Indole Alkaloids with New Skeleton Activating Neural Stem Cells

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Supporting Information

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SI-1. The ^{1}H and ^{13}C NMR spectral data of the new compounds

Table S1. ^{13}C (150 MHz) NMR spectral data (δ in ppm) of compounds **1**–**5**

No.	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[a]
C-2	136.9, s	133.8, s	133.9, s	136.7, s	137.0, s
C-3	47.3, t	46.3, t	46.0, t	47.1, t	47.1, t
C-6	8.1, q	9.1, q	9.0, q	9.8, q	8.0, q
C-7	105.0, s	105.3, s	105.1, s	107.1, s	105.0, s
C-8	130.2, s	129.6, s	129.5, s	130.8, s	130.2, s
C-9	118.7, d	118.4, d	118.3, d	118.7, d	118.6, d
C-10	119.8, d	119.4, d	119.3, d	120.1, d	119.8, d
C-11	121.3, d	121.2, d	121.1, d	121.7, d	121.3, d
C-12	111.3, d	110.8, d	111.1, d	110.9, d	111.6, d
C-13	138.5, s	137.3, s	137.5, s	138.1, s	138.7, s
C-14	31.1, t	29.0, t	28.7, t	28.5, t	31.0, t
C-15	34.5, d	39.2, d	33.5, d	33.1, d	28.5, d
C-16	35.8, d	48.8, s	49.1, s	50.8, s	36.0, d
C-17	74.3, t	74.8, t	68.9, t	70.6, t	68.8, t
C-18	18.7, q	18.2, q	17.9, q	18.2, q	18.3, q
C-19	75.3, d	74.5, d	72.1, d	72.9, d	73.3, d
C-20	43.0, d	42.5, d	42.9, d	42.9, d	43.4, d
C-21	67.7, d	66.9, d	71.8, d	71.9, d	72.4, d
N-CH_3	45.6, q	45.1, q	45.0, q	44.5, q	45.5, q
-CO-		173.3, s	173.4, s	179.3, s	
OCH_3		52.1, q	52.2, q		

[a]Recorded in methanol-d_4. [b]Recorded in acetone-d_6.

Table S2. ^{1}H (600 MHz) NMR spectral data (δ in ppm, J in Hz) of compounds **1**–**5**

No.	1[a]	2[b]	3[b]	4[a]	5[a]
H-3	2.30, dd (12.0, 6.4)	2.38, m	2.34, m	2.46, brd (10.5)	2.33, dd (11.3, 6.8)
	1.84, td (12.0, 4.0)	2.22, td (12.0, 4.5)	2.18, m		1.85, td (11.3, 4.0)
H-6	2.21, s	2.10, s	2.06, s	2.21, s	2.21, s
H-9	7.42, d (7.9)	7.48, d (7.9)	7.44, d (7.9)	7.44, d (7.9)	7.41, d (8.0)
H-10	6.98, t (7.9)	7.02, t (7.9)	6.98, t (7.9)	7.00, t (7.9)	6.99, t (8.0)
H-11	7.04, t (7.9)	7.10, t (7.9)	7.05, t (7.9)	7.07, t (7.9)	7.04, t (8.0)
H-12	7.50, d (7.9)	7.66, d (7.9)	7.52, d (7.9)	7.48, d (7.9)	7.49, d (8.0)
H-14	2.03, m	1.96, m	1.88, m	2.24, overlap	Hα 2.03, m
	1.74, brd (13.6)	1.93, m		1.85, m	Hβ 1.81, m
H-15	2.18, m	2.41, m	2.57, m	2.57, brs	2.50, m
H-16	3.03, brs				3.09, brs
H-17	β 3.60, brd (10.2)	3.85, d (10.5)	β 4.04, d (10.5)	β 4.10, d (10.5)	β 3.92, dd (10.2, 1.9)
	α 3.57, dd (10.2, 2.6)	3.75, d (10.5)	α 3.48, d (10.5)	α 3.50, d (10.5)	α 3.37, dd (10.2, 2.6)
H-18	1.22, d (6.8)	1.16, d (6.4)	1.28, d (6.8)	1.33, d (6.8)	1.32, d (6.8)
H-19	3.69, qd (6.8, 3.0)	3.80, dq (6.4, 3.4)	3.94, q (6.8)	3.94, q (6.8)	4.04, q (6.8)
H-20	2.12, m	2.33, m	2.28, t (3.0)	2.27, brs	2.16, brs
H-21	5.47, d (2.6)	5.57, d (2.3)	5.38, d (3.0)	5.49, d (2.3)	5.37, d (2.3)
N-CH_3	2.24, s	2.35, s	2.31, s	2.38, s	2.26, s
OCH_3		3.87, s	3.82, s		

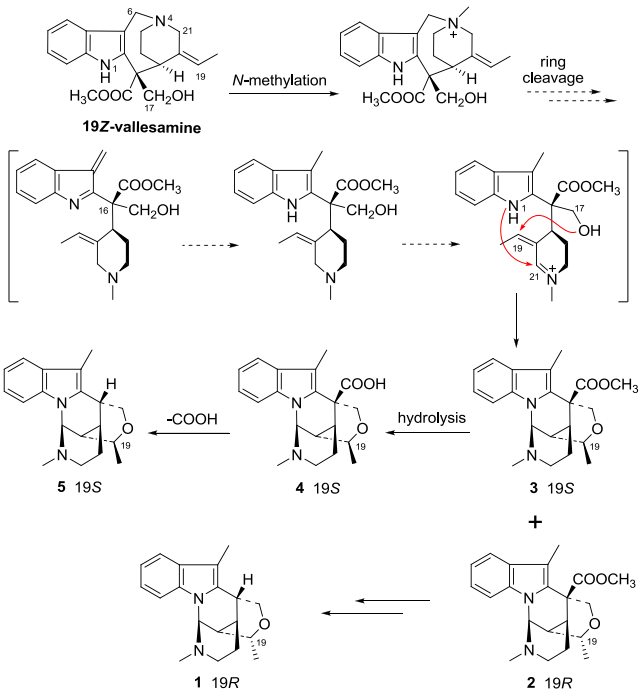
[a]Recorded in methanol-d_4. [b]Recorded in acetone-d_6.

SI-2. The plausible biosynthesis pathways of 1–5

Biosynthetically, compounds **1**–**5** might be derived from 19*Z*-vallesamine, the main MIA of *A. scholaris*. The N-4 methylation of 19*Z*-vallesamine to form *N*-methylvallesamine[1] in which the cleavage of C-6/N-4 was presumed to afford the key intermediate with imine moiety. Subsequently, the addition reaction between 17-OH and C-19, together with addition between N-1 and the imine carbon (C-21) were proceeded to construct the architecture of complicated fused rings, afforded a pair of 19-epimers (**2** and **3**). Then, compound **4** could be derived from **3** by hydrolysis, while compounds **1** and **5** were decarboxymethyl derivatives of **2** and **3**, respectively (Scheme S1).

Reference

(1) Walser, A.; Djerassi, C. *Helv. Chim. Acta* **1964**, *47*, 2072−2086.



Scheme S1. Plausible biosynthesis pathways of **1**–**5.**

SI-3. General experimental procedures, extraction and isolation

3.1 General experimental procedures

Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were detected on a SHIMADZU UV-2401PC spectrometer. IR spectra were determined on a Bruker FT-IR Tensor-27 infrared spectrophotometer with KBr disks. All the 1D and 2D NMR spectra were recorded on Bruker DRX-600 spectrometers using TMS as an internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESI-MS and HR-EI-MS analysis were carried out on Waters Xevo TQS and Waters AutoSpec Premier P776 mass spectrometers, respectively. Semi-preparative HPLC was performed on an Agilent 1100 HPLC with a ZORBAX SB-C_{18} (9.4 × 250 mm) column. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co., Ltd., PR China), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography. Fractions were monitored by TLC (GF 254, Qingdao Marine Chemical Co., Ltd.), and spots were visualized by Dragendorff ’s reagent.

3.2 Extraction and isolation

The leaves of *A. scholaris* were collected in Xishuangbanna, Yunnan Province, P. R. China, in October 2006. This material (18 kg) was extracted with EtOH (40 L × 3) under reflux conditions, and the solvent was evaporated in vacuum. The residue was dissolved in 0.37% HCl, and the solution was subsequently basified using ammonia water to pH 9–10. The basic solution was partitioned with EtOAc, affording a two-phase mixture including the aqueous phase and the EtOAc/organic phase. The organic fraction (total alkaloids, 180 g) was collected and then dissolved in MeOH, and the resulting solution was subjected to column chromatography over silica gel eluting with $CHCl_3$-MeOH (from 1:0 to 2:8) to afford six fractions (Fr. A–F). Fr. A (25 g) was further chromatographed using $CHCl_3$-Me_2CO (from 9:1 to 7:3) as eluent to give five fractions (Fr. A1–A5). Fr. A3 (1.6 g) was further purified by preparative HPLC (CH_3CN-H_2O, 1:1) to afford **1** (103 mg), **2** (8 mg), **3** (35 mg), and **5** (26 mg). In the same way, compound **4** (15 mg) was obtained from Fr. C (15 g).

SI-4. The physical data of the new compounds

Alstoscholarisine A (1): colorless block crystal from MeOH; mp 151–152 ℃; $[\alpha]^{25}_{D}$ −184.6 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) = 203 (4.08), 233 (4.30), 287 (3.66) nm; IR (KBr) ν_{max} 2918, 2852, 1631, 1460, 1319, 1148, 1091, 738 cm^{-1}; CD (0.0004 M, MeOH) λ_{max} ($\Delta\varepsilon$) 197 (+7.6), 206 (−7.6), 233 (−27.3), 280 (−4.7) nm; for ^1H and ^{13}C NMR data, see Tables S1 and S2; (+)-ESIMS *m*/*z* 297 $[M + H]^+$; HREIMS *m*/*z* 296.1890 $[M]^+$ (calcd for $C_{19}H_{24}N_2O$, 296.1889).

Alstoscholarisine B (2): colorless needle crystal from MeOH; mp 168–169 ℃; $[\alpha]^{25}_{D}$ −162.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) = 204 (4.31), 229 (4.48), 287 (3.83) nm; IR (KBr) ν_{max} 2973, 2927, 2862, 1730, 1679, 1456, 1324, 1161, 1205, 1042, 744 cm^{-1}; CD (0.0003 M, MeOH) λ_{max} ($\Delta\varepsilon$) 197 (+7.3), 231 (−22.0), 279 (−2.6) nm; for ^1H and ^{13}C NMR data, see Tables S1 and S2; (+)-ESIMS *m*/*z* 355 $[M + H]^+$; HREIMS *m*/*z* 354.1940 $[M]^+$ (calcd for $C_{21}H_{26}N_2O_3$, 354.1943).

Alstoscholarisine C (3): colorless block crystal from MeOH; mp 98–101 ℃; $[\alpha]^{25}_{D}$ −213.3 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) = 202 (4.31), 230 (4.51), 287 (3.86) nm; IR (KBr) ν_{max} 2932, 1737, 1631, 1461, 1247, 1197, 1124, 1083, 748 cm^{-1}; CD (0.0003 M, MeOH) λ_{max} ($\Delta\varepsilon$) 197 (+11.5), 231 (−40.0), 278 (−3.6) nm; for ^1H and ^{13}C NMR data, see Tables S1 and S2; (+)-ESIMS *m*/*z* 355 $[M + H]^+$; HREIMS *m*/*z* 354.1946 $[M]^+$ (calcd for $C_{21}H_{26}N_2O_3$, 354.1943).

Alstoscholarisine D (4): white powder; $[\alpha]^{23}_{D}$ −148.5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) =

203 (4.24), 232 (4.42), 288 (3.79) nm; IR (KBr) ν_{max} 3418, 2922, 1574, 1460, 1383, 1346, 1203, 1043, 739 cm^{-1}; CD (0.0004 M, MeOH) λ_{max} ($\Delta\varepsilon$) 196 (+9.3), 231 (−31.4), 280 (−2.8) nm; for ^{1}H and ^{13}C NMR data, see Tables S1 and S2; (–)-ESIMS *m*/*z* 339 $[M - H]^-$; HREIMS *m*/*z* 340.1780 $[M]^+$ (calcd for $C_{20}H_{24}N_2O_3$, 340.1787).

Alstoscholarisine E (5): colorless block crystal from MeOH; mp 138–140 ℃; $[\alpha]^{25}_D$ −340.7 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) = 203 (4.31), 233 (4.51), 287 (3.88) nm; IR (KBr) ν_{max} 2965, 2927, 2860, 1631, 1456, 1382, 1319, 1135, 1077, 748 cm^{-1}; CD (0.0004 M, MeOH) λ_{max} ($\Delta\varepsilon$) 196 (+7.8), 208 (−7.3), 233 (−20.2), 280 (−3.7) nm; for ^{1}H and ^{13}C NMR data, see Tables S1 and S2; (+)-ESIMS *m*/*z* 297 $[M + H]^+$; HREIMS *m*/*z* 296.1881 $[M]^+$ (calcd for $C_{19}H_{24}N_2O$, 296.1889).

Crystal data for 1: $C_{19}H_{24}N_2O$, M = 296.40, monoclinic, a = 10.2476(4) Å, b = 7.3019(3) Å, c = 10.2973(4) Å, α = 90.00 °, β = 94.7650(10) °, γ = 90.00 °, V = 767.85(5) $Å^3$, T = 100(2) K, space group $P21$, Z = 2, μ(CuKα) = 0.618 mm^{-1}, 9885 reflections measured, 2576 independent reflections (R_{int} = 0.0418). The final R_1 values were 0.0510 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1448 ($I > 2\sigma(I)$). The final R_1 values were 0.0510 (all data). The final $wR(F^2)$ values were 0.1448 (all data). The goodness of fit on F^2 was 1.174. Flack parameter = -0.3(3). The Hooft parameter is 0.13(7) for 1105 Bijvoet pairs.

Crystal data for 3: $C_{21}H_{26}N_2O_3$, M = 354.44, monoclinic, a = 10.5788(10) Å, b = 7.7312(7) Å, c = 10.8099(10) Å, α = 90.00 °, β = 90.3850(10) °, γ = 90.00 °, V = 884.09(14) $Å^3$, T = 100(2) K, space group $P21$, Z = 2, μ(MoKα) = 0.089 mm^{-1}, 9423 reflections measured, 4855 independent reflections (R_{int} = 0.0182). The final R_1 values were 0.0310 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0836 ($I > 2\sigma(I)$). The final R_1 values were 0.0318 (all data). The final $wR(F^2)$ values were 0.0843 (all data). The goodness of fit on F^2 was 1.080. Flack parameter = 0.2(6).

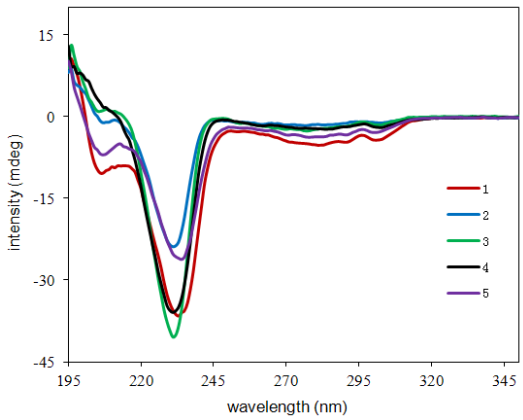


Figure S1. The overlapped experimental CD of compounds **1**–**5**.

SI-5. Detail description of NSCs biological functional studies

5.1 NSCs culture and Sulforhodamine B colorimetric (SRB) assay

We used only early passage cells (between passage 4 and 10) and only the same passage numbers of cells for indicated compounds, growth factors or DMSO. 20,000 adult neural stem cells (NSCs) were evenly seeded into 96 well plate, cells were grown for 24 hrs in serum-free growth medium (DMEM/F12 1:1; Hyclone) containing 20 ng/mL epidermal growth factor (EGF), 20 ng/mL fibroblast growth factor (bFGF), 1% penicillin/streptavidin, 1% N2 supplement (Gibco), 1XB27 (Gibco) and 10 μg/mL heparin as previously described.[1] bFGF and EGF have been identified to affect NSCs in various ways.[2] The resulting multipotent neurospheres were passaged every 3 to 4 days to single-cell suspension for continued growth and enrichment of stem cells. NSCs proliferation rate was measured by SRB assay according to the standard protocol.[3] Simply, cells were incubated with DMSO and different compounds at different concentration for 48 hrs, cells were gently fixed with 16% (w/v) TCA to each well, the plates were incubated at 4 ^{o}C for 1 hr. Plates were washed 5 times with water and air dried, 100 μL of sulforhodamine B solution 0.4% (w/v) in 1% acetic acid was added into each well, the plate was incubated at RT for 10 min. After staining, unbound dye is removed by washing 5 times with 1% acetic acid and plates were air dried, the bound stain is solubilized with 50 μL 10 mM Tris-base, the absorbance were read on automated plate reader (Epoch, Biotek) at 515 nm. Each compound treats 3 independent wells per assay, and the assay was repeated at least 3 times.

5.2 Identifying compounds promoting hippocampal NSCs proliferation

NSCs were cultured in media: DMEM/F12 1:1 containing 1% penicillin/streptavidin, 1% N2 supplement (Gibco), 1XB27 (Gibco) and 10 μg/mL heparin. Basal also positive control: media + 10 ng/mL fibroblast growth factor (bFGF). Another positive control (bFGF + EGF): media + 20 ng/mL EGF + 20 ng/mL bFGF. The value of "Basal" was set to 1, and the other values were normalized to "Basal". Negative control: 10μg/mL puromycin.[4]

5.3 BrdU staining

For the cell proliferation BrdU staining, NSCs were plated on poly-D-lysine- and

laminin-coated plates and pulsed with 10 μM 5-bromo-2-deoxyuridine (BrdU) for 20 minutes to label dividing cells in NSCs growth medium as described above. NSCs were fixed and pretreated with 1 N HCl for 30 min at 37 ^{o}C, washed with borate buffer, pH 8.5 for 10 min, and then stained with BrdU antibody from Abcam (1:500) following the standard Immunocytochemistry protocol online. Quantification of proliferation was performed manually in a blinded manner (Student's *t*-test) with Image J software.

5.4 NSCs/Neuro2a differentiation assay

At 24 hrs post-plating, NSCs were changed into differentiation medium: DMEM/F12 1:1 containing 1% penicillin/streptavidin, 1% N2 supplement (Gibco), 1XB27 (Gibco) and 10 μg/ml heparin containing DMSO, compound **1** or Forskolin for 3 days, followed by fixation with 4% paraformaldehyde for 20 min at RT, then washing with Phosphate-Buffered Saline, pH 7.4 (PBS) for 3 × 10 min.[5] Immunocytochemistry staining was carried out following the standard protocol online. Briefly, cells were preblocked using PBS containing 10% normal donkey serum and 0.1% Tween 20 for 1 h, followed by overnight incubation with primary antibodies: mouse Tuj1 (1:100, Sigma), rabbit glial fibrillary acidic protein (GFAP, 1:2000, DAKO). After washing with PBS containing 0.1% Tween 20, cells were incubated with secondary antibodies that included donkey anti-mouse or donkey anti-rabbit Cy3 (1:500, Jackson Immuno Research), followed by staining with the fluorescent nuclear dye 4',6-dimidino-2'-phenylindole dihydrochloride (DAPI, Sigma). The numbers of Tuj1+ and GFAP+ cells were quantified with Image J software by Student *t*-test. The percentage of differentiated cells was calculated as the number of Tuj1+ or GFAP+ cells divided by the total number of cells stained with DAPI. Neuro2a cells were changed into DMEM basal medium without Fetal Bovine Serum supplied with DMSO or compound **1** to let the cells differentiate, 48 hrs later, cells were fixed with 4% paraformaldehyde for 20 min at RT, then washed with Phosphate-Buffered Saline, pH 7.4 (PBS) for 3 × 10 min. Images of the Neuro2a cells were taken under the microscope (ECLIPSE Ti-S, Nikon), and the neurite bearing cell number were quantified in a blinded manner (Student's *t*-test) with Image J software.

5.5 Cytosol and Nuclear fractionation

NSCs cells after different treatment were collected and resuspended in buffer A (20 mM Tris (pH 7.5); 20% Glycerol; 10 mM NaCl; 1.5 mM MgCl2; 5 mM EDTA (pH 7.5); 0.1% NP40 and CompleteTM protease inhibitors), cells were incubated on ice for 5 min and then passed through a

0.4-mm needlepoint to break down the cell membrane and were centrifuged at 800 g at 4 ^{o}C for 4 min. The supernatant was centrifuged at 100,000g at 4 ^{o}C for 1 h. The supernatant after ultracentrifugation was collected as cytosolic fraction. The pellet from 800 g centrifugation was washed by buffer A, resuspended in buffer B (20 mM Tris (pH 7.5); 10% Glycerol; 100 mM NaCl; 1.5mM $MgCl_2$; 5 mM EDTA (pH 7.5); 1% NP40 and CompleteTM protease inhibitors) and incubated on ice for 30 min. Nuclear fractions were extracted as the supernatants after centrifugation at 100,000 g at 4 ^{o}C for 1 h. Antibodies used for Immunoblotting are: anti-β-catenin (1:5000, cell signaling Technology), anti-SP1 (1:500, Sigma).

5.6 Annexin V/PI staining

NSCs treated by 20 ng/mL TNFα with DMSO or compound **1** additional treatment were collected, phosphatidylserine exposure was determined using an annexin V-FITC kit (Roche) in combination with PI (Sigma-Aldrich Chemical Co). Cells were washed and labeled with annexin V-FITC for 30 min on ice and subsequently with 1 mg/mL PI. Annexin V/PI staining was analyzed on a BD FACScanTM using Cell Quest software. Apoptotic cells were examined on the basis of hypodiploid DNA content.

5.7 Microarray assay

Total RNA from cultured mice hippocampal adult NSCs treated with compound **1** or DMSO was isolated with Rneasy Mini Kit (Qiagen p/n 74104), and the RNA quantification and quality (RNA-QC) were well controlled. To minimize the variation, RNAs from 3 independent compound **1** or DMSO treated NSCs were extracted, respectively, and pooled together for the following experiments. The assay was performed according to the standard protocol of "Agilent One-Color Micorarray-Based Gene Expression Analysis". Briefly, the cRNA were synthesized, amplified, purified, fragmented and hybridized following *Agilent Microarray Hybridization Chamber User Guide* (G2534-90001) using Agilent Gene Expression Hybridization Kit (Agilent p/n 5188-5242). The Microarray was washed and scanned (Agilent p/n G2505C). mRNAs enriched ≥ 1.5 fold in compound **1** over DMSO were selected for further analyses.

5.8 Luciferase reporter assay

The day before transfection, cells were seeded at a density of 1–2X105 cells/mL, cells were transfected with a 4:1 ratio of 8XGliBS-luciferase vector or TOPFlash: pRL-TK and other transgene constructs with X-tremeGENE (Roche) according to the manufacturer's instructions. 1

days after transfection, cells were further cultured with or without additional treatments as indicated in different figures, cells were harvested and luciferase activities were determined, individual sample was performed in triplicate and all the assays were repeated for at least 3 times.

5.9 Quantitative RT-PCR with reverse transcription

The total RNA was isolated with RNAiso plus 9108 kit (Takara). RNA reverse transcription was preformed using IScript^{TM} cDNA Synthesis kit (Biorad, #170-8891) according to the manufacturer's instructions. Quantitative RT-PCR for *Axin2*, *CyclinD1*, *MYC*, *Gli1*, *Ptch1*, *KLF4*, *Sox2*, *Nanog*, *Nestin*, *NeuroD*, *Oligo1*, *GFAP* and *GAPDH* was performed with Fast Start Universal SYBR Green Master (Rox) (Roche, #10476600) kit on 7900HT Fast Real-Time PCR system (Applied Biosystems). The specific primers used for detecting the genes are listed in Table S3.

Table S3: Summary of the differentially expressed genes in **1** treated NSCs

Sample	Total # genes upregulated (≥ 1.5 fold change)	Total # genes downregulated (≥ 1.5 fold change)	Total # genes (≥ 1.5 fold change)
1 vs DMSO	1847	1302	3149
		Signature genes affected	
Hh signaling pathway		Ptch1, Gli1, Gli2, Kif7, Bmp2, Lrp2	
Wnt signaling pathway		Dvl2, Wnt5a,Wnt8b, Wnt9a, Wnt9b, Nfatc1, Nfatc2	
Other interested genes		KLF4, Nanog, NeuroD	

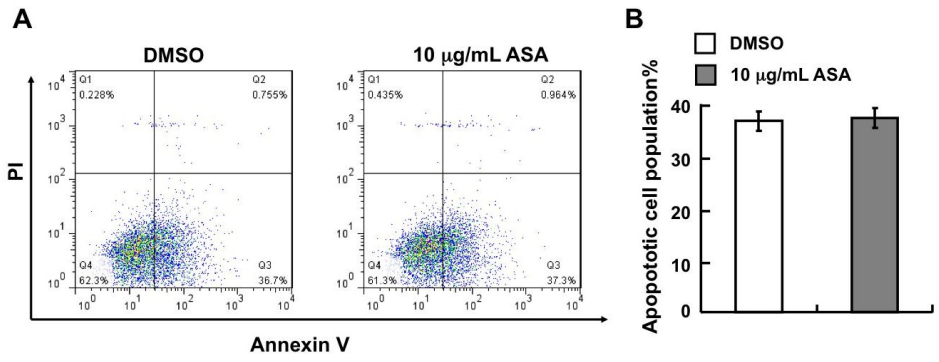


Figure S2. alstoscholarisine A (**ASA**) does not affect NSCs apoptotic signaling pathway. NSCs treated by 20 ng/mL TNFα with DMSO or **1** additional treatment were collected, annexin V in combination with PI staining was performed. **B** is the quantification results for **A**. Individual sample was performed in triplicate and all the assays were repeated for at least 3 times.

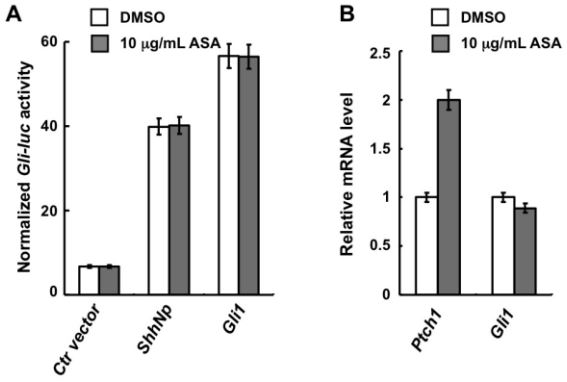


Figure S3. The Hedgehog (Hh) signaling pathway was not affected by alstoscholarisine A (**ASA**). **A**: **1** did not activate Hh signaling response luciferase reporter *Gli-luc*. **B: 1** did not activate Hh signaling downstream genes *Ptch1* and *Gli1*. qRT-PCR was normalized to *GAPDH* expression, Ctr = Control. Individual sample was performed in triplicate and all the assays were repeated for at least 3 times.

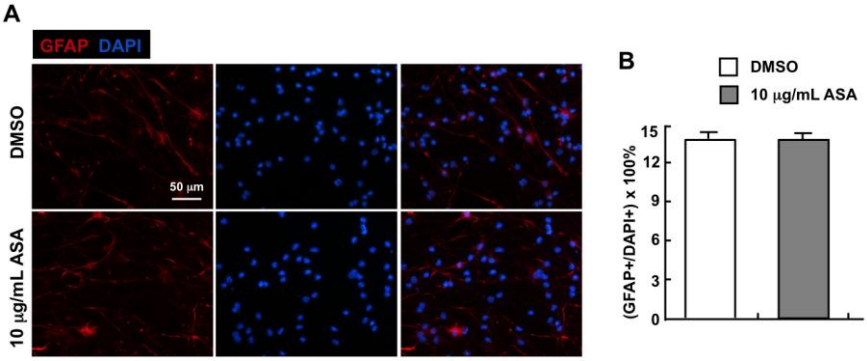


Figure S4. Alstoscholarisine A (**ASA**) does not promote NSC differentiation into GFAP+ astrocytes. **A** and **B**: Hippocampal NSCs differentiation assay was performed and the cells were stained for GFAP and DAPI, **B** is the quantification results for **A**, scale bar, 50 μm. Individual sample was performed in triplicate and all the assays were repeated for at least 3 times.

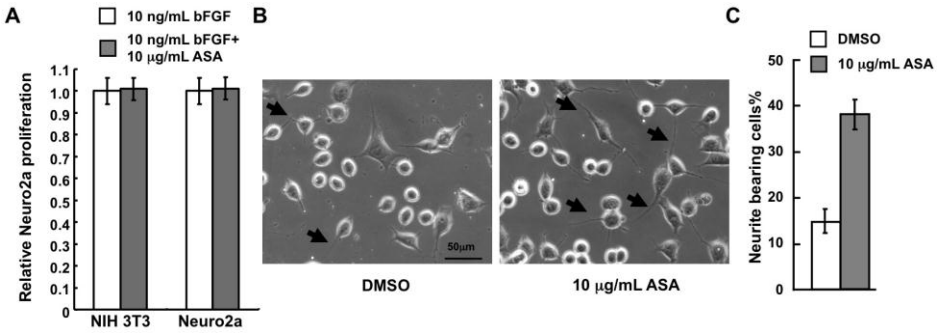


Figure S5. The neuronal differentiation is increased treated by alstoscholarisine A (**ASA**). **A**: mouse neuroblastoma cells (Neuro2a) and fibroblast cells (NIH 3T3) proliferation assay by SRB. The relative proliferation is shown compared to the 10 ng/mL bFGF, which is set to 1. **B** and **C:** Neuro2a cell differentiation assay. The dark arrows are pointing to the standard neurites. **C** is the quantification results for **B**. Individual sample was performed in triplicate and all the assays were repeated for at least 3 times.

Table S4. Primers for qRT-PCR

Genes name	Primer sequences
*Axin2*_F	5’-AGTCAGCAGAGGGACAGGAA-3’
*Axin2*_R	5’-CTTCGTACATGGGGAGCACT-3’
*CyclinD1*_F	5'-GGCACCTGGATTGTTCTGTT- 3'
*CyclinD1*_R	5'-CAGCTTGCTAGGGAACTTGG- 3'
*MYC*_F	5’- AATCCTGTACCTCGTCCGAT-3’
*MYC*_R	5’-TCTTCTCCACAGACACCACA-3’
*Gli1*_F	5'-GCTGTCGGAAGTCCTATT-3'
*Gli1*_R	5'-ACTGGCATTGCTAAAGG-3'
*Ptch1*_F	5'-CTCAGGGAATACGAAGCACA- 3'
*Ptch1*_R	5'-GACAAGGAGCCAGAGTCCAG- 3'
*KLF4*_F	5’-CACACAGGCGAGAAACCTTACC-3’
*KLF4*_R	5’-CGGAGCGGGCGAATTT-3’
*Sox2*_F	5’-CACAGATGCAACCGATGCA-3’
*Sox2*_R	5’-GGTGCCCTGCTGCGAGTA-3’
*Nanog*_F	5’- AGGATGAAGTGCAAGCGGTG-3’
*Nanog*_R	5’- TGCTGAGCCCTTCTGAATCAG-3’
*Nestin*_F	5'-CCAGAGCTGGACTGGAACTC- 3'
*Nestin*_R	5'-ACCTGCCTCTTTTGGTTCCT- 3'
*NeuroD*_F	5'-CAAAGCCACGGATCAATCTT- 3'
*NeuroD*_R	5'-CCCGGGAATAGTGAAACTGA- 3'
*Oligo1*_F	5'- GCAACTACATCCTGCTGCTG -3'
*Oligo1*_R	5'- CACCAGCTGGGAGAGAGAAC -3'
*GFAP*_F	5'-ACCAAATCCGTGTCAGAAGG- 3'
*GFAP*_R	5'-CAGAAGGAAGGGAAGTGCTG- 3'
*GAPDH*_F	5'-CTCAACTACATGGTCTACATGTTCCA- 3'
*GAPDH*_R	5'-CCATTCTCGGCCTTGACTGT- 3'

References

(1) Yang, C. P.; Gilley, J. A.; Zhang, G.; Kernie, S. G. *Development* **2011**, *138*, 4351–4362.

(2) Khalifa, S. A. M.; Medina P.; Erlandsson, A.; El-Seedi, H. R.; Silvente-Poirot, S.; Poirot, M. *Biochem. Biophys. Res. Commun*. **2014**, *446*, 681–686.

(3) Vichai, V.; Kirtikara, K. *Nat. Protoc*. **2006**, *1*, 1112–1116.

(4) Liu, Y.; Lacson, R.; Cassaday, J.; Ross, D. A.; Kreamer, A.; Hudak, E.; Peltier, R.; Mclaren, D.; Mu ñoz-Sanjuan, I.; Santini, F.; Strulovici, B.; Ferrer, M. *J. Biomol. Screen.* **2009**, *14*, 319–329.

(5) Hsieh, J.; Aimone, J. B.; Kaspar, B. K.; Kuwabara, T.; Nakashima, K.; Gage, F. H. *J. Cell biol.* **2004**, *164*, 111–122.

SI-6. The original NMR and MS spectra of the new isolates

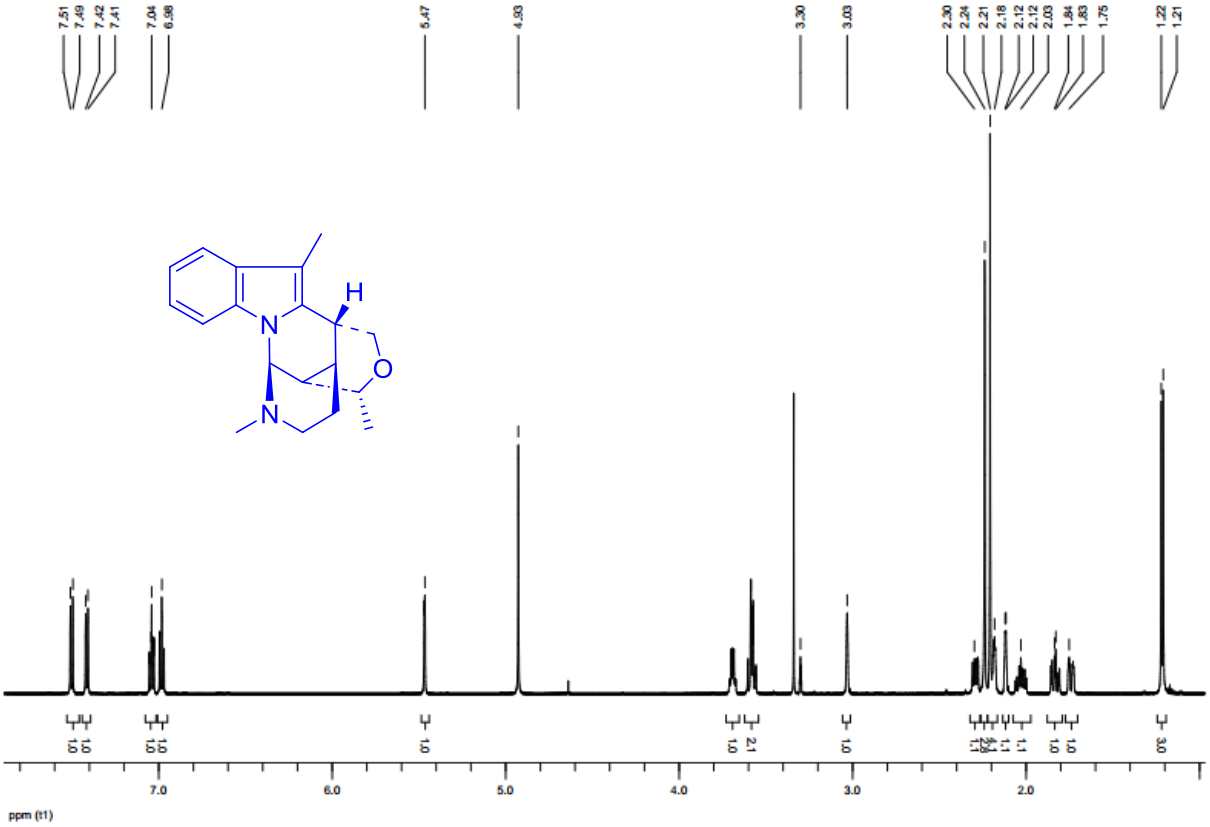


Figure S6. ^{1}H (in methanol-d_4) spectrum of alstoscholarisine A (**1**)

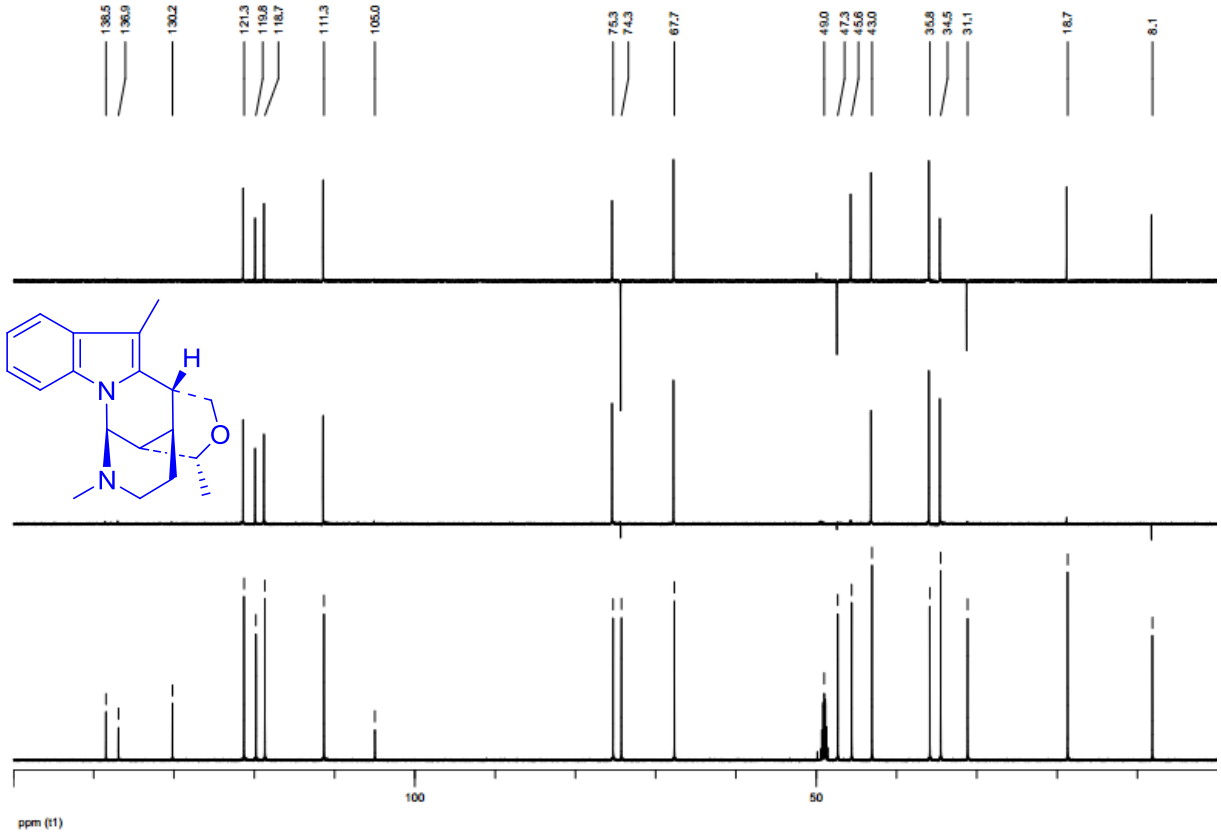


Figure S7. ^{13}C-DEPT (in methanol-d_4) spectrum of alstoscholarisine A (**1**)

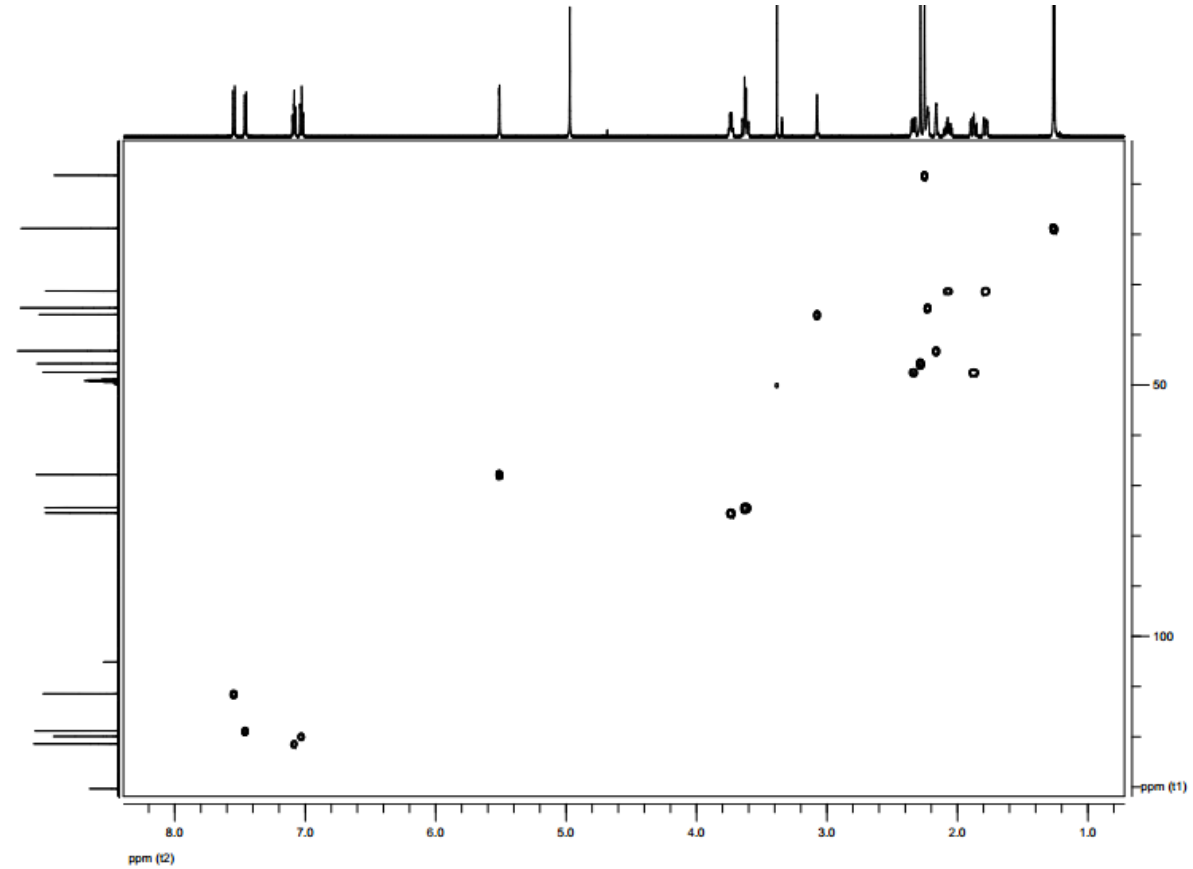


Figure S8. HSQC spectrum of alstoscholarisine A (**1**)

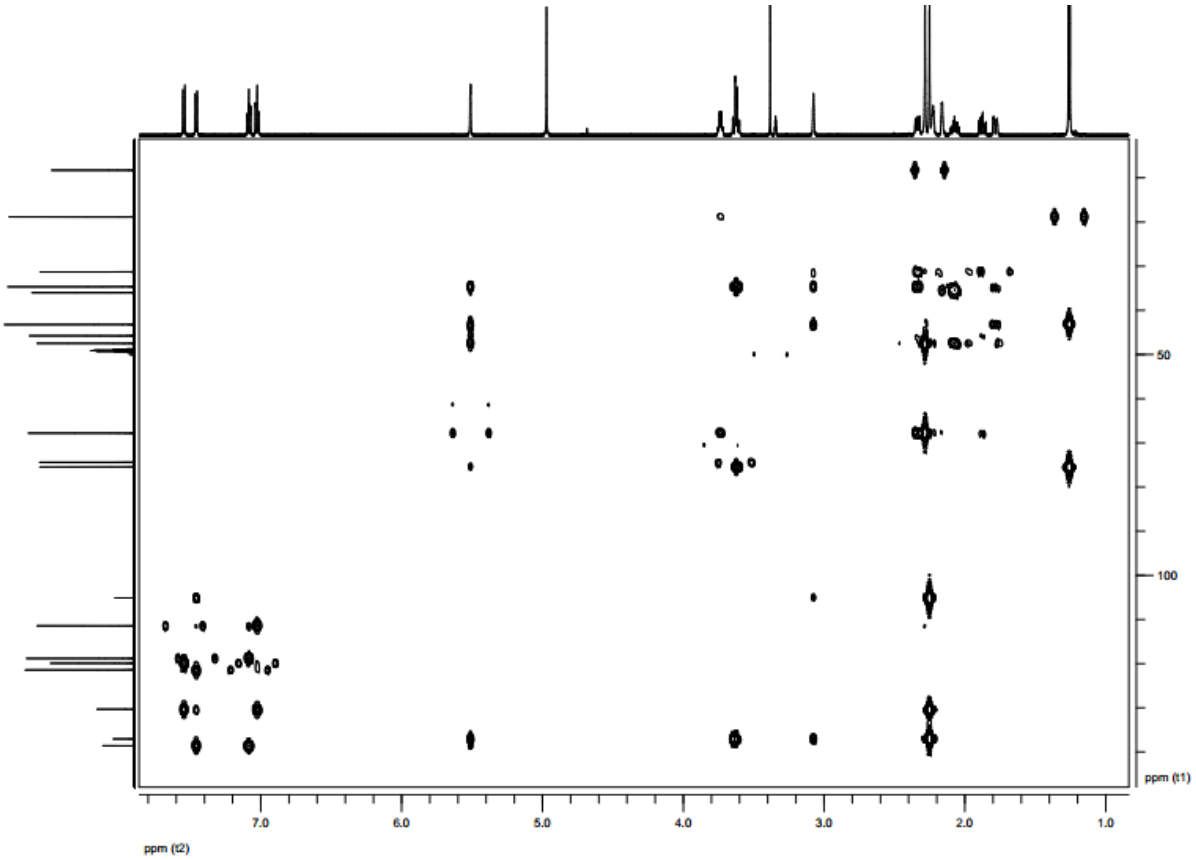


Figure S9. HMBC spectrum of alstoscholarisine A (**1**)

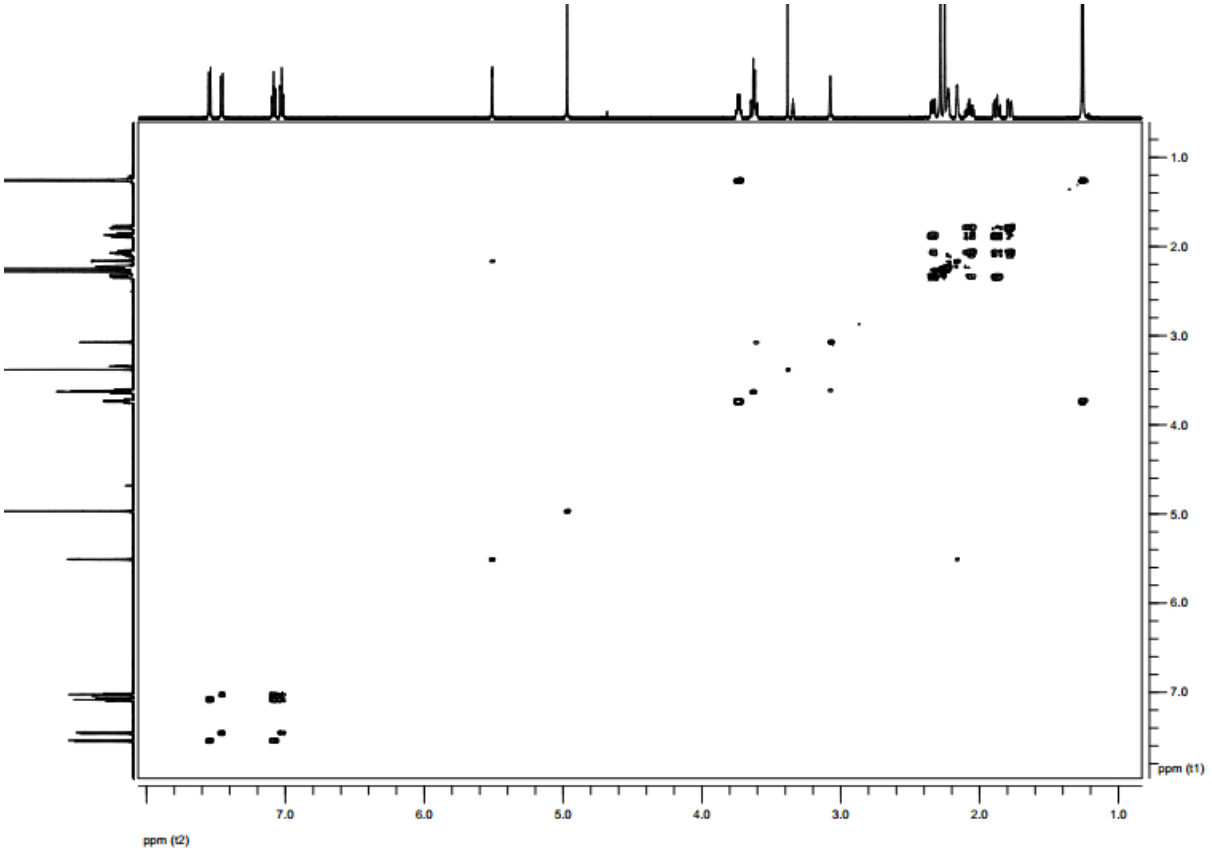


Figure S10. ^{1}H–^{1}H COSY spectrum of alstoscholarisine A (**1**)

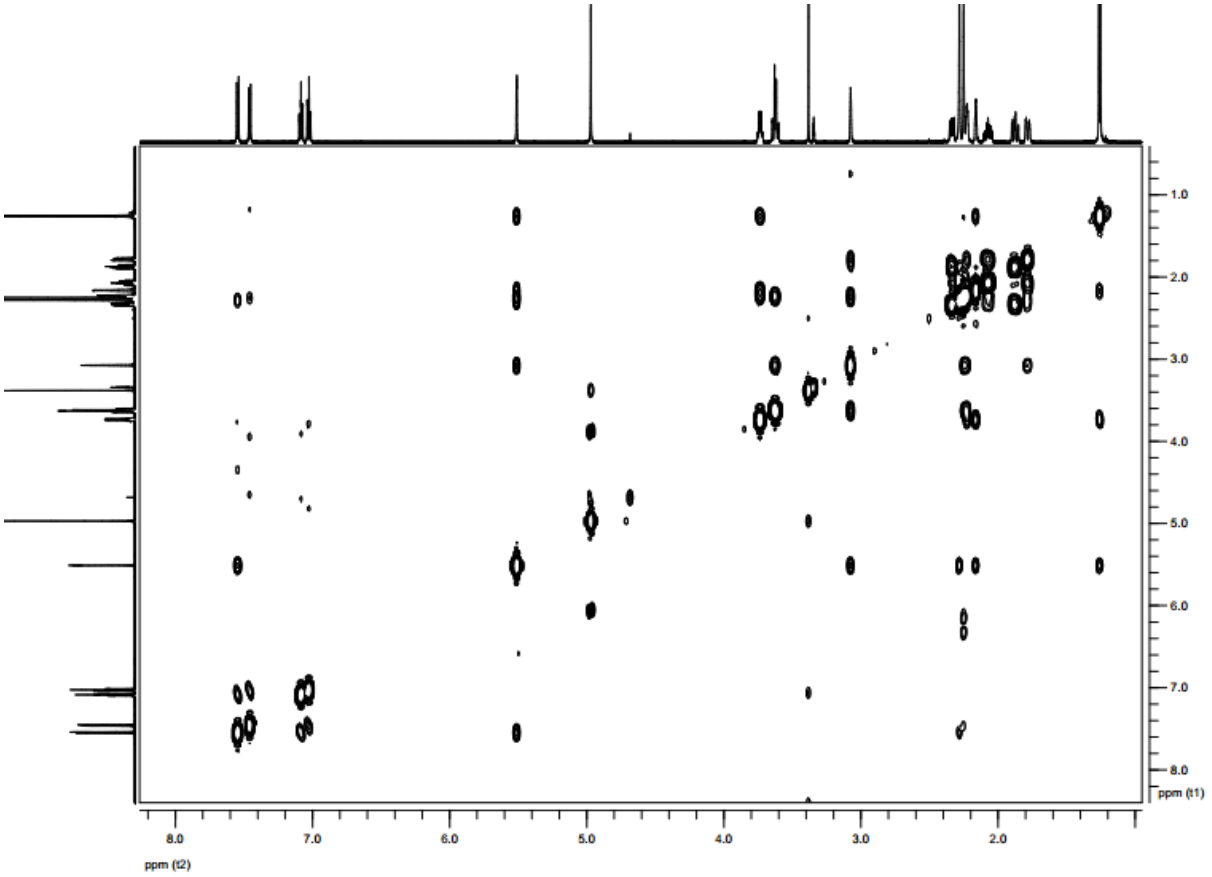


Figure S11. ROESY spectrum of alstoscholarisine A (**1**)

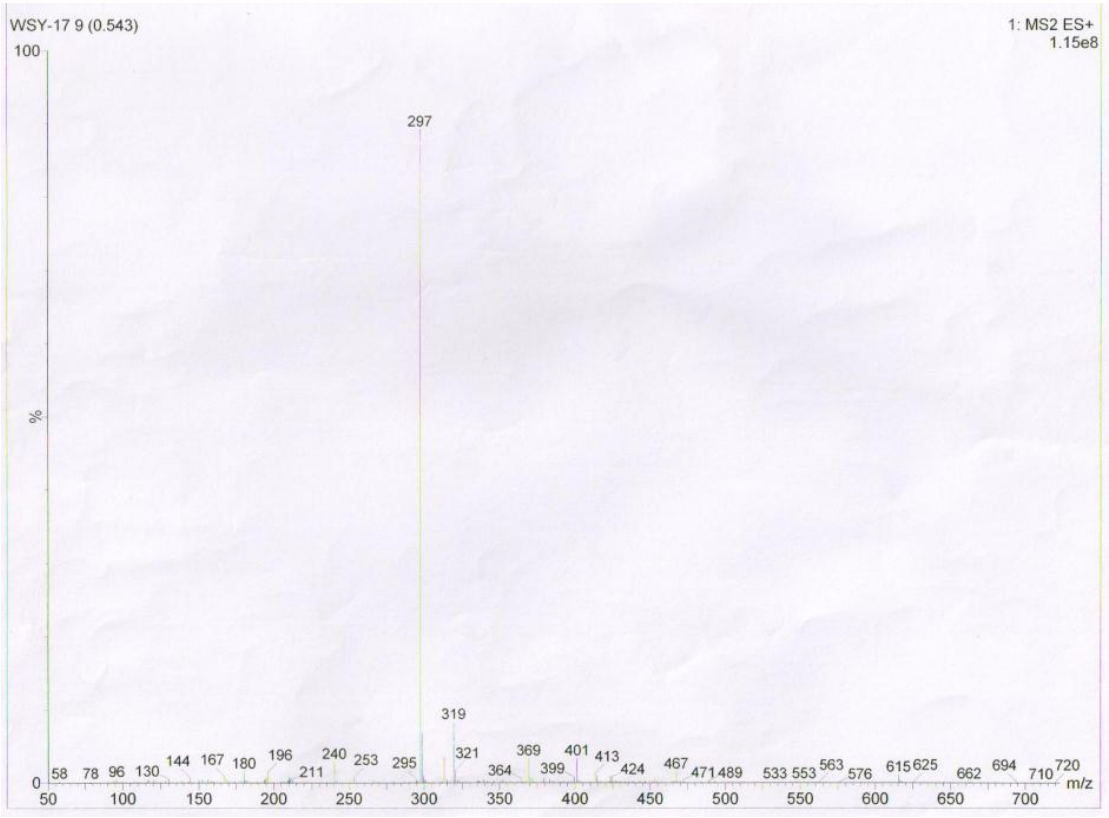
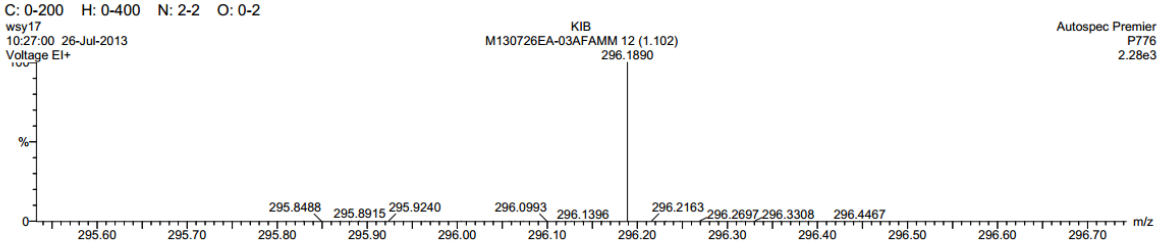


Figure S12. ESI-MS spectrum of alstoscholarisine A (**1**)

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
17 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0-200 H: 0-400 N: 2-2 O: 0-2



Minimum:				-10.0		
Maximum:		200.0	10.0	120.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
296.1890	296.1889	0.1	0.3	9.0	5547149.5	C19 H24 N2 O

Figure S13. HR-EI-MS spectrum of alstoscholarisine A (**1**)

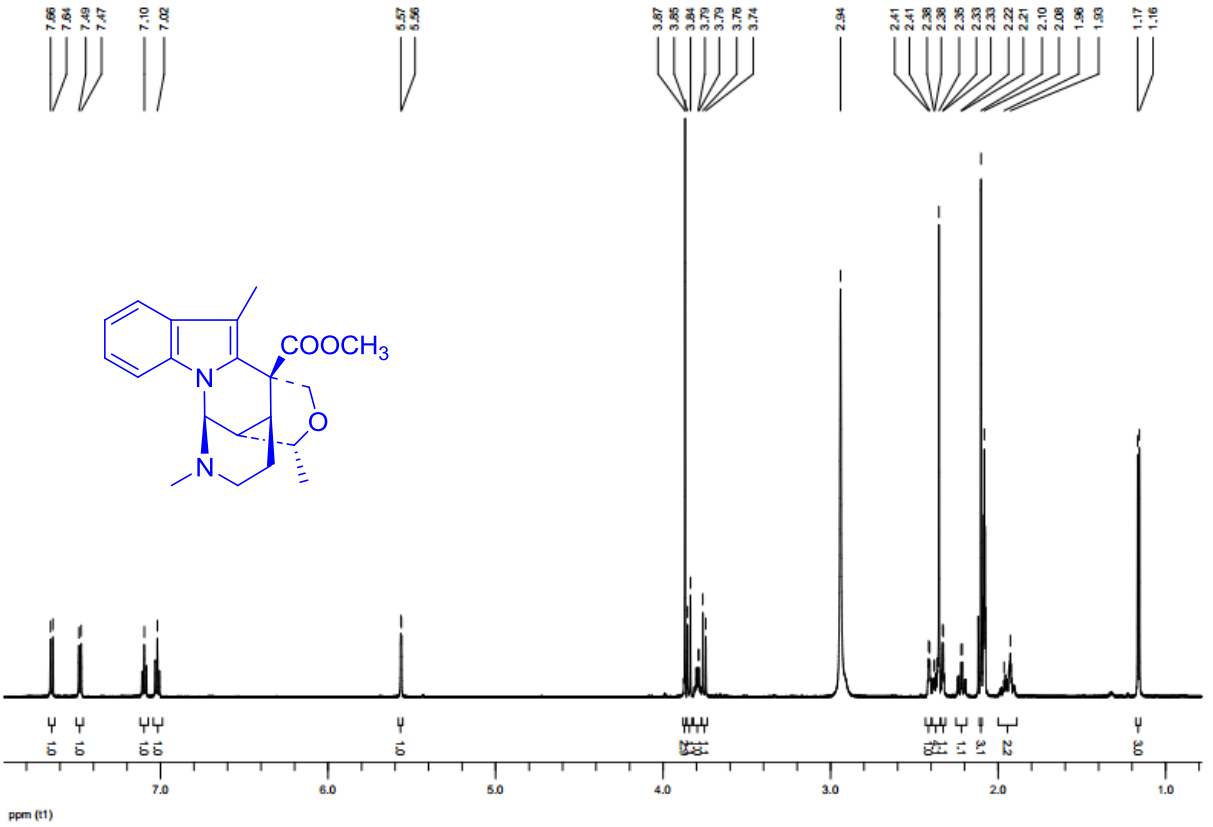


Figure S14. ^{1}H (in acetone-d_6) spectrum of alstoscholarisine B (**2**)

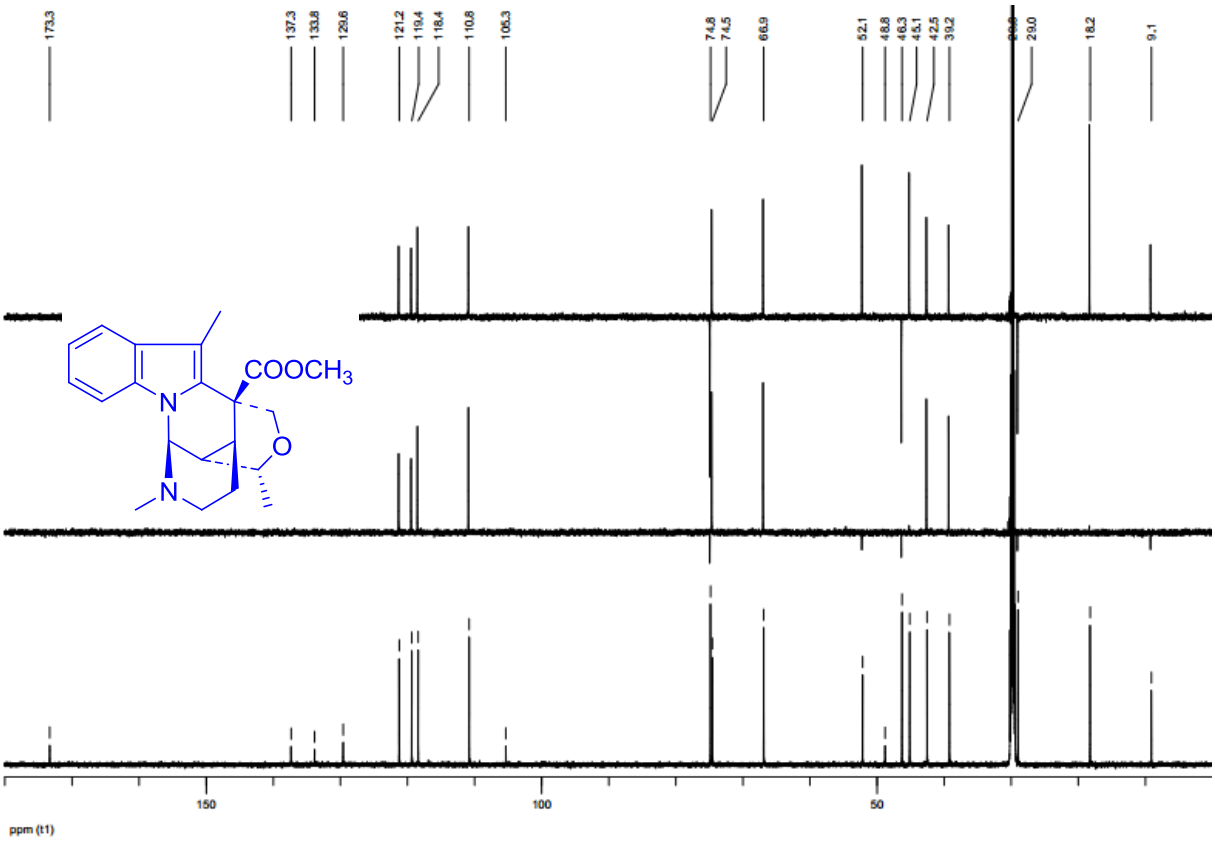


Figure S15. ^{13}C-DEPT (in acetone-d_6) spectrum of alstoscholarisine B (**2**)

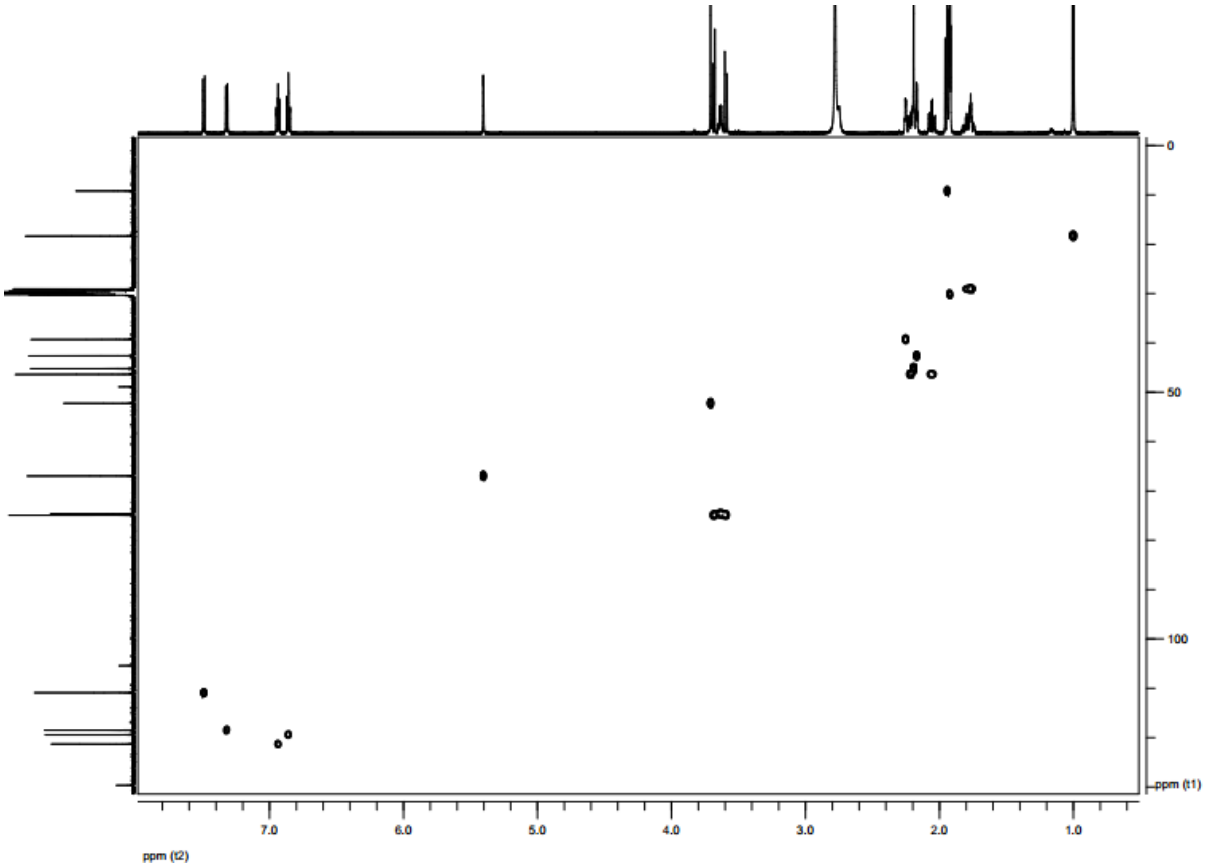


Figure S16. HSQC spectrum of alstoscholarisine B (**2**)

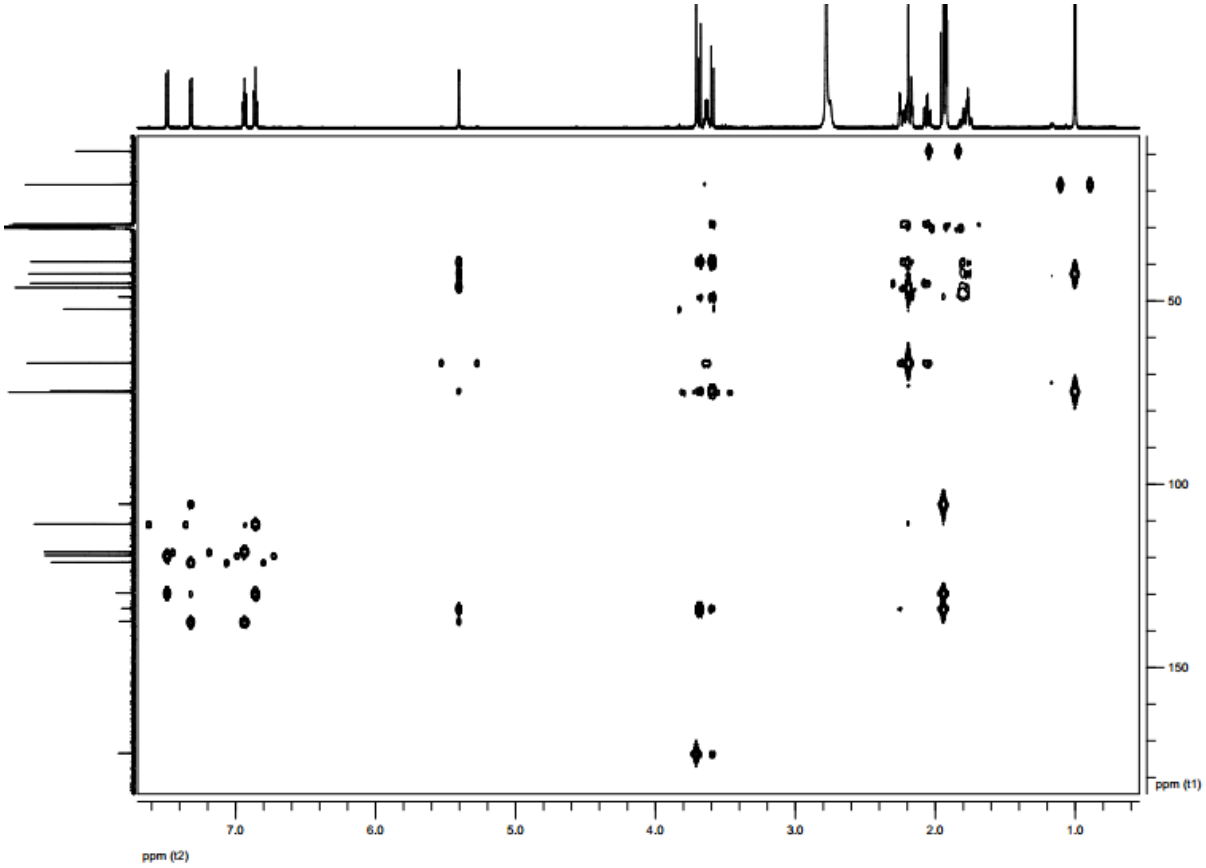


Figure S17. HMBC spectrum of alstoscholarisine B (**2**)

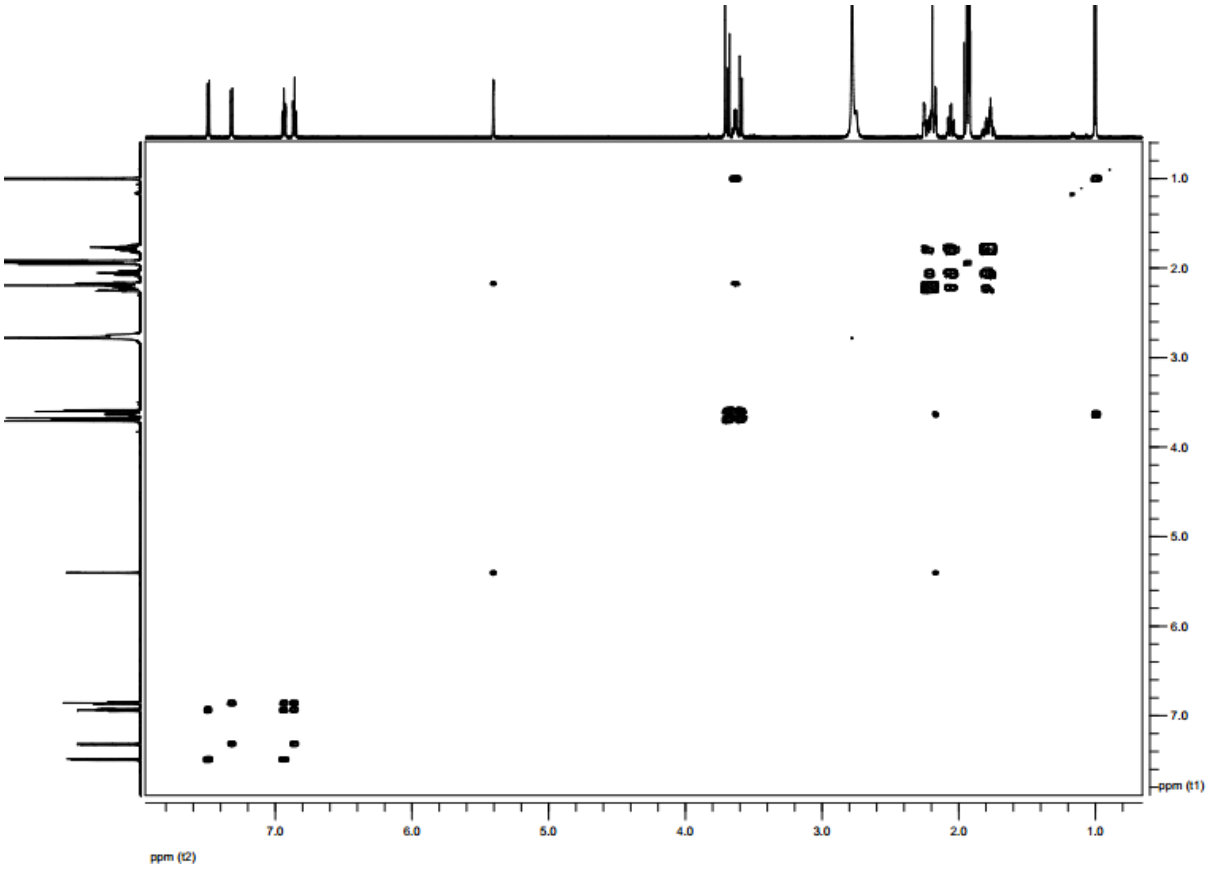


Figure S18. ^{1}H–^{1}H COSY spectrum of alstoscholarisine B (**2**)

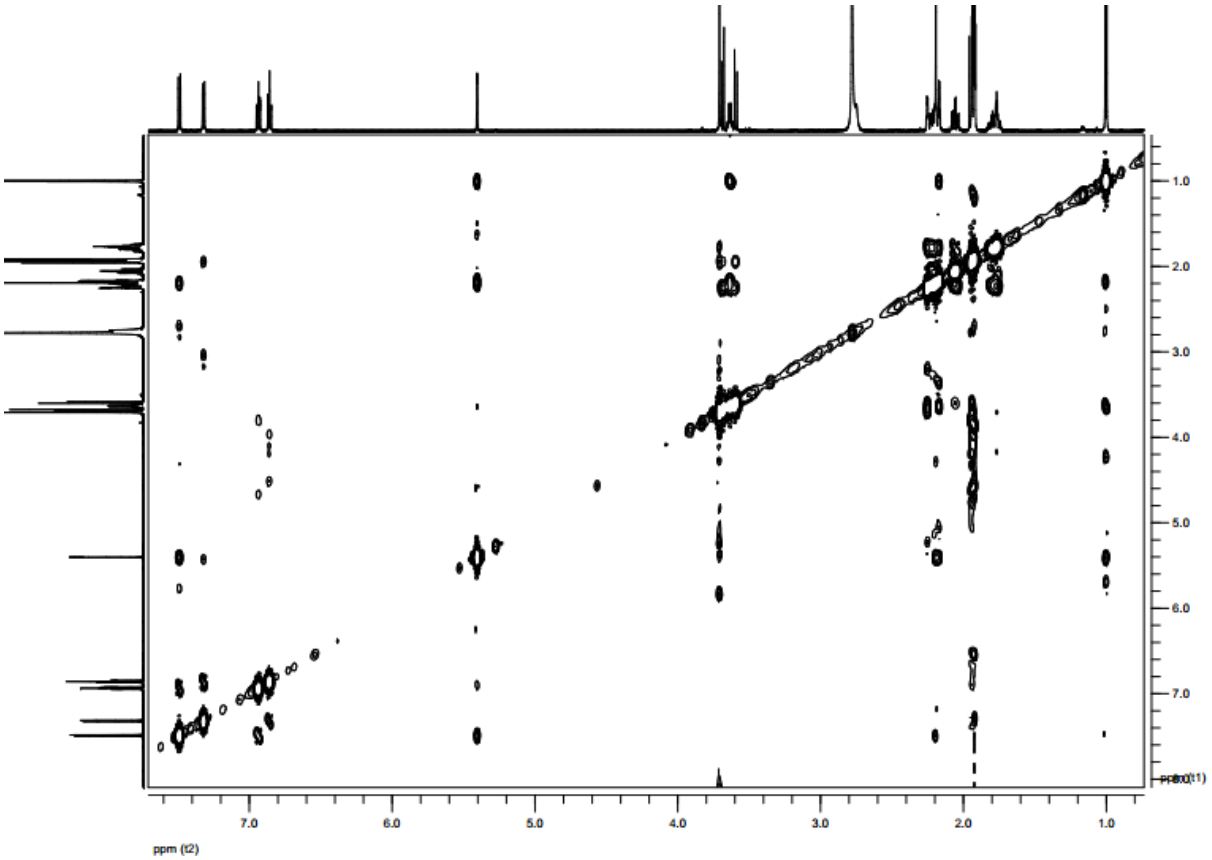


Figure S19. ROESY spectrum of alstoscholarisine B (**2**)

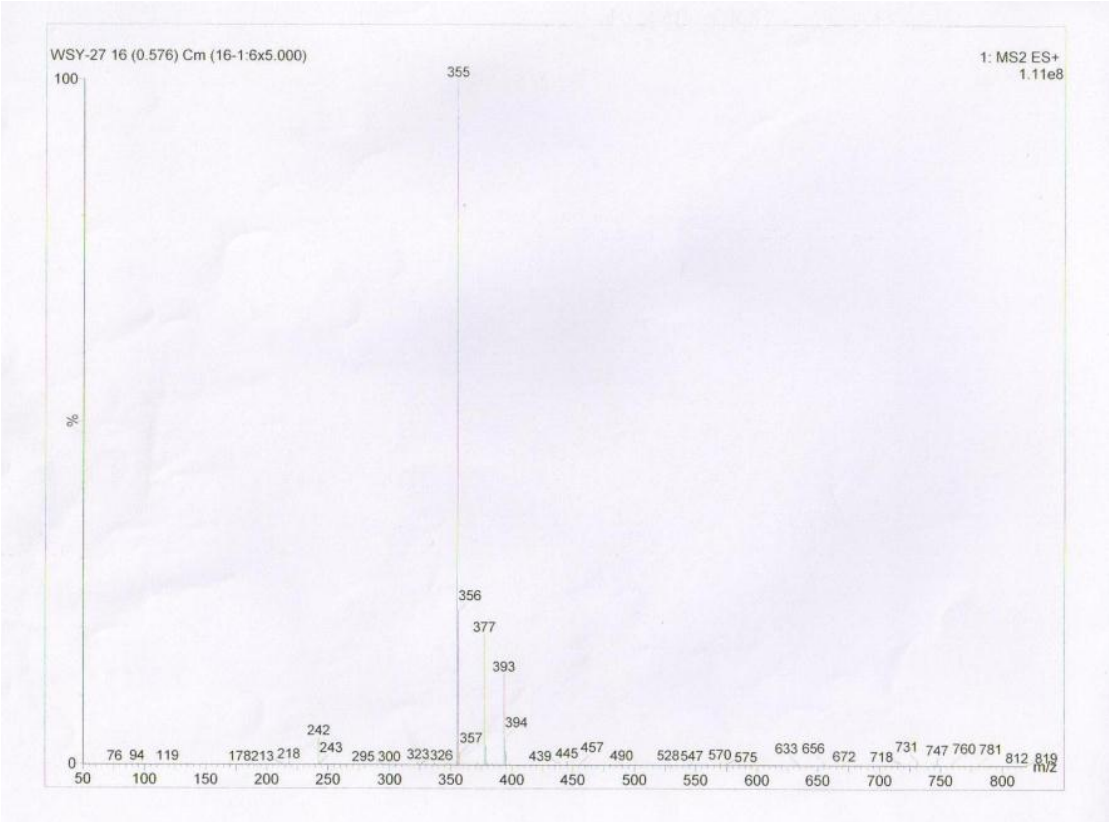
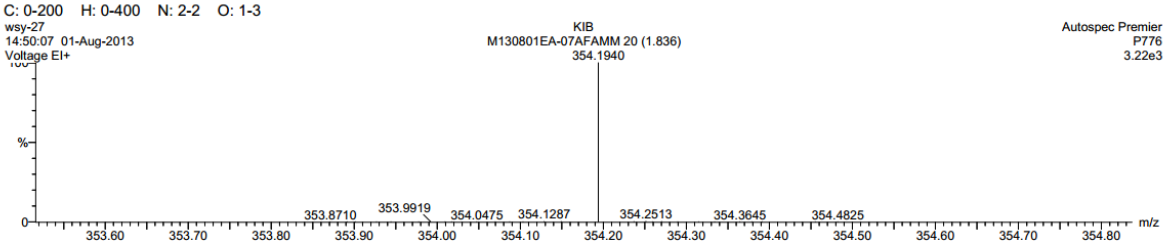


Figure S20. ESI-MS spectrum of alstoscholarisine B (**2**)

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
18 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0-200 H: 0-400 N: 2-2 O: 1-3



Minimum:			-10.0			
Maximum:		200.0	10.0	120.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
354.1940	354.1943	-0.3	-0.8	10.0	5547625.0	C21 H26 N2 O3

Figure S21. HR-EI-MS spectrum of alstoscholarisine B (**2**)

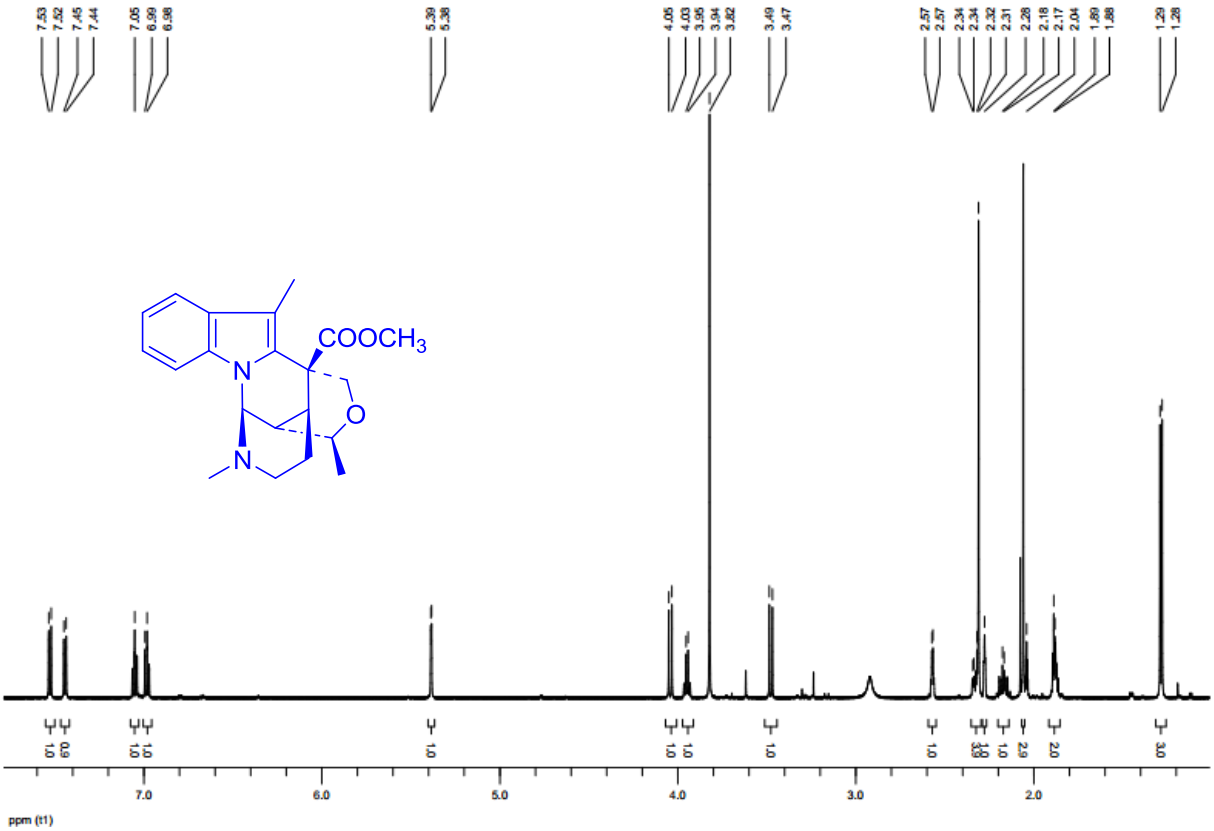


Figure 22. ^{1}H (in acetone-d_6) spectrum of alstoscholarisine C (**3**)

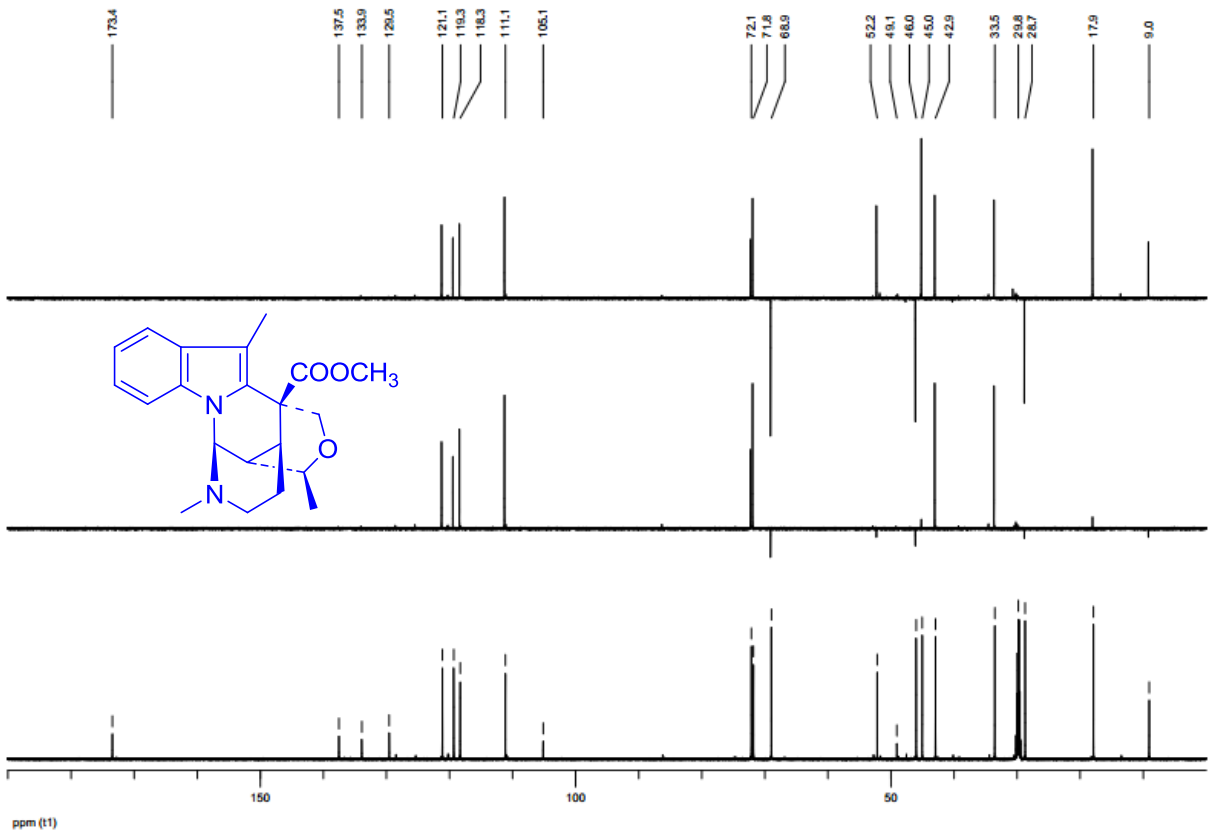


Figure S23. ^{13}C-DEPT (in acetone-d_6) spectrum of alstoscholarisine C (**3**)

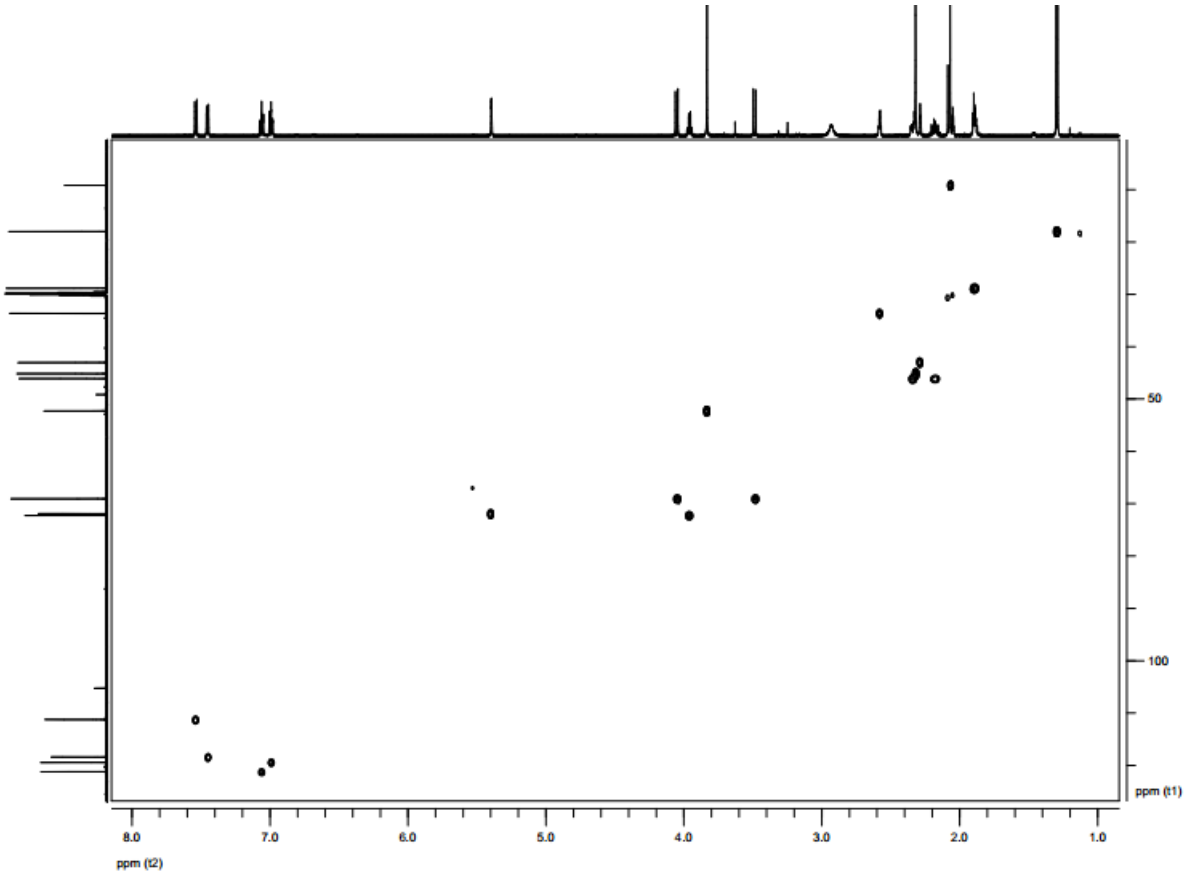


Figure S24. HSQC spectrum of alstoscholarisine C (**3**)

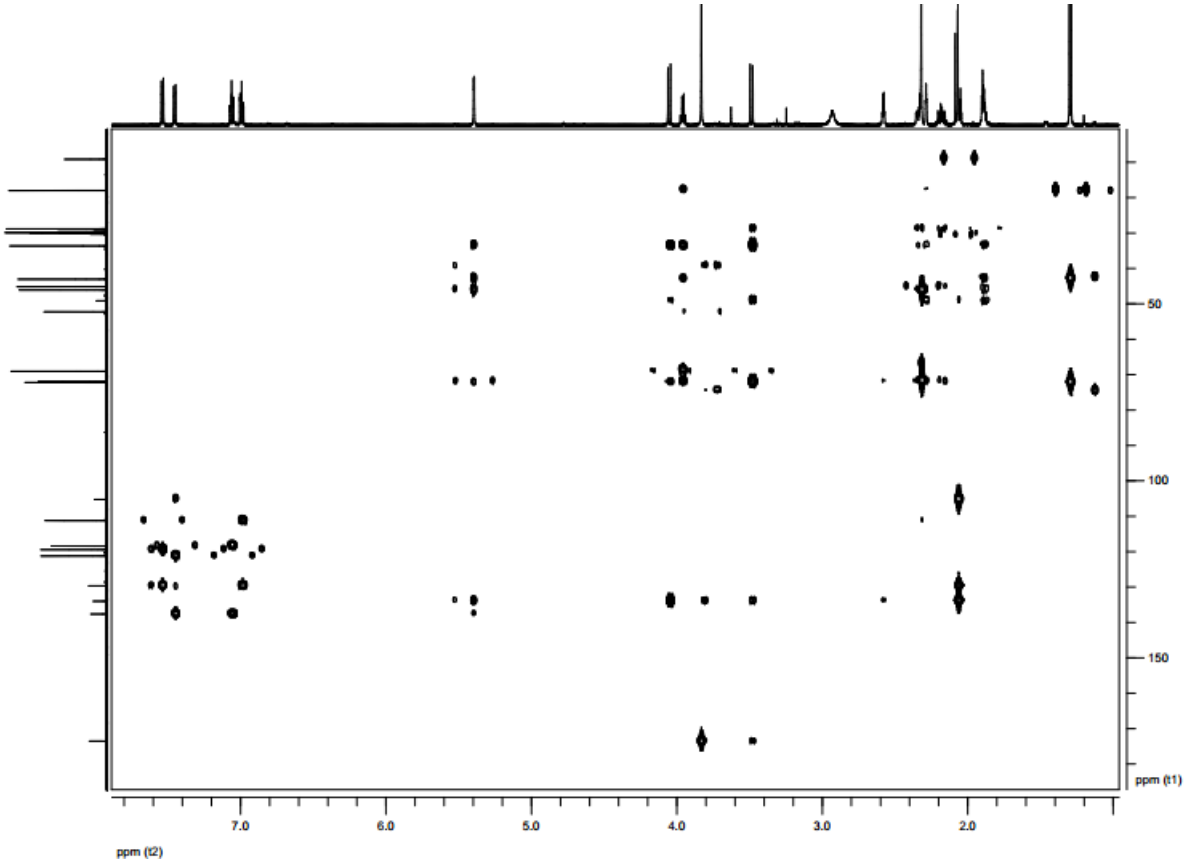


Figure S25. HMBC spectrum of alstoscholarisine C (**3**)

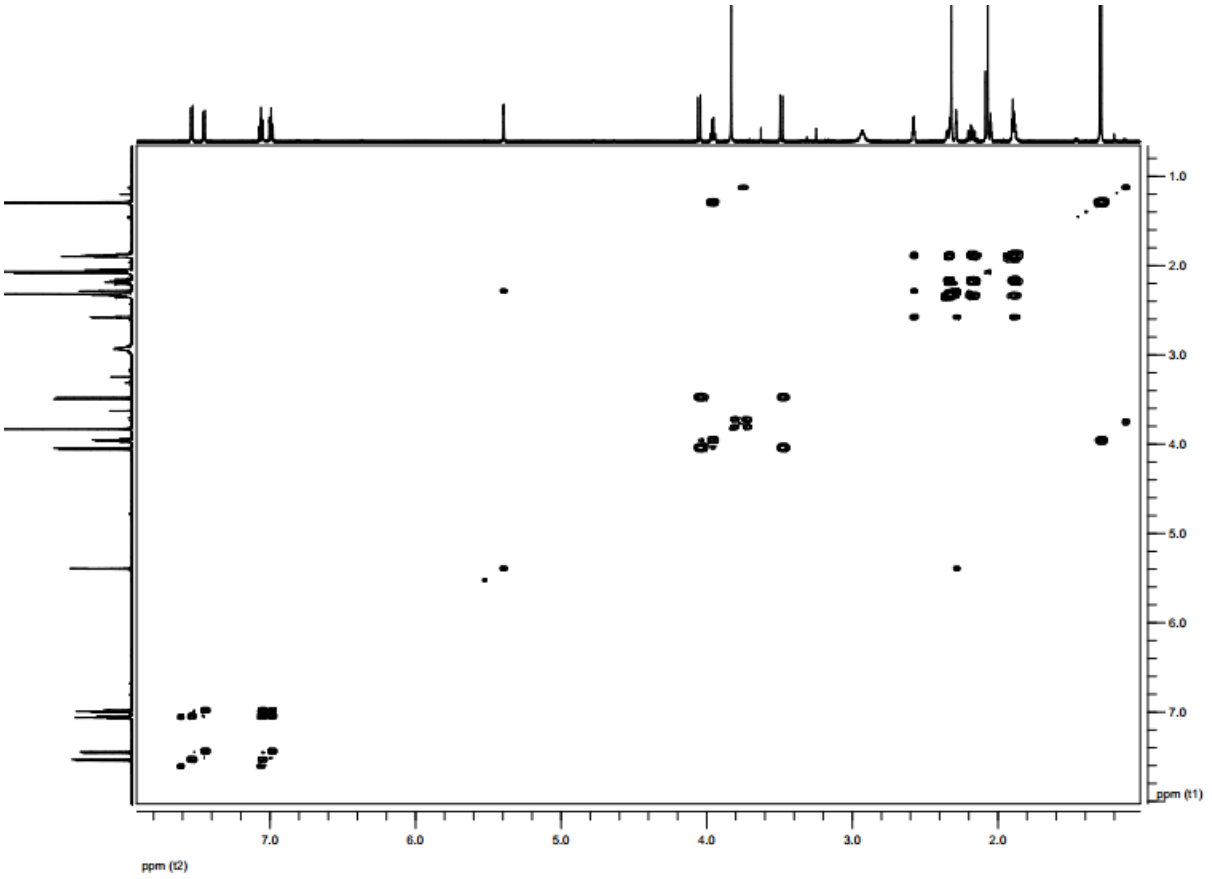


Figure S26. $^{1}H–^{1}H$ COSY spectrum of alstoscholarisine C (**3**)

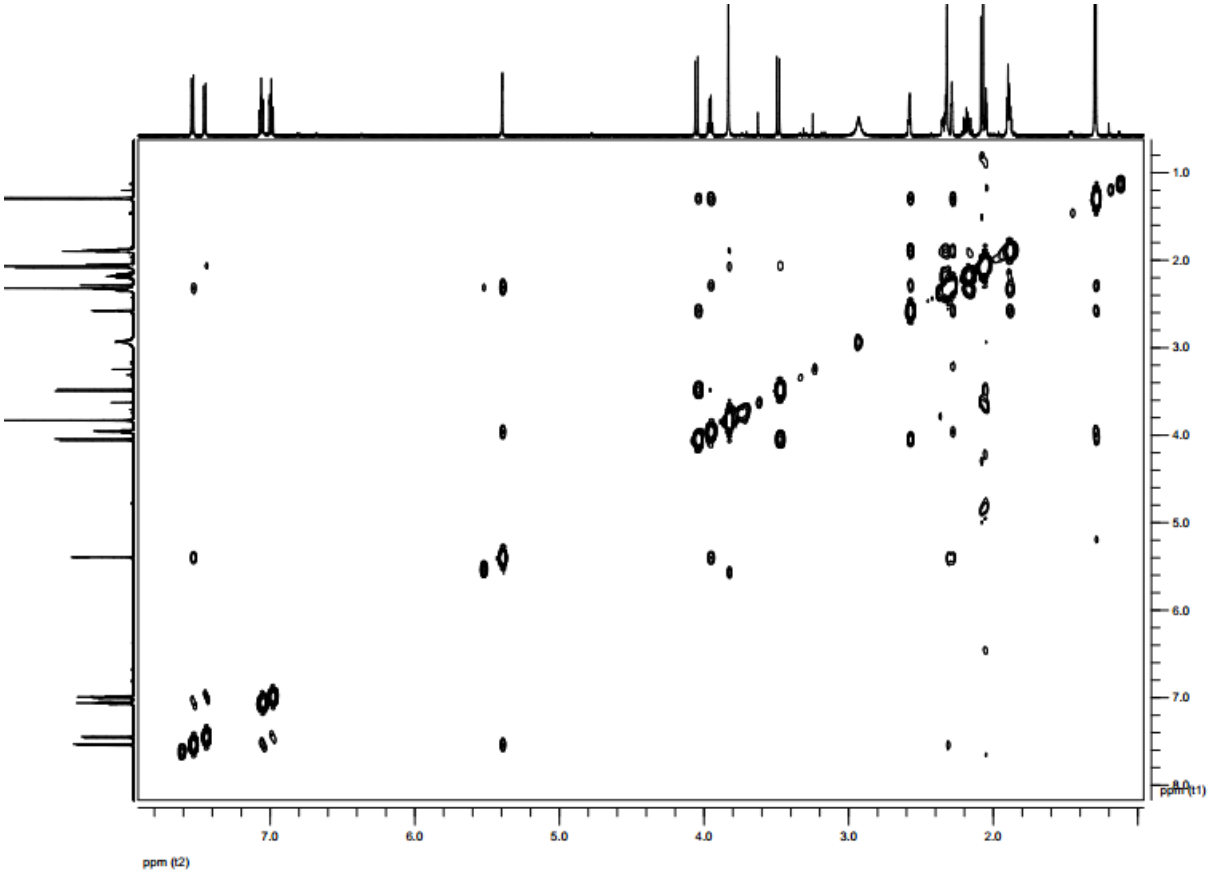


Figure S27. ROESY spectrum of alstoscholarisine C (**3**)

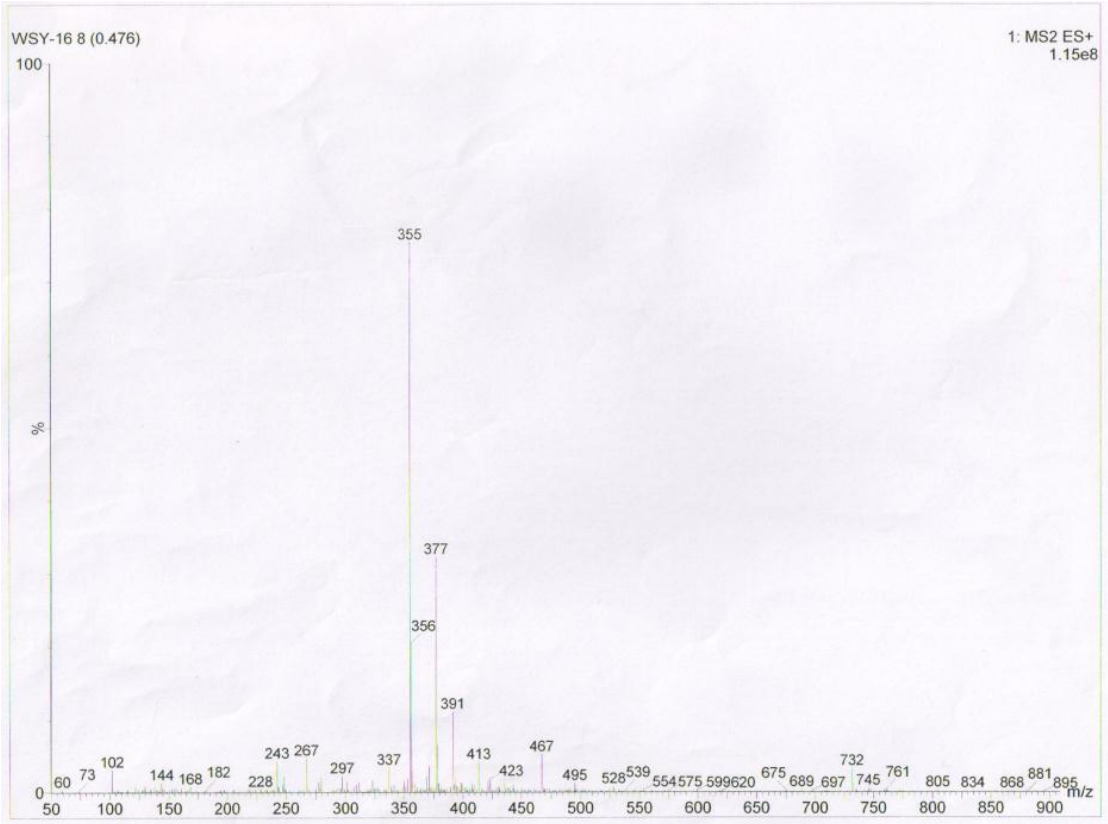


Figure S28. ESI-MS spectrum of alstoscholarisine C (**3**)

Elemental Composition Report **Page 1**

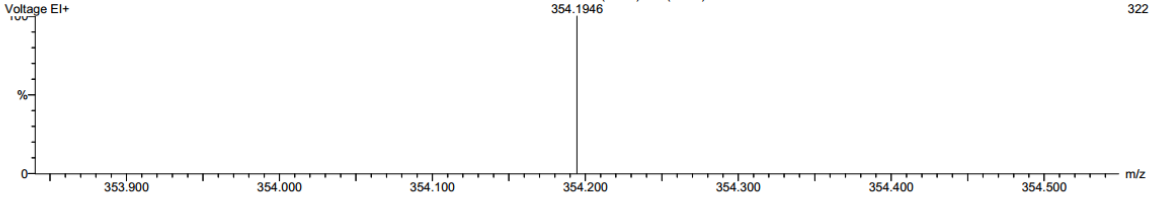
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
17 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0-200 H: 0-400 N: 2-2 O: 2-4

wsy16
10:20:09 26-Jul-2013
Voltage EI+

KIB
M130726EA-02AFAMM 19 (1.745) Cm (19:20)
354.1946

Autospec Premier
P776
322



Minimum:				-10.0		
Maximum:		200.0	10.0	120.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
354.1946	354.1943	0.3	0.8	10.0	5546174.5	C21 H26 N2 O3

Figure S29. HR-EI-MS spectrum of alstoscholarisine C (**3**)

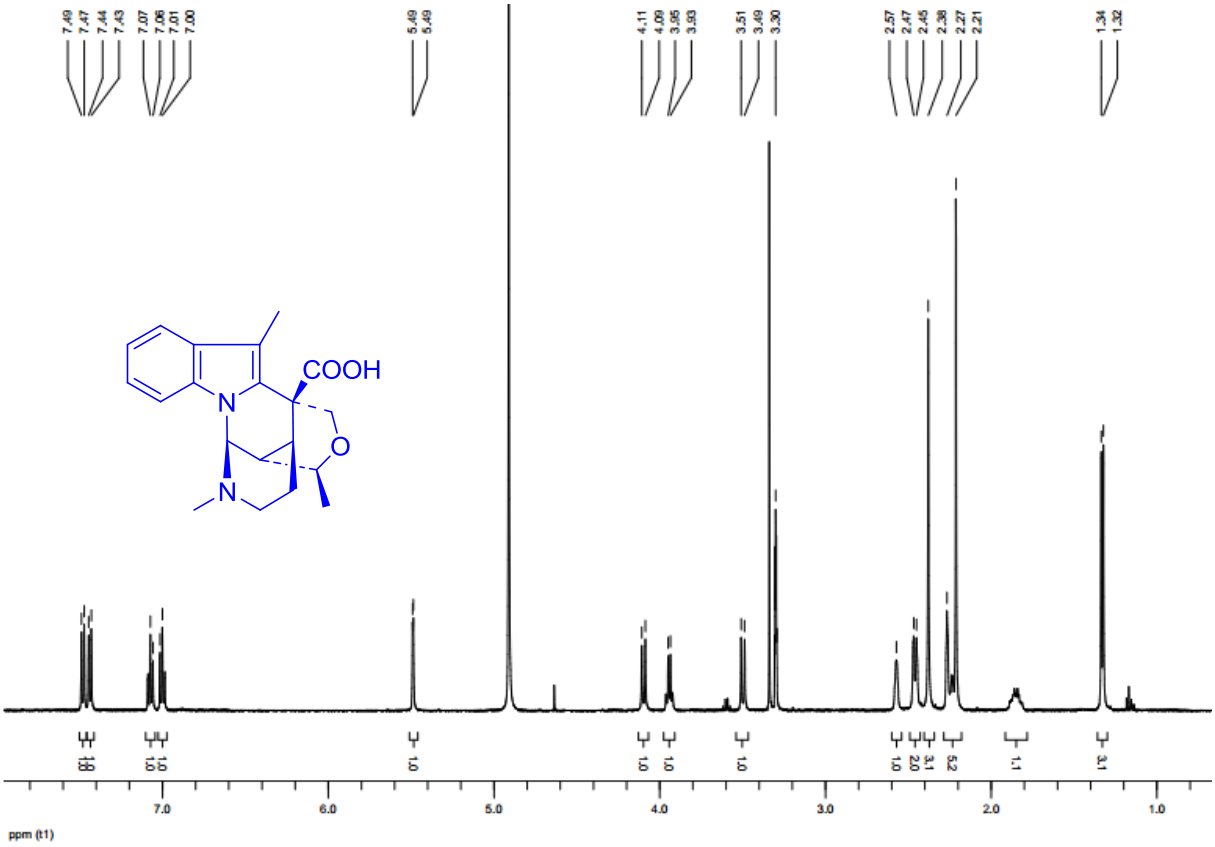


Figure S30. ^{1}H (in methanol-d_4) spectrum of alstoscholarisine D (**4**)

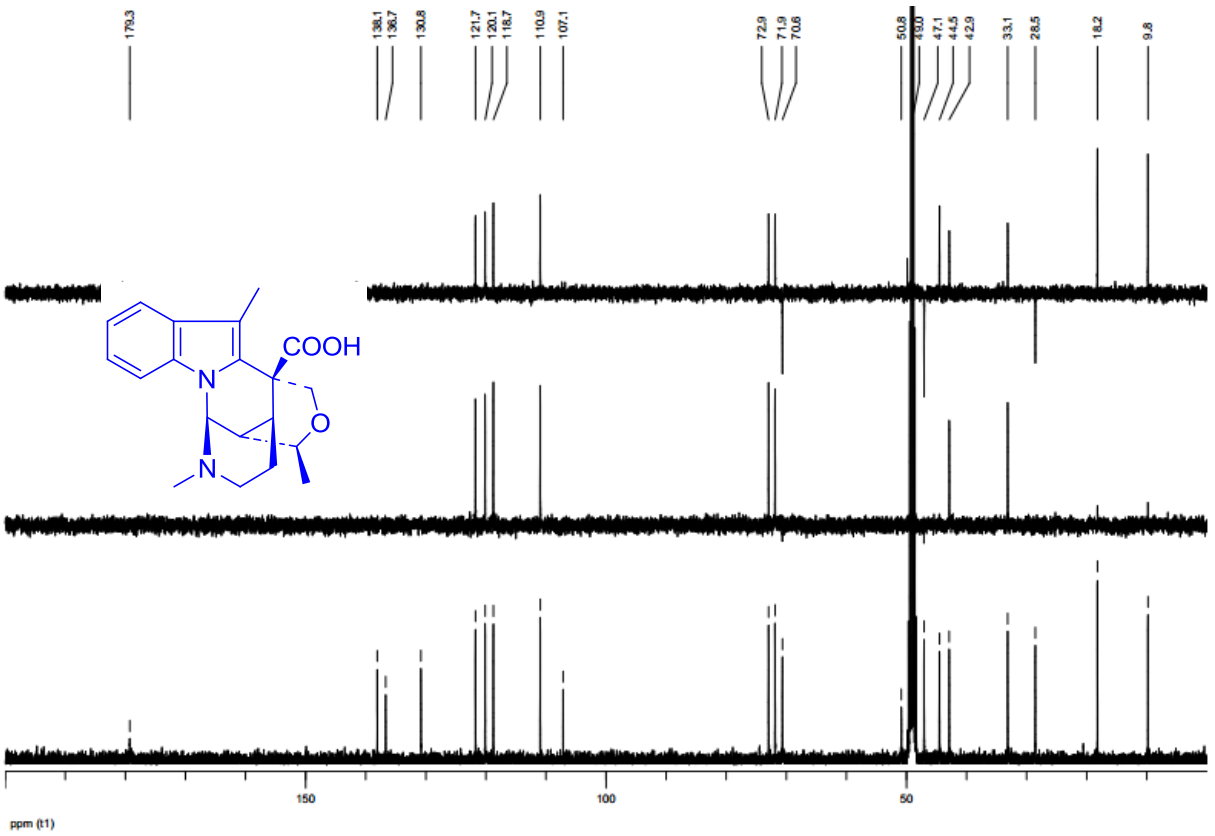


Figure S31. ^{13}C-DEPT (in methanol-d_4) spectrum of alstoscholarisine D (**4**)

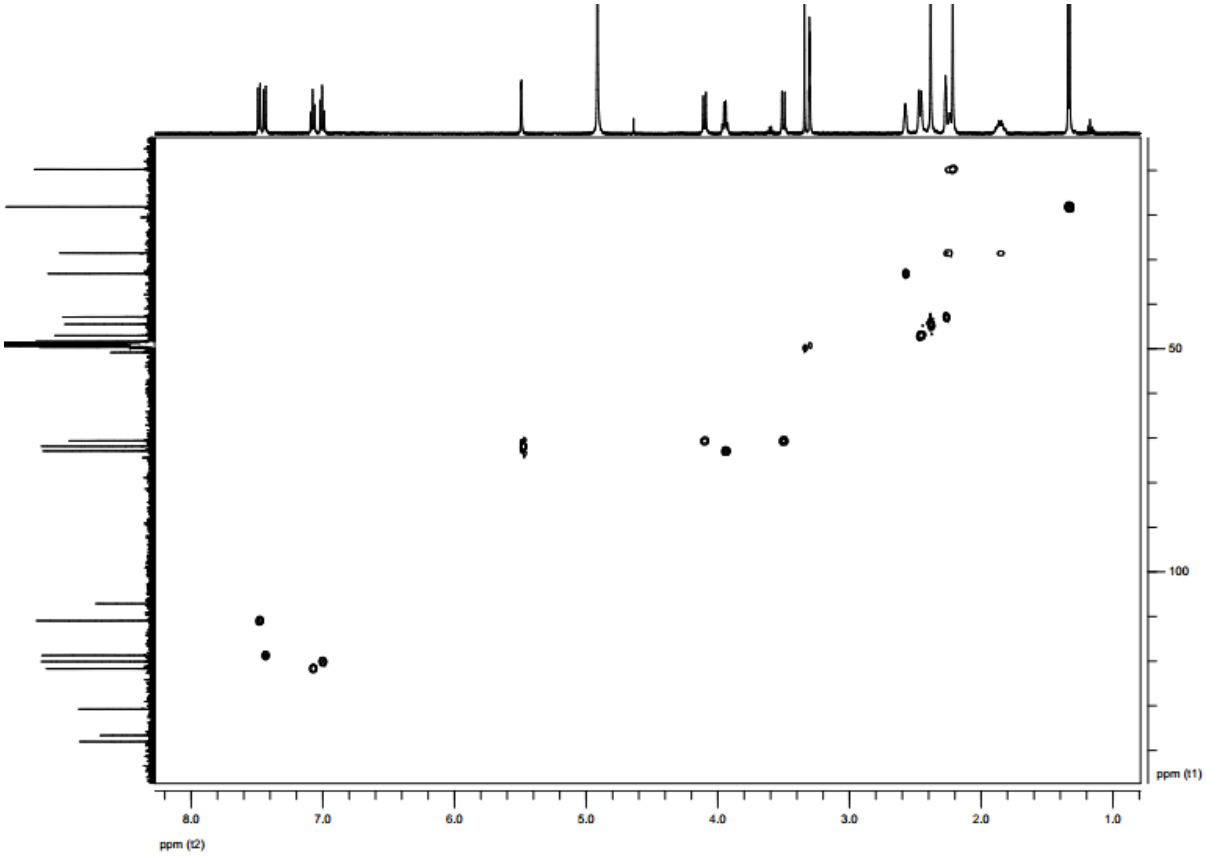


Figure S32. HSQC spectrum of alstoscholarisine D (**4**)

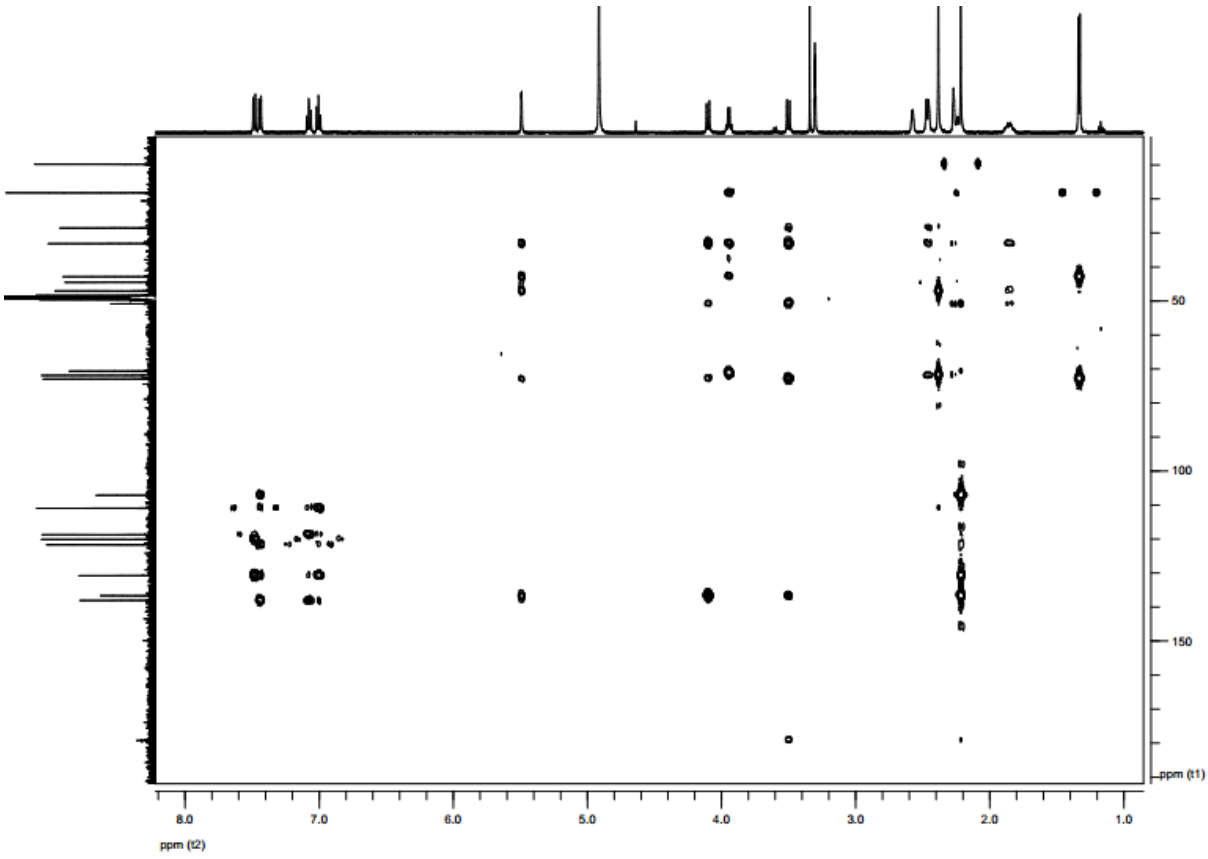


Figure S33. HMBC spectrum of alstoscholarisine D (**4**)

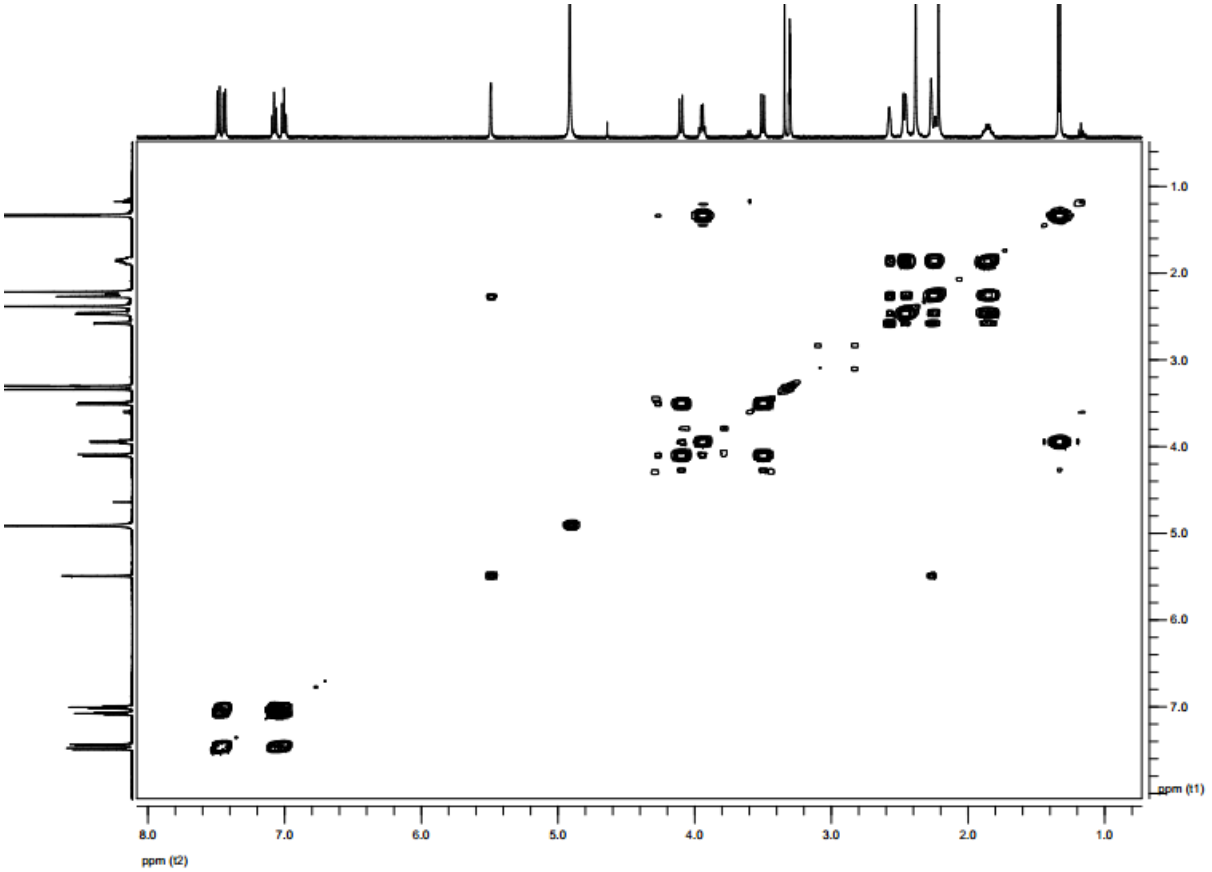


Figure S34. $^{1}H–^{1}H$ COSY spectrum of alstoscholarisine D (**4**)

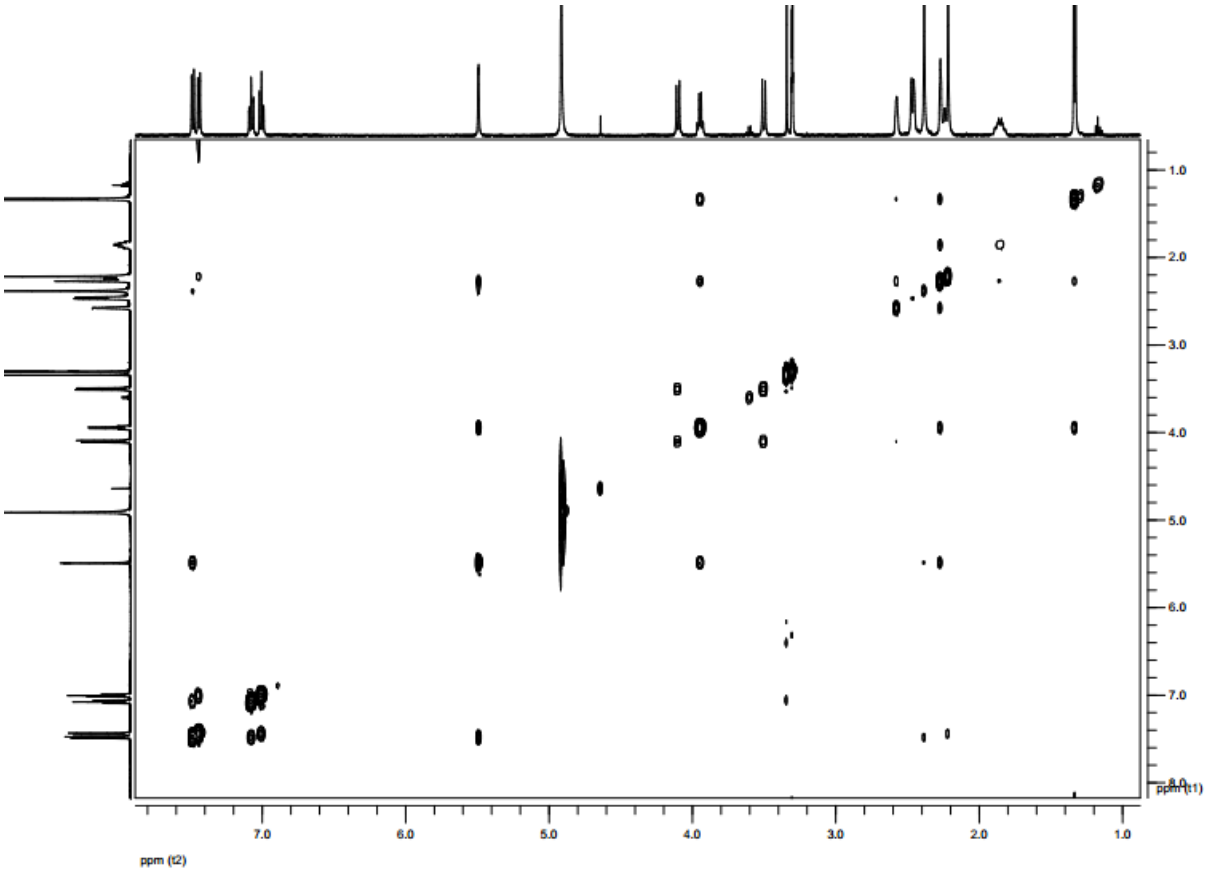


Figure S35. ROESY spectrum of alstoscholarisine D (**4**)

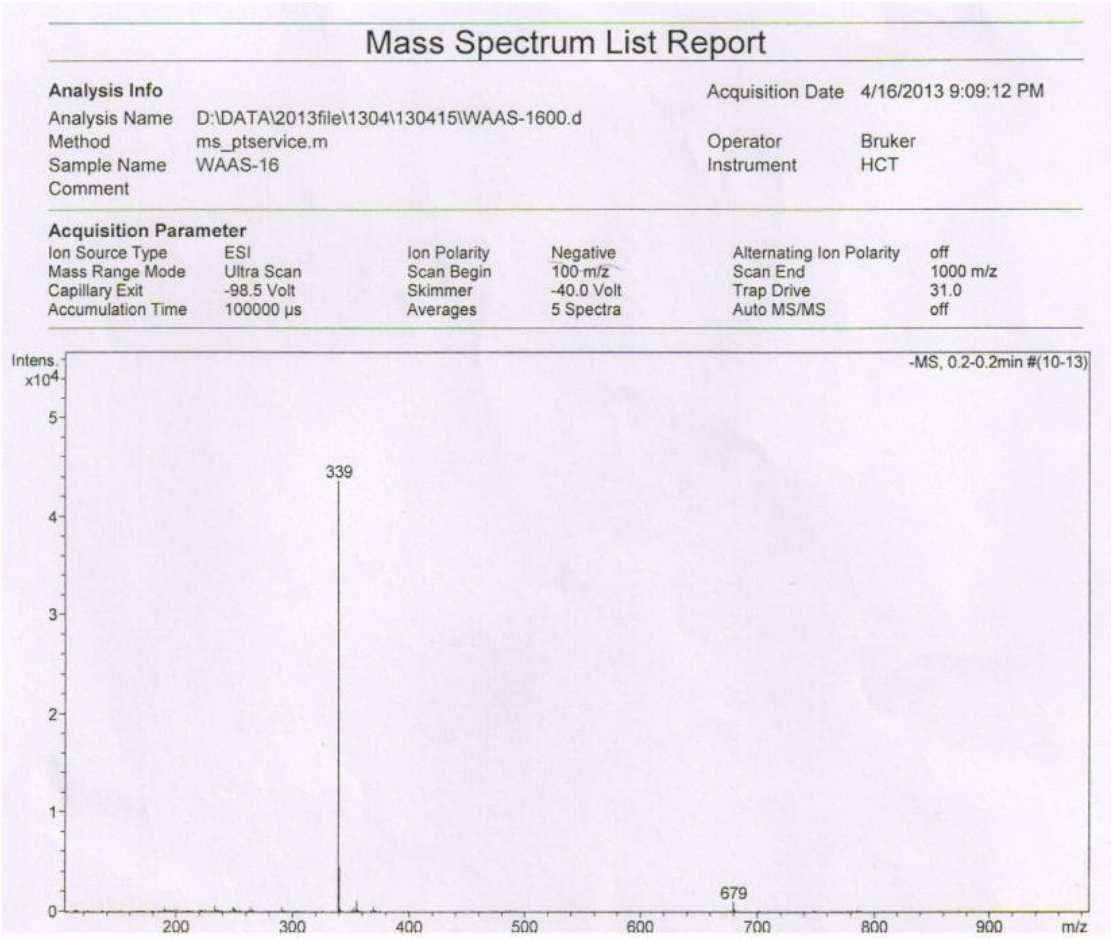


Figure S36. ESI-MS spectrum of alstoscholarisine D (**4**)

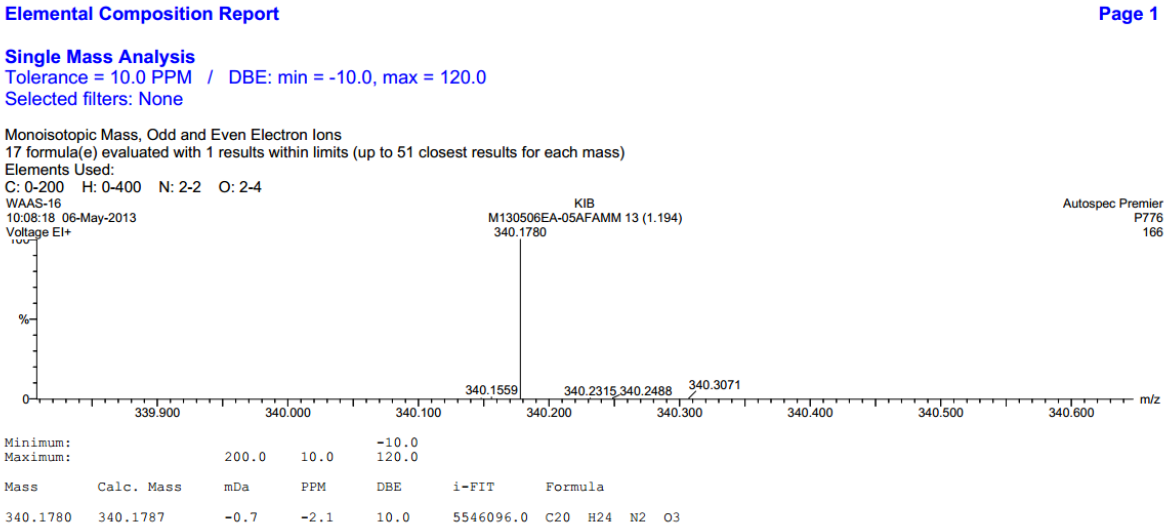


Figure S37. HR-EI-MS spectrum of alstoscholarisine D (**4**)

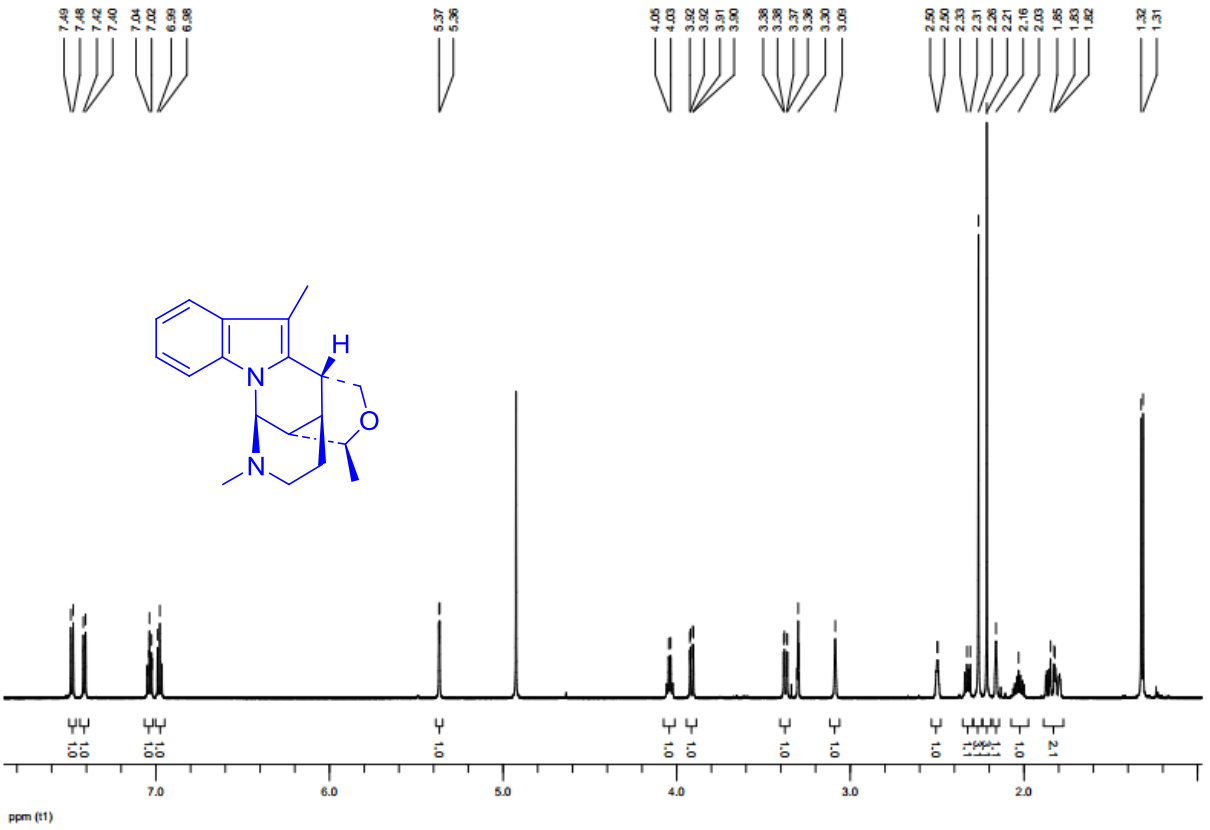


Figure S38. ^{1}H (in methanol-d_4) spectrum of alstoscholarisine E (**5**)

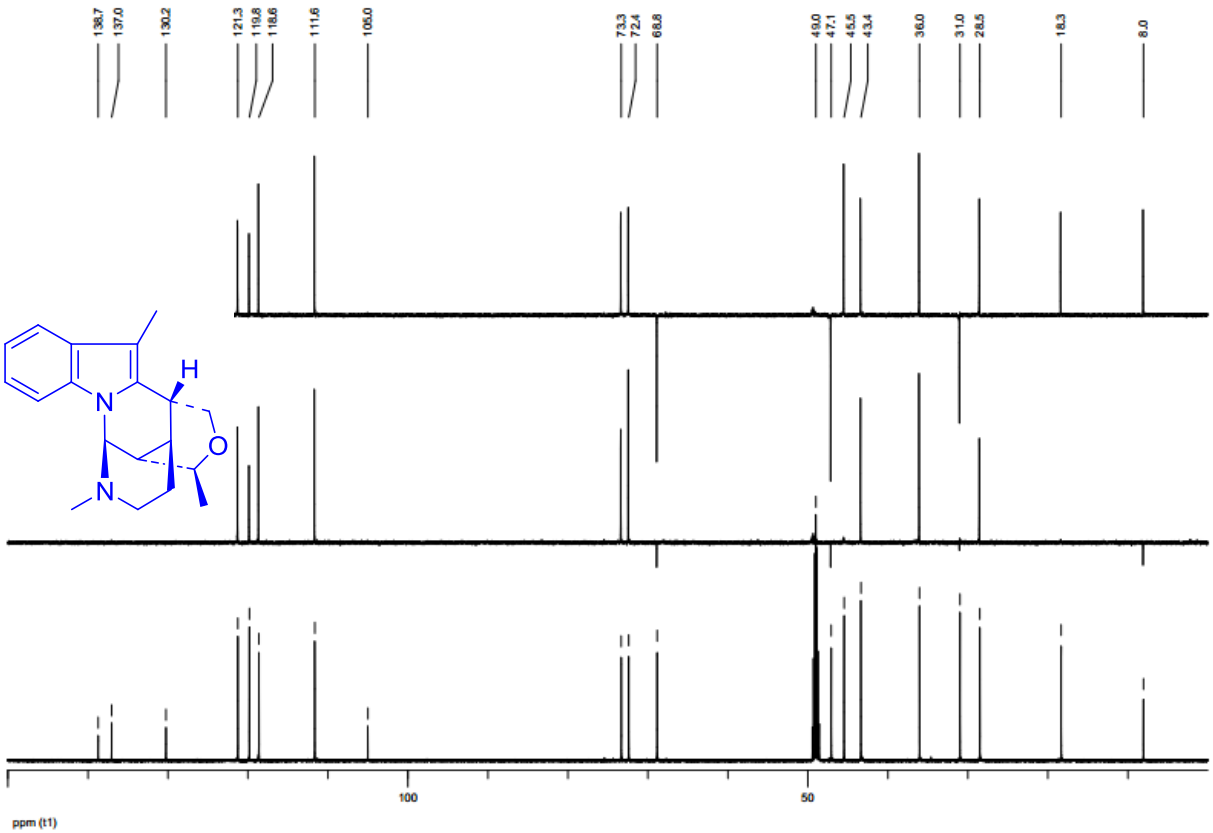


Figure S39. ^{13}C-DEPT (in methanol-d_4) spectrum of alstoscholarisine E (**5**)

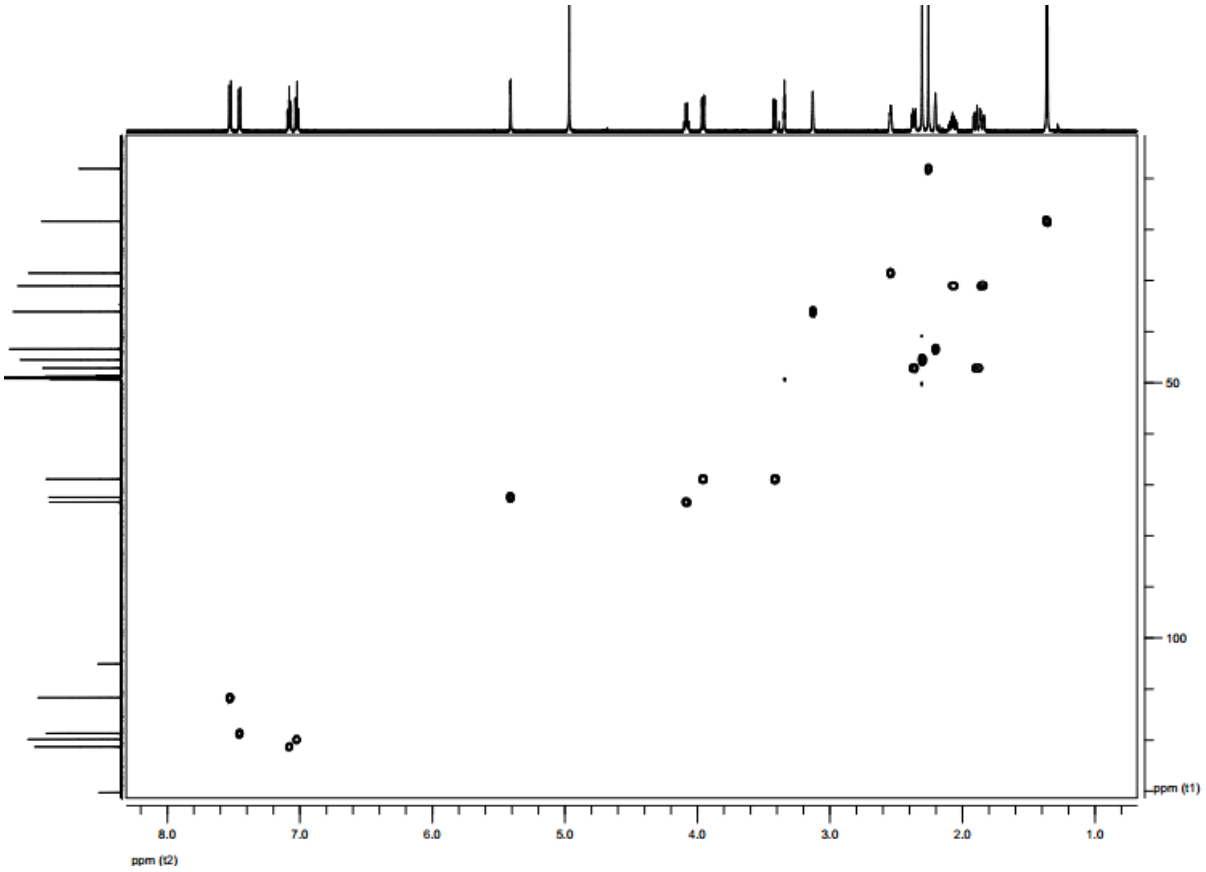


Figure S40. HSQC spectrum of alstoscholarisine E (**5**)

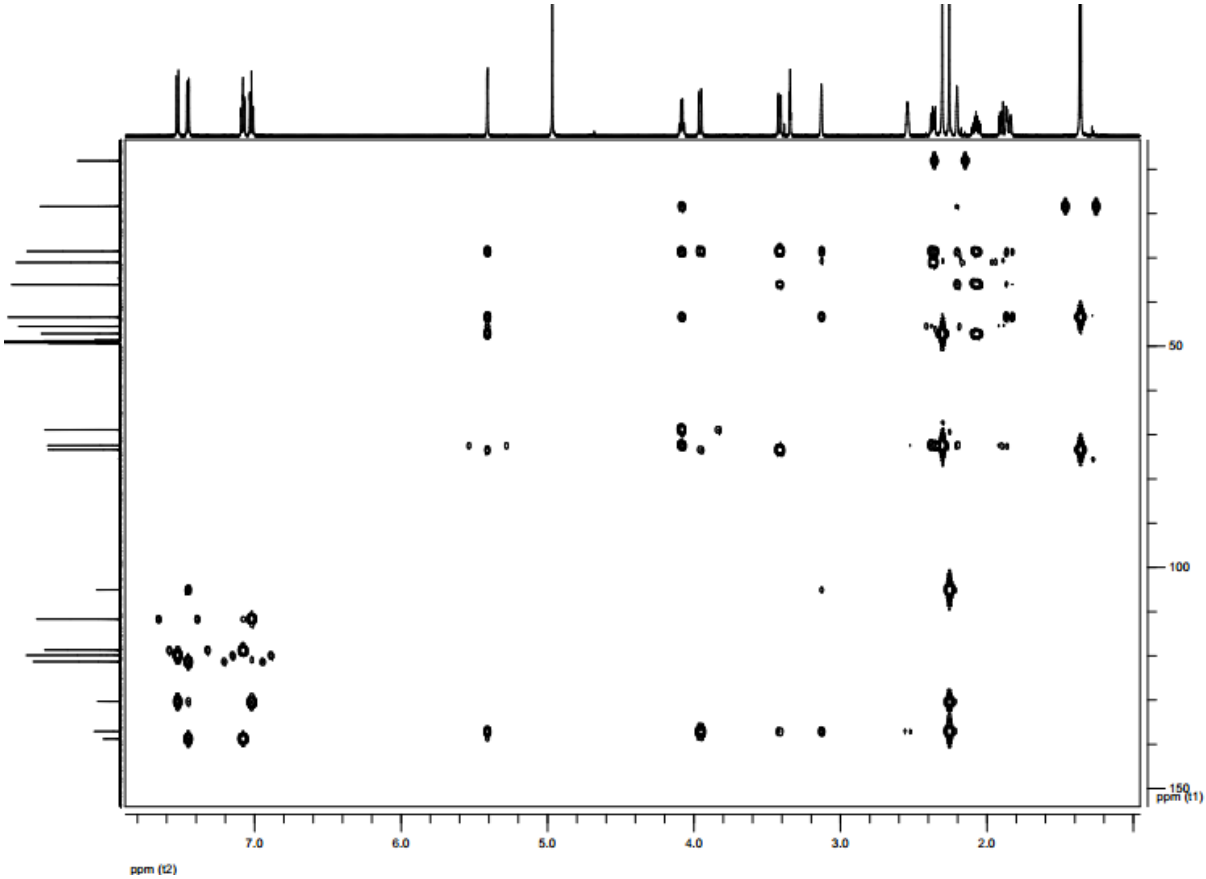


Figure S41. HMBC spectrum of alstoscholarisine E (**5**)

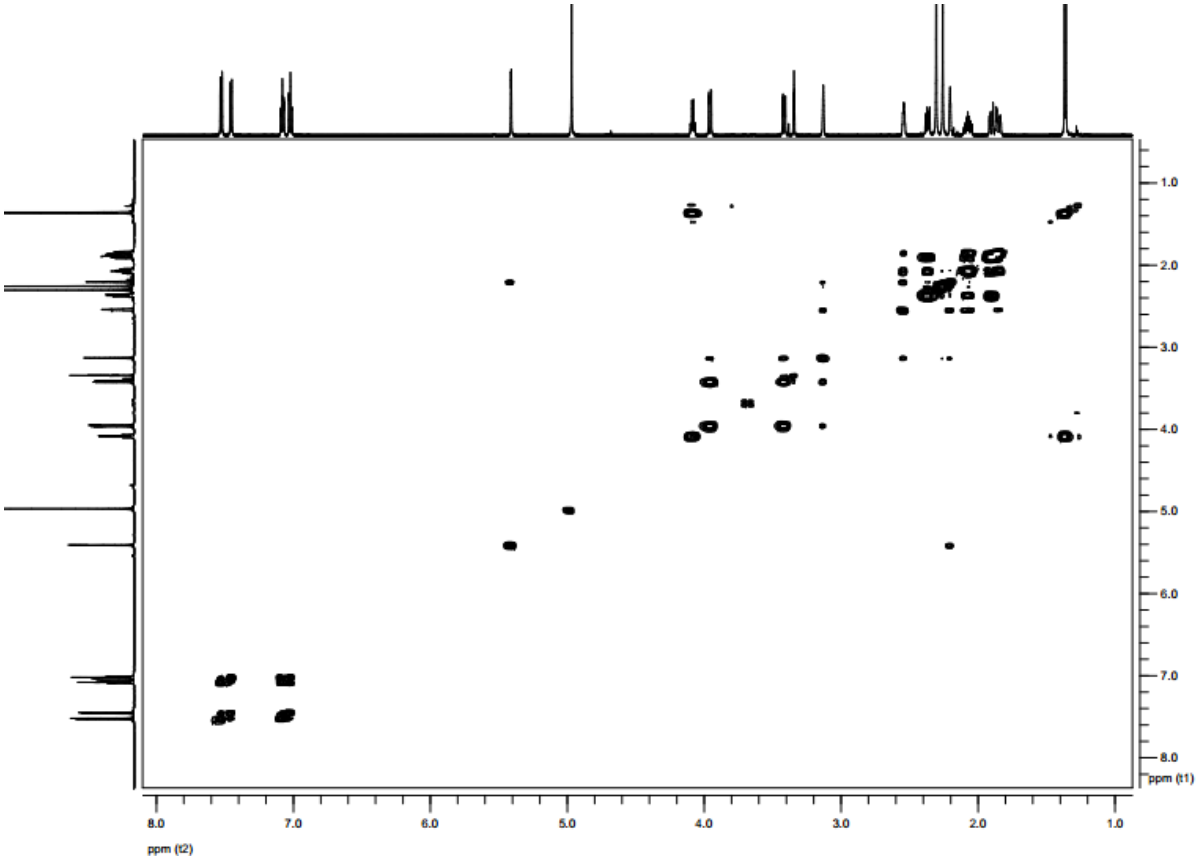


Figure S42. ^{1}H–^{1}H COSY spectrum of alstoscholarisine E (**5**)

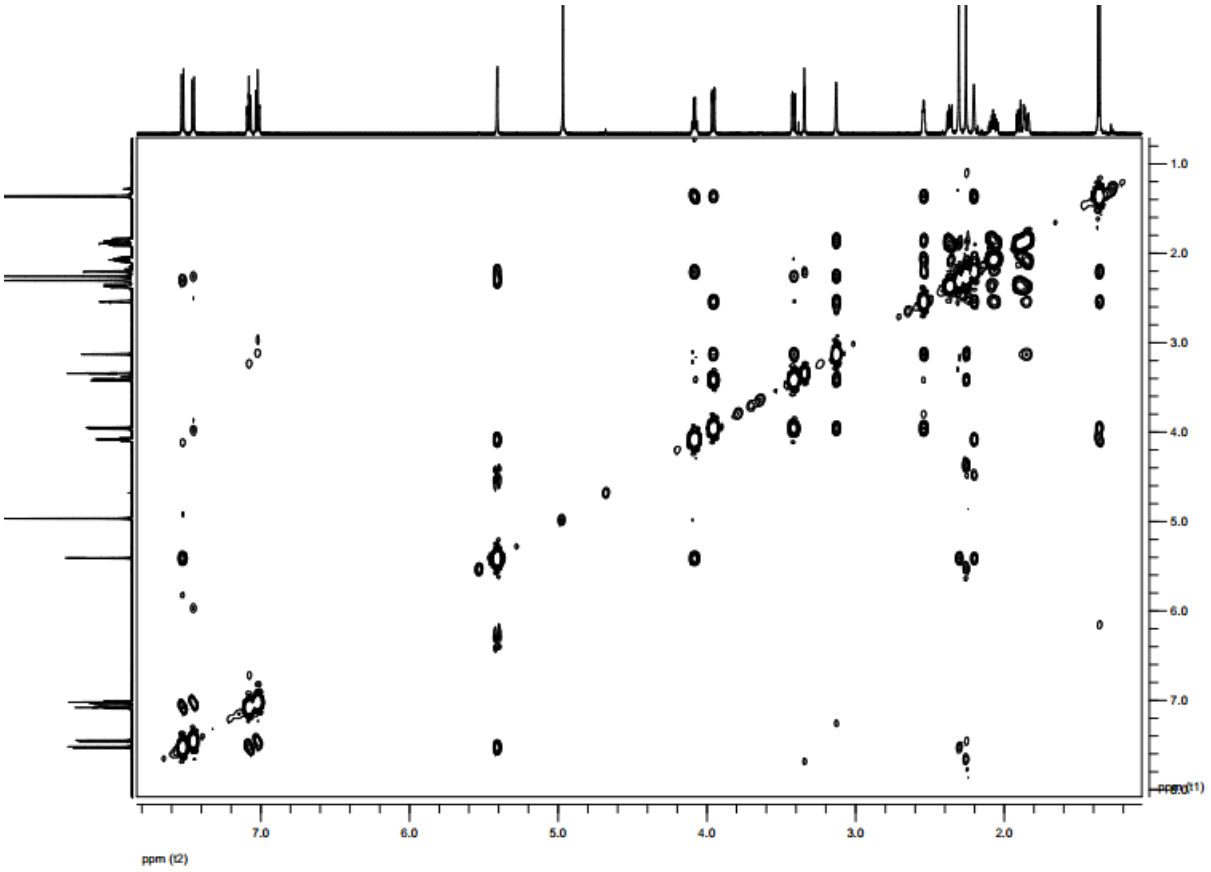


Figure S43. ROESY spectrum of alstoscholarisine E (**5**)

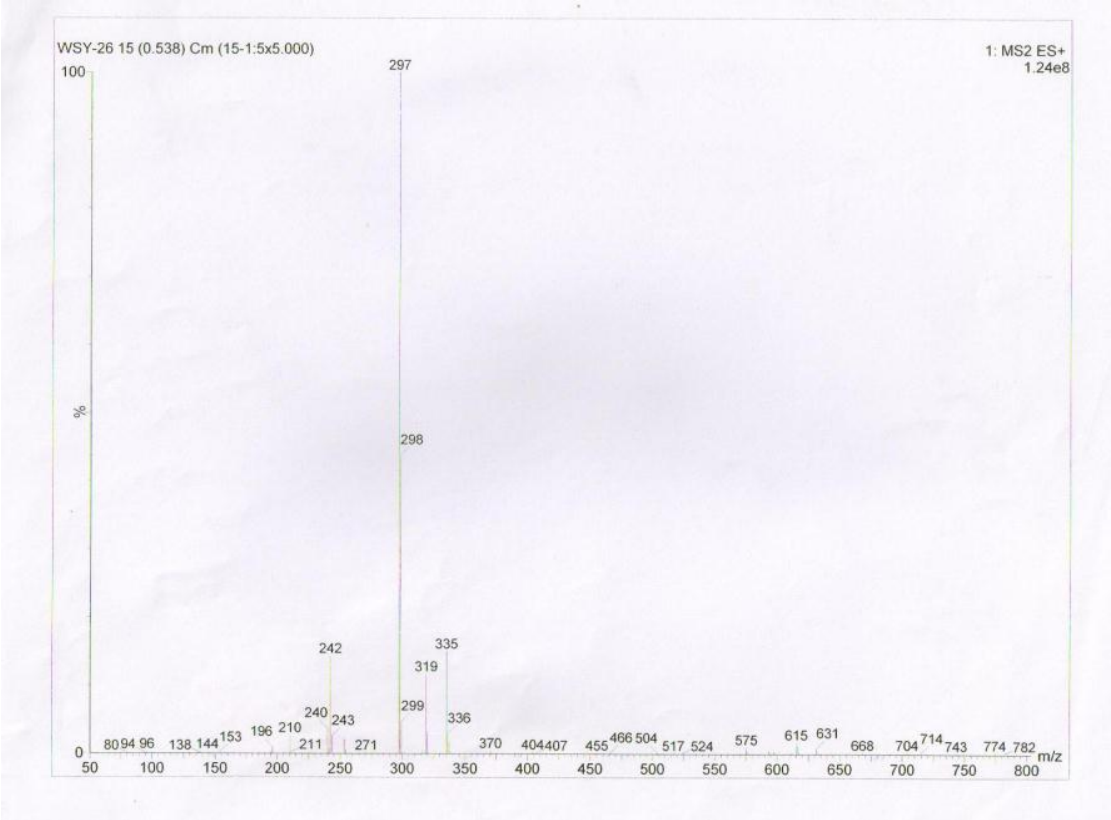
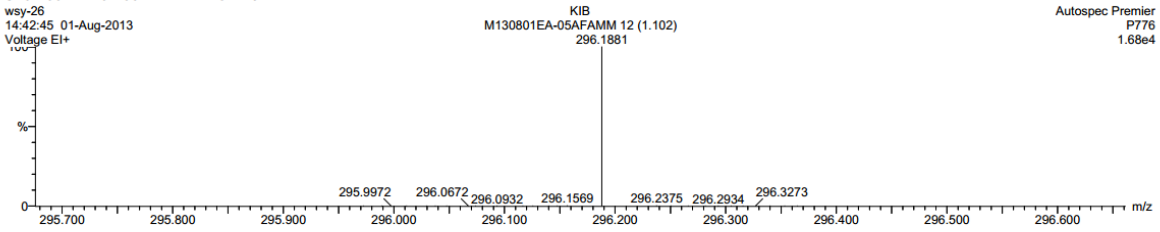


Figure S44. ESI-MS spectrum of alstoscholarisine E (**5**)

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0
Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions
16 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)
Elements Used:
C: 0-200 H: 0-400 N: 2-2 O: 1-3



Minimum:			-10.0			
Maximum:		200.0	10.0	120.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
296.1881	296.1889	-0.8	-2.7	9.0	5554431.0	C19 H24 N2 O

Figure S45. HR-EI-MS spectrum of alstoscholarisine E (**5**)