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Carbapenem and Penem Antibiotics. I. Total Synthesis and Antibacterial Activity of *dl*-Asparenomycins A, B and C and Related Carbapenem Antibiotics[1)]

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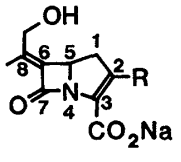
Racemic carbapenem antibiotics having a 1-(hydroxymethyl)ethylidene side-chain at C-6 [*dl*-asparenomycins A (**54**), B (**37**), C (**53**) and related compounds, **55**, **56**, **38**, **59** and **45**] were synthesized starting from the common intermediates **9a** and **9b**, and their antibacterial activities were examined. The synthesis involves transformation of a cyclic carbonate group into the 1-(hydroxymethyl)ethylidene moiety with a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in an appropriate solvent, and deblocking of the *p*-methoxybenzyl ester group by the $AlCl_3$–anisole method.

Keywords——β-lactam antibiotic; carbapenem antibiotic; asparenomycin; 6643-X; allylazetidinone; cyclic carbonate; intramolecular Wittig reaction; carbapenem antibiotic carboxy deprotection; antibacterial activity

An extensive search for new β-lactam antibiotics at our laboratories resulted in the isolation of a novel carbapenem antibiotic named asparenomycin A (**1**) from a fermentation broth of *Streptomyces tokunonensis* sp. nov. and also that of *St. argenteolus*.[2a,b)] Asparenomycin A is active against a broad range of Gram-positive and Gram-negative bacteria including β-lactamase-producing organisms, and shows potent inhibitory activity against various types of β-lactamases.[2a,b)] Subsequent structure elucidation studies, involving chemical degradation, spectroscopy and X-ray crystallography, revealed that the antibiotic had a carbapenem skeleton having a hitherto unknown 1-(hydroxymethyl)ethylidene side-chain at C-6.[2a,c)] Two related antibiotics, asparenomycins B (**3**) and C (**2**), were isolated from the same source in minute amounts.[2)] Very recently, isolation of 6643-X (**4**), the fourth antibiotic belonging to this family, was reported by a Kowa group.[3)]



1:asparenomycin A (ASM-A) R=S(=O)–NHAc

2:asparenomycin C (ASM-C) R=S–NHAc

3:asparenomycin B (ASM-B) R=S(=O)–NHAc

4:6643-X R=S–NHAc

Chart 1

Before the complete structure of asparenomycin A was elucidated by X-ray analysis, we had started a synthetic program having the following objectives in mind. 1) Confirm the initially proposed structures by total synthesis and provide a sufficient amount of material for further biological evaluation. 2) Discover more potent and yet more biologically stable (therefore clinically usable) compounds by chemical modification of the parent structure at

position(s) 1, 2 and 8. In this and the following papers we describe our work along these lines and present antibacterial activity data for several compounds that we have prepared.

Very recently, Ohno and his associates reported the total synthesis of natural asparenomycin C, starting from an optically active compound.[4]

Chemistry

From our previous experience in 1-oxacephem[5] and 1-carbacephem[6] syntheses, we selected the azetidinone **5** as a common intermediate for our purpose, since the cyclic carbonate of the α-glycol would serve as a suitable precursor of the 1-(hydroxymethyl)ethylidene part, and the allyl group would eventually become the C-1 and C-2 (and C-2′) part of the carbapenem nucleus. The stereochemistry of C-6 and C-8 in **5** (carbapenem numbering) was expected to control the geometry of the resulting C-6–C-8 double bond as discussed later.

The objective common intermediates **9a** and **9b** corresponding to **5** were prepared in the following way. *N*-Silylated allylazetidinone **6**,[7] a popular starting material for carbapenem synthesis, was deprotonated with lithium diisopropyl amide (LDA) in tetrahydrofuran (THF) at −70 °C and then reacted with trimethylsilyloxyacetone, and the products were *O*-desilylated with acetic acid in methanol to give a mixture of C-5, 6 *trans* azetidinones **7a** and **7b**, diastereoisomeric at C-8, in a ratio of 2 to 1 in good yield. Separation of the mixture could be achieved by silica gel chromatography. No *cis* compounds were obtained in this reaction. Treatment of the glycols **7a** and **7b** with phosgene and pyridine in methylene dichloride at 0 °C afforded cyclic carbonates **8a** and **8b**, which were then *N*-desilylated with tetrabutylammonium fluoride [$(n\text{-Bu})_4$NF] in THF containing acetic acid to afford crystalline azetidinones **9a** and **9b**, respectively, in *ca.* 75% overall yields.

X-Ray crystallographic analysis[8] showed that **9a**, derived from the major product **7a**, had a 5*R**,6*S**,8*R** stereo structure, and therefore the diastereoisomer **9b** should have a 5*R**,6*S**,8*S** structure. Hereafter, **a** and **b** are used to indicate the same stereochemical structures throughout this and the following papers.

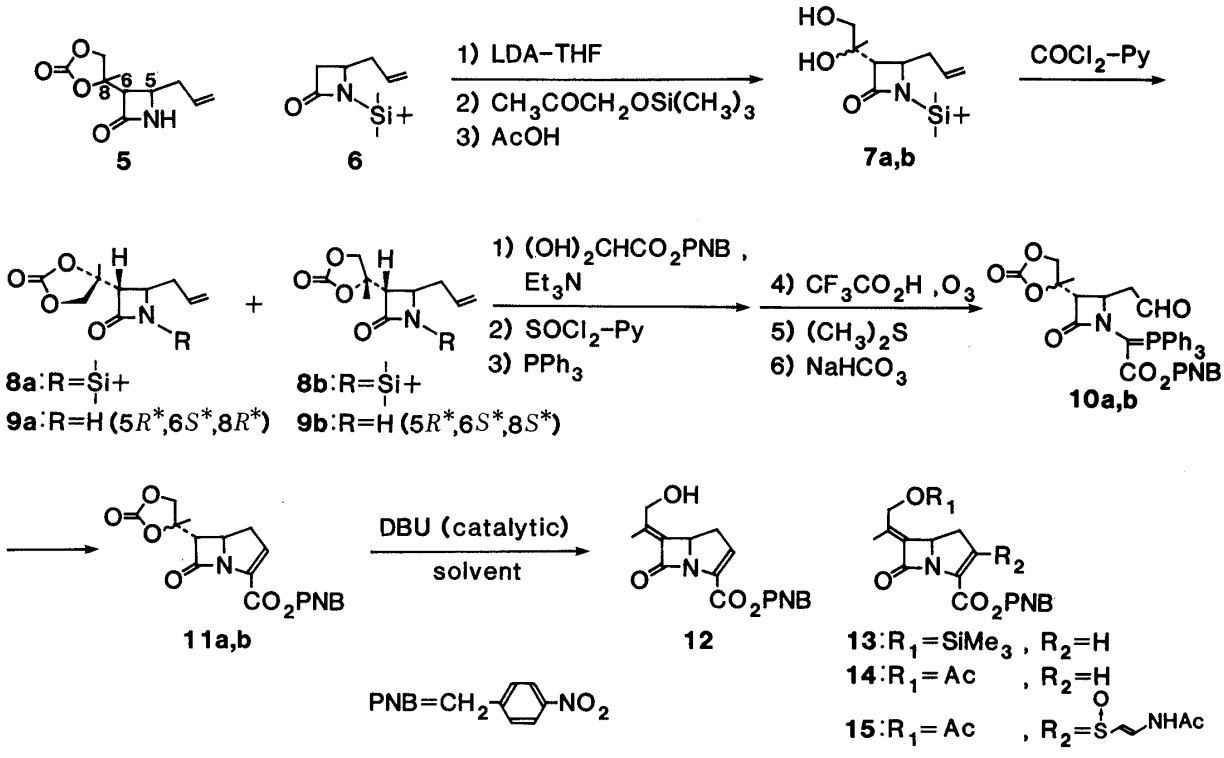


Chart 2

Having the intermediates **9a** and **9b** in hand, we then directed our attention to the establishment of a suitable method for converting the carbonate grouping into the 1-(hydroxymethyl)ethylidene structure using simple carbapenems as model compounds.

Preparation of *p*-nitrobenzyl (PNB) esters of carbapenem **11a** and **11b** from the azetidinone **9a** and **9b** was easily achieved by the standard procedure involving an intramolecular Wittig reaction of ylides **10a** and **10b.**[7] It was gratifying to find that the transformation of the carbonate side-chain to the asparenomycin-type allylic alcohol moiety in **12** could be realized easily not only from **11b** (having a favorable stereochemical arrangement for the *E*2 elimination reaction) but also from the stereochemically unfavorable epimer **11a** by the following simple operation. Thus, a solution of **11b** in acetonitrile was treated with a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at 0 °C for 30 min to give the desired compound **12** as a major product. On the other hand, conversion of the diastereoisomer **11a** into **12** was effected by changing the solvent from acetonitrile to methylene dichloride or chloroform. When this reaction was carried out in the presence of bis(trimethylsilyl)acetamide (BSA), the product was trapped as an *O*-silyl compound **13**, which was purified by silica gel chromatography. Subsequent desilylation with $(n\text{-Bu})_4NF$ in THF containing acetic acid afforded **12** in a pure state. Acetylation of **12** with acetyl chloride and pyridine gave the *O*-acetate **14**.

The structure assignment of **12**, especially the *E* geometry of the C-6, 8 double bond, is based on a comparison of the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the acetate **14** with that of *O*-acetyl-asparenomycin A PNB ester (**15**), whose structure was determined by X-ray analysis at the time.[2c] Two characteristic signals of **14** at 2.12 (s, 3H) and 4.63 ppm (br s, 2H) ascribed to the methyl and the hydroxymethyl group at C-8, respectively, were in good agreement with those of the asparenomycin A derivative **15** at 2.13 (s, 3H) and 4.58, 4.71 ppm (ABq, J = 16.1 Hz, 2H).[2c]

We assume that this interesting elimination reaction goes predominantly through a typical *E*2 mechanism in a polar solvent, whereas in a nonpolar solvent the leaving ability of the carbonate group is insufficient for effecting the *E*2 elimination, thereby allowing an *E*1cB-type mechanism to occur[9] *via* a β-lactam enolate or C-6 carbanion, leading in the opposite direction. The corresponding monocyclic β-lactam **8a** and **8b**, in contrast, did not give the 1-(hydroxymethyl)ethylidene compound under the same conditions.

Since attempted carboxy deprotection of the PNB ester **12** to produce the carboxylate by catalytic hydrogenolysis resulted in decomposition of the β-lactam ring system, we then prepared an enzymatically removable pivaloyloxymethyl (POM) ester **18** *via* the carbonates **17a** and **17b**. The intermediates **16a** and **16b** were prepared conveniently from **9a** and **9b** by

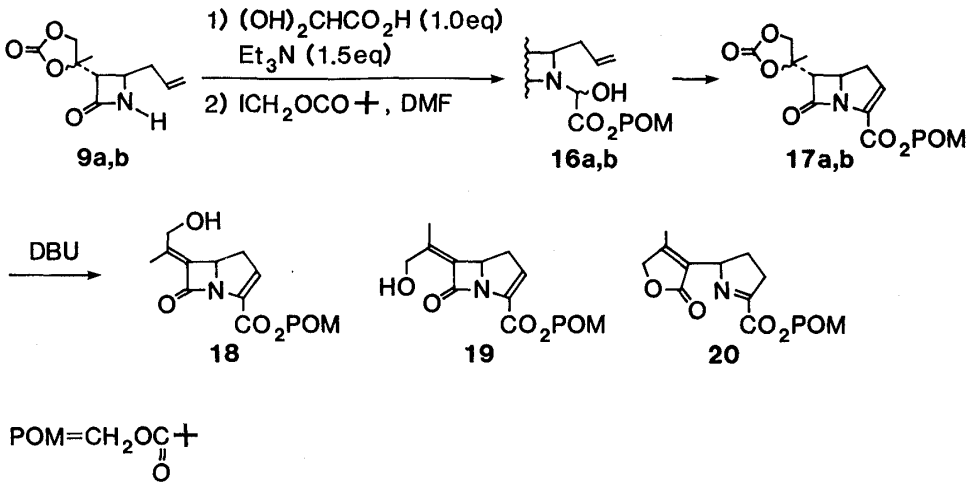


Chart 3

treatment with glyoxylic acid (1.0 eq) and triethylamine (1.5 eq) in dimethylformamide (DMF) at room temperature for several hours followed by addition of iodomethyl pivalate.

The carbapenem **18** has antibacterial activity similar to that of **45**, as indicated in Table I, when assayed in the presence of horse serum. We therefore attempted to prepare the geometrical isomer (*Z*-isomer) **19** for comparison of the antibacterial activity with that of **18**. Because all attempts to obtain the *Z*-isomer **19** from either **17a** or **17b** under the influence of DBU resulted in failure, we studied this reaction in some detail in deuterated solvents by ^{1}H-NMR spectroscopy. On addition of a catalytic amount of DBU to a solution of **17b** in $CDCl_3$, signals at 1.68 (s, 3H) and 4.22, 4.46 ppm (ABq, J=9 Hz, 2H) corresponding to the C-8 methyl and methylene groups disappeared and new signals appeared instead at 1.85 (s, 3H) and 4.44 ppm (s, 2H), which were ascribed to the C-8 methyl and hydroxymethyl groups of the *Z*-isomer **19**. Aqueous work-up, however, did not give the expected *Z*-isomer **19**, but provided another product showing signals at 2.14 (s, 3H) and 4.68 ppm (s, 2H), which could be assigned to the methyl and methylene groups of a lactone compound **20**, respectively.

On the other hand, NMR analysis demonstrated that **17a** in CD_3CN, a more polar solvent, gave, on treatment with DBU, the decomposed compound **20** as a major product together with a small amount of the *Z*-isomer **19** in accordance with the mechanism discussed above. It was apparent that the *Z*-isomer was too unstable to be subjected to

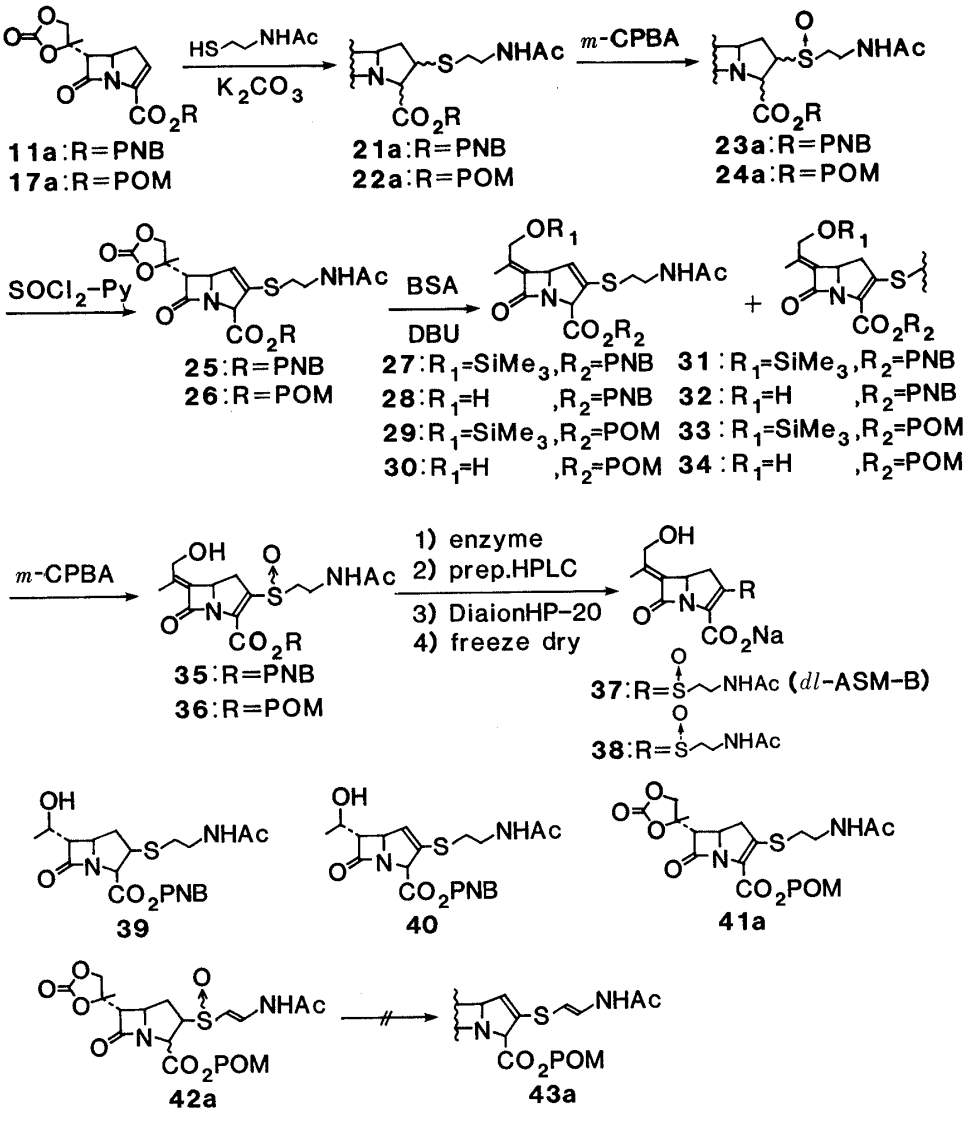


Chart 4

antibacterial assay. In any event we were now able to utilize both intermediates **17a** and **17b** for synthesizing asparenomycin-type carbapenems. We thus focused our efforts on the synthesis of the natural products starting from the carbapenem carbonates **9a** and **9b**.

Conjugate addition of *N*-acetyl-*β*-aminoethanethiol to **11a** in the presence of potassium carbonate in DMF gave a mixture of three adducts **21a**[10] which were, without separation, oxidized to a mixture of sulfoxides **23a** with *m*-chloroperbenzoic acid (*m*-CPBA). The sulfoxides **23a** were then treated with thionyl chloride and pyridine in methylene dichloride to afford, contrary to expectation, a carbapen-1-em **25a** exclusively. This result is similar to that of an independent study by Beecham chemists, in which a carbapenam-sulfide **39** having the hydroxyethyl side-chain at C-6 was transformed into the carbapen-1-em-sulfide **40** on oxidation with iodobenzene dichloride under anhydrous conditions.[11]

Exposure of **25a** to DBU[11] in $CDCl_3$ in the presence of BSA gave a mixture of the *O*-silyl carbapen-1-em **27** and the *O*-silyl carbapen-2-em **31**, which were separated by silica gel chromatography and then desilylated by allowing them to stand in aqueous methanol to give **28** and **32**, respectively. Since the above double bond isomerization reaction requires a higher concentration of DBU than that for the side-chain transformation, trapping the products as *O*-silylated compounds is preferable. The desired carbapen-2-em **32** was then oxidized with *m*-CPBA to a mixture of sulfoxides **35**, which were separated by preparative thin-layer chromatography (TLC) (silica gel). The sulfoxide with smaller *Rf* value was indistinguishable from asparenomycin B PNB ester by TLC comparison.

The same sequence of reactions starting from the POM ester **17a** was repeated and the carbapen-2-em esters **36** were obtained as a mixture of sulfoxides. In the reaction of **24a** to **26a** a small amount of a carbapen-2-em **41a** was isolated, and was transformed into **34** as before.

The carboxy deprotection of the POM ester **36** (27 mg) was effected by treatment with a commercially available porcine liver esterase (Sigma) in a mixture of methanol and phosphate buffer (pH 7) for 2 h at room temperature. The reaction mixture was diluted with buffer solution and poured into a column of Diaion HP-20AG, which was eluted with water. Fractions containing a mixture of the sodium salts **37** and **38** were collected and the sulfoxide isomers were separated by preparative high performance liquid chromatography (HPLC). The subsequent desalination on an HP-20AG column and freeze-drying provided a minute amount of **37** (1 mg), which gave the same retention times as asparenomycin B in HPLC analysis. The other sulfoxide isomer **38** (2.7 mg) was similarly obtained. It thus became clear that this enzymatic carboxy deprotection was not effective for preparing sufficient amounts of the antibiotics for further biological evaluations. Moreover, the sulfoxides **42a**, synthesized in the same way from **17a**, failed to afford the carbapenem **43a**.

Therefore we needed to develop a useful carboxy deblocking procedure applicable to asparenomycin-type structures and also a method for synthesizing the 2-functionalized carbapenem skeleton. During the course of studies on *β*-lactam antibiotics, a very convenient carboxy deblocking technique using $AlCl_3$ and anisole was developed at our laboratories and has been used extensively for cephem,[12] 1-oxacephem[5] and 1-carbacephem[6] syntheses. Very recently, this useful procedure was found to be applicable to certain carbapenem systems having a *p*-methoxybenzyl (PMB) group for carboxy protection instead of a conventional benzyl or benzhydril group.[13]

We found that this procedure was also applicable to the asparenomycin-type carbapenem **44**. Thus, the PMB ester **44**, prepared in a similar manner to that used for **12** and **18**, was dissolved in methylene dichloride containing anisole and treated with $AlCl_3$ (2.5 mol eq) at $-60\,^{\circ}C$ for 20 min; then the reaction mixture was quenched with an aqueous sodium bicarbonate solution. The aqueous phase was separated, washed with methylene dichloride, desalinated by HP-20AG column chromatography and finally freeze-dried to give the sodium salt **45** as an amorphous powder. Having this convenient carboxy deprotection method in

hand, we were ready to prepare asparenomycin B from the PMB ester **46a** by the sequence of reactions previously described, when a very efficient carbapenem synthesis *via* the 2-oxo derivatives was disclosed by Merck chemists.[14] We therefore prepared the PMB esters of the carbapenem skeleton with the carbonate side-chain by the Merck route.

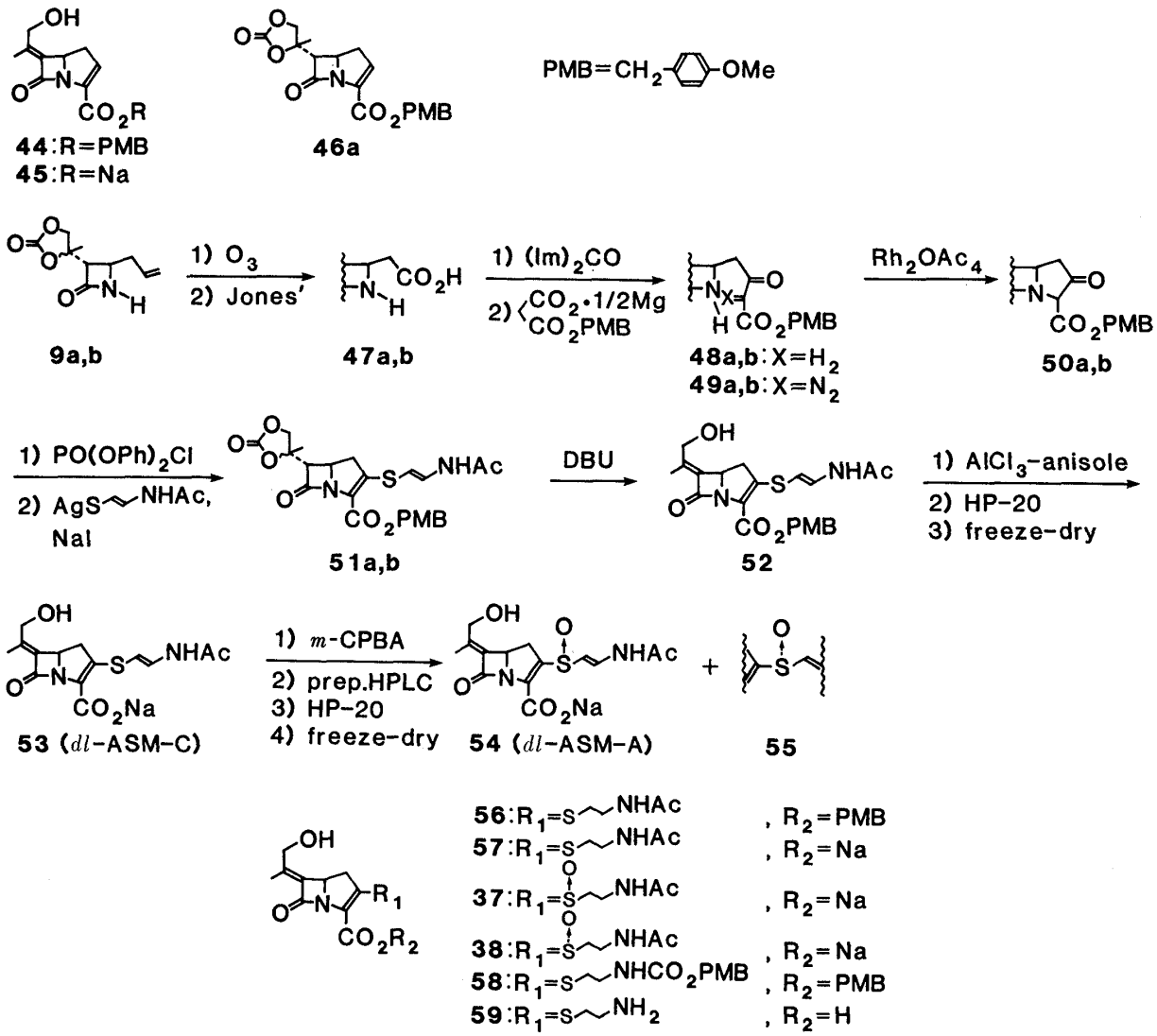


Chart 5

The allylazetidinones **9a** and **9b** were converted into the acids **47a** and **47b** by ozonolysis and subsequent Jones' oxidation in 90% yields, and the latter compounds were then transformed to bicyclic ketoesters **50a** and **50b** *via* β-keto PMB esters **48a** and **48b** and diazo derivatives **49a** and **49b** in *ca.* 50% overall yields. Introduction of the alkylthio group of asparenomycin C-type furnished carbapenems **51a** and **51b** in *ca.* 70% yields, and these were converted into asparenomycin C PMB ester (**52**) by treatment with DBU in an appropriate solvent, *i.e.*, **51a** in a mixed solvent of benzene and methylene dichloride and **51b** in acetonitrile. Removal of the PMB protective group was effected cleanly by the above-mentioned $AlCl_3$–anisole method to give the sodium salt **53** in pure form as an amorphous powder in 40% yield from **51b**. Oxidation of **53** with *m*-CPBA in a two-phase solvent system of methylene dichloride and phosphate buffer (pH 7) afforded a mixture of isomeric sulfoxides **54** and **55** in a ratio of 2 to 1; the components were separated by preparative HPLC. The pure sodium salt **54** and the sulfoxide isomer **55** were obtained by HP-20AG chromatography followed by freeze-drying in 59% and 30% yields, respectively. The synthetic materials **53** and

54 were proved to be *dl*-asparenomycins C and A, respectively, by the identity of their spectral data and HPLC retention times with those of authentic samples.

By following the same sequence of reactions, *dl*-asparenomycin B (**37**) and its sulfoxide isomer **38** were synthesized *via* **57** (*dl*-6643-X) from the PMB ester **56**.

We assigned the sulfoxide stereochemistry of asparenomycin B (**3**) as *R* based upon the following observation. In the ^{1}H-NMR spectra obtained in D_2O, signals of the C-1 methylene protons of both *dl*-asparenomycins A (**54**) and B (**37**) appeared as two broad singlets at 3.57 and 3.66 ppm while those of the isomeric sulfoxides **55** and **38** appeared as a pair of doublets at 3.60 ($J=8$ Hz), 3.64 ($J=10$ Hz) and 3.61 ($J=8$ Hz), 3.66 ppm ($J=7$ Hz), respectively. On the other hand, in HPLC analysis using a Nucleosil column, the sulfoxides **54** and **37** with the natural stereochemistry gave shorter retention times than the corresponding sulfoxides **55** and **38** having unnatural configuration. Since the C-5 configuration of asparenomycin B should be *R*, as in all the known naturally occurring bicyclic β-lactam antibiotics, asparenomycin B should have the same *R* sulfoxide configuration as asparenomycin A (whose structure was clearly determined by X-ray analysis).

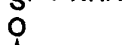
In this way we confirmed the proposed structures by total synthesis and at the same time provided materials for further biological evaluations. We also prepared a naturally-nonoccurring derivative **59** with the thienamycin aminoethylthio group at C-2 by using *p*-methoxybenzyloxycarbonyl as an amino-protecting group. The *O*,*N*-bisprotected carbapenem **58** was cleanly deblocked to **59** by the $AlCl_3$–anisole method.

Antibacterial Activity

The antibacterial activities of the above racemic carbapenems having the asparenomycin-type structure are shown in Table I.

The antibacterial activity of the synthetic asparenomycin A (**54**) was half that of the natural one (**1**), indicating that the natural configuration is indispensable for activity.

TABLE I. *In Vitro* Antibacterial Activity[a)]

R		MIC (μg/ml)					
		S. aureus C-14(R)	*S. pyogenes* C-203	*E. coli* EC-14	*K. Pneumoniae* SRL-1	*P. vulgaris* CN-329	*S. marcescens* A13880
	**1**	3.13	—	0.39	0.39	12.5	6.25
	**54** (*dl*-**1**)	6.25	6.25	0.39	1.56	25	12.5
	**55**	12.5	6.25	6.25	12.5	100	50
	53 (*dl*-**2**)	12.5	3.13	0.39	0.78	6.25	6.25
	56 (*dl*-**4**)	6.25	3.13	0.2	0.39	3.13	6.25
	**37** (*dl*-**3**)	12.5	6.25	0.78	3.13	100	25
	**38**	12.5	6.25	50	50	>100	>100
	59 (H)	1.56	0.78	1.56	3.13	25	100
H	**45**	1.56	1.56	1.56	3.13	12.5	12.5

a) MICs (Minimum inhibitory concentrations) were determined by the agar dilution method. Inoculation was performed with one loopful of 10^6 cells per ml.

Comparison of the activities of two other compounds, **37** and **53**, with those of asparenomycins B and C,[2d] respectively, also supports this conclusion.

The configuration of sulfoxide is important for the activity: compounds **37** and **54** having the natural sulfoxide configuration (*R*) exhibited better Gram-negative activity and similar Gram-positive activity as compared with the corresponding (*S*)-epimers **38** and **55**, respectively.

Experimental

All reactions were carried out under a nitrogen atmosphere using dry solvents under anhydrous conditions unless otherwise stated. Melting points were determined on a Yanagimoto apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Hitachi 260-10 spectrophotometer in $CHCl_3$ as a solvent or a JASCO DS-403G spectrophotometer in KBr unless otherwise noted. NMR spectra were recorded on a Varian T-60A or a Varian EM-390 (90 MHz) spectrometer for ^{1}H-NMR in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard and a Varian XL-100A (100 MHz) in D_2O with TMS as an external standard unless otherwise stated. Ultraviolet (UV) spectra were obtained on a Hitachi EPS-3T or EPS-2 spectrometer. Mass spectra (MS) were obtained on a Hitachi RUM8-GN (FD-Mass) or M-68 (SIMS) mass spectrometer. Elemental analysis values obtained were within 0.3% of those calculated for the formula given. Medium pressure liquid chromatographies were performed on Merck "Lobar®" prepacked columns packed with LiChroprep Si 60; size A (240—10 mm, 40—60 μm), size B (310—25 mm, 40—63 μm) and size C (440—37 mm, 63—125 μm). Organic solvents were dried with $MgSO_4$ and removed by evaporation under reduced pressure using a rotary evaporator.

(3*S,4*R**)-4-Allyl-1-*tert*-butyldimethylsilyl-3-(1,2-dihydroxy-2-propyl)-2-azetidinone (7a, b)**——A solution of *n*-butyllithium in hexane (1.6 N, 17.5 ml, 1.4 eq) was added to a solution of diisopropylamine (4.2 ml, 1.5 eq) in THF (50 ml) at −70 °C. The mixture was stirred at 0 °C for 1 h then cooled to −70 °C. A solution of **6** (4.50 g, 20.0 mmol) in THF (10 ml) was added dropwise to the above solution and the mixture was stirred for 40 min. To this mixture was added trimethylsilyloxyacetone (7.5 ml, 2.3 eq), and the whole was stirred for 30 min at the same temperature (−70 °C). The reaction mixture was diluted with brine and extracted with EtOAc. The organic extracts were dried, filtered and evaporated to give an oily residue, which was dissolved in MeOH (30 ml) containing acetic acid (3 ml). The solution was allowed to stand overnight at room temperature and then concentrated. The residue was chromatographed on a Lobar column (size C, benzene–EtOAc 1 : 1) to give **7a** (3.02 g, 51%) and **7b** (1.30 g, 22%). **7a**: mp 63—64 °C (hexane–ether). IR: 3410 (br), 2925, 2850, 1725 cm^{-1}. ^{1}H-NMR δ: 0.23 (3H, s, SiMe), 0.30 (3H, s, SiMe), 1.00 (9H, s, *tert*-Bu), 1.08 (3H, s, Me), 2.0—2.8 (2H, m, C=CCH_2), 2.85 (1H, d, J=2 Hz, C-6H), 3.0—4.7 (5H, m), 5.0—6.2 (3H, m, –CH=CH_2). *Anal.* Calcd for $C_{15}H_{28}NO_3Si$: C, 60.16; H, 9.76; N, 4.68. Found: C, 60.14; H, 9.78; N, 4.67. **7b**: mp 60—63 °C (hexane–ether). IR: 3410 (br), 2925, 2850, 1725 cm^{-1}. ^{1}H-NMR δ: 0.23 (3H, s, SiMe), 0.27 (3H, s, SiMe), 1.00 (9H, s, *tert*-Bu), 1.30 (3H, s, Me), 1.9—4.0 (8H, m), 4.9—6.2 (3H, m, –CH=CH_2). *Anal.* Calcd for $C_{15}H_{29}NO_3Si$: C, 60.16; H, 9.76; N, 4.68. Found: C, 60.23; H, 9.76; N, 4.62.

(3*S,4*R**)-4-Allyl-1-*tert*-butyldimethylsilyl-3-(4-methyl-2-oxo-1,3-dioxolan-4-yl)-2-azetidinone (8a, b)**——A solution of phosgene in toluene (3 M, 3.6 ml, 1.1 eq) was added to a solution of the above crude diol **7a** (3.02 g, 10.1 mmol) in CH_2Cl_2 (20 ml) containing pyridine (1.77 ml, 2.2 eq) under ice-cooling, and the mixture was stirred for 30 min, then diluted with EtOAc, washed with water, dried and concentrated to give the crude carbonate **8a** (3.10 g, 95%); mp 82—84 °C (hexane–ether). IR: 2925, 2850, 1805, 1740 cm^{-1}. ^{1}H-NMR (CCl_4) δ: 0.23 (3H, s, SiMe), 0.30 (3H, s, SiMe), 1.00 (9H, s, *tert*-Bu), 1.33 (3H, s, Me), 2.0—2.95 (2H, m, C=CCH_2), 3.10 (1H, d, J=3 Hz, C-6H), 3.50—3.83 (1H, m, C-5H), 4.07 and 4.67 (2H, ABq, J=8 Hz, OCH_2), 5.2—6.2 (3H, m, –CH=CH_2). *Anal.* Calcd for $C_{16}H_{27}NO_4Si$: C, 59.04; H, 8.36; N, 4.30. Found: C, 59.04; H, 8.41; N, 4.31.

Similarly, **7b** (1.30 g, 4.34 mmol) gave **8b** (1.54 g, 100%); mp 61—62 °C (hexane–ether). IR: 2925, 2850, 1805, 1740 cm^{-1}. ^{1}H-NMR (CCl_4) δ: 0.13 (3H, s, SiMe), 0.19 (3H, s, SiMe), 0.92 (9H, s, *tert*-Bu), 1.53 (3H, s, Me), 2.0—2.8 (2H, m, C=CCH_2), 3.06 (1H, d, J=3 Hz, C-6H), 3.3—3.7 (1H, m, C-5H), 4.02 and 4.21 (2H, ABq, J=8 Hz, OCH_2), 4.9—6.1 (3H, m, –CH=CH_2). *Anal.* Calcd for $C_{16}H_{27}NO_4Si$: C, 59.04; H, 8.36; N, 4.30. Found: C, 59.03; H, 8.36; N, 4.26.

(3*S,4*R**)-4-Allyl-3-(4-methyl-2-oxo-1,2-dioxolan-4-yl)-2-azetidinone (9a, b)**——A solution of **8a** (4.50 g, 13.8 mmol) in THF (30 ml) and acetic acid (1.6 ml, 2 eq) was treated with (n-Bu)$_4$NF (5.4 g, 1.5 eq), and the mixture was stirred at room temperature for 1 h, then diluted with EtOAc, washed with brine, dried and concentrated. The residue was chromatographed on a Lobar column (size B, benzene–EtOAc 1 : 1) to give **9a** (2.81 g, 96%). Recrystallization from CH_2Cl_2–ether gave a pure material; mp 91—92 °C. IR: 3410, 1810, 1775 cm^{-1}. ^{1}H-NMR δ: 1.55 (3H, s, Me), 2.3—2.6 (2H, m, C=CCH_2), 3.07 (1H, d, J=2 Hz, C-6H), 3.6—3.8 (1H, m, C-5H), 4.14 and 4.68 (2H, ABq, J=8 Hz, OCH_2), 4.9—6.1 (3H, m, –CHC=CH_2), 6.6 (1H, br, NH). *Anal.* Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63; O, 30.30. Found: C, 56.82; H, 6.19; N, 6.56; O, 30.25.

Under the same conditions, **8b** (0.69 g, 2.12 mmol) gave **9b** (0.45 g, 100%); mp 76—81 °C (CH_2Cl_2–ether). IR:

3410, 1815, 1780 cm^{-1}. ^{1}H-NMR (90 MHz) δ: 1.64 (3H, s, Me), 2.41 (1H, br d, J=6 Hz, C=CCH_2), 2.48 (1H, br d, J=6 Hz, C=CCH_2), 3.14 (1H, d, J=3 Hz, C-6H), 3.69 (1H, ddd, J=3, 6, 6 Hz, C-5H), 4.18 and 4.39 (2H, ABq, J=9 Hz, OCH_2), 5.0—5.3 and 5.5—6.1 (3H, m, $CH=CH_2$), 6.25 (1H, br, NH). *Anal.* Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63; O, 30.30. Found: C, 56.58; H, 6.10; N, 6.70; O, 30.28.

***p*-Nitrobenzyl (5*R**,6*S**)-6-(4-Methyl-2-oxo-1,3-dioxolan-4-yl)carbapen-2-em-3-carboxylate (11a,b). *p*-Nitrobenzyl α-[(3*S**,4*R**)-4-Allyl-3-(4-methyl-2-oxo-1,3-dioxolan-4-yl)-2-azetidinon-1-yl]-α-triphenylphosphoranylideneacetate**——A mixture of **9a** (1.79 g, 8.48 mmol), glyoxylic acid PNB ester (1.93 g, 1.0 eq), and triethylamine (40 μl, 0.03 eq) in THF (30 ml) was allowed to stand overnight at room temperature and then concentrated to dryness.

The residual glycolates were dissolved again in THF (25 ml) and the solution was cooled to −35 °C. 2,6-Lutidine (1.88 ml, 1.90 eq) and thionyl chloride (0.89 ml, 1.43 eq) were added, and the whole was stirred at −35 to −20 °C for 1.5 h then concentrated under reduced pressure to remove the solvent and the reagents.

The residue (chlorides) was dissolved in dioxane (25 ml) and treated with 2,6-lutidine (1.0 ml, 1.0 eq) and triphenylphosphine (3.0 g, 1.35 eq) at room temperature overnight. The reaction mixture was diluted with EtOAc, washed with water, dried and concentrated. The residue was purified by chromatography on Lobar columns (size B×2, benzene–EtOAc 1 : 1) to give the title compound. IR: 1810, 1745, 1350 cm^{-1}.

Ozone was passed through a solution of the above ylid (1.10 g, 1.66 mmol) in CH_2Cl_2 (30 ml) containing trifluoroacetic acid (2 ml) at −60 °C until a blue color persisted. Excess ozone was removed by passing nitrogen gas through the reaction mixture, then dimethylsulfide (2 ml) was added and the whole was stirred at room temperature for 1 h and concentrated.

The residue was dissolved in EtOAc (20 ml) and the solution was stirred wtih aqueous saturated $NaHCO_3$ (20 ml) for 1 h at room temperature. The organic layer was separated, washed with water, dried and concentrated. The residue was crystallized from a mixture of ether and benzene to give the carbapenem **11a** (0.35 g, 54%); mp 159—160 °C. IR: 1810, 1788, 1730 cm^{-1}. ^{1}H-NMR δ: 1.65 (3H, s, Me), 2.9—3.1 (2H, m, C-1H_2), 3.61 (1H, d, J=3 Hz, C-6H), 4.26 and 4.68 (2H, ABq, J=9 Hz, C-8CH_2), 4.37 (1H, ddd, J=3, 9, 9 Hz, C-5H), 5.32 and 5.45 (2H, ABq, J=14 Hz, CO_2CH_2), 6.64 (1H, dd, J=2, 2.5 Hz, C-2H), 7.60 and 8.22 (4H, A_2B_2q, J=9 Hz, Ar). *Anal.* Calcd for $C_{18}H_{16}N_2O_8 \cdot 1/10H_2O$: C, 55.42; H, 4.19; N, 7.18. Found: C, 55.29; H, 4.13; N, 7.13.

Using the same procedure as above, the carbapenem **11b** was prepared from **9b**. **11b**: mp 175—177 °C (CH_2Cl_2–ether). IR: 1805, 1785, 1725 cm^{-1}. ^{1}H-NMR δ: 1.70 (3H, s, Me), 2.8—3.1 (2H, m, C-1H_2), 3.58 (1H, d, J=3 Hz, C-6H), 4.24 and 4.44 (2H, ABq, J=9 Hz, C-8CH_2), 4.1—4.5 (1H, m, C-5H), 5.29 and 5.41 (2H, ABq, J=14 Hz, CO_2CH_2), 6.62 (1H, t, J=2 Hz, C-2H), 7.61 and 8.24 (4H, A_2B_2q, J=9 Hz, Ar). *Anal.* Calcd for $C_{18}H_{16}N_2O_8$: C, 55.67; H, 4.15; N, 7.21; O, 32.96. Found: C, 55.66; H, 4.07; N, 7.19; O, 32.66.

***p*-Nitrobenzyl 6-[(*E*)-1-(Hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (12). (A) From the Carbonates 11a, b**——A solution of DBU in toluene (1 N, 36 μl) was added to a solution of **11a** (70 mg, 0.18 mmol) in CH_2Cl_2 (2.7 ml), and the mixture was stirred at room temperature for 5 min, then diluted with EtOAc, washed with water, dried and concentrated to give **12** (60 mg, crude, 97%) as an oil. ^{1}H-NMR δ: 2.04 (3H, s, Me), 2.27 (1H, br s, OH), 2.93 (2H, dd, J=2.5, 9 Hz, C-1H_2), 4.32 (2H, s, C-8CH_2), 5.04 (1H, br t, J=9 Hz, C-5H), 5.32 and 5.49 (2H, ABq, J=14 Hz, CO_2CH_2), 6.58 (1H, br t, J=2.5 Hz, C-2H), 7.68 and 8.27 (4H, A_2B_2q, J=9 Hz, Ar).

A solution of **11b** (58 mg, 0.15 mmol) in acetonitrile (3 ml) was treated with a solution of DBU in toluene (1 M, 30 μl, 0.2 eq) under ice-cooling for 30 min. Work-up as for **11a** gave crude **12** (49 mg).

(B) From 11b *via* the *O*-Silyl Ether 13. *p*-Nitrobenzyl 6-[(*E*)-1-(Trimethylsilyloxymethyl)ethylidene]carbapen-2-em-3-carboxylate (13)——A solution of DBU in toluene (1 M, 40 μl, 0.2 eq) was added to a solution of **11b** (78 mg, 0.2 mmol) and BSA (60 μl, 1.2 eq) in acetonitrile (4 ml) at room temperature over 10 min. The reaction mixture was passed through a Lobar column (size A, pre-treated with pyridine, *n*-hexane–EtOAc 1 : 1 and CH_2Cl_2–EtOAc 1 : 1) to give the silyl ether **13** (45 mg, 54%) and **12** (12 mg, 20%). **13**: ^{1}H-NMR δ: 0.18 (9H, s, $SiMe_3$), 2.02 (3H, s, Me), 2.88 (2H, dd, J=2.5, 9 Hz, C-1H_2), 4.23 (2H, br s, C-8CH_2), 5.00 (1H, br t, J=9 Hz, C-5H), 5.31 and 5.48 (2H, ABq, J=14 Hz, CO_2CH_2), 6.54 (1H, br t, J=2.5 Hz, C-2H), 7.64 and 8.25 (4H, A_2B_2q, J=9 Hz, Ar).

A solution of the silyl ether **13** (74 mg, 0.18 mmol) in THF (1 ml) was treated with tetraethylammonium fluoride hydrate (40 mg, 1.2 eq) and the mixture was stirred at room temperature for 1 h, then diluted with EtOAc, filtered and concentrated. The residue was dissolved in benzene–EtOAc (1 : 1) and the solution was passed through a short column of silica gel to give **12** (40 mg, 66%).

***p*-Nitrobenzyl 6-[(*E*)-1-(Acetoxymethyl)ethylidene]carbapen-2-em-3-carboxylate (14)**——A mixture of the crude allylic alcohol **12** (33 mg, 0.1 mmol), acetyl chloride (9.6 μl, 1.4 eq) and pyridine (17 μl, 2.2 eq) in CH_2Cl_2 (1 ml) was stirred at −28 °C for 30 min. The mixture was diluted with EtOAc, washed with water, dried and concentrated. The residue was chromatographed on a Lobar column (size A,CH_2Cl_2–EtOAc 1 : 1) to give **14** (21 mg, 57%) as an oily product. **14**: IR: 1765, 1732, 1608 cm^{-1}. ^{1}H-NMR (90 MHz) δ: 2.07 (3H, s, C-8Me), 2.10 (3H, s, Ac), 2.90 (2H, dd, J=3, 9 Hz, C-1H_2), 4.63 (2H, br s, C-8CH_2), 4.90 (1H, br t, J=9 Hz, C-5H), 5.28 and 5.43 (2H, ABq, J=14 Hz, CO_2CH_2), 6.53 (1H, br t, J=2.5 Hz, C-2H), 7.62 and 8.20 (4H, A_2B_2q, J=9 Hz, Ar).

Pivaloyloxymethyl α-[(3*S,4*R**)-4-Allyl-3-(4-methyl-2-oxo-1,3-dioxolan-4-yl)-2-azetidinon-1-yl]glycolate (16a, b)**——A mixture of the azetidinone **9a** (211 mg, 1.0 mmol), glycolic acid hydrate (97 mg, 1.05 eq) and triethylamine (153 μl, 1.1 eq) in DMF (1 ml) was stirred at room temperature overnight, then iodomethyl pivalate (*ca.*

0.4 g) was added and the whole was stirred for 2 h. The mixture was diluted with EtOAc, washed with water, dried and concentrated to give crude **16a** (370 mg). ^{1}H-NMR δ: 1.20 (9H, s, *tert*-Bu), 1.55 (3H, s, Me), 2.2—2.8 (2H, m, C=CCH$_2$), 3.00 (1H, d, J=2 Hz, C-6H), 3.6—4.1 (1H, m, C-5H), 4.13 and 4.57 (2H, ABq, J=8 Hz, OCH$_2$), 5.0—6.2 (4H, m, CH=CH$_2$ and CHOH), 5.77 (2H, m, CO$_2$CH$_2$).

Similarly **9b** (363 mg) gave crude **16b** (740 mg) under the same conditions. **16b**: ^{1}H-NMR δ: 1.20 (9H, s, *tert*-Bu), 1.62 and 1.63 (3H, s, Me), 2.3—2.8 (2H, m, C=CCH$_2$), 3.2—3.4 (1H, m, C-6H), 3.6—4.1 (1H, m, C-5H), 4.19 and 4.38 (2H, ABq, J=8 Hz, OCH$_2$), 5.0—6.2 (4H, m, CH=CH$_2$, CHOH), 5.80 (2H, m, CO$_2$CH$_2$).

Pivaloyloxymethyl (5*R,6*S**)-6-(4-Methyl-2-oxo-1,3-dioxolan-4-yl)carbapen-2-em-3-carboxylate (17a, b). Pivaloyloxymethyl α-[(3*S**,4*R**)-4-Allyl-3-(4-methyl-2-oxo-1,3-dioxolan-4-yl)-2-azetidinon-1-yl]-α-triphenylphosphoranilideneacetate**——2,6-Lutidine (234 μl, 2.2 eq) and thionyl chloride (147 μl, 2.0 eq) were added to a solution of **16a** (370 mg, 0.93 mmol) in THF (2 ml) at −30 °C, and the mixture was stirred for 30 min, then diluted with THF and insoluble material was removed by filtration. Concentration of the filtrate gave crude chlorides, which were dissolved in dioxane (2 ml) and treated with triphenylphosphine (0.45 g, 1.84 eq) and 2,6-lutidine (150 μl, 1.4 eq) at room temperature overnight. The reaction mixture was diluted with EtOAc, washed with water, dried and concentrated. The residue was chromatographed on a Lobar column (size A, benzene–EtOAc 2:1) to give the title ylid (428 mg, 66% from **9a**). IR: 1800, 1740 cm^{-1}.

This compound (428 mg) was converted into the carbapenem **17a** (162 mg, 67%) by using the same procedure as described for **11a**. Recrystallization from CH$_2$Cl$_2$–ether gave pure **17a**: mp 119—121 °C. IR: 1810, 1790, 1750 cm^{-1} ^{1}H-NMR δ: 1.22 (9H, s, *tert*-Bu), 1.62 (3H, s, Me), 2.9—3.1 (2H, m, C-1H$_2$), 3.63 (1H, d, J=3 Hz, C-6H), 4.23 and 4.64 (2H, ABq, J=9 Hz, C-8CH$_2$), 4.33 (1H, br dt, J=3, 9 Hz, C-5H), 5.87 (2H, br s, CO$_2$CH$_2$), 6.60 (1H, br t, J=2 Hz, C-2H). *Anal.* Calcd for C$_{17}$H$_{21}$NO$_8$·2/3H$_2$O: C, 53.82; H, 5.93; N, 3.69. Found: C, 53.94; H, 5.76; N, 3.53.

Similarly **17b** was prepared from **9b** in 36% overall yield. **17b**: mp 186—188 °C (CH$_2$Cl$_2$–ether). IR: 1810 (sh), 1790, 1755 cm^{-1}. ^{1}H-NMR δ: 1.22 (9H, s, *tert*-Bu), 1.68 (3H, s, Me), 2.8—3.1 (2H, m, C-1H$_2$), 3.57 (1H, d, J=3 Hz, C-6H), 4.1—4.5 (1H, m, C-5H), 4.22 and 4.46 (2H, ABq, J=9 Hz, C-8CH$_2$), 5.86 (2H, br s, CO$_2$CH$_2$), 6.57 (1H, br t, J=2.5 Hz, C-2H). *Anal.* Calcd for C$_{17}$H$_{21}$NO$_8$: C, 55.58; H, 5.76; N, 3.81. Found: C, 55.39; H, 5.72; N, 3.83.

Pivaloyloxymethyl 6-[(*E*)-1-(Hydroxymethyl)ethylidene]carbapen-2-em-3-carbaoxylate (18)——A solution of DBU in toluene (1 M, 8 μl, 0.1 eq) was added to a solution of **17a** (32 mg, 0.087 mmol) in CDCl$_3$ (0.4 ml) and the mixture was allowed to stand for 1 h at room temperature. Work-up as for **11a** gave **18** (27 mg, 96%) as an oily residue. IR: 3550, 2970, 1760, 1610 cm^{-1}. ^{1}H-NMR δ: 1.23 (9H, s, *tert*-Bu), 2.02 (3H, s, Me), 2.87 (1H, br d, J=9 Hz, C-1H), 2.91 (1H, br d, J=9H, C-1H), 2.65 (1H, br s, OH), 4.28 (2H, br s, C-8CH$_2$), 4.99 (1H, br dd, J=9, 9 Hz, C-5H), 5.90 (2H, br s, CO$_2$CH$_2$), 6.59 (1H, br t, J=9 Hz, C-2H).

Compound **17b** (28 mg, 0.076 mmol) in CD$_3$CN (0.4 ml) was treated with a solution of DBU in toluene (1 M, 15 μl) at 0 °C for 10 min. Work-up as above gave **18** (20 mg, 81%).

Pivaloyloxymethyl 6-[(*Z*)-1-(Hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (19)——A solution of DBU in toluene (1 M, 8.6 μl) was added to a solution of **17b** (28 mg) in CDCl$_3$ (0.45 ml) in an NMR tube at room temperature, and after 5 min the NMR spectrum was measured. ^{1}H-NMR δ: 1.23 (9H, s, *tert*-Bu), 1.85 (3H, s, Me), 2.91 (2H, dd, J=3, 9 Hz, C-1H$_2$), 3.27 (1H, br s, OH), 4.40 (2H, s, C-8CH$_2$), 4.81 (1H, br t, J=9 Hz, C-5H), 5.90 (2H, br s, CO$_2$CH$_2$), 6.58 (1H, br t, J=3 Hz, C-2H).

The above reaction mixture was diluted with EtOAc, washed with water, dried and concentrated to give **20**. IR: 2970, 1750 cm^{-1}. ^{1}H-NMR δ: 1.25 (9H, s, *tert*-Bu), 1.8—2.6 (2H, m, CH$_2$), 2.12 (3H, s, Me), 2.6—3.4 (2H, m, CH$_2$), 4.67 (2H, s, OCH$_2$), 5.0—5.5 (1H, m, =NCH), 5.92 (2H, s, CO$_2$CH$_2$).

***p*-Nitrobenzyl (5*R**,6*S**)-2-(2-Acetamidoethyl)thio-6-[(4*R**)-4-methyl-2-oxo-1,3-dioxolan-4-yl]carbapenam-3-carboxylate (21a)**——*N*-Acetylcysteamine (0.34 ml, 1.05 eq) and K$_2$CO$_3$ (207 mg, 0.5 eq) were added to a solution of carbapenem **11a** (1.17 g, 3.00 mmol) in a mixture of THF (30 ml) and DMF (4.5 ml) and the mixture was stirred at room temperature for 1.5 h, then diluted with EtOAc, washed with water, dried and concentrated to give a mixture of the sulfides **21a** (1.7 g), which were used without separation for the next reaction.

***p*-Nitrobenzyl (5*S**,6*S**)-2-(2-Acetamidoethyl)thio-6-[(4*R**)-4-methyl-2-oxo-1,3-dioxolan-4-yl]carbapen-1-em-3-carboxylate (25a)**——A solution of the carbapenam mixture **21a** (910 mg, crude, 1.53 mmol) in CH$_2$Cl$_2$ (30 ml) was treated with *m*-CPBA (85%, 312 mg) under ice cooling, and the mixture was stirred for 30 min, then washed with NaHCO$_3$ solution and brine, dried and concentrated to give crude sulfoxides **23a** (869 mg).

The products were dissolved in CH$_2$Cl$_2$ (15 ml) and treated with thionyl chloride (150 μl, 1.4 eq) and pyridine (410 μl, 3.3 eq) under ice cooling for 30 min. The reaction mixture was diluted with CH$_2$Cl$_2$, washed with NaHCO$_3$ solution and water, dried and concentrated. The residue was chromatographed on a Lobar column (size B, 15% isopropanol–EtOAc) to give **25a** (421 mg, 54%) and one of the stereoisomers of the starting material **21a** (63 mg, 9%). **25a**: IR: 1810, 1780, 1750 (sh), 1675 cm^{-1}. ^{1}H-NMR δ: 1.63 (3H, s, Me), 1.98 (3H, s, NAc), 2.5—3.7 (4H, m, SC$_2$H$_4$), 3.41 (1H, d, J=3 Hz, C-6H), 4.23 and 4.68 (2H, ABq, J=8.5 Hz, C-8CH$_2$), 4.5—4.8 (1H, m, C-5H), 5.18 (1H, m, C-3H), 5.33 (2H, s, CO$_2$CH$_2$), 6.13 (1H, t, J=1.5 Hz, C-1H), 6.31 (1H, br, NH), 7.59 and 8.21 (4H, A$_2$B$_2$q, J=9 Hz, Ar).

***p*-Nitrobenzyl 2-(2-Acetamidoethyl)thio-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (32)**——BSA (137 μl, 1.1 eq) and DBU (25 μl, 0.3 eq) were added to a solution of the carbapen-1-em **25a** (257 mg,

0.51 mmol) in CH_2Cl_2 (2.5 ml). The mixture was stirred at room temperature for 2 h, then charged on a Lobar column (size A), and the column was eluted with a mixture of *n*-hexane–CH_2Cl_2–EtOAc–CH_3CN (1 : 1 : 1 : 1) to give **31** (171 mg, 41%) and the carbapen-1-em **27** (32 mg, 8%). **31**: IR: 1750, 1700 (sh), 1675 cm^{-1}. ^{1}H-NMR δ: 0.18 (9H, s, $SiMe_3$), 1.80 (6H, s, C-8Me, NAc), 2.7—3.7 (6H, m, C-1H_2, SC_2H_4), 4.23 (2H, s, C-8CH_2), 4.91 (1H, br t, J=9 Hz, C-5H), 5.25 and 5.57 (2H, ABq, J=14 Hz, CO_2CH_2), 6.2 (1H, br, NH), 7.72 and 8.24 (4H, A_2B_2q, J=9 Hz, Ar). **27**: ^{1}H-NMR δ: 0.17 (9H, s, $SiMe_3$), 1.97 (6H, s, C-8Me, NAc), 2.8—3.6 (4H, m, SC_2H_4), 4.22 (2H, br s, C-8CH_2), 5.0—5.5 (2H, m, C-3H, C-5H), 5.32 (2H, s, CO_2CH_2), 5.95 (1H, s, C-1H), 6.1 (1H, br, NH), 7.55—8.12 (4H, A_2B_2q, J=9 Hz, Ar).

A solution of the silyl ether **31** (171 mg) in a mixture of methanol (1.5 ml) and water (0.15 ml) was allowed to stand at room temperature for 3 h. Evaporation of the solvent and trituration of the residue with CH_2Cl_2 and ether gave **32** (64 mg, 43%); mp 162—165 °C. IR (KBr): 3355, 3300, 2925, 1750, 1705, 1695, 1655 cm^{-1}. ^{1}H-NMR ($CDCl_3$+C_5D_5N) δ: 1.95 (3H, s, NAc), 2.02 (3H, s, C-8Me), 2.6—3.6 (6H,m, C-1H_2, S-C_2H_4), 4.29 (2H, s, C-8CH_2), 5.01 (1H, br dd, J=9, 9 Hz, C-5H), 5.22 and 5.52 (2H, ABq, J=14 Hz, CO_2CH_2), 7.68 and 8.17 (4H, A_2B_2q, J=9 Hz, Ar). UV (EtOH): 267, 300, 334 nm. MS (FD) *m/e*: 461 (M^+).

***p*-Nitrobenzyl 2-(2-Acetamidoethyl)sulfinyl-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (35)**——A solution of *m*-CPBA (85%, 4.4 mg, 1 eq) in CH_2Cl_2 (2 ml) was added to a solution of **32** (10 mg) in CH_2Cl_2 (10 ml), and the mixture was stirred under ice cooling for 10 min, then washed with aqueous $NaHCO_3$ solution, dried and concentrated to give a residue, which was triturated with ether to afford **35** as a mixture of sulfoxide isomers. This product was separated by preparative TLC (E. Merck, silica gel, $CHCl_3$–MeOH 9 : 1) to a more polar product with *Rf* 0.19 and a less polar product with *Rf* 0.24. The more polar product gave the same *Rf* value as asparenomycin B PNB ester on silica gel TLC plates with several different solvent systems. MS (FD) *m/e*: 477 (M^+).

Pivaloyloxymethyl (5*R,6*S**)-2-(2-Acetamidoethyl)thio-6-[(4*R**)-4-methyl-2-oxo-1,3-dioxolan-4-yl]-carbapenam-3-carboxylate (22a)**——*N*-Acetylcysteamine (0.12 ml, 1.1 eq) and K_2CO_3 (69 mg, 0.5 eq) were added to a solution of the carbapenem **17a** (368 mg, 1.00 mmol) in a mixture of THF (10 ml) and DMF (1.5 ml), and the mixture was stirred for 15 min at room temperature, then diluted with EtOAc, washed with $NaHCO_3$ solution, dried and concentrated. The residue was chromatographed on a Lobar column (size A, CH_3CN) to give **22a** (522 mg, 83%) as a mixture of stereoisomers.

Pivaloyloxymethyl (5*S,6*S**)-2-(2-Acetamidoethyl)thio-6-[(4*R**)-4-methyl-2-oxo-1,3-dioxolan-4-yl]-carbapen-1-em-3-carboxylate (26a)**——The penams **22a** (420 mg, 0.86 mmol) were oxidized with *m*-CPBA (175 mg, 1.0 eq) in CH_2Cl_2 under ice cooling to sulfoxides **24a** (411 mg), which were treated with thionyl chloride (72 μl, 1.2 eq) and pyridine (230 μl, 3.5 eq) in CH_2Cl_2 (8.2 ml) under ice cooling for 30 min to give, after chromatography on a Lobar column (size A, $CHCl_3$–MeOH), **26a** (236 mg, 60%) and the carbapen-2-em **41a** (49 mg, 12%). **26a**: IR: 1810, 1780, 1670 cm^{-1}. ^{1}H-NMR δ: 1.23 (9H, s, *tert*-Bu), 1.63 (3H, s, C-8Me), 2.00 (3H, s, NAc), 2.5—3.6 (5H, m, C-6H, SC_2H_4), 4.20 and 4.63 (2H, ABq, J=8.5 Hz, C-8CH_2), 4.4—4.5 (1H, m, C-5H), 5.11 (1H, m, C-3H), 5.81 (2H, br s, CO_2CH_2), 6.06 (1H, br s, C-1H), 6.1—6.6 (1H, m, NH). UV (EtOH): 245 nm. **41a**: IR: 1810, 1790, 1750, 1675 cm^{-1}. ^{1}H-NMR δ: 1.22 (9H, s, *tert*-Bu), 1.63 (3H, s, C-8Me), 1.98 (3H, s, NAc), 2.8—3.7 (6H, m, C-1H_2, SC_2H_4), 3.56 (1H, d, J=3 Hz, C-6H), 4.1—4.5 (1H, m, C-5H), 4.20 and 4.63 (2H, ABq, J=9 Hz, C-8CH_2), 5.80 and 5.97 (2H, ABq, J=5.5 Hz, CO_2CH_2), 6.45 (1H, br, NH). UV (EtOH): 323 nm.

Pivaloyloxymethyl 2-(2-Acetamidoethyl)thio-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (34)——In a manner similar to that used for the corresponding PNB ester **25a**, the POM ester **26a** (164 mg, 0.39 mmol) was treated with BSA (165 μl, 2.0 eq) and DBU (17 μl, 0.3 eq) in CH_2Cl_2 to give a mixture of **29** and **33** (total 170 mg), which was dissolved in methanol (0.4 ml) containing water (40 μl). After standing at room temperature for 3 h, the reaction mixture was worked up and the residue was chromatographed on a Lobar column (size A, $CHCl_3$–acetonitrile) to give **34** (26 mg, 12%) and **30** (29 mg, 16%). **34**: IR: 1755, 1710, 1670 cm^{-1}. ^{1}H-NMR δ: 1.23 (9H, s, *tert*-Bu), 1.98 (6H, s, C-8Me, NAc), 2.6—3.7 (6H, m, C-1H_2, SC_2H_4), 4.25 (2H, br s, C-8CH_2), 4.92 (1H, br dd, J=8.5 Hz, C-5H), 5.83 and 5.94 (2H, ABq, J=5 Hz, CO_2CH_2), 6.4—6.7 (1H, m, NH). UV (EtOH): 312, 340 nm.

Pivaloyloxymethyl 2-(2-Acetamidoethyl)sulfinyl-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (36)——The sulfide **34** (88 mg, 0.20 mmol) was oxidized with *m*-CPBA (41 mg, 1.0 eq) in CH_2Cl_2 under ice cooling to give **36** (91 mg, 100%) as a mixture of sulfoxide isomers. **36**: IR: 3400 (br), 1780 (sh), 1760, 1705, 1675 cm^{-1}. ^{1}H-NMR δ: 1.25 (9H, s, *tert*-Bu), 2.03 (6H, s, C-8Me, NAc), 3.0—4.0 (6H, m, C-1H_2, SC_2H_4), 4.30 (2H, br s, C-8CH_2), 4.8—5.4 (1H, m, C-5H), 5.88 (2H, br s, CO_2CH_2), 5.6—6.9 (1H, m, NH).

***dl*-Asparenomycin B (37) and Its Sulfoxide Isomer 38 from the POM Esters 36**——A solution of the POM ester **36** (27 mg, 0.059 mmol) in MeOH (0.45 ml) was diluted with 0.05 M pH 7 phosphate buffer (3.1 ml). Esterase (Sigma, Type 1, from porcine liver, 1380 units/ml suspension, 0.83 ml, 1150 units) was then added, and the mixture was diluted with the phosphate buffer, (11 ml). The reaction mixture was stirred at room temperature for 2 h and chromatographed on a Diaion HP-20AG column (25 mm × 310 mm) using deionized water as the eluting solvent to give, after concentration and freeze-drying of the eluate, a mixture of **37** and **38** (*ca.* 4 mg) and almost pure **38** (2 mg). The mixture was separated by preparative HPLC (Nucleosil 30C_{18}, 20 mm × 250 mm, 0.01 M phosphate buffer), desalinated (HP-20AG, 25 mm × 310 mm, water) and freeze-dried to give pure **37** (1.0 mg, 5%) and **38** (total 2.7 mg,

13%). **37** showed the same retention times as the authentic asparenomycin B ester on HPLC analysis (Nucleosil columns).

***p*-Methoxybenzyl (5*R**,6*S**)-6-(4-Methyl-2-oxo-1,3-dioxolan-4-yl)carbapen-2-em-3-carboxylate (46a)**——The PMB ester **46a** was prepared by the method described for **11a, b** and **17a, b** from **9a**. **46a**: mp 131—134 °C. IR: 1810, 1790, 1730 cm^{-1}. ^{1}H-NMR (90 MHz) δ: 1.62 (3H, s, C-8Me), 2.77—3.05 (2H, m, C-1H_2), 3.51 (1H, d, J=3 Hz, C-6H), 3.80 (3H, s, OMe), 4.23 (1H, ddd, J=3, 9, 9 Hz, C-5H), 4.17 and 4.59 (2H, ABq, J=9 Hz, C-8CH_2), 5.18 (2H, s, CO_2CH_2), 6.47 (1H, dd, J=2.3, 2.5 Hz, C-2H), 6.88 and 7.34 (4H, A_2B_2q, J=9 Hz, Ar). UV (EtOH): 227, 274, 280 (sh) nm. *Anal.* Calcd for $C_{19}H_{19}NO_7 \cdot 1/5H_2O$: C, 60.54; H, 5.19; N, 3.72. Found: C, 60.54; H, 5.00; N, 3.89.

***p*-Methoxybenzyl 6-[(*E*)-1-(Hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (44)**——Compound **46a** (114 mg, 0.30 mmol) in CH_2Cl_2 was treated with DBU in toluene (1 M, 90 μl, 0.3 eq) at room temperature for 10 min to give **44** (87 mg, 88%) after the same work-up as for the PNB ester **12** and chromatography on a Lobar column (size A, benzene–EtOAc 1 : 2). **44**: IR: 3340 (br), 1760, 1718, 1615 cm^{-1}. ^{1}H-NMR (90 MHz) δ: 1.99 (3H, s, C-8Me), 2.83 (2H, dd, J=3, 9 Hz, C-1H_2), 3.79 (3H, s, OMe), 4.23 (2H, s, C-8CH_2), 4.94 (1H, br t, J=9 Hz, C-5H), 5.20 (2H, s, CO_2CH_2), 6.41 (1H, t, J=3 Hz, C-2H), 6.88 and 7.31 (4H, A_2B_2q, J=9 Hz, Ar).

Carboxy Deprotection of 44. Sodium 6-[(*E*)-1-(Hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (45)——A solution of **44** (80 mg, 0.25 mmol) in anisole (1 ml) was added to a solution of $AlCl_3$ (82 mg, 2.5 eq) in a mixture of anisole (1.8 ml) and CH_2Cl_2 (0.1 ml), at -30 °C and the mixture was stirred for 20 min at the same temperature, then poured into a suspension of $NaHCO_3$ (200 mg) in water (10 ml) with vigorous stirring. The insoluble materials were filtered off and washed with a small amount of water, and the filtrate was extracted with CH_2Cl_2. The aqueous phase was poured into a column packed with Diaion HP-20AG (1 cm × 20 cm) and it was eluted with deionized water. The fractions were checked by HPLC (Nucleosil 10C_{18}, 0.02 N, pH 7 phosphate buffer) and the $AgNO_3$ test. Fractions containing the product but no NaCl were collected and concentrated under reduced pressure to *ca.* 2 ml, then freeze-dried to give **45** (18 mg, 32%) as an amorphous powder. IR: 3400 (br), 1745, 1705 cm^{-1}. ^{1}H-NMR (D_2O, ext. TMS) δ: 2.10 (3H, s, C-8Me), 2.93 (2H, dd, J=3, 9 Hz, C-1H_2), 4.30 (2H, s, C-8CH_2), 5.02 (1H, br t, J=9 Hz, C-5H), 6.28 (1H, t, J=3 Hz, C-2H). UV (H_2O): 236 (12300), 308 (1200) nm. MS (SIMS, glycerol) *m/e*: 254 $(M+Na)^+$.

(3*S,4*R**)-3-(4-Methyl-2-oxo-1,3-dioxolan-4-yl)-4-carboxymethyl-2-azetidinone (47a, b)**——Ozone was introduced into a solution of **9a** (4.41 g, 20.9 mmol) in a mixture of CH_2Cl_2 (150 ml) and methanol (100 ml) at -70 °C until a blue color persisted. Excess ozone was removed by bubbling with nitrogen. Dimethyl sulfide (10 ml) was added, and the reaction mixture was stirred for 1 h, then washed with water, dried and concentrated.

The residue was dissolved in acetone (50 ml) and treated with a small excess of Jones' reagent at room temperature for 30 min. After the excess reagent had been decomposed with methanol, the reaction mixture was diluted with EtOAc, filtered and extracted with 5% aqueous $NaHCO_3$. The aqueous extract was back-washed with CH_2Cl_2, acidified with dil. HCl, saturated with NaCl and extracted with methyl ethyl ketone. The organic extract was washed with saturated brine, dried and concentrated to give a residue, which was triturated with ether to give **47a** (4.79 g, 90%). Recrystallization from EtOAc–ether gave pure **47a**; mp 145—146 °C. IR (Nujol): 3300, 1795 (sh), 1780, 1735, 1700 cm^{-1}. ^{1}H-NMR (90 MHz, CD_3CN + MDSO-d_6) δ: 1.51 (3H, s, Me), 2.6—2.7 (2H, m, CH_2CO_2), 3.24 (1H, d, J=2.5 Hz, C-6H), 3.87 (2H, m, C-5H), 4.21 and 4.56 (2H, ABq, J=8 Hz, OCH_2), 7.4 (2H, br, NH, CO_2H).

Similarly **9b** (2.40 g) was converted into **47b** (2.77 g); mp 176—177 °C. IR (Nujol): 3240 (br), 1795, 1735, 1700 cm^{-1}. ^{1}H-NMR (90 MHz, CD_3OD + acetone-d_6) δ: 1.66 (3H, s, Me), 2.73 (2H, d, J=7 Hz, CH_2CO_2H), 3.34 (1H, d, J=2.5 Hz, C-6H), 3.87 (1H, dt, J=2.5, 7 Hz, C-5H), 4.23 and 4.54 (2H, ABq, J=9 Hz, OCH_2).

(3*S,4*R**)-3-(4-Methyl-2-oxo-1,3-dioxolan-4-yl)-4-(3-*p*-methoxybenzyloxycarbonyl-2-oxopropyl)-2-azetidinone (48a, b)**——*N*,*N*′-Carbonyldiimidazole (0.79 g, 1.1 eq) was added to a solution of **47a** (1.02 g, 4.45 mmol) in THF (22 ml), and the mixture was stirred at room temperature for 1.5h. Then a solution of malonic acid mono-*p*-methoxybenzyl ester monomagnesium salt in THF [prepared from the malonic acid mono-PMB ester (1.05 g, 1.05 eq) and magnesium ethoxide (3 N in EtOH, 1.56 ml, 1.05 eq)] was added. The reaction mixture was stirred for 5 h at room temperature, diluted with EtOAc, washed with water, dried and concentrated. The residue was chromatographed on a Lobar column (size B, benzene–EtOAc 1 : 2) to give **48a** (1.13 g, 65%). ^{1}H-NMR (90 MHz) δ: 1.48 (3H, s, Me), 2.7—3.2 (2H, m, CH_2CO), 3.02 (1H, d, J=2.5 Hz, C-6H), 3.49 (2H, s, $COCH_2CO_2$), 3.77 (3H, s, OMe), 3.7—4.2 (1H, m, C-5H), 4.09 and 4.60 (2H, ABq, J=9 Hz, OCH_2), 5.07 (2H, s, CO_2CH_2), 6.8 (1H, br, NH), 6.88 and 7.28 (4H, A_2B_2q, J=9 Hz, Ar).

Similarly, **47b** (3.00 g, 13.0 mmol) gave **48b** (3.43 g, 67%). ^{1}H-NMR δ: 1.58 (3H, s, Me), 2.7—3.3 (2H, m, CH_2CO), 3.08 (1H, d, J=2.5 Hz, C-6H), 3.48 (2H, s, $COCH_2CO_2$), 3.77 (3H, s, OMe), 3.7—4.1 (1H, m, C-5H), 4.11 and 4.43 (2H, ABq, J=9 Hz, OCH_2), 5.07 (2H, s, CO_2CH_2), 6.7 (1H, br s, NH), 6.83 and 7.22 (4H, A_2B_2q, J=9 Hz, Ar).

(3*S,4*R**)-3-(4-Methyl-2-oxo-1,3-dioxolan-4-yl)-4-(3-diazo-3-*p*-methoxybenzyloxycarbonyl-2-oxopropyl)-2-azetidinone (49a, b)**——A solution of **48a** (790 mg, 2.02 mmol) in CH_3CN (10 ml) containing triethylamine (0.37 ml, 1.3 eq) was treated with *p*-toluenesulfonyl azide (520 mg, 1.3 eq) under ice cooling, and the mixture was stirred for 30 min, then diluted with EtOAc, washed with water, dried and concentrated. The residue was chromatographed on a Lobar column (size B, benzene–EtOAc 1 : 2) to give **49a** (750 mg, 89%); mp 168—169 °C (CH_2Cl_2–ether). IR (Nujol):

2140, 1790, 1765, 1735 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3+CD_3CN$) δ: 1.54 (3H, s, C-8Me), 3.0—3.4 (3H, m, C-6H, CH_2CO), 3.30 (3H, s, OMe), 3.9—4.2 (1H, m, C-5H), 4.15 and 4.66 (2H, ABq, J=9 Hz, C-8CH_2), 5.20 (2H, s, CO_2CH_2), 6.2—6.4 (1H, m, NH), 8.90 and 7.31 (4H, A_2B_2q, J=9 Hz, Ar). *Anal.* Calcd for $C_{19}H_{19}N_3O_8$: C, 54.68; H, 4.59; N, 10.07. Found: C, 54.54; H, 4.51; N, 10.28.

Similarly **48b** (227 mg) gave **49b** (228 mg, 94%). IR: 3405, 2140, 1805, 1765, 1708, 1645 cm^{-1}. ^{1}H-NMR δ: 1.63 (3H, s, Me), 3.1—3.4 (3H, m, C-6H, CH_2CO), 3.78 (3H, s, OMe), 3.8—4.2 (1H, m, C-5H), 4.13 and 4.47 (2H, ABq, J=9 Hz, C-8CH_2), 5.17 (2H, s, CO_2CH_2), 6.55 (1H, br s, NH), 6.83 and 7.22 (4H, A_2B_2q, J=9 Hz, Ar).

***p*-Methoxybenzyl (5*R**,6*S**)-6-(4-Methyl-2-oxo-1,3-dioxolan-4-yl)-2-oxocarbapenam-3-carboxylate (50a, b)**——A suspension of **49a** (469 mg, 1.12 mmol) and rhodium (II) acetate (22 mg) in benzene (100 ml) was stirred at 80 °C for 10 min. The reaction mixture was filtered and concentrated. The residue was triturated with ether to give **50a** (349 mg, 80%). Recrystallization from a CH_2Cl_2–ether mixture gave pure **50a**; mp 124—126 °C. IR: 1810, 1780, 1750 cm^{-1}. ^{1}H-NMR (90 MHz) δ: 1.60 (3H, s, C-8Me), 2.4—3.0 (2H, m, C-1H_2), 3.52 (1H, d, J=2.4 Hz, C-6H), 3.77 (3H, s, OMe), 4.08 (1H, m, C-5H), 4.19 and 4.59 (2H, ABq, J=9 Hz, C-8CH_2), 4.64 (1H, s, C-3H), 5.10 (2H, s, CO_2CH_2), 6.87 and 7.27 (4H, A_2B_2q, J=9 Hz, Ar). UV (EtOH): 274, 280 nm.

Similarly, **49b** (228 mg) gave **50b** (168 mg, 79%); mp 153—155 °C (CH_2Cl_2–ether). IR: 1810, 1780, 1740 cm^{-1}. ^{1}H-NMR δ: 1.68 (3H, s, C-8Me), 2.5—2.9 (2H, m, C-1H_2), 3.55 (1H, d, J=2 Hz, C-6H), 3.78 (3H, s, OMe), 3.9—4.2 (1H, m, C-5H), 4.19 and 4.41 (2H, ABq, J=9 Hz, C-8CH_2), 4.67 (1H, s, C-3H), 5.10 (2H, s, CO_2CH_2), 6.83 and 7.22 (A_2B_2q, J=9 Hz, 4H, Ar). UV (EtOH): 274, 280 nm.

***p*-Methoxybenzyl (5*R**,6*S**)-2-(*E*)-(2-Acetamidoethenyl)thio-6-(4-methyl-2-oxo-1,3-dioxolan-4-yl)carbapen-2-em-3-carboxylate (51a, b)**——A mixture of **50a** (292 mg, 0.75 mmol), diphenylchlorophosphate (171 μl, 1.1 eq) and diisopropylethylamine (144 μl, 1.1 eq) in CH_3CN (19 ml) was stirred for 10 min under ice cooling, and then silver (*E*)-2-acetamido-1-ethenethiolate (420 mg, 2.5 eq) and NaI (280 mg, 2.5 eq) were added. After being stirred at room temperature for 50 min, the reaction mixture was diluted with EtOAc, washed with water, dreid and concentrated. The residue was chromatographed on a Lobar column (size B, benzene–EtOAc 1:2 and hexane–CH_2Cl_2–EtOAc–CH_3CN 1:1:1:1) to give **51a** (168 mg, 46%). IR: 1805, 1795, 1730, 1610 cm^{-1}. UV (EtOH): 268, 278, 323 nm.

Similarly **50b** (350 mg) gave **51b** (308 mg, 70%). IR: 1805, 1782, 1700, 1620 cm^{-1}. ^{1}H-NMR δ: 1.59 (3H, s, C-8Me), 1.97 (3H, s, NAc), 3.2 (2H, m, C-1H_2), 3.63 (1H, d, J=3 Hz, C-6H), 3.77 (3H, s, OMe), 4.08 (1H, dt, J=3, 9 Hz, C-5H), 4.22 and 4.29 (2H, ABq, J=9 Hz, C-8CH_2), 5.16 (2H, s, CO_2CH_2), 5.89 (1H, d, J=14 Hz, SCH=), 6.8—7.5 (5H, m, =CHN, Ar), 8.68 (1H, br d, J=10 Hz, NH). UV (EtOH): 228, 268, 278, 323 nm. MS (SIMS, glycerol) *m/e*: 489 $(M+H)^+$.

***p*-Methoxybenzyl 2-(*E*)-(2-Acetamidoethenyl)thio-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (52)**——A solution of DBU in toluene (1 M, 40 μl, 0.15 eq) was added to a solution of **51b** (133 mg, 0.27 mmol) in CD_3CN (1 ml), and the mixture was stirred at room temperature for 30 min, then diluted with EtOAc, washed with water, dried and concentrated. The residue was chromatographed on a Lobar column (size A, hexane–EtOAc–CH_2Cl_2–CH_3CN 1:1:1:1) to give **52** (70 mg, 58%). IR: 3400—3300 (br), 1740, 1695, 1620 cm^{-1}. ^{1}H-NMR δ: 1.93 (3H, s, C-8Me), 1.98 (3H, s, NAc), 2.9—3.2 (2H, m, C-1H_2), 3.73 (3H, s, OMe), 4.15 (2H, br s, C-8CH_2), 4.79 (1H, br t, J=8 Hz, C-5H), 5.16 (2H, s, CO_2CH_2), 5.72 (1H, d, J=14 Hz, SCH=), 6.7—7.4 (5H, m, =CHN, Ar), 8.57 (1H, br d, J=10 Hz, NH). UV (EtOH): 226, 273, 280 (sh), 312 nm. MS (SIMS, glycerol) *m/e*: 445 $(M+H)^+$.

A suspension of **51a** (90 mg, 0.18 mmol) in a mixture of CH_2Cl_2 and benzene (5:1, 9 ml) was treated with DBU (30 μl) at room temperature for 3 h to give, after usual work-up, crude **52** (61 mg).

Sodium 2-(*E*)-(2-Acetamidoethenyl)thio-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (*dl*-Asparenomycin C) (53)——A solution of crude **52** (295 mg) [prepared from **51b** (234 mg, 0.48 mmol) in CH_3CN (9.6 ml) with DBU (14 μl)] in a mixture of CH_2Cl_2 (2.4 ml) and anisole (2.4 ml) was added to a solution of $AlCl_3$ in a mixture of anisole (4.8 ml) and CH_2Cl_2 (0.48 ml) at −60 °C, and the whole stirred at the same temperature for 10 min. A solution of $NaHCO_3$ (453 mg) in water (19 ml) was added, and the reaction mixture was stirred vigorously for 30 min at room temperature then filtered to remove insoluble materials. The aqueous filtrate was washed with CH_2Cl_2 and poured into a column of Diaion HP-20AG (20 × 250 mm), which was eluted with deionized water. Fractions containing the product (HPLC) but no NaCl ($AgNO_3$ test) were collected, concentrated to a small volume and freeze dried to give **53** (66 mg, 40% from **51b**) as an amorphous powder. This material was identical with an authentic sample of asparenomycin C (**2**) based on IR (KBr), NMR (D_2O) and HPLC (Nucleosil 10C_{18}, pH 7 phosphate buffer) comparisons. **53**: IR (KBr): 3400 (br), 1745, 1675, 1620 cm^{-1}. ^{1}H-NMR (100 MHz, DSS) δ: 1.98 (3H, s, C-8Me), 2.06 (3H, s, NAc), 8.10 (1H, d, J=8.5 Hz, C-1H), 3.11 (1H, d, J=9.5 Hz, C-1H), 4.22 (2H, s, C-8CH_2), 4.7—5.0 (1H, m, C-5H), 6.01 (1H, d, J=14 Hz, SCH=), 7.13 (1H, d, J=14 Hz, NCH=). UV (H_2O): 234 (26100), 296 (12900), 340 (5600) nm. MS (SIMS, glycerol) *m/e*: 369 $(M+Na)^+$.

Sodium 2-(*E*)-(2-Acetamidoethenyl)sulfinyl-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (54 and 55)——A solution of **53** (52 mg) in pH 7 phosphate buffer (0.02 M, 15 ml) and a solution of *m*-CPBA (85%, 46 mg) in CH_2Cl_2 (18 ml) were agitated together vigorously under ice cooling for 2 h, then the aqueous phase was separated, washed with CH_2Cl_2, concentrated to a small volume and subjected to preparative HPLC (Nucleosil 30C_{18}, 20 × 250 mm, 0.01 M pH 7 phosphate buffer). Fractions containing **54**, which were eluted first, were combined and concentrated to a small volume and desalinated by HP-20AG column chromatography. Concentration and

subsequent freeze–drying of the eluate gave **54** (32 mg, 59%) as an amorphous powder, which was identical with authentic asparenemycin A (**1**) based on IR (KBr), ^{1}H-NMR (D_2O) and HPLC comparisons. **54**: IR (KBr): 3400 (br), 1747, 1700, 1643 cm^{-1}. ^{1}H-NMR (100 MHz) δ: 2.45 (3H, s, C-8Me), 2.57 (3H, s, NAc), 3.61 (1H, d, J=9.5 Hz, C-1H), 3.62 (1H, d, J=8.5 Hz, C-1H), 4.71 (2H, s, C-8CH_2), 5.3—5.5 (1H, m, C-5H), 6.80 (1H, d, J=14 Hz, SCH=), 7.99 (1H, d, J=14 Hz, NCH=). UV (H_2O): 240 (23800), 270 (13800), 308 (6000) nm. MS (SIMS, glycerol) m/e: 385 $(M+Na)^+$.

Similarly, from preparative HPLC fractions containing **55**, pure **55** (16 mg, 29%) was obtained as an amorphous powder. **55**: IR (KBr): 3400 (br), 1746, 1700, 1623 cm^{-1}. ^{1}H-NMR (100 MHz) δ: 2.43 (3H, s, C-8Me), 2.55 (3H, s, NAc), 3.60 (1H, d, J=8 Hz, C-1H), 3.64 (1H, d, J=10 Hz, C-1H), 4.71 (2H, s, C-8CH_2), 5.4—5.6 (1H, m, C-5H), 6.74 (1H, d, J=14 Hz, SCH=), 7.96 (1H, d, J=14 Hz, NCH=). UV (H_2O): 240 (21100), 267 (13000), 309 (4100) nm. MS (SIMS, grycerol) m/e: 385 $(M+Na)^+$.

***p*-Methoxybenzyl 2-(2-Acetamidoethyl)thio-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (56)**——Compound **56** was prepared from **50a, b** by a method similar to that used for **52** in about 70% yield. **56**: IR: 3450, 3360, 1755, 1700 (sh), 1670, 1615 cm^{-1}. ^{1}H-NMR (90 MHz) δ: 1.82 (3H, s, C-8Me), 1.91 (3H, s, NAc), 2.91 (2H, br d, J=6.3 Hz, C-1H_2), 3.1—3.5 (4H, m, SC_2H_4), 3.77 (3H, s, OMe), 4.13 (2H, br s, C-8CH_2), 4.83 (1H, br t, J=9 Hz, C-5H), 5.14 (2H, s, CO_2CH_2), 6.7—7.1 (1H, m, NH), 6.91 and 7.37 (4H, A_2B_2q, J=9 Hz, Ar). UV (EtOH): 274 (sh), 280 (sh), 307, 330 (sh) nm.

Sodium 2-(2-Acetamidoethyl)thio-6-[(*E*)-1-(hydroxyethyl)ethylidene]carbapen-2-em-3-carboxylate (*dl*-6643-X) (57)——Compound **56** (290 mg 0.65 mmol) was converted into the sodium salt **57** (98 mg, 68%) by the same procedure as used for **53**. **57**: IR (KBr): 3400 (br), 1740, 1625 cm^{-1}. ^{1}H-NMR (100 MHz) δ: 2.42 (6H, s, C-8Me, NAc), 3.26—3.90 (6H, m, C-1H_2, SC_2H_4), 4.68 (2H, s, C-8CH_2), 5.31 (1H, br t, J=9 Hz, C-5H). UV (H_2O): 236 (14200), 284 (6200), 325 (3300) nm. MS (SIMS, glycerol) m/e: 371 $(M+Na)^+$.

Sodium 2-(2-Acetamidoethyl)sulfinyl-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylate (*dl*-Asparenomycin B) (37 and 38)——Compound **57** (30 mg, 0.09 mmol) was oxidized with *m*-CPBA in a manner similar to that used for **54** and **55** to give **37** (16 mg, 51%) and **38** (7 mg, 22%) as amorphous powders. **37** was identical with authentic asparenomycin B based on IR (KBr), ^{1}H-NMR (D_2O) and HPLC comparisons. **37**: IR (KBr): 3420 (br), 1772, 1752, 1660, 1620, 1600 cm^{-1}. ^{1}H-NMR (100 MHz) δ: 2.46 (6H, s, C-8Me, NAc), 3.62 (2H, br d, J=9 Hz, C-1H_2), 3.7—4.1 (4H, m, SC_2H_4), 4.72 (2H, s, C-8CH_2), 5.50 (1H, br t, J=9 Hz, C-5H). UV (H_2O): 238 (14000), 313 (3100) nm. MS (SIMS, glycerol) m/e: 387 $(M+Na)^+$. **38**: ^{1}H-NMR (100 MHz) δ: 2.43 (3H, s, C-8Me), 2.46 (3H, s, NAc), 3.5—3.8 (4H, m, C-1H_2, SCH_2), 3.9—4.2 (2H, m, CH_2N), 4.73 (s, 2H, C-8CH_2), 5.54 (1H, br t, J=9 Hz, C-5H). MS (SIMS, grycerol) m/e: 387 $(M+Na)^+$.

***p*-Methoxybenzyl 2-(2-*p*-Methoxybenzyloxycarbonylaminoethyl)thio-6-[(*E*)-1-(hydroxymethyl)ethylidene]-carbapen-2-em-3-carboxylate (58)**——Compound **58** (187 mg, 58%) was prepared from **50a** (219 mg) in a manner similar to that used for **52**. **58**: ^{1}H-NMR δ: 1.97 (3H, s, C-8Me), 2.5—3.5 (6H, m, C-1H_2. SC_2H_4), 3.75 (6H, s, OMe×2), 4.17 (2H, br s, C-8CH_2), 4.80 (1H, br t, J=9 Hz, C-5H), 4.97 and 5.18 (4H, s×2, CO_2CH_2×2), 6.8—7.5 (8H, m, Ar).

2-(2-Aminoethyl)thio-6-[(*E*)-1-(hydroxymethyl)ethylidene]carbapen-2-em-3-carboxylic Acid (59)——A solution of **58** (130 mg, 0.23 mmol) in a mixture of anisole (2 ml) and CH_2Cl_2 (0.2 ml) was added to a solution of $AlCl_3$ (151 mg, 5 eq) in a mixture of anisole (4.5 ml) and CH_2Cl_2 (0.45 ml) at −50 °C, and the whole was stirred for 40 min. A solution of $NaHCO_3$ (430 mg) in water (9 ml) was added, and the reaction mixture was stirred vigorously for 30 min at room temperature. The mixture was filtered to remove the insoluble material, then the aqueous phase was washed with CH_2Cl_2 and chromatographed on an HP-20AG column (1×10 cm). Elution with deionized water followed by concentration and freeze–drying of the eluate gave **59** (23 mg, 36%) as a powder. **59**: IR (KBr): 3400 (br), 1740, 1705 cm^{-1}. ^{1}H-NMR (100 MHz) δ: 2.43 (3H, s, C-8Me), 3.2—3.8 (6H, m, C-1H_2, SC_2H_4), 4.67 (2H, s, C-8CH_2), 5.33 (1H, br t, J=9 Hz, C-5H). UV (H_2O): 236 (12500), 282 (5400), 320 (3100) nm.

Determination of Minimum Inhibitory Concentrations (MICs)——MICs were determined by the agar dilution method using sensitivity test agar (Eiken, Japan). An overnight culture of bacteria in tryptosoy broth (Eiken, Japan) was diluted to about 10^6 cells/ml with the same broth and inoculated with an inoculating device onto agar containing serial twofold dilutions of the test compound. Organisms were incubated at 37 °C for 18—20 h.

The MIC of a compound was defined as the lowest concentration that visibly inhibited growth.

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