ANTIBACTERIAL ACTIVITY AND CHEMICAL MODIFICATIONS OF PS-5 AT THE C-3 SIDE CHAIN

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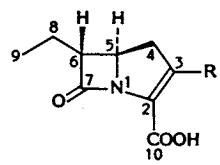
Using PS-5 as starting material, the effects of chemical modification at the C-3 side chain were studied on the antibacterial activity against Gram-positive and Gram-negative bacteria including β-lactamase-producers. Among 35 side chains tested, 4-pyridylthio showed the highest antibacterial activity against the Gram-positive bacteria, and D-cysteinyl against the Gram-negative microbes. In general, compared with acetamidoethylthio in PS-5, basic side chains showed improved antibacterial activity against the staphylococci and pseudomonads, whereas the antibiotic activity against the Gram-negative bacteria decreased with bulky side chains. The introduction of 6-aminopenicillanate and 7-aminocephalosporanate to the C-3 side chain of carbapenem significantly reduced the antibacterial activity against the β-lactamase-producing microbes.

Naturally-occurring carbapenem compounds such as PS-5 have a marked β-lactamase-inhibitory activity[1] together with a broad spectrum of potent antimicrobial activity against Gram-positive and Gram-negative bacteria.[2] However they are inferior to penicillins and cephalosporins in physico-chemical and biological stability, which hampers their clinical use.

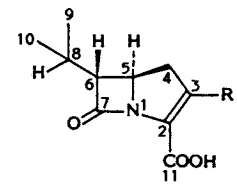
PS-series carbapenems such as PS-5[2], PS-6[3], PS-7[3] and PS-8[4] are structurally characterized by their side chains at C-3 and C-6 (Fig. 1). Like other naturally-occurring carbapenem compounds, PS-5 is

Fig. 1. Strucrures of PS-series carbapenem and related compounds.

PS-series carbapenems

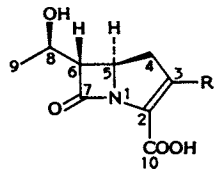


PS-5 $R=SCH_2CH_2NHCOCH_3$
PS-7 $R=SCH=CHNHCOCH_3$



PS-6 $R=SCH_2CH_2NHCOCH_3$
PS-8 $R=SCH=CHNHCOCH_3$

Thienamycins



Thienamycin $R=SCH_2CH_2NH_2$
Imipenem $R=SCH_2CH_2NH-CH=NH$

88617

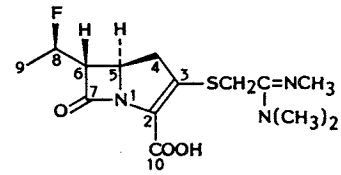
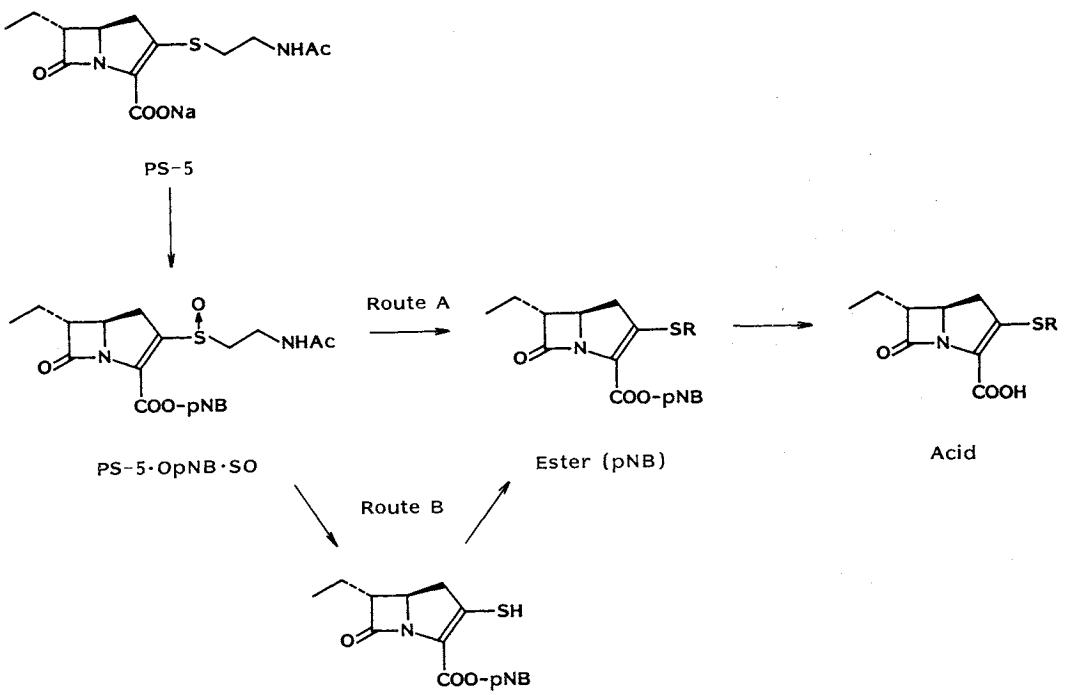


Fig. 2. Sulfoxide method (Route A) and its modification (Route B).



also physico-chemically unstable and susceptible to dehydropeptidase-I (DHP-I) *in vitro*[5] and *in vivo*[6], resulting in poor urinary recovery after parenteral administration. PS-5 is antimicrobially inferior to thienamycin in anti-pseudomonal activity. As is the case with the penicillin and cephalosporin antibiotics, however, the above clinical disadvantages of PS-5 and related carbapenem compounds may be overcome by chemical and biological modifications. To pursue this line of approach, a practically useful synthetic process named "the sulfoxide method" has been developed. It consists of treatment of carbapenem ester sulfoxides with a variety of thiol compounds, and it offers an efficient replacement of the C-3 side chain of carbapenem compounds (Fig. 2)[7,8].

This paper describes the structure-antimicrobial activity relationships of 34 PS-5 carbapenem derivatives which were prepared by the sulfoxide method and its modification, with particular reference to the antibacterial activity against Gram-positive and Gram-negative bacteria including β-lactamase-producing strains.

Results and Discussion

Structures and Derivation Yields of PS-5 Derivatives

Table 1 shows the side chain structures and derivation yields of PS-5 derivatives modified at the C-3 side chain.

For ease of comparison, the 35 PS-5 derivatives are divided into 4 groups depending on the common structures of the C-3 side chain, and their antibacterial activities were examined against Gram-positive and Gram-negative test microorganisms including β-lactamase-producers.

Table 1. Recovery yields of *S*-substituted carbapenem derivatives.

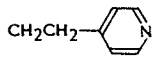
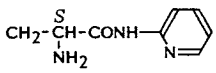
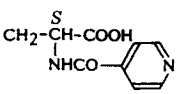
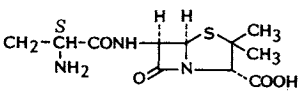
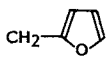
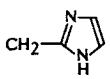
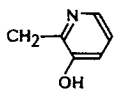
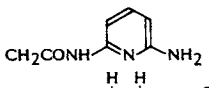
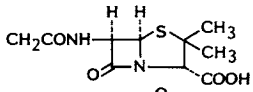
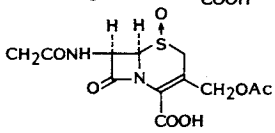
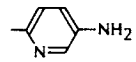
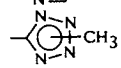
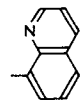
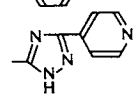
Derivative	R	Ester (pNB)		Acid	
		Route	Yield (%)	Form	Yield (%)
1	$-CH_2CH_2NHAc$ (PS-5)	—	—	Na	—
2	$-CH_2CH_2CH_2CH_3$	A	67	Na	72
3	$-CH_2CH_2OH$	A	70	Na	39
4	$-CH_2CH_2COOH$	A	82	Na	87
5	$-CH_2CH_2NH_2$	A	58	H	20
6	$-CH_2CH_2N(CH_3)_2$	A	75	H	39
7		A	79	Na	34
8	$-CH_2CH_2NHPO(OH)_2$	A	25	Na	25
9		A	84	H	51
10	$-CH_2\overset{R}{C}HCOOH$ NH_2	A	81	Na	25
11	$-CH_2\overset{S}{C}HCOOH$ NH_2	A	68	Na	47
12	$-CH_2\overset{S}{C}HCH_2OH$ NH_2	A	93	H	39
13	$-CH_2\overset{S}{C}HCONH_2$ NH_2	A	78	H	32
14		A	61	H	28
15	$-CH_2\overset{S}{C}HCOOH$ $N(CH_3)_2$	A	85	Na	73
16	$-CH_2\overset{S}{C}HCOOH$ $NHAc$	A	31	Na	69
17		A	50	Na	65
18		A	45	Na	45
19	$-C(CH_3)_2\overset{S}{C}HCOOH$ NH_2	A	61	Na	33
20		A	68	Na	27
21		B	67	H	46
22		B	53	H	50
23	$-CH_2CONHC(CH_3)_2COOH$	B	52	Na	35

Table 1. (Continued)

Derivative	R	Ester (pNB)		Acid	
		Route	Yield (%)	Form	Yield (%)
24		B	34	H	25
25		B	50	Na	44
26		B	55	Na	67
27		A	61	Na	33
28		A	65	Na	36
29		A	31	Na	42
30		A	30	H	40
31		B	55	H	52
32		A	32	H	39
33		A	24	Na	52
34		A	78	H	29
35		A	19	H	34

Antibacterial Activity against Standard Strains of Gram-positive and Gram-negative Bacteria

In Table 2, a common structure of "$-S-CH_2-CH_2$" in the C-3 side chain of PS-5 is modified with a variety of substituents. Compared with acetamidoethylthio in PS-5 (parent; derivative **1**), the elongation of the common structure by ethyl (derivative **2**) results in overall reduction of the antibiotic potency. The introduction of a hydroxyl function (derivative **3**) has no influence on the antibacterial activity against the Gram-positive microbes, but slightly reduces the activity against the Gram-negative microbes. Carboxylation (derivative **4**) decreases the antibacterial activity against not only the Gram-positive but also the Gram-negative microorganisms. Derivative **5** (deacetyl PS-5 or NS-5) is chemically comparable to thienamycin, as both have a free amino group at the terminus of the C-3 side chain. Like thienamycin, NS-5 has better antibacterial activity than PS-5 against the Gram-positive bacteria, because of higher basicity. It is interesting to indicate that, as far as the Gram-negative microbes are concerned, only the anti-pseudomonal activity is improved by deacetylation of PS-5, while the other test microbes show less

Table 2. Comparative antibacterial activities of PS-5 derivatives (**1**) MIC (μg/ml).

	Derivative							
	1 (PS-5)	**1**[a]	**2**	**3**	**4**	**5**	**6**	**7**
Gram-positive bacteria:								
Bacillus subtilis ATCC 6633	0.05	0.10	3.75	0.10	0.39	0.05	0.10	0.06
Micrococcus luteus ATCC 9341	0.05	0.10	1.88	0.10	1.56	0.024	0.20	0.03
Staphylococcus aureus FDA 209P	0.024	0.024	0.24	0.05	0.78	0.0001	<0.006	0.01
S. aureus Smith	0.10	0.10		0.10	1.56	0.003	0.024	0.10
S. aureus Russel[b]	0.10	0.10		0.20	1.56	0.024	0.20	0.10
S. epidermidis	0.20	0.20	0.12	0.20	1.56	0.05	0.20	0.20
Gram-negative bacteria:								
Alcaligenes faecalis A1	0.39	0.78	15.0	1.56	0.78	1.56	3.13	0.86
Citrobacter freundii GN346[c]	3.13	3.13	>60	3.13	25	6.25	12.5	27.5
Comamonas terrigena B-996	0.024	0.012	0.24	0.024	0.024	0.05	0.05	<0.007
Enterobacter aerogenes E19[c]	3.13	1.56	>60	3.13	6.25	6.25	25	27.5
E. cloacae 45[c]	3.13	3.13		6.25	25	12.5	25	55
Enterobacter sp. E8[c]	3.13	3.13	>60	3.13	3.13	6.25	12.5	6.88
Escherichia coli K-12	1.56	1.56	>60	3.13	3.13	6.25	12.5	3.43
E. coli RGN823[b]	3.13	1.56	>60	12.5	6.25	3.13	12.5	13.8
Klebsiella pneumoniae K13[b]	3.13	3.13	>60	12.5	6.25	6.25	25	27.5
Proteus mirabilis P6	6.25	6.25	>60	12.5	6.25	12.5	50	6.88
P. rettgeri P7[c]	3.13	3.13	>60	12.5	3.13	12.5	50	13.8
P. vulgaris GN76[c]	6.25	6.25	>60	25	6.25	12.5	50	55
Proteus sp. P22[c]	6.25	12.5	>60	50	6.25	12.5	50	27.5
Providencia sp. P8	3.13	1.56	30	3.13	3.13	6.25	12.5	3.43
Pseudomonas aeruginosa IFO 3445	12.5	12.5	>60	12.5	12.5	3.13	6.25	55
P. aeruginosa NCTC 10490	12.5	25	>60	12.5	12.5	0.78	3.13	13.8
Serratia marcescens S18[c]	3.13	3.13	>60	6.25	12.5	12.5	50	27.5
S. marcescens T55[c]	3.13	6.25	>	12.5	12.5	12.5	50	110

Inoculum size: 10^6 cells/ml.
Medium: (No mark) Heart infusion agar; [a] Mueller-Hinton agar.
[b] Penicillinase-producer. [c] Cephalosporinase-producer.
THM: Thienamycin. IPM: Imipenem. CEZ: Cefazolin. ABPC: Ampicillin.

susceptibility, presumably because NS-5 becomes physico-chemically more labile than PS-5, as is reported for MM 4550 (sulfoxide)[9]. Dimethylation of NS-5 (derivative **6**), however, results in diminished antibiotic activity. Phenylacetylation (derivative **7**) and phosphorylation (derivative **8**) of NS-5 reduce the antibacterial activity against the Gram-negative microbes only. As is observed with NS-5, the introduction of a 4-pyridyl group (derivative **9**) improves the antibacterial activity against the Gram-positive pathogens owing to elevated basicity, while its activity falls against the Gram-negative bacteria including the pseudomonads.

Table 3 deals with "$-S-CH_2-CH(NHX)-$" as common structure of the C-3 side chain of PS-5. Replacement of the acetamidoethylthio side chain (PS-5; derivative **1**) by optically active cysteines (derivatives **10** and **11**) leads to the significantly reduced activities against the Gram-positive microbes, while the improved activity against the Gram-negative bacteria is higher with the D-cysteinyl side chain (derivative **11**) than with the L-cysteinyl (derivative **10**). Accordingly, all the rest derivatives in Table 3 have the amino group in the (*S*)-configuration.

The introduction of a hydroxymethyl group (derivative **12**) into NS-5 (derivative **5**) results in the

Table 2. (Continued)

	Derivative						
	8	**9**	THM	IPM	CEZ	ABPC	88617[a,22]
Gram-positive bacteria:							
Bacillus subtilis ATCC 6633	0.10	0.03	0.024	0.006	0.10	<0.003	<0.006
Micrococcus luteus ATCC 9341	0.10	0.008	0.024	0.012	0.78	<0.003	0.012
Staphylococcus aureus FDA 209P	0.012	0.008	0.012	0.006	0.10	<0.003	<0.006
S. aureus Smith	0.012	0.06	0.024	0.006	0.10	0.20	0.012
S. aureus Russell[b]	0.012	0.03	0.024		0.10	0.05	
S. epidermidis	0.10	0.03	0.024		0.20	<0.003	
Gram-negative bacteria:							
Alcaligenes faecalis A1	3.13	0.25	0.39		3.13	0.10	0.39
Citrobacter freundii GN346[c]	12.5	8.1	0.39	0.20	>400	>100	0.78
Comamonas terrigena B-996	0.05	<0.008	0.05	0.024	0.05	0.10	0.024
Enterobacter aerogenes E19[c]	12.5	16.2	0.39	0.20	>400	>100	0.78
E. cloacae 45[c]	12.5	130	0.78	0.39	>400	>100	1.56
Enterobacter sp. E8[b]	6.25	4.1	0.39		0.78	12.5	
Escherichia coli K-12	6.25	4.1	0.20	0.10	1.56	3.13	0.39
E. coli RGN823[b]	6.25	8.1	0.78	0.10	12.5	>100	0.78
Klebsiella pneumoniae K13[b]	12.5	32.4	1.56	0.10	12.5	>100	0.39
Proteus mirabilis P6	25	4.1	3.13		12.5	3.13	
P. rettgeri P7[c]	12.5	4.1	1.56		0.78	1.56	
P. vulgaris GN76[c]	25	32.4	3.13	3.13	>400	>100	1.56
Proteus sp. P22[c]	25	16.2	3.13		>400	>100	3.13
Providencia sp. P8	6.25	1.0	0.78		12.5	50	
Pseudomonas aeruginosa IFO 3445	12.5	130	0.78	3.13	>400	12.5	
P. aeruginosa NCTC 10490	3.13	16.2	0.78	0.39	>400	12.5	0.39
Serratia marcescens S18[c]	25	16.2	1.56		>400	>100	0.10
S. marcescens T55[c]	25	130	1.56	0.78	>400	50	1.56

diminished antibacterial activity. Amidation (derivative **13**) and pyridylamidation (derivative **14**) of the terminal carboxyl in the C-3 side chain of derivative **11** improves the antibacterial activity against the Gram-positive bacteria, but reduces the activity against the Gram-negative microbes. Dimethylation (derivative **15**), acetylation (derivative **16**) and *iso*-nicotinylation (derivative **17**) of the free amino group of derivative **11** clearly cause the activity to drop. Coupling of derivative **11** with 6-aminopenicillanate (derivative **18**) decreases the antibiotic activity, particularly against the Gram-negative bacteria. Dimethylation of the C-3 side chain at C-11 (derivative **19**) also diminishes antibacterial activity.

It is noteworthy that the configuration of the amino group of the C-3 side chain (derivatives **10** and **11**) is important not only for expression of antimicrobial activity, but also stabilization to DHP-I (see a separate paper)[10].

Table 4 contains the PS-5 derivatives which have "$-S-CH_2-$" as common structure of the C-3 side chain. In structural comparison with the C-3 side chain of PS-5, no plausible explanation is available about the good antibacterial activity of derivative **20** against the Gram-positive test microbes, while its activity against the Gram-negative organisms falls. The introduction of basic groups such as imidazolyl, hydroxypyridyl and aminopyridylcarbamoyl (derivatives **21**, **22** and **24**) leads to improved antibacterial activity against the Gram-positive pathogens, as is observed above with NS-5 (derivative **5**), and to reduced activity against the Gram-negative microorganisms. The other carbapenems (derivatives **23**, **25** and **26**)

Table 3. Comparative antibacterial activities of PS-5 derivatives (2) MIC (μg/ml).

	Derivative												
	10	**11**	**12**[a]	**13**[a]	**14**[a]	**15**[a]	**16**	**17**	**18**	**19**	**1 (PS-5)**	**4**	**5**
Gram-positive bacteria:													
Bacillus subtilis ATCC 6633	0.78	0.20	0.20	0.20	0.10	0.20	0.39	0.39	0.20	0.39	0.05	0.39	0.05
Micrococcus luteus ATCC 9341	0.78	1.56	0.20	0.39	0.10	0.39	3.13	3.13	0.20	1.56	0.05	1.56	0.024
Staphylococcus aureus FDA 209P	0.39	0.78	0.012	0.05	0.024	0.10	3.13	3.13	0.78	0.78	0.024	0.78	0.001
S. aureus Smith	0.39	0.39	0.05	0.39	0.20	0.39	6.25	3.13	3.13	0.39	0.10	1.56	0.003
S. aureus Russell[b]	0.39	0.39	0.05	0.39	0.39	0.39	6.25	6.25	1.56	0.39	0.10	1.56	0.024
S. epidermidis	0.78	1.56	0.10	0.39	0.39	0.39	6.25	3.13	0.78	1.56	0.20	1.56	0.05
Gram-negative bacteria:													
Alcaligenes faecalis A1	3.13	1.56	1.56	3.13	1.56	1.56	3.13	1.56	6.25	3.13	0.39	0.78	1.56
Citrobacter freundii GN346[c]	6.25	1.56	6.25	6.25	12.5	6.25	>100	>100	200	>100	3.13	25	6.25
Comamonas terrigena B-996	0.20	0.024	0.012	0.012	0.05	0.05	0.10	0.10	0.10	0.20	0.024	0.024	0.05
Enterobacter aerogenes E19[c]	6.25	0.39	12.5	12.5	6.25	6.25	50	>100	100	100	3.13	6.25	6.25
E. cloacae 45[c]	12.5	3.13	12.5	12.5	12.5	12.5	50	>100	>200	>100	3.13	25	12.5
Enterobacter sp. E8[c]	3.13	0.05	6.25	12.5	6.25	6.25	12.5	50	12.5	50	3.13	3.13	6.25
Escherichia coli K-12	3.13	0.024	6.25	6.25	3.13	6.25	12.5	50	6.25	50	1.56	3.13	6.25
E. coli RGN823[b]	6.25	0.05	3.13	6.25	3.13	6.25	12.5	25	6.25	50	3.13	6.25	3.13
Klebsiella pneumoniae K13[b]	12.5	0.39	12.5	12.5	12.5	6.25	100	>100	100	50	3.13	6.25	6.25
Proteus mirabilis P6	25	0.78	25	12.5	12.5	25	25	50	6.25	50	6.25	6.25	12.5
P. rettgeri P7[c]	12.5	3.13	12.5	12.5	6.25	25	25	25	6.25	100	3.13	3.13	12.5
P. vulgaris GN76[c]	12.5	0.78	25	25	12.5	25	25	50	25	25	6.25	6.25	12.5
Proteus sp. P22[c]	12.5	1.56	25	12.5	12.5	25	25	50	25	50	6.25	6.25	12.5
Providencia sp. P8	6.25	0.10	12.5	3.13	1.56	12.5	6.25	6.25	6.25	12.5	3.13	3.13	6.25
Pseudomonas aeruginosa IFO 3445	25	3.13	3.13	12.5	50	25	>100	>100	100	>100	12.5	12.5	3.13
P. aeruginosa NCTC 10490	25	6.25	3.13	25	12.5	25	>100	>100	100	>100	12.5	12.5	0.78
Serratia marcescens S18[c]	3.13	0.39	25	12.5	12.5	25	>100	>100	50	>100	3.13	12.5	12.5
S. marcescens T55[c]	25	3.13	25	12.5	12.5	25	>100	>100	50	>100	3.13	12.5	12.5

Inoculum size: 10^6 cells/ml.

Medium: (No mark) Heart infusion agar; [a] Mueller-Hinton agar.

[b] Penicillinase-producer.

[c] Cephalosporinase-producer.

Table 4. Comparative antibacterial activities of PS-5 derivatives (3) MIC (μg/ml).

	Derivative									
	20	**21**	**22**	**23**	**24**	**25**	**26**	**1** (PS-5)	**3**	**4**
Gram-positive bacteria:										
Bacillus subtilis ATCC 6633	<0.012	0.006	0.05	3.13	0.05	0.39	1.56	0.05	0.10	0.39
Micrococcus luteus ATCC 9341	<0.012	0.006	0.05	12.5	0.012	0.39	3.13	0.05	0.10	1.56
Staphylococcus aureus FDA 209P	<0.012	0.0015	0.10	3.13	0.012	0.39	3.13	0.24	0.05	0.78
S. aureus Smith	<0.012	0.05	0.10	1.56	0.024	1.56	1.56	0.10	0.10	1.56
S. aureus Russell[b]	<0.012	0.006	0.10	3.13	0.024	0.78	3.13	0.10	0.20	1.56
S. epidermidis	<0.012	0.012	0.10	6.25	0.05	0.78	3.13	0.20	0.20	1.56
Gram-negative bacteria:										
Alcaligenes faecalis A1	6.25	0.20	0.39	3.13	0.39	6.25	3.13	0.39	1.56	0.78
Citrobacter freundii GN346[c]	25	1.56	6.25	100	12.5	>50	50	3.13	3.13	25
Comamonas terrigena B-996	<0.012	<0.0008	0.024	0.20	0.006	0.10	0.024	0.024	0.024	0.024
Enterobacter aerogenes E19[c]	25	3.13	12.5	50	25	>50	12.5	3.13	3.13	6.25
E. cloacae 45[c]	50	1.56	12.5	100	25	>50	50	3.13	6.25	25
Enterobacter sp. E8[c]	6.25	0.78	3.13	12.5	12.5	25	6.25	3.13	3.13	31.3
Escherichia coli K-12	3.13	0.78	3.13	12.5	6.25	12.5	3.13	1.56	3.13	3.13
E. coli RGN823[b]	6.25	1.56	1.56	12.5	3.13	>50	3.13	3.13	12.5	6.25
Klebsiella pneumoniae K13[b]	12.5	1.56	6.25	25	25	>50	25	3.13	12.5	6.25
Proteus mirabilis P6	6.25	6.25	6.25	50	12.5	6.25	25	6.25	12.5	6.25
P. rettgeri P7[c]	3.13	1.56	1.56	25	6.25	6.25	6.25	3.13	12.5	3.13
P. vulgaris GN76[c]	12.5	6.25	6.25	50	12.5	50	25	6.25	25	6.25
Proteus sp. P22[c]	12.5	6.25	12.5	50	12.5	50	25	6.25	50	6.25
Providencia sp. P8	1.56	1.56	1.56	12.5	3.13	6.25	3.13	3.13	3.13	3.13
Pseudomonas aeruginosa IFO 3445	25	6.25	12.5	>100	>100	>50	>100	12.5	12.5	12.5
P. aeruginosa NCTC 10490	25	0.78	6.25	>100	100	25	>100	12.5	12.5	12.5
Serratia marcescens S18[c]	12.5	1.56	6.25	25	25	>50	12.5	3.13	6.25	12.5
S. marcescens T55[c]	50	1.56	12.5	50	100	>50	25	3.13	12.5	12.5

Inoculum size: 10^6 cells/ml.
Medium: Heart infusion agar.
[b] Penicillinase-producer.
[c] Cephalosporinase-producer.

Table 5. Comparative antibacterial activities of PS-5 derivatives (**4**) MIC (μg/ml).

	Derivative												
	27	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**1** (PS-5)	**2**	**5**	**9**
Gram-positive bacteria:													
Bacillus subtilis ATCC 6633	0.09	0.07	0.012	<0.007	0.05	<0.012	0.024	0.25	0.39	0.05	3.75	0.05	0.03
Micrococcus luteus ATCC 9341	<0.01	<0.01	<0.001	<0.007	0.0015	<0.012	0.024	0.12	0.10	0.05	1.88	0.024	<0.008
Staphylococcus aureus FDA 209P	<0.01	<0.01	0.006	<0.007	0.05	<0.012	0.024	0.12	0.39	0.024	0.24	0.001	<0.008
S. aureus Smith	0.04	0.10	0.012	<0.007	0.10	0.024	0.05	0.25	0.78	0.10		0.003	0.06
S. aureus Russell[b]	<0.01	<0.01	0.012	<0.007	0.05	<0.012	0.05	0.25	0.78	0.10		0.024	0.03
S. epidermidis	0.04	<0.01	0.012	<0.007	0.05	0.024	0.05	0.25	0.78	0.20	0.12	0.05	0.03
Gram-negative bacteria:													
Alcaligenes faecalis A1	5.6	4.7	0.10	<0.007	0.39	0.20	0.20	63.5	6.25	0.39	15.0	1.56	0.25
Citrobacter freundii GN346[c]	180	150	25	29.5	25	25	12.5	>127	>50	3.13	>60	6.25	8.1
Comamonas terrigena B-996	<0.01	<0.01	0.003	<0.007	0.003	<0.012	0.006	0.5	0.10	0.024	0.24	0.05	<0.008
Enterobacter aerogenes E19[c]	>180	150	25	7.4	50	12.5	6.25	>127	>50	3.13	>60	6.25	16.2
E. cloacae 45[c]	>180	150	50	29.5	50	25	50	>127	>50	3.13		12.5	130
Enterobacter sp. E8[c]	>180	75	12.5	0.46	12.5	3.13	12.5	127	>50	3.13	>60	6.25	4.1
Escherichia coli K-12	180	37.5	6.25	0.92	6.25	1.56	6.25	63.5	>50	1.56	>60	6.25	4.1
E. coli RGN823[b]	180	37.5	6.25	14.8	3.13	3.13	3.13	127	>50	3.13	>60	3.13	8.1
Klebsiella pneumoniae K13[b]	>180	>150	25	14.8	25	12.5	25	>127	>50	3.13	>60	6.25	32.4
Proteus mirabilis P6	45	75	3.13	1.8	12.5	3.13	6.25	>127	>50	6.25	>60	12.5	4.1
P. rettgeri P7[c]	180	75	6.25	1.8	3.13	3.13	3.13	63.5	>50	3.13	>60	12.5	4.1
P. vulgaris GN76[c]	180	150	12.5	29.5	12.5	6.25	12.5	>127	>50	6.25	>60	12.5	32.4
Proteus sp. P22[c]	90	75	6.25	29.5	25	12.5	25	>127	>50	6.25	>60	12.5	16.2
Providencia sp. P8	180	18.8	1.56	0.92	3.13	0.78	3.13	127	>50	3.13	30	6.25	1.0
Pseudomonas aeruginosa IFO 3445	180	18.8	12.5	0.92	25	12.5	25	>127	>50	12.5	>60	3.13	130
P. aeruginosa NCTC 10490	22.5	4.7	25	0.92	6.25	12.5	6.25	63.5	>50	12.5	>60	0.78	16.2
Serratia marcescens S18[c]	>180	75	12.5	29.5	25	12.5	50	>127	>50	3.13	>60	12.5	16.2
S. marcescens T55[c]	>180	150	25	29.5	25	25	25	>127	>50	3.13		12.5	130

Inoculum size: 10^6 cells/ml.
Medium: Heart infusion agar.
[b] Penicillinase-producer.
[c] Cephalosporinase-producer.

show the significantly decreased antibacterial activities against the test microbes.

Table 5 exhibits the antibacterial activities of the carbapenem derivatives which are collectively included in the group having no alkyl linkage between the sulfur atom and the substituents in the C-3 side chain. It is interesting to note that this type of the C-3 side chain modification significantly reduces the antibacterial activity against the Gram-negative microbes only, which seems to indicate that a short alkyl linkage between the sulfur atom and the substituents is necessary for expression of the antibacterial activity against the Gram-negative pathogens, whereas the antibacterial activity against the Gram-positive microbes is not affected by its absence.

Antibacterial Activity against β-Lactamase-producing Bacteria

PS-5 is resistant to almost all the β-lactamases known in the literature excluding several metal-containing ones. Furthermore, it inactivates them, leading to strong synergistic antimicrobial activity[1] with the penicillins and cephalosporins. All the naturally-occurring carbapenem compounds including PS-5 and thienamycin, on the other hand, are susceptible *in vivo* and *in vitro* to DHP-I by the same mechanism as is observed in the reaction of penicillins and cephalosporins with β-lactamase[11], while DHP-I attacks no penicillins and cephalosporins at all. Accordingly, it is important to examine the influence of chemically modified C-3 side chains on β-lactamase and DHP-I susceptibilities to see if they differ from those having the acetamidoethylthio group in this position.

The antibacterial activities of carbapenem, penicillin and cephalosporin derivatives on β-lactamase-producing microorganisms largely depends on the β-lactamase susceptibilities, β-lactamase-inactivating activities, and cell wall and membrane permeability. Except for *Bacillus cereus* type II β-lactamase[12] and *Pseudomonas maltophilia* β-lactamase[13], no enzymes are known to inactivate carbapenems. Accordingly it seemed acceptable to postulate that MICs were a useful indicator for β-lactamase susceptibility. In this paper, the effects of the C-3 side chain modification on β-lactamases were indirectly examined by the antibacterial activity against the β-lactamase-producing Gram-negative microorganisms. In a subsequent paper, the β-lactamase-susceptibility and β-lactamase-inhibitory activity of these and related carbapenem derivatives will be studied *in vitro* by purified β-lactamase preparations.

To antibacterial activity of PS-5 against β-lactamase-producing Gram-positive bacteria except *B. cereus* is not influenced by the introduction of a variety of side chains at C-3 (*Staphylococcus aureus* Russell in Tables 2~5 and unpublished results).

As is reported by SAWAI *et al.*[14], the β-lactamase-susceptibility and the cell wall and membrane permeability of penicillins and cephalosporins are key factors in expression of their antibiotic activity against β-lactamase-producing Gram-negative microbes. PS-5 (parent compound) shows strong antibacterial activity against the β-lactamase-producing Gram-negative bacteria (Table 2), suggesting seemingly complete resistance of PS-5 to these β-lactamases *in vitro*. The C-6 ethyl and C-3 acetamidoethylthio side chains and the stereochemistry at C-6 of PS-5 are known to be very important for expression of its antibacterial activity against Gram-positive and Gram-negative bacteria.

An examination of Tables 2~5 reveals that the *Enterobacter*, *Proteus*, *Pseudomonas* and *Serratia* strains are significantly responsive to the chemical modification of the C-3 side chain of PS-5. For example, in the antimicrobial activity against the 3 *Enterobacter* strains, derivatives **11** and **21** seem to differ from derivatives **4**, **7**, **10**, **18**, **20**, **22**, **23**, **26**, **29**, **30**, **31**, **32** and **33**. In particular, compared with the acetamidoethylthio side chain of the parent compound, only the D-cysteinyl (derivative **11**) and imidazolyl

(derivative **21**) groups increase the specific antibacterial potencies against *Enterobacter cloacae* 45 and *Enterobacter* sp. E8. In a similar context, derivatives **11**, **30** and **32** may be different from derivatives **7**, **8**, **22**, **25**, **27**, **29**, **31** and **33** in the pattern of influence of the side chain modification on the antibacterial activity against the 4 *Proteus* species. Synergism of PS-5 with penicillins and cephalosporins in their antibacterial activity against *Proteus vulgaris* GN76 was previously reported.[1)]

The two strains of *P. aeruginosa* show different responses to the modified C-3 side chains (derivatives **5**, **7**, **14**, **21**, **27**, **28**, **31** and **33**), presumably indicating that the β-lactamases involved may have distinct substrate profiles. The same observation as above is also seen for derivatives **7**, **10**, **11**, **20** and **24** against the two strains of *Serratia marcescens*.

PS-5 is a potent inhibitor of a variety of β-lactamases[15,16)], so it seemed reasonable to assume that binding of PS-5 with 6-aminopenicillanate (6-APA) and 7-aminocephalosporanate (7-ACA) with a covalent bond might protect the penicillin and cephalosporin from β-lactamase attack by virtue of the carbapenem. Contrary to the expectation, however, the introduction of PS-5 to 6-APA (derivatives **18** and **25**)[17)] and 7-ACA (derivative **25**) seems to result in rather increased sensitivity of penicillin and cephalosporin to β-lactamases.

Experimental

Preparation of Antibiotics

PS-5 (sodium salt) was obtained by fermentation as detailed in a previous paper[18)]. Derivative **5** (NS-5) was prepared from PS-5 by microbial deacetylation[19)]. Thirty-three PS-5 derivatives with modified C-3 side chains were synthesized by the sulfoxide method through Route A or B (Fig. 2). More particularly, derivatives **21**～**26** and **31** were prepared through Route B, and the other derivatives through Route A. Of these derivatives, **2**～**6**, **9**, **21**, **22** and **27**～**34** were described in a previous report[8)]. Thienamycin (THM)[20)] was produced by fermentation; and imipenem (IPM)[21)] and 88617[22)] were synthesized according to reported procedures. Cefazolin (CEZ) and ampicillin (ABPC) were purchased from Fujisawa Pharmaceuticals Co., Ltd. and Toyo Jozo Co., Ltd., respectively.

Antibacterial Evaluation

Susceptibility testing was performed by the standard agar dilution technique[23)] using Mueller-Hinton agar and Heart infusion agar (Difco). Overnight cultures of test organisms were diluted in saline. Final inocula of approximately 10^6 cells/ml were applied on agar medium by a multipoint spot inoculator. Agar plates were examined after 18 hours of incubation at 35°C.

General Methodology

UV and IR spectra were recorded on a Hitachi 200-20 and a Hitachi 260-30 spectrophotometer, respectively. NMR spectra were obtained in $CDCl_3$ with a Varian EM-390 or a Jeol PS-100 spectrometer, using TMS as internal standard, unless otherwise stated. MS data were obtained with Hitachi EM-80 and RMU-7 mass spectrometers. Specific rotations were measured with a Jasco DIP-181 digital polarimeter. Pre-coated silica gel plates F_{254} and Silica gel 60 (70～300 mesh, E. Merck, Darmstadt) were used for TLC and column chromatography, respectively. All carbapenem *p*-nitrobenzyl esters were deprotected by hydrogenation and chromatographed on a column of QAE-Sephadex A-25 or Diaion CHP-20AG followed by freeze-drying to yield corresponding carbapenem derivatives.

Chemical Derivation

p-Nitrobenzyl 3-(2-Phenylacetylaminoethyl)thio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **7**; A Typical Example of Derivation Through Route A): To a solution of 150 mg (0.334 mmol) of PS-5 *p*-nitrobenzyl ester sulfoxide in 40 ml of DMF were dropwise added 51 μl (0.36 mmol) of triethylamine and 72 mg (0.369 mmol) of *N*-phenylacetylcysteamine in 1 ml of DMF at −50°C under stirring. After agitation for 1 hour at −50°C, the reaction mixture was poured into 80 ml

of benzene and washed three times with 0.1 M phosphate buffer, pH 6.8. The organic layer was separated, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to dryness under reduced pressure to give a pale-yellow oily product. The product was dissolved in a small volume of methylene chloride and passed through a column of silica gel (20 g) which had been wetted with a solvent mixture of benzene and acetone (1 : 1). The product was developed with the same solvent system. From the eluate 134 mg of the title compound (79% yield) was recovered. On a silica gel TLC plate this derivative showed an Rf value of 0.66 with a solvent system of benzene-acetone (1 : 2). $[\alpha]_D^{22}$ 50.4° (*c* 0.8, THF); UV λ_{max}^{THF} nm (ε) 268 (9,800), 321 (11,300); IR ν_{max} ($CHCl_3$) cm^{-1} 1775 (β-lactam), 1700 (ester); ^1H NMR ($CDCl_3$) δ 1.07 (3H, t, J=7.2 Hz, CH_2CH_3), 1.70~2.00 (2H, m, $-CH_2CH_3$), 2.80~3.55 (7H, m, 4-H×2, 6-H, SCH_2CH_2N), 3.56 (2H, s, Ar-CH_2CO), 3.97 (1H, dt, J=3 and 9 Hz, 5-H), 5.22 (1H, d, J=14 Hz, CHH-Ar), 5.51 (1H, d, J=14 Hz, CHH-Ar), 5.82 (1H, m, NH), 7.30 (5H, s, Ar), 7.65 (2H, d, J=9 Hz, Ar), 8.21 (2H, d, J=9 Hz, Ar); MS (*m/z*) 509 (M^+), 439 ($M^+ - EtCH=C=O$).

Sodium 3-(2-Phenylacetylaminoethyl)thio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **7**): *p*-Nitrobenzyl 3-(2-phenylacetylaminoethyl)thio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (120 mg) was dissolved in a solvent mixture of 10.2 ml of THF and 9.4 ml of 0.1 M phosphate buffer, pH 7.0, and then hydrogenated in the presence of 170 mg of Adams catalyst at room temperature for 5 hours in a Paar apparatus (hydrogen pressure 4 kg/cm^2). The catalyst was removed by filtration and washed with 0.01 M phosphate buffer, pH 6.8. The filtrate and the phosphate wash were combined. The solution was applied on a column of QAE-Sephadex A-25 (1.1×20 cm) and developed with a linear concentration gradient of sodium chloride in 0.01 M phosphate buffer, pH 6.8, from 0 to 0.4 M. Each eluate fraction was monitored with a spectrophotometer and fractions having an absorption maximum at 300 nm were combined. After 2% sodium chloride was added, the solution was charged on a column of Diaion CHP-20AG (1.1×20 cm). Elution was carried out with a linear concentration gradient of acetone in water from 0 to 30% (total eluant 200 ml). Eluate fractions possessing a UV absorption maximum at 300 nm were collected and freeze-dried to give 31.8 mg of the target derivative (yield 34%).

UV $\lambda_{max}^{H_2O}$ nm (ε) 301 (4,800); ^1H NMR (D_2O) δ 1.02 (3H, t, J=7.5 Hz, CH_2CH_3), 1.80 (2H, m, $-CH_2CH_3$), 2.7~3.35 (5H, m, 4-H×2, 6-H, $NHCH_2-$), 3.43 (2H, t, J=6 Hz, $-SCH_2-$), 3.62 (2H, s, Ar-CH_2CO), 3.90 (1H, m, 5-H), 7.28 (5H, s, Ar).

p-Nitrobenzyl 6-Ethyl-3-[2-(di-*p*-nitrobenzyloxyphosphoryl)aminoethyl]thio-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **8**): Cysteamine (76.5 mg) was dissolved in 3 ml of chloroform and cooled to −20°C. A solution of 297 mg of PS-5 *p*-nitrobenzyl ester *S*-oxide in 20 ml of DMF was added to the cysteamine solution under stirring. Triethylamine (369 μl) and then a solution of 511 mg of di-*p*-nitrobenzyloxyphosphoryl chloride in 2 ml of DMF were added dropwise to the said reaction mixture at −20°C. After the same work-up as described for derivative **7**, the crude product was subjected to purification by silica gel column chromatography using a solvent system of benzene and acetone (2 : 1). By silica gel TLC monitoring with a developing solvent mixture of benzene and acetone (1 : 2), eluate fractions were collected which contained a UV-absorbing compound at Rf 0.74. The evaporation of the solvent under reduced pressure resulted in 122.7 mg of the title product (yield 25%). $[\alpha]_D^{24}$ 37.8° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 268 (31,000), 318 (15,300); IR ν_{max} ($CHCl_3$) cm^{-1} 1772 (β-lactam), 1708 (ester); ^1H NMR ($CDCl_3$) δ 1.05 (3H, t, J=7.5 Hz, CH_2CH_3), 1.50~2.10 (2H, m, CH_2CH_3), 2.70~3.40 (7H, m, 4-H×2, 6-H, $-SCH_2CH_2$), 3.90 (1H, dt, J=3 and 9 Hz, 5-H), 5.12 (4H, d, J=8 Hz, O=P-OCH_2-Ar×2), 5.20 (1H, d, J=14 Hz, $COOCH$H-Ar), 5.50 (1H, d, J=14 Hz, $COOCH$H-Ar), 7.51 (4H, d, J=9 Hz, Ar), 7.66 (2H, d, J=9 Hz, Ar), 8.21 (6H, d, J=9 Hz, Ar).

Trisodium 6-Ethyl-3-(2-phosphonatoaminoethyl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **8**): Derivative **8** was prepared in a manner similar to derivative **7**. ^1H NMR (D_2O, external standard TMS) δ 1.46 (3H, t, J=7.5 Hz, CH_2CH_3), 1.90~2.40 (2H, m, CH_2CH_3), 3.25~4.0 (7H, m, 4-H×2, 6-H, SCH_2CH_2N), 4.50 (1H, dt, J=3 and 9 Hz, 5-H).

p-Nitrobenzyl 6-Ethyl-3-[(2*R*)-2-amino-2-*p*-nitrobenzyloxycarbonyl]ethylthio-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-carboxylate (Protected Derivative **10**): IR ν_{max} ($CHCl_3$) cm^{-1} 1775 (β-lactam), 1740 (ester); ^1H NMR ($CDCl_3$) δ 1.03 (3H, t, J=6.9 Hz, CH_2CH_3), 1.83 (2H, m, CH_2CH_3), 2.32 (2H, br, NH_2), 2.76~3.53 (5H, m, 4-H×2, 6-H, 1′-H×2), 3.67~4.08 (2H, m, 5-H, 2′-H), 5.15 (1H, d, J=14.5 Hz, CHH-Ar), 5.24 (2H, s, CH_2-Ar), 5.48 (1H, d, J=14.5 Hz, CHH-Ar), 7.49 (2H, d, J=8.5 Hz, Ar), 7.62 (2H, d, J=8.5 Hz, Ar), 8.18 (4H, d, J=8.5 Hz, Ar).

Sodium 6-Ethyl-3-[(2*R*)-2-amino-2-carboxy]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **10**): UV λ_{max} nm (ε) 298; ^{1}H NMR (D_2O, external standard TMS) δ 1.43 (3H, t, J=7.5 Hz, CH_2CH_3), 2.19 (2H, m, CH_2CH_3), 3.30~4.10 (5H, m, 4-H×2, 6-H, SCH_2–), 4.15~4.72 (2H, m, 5-H, 2'-H).

Sodium 6-Ethyl-3-[(2*S*)-2-amino-2-carboxy]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **11**): UV λ_{max} nm (ε) 298.5 (7,900) (in 0.01 M phosphate buffer, pH 7.5); ^{1}H NMR (D_2O, external standard TMS) δ 1.46 (3H, t, J=7.5 Hz, CH_2CH_3), 2.0~2.5 (2H, m, $-CH_2CH_3$), 3.4~4.1 (5H, m, 4-H×2, 6-H, 1'-H×2), 4.34 (1H, dd, J=4 and 7.5 Hz, 2'-H), 4.49 (1H, dt, J=3 and 9 Hz, 5-H).

p-Nitrobenzyl 3-[(2*S*)-3-Hydroxy-2-*p*-nitrobenzyloxycarbonylamino]propylthio-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **12**): Rf (silica gel TLC): 0.34 (benzene-acetone, 3:1); $[\alpha]_D^{22}$ 89.5° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 268 (20,300), 320.5 (12,700); IR ν_{max} (KBr) cm^{-1} 1765 (β-lactam), 1720 (ester), 1690 (urethane); ^{1}H NMR (DMSO-d_6) δ 0.96 (3H, t, J=7.5 Hz, CH_2CH_3), 1.72 (2H, m, $-CH_2CH_3$), 2.8~3.9 (8H, m, 4-H×2, 6-H, SCH_2CHCH_2O), 3.93 (1H, dt, J=3 and 9 Hz, 5-H), 4.88 (1H, t, J=5.5 Hz, –OH), 5.17 (2H, s, OCH_2-Ar), 5.25 (1H, d, J=14.5 Hz, OCHH-Ar), 5.47 (1H, d, J=14.5 Hz, OCHH-Ar), 7.4 (1H, d, J=8 Hz, NH), 7.56 (2H, d, J=9 Hz, Ar), 7.68 (2H, d, J=9 Hz, Ar), 8.17 (4H, d, J=9 Hz, Ar), MS (*m/z*) 600 (M^+), 530 ($M^+ - CH_3CH_2C{=}C{=}O$).

3-[(2*S*)-2-Amino-3-hydroxy]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (Derivative **12**): UV λ_{max} nm (ε) 300 (6,600) (in 0.01 M phosphate buffer, pH 7.0); ^{1}H NMR (D_2O) δ 0.97 (3H, t, J=7.5 Hz, $-CH_2CH_3$), 1.4~1.9 (2H, m, $-CH_2CH_3$), 2.6~4.1 (9H, m, 4-H×2, 5-H, 6-H, SCH_2CHCH_2O).

p-Nitrobenzyl 6-Ethyl-3-[(2*S*)-2-carbamoyl-2-*p*-nitrobenzylcarbonylamino]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **13**): Rf (silica gel TLC): 0.3 (benzene-acetone, 2:1); IR ν_{max} (KBr) cm^{-1} 1780 (β-lactam), 1720 (ester), 1695 (urethane), 1670 (amide); ^{1}H NMR ($(CD_3)_2CO$) δ 1.03 (3H, t, J=7.5 Hz, CH_2CH_3), 1.55~1.95 (2H, m, $-CH_2CH_3$), 2.85~3.50 (5H, m, 4-H×2, 6-H, 1'-H×2), 3.98 (1H, dt, J=3 and 9 Hz, 5-H), 4.25~4.55 (1H, m, 2'-H), 5.24 (2H, s, $-OCH_2$-Ar), 5.25 (1H, d, J=14 Hz, CHH-Ar), 5.52 (1H, d, J=14 Hz, CHH-Ar), 6.40~6.95 (2H, br, $CONH_2$), 6.95~7.25 (1H, br, NH), 7.61 (2H, d, J=9 Hz, Ar), 7.75 (2H, d, J=9 Hz, Ar), 8.20 (4H, d, J=9 Hz, Ar).

3-[(2*S*)-2-Amino-2-carbamoyl]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (Derivative **13**): UV λ_{max} nm (ε) 300 (3,500); ^{1}H NMR (D_2O) δ 0.99 (3H, t, J=7.5 Hz, CH_2CH_3), 1.4~1.9 (2H, m, $-CH_2CH_3$), 2.6~3.4 (5H, m, 4-H×2, 6-H, 1'-H×2), 3.4~3.8 (1H, m, 2'-H), 3.97 (1H, dt, J=3 and 9 Hz, 5-H).

p-Nitrobenzyl 6-Ethyl-3-[(2*S*)-2-*p*-nitrobenzyloxycarbonylamino-2-N-(pyridin-2-yl)carbamoyl]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carxoxylate (Protected Derivative **14**): Rf (silica gel TLC): 0.41 (benzene-acetone, 5:1); $[\alpha]_D^{23}$ 25.5° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 269 (24,400), 318 (12,500); IR ν_{max} ($CHCl_3$) cm^{-1} 1770 (β-lactam), 1715 (ester), 1695 (urethane, amide); ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, t, J=7.5 Hz, CH_2CH_3), 1.60~2.00 (2H, m, $-CH_2CH_3$), 2.80~3.50 (5H, m, 4-H×2, 6-H, 1'-H×2), 3.82 (1H, dt, J=3 and 9 Hz, 5-H), 4.50~4.80 (1H, m, 2'-H), 5.14 (1H, d, J=13.5 Hz, –OCHH-Ar), 5.18 (2H, s, $-OCH_2$-Ar), 5.43 (1H, d, J=13.5 Hz, –OCHH-Ar), 6.40 (1H, d, J=8 Hz, NH), 7.00 (1H, dd, J=5 and 7.5 Hz, 5'-H), 7.41 (2H, d, J=9 Hz, Ar), 7.53 (2H, d, J=9 Hz, Ar), 7.50~7.8 (1H, m, 4'-H), 8.10 (4H, d, J=9 Hz, Ar), 7.90~8.3 (2H, m, 3'-H, 6'-H), 9.47 (1H, br, NH).

3-[(2*S*)-2-Amino-2-*N*-(pyridin-2-yl)carbamoyl]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (Derivative **14**): UV λ_{max} nm 233, 276, 299.5.

p-Nitrobenzyl 3-[(2*S*)-2-Dimethylamino-2-*p*-nitrobenzyloxycarbonyl]ethylthio-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **15**): Rf (silica gel TLC): 0.45 (benzene-acetone, 3:1); $[\alpha]_D^{22}$ 16.7° (*c* 0.7, THF); UV λ_{max}^{THF} nm (ε) 266 (19,600), 321 (13,200); IR ν_{max} ($CHCl_3$) cm^{-1} 1770 (β-lactam), 1730 (ester); ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, J=7.5 Hz, CH_2CH_3), 1.5~2.0 (2H, m, $-CH_2CH_3$), 2.34 (6H, s, $N(CH_3)_2$), 2.9~3.3 (5H, m, 4-H×2, 6-H, 1'-H×2), 3.45 (1H, dd, J=7 and 8 Hz, 2'-H), 3.95 (1H, dt, J=3 and 9 Hz, 5-H), 5.17 (1H, d, J=13 Hz, OCHH-Ar), 5.25 (2H, s, OCH_2-Ar), 5.48 (1H, d, J=13 Hz, OCHH-Ar), 7.50 (2H, d, J=9 Hz, Ar), 7.63 (2H, d, J=9 Hz, Ar), 8.18 (4H, d, J=9 Hz, Ar), MS (*m/z*) 598 (M^+), 418 ($M^+ - COOpNB$).

Sodium 3-[(2*S*)-2-Carboxy-2-dimethylamino]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **15**): UV λ_{max} nm (ε) 299 (8,000) (in 0.01 M phosphate buffer, pH 7.5); ^{1}H NMR (D_2O) δ 0.98 (3H, t, J=7.5 Hz, CH_2CH_3), 1.55~1.95 (2H, m, $-CH_2CH_3$), 2.84 (6H, s, $N(CH_3)_2$),

3.05~3.4 (3H, m, 4-H×2, 6-H), 3.32 (2H, d, J=5.5 Hz, 1'-H×2), 4.02 (1H, dt, J=3 and 9 Hz, 5-H), 4.18 (1H, t, J=5.5 Hz, 2'-H).

p-Nitrobenzyl 3-[(2*S*)-2-Acetylamino-2-*p*-nitrobenzyloxycarbonyl]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **16**): Rf (silica gel TLC): 0.60 (benzene-acetone, 1:1); $[\alpha]_D^{24}$ 37.0° (*c* 1.0, CH_2Cl_2); UV $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 269 (24,500), 317 (14,700); IR ν_{max} (CH_2Cl_2) cm^{-1} 1775 (β-lactam), 1748 and 1700 (ester), 1680 (amide); ^1H NMR ($CDCl_3$) δ 1.03 (3H, t, J=7 Hz, CH_2CH_3), 1.67~2.00 (2H, m, $-CH_2CH_3$), 2.00 (3H, s, $COCH_3$), 2.80~3.53 (5H, m, 4-H×2, 6-H, 1'-H×2), 3.90 (1H, dt, J=3 and 9 Hz, 5-H), 4.70~4.85 (1H, m, 2'-H), 5.15 (1H, d, J=14 Hz, CHH-Ar), 5.23 (2H, s, CH_2-Ar), 5.47 (1H, d, J=14 Hz, CHH-Ar), 6.25 (1H, d, J=8 Hz, NH), 7.47 (2H, d, J=9 Hz, Ar), 7.60 (2H, d, J=9 Hz, Ar), 8.17 (4H, d, J=9 Hz, Ar).

Disodium 3-[(2*S*)-2-Acetylamino-2-carboxy]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **16**): UV λ_{max} nm (ε) 301.5 (9,200) (in 0.01 M phosphate buffer, pH 7.5); ^1H NMR (D_2O) δ 0.98 (3H, t, J=7.5 Hz, CH_2CH_3), 1.6~1.9 (2H, m, $-CH_2CH_3$), 2.02 (3H, s, $COCH_3$), 2.8~3.5 (5H, m, 4-H×2, 6-H, 1'-H), 3.98 (1H, dt, J=3 and 9 Hz, 5-H), 4.37 (1H, dd, J=5 and 7 Hz, 2'-H).

p-Nitrobenzyl 6-Ethyl-3-[(2*S*)-2-*p*-nitrobenzyloxycarbonyl-2-(pyridin-4-yl)carbonylamino]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **17**): Rf (silica gel TLC): 0.6 (benzene-acetone, 1:1); $[\alpha]_D^{22}$ 25.9° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 265.5 (21,200), 318.5 (11,700); IR ν_{max} ($CHCl_3$) cm^{-1} 1775 (β-lactam), 1750 (ester), 1670 (amide); ^1H NMR ($CDCl_3$) δ 1.02 (3H, t, J=7.5 Hz, CH_2CH_3), 1.6~1.9 (2H, m, $-CH_2CH_3$), 2.8~3.3 (3H, m, 4-H×2, 6-H), 3.30~3.60 (2H, m, 1'-H×2), 3.82 (1H, dt, J=3 and 9 Hz, 5-H), 4.90~5.20 (1H, m, 2'-H), 5.15 (1H, d, J=14 Hz, $-OCHH$-Ar), 5.30 (2H, s, $-OCH_2$-Ar), 5.45 (1H, d, J=14 Hz, $-OCHH$-Ar), 7.2~7.4 (1H, br, NH), 7.40~7.70 (6H, m, Ar), 8.17 (4H, d, J=9 Hz, Ar), 8.60~8.80 (2H, m, Ar).

Disodium 3-[(2*S*)-2-Carboxy-2-(pyridin-4-yl)carbonylamino]ethyl-6-ethyl-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate (Derivative **17**): UV λ_{max} nm (ε) 302 (8,200) (in 0.01 M phosphate buffer, pH 7.5); ^1H NMR (D_2O) δ 0.90 (3H, t, J=7.5 Hz, CH_2CH_3), 1.50~1.90 (2H, m, $-CH_2CH_3$), 2.80~3.20 (3H, m, 4-H×2, 6-H), 3.20~3.45 (2H, m, 1'-H×2), 3.77 (1H, dt, J=3 and 9 Hz, 5-H), 4.63 (1H, dd, J=4.5 and 7.5 Hz, 2'-H), 7.72 (2H, dd, J=4.5 and 2 Hz, pyridine-β×2), 8.65 (2H, dd, J=4.5 and 2 Hz, pyridine-α×2).

p-Nitrobenzyl 6-[(2*S*)-2-Amino-3-((5*R*,6*R*)-6-ethyl-2-*p*-nitrobenzyloxycarbonyl-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-3-yl)thio]propionylaminopenicillanate (Protected Derivative **18**): Rf (silica gel TLC): 0.37 (benzene-acetone, 4:1); $[\alpha]_D^{24}$ 110.6° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 267 (27,800), 320 (11,100); IR ν_{max} ($CHCl_3$) cm^{-1} 1775 (β-lactam), 1750 (ester), 1720 (urethane), 1680 (amide); ^1H NMR ($CDCl_3$) δ 1.05 (3H, t, J=7.5 Hz, CH_2CH_3), 1.38 (3H, s, CH_3CCH_3), 1.55 (3H, s, CH_3CCH_3), 1.60~2.10 (2H, m, $-CH_2CH_3$), 2.80~3.60 (5H, m, 4-H×2, 6-H, SCH_2-), 3.97 (1H, dt, J=3 and 9 Hz, 5-H), 4.20~4.50 (1H, m, $-SCH_2CHCO$), 4.44 (1H, s, 3'-H), 5.18 (1H, d, J=14 Hz, $OCHH$-Ar), 5.20 (2H, s, OCH_2-Ar), 5.27 (2H, s, OCH_2-Ar), 5.48 (1H, d, J=14 Hz, $OCHH$-Ar), 5.40~5.70 (2H, m, 5'-H, 6'-H), 5.88 (1H, d, J=8 Hz, NH), 7.00~7.4 (1H, m, NH), 7.48 (2H, d, J=9 Hz, Ar), 7.53 (2H, d, J=9 Hz, Ar), 7.63 (2H, d, J=9 Hz, Ar), 8.20 (4H, d, J=9 Hz, Ar), 8.23 (2H, d, J=9 Hz, Ar).

Sodium 6-[(2*S*)-2-Amino-3-((5*R*,6*R*)-2-carboxy-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-3-yl)-thio]propionylaminopenicillanate (Derivative **18**): UV λ_{max} nm (ε) 300 (8,100) (in 0.01 M phosphate buffer, pH 6.8); ^1H NMR (D_2O, external standard TMS) δ 1.44 (3H, t, J=7.5 Hz, CH_2CH_3), 1.96 (3H, s, CH_3CCH_3), 2.07 (3H, s, CH_3CCH_3), 1.80~2.40 (2H, m, $-CH_2CH_3$), 3.40~3.90 (5H, m, 4-H×2, 6-H, $-SCH_2CH$), 4.10~4.40 (1H, m, SCH_2CH-), 4.44 (1H, dt, J=3 and 9 Hz, 5-H), 4.66 (1H, s, 3'-H), 5.80~6.10 (2H, m, 5'-H, 6'-H).

p-Nitrobenzyl 6-Ethyl-3-[(2*S*)-1,1-dimethyl-2-*p*-nitrobenzyloxycarbonylamino-2-*p*-nitrobenzyloxycarbonyl]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **19**): Rf (silica gel TLC): 0.53 (benzene-acetone, 5:1); $[\alpha]_D^{23}$ 33.6° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 264 (24,700), 320 (9,000); IR ν_{max} ($CHCl_3$) cm^{-1} 1772 (β-lactam), 1720 (ester, urethane); ^1H NMR ($CDCl_3$) δ 1.06 (3H, t, J=7.5 Hz, CH_2CH_3), 1.47 (3H, s, CH_3CCH_3), 1.52 (3H, s, CH_3CCH_3), 1.60~2.10 (2H, m, $-CH_2CH_3$), 2.90~3.55 (3H, m, 4-H×2, 6-H), 3.91 (1H, dt, J=3 and 9 Hz, 5-H), 4.62 (1H, d, J=9 Hz, 2'-H), 5.17 (1H, d, J=13.5 Hz, CHH-Ar), 5.18 (2H, s, CH_2-Ar), 5.23 (2H, s, CH_2-Ar), 5.46 (1H, d, J=13.5 Hz, CHH-Ar), 5.95 (1H, d, J=9 Hz, NH), 7.48 (2H, d, J=9 Hz, Ar), 7.49 (2H, d, J=9 Hz, Ar), 7.62 (2H, d, J=9 Hz, Ar), 8.17 (2H, d, J=9 Hz, Ar).

Sodium 3-[(2*S*)-2-Amino-2-carboxy-1,1-dimethyl]ethylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **19**): UV λ_{max} nm (ε) 283 (5,600) (in 0.01 M phosphate buffer, pH 7.2); ^{1}H NMR (D_2O, external standard TMS) δ 1.44 (3H, t, J=7.5 Hz, CH_2CH_3), 1.78 (3H, s, CH_3CCH_3), 2.12 (3H, s, CH_3CCH_3), 1.90~2.4 (2H, m, $-CH_2CH_3$), 3.43 (1H, dd, J=10 and 18 Hz, 4-Ha), 3.70 (1H, dd, J=8 and 18 Hz, 4-Hb), 3.85 (1H, dt, J=3 and 7 Hz, 6-H), 4.04 (1H, s, 2'-H), 4.54 (1H, ddd, J=3, 8 and 10 Hz, 5-H).

p-Nitrobenzyl 6-Ethyl-3-furfurylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **20**): $[\alpha]_D^{22}$ 18.6° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 268 (14,500), 320 (14,800); IR ν_{max} ($CHCl_3$) cm^{-1} 1775 (β-lactam), 1700 (ester); ^{1}H NMR ($CDCl_3$) δ 1.04 (3H, t, J=8.0 Hz, CH_2CH_3), 1.82 (2H, m, $-CH_2CH_3$), 3.06 (1H, dd, J=18 and 9 Hz, 4-Ha), 3.10 (1H, m, 6-H), 3.38 (1H, dd, J=18 and 9 Hz, 4-Hb), 3.96 (1H, dt, J=3 and 9 Hz, 5-H), 4.02 (2H, s, CH_2-fur), 5.21 (1H, d, J=14 Hz, CHH-Ar), 5.50 (1H, d, J=14 Hz, CHH-Ar), 6.20~6.40 (2H, m, 3'-H, 4'-H), 7.32 (1H, m, 5'-H), 7.63 (2H, d, J=9 Hz, Ar), 8.18 (2H, d, J=9 Hz, Ar) MS (*m/z*) 428 (M^+).

Sodium 6-Ethyl-3-furfurylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Derivative **20**): UV λ_{max} nm (ε) 301 (10,900); ^{1}H NMR (D_2O) δ 1.00 (3H, t, J=7.5 Hz, CH_2CH_3), 1.80 (2H, m, $-CH_2CH_3$), 3.04 (1H, dd, J=9 and 18 Hz, 4-Ha), 3.23 (1H, m, 6-H), 3.32 (1H, dd, J=9 and 18 Hz. 4-Hb), 3.99 (1H, dt, J=3 and 9 Hz, 5-H), 4.08 (2H, s, CH_2-fur), 6.40 (2H, m, 3'-H, 4'-H), 7.49 (1H, d, J=1.5 Hz, 5'-H).

p-Nitrobenzyl 6-Ethyl-3-[*N*-(2-methyl-1-*p*-nitrobenzyloxycarbonyl-1-propenyl)carbamoyl]methylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **23**): Rf (silica gel TLC): 0.27 (benzene-acetone, 5:1); $[\alpha]_D^{23}$ 16.7° (*c* 1.0, THF), UV λ_{max}^{THF} nm (ε) 243 (16,300), 265 (22,500), 318 (12,900); IR ν_{max} ($CHCl_3$) cm^{-1} 1775 (β-lactam), 1720, 1700 (ester), 1685 (amide); ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t, J=7.5 Hz, CH_2CH_3), 1.6~2.0 (2H, m, $-CH_2CH_3$), 1.85 (3H, s, $CH_3\overset{\|}{C}CH_3$), 2.17 (3H, m, $CH_3\overset{\|}{C}CH_3$), 2.9~3.3 (3H, m, 4-H×2, 6-H), 3.55 (2H, s, $-SCH_2CO$), 3.89 (1H, dt, J=3 and 9 Hz, 5-H), 5.22 (1H, d, J=14 Hz, CHH-Ar), 5.27 (2H, s, CH_2-Ar), 5.50 (1H, d, J=14 Hz, CHH-Ar), 7.52 (2H, d, J=9 Hz, Ar), 7.4~7.7 (1H, m, –NH–), 8.18 (4H, d, J=9 Hz, Ar), MS (*m/z*) 568 ($M^+ - EtCH{=}C{=}O$).

Disodium 6-Ethyl-3-[*N*-(1-carboxy-2-methyl-1-propenyl)carbamoyl]methylthio-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (Derivative **23**): UV λ_{max} nm (ε) 299 (8,800) (in 0.01 M phosphate buffer, pH 7.5), ^{1}H NMR (D_2O, external standard TMS) δ 1.43 (3H, t, J=7.5 Hz, $-CH_2CH_3$), 2.0~2.4 (2H, m, $-CH_2CH_3$), 2.17 (3H, s, $CH_3\overset{\|}{C}CH_3$), 2.42 (3H, s, $CH_3\overset{\|}{C}CH_3$), 3.3~3.9 (3H, m, 4-H×2, 6-H), 4.12 (2H, s, $-SCH_2CO$), 4.45 (1H, dt, J=3 and 9 Hz, 5-H).

p-Nitrobenzyl 3-[*N*-(2-Aminopyridin-6-yl)carbamoyl]methylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate (Protected Derivative **24**): Rf (silica gel TLC): 0.37 (benzene-acetone, 2:1); $[\alpha]_D^{24}$ −12.3° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 255 (sh, 11,600), 259 (12,700), 314 (18,500); IR ν_{max} ($CHCl_3$) cm^{-1} 1778 (β-lactam), 1710 (ester), 1690 (amide); ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, t, J=7.5 Hz, CH_2CH_3), 1.6~2.0 (2H, m, $-CH_2CH_3$), 2.8~3.45 (3H, m, 4-H×2, 6-H), 3.60 (2H, s, SCH_2CO), 3.93 (1H, dt, J=3 and 9 Hz, 5-H), 4.2~4.65 (2H, br, NH_2), 5.21 (1H, d, J=14 Hz, CHH-Ar), 5.47 (1H, d, J=14 Hz, CHH-Ar), 6.22 (1H, dd, J=3 and 6 Hz, 4'-H), 7.30~7.45 (2H, m, 3'-H, 5'-H), 7.62 (2H, d, J=9 Hz, Ar), 8.16 (2H, d, J=9 Hz, Ar), 8.70 (1H, s, NH), MS (*m/z*) 497 (M^+).

3-[*N*-(2-Aminopyridin-6-yl)carbamoyl]methylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (Derivative **24**): ^{1}H NMR (D_2O, external standard TMS) δ 1.38 (3H, t, J=7.5 Hz, CH_2CH_3), 2.19 (2H, m, CH_2CH_3), 3.3~3.8 (3H, m, 4-H×2, 6-H), 4.16 (2H, s, SCH_2CO), 4.39 (1H, dt, J=3 and 9 Hz), 6.90 (1H, d, J=8 Hz, 3'-H), 7.40 (1H, d, J=7.5 Hz, 5'-H), 7.98 (1H, dd, J=7.5 and 8 Hz, 4'-H).

p-Nitrobenzyl 6-[(5*R*,6*R*)-6-Ethyl-2-nitrobenzyloxycarbonyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-3-yl]thioacetylaminopenicillanate (Protected Derivative **25**; A Typical Example of Derivation Through Route B): PS-5 *p*-nitrobenzyl ester *S*-oxide (20 mg) was dissolved in 10 ml of dry DMF and cooled to −35°C. To the solution, 0.36 ml of sodium bisulfide hydrate in DMF (10 mg/ml of NaSH xH_2O) was slowly added under agitation and stirred for 10 minutes at −35°C. Then 25 μl of triethylamine was added to the solution and warmed to room temperature. *p*-Nitrobenzyl 6-chloroacetylaminopenicillanate (22.8 mg) in 1.5 ml of DMF was gradually added into the said solution and allowed to react for 2 hours under agitation. The reaction mixture was poured in 150 ml of benzene and then washed with 0.1 M phosphate

buffer, pH 6.8 (60 ml × 3). The benzene phase was dried over anhydrous sodium sulfate and condensed to 1.5 ml *in vacuo*. The condensate was charged on a silica gel column (20 g). The elution was performed with a solvent mixture of benzene and acetone (10 : 1). By silica gel TLC monitoring with a solvent system of benzene and acetone (5 : 1), eluate fractions were collected which contained a UV-absorbing material at Rf 0.25. The evaporation of the solvent *in vacuo* yielded 166 mg of the title compound (yield 50.4%). $[\alpha]_D^{25}$ 118.8° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 267.5 (21,000), 317 (11,800); IR ν_{max} ($CHCl_3$) cm^{-1} 1780 (β-lactam), 1750 (ester), 1687 (amide); ^1H NMR ($CDCl_3$) δ 1.04 (3H, t, *J*=7 Hz, CH_2CH_3), 1.39, 1.48 (3H, each, s, 2'-$(CH_3)_2$), 1.6~2.1 (2H, m, $-CH_2CH_3$), 2.9~3.3 (3H, m, 4-H × 2, 6-H), 3.53 (2H, s, $-SCH_2CO$), 3.96 (1H, dt, *J*=3 and 9 Hz, 5-H). 4.47 (1H, s, 3'-H), 5.21 (1H, d, *J*=14 Hz, $-OCHH$-Ar), 5.27 (2H, s, $-OCH_2$-Ar), 5.50 (1H, d, *J*=14 Hz, $-OCHH$-Ar), 5.5~5.7 (2H, m, 5'-H, 6'-H), 7.0~7.4 (1H, m, NH), 7.56 (2H, d, *J*=9 Hz, Ar), 7.65 (2H, d, *J*=9 Hz, Ar), 8.21 (2H, d, *J*=9 Hz, Ar).

Disodium 6-[(5*R*,6*R*)-2-Carboxy-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-3-yl]thioacetylaminopenicillanate (Derivative **25**): UV λ_{max} nm (ε) 299.5 (7,400) (in 0.1 M phosphate buffer, pH 6.8); ^1H NMR (D_2O, external standard TMS) δ 1.41 (3H, t, *J*=7.5 Hz, $-CH_2CH_3$), 1.95, 2.0 (3H, each, s, 2'-$(CH_3)_2$), 1.9~2.4 (2H, m, $-CH_2CH_3$), 3.4~3.9 (3H, m, 4-H × 2, 6-H), 4.1 (2H, s, $-SCH_2CO$), 4.22 (1H, dt, *J*=3 and 9 Hz, 5-H), 4.7 (1H, s, 3'-H), 5.9 (1H, d, *J*=4 Hz, 5'-H or 6'-H), 6.0 (1H, d, *J*=4 Hz, 5'-H or 6'-H).

p-Nitrobenzyl 7-[(5*R*,6*R*)-6-Ethyl-2-*p*-nitrobenzyloxycarbonyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-3-yl]thioacetylaminocephalosporanate-1-oxide (Protected Derivative **26**): Rf (silica gel TLC): 0.3 (benzene-acetone, 1 : 1); UV λ_{max}^{THF} nm (ε) 268 (35,900), 282 (26,700), 316 (16,800); IR ν_{max} (KBr) cm^{-1} 1785 (β-lactam), 1735, 1720, 1690 (ester), 1665 (amide); ^1H NMR (DMSO-d_6) δ 0.97 (3H, t, *J*=7.5 Hz, $-CH_2CH_3$), 1.5~1.9 (2H, m, $-CH_2CH_3$), 2.02 (3H, $COCH_3$), 2.7~3.5 (3H, m, 4-H × 2, 6-H), 3.5~4.2 (5H, m, 5-H, SCH_2CO, 2'-H × 2), 4.65 (1H, d, *J*=13.5 Hz, $-CHH$-OAc), 4.97 (1H, d, *J*=4 Hz, 6'-H), 5.26 (1H, d, *J*=13.5 Hz, $-CHH$-OAc), 5.28 (1H, d, *J*=14 Hz, CHH-Ar), 5.47 (2H, s, $-OCH_2$-Ar), 5.49 (1H, d, *J*=14 Hz, $-OCHH$-Ar), 5.92 (1H, dd, *J*=4 and 9 Hz, 7'-H), 7.72 (4H, d, *J*=9 Hz, Ar), 8.25 (4H, d, *J*=9 Hz, Ar), 8.63 (1H, d, *J*=9 Hz, NH).

Disodium 7-[(5*R*,6*R*)-2-Carboxy-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-3-yl]thioacetylaminocephalosporanate-1-oxide (Derivative **26**): UV λ_{max} nm (ε) 260 (11,900), 299 (7,700) (in 0.01 M phosphate buffer, pH 7.5); ^1H NMR (D_2O, external standard TMS) δ 1.45 (3H, t, *J*=7.5, $-CH_2CH_3$), 2.0~2.5 (2H, m, CH_2CH_3), 2.56 (3H, s, $COCH_3$), 3.3~3.85 (3H, m, 4-H × 2, 6-H), 4.16 (2H, s, SCH_2CO), 3.85~4.6 (2H, m, 2'-H × 2), 4.48 (1H, dt, *J*=3 and 9 Hz, 5-H), 5.14 (1H, d, *J*=13 Hz, CHH-OAc), 5.39 (1H, d, *J*=4.5 Hz, 6'-H), 5.52 (1H, d, *J*=13 Hz, CHH-OAc), 6.36 (1H, d, *J*=4.5 Hz, 7'-H).

p-Nitrobenzyl 6-Ethyl-3-(5-nitropyridin-2-yl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **31**): Rf (silica gel TLC): 0.39 (benzene-acetone, 10 : 1); $[\alpha]_D^{23}$ 33.0° (*c* 0.5, THF), UV λ_{max}^{THF} nm (ε) 269 (16,300), 356 (15,200); IR ν_{max} ($CHCl_3$) cm^{-1} 1780 (β-lactam), 1720 (ester); ^1H NMR ($CDCl_3$) δ 1.06 (3H, t, *J*=7.5 Hz, CH_2CH_3), 1.66~2.07 (2H, m, $-CH_2CH_3$), 3.03 (1H, dd, *J*=9 and 18 Hz, 4-Ha), 3.00~3.33 (1H, m, 6-H), 3.58 (1H, dd, *J*=9 and 18 Hz, 4-Hb), 4.03 (1H, dt, *J*=3 and 9 Hz, 5-H), 5.22 (1H, d, *J*=14 Hz, CHH-Ar), 5.49 (1H, d, *J*=14 Hz, CHH-Ar), 7.45 (1H, d, *J*=9 Hz, 3'H), 8.30 (1H, dd, *J*=3 and 9 Hz, 4'-H), 9.24 (1H, d, *J*=3 Hz, 6'-H), MS (*m/z*) 470 (M^+).

3-(5-Aminopyridin-2-yl)thio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (Derivative **31**): UV λ_{max} nm (ε) 261.5 (10,900), 304.5 (12,500) (in 0.1 M phosphate buffer, pH 7.2); ^1H NMR (D_2O, external standard TMS) δ 1.36 (3H, t, *J*=7.5 Hz, CH_2CH_3), 1.90~2.32 (2H, m, CH_2CH_3), 2.80~3.80 (3H, m, 4-H × 2, 6-H), 4.00~4.40 (1H, m, 5-H), 7.63 (1H, dd, *J*=3 and 8 Hz, 4'-H), 7.92 (1H, d, *J*=8 Hz, 3'-H), 8.50 (1H, d, *J*=3 Hz, 6'-H).

p-Nitrobenzyl 6-Ethyl-3-[5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl]thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (Protected Derivative **35**): Rf (silica gel TLC): 0.4 (benzene-acetone, 1 : 1); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 262 (18,400), 310 (13.900); IR ν_{max} ($CHCl_3$) cm^{-1} 1775 (β-lactam), 1705 (ester); ^1H NMR ($CDCl_3$) δ 0.98 (3H, t, *J*=7 Hz, $-CH_2CH_3$), 1.77 (2H, m, $-CH_2CH_3$), 2.9~3.2 (3H, m, 4-H × 2, 6-H), 3.90 (1H, dt, *J*=3 and 9 Hz, 5-H), 5.25 (1H, d, *J*=14 Hz, CHH-Ar), 5.26 (1H, br, NH), 5.48 (1H, d, *J*=14 Hz, CHH-Ar), 7.57 (2H, d, *J*=9 Hz, Ar), 7.92 (2H, d, *J*=6 Hz, 3'-H, 5'-H), 8.12 (2H, d, *J*=9 Hz, Ar), 8.68 (2H, d, *J*=6 Hz, 2'-H, 6'-H).

6-Ethyl-3-[5-(4-pyridyl)-1*H*-1,2,4-triazol-3-yl]thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (Derivative **35**): As the amount of derivative **35** was not enough for measuring the physico-chemical properties, only its biological properties were evaluated.

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References

1) SAKAMOTO, M.; T. ISHIKURA & Y. FUKAGAWA: Synergism of PS-5 with penicillins and cephalosporins in antimicrobial activity against β-lactamase-resistant Gram-negative microorganisms. J. Antibiotics 37: 1414~1422, 1984
2) SAKAMOTO, M.; H. IGUCHI, K. OKAMURA, S. HORI, Y. FUKAGAWA, T. ISHIKURA & J. LEIN: PS-5, a new β-lactam antibiotic. II. Antimicrobial activity. J. Antibiotics 32: 272~279, 1979
3) SAKAMOTO, M.; N. SHIBAMOTO, H. IGUCHI, K. OKAMURA, S. HORI, Y. FUKAGAWA, T. ISHIKURA & J. LEIN: PS-6 and PS-7, new β-lactam antibiotics. *In vitro* and *in vivo* evaluation. J. Antibiotics 33: 1138~1145, 1980
4) SHIBAMOTO, N.; M. NISHINO, K. OKAMURA, Y. FUKAGAWA & T. ISHIKURA: PS-8, a minor carbapenem antibiotic. J. Antibiotics 35: 763~765, 1982
5) SHIBAMOTO, N.; M. SAKAMOTO, H. IGUCHI, H. TONE, Y. FUKAGAWA & T. ISHIKURA: Pharmacological studies on carbapenem antibiotics. I. Metabolism of PS-5 in animal tissues. J. Antibiotics 35: 721~728, 1982
6) SHIBAMOTO, N.; M. SAKAMOTO, Y. FUKAGAWA & T. ISHIKURA: Pharmacological studies on carbapenem antibiotics. II. Isolation of a PS-5-inactivating factor from the rat kidney. J. Antibiotics 35: 729~735, 1982
7) YAMAMOTO, K.; T. YOSHIOKA, Y. KATO, K. ISSHIKI, M. NISHINO, F. NAKAMURA, Y. SHIMAUCHI & T. ISHIKURA: Versatile chemical modification of the C-2 side chain of carbapenem antibiotics. Tetrahedron Lett. 23: 897~900, 1982
8) YAMAMOTO, K.; T. YOSHIOKA, Y. KATO, K. ISSHIKI, M. NISHINO, F. NAKAMURA, Y. SHIMAUCHI & T. ISHIKURA: Chemical modification of carbapenem antibiotics. Versatile methods for displacement of the C-3 sulfur side chain of carbapenems with other thiol groups. J. Antibiotics 36: 407~415, 1983
9) BASKER, M. J.; R. J. BOON & P. A. HUNTER: Comparative antibacterial properties *in vitro* of seven olivanic acid derivatives: MM 4550, MM 13902, MM 17880, MM 22380, MM 22381, MM 22382 and MM 22383. J. Antibiotics 33: 878~884, 1980.
10) SAKAMOTO, M.; YAMAMOTO, H. IGUCHI, H. TONE, T. ISHIKURA, T. YOSHIOKA & Y. FUKAGAWA: Comparative *in vitro* and *in vivo* dehydropeptidase-I stabilities of PS-5 derivatives modified at the C-3 side chain. Chem. Pharm. Bull., to submitted
11) SHIBAMOTO, N.; T. YOSHIOKA, M. SAKAMOTO, Y. FUKAGAWA & T. ISHIKURA: Pharmacological studies on carbapenem antibiotics. III. Chemical structure of PS-5D III, the primary renal metabolite of PS-5. J. Antibiotics 35: 736~741, 1982
12) DAVIS, R. B. & E. P. ABRAHAM: Metal cofactor requirements of β-lactamase. II. Biochem. J. 143: 129~135, 1974
13) SAINO, Y.; F. KOBAYASHI, M. INOUE & S. MITSUHASHI: Purification and properties of inducible penicillin β-lactamase isolated from *Pseudomonas maltophilia*. Antimicrob. Agents Chemother. 22: 564~570, 1982
14) SAWAI, T.; K. MATSUBA, A. TAMURA & S. YAMAGISHI: The bacterial outer-membrane permeability of β-lactam antibiotics. J. Antibiotics 32: 59~65, 1979
15) OKAMURA, K.; M. SAKAMOTO, Y. FUKAGAWA, T. ISHIKURA & J. LEIN: PS-5, a new β-lactam antibiotic. III. Synergistic effects and inhibitory activity against a β-lactamase. J. Antibiotics 32: 280~286, 1979
16) FUKAGAWA, Y.; T. TAKEI & T. ISHIKURA: Inhibition of β-lactamase of *Bacillus licheniformis* 749/C by compound PS-5, a new β-lactam antibiotic. Biochem. J. 185: 177~188, 1980
17) HUNTER, P. A.; K. COLEMAN, J. FISHER & D. TAYLOR: *In vitro* synergistic properties of clavulanic acid, with ampicillin, amoxycillin and ticarcillin. J. Antimicrob. Chemother. 6: 455~470, 1980
18) OKAMURA, K.; A. KOKI, M. SAKAMOTO, K. KUBO, Y. MUTOH, Y. FUKAGAWA, K. KOUNO, Y. SHIMAUCHI, T. ISHIKURA & J. LEIN: Microorganisms producing a new β-lactam antibiotic. J. Ferment. Technol. 57: 265~272, 1979
19) FUKAGAWA, Y.; K. KUBO, T. ISHIKURA & K. KOUNO: Deacetylation of PS-5, a new β-lactam compound. I. Microbial deacetylation of PS-5. J. Antibiotics 33: 543~549, 1980
20) KAHAN, J. S.; F. M. KAHAN, R. GOEGELMAN, S. A. CURRIE, M. JACKSON, E. O. STAPLEY, T. W. MILLER, A. K. MILLER, D. HENDLIN, S. MOCHALES, S. HERNANDEZ, H. B. WOODRUFF & J. BIRNBAUM: Thienamycin, a new β-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. J. Antibiotics 32: 1~12, 1979
21) LEANZA, W. J.; K. J. WINDONGER, T. W. MILLER & B. G. CHRISTENSEN: N-Acetimidoyl- and *N*-formimidoylthienamycin derivatives: Antipseudomonal β-lactam antibiotics. J. Medicinal Chem. 22: 1435~1436, 1979
22) MAK, C. P. & H. FLIRI (Sandoz): Fluoralkylated carbapenem derivatives. U.S. 4720490, Jan. 19, 1988
23) Japan Society of Chemotherapy: The revised method of determination of MIC values. Chemotherapy 29: 76~79, 1981