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Synthesis and Antibacterial Evaluation of 1β-Methyl-2-(5-substituted heterocyclic carbamoyl)pyrrolidin-3-ylthio)carbapenem Derivatives

The synthesis of a new series of 1β-methylcarbapenems having a substituted heterocyclic carbamoyl pyrrolidine moiety is described. Their *in vitro* antibacterial activity against both gram-positive and gram-negative bacteria was tested, and the effect of heterocyclic substituents on the pyrrolidine was investigated. One particular compound ($\mathbf{III}_d$) having a substituted oxadiazole moiety showed the most potent antibacterial activity.

Keywords: β-Methylcarbapenem; Antibacterial activity; Substituent effect

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Introduction

Carbapenem compounds that have a (3S)-pyrrolidin-3-ylthio group at the C-2 position in the carbapenem skeleton are noted for their broad and potent antibacterial activity [1], and a large number of derivatives have been synthesized and investigated. Among these compounds, panipenem [2] and meropenem [3] were the first to be successfully launched in the market, and clinical evaluations are in progress for S-4661 [4], BO-2727 [5] and DX-8739 [6], which show enhanced metabolic stability to renal dehydropeptidase-1 (DHP-1) due to the introduction of a 1b-methyl group in the carbapenem skeleton for high antibacterial potency. We were also interested in this pyrroldin-3-ylthio group and 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 and investigated [7–15].

In this paper, we describe the synthesis and structure-activity relationships of the 1β-methylcarbapenems having a substituted heterocyclic carbamoyl pyrrolidin-3′-ylthio group as C-2 side chain, and our approach for the improvement of antibacterial activity of the carbapenems is discussed.

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Results and discussion

Chemistry

Our synthetic route leading to the new carbapenems commences with preparation of appropriately substituted pyrrolidine carrying a thiol group at the 3-position. The intermediates thus prepared were then coupled with carbapenem diphenylphosphates, and the protecting groups were removed by the usual techniques.

The thiol compounds $\mathbf{I}_a$ and $\mathbf{I}_b$ were prepared by the sequence shown in Scheme 1. The hydroxyethyl carbamoyl compound **1** [15] was converted to the aldehyde compound **2** by Swern oxidation. The formation of thiazolidine (**3**) and 2′-ester substituted thiazolidine (**4**) were accomplished by the reaction of aldehyde **2** with L-cysteamine and L-cysteine ethyl ester in 90% aqueous ethanol. Deprotection of the trityl group to thiol compounds ($\mathbf{I}_a$ and $\mathbf{I}_b$) was achieved by treatment of **3** and **4** with trifluoroacetic acid in the presence of triethylsilane (Scheme 1). *N*-Protected proline carboxylic acid (**5**) [15] was converted to cyanomethyl carbamoyl **6** by treatment with ethyl chloroformate and aminoacetonitrile and subsequently hydrolyzed with sulfuric acid in THF to provide amide **7**. The synthesis of 4′-ester substituted thiazole (**9**) was accomplished by the reaction of thioamide **8** with ethyl bromopyruvate in ethanol (Scheme 2). The formation of 5′-ester substituted oxadiazole (**12**) was accomplished by the reaction of *N*-hydroxyacetamidine with ethyl oxalyl chloride in THF. The 4′-ester substituted oxadiazole (**12**) was converted to the amide **13** by treatment with ammonium hydroxide in ethanol (Scheme 3). *N*-Protected acetimidate **16** was allowed to react with L-cysteine ethyl ester to give 4′-ester substituted thiazoline

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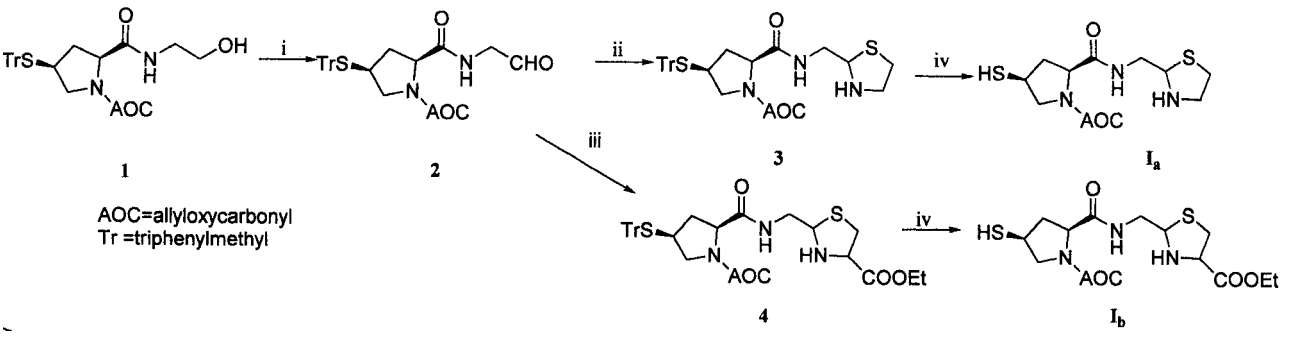
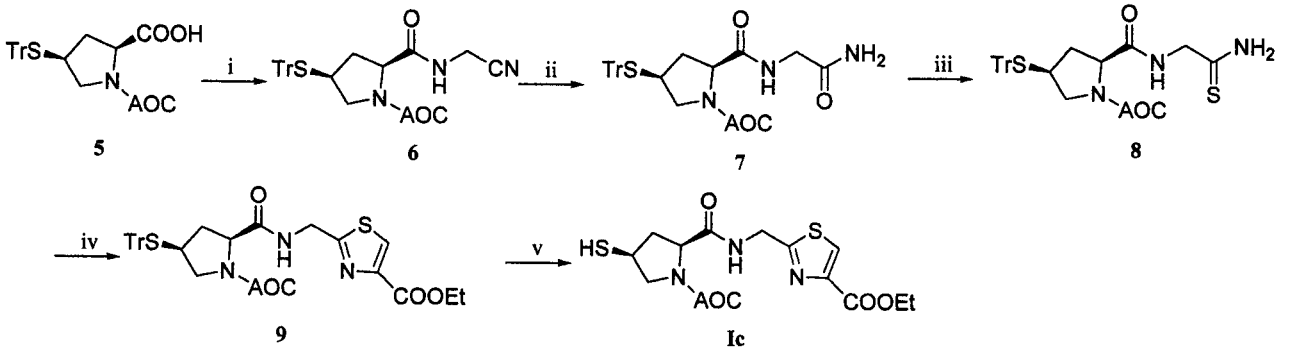
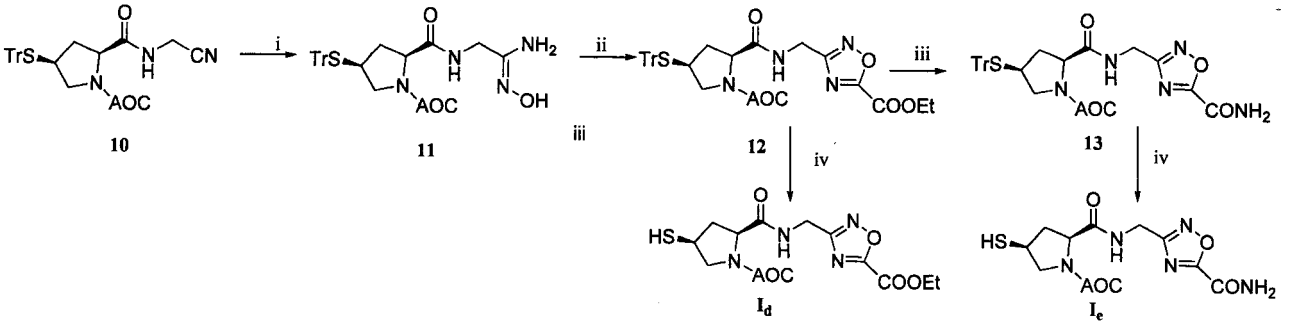


Scheme 1. i) Oxalyl chloride, DMSO, TEA, CH_2Cl_2; ii) Cysteamine hydrochloride, 90% EtOH; iii) Cysteine ethyl ester, 90% EtOH; iv) Trifluoroacetic acid, triethyl silane, CH_2Cl_2.



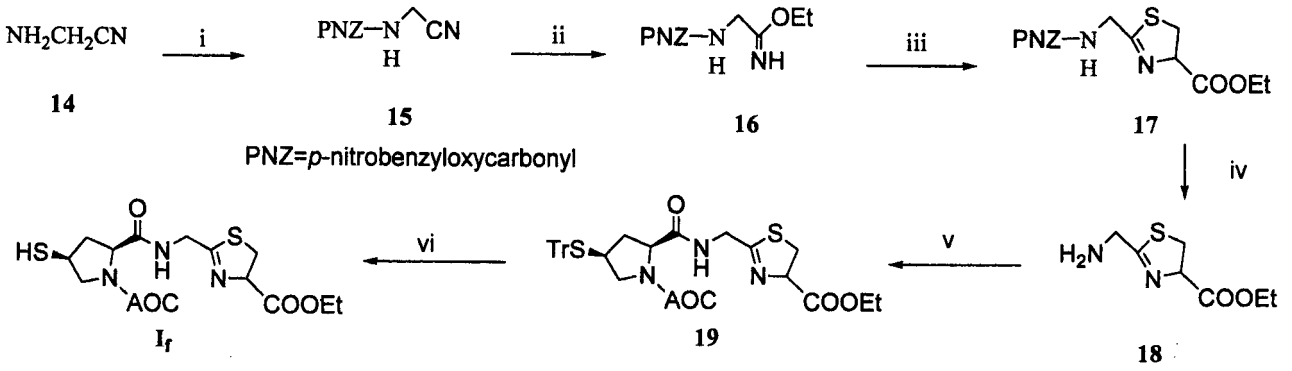
Scheme 2. i) Ethyl chloroformate NH_2CH_2CN, TEA, CH_2Cl_2; ii) H_2SO_4, 4*N*-NaOH, THF; iii) Lawessen's reagent, THF; iv) Ethyl bromopyruvate, EtOH; v) Trifluoroacetic acid, triethyl silane, CH_2Cl_2.



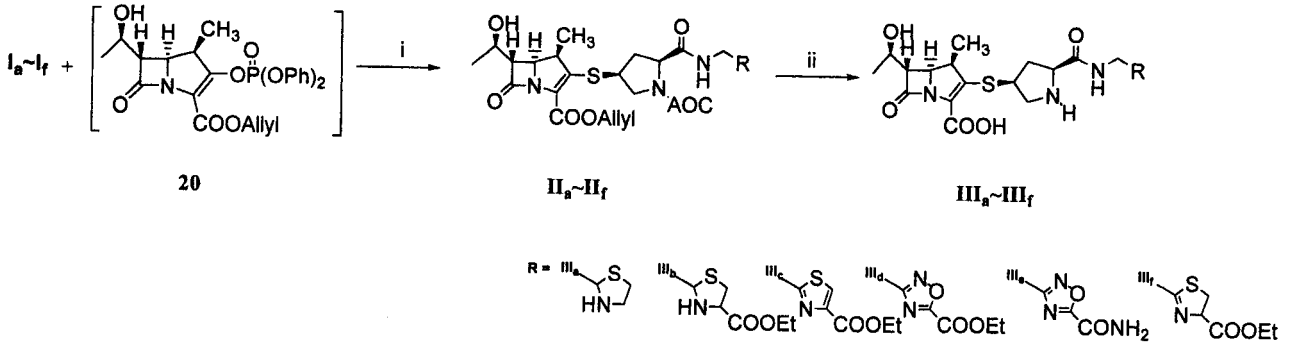
Scheme 3. i) Hydroxyl amine, EtOH; ii) Ethyloxaly chloride, THF; iii) NH_4OH, EtOH; iv) Trifluoroacetic acid, triethyl silane, CH_2Cl_2.

(**17**); the following deprotection with palladium in methanol led to the amino compound **18**. Synthesis of **19** was accomplished by the reaction of *N*-protected proline carboxylic acid (**5**) with ethyl chloroformate in CH_2Cl_2 (Scheme 4). Also, the thiols $\mathbf{I}_{c-f}$ were prepared by a method similar to that described for the preparation of $\mathbf{I}_a$.

Finally, the reaction of **20** [16] with thiols ($\mathbf{I}_{a-f}$) in the presence of diisopropylethylamine provided the 2-sub-



Scheme 4. i) 4-Nitrobenzyl chloroformate, TEA, CH_2Cl_2CN; ii) HCl gas, EtOH; iii) *L*-Cysteine ethyl ester HCl, TEA, EtOH; iv) Pd/C, MeOH:THF = 1:1; v) **5**, Ethyl chloroformate, TEA, CH_2Cl_2; vi) Trifluoroacetic acid, triethyl silane, CH_2Cl_2.



Scheme 5. i) *N,N*-Diisopropylethyl amine, CH_3CN; ii) $Pd(OH)_2$, THF, H_2O.

stituted carbapenem ($\mathbf{II_{a-f}}$). Deprotection of these compounds by catalytic hydrogenation gave the crude products that were purified by HP-20 column to give the pure carbapenems ($\mathbf{III_{a-f}}$) (Scheme 5).

Biological assay

Measurement of in vitro antibacterial activity

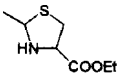
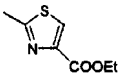
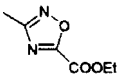
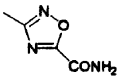
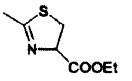
MIC were determined by the agar dilution method using test agar. An overnight culture of bacteria in tryptosoy broth was diluted to about 10^6 cells/mL with the same broth and inoculated with an inoculating device onto agar containing serial twofold dilutions of the test compounds. Organisms were incubated at 37 °C for 18–20 h. The MIC of a compound was defined as the lowest concentration that visibly inhibited growth.

Antibacterial activity studies

The *in vitro* antibacterial activities of the new carbapenems ($\mathbf{III_{a-f}}$), prepared as described above, against gram-positive and gram-negative bacteria are listed in Table 1. For comparison, the MIC values of Imipenem and Meropenem are also listed. One particular compound ($\mathbf{III_d}$) having a substituted oxadiazole moiety displayed superior or similar antibacterial activities against gram-positive bacteria compared to Meropenem, and against gram-negative bacteria compared to Imipenem.

The non-substituted thiazolidine compound ($\mathbf{III_a}$) showed better antibacterial activities against gram-positive and gram-negative bacteria than the ester substituted compounds ($\mathbf{III_b}$). Comparing the compounds ($\mathbf{III_{b-d}}$, $\mathbf{III_f}$) containing an ester substituted heterocyclic carbamoyl moiety at C′-5 of pyrrolidine,

Table 1. *In vitro* antibacterial activities of carbapenem derivatives.

R =							Imipenem	Meropenem
Organism	III_a	III_b	III_c	III_d	III_e	III_f		
Streptococcusp. 308A[a]	0.05	0.10	0.01	0.03	0.10	0.05	< 0.01	0.01
Streptococcus p. 77A[b]	0.03	0.10	0.01	0.01	0.05	0.03	< 0.01	0.01
Streptococcusa. SG511[c]	0.20	0.80	0.10	0.10	0.20	0.10	0.01	0.10
Escherichia c. DC2[d]	0.10	0.20	0.10	0.05	0.20	0.10	0.10	0.03
Escherichia c. TEM[e]	0.20	0.40	0.40	0.05	0.40	0.20	0.20	0.03
Pseudomonas a. 9027[f]	1.56	3.10	3.10	0.80	1.56	1.56	0.80	0.40
Enterobacter c. 1321E[g]	0.10	0.80	0.40	0.10	0.20	0.10	0.10	0.04

MIC (μg/mL) determined by agar dilution method. a: *Streptococcus pyogenes* 308A; b: *Streptococcus pyogenes* 77A; c: *Streptococcus aureus* SG511; d: *Escherichia coli* DC2; e: *Escherichia coli* TEM; f: *Pseudomonas aeruginosa* 9027; g: *Enterobacter cloacae* 1321E.

similar activity was observed against all bacteria tested. Among these compounds, **III_d** having a substituted oxadiazole moiety exhibited the most potent and well balanced activity.

Experimental

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

(2S,4S)-1-Allyloxycarbonyl-2-N-(formylmethyl)carbamoyl-4-tritylthiopyrrolidine (2)

Dry DMSO (1.6 mL, 9.8 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise over 20 min to a stirred solution of oxalyl chloride (1.2 g, 13.7 mmol) in dry CH_2Cl_2 (20 mL), under an argon atmosphere at −45 °C. After stirring for an additional 15 min, compound **1** (5.1 g, 9.8 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise over 45 min. The mixture was stirred for a further 45 min at −45 °C, and triethylamine was then added dropwise over 30 min, followed by stirring for a further 2 h at −45 °C. The mixture was washed with brine and dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **2** (3.7 g, 73.4%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.05−2.27 (m, 2H), 2.95 (m, 1H), 3.12 (bs, 2H), 4.07 (d, 2H, *J* = 1.6 Hz), 4.45 (m, 2H), 5.32 (d, 2H, *J* = 7.6 Hz), 5.90 (bs, 1H), 7.29 (m, 9H), 7.55 (d, 6H, *J* = 8.1 Hz), 9.60 (s, 1H).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(thiazolidin-2′-yl)methyl]-carbamoyl-4-tritylthiopyrrolidine (**3**)*

A solution of **2** (6.2 g, 12.0 mmol) in 90% aqueous ethanol (60 mL) was added slowly to a solution of cysteamine hydrochloride (1.3 g, 12.0 mmol) in water (20 mL) and $NaHCO_3$ (0.9 g, 12.0 mmol) at −5 °C. The reaction mixture was stirred for 24 h at room temperature and then concentrated *in vacuo* to give a white solid, which was diluted in ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **3** (3.0 g, 43.3%) as a light yellowish oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.20 (m, 2H), 2.80 (m, 1H), 2.93 (bs, 2H), 3.21 (m, 2H), 3.46 (m, 2H), 3.72 (m, 2H), 3.94 (m, 2H), 4.48 (d, 2H, *J* = 6.2 Hz), 5.21 (d, 2H, *J* = 3.2 Hz), 5.82 (m, 1H), 7.29 (m, 9H), 7.32 (d, 6H, *J* = 7.8 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(4′-ethoxycarbonylthiazolidin-2′-yl)methyl]carbamoyl-4-trityl thiopyrrolidine (**4**)*

A solution of **2** (2.7 g, 5.3 mmol) in 90% aqueous ethanol (30 mL) was added slowly to a solution of cysteine ethyl ester hydrochloride (1.0 g, 5.3 mmol) in water (10 mL) and 10% $NaHCO_3$ (0.4 g, 5.3 mmol) at −5 °C. The reaction mixture

was stirred for 24 h at room temperature and then concentrated *in vacuo* to give a white solid, which was diluted in ethyl acetate and water. The organic layer was dried over anhydrous $NaSO_4$. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **4** (1.7 g, 50.4%) as a light yellowish oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.27–1.34 (t, 3H, J = 3.6 Hz), 2.21–2.37 (bs, 2H), 2.70 (bs, 1H), 2.80 (d, 2H, J = 8.6 Hz), 3.12 (m, 2H), 3.26 (m, 1H), 3.27–3.36 (bs, 1H), 4.01 (bs, 2H), 4.19 (q, 2H, J = 3.6 Hz), 4.44 (d, 2H, J = 4.2 Hz), 5.21–5.26 (d, 2H, J = 5.2 Hz), 5.81 (m, 2H), 7.30 (m, 9H), 7.44 (d, 6H, J = 7.6 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(thiazolidine-2′-yl)methyl]-carbamoyl-4-mercaptanpyrrolidine (**I_a**)*

To a solution of **3** (1.3 g, 2.3 mmol) in CH_2Cl_2 was added dropwise trifluoroacetic acid (2 mL) at 0 °C, then triethylsilane (0.4 mL, 2.6 mmol). After stirring for 30 min at room temperature, the mixture was evaporated under reduced pressure. The residue was washed with 10% $NaHCO_3$ and brine. The organic layer was concentrated *in vacuo* to give a residue which was used without further purification.

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(4′-ethoxycarbonythiazol-xidin-2′-yl)methyl]carbamoyl-4-mercaptanpyrrolidine (**I_b**)*

I_b was prepared from **4** by a method similar to that described for the preparation of **I_a**.

*(2S,4S)-1-Allyloxycarbonyl-2-(N-cyanomethyl)carbamoyl-4-tritylthiopyrrolidine (**6**)*

A solution of **5** (20.0 g, 42.3 mmol) and triethylamine (6.5 mL, 46.5 mmol) in dry CH_2Cl_2 (150 mL) was cooled to 0 °C under nitrogen and treated with ethyl chloroformate (4.5 mL, 46.5 mol). After stirring for 30 min at 0 °C, aminoacetonitrile (5.2 mL, 84.6 mmol) was added, and the mixture was stirred for 1 h at 0 °C. The mixture was washed with 10% $NaHCO_3$ and brine and dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **6** (20.1 g, 93.0%) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.21 (s, 2H), 2.94 (d, 2H, J = 7.5 Hz), 3.31 (bs, 1H), 4.02 (s, 3H), 4.54 (d, 2H, J = 6.8 Hz), 5.28 (d, 2H, J = 5.2 Hz), 5.89 (s, 1H), 7.23 (m, 9H), 7.50 (d, 6H, J = 8.2 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-(N-carbamoylmethyl)carbamoyl-4-tritylthiopyrrolidine (**7**)*

At 0 °C H_2SO_4 (20 mL) was added slowly to a solution of **6** (4.4 g, 8.5 mmol) in THF (50.0 mL), and the mixture was stirred for 1 h at 0 °C. The mixture was neutralized with 1 N NaOH, diluted with ethyl acetate (60 mL) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **7** (3.8 g, 83.1%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.92–2.16 (m, 2H), 2.87 (m, 1H), 3.07 (bs, 2H), 3.89 (m, 1H), 4.04 (bs, 2H), 4.52 (t, 2H, J = 6.3 Hz), 5.26 (d, 2H, J = 7.6 Hz), 5.91 (m, 1H), 7.28 (m, 9H), 7.44 (d, 6H, J = 4.2 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-(N-thiocarbamoylmethyl)-carbamoyl-4-tritylthiopyrrolidine (**8**)*

A mixture of **7** (1.2 g, 2.2 mmol) and Lawesson's reagent (1.8 g, 4.4 mmol) was dissolved in THF (30 mL), and the solution was refluxed for 2 h. Evaporation of the resulting solution *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **8** (0.6 g, 48.8%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.14–2.18 (d, 2H, J = 7.2 Hz), 2.86 (m, 2H), 3.03 (d, 1H, J = 4.2 Hz), 4.36 (s, 2H), 4.53 (t, 2H, J = 3.6 Hz), 4.93 (d, 1H, J = 6.2 Hz), 5.22 (q, 2H), 5.77 (m, 1H), 7.22 (m, 9H), 7.42 (d, 6H, J = 7.2 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(4′-ethoxycarbonylmethyl-thiazol-2′-yl)methyl]carbamoyl-4-tritylthiopyrrolidine (**9**)*

Ethyl bromopyruvate (0.5 g, 2.2 mmol) was added dropwise to a stirred solution of **8** (1.0 g, 1.8 mmol) in ethanol (30 mL), and the mixture was stirred for 20 h at room temperature. The resulting solution was evaporated and diluted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the resulting solution *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **9** (0.6 g, 51.0%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.35 (t, 3H, J = 3.7 Hz), 2.03 (bs, 2H), 2.57 (m, 1H), 2.91 (t, 2H, J = 5.7 Hz), 4.30 (q, 5H), 4.55 (t, 2H, J = 6.2 Hz), 5.26 (bs, 2H), 5.81 (bs, 1H), 7.22 (m, 9H), 7.40 (d, 6H, J = 7.2 Hz), 8.03 (s, 1H).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(4′-ethoxycarbonylmethyl-thiazol-2′-yl)methyl]carbamoyl-4-mercaptanpyrrolidine (**I_c**)*

I_c was prepared from **9** by a method similar to that described for the preparation of **I_a**. The above compound was used without further purification.

*(2S,4S)-1-Allyloxycarbonyl-2-(N-hydroxyacetamidine)-carbamoyl-4-tritylthiopyrrolidine (**11**)*

Hydroxylamine hydrochloride (1.7 g, 23.5 mmol) was added slowly to a solution of **6** (10.0 g, 19.6 mmol) in ethanol at room temperature, and the mixture was stirred for 20 h at 60 °C. The resulting solution was evaporated, diluted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the resulting solution *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **11** (8.5 g, 79.6%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.20–2.24 (m, 2H), 2.78 (m, 1H), 3.02–3.14 (m, 2H), 3.85 (bs, 2H), 4.08 (t, 1H, J = 6.2 Hz), 4.49 (m, 2H), 5.26 (d, 2H, J = 5.4 Hz), 5.80 (m, 1H), 7.25 (m, 9H), 7.43 (d, 6H, J = 7.2 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(5′-ethoxycarbonyl-1′,2′,4′-oxadiazol-3′-yl)methyl]carbamoyl-4-tritylthiopyrrolidine (**12**)*

Ethyl oxalylchloride (0.6 mL, 5.5 mmol) was added dropwise to a solution of **11** (3.0 g, 5.5 mmol) in THF (40 mL), and the resulting solution was refluxed for 12 h. The reaction mixture was poured into 10% $NaHCO_3$ and extracted with ethyl acetate (60 mL). Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **12** (1.6 g, 46.2%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.44 (t, 3H, J = 6.2 Hz), 2.01 (m, 2H), 2.42 (bs, 1H), 2.98 (bs, 2H), 4.11 (bs, 1H), 4.49 (m, 4H), 4.65 (m, 2H), 5.28 (bs, 2H), 5.82 (bs, 1H), 7.26 (m, 9H), 7.43 (d, 6H, J = 7.2 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(5′-ethoxycarbonyl-1′,2′,4′-oxadiazole-3′-yl)methyl]carbamoyl-4-thiopyrrolidine (**I_d**)*

I_d was prepared from **12** by a method similar to that described for the preparation of **I_a**. The above compound was used without further purification.

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(5′-carbamoyl-1′,2′,4′-oxadiazole-3′-yl)methyl]carbamoyl-4-tritylthiopyrrolidine (**13**)*

Ammonium hydroxide (28%, 10 mL) was added to a stirred solution of **12** (1.5 g, 2.4 mmol) in ethanol (20 mL), and the mixture was stirred for 3 h at 60°C. The mixture was neutralized with 6 N HCl, diluted with ethyl acetate (50 mL) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **13** (1.1 g, 77.9%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.13 (bs, 2H), 2.43 (m, 1H), 3.00 (bs, 2H), 4.12 (t, 1H, *J* = 5.2 Hz), 4.46 (s, 2H), 4.60 (d, 2H, *J* = 6.5 Hz), 5.24 (d, 2H, *J* = 7.4 Hz), 5.81 (bs, 1H), 7.25 (m, 9H), 7.46 (d, 6H, *J* = 4.6 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-[N-(5′-carbamoyl-1′,2′,4′-oxadiazole-3′-yl)-methyl]carbamoyl-4-mercaptanpyrrolidine (**I$_e$**)*

I$_e$ was prepared from **12** by a method similar to that described for the preparation of **I$_a$**. The above compound was used without further purification.

*p-Nitrobenzyloxycarbonyl)aminoacetonitrile (**15**)*

A solution of **14** (2.9 g, 51.1 mmol) and triethylamine (7.1 mL, 51.1 mmol) in CH_2Cl_2 (80 mL) was cooled to 0°C under nitrogen and treated with *p*-nitrobenzyl chloroformate (13.5 g, 61.3 mmol). The mixture was stirred for 1 h at 0°C, diluted with ethyl acetate (50 mL) and washed with aqueous 10% $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the resulting solution *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **15** (8.6 g, 71.3%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.72 (s, 2H), 4.08 (s, 2H), 7.52 (d, 2H, *J* = 7.2 Hz), 8.17 (d, 2H, *J* = 8.5 Hz).

*Ethyl(p-nitrobenzyloxycarbonyl)aminoacetimidate (**16**)*

Gaseous HCl was bubbled into a solution of **15** (12.0 g, 51.0 mmol) in ethanol (40 mL) and stirred for 1 h at 0°C. The resulting solution was evaporated to give **16** (13.3 g, 82.2%) as a white powder. Mp = 95–99°C (dec.). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.30 (bs, 3H), 3.24 (s, 2H), 4.00 (d, 2H, *J* = 6.8 Hz), 4.25 (m, 2H), 7.54 (d, 2H, *J* = 14.2 Hz), 8.24 (d, 2H, *J* = 12.3 Hz).

*2-(p-Nitrobenzyloxycarbonyl)aminomethyl-4-ethoxycarbonyl-thiazoline (**17**)*

L-cysteine ethyl ester hydrochloride (2.1 g, 11.1 mmol) was added to a solution of **16** (3.3 g, 11.1 mmol) and triethylamine (1.5 mL, 11.1 mmol) in dry methanol (50 mL), and the mixture was stirred for 2 h at room temperature. The resulting solution was evaporated, diluted with ethyl acetate and washed with brine. The organic layer was dried over anhydrous $MgSO_4$. Evaporation of the resulting solution *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **17** (1.8 g, 45.0%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.33 (t, 3H, *J* = 9.3 Hz), 1.71 (s, 3H), 3.57 (bs, 2H), 4.29 (m, 2H), 5.09 (s, 2H), 7.44 (d, 2H, *J* = 14.6 Hz), 8.24 (d, 2H, *J* = 12.4 Hz).

*2-Aminomethyl-(4′-ethoxycarbonyl)thiazoline (**18**)*

Compound **17** (1.44 g, 4.5 mmol) and 0.5 g of Pd/C (10% [wt/wt]) were dissolved in THF:MeOH (1:1) (10 mL each). The mixture was hydrogenated at 45 psi for 2 h and subsequently filtered through celite. The combined filtrates were dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave **18** as a brown oil. The above compound was used without further purification.

*(2S,4S)-1-Allyloxycarbonyl-2-{[N-(4′-ethoxycarbonyl)thiazolin-2′-yl]methyl}carbamoyl-4-trityl thiopyrrolidine (**19**)*

A solution of **5** (3.0 g, 6.3 mmol) and triethylamine (0.9 mL, 6.9 mmol) in dry CH_2Cl_2 (50 mL) was cooled to 0°C under nitrogen and treated with ethyl chloroformate (0.7 mL, 6.9 mmol). The mixture was stirred at 0°C for 30 min, compound **18** (1.2 g, 6.3 mmol) was added, and the resulting mixture was stirred at 0°C for 1 h. The mixture was washed with 10% $NaHCO_3$ and brine and dried over anhydrous $MgSO_4$. Evaporation of the solvent *in vacuo* gave a crude residue which was purified by silica gel column chromatography to give **19** (2.1 g, 52.8%) as a pale yellowish oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.31 (m, 3H), 1.62 (bs, 2H), 2.62 (bs, 2H), 2.90 (d, 2H, *J* = 6.2 Hz), 3.20 (bs, 2H), 3.55 (m, 2H), 4.29 (d, 1H, *J* = 7.2 Hz), 4.48 (m, 2H), 5.20 (m, 2H), 5.92 (bs, 1H), 7.26 (m, 9H), 7.46 (d, 6H, *J* = 8.4 Hz).

*(2S,4S)-1-Allyloxycarbonyl-2-{[N-(4′-ethoxycarbonyl)thiazolin-2′-yl]methyl}carbamoyl-4-mercaptanpyrrolidine (**I$_f$**)*

I$_f$ was prepared from **19** by a method similar to that described for the preparation of **I**$_a$. The above compound was used without further purification.

*Allyl(1R,5S,6S)-6-[(1R)-1-hydroxyethyl]-2-{(3S,5S)-5-[N-(thiazolidin-2-yl)methyl]carbamoyl-1-(allyloxycarbonyl)-pyrrolidin-3-ylthio}-1-methylcarbapen-2-em-3-carboxylate (**II$_a$**)*

A solution of allyl(1*R*,5*S*,6*S*)-2-(diphenylphosphoryloxy)-6-[(1*R*)-1-hydroxymethyl]-1-methylcarbapen-2-em-3-carboxylate (**20**) (0.5 g, 1.0 mmol) in CH_3CN (10 mL) was cooled to 0°C under nitrogen. This solution was added to diisopropylethylamine (0.2 g, 1.0 mmol) and a solution of the thiol compound **I**$_a$ in CH_3CN (5 mL). After stirring for 6 h, the mixture was diluted with ethyl acetate, washed with 10% $NaHCO_3$ and brine and dried over anhydrous $MgSO_4$. Evaporation of the solvents *in vacuo* gave a residue which was purified by silica gel column chromatography to give **II**$_a$ (0.3 g, 43.2%) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.9–1.26 (m, 6H), 1.53 (m, 2H), 2.32 (bs, 3H), 2.74 (bs, 3H), 3.12 (bs, 3H), 3.40 (m, 1H), 3.62 (bs, 2H), 3.84 (m, 2H), 4.21 (m, 1H), 4.60 (m, 6H), 5.25 (bs, 6H), 5.92 (m, 3H).

Synthesis of compounds **II$_b$**–**II$_f$** was carried out by the same procedure as described for the preparation of **II$_a$**.

II$_b$: Yield 53.4%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.21–1.43 (m, 9H), 2.40 (bs, 2H), 2.42 (bs, 3H), 2.88 (bs, 3H), 3.22 (m, 1H), 3.29 (m, 3H), 3.52 (bs, 3H), 3.93 (bs, 1H), 4.24 (m, 2H), 4.60 (m, 4H), 5.27 (m, 4H), 5.93 (m, 2H).
II$_c$: Yield 49.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (m, 9H), 2.81 (m, 2H), 2.90 (bs, 2H), 3.31 (bs, 2H), 3.74 (bs, 2H), 4.13 (m, 1H), 4.36 (bs, 2H), 4.47 (bs, 2H), 4.50 (m, 1H), 4.89 (m, 4H), 5.30 (m, 4H), 5.89 (m, 2H), 8.09 (t, 1H, *J* = 7.6 Hz).
II$_d$: Yield 57.2%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.23–1.32 (m, 6H), 1.43 (t, 3H, *J* = 7.2 Hz), 2.17 (bs, 2H), 2.78 (bs, 2H), 3.24 (d, 2H, *J* = 6.4 Hz), 3.27 (m, 2H), 3.74 (t, 1H, *J* = 6.6 Hz), 4.12 (m, 2H), 4.45 (m, 2H), 4.57 (m, 4H), 4.92 (m, 1H), 5.45 (m, 4H), 5.93 (m, 2H).
II$_e$: Yield 52.2%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.20–1.63 (m, 6H), 2.06 (m, 1H), 2.62 (m, 2H), 3.22 (m, 2H), 3.54 (bs, 1H), 3.84 (bs, 3H), 4.12 (bs, 1H), 4.27 (d, 2H, *J* = 3.8 Hz), 4.62 (d, 4H, *J* = 7.8 Hz), 5.25–5.47 (m, 4H), 5.91 (m, 2H).

II_f: Yield 45.6%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.24−1.38 (m, 9H), 1.86 (bs, 2H), 2.32 (m, 2H), 2.98 (bs, 2H), 3.21 (m, 4H), 3.30 (m, 4H), 4.23−4.27 (m, 2H), 4.64−4.87 (m, 4H), 5.24−5.41 (m, 4H), 5.91 (m, 2H).

*(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(3S,5S)-5-(N-thiazolidin-2-ylmethyl)carbamoylpyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylic acid (**III_a**)*

Compound **II_a** (0.31 g, 0.5 mmol) and 0.1 g of $Pd(OH)_2$ (10%) were dissolved in THF-phosphate buffer (pH 7) (1:1; 10 mL each). The mixture was hydrogenated at 50 psi for 2 h. The solution was filtered through celite and washed with water (2 × 10 mL). The combined filtrates were washed with ethyl ether (2 × 20 mL) and lyophilized to give a yellow powder, which was purified on a Diaion HP-20 column and eluted with 2% THF in water. Fractions showing UV absorption at 298 nm were collected and lyophilized again to give the title compound **III_a** (27 mg, 18.0%) as a white powder. Mp = 155−159 °C (dec.). ^{1}H-NMR (300 MHz, D_2O) δ 0.90−1.26 (m, 6H), 1.53 (m, 2H), 2.32 (bs, 3H), 2.74 (bs, 3H), 3.12 (bs, 3H), 3.40 (m, 1H), 3.62 (bs, 2H), 3.84 (m, 2H), 4.21 (m, 1H). IR (KBr): 3480 (OH), 1670 (C=O) cm^{-1}. MS *m/z* 457 (M^+).

Synthesis of compounds **III_b**−**III_f** was carried out by the same procedure as described for the preparation of **III_a**.

III_b: Yield 15.2%. λ_{max}: 298 nm. Mp = 160−163 °C (dec.). ^{1}H-NMR (300 MHz, D_2O) δ 0.93−1.20 (bs, 9H), 2.10 (bs, 2H), 2.54 (m, 1H), 3.22 (m, 3H), 3.52 (m, 2H), 3.84 (bs, 4H), 3.97 (bs, 3H), 4.23 (bs, 3H). IR (KBr): 3480 (OH), 1710, 1670 (C=O) cm^{-1}. MS *m/z* 529 (M^+).
III_c: Yield 9.4%. λ_{max}: 298 nm. Mp = 140−143 °C (dec.). ^{1}H-NMR (300 MHz, D_2O) δ 1.02 (bs, 3H), 1.13 (bs, 3H), 1.22 (bs, 2H), 1.89 (t, 2H, *J* = 7.2 Hz), 2.32 (m, 1H), 2.84 (bs, 1H), 3.02−3.24 (bs, 3H), 3.50 (m, 1H), 3.81 (m, 2H), 4.02 (bs, 2H) 4.21 (bs, 2H), 8.23 (s, 1H). IR (KBr): 3480 (OH), 1730, 1670 (C=O) cm^{-1}. MS *m/z* 525 (M^+).
III_d: Yield 21.4%. λ_{max}: 298 nm. Mp = 175−179 °C (dec.). ^{1}H-NMR (300 MHz, D_2O) δ 1.07−1.20 (m, 9H), 1.54 (m, 2H), 2.70 (m, 1H), 3.12 (bs, 3H), 3.45 (bs, 2H), 3.92 (m, 2H), 4.02−4.21 (m, 4H). IR (KBr): 3440 (OH), 1700, 1670 (C=O) cm^{-1}. MS *m/z* 510 (M^+).
III_e: Yield 15.2%. λ_{max}: 298 nm. Mp = 120−125 °C (dec.). ^{1}H-NMR (300 MHz, D_2O) δ 1.17−1.26 (m, 6H), 1.79 (m, 2H), 2.59−2.71 (m, 2H), 3.31 (m, 4H), 3.80 (m, 2H), 4.12 (m, 2H). IR (KBr): 3480 (OH), 1690, 1660 (C=O) cm^{-1}. MS *m/z* 481 (M^+).
III_f: Yield 10.6%. λ_{max}: 298 nm. Mp = 140−146 °C (dec.). ^{1}H-NMR (300 MHz, D_2O) δ 1.09−1.21 (m, 6H), 2.21 (bs, 2H), 2.59 (m, 2H), 3.02 (m, 5H), 3.20 (m, 3H), 3.73 (bs, 3H), 4.48 (m, 2H). IR (KBr): 3490 (OH), 1705, 1660 (C=O) cm^{-1}. MS *m/z* 527 (M^+).

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