Pergamon

Tetrahedron 57 (2001) 10181–10189

TETRAHEDRON

Novel tryptophan-derived dipeptides and bioactive metabolites from the sea hare *Aplysia dactylomela*

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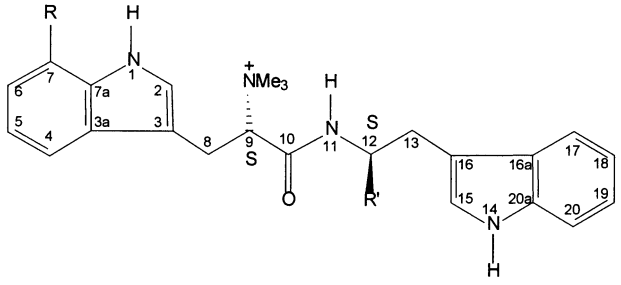
Received 27 July 2001; revised 2 October 2001; accepted 25 October 2001

Abstract—Dactylamides A (**1**) and B (**2**), two new tryptophan-derived dipeptides were isolated from the sea hare *Aplysia dactylomela* and structurally characterised by spectroscopic methods and synthesis of deoxy-analogues. Isolaurenisol, allolaurinterol, their respective acetates and aplysioviolin, isolated as the bioactive constituents of the sea hare, were also structurally characterised and evaluated in a range of biological assays.

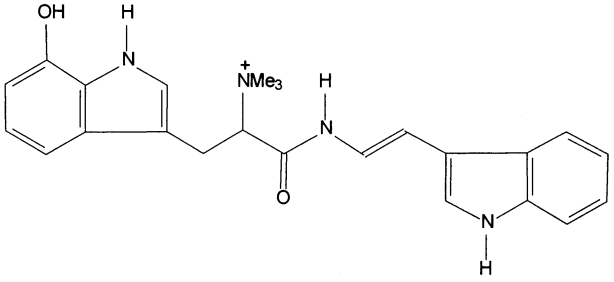
1. Introduction

Opisthobranch molluscs (sea hares) are known to be a rich source of bioactive secondary metabolites,[1–4] the majority of which are sequestered from the algae that make up their diet.[4–6] In many cases, there appears to be an ecological role played by the sequestered metabolites, ranging from feeding deterrence to camouflage.[5,7,8]

As there have been no reports of studies of chemical constituents of sea hares collected in New Zealand, we have investigated a single specimen of *Aplysia dactylomela* Rang 1828 (Order Anaspidea, Family Aplysiidae) collected near Leigh Harbour, Northland, New Zealand. Here we report the isolation and characterisation of two novel tryptophan derived dipeptides, dactylamide A (**1**) and dactylamide B (**2**). Synthesis of related analogues and comparison of circular-dichroism spectra secured the absolute stereochemistry of **1**. Dipeptides **1** and **2** are putative biosynthetic precursors to the chondriamides, metabolites reported from red algae of the genus *Chondria*.[9,10] Also identified in the organism were the biologically active components isolaurenisol (**3**) and allolaurinterol (**4**), their respective acetates **5** and **6** and the ink pigment aplysioviolin (**7**), which was characterised by NMR spectroscopy for the first time, and shown to be comprised of two inseparable diastereomers.



1 R=OH, R'=CO_2^-
8 R=OCH_3, R'=CO_2CH_3
9 R=H, R'=CO_2^-, (9*S*, 12*S*)
10 R=H, R'=CO_2^-, (9*S*, 12*R*)



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2. Results and discussion

2.1. Isolation

The crude organic extract of *A. dactylomela* exhibited cytotoxicity towards the P388 murine leukaemia cell line, the nonmalignant cell line BSC-1, and anti-microbial activity

Keywords: sea hare metabolites; dactylamide; biologically active compounds; sea hare ink; laurene derivatives.
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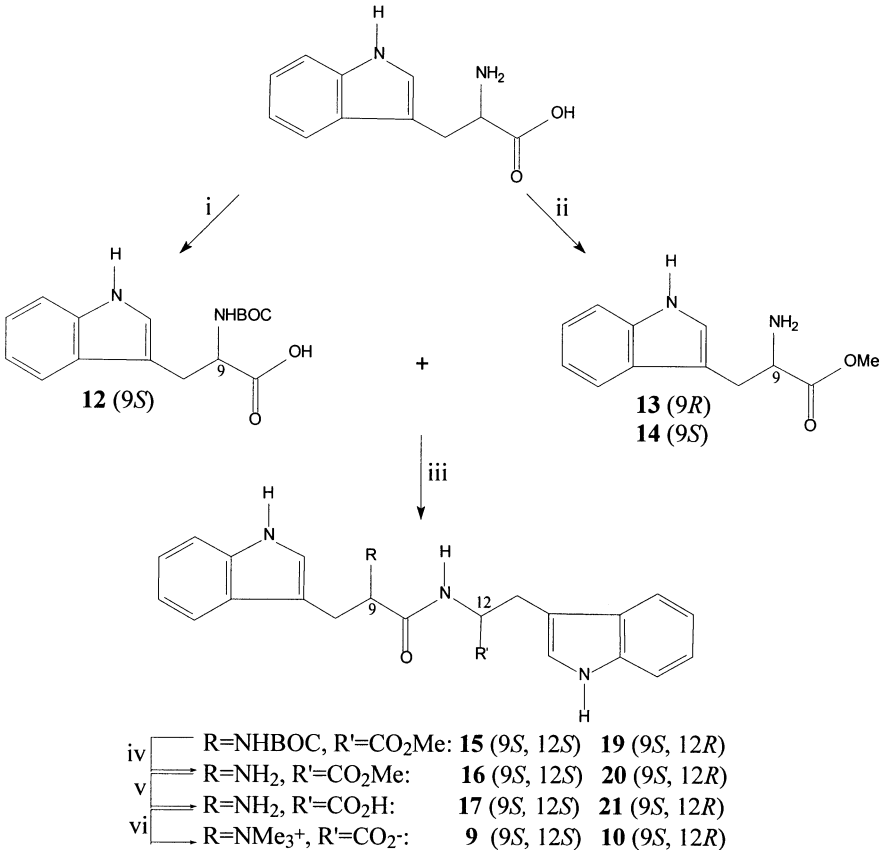
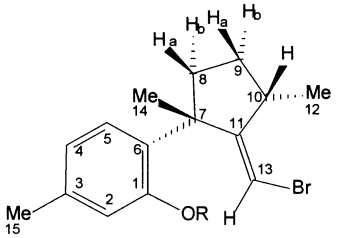
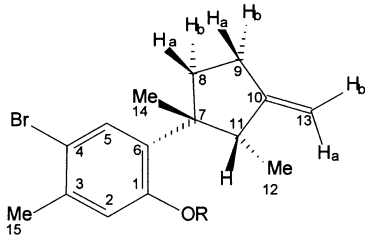
PII: S0040-4020(01)01044-4



Scheme 1. *Reagents and conditions*: (i) $(BOC)_2O$, Et_3N, MeOH, N_2, room temperature, 15 h, 100%; (ii) $SOCl_2$, MeOH, reflux, 4 h, 100%; (iii) BOP, Et_3N, CH_2Cl_2, N_2, room temperature, 4.5 h, 98%; (iv) TFA/CH_2Cl_2 (1:9), N_2, room temperature, 1.5 h, 98%; (v) KOH, MeOH/H_2O (2:3), room temperature, 5 h, 80%; (vi) MeI, $KHCO_3$, MeOH, room temperature, 2.5 d, 78%.



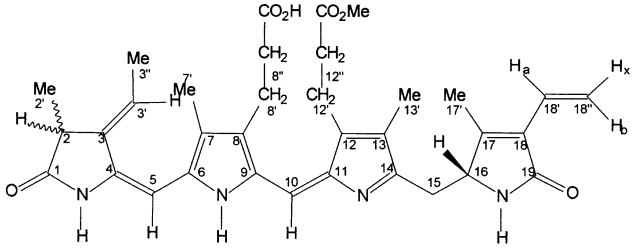
3 R=H
5 R=Ac



4 R=H
6 R=Ac

towards *Bacillus subtilis* (Gram positive bacteria) and *Trichophyton mentagrophytes* (fungi). Crude extract was partitioned between H_2O and CH_2Cl_2 with all biological activity being concentrated in the CH_2Cl_2 fraction. Repeated C18 flash chromatography and semi-preparative HPLC of the aqueous fraction afforded two novel dipeptides **1** and **2**, while bioassay guided fractionation of the organic partition yielded the known algal metabolites isolaurenisol (**3**)[11] and allolaurinterol (**4**),[12–14] their respective acetates **5**[11] and **6**[4,12] and the ink pigment aplysioviolin (**7**)[1,8] as the bioactive components of the extract. Isolaurenisol (**3**) and allolaurinterol (**4**) have previously been reported from algae; this is the first report of their isolation from a sea hare. Acetate **5** has been prepared synthetically from **3**[11] but has not previously been isolated from a natural source.

Compound **1** had a molecular formula $C_{25}H_{28}N_4O_4$ as



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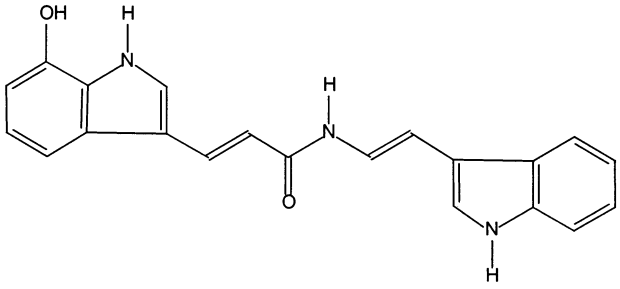
established by HRFABMS. UV–Vis and ^{1}H NMR spectroscopy suggested the presence of two different 3-substituted indole systems, one also being substituted on the benzene ring at either the 4- or 7-position. The presence of two carbonyl resonances in the ^{13}C NMR spectrum (δ 174.1 and 164.4), an amide resonance in the ^{1}H NMR spectrum (δ 8.43) and two separate $CHCH_2$ spin-systems suggested the compound was a tryptophan-derived dipeptide. The observation of a ^{1}H NMR resonance at δ 2.89 (9H, s) and corresponding ^{13}C NMR resonance at δ 51.0 indicated the presence of a trimethyl quaternary nitrogen functional group while the molecular formula also required the presence of a hydroxyl group. The position of the hydroxyl group, quaternary nitrogen and linkage of the amino acid portions were established by standard 2-D NMR techniques. To further confirm the position of the hydroxyl group, **1** was methylated using CH_2N_2 to yield the dimethyl analogue **8**. The observation of a HMBC NMR correlation from one of the new methyl ether resonances at δ 3.89 to C-7 (δ 146.1) allowed placement of the phenolic hydroxyl group at the 7-position, establishing the planar structure of **1**.

Attempts to secure the absolute stereochemistry of **1** at C-9 and C-12 were made using both acid and base hydrolysis and by digestion with carboxypeptidase. Both acid and base methods gave a complex mixture of products with analytical HPLC of the product mixtures being unable to provide evidence of the expected tryptophan or 7-hydroxyhyparphorine fragments. Alkaloid **1** was resistant to hydrolysis by carboxypeptidase.

Two deoxy-analogue diastereomers **9** (9*S*,12*S*) and **10** (9*S*,12*R*) were then prepared in order to use optical rotation and circular-dichroism techniques to infer the stereochemistry of **1**. The stereochemically-defined tryptophan dipeptides were synthesised using standard methods, with subsequent deprotection and methylation using MeI/$KHCO_3$ yielding diastereomers **9** and **10** (see Scheme 1). Close similarity between the ^{1}H NMR and chiroptical $[\alpha]_D$ data observed for synthetic **9** vs. natural product **1** (see Section 3) suggested the natural product had (9*S*,12*S*) or (9*R*,12*R*) stereochemistry rather than (9*S*,12*R*) or (9*R*,12*S*). Final confirmation of the absolute stereochemistry was achieved by comparison of circular-dichroism spectra of **1** with those obtained for **9** and **10**. Near identity of data observed for **1**, which had a negative Cotton effect at 222 nm [λ 217 nm, ΔCD +6.1; λ 226 nm, ΔCD −2.9; *A* 9.0] and **9** which also had a negative Cotton effect at 222 nm [λ 217 nm, ΔCD +2.8; λ 227 nm, ΔCD −2.1; *A* 4.5] concludes that **1** is (9*S*,12*S*). Compound **10** had a positive Cotton effect at 220 nm [λ 215 nm, ΔCD −0.3; λ 225 nm, ΔCD +3.5; *A* 3.8].

The second water-soluble alkaloid **2** had a molecular formula of $C_{24}H_{27}N_4O_2$ as determined by HRFABMS. Direct comparison with the NMR data observed for **1** indicated the absence of the carboxylate group and that one $CHCH_2$ spin-system was replaced by an *E*-geometry α,β-unsaturated amide system (δ 6.32 (d, J=14.9 Hz), 7.18 (dd, J=14.8, 9.8 Hz), 10.56 (d, J=9.7 Hz, NH)). UV–Vis spectra of **1** and **2** were similar except for a new absorption at longer wavelength, λ_{max} 313 nm in **2**, consistent with the presence of extended conjugation. 2-D NMR techniques were again used to confirm the structure and fully assign NMR data. The absolute stereochemistry at C-9 was not deduced.

Structurally, compounds **1** and **2** are saturated analogues of chrondriamide B (**11**), a metabolite of the red alga genus *Chondria*.[9,10] The conversion of tryptophan to indoleacrylic acid via a quaternised amine is a known metabolic pathway in plants;[15–17] thus, it is possible that **1** and **2** are present in algae as biosynthetic precursors to **11**.



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Isolated from the bioactive CH_2Cl_2 partition were the sesquiterpenes isolaurenisol (**3**), allolaurinterol (**4**), and their respective acetates **5** and **6**. Complete NMR spectral assignment of **3**, **5** and **6** was achieved using standard 2-D NMR techniques. Full assignment of **4** has been recently reported by König and Wright.[14]

Isolaurenisol (**3**) has been previously reported from the New Zealand alga *Laurencia distichophylla*.[11] In their original report, Blunt et al. were unable to assign the stereochemistry of the $\Delta^{11,13}$ bond. Our observation of strong ROE correlations between H-14 methyl protons and H-13 establishes the $\Delta^{11,13}$ bond to be *E*-configuration. Also of note was a significant difference between the optical rotation measured for our isolated natural product **3** ($[\alpha]^{20}_D$=+7) compared to the previously reported value ($[\alpha]^{25}_D$=−42).[11] Acetylation of our isolated **3** using Ac_2O/pyridine (1:1) yielded **5** that had identical optical properties to those of the natural product isolated in the present study and that reported previously for semi-synthetic material.[11] Final confirmation of the optical rotatory properties of **3** was made by examination of a fresh specimen of *L. distichophylla*, collected from the same locale as the original report and the same locale as the sea hare specimen used in this study. Terpene **3** isolated from the algae was spectroscopically and chiroptically identical to **3** isolated from *A. dactylomela*. We noted complete lack of phenol **4** and acetates **5** and **6** in the algae extract, which suggests that *A. dactylomela* has a wide range of dietary algae[18] and that the sea hare may well be effecting the conversion of **3** and **4** to acetates **5** and **6**. A previous example of such chemical conversion by opisthobranch molluscs is the metabolite stylocheilamide, an acetate derivative of malyngamide I,[3] which was isolated from the hare *Stylocheilus longicauda* that feeds upon the blue–green alga *Lyngbya majuscula*, a known producer of malyngamide I.[19]

Also isolated from the CH_2Cl_2 partition, the structure of the bioactive metabolite **7** was deduced as being aplysioviolin, an intensely purple pigment previously reported from sea

Table 1. ^{1}H and ^{13}C NMR data (DMSO-d_6) for (2*S*,16*R*)- and (2*R*,16*R*)-diastereomers of aplysioviolin (**7**). Chemical shifts of the diastereomers are separated by '/' where different

Atom no.	δ ^{13}C	δ ^{1}H[a]	(Mult, *J* (Hz))	HMBC corr (H→C)
1	177.8	–		
2	37.3/37.4	3.31	(bq, 7.4)	1,2′,3
2′	15.76/15.81	1.27/1.29	(each d, 7.8)	1,2,3
3	135.6	–		
3′	123.0/123.1	6.55	(qd, 7.3, 2.4)	2,4
3″	14.5	1.87	(d, 7.2)	3,3′,4
4	145.3	–		
5	87.1	6.00	(s)	3,4,6,7
6	164.8/165.0	–		
7	130.56/130.60	–		
7′	9.3	2.01	(s)	6,7,8,9
8	142.6	–		
8′	19.5	2.80	(t, 7.4)	7,8,8″,9, *C*O_2H
8″	35.5	2.38	(t, 7.4)	8,8′, *C*O_2H
9	146.0/146.1	–		
10	113.9	6.78	(s)	8,11,12
11	126.6	–		
12	130.7/130.8	–		
12′	19.4	2.82	(t, 7.4)	11,12,12″,13, *C*$O_2$$CH_3$
12″	34.8	2.48	(t, 7.2)	12,12′,*C*$O_2$$CH_3$
13	118.26/118.29	–		
13′	8.8	1.91	(s)	12,13,14
14	134.5/134.7	–		
15	29.3/29.4	3.19/3.21	(each m)	13,14,16,17
		2.64/2.67	(each m)	13,14,16,17
16	58.9/59.0	4.34	(bm)	17
17	154.8/154.9	–		
17′	11.9	2.00	(s)	16,17,18,19
18	126.9/127.0	–		
18′a	126.6	6.42/6.43	(each dd, 17.6, 11.5)	17,18,19
18″b	117.7	6.17/6.18	(each dd, 17.6, 3.1)	18
18″x		5.25/5.26	(each dd, 11.7, 2.4)	18
19	171.7	–		
19-N*H*	–	8.02	(d, 9.1)	16,17,18,19
CO_2H	173.6			
C$O_2$$CH_3$	172.6			
CO_2*C*H_3	51.2	3.55	(s)	*C*$O_2$$CH_3$

[a] Two N*H* and one CO_2*H* resonances not observed.

hares.[1,8] A molecular formula of $C_{34}H_{40}N_4O_6$ was deduced by FAB mass spectroscopy, combined with detailed analysis of ^{1}H and ^{13}C NMR spectra. Complete assignment of all ^{1}H and ^{13}C resonances was achieved by standard 2-D NMR techniques (Table 1). The observation of a 1:1 doubling of some of the proton and carbon signals in the NMR spectra indicated that a mixture of diastereomers was present. Chemical shifts of the diastereomers were comparable to ^{1}H NMR data reported for diastereomers of the structurally related dimethyl ester of phycoerythrobilin synthesised by

Table 2. In vitro antitumour, antimicrobial and cytotoxicity activity of compounds **1**–**7**

	P388[a]	BSC-1[b]	GI_{50}[c]	TGI[c]	LC_{50}[c]	*B. s*[d]	*C. a*[d]	*T. m*[d]	*M. t.*[e]
1	>56	−	n.t.[f]	n.t.	n.t.	0	0	0	0
2	>62	−	n.t.	n.t.	n.t.	2	0	0	n.t.
3	17.3	2+	4.2 (0.8)	15 (0.9)	45 (1.0)	9	1	6	38
4	23.4	3+	n.t.	n.t.	n.t.	10	1	7	n.t.
5	>74	2+	4.3 (0.8)	14 (0.9)	45 (1.0)	0	0	8	18
6	>74	1+	13 (0.7)	27 (0.4)	58 (0.4)	0	0	3	9
7	16.5	4+	n.t.	n.t.	n.t.	1	0	0	2

[a] IC_{50} (μM) against the P388 D1 murine leukemia cell line.
[b] The test compound (120 μg) was applied to a 6 mm paper disc and incubated with the BSC cell line growing in continuous culture in a 16 mm well for 24 h at 36°C in an atmosphere containing 5% CO_2. Zones of cytotoxicity were measured microscopically as excess radii from the disc and indicated by −, none detectable;+, 1–2 mm; 2+, 2–3.5 mm; 3+, 3.5–4.5 mm; 4+, greater than 4.5 mm.
[c] GI_{50} (50% growth inhibition), total growth inhibition (TGI) and LC_{50} (50% cell kill) data are averaged calculated mean micro-molar values obtained from two experiments at the NCI. Value in parenthesis is the observed range of data, being the number of $\log_{10}$ units between the most and least sensitive cell line(s) in the panel.
[d] Zone of microbial inhibition against *B. subtilis*, *C. albicans* and *T. mentagrophytes* for 120 μg of test compound on a 6 mm paper disc. Incubation for 18 h at 35°C. Zones measured as excess radii in mm.
[e] Growth inhibition (%) of *M. tuberculosis* H_{37}Rv when compound tested at 6.25 μg/mL.
[f] n.t. not tested.

Gossauer and Weller.[20] The H-2 proton of phycoerythrobilin has been reported as being labile[21,22] further confirming the conclusion that C-2 diastereomers have been isolated in this case. The stereochemistry at C-16 has been previously deduced as *R*.[20,22]

2.2. Biological activity

Natural products **1**–**7** were assayed for a range of cytotoxic and antimicrobial–antimycobacterial properties (Table 2). The free phenol sesquiterpenes **3** and **4**[14,23] exhibited moderate P388 and BSC-1 activity, but also exhibited significant *B. subtilis* activity (zone sizes, **3** 9 mm; **4** 10 mm, 120 μg loading) and moderate activity against the fungus *T. mentagrophytes*. P388 and *B. subtilis* activity was greatly reduced for the acetates **5** and **6**. The major component of the ink, **7** showed moderate P388 activity and BSC-1 cytotoxicity along with some antibacterial activity. This result is consistent with results found for crude ink fractions reported previously.[1,7] Compounds **1** and **2** showed no significant activity in any assay, and none of the compounds tested exhibited any significant activity towards *Mycobacterium tuberculosis* $H_{37}Rv$ or human solid tumour cell lines.

3. Experimental

3.1. General

Details of general procedures and analytical HPLC conditions have been reported previously.[24]

3.2. Animal material

A single specimen (collection number 2000LL1-1) of *A. dactylomela* was collected in April 2000 near Leigh Harbour, Northland, New Zealand and identified by Dr Mary Sewell, School of Biological Sciences, the University of Auckland.

3.3. Extraction and isolation

Animal tissue was freeze-dried (dry weight 70.38 g) and extracted with MeOH (2×200 mL) then CH_2Cl_2 (2×200 mL). Solvents were removed in vacuo to give a dark purple extract (16.12 g). A portion of extract (4.91 g) was partitioned between H_2O and CH_2Cl_2. Biological assay showed all activity had been concentrated in the CH_2Cl_2 partition (0.73 g). The H_2O partition (4.01 g) contained two major compounds by analytical HPLC. A portion (1.97 g) was subjected to C_{18} reverse phase column chromatography. After first eluting with MeOH/H_2O (20:80), and then with MeOH/H_2O (0.05% trifluoroacetic acid (TFA)) (30:70, 300 mL) a clean mixture of the two major compounds was achieved. The compounds were then separated on a second C_{18} column by eluting with MeOH/H_2O (30:70, 200 mL) to yield **1** (110 mg, 1.0% dry weight). Compound **2** (38 mg, 0.51% dry weight) was then eluted with MeOH/H_2O (0.05% TFA) (40:60, 100 mL). A portion of the bioactive CH_2Cl_2 partition (470 mg) was fractionated on a C_{18} reverse phase column using a steep gradient from MeOH/H_2O (50:50) through to MeOH. Biological activity (BSC-1 cytotoxicity) was traced to the 70–80% MeOH fractions. The active fraction was further partitioned on Sephadex LH20 eluting with MeOH to yield three fractions. Fraction 1 was subjected to amino-propyl silica gel chromatography eluting with $CHCl_3$ followed by MeOH/H_2O (0.05% TFA). The colourless $CHCl_3$ fraction contained two compounds that were separated using semi-preparative HPLC (C_{18}, MeOH/H_2O, 88:12, 5 mL/min) yielding **5** (6.9 mg, 0.057% dry weight) and **6** (4.8 mg, 0.035% dry weight). The MeOH/H_2O fraction was pure in the purple pigment **7** (20.5 mg, 0.15% dry weight). LH20 column fractions 2 and 3 contained two other compounds, separation of which was achieved using semi-preparative HPLC (C_{18}, MeOH/H_2O, 85:15, 5 mL/min) on a portion (49 mg) yielding **3** (13.5 mg, 0.17% dry weight) and **4** (10.0 mg, 0.13% dry weight).

3.3.1. Dactylamide A (1). Compound **1** was obtained as a white amorphous solid. Mp 204–210°C (dec); $[\alpha]^{20}_D$=+7 (*c* 0.68, MeOH/H_2O (1:1)); UV (MeOH) λ_{max} (log ε) 220 (4.34), 272 (3.56), 286 (3.53), 290 (3.45); IR (smear) ν_{max} 3304, 1649, 1594, 1579 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ 12.35 (1H, bs, H-1), 11.25 (1H, bs, O*H*), 10.80 (1H, bs, H-14), 8.43 (1H, bd, *J*=5.0 Hz, H-11), 7.55 (1H, d, *J*=7.8 Hz, H-17), 7.28 (1H, d, *J*=8.1 Hz, H-20), 7.26 (1H, bs, H-2), 7.16 (1H, bs, H-15), 7.03 (1H, t, *J*=7.8 Hz, H-19), 6.99 (1H, d, *J*=8.4 Hz, H-4), 6.94 (1H, t, *J*=7.4 Hz, H-18), 6.81 (1H, t, *J*=7.7 Hz, H-5), 6.43 (1H, d, *J*=7.7 Hz, H-6), 4.38 (1H, bm, H-12), 4.30 (1H, bd, *J*=10.7 Hz, H-9), 3.36 (1H, obsc, H-13), 3.35 (1H, obsc, H-8), 3.24 (1H, bd, *J*=11.1 Hz, H-8), 3.06 (1H, obsc, H-13), 2.89 (9H, s, $^+N(CH_3)_3$); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 174.1 (s, CO_2^-), 164.4 (s, C-10), 144.3 (s, C-7), 135.8 (s, C-20a), 128.8 (s, C-3a), 127.5 (s, C-16a), 126.6 (s, C-7a), 123.4 (d, *J*=184 Hz, C-2), 123.4 (d, *J*=184 Hz, C-15), 120.6 (d, *J*=159 Hz, C-19), 119.1 (d, *J*=154 Hz, C-5), 118.3 (d, *J*=159 Hz, C-17), 118.0 (d, *J*=159 Hz, C-18), 111.0 (d, *J*=164 Hz, C-20), 110.8 (s, C-16), 108.2 (d, *J*=154 Hz, C-4), 106.6 (s, C-3), 106.5 (d, *J*=154 Hz, C-6), 73.4 (d, *J*=149 Hz, C-9), 55.2 (d, *J*=139 Hz, C-12), 51.0 ($^+N(CH_3)_3$), 27.4 (dd, *J*=124, 119 Hz, C-13), 21.8 (dd, *J*=134, 129 Hz, C-8); FABMS *m/z* 449 $[M+H]^+$; HRFABMS *m/z* 449.2180 (calcd for $C_{25}H_{29}N_4O_4$ 449.2189).

3.3.2. Dactylamide B (2). Compound **2** was obtained as a light yellow amorphous solid. Mp 160–163°C (dec); $[\alpha]^{20}_D$=+160 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.44), 282 (3.85), 292 (3.83), 313 (3.85); IR (smear) ν_{max} 3282, 1671, 1202, 1135 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ 11.21 (1H, d, *J*=2.0 Hz, H-14), 10.83 (1H, d, *J*=2.2 Hz, H-1), 10.56 (1H, d, *J*=9.7 Hz, H-11), 9.57 (1H, bs, O*H*), 7.59 (1H, d, *J*=7.8 Hz, H-17), 7.44 (1H, d, *J*=2.6 Hz, H-15), 7.37 (1H, d, *J*=7.9 Hz, H-20), 7.18 (1H, dd, *J*=14.8, 9.8 Hz, H-12), 7.12 (1H, td, *J*=7.7, 0.9 Hz, H-19), 7.07 (1H, td, obsc, H-18), 7.06 (1H, d, *J*=7.3 Hz, H-4), 7.05 (1H, bs, H-2), 6.83 (1H, t, *J*=7.8 Hz, H-5), 6.51 (1H, d, *J*=7.2 Hz, H-6), 6.32 (1H, d, *J*=14.9 Hz, H-13), 4.25 (1H, dd, *J*=11.4, 2.8 Hz, H-9), 3.51 (1H, dd, *J*=13.7, 2.6 Hz, H-8), 3.39 (1H, dd, *J*=13.4, 11.7 Hz, H-8), 3.30 (9H, s, $^+N(CH_3)_3$); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 162.2 (C-10), 143.6 (C-7), 136.7 (C-20a), 128.5 (C-3a), 126.2 (C-7a), 124.6 (C-15), 124.5 (C-16a), 123.5 (C-2),

121.5 (C-19), 119.4 (C-5), 119.4 (C-18), 118.9 (C-17), 117.7 (C-12), 111.9 (C-20), 110.6 (C-16), 109.6 (C-13), 109.1 (C-4), 106.5 (C-3), 105.7 (C-6), 74.0 (C-9), 51.9 ($^{+}N(CH_3)_3$), 22.4 (C-8); FABMS *m/z* 403 $[M]^+$; HRFABMS *m/z* 403.2145 (calcd for $C_{24}H_{27}N_4O_2$ 403.2134).

3.3.3. Isolaurenisol (3). Compound **3** was obtained as a colourless oil.[11] $[\alpha]^{20}_D=+7$ (*c* 1.5, $CHCl_3$) (lit.[11] $[\alpha]^{25}_D=-42$ (*c* 2.5, $CHCl_3$)); UV (MeOH) λ_{max} (log ε) 205 (4.52), 275 (3.91) nm; IR (smear) ν_{max} 3507, 2959, 1617, 1412, 1122, 810 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.17 (1H, d, *J*=7.9 Hz, H-5), 6.69 (1H, dd, *J*=7.8, 1.1 Hz, H-4), 6.58 (1H, s, H-2), 6.01 (1H, d, *J*=2.0 Hz, H-13), 5.10 (1H, bs, O*H*), 3.01 (1H, m, H-10), 2.58 (1H, m, H-8b), 2.27 (3H, s, H-15), 2.04 (1H, m, H-9a), 1.61 (1H, m, H-8a), 1.47 (3H, s, H-14), 1.42 (1H, m, H-9b), 1.24 (3H, d, *J*=7.3 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.2 (C-11), 153.3 (C-1), 138.0 (C-3), 128.7 (C-6), 128.1 (C-5), 121.3 (C-4), 118.1 (C-2), 101.3 (C-13), 52.0 (C-7), 39.23 (C-10), 39.17 (C-8), 31.0 (C-9), 26.8 (C-14), 20.7 (C-15), 19.1 (C-12); EIMS *m/z* 294/296 $[M]^+$, 279/281 $[M-Me]^+$, 215 $[M-Br]^+$; HREIMS *m/z* 294.0620 (calcd for $C_{15}H_{19}{}^{79}BrO$ 294.0619), 296.0605 (calcd for $C_{15}H_{19}{}^{81}BrO$ 296.0599).

3.3.4. Allolaurinterol (4). $[\alpha]^{20}_D=+29$ (*c* 1.5, $CHCl_3$) (lit.[12] $[\alpha]_D=+22$ (*c* 1.66, $CHCl_3$)); all remaining physical and spectral properties of **4** were in agreement with those published.[12–14]

3.3.5. Isolaurenisol acetate (5). Compound **5** was obtained as a clear glass.[11] $[\alpha]^{20}_D=-7$ (*c* 0.15, MeOH) (lit.[11] $[\alpha]^{25}_D=-6$ (*c* 5.5, $CHCl_3$)); UV (MeOH) λ_{max} (log ε) 205 (4.28), 273 (2.3) nm; IR (smear) ν_{max} 2960, 1768, 1369, 1199, 1107 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.25 (1H, d, *J*=8.1 Hz, H-5), 6.94 (1H, dd, *J*=8.4, 1.3 Hz, H-4), 6.81 (1H, d, *J*=1.3 Hz, H-2), 6.00 (1H, d, *J*=2.0 Hz, H-13), 2.95 (1H, m, H-10), 2.37 (1H, m, H-8b), 2.32 (3H, s, H-17), 2.31 (3H, s, H-15), 1.94 (1H, m, H-9a), 1.72 (1H, m, H-8a), 1.43 (3H, s, H-14), 1.30 (1H, m, H-9b), 1.10 (3H, d, *J*=7.1 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.5 (C-16), 158.3 (C-11), 148.5 (C-1), 137.6 (C-3), 134.3 (C-6), 128.9 (C-5), 126.3 (C-4), 124.9 (C-2), 101.9 (C-13), 52.1 (C-7), 40.0 (C-8), 39.6 (C-10), 30.9 (C-9), 27.3 (C-14), 21.7 (C-17), 20.6 (C-15), 18.5 (C-12); EIMS *m/z* 336/338 $[M]^+$, 294/296 $[M-CH_2CO]^+$, 279/281, 257 $[M-Br]^+$, 215; HREIMS *m/z* 336.0723 (calcd for $C_{17}H_{21}{}^{79}BrO_2$ 336.0725), 338.0696 (calcd for $C_{17}H_{21}{}^{81}BrO_2$ 338.0704).

3.3.6. Allolaurinterol acetate (6). Compound **6** was obtained as a clear glass. $[\alpha]^{20}_D=+47$ (*c* 0.075, MeOH) (lit.[12] $[\alpha]_D=+48.2$ (*c* 1.03, $CHCl_3$)); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40 (1H, s, H-5), 6.95 (1H, s, H-2), 4.98 (1H, bs, H-13a), 4.89 (1H, m, H-13b), 2.74 (1H, q, *J*=7.2 Hz, H-11), 2.47 (2H, m, H-9a, H-9b), 2.35 (3H, s, H-15), 2.33 (3H, s, H-17), 2.24 (1H, m, H-8b), 1.82 (1H, m, H-8a), 1.14 (3H, d, *J*=0.6 Hz, H-14), 0.70 (3H, d, *J*=7.2 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.0 (C-16), 157.1 (C-10), 147.3 (C-1), 138.5 (C-6), 136.4 (C-3), 132.2 (C-5), 125.4 (C-2), 121.5 (C-4), 106.7 (C-13), 49.3 (C-11), 48.6 (C-7), 34.4 (C-8), 27.5 (C-9), 26.8 (C-14), 22.4 (C-15), 21.6 (C-17), 19.5 (C-12). All remaining physical and spectral properties of **6** were in agreement with those published.[4,12]

3.3.7. (2*R*,16*R*)- and (2*S*,16*R*)-Aplysioviolin (7). The 1:1 mixture of (2*R*,16*R*)- and (2*S*,16*R*)—**7** was obtained as a purple amorphous solid. $[\alpha]^{20}_{436}=+730$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.29), 329 (4.18), 595 (4.46) nm; IR (smear) ν_{max} 3326, 1681, 1603, 1532, 1245, 1166, 1135, 1104 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; FABMS *m/z* 601 $[M+H]^+$; HRFABMS *m/z* 601.3028 (calcd for $C_{34}H_{41}N_4O_6$ 601.3026).

3.3.8. Dactylamide A-methyl ether-methyl ester (8). Compound **1** (9 mg) was suspended in MeOH (2 mL). Freshly prepared diazomethane/diethyl ether solution (5 mL) was added. The mixture was then left at room temperature to allow the excess diazomethane/diethyl ether to evaporate. Purification was achieved by C_{18} flash column chromatography, eluting with MeOH/H_2O (0.05% TFA) (60:40 to 100:0). Final purification using semi-preparative HPLC (C_{18}, MeOH/H_2O (0.05% TFA), 75:25, 4 mL/min), yielded **8** (3.7 mg) as a white amorphous solid. $[\alpha]^{20}_D=+3$ (*c* 0.06, MeOH); UV (MeCN/H_2O (0.05% TFA)) λ_{max} 220, 272 nm; ^1H NMR ($(CD_3)_2SO$, 400 MHz) δ 11.12 (1H, bd, *J*=2 Hz, H-1), 10.85 (1H, bs, H-14), 8.74 (1H, d, *J*=8.3 Hz, H-11), 7.45 (1H, d, *J*=7.9 Hz, H-17), 7.31 (1H, d, *J*=8.0 Hz, H-20), 7.11 (1H, d, *J*=8.4 Hz, H-4), 7.06 (1H, td, *J*=8.1, 0.9 Hz, H-19), 7.03 (1H, s, H-15), 6.97 (1H, s, H-2), 6.97 (1H, td, *J*=7.5, 0.7 Hz, H-18), 6.93 (1H, t, *J*=7.9 Hz, H-5), 6.66 (1H, d, *J*=7.5 Hz, H-6), 4.68 (1H, ddd, *J*=8.7, 8.3, 5.7 Hz, H-12), 4.10 (1H, dd, *J*=11.4, 2.7 Hz, H-9), 3.89 (3H, s, 7-OCH_3), 3.45 (3H, s, CO_2CH_3), 3.42 (1H, dd, *J*=13.4, 2.7 Hz, H-8), 3.22 (1H, dd, *J*=13.4, 11.4 Hz, H-8), 3.11 (1H, dd, *J*=14.8, 5.5 Hz, H-13), 2.94 (1H, obsc, H-13), 2.96 (9H, s, $^{+}N(CH_3)_3$); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 170.7 (CO_2Me), 165.0 (C-10), 146.1 (C-7), 135.9 (C-20a), 128.1 (C-3a), 126.7 (C-16a), 126.1 (C-7a), 124.0 (C-15), 123.9 (C-2), 121.0 (C-19), 119.1 (C-5), 118.3 (C-18), 118.0 (C-17), 111.3 (C-20), 110.7 (C-4), 108.6 (C-16), 106.4 (C-3), 101.6 (C-6), 73.5 (C-9), 55.0 (7-OCH_3), 52.5 (C-11), 51.8 (CO_2CH_3), 51.4 ($^{+}N(CH_3)_3$), 27.0 (C-13), 22.0 (C-8); FABMS *m/z* 477 $[M]^+$; HRFABMS *m/z* 477.2504 (calcd for $C_{27}H_{33}N_4O_4$ 477.2502).

3.3.9. Isolaurenisol acetylation. Compound **3** (4 mg, 0.014 mmol) was stirred in pyridine/Ac_2O (1:1, 0.5 mL) at room temperature for 5 h. CH_2Cl_2 (40 mL) was added then washed with water (2×30 mL) and brine (1×30 mL). The solvent was then removed in vacuo. Silica flash column chromatography eluting with $CHCl_3$, yielded a compound (4.5 mg, 97%) identical to **5** in all respects.

3.4. Synthesis of dactylamide A (1) analogues

3.4.1. *N*-*t*-Butyloxycarbonyl-L-tryptophan (12). To a solution of L-tryptophan (0.800 g, 3.92 mmol) in MeOH (30 mL) and Et_3N (1.116 mL, 8 mmol), di-*t*-butyloxycarbonate (1.136 g, 5.2 mmol) was added. The mixture was then stirred at room temperature under N_2 for 15 h. The solvent was then removed in vacuo yielding **12** (1.22 g, 100%) as a white amorphous solid. Mp 138–139°C (lit.[25] 136–140°C); $[\alpha]^{20}_D=-18$ (*c* 0.8, AcOH) (lit.[25] $[\alpha]^{25}_D=-18.2$ (*c* 2.0, AcOH)); UV (MeCN/H_2O (0.05% TFA)) λ_{max} 220, 281 nm; IR (smear) ν_{max} 3410, 2979, 1718, 1504, 1368, 1162, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.15 (1H,

bs, H-1), 7.60 (1H, d, J=7.9 Hz, H-4), 7.34 (1H, d, J=8.0 Hz, H-7), 7.20 (1H, t, J=7.5 Hz, H-6), 7.11 (1H, t, J=7.4 Hz, H-5), 7.01 (1H, bs, H-2), 5.08 (1H, bs, N*H*CO_2), 4.65 (1H, bm, H-9), 3.32 (2H, bm, H-8), 1.43 (9H, s, OC(CH_3)$_3$), (CO_2H not observed); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.0, 155.6, 136.1, 127.7, 123.0, 122.1, 119.6, 118.8, 111.2, 110.0, 80.3, 54.2, 28.3, 27.4; EIMS m/z 304 $[M]^+$; HREIMS m/z 304.1425 (calcd for $C_{16}H_{20}N_2O_4$, 304.1423).

3.4.2. D-Tryptophan methyl ester (13). To a stirred solution of D-tryptophan (0.400 g, 1.96 mmol) in MeOH (20 mL), $SOCl_2$ (0.36 mL, 4.9 mmol) was added dropwise at 0°C. The solution was then heated to reflux for 4 h. The solvent was then removed in vacuo yielding **13** as the HCl salt (0.514 g, 100%). Mp 211–215°C (lit.[27] 214°C); $[\alpha]^{20}_D$=−17 (c 0.42, MeOH) (lit.[26] $[\alpha]^{20}_D$=−16.7 (c 1.5, MeOH)); UV (MeCN/H_2O (0.05% TFA)) λ_{max} 215, 277 nm; IR (smear) ν_{max} 3275, 2862, 1746, 1435, 1228, 730 cm^{-1}; ^{1}H NMR ((CD_3)$_2$SO, 400 MHz) δ 11.14 (1H, bs, H-1), 8.69 (3H, bs, $^+$NH_3), 7.52 (1H, d, J=7.9 Hz, H-4), 7.37 (1H, d, J=8.1 Hz, H-7), 7.25 (1H, d, J=2.3 Hz, H-2), 7.09 (1H, btd, J=8.0, 1.0 Hz, H-6), 7.00 (1H, td, J=7.8, 0.8 Hz, H-5), 4.19 (1H, bm, H-9), 3.64 (3H, s, CO_2CH_3), 3.36 (1H, dd, J=14.9, 5.5 Hz, H-8), 3.29 (1H, dd, J=14.9, 6.8 Hz, H-8); ^{13}C NMR ((CD_3)$_2$SO, 100 MHz) δ 169.6 (C-10), 136.1 (C-7a), 126.8 (C-3a), 124.9 (C-2), 121.0 (C-6), 118.5 (C-5), 117.9 (C-4), 111.5 (C-7), 106.2 (C-3), 52.6 (C-9), 52.5 (CO_2CH_3), 26.0 (C-8); EIMS m/z 218 $[M]^+$; HREIMS m/z 218.1055 (calcd for $C_{12}H_{14}N_2O_2$, 218.1055).

3.4.3. L-Tryptophan methyl ester (14). Using the method described earlier for the preparation of **13**, compound **14** (0.517 g, 100%) was prepared as the HCl salt. Mp 211–214°C (lit.[27] 214°C); $[\alpha]^{20}_D$=+17 (c 0.47, MeOH) (lit.[27] $[\alpha]^{20}_D$=+17.1 (c 2.1, MeOH)); IR (smear) ν_{max} 3369, 2920, 1747, 1588, 1429, 1020 cm^{-1}; EIMS m/z 218 $[M]^+$; HREIMS m/z 218.1054 (calcd for $C_{12}H_{14}N_2O_2$, 218.1055); ^{1}H NMR data was identical to that of **13** listed earlier.

3.4.4. *N*-*t*-Butyloxycarbonyl-L-tryptophyl-L-tryptophan-methyl ester (15). Compounds **14** (59 mg, 0.23 mmol) and **12** (70 mg, 0.23 mmol) were dissolved in dry CH_2Cl_2 (5 mL) and dry Et_3N (80 μL, 0.58 mmol). To this, benzotriazol-1-yloxytri(dimethylamine) phosphonium hexafluorophosphate (BOP) (132 mg, 0.30 mmol) was added and the mixture was stirred under N_2 at room temperature for 3.5 h. CH_2Cl_2 (20 mL) was then added and the solution was washed with 2N HCl (1×25 mL), H_2O (1×25 mL), 5% sat. $NaHCO_3$ (1×25 mL) and brine (1×25 mL). It was then dried with anhydrous $MgSO_4$ and the solvent was then removed in vacuo. The product was purified with silica flash column chromatography eluting with CH_2Cl_2/MeOH (9:1) yielding **15** (118 mg, 93%) as a clear glass. $[\alpha]^{20}_D$=−15 (c 0.89, MeOH) (lit.[28] $[\alpha]^{25}_D$=−15.3 (c 1.0, MeOH)); IR (smear) ν_{max} 3406, 3326, 2978, 1739, 1696, 1662, 1492, 1367, 1165, 742 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94, 7.90 (2H, each s, H-1, H-14), 7.66–7.11 (8H, series of doublets and triplets, H-4, H-5, H-6, H-7, H-17, H-18, H-19, H-20), 6.90, 6.59 (2H, each s, H-2, H-15), 6.24 (1H, bs, H-11), 5.09 (1H, bs, N*H*CO_2), 4.81 (1H, m, H-12), 4.43 (1H, bm, H-9), 3.59 (3H, s, CO_2CH_3), 3.29 (1H, bm, H-8), 3.20–3.10 (2H, m, H-13), 3.09 (1H, m, H-8), 1.38 (9H, s, OC(CH_3)$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.8, 171.2, 155.4, 136.2, 136.0, 127.6, 127.4, 123.5, 122.9, 122.2, 119.8, 119.6, 119.0, 118.4, 111.2, 111.1, 109.6, 80.0, 55.1, 52.7, 52.3, 28.2, 27.6, (plus three carbon resonances either obscured or not observed); FABMS m/z 505 $[M+H]^+$; HRFABMS m/z 505.2438 (calcd for $C_{28}H_{33}N_4O_5$ 505.2451).

3.4.5. L-Tryptophyl-L-tryptophan-methyl ester (16). To a solution of **15** (100 mg, 0.20 mmol) in CH_2Cl_2 (9 mL), TFA (1 mL) was added. The mixture was stirred at room temperature under N_2 for 1.5 h. The solvent was then removed in vacuo. The resulting product was dissolved in CH_2Cl_2 (30 mL), washed with 5% sat. $NaHCO_3$ (1×25 mL), H_2O (1×25 mL) and brine (1×25 mL) then dried with anhydrous $MgSO_4$. The solvent was removed in vacuo yielding **16** (78 mg, 98%) as a clear glass. $[\alpha]^{25}_D$=−11 (c 0.75, MeOH); IR (smear) ν_{max} 3400, 2922, 1738, 1654, 1516, 1436, 1221, 743 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03, 7.96 (2H, each s, H-1, H-14), 7.75 (1H, d, J=8.5 Hz, H-11), 7.62–6.97 (8H, series of doublets and triplets, H-4, H-5, H-6, H-7, H-17, H-18, H-19, H-20), 6.87, 6.73 (2H, each d, J=2.3 Hz, H-2, H-15), 4.94 (1H, m, H-12), 3.70 (1H, m, H-9), 3.67 (3H, s, CO_2CH_3), 3.21, 3.20 (2H, each dd, obsc, H-13), 3.19, 2.92 (2H, each dd, obsc, H-8), (NH_2 not observed); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 172.4, 136.3, 136.1, 127.7, 127.6, 123.2, 122.7, 122.2, 122.1, 119.6, 119.5, 119.0, 118.7, 111.3, 111.2, 110.2, 55.3, 52.4, 52.3, 30.2, 27.8, (plus one carbon resonance either obscured or not observed); FABMS m/z 405 $[M+H]^+$; HRFABMS m/z 405.1930 (calcd for $C_{23}H_{25}N_4O_3$ 405.1927).

3.4.6. L-Tryptophyl-L-tryptophan (17). To a solution of **16** (72 mg, 0.18 mmol) in MeOH (2 mL), KOH (0.56 g, 10 mmol) in H_2O (3 mL) was added. The mixture was stirred at room temperature for 5 h. The resulting mixture was neutralised with 2N HCl and the solvent was removed in vacuo. The product was purified with C_{18} flash column chromatography by eluting with H_2O and then with MeOH/H_2O (40:60) yielding **17** (55 mg, 80%) as a white amorphous solid. Mp 183–186°C (lit.[29] 186°C); $[\alpha]^{20}_D$=−19 (c 1.0, MeOH) (lit.[29] $[\alpha]^{25}_D$=−11.0 (c 0.426, EtOH)); IR (smear) ν_{max} 3400, 1638, 1590, 1523, 1396, 1093 cm^{-1}; ^{1}H NMR ((CD_3)$_2$SO, 400 MHz) δ 10.97, 10.80 (2H, each s, H-1, H-14), 8.04 (1H, d, J=7.2 Hz, H-11), 7.55–6.81 (8H, series of doublets and triplets, H-4, H-5, H-6, H-7, H-17, H-18, H-19, H-20), 7.14, 7.03 (2H, each d, J=2.1, 2.5 Hz resp, H-2, H-15), 4.31 (1H, m, H-12), 3.43 (1H, dd, J=8.8, 4.0 Hz, H-9), 3.15, 3.12 (2H, each dd, obsc, H-13), 3.10 (1H, dd, obsc, H-8), 2.63 (1H, dd, J=14.4, 9.0 Hz, H-8), (NH_2 and CO_2H not observed); ^{13}C NMR ((CD_3)$_2$SO, 100 MHz) δ 173.9, 173.0, 136.2, 135.8, 128.0, 127.4, 123.8, 123.4, 120.8, 120.3, 118.7, 118.3, 118.1, 117.7, 111.3, 110.9, 110.8, 110.5, 55.1, 54.1, 30.4, 27.6; FABMS m/z 391 $[M+H]^+$; HRFABMS m/z 391.1756 (calcd for $C_{22}H_{23}N_4O_3$ 391.1770).

3.4.7. *N*,*N*,*N*-Trimethylammonium-L-tryptophyl-L-tryptophan inner salt (9). To a solution of **17** (36 mg, 0.09 mmol) in MeOH (5 mL), MeI (120 μL, 1.9 mmol) and $KHCO_3$ (80 mg, 0.80 mmol) were added. The mixture was then stirred at room temperature for 2.5 d. Two drops of

H_2O were added and the solvent was removed in vacuo. Purification was achieved using C_{18} flash column chromatography. After eluting with H_2O and then MeOH/H_2O (40:60), the product **9** (31 mg, 78%) was eluted with MeOH/H_2O (50:50). The solvent was removed in vacuo yielding a white amorphous solid. Mp 188–192°C; $[\alpha]^{20}_D$=−6 (*c* 0.69, MeOH/H_2O (1:1)); UV (MeOH) λ_{max} (log ε) 220 (4.72), 274 (3.95), 281 (3.97), 290 (3.90) nm; IR (smear) ν_{max} 3043, 1668, 1617, 1548, 1202, 1131, 745 cm^{-1}; ^1H NMR (($CD_3)_2SO$, 400 MHz) δ 11.26 (1H, bs, H-1), 10.83 (1H, bs, H-14), 8.40 (1H, bs, H-11), 7.55 (1H, d, *J*=7.9 Hz, H-4), 7.51 (1H, d, *J*=7.9 Hz, H-17), 7.34 (1H, d, *J*=7.9 Hz, H-7), 7.27 (1H, d, *J*=8.0 Hz, H-20), 7.21 (1H, bs, H-2), 7.11 (1H, bs, H-15), 7.06 (1H, t, *J*=7.7 Hz, H-6), 7.00 (1H, t, *J*=7.2 Hz, H-5), 7.00 (1H, t, *J*=7.2 Hz, H-19), 6.91 (1H, t, *J*=7.4 Hz, H-18), 4.49 (1H, bm, H-9), 4.08 (1H, bm, H-12), 3.34 (1H, obsc, H-13), 3.31 (1H, obsc, H-8), 3.23 (1H, dd, obsc, H-8), 3.08 (1H, dd, *J*=14.8, 5.8 Hz, H-13), 2.88 (9H, s, $^+N(CH_3)_3$); ^{13}C NMR (($CD_3)_2SO$, 100 MHz) δ 171.7 (CO_2^-), 163.8 (C-10), 136.0 (C-7a), 135.7 (C-20a), 127.8 (C-16a), 126.7 (C-3a), 124.0 (C-2), 123.3 (C-15), 120.9 (C-6), 120.3 (C-19), 118.3 (C-17), 118.2 (C-5), 117.9 (C-4), 117.7 (C-18), 111.5 (C-7), 111.1 (C-16), 110.9 (C-20), 106.6 (C-3), 73.1 (C-9), 55.5 (C-12), 51.0 ($^+N(CH_3)_3$), 27.1 (C-13), 21.6 (C-8); FABMS *m/z* 433 $[M+H]^+$; HRFABMS *m/z* 433.2253 (calcd for $C_{25}H_{29}N_4O_3$ 433.2240).

3.4.8. *N*-*t*-Butyloxycarbonyl-L-tryptophyl-D-tryptophan-methyl ester (19). Compounds **13** (100 mg, 0.39 mmol) and **12** (119 mg, 0.39 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and dry Et_3N (136 μL, 0.98 mmol). To this, BOP (190 mg, 0.43 mmol) was added and the mixture was stirred under N_2 at room temperature for 4.5 h. CH_2Cl_2 (20 mL) was then added and the solution was washed with 2N HCl (1×25 mL), H_2O (1×25 mL), 5% sat. $NaHCO_3$ (1×25 mL) and brine (1×25 mL). It was then dried with anhydrous $MgSO_4$ and the solvent was removed in vacuo. The product was purified with silica flash column chromatography eluting with CH_2Cl_2/MeOH (95:5) yielding **19** (194 mg, 98%) as a clear glass. $[\alpha]^{20}_D$=+3 (*c* 1.0, MeOH); IR (smear) ν_{max} 3333, 2928, 1704, 1666, 1502, 1366, 1166, 742 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.06 (2H, bs, H-1, H-14), 7.64–7.02 (8H, series of doublets and triplets, H-4, H-5, H-6, H-7, H-17, H-18, H-19, H-20), 6.77, 6.41 (2H, each s, H-2, H-15), 6.24 (1H, d, *J*=7.7 Hz, H-11), 5.18 (1H, bs, N*H*CO_2), 4.76 (1H, bm, H-12), 4.43 (1H, bm, H-9), 3.56 (3H, s, CO_2CH_3), 3.20 (1H, bm, H-8), 3.14–3.09 (2H, m, H-8, H-13), 2.92 (1H, m, H-13), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.0, 171.4, 155.4, 136.14, 136.07, 127.5, 127.2, 123.2, 122.9, 122.1, 119.7, 119.5, 118.9, 118.4, 111.2, 110.5, 109.3, 80.0, 55.2, 52.4, 52.3, 28.6, 28.3, 27.3, (plus two carbon resonances either obscured or not observed); FABMS *m/z* 505 $[M+H]^+$; HRFABMS *m/z* 505.2444 (calcd for $C_{28}H_{33}N_4O_5$ 505.2451).

3.4.9. L-Tryptophyl-D-tryptophan-methyl ester (20). To a solution of **19** (170 mg, 0.34 mmol) in CH_2Cl_2 (9 mL), TFA (1 mL) was added. The mixture was then stirred at room temperature under N_2 for 1.5 h. The solvent was then removed in vacuo. The resulting product was dissolved in CH_2Cl_2 (20 mL), washed with 5% sat. $NaHCO_3$ (1×25 mL), H_2O (1×25 mL) and brine (1×25 mL) then dried with anhydrous $MgSO_4$. The solvent was the removed in vacuo yielding **20** (131 mg, 96%) as a clear glass. $[\alpha]^{20}_D$=+15 (*c* 1.0, MeOH); IR (smear) ν_{max} 3401, 2924, 1738, 1659, 1515, 1435, 1342, 1221, 743 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.16, 8.11 (2H, each s, H-1, H-14), 7.61 (1H, d, *J*=7.9 Hz, H-11), 7.62–7.04 (8H, series of doublets and triplets, H-4, H-5, H-6, H-7, H-17, H-18, H-19, H-20), 6.93, 6.84 (2H, each d, *J*=2.2, 2.4 Hz resp, H-2, H-15), 4.92 (1H, m, H-12), 3.66 (3H, s, CO_2CH_3), 3.64 (1H, m, H-9), 3.32 (1H, dd, *J*=14.6, 4.5 Hz, H-8), 3.27 (2H, d, *J*=5.7 Hz, H-13), 2.84 (1H, dd, *J*=14.6, 9.1 Hz, H-8), (NH_2 not observed); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.6, 172.4, 136.4, 136.1, 127.6, 127.4, 123.1, 122.7, 122.17, 122.15, 119.6, 119.5, 118.9, 118.6, 111.7, 111.24, 111.20, 110.2, 55.6, 52.6, 52.3, 30.6, 27.6; FABMS *m/z* 405 $[M+H]^+$; HRFABMS *m/z* 405.1935 (calcd for $C_{23}H_{25}N_4O_3$ 405.1927).

3.4.10. L-Tryptophyl-D-tryptophan (21). To a solution of **20** (121 mg, 0.30 mmol) in MeOH (2 mL), KOH (0.56 g, 10 mmol) in H_2O (3 mL) was added. The mixture was stirred at room temperature for 5 h. The resulting mixture was neutralised with 2N HCl, and the solvent was removed in vacuo. The product was purified with C_{18} flash column chromatography by eluting with H_2O and then with MeOH/H_2O (40:60) to yield **21** (85 mg, 73%) as a white amorphous solid. Mp 184–188°C; $[\alpha]^{20}_D$=+43 (*c* 0.89, MeOH); IR (smear) ν_{max} 3400, 3050, 1729, 1673, 1457, 1338, 1230, 743 cm^{-1}; ^1H NMR (($CD_3)_2SO$, 400 MHz) δ 11.01, 10.92 (2H, each d, *J*=1.9, 1.8 Hz resp, H-1, H-14), 8.94 (1H, d, *J*=8.0 Hz, H-11), 7.58–6.79 (8H, series of doublets and triplets, H-4, H-5, H-6, H-7, H-17, H-18, H-19, H-20), 7.15, 7.10 (2H, each d, *J*=2.3 Hz, H-2, H-15), 4.52 (1H, m, H-12), 4.01 (1H, dd, *J*=8.3, 5.2 Hz, H-9), 3.15 (1H, dd, *J*=14.4, 5.2 Hz, H-13), 3.07 (1H, dd, *J*=14.7, 5.1 Hz, H-8), 3.01 (1H, dd, *J*=14.4, 8.5 Hz, H-13), 2.84 (1H, dd, *J*=14.6, 8.3 Hz, H-8), (NH_2 and CO_2H not observed); ^{13}C NMR (($CD_3)_2SO$, 100 MHz) δ 173.0, 168.7, 136.2, 136.0, 127.1, 127.0, 124.8, 123.8, 120.9, 120.8, 118.5, 118.3, 118.2, 118.1, 111.3, 111.2, 109.4, 106.9, 53.3, 52.5, 27.5, 27.3; FABMS *m/z* 391 $[M+H]^+$; HRFABMS *m/z* 391.1785 (calcd for $C_{22}H_{23}N_4O_3$ 391.1770).

3.4.11. *N*-,*N*-,*N*-Trimethylammonium-L-tryptophyl-D-tryptophan inner salt (10). To a solution of **21** (40 mg, 0.10 mmol) in MeOH (5 mL), MeI (128 μL, 2.0 mmol) and $KHCO_3$ (102 mg, 1.02 mmol) were added. The mixture was then stirred at room temperature for 2 d. Two drops of H_2O were added and the solvent was removed in vacuo. Purification was achieved using C_{18} flash chromatography. After eluting with H_2O and then MeOH/H_2O (40:60), the product **10** (33 mg, 75%) was eluted with MeOH/H_2O (50:50). The solvent was then removed in vacuo yielding a white amorphous solid. Mp 198–203°C; $[\alpha]^{20}_D$=+52 (*c* 0.48, MeOH/H_2O (1:1)); UV (MeOH) λ_{max} (log ε) 219 (4.72), 273 (3.96), 281 (3.99), 290 (3.91) nm; IR (smear) ν_{max} 3246, 1671, 1596, 1454, 1385, 1100, 742 cm^{-1}; ^1H NMR (($CD_3)_2SO$, 400 MHz) δ 11.55 (1H, bs, H-1), 11.02 (1H, bs, H-14), 8.42 (1H, d, *J*=7.8 Hz, H-11), 7.57 (1H, d, *J*=7.9 Hz, H-4), 7.43 (1H, d, *J*=8.1 Hz, H-7), 7.38 (1H, d, *J*=7.9 Hz, H-17), 7.29 (1H, d, *J*=8.1 Hz, H-20), 7.08 (1H, bs, H-2), 7.06 (1H, td, *J*=8.1, 0.8 Hz, H-6), 6.99 (1H, t, *J*=7.3 Hz, H-5), 6.95 (1H, t, *J*=7.3 Hz, H-19), 6.83 (1H, t, *J*=7.4 Hz, H-18), 6.46 (1H, bs, H-15), 4.55 (1H, dd, *J*=10.3,

3.0 Hz, H-9), 4.21 (1H, m, H-12), 3.36, 3.35 (2H, each obsc, H-8), 3.27 (9H, s, $^{+}N(CH_3)_3$), 2.99 (1H, dd, J=15.0, 4.4 Hz, H-13), 2.73 (1H, dd, J=15.0, 8.2 Hz, H-13); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 173.0 (CO_2^-), 164.3 (C-10), 136.2 (C-7a), 135.9 (C-20a), 127.4 (C-16a), 126.6 (C-3a), 124.1 (C-2), 122.7 (C-15), 120.9 (C-6), 120.2 (C-19), 118.3 (C-5), 118.2 (C-17), 118.1 (C-4), 117.7 (C-18), 111.8 (C-7), 111.4 (C-16), 111.1 (C-20), 106.6 (C-3), 73.2 (C-9), 56.1 (C-12), 51.7 ($^{+}N(CH_3)_3$), 27.6 (C-13), 21.6 (C-8); FABMS m/z 433 $[M+H]^+$; HRFABMS m/z 433.2235 (calcd for $C_{25}H_{29}N_4O_3$ 433.2240).

Acknowledgements

We would like to thank the Auckland Medical Research Foundation and the University of Auckland Research Committee for funding, the University of Auckland for the award of a Doctoral Scholarship to DRA, Mrs Gill Ellis for cytotoxicity and antimicrobial assays and Dr V. Narayanan (NCI, Bethesda) for testing of compounds against the NCI human tumour cell line panel. Antimycobacterial testing was conducted by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) through a research and development contract with the US National Institute of Allergy and Infectious Diseases. We are also extremely grateful to Professor Roberto Berlinck of the Universidade de Sao Paulo for obtaining circular-dichroism data that was crucial to the success of this project. We would also like to thank Dr Mary Sewell (School of Biological Sciences, the University of Auckland) for identifying the sea hare and Nick Shears (Leigh Marine Laboratory, the University of Auckland) for collection and identification of *L. distichophylla*.

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