.1	0.8	3.1	1.6	0.8	<0.1	<0.1	0.8	0.2	<0.1	42
MM 22382	—	—	—	3.1	0.2	25	0.4	0.8	0.8	>100	—	—	—	3.1	0.4	0.4	6.2	1.6	0.05	0

Abbreviations: *E.cl.*, *Enterobacter cloacae*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *P.v.*, *P. vulgaris*; *P.a.*, *Pseudomonas aeruginosa*; *S.m.*, *Serratia marcescens*; *S.a.*, *Staphylococcus aureus*; *S.f.*, *Streptococcus faecalis*; *S.p.*, *S. pneumoniae*; R⁺, denotes resistance to ampicillin.

Table 2. *In vitro* antibacterial activity of (5R,6S,8R) derivatives.

Compound	R ₁	R ₂	R ₃	Antibacterial activity (μg/ml)															Human kidney stability (% left after 1 hour)	
				<i>E.cl.</i> N1	<i>E.c.</i> 0111	<i>E.c.</i> JT39 (R ⁺)	<i>K.p.</i> A	<i>P.m.</i> 977	<i>P.v.</i> W091	<i>P.a.</i> A	<i>P.a.</i> Fr11	<i>P.a.</i> NCTC 10662	<i>P.a.</i> T447B	<i>S.m.</i> US20	<i>S.a.</i> Ox	<i>S.a.</i> Ru (R ⁺)	<i>S.a.</i> 1517	<i>S.f.</i> I		<i>S.p.</i> CN10
8c	H	H	CH ₂ NH ₂	1.2	0.3	0.3	0.3	2.5	1.2	25	—	6.2	3.1	1.6	0.04	0.08	0.4	12.5	≤0.01	40
8d	H	CH ₂ NH ₂	H	1.6	0.4	0.2	0.4	3.1	1.6	12.5	1.6	12.5	—	0.4	≤0.1	≤0.1	0.8	0.4	≤0.1	8
19c	CH ₃	H	CH ₂ NH ₂	0.4	0.1	3.1	0.4	6.2	12.5	25	12.5	25	3.1	—	<0.05	0.1	6.2	0.8	≤0.05	56
19d	CH ₃	CH ₂ NH ₂	H	3.1	0.4	0.4	0.8	6.2	1.6	25	12.5	25	—	1.6	≤0.1	≤0.1	12.5	1.6	≤0.1	23
20c	Et	H	CH ₂ NH ₂	6.2	<0.1	0.8	0.2	3.1	1.6	50	25	25	—	1.6	<0.1	<0.1	6.2	0.8	<0.1	28
26c	H	CH ₃	CH ₂ NH ₂	3.1	0.2	0.4	1.6	6.2	3.1	25	25	25	—	1.6	<0.1	<0.1	25	<0.1	<0.1	1
26d	H	CH ₂ NH ₂	CH ₃	3.1	0.2	0.8	0.8	6.2	3.1	25	25	25	—	—	<0.1	<0.1	0.2	0.4	0.8	14
27c	—	(CH ₂) ₃	CH ₂ NH ₂	0.2	1.6	1.6	1.6	12.5	3.1	50	—	—	—	25	<0.1	<0.1	<0.1	0.8	<0.1	4
MM 22383	—	—	—	25	12.5	12.5	6.2	12.5	6.2	>100	—	—	—	12.5	6.2	6.2	100	50	1.6	0
MK 0787	—	—	—	—	<0.1	0.4	0.4	6.2	1.6	3.1	0.8	3.1	1.6	0.8	<0.1	<0.1	0.8	0.2	<0.1	42

Abbreviations: See footnote in Table 1.

Results and Discussion

The antibacterial activities and the stabilities to human kidney homogenate of the aminoalkenylthio-carbapenem derivatives with the (5*R*,6*R*,8*S*)- and (5*R*,6*S*,8*R*)-stereochemistries are shown in Tables 1 and 2, respectively. Most of these compounds were highly active against a broad range of penicillin-sensitive and penicillin-resistant Gram-positive and Gram-negative bacteria and showed significant improvements over the parent compounds, MM 22382 and MM 22383, particularly in the latter series.

Of this new class of compounds, the unsubstituted 3-aminoprop-1-en-1-ylthio-derivatives (**8a**~**8d**) displayed the most potent antibacterial activity. With the exception of *Pseudomonas aeruginosa*, their level of activity was similar to that of MK 0787; in general they were 2- to 4-fold less active against *Pseudomonas* sp. Unlike other series,^{8,14} little difference in antibacterial activity was observed between the (*Z*)-isomers **8a** and **8c** and the (*E*)-isomers **8b** and **8d**. A striking difference was however observed in their stabilities to human kidney homogenate. In the case of the (*Z*)-isomers (**8a** and **8c**), 40% of each compound remained after 1 hour when incubated with human kidney homogenate. In contrast, only 7% and 8%, respectively of the (*E*)-isomers (**8b** and **8d**) remained in a similar test.

Preparation of the amidines (**47** and **48**) failed to improve either the antibacterial activity or the tissue stability of the parent amine (**8a**).

Whilst substitution α - to the sulfur atom in the C-2 aminoalkenylthio-derivatives provided compounds with improved stability to human kidney homogenate, they were generally less active *in vitro*, particularly against *Pseudomonas* sp. For example the 1-methyl substituted derivative with the (*Z*)-configuration (**19c**) showed 56% recovery in the tissue stability test (compared with 42% for MK 0787), but was approximately 8-fold less active than MK 0787 against *P. aeruginosa*.

Substitution β - to the sulfur atom of the aminoalkenylthio side chain produced compounds not only with reduced antibacterial potency, but also with inferior stability to human kidney homogenate (**26a**~**26d**).

The disubstituted derivative (**27c**) also displayed poor stability to human kidney homogenate, as well as poor activity against *Pseudomonas* organisms.

Conclusion

In the unsubstituted derivatives (**8a**, **8c**) and the 1-methyl substituted derivatives (**19a**, **19c**) we had achieved our objective of preparing compounds which combined potent antibacterial activity with improved stability to kidney homogenate. Activities against *P. aeruginosa* were significantly better than those of MM 22382 and MM 22383. However, the overall profile of these compounds offered little advantage over MK 0787 and for this reason were not progressed.

Experimental

MM 22382 and MM 22383²⁾ were prepared by fermentation of *Streptomyces olivaceus* ATCC 31365 as described previously. MK 0787 was a gift from Merck Sharp & Dohme Laboratories, Rahway, New Jersey, U.S.A.

MIC's

The compounds were serially diluted in 0.05 ml volumes of Nutrient broth No. 2 (Oxoid) using microtitre equipment (Dynatech). All microtitre trays were inoculated with a multipoint inoculator (Denley) which delivered 0.001 ml of a 1/10 dilution of an overnight broth culture of the test organism; an

inoculum equivalent to 10^6 cfu/ml. The MIC was determined as the lowest concentration of antibiotic preventing visible microbial growth after incubation at 37°C for 18 hours.

Tissue Stability Studies

30% w/v Human kidney was homogenised and sonicated in 0.02 M Tris-HCl pH 7.0 buffer containing 5% Triton X-100 and then centrifuged at $1,500 \times g$ for 15 minutes. The supernatant was stored at -20°C until used. The kidney preparation and the β -lactam derivative were warmed separately to 37°C for 5 minutes before mixing equal volumes of each (80 μl) at time 0. After incubation for 60 minutes at 37°C samples were assayed by HPLC (for conditions see preceding paper) and the results expressed as the percentage of compound remaining. Compounds were tested at a final concentration of 250 μM .

Chemistry: General

The *p*-nitrobenzyl ester of *N*-acetyldehydrothienamycin (2) was prepared from MM 22383 by the previously reported method.⁹⁾ Other spectroscopic and experimental techniques are described in the preceding paper.⁸⁾ Numbering in the experimental section is based on the 7-oxo-1-azabicyclo[3.2.0]hept-2-ene ring system according to systematic IUPAC nomenclature.¹⁵⁾ The purities of the sodium salts and zwitterions were determined by assay using the characteristic olivanic acid/thienamycin chromophore at λ_{max} nm 295~307 in the UV spectrum and were based on an estimated ϵ 12,000 for C-2 aminoalkenylthio-compounds.

General Procedure for Scheme 1, Method A

p-Nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-3-Hydroxyprop-1-en-1-ylthio]-6-[(*S*)-1-trimethylsilyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (6a)

p-Nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-2-ethoxycarbonylethenylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (5a) (0.757 g), prepared from MM 22382 by the general procedure outlined in the preceding paper,⁸⁾ was dissolved in pyridine (5 ml) and stirred at room temperature for 30 minutes with trimethylsilyl chloride (1.068 g). The solvent was evaporated at reduced pressure and the residue was partitioned between ethyl acetate and water. The ethyl acetate solution was washed with satd sodium chloride solution, dried (MgSO_4) and evaporated at reduced pressure. The crude product was chromatographed over silica gel. Elution with 75% ethyl acetate-hexane afforded the trimethylsilyl ether as a pale yellow oil (0.666 g; 76%); IR ν_{max} (CHCl_3) cm^{-1} 1782, 1708, 1608, 1580, 1559, 1521.

A portion of the above trimethylsilyl ether (0.360 g) was dissolved in dry THF (30 ml) and cooled to 0°C under an argon atmosphere. A solution of diisobutylaluminium hydride in toluene (25% w/v; 1.15 ml) was added to the stirred solution and stirring was continued for 1.5 hours. The reaction was quenched by the addition of aq ethanol and the solution was partitioned between ethyl acetate and water and filtered. The organic solution was washed with water, satd sodium chloride solution, dried (MgSO_4) and evaporated at reduced pressure. The residue was chromatographed over silica gel. Elution with 50% ethyl acetate-hexane afforded a quantity of unreacted starting material (0.040 g) followed by the desired *p*-nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-3-hydroxyprop-1-en-1-ylthio]-6-[(*S*)-1-trimethylsilyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (6a) (0.100 g; 30%) as an oil; IR ν_{max} (CHCl_3) cm^{-1} 3600, 1782, 1701, 1609, 1559, 1521; ^1H NMR (CDCl_3) δ 1.39 (3H, d, $J=7$ Hz, CH_3CH), 3.11 (1H, dd, $J=9$ and 18 Hz, 4- CH_a), 3.35~3.75 (2H, m, 4- CH_b + 6- CH), 4.0~4.5 (4H, m, $\text{CH}_2\text{OH} + 5\text{-CH} + 8\text{-CH}$), 5.22 (1H, d, $J=13.5$ Hz) and 5.49 (1H, d, $J=13.5$ Hz) (CH_2Ar), 6.06 (1H, dt, $J=6.5$ and 10 Hz, $\text{C}=\text{CHCH}_2$), 6.30 (1H, t, $J=10$ Hz, $\text{SCH}=\text{C}$), 7.62 (2H, d, $J=8.5$ Hz, Ar), 8.20 (2H, d, $J=8.5$ Hz, Ar).

p-Nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-3-Azidoprop-1-en-1-ylthio]-6-[(*S*)-1-trimethylsilyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (7a)

The alcohol 6a (0.078 g) was dissolved in dry THF (25 ml) and cooled to 0°C . Triphenylphosphine (0.137 g), hydrazoic acid (0.3 ml; 1.7 M solution in toluene) and diisopropylazodicarboxylate (0.106 g) were added to the solution, which was then stirred at room temperature for 2.25 hours. The solution was partitioned between ethyl acetate and water. The organic solution was washed with sodium bicarbonate solution, satd sodium chloride solution, dried (MgSO_4) and evaporated at reduced pressure. The crude

product was chromatographed over silica gel. Elution with 40% ethyl acetate-hexane afforded the title compound **7a** as a pale yellow oil (0.041 g; 50%); UV $\lambda_{\max}^{\text{EtOH}}$ nm 263, 322; IR ν_{\max} (CHCl₃) cm⁻¹ 2100, 1783, 1704, 1562, 1521; ¹H NMR (CDCl₃) δ 1.22 (3H, d, H₃CCH), 2.93 (1H, dd, *J* = 10 and 17 Hz, 4-CH_a), 3.4~4.0 (3H, m, 4-CH_b + CH₂N₃), 4.05~4.4 (2H, m, 5-CH + 8-CH), 5.15 (1H, d, *J* = 13.5 Hz) and 5.42 (1H, d, *J* = 13.5 Hz) (CH₂Ar), 5.89 (1H, dt, *J* = 7 and 9.5 Hz, C=CHCH₂), 6.45 (1H, d, *J* = 9.5 Hz, SCH=C), 7.55 (2H, d, *J* = 8.5 Hz, Ar), 8.11 (2H, d, *J* = 8.5 Hz, Ar).

(5*R*,6*R*)-3-[(*Z*)-3-Aminoprop-1-en-1-ylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (**8a**)

The azido-derivative (**7a**) (0.081 g) was dissolved in 1,4-dioxan (36 ml), water (8 ml) and 0.05 M, pH 7.0 phosphate buffer (12 ml) and shaken with hydrogen at ambient temperature and pressure for 1.5 hours in the presence of 5% palladium on carbon catalyst (0.120 g).

The suspension was then filtered over Celite, washing well with water (80 ml). The filtrate was concentrated to approximately 20 ml and washed with ethyl acetate (3 × 150 ml). The pH of the aqueous solution was adjusted to 2.5 by the addition of 1N HCl and immediately neutralised by the addition of 1N NaOH solution. The solution was then concentrated to small volume at reduced pressure and chromatographed over Diaion HP-20SS, eluting with water. Fractions containing the amino acid **8a**, identified by the absorption at λ_{\max} nm 301 in the UV spectrum, were combined to provide an aqueous solution of the product (approx 0.012 g based on ϵ 12,000; 27%).

(5*R*,6*R*)-3-[(*Z*)-3-Acetimidoylamino-prop-1-en-1-ylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (**48**)

An aqueous solution of (5*R*,6*R*)-3-[(*Z*)-3-aminoprop-1-en-1-ylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (**8a**) (12 mg in 5 ml) was cooled in an ice bath with stirring. The pH of the solution was adjusted to 9.0 by the addition of dilute sodium hydroxide solution. Ethyl acetimidate hydrochloride (0.101 g) was added portionwise over a period of 5 minutes, whilst maintaining the pH of the solution at 9.0. Stirring was continued for 30 minutes. The pH of the solution was then readjusted to 7.0 by the addition of dilute hydrochloric acid and chromatographed over Diaion HP-20, eluting with a gradient of 0~10% ethanol-water. Those fractions containing the product, identified by the absorption at λ_{\max} nm 302 in the UV spectrum, were combined to afford **48** as a white solid (0.015 g); UV $\lambda_{\max}^{\text{H}_2\text{O}}$ nm (ϵ) 302 (6,100); IR ν_{\max} (KBr) cm⁻¹ 3300 (br), 1750, 1685, 1640, 1585.

General Procedure for the Addition of the C-2 Thiol to Acetylenic Aldehydes and NaBH₄ Reduction of the Adduct (Scheme 1, Method B)

p-Nitrobenzyl (5*R*,6*R*)-3-[3-Oxo-1-methylprop-1-en-1-ylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**10a** and **10b**)

2-Butyn-1-ol (1.0 g) was dissolved in dry DMF (20 ml) and stirred vigorously for 16 hours at room temperature with activated manganese dioxide (5 g). After removal of the manganese dioxide by centrifuging, the crude solution of 2-butyne-1-ol was used in the next step.

p-Nitrobenzyl (5*R*,6*R*)-3-[(*E*)-2-acetamidoethenylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**1**) (0.250 g) was dissolved in 1,4-dioxan (10 ml) containing water (15 drops). A solution of *N*-bromoacetamide (0.078 g) in 1,4-dioxan (5 ml) was added and the solution was stirred at room temperature for 5 minutes. Chloroform (100 ml) was added and the organic solution washed with pH 7.0 phosphate buffer, brine and dried (MgSO₄). Removal of the solvent at reduced pressure afforded the crude thiol (**3**) as a colourless oil. This oil was dissolved in the solution of but-2-yn-1-ol in DMF. Anhydrous potassium carbonate (0.039 g) was added and the solution stirred at room temperature for 20 minutes. The solution was then partitioned between ethyl acetate and brine. The organic layer was dried (MgSO₄) and the solvent removed at reduced pressure to afford the crude product, which was chromatographed over silica gel. Elution with 80% ethyl acetate-hexane gave a mixture of *p*-nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-3-oxo-1-methylprop-1-en-1-ylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**10a**) and the corresponding (*E*)-isomer (**10b**) in the approximate ratio of 5 : 1, as a pale yellow gum (0.159 g; 62%); UV $\lambda_{\max}^{\text{EtOH}}$ nm 264, 317; IR ν_{\max} (CHCl₃) cm⁻¹ 3450, 1785, 1720, 1681, 1612,

1563, 1523; $^1\text{H NMR}$ (CDCl_3) δ 1.40 (3H, d, $J=6.5$ Hz, CH_3CH), 2.32 (major) and 2.46 (minor) (3H, each d, $J=1$ Hz, $\text{CH}_3\text{C}=\text{C}$), 2.98 (major) and 3.11 (minor) (1H, each dd, $J=9$ and 18 Hz, 4-CH_a), 3.52 (1H, dd, $J=9$ and 18 Hz, 4-CH_b), 3.64 (1H, dd, $J=6$ and 7.5 Hz, 6-CH), 4.15~4.50 (2H, m, $5\text{-H}+8\text{-H}$), 5.28 (1H, d), and 5.51 (1H, d) (CH_2Ar), 6.00 (minor) and 6.36 (major) (1H, each dd, $J=1$ and 7 Hz, $\text{CH}=\text{C}$), 7.64 (2H, d, $J=8.5$ Hz, Ar), 8.24 (2H, d, $J=8.5$ Hz, Ar), 9.83 (minor) and 10.08 (major) (1H, each d, $J=7$ Hz, CHO).

p-Nitrobenzyl (5*R*,6*R*)-3-[3-Hydroxy-1-methylprop-1-en-1-ylthio]-6-[(*S*)-1-trimethylsilyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (13*a*; *Z*-Isomer and 13*b*; *E*-Isomer)

The mixture of *p*-nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-3-oxo-1-methylprop-1-en-1-ylthio]-6-[(*S*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (10*a*) and the corresponding (*E*)-isomer (10*b*) (1.145 g) was dissolved in pyridine (10 ml) and stirred at room temperature for 1 hour with trimethylsilyl chloride (2.2 ml). The solvent was then evaporated at reduced pressure and the residue partitioned between ethyl acetate and water. The organic layer was washed with brine, dried (MgSO_4) and evaporated at reduced pressure to yield the crude product, which was chromatographed over silica gel. Elution with ethyl acetate-hexane (1:1) afforded the isomeric mixture of trimethylsilyl ethers as an oil (0.819 g); UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm 263, 318; IR ν_{max} (CHCl_3) cm^{-1} 1788, 1722, 1678, 1610, 1562, 1522, 842.

The above product was dissolved in THF (100 ml) and cooled to -40°C . A solution of sodium borohydride (0.302 g) in 20% aqueous THF (100 ml) was added and stirring was continued at this temperature for 15 minutes. The solution was then partitioned between ethyl acetate and water. The organic layer was washed with brine, dried (MgSO_4) and evaporated at reduced pressure. The residual gum was chromatographed over silica gel (50 g). Elution with a gradient of 25~50% ethyl acetate-hexane afforded the pure *p*-nitrobenzyl (5*R*,6*R*)-3-[(*Z*)-3-hydroxy-1-methylprop-1-en-1-ylthio]-6-[(*S*)-1-trimethylsilyloxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (13*a*) (0.517 g; 39%) as an oil; UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm 267, 316; IR ν_{max} (CHCl_3) cm^{-1} 1786, 1709, 1612, 1558, 1522, 848; $^1\text{H NMR}$ (CDCl_3) δ 0.11 (9H, s, $(\text{CH}_3)_3\text{Si}$), 1.32 (3H, d, $J=6.5$ Hz, CH_3CH), 1.81 (1H, br resonance, OH), 2.13 (3H, d, $J=1$ Hz, $\text{CH}_3\text{C}=\text{C}$), 2.87 (1H, dd, $J=10$ and 18 Hz, 4-CH_a), 3.56 (dd, $J=9$ and 18 Hz, 4-CH_b) and 3.59 (t, $J=6$ Hz, 6CH) (2H), 4.20~4.32 (2H, m, $5\text{-CH}+8\text{-CH}$), 4.36 (2H, dd, $J=7$ and ca. 1 Hz, CH_2OH), 5.27 (1H, d, $J=14.5$ Hz) and 5.51 (1H, d, $J=14.5$ Hz) (CH_2Ar), 6.21 (1H, dt, $J=1.5$ and 6.5 Hz, $\text{C}=\text{CH}$), 7.67 (2H, d, $J=8.5$ Hz, Ar) and 8.22 (2H, d, $J=8.5$ Hz, Ar).

Continued elution gave the (*E*)-isomer (13*b*) (0.169 g; 13%) as an oil; UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm 265, 317; IR ν_{max} (CHCl_3) cm^{-1} 3500 (br), 1780, 1705, 1611, 1555, 1522; $^1\text{H NMR}$ (CDCl_3) δ 1.30 (3H, d, $J=6$ Hz, CH_3CH), 2.02 (3H, s, $\text{CH}_3\text{C}=\text{C}$), 2.86 (1H, dd, $J=9.5$ and 18 Hz, 4-CH_a), 3.35~3.90 (2H, m, $4\text{-CH}_b+6\text{-CH}$), 4.05~4.40 (4H, m, $5\text{-CH}+8\text{-CH}+\text{CH}_2\text{OH}$), 5.21 (1H, d, $J=15$ Hz) and 5.48 (1H, d, $J=15$ Hz) (CH_2Ar), 6.21 (1H, br t, $J=6.5$ Hz, $\text{C}=\text{CH}$), 7.64 (2H, d, $J=8.5$ Hz, Ar), 8.19 (2H, d, $J=8.5$ Hz, Ar).

The above alcohols (13*a* and 13*b*) were then elaborated to the amino acids (19*a*; 13% and 19*b*; 16%), via the azido-derivatives (16*a*; 47% and 16*b*; 40%) by the procedure described for the preparation of 8*a* from 6*a*.

General Procedure for the Sulfoxide Displacement Reaction (Scheme 2)

p-Nitrobenzyl (5*R*,6*S*)-3-[(*E*)-2-Methyl-3-*N*-*p*-nitrobenzylxycarbonylamino-prop-1-en-1-ylthio]-6-[(*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (24*d*)

a) (*E*)-1-Tritylthio-2-methyl-3-*N*-*p*-nitrobenzylxycarbonylamino-prop-1-ene (0.283 g) (36) was dissolved in dry methanol (50 ml) and stirred at room temperature under an atmosphere of argon in the presence of pyridine (0.053 ml) and powdered silver nitrate (0.111 g). The yellow precipitate was collected by centrifugation, washed with methanol ($\times 2$), diethyl ether ($\times 2$) and dried under vacuum. The silver thiolate (38) thus obtained was sufficiently pure to use in the next stage.

b) The *p*-nitrobenzyl ester of *N*-acetyldehydrothienamycin (0.225 g) (2) was dissolved in 20% ethanol-dichloromethane (50 ml) and stirred at 5°C for 30 minutes with *m*-chloroperbenzoic acid (0.126 g; 80%). Triethylamine (0.047 ml) was added and the solvent was evaporated at reduced pressure. The residue was applied to a silica gel column and elution with a gradient of 0~10% ethanol-chloroform provided the sulfoxide (23) which was used in the next stage.

c) The sulfoxide (**23**) and the silver thiolate (**38**) were dissolved in dry acetonitrile (20 ml) and the solution cooled to 5°C. Sodium iodide (0.729 g) was added to the stirred solution and stirring was continued at 5°C for 20 minutes. The solution was partitioned between ethyl acetate and water. The organic phase was washed with satd sodium chloride solution, dried (MgSO₄) and evaporated at reduced pressure and the residue chromatographed over silica gel. Elution with a gradient of 50~75% ethyl acetate-hexane provided the title compound (**24d**) (0.067 g; 22%); UV $\lambda_{\text{max}}^{\text{CH}_3\text{CN}}$ nm (ϵ) 266 (24,888), 327 (17,748); IR ν_{max} (KBr) cm^{-1} 3415, 1776, 1700, 1606, 1518, 1347; ¹H NMR (DMF-*d*₇) δ 1.23 (3H, d, *J*=6 Hz, CH₃CH), 1.84 (3H, s, CH₃C=C), 3.2~3.5 (m, 4-CH₂), 3.43 (dd, *J*=2.5 and 6.5 Hz, 6-CH), 3.85 (2H, d, *J*=5.5 Hz, CH₂NH), 4.09 (1H, t, *J*=6 Hz, 8-CH), 4.29 (1H, br t, 5-CH), 5.28 (3H, s+br resonance, CH₂Ar+OH), 5.38 and 5.58 (each 1H, d, *J*=14 Hz, CH₂Ar), 6.38 (1H, s, CH=C), 7.70 (2H, d, *J*=8.5 Hz, Ar), 7.76 (1H, br resonance, NH), 7.85 (2H, d, *J*=8.5 Hz, Ar), 8.28 (4H, 2×d, *J*=8.5 Hz, Ar).

(5*R*,6*S*)-3-[(*E*)-2-Methyl-3-aminoprop-1-en-1-ylthio]-6-[(*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylic Acid (**26d**)

The *p*-nitrobenzyl protected intermediate (**24d**) (0.025 g) was deprotected as described for the preparation of **8a**, to yield an aqueous solution containing the zwitterion (**26d**) (estimated yield 0.004 g, 27%, based on ϵ 12,000 at λ_{max} nm 307 in UV spectrum).

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