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Laboratory Note

Synthesis and antibacterial activity of 1β-methyl-2-(5-substituted thiazolo pyrrolidin-3-ylthio)carbapenem derivatives

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Abstract

The synthesis of a new series of 1β-methylcarbapenems having the substituted thiazolopyrrolidine moiety is described. Their in vitro antibacterial activities against both Gram-positive including MRSA and Gram-negative bacteria were tested and the effect of substituent on the thiazole ring was investigated. A particular compound (**28c**) having 4′-amide substituted thiazole moiety showed the most potent antibacterial activity. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: 1β-Methylcarbapenem; Antibacterial activity; Substituent effect

1. Introduction

A number of carbapenem antibiotics, for example imipenem, panipenem and meropenem, are currently in clinical use due to their broad antibacterial spectra and potent bactericidal effects [1]. However, a number of problems still remain with these agents, in particular, activity against resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and the Gram-negative pathogen *Pseudomonas aeruginosa*, is relatively weak. During the past decade, extensive synthetic efforts have been made to confer anti-MRSA activity on β-lactams such as a cephalosporin [2] or a carbapenem [3]. As a result, some cephalosporin and carbapenem derivatives with potent in vitro anti-MRSA activity were identified by introducing hydrophobic functional groups into the C-3 or C-7 side chain of the cephalosporin nucleus or the C-2 side chain of the carbapenem nucleus.

From the literature of carbapenem antibiotics, especially the SAR related to meropenem [4,5] and panipenem [6], the importance of a pyrrolidine ring for potent activity and high PBP affinity were noted. Thus we postulated that a combination of these two factors in a single side chain might lead to agents with a broader spectrum of activity and anti-MRSA activity.

Previously, we reported that the carbapenem compounds which have 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 synthesised and investigated [7–11].

In this paper, we describe the synthesis and structure–activity relationships of the 1β-methylcarbapenems having a 5′-substituted thiazolo pyrrolidine-3′-ylthio group as C-2 side chain and our approach for improvement of anti-MRSA and antibacterial activity of the carbapenems is discussed.

2. Results and discussion

2.1. Chemistry

Our general synthetic route leading to new carbapenems involved the preparation of appropriately protected thiols containing thiazole 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.

2-(4-Substituted thiazole)pyrrolidine derivatives

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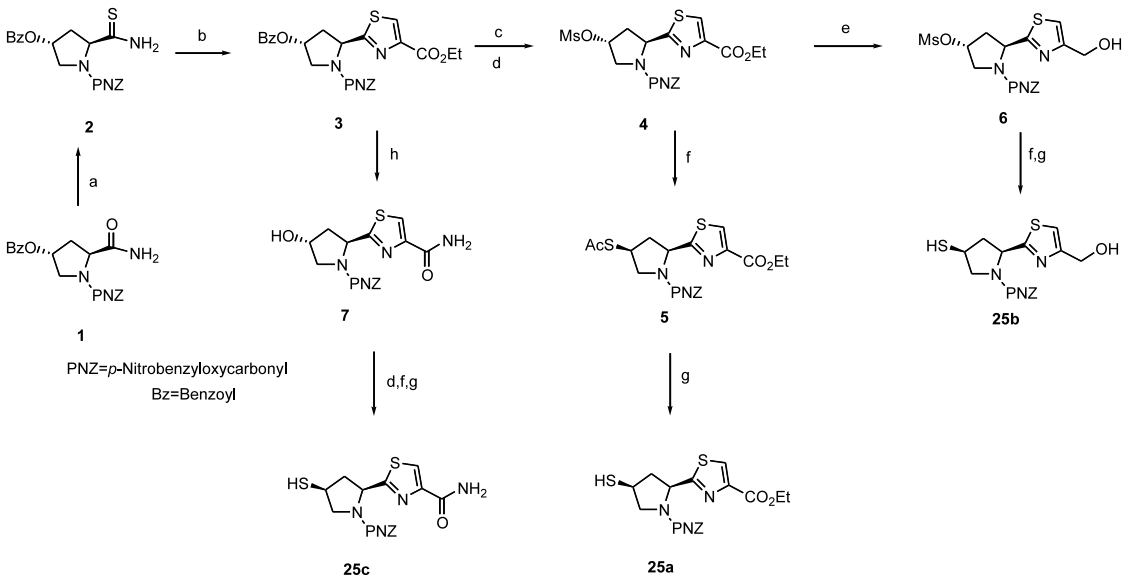

Fig. 1. (a) Lawesson's reagent, THF; (b) Ethyl bromopyruvate, EtOH; (c) 4*N*-NaOH, MeOH; (d) MsCl, TEA, CH_2Cl_2; (e) $NaBH_4$, EtOH; (f) AcSK, DMF–toulene = 1:1; (g) 4*N*-NaOH, MeOH; (h) 28% NH_4OH, MeOH.

containing the ester, hydroxy, amide, amine, aminosulfonmethyl and cyano group (**25a**–**f**) were prepared by the sequence shown in Figs. 1 and 2. The formation of 4′-substituted thiazole ring was accomplished by the reaction of thioamide **2** with ethyl bromopyruvate in EtOH solution. Debenzolylation of **3** with potassium hydroxide gave the hydroxyl compound, which was converted to the *O*-mesylated compound **4** by treatment of mesyl chloride. Treatment of **4** with potassium thioacetate in DMF and followed by hydrolysis of the resulting acetylthio group with 4*N*-NaOH in methanol led to the thiol compound (**25a**). Thiazole derivative **4** was reduced with sodium borohydride in EtOH to give **6**. The 4′-ester substituted thiazole (**3**) was converted to the amide **7** by aminolysis with aqueous ammonia in MeOH. Compounds **25b** and **25c** were prepared from **6** and **7** by similiar methods to that described for the preparation of **25a** from **4** (Fig. 1).

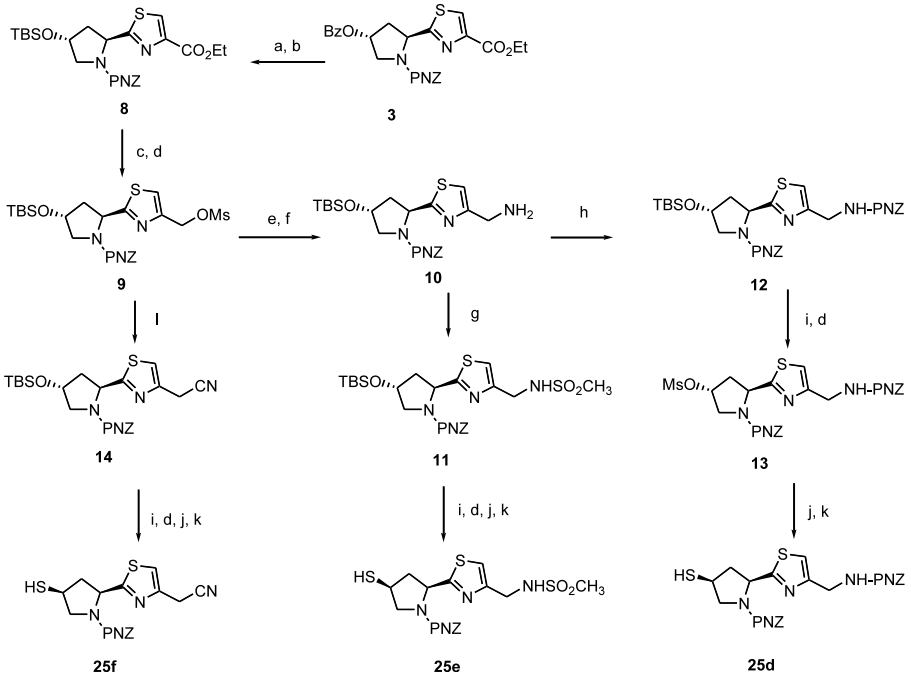

Fig. 2. (a) 4*N*-NaOH, MeOH; (b) TBSCl, imidazole, DMF; (c) $NaBH_4$, EtOH; (d) MsCl, TEA, CH_2Cl_2; (e) potassium pthalimide, DMF; (f) $N_2H_4 \cdot H_2O$, MeOH; (g) MsCl, TEA, CH_2Cl_2; (h) PNZ-Cl TEA, CH_2Cl_2; (i) 1*N*-tetrabutylammonium fluoride, THF; (j) AcSK, DMF–toulene = 1:1; (k) 4*N*-NaOH, MeOH (l) NaCN, DMSO.

The protecting group of **3** converted into the silyl group by the reaction with *t*-butyldimethylsilylchloride (TBSCl) and imidazole in DMF. The silyl compound **8** was reduced with sodium borohydride in EtOH and subsequently mesylated to give **9**, which was successfully converted into the amine **10** via phthalimide derivative by the usual method. Sulfonylation of the amine **10** with mesyl chloride proceeded to afford **11**. The treatment of the mesylated **9** with sodium cyanide in DMSO gave the cyano substituted thiazole derivative **14**.

Deprotection of the silyl derivatives (**11**, **12**, **14**) carried out with tetrabutylammonium fluoride in THF and the corresponding thiols (**25d**–**f**) were prepared by a similar procedure of preparation of **25a** (Fig. 2).

The preparation of 2-methoxycarbonylmethylthiazole (**15**) and 5-methylthiazole (**18**) were carried out using methyl 4-chloroacetoacetate and methyl 2-chloroacetoacetate by a similiar method for the preparation of **3**, respectively. Also the preparation of the corresponding thiols (**25g**–**i**) were obtained by above similar manners (Fig. 3).

On the other hand, the pyrrolidine intermediate **20** for one carbon homologated compound ($n = 1$) was obtained from *trans*-hydroxy-L-proline via several steps [11].

The other thiols (**25j**–**n**) were prepared by similar methods as shown in Figs. 1 and 2 (Fig. 4). Finally, the reaction of **26** [9] with thiols (**25a**–**n**) in the presence of diisopropylethylamine provided the 2-substituted carbapenem (**27a**–**n**). Deprotection of these compounds by catalytic hydrogenation gave the crude products, which were purified by HP-20 column to give the pure carbapenems (**28a**–**n**) (Fig. 5).

2.2. Biological assay

2.2.1. 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^{-1} 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 MICs of a compound was defined as the lowest concentration that visibly inhibited growth.

2.2.2. 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^{-1}) 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.

2.2.3. Antibacterial activity studies

The in vitro antibacterial activities of the new carbapenems (**28a**–**n**) prepared above against Gram-positive and negative bacteria including strains of MRSA are listed in Table 1. For comparison, the MIC values of imipenem, meropenem and vancomycin are also listed. All compounds (**28a**–**n**) displayed superior or similar antibacterial activities against Gram-

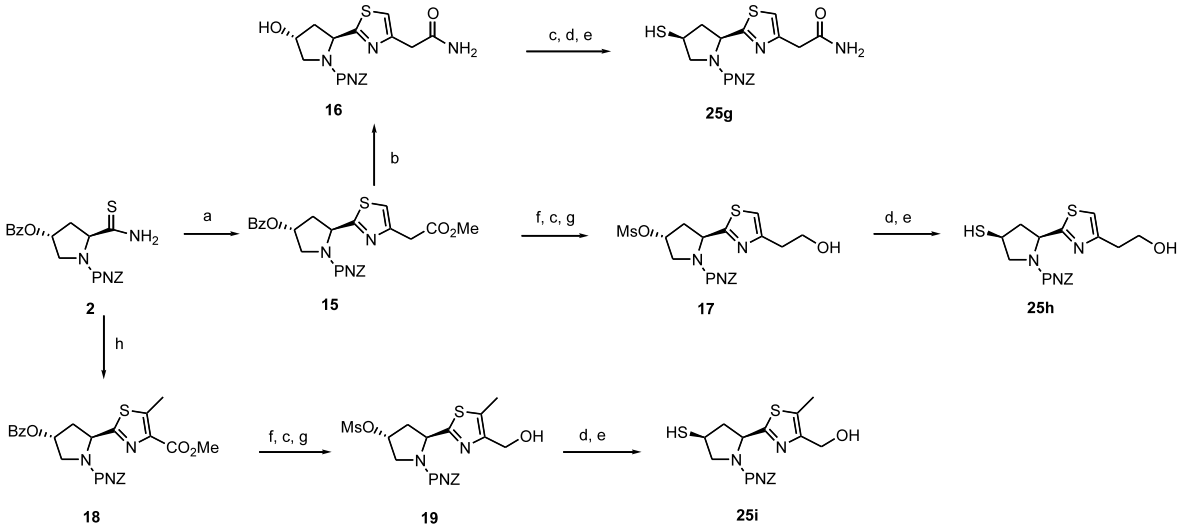

Fig. 3. (a) Methyl-4-chloroacetoacetate, EtOH; (b) 28% NH_4OH, MeOH; (c) MsCl, TEA, CH_2Cl_2; (d) AcSK, DMF–toulene = 1:1; (e) 4*N*-NaOH, MeOH; (f) 4*N*-NaOH, MeOH; (g) $NaBH_4$, EtOH; (h) methyl-2-chloroacetoacetate, EtOH.

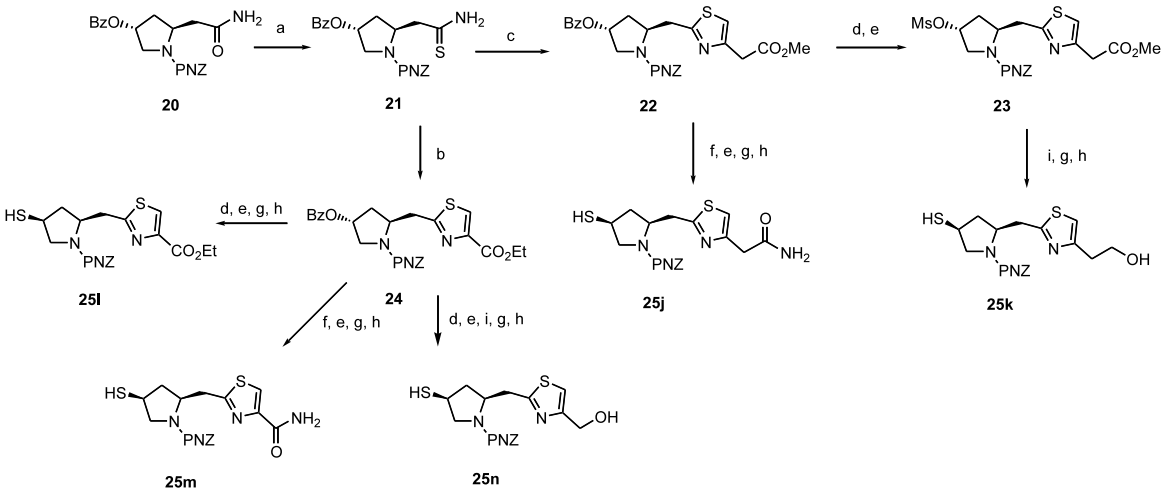

Fig. 4. (a) Lawesson's reagent, THF; (b) ethyl bromopyruvate, EtOH; (c) methyl-4-chloroacetoacetate, EtOH; (d) 4*N*-NaOH, MeOH; (e) MsCl, TEA, CH_2Cl_2; (f) 28% NH_4OH, MeOH; (g) AcSK, DMF–toulene = 1:1; (h) 4*N*-NaOH, MeOH; (i) $NaBH_4$, EtOH.

positive to meropenem, and against Gram-negative bacteria except *P. aeruginosa* to imipenem.

Comparing the compounds **28a**–**f** having amide, ester, hydroxy, amine, cyano group at C′-4 position of thiazole showed slight differences in the antibacterial activities against Gram-positive and -negative bacteria. As expected, the 4′-amide substituted compound **28c** exhibited the most potent and well balanced activity. The ester substituted compounds (**28a**, **28l**) showed better antibacterial activities especially against Gram-positive bacteria than the corresponding analogues (**28b**–**e**).

As an extent of alkyl group in the thiazole ring increased, antibacterial activity generally decreased against Gram-positive and -negative bacteria including strains of MRSA, as shown in compounds (**28b**, **c** vs. **28h**, **g**). Also introduction of a methyl group in the thiazole ring **28i** significantly lowered the antibacterial activity compared to compound **28b**. On the other hand, comparison with **28a**–**c** and **28j**–**n** shows that introduction of carbon between pyrrolidine and thiazole does not enhance antibacterial activities but showed high stability to renal DHP-I. From these observations, the length of the spacer part seems to play important roles improving stability while decreasing antibacterial activity.

The amide substituted compounds (**28c**, **28m**) showed more improved stability to DHP-I than the corresponding analogues (**28a**, **b**, **l**, **n**).

3. Experimental

Melting point (m.p.): 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.

*3.1. (2S,4R)-4-Benzoyloxy-2-thiocarbamoyl-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**2**)*

Compound **1** [11] (7.02 g, 17.0 mmol) and Lawesson's reagent (4.10 g, 10.0 mmol) were dissolved in THF (50 mL) and the solution was heated at reflux for 2 h. Evaporation of the resulting solution in vacuo gave a crude residue, which was purified by silica gel column chromatography to give **2** (6.91 g, 94.7%) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$): δ 2.47 (bs, 1H), 2.72 (bs, 1H), 3.86 (bs, 1H), 4.01 (bs, 1H), 4.55 (bs, 2H), 5.05

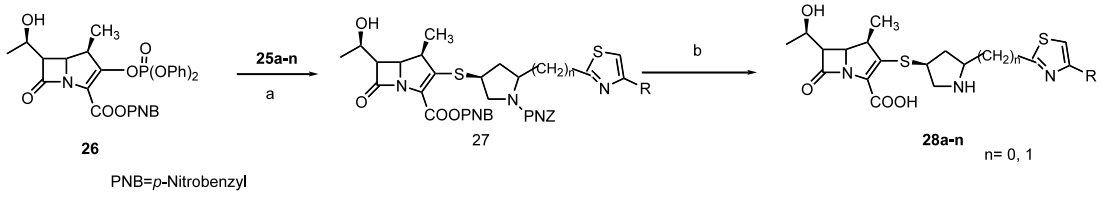

Fig. 5. (a) *N*,*N*-Diisopropylethylamine, CH_3CN; (b) H_2, Pd–C, THF–H_2O.

Table 1
In vitro antibacterial activity (MIC, μg mL^{-1}) and DHP-I stability of the carbapenem derivatives

Strains	**28a**	**28b**	**28c**	**28d**	**28e**	**28f**	**28g**	**28h**	**28i**	**28j**	**28k**	**28l**	**28m**	**28n**	IPM	MPM	VCM
S. pyogenes 308A	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.10	0.02	0.02	0.02	0.01	< 0.01	< 0.01	0.01	< 0.01	0.01	–
S. pyogenes 77A	< 0.01	0.01	< 0.01	0.01	0.01	0.01	0.10	0.02	0.02	0.02	0.01	< 0.01	< 0.01	0.01	< 0.01	< 0.01	–
S. aureus SG511	0.03	0.03	0.03	0.05	0.05	0.05	0.05	0.05	0.10	0.20	0.10	0.03	0.03	0.05	0.01	0.10	–
S. aureus 285	0.03	0.05	0.05	0.10	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.03	0.05	0.10	0.01	0.10	–
S. aureus SNU5 [a]	0.10	0.10	0.10	0.20	0.20	0.20	0.80	3.10	6.20	12.5	12.5	12.5	25.0	12.5	–	–	0.80
S. aureus SNU12 [a]	0.40	0.40	0.20	0.40	0.40	0.40	0.40	0.40	0.80	1.56	1.56	6.20	0.40	0.80	–	–	0.80
S. aureus SNU16 [a]	6.20	3.10	6.20	6.20	6.20	12.5	3.10	25.0	12.5	25.0	25.0	12.5	3.10	12.5	–	–	0.40
Escherichia coli DC2	0.01	0.01	0.01	0.05	0.03	0.01	0.03	0.03	0.03	0.10	0.20	0.03	0.03	0.05	0.40	0.03	–
E. Coli TEM	0.05	0.01	0.01	0.05	0.03	0.03	0.05	0.05	0.05	0.10	0.20	0.03	0.03	0.10	0.20	0.03	–
P. aeruginosa 1592E	1.56	1.56	1.56	3.10	12.5	6.10	6.10	6.10	12.5	6.10	12.5	1.56	1.56	6.20	0.80	0.20	–
S. typhimurium	0.05	0.02	0.02	0.10	0.05	0.03	0.03	0.05	0.10	0.40	0.20	0.05	0.05	0.05	0.80	0.03	–
K. aerogenes 1522E	0.03	0.05	0.03	0.10	0.05	0.05	0.05	0.03	0.10	0.40	0.20	0.05	0.05	0.05	0.10	0.05	–
E. cloacae 1321E	0.01	0.01	0.01	0.05	0.02	0.01	0.01	0.03	0.05	0.20	0.10	0.03	0.03	0.05	0.10	0.03	–
DHP-1	0.98	1.11	1.59	1.27	1.05	1.61	0.93	0.90	1.57	1.54	1.25	1.71	2.05	1.33	0.18	1.00	–

[a] Methicillin resistant. IPM, imipenem; MPM, meropenem; VCM, vancomycin.

and 5.32 (2H, s and dd, $J = 12.6$ Hz), 5.80, 6.12 (bs, 2H), 7.49 (m, 4H), 7.64 (d, 1H, $J = 7.1$ Hz), 7.94 (d, 2H, $J = 7.1$ Hz), 8.11 (d, 2H, $J = 7.1$ Hz).

3.2. (2S,4R)-4-Benzoyloxy-2-(4-ethoxycarbonythiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (3)

To a stirred solution of **2** (4.29 g, 10.0 mmol) in EtOH (60 mL) was added dropwise ethyl bromopyruvate (1.80 mL, 13.0 mol) and was stirred for 20 h at room temperature (r.t.). The resulting solution was evaporated and diluted with EtOAc, and washed with 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 **3** (4.69 g, 89.2%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 1.43 (t, 3H, $J = 7.9$ Hz), 2.75–2.81 (bs, 2H), 3.98–4.07 (bs, 2H), 4.17 (q, 2H, $J = 7.9$ Hz), 4.95 (bs, 1H), 5.15 (bs, 1H), 5.61 (bs, 2H), 7.27 (s, 1H), 7.49 (t, 3H, $J = 8.5$ Hz), 7.66 (t, 1H, $J = 8.5$ Hz), 7.96 (d, 2H, $J = 8.5$ Hz), 8.11 (d, 2H, $J = 8.5$ Hz).

3.3. (2S,4R)-2-(4-Ethoxycarbonythiazole-2-yl)-4-mesyloxy-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (4)

To a solution of **3** (5.50 g, 10.5 mmol) in MeOH (20 mL) was added 0.59 g of KOH (10.5 mmol) at ice bath. After stirring for 2 h, the reaction mixture was neutralised with 4*N*-HCl and was diluted with EtOAc, washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to give a yellow oil. A solution of above and Et_3N (1.41 mL, 10.5 mmol) in dry CH_2Cl_2 was cooled to 0 °C under nitrogen and treated with methanesulfonyl chloride (1.20 g, 10.5 mmol). The mixture was stirred at 0 °C for 1 h, diluted with EtOAc (50 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 **4** (4.61 g, 87.9%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 1.34 (t, 3H, $J = 7.7$ Hz), 2.25 (bs, 1H), 2.81 (bs, 1H), 3.01 (s, 3H), 3.98–4.07 (bs, 2H), 4.11 (q, 2H, $J = 7.7$ Hz), 4.81 (bs, 1H), 5.15 (bs, 1H), 5.41 (bs, 2H), 7.05 (s, 1H), 7.35 (d, 1H, $J = 7.5$ Hz), 7.60 (d, 1H, $J = 7.5$ Hz), 7.96 (d, 1H, $J = 7.5$ Hz), 8.11 (d, 1H, $J = 7.5$ Hz).

3.4. (2S,4S)-4-Acetylthio-2-(4-ethoxycarbonythiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrroli dine (5)

A mixture of **4** (0.58 g, 1.15 mmol) and potassium thioacetate (0.39 g, 3.45 mmol) in DMF (20 mL) and $C_6H_5CH_3$ (20 mL) was stirred at 70 °C for 3 h under N_2 gas. After cooling, the reaction mixture was diluted with EtOAc (50 mL), water (50 mL) and the aq. layer was washed with EtOAc (20 × 2 mL). 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 EtOAc–*n*-C_6H_{14} (1:1) to give **5** (0.47 g, 85.0%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 1.34 (t, 3H, $J = 7.7$ Hz), 2.18 (bs, 1H), 2.31 (s, 3H), 2.87 (bs, 1H), 3.98–4.07 (bs, 2H), 4.11 (q, 2H, $J = 7.7$ Hz), 4.65 (bs, 1H), 5.15 (bs, 1H), 5.31 (bs, 2H), 7.05 (s, 1H), 7.35 (d, 1H, $J = 7.5$ Hz), 7.60 (d, 1H, $J = 7.5$ Hz), 7.99 (d, 1H, $J = 7.5$ Hz), 8.09 (d, 1H, $J = 7.5$ Hz).

3.5. (2S,4S)-2-(4-Ethoxycarbonylthiazole-2-yl)-4-mercapto-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (25a)

To a solution of **5** (1.11 g, 2.32 mmol) in MeOH (15 mL) was added 0.58 mL of 4*N*-NaOH at ice bath. After stirring for 20 min at the same temperature, 0.58 mL of 4*N*-HCl was added to this solution and diluted with EtOAc, washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to give **25a** (0.96 g, 94.9%) as a yellow oil. ^{1}H-NMR ($CDCl_3$): δ 1.34 (t, 3H, $J = 7.3$ Hz), 2.08 (bs, 1H), 2.77 (bs, 1H), 3.89–4.08 (bs, 2H), 4.14 (q, 2H, $J = 7.3$ Hz), 5.15–5.26 (bs, 2H), 5.45 (bs, 2H), 7.01 (s, 1H), 7.35 (d, 1H, $J = 7.5$ Hz), 7.60 (d, 1H, $J = 7.5$ Hz), 7.95 (d, 1H, $J = 7.5$ Hz), 8.11 (d, 1H, $J = 7.5$ Hz).

3.6. (2S,4R)-2-(4-Hydroxymethylthiazole-2-yl)-4-mesyloxy-1-(p-nitrobenzyloxycarbonyl)pyrroli dine (6)

To a solution of **4** (2.40 g, 4.80 mmol) in EtOH (50 mL) was added slowly $NaBH_4$ (0.54 g 14.3 mmol) at 0 °C. After 5 h, the mixture was diluted with H_2O (20 mL), 1*N*-HCl and EtOAc (50 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and the resulting residue was purified by silica gel column chromatography to give **6** (1.48 g, 67.3%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 2.27 (bs, 1H), 2.85 (bs, 1H), 3.05 (s, 3H), 3.50 (s, 2H), 3.88 (bs, 1H), 4.05 (bs, 1H), 4.81 (bs, 1H), 5.19–5.26 (bs, 2H), 5.45 (bs, 1H), 7.05 (s, 1H), 7.25 (d, 1H, $J = 7.2$ Hz), 7.65 (d, 1H, $J = 7.2$ Hz), 8.12 (d, 1H, $J = 7.2$ Hz), 8.24 (d, 1H, $J = 7.2$ Hz).

3.7. (2S,4S)-2-(4-Hydroxymethylthiazole-2-yl)-4-mercapto-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (25b)

Compound **25b** was prepared from **6** by a similiar method to that described for the preparation of **25a** from **4**. ^{1}H-NMR ($CDCl_3$): δ 2.20 (bs, 1H), 2.89 (bs, 1H), 3.51 (s, 2H), 3.88 (bs, 1H), 4.00 (bs, 1H), 4.77 (bs, 1H), 5.16–5.28 (bs, 2H), 5.45 (bs, 1H), 7.05 (s, 1H), 7.25

(d, 1H, $J = 7.1$ Hz), 7.68 (d, 1H, $J = 7.1$ Hz), 8.10 (d, 1H, $J = 7.1$ Hz), 8.22 (d, 1H, $J = 7.1$ Hz).

3.8. (2S,4R)-2-(4-Carbamoylthiazole-2-yl)-4-hydroxy-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (7)

To a stirred solution of **3** (1.46 g, 2.78 mmol) in MeOH (20 mL) was added NaOH (28%, 20 mL) and was stirred for 20 h at r.t. The mixture was neutralised with 6*N*-HCl, diluted with EtOAc (50 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4, which was purified by silica gel column chromatography to give **7** (0.85 g, 78.1%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 2.50 (bs, 1/2H), 2.75 (bs, 1H), 2.88 (bs, 1/2H), 3.88 (bs, 1H), 4.02 (bs, 1H), 5.19–5.31 (bs, 2H), 5.45 (bs, 2H), 6.50–6.62 (2bs, 2H), 7.04 (s, 1H), 7.25 (d, 1H, $J = 7.4$ Hz), 7.63 (d, 1H, $J = 7.4$ Hz), 8.01 (d, 1H, $J = 7.4$ Hz), 8.12 (d, 1H, $J = 7.4$ Hz).

3.9. (2S,4S)-2-(4-Carbamoylthiazole-2-yl)-4-mercapto-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**25c**)

Compound **25c** was prepared from **7** by a similiar method to that described for the preparation of **25a**. ^{1}H-NMR ($CDCl_3$): δ 2.20 (bs, 1H), 2.88 (bs, 1H), 3.86 (bs, 1H), 4.04 (bs, 1H), 5.19–5.31 (bs, 2H), 5.49 (bs, 2H), 6.50–6.62 (2bs, 2H), 7.01 (s, 1H), 7.23 (d, 1H, $J = 7.3$ Hz), 7.66 (d, 1H, $J = 7.3$ Hz), 8.06 (d, 1H, $J = 7.4$ Hz), 8.14 (d, 1H, $J = 7.4$ Hz).

3.10. (2S,4R)-4-(t-Butyldimethylsilanyloxy)-2-(4-ethoxycarbonylthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**8**)

To a solution of **3** (5.0 g, 9.52 mmol) in MeOH (20 mL) was added 0.53 g of KOH (9.52 mmol) at ice bath. After stirring for 2 h, the reaction mixture was neutralised with 4*N*-HCl and was diluted with EtOAc, washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to give a yellow oil. To mixture of above compound and imidazole (13.26 g, 22.5 mmol) in DMF (40 mL) was added TBSCl (13.56 g, 9.0 mmol) and stirred at r.t. for 15 h under N_2 gas. The reaction mixture was diluted with EtOAc (50 mL), water (50 mL) and the aqueous layer was washed with EtOAc (30×2 mL). The combined solvent was washed with water (30 mL $\times$ 3), brine and dried over anhydrous Na_2SO_4. Removal of the solvent gave a crude residue, which was chromatographed on silica gel using EtOAc–n-C_6H_{14} (1:1) to give **8** (3.93 g, 77.1%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 0.01 (s, 6H), 0.99 (s, 9H), 1.34 (t, 3H, $J = 7.0$ Hz), 2.75–2.81 (bs, 2H), 3.88–4.07 (bs, 2H), 4.12 (q, 2H, $J = 7.0$ Hz), 4.95 (bs, 1H), 5.15–5.30 (s and dd, 2H, $J = 7.9$ Hz), 5.61 (bs, 1H), 7.11 (s, 1H), 7.46 (d, 2H, $J = 7.2$ Hz), 8.16 (d, 2H, $J = 7.2$ Hz).

3.11. (2S,4R)-4-(t-Butyldimethylsilanyloxy)-2-(4-mesyloxymethylthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**9**)

To a solution of **8** (5.71 g, 10.0 mmol) in EtOH (80 mL) was added slowly $NaBH_4$ (1.14g 30.0 mmol) at 0 °C. After 6 h, the mixture was diluted with H_2O (30 mL), 1*N*-HCl and EtOAc (80 mL). The organic layer was concentrated in vacuo to give a residue, which was used without further purification. A solution of above and Et_3N (1.21 mL, 9.0 mmol) in dry CH_2Cl_2 was cooled to 5 °C under nitrogen and treated with methanesulfonyl chloride (1.03 g, 9.0 mmol). The mixture was stirred at 0 °C for 1 h, diluted with EtOAc (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 **9** (4.11 g, 71.9%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 0.01 (s, 6H), 0.99 (s, 9H), 2.25 (bs, 1H), 2.81 (bs, 1H), 3.03 (s, 3H), 3.37 (s, 2H), 3.88–4.03 (bs, 2H), 4.93 (bs, 1H), 5.15–5.30 (s and dd, 2H, $J = 7.9$ Hz), 5.51 (bs, 1H), 7.11 (s, 1H), 7.49 (d, 2H, $J = 7.2$ Hz), 8.18 (d, 2H, $J = 7.2$ Hz).

3.12. (2S,4R)-2-(4-Aminomethylthiazole-2-yl)-4-(t-butyldimethylsilanyloxy)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**10**)

A mixture of **9** (2.31 g, 4.01 mmol) and potassium phthalimide (1.48 g, 8.02 mmol) in DMF (30 mL) was heated at 100 °C for 1 h. The reaction mixture was poured into ice water and extracted with EtOAc. The residue was purified by silica gel column chromatography ($C_6H_5CH_3$–EtOAc = 8:1) to give phthalimidate compound. To a solution of above compound in a mixture of CH_2Cl_2 (10 mL) and MeOH (30 mL), hydrazine hydrate (0.40 g, 8.02 mmol) was added. After being heated at 60 °C for 5 h, the reaction mixture was evaporated in vacuo to dryness. The residue was dissolved in CH_2Cl_2 and the resulting precipitates were filtered off. The filtrate was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography to give **10** (1.36 g, 69.1%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 0.01 (s, 6H), 0.99 (s, 9H), 2.25 (bs, 1H), 2.31 (bs, 1H), 3.68–3.86 (bs, 2H), 4.53 (bs, 3H), 5.19–5.33 (s and dd, 2H, $J = 7.5$ Hz), 5.51 (bs, 1H), 7.01 (s, 1H), 7.51 (d, 2H, $J = 7.1$ Hz), 8.29 (d, 2H, $J = 7.1$ Hz).

3.13. (2S,4R)-4-(t-Butyldimethylsilanyloxy)-2-(4-methanesulfonylaminomethylthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**11**)

A solution of **10** (2.0 g, 4.05 mmol) and Et_3N (0.65 mL, 4.86 mmol) in dry CH_2Cl_2 was cooled to 0 °C under nitrogen and treated with methanesulfonyl chloride (0.56 g, 4.86 mmol). The mixture was stirred at 0 °C for 1 h, diluted with EtOAc (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 **11** (2.10 g, 91.1%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 0.01 (s, 6H), 0.99 (s, 9H), 2.28–2.32 (bs, 2H), 2.98 (bs, 3H), 3.68 (m, 1H), 3.88 (bs, 1H), 4.11 (bs, 1H), 4.31 (bs, 1H), 4.45 (s, 2H), 5.23–5.38 (s and dd, 2H, $J = 7.5$ Hz), 5.88 (bs, 1H), 7.15 (s, 1H), 7.35 (d, 1H, $J = 7.1$ Hz), 7.58 (d, 1H, $J = 7.1$ Hz), 8.03 (d, 1H, $J = 7.1$ Hz), 8.28 (d, 1H, $J = 7.1$ Hz).

3.14. (2S,4R)-4-(t-Butyldimethylsilanyloxy)-2-[4-(p-nitrobenzyloxycarbonylaminomethylthizole-2-yl)]-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**12**)

A solution of **10** (1.42 g, 2.88 mmol) and Et_3N (0.46 mL, 3.46 mmol) in dry CH_2Cl_2 was cooled to 0 °C under nitrogen and treated with *p*-nitrobenzylchloroformate (0.75 g, 3.46 mmol). The mixture was stirred at 0 °C for 1 h, diluted with EtOAc (50 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 **12** (1.82 g, 94.2%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 0.01 (s, 6H), 0.99 (s, 9H), 2.25 (bs, 1H), 2.32 (bs, 1H), 3.68–3.88 (bs, 2H), 4.53 (bs, 3H), 5.19–5.33 (s and dd, 2H, $J = 7.5$ Hz), 5.51 (bs, 1H), 7.01 (s, 1H), 7.15 (d, 1H, $J = 7.1$ Hz), 7.55 (d, 3H, $J = 7.1$ Hz), 8.07 (d, 1H, $J = 7.1$ Hz), 8.25 (d, 3H, $J = 7.1$ Hz).

3.15. (2S,4R)-4-Mesyloxy-2-[4-(p-nitrobenzyloxycarbonylaminomethyl)thiazole-2-yl)]-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**13**)

To a solution of **12** (1.13 g, 1.66 mmol) in THF (30 mL) was added slowly a solution of 1 M nBu$_4$NF (2.49 mL, 2.49 mmol) in THF, and then was stirred for 2 h at r.t. The solvent was concentrated in vacuo to give a residue, which was used without further purification. A solution of above (0.73 g, 1.57 mmol) and Et_3N (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 EtOAc (50 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 **13** (0.80 g, 75.8%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 2.21 (bs, 1H), 2.35 (bs, 1H), 3.01 (s, 3H), 3.68–3.90 (bs, 2H), 4.45–4.56 (bs, 3H), 5.19–5.33 (s and dd, 2H, $J = 7.5$ Hz), 5.90 (bs, 1H), 7.05 (s, 1H), 7.13 (d, 1H, $J = 6.8$ Hz), 7.53 (d, 3H, $J = 6.8$ Hz), 8.05 (d, 1H, $J = 6.8$ Hz), 8.25 (d, 3H, $J = 6.8$ Hz).

3.16. (2S,4R)-4-(t-Butyldimethylsilanyloxy)-2-(4-cyanomethylthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**14**)

A mixture of **9** (1.50 g, 2.62 mmol) and sodium cyanide (0.39 g, 7.86 mmol) in DMSO (20 mL) was stirred at 70 °C for 8 h under N_2 gas. After cooling, the reaction mixture was diluted with EtOAc (50 mL), water (50 mL) and the aqueous layer was washed with EtOAc (20×2 mL). 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 EtOAc–n-C_6H_{14} (1:2) to give **14** (1.03 g, 78.5%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 0.01 (s, 6H), 0.99 (s, 9H), 2.28–2.32 (bs, 2H), 3.68 (m, 1H), 3.88 (bs, 1H), 4.11 (bs, 1H), 4.17–4.31 (bs, 3H), 5.23–5.38 (s and dd, 2H, $J = 7.5$ Hz), 7.11 (s, 1H), 7.35 (d, 1H, $J = 7.0$ Hz), 7.52 (d, 1H, $J = 7.0$ Hz), 8.01 (d, 1H, $J = 7.0$ Hz), 8.21 (d, 1H, $J = 7.0$ Hz).

3.17. (2S,4S)-4-Mercapto-2-[4-(p-nitrobenzyloxycarbonylaminomethyl)thiazole-2-yl)]-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**25d**)

Compound **25d** was prepared from **13** by a similiar method to that described for the preparation of **25a** from **4**. ^{1}H-NMR ($CDCl_3$): δ 2.09 (bs, 1H), 2.85 (bs, 1H), 3.68–3.90 (bs, 2H), 4.45–4.56 (bs, 3H), 5.20–5.35 (s and dd, 2H, $J = 7.9$ Hz), 5.90 (bs, 1H), 7.09 (s, 1H), 7.13 (d, 1H, $J = 6.9$ Hz), 7.55 (d, 3H, $J = 6.8$ Hz), 8.08 (d, 1H, $J = 6.9$ Hz), 8.28 (d, 3H, $J = 6.8$ Hz).

3.18. (2S,4S)-4-Mercapto-2-(4-methanesulfonylaminomethylthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**25e**)

Compound **25e** was prepared from **11** by a similiar method to that described for the preparation of **25d** from **12**. ^{1}H-NMR ($CDCl_3$): δ 2.28–2.32 (bs, 1H), 3.68 (m, 1H), 3.88 (bs, 1H), 4.11 (bs, 1H), 4.31 (bs, 1H), 4.45 (s, 2H), 5.22–5.38 (s and dd, 2H, $J = 7.5$ Hz), 5.50 (bs, 1H), 7.11 (s, 1H), 7.30 (d, 1H, $J = 7.4$ Hz), 7.59 (d, 1H, $J = 7.4$ Hz), 8.09 (d, 1H, $J = 7.4$ Hz), 8.28 (d, 1H, $J = 7.4$ Hz).

3.19. (2S,4S)-2-(4-Cyanomethylthiazole-2-yl)-4-mercapto-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (25f)

Compound **25f** was prepared from **14** by a similiar method to that described for the preparation of **25d** from **12**. ^{1}H-NMR ($CDCl_3$): δ 2.28–2.32 (bs, 1H), 3.68 (m, 1H), 3.88 (bs, 1H), 4.11 (bs, 1H), 4.31 (bs, 1H), 4.45 (s, 2H), 5.22–5.38 (s and dd, 2H, $J = 7.5$ Hz), 5.50 (bs, 1H), 7.11 (s, 1H), 7.30 (d, 1H, $J = 7.4$ Hz), 7.59 (d, 1H, $J = 7.4$ Hz), 8.09 (d, 1H, $J = 7.4$ Hz), 8.28 (d, 1H, $J = 7.4$ Hz).

3.20. (2S,4R)-4-Benzoyloxy-2-(4-methoxycarbonylmethylthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (15)

To a stirred solution of **2** (5.0 g, 11.64 mmol) in EtOH (70 mL) was added dropwise methyl 4-chloroacetoacetate (1.64 mL, 13.97 mmol) and was stirred for 20 h at reflux. The resulting solution was evaporated and diluted with EtOAc, and washed with 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 **15** (4.78 g, 78.2%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): δ 2.75–2.85 (bs, 2H), 3.55 (s, 3H), 3.64–4.01 (bs, 4H), 4.95 (bs, 1H), 5.15–5.38 (bs and s, 2H), 5.63 (bs, 1H), 7.05 (s, 1H), 7.25 (t, 2H, $J = 8.1$ Hz), 7.49 (d, 1H, $J = 7.9$ Hz), 7.66 (t, 1H, $J = 7.8$ Hz), 7.78 (m, 3H), 8.13 (d, 1H, $J = 7.9$ Hz), 8.30 (d, 1H, $J = 7.8$ Hz).

3.21. (2S,4R)-2-(4-Carbamoylmethylthiazole-2-yl)-4-hydroxy-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (16)

Compound **16** was prepared from **15** by a similiar method to that described for the preparation of **7** from **3** (79.9%). ^{1}H-NMR ($CDCl_3$): δ 2.35 (bs, 1/2H), 2.72 (bs, 1H), 2.88 (bs, 1/2H), 3.81–4.05 (bs, 3H), 4.02 (bs, 1H), 5.18–5.34 (bs, 2H), 5.45 (bs, 2H), 6.50–6.62 (2bs, 2H), 7.01 (s, 1H), 7.29 (d, 1H, $J = 7.4$ Hz), 7.62 (d, 1H, $J = 7.4$ Hz), 8.02 (d, 1H, $J = 7.4$ Hz), 8.15 (d, 1H, $J = 7.4$ Hz).

3.22. (2S,4S)-2-(4-Carbomoylmethylthiazole-2-yl)-4-mercapto-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (25g)

Compound **25g** was prepared from **16** by a similiar method to that described for the preparation of **25c**. ^{1}H-NMR ($CDCl_3$): δ 2.25 (bs, 1H), 2.85 (bs, 1H), 3.64–4.01 (bs, 4H), 4.95 (bs, 1H), 5.15–5.38 (bs and s, 2H), 5.63 (bs, 1H), 5.99 (bs, 1H), 6.17 (bs, 1H), 7.02 (s, 1H), 7.46 (d, 2H, $J = 7.9$ Hz), 8.13 (d, 2H, $J = 7.9$ Hz).

3.23. (2S,4R)-2-[4-(2-Hydroxyethyl)thiazole-2-yl]-4-mesyloxy-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (17)

Compound **17** was prepared from **15** by a similiar method to that described for the preparation of **6** from **3**. ^{1}H-NMR ($CDCl_3$): δ 2.18 (bs, 1H), 2.70–2.89 (bs, 3H), 3.01 (s, 3H), 3.66–4.01 (bs, 4H), 4.95 (bs, 1H), 5.15–5.38 (bs and s, 2H), 5.60 (bs, 1H), 7.03 (s, 1H), 7.51 (d, 2H, $J = 7.4$ Hz), 8.12 (d, 2H, $J = 7.4$ Hz).

3.24. (2S,4S)-2-[4-(2-Hydroxyethyl)thiazole-2-yl]-4-mercapto-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (24h)

Compound **24h** was prepared from **17** by a similiar method to that described for the preparation of **25b**. ^{1}H-NMR ($CDCl_3$): δ 2.21 (bs, 1H), 2.69–2.85 (bs, 3H), 3.69–4.05 (m, 4H), 4.95 (bs, 1H), 5.15–5.38 (bs and s, 2H), 5.63 (bs, 1H), 7.01 (s, 1H), 7.55 (d, 2H, $J = 7.4$ Hz), 8.11 (d, 2H, $J = 7.4$ Hz).

3.25. (2S,4R)-4-Benzoyloxy-2-(4-methoxycarbonyl-5-methylthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (18)

The compound **18** was achieved using methyl 2-chloroacetoacetate by the same method as described for the preparation of **15** (84.5%). ^{1}H-NMR ($CDCl_3$): δ 2.51 (s, 3H), 2.75–2.85 (bs, 2H), 3.55 (s, 3H), 3.88–4.01 (bs, 2H), 4.95 (bs, 1H), 5.15–5.40 (bs and s, 2H), 5.69 (bs, 1H), 7.27 (t, 2H, $J = 8.1$ Hz), 7.49 (d, 1H, $J = 8.1$ Hz), 7.66 (t, 1H, $J = 8.1$ Hz), 7.78 (m, 3H), 8.11 (d, 1H, $J = 8.1$ Hz), 8.31 (d, 1H, $J = 8.1$ Hz).

3.26. (2S,4R)-2-(4-Hydroxymethyl-5-methylthiazole-2-yl)-4-mesyloxy-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (19)

Compound **19** was prepared from **18** by a similiar method to that described for the preparation of **6** from **3**. ^{1}H-NMR ($CDCl_3$): δ 2.39 (s, 3H), 2.75–2.89 (bs, 2H), 3.02 (s, 3H), 3.88–4.01 (bs, 3H), 4.51 (bs, 1H), 5.02 (bs, 2H), 5.15–5.40 (bs and s, 2H), 7.48 (d, 2H, $J = 7.8$ Hz), 8.23 (d, 2H, $J = 7.8$ Hz).

3.27. (2S,4S)-2-(4-Hydroxymethyl-5-methylthiazole-2-yl)-4-mercaptan-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (25i)

Compound **25i** was prepared from **19** by a similiar method to that described for the preparation of **25b**. ^{1}H-NMR ($CDCl_3$): δ 2.35 (s, 3H), 2.75–2.85 (bs, 2H), 3.88–4.01 (bs, 3H), 4.55 (bs, 1H), 5.09 (bs, 2H), 5.15–5.40 (bs and s, 2H), 7.49 (d, 2H, $J = 7.8$ Hz), 8.23 (d, 2H, $J = 7.8$ Hz).

*3.28. (2S,4R)-4-Benzoyloxy-2-thiocarbamoylmethyl-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**21**)*

The compound **21** from **20** [11] was achieved by the same method as described for the preparation of **2** (89.9%). ^{1}H-NMR($CDCl_3$): δ 2.42 (bs, 1H), 2.75–2.99 (bs, 3H), 3.86 (bs, 1H), 4.01 (bs, 1H), 4.55 (bs, 2H), 5.09 and 5.32 (s and dd, 2H, $J = 11.2$ Hz), 5.78 and 6.55 (bs, 2H), 7.49 (m, 4H), 7.69 (d, 1H, $J = 7.0$ Hz), 7.94 (d, 2H, $J = 7.0$ Hz), 8.13 (d, 2H, $J = 7.0$ Hz).

*3.29. (2S,4R)-4-Benzoyloxy-2-[(4-methoxycarbonylmethyl)thiazole-2-ylmethyl]-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**22**)*

The compound **22** was achieved by the same method as described for the preparation of **14** (84.8%). ^{1}H-NMR ($CDCl_3$): δ 2.75–2.85 (bs, 2H), 3.41 (bs, 2H), 3.55 (s, 3H), 3.64–4.01 (bs, 4H), 4.95 (bs, 1H), 5.15–5.38 (bs and s, 2H), 5.69 (bs, 1H), 7.05 (s, 1H), 7.29 (t, 2H, $J =$ 8.1 Hz), 7.49 (d, 1H, $J = 8.0$ Hz), 7.62 (t, 1H, $J = 8.0$ Hz), 7.78 (m, 3H), 8.17 (d, 1H, $J = 7.9$ Hz), 8.29 (d, 1H, $J = 8.0$ Hz).

*3.30. (2S,4S)-2-(4-Carbamoylmethylthiazole-2-ylmethyl)-4-mercaptan-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**25j**)*

Compound **25j** was prepared from **22** by a similiar method to that described for the preparation of **25c**. ^{1}H-NMR ($CDCl_3$): δ 2.19 (bs, 1H), 2.80 (bs, 1H), 3.30 (bs, 2H), 3.65–4.04 (bs, 4H), 4.91 (bs, 1H), 5.15–5.38 (bs and s, 2H), 5.45 (bs, 1H), 5.99 (bs, 1H), 6.17 (bs, 1H), 7.02 (s, 1H), 7.48 (d, 2H, $J = 7.6$ Hz), 8.17 (d, 2H, $J =$ 7.6 Hz).

*3.31. (2S,4R)-4-Mesyloxy-2-[(4-methoxycarbonylmethyl)thiazole-2-ylmethyl]-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**23**)*

The compound **23** was achieved by the same method as described for the preparation of **4**. ^{1}H-NMR ($CDCl_3$): δ 2.15 (bs, 1H), 2.83 (bs, 1H), 3.02 (s, 3H), 3.43 (bs, 2H), 3.55 (s, 3H), 3.64–4.01 (bs, 4H), 4.95 (bs, 1H), 5.15–5.35 (bs and s, 2H), 5.69 (bs, 1H), 7.02 (s, 1H), 7.49 (d, 2H, $J = 8.0$ Hz), 8.17 (d, 2H, $J = 7.9$ Hz).

*3.32. (2S,4S)-2-[4-(2-Hydroxyethyl)thiazole-2-ylmethyl]-4-mercaptan-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**25k**)*

Compound **25k** was prepared from **23** by a similiar method to that described for the preparation of **25b**. ^{1}H-NMR ($CDCl_3$): δ 2.11 (bs, 1H), 2.69–2.89 (bs, 3H), 3.28 (bs, 2H), 3.69–4.05 (bs, 4H), 4.95 (bs, 1H), 5.17–5.39 (bs and s, 2H), 5.45 (bs, 1H), 7.03 (s, 1H), 7.50 (d, 2H, $J = 7.6$ Hz), 8.14 (d, 2H, $J = 7.6$ Hz).

*3.33. (2S,4R)-4-Benzoyloxy-2-[(4-ethoxycarbonyl)thiazole-2-ylmethyl]-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**24**)*

The compound **24** was achieved by the same method as described for the preparation of **3**. ^{1}H-NMR ($CDCl_3$): δ 1.40 (t, 3H, $J = 7.3$ Hz), 2.75–2.81 (bs, 2H), 3.05 (bs, 2H), 3.98–4.07 (bs, 2H), 4.16 (q, 2H, $J =$ 7.3 Hz), 4.95 (bs, 1H), 5.15 (bs, 1H), 5.61 (bs, 2H), 7.15 (s, 1H), 7.51 (t, 3H, $J = 8.5$ Hz), 7.68 (t, 1H, $J = 8.5$ Hz), 7.96 (d, 2H, $J = 8.5$ Hz), 8.14 (d, 2H, $J = 8.5$ Hz).

*3.34. (2S,4S)-2-(4-Ethoxycarbonylthiazole-2-ylmethyl)-4-mercaptan-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**25l**)*

Compound **25l** was prepared from **24** by a similiar method to that described for the preparation of **25a**. ^{1}H-NMR ($CDCl_3$): δ 1.30 (t, 3H, $J = 7.1$ Hz), 2.22 (bs, 1H), 2.88 (bs, 1H), 3.28 (bs, 2H), 3.86 (bs, 1H), 4.04 (bs, 1H), 4.12 (q, 2H, $J = 7.1$ Hz), 5.19–5.36 (bs, 2H), 5.49 (bs, 2H), 6.50 and 6.62 (2bs, 2H), 7.02 (s, 1H), 7.27 (d, 1H, $J = 7.1$ Hz), 7.69 (d, 1H, $J = 7.1$ Hz), 8.09 (d, 1H, $J = 7.1$ Hz), 8.19 (d, 1H, $J = 7.1$ Hz).

*3.35. (2S,4S)-2-(4-Carbamoylthiazole-2-ylmethyl)-4-mercaptan-1-(p-nitrobenzyloxycarbonyl)pyrroIidine (**25m**)*

Compound **25m** was prepared from **24** by a similiar method to that described for the preparation of **25c**. ^{1}H-NMR ($CDCl_3$): δ 2.20 (bs, 1H), 2.89 (bs, 1H), 3.44 (bs, 2H), 3.88 (bs, 1H), 4.00 (bs, 1H), 4.77 (bs, 1H), 5.16–5.28 (bs, 2H), 5.45 (bs, 1H), 7.09 (s, 1H), 7.25 (d, 1H, $J = 7.1$ Hz), 7.69 (d, 1H, $J = 7.1$ Hz), 8.12 (d, 1H, $J = 7.1$ Hz), 8.23 (d, 1H, $J = 7.1$ Hz).

*3.36. (2S,4S)-2-(4-Hydroxymethylthiazole-2-ylmethyl)-4-mercaptan-1-(p-nitrobenzyloxycarbonyl)pyrrolidine (**25n**)*

Compound **25n** was prepared from **24** by a similiar method to that described for the preparation of **25b**. ^{1}H-NMR ($CDCl_3$): δ 2.22 (bs, 1H), 2.88 (bs, 1H), 3.28 (bs, 2H), 3.86 (bs, 1H), 4.04–4.31 (bs, 3H), 5.19–5.36 (bs, 2H), 5.49 (bs, 2H), 6.50 and 6.62 (2bs, 2H), 7.02 (s, 1H), 7.27 (d, 1H, $J = 7.1$ Hz), 7.69 (d, 1H, $J = 7.1$ Hz), 8.09 (d, 1H, $J = 7.1$ Hz), 8.19 (d, 1H, $J = 7.1$ Hz)

3.37. p-Nitrobenzyl (1R,5S,6S)-6-[(1R)-hydroxyethyl]-3-[5-(4-ethoxycarbonyllthiazole-2-yl)-1-(p-nitrobenzyloxycarbonyl)pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate (**27a**)

A solution of *p*-nitrobenzyl (1*R*,5*S*,6*S*)-3-(diphenylphosphoryloxy)-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**26**, 0.54 g, 0.91 mmol) in CH_3CN (50 mL) was cooled to 0 °C under N_2. To this solution was added diisopropylethylamine (0.13 g, 1.0 mmol) and a solution of the mercapto compound **25a** (0.40 g, 0.91 mmol) in CH_3CN (10 mL). After stirring for 2 h, the mixture was diluted with EtOAc, 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 to give **27a** as a yellow foam solid. Yield: 79.3%. ^{1}H-NMR ($CDCl_3$): δ 1.25 (d, 3H, $J = 6.6$ Hz), 1.31–1.37 (dd, 6H, $J = 6.6$ Hz), 1.98 (bs, 1H), 2.14 (m, 1H), 2.46 (m, 1H), 2.95 (m, 2H), 3.45 (d, 2H, $J = 9.6$ Hz), 4.01–4.18 (m, 4H), 4.43 (bs, 1H), 5.11–5.55 (m, 2H), 7.09 (s, 1H), 7.23 (d, 2H, $J = 7.3$ Hz), 7.45 (d, 2H, $J = 7.3$ Hz), 8.21 (d, 4H, $J = 7.3$ Hz). IR (KBr): 3410 (OH), 3230 (NH), 1720, 1705, 1660 (C=O) cm^{-1}.

3.38. (1R,5S,6S)-6-[(1R)-Hydroxyethyl]-3-[5-(4-ethoxycarbonylthiazole-2-yl)pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylic acid (**28a**)

Compound **27a** (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 50 psi 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 lyophilised 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 lyophilised again to give the title compound **27a** as a white powder. Yield: 47.9%. UV: λ_{max} 298 nm. M.p. 145–150 °C (dec.). ^{1}H-NMR (D_2O): δ 1.06 (d, 3H, $J = 6.5$ Hz), 1.15 (d, 3H, $J = 5.7$ Hz), 1.29 (t, 3H, $J = 6.1$ Hz), 2.04 (bs, 1H), 2.86 (m, 1H), 3.01–3.13 (bs, 2H), 3.53 (bs, 2H), 3.70 (bs, 1H), 4.10 (m, 2H), 4.15 (q, 2H, $J = 6.1$ Hz), 4.51 (bs, 1H), 7.11 (s, 1H). IR (KBr): 3470 (OH), 3230 (NH), 1710, 1690, 1660 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{20}H_{26}N_3O_6S_2$: 468.1263. Found $[M+H]^+$: 468.1272%.

Compound **28b**: Yield: 35.0%. UV: λ_{max} 298. M.p. 145–151 °C (dec.). ^{1}H-NMR (D_2O): δ 1.16 (d, 3H, $J = 6.4$ Hz), 1.21 (d, 3H, $J = 6.4$ Hz), 2.14 (m, 1H), 2.86 (m, 1H), 2.75 (m, 2H), 3.01–3.13 (bs, 2H), 3.53 (dd, 1H, $J = 5.4$ and 5.8 Hz), 3.97 (bs, 1H), 4.10 (m, 2H), 4.43 (m, 1H), 4.91 (t, 1H, $J = 6.6$ Hz), 7.11 (s, 1H). IR (KBr): 3510 (OH), 3300 (NH), 2990, 1710, 1690 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{18}H_{24}N_3O_5S_2$: 426.1157. Found $[M+H]^+$: 426.1162%.

Compound **28c**: Yield: 41.2%. UV: λ_{max} 298 nm. M.p. 115–123 °C (dec.). ^{1}H-NMR (D_2O): δ 1.08 (d, 3H, $J = 7.1$ Hz), 1.22 (d, 3H, $J = 6.0$ Hz), 2.23 (m, 1H), 2.89 (m, 1H), 3.11 (bs, 2H), 3.53 (dd, 1H, $J = 6.0$ Hz), 3.85 (m, 1H), 4.01 (bs, 2H), 4.55 (m, 1H), 5.15 (t, 1H, $J = 8.1$ Hz), 8.11 (s, 1H). IR (KBr): 3480 (OH), 3260 (NH), 1670 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{18}H_{23}N_4O_5S_2$: 439.1110. Found $[M+H]^+$: 439.1104%.

Compound **28d**: Yield: 37.9%. UV: λ_{max} 298 nm. M.p. 132–142 °C (dec.). ^{1}H-NMR (D_2O): δ 1.05 (d, 3H, $J = 6.7$ Hz), 1.15 (d, 3H, $J = 6.0$ Hz), 1.96 (bs, 1H), 2.79 (m, 1H), 2.98 (bs, 1H), 3.22–3.29 (bs, 2H), 3.57 (q, 1H, $J = 5.9$ Hz), 3.82 (m, 1H), 4.11 (m, 2H), 4.23 (s, 2H), 4.44 (bs, 1H), 7.59 (s, 1H). IR (KBr): 3540 (OH, NH_2), 3270 (NH), 1705, 1665 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{18}H_{25}N_4O_4S_2$: 425.1317. Found $[M+H]^+$: 425.1316%.

Compound **28e**: Yield: 34.5%. UV: λ_{max} 296 nm. M.p. 133–134 °C (dec.). ^{1}H-NMR (D_2O): δ 1.07 (d, 3H, $J = 6.7$ Hz), 1.18 (d, 3H, $J = 6.0$ Hz), 1.90 (m, 1H), 2.79–2.88 (m, 1H), 3.01 (s, 3H), 3.13 (dd, 1H, $J = 4.1$ Hz), 3.32–3.38 (bs, 2H), 3.57 (q, 1H, $J = 5.9$ Hz), 3.85 (m, 1H), 4.11–4.20 (m, 2H), 4.23 (s, 2H), 4.48 (bs, 1H), 7.51 (s, 1H). IR (KBr): 3510 (OH), 3300 (NH), 1710, 1690 (C=O), 1220 (S=O) cm^{-1}. HRMS (FAB): Calc. for $C_{19}H_{27}N_4O_6S_3$: 503.1093. Found $[M+H]^+$: 503.1099%.

Compound **28f**: Yield: 23.8%. UV: λ_{max} 298 nm. M.p. 156–160 °C (dec.). ^{1}H-NMR (D_2O): δ 1.09 (d, 3H, $J = 6.5$ Hz), 1.16 (d, 3H, $J = 6.0$ Hz), 2.21 (m, 1H), 2.98 (m, 1H), 3.57–3.76 (m, 3H), 3.82 (m, 2H), 4.09–4.23 (m, 3H), 4.44 (bs, 1H), 5.10 (t, 1H, $J = 6.6$ Hz), 7.49 (s, 1H). IR (KBr): 3510 (OH), 3270 (NH), 2210 (C=N), 1710, 1680 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{19}H_{23}N_4O_4S_2$: 435.1161. Found $[M+H]^+$: 435.1154%.

Compound **28g**: Yield: 40.1%. UV: λ_{max} 298 nm. ^{1}H-NMR (D_2O): δ 1.08 (d, 3H, $J = 7.8$ Hz), 1.22 (d, 3H, $J = 7.0$ Hz), 2.16 (m, 1H), 2.93 (m, 1H), 3.23–3.43 (m, 2H), 3.65 (m, 1H), 3.85 (s, 2H), 4.01 (bs, 1H), 4.25 (bs, 2H), 4.55 (bs, 1H), 5.01 (t, 1H, $J = 8.3$ Hz), 7.38 (s, 1H). IR (KBr): 3480 (OH), 3260 (NH), 1730, 1690, 1670 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{19}H_{25}N_4O_5S_2$: 453.1266. Found $[M+H]^+$: 453.1278%.

Compound **28h**: Yield: 32.9%. UV: λ_{max} 298 nm. M.p. 155–159 °C (dec.). ^{1}H-NMR (D_2O): δ 1.16 (d, 3H, $J = 7.2$ Hz), 1.21 (d, 3H, $J = 6.4$ Hz), 2.14 (m, 1H), 2.86 (m, 1H), 2.96 (t, 2H, $J = 6.4$ Hz), 3.21–3.39 (m, 3H), 3.59 (dd, 1H, $J = 5.4$ and 5.8 Hz), 3.76 (t, 2H, $J = 6.4$ Hz), 3.90 (bs, 1H), 4.10 (m, 1H), 4.43 (m, 1H), 4.95 (t, 1H, $J = 6.6$ Hz), 7.24 (s, 1H). IR (KBr): 3500 (OH), 3320 (NH), 1710, 1690 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{19}H_{26}N_3O_5S_2$: 440.1314. Found $[M+H]^+$: 440.1315%.

Compound **28i**: Yield: 29.8%. UV: λ_{max} 298 nm. M.p. 127–130 °C (dec.). ^{1}H-NMR (D_2O): δ 1.12 (d, 3H, $J = 7.2$ Hz), 1.21 (d, 3H, $J = 6.4$ Hz), 2.24 (s, 3H), 2.46 (m, 1H), 2.66 (m, 1H), 3.11–3.39 (m, 3H), 3.59 (dd, 1H, $J =$

5.4 and 5.8 Hz), 3.79 (bs, 1H), 3.90 (bs, 1H), 4.15 (m, 2H), 4.43 (m, 1H), 4.95 (t, 1H, $J = 6.6$ Hz). IR (KBr): 3500 (OH), 3320 (NH), 1710, 1680 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{19}H_{26}N_3O_5S_2$: 440.1314. Found $[M+H]^+$: 440.1319%.

Compound **28j**: Yield: 31.3%. UV: λ_{max} 298 nm. ^{1}H-NMR (D_2O): δ 1.09 (d, 3H, $J = 7.7$ Hz), 1.20 (d, 3H, $J = 7.0$ Hz), 2.11 (m, 1H), 2.90 (m, 1H), 3.21–3.47 (m, 4H), 3.65 (m, 1H), 3.85 (s, 2H), 3.99 (bs, 1H), 4.21 (bs, 2H), 4.55 (bs, 1H), 5.01 (t, 1H, $J = 8.3$ Hz), 7.35 (s, 1H). IR (KBr): 3500 (OH), 3320 (NH), 1710, 1690, 1665 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{20}H_{27}N_4O_5S_2$: 467.1423. Found $[M+H]^+$: 467.1432%.

Compound **28k**: Yield: 32.9%. UV: λ_{max} 298 nm. ^{1}H-NMR (D_2O): δ 1.16 (d, 3H, $J = 7.2$ Hz), 1.21 (d, 3H, $J = 6.4$ Hz), 2.10 (m, 1H), 2.85 (m, 1H), 2.99 (t, 2H, $J =$ 6.5 Hz), 3.22–3.51 (m, 5H), 3.59 (dd, 1H, $J = 5.4$ and 5.8 Hz), 3.71 (t, 2H, $J = 6.5$ Hz), 3.90 (bs, 1H), 4.11 (m, 1H), 4.43 (m, 1H), 4.98 (t, 1H, $J = 6.9$ Hz), 7.25 (s, 1H). IR (KBr): 3500 (OH), 3320 (NH), 1710, 1690 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{20}H_{28}N_3O_5S_2$: 454.1470. Found $[M+H]^+$: 454.1463%.

Compound **28l**: Yield: 29.5%. UV: λ_{max} 298 nm. ^{1}H-NMR (D_2O): δ 1.07 (d, 3H, $J = 6.9$ Hz), 1.15 (d, 3H, $J = 5.9$ Hz), 1.30 (t, 3H, $J = 6.1$ Hz), 2.04 (bs, 1H), 2.86 (m, 1H), 3.01–3.13 (bs, 2H), 3.53–3.77 (bs, 4H), 3.70 (bs, 1H), 4.10 (m, 2H), 4.11 (q, 2H, $J = 6.1$ Hz), 4.51 (bs, 1H), 7.11 (s, 1H). IR (KBr): 3470 (OH), 3230 (NH), 1710, 1690, 1660 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{21}H_{28}N_3O_6S_2$: 482.1420. Found $[M+H]^+$: 482.1433%.

Compound **28m**: Yield: 23.0%. UV: λ_{max} 298 nm. ^{1}H-NMR (D_2O): δ 1.08 (d, 3H, $J = 7.1$ Hz), 1.20 (d, 3H, $J = 6.2$ Hz), 2.20 (m, 1H), 2.89 (m, 1H), 3.11 (bs, 2H), 3.38–3.53 (m, 3H), 3.82 (m, 1H), 4.02 (bs, 2H), 4.50 (m, 1H), 5.11 (t, 1H, $J = 8.1$ Hz), 7.47 (s, 1H). IR (KBr): 3510 (OH), 3320 (NH), 1710, 1690, 1660 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{19}H_{25}N_4O_5S_2$: 453.1266. Found $[M+H]^+$: 453.1269%.

Compound **28n**: Yield: 30.9%. UV: λ_{max} 298 nm. ^{1}H-NMR (D_2O): δ 1.16 (d, 3H, $J = 6.4$ Hz), 1.21 (d, 3H, $J = 6.4$ Hz), 2.14 (m, 1H), 2.86 (m, 1H), 2.75 (m, 2H), 3.01–3.13 (bs, 2H), 3.31–3.55 (m, 3H), 3.93 (bs, 1H), 4.11 (m, 2H), 4.45 (m, 1H), 4.99 (t, 1H, $J = 6.6$ Hz), 7.17 (s, 1H). IR (KBr): 3500 (OH), 3320 (NH), 1710, 1690 (C=O) cm^{-1}. HRMS (FAB): Calc. for $C_{19}H_{26}N_3O_5S_2$: 440.1314. Found $[M+H]^+$: 440.1320%.

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