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

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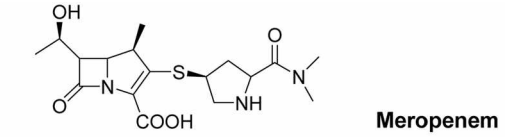
The synthesis of a new series of 1β-methylcarbapenems having spiro[2,4]heptane moieties is described. Their *in vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria were tested and the effect of substituents at the pyrrolidine ring was investigated. Most of the compounds 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.

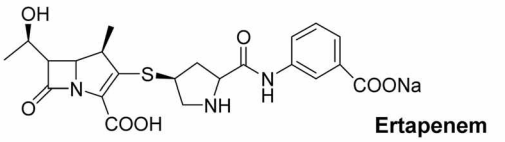
Key Words : 1β-Methylcarbapenems, Antibacterial activity, Spiro[2,4]heptane, Substituent effects

Introduction

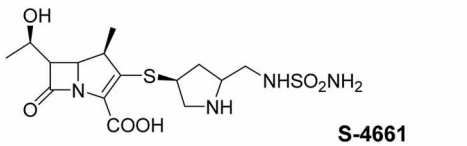
Carbapenems are one of the most potent types of antibacterial agents and are among those used as last resort against infections in the clinical field. Three carbapenems, imipenem,[1,2] meropenem[3] and ertapenem[4] have been marketed so far. It was revealed that 1β-methylcarbapenems showed not only a 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).

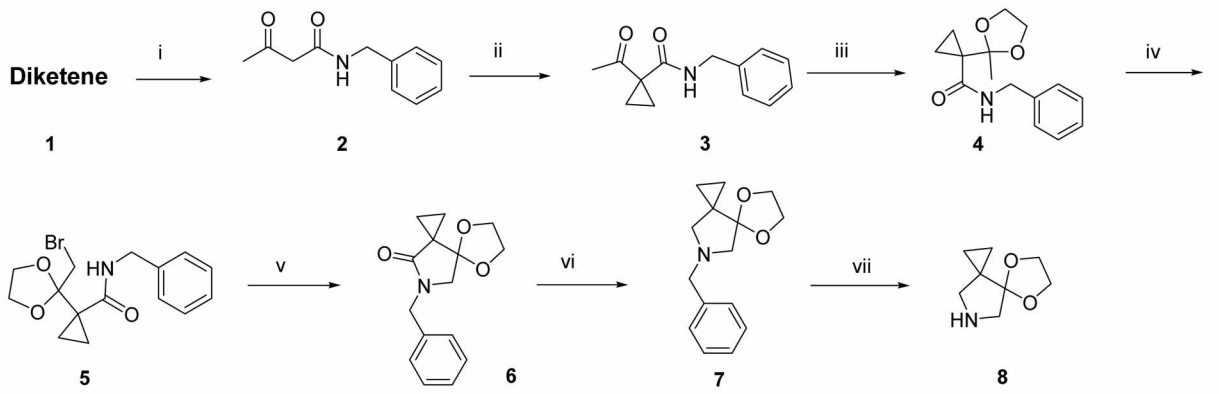
The oxime **13** was converted to the methoxyimino **14**, ethyloxyimino **15** and allyloxyimino **16** by treatment with methyl iodide, ethyl bromide and allyl bromide respectively, in the presence of potassium hydroxide (Scheme 3).

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).

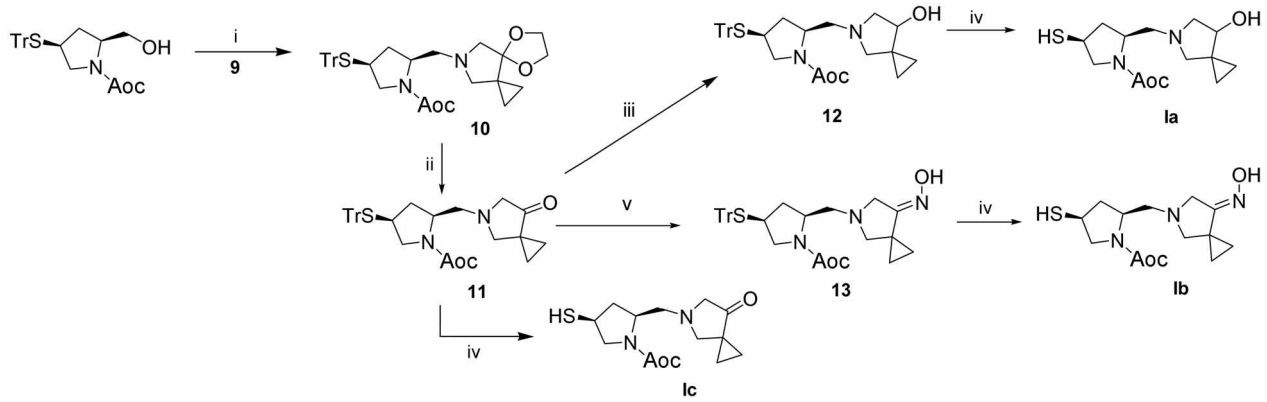
The oxime **13** was converted to the intermediate amine by reduction with lithium aluminum hydride in THF and subsequent treatment with allyl chloroformate to provide **18** (Scheme 5).

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

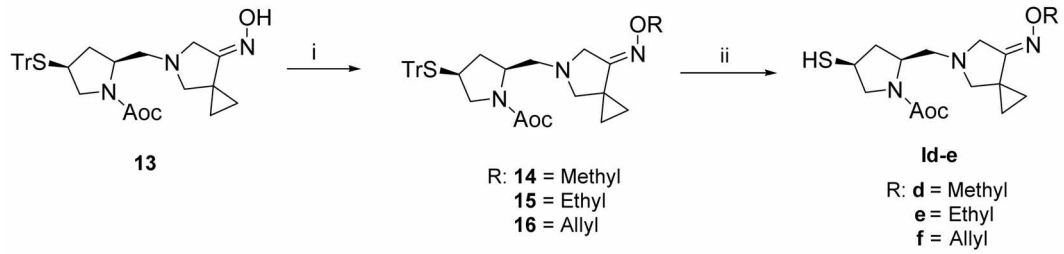
Finally, the reaction of **19** with thiols **Ia-h** in the presence of diisopropylethylamine gave the corresponding 2-substituted carbapenems (**IIa-h**). Deprotection of **IIa-h** by



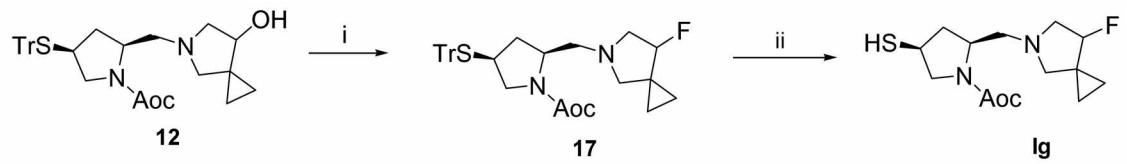
Scheme 1. i) benzylamine, EDC. ii) dibromoethane, K_2CO_3, DMF. iii) ethylenglycol, *p*-toluensulfonic acid, Benzene. iv) Br_2, dioxane, ether. v) NaH, DMF. vi) LAH, THF. vii) Pd/C, H_2, EtOH.



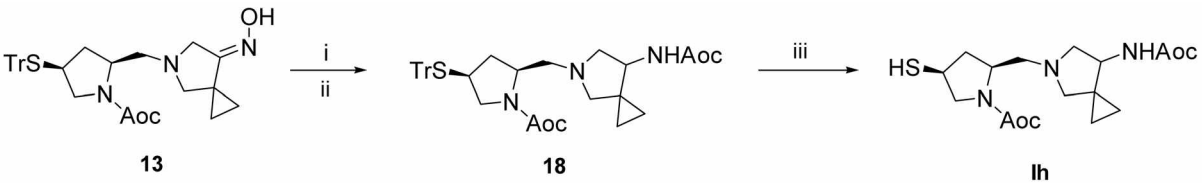
Scheme 2. (i) 1. *p*-toluene sulfonyl chloride, TEA, CH_2Cl_2. 2. **8**, TEA, CH_2Cl_2. (ii) 1 N-HCl. (iii) $NaBH_4$, THF. (iv) trifluoroacetic acid, triethylsilane, CH_2Cl_2. (v) hydroxyl amine hydrochloride, TEA, EtOH.



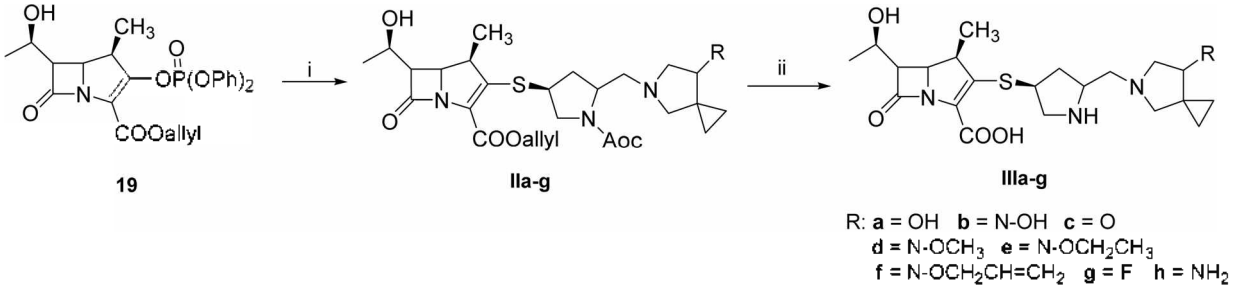
Scheme 3. (i) **14**: methyl iodide, **15**: ethyl bromide, **16**: allyl bromide, KOH, DMF. (ii) trifluoro acetic acid, triethylsilane, CH_2Cl_2.



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, μg/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 °C 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, $DMSO_{d6}$): δ 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 ($DMSO_{d6}$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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 ($CDCl_3$): δ 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, $CDCl_3$): δ 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, $CDCl_3$): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (D_2O): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 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 λ_{max}: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 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.