SUPPLEMENTARY MATERIAL

A New Dimeric Protoberberine Alkaloid and Other Compounds from the Tubers of
Tinospora dentata

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Abstract: A new dimeric quaternary protoberberine alkaloid, bispalmatrubine (**1**), and thirteen known compounds (**2-14**) were purified from the tubers of *Tinospora dentata*. Their structures were determined by spectroscopic and spectrometric analytical methods. Among the isolates, eight compounds were examined for their *in vitro* anti-inflammatory potential and several tested alkaloids displayed moderate inhibitory effects of *N*-formyl-methionyl-leucyl-phenylalanine/cytochalasin B (fMLP/CB)-induced superoxide anion generation and elastase release.

Contents

Experimental section. Anti-inflammatory Bioactivity Experimental Procedures.

Preparation of human neutrophils

Human neutrophils study conducted according to the Declaration of Helsinki (2013) was approved by the Institutional Review Board at Chang Gung Memorial Hospital, Taoyuan, Taiwan. Blood was drawn from healthy human donors (20–30 years old) after the written informed consent was obtained. Base on a protocol approved by the Institutional Review Board at Chang Gung Memorial Hospital, blood was drawn by venipuncture into heparin-coated vacutainer tubes. Blood samples were mixed with an equal volume of 3 % dextran solution gently. Neutrophils were isolated with a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes. After sedimentation of the red cells for 30 min at room temperature, the leukocyte-rich plasma was collected and transferred to 20 mL Ficoll solution (1.077 g/mL) and spun down at 400 g for 40 min at 20 °C. The granulocyte/erythrocyte pellets were resuspended in ice-cold 0.2 % sodium chloride to lyse erythrocytes. After 30 sec, the same volume of 1.6 % sodium chloride solution was added to reconstitute the isotonic condition. Purified neutrophils were pelleted and then resuspended in a calcium-free Hank's balanced salt solution (HBSS) buffer at pH 7.4, and were maintained at 4 °C before use.

Measurement of superoxide anion generation and elastase release

The generation of superoxide anion assay was based on the *superoxide dismutase* (SOD)-inhibitable reduction of ferricytochrome c (Yu et al., 2011; Yang et al., 2013). After supplementation with 0.5 mg/mL ferricytochrome c and 1 mM Ca^{2+}, neutrophils (6×10^5 cells/mL) were equilibrated at 37 °C for 2 min and then incubated with samples (10 μM) or vehicle (0.1 % Dimethyl sulfoxide, negative control) for 5 min. During the preincubation of 1

μg/mL cytochalasin B (fMLP/CB) for 3 min, cells were activated with 100 nM fMLP. Changes in the absorbance with a reduction in ferricytochrome c at 550 nm were continuously monitored in a double-beam spectrophotometer with constant stirring. Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome c ($\varepsilon = 21.1$/mM/10 mm). Degranulation of azurophilic granules was determined by elastase release as described previously (Yu et al., 2011; Yang et al., 2013). In this experiments, MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide was using as the elastase substrate. After supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with test compounds (10 μM) or a vehicle (0.1 % Dimethyl sulfoxide, negative control) for 5 min. Cells were activated by 100 nM fMLP and 0.5 μg/mL cytochalasin B. The changes in absorbance at 405 nm were continuously monitored to assay elastase release. The results were expressed as the percent of elastase release in the fMLP/CB-activated, drug-free control system. For compounds **5**, **7**, and genistein, IC_{50} values were calculated from a series of different examined concentrations of 1-10 μM (**5** and **7**) and 1-50 μM (genistein), respectively.

Statistical analysis

Results were expressed as mean ± S.E.M. Calculations of 50 % inhibitory concentrations (IC_{50}) were computer-assisted (PHARM/PCS v.4.2). Statistical comparisons were made between groups using the Student's *t* test. Values of *p* less than 0.05 were considered to be statistically significant, and ** $p < 0.01$, *** $p < 0.001$, respectively.

Table S1. NMR spectroscopic data of compound **1**.

Position	δ_H	δ_C	HMBC (H→C)	COSY	NOESY
1, 1'	7.80 *s*	109.5 *d*	129.9, 140.3, 149.5,		4.12, 8.88
2, 2'		149.5 *s*			
3, 3'		145.6 *s*			
4, 4'		122.2 *s*			
4a, 4a'		119.7 *s*			
5, 5'	2.86 *m*	25.6 *t*		3.00, 4.90	
	3.00 *m*			2.86, 4.90	
6, 6'	4.90 m	57.2 *t*		2.86, 3.00	
8, 8'	9.71 *s*	145.9 *d*	57.2, 123.2, 135.4,		
			140.4, 145.6		
8a, 8a'		135.4 *s*			
9, 9'		146.0 *s*			
10, 10'		151.9 *s*			
11, 11'	8.05 *d* (9.1)	124.5 *d*	121.4, 123.2, 151.9	8.13	4.12, 8.13
12, 12'	8.13 *d* (9.1)	128.1 *s*	135.4, 145.9, 151.9	8.05	8.05, 8.88
12a, 12a'		123.2 *s*			
13, 13'	8.88 *s*	121.4 *d*	119.7, 123.3, 140.4		7.80, 8.13
14, 14'		140.3 *s*			
14a, 14a'		129.9 *s*			
OCH$_3$-2, 2'	4.12 *s*	57.1 *q*	149.5		
OCH$_3$-3, 3'	4.20 *s*	62.5 *q*	145.6		
OCH$_3$-10, 10'	4.12 *s*	57.7 *q*	151.9		

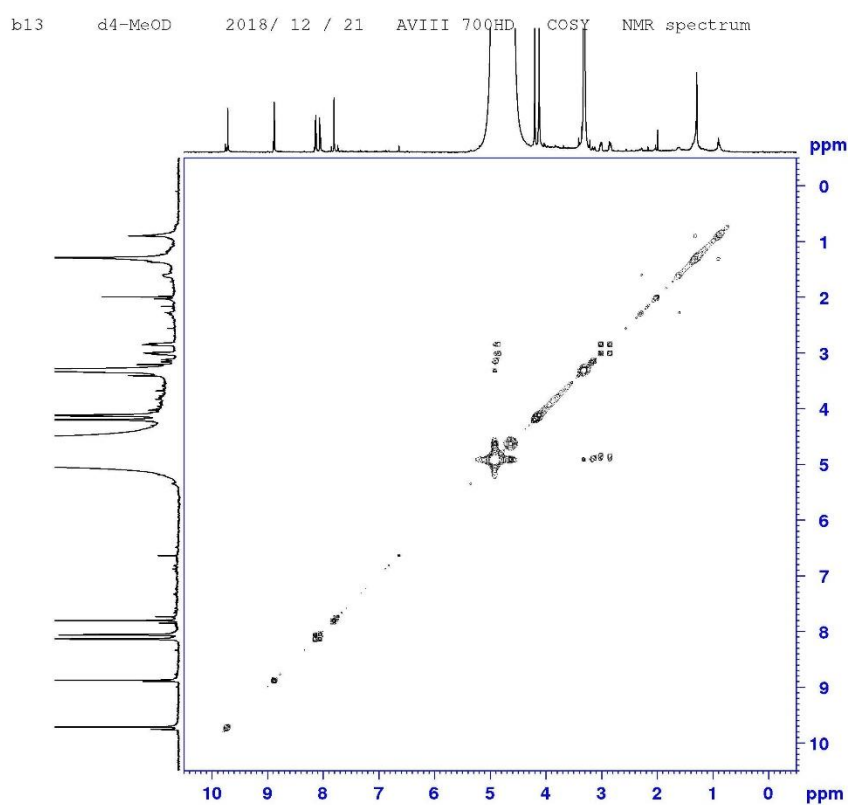^{1}H and ^{13}C NMR data measured in CD$_3$OD at 700 MHz and 175 MHz, respectively.

Figure S1. ^{1}H NMR spectrum of **1**.
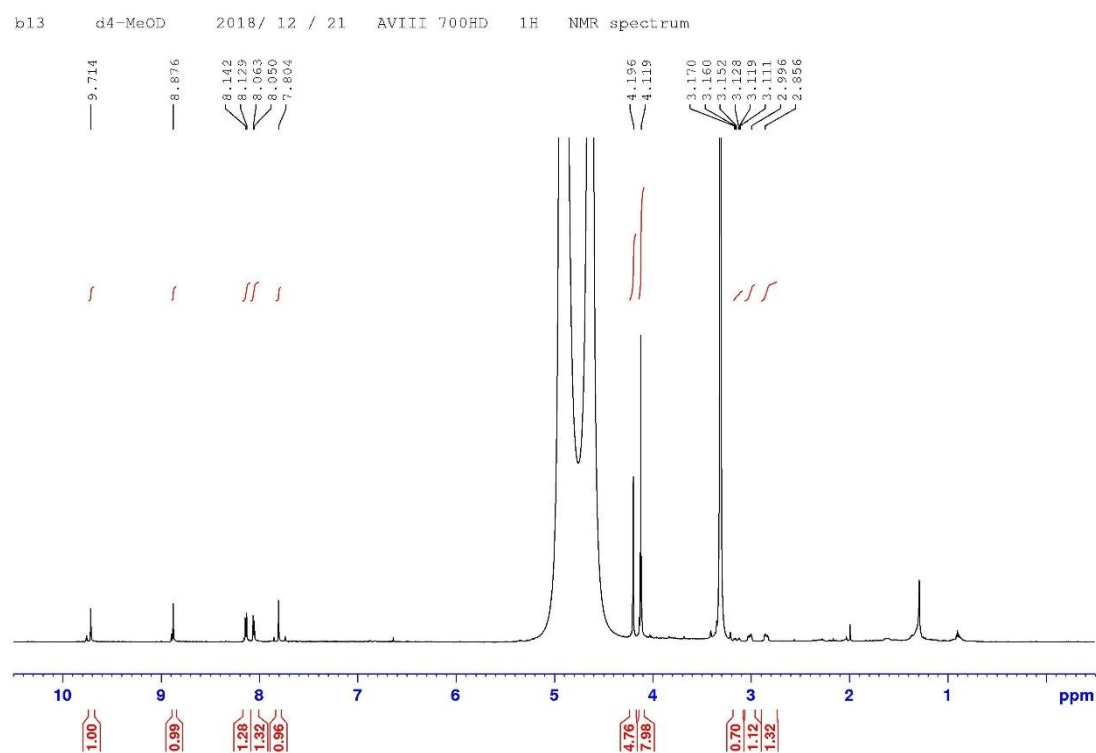
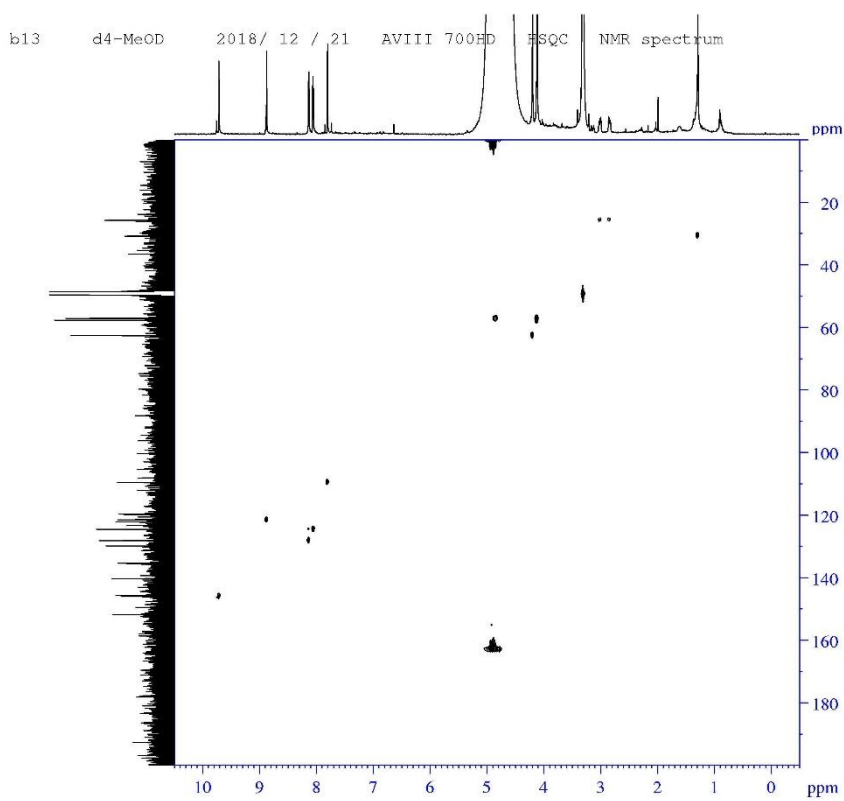

Figure S2. ^{13}C NMR spectrum of **1**.
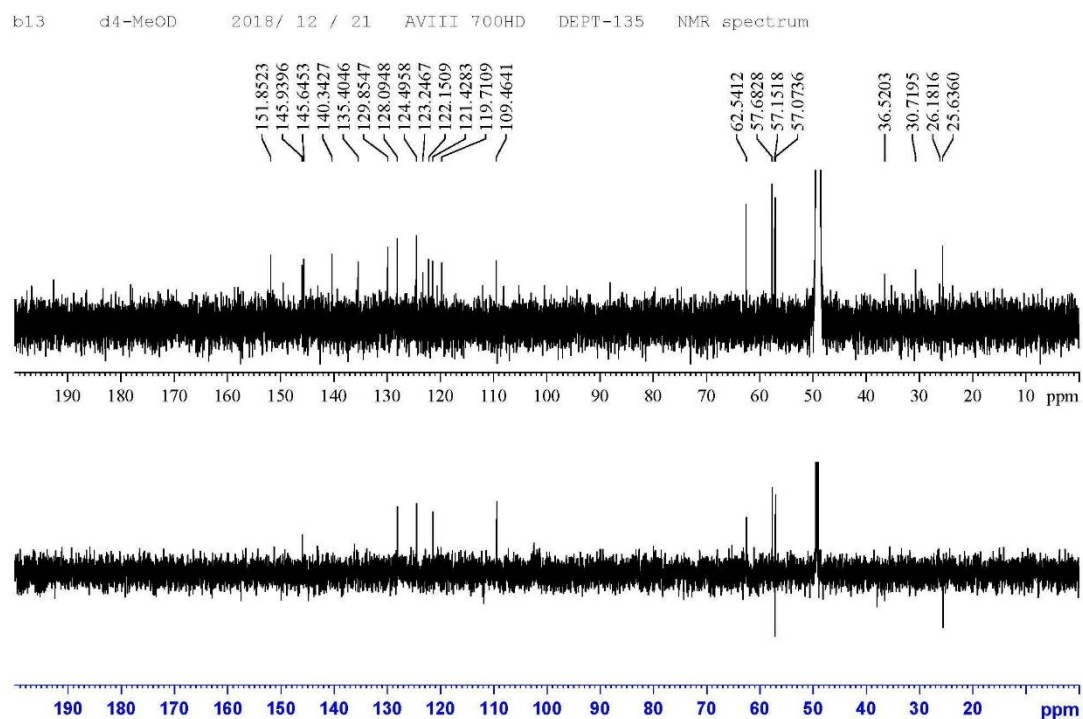
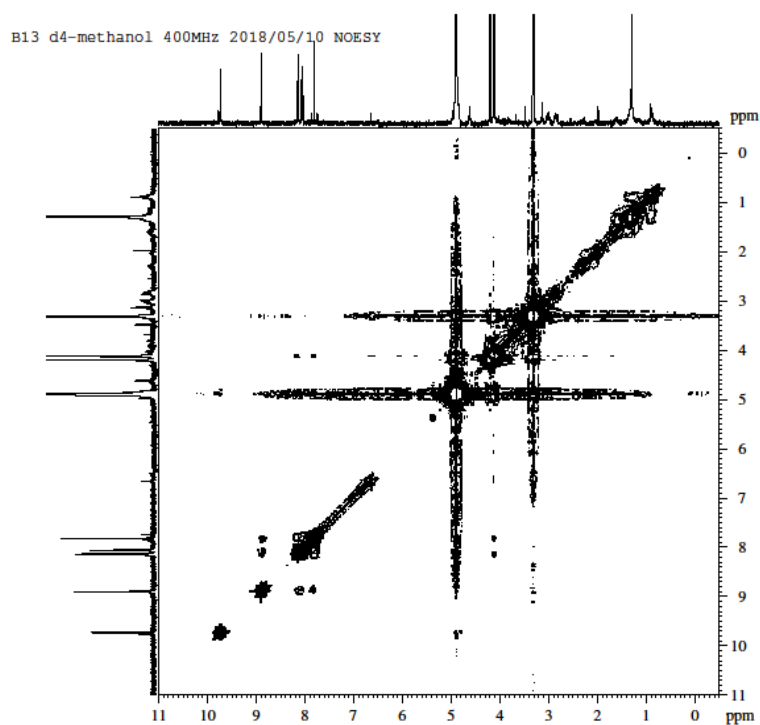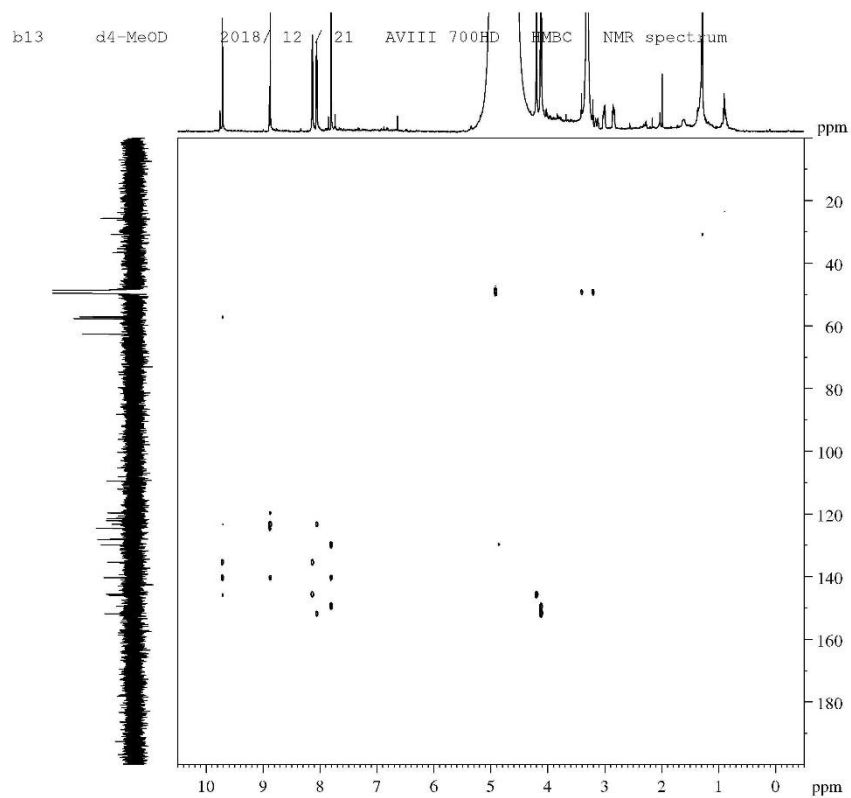**Figure S3.** COSY spectrum of **1**.

Figure S4. HSQC spectrum of **1**.

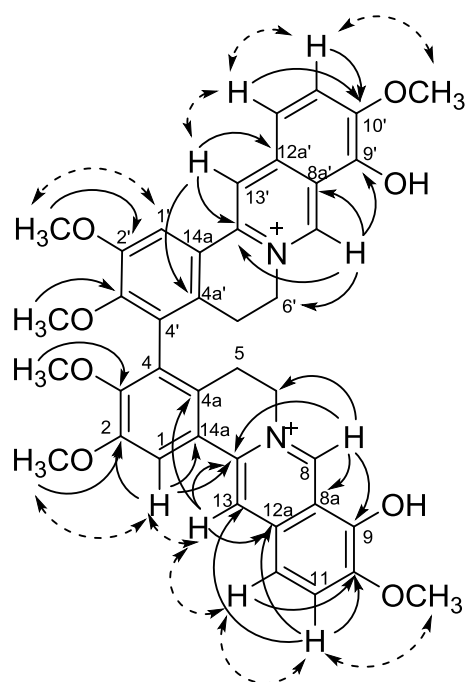**Figure S6.** NOESY spectrum of **1**.

B13 d4-methanol 400MHz 2018/05/10 NOESY

Figure S6. HMBC spectrum of **1**.
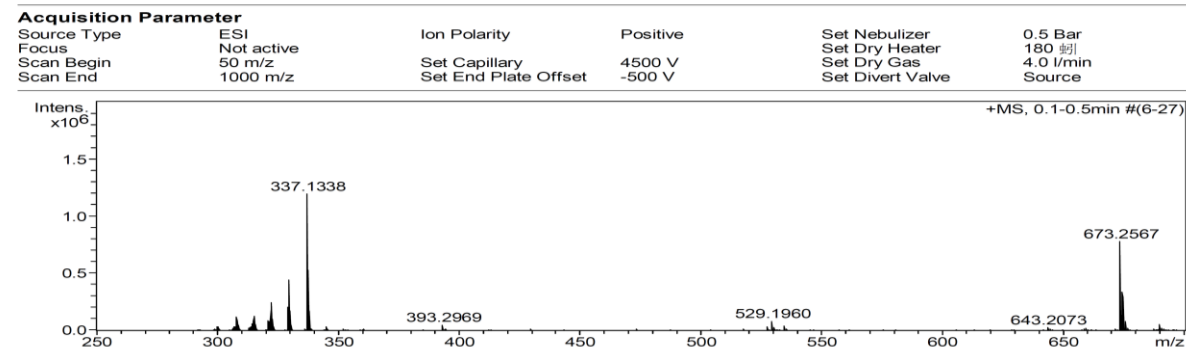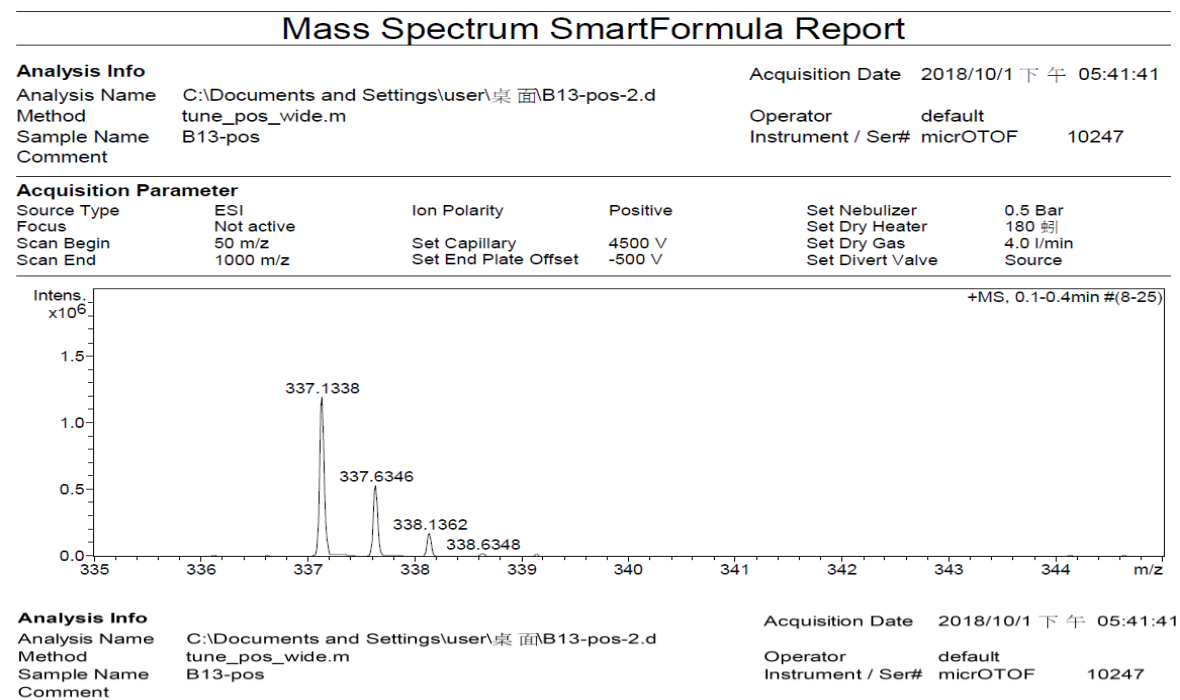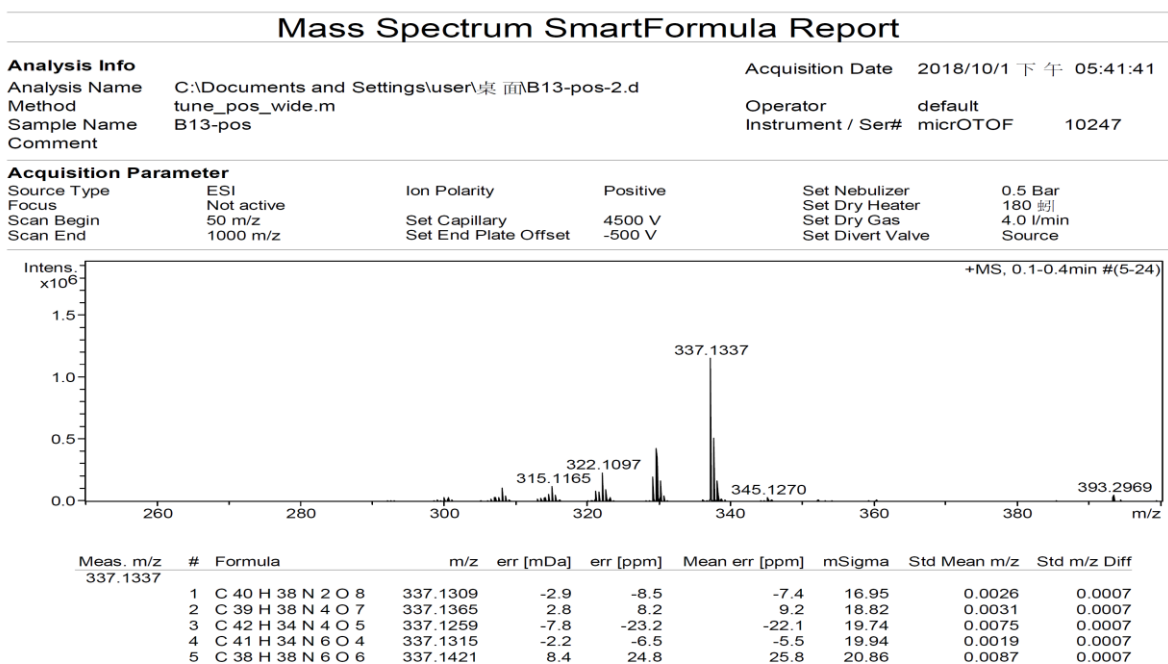

b13   d4-MeOD   2018/ 12 / 21   AVIII 700HD   HMBC   NMR spectrum

Figure S7. Significant HMBC (→)/NOESY (↔) correlations of compound **1**.

Figure S8. ESI-MS spectrum of **1**.

FT-MS
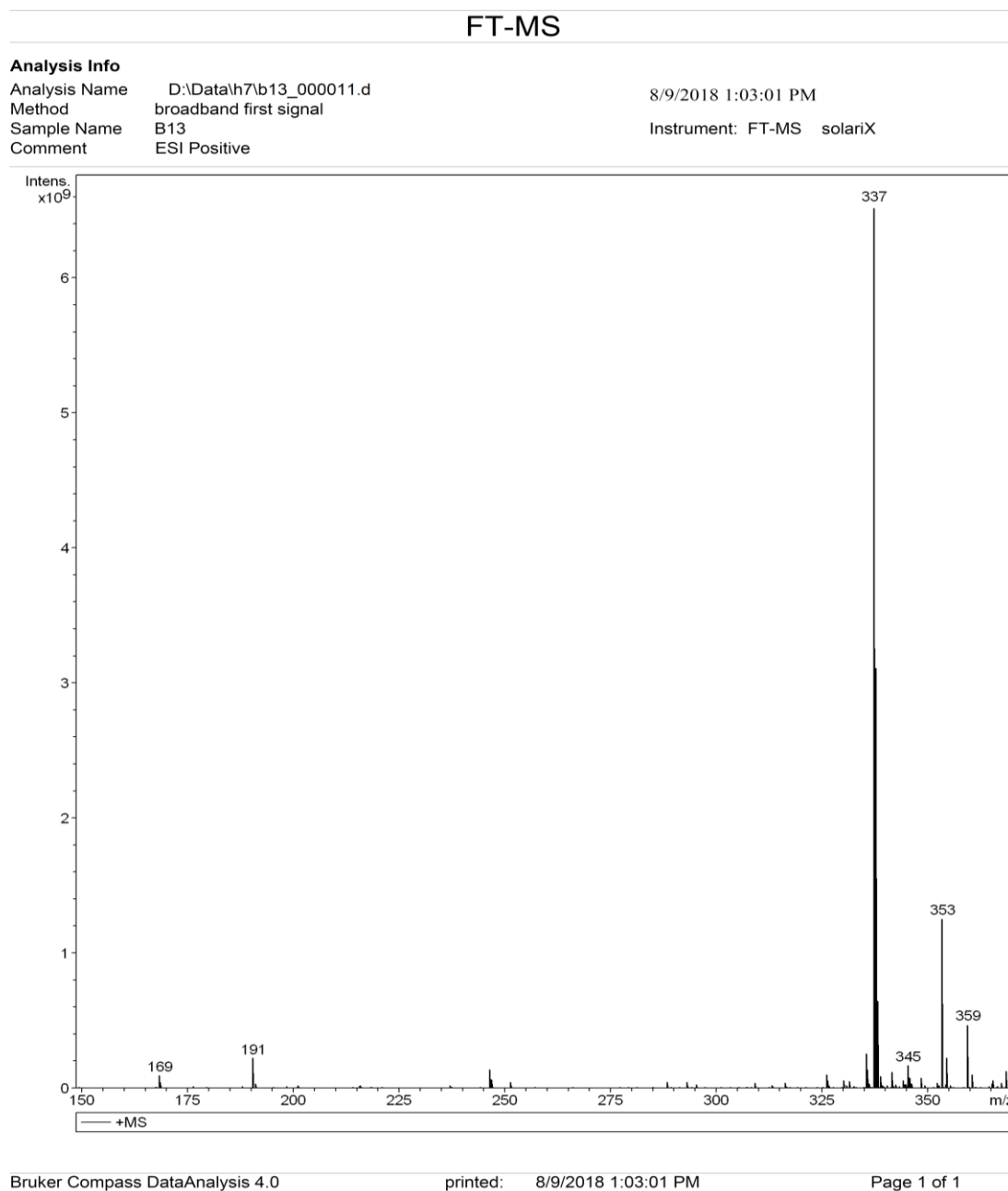
Figure S9. HR-MS spectrum of **1**.

Mass Spectrum SmartFormula Report

Meas. m/z	#	Formula	m/z	err [mDa]	err [ppm]	Mean err [ppm]	mSigma	Std Mean m/z	Std m/z Diff
337.1337									
	1	C 40 H 38 N 2 O 8	337.1309	-2.9	-8.5	-7.4	16.95	0.0026	0.0007
	2	C 39 H 38 N 4 O 7	337.1365	2.8	8.2	9.2	18.82	0.0031	0.0007
	3	C 42 H 34 N 4 O 5	337.1259	-7.8	-23.2	-22.1	19.74	0.0075	0.0007
	4	C 41 H 34 N 6 O 4	337.1315	-2.2	-6.5	-5.5	19.94	0.0019	0.0007
	5	C 38 H 38 N 6 O 6	337.1421	8.4	24.8	25.8	20.86	0.0087	0.0007

Mass Spectrum SmartFormula Report

Figure S10. Possible mass fragmentation of **1**.