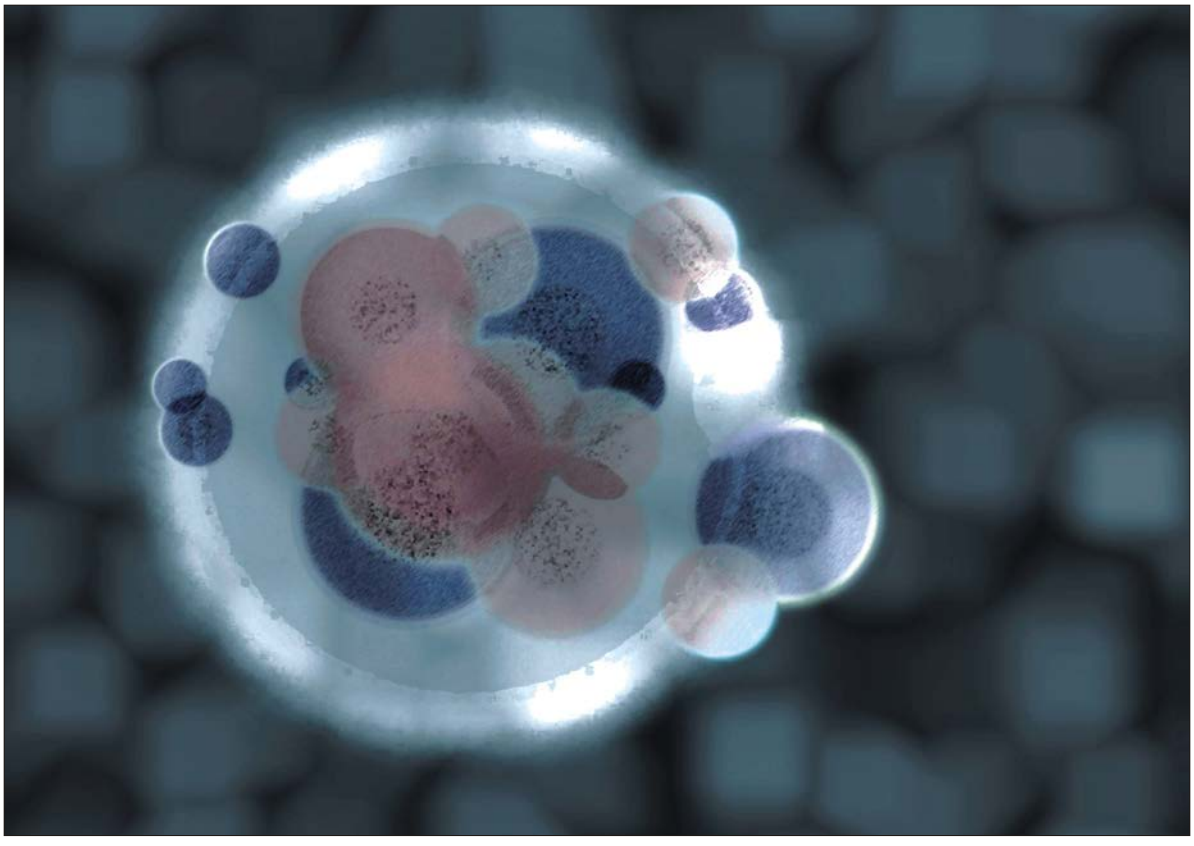

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Theonellapeptolide Id: Structure Identification of Cytotoxic Constituent from *Kaliapsis* sp. Sponge (Bowerbank) Collected from West Bali Sea Indonesia

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Abstract: Structure identification of cytotoxic of isolated compound from *Kaliapsis* sp. sponge collected from North west Bali sea was conducted. The identity of the structure was analyzed based on physical and spectral data, namely, ultraviolet, MS, one- and two-dimensional ^{1}H-NMR and ^{13}C-NMR and comparison to published values. The isolated compound was confirmed as Theonellapeptolide Id.

Key words: Cyclodepsipeptide, *Kaliapsis* sp. sponge, cytotoxic constituent

INTRODUCTION

Sponge is the lowest rank multicellular organism (metazoa) and considered as the oldest multicellular organism on earth. Sponge is belonging to Porifera phylum. Porifera is a Latin word for phorus (small porous matter) and ferre (to bear). Therefore, Porifera means organism having porous main organ part (Castro and Huber, 1997; Leys *et al*., 2005).

Almost 98% of sponges grow and are found in the sea from equator to south and north pole, either in deep or shallow seas. As compared to metazoa other members, sponge does not have any special organs for reproduction, digestion, respiration, sensory or excretory. The response from environment is individual. Self defense against parasite organism or microbe is done based on secondary metabolites resulted by sponge (Hooper and Soest, 2002; Colin and Anderson, 1995).

In this study, *Kaliapsis* sp. (*Kaliapsis*, Bowerbank) sponge was collected from the sea around Menjangan island, West Bali, Indonesia. *Kaliapsis* sp. sponge is sticky and elastic. Megasclere consists of desmas tetraclone (tetracrepidial), phyllotriaenes, Short shafe triane, Orthotriaene and Triaeniform and mikrosclere consists of *Microstrongyle*, *Microxea*, *Amphiaster streptaster*. Sponge belongs to Animalia Kingdom, Porifera phylum, Class of Demospongiae, Ordo Lithisda, Sub-ordo Triaenosina, Family of Theonellidae and Genus of *Kaliapsis*, Bowerbank, 1868 (Hooper, 1997).

By bioactivity guided fractionation and isolation, various cytotoxic constituents we have isolated (Setyowati *et al*., 2007a, b). *In vitro* cytotoxic assay of further isolated compound 5M74 using Myeloma cells showed its IC_{50} of 10.3 μg mL^{-1}. On this occasion we are reporting the structure identification of 5M74.

MATERIALS AND METHOD

Materials: *Kaliapsis* sp. sponge (collected from Menjangan Island, West Bali National Park at 20 m bellow sea surface, on October 15, 2004). Sample specimen was deposited at Gadjah Mada University Laboratory.

Analytical apparatus: Infra red spectrometer (FTIR 8201 PC Shimadzu), EIMS (Electron Impact Mass Spectroscopy) dari INCOS 50 (Finigan MT). Nuclear Magnetic Resonance (NMR) 500 MHZ (Jeol) with radiofrequency strength for ^{13}C at 125 MHZ. Ultraviolet spectrometer (UV) (Milton Roy 3000).

Method for identification of bioactive compound: Identification of bioactive compound was conducted based on its physical, spectral data (IR, UV and MS, one- and two-dimension ^{1}H-^{13}C NMR) and comparison to published values.

Isolation procedure: The bioactivity guided extraction, fractionation and isolation of the active isolate were conducted based on standard procedure as reported previously (Setyowati *et al*., 2007b; Houssen and Jaspars, 2005).

Cytotoxic evaluation: The cytotoxic evaluation was conducted based on standard protocols (Doyle and Griffiths, 1998).

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RESULTS AND DISCUSSION

Isolated 5M74, white orthorhomic crystals, mp 67-169°C, was chloroform and methanol soluble. On TLC plate (its R_f value of 0.57 solvent system methanol:ethyl acetate 7:2 v/v), showed blue color at UV λ 366 nm and orange color on iodium vapor.

Its infra red spectrum, 5M74 showed having functional group of OH or amide (v 3448 cm^{-1}). Functional of alkyl groups were represented of its peaks at v 2800 cm^{-1}, 2962, 2873.9, 1465.8 and 1419.5 cm^{-1} for its stretching vibration. The ether functional groups were indicated by v 1732-1740, 1253.6 and 1199.6 cm^{-1}. Infrared absorption of v 1624.5 cm^{-1} was specific for amide carbonyl functional group. The secondary amide was represented by v 1546.8 cm^{-1}. From above data the isolate 5M74 might have -OH, NH, -N-C=O-, HO-C=O functional groups (Silverstein and Webster, 2000).

Mass spectra of 5M74 was obtained from Maldi Tof High Resolution Electrospray Ionisation (HRESI-MS) LCMS. Its fragment ions were m/z 1404.5565 (M^++1)(30%); 711.3475 (8%), 704.9012 (8%), 702.9031 (100%, base peak), 463.5227 (1%), 179.8647 (1%). The molecular ion was found at m/z 1404.5565 indicating its molecular weight. Therefore, the molecular formula of 5M74 was $C_{70}H_{125}N_{13}O_{16}$ calculated for 1403.9374.

UV spectra of 5M74 showed maximum absorption at λ 205 nm in methanol.

Figure 1 showed the ^{1}H-NMR spectrum of 5M74. The proton resonances at δ 5, 4-δ_H 3,6 ppm indicated the alfa proton of an aminoacid. The aliphatic protons were at the highfield area of δ_H 1.5-2.5 ppm. The methyl groups were showed at <δ_H 1, 5 ppm.

^{13}C-NMR spectra were indicating 5M74 (Fig. 2a) had 69 atom carbons, in which the down field carbonyls being 13 atoms of quaternary carbonyls from δ_C 176.3 ppm to δ_C 168.5 ppm (Fig. 2b).

The expansion of certain areas of ^{13}C-NMR spectra of 5M74 were showed at Fig. 2c-e. The Distortionless Enhancement by Polarization Transfer (DEPT) result, showed that 5M74 had 14 quaternary, 17 methine, 16 methylene and 22 methyl carbons. Table 1 showed the chemical shifts of ^{13}C-NMR spectra of 5M74 resulted from DEPT experiment.

Application of two-dimensional NMR, HMQC (Heteronuclear Multiple Quantum Coherence) (Fig. 3) will resolved the proton carbon chemical shifts correlation of 5M74. Table 2 showed that the NMR data of 5M74 were indeed in accordance with those of Theonellapeptolide 1d (Roy *et al*., 2000).

Long-range proton-carbon correlation of 5M74 were analyzed using HMBC (Heteronuclear Multiple Bond

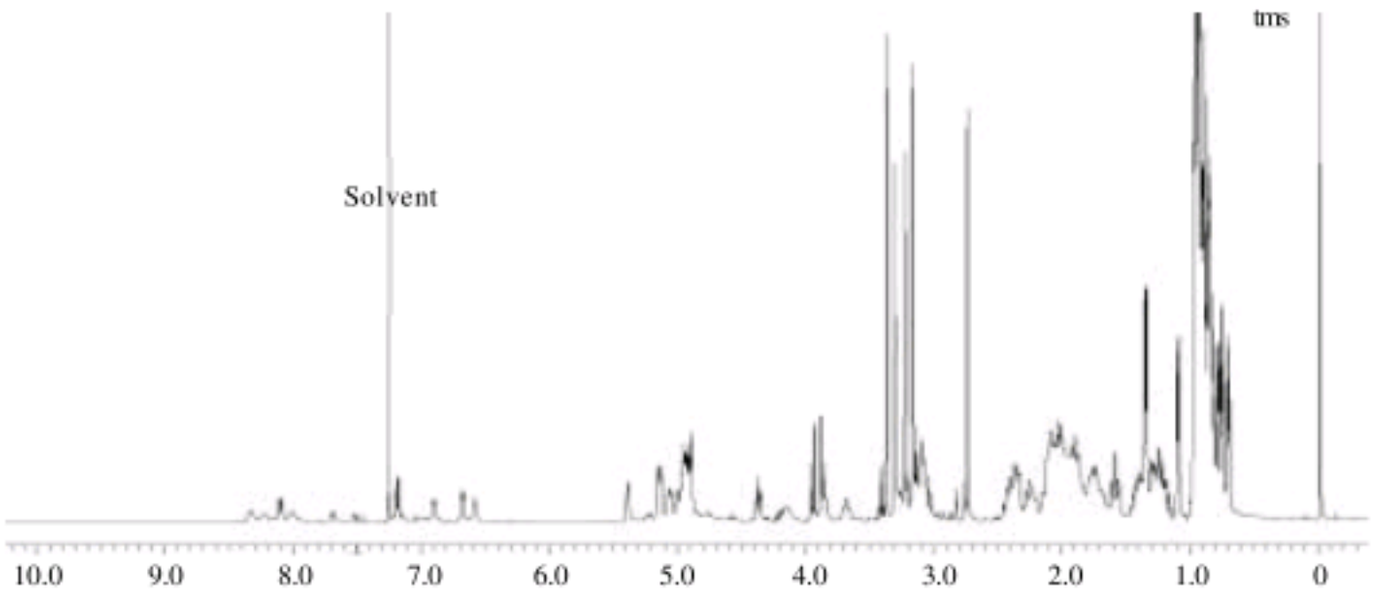

Fig. 1: ^{1}HNMR spectra of 5M74

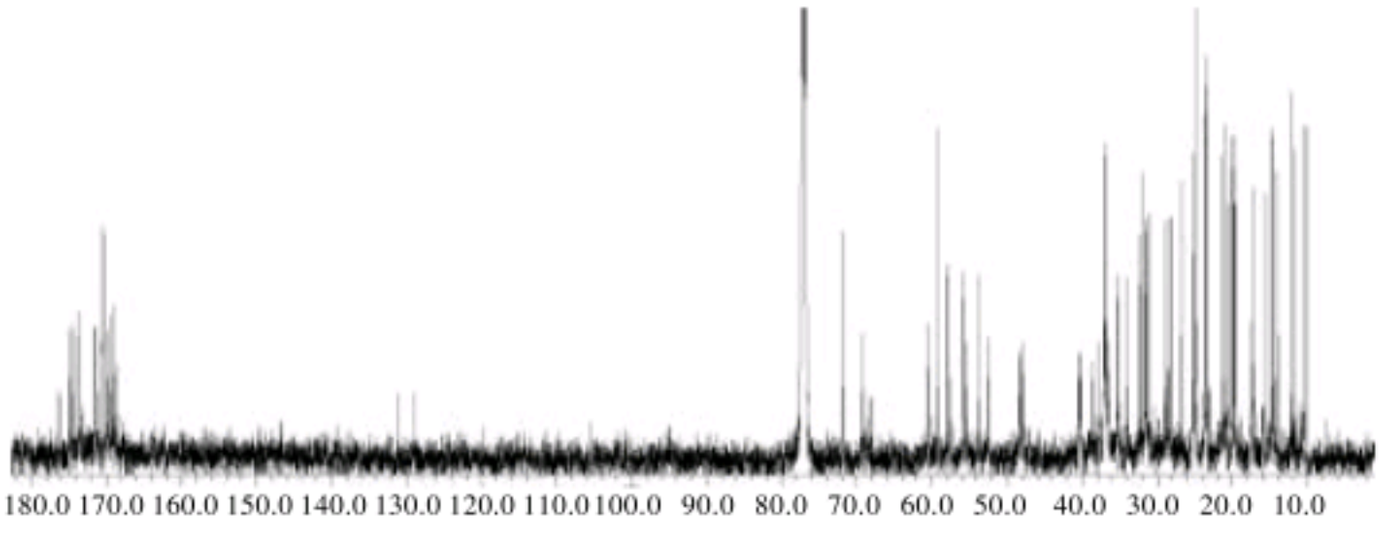

Fig. 2a: ^{13}C-NMR spectra of 5M74

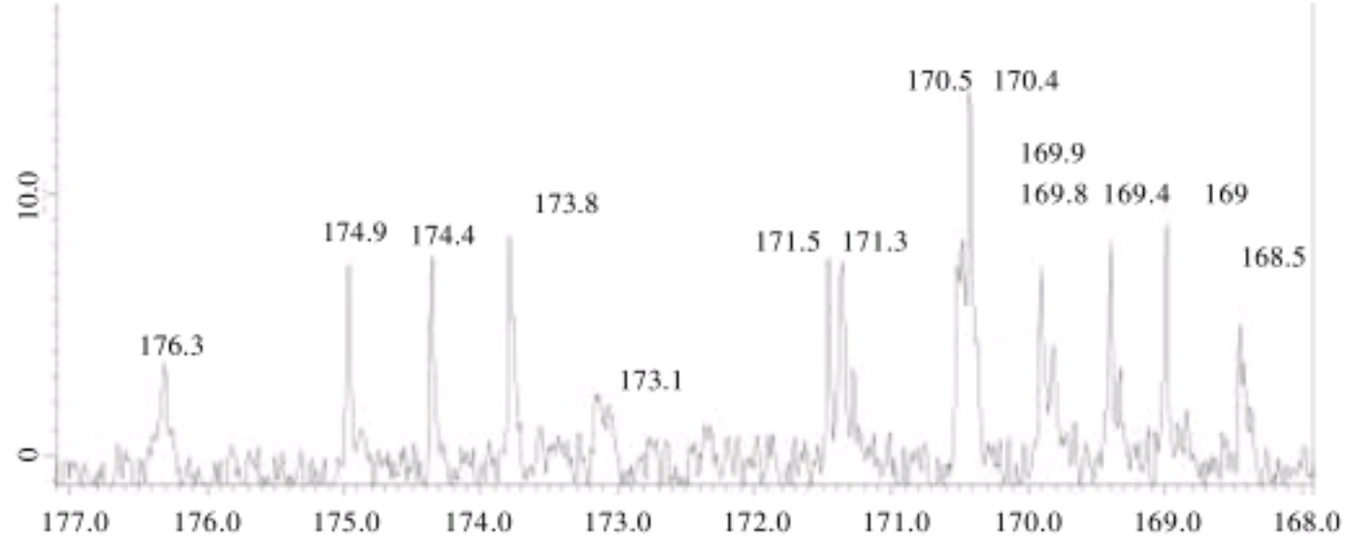

Fig. 2b: ^{13}C-NMR spectra of 5M74 at the carbonyl area

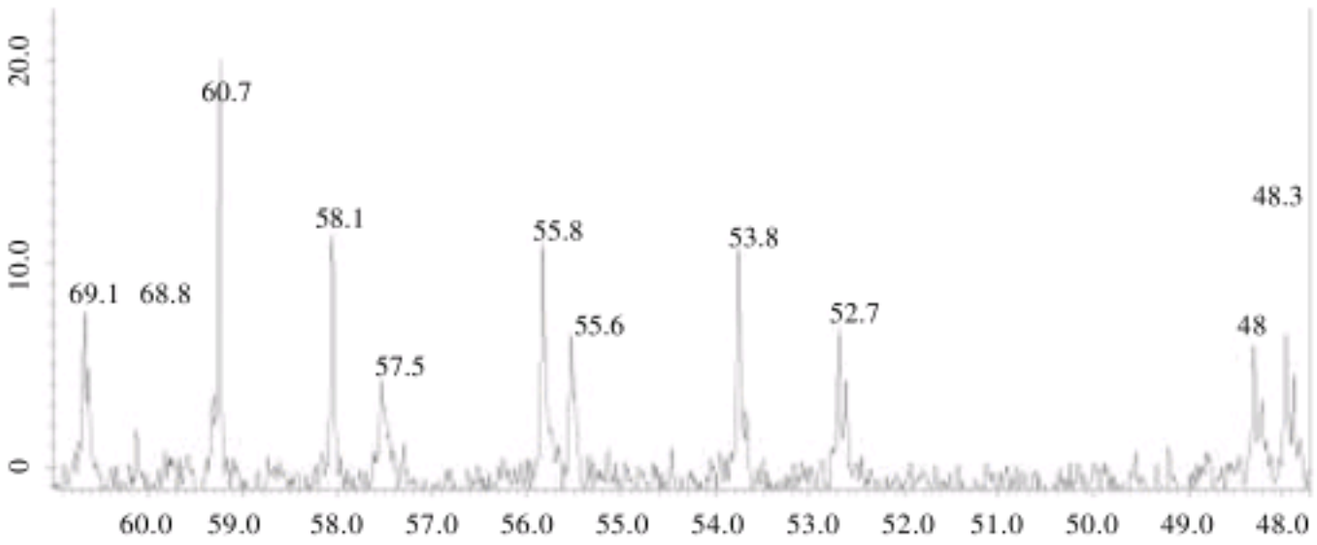

Fig. 2c: ^{13}C-NMR spectra of 5M74 from δ 48-60 ppm

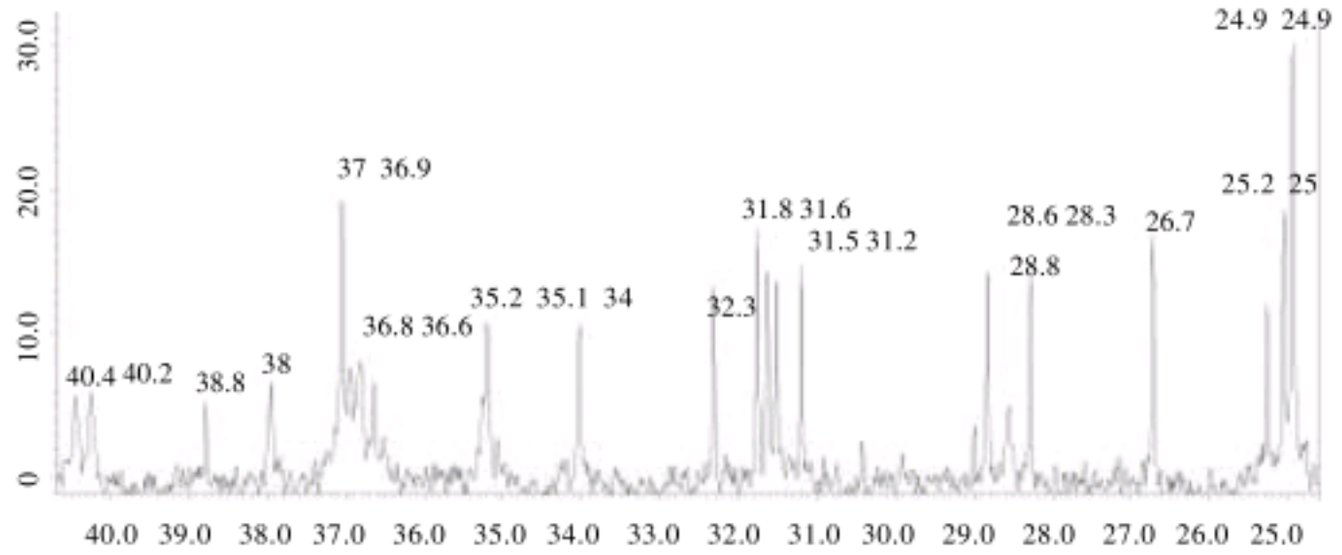

Fig. 2d: ^{13}C-NMR spectra of 5M74 at aliphatic (upfield) areas

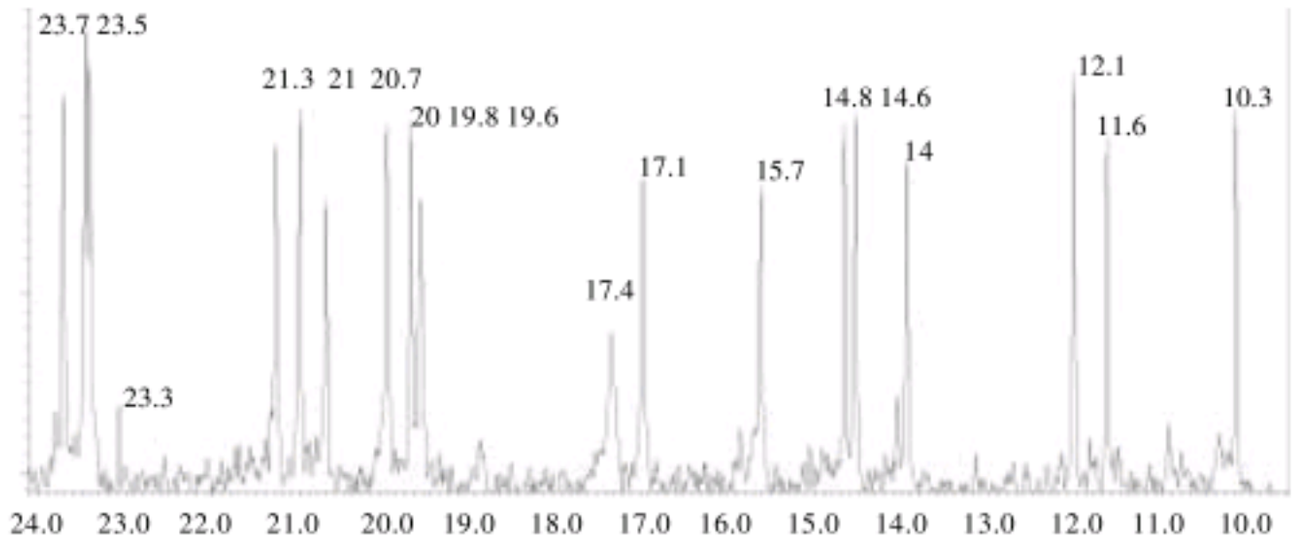

Fig. 2e: ^{13}C-NMR spectra of 5M74 at aliphatic areas (continued)

Table 1: ^{13}C-NMR of 5M74 in $CDCl_3$

δ_C (ppm)		δ_C (ppm)	
176.3	C	35.2	CH_2
174.9	C	35.1	CH_2
174.4	C	34.0	CH
173.8	C	32.3	CH
173.1	C	31.8	CH_3
171.5	C	31.6	CH
171.3	C	31.5	CH_3
170.5	C	31.2	CH_3
170.4	C	28.8	CH_3
170.0	C	28.6	CH_2
169.9	C	28.3	CH
169.4	C	26.7	CH_3
169.0	C	25.2	CH
168.5	C	25.0	CH
71.8	C	24.9	CH_2
69.1	CH_2	24.9	CH
68.8	CH	23.7	CH_3
60.7	CH	23.5	CH_3
59.2	CH	23.3	CH_3
58.1	CH_3	21.3	CH_3
57.5	CH	21.0	CH_3
55.8	CH	20.7	CH_2
55.6	CH	20.0	CH_3
53.8	CH	19.8	CH_3
52.7	CH	19.6	CH_3
48.3	CH_2	17.4	CH_3
48.0	CH	17.1	CH_3
40.4	CH_2	15.7	CH_3
40.2	CH_2	14.8	CH_3
38.8	CH_2	14.6	CH_3
38.0	CH	14.0	CH_3
37.0	CH_2	12.1	CH_3
36.9	CH_2	11.7	CH_2
36.8	CH	10.3	CH_3
36.6	CH_2		

Table 2: NMR ($CDCL_3$) data of 5M74

Amino acid	No.	13C-NMR (mult., J/Hz) 5M74	1H-HMR (mult., J/Hz) 5M74
L-Thr (1)	1	168.5 (s)	
	2	57.5	4.37 (dd)
	3	69.1	5.12 (m)
	4	17.4	1.09 (d)
	2-NH		8.32 (brd)
	OH		
D-allo-Me-Ile (2)	5	170.5 (s)	
	6	68.8	5.11 (br)
	7	34	2.4 (m)
	8	28.6	1.89 (m)
	8’		
	9	11.7	0.94 (t)
	10	14.6	0.75 (d)
	6-NMe	38.8	3.23 (s)
D-leu (3)	11	174.4 (s)	
	12	48	5.0 (ddd)
			1.55 (br)
	13	40.4	1.22 (m)
	13’		1.7 (m)
	14	24.9	0.88 (d)
	15	23.7	0.91 (d)
	16	21	8.1 (d)
	12-NH		
β-Ala (4)	17	170.4 (s)	
	18	36.8	2.33 (m)
	18’		2.04 (m)
	19	37	3.85 (m)
	19’		3.05 (m)

Table 2: Continued

Amino acid	No.	13C-NMR (mult.. J/Hz) 5M74	1H-HMR (mult.. J/Hz) 5M74
	19-NH		6.68 (dd)
L-Me-Ala (5)	20	169 (s)	
	21	55.8	4.89 (q)
	22	14.8	1.34 (d)
	21-NMe	28.8	2.74 (s)
L-Me-Val (6)	23	169.9 (s)	
	24	58.1	4.91 (d)
	25	28.3	2.35 (m)
	26	20	0.89 (d)
	27	19.7	0.86 (d)
	24-NMe	31.6	3.3 (s)
D-allo-Ile (7)	28	176.3 (s)	
	29	52.7	5.38 (dd)
	30	36.9	1.73 (m)
	31	26.7	1.43 (m)
	31’		1.2 (m)
	32	12.1	0.96 (t)
	33	14	0.7 (d)
	29-NH		8.2 (brs)
β-Ala (8)	34	171.3 (s)	
	35	35.2	2.37 (m)
	35’		2.17 (m)
	36	35.1	4.2 (m)
	36’		3.09 (m)
	36-NH		6.89 (dd)
L-Me-Ile (9)	37	169.9 (s)	
	38	60.7	4.94 (d)
	39	32.3	2.08 (m)
	40	24.9	1.3 (m)
	40’		0.96 (m)
	41	10.3	0.83 (t)
	42	15.7	0.93 (d)
	38-NMe	31.2	3.16 (s)
D-Leu (10)	43	174.9 (s)	
	44	48.3	5.06 (ddd)
	45	40.2	1.58 (brt)
	45’		1.25 (m)
	46	25.2	1.77 (d)
	47	23.5	0.90 (d)
	48	21.3	0.94 (brs)
	44-NH		8
β-Ala (11)	49	171.5 (s)	
	50	36.8	2.24 (m)
	50’		2.09 (m)
	51	36.6	3.68 (m)
	51’		3.3 (m)
	51-NH		6.58 (brt)
D-Me-Leu (12)	52	173.1 (s)	
	53	55.6	5.12 (m)
	54	38	1.92 (brt)
	54’		1.39 (m)
	55	25.2	1.34(m)
	56	23.5	0.92 (d)
	57	20.7	0.78 (d)
	53-NMe	31.8	3.16 (s)
L-Val (13)	58	173.8 (s)	
	59	53.8	4.92 (dd)
	60	31.6	2.01 (d)
	61	19.8	0.97 (d)
	62	17.1	0.85 (d)
	59-NH		7.19 (d)
MeOAc (14)	63	169.4 (s)	
	64	71.8	3.97 (d)
	64’		3.87 (d)
	64-OMe	59.2	3.37 (s)

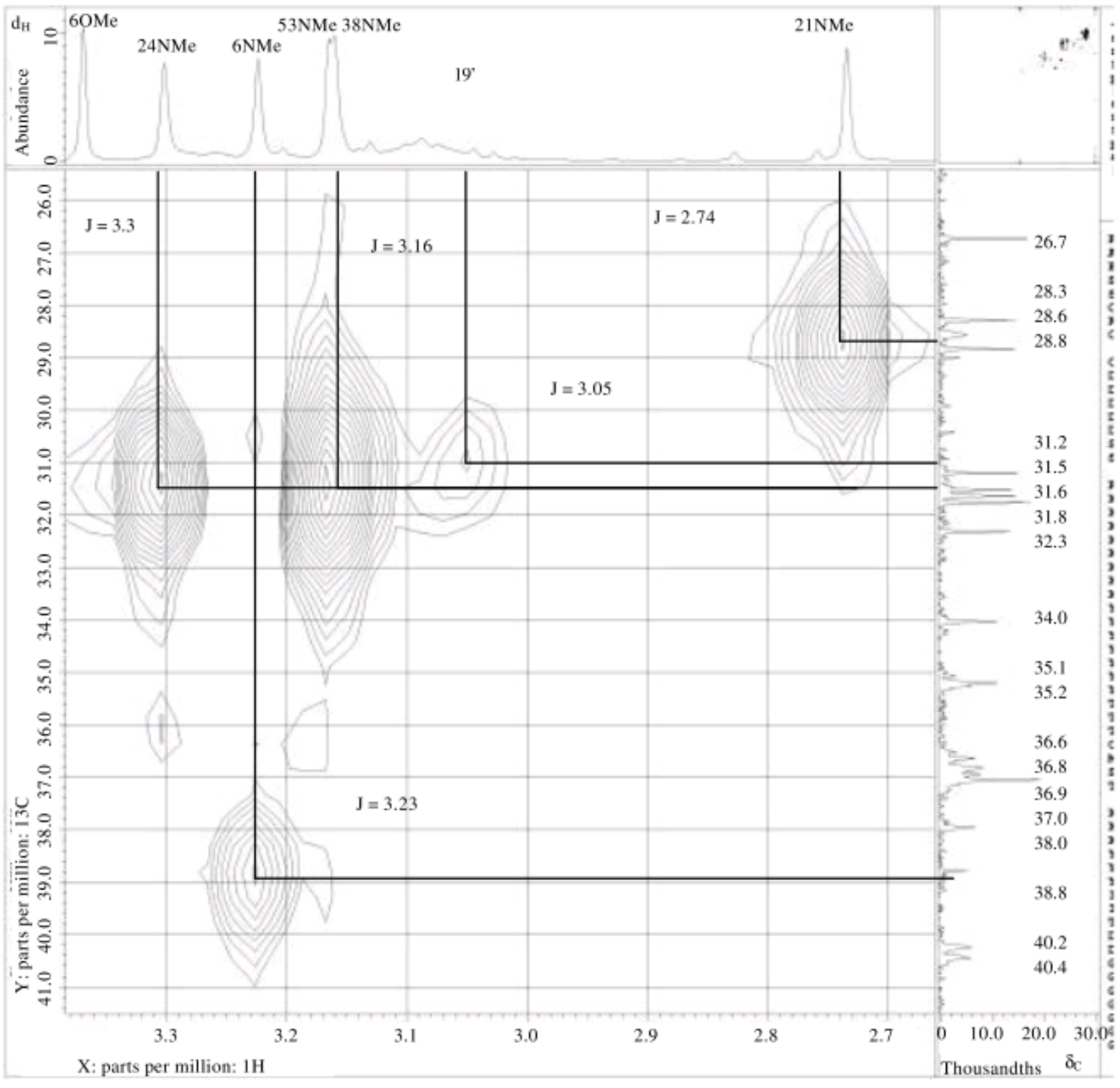

Fig. 3: HMQC spectra of 5M74

Coherence) spectrum (Fig. 4). Figure 5 showed the proton-carbon long-range correlation, deducted from HMBC spectrum.

The ^{1}H-^{1}H COSY (Correlation Spectroscopy) gave information on proton-proton correlation either geminal (^{2}J) or vicinal (^{3}J) in a molecule (Jenie *et al*., 2006; Silverstein and Webster, 2000). The proton-proton network of 5M74 was showed at COSY spectrum (Fig. 6).

The bold numbers indicated proton correlations of amino acid moiety of 5M74 (Fig. 6). As an example, number 1 showed the L-Thr (L-threonin; Table 2) amino acid which showed the proton correlation of δ_H 8.32 ppm (2NH) to H-2 proton (δ_H 4.37 ppm) and H-3 proton (δ_H 5.12 ppm).

From all above data, it was concluded that 5M74 was Theonellapeptolide 1d as showed in Fig. 7.

The 5M74, a tridecapeptide lactone, was characterized with its a number of D-amino acid, N-methyl amino acid and β-amino acid. From above data it was concluded that 5M74 was Theonellapeptolide 1d that had been isolated from *Theonella swinhoe* (Roy *et al*., 2000). This compound showed various activities, namely, cytotoxic, Na^{+}, K^{+} ions transport and inhibitory activity on ATP ase (Roy *et al*., 2000). On this occasion we are reporting the Theonellapeptolide 1d from *Kaliapsis* sp. The cytotoxic data on this experiment using various cancer cell lines were following (Table 3).

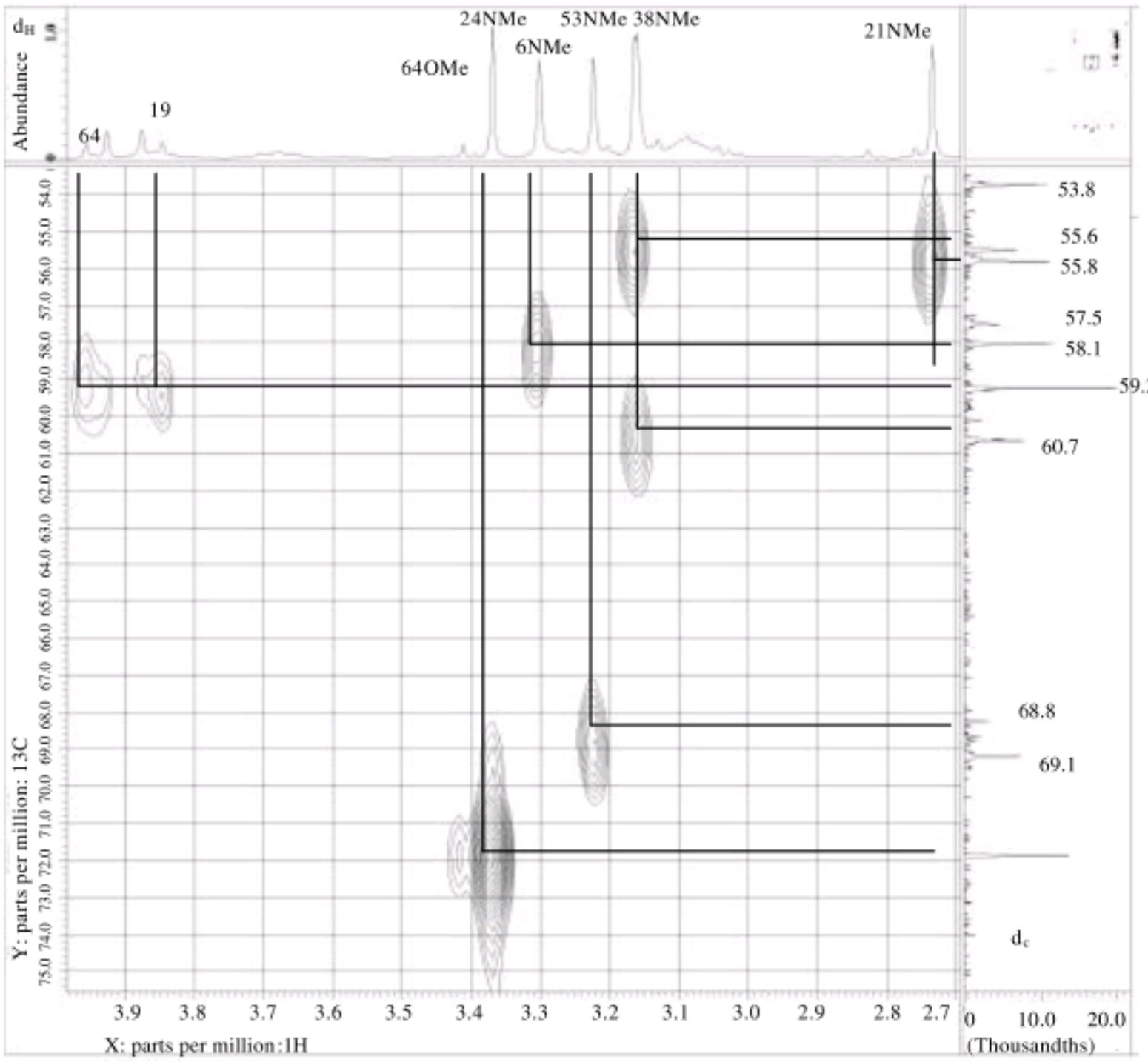

Fig. 4: HMBC spectrum for proton-carbon long-range correlation of 5M74

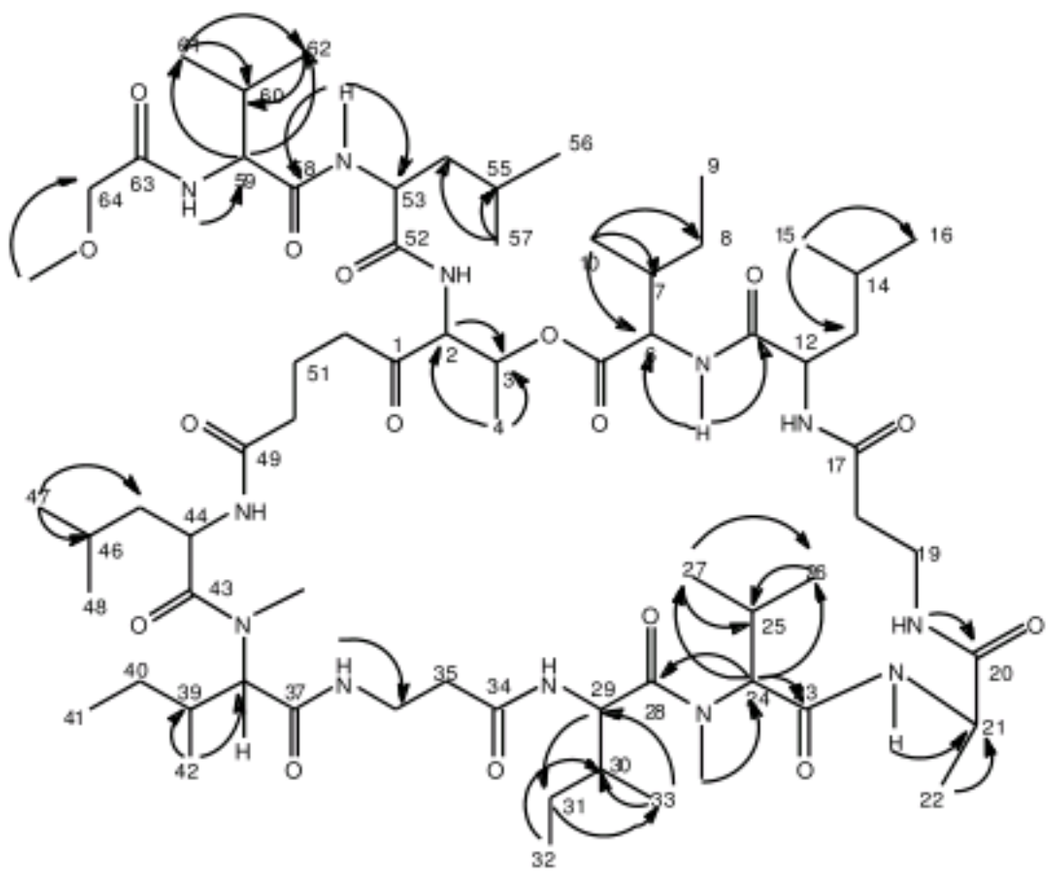

Fig. 5: HMBC long-range correlation of 5M74

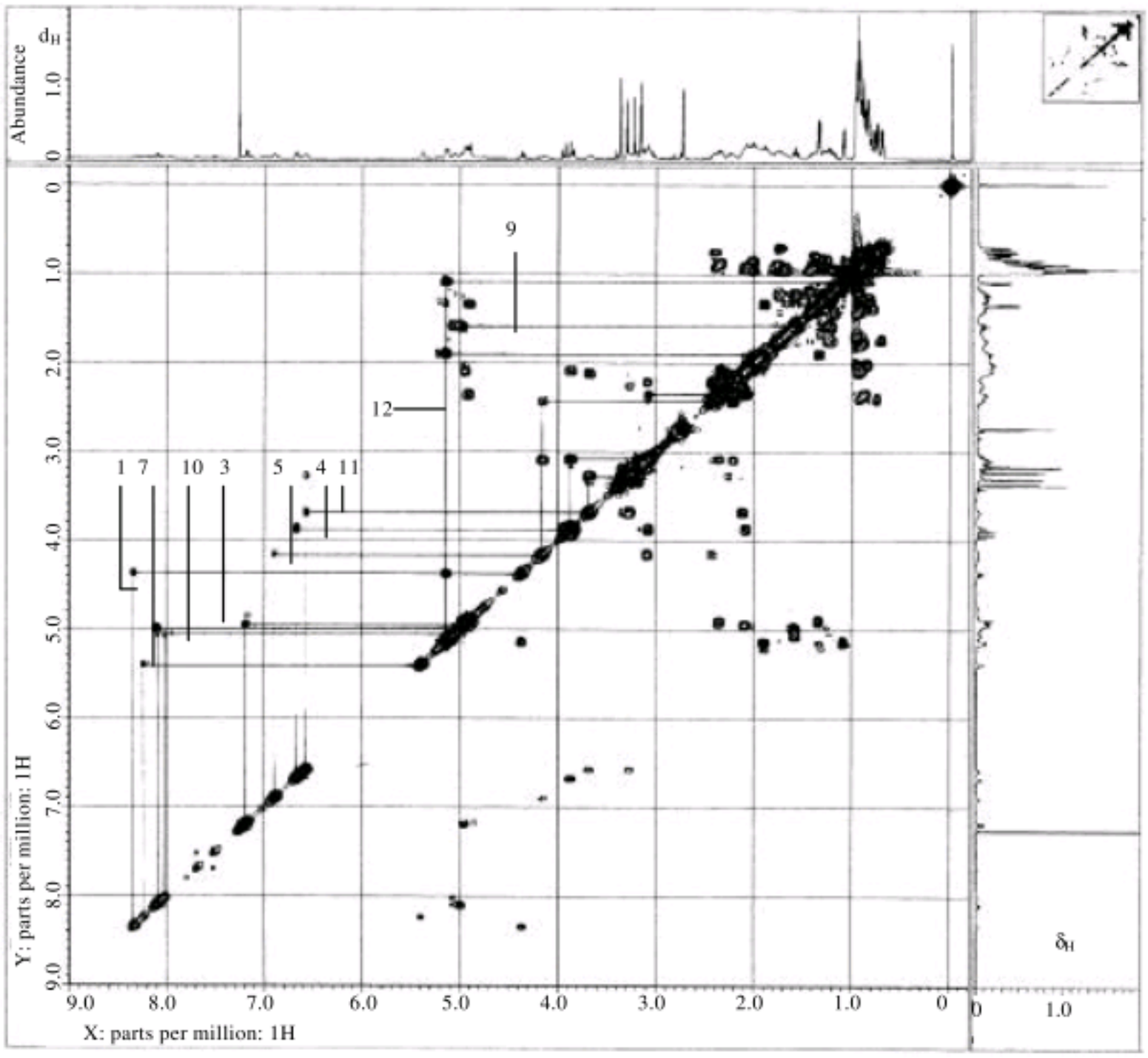

Fig. 6: ^{1}H-^{1}H COSY spectrum of 5M74

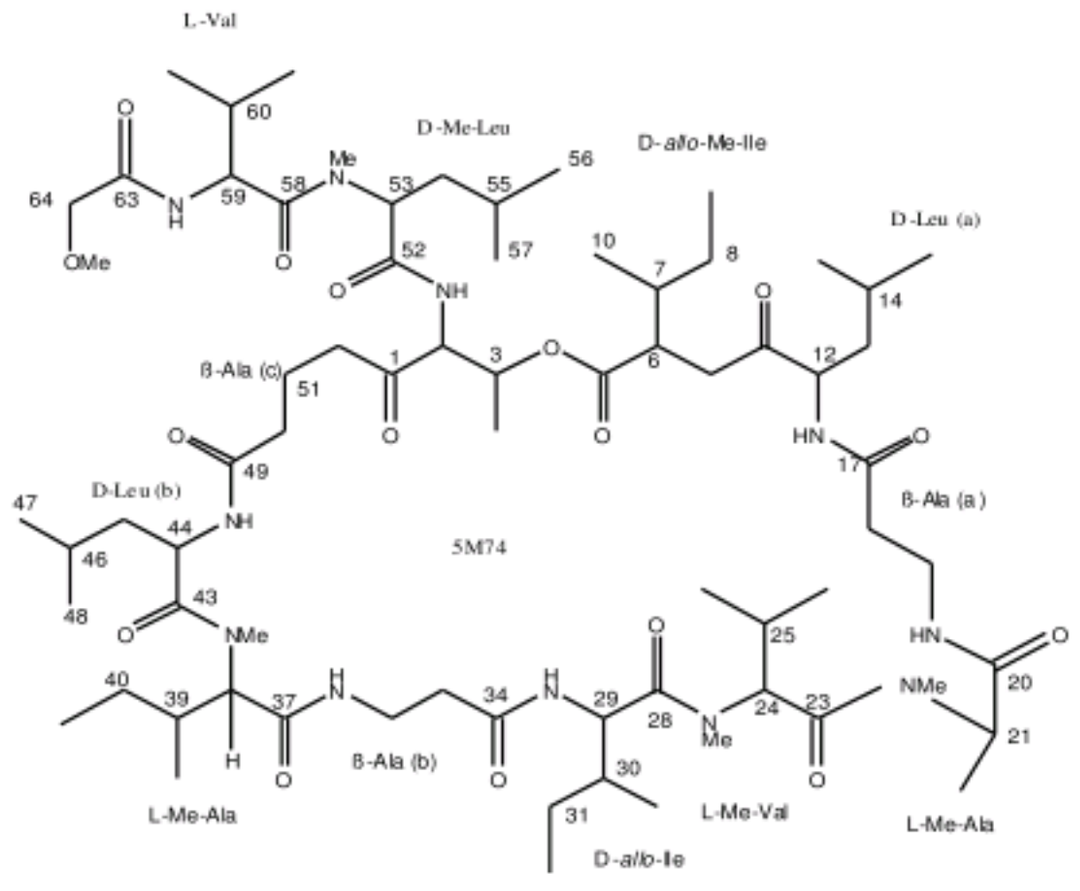

Fig. 7: Structure of 5M74 (Theonellapeptolide Id)

Table 3: Cytotoxic effect of 5M74 against several cell lines

Name of compound	Myeloma cell IC_{50} µg mL^{-1}	T47D cell IC_{50} µg mL^{-1}	HeLa cell IC_{50} µg mL^{-1}	Raji cell IC_{50} µg mL^{-1}
5M74	10.3	8.3	16.5	7.8

This data showed that marine organisms are rich in bioactive compounds, including those are potential for anticancer.

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