Structural Assignment of the Peptide Antibiotic LP237-F8, a Metabolite of *Tolypocladium geodes*

Youla S. Tsantrizos,*,† Sotiria Pischos,† and Françoise Sauriol‡

Department of Chemistry and Biochemistry, Concordia University, Montréal, Québec H3G 1M8, and Department of Chemistry, McGill University, Montréal, Québec H3A 2K6, Canada

Received October 12, 1995 (Revised Manuscript Received December 14, 1995⊗)

Antibiotic LP237-F8 is the main cytotoxic metabolite isolated from the liquid cultures of the fungus *Tolypocladium geodes*. Complete ^{1}H and ^{13}C NMR resonance assignments and sequencing of the peptide was achieved by extensive high-field 2D NMR spectroscopy. The N- and C-terminals of LP237-F8 are protected with a capryloyl unit and the amino alcohol leucinol, respectively. This linear peptide is a new member of the peptaibol class of natural products, containing the unusual amino acids α-aminoisobutyric acid (Aib) and α-amino-α-ethyl-*n*-pentanoic acid (α-ethylnorvaline, α-EtNor).

Introduction

In the course of our screening program for novel fungal metabolites exhibiting antitumor activity, the *Tolypocladium geodes* isolate LP237 was found to produce metabolites exhibiting high levels of cytotoxicity and antibacterial activity. This isolate of *T. geodes* was originally found in soil samples collected at the Pennine mountains of England at an altitude of 600 m.[1] Fungi of the *Tolypocladium* genus are commonly found in cold climates; these include the well known species of *T. cylindrosporum* and *T. niveum*. The *T. geodes* species is a much more rare organism and very sensitive to warm temperatures.[2] In the past, several biologically active peptides have been isolated from *Tolypocladium* fungi, including the cyclosporins,[3] the efrapeptins,[4] and elvapeptins.[5] This report describes the novel peptaibol metabolite LP237-F8 (**1**), isolated from the liquid culture of *T. geodes*. The structural assignment of **1** was based on amino acid analysis, mass spectral and IR data, and extensive investigation by 1D and 2D NMR.

Results and Discussion

A bioassay-guided purification scheme was developed for the isolation of several extracellular cytotoxic peptides produced by *T. geodes* in liquid culture. The presence of these metabolites in fractions collected from each purification step was identified using the SOS Chromotest,[6] a bacterial colorimetric assay which is widely used as a reliable test for the detection of genotoxic compounds (DNA-damaging compounds). The crude metabolite mixture of *T. geodes* was partitioned by Diaion HP 20 resin chromatography, followed by size exclusion chromatography on Sephadex LH-20 and finally by reversed phase flash column chromatography.[7] This led to the isolation of more than 10 cytotoxic peptides,[8] exhibiting an IC_{50} value in the range of 0.5−50 μg/mL on P388D1 murine leukemia cells. The structural identity of the main component, metabolite LP237-F8 (**1**), has now been determined and those of the remaining peptides is currently in progress.[8]

Both MALDI and FAB MS of metabolite **1** suggested a molecular formula of $C_{65}H_{108}N_{14}O_{15}$ [MALDI MS *m*/*z* 1348.4 $(M + Na)^+$, FAB MS (NBA + NaCl matrix) *m*/*z* 1347.74 $(M + Na)^+$, calculated mass for $C_{65}H_{108}N_{14}O_{15}$-Na 1347.80]. A strong absorption at 1649 cm^{-1}, together with an absorption at 3436 and 1541 cm^{-1}, in the IR spectrum suggested the presence of a peptide linkage along with an intermolecularly hydrogen-bonded alcohol (3329 cm^{-1}) or a primary amide. The presence of 14 amide or ester moieties was confirmed by ^{13}C NMR (125 MHz). Complete acid hydrolysis and amino acid analysis[9] of LP237-F8 (**1**) indicated the presence of Phe, Ala, Pro, and Glx in a ratio of 1:1:1:3. Metabolite **1** was resistant to methylation with diazomethane and gave a negative ninhydrin test, suggesting the absence of free carboxyl and amino functionalities. The ^{1}H NMR (500 MHz) of **1** in DMSO-d_6 (Table 1) clearly showed the presence of 16 NH resonances in the downfield region of

† Concordia University.
‡ McGill University.
* Author to whom correspondence may be addressed: Department of Chemistry and Biochemistry, Concordia University, 1455 de Maisonneuve Blvd West, Montréal, Québec H3G 1M8, Canada. Tel: (514) 848-3335, FAX: (514) 848-2868, e-mail: Youla@Vax2.Concordia.ca.
⊗ Abstract published in *Advance ACS Abstracts,* March 1, 1996.

(1) (a) *T. geodes* isolate LP237 was originally isolated by P. Widden in 1982; Widden, P. *Mycologia* **1987**, *79*, 298. (b) The culture was deposited at the Biosystematics Research Institute (Ottawa, Canada) and its taxonomic identity was confirmed by J. Bissett, Bissett, J. *Can. J. Bot.* **1983**, *61*, 1311.

(2) Dowding, P.; Widden, P. In *Soil Organisms and Decomposition in Tundra*; Holding, A. J., Ed.; Tundra Biome Streering Committee: Stockholm, 1974; pp 123−150.

(3) Review: Ovchinnikov, Y. A.; Ivanov, V. T. The Cyclic Peptides: Structure, Conformation, and Function. In *The Proteins*; Neurath, H., Hill, R. L., Eds.; Academic Press: New York, erd ed. 1982; Vol. V, pp 547−555.

(4) (a) Jackson, C. G.; Linnett, P. E.; Beechey, R. B.; Henderson, P. J. F. *Biochem. Soc. Trans.* **1979**, *7*, 224. (b) Bullough, D. A.; Jackson, C. G.; Henderson, P. J. F.; Cottee, F. H.; Beechey, R. B.; Linnett, P. E. *Biochem. Int.* **1982**, *4*, 543. (c) Gupta, S.; Krasnoff, S. B.; Roberts, D. W.; Renwick, J. A. A. *J. Am. Chem. Soc.* **1991**, *113*, 707. (d) Krasnoff, S. B.; Gupta, S.; St. Leger, R. J.; Renwick, J. A. A.; Roberts.D. W. *J. Invertebr. Path.* **1991**, *58*, 180. (e) Gupta, S.; Krasnoff, S. B.; Roberts, D. W.; Renwick, J. A. A.; Brinen, L. S.; Clardy, J. *J. Org. Chem.* **1992**, *57*, 2306.

(5) Bullough, D. A.; Jackson, C. G.; Henderson, P. J. F.; Beechey, R. B. ; Linnett, P. E. *FEBS Lett.* **1982**, *145*, 258.

(6) Review: Quillardet, P.; Hofnung, M. *Mutat. Res.* **1993**, *297*, 235.

(7) (a) Blunt, J. W.; Calder, V. L.; Fenwick, G. D.; Lake, R. J.; McCombs, J. D.; Munro, M. H. G.; Perry, N. B. *J. Nat. Prod.* **1987**, *50*, 290. (b) Tsantrizos, Y. S.; Ogilvie, K. K.; Watson, A. K. *Can. J. Chem.* **1992**, *70*, 2276.

(8) Tsantrizos, Y. S.; Pischos, S.; Sauriol, F.; Widden, P. *Can. J. Chem.* **1996**, *74*, in press.

(9) Spackman, D. H.; Stein, W. H.; Moore, S. *Anal. Chem.* **1958**, *30*, 1190.

0022-3263/96/1961-2118$12.00/0

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 1 in DMSO-d_6

	assignment	^{13}C (δ)	^{1}H (δ)	int, mult, J (Hz)
^{1}Lol	α	48.38	3.772	1H, m
	β	39.80[a]	1.32[b]	2H, m
	γ	24.00	1.57[b]	1H, m
	δ	23.34 & 21.53	0.775 & 0.793	2 × 3H, d, J = 6.3, J = 6.8
	CH_2-OH	64.11	3.124 & 3.244	2H, m
	OH		4.411	1H, t, J = 5.86
	NH		6.655	1H, d, J = 9.3 Hz
^{2}Ala	α	49.29	4.015	1H, m
	β	17.19	1.259	3H, d, J = 7.3
	CO	172.2[c]		
	NH		7.526	1H, d, J = 7.3
^{3}Gln	α	54.38	3.817	1H, m
	β	26.28	1.99[b] & 1.93[b]	2H, m
	γ	31.79	2.30[b] & 2.19[b]	2H, m
	δ CO	174.0[c]		
	ϵNH_2		6.759 & 7.163	2H, br s
	CO	172.2[c]		
	NH		7.575	1H, d, J = 5.4
^{4}EtNor	α	58.74		
	β	~26[a]	1.31[b]	2H, m
	γ	28.66	1.18[b]	2H, m
	δ	13.91	0.80[b]	3H, t, J = 6.8
	CO	176.8[c]		
	NH		7.447	1H, s
	β'	26.09	2.19 & 1.63[b]	2H, m
	γ'	7.02	0.696	3H, t, J = 7.5
^{5}Aib	α	56.07		
	β	22.74	1.287	3H, s
	β	26.04	1.347	3H, s
	CO	175.5[c]		
	NH		7.925	1H, s
^{6}Gln	α	55.04[d]	3.933	1H, m
	β	26.09	1.97[b]	2H, m
	γ	31.38[e]	2.19[b] & 2.10[b]	2H, m
	δ CO	174.0[c]		
	ϵ NH_2		6.802 & 7.266	2H, br s
	CO	173.6[c]		
	NH		7.785	1H, d, J = 6.6
^{7}Gln	α	55.18[d]	3.899	1H, dt, J = 5.9,8.3
	β	26.09	1.91[b]	2H, m
	γ	31.46[e]	2.19[b]	2H, m
	δ CO	174.0[c]		
	ϵ NH_2		6.819 & 7.275	2H, br s
	CO	175.9[c]		
	NH		7.748	1H, d, J = 5.4
^{8}Aib	α	55.94[f]		
	β	22.63	1.347	3H, s
	β	26.36	1.466	3H, s
	CO	175.7[c]		
	NH		7.580	1H, s
^{9}Phe	α	55.29	4.351	1H, ddd, J = 11.2, 8.3, 4.9
	β	34.73	3.21[b] & 2.88	2H, m & dd, J = 13.7, 11.2
	1	137.61		
	2,6	128.87	7.180	2H, m
	3,5	128.13	7.22[b]	2H, m
	4	126.34	7.16	1H, m
	CO	175.9[c]		
	NH		7.805	1H, d, J = 8.3
^{10}Pro	α	62.52	4.191	1H, t, J = 7.5
	β	28.10	2.015, 1.216	2H, m
	γ	25.28	1.637, 1.731	2H, m
	δ	48.11	3.699, 3.278	2H, m
	CO	172.9[c]		
^{11}Aib	α	55.81[f]		
	β	23.54	1.314	3H, s
	β	25.85	1.398	3H, s
	CO	173.3[c]		
	NH		8.786	1H, s
^{12}Oc	CO-1′	174.2[c]		
	2′	35.93	2.26	2H, m
	3′	25.11	1.55	2H, m
	4′	28.81	1.22	2H, m
	5′	28.59	1.22	2H, m
	6′	31.20	1.17	2H, m
	7′	22.03	1.18	2H, m
	8′	13.91	0.81	3H, ot

[a] Due to overlapping ^{13}C resonances, these chemical shifts were assigned based on the DEPT NMR data. [b] Due to overlapping resonances in ^{1}H NMR spectrum, the exact chemical shift for each proton could not be determined; the values given were obtained from the HMQC or HMBC data. [c] Chemical shift assignment was based on the HMBC NMR data. [d]–[f] These chemical shifts could be interchanged.

the spectrum which are exchangeable in CD_3OD (NH δ 6.6−8.8, overlapping aromatic protons of Phe δ 7.16−7.22). Chemical shift assignments for each amino acid unit were based on the combined ^1H, ^{13}C, COSY, DEPT, HMQC, and HMBC NMR data (Table 1). For example, coupling of the α-H to the NH resonance of Phe (δ 4.35 to 7.81), Ala (δ 4.02 to 7.53), ^{6}Glx (δ 3.93 to 7.79), ^{7}Glx (δ 3.90 to 7.75), ^{3}Glx (δ 3.82 to 7.57), and leucinol (Lol, δ 3.77 to 6.65) were clearly observed in the COSY spectrum. Three other NH resonances at δ 6.76, 6.80, and 6.82 were observed which were coupled to those at δ ~7.16, 7.27 and 7.27, respectively, and showed positive NOE effects on the γ-CH_2 resonances of the Glx units. Thus, these resonances were assigned to ϵNH_2 moieties of Gln, establishing the identity of the three Glx units of LP237-F8 (**1**). Further analyses of the NMR data of **1** revealed the presence of one amino alcohol unit, leucinol (Lol), and three units of the unusual amino acid α-aminoisobutyric acid (Aib); both are commonly found in peptaibol metabolites.

^{6}Gln — ^{5}Aib — ^{4}EtNor — ^{3}Gln — ^{2}Ala — ^{1}Lol
|
^{7}Gln — ^{8}Aib — ^{9}Phe — ^{10}Pro — ^{11}Aib — ^{12}Oc

(**1**)

Based on the amino acid analysis and the overall MS and NMR spectral data of LP237-F8 (**1**), the unequivocal assignment of most amino acid units was achieved. The structural identity of the novel α-ethylnorvaline unit (EtNor, α-amino-α-ethyl-*n*-pentanoic), and the octyl fatty acid chain (Oc) proved to be the more challenging aspect of the structural assignment of metabolite **1**. Initially, two plausible structures were considered: (1) the structure containing an EtNor and an Oc chain as shown in **1** and (2) an alternate structure having an isovaline amino acid in the place of the EtNor unit and a decanoyl fatty acid in the place of Oc. However, the alternate structure was rejected after further analysis of the NMR data. For example, the ^{1}H NMR of LP237-F8 clearly showed a total of five *shielded* methyl groups: four methyls between δ 0.76−0.81 (assigned to the 2 δ-CH_3 of Lol, the C8-CH_3 of Oc, and the δ-CH_3 of EtNor) and a fifth methyl at δ 0.696 (assigned to the γ'-CH_3 of EtNor). The expected chemical shift of a methyl group attached to an α-carbon, as in the case of isovaline, would be at δ ~1.3−1.4, whereas that of metabolite **1** is at δ ~0.8, consistent with the presence of the *n*-propyl group in EtNor. The methyl group of the fatty acid chain (C8-Oc) and the δ-CH_3 of EtNor nearly overlap at δ ~0.80, and they are both coupled to methylene protons at δ 1.20−1.25; a chemical shift of δ ~1.25 was assigned to the γ-CH_2 of EtNor based on the COSY and NOESY NMR data. The HMBC spectrum of **1** shows clear correlation between the γ'-CH_3 and its neighboring methylene carbon (β'-C) at δ 26.09, as well as the quaternary α-carbon at δ 58.74. In addition, the HMBC spetrum showed correlation between the β'-CH_2, the quaternary α-carbon (δ 58.74), and the β-CH_2 at δ 23.4. Due to extensive overlap of signals in the HMBC spectrum, the correlations between the β, γ, and δ proton and carbon resonances could not be clearly assigned. However, neither of the two methyls at δ ~0.8 show a correlation to the α-quaternary carbon at δ 58.74, which strongly suggests (but does not prove) that those two methyls are located on carbon chains longer than a $(CH_2)_2$ unit.

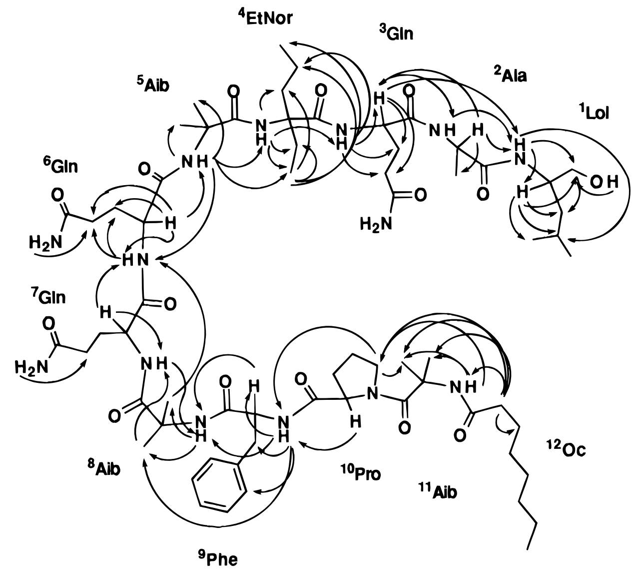

Figure 1. Structure of LP237-F8 (**1**) and NOEs observed in the NOESY NMR experiment.

Although distinct HMQC and HMBC NMR correlations between the protons and carbons of the propyl chain were somewhat difficult to confirm due to extensive overlapping of signals, the observed data was consistent with the proposed presence of an EtNor unit, located between ^{3}Gln and ^{5}Aib. Careful examination of the NOESY data associated with the EtNor unit showed NOE correlations between the δ-CH_3 and the γ'-CH_3, further suggesting that these two shielded methyls are part of the same amino acid. The presence of NOE correlations between the γ'-CH_3 of EtNor and its neighboring protons of the β-CH_2 (δ 1.31), β'-CH_2 (δ 1.63 and 2.19), and δ-CH_3 (δ 0.80) were clearly observed. Finally, the EtNor-NH (δ 7.45) displays NOE interactions with neighboring NH groups (^{3}Gln and ^{5}Aib), the α-H of ^{3}Gln, and some of the other protons within the EtNor unit. Thus, the structure of an α-amino-α-ethyl-*n*-pentanoic acid unit (α-ethylnorvaline, EtNor) was confirmed by the combined NMR data. This amino acid is exceedingly rare, found only in extraterrestrial sediments such as the Murchison meteorite.[10] It has also been synthesized and shown to be a competitive inhibitor of methionine.[11] To our knowledge, EtNor has not been previously reported as a constituent of a natural product.

Finally, the presence of an octanoyl (Oc) moiety at the N-terminal of the peptide was confirmed by the NMR data. DEPT NMR analysis of **1** indicated the presence of 17 CH_2 [including the CH_2OH of ^{1}Lol (δ 64.11)] and 11 CH_3 carbons; the chemical shifts of the overlapping ^{13}C resonances (CH_2 resonances of Oc overlapping at δ 1.17−1.22) were identified from the HMQC and HMBC experiments.

The sequence assignment of the peptide backbone was based primarily on the NOESY NMR data (Figure 1). Strong NOE effects were observed between amide protons and the α-protons of neighboring residues and also between amide protons and the side chains of the neighboring amino acids (β-, γ-, and δ-protons) (Figure 1). Furthermore, the fragmentation ions observed by FAB MS and ES MS/MS were consistent with the proposed structure of metabolite **1**.

(10) Cronin, J. R.; Pizzarello, S. *Geochim. Cosmochim. Acta* **1986**, *50*, 2419.
(11) Abshire, C. J.; Planet, G. *J. Med. Chem.* **1972**, *15*, 226.

Linear peptides containing a C-terminal amino alcohol, Lol,[12] Phol (phenylalaninol),[13] or Trpol (tryptophanol)[14] and a high proportion of the unusual amino acid Aib[15] belong to the class of natural products known as peptaibols. The *Trichoderma* metabolites trichorzianine A,[14] trichosporin B,[13b−d] tricholongin BI and BII,[12a] trichogin A IV,[12b] trichodecenins-I and -II[12d], and the alamethicins[13f] are among the best known peptaibols. The zervamicins,[16] emerimicins[7a,16] and hypelcin A,[12c,e] represent examples of peptaibols produced by a few other fungi. The N-terminal amino acid of peptaibols is usually protected by an acetyl group; however, in trichogin A IV[12b] and the trichodecenins-I and II[12d] it is protected with an octanoyl and a *cis*-4-decenoyl group, respectively.

The novel peptide LP237-F8 (**1**) is the first peptaibol to be isolated from the *Tolypocladium* genus and the first bioactive metabolite to be isolated from *T. geodes*. Peptaibols are known to exhibit many unique biological activities, including the formation of voltage-gated ion channels in lipid bilayer membranes,[17,12b] induction of catecholamine secretion from adrenal chromaffin cells,[18] uncoupling of oxidative phosphorylation in mitochondria,[19] and the inhibition of cell multiplication in ameba.[20] The details of the biological properties of LP237-F8 (**1**) are currently under further investigation.

Experimental Section

Isolation of Metabolite 1. Stock cultures of *T. geodes* were maintained on 2% malt extract (Difco) plus 1.5% agar in slant tubes at 4 °C. A small amount of mycelium was used to inoculate 2% malt extract agar plates which were then incubated in the dark, at room temperature for a period of 14 days. Plugs (∼15 plugs/flask) of actively growing mycelium were used to inoculate 2% malt extract liquid medium (6 × 500 mL culture in 3 L flasks). The fermentation flasks were then incubated at 20 °C on a rotary shaker at 120 rpm for 7 days. The mycelium was removed by filtration, and the resulting filtrate was absorbed onto a Diaion HP 20 column (60 mm × 25 cm) which was eluted with a linear gradient from 100% H_2O to 100% MeOH to 100% acetone, at a flow rate of 10 mL/min. The cytotoxic fractions, which eluted from ∼75% aqueous MeOH to 100% MeOH, were combined and evaporated to dryness to give ∼1.5 g of a brown gum. The active crude was subsequently dissolved in 2−5 mL of MeOH, loaded on a Sephadex LH-20 column (25 mm × 100 cm) and partitioned into 90 fractions (∼8 mL each) by eluting with degassed MeOH at a flow rate of 0.7 mL/min. Fractions 22−30 exhibited strong cytotoxic activity (IC_{50} = 52 ng/mL) and gave a strong positive SOS Chromotest. These fractions were combined, evaporated to dryness (∼230 mg), and further purified by flash column chromatography on a C_{18} reversed phase column (20 mm × 15 cm), using a linear solvent gradient from 100% H_2O to 100% MeOH to 100% CH_2Cl_2 at a flow rate of 2 mL/min. The active fractions were once again combined, evaporated to dryness to obtain ∼40 mg of cytotoxic crude which was further analyzed by semipreparative C_{18} reversed phase HPLC. In a solvent mixture of MeOH:H_2O:MeCN (70:20:10, with 0.1% TFA in all solvent) and a flow rate of 4 mL/min, peptide **1** had a retention time of 23.6 min. Pure metabolite **1** was isolated as an amorphous white solid (∼4 mg) after a secong purification by HPLC under the same conditions. UV (MeOH, nm): max 226. $[\alpha]_D$ +9.88 (*c* 0.0016, MeOH). NMR data are given in Table 1; IR and MS data are reported in the discussion.

Acknowledgment. This work was supported by grants to Y.S.T. from the Natural Sciences and Engineering Research Council of Canada and le Fonds pour la Formation de Chercheurs et l'Aide à la Recherche du Québec, Établissement de Nouveaux Chercheurs. We are grateful to Dr. Orvil A. Mamer and Dr. Daniel Boismenu of the Biomedical Mass Spectrometry Unit for assistance with the MS analysis. We also wish to thank Mr. Nick Plavac, Chemistry Department, University of Toronto, for recording the ^{13}C and DEPT (125-MHz) NMR spectra. Finally, we thank Mr. Bernard Gibbs, Biotechnology Research Institute, NRC, for the amino acid analysis.

Supporting Information Available: ^1H (500 MHz), ^{13}C (125 MHz), DEPT, COSY, NOESY, HMQC, and HMBC NMR spectra of **1** in DMSO-d_6 (103 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO951837T

(12) (a) Rebuffat, S.; Prigent, Y.; Auvin-Guette, C.; Bodo, B. *Eur. J. Biochem.* **1991**, *201*, 661. (b) Auvin-Guette, C.; Rebuffat, S.; Prigent, Y.; Bodo, B. *J. Am. Chem. Soc.* **1992**, *114*, 2170. (c) Matsura, K.; Yesilada, A.; Iida, A.; Takaishi, Y.; Kanai, M.; Fujita, T. *J. Chem. Soc. Perkin Trans. 1* **1993**, 381. (d) Fujita, T.; Wada, S.; Iida, A.; Nishimura, T.; Kanai, M.; Toyama, N. *Chem. Pharm. Bull.* **1994**, *42*, 489. (e) Fujita, T.; Takaishi, Y. Shiromoto, T. *J. Chem. Soc., Chem. Commun.* **1979**, 413.

(13) (a) Pandey, R. C.; Cook, J. C., Jr.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 5205. (b) Fujita, T.; Iida, A,; Uesato, S.; Takaishi, Y.; Shingu, T.; Saito, M.; Morita, M. *J. Antibiot.* **1988**, *41*, 814. (c) Iida, A.; Okuda, M.; Uesato, S.; Takaishi, Y.; Shingu, T.; Morita, M.; Fujita, T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 3249. (d) Nagaoka, Y.; Iida, A,; Fujita, T *Chem. Pharm. Bull.* **1994**, *42*, 1258. (e) Huang, Q.; Tezuka, Y.; Kikuchi, T.; Nishi, A.; Tubaki, K.; Tanaka, K. *Chem. Pharm. Bull.* **1995**, *43*, 223. (f) Pandey, R. C.; Cook, J. C., Jr.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 8469.

(14) Bodo, B.; Rebuffat, S.; El Hajji, M.; Davoust, D. *J. Am. Chem. Soc.* **1985**, *107*, 6011.

(15) Shaw, I. M.; Taylor. A. *Can. J. Chem.* **1986**, *64*, 164.

(16) (a) Argoudelis, A. D.; Dietz, A.; Johnson, L. E. *J. Antibiot.* **1974**, *27*, 321. (b) Rinehart, K. L., Jr.; Gaudioso, L. A.; Moore, M. L.; Pandey, R. C.; Cook, J. C., Jr. *J. Am. Chem. Soc.* **1981**, *103*, 6517.

(17) (a) Fox, R. O., Jr.; Richards, F. M. *Nature* **1982**, *300*, 325. (b) Menestrina, G.; Voges, K. P.; Jung, G.; Boheim, G. *J. Mem. Biol.* **1986**,*93*, 111. (c) Rebuffat, S.; Duclohier, H.; Auvin-Guette, C.; Molle, G.; Spach, G.; Bodo, B. *FEMS Microbiol. Immunol.* **1992**, *105*, 151. (d) Sansom, M. S. P. *Eur. Biophys. J.* **1993**, *22*, 105.

(18) (a) Artalejo, A. R.; Montiel, C.; Sanchez-Garcia, P.; Uceda, G.; Guantes, J. M.; Garcia, A. G. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 1204. (b) Tachikawa, E.; Takahashi, S.; Furumachi, K.; Kashimoto, T.; Iida, A.; Nagaoka, Y.; Fujita, T.; Takaishi, Y. *Mol. Pharmacol.* **1991**, *40*, 790. (c) Fonteriz, R. I.; Lopez, M. G.; Garcia-Sancho, J.; Garcia, A. G. *FEBS Lett.* **1991**, *283*, 89.

(19) (a) Takaishi, Y.; Terada, Y.; Fujita, T. *Experientia* **1980**, *36*, 550. (b) Mathew, M. K.; Nagaraj, R.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1981**, *98*, 548. (c) Das, M. K.; Raghothama, S.; Balaram, P. *Biochemistry* **1986**, *25*, 7110. (d) Okuda, M.; Iida, A.; Uesato, S.; Nagaoka, Y.; Fujita, T.; Takaishi, Y.; Terada, H. *Biol. Pharm. Bull.* **1994**, *17*, 482.

(20) Hajji, M. E.; Rebuffat, S.; Doan, T. L.; Klein, G.; Satre, M.; Bodo, B. *Biochim. Biophys. Acta* **1989**, *978*, 97.