Full Paper

Synthesis and *In-Vitro* Activity of Novel 1β-Methylcarbapenems Having Spiro[2,4]heptane Moieties

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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 on the pyrrolidine ring was investigated. Most compounds were shown to be more active than the compared meropenem and imipenem against *Escherichia coli*. One particular compound, **IIIb**, having hydroxy a moiety showed the most potent antibacterial activity.

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

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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] (Fig. 1), and ertapenem [4] (Fig. 1) have been marketed so far. In particular, 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 which have a (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 a large number of derivatives have been synthesized and investigated. At present, several carbapenem derivatives such as S-4661 [8] (Fig. 1), BO-2727 [9], and E-1010 [10] are under clinical or preclinical studies since the launch of meropenem.

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Abbreviations: diethylaminosulfur trifluoride (DAST)

We were also interested in this pyrroldin-3-ylthio group and reported that the carbapenem compounds having a pyrrolidin-3-ylthio group at the C-2 position in the carbapenem skeleton are noted for their broad and potent antibacterial activity, and a large number of derivatives have been synthesized and investigated [11 – 15]. In this paper, we describe the synthesis and structure-activity relationships of carbapenem having spiro[2,4]heptane moieties and our approach for improvement of antibacterial activity of the carbapenem is discussed. It is revealed that a 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 the facts, a positive effect of a spiro[2,4]heptane moiety on the activity of carbapenem was anticipated.

Results and discussions

Chemistry

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

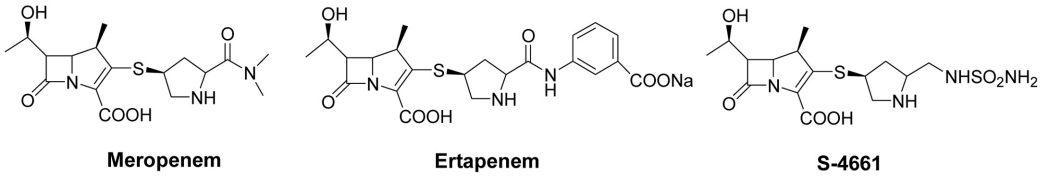
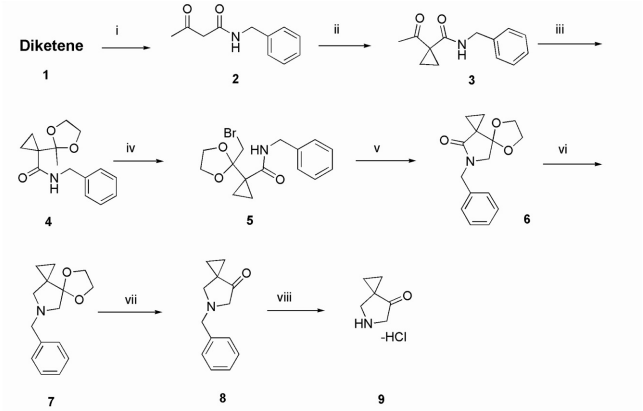
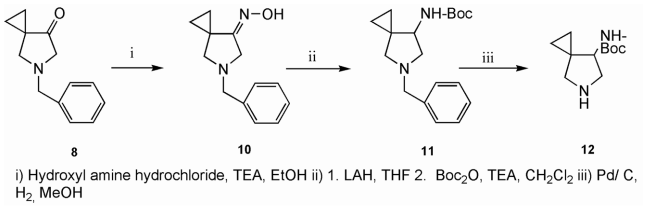


Figure 1. Structures of several carbapenems.



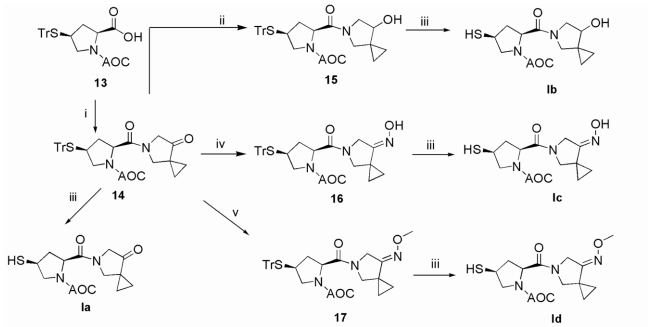
i) Benzylamine, EDC ii) Dibromoethane, K_2CO_3, DMF iii) Ethylenglycol, *p*-Toluensulfonic acid, Benzene
iv) Br_2, Dioxane, Ether v) NaH, DMF vi) LAH, THF vii) 1N HCl, Acetone viii) Pd/C, HCl, H_2, EtOH

Scheme 1. Synthesis route of compounds **1–9**.



i) Hydroxyl amine hydrochloride, TEA, EtOH ii) 1. LAH, THF 2. Boc_2O, TEA, CH_2Cl_2 iii) Pd/ C, H_2, MeOH

Scheme 2. Synthesis route of compounds **8–12**.



i) 1.Oxalyl chloride, CH_2Cl_2 2. **9**, TEA, CH_2Cl_2 ii) $NaBH_4$, THF iii) Trifluoroacetic acid, triethylsilane, CH_2Cl_2
iv) Hydroxyl amine hydrochloride, TEA, EtOH v) Methoxylamine hydrochloride, TEA, MeOH

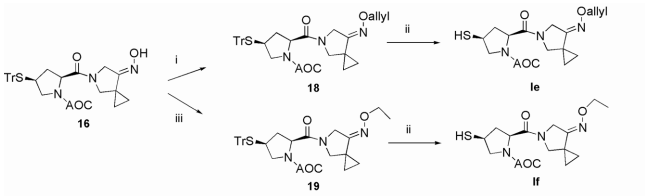
Scheme 3. Synthesis route of compounds **13–17** and **Ia–d**.

7-Oxo-5-azaspiro[2,4]heptane hydrochloride **9** was prepared via eight steps from diketene and benzylamine as shown in Scheme 1. Aceto compound **2** was obtained from diketene and benzylamine, which was then cyclized to compound **3** using 1,2-dibromoethane. The carbonyl group of **3** was protected by ethylenglycol; bromide **5** was prepared by bromination with Br_2, and was converted to spiro[2,4]heptane **6** using sodium hydride. Cyclized compound **6** was reduced with lithium aluminium hydride to give **7**, which was hydrolyzed to ketone **8** with hydrochloric acid. The key compound **9** was obtained by hydrogenation of **8** in the presence of palladium carbon.

Preparation of the oxime **10** was accomplished by treatment of **8** with hydroxyl amine hydrochloride. Then, the reduction of **10** with lithium aluminium hydride in the THF afforded the amine. Since the purification using silica gel column chromatography was difficult, *t*-butoxycarbonylation was carried out by a general method; the product **11** was purified using silica gel column chromatography. Next, the fraction was subjected to deprotection by hydrogenation of **11** in the presence of palladium carbon to give intermediate **12** (Scheme 2).

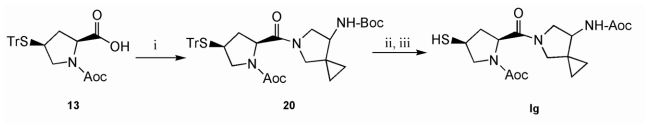
Compound **14** was obtained by treatment of carboxylic acid **13** and **9** using oxalyl chloride in CH_2Cl_2. Reduction of **14** with sodium borohydride in THF gave the hydroxyl compound **15**. Preparation of the oxime **16** and methoxyimino compound **17** were accomplished by treatment of the carbonyl group **14** with hydroxyl and methoxyl amine (Scheme 3). The oxime **16** was converted to the allyloxyimino **18** and ethyloxyimino **19** by treatment of allyl bromide and bromoethane, respectively, in the presence of potassium hydroxide (Scheme 4). The synthesis of **20** was carried out by the same procedure as described for the preparation of **14** using the compound **12**. Replacement of hydroxyl group in compound **15** to the fluoro group in compound **21** was accomplished by treatment of **15** with diethylaminosulfur trifluoride (DAST) in CH_2Cl_2 (Schemes 5 and 6).

Deprotection of the trityl group to mercaptanes **Ia–h** was achieved by treatment of **14–21** with trifluoroacetic acid in the presence of triethylsilane. Finally, the reaction of **22** with thiols **Ia–h** in the presence of diisopropylethylamine gave the corresponding 2-substituted carbapenems **IIa–h**. Deprotection of **IIa–h** by treatment of tet-



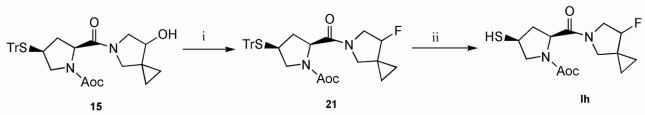
i) Allylbromide, potassium hydroxide, DMF ii) Trifluoroacetic acid, triethylsilane, CH_2Cl_2 iii) Bromoethane, Potassium hydroxide, DMF

Scheme 4. Synthesis route of compounds **16–19** and **Ie–f**.



i) 1.Oxalyl chloride, CH_2Cl_2 2. **12**, TEA, CH_2Cl_2 ii) 1. Trifluoroacetic acid, CH_2Cl_2 2. allylchloroformate, TEA, CH_2Cl_2 iii) Trifluoroacetic acid, triethylsilane, CH_2Cl_2

Scheme 5. Synthesis route of compounds **13, 20** and **Ig**.



i) DAST, CH_2Cl_2 ii) Trifluoroacetic acid, triethylsilane, CH_2Cl_2

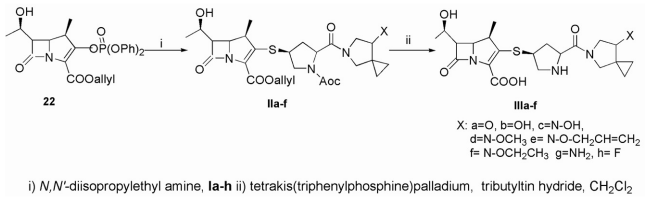
Scheme 6. Synthesis route of compounds **15, 21** and **Ih**.

rakis(triphenylphosphine)palladium(0) and tributyltin hydride gave the crude products, which were purified on a HP-20 column to give the pure carbapenems **IIIa–h** (Scheme 7).

Antibacterial activity

The MICs were 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 twofold 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 the 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 and meropenem are also listed. All the compounds displayed superior or similar antibacterial activities against *gram*-positive bacteria to meropenem, and *gram*-negative bacteria to imipenem. In particular, against *Escherichia coli,* most compounds were shown to be more active than the compared meropenem and imipenem. The compounds **IIIb** and **IIIc**, having the hydroxy group and oxime group, were generally more potent than other groups. As a results, among them, compound **IIIb** having 7-hydroxy-5-azaspiro[2,4]-heptane moiety showed the most potent antibacterial activity. As expected, the amino-substituted compound **IIIg** exhibited the most potent activity against *Pseudomonas aeruginosa*.

Comparative *in-vitro* activities of **IIIb**, meropenem, and imipenem against 40 bacterial strains are summarized in Table 2. The selected carbapenem **IIIb** possessed excel-



i) *N,N'*-diisopropylethyl amine, **Ia-h** ii) tetrakis(triphenylphosphine)palladium, tributyltin hydride, CH_2Cl_2

Scheme 7. Synthesis route of compounds **22, IIa–f** and **IIIa–f**.

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]	MPM[b]
Staphylococcus aureus 1218	6.25	1.560	1.560	3.125	6.25	6.25	1.56	3.125	1.560	6.250
Coagulase negative staphylococci	0.198	0.198	0.098	0.198	0.391	0.198	0.198	0.198	0.049	0.098
Enterococcus faecalis 2347	6.25	3.125	3.125	6.25	6.25	6.25	6.25	6.25	1.560	12.50
Streptococcus pyogenes 9889	0.025	0.013	0.013	0.049	0.049	0.025	0.025	0.025	<0.01	0.013
Streptococcus agalaciae 32	0.049	0.025	0.013	0.049	0.049	0.049	0.025	0.049	0.01	0.049
Streptococcus pneumoniae 0025	0.025	0.013	0.013	0.049	0.098	0.049	0.013	0.025	<0.01	0.01
Haemophilus influenzae 1210	3.125	1.560	1.560	3,125	6.25	6.25	6.25	3.125	6.250	3.125
Escherichia coil 04	0.025	0.025	0.013	0.049	0.195	0.195	0.049	0.025	0.391	0.098
Klebsiella peneumoniae 523	0.049	0.025	0.025	0.098	0.391	0.781	0.098	0.049	0.781	0.025
Citrobacter freundii 323	0.025	0.013	0.025	0.098	0.781	0.781	0.098	0.025	0.391	0.025
Enterobactor cloacae 34	0.098	0.049	0.195	0.098	0.391	0.781	0.098	0.049	0.781	0.025
Serratia marcescens 3349	0.098	0.049	0.098	0.391	0.391	0.781	0.098	0.049	0.781	0.049
Acinetobacter baumannii 2289	12.5	12.5	6.25	12.5	25.0	50.0	12.5	6.25	12.500	12.5
Psudemonas aeruginosa 5455	6.25	3.125	6.25	12.5	50	50	0.781	6.25	3.125	1.563

[a] imipenem.
[b] meropenem.

Table 2. Comparative *in-vitro* antibacterial activity of **IIIb**, meropenem, and imipenem against 40 strains (MIC, μg/mL).

Organism	IIIb	IPM	MPM	Organism	IIIb	IPM	MPM
SStaphylococcus aureus giorgio	0.02	0.01	0.10	*Salmonella paratyphi A*	0.10	0.10	0.03
SStaphylococcus aureus 209P	0.05	0.01	0.10	*Salmonella typhimurium*	0.10	0.40	0.05
SStaphylococcus aureus 503	0.02	<0.01	0.05	*Salmonella oranienberg*	0.10	0.40	0.05
Micrococcus luteus ATCC 9341	0.01	0.01	0.05	*Salmonella Typhi*	0.03	0.05	0.01
Streptococcus facium 77A	<0.01	<0.01	0.01	*Salmonella orion*	0.10	0.20	0.10
Streptococcus agalctiae B	0.01	0.01	0.05	*Salmonella give*	0.10	0.20	0.03
Streptococcus durans D	0.10	0.10	0.80	*Klebsiella pneumonise 477*	0.05	0.20	0.05
Bacillus subtilts ATCC 6633	0.05	0.03	0.05	*Enterobacter cloacae*	0.01	0.10	0.01
Bacillus megatherium	0.05	0.03	0.05	*Enterobacter cloacae 417*	0.02	0.10	0.01
Pseudomonas aeruginosa 9027	1.60	0.80	0.40	*Serratia marcescens 370*	0.20	0.20	0.05
Pseudomonas aeruginosa 77/2	1.60	0.80	0.80	*Serratia marcescens 6093*	0.20	0.40	0.05
Pseudomonas aeruginosa 110/2	0.80	0.80	0.40	*Serratia marcescens 14273*	0.40	0.80	0.20
Pseudomonas aeruginosa 880/2	0.40	0.80	0.20	*Proteus mirabilis 112/3*	0.20	0.20	0.10
Pseudomonas cepacia	0.80	0.80	0.40	*Proteus mirabilis 174/3*	0.20	0.10	0.10
Escherchia coil 086	0.02	0.10	0.05	*Proteus vulgaris 868*	0.40	0.10	0.10
Escherchia coil 0114	0.02	0.10	0.05	*Proteus rettgeri 936*	0.40	0.20	0.10
Escherchia coil 0126	0.02	0.10	0.05	*Proteus rettgeri 937*	0.40	0.20	0.05
Escherchia coil V6311/65	0.02	0.05	0.05	*Pasteurella multocida*	0.05	<0.01	0.05
Escherchia coil TEM	0.01	0.20	0.02	*Corynebacterium diphtheriae*	0.01	0.02	0.05
Escherchia coil 1507	0.02	0.10	0.02	*Corynebacterium pyogenes*	0.01	0.01	0.03

lent *in-vitro* activity against 40 target pathogens except *P. aeruginosa* and superior or similar antibacterial activities against *gram*-positive bacteria to meropenem, and against *gram*-negative bacteria to imipenem. Against *Escherichia coli, Corynebacterium diphtheriae,* **IIIb** was 2 to 3 times more active than the compared meropenem and imipenem.

We would like to thank Hawon Pharmaceuticals Co. which supported us with fund.

Experimental

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

N-Benzyl-3-oxo-butyramide 2

To a solution of benzylamine (79.6 g, 0.74 mol) in dry dichloroethane (250 mL) was added dropwise fresh diketene (58.8 g, 0.68 mol) for 2 h at 0°C and was stirred for 15 h at 5°C. The mixture was evaporated under reduced pressure. After the residue was washed with isopropyl ether, the solid was filtered and washed with isopropyl ether, and dried in air to give **2** (115.6 g, 89%) as a white solid. Mp. 97 – 98°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H), 3.44 (s, 2H), 4.45 (d, 2H, *J* = 4.2 Hz), 7.22 – 7.35 (m, 5H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 31.13, 43.51, 49.45, 127.53, 127.72, 128.72, 137.89, 165.45, 204.70.

1-Acetyl-1-benzylamide-cyclopropane 3

To a solution of **2** (3.0 g, 5.2 mmol) and potassium carbonate (4.3 g, 31.4 mmol) in dry DMF (20 mL) was added 1,2-dibromoethane (3.8 g, 20.4 mmol) and was stirred for 12 h at room temperature. The reaction mixture was poured into cold ice water, and then extracted with ethyl acetate, and washed with water and brine. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 4) to give **3** (3.1 g, 92%) as a white solid. Mp. 69 – 71°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.50 – 1.56 (m, 2H), 1.83 – 1.92 (m, 2H), 1.95 (s, 3H), 4.49 (d, 2H, *J* = 5.7 Hz), 7.22 – 7.35 (m, 5H), 9.23 (bs, 1H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 19.32, 25.12, 34.32, 43.66, 127.24, 127.61, 128.61, 138.48, 168.94, 207.66.

1-(1,1-Ethylenedioxyethyl)-1-benzylamidecyclopropane 4

A solution containing compound **3** (95.1 g, 0.44 mol), ethyleneglycol (129.1 mL, 1.9 mol) and *p*-toluenesulfonic acid (5.0 g, 26.2 mmol) in benzene (400 mL) was heated at reflux temperature overnight using a Dean – Stark system. The reaction mixture was washed with cold aqueous $NaHCO_3$ and the aqueous layer extracted with EtOAc. The combined organic layers were washed with brine, dried, and concentrated under reduced pressure. Flash chromatography, eluting with (EtOAc : *n*-hexane; 1 : 5) gave **4** (90.5 g, 79%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.85 – 0.89 (m, 2H), 1.17 – 1.12 (m, 2H), 1.50 (s, 3H), 3.94 (s, 4H), 4.5 (d, 2H, *J* = 5.7 Hz), 7.24-7.37 (m, 5H), 7.77 (bs, 1H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 11.03, 24.26, 31.47, 43.73, 65.18, 108.80, 127.18, 127.49, 128.61, 138.90, 171.45.

1-(2-Bromo-1,1-ethylenedioxyethyl)-1-benzylamidecyclopropane 5

To a solution of **4** (10.0 g, 38.3 mmol) in dry dioxane (80 mL) was added dropwise bromine (3.8 mL, 42.0 mmol) at ice bath and was stirred for 5 h at room temperature. The mixture was evaporated under reduced pressure. The residue was dissolved with chloroform and washed with 2.5% sodium thiosulfate, water and dried over anhydrous Na_2SO_4. The organic layer was concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 5) to give **5** (8.9 g, 68%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.90–0.93 (m, 2H), 1.20–1.13 (m, 2H), 3.75 (bs, 1H), 4.01–4.24 (m, 2H), 4.06–4.24 (m, 2H), 4.46 (d, 2H, *J* = 5.7 Hz), 7.26–7.37 (m, 5H), 7.70 (bs, 1H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 11.34, 29.68, 36.94, 43.78, 66.89, 107.43, 127.32, 127.46, 128.70, 138.60, 170.50.

5-Benzyl-7,7-ethylenedioxy-4-oxo-5-azaspiro[2.4]heptane 6

To a solution of **5** (8.9 g, 26.1 mmol) in dry DMF (40 mL) was added sodium hydride (1.8 g, 44.4 mmol, 60%) in an ice bath and was stirred for 2 h at the same temperature. The reaction mixture was poured into ice-cold water, and then extracted with ethyl acetate, and washed with water and brine. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 5) to give **6** (6.7 g, 98%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.08–1.12 (m, 2H), 1.17–1.24 (m, 2H), 3.36 (s, 2H), 3.85 (s, 4H), 4.54 (s, 2H), 7.22-7.36 (m, 5H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 11.38, 29.20, 46.47, 56.64, 64.82, 108.96, 127.55, 128.02, 128.73, 136.11, 137.73.

5-Benzyl-7,7-ethylenedioxy-5-azaspiro[2.4]heptane 7

To a solution of **6** (6.0 g, 23.1 mmol) in dry THF (40 mL) was added dropwise lithium hydride (3.3 g, 87.9 mmol) in an ice bath and was refluxed for 4 h. The reaction mixture was added to cold ice water, 15% NaOH (3.4 mL) and filtered off and the filtrate was concentrated under reduced pressure. After the residue was dissolved with chloroform and washed with water, brine and concentrated. Column chromatography using 15% EtOAc/hexane as eluent afforded **7** (5.6 g, 99%) as a pale clear oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 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, 4H), 7.22-7.36 (m, 5H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 9.71, 26.68, 60.76, 61.75, 64.21, 64.60, 113.88, 126.98, 128.16, 128.97, 138.33.

5-Benzyl-7-oxo-5-azaspiro[2.4]heptane 8

To a solution of **7** (3.0 g, 12.2 mmol) in acetone (80 mL) was added dropwise 1N HCl (36.3 mL, 36.3 mmol) and was refluxed for 15 h. The mixture was evaporated under reduced pressure. To the residue was added ice-cold water (50 mL) and neutralized with 10% sodium hydrogen carbonate, and then extracted with chloroform. The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude residue was purified by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 7) to give **8** (2.4 g, 96%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.96–0.99 (m, 2H), 1.25–1.28 (m, 2H), 2.97 (s, 2H), 3.19 (s, 2H), 3.76 (s, 2H), 7.26–7.35 (m, 5H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 16.94, 29.54, 58.92, 61.08, 62.47, 127.41, 128.45, 128.87, 137.55, 214.27.

7-Oxo-5-azaspiro[2.4]heptane hydrochlorid 9

To a solution of **8** (1.2 g, 6.0 mmol) and 0.6 g of Pd/C (10%) in EtOH (30 mL) was added dropwise conc. HCl (0.7 mL, 8.4 mmol) and was hydrogenated at 50 psi for 2.5 h. The solution was filtered through celite and was evaporated under reduced pressure. After the residue was washed with isopropyl ether, the solid was filtered and washed with isopropyl ether, and dry in air to give **9** (0.8 g, 91%) as a brown solid. ^{1}H-NMR (300 MHz, CD_3OD): δ 1.31–1.35 (m, 2H), 1.37–1.41 (m, 2H), 3.81 (bs, 2H), 3.89 (bs, 2H). ^{13}C-NMR (300 MHz, CD_3OD): δ 18.33, 27.74, 50.67, 51.70, 207.27.

5-Benzyl-7-hydroxyimino-5-azaspiro[2.4]heptane 10

To a stirred solution of **8** (3.96 g, 18.4 mmol) in EtOH (40 mL) was added hydroxylamine hydrochloride (4.2 g, 64.4 mmol), triethylamine (8.7 mL, 64.4 mmol) and was stirred for 7 h at 60°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. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 1) to give **10** (4.1 g, 96%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.82–0.92 (m, 2H), 1.00–1.24 (m, 2H), 2.75 (bs, 2H), 3.56 (bs, 2H), 3.73 (bs, 2H), 7.24–7.36 (m, 5H), 8.02 (bs, 1H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 15.30, 23.39, 54.71, 60.69, 61.10, 127.29, 128.39, 129.98, 137.86, 165.19.

5-Benzyl-7-(*t*-butyloxycarbonylamino)-5-azaspiro[2.4]heptane 11

To a solution of **10** (2.8 g, 13.1 mmol) in dry THF (40 mL) was added dropwise lithium aluminium hydride (2.0 g, 52.3 mmol) in an ice bath and was refluxed for 4 h. To the reaction mixture was added ice-cold water (2.0 mL), 15% NaOH (2.0 mL) and filtered off and the filtrate was concentrated under reduced pressure. After the residue was dissolved with 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 above residue in dry CH_2Cl_2 (20 mL) was cooled to 0°C under nitrogen and treated with di-*t*-butyldicarbonate (2.0 g, 9.0 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 by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 3) gave **11** (1.9 g, 76%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.42–0.45 (m, 1H), 0.57–0.58 (m, 1H), 0.71–0.78 (m, 2H), 1.41 (s, 9H), 2.33 (d, 1H, *J* = 8.9 Hz), 2.66 (d, 2H, *J* = 8.9 Hz), 2.87–2.92 (m, 1H), 3.53-3.65 (q, 2H), 3.82–3.84 (m, 1H), 4.98 (d, 1H, *J* = 5.0 Hz), 7.20–7.31 (m, 5H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 8.78, 14.16, 26.60, 28.38, 55.89, 60.42, 61.98, 62.02, 79.06, 127.06, 128.26, 128.80, 138.57, 155.67.

7-(*t*-Butyloxycarbonylamino)-5-azaspiro[2.4]heptane 12

To a solution of **11** (0.5 g, 1.58 mmol) and 0.4 g of Pd/C (10%) in EtOH (30 mL) was hydrogenated at 45 psi for 2.5 h. The solution was filtered through celite and it was evaporated under reduced pressure. After the residue was washed with isopropyl ether, the solid was filtered and washed with isopropyl ether, and dry in air to give **12** (0.32 g, 95%) as clear liquid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.51–0.62 (m, 2H), 0.79 (s, 2H), 1.43 (s, 9H), 2.72–3.17 (m, 4H), 3.33 (s, 1H), 3.68 (s, 1H), 4.50 (s, 1H). ^{13}C-NMR (300 MHz, CD_3Cl): δ 6.94, 13.94, 26.99, 28.30, 53.91, 56.86, 79.24, 155.63.

(2*S*,4*S*)-2-[(7-Oxo-5-aza-spiro[2.4]heptane)carbonyl]-4-tritylthio-1-(allyloxycarbonyl) pyrrolidine 14

To a solution of **13** (2.0 g, 4.2 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise oxalyl chloride (3.8 mL, 42.0 mmol) and was

stirred for 2 h at room temperature. The mixture was evaporated under reduced pressure. To an ice-cold solution of **9** (0.8 g, 4.2 mmol) and triethylamine (1.4 mL, 10.6 mmol) in dry CH_2Cl_2 (20 mL) was slowly added to the above solution at 0°C and mixture was stirred for 30 min at room temperature. The mixture was diluted with H_2O (50 mL) and CH_2Cl_2 (100 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and the resulting residue was purified by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 3) to give **14** (2.1 g, 83%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.11–1.16 (m, 2H), 1.43–1.47 (m, 2H), 1.88–2.10 (m, 2H), 2.60–2.85 (m, 2H), 3.10–3.25 (m, 2H), 3.80–3.92 (m, 2H), 3.95–4.20 (m, 2H), 4.32 and 4.38 (2s, 1H), 4.45–4.52 (m, 2H), 5.20–5.31 (m, 2H), 5.82–5.86 (m, 1H), 7.21–7.47 (m, 15H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 19.37, 19.79, 27.62, 36.11, 41.72, 50.62, 52.00, 53.72, 57.26, 65.94, 67.27, 117.27, 126.87, 128.09, 129.48, 132.58, 144.55, 154.13, 170.49, 210.10.

(2*S*,4*S*)-2-[(7-Hydroxy-5-aza-spiro[2.4]heptane)carbonyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 15

To a solution of **14** (1.5 g, 2.5 mmol) in THF (30 mL) was added slowly $NaBH_4$ (0.2 g, 4.9 mmol) at 0°C and was stirred for 2 h at room temperature. The reaction mixture was poured into ice-cold 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; 1 : 2) to give **15** (1.2 g, 78%) as a pale yellow oil.^{1}H-NMR ($CDCl_3$): δ 0.50–0.78 (m, 3H), 0.79–1.00 (m, 1H), 1.81–2.05 (m, 2H), 2.72–2.75 (m, 1H), 3.02–3.09 (m, 2H), 3.56–3.61 (m, 1H), 3.67–3.73 (m, 2H), 3.82–4.01 (m, 1H), 4.10–4.15 (m, 2H), 4.40–4.53 (m, 2H), 5.18–5.24 (m, 2H), 5.82–5.85 (m, 1H), 7.22–7.64 (m, 15H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 12.76, 13.07, 26.24, 36.08, 41.67, 52.00, 52.21, 54.54, 56.85, 67.23, 74.99, 117.00, 126.88, 128.09, 129.52, 132.76, 144.65, 154.05, 169.91.

(2*S*,4*S*)-2-[(7-Hydroxyimino-5-aza-spiro[2.4]heptane)-carbonyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 16

To a stirred solution of **14** (1.8 g, 3.2 mmol) in EtOH (20 mL) was added hydroxylamine hydrochloride (0.7 g, 11.3 mmol), triethylamine (1.5 mL, 11.3 mmol) and was stirred for 7 h at 60°C. The reaction mixture was diluted with ethyl acetate (50 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 (EtOAc : *n*-hexane; 1 : 1) afforded **16** (1.6 g, 82%) as a pale yellow oil. ^{1}H-NMR (300 MHz $CDCl_3$): δ 0.93–0.94 (m, 2H), 1.16–1.26 (m, 2H), 1.72–2.05 (m, 2H), 2.72–2.88 (m, 1H), 3.07–3.12 (m, 2H), 3.61–3.74 (m, 2H), 4.15–4.20 (m, 2H), 4.41–4.48 (m, 2H), 5.18–5.29 (m, 2H), 5.71–5.95 (m, 1H), 7.20–7.47 (m, 15H), 8.25–8.71 (m, 1H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 15.79, 17.40, 22.35, 35.90, 44.70, 47.41, 52.08, 53.58, 56.95, 60.43, 66.03, 67.29, 117.25, 126.95, 128.13, 129.48, 132.62, 144.59, 154.28, 160.88, 170.29.

(2*S*,4*S*)-2-[(7-Methoxyimino-5-aza-spiro[2.4]heptane)carbonyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 17

To a solution of **14** (1.0 g, 1.8 mmol) in dry MeOH (20 mL) was added dropwise methoxylamine hydrochloride (1.1 mL, 5.3 mmol, 35%), triethylamine (0.8 mL, 5.7 mmol) and was stirred for 10 h 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 (EtOAc : hexane; 1 : 2) to give **17** (0.7 g, 68%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 0.92–0.97 (m, 2H), 1.18–1.26 (m. 2H), 1.87–2.14 (m, 2H), 2.72–2.76 (m, 1H), 3.08–3.11 (m, 2H), 3.21–3.61 (m, 2H), 3.77–3.81 (m, 3H), 4.15–4.21 (m, 2H), 4.29 and 4.34 (2s, 1H), 4.40–4.54 (m, 2H), 5.18–5.21 (m, 2H), 5.81–5.85 (m, 1H), 7.19–7.47 (m, 15H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 15.56, 16.36, 19.75, 35.06, 40.70, 46.70, 51.10, 52.23, 55.79, 60.88, 64.78, 66.23, 116.04, 125.82, 127.05, 128.45, 131.67, 143.57, 153.00, 159.88, 169.28.

(2*S*,4*S*)-2-[(7-Allyloxyimino-5-aza-spiro[2.4]heptane)carbonyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 18

Oxime **16** (0.6 g, 1.0 mmol), anhydrous DMF (15 mL), and allyl bromide (0.43 mL, 5.5 mol), were placed in a 100 mL round-bottomed flask under argon. The reaction flask was then cooled in ice, and 0.1 g (about 2.0 mmol) of 85% KOH pellets crushed into a fine powder were added all at once. The reaction flask was then heated in an oil bath at ~40°C for 3 h with vigorous stirring. After this period, the reaction mixture was poured into 20 mL of water and the resulting mixture was extracted with EtOAc (2 × 20 mL) and washed with saturated brine, dried over $MgSO_4$, and concentrated *in vacuo* to afford 612 mg. It was purified by silica gel column chromatography (EtOAc : *n*-hexane; 1 : 2) to give **18** (0.5 g, 83%) as a pale yellow oil. ^{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), 2.66–2.69 (m, 1H), 3.01–3.05 (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, 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, 169.12.

(2*S*,4*S*)-2-[(7-Ethyloxyimino-5-aza-spiro[2.4]-heptane)carbonyl]-4-tritylthio-1-(allyloxycarbonyl)-pyrrolidine 19

Oxime **16** (0.6 g, 1.0 mmol), anhydrous DMF (10 mL), and allyl bromoethane (0.3 mL, 4.1 mol), were placed in a 50 mL round-bottomed flask under argon. The reaction flask was then cooled in ice, and 98.4 mg (about 1.8 mmol) of 85% KOH pellets crushed into a fine powder were added all at once. The reaction flask was then heated in an oil bath at ~40°C for 3 h with vigorous stirring. After this period, the reaction mixture was poured into 20 mL of water and the resulting mixture was extracted and then washed with saturated brine, dried over $MgSO_4$, and concentrated *in vacuo* to afford 0.6 g. It was purified by silica gel column chromatography (EtOAc : hexane; 1 : 4) to give **19** (0.5 g, 85%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.88–0.95 (m, 2H), 1.15–1.27 (m, 5H), 1.75–2.07 (m, 2H), 2.71–2.81 (m, 1H), 3.08–3.12 (m, 2H), 3.62–3.72 (m, 2H), 3.99–4.06 (m, 2H), 4.13–4.21 (m, 2H), 4.36–4.55 (m, 2H), 5.14–5.26 (m, 2H), 5.93–5.77 (m, 1H), 7.19–7.49 (m, 15H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 14.66, 16.51, 16.84, 20.84, 36.11, 41.75, 47.90, 52.14, 52.75, 56.87, 65.83, 67.28, 69.72, 117.09, 126.89, 128.10, 129.51, 132.73, 144.62, 154.08, 160.45, 170.18.

(2*S*,4*S*)-2-[7-(*t*-Butyloxycarbonylamino-5-aza-spiro[2.4]heptane)carbonyl]-4-tritylthio-1-(allyloxy-carbonyl)pyrrolidine 20

The synthesis of compound **12** was carried out by the same procedure as described for the preparation of **9**. The synthesis of compound **20** was carried out by the same procedure as described for the preparation of **14**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.56–0.95 (m, 4H), 1.40–1.50 (m, 9H), 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), 4.40–4.56 (m, 2H), 5.13–5.29 (m, 2H), 5.13–5.29 (m, 2H), 5.81–5.86 (m, 1H), 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.

(2*S*,4*S*)-2-[(7-Fluoro-5-aza-spiro[2.4]heptane)-carbonyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine 21

To a suspension of compound **15** (1.11 g, 1.95 mmol) in CH_2Cl_2 was added DAST (0.35 g, 2.15 mmol) at −70°C. The mixture was stirred at −70°C for 45 min and then allowed to warm to room temperature. At this time, 4 mL of methanol were added to quench the reaction. The solvent was evaporated *in vacuo* and the resulting oil dissolved in ethyl acetate, neutralized (pH = 7-8) by addition of a 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 : *n*-hexane; 1 : 3) to give **21** (0.7 g, 58%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.15–1.40 (m, 2H), 1.67–1.86 (m, 2H), 2.24 (bs, 1H), 2.35–2.47 (m, 1H), 2.77 (bs, 1H), 3.02–3.27 (m, 3H), 3.44–3.50 (m, 1H), 3.58–3.81 (m, 2H), 4.00–4.21 (m, 2H), 4.11–4.57 (m, 2H), 5.10–5.28 (m, 2H), 5.77–5.91 (m, 1H), 7.22–7.41 (m, 15H). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 16.00, 30.98, 36.29, 37.24, 41.10, 41.70, 50.88, 52.04, 57.21, 65.88, 67.23, 117.03, 126.86, 128.08, 129.52, 132.76, 144.61, 153.30, 154.10.

Allyl(1*R*,5*S*,6*S*)-6-[(1*R*)-hydroxyethyl]-2-[[5-(7-oxo-5-aza-spiro[2.4]heptane) carbonyl]-1-(allyloxy-carbonyl)pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate IIa

To a solution of **14** (0.6 g, 1.0 mmol) in CH_2Cl_2 (3 mL) was added dropwise triethylsilane (0.20 mL, 1.2 mmol) at 5°C, and then TFA (1.2 mL). 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% $NaHCO_3$, 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-carb-oxylate (**22**, 0.60 g, 1.2 mmol) in CH_3CN (10 mL) was cooled to 0°C 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** (0.15 g, 31%) as a yellow amorphous solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.25–1.31 (m, 6H), 1.36 (d, 3H, *J* = 4.3 Hz), 1.95–2.05 (m, 1H), 2.60–2.65 (bs, 1H), 2.95–3.06 (m, 1H), 3.24–3.28 (bs, 1H), 3.48–3.65 (m, 3H), 3.69–3.78 (m, 2H), 3.88–4.02 (m, 2H), 4.12–4.25 (m, 5H), 4.46–4.58 (m, 4H), 4.70 (dd, 1H, *J* = 5.7 and 5.3 Hz), 4.82 (dd, 1H, *J* = 5.3 and 5.3 Hz), 5.19–5.34 (m, 3H), 5.42 and 5.47 (2s, 1H), 5.87–6.04 (m, 2H).

The syntheses of compounds **IIb-h** were carried out by the same procedure as described for the preparation of **IIa**.

IIb: Yield 39%. ^{1}H-NMR ($CDCl_3$): δ 0.61–0.77 (m, 2H), 0.88–0.94 (m, 2H), 1.21–1.29 (m, 3H), 1.34–1.37 (m, 3H), 2.02–2.13 (m, 2H), 2.64–2.68 (m, 1H), 3.24–3.26 (m, 1H), 3.37–3.50 (m, 2H), 3.60–3.64 (m, 2H), 3.72–3.79 (m, 2H), 4.02–4.05 (m, 1H), 4.12–4.14 (m, 1H), 4.23–4.26 (m, 2H), 4.51–4.58 (m, 3H), 4.69 (dd, 1H, *J* = 5.4 and 5.6 Hz), 4.82 (dd, 1H, *J* = 5.4 and 5.5 Hz), 5.17–5.34 (m, 3H), 5.42 and 5.48 (2s, 1H), 5.90–6.02 (m, 2H).

IIc: Yield 33%. ^{1}H-NMR ($CDCl_3$): δ 0.93 (s, 2H), 1.11–1.28 (m, 8H), 1.98–2.02 (m, 2H), 2.67–2.69 (m, 1H), 3.37–3.42 (m, 3H), 3.56–3.64 (m, 1H), 3.72–3.74 (m, 3H), 3.98–4.06 (m, 3H), 4.08–4.19 (m, 1H), 4.26–4.32 (m, 1H), 4.38–4.51 (m, 4H), 4.62 (dd, 1H, *J* = 5.5 and 5.6 Hz), 4.74 (dd, 1H, *J* = 4.0 and 5.3 Hz), 5.10–5.27 (m, 3H) 5.34 and 5.40 (2s, 1H), 5.71–5.96 (m, 2H).

IId: Yield 34%. ^{1}H-NMR ($CDCl_3$): δ 0.93 (bs, 1H), 1.03–1.23 (m, 6H), 1.27–1.29 (m, 2H), 1.89–1.98 (m, 2H), 2.56–2.67 (m, 1H), 3.18–3.20 (m, 1H), 3.54–3.65 (m, 2H), 4.02–4.11 (m, 2H), 4.16–4.20 (m, 2H), 4.29–4.34 (m, 1H), 4.40–4.55 (m, 4H), 4.59–4.64 (m, 1H), 4.74 (dd, 1H, *J* = 5.3 and 5.4 Hz), 5.10–5.27 (m, 3H), 5.35–5.40 (2s, 1H) 5.75–5.96 (m, 1H), 8.20–8.50 (m, 1H).

IIe: Yield 29%. ^{1}H-NMR ($CDCl_3$): δ 0.81–0.94 (m, 2H), 1.11–1.22 (m, 6H), 1.28–1.30 (m, 2H), 1.92–2.13 (m, 2H), 2.52–2.64 (m, 1H), 3.18–3.19 (m, 1H), 3.32–3.37 (m, 2H), 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), 5.12–5.27 (m, 5H), 5.35 and 5.41 (2s, 1H), 5.72–5.95 (m, 3H).

IIf: Yield 31%. ^{1}H-NMR ($CDCl_3$): δ 0.99–1.00 (m, 2H), 1.17–1.23 (m, 3H), 1.26–1.32 (m, 6H), 1.35–1.37 (m, 2H), 1.99–1.53 (m, 2H), 2.60–2.72 (m, 1H), 3.24–3.25 (m, 1H), 3.38–3.51 (m, 2H), 3.60–3.82 (m, 2H), 3.99–4.09 (m, 2H), 4.14–4.19 (m, 1H), 4.21–4.27 (m, 2H), 4.31–4.37 (m, 1H), 4.48–4.59 (m, 4H), 4.67–4.73 (m, 1H), 4.82 (dd, 1H, *J* = 5.4 and 5.4 Hz), 5.17–5.38 (m, 3H), 5.42 and 5.48 (2s, 1H), 5.79–6.03 (m, 2H).

IIg: Yield 28%. ^{1}H-NMR ($CDCl_3$): δ 0.63–0.95 (m, 4H), 1.26–1.38 (m, 6H), 1.90–2.28 (m, 2H), 2.30–2.64 (m, 2H), 2.87–2.95 (m, 1H), 2.97–3.14 (m, 1H), 3.19–3.49 (m, 2H), 3.54–3.85 (m, 3H), 3.96–4.08 (m, 2H), 4.16–4.25 (m, 1H), 4.39–4.56 (m, 5H), 4.68 (dd, 1H, *J* = 5.3 and 4.6 Hz), 4.83 (dd, 1H, *J* = 5.7 and 3.8 Hz), 5.18–5.37 (m, 5H), 5.42 and 5.48 (2s, 1H), 5.67–5.98 (m, 3H).

IIh: Yield 30%. ^{1}H-NMR ($CDCl_3$): δ 0.71–0.88 (m, 4H), 1.07–1.51 (m, 6H), 2.04–2.09 (m, 1H), 2.52–2.60 (m, 1H), 3.26–3.49 (m, 3H), 3.54–3.67 (m, 1H), 3.76–3.80 (m, 1H), 3.84–3.90 (m, 2H), 3.95–4.10 (m, 2H), 4.17–4.30 (m, 1H), 4.41–4.60 (m, 5H), 4.69 (dd, 1H, *J* = 5.52 and 5.88 Hz), 4.82 (dd, 1H, *J* = 5.4 and 5.6 Hz) 5.12–5.34 (m, 3H), 5.42 and 5.48 (2s, 1H), 5.88–6.00 (m, 2H).

(1*R*,5*S*,6*S*)-6-[(1*R*)-Hydroxyethyl]-2-[5-(7-oxo-5-aza-spiro[2.4]heptane)carbonyl]-pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylic acid IIIa

To a stirred solution of **IIa** (0.1 g, 0.2 mmol) and $Pd(PPh_3)_4$ (30 mg) in CH_2Cl_2 (10 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. To the resulting solution was diluted with water (10 mL) and the organic layers was 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, eluting with 2%

THF in water. Fractions having UV absorption at 298 nm were collected and lyophilized again to give the title compound **IIIa** as a amorphous solid. Yield 22%. UV λ max: 298 nm. ^{1}H-NMR (300 MHz, D_2O): δ 1.11 (d, 3H, *J* = 7.2 Hz), 1.19 (d, 3H, *J* = 6.3 Hz), 1.25 – 1.30 (m, 2H), 1.36 – 1.38 (m, 2H), 1.88 – 1.96 (m, 1H), 2.95 – 2.97 (m, 1H), 3.28 – 3.38 (m, 3H), 3.63 – 3.69 (m, 2H), 3.91 – 4.06 (m, 3H), 4.10 – 4.20 (m, 3H), 4.54 – 4.66 (m, 1H). IR (KBr): 3430, 2970, 1740, 1650 cm^{-1}. HRMS(FAB) Calcd. for $C_{21}H_{27}N_3O_6S$ 449.1621, Found 449.1619.

The syntheses of compounds **IIIb – h** were carried out by the same procedure as described for the preparation of **IIIa**.

IIIb: Yield 33%. UV λ max: 298 nm. ^{1}H-NMR (D_2O): δ 0.27 – 0.54 (m, 4H), 0.82 (d, 3H, *J* = 5.8 Hz), 0.90 (d, 3H, *J* = 6.0 Hz), 1.55 – 1.58 (m, 1H), 2.59 – 2.66 (m, 1H), 2.80 – 2.87 (m, 1H), 2.90 – 3.05 (m, 3H), 3.17 – 3.36 (m, 2H), 3.44 – 3.56 (m, 3H), 3.63 – 3.64 (m, 1H), 3.84 (d, 2H, *J* = 6.8 Hz), 4.15 – 4.30 (m, 1H). IR (KBr): 3410, 2970, 1760, 1650 cm^{-1}. HRMS(FAB) Calcd. for $C_{21}H_{29}N_3O_6S$ 451.1777, Found 451.1775.

IIIc: Yield 29%. UV max: 298 nm. ^{1}H-NMR (D_2O): δ 0.83 – 1.16 (m, 10H), 1.87 – 1.89 (m, 1H), 2.92 – 2.96 (m, 1H), 2.96 – 3.33 (m, 2H), 3.64 – 3.69 (m, 3H), 3.93 (bs, 1H), 4.10 – 4.12 (d, 2H, *J* = 6.1 Hz), 4.26 – 4.58 (m, 4H). IR (KBr): 3380, 2970, 1750, 1650 cm^{-1}. HRMS(FAB) Calcd. for $C_{21}H_{28}N_4O_6S$ 464.1730, Found 464.1728.

IIId: Yield 31%. UV λ max: 298 nm. ^{1}H-NMR (D_2O): δ 1.07 – 1.12 (m, 7H), 1.19 (d, 3H, *J* = 6.0), 1.85 – 1.95 (m, 1H), 2.81 – 2.98 (m, 1H), 3.28 – 3.34 (m, 3H), 3.41 – 3.46 (m, 1H), 3.55 – 3.64 (m, 5H), 3.90 – 4.00 (m, 1H), 4.13 (d, 2H, *J* = 7.2 Hz), 4.31 – 4.54 (m, 3H). IR (KBr): 3420, 2970, 1750, 1650 cm^{-1}. HRMS(FAB) Calcd. for $C_{22}H_{30}N_4O_6S$ 478.1886, Found 478.1888.

IIIe: Yield 29%. UV λ max: 298 nm. ^{1}H-NMR (D_2O): δ 0.85 – 1.28 (m, 10H), 1.79 – 2.01 (m, 1H), 2.25 – 2.31 (m, 1H), 2.31 – 2.73 (m, 2H), 2.86 – 3.16 (m, 1H), 3.35 – 3.73 (m, 5H), 3.95 – 4.16 (m, 2H), 4.16 – 4.49 (m, 3H), 4.52 – 4.54 (m, 1H), 5.13 – 5.37 (m, 2H), 5.84 – 5.87 (m, 1H). IR (KBr): 3410, 2970, 1740, 1650 cm^{-1}. HRMS(FAB) Calcd. for $C_{24}H_{32}N_4O_6S$ 504.2043, Found 504.2038.

IIIf: Yield 43%. UV max: 298 nm. ^{1}H-NMR (D_2O): δ 1.05 – 1.16 (m, 8H), 1.27 (d, 2H, *J* = 5.5 Hz), 2.15 – 2.45 (m, 1H), 2.45 – 2.75 (m, 1H), 2.86 – 3.05 (m, 1H), 3.16 – 3.41 (m, 1H), 3.49 – 3.74 (m, 4H), 3.95 – 4.04 (m, 1H), 4.04 – 4.14 (m, 1H), 4.24 – 4.35 (m, 1H), 4.41 (d, 2H, *J* = 5.1 Hz), 4.49 – 4.76 (m, 3H), 5.13 – 5.39 (m, 2H), 5.84 – 5.87 (m, 1H). IR (KBr): 3420, 2980, 1750, 1650 cm^{-1}. HRMS(FAB) Calcd. for $C_{23}H_{32}N_4O_6S$ 492.2043, Found 492.2040.

IIIg: Yield 35%. UV λ max: 298 nm. ^{1}H-NMR (D_2O): δ 0.61 – 0.89 (m, 4H), 1.08 (d, 3H, *J* = 7.27 Hz), 1.18 (d, 3H, *J* = 6.30 Hz), 1.57 (bs, 1H), 1.76 (bs, 1H), 2.59 (bs, 1H), 2.92 – 2.99 (m, 1H), 3.04 – 3.08 (m, 2H), 3.16 – 3.26 (m, 2H), 3.29 – 3.32 (m, 1H), 3.44 – 3.58 (m, 1H), 3.61 – 3.74 (m, 2H), 3.78 – 3.97 (m, 2H), 4.05 – 4.15 (m, 1H), 7.50 (bs, 2H). IR (KBr): 3410, 2970, 1750, 1640 cm^{-1}. HRMS(FAB) Calcd. for $C_{21}H_{30}N_4O_5S$ 450.1937, Found 450.1927.

IIIh: Yield 33%. UV λ max: 298 nm. ^{1}H-NMR (D_2O): δ 0.60 – 0.98 (m, 4H), 1.06 (d, 3H, *J* = 7.1 Hz), 1.14 (d, 3H, *J* = 6.3 Hz), 1.69 – 1.75 (m, 1H), 2.70 – 2.85 (m, 1H), 3.04 – 3.24 (m, 3H), 3.30 – 3.32 (m, 1H), 3.37 – 3.42 (m,1H), 3.55 – 3.64 (m, 1H), 3.70 – 3.74 (m, 1H), 3.79 – 3.94 (m, 3H), 3.99 – 4.12 (m, 2H), 4.20 – 4.46 (m, 1H). IR (KBr): 3420, 2970, 1750, 1650, 1255 cm^{-1}. HRMS(FAB) Calcd. for $C_{21}H_{28}FN_3O_5S$ 453.1734, Found 453.1730.

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