Pergamon

Tetrahedron, Vol. 52, No. 26, pp. 8763-8774, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00417-6

Chemical Modification of the GE 2270A Macrocycle (MDL 62,879)

Paolo Tavecchia*, Michael Kurz§, Luigi Colombo, Riccardo Bonfichi, Enrico Selva, Sergio Lociuro, Ettore Marzorati and Romeo Ciabatti

Lepetit Research Center, Via R. Lepetit 34, 21040 Gerenzano (VA), Italy
§ Central Pharma Research, HOECHST AG, 65926 Frankfurt, Germany

Abstract: Controlled acid hydrolysis of compound **2** resulted in the selective opening of the macrocycle while in basic condition a retro-aldol reaction occurred at the level of phenylserine thiazole. Compound **2** can be easily prepared from the natural antibiotic MDL 62,879. All the compounds prepared resulted less active than MDL 62,879. Copyright © 1996 Elsevier Science Ltd

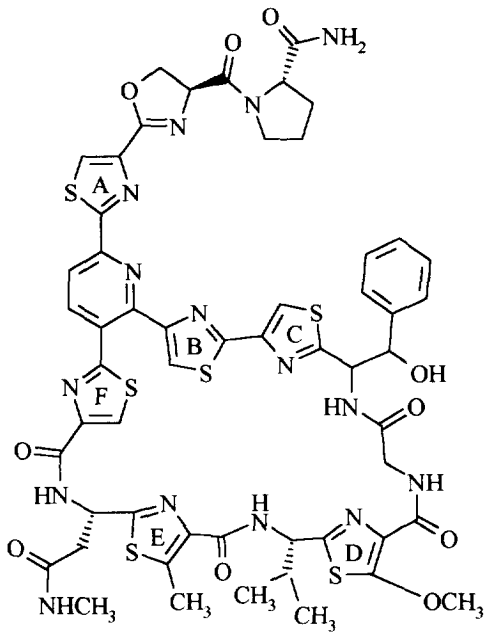
MDL 62,879 **1** (GE 2270A; Fig. 1) is a new highly modified peptide which is produced by Planobispora rosea[1] and belongs to the thiazolyl peptide group of antibiotics[2]. It is a specific inhibitor of the Elongation Factor-Tu (EF-Tu), a bacterial protein essential for protein synthesis[3,4]. EF-Tu is also the target of Kirromycin-type of antibiotics[5] and pulvomycin[6,7]. However, these antibiotics differ from MDL 62,879 in the spectra of antibacterial activity, structures and mode of action.

MDL 62,879 was demonstrated to be active against Gram-positive bacteria and anaerobes including pathogens resistant to antibiotics currently used in therapy. Its chemical structure has been recently elucidated through analytical methods, chemical degradation of the natural compound and partial chemical synthesis[8]. The absolute configuration of proline, oxazoline (serine), asparagine-thiazole E and valine-thiazole D amino acids was assigned while the absolute configuration of the two stereocenters of the phenylserine-thiazole C is still unknown. New cyclic thiazolyl peptides GE 37468[9] and Amythiamicin[10] have been recently discovered both acting on EF-Tu. Their structures closely resemble MDL 62,879 sharing a thiazolyl peptide macrocycle of similar size and a pyridine ring bearing a peptide side chain. In particular, amythiamycin differs from MDL 62,879 for the presence of the thiazolyl-valine aminoacid instead of the thiazolyl-phenylserine at the same position in the macrocycle ring.

A program of chemical modification of MDL 62,879 was aimed at acquiring information on structure-activity relationship of this new antibiotic. Here we report on the results of the chemical manipulation inducing selective modifications of the MDL 62,879 macrocycle.

RESULTS AND DISCUSSION

As previously described[8], the treatment of compound **1** under strong acid hydrolytic condition led to extensive fragmentation of the molecule and to racemization of the thiazolyl amino acids. Milder hydrolytic acid conditions produced modifications only in the side chain of compound **1** leaving untouched the macrocycle

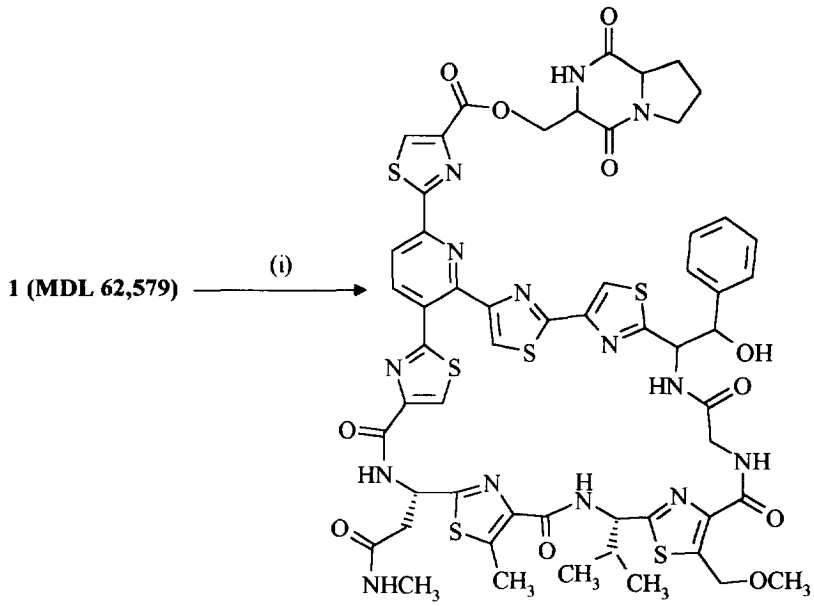


1 (MDL 62,879)

Figure 1

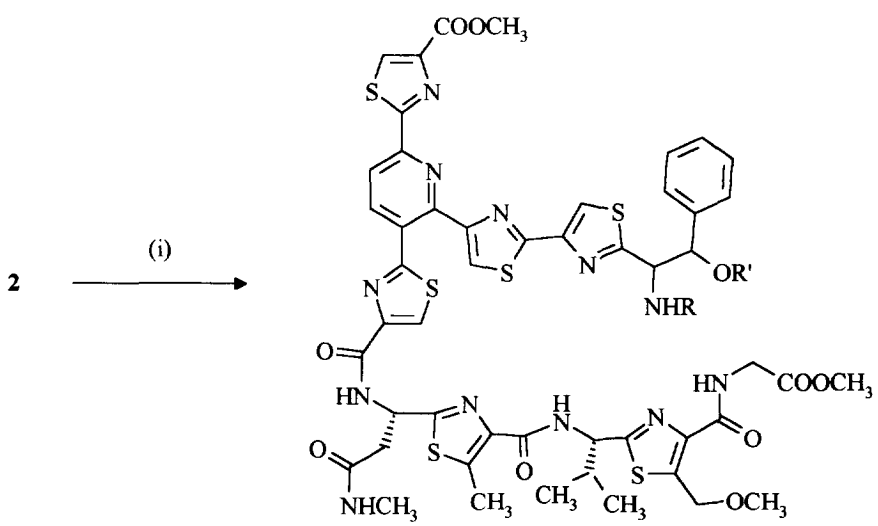
thiazole amino acids. In fact, acid hydrolysis of compound **1** in a mixture of dioxane, water and formic acid (10:1:1) yielded the rearranged compound **2** as a consequence of oxazoline opening, N-O acyl shift and diketo piperazine formation (Scheme 1). The stereocenters present in the macrocycle were not affected under these reaction conditions as confirmed by chiral HPLC.

Moreover, methanolysis of compound **2** in the presence of 5% of sulphuric acid gave the dimethyl ester **3** as the main product in an acceptable yield (Scheme 2). This reaction give rise to the removal of the diketo piperazine moiety and to the regioselective opening of the thiazolyl peptide macrocycle as demonstrated by NMR structural studies. Indeed, the signal at 9.0 ppm present in the NMR spectrum of compound **2,** attributed to the amidic proton of the amide between thiazole phenylserine and glycine, disappeared. It was replaced in compound **3** by broad signal at 2.22 ppm consistent with the presence of two protons (NH_2). The methyl ester at 3.64 ppm was assigned to the glycine moiety by 2D-NMR (see experimental) while the signal at 3.91 ppm was assigned to the methyl ester of thiazole A connected to the pyridine ring. Additional evidence was provided by MS studies. The sequence information of MDL 62,879 were limited to the side chain. In fact, linear peptides containing natural aminoacids can be sequenced efficiently using FAB and ESI MS/MS[11], while in the case of cyclic and/or highly modified peptides little or no information can be deduced from collision induced dissociation MS/MS experiments. However, the opening of the cyclic part of the natural antibiotic gives rise to a product that can be fragmented under MS/MS conditions. In the case of compound **3** the tandem MS spectrum showed all the fragments corresponding to the breakage of the amidic bonds of the linear modified peptide. Both NMR studies and MS experiments are consistent



(i) Dioxane/water/formic acid (10:1:1), 80° C, overnight

Scheme 1



(i) $MeOH/H_2SO_4$ 5%

(ii) acetic anhydride/pyridine

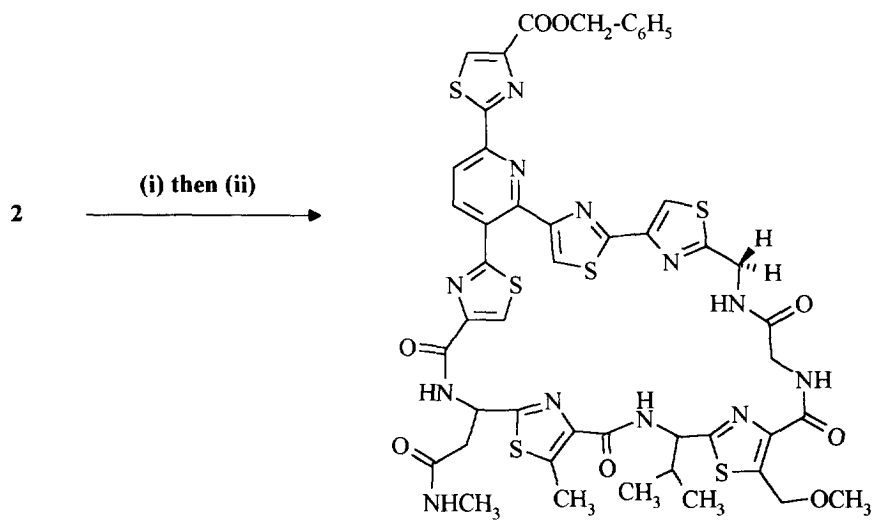
R = R' = H **3** (ii)

R = R' = $-COCH_3$ **4**

Scheme 2

with the cleavage of the amidic bond between the thiazolyl phenylserine and the adjacent glycine. Furthermore, the chiral centers appeared not to be affected as **3** was observed as a single compound in chiral HPLC analysis and in spectroscopic studies. To confirm the structure, compound **3** was treated overnight with acetic anhydride/pyridine mixture to give compound **4**. The protons at 2.22 ppm (NH_2) and at 5.81 ppm (OH) in compound **3** were lacking in the NMR spectrum of **4**. Signals at 8.83 ppm (NH), 1.99/1.75 ppm (two acetyl residue) and the down shift of the hydrogens on α/β carbons of the phenyl serine moiety were in agreement with the proposed structure of **4**.

The chemical behaviour of compound **2** was also evaluated in basic conditions. In the presence of cesium carbonate in water - dioxane mixture, compound **2** was completely transformed. The crude material appeared as one peak in HPLC (RP18 stationary phase), but when was treated with benzyl bromide in dimethylformamide yielded two peaks with 13.8 and 14.8 HPLC retention times corresponding to benzyl esters **5** and **6** respectively (Scheme 3). These compounds were separated on silica gel (see experimental) and identified as two isomers having molecular weight of m/z 1109.2 [MH^+]. The MW and NMR spectra were consistent with the loss of the diketo piperazine (ester hydrolysis), the formation of the benzyl ester and benzaldehyde elimination[12] (retro aldol reaction). Their optical rotation values were different suggesting a possible racemization at one of the two stereocenters leading to diastereoisomers formation. The NMR spectra of compounds **5** and **6** showed different chemical shifts mainly concerning the methylene protons of thiazole asparagine. The correct stereochemistry of **5** and **6** has still to be defined.



(i) Cs_2CO_3, dioxane/water (1:1), 80° C.

(ii) Benzyl bromide / dimethyl formamide

Scheme 3

Compound **1** (MDL 62,879) inhibits a cell free system[9] of bacterial protein synthesis with an IC_{50} of 0.8 μg/ml and is very active against a panel of Gram positive microorganisms[1]. Compound **2** and its methyl ester were active although they lost activity against some strains of streptococci. Compounds **3** was less active than **1** against a

selected panel of Gram positive microorganisms while compounds **4**, **5** and **6** were inactive (MICs > 128 μg/ml). On the basis of the above results it seems that the moiety connected to thiazole C is important for the biological activity of MDL 62,879 but it does not appear to be a stringent structural requirement. Indeed, amythiamicin bearing an isopropyl group at the position of the hydroxy benzyl group in MDL 62,879, has biological activity comparable to MDL 62,879.

CONCLUSION

Thiazolyl peptide antibiotics acting on the elongation factor Tu seem to be attractive for their antibacterial activity in particular against multi resistant pathogens. Here, we have reported on the preparation of MDL 62,879 derivatives having a modified macrocycle ring. All the compounds prepared have lost significantly their capability to inhibit the growth of the bacteria susceptible to MDL 62,879. These results have represented an important preliminary structure activity relationship of these thiazolyl peptide antibiotics and they are useful for addressing further chemical modifications.

EXPERIMENTAL

The solvents and reagent were purified and dried by standard techniques. Melting points were determined with Buchi Model 510 capillary apparatus and are uncorrected.

The IR absorption spectra were recorded with I.F.S. 48 Bruker F.T.I.R. spectrophotometer. The UV absorption spectra were recorded with Perkin-Elmer spectrophotometer Mod. Lambda 16 (200-800 nm).

Optical rotation were measured with a Perkin-Elmer Model 241 polarimeter.

All NMR spectra have been recorded on a Bruker AMX 600 at 30°C. The data were processed on an Aspect station with the UXNMR software from Bruker. All homonuclear experiments (DQF-COSY, TOCSYand ROESY were performed with a spectral width of 12 ppm. In all the experiments, spectra were recorded with 512 increments in t_1 and 4096 complex data points in t_2. For the ROESY spectra 48 transients were averaged for each t_1 value, for COSY and TOCSY 16 transients. Mixing times of 70 or 150 ms were used for TOCSY and ROESY spectra, respectively. For HMQC spectra 512 increments (64 scans) with 2048 complex data points in t_2 were collected using a sweep width of 12 ppm in the proton and 165 ppm in the carbon dimension. A BIRD pulse was applied to suppress magnetization of protons connected to ^{12}C (recovery delay of 200 ms). The HMBC spectra were acquired with a sweep width of 12 ppm in the proton and 165 ppm in the carbon dimension. A total of 96 transients were averaged for each of 512 increments in t_1, and 2048 complex points in t_2 were recorded. A delay of 3.3 ms was used to supress 1-J couplings and 70 ms were taken for the development of long range correlations. After Fourier transformation the strong t_1 noise was reduced by a mean row subtraction using the AURELIA program (Bruker).

An API III+ triple quadrupole mass spectrometer (PE-Sciex, Thornhill, ON, Canada) equipped with an articulated ion spray interface was employed. Tuning and calibration were performed on both the first (Q1) and the third quadrupole using a solution of polypropylene glycols (PPPG's) in 3 mM ammonium acetate. Samples were infused via a 75 μm i.d. fused silica capillary tubing to the ion spray tip which was held at a potential of +5.8 kV.

A syringe pump (Model 22, Harvard Apparatus, MA, USA) controlled the delivery of the sample at a rate of 5.0 μL/min. Zero grade compressed air was used as nebulizer gas (pressure set at 55 psi). A curtain gas (99.999% UHP nitrogen) flow of 0.8 L/min. was employed. The interface heater was set at 60°C. Mass spectra were obtained at a dwell time of 1.00 msec (Q1 scan range 300-1500 u, 10 scans averaged) and a step size of 0.10 u. The orifice voltage was maintained at 80 V. All the MS/MS experiments were carried out using a 90:10 mixture of argon and nitrogen as collision gas. All the samples (c.a. 100 μg/mL) were dissolved immediately prior to analysis in a 1:1:1 mixture of water, added with 0.1% of trifluoroacetic acid, methanol and tetrahydrofuran.

The "flash chromatography" purifications were performed as reported[13].

HPLC analysis: Varian 5000 Liquid Chromatograph equipped with a 3390 A Hewlett Packard integrator. Detection UV at 254 nm. Column Lichrocart 125-4 (Merck) - Lichrospher 100 RP18 (5 μm). Mobile phase A : ammonium formate 0.05 M; mobile phase B : acetonitrile. Flow rate : 1 ml/min.

Chiral HPLC for compound **2** : Liquid chromatograph Hewlett Packard Mod. 1090 equipped with diode-array detector. Bakerbond prepacked column (J.T. Baker 250 mm x 4.6 mm; 5 μm). Chiral stationary phase : DNBPG covalently linked. Mobile phase A : n-hexane; Mobile phase B : isopropyl alcohol.

Chiral HPLC for compounds **3**, **4**, **5** and **6** : Beckman System Gold Mod. 126 equipped with a Mod. 166 UV variable wavelenght detector. Supelco prepacked column (250 mm x 4.6 mm, 5 μm). Chiral stationary phase: DNBPG(R) covalently linked. The mobile phase was a ternary mixture consisting of n-hexane, isopropylic alcohol and acetonitrile or chloroform. For each sample were injected 20 μL of a ca. 1 mg/mL solution in the corresponding mobile phase.

Compound **2.** - A solution of 5 g of **1** (3.87 mmoles) in 60 ml of dioxane, 6 ml of water and 6 ml of formic acid 99% was left overnight at 80° C under stirring. After cooling, the solvent was evaporated and concentrated *in vacuo* through azeotropical removal of water with toluene. The yellow solid residue was chromatographed on silica gel ($CHCl_3$-MeOH 95:5) to give 4.07 g of pure **2** as a pale yellow solid (3.15 mmoles, yield 81%); UV (MeOH) λ_{max}= 306 nm; υ_{max} (nujol)/cm^{-1} 1725, 1655, 1410; δ_H (DMSO-d_6) 9.00 (1H, d 8Hz), 8.69 (1H, d 8.6 Hz), 8.68 (1H, d 8Hz), 8.61 (1H, s) 8.46 (1H, s), 8.45 (1H, t), 8.30 (1H,s), 8.27 (1H, d 8Hz), 7.37 (1H, q), 7.36 (1H, s), 7.33-7.26 (4H, m), 6.01 (1H, d 4.3 Hz), 5.28 (1H, m), 5.24 (1H, t 7.3 Hz), 5.21 (1H, dd 8.3, 4.6 Hz), 5.01 (1H, t 5.6 Hz), 4.98 (2H, s), 4.68 (1H, dd 9.6, 2.3 Hz), 4.53 (2H, m), 4.35-4.28 (2H, m), 3.77 (1H, dd 16.9, 4.0 Hz), 3.55 (1H, m), 3.39 (3H, s), 2.70 (1H, dd 16.6, 3.0 Hz), 2.59 (3H, s), 2.47 (3H, d 4.6 Hz), 2.23-2.13 (2H, m), 1.91-1.80 (3H, m), 1.35 (1H, d 12.9 Hz), 0.88 (3H, d 7.0 Hz), 0.84 (3H, d 7.0 Hz); $[\alpha]_D^{25}$ = + 126.1 (c= 1 $CHCl_3$-MeOH 9:1); ESI-MS m/z 1291 (MH^+, 100%), *Found*: C, 52.05%, H, 4.34, N, 15.11; *calculated for* $C_{56}H_{54}N_{14}O_{11}S_6$: C, 52.09%, H, 4.18, N, 15.9; t_R (HPLC): 7.88 min., mobile phase B 44%.

Compound **3.** - A solution of 2.5 g of **2** (1.93 mmoles) in 47.5 ml of methanol and 2.5 of conc. sulphuric acid was left stirring at 60° C for 40 hrs. After cooling, the reaction mixture was neutralized by adding solid sodium bicarbonate in several portions. The resulted suspension was concentrated, diluted with 20 ml of chloroform and then filtered. After evaporation of the solvent *in vacuo*, the crude solid material was washed with 50 ml of ethyl ether and then purified by "flash chromatography" on silica gel ($CHCl_3$- MeOH from 99:1 to 97:3) yielding 1.0 g of compound **3** (0.85 mmoles). An analytical sample was obtained by crystallization from chloroform - isopropyl alcohol : m.p. 177° C (dec.); υ_{max} ($CDCl_3$)/cm^{-1} 1733, 1668, 1558, 1530, 1506, 1221; ESI-MS m/z 1171.3 (MH^+, 100%); ^1H and

^{13}C NMR, see Table 1; $[\alpha]_D^{25}$ = - 8.8 (c= 0.17, $CDCl_3$); *Found*: C, 51.06%, H, 4.32, N, 14.16; *calculated for* C_{50} H_{50} N_{12} O_{10} S_6: C, 51.28%, H, 4.27, N, 14.35; t_R (HPLC): 5.7 min., mobile phase B 60%.

Chiral HPLC : t_R = 8.4 min. mobile phase : n-hexane/isopropyl alcohol/chloroform (40:24:36 v/v/v), flow 0.9 ml/min.

Compound **4**. - To a solution of compound **3** (200 mg; 0.17 mmoles) in 2 ml of pyridine was added at 0° C 0.5 ml of acetic anhydride. After stirring at R.T. overnight the reaction was cooled at 0° C, acidified with hydrochloric acid 1N (pH = 5), diluted whit 20 ml of water and then extracted with chloroform (2x20 mL). The organic phase was washed with hydrochloric acid 1N (10 mL), saturated sodium chloride solution (10 mL) and water (10 mL). The crude material obtained after drying with sodium sulfate and concentration *in vacuo* was purified by flash chromatography on silica gel ($CHCl_3$- MeOH from 99:1 to 98.5:1.5) to give 170 mg of pure **4** (0.13 mmoles, yield 79%). An analytical sample was obtained by crystallization from chloroform - isopropyl alcohol : m.p. 225° C; υ_{max} ($CDCl_3$)/cm^{-1} 3402, 3121, 2957, 1739, 1666, 1501, 1225; ESI-MS m/z 1255.2 (MH^+, 100%); ^1H and ^{13}C NMR see Table 1; $[\alpha]_D^{25}$ = 0 (c= 0.16, $CDCl_3$); *Found*: C, 51.25%, H, 4.36, N, 13.25; *calculated for* C_{54} H_{54} N_{12} O_{12} S_6: C, 51.67%, H, 4.30, N, 13.39; t_R (HPLC): 10.8 min., gradient profile time (min.) 0 (B 50%), 5 (B 50%), 10 (B 70%).

Chiral HPLC : t_R = 10.8 min. mobile phase : n-hexane/isopropyl alcohol/acetonitrile (40:24:36 v/v/v), flow 0.5 ml/min.

Compounds **5** and **6**. - 1.29 g of compound **2** (1 mmoles) and 977 mg of cesium carbonate (3 mmoles) in 20 ml of water/dioxane (1:1) were left at 80° C for 65 h. After cooling, the solvent was evaporated and concentrated in vacuo through azeotropical removal of water with ethanol. The crude solid material was washed with 30 mL of ethyl acetate , dried *in vacuo* and then suspended in 10 mL of dimethylformamide. Benzyl bromide (0.3 mL) was added to the suspension and the reaction stirred overnight. The reaction mixture was then diluted with water (40 mL) and acidified at 0° C with hydrochloric acid 1N. The reulting precipitate was filtered, washed with water, ethyl ether and dried under vacuum to give 1.15 g of a pale yellow solid (crude mixture of compounds **5** and **6**). The purification by flash chromatography on silica gel (CH_2Cl_2 / THF 75:25) provided 250 mg of compound **5** (0.22 mmoles), 300 mg of compound **6** (0.27 mmoles) and 200 mg of the mixture of compounds **5** and **6**.

Compound **5** - An analitical sample was obtained by crystallization from chloroform - isopropyl alcohol; t_R (HPLC) : 13.8 min., gradient profile time (min.) 0 (B 50%), 5 (B 50%), 10 (B 70%); m.p.239 (dec.); υ_{max} ($CDCl_3$)/cm^{-1} 3392, 1730, 1664, 1529, 1504, 1212; $[\alpha]_D^{25}$ = +207° (c= 0.17, $CDCl_3$); ESI-MS m/z 1109.2 (MH^+, 100%); ^1H and ^{13}C NMR see Table 2; Found: C, 51.39%, H, 4.03, N, 14.78; *calculated for* C_{48} H_{44} N_{12} O_8 S_6: C, 51.98%, H, 3.97, N, 15.16.

Chiral HPLC : t_R = 14.5 min. mobile phase n-hexane/isopropyl alcohol/chloroform (50:10:40 v/v/v), flow 0.5 ml/min.

Compound **6** - An analitical sample was obtained by preparative TLC on silica gel 60 F254 (Merck 20x20; 1 mm) : t_R (HPLC) : 14.8 min., gradient profile time (min.) 0 (B 50%), 5 (B 50%), 10 (B 70%); m.p.186; υ_{max} ($CDCl_3$)/cm^{-1} 3391, 1725, 1664, 1529, 1504, 1212; $[\alpha]_D^{25}$ = - 164° (c= 0.13, $CDCl_3$); ESI-MS m/z 1109.2 (MH^+, 100%); ^1H and ^{13}C NMR see Table 2; *Found*: C, 50.95%, H, 4.0, N, 14.32; *calculated for* C_{48} H_{44} N_{12} O_8 S_6: C, 51.98%, H, 3.97, N, 15.16.

Table 1. Chemical Shifts of Compounds **3** and **4**[a)]

	3	**3**	**4**	**4**
	^1H	^{13}C	^1H	^{13}C
Gly-NH	8.62 (t 6.2)	-	8.62 (t 6.2)	-
Gly-α	4.00 (t 5.5)	40.7	4.00 (t 5.5)	40.7
Gly-C'	-	170.1	-	170.1
Gly-OMe	3.64 (s)	51.7	3.64 (s)	51.7
Val-NH	8.33 (d 8.9)	-	8.34 (d 8.3)	-
Val-α	5.09 (dd 8.9, 7.3)	56.2	5.09 (dd 8.3, 7.0)	56.2
Val-β	2.44 (m)	32.1	2.45 (m)	32.1
Val-γ	0.96 (d 6.9)	18.1	0.96 (d 7.0)	18.1
Val-γ'	0.94 (d 6.9)	19.5	0.95 (d 7.0)	19.5
ThiazoleD-2	-	169.7	-	169.7
ThiazoleD-4	-	143.4	-	143.4
ThiazoleD-5	-	141.1	-	141.1
ThiazoleD-OCH_2	4.94 (d 2.0)	67.3	4.94 (d 2.0)	67.3
ThiazoleD-OCH_3	3.35 (s)	58.4	3.35 (s)	58.4
ThiazoleD-C'	-	161.7	-	161.7
Asn-NH	9.48 (d 8.6)	-	9.50 (d 8.6)	-
Asn-α	5.68 (m)	48.1	5.68 (m)	48.2
Asn-β	3.04/2.91 (dd 15.3,	38.3	3.05/2.92 (dd 15.3,	38.3
Asn-γ-C'	-	170.1	-	170.1
Asn-δ-NH	8.03 (q)	-	8.03	-
Asn-ϵ-CH_3	2.56 (d 4.5)	25.5	2.55	25.5
ThiazoleE-2	-	167.8	-	167.7
ThiazoleE-4	-	141.2[b]	-	141.2[b]
ThiazoleE-5	-	141.1[b]	-	141.1[b]
ThiazoleE-CH_3	2.65 (s)	12.2	2.67 (s)	12.2
ThiazoleE-C'	-	161.6	-	161.6
ThiazoleF-2	-	164.0	-	163.9
ThiazoleF-4	-	148.8	-	148.8
ThiazoleF-5	8.49 (s)	127.6	8.47 (s)	127.6

Table 1. continued

ThiazoleF-C'	-	160.0	-	160.1
Pyridine-2	-	150.9[c]	-	150.8[c]
Pyridine-3	-	129.2	-	129.2
Pyridine-4	8.65 (d 8.2)	140.0	8.67 (d 8.2)	140.0
Pyridine-5	8.35 (d 8.2)	118.9	8.36 (d 8.2)	119.0
Pyridine-6	-	150.0[c]	-	150.0[c]
ThiazoleB-2	-	162.3	-	161.8
ThiazoleB-4	-	152.5	-	152.6
ThiazoleB-5	8.30 (s)	122.7	8.33 (s)	123.1
ThiazoleC-2	-	176.7	-	170.2
ThiazoleC-4	-	147.1	-	147.2
ThiazoleC-5	7.69 (s)	117.1	7.84 (s)	117.8
PheSer-NH_2/NH	2.22 (broad)	-	8.83 (d 8.7)	-
PheSer-NH-C'	-	-	-	169.0
PheSer-NH-C'CH_3	-	-	1.99 (s)	20.8
PheSer-α	4.44 (d 5.1)	59.5	5.58 (t 8.3)	54.2
PheSer-β	4.96 (t 4.2)	76.0	6.18 (d 7.8)	74.6
PheSer-β-OH	5.81 (d 4.2)	-	-	-
PheSer-β-C'	-	-	-	169.1
PheSer-β-CH_3	-	-	1.75 (s)	22.2
PheSer-1	-	141.3	-	136.9
PheSer-2,6	7.24 (m)	126.6	7.38 (m)	127.1
PheSer-3,5	7.21 (m)	127.6	7.35 (m)	128.2
PheSer-4	7.19 (m)	126.8	7.31 (m)	128.3
ThiazolA-2	-	167.7	-	167.7
ThiazolA-4	-	147.3	-	147.3
ThiazolA-5	8.77 (s)	132.3	8.77 (s)	132.3
ThiazolA-C'	-	161.1	-	161.1
ThiazolA-OCH_3	3.91 (s)	52.2	3.91 (s)	52.2

a) Chemical shifts in DMSO-d at 303 K. Chemical shifts are referenced to DMSO at 2.50 and 39.5 ppm, respectively. Coupling constants are given in Hz.
b) Carbon resonances of ThiazoleE-4 and ThiazoleE-5 could not be distinguished.
c) Carbon resonances of Pyridine-2 and Pyridine-6 could not be distinguished.

Table 2. Chemical Shifts of Compounds **5** and **6**[a)]

	5	**5**	**6**	**6**
	^{1}H	^{13}C	^{1}H	^{13}C
Gly^1-NH[b)]	9.07 (b)	-	9.20 (t 5.7)	-
Gly^1-α	4.69/4.49 (dd 15.8, 5.6/dd 15.8, 5.9)	41.0	4.61 (d 5.7)	40.9
Gly^2-NH[b)]	8.36 (b)	-	8.40 (b)	-
Gly^2-α	4.19/3.92 (dd 17.0, 6.8/dd 17.0, 4.4)	41.6	4.32/3.90 (dd 17.0, 7.2/dd 17.0, 4.5)	41.4
Gly^2-C'	-	169.4	-	169.8
Val-NH	8.33 (d 8.4)	-	8.45 (d 8.5)	-
Val-α	5.17 (dd 8.4, 6.0)	55.4	5.20 (dd 8.5, 6.0)	55.1
Val-β	2.24 (m)	33.3	2.22 (m)	33.7
Val-γ	0.93 (d 6.8)	17.9	0.92 (d 6.8)	18.0
Val-γ'	0.90 (d 6.8)	18.8	0.88 (d 6.8)	18.7
ThiazoleD-2	-	166.4	-	166.1
ThiazoleD-4	-	143.2	-	143.4
ThiazoleD-5	-	141.0	-	141.1
ThiazoleD-OCH_2	5.00 (s)	67.1	5.00 (s)	67.2
ThiazoleD-OCH_3	3.40 (s)	58.4	3.40 (s)	58.4
ThiazoleD-C'	-	161.1	-	161.2
Asn-NH	9.09 (b)	-	8.86 (d 8.1)	-
Asn-α	5.33	48.5	5.31	48.4
Asn-β	2.78/2.31 (dd 15.8, 5.3/15.8, 5.0)	38.4	2.56/2.07 (dd 15.8, 4.5/broad)	37.9
Asn-γ-C'	-	169.7	-	169.6
Asn-δ-NH	7.76 (q)	-	7.57 (q)	-
Asn-ϵ-CH_3	2.49 (c)	25.5	2.45 (d 4.5)	25.5
ThiazoleE-2	-	168.4	-	167.9
ThiazoleE-4	-	142.5	-	141.8
ThiazoleE-5	-	139.2	-	139.7
ThiazoleE-CH_3	2.54 (s)	12.0	2.61 (s)	12.0
ThiazoleE-C'	-	161.5	-	161.1

Table 2. continued

ThiazoleF-2	-	164.8	-	164.5
ThiazoleF-4	-	149.4	-	149.4
ThiazoleF-5	8.57 (s)	126.8	8.55 (s)	126.7
ThiazoleF-C'	-	160.0	-	160.1
Pyridine-2	-	150.5	-	150.3
Pyridine-3	-	128.3	-	128.2
Pyridine-4	8.39 (d 8.1)	140.8	8.41 (d 8.1)	140.9
Pyridine-5	8.30 (d 8.1)	118.4	8.31 (d 8.1)	118.6
Pyridine-6	-	150.1	-	150.1
ThiazoleB-2	-	160.3	-	160.3
ThiazoleB-4	-	153.2	-	153.2
ThiazoleB-5	8.37 (s)	122.7	8.26 (s)	122.6
ThiazoleC-2	-	171.3	-	171.2
ThiazoleC-4	-	147.4	-	147.4
ThiazoleC-5	7.35 (s)	116.5	7.43 (s)	116.4
ThiazolA-2	-	168.0	-	167.8
ThiazolA-4	-	147.2	-	147.3
ThiazolA-5	8.84 (s)	132.6	8.83 (s)	132.6
ThiazolA-C'	-	160.4	-	160.4
Benz-CH_2	5.42 (s)	66.3	5.42 (s)	66.3
Benz-1	-	135.9	-	135.9
Benz-2,6	7.52 (m)	128.2	7.52 (m)	128.2
Benz-3,5	7.44 (m)	128.5	7.44 (m)	128.5
Benz-4	7.38 (m)	128.2	7.38 (m)	128.2

a) Chemical shifts in DMSO-d at 303 K. Chemical shifts are referenced to DMSO at 2.50 and 39.5 ppm, respectively. Coupling constants are given in Hz.

b) Gly^1 refers to the new thiazolyl-glycine obtained and Gly^2 refers to glycine already present in the natural compound.

Chiral HPLC : t_R = 11.0 min. mobile phase n-hexane/isopropyl alcohol/chloroform (50:20:30 v/v/v), flow 0.5 ml/min.

Acknowledgements

We would like to thank Dr. E. Restelli for the supply of the natural antibiotic, G. Tamborini for recording the analytical spectra and Dr. M. Nebuloni for the microanalysis.

REFERENCES AND NOTES

1. Selva, E. ; Beretta, G.; Montanini, N.; Gastaldo, L.; Ferrari, P.; Lorenzetti, R.; Landini, P.; Goldstein, B.P.; Berti, M.; Montanaro, L.; Denaro, M. *J. Antibiotics* **1991**, 44, 693.
2. Bèrdy, J. *Hanbook of Antibiotiocs Compounds*, CRC Press Inc. **1980**.
3. Anborg, P.H., Parmeggiani, A. *The EMBO Journal*, **1991**, 10, 779.
4. Landini, P.; Bandera, M.; Goldstein, B.P.; Ripamonti, F.; Soffientini, A.; Islam, K.; Denaro, M. *J. Biochem.* **1992**, 283, 649.
5. Parmeggiani, A.; Swart, G.W.M. *Annu. Rev. Microbiol.* **1985**, 39, 557.
6. Smith, R.J.; Williams, D.H.; Barna, C.J.; McDermot, I.R.; Haegele, K.D.; Piriou, F.; Wagner, J.; Higgins, W. *J. Am. Chem. Soc.* **1985**, 107, 2849.
7. Wolf, H.; Hassmann, D.; Fisher, E. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5324.
8. Tavecchia, P.; Gentili, P.; Kurz, M., Sottani, C.; Bonfichi, R.; Selva, E.; Lociuro, S.; Restelli, E.; Ciabatti, R. *Tetrahedron* **1995**, 51, 4890.
9. Stella, S.; Montanini, N.; Le Monnier, F.; Ferrari, P.; Colombo, L.; Marinelli, F.; Landini, P.; Ciciliato, I.; Goldstein, B.P.; Selva, E.; Denaro, M. *J. Antibiotics* **1995**, 48, 780.
10. Shimanaka, K.; Iinuma, H.; Hamada, M.; Ikeno, S.; Tsuchiya, K.S.; Arita, M.; Hori, M. *J. Antibiotics* **1995**, 48, 182.
11. Bush, K.L.; Glish, G.L.; McLuckey, S.A. *Mass Spectrometry/Mass Spectrometry*, VCH, New York, **1988**, 82.
12. The HPLC profile of the reaction shows a peak which retention time corresponds to a pure sample of benzaldhyde.
13. Still, W.C. *J. Org. Chem.* **1978**, 43, 2923.

(*Received in UK* 20 *February* 1996; *revised* 26 *April* 1996; *accepted* 2 *May* 1996)