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Synthesis and Antibacterial Activity of 1β-Methyl-2-(5-substituted thiazolidinopyrrolidin-3-ylthio)carbapenems and Related Compounds

The synthesis of a new series of 1β-methylcarbapenems containing the substituted thiazolidinopyrrolidine moiety is described. Their *in vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria were tested and the effect of substituent on the thiazolidine ring was investigated. A particular compound (**18c**) having a 2-amide substituted thiazolidine moiety showed the most potent antibacterial activity.

Keywords: Carbapenem; Antibiotics

Received: October 1, 2001 [FP637]

Introduction

Imipenem [1], the first marketed carbapenem antibiotic, is highly valued in the clinic for its efficacy against serious bacterial infections. However, due to its instability to renal dehydropeptidase-I(DHP-I), it is used in combination with cilastatin, a DHP-I inhibitor. In 1984, it was reported by Merck researchers [2] that the introduction of a methyl group on the carbapenem nucleus resulted in a great improvement of both the chemical and metabolic stabilities.

The carbapenem compounds which have a pyrrolidin-3-ylthio group at the C-2 position in the carbapenem skeleton are noted for their broad and potent antibacterial activity [3], and a large number of derivatives have been synthesized and investigated [4–6]. Previously, we reported the synthesis and biological properties of carbapenem compounds having the heterocyclic [7] and bicyclic moiety [8, 9].

As part of our program directed toward a new parenteral 1β-methylcarbapenem agent with improved properties including antibacterial activity and stability to DHP-I, 1β-methyl-2-(5-substituted pyrrolidin-3-ylthio)carbapenems, bearing 2-substituted thiazolidine or *N*-substituted thiazolidine, were prepared.

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Chemistry

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

2-Substituted thiazolidine derivatives (**6a**–**e**) were prepared by the sequence shown in Scheme 1. Formation of the 2-substituted thiazolidine ring was accomplished by the reaction of the aldehydic compound **1** [10] with L-cysteine ethyl ester in 60% aq EtOH solution. Thiazolidine **2** was reduced with sodium borohydride in EtOH/THF, and subsequently *O*-acetylation with acetic anhydride was performed to give 4. Deprotection of the silyl ether with TBAF gave the hydroxyl compound, which was converted into the *O*-mesylated compound (**5**, **7**, **9**) by treatment with mesyl chloride. Treatment of **5** with potassium thioacetate in DMF followed by hydrolysis of the resulting acetylthio group with 4N-NaOH in methanol lead to the thiol compound (**6a**). Also, preparation of the 2-thiazinanecarboxylic acid (**10**) was carried out with homocysteine using the same method as for the preparation of **2** and esterfication of 10 with EtOH in the presence of acid gave the ethyl 2-thiazinanecarboxlic ester (**11**).

The 2′-thiazolidinecarboxylic ester (2) was converted to the amide (8) by aminolysis with aqueous ammonia in MeOH. Preparation of thiols (**6b**–**e**) was carried out by a method similar to that used for the preparation of **6a**.

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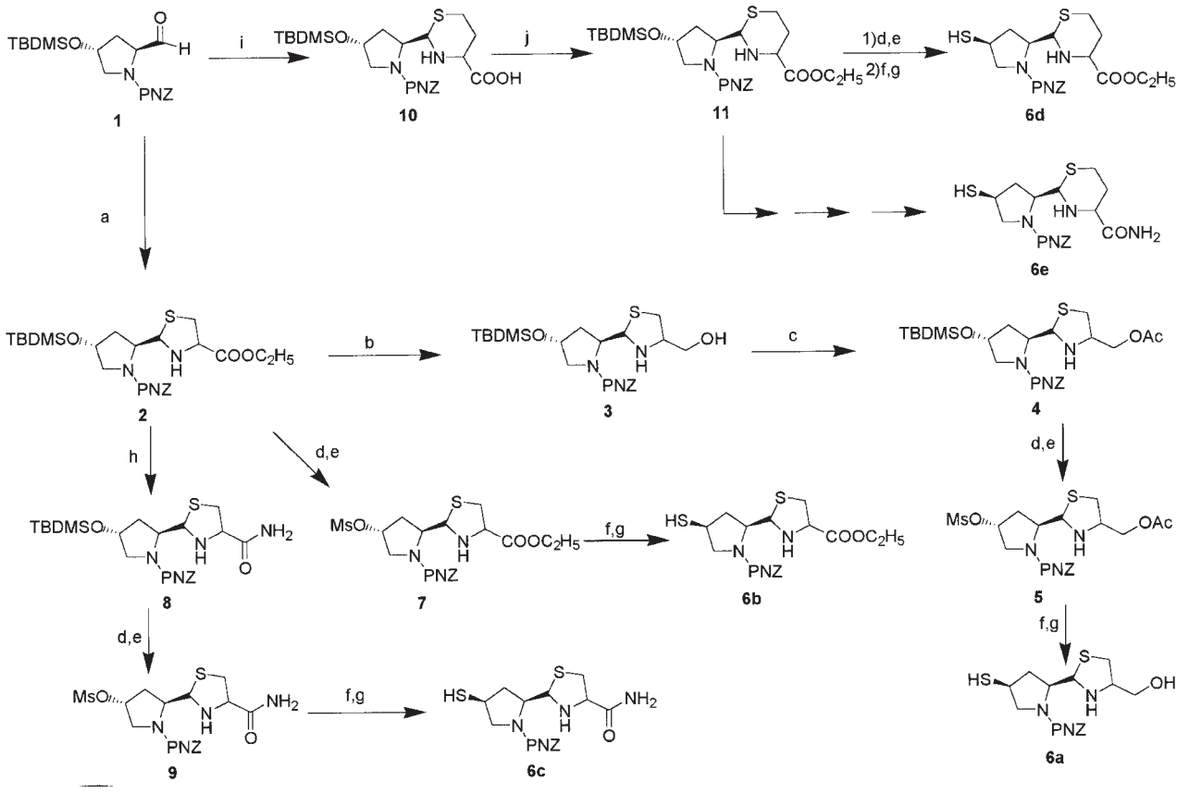
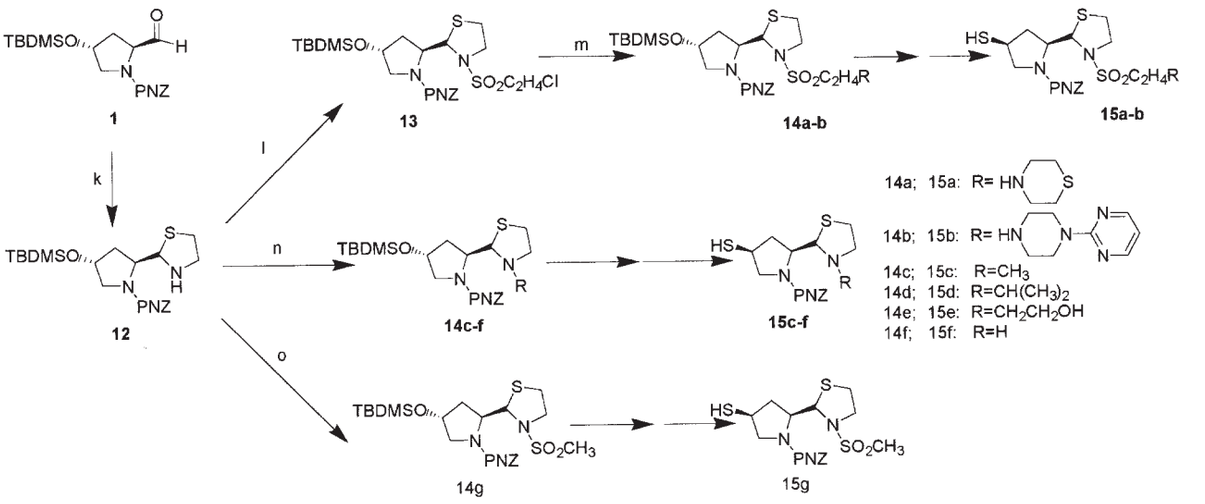


Scheme 1. (a) L-Cysteine ethyl ester, 60% ethanol, 0 °C. (b) $NaBH_4$, THF : EtOH = 4 : 6, 0 °C. (c) $(CH_3CO)_2O$, Et_3N, CH_2Cl_2, 0 °C. (d) TBAF, THF. (e) MsCl, Et_3N, CH_2Cl_2. (f) AcSK, DMF : Toluene = 1 : 1, 70 °C. (g) 4N NaOH, MeOH. (h) NH_4OH, MeOH, rt. (i) *L*-Homocysteine, 60% ethanol. (j) EtOH, H^+.



Scheme 2. (k) L-Cysteine, 60% ethanol. 0 °C. (l) $BrCH_2CH_2SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C. (m) cyclic amine, K_2CO_3, DMF, 90 °C (n) RX, K_2CO_3, CH_3CN, (o) $ClSO_2CH_3$, Et_3N, CH_2Cl_2, 0 °C.

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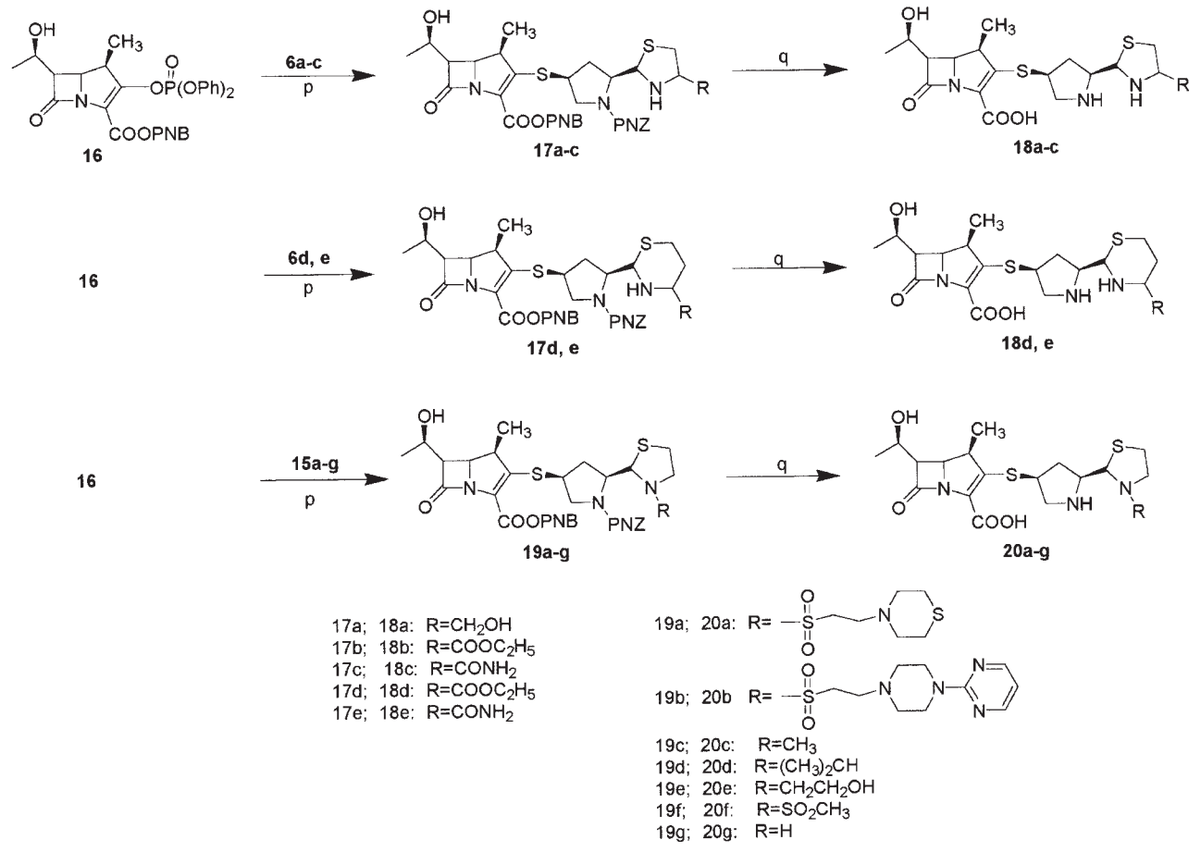

Scheme 3. (p) Diisopropylethylamine, CH_3CN, 0 °C. (q) H_2, Pd/C, THF : H_2O = 1 : 1.

The *N*-alkylated and acylated thiazolidine thiol derivatives (**15a**–**g**) were prepared as shown in Scheme 2. Treatment of **12** with bromoethanesulfonyl chloride provided **13**, which was reacted with cyclic amines to afford corresponding compounds (**14a**–**b**). Also treatment of **12** with alkyl halides gave the corresponding *N*-alkylated thiazolidine derivatives (**14c**–**g**). Other thiols (**14a**–**g**) were prepared by a procedure similar to the preparation of **6a**.

Reaction of **16** [9] with thiol (**6a**–**e**, **15a**–**g**) in the presence of diisopropylethylamine provided the 2-substituted carbapenem (**18a**–**e**, **20a**–**g**) (Scheme 3). Deprotection of these compounds by catalytic hydrogenation gave the crude products, which were purified on a HP-20 column to give the pure carbapenems.

Results and discussion

The *in vitro* antibacterial activities of the new carbapenems (**18a**–**e**, **20a**–**g**) prepared above against Gram-positive and negative bacteria are listed in Table 1. For comparision, the MIC values of Imipenem and Meropenem are also listed. Comparison of the compounds (**18a**–**c**) having an amide, ester, or hydroxy group at the 2′ position of thiazolidine showed little difference in the antibacterial activities against Gram-positive and Gram-negative bacteria; however, the substituted thiazolidines exhibited superior antibacterial activities to the non-substituted compound (**20g**). As expected, the 2-amide-substituted compound (**18c**) showed the most potent and well balanced activity. The ester-substituted compound (**18a**) showed better antibacterial activity, especially against Gram-positive bacteria than the corresponding analogues (**18b**–**c**, **20g**). On the other hand, in the *N*-alkylated thiazolidine series, the *N*-mesylated compound (**20f**) showed the most potent activities against Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa.* However, most *N*-alkylated thiazolidine compounds (**20a**–**e**) displayed inferior or similar antibacterial activities to the compound **20g**, especially against Gram-positive bacteria includ-

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

Strains	**18a**	**18b**	**18c**	**18d**	**18e**	**20a**	**20b**	**20c**	**20d**	**20e**	**20f**	**20g**	Imi-penem	Mero-penem
1 *Streptococcus pyogenes* 308A	<0.01	<0.01	0.01	<0.01	0.01	0.20	0.01	0.01	0.01	0.01	<0.01	0.01	<0.01	0.01
2 *Streptococcus pyogenes* 77A	<0.01	<0.01	0.01	<0.01	0.01	0.10	0.01	0.01	0.01	0.01	<0.01	0.01	<0.01	<0.01
3 *Staphylococcus aureus* SG511	0.05	0.05	0.10	0.05	0.10	0.80	0.10	0.20	0.20	0.05	0.03	0.20	0.01	0.10
4 *Staphylococcus aureus* 285	0.10	0.20	0.20	0.05	0.10	0.80	0.20	0.40	0.40	0.10	0.05	0.40	0.01	0.10
5 *Escherichia coli* DC2	0.01	0.03	0.03	0.03	0.05	0.10	0.40	0.80	0.40	0.05	0.01	0.40	0.40	0.03
6 *Escherichia coli* TEM	0.01	0.10	0.10	0.05	0.05	0.40	1.56	0.20	0.20	0.10	0.03	0.40	0.20	0.03
7 *Pseudemonas aeroginosa* 1592E	1.56	12.5	0.80	6.10	1.56	25	100	25.0	50.0	1.56	3.10	6.25	0.80	0.20
8 *Salmonella typhimurium*	0.20	0.10	0.05	0.80	0.05	0.40	0.20	0.40	0.40	0.20	0.03	0.40	0.80	0.03
9 *Klebsiella aerogenes* 1522E	0.05	0.20	0.05	0.10	0.05	0.80	3.10	0.40	0.40	0.20	0.03	0.40	0.10	0.05
10 *Enterobacter cloacae* 132lE	0.01	0.05	0.01	0.05	0.02	0.20	0.80	0.20	0.20	0.05	0.03	0.20	0.10	0.03
DHP-1	10.80	11.14	13.80	13.17	14.43	11.91	13.9	11.01	11.50	9.40	9.81	10.30	1.84	9.13

ing *Pseudomonas aeruginosa*. Comparing the compounds (**18b**, **c**, **d**, **e**) with 2-substituted thiazolidine or 2-substituted thiazinane moiety at C′-5 of pyrrolidine, similar activity was observed against all tested bacteria. Among these compounds, **18b**, **18d**, and **20f** showed superior or similar antibacterial activity against Gram-positive bacteria to Meropenem, and **18c** and **20f** exhibited improved antibacterial activity against Gram-negative bacteria compared to Imipenem, except in the case of *P. aeruginosa.* As for DHP-I stability, most of the amide substituted thiazolidine compounds, especially **18c** and **18e**, showed high stability to renal DHP-I.

Experimental part

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.

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

4-(tert-Butyldimethylsilanyloxy)-2-[(4-ethoxycarbonymethyl)-thiazolidin-2-yl]-1-(p-nitrobenzyl oxycarbonyl)pyrrolidine (**2**)

To a solution of **1** (7.38 g, 18.0 mmol) in 90 % aq. EtOH (50 mL) was added slowly a solution of L-cysteine ethyl ester hydrochloride (3.34 g, 18.0 mmol) in water (20 mL) and $NaHCO_3$ (1.52 g, 18.0 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 ethyl acetate and water. Organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give **2** as a light yellowish oil. Yield 77.3 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.05 (s, 6 H), 0.98 (s, 9 H), 1.20 (t, 3 H, *J* = 7.0 Hz), 2.01 (bs, 2 H), 2.88 (m, 1 H), 3.02–3.17 (bs, 4 H), 3.25 (bs, 1 H), 4.11 (q, 2 H, *J* = 7.0 Hz), 4.23 (bs, 1 H), 4.35 (bs, 1 H), 5.06–5.19 (m, 2 H), 7.46 (d, 2 H, *J* = 7.2 Hz), 8.16 (d, 2 H, *J* = 7.2 Hz). – IR (KBr) 3220 (NH), 1710, 1690 (C=O) cm^{-1}.

4-(tert-Butyldimethylsilanyloxy)-2-[(4-hydroxymethyl)thiazolidin-2-yl]-1-(p-nitrobenzyl oxycarbonyl)pyrrolidine (**3**)

To a solution of **2** (2.55 g, 4.80 mmol) in EtOH (50 mL) was added slowly NaBH4 (0.54 g 14.3 mmol) at 0 °C. After 5 h, the mixture was diluted with H_2O (20 mL), 1N-HCl, and ethyl acetate (50 mL). The organic layer was dried over anhydrous, concentrated Na_2SO_4, and the resulting residue was purified by silica gel column chromatography to give **3** as a pale yellow oil. Yield 67.3 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.05 (s, 6 H), 0.98 (s, 9 H), 2.01 (bs, 2 H), 2.88 (m, 1 H), 3.02–3.17 (bs, 3 H), 3.25–3.45 (m, 4 H), 4.23 (bs, 1 H), 4.35 (bs, 1 H), 5.06–5.19 (m, 2 H), 7.56 (d, 2 H, *J* = 7.2 Hz), 8.12 (d, 2 H, *J* = 7.2 Hz). – IR (KBr) 3318 (OH), 3220 (NH), 1690 (C=O) cm^{-1}.

2-[(4-Acetoxymethyl)thiazolidin-2-yl]-4-(tert-butyldimethylsilanyloxy)-1-(p-nitrobenzyl oxycarbonyl)pyrrolidine (**4**)

A solution of **3** (1.30 g, 2.71 mmol) and triethylamine (0.57 mL, 4.07 mmol) in dry CH_2Cl_2 was cooled to –5 °C under nitrogen and treated with acetic anhydride (0.38 mL, 4.07 mmol). The mixture was stirred at 0 °C for 1 h, diluted with ethyl acetate (50 mL), and washed with cold water and brine. The organic

layer was dried over anhydrous Na_2SO_4, concentrated, and the resulting residue was purified by silica gel column chromatograhy to give **4** as a pale yellow oil. Yield 95. %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.05 (s, 6 H), 1.98 (bs, 2 H), 2.15 (s, 3 H), 2.98 (m, 1 H), 3.03–3.21 (bs, 3 H), 3.25–3.45 (m, 4 H), 4.21 (bs, 1 H), 4.37 (bs, 1 H), 5.06–5.13 (m, 2 H), 7.50 (d, 2 H, *J* = 7.2 Hz), 8.15 (d, 2 H, *J* = 7.2 Hz). – IR (KBr) 3220 (H), 1705, 1690 (C=O) cm^{-1}.

2-[(4-Acetoxymethyl)thiazolidin-2-yl]-4-methanesulfonyloxy-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**5**)

To a solution of **4** (0.89 g, 1.66 mmol) in THF (50 mL) was added slowly a solution of 1 M tetrabutylammonium fluoride (2.49 mL, 2.49 mmol) in HF, and then was stirred for 2 H at room temperature. The solvent was concentrated *in vacuo* to give a residue, which was used without further purification. A solution of this residue (0.67 g, 1.57 mmol) and triethylamine (0.23 mL, 1.73 mmol) in dry CH_2Cl_2 was cooled to –5 °C under nitrogen and treated with methanesulfonyl chloride (0.20 g, 1.73 mmol). The mixture was stirred at 0 °C for 1 h, diluted with ethyl acetate (50 mL), and washed with cold water 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 **5** as a pale yellow oil. Yield 69.0 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.98 (bs, 2 H), 2.15 (s, 3 H), 2.96 (m, 1 H), 3.01 (s, 3 H), 3.06–3.17 (bs, 3 H), 3.25–3.45 (m, 4 H), 4.21 (bs, 1 H), 4.37 (bs, 1 H), 5.06–5.13 (m, 2 H), 7.54 (d, 2 H, *J* = 6.9 Hz), 8.22 (d, 2 H, *J* = 6.9 Hz). – IR (KBr) 3230 (NH), 1705, 1690 (C=O), 1220 (S=O) cm^{-1}.

2-(4-Hydroxymethylthiazolidin-2-yl)-4-mercapto-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**6 a**)

A mixture of **5** (0.58 g, 1.15 mmol) and potassium thioacetate (0.39 g, 3.45 mmol) in DMF (20 mL) and toluene (20 mL) was stirred at 70 °C for 3 h under N_2 gas. After cooling, the reaction mixture was diluted with ethyl acetate (50 mL), water (50 mL), and the aqueous layer was washed with ethyl acetate (20 mL × 2). The combined solvent was washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent gave a crude residue, which was chromatographed on silica gel using ethyl acetate/*n*-hexane (1 : 1) to give thioacetyl compound as a pale yellow oil. To a solution of the above acetyl compound in MeOH (15 mL) was added 0.58 mL of 4N-NaOH in an ice bath. After stirring for 20 min, 0.58 mL of 4N-HCl was added to this solution and the mixture diluted with ethyl acetate, washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated *in vacuo* to give **6 a** as a yellow oil. Yield 78.9 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.05 (bs, 2 H), 2.96 (m, 1 H), 3.06–3.17 (bs, 3 H), 3.25–3.45 (m, 4 H), 4.21 (bs, 1 H), 4.37 (bs, 1 H), 5.06–5.13 (m, 2 H), 7.41 (d, 2 H, *J* = 6.9 Hz), 8.18 (d, 2 H, *J* = 6.9 Hz). – IR (KBr) 3360 (OH), 3230 (NH), 2580 (SH), 1670 (C=O) cm^{-1}.

Compounds **6 b**–**e** were prepared by a similar procedure to that described for the preparation of **6 a**.

4-(tert-Butyldimethylsilanyloxy)-2-[(4-carbamoyl)thiazolidin-2-yl]-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**8**)

To a stirred solution of **2** (1.50 g, 2.78 mmol) in MeOH (20 mL) was added ammonium hydroxide (28 %, 20 mL) and was stirred for 20 h at room temperature. The mixture was neutralized with 6N-HCl, diluted with ethyl acetate (50 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4, and was purified by silica gel column chromatography to give **8** as a pale yellow oil. Yield 87.1 %. – ^{1}H-NMR ($CDCl_3$): δ = 0.05 (s, 6 H), 0.98 (s, 9 H), 2.01 (bs, 2 H), 2.99 (m, 1 H), 3.02–3.17 (bs, 2 H), 3.25 (bs, 1 H), 4.23 (bs, 1 H), 4.35 (bs, 1 H), 5.06–5.19 (m, 2 H), 5.91 and 6.08 (2 s, 2 H), 7.46 (d, 2 H, *J* = 7.2 Hz), 8.16 (d, 2 H, *J* = 7.2 Hz). – IR (KBr) 3230 (NH), 1705, 1670 (C=O) cm^{-1}

2-[4-(tert-Butyldimethylsilanyloxy)-1-(p-nitrobenzyloxycarbonyl)pyrrolidin-2-yl]-[1,3]thiazinane-4-carboxylic acid ethyl ester (**11**)

L-Homocysteine hydrochloride (0.71 g, 4.13 mmol) was dissolved in H_2O (10 mL). NaOH (0.17 g, 4.13 mmol) in H_2O (4 mL) was added, followed by a solution of **2** (1.65 g, 4.13 mmol) in EtOH (95 %, 20 mL). The reaction mixture was stirred overnight at room temperature and then concentrated *in vacuo* to give a residue, which was diluted with ethyl acetate and water. Organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo* to give **10** as a light yellowish oil. This material was used without further purification. To a stirred solution of **10** in EtOH (20 mL) was added three drops of sulfuric acid. The reaction mixture was refluxed for 4 h and then was cooled to 0 °C. The mixture was neutralized with 10 % $NaHCO_3$, diluted with ethyl acetate (50 mL) and H_2O (20 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4. Removal of the solvent gave a crude residue, which was purified by silica gel column chromatography to give **11** as a pale yellow oil. Yield 75.6 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.05 (s, 6 H), 0.98 (s, 9 H), 1.20 (t, 3 H, *J* = 7.0 Hz), 1.68–1.85 (bs, 2 H), 2.01 (bs, 2 H), 2.78 (m, 1 H), 3.02–3.17 (bs, 3 H), 3.25 (bs, 2 H), 4.11 (q, 2 H, *J* = 7.0 Hz), 4.23 (bs, 1 H), 4.35 (bs, 1 H), 5.06–5.19 (m, 2 H), 7.56 (d, 2 H, *J* = 7.2 Hz), 8.14 (d, 2 H, *J* = 7.2 Hz). – IR (KBr) 3250 (NH), 1720, 1680 (C=O) cm^{-1}.

4-(tert-Butyldimethylsilanyloxy)-2-(thiazolidin-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**12**)

Synthesis of **12** from **1** was carried out by the same procedure using aminoethane thiol as described for the preparation of **2**. Yield 87.2 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.03 (s, 6 H), 0.95 (s, 9 H), 2.01 (bs, 2 H), 2.88 (m, 1 H), 3.02 (m, 1 H), 3.14–3.30 (bs, 5 H), 4.25 (bs, 1 H), 4.35 (bs, 1 H), 5.06 (q, 2 H), 7.46 (d, 2 H, *J* = 7.2 Hz), 8.13 (d, 2 H, *J* = 7.2 Hz). – IR (KBr) 3250 (NH), 1680 (C=O) cm^{-1}.

2-[3-(2-Chloroethanesulfonyl)thiazolidin-2-yl]-4-(tert-butyldimethylsilanyloxy)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**13**)

A solution of **12** (2.27 g, 4.85 mmol) and triethylamine (0.81 mL, 5.82 mmol) in dry CH_2Cl_2 was cooled to –5 °C under nitrogen and treated with chloroethanesulfonyl chloride (0.61 mL, 5.82 mmol). The mixture was stirred at 0 °C for 1 h, diluted with ethyl acetate (50 mL), and washed with 10 % $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4. Removal of the solvent gave a crude residue, which was purified by silica gel column chromatography to give **13** as a pale yellow oil. Yield 76.9 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.05 (s, 6 H), 0.94 (s, 9 H), 1.98 (bs, 2 H), 2.94 (m, 1 H), 3.02 (m, 1 H), 3.17–3.29 (bs, 5 H), 3.45 (t, 2 H, *J* = 5.8 Hz), 3.64 (t, 2 H, *J* = 5.8 Hz), 4.23 (bs, 1 H), 4.35 (bs, 1 H), 5.06 (q, 2 H), 7.55 (d, 2 H, *J* = 6.9 Hz), 8.18 (d, 2 H, *J* = 6.9 Hz). – IR (KBr) 1690 (C=O), 1220 (S=O) cm^{-1}.

4-(tert-Butyldimethylsilanyloxy)-2-[3-(2-thiomorpholin-4-yl-ethanesulfonyl)thiazolidin-2-yl]-1-(p-nitrobenzyloxycarbonyl)-pyrrolidine (**14 a**)

Compound **13** (1.11 g, 1.87 mmol), potassium carbonate (0.28 g, 2.05 mmol), and thiomorpholine (0.21 g, 2.05 mmol) were dissolved in DMF (20 mL) and the solution was heated at 90 °C for 10 h. The resulting solution was diluted with ethyl acetate. The organic layer was washed with water, 1N-HCl and brine. Evaporation of the solvent in vacuo gave a crude residue,

which was chromatographed on silica gel using ethyl acetate/hexane (1:1) as eluent to give **14a** as a pale yellow oil. Yield 80.1%. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.01 (s, 6H), 0.95 (s, 9H), 1.98 (bs, 2H), 2.94 (m, 1H), 3.02–3.31 (bs, 8H), 3.40–3.62 (bs, 8H), 3.68 (t, 2H, *J* = 5.8 Hz), 4.23 (bs, 1H), 4.35 (bs, 1H), 5.01 (q, 2H), 7.42 (d, 2H, *J* = 7.2 Hz), 8.11 (d, 2H, *J* = 7.2 Hz).

Compounds **14b** from **13** were prepared by the same procedure as described for the preparation of **14a**.

4-(tert-Butyldimethylsilanyloxy)-2-(3-methylthiazolidin-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**14c**)

To a solution of potassium carbonate (0.54 g, 3.96 mmol) in dry CH_3CN (50 mL) was added slowly a solution of **12** (1.85 g, 3.96 mmol) in dry CH_3CN (10 mL) under N_2 gas. After 1 h, methyl iodide (2.81 g, 19.8 mmol) was added dropwise to the reaction mixture, which was stirred for 24 h at room temperature. The resulting mixture was diluted with ethyl acetate (50 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent gave a crude residue, which was chromatographed on silica gel using ethyl acetate as eluent to give **14c** as a pale yellow oil. Yield 82.0%. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.05 (s, 6H), 0.98 (s, 9H), 2.06 (bs, 2H), 2.99 (m, 1H), 3.13–3.25 (bs, 6H), 3.30 (s, 3H), 4.24 (bs, ^{1}H), 4.54 (bs, 1H), 5.11 (q, 2H), 7.46 (d, 2H, *J* = 7.2 Hz), 8.13 (d, 2H, *J* = 7.2 Hz).

4-(tert-Butyldimethylsilanyloxy)-2-(3-methanesulfonylthiazolidin-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**14g**)

A solution of **12** (0.73 g, 1.57 mmol) and triethylamine (0.23 mL, 1.73 mmol) in dry CH_2Cl_2 was cooled to 0 °C under nitrogen and treated with methanesulfonyl chloride (0.20 g, 1.73 mmol). The mixture was stirred at 0 °C for 1 h, diluted with ethyl acetate (50 mL), and washed with cold water 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 **14g** as a pale yellow oil. Yield 89.0%. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.01 (s, 6H), 0.98 (s, 9H), 2.05 (bs, 2H), 2.82 (m, 1H), 3.05 (s, 3H), 3.22 (m, 1H), 3.14–3.30 (bs, 5H), 4.25 (bs, 1H), 4.35 (bs, 1H), 5.06–5.11 (q, 2H), 7.43 (d, 2H, *J* = 7.1 Hz), 8.13 (d, 2H, *J* = 7.1 Hz). – IR (KBr) 3250 (NH), 1680 (C=O), 1225 (S=O) cm^{-1}.

Compounds **15b**–**f** from **14b**–**f** were carried out by the same procedure as described for the preparation of **6a**.

p-Nitrobenzyl(1R,5S,6S)-6-[(1R)-hydroxyethyl)]-2-[5-(4-hydroxymethylthiazolidin-2-yl)-1-(p-nitrobenzyloxycarbonyl)-pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylate (**17a**)

A solution of *p*-nitrobenzyl-(1*R*,5*S*,6*S*)-3-(diphenylphosphoryloxy)-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**16**, 0.54 g, 0.91 mmol) in CH_3CN (50 mL) was cooled to 0 °C under N_2. To this solution was added diisopropyl ethylamine (0.13 g, 1.0 mmol) and a solution of the mercapto compound **6a** (0.36 g, 0.91 mmol) in CH_3CN (10 mL). After stirring for 2 h, the mixture was diluted with ethyl acetate, washed with 10% $NaHCO_3$, brine, and dried over $MgSO_4$. Evaporation *in vacuo* gave a foam, which was purified by silica gel chromatography to give **17a** as a yellow foam solid. Yield 79.3%. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.25 (d, 3H, *J* = 6.6 Hz), 1.33 (d, 3H, *J* = 6.2 Hz), 1.88 (bs, 1H), 2.14 (m, 1H), 2.46 (m, 1H), 2.95 (m, 2H), 3.33 (bs, 2H), 3.45 (d, 2H, *J* = 9.6 Hz), 3.87 (m, 2H) 4.01–4.18 (m, 3H), 4.25–4.35 (m, 2H), 4.43 (bs, 1H), 5.11–5.55 (m, 4H), 7.21 (d, 2H, *J* = 7.4 Hz), 7.41 (d, 2H, *J* = 7.4 Hz), 8.18 (d, 4H, *J* = 7.4 Hz). – IR (KBr): 3410 (OH), 3230 (NH), 1720, 1705, 1660 (C=O) cm^{-1}.

(1R,5S,6S)-6-[(1R)-Hydroxyethyl)]-3-[5-(4-hydroxymethylthiazolidin-2-yl)pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid (**18a**)

Compound **17a** (0.24 g, 0.06 mmol) and 0.1 g of Pd/C (10%) were dissolved in THF/phosphate buffer (pH = 7) (1:1, 10 mL each). The mixture was hydrogenated at 345 kPa for 1 h. The solution was filtered through celite and washed with water (2 × 10 mL). The combined filtrate was washed with ethyl ether (2 × 20 mL) and lyophilized to give a yellow powder which was purified on reversed phase column chromatography, eluting with 5% THF in water. Fractions having UV absorption at 298 nm were collected and lyophilized again to give the title compound **18a** as a white powder. Yield 47.9%. – UV λ_{max}: 298 nm. – Mp 145–150 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.06 (d, 3H, *J* = 6.5 Hz), 1.15 (d, 3H, *J* = 5.7 Hz), 1.56 (bs, 1H), 2.04 (bs, 1H), 2.46 (m, 1H), 3.05 (bs, 2H), 3.23 (bs, 2H), 3.40 (bs, 2H), 3.56 (bs, 2H), 3.77 (m, 2H), 4.10 (m, 2H), 4.39 (bs, 1H). – IR (KBr): 3470 (OH), 3230 (NH), 1710, 1690 (C=O) cm^{-1}. – FABMS: *m/z* 430 $(M + H)^+$.

18b: Yield 42.3%. – UV λ_{max}: 297 nm. – Mp 139–144 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.06 (d, 3H, *J* = 6.5 Hz), 1.15 (d, 3H, *J* = 5.7 Hz), 1.23 (t, 3H, *J* = 7.0 Hz), 1.66 (bs, 1H), 2.04 (bs, 1H), 2.46 (m, 1H), 3.05 (bs, 2H), 3.23 (bs, 2H), 3.40 (bs, 2H), 3.77 (m, 2H), 4.10 (m, 2H), 4.11 (q, 2H, *J* = 7.0 Hz), 4.39 (bs, 1H). – IR (KBr): 3450 (OH), 3250 (NH), 1720, 1705 (C=O) cm^{-1}. – FABMS: *m/z* 472 $(M + H)^+$.

18c: Yield 34.6%. – UV λ_{max}: 298 nm. – Mp 172–180 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.09 (d, 3H, *J* = 6.3 Hz), 1.16 (d, 3H, *J* = 5.9 Hz), 1.70 (bs, 1H), 2.04 (bs, 1H), 2.55 (m, 1H), 3.05 (bs, 2H), 3.23 (bs, 2H), 3.40 (bs, 2H), 3.77 (m, 2H), 4.10 (m, 2H), 4.39 (bs, 1H). – IR (KBr): 3450 (OH), 3240 (NH), 1690, 1660 (C=O) cm^{-1}. – FABMS: *m/z* 443 $(M + H)^+$.

18d: Yield 36.9%. – UV λ_{max}: 297 nm. – Mp 125–130 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.06 (d, 3H, *J* = 6.5 Hz), 1.15 (d, 3H, *J* = 5.7 Hz), 1.23 (t, 3H, *J* = 7.0 Hz), 1.66–1.78 (bs, 3H), 2.02 (bs, 1H), 2.56 (m, 1H), 3.05 (bs, 2H), 3.23 (bs, 2H), 3.40 (bs, 2H), 3.77 (m, 2H), 4.10–4.16 (m, 4H), 4.39 (bs, 1H). – IR (KBr): 3480 (OH), 3210 (NH), 1705, 1690 (C=O) cm^{-1}. – FABMS: *m/z* 472 $(M + H)^+$.

18e: Yield 27.3%. – UV λ_{max}: 298 nm. – Mp 189–192 °C (dec.). – ^{1}H–NMR (D_2O): δ (ppm) = 1.11 (d, 3H, *J* = 6.2 Hz), 1.21 (d, 3H, *J* = 5.8 Hz), 1.59–1.78 (bs, 3H), 2.01 (bs, 1H), 2.78 (m, 1H), 3.05 (bs, 2H), 3.23 (bs, 2H), 3.42 (bs, 2H), 3.87 (m, 2H), 4.12 (m, 2H), 4.34 (bs, 1H). – IR (KBr): 3430 (OH), 3250 (NH), 1685, 1665 (C=O) cm^{-1}. – FABMS: *m/z* 457 $(M + H)^+$.

20a: Yield 66.0%. – UV λ_{max}: 296 nm. – ^{1}H-NMR (D_2O): δ (ppm) = 1.13 (d, 3H, *J* = 6.2 Hz), 1.21 (d, 3H, *J* = 5.8 Hz), 1.59 (bs, 1H), 2.01 (bs, 1H), 2.78–3.05 (m, 6H), 3.15 (bs, 2H), 3.23–3.35 (bs, 6H), 3.42–3.66 (bs, 6H), 3.87 (m, 2H), 4.11 (m, 2H), 4.39 (bs, 1H). – IR (KBr): 3510 (OH), 3300 (NH), 1690 (C=O) cm^{-1}. – FABMS: *m/z* 529 $(M + H)^+$.

20b: Yield 35.0%. – UV λ_{max}: 298, 310 nm. – Mp 145–151 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.13 (d, 3H, *J* = 6.2 Hz), 1.21 (d, 3H, *J* = 5.8 Hz), 1.59 (bs, 1H), 2.21 (bs, 1H), 2.78–3.05 (m, 2H), 3.15 (bs, 2H), 3.24–3.55 (bs, 10H), 3.42–3.66 (bs, 6H), 3.87 (m, 2H), 4.05 (m, 2H), 4.49 (bs, 1H), 7.31 (t, ^{1}H, *J* = 6.4 Hz), 8.68 (d, 2H, *J* = 6.4 Hz). – IR (KBr): 3510 (OH), 3300 (NH), 2990, 1690 (C=O) cm^{-1}. – FABMS: *m/z* 590 $(M + H)^+$.

20c: Yield 41.2%. – UV λ_{max}: 298 nm. – Mp 115–123 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.11 (d, 3H, *J* = 6.2 Hz), 1.18 (d, 3H, *J* = 5.9 Hz), 1.59 (bs, 1H), 2.03 (bs, 1H), 2.84 (m, 1H), 3.05 (bs, 2H), 3.23–3.42 (bs, 5H), 3.55 (s, 3H), 3.77 (m, 2H), 4.12 (m,

2H), 4.45 (bs, 1H). – IR (KBr): 3480 (OH), 3260 (NH), 1670 (C=O) cm^{-1}. – FABMS: *m/z* 414 $(M + H)^+$.

20d: Yield 37.9%. – UV λ_{max}: 298 nm. – Mp 132–142 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 0.96 (d, 6H, *J* = 7.6 Hz), 1.16 (d, 3H, *J* = 6.2 Hz), 1.30 (d, 3H, *J* = 5.9 Hz), 1.69 (bs, 1H), 2.03 (bs, 1H), 2.94 (m, 1H), 3.05 (bs, 2H), 3.22–3.51 (bs, 5H), 3.77 (m, 2H), 4.13 (m, 2H), 4.28 (m, 1H), 4.51 (bs, 1H). – IR (KBr): 3440 (OH), 3270 (NH), 1665 (C=O) cm^{-1}. – FABMS: *m/z* 442 $(M + H)^+$.

20e: Yield 34.5%. – UV λ_{max}: 296 nm. – Mp 133–134 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.16 (d, 3H, *J* = 6.9 Hz), 1.28 (d, 3H, *J* = 5.4 Hz), 1.58 (bs, 1H), 2.01 (bs, 1H), 2.79 (m, 1H), 3.03 (bs, 2H), 3.22–3.59 (bs, 9H), 3.77 (m, 2H), 4.13 (m, 2H), 4.44 (bs, 1H). – IR (KBr): 3510 (OH), 3300 (NH), 1690 (C=O) cm^{-1}. – FABMS: *m/z* 444 $(M + H)^+$.

20f: Yield 40.1%. – UV λ_{max}: 298 nm. – ^{1}H-NMR (D_2O): δ (ppm) = 1.13 (d, 3H, *J* = 6.2 Hz), 1.19 (d, 3H, *J* = 5.9 Hz), 1.59 (bs, 1H), 2.03 (bs, 1H), 2.88 (m, 1H), 2.99 (s, 3H), 3.08 (bs, 2H), 3.23–3.42 (bs, 5H), 3.77 (m, 2H), 4.12 (m, 2H), 4.45 (bs, 1H). – IR (KBr): 3510 (OH), 3230 (NH), 1690 (C=O), 1220 (S=O) cm^{-1}. – FABMS: *m/z* 478 $(M + H)^+$.

20g: Yield 34.7%. – UV λ_{max}: 298 nm. – Mp 111–115 °C (dec.). – ^{1}H-NMR (D_2O): δ (ppm) = 1.13 (d, 3H, *J* = 6.2 Hz), 1.19 (d, 3H, *J* = 5.9 Hz), 1.59 (bs, 1H), 2.03 (bs, 1H), 2.88 (m, 1H), 3.08 (bs, 2H), 3.18 (m, 2H), 3.30–3.55 (bs, 3H), 3.77 (m, 2H), 4.12 (m, 2H), 4.41 (bs, 1H). – IR (KBr): 3510 (OH), 3350 (NH), 1680 (C=O) cm^{-1}. – FABMS: *m/z* 400 $(M + H)^+$.

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