SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW 2-SUBSTITUTED PENEMS. I

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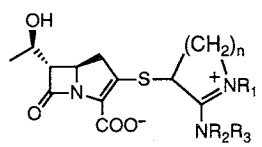
A new type of penem derivative (**3**~**6**) having a cyclic amidine moiety or a quaternary heterocycle moiety at the C-2 position was prepared. The susceptibility to renal dehydropeptidase-I (DHP-1) and the antimicrobial activity of these compounds were determined. Some of these compounds (**5**, **6**) showed a broad spectrum of antibacterial activity, including activity against *Pseudomonas aeruginosa*.

Penems are known for their broad spectrum of antimicrobial activity and their stability to various β-lactamases[1,2]. Since the first synthesis of the penem nucleus[3], a variety of derivatives have been synthesized; among these, Sch 34343[4], FCE 22101[5], SUN-5555[6] and CP-70429[7] have been investigated in clinical trials. Although these penems have shown strong activity against Gram-positive becteria, their anti-pseudomonal activity has not been sufficient for commercialization.

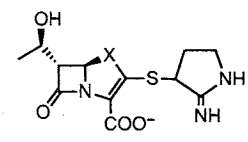
We have reported[8] that carbapenem derivatives (**1**) having a cyclic amidine moiety at the *S*-α-position of the C-2 side chain showed good activity comparable to that of imipenem[9~11] against Gram-positive and Gram-negative bacteria, as well as improved stability to hydrolysis by DHP-1. As part of our study to find new penem derivatives, we prepared compound **3**, which has the same substituent at the C-2 position as carbapenem **2**, one of the most active compounds in previous study[8] (Fig. 1), and examined the activity and stability to DHP-1. Compound **3** showed significantly more resistance to hydrolysis by DHP-1 than carbapenem compound **2**, but was less active against Gram-negative bacteria than carbapenem compounds, **2** and imipenem.

Recently, Bristol-Myers chemists reported[12] that carbapenem derivatives with pyridinium methyl thio groups as a positive charge at the C-2 side chain showed potent activities against Gram-negative bacteria. This finding suggested that the introduction of a quaternary pyridinium group to penem derivatives would enhance activity against Gram-negative bacteria. In our search for new penem derivatives with potent antimicrobial activity as well as good resistance to DHP-1, we therefore designed the new bicyclic pyridinium compounds (**5**~**7**) having a ring structure and quaternary pyridinium moiety as a C-2 substituent of penem derivatives. The present paper deals with the synthesis of these derivatives and biological data in this series.

Fig. 1.



1
(n=2, 3)



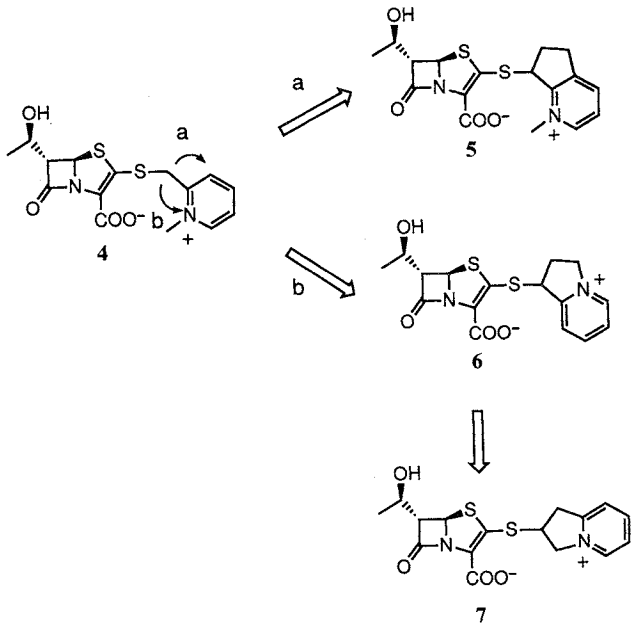
2 X=C
3 X=S

Chemistry

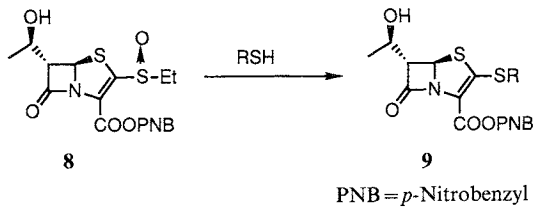
Sulfoxide **8**[13], which was used as a key intermediate for the synthesis of new penems, was treated with various thiols in the presence of *N*,*N*-diisopropylethylamine to give 2-substituted penem derivative **9** (Scheme 1).

Treatment of sulfoxide **8** and thiol **10**[8] under the same conditions as above, followed by catalytic hydrogenation of the resulting reaction products in the presence of PtO_2 gave compound **3** after purification by HPLC. As we had already reported in a previous paper[8], these cyclic amidine derivatives epimerized to a mixture of diastereomers. Compound **3** was not separated, although it was a mixture of diastereomers. Mitsunobu reaction[14] of alcohol **11**[15] using thiobenzoic acid, followed by deprotection of the resulting compound (**12**) with ammonium hydroxide gave thiol **13**, Treatment of sulfoxide **8** with thiol **13** gave

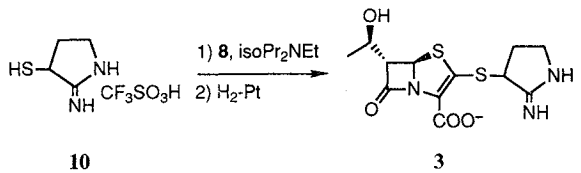
Fig. 2.



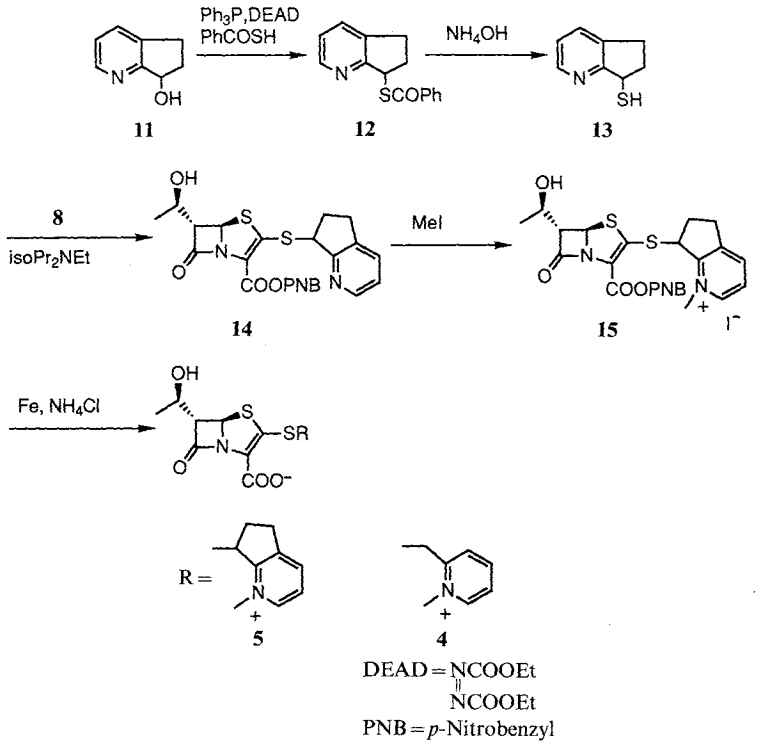
Scheme 1.



PNB = *p*-Nitrobenzyl



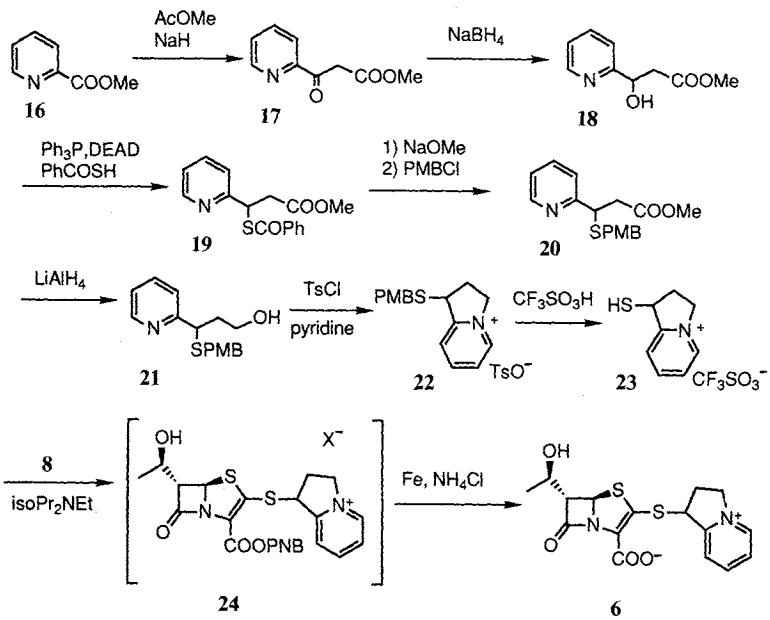
Scheme 2.



DEAD = NCOOEt
‖
NCOOEt

PNB = *p*-Nitrobenzyl

Scheme 3.

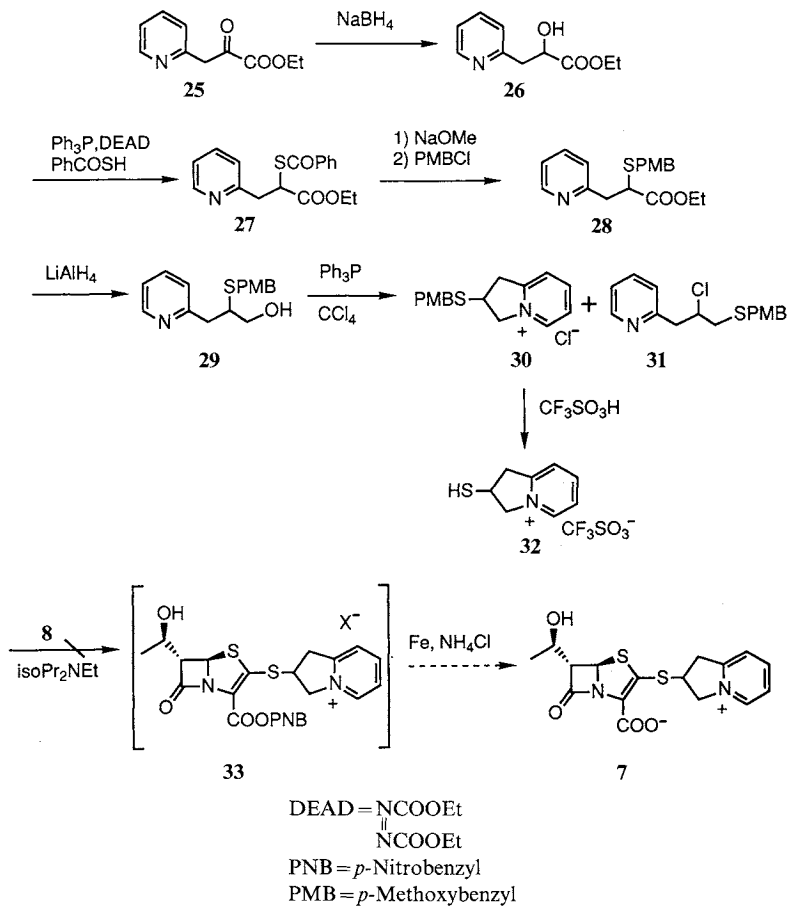


DEAD = NCOOEt
‖
NCOOEt

PNB = *p*-Nitrobenzyl

PMB = *p*-Methoxybenzyl

Scheme 4.



DEAD = $\begin{matrix} \text{NCOOEt} \\ \| \\ \text{NCOOEt} \end{matrix}$

PNB = *p*-Nitrobenzyl

PMB = *p*-Methoxybenzyl

penem **14**, which was then treated with methyl iodide to give a quaternary intermediate (**15**). Although catalytic hydrogenation of **15** over Pd-C catalyst gave compound **5** in low yield, reaction of **15** by use of Fe and NH_4Cl[16] gave compound **5** in 20% yield after purification by HPLC. The NMR spectrum of compound **5** showed the presence of two diastereomers resulting from the asymmetric carbon on the C-2 substituent of **5**, although they were not separated by HPLC in several conditions. Compound **4** was prepared similarly from sulfoxide **8** and 2-pyridinemethanthiol[17] (Scheme 2).

Methyl picolinate (**16**) was treated with MeOAc in the presence of NaH to give *β*-ketoester **17**. Reduction of **17** with $NaBH_4$ followed by Mitsunobu reaction of alcohol **18** using thiobenzoic acid gave thioester **19**. Treatment of **19** with sodium methoxide followed by protection with *p*-methoxybenzylchloride gave **20**, which was further converted into alcohol **21** by use of $LiAlH_4$. Alcohol **21** was treated with tosyl chloride in pyridine[18] to give a quaternary compound (**22**). Treatment of **22** with trifluoromethanesulfonic acid[19] gave thiol **23**. The replacement reaction of sulfoxide **8** with thiol **23** gave crude **24**, which was then deprotected in a similar manner to that described above to give compound **6** in 20% yield after purification by HPLC. Although the diastereomers of compound **3** were separated by HPLC (retention time 13.0 and 13.6 minutes, solvent 7% aq CH_3CN, flow rate 3.65 ml/minute), they came to the equilibrium state during concentration of the separated solution and became a mixture of diastereomers (Scheme 3).

Table 1. Antimicrobial activity (MIC μg/ml) and DHP-1 stability of penems and imipenem.

Organisms	2	3	4	5	6	Sch 34343	Imipenem
Escherichia coli NIHJ	<0.10	0.20	0.78	0.20	0.20	0.39	0.20
Citrobacter freundii IID 976	<0.10	0.78	0.78	0.10	0.10	0.39	0.10
Proteus vulgaris 08601	0.20	0.78	6.25	0.39	0.20	0.39	0.39
P. mirabilis IFO 3849	0.20	0.78	1.56	0.39	0.20	0.39	0.10
Klebsiella pneumoniae Type 1	<0.10	0.20	0.78	0.10	0.10	0.39	0.10
Enterobacter cloacae 12005	0.10	0.78	3.13	0.20	0.39	1.56	0.78
Serratia marcescens 10100	0.20	0.78	3.13	0.78	0.39	1.56	0.78
Pseudomonas aeruginosa 32233	0.78	1.56	12.5	12.5	3.13	100	1.56
Staphylococcus aureus 209P	<0.10	<0.10	<0.10	<0.10	<0.10	0.10	<0.10
S. epidermidis 56500	<0.10	<0.10	<0.10	<0.10	<0.10	0.39	0.10
Streptococcus pyogenes G-36	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
S. faecalis ATCC 19433	0.78	6.25	6.25	3.13	3.13	6.25	0.78
DHP-1 susceptibility[a]	46	<2	<2	<2	<2	30	100

[a] DHP-1 susceptibility is given relative to imipenem = 100.

Alcohol **29**, which was prepared from **25**[20] using a similar method to that described above (Scheme 3), was treated with tosyl chloride in pyridine[17] in a similar manner as above; however, in this case, β-elimination occurred after cyclization under the basic conditions, and compound **30** was not obtained. Using triphenylphosphine and carbon tetrachloride[21] as a neutral condition, the cyclized compound (**30**) and rearranged compound (**31**) were prepared in 51% and 6% yields, respectively. Treatment of **30** with trifluoromethanesulfonic acid gave thiol **32**. When the replacement reaction of sulfoxide **8** with thiol **32** in the presence of *N,N*-diisopropylethylamine was run, evolution of hydrogen sulfide was observed during the course of the reaction, and most of the starting material (**8**) remained. Deprotection and purification of the reaction mixture as above did not give targeted compound **7**, although its UV spectrum showed an absorption band at 322 nm based on the double bond of the deprotected penem molecule (Scheme 4).

Biological Properties and Discussion

The susceptibility to DHP-1 and MICs of the prepared new penems are shown in Table 1, and compared with those of Sch 34343[4] and imipenem[9~11].

All of the prepared compounds (**3**~**6**) were over 50 times and over 15 times more resistant than imipenem and Sch 34343 in resistance to hydrolysis by DHP-1 of swine, respectively. A Sumitomo group has reported[22] that penem compounds had greater resistance against hydrolysis by DHP-1 than carbapenems having the same C-2 side chain. A similar result was observed between carbapenem **2** and penem **3**. As we expected, penem derivatives **3**, **5** and **6**, having a ring structure at the *S*-α-position, showed considerably higher resistance to DHP-1 than Sch 34343, with a linear side chain, a finding compatible with our previous study[8]. On the other hand, non-cyclic quaternary penem compound **4** also showed good resistance to DHP-1, as has been reported for carbapenems with a similar side chain[12]. These results demonstrated that both a ring structure at the *S*-α-position and a quaternary structure contributed to resistance to hydrolysis by DHP-1.

Compounds **3**~**6** possessed potent antimicrobial activities against Gram-positive organisms, closely similar to those of Sch 34343 and imipenem. Against Gram-negative bacteria, penem derivatives showed lower activity than carbapenem derivatives; penem derivative **3** was 2- to ≥8-fold less active than carbapenem derivative **2** and imipenem. We have found that bicyclic pyridinium compounds show good antimicrobial

activities almost equal to imipenem. As for anti-pseudomonal activity, quaternary compounds with a pyridinium moiety were generally superior to Sch 34343, although a diminution of activity was confirmed in comparison with cyclic amidine derivative **3**. Interestingly, the site of the nitrogen atom as a positive charge of the bicyclic pyridinium substituent played an important role in improving activity against *Pseudomonas aeruginosa*; compound **6** had 4-fold higher activity than compound **5**. However, no significant difference was observed between **5** and **6** against other organisms. Similar results against *Pseudomonas aeruginosa* were observed in a previous report[12] on carbapenems with a non-cyclic pyridinium moiety. Further, against other Gram-negative bacteria, the introduction of a bicyclic pyridinium group at the *S*-α-position improved activity: compounds **5** and **6** were 32-fold and 8-fold more active than non-cyclic compound **4** and cyclic amidine derivative **3**, respectively. Bicyclic pyridinium groups are thus useful substituents of penem derivatives to improve activity against Gram-negative bacteria, including *Pseudomonas aeruginosa*.

In our study, indolizinium derivative **6**, having a new type of quaternary hetero-bicyclic moiety at the C-2 position, had good DHP-1 resistance, and MIC values were comparable to those of imipenem. These results show a new direction in the search for novel penems with high resistance to DHP-1 and more potent activity than carbapenems.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and were uncorrected. IR spectra were obtained using Hitachi Models 260-30 and 270-30. ^{1}H NMR spectra were obtained on a Hitachi R-40 (90 MHz) or a JEOL FX-90Q (90 MHz) spectrometer, in the designated solvent, using tetramethylsilane or residual HOD (δ 4.80) as an internal reference. UV spectra were measured on a Hitachi 323 spectrometer. HPLC purifications were performed using Sensyu-Pack Nucleosil 7C18 (Sensyu Kagaku Co., Ltd.).

Measurement of *In Vitro* Antibacterial Activity

Minimal inhibitory concentrations (MICs) were measured according to the 2-fold broth dilution method using Mueller-Hinton broth (Difco Laboratories, Detroit, Mich., U.S.A.). The inoculum size was about 10^5 cfu/ml. The MIC was defined as the lowest concentration that prevented visual growth of bacteria after incubation at 37°C for 18 hours.

Test of Stability of Penem Compounds against Hydrolysis by DHP-1

The rate of hydrolysis of each derivative by DHP-1 of swine was determined as described in a preceding paper[23]. Resistance of compounds to hydrolysis by the enzyme was represented in terms of the hydrolysis rate relative to that of the control compound, imipenem, represented as 100. The sample of DHP-1 used here was the same as that used in the preceding report.

(5*R*,6*S*,8*R*)-6-(1-Hydroxyethyl)-2-(2-iminopyrrolidin-3-ylthio)penem-3-carboxylic acid (**3**)

To a solution of **8** (90 mg, 0.21 mmol) and **10** (73 mg, 0.27 mmol) in DMF (1.5 ml) was added *N*,*N*-diisopropylethylamine (35 mg, 0.27 mmol) at −40°C under argon. After stirring for 20 minutes at the same temperature, the reaction mixture was added to a solution of THF (10 ml) and 0.1 M phosphate buffer (pH 6.0, 10 ml), and subjected to catalytic hydrogenation under 4 atm for 1 hour at room temperature in the presence of PtO_2 (90 mg). The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to remove organic solvents and chromatographed on a column of Diaion HP-20. Fractions eluted with 5% aq THF were concentrated under reduced pressure and were purified by HPLC eluting with 5% aq acetonitrile. Fractions having UV absorption at 325 nm were combined and lyophilized to give **6** (13 mg, 19%) as a colorless powder. IR (KBr) 1770, 1700, 1590 cm^{-1}; ^{1}H NMR (D_2O) δ 1.39

(3H, d, J=7 Hz), 2.2~2.6 (1H, m), 2.6~3.2 (1H, m), 3.7~4.0 (2H, m), 4.04 (1H, dd, J=2 and 7 Hz), 4.35 (1H, quinted, J=7 Hz), 5.80 (1H, d, J=2 Hz); UV λ_{max} (H_2O) 255, 325 nm.

7-Benzoylthio-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine (**12**)

A solution of *N*,*N*-diethylazodicarboxylate (13.9 g, 0.08 mol) in THF (20 ml) was added to a solution of Ph_3P (21 g, 0.08 mol) in THF (45 ml) and the mixture was stirred at 0~5°C for 30 minutes. To the reaction mixture was added a solution of **11** (5.4 g, 0.04 mol) and thiobenzoic acid (11.1 g, 0.08 mol) in THF (80 ml) at 0~5°C under argon. The mixture was stirred at 0~5°C for 1.5 hours under argon, then was concentrated under reduced pressure and the residue was extracted with EtOAc. The extract was washed with aq NaCl, aq $NaHCO_3$ and aq NaCl, and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with benzene to give **12** (9.7 g, 94%) as a yellow syrup. ^{1}H NMR ($CDCl_3$) δ 2.0~2.5 (1H, m), 2.6~3.3 (3H, m), 5.27 (1H, dd, J=5 and 7 Hz), 7.11 (1H, dd, J=5 and 8 Hz), 7.3~7.7 (4H, m), 7.99 (2H, dd, J=2 and 7 Hz), 8.45 (1H, d, J=4 Hz).

7-Mercapto-6,7-dihydro-5*H*-cyclopenta[*b*]pyridine (**13**)

To a solution of compound **12** (9.6 g, 38 mmol) in EtOH (300 ml) was added NH_4OH (50 ml). After stirring at room temperature for 15 hours under argon, the reaction mixture was neutralized to pH 6 with 10% HCl and concentrated under reduced pressure. The residue was extracted with EtOAc, washed with water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue obtained was chromatographed on silica gel eluting with benzene - EtOAc (5 : 1) to give **13** (3.45 g, 64%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.8~2.4 (2H, m), 2.4~2.8 (1H, m), 2.8~3.3 (2H, m), 4.3~4.7 (1H, m), 7.08 (1H, dd, J=5 and 8 Hz), 7.54 (1H, d, J=8 Hz), 8.42 (1H, d, J=5 Hz).

p-Nitrobenzyl (5*R*,6*S*,8*R*)-2-[(6,7-Dihydro-5*H*-cyclopenta[*b*]pyridin-7-yl)thio]-6-(1-hydroxyethyl)-penem-3-carboxylate (**14**)

To a solution of **8** (341 mg, 0.8 mmol) and **13** (242 mg, 1.6 mmol) in DMF was added *N*,*N*-diisopropylethylamine at −40°C under nitrogen. After stirring at the same temperature for 30 minutes, the reaction mixture was diluted with EtOAc, washed with aq NaCl, aq citric acid, aq $NaHCO_3$ and aq NaCl, and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave a yellow syrup which was chromatographed on silica gel eluting with benzene - EtOAc (3 : 1) to give **14** (364 mg, 91%) as a yellow syrup. ^{1}H NMR ($CDCl_3$) δ 1.38 (3/2H, d, J=6 Hz), 1.39 (3/2H, d, J=6 Hz), 2.1~3.3 (4H, m), 3.77 (1/2H, dd, J=1.5 and 7 Hz), 3.84 (1/2H, dd, J=1.3 and 7 Hz), 4.0~4.5 (1H, m), 4.8~5.0 (1H, m), 5.30 (2/2H, ABq, J=13 Hz), 5.33 (2/2H, ABq, J=12 Hz), 5.71 (1/2H, d, J=1.3 Hz), 5.73 (1/2H, d, J=1.5 Hz), 7.11 (1/2H, dd, J=6 and 9 Hz), 7.13 (1/2H, dd, J=6 and 9 Hz), 7.4~7.8 (3H, m), 8.18 (2H, d, J=9 Hz), 8.45 (1H, d, J=5 Hz).

(5*R*,6*S*,8*R*)-2-[(1-Methyl-6,7-dihydro-5*H*-cyclopenta[*b*]pyridinium-7-yl)thio]-6-(1-hydroxyethyl)-penem-3-carboxylate (**5**)

To a solution of compound **14** (203 mg, 0.41 mmol) in acetone (12 ml) was added MeI (1.26 ml, 20.3 mmol). After the reaction mixture was stirred at room temperature for 16 hours under argon, the solvent was evaporated, and the residue was washed with ether to give **15** (251 mg, 96%) as a yellow solid which was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, d, J=7 Hz), 2.2~4.2 (7H, m), 4.36 (3H, s), 5.34 (2H, d, J=7 Hz), 5.80 (1/2H, d, J=1.7 Hz), 5.85 (1/2H, d, J=1.7 Hz), 7.65 (2H, d, J=9 Hz), 8.00 (1H, dd, J=7 and 9 Hz), 8.22 (2H, d, J=9 Hz), 8.51 (1H, d, J=9 Hz), 8.86 (1H, d, J=7 Hz).

To a solution of compound **15** (50 mg, 0.08 mmol) in THF (4 ml) and water (4 ml) was added NH_4Cl (840 mg) and Fe powder (420 mg, 100 mesh). After vigorous stirring at 5~10°C for 70 minutes, the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and chromatographed on a column of Diaion HP-20. Fractions eluted with 5% aq THF were concentrated under reduced pressure and were purified by HPLC eluting with 10% aq acetonitrile. Fractions having UV absorption at 320 nm were combined and lyophilized to give **5** (6 mg, 20%) as a yellow powder. IR (KBr) 3420, 1765, 1600, 1360 cm^{-1}; ^{1}H NMR (D_2O) δ 1.30 (3H, d, J=7 Hz), 2.5~3.6 (4H, m), 3.98 (1H, dd, J=1.8 and 7 Hz), 4.1~4.4 (1H, m), 4.41 (3H, s), 5.0~5.3 (1H, m), 5.62 (1/2H, d, J=1.8 Hz), 5.69 (1/2H, d, J=1.8 Hz), 7.87

(1H, dd, J=7 and 8 Hz), 8.38 (1H, d, J=8 Hz), 8.58 (1H, d, J=7 Hz); UV λ_{max} (H_2O) 280, 323 nm.

4 was prepared in a similar manner, with spectroscopic data as follows:

4: ^{1}H NMR (D_2O) δ 1.31 (3H, d, J=6 Hz) 3.90 (1H, dd, J=2 and 7 Hz), 4.0~4.4 (1H, m), 4.44 (3H, s), 4.62 (2H, d, J=5 Hz), 5.63 (1H, d, J=2 Hz), 7.8~8.2 (2H, m), 8.50 (1H, t, J=8 Hz), 8.82 (1H, d, J=6 Hz); UV λ_{max} (H_2O) 266, 320 nm.

Methyl Picolinoylacetate (**17**)

To a suspension of NaH (0.72 g, 15 mmol) in DMF (15 ml) was added MeOAc (1.12 g, 15 mmol) and compound **16** (1.37 g, 10 mmol) in DMF (10 ml) slowly at room temperature. The reaction mixture was stirred at 50°C for 45 minutes. The mixture was cooled and neutralized with AcOH (0.9 g, 15 mmol) and diluted with EtOAc, washed with water and dried over Na_2SO_4. Evaporation of the solvent gave **17** (1.0 g, 56%) as a yellow syrup. ^{1}H NMR ($CDCl_3$) δ 3.72 (3H, s), 4.20 (2H, s), 7.3~7.6 (1H, m), 7.7~8.2 (2H, m), 8.65 (1H, d, J=4 Hz).

Methyl 3-Hydroxy-3-(2-pyridyl)propionate (**18**)

To a solution of compound **17** (1.0 g, 5.6 mmol) in MeOH (20 ml) was added sodium borohydride (0.15 g, 4.0 mmol) at 0~5°C. After the mixture was stirred for 10 minutes at the same temperature, water was added to the mixture and evaporated under reduced pressure. The residue was diluted with EtOAc, washed with water and dried over Na_2SO_4. Evaporation of the solvent gave **18** (0.67 g, 66%) as a yellow syrup. ^{1}H NMR ($CDCl_3$) δ 2.8~3.0 (2H, m), 3.72 (3H, s), 5.18 (1H, dd, J=5 and 8 Hz), 7.1~7.5 (2H, m), 7.6~7.9 (1H, m), 8.52 (1H, d, J=6 Hz).

Methyl 3-Benzoylthio-3-(2-pyridyl)propionate (**19**)

A solution of *N*,*N*-diethylazodicarboxylate (1.17 g, 6.7 mmol) in THF (5 ml) was added to a solution of Ph_3P (1.76 g, 6.7 mmol) in THF (20 ml). After the mixture was stirred at 0~5°C for 30 minutes, to the reaction mixture was added a solution of **18** (0.62 g, 3.4 mmol) and thiobenzoic acid (0.93 g, 6.7 mmol) in THF (15 ml) at 0~5°C under argon. The mixture was then stirred at room temperature for 2 hours under argon. The reaction mixture was concentrated under reduced pressure and the residue was extracted with benzene. The extract was washed with aq $NaHCO_3$ and water, and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with benzene-EtOAc (10:1) to give **19** (0.60 g, 58%) as a yellow syrup. IR (KBr) 1735, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.20 (1H, dd, J=2 and 6 Hz), 3.32 (1H, dd, J=2 and 9 Hz), 3.63 (3H, s), 5.38 (1H, dd, J=6 and 9 Hz), 7.0~7.8 (6H, m), 7.92 (2H, d, J=8 Hz), 8.56 (1H, d, J=5 Hz).

Methyl 3-*p*-Methoxybenzylthio-3-(2-pyridyl)propionate (**20**)

To a solution of sodium methoxide (0.49 g, 9.1 mmol) in MeOH (40 ml) was added compound **19** (2.74 g, 9.1 mmol) at 0~5°C under argon. After stirring at the same temperature for 3 hours, the mixture was neutralized with AcOH (0.54 g, 9.0 mmol) and concentrated under reduced pressure. The residue was diluted with EtOAc and washed with aq $NaHCO_3$, water and dried over Na_2SO_4. Evaporation of the solvent gave crude thiol.

To a solution of the crude thiol in benzene (100 ml) was added *p*-methoxybenzylchloride (1.41 g, 9.1 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-en (DBU) (1.39 g, 9.1 mmol). The mixture was stirred at room temperature for 1 hour under argon, then was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with benzene-EtOAc (5:1) to give **20** (1.70 g, 59%) as a yellow syrup. IR (KBr) 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.7~3.5 (2H, m), 3.60 (5H, s), 3.79 (3H, s), 4.29 (1H, dd, J=7 and 8 Hz), 6.80 (2H, d, J=9 Hz), 7.1~7.4 (4H, m), 7.60 (1H, t, J=7 Hz), 8.54 (1H, d, J=5 Hz).

3-*p*-Methoxybenzylthio-3-(2-pyridyl)propanol (**21**)

To a suspension of $LiAlH_4$ (39 mg, 1.0 mmol) in Et_2O (10 ml) was added a solution of **20** (463 mg, 1.4 mmol) in Et_2O (10 ml) slowly at room temperature under argon. The reaction mixture was refluxed for 4 hours. After cooling, 20% aq NH_4Cl (10 ml) was added and filtered through Celite. The filtrate was separated into the organic and aqueous layers, and the organic layer was washed with water and dried

over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with EtOAc to give **21** (344 mg, 82%) as a yellow syrup. IR (KBr) 3350, 1610, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.0~2.3 (2H, m), 3.56 (2H, s), 3.5~3.8 (2H, m), 3.78 (3H, s), 4.08 (1H, t, J=7 Hz), 6.80 (2H, d, J=8 Hz), 7.0~7.5 (4H, m), 7.68 (1H, t, J=8 Hz), 8.54 (1H, d, J=4 Hz).

2,3-Dihydro-1-(*p*-methoxybenzylthio)-1*H*-indolizinium *p*-Toluenesulfonate (**22**)

To a mixture of **21** (579 mg, 2 mmol) and pyridine (3 ml) was added *p*-toluenesulfonyl chloride (819 mg, 4.3 mmol) at 0~5°C. The reaction mixture was stirred at 0~5°C for 4.5 hours. Ice water (10 ml) was added and the pH was adjusted to 9.2 with 6 N NaOH, and the solution was continuously extracted in Et_2O for 5 hours. The aqueous phase was washed with benzene and EtOAc, then adjusted to pH 1.8 with conc HCl and evaporated to dryness. The residue was added to EtOH and filtered. The filtrate was concentrated under reduced pressure, washed with Et_2O and dried under reduced pressure to give **22** (755 mg, 85%) as a yellow oil. ^1H NMR (DMSO-d_6) δ 2.26 (3H, s), 2.2~2.6 (1H, m), 2.6~3.1 (1H, m), 3.70 (2H, s), 3.90 (3H, s), 3.9~4.0 (1H, m), 4.5~5.1 (2H, m), 6.82 (2H, d, J=9 Hz), 7.06 (2H, d, J=8 Hz), 7.23 (2H, d, J=9 Hz), 7.42 (2H, d, J=8 Hz), 7.8~8.1 (2H, m), 8.41 (1H, t, J=9 Hz), 8.91 (1H, d, J=6 Hz).

2,3-Dihydro-1-mercapto-1*H*-indolizinium Trifluoromethanesulfonate (**23**)

To a solution of **22** (640 mg, 1.44 mmol) and anisole (779 mg, 7.2 mmol) in trifluoroacetic acid (5 ml) was added trifluoromethanesulfonic acid (350 mg, 2.33 mmol) at 0~5°C. The reaction mixture was stirred at room temperature for 1 hour, and evaporated under reduced pressure. The residue was washed with petroleum ether, isopropyl ether (IPE) and Et_2O to give **23** (489 mg, 100%) as a yellow oil. ^1H NMR (DMSO-d_6) δ 2.0~2.5 (1H, m), 2.6~3.2 (1H, m), 3.7~4.1 (1H, m), 4.5~5.0 (2H, m), 7.8~8.2 (2H, m), 8.46 (1H, t, J=9 Hz), 8.90 (1H, d, J=6 Hz).

(5*R*,6*S*,8*R*)-2-[(2,3-Dihydro-1*H*-indolizinium-1-yl)thio]-6-(1-hydroxyethyl)penem-3-carboxylate (**6**)

To a solution of compound **8** (180 mg, 0.42 mmol) and compound **23** (253 mg, 0.84 mmol) in DMF (3 ml) was added *N*,*N*-diisopropylethylamine (108 mg, 0.84 mmol) at −40°C under argon. After stirring for 30 minutes at the same temperature, Et_2O (50 ml) was added to the mixture. The ether phase was removed to give crude **24** having UV absorption at 340 nm, which was used in the next reaction without further purification.

To a solution of **24** above in THF (20 ml) and water (20 ml) was added NH_4Cl (4.16 g) and Fe powder (2.08 g, 100 mesh). After vigorous stirring at 5~10°C for 50 minutes, the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and chromatographed on a column of Diaion HP-20. Fractions eluted with 5% aq THF were concentrated under reduced pressure and were purified by HPLC eluting with 7% aq acetonitrile. Fractions having UV absorption at 325 nm were combined and lyophilized to give **6** (20 mg, 20%) as a yellow powder. IR (KBr) 1760, 1590 cm^{-1}; ^1H NMR (D_2O) δ 1.33 (3H, d, J=6 Hz), 2.5~2.9 (1H, m), 2.9~3.4 (1H, m), 3.9~5.2 (5H, m), 5.64 (1/2H, d, J=1.8 Hz), 5.76 (1/2H, d, J=1.8 Hz), 7.9~8.3 (2H, m), 8.58 (1H, t, J=8 Hz), 8.88 (1H, d, J=7 Hz); UV λ_{max} (H_2O) 265, 325 nm.

Ethyl 2-Hydroxy-3-(2-pyridyl)propionate (**26**)

A solution of compound **25** (4.31 g, 22.3 mmol) in EtOH (30 ml) was treated with sodium borohydride (0.30 g, 7.9 mmol) at 0~5°C for 30 minutes. Water was added to the mixture and evaporated under reduced pressure. The residue was diluted with EtOAc, washed with water and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with benzene-EtOAc (1 : 1) to give **26** (3.84 g, 88%) as a light yellow syrup. ^1H NMR ($CDCl_3$) δ 1.22 (3H, t, J=7 Hz), 3.18 (1H, dd, J=7 and 14 Hz), 3.25 (1H, dd, J=4 and 14 Hz), 4.17 (2H, q, J=7 Hz), 4.60 (1H, dd, J=4 and 7 Hz), 4.7~5.0 (1H, m), 7.0~7.2 (2H, m), 7.56 (1H, t, J=8 Hz), 8.41 (1H, d, J=5 Hz).

Ethyl 2-Benzoylthio-3-(2-pyridyl)propionate (**27**)

A solution of *N*,*N*-diethylazodicarboxylate (6.69 g, 38.4 mmol) in THF (15 ml) was added to a Ph_3P (10.34 g, 38.4 mmol) in THF (150 ml). The mixture was then stirred at 0~5°C for 30 minutes. To the reaction mixture was added a solution of **26** (2.96 g, 20.3 mmol) and thiobenzoic acid (3.70 g, 26.8 mmol) in THF

(50 ml) at 0~5°C under argon. After mixture was stirred at room temperature for 2 hours under argon, the reaction mixture was concentrated under reduced pressure and the residue was extracted with benzene. The extract was washed with aq $NaHCO_3$ and water, and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with benzene - EtOAc (9 : 1) to give **27** (5.92 g, 93%) as a yellow syrup. IR (neat) 1730, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (3H, t, J=7 Hz), 3.39 (1H, dd, J=7 and 15 Hz), 3.50 (1H, dd, J=7 and 15 Hz), 4.15 (2H, q, J=7 Hz), 4.88 (1H, t, J=7 Hz), 7.0~7.6 (6H, m), 7.86 (2H, d, J=8 Hz), 8.46 (1H, d, J=5 Hz).

Ethyl 2-*p*-Methoxybenzylthio-3-(2-pyridyl)propionate (**28**)

To a solution of sodium methoxide (1.01 g, 18.7 mmol) in MeOH (50 ml) was added compound **27** (5.90 g, 18.7 mmol) at 0~5°C under argon. After stirring at the same temperature for 15 minutes, the mixture was neutralized with AcOH (1.12 g, 18.7 mmol) and concentrated under reduced pressure. The residue was diluted with EtOAc and washed with aq $NaHCO_3$ and water and dried over Na_2SO_4. Evaporation of the solvent gave crude thiol.

To a solution of the crude thiol in benzene (20 ml) was added *p*-methoxybenzylchloride (2.93 g, 18.7 mmol) and DBU (2.85 g, 18.7 mmol). The mixture was stirred at room temperature for 1 hour under argon, then was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with benzene - EtOAc (5 : 1) to give **28** (4.76 g, 77%) as a colorless oil. IR (neat) 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (3H, t, J=7 Hz), 3.10 (1H, dd, J=7 and 15 Hz), 3.27 (1H, dd, J=7 and 15 Hz), 3.75 (3H, s), 3.77 (2H, s), 3.7~3.9 (1H, m), 4.12 (2H, q, J=7 Hz), 6.76 (2H, d, J=9 Hz), 6.9~7.3 (4H, m), 7.50 (2H, t, J=9 Hz), 8.40 (1H, d, J=5 Hz).

2-*p*-Methoxybenzylthio-3-(2-pyridyl)propanol (**29**)

To a suspension of $LiAlH_4$ (115 mg, 3.0 mmol) in Et_2O (5 ml) was added a solution of **28** (994 mg, 3.0 mmol) in Et_2O (20 ml) slowly at 0~5°C under argon. After the mixture was stirred at the same temperature for 1 hour, 20% aq NH_4Cl (10 ml) was added and filtered through Celite. The filtrate was separated into organic and aqueous layers, and the organic layer was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel eluting with EtOAc to give **29** (694 mg, 80%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 3.0~3.5 (2H, m), 3.5~4.3 (3H, m), 3.64 (2H, s), 3.76 (3H, s), 6.76 (2H, d, J=9 Hz), 7.0~7.3 (2H, m), 7.12 (2H, d, J=9 Hz), 7.54 (1H, ddd, J=2, 7 and 7 Hz), 8.40 (1H, dd, J=2 and 7 Hz).

2,3-Dihydro-2-(*p*-methoxybenzylthio)-1*H*-indolizinium Chloride (**30**)

To a solution of the alcohol **29** (0.80 g, 2.76 mmol) in CCl_4 (40 ml) was added triphenylphosphine (1.45 g, 5.52 mmol). The reaction mixture was then refluxed for 20 hours. After cooling, the supernatant was separated, and the residue was diluted with water, washed with $CHCl_3$ and decolorized by activated charcoal powder. Evaporation of the solvent gave **30** (0.53 g, 51%) as a colorless oil. The supernatant was chromatographed on silica gel eluting with EtOAc to give **31** (66 mg, 6%) as a colorless oil. ^1H NMR (DMSO-d_6) δ 3.2~3.7 (2H, m), 3.77 (3H, s), 3.97 (2H, s), 3.7~4.0 (1H, m), 4.83 (1H, dd, J=5 and 13 Hz), 5.10 (1H, dd, J=7 and 13 Hz), 6.92 (2H, d, J=9 Hz), 7.32 (2H, d, J=9 Hz), 7.8~8.2 (2H, m), 8.54 (1H, t, J=8 Hz), 9.00 (1H, d, J=8 Hz).

31: ^1H NMR ($CDCl_3$) δ 2.85 (2H, s), 3.1~3.5 (2H, m), 3.75 (2H, s), 3.78 (3H, s), 4.3~4.6 (1H, m), 6.78 (2H, d, J=9 Hz), 7.0~7.4 (4H, m), 7.56 (1H, t, J=8 Hz), 8.50 (1H, d, J=6 Hz).

2,3-Dihydro-2-mercapto-1*H*-indolizinium Trifluoromethanesulfonate (**32**)

To a solution of **30** (535 mg, 1.74 mmol) and anisole (940 mg, 8.70 mmol) in trifluoroacetic acid (3 ml) was added trifluoromethanesulfonic acid (672 mg, 4.48 mmol) at 0~5°C. The reaction mixture was stirred at room temperature for 1 hour, then evaporated under reduced pressure. The residue was washed with petroleum ether, IPE and Et_2O to give **32** (524 mg, 100%) as a light brown oil. ^1H NMR (D_2O) δ 3.2~4.4 (3H, m), 4.7~5.0 (1H, m), 5.1~5.4 (1H, m), 7.7~8.1 (2H, m), 8.52 (1H, ddd, J=2, 8 and 8 Hz), 8.84 (1H, dd, J=2 and 8 Hz).

(5*R*,6*S*,8*R*)-2-[(2,3-Dihydro-1*H*-indolizinium-2-yl)thio]-6-(1-hydroxyethyl)penem-3-carboxylate (7)

To a solution of compound **8** (150 mg, 0.35 mmol) and compound **32** (212 mg, 0.70 mmol) in DMF (3 ml) was added *N*,*N*-diisopropylethylamine (181 mg, 1.40 mmol) at −40°C under argon. After stirring for 1.5 hours at the same temperature, the mixture was examined by TLC. Although the thiol **32** was not observed, most of the sulfoxide **8** was observed. Et_2O (50 ml) was added to the mixture. The ether phase was removed to give crude products having UV absorption at 340 nm, which were used in the next reaction without further purification.

To a solution of the above products in THF (20 ml) and water (20 ml) was added NH_4Cl (4.1 g) and Fe powder (2.0 g, 100 mesh). After vigorous stirring at 5~10°C for 1 hour, the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and chromatographed on a column of Diaion HP-20. Fractions eluted with 5% aq THF were concentrated under reduced pressure and were purified by HPLC eluting with 7% aq acetonitrile. Fractions having UV adsorption at 322 nm based on a penem skeleton were combined and lyophilized to give a trace amount of compound; however, the other spectroscopic data could not be measured.

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