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Unusual Anti-allergic Diterpenoids from the Marine Sponge *Hippospongia lachne*

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Hipposponlachnins A (1) and B (2), possessing an unprecedented tetracyclo [9.3.0.0^{2,8}.0^{3,7}] tetradecane ring system, and the probable biogenetic precursor [3, (1*R,2*E*,4*R**,7*E*,10*S**,11*S**,12*R**)-10, 18-diacetoxydolabella-2,7-dien-6-one] of 1–2 were isolated from the South China Sea marine sponge *Hippospongia lachne*. The structures of the novel compounds were determined using integrated spectroscopic methods in combination with single-crystal X-ray diffraction analysis. Compounds 1–2 showed potent inhibitory activity on the release of β-hexosaminidase, a biomarker for degranulation, as well as the production of pro-inflammatory cytokine IL-4 and lipid mediator LTB$_4$ in DNP-IgE-stimulated RBL-2H3 cells.**

Allergy is an abnormal adaptive immune response against allergens, such as pollen, food, dust mites, cosmetics, mold spores, and animal hairs[1,2]. β-Hexosaminidase, typically released in addition to histamine by mast cells after stimulation with antigen, has been used as a biomarker for antigen-induced degranulation in rat basophilic leukemia (RBL-2H3) cells[3]. Thus, the release of β-hexosaminidase in RBL-2H3 cells has commonly been used as a reliable indicator of inhibitory activity against allergic reactions induced by natural products[4]. Natural products have been proved to be an important source for drug discovery and development. More than three hundreds natural compounds were reported to exhibit diverse biological properties, such as antitumor, antibacterial, antifungal, and a range of other bioactivities[5]. However, only a few natural compounds with anti-allergic activity have been isolated to date[5]. Therefore, there is a great opportunity in search for leads to treat allergy effectively.

Marine invertebrates such as sponges are well-known sources of bioactive natural products[6]. Sponges of the genus *Hippospongia* are reported to be rich sources of polyketides[7], sesquiterpenes[8], sesterterpenes and sulfates[9], furanoterpenes[10], triterpenoic acids[11], fatty acids[12], and sterols[13]. These metabolites exhibited a broad spectrum of biological activities, including cytotoxic[9], antifungal[7], anti-inflammatory[8], coronary vasodilating[14], and RCE-protease inhibitory activities[11]. A literature survey revealed that no anti-allergic metabolites have been isolated from sponges belonging to this genus. As part of our searching for anti-allergic secondary metabolites from marine sponges, we investigated the sponge *Hippospongia lachne*, collected from the Xisha Islands of China in March 2013. The 95% ethanol extract of *H. lachne* showed promising β-hexosaminidase inhibitory activity with IC_{50} value of 7.8 μg/mL. Further bioactivity-guided fractionation of this extract led to the isolation of compounds **1–3** (Fig. 1). Of these three metabolites, hipposponlachnins A (**1**) and B (**2**) possessed a rare tetracyclo [9.3.0.0^{2,8}.0^{3,7}] tetradecane carbon skeleton, exemplifying the first diterpene scaffold obtained from marine source, and showed inhibitory activity on the release of β-hexosaminidase in anti-murine DNP-IgE-stimulated RBL-2H3 cells. Herein, we report the isolation, structure elucidation and anti-allergic activity of the two unprecedented diterpenoids. In addition, biogenetic relationship between these compounds was proposed, suggesting **1–2** may be obtained from **3** via an intramolecular [2 + 2] cycloaddition reaction.

Results and Discussion

Isolation and structure elucidation. Air dried specimen of *H. lachne* were extracted with 95% ethanol and after evaporation under reduced pressure, the aqueous residue was extracted with EtOAc. The EtOAc

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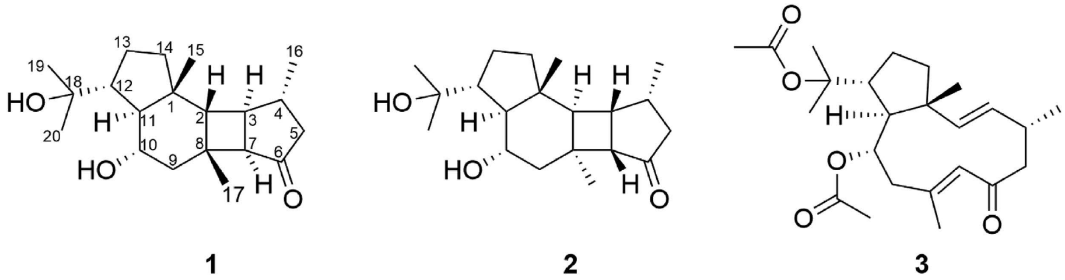

Figure 1. Structures of compounds 1–3.

position	1		2	
	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)
1	45.9, C		43.1, C	
2	54.01, CH	1.84, d (7.5)	50.4, CH	2.10, d (7.0)
3	41.0, CH	2.47, t (7.5)	39.2, CH	2.90, q (7.0)
4	34.2, CH	2.10, m	32.3, CH	2.33, m
5α	47.1, CH_2	2.00, d (19.0)	46.0, CH_2	2.17, m
5β		2.75, dd (19.0, 7.5)		2.34, m
6	220.6, C		220.4, C	
7	53.98, CH	2.34, d (7.5)	56.1, CH	2.32, m
8	39.3, C		38.0, C	
9α	48.8, CH_2	1.52, dd (21.0, 10.0)	40.9, CH_2	1.65, d (15.0)
9β		2.10, m		2.15, m
10	67.2, CH	3.72, td (10.0, 4.0)	70.2, CH	3.92, dd (9.5, 7.5)
11	50.3, CH	1.53, dd (10.0, 4.0)	49.4, CH	1.54, t (9.5)
12	51.2, CH	2.10, m	52.8, CH	2.03, m
13α	26.0, CH_2	1.28, m	27.2, CH_2	1.31, m
13β		1.94, m		1.96, m
14α	33.1, CH_2	1.28, m	42.3, CH_2	1.22, d (11.0)
14β		1.24, m		1.60, dd (11.0, 8.5)
15	22.7, CH_3	0.85, s	19.6, CH_3	0.93, s
16	21.1, CH_3	0.97, d (7.0)	15.5, CH_3	1.01, d (6.0)
17	26.5, CH_3	1.11, s	26.2, CH_3	1.12, s
18	72.7, C		73.2, C	
19	30.8, CH_3	1.28, s	30.9, CH_3	1.27, s
20	23.0, CH_3	1.24, s	23.0, CH_3	1.19, s

Table 1. ^{1}H and ^{13}C NMR Data for 1 and 2 (500, 125 MHz, $CDCl_3$, TMS, δ ppm).

layer was repeatedly chromatographed on Sephadex LH-20, silica gel, ODS column and reversed phase high performance liquid chromatography (HPLC) to yield hipposponlachnin A (**1**), hipposponlachnin B (**2**), and (1*R**,2*E*,4*R**,7*E*,10*S**,11*S**,12*R**)-10,18-diacetoxydolabella-2,7-dien-6-one (**3**) (Fig. 1).

Hipposponlachnin A (**1**) was obtained as a colorless crystal. Its molecular formula was determined to be $C_{20}H_{32}O_3$ by HRESIMS (*m/z* 343.2251 $[M+Na]^+$), indicating five degrees of unsaturation. The IR spectrum featured characteristic absorptions of hydroxyl (3280 cm^{-1}) and carbonyl (1732 cm^{-1}) groups. The ^{1}H NMR spectrum revealed the presence of four methyl singlets (δ_H 0.85, 1.11, 1.24, and 1.28), one methyl doublet (δ_H 0.97, d, $J = 7.0$ Hz), and one oxymethine proton (δ_H 3.72, td, $J = 10.0$, 4.5 Hz). The ^{13}C and DEPT135 NMR spectra displayed 20 carbon resonances (Table 1), including five methyls, four methylenes, seven methines (one oxygenated), and four quaternary carbons (one oxygenated and one carbonyl). The aforementioned data accounted for one degree of unsaturation. The remaining four degrees of unsaturation suggested a tetracyclic core in **1**.

The planar structure of **1** was elucidated via a detailed analysis of 2D NMR data (Fig. 2). The COSY spectrum readily revealed the presence of four isolated spin systems: (*a*) C-2–C-3–C-7, (*b*) C-5–C-4–C-16, (*c*) C-9–C-10–C-11, and (*d*) C-12–C-13–C-14. The observed HMBC correlations from H_3-16 to C-3, C-4, and C-5 and from H-12 to C-11 revealed the connectivities of C-3 and C-4 and of C11 and C-12, respectively. The HMBC correlations from H-3, H-4, H-5, and H-7 to C-6 indicated that C-5 and C-7 were connected via the carbonyl carbon C-6. The additional HMBC correlations of H_3-17/C-2, C-7, C-8, and C-9, H_3-15/C-1, C-2, C-11, and C-14, and H-12/C-10, tethered the remaining three fragments *a*, *c*, and *d* by inserting the "loose ends" of the quaternary

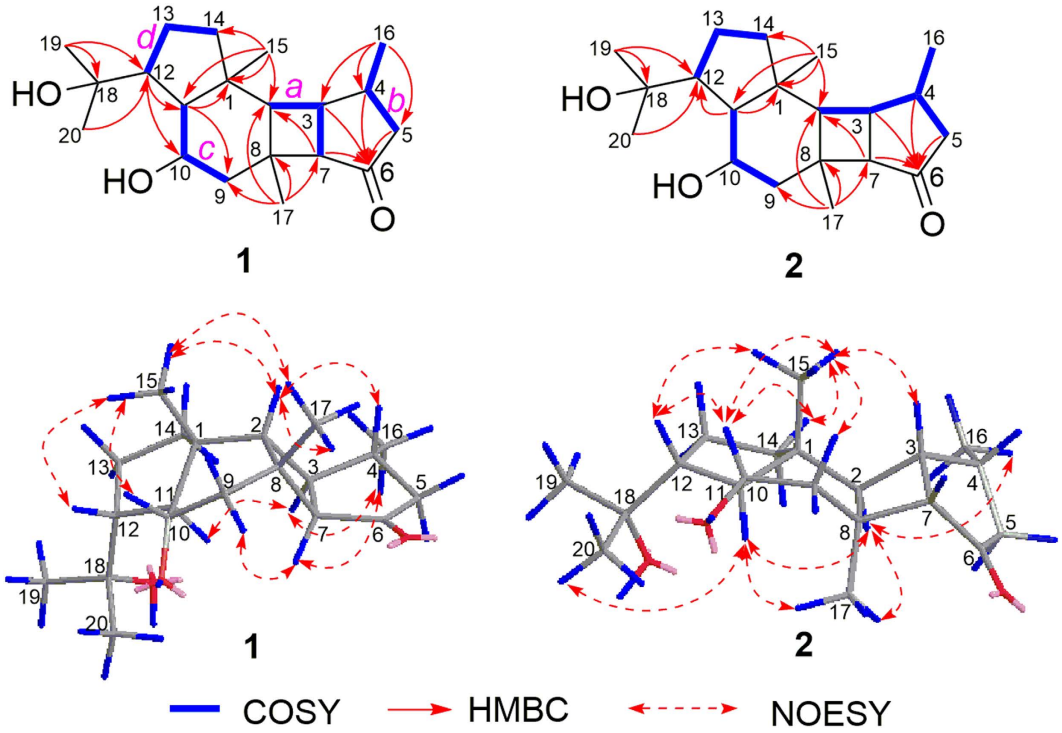

Figure 2. Key HMBC and COSY and selected NOE correlations of 1 and 2.

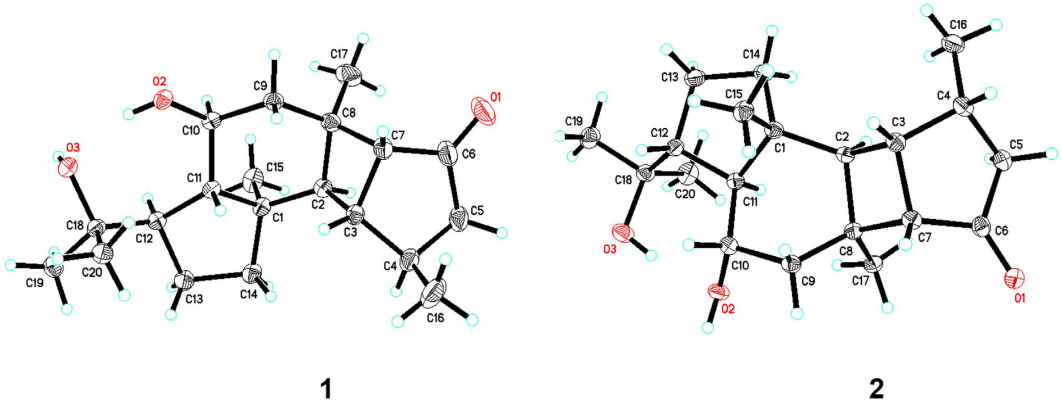

Figure 3. X-ray crystallographic structures of 1 and 2.

carbons C-1 and C-8, and located the three methyl groups at C-1, C-4, and C-8, respectively, demonstrating a tetracyclo [9.3.0.0^{2,8}.0^{3,7}] tetradecane core in **1**. Moreover, the presence of a hydroxy isopropyl group at C-12 was supported by the HMBC correlations from the two remaining methyl groups, H_3-19 and H_3-20, to C-12 and the oxygenated quaternary carbon at δ_C 72.7 (C-18). The unassigned hydroxyl group was attached at the downfield-shifted carbon at (δ_C 67.2, C-10). Thus, the planar structure of hipposponlachnin A (**1**) was established as shown in Fig. 2.

The relative configuration of **1** was determined by NOESY data (Fig. 2). The NOESY correlations of H_3-15/H-2, H_3-15/H_3-17, H_3-15/H-10, H_3-15/H-12, H-2/H-4, and H-2/H_3-17 indicated that these protons and methyl groups were cofacially oriented, whereas the NOESY correlations of H-11/H-3, H-3/H_3-16, H_3-16/H-7, and H-7/H-9α suggested that they were oriented in opposite directions. Therefore, the configuration of **1** was determined and confirmed by single-crystal X-ray diffraction analysis with Cu Kα irradiation as shown in Fig. 3.

Hipposponlachnin B (**2**) was also obtained as a colorless crystal. Its molecular formula of $C_{20}H_{32}O_3$ was deduced from its HRESIMS data, consistent with that of **1**. The overall appearance of the NMR spectrum of **2** revealed close structural similarity between **1** and **2**, indicating the presence of the same tetracyclo [9.3.0.0^{2,8}.0^{3,7}] tetradecane skeleton in both compounds. Further analysis of 1D and 2D NMR spectra of **2** established the planar structure of **2**, which was identical to that of **1** (Fig. 2). Moreover, the almost mirror-image CD spectra of **1** and **2** (see Supplementary Fig. S21) indicated that these two compounds must be a pair of diastereoisomers with

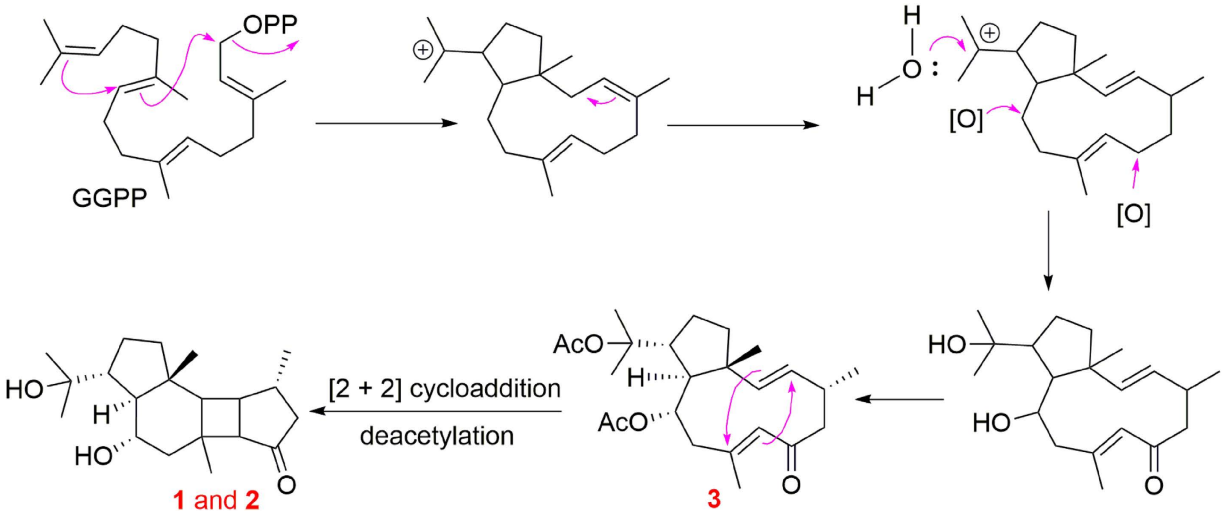

Figure 4. Proposed Biosynthetic Pathway of 1–3.

a variation of configurations at the chiral centers around the carbonyl chromophore. Detailed analysis of the single-crystal X-ray diffraction patterns of **2** confirmed this hypothesis and established the absolute configuration of **2** as 1*R*,2*R*,3*S*,4*R*,7*S*,8*S*,10*S*,11*S*,12*R* (Fig. 3).

Compound **3** was identified as a known dolabellane diterpene, named (1*R**,2*E*,4*R**,7*E*,10*S**,11*S**,12*R**)-10,18-diacetoxydolabella-2,7-dien-6-one, by comparing its physical and spectroscopic features with the data reported in the literature[15,16].

In addition, the biosynthetic pathway for **1–3** was proposed as shown in Fig. 4. **3** could be produced from geranylgeranyl diphosphate (GGPP) following a series of biosynthetic cyclization, migration, oxidation, and acetylation processes[17–19]. The four-membered ring in **1** and **2** might be formed from **3** via an intramolecular [2 + 2] cycloaddition reaction between two double bonds ($\Delta^{2,3}$ and $\Delta^{7,8}$)[7,20]. The deacetylation could be carried out by an esterase[21]. What's more, compound **3** with 7, 8-E geometry rather than 7,8-Z isomer is the predicted precursor of **1** and **2**, because the high rotational barrier around the C_7-C_8 bond in the intermediate of the 7, 8-Z isomer in the [2 + 2] cycloaddition process makes it difficult to form stable *trans*-cyclobutane products (**1** and **2**) (see Supplementary Fig. S26).

Anti-allergic activity evaluation. Subsequently, RBL-2H3 cells were used as a model system to evaluate the anti-allergic activity of **1–2**[22]. As indicated in Fig. 5A, no significant cytotoxicity was observed in RBL-2H3 cells after 24 h of treatment with **1** or **2**. **1** and **2** exhibited higher activity (IC_{50} 49.37 and 23.91 μM, respectively) in the release of β-hexosaminidase inhibition (Fig. 5B), compared with the market-available anti-asthmatic drug, ketotifen fumarate ($IC_{50} = 63.88\ \mu$M). In addition, **1** and **2** suppressed IL-4 production in a dose-dependent manner (Fig. 5C) and significantly inhibited LTB4 release in activated RBL-2H3 cells compared with untreated control (Fig. 5D). The results indicated that **1** and **2** are promising new anti-allergic lead compounds.

In summary, two novel tetracyclic diterpenes (**1–2**) were isolated from the marine sponge *H. lachne*, together with their probable biogenetic precursor (**3**). The structures of new compounds were elucidated by spectroscopic and single-crystal X-ray diffraction analysis. To our knowledge, only one diterpenoid with a similar skeleton, namely vulgarisin A, derived from the Chinese Medicinal Plant *Prunella vulgaris*, has been reported to date[19]. These two novel compounds represented an unprecedented tetracyclo [9.3.0.0^{2,8}.0^{3,7}] tetradecane ring system from marine source for the first time. Moreover, the potent inhibitory activity on the release of β-hexosaminidase for **1–2** suggested that these two bioactive diterpenoids can be the potential therapeutic agents for the treatment of allergy.

Methods

General Experimental Procedures. Optical rotation data were recorded on a PerkinElmer model 341 polarimeter with a 10 cm length cell at room temperature. UV and IR (KBr) spectra were obtained on a Hitachi U-3010 spectrophotometer and Jasco FTIR-400 spectrometer, respectively. CD spectra were obtained on a Jasco J-715 spectropolarimeter in MeCN. NMR spectra including 1D and 2D spectra were acquired at room temperature on Bruker AMX-500 instrument. HRESIMS data were obtained on a Waters Q-Tof micro YA019 mass spectrometer. Reversed-phase HPLC was performed on YMC-Pack Pro C18 RS (5 μm) columns with a Waters 1525 separation module equipped with a Waters 2998 photodiode array detector. Purifications by column chromatography were performed on silica gel 60 (200–300 mesh; Yantai, China), Sephadex LH-20 (18–110 μm, Pharmacia Co.), and ODS (50 μm, YMC Co.). Analytical thin-layer chromatography was carried out using HSGF 254 plates and visualized by spraying with anisaldehyde-H_2SO_4 reagent.

Animal Material. The marine sponge *H. lachne* was collected from the Xisha Islands in the South China Sea in March 2013, and identified by Prof. Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, China). The voucher specimens of *H. lachne* (RM-2013) is deposited at Research Center for Marine Drugs, State

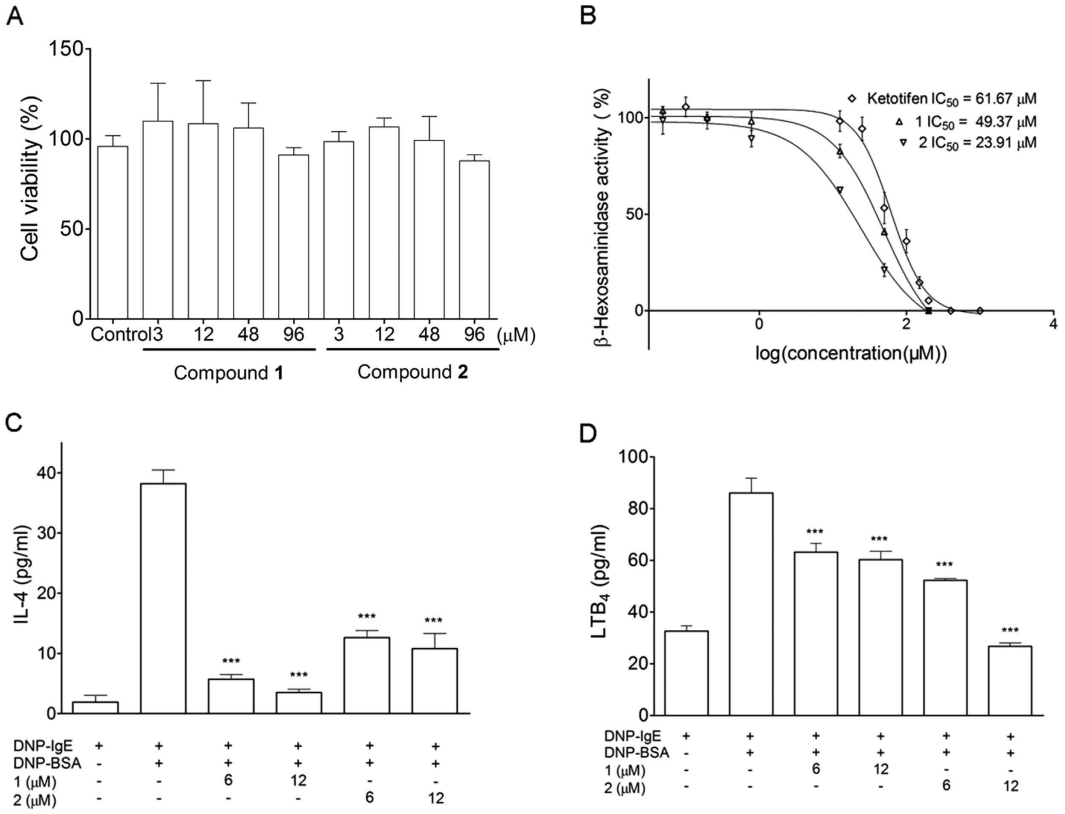

Figure 5. (**A**) Cytotoxic effects of **1** and **2** on RBL-2H3 cells. (**B**) Inhibitory effects of **1** and **2** on the release of β-hexosaminidase in DNP-IgE-activated RBL-2H3 cells. (**C**) Inhibition of IL-4 production of **1** and **2** in RBL-2H3 cells. (**D**) Inhibitory activity on lipid mediator LTB_4 secretion of **1** and **2** in RBL-2H3 cells. Data are presented as the mean ± SD values of triplicate determinations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus DNP-BSA-treated group (n = 3). Data are analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Post-Test (GraphPad Prism 5.0).

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Extraction and Isolation. The sponge *H. lachne* (1.2 kg, dry weight) was cut into small pieces and exhaustively extracted by percolation with 95% EtOH at room temperature to give 56.0 g extract, which was suspended in H_2O and extracted sequentially with EtOAc to afford EtOAc-soluble extract (27.5 g). The EtOAc-soluble extract was dissolved in 90% aqueous MeOH, and extracted with petroleum ether to yield petroleum ether-soluble extract (15.2 g). The 90% aqueous MeOH phase was diluted to 60% MeOH with H_2O, and extracted with CH_2Cl_2 to yield CH_2Cl_2-soluble extract (10.0 g). The CH_2Cl_2-soluble extract was subjected to column chromatography on silica gel with a gradient elution of MeOH in CH_2Cl_2 to give six fractions (A–F). Fraction C was subjected to column chromatography (CC) on Sephadex LH-20 eluting with CH_2Cl_2–MeOH (1:1) to afford three subfractions (C1–C3). Subfraction C2 was separated by CC on ODS to give nine subfractions (C2A–C2I) with 80% MeOH-H_2O as elution. Fr. C2C was purified by reversed-phase semipreparative HPLC (65% MeCN/H_2O, 2.0 mL/min, 250 nm) to obtain **3** (3.0 mg, $t_R = 51.0$ min). Fr. C2D was further purified by reversed-phase semipreparative HPLC (50% MeCN/H_2O, 2.0 mL/min, 210 nm) to obtain **1** (2.4 mg, $t_R = 15.2$ min) and **2** (2.8 mg, $t_R = 18.0$ min).

Chemical structure data. All compounds were ≥98% pure, which were supported by the NMR spectra of the compounds provided in the Supporting Information.

*Hipposponlachnin A (**1**).* Colorless crystal; $[\alpha]_D^{30} + 49$ (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.37) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$) 200 (+0.28), 308 (+0.16) nm; IR (KBr) ν_{max} 3280, 3167, 2943, 2922, 2861, 1732, 1453, 1410, 1377, 1339, 1306, 1252, 1147, 1122, 1076, 1045, 1025, 962, 942, 905, 858, 806, 672 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 343.2251 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2249).

*Hipposponlachnin B (**2**).* Colorless crystal; $[\alpha]_D^{30} - 44$ (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.47) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$) 208 (−0.13), 308 (−0.25) nm; IR (KBr) ν_{max} 3375, 3331, 2953, 2926, 2900, 1726, 1704, 1456, 1412, 1377, 1360, 1329, 1263, 1240, 1171, 1130, 1088, 1032, 1009, 982, 957, 906, 874, 839, 804 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 343.2247 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2249).

(1R,2E,4R*,7E,10S*,11S*,12R*)-10,18-diacetoxydolabella-2,7-dien-6-one (**3**).* Colorless oil; $[\alpha]_D^{30} - 23$ (*c* 0.15, MeOH); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.05 (1H, s, H-7), 5.53 (1H, dd, $J = 16.2$ and 6.0 Hz, H-3), 4.99 (1H, d, $J = 16.2$ Hz, H-2), 4.81 (1H, dd, $J = 10.2$ and 3.0 Hz, H-10), 3.13 (1H, m, H-12), 2.69 (1H, dd, $J = 11.4$ and 3.6 Hz, H-5a), 2.55 (1H, m, H-4), 2.33 (3H, m, H-5b and H-9), 2.06 (3H, s, H-10-OAc), 2.01 (3H, br s, H-17), 1.95 (1H, m, H-13a), 1.92 (3H, s, H-18-OAc), 1.64 (1H, d, $J = 10.2$ Hz, H-11), 1.61 (3H, s, H-19), 1.49 (2H, m, H-13b and H-14a), 1.44 (1H, m, H-14b), 1.39 (3H, s, H-20), 1.02 (3H, d, $J = 6.6$ Hz, H-16), 0.86 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 201.5 (C, C-6), 170.2 (C, C-10-OAc), 170.0 (C, C-18-OAc), 146.3 (C, C-8), 138.2 (CH, C-2), 132.1 (CH, C-3), 131.3 (CH, C-7), 84.7 (C, C-18), 71.9 (CH, C-10), 53.8 (CH, C-11), 51.1 (CH_2, C-5), 47.8 (C, C-1), 44.9 (CH, C-12), 43.8 (CH_2, C-9), 39.1 (CH_2, C-14), 34.1 (CH, C-4), 26.7 (CH_3, C-19), 26.2 (CH_2, C-13), 23.3 (CH_3, C-20), 23.0 (CH_3, C-18-OAc), 21.5 (CH_3, C-17), 21.0 (CH_3, C-10-OAc), 18.3 (CH_3, C-16), 17.5 (CH_3, C-15); HRESIMS *m/z* 427.2451 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_5Na$, 427.2460).

X-ray Crystallographic Analysis of 1 and 2. Hipposponlachnins A (**1**) and B (**2**) were crystallized from MeOH at room temperature. The X-ray crystallographic data of them were measured on a Bruker Apex-II CCD diffractometer employing graphite monochromated Cu-Kα radiation ($\lambda = 1.54178$ Å) at 140(2) K (operated in the φ-ω scan mode). The structures were solved by direct method using SHELXS-97 program and refined with full-matrix least-squares calculations on F^2 using SHELXL-97. Crystallographic data for **1** and **2** have been deposited at the Cambridge Crystallographic Data Centre. Copies of these data can be obtained free of charge via the internet at www.ccdc.cam.ac.uk/conts/retrieving.html or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Tel: (+44) 1223-336-408; Fax: (+44) 1223-336-033; E-mail: deposit@ccdc.cam.ac.uk].

*Crystallographic data of **1**.* Colorless crystal, $C_{20}H_{32}O_3$, $M_r = 320.45$, orthorhombic, space group $P2_12_12_1$, $a = 6.62210(10)$ Å, $b = 15.2395(3)$ Å, $c = 18.4069(3)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 1857.58(6)$ Å^3, $T = 140$ K, $Z = 4$, $D_{calcd} = 1.146$ g/cm^3, crystal size $0.20 \times 0.12 \times 0.05$ mm^3, $\mu = 0.588$ mm^{-1}, F(000) = 704, 8888 reflections measured ($7.53° < 2\theta < 139.318°$), 3177 unique ($R_{int} = 0.0311$, $R_{sigma} = 0.0335$). The final R_1 value is 0.0354 [$I > 2\sigma(I)$] and $wR_2 = 0.0953$ (all data). The goodness of fit on F^2 was 1.060. Flack parameter = 0.13 (13). CCDC deposition number: 1501503.

*Crystallographic data of **2**.* Colorless crystal, $C_{20}H_{32}O_3$, $M_r = 320.45$, monoclinic, space group $P2_1$, $a = 8.17720(10)$ Å, $b = 9.71010(10)$ Å, $c = 11.2700(2)$ Å, $\alpha = \gamma = 90°$, $\beta = 92.8110(10)°$, $V = 893.78(2)$ Å^3, $T = 140$ K, $Z = 2$, $D_{calcd} = 1.191$ g/cm^3, crystal size $0.20 \times 0.10 \times 0.02$ mm^3, $\mu = 0.611$ mm^{-1}, F(000) = 352, 6940 reflections measured ($7.854° < 2\theta < 139.132°$), 2953 unique ($R_{int} = 0.0248$, $R_{sigma} = 0.0310$). The final R_1 value is 0.0307 [$I > 2\sigma(I)$] and $wR_2 = 0.0818$ (all data). The goodness of fit on F^2 was 1.066. Flack parameter = −0.03 (9). CCDC deposition number: 1501504.

Anti-allergic assay. Cell viability were determined using the MTT method. RBL-2H3 cells were plated into a 96-well plate at 5×10^5 cells per well (100 μL/well) for 24 h. Subsequently, cells were incubated with hipposponlachnins A (**1**) and B (**2**) for 24 hours and medium was replaced with MTT solution (250 μg/mL) and incubated at 37 °C for 4 h. The medium was carefully discarded and formazan was resuspended in 150 μL of dimethyl sulfoxide (DMSO). The absorbance was measured at 490 nm using a microplate reader. Values measured from untreated cells were considered to represent 100% viability. RBL-2H3 cells were seeded in a 24-well plate at 5×10^5 cells per well, and sensitized with dinitrophenyl (DNP)-specific IgE (DNP-IgE) (1 μg/mL) at 37 °C overnight. DNP-IgE-sensitized cells were preincubated with hipposponlachnins A (**1**) and B (**2**) for 30 min, and then stimulated with DNP-BSA for 1.5 h. To measure the activity of β-hexosaminidase released from the cells, cultured media were centrifuged (17,000 g, 10 min) at 4 °C. The supernatant (50 μL) was mixed with 50 μL of 0.1 M sodium citrate buffer (pH 4.5) containing 10 mM 4-nitrophenyl N-acetyl-β-D-glucosaminide in a 96-well plate, and then incubated for 90 min at 37 °C. The absorbance was measured at 405 nm after terminating the reaction by 0.2 M glycine (pH 10.0). To measure Interleukin-4 (IL-4) and Leukotriene B_4 (LTB_4) level in cultured media, all cultured media were centrifuged at 4 °C, and hipposponlachnins A (**1**) and B (**2**) were stored at −80 °C until assay. IL-4 and LTB4 were quantified using an ELISA kit according to the manufacturer's instructions. The data were analyzed using a one-way ANOVA followed by Dunnett's Multiple Comparison Test with GraphPad Prism software (GraphPad Prism version 5.01 for Windows, San Diego, CA, USA). The values are expressed as the means ± SD. The differences with $p < 0.05$ were considered significant.

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Author Contributions

L.-L.H. conducted the experiments. L.-L.H. and H.-B.Y. wrote the manuscript; J.W. assisted extraction and isolation; W.-H.J. and B.-H.C. assisted the biological test; W.-H.J., F.Y., B.-B.G., and Y.-J. Z. assisted the structure elucidation and analysed the data. S.-J.S. and H.-W.L. revised and polished the manuscript. All authors reviewed the manuscript.

Additional Information

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Supplementary Information

Unusual Anti-allergic Diterpenoids from the Marine Sponge

Hippospongia lachne

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Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of hipposponlachnin A (**1**)

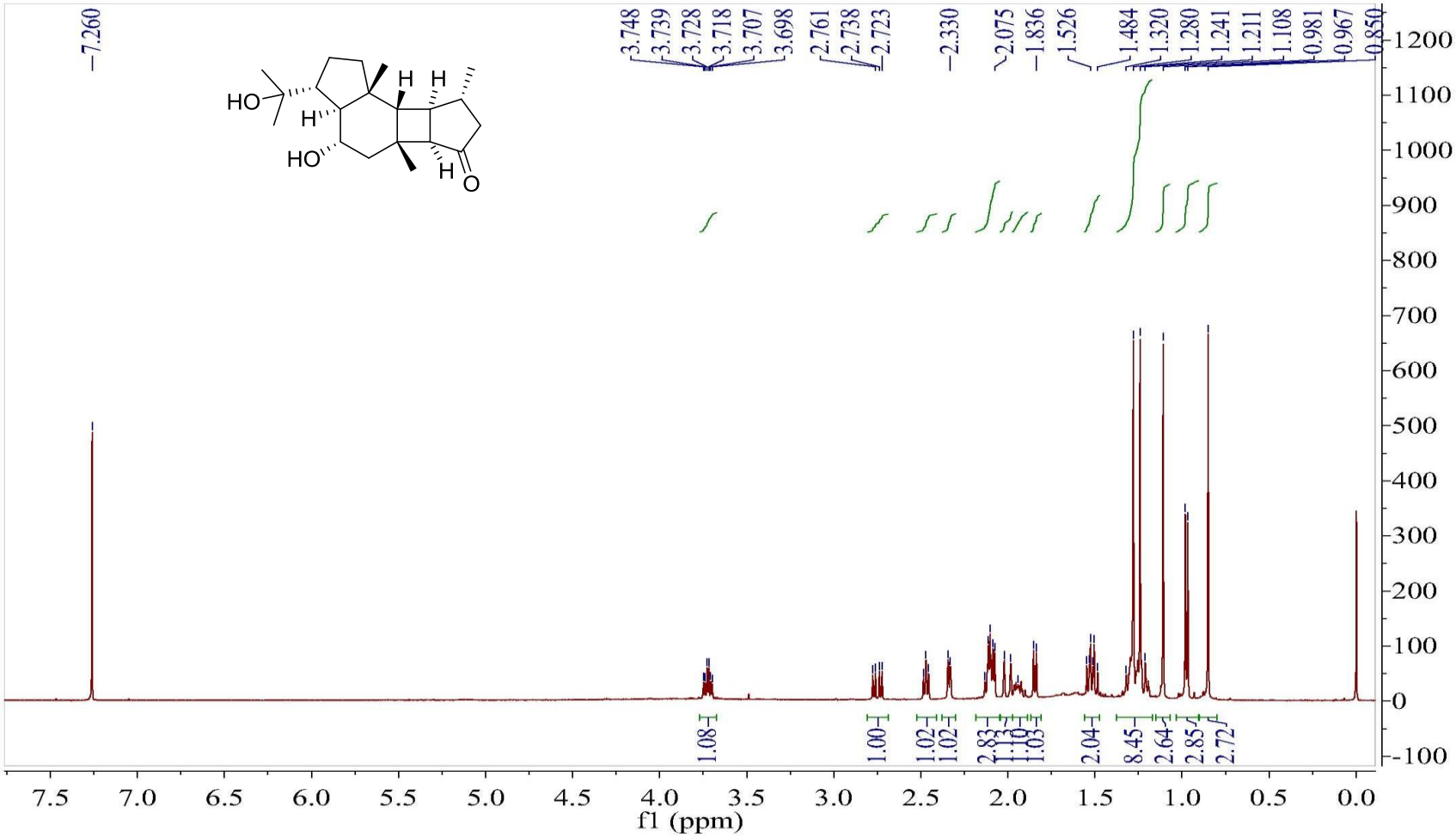


Figure S2. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of hipposponlachnin A (**1**)

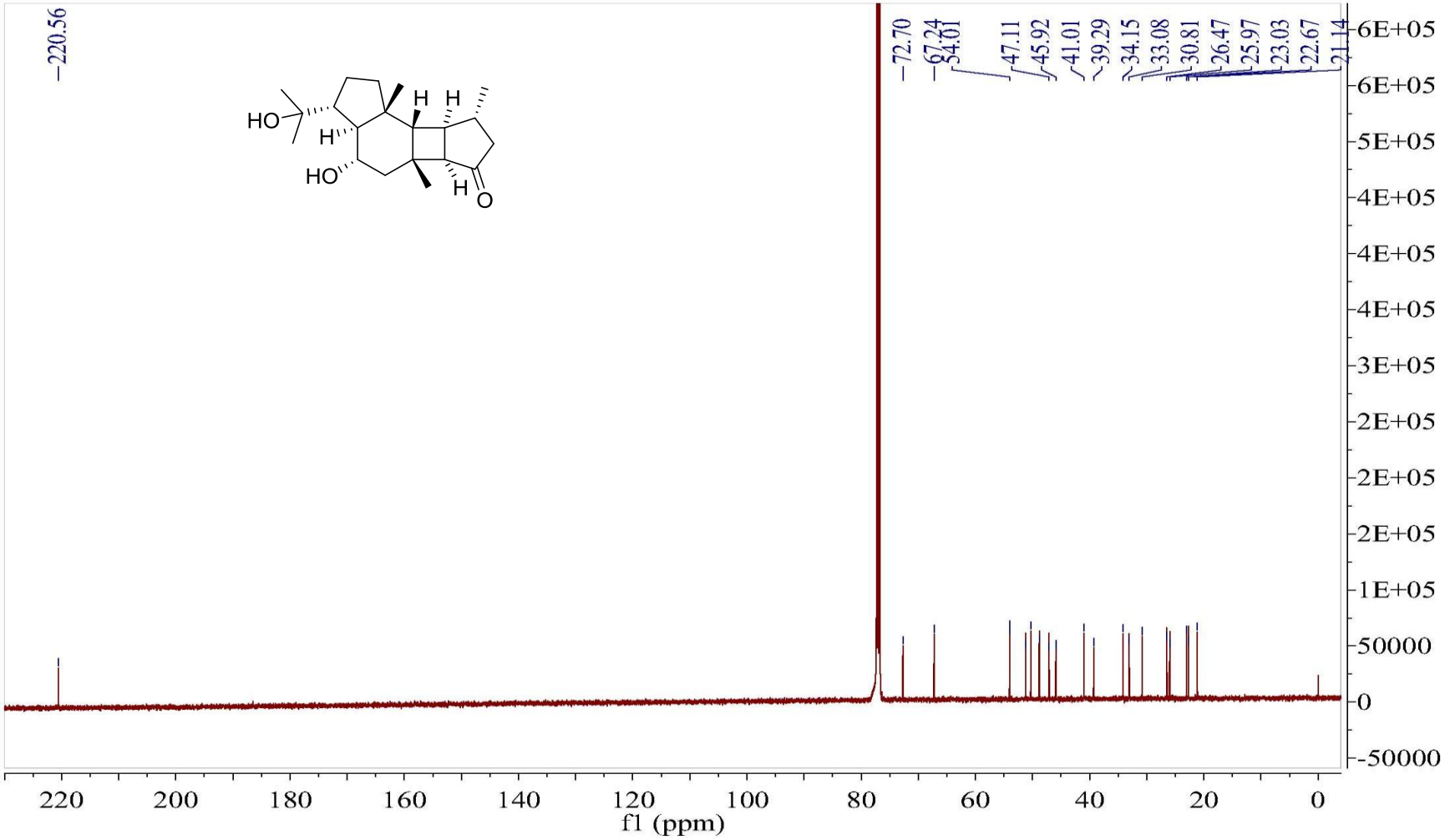


Figure S3. DEPT135 spectrum of hipposponlachnin A (**1**) in $CDCl_3$

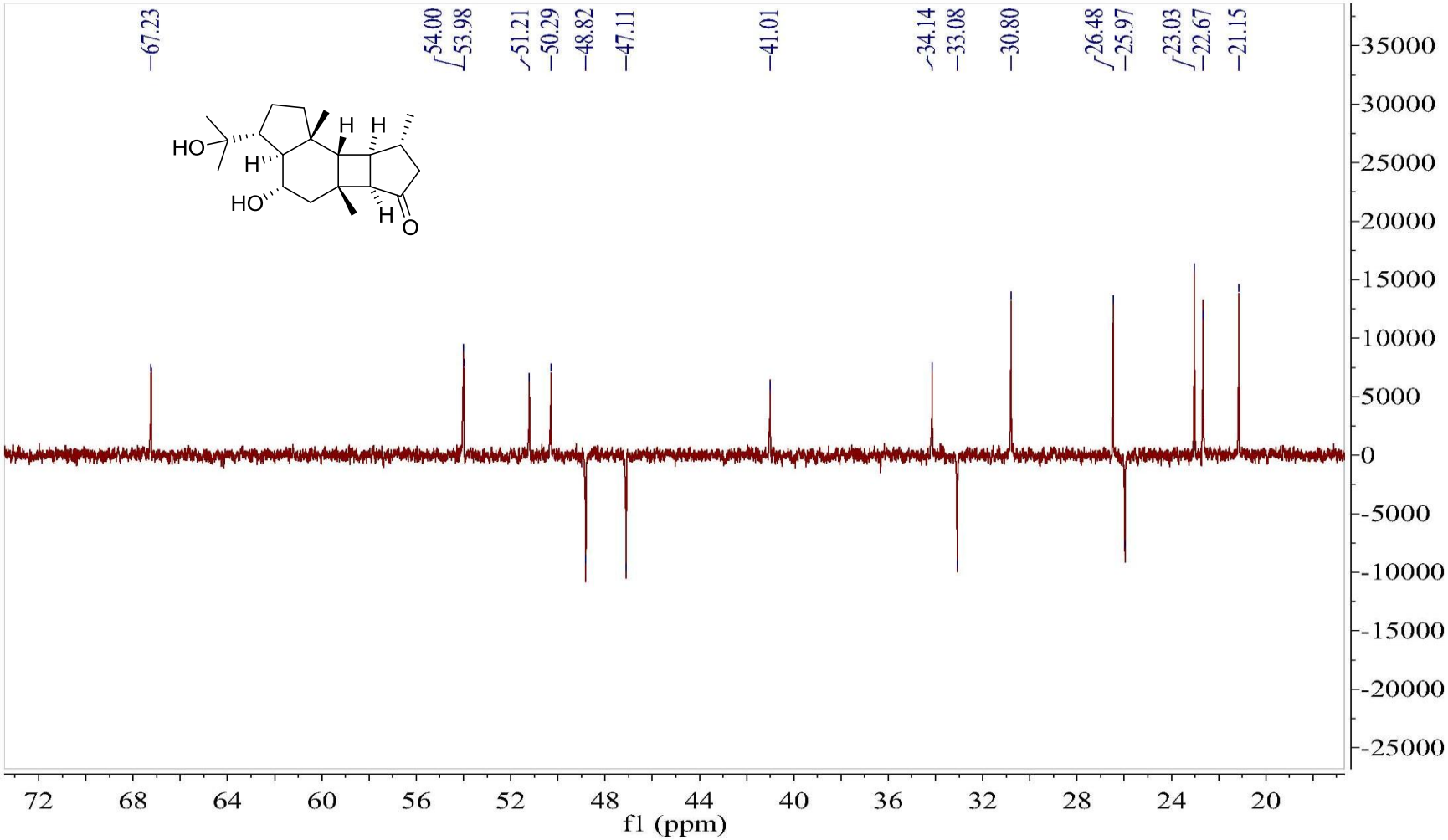


Figure S4. HSQC spectrum of hipposponlachnin A (**1**) in $CDCl_3$

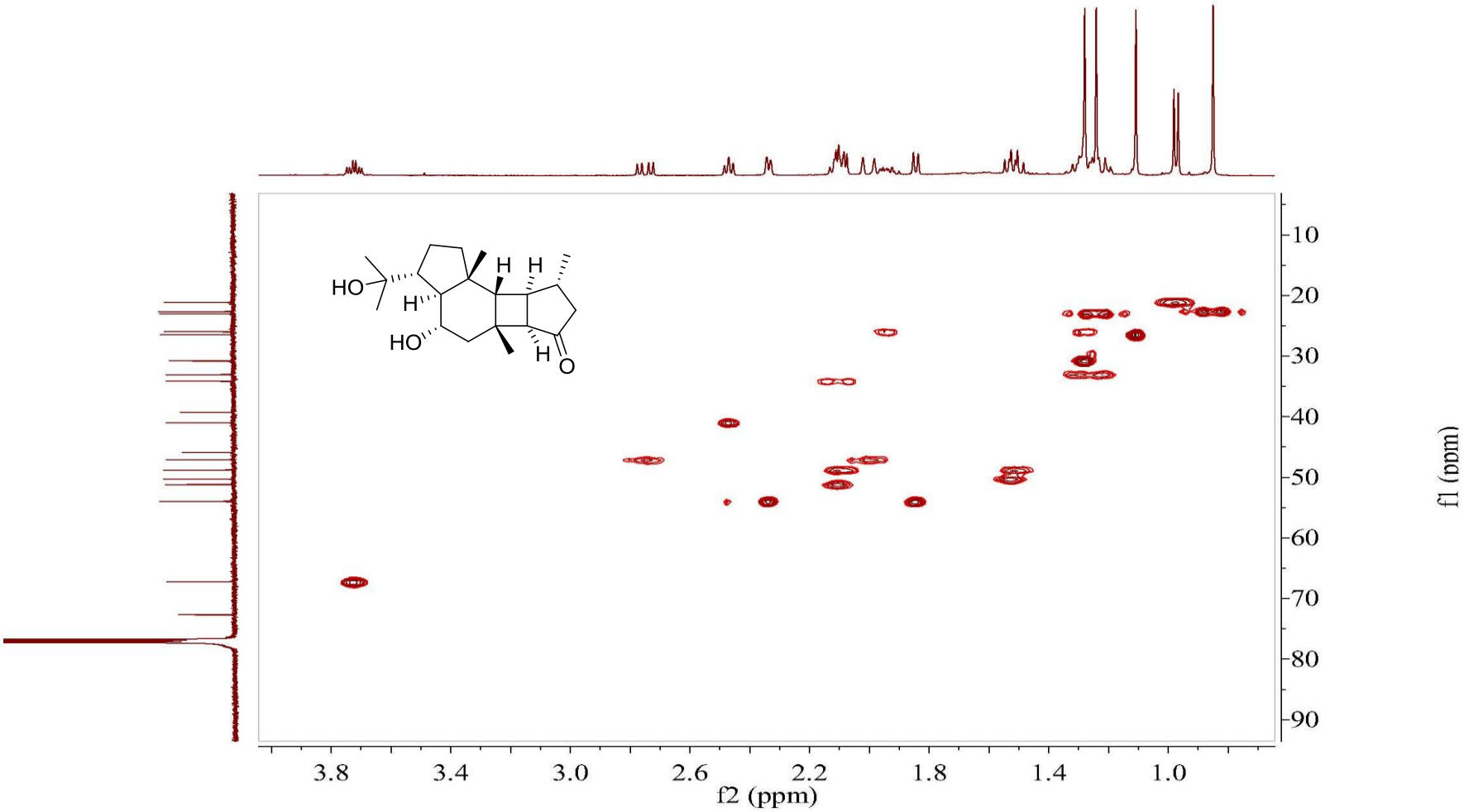


Figure S5. COSY spectrum of hipposponlachnin A (**1**) in $CDCl_3$

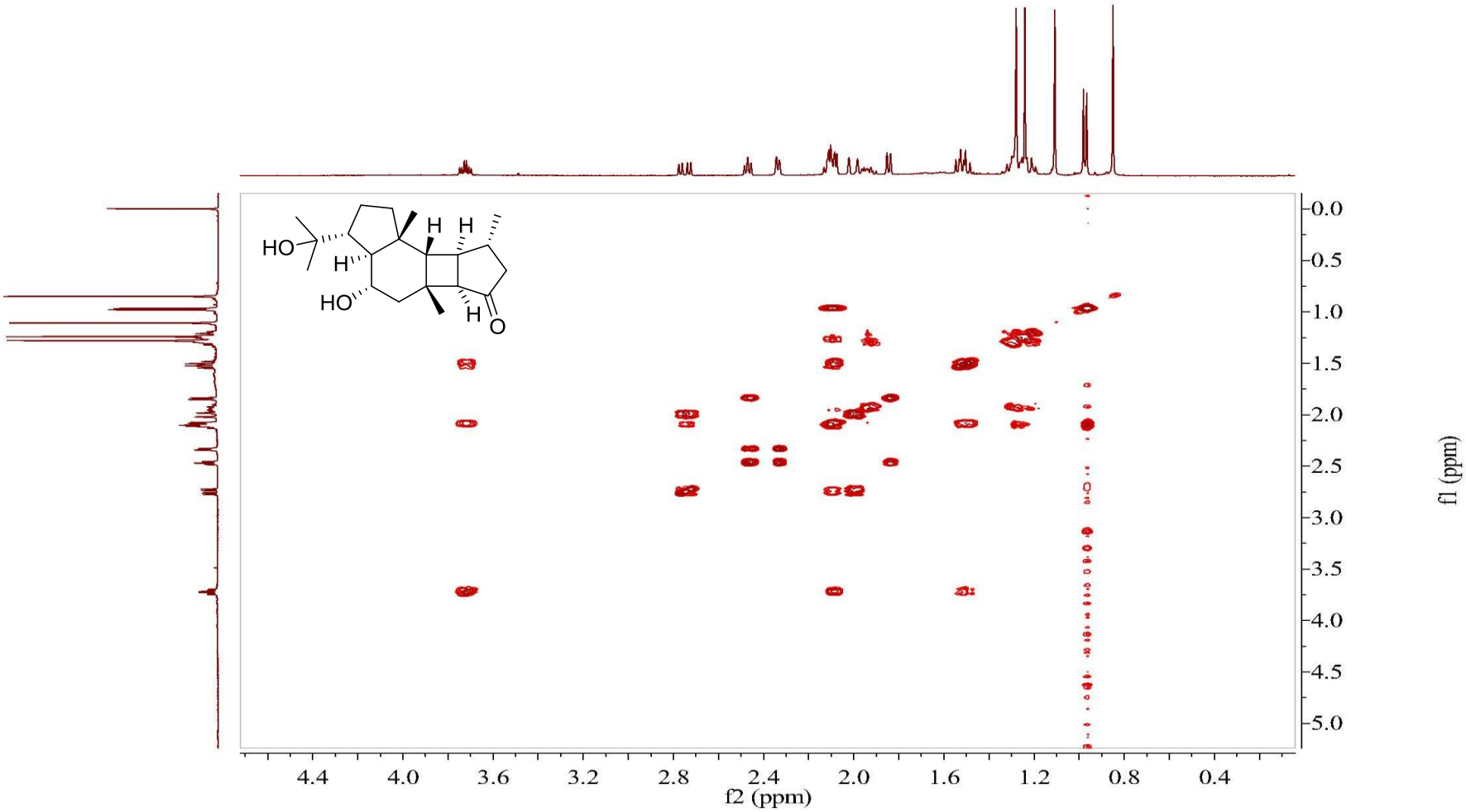


Figure S6. HMBC spectrum of hipposponlachnin A (**1**) in $CDCl_3$

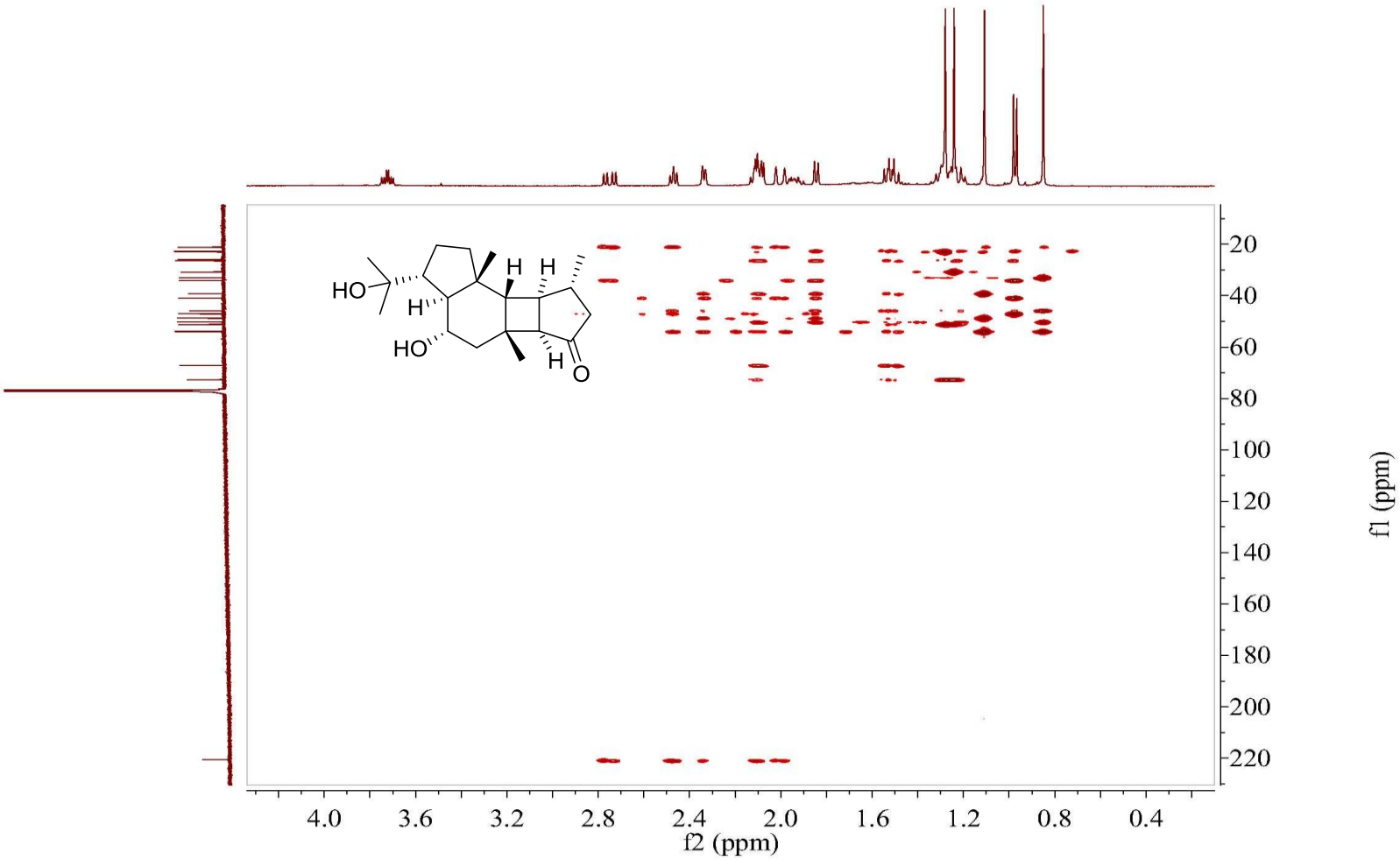


Figure S7. NOESY spectrum of hipposponlachnin A (**1**) in $CDCl_3$

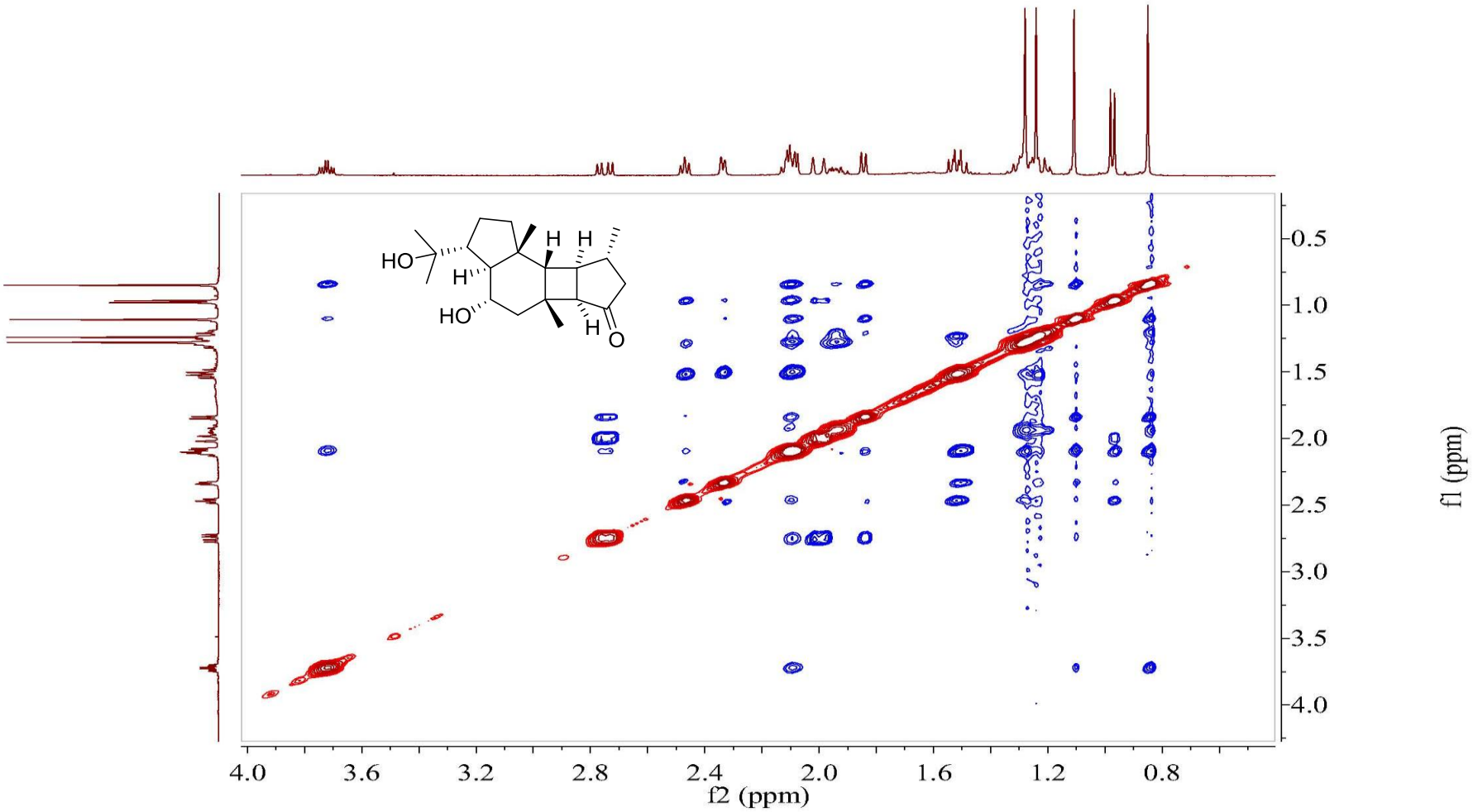
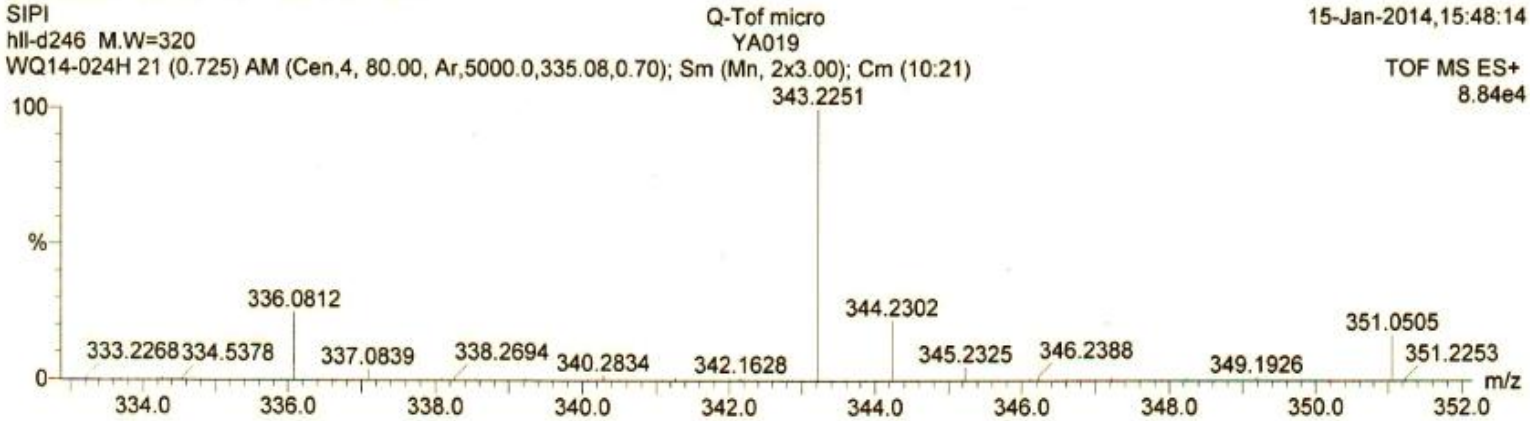


Figure S8. HRESIMS of hipposponlachnin A (**1**)

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
8 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 10-22 H: 10-40 O: 1-4 Na: 1-1



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Maximum:	100.00		5.0	10.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
343.2251	100.00	343.2249	0.2	0.6	4.5	295.3	C20 H32 O3 Na

Figure S9. UV spectrum of hipposponlachnin A (**1**) in MeOH

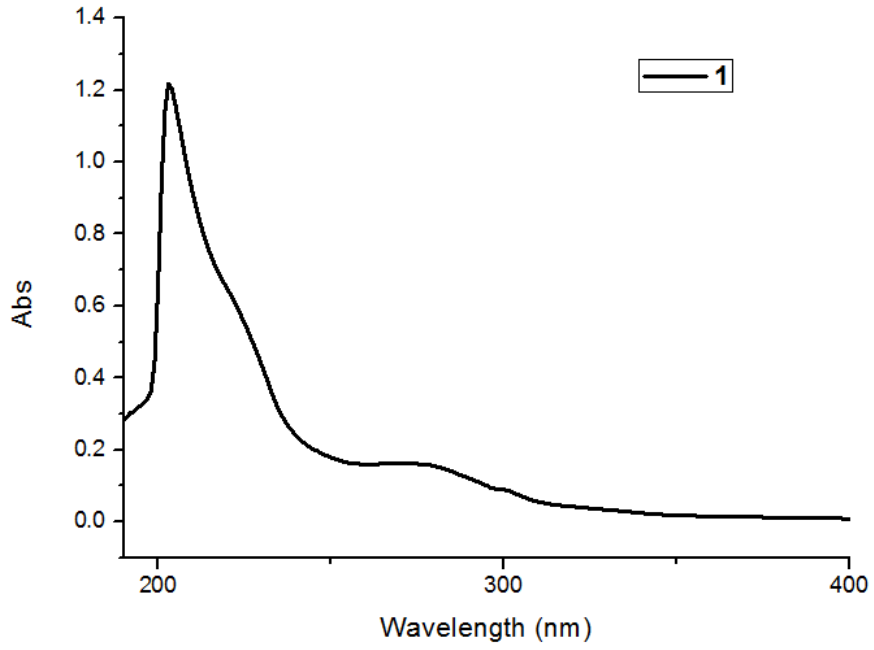


Figure S10. IR spectrum of hipposponlachnin A (**1**)

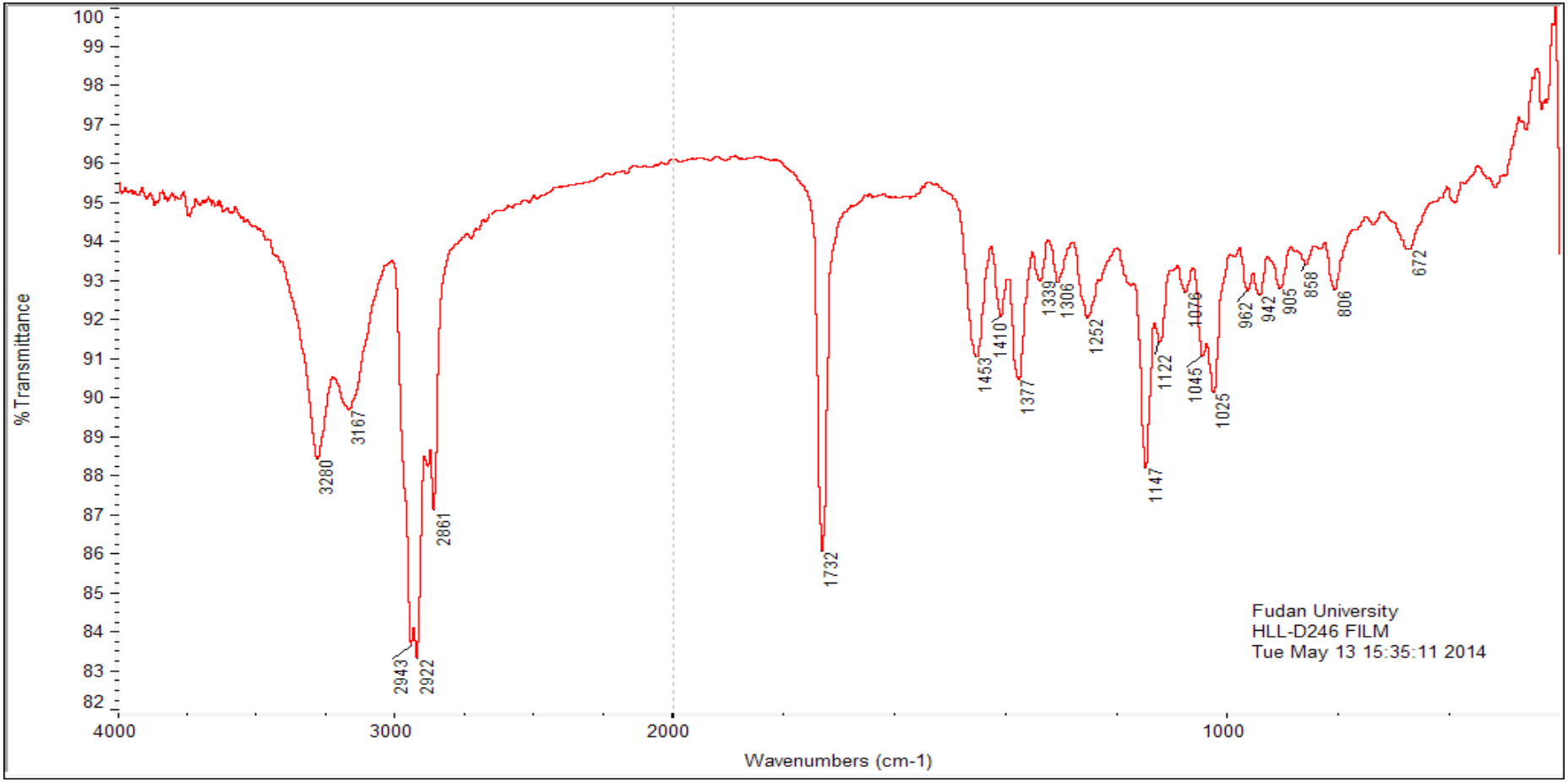


Figure S11. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of hipposponlachnin B (**2**)

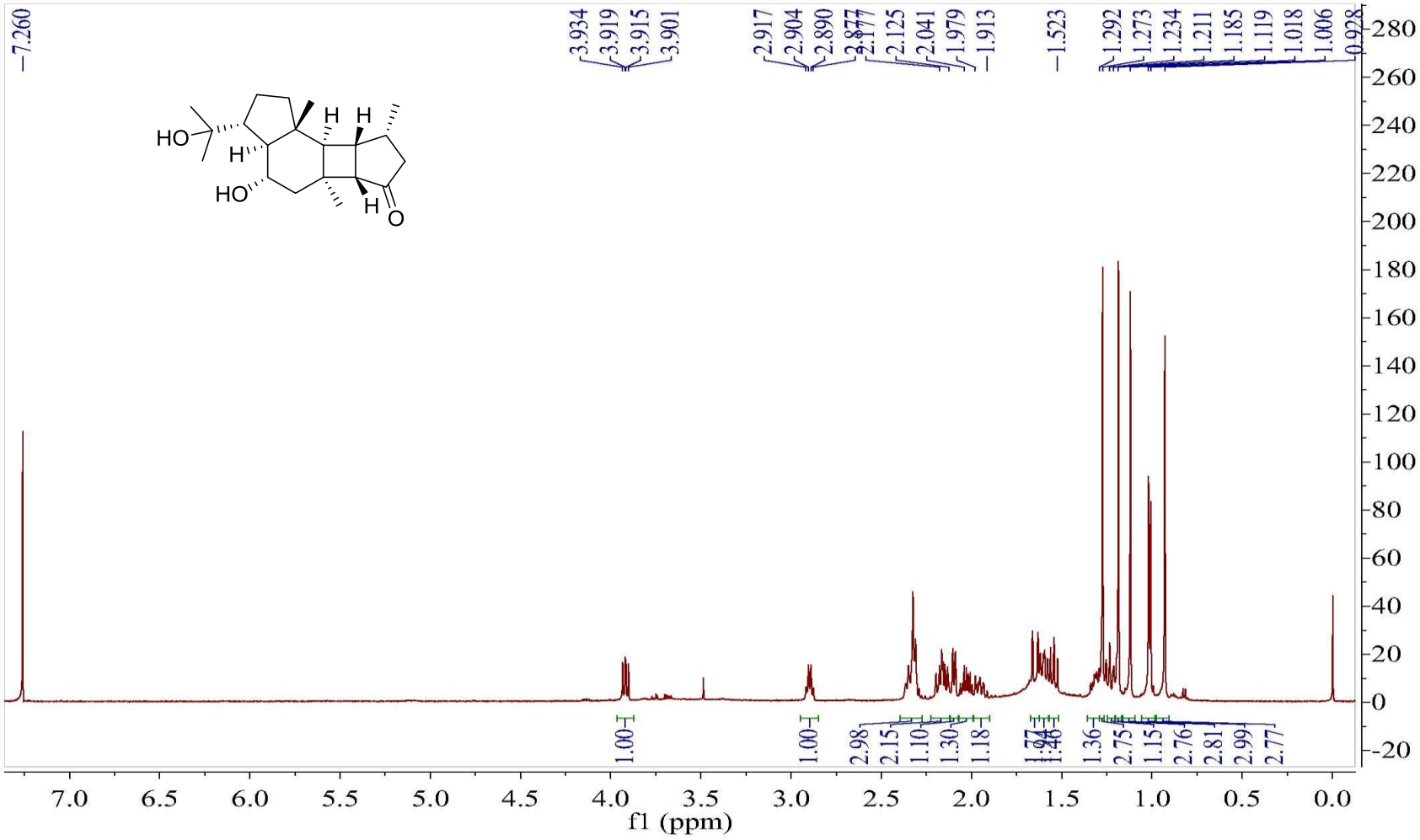


Figure S12. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of hipposponlachnin B (**2**)

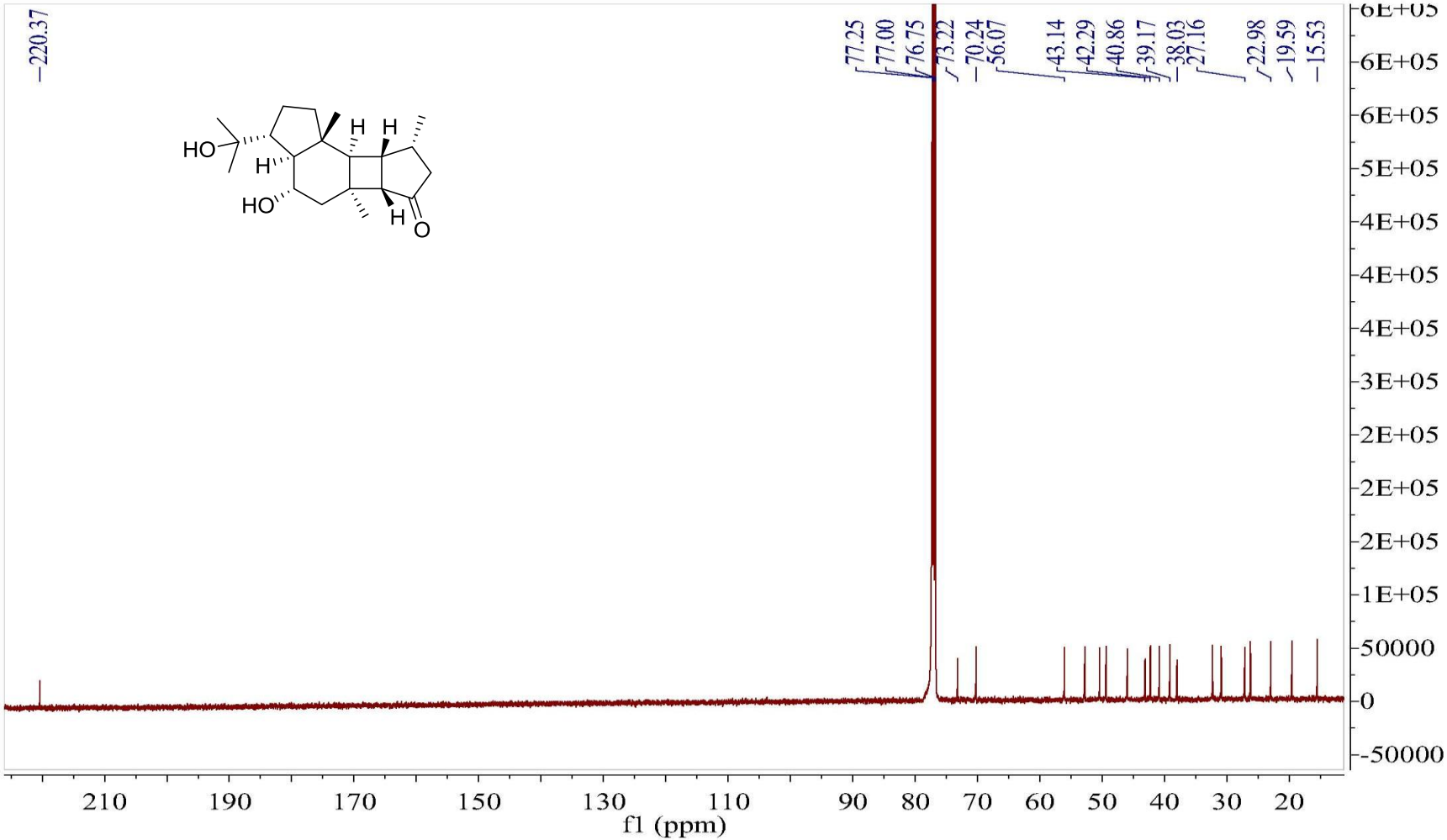


Figure S13. DEPT135 spectrum of hipposponlachnin B (**2**) in $CDCl_3$

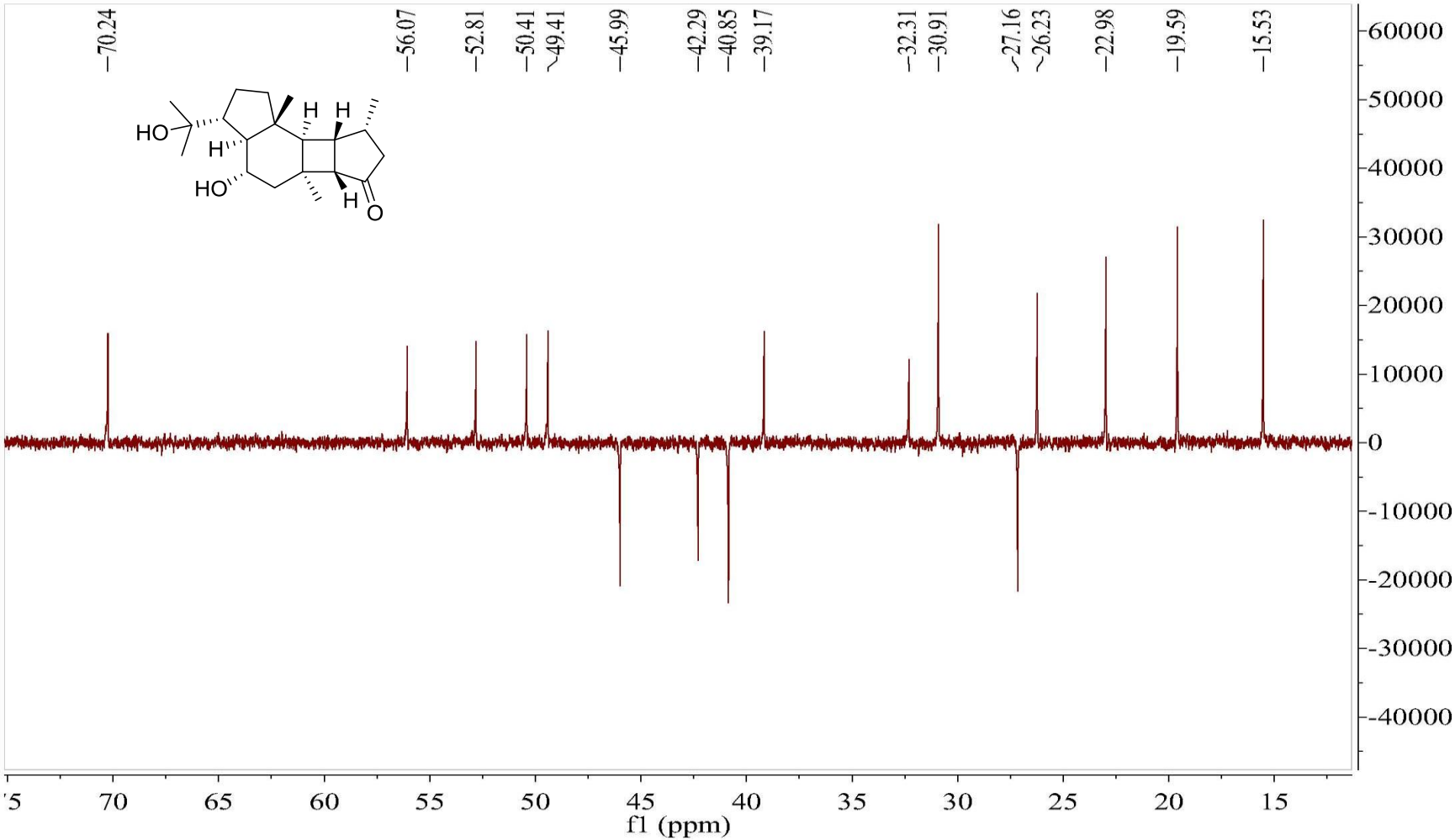


Figure S14. HSQC spectrum of hipposponlachnin B (**2**) in $CDCl_3$

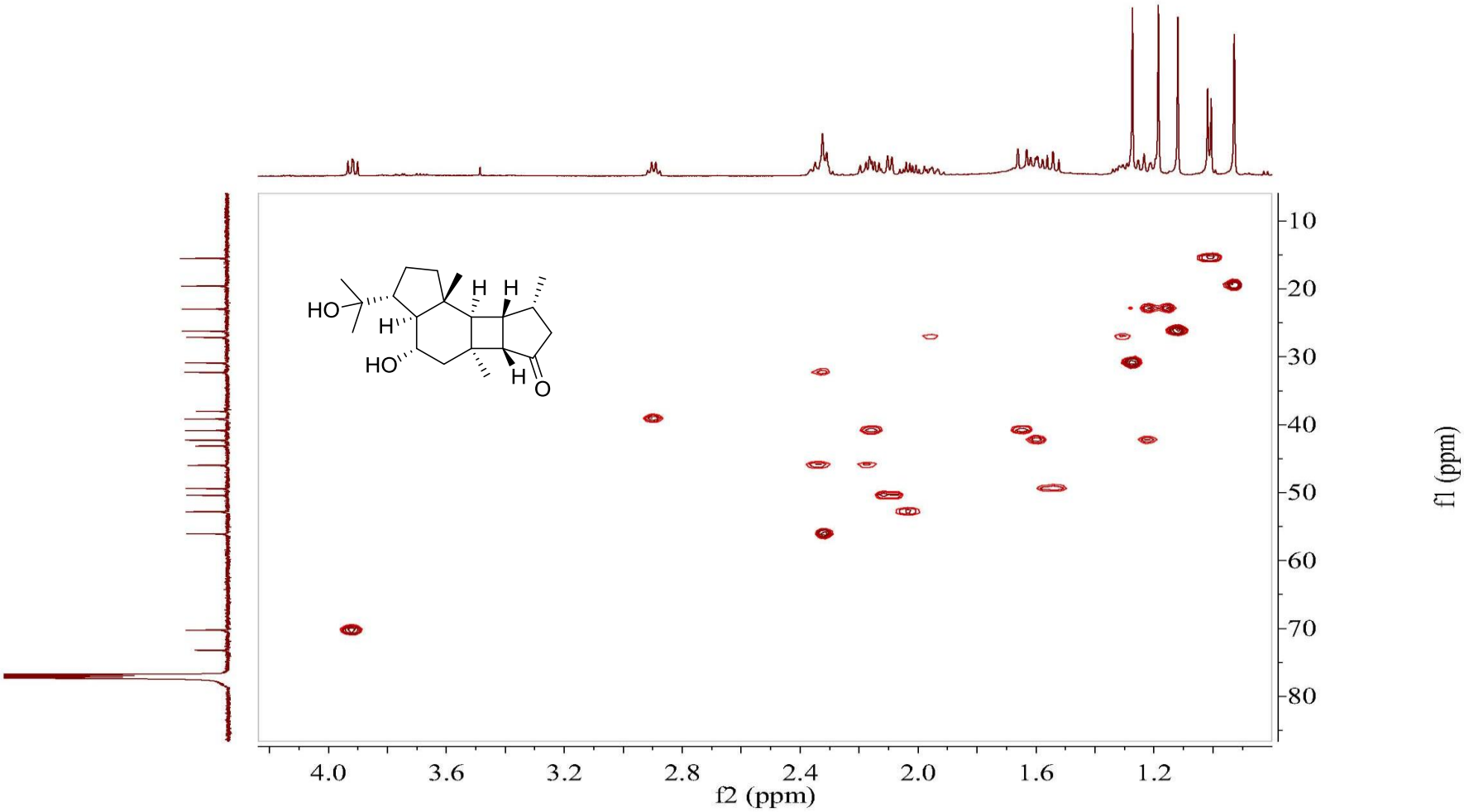


Figure S15. COSY spectrum of hipposponlachnin B (**2**) in $CDCl_3$

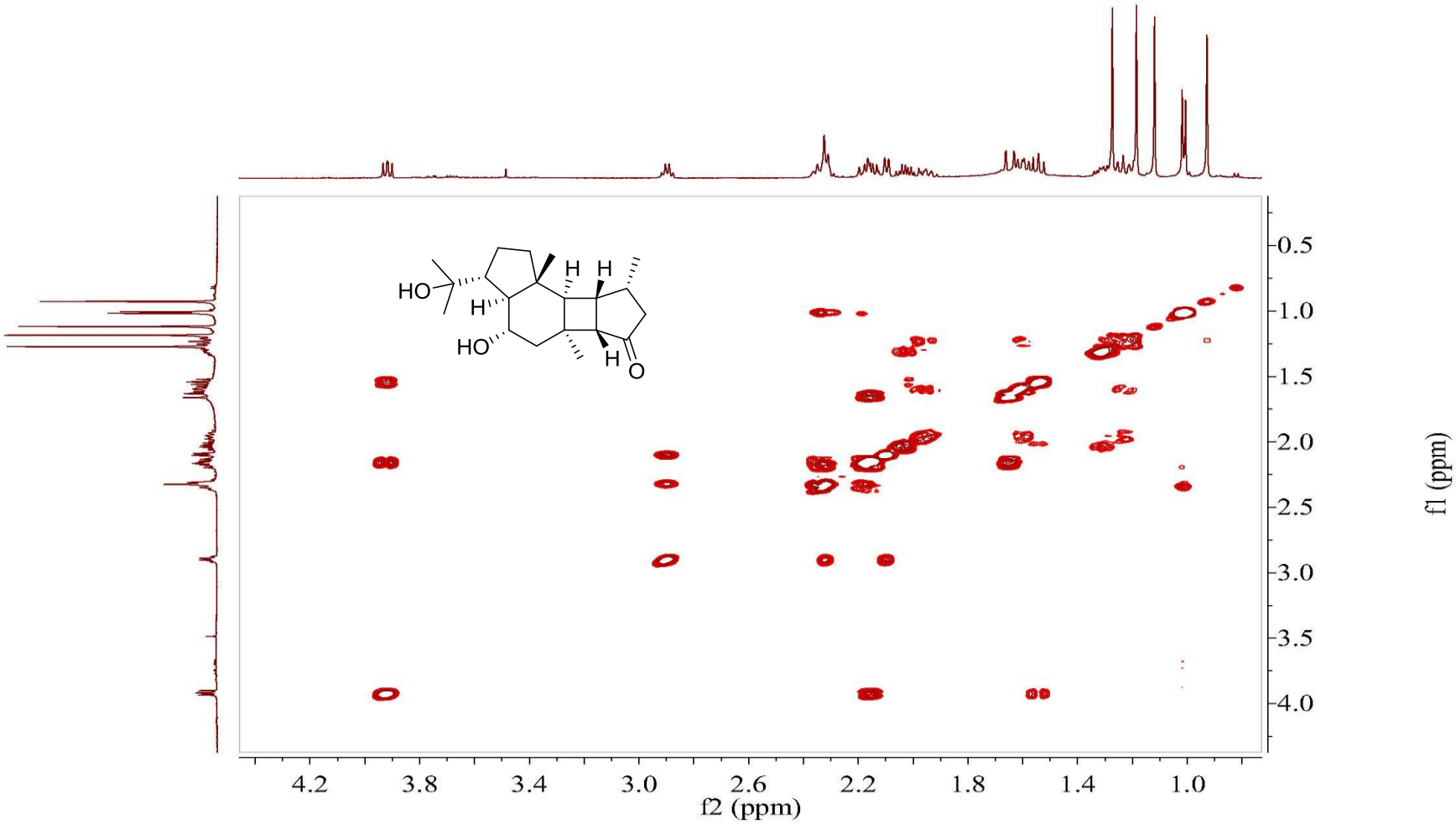


Figure S16. HMBC spectrum of hipposponlachnin B (**2**) in $CDCl_3$

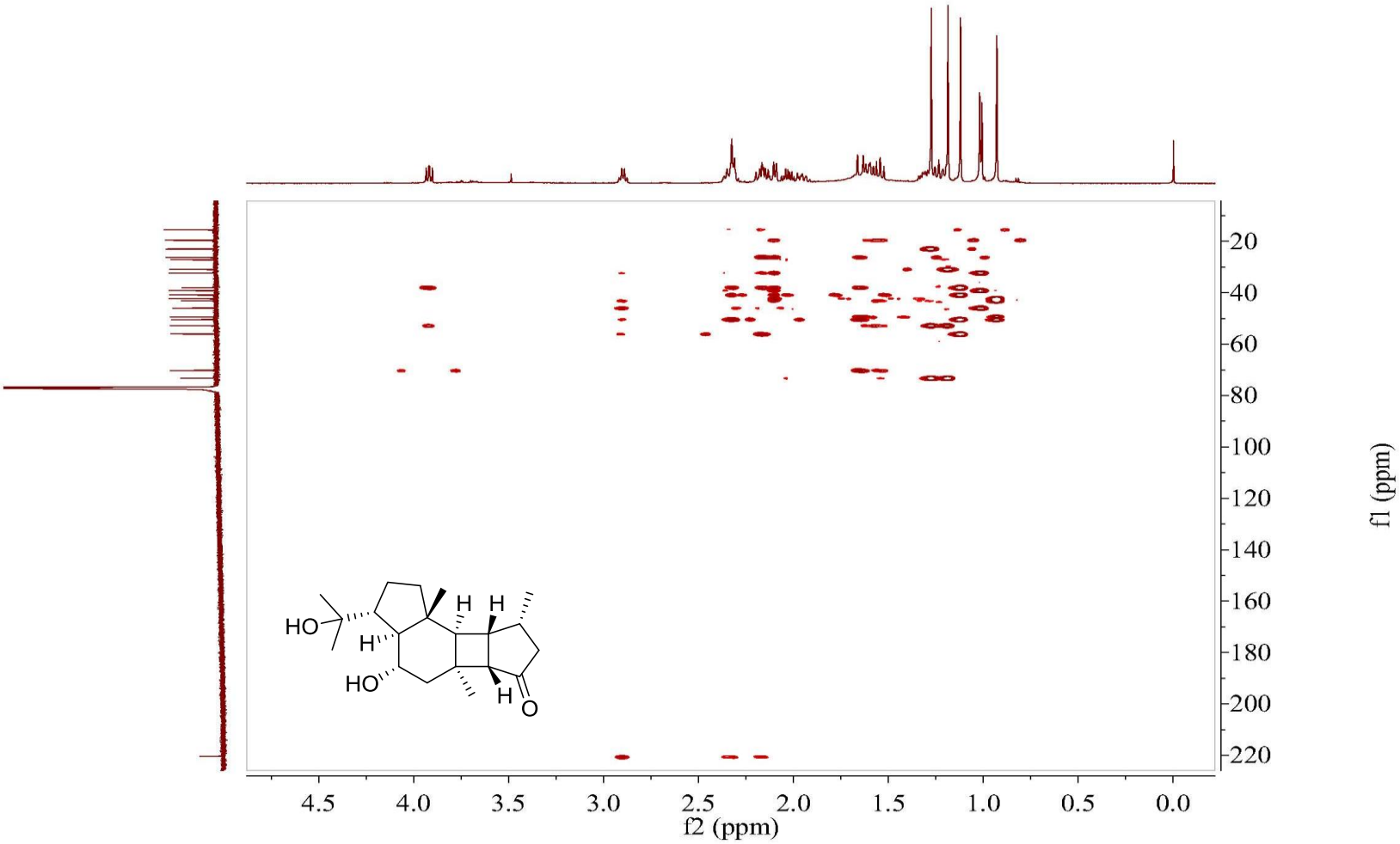


Figure S17. NOESY spectrum of hipposponlachnin B (**2**) in $CDCl_3$

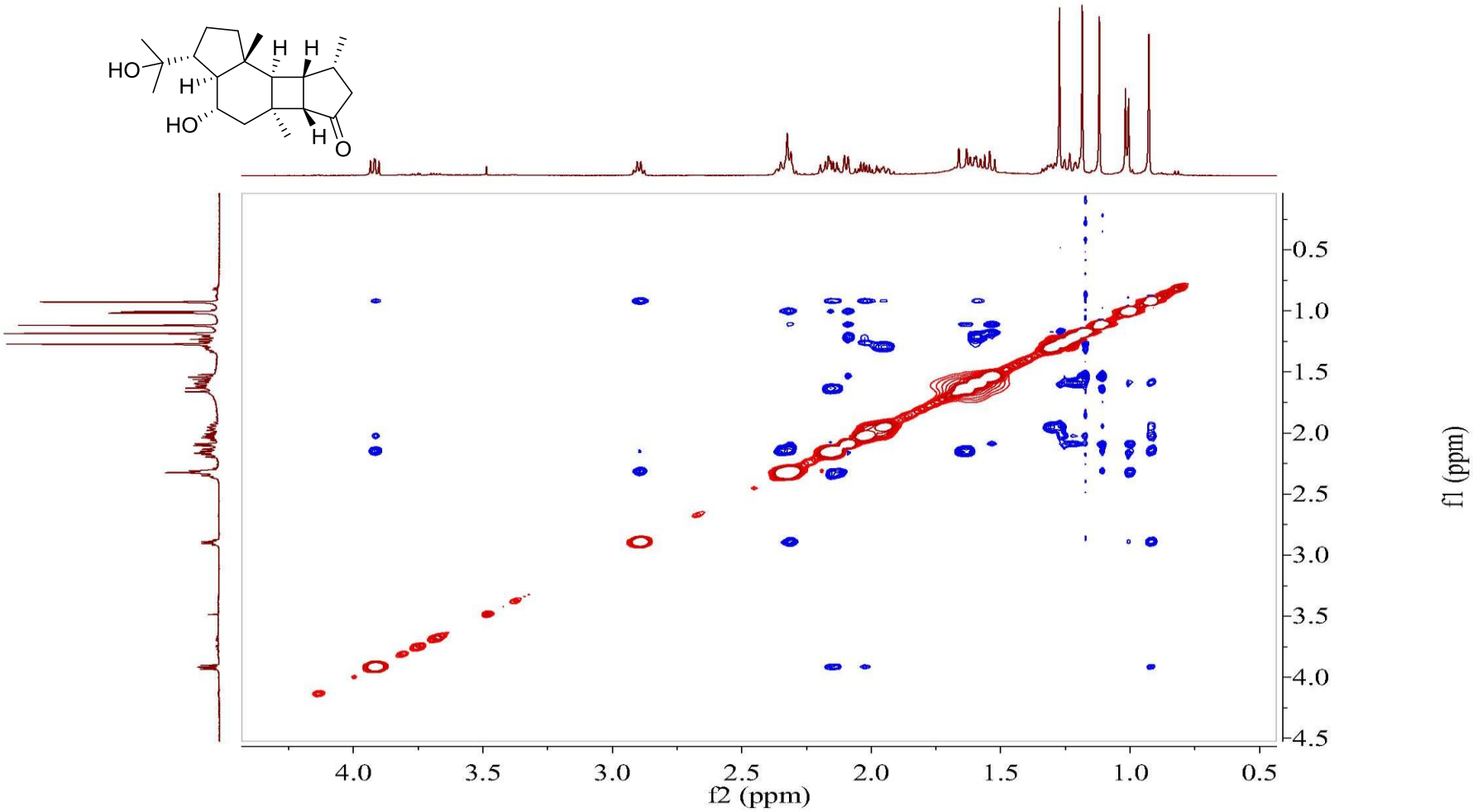
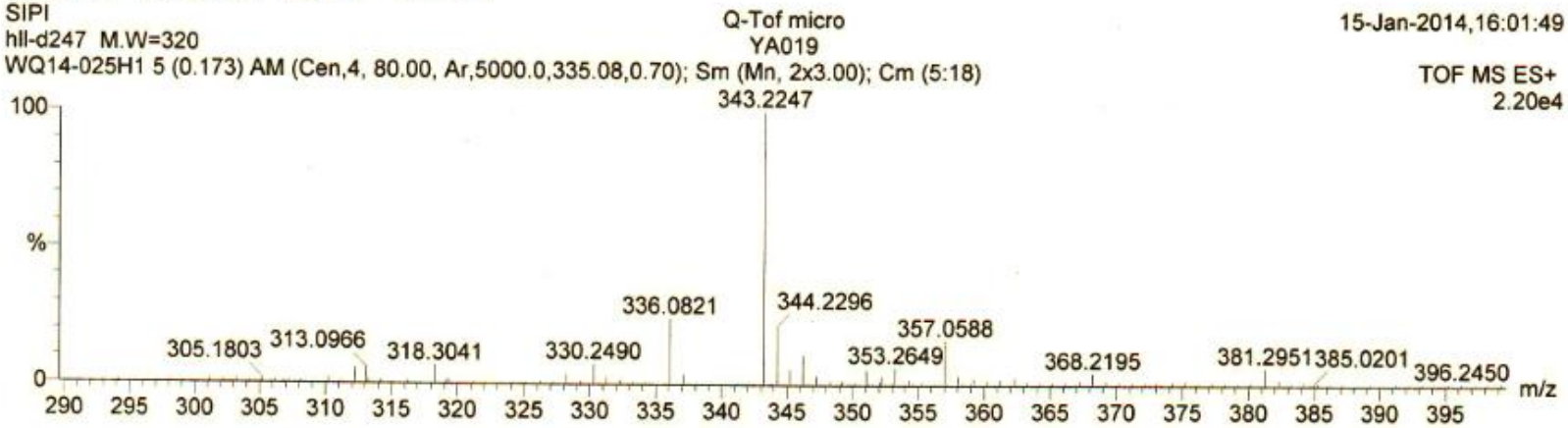


Figure S18. HRESIMS of hipposponlachnin B (**2**)

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
8 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 10-22 H: 10-40 O: 1-4 Na: 1-1



Minimum:	62.00				-1.5		
Maximum:	100.00		5.0	10.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
343.2247	100.00	343.2249	-0.2	-0.6	4.5	130.2	C20 H32 O3 Na

Figure S19. UV spectrum of hipposponlachnin B (**2**) in MeOH

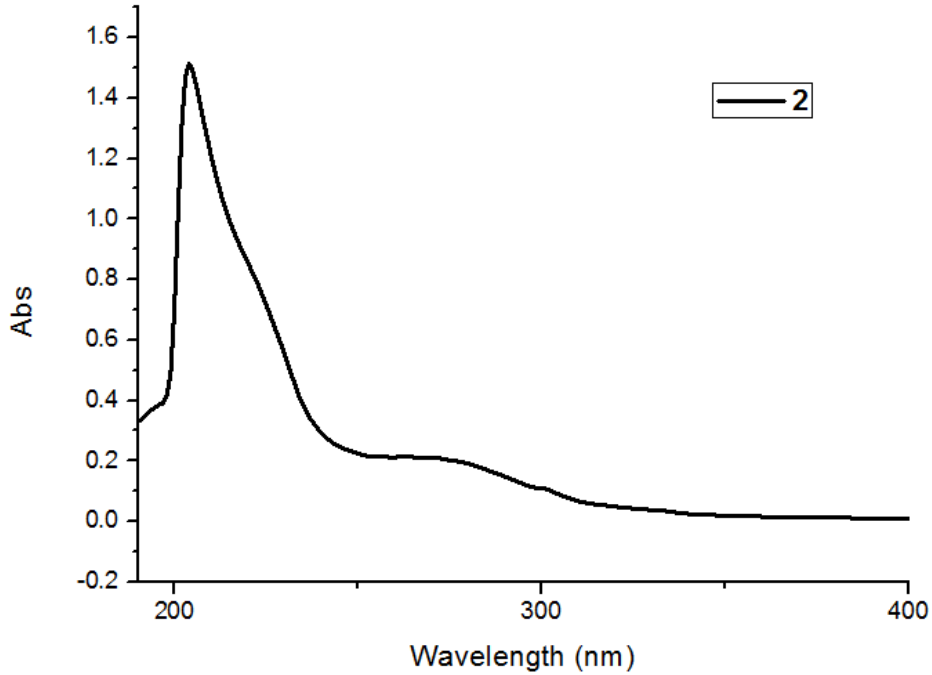


Figure S20. IR spectrum of hipposponlachnin B (**2**)

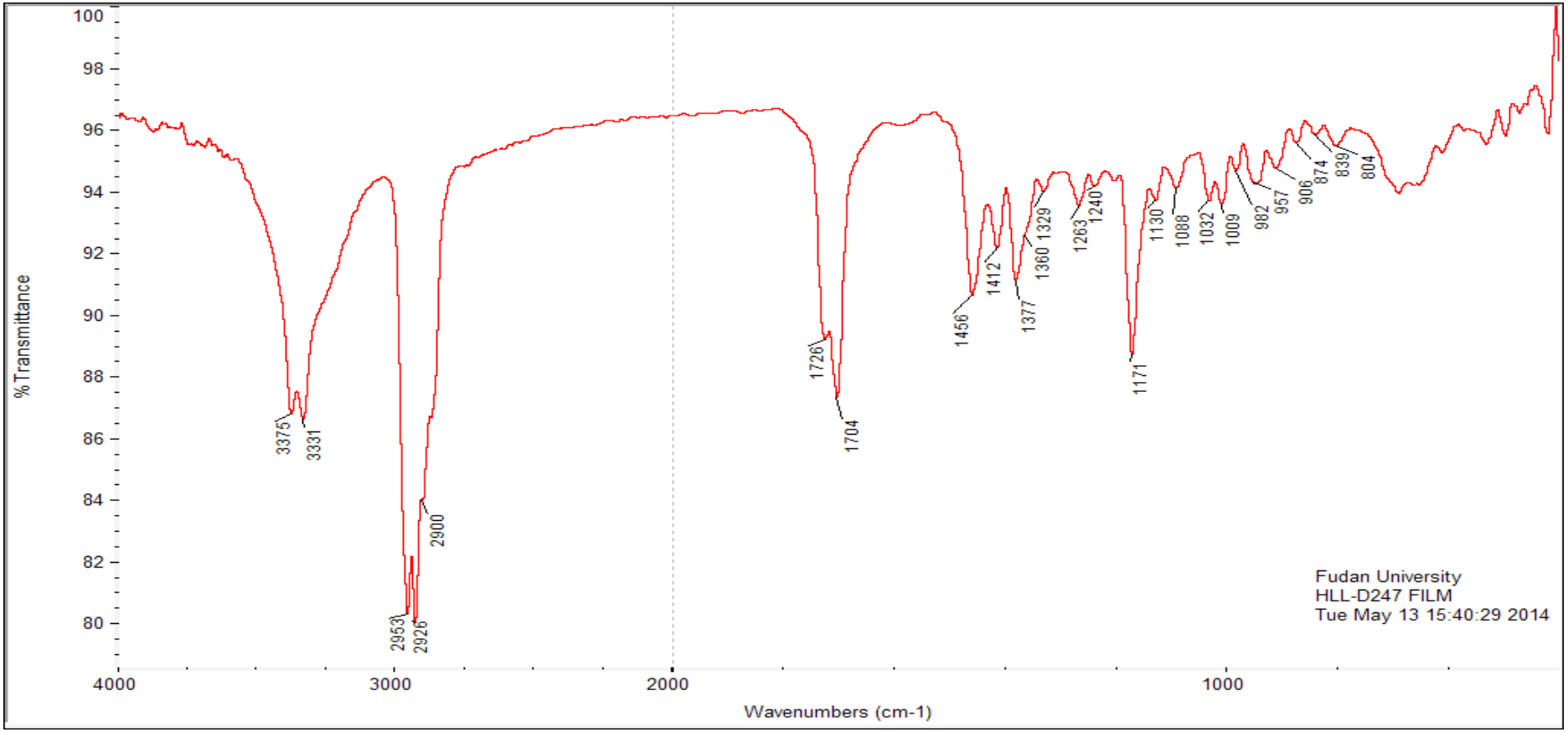


Figure S21. CD spectra of hipposponlachnin A **(1)** and B (**2**) in MeCN.

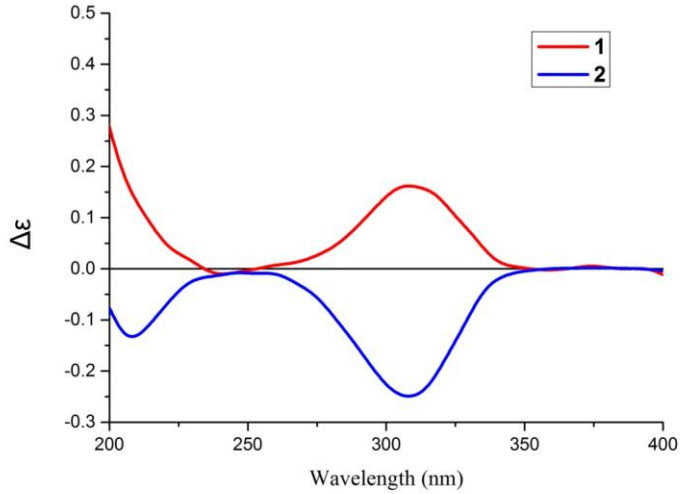


Figure S22. ESIMS of **3**.

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
263 formula(e) evaluated with 7 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O	Na
427.2451	427.2460	-0.9	-2.1	6.5	C24 H36 O5 Na	1756.7	0.207	81.34	24	36	5	1
	427.2436	1.5	3.5	3.5	C22 H37 O5 Na2	1758.3	1.799	16.54	22	37	5	2
	427.2484	-3.3	-7.7	9.5	C26 H35 O5	1760.7	4.212	1.48	26	35	5	
	427.2378	7.3	17.1	12.5	C29 H33 Na2	1762.2	5.727	0.33	29	33		2
	427.2543	-9.2	-21.5	0.5	C19 H39 O10	1762.7	6.218	0.20	19	39	10	
	427.2402	4.9	11.5	15.5	C31 H32 Na	1763.6	7.113	0.08	31	32		1
	427.2426	2.5	5.9	18.5	C33 H31	1764.6	8.128	0.03	33	31		

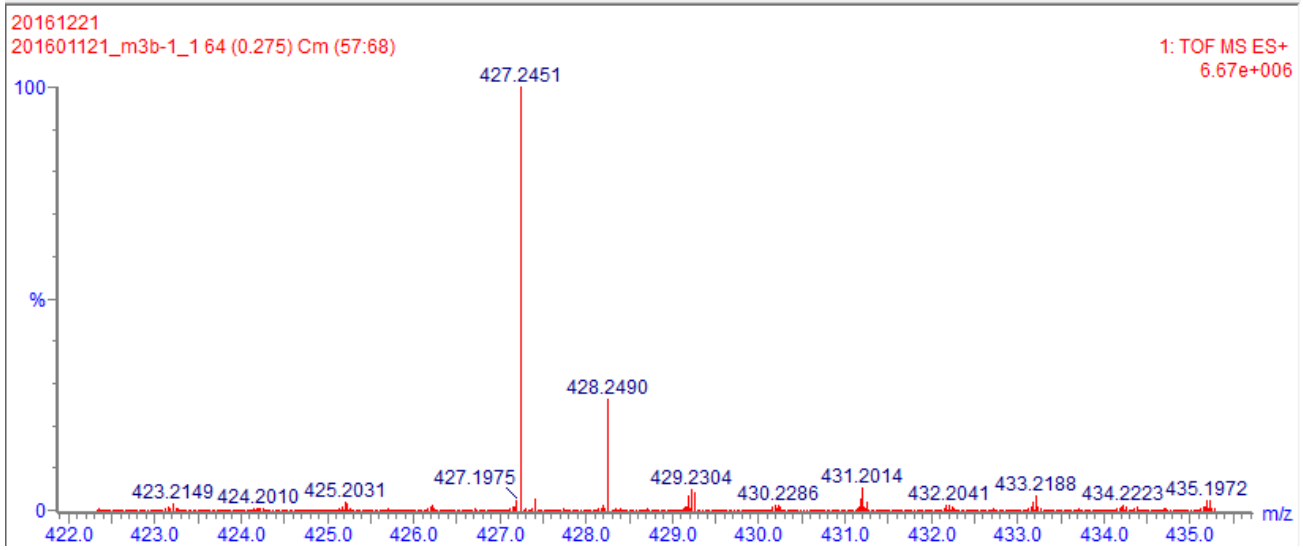


Figure S23. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **3**.

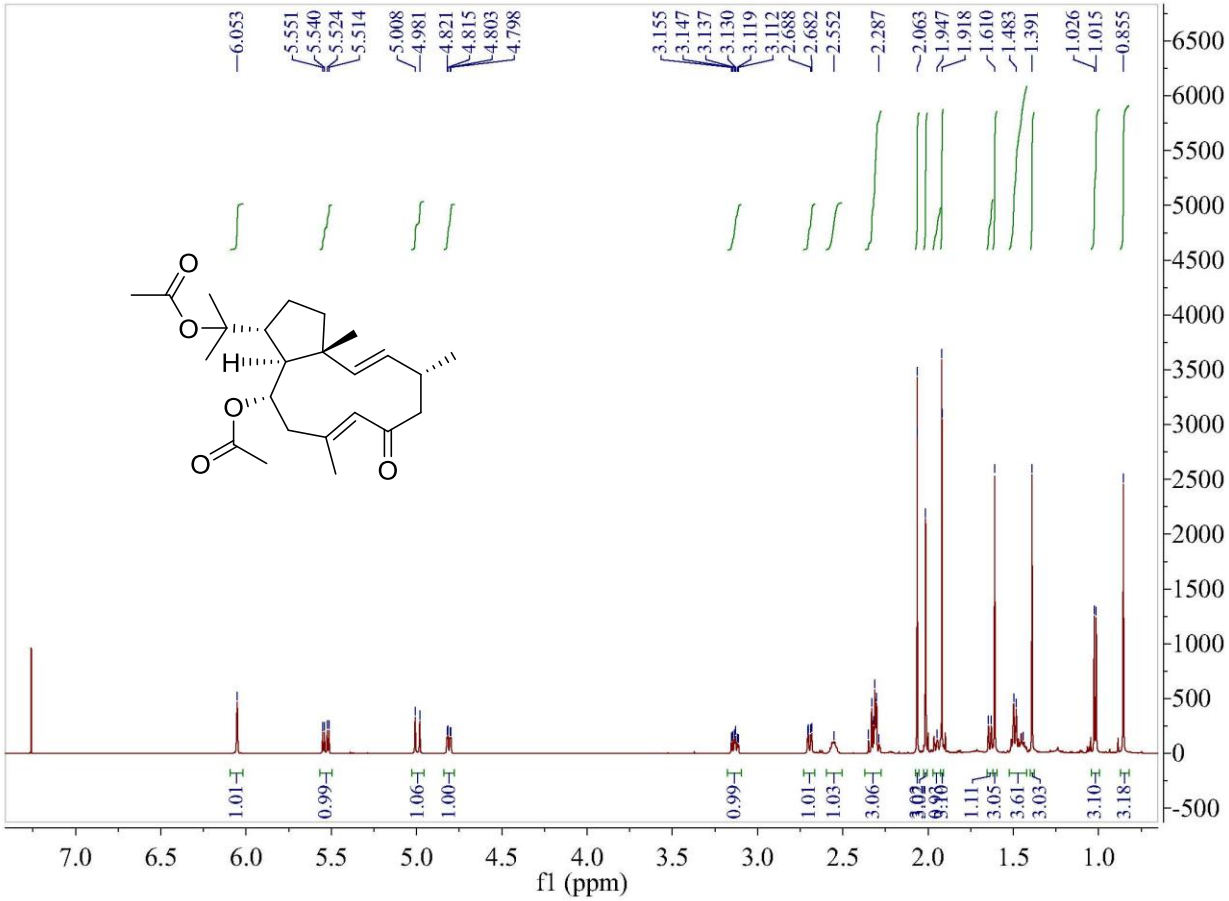


Figure S24. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of **3**.

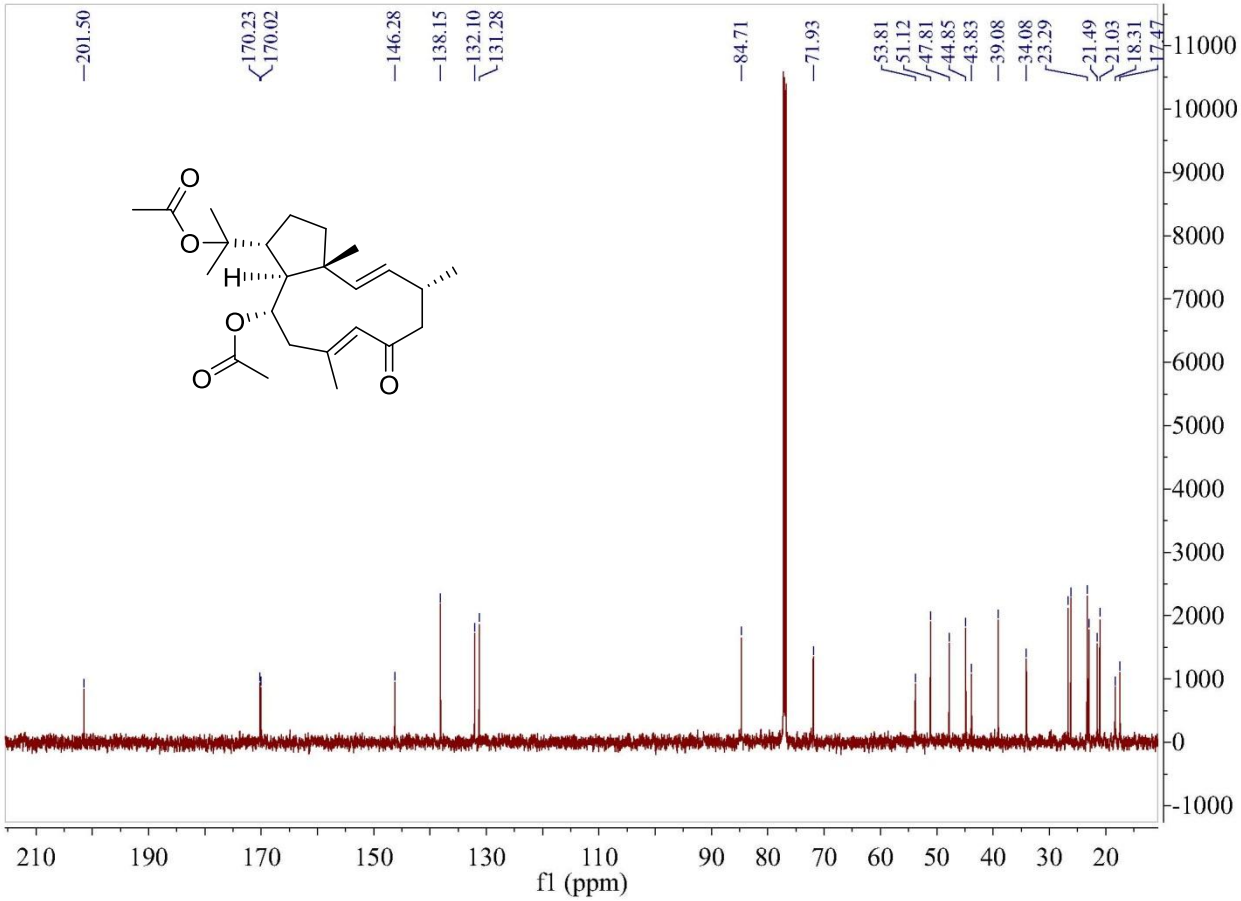


Figure S25. DEPT135 spectrum of **3** in $CDCl_3$.

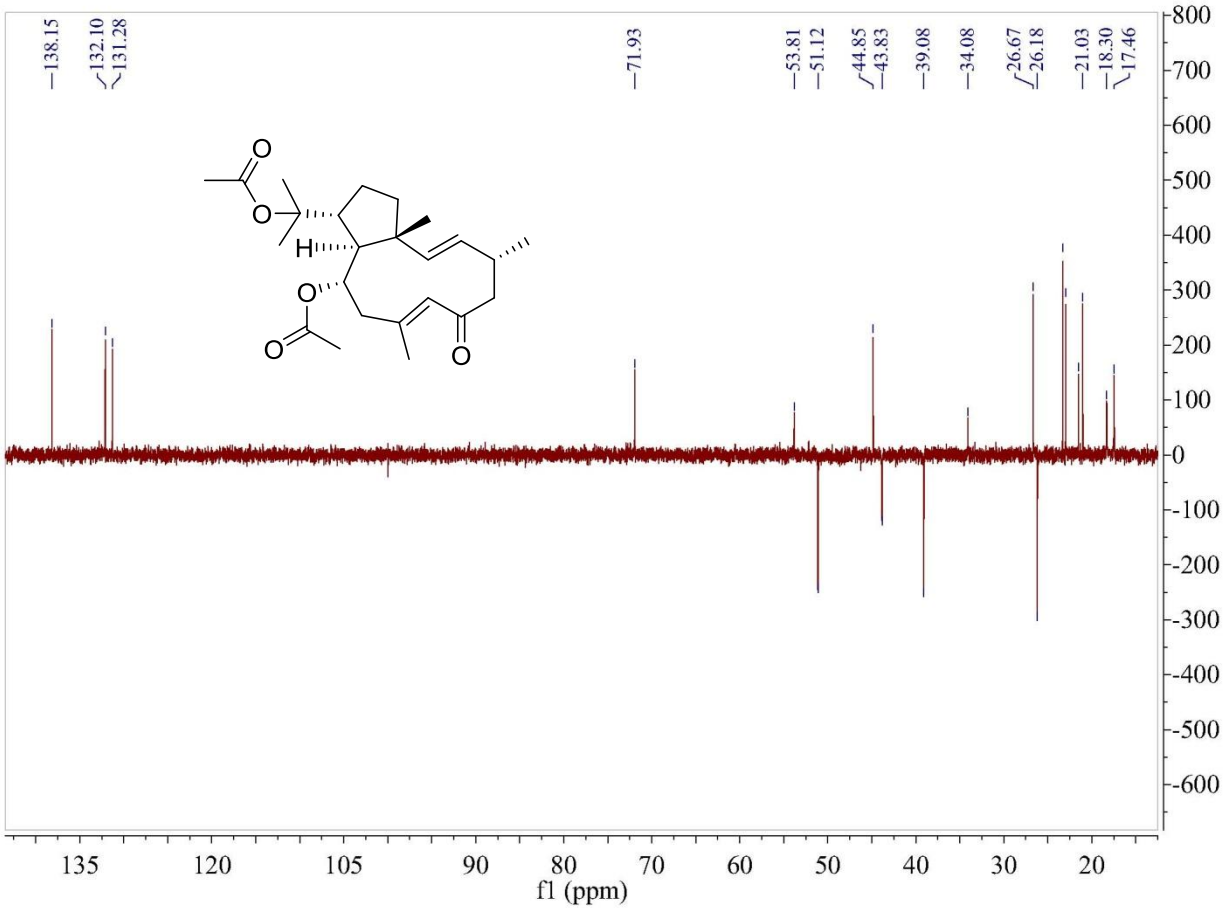


Figure S26. Proposed mechanism of the [2 + 2] cycloaddition of **3** and its 7,8-Z isomer

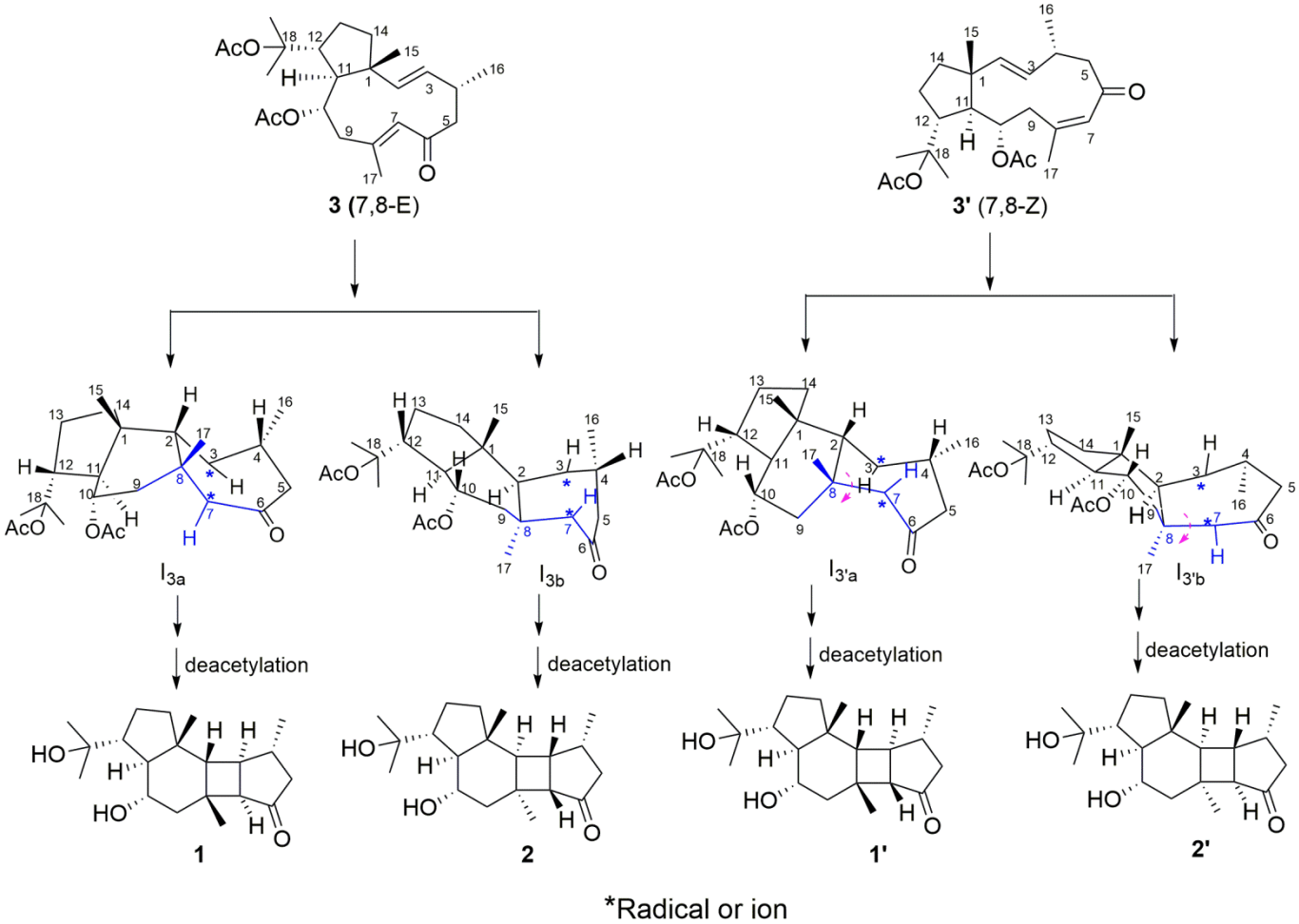


Table S1. Crystal data and structure refinement for hipposponlachnin A (**1**)

Identification code	Cu_dm14258_0m	
Empirical formula	C20 H32 O3	
Formula weight	320.45	
Temperature	140(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 6.62210(10) Å	α = 90 °.
	b = 15.2395(3) Å	β = 90 °.
	c = 18.4069(3) Å	γ = 90 °.
Volume	1857.58(6) $Å^3$	
Z	4	
Density (calculated)	1.146 mg/m^3	
Absorption coefficient	0.588 mm^{-1}	
F(000)	704	
Crystal size	0.200 x 0.120 x 0.050 mm^3	
Theta range for data collection	3.765 to 69.659 °.	
Index ranges	-7<=h<=4, -18<=k<=18, -21<=l<=22	
Reflections collected	8888	
Independent reflections	3177 [R(int) = 0.0311]	
Completeness to theta = 67.679 °	99.4 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7532 and 0.6136	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3177 / 0 / 215	
Goodness-of-fit on F^2	1.060	
Final R indices [I>2sigma(I)]	R1 = 0.0354, wR2 = 0.0936	
R indices (all data)	R1 = 0.0377, wR2 = 0.0953	
Absolute structure parameter	0.13(13)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.131 and -0.188 e.$Å^{-3}$	

Table S2. Crystal data and structure refinement for hipposponlachnin B (**2**)

Identification code	cu_dm14257_0m	
Empirical formula	C20 H32 O3	
Formula weight	320.45	
Temperature	140(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 8.17720(10) Å	α = 90 °.
	b = 9.71010(10) Å	β = 92.8110(10) °.
	c = 11.2700(2) Å	γ = 90 °.
Volume	893.78(2) $Å^3$	
Z	2	
Density (calculated)	1.191 mg/m^3	
Absorption coefficient	0.611 mm^{-1}	
F(000)	352	
Crystal size	0.200 x 0.100 x 0.020 mm^3	
Theta range for data collection	3.927 to 69.566 °.	
Index ranges	-9<=h<=9, -11<=k<=11, -13<=l<=13	
Reflections collected	6940	
Independent reflections	2953 [R(int) = 0.0248]	
Completeness to theta = 67.679 °	96.3 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7532 and 0.6733	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	2953 / 1 / 215	
Goodness-of-fit on F^2	1.061	
Final R indices [I>2sigma(I)]	R1 = 0.0308, wR2 = 0.0827	
R indices (all data)	R1 = 0.0312, wR2 = 0.0830	
Absolute structure parameter	-0.03(9)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.188 and -0.143 $e.Å^{-3}$	

CheckCIFPLATON report of hipposponlachnin A (**1**)

You have not supplied any structure factors. As a result the full set of tests cannot be run.

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found.
Please wait while processing ....

CIF dictionary
Interpreting this report

Datablock: cu_dm14258_0m

Bond precision: C-C = 0.0030 A Wavelength=1.54178

Cell: a=6.6221(1) b=15.2395(3) c=18.4069(3)
alpha=90 beta=90 gamma=90

Temperature: 140 K

	Calculated	Reported
Volume	1857.58(6)	1857.58(6)
Space group	P 21 21 21	P 21 21 21
Hall group	P 2ac 2ab	P 2ac 2ab
Moiety formula	C20 H32 O3	C20 H32 O3
Sum formula	C20 H32 O3	C20 H32 O3
Mr	320.46	320.45
Dx, g cm-3	1.146	1.146
Z	4	4
Mu (mm-1)	0.588	0.588
F000	704.0	704.0
F000'	705.96	
h,k,lmax	8,18,22	7,18,22
Nref	3488[2025]	3177
Tmin,Tmax	0.919,0.971	0.614,0.753
Tmin'	0.889	

Correction method= # Reported T Limits: Tmin=0.614 Tmax=0.753 AbsCorr = MULTI-SCAN

Data completeness= 1.57/0.91 Theta(max)= 69.659

R(reflections)= 0.0354(2970) wR2(reflections)= 0.0953(3177)

S = 1.060 Npar= 215

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

PLAT480_ALERT_4_C Long H...A H-Bond Reported H19B .. O1 .. 2.66 Ang.

Alert level G

PLAT007_ALERT_5_G Number of Unrefined Donor-H Atoms ..... 2 Report
PLAT791_ALERT_4_G The Model has Chirality at C1 (Chiral SPGR) R Verify
And 8 other PLAT791 Alerts
More ...

0 **ALERT level A** = Most likely a serious problem - resolve or explain
0 **ALERT level B** = A potentially serious problem, consider carefully
1 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight
10 **ALERT level G** = General information/check it is not something unexpected

0 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
0 ALERT type 2 Indicator that the structure model may be wrong or deficient
0 ALERT type 3 Indicator that the structure quality may be low
10 ALERT type 4 Improvement, methodology, query or suggestion
1 ALERT type 5 Informative message, check

It is advisable to attempt to resolve as many as possible of the alerts in all categories. Often the minor alerts point to easily fixed oversights, errors and omissions in your CIF or refinement strategy, so attention to these fine details can be worthwhile. In order to resolve some of the more serious problems it may be necessary to carry out additional measurements or structure refinements. However, the purpose of your study may justify the reported deviations and the more serious of these should normally be commented upon in the discussion or experimental section of a paper or in the "special_details" fields of the CIF. checkCIF was carefully designed to identify outliers and unusual parameters, but every test has its limitations and alerts that are not important in a particular case may appear. Conversely, the absence of alerts does not guarantee there are no aspects of the results needing attention. It is up to the individual to critically assess their own results and, if necessary, seek expert advice.

Publication of your CIF in IUCr journals

A basic structural check has been run on your CIF. These basic checks will be run on all CIFs submitted for publication in IUCr journals (*Acta Crystallographica*, *Journal of Applied Crystallography*, *Journal of Synchrotron Radiation*); however, if you intend to submit to *Acta Crystallographica Section C* or *E*, you should make sure that full publication checks are run on the final version of your CIF prior to submission.

Publication of your CIF in other journals

Please refer to the *Notes for Authors* of the relevant journal for any special instructions relating to CIF submission.

CheckCIFPLATON report of hipposponlachnin B (**2**)

You have not supplied any structure factors. As a result the full set of tests cannot be run.

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found.
Please wait while processing ....

CIF dictionary
Interpreting this report

Datablock: cu_dm14257_0m

Bond precision: C-C = 0.0028 A Wavelength=1.54178

Cell: a=8.1772(1) b=9.7101(1) c=11.2700(2)
alpha=90 beta=92.811(1) gamma=90

Temperature: 140 K

	Calculated	Reported
Volume	893.78(2)	893.78(2)
Space group	P 21	P 1 21 1
Hall group	P 2yb	P 2yb
Moiety formula	C20 H32 O3	C20 H32 O3
Sum formula	C20 H32 O3	C20 H32 O3
Mr	320.46	320.45
Dx, g cm-3	1.191	1.191
Z	2	2
Mu (mm-1)	0.611	0.611
F000	352.0	352.0
F000'	352.98	
h, k, lmax	9, 11, 13	9, 11, 13
Nref	3352[1782]	2953
Tmin, Tmax	0.929, 0.988	0.673, 0.753
Tmin'	0.885	

Correction method= # Reported T Limits: Tmin=0.673 Tmax=0.753 AbsCorr = MULTI-SCAN

Data completeness= 1.66/0.88 Theta(max)= 69.566

R(reflections)= 0.0307(2906) wR2(reflections)= 0.0818(2953)

S = 1.066 Npar= 215

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

PLAT029_ALERT_3_C _diffrn_measured_fraction_theta_full Low ..... 0.963 Note

Alert level G

PLAT007_ALERT_5_G Number of Unrefined Donor-H Atoms ..... 2 Report
PLAT791_ALERT_4_G The Model has Chirality at C1 (Chiral SPGR) R Verify
And 8 other PLAT791 Alerts
More ...

0 **ALERT level A** = Most likely a serious problem - resolve or explain
0 **ALERT level B** = A potentially serious problem, consider carefully
1 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight
10 **ALERT level G** = General information/check it is not something unexpected

0 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
0 ALERT type 2 Indicator that the structure model may be wrong or deficient
1 ALERT type 3 Indicator that the structure quality may be low
9 ALERT type 4 Improvement, methodology, query or suggestion
1 ALERT type 5 Informative message, check

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