Synthesis and Antibacterial Activity of 1β-Methyl-2-[5-(α,β-disubstituted ethyl)pyrrolidin-3-ylthio]carbapenem Derivatives. Part II

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The synthesis of a new series of 1β-methylcarbapenems having 5-(α,β-disubstituted ethyl)pyrrolidine moiety 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. A particular compound (**VIh**) having α-hydroxy-β-piperazinylethyl substituted moiety showed the most potent activity.

Key Words : 1β-Methylcarbapenem, Antibacterial activity, DHP-I stability

Introduction

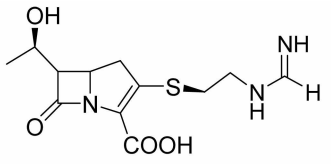
Carbapenems are one of the most potent types of antibacterial agents and are among those used as last resort against infections in the clinical field. Three carbapenems, imipenem (**1**),[1,2] meropenem (**2**),[3] and ertapenem (**3**)[4] have been marketed so far. In particular, it was revealed that 1β-methylcarbapenems showed not only a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria but also high stability to 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] BO-2727[9] and E-1010[10] are under clinical or preclinical studies since the launch of meropenem.

We have previously reported that the carbapenem compounds having a pyrrolidine-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.[11-14] We considered that introduction of additional methoxyimine and oxime moieties into pyrrolidine side chain will contribute to the improvements of activity, as methoxyimine and oxime moieties led to the inhanced the drug activity in general. In this paper, we described the synthesis and structure-activity relationships of the 1β-methylcarbapenems having a 5'-(1,2-disubstituted ethyl)pyrrolidin-3'-ylthio group as a C-2 side chain and our approach to the improve the antibacterial activity of the carbapenems are discussed.

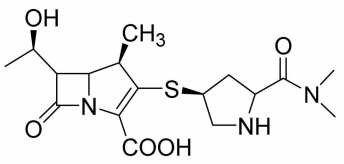
Results and Discussion

Synthesis. Our general synthetic route leading to new carbapenems involves successively the preparation of the appropriately protected thiols containing pyrrolidine ring as a side chain and subsequent coupling reaction with the carbapenem diphenylphosphates, followed by deprotection of the resulting protected carbapenems in a usual manner. 5-(1,2-Disubstituted ethyl)pyrrolidinethiol derivatives (**Ia-c-IVa-c**) were prepared by the sequence shown in Scheme 1. *N*-Protected proline methyl ester **1** was converted to the *O*-mesylated compound **2** by treatment of mesyl chloride and subsequently treated with sodium triphenylmethylthioate, which was generated *in situ* from triphenylmethylmercaptan and sodium hydride in DMF, to provide **3** with the inversion of C-4 configuration. The ester **3** was hydrolyzed to the carboxylic acid **4** by treatment of 4N-NaOH and subsequently reacted with ethyl chloroformate and diazomethane to provide the diazomethyl ketone **5**. The key intermediate **6** was obtained by reaction of diazomethyl ketone **5** with corresponding cyclic amine in the presence of chrolocyclopentadienylbis(triphenylphosphine)ruthenium(II)[15] and subsequently reduced with sodium borohydride to provide α-hydroxy compound **7**. Preparation of the oxime **8** and methoxyimino compound **9** was accomplished by reaction of the carbonyl compound **6** with hydroxyl and methoxy amines. Deprotection of the trityl group to mercaptans (**Ia-c-IVa-c**) was achieved by treatment of **6-8a-c** with trifluoroacetic acid in the presence of triethylsilane (Scheme 1).

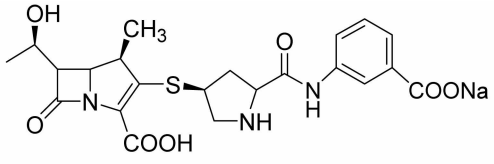
Finally, the reaction of **10**[5] with thiols (**Ia-c-IVa-c**) in the presence of diisopropylethylamine provided the 2-substitut-



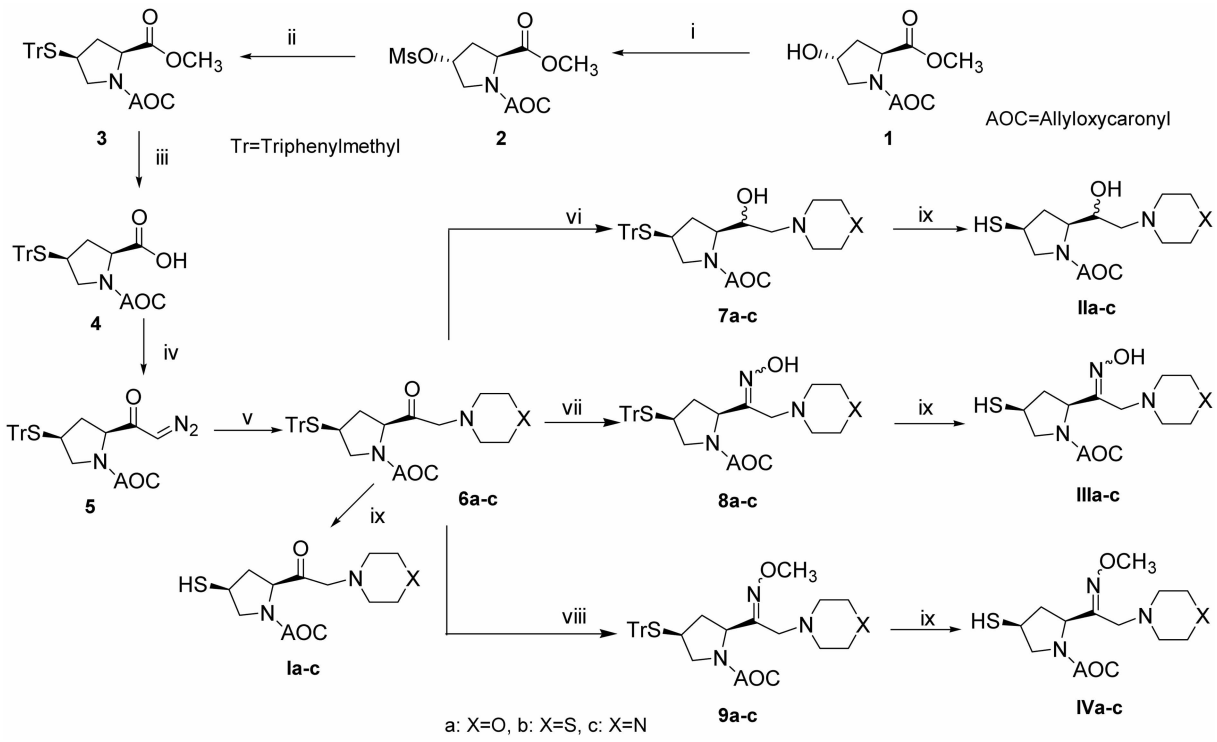
1, Imipenem



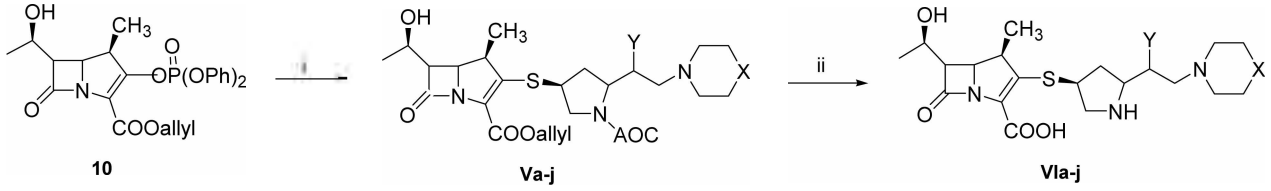
2, Meropenem



3, Ertapenem



Scheme 1. (i) Methanesulfonyl chloride, TEA, CH_2Cl_2 (ii) Triphenylmethylthiol, NaH, DMF (iii) 4N-NaOH, MeOH (iv) 1. Ethyl chloroformate, TEA, THF 2. Diazomethane (v) Ru-complex, cyclic amine, $CHCl_3$ (vi) $NaBH_4$, THF (vii) Hydroxyl amine, EtOH (viii) Methoxylamine hydrochloride, CH_2Cl_2.



a: X=O, Y=O
b: X=O, Y=OH
c: X=O, Y=N-OH
d: X=O, Y=N-OCH_3
e: X=S, Y=O
f: X=S, Y=N-OCH_3
g: X=N-AOC, Y=O
h: X=N-AOC, Y=OH
i: X=N-AOC, Y=N-OH
j: X=N-AOC, Y=N-OCH_3

a: X=O, Y=O
b: X=O, Y=OH
c: X=O, Y=N-OH
d: X=O, Y=N-OCH_3
e: X=S, Y=O
f: X=S, Y=N-OCH_3
g: X=NH, Y=O
h: X=NH, Y=OH
i: X=NH, Y=N-OH
j: X=NH, Y=N-OCH_3

Scheme 2. (i) *N,N*-Diisopropylethyl amine, **HV** (ii) Tetrakis(triphenylphosphine)palladium, tributyltin hydride, CH_2Cl_2.

ed carbapenems (**Va-j**). Deprotection of these compounds by treating with tetrakis(triphenylphosphine)palladium and tributyltin hydride gave the crude products, which were purified by HP-20 column to give the pure carbapenems (**VIa-j**) (Scheme 2).

Biological Assay.

Measurement of *in vitro* antibacterial activity: The MICs (minimum inhibitory concentrations) 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.

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

Table 1. *In vitro* antibacterial activity (MIC, μg/mL) of the carbapenem derivatives

STRAINS	VIa	VIb	VIc	VId	VIe	VIf	VIg	VIh	VIi	VIj	IPM[a]	MPM[b]
Staphylococcus aureus 1218	6.25	6.25	3.125	6.25	6.25	3.125	6.25	6.25	25	3.125	1.560	6.250
Coagulase negative staphylococci	0.391	0.195	0.195	0.195	0.391	0.195	0.391	0.098	0.781	0.391	0.025	0.098
Enterococcus faecalis 2347	6.25	6.25	6.25	6.25	6.25	3.125	6.25	6.25	50	12.5	1.563	12.500
Streptococcus pyogenes 9889	0.025	<0.01	0.013	0.013	0.025	<0.01	0.025	<0.01	0.025	<0.01	<0.01	0.013
Streptococcus agalactiae 32	0.098	0.025	0.049	0.025	0.098	0.025	0.049	0.025	0.098	0.049	0.013	0.049
Streptococcus pneumoniae 0025	0.025	<0.01	0.013	<0.01	0.025	0.013	0.025	0.025	0.098	<0.01	<0.01	0.01
Haemophilus influenzae 1210	25	12.500	12.5	12.500	12.5	12.5	12.500	12.5	25	25	6.250	3.125
Escherichia coil 04	0.098	0.195	0.098	0.781	0.049	0.198	0.195	0.098	0.781	0.195	0.195	0.049
Klebsiella peneumoniae 523	0.195	0.391	0.195	0.781	0.195	0.781	0.781	0.098	1.560	0.195	0.781	0.025
Citrobacter freundii 323	0.195	0.195	0.195	0.391	0.781	0.391	0.195	0.049	0.781	0.195	0.391	0.025
Enterobactor cloacae 34	0.195	0.195	0.195	0.195	0.098	0.195	0.391	0.195	0.781	0.391	0.781	0.025
Serratia marcescens 3349	0.391	0.781	1.560	1.560	0.098	1.560	0.391	0.391	1.560	0.391	0.781	0.049
Acinetobacter baumannii 2289	3.125	12.5	50	50	12.5	50	6.25	12.5	50	12.5	12.500	6.250
Psudemonas aeruginosa 5455	3.125	12.5	25	50	1.560	50	6.25	3.125	50	12.5	3.125	3.125

a= Imipenem, b=Meropenem

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

Organisms	VIh	IPM	MPM	Organism	VIh	IPM	MPM
Staphylococcus aureus giorgio	0.03	0.01	0.10	*Salmonella paratyphi A*	0.10	0.10	0.03
Staphylococcus aureus 209P	0.03	0.01	0.10	*Salmonella typhimurium*	0.20	0.40	0.05
Staphylococcus aureus 503	0.01	<0.01	0.05	*Salmonella oranienberg*	0.20	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 agalactiae B	0.01	0.03	0.05	*Salmonella give*	0.10	0.20	0.03
Streptococcus durans D	0.10	0.10	0.80	*Klebsiella pneumonise 477*	0.20	0.20	0.05
Bacillus subtilts ATCC 6633	0.03	0.03	0.05	*Enterobacter cloacae*	0.03	0.10	0.01
Bacillus megatherium	0.01	0.03	0.05	*Enterobacter cloacae 417*	0.05	0.10	0.01
Pseudomonas aeruginosa 9027	0.80	0.80	0.40	*Serratia marcescens 370*	0.20	0.20	0.05
Pseudomonas aeruginosa 77/2	0.80	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	0.10	0.80	0.40	*Proteus mirabilis 174/3*	0.20	0.10	0.10
Escherchia coil 086	0.05	0.10	0.03	*Proteus vulgaris 868*	0.40	0.10	0.10
Escherchia coil 0114	0.05	0.10	0.01	*Proteus rettgeri 936*	0.40	0.20	0.10
Escherchia coil 0126	0.05	0.10	0.03	*Proteus rettgeri 937*	0.40	0.20	0.05
Escherchia coil V6311/65	0.05	0.05	0.01	*Pasteurella multocida*	0.05	<0.01	0.05
Escherchia coil TEM	0.10	0.20	0.03	*Corynebacterium diphtheriae*	0.01	0.01	0.05
Escherchia coil 1507	0.10	0.10	0.03	*Corynebacterium pyogenes*	0.01	<0.01	0.03

Table 3. DHP-I stablity of **VIb**, **VIe**, **VIh** and **VIj**

	VIb	VIe	VIh	VIj	Meropenem	Imipenem
DHP-I	1.49	2.07	1.88	1.49	1.00	0.20

μM, was incubated at 35 °C in 50 mM MOPS buffer, pH 7.0.

Antibacterial activity studies: The *in vitro* antibacterial activities of the new carbapenems (**VIa-j**) prepared above against both Gram-positive and Gram-negative bacteria are listed in Table 1. For comparison, the MIC values of imipenem, meropenem are also listed. Among these compounds, **VIh** showed superior or similiar antibacterial activity against Gram-positive bacteria to Meropenem, and exhibited improved antibacterial activity against Gram-negative bacteria than Imipenem. Regarding Gram-negative bacteria, the MICs of **VIa**, **VIe** and **VIh** against *Escherichia coli*, *Klebsiella pneumoniae*, *Enterbacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* were comparable or better than those of imipenem

As to the substituents of the 1,2-disubstituted ethyl side chain, in the case of introduction of piperazine and morpholine on the β-position, the compounds **VIb** and **VIh** having hydroxy group exhibited more potent activity than oxime

and methoxyimine groups. Meanwhile, in the case of carbonyl group on the α-position, the compound **VIe** having thiomorpholine was better than morpholine (**VIa**) and piperazine (**VIg**) against Gram-negative bacteria such as *Escherichia coli*, *Enterbacter cloacae* and *Serratia marcescens*. On the other hand, methoxy, piperazine moiety (**VIj**) exhibited more potent activity than morpholine (**VId**) and thiomorpholine (**VIf**) against Gram-negative bacteria. Unfortunately, in this study, we did not obtain satisfactory results for the introduce of oxime and methoxy group on the α-position.

Comparative *in vitro* activities of **VIh**, meropenem, and imipenem against 40 bacterial strains were summarized in Table 2. The selected carbapenem **VIh** possessed excellent *in vitro* activity against 40 target pathogens except *P. aeruginosa*, and superior or similar antibacterial activities against Gram-positive to meropenem, and against Gram-negative bacteria to imipenem. Against *Bacillus megatherium* and *Streptococcus agalctiae B*, **VIh** was 2-3 times more active than the compared meropenem and imipenem.

The stability to DHP-I for the potent compound was tested and all the compounds were more stable than meropenem. In particular, the compound **VIe** showed the most stability.

Experimental Section

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

(2*S*,4*R*)-4-Mesyloxy-1-(allyloxycarbonyl)pyrrolidine-2-carboxylic acid methyl ester (2). A solution of **1** (93.9 g, 0.41 mol) and triethylamine (68.0 mL, 0.49 mol) in dry CH_2Cl_2 (600 mL) was cooled to 0 °C under nitrogen and treated with methanesulfonyl chloride (56.0 g, 0.49 mol). The mixture was stirred at 0 °C for 1 h, diluted with CH_2Cl_2 (500 mL), and washed with 10% $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography to give **2** (117.3 g, 93.2%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 2.27 (m, 1H), 2.75 (m, 1H), 3.06 (s, 3H), 3.77 and 3.80 (2s, 3H), 3.82-3.97 (m, 2H), 4.42 (m, 1H), 4.57 (d, 2H, J = 5.8 Hz), 5.25 (m, 3H), 5.92 (m, 1H).

(2*S*,4*S*)-4-Tritylthio-1-(allyloxycarbonyl)pyrrolidin-2-carboxylic acid methyl ester (3). To a stirred solution of triphenylmethylmercaptan (80.0 g, 0.29 mol) in dry DMF (600 mL) was added portionwise sodium hydride (11.6 g, 0.29 mol, 60% oil suspension) at 0 °C and was stirred for 1 h at room temperature. To the resulting solution was added **2** (75.7 g, 0.25 mol) in dry DMF (150 mL) at 0 °C and was stirred for 3 h at room temperature. The reaction mixture was poured into cold dilute HCl and extracted with ethyl acetate. The organic layer was successively washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent in vacuo gave a crude residue, which was purified by silica gel column chromatography to give **3** (100.6 g, 82.5%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 2.01 (m, 1H), 2.55 (m, 1H), 3.16 (bs, 1H), 3.54 (bs, 1H), 3.77 and 3.80 (2s, 3H), 3.97 (m, 1H), 4.42 (m, 1H), 4.55 (d, 2H, J = 5.5 Hz), 5.26 (m, 2H), 5.98 (m, 1H) 7.23 (m, 9H), 7.47 (d, 6H, J = 7.2 Hz).

(2*S*,4*S*)-4-Tritylthio-1-(allyloxycarbonyl)pyrrolidin-2-carboxylic acid (4). To a solution of **3** (48.7 g, 0.10 mol) in MeOH (500 mL) was added slowly 4N-NaOH (38 mL, 0.15 mol) at 0 °C and was stirred for 5 h at room temperature. The mixture was neutralized with 4N-HCl (38 mL) and concentrated, and the resulting residue was diluted with water (300 mL) and ethyl acetate (200 mL). The resulting precipitates were filtered and the solid was washed water, and dried in air to give **4** (39.4 g, 83.2%) as a white solid. -mp 202-203 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ 1.98 (m, 1H), 2.75-2.82 (m, 1H), 3.01 (m, 1H), 3.55 (bs, 2H), 3.98 (m, 1H), 4.55 (d, 2H, J = 5.9 Hz), 5.25 (m, 2H), 5.90 (m, 1H), 7.27 (m, 9H), 7.47 (d, 6H, J = 7.2 Hz).

(2*S*,4*S*)-2-[(1-Oxo-2-diazo)ethyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine (5). A solution of **4** (44.5 g, 94.0 mmol) and triethylamine (31.4 mL, 188.0 mmol) in dry THF (500 mL) was cooled to −20 °C under nitrogen and treated with ethyl chloroformate (10.8 mL, 112.9 mmol). After 30 min, a solution of CH_2N_2 in ether was added at −20 °C until the pale yellow color persisted and was stirred for 3 h at room temperature. The excess of CH_2N_2 was destroyed with acetic acid (25 mL), and washed with 10% $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography (EtOAc : *n*-Hexane = 1 : 3) to give **5** (40.1 g, 85.9%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.77-2.01 (bs, 2H), 2.76-2.83 (m, 1H), 2.94-3.09 (m, 2H), 4.05 (bs, 1H), 4.02-4.50 (m, 2H), 5.15-5.25 (m, 2H), 5.33-5.35 (m, 1H), 5.83-5.86 (m, 1H), 7.21 -7.33 (m, 9H), 7.47 (d, 6H, J = 7.2 Hz).

(2*S*,4*S*)-2-[(1-Oxo-2-(morpholin-4-yl)ethyl)]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine (6a). A mixture of **5** (2.0 g, 4.02 mmol), Ru(II)-complex (catalytic amount) and morpholine (0.63 mL, 7.24 mmol) in $CHCl_3$ (40 mL) was stirred for 12 h under reflux. The mixture was diluted with water (50 mL) and $CHCl_3$ (50 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and the resulting residue was purified by silica gel column chromatography (EtOAc : Hexane = 3 : 1) to give **6a** (1.1 g, 46.4%). ^{1}H-NMR ($CDCl_3$) δ 1.55 (m, 1H), 2.03 (m, 2H), 2.45 (s, 4H), 2.83 (m, 1H), 3.03-3.25 (m, 3H), 3.70 (s, 4H), 4.20-4.28 (m, 1H), 4.47 (bs, 2H), 5.13-5.28 (m, 2H), 5.84 (m, 1H), 7.22-7.29 (m, 9H), 7.46 (d, 6H, J = 7.5 Hz).

The synthesis of compounds **6b-c** was carried out by the same procedure as described for the preparation of **6a**.

6b: Yield 42.1%. ^{1}H-NMR ($CDCl_3$) δ 1.51 (m, 1H), 1.97 (m, 1H), 2.67 (s, 8H), 2.71 (m, 1H), 3.08-3.23 (m, 4H), 4.29 (m, 1H), 4.49 (m, 2H), 5.23 (m, 2H), 5.84 (m, 1H), 7.21-7.33 (m, 9H), 7.45 (d, 6H, J = 7.8 Hz).

6c: Yield 39.6%. ^{1}H-NMR ($CDCl_3$) δ 1.55 (q, 1H, J = 10.2 Hz), 2.03 (m, 1H), 2.43 (m, 4H), 2.84 (m, 1H), 3.03-3.28 (m, 4H), 3.51 (bs, 4H), 4.32 (m, 1H), 4.47 (m, 2H), 4.60 (d, 2H, J = 5.1 Hz), 5.18-5.33 (m, 4H), 5.90-5.96 (m, 2H), 7.22-7.33 (m, 9H), 7.44 (d, 6H, J = 7.5 Hz).

(2*S*,4*S*)-2-[(1-Hydroxy-2-(morpholin-4-yl)ethyl)]-4-tritylthio-1-(allyloxycarbonyl)pyrrololidine (7a). To a solution of **6a** (0.80 g, 1.4 mmol) in THF (30 mL) was added slowly $NaBH_4$ (0.05 g, 1.4 mmol) at 0 °C and was stirred for 2 h at room temperature. The reaction mixture was poured into 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 = 1 : 2) to give **7a** (0.54 g, 68.0%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.50 (m, 1H), 1.95-2.06 (m, 1H), 2.37 (m, 4H), 2.56 (m, 2H), 2.71 (bs, 1H), 3.16 (m, 2H), 3.69 (s, 4H), 3.87 (bs, 1H), 4.12 (m, 1H), 4.50 (m, 2H), 5.21-5.31 (m, 2H), 5.82-5.91 (m, 1H), 7.21-7.33 (m, 9H), 7.45 (d, 6H, J = 7.8 Hz).

The synthesis of compound **7c** was carried out by the same procedure as described for the preparation of **7a**.

7c: Yield 49.1%. ^{1}H-NMR ($CDCl_3$) δ 1.57 (m, 1H), 1.98-2.06 (m, 1H), 2.19 (m, 2H), 2.35 (m, 2H), 2.50 (m, 2H), 2.73 (m, 1H), 3.13 (m, 2H), 3.47 (bs, 4H), 3.70 (bs, 1H), 3.91 (m, 1H), 4.50 (m, 2H), 4.59 (d, 2H, J = 6.0 Hz), 5.19-5.32 (m, 4H), 5.87-5.96 (m, 2H), 7.21-7.31 (m, 9H), 7.46 (d, 6H, J = 7.2 Hz).

(2*S*,4*S*)-2-[(1-Hydroxyimine-2-(morpholin-4-yl)ethyl)]-4-tritylthio-1-(allyloxycarbonyl) pyrrolidine (8a). To a stirred solution of **6a** (1.2 g, 2.2 mmol) in pyridine (20 mL) was added dropwise 50% aqueous hydroxylamine (0.16 mL, 2.4 mmol) and was stirred for 12 h at 50 °C. The mixture was evaporated under reduced pressure. The residue was dissolved with ethyl acetate and washed with 1N-HCl, 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 : 1) to give **8a** (0.75 g, 61.0%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.66 (bs, 2H), 2.42 (s, 4H), 2.80 (m, 1H), 2.98-3.28 (m, 3H), 3.34 (m, 1H), 3.69 (m, 4H), 4.24 (bs, 1H), 4.46-4.53 (m, 2H), 5.30 (m, 2H), 5.85 (m, 1H), 7.20-7.33 (m, 9H), 7.45 (d, 6H, J = 7.5 Hz).

The synthesis of compound **8c** was carried out by the same procedure as described for the preparation of **8a**.

8c: Yield 71.7%. ^{1}H-NMR ($CDCl_3$) δ 1.59 (m, 1H), 2.04 (bs, 1H), 2.40 (m, 4H), 2.79 (m, 1H), 2.85-3.18 (m, 3H), 3.32 (d, 1H, J = 12.0 Hz), 3.45 (d, 4H, J = 18.0 Hz), 4.28 (m, 1H), 4.46-4.52 (m, 2H), 4.60 (d, 2H, J = 6.0 Hz), 5.21-5.33 (m, 4H), 5.86-5.94 (m, 2H), 7.21-7.32 (m, 9H), 7.45 (d, 6H, J = 7.5 Hz).

(2*S*,4*S*)-2-[(1-Methoxyimine-2-(morpholin-4-yl)ethyl)]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine (9a). To a solution of **6a** (0.60 g, 1.1 mmol) in dry pyridine (20 mL) was added dropwise methoxylamine hydrochloride (0.23 mL, 1.3 mmol, 35%) and was stirred for 10 h at 50 °C. The mixture was evaporated under reduced pressure. The residue was dissolved with ethyl acetate and washed with 1N-HCl, 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 : 1) to give **9a** (0.53 g, 83.8%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.69 (m, 1H), 2.05 (m, 1H), 2.29-2.51 (m, 4H), 2.80 (m, 2H), 3.01 (m, 2H), 3.37 (m, 1H), 3.67 (bs, 4H), 3.84 (s, 3H), 4.49 (m, 2H), 4.75 (m, 1H), 5.16-5.28 (m, 2H), 5.83 (1H), 7.24-7.34 (m, 9H), 7.47 (d, 6H, J = 7.5 Hz).

The synthesis of compounds **9b-c** was carried out by the same procedure as described for the preparation of **9a**.

9b: Yield 72.7%. ^{1}H-NMR ($CDCl_3$) δ 1.65-2.06 (m, 2H), 2.70 (bs, 8H), 2.89 (m, 1H), 2.99-3.39 (m, 4H), 3.84 (s, 3H), 4.53 (m, 3H), 5.16-5.28 (m, 2H), 5.84 (m, 1H), 7.23-7.34 (m, 9H), 7.48 (d, 6H, J = 7.8 Hz).

9c: Yield 70.2%. ^{1}H-NMR ($CDCl_3$) δ 1.55 (m, 1H), 2.03 (bs, 1H), 2.28-2.46 (m, 4H), 2.70-2.97 (m, 2H), 3.09 (m, 3H), 3.47 (s, 4H), 3.80 (d, 3H, J = 15.6 Hz), 4.50 (m, 2H), 4.59 (d, 2H, J = 5.7 Hz), 4.75 (m, 1H), 5.19-5.33 (m, 4H), 5.88-5.99 (m, 2H), 7.19-7.33 (m, 9H), 7.46 (d, 6H, J = 7.5 Hz).

Allyl (1*R*,5*S*,6*S*)-6-[(1*R*)-hydroxyethyl]-2-{5-[(1-oxo-2-(morpholin-4-yl)ethyl)]-1-(allyloxycarbonyl)pyrrolidin-3-ylthio}-1-methylcarbapen-2-em-3-carboxylate (Va). To a solution of **6a** (0.70 g, 1.3 mmol) in CH_2Cl_2 (2 mL) was added dropwise triethylsilane (0.25 mL, 1.5 mmol) at 5 °C, and then TFA (1.5 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-carboxylate (7, 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 7 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 **Va** (0.24 g, 34.1%) as a yellow amorphous solid. ^{1}H-NMR ($CDCl_3$) δ 1.25 (m, 3H), 1.36 (d, 3H, J = 6.0 Hz), 1.91 (m, 1H), 2.05 (bs, 1H), 2.54 (m, 4H), 2.63 (m, 1H), 3.24-3.40 (m, 4H), 3.74 (t, 4H, J = 4.5 Hz), 4.01 (m, 1H), 4.24 (t, 2H, J = 6.6 Hz), 4.56 (m, 4H), 4.72 (dd, 1H, J = 5.4 and 13.5 Hz), 4.80 (dd, 1H, J = 5.4 and 13.5 Hz), 5.23-5.30 (m, 3H), 5.47 (dd, 1H, J = 1.5 and 17.1 Hz), 5.95 (m, 2H).

The synthesis of compounds **Vb-j** was carried out by the same procedure as described for the preparation of **Va**.

Vb: Yield 22.2%. ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 3H), 1.38 (d, 3H, J = 6.3 Hz), 2.18 (m, 2H), 2.45 (m, 4H), 2.66 (m, 3H), 3.27 (m, 1H), 3.45 (m, 2H), 3.79 (bs, 4H), 3.95 (m, 2H), 4.26 (m, 3H), 4.59 (bs, 2H), 4.73 (dd, 1H, J = 5.4 and 13.5 Hz), 4.82 (dd, 1H, J = 5.4 and 13.5 Hz), 5.21-5.33 (m, 3H), 5.48 (dd, 1H, J = 1.5 and 17.1 Hz), 5.91-5.96 (m, 2H).

Vc: Yield 18.2%. ^{1}H-NMR ($CDCl_3$) δ 1.27 (m, 3H), 1.37

(d, 3H, J = 6.0 Hz), 1.90 (m, 1H), 2.05 (m, 1H), 2.39 (bs, 2H), 2.50 (bs, 2H), 2.78 (m, 2H), 3.12 (m, 1H), 3.29 (d, 1H, J = 6.9 Hz), 3.38-3.47 (m, 2H), 3.70 (bs, 4H), 3.90 (m, 1H), 4.28 (m, 2H), 4.57-4.67 (m, 3H), 4.74 (dd, 1H, J = 5.4 and 13.5 Hz), 4.82 (dd, 1H, J = 5.4 and 13.5 Hz), 5.22-5.30 (m, 3H), 5.49 (dd, 1H, J = 1.5 and 17.1 Hz), 5.92-6.00 (m, 2H).

Vd: Yield 22.1%. ^{1}H-NMR ($CDCl_3$) δ 1.28 (d, 3H, J = 6.3 Hz), 1.37 (m, 3H), 1.45 (m, 1H), 1.96 (m, 1H), 2.41 (bs, 2H), 2.51 (bs, 2H), 2.87 (m, 1H), 2.99 (m, 1H), 3.11 (m, 1H), 3.41 (m, 2H), 3.69 (s, 4H), 3.86 (s, 3H), 4.27 (m, 2H) 4.58 (bs, 4H), 4.72 (dd, 1H, J = 5.4 and 13.5 Hz), 4.83 (dd, 1H, J = 5.4 and 13.5 Hz), 5.01 (m, 1H), 5.24-5.31 (m, 3H), 5.49 (dd, 1H, J = 1.5 and 17.1 Hz), 5.94-5.97 (m, 2H).

Ve: Yield 16.5%. ^{1}H-NMR ($CDCl_3$) δ 1.28 (m, 3H), 1.38 (d, 3H, J = 6.0 Hz), 1.78-1.93 (m, 2H), 2.73 (bs, 8H), 3.25-3.44 (m, 5H), 3.73 (m, 1H), 4.01 (m, 1H), 4.28 (m, 2H), 4.63 (m, 3H), 4.73 (dd, 1H, J = 5.4 and 13.5 Hz), 4.81 (dd, 1H, J = 5.4 and 13.5 Hz), 5.24-5.31 (m, 3H), 5.49 (dd, 1H, J = 1.5 and 17.1 Hz), 5.80-6.03 (m, 2H).

Vf: Yield 14.2%. ^{1}H-NMR ($CDCl_3$) δ 1.28 (m, 3H), 1.38 (d, 3H, J = 6.0 Hz), 1.62 (bs, 2H), 1.85 (m, 2H), 2.64 (bs, 5H), 2.78 (d, 3H, J = 8.7 Hz), 3.12 (m, 1H), 3.26 (bs, 1H), 3.42 (m, 2H), 3.56 (m, 1H), 3.86 (s, 3H), 4.24 (bs, 2H), 4.58 (bs, 2H), 4.75 (dd, 1H, J = 5.4 and 13.5 Hz), 4.82 (dd, 1H, J = 5.4 and 13.5 Hz), 5.03 (m, 1H), 5.19-5.35 (m, 3H), 5.49 (dd, 1H, J = 1.5 and 17.1 Hz), 5.84-6.03 (m, 2H).

Vg: Yield 35.7%. ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 3H), 1.35 (d, 3H, J = 6.0 Hz), 1.90 (m, 1H), 2.25 (bs, 1H), 2.49 (bs, 5H), 3.23-3.47 (m, 4H), 3.53 (bs, 4H), 3.97 (m, 1H), 4.24 (m, 2H), 4.59 (d, 6H, J = 5.4 Hz), 4.70 (dd, 1H, J = 5.4 and 13.5 Hz), 4.78 (dd, 1H, J = 5.4 and 13.5 Hz), 5.19-5.31 (m, 5H), 5.46 (dd, 1H, J = 1.5 and 17.1 Hz), 5.88-5.97 (m, 3H).

Vh: Yield 11.2%. ^{1}H-NMR ($CDCl_3$) δ 1.26 (bs, 3H), 1.38 (d, 3H, J = 6.0 Hz), 1.74 (m, 2H), 2.37 (m, 2H), 2.59 (bs, 2H), 3.26 (m, 2H), 3.52 (bs, 4H), 3.70 (m, 1H), 3.96 (m, 1H), 4.14 (m, 1H), 4.28 (m, 4H), 4.61 (d, 6H, J = 5.4 Hz), 4.73 (dd, 1H, J = 5.4 and 13.5 Hz), 4.82 (dd, 1H, J = 5.4 and 13.5 Hz), 5.21-5.34 (m, 5H), 5.49 (dd, 1H, J = 1.5 and 17.1 Hz), 5.90-6.00 (m, 3H).

Vi: Yield 13.5%. ^{1}H-NMR ($CDCl_3$) δ 1.26 (bs, 3H), 1.38 (d, 3H, J = 6.0 Hz), 1.70 (m, 2H), 2.37 (m, 2H), 2.50 (m, 2H), 2.84-2.91 (m, 2H), 3.27 (m, 2H), 3.50 (bs, 4H), 4.08 (bs, 1H), 4.25 (m, 3H), 4.60 (d, 6H, J = 5.4 Hz), 4.71 (dd, 1H, J = 5.4 and 13.5 Hz), 4.80 (dd, 1H, J = 5.4 and 13.5 Hz), 5.13-5.33 (m, 5H), 5.47 (dd, 1H, J = 1.5 and 17.1 Hz), 5.89-6.00 (m, 3H).

Vj: Yield 18.5%. ^{1}H-NMR ($CDCl_3$) δ 1.26 (bs, 3H), 1.38 (d, 3H, J = 6.0 Hz), 1.64 (m, 1H), 1.87 (m, 1H), 2.34 (bs, 2H), 2.51 (m, 2H), 2.75-2.96 (m, 2H), 3.17 (m, 1H), 3.25 (bs, 1H), 3.48 (bs, 4H), 3.86 (s, 3H), 4.09 (m, 1H), 4.24 (m, 2H), 4.60 (d, 6H, J = 5.4 Hz), 4.71 (dd, 1H, J = 5.4 and 13.5 Hz), 4.84 (dd, 1H, J = 5.4 and 13.5 Hz), 4.98-5.06 (m, 1H), 5.19-5.32 (m, 5H), 5.46 (dd, 1H, J = 1.5 and 17.1 Hz), 5.84-5.99 (m, 3H).

(1*R*,5*S*,6*S*)-6-[(1R)-Hydroxyethyl]-2-{5-[(1-oxo-2-(morpholin-4-yl)ethyl)]pyrrolidin-3-ylthio}-1-methylcarbapen-2-em-3-carboxylic acid (VIa). To a stirred solution of **Va** (0.24 g, 0.43 mol) and $Pd(PPh_3)_4$ (30 mg) in CH_2Cl_2 (10 mL) was added dropwise *n*-tributyltin hydride (0.2 mL, 0.5 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 **VIa** (57.0 mg, 35.7%) as a amorphorus solid. -UV max : 298 nm. -^{1}H-NMR (D_2O) δ 1.10 (d, 3H, J = 7.2 Hz), 1.18 (d, 3H, J = 6.3 Hz), 1.90 (m, 1H), 2.52 (bs, 4H), 3.20 (t, 3H, J = 5.1 Hz), 3.33 (m, 2H), 3.56 (m, 1H), 3.67 (bs, 4H), 3.84 (t, 3H, J = 5.1 Hz), 4.14 (m, 2H). -IR (KBr): 3480, 1720, 1700, 1670 cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{29}N_3O_6S$ 439.1777, Found 439.1770.

The synthesis of compounds **VIb-j** was carried out by the same procedure as described for the preparation of **VIa**.

VIb: Yield 29.2%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.07 (d, 3H, J = 6.6 Hz), 1.14 (d, 3H, J = 6.3 Hz), 1.69 (m, 1H), 2.01 (m, 2H), 2.39 (bs, 2H), 2.49 (bs, 4H), 3.24-3.37 (m, 4H), 3.61 (d, 4H, J = 4.2 Hz), 3.97 (m, 2H), 4.08 (m, 2H). -IR (KBr): 3460, 1730, 1690, cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{31}N_3O_6S$ 441.1934, Found 441.1940.

VIc: Yield 43.3%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.07 (d, 3H, J = 7.2 Hz), 1.13 (d, 3H, J = 6.3 Hz), 1.92 (m, 1H), 2.47 (t, 4H, J = 4.5 Hz), 2.84 (m, 2H), 3.14 (m, 1H), 3.34 (m, 3H), 3.39-3.53 (m, 2H), 3.61 (bs, 4H), 3.86 (m, 1H), 4.10 (m, 2H). -IR (KBr): 3460, 1740, 1710, 1610 cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{30}N_4O_6S$ 454.1886, Found 454.1889.

VId: Yield 26.9%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.09 (d, 3H, J = 7.2 Hz), 1.16 (d, 3H, J = 6.0 Hz), 1.82 (m, 1H), 2.48 (m, 4H), 2.74 (m, 2H), 3.11-3.28 (m, 5H), 3.32-3.44 (m, 2H), 3.65 (bs, 4H), 3.79 (s, 3H), 4.11 (m, 2H). -IR (KBr): 3490, 1735, 1710, 1670 cm^{-1}. -HRMS (FAB) Calcd. for $C_{21}H_{32}N_4O_6S$ 468.2043, Found 468.2044.

VIe: Yield 41.6%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.02 (d, 3H, J = 7.2 Hz), 1.10 (d, 3H, J = 6.3 Hz), 1.81-1.95 (m, 1H), 2.33 (m, 1H), 2.59 (m, 2H), 2.66-2.75 (m, 5H), 3.18 (m, 1H), 3.26 (t, 4H, J = 5.4 Hz), 3.46-3.56 (m, 2H), 3.81 (m, 1H), 4.01-4.07 (m, 2H), 4.41 (m, 1H). -IR (KBr): 3540, 1720, 1700, 1670 cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{29}N_3O_5S_2$ 455.1549, Found 455.1546.

VIf: Yield 18.6%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.12 (d, 3H, J = 7.2 Hz), 1.18 (d, 3H, J = 6.3 Hz), 1.81-1.95 (m, 1H), 2.38 (m, 1H), 2.66 (bs, 2H), 2.71-3.04 (m, 6H), 3.22-3.40 (m, 4H), 3.61 (m, 2H), 3.81 (m, 1H), 3.85 (s, 3H), 3.92 (m, 1H), 4.16 (m, 2H). -IR (KBr): 3510, 1730, 1710, 1660 cm^{-1}. -HRMS (FAB) Calcd. for $C_{21}H_{32}N_4O_5S_2$ 484.1814, Found 484.1811.

VIg: Yield 48.2%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.00 (bs, 3H), 1.10 (d, 3H, J = 6.0 Hz), 1.71 (m, 1H), 1.95 (m, 1H), 2.36 (bs, 1H), 2.62 (bs, 4H), 3.08 (bs, 4H), 3.23 (m, 2H), 3.43-3.80 (m, 3H), 4.04 (m, 2H), 4.44 (m, 2H). -IR (KBr): 3440, 1710, 1690, 1630 cm^{-1}. -HRMS (FAB) Calcd.

for $C_{20}H_{30}N_4O_5S$ 438.1937, Found 438.1940.

VIh: Yield 33.7%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.11 (d, 3H, J = 7.2 Hz), 1.17 (d, 3H, J = 6.3 Hz), 1.57-1.60 (m, 1H), 1.78 (m, 1H), 2.51 (m, 2H), 2.73 (d, 3H, J = 4.8 Hz), 3.16 (t, 4H, J = 4.8 Hz), 3.23 (m, 1H), 3.32-3.35 (m, 2H), 3.48-3.62 (m, 3H), 3.71-3.99 (m, 2H), 4.12 (m, 2H). -IR (KBr): 3490, 1710, 1670 cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{32}N_4O_5S$ 440.2093, Found 440.5580.

VIi: Yield 28.2%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.14 (m, 3H), 1.20 (d, 3H, J = 6.3 Hz), 1.60 (m, 1H), 1.79 (m, 1H), 2.51 (m, 1H), 2.66 (bs, 4H), 3.21 (bs, 4H), 3.27-3.39 (m, 2H), 3.43-3.69 (m, 3H), 3.72-4.00 (m, 2H), 4.15 (m, 2H). -IR (KBr): 3440, 1710, 1690, 1630 cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{31}N_5O_5S$ 453.2046, Found 453.2049.

VIj: Yield 43.1%. -UV λ_{max}: 298 nm. -^{1}H-NMR (D_2O) δ 1.11 (d, 3H, J = 7.2 Hz), 1.21 (d, 3H, J = 6.3 Hz), 1.51-1.57 (m, 1H), 1.78 (m, 1H), 2.51 (m, 1H), 2.66 (bs, 4H), 2.80 (m, 1H), 3.12-3.21 (m, 4H), 3.25-3.34 (m, 2H), 3.43 (m, 1H), 3.56 (m, 1H), 3.78 (s, 3H), 3.92 (m, 1H), 4.13 (m, 2H), 4.15 (m, 1H). -IR (KBr): 3490, 1710, 1670, 1590 cm^{-1}. -HRMS (FAB) Calcd. for $C_{21}H_{33}N_5O_5S$ 467.2202, Found 467.2204.

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