# Concise total synthesis of aplysinellamides A and B

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Concise and efficient total syntheses of bromotyrosine-derived metabolites aplysinellamides A and B, isolated from Australian marine sponge *Aplysinella* sp., have been accomplished in seven steps. A condensation between cinnamic acid and Boc-D-lysine methyl ester was applied to form the amide skeleton as a key step.

**Keywords:** bromotyrosine, marine sponge, aplysinella species

Among more than 20,000 marine natural products, 2% of them were discovered from deep-water marine organisms.[1, 2] Deep-water organisms are expected to produce metabolites having unique structures with interesting bioactivities, such as cytotoxic,[3] anti-inflammatory,[4] anti-bacterial,[5] anti-malarial,[6] anti-viral,[7] anti-thrombotic,[8] anti-trypanosomal,[9] anti-neurodegenerative[10] and estrogen.[11] For example, tetracyclic compound halenaquinone exhibits extremely potent activity towards farnesyltransferases ($IC_{50}$ 0.017–0.031 μM) and malaria ($IC_{50}$ 0.53–0.62 μM) and meroterpene sulfate fascioquinols A and B displayed promising Gram-positive selective antibacterial activity towards Staphylococcus aureus ($IC_{50}$ 0.9–2.5 μM).[5]

In 2014, Quinn *et al* isolated four new bromotyrosine derivatives aplysinellamides A–C (**1–3**) and aplysamine-1-N-oxide (**4**) with ApoE modulation activity from the Australian marine sponge *Aplysinella* sp. and assigned their structures by extensive NMR experiments (Fig. 1).[12] The unique structure of them intrigues many chemists. We initiated the total synthesis of aplysinellamides in order to investigate the potential bioactivity of the derivatives. We now report the first efficient total syntheses of aplysinellamides A (**1**) and B (**2**) from the accessible starting material D-lysine (**5**) in seven steps.

## Results and discussion

Our initial retrosynthetic analysis of **1** and **2** is outlined in Scheme 1. We envisaged that the cinnamamide skeleton of **1** and **4** could be constructed through a coupling between amine **9** and the respective acids **11** and **16** (see Scheme 2). Amine **9**, in turn, could be obtained from a cheap starting material D-lysine (**5**). Intermediates including methyl $N^{\alpha}$-Boc-D-lysinate **9** and the cinnamic acids **11** and **16** were synthesised according to reported literature procedures.[13-18] Accordingly, D-lysine (**5**) was reacted with ClCOOBn in the presence of $CuSO_4$ to form D-lys(Z)-OH **6** in 91% yield, followed by N-protection with $(Boc)_2O$ to afford acid **7** in 96% yield.[13] Acid **7** was alkylated with $CH_3I$ smoothly and gave methyl ester **8** in 85% yield.[14] Removal of the Cbz group from **8** by hydrogenation with 10% Pd/C in MeOH smoothly provided methyl $N^{\alpha}$-Boc-D-lysinate **9** in 90% yield.[13] Cinnamic acid **11** was prepared through a two-step sequence. Veratraldehyde was mono-brominated with $Br_2$/AcOH to give 3-bromo-4-methoxybenzaldehyde **10** in 70% yield, which was subjected to Doebner–Knoevenagel condensation with malonic acid in the presence of pyridine and piperidine to afford cinnamic acid **11** in 92% yield.[15,16] Similarly, 4-hydroxybenzaldehyde was dibrominated to afford

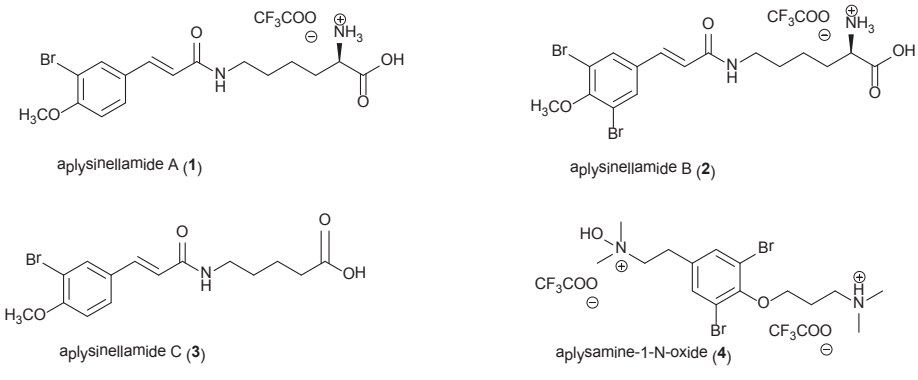
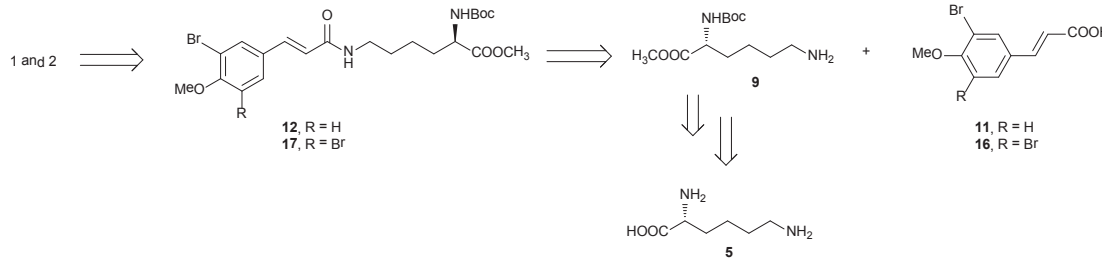



**Fig. 1** Chemical structures of aplysinellamides A-C (**1–3**) and aplysamine-1-N-oxide (**4**).




**Scheme 1** Retrosynthetic analysis of aplysinellamides A (**1**) and B (**2**).

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3,5-dibromo-4-hydroxybenzaldehyde **14** in 96% yield,[17] which was methylated with $CH_3I$ to give 3,5-dibromo-4-methoxybenzaldehyde **15** in 97% yield.[18] Then, aldehyde **15** was subjected to Doebner–Knoevenagel condensation to provide cinnamic acid **16** in 79% yield.[16] Coupling of **9** with cinnamic acid **11** was carried out under similar conditions to give cinnamamide **12**, which was saponified with 1M LiOH/MeOH to give amino acid **13** in 61% yield in two steps.[19] Finally, removal of the Boc group from **13** with TFA in DCM smoothly provided aplysinellamide A (**1**) in 91% yield as a white solid ($[\alpha]_D^{23}$ –3.2°, c 0.007 in MeOH). Likewise, coupling of **9** with cinnamic acid **16** was carried out under similar conditions to give cinnamamide **17** in a yield of 70%, which was saponified to give amino acid **18**. Subsequently, removal of the Boc group from **18** with TFA in DCM afforded aplysinellamide B (**2**) in 61% yield in two steps as a white solid ($[\alpha]_D^{23}$ +0.30°, c 0.017 in MeOH) (Scheme 2). Spectral data of **1** and **2** were consistent with those described for the natural products.[12]

## Conclusions

In summary, we have developed a very concise route for the first total syntheses of aplysinellamides A and B starting from D-lysine in seven steps (overall yields: 39% for aplysinellamide A; 31% for aplysinellamide B). Syntheses of these natural products and their derivatives on a large scale could be realised by this route, which will facilitate further biological studies. Studies towards the structure modifications of these natural products for further pharmacological investigation are ongoing and will be reported elsewhere.

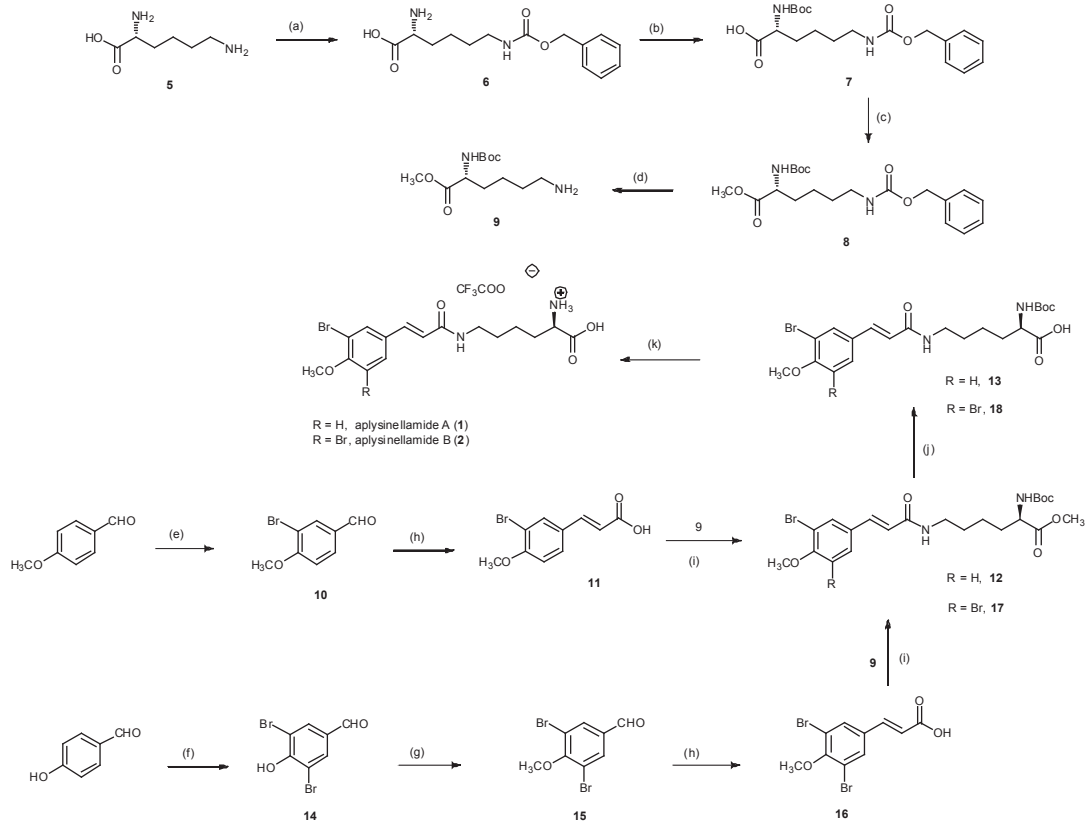
## Experimental

Melting points were measured on a microscopic melting point apparatus. The IR spectra were recorded on a Bruker Tensor 27 FTIR spectrometer with a KBr disk or film. $^1$H NMR and $^{13}$C NMR spectra were taken on a Bruker AV 300 or AV 400 MHz and 75 or 100 MHz spectrometer in DMSO-$d_6$ or $CD_3OD$, chemical shifts are given in parts per million (ppm) relative to TMS as an internal standard. Mass spectra and high resolution mass spectra were performed on Agilent Q TOF 6520 mass spectrometer with electron spray ionisation (ESI) as the ion mode. Optical rotations were recorded using a sodium lamp with a Rudolph Autopol I Automatic Polarimeter with 1 dm tube.

*(*R*)-Methyl 6-amino-2-(tert-butoxycarbonylamino)hexanoate* (**9**): Removal of Cbz group from **8** by hydrogenation with 10% Pd/C in MeOH for 14 h at room temperature smoothly provided methyl N$^{\alpha}$-Boc-D-lysinate **9** as an oil;[13] yield 90%. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 7.23 (br d, *J* = 7.7 Hz, 1H, C2NH), 3.88–3.94 (m, 1H, C2H), 3.61 (s, 3H, 1-$COOCH_3$), 3.07–3.15 (m, 2H, C6-$NH_2$), 1.53–1.58 (m, 2H, C1H), 1.37 (s, 9H, $C(CH_3)_3$), 1.23–1.32 (m, 6H, C3,4,5).

*(*E*)-3-(3-Bromo-4-methoxyphenyl)acrylic acid* (**11**): 3-Bromo-4-methoxybenzaldehyde (**10**) was subjected to Doebner–Knoevenagel condensation with malonic acid in the presence of pyridine and piperidine to afford cinnamic acid **11** as a yellow solid; yield 92%;[16] m.p. 240–242 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 12.31 (br s, 1H, COOH), 7.96 (s, 1H, C2'H), 7.70 (d, *J* = 8.6 Hz, 1H, C6'H), 7.51 (d, *J* = 16.0 Hz, 1H, C3H), 7.14 (d, *J* = 8.6 Hz, 1H, C5'H), 6.46 (d, *J* = 16.0 Hz, 1H, C2H), 3.89 (s, 3H, 4'-$OCH_3$).

*(*R,E*)-6-[3-(3-bromo-4-methoxyphenyl)acrylamido]-2-[(*tert-*butoxycarbonyl)amino]hexanoic acid* (**13**): Compound **11** (0.08 g, 0.3 mmol) was dissolved in anhydrous DMF (2 mL) and anhydrous




**Scheme 2** Synthesis of aplysinellamide A and B. Reagents and conditions: (a) $CuSO_4$, aq. NaOH, r.t., 5h; ClCOOBn, EDTA, r.t., 3h, 91%; (b) $(Boc)_2O$, 4M NaOH, Dioxane, $H_2O$, r.t., 14h; MeOH, r.t., 12h, 96%; (c) $NaHCO_3$, $CH_3I$, DMF, r.t., 36h, 85%; (d) 10% Pd/C (15 w/w %, MeOH, r.t., 14h, 95%; (e) $Br_2$, AcOH, r.t., 14h, 70%; (f) $Br_2$, AcOH, NaOAc, r.t., 1h, 96%; (g) $CH_3I$, $K_2CO_3$, DMF, 55 °C, 3h, 97%; (h) Malonic acid, pyridine, piperidine, reflux 12h, 92% for **11** and 79% for **16**; (i) EDC.HCl, HOBt, TEA, dry DMF, dry DCM, r.t., 16h, 70% for **17**; (j) LiOH.$H_2O$, MeOH, r.t., 16h, 61% for **13** in two steps; (k) TFA, dry DCM, r.t., 40min, 91% for **1**; 62% for **2** in two steps.

DCM (2 mL) and cooled to 0 °C. 1-Hydroxy-benzotriazole hydrate (0.05 g, 0.37 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.07 g, 0.37 mmol) and TEA (0.11 mL, 0.74 mmol) were added to the solution and the mixture was stirred at 0 °C for 1 h. Then compound **9** (0.07 g, 0.27 mmol) was dissolved in anhydrous DCM (2 mL) and added to the mixture. The mixture was stirred while warming to room temperature for 16 h. The reaction mixture was diluted with DCM (2mL) and washed with saturated $NaHCO_3$ (2×4 mL). The DCM layer was washed with water (4 mL), brine (4 mL), dried ($Na_2SO_4$), filtered, and evaporated under reduced pressure, yielding 0.1 g crude product (**12**) which was used for next step without further purification. The residue was dissolve in methanol (3 mL), water (1 mL) and cooled to 0 °C. $LiOH.H_2O$ (0.04 g, 0.98 mmol) was added to the solution and stirred at room temperature for 16 h. The methanol was evaporated under reduced pressure, and the pH of the remaining water was adjusted to 3 with 3 M HCl. The aqueous solution was extracted with DCM (2×10 mL). The DCM layers were dried with $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to give **13** (0.084 g, 61% yield in two steps) as a white solid; m.p. 119–120 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 8.00 (t, *J* = 5.6 Hz, 1H, N1H), 7.78 (d, *J* = 2.0 Hz, 1H, C2"H), 7.55 (dd, *J* = 8.8, 2.0 Hz, 1H, C6"H), 7.32 (d, *J* = 15.8 Hz, 1H, C3'H), 7.15 (d, *J* = 8.8 Hz, 1H, C5"H), 6.97–7.03 (m, 1H, N2H), 6.52 (d, *J* = 15.8 Hz, 1H, C2'H), 3.88 (s, 3H, $OCH_3$), 3.80–3.84 (m, 1H, C2H), 3.12–3.17 (m, 2H, C6H), 1.55–1.65 (m, 2H, C3H), 1.41–1.47 (m, 2H, C5H), 1.37 (s, 9H, $C(C\underline{H}_3)_3$), 1.35–1.32 (m, 2H, C4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 174.3 (C1), 164.8 (C1'), 156.1 (C4"), 155.6 (C=O of Boc), 136.6 (C3'), 131.6 (C2"), 129.1 (C1"), 128.4 (C6"), 121.4 (C2'), 112.9 (C3"), 111.1 (C5"), 77.9 ($\underline{C}(CH_3)_3$), 56.4 (C2), 53.5 (C4"-$OCH_3$), 38.4 (C6), 30.5 (C3), 28.8 ($C(\underline{C}H_3)_3$), 28.2 (C5), 23.1 (C4); HRMS (ESI) calcd for $C_{21}H_{29}BrN_2O_6Na$ (M+Na) *m/z* 507.1101; found: 507.1123.

*(*R,E*)-Methyl 2-[(*tert*-butoxycarbonyl)amino]-6-[3-(3,5-dibromo-4-methoxyphenyl)acrylamido] hexanoate (***17***):* Compound **16** (0.20 g, 0.6 mmol) was dissolved in anhydrous DMF (3 mL) and anhydrous DCM (3 mL) and cooled to 0 °C. 1-Hydroxy-benzotriazole hydrate (0.10 g, 0.73 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.14 g, 0.73 mmol) and TEA (0.20 mL, 1.4 mmol) were added to the solution and the mixture was stirred at 0 °C for 1 h. Then compound **9** (0.19 g, 0.72 mmol) was dissolved in anhydrous DCM (2 mL) and added to the mixture. The mixture was stirred while warming to room temperature for 16 h. The reaction mixture was diluted with DCM (5 mL) and washed with saturated $NaHCO_3$ (2×5 mL). The DCM layer was washed with water (10 mL) and brine (10 mL), dried ($Na_2SO_4$), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel to give **17** (0.24 g, 70% yield) as a white solid; m.p. 100–102 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 8.04 (t, *J* = 5.8 Hz, 1H, N1H), 7.87 (s, 2H, C2",6"H), 7.32 (d, *J* = 15.8 Hz, 1H, C3'H), 7.23 (d, *J* = 8.0 Hz, 1H, N2H), 6.63 (d, *J* = 15.8 Hz, 1H, C2'H), 3.87–3.96 (m, 1H, C2H), 3.82 (s, 3H, C4"$OCH_3$), 3.61 (s, 3H, C1$OCH_3$), 3.12–3.17 (m, 2H, C6H), 1.53–1.66 (m, 2H, C3H), 1.41–1.48 (m, 2H, C5H), 1.37 (s, 9H, $C(C\underline{H}_3)_3$), 1.30–1.33 (m, 2H, C4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 173.2 (C1), 164.2 (C1'), 155.5 (C=O of Boc), 153.8 (C4"), 134.9 (C3'), 134.4 (C1"), 131.5 (C2",6"), 124.6 (C2'), 117.9 (C3",5"), 78.1 ($\underline{C}(CH_3)_3$), 60.5 (C4"-$O\underline{C}H_3$), 53.5 (C2), 51.7 (C1-$O\underline{C}H_3$), 38.4 (C6), 30.3 (C3), 28.6 ($C(\underline{C}H_3)_3$), 28.1 (C5), 23.0 (C4); HRMS (ESI) calcd for $C_{22}H_{30}Br_2N_2O_6Na$ (M+Na) *m/z* 599.0363; found: 599.0398.

*Aplysinellamide A* (**1**): Compound **13** (0.15 g, 0.31 mmol) was dissolved in DCM: TFA (1 mL: 1 mL) and the reaction was stirred at r.t. for 40 min. The solvents were subsequently evaporated and the product crystallised with diethyl ether to give aplysinellamide A (**1**) (0.13 g, 91% yield) as a pale powder; m.p.>250 °C; $[\alpha]_D^{23}$ –3.2 (c 0.007 MeOH) (lit. [12] $[\alpha]_D^{23}$ –3.1 (c 0.007, MeOH); IR (KBr): 2923, 2854, 1650, 1595, 1499, 1260, 1050 cm$^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$ and one drop of TFA): δ 7.77 (d, *J* = 2.0 Hz, 1H, C2"H), 7.50 (dd, $J_1$= 8.6 Hz, $J_2$ = 2.0 Hz, 1H, C6"H), 7.43 (d, *J* = 15.6 Hz, 1H, C3'H), 7.04 (d, *J* = 8.6 Hz, 1H, C5"H), 6.47 (d, *J* = 15.6 Hz, 1H, C2'H), 3.95–3.98 (m, 1H, C2H), 3.90 (s, 3H, $OCH_3$), 3.33–3.35 (m, 2H, C6H), 1.90–2.01 (m, 2H, C3H), 1.57–1.65 (m, 2H, C5H), 1.43–1.54 (m, 2H, C4H); $^{13}$C NMR (100 MHz, $CD_3OD$ and one drop of TFA): δ 172.0 (C1), 169.0 (C1'), 158.8 (C4"), 140.4 (C3'), 133.4 (C2"), 130.5 (C1"), 130.1 (C6"), 120.8 (C2'), 113.5 (C5"), 113.3 (C3"), 57.1 (4"-$OCH_3$), 54.0 (C2), 40.2 (C6), 31.4 (C3), 30.3 (C5), 23.6 (C4); HRMS (ESI) calcd for $C_{16}H_{22}BrN_2O_4$ (M+H) *m/z* 385.0765; found: 385.0775.

*Aplysinellamide B* (**2**): Compound 17 (0.10 g, 0.17 mmol) was dissolved in methanol (3 mL) and water (1 mL) and cooled to 0 °C. $LiOH.H_2O$ (0.04 g, 0.98 mmol) was added to the solution and the mixture was stirred at room temperature for 16 h. The methanol was evaporated under reduced pressure and the pH of the remaining water was adjusted to 3 with 3 M HCl. The aqueous solution was extracted with DCM (2×10 mL). The DCM layers were dried with $Na_2SO_4$ and evaporated under reduced pressure, yielding crude product (0.08 g). The residue was dissolved in DCM: TFA (1 mL:1 mL) and the reaction was stirred at r.t. for 40 min. The solvents were subsequently evaporated and the product crystallised with diethyl ether to give aplysinellamide B (**2**) (0.056 g, 62% yield in two steps) as a pale powder; m.p.>250 °C; $[\alpha]_D^{23}$ +0.30 (c 0.017, MeOH) (lit.[12] $[\alpha]_D^{23}$ +0.29 (c 0.017, MeOH); IR (KBr): 2927, 2857, 1634, 1419, 1260, 1205cm$^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$ and one drop of TFA): δ 7.79 (s, 2H, C2",6"H), 7.39 (d, *J* = 15.8 Hz, 1H, C3'H), 6.56 (d, *J* = 15.8 Hz, 1H, C2'H), 3.97 (t, *J* = 6.4 Hz, 1H, C2H), 3.88 (s, 3H, C4"–$OCH_3$), 3.31–3.33 (m, 2H, C6H), 1.92–2.00 (m, 2H, C3H), 1.62–1.66 (m, 2H, C5H), 1.46–1.58 (m, 2H, C4H); $^{13}$C NMR (100 MHz, $CD_3OD$ and one drop of TFA): δ 172.0 (C1), 168.2 (C1'), 156.6 (C4"), 138.4 (C3'), 135.6 (C1"), 133.2 (C2",6"), 124.1 (C2'), 119.8 (C3",5"), 61.4 (4"-$OCH_3$), 54.0 (C2), 40.2 (C6), 31.3 (C3), 30.2 (C5), 23.6 (C4); HRMS (ESI) calcd for $C_{16}H_{20}Br_2N_2O_4Na$ (M+Na) *m/z* = 484.9679; found: 484.9672.

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## Electronic Supplementary Information

Supplementary information including $^1$H NMR and $^{13}$C NMR data and spectra of aplysinellamide A (**1**), aplysinellamide B (**2**) and compounds **13** and **17**, has been deposited online, at stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

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