Full Paper

Synthesis and *In-Vitro* Activity of New 1β-Methylcarbapenem Derivatives as Antibacterial Agents

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The synthesis of a new series of 1β-methylcarbapenems having pyrrolidine and piperidine moieties is described. Their *in-vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria were tested and the effect of substituents on the pyrrolidine ring was investigated. A particular compound **III b** having an oxime-pyrrolidine moiety showed the most potent antibacterial activity.

Keywords: Antibacterial activity / 1β-Methylcarbapenems / Substituent effects

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Introduction

Carbapenems are one of the most potent types of antibacterial agents and are among those used as last resort against infections in the clinical field. Three carbapenems, imipenem [1, 2], meropenem [3], and ertapenem [4] have been marketed so far. It was revealed that 1β-methylcarbapenems showed not only a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria but also high stability to human renal DHP-I [5; 6]. The carbapenem compounds having a (3*S*)-pyrrolidin-3-ylthio group at the C-2 position in the carbapenem skeleton are noted for their broad and potent antibacterial activity [7], and, therefore, a large number of these derivatives have been synthesized and investigated [8–12]. At present, several carbapenem derivatives such as S-4661 [13], BO-2727 [14], and E-1010 [15] are under clinical or preclinical studies since the launch of meropenem.

In this paper, we described the synthesis and structure-activity relationship of the lβ-methylcarbapenems having a pyrrolidin-3-ylthio moiety at C-2 that is substituted either with a piperidinylmethyl or a pyrrolidinylmethyl group at 5′ position. Furthermore, our approach to improve the antibacterial activity of the carbapenems is also discussed.

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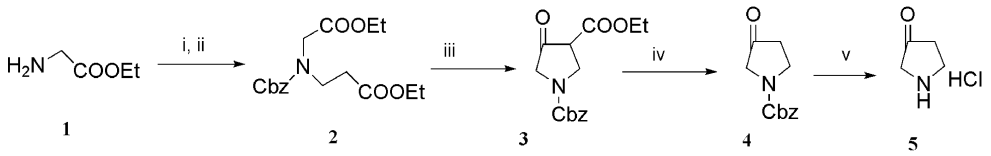
Results and discussions

Chemistry

Our general synthetic route for the new carbapenems involved the preparation of appropriately protected thiols containing a pyrrolidine ring as a side chain and a subsequent coupling reaction with the carbapenem diphenylphosphates, followed by deprotection of the resulting protected carbapenems, following a usual decoupling procedure.

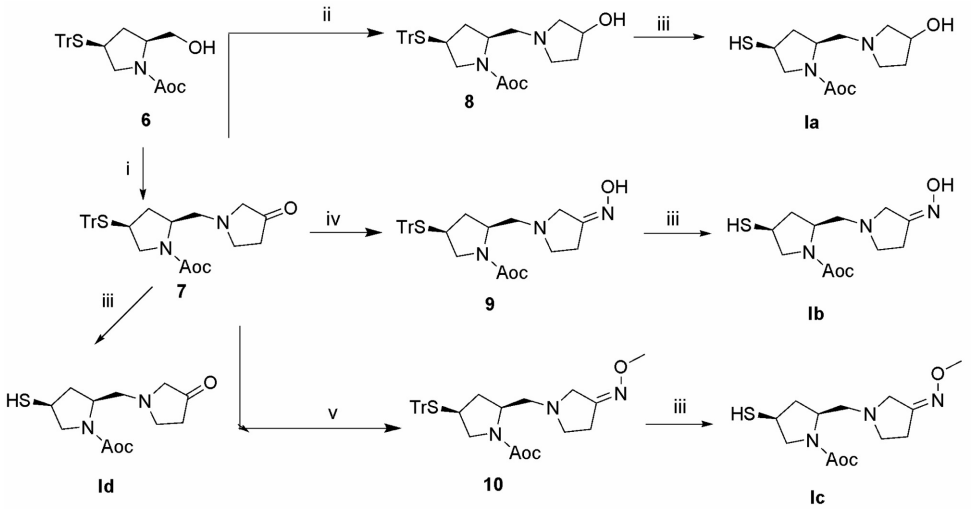
The β-ketoester **3** was prepared in three steps from glycin ester and ethyl acrylate using the Dieckmann condensation method [16]. The intermediate **4** was obtained by reaction of **3** with 10% hydrochloric acid [17] and was subsequently subjected to hydrogenation in the presence of palladium carbon to provide the key compound **5** (Scheme 1) [18].

The intermediate **7** was obtained by treatment of the hydroxy compound **6** [19] with β-keto amine **5** using trifluoromethane sulfonic anhydride. The intermediate **7** was converted to the hydroxy compound **8** by treatment with sodium borohydride in THF. Preparation of the oxime **9**, and methoxyimino compound **10** was accomplished by treatment of compound **7** with hydroxyl-



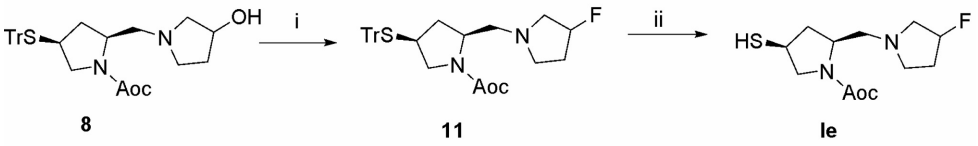
Reactions and conditions: (i) Ethyl acrylate, TEA, EtOH; (ii) Benzyl chloroformate, TEA, CH_2Cl_2; (iii) Potassium *t*-butoxide, toluene; (iv) 10% HCl; (v) Pd/C, H_2, EtOH.

Scheme 1. Synthesis of key compound **5**.



Reactions and conditions: (i) 1.) Trifluoromethane sulfonic anhydride, CH_2Cl_2, 2.) **5**, TEA, CH_2Cl_2; (ii) $NaBH_4$, THF; (iii) Trifluoroacetic acid, triethylsilane, CH_2Cl_2; (iv) Hydroxylamine, EtOH; (v) Methoxylamine hydrochloride, pyridine.

Scheme 2. Synthesis of the mercaptans **Ia–d**.



Reactions and conditions: (i) DAST, CH_2Cl_2; (ii) Trifluoroacetic acid, triethylsilane, CH_2Cl_2.

Scheme 3. Synthesis of mercaptan **Ie**.

amine and methoxylamine, respectively. Deprotection of the trityl group to mercaptans **Ia–d** was achieved by treatment of **7–10** with trifluoroacetic acid in the presence of triethylsilane (Scheme 2).

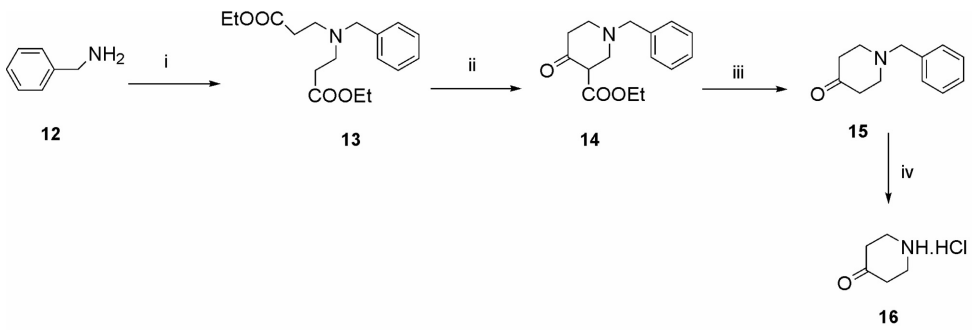
Preparation of the fluoro compound **11** was accomplished by treatment of the hydroxyl compound **8** with DAST (diethylaminosulfur trifluorid).

The reaction of benzylamine with excess of ethylacrylate in the presence of triethylamine gave **13**, through a bis-addition, in excellent yield. Compound **13** was subjected to Dieckmann cyclization with sodium hydride to give ethyl-*N*-benzyl-4-oxo-piperidinecarboxylate **14** in moderate yield [20].

Compounds **15** and **16** were prepared by a similar manner to that described for the preparation of **5** (Scheme 4).

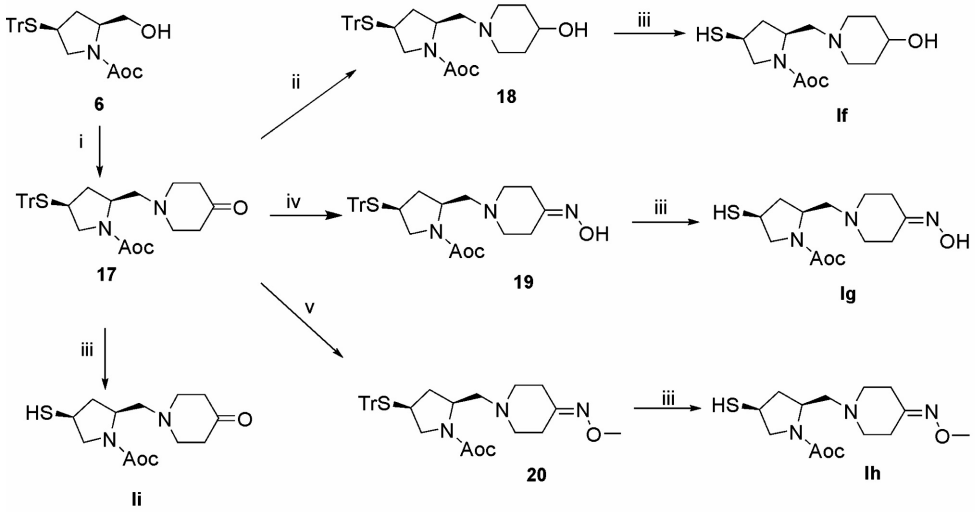
The treatment of the hydroxy compound **6** with the piperidine derivative **16** using trifluoromethane sulfonic anhydride gave the intermediate **17**, which was successfully converted into derivatives **18–20**, using the same procedure described for the preparation of compounds **7–10** (Scheme 5).

Finally, the reaction of **21** with the thiols (**Ia–i**) in the presence of diisopropylethylamine gave the corresponding 2-substituted carbapenems (**IIa–i**). Deprotection of these compounds by treatment with tetrakis(triphenylphosphine)palladium(0) and tributyltin hydride gave the



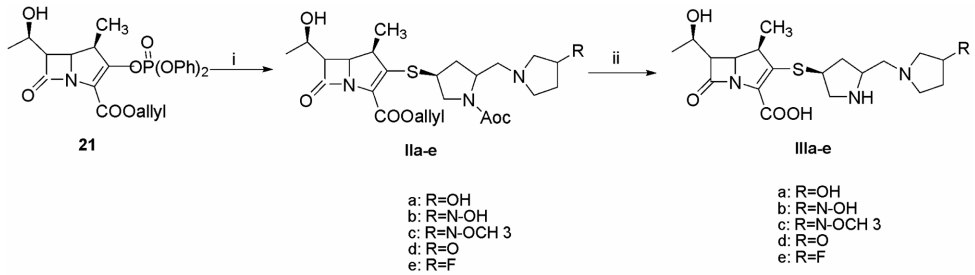
Reactions and conditions: (i) Ethyl acrylate, TEA, EtOH; (ii) NaH, toluene; (iii) 10% HCl; (iv) Pd/C, H_2, EtOH.

Scheme 4. Synthesis of key compound **16**.



Reactions and conditions: (i) 1.) Trifluoromethane sulfonic anhydride, CH_2Cl_2, 2.) **16**, TEA, CH_2Cl_2; (ii) $NaBH_4$, THF; (iii) Trifluoroacetic acid, triethylsilan, CH_2Cl_2; (iv) Hydroxylamine, EtOH; (v) Methoxylamine hydrochloride, pyridine.

Scheme 5. Synthesis of mercaptans **If**–**Ii**.



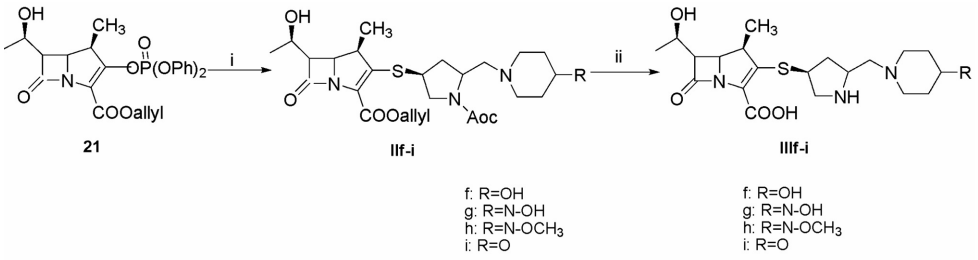
Reactions and conditions: (i) *N,N*-Diisopropylethyl amine, **Ia–e**; (ii) Tetrakis(triphenylphosphine)palladium, tributyltin hydride, CH_2Cl_2.

Scheme 6. Synthesis of the carbapenems **IIIa**–**e**.

crude products, which were purified on a HP-20 column to give the pure carbapenems (**IIIa**–**i**) (Schemes 6 and 7).

Biological studies

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 MIC of a compound was defined as the lowest concentration that visibly inhibited growth.



Reactions and conditions: (i) *N,N′*-Diisopropylethyl amine, **If–i**; (ii) Tetrakis(triphenylphosphine)palladium, tributyltin hydride, CH_2Cl_2.

Scheme 7. Synthesis of the carbapenems **IIIf–i**.

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

STRAINS	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIg	IIIh	IIIi	IPM[a)]
Staphylococcus aureus 1218	6.25	3.12	3.12	6.25	3.12	3.12	25	6.25	12.5	1.56
Coagulase negative staphylococci	0.391	0.098	0.098	0.391	0.195	0.391	0.781	0.195	0.391	0.025
Enterococcus faecalis 2347	6.25	1.563	3.12	6.25	6.25	12.5	25	12.5	12.5	1.56
Streptococcus pyogenes 9889	0.098	0.013	0.025	0.098	0.049	0.098	0.198	0.049	0.049	<0.01
Streptococcus agalaciae 32	0.049	0.025	0.025	0.098	0.049	0.098	0.098	0.049	0.049	0.01
Streptococcus pneumoniae 0025	0.098	0.013	0.025	0.098	0.025	0.098	0.098	0.025	0.025	<0.01
Haemophilus influenzae 1210	6.25	3.12	3.12	6.25	6.25	6.25	12.5	6.25	12.5	6.25
Escherichia coil 04	0.098	0.025	0.098	0.195	0.195	0.391	0.391	0.098	0.195	0.391
Klebsiella peneumoniae 523	0.098	0.049	0.098	0.195	0.391	0.781	0.781	0.195	0.391	0.781
Citrobacter freundii 323	0.098	0.025	0.098	0.195	0.195	0.391	0.391	0.098	0.195	0.391
Enterobactor cloacae 34	0.098	0.049	0.098	0.195	0.195	0.781	1.563	0.391	0.391	0.781
Serratia marcescens 3349	0.195	0.098	0.098	0.391	0.391	0.781	1.563	0.391	0.781	0.781
Acinetobacter baumannii 2289	12.5	3.12	6.25	12.5	25	50	25	12.5	12.5	12.5
Psudemonas aeruginosa 5455	12.5	3.12	6.25	6.25	6.25	50	50	12.5	50	3.12

[a)] Imipenem.

The *in-vitro* antibacterial activities of the new carbapenems (**IIIa–i**) prepared above against Gram-positive and negative bacteria are listed in Table 1. For comparison, the MIC values of imipenem are also listed. All compounds displayed superior or similar antibacterial activities against Gram-negative bacteria to imipenem. In particular, against *Escherichia coli,* most of the compounds showed higher activity when compared to imipenem. By comparing the activity of derivatives substituted with pyrrolidine and piperidine moieties at C-5 of the pyrrolidine side chain, it was found that the derivatives with pyrrolidine moieties **IIIa–e** were generally more potent than the derivatives with piperidine moieties **IIIf–i**. The effects of substituents on the pyrrolidine and piperidine ring were also investigated. Compounds **IIIb** and **IIIg** having the oxime and fluoro group were generally more potent than those with hydroxy or methoxy imine groups. As a result, among all of these derivatives, compound **IIIb** having an oxime-pyrrolidine moiety showed the most potent antibacterial activity.

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The authors have declared no conflict of interest.

Experimental

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

Chemistry

*(2S,4S)-2-[(4-oxopyrrolidinyl)methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine **7***

To a solution of **6** (1.5 g, 3.3 mmol) in dry CH_2Cl_2 (10 mL), triethylamine (0.9 mL, 6.6 mmol) and trifluoromethane sulfonic

anhydride (0.66 mL, 4.0 mmol) were added dropwise and the solution was stirred for 45 min at −70°C. An ice-cooled solution of **5** (1.2 g, 9.9 mmol) and triethylamine (2.3 mL, 24.2 mmol) in dry CH_2Cl_2 was slowly added to the above solution at −70°C and the mixture was stirred for 2 h at room temperature. The mixture was diluted with H_2O (50 mL) and CH_2Cl_2 (100 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated, and the resulting residue was purified by silica gel column chromatography (EtOAc / hexane = 1 : 1) to give **7** (0.85 g, 49%). ^{1}H-NMR ($CDCl_3$) δ: 1.66–1.72 (m, 2H), 2.08–2.16 (m, 2H), 2.47–2.54 (m, 2H), 2.65–2.84 (m, 6H), 2.98 (q, *J* = 6.9 Hz, 1H), 3.66–6.68 (bs, 1H), 4.37–4.43 (m, 2H), 5.12–5.18 (bs, 2H), 5.73–5.79 (m, 1H), 7.20–7.33 (m, 9H), 7.37 (m, 6H). ^{13}C-NMR (300 MHz; $CDCl_3$) δ: 8.6, 14.6, 29.6, 36.9, 37.8, 41.4, 45.7, 52.2, 52.5, 55.7, 59.0, 65.6, 67.3, 117.2, 126.8, 128.0, 129.5, 132.8, 144.6, 144.7, 154.3, 214.4.

*(2S,4S)-2-[(4-Hydroxypyrrolidinyl)methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine **8***

To a solution of **7** (1.3 g, 2.5 mmol) in THF (30 mL), $NaBH_4$ (0.2 g, 4.9 mmol) was added slowly at 0°C and the resulted mixture was stirred for 2 h at room temperature. The reaction mixture was poured into ice water, acidified to pH = 4–5 with acetic acid, and then extracted with ethyl acetate. Evaporation of the solvent *in vacuo* gave a crude residue, which was purified by silica gel column chromatography (EtOAc / Hexane; 1 : 2) to give **8** (0.9 g, 71%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.67–1.76 (m, 2H), 2.05–2.10 (m, 4H), 2.46–2.05 (m, 2H), 2.53–3.24 (m, 6H), 3.77 (bs, 1H), 4.17–4.26 (s, 1H), 4.47 (bs, 2H), 5.12–5.31 (m, 2H), 5.83–5.89 (m, 1H), 7.21–7.33 (m, 9H), 7.46 (d, *J* = 6.8 Hz, 6H). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 14.2, 34.8, 37.2, 41.0, 41.5, 52.5, 55.9, 58.6, 59.4, 63.4, 65.5, 67.3, 71.4, 117.1, 126.8, 127.0, 128.0, 128.4, 129.2, 129.5, 129.8, 130.1, 132.9, 144.7, 154.3.

*(2S,4S)-2-[(4-Hydroxyiminopyrrolidinyl)methyl]-4-tritylthio-1-(allyloxycarbonyl) pyrrolidine **9***

To a stirred solution of **7** (1.3 g, 2.4 mmol) in EtOH (20 mL), hydroxylamine hydrochloride (0.1 g, 8.3 mmol) and triethylamine (1.1 mL, 8.3 mmol) was added dropwise, and the resulted mixture was stirred for 7 h at 60°C. The reaction mixture was diluted with ethyl acetate (30 mL) and water (50 mL), and then the organic layer was dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and purification by flash chromatography (EtOAc / hexane; 1 : 2) afforded **9** (0.91 g, 70%). ^{1}H-NMR ($CDCl_3$) δ: 1.66–1.80 (m, 1H), 2.12–2.34 (m, 2H), 2.50–2.63 (m, 3H), 2.76–2.92 (m, 6H), 3.23–3.30 (bs, 1H), 3.41–3.46 (bs, 1H), 3.72–3.83 (bs, 1H), 4.40–4.62 (bs, 2H), 5.22–5.28 (m, 2H), 5.87–5.89 (m, 1H), 7.21–7.33 (m, 9H), 7.47 (d, *J* = 7.1 Hz, 6H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 14.1, 22.7, 26.8, 29.3, 29.6, 30.0, 37.1, 52.5, 55.8, 56.3, 65.6, 67.3, 117.1, 126.8, 127.0, 128.0, 128.3, 129.2, 129.5, 129.9, 132.8, 144.3, 144.7, 145.1, 151.8, 154.3, 162.5.

*(2S,4S)-2-[(4-Methoxyiminopyrrolidinyl)methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine **10***

To a solution of **7** (1.1 g, 2.1 mmol) in dry pyridine (20 mL), methoxylamine hydrochloride (0.6 mL, 3.2 mmol, 35%) was added dropwise, and the resulted mixture was stirred for 10 h at 50°C. The mixture was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with 1 N HCl, 10% $NaHCO_3$, and brine, respectively. The organic layer was concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatograph (EtOAc / hexane = 1 : 2) to give **10** (0.7 g, 60%). ^{1}H-NMR ($CDCl_3$) δ: 1.25–1.33 (m, 1H), 1.74–1.84 (m, 1H), 2.18–2.22 (bs, 1H), 2.51–2.59 (m, 3H), 2.60–2.91 (m, 6H), 3.27 (m, 2H,), 3.88 (m, 3H), 4.40–4.60 (bs, 2H), 5.19–5.29 (m, 2H), 5.81–5.92 (m, 1H), 7.21–7.33 (m, 9H), 7.47 (d, *J* = 7.5 Hz, 6H). ^{13}C-NMR (300 MHz $CDCl_3$) δ: 14.2, 27.2, 29.6, 37.2, 41.4, 54.2, 55.9, 56.4, 58.8, 59.6, 61.5, 65.5, 67.3, 117.1, 126.8, 127.8, 128.0, 129.5, 130.1, 132.8, 144.7, 154.2, 161.8.

*(2S,4S)-2-[(4-Fluoropyrrolidinyl)methyl]-4-tritylthio-1-(allyloxycarbonyl)pyrrolidine **11***

To a suspension of compound **8** (0.9 g, 1.7 mmol) in dry CH_2Cl_2 was added diethylamine sulfur trifluoride (0.4 mL, 2.7 mmol) at −70°C, the mixture was stirred at −70°C for 45 min and then allowed to warm to room temperature. At this time, 4 mL of methanol were added to quench the reaction. The solvent was evaporated *in vacuo* and the resulting oil dissolved in ethyl acetate, neutralized (pH = 7–8) by addition of 32% ammonia solution, and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$, and then evaporated. The crude residue was purified by silica gel column chromatography (EtOAc / hexane = 1 : 2) to give **11** (0.4 g, 48%). ^{1}H-NMR ($CDCl_3$) δ: 1.12–1.33 (m, 3H), 1.78–1.90 (m, 1H), 2.00–2.10 (m, 2H), 2.44–2.56 (m, 2H), 2.65–2.81 (m, 6H), 3.68–3.80 (bs, 1H), 4.30–4.50 (bs, 2H), 5.18–5.31 (m, 2H), 5.82–5.88 (m, 1H), 7.09–7.21 (m, 9H), 7.46 (d, *J* = 6.9 Hz, 6H). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 32.6, 32.9, 41.0, 52.7, 65.5, 67.3, 76.5, 92.2, 94.5, 126.8, 128.0, 129.5, 132.9, 144.7, 154.2.

*Preparation of compounds **17–20***

Compounds **17**, **18**, **19** and **20** were prepared from **6**, as described for the preparation of **7**, **8**, **9** and **10**, respectively.

17: ^{1}H-NMR ($CDCl_3$) δ: 0.82–0.90 (m, 2H), 1.32–1.44 (m, 1H), 1.76–1.84 (m, 2H), 2.71–2.83 (m, 7H), 2.90–2.99 (m, 2H), 3.16–3.23 (m, 1H), 3.63–3.85 (bs, 1H), 4.33–4.51 (m, 2H), 5.17–5.22 (bs, 2H), 5.81–5.92 (m, 1H), 7.21–7.31 (m, 9H), 7.43–7.47 (m, 6H). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 11.4, 14.2, 21.0, 22.6, 29.0, 31.5, 38.4, 41.3, 43.7, 52.1, 53.5, 55.5, 58.7, 59.5, 60.3, 65.5, 67.1, 67.4, 67.5, 117.1, 126.9, 127.1, 128.3, 129.2, 129.4, 132.8, 144.4, 144.6, 144.8, 154.2, 160.8, 171.0, 209.1.

18: ^{1}H-NMR ($CDCl_3$) δ: 1.65–1.83 (m, 6H), 2.42–2.61 (m, 4H), 2.66–2.82 (m, 3H), 3.14–3.17 (m, 4H), 3.62–3.76 (m, 1H), 4.46–4.51 (m, 2H), 5.18–5.31 (m, 2H), 5.80–5.93 (m, 1H), 7.22–7.32 (m, 9H), 7.45-7.48 (m, 6H). ^{13}C-NMR (300 MHz, $CDCl_3$) δ: 14.5, 21.0, 29.7, 32.5, 33.4, 37.3, 38.4, 41.6, 43.7, 47.4, 51.0, 52.1, 52.5, 55.3, 58.7, 60.4, 65.53, 66.9, 67.1, 67.3, 76.6, 98.4, 117.0, 126.8, 126.9, 128.0, 129.4, 129.5, 130.1, 132.9, 144.4, 144.6, 154.2.

19: ^{1}H-NMR ($CDCl_3$) δ: 1.24–1.29 (m, 1H), 1.70–1.79 (m, 1H), 2.31–2.39 (m, 4H), 2.51–2.61 (m, 6H), 2.75–2.80 (m, 3H), 3.21–3.26 (bs, 1H), 3.75–3.81 (bs, 1H), 4.47–4.53 (m, 2H), 5.21–5.26 (m, 2H), 5.80–5.93 (m, 1H), 7.20–7.33 (m, 9H), 7.45–7.48 (m, 6H).

20: ^{1}H-NMR ($CDCl_3$) δ: 0.84–0.91 (m, 2H), 1.27–1.32 (m, 2H), 2.29–2.31 (m, 3H), 2.52–2.57 (m, 6H), 2.82–2.91 (m, 3H), 3.82–3.86 (s, 3H), 4.12–4.17 (m, 2H), 5.21–5.31 (m, 2H), 5.72–5.91 (m, 1H), 7.20–7.33 (m, 9H), 7.45–7.47 (m, 6H).

Allyl (1R,5S,6S)-6-[(1R)-hydroxyethyl]-2-[[5-(4-hydroxypyrrolidinyl)methyl]-1-(allyloxycarbonyl)pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate ***IIa***

To a solution of **8** (0.6 g, 1.2 mmol) in CH_2Cl_2 (3 mL) was added dropwise triethylsilane (0.2 mL, 1.2 mmol) at 5°C, and then TFA (1.2 mL). After stirring for 30 min at room temperature, the mixture was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with 10% $NaHCO_3$, brine. The organic layer was concentrated *in vacuo* to give a residue **Ia**, which was used without further purification. A solution of allyl (1*S*,5*S*,6*S*)-2-(diphenylphosphoryloxy)-6-[(1*R*)-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate **21** (0.6 g, 1.2 mmol) in CH_3CN (10 mL) was cooled to 0°C under N_2. To this solution was added diisopropylethyl amine (0.13 g, 1.0 mmol) and a solution of the mercapto compound **Ia** in CH_3CN (5 mL). After stirring for 5 h, the mixture was diluted with ethyl acetate, washed with 10% $NaHCO_3$, brine, and dried over anhydrous $MgSO_4$. Evaporation *in vacuo* gave a foam, which was purified by silica gel chromatography (EtOAc / MeOH = 10 : 1) to give **IIa** (99.0 mg, 21%). ^{1}H-NMR ($CDCl_3$) δ: 0.83 – 0.94 (m, 3H), 0.95 – 1.24 (m, 4H), 1.60 – 1.76 (m, 3H), 2.02 – 2.17 (m, 2H), 2.18 – 2.32 (m, 2H), 2.35 – 2.53 (m, 2H), 2.80 – 2.93 (m, 3H), 3.21 – 3.38 (m, 2H), 3.45 – 3.55 (m, 1H), 3.75 – 3.89 (m, 1H), 3.89 – 4.09 (m, 3H), 4.32 – 4.57 (bs, 3H), 4.70 – 4.74 (dd, *J* = 5.5 Hz, 1H) 4.74 – 4.78 (dd, *J* = 5.7 Hz, 1H), 5.20 – 5.30 (m, 2H), 5.41 (s, 1H), 5.47 (s, 1H), 5.89 – 5.99 (m, 2H).

Synthesis of compounds ***IIb – i***

The synthesis of compounds **IIb – i** was carried out by the same procedure as described for the preparation of **IIa**.

IIb: Yield 32%. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (m, 3H), 1.35 (d, *J* = 6.2 Hz, 3H), 1.50 – 1.62 (s, 4H), 2.47 – 2.53 (m, 2H), 2.63 – 2.82 (m, 6H), 3.18 – 3.49 (m, 2H), 3.57 – 3.59 (m, 2H), 4.00 – 4.17 (m, 1H), 4.18 – 4.30 (m, 2H), 4.52 – 4.62 (m, 3H), 4.65 – 4.73 (dd, *J* = 5.4 Hz, 1H), 4.80 – 4.88 (dd, *J* = 5.5 Hz, 1H), 5.20 – 5.30 (m, 2H), 5.42 (m, 1H), 5.47 (d, *J* = 1.5 Hz, 1H), 5.92 – 5.99 (m, 2H).

IIc: Yield 27%. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (m, 3H), 1.34 (d, *J* = 6.2 Hz, 3H), 1.53 – 1.65 (s, 4H), 2.53 – 2.55 (m, 2H), 2.70 – 2.94 (m, 6H), 3.25 – 3.47 (m, 2H), 3.56 – 3.61 (m, 1H), 3.84 – 3.85 (s, 3H), 4.00 – 4.15 (m, 2H), 4.21 – 4.24 (m, 1H), 4.52 – 4.64 (m, 3H), 4.66 – 4.72 (dd, *J* = 5.4 Hz, 1H), 4.80 – 4.82 (dd, *J* = 5.5 Hz, 1H), 5.21 – 5.48 (m, 4H), 5.89 – 6.00 (m, 2H).

IId: Yield 24%. ^{1}H-NMR($CDCl_3$) δ: 1.26 (m, 3H), 1.35 (d, *J* = 9.4 Hz, 3H), 1.58 – 1.68 (bs, 2H), 1.99 – 2.12 (m, 1H), 2.38 (t, *J* = 6.9 Hz, 3H), 2.83 – 3.02 (m, 6H), 3.23 – 3.20 (m, 1H), 3.32 – 3.47 (m, 2H), 3.98 – 4.20 (m, 3H), 4.58 – 4.60 (m, 2H), 4.59-4.60 (dd, *J* = 5.4 Hz, 1H), 4.81 – 4.82 (dd, *J* = 5.5 Hz, 1H), 5.21 – 5.35 (m, 2H), 5.47 (s, 1H), 5.48 (s, 1H), 5.92 – 6.02 (m, 2H).

IIe: Yield 21%. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (m, 3H), 1.34 (d, *J* = 6.2 Hz, 3H), 1.56 – 1.58 (s, 3H), 1.99 – 2.15 (m, 3H), 2.66 – 2.84 (m, 6H), 3.22 – 3.26 (m, 1H), 3.30 – 3.40 (m, 2H), 3.44 (t, *J* = 6.9 Hz, 1H), 3.97 – 4.08 (m, 2H), 4.13 – 4.28 (m, 1H), 4.50 – 4.58 (m, 3H), 4.60 – 4.71 (dd, *J* = 5.5 Hz, 1H), 4.72 – 4.83 (dd, *J* = 5.6 Hz, 1H), 5.20 – 5.48 (m, 4H), 5.89-5.99 (m, 2H).

IIf: Yield 20%. ^{1}H-NMR ($CDCl_3$) δ: 1.20 – 1.35 (m. 3H), 2.03 – 2.18 (m, 3H), 2.37 – 2.51 (m, 9H), 2.67 – 2.87 (m, 6H), 3.26 – 3.50 (m, 4H), 3.90 – 4.17 (m, 3H), 4.58 – 4.62 (m, 3H), 4.66 – 4.71 (dd, *J* =5.7 Hz, 1H), 4.81 – 4.86 (dd, *J* = 5.5 Hz, 1H), 5.21 – 5.36 (m, 4H), 5.87-6.00 (m, 2H).

IIg: Yield 23%.^{1}H-NMR ($CDCl_3$) δ: 1.24 – 1.27 (d, *J* = 9.0 Hz, 3H), 1.36 – 1.38 (m, 2H), 2.04 – 2.07 (m, 1H), 2.09 – 2.11 (m, 1H), 2.32 – 2.40 (m, 2H), 2.42 – 2.49 (m, 2H), 2.54 – 2.80 (m, 9H), 3.25 – 3.47 (m, 3H), 3.54 – 3.63 (m, 2H), 4.07 – 4.22 (m, 3H), 4.60 – 4.66 (m, 3H), 4.66-4.68 (dd, *J* = 5.5 Hz, 1H), 4.86 – 4.88 (dd, *J* = 5.4 Hz, 1H), 5.29 – 5.35 (m, 3H), 5.92 – 6.03 (m, 2H).

IIh: Yield 19%. ^{1}H-NMR ($CDCl_3$) δ: 1.26 – 1.36 (d, *J* = 6.1 Hz, 3H), 1.36 – 1.38 (m, 2H), 2.08 – 210 (m, 1H), 2.13 – 2.15 (m, 1H), 2.31 – 2.37 (m, 2H), 2.41 – 2.57 (m, 8H), 2.59 – 2.71 (m, 2H), 3.37 – 3.53 (m, 3H), 3.82 – 3.84 (s, 3H), 4.05 – 4.19 (m, 3H), 4.22 – 4.27 (m, 2H), 4.59 – 4.66 (m, 3H), 4.68 – 4.72 (dd, *J* = 5.5 Hz, 1H), 4.81 – 4.85 (dd, *J* = 5.3 Hz, 1H), 5.21 – 5.34 (m, 3H), 5.89 – 6.02 (m, 2H).

IIi: Yield 22%.^{1}H-NMR($CDCl_3$) δ: 1.17 – 1.18 (m, 2H), 1.30 – 1.39 (m, 2H), 1.57 – 1.68 (bs, 2H), 1.69 – 1.71 (m, 2H) 2.03 – 2.07 (m, 1H), 2.21 – 2.22 (m, 1H), 2.35 – 2.54 (m, 3H), 3.27 – 3.45 (m, 3H), 3.65 – 3.79 (m, 3H), 3.99 – 4.17 (m, 4H), 4.19 – 4.27 (m, 2H), 4.61 – 4.63 (m, 4H), 4.71 – 4.75 (dd, *J* = 5.7 Hz, 1H), 4.81 – 4.85 (dd, *J* = 5.5 Hz, 1H), 5.24 – 5.31 (m, 3H), 5.43 – 5.45 (bs, 1H), 5.91 – 6.02 (m, 2H).

(1R,5S,6S)-6-[(1R)-Hydroxyethyl]-2-[[5-(4-hydroxypyrrolidinyl)methyl]pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylic acid ***IIIa***

To a stirred solution of **IIa** (40 mg, 0.1 mmol) and $Pd(PPh_3)_4$ (30 mg) in CH_2Cl_2 (10 mL) was added dropwise *n*-tributytin hydride (0.1 mL, 0.15 mmol) at 0°C and was stirred for 1 h at same temperature. To the resulting solution was diluted with water (10 mL) and organic layers was washed with water (2 × 10 mL). The combined aqueous layers were washed with ethyl ether (2 × 10 mL) and lyophilized to give a yellow power which was purified on a Diaion HP-20 column, eluting with 2% THF in water. **IIIa** as an amorphous solid. Yield (24%). ^{1}H-NMR (D_2O) δ: 1.10 – 1.13 (d, *J* = 7.0 Hz, 3H), 1.19 – 1.22 (d, *J* = 6.2 Hz, 3H), 1.56 – 1.66 (m, 2H), 1.79 – 1.81 (m, 1H), 1.87 – 1.92 (m, 1H), 1.93 – 1.98 (m, 2H), 2.21 – 2.37 (m, 1H), 2.38 – 2.70 (m, 3H), 2.82 – 2.91 (m, 3H), 3.02 – 3.05 (m, 1H), 3.06 – 3.15 (m, 1H), 3.21 – 3.25 (m, 5H), 3.59 – 3.63 (m, 2H), 3.65 – 3.79 (m, 1H). -IR (KBr): 3470, 1710, 1650 cm^{-1}. – HRMS (FAB) Calcd. for $C_{19}H_{29}N_3O_5S$: 411.1828. Found: 411.1827.

Synthesis of compounds ***IIIb – i***

The synthesis of compounds **IIIb – i** was carried out by the same procedure as described for the preparation of **IIIa**.

IIIb: Yield 22%. UV λmax: 298 nm. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (m, 3H), 1.35 (d, *J* = 6.2 Hz, 3H), 1.50 – 1.62 (s, 4H), 2.47 – 2.53 (m, 2H), 2.63 – 2.82 (m, 6H), 3.18 – 3.49 (m, 2H), 3.57 – 3.59 (m, 2H), 4.00 – 4.17 (m, 1H), 4.18 – 4.30 (m, 2H), 4.52 – 4.62 (m, 3H), 4.65 – 4.73 (dd, *J* = 5.4 Hz, 1H), 4.80 – 4.88 (dd, *J* =5.5 Hz, 1H), 5.20 – 5.30 (m, 2H), 5.42 (m, 1H), 5.47 (d, *J* =1.5 Hz, 1H), 5.92 – 5.99 (m, 2H). -IR (KBr): 3450, 1740, 1670 cm^{-1}.– HRMS (FAB) Calcd. for $C_{19}H_{28}N_4O_5S$: 424.1780. Found: 424.1760.

IIIc : Yield 24%. UV λmax: 298 nm. ^{1}H-NMR($CDCl_3$) δ: 1.25 (m, 3H), 1.34 (d, *J* = 6.2 Hz, 3H), 1.53 – 1.65 (s, 4H), 2.53 – 2.55 (m, 2H), 2.70 – 2.94 (m, 6H), 3.25 – 3.47 (m, 2H), 3.56 – 3.61 (m, 1H), 3.84 – 3.85 (s, 3H), 4.00 – 4.15 (m, 2H), 4.21 – 4.24 (m, 1H), 4.52 – 4.64 (m, 3H), 4.66 – 4.72 (dd, *J* = 5.4 Hz, 1H), 4.80 – 4.82 (dd, *J* = 5.5 Hz, 1H), 5.21 – 5.48 (m, 4H), 5.89 – 6.00 (m, 2H). – IR (KBr): 3470, 1710, 1680 cm^{-1}. – HRMS (FAB) Calcd. for $C_{20}H_{30}N_4O_5S$: 438.1937. Found: 438.1937.

IIId: Yield 18%. UV λmax: 298 nm. ^{1}H-NMR (D_2O) δ: 1.08 – 1.12 (d, *J* = 7.1 Hz, 3H), 1.13 – 1.17 (d, *J* = 6.3 Hz, 3H), 1.48 – 1.63 (m, 1H), 2.34 – 2.36 (m, 3H), 2.48 – 2.60 (m, 1H), 2.60 – 2.64 (m, 1H), 2.65 – 3.79 (m, 2H), 2.80 – 3.08 (m, 2H), 3.24 – 3.33 (m, 4H), 3.34 – 3.36 (m, 2H), 3.60 – 3.79 (m, 2H), 3.81 – 3.93 (m, 1H), 4.09 – 4.13 (m, 2H).

-IR (KBr): 3460, 1740, 1710, 1670 cm^{-1}. –HRMS (FAB) Calcd. for $C_{19}H_{27}N_3O_5S$: 409.1671. Found: 409.1670.

IIIe: Yield 26%. UV λmax: 298 nm. ^{1}H-NMR (D_2O) δ: 1.09–1.11 (d, *J* = 7.1 Hz, 3H), 1.16–1.18 (d, *J* = 6.3 Hz, 3H), 1.92–2.10 (m, 3H), 2.46–2.51 (m, 3H), 2.60–2.88 (m, 6H), 2.95–3.21 (m, 2H), 3.33–3.40 (m, 2H), 3.55–3.62 (m, 2H), 3.80–3.98 (m, 2H), 4.11–4.13 (m, 2H). -IR (KBr): 3460, 1710, 1650 cm^{-1}. –HRMS (FAB) Calcd. for $C_{19}H_{28}FN_3O_4S$: 413.1785. Found: 413.1780.

IIIf: Yield 24%. UV λmax: 298 nm. ^{1}H-NMR(D_2O) δ: 1.02–1.09 (d, *J* = 6.0 Hz, 3H), 1.11–1.18 (d, *J* = 7.0 Hz, 3H), 2.42–2.46 (m, 6H), 2.57–2.62 (m, 4H), 2.73–2.81 (m, 3H), 3.06–3.12 (m, 3H) 3.23–3.32 (m, 3H), 3.36–3.68 (m, 3H), 4.05–4.08 (m, 3H). -IR (KBr): 3460, 1710, 1650 cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{31}N_3O_5S$: 425.1984. Found: 425.1981.

IIIg: Yield 19%. UV λmax: 298 nm. ^{1}H-NMR (D_2O) δ: 1.13–1.15 (d, *J* = 6.2 Hz, 3H), 1.16–1.18 (d, *J* = 7.1 Hz, 3H), 1.51–1.72 (m, 3H), 2.42–2.53 (m, 3H), 2.53–2.76 (m, 9H), 3.07–3.15 (m, 1H), 3.17–3.21 (m, 2H), 3.24–3.30 (m, 3H), 3.51–5.72 (m, 3H). -IR (KBr): 3460, 1710, 1650 cm^{-1}. -HRMS (FAB) Calcd. for $C_{20}H_{30}N_4O_5S$: 438.1937. Found: 438.1934.

IIIh: Yield 24%. UV λmax: 298 nm. ^{1}H-NMR(D_2O) δ: 1.06–1.08 (d, *J* = 6.2 Hz, 3H), 1.24–1.26 (d, *J* = 7.1 Hz, 3H), 1.41–1.58 (m, 2H), 2.22–2.24 (m, 2H), 2.35–2.55 (m, 5H), 2.57–2.73 (m, 6H), 3.10–3.15 (m, 1H), 3.16–3.21 (m, 1H), 3.30–3.32 (m, 1H), 3.37–3.44 (m, 2H), 3.64–3.68 (s, 3H), 3.78–3.82 (m, 1H), 4.09–4.17 (m, 2H). -IR (KBr): 3460, 1710, 1650 cm^{-1}. –HRMS (FAB) Calcd. for $C_{21}H_{32}N_4O_5S$: 452.2093. Found: 452.2091.

IIIi: Yield 34%. UV λmax: 298 nm. ^{1}H-NMR(D_2O) δ: 1.04–1.10 (d, *J* = 6.3 Hz, 3H), 1.21–1.25 (d, *J* = 7.1 Hz, 3H), 1.57–1.79 (m, 2H), 2.12–2.16 (m, 1H), 2.47–2.61 (m, 2H), 2.77–2.93 (m, 1H), 3.24–3.38 (m, 2H), 3.39–3.47 (m, 3H), 3.49–3.80 (m, 9H), 4.04–4.24 (m, 3H). -IR (KBr): 3460, 1740, 1710, 1650 cm^{-1}. –HRMS (FAB) Calcd. for $C_{20}H_{29}N_3O_5S$: 423.1828. Found 423.1821.

References

[1] W. Leanza, K. Wildonger, T. W. Miller, B. G. Christensen, *J. Med. Chem.* **1979**, *22*, 1435–1436.

[2] J. Birnbaum, F. M. Kahan, J. S. MacDonald, *Am. J. Med.* **1985**, *78*, (Suppl. 6A), 3–21.

[3] M. Sunagawa, H. Matsumure, T. Inoue, M. Kato, *J. Antibiot.* **1990**, *43*, 519–532.

[4] C. J. Gill, J. J. Jackson, L. S. Gerckens, B. A. Pelak, *et al., Antimicrob. Agents Chemother*. **1998**, *42*, 1996–2001.

[5] D. H. Shih, F. L. Baker, B. G. Christensen, *Heterocycles* **1984**, *21*, 29–40.

[6] D. H. Shih, F. L. Baker, B. G. Christensen, *Tetrahedron Lett*. **1985**, *26*, 587–590.

[7] M. Sato, M. Takemura, S. Atarashi, K. Higashi, *et al.*, *J. Antibiot*. **1987**, *40*, 1292–1302.

[8] C.-H. Oh, J.-H. Cho, *J. Antibiot*. **1994**, *47*, 126–128.

[9] C. B. Jin, I. S. Jung, H.-J. Ku, J. W. Yook, *et al.*, *Toxicology* **1999**, *138*, 59–67.

[10] C.-H. Oh, H.-W. Cho, I.-K. Lee, J.-Y. Gong, *et al.*, *Arch. Pharm. Pharm. Med. Chem*. **2002**, *335*, 152–158.

[11] C.-H. Oh, H.-G. Dong, H.-W. Cho, S. J. Park, *et al.*, *Arch. Pharm. Pharm. Med. Chem*. **2002**, *335*, 200–206.

[12] C.-H. Oh, H.-W. Cho, J.-H. Cho, *Eur. J. Med. Chem.* **2002**, *37*, 743754.

[13] Y. Iso, T. Irie, Y. Nishino, K. Motokawa, Y. Nishitani, *J. Antibiot.* **1996**, *49*, 199–209.

[14] K. Inoue, Y. Hamana, T. Inoue, M. Fukasawa, M. Kato, Abstracts of Papers of 34th Intersci. Conf. on *Antimicrob. Agents Chemother.*, No. 1, Orlando, **1994.**

[15] N. Sato, F. Ohba, *Drugs Future* **1996**, *21*, 361–365.

[16] M. Sunagawa, M. Itho, K. Kubota, A. Sasaki, Y. Ueda, *J. Antibiot*. **2002**, *55*, 722–757.

[17] B. Nicolae, B. Constantin, B. Emila, C. Olga, B. Rom, *Revistade Chimie* **1983**, *34*, 699–706.

[18] M. Solymar, E. Forro, F. Fulop, *Tetrahedron Asymmetry* **2004**, *15*, 3281–3287.

[19] C.-H. Oh, H.-W. Cho, J.-H. Cho, *Eur. J. Med. Chem.* **2006**, *41*, 825–832.

[20] N. S. Shaikh, V. H. Deshpande, A. V. Bedekar, *Tetrahedron* **2001**, *57*, 9045–9048.

