Antimicrobial Activities of Chemically Modified Thiazolyl Peptide Antibiotic MDL 62,879 (GE2270A)

SERGIO LOCIURO,*,[a] PAOLO TAVECCHIA, ETTORE MARZORATI, PAOLO LANDINI,[b] BETH P. GOLDSTEIN,[c] MAURIZIO DENARO[d] and ROMEO CIABATTI

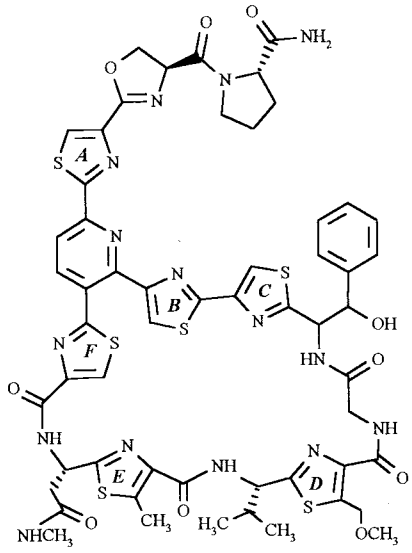
Biosearch Italia S.p.A.,
Via R. Lepetit 34, 21040 Gerenzano (Va), Italy

(Received for publication September 26, 1996)

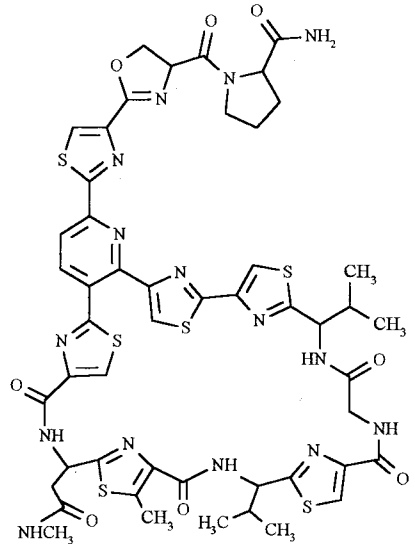
MDL 62,879 (GE2270A) **1** is a new inhibitor of elongation factor-Tu (EF-Tu) and belongs to the class of thiazolyl peptide antibiotics. Controlled acid hydrolysis of **1** followed by treatment with base resulted in the lost of the two terminal amino acids and in the formation of water-soluble MDL 62,935 **2**. Although less active *in vitro* than its parent compound, **2** was able to inhibit by 50% an *Escherichia coli* cell-free protein synthesis system at roughly the same concentration of **1**. MDL 62,935 **2** was subjected to further modification at the β-phenylserine residue. Derivatives obtained from **2** were less active in both antimicrobial (MIC) and enzymatic (IC_{50}) assays. This suggests that β-phenylserine plays an important role for the inhibition of EF-Tu by **1** and **2**.

MDL 62,879 (GE2270A) **1** is a thiazolyl peptide antibiotic isolated from fermentation of *Planobispora rosea* ATCC 53733.[1] Although the structure of this highly modified cyclic peptide has been recently revised,[2] the chirality of the two stereo centers of the β-phenylserine still remains undetermined. Recently, two new thiazolyl peptide antibiotics have been discovered: GE37468[3] and amythiamicin.[4] Their structures resemble very closely that of MDL 62,879 in sharing a thiazolyl peptide macrocycle of similar size and a pyridine from which a peptide side chain departs. The major difference between amythiamicin[5] and MDL 62,879[2] is the presence of thiazolylvaline in place of the thiazolyl-β-phenylserine of MDL 62,879. MDL 62,879,[6,7] GE37468[3] and amythiamicin[8] act specifically on the elongation factor-Tu (EF-Tu), a protein essential for bacterial protein syn-

Fig. 1.



MDL 62,879 (GE2270A)
1



Amythiamicin

Present address: [a] Glaxo Wellcome Research Center, Via Fleming 4, 37135 Verona, Italy.
[b] School of Biochemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.
[c] Applied Microbiology Inc., 771 Old Saw Mill River Road, Tarrytown, New York 10591, U.S.A.
[d] Amylin Pharmaceuticals Inc, 9373 Towne Centre Drive, San Diego, California 92121, U.S.A.

thesis. EF-Tu is also the target of the kirromycin class of antibiotics[9] and pulvomycin.[10] These antibiotics differ from MDL 62,879 in structure, mode of action and spectrum of antibacterial activity.[11] Despite being very active in cell-free protein synthesis assays using EF-Tu extracts from Gram-negative microorganisms (*e.g. E. coli*), MDL 62,879 lacks activity against the intact bacteria because it is unable to enter the cell.[1] In contrast, it is very active against Gram-positive bacteria, including pathogens resistant to antibiotics currently available on the market, both *in vitro*[12,13] and in experimental infection models in rodents.[14] However, its very poor solubility in water makes formulation for systemic administration to humans difficult. We initiated studies aimed at identifying which parts of the molecule could be chemically modified to increase solubility while maintaining antimicrobial activity.

We now report on MDL 62,935 **2**, a water-soluble hydrolysis product of **1**, and on some of its derivatives at the β-phenylserine residue. We based our preliminary Structure Activity Relationship analyses both on the antimicrobial activities (MICs) and on the inhibitory concentrations (IC_{50}) in protein synthesis assays in cell-free extracts from *E. coli*.

Results and Discussion

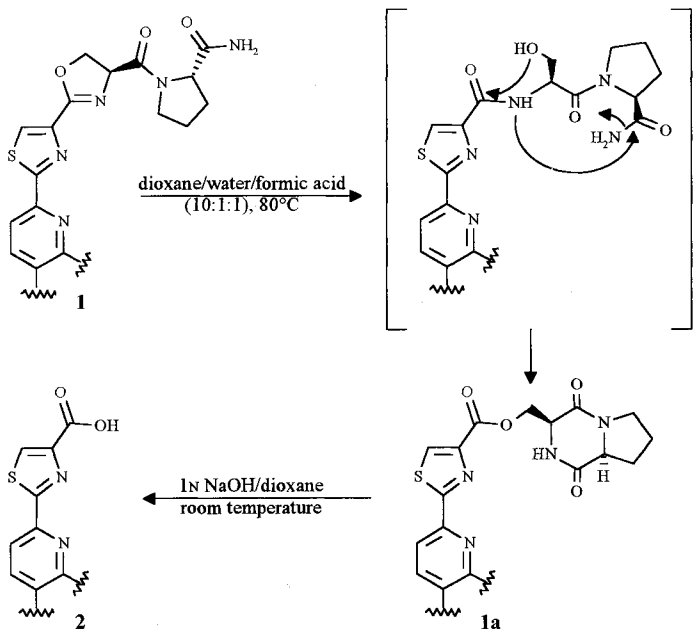
As previously described,[2] under mild acid conditions the oxazoline ring of antibiotic **1** opened to generate a serine which in turn underwent N→O shift. Nucleophilic attack of the serine nitrogen to the proline carboxamide led to the diketopiperazine ring closure of ester **1a**. Hydrolysis of ester **1a** with 1 N NaOH produced the acid derivative **2** (Scheme 1). The presence of a carboxylic group allowed dissolution of the sodium salt of **2** in water at concentrations superior to 50 mg/ml.

As reported elsewhere,[15] treatment of **1a** with Cs_2CO_3 in dioxane - water 1 : 1 at 80°C induced hydrolysis of the ester and retro-aldol reaction with lost of benzaldehyde from thiazolyl-β-phenylserine to produce **3** (Scheme 2). It was also shown by esterification of compound **3** with benzyl bromide that **3** was a mixture of two diastero-isomers, probably at the chiral center of thiazolyl-*N*-methylasparagine.

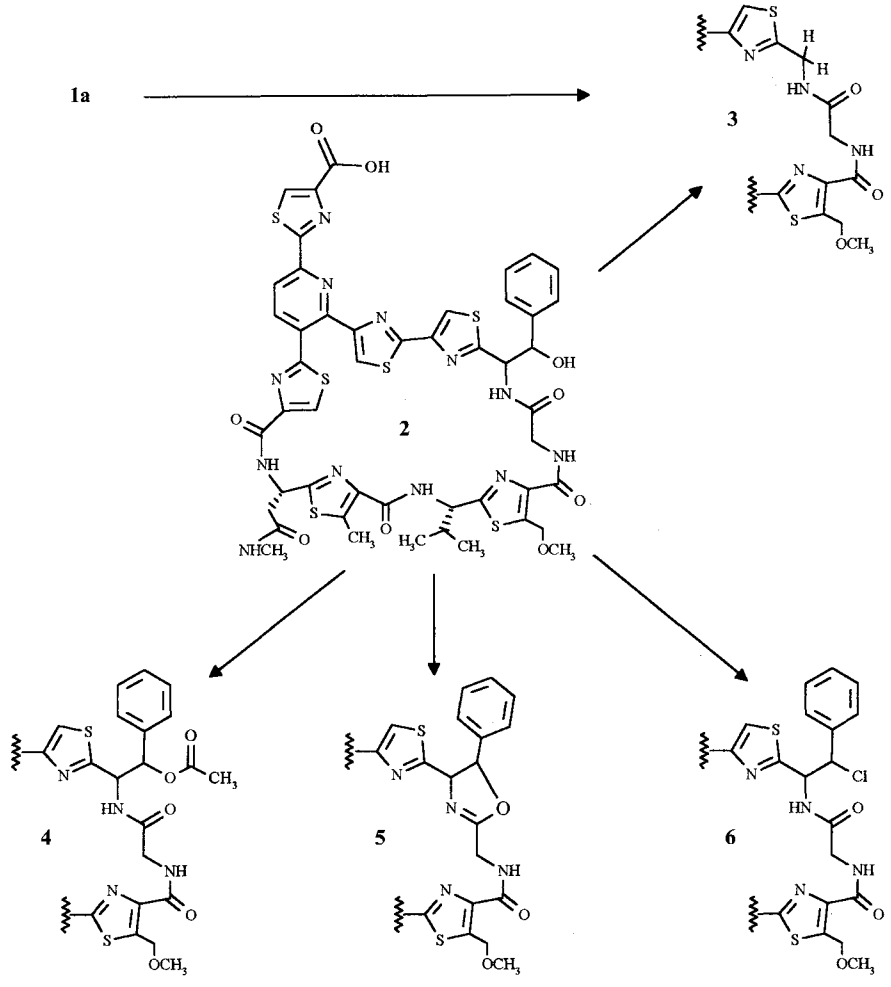
Acetylation of **2** with acetic anhydride in the presence of 33% HBr in acetic acid at room temperature yielded compound **4** (Scheme 2), quantitatively. The disappearance of the doublet of the hydroxy proton at 6.02 ppm, the simplification to a doublet and downfield shift of the β-proton of thiazolyl-β-phenylserine of 2.15 ppm and the appearance of a new sharp singlet at 1.98 ppm confirmed its structure.

Mesylation of **2** in dichloromethane with a large excess of methansulfonic anhydride and TEA at room temperature resulted in the direct formation of oxazoline **5** (Scheme 2) in 50% yield, the remaining material being unreacted starting compound **2**. (On the methyl ester of **2** the same reaction proceeded quantitatively; results not shown.) When the same reaction was conducted with an amount of TEA insufficient to neutralize the acidity of

Scheme 1.



Scheme 2.

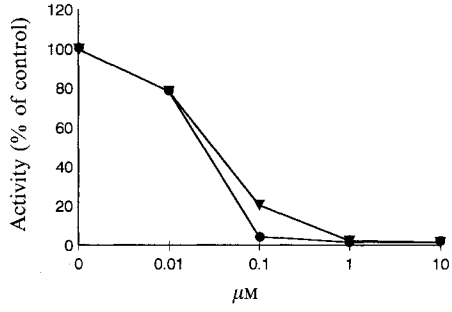


the methansulfonic acid being generated, a compound presumed to be the intermediate mesylate was observed by HPLC analysis. Addition of TEA to basic pH quantitatively converted this chromatographic peak to oxazoline **5** (results not shown). The structure of compound **5** was confirmed both by FAB-MS and ^{1}H NMR analysis. In particular, the disappearance of the hydroxy and amide protons of thiazolyl-β-phenylserine at 6.02 and 9.01 ppm, respectively, together with a significant change in chemical shift and coupling constant of the glycine protons were of diagnostic value.

In contrast, treatment of **2** with neat thionyl chloride at room temperature yielded quantitatively chloro-derivative **6** (Scheme 2) as confirmed by FAB-MS and ^{1}H NMR analysis. The disappearance of the doublet of the hydroxy proton at 6.02 ppm and the simplification to a doublet and upfield shift of 0.54 ppm of the β-proton of thiazolyl-β-phenylserine were in agreement with the structure. Concerning the stereochemical outcome of the reaction, NMR analyses did not allow to establish whether it proceeded with retention or inversion of configuration.

Fig. 2. Poly(U)-dependent poly(phe) synthesis inhibition by **1** (MDL 62,879) and **2** (MDL 62,935).

● **1**, ▼ **2**.



1 and **2** inhibited cell-free polyphenylalanine synthesis in the *E. coli* system (Fig. 2) at the same concentration (IC_{50} 0.02 μM), thus suggesting that the removal of the

oxazolinylprolinamide portion of **1** does not affect the binding of the antibiotic to the elongation factor-Tu (EF-Tu) of *E. coli*. However, the antimicrobial activity of **2** was somewhat less than that of **1** (Table 1). This may be due either to small differences in affinity of the two molecules for the EF-Tu of Gram-positive bacteria or to a lesser ability of **2** to diffuse through the membrane of these microorganisms.

When the β-phenylserine residue of **2** was modified, as in compounds **3**, **4**, **5** and **6**, both antibacterial activity and activity in the cell-free protein synthesis assay were reduced, although to a different extent, as shown in Table 2 and Fig. 3, respectively. These results suggest that this part of the molecule plays an important role in the interaction of the molecules with the elongation factor-Tu. Somewhat at odds with these findings is the observation that amythiamicin, in which thiazolylvaline replaces thiazolyl-β-phenylserine (Fig. 1), inhibits cell-free protein synthesis to the same extent as **1** and **2** (MONTI, F.; K. ISLAM, personal communication). One hypothesis that could explain the results is that the valine and the β-phenylserine side-chains, respectively, of amythiamicin and of **1** and **2**, are inserted into a cavity of the EF-Tu, giving rise to a strong lipophilic interaction. When the side-chain is removed as in compound **3** or the radius of the residue is increased as for compounds **4** and **6**, such an interaction would be less favored, resulting in a significant decrease in activity.

Table 1. Antibacterial activity of MDL 62,935 (**1**) and MDL 62,935 (**2**).

Strain No.	MIC (μg/ml)	
	1	**2**
Staphylococcus aureus Tour	0.03	0.13
S. epidermidis ATCC 122228	0.06	0.13
S. haemolyticus clin. isolate	0.13	0.5
Streptococcus pyogenes C203	0.25	2
S. pneumoniae UC41	0.06	1
Enterococcus faecalis ATCC 7080	0.008	0.13
Escherichia coli SKF 12140	>128	>128

The reduced activity of compounds **3**, **4**, **5** and **6** in the cell-free polyphenylalanine synthesis assay is reflected in their minimal inhibitory concentrations (MIC) (Table 2). Why certain compounds are more or less active against particular bacterial species is not yet clear.

Experimental

^{1}H NMR spectra were recorded on either a Bruker AM 500 or a Bruker AMX 600 spectrometer equipped with a X32 computer. Chemical shifts (DMSO-d_6 at 303 K) are reported in δ units (ppm) and are referenced to DMSO at 2.50 ppm. Coupling constants (J) are reported in hertz (Hz). The FAB-MS spectra were obtained with a triple stage quadrupole spectrometer TSQ 700 Finningan under the following conditions: saddle field atom gun with Xe gas at 8 kV voltage and 1 mA current, matrix NBA or thioglycerol. The elemental analysis was carried out with Carlo Erba Mod. 1106 equipped with Eager 200 computer. All commercially available solvents and reagents were used without further purification. Solvent removal was accomplished by a rotary evaporator operating at house vacuum (40~50 Torr). HPLC analyses were carried out with a Varian Star 9010 solvent delivery system equipped with a Varian Star 9065 Polychrom diode array detector. Detection: 254 nm and/or 306 nm. Column: Lichrocart 125-4 (Merck)—Lichrospher 100 RP18 (5 μm). Mobile phase A: 3.15 g/liter of ammonium formate; mobile phase B:

Fig. 3. Poly(U)-dependent poly(phe) synthesis inhibition by **2** (MDL 62,935) and derivatives.

▼ **2**, ◆ **3**, ▲ **4**, ○ **5**, □ **6**.

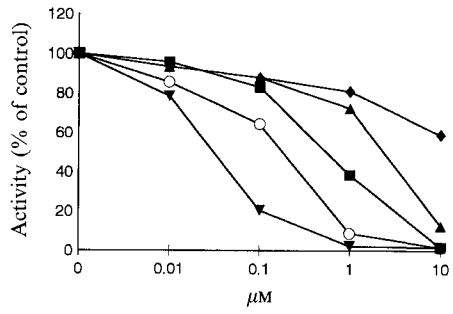


Table 2. Antibacterial activity of **2** (MDL 62,935) and of its derivatives at the β-phenylserine residue.

Strain No.	MIC (μg/ml)				
	2	**3**	**4**	**5**	**6**
Staphylococcus aureus Tour	0.13	16	2	2	1
S. epidermidis ATCC 122228	0.13	>128	8	4	4
S. haemolyticus clin. isolate	0.5	>128	16	8	8
Streptococcus pyogenes C203	2	64	>128	16	8
S. pneumoniae UC41	1	16	32	16	8
Enterococcus faecalis ATCC 7080	0.13	>128	1	1	2
E. coli SKF 12140	>128	>128	>128	>128	>128

acetonitrile. Flow-rate: 0.7 ml/minute. Gradient profile: time (minute) 0 (B 37%), 20 (B 50%), 30 (B 80%).

Preparation of Compound **2**

As previously described,[2] overnight treatment of compound **1** with dioxane - water - formic acid (10 : 1 : 1) at 80°C produced ester **1a** in 81% yield.

In turn, compound **1a** (2 g, 1.55 mmol) in dioxane (30 ml) was hydrolyzed by an overnight treatment at room temperature with 1 N NaOH (2.32 ml, 2.32 mmol). Acidification of the solution to pH 3 with 1 N HCl and precipitation with water yielded a whitish solid. Purification by flash chromatography on silica gel 60 ($CHCl_3$ - MeOH 9 : 1) produced 1.57 g (90%) of **2** as a white powder. HPLC Rt (7.5 minute); FAB-MS *m/z* 1125 (MH^+, 100%); ^1H NMR (DMSO-d_6, 600 MHz): δ 9.01 (d, J=7.3 Hz, pheser-NH), 8.69 [m, asn-αNH, val-NH, th(A)-H], 8.61 [s, th(F)-H], 8.44 (m, gly-NH), 8.43 [d, J=8.1 Hz, py(4)-H], 8.30 [s, th(B)-H], 8.29 [d, J=8.1 Hz, py(5)-H], 7.38 ~ 7.21 (m, 5H phenyl, asn-ΔNH), 7.36 [s, th(C)-H], 6.02 (d, J=4.5 Hz, OH), 5.30 (m, asn-α), 5.24 (t, J=7.3 Hz, pheser-α), 5.20 (dd, J=8.1 Hz, J'=4.7 Hz, val-α), 5.00 (dd, J=7.3 Hz, J'=4.5 Hz, pheser-β), 4.98 [s, th(E)-CH_2], 4.28 (dd, J=17.0 Hz, J'=8.8 Hz, gly-α), 3.79 (dd, J=17.0 Hz, J'=3.7 Hz, gly-α'), 3.39 [s, th(E)-OCH_3], 2.71 (dd, J=16.5 Hz, J'=3.6 Hz, asn-β), 2.59 [s, th(D)-CH_3], 2.48 (d, J=4.7 Hz, asn-εCH_3), 2.17 (m, val-β), 1.28 (br d, asn-β'), 0.88 (d, J=7.0 Hz, val-γ), 0.85 (d, J=7.0 Hz, val-γ').

Anal Calcd for $C_{48}H_{44}N_{12}O_9S_6$:
C 51.23, H 3.94, N 14.93
Found:
C 51.27, H 4.02, N 14.94

Preparation of Compound **3**

Compound **3** was prepared and characterized as previously described[15] upon treatment of ester **1a** with cesium carbonate in water/dioxane at 80°C for 65 hours.

Preparation of Compound **4**

A solution of **2** (100 mg, 0.089 mmol) in acetic anhydride (0.5 ml) and 33% HBr in acetic acid (0.5 ml) was stirred at room temperature. In a few minutes, HPLC analysis revealed the reaction to be completed and the solution was poured into ice-water. The precipitate that formed was filtered, washed over the filter with more water and then allowed to dry in air to yield 103.7 mg (100%) of **4** as a white powder. HPLC Rt (10.4 minutes); FAB-MS *m/z* 1167 (MH^+, 100%); ^1H NMR (DMSO-d_6, 500 MHz): δ 9.21 (d, J=7.6 Hz, pheser-NH), 8.80 (d, J=8.3 Hz, asn-αNH), 8.69 [s, th(A)-H], 8.62 (d, J=8.1 Hz, val-NH), 8.59 [s, th(F)-H], 8.43 [m, gly-NH, py(4)-H], 8.30 [m, th(B)-H, py(5)-H], 7.43 [s, th(C)-H], 7.41 (m, asn-ΔNH), 7.35 (m, 5H phenyl), 6.15 (d, J=7.6 Hz, pheser-β), 5.48 (t, J=7.6 Hz, pheser-α), 5.30 (m, asn-α), 5.20 (dd, J=8.1 Hz, J'=5.0 Hz, val-α), 4.98 [s, th(E)-CH_2], 4.27 (dd, J=16.8 Hz, J'=8.5 Hz, gly-α), 3.74 (dd, J=16.8 Hz, J'=3.6 Hz, gly-α'), 3.39 [s, th(E)-OCH_3], 2.69 (dd, J=16.5 Hz, J'=3.6 Hz, asn-β), 2.60 [s, th(D)-CH_3], 2.47 (d, J=4.5 Hz, asn-εCH_3), 2.19 (m, val-β), 1.98 (s, $OCOCH_3$), 1.52 (br d, asn-β'), 0.89 (d, J=6.8 Hz, val-γ), 0.85 (d, J=6.8 Hz, val-γ').

Anal Calcd for $C_{50}H_{46}N_{12}O_{10}S_6$:
C 51.45, H 3.94, N 14.40
Found:
C 50.98, H 3.86, N 14.29

Preparation of Compound **5**

To a stirred solution of **2** (300 mg, 0.27 mmol) in dichloromethane (10 ml) and TEA (376 μl, 2.7 mmol) cooled at 0°C, methansulfonic anhydride (235 mg, 1.35 mmol) was slowly added. At the end of the addition, the ice cooling bath was removed and stirring was continued at room temperature. HPLC analysis of the reaction mixture revealed the presence of about 50% starting material **2** even after 16 hours. Further addition of TEA (376 μl, 2.7 mmol) and methansulfonic anhydride (235 mg, 1.35 mmol) did not make the reaction proceed any further as revealed by the HPLC chromatogram. The reaction mixture was then diluted with more dichloromethane (10 ml) and washed three times with water (10 ml). The organic phase was dried with anhydrous Na_2SO_4 and then the solvent evaporated to dryness *in vacuo*. The residual syrup was chromatographed on preparative silica gel TLC plates (CH_2Cl_2 - MeOH 9 : 1) to yield 62 mg (21%) of **5** as a white powder. HPLC Rt (15.2 minutes); FAB-MS *m/z* 1107 (MH^+, 100%); ^1H NMR (DMSO-d_6, 600 MHz): δ 8.99 (d, J=8.2 Hz, asn-αNH), 8.64 [m, val-NH, th(A)-H], 8.58 [s, th(F)-H], 8.39 [d, J=8.1 Hz, py(4)-H], 8.30 [m, gly-NH, py(5)-H], 7.80 (m, asn-ΔNH), 7.53 [s, th(C)-H], 7.49 ~ 7.41 (m, 5H phenyl), 5.90 (d, J=7.5 Hz, pheser-β), 5.43 (d, J=7.5 Hz, pheser-α), 5.39 (m, asn-α), 5.18 (dd, J=8.3 Hz, J'=6.4 Hz, val-α), 5.02 [s, th(E)-CH_2], 4.51 (dd, J=17.1 Hz, J'=6.3 Hz, gly-α), 4.23 (dd, J=17.1 Hz, J'=4.7 Hz, gly-α'), 3.41 [s, th(E)-OCH_3], 2.72 (dd, J=16.0 Hz, J'=4.9 Hz, asn-β), 2.63 (br d, asn-β'), 2.53 [s, th(D)-CH_3], 2.43 (d, J=4.6 Hz, asn-εCH_3), 2.24 (m, val-β), 0.94 (d, J=6.8 Hz, val-γ), 0.91 (d, J=6.8 Hz, val-γ').

Anal Calcd for $C_{48}H_{42}N_{12}O_8S_6$:
C 52.07, H 3.79, N 15.18
Found:
C 51.60, H 3.74, N 15.06

Preparation of Compound **6**

Neat thionyl chloride (16 ml) was added to **2** (1 g, 0.89 mmol) and the resulting solution was stirred for 3 hours at room temperature. At this time, HPLC analysis revealed the completion of the reaction. The solution was concentrated to a smaller volume and ethyl ether was added to induce precipitation of a yellow solid which was filtered, washed over the filter with more ethyl ether and allowed to dry in air to yield 1.015 g (100%) of **6** as a yellow powder. HPLC Rt (14.7 minutes); FAB-MS *m/z* 1143 (MH^+, 100%); ^1H NMR (DMSO-d_6, 600 MHz): δ

9.13 (d, J=8.9 Hz, pheser-NH), 8.79 (d, J=8.5 Hz, asn-αNH), 8.69 [s, th(A)-H], 8.61 [s, th(F)-H], 8.58 (d, J=8.1 Hz, val-NH), 8.45 (m, gly-NH), 8.44 [d, J=8.1 Hz, py(4)-H], 8.35 [s, th(B)-H], 8.30 [d, J=8.1 Hz, py(5)-H], 7.50~7.39 [m, 5H phenyl, asn-ΔNH, th(C)-H], 5.72 (t, J=8.9 Hz, pheser-α), 5.54 (d, J=8.9 Hz, pheser-β), 5.34 (m, asn-α), 5.20 (dd, J=8.1 Hz, J'=4.8 Hz, val-α), 4.97 [d, J=14.7 Hz, th(E)-CH], 4.94 [d, J=14.7 Hz, th(E)-CH'], 4.08 (dd, J=16.8 Hz, J'=8.6 Hz, gly-α), 3.62 (dd, J=16.8 Hz, J'=3.8 Hz, gly-α'), 3.38 [s, th(E)-OCH_3], 2.69 (dd, J=16.5 Hz, J'=3.6 Hz, asn-β), 2.17 (m, val-β), 1.60 (br d, asn-β'), 0.86 (d, J=6.8 Hz, val-γ), 0.84 (d, J=6.8 Hz, val-γ').

Anal Calcd for $C_{48}H_{43}ClN_{12}O_8S_6$:
C 50.43, H 3.76, N 14.71
Found:
C 50.07, H 3.72, N 14.67

Antimicrobial Activity

Minimal inhibitory concentrations (MIC) were determined by broth microdilution methodology. Inocula were 10^4 CFU/ml. Media used were: Oxoid Iso-Sensitest broth for staphylococci, *E. faecalis* and *E. coli* and Difco Todd-Hewitt broth for streptococci.

Cell-free Protein Synthesis

The cell-free protein synthesis assay was performed as poly(U)-directed polyphenylalanine synthesis using ribosomes and protein synthesis factors from *E. coli* K12 HfrC as described by TRAUB *et al.*[16] Assays were performed as previously described.[11]

Acknowledgments

The authors are indebted to P. FERRARI, L. COLOMBO and M. NEBULONI for the analytical characterization of the compounds.

References

1) SELVA, E.; G. BERETTA, N. MONTANINI, G. S. SADDLER, L. GASTALDO, P. FERRARI, R. LORENZETTI, P. LANDINI, F. RIPAMONTI, B. P. GOLDSTEIN, M. BERTI, L. MONTANARO & M. DENARO: Antibiotic GE2270A: A novel inhibitor of bacterial protein synthesis. I. Isolation and characterization. J. Antibiotics 44: 693~701, 1991
2) TAVECCHIA, P.; P. GENTILI, M. KURZ, C. SOTTANI, R. BONFICHI, E. SELVA, S. LOCIURO, E. RESTELLI & R. CIABATTI: Degradation studies of antibiotic MDL 62,879 (GE2270A) and revision of the structure. Tetrahedron 51: 4867~4890, 1995
3) STELLA, S.; N. MONTANINI, F. LE MONNIER, P. FERRARI, L. COLOMBO, F. MARINELLI, P. LANDINI, I. CICILIATO, B. P. GOLDSTEIN, E. SELVA & M. DENARO: Antibiotic GE37468A: A new inhibitor of bacterial protein synthesis. I. Isolation and characterization. J. Antibiotics 48: 780~786, 1995
4) SHINAMAKA, K.; N. KINOSHITA, H. IINUMA, M. HAMADA & T. TAKEUCHI: Novel antibiotics, amythiamicin. I. Taxonomy, fermentation, isolation, physico-chemical properties, and antimicrobial activity. J. Antibiotics 47: 668~674, 1994
5) SHINAMAKA, K.; Y. TAKAHASHI, H. IINUMA, H. NAGANAWA & T. TAKEUCHI: Novel antibiotics, amythiamycin. III. Structure elucidation of amythiamicins A, B and C. J. Antibiotics 47: 1153~1159, 1994
6) LANDINI, P.; M. BANDERA, B. P. GOLDSTEIN, F. RIPAMONTI, A. SOFFIENTINI, K. ISLAM & M. DENARO: Inhibition of bacterial protein synthesis by elongation-factor-Tu-binding antibiotics MDL 62,879 and efrotomycin. Biochem. J. 283: 649~652, 1992
7) ANBORGH, P. H. & A. PARMEGGIANI: New antibiotic that acts specifically on the GTP-bound form of elongation factor Tu. EMBO J. 10: 779~784, 1991
8) SHIMANAKA, K.; H. IINUMA, M. HAMADA, S. IKENO, K. S.-TSUCHIYA, M. ARITA & M. HORI: Novel antibiotics, amythiamicins. IV. A mutation in the elongation factor Tu gene in a resistant mutant of *B. subtilis*. J. Antibiotics 48: 182~184, 1995
9) PARMEGGIANI, A. & G. W. M. SWART: Mechanism of action of kirromycin-like antibiotics. Ann. Rev. Microbiol. 39: 557~577, 1985
10) WOLF, H.; D. ASSMANN & E. FISCHER: Pulvomycin, an inhibitor of protein biosynthesis preventing ternary complex formation between elongation factor Tu, GTP, and aminoacyl-tRNA. Proc. Natl. Acad. Sci. USA 75: 5324~5328, 1978
11) LANDINI, P.; M. BANDERA, A. SOFFIENTINI & B. P. GOLDSTEIN: Sensitivity of elongation factor Tu (EF-Tu) from different bacterial species to the antibiotics efrotomycin, pulvomycin and MDL 62879. J. Gen. Microbiol. 139: 769~774, 1993
12) GOLDSTEIN, B. P.; M. BERTI, F. RIPAMONTI, A. RESCONI, R. SCOTTI & M. DENARO: *In vitro* antimicrobial activity of a new antibiotic, MDL 62,879 (GE2270A). Antimicrob. Agents Chemother. 37: 741~745, 1993
13) KING, A.; L. BETHUNE & I. PHILLIPS: *In vitro* activity of MDL 62,879 (GE2270A) against aerobic gram-positive and anaerobic bacteria. Antimicrob. Agents Chemother. 37: 746~749, 1993
14) BERTI, M.; G. CANDIANI, B. P. GOLDSTEIN & M. DENARO: Experimental streptococcal endocarditis in the rat. Activity of various antibiotics. Zentralbl. Bakteriol. Int. J. Med. Microbiol. (Suppl.) 22: 442~443, 1992
15) TAVECCHIA, P.; M. KURZ, L. COLOMBO, R. BONFICHI, E. SELVA, S. LOCIURO, E. MARZORATI & R. CIABATTI: Chemical modification of the GE2270A macrocycle (MDL 62,879). Tetrahedron 52: 8763~8774, 1996
16) TRAUB, P.; S. MIZUSHIMA, C. V. LOWRY & M. NOMURA: Reconstitution of ribosomes from subribosomal components. Methods in Enzymology 20: 306~316, 1971