Chang-Hyun Oh[a], **Hyun-Gu Dong**[a], **Joo-Shin Lee**[a], **Su-Chul Lee**[b], **Joon Hee Hong**[c], **Jung-Hyuck Cho**[a]

[a] Medicinal Chemistry Research Center, Korea Institute of Science and Technology, Seoul 130-650, Korea
[b] Hawon pharm. Co., Seoul 135-080, Korea
[c] College of Pharmacy, Chosun University, Kwangju 501-759, Korea

Synthesis and Antibacterial Activity of 1β-Methyl-2-(5-substituted oxadiazolo pyrrolidin-3-yl-thio)carbapenem Derivatives

Synthesis of a new series of 1β-methylcarbapenems with a substituted oxadiazolo-pyrrolidine moiety is described. Their *in vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria were tested and the effect of the substituent on the oxadiazole ring investigated. In particular, compounds **13a** and **13c** with ester and carbamoyl substituted oxadiazole moieties showed the most potent antibacterial activity.

Keywords: 1β-Methylcarbapenem; Antibacterial activity; Substituent effect

Received: January 24, 2003; Accepted: August 6, 2003 [FP768]
DOI 10.1002/ardp.200300768

Introduction

Discovery of lβ-methyl carbapenem [1] by the Merck group was a milestone in that it proffered the possibility of its clinical use as a single agent without dehydropeptidase-I (DHP-I) inhibitor, due to improved chemical and metabolic stability. Carbapenem compounds which have a pyrrolidin-3-ylthio group at the C-2 position in the carbapenem skeleton are noted for their broad and potent antibacterial activity. A large number of derivatives have been synthesized and investigated with enthusiasm. Meropenem [2, 3] and biapenem [4, 5] have been reported to be under development as a single agent.

We have previously reported that carbapenem compounds with a pyrrolidin-3-ylthio group at the C-2 position in the carbapenem skeleton have an outstanding broad and potent antibacterial activity. Consequently a large number of derivatives have been synthesized and investigated [6–13].

In this paper, we describe the synthesis and structure-activity relationships of 1β-methylcarbapenems with a 5′-substituted oxadiazolo pyrrolidine-3′-ylthio group as C-2 side chain. The antibacterial activity of these carbapenem derivatives are also discussed.

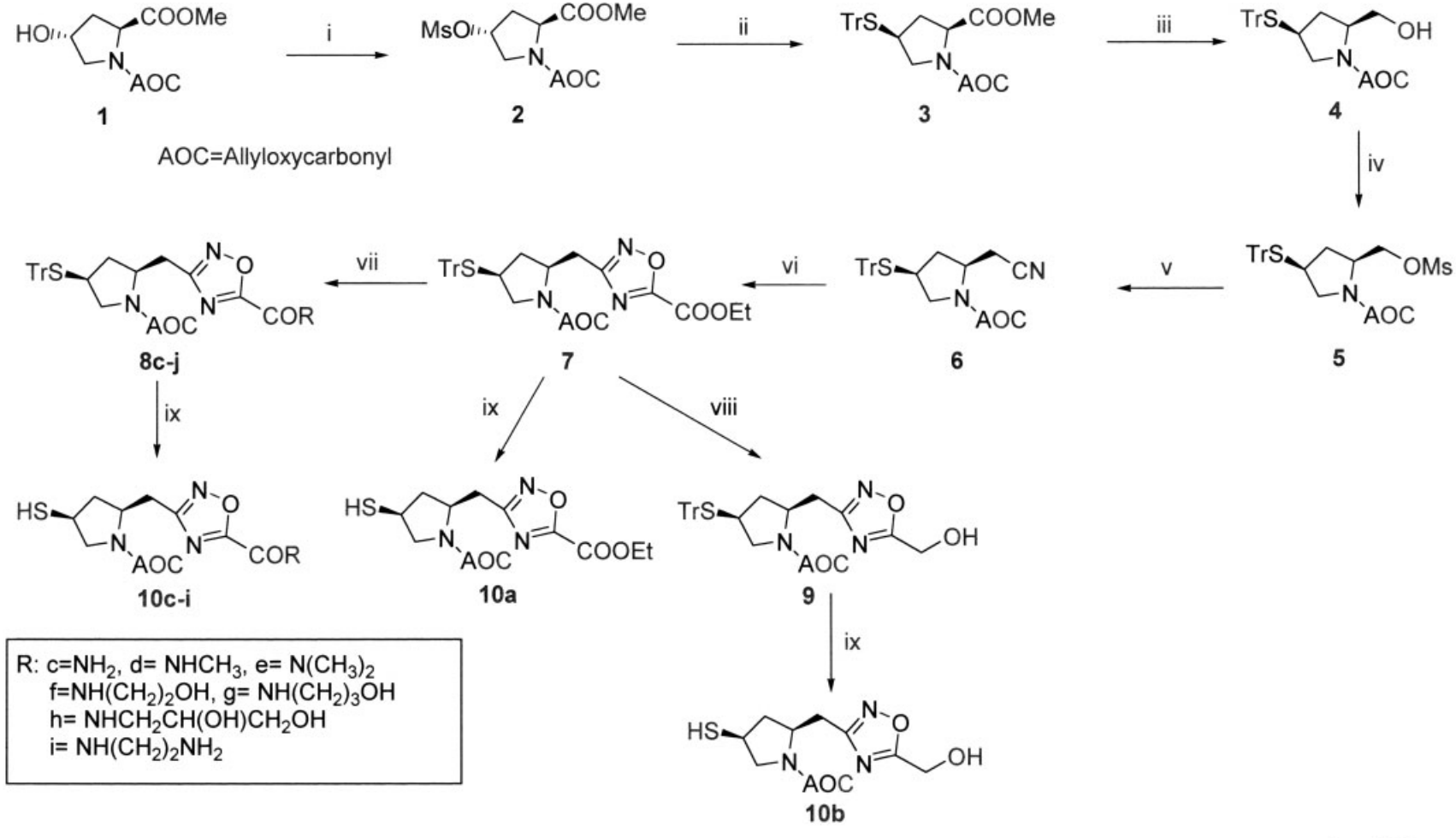
Chemistry

Our general synthetic route leading to new carbapenems, involved preparation of appropriately protected thiols containing pyrrolidine ring as a side chain and subsequent coupling reaction with the carbapenem diphenylphosphates, followed by deprotection of the resulting protected carbapenems in a usual manner.

2-(5-Substituted oxadiazole) pyrrolidine derivatives (**10a–i**) were prepared by the sequence shown in Scheme 1. N-Protected proline methyl ester was converted to *O*-mesylate **2** by treatment of mesyl chloride, and subsequently treated with sodium triphenylmethylthioate, which was generated *in situ* from triphenylmethylmercaptan and sodium hydride in DMF to provide **3** with inversion of the C-4 configuration. **3** was reduced with lithium borohydride in THF-EtOH and subsequently mesylated to give **5**. The treatment of mesylate **5** with sodium cyanide in DMSO gave the cyano compound **6**, which was successfully converted into *N*-hydroxyacetamidine as an intermediater by reaction of hydroxylamine. The formation of 5′-substituted oxadiazole ring **7** was accomplished by reaction of *N*-hydroxyacetamidine with ethyl oxalyl chloride in THF solution. The 4′-ester substituted oxadiazole **7** was converted to the amide **8** by treatment of corresponding amine in EtOH. **7** was also reduced with sodium borohydride in EtOH to give **9**. Deprotection of the trityl group to mercaptan (**10a–i**) was achieved by treatment of **7**, **8c–i** and **9** with trifluoroacetic acid in the presence of triethylsilane.

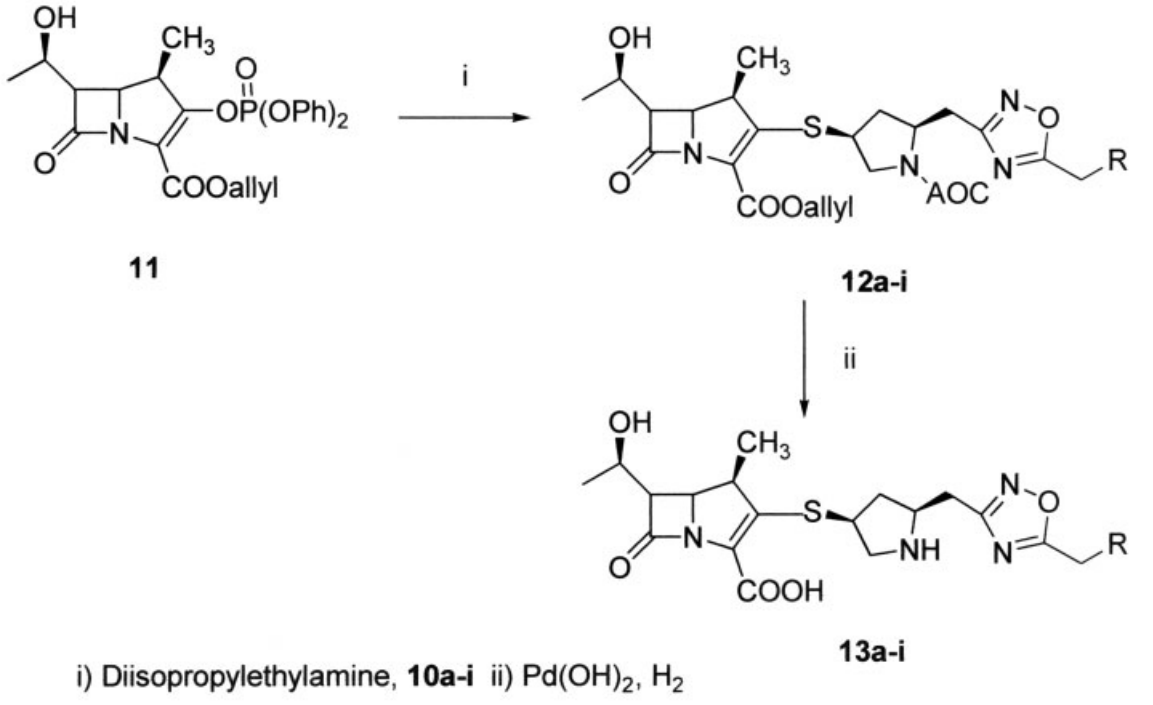
Finally, the reaction of **11** [9] with thiols (**10a–i**) in the presence of diisopropylethylamine, provided 2-substituted carbapenem (**12a–i**). Deprotection of these compounds by catalytic hydrogenation gave the crude products, which were purified by HP-20 column to give the pure carbapenems (**13a–i**) (Scheme 2).

Correspondence: Jung-Hyuck Cho, Medicinal Chemistry Research Center, Korea Institute of Science and Technology, Seoul 130-650, Korea; Phone: +82 2 958-5150 or -5160, Fax: +82 2 958-5189; email: choh@kist.re.kr and hwcho@kist.re.kr



i) MsCl, TEA, CH_2Cl_2 ii) TrSH, NaH, DMF c) $LiBH_4$, EtOH/THF iv) MsCl, TEA, CH_2Cl_2 v) NaCN, DMSO vi)1) Hydroxyamine.HCl , EtOH 2) Ethyloxalyl chloride, THF, reflux vii) R-NH_2, EtOH, reflux viii) $NaBH_4$, THF:EtOH=1;2 ix) Trifluoroacetic acid, Triethyl silane, CH_2Cl_2

Scheme 1.



Scheme 2.

Table 1. *In vitro* antibacterial activity (MIC, μg/mL) and DHP-I stability of carbapenem derivatives.

	STRAINS	**13a**	**13b**	**13c**	**13d**	**13e**	**13f**	**13g**	**13h**	**13i**	Imi-penem	Mero-penem
1	Streptococcus pyogenes 308A	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.02	0.04	0.04	<0.01	0.01
2	Streptococcus pyogenes 77A	<0.01	<0.01	0.01	0.01	0.02	<0.0	0.02	0.04	0.04	<0.01	<0.01
3	Staphylococcus aureus SG511	0.05	0.05	0.05	0.20	0.20	0.10	0.40	0.40	0.40	0.01	0.10
4	Staphylococcus aureus 285	0.10	0.10	0.10	0.40	0.40	0.20	0.80	0.80	0.40	0.01	0.10
5	Eschericihia coli DC2	0.10	0.10	0.20	0.80	0.40	0.10	0.40	0.40	0.40	0.40	0.03
6	Eschericihia coli TEM	0.05	0.10	0.10	0.40	0.20	0.05	0.40	0.40	0.40	0.20	0.03
7	Psudemonas aeruginosa 9027	0.80	6.10	3.10	25.0	12.5	6.10	50	50	50	0.80	0.20
8	Salmonella typhimurium	0.20	0.10	0.10	0.40	0.40	0.20	0.40	0.40	0.40	0.80	0.03
9	Klebsiella aerogenes 1522E	0.10	0.20	0.20	0.40	0.40	0.10	0.4	0.40	0.40	0.10	0.05
10	Enterobactor cloacae 1321E	0.05	0.10	0.10	0.20	0.20	0.10	0.40	0.40	0.80	0.10	0.03
	DHP-I	1.48	2.73	1.42	2.18	2.53	1.85	1.24	3.13	2.40	0.20	1.00

Antibacterial activity studies

The *in vitro* antibacterial activities of the new carbapenems (**13a–i**) described above, against Gram-positive and negative bacteria are listed in Table 1. For comparison, the MIC values of Imipenem and Meropenem are also listed. Among these compounds, **13a**, **13b** and **13c** showed superior or similar antibacterial activity against Gram-positive bacteria compared to Meropenem. Furthermore, they exhibited improved antibacterial activity against Gram-negative bacteria in comparison to Imipenem, except against *P. aeruginosa*.

Compounds **13a–l,** with amide, ester and hydroxy groups at C′-5 position of oxadiazole, showed slight differences in their antibacterial activities against Gram-

positive and Gram-negative bacteria. As expected, the 5′-carbamoyl, ester, hydroxy substituted compound **(13 a–c)** exhibited the most potent and well balanced activity. The effects of substituent on the oxadiazole ring were investigated. Results showed that the larger the size of the substituent, the lower the activity against *Pseudomonas aeruginosa*. The amides series, also show that the larger the size of the 5′-amide substituents, the lower the activity against Gram-positive and negative bacteria.

The stability to DHP-I of most compounds was tested and all compounds were found to be more stable than DHP-I compared to Meropenem. In particular, compound **13 b** and **13 h** exhibited the highest stability.

Experimental

Melting point (mp): Thomas Hoover apparatus, uncorrected. UV spectra: Hewlett Packard 8451A UV-VIS spectrophotometer. IR spectra: Perkin Elmer 16F-PC FT-IR. NMR spectra: Varian Gemini 300 spectrometer, tetramethylsilane (TMS), as an internal standard. The mass spectrometry system was based on a HP5989A MS Engine (Palo Alto, CA, USA) mass spectrometer with a HP Model 59987A.

Measurement of in vitro antibacterial activity

The minimum inhibitory concentrations (MICs) were determined by the agar dilution method using test agar. An overnight culture of bacteria in Tryptosoy broth was diluted to a final concentration of approximately 10^6 cells/mL with the same broth, and inoculated aseptically onto agar, containing serial two fold dilutions of test compounds. Organisms were incubated at 37 °C for 18–20 h. The MICs of a compound was defined as the lowest concentration that visibly inhibited growth.

Determination of susceptibility to renal dehydropeptidase-I (DHP-I)

The relative hydrolysis rate of carbapenems was determined by porcine renal DHP-I, taking the initial hydrolysis rate of Imipenem to be 1.0. Partially purified porcine DHP-I (final concentration 0.3 U/mL) was incubated with 50 μM carbapenem at 35 °C in 50 mM MOPS buffer, pH 7.0. The initial hydrolysis rate was monitored by spectrophotometric methods. One unit of activity was defined as the amount of enzyme required to hydrolyze 1 μM of glycyldehydrophenylalanine per min, when 50 μM of substrate was incubated at 35 °C in 50 mM MOPS buffer, pH 7.0.

(2S,4R)-4-Mesyloxy-1-(allyloxycarbonyl)pyrrolidine-2-carboxylic acid methyl ester (**2**)

A solution of **1** (94.0 g, 0.41 mol) and triethylamine (65.0 mL, 0.49 mol) in dry CH_2Cl_2 (600 mL) was cooled to 0 °C under nitrogen and treated with methanesulfonyl chloride (56.0 g, 0.49 mol). The mixture was stirred at 0 °C for 1 h, diluted with CH_2Cl_2 (500 mL), and washed with 10 % NaHCO3 and brine. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **2** (117.5 g, 93.2 %), as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 2.27 (m, 1 H), 2.75 (m, 1 H), 3.02 (s, 3 H), 3.77 and 3.80 (2 s, 3 H), 3.82–3.97 (m, 2 H), 4.42 (m, 1 H), 4.57 (d, 2 H, *J* = 5.8 Hz), 5.25 (m, 3 H), 5.92 (m, 1 H).

(2S,4S)-4-Tritylthio-1-(allyloxycarbonyl)pyrrolidine-2-carboxylic acid methyl ester (**3**)

To a stirred solution of triphenylmethylmercaptan (80.0 g, 0.29 mol) in dry DMF (600 mL), sodium hydride (11.6 g, 0.29 mol, 60 % oil suspension) was added dropwise at 0 °C, and stirred for 1 h at room temperature. To the resulting solution was added solution **2** (76.7 g, 0.25 mol), in dry DMF (150 mL) at 0 °C and stirred for 3 h at room temperature. The reaction mixture was poured into cold dilute HCl and extracted with ethyl acetate. The organic layer was successively washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography to give **3** (100.6 g, 82.5 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 2.01 (m, 1 H), 2.55 (m, 1 H), 3.16 (bs, 1 H), 3.54 (bs, 1 H), 3.77 and 3.82 (2 s, 3 H), 3.97 (m, 1 H), 4.42 (m, 1 H), 4.55 (d, 2 H, *J* = 5.5 Hz), 5.26 (m, 2 H), 5.98 (m, 1 H), 7.23 (m, 9 H), 7.48 (m, 6 H).

(2S,4S)-2-Hydroxymethyl-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine (**4**)

To a solution of **3** (107.3 g, 0.22 mol) in THF (800 mL), $LiBH_4$ (4.79 g 0.22 mol) was added slowly at 0 °C and stirred for 25 h at room temperature. The mixture was diluted with H_2O (200 mL), 1 N HCl (200 mL) and ethyl acetate (800 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and the resulting residue purified by silica gel column chromatography to give **4** (79.4 g, 78.5 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.98 (m, 1 H), 2.75–2.82 (m, 2 H), 3.01 (m, 1 H), 3.55 (bs, 2 H), 3.78 (m, 1 H), 4.55 (d, 2 H, *J* = 5.9 Hz), 5.25 (m, 3 H), 5.90 (m, 1 H), 7.27 (m, 9 H), 7.47 (m, 6 H).

(2S,4S)-2-Mesyloxymethyl-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine (**5**)

A solution of **4** (68.9 g, 0.15 mol) and triethylamine (24.2 mL, 0.18 mol) in dry CH_2Cl_2 (400 mL) was cooled to 0 °C under nitrogen and treated with methanesulfonyl chloride (20.6 g, 0.18 mol). The mixture was stirred at 0 °C for 1 h, diluted with CH_2Cl_2 (200 mL), and washed with 10 % $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography to give **5** (76.5 g, 94.8 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.91 (bs, 1 H), 2.11 (bs, 1 H), 2.75–2.82 (bs, 2 H), 2.99 (s, 3 H), 3.95 (bs, 1 H), 4.01 (m, 1 H), 4.22 (bs, 1 H), 4.55 (bs, 3 H), 5.31 (m, 2 H), 5.91 (m, 1 H), 7.27 (m, 9 H), 7.48 (m, 6 H).

(2S,4S)-2-Cyanomethyl-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine (**6**)

A mixture of **5** (59.0 g, 0.11 mol) and sodium cyanide (10.8 g, 0.22 mol) in DMSO (300 mL) was heated at 75 °C for 5 h. The reaction mixture was poured into ice water and extracted with ethyl acetate (300 mL × 2). The organic layer was successively washed with water (200 mL, × 2) and brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **6** (45.9 g, 89.1 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.88 (m, 1 H), 2.19 (m, 1 H), 2.82 (d, 2 H), 2.85–3.01 (m, 2 H), 3.88 (m, 1 H), 4.55 (d, 2 H, *J* = 5.9 Hz), 5.29 (m, 2 H), 5.88 (m, 1 H), 7.27 (m, 9 H), 7.47 (m, 6 H).

(2S,4S)-2-[(5-Ethoxycarbonyl)-1,2,4-oxadiazole-3-ylmethyl]-4-tritylthio-1-(allyloxycarbonyl) pyrrolidine (**7**)

To a solution of **6** (23.4 g, 0.05 mol) and hydroxylamine hydrochloride (10.4 g, 0.15 mol) in EtOH (250 mL) a solution of Na_2CO_3 (15.9 g, 0.15 mol) in water (70 mL) was added slowly at room temperature and stirred for 20 h at 60 °C. The mixture was

diluted with H_2O (200 mL), then neutralized with 6 N HCl, diluted with ethyl acetate (300 mL), and washed with brine. Evaporation of the solvent *in vacuo* gave a crude residue, which was used without further purification.

To the above solution in THF (200 mL), ethyloxalyl chloride (5.60 mL, 0.05 mol) was added dropwise and heated at reflux for 2 h. The reaction mixture was poured into 10 % $NaHCO_3$ and extracted with ethyl acetate (200 mL). Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography to give **7** (22.3 g, 76.5 %) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.48 (t, 3 H, *J* = 7.4 Hz), 1.85 (bs, 1 H), 2.16 (bs, 1 H), 2.85–2.98 (bs, 3 H), 3.55 (bs, 1 H), 3.89 (bs, 1 H), 4.01 (bs, 1 H), 4.26–4.51 (bs, 4 H), 5.31 (m, 2 H), 5.91 (m, 1 H), 7.26 (m, 9 H), 7.47 (m, 6 H).

(2S,4S)-2-[(5-Carbamoyl)-1,2,4-oxadiazole-3-ylmethyl]-4-tritylthio-1-(allyloxycarbonyl) pyrrolidine (**8 c**)

To a stirred solution of **7** (1.17 g, 2.0 mmol) in EtOH (20 mL), ammonium hydroxide (28 %, 10 mL) was added and stirred for 3 h at 60 °C. The mixture was neutralized with 6 N HCl, diluted with ethyl acetate (50 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4, which was purified by silica gel column chromatography to give **8 c** (0.87 g, 78.1 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.82 (bs, 1 H), 2.11 (bs, 1 H), 2.71–2.91 (bs, 3 H), 3.25 (bs, 2 H), 4.01 (bs, 1 H), 4.51 (bs, 2 H), 5.35 (m, 2 H), 5.90 (m, 1 H), 6.55 (bs, 1 H), 6.78 (bs, 1 H), 7.26 (m, 9 H), 7.47 (m, 6 H).

8 d–**i** were also prepared as described for preparation of **8 c** using the corresponding amines.

8 d: Yield 82.5 %. ^{1}H-NMR ($CDCl_3$) δ 1.80 (bs, 1 H), 2.11 (bs, 1 H), 2.71–2.94 (bs, 3 H), 3.03 (s, 3 H), 3.25 (bs, 2 H), 4.01 (bs, 1 H), 4.53 (bs, 2 H), 5.35 (m, 2 H), 5.91 (m, 1 H), 6.59 (bs, 1 H), 7.26 (m, 9 H), 7.47 (m, 6 H).

8 e: Yield 77.8 %. ^{1}H-NMR ($CDCl_3$) δ 1.77 (bs, 1 H), 2.16 (bs, 1 H), 2.73–2.91 (bs, 3 H), 3.01 (s, 6 H), 3.29 (bs, 2 H), 4.11 (bs, 1 H), 4.55 (bs, 2 H), 5.35 (m, 2 H), 5.88 (m, 1 H), 6.39 (bs, 1 H), 7.25 (m, 9 H), 7.46 (m, 6 H).

8 f: Yield 73.5 %. ^{1}H-NMR ($CDCl_3$) δ 1.84 (bs, 1 H), 2.28 (bs, 1 H), 2.54 (m, 1 H), 2.60–2.72 (bs, 3 H), 3.55–3.68 (t, 2 H, *J* = 7.0 Hz), 3.77–3.85 (m, 3 H), 4.05 (bs, 1 H), 4.50 (bs, 2 H), 5.25 (m, 2 H), 5.86 (m, 1 H), 7.24 (m, 9 H), 7.46 (m, 6 H).

8 g: Yield 80.7 %. ^{1}H-NMR ($CDCl_3$) δ 1.80 (bs, 1 H), 1.95 (bs, 2 H), 2.06 (bs, 1 H), 2.65–2.79 (bs, 3 H), 3.01 (bs, 1 H), 3.22 (bs, 1 H), 3.55 (bs, 2 H), 3.78 (bs, 2 H), 3.94 (bs, 1 H), 4.55 (bs, 2 H), 5.25 (m, 2 H), 5.96 (m, 1 H), 7.23 (m, 9 H), 7.46 (m, 6 H).

8 h: Yield 69.9 %. ^{1}H-NMR ($CDCl_3$) δ 1.75 (bs, 1 H), 2.05 (bs, 1 H), 2.62–2.87 (bs, 2 H), 3.01–3.38 (bs, 4 H), 3.55 (bs, 2 H), 3.78 (bs, 2 H), 3.94 (bs, 1 H), 4.55 (bs, 2 H), 5.25 (m, 2 H), 5.92 (m, 1 H), 7.23 (m, 9 H), 7.44 (m, 6 H).

8 i: Yield 76.2 %. ^{1}H-NMR ($CDCl_3$) δ 1.70 (bs, 1 H), 2.19 (bs, 1 H), 2.27 (m, 1 H), 2.63–2.90 (bs, 3 H), 3.33–3.69 (bs, 3 H), 3.77–3.83 (bs, 3 H), 4.49 (bs, 2 H), 5.26 (m, 2 H), 5.88 (m, 1 H), 7.27 (m, 9 H), 7.47 (m, 6H).

(2S,4S)-2-[(5-Hydroxymethyl)-1,2,4-oxadiazole-3-ylmethyl]-4-tritylthio-1-(allyloxycarbonyl) pyrrolidine (**9**)

To a solution of **7** (11.67 g, 20.0 mmol) in EtOH (50 mL) and THF (50 mL), $NaBH_4$ (1.14 g, 30.0 mmol) was added slowly at 0 °C. After 1 h the mixture was diluted with H_2O (40 mL), 1 N HCl and ethyl acetate (100 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and the resulting residue purified by silica gel column chromatography to give **9** (7.30 g, 66.5 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.72 (bs, 1 H), 2.12 (bs, 1 H), 2.75–2.99 (bs, 5 H), 3.25 (bs, 2 H), 4.05 (bs, 1 H), 4.53 (bs, 2 H), 5.35 (m, 2 H), 5.90 (m, 1 H), 7.26 (m, 9 H), 7.47 (m, 6 H).

(2S,4S)-2-[(5-Ethoxycarbonyl)-1,2,4-oxadiazole-3-ylmethyl]-4-mercapto-1-(allyloxycarbonyl) pyrrolidine (**10 a**)

To a solution of **9 a** (0.58 g, 1.0 mmol) in CH_2Cl_2 (2 mL), triethylsilane (0.13 g, 1.1 mmol) was added dropwise at 5 °C, followed by TFA (2 mL) addition. After stirring for 30 min at room temperature, the mixture was evaporated under reduced pressure. The residue was dissolved with ethyl acetate and washed with 10 % $NaHCO_3$ and brine. The organic layer was concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography to give **10 a** (0.25 g, 72.0 %) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.41 (t, 3 H, *J* = 7.4 Hz), 1.80 (bs, 1 H), 1.94 (bs, 1 H), 2.55–2.72 (bs, 2 H), 3.01–3.35 (bs, 2 H), 3.55 (bs, 1 H), 3.89 (bs, 1 H), 4.01 (bs, 1 H), 4.14 (q, 2 H, *J* = 7.4 Hz), 4.53 (bs, 2 H), 5.31 (m, 2 H), 5.91 (m, 1 H).

Compounds **10 b**–**i** were prepared by the same procedure as described for **10 a.**

Allyl (1R,5S,6S)-6-[(1R)-hydroxyethyl]-3-[5-(4-ethoxycarbonyl-1,2,4-oxadiazole-2-ylmethyl)-1-(allyloxycarbonyl)pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate (**12 a**)

A solution of allyl (1*R*,5*S*,6*S*)-3-(diphenylphosphoryloxy)-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**11**, 0.45 g, 0.91 mmol) in CH_3CN (50 mL) was cooled to 0 °C under N_2. To this, diisopropylethylamine (0.13 g, 1.0 mmol) and a solution of the mercapto compound **10 a** (0.31 g, 0.91 mmol) in CH_3CN (10 mL), were added. After stirring for 2 h, the mixture was diluted with ethyl acetate, washed with 10 % $NaHCO_3$, brine, and dried over anhydrous $MgSO_4$. Evaporation *in vacuo* gave a foam, which was purified by silica gel chromatography to give **12 a** (0.41 g, 71.4 %) as a yellow foam solid. ^{1}H-NMR ($CDCl_3$) δ 1.25 (d, 3 H, *J* = 6.6 Hz), 1.31–1.37 (dd, 6 H, *J* = 6.6 Hz), 1.98 (bs, 1 H), 2.14 (m, 1 H), 2.46 (m, 1 H), 2.95 (m, 2 H), 3.45 (d, 2 H, *J* = 9.6 Hz), 3.79–3.96 (bs 2 H), 4.01–4.18 (m, 2 H), 4.43 (bs, 1 H), 4.51–4.64 (bs, 4 H), 5.32–5.48 (m, 4 H), 5.90–5.98 (m, 2 H). IR (KBr): 3410 (OH), 3230 (NH), 1720, 1705, 1660 (C=O) cm^{-1}.

(1R,5S,6S)-6-[(1R)-Hydroxyethyl]-3-[5-(4-ethoxycarbonyl-1,2,4-oxadiazole-2-ylmethyl)pyrro lidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylic acid (**13 a**)

Compound **12 a** (0.24 g, 0.38 mmol) and 0.1 g of $Pd(OH)_2$ (20 %) were dissolved in THF/phosphate buffer (pH 7) (1:1, 10 mL each). The mixture was hydrogenated at 50 psi for 1 h. The solution was filtered through Celite and washed with water (2 × 10 mL). The combined filtrate was washed with ethyl ether (2 × 20 mL), lyophilized to give a yellow powder which was purified on a Diaion HP-20 column, and eluted with 2 % THF in water. Fractions with UV absorption at 298 nm were collected and lyophilized again to give the title compound **13 a** (32.0 mg, 17.9 %) as a white powder. UV λ_{max}: 298 nm. ^{1}H-NMR (D_2O) δ 1.06 (d, 3 H, *J* = 6.5 Hz), 1.15 (d, 3 H, *J* = 5.7 Hz), 1.29 (t, 3 H, *J* = 6.1 Hz), 2.04 (bs, 1 H), 2.86 (m, 1 H), 3.01–3.13 (bs, 2 H), 3.53 (bs, 2 H), 3.70 (bs, 1 H), 4.10 (m, 2 H), 4.15 (q, 2 H, *J* = 6.1 Hz), 4.51 (bs, 1 H). IR (KBr): 3470 (OH), 3230 (NH), 1710, 1690, 1660 (C=O) cm^{-1}. FABMS *m/z* 467 $(M + H)^+$.

The preparation of **13 b**–**i** was carried out by a procedure similar to that described for **13 a**

13 b: Yield 25.0 %. UV λ_{max}: 298 nm. ^{1}H-NMR (D_2O) δ 1.11 (d, 3 H, *J* = 6.9 Hz), 1.19 (d, 3 H, *J* = 6.2 Hz), 1.85 (m, 1 H), 2.46–2.67 (bs, 2 H), 2.90 (m, 1 H), 3.01–3.13 (bs, 2 H), 3.20–3.30 (m,

3 H), 3.53 (dd, 1 H, *J* = 5.4 and 5.8 Hz), 3.97 (bs, 1 H), 4.10 (bs, 2 H), 4.43 (m, 1 H). IR (KBr): 3510 (OH), 3300 (NH), 1710, 1690 (C=O) cm^{-1}. FABMS *m/z* 425 $(M + H)^+$.

13 c: Yield 21.1 %. UV λ_{max}: 298 nm. mp 125–129 °C (dec.). ^{1}H-NMR (D_2O) δ 1.08 (d, 3 H, *J* = 7.2 Hz), 1.17 (d, 3 H, *J* = 6.3 Hz), 1.71 (m, 1 H), 2.55–2.66 (m, 2 H), 3.05 (dd, 1 H, *J* = 6.9 and 6.9 Hz), 3.23 (t, 1 H, *J* = 7.5 Hz), 3.32 (bs, 2 H), 3.55 (q, 1 H, *J* = 5.9 Hz), 3.75 (t, 1 H, *J* = 7.1 Hz), 4.11 (bs, 2 H), 4.43 (m, 1 H). IR (KBr): 3480(OH), 3260 (NH), 1730, 1690, 1670 (C=O) cm^{-1}. FABMS *m/z* 438 $(M + H)^+$.

13 d: Yield 24.3 %. UV λ_{max}: 298 nm. ^{1}H-NMR (D_2O) δ 1.07 (d, 3 H, *J* = 6.9 Hz), 1.16 (d, 3 H, *J* = 6.4 Hz), 1.69 (m, 1 H), 2.55–2.72 (m, 2 H), 2.88 (s, 3 H), 3.05 (dd, 1 H, *J* = 6.9 and 6.9 Hz), 3.25 (t, 1 H, *J* = 7.5 Hz), 3.34 (bs, 1 H), 3.57 (q, 1 H, *J* = 5.9 Hz), 3.75 (t, 1 H, *J* = 7.1 Hz), 4.11 (bs, 3 H), 4.44 (m, 1 H). IR (KBr): 3540 (OH), 3270 (NH), 1705, 1680, 1665 (C=O) cm^{-1}. FABMS *m/z* 452 $(M + H)^+$.

13 e: Yield 19.1 %. UV λ_{max}: 296 nm. mp 133–134 °C (dec.). ^{1}H-NMR (D_2O) δ 1.07 (d, 3 H, *J* = 6.9 Hz), 1.16 (d, 3 H, *J* = 6.4 Hz), 1.79 (m, 1 H), 2.65–2.78 (m, 2 H), 2.92 and 2.96 (2 s, 6 H), 3.09 (dd, 1 H, *J* = 6.7 and 6.7 Hz), 3.27 (t, 1 H, *J* = 7.5 Hz), 3.34 (bs, 1 H), 3.57 (q, 1 H, *J* = 5.9 Hz), 3.75 (t, 1 H, *J* = 7.1 Hz), 3.99 (m, 1 H), 4.11 (bs, 2 H), 4.44 (m, 1 H). IR (KBr): 3510 (OH), 3300 (NH), 1710, 1690 (C=O) cm^{-1}. FABMS *m/z* 466 $(M + H)^+$.

13 f: Yield 23.8 %. UV λ_{max}: 298 nm. ^{1}H-NMR (D_2O) δ 1.09 (d, 3 H, *J* = 6.5 Hz), 1.16 (d, 3 H, *J* = 6.0 Hz), 1.68 (m, 1 H), 1.98 (m, 1 H), 2.81 (m, 1 H), 3.23–3.36 (bs, 2 H), 3.57–3.64 (m, 2 H), 3.67–3.77 (m, 2 H), 3.81 (t, 2 H, *J* = 7.1 Hz), 3.98 (m, 2 H), 4.09 (m, 2 H), 4.44 (bs, 1 H). IR (KBr): 3510 (OH), 3270 (NH), 1710, 1680 (C=O) cm^{-1}. FABMS *m/z* 482 $(M + H)^+$.

13 g: Yield 16.9 %. UV λ_{max}: 298 nm. ^{1}H-NMR (D_2O) δ 1.08 (d, 3 H, *J* = 7.8 Hz), 1.22 (d, 3 H, *J* = 7.0 Hz), 1.76–2.04 (bs, 4 H), 2.93 (m, 1 H), 3.23–3.43 (m, 2 H), 3.54–3.65 (m, 3 H), 3.71–3.95 (m, 4 H), 4.01 (bs, 1 H), 4.21–4.29 (bs, 2 H), 4.55 (bs, 1 H). IR (KBr): 348 (OH), 326 (NH), 1730, 1670 (C=O) cm^{-1}. FABMS *m/z* 496 $(M + H)^+$.

13 h: Yield 15.9 %. UV λ_{max}: 298 nm. ^{1}H-NMR (D_2O) δ 1.08 (d, 3 H, *J* = 7.7 Hz), 1.22 (d, 3 H, *J* = 7.1 Hz), 1.77 (m, 1 H), 1.98 (m, 1 H), 2.93 (m, 1 H), 3.23–3.43 (m, 2 H), 3.54–3.65 (m, 3 H), 3.71–3.95 (m, 5 H), 4.01 (bs, 1 H), 4.20–4.28 (bs, 2 H), 4.55 (bs, 1 H). IR (KBr): 3480 (OH), 3260 (NH), 1730, 1670 (C=O) cm^{-1}. IR (KBr): 3500 (OH), 3320 (NH), 1710, 1690 (C=O) cm^{-1}. FABMS *m/z* 512 (M + H)+.

13 i: Yield 20.8 %. UV λ_{max}: 298 nm. ^{1}H-NMR (D_2O) δ 1.09 (d, 3 H, *J* = 6.7 Hz), 1.14 (d, 3 H, *J* = 6.1 Hz), 1.75 (m, 1 H), 2.01 (m, 1 H), 2.83 (m, 1 H), 3.20–3.37 (bs, 2 H), 3.53–3.69 (m, 2 H), 3.67–3.79 (m, 2 H), 3.81 (t, 2 H, *J* = 6.9 Hz), 3.98 (m, 2 H), 4.09 (m, 2 H), 4.44 (bs, 1 H). IR (KBr): 3510 (OH), 3270 (NH), 1710, 1680, 1660 (C=O) cm^{-1}. FABMS *m/z* 481 $(M + H)^+$.

Reference

[1] D. H. Shih, F. Baker, L. Cama, B. G. Christensen, *Heterocycles* **1984,** *21*, 29–40.

[2] I. Sunagawa, H. Matsumura, T. Inoue, M. Fukasawa, M. Kato, *J. Antibiot.* **1990**, *43*, 519–532.

[3] D. E. Sentochnick, G. M. Elipoulos, M. J. Ferraro, R. C. Moellering, *Antimicrob. Agents Chemother.* **1990**, *33*, 1232–1236.

[4] Y. Nagao, Y. Nagase, T. Kumagai, T. Hayashi, Y. Inoue, *J. Org. Chem.* **1992**, *57*, 4243–4249.

[5] K. Ubukata, M. Hikida, M. Yoshida, K. Hishiki, M. Konno, S. Mitsuhashi, *Antimicrob. Agents Chemother.* **1992**, *36*, 994–1000.

[6] C.-H. Oh, S.-Y, Hong, J.-H. Cho, *Korean J. Med. Chem.* **1992**, *2*, 7–16.

[7] C.-H. Oh, S.-Y, Hong, K.-H. Nam, J.-H. Cho, *Korean J. Med. Chem.* **1993**, *3*, 82–92.

[8] C.-H. Oh, E.-R. Woo, S.-Y, Hong, K.-H. Nam, D. Y. Kim, J.-H. Cho, *Korean J. Med. Chem.* **1994**, *4*, 26–34.

[9] C.-H. Oh, J.-H. Cho*, J. Antibiot.* **1994**, *47*, 126–128.

[10] C. B. Jin, I. S. Jung, H.-J. Ku, J. W. Yook, D.-H. Kim, J.-H. Cho, M.S. Kim, C.-H. Oh, *Toxicology* **1999**, *138*, 59–67.

[11] C.-H. Oh, H.-W. Cho, I.-K. Lee, J.-Y. Gong, J.-H. Choi, J.-H. Cho, *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 152–158.

[12] C.-H. Oh, H.-G. Dong, H.-W. Cho, S. J. Park, J. H. Hong, J.-H. Cho, *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 200–206.

[13] C.-H. Oh, H.-W. Cho, J.-H. Cho, *European J. of Med. Chem.* **2002**, *37*, 743–754.