Expedited Articles

5,6-*cis*-Penems: Broad-Spectrum Anti-Methicillin-Resistant *Staphylococcus aureus* β-Lactam Antibiotics

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5,6-*cis*-Penem derivatives have been synthesized and evaluated as anti-MRSA antibiotics. The *cis*-penems **5** and **6** showed potent activities against not only MRSA but also a wide variety of bacteria including β-lactamase-producing microorganisms. These compounds were designed to have high affinity to the penicillin-binding protein 2a of MRSA and to form stable acyl intermediates with β-lactamases by blocking the deacylating water molecule.

Introduction

Among known resistance mechanisms of bacteria against antibiotics, alteration of the target site structures is a major concern in the field of β-lactam-interacting enzymes, such as β-lactamases[1,2] and penicillin-binding proteins (PBPs).[3] β-Lactamases change their active site structures to gain higher affinities to various β-lactam antibiotics for degradation of the β-lactam structure, while the penicillin-binding protein of methicillin-resistant *Staphylococcus aureus* (MRSA) has an altered active site structure having a reduced affinity toward β-lactam antibiotics. Since amino acid sequences for the mutant β-lactamases have been elucidated from their gene sequences, it is possible to examine a plausible contribution of the mutated amino acids for the resistance through three-dimensional structures of β-lactamases.[4,5] Recently, a three-dimensional structure of transpeptidase PBP2x, a high molecular weight penicillin-binding protein of *Klebsiella pneumoniae*, has been reported,[6] and it has been suggested that the high molecular weight transpeptidases have active site residues similar to the class A β-lactamases. Thus, the reaction of β-lactam antibiotics on PBPs such as the PBP2a of MRSA and the PBP2 of *Escherichia coli* would be analogous to that of β-lactam antibiotics with class A β-lactamases in Michaelis complex formation and acylation.

Recently developed penem and carbapenem antibiotics are essentially inactive against MRSA. Those antibiotics have the characteristic 5,6-*trans*-hydroxyethyl side chain at C-6 and a variety of C-2 side chains. A recent crystallographic structure of an acyl intermediate of TEM-1 β-lactamase with 6α-(hydroxymethyl)-penicilloic acid[7] indicated that the hydroxyethyl moiety should reside in the binding site similar to that of the C-6 acyl moiety of penicillins (Figure 1). On the other hand, the crystallographic structure analysis on penem derivatives has inferred that the side chains at C-2 of penem derivatives are required to have a specific conformation at another side of the binding site.[8] Taking account of the importance of the interaction of the carboxylate of β-lactam antibiotics with the Ser130 residue (Ambler numbering for class A β-lactamases is used for residue number unless otherwise specified)[9] of class A β-lactamases,[10] the substituents at C-2 and C-6 may play a crucial role in adequate disposition of the C-3 carboxylate for initiation of the acylation. Hence, alteration of amino acid residues proximal to those binding sites of the β-lactam-interacting enzymes would interrupt or facilitate the interaction of the carboxylate for the acylation.

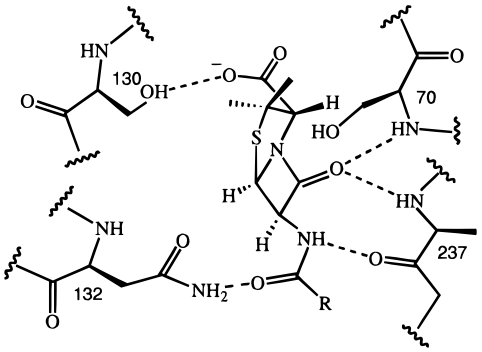

Figure 1. Illustration of a putative binding of penicillin derivative at the active site of class A β-lactamase. Putative hydrogen bondings between penicillin and β-lactamase are shown by broken lines. Ser70 is the active center for the catalysis. R: acyl substituent at C-6.

It has been reported that through introduction of a fairly large substituent at C-2 such as carbolinyl,[11] thiazolobenzimidazoylmethylthio,[12] and 4-arylthiazoylthio[13] moieties, carbapenem derivatives show an improved activity against MRSA, suggesting the alteration of the binding pocket for C-2 substituents. On the other hand, except the *trans*-hydroxyethyl group, C-6 side chains effective for anti-MRSA activity have not been reported for penem or carbapenem derivatives. Antimicrobial activity of 5,6-*cis*-penems bearing the hydroxyethyl at C-6 (e.g., compound **22**) indicated that the *S* configuration of the hydroxyethyl group is preferable for the interaction with PBPs, although both isomers were not stable to β-lactamases.[14] The susceptibility of the *cis*-penems to β-lactamases implies instability of an acyl intermediate against attack of a

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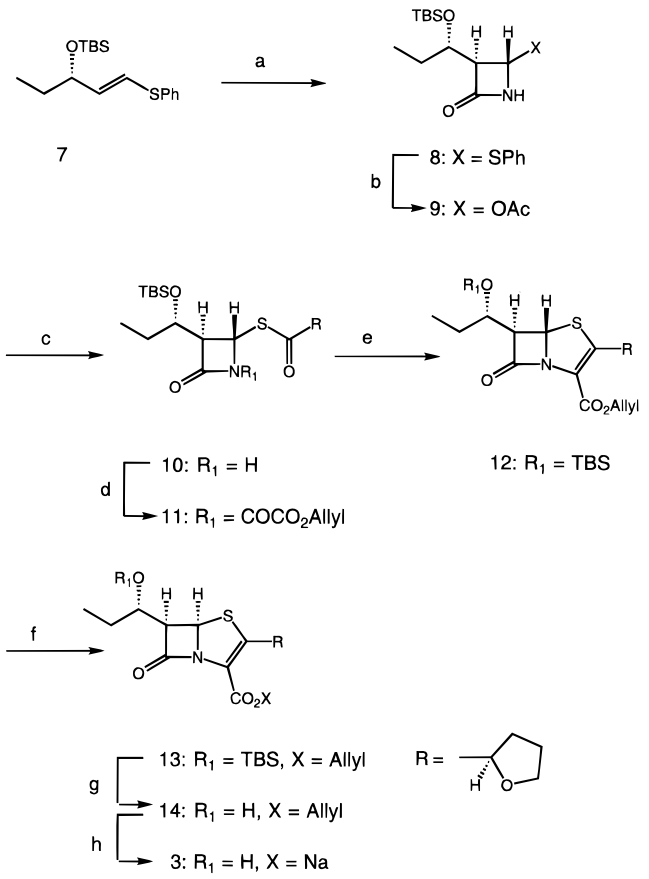
Scheme 1. Synthesis of 5,6-*cis*-Penem Bearing Carbon at C-2[a]



[a] (a) Chlorosulfonyl isocyanate, *i*-Pr_2O then CH_3COSH; (b) Cu_2O, AcOH; (c) RCOSH (R = (2*R*)-tetrahydrofuranyl), NaOH; (d) $ClCOCO_2$allyl, Et_3N; (e) $P(OEt)_3$, toluene, reflux; (f) *hν*; (g) Bu_4NF, AcOH; (h) $Pd(OAc)_2$.

hydrolyzing water molecule, a crucial problem to be overcome for design of broad spectrum *cis*-penem antibiotics. Herein, we report that novel 5,6-*cis*-penem derivatives show a potent antimicrobial activity against MRSA and note that the structure−activity relationship of the *cis*-penems against *β*-lactamase-producing bacteria shows a significant influence of the C-2 side chain structures for the deacylation in the acyl enzymes.

Chemistry

The *cis*-penem **3** was synthesized as shown in Scheme 1. Starting from commercially available ethyl (*S*)-3-hydroxypentanoate, the vinyl sulfide **7** was prepared by the same method as previously described for the preparation of (*R*)-3-[(*tert*-butyldimethylsilyl)oxy]-1-(phenylthio)-1-pentene.[15] The coupling of the vinyl sulfide **7** with chlorosulfonyl isocyanate in diisopropyl ether at room temperature afforded the (3*R*,4*S*)-azetidinone **8** as a major product (3:1 mixture of the diastereomeric 3,4-*trans* isomers), which was then converted to the acetoxyazetidinone **9** in acetic acid using cupric acetate at 100 °C.[16] The penem skeleton was built by a conventional method in three steps: reaction of the acetoxyazetidinone **9** with sodium acylthiolates (R = (2*R*)-tetrahydrofuranoyl) to afford the ester **10**; formation of the oxamate **11** by reaction with allyl chlorooxalate; penem ring formation by intramolecular Wittig reaction of the phosphorane generated *in situ* by treatment of **11** with triethyl phosphite.[17] Then, the 5,6-

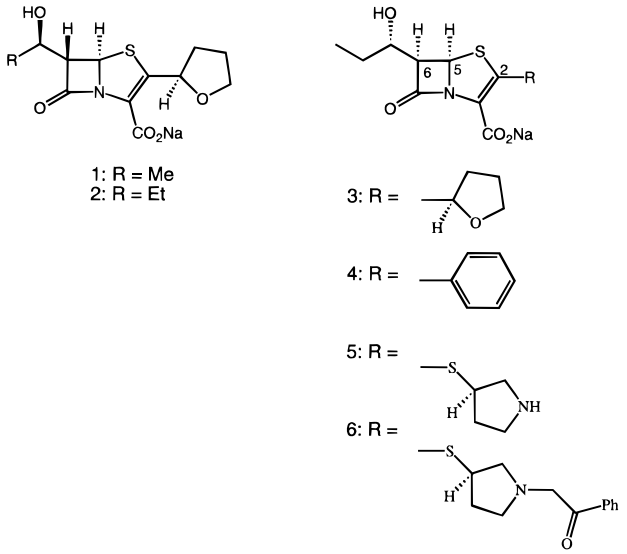


Figure 2. 5,6-*trans*- and -*cis*-penem derivatives.

trans-penem **12** was isomerized to a mixture of the 5,6-*cis*-penem **13** and the starting *trans*-penem **12** (1:2 ratio) by photoirradiation.[18] After separation of the *cis*-penem **13** from the mixture by silica gel chromatography, removal of the TBS group of **13** and subsequent deprotection of **14** provided the desired *cis*-penem **3**.

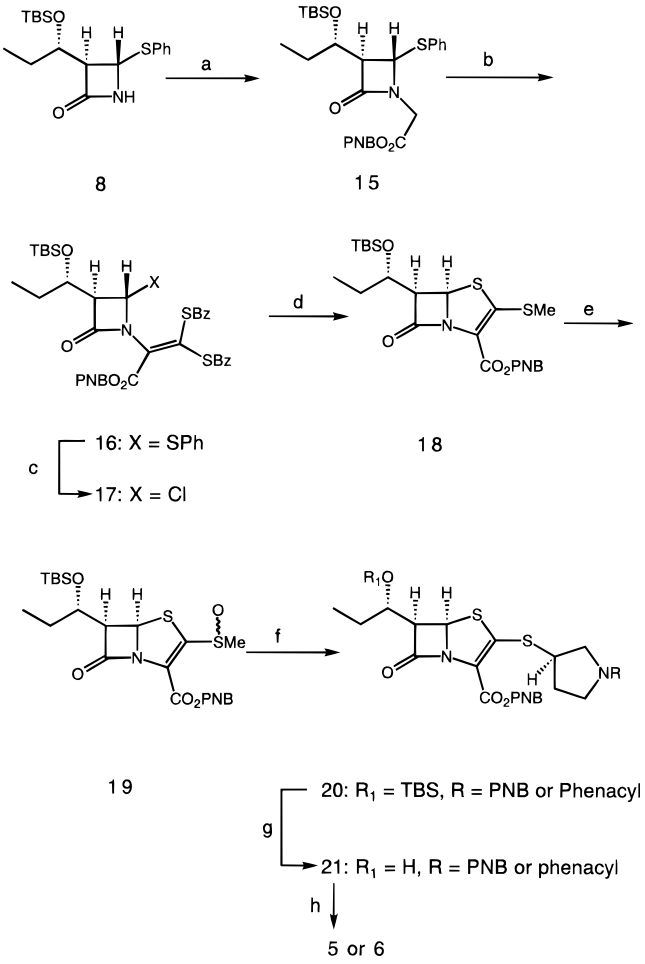
The *cis*-penems **5** and **6** were synthesized by another route, starting from the (3*R*,4*S*)-azetidinone **8** (Scheme 2). The N-alkylation of the azetidinone **8** with *p*-nitrobenzyl iodoacetate gave the *N*-alkylazetidinone **15**. Treatment of the azetidinone **15** with lithium diisopropylamide and successive reactions with carbon disulfide followed by treatment with benzoyl chloride afforded the ketene dithioacetal derivative **16**. After generation of the crude chloride **17** by treatment with sulfuryl chloride, the chloride **17** was reacted with morpholine and methyl iodide to provide the 5,6-*cis*-penem **18.**[19] Oxidation of the methylthio group to the sulfoxide **19** and then substitution of the sulfoxide moiety with pyrrolidinylthiol derivatives furnished the protected penem **20**. Deprotection of the hydroxyl group and carboxylic acid gave the desired 5,6-*cis*-penem derivative **5** (or **6**).

Results and Discussion

Hydroxypropyl derivatives **2**−**6** were synthesized to examine an effect on the antimicrobial activity by the alteration of the bulkiness of the C-6 side chain. The *cis*-penem derivatives **3** and **4** retained the antimicrobial activity against Gram-positive bacteria including MRSA as shown in Table 1. The methyl group of the hydroxypropyl moiety did not reduce the activity of the *cis*-penem but rather enhanced the activity against MRSA (compounds **3** and **22**). The corresponding *trans*-penem **2** greatly reduced the antimicrobial activity against all organisms tested. This result implies that the hydroxypropyl moiety of the *cis*-penems interacts at a less hindered site, whereas the hydroxyethyl moiety of the *trans*-penems binds at a limited space which does not accommodate the additional methyl group of the hydroxypropyl moiety.

The *cis*-penems **3** and **4** were unstable against the *β*-lactamase-producing bacteria. A study on hydrolysis of the *cis*-penem **22** by class A *β*-lactamases indicated

Scheme 2. Synthesis of 5,6-*cis*-Penem Bearing Sulfur at C-2[a]



[a] (a) $BrCH_2CO_2PNB$, K_2CO_3; (b) $LiN(i\text{-}Pr)_2$, CS_2 then benzoyl chloride; (c) SO_2Cl_2; (d) morpholine then MeI; (e) *m*-ClPBA; (f) 1-(*p*-nitrobenzyl)-3-mercaptopyrrolidine; (g) TBAF; (h) H_2/Pd−C.

that **22** was hydrolyzed more rapidly (>1000 times) than the corresponding *trans*-penem **1**,[15] opening the tetrahydrofuran ring to give rise to the hydroxybutylidene derivative **23** as observed in the hydrolysis of the *trans*-penem (Scheme 3).[20] This indicates that the acyl enzymes formed by the *cis*-penems are not resistant to the hydrolytic attack of the deacylating water, while *trans*-penem derivatives form stable acyl enzymes with class A and C β-lactamases.[21]

Since after formation of the acyl enzyme, the (6*R*,8*S*)-hydroxyethyl or -hydroxypropyl moiety of the *cis*-penems would loosely reside at the binding site, it would allow the acyl moiety to have a more mobile conformation which will permit the deacylating water molecule to enter at the site for the deacylation in a similar mode proposed for the penicillin hydrolysis.[10] On the other hand, the fir mLy bound hydroxyethyl moiety of the *trans*-penems would keep the conformation of the rest of the acyl group unchanged to block the attack of the deacylating water molecule.

The *cis*-penems **5** and **6** showed good activity against β-lactamase-producing bacteria as well as MRSA (Table 1). We assume that thioalkyl moieties (SR in compound **24**) would be converted to the α-configuration via β-lactamase-catalyzed tautomerization of the Δ^2- to Δ^3-dihydrothiazole (Scheme 4), as observed in the carbapenems bearing a sulfur atom at C-2.[22] The α-oriented substituents at C-2 should bind firmly at the binding site and block the deacylating water molecule. Thus, the present result would become good evidence for the deacylation mechanism proposed by us.[10] Although another process of the deacylation proposed by Herzberg[23] can explain the instability of the *cis*-penems **3** and **4**, it does not account for the stability of the alkylthio-substituted *cis*-penems **5** and **6**. Recently, Strynadka *et al*. have reported a crystal structure of TEM-1 β-lactamase complexed with a borane derivative and postulated that the complex structure is a transition-state mimic based on Herzberg's assumption.[24] However, the present finding suggests that it will be necessary to investigate if the complex structure represents the true transition state for the deacylation.

The alkylthio-bearing *cis*-penems retained the activity against MRSA. Conformational analysis[25] of the hydroxypropyl (or hydroxyethyl) group indicated that the *cis*-penem has a single local energy minimum at the torsion angle (160°) for $C_7-C_6-C_8-O_{11}$, while the *trans*-penem has two local minima at −160° and −50° where the individual conformations are found in the crystal structures.[8] This infers that in the Michaelis complex formation of the *cis*-penems, the conformationally constrained substituents at C-6 and the carbonyl oxygen of β-lactam are sufficient to determine the disposition of the C-3 carboxylate group suitable for the interaction with the Ser130 residue irrespective of the C-2 side chains. For the *trans*-penems, not only the hydroxyethyl group at C-6 but also the side chains at C-2 would play an important role in locating the carboxylate proximal to the Ser130 residue due to the conformational flexibility of the hydroxyethyl group. Although it is hard to explore the binding site model for PBP2a through model building due to the lack of the structural details of the PBPs, the present results imply that the PBP2a of MRSA has a larger binding pocket at the C-2 substituent-binding region of PBP2a than the other PBPs such as the PBP2 of *E. coli*.

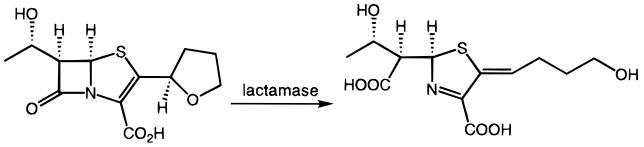
In conclusion, it will be conceivable that the *trans*-penems lose the effective interaction at the C-2 substituent-binding site of PBP2a and the resulting enzyme−substrate complex would not have the suitable interaction between the carboxylate and the Ser130 residue for the acylation. On the other hand, the carboxylate of the *cis*-penems would be located at the suitable position for the acylation only through the interaction of the C-6 substituents and the β-lactam carbonyl oxygen at the oxyanion hole. The stability of the acyl enzymes of β-lactamases is largely dependent on not only the C-6 substituents but also the C-2 moieties. The putative deacylating water which enters at the binding site for the C-2 moieties clearly explains the distinct stability between **3** and **5**. Thus, the *cis*-penems **5** and **6** showed potent activities against a wide variety of bacteria such as MRSA and *Pseudomonas aeruginosa* including β-lactamase-producing microorganisms. The present findings should provide a better understanding about the interaction of β-lactam antibiotics with penicillin-interacting enzymes as well as the hydrolytic mechanism of β-lactamases and thus afford important information for design of anti-MRSA and β-lactamase-resistant β-lactam antibiotics.

Table 1. *In Vitro* Antibacterial Activity (MIC, μg/mL)[a] of Penem Derivatives

	compound no.							
microorganisms	**1**	**2**	**3**	**4**	**5**	**6**	**22**	IPM[o]
S. a.[c]	0.05	0.78	0.39	0.78	0.1	0.1	0.78	<0.025
S. a.[d]	>100	>100	6.25	12.5	3.13	3.13	25	>100
E. f.[e]	0.78	25	0.39	0.20	1.56	0.78	0.39	0.78
B. s.[f]	<0.025	0.78	0.05	0.20	0.05	<0.025	0.05	<0.025
E. c.[g]	0.1	100	3.13	25	0.2	0.2	0.78	0.2
E. c.[b,h]	0.1	12.5	>100	50	0.78	0.78	25	0.1
C. f.[b,i]	25	>100	>100	>100	6.25	6.25	>100	0.39
K. p.[j]	0.2	12.5	1.56	6.25	0.39	0.1	0.78	0.2
S. m.[k]	6.25	>100	25	50	3.13	1.56	25	0.78
E. c.[b,l]	1.56	>100	>100	>100	0.78	0.39	100	0.2
P. v.[m]	3.13	25	1.56	1.56	0.78	0.78	1.56	3.13
P. s.[n]	>100	>100	>100	100	12.5	6.25	>100	1.56

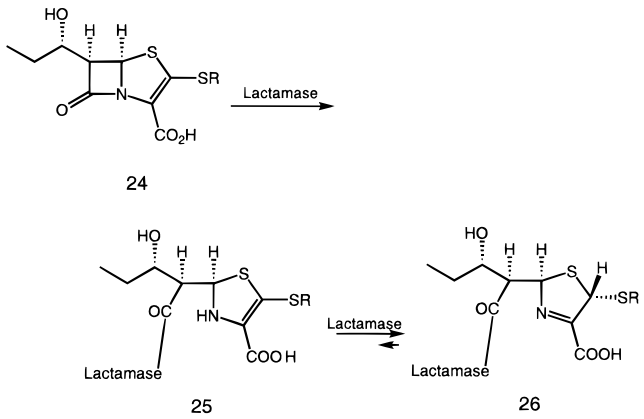
[a] Minimum inhibitory concentration was determined in heart infusion agar medium. Inoculum size: 10^6 cells/mL. Incubation: 24 h at 37 °C. [b] β-Lactamase-producing organism. [c] *Staphylococcus aureus* 209P JC-1. [d] *Staphylococcus aureus* (MRSA). [e] *Enterococcus faecalis* ATCC 19433. [f] *Bacillus subtilis* ATCC 6633. [g] *Escherichia coli* NIHJ JC-2. [h] *Escherichia coli* KC-14/RGN 823. [i] *Citrobacter freundii* GN 7391. [j] *Klebsiella pneumoniae* PCI 602. [k] *Serratia marcescens* IAM 1136. [l] *Enterobacter cloacae* NTCC9394. [m] *Proteus vulgaris* GN 7919. [n] *Pseudomonas aeruginosa* PAO-1. [o] Imipenem.

Scheme 3. Degradation Product of 5,6-*cis*-Penem



22 23

Scheme 4. Plausible Tautomerization ($\Delta^2 \rightarrow \Delta^3$) of Acyl Enzyme



R: alkyl derivatives

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes with a Buchi 535 apparatus. The NMR spectra were recorded on JEOL JNM-GX270 and Brucker ARX 400 FT NMR spectrometers in either $CDCl_3$ solution with tetramethylsilane as an internal standard or D_2O solution with 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt (TSP) as an internal standard. Chemical shifts are reported in ppm relative to the standards. Positive ion fast atom bombardment mass spectra (FABMS) were recorded on a JEOL JMS-AX5000 instrument, and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-HX/HX110A instrument.

All reactions were performed under a positive atmosphere of argon. Organic solutions obtained after workup were dried over anhydrous Na_2SO_4 unless otherwise specified. Flash column chromatography was conducted with Merck silica gel 60 Art 9385 (230−400 mesh), and preparative TLC of Merck Kieselgel 60F254 Art 13895 (1 mm) was used. Compounds showed satisfactory purity by TLC on Merck Kieselgel 60F254 Art 5714 plates (visualized by UV light at 254 nm and/or by 6.3 w/v % phosphomolybdic acid in ethanol). FT IR spectrophotometer: only selected absorptions are reported. For analysis of carboxylic acids or their salts, reverse phase HPLC analysis was carried out using a TSK ODS-80Tm column (4.6 mm i.d. × 150 mm) and a Shimazu liquid chromatograph 6A system. An acetonitrile/water (including 0.1 v/v % trifluoroacetic acid) was employed to elute compounds from the column (flow rate 1 mL/min). Eluting materials were also detected by measuring their UV absorbance with a Waters 991J photodiode array detector. For purification of carboxylic acids or their salts, reverse phase column chromatography on an ODS Chromatorex (100−200 mesh; (Fuji Davison Chemical Ltd.) or preparative HPLC separations on a YMC SH-343-5 S-5 120A AM ODS column (20 mm i.d. × 250 mm), elution with acetonitrile/water (including 0.1 v/v % acetic acid, flow rate 10 mL/min), was carried out.

(3*R*,4*S*)-3-[(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]propyl]-4-(phenylthio)azetidin-2-one (8). To a solution of chlorosulfonyl isocyanate (6.6 mL, 75.8 mmol) in diisopropyl ether (117ml) was added **7** (15.8 g, 51.2 mmol) in diisopropyl ether (31 mL) under Ar at room temperature. The reaction mixture was stirred at room temperature for 4 h. The mixture was cooled to −50 °C and treated with thiophenol (13.0 mL, 127 mmol) and then pyridine (10.2 mL, 127 mmol) followed by stirring at −20 °C for 30 min. The mixture was poured into saturated $KHSO_4$ (100 mL) and extracted with AcOEt (100 mL). The organic phase was washed with saturated $NaHCO_3$ (100 mL) and then saturated Na_2SO_4 (100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided 7.67 g (29%) of a mixture of **8** and (3*S*,4*R*)-3-[(*S*)-1-[(*tert*-butyldimethylsilyl)oxy]propyl]-4-(phenylthio)azetidin-2-one in a 3:1 ratio. **8** (white solid recrystallized from *n*-hexane−toluene): mp 123−124 °C; IR (cm^{-1}) (KBr) 3158 (NH), 1762 (β-lactam C=O); ^{1}H NMR ($CDCl_3$) δ 7.42−7.52 (2H, m), 7.33−7.42 (3H, m), 6.03 (1H, brs), 5.09 (1H, d, J = 2.6 Hz), 4.00−4.08 (1H, m), 3.14 (1H, t, J = 2.6 Hz), 1.45−1.67 (2H, m), 0.87 (9H, s), 0.06 (3H, s), 0.05 (3H, s, SiMe). Anal. ($C_{18}H_{29}NO_2SSi$) C, H, N.

(3*R*,4*S*)-3-[(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]propyl]-4-acetoxyazetidin-2-one (9). To a solution of **8** (4.92 g, 14.0 mmol) in AcOH (30 mL) was added $Cu(OAc)_2 \cdot H_2O$ (2.00 g, 10.0 mmol) at room temperature, and the mixture was heated at 100 °C for 75 min. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure. The residue was diluted with AcOEt (100 mL) and poured into water (100 mL). The organic phase was washed with saturated $NaHCO_3$ (100 mL) and then saturated Na_2SO_4 (100 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided 3.37 g of **9** as a white solid in 80% yield. **9**: mp 72−74 °C; IR (cm^{-1}) (KBr) 3170, 1780, 1748; ^{1}H NMR ($CDCl_3$) δ 6.46 (1H, brs), 5.84 (1H, s), 4.01−4.10 (1H, m), 3.31 (1H, t, J = 4.3 Hz), 2.11 (3H, s), 1.53−1.68 (2H, m), 0.87 (9H,

s), 0.07 (3H, s), 0.06 (3H, s); HRMS (FAB) *m/z* (M + 23) calcd for $C_{14}H_{27}NO_4NaSi$ 324.1607, found 324.1605.

(3*R*,4*S*)-3-[(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]propyl]-4-((*R*)-tetrahydro-2-furyl)azetidin-2-one (10). (*R*)-Tetrahydro-2-furanthiolcarboxylic acid (1.19 g, 9.0 mmol) was buffered at pH 9−10 with 1 N NaOH. To this solution was added **9** (1.81 g, 6.0 mmol) in acetone (6 mL) at room temperature. The reaction mixture was stirred at 50 °C for 10 min and buffered at pH 8−9 with 1 N NaOH followed by stirring for 2 h. The residue was diluted with AcOEt (30 mL) and poured into water (30 mL). The organic phase was washed with saturated $NaHCO_3$ (30 mL) and then saturated Na_2SO_4 (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided 1.79 g as a colorless oil in 80% yield. **10**: IR (cm^{-1}) (KBr) 3158, 1770, 1698; ^1H NMR ($CDCl_3$) δ 6.28, 6.27 (1H, brs), 5.24, 5.21 (1H, d, J = 2.6 Hz), 4.43−4.52 (1H, m), 3.91−4.16 (3H, m), 3.30 (1H, dd, J = 2.6, 2.6 Hz), 2.18−2.37 (1H, m), 1.85−2.18 (3H, m), 1.42−1.68 (2H, m), 0.89 (9H, s), 0.08 (6H, s); HRMS (FAB) *m/z* (M + 1) calcd for $C_{17}H_{32}NO_4SSi$ 374.1821, found 374.1822.

(5*S*,6*R*)-6-[(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]propyl]-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylic Acid Allyl Ester (12). To a solution of **10** (1.49 g, 4.0 mmol) in CH_2Cl_2 (2 mL) were added monoallyl oxalyl chloride (0.99 g, 6.7 mL) in CH_2Cl_2 (1.6 mL) and then triethylamine (0.69 g, 6.8 mmol) in CH_2Cl_2 (1.6 mL) at −20 °C. The reaction mixture was stirred at the same temperature for 1.5 h. The mixture was poured into H_2O (30 mL) and extracted with CH_2Cl_2 (30 mL). The organic phase was washed with saturated $NaHCO_3$ (30 mL) and then saturated Na_2SO_4 (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. After azeotropic drying with toluene, to the residue was added triethyl phosphite (5.5 mL, 32.0 mmol) at room temperature. The reaction mixture was stirred at 70 °C for 1 h. After removal of the excess of triethyl phosphite in vacuo, the reaction mixture was stirred under xylene (20 mL) reflux for 3 h. The mixture was poured into H_2O (30 mL) and extracted with *n*-hexane (30 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided 1.04 g of the product **12** as a yellow oil (58% yield). **12**: IR (cm^{-1}) (neat) 2955, 1790, 1707; ^1H NMR ($CDCl_3$) δ 5.84−6.02 (1H, m), 5.58 (1H, d, J = 1.3 Hz), 5.32−5.49 (2H, m), 5.24 (1H, d, J = 10.6 Hz), 4.59−4.82 (2H, m), 4.00−4.11 (1H, m), 3.89−4.00 (1H, m), 3.73−3.89 (2H, m), 2.33−2.50 (1H, m), 1.87−2.05 (2H, m), 1.69−1.87 (1H, m), 1.45−1.69 (2H, m), 0.89 (9H, s), 0.08 (3H, s), 0.07 (3H, s); CD (CH_3CN) λ_{max} 251 nm ($\theta = -0.84 \times 10^5$).

(5*R*,6*R*)-6-[(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]propyl]-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylic Acid Allyl Ester (13). A solution of **12** (343 mg, 1.0 mmol) in AcOEt (200 mL) was irradiated by use of a high-pressure UV lamp (200 W; Ishiirikaseisakusho) through a Pyrex filter for 1 h. The solvent was removed, and purification of the residue by chromatography on silica gel provided 106 mg of **13** in 32% yield as a colorless oil together with 191 mg of unchanged material in 56% yield. **13**: mp 80−83 °C; IR (cm^{-1}) (neat) 3500, 1790, 1704; ^1H NMR ($CDCl_3$) δ 5.85−6.02 (1H, m), 5.55 (1H, d, J = 4.0 Hz), 5.20−5.48 (3H, m), 4.58−5.82 (2H, m), 4.31−4.42 (1H, m), 3.79−4.09 (3H, m), 2.34−2.53 (1H, m), 1.71−2.10 (5H, m), 0.97 (3H, t, J = 7.9 Hz), 0.87 (9H, s), 0.11 (3H, s), 0.12 (3H, s); HRMS (FAB) *m/z* (M + 23) calcd for $C_{22}H_{35}NO_5SNaSi$ 476.1903, found 476.1889.

(5*R*,6*R*)-6-((*S*)-1-Hydroxypropyl)-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylic Acid Allyl Ester (14). To a solution of **13** (138 mg, 0.30 mmol) in THF (0.61 mL) were added AcOH (0.069 mL, 1.2 mmol) and then 1.0 M tetra-*n*-butylammonium fluoride in THF (0.89 mL, 0.89 mmol) at room temperature. The reaction mixture was stirred at 50 °C for 6 h. The reaction mixture was poured into H_2O (10 mL) and extracted with AcOEt (10 mL). The organic phase was washed with saturated $KHSO_4$ (10 mL), saturated $NaHCO_3$ (10 mL), and saturated Na_2SO_4 (10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided 79 mg of **14** in 77% yield as a pale yellow oil. **14**: IR (cm^{-1}) (neat) 2955, 1790, 1704; ^1H NMR ($CDCl_3$) δ 5.86−6.03 (1H, m), 5.58 (1H, d, J = 4.0 Hz), 5.22−5.47 (3H, m), 4.77 (1H, dd, J = 5.3, 13.9 Hz), 4.63 (1H, dd, J = 5.3, 13.9 Hz), 4.12−4.27 (1H, m), 3.95−4.06 (1H, m), 3.82−3.95 (2H, m), 2.39−2.55 (1H, m), 1.76−2.10 (5H, m), 1.03 (3H, t, J = 7.3 Hz).

(5*R*,6*R*)-6-((*S*)-1-Hydroxypropyl)-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylic Acid (3). To a solution of **14** (20 mg, 0.059 mmol) in CH_2Cl_2 (0.5 mL) were added $Pd(PPh_3)_4$ (0.7 mg, 0.0006 mmol), PPh_3 (0.8 mg, 0.003 mmol), and sodium 2-ethylhexanoate (11 mg, 0.067 mmol) at room temperature, and the mixture was stirred at the same temperature for 2 h. After completion of reaction, the reaction mixture was diluted with AcOEt (5 mL) and extracted with H_2O (5 mL) twice. The combined water phase was lyophilized, and the residue was purified with HPLC (HPLC conditions: column, YMC D-ODS-5 120A AM type i.d. 20 × 250 mm; eluent A, H_2O:CH_3CN:AcOH = 950:50:1; eluent B, H_2O:CH_3CN:AcOH = 50:950:1; A:B = 100:0 to 0:60 linear gradient for 60 min; flow rate, 9 mL/min; temperature, room temperature; detected at 254 nm); 9 mg of **3** was obtained as an off-white powder after lyophilization of the fraction containing the desired product. **3**: IR (cm^{-1}) (KBr) 3401, 1771; ^1H NMR ($CDCl_3$) δ 5.60 (1H, d, J = 4.0 Hz), 5.34 (1H, t, J = 7.3 Hz), 4.12−4.25 (1H, m), 3.93−4.07 (1H, m), 3.81−3.93 (2H, m), 2.40−2.59 (1H, m), 1.78−2.12 (4H, m), 1.48−1.63 (1H, m), 1.04 (3H, t, J = 7.3 Hz). Anal. ($C_{13}H_{16}NO_5SNa$) C, H, N.

2-[(3*R*,4*S*)-3-[1(*S*)-[(*tert*-Butyldimethylsilyl)oxy]propyl]-4-(phenylthio)-2-oxoazetidin-1-yl]acetic Acid *p*-Nitrobenzyl Ester (15). To a solution of (3*R*,4*S*)-3-[1(*S*)-[(*tert*-butyldimethylsilyl)oxy]propyl]-4-(phenylthio)-2-azetidinone (**8**; 1.53 g, 4.35 mmol) in anhydrous DMF (6.7 mL) were added iodoacetic acid *p*-nitrobenzyl ester (1.66 g, 4.78 mmol) and K_2CO_3 (1.82 g, 13.2 mmol). The reaction mixture was heated at 50−55 °C for 4.5 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (100 mL and then 50 mL). The combined organic phase was washed with saturated NaCl (100 mL) and dried, followed by concentration under reduced pressure. Purification of the residue by chromatography on silica gel (38 g, AcOEt:*n*-hexane = 1:8) provided 2.20 g of **15** as a pale yellow oil (93% yield). **15**: ^1H NMR ($CDCl_3$) δ 8.22 (2H, d, J = 8.7 Hz), 7.47 (2H, d, J = 8.7 Hz), 7.40−7.50 (2H, m), 7.25−7.35 (3H. m), 5.30 (1H, d, J = 2.1 Hz), 5.22 (1H, d, J = 13.2 Hz), 5.16 (1H, d, J = 13.2 Hz), 4.25 (1H, d, J = 17.8 Hz), 4.05−4.10 (1H, m), 3.93 (1H, d, J = 17.8 Hz), 3.17 (1H, dd, J = 2.1, 2.8 Hz), 1.55−1.70 (2H, m), 0.91 (3H, t, J = 7.5 Hz), 0.86 (9H, s), 0.06 (3H, s), 0.01 (3H, s).

2-[Bis(benzoylthio)methylidene]-2-[(3*R*,4*S*)-3-[1(*S*)-[(*tert*-butyldimethylsilyl)oxy]propyl]-4-(phenylthio)-2-oxoazetidin-1-yl]acetic Acid *p*-Nitrobenzyl Ester (16). To a solution hexamethyldisilazane (1.65 g, 10.2 mmol) in THF (25 mL) was added 1.71 N *n*-BuLi in *n*-hexane (5.3 mL, 9.06 mmol) at room temperature. The reaction mixture was stirred at room temperature for 30 min and then cooled to −78 °C. To this solution was added a solution of **15** (2.50 g, 4.59 mmol) in THF (5 mL) at −78 °C. The reaction mixture was stirred at the same temperature for 10 min, and to this solution was added CS_2 (0.55 mL, 9.14 mmol) followed by a solution of benzoyl chloride (1.6 mL, 13.8 mmol) in THF (5 mL). The reaction mixture was stirred for 10 min, and to this solution was added acetic acid (0.45 mL, 7.84 mmol). The reaction mixture was diluted with AcOEt (200 mL) and washed with saturated NaCl (100 mL) saturated $NaHCO_3$ (100 mL), and saturated NaCl (100 mL). The organic phase was dried, and the solvent was removed in vacuo. Purification of the residue by chromatography on silica gel (50 g, AcOEt:*n*-hexane = 1:3) provided 3.37 g of **16** as a pale yellow oil (88% yield). **16**: ^1H NMR ($CDCl_3$) δ 7.99 (2H, d, J = 8.7 Hz), 7.92 (2H, d, J = 8.7 Hz), 7.80 (2H, d, J = 7.1 Hz), 7.69 (2H, d, J = 7.1 Hz), 7.50−7.60 (2H, m), 7.40−7.50 (4H, m), 7.35−7.40 (2H, m), 7.20−7.35 (3H, m), 5.85 (1H, d, J = 2.8 Hz), 5.30 (1H, d, J = 12.9 Hz), 5.24 (1H, d, J = 12.9 Hz), 3.95−4.00 (1H, m), 3.12 (1H, dd, J = 2.5, 2.8 Hz), 1.50−1.65 (2H, m), 0.90 (3H, t, J = 7.5 Hz), 0.83 (9H, s), 0.02 (3H, s) −0.01 (3H, s). Anal. ($C_{42}H_{44}N_2O_8S_3Si$) C, H, N.

(5*R*,6*R*)-6-[(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]propyl]-

2-(methylthio)penem-3-carboxylic Acid *p*-Nitrobenzyl Ester (18). To a solution of **16** (2.43 g, 2.93 mmol) in CH_2Cl_2 (40 mL) was added sulfuryl chloride (0.44 mL, 4.41 mmol) at −5 °C. The reaction mixture was stirred at the same temperature for 15 min, and to this solution was added allyl acetate (1.6 mL, 14.8 mmol) followed by diphenyl disulfide (639 mg, 2.93 mmol). The reaction mixture was stirred at room temperature for 5 min. After cooling with an ice bath for 20 min, to the solution were added a solution of morpholine (0.77 mL, 8.80 mmol) in CH_2Cl_2 (4 mL) and triethylamine (0.60 mL, 4.30 mmol) and after 10 min a mixture of methyl iodide (0.70 mL, 11.2 mmol) and triethylamine (0.40 mL, 2.87 mmol). The reaction mixture was stirred at room temperature for 1 h. The mixture was diluted with AcOEt (200 mL) and washed with saturated $KHSO_4$ (100 mL), saturated $NaHCO_3$ (100 mL), and saturated NaCl (100 mL). The organic phase was dried, and the solvent was removed in vacuo. Purification of the residue by chromatography on silica gel (50 g, AcOEt:*n*-hexane = 1:8) provided 1.07 g of **18** as a pale yellow powder (70% yield). **18**: ^{1}H NMR ($CDCl_3$) δ 8.21 (2H, d, J = 8.7 Hz), 7.61 (2H, d, J = 8.7 Hz), 5.73 (1H, d, J = 4.0 Hz), 5.47 (1H, d, J = 13.8 Hz), 5.21 (1H, d, J = 13.8 Hz), 4.35 (1H, dt, J = 4.5, 9.5 Hz), 4.09 (1H, dd, J = 4.0, 9.5 Hz), 2.56 (3H, s), 1.75−1.90 (2H, m), 0.99 (3H, t, J = 7.5 Hz), 0.88 (9H, s), 0.12 (3H, s), 0.12 (3H, s). Anal. ($C_{23}H_{32}N_2O_6S_2Si$) C, H, N.

(5*R*,6*R*)-6-[(*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]propyl]-2-(methylsulfinyl)penem-3-carboxylic Acid *p*-Nitrobenzyl Ester (19). To a solution of **18** (1.068 g, 2.03 mmol) in CH_2Cl_2 (20 mL) was added *m*-chloroperbenzoic acid (393 mg, 2.28 mmol) at −30 °C. The reaction mixture was stirred for 1 h. The mixture was diluted with AcOEt (200 mL) and washed with 0.01 N sodium thiosulfate (30 mL), saturated $NaHCO_3$ (100 mL), and saturated NaCl (100 mL). The organic phase was dried, and the solvent was removed in vacuo. Purification of the residue by chromatography on silica gel (50 g, AcOEt:*n*-hexane = 1:1) provided 793 mg of **19** as a pale yellow powder (72% yield). **19**: ^{1}H NMR ($CDCl_3$) δ 8.24 (2H, d, J = 8.7 Hz), 7.60 (0.8H, d, J = 8.7 Hz), 7.58 (1.2H, d, J = 8.7 Hz), 5.93 (0.6H, d, J = 4.2 Hz), 5.78 (0.4H, d, J = 4.2 Hz), 5.44 (0.6H, d, J = 13.5 Hz), 5.24 (0.4H, d, J = 13.5 Hz), 5.23 (0.6H, d, J = 13.5 Hz), 4.35−4.45 (1H, m), 4.18 (1H, dt, J = 4.2, 10.2 Hz), 2.95 (3H, s), 1.75−1.85 (2H, m), 1.00 (3H, t, J = 7.4 Hz), 0.88 (9H, s), 0.13 (6H, s). Anal. ($C_{23}H_{32}N_2O_7S_2Si$) C, H, N.

(5*R*,6*R*)-2-[[(*S*)-1-(*p*-Nitrobenzyl)pyrrolidin-3-yl]thio]-6-[(*S*)-1-[(*tert*-butyldimethylsilyl)oxy]propyl]penem-3-carboxylic Acid *p*-Nitrobenzyl Ester (20). To a solution of **19** (179 mg, 0.33 mmol) in dimethylformamide (6 mL) were added a solution of diisopropylamine (75 mL, 0.43 mmol) in dimethylformamide (2 mL) and a solution of (*S*)-3-mercapto-1-(*p*-nitrobenzyl)pyrrolidine (84 mg, 0.43 mmol) in dimethylformamide (2 mL). After 20 min the reaction mixture was diluted with ethyl acetate (100 mL), and the solution was washed with saturated $KHSO_4$ (50 mL), saturated $NaHCO_3$ (50 mL), and saturated NaCl (50 mL) successively. The organic phase was dried, and the solvent was removed in vacuo. Purification of the residue by chromatography on silica gel (10 g, AcOEt:*n*-hexane = 1:8) provided 110 mg of **20** as a pale yellow oil (50% yield). **20**: ^{1}H NMR ($CDCl_3$) δ 8.21 (2H, d, J = 8.7 Hz), 7.61 (2H, d, J = 8.7 Hz), 5.69 (1H, d, J = 4.0 Hz), 5.46 (1H, d, J = 13.8 Hz), 5.20 (1H, d, J = 13.8 Hz), 4.3−4.4 (1H, m), 4.08 (1H, dd, J = 4.0, 9.8 Hz), 3.75−3.9 (1H, m), 3.63 (2H, s), 3.1−3.2 (1H, m), 2.65−2.75 (1H, m), 2.45−2.55 (1H, m), 2.3−2.45 (1H, m), 1.7−1.95 (3H, m), 0.99 (3H, t, J = 7.4 Hz), 0.87 (9H, s), 0.11 (3H, s), 0.10 (3H, s).

(5*R*,6*R*)-2-[[(*S*)-1-(*p*-Nitrobenzyl)pyrrolidin-3-yl]thio]-6-((*S*)-1-hydroxypropyl)penem-3-carboxylic Acid *p*-Nitrobenzyl Ester (21, R = PNB). To a solution of **20** (140 mg, 0.20 mmol) in THF (0.5 mL) were added AcOH (0.045 mL, 0.8 mmol) and then 1.0 M tetra-*n*-butylammonium fluoride in THF (0.55 mL, 0.55 mmol) at room temperature. The reaction mixture was stirred at 50 °C for 6 h. The reaction mixture was poured into H_2O (10 mL) and extracted with AcOEt (10 mL). The organic phase was washed with saturated $KHSO_4$ (10 mL), saturated $NaHCO_3$ (10 mL), and saturated Na_2SO_4 (10 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided 110 mg of **21** in 90% yield as a pale yellow powder. **21**: IR (cm^{-1}) (NaCl) 3447, 1785, 1684, 1552; ^{1}H NMR ($CDCl_3$) δ 8.22 (2H, d, J = 8.8 Hz), 8.21 (2H, d, J = 8.7 Hz), 7.61 (2H, d, J = 8.5 Hz), 7.51 (2H, d, J = 8.5 Hz), 5.78 (1H, d, J = 4.0 Hz), 5.60 (2H, s), 5.47 (1H, d, J = 13.7 Hz), 5.20 (1H, d, J = 13.8 Hz), 4.07−4.18 (1H, m), 3.87−4.00 (2H, m), 3.45−3.71 (4H, m), 2.32−2.48 (1H, m), 1.94−2.15 (2H, m), 1.50−1.70 (1H, m), 1.07 (3H, t, J = 7.5 Hz). Anal. ($C_{27}H_{28}N_4O_8S_2$) C, H, N.

(5*R*,6*R*)-2-[[(*S*)-1-Phenacylpyrrolidin-3-yl)thio]-6-((*S*)-1-hydroxypropyl)penem-3-carboxylic Acid *p*-Nitrobenzyl Ester (21, R = phenacyl). In the manner described above, compound **21** (R = phenacyl) was prepared from the sulfinyl derivative **19** in 79% yield. **21**: IR (cm^{-1}) (NaCl) 3400, 1782, 1690; ^{1}H NMR ($CDCl_3$) δ 8.21 (2H, d, J = 8.8 Hz), 7.96 (2H, d, J = 7.2 Hz), 7.54−7.70 (3H, m), 7.37−7.54 (2H, m), 5.75 (1H, d, J = 4.0 Hz), 5.46 (1H, d, J = 13.7 Hz), 5.21 (1H, d, J = 13.7 Hz), 4.07−4.20 (1H, m), 4.00 (2H, s), 3.85−3.96 (1H, m), 3.94 (1H, dd, J = 4.1, 10.5 Hz), 3.49 (1H, dd, J = 7.3, 10.0 Hz), 2.90−3.01 (1H, m), 2.62−2.85 (2H, m), 1.40−1.53 (1H, m), 1.87−2.09 (2H, m), 1.51−1.70 (1H, m), 1.06 (3H, t, J = 7.5 Hz).

(5*R*,6*R*)-2-[[(*S*)-Pyrrolidin-3-yl)thio]-6-((*S*)-1-hydroxypropyl)penem-3-carboxylic Acid (5). A solution of the ester **21** (R = PNB; 68 mg, 0.11 mmol) in tetrahydrofuran (5.5 mL) was added to a suspension of palladium/carbon (10%, 110 mg) in phosphate buffer (pH 7.0, 0.1 M, 5.5 mL) under hydrogen atmosphere. After vigorous stirring at room temperature for 3 h, the catalyst was removed by filtration. The resulting solution was evaporated under reduced pressure and then lyophilized. The residue was purified by use of reverse phase ODS-HPLC using water−acetonitrile (1 mM ammonium formate) as mobile phase. Pure product **5** was obtained as a white powder (22 mg, 58% yield). **5**: IR (cm^{-1}) (KBr) 3420, 1764, 1596; ^{1}H NMR (D_2O) δ 5.81 (1H, d, J = 3.6 Hz), 4.03−4.22 (3H, m), 3.78 (1H, dd, J = 6.5, 12.8 Hz), 3.39−3.61 (4H, m), 2.48−2.61 (1H, m), 1.80−1.94 (1H, m), 1.49−1.65 (1H, m), 1.00 (3H, t, J = 7.4 Hz). Anal. ($C_{13}H_{17}N_2O_4S_2Na$) C, H, N.

(5*R*,6*R*)-2-[[(*S*)-1-Phenacylpyrrolidin-3-yl)thio]-6-((*S*)-1-hydroxypropyl)penem-3-carboxylic Acid (6). A solution of the ester **21** (R = phenacyl; 59 mg, 0.1 mmol) in tetrahydrofuran (2.5 mL) was added to a suspension of palladium/carbon (10%, 100 mg) in phosphate buffer (pH 7.0, 0.1 M, 1.6 mL) under hydrogen atmosphere. After vigorous stirring at room temperature, the catalysis was removed by filtration. The resulting solution was lyophilized, and the residue was purified by use of reverse phase ODS-HPLC using water−acetonitrile (1 mM ammonium formate) as mobile phase. Pure product **6** was obtained as a white solid (9.0 mg, 20% yield). **6**: IR (cm^{-1}) (NaCl) 3430, 1772, 1694, 1595, 1376; ^{1}H NMR (CD_3OD) δ 8.00 (2H, d, J = 7.0 Hz), 7.66 (1H, t, J = 7 Hz), 7.53 (2H, t, J = 7 Hz), 5.72 (1H, d, J = 3.0 Hz), 4.58 (2H, s), 3.90−4.08 (3H, m), 3.62−3.70 (1H, m), 3.20−3.30 (3H, m), 2.50−2.60 (1H, m), 1.95−2.05 (1H, m), 1.85−1.95 (1H, m), 1.50−1.60 (1H, m), 1.03 (3H, t, J = 7.5 Hz). Anal. ($C_{21}H_{23}N_2O_5S_2Na$) C, H, N.

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