Carbapenem and Penem Antibiotics. VII. Synthesis and Antibacterial Activity of 1β-Methyl-2-(Quaternary Heteroaromatic-thiomethyl) Carbapenems[1)]

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The synthesis and antibacterial activity of a series of 1β-methylcarbapenems having quaternary heteroaromatic-thiomethyl groups at the C-2 position are described. Both 2-hydroxymethyl and 2-chloromethyl carbapenems (1 and 7) respectively served as the common key intermediates for the preparation of these compounds. Of these, the 4-pyridiniothiomethyl derivatives exhibited the best antibacterial properties and turned out to possess high *in vivo* efficacy as well.

Keywords β-lactam antibiotic; carbapenem antibiotic; 1β-methyl carbapenem antibiotic; 2-(quaternary heteroaromatic-thiomethyl) carbapenem antibiotic; antibacterial activity; methicillin-resistant *S. aureus* (MRSA)

In the preceding paper[1)] we described that the 1β-methyl 2-functionalized-methyl carbapenems, particularly 2-heteroaromatic-thiomethyl derivatives which are definitely distinguished from the well established 2-alkylthio carbapenems such as thienamycin family,[2)] exhibited high and well-balanced *in vitro* antibacterial activity except for *Pseudomonas aeruginosa*. The minimal inhibitory concentration (MIC) values of these compounds, however, were not reflected well in their protective effect on intraperitoneal infection in mice. In this respect, a similar tendency observed with some penem compounds has also been reported.[3)] To overcome this drawback, as well as to improve the activity against *P. aeruginosa*, we decided to continue our modification studies on 1β-methyl carbapenems by chemical manipulation at the C-2′ position.

In the field of cephalosporins, introduction of quaternary heterocyclic methyl groups at the C-3 position had led to a new class of cephalosporins such as ceftazidime[4)] and cefpirome,[5)] which are characterized by their potent activity against *P. aeruginosa*. Recently it has been reported that a successful application of this strategy for the 2-alkylthio carbapenems resulted in a high anti-pseudomonal activity.[6)] Since the 2-functionalized-methyl carbapenems might be regarded as a hybrid of the naturally occurring carbapenem and cephalosporin structures, we therefore became interested in the synthesis of a number of the title compounds to investigate the effect of quaternized heteroaromatic-thiomethyl groups on antibacterial properties.

Our synthetic route involves direct introduction of various heteroaromatic-thio groups into the C-2′ position of either 2-hydroxymethyl or 2-chloromethyl carbapenems (**1** and **7**) employed as the key intermediates.

Among these compounds prepared, the N-substituted 4-pyridinio-thiomethyl derivatives (**4a**, **b**, **c**, **e**, **k** and **4l**) exhibited the best balanced antibacterial spectrum. Furthermore, these derivatives were found to possess high *in vivo* activity against both gram-positive and gram-negative bacteria including *P. aeruginosa*. Of these, **4a** as a representative compound was subjected to pharmacokinetic investigations and proved to be more stable than imipenem in various kinds of kidney tissue homogenates, respectively. In this paper, we now describe details of the synthetic studies on this new class of carbapenems. Their antibacterial properties will also be discussed.

Chemistry Although the nature of a highly strained and reactive carbapenem nucleus requires that a minimum number of synthetic operations be performed on the carbapenem structure once it is formed, our synthetic strategy for the present modification studies was to introduce various C-2′ functional groups onto this constructed bicyclic system at a late stage of the synthesis. For this purpose, we employed the 2-hydroxymethyl carbapenem **1** as the key intermediate which can now be readily prepared in large quantity by our procedure reported in the preceding paper.[1)]

Since quaternization of pyridine ring nitrogen by a Menschutkin-type reaction[7)] has been well established, we first chose mercaptopyridines as reagents to react with the above compound **1**. As shown in Chart 1, incorporation of a variety of pyridylthio groups into the C-2′ position were readily carried out by either the Mitsunobu reaction[8)] or the analogous reaction[9)] in good yields, as exemplified by the synthesis of **2**.

Quaternary methylation at the pyridine nitrogen of the compound **2** and also in the case of the corresponding

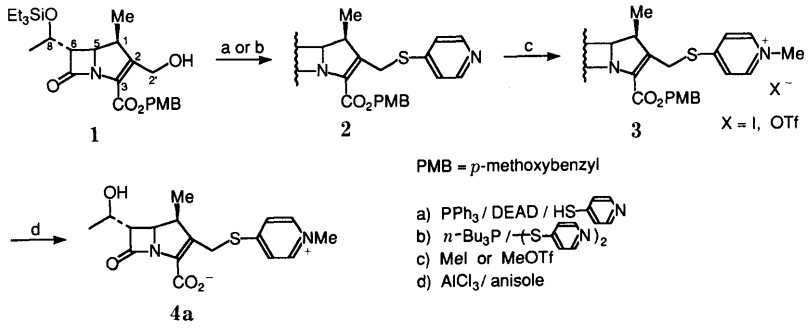


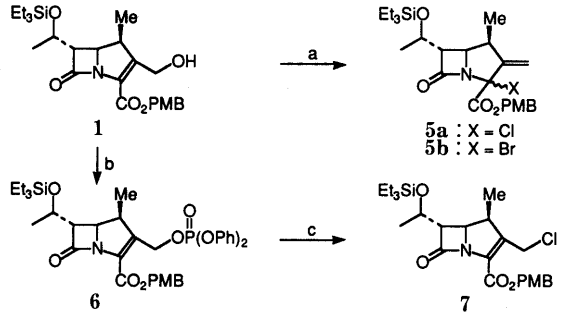
Chart 1

3-pyridyl (meta pyridine) ring was successfully accomplished by reaction with methyl iodide in acetonitrile at room temperature in almost quantitative yield. The same method, however, could not be applied to the 2-pyridyl (ortho pyridine ring) derivatives. This compound was found to be quaternized by treatment with methyl trifluoromethanesulfonate in CH_2Cl_2 under an ice-cooling condition. It should be noted that in both cases there was no detectable amount of product derived from alkylation at the sulfur atom of the C-2 side chains.

As far as the final deprotection step is concerned, the removal of the triethylsilyl (TES) group at the C-8 position, together with the *p*-methoxybenzyl (PMB) moiety, was successfully carried out by the conventional method using $AlCl_3$–anisole[10] to give the corresponding zwitterionic products as amorphous solids.

At this stage, the N-methyl-4-pyridinio-thiomethyl derivative **4a** thus obtained showed extremely potent overall activity as shown in Table I. Consequently, this derivative served as a lead compound and prompted us to explore the congeners in this area more extensively.

Therefore, we next attempted to examine the N-substituent effect on the activity of the pyridinio-thiomethyl derivatives. Quaternization of the pyridine moiety in such a carbapenem system by the Menschutkin-type reaction seemed to be limited, wherein only the simple alkyl halides as described above turned out to be applicable. This would in turn necessitate a more versatile strategy for introduction of the suitably functionalized substituents. Toward this end, our knowledge concerning cephalosporin chemistry[11] suggested that the reaction of the 2-chloromethyl carbapenem **7** with N-substituted thiopyridone would be one of the most efficient ways to generate the desired pyridinio-thiomethyl derivatives.



a) $SOCl_2$ / pyridine or PPh_3 / CCl_4 or $SOBr_2$ / pyridine
b) $ClPO(OPh)_2$ / DMAP
c) LiCl or Me_3SiCl

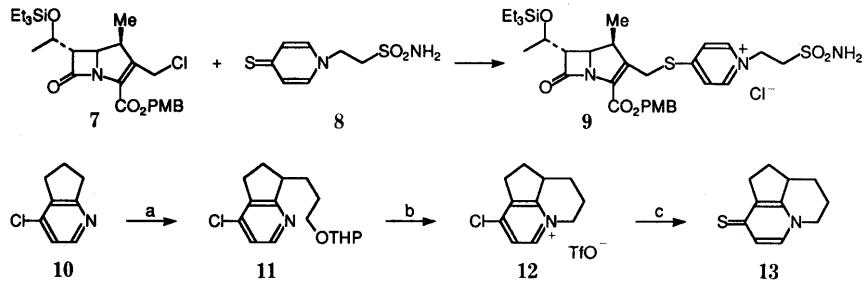
Chart 2

Chlorination of **1** by the ordinary method ($SOCl_2$/pyridine or PPh_3/CCl_4) as shown in Chart 2 gave rise to the undesired 3-chlorinated carbapenams **5a** in a regioselective manner. Similarly, bromination of **1** by the same kind of reaction ($SOBr_2$/pyridine) also afforded only the corresponding 3-brominated carbapenams **5b**. Our attempted elaborations to solve this problem finally resulted in the finding of an alternative route which involved the phosphonate intermediate **6**. Treatment of **1** with diphenylphosphoryl chloride in the presence of 4-dimethylaminopyridine at $-50\,°C$ generated the compound **6** as a pale yellow oil almost quantitatively. Subsequent treatment of this compound with either LiCl or Me_3SiCl in CH_2Cl_2 at room temperature gave rise to the desired 2-chloromethyl derivative **7** as a sole product in fairly good yield.[12] Although this compound was isolated as a relatively unstable form, it turned out that this ultimate intermediate could be utilized without purification to provide an entire series of target compounds. Thus, nucleophilic displacement of the chlorine by a variety of N-substituted thiopyridones such as **8** afforded the corresponding quaternized carbapenems in one step, as shown in Chart 3. According to this procedure, even bicyclic or tricyclic substrates such as **13** could be readily incorporated.

As for the synthesis of the unique tricyclic thiopyriridone **13**, we undertook an efficient synthetic route involving an intramolecular quaternization reaction of the trifluoromethane sulfonate intermediate (**11** to **12**) as depicted in Chart 3.

Finally, we investigated the antibacterial effect of a catechol moiety in the carbapenem molecule by synthesizing the compound **14**, since penicillin[13] and cephalosporin[14] derivatives containing a catechol residue are known to exhibit remarkable activity against *P. aeruginosa*. After several unsuccessful trials, we eventually synthesized the objective compound by the quaternization of **2** with 3,4-di-*p*-methoxybenzyloxybenzyl bromide, followed by the $AlCl_3$–anisole deblocking procedure under slightly acidic conditions (*ca.* pH 5), albeit in low yield. The derivative **14** displayed disappointingly reduced activity compared to the corresponding N-methyl compound **4a**, probably due to its chemical instability.

***In Vitro* Antibacterial Activity** The MIC values of these new carbapenems (Chart 5, 6 and 7) against selected strains of gram-positive and gram-negative bacteria are shown in Table I. As we expected, the positive charge in the quaternized heterocyclic ring resulted in significantly



a) 1) BuLi, 2) $ICH_2CH_2CH_2OTHP$
b) 1) TsOH, 2) Tf_2O / Hünig base
c) 1) NaSH

Chart 3

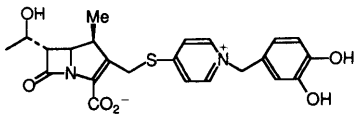


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Chart 4

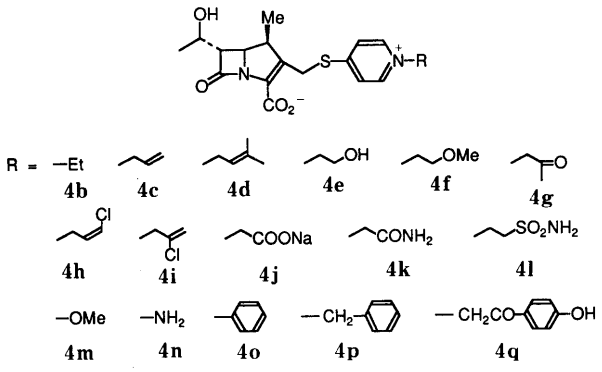

Chart 5

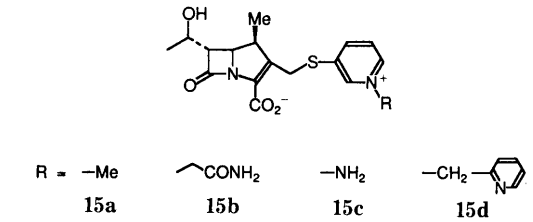

Chart 6

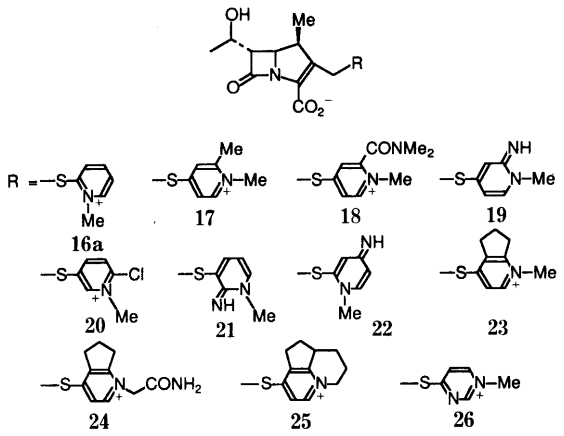

Chart 7

TABLE I. *In Vitro* Antibacterial Activity

Comp. No.	MIC (μg/ml)					
	S. aureus SR14	*S. pyogenes* C-203	*E. coli* EC-14	*K. pneumoniae* SR1	*P. vulgaris* CN-329	*S. marcescens* ATCC 13880
4a	<0.01	<0.003	0.1	0.1	0.2	0.2
4b	0.01	0.003	0.1	0.1	0.2	0.2
4c	0.01	<0.003	0.1	0.2	0.2	0.2
4d	0.01	<0.003	0.2	0.2	0.4	0.8
4e	0.02	<0.003	0.1	0.2	0.4	0.4
4f	0.02	<0.003	0.2	0.2	0.4	0.4
4g	0.02	0.006	0.1	0.2	0.4	0.8
4h	0.01	<0.003	0.1	0.2	0.2	0.4
4i	0.01	<0.003	0.2	0.2	0.2	0.4
4j	0.05	0.01	0.1	0.1	0.2	0.4
4k	0.02	0.006	0.1	0.2	0.4	0.4
4l	0.02	<0.003	0.1	0.1	0.2	0.2
4m	0.8	0.2	12.5	6.3	6.3	50
4n	0.01	<0.003	0.1	0.1	0.2	0.4
4o	0.01	<0.003	0.2	0.2	0.2	0.8
4p	0.01	<0.003	0.2	0.2	0.4	0.8
4q	0.02	0.006	0.4	0.4	0.4	3.1
15a	0.02	0.006	0.2	0.2	0.4	0.8
15b	0.05	0.006	0.2	0.4	0.4	0.8
15c	0.02	0.006	0.2	0.4	0.4	0.8
15d	0.02	<0.003	0.4	0.4	0.4	1.6
16a	0.8	0.4	3.1	12.5	12.5	25
17	0.02	0.006	0.1	0.2	0.4	0.4
18	0.05	0.006	0.2	0.4	0.8	0.8
19	0.01	<0.003	0.2	0.2	0.4	0.8
20	0.02	0.006	0.4	0.4	0.4	0.8
21	0.05	0.01	0.8	0.8	0.8	1.6
22	0.02	0.006	0.4	0.4	0.8	1.6
23	0.01	<0.003	0.8	0.4	0.4	6.3
24	0.02	<0.003	0.8	0.4	0.4	6.3
25	0.01	<0.003	6.3	1.6	3.1	25
26	0.1	0.01	0.1	0.1	0.1	0.4
Imipenem	0.025	0.013	0.1	0.2	0.78	0.39

TABLE II. *In Vitro* Antibacterial Activity

Compound No.	Mean MIC (μg/ml)			
	L-MRSA[a] (22 strains)	H-MRSA[b] (22 strains)	*E. faecalis* (22 strains)	*P. aeruginosa* (21 strains)
4a	0.067	7.5	0.24	12.0
4b	0.069	10.6	0.25	17.3
4c	0.063	8.8	0.25	17.3
4e	0.11	12.5	0.34	16.2
4k	0.098	11.7	0.27	11.7
4l	0.13	11.7	0.31	9.28
Imipenem	0.095	48.4	0.83	1.41

a) Low-resistance groups of methicillin-resistant *S. aureus*. *b*) High-resistance groups of methicillin-resistant *S. aureus*.

enhanced overall activity relative to their unquaternized partners.[1)]

The correlation between the structure of heterocyclic rings and their activity became clear. For example, additional substituents at various positions on the pyridine ring (Chart 7) had a negative effect on the activity in comparison with the parent derivatives (Chart 5 and 6), indicating that the electronic factor of the substituents seems to be less effective. With saturated rings fused to the pyridine moiety (**23**, **24** and **25**), the activity against gram-negative bacteria was reduced, thus implying that the steric bulkiness surrounding the cationic center and/or the increasing lipophilicity may reduce the permeability of the bacterial outer membrane. Introduction of an additional nitrogen atom on the pyridine ring tended to decrease the activity against gram-positive bacteria, as exemplified by the N-methyl pyrimidine derivative **26**.

Among the N-substituted pyridinio-thiomethyl series bearing no substituents on the ring, the 4-pyridinio (*para* pyridinium ring) derivatives (Chart 5) proved to be the most active, while the 3-pyridinio (*meta* pyridinium ring) groups (Chart 6) were a little weak and the 2-pyridinio (*ortho* pyridinium ring) one (**16a**) had considerably diminished activity. Thus, the position of the positive charge on the pyridine ring played an important role in the activity on the whole. It is significant to note that the effect of variation of the N-substituents in the 4-pyridinio series (Chart 5) did

not influence the activity to a great extent. The MIC values (not including *P. aeruginosa*) of all the compounds except for **4m** in this group were comparable to those of imipenem.

Although the anti-pseudomonal activity of the quaternized derivatives has been enhanced to some extent as compared with the corresponding unquaternized counterparts, none of the compounds reached the same level as that of imipenem.

On the other hand, several derivatives (**4a**, **b**, **c**, **e**, **k** and **4l**) in the 4-pyridinio-thiomethyl series (Chart 5) were found to possess beneficial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) which has recently been recognized as an increasing pathogen (Table II). Against low-resistance groups of methicillin-resistant *S. aureus* (L-MRSA),[15] these compounds showed about the same activity as that of imipenem in terms of the mean MIC values (22 strains). While imipenem showed considerably weak activity against high-resistance groups of methicillin-resistant *S. aureus* (H-MRSA), the above compounds exhibited mean MIC values of less than 12.5 μg/ml.

As another specific feature of these compounds, a fairly good activity against *E. faecalis* was demonstrated by the mean MIC values (22 strains) of less than 0.34 μg/ml.

***In Vivo* Activity** Because of the high levels of *in vitro* antibacterial activity against a wide variety of organisms, the above compounds were further examined for their *in vivo* activity. The ED_{50} values of these compounds in the mouse infection models against selected strains of gram-positive and gram-negative bacteria were determined and compared to those of imipenem, as shown in Table III. The *in vivo* activity of these compounds definitely reflected the corresponding *in vitro* antibacterial activity. Particularly against *P. aeruginosa*, these compounds exhibited much improved activity; thus, **4a** and **4k** became as active as imipenem on the basis of ED_{50} values (less than 1 mg/kg/dose). A good correlation between *in vitro* and *in vivo* activity of the quaternized heteroaromatic-thiomethyl derivatives was in marked contrast to the corresponding unquaternized compounds reported in the preceding paper.

TABLE III. Protective Effect on Intraperitoneal Infection in Mice

Compound No.	ED_{50} (mg/kg/dose)/MIC (μg/ml)			
	S. aureus SMITH	*S. pyogenes* C-203	*E. coli* EC-14	*P. aeruginosa* SR24
4a	0.0065/0.0125	0.012/<0.003	0.19/0.1	0.65/6.25
4b	0.0076/0.0125	— / 0.003	0.21/0.1	—/6.25
4c	0.011 /0.0125	0.016/<0.003	0.24/0.1	1.03/6.25
4e	0.009 /0.0125	0.016/<0.003	0.29/0.1	2.86/6.25
4k	0.0078/0.025	0.018/ 0.006	0.11/0.1	0.63/6.25
4l	0.009 /0.0125	0.016/<0.003	0.14/0.1	1.38/6.25
Imipenem	0.021 /0.0125	0.028/<0.013	0.36/0.1	0.98/1.56

ED_{50} was determined by survival rate 7 d after challenge.

TABLE IV. Stability in Kidney Tissue Homogenate

Compound No.	Remaining activity % in tissue homogenate[a]					
	Mouse	Rat	Rabbit	Dog	Monkey	Human
4a	54	43	46	59	46	70
Imipenem	37	32	33	36	33	54

a) Each compound was tested at a final concentration of 100 μg/ml and remaining activity % was determined after 1 h incubation at 37 °C.

Pharmacokinetic Properties of the Compound 4a As a representative of the above 6 compounds, **4a** was tested for stability in various kinds of kidney tissue homogenates respectively. In each species, this compound was found to be considerably more stable than imipenem (Table IV).

The urinary recovery of **4a** was next examined. In the case of mice (20 mg/kg, s.c.) and monkeys (10 mg/kg, i.v.), the average percentage of compound **4a** excreted for 24 h after dosing was 67% and 65%, respectively. These results were much better than those of imipenem (whose recovery values were in the range of about 25—30%) reflecting the above observed intrinsic stability of **4a** to mouse and monkey kidney enzyme preparations.

Experimental

General Procedures All reactions involving air-sensitive reactants or products were carried out under a nitrogen atmosphere using dry solvents. Infrared (IR) spectra were recorded on a Hitachi 260-10 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Varian EM-390 (90 MHz) and VXR-200 (200 MHz). Unless otherwise stated, chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal (in organic solvent) or external (in D_2O) standard. Ultraviolet (UV) spectra were taken on a Hitachi EPS-3T spectrometer. Specific optical rotations ($[\alpha]_D$) were taken at 25 °C on a Perkin–Elmer 241 Polarimeter. Mass spectra (MS) were obtained on a Hitachi M-90 (SIMS) mass spectrometer. Medium pressure liquid chromatographies were performed on Merck 'Lobar' prepacked columns packed with LiChroprep Si 60; size A (240—10 mm, 40—60 μm), size B (310—25 mm, 40—60 μm) and size C (440—37 mm, 63—125 μm). Most of the new compounds reported here are either non-crystalline solids or forms, or unstable. Hence, their analytical data could not be obtained.

***p*-Methoxybenzyl (1*S*,5*R*,6*S*)-2-Hydroxymethyl-1-methyl-6-[(*R*)-1-triethylsilyloxyethyl]carbapen-2-em-3-carboxylate (1)** The title compound was prepared by essentially the same method described in the preceding paper.

***p*-Methoxybenzyl (1*S*,5*R*,6*S*)-1-Methyl-2-(4-pyridyl)thiomethyl-6-[(*R*)-1-triethylsilyloxyethyl]carbapen-2-em-3-carboxylate (2)** Method I: To a solution of **1** (476 mg, 1 mmol), triphenylphosphine (315 mg, 1.2 eq) and 4-mercaptopyridine (133 mg, 1.2 eq) in tetrahydrofuran (THF, 10 ml) was added diethyl azodicarboxylate (209 mg, 1.2 eq), and the mixture was stirred at room temperature for 1 h. After the usual work up, the residue was chromatographed on a Lobar column (size B, toluene–EtOAc 1 : 2) to give **2** (410 mg, 72%). IR ($CHCl_3$) cm^{-1}: 1780, 1615. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.52—0.68 (6H, m), 0.85—1.00 (9H, m), 1.15 (3H, d, J=8.7 Hz), 1.23 (3H, d, J=6 Hz), 3.18 (1H, dd, J=2.2, 6 Hz), 3.09—3.30 (1H, m), 3.46 and 4.91 (2H, ABq, J=14 Hz), 3.78 (3H, s), 4.09 (1H, dd, J=2.2, 13 Hz), 4.10—4.30 (1H, m), 5.21 (2H, s), 6.85 and 7.30 (4H, ABq, J=9 Hz), 7.06 and 8.30 (4H, ABq, J=6 Hz).

Method II: A solution of **1** (476 mg, 1 mmol), tributylphosphine (223 mg, 1.1 eq) and 4,4′-dipyridyl disulfide (242 mg, 1.1 eq) in CH_2Cl_2 (10 ml) was stirred at room temperature for 1 h. The reaction mixture was washed with aqueous $NaHCO_3$ solution, dried, concentrated and chromatographed as described above to give **2** (444 mg, 78%).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-methyl-4-pyridinio)-thiomethylcarbapen-2-em-3-carboxylate (4a) and Related Compounds A solution of **2** (569 mg, 1 mmol) and iodomethane (1.42 g, 10 eq) in acetonitrile (10 ml) was stirred at room temperature for 2 h and then concentrated to give **3** (710 mg, 100%). The PMB ester **3** (710 mg, 1 mmol) was added to a solution of $AlCl_3$ (400 mg, 3 eq) in a mixture of anisole (7 ml) and CH_2Cl_2 (4 ml) at −40 °C, and the mixture was stirred for 1 h at the same temperature. A solution of $NaHCO_3$ (336 mg, 4 eq) in water (5 ml) and CH_2Cl_2 (20 ml) were added, and the reaction mixture was stirred under ice cooling for 10 min and filtered. The aqueous filtrate was chromatographed on an HP-20AG column (20 × 250 mm, H_2O) and the fractions containing the product were concentrated and freeze-dried to give **4a** as a yellow powder (233 mg, 67%). ^{1}H-NMR (90 MHz, D_2O) δ: 1.58 (3H, d, J=7 Hz), 1.65 (3H, d, J=6 Hz), 3.55—3.70 (1H, m), 3.85 (1H, dd, J=2, 6 Hz), 4.30 and 5.48 (2H, ABq, J=14 Hz), 4.35—4.75 (2H, m), 4.62 (3H, s), 8.20 and 8.75 (4H, A_2B_2q, J=8 Hz). UV (H_2O) nm: 231,

305. MS (SIMS, glycerol) *m/z*: 349 $(M+H)^+$, 697 $(2M+H)^+$. *Anal.* Calcd for $C_{17}H_{20}N_2O_4S \cdot 2H_2O$: C, 53.11; H, 6.29; N, 7.29; S, 8.34. Found: C, 52.94; H, 6.16; N, 7.50; S, 8.31.

The following compounds were prepared by the same or a slightly modified procedure.

(1*S*,5*R*,6*S*)-2-(1-Ethyl-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4b) **4b**: ^{1}H-NMR (90 MHz, D_2O) δ: 1.62 (3H, d, J=7 Hz), 1.73 (3H, d, J=6 Hz), 2.02 (3H, t, J=8 Hz), 3.70—3.80 (1H, m), 3.88 (1H, dd, J=2, 6 Hz), 4.30 and 5.52 (2H, ABq, J=14 Hz), 4.50—4.90 (2H, m), 4.80 (2H, q, J=8 Hz), 8.25 and 8.95 (4H, A_2B_2q, J=8 Hz). UV (H_2O) nm: 232, 305.

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[1-(prop-2-enyl)-4-pyridinio]thiomethylcarbapen-2-em-3-carboxylate (4c) **4c**: ^{1}H-NMR (90 MHz, D_2O) δ: 1.55 (3H, d, J=7 Hz), 1.70 (3H, d, J=6 Hz), 3.70—3.90 (2H, m), 4.30 and 5.62 (2H, ABq, J=14 Hz), 4.40—4.80 (2H, m), 5.50 (1H, d, J=6 Hz), 5.91 (1H, m), 8.25 and 8.90 (4H, A_2B_2q, J=8 Hz). UV (H_2O) nm: 230, 307. MS (SIMS, glycerol) *m/z*: 375 $(M+H)^+$, 749 $(2M+H)^+$. *Anal.* Calcd for $C_{19}H_{22}N_2O_4S \cdot H_2O$: C, 58.15; H, 6.16; N, 7.14; S, 8.17. Found: C, 58.40; H, 6.27; N, 7.26; S, 8.09.

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[1-(3-methylbut-2-enyl)-4-pyridinio]thiomethylcarbapen-2-em-3-carboxylate (4d) **4d**: ^{1}H-NMR (200 MHz, D_2O, sodium 4,4-dimethyl-4-silapentanesulfonate (DSS) as standard) δ: 1.16 (3H, d, J=7.4 Hz), 1.25 (3H, d, J=6.4 Hz), 1.82 (3H, s), 1.84 (3H, s), 3.29—3.38 (1H, m), 3.42 (1H, dd, J=2.8, 6 Hz), 3.85 and 5.07 (2H, ABq, J=15 Hz), 4.12—4.28 (1H, m), 4.99—5.10 (2H, m), 5.43—5.51 (1H, m), 7.75 and 8.40 (4H, A_2B_2q, J=7.2 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[1-(2-hydroxy)ethyl-4-pyridinio]-thiomethyl-1-methylcarbapen-2-em-3-carboxylate (4e) **4e**: ^{1}H-NMR (200 MHz, D_2O) δ: 0.98 (3H, d, J=7.4 Hz), 1.07 (3H, d, J=6.4 Hz), 3.10—3.25 (1H, m), 3.27 (1H, dd, J=2, 6 Hz), 3.69 and 4.90 (2H, ABq, J=14 Hz), 3.80—3.90 (2H, m), 3.92 (1H, dd, J=2, 9 Hz), 3.95—4.05 (1H, m), 4.30—4.40 (2H, m), 7.62 and 8.27 (4H, A_2B_2q, J=7.2 Hz). MS (SIMS, glycerol) *m/z*: 379 $(M+H)^+$, 757 $(2M+H)^+$.

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[1-(2-methoxy)ethyl-4-pyridinio]-thiomethyl-1-methylcarbapen-2-em-3-carboxylate (4f) **4f**: IR (KBr) cm^{-1}: 3450, 1749, 1630, 1592, 1546, 1104. ^{1}H-NMR (200 MHz, D_2O, DSS as standard) δ: 1.17 (3H, d, J=7.2 Hz), 1.26 (3H, d, J=6.4 Hz), 3.22—3.40 (1H, m), 3.43 (1H, dd, J=3, 6 Hz), 3.35 (3H, s), 3.87 and 5.08 (2H, ABq, J=15 Hz), 3.81—3.91 (2H, m), 4.07 (1H, dd, J=3, 10 Hz), 4.17—4.24 (1H, m), 4.58—4.62 (2H, m), 7.79 and 8.44 (4H, A_2B_2q, J=7 Hz). UV (H_2O) nm: 230.5, 307.

(1*S*,5*R*,6*S*)-2-(1-Acetylmethyl-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4g) **4g**: ^{1}H-NMR (200 MHz, D_2O, DSS as standard) δ: 1.49 (3H, d, J=7.4 Hz), 1.58 (3H, d, J=6.4 Hz), 2.70 (3H, s), 3.6—3.8 (1H, m), 3.76 (1H, dd, J=3, 6 Hz), 4.21 and 5.41 (2H, ABq, J=13 Hz), 4.40 (1H, dd, J=3, 10 Hz), 4.5—4.6 (1H, m), 4.17—4.24 (1H, m), 5.88 (2H, s), 8.14 and 8.56 (4H, A_2B_2q, J=7.4 Hz).

(1*S*,5*R*,6*S*)-2-[1-(3-Chloroprop-2-enyl)-4-pyridinio]thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4h) **4h**: ^{1}H-NMR (200 MHz, D_2O, DSS as standard) δ: 1.16 (3H, d, J=7.2 Hz), 1.25 (3H, d, J=6.2 Hz), 3.26—3.38 (1H, m), 3.43 (1H, dd, J=2.4, 6 Hz), 3.87 and 5.08 (2H, ABq, J=15 Hz), 4.07 (1H, dd, J=2.4, 10 Hz), 4.14—4.27 (1H, m), 5.22—5.28 (2H, m), 6.16—6.28 (1H, m), 6.64—6.76 (1H, m), 7.79 and 8.44 (4H, A_2B_2q, J=6.8 Hz).

(1*S*,5*R*,6*S*)-2-[1-(2-Chloroprop-2-enyl)-4-pyridinio]thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4i) **4i**: ^{1}H-NMR (200 MHz, D_2O, DSS as standard) δ: 1.17 (3H, d, J=7.4 Hz), 1.26 (3H, d, J=6.4 Hz), 3.27—3.39 (1H, m), 3.43 (1H, dd, J=2.8, 6 Hz), 3.90 and 5.10 (2H, ABq, J=14.8 Hz), 4.08 (1H, dd, J=2.8, 9.8 Hz), 4.18—4.28 (1H, m), 5.26 (2H, s), 5.72 (1H, d, J=2.4 Hz), 5.83 (1H, d, J=2.4 Hz), 7.84 and 8.50 (4H, A_2B_2q, J=7.2 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-sodiumcarboxymethyl-4-pyridinio)thiomethylcarbapen-2-em-3-carboxylate (4j) **4j**: ^{1}H-NMR (200 MHz, D_2O) δ: 1.00 (3H, d, J=7.0 Hz), 1.09 (3H, d, J=6.6 Hz), 3.1—3.3 (1H, m), 3.27 (1H, dd, J=2.8 and 5.8 Hz), 3.73 and 4.92 (2H, ABq, J=15 Hz), 3.9—4.2 (1H, m), 3.92 (1H, dd, J=2.8 and 8.8 Hz), 4.17—4.24 (1H, m), 4.86 (2H, s), 7.63 and 8.20 (4H, A_2B_2q, J=7 Hz).

(1*S*,5*R*,6*S*)-2-(1-Carbamoylmethyl-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4k) **4k**: ^{1}H-NMR (200 MHz, D_2O) δ: 0.98 (3H, d, J=7.2 Hz), 1.07 (3H, d, J=6.4 Hz), 3.1—3.2 (1H, m), 3.24 (1H, dd, J=3, 6 Hz), 3.71 and 4.90 (2H, ABq, J=14.2 Hz), 3.88 (1H, dd, J=2.8, 8.8 Hz), 3.9—4.1 (1H, m), 5.12 (2H, s), 7.64 and 8.20 (4H, A_2B_2q, J=6.6 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-methoxyl-4-pyridinio)thiomethylcarbapen-2-em-3-carboxylate (4m) **4m**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 1.16 (3H, d, J=7.4 Hz), 1.24 (3H, d, J=6.3 Hz), 3.10—3.30 (1H, m), 3.24 (1H, dd, J=2.9, 7.1 Hz), 3.72 (1H, d, J=15 Hz), 3.89 (1H, dd, J=2.8, 9.8 Hz), 3.90—4.10 (1H, m), 4.15 (3H, s), 4.62 (DHO), 4.89 (1H, d, J=15 Hz), 7.62 (2H, d, J=7.2 Hz), 8.59 (2H, d, J=7.2 Hz).

(1*S*,5*R*,6*S*)-2-(1-Benzyl-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4p) **4p**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 0.94 (3H, d, J=7.4 Hz), 1.05 (3H, d, J=6.2 Hz), 3.05—3.19 (1H, m), 3.22 (1H, dd, J=2.8, 6 Hz), 3.64 and 4.85 (2H, ABq, J=14.6 Hz), 3.84 (1H, dd, J=2.8, 10 Hz), 3.93—4.25 (1H, m), 4.62 (DHO), 5.39 (2H, s), 7.24 (5H, s), 7.56 and 8.31 (4H, A_2B_2q, J=7.2 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-methyl-3-pyridinio)-thiomethylcarbapen-2-em-3-carboxylate (15a) **15a**: IR (KBr) cm^{-1}: 3400, 1750, 1595, 1500, 1282, 1255. ^{1}H-NMR (200 MHz, D_2O) δ: 0.96 (3H, d, J=7.4 Hz), 1.08 (3H, d, J=6.4 Hz), 3.1—3.3 (1H, m), 3.22 (1H, dd, J=2.6, 6 Hz), 3.46 and 4.69 (2H, ABq, J=14 Hz), 3.87 (1H, dd, J=2.6, 9.8 Hz), 3.9—4.1 (1H, m), 4.14 (2H, s), 7.72 (1H, dd, J=6, 8.4 Hz), 8.25 (1H, d, J=8.4 Hz), 8.43 (1H, d, J=6.0 Hz), 8.59 (1H, s). MS (SIMS, glycerol) *m/z*: 349 $(M+H)^+$, 697 $(2M+H)^+$. *Anal.* Calcd for $C_{17}H_{20}N_2O_4S \cdot 0.7H_2O$: C, 56.56; H, 5.97; N, 7.76; S, 8.88. Found: C, 56.84; H, 6.23; N, 7.96; S, 8.75.

(1*S*,5*R*,6*S*)-2-(1-Carbamoylmethyl-3-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (15b) **15b**: ^{1}H-NMR (200 MHz, D_2O, DSS as standard) δ: 1.13 (3H, d, J=7.4 Hz), 1.25 (3H, d, J=6.4 Hz), 3.26—3.40 (2H, m), 4.03 (1H, dd, J=2.9, 9.6 Hz), 4.11—4.24 (1H, m), 3.69 and 4.92 (2H, ABq, J=14.8 Hz), 5.43 (2H, s), 7.98 (1H, dd, J=6, 8.4 Hz), 8.52 (1H, dd, J=6 Hz), 8.63 (1H, d, J=8.4 Hz), 8.85 (1H, s).

(1*S*,5*R*,6*S*)-2-(1-Amino-3-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (15c) **15c**: ^{1}H-NMR (200 MHz, D_2O, DSS as standard) δ: 1.14 (3H, d, J=7.4 Hz), 1.26 (3H, d, J=6.4 Hz), 3.23—3.47 (1H, m), 3.42 (1H, dd, J=3, 6.4 Hz), 3.64 and 4.84 (2H, ABq, J=14 Hz), 4.08 (1H, dd, J=3, 9.8 Hz), 4.17—4.23 (1H, m), 7.85 (1H, dd, J=6.4, 8 Hz), 8.26 (1H, d, J=8.4 Hz), 8.57 (1H, dd, J=2 Hz), 8.72 (1H, s).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[1-(2-pyridyl)methyl-3-pyridinio]thiomethylcarbapen-2-em-3-carboxylate (15d) **15d**: ^{1}H-NMR (200 MHz, D_2O, DSS as standard) δ: 1.10 (3H, d, J=7.2 Hz), 1.25 (3H, d, J=6.2 Hz), 3.27—3.38 (2H, m), 3.66 and 4.94 (2H, ABq, J=14.7 Hz), 3.90 (1H, dd, J=2.8, 9.8 Hz), 4.15—4.21 (1H, m), 5.88 (2H, s), 7.45—7.59 (2H, m), 7.93—8.01 (2H, m), 8.48—8.52 (2H, m), 8.76 (1H, d, J=5.8 Hz), 8.91 (1H, m).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-methyl-6-chloro-3-pyridinio)thiomethylcarbapen-2-em-3-carboxylate (20) **20**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 0.97 (3H, d, J=7.2 Hz), 1.07 (3H, d, J=6.2 Hz), 3.12—3.19 (1H, m), 3.24 (1H, dd, J=2.8, 6.2 Hz), 3.51 and 4.76 (2H, ABq, J=14.5 Hz), 3.87 (1H, dd, J=2.8, 9.8 Hz), 3.95—4.08 (1H, m), 4.14 (3H, s), 4.61 (DHO), 8.33 (1H, s), 8.57 (1H, s), 8.63 (1H, s).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-(2-imino-1-methyl-1,2-dihydropyridin-3-yl)thiomethyl-1-methylcarbapen-2-em-3-carboxylate (21) **21**: IR (KBr) cm^{-1}: 3400, 1748, 1645, 1585, 1515, 1240. ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 1.11 (3H, d, J=7.2 Hz), 1.27 (3H, d, J=6.3 Hz), 3.30—3.47 (1H, m), 3.33 and 4.49 (2H, ABq, J=13.4 Hz), 3.38 (1H, dd, J=2.8, 6 Hz), 3.84 (3H, s), 4.13 (1H, dd, J=2.8, 9.9 Hz), 4.15—4.28 (1H, m), 4.80 (DHO), 6.83 (1H, t, J=7 Hz), 7.87 (1H, dd, J=1.4, 6.6 Hz), 7.99 (1H, dd, J=1.4, 7.4 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-(4-imino-1-methyl-1,4-dihydropyridin-2-yl)thiomethyl-1-methylcarbapen-2-em-3-carboxylate (22) **22**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 0.98 (3H, d, J=7.2 Hz), 1.09 (3H, d, J=6.2 Hz), 3.12—3.21 (1H, m), 3.23 (1H, dd, J=3, 6.2 Hz), 3.35—3.49 (1H, m), 3.58 and 4.68 (2H, ABq, J=14.6 Hz), 3.70 (3H, s), 3.92 (1H, dd, J=3, 10 Hz), 4.00—4.10 (1H, m), 6.47 (1H, dd, J=2.6, 7.2 Hz), 4.62 (DHO), 6.56 (1H, d, J=2.6 Hz), 7.72 (1H, d, J=7.2 Hz).

(1*S*,5*R*,6*S*)-2-(1-Amino-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4n) **4n**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 1.15 (3H, d, J=7.4 Hz), 1.25 (3H, d, J=6.3 Hz), 3.20—3.50 (1H, m), 3.45 (1H, dd, J=2.9, 7.1 Hz), 3.83 (1H, d, J=15.2 Hz), 4.06 (1H, dd, J=2.9, 9.9 Hz), 4.20 (1H, quintet, J=6.3 Hz), 4.81 (DHO), 5.06 (1H, d, J=15 Hz), 7.74 (2H, d, J=7.3 Hz), 8.41 (2H, d, J=7.3 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-methyl-2-pyridinio)-thiomethylcarbapen-2-em-3-carboxylate (16a) **16a**: ^{1}H-NMR (200 MHz, D_2O) δ: 0.95 (3H, d, J=7.2 Hz), 1.11 (3H, d, J=6.4 Hz), 3.02—3.15 (1H, m), 3.24 (1H, dd, J=2, 6 Hz), 3.47 and 4.70 (2H, ABq, J=14 Hz), 3.80 (3H, s), 3.89 (1H, dd, J=3, 9.6 Hz), 3.9—4.2 (1H, m), 7.70—8.70 (4H, m).

(1*S*,5*R*,6*R*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-methyl-4-pyrimidinio)thiomethylcarbapen-2-em-3-carboxylate (26) **26**: ^{1}H-NMR

(200 MHz, D_2O) δ: 0.96 (3H, d, J=7.2 Hz), 1.06 (3H, d, J=6.4 Hz), 3.05—3.14 (1H, m), 3.25 (1H, dd, J=2, 6 Hz), 3.78 and 4.80 (2H, ABq, J=14.2 Hz), 3.90 (1H, dd, J=2, 9 Hz), 3.94 (3H, s), 3.98—4.04 (1H, m), 7.67 (1H, d, J=7 Hz), 8.32 (1H, d, J=7 Hz), 8.90 (1H, s).

(1*S*,5*R*,6*S*)-2-[1-(3′,4′-Dihydroxy)benzyl-4-pyridinio]thiomethyl-6-[(*R*)-1-Hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (14) A solution of **2** (156 mg, 0.34 mmol) and 3,4-di-*p*-methoxybenzyloxybenzyl bromide (302 mg, 2 eq) in acetonitrile (5 ml) was stirred at room temperature for 12 h and then condensed to give a pyridinium salt which was washed with diethyl ether (284 mg, 92%). IR ($CHCl_3$) cm^{-1}: 2950, 1780, 1720, 1615. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.53—0.67 (6H, m), 0.85—1.00 (9H, m), 1.17 (3H, d, J=8.7 Hz), 1.23 (3H, d, J=6 Hz), 3.10 (1H, dd, J=2.2, 6 Hz), 3.15—3.30 (1H, m), 3.50 and 4.80 (2H, ABq, J=14 Hz), 3.75 (3H, s), 3.77 (3H, s), 3.80 (3H, s), 4.10 (1H, dd, J=2.2, 13 Hz), 4.15—4.30 (1H, m), 4.95—5.10 (4H, m), 5.20 (2H, s), 5.95 (2H, s), 6.80—7.45 (15H, m), 7.65—9.20 (4H, m).

The pyridinium salt (280 mg, 0.31 mmol) was added to a solution of $AlCl_3$ (186 mg, 4.5 eq) in a mixture of anisole (4 ml) and CH_2Cl_2 (3 ml) at −40 °C, and the mixture was stirred for 1 h at the same temperature. The mixture was poured into water and stirred under ice cooling for 10 min and filtered. The aqueous filtrate was chromatographed on an HP-20AG column (20 × 250 mm, H_2O) and the fractions containing the product were concentrated and freeze-dried to give **14** as yellow powder (13 mg, 9%). ^{1}H-NMR (90 MHz, D_2O) δ: 1.07 (3H, d, J=7.2 Hz), 1.12 (3H, d, J=6.4 Hz), 3.25—3.31 (1H, m), 3.45 (1H, dd, J=2, 6 Hz), 3.90 and 5.05 (2H, ABq, J=14 Hz), 4.00—4.18 (2H, m), 4.70 (2H, s), 6.80—8.50 (7H, m).

***p*-Methoxybenzyl (1*S*,5*R*,6*S*)-2-Diphenylphosphorylmethyl-1-methyl-6-[(*R*)-1-triethylsilyloxyethyl]carbapen-2-em-3-carboxylate (6)** To solution **1** (220 mg, 0.46 mmol) and 4-dimethylaminopyridine (62 mg, 1.1 eq) in CH_2Cl_2 (2 ml) was added chlorodiphenylphosphate (0.1 ml, 1.05 eq) at −50 °C, and the mixture was stirred for 30 min at the same temperature. The reaction mixture was washed with aqueous $NaHCO_3$ solution, 0.1 N HCl and water, and dried and concentrated to give **6** as a pale yellow oil (307 mg, 92%). IR ($CHCl_3$) cm^{-1}: 2900, 1770, 1700. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.53—0.68 (6H, m), 0.86—1.01 (9H, m), 1.18 (3H, d, J=7.2 Hz), 1.29 (3H, d, J=6.5 Hz), 3.05—3.31 (2H, m), 3.80 (3H, s), 4.00—4.30 (2H, m), 4.80 and 5.08 (2H, ABq, J=14 Hz), 5.18 (2H, s), 6.86 and 7.38 (4H, A_2B_2q, J=6.8 Hz), 6.90—7.50 (10H, m).

***p*-Methoxybenzyl (1*S*,5*R*,6*S*)-2-Chloromethyl-1-methyl-6-[(*R*)-1-trimethylsilyloxyethyl]carbapen-2-em-3-carboxylate (7)** To a solution of **6** (300 mg, 0.41 mmol) in CH_2Cl_2 (2 ml) was added LiCl (37 mg, 2 eq), followed by stirring at room temperature for 1 h. The reaction mixture was washed with aqueous $NaHCO_3$ solution. 0.1 N HCl and water, and dried and concentrated to give **7** as a colorless oil (178 mg, 78%). IR($CHCl_3$) cm^{-1}: 2950, 1770, 1620. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.54—0.65 (6H, m), 0.90—1.01 (9H, m), 1.02 (3H, d, J=7.2 Hz), 1.22 (3H, d, J=6.4 Hz), 2.71—2.82 (1H, m), 2.85—3.05 (1H, m), 3.80 (3H, s), 4.20—4.42 (2H, m), 5.15 and 5.40 (2H, ABq, J=14 Hz), 5.23 (2H, s), 6.87 and 7.39 (4H, A_2B_2q, J=6.8 Hz).

As for the direct chlorination of **1**, the following results were obtained. To a solution of **1** (128 mg, 0.27 mmol) and pyridine (0.052 ml, 2 eq) in THF (2 ml) at −30 °C was added thionyl chloride (0.082 ml, 1.2 eq). The mixture was stirred for 1 h at the same temperature. The reaction mixture was diluted with EtOAc (10 ml), washed with aqueous $NaHCO_3$ solution and water, and dried, concentrated and chromatographed on a Lobar column (size A, toluene–EtOAc 4 : 1) to give the 3-chloro-carbapenam **5a** as a 3 : 2 epimeric mixture (yellow oil, 92 mg, 68%). IR ($CHCl_3$) cm^{-1} of the mixture of two epimers: 1765, 1720, 1605. ^{1}H-NMR (200 MHz, $CDCl_3$) of the main epimer δ: 0.52—0.70 (6H, m), 0.85—1.00 (9H, m), 1.21 (3H, d, J=7.2 Hz), 1.36 (3H, d, J=7 Hz), 2.80—3.30 (2H, m), 3.80 (3H, s), 4.05—4.40 (2H, m), 5.20 (2H, s), 5.40 and 5.82 (2H, dd, J=2.5 Hz, vinyl H), 6.87 and 7.40 (4H, A_2B_2q, J=6.8 Hz).

When **1** (60 mg, 0.13 mmol) was treated with triphenylphosphine (80 mg, 2 eq) and CCl_4 (0.15 ml, 10 eq) in acetonitrile (2 ml) at room temperature, in this case also, only **5a** (3 : 2 epimeric mixture, 70%) was obtained.

Similarly, bromination of **1** (190 mg, 0.40 mmol) using thionyl bromide (0.041 ml, 1.2 eq) and pyridine (0.071 ml, 2 eq) afforded the 3-bromo-carbapenam **5b** as a 3 : 1 epimeric mixture (brownish oil, 138 mg, 62%). ^{1}H-NMR (200 MHz, $CDCl_3$) of the main epimer δ: 0.52—0.70 (6H, m), 0.85—1.00 (9H, m), 1.23 (3H, d, J=7.2 Hz), 1.38 (3H, d, J=7.0 Hz), 2.80—3.30 (2H, m), 3.80 (3H, s), 4.03—4.44 (2H, m), 5.20 (2H, s), 5.40 and 5.84 (2H, dd, J=2.5 Hz, vinyl H), 6.88 and 7.40 (4H, A_2B_2q, J=6.8 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-sulfamoylethyl-4-pyridinio)thiomethylcarbapen-2-em-3-carboxylate (41) and Related Compounds To a solution of **7** (256 mg, 0.5 mmol) in acetonitrile (2 ml) was added **8** (131 mg, 1.2 eq), and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with EtOAc (10 ml), washed with water, dried and concentrated to give tyhe pyridinio-thiomethyl derivative **9** (325 mg, 89%) which was converted to the titled compound by the $AlCl_3$–anisole method. **41**: ^{1}H-NMR (200 MHz, D_2O) δ: 1.15 (3H, d, J=7.4 Hz), 1.25 (3H, d, J=6.4 Hz), 3.37 (1H, m), 3.44 (1H, dd, J=2, 6 Hz), 3.80—3.95 (2H, m), 4.07 (1H, dd, J=2.4, 9 Hz), 4.20 (1H, m), 3.90 and 5.02 (2H, ABq, J=14 Hz), 4.85—4.95 (2H, m), 7.80 and 8.53 (4H, A_2B_2q, J=8 Hz).

The following compounds were also prepared by the same method.

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-(1-phenyl-4-pyridinio)-thiomethylcarbapen-2-em-3-carboxylate (4o) **4o**: ^{1}H-NMR (200 MHz, D_2O) δ: 0.99 (3H, d, J=7.2 Hz), 1.08 (3H, d, J=6.4 Hz), 3.12—3.20 (1H, m), 3.27 (1H, dd, J=2, 6 Hz), 3.78 and 4.96 (2H, ABq, J=14 Hz), 3.92 (1H, dd, J=2, 9 Hz), 4.01—4.10 (1H, m), 6.98—7.51 (5H, m), 7.76 and 8.51 (4H, A_2B_2q, J=7 Hz).

(1*S*,5*R*,6*S*)-2-[1-(4-Hydroxy)benzoylmethyl-4-pyridinio]thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (4q) **4q**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 1.05 (3H, d, J=7.0 Hz), 1.13 (3H, d, J=6.5 Hz), 3.10—3.30 (1H, m), 3.25 (1H, dd, J=2.8, 7.2 Hz), 3.65 (1H, d, J=14.8 Hz), 3.95 (1H, dd, J=2.8, 10 Hz), 4.00—4.20 (1H, m), 4.60—4.90 (3H, m), 4.61 (DHO), 6.50—6.60 (2H, m), 7.60—7.80 (4H, m), 8.10—8.20 (2H, m).

(1*S*,5*R*,6*S*)-2-(1,2-Dimethyl-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (17) **17**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 0.96 (3H, d, J=7.4 Hz), 1.07 (3H, d, J=6.3 Hz), 2.45 (3H, s), 3.00—3.30 (1H, m), 3.22 (1H, dd, J=3.2, 5.2 Hz), 3.60 (1H, d, J=15 Hz), 3.86 (1H, dd, J=3.2, 9 Hz), 4.01 (1H, quintet, J=6.4 Hz), 4.61 (DHO), 4.88 (1H, d, J=15 Hz), 7.30—7.50 (2H, m), 8.10 (1H, d, J=6.8 Hz).

(1*S*,5*R*,6*S*)-2-(2-Dimethylcarbamoyl-1-methyl-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (18) **18**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 0.97 (3H, d, J=7.2 Hz), 1.06 (3H, d, J=6.2 Hz), 2.79 (3H, s), 2.98 (3H, s), 3.00—3.30 (1H, m), 3.21 (1H, dd, J=2.8, 6.1 Hz), 3.68 (1H, d, J=15 Hz), 3.50—3.70 (1H, m), 3.87 (3H, s), 4.00 (1H, quintet, J=6.1 Hz), 4.61 (DHO), 4.99 (1H, d, J=15 Hz), 7.60—7.80 (2H, m), 8.26 (1H, d, J=6.8 Hz).

(1*S*,5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-(2-imino-1-methyl-1,2-dihydropyridin-4-yl)thiomethyl-1-methylcarbapen-2-em-3-carboxylate (19) **19**: ^{1}H-NMR (200 MHz, D_2O, DHO as standard) δ: 0.94 (3H, d, J=7.2 Hz), 1.06 (3H, d, J=6.4 Hz), 3.00—3.30 (1H, m), 3.20 (1H, dd, J=2.9, 6.2 Hz), 3.48 (1H, d, J=14.8 Hz), 3.49 (3H, s), 3.87 (1H, dd, J=2.9, 9 Hz), 3.98 (1H, quintet, J=6.4 Hz), 4.61 (DHO), 4.70 (1H, d, J=14.8 Hz), 6.50—6.60 (2H, m), 7.42 (1H, d, J=7.7 Hz).

(1*S*,5*R*,6*S*)-2-(2,3-Cyclopenteno-1-methyl-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (23) **23**: ^{1}H-NMR (200 MHz, D_2O) δ: 1.58 (3H, d, J=7.2 Hz), 1.72 (3H, d, J=6.4 Hz), 2.65—2.90 (2H, m), 3.40—3.55 (2H, m), 3.60—3.90 (4H, m), 4.20—4.50 (2H, m), 4.25 and 5.30 (2H, ABq, J=14 Hz), 4.51 (3H, s), 8.00 (1H, d, J=7 Hz), 8.68 (1H, d, J=7 Hz). MS (SIMS, glycerol) *m/z*: 389 $(M+H)^+$, 427 $(M+K)^+$.

(1*S*,5*R*,6*S*)-2-(1-Carbamoylmethyl-2,3-cyclopenteno-4-pyridinio)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (24) **24**: ^{1}H-NMR (200 MHz, D_2O) δ: 0.98 (3H, d, J=7.4 Hz), 1.08 (3H, d, J=6.4 Hz), 2.12—2.20 (2H, m), 2.80—3.06 (4H, m), 3.22 (1H, dd, J=2, 6 Hz), 3.10—3.20 (1H, m), 3.70 and 4.92 (2H, ABq, J=14.6 Hz), 3.90 (1H, dd, J=2, 9 Hz), 4.00—4.10 (1H, m), 5.04 (2H, s), 7.40 (1H, d, J=6.6 Hz), 8.02 (1H, d, J=8.6 Hz).

(1*S*,5*R*,6*S*)-2-(1,9-Ethano-1,2,3,4-tetrahydroquinolizino-8-yl)thiomethyl-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (25) **25**: ^{1}H-NMR (200 MHz, D_2O) δ: 1.07 (3H, d, J=7.2 Hz), 1.12 (3H, d, J=6.4 Hz), 3.25—3.31 (1H, m), 3.45 (1H, dd, J=2, 6 Hz), 3.90 and 5.05 (2H, ABq, J=14 Hz), 4.00—4.18 (2H, m), 4.70 (2H, s), 6.80—8.50 (7H, m).

1,9-Ethano-6,7,8,9-tetrahydro-2-thioxo-2*H*-quinolizine (13) To a solution of 4-chloro-2,3-cyclopentenopyridine (614 mg, 4 mmol) in THF (4 ml), *n*-butyllithium (1.6 N, 2.75 ml, 4.4 mmol) was added dropwise at −78 °C, and after stirring for 10 min, hexamethyl phosphoric triamide (HMPA, 0.6 ml) was added. To the resulting mixture, 3-iodopropanol 2-tetrahydropyranyl (THP) ether (1.134 g, 4.2 mmol) in THF (2 ml) was added dropwise and the temperature was allowed to rise to 25 °C over a 1 h period. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and dried over $MgSO_4$. The extracts was condensed and purified by chromatography (hexane–EtOAc 2 : 1) to give **11** (781 mg, 66%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.40—2.50

(12H, m), 2.80—3.10 (2H, m), 3.10—3.30 (1H, m), 3.40—3.60 (2H, m), 3.70—3.90 (2H, m), 4.50—4.60 (1H, m), 7.05 (1H, d, $J=5.4$ Hz), 8.25 (1H, d, $J=5.4$ Hz). *p*-Toluenesulfonic acid monohydrate (796 mg, 4.19 mmol) was added to a solution of **11** (739 mg, 3.49 mmol) in MeOH (10 ml). After stirring for 20 min, the mixture was poured into aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with water, dried, and concentrated. The residue was chromatographed (EtOAc as eluent) to give an alcohol (432 mg, 58%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.50—2.20 (5H, m), 2.30—2.50 (1H, m), 2.80—3.30 (3H, m), 3.71 (2H, t, $J=7.1$ Hz), 7.08 (1H, d, $J=5.4$ Hz), 8.24 (1H, d, $J=5.4$ Hz). Trifluoromethanesulfonic anhydride (0.293 ml, 1.74 mmol) was added dropwise to a solution of the above alcohol (351 mg, 1.66 mmol) and diisopropylethylamine (0.346 ml, 1.99 mmol) in CH_2Cl_2 (5 ml) at -78 °C. The temperature was raised to 25 °C and then refluxed for 1 h. The mixture was condensed under reduced pressure to give the pyridinium salt **12** as a yellow oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.20—3.90 (9H, m), 4.50—5.30 (2H, m), 7.65 (1H, d, $J=6.6$ Hz), 8.46 (1H, d, $J=6.6$ Hz). To the solution of the salt **12** in EtOH (7 ml), sodium hydrosulfide (531 mg) in water (3 ml) was added under ice cooling and then stirred for 30 min at room temperature. The resulting solution was extracted with $CHCl_3$, washed with water, dried and chromatographed to give **13** a yellow oil which was then triturated with ether to make a pale yellow solid (280 mg, 88%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.40—2.50 (7H, m), 2.60—2.80 (1H, m), 2.80—3.20 (2H, m), 3.70—4.20 (2H, m), 7.03 (1H, d, $J=6.9$ Hz), 7.30 (1H, d, $J=6.9$ Hz).

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

***In Vivo* Activity (Determination of 50% Effective Doses (ED_{50}) Values)** Five-week-old male strain ICR mice weighing 19 to 23 g, in groups of 5—10 mice, were injected intraperitoneally with 10 to 100 times the 50% lethal dose of bacteria, which was suspended in heart infusion broth (HIB) or HIB containing 5% gastric mucin. The compounds were administered 1 and 5 h after infection. The ED_{50} (in milligram per killogram) were determined by the Probit method using survival rates at 7 days post-infection, and were expressed as a single dose.

Stability Test The kidneys were dissected out and each tissue homogenate was prepared at 10% final concentration with a phosphate buffer solution (pH 7.0, 0.1 M). A mixture of 0.1 mg of the test compound and 1 ml of tissue homogenate was incubated at 37 °C. Aliquots were collected at intervals, and after centrifugation the supernatant was subjected to bioassay.

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