SUPPORTING INFORMATION

Callyspongisines A–D: Bromopyrrole alkaloids from an Australian marine sponge, *Callyspongia* sp.

Fabien Plisson, Pritesh Prasad, Xue Xiao, Andrew M. Piggott,
Xiao-cong Huang, Zeinab Khalil, Robert J. Capon*

Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD 4072, Australia

* Corresponding author. Tel: (+617) 3346 2979. Fax: (+617) 3346 2090. r.capon@uq.edu.au

TABLE OF CONTENTS

1. General Experimental Details

Optical measurements ($[\alpha]_D$) were obtained on a JASCO P-1010 polarimeter in a 10 cm cell. Ultraviolet-visible (UV-vis) absorption spectra were obtained using a CARY50 UV-vis spectrophotometer in a 1 cm quartz cell. NMR experiments were performed on a Bruker Avance DRX600 spectrometer and referenced to residual signals in the deuterated solvents. Electrospray ionization mass spectra (ESIMS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 series LC/MSD mass detector in both positive and negative ion modes. High-resolution (HR) ESIMS measurements were obtained on a Bruker micrOTOF mass spectrometer with an ESI probe by direct infusion in acetonitrile at 3 μL/min using sodium formate clusters as an internal calibrant. All HPLC analyses and separations were performed on Agilent 1100 series LC instruments with corresponding detectors, collectors and software.

2. Sponge Taxonomy

A description of the specimen is as follows: Growth form stalked, flabelliform (5–10 mm thick); colour on deck yellow, colour in EtOH beige; texture spongy, tough to tear, oscules small (1–2 mm), discrete, conspicuous, scattered; surface porous, optically smooth; spicules megascleres oxeas centrangulate hastate-mammilliform, 75–200 × 1–12 μm; ectosome paratangential reticulation of brushes of spicules protruding from choanosomal primary fibres; choanosome rectangular reticulation of multispicular primary tracts becoming plumose in the subectosomosal region with uni- or bi-spicular connecting tracts. Spongin evident.

3. Spectroscopic Data

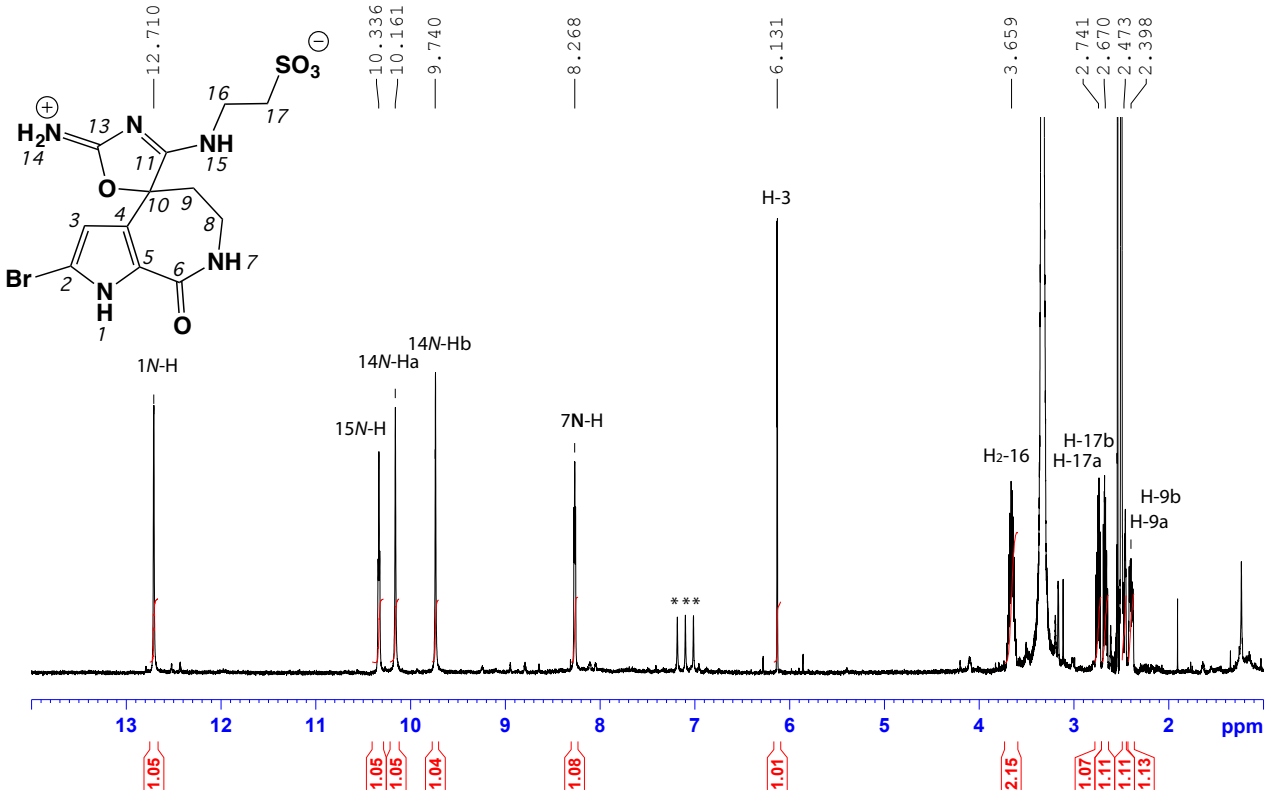


Figure S1a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of callyspongisine A (**1**). [*** = $^{14}NH_4^+$]

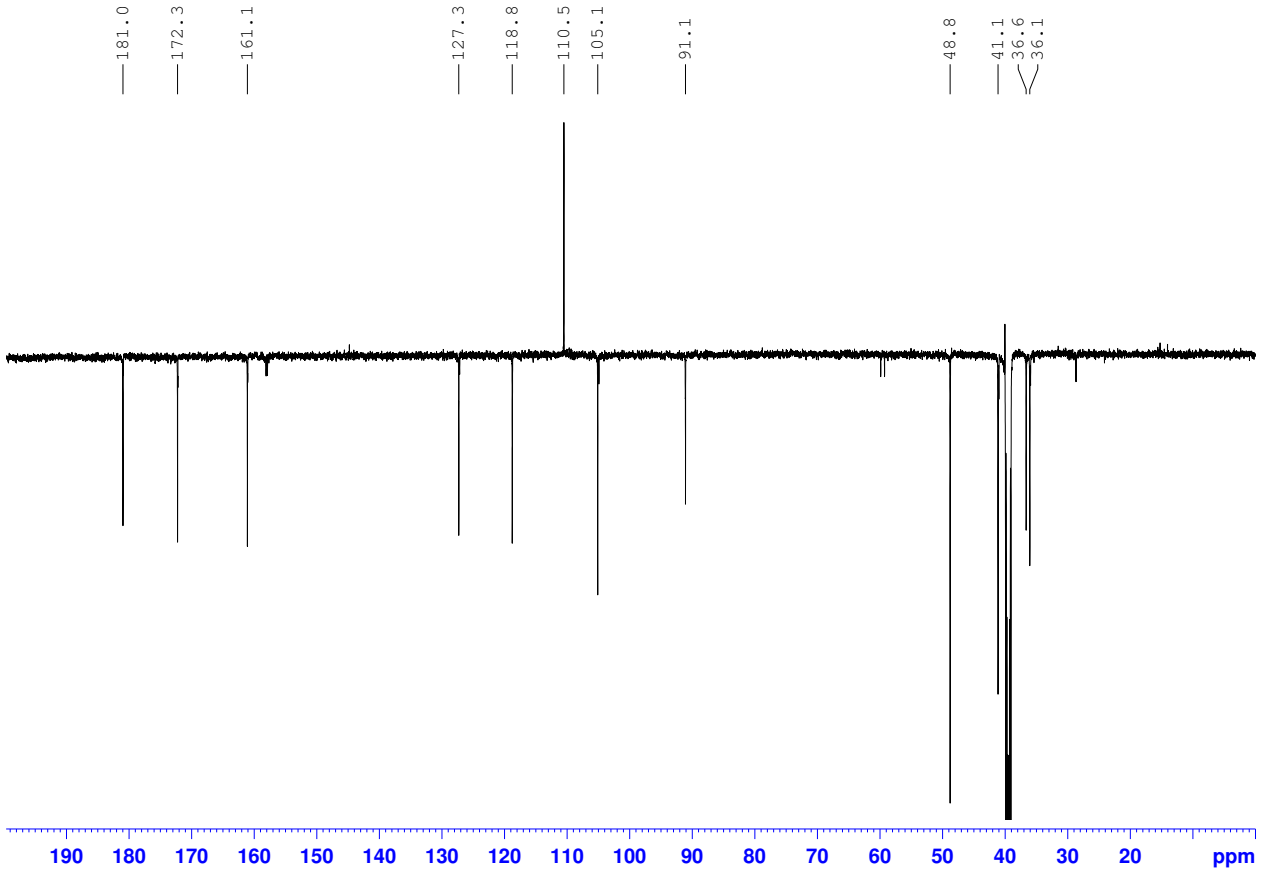
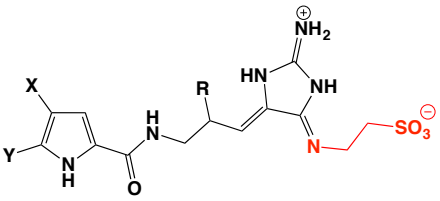
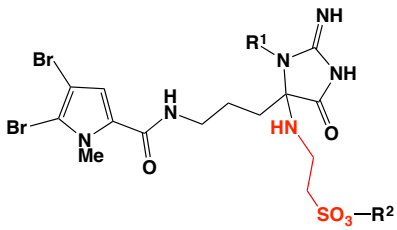


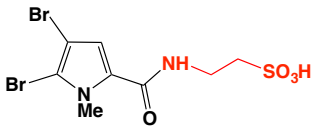
Figure S1b. ^{13}C NMR (150 MHz, DMSO-d_6) J-Mod spectrum of callyspongisine A (**1**).



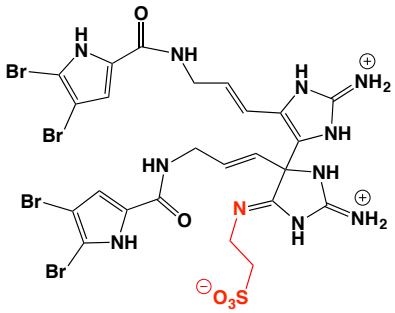
tauroacidin A: X=Y=Br, R=OH (9S/9R =6:4)
tauroacidin B: X=Br, Y=H, R=OH (9S/9R =1:1)
taurodispacamide A: X=Y=Br, R=H
debromotaurodispacamide A: X=Br, Y=R=H



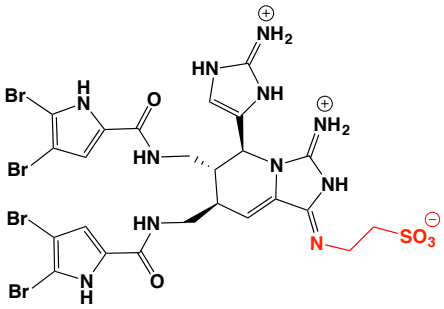
mauritamide A: R^1=H, R^2=Me
mauritamide B: R^1=R^2=H
mauritamide C: R^1=Et, R^2=H



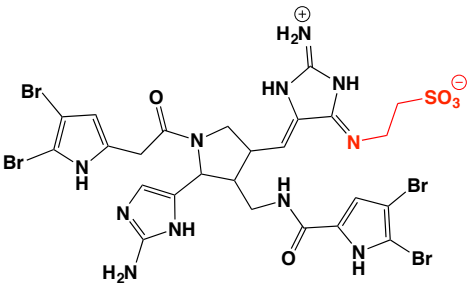
mauritamide D



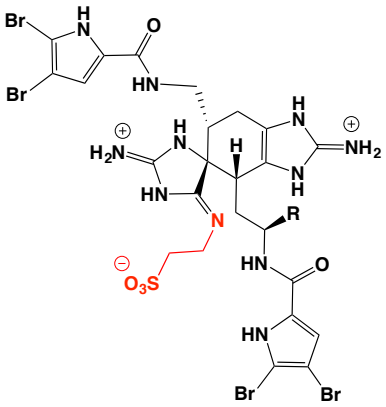
nagelamide H



nagelamide K



nagelamide Q



nagelamide X: R=OH
nagelamide Y: R=H

Figure S2. Previously reported taurinyl-2-aminoimidazoles (to September 2013).

Table S1. NMR (DMSO-d_6) data comparison between **1** and known taurinyl-2-aminoimidazoles.

Compound	H_2-16		H_2-17		Ref
	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C	
callyspongisine A (**1**)	3.66, ddd (7.0, 7.0, 5.4)	41.1	2.67-2.74, dt (13.0, 7.0)	48.8	
tauroacidin A	3.67, dt (7.2, 3.0)	39.8	2.75, t (7.2)	49.1	A
tauroacidin B	3.67, m	39.5	2.74, t (7.5)	49.2	A
mauritamide A	3.63, m	38.9	2.76, t	49.2	B
mauritamide B	3.60, m	39.7	2.74, t (7.2)	49.2	C
mauritamide C	3.63, m	39.7	2.75, t (7.2)	49.0	C
mauritamide D	3.44, dd (13.0, 6.2)	35.1	2.62, dd (7.6, 7.0)	49.7	C
nagelamide H	3.56, t (7.1)	40.3	2.81, t (7.1)	48.2	D
nagelamide K	3.69, -	40.4	2.82, -	49.6	E
nagelamide Q	3.64, m	40.6	2.75, m	49.3	F
nagelamide X	3.74 and 3.47, m	40.0	2.70, m	48.5	G
nagelamide Y	3.71 and 3.53, m	40.5	2.75, t (6.2)	48.5	G

A Kobayashi, J.; Inaba, K.; Tsuda, M. *Tetrahedron* **1997**, *53*, 16679

B Jimenez, C.; Crews, P. *Tetrahedron Lett* **1994**, *35*, 1375

C Hertiani, T.; Edrada-Ebel, R.; Ortlepp, S.; Van Soest, R. W. M.; de Voogd, N. J.; Wray, V.; Hentschel, U.; Kozytska, S.; Muller, W. E. G.; Proksch, P. *Bioorg Med Chem* **2010**, *18*, 1297

D Endo, T.; Tsuda, M.; Okada, T.; Mitsuhashi, S.; Shima, H.; Kikuchi, K.; Mikami, Y.; Fromont, J.; Kobayashi, J. *J Nat Prod* **2004**, *67*, 1262

E Araki, A.; Kubota, T.; Tsuda, M.; Mikami, Y.; Fromont, J.; Kobayashi, J. *Org Lett* **2008**, *10*, 2099

F Araki, A.; Kubota, T.; Aoyama, K.; Mikami, Y.; Fromont, J.; Kobayashi, J. *Org Lett* **2009**, *11*, 1785.

G Tanaka. N.; Kusama, T.; Takahashi-Nakaguchi, A.; Gonoi, T.; Fromont, J.; Kobayashi, J. *Org Lett* **2013**, *15*, 3262.

Table S2. NMR data (600 MHz, DMSO-d_6) from callyspongisine A (**1**).

#	δ_H, mult. (*J* in Hz)	δ_C	HMBC	COSY	ROESY
1*N*-H	12.71, s		3, 4, 5	3	8
2		105.1			
3	6.13, s	110.5	2, 4, 5, 10	1*N*-H	16, 17_a, 17_b
4		118.8			
5		127.3			
6		161.1			
7*N*-H	8.27, t (5.4)		5, 8	8	8, 9
8	3.30[A]	36.1	6, 10	9, 7*N*-H	
9_a	2.47, m	36.6	4, 10, 13	8	
9_b	2.39, m		8		
10		91.1			
11		181.0			
13		172.3			
14*N*-H_a	10.16, s		13		14*N*-H_b, 15
14*N*-H_b	9.74, s		11, 13		14*N*-H_a, 15
15*N*-H	10.34, t (5.4)		10, 11	16	8, 9, 14*N*-H_a, 14*N*-H_b, 16
16	3.66, ddd (7.0, 7.0, 5.4)	41.1	11, 17	17, 15*N*-H	
17_a	2.74, dt (13.0, 7.0)	48.8	16	16	
17_b	2.67, dt (13.0, 7.0)				

[A] Overlapped by H_2O signal.

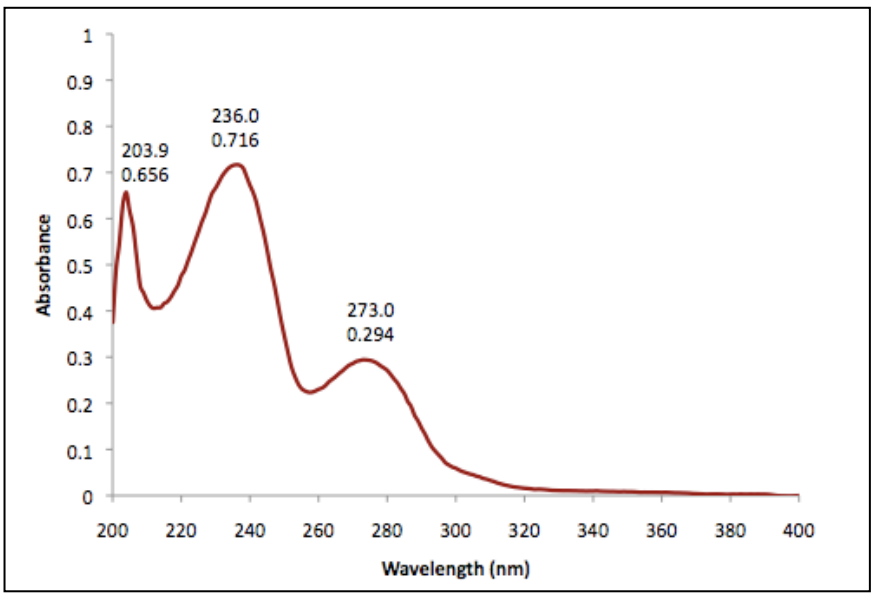


Figure S3. UV-vis (MeOH) spectrum of callyspongisine A (**1**)

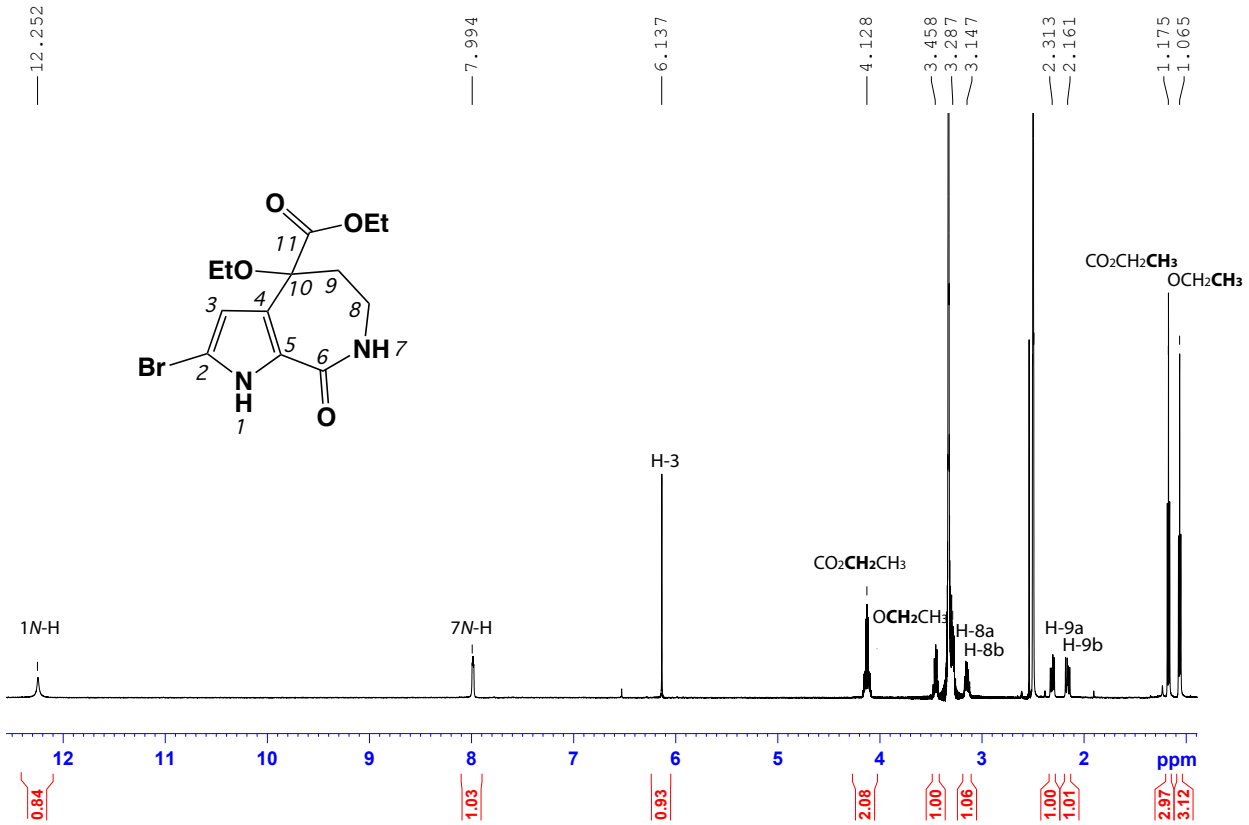


Figure S4. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of callyspongisine C (**3**)

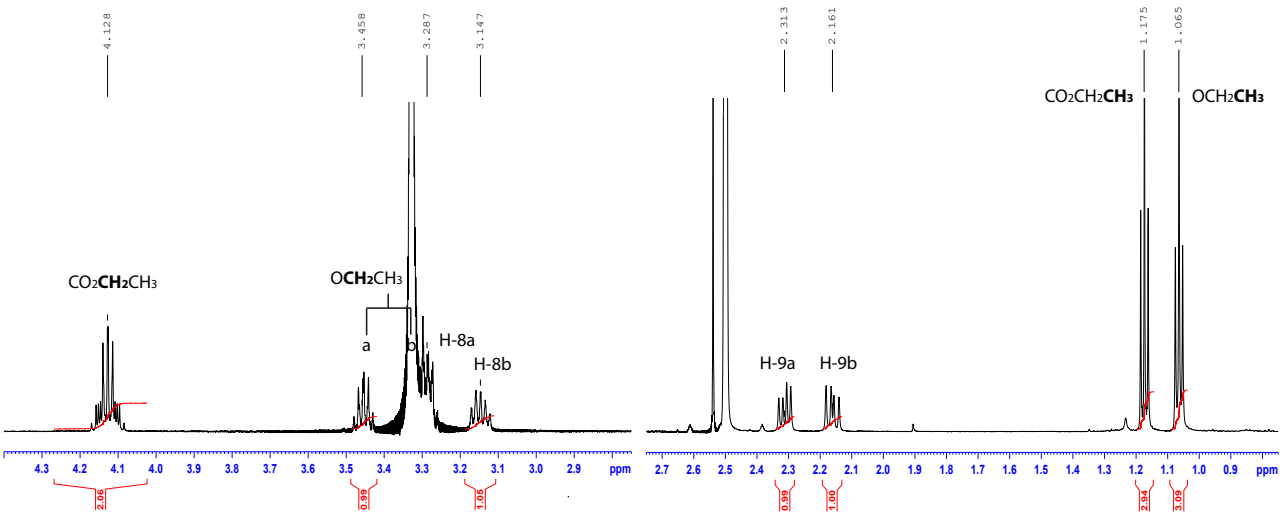


Figure S5. Expansion of ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of callyspongisine C (**3**)

Table S3. NMR assignments (600 MHz, DMSO-d_6) of callyspongisine C (**3**)

#	δ_H, mult. (*J* in Hz)	δ_C[B]	HMBC	COSY
1*N*-H	12.25, s			
2		103.6		
3	6.13, s	112.6	2, 5	
4		124.6		
5		125.4		
6		161.7		
7*N*-H	7.99, t (1.0)		5	8a, 8b
8	a 3.28, m b 3.15, ddd (7.7, 7.7, 1.0)	35.5	6, 9, 10	7*N*-H, 9b 7*N*-H, 9a
9	a 2.31, dd (15.0, 7.7)	34.7	4, 8, 10, 11	8b
9	b 2.16, dd (15.0, 7.7)			8a
10		79.3		
11		172.4		
$CO_2\underline{CH_2}CH_3$	a 4.15, dq (14.3, 7.0) b 4.11, dq (14.3, 7.0)	61.2	11, $CO_2\underline{CH_2}\underline{CH_3}$	$CO_2CH_2\underline{CH_3}$
$O\underline{CH_2}CH_3$	a 3.45, dq (14.2, 7.0) b 3.30[A]	60.0	10, $O\underline{CH_2}\underline{CH_3}$	$OCH_2\underline{CH_3}$
$CO_2CH_2\underline{CH_3}$	1.17, t (7.0)	14.2	$CO_2\underline{CH_2}CH_3$	$CO_2\underline{CH_2}CH_3$
$OCH_2\underline{CH_3}$	1.06, t (7.0)	15.8	$O\underline{CH_2}CH_3$	$O\underline{CH_2}CH_3$

[A] Overlapped by H_2O signal.
[B] Assignments supported by 2D NMR correlations.

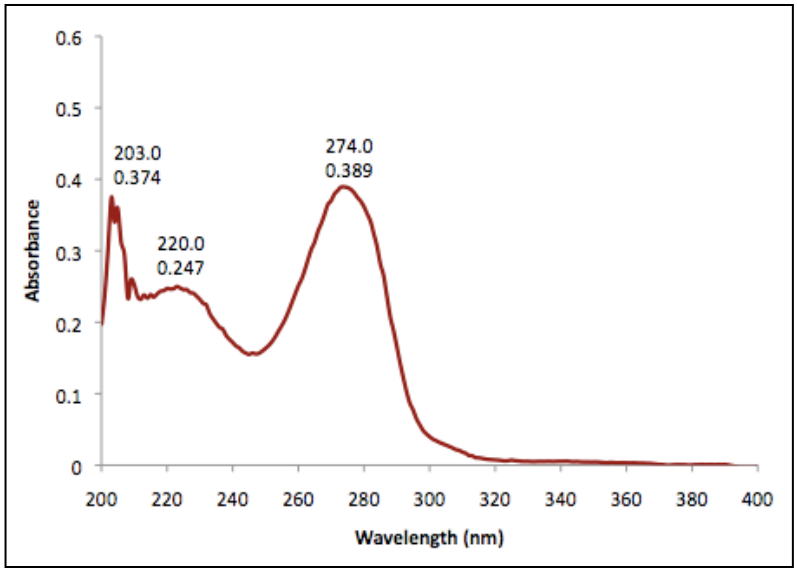
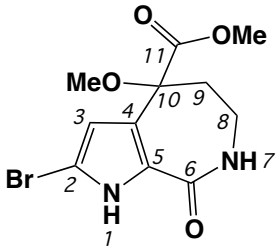


Figure S6. UV-vis (MeOH) spectrum of callyspongisine C (**3**)



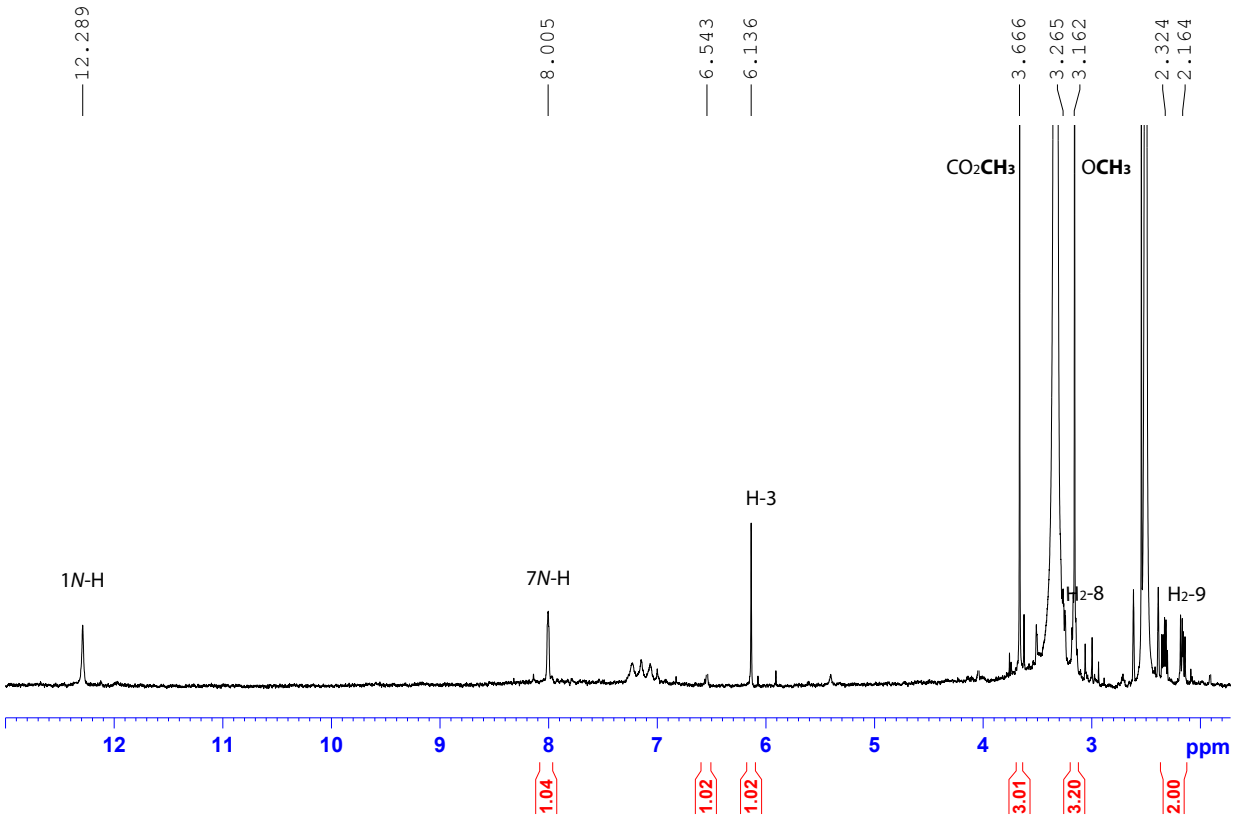


Figure S7. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of callyspongisine D (**4**)

Table S4. NMR (600 MHz, DMSO-d_6) data for callyspongisine D (**4**)

#	δ_H, mult. (*J* in Hz)	δ_C[B]	HMBC
1*N*-H	12.29, s		
2		103.6	
3	6.13, s	112.4	5
4		124.1	
5		125.7	
6		161.6	
7*N*-H	8.00, s		5
8	a 3.26[A], m b 3.16[A], m	35.5	6, 10
9	a 2.32, dd (15.0, 7.7) b 2.16, dd (15.0, 7.7)	34.4	4, 10
10		79.7	
11		172.7	
$CO_2\underline{CH_3}$	3.67, s	52.6	11
$O\underline{CH_3}$	3.16, s	52.2	10

[A] Overlapped by H_2O signal and signal (H-13) at 3.16 ppm
[B] Assignments supported by 2D NMR correlations (HSQC/HMBC).

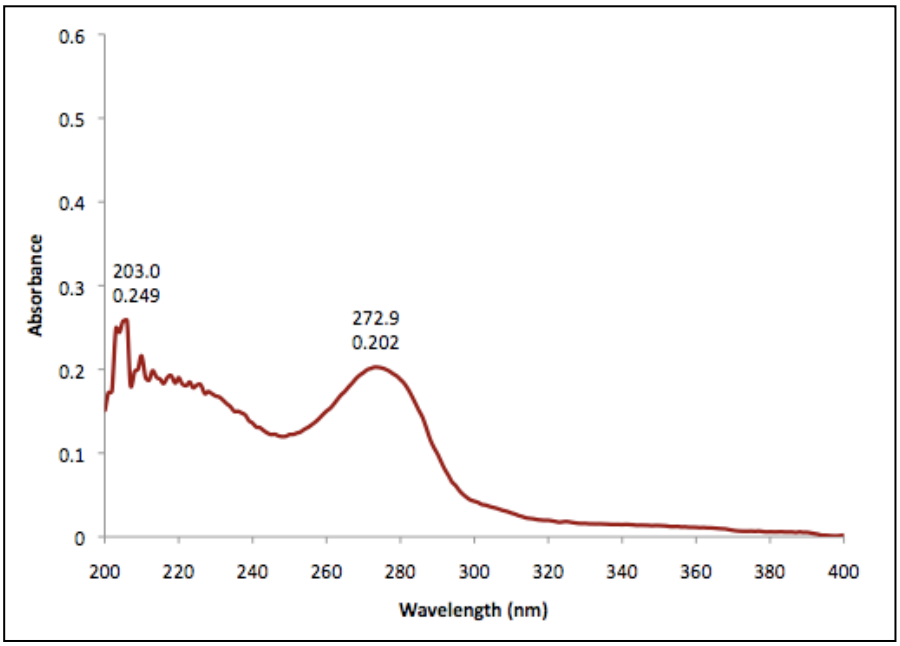


Figure S8. UV-vis (MeOH) spectrum of callyspongisine D (**4**)

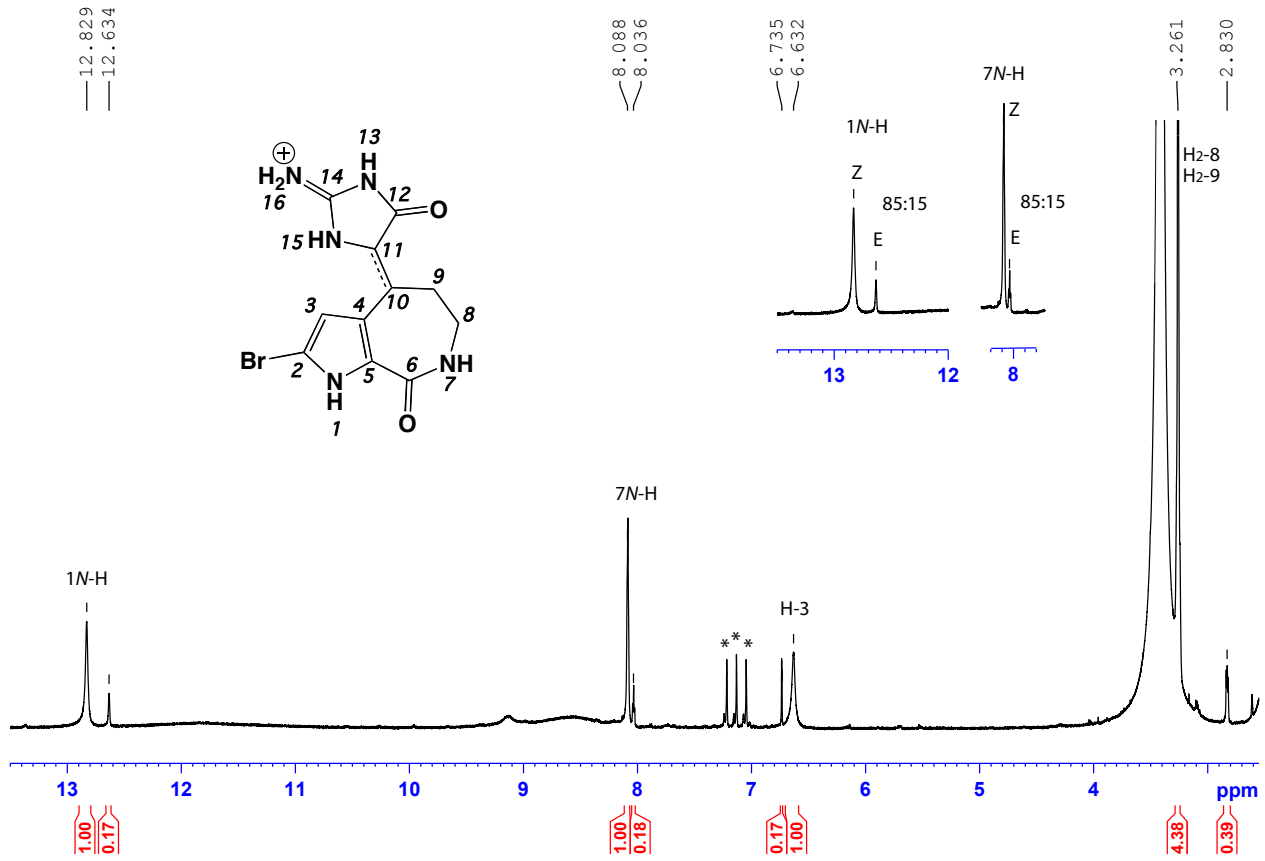


Figure S9. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of hymenialdisine (**5**)

Table S5. NMR (600 MHz, DMSO-d_6) data for hymenialdisine (**5**)

#	10*Z*-hymenialdisine		10*E*-hymenialdisine	
	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C[B]
1*N*-H	12.83, s		12.63, s	
2		104.9		102.2
3	6.63, s	111.2	6.74, s	113.9
4		121.6		119.9
5		128.3		127.6
6		162.3		163.0
7*N*-H	8.09, s		8.03, s	
8	3.26, s	38.9	3.26, s	38.0
9	3.26, s	31.8	2.83, s	36.6
10		128.3		128.1
11		121.6		123.5
12		163.7		161.5
13*N*-H	[A]		[A]	
14		154.6		153.8
15*N*-H	[A]		[A]	
16*N*-H_2	[A]		[A]	

[A] Not detected.
[B] Williams, D. H.; Faulkner, D. J. *Nat Prod Lett* **1996**, *9*, 57

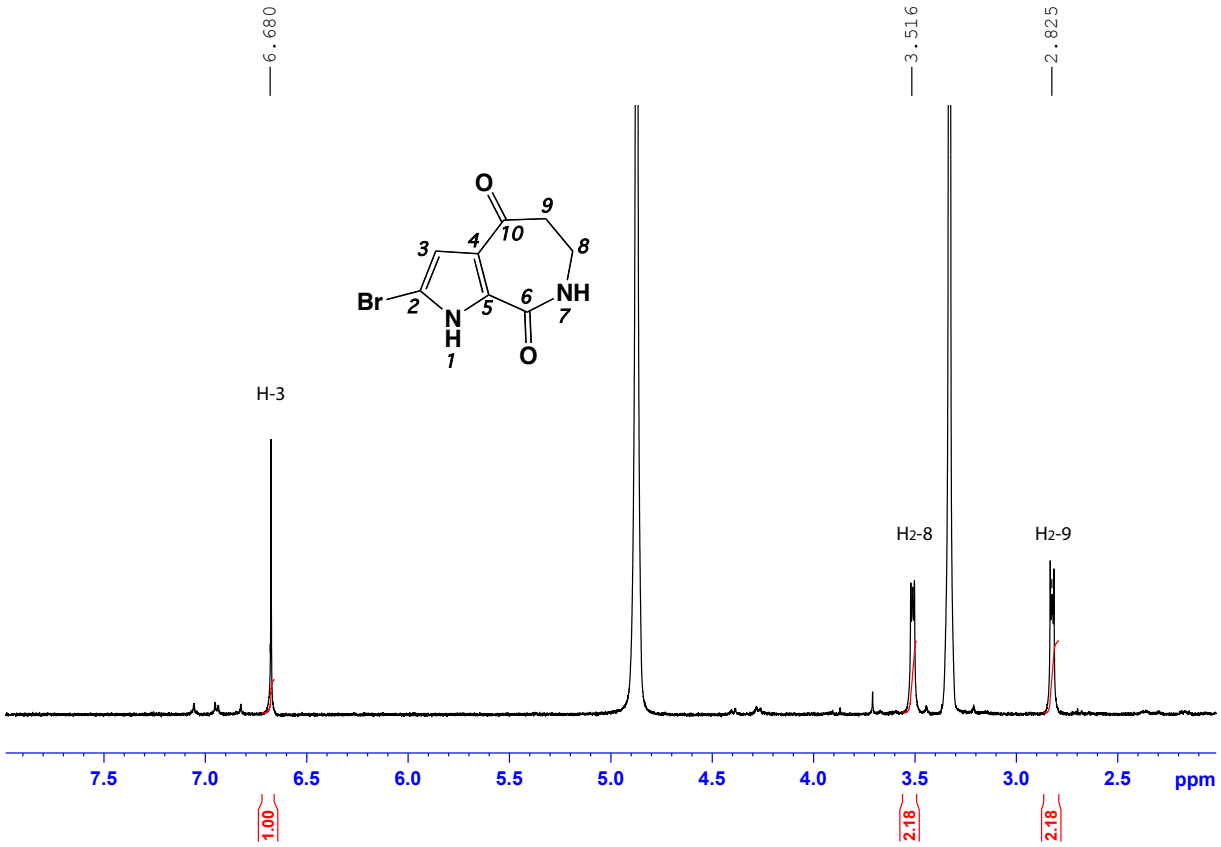


Figure S10. ^{1}H NMR (600 MHz, MeOH-d_4) spectrum of 2-bromoaldisine (**6**)

Table S6. NMR (600 MHz, MeOH-d_4) data for 2-bromoaldisine (**6**)

#	isolated 2-bromoaldisine (**6**)		reported 2-bromoaldisine [A]	
	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C
1*N*-H				
2		107.2		104.2
3	6.68, s	113.3	6.65, s	113.2
4		126.9		126.8
5		130.6		130.6
6		164.1		164.2
7*N*-H				
8	3.51, m	38.2	3.50, m	38.0
9	2.82, m	44.7	2.81, m	44.6
10		196.2		196.2

[A] Schmitz, F. J.; Gunasekera, S. P.; Lakshmi, V.; Tillekeratine, L. M. V. *J Nat Prod* **1985**, *48*, 47.

4. Bioassays

4.1 Kinase Assays

4.1.1 CDK5 Inhibition Assay: Test compounds (1% DMSO) were added in duplicate to a 384-well plate at desired concentrations. CDK5/p25 (0.8 ng/mL) was dispensed into wells and the plate incubated for 10 min at room temperature. ATP (4 μM) and histone (300 μg/mL) were added to a final assay volume of 25 μL, the plate was then covered with Parafilm and incubated at 27 °C for 60 min. After equilibrating to room temperature, Kinase Glo reagent (20 μL) was added to each well and the plate incubated for a further 10 min before measuring luminescence with a POLARstar Omega plate reader (BMG LABTECH, Offenburg, Germany). Concentrations given are for final assay conditions. The assay buffer contains: 6.25 mM MOPS, pH 7.2, 6.25 mM $MgCl_2$, 1.25 mM EGTA, 1.25 mM EDTA, 0.25% glycerol. Assay components: CDK5/p25 (Sigma Aldrich, C0745), ATP (Sigma Aldrich, A7699), histone (Sigma Aldrich, H4524), Kinase Glo (Promega, V6712), 384 well plates (Perkin Elmer, 6007290). Controls included a 'No Kinase' control (ATP, histone only), 'Kinase' control (CDK5/p25, ATP and histone) and a 'vehicle' control (CDK5/p25, ATP, histone and DMSO). Percent inhibition was calculated according to Equation (1). IC_{50} were calculated using Prism 5.0.

$$\%\, \boldsymbol{I} = \frac{(\textbf{CPS compound} - \textbf{CPS kinase control})}{(\textbf{CPS no kinase control} - \textbf{CPS kinase control})} \times \mathbf{100} \qquad \textbf{(1)}$$

4.1.2 CK1δ Inhibition Assay: Test compounds (1% DMSO) were added in duplicate to a 384-well plate at desired concentrations. CK1δ (0.4 ng/μL) was dispensed into wells and the plate incubated for 10 min at room temperature. ATP (6 μM) and CK1tide (125 μM) were added to a final assay volume of 25 μL. The method then proceeded as described above for the CDK5 assay. Assay buffer: 7.5 mM MOPS, pH 7.0, 0.25 mM EDTA, 0.003% Brij-35, 1% glycerol, 0.03% BME, 0.5 mg/mL BSA, 12.5 mM $Mg(OAc)_2$. Assay components: CK1δ (Millipore, 14-520), Ck1tide (Millipore, 12-529), ATP (Sigma Aldrich, A7699), Kinase Glo (Promega, V6712), 384-well plates (Perkin Elmer, 6007290).

4.1.3 GSK3β Inhibition Assay: Test compounds (1% DMSO) were added in duplicate to a 384-well plate at desired concentrations. GSK3β (0.4 ng/μL) was dispensed into wells and the plate incubated for 10 min at room temperature. ATP (0.4 μM) and GSK3β substrate (15 μM) were added to a final assay volume of 25 μL. The method then proceeded as described above for the CDK5 assay. Assay buffer: 50 mM HEPES, pH 7.5, 1 mM EDTA, 1 mM EGTA, 15 mM $Mg(OAc)_2$. Assay components: GSK3β (Millipore, 14-306), GSK3β substrate C-terminal fragment of GS-2 peptide [His-Ser-Ser-Pro-His-Gln-Ser(PO_3H_2)-Glu-Asp-Glu-Glu-Glu] (Auspep), ATP (Sigma Aldrich, A7699), Kinase Glo (Promega, V6712), 384-well plates (Perkin Elmer, 6007290).

Table S7. Results of kinase inhibition assays

Compound	Kinase inhibition – IC_{50} (μM)		
	CK1δ	CDK5/p25	GSK3β
callyspongisine A (**1**)	9.5	>30	>10
callyspongisine C (**3**)	8	>30	>10
callyspongisine D (**4**)	>100	>30	>10
hymenialdisine (**5**)	0.03	0.16	0.07
2-bromoaldisine (**6**)	6	>30	>10

4.2 Cytotoxicity Assays

4.2.1 Cell Lines: The human colon cancer cell line SW620, the human large cell lung cancer cell line NCI-H460 and the human glioblastoma cell line SF-295 were provided by Susan E. Bates and Robert W. Robey of the National Cancer Institute (NCI), Bethesda, MD. The multidrug-resistant (MDR) cell line SW620 Ad300, which overexpresses P-gp, was selected from SW620 by growth in the presence of increasing concentrations of doxorubicin. These four cell lines were cultured in RPMI medium 1640 (Invitrogen, Carlsbad, CA) and SW620 Ad300 cells were maintained in the presence of 300 ng/mL doxorubicin. The human epidermoid carcinoma cell line KB-3-1 was provided by Michael M. Gottesman (NCI, MD) and maintained in Dulbecco modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA).

4.2.2 P-gp Inhibition (Calcein AM) Assay: The calcein AM accumulation was used to detected P-gp inhibitory activity of tested compounds as previously described[1]. Briefly, cells that overexpress P-gp (SW620 Ad300) were harvested with trypsin and resuspended in RPMI 1640 to give a final concentration of 50×10^4 cells/mL. Cells (100 μL/well) were then plated in a 96-well, flat clear-bottom and black-well micro-plate (353219, BD Falcon, NJ) and incubated at 37 °C in 5% CO_2. After 48 h, each well was washed twice and replaced with 50 μL warm RPMI 1640 (phenol red-free). Subsequently, compounds (final concentration 20 μM), verapamil (final concentration 100 μM, positive control) or PBS (negative control and background) were distributed to designated wells (25 μL/well) in duplicate and incubated at 37 °C in 5% CO_2. After 15 min, calcein acetoxymethyl ester (calcein AM; 25 μL; final concentration 0.25 μM) was added to each well, except the background well, which contained only 25 μL of PBS. The plate was then incubated at 37 °C 5% CO_2 for a further 30 min, after which the fluorescence from accumulated intracellular calcein was detected using a POLARstar Omega plate reader (BMG LABTECH, Offenburg, Germany) at an excitation wavelength of 490 nm and emission of 510 nm. Data were analysed using Prism 5.0. Maximum inhibition (I_{max}) was calculated as a percentage from the fluorescence in the presence of 20 μM test compound ($RFU_{compound}$), 100 μM verapamil ($RFU_{positive}$), and PBS alone ($RFU_{negative}$) according Equation (2) as follows. A compound was deemed to inhibit P-gp when the I_{max} value was >30%.

$$I\text{max} = \frac{(\text{RFU compound} - \text{RFU negative})}{(\text{RFU positive} - \text{RFU negative})} \times 100 \qquad (2)$$

[1] Huang, X. C.; Sun, Y. L.; Salim, A. A.; Chen, Z. S.; Capon, R. J. *Biochem. Pharmacol.* **2013**, *85*, 1257

Table S8. Results of P-gp inhibition assays

Compound[A]	P-gp inhibitory activity[B]
callyspongisine A (**1**)	< 1%
callyspongisine C (**3**)	< 1%
callyspongisine D (**4**)	< 1%
hymenialdisine (**5**)	< 1%
2-bromoaldisine (**6**)	< 1%

[A] tested at 20 μM. [B] activity (%) from 100 verapamil

4.2.3 Cytotoxicity (MTT) Assay: The MTT assay was used to evaluate the cytotoxicity of compounds against cancer cell lines as previously described[1]. Briefly, cells (2,000/well in 180 μL of RPMI 1640 supplemented with 10% FBS) were seeded evenly in a 96-well micro-plate, and the plate was incubated for 18 h (37 °C; 5% CO_2) to allow cells to attach. Compounds to be tested were dissolved in 5% DMSO (v/v) and diluted from 300 μM–1 μM. Aliquots (20 μL) of each dilution (or of 5% DMSO for control wells) were added to the plate in duplicate. After 68 h incubation (37 °C; 5% CO_2), a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, USA) in PBS was added to each well to a final concentration of 0.4 mg/mL and the plate was incubated for a further 4 h (37 °C; 5% CO_2). The medium was then carefully aspirated and precipitated formazan crystals were dissolved in DMSO (100 μL/well). Finally, the absorbance of each well at 580 nm was measured with a PowerWave XS Microplate Reader from Bio-Tek Instruments Inc. (Vinooski, VT). IC_{50} values (the concentration of the compound required for 50% inhibition of the cancer cells) were calculated using Prism 5.0 from GraphPad Software Inc. (La Jolla, CA).

Table S9. Results of cytotoxicity (MTT) assays

Compound	Cytotoxicity - IC_{50} (μM)			
	SF-295	SW620	NCI-H460	KB-3-1
callyspongisine A (**1**)	24.0	>30	>30	17.6
callyspongisine C (**3**)	23.4	>30	>30	18.4
callyspongisine D (**4**)	23.8	>30	>30	21.9
hymenialdisine (**5**)	>30	3.1	>30	2.0
2-bromoaldisine (**6**)	26.8	>30	>30	27.3

[1] Huang, X. C.; Sun, Y. L.; Salim, A. A.; Chen, Z. S.; Capon, R. J. *Biochem. Pharmacol.* **2013**, *85*, 1257.

4.3 Antibiotic Assays

4.3.1 Antibacterial Assay: The bacterium to be tested was streaked onto a tryptic soy agar plate and was incubated at 37 °C for 24 h. One colony was then transferred to fresh tryptic soy broth (15 mL) and the cell density was adjusted to 10^4-10^5 cfu/mL. The compounds to be tested were dissolved in DMSO and diluted with H_2O to give 300 µM stock solutions (10% DMSO). The stock solutions were then serially diluted with 10% DMSO to give final concentrations of 30 µM to 0.01 µM in 1% DMSO. An aliquot (20 µL) of each dilution was transferred to a 96-well microtitre plate and freshly prepared microbial broth (180 µL) was added to each well. The plates were incubated at 37 °C for 24 h and the optical density of each well was measured spectrophotometrically at 600 nm using POLARstar Omega plate (BMG LABTECH, Offenburg, Germany). Each test compound was screened against the Gram-negative bacteria *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145) and the Gram-positive bacteria *Staphylococcus aureus* (ATCC 9144 and ATCC 25923) and *Bacillus subtilis* (ATCC 6633 and ATCC 6051). The IC_{50} value was calculated as the concentration of the compound or anticancer drug required for 50% inhibition of the cancer cells using Prism 5.0 from GraphPad Software Inc. (La Jolla, CA).

4.3.2 Antifungal Assay: The fungus to be tested was streaked onto a Sabouraud agar plate and was incubated at 26.5 °C for 48 h. One colony was then transferred to fresh Sabouraud broth (15 mL) and the cell density was adjusted to 10^4-10^5 cfu/mL. Test compounds were dissolved in DMSO and diluted with H_2O to give a 300 µM stock solution (10% DMSO). The stock solution was then serially diluted with 10% DMSO to give final concentrations of 30 µM to 0.01 µM in 1% DMSO. An aliquot (20 µL) of each dilution was transferred to a 96-well microtiter plate and freshly prepared microbial broth (180 µL) was added to each well. The plates were incubated at 26.5 °C for 48 h and the optical density of each well was measured spectrophotometrically at 600 nm using POLARstar Omega plate (BMG LABTECH, Offenburg, Germany). Each test compound was screened against the fungus *Candida albicans* (ATCC 90028). The IC_{50} value was calculated as the concentration of the compound or anticancer drug required for 50% inhibition of the cancer cells using Prism 5.0 from GraphPad Software Inc. (La Jolla, CA).

4.3.3 *Mycobacterium bovis* culture: *Mycobacterium bovis*, Bacille Calmette Guerin (BCG) (strain Pasteur, ATCC) was grown until early-mid log phase in 7H9 liquid medium (Difco) containing 0.2% glycerol, 0.05% Tween80, 0.5% bovine serum albumin (BSA), 0.2% dextrose, and 0.085% sodium chloride. Single cell suspensions of independent cultures were prepared diluting the culture to an optical density (OD; 600 nm) of 0.02. Test compounds were dissolved in DMSO and an aliquot (5 µL) from callyspongisines A–D (**1**, **3**–**4**), hymenialdisine (**5**) and 2-bromoaldisine (**6**) (10 µL aliquots) was transferred to a 96-well microtiter plate containing BCG (195 µL). The plate was incubated at 37 °C, 5% CO_2 for 7 days and was measured spectrophotometrically at 600 nm. To analyse bacterial survival, serial dilutions of culture material were plated on Middlebrook 7H10 plates supplemented with 10% oleic acid/albumin/dextrose catalase (OADC; Middlebrook) and 0.5% glycerol. Plates were incubated at 37 °C for 3 weeks and colonies enumerated to calculate colony forming units (CFU). Isoniazid (INH) was used as a positive control (20 µg/mL in 10% DMSO). All compounds were inactive at concentration > 30 µM.

5. Synthetic transformations

*Synthesis of pemoline (**13**):* To a solution of guanidine hydrochloride (531 mg, 5.56 mmol) in ethanol (5 mL) was added a solution of NaOH (223 mg, 5.56 mmol) in ethanol (5 mL), and the mixture was refluxed for 5 min. A solution of ethyl mandelate (500 mg, 2.78 mmol) was added to the reaction, which was then refluxed for a further 30 min. The solution was cooled, diluted with water (15 mL) and adjusted to pH 7 with acetic acid. The precipitated white solid was collected, washed with water (10 mL), ether (10 mL) and dried *in vacuo* to afford pure pemoline (400 mg, 82%). ^{1}H NMR (600 MHz, DMSO-d_6): δ_H 8.75 (br, 1H), 8.53 (br, 1H), 7.37–7.43 (m, 3H), 7.28 (m, 2H), 5.71 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ_C 186.4, 176.8, 134.6, 128.7, 128.7, 126.3, 82.3; ESI(+)MS *m/z* 177 $[M+H]^+$.

*Hydrolysis of callyspongisine A (**1**):* A sample of **1** (10 μg) in 0.5% TFA/MeOH (50 μL) maintained at 40 °C for 2 days and 65 °C for further 3 days was analyzed periodically (2, 4 and 5 days) by HPLC-DAD-MS (Agilent Zorbax SB-C_8, 5 μm, 150 × 4.6 mm, 1.0 mL/min gradient elution from 10–100% MeCN/H_2O over 15 min with isocratic 0.05% formic acid modifier) (Figure S11).

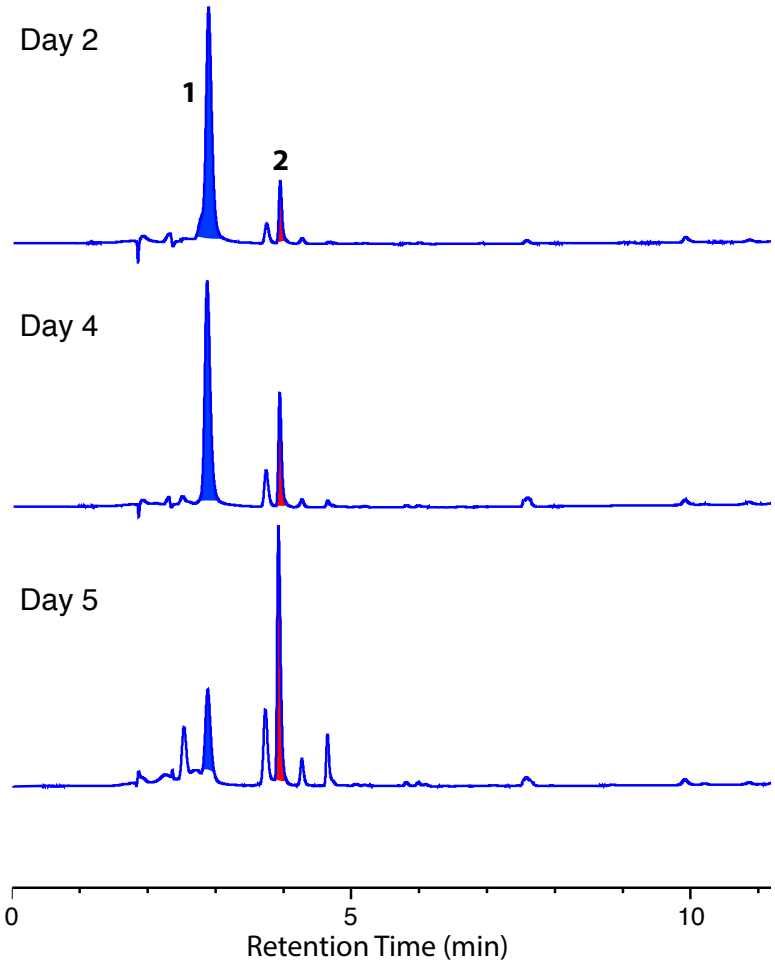


Figure S11. Hydrolysis of callyspongisine A (**1**) in 0.5% TFA/MeOH at 40 °C for 2 days and then 65 °C for a further 2 days and 3 days.

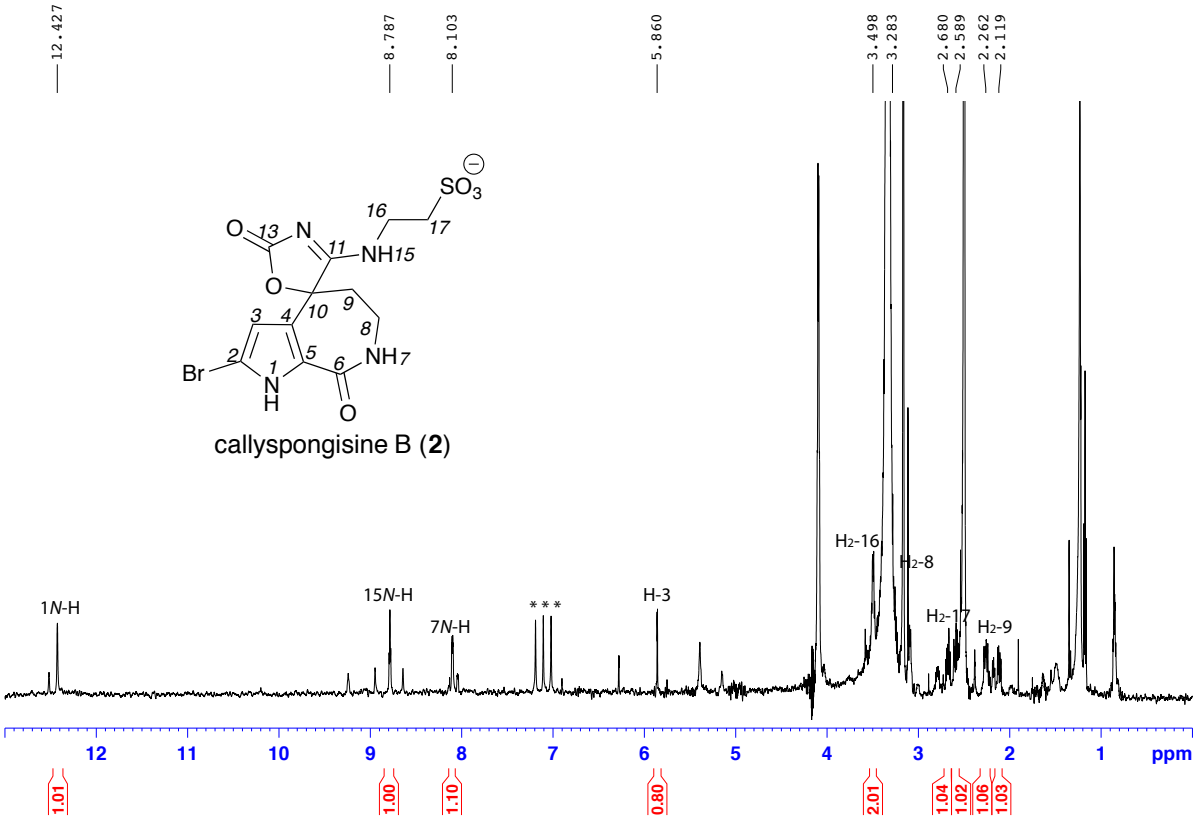


Figure S12a. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of callyspongisine B (**2**). [*** = $^{14}NH_4^+$]

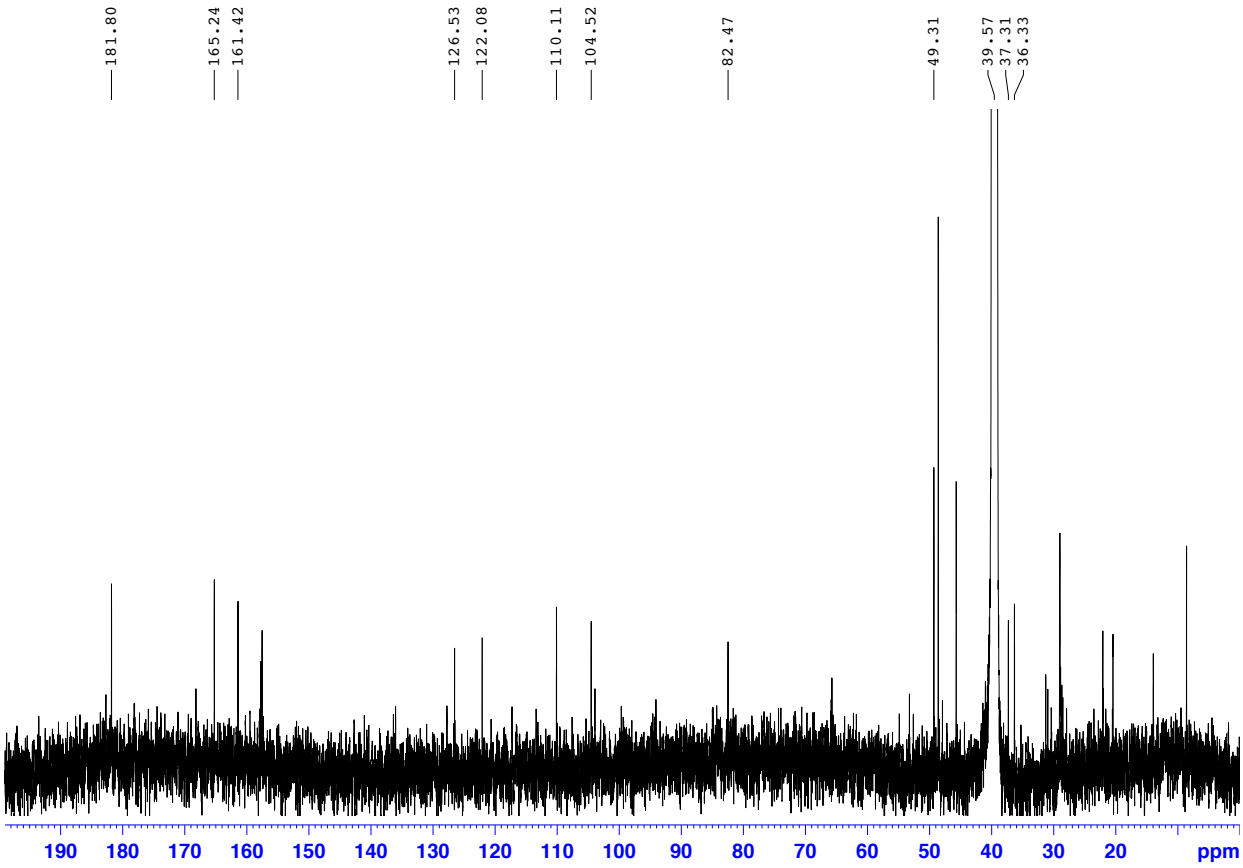


Figure S12b. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of callyspongisine B (**2**).

Table S10. NMR data (600 MHz, DMSO-d_6) from callyspongisine B (**2**).

#	δ_H, mult. (*J* in Hz)	δ_C	HMBC	COSY
1*N*-H	12.43, s		3, 4	
2		104.5		
3	5.86, s	110.1	2, 4, 5	
4		122.1		
5		126.5		
6		161.4		
7*N*-H	8.10, dd (6.9, 2.8)		5	
8	3.28[A]	36.3	6, 8	9
9	a 2.26, dd (14.8, 10.0) b 2.12, dd (14.8, 6.8)	37.3	4, 10	8
10		82.5		
11		181.8		
13		165.2		
15*N*-H	8.79, t (5.5)			16
16	3.50, m	39.6[B]	11, 17	15N-H, 17
17	a 2.68, m b 2.59, m	49.3		16

[A] Overlapped by H_2O signal. [B] Overlapped by DMSO signal

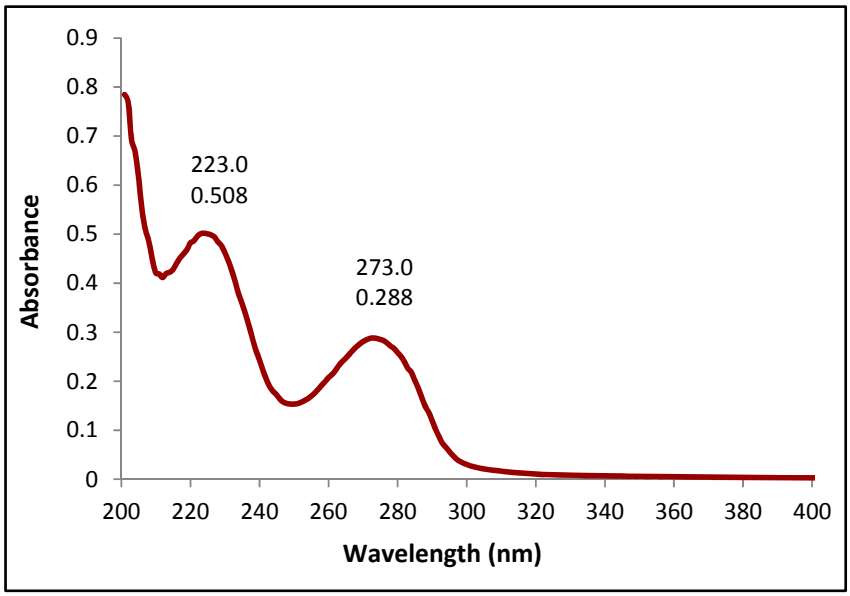


Figure S13. UV-vis (MeOH) spectrum of callyspongisine B (**2**).