# SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF A NEW PENEM, SODIUM (5*R*,6*S*)-2-(2-FLUOROETHYLTHIO)-6-[(1*R*)-1-HYDROXYETHYL]PENEM-3-CARBOXYLATE†

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The synthesis and *in vitro* antimicrobial activity of a new penem antibiotic, sodium (5*R*, 6*S*)-2-(2-fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate (**1**), are reported. The MIC values of **1** are compared with those of some related 2-halcalkylthio penems prepared in this work, and also Sch 29482 and thienamycin.

Nonclassical $\beta$-lactam antibiotics, penems and carbapenems have received extensive attention since the pioneering synthetic work of WOODWARD[2] and the discovery of thienamycin (THM)[3~6]. Among penems, Sch 29482[7] and FCE 22101[8] have been reported to be potent broad-spectrum antibiotics. From the viewpoint of molecular modification, new penem derivatives having the fluoroalkylthio group at 2-position were of particular interest to us since the introduction of fluorine atom does influence biological properties such as antimicrobial activity, pharmacokinetics and metabolism of those penems[9,10]. As a result of extensive syntheses of 2-fluoroalkylthio penems, we ultimately obtained a new penem, sodium (5*R*,6*S*)-2-(2-fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate (**1**), having potent *in vitro* and *in vivo* activity against wide range of bacteria.

## Synthesis

For the synthesis of **1**, we utilized thioxopenam which was developed by MIYADERA *et al.* in our research laboratories[11]. Alkylation reaction of *p*-nitrobenzyl (5*S*,6*S*)-6-[(1*R*)-1-*tert*-butyldimethylsilyloxyethyl]-2-thioxopenam-3-carboxylate (**2**) with 1-bromo-2-fluoroethane was carried out in nitromethane in the presence of triethylamine at room temperature for 3 days to give *cis* 2-fluoroethylthio penem **3** in 23% yield as shown in Chart 2. The *cis* penem **3** isomerized to the *trans* penem **4** by heating in xylene containing a small amount of hydroquinone at 135°C for 3 hours and reached equilibrium in which the ratio of **3**:**4** was *ca.* 1:2.6. After separation of **3** and **4** by chromatography through a Lobar column, **4** was subjected to equilibration again and eventually **4** was obtained in 75% yield. Presumably, this equilibrium would occur *via* betaine intermediate **6**. The deprotection of *tert*-butyldimethylsilyl group with tetrabutylammonium fluoride in THF furnished the hydroxy penem **5** in 81% yield. The conversion of **5** into **1** was achieved by hydrogenolysis over 10% Pd-C in THF-phosphate buffer in 90% yield. Other structurally related 2-haloalkylthio penems **7~12** were prepared by alkylation reactions of the thioxopenam **2** with halogen substituted alkylhalide, modified MITSUNOBU reactions[12] of **2** with alcohols, and intramolecular Wittig-type reactions between phosphoranes and trithiocarbonates which were developed by WOODWARD *et al.*[13]. The details of

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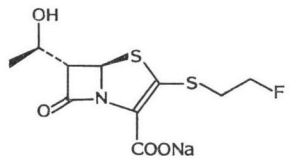
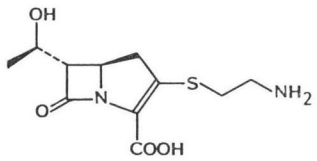
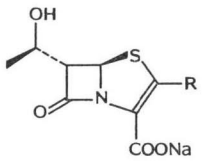
Chart 1.



1



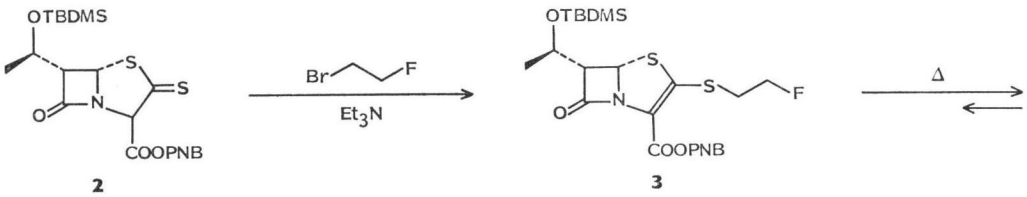
THM



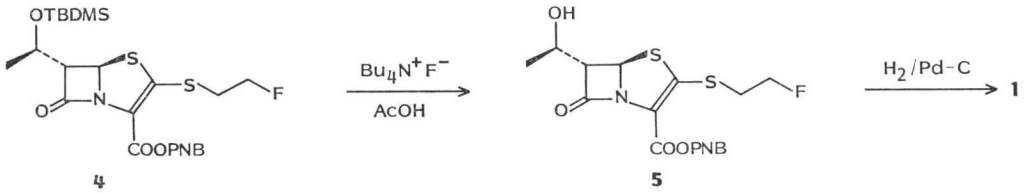
Sch 29482 R = $SCH_2CH_3$

FCE 22101 R = $CH_2OCONH_2$

Chart 2.



2 3



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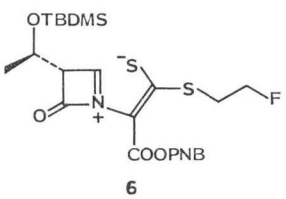
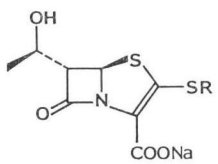


6



7 R = $CH(CH_3)CH_2F$
8 R = $CH(CH_2F)_2$
9 R = $CH_2CH(F)CH_3$
10 R = $(CH_2)_3F$
11 R = $CH_2CH_2Cl$
12 R = $CH_2CH_2Br$

TBDMS = *tert*-Butyldimethylsilyl
PNB = *p*-Nitrobenzyl

the syntheses will be described elsewhere.

## Antimicrobial Activity

The *in vitro* antimicrobial activity was tested by the serial agar dilution method. The minimal inhibitory concentration (MIC) against a variety of Gram-positive and Gram-negative bacteria are listed in Table 1 and compared with those of related 2-haloalkylthio penems **7**~**12**, Sch 29482 and THM. The penem **1** shows excellent antimicrobial activity comparable to or better than those of the structurally related 2-haloalkylthio penems **7**~**12** against all species shown in Table 1. In particular, **1** is the most active compound against *Escherichia coli*. Also, **1** has *in vitro* activity 2 to 8 times

Table 1. Antimicrobial activities of **1** and other β-lactam antibiotics against Gram-positive and Gram-negative bacteria.

| Organism | MIC (μg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **1** | **7** | **8** | **9** | **10** | **11** | **12** | Sch 29482 | THM |
| *Bacillus subtilis* PCI 219 | ≦0.01 | ≦0.01 | ≦0.01 | ≦0.01 | 0.02 | ≦0.01 | 0.02 | ≦0.01 | ≦0.01 |
| *Staphylococcus aureus* 209P | ≦0.01 | ≦0.01 | ≦0.01 | ≦0.01 | 0.02 | 0.02 | 0.05 | 0.02 | ≦0.01 |
| *S. aureus* 56* | 0.02 | 0.02 | 0.05 | 0.02 | 0.02 | 0.02 | 0.05 | 0.05 | ≦0.01 |
| *Escherichia coli* NIHJ | 0.1 | 0.2 | 0.4 | 0.8 | 0.8 | 0.2 | 0.4 | 0.4 | 0.1 |
| *E. coli* 609** | 0.1 | 0.4 | 0.4 | 0.8 | 1.5 | 0.4 | 0.4 | 0.8 | 0.1 |
| *Salmonella enteritidis* Gaertner | 0.1 | 0.1 | 0.2 | 0.4 | 0.4 | 0.2 | 0.4 | 0.2 | 0.2 |
| *Shigella flexneri* 2a Komagome | 0.05 | 0.05 | 0.05 | 0.2 | 0.2 | 0.4 | 0.2 | 0.2 | 0.1 |
| *Klebsiella pneumoniae* 806 | 0.1 | 0.2 | 0.2 | 0.4 | 0.8 | 0.4 | 0.4 | 0.4 | 0.1 |
| *Enterobactor cloacae* 963 | 1.5 | 6.2 | 6.2 | 6.2 | 6.2 | 3.1 | 3.1 | 6.2 | 3.1 |
| *Serratia marcescens* 1850 | 0.4 | 0.4 | 0.8 | 1.5 | 1.5 | 0.8 | 0.8 | 0.8 | 0.2 |
| *Proteus vulgaris* 1420 | 0.8 | 0.8 | 1.5 | 0.8 | 1.5 | 1.5 | 0.8 | 1.5 | 3.1 |
| *Pseudomonas aeruginosa* 1001 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | 6.2 |

* Penicillinase producer.

** Cephalosporinase producer.

Nutrient agar: Inocula were diluted 100-fold after overnight culture. Final inoculum size was one-loopful of $10^7$ cfu/ml.

higher than the known compound, Sch 29482. Although **1** is inactive against *Pseudomonas aeruginosa*, the MIC values of **1** against other organisms are comparable to those of THM.

It is known that the low urinary recovery of THM resulted from the hydrolysis of the β-lactam ring by dehydropeptidase-I (DHP-I), and Sch 29482 was less susceptible than THM to DHP-I and the urinary recovery of Sch 29482 was higher than that of THM[14~16]. Thus, the urinary recovery of **1** was compared with Sch 29482 under identical conditions. The urinary recovery of **1** in mice after po administration was 7.3% during 0~24 hours which was nearly equal to that of Sch 29482 (7.4%). After sc administration the urinary recovery of **1** was 42.5% during 0~24 hours which was slightly higher than that of Sch 29482 (37.2%).

The *in vivo* activity of the new penem **1** was compared with that of Sch 29482. Against *Staphylococcus aureus* infection, the penem **1** was superior to Sch 29482, while against *Klebsiella pneumoniae* and *Proteus vulgaris* its efficacy was equivalent to the reference compound after po administration. However, after sc administration the penem **1** appeared to be remarkably superior to Sch 29482 against evaluated all species, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. vulgaris* and *Serratia marcescens*. Furthermore, in rat the odors of collected urine of **1** were compared with those of the control and Sch 29482 by sensory test after po and sc administrations. The urinary odors of **1** was nearly similar to those of the control and clearly distinct from those of Sch 29482. The details of the *in vivo* activity and the sensory test of the urinary odors of **1** will be described elsewhere. Further evaluation of **1** is of interest in order to establish the efficacy of this new penem antibiotic.

## Experimental

Melting points were determined on a Yanaco melting point apparatus and were uncorrected. IR spectra were recorded on a Jasco A-2 spectrometer and UV spectra were obtained on a Cary 14 CM-50 (Serial 1258) spectrometer. NMR spectra were recorded on a Varian XL-100A or a EM-360L spectrometer. Chemical shifts are reported in ppm ($\delta$) using, unless otherwise specified, tetramethylsilane (TMS) as an internal standard. Rotation were determined on a Perkin-Elmer 241 polarimeter.

*p*-Nitrobenzyl (5*S*,6*S*)-6-[(1*R*)-*tert*-Butyldimethylsilyloxyethyl]-2-(2-fluoroethylthio)penem-3-carboxylate (**3**)

To a solution of **2** (447 mg, 0.90 mmol) in nitromethane (5 ml) was added a solution of 1-bromo-2-fluoroethane (171 mg, 1.35 mmol) in nitromethane (1 ml) and triethylamine (138 μl, 0.99 mmol) at 0~5°C under nitrogen. The mixture was stirred at room temperature for 3 days. The reaction mixture was concentrated *in vacuo* and the residue was extracted with methylene chloride. The extract was washed with satd NaCl and then dried over $MgSO_4$. After evaporation of the solvent, the residue was subjected to column chromatography on silica gel using a mixture of benzene - EtOAc (20: 1). The crude product was chromatographed on silica gel eluted with a mixture of hexane - EtOAc (5 : 1) to give **3** (110 mg, 23%) as an oil: IR (liquid film) $cm^{-1}$ 1790, 1680; NMR ($CDCl_3$) $\delta$ 0.90 (9H, s), 1.45 (3H, d, $J$=6.0 Hz), 3.30 (2H, dt, $J$=19, 6.0 Hz), 3.90 (1H, dd, $J$=10.0, 4.0 Hz), 4.20~4.60 (1H, m), 4.65 (2H, dt, $J$=47.0, 6.0 Hz), 5.20, 5.50 (2H, ABq, $J$=14.0 Hz), 5.70 (1H, d, $J$=4.0 Hz), 7.62, 8.23 (4H, $A_2B_2$, $J$=9.0 Hz).

*p*-Nitrobenzyl (5*R*,6*S*)-6-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-2-(2-fluoroethylthio)penem-3-carboxylate (**4**)

To a solution of **3** (110 mg, 0.20 mmol) in xylene (18 ml) was added hydroquinone (2.0 mg) and the mixture was heated at 135°C under nitrogen for 3 hours. After evaporation of the solvent the residue was purified by chromatography through a Lobar column (LiChroprep Si 60, size B) eluted with a mixture of benzene - EtOAc (25: 1) to give, in separate fractions, **3** (26 mg), and **4** (67 mg).

Recovered **3** (26 mg) was then treated in the same way as described above to give **4** (total yield, 82 mg, 75%): MP 118~119°C; IR ($CHCl_3$) $cm^{-1}$ 1790, 1690, 1605; NMR ($CDCl_3$) $\delta$ 0.09 (9H, s), 1.35 (3H, d, $J$=6.0 Hz), 3.32 (2H, dt, $J$=19.0, 6.0 Hz), 3.80 (1H, dt, $J$=4.0, 2.0 Hz), 4.00~4.60 (1H, m), 4.70 (2H, dt, $J$=47.0, 6.0 Hz), 5.25, 5.50 (2H, ABq, $J$=14.0 Hz), 5.72 (1H, d, $J$=2.0 Hz), 7.68, 8.24 (4H, $A_2B_2$, $J$=9.0 Hz).

*p*-Nitrobenzyl (5*R*,6*S*)-2-(2-Fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate (**5**)

To a solution of **4** (1.135 g, 2.09 mmol) in THF (30 ml) was added acetic acid (1.2 ml, 20.9 mmol) and 1 M tetrabutylammonium fluoride in THF (8.36 ml, 8.36 mmol) and the mixture was stirred at 25~30°C for 18 hours. The mixture was diluted with EtOAc and washed successively with aq NaCl, aq $NaHCO_3$ and aq NaCl, and dried over $MgSO_4$. After evaporation of the solvent the residue was treated with a small amount of EtOAc to give **5** (542 mg) as a powder. The filtrate was purified by chromatography through a Lobar column (LiChroprep Si 60, size B) eluted with a mixture of benzene - EtOAc (3: 2) to give additional amount of **5** (184 mg, total yield 81%): MP 168~170°C; IR (KBr) $cm^{-1}$ 3430, 1765, 1675, 1605; UV $\lambda_{max}^{EtOH}$ nm 261, 339; NMR (DMSO-$d_6$) $\delta$ 1.18 (3H, d, $J$=6.0 Hz), 2.90~3.70 (2H, m), 3.86 (1H, dd, $J$=6.0, 2.0 Hz), 3.80~4.15 (1H, m), 4.66 (2H, dt, $J$=47.0, 6.0 Hz), 5.19 (1H, d, $J$=4.0 Hz), 5.30, 5.48 (2H, ABq, $J$=14.0 Hz), 5.77 (1H, d, $J$=2.0 Hz), 7.72, 8.25 (4H, $A_2B_2$, $J$=9.0 Hz).

Sodium (5*R*,6*S*)-2-(2-Fluoroethylthio)-6-[(1*R*)-1-hydroxyethyl]penem-3-carboxylate (**1**)

A mixture of **5** (720 mg, 1.68 mmol) in a solution of THF (50 ml) and 0.1 M phosphate buffer (pH 7.1, 50 ml) was stirred with 10% Pd-C (1.44 g) for 2.5 hours under a $H_2$ atmosphere. After removal of the catalyst by filtration through celite, the filtrate was washed with EtOAc. The resulting aqueous layer was concentrated *in vacuo* to *ca.* 10 ml and chromatographed on a column of Diaion HP-20AG (Mitsubishi Chemical Industries Limited.). Fractions eluted with 5% aq acetone were concentrated *in vacuo* at 15~20°C and lyophilized to give **1** (477 mg, 90%) as a slightly hygroscopic powder: MP 159~161°C (dec); $[\alpha]_D^{25}$ +216.1° (*c* 1.0, $H_2O$); IR (KBr) $cm^{-1}$ 3425, 1765, 1600; UV $\lambda_{max}^{H_2O}$ nm ($\varepsilon$) 250.7 (5,906), 320.2 (7,456); NMR ($D_2O$) $\delta$ 1.30 (3H, d, $J$=6.0 Hz), 2.90~3.50 (2H, m), 3.90 (1H, dd, $J$= 6.0, 2.0 Hz), 4.00~4.45 (1H, m), 4.70 (2H, dt, $J$=47.0, 6.0 Hz), 5.69 (1H, d, $J$=2.0 Hz).

*Anal* Calcd for $C_{10}H_{11}NO_4S_2 \cdot H_2O$: C 36.61, H 3.65, N 4.21.
Found: C 36.03, H 3.93, N 4.20.

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