CHEMICAL MODIFICATION OF CARBAPENEM ANTIBIOTICS

VERSATILE METHODS FOR DISPLACEMENT OF THE C-3 SULFUR SIDE CHAIN OF CARBAPENEMS WITH OTHER THIOL GROUPS

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Carbapenem derivatives substituted at the C-3 position were synthesized (1) by treating carbapenem *S*-oxides with various mercaptans in the presence of a base (Fig. 3), or (2) by alkylating or acylating 3-thiol carbapenems (Fig. 4).

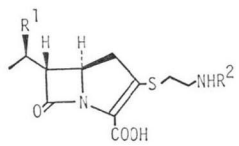
Since the epoch-making discovery of thienamycin,[1] 35 carbapenem compounds including epithienamycins,[2] olivanates[3] and the PS series[4] have been isolated from many streptomycetes. This family of β-lactam compounds are characterized by a common nucleus of 7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylic acid and differ in the C-3 and C-6 side chains and the stereochemistry at C-6 and C-8.

Compared with penicillins and cephalosporins, carbapenem compounds have an unexpectedly broad spectrum of potent antimicrobial activity as well as a strong β-lactamase-inactivating property.[5~8] However, as they are labile *in vitro* and *in vivo*,[9,10] naturally occurring carbapenem compounds must chemically be modified to clinically useful carbapenem derivatives having satisfactory chemical and biological stability. Recent papers have shown that chemical modifications of carbapenem compounds result in the improvement of their antimicrobial spectrum.[11,12]

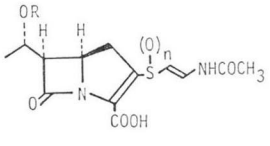
In the total synthesis of thienamycin Merck scientists have described the preparation of C-3 substituted carbapenems by treatment of the C-3-*O*-tosylate or C-3-*O*-phosphate of carbapenem with thiol compounds.[13] Recently CORBETT has reported a method of C-3 modification with other thiol groups which is limitedly operable in carbapenem compounds having a double bond in the C-3 side chain such as MM 13902 and epithienamycins B and D.[14]

In this paper we describe convenient and versatile methods of C-3 displacement which are commonly employable for naturally occurring carbapenem compounds. These methods involve the initial *S*-oxidation of carbapenem compounds followed by displacement of the sulfinyl group in carba-

Fig. 1. Naturally occurring carbapenem compounds.



R^1=OH, R^2=H
Thienamycin
R^1=H, R^2=$COCH_3$
PS-5



R=H, n=0
Epithienamycin B
R=SO_3H, n=1
Olivanic acid
MM 4550

Fig. 2.

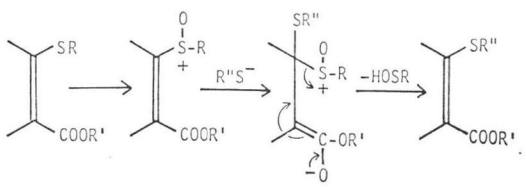
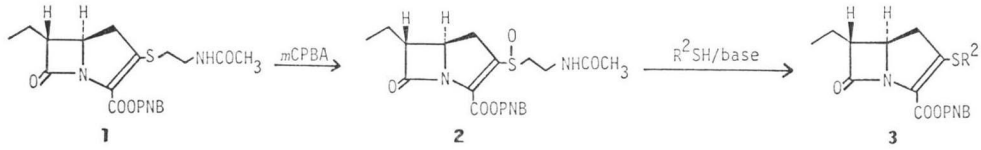


Fig. 3.



penem *S*-oxide with different sulfenyl groups.

The thiene moiety of carbapenem, particularly after activation by *S*-oxidation of the side chain, is susceptible to nucleophilic addition, for example, with thiolate anions. The final displacement occurs as shown in Fig. 2, as the *S*-oxide side chain is a better leaving group than the sulfenyl. In other words, the *S*-oxide is useful not only to activate the thiene moiety but also to serve as a good leaving group. Some of the naturally occurring carbapenems are present in the form of *S*-oxide, and non-*S*-oxide type members are easily converted to *S*-oxides.

The *S*-oxide of PS-5 *p*-nitrobenzyl ester (**2**) was prepared in excellent yield by treatment of PS-5 *p*-nitrobenzyl ester (**1**) with *m*-chloroperbenzoic acid in methylene chloride at $-35°C$ for one hour. The subsequent reaction of S-oxide (**2**) with mercaptans under basic conditions resulted in C-3 substituted PS-5 derivatives. For example, *S*-oxide (**2**) (1.0 equiv.) was allowed to react with 1.1 equiv. of *n*-butyl mercaptan together with 1.1 equiv. of triethylamine in *N*,*N*-dimethylformamide at $-65°C$ for 10 minutes to provide 3-*n*-butylthio substituted PS-5 ester (**6**) in an isolated yield of 67%.

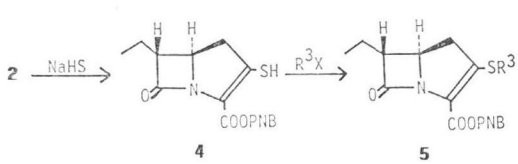
Table 1 summarizes the C-3 substituted carbapenem derivatives prepared by reaction of carbapenem *S*-oxides with mercaptans.

With more nucleophilic mercaptans such as alkyl and phenyl mercaptans, the C-3 substitution was completed in a short period of less than 15 minutes, showing a good yield of reaction, whereas less nucleophilic compounds such as 4-pyridyl and 2-pyrimidyl mercaptans required a stronger base (for example, sodium hydride) and a longer period of reaction (more than 30 minutes).

The reaction of *S*-oxide (**2**) with sodium hydrosulfide provided 3-mercapto compound (**4**) as a single product without the thioxo compound, because its IR spectrum revealed two sharp single absorption bands at 1778 and 1690 cm^{-1} which were attributable to β-lactam and ester carbonyls, respectively; and because its NMR spectrum showed no signal assignable to the C-2 proton.

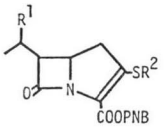
3-Mercapto carbapenem (**4**) is also a useful intermediate for preparation of C-3 substituted carbapenem derivatives. Alkylation and acylation of 3-mercapto carbapenem give alternative methods for C-3 substitued carbapenem, especially in the case where a poor reaction yield is obtained by the aforementioned methods. In practice, it is more advantageous to carry out the reaction in one pot without isolation of 3-mercapto compound (**4**), as it easily decomposes during isolation and purification. *S*-Oxide (**2**) was treated at $-40°C$ for 30 minutes with sodium hydrosulfide in dimethylformamide, and then at $-40°C$ for 30 minutes with imidazolylmethyl chloride, giving 3-imidazolylmethylthiosubstituted PS-5 in a yield of 67.2%. Table 2 presents the results of alkylation and acylation of 3-mercapto carbapenems.

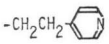
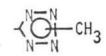
Fig. 4.



After the *p*-nitrobenzyl group was removed by hydrogenolysis in the presence of platinum

Table 1. C-3 Substituted carbapenems.



Compound	R^1	R^2	Base	Time (minutes)	Temperature (°C)	Isolated yield (%)
6	H	$-CH_2CH_2CH_2CH_3$	TEA[1]	10	−65	67.0
7	H	$-CH_2CH_2OH$	TEA	15	−50	70.0
8	H	$-CH_2CH_2N(CH_3)_2$	TEA	10	−50	75.1
9	H	$-CH_2CH_2CO_2PNB$[2]	TEA	10	−35	81.9
10	H	$-CH_2CHCOOPNB$ NHCOOPNB	TEA	15	−30	68.0
11	H		TEA	15	−10	83.7
12	H		TEA	10	−35	61.0
13	H		TEA	10	−50	65.2
14	H		TEA	15	−10	77.5
15	OH[3]		TEA	60	−50	53.6
16	H		NaH	40	−50	28.0
17	H		NaH	40	−50	28.5
18	H		NaH	30	−50	11.4
19	H		NaH	60	−30	43.0
4	H	H	—	30	−40	25.8

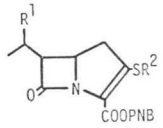
[1] TEA: Triethylamine.
[2] PNB: *p*-Nitrobenzyl.
[3] Epithienamycin C was used as the starting material.

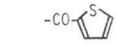
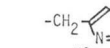
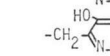
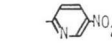
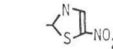
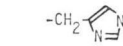
dioxide, new carbapenem derivatives listed in Tables 1 and 2 showed an antimicrobial activity superior or comparable to the parent carbapenem compounds. The structure-activity relationships of these new carbapenem derivatives will be discussed in a forthcoming publication.

Experimental

UV spectra were taken with a Hitachi 200-20 spectrophotometer. IR spectra were recorded in chloroform solution on a Hitachi 260-30 spectrophotometer. NMR spectra were obtained in $CDCl_3$ with a Varian EM-390 or a JEOL PS-100 spectrometer, using tetramethylsilane as internal standard, unless otherwise stated. Mass spectroscopic data were collected on a Hitachi EM-80 spectrometer. Specific rotations were measured with a JASCO DIP-181 digital polarimeter.

Table 2. C-3 substituted carbapenems *via* 3-mercapto compound.



Compound	R^1	R^2	Reagent	Time (minutes)	Temperature (°C)	Isolated yield (%)
20	H	$-CH_3$	CH_2N_2	5	0	58.5
21	H	$-COCH_3$	$(CH_3CO)_2O$	30	−35	56.3
22	H			30	−35	64.0
23	H			40	−35	67.2
24	H			30	−45	53.0
25	H			120	25	55.2
26	H			30	−35	87.3
27	OH*			30	−35	63.2

* *N*-Acetylthienamycin was used as the starting material.

p-Nitrobenzyl 3-Acetamidoethylsulfinyl-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (PS-5 *S*-oxide, **2**)

PS-5 *p*-nitrobenzyl ester (**1**) (240 mg, 0.554 mmole) in dry methylene chloride (25 ml) was treated with *m*-chloroperbenzoic acid (104.9 mg, 0.608 mmole) in dry methylene chloride (12 ml) at −35°C for 60 minutes. The reaction solution was poured into 100 ml of benzene and washed with aqueous sodium bicarbonate (50 ml×3) and brine (50 ml×3). The organic phase was dried with anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel with mixtures of benzene and acetone (1:1 and 1:3, v/v) to yield 211 mg of the *S*-oxide (**2**) (90%).

$[\alpha]_D^{21}$ +18.0° (*c* 1.0, $CHCl_3$); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 267 (13,100), 310 (7,800); IR ν (max) cm^{-1} 3460, 1785, 1720; NMR δ 1.08 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.84 (2H, m, CH_2CH_3), 2.00 (3H, s, $NHCOCH_3$), 2.90~3.92 (7H, m, C-4H, C-6H, 2×CH_2), 3.92~4.23 (1H, m, C-5H), 5.22 (1H, d, *J* = 14.0 Hz, ArCHH), 5.48 (1H, d, *J* = 14.0 Hz, ArCHH), 6.40 (1H, brs, NH), 7.53~7.72 (2H, d, *J* = 9.0 Hz, ArH), 8.12~8.28 (2H, d, *J* = 9.0 Hz, ArH); MS (*m*/*z*) 449 (M^+) (FD MS).

p-Nitrobenzyl 3-*n*-Butylthio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**6**)

S-Oxide of PS-5 *p*-nitrobenzyl ester (**2**) (45.0 mg, 0.100 mmole) was dissolved in 30 ml of dry *N*,*N*-dimethylformamide (DMF) and cooled at −65°C. *n*-Butylmercaptan (9.9 mg, 0.110 mmole) and 11.1 mg (0.110 mmole) of triethylamine (TEA) were added to the solution under agitation and allowed to react for 10 minutes. The reaction solution was diluted with 100 ml of benzene, washed with brine (50 ml×3) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was chromatographed by silica gel with a mixture of benzene and acetone (50:1, v/v) to give 27 mg of **6**.

$[\alpha]_D^{22}$ +39.4° (*c* 0.197, THF); UV λ_{max}^{THF} nm (ε) 322.5 (11,300), 270 (9,900); IR ν (max) cm^{-1} 1765, 1695; NMR δ 0.92 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.06 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.60 (4H, m, 2×CH_2), 1.88 (2H, m, CH_2CH_3), 2.84 (2H, t, *J* = 7.0 Hz, SCH_2), 2.96 (1H, dd, *J* = 8.0, 18.0 Hz, C-4H), 3.12 (1H, dt, *J* = 2.5, 7.0 Hz, C-6H), 3.29 (1H, dd, *J* = 9.0, 18.0 Hz, C-4H), 3.94 (1H, m, C-5H), 5.19

(1H, d, J = 14.0 Hz, ArC*H*H), 5.49 (1H, d, J = 14.0 Hz, ArCH*H*), 7.62 (2H, d, J = 9.0 Hz, ArH), 8.18 (2H, d, J = 9.0 Hz, ArH); MS (m/z) 404 (M^+), 334 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(2-hydroxy)ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**7**)

$[\alpha]_D^{22}$ +33.2° (*c* 1.0, THF); UV λ_{max}^{THF}nm (ε) 320 (10,400), 270 (10,600); IR ν (max) cm^{-1} 3450, 1790, 1700; NMR δ 1.04 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.75~2.05 (2H, m, CH_2CH_3), 2.85~3.30 (5H, m, C-4H, C-6H, SCH_2), 3.70~4.10 (3H, m, C-5H, CH_2OH), 5.18 (1H, d, J = 14 Hz, ArC*H*H), 5.49 (1H, d, J = 14.0 Hz, ArCH*H*), 7.80 (2H, d, J = 9.0 Hz, ArH), 8.16 (2H, d, J = 9.0 Hz); MS (m/z) 392 (M^+), 322 (M^+ − EtCH=C=O), 304 (M^+ − EtCH=C=O − H_2O).

p-Nitrobenzyl 6-Ethyl-3-(2-*N*,*N*-dimethylamino)ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**8**)

$[\alpha]_D^{22}$ +38.5° (*c* 1.0, THF); UV λ_{max}^{THF}nm (ε) 322 (12,100), 270 (10,700); IR ν (max) cm^{-1} 1779, 1705; NMR δ 1.04 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.70~2.00 (2H, m, CH_2CH_3), 2.28 (6H, s, $N(CH_3)_2$), 2.56 (2H, m, CH_2N=), 2.84 (1H, dd, J = 9.0, 18.0 Hz, C-4H), 3.05 (1H, dt, J = 3.0, 7.5 Hz, C-6H), 3.20 (2H, m, CH_2S), 3.32 (1H, dd, J = 9.0, 18.0 Hz, C-4H), 3.98 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.24 (1H, d, J = 14.0 Hz, ArC*H*H), 5.54 (1H, d, J = 14.0 Hz, ArCH*H*), 7.68 (2H, d, J = 9.0 Hz, ArH), 8.24 (2H, d, J = 9.0 Hz, ArH); MS (m/z) 419 (M^+), 404 (M^+ − CH_3), 349 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(2-*p*-nitrobenzyloxycarbonyl)ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9**)

$[\alpha]_D^{22}$ +29.2° (*c* 1.0, THF); UV λ_{max}^{THF}nm (ε) 319 (13,800), 268 (22,400); IR ν (max) cm^{-1} 1772, 1740, 1700; NMR δ 1.05 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.6~2.1 (2H, m, CH_2CH_3), 2.72 (2H, t, J = 7.0 Hz, SCH_2CH_2CO), 3.0~3.3 (5H, m, SCH_2CH_2, C-4H, C-6H), 3.96 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.21 (1H, d, J = 14.0 Hz, ArC*H*H), 5.22 (2H, s, $ArCH_2$), 5.50 (1H, d, J = 14.0 Hz, ArCH*H*), 7.50 (2H, d, J = 9.0 Hz, ArH), 7.65 (2H, d, J = 9.0 Hz, ArH), 8.21 (4H, d, J = 9.0 Hz, ArH); MS (m/z) 485 (M^+ − CH_3CH_2CH=C=O).

p-Nitrobenzyl 6-Ethyl-3-[(2*S*)-2-*p*-nitrobenzyloxycarbonylamino-2-*p*-nitrobenzyloxy-carbonyl]-ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**10**)

$[\alpha]_D^{22}$ +31.8° (*c* 1.0, THF); UV λ_{max}^{THF}nm (ε) 320 (12,000), 266 (30,000); IR ν (max) cm^{-1} 1775, 1740, 1720, 1520, 1350; NMR δ 1.05 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.40~2.10 (2H, m, CH_2CH_3), 2.80~3.40 (3H, m, C-4H, C-6H), 3.30 (2H, d, J = 4.5 Hz, SCH_2), 3.90 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 4.67 (1H, dt, J = 4.5, 7.0 Hz, SCH_2CH=), 5.17 (IH, d, J = 14.0 Hz, C*H*HAr), 5.20 (2H, s, OCH_2Ar), 5.27 (2H, s, OCH_2Ar), 5.50 (1H, d, J = 14.0 Hz, CH*H*Ar), 5.70 (1H, d, J = 7.0 Hz, NH), 7.47 (2H, d, J = 9.0 Hz, ArH), 7.50 (2H, d, J = 9.0 Hz, ArH), 7.63 (2H, d, J = 9.0 Hz, ArH), 8.20 (2H, d, J = 9.0 Hz, ArH); MS (m/z) 749 (M^+), 679 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-[2-(pyridin-4-yl)]ethylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**11**)

$[\alpha]_D^{22}$ +31.4° (*c* 1.0, THF); UV λ_{max}^{THF} nm(ε) 322 (15,000), 266 (13,400); IR ν (max) cm^{-1} 1775, 1700; NMR δ 1.05 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.6~2.1 (2H, m, CH_2CH_3), 2.8~3.2 (7H, m, SCH_2CH_2–Py, C-4H, C-6H), 3.90 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.2 (1H, d, J = 14.0 Hz, ArC*H*H), 5.5 (1H, d, J = 14.0 Hz, ArCH*H*), 7.08 (2H, d, J = 6.0 Hz, PyH), 7.56 (2H, d, J = 9.0 Hz, ArH), 8.15 (2H, d, J = 9.0 Hz, ArH), 8.48 (2H, d, J = 6.0 Hz, PyH).

p-Nitrobenzyl 6-Ethyl-3-cyclohexylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**12**)

$[\alpha]_D^{22}$ +42.6° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 325 (15,600), 270 (12,800); IR ν (max) cm^{-1} 1770, 1695; NMR δ 1.08 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.2~2.1 (12H, m, CH_2CH_3, cyclohexyl CH_2), 3.00 (1H, dd, J = 8.0, 18.0 Hz, C-4H), 3.13 (1H, dt, J = 3.0, 7.0 Hz, C-6H), 3.20 (1H, t, J = 9.0 Hz, –SCH), 3.32 (1H, dd, J = 9.0, 18.0 Hz, C-4H), 3.94 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.18 (1H, d, J = 14.0 Hz, ArC*H*H), 5.50 (1H, d, J = 14.0 Hz, ArCH*H*), 7.62 (2H, d, J = 9.0 Hz, ArH), 8.18 (2H, d, J = 9.0 Hz, ArH); MS (m/z) 430 (M^+), 360 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-phenylthio-7-oxo-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**13**)

$[\alpha]_D^{22}$ +3.0° (*c* 1.0, THF); UV λ_{max}^{THF} nm(ε) 320 (11,000), 271 (9,100); IR ν (max) cm^{-1} 1780, 1710;

NMR δ 1.00 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.60~1.90 (2H, m, CH_2CH_3), 2.66 (2H, d, J = 9.0 Hz, C-4H), 3.02 (1H, dt, J = 3.0, 7.5 Hz, C-6H), 3.82 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.24 (1H, d, J = 14.0 Hz, ArCHH), 5.54 (1H, d, J = 14.0 Hz, ArCHH), 7.28~7.60 (5H, m, ArH), 7.68 (2H, d, J = 9.0 Hz, ArH), 8.24 (2H, d, J = 9.0 Hz, ArH); MS (m/z) 424 (M^+), 354 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-quinolinylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**14**)

$[\alpha]_D^{22}$ +5.1° (c 1.0, THF); UV λ_{max}^{THF} nm (ε) 317.5(10,200), 266 (11,000); IR ν (max) cm^{-1} 1775, 1700; NMR δ 0.94 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.79 (2H, m, CH_2CH_3), 2.44 (1H, dd, J = 9.0, 17.0 Hz, C-4H), 2.68 (1H, dd, J = 9.0, 17.0 Hz, C-4H), 2.94 (1H, dt, J = 3.0, 7.5 Hz, C-6H), 3.74 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.28 (1H, d, J = 14.0 Hz, ArCHH), 5.56 (1H, d, J = 14.0 Hz, ArCHH), 7.20~8.20 (10H, m, ArH); MS (m/z) 475 (M^+), 405 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Hydroxyethyl-3-phenylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**15**)

IR ν (max) cm^{-1} 1775, 1710; NMR δ 1.32 (3H, d, J = 6.4 Hz, =CHCH$_3$), 2.68 (2H, d, J = 9.2 Hz, C-4H), 3.18 (1H, dd, J = 2.8, 5.2 Hz, C-6H), 3.99 (1H, dt, J = 2.8, 9.2 Hz, C-5H), 4.12 (1H, dq, J = 5.2, 6.4 Hz, =CH–CH$_3$), 5.29 (1H, d, J = 14.0 Hz, ArCHH), 5.54 (1H, d, J = 14.0 Hz, ArCHH), 7.68 (2H, d, J = 9.2 Hz, ArH), 8.23 (2H, d, J = 9.2 Hz, ArH); MS (m/z) 440 (M^+), 354 (M^+ − 86).

p-Nitrobenzyl 6-Ethyl-3-(4-pyridyl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**16**)

S-Oxide of PS-5 *p*-nitrobenzyl ester (**2**) (150 mg, 0.372 mmole) was dissolved in 40 ml of dry DMF and treated with a solution of 4-mercaptopyridine (53.6 mg, 0.483 mmole) and sodium hydride (11.6 mg, 0.483 mmole) in 10 ml of dry DMF at −50°C for 40 minutes. The reaction solution was poured into 150 ml of benzene and washed with 0.1 M phosphate buffer, pH 8.5 (70 ml × 3). After drying the organic layer over anhydrous sodium sulfate, the solvent was removed by evaporation under reduced pressure. The resulting oil was subjected to silica gel column chromatography (benzene - acetone, 5:1, v/v), providing 46 mg of **16**.

$[\alpha]_D^{22}$ +18.4° (c 1.0, THF); UV λ_{max}^{THF} nm (ε) 322 (7,900), 267 (8,700); IR ν (max) cm^{-1} 1785, 1720; NMR δ 1.10 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.64~2.04 (2H, m, CH_2CH_3), 2.60~3.24 (3H, m, C-4H, C-6H), 3.92 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.30 (1H, d, J = 14.0 Hz, ArCHH), 5.58 (1H, d, J = 14.0 Hz, ArCHH), 7.40 (2H, dd, J = 2.0, 6.0 Hz, PyH), 7.68 (2H, d, J = 9.0 Hz, ArH), 8.24 (2H, d, J = 9.0 Hz, ArH), 8.60 (2H, dd, J = 2.0, 6.0 Hz, PyH); MS (m/z) 425 (M^+), 355 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(2-pyridyl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**17**)

$[\alpha]_D^{22}$ +16.2° (c 1.0, THF); UV λ_{max}^{THF} nm (ε) 325 (16,900), 269 (12,100); IR ν (max) cm^{-1} 1780, 1710; NMR δ 1.00 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.60~2.10 (2H, m, CH_2CH_3), 2.92 (1H, dd, J = 9.0, 18.0 Hz, C-4H), 3.07 (1H, dt, J = 3.0, 7.0 Hz, C-6H), 3.22 (1H, dd, J = 9.0, 18.0 Hz, C-4H), 3.92 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.26 (1H, dd, J = 14.0 Hz, ArCHH), 5.53 (1H, dd, J = 14.0 Hz, ArCHH), 7.40~7.80 (3H, m, PyH), 7.63 (2H, d, J = 8.0 Hz, ArH), 8.20 (2H, d, J = 8.0 Hz, ArH), 8.58 (1H, dd, J = 2.0, 5.0 Hz, PyH); MS (m/z) 425 (M^+), 355 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(2-pyrimidinyl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**18**)

$[\alpha]_D^{22}$ +38.4° (c 0.47, THF); UV λ_{max}^{THF} nm (ε) 322 (9,800), 268 (10,300); IR ν (max) cm^{-1} 1780, 1715; NMR δ 1.08 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.68~2.00 (2H, m, CH_2CH_3), 3.17 (1H, dd, J = 8.5, 18.0 Hz, C-4H), 3.22 (1H, dt, J = 3.0, 7.0 Hz, C-6H), 3.42 (1H, dd, J = 9.0, 18.0 Hz, C-4H), 4.05 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.28 (1H, d, J = 14.0 Hz, ArCHH), 5.52 (1H, d, J = 14.0 Hz, ArCHH), 7.04 (1H, t, J = 6.0 Hz, C-5′H), 7.62 (2H, d, J = 9.0 Hz, ArH), 8.20 (2H, d, J = 9.0 Hz, ArH), 8.56 (2H, d, J = 6.0 Hz, C-4′H, C-6′H); MS (m/z) 426 (M^+), 356 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-methyltetrazolylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**19**)

$[\alpha]_D^{22}$ +32.0° (c 1.0, THF); UV λ_{max}^{THF} nm (ε) 307 (12,200), 269.5 (11,000); IR ν (max) cm^{-1} 1775, 1700; NMR δ 1.01 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.83 (2H, m, CH_2CH_3), 2.90~3.30 (3H, m, C-4H, C-6H), 3.92 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 4.06, 4.38 (3H, each s, N-CH$_3$), 5.28 (1H, d, J = 14.0 Hz, ArCHH), 5.55 (1H, d, J = 14.0 Hz, ArCHH), 7.68 (2H, d, J = 7.0 Hz, ArH), 8.23 (2H, d, J = 7.0 Hz, ArH); MS (m/z) 430 (M^+), 360 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-mercapto-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**4**)

S-Oxide of PS-5 *p*-nitrobenzyl ester (**2**) (49.5 mg, 0.110 mmole) in 15 ml of dry DMF was reacted with 9.4 mg of sodium hydrosulfide ($NaSH \cdot xH_2O$ about 70% pure, 0.117 mmole) at −40°C for 30 minutes. The reaction solution was diluted with 70 ml of benzene and rinsed with 0.1 M phosphate buffer, pH 6.86 (30 ml × 3). The organic layer was dried over anhydrous sodium sulfate, concentrated to 0.3 ml under reduced pressure and purified by Bio-Beads S × 3 column chromatography using benzene as eluant to provide 10.3 mg (25.8%) of **4**.

UV λ_{max}^{THF} nm 310 (sh.), 269.5; IR ν (max) cm^{-1} 1778, 1690; NMR δ 1.06 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.59 (1H, s, SH), 1.55 ~ 2.05 (2H, m, CH_2CH_3), 2.07 ~ 3.06 (3H, m, C-4H, C-6H), 3.92 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.22(1H, d, J = 14.0 Hz, ArCHH), 5.53(1H, d, J = 14.0 Hz, ArCHH), 7.65(2H, d, J = 9.0 Hz, ArH), 8.22 (2H, d, J = 9.0 Hz, ArH).

p-Nitrobenzyl 6-Ethyl-3-methylthio-7-oxo-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**20**)

Sodium hydrosulfide (about 70%, pure, 4.3 mg, 0.053 mmole) was added to a solution of *S*-oxide of PS-5 *p*-nitrobenzyl ester (**2**) (23 mg, 0.051 mmole) in 10 ml of DMF at −35°C and stirred for 30 minutes. The reaction mixture was diluted with ethyl acetate (50 ml), washed with 0.1 M phosphate buffer, pH 6.86 and dried over anhydrous sodium sulfate. The organic phase containing crude 3-mercapto compound (**4**) was concentrated to 10 ml under reduced pressure and mixed with an excess of diazomethane in ethyl ether at 0°C. After 5 minutes of reaction, the mixture was evaporated to dryness under reduced pressure and the residue was charged on a silica gel column using a solvent of benzene and acetone (30:1) as an eluant. The yield of **20** was 10 mg.

$[\alpha]_D^{22}$ +49.8° (*c* 0.5, THF); UV λ_{max}^{THF} nm (ε) 320 (9,600), 269 (9,400); IR ν (max) cm^{-1} 1778, 1702; NMR δ 1.04 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.85 (2H, m, CH_2CH_3), 2.36 (3H, s, SCH_3), 3.12 (3H, m, C-6H, C-4H), 3.94 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.20 (1H, d, J = 15.0 Hz, ArCHH), 5.50 (1H, d, J = 15.0 Hz, ArCHH), 7.60 (2H, d, J = 8.0 Hz, ArH), 8.16 (2H, d, J = 8.0 Hz, ArH); MS (*m/z*) 362 (M^+), 292 (M^+ − EtCH=C=O).

p-Nitrobenzyl 3-Acetylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**21**)

3-Mercapto compound (**4**) was similarly prepared from 23 mg (0.051 mmole) of *S*-oxide of PS-5 *p*-nitrobenzyl ester. The compound was dissolved in 10 ml of DMF and treated with 40 μl of acetic anhydride (0.4 mmole) at −35°C for 30 minutes. The reaction solution was poured in benzene (50 ml), washed with 0.1 M phosphate buffer, pH 8.4 and dried over anhydrous sodium sulfate. After evaporation of the organic layer under reduced pressure, the residue was applied on a silica gel column using benzene - acetone mixture of 30:1 as eluant to give 11.2 mg of **21**.

$[\alpha]_D^{22}$ +8.5° (*c* 0.5, THF); UV λ_{max}^{THF} nm (ε) 310 (sh., 11,500), 269 (17,200); IR ν (max) cm^{-1} 1779, 1720, 1708; NMR δ 1.05 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.60 ~ 2.00 (2H, m, CH_2CH_3), 2.35 (3H, s, $SCOCH_3$), 2.90 ~ 4.20 (4H, m, C-4H, C-5H, C-6H), 5.20 (2H, d, J = 14.0 Hz, ArCHH), 5.45 (2H, d, J = 14.0 Hz, ArCHH), 7.60 (2H, d, J = 9.0 Hz, ArH), 8.18 (2H, d, J = 9.0 Hz, ArH); MS (*m/z*) 390 (M^+), 320 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(2-thiophenecarbonyl)thio-7-oxo-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**22**)

3-Mercapto compound (**4**) prepared from 22.7 mg (0.05 mmole) of *S*-oxide of PS-5 *p*-nitrobenzyl ester (**2**) was treated with thenoyl chloride (8 μl, 0.075 mmole) in the presence of triethylamine (8.2 mg, 0.081 mmole) at −35°C for 30 minutes. The reaction mixture was diluted with benzene (70 ml), rinsed with 0.1 M phosphate buffer, pH 6.8 (70 ml × 2) and dried over anhydrous sodium sulfate. After removal of the benzene under reduced pressure, the residue was subjected to silica gel column chromatography (benzene - acetone, 100:1), giving 14.8 mg of **22**.

$[\alpha]_D^{23}$ +10.6° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 315 (sh., 14,300), 266.5 (20,100); IR ν (max) cm^{-1} 1773, 1718; NMR δ 1.02 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.70 ~ 2.10 (2H, m, CH_2CH_3), 2.80 ~ 4.00 (3H, m, C-4H, C-6H), 4.12 (1H, dt, J = 3.0, 9.0 Hz, C-5H), 5.30 (1H, d, J = 14.0 Hz, ArCHH), 5.55 (1H, d, J = 14.0 Hz, ArCHH), 7.21 (1H, t, J = 4.5 Hz, C-4′H), 7.72 (2H, d, J = 9.0 Hz, ArH), 7.79 (1H, d, J = 4.5 Hz, C-5′H), 7.93 (1H, d, J = 4.5 Hz, C-3′H), 8.30 (2H, d, J = 9.0 Hz, ArH); MS (*m/z*) 458 (M^+), 388 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(imidazol-4-yl)methylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**23**)

To a solution in 50 ml of DMF of 3-mercapto compound (**4**) prepared from 147 mg (0.327 mmole) of *S*-oxide of PS-5 *p*-nitrobenzyl ester (**2**), 75.1 mg (0.491 mmole) of 4-chloromethylimidazole hydrochloride in 1.5 ml of DMF and 132.7 mg (1.311 mmole) of triethylamine were added at −35°C and allowed to react for 40 minutes. The reaction mixture was diluted with benzene (200 ml), rinsed with 0.1 M phosphate buffer, pH 8.4 (200 ml × 3) and dried with sodium sulfate. The solvent was removed *in vacuo* by evaporation. The residue was purified by column chromatography on Sephadex LH-20 (Pharmacia Fine Chemicals AB) using acetone as eluant to provide 94.1 mg of **23**.

$[\alpha]_D^{23}$ +10.3° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 323.5 (15,300), 269.2 (11,900); IR ν (max) cm^{-1} 1778, 1703; NMR δ 1.03 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.06~2.00 (2H, m, CH_2CH_3), 2.80~3.50 (3H, m, C-4H, C-6H), 3.91 (1H, dt, *J* = 3.0, 9.0 Hz, C-5H), 4.03 (2H, s, SCH_2), 5.18 (1H, d, *J* = 14.0 Hz, ArCHH), 5.44 (1H, d, 14.0 Hz, ArCHH), 5.55~6.00 (1H, brs, NH), 6.93 (1H, s, Imd-5'H), 7.55 (1H, s, Imd-2'H), 7.60 (2H, d, *J* = 8.0 Hz, ArH), 8.15 (2H, d, *J* = 8.0 Hz, ArH); MS (*m/z*) 428 (M^+), 358 (M^+ −EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(3-hydroxypyridin-2-yl)methylthio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**24**)

$[\alpha]_D^{24}$ −17.0° (*c* 1.0, THF); UV λ_{max}^{THF} nm(ε) 323.5 (9,600), 276 (14,400); IR ν (max) cm^{-1} 3250, 1775, 1698; NMR (acetone-d_6) δ 0.82 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.40~1.80 (2H, m, CH_2CH_3), 2.90~3.90 (4H, m, C-4H, C-5H, C-6H), 4.07 (2H, s, SCH_2–Py), 5.08 (1H, d, *J* = 14.0 Hz, ArCHH), 5.32 (1H, d, *J* = 14.0 Hz, ArCHH), 6.90~7.18 (2H, m, Py-4'H, 5'H), 7.62 (2H, d, *J* = 8.5 Hz, ArH), 7.82~7.97 (1H, m, Py-6'H), 8.10 (2H, d, *J* = 8.5 Hz, ArH); MS (*m/z*) 455 (M^+), 385 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(5-nitropyridin-2-yl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**25**)

3-Mercapto compound (**4**) prepared from *S*-oxide of PS-5 *p*-nitrobenzyl ester (149.6 mg, 0.333 mmole) was dissolved in 50 ml of DMF and then treated with triethylamine (67.3 mg, 0.664 mmole) and 2-bromo-5-nitropyridine (102 mg, 0.502 mmole) at −35°C for 5 minutes. After stirring for 2 hours at room temperature, the reaction mixture was diluted with benzene (200 ml), rinsed with 0.1 M phosphate buffer, pH 8.4 (200 ml × 3), and dried over anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* yielded a residue which was purified by column chromatography on silica gel (benzene - acetone, 10:1) to give 86.5 mg of **25**.

$[\alpha]_D^{23}$ +33.0° (*c* 0.5, THF); UV λ_{max}^{THF} nm (ε) 326 (15,200), 269 (16,300); IR ν (max) cm^{-1} 1781, 1720; NMR 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* = 9.0, 18.0 Hz, C-4H), 3.00~3.33 (1H, m, C-6H), 3.58 (1H, dd, *J* = 9.0, 18.0 Hz, C-4H), 4.03 (1H, dt, *J* = 3.0, 9.0 Hz, C-5H), 5.22 (1H, d, *J* = 14.0 Hz, ArCHH), 5.49 (1H, d, *J* = 14.0 Hz, ArCHH), 7.45 (1H, d, *J* = 9.0 Hz, Py-3'H), 8.30 (1H, dd, *J* = 3.0, 9.0 Hz, Py-4'H), 9.24 (1H, d, *J* = 3.0 Hz, Py-6'H); MS (*m/z*) 470 (M^+), 400 (M^+ − EtCH=C=O).

p-Nitrobenzyl 6-Ethyl-3-(5-nitrothiazol-2-yl)thio-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**26**)

$[\alpha]_D^{23}$ −10.0° (*c* 1.0, THF); UV λ_{max}^{THF} nm (ε) 373.5 (8,300), 301 (14,800), 270 (15,000); IR ν (max) cm^{-1} 1775, 1700; NMR δ 1.05 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.67~2.10 (2H, m, CH_2CH_3), 3.06 (1H, dd, *J* = 9.0, 18.0 Hz, C-4H), 3.10~3.40 (1H, m, C-6H), 3.40 (1H, dd, *J* = 9.0, 18.0 Hz, C-4H), 4.04 (1H, dt, *J* = 3.0, 9.0 Hz, C-5H), 5.28 (1H, d, *J* = 14.0 Hz, ArCHH), 5.54 (1H, d, *J* = 14.0 Hz, ArCHH), 7.66 (2H, d, *J* = 9.0 Hz, ArH), 8.23 (2H, d, *J* = 9.0 Hz, ArH), 8.46 (1H, s, Thi-4'H); MS (*m/z*) 476 (M^+), 406(M^+ − EtCH=C=O).

5*R*,6*S*,8*R* *p*-Nitrobenzyl 6-Hydroxyethyl-3-(imidazol-4-yl)methylthio-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate (**27**)

$[\alpha]_D^{23}$ +23.0° (*c* 0.25, MeOH); UV λ_{max}^{EtOH} nm (ε) 322 (13,500), 267 (12,300); IR (KBr) ν (max) cm^{-1} 1765, 1695; NMR (acetone-d_6) δ 1.28 (3H, d, *J* = 6.5 Hz, C-9H), 3.15~3.90 (3H, m, C-4H, C-6H), 3.90~4.40 (4H, m, C-5H, C-8H, $-SCH_2$-Imd), 5.23 (1H, d, *J* = 15.0 Hz, ArCHH), 5.51 (1H, d, *J* =

15.0 Hz, ArCH*H*), 7.08 (1H, s, C-5′H), 7.62 (1H, s, C-2′H), 7.76 (2H, d, J = 9.0 Hz, ArH), 8.22 (2H, d, J = 9.0 Hz, ArH); MS (m/z) 358 ($M^+ - CH_3CH(OH)CH=C=O$).

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