Syntheses and Biological Properties of New 8-Fluorocarbapenem Derivatives

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8-Fluorocarbapenem derivatives having various C-3 side chains were synthesized to study for the structure–activity relationship of carbapenems by *in vitro* biological evaluation. The introduction of fluorine at C-8 of racemic PS-5 led to slight improvements of the antimicrobial activity and the stability to renal dehydropeptidase-I. When D-cysteine was additionally introduced to the C-3 position of (±)-8-fluorocarbapenem, the diastereomeric separation of the 8-fluorocarbapenems became feasible. As expected from penicillins and cephalosporins, (+)-8-fluoro-3-D-cysteinylcarbapenem (+)-7a was antimicrobially active, whereas (−)-7b was inactive. It is worth noting, however, that (+)-7a was significantly more sensitive to renal dehydropeptidase-I than (−)-7b. Irrespective of the presence of fluorine at C-8, basic S-side chains at C-3, such as the pyridyl and pyrrolidyl groups, significantly improved in antimicrobial activity and dehydropeptidase-I stability. The combination of 8-fluorination with C-3 basic side chains in 7c—g resulted in a marked improvement of antimicrobial activity and dehydropeptidase-I stability.

Keywords 8-fluorocarbapenem; carbapenem; PS-5; diastereomeric separation; sulfoxide displacement method; antimicrobial activity; dehydropeptidase-I stability

Since the isolation of PS-5 and related carbapenem compounds from streptomycetes,[2] we have continued development of a clinically acceptable carbapenem derivative which has improved physicochemical stability and is resistant to dehydropeptidase-I (DHP-I) *in vivo*. As a result, chemical modification of PS-5 at C-3 by displacement methods *via* sulfoxide provided a series of resistant carbapenem derivatives, but without practically satisfactory stability in mice to date.[3] Among these derivatives, the D-cysteinyl derivative **1** and the 4-pyridylthio derivative **2** were found to be most promising in antimicrobial activity and dehydropeptidase-I stability (Fig. 1).

Recently, imipenem has become available in the market as the first carbapenem drug, but it still has some inconveniences such as insufficient *in vivo* stability which requires coadministration of cilastatin; and also poor water solubility, which limits its clinical use to infusion only.[4] Mak and Fliri have reported the chemical synthesis of 8-fluorocarbapenem derivatives[5] as racemates through the Melillo lactone route.[6] Among them, the racemate of (5*R*,6*R*)-6-[(*R*)-1-fluoroethyl]-7-oxo-3-[(*N*,*N*,*N*′-trimethylcarbamimidoyl)methyl]thio-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (88.617) showed excellent antimicrobial activity and a high resistance to renal DHP-I.[5] Starting from OA-6129B_2, a fermentation carbapenem product, we reported an efficient method of derivation of optically active 88.617 by stereoselective fluorination with DAST.[7] In due course, we thought that the combination of 8-fluorination with a suitable C-3 side chain might bring about a therapeutically more valuable carbapenem derivative which, unlike imipenem, is sufficiently stable *in vivo* without DHP-I inhibitors and is administrable by various routes in addition to infusion.

Using PS-5 as a starting compound, we showed that the chemical introduction of the D-cysteinyl and 4-pyridylthio groups at C-3 of PS-5 resulted in significantly improved antimicrobial activity and DHP-I stability.[3] Accordingly, we began this study with the derivation of the 8-fluoro-3-D-cysteinyl carbapenem derivative **7ab** from the racemic diazo compound (±)-**4**,[5] as illustrated in Fig. 2. In particular, the diazo group of (±)-**4** was treated with a catalytic amount of rhodium diacetate, resulting in the slective N–H insertion of carbenoid.[8] Without purification, the bicyclic keto-ester (±)-**5** was converted to a tris-protected 8-fluorocarbapenem

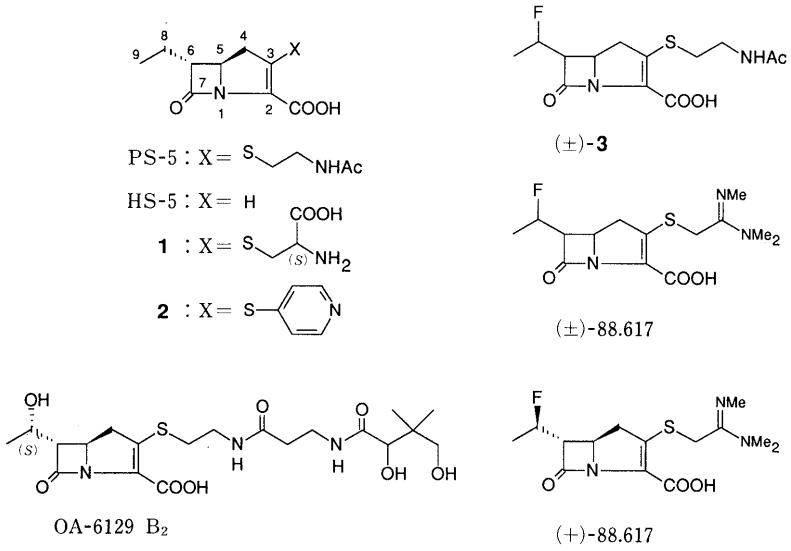

Fig. 1. PS-5 and Related Carbapenem Compounds

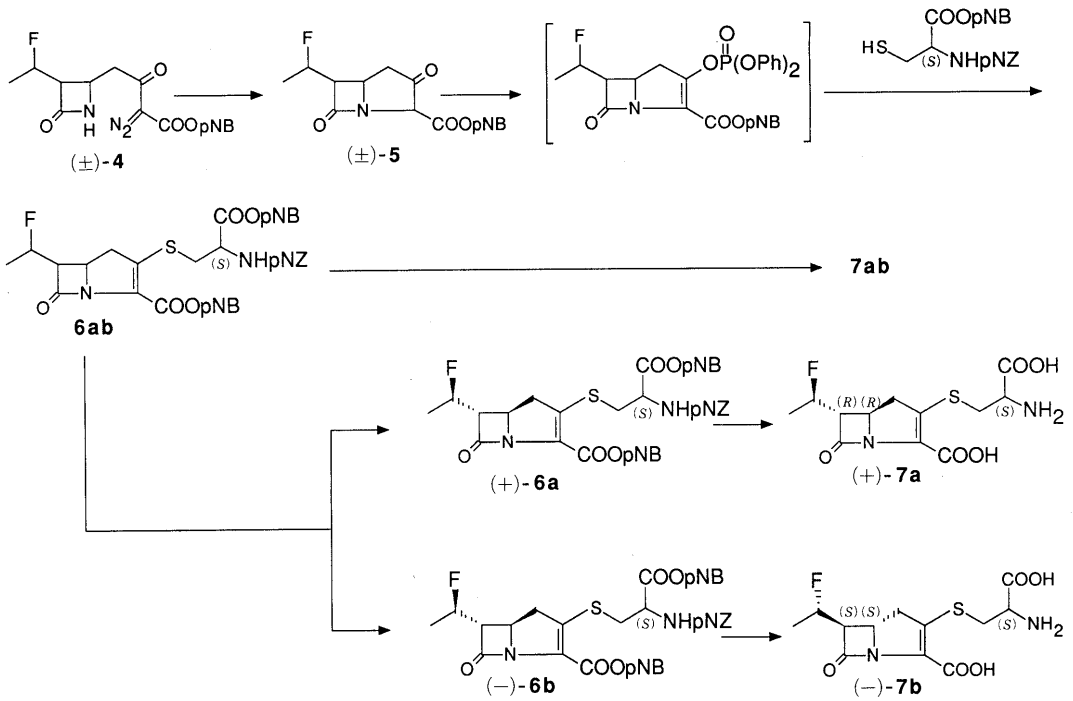

Fig. 2. Derivation and Diastereomeric Separation of **7ab**, (+)-**7a** and (−)-**7b**

TABLE I. Comparative Antimicrobial Activities of PS-5, 88.617 and Related Carbapenem Derivatives

Microorganism	CEZ	PS-5	(±)-**3**	**1**	**7ab**	(+)-**7a**	(−)-**7b**	**2**	**7c**	88.617
Gram-positive bacteria										
Bacillus subtilis ATCC6633	0.1	0.10	0.05	0.20	0.10	0.05	0.39	<0.007	<0.003	0.003
Micrococcus luteus ATCC9341	0.78	0.20	0.10	1.56	0.39	0.20	1.56	<0.007	<0.003	0.006
Staphylococcus aureus FDA209P	0.05	0.05	0.024	0.39	0.10	0.05	0.39	<0.007	<0.003	0.0008
Staphylococcus aureus SMITH	0.20	0.20	0.10	0.39	0.39	0.20	1.56	<0.007	0.006	0.006
Staphylococcus epidermidis	0.20	0.20	0.10	1.56	0.39	0.39	3.13	<0.007	—	—
Gram-negative bacteria										
Citrobacter freundii GN346[a]	>100	1.56	25	1.56	3.13	1.56	25	29.5	25	0.39
Comamonas terrigena B996	0.05	0.024	0.05	0.024	0.05	0.024	0.39	<0.007	<0.003	0.012
Enterobacter aerogenes E-19[a]	>100	1.56	6.25	0.39	0.78	0.78	6.25	7.4	0.78	0.78
Enterobacter cloacae 45[a]	>100	3.13	50	3.13	1.56	1.56	25	29.5	6.25	0.78
Escherichia coli K-12	0.78	1.56	0.78	0.024	0.39	0.20	1.56	0.92	0.10	0.39
Klebsiella pneumoniae 130[a]	50	6.25	1.56	0.39	0.39	0.39	3.13	14.8	1.56	0.78
Proteus vulgaris GN76[a]	>100	6.25	>100	0.78	1.56	1.56	25	29.5	3.13	3.13
Proteus sp. P-22[a]	>100	6.25	>100	1.56	1.56	1.56	25	29.5	1.56	1.56
Providencia sp. P-8	0.78	1.56	6.25	0.10	0.39	0.20	3.13	0.92	0.05	0.78
Pseudomonas aeruginosa IFO3445	>100	25	>100	3.13	25	12.5	>100	0.92	0.20	0.39
Pseudomonas aeruginosa NCTC10490	>100	25	50	6.25	50	50	>100	0.92	0.10	0.20
Serratia marcescens T55[a]	1.56	1.56	>100	3.13	3.13	1.56	25	29.5	12.5	1.56

a) Beta-lactamase producer. Diastereomeric purities of (+)-**7a** and (−)-**7b** were about 80% and 95%, respectively.

mixture **6ab** as a diastereomeric mixture with *N-p*-nitrobenzyloxycarbonyl-D-cysteine *p*-nitrobenzyl ester *via* an enol phosphate. After purification by silica gel column chromatography, the diastereomeric mixture **6ab** was subjected to fractional precipitation with chloroform to give (+)-**6a** and (−)-**6b** which contained small amounts of the corresponding diastereomers, respectively. After hydrogenation, diastereomerically about 80% pure (+)-**7a** and about 95% pure (−)-**7b** (by high performance liquid chromatography (HPLC) analysis) were produced for biological evaluation (Tables I and II).

Table I compares the antimicrobial activities of cefazolin (CEZ), PS-5,8-fluoro-PS-5 ((±)-**3**), 3-D-cysteinyl-HS-5 (**1**), **7ab**, (+)-**7a**, (−)-**7b**, 3-pyridyl-HS-5 (**2**), 8-fluoro-3-pyridyl-HS-5 (**7c**) and 88.617. Although the tested compounds (+)-**7a** and (−)-**7b** were not diastereomerically pure, it is still clear that (+)-**7a** is antimicrobially active, whereas (−)-**7b** seems to be inactive, as is the case in penicillins and cephalosporins. Retrospectively, the absolute configurations at C-5 of (+)-**7a** and (−)-**7b** are considered to be *R* and *S*, respectively. Furthermore, since the relative configuration of the beta-lactam hydrogens of (+)-**7a** and (−)-**7b** is *trans*, the full structures of (+)-**6a**, (−)-**6b**, (+)-**7a** and (−)-**7b** are concluded to be as shown in Fig. 2. Considering from the 95% diastereomeric purity of the (−)-**7b** preparation, it is reasonable to conclude that (−)-

diastereomers of carbapenems have no antimicrobial activity at all. In addition, as previously reported, the introduction of D-cysteine or 4-mercaptopyridines at C-3 of HS-5 leads to improved activity against the gram-positive pathogens,[3] while 8-fluorination has insignificant influence on the antimicrobial activity of PS-5 (Table I).

The currently inexplicably high susceptibility of carbapenems to DHP-I is a serious concern of organic chemists from the viewpoint of development of clinically useful carbapenem derivatives.[9] Figure 3 shows the comparative time courses of *in vitro* hydrolysis of the carbapenem derivatives, PS-5, **1**, **2** and (±)-**3**, by dog DHP-I.

TABLE II. Comparative DHP-I Stabilities of PS-5, 88.617 and Related Carbapenem Derivatives against Mouse, Dog and Man Dehydropeptidase-I

Animal	PS-5	(±)-3	1	7ab	2	7c	88.617
Mouse	1.0	3.1	7.7	8.7	21.6	8.3	25.9
Dog	1.0	1.0	1.7	5.4	—	15.8	4.6
Man	1.0	1.6	2.3	1.9	1.3	2.0	4.2

It is apparent that PS-5 is the most susceptible to mouse DHP-I *in vitro*; and that 8-fluorination, 3-cysteinylation or 3-pyridylthionylation of carbapenem results in significant stabilization of carbapenem to DHP-I.

As shown in Fig. 4, it is contrary to our expectations, (+)-**7a** in which the C-5 has the *R*-configuration is more susceptible to DHP-I than (−)-**7b** in which the C-5 has the *S*-configuration; also, the time course of hydrolysis of **7ab** is not the arithmetic mean of those of (+)-**7a** and (−)-**7b**. At present, our enzymological observations collected under various reaction conditions seem to suggest that, (−)-**7b** is a relatively poor DHP-I substrate, but acts as a kind of DHP-I inhibitor in **7ab** (unpublished results).

Based on the above-described findings, it is reasonable to conclude that racemic or diastereomeric mixtures of carbapenem derivatives should not be employed for biological evaluation of antimicrobial activity and DHP-I stability of carbapenems. So we have restricted the subsequent derivation of carbapenems to the optically active series starting from optically active (+)-**4** as follows (Fig. 5):

4-Mercaptopyridine and 4-mercaptopyrrolidines were se-

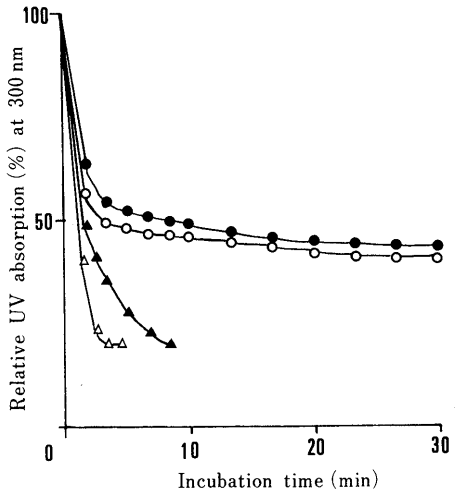

Fig. 3. Comparative Time Courses of Hydrolysis of PS-5 (△—△), **1** (●—●), **2** (○—○), and (±)-**3** (▲—▲) by Dehydropeptidase-I

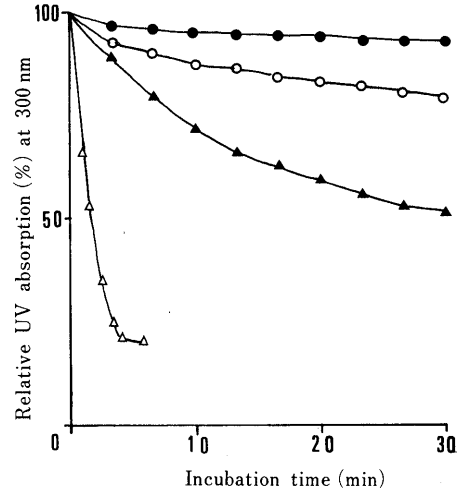

Fig. 4. Comparative Time Courses of Hydrolysis of PS-5 (△—△), **7ab** (○—○), (+)-**7a** (▲—▲) and (−)-**7b** (●—●) by Dehydropeptidase-I

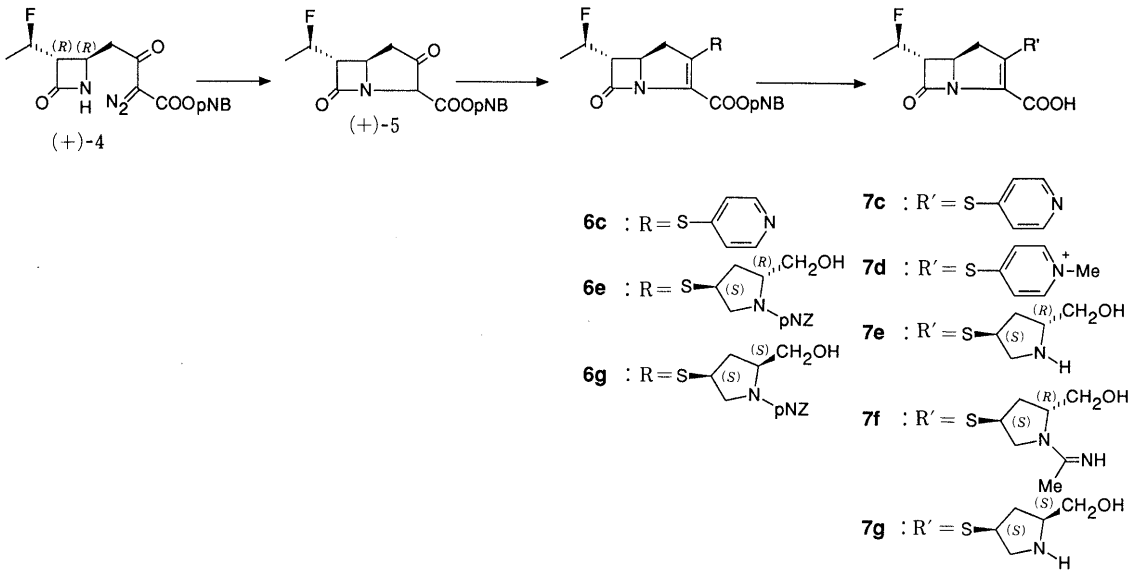

Fig. 5. Derivation of New 8-Fluorocarbapenem Derivatives

TABLE III. Antimicrobial Activities and Dehydropeptidase-I Stabilities of New 8-Fluorocarbapenem Derivatives

Microorganism	CEZ	PS-5	(±)-3	7c	7d	7e	7f	7g	88.617
Gram-positive bacteria									
Bacillus subtilis ATCC6633	0.10	0.10	0.05	<0.003	0.006	0.003	0.012	0.003	0.003
Micrococcus luteus ATCC9341	0.78	0.20	0.10	<0.003	0.003	0.012	0.012	0.006	0.006
Staphylococcus aureus FDA209P	0.05	0.05	0.024	<0.003	<0.003	0.0015	<0.0015	0.0015	0.0008
Staphylococcus aureus SMITH	0.20	0.20	0.10	0.006	0.006	0.012	0.006	0.003	0.006
Gram-negative bacteria									
Alcaligenes faecalis ATCC8750	12.5	1.56	0.39	0.012	0.006	0.39	0.20	0.20	0.78
Citrobacter freundii GN346[a]	>100	1.56	25	25	0.78	0.78	0.78	0.78	0.39
Comamonas terrigena B996	0.05	0.024	0.05	<0.003	0.006	0.012	0.012	0.003	0.012
Enterobacter aerogenes E-19[a]	>100	1.56	6.25	0.78	1.56	1.56	0.78	0.78	0.78
Enterobacter cloacae 45[a]	>100	3.13	50	6.25	3.13	1.56	1.56	1.56	0.78
Escherichia coli K-12	0.78	1.56	0.78	0.10	0.20	0.78	0.20	0.39	0.39
Escherichia coli RGN238[a]	1.56	1.56	1.56	0.10	0.39	0.78	0.39	0.78	0.78
Klebsiella pneumoniae 130[a]	50	6.25	0.78	1.56	0.78	0.78	0.78	0.78	0.78
Proteus morganii 111[a]	50	6.25	6.25	0.20	0.78	1.56	0.39	0.78	1.56
Proteus rettgeri P-48	50	3.13	12.5	0.78	3.13	1.56	0.78	1.56	1.56
Proteus vulgaris GN76[a]	>100	6.25	>100	3.13	3.13	3.13	3.13	3.13	3.13
Proteus sp. P-22[a]	>100	6.25	>100	1.56	3.13	3.13	3.13	3.13	1.56
Providencia sp. P-8	0.78	1.56	6.25	0.05	0.20	0.78	0.78	0.78	0.78
Pseudomonas aeruginosa IFO3445	>100	12.5	>100	0.20	0.39	0.20	0.39	0.39	0.39
Pseudomonas aeruginosa NCTC10490	>100	12.5	50	0.10	0.39	0.20	0.39	0.39	0.20
Pseudomonas aeruginosa E2[a]	>100	>100	>100	3.13	3.13	3.13	6.25	3.13	3.13
Salmonella gallinarum ATCC9184	0.78	1.56	1.56	0.10	0.20	0.78	0.39	0.78	0.39
Serratia marcescens IFO3736	>100	3.13	>100	6.25	1.56	3.13	0.78	1.56	1.56
Serratia marcescens T55[a]	1.56	6.25	>100	12.5	3.13	3.13	3.13	1.56	1.56
Shigella sonnei EW33	3.13	3.13	6.25	0.10	0.78	1.56	0.78	0.78	0.78
Dog dehydropeptidase-I (% activity after reaction[b])	//	21.1	—	88.7	93.9	91.8	84.5	90.0	97.1

a) Beta-lactamase producer. *b*) Incubation at 37 °C for 60 min.

lected as C-3 side chains for combination with 8-fluorination, as they seemed promising from the viewpoints of antimicrobial activity and DHP-I stability.[3]

Optically active (+)-**4**, which was prepared by a procedure including the resolution of the Melillo lactone,[10] was converted to a bicyclic ester (+)-**5** as described for (±)-**4**. The enol phosphate of (+)-**5** was then condensed with 4-mercaptopyridine to give **6c** in 58% yield, which was further led to **7c** by hydrogenation. **7d** was derived from **7c** by *N*-methylation with methyl iodide.

8-Fluorocarbapenem derivatives having pyrrolidine side chains at C-3,[11] **6e** and **6g**, were prepared by treating the enol phosphate of (+)-**5** with (2*R*,4*S*)-2-hydroxymethyl-4-mercapto-*N*-*p*-nitrobenzyloxycarbonylpyrrolidine.[12] Hydrogenation of **6e** resulted in **7e**. Treatment of **7e** with ethyl acetimidate hydrochloride at 0 °C under weakly alkaline conditions yielded the acetimidate **7f** in 72% yield. **6g**, a C-2′ epimer of **6e**, was hydrogenated to give **7g**.

Table III summarizes the antimicroial activities of new 8-fluorocarbapenem derivatives having pyridylthio and pyrrolidinylthio side chains at C-3. As in the case with thienamycin derivatives,[13] increased basicity of the C-3 side chains clearly resulted in significantly improved antimicrobial activity against both the gram-positive bacteria and the gram-negative. Table III also compares the *in vitro* dog DHP-I stability of the new carbapenem derivatives, which demonstrates that the pyridylthio and pyrrolidinylthio side chains at C-3 are more effective than 8-fluorination for stabilization of carbapenem toward DHP-I.

The results of *in vitro* biological evaluation in Tables I—III allow the following conclusions: 1) the *R*-configuration at C-5 is essential for antimicrobial activity, whereas the *S*-configuration at C-5 seems to be useful for DHP-I inhibition or resistance activity, but without antimicrobial activity (Table I and Fig. 4); 2) 8-fluorination slightly improves the antimicrobial activity and DHP-I stability, but also increases susceptibility to some beta-lactamases (Table I and Fig. 3); 3) basic side chains at C-3 lead to significant improvement in antimicrobial activity and DHP-I stability, but again make carbapenem somewhat more sensitive to beta-lactamases (Tables I and II); and 4) the combination of 8-fluorination with basic side chains at C-3 results in synergistic improvement of antimicrobial activity and DHP-I stability (Table III).

8-Fluorination shows another advantage by making markedly water-soluble generally least water-soluble 8-hydroxycarbapenem molecules with strong basic side chains at C-3.[4] For example, imipenem has a water-solubility as low as 1%, which limits the clinical use of imipenem to infusion only. In contrast, 8-fluorocarbapenem derivatives with a strong basic side chain at C-3, such as optically active 88.617, **7c**, **7e** and **7f**, show good water-solubility (over 30%). The new 8-fluorocarbapenem derivatives will be further evaluated by *in vivo* animal studies, including pharmacokinetics and toxicology, in a variety of animals including man.

Experimental

General Methodology Silica gel thin-layer chromatography (TLC) and column chromatography were carried out by using pre-coated TLC plate Silica gel 60 F_{254} (E. Merck) and silica gel 60 (70—230 mesh; E. Merck), respectively. Infrared (IR), ultraviolet (UV) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with a Hitachi 260-30 infrared

spectrophotometer, a Hitachi 200-20 UV/visible spectrophotometer and a Varian EM-390 (90 MHz) spectrometer, respectively. Chemical shifts are given in ppm from internal tetramethylsilane (TMS) in $CDCl_3$ and pyridine-d_5 or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) in D_2O. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. Melting points were determined in a YANACO apparatus, and are uncorrected.

PS-5 This carbapenem was isolated from the fermentation broth of *Streptomyces cremeus* subsp. *auratilis* A271, a soil microorganism (ATCC 31358).[2a)]

(5*R*,6*R*)-3-[(2*S*)-2-Amino-2-carboxy]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (3-D-Cysteinyl-HS-5: 1) and (5*R*,6*R*)-6-Ethyl-3-(pyridin-4-yl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (3-Pyridyl-HS-5: 2) 3-D-Cysteinyl-HS-5 (**1**) and 3-pyridyl-HS-5 (**2**) were prepared from the sulfoxide of PS-5 *p*-nitrobenzyl ester by the methods for displacing the C-3 sulfur side chains of carbapenems.[3c)]

(5*RS*,6*RS*)-3-Acetamidoethylthio-6-[(*RS*)-1-fluoroethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (8-Fluoro-PS-5: (±)-3) 8-Fluoro-PS-5 was prepared according to the method of Mak and Fliri.[5a)]

***p*-Nitrobenzyl (5*RS*,6*RS*)-3,7-Dioxo-6-[(*RS*)-1-fluoroethyl]-1-azabicyclo[3.2.0]heptane-2-carboxylate ((±)-5)** A suspension of 394 mg of *p*-nitrobenzyl 4-[(3*RS*,4*RS*)-3-[(*RS*)-1-fluoroethyl]-2-oxoazetidinin-4-yl]-2-diazo-3-oxobutyrate ((±-**4**) and 8.0 mg of rhodium (II) acetate dimer in 40 ml of benzene was heated at 80 °C for 1 h under a nitrogen atmosphere, cooled to room temperature and filtered. The filtrate was concentrated to dryness *in vacuo* to afford 356 mg (100% yield) of (±)-**5** which was employed in the next step without purification. IR (KBr): 1780 (shoulder), 1760, 1745 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 266.5 (10200). ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, dd, *J*=6.0, 24.0 Hz), 2.49 (1H, dd, *J*=8.0, 18.0 Hz), 2.98 (1H, dd, *J*=8.0, 18.0 Hz), 3.34 (1H, m), 4.20 (1H, dt, *J*=2.0, 8.0 Hz), 4.7—5.55 (1H, m), 4.80 (1H, s), 5.26 (1H, d, *J*=14.0 Hz), 5.40 (1H, d, *J*=14.0 Hz), 7.50 (2H, d, *J*=9.0 Hz), 8.25 (2H, d, *J*=9.0 Hz).

Optically active (+)-**5** was obtained from optically active (+)-**4** by the reaction procedure as described above for (±)-**5**. $[\alpha]_D^{20}$ +185.7° (*c*=1.0, $CHCl_3$).

***p*-Nitrobenzyl (5*RS*,6*RS*)-6-[(*RS*)-1-Fluoroethyl]-3-[(2*S*)-2-*p*-nitorobenzyloxycarbonyl-2-*p*-nitrobenzyloxycarbonylamino]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (6ab) and Separation of the Two Diastereomers ((+)-6a and (−)-6b)** A racemic compound (±)-**5** (340 mg) was dissolved at −30 °C in 10 ml of *N*,*N*-dimethylformamide and then mixed with 0.253 ml of diisopropylethylamine and 0.243 ml of diphenylphosphoryl chloride at −30 °C. The mixture was stirred for 30 min at −30 °C. Diisopropylethylamine (0.202 ml) and a solution of 50.8 mg of *N*-*p*-nitrobenzyloxycarbonyl-D-cysteine *p*-nitrobenzyl ester in 2 ml of *N*,*N*-dimethylformamide were added dropwise to the reaction mixture and then stirred for 30 min. After mixed with 100 ml of dichloromethane, the dilution was washed with 70 ml each of 0.1 M phosphate buffers of pH 8.40, 6.90 and 8.40. The washed solution was dried over sodium sulfate and then evaporated to dryness *in vacuo* to provide an oil, which was subjected to silica gel (20 g) column chromatography using benzene–ethyl acetate (3:1) as an eluent. Eluate fractions containing the desired product were combined and concentrated to dryness *in vacuo* to afford a diastereomeric mixture **6ab** (520 mg, 70% yield) as a white precipitate. IR (KBr): 1780, 1735, 1690 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 268.5 (35000). ^{1}H-NMR (pyridine-d_5) δ: 1.37 (3H, dd, *J*=6.0, 24.0 Hz), 3.3—4.05 (5H, m), 4.29 (1H, dt, *J*=2.5, 8.0 Hz), 4.45—5.65 (8H, m), 7.3—7.8 (6H, m), 8.10 (6H, d, *J*=9.0 Hz), 9.80 (1H, d, *J*=9.0 Hz). *Anal.* Calcd for $C_{34}H_{30}FN_5O_{13}S$: C, 54.32; H, 4.02; N, 24.23. Found: C, 54.59; H, 4.23; N, 23.95.

The precipitate was washed with 20 ml of chloroform, leaving (−)-**6b** 227 mg, $[\alpha]_D^{23}$ −27.7° (*c*=0.5, CH_2Cl_2) as a major diastereoisomer. The solid was further purified by precipitation from a mixture of CH_2Cl_2 and $CHCl_3$. The final preparation $[\alpha]_D^{23}$ −36.8° (*c*=0.5, CH_2Cl_2) of (−)-**6b** was found to be diastereomerically 80% pure.

The chloroform wash was concentrated to dryness under reduced pressure, yielding a (+)-**6a**-rich preparation $[\alpha]_D^{23}$ +17.5° (*c*=1.0, CH_2Cl_2). After repeated crystallization from chloroform, (+)-**6a** 44 mg, $[\alpha]_D^{23}$ +23.9° (*c*=0.25, CH_2Cl_2) was obtained as a 95%-pure preparation.

(5*RS*,6*RS*)-3-[(2*S*)-2-Amino-2-carboxy]ethylthio-6-[(*RS*)-1-fluoroethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-caroxylic Acids (7ab) The diastereomer **6ab** (135 mg) was dissolved in a mixture of 4 ml of tetrahydrofuran, 3 ml of dioxane and 3 ml of 0.1 M phosphate buffer, pH 8.40; it was then hydrogenated in the presence of 100 mg of platinum dioxide at room temperature for 3.5 h at a hydrogen pressure of 4 kg/cm^2. After filtration, the filtrate was subjected to successive column chromatography on QAE-Sephadex A-25 (Pharmacia Fine Chemicals AB, Sweden) and Diaion CHP-20P (Mitsubishi Chemical Industries Ltd., Japan). Eluate fractions which showed a maximum UV absorption at 300 nm were combined and lyophilized to provide a white powder of diastereomer **7ab** (21.6 mg, 36% yield). IR (KBr): 1760 dm^{-1}. UV $\lambda_{max}^{0.001\,M\,PBS(pH\,7.0)}$ nm (ε): 298 (5600). ^{1}H-NMR (D_2O) δ: 1.41 (3H, dd, *J*=6.0, 25.0 Hz), 2.8—3.65 (5H, m), 3.75—4.0 (1H, m), 4.29 (1H, dt, *J*=2.5, 8.5 Hz), 5.11 (1H, ddd, *J*=6.0, 7.5, 48.4 Hz).

(5*R*,6*R*)-3-[(2*S*)-2-Amino-2-carboxy]ethylthio-6-[(*R*)-1-fluoroethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid ((+)-7a) and (5*S*,6*S*)-3-[(2*S*)-2-Amino-2-carboxylethylthio-6-[(*S*)-1-fluoroethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid ((−)-7b) Under the same reaction conditions as described for the diastereomeric mixture, (+)-**6a** and (−)-**6b** were hydrogenated to give (+)-**7a** and (−)-**7b**, respectively. The diastereomeric purities of (+)-**7a** and (−)-**7b** were determined by HPLC. The conditions for HPLC were as follows: column, μ-Bondapak C_{18} (3.7 i.d. × 300 mm, Waters Associates); solvent, MeOH–0.01 M phosphate buffer (pH 7.5) (1:9); flow rate, 2.0 ml/min; temperature, 25 °C; detection, UV 300 nm. The retention times of (+)-**7a** and (−)-**7b** were 22.5 and 21.0 min, respectively.

***p*-Nitrobenzyl (5*R*,6*R*)-6-[(*R*)-1-Fluoroethyl]-7-oxo-3-(pyridin-4-yl)thio-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (6c)** Treatment of the enol phosphate of (+)-**5** (374 mg) with 4-mercaptopyridine sodium salt gave a mercaptopyridine derivative **6c** (277 mg, 58% yield) as an amorphous solid. IR (KBr): 1796, 1714 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 321 (15000), 268 (15500). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, dd, *J*=6.0, 24.0 Hz), 2.86 (2H, d, *J*=9.0 Hz), 3.31 (1H, ddd, *J*=3.0, 7.5, 19.5 Hz), 4.21 (1H, dt, *J*=3.0, 9.0 Hz), 4.5—5.45 (1H, m), 5.28 (1H, d, *J*=13.5 Hz), 5.54 (1H, d, *J*=13.5 Hz), 7.40 (2H, d, *J*=5.0 Hz), 7.66 (2H, d, *J*=8.5 Hz), 8.22 (2H, d, *J*=8.5 Hz), 8.62 (2H, d, *J*=5.0 Hz). *Anal.* Calcd for $C_{21}H_{18}FN_3O_5S$: C, 56.87; H, 4.09; N, 9.48. Found: C, 57.13; H, 4.14; N, 9.58.

(5*R*,6*R*)-6-[(*R*)-1-Fluoroethyl]-7-oxo-3-(pyridin-4-yl)thio-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (7c) Under reaction conditions similar to those for **7ab**, 275 mg of *p*-nitrobenzyl ester **6c** was hydrogenated to afford 51.8 mg of **7c** (27% yield) as a colorless powder. IR (KBr): 1766 cm^{-1}. UV $\lambda_{max}^{0.001\,M\,PBS(pH\,7.0)}$ nm (ε): 303.5 (8800). ^{1}H-NMR (D_2O) δ: 1.39 (3H, dd, *J*=6.5, 24.5 Hz), 2.91 (2H, d, *J*=9.5 Hz), 3.4—3.85 (1H, m), 4.26 (1H, dt, *J*=3.0, 9.5 Hz), 4.65—5.55 (1H, m), 7.45 (2H, d, *J*=5.0 Hz), 8.40 (2H, d, *J*=5.0 Hz).

(5*R*,6*R*)-6-[(*R*)-1-Fluoroethyl]-3-(1-methyl-4-pyridinio)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (7d) One milliliter of methyl iodide was added at 0 °C to a solution of 27.5 mg of **7c** in a mixture of 2.0 ml of a 0.01 M phosphate buffer, pH 8.3 and 2.0 ml of dioxane, and stirred for 12 h at room temperature. After being diluted with 10 ml of the phosphate buffer, the dilution was concentrated to about 5 ml *in vacuo*. The concentrate was again diluted with 10 ml of deionized water and washed twice, each time with 10 ml of ethyl acetate. The aqueous layer was recovered and subjected to column chromatography on Diaion CHP-20P using a linear concentration gradient of isopropyl alcohol from 0 to 50%. Active eluate fractions were combined and freeze-dried to produce 18.8 mg of **7d** (65% yield) as a colorless powder. IR (KBr): 1773 cm^{-1}. UV $\lambda_{max}^{0.001\,M\,PBS(pH\,7.0)}$ nm (ε): 305 (8000). ^{1}H-NMR (D_2O) δ: 1.43 (3H, dd, *J*=6.0, 24.5 Hz), 3.14 (2H, d, *J*=9.5 Hz), 3.83 (1H, ddd, *J*=3.0, 4.5, 28.5 Hz), 4.24 (3H, s), 4.48 (1H, dt, *J*=3.0, 9.5 Hz), 4.8—5.55 (1H, m), 7.80 (2H, d, *J*=6.0 Hz), 8.45 (2H, d, *J*=6.0 Hz).

***p*-Nitrobenzyl (5*R*,6*R*)-6-[(*R*)-1-Fluoroethyl]-3-[(2*R*,4*S*)-2-hydroxymethyl-1-*p*-nitrobenzyloxycarbonylpyrrolidin-4-yl]thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (6e)** Treatment of the enol phosphate of (+)-**5** (375 mg) with 401 mg of (2*R*,4*R*)-2-hydroxymethyl-4-mercapto-1-*p*-nitrobenzyloxycarbonylpyrrolidine yielded 499 mg of *p*-nitrobenzyl ester **6e** (72% yield) as an amorphous solid. IR ($CHCl_3$): 1785, 1700, 1670 cm^{-1}. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 270 (21000), 318 (13000). ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, dd, *J*=6.0, 23.0 Hz), 1.85—2.4 (2H, m), 3.27 (2H, d, *J*=9.5 Hz), 3.35—4.25 (8H, m), 4.28 (1H, dt, *J*=3.5, 9.5 Hz), 4.55—5.45 (1H, m), 5.21 (1H, d, *J*=13.5 Hz), 5.25 (2H, s), 5.52 (1H, d, *J*=13.5 Hz), 7.50 (2H, d, *J*=8.5 Hz), 7.67 (2H, d, *J*=8.5 Hz), 8.22 (2H, d, *J*=8.5 Hz). $[\alpha]_D^{24}$ +57.9° (*c*=1.0, $CHCl_3$). *Anal.* Calcd for $C_{29}H_{29}FN_4O_{10}S$: C, 54.02; H, 4.53; N, 8.69. Found: C, 53.79; H, 4.65; N, 8.74.

(5*R*,6*R*)-6-[(*R*)-1-Fluoroethyl]-3-[(2*R*,4*S*)-2-hydroxymethylpyrrolidin-4-yl]thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (7e) Under the same reaction conditions as for **7ab**, 456 mg of *p*-nitrobenzyl ester **6e** was hydrogenated to produce 97 mg of **7e** (42% yield) as a colorless powder. IR (KBr): 1768 cm^{-1}. UV $\lambda_{max}^{0.01\,M\,PBS(pH\,7.0)}$ nm (ε): 298.5 (10000). ^{1}H-NMR (D_2O) δ: 1.42 (3H, dd, *J*=6.0, 24.5 Hz), 2.05—2.45 (2H, m),

3.21 (2H, d, J=9.0 Hz), 3.3—4.15 (7H, m), 4.26 (1H, dt, J=3.0, 9.0 Hz), 4.7—5.5 (1H, m). $[\alpha]_D^{24}$ +55.6° (c=1.0, H_2O).

(5*R*,6*R*)-6-[(*R*)-1-Fluoroethyl]-3-[(2*R*,4*S*)-1-acetoimidoyl-2-hydoroxymethylpyrrolidin-4-yl]thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (7f) Ethyl acetimidate hydrochloride (72 mg) was slowly added at 0 °C to a solution of 38 mg of the pyrrolidine derivative **7e** in 15 ml of a 0.1 M phosphate buffer, pH 8.4, while the pH of the mixture was maintained in the range from 8.5 to 9.0 with 1 N NaOH. After stirring for 30 min, the pH of the reaction mixture was adjusted to 8.0 with 1 N HCl. The solution was subjected to column chromatography on Diaion CHP-20P, giving 31 mg of **7f** (72% yield) as a colorless powder. IR (KBr): 1768 cm^{-1}. UV $\lambda_{max}^{0.01\,M\,PBS\,(pH\,7.0)}$ nm (ε): 295.5 (11000). ^{1}H-NMR (D_2O) δ: 1.42 (3H, dd, J=6.0, 24.0 Hz), 1.95—2.55 (2H, m), 1.27 and 1.37 (total 3H, s and s), 3.22 (2H, d, J=9.0 Hz), 3.4—4.25 (7H, m), 4.27 (1H, dt, J=3.0, 9.0 Hz), 4.65—5.55 (1H, m). $[\alpha]_D^{24}$ +37.5° (c=1.0, H_2O).

(5*R*,6*R*)-6-[(*R*)-1-Fluoroethyl]-3-[(2*S*,4*S*)-2-hydroxymethylpyrrolidin-4-yl]thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (7g) Under reaction conditions similar to those for **7e**, **7g** was given as a colorless powder. IR (KBr): 1765 cm^{-1}. UV $\lambda_{max}^{0.01\,M\,PBS\,(pH\,7.0)}$ nm (ε): 298.5 (12000). ^{1}H-NMR (D_2O) δ: 1.40 (3H, dd, J=6.0, 25 Hz), 1.6—2.0 (1H, m), 2.4—4.05 (10H, m), 4.23 (1H, dt, J=3.0, 9.0 Hz), 4.65—5.4 (1H, m).

Biological Assays Antimicrobial activities against gram-positive and gram-negative bacteria were determined by the standard agar dilution technique using Mueller-Hinton agar (Difco Co.).[2]

Time course assay of relative DHP-I stability of a carbapenem derivative at 37 °C was run by UV-tracing the reaction mixture containing 0.2 ml of 100 μg/ml of PS-5 (reference carbapenem) (or a carbapenem derivative at the corresponding concentration) and 0.2 ml of a kidney homogenate (man, dog or mouse kidney homogenate) at 300 nm (for PS-5) or appropriate wavelengths (Table II).[3] Comparative DHP-I stability assay in Table III was carried out at 37 °C for 60 min by incubating 0.1 ml each of a test carbapenem solution (PS-5 at 100 μg/ml and other carbapenem derivatives at equivalent concentrations) and 0.1 ml of dog DHP-I homogenate in a 0.1 M Tris–HCl buffer, pH 7.0. After being heated at 100 °C for 15 s, the concentration of the carbapenem derivative remaining in the reaction mixture was bioassayed by the disc agar diffusion method using *Comamonas terrigena* B996.[3]

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