# Synthesis and *in vitro* Activity of Novel 1$\beta$-Methylcarbapenems Having Spiro[2,4]heptane Moieties. Part II

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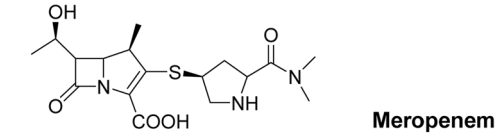
The synthesis of a new series of 1$\beta$-methylcarbapenems having spiro[2,4]heptane moieties is described. Their *in vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria were tested and the effect of substituents at the pyrrolidine ring was investigated. Most of the compounds were found to be more active compared to imipenem against Gram-negative bacteria. A particular compound (**IIIc**) having 7-oxo-5-azaspiro[2,4]heptane moiety showed the most potent antibacterial activity.

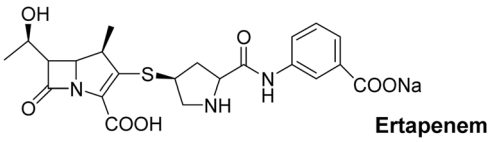
**Key Words :** 1$\beta$-Methylcarbapenems, Antibacterial activity, Spiro[2,4]heptane, Substituent effects

## Introduction

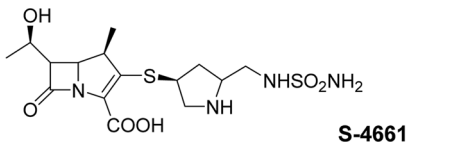
Carbapenems are one of the most potent types of antibacterial agents and are among those used as last resort against infections in the clinical field. Three carbapenems, imipenem,[1,2] meropenem[3] and ertapenem[4] have been marketed so far. It was revealed that 1$\beta$-methylcarbapenems showed not only a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria but also high stability to human renal DHP-I.[5,6] The carbapenem compounds having (3*S*)-pyrrolidin-3-ylthio group at the C-2 position in the carbapenem skeleton are noted for their broad and potent antibacterial activity,[7] and therefore a large number of these derivatives have been synthesized and investigated.[8-12] At present, several carbapenem derivatives such as S-4661,[13] BO-2727[14] and E-1010[15] are under clinical or preclinical studies since the launch of meropenem.



**Meropenem**



**Ertapenem**



**S-4661**

In this paper, we describe the synthesis and structure-activity relationships of carbapenems having spiro[2,4]-heptane moieties and our approach for improvement of the antibacterial activity of the carbapenem is discussed. It has been reported that an spiro[2,4]heptane substituent could enhance largely the activity of quinolone antibiotics especially against Gram-positive and Gram-negative bacteria.[16,17] Based on this fact, a positive effect of a spiro[2,4]heptane moiety on the activity of carbapenem was anticipated.

## Results and Discussion

**Chemistry.** Our synthetic route for the new carbapenems involved the preparation of appropriately protected thiols containing pyrrolidine ring as a side chain and subsequent coupling reaction with a carbapenem diphenylphosphate, followed by deprotection of the resulting protected carbapenems.

7,7-Ethylenedioxy-5-azaspiro[2,4]heptane (**8**) was prepared *via* seven steps from diketene and benzylamine as shown in Scheme 1.[18]

The intermediate **11** was obtained by treatment of the hydroxy compound **9**[19] with 7,7-ethylenedioxy-5-azaspiro-[2,4]heptane (**8**) using *p*-toluenesulfonyl chloride, followed by hydrolysis with acid. The intermediate **11** was converted to the hydroxy compound **12** by treatment of sodium borohydride in THF. Preparation of the oxime **13** was accomplished by treatment of compound **11** with hydroxyl amine (Scheme 2).

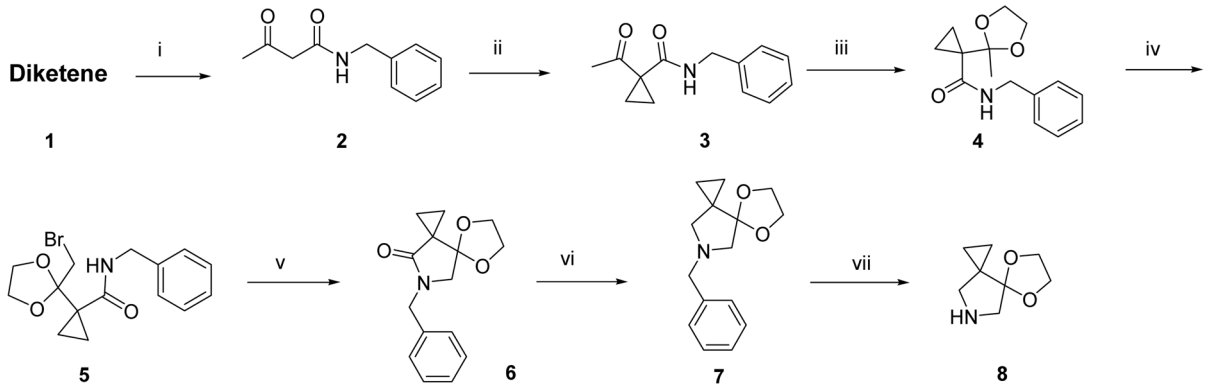
The oxime **13** was converted to the methoxyimino **14**, ethyloxyimino **15** and allyloxyimino **16** by treatment with methyl iodide, ethyl bromide and allyl bromide respectively, in the presence of potassium hydroxide (Scheme 3).

Replacement of the hydroxy group in compound **12** with fluoro moiety in compound **17** was accomplished by treatment of **12** with DAST in $CH_2Cl_2$ (Scheme 4).

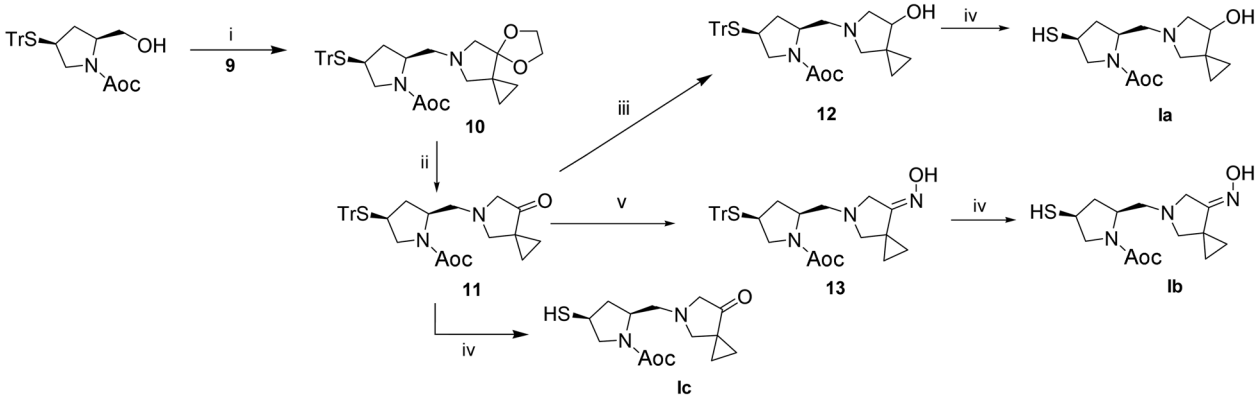
The oxime **13** was converted to the intermediate amine by reduction with lithium aluminum hydride in THF and subsequent treatment with allyl chloroformate to provide **18** (Scheme 5).

Deprotection of trityl group in mercaptanes **Ia-h** was achieved by treatment of **11-18** with trifluoroacetic acid in the presence of triethylsilane.

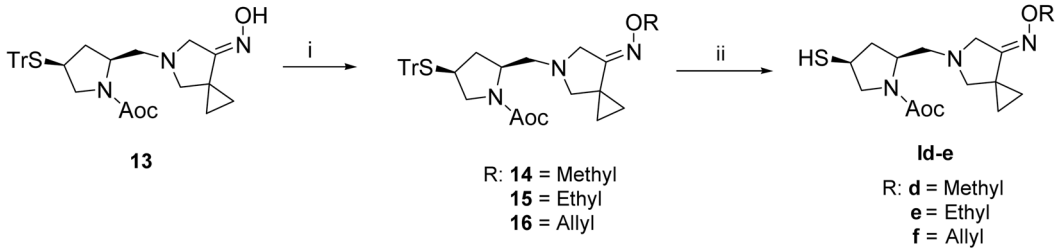
Finally, the reaction of **19** with thiols **Ia-h** in the presence of diisopropylethylamine gave the corresponding 2-substituted carbapenems (**IIa-h**). Deprotection of **IIa-h** by



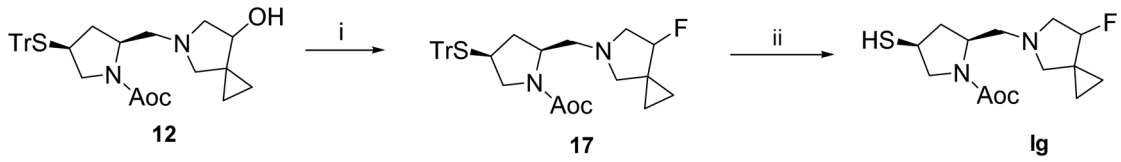

**Scheme 1**. i) benzylamine, EDC. ii) dibromoethane, $K_2CO_3$, DMF. iii) ethylenglycol, *p*-toluensulfonic acid, Benzene. iv) $Br_2$, dioxane, ether. v) NaH, DMF. vi) LAH, THF. vii) Pd/C, $H_2$, EtOH.



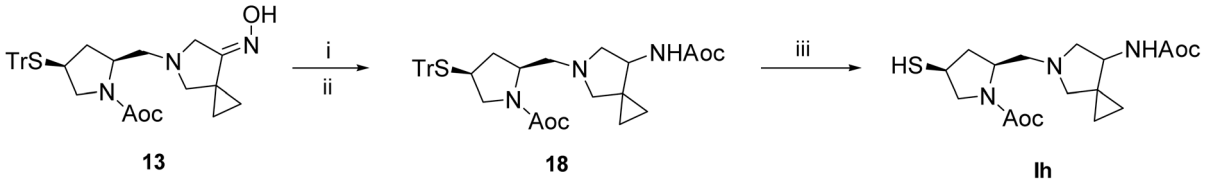

**Scheme 2**. (i) 1. *p*-toluene sulfonyl chloride, TEA, $CH_2Cl_2$. 2. **8**, TEA, $CH_2Cl_2$. (ii) 1 N-HCl. (iii) $NaBH_4$, THF. (iv) trifluoroacetic acid, triethylsilane, $CH_2Cl_2$. (v) hydroxyl amine hydrochloride, TEA, EtOH.



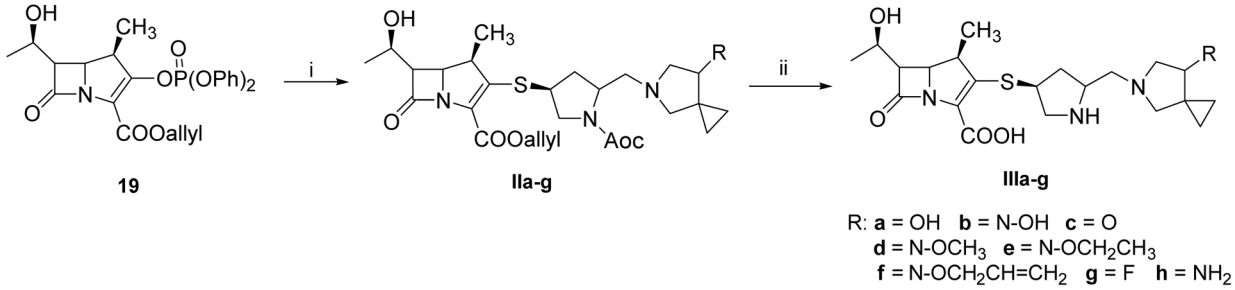

**Scheme 3**. (i) **14**: methyl iodide, **15**: ethyl bromide, **16**: allyl bromide, KOH, DMF. (ii) trifluoro acetic acid, triethylsilane, $CH_2Cl_2$.




**Scheme 4**. (i) DAST, $CH_2Cl_2$. (ii) trifluoroacetic acid, triethylsilane, $CH_2Cl_2$.




**Scheme 5**. (i) lithiumaluminium hydride, THF. (ii) allyl chloroformate, TEA, $CH_2Cl_2$. (iii) trifluoroacetic acid, triethylsilane, $CH_2Cl_2$.




**Scheme 6**. (i) *N,N'*-diisopropylethyl amine, **1a-h**. (ii) tetrakis(triphenylphosphine)palladium, tributyltin hydride, $CH_2Cl_2$.

**Table 1**. *In vitro* antibacterial activity (MIC, $\mu$g/mL) of the carbapenem derivatives (**IIIa-h**)

| STRAINS | **IIIa** | **IIIb** | **IIIc** | **IIId** | **IIIe** | **IIIf** | **IIIg** | **IIIh** | **IPM**[a] |
|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* 1218 | 3.12 | 6.25 | 3.12 | 3.12 | 6.25 | 6.25 | 3.12 | 6.25 | 1.560 |
| *Coagulase negative staphylococci* | 0.198 | 0.198 | 0.098 | 0.098 | 0.391 | 0.391 | 0.098 | 0.098 | 0.049 |
| *Enterococcus faecalis* 2347 | 6.25 | 12.5 | 6.25 | 6.25 | 6.25 | 12.5 | 6.25 | 12.5 | 1.560 |
| *Streptococcus pyogenes* 9889 | 0.025 | 0.025 | 0.013 | 0.013 | 0.049 | 0.098 | 0.013 | 0.013 | < 0.01 |
| *Streptococcus agalaciae* 32 | 0.025 | 0.049 | 0.013 | 0.013 | 0.049 | 0.049 | 0.013 | 0.013 | 0.01 |
| *Haemophilus influenzae* 1210 | 12.5 | 25.0 | 12.5 | 12.5 | 6.25 | 6.25 | 1.56 | 12.5 | 6.250 |
| *Escherichia coil* 04 | 0.098 | 0.198 | 0.198 | 0.198 | 1.56 | 0.195 | 0.098 | 0.781 | 0.391 |
| *Klebsiella peneumoniae* 523 | 0.391 | 0.391 | 0.098 | 0.391 | 3.12 | 1.56 | 0.198 | 0.781 | 0.781 |
| *Citrobacter freundii* 323 | 0.049 | 0.198 | 0.049 | 0.198 | 1.56 | 0.781 | 0.098 | 0.781 | 0.195 |
| *Enterobactor cloacae* 34 | 0.098 | 0.391 | 0.049 | 0.198 | 3.12 | 1.56 | 0.198 | 0.781 | 0.391 |
| *Serratia marcescens* 3349 | 0.781 | 0.781 | 0.098 | 1.563 | 3.12 | 0.781 | 0.391 | 1.563 | 0.391 |
| *Acinetobacter baumannii* 2289 | 12.5 | 50 | 12.5 | 25 | 50 | 50.0 | 12.5 | 50 | 12.5 |
| *Psudemonas aeruginosa* 5455 | 12.5 | 50 | 6.25 | 25 | 50 | 50 | 12.5 | 50 | 12.5 |

[a]Imipenem

treatment with tetrakis(triphenylphosphine)palladium(0) and tributyltin hydride gave the crude products, which were purified by HP-20 column to give the pure carbapenems (**IIIa-h**) (Scheme 6).

**Antibacterial activity.** The MIC was determined by the agar dilution method using test agar. An overnight culture of bacteria in tryptosoy broth was diluted to about $10^6$ cells/mL with the same broth and inoculated with an inoculating device onto agar containing serial two fold dilutions of the test compounds. Organisms were incubated at 37 $^oC$ for 18-20 hours. The MICs of a compound were defined as the lowest concentration that visibly inhibited growth.

The *in vitro* antibacterial activities of new carbapenems (**IIIa-h**) prepared above against both Gram-positive and Gram-negative bacteria are listed in Table 1. For comparison, the MIC values of imipenem are also listed. All the compounds displayed superior or similar antibacterial activities to imipenem against Gram-negative bacteria except **IIIe** and compounds **IIIc** and **IIIg** showed similar antibacterial activities to imipenem against Gram-positive bacteria. In particular, against *Escherichia coli, Klebsiella peneumoniae*, *Citrobacter freundii* and *Enterobactor cloaca*, most of the compounds except compounds **IIIe-f** and **IIIh** were showed to be 2-4 times more active than imipenem.

By comparing the effect of sustituents at C-5 of the pyrrolidine side chain on activity, it was found Unexpectedly that compounds **IIIa** and **IIIh** having hydroxy and amino groups respectively didn't show good activity compared to other compounds. In case of compounds **IIId**, **IIIe**, and **IIIf**, with increasing of bulkiness from methoxy, ethoxy to allyloxy, their activities decreased in order.

As a result, among all of these derivatives, compound **IIIc** having 7-oxo-5-azaspiro[2,4]heptane moiety showed the most potent antibacterial activity while the fluoro substituted compound **IIIg** exhibited the most potent activity against *Haemophilus influenzae*.

## 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 LC/MS system consisted of an HP 1100 series binary pump HPLC system (Agilent, Palo Alto, CA, USA) and an LC/MSD iontrap mass spectrometer equipped with an electrospray ionization source (Agilent, Palo Alto, CA, USA).

**7,7-Ethylenedioxy-4-oxo-5-azaspiro[2.4]heptane 8.** A solution of **7** (2.0 g, 8.0 mmol) and 1.0 g of Pd/C (10%) in EtOH (50 mL) was hydrogenated at 45 psi for 4 h. The solution was filtered through celite and was then evaporated under reduced pressure. The solid was filtered and washed with isopropyl ether, and dry in air to give **8** (1.20 g, 95%) as

clear liquid. $^1$H-NMR (300 MHz, $DMSO_{d6}$): $\delta$ 0.52-0.54 (m, 2H), 0.83-0.89 (m, 2H), 2.61 (s, 2H), 2.66 (s, 2H), 3.63 (s, 2H), 3.79 (s, 2H). $^{13}$C-NMR ($DMSO_{d6}$): $\delta$ 9.71, 26.68, 60.76, 61.75, 64.21, 64.60, 113.88. ESI-MS: 156.0 $[M+H]^+$.

**(2*S*,4*S*)-2-[(7,7-Ethylenedioxy-5-azaspiro[2.4]heptane)-methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 10.** To a solution of **9** (5.4 g, 11.8 mmol) in dry $CH_2Cl_2$ (10 mL) was added drop-wise triethylamine (2.5 mL, 17.7 mmol) and *p*-toluensulfonyl chloride (2.7 g, 14.2 mmol) at 0 °C and mixture was stirred for 6 hr at the same temperature. The reaction solution was neutralized with 10% $NaHCO_3$ and washed with water, brine, and dried over $MgSO_4$. The organic layer was concentrated *in vacuo* to give a residue, which was used without further purification. To a solution of **8** (3.1 g, 20.0 mmol) in dry DMF (30 mL) was added drop-wise triethylamine (3.0 mL, 22.0 mmol) and tosyl residues, and then was stirred for 10 h at 80 °C. The reaction mixture was diluted with ethyl acetate (100 mL) and water (50 mL), and then the organic layer was dried over anhydrous $Na_2SO_4$. The solvent was removed *in vacuo*, and purification was achieved by flash chromatography (EaOAC:hexane = 1:3) to afford **10** (5.0 g, 70%). $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.53 (d, 2H, *J* = 0.5 Hz), 0.85-0.88 (m, 2H), 1.79-1.88 (m, 1H), 2.17-2.46 (m, 2H), 2.67-2.79 (m, 8H), 3.70 (bs, 1H), 3.81 (d, 4H, *J* = 1.7 Hz), 4.44 (bs, 2H), 5.19 (bs, 2H), 5.79-5.91 (m, 1H), 7.19-7.31 (m, 9H), 7.45 (d, 6H, *J* = 7.2 Hz).

**(2*S*,4*S*)-2-[(7-Oxo-5-azaspiro[2.4]heptane)methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 11.** To a solution of **10** (1.8 g, 3.0 mmol) in acetone (50 mL) was added drop-wise 1 *N*-HCl (10.5 mL, 0.13 mol) and was stirred for 8 h at 80 °C. The solution was evaporated under reduced pressure, and 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 (EtOAc:Hexane = 1:5) to give **11** (1.3 g, 78%) as a pale yellow foamy solid. mp: 72-73 °C. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.97-1.00 (m, 2H), 1.25-1.28 (m, 2H), 1.77-1.86 (m, 1H), 2.05-2.21 (m, 1H), 2.58-2.98 (m, 7H), 3.17 (s, 2H), 3.75 (bs, 1H), 4.45 (bs, 2H), 5.20 (bs, 2H), 5.88-5.92 (m, 1H), 7.20-7.32 (m, 9H), 7.45 (d, 6H, *J* = 7.5 Hz). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 17.04, 29.25, 37.11, 41.48, 52.61, 55.76, 59.37, 59.97, 62.83, 65.63, 67.40, 117.20, 144.70, 154.29, 214.45.

**(2*S*,4*S*)-2-[(7-Hydroxyl-5-azaspiro[2.4]heptane)methyl]-4-tritylthio1-(allyloxycarbonyl)pyrrolidine 12.** To a solution of **11** (0.5 g, 0.9 mmol) in THF (10 mL) was added slowly $NaBH_4$ (38 mg, 1.0 mmol) at 0 °C and was stirred for 24 h at 60 °C. The reaction mixture was poured into cold ice water, acidified to pH 4-5 with acetic acid, and then extracted with ethyl acetate. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography (EtOAc:Hexane = 2:1) to give **11** (0.9 g, 72%) as a pale yellow solid.

mp: 58-59 °C. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.51-0.57 (m, 2H), 0.69-0.72 (m, 1H), 0.87-0.91 (m, 1H), 1.73-1.82 (m, 1H), 2.16-2.54 (m, 3H), 2.65-2.78 (m, 7H), 3.58-3.61 (m, 1H), 3.73 (bs, 1H), 4.45 (bs, 2H), 5.19 (bs, 2H), 5.79-5.92 (m, 1H), 7.19-7.31 (m, 9H), 7.60 (d, 6H, *J* = 7.5 Hz), 8.61 (d, 1H, *J* = 4.2 Hz). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 8.05, 13.54, 28.11, 38.18, 41.56, 52.52, 55.89, 61.89, 63.85, 64.36, 65.55, 67.35, 117.11, 126.84, 129.52, 132.93, 144.70, 154.29.

**(2*S*,4*S*)-2-[(7-Hydroxyimino-5-azaspiro[2.4]heptane)-methyl]-4-tritylthio1-(allyloxycarbonyl)pyrrolidine 13.** To a stirred solution of **11** (0.5 g, 0.90 mmol) in EtOH (10 mL) was added dropwise hydroxylamine hydrochloride (0.24 g, 3.2 mmol), triethylamine (0.5 mL, 3.2 mmol) and was stirred for 30 h at 70 °C. The reaction mixture was diluted with ethyl acetate (30 mL) and water (50 mL), and then the organic layer was dried over anhydrous $Na_2SO_4$. The solvent was removed *in vacuo*, and purification by flash chromatography (EaOAC:hexane = 1:2) afforded **13** (0.31 g, 60%). mp: 75-76 °C. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.73-0.80 (m, 2H), 0.93-1.02 (m, 2H), 1.69-1.80 (m, 1H), 2.05-2.22 (m, 1H), 2.37-2.47 (m, 1H), 2.66-2.81 (m, 6H), 3.31-3.57 (m, 2H), 3.67 (bs, 1H), 4.37-4.44 (m, 2H), 5.12 (bs, 2H), 5.73-5.82 (m, 1H), 7.02-7.22 (m, 9H), 7.38 (d, 6H, *J* = 5.8 Hz), 8.46 (bs, 1H). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 15.56, 23.25, 37.35, 41.56, 52.52, 55.36, 55.81, 59.29, 62.12, 65.64, 67.35, 117.14, 126.86, 128.08, 129.53, 132.86, 144.71, 154.39, 164.88.

**(2*S*,4*S*)-2-[(7-Methoxyimino-5-azaspiro[2.4]heptane)-methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 14.** To a solution of **13** (0.5 g, 0.8 mmol) in dry DMF (10 mL) was added portionwise KOH (82.6 mg, 1.4 mmol), methyl iodide (0.05 mL, 0.78 mmol) and the mixture was stirred for 2 h at ice bath. The reaction mixture was diluted with ethyl acetate (50 mL) and water (30 mL), and then the organic layer was dried over anhydrous $Na_2SO_4$. The solvent was removed *in vacuo*, and purification was achieved by flash chromatography (EaOAC:hexane = 1:5) to afford **14** (0.44 g, 71%). $^1$H-NMR (300 MHz, $CDCl_3$): $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.74-0.85 (m, 2H), 0.96-1.03 (m, 2H), 1.69-1.81 (m, 1H), 2.03-2.16 (m, 1H), 2.40-2.45 (m, 1H), 2.65 (bs, 5H), 2.78 (bs, 1H), 3.25-3.40 (m, 2H), 3.64 (bs, 1H), 3.69 (s, 3H), 4.37 (bs, 2H), 5.13 (bs, 2H), 5.73-5.83 (m, 1H), 7.12-7.23 (m, 9H), 7.38 (d, 6H, *J* = 6.0 Hz). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 15.51, 23.31, 31.62, 38.17, 41.06, 55.97, 59.20, 60.02, 61.60, 62.11, 65.54, 67.35, 117.60, 126.87, 127.80, 129.54, 132.93, 144.72, 154.27, 164.21.

The synthesis of compounds **15** and **16** was carried out by the same procedure described for the preparation of **14** using ethyl bromide and allyl bromide, respectively.

**15**: Yield 62%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.82-0.88 (m, 2H), 1.07-1.10 (m, 2H), 1.20 (t, 3H, *J* = 7.1 Hz), 1.75-1.85 (m, 1H), 2.24 (bs, 1H), 2.46-2.53 (m, 1H), 2.72 (s, 5H), 2.85 (bs, 1H), 3.48 (bs, 2H), 3.72 (bs, 1H), 4.00 (q, 2H, *J* = 7.0 Hz), 4.46 (bs, 2H), 5.17-5.25 (m, 2H), 5.79-5.92 (m, 1H), 7.19-7.44 (m, 9H), 7.47 (d, 6H, *J* = 1.3 Hz). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 14.67, 15.23, 15.65, 23.33, 37.39, 41.55, 52.55, 53.43, 55.82, 59.32, 62.15, 65.55, 67.35, 69.20, 117.58, 126.38, 128.06, 129.53, 132.92, 144.72, 154.27, 163.75.

**16**: Yield 57%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.85-0.89

(m, 2H), 1.12-1.20 (m, 2H), 1.63-1.95 (m, 2H), 2.60-2.67 (m, 1H), 3.01-3.08 (m, 2H), 3.32-3.42 (m, 2H), 3.51-3.65 (m, 2H), 4.04-4.08 (m, 2H), 4.38-4.45 (m, 5H), 5.11-5.19 (m, 4H), 5.78-5.84 (m, 2H), 7.14-7.40 (m, 15H). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 15.71, 16.38, 19.84, 35.07, 40.72, 45.4, 46.93, 51.06, 51.69, 55.85, 64.82, 66.19, 74.16, 116.07, 116.75, 125.86, 127.06, 128.48, 131.69, 133.10, 143.58, 153.05, 160.15.

**(2*S*,4*S*)-2-[(7-Fluoro-5-azaspiro[2.4]heptane)methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 17.** To a suspension of compound **12** (0.43 g, 0.78 mmol) in dry $CH_2Cl_2$ (10 mL) was added diethylamine sulfur trifluoride (0.12 mL, 0.94 mmol) at −70 °C, the mixture was stirred at −70 °C for 45 min and then allowed to warm to room temperature. 4 mL of methanol were added to quench the reaction. The solvent was evaporated *in vacuo* and the resulting oil was dissolved in ethyl acetate, neutralized (pH = 7-8) by addition of 32% ammonia solution, and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$ and then evaporated. The crude residue was purified by silica gel column chromatography (EtOAc:hexane = 1:7) to give **17** (0.16 g, 36%). $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.76-1.39 (m, 4H), 1.73-1.76 (m, 1H), 1.89-1.93 (m, 2H), 1.97-2.14 (m, 2H), 2.20-2.50 (m, 4H), 2.55-2.58 (m, 1H), 2.61-2.73 (m, 4H), 2.89-2.93 (m, 1H), 3.65 (bs, 1H), 4.39 (bs, 2H), 5.10-5.21 (m, 2H), 5.76-5.85 (m, 1H), 7.12-7.24 (m, 9H), 7.39 (d, 6H, $J$ = 7.4 Hz). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 16.84, 29.70, 31.17, 37.96, 43.51, 52.50, 56.01, 59.08, 59.96, 65.57, 67.25, 99.46, 102.34, 117.15, 127.23, 128.04, 129.54, 132.96, 144.75, 154.20.

**(2S,4S)-2-[(7-(Allyloxycarbonylamino)-5-azaspiro[2.4]-heptane)methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 18.** To a solution of **13** (0.74 g, 1.3 mmol) in dry THF (10 mL) was added dropwise lithium aluminium hydride (0.2 g, 5.2 mmol) at ice bath and was refluxed for 4 h. The reaction mixture was added ice water (2.0 mL), 15% NaOH (2.0 mL) and filtered off. The filtrate was concentrated under reduced pressure, and the residue was dissolved in chloroform and washed with water and brine. Evaporation of the solvent *in vacuo* gave a crude residue, which was used without further purification. A solution of the above residue in dry $CH_2Cl_2$ (20 mL) was cooled to 0 °C under nitrogen and treated with allyl chloroformate (0.2 g, 1.5 mmol). The mixture was stirred at room temperature 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 $Na_2SO_4$. Purification was achieved by silica gel column chromatography (EtOAc:*n*-Hexane = 1:3) to give **18** (0.46 g, 56%) as a pale yellow oil. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.56-0.95 (m, 4H), 1.73-1.91 (m, 2H), 2.72-2.75 (m, 1H), 3.04-3.21 (m, 3H), 3.45-3.82 (m, 4H), 4.01-4.05 (m, 1H), 4.40-4.59 (m, 4H), 5.13-5.29 (m, 2H), 5.18-5.39 (m, 4H), 5.87-5.98 (m, 2H), 7.20-7.47 (m, 15H). $^{13}$C-NMR (300 MHz, $CDCl_3$): $\delta$ 5.65, 13.34, 24.10, 24.93, 28.33, 36.21, 41.12, 41.65, 52.42, 53.02, 56.91, 65.83, 67.21, 80.06, 117.23, 126.87, 128.08, 129.50, 130.09, 132.72, 144.61, 154.05, 155.31, 169.92.

**Allyl(1*R*,5*S*,6*S*)-6-[(1*R*)-hydroxyethyl]-2-[[5-(7-hydroxy-5-aza-spiro[2.4]heptane)methyl]-1-(allyloxycarbonyl)-pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate IIa.** To a solution of **12** (0.5 g, 0.9 mmol) in $CH_2Cl_2$ (5 mL) triethylsilane (0.35 mL, 2.2 mmol) was added dropwise followed by TFA (1.2 mL) at 5 °C. 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 (**Ia**), which was used without further purification. A solution of allyl (1*R*,5*S*,6*S*)-2-(diphenylphosphoryloxy)-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**19**, 0.50 g, 1.0 mmol) in $CH_3CN$ (10 mL) was cooled to 0 under $N_2$. To this solution was added diisopropylethyl amine (0.13 g, 1.0 mmol) and a solution of the mercapto compound **Ia** in $CH_3CN$ (5 mL). After stirring for 5 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 (EtOAc:*n*-Hexane = 3:1) to give **IIa** (46 mg, 19%) as a yellow amorphous solid. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.54-0.62 (m, 2H), 0.85-0.97 (m, 2H), 1.27 (d, 3H, $J$ = 7.1 Hz), 1.36 (d, 3H, $J$ = 6.1 Hz) 2.00-2.03 (m, 1H), 2.40 (bs, 1H), 2.52-2.59 (m, 1H), 2.64-2.74 (m, 1H), 2.87-3.08 (m, 3H), 3.19-3.25 (m, 2H), 3.36 (bs, 1H), 3.65 (bs, 2H), 4.03-4.08 (m, 2H), 4.22-4.26 (m, 2H), 4.59-4.65 (m, 3H), 4.69 (dd, 1H, $J$ = 5.5 Hz), 4.83 (dd, 1H, $J$ = 5.1 and 4.9 Hz), 5.22-5.33 (m, 3H), 5.43 and 5.47 (2s, 1H), 5.90-5.99 (m, 2H).

The synthesis of compounds **IIb-h** was carried out by the same procedure described for the preparation of **IIa**.

**IIb**: Yield 13%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.84-0.90 (m, 2H), 0.97-1.08 (m, 2H), 1.28 (d, 3H, $J$ = 7.1 Hz), 1.37 (d, 3H, $J$ = 6.2 Hz), 2.02-2.18 (m, 1H), 2.44-2.61 (m, 1H), 2.69-2.81 (m, 4H), 3.24-3.30 (m, 1H), 3.34-3.48 (m, 2H), 3.49-3.69 (m, 3H), 4.04-4.14 (m, 2H), 4.21-4.28 (m, 1H), 4.61 (bs, 3H), 4.70 (dd, 1H, $J$ = 5.5 and 5.6 Hz), 4.84 (dd, 1H, $J$ = 5.6 and 5.4 Hz) 5.22-5.36 (m, 3H), 5.43 and 5.49 (2s, 1H), 5.89-6.03 (m, 2H), 7.32 (bs, 1H).

**IIc**: Yield 21%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.93-0.94 (m, 2H), 1.17-1.22 (m, 5H), 1.29 (d, 3H, $J$ = 6.2 Hz), 1.62-1.72 (m, 2H), 1.95-2.04 (m, 1H), 2.46-2.52 (m, 1H), 2.73-2.85 (m, 1H), 2.97 (bs, 2H), 3.16-3.33 (m, 4H), 3.55 (bs, 1H), 3.99-4.09 (m, 2H), 4.16-4.20 (m, 2H), 4.53 (bs, 2H), 4.62 (dd, 1H, $J$ = 5.5 Hz), 4.77 (dd, 1H, $J$ = 5.4 Hz), 5.15-5.28 (m, 3H), 5.36 and 5.41 (2s, 1H), 5.82-5.95 (m, 2H).

**IId**: Yield 12%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.87-0.90 (m, 2H), 1.09-1.11 (m, 2H), 1.27 (d, 3H, $J$ = 7.3 Hz), 1.36 (d, 3H, $J$ = 6.2 Hz), 2.00-2.10 (m, 1H), 2.49-2.58 (m, 1H), 2.55-2.88 (m, 4H), 3.24-3.28 (m, 2H), 3.33-3.54 (m, 3H), 3.77 (s, 1H), 4.02-4.13 (m, 2H), 4.23 (bs, 2H), 4.60 (bs, 2H), 4.69 (dd, 1H, $J$ = 5.5 and 5.6 Hz), 4.84 (dd, 1H, $J$ = 5.4 Hz), 5.21-5.34 (m, 3H), 5.43-5.48 (2s, 1H), 5.89-6.02 (m, 2H).

**IIe**: Yield 18%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.79-0.82 (m, 2H), 1.02-1.03 (m, 2H), 1.11-1.16 (m, 3H), 1.20 (d, 3H, $J$ = 6.4 Hz), 1.29 (d, 3H, $J$ = 6.2 Hz), 1.94-2.03 (m, 1H), 2.43-2.52 (m, 1H), 2.59-2.81 (m, 4H), 3.17-3.19 (m, 2H),

3.26-3.34 (m, 1H), 3.38-3.42 (m, 1H), 3.43-3.54 (m, 2H), 3.83-4.02 (m, 4H), 4.12-4.20 (m, 2H), 4.53 (bs, 2H), 4.62 (dd, 1H, *J* = 5.5 and 5.5 Hz), 4.77 (dd, 1H, *J* = 5.4 and 5.3 Hz), 5.14-5.27 (m, 3H), 5.36 and 5.41 (2s, 1H), 5.80-5.97 (m, 2H).

**IIf**: Yield 19%. $^1$H-NMR ($CDCl_3$): $\delta$ 0.81-0.90 (m, 2H), 1.08-1.20 (m, 6H), 1.28-1.30 (m, 2H), 1.92-2.13 (m, 2H), 2.52-2.60 (m, 1H), 3.13-3.19 (m, 1H), 3.25-3.37 (m, 4H), 3.40-3.44 (m, 2H), 3.55-3.65 (m, 1H), 3.94-4.09 (m, 2H), 4.17-4.19 (m, 2H), 4.27-4.32 (m, 1H), 4.37-4.44 (m, 4H), 4.50-4.52 (m, 2H), 4.65 (dd, 1H, *J* = 4.2 and 6.8 Hz), 4.75 (dd, 1H, *J* = 5.4 and 5.3 Hz), 5.10-5.29 (m, 5H), 5.35 and 5.41 (2s, 1H), 5.72-5.99 (m, 3H).

**IIg**: Yield 22%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 1.19-1.30 (m, 10H), 1.78-2.13 (m, 3H), 2.22-2.53 (m, 3H), 2.56-2.73 (m, 4H), 2.90-2.92 (m, 1H), 2.98-3.01 (m, 1H), 3.17-3.31 (m, 2H), 3.45-3.71 (m, 2H), 4.01-4.18 (m, 3H), 4.53 (bs, 1H), 4.62 (dd, 1H, *J* = 5.6 and 5.5 Hz), 4.77 (dd, 1H, *J* = 5.4 and 5.6 Hz), 5.15-5.28 (m, 2H), 5.43 and 5.47 (2s, 1H), 5.82-5.95 (m, 2H).

**IIh**: Yield 15%. $^1$H-NMR (300 MHz, $CDCl_3$): $\delta$ 0.49-0.60 (m, 2H), 0.74-0.81 (m, 2H), 1.27 (d, 3H, *J* = 7.2 Hz), 1.37 (d, 3H, *J* = 6.2 Hz), 1.88-2.21 (m, 1H), 2.40 (bs, 1H), 2.51-2.65 (m, 1H), 2.77-2.88 (m, 5H), 3.24-3.25 (m, 1H), 3.35 -3.43 (m, 2H), 3.60-3.61 (m, 1H), 3.79 (bs, 1H), 4.00 (bs, 2H), 4.22-4.27 (m, 1H), 4.53-4.59 (m, 4H), 4.69 (dd, 1H, *J* = 5.5 Hz), 4.83 (dd, 1H, *J* = 5.4 Hz), 5.20-5.32 (m, 6H), 5.52 and 5.57 (2s, 1H), 5.87-6.02 (m, 3H).

**(1*R*,5*S*,6*S*)-6-[(1*R*)-Hydroxyethyl]-2-[5-(7-hydroxy-5-aza-spiro[2.4]heptane)methyl]-pyrrolidin-3-ylthio]-1-methyl-carbapen-2-em-3-carboxylic acid IIIa.** To a stirred solution of **IIa** (46 mg, 0.08 mmol) and $Pd(PPh_3)_4$ (10 mg) in $CH_2Cl_2$ (5 mL) was added dropwise *n*-tributyltin hydride (0.1 mL, 0.25 mmol) at 0 °C and was stirred for 1 h at same temperature. The resulting solution was diluted with water (10 mL) and the organic layer was further washed with water (2 × 10 mL). The combined aqueous layers were washed with ethyl ether (2 × 10 mL) and lyophilized to give a yellow powder which was purified on a Diaion HP-20 column using eluting system of 2% THF in water. Fractions having UV absorption at 298 nm were collected and lyophilized again to give the title compound **IIIa** as a amorphorus solid. Yield 34%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR (300 MHz, $D_2O$): $\delta$ 0.39-0.96 (m, 10H), 1.17-1.34 (m, 1H), 2.32-2.60 (m, 2H), 2.74-2.96 (m, 3H), 3.07-3.29 (m, 4H), 3.36-3.41 (m, 2H), 3.50-3.66 (m, 3H), 3.86-3.90 (m, 2H). -IR (KBr): 3392, 2968, 2932, 1751 cm$^{-1}$. ESI-MS: 438.0 $[M+H]^+$.

The synthesis of compounds **IIIb-h** was carried out by the same procedure as described for the preparation of **IIIa**.

**IIIb**: Yield 28%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR (300 MHz, $D_2O$): $\delta$ 0.89-1.24 (m, 10H), 1.35-1.50 (m, 2H), 2.38-2.50 (m, 2H), 2.66-2.79 (m, 2H), 3.04-3.14 (m, 2H), 3.21-3.34 (m, 2H), 3.36-3.49 (m, 2H), 3.52-3.61 (m, 2H), 3.77-3.88 (m, 1H), 4.08-4.15 (m, 1H). -IR (KBr): 3369, 3275, 2964, 2928, 1752 cm$^{-1}$. ESI-MS: 451.0 $[M+H]^+$.

**IIIc**: Yield 32%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR (300 MHz, $D_2O$): $\delta$ 1.06-1.18 (m, 10H), 1.55-1.59 (m, 1H), 2.06 (m, 1H), 2.82-2.88 (m 1H), 2.96-3.07 (m, 3H), 3.17-3.33 (m, 4H), 3.47-3.51 (m, 1H), 3.73-3.88 (m, 3H), 4.06-4.12 (m, 2H). -IR (KBr): 3420.3, 2967.8, 2930.8, 1740.2 cm$^{-1}$. ESI-MS: 436.0 $[M+H]^+$.

**IIId**: Yield 25%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR (300 MHz, $D_2O$): $\delta$ 0.83-1.02 (m, 4H), 1.11 (d, 3H, *J* = 7.1 Hz), 1.18 (d, 3H, *J* = 6.4 Hz), 1.57-1.80 (m, 1H), 2.62-2.69 (m, 1H), 2.76-2.87 (m, 3H), 2.96-3.03 (m, 1H), 3.24-3.37 (m, 3H), 3.48-3.61 (m, 3H), 3.63-3.71 (m, 3H), 3.75-3.62 (m, 1H), 3.88-3.94 (m, 1H), 4.06-4.16 (m, 2H). -IR (KBr): 3413, 2967, 2936, 1756, 1051 cm$^{-1}$. ESI-MS: 465.0 $[M+H]^+$.

**IIIe**: Yield: 33%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR (300 MHz, $D_2O$): $\delta$ 0.89-1.25 (m, 13H), 1.54-1.63 (m, 1H), 2.62-2.82 (m, 4H), 2.92-3.02 (m, 2H), 3.22-3.33 (m, 2H), 3.50-3.57 (m, 2H), 3.65-3.68 (m, 1H), 3.73-3.76 (m, 1H), 3.85-3.92 (m, 2H), 3.99-4.12 (m, 3H). -IR (KBr): 3414, 2974, 2933, 1733, 1052 cm$^{-1}$. ESI-MS: 479.0 $[M+H]^+$.

**IIIf**: Yield 29%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR ($D_2O$): $\delta$ 0.83-1.29 (m, 10H), 1.78-2.07 (m, 1H), 2.21-2.30 (m, 1H), 2.35-2.70 (m, 2H), 2.86-3.15 (m, 1H), 3.33-3.70 (m, 7H), 3.95-4.16 (m, 2H), 4.11-4.43 (m, 3H), 4.50-4.56 (m, 1H), 5.15-5.34 (m, 2H), 5.81-5.89 (m, 1H). -IR (KBr): 3410, 2970, 1740, 1650 cm$^{-1}$. ESI-MS: 491.0 $[M+H]^+$.

**IIIg**: Yield 36%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR (300 MHz, $D_2O$): $\delta$ 0.98-1.17 (m, 10H), 1.25-1.27 (m, 1H), 1.55-1.62 (m, 1H), 1.96-2.33 (m, 2H), 2.60-2.84 (m, 4H), 2.93-3.11 (m, 1H), 3.67-3.69 (m, 1H), 3.77-3.90 (m, 1H), 3.98-4.14 (m, 3H). -IR (KBr): 3412, 2973, 2941, 1736, 1271, 1248 cm$^{-1}$. ESI-MS: 440.0 $[M+H]^+$.

**IIIh**: Yield 27%. UV $\lambda_{max}$: 298 nm. $^1$H-NMR (300 MHz, $D_2O$): $\delta$ 0.66-1.24 (m, 10H), 1.25-1.55 (m, 1H), 2.31-2.58 (m, 2H), 2.67-2.84 (m, 3H), 2.95-3.04 (m, 1H), 3.08-3.50 (m, 6H), 3.74-3.90 (m, 2H), 4.01-4.14 (m, 2H). -IR (KBr): 3411, 2964, 2927, 1751 cm$^{-1}$. ESI-MS: 437.0 $[M+H]^+$.

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## References

1. Leanza, W.; Wildonger, K.; Miller, T. W.; Christensen, B. G. *J. Med. Chem.* **1979**, *22*, 1435.
2. Birnbaum, J.; Kahan, F. M.; MacDonald, J. S. *Am. J. Med.* **1985**, *78*, (Suppl. 6A), 3.
3. Sunagawa, M.; Matsumure, H.; Inoue, T.; Kato, M. *J. Antibiot.* **1990**, *43*, 519.
4. Gill, C. J.; Jackson, J. J.; Gerckens, L. S.; Pelak, B. A.; Thompson, R. K.; Sundelof, J. G.; Kropp, H.; Rosen, H. *Antimicrob Agents Chemother.* **1998**, *42*, 1996.
5. Shih, D. H.; Baker, F. L.; Christensen, B. G. *Heterocycles* **1984**, *21*, 29.
6. Shih, D. H.; Baker, F. L.; Christensen, B. G. *Tetrahedron Lett.* **1985**, *26*, 587.
7. Sato, M.; Takemura, M.; Atarashi, S.; Higashi, K.; Nagahara, T.; Furukawa, M.; Ikeuchi, T.; Osada, Y. *J. Antibiot.* **1987**, *40*, 1292.
8. Oh, C.-H.; Cho, J.-H. *J. Antibiot.* **1994**, *47*, 126.
9. Jin, C. B.; Jung, I. S.; Ku, H.-J.; Yook, J. W.; Kim, D.-H.; Cho, J.-

H.; Kim, M. S.; Oh, C.-H. *Toxicology* **1999**, *138*, 59.
10. Oh, C.-H.; Cho, H.-W.; Lee, I.-K.; Gong, J.-Y.; Choi, J.-H.; Cho, J.-H. *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 152.
11. Oh, C.-H.; Dong, H.-G.; Cho, H.-W.; Park, S. J.; Hong, J. H.; Cho, J.-H. *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 200.
12. Oh, C.-H.; Cho, H.-W.; Cho, J.-H. *Eur. J. Med. Chem.* **2002**, *37*, 743.
13. Iso, Y.; Irie, T.; Nishino, Y.; Motokawa, K.; Nishitani, Y. *J. Antibiot.* **1996**, *49*, 199.
14. Inoue, K.; Hamana, Y.; Inoue, T.; Fukasawa, M.; Kato, M. *Abstracts of Papers of 34th Intersci. Conf. on Antimicro. Agents Chemother., No. 1*, Orlando, 1994.
15. Sato, N.; Ohba, F. *Drugs of Future* **1996**, *21*, 361.
16. Nada, J. *Cur. Opin. Invest. Drugs* **2000**, *1*(1), 31.
17. Youichi, K.; Shohgo, A.; Katsuhiro, K.; Kenichi, S.; Isao, H. *J. Med. Chem.* **1994**, *37*(20), 3344.
18. Oh, C.-H.; Park, H. B.; Cho, J.-H. *Archive der Pharmazie* **2007**, *340*, 530.
19. Oh, C.-H.; Cho, H.-W.; Cho, J.-H. *Eur. J. Med. Chem.* **2006**, *41*, 825.