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## ARTICLE

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

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An extract of the Great Australian Bight marine sponge *Callyspongia* sp. (CMB-01152) displayed inhibitory activity against the neurodegenerative disease kinase targets casein kinase 1 (CK1), cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 (GSK3 $\beta$ ). Chemical investigation, employing HPLC-DAD-MS single ion extraction protocols, facilitated identification of the new bromopyrrole alkaloids, callyspongisines A–D (1–4), and two known co-metabolites, hymenialdisine (5) and 2-bromoaldisine (6). Structure elucidation of 1–6 was supported by detailed spectroscopic analysis and chemical interconversion, as well as biosynthetic and synthetic considerations. Callyspongisine A (1) is only the second reported example of a natural imino-oxazoline, and the first to feature a *spiro* heterocyclic framework, while callyspongisines B–D (2–4) were speculated to be storage and handling artefacts of 1. The kinase inhibitory activity detected in *Callyspongia* sp. (CMB-01152) was attributed to 5.

### Introduction

Natural products can be viewed as bioactive chemicals produced by organisms, and optimized by evolution, to deliver a survival advantage. Knowledge of natural product chemistry, ecology and biology has inspired generations of researchers, informing our understanding of the natural world, and inspiring the development of many of the most successful medicines. Notwithstanding past successes, there remains an urgent need to continue natural products discovery, to deliver new and improved therapeutics capable of treating a broad array of existing and emerging diseases. For example, the very fact that healthcare programs successfully extend life has the alarming corollary of increasing the incidence of neurodegenerative disorders (NDs) in an aging population. Patients suffering from the archetypal ND, Alzheimer's disease (AD), experience severe and irreversible deterioration of cognitive ability and loss of quality of life, driven by a highly distinctive amyloid and tau brain pathology. Existing wisdom has amyloid pathology caused by the protease  $\beta$ -secretase (BACE) abnormally processing the essential neuronal transmembrane amyloid precursor protein (APP) to generate amyloid (A $\beta$ ) plaques. Likewise, tau pathology is believed to be caused by a host of kinases, including casein kinase 1 (CK1), cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 (GSK3 $\beta$ ), abnormally hyperphosphorylating the highly soluble microtubule-associated tau protein to generate neurofibrillary tangles (NFT).<sup>1</sup> The development of clinically useful treatments for AD (and other NDs) has included a search for safe, potent and selective inhibitors of amyloid and tau pathology.

In collaboration with the Spanish pharmaceutical company Noscira, we screened a library of ~2,600 southern Australian and Antarctic marine organisms to successfully detect extracts that inhibited BACE (27, 1%), CK1 (44, 1.7%), CDK5 (26, 1%) and GSK3 $\beta$  (69, 2.6%), and that contained rare and new natural product scaffolds. A particularly promising extract, from a Great Australian Bight marine sponge *Callyspongia* sp. (CMB-01152), displayed inhibitory activity against all three kinases, with preliminary chemical profiling (HPLC-DAD-MS and <sup>1</sup>H NMR) indicating the presence of brominated alkaloids.

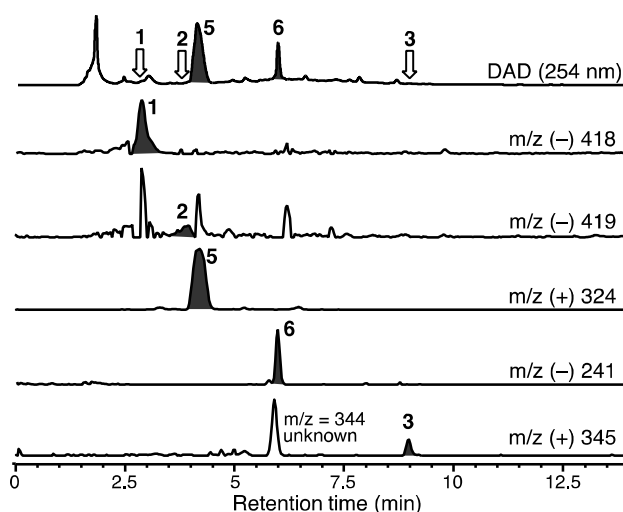


Fig. 1. HPLC-DAD-MS analysis of *Callyspongia* sp. (CMB-01152) crude extract showing HPLC-DAD trace at 254 nm and extracted ion mass chromatograms corresponding to quasi-molecular ions for 1, 2, 5, 6 and 3.

## Results and Discussion

A portion of the aqueous EtOH extract of *Callyspongia* sp. (CMB-01152) was decanted, concentrated *in vacuo* and subjected to solvent partitioning and trituration. Analytical HPLC-DAD-MS analysis (Fig. 1) revealed two major products (**5** and **6**), while careful analysis of the very minor co-metabolites (utilizing single ion extraction protocols) detected four additional brominated co-metabolites (**1–4**). Subsequent semi-preparative reversed-phase HPLC fractionation yielded the new minor bromopyrrole alkaloids, callyspongisines A (**1**) and C–D (**3–4**), and the known major bromopyrrole alkaloids, hymenialdisine (**5**)<sup>2</sup> and 2-bromoaldisine (**6**)<sup>3</sup> (Fig. 2). Although callyspongisine B (**2**) was only detected in the crude extract at levels that precluded isolation and detailed spectroscopic analysis, hydrolysis of a portion of **1** yielded a sufficient quantity of **2** to permit assignment of the structure, as shown in Fig. 2. The known bromopyrroles **5** and **6** were identified by detailed spectroscopic analysis with comparison to literature data,<sup>2–3</sup> while the structure elucidation of **1–4** is outlined below.

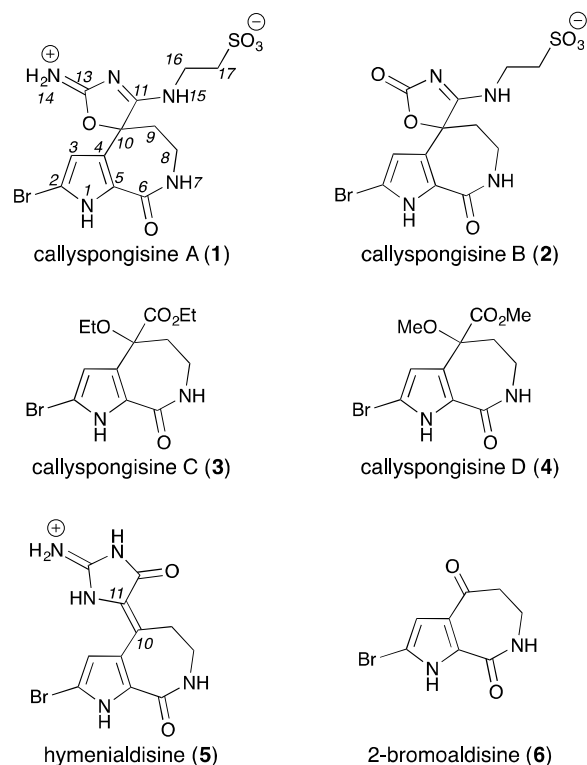


Fig. 2. *Callyspongia* sp. (CMB-01152) chemistry

HRESI(–)MS analysis of **1** revealed a quasi-molecular ion  $[M-H]^-$  consistent with the molecular formula  $C_{12}H_{14}BrN_5O_5S$  ( $\Delta m_{mu} -1.5$ ), while analysis of the NMR (DMSO-*d*<sub>6</sub>) data (Table 1) permitted assembly of structure fragments A, B and C (Fig. 3). Fragment A (N-1 to C-10) was identified as a 2-bromopyrrole fused to a 7-membered lactam, with the 2-bromo regiochemistry supported by comparison of NMR chemical shifts in **1** ( $\delta_H$  6.13, s, H-3;  $\delta_C$  110.5, C-3) with those for the

known 2-bromopyrrole co-metabolites hymenialdisine (**5**) ( $\delta_H$  6.63, s, H-3;  $\delta_C$  111.2, C-3), 2-bromoaldisine (**6**) ( $\delta_H$  6.68, s, H-3;  $\delta_C$  113.3, C-3) and spongiacidin D (**7**) ( $\delta_H$  6.53, s, H-3;  $\delta_C$  111.7, C-3),<sup>4</sup> and the known 3-bromopyrrole analogues debromostevensine (**8**) ( $\delta_H$  7.20, d, H-2;  $\delta_C$  123.0, C-2),<sup>5</sup> debromohymenin (**9**) ( $\delta_H$  7.08, d, H-2;  $\delta_C$  122.1, C-2)<sup>5</sup> and spongiacidin B (**10**) ( $\delta_H$  7.28, H-2;  $\delta_C$  123.2, C-2)<sup>4</sup> (Fig. 4). Fragment B (N-15 to C-17, plus C-11) was identified as a taurinyl residue fused to an  $sp^2$  hybridized deshielded quaternary carbon ( $\delta_C$  181.0, C-11), with the latter more consistent with an imidazoline (e.g. nagelamide X (**11**),  $\delta_C$  179.1)<sup>6</sup> than an amide (e.g. mauritamide D (**12**),  $\delta_C$  158.8)<sup>7</sup> (Fig. 4). The presence of a taurinyl moiety was further supported by comparison of NMR data with an array of known sponge taurinyl-2-aminoimidazoles (ESI Fig. S1 and Table S1). A diagnostic HMBC correlation from 15-NH to C-10 supported assembly of fragments A and B (Fig. 3), and necessitated that fragment C (CH<sub>2</sub>NO) be incorporated into **1** as a *spiro* heterocyclic moiety. The deshielded <sup>13</sup>C NMR shift for C-10 in **1** ( $\delta_C$  91.1) favoured substitution by oxygen (e.g. pemoline (**13**),  $\delta_C$  82.3, and indolmycin (**14**),  $\delta_C$  85.3), versus nitrogen (e.g. 5-phenyl-hydantoin (**15**),  $\delta_C$  61.2)<sup>8</sup> (Fig. 4). Diagnostic HMBC correlations from 14-NH<sub>2</sub> to C-11 and from H-9 to C-13 supported assembly of fragments A, B and C (Fig. 3). Thus callyspongisine A (**1**) was identified as the first example of a natural product bearing a *spiro*-imino-oxazoline moiety, and was assigned the structure as shown (Fig. 2). Further support for this assignment is outlined below.

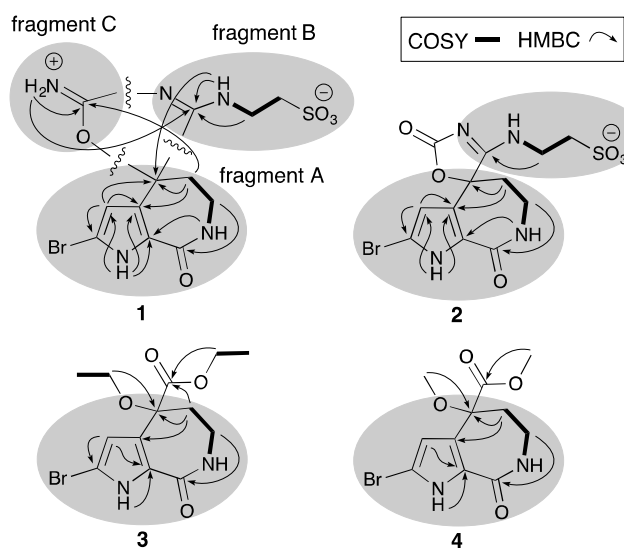


Fig. 3. Diagnostic 2D NMR correlations and structure fragments for **1–4**

HPLC-DAD-MS analysis of a sample of **1** ( $m/z$  418  $[M-H]^-$ ) stored for >12 months at  $-30$  °C in DMSO revealed ~10% conversion to a less polar analogue **2** ( $m/z$  419  $[M-H]^-$ ) with a near identical UV-vis spectrum. HPLC-HRESI(–)MS analysis on this mixture established a molecular formula for **2** ( $C_{12}H_{13}BrN_4O_6S$ ,  $\Delta m_{mu} -0.6$ ) indicative of a hydrolysis product of **1**. Supportive of this hypothesis, a sample of **1** in

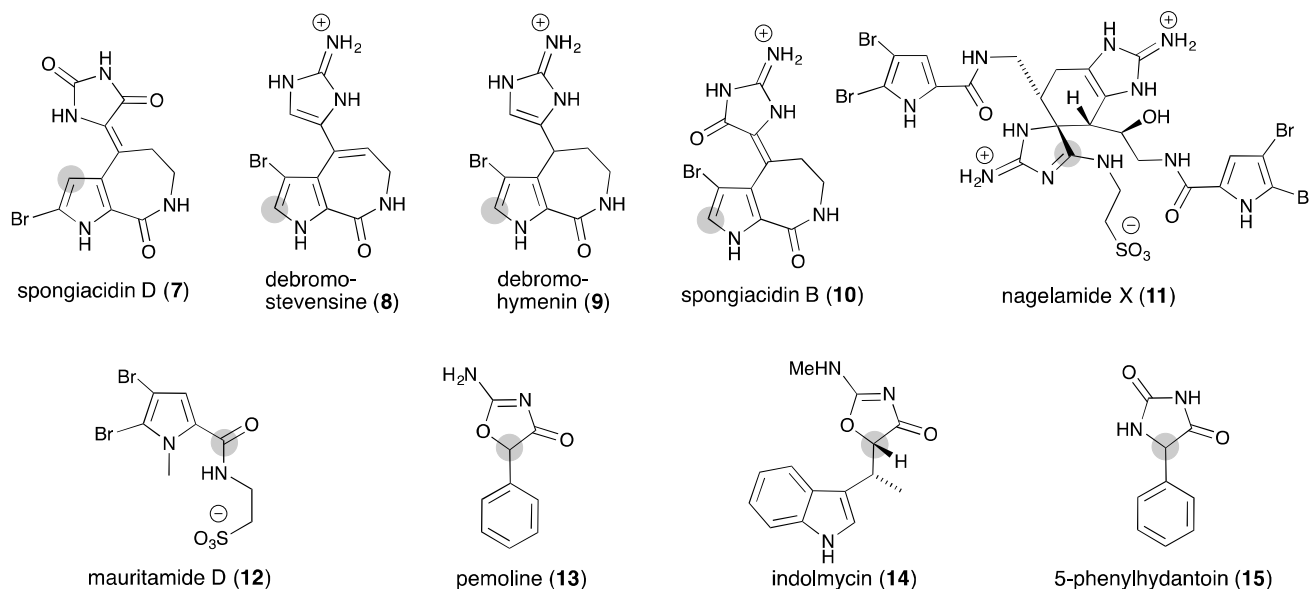


Fig. 4. Known model compounds 7–15 with key carbons highlighted

0.5% TFA/MeOH maintained at 65 °C for 3 days was observed to undergo conversion to >60% **2**, with a plausible mechanism for this transformation outlined in Fig. 5. Analysis of the NMR (DMSO-*d*<sub>6</sub>) data for **2** (Table 1 and Fig. 3) confirmed the presence of structure fragments A and B, while the absence of resonances associated with 14-NH<sub>2</sub> and a more upfield resonance for C-13 ( $\delta_C$  165.2 in **2** vs. 172.3 in **1**) indicated the presence of a *spiro*-oxazolone in place of the *spiro*-imino-oxazoline in **1**. These observations permitted assignment of the structure for callyspongisine B (**2**) as shown in Fig. 2.

HRESI(+)-MS analysis of the co-metabolites **3** and **4** revealed quasi-molecular and adduct ions consistent with the molecular formulae C<sub>13</sub>H<sub>17</sub>BrN<sub>2</sub>O<sub>4</sub> ([M+H]<sup>+</sup>,  $\Delta m_{mu}$  +0.2) and C<sub>11</sub>H<sub>13</sub>BrN<sub>2</sub>O<sub>4</sub> ([M+Na]<sup>+</sup>,  $\Delta m_{mu}$  -0.6) respectively. Analysis of NMR (DMSO-*d*<sub>6</sub>) data (Table 1) confirmed that **3–4** share fragment A in common with **1** (Fig. 3). This analysis also revealed that where **3** features 11-OEt ( $\delta_H$  1.06 and 3.30/3.45;  $\delta_C$  15.8 and 60.0) and 11-CO<sub>2</sub>Et ( $\delta_H$  1.17 and 4.11/4.15;  $\delta_C$  14.2, 61.2 and 172.4) moieties, **4** features 11-OMe ( $\delta_H$  3.16;  $\delta_C$  52.2) and 11-CO<sub>2</sub>Me ( $\delta_H$  3.67;  $\delta_C$  52.6 and 172.7) moieties. These considerations permitted assignment of structures to callyspongisines C (**3**) and D (**4**) as shown (Fig. 2).

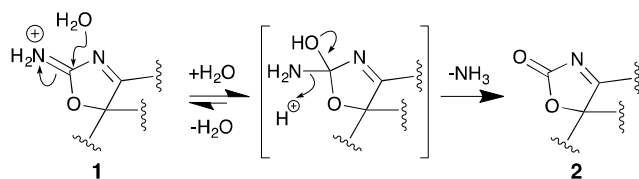


Fig. 5. Mechanism for hydrolysis of **1** to **2**

A plausible biosynthetic/chemical relationship linking **1–6** (Fig. 6) invokes oxidative processing of  $\Delta^{10,11}$  in **5** to yield **6** and the hydroxy acid precursor (i) that can later undergo conjugation with guanidine to yield a *spiro*-imino-oxazoline intermediate (ii), which in turn forms the taurinyl Schiff base **1**. Supportive of the proposed guanidine conjugation step, treatment of mandelic acid ethyl ester with guanidine.HCl (2 eq.) and NaOH (2 eq.) delivered a high yield (82%) of the known imino-oxazoline CNS stimulant pemoline (**13**). Subsequent efforts to form a pemoline-*taurinyl* Schiff base proved unsuccessful, presumably due to unfavourable keto-enol tautomerism. During long-term storage in EtOH we propose that **1** undergoes partial hydrolysis to **2**, and (i) undergoes ethanolysis to **3**. As **4** was only detected/isolated following HPLC (H<sub>2</sub>O/MeOH/TFA) fractionation we propose it is a methanolysis artefact of **3**. In summary, we present callyspongisine A (**1**) as a natural product, and callyspongisines B–C (**2–3**) and callyspongisine D (**4**) as possible storage and isolation artefacts respectively.

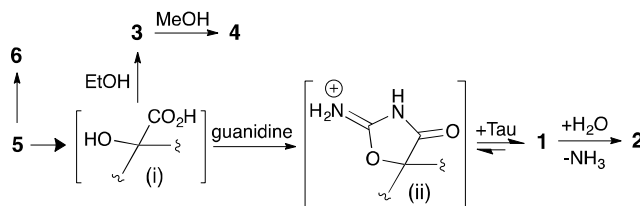


Fig. 6. Plausible biosynthetic/chemical relationship between **1–6**

Imino-oxazolines such as **1** are exceptionally rare in the natural products literature, with the only reported example being indolmycin (**14**), first reported in 1961 from *Streptomyces albus*<sup>9</sup> and again in 1978 from *Streptomyces griseus*.<sup>10</sup> Renewed interest in the biological properties of **14** resurfaced recently, with 2001 and 2004 accounts of antibacterial activity

**Table 1.** <sup>1</sup>H (600 MHz) and <sup>13</sup>C (150 MHz) NMR data for callyspongisines A–D (1–4) in DMSO-*d*<sub>6</sub>

Pos.	callyspongisine A (1) δ <sub>H</sub> , mult ( <i>J</i> in Hz)	δ <sub>C</sub>	callyspongisine B (2) δ <sub>H</sub> , mult ( <i>J</i> in Hz)	δ <sub>C</sub>	callyspongisine C (3) δ <sub>H</sub> , mult ( <i>J</i> in Hz)	δ <sub>C</sub> <sup>C</sup>	callyspongisine D (4) δ <sub>H</sub> , mult ( <i>J</i> in Hz)	δ <sub>C</sub> <sup>C</sup>
1-NH	12.71, s		12.43, s		12.25, s		12.29, s	
2		105.1		104.5		103.6		103.6
3	6.13, s	110.5	5.86, s	110.1	6.13, s	112.6	6.13, s	112.4
4		118.8		122.1		124.6		124.1
5		127.3		126.5		125.4		125.7
6		161.1		161.4		161.7		161.6
7-NH	8.27, t (5.4)		8.10, dd (6.9, 2.8)		7.99, t (1.0)		8.00, s	
8	3.30 <sup>A</sup>	36.1	3.28 <sup>A</sup>	36.3	a 3.28, m b 3.15, ddd (7.7, 7.7, 1.0)	35.5	a 3.26 <sup>A</sup> , m b 3.16 <sup>A</sup> , m	35.5
9	a 2.47, m b 2.39, m	36.6	a 2.26, dd (14.8, 10.0) b 2.12, dd (14.8, 6.8)	37.3	a 2.31, dd (15.0, 7.7) b 2.16, dd (15.0, 7.7)	34.7	a 2.32, dd (15.0, 7.7) b 2.16, dd (15.0, 7.7)	34.4
10		91.1		82.5		79.3		79.7
11		181.0		181.8		172.4		172.7
13		172.3		165.2				
14-NH <sub>2</sub>	a 10.16, s b 9.74, s							
15-NH	10.34, t (5.4)		8.79, t (5.5)					
16	3.66, ddd (7.0, 7.0, 5.4)	41.1	3.50, m	39.6 <sup>B</sup>				
17	a 2.74, dt (13.0, 7.0) b 2.67, dt (13.0, 7.0)	48.8	a 2.68, m b 2.59, m	49.31				
CO <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>					a 4.15, dq (14.3, 7.0) b 4.11, dq (14.3, 7.0)	61.2		
OCH <sub>2</sub> CH <sub>3</sub>					a 3.45, dq (14.2, 7.0) b 3.30 <sup>A</sup>	60.0		
CO <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>					1.17, t (7.0)	14.2		
OCH <sub>2</sub> CH <sub>3</sub>					1.06, t (7.0)	15.8		
CO <sub>2</sub> CH <sub>3</sub>							3.67, s	52.6
OCH <sub>3</sub>							3.16, s	52.2

<sup>A</sup> Overlapped by H<sub>2</sub>O resonance. <sup>B</sup> Overlapped by DMSO resonance. <sup>C</sup> Assignments supported by 2D NMR correlations.

against *Helicobacter pylori*<sup>11</sup> and methicillin-resistant *Staphylococcus aureus* (MRSA),<sup>12</sup> and an unpublished 2013 observation in our laboratory that indolmycin exhibits growth inhibitory activity against the *Mycobacterium tuberculosis* surrogate Bacillus Calmette-Guérin (BCG). These observations prompted us to extend our biological evaluations to assess the kinase-inhibitory, cytotoxic and antibiotic properties of **1** and **3–6**.

The kinase inhibitory activity detected in the *Callyspongia* sp. extract was attributed to the major metabolite hymenialdisine (**5**) (IC<sub>50</sub> 0.03 μM (CK1δ), 0.16 μM (CDK5/p25) and 0.07 μM (GSK-3β)), which is a well known kinase inhibitor.<sup>13</sup> During cytotoxicity screening, **5** displayed moderate growth-inhibitory activity against human colon adenocarcinoma cell line SW620 (IC<sub>50</sub> 3.1 μM) and epidermoid carcinoma cell line KB-3-1 (IC<sub>50</sub> 2.0 μM), while all tested compounds were inactive (IC<sub>50</sub> >30 μM) against human multiform glioblastoma cell line SF-295 and large cell lung cancer cell line NCI-H460. None of the tested compounds inhibited the efflux properties of the transmembrane transporter P-glycoprotein (P-gp) in the adriamycin and vinblastine selected multidrug-resistant cell lines, SW620 Ad300 and KB-V1, nor did they inhibit growth of the Gram-positive bacteria *Staphylococcus aureus* (ATCC 9144 and ATCC 25923), *Bacillus subtilis* (ATCC 6051 and ATCC 6633) and BCG (ATCC 35733), the Gram-negative bacteria *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145), or the fungus *Candida albicans* (ATCC 90028).

## Conclusions

In summary, during the course of our chemical investigation of the Great Australian Bight sponge *Callyspongia* sp. (CMB-01152), we identified the major metabolites as the known bromopyrrole alkaloids hymenialdisine (**5**) and 2-bromoaldisine (**6**). Through the application of highly sensitive HPLC-DAD-MS single ion extraction protocols, we extended these discoveries to detect and identify exceptionally minor callyspongisines **1–4**. Callyspongisine A (**1**) is only the second reported example of a natural imino-oxazoline, and the first to feature a *spiro* heterocyclic framework, while callyspongisines B–D (**2–4**) were speculated to be storage and handling artefacts of **1**. Structure elucidation of **1–6** was supported by detailed spectroscopic analysis and chemical interconversion, as well as biosynthetic and synthetic considerations. The potent kinase inhibitory activity detected in *Callyspongia* sp. (CMB-01152) was attributed to hymenialdisine (**5**), while the callyspongisines proved to be non-cytotoxic against a range of prokaryotic, eukaryotic and mammalian cell lines.

## Experimental section

### Collection

The sponge material identified as *Callyspongia* sp. (UQ code: CMB-01152, Museum Victoria Registry No. MVF166266) was collected in January 1991 during a scientific expedition to the Great Australian Bight aboard the RV Franklin between Adelaide and Portland, SA. Freshly collected samples were frozen (−4 °C) for shipping to the laboratory, where they were thawed, catalogued, diced, and steeped in aqueous EtOH at −30 °C for prolonged storage.

### Extraction and isolation

An aliquot (100 mL) of the aqueous EtOH extract of specimen CMB-01152 was decanted, concentrated *in vacuo*, and the residue (366.4 mg) partitioned between H<sub>2</sub>O and *n*-BuOH. The *n*-BuOH-soluble fraction was concentrated *in vacuo* (108.1 mg) and the residue further triturated into light petroleum (18.9 mg), CH<sub>2</sub>Cl<sub>2</sub> (14.0 mg), and MeOH (74.3 mg) solubles. The H<sub>2</sub>O-soluble fraction was concentrated *in vacuo* (258.3 mg) and the residue further triturated into MeOH (168.4 mg) and H<sub>2</sub>O (88.4 mg) solubles. Kinase inhibition assays localized inhibitory activity in the CH<sub>2</sub>Cl<sub>2</sub> and MeOH solubles.

The CH<sub>2</sub>Cl<sub>2</sub> solubles (14.0 mg) were subjected to HPLC fractionation (Agilent Zorbax Phenyl 5 μm, 150 × 4.6 mm analytical column, 1 mL/min, isocratic 0.01% TFA modifier, gradient elution from 90% H<sub>2</sub>O/MeCN to 100% MeCN over 18 min, then a 4 min hold at 100% MeCN), to yield 2-bromoaldisine (**6**) (t<sub>R</sub> = 9.2 min, 0.3 mg) and callyspongisine C (**3**) (t<sub>R</sub> = 13.0 min, 0.9 mg).

A portion (37.1 mg) of the MeOH solubles derived from the *n*-BuOH solubles was subjected to HPLC fractionation (Agilent Zorbax C<sub>3</sub> 5 μm, 250 × 9.4 mm semi-preparative column, 4 mL/min, isocratic 0.01% TFA modifier, isocratic elution of 90% H<sub>2</sub>O/MeCN for 5 min, then a gradient elution from 90% H<sub>2</sub>O/MeCN to 40% H<sub>2</sub>O/MeCN over 15 min, then a 4 min hold at 100% MeCN, to yield hymenialdisine (**5**) (t<sub>R</sub> = 10.6 min, 7.1 mg) and 2-bromoaldisine (**6**) (t<sub>R</sub> = 13.0, 5.0 mg).

The remainder of the MeOH solubles (37.2 mg) derived from the *n*-BuOH solubles was subjected to HPLC fractionation (Agilent Zorbax Eclipse C<sub>8</sub> 5 μm, 250 × 9.4 mm semi-preparative column, 4 mL/min, isocratic 0.01% TFA modifier, gradient elution from 90% H<sub>2</sub>O/MeOH to 64% H<sub>2</sub>O/MeOH over 10 min, then from 64% H<sub>2</sub>O/MeOH to 22% H<sub>2</sub>O/MeOH over 16.4 min, then from 22% H<sub>2</sub>O/MeOH to 100% MeOH over 1.6 min, then a 3 min hold at 100% MeOH, to yield hymenialdisine (**5**) (t<sub>R</sub> = 10.1 min, 3.0 mg), 2-bromoaldisine (**6**) (t<sub>R</sub> = 13.2 min, 3.9 mg), callyspongisine D (**4**) (t<sub>R</sub> = 15.8 min, 1.4 mg) and callyspongisine C (**3**) (t<sub>R</sub> = 21.6 min, 0.9 mg).

The MeOH solubles (168.4 mg) derived from H<sub>2</sub>O solubles were subjected to HPLC fractionation (Agilent Zorbax C<sub>3</sub> 5 μm, 250 × 9.4 mm semi-preparative column, 4.2 mL/min, isocratic 0.01% TFA modifier, gradient elution from 90% H<sub>2</sub>O/MeOH to 75% H<sub>2</sub>O/MeOH over 8 min, then from 75% H<sub>2</sub>O/MeOH to 40% H<sub>2</sub>O/MeOH over 10 min, then from 40% H<sub>2</sub>O/MeOH to 100% MeOH over 2 min, then a 3 min hold at 100% MeOH, to yield callyspongisine A (**1**) (t<sub>R</sub> = 8.3 min, 1.5 mg), hymenialdisine (**5**) (t<sub>R</sub> = 13.4 min, 1.1 mg) and 2-bromoaldisine (**6**) (t<sub>R</sub> = 16.4 min, 0.7 mg).

% yields for **1** (0.41%), **3** (0.49%), **4** (0.38%), **5** (3.1%) and **6** (2.7%) are calculated as weight-to-weight estimate against the crude extract (366.4 mg).

### Characterisation of compounds

*Callyspongisine A* (**1**). Amorphous solid. [α]<sub>D</sub><sup>23</sup> -3.0 (*c* 0.14, MeOH); UV (MeOH) λ<sub>max</sub> (log ε) 204 (3.96), 236 (3.99), 274 (3.61) nm; NMR (600 MHz, DMSO-*d*<sub>6</sub>) see Table 1 and ESI Table S2; HRESI(-)MS *m/z* 417.9841 [M-H]<sup>-</sup> (calcd for C<sub>12</sub>H<sub>13</sub><sup>79</sup>BrN<sub>5</sub>O<sub>5</sub>S<sup>-</sup>, 417.9826).

*Callyspongisine B* (**2**). Amorphous solid. UV (MeOH) λ<sub>max</sub> (log ε) 224 (3.80), 273 (3.56) nm; NMR (600 MHz, DMSO-*d*<sub>6</sub>) see Table 1 and ESI Table S10; HRESI(-)MS *m/z* 418.9665 [M-H]<sup>-</sup> (calcd for C<sub>12</sub>H<sub>12</sub><sup>79</sup>BrN<sub>4</sub>O<sub>6</sub>S<sup>-</sup>, 418.9666). Insufficient quantities of **2** were available to obtain a stable optical rotation measurement.

*Callyspongisine C* (**3**). [α]<sub>D</sub><sup>21</sup> -10 (*c* 0.1, EtOH); Amorphous solid. UV (MeOH) λ<sub>max</sub> (log ε) 203 (3.63), 220 (3.45), 274 (3.64) nm; NMR (600 MHz, DMSO-*d*<sub>6</sub>) see Table 1 and ESI Table S3; HRESI(+MS) *m/z* 345.0442 [M+H]<sup>+</sup> (calcd for C<sub>13</sub>H<sub>18</sub><sup>79</sup>BrN<sub>2</sub>O<sub>4</sub><sup>+</sup>, 345.0444).

*Callyspongisine D* (**4**). [α]<sub>D</sub><sup>21</sup> -13 (*c* 0.1, EtOH); Amorphous solid. UV (MeOH) λ<sub>max</sub> (log ε) 203 (3.42), 273 (3.33) nm; NMR (600 MHz, DMSO-*d*<sub>6</sub>) data see Table 1 and ESI Table S4; HRESI(+MS) *m/z* 338.9957 [M+Na]<sup>+</sup> (calcd for C<sub>11</sub>H<sub>13</sub><sup>79</sup>BrN<sub>2</sub>O<sub>4</sub>Na<sup>+</sup>, 338.9951).

*Hymenialdisine* (**5**). A yellow solid. NMR (DMSO-*d*<sub>6</sub>, 600 MHz) data see ESI Table S5; HRESI(+MS) *m/z* 324.0097 [M+H]<sup>+</sup> (calc. for C<sub>11</sub>H<sub>11</sub>BrN<sub>5</sub>O<sub>2</sub><sup>+</sup>, 324.0091).

*2-Bromoaldisine* (**6**). A yellow oil. NMR (DMSO-*d*<sub>6</sub>, 600 MHz) data see ESI Table S6; HRESI(-)MS *m/z* 240.9627 [M-H]<sup>-</sup> (calc. for C<sub>8</sub>H<sub>6</sub><sup>79</sup>BrN<sub>2</sub>O<sub>2</sub><sup>-</sup>, 240.9618).

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## Notes and references

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