

# Structure of Thioviridamide, a Novel Apoptosis Inducer from *Streptomyces olivoviridis*

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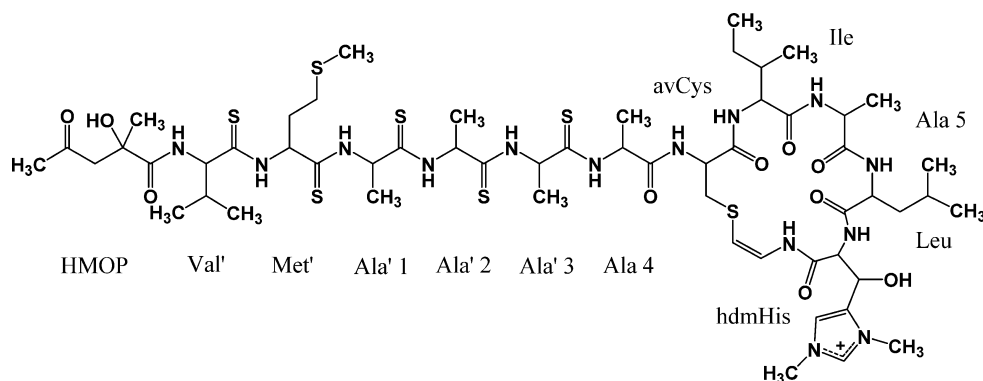
**Abstract** A novel apoptosis inducer, thioviridamide, was isolated from an actinomycete identified as *Streptomyces olivoviridis*. The molecular formula of thioviridamide was determined to be  $C_{56}H_{93}N_{14}O_{10}S_7^+$  from high-resolution FAB-MS. The structure of thioviridamide was analyzed by NMR spectral analysis including a variety of two-dimensional techniques. COSY and HMBC experiments revealed the presence of a 2-hydroxy-2-methyl-4-oxopentanoyl group and eleven amino acid residues including two novel amino acids,  $\beta$ -hydroxy- $N^1, N^3$ -dimethylhistidinium and  $S$ -(2-aminovinyl)cysteine.  $^1H$ - $^{13}C$  long-range correlations observed in the HMBC, CT-HMBC and HMBC-HOHAHA spectra connected the partial structures with seven amide linkages and five thioamide linkages to establish the planar structure of thioviridamide

as a unique cyclic peptide.

**Keywords** thioviridamide, antitumor antibiotics, cyclic peptide, thioamide

## Introduction

In the course of screening for apoptosis inducers in transformed cells, a new active substance, thioviridamide (**1**, Fig. 1), was isolated from the fermentation broth of an actinomycete identified as *Streptomyces olivoviridis*. Thioviridamide induced apoptotic cell death selectively in 3Y1 rat fibroblasts transformed with adenovirus oncogenes. In the preceding paper [1], we have described the



**Fig. 1** Structures of thioviridamide (**1**).

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**Table 1** NMR data summary for thioviridamide in CD<sub>3</sub>OD

	$\delta_C$	$\delta_H$ (Hz)		$\delta_C$	$\delta_H$ (Hz)
HMOP			avCys		
1	180.2		1	173.4	
2	75.0		2	52.9	3.75 d (10.5)
3	53.5	3.05 2H s	3	36.4	3.21 dd (12.0, 10.5)
4	212.9				2.91 d (12.0)
5	32.4	2.24 3H s	5	99.7	5.44 d (7.0)
2-Me	27.5	1.41 3H s	6	135.0	7.30 d (7.0)
Val'			Ile		
1	206.0		1	174.8	
2	68.8	4.50 d (4.5)	2	63.5	3.96 d (10.0)
3	33.1	2.57 m	3	37.2	1.98 m
4	20.1	1.10 3H d (7.0)	4	27.5	1.74 m
5	17.4	1.00 3H d (7.0)			1.31 m
Met'			5	11.1	0.99 3H t (7.0)
1	205.6		6	15.8	1.01 3H d (6.5)
2	67.3	5.59 dd (8.5, 6.0)	Ala 5		
3	33.3	2.49 2H m	1	176.2	
4	31.5	2.77 m	2	53.7	4.01 q (7.0)
		2.63 m	3	17.4	1.44 3H d (7.0)
6	14.9	2.11 3H s	Leu		
Ala' 1			1	174.9	
1	206.8		2	52.8	4.02 m
2	64.1	5.24 q (7.0)	3	40.5	1.47 2H m
3	19.9	1.59 3H d (7.0)	4	25.8	1.71 m
Ala' 2			5	23.8	0.84 3H d (6.5)
1	207.11		6	20.7	0.79 3H d (6.5)
2	64.5	5.24 q (7.0)	hdmHis		
3	20.0	1.73 3H d (7.0)	CO	167.6	
Ala' 3			$\alpha$	58.9	4.19 d (9.5)
1	207.08		$\beta$	62.3	6.00 d (9.5)
2	64.5	5.30 q (7.0)	2	138.7	8.77 s
3	20.1	1.79 3H d (7.0)	4	135.0	
Ala 4			5	124.1	7.66 s
1	174.0		N <sup>1</sup> -Me	36.5	3.94 3H s
2	57.9	4.73 q (7.5)	N <sup>3</sup> -Me	34.9	4.00 3H s
3	16.9	1.60 3H d (7.5)			

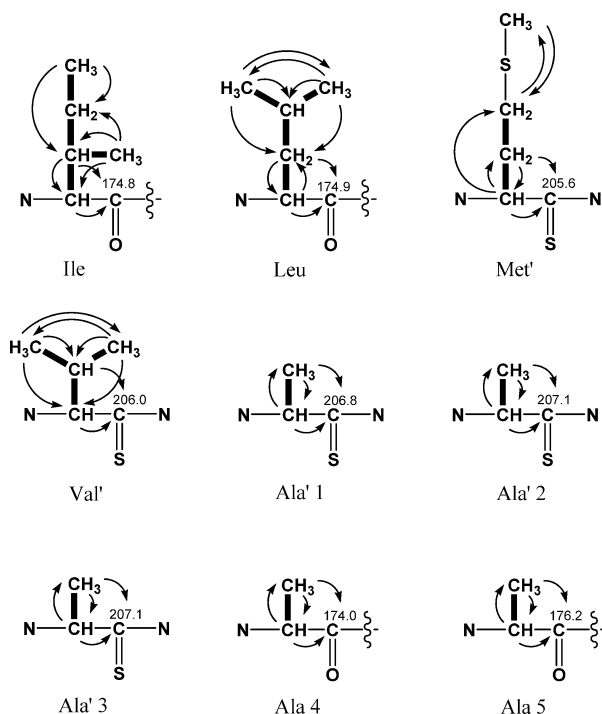
fermentation, isolation, physico-chemical properties and biological activities of **1**, as well as the taxonomy of the producing organism. This paper describes the structure elucidation of **1**.

## Results and Discussion

### Structure Elucidation

The molecular formula of thioviridamide (**1**) was determined to be C<sub>56</sub>H<sub>93</sub>N<sub>14</sub>O<sub>10</sub>S<sub>7</sub><sup>+</sup> (ion) from high-

resolution FAB-MS (*m/z* found: 1345.5215, calcd.: 1345.5244). The strong IR absorption peaks at 3350, 1670 and 1530 cm<sup>-1</sup> due to amide groups indicated that **1** is a peptide antibiotic. The <sup>13</sup>C NMR spectrum confirmed the presence of 56 carbons and the heteronuclear multiple-quantum coherency (HMQC) [2] spectrum established all one-bond <sup>1</sup>H-<sup>13</sup>C connectivities (Table 1). COSY and heteronuclear multiple-bond correlation (HMBC) [3] experiments revealed proton spin networks and <sup>1</sup>H-<sup>13</sup>C long-range correlations as shown in Fig. 2. These data identified two alanine residues (Ala 4, Ala 5), an isoleucine

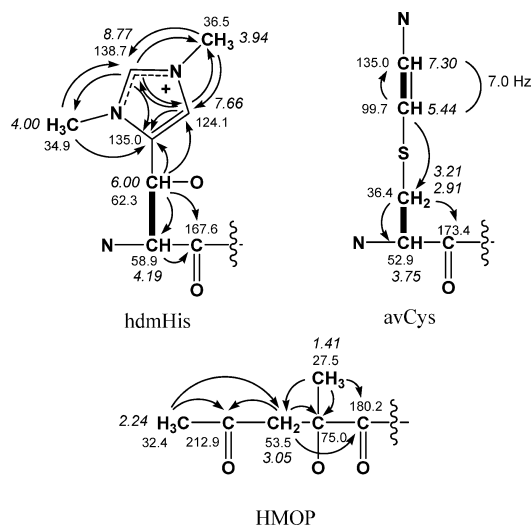


**Fig. 2** Amino acid residues in **1**.

Bold lines show  $^1\text{H}$  spin networks, arrows indicate  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations.

residue (Ile) and a leucine residue (Leu). Three alanine-like moieties (Ala' 1, Ala' 2, Ala' 3), a valine-like moiety (Val') and a methionine-like moiety (Met') contained quaternary carbons ( $\delta$  205.6~207.1), which were assigned to thioamide groups from their chemical shifts (thioacetamide:  $\delta$  207.6) and the molecular formula of **1**. Acid hydrolysis of **1** for amino-acid analysis yielded alanine, valine, isoleucine and leucine in a molar ratio of 3.8 : 0.6 : 1.0 : 1.2. Neither methionine nor methionine *S*-oxide was detected in the hydrolysate, while  $^{13}\text{C}$  NMR data for Met' are closely similar to those of methionine. The lack of methionine and the low recovery of valine and alanine might be ascribed to the resistance of thioamide bonds to acid hydrolysis.

In the HMBC spectrum,  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations were observed among two *N*-methyl groups ( $\delta_{\text{C}}$  34.9,  $\delta_{\text{H}}$  4.00 and  $\delta_{\text{C}}$  36.5,  $\delta_{\text{H}}$  3.94), two aromatic methines ( $\delta_{\text{C}}$  138.7,  $\delta_{\text{H}}$  8.77 and  $\delta_{\text{C}}$  124.1,  $\delta_{\text{H}}$  7.66) and an aromatic quaternary carbon ( $\delta$  135.0), indicating the presence of a 1,3-dimethylimidazolium moiety (Fig. 3). This substructure was expanded to include a  $\beta$ -hydroxy acid due to long-range couplings from an oxymethine proton ( $\delta$  6.00) to the two aromatic carbons ( $\delta$  135.0 and 124.1), a methine carbon ( $\delta$  58.9) and a carbonyl carbon ( $\delta$  167.6). These data including the  $^{13}\text{C}$  chemical shift of the  $\alpha$ -methine

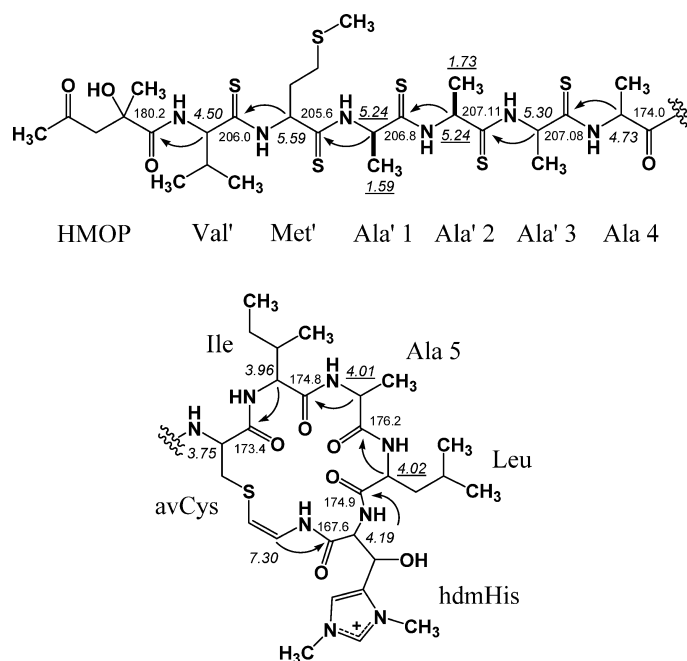


**Fig. 3**  $\beta$ -Hydroxy- $N^1,N^3$ -dimethylhistidinium (hdmHis), *S*-(2-aminovinyl)cysteine (avCys) and 2-hydroxy-2-methyl-4-oxopentanoyl (HMOP) moieties in **1**.

Bold lines show  $^1\text{H}$  spin networks, arrows indicate  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations. Plain and italic numbers show  $^{13}\text{C}$  and  $^1\text{H}$  chemical shifts, respectively.

established the structure of a novel amino acid,  $\beta$ -hydroxy- $N^1,N^3$ -dimethylhistidinium (hdmHis). Both the singlet methyl ( $\delta_{\text{H}}$  1.41) and the singlet methylene ( $\delta_{\text{H}}$  3.05) were coupled with an oxygenated quaternary carbon ( $\delta$  75.0) and an amide or ester carbonyl carbon ( $\delta$  180.2). An acetyl substituent on the methylene was required by long-range couplings from a singlet methyl ( $\delta_{\text{H}}$  2.24) to a ketone carbonyl ( $\delta$  212.9) and the methylene ( $\delta$  53.5), thereby showing the presence of a 2-hydroxy-2-methyl-4-oxopentanoyl group (HMOP). In the remaining part, a cysteine-like moiety and a *cis* olefin were established by NMR data as shown in Fig. 3. A sulfide linkage between the two partial structures was required by a long-range coupling from an olefin proton ( $\delta$  5.44) to the  $\beta$ -methylene carbon ( $\delta$  36.4) in the cysteine-like moiety, no coupling between the olefin proton and the  $\beta$ -methylene protons ( $\delta$  3.21, 2.91) and the molecular formula of **1** to generate an *S*-(2-aminovinyl)cysteine moiety (avCys).

Five amide bonds between Val' and HMOP, between Ile and avCys, between Ala 5 and Ile, between Leu and Ala 5, and between hdmHis and Leu were identified by three-bond couplings from  $\alpha$ -methine protons to amide carbonyl carbons (Fig. 4). In this analysis, a CT-HMBC [4] experiment enabled us to distinguish the two overlapping  $\alpha$ -methine protons (Leu:  $\delta$  4.02, Ala 5:  $\delta$  4.01). Three-bond correlations from  $\alpha$ -methine protons to thioamide carbons established five thioamide linkages between Ala 4 and Ala' 3, between Ala' 3 and Ala' 2, between Ala' 2 and



**Fig. 4** Amide and thioamide linkages in **1**.

Arrows indicate  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations. Plain and italic numbers show  $^{13}\text{C}$  and  $^1\text{H}$  chemical shifts, respectively.

Ala' 1, between Ala' 1 and Met', and between Met' and Val'. Although each  $\alpha$ -methine proton of Ala' 1 and Ala' 2 was assigned to the same chemical shift ( $\delta$  5.24), an HMBC-HOHAHA [5] experiment revealed four-bond correlations between  $\beta$ -methyl protons and thioamide carbons by moving the magnetization of  $\alpha$ -methine protons to the corresponding  $\beta$ -methyl protons (Ala' 1:  $\delta$  1.59 and Ala' 2:  $\delta$  1.73). A long-range coupling from an olefin proton ( $\delta$  7.30) to a carbonyl carbon ( $\delta$  167.6) indicated an additional amide linkage between the carbonyl group of hdmHis and the  $\varepsilon$ -amino group of avCys. The two partial structures thus obtained (Fig. 4) needed to be directly joined from the molecular formula of **1**. Negative ninhydrin reaction for **1** required an amide linkage between avCys and Ala 4 to establish the planar structure of **1** as shown in Fig. 1.

Thioviridamide (**1**) is a unique cyclic peptide containing five thioamide bonds and novel amino acids,  $\beta$ -hydroxy- $N^1,N^3$ -dimethylhistidinium (hdmHis) and *S*-(2-aminovinyl)cysteine (avCys). Thioamide-containing natural products, (4-methoxyphenyl)-*N*-methyl-2-oxothioacetamide [6] and cycastioamide [7] have been isolated from a tunicate *Polycarpa aurata* and a cycad *Cycas revoluta*, respectively. Each of these compounds, however, contains only one thioamide group. The unique structural features of thioviridamide combined with its interesting activity make it a possible candidate for anticancer therapeutics

and expand the area of natural product chemistry. Stereochemical studies on thioviridamide are in progress.

## Experimental

### General

Mass spectra were measured on a JEOL HX-110 spectrometer in the FAB mode using *m*-nitrobenzyl alcohol as matrix and polyethylene glycol as internal standard. IR spectra were recorded on JASCO FT/IR-470plus spectrometers. NMR spectra were obtained on a JEOL JNM-A500 spectrometer or a Varian INOVA 500 spectrometer with  $^1\text{H}$  NMR at 500 MHz and with  $^{13}\text{C}$  NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard. HMQC and HMBC spectra were measured using pulse field gradient programs.

### Amino Acid Analysis

A 6 M HCl solution of **1** (2.4 mg) was heated at 110°C for 24 hours. After evaporating, the residue was dissolved in 25 ml of water, and 50  $\mu\text{l}$  of the solution were analyzed using a Hitachi 835 amino-acid analyzer.

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## References

1. Hayakawa Y, Sasaki K, Adachi H, Furihata K, Nagai K, Shin-ya K. Thioviridamide, a novel apoptosis inducer in transformed cells from *Streptomyces olivoviridis*. *J Antibiot* 59: 1–5 (2006)
2. Summers MF, Marzilli LG, Bax A. Complete  $^1\text{H}$  and  $^{13}\text{C}$  assignments of coenzyme B12 through the use of new two-dimensional NMR experiments. *J Am Chem Soc* 108: 4285–4294 (1986)
3. Bax A, Summers MF.  $^1\text{H}$  and  $^{13}\text{C}$  assignments from sensitivity enhanced detection of heteronuclear multiple-bond connectivity by multiple quantum NMR. *J Am Chem Soc* 108: 2093–2094 (1986)
4. Furihata K, Seto H. Constant time HMBC (CT-HMBC), a new HMBC technique useful for improving separation of cross peaks. *Tetrahedron Lett* 39: 7337–7340 (1998)
5. Furihata K, Seto H. HMBC-COSY and HMBC-HOHAHA, new application of HMBC method. Abstract Papers of 15th NMR Symposium 5~8 (1992)
6. Abas SA, Hossain MB, van der Helm D, Schmitz FJ, Laney M, Cabuslay R, Schatzman RC. Alkaloids from the Tunicate *Polycarpa aurata* from Chuuk Atoll. *J Org Chem* 61: 2709–2712 (1996)
7. Pan M, Mabry TJ, Beale JM, Mamiya BM. Nonprotein amino acids from *Cycas revoluta*. *Phytochemistry* 45: 517–519 (1997)