

## Supporting Information

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### A New Diketopiperazine from the Marine Sponge *Callyspongia* Species

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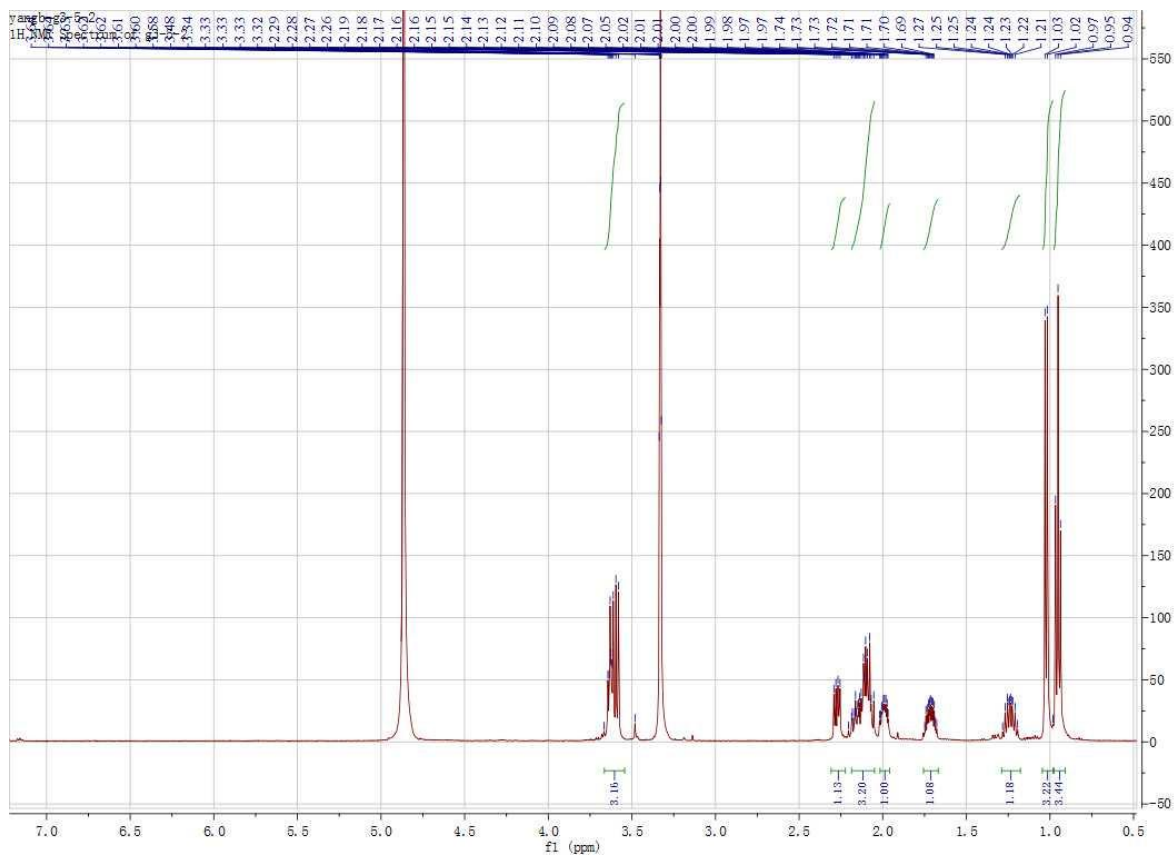
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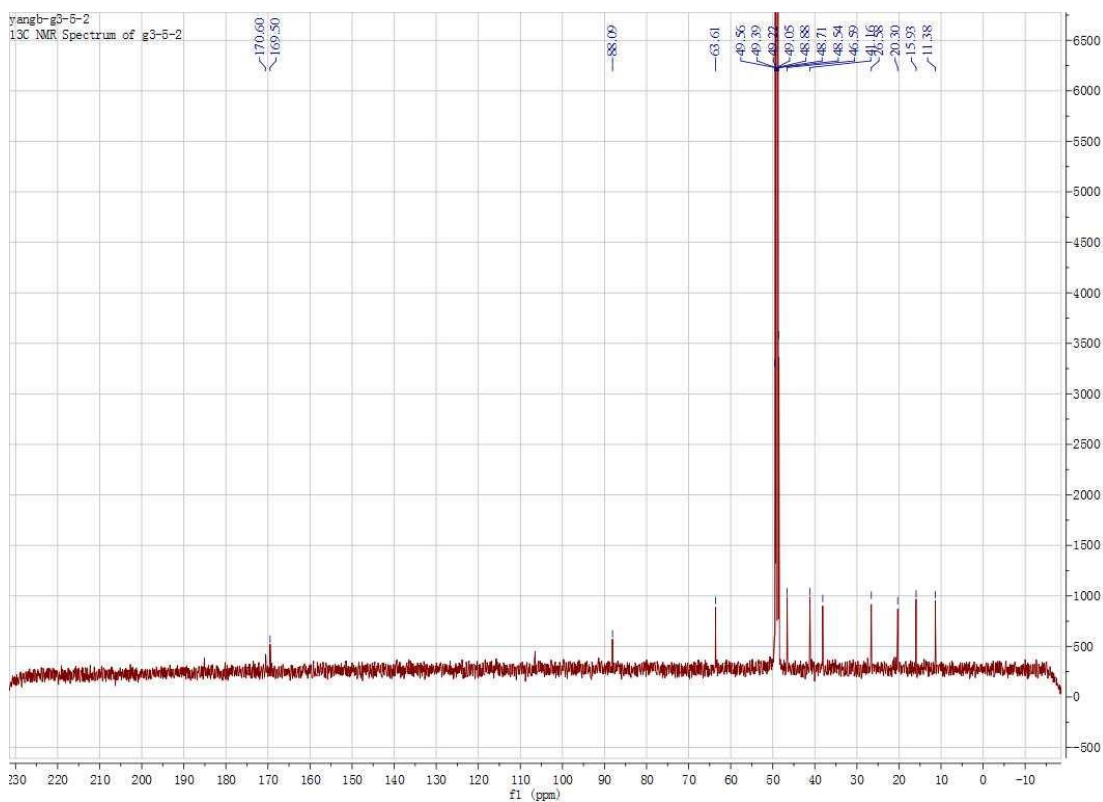
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## Extraction and Isolation

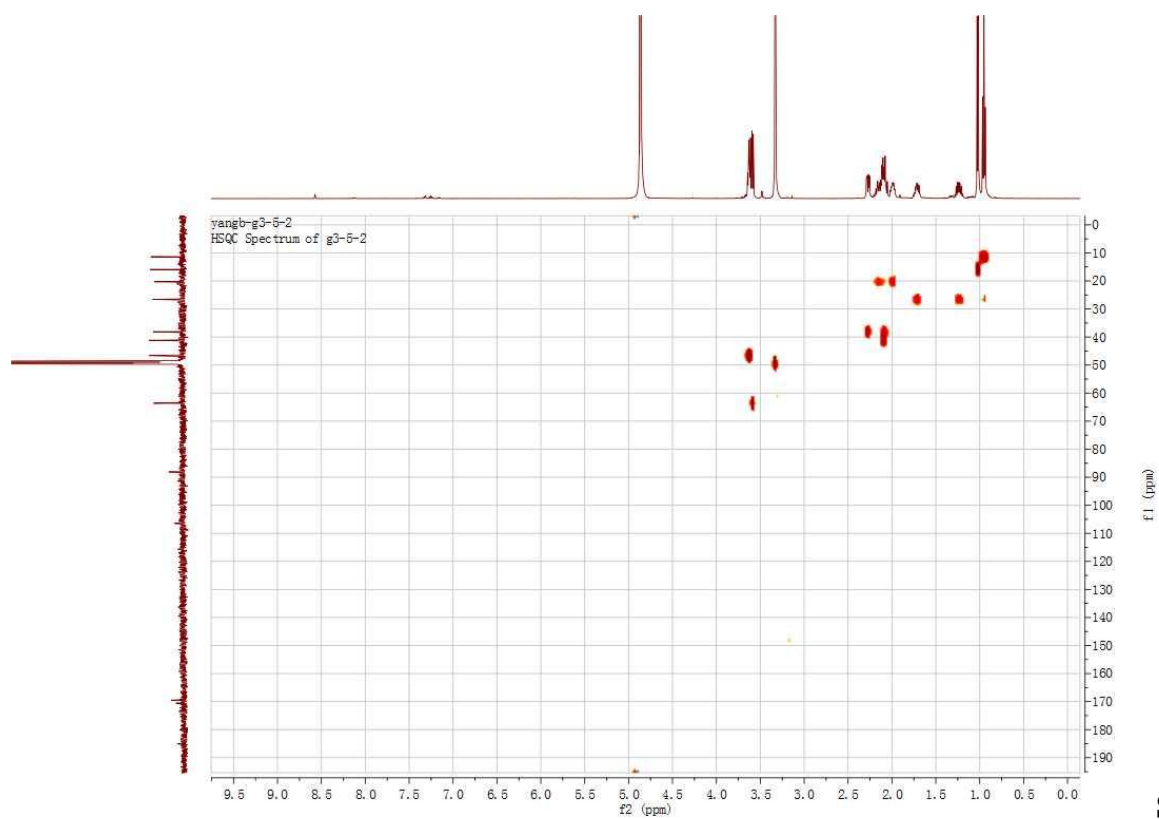
The 85% EtOH fraction was subjected to reversed-phase flash column chromatography, eluting with a solvent system of 40→100% MeOH, to afford 25 fractions. *Fr. 2* (2.2 g) was further separated by reverse phase flash column chromatography, eluting with 10→50% MeOH, to afford 12 subfractions (*Fr.g1–g12*). *Fr.g5* was separated by semi-preparative HPLC, eluting with MeOH-H<sub>2</sub>O (12:88) at a flow rate of 2 ml min<sup>-1</sup>, to provide compounds **1** (4.0 mg), **2** (5.2 mg). *Fr.g7* were combined and was separated by semi-preparative HPLC, eluting with MeOH-H<sub>2</sub>O (30:70) at a flow rate of 2 ml min<sup>-1</sup>, to provide compounds **3** (5.2 mg) and **4** (7.6 mg). The *n*-BuOH layer was subjected to reversed-phase flash column chromatography, eluting with a solvent system of 0→50% MeOH, to afford 19 fractions (*Fr.n1–n19*). *Fr.n12* was further purified with silica gel column chromatography, eluting with PE-EtOAc (1: 2), to provide compounds **5** (2.2 mg) and **6** (3.7 mg). *Fr.n5* was subjected to Sephadex LH-20 (MeOH) to afford 5 subfractions (*Fr.n5-1–n5-5*). *Fr.n5-1* was further purified with silica gel column chromatography, eluting with MeOH-CHCl<sub>3</sub> (10:90), to provide compound **7** (2.2 mg). *Fr.n1* was further purified with semi-preparative HPLC, eluting with MeOH-H<sub>2</sub>O (7:93) at a flow rate of 1.5 ml min<sup>-1</sup>, to provide compound **8** (6.3 mg). *Fr.n2* was subjected to Sephadex LH-20 (MeOH) to afford 5 fractions (*Fr.n2-1–n2-5*). *Fr.n2-3* was further purified with semi-preparative HPLC eluting with MeOH-H<sub>2</sub>O (22:78) at a flow rate of 1.5 ml min<sup>-1</sup>, to provide compounds **9** (14.2 mg) and **10** (3.5 mg).



**S1:**  $^1\text{H-NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ ) Spectrum of Compound **1**

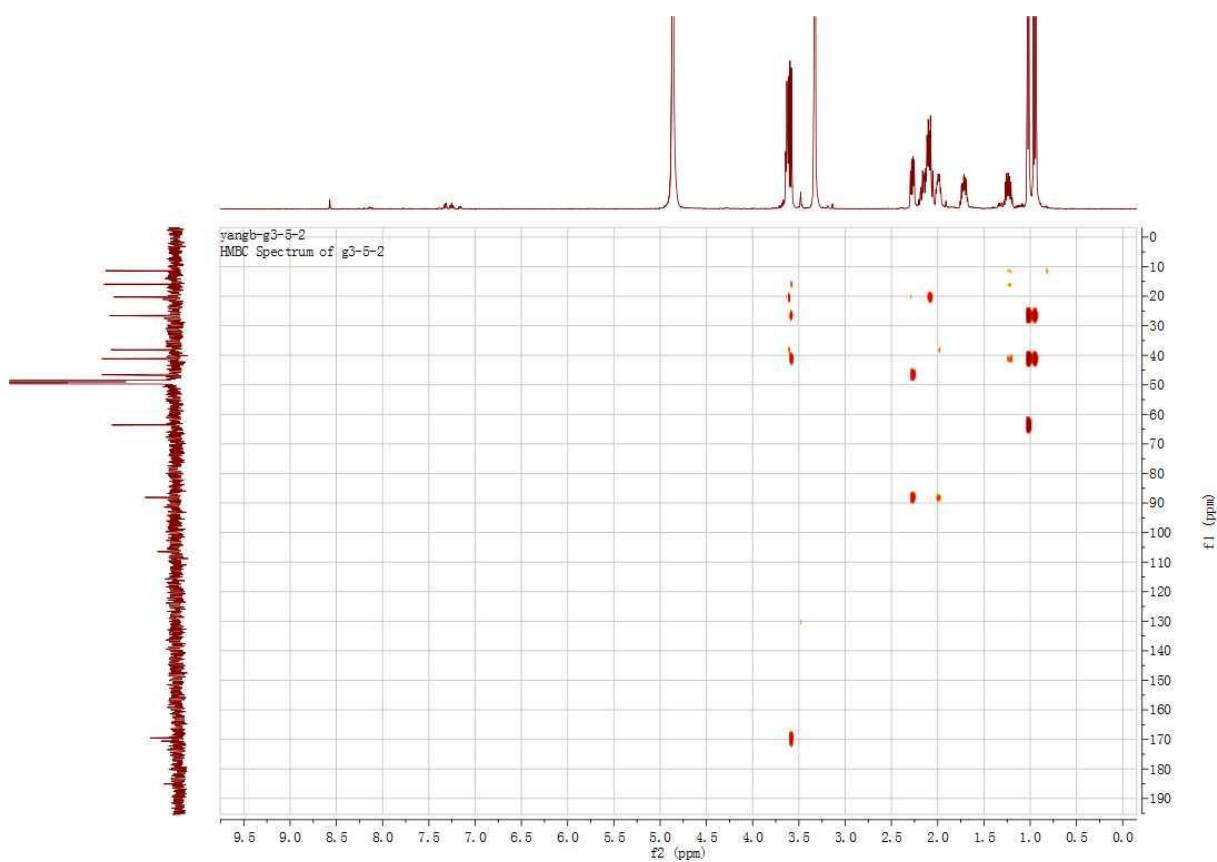


**S2:**  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CD}_3\text{OD}$ ) Spectrum of Compound **1**

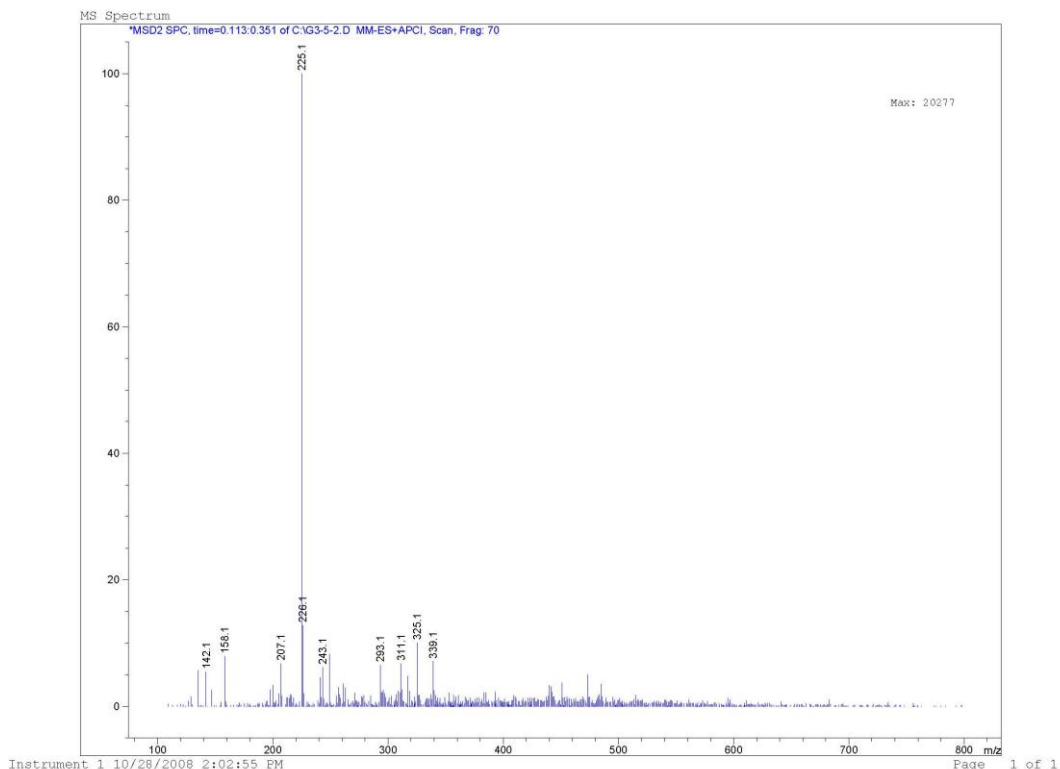


S3:

HMQC (500 MHz) Spectrum of Compound 1



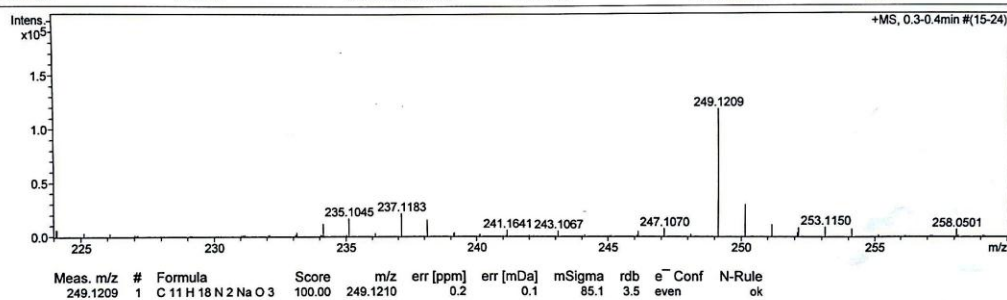
S4: HMBC (500 MHz) Spectrum of Compound 1



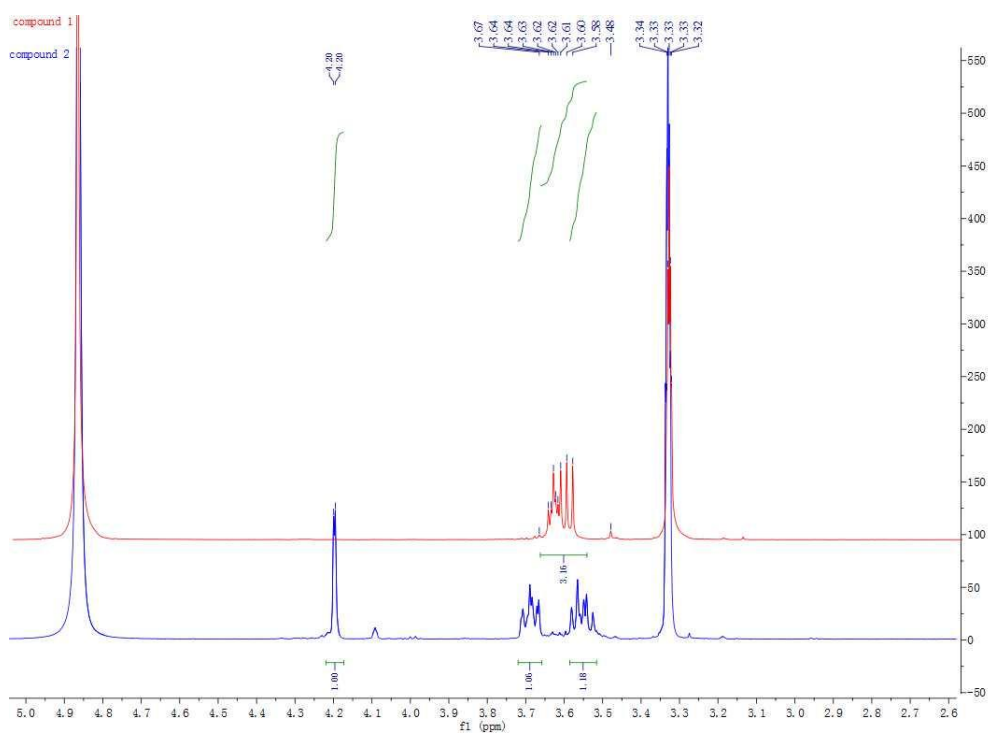
### S5: ESI-MS Spectrum of Compound 1

#### Mass Spectrum SmartFormula Report

Analysis Info		Acquisition Date	
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Method	tune_low_pos_100-500mz.m	Operator	SCSIO
Sample Name	SCSIO	Instrument / Ser#	maXis 29
Comment			
Acquisition Parameter			
Source Type	ESI	Ion Polarity	Positive
Focus	Active	Set Capillary	4500 V
Scan Begin	50 m/z	Set End Plate Offset	-500 V
Scan End	1500 m/z	Set Collision Cell RF	450.0 Vpp
		Set Nebulizer	0.3 Bar
		Set Dry Heater	180 °C
		Set Dry Gas	4.0 l/min
		Set Divert Valve	Waste



### S6: HR-ESI-MS Spectrum of Compound 1



**S7:  $^1\text{H-NMR}$  Comparative Analysis of Compounds 1 and 2**