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# Further Tolyporphins from the Blue-Green Alga Tolypothrix nodosa.

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Abstract: Eight new porphinoids, tolyporphins B-I (2-9), along with the known tolyporphin A (1) have been isolated from a lipophilic extract of the Pacific cyanophyte, *Tolypothrix nodosa*. Their structures have been elucidated using 1- and 2-D NMR spectroscopic experiments and the tolyporphins showed varying activities in drug accumulation and competitive binding assays for multidrug resistance (MDR) reversal

As part of our ongoing search for new anticancer agents from microalgae, we investigated the lipophilic extract of *Tolypothrix nodosa* Bharadwaja (UH strain HT-58-2), a cyanophyte isolated from a soil sample collected in Pohnpei. We have already reported the isolation and structural elucidation of tolyporphin (1), hereafter known as tolyporphin A, from this source. The substitution pattern of tolyporphin A was unique among naturally occurring porphinoids and tolyporphin A reversed multidrug resistance (MDR) in a vinblastine-resistant subline of a human ovarian adenocarcinoma line. We now report the isolation and structural elucidation of eight additional tolyporphins B-I (2-9).

An extract (CH<sub>2</sub>Cl<sub>2</sub>/2-propanol, 1:1) of the cultured alga was subjected to repeated reversed phase (C18) and normal phase (silica gel) flash column chromatography to give tolyporphin A (1), yield 0.1 % and eight additional compounds, tolyporphins B-I (2-9) that were clearly structurally related to tolyporphin A (1), in yields of 0.032, 0.008, 0.005, 0.049, 0.013, 0.004, 0.002 and 0.002 % respectively. Structures of the new tolyporphins were determined by comparison of their <sup>1</sup>H and <sup>13</sup>C NMR spectra with those of tolyporphin A (1) and by extensive use of 2-D NMR techniques such as COSY, HMOC<sup>2</sup> and HMBC.<sup>3</sup>

Pairs of tolyporphins B and C (2-3) and G and H (7-8) could not be separated chromatographically and appeared as one spot on tlc and one peak in hplc in a variety of solvent systems. The signals in the <sup>1</sup>H and <sup>13</sup>C NMR spectra of the mixtures (Tables 1 and 2) were sufficiently resolved however to permit signal assignment.

The <sup>1</sup>H NMR spectrum of combined tolyporphins B and C (2-3) revealed a 4:1 mixture in favour of tolyporphin B (2). The spectrum resembled that of tolyporphin A (1), except that both tolyporphins B and C exhibited only one acetate methyl resonance (at 0.71 and 0.36 ppm respectively). Similarly to the spectrum of 1, these signals are considerably upfield from those expected for acetate methyl protons, due to their projection over the porphinoid ring system. The <sup>13</sup>C NMR spectrum of the mixture of 2 and 3 showed 38 signals for each, with one carbonyl resonance per structure. This data indicated that 2 and 3 were identical to 1 in most structural features but that each lacked an acetate on one C-glycosyl ring. Tolyporphin B (2) lacked the acetate in the C17 substituent while tolyporphin C (3) lacked the acetate in the C7 substituent. High resolution positive ion FAB mass spectrometry confirmed the structures with a molecular formula for each compound of  $C_{38}H_{44}N_4O_9$  with m/z 700.3133,  $\Delta$  2.5 mmu.

The  $^1H$  NMR spectrum of tolyporphin D (4) exhibited similar resonances to the other compounds but lacked acetate methyl resonances entirely. The  $^{13}C$  NMR spectrum contained 36 signals and high resolution positive ion FAB mass spectrometry confirmed a molecular formula of  $C_{36}H_{42}N_4O_8$  with m/z 658.3036,  $\Delta$  3.4 mmu. Thus tolyporphin D (4) was a tetrahydroxy analogue of tolyporphin A (1).

 $^{1}$ H and  $^{13}$ C NMR spectra of tolyporphins E and F (5-6) indicated that each contained only one attached C-glycosyl ring. The  $^{13}$ C NMR spectrum of each compound lacked one of the characteristic quaternary carbon signals at ~ 56 ppm and contained instead quaternary carbon signals at 80.79 and 77.59 ppm respectively, indicative of attached oxygen atoms. A quaternary carbon signal in the  $^{13}$ C NMR spectrum of tolyporphin E (5) at 167.99 ppm and a methyl carbon signal at 20.30 ppm that were correlated to a  $^{1}$ H NMR signal at 2.27 ppm by HMQC and HMBC experiments respectively, indicated that an acetate was the functional group replacing one of the C-glycosyl rings. HREIMS confirmed a molecular formula of  $^{13}$ C NMR spectrum of tolyporphin F (6) contained no additional signals and a molecular formula of  $^{13}$ C NMR spectrum of tolyporphin F (6) contained by high resolution positive ion FAB mass spectrometry, indicating that a hydroxyl group replaced a C-glycosyl unit in this case.

The <sup>1</sup>H NMR spectrum of combined tolyporphins G and H (7-8) revealed a 2:1 mixture in favour of tolyporphin G (7). The spectrum was somewhat simpler than that of the other tolyporphins, with no shielded acetate methyl signals or C-glycosyl ring heteroatomic proton signals. The <sup>13</sup>C NMR spectrum contained twenty six resonances for each compound and the chemical shifts indicated that the C7 and C17 substituents were an acetate and an hydroxyl group respectively for tolyporphin G (7), and vice versa for tolyporphin H

(8). High resolution positive ion FAB mass spectrometry of the mixture confirmed a molecular formula for each of  $C_{26}H_{24}N_4O_5$  with m/z 472.1736,  $\Delta$  2.3 mmu.

NMR spectra of tolyporphin I (9) indicated that the only structural difference between 9 and combined 7-8 was an additional acetate functionality. A molecular formula of  $C_{28}H_{26}N_4O_6$  with m/z 514.1848,  $\Delta$  0.8 mmu was confirmed for tolyporphin I (9) by high resolution positive ion FAB mass spectrometry.

Complete spectral assignment of all <sup>1</sup>H and <sup>13</sup>C NMR spectra of tolyporphins B-I (2-9) was performed using COSY, HMBC and HMQC 2D-NMR techniques (See Tables 1 and 2). Coupling constants could only be determined for those signals which were sufficiently resolved and are given if determined in Table 1.

Assays designed to detect potential MDR reversing compounds, namely a [ $^3$ H] azidopine photolabelling assay and a [ $^3$ H] vinblastine accumulation assay, were performed by methods that we have described elsewhere. The [ $^3$ H] azidopine photolabelling assay indicates the abilities of compounds to directly bind to P-glycoprotein, a transmembrane protein that acts as an energy-dependent drug efflux pump which actively removes a variety of important anticancer drugs from cells. It is a competitive binding assay where test compound competes with azidopine for binding to P-glycoprotein in membranes isolated from MCF-7/ADR cells. In these experiments, tolyporphins were tested at 10  $\mu$ g/ml and compared with 10  $\mu$ M verapamil, a P-glycoprotein antagonist.

Tolyporphin D (4) and a mixture of tolyporphins G and H (7-8) competed more efficiently for the azidopine binding site on P-glycoprotein than did verapamil, while tolyporphins A-C (1-3) and F (6) competed less well and tolyporphins E (5) and I (9) competed very poorly for the binding site. The ability of a compound to increase the extent of  $[^3H]$  vinblastine accumulation by MCF-7/ADR cells indicates its effectiveness in antagonizing drug transport by P-glycoprotein, which is a good indication of its potential utility as a clinical MDR reversing agent. In this assay, tolyporphin F (6) and a mixture of tolyporphins G and H (7-8) exhibited the greatest effects on vinblastine accumulation, producing approximately 5-fold increases at 0.5  $\mu$ g/ml. Therefore, these compounds are approximately 10-fold more potent for antagonism of P-glycoprotein than is verapamil.

Tolyporphins A (1), E (5) and I (9) had intermediate effects on vinblastine accumulation (3- to 6-fold increases at  $10 \mu g/ml$ ), while tolyporphins B-D (2-4) were inactive up to at least  $10 \mu g/ml$ . The discrepancies in the efficacies of the different tolyporphins in the azidopine photolabelling assay and the [ $^3H$ ] vinblastine accumulation assay may be due to differences in the permeabilities of the compounds, differential metabolism of the compounds or interactions with different drug binding sites on P-glycoprotein. All of the tolyporphins demonstrated quite high cytotoxicity (50 % inhibition of cell proliferation at <0.1  $\mu g/ml$ ), which precluded demonstration of MDR reversal using the standard cytotoxicity assay. However, since the cytotoxicities of these compounds are light-dependent, they may demonstrate useful MDR reversing activity *in vivo*.

Table 1: <sup>1</sup>H NMR Data (ppm in CDCl<sub>3</sub>) for Tolyporphins B-I (2-9).

Tolyporphin I		× × × ×	9.70	9.66	8.92	9.13	80.6	3.50	,	-11.7	3.38	2.134	-2.80 br	-2.80 br	2.23											,	2.25		•	:.≊ O-	0\	= 1	->>	, oğo	in No.	B	Z Aco	~~ ~	₹
Tolyporphin C Tolyporphin D Tolyporphin E Tolyporphin F Tolyporphin G Tolyporphin H $(3)$ $(4)$ $(5)$ $(6)$		8.80	2.50	80.0	8.76	90.6	09.6	3.49	200	7.07	0.40	50.7	opscnred	opscnred												!	2.27	;	<b>Me</b> 23	2 2 2		ER.		115	7	N N		20 19 17 Me	=0
Tolyporphin (7)		0.04	25.0	C/.6	8.80	9.23	9.50	3.56	2 12	3 22	200	2.00	-2.86	-2.81	7.78													•	o= ឧ;	Me	×	\ \ \ E		<b>»</b> s		₩.	Zi We 🕆	<u>)</u> "	
Tolyporphin F (6)	70 0	0 33	66.0	0.04	0.00	9.42	9.56	3.57	2.13	3 20	2.27	2,008	-3.00	-7.90													4.46 d (10.0) 4.10 ddd						3.78 hr			(6.2.1.3)			
Tolyporphin E (5)	00	9.15	07.0	8 87	0.07	5.0	\$	3.57	2.19	3.564	211	7 02b hr	2 0 10 L	10 - 16.7-	77.7											4 50 d (0 0)	4.18 ddd	(9 9 10 7 4 9)	2.03 ddd	(13.2.10.7.4.9)	1 \$7 ddd	(13 2 2 0 3 2)	3.73 br	(2008)	3.98 da	(6.6,0.8)	0.73	1.63 d (6.6)	
Tolyporphin D (4)	8.71	9.50	99.6	8.75	9,0	0.4.0	24.5	3.43	2.06	3.47			.2 98a br		2.68b hr		1.40 hr	5	1.21 br		3.35 br	3.71 br			1.49° d (6.3)	3.94 hr	2.85 <sup>b</sup> br		1.40 br		1.21 br	;	3.35 br		3.71 br			1.25° d (6.3)	e interchanged
Tolyporphin C (3)	8.84	9.41	9.59	8.69	9.41	65.0	10.0	3.43	1.94	3.18	5.04	-3.09 br	-2.97 br	3.92 d (10.4)	2.32 hr		0.68 br		0.92 br		2.81 br	3.45 br			1.33 d (6.2)	4.45 d (9.5)	4.19 ddd	(9.5, 10.8, 4.6)	1.94	opscured	1.49 br d	(11.2)	3.66 br		3.90 br d	(9.9)	0.36	1.60 d (6.6)	he same superscript may be interchanged
Proton Tolyporphin B (2)	8.10	9.48	9.64	8.77	9.28	9.37	200	7.7	40.7	3.50	1.97	-3.12 br	-3.03 br	4.42 d (9.9)	4.01 ddd	(9.9, 4.9, 10.7)	1.88 ddd	(12.8, 10.7, 3.3)	1.44 dd	(12.8,4.9)	3.62 br	3.85 br q	(6.2)	0.71	1.57 d (6.2)	3.99 d (9.5)	2.51 br m		0.68 br m		0.92 br m		2.95	opscnred	3.54 br q	(6.2)		1.37 d (6.2)	column with the san
Proton	7	<b>S</b>	10	13	1.5	2.0	7.		7 (	2.3	2.4	H H	7	R1 1	7,		3,					Š		5		R2 1'	. 7		3,				. 4		5.	7	7	:	Values within a column with t

Values within a column with the same superscript may be interchanged.

Table 2: <sup>13</sup>C NMR Data (ppm in CDCl<sub>3</sub>) for Tolyporphins B-I (2-9).

13.89   13.49   13.54   13.5	4.6	Tolundar D	Tolumbin	C Telementin	D Telementhia	T. T. Innertal to	Tolundaria Company	T.lungumbin U	Tolunounhin
134.99   135.59   135.12   135.88   135.39   136.53   136.64   1	-	01yporpun p (2)	1 of y por purit. (3)	(4)	oryporpum (5)	E tonyporpum r (6)	(7)	(8)	(6)
1490   125.18   125.60   125.97     146.60   136.61*   136.94*   137.46   125.60     156.18   136.61*   135.71*   136.58   135.82*     156.18   136.61*   135.71*   136.58   135.82*     157.75   136.33   136.49   159.94   156.63     157.75   136.33   136.49   156.63     145.65*   145.93*   143.55   145.85   143.39*     145.65*   145.93*   143.55   146.85   143.39*     145.65*   145.93*   143.55   146.85   143.39*     156.81   136.32   137.49*   135.65   137.49*     156.81   136.32   137.49*   137.45*     156.81   136.32   137.49*   137.45*     156.33   136.32   137.49*   137.45*     156.33   136.32   137.49*   137.45*     156.34   135.74   137.49*   137.45*     156.35   136.32   137.49*   137.45*     156.31   161.33   161.03   137.44     156.32   136.32   146.04   137.74     156.33   146.94*   146.04     156.34   13.27   13.27   13.39*     156.35   12.86   13.38*     156.36   13.38*   146.89     156.46   40.16*   13.38*     157.48   13.34*   13.34*     157.49   16.73   14.73*     157.40   16.73   14.73*     157.41   13.70   13.34*     157.42   13.34*   14.83*     157.44   13.70   17.04     168.13   16.75   13.34*     168.14   16.75   13.34*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   14.73*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   17.01   17.06     168.19   17.01   17.01     17.06   17.01   17.01     17.06   17.01   17.01     17.06   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.75   16.90*     168.19   16.70   16.90*     168.19   16.70   16.90*     168.19   16.70   16.90*     168.19   16.70   16.90*     168.19   16.70   16.90*     168.10   16.70   16.90*     168.10   16.70   16.9		135.89	134.99	135.59	135.12	135.88	135.39	136.53	137.02
156.60   136.04*   136.94*   137.45   136.94*   137.72*   136.14		124.86	124.90	125.18	125.26	125.46	125.60	125.97	124.74
156.18   136.64   135.71   136.58   135.82   136.94   136.18   136.18   136.18   136.18   136.18   136.18   136.18   136.18   136.18   136.18   136.18   136.24   136.25   160.35   160.35   160.35   160.35   160.35   160.35   160.35   160.35   144.76   145.55   145.36   145.36   145.36   145.36   145.36   145.37   136.27   136.38   136.39   136.38   136.39   1		$136.84^{a}$	136.60	$136.06^{a}$	136.94ª	137.45	136.95	$137.72^{a}$	136.03 <sup>a</sup>
97.51         97.05         93.43         94.87         93.82         95.06           160.55         160.83         159.44         189.82         160.43           55.70         25.92         80.87         77.59         480.83         77.85           207.06         202.36         202.36         202.19         207.64         447.76           145.68         145.93         143.59         144.76         144.76         144.76           145.64         145.93         145.93         144.76         144.76         144.76           13.46         135.57         136.32         137.82         137.14         135.27           13.5         136.32         137.82         137.44         136.22         137.44         136.22           13.5         144.86         135.57         137.63         137.44         136.29         137.44         136.29         137.44         136.29         137.44         136.29         137.44         136.29         137.44         136.29         137.44         136.29         137.44         136.29         146.61         146.31         146.31         147.33         147.33         147.33         147.34         147.33         147.33         147.23         147.23		136.38ª	136.18	136.61 <sup>a</sup>	135.71a	136.58	135.82ª	136.94*	137.578
160.95   160.83   156.49   159.94   156.63   160.43     160.95   160.83   156.49   159.94   156.63   160.43     145.64   145.93   143.55   143.55   145.85   143.39   144.76     145.64   145.93   143.55   146.85   143.39   144.76     145.64   145.93   143.55   146.85   136.75     135.94   135.55   136.39   136.76     135.94   135.55   136.39   136.76     136.31   124.86   137.49   135.65   137.14     155.23   136.32   137.49   137.14     155.33   136.32   137.18   137.19     150.91   161.33   161.05   161.01     150.91   161.33   161.05   161.01     150.91   166.91   146.91   146.49   142.24     146.33   146.91   146.49   146.49     13.04   13.27   13.27   13.36     13.04   13.10   13.38   13.42   13.39     15.04   16.75   12.34   13.37     16.05   16.05   13.37     17.00   16.75   16.79   13.42     17.00   16.75   14.70     16.75   14.70   14.70     17.00   16.75   14.70     16.71   14.70   14.70     16.72   14.70   14.70     16.73   14.70   14.70     16.74   14.70   14.70     16.75   14.70   14.70     16.75   14.70   14.70     16.77   14.70   14.70     16.70   14.70   14.70     16.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70   14.70     17.70   14.70     17.70   14.70     17.70   14.70     17.70   14.70     17.70   14.70		97.56	97.51	97.05	93.43	94.87	93.82	92.06	95.71 <sup>b</sup>
55.70         55.92         80.87         77.59         80.83         77.85           207.06         202.36         202.36         202.19         207.64           145.63         145.93         143.53         144.76           145.64         145.93         143.55         146.85         144.76           145.64         145.94         144.76         145.73         144.76           135.94         135.55         136.53         136.73         137.19         144.76           136.91         136.74         136.37         137.19         137.14         136.22         137.44         136.59           136.92         136.32         137.19         137.14         136.59         137.13         136.99         137.13         137.35         137.35         137.35         137.35         137.35         137.35         137.35         137.35         137.35         144.224         136.99         137.35 <td< td=""><th></th><td>160.20</td><td>160.95</td><td>160.83</td><td>156.49</td><td>159.94</td><td>156.63</td><td>160.43</td><td>158.26€</td></td<>		160.20	160.95	160.83	156.49	159.94	156.63	160.43	158.26€
107.06         207.06         207.06         207.06         207.06         207.06         207.06         207.06         207.06         207.06         207.06         207.06         207.06         44.75         94.05         95.38         95.38         95.62         95.38         95.78         144.76         95.30         95.38         95.67         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         135.27         137.44         135.27         137.44         135.27         137.44         136.69         95.89         137.14         95.89         137.14         95.89         137.14         95.89         144.44         146.69         146.04         146.04         146.04         146.04         146.49         146.04         146.49         146.04         146.49         146.04         146.49         146.04         146.49         146.49         146.04         146.49         146.04         146.49         146.04         13.27         13.39         13.39         13.39         13.39         13.39         13.39         13.39         13.39         13.39         13.39         13.39         13.39 <th></th> <td>55.84</td> <td>55.70</td> <td>55.92</td> <td>80.87</td> <td>77.59</td> <td>80.83</td> <td>77.85</td> <td>80.54</td>		55.84	55.70	55.92	80.87	77.59	80.83	77.85	80.54
145.65a         145.93b         143.55         146.85         143.39b         144.76b           195.64         94.55         95.30         95.30         95.38         195.62           135.94         135.55         136.78         136.78         135.57         135.27           135.94         135.57         136.38         136.78         137.14         135.52         137.14         136.72         137.14         136.73         137.14         136.73         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89         137.14         136.89 <t< td=""><th></th><td>206.36</td><td>207.06</td><td>207.06</td><td>202.36</td><td>207.67</td><td>202.19</td><td>207.64</td><td>202.19</td></t<>		206.36	207.06	207.06	202.36	207.67	202.19	207.64	202.19
94.65         94.75         95.30         95.38         95.62           135.94         135.54         136.78         136.78         136.79         133.27           136.81         136.74         136.74         137.29         137.14         137.27           136.81         136.47         125.34         125.22         137.14         137.14           125.23         137.08         137.08         137.44         95.89         137.44         136.69           160.91         161.33         161.33         161.05         161.01         137.44         95.89           160.91         161.33         161.33         161.05         161.01         157.35         206.14         95.89         99.72         97.144         95.89         206.54         206.23         207.14         167.34         167.35         167.34         167.35         167.34         113.37         1167.44         113.37         1167.34         <		144.65 <sup>b</sup>	145.65a	145.93b	143.55	146.85	143.39b	144.76b	143.41 <sup>d</sup>
135.94   135.55   136.53   136.74   135.57   135.27°   135.27°   135.23   136.32   136.32   136.32   137.42   137.42   137.82   137.42   137.82   137.14   137.42   137.42   137.82   137.14   136.32   136.32   137.42   137.22   124.46   136.32   137.34   137.14   136.53   137.14   137.14   136.69   160.91   161.33   161.33   161.01   157.35   161.01   157.35   160.91   161.33   164.91   146.91   146.94   146.49   146.49   146.49   146.49   146.49   146.49   146.49   13.27   13.27   13.27   13.27   13.27   13.27   13.27   13.36   13.37   12.87   13.37   12.87   13.37   13.36   167.99   20.40   20.40		94.13	94.65	94.75	95.30	95.30	95.38	95.62	97.27
136.81   136.74°   137.49°   135.65   137.82°   137.14°   136.35   125.22   124.46   136.35   125.22   124.46   136.35   136.34   135.22   124.46   136.35   136.35   136.35   136.32   137.19   137.19   137.19   137.19   137.19   137.19   137.15   136.59   137.22   137.15   146.31°   13.27°   13.37°   13.37°   13.37°   13.37°   13.37°   13.37°   13.37°   13.37°   13.37°   13.31°   167.39°   13.42°   13.34°   13.37°   146.31°   167.39°   169.70°   169.		$136.30^{\circ}$	135.94	135.55°	136.53 <sup>b</sup>	136.78	136.57°	135.27°	137.60°
125.23   124.86   125.57   125.24   125.22   124.46   136.59   99.59   99.7144   95.89   99.7134   156.69   99.59   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7144   95.89   99.7146   95.89   99.60.27   206.69   206.27   206.27   206.27   206.27   206.29   96.29   97.084   95.89   95.07   13.27   13.27   13.27   13.27   13.39   140.29   169.70   17.70   1		134.85°	136.81	$136.74^{\circ}$	137.49 <sup>b</sup>	135.65	137.82°	137.14°	137.31
136.35         136.32         137.08         137.19         137.44         136.69           160.91         19.35         197.19         137.44         136.69           160.91         161.33         161.33         161.01         157.35           160.91         161.33         161.33         161.01         157.35           55.72         55.67         55.67         55.67         157.35           160.94         206.99         226.27         206.22         207.46         202.11           146.34         146.91         146.04         146.49         147.39         96.89           95.67         13.27         13.27         13.27         13.27         13.27           12.87         13.27         13.27         13.39         13.37           20.70         20.49         20.68         20.71         20.38           12.87         13.27         13.27         13.34         13.37           20.59         20.49         20.68         20.71         20.34         21.86           88.22         40.16         65.25         20.30         24.58         24.68           17.00         16.75         83.71         83.78         67.88 <th></th> <td>124.96</td> <td>125.23</td> <td>124.86</td> <td>125.57</td> <td>125.34</td> <td>125.22</td> <td>124.46</td> <td>125.89</td>		124.96	125.23	124.86	125.57	125.34	125.22	124.46	125.89
99.25 98.84 99.59 99.72 97.14 <sup>d</sup> 95.89 160.91 161.33 161.35 161.05 161.01 157.35 55.72 55.67 57.2 56.64 77.63 80.65 206.46 206.99 206.27 206.22 207.46 202.11 146.33* 146.91* 146.04 146.49 143.73* 142.24* 14		136.66	136.35	136.32	137.08	137.19	137.44	136.69	136.15
160.91         161.33         161.05         161.01         157.35           25.72         55.67         55.72         25.64         77.63         80.65           206.46         206.99         206.27         206.29         207.46         202.11           146.33*         146.91*         146.04         146.49         143.73*         142.24*           15.04         13.27         13.27*         13.39*         142.24*           15.04         13.27         13.37*         13.39*         142.24*           20.70         20.66         21.84         21.93         20.28           20.71         13.34*         21.34         21.36         13.37           20.59         20.68         20.71         20.34         21.86           86.25         40.16*         20.30         20.71         20.34         21.86           88.33         167.99         20.71         20.34         21.86           68.93         69.16         76.34         24.58         24.58           76.16         76.34         37.02         67.58         24.68           83.43         40.44*         37.00         37.02         24.68           66.91 <td< td=""><th></th><td>99.38</td><td>99.25</td><td>98.84</td><td>99.59</td><td>99.72</td><td>97.14<sup>d</sup></td><td>68.86</td><td>93.69</td></td<>		99.38	99.25	98.84	99.59	99.72	97.14 <sup>d</sup>	68.86	93.69
55.72     55.67     55.72     55.64     77.63     80.65       206.46     206.49     266.27     206.22     207.46     202.11       146.33***     146.91b     146.49     146.49     142.24b     142.24b       95.67     96.01     96.23     95.92     97.084     96.89       13.04     13.27     13.27**     13.39**     13.23       12.87     13.10     13.38**     13.34**     13.37       20.50     20.49     20.48     20.193     20.28       86.22     86.33     167.99     20.71     20.34     21.86       86.93     69.16     65.25*     20.30     24.58     21.86       86.93     69.16     76.31     83.78     169.70       83.43     86.22     83.71     83.78     169.70       86.83     69.30     69.01     69.09     69.09       168.19     77.54     77.54     17.54       168.19     16.90     19.80       168.19     16.90     19.80       168.19     19.80     19.80		161.60	160.91	161.33	161.33	161.05	161.01	157.35	157.56°
206.46         206.99         206.27         207.46         202.11           146.33***         146.91b         146.04         146.49         143.73b         142.24b           95.67         96.01         96.23         97.08*         96.89           13.04         13.27*         13.27*         13.37*         13.38*           13.07         20.66         21.84         24.68         20.28           12.87         13.10         13.38*         13.34*         13.37*           20.70         20.49         20.68         20.71         20.34         20.28           86.22         86.33         167.99         20.71         169.74         21.86           86.25         65.25*         20.30         24.58         21.86           89.36         69.16         76.31         167.39         169.74           76.16         76.31         83.78         83.78         169.70           83.43         86.22         83.71         83.78         24.68           86.83         69.30         69.01         69.09         77.54           168.19         16.90         19.80         19.80           168.19         17.50         17.54		55.55	55.72	55.67	55.72	55.64	77.63	80.65	80.54
146.33**     146.91b     146.04     146.49     143.73b     142.24b       95.67     96.01     96.23     95.92     97.08d     96.89       13.04     13.27     13.77     13.39°     13.23       20.70     20.66     21.84     24.68     21.93       20.70     20.68     20.71     20.34     21.86       86.25     86.33     167.99     20.34     21.86       86.26     65.25d     20.30     24.58     21.86       86.93     69.16     20.30     24.58     169.70       76.16     76.31f     83.78     169.70       83.43     86.22     83.71     83.78     169.70       83.83     69.30     69.01     69.00     37.02     24.68       86.83     69.30     69.01     69.00     169.00       168.19     169.00     17.54     17.64       168.19     169.00     19.80       168.42     17.01     17.64		206.84	206.46	206.99	206.27	206.22	207.46	202.11	206.65
95.67         96.01         96.23         95.92         97.08 <sup>d</sup> 96.89           13.04         13.27         13.27         13.23         13.23         13.23           20.70         20.66         21.84         24.68         21.93         20.28           12.87         13.10         13.38 <sup>e</sup> 13.42 <sup>a</sup> 21.93         20.28           12.87         13.10         13.38 <sup>e</sup> 13.42 <sup>a</sup> 21.93         20.28           8.6.22         86.33         167.99         20.71         20.34         21.86           64.66         65.254         20.30         24.58         21.86           88.93         69.16         20.30         24.58         24.58           76.16         76.14         67.65         67.58         24.58           17.00b         16.75         83.71         83.78         169.70           83.43         40.41 <sup>e</sup> 37.00         37.02         24.68           66.91         67.91         67.65         67.58         24.68           88.43         40.41 <sup>e</sup> 37.00         37.02         24.68           88.83         69.30         69.01         69.09         67.58		147.53 <sup>b</sup>	146.33ª	146.91 <sup>b</sup>	146.04	146.49	143.73b	142.24b	144.46 <sup>d</sup>
13.04   13.27   13.27°   13.27°   13.23     13.04   13.27   13.27°   13.27°   13.23     12.87   20.66   21.84   24.68   21.93   20.28     12.87   13.10   13.38°   13.42°   13.34°   13.37     20.59   20.49   20.68   20.71   20.34   21.86     86.22   86.33   167.99   20.71   169.74     20.56   40.16°   20.30   24.58     39.56   40.16°   20.30   24.58     76.16   76.31°   26.34°   67.65   67.58     17.00°   16.75   67.58   67.58     36.81   40.41°   37.00   69.09     168.19   16.90   19.80     168.19   16.90     168.19   16.90     168.19   16.90     16.80   16.90     16.80   16.90     16.80   16.90     16.80   16.90     16.80   16.90     16.80   16.90     16.80   16.90     16.80   16.90     16.80   16.90     16.80   17.50   17.54     16.80   17.50   17.54     16.80   17.50   17.54     16.80   17.50   17.54     16.80   16.90     16.80   17.50   17.50     16.80   17.50   17.50     16.80   17.50   17.50     16.80   17.50   17.50     16.80   17.50   17.50     16.80   17.50   17.50     16.80   17.50   17.50     16.80     16.80   17.50     16.80   17.50     16.80   17.50     16.80   17.50     16.80     16.80   17.50     16.80   17.50     16.80   17.50     16.80     16.80   17.50     16.80     16.80   17.50     16.80     16.80     16.80   17.50     16.80		68.86	95.67	96.01	96.23	95.92	97.08 <sup>d</sup>	68.96	95.79b
20.70     20.66     21.84     24.68     21.93     20.28       20.70     13.38     13.42a     13.34     13.37       20.59     20.49     20.49     20.71     20.34     21.86       86.22     86.33     167.99     20.34     21.86       86.24     86.35     20.30     24.58     21.86       39.56     69.16     24.58     24.58       68.93     69.16     69.70     24.58       17.00b     16.75     83.71     83.78     169.70       83.43     86.22     83.71     83.78     169.70       67.91     65.44     67.65     67.58     24.68       36.81     69.30     69.01     69.09       77.43     76.59     77.54     17.54       168.19     169.00     19.80       16.84b     17.01     17.01     17.01		12.59	13.04	13.27	13.27°	13.27 <sup>a</sup>	13.39	13.23	13.44
12.87     13.10     13.38°     13.42³     13.34°     13.37       05.59     20.49     20.68     20.71     20.34     21.86       86.22     86.33     167.99     20.71     20.34     21.86       64.66     65.25⁴     20.30     24.58     21.86       39.56     40.16°     20.30     24.58     24.58       68.93     69.16     67.51     67.58     169.70       17.00b     16.75     83.71     83.78     169.70       83.43     86.22     83.71     83.78     169.70       67.91     65.44°     67.65     67.58     24.68       67.91     65.44°     67.65     67.58     24.68       88.83     69.30     69.01     69.09     77.54       168.19     77.43     77.54     17.60       168.19     17.60     17.60     17.60       16.84     17.01     17.01     17.60		20.59	20.70	20.66	21.84	24.68	21.93	20.28	21.848
20.59 20.49 20.68 20.71 20.34 21.86  86.22 86.33 167.99 169.74  64.66 65.25 <sup>4</sup> 20.30 169.74  39.56 40.16  68.93 69.16  17.00 <sup>b</sup> 16.75 83.71 83.78  67.91 65.44 67.65 67.58  68.83 69.30 69.01 69.09  168.19 168.19 169.00  168.19 169.00  168.19 169.00  168.19 169.00		13.21	12.87	13.10	13.38°	$13.42^{a}$	13.34e	13.37	13.391
86.22     86.33     167.99     169.74       64.66     65.254     20.30     24.58       39.56     40.16*     20.30     24.58       68.93     69.16     68.93     169.70       76.16     76.16     76.31     169.70       17.00b     16.75     83.71     83.78     169.70       83.43     86.22     83.71     83.78     24.68       67.91     65.44*     67.65     67.58     24.68       36.81     40.41*     37.00     37.02     24.68       68.83     69.30     69.01     69.09       168.19     77.50     77.54     169.00       168.19     169.00     19.80       16.84b     17.01     17.01     17.01		20.59	20.59	20.49	20.68	20.71	20.34	21.86	21.90
64.66 65.25 <sup>d</sup> 20.30 24.58 39.56 40.16 <sup>e</sup> 69.16 76.16 76.31 <sup>f</sup> 169.70 17.00 <sup>b</sup> 16.75 83.71 83.78 169.70 67.91 65.44 <sup>d</sup> 67.65 67.58 24.68 36.81 69.30 69.01 69.09 77.43 76.59 <sup>f</sup> 77.50 17.54 168.19 16.900 168.19 17.01 17.01		83.09	86.22	86.33	167.99		169.74		169.51h
39.56 40.16° 68.93 69.16 76.16 76.31¹ 17.00 <sup>b</sup> 16.75 83.71 83.78 169.70 67.91 65.44° 67.65 67.58 24.68 36.81 40.41° 37.00 69.01 68.83 69.30 69.01 69.09 77.43 76.59¹ 77.50 169.00 168.19 16.90 17.60		67.63	64.66	65.25 <sup>d</sup>	20.30		24.58		20.818
68.93 69.16 76.16 76.31f 17.00b 16.75 83.71 83.78 169.70 83.43 86.22 83.71 83.78 169.70 67.91 65.44 67.65 67.58 24.68 36.81 40.41¢ 37.00 37.02 68.83 69.30 69.01 69.09 168.19 17.50 17.54 168.19 17.50 17.54 17.51 17.51 17.51		36.56	39.56	$40.16^{e}$					
76.16 76.31 <sup>f</sup> 17.00 <sup>b</sup> 16.75 83.71 83.78 169.70 1  83.43 86.22 83.71 83.78 169.70 1  67.51 65.44 67.65 67.58 24.68  36.81 40.41 <sup>e</sup> 37.00 69.09  77.43 76.59 <sup>f</sup> 77.50 17.54  188.9 169.00  19.80 17.01 17.01		68.57	68.93	69.16					
17.00b 16.75 83.71 83.78 169.70 1 83.43 86.22 83.71 83.78 169.70 1 83.43 86.22 83.71 83.78 169.70 1 83.43 86.22 83.71 83.70 24.68 24		76.83	76.16	76.31 <sup>f</sup>					
17.00b 16.75 83.71 83.78 169.70 1 83.43 86.22 83.71 83.78 169.70 1 83.43 86.22 83.71 83.78 169.70 1 83.44 67.65 67.58 24.68 24.68 26.81 40.41° 37.00 69.09 69.09 77.43 76.59° 77.50 169.00 168.19 15.80 17.01 17.61 17.61		168.24							
83.43 86.22 83.71 83.78 169.70 1 1 65.44 67.65 67.58 24.68 24.68 26.91 65.44 67.65 67.58 24.68 24.68 25.81 69.30 69.01 69.09 77.43 76.59 77.50 169.00 168.19 168.19 17.84 17.84 17.84 17.81 17.8		19.70	17 000	37 31					
67.91 65.44 67.65 67.58 24.68 36.81 40.41° 37.00 37.02 68.83 69.30 69.01 69.09 77.43 76.59f 77.50 77.54 168.19 169.00 19.80 17.01 17.00		10.01	83.43	66.93	93.71	63 78		169.70	169 56h
36.81 40.41 37.00 37.02 68.83 69.30 69.01 69.09 77.43 76.59 77.50 77.54 168.19 169.00 14.2 17.80 17.80		64.80	67.91	65 44 <sup>d</sup>	67.65	67.58		24.68	21.138
68.83 69.30 69.01 77.43 76.59 <sup>4</sup> 77.50 168.19 76.59 77.50 1 16.8.19 16.98 17.01		39.98	36.81	40.41	37.00	37.02			
77.43 76.59f 77.50 168.19 16.59 168.19 16.84 17.01		69.14	68.83	69.30	69.01	60.69			
168.19 19.42 16.83b 16.98 17.01		76.61	77.43	76.59 <sup>f</sup>	77.50	77.54			
19.42 16.83b 16.98 17.01			168.19			169.00			
		16.08	14.83b	16.08	17.01	17.06			

Values within a column with the same superscript may be interchanged.

#### EXPERIMENTAL

NMR spectra were determined in CDCl<sub>3</sub> at 500 MHz. Chemical shifts are referenced to solvent peaks:  $\delta_H$  7.26 (residual CHCl<sub>3</sub>) and  $\delta_C$  77.0 for CDCl<sub>3</sub>. Homonuclear  $^1H$  connectivities were determined with the COSY experiment and heteronuclear  $^1H$ - $^{13}C$  connectivities were determined by HMQC<sup>2</sup> and HMBC<sup>3</sup> experiments.

High resolution mass spectra of tolyporphins E-I (5-9) were determined in positive ion FAB mode with a matrix of nitrobenzyl alcohol. High resolution mass spectra of tolyporphins B-D (2-4) were also determined in positive ion FAB mode but with a matrix of nitrobenzyl alcohol and potassium chloride.

Culture conditions: A clonal culture, designated UH strain HT-58-2 and identified as *Tolypothrix nodosa*, was obtained from a soil sample at Nan Madol, Pohnpei. Repeated subculture on a solidified medium was used to purify the alga. Unialgal, nonaxenic cultures of HT-58-2 were grown on BG-11 medium in 20-litre glass bottles as described elsewhere.<sup>5</sup> After 24 to 30 days, the alga was harvested by filtration and freezedried. Yields of lyophilized alga averaged 0.2 g/litre.

**Isolation and Characterisation:** The freeze-dried alga (93 g) was extracted in a blender with 1 litre portions of CH<sub>2</sub>Cl<sub>2</sub>/2-propanol (1:1) three times. The combined extract was filtered and the solvent removed *in vacuo*. The crude extract (7.5 g) was fractionated by reversed-phase flash chromatography on a column of C18 silica (YMC gel ODS 120 Å, 80 g) using a steep-stepped gradient from H<sub>2</sub>O to MeOH to CH<sub>2</sub>Cl<sub>2</sub>.

Column chromatography on silica gel (Davisil 200-425 mesh, 60 Å) using a hexane/EtOAc gradient, followed by further reversed phase flash column chromatography with a H<sub>2</sub>O/MeOH gradient yielded pure tolyporphin A (1) (123 mg). A similar procedure yielded tolyporphin F (6) (12 mg). A further step of reversed-phase hplc, using an Alltech semipreparative C18 column, with H<sub>2</sub>O/MeOH (1:9) as solvent at a flowrate of 2 ml/min. and UV detection at 220 nm, yielded a fraction (38 mg) pure in tolyporphins B and C (2-3). This same hplc procedure yielded tolyporphin D (4) (5 mg).

Tolyporphin E (5) (46 mg) was isolated by repeated column chromatography of a fraction from the initial reversed phase column on silica gel (Davisil 200-425 mesh, 60 Å) using a hexane/EtOAc gradient. After the initial C18 column and subsequent two Davisil columns, a further step of reversed-phase hplc, using conditions as described above, was necessary to purify tolyporphins G and H (7-8). Tolyporphin I (9) (1.5 mg) was isolated by reversed phase flash column chromatography using a steep-stepped gradient as previously.

**Tolyporphin A** (1): Amorphous purple solid,  $[\alpha]_D + 3$  (c, 0.1; MeOH), CD (MeOH)  $\Delta\epsilon$  (nm) +0.18 (675), 0 (660), -0.32 (642), 0 (604), +0.32 (575), 0 (551), -1.57 (525), 0 (480), +0.2 (465), 0 (419), -0.09 (415), 0 (412), +4.99 (363), 0 (325), -2.59 (305), 0 (290), +8.94 (245).

**Tolyporphins B** (2) and C (3): Amorphous red-purple solids,  $[\alpha]_D$  (mixture) -35 (c, 0.1; MeOH), CD (MeOH) Δε (nm) -0.42 (646), 0 (625), +0.48 (563), 0 (543), -0.96 (525), 0 (502), 0.74 (467), +0.32 (411), +1.49 (397), +0.64 (384), +2.76 (379), 0 (376), +9.44 (371), +0.21 (365), +2.76 (347), 0 (321), -0.85 (305), 0 (297), +7.74 (251), UV (MeOH)  $\lambda_{max}$  278 nm (ε 14000), 296 (12000), 378 (2500), 406 sh (2100), 480 (3200), 502 (5000), 546 (4700), 566 sh (2000), 614 (4000), 620 (3900), 644 (4100), 680 (13000), ms (FAB+): C<sub>38</sub>H<sub>44</sub>N<sub>4</sub>O<sub>9</sub>; m/z 700.3133, Δ 2.5 mmu. For <sup>1</sup>H and <sup>13</sup>C NMR spectra see Tables 1 and 2.

**Tolyporphin D** (4): Amorphous red-brown solid,  $[\alpha]_D$  +45 (c, 0.1; MeOH), CD (MeOH)  $\Delta \varepsilon$  (nm) +0.60 (656), 0 (653), -0.65 (648), 0 (617), +0.40 (570), 0 (539), -0.80 (521), 0 (501), +2.99 (379), 0 (373), -1.70

(370), 0 (369), +3.24 (352), 0 (320), -1.00 (300), 0 (291), +6.18 (248), UV (MeOH)  $\lambda_{max}$  278 nm ( $\epsilon$  13000), 296 sh (11000), 368 (24000), 478 (3300), 502 (5000), 544 (4500), 614 (3700), 622 (3600), 646 (3700), 680 (12000), ms (FAB+): C<sub>36</sub>H<sub>42</sub>N<sub>4</sub>O<sub>8</sub>; m/z 658.3036,  $\Delta$  3.4 mmu. For <sup>1</sup>H and <sup>13</sup>C NMR spectra see Tables 1 and 2.

Tolyporphin E (5): Amorphous dark red solid,  $[\alpha]_D$  +52 (c, 0.1; MeOH), CD (MeOH) Δε (nm) +0.15 (650), +0.61 (531), +0.46 (451), +1.64 (405), +0.76 (392), +1.52 (386), 0 (375), -0.64 (363), -0.15 (328), -0.49 (309), 0 (297), +0.88 (274), +0.67 (276), +1.10 (241), UV (MeOH)  $\lambda_{max}$  284 nm (ε 5000), 312 sh (4300), 388 (16000), 504 (1500), 548 (1400), 624 (1200), 686 (7300), ms (FAB+):  $C_{34}H_{36}N_4O_8$ ; m/z 628.2545, Δ 1.2 mmu. For  $^1H$  and  $^{13}C$  NMR spectra see Tables 1 and 2.

**Tolyporphin F** (6): Amorphous red-brown solid,  $\{\alpha\}_D$  +20 (c, 0.05, MeOH), CD (MeOH) Δε (nm) 0 (632), +1.28 (532), +0.71 (445), +5.06 (406), +2.93 (396), +8.35 (380), 0 (355), -0.71 (322), -2.31 (306), 0 (296), +6.22 (250), UV (MeOH)  $\lambda_{max}$  282 nm (ε 16000), 306 (14000), 386 (39000), 506 (4700), 548 (4400), 624 (3900), 684 (22000), ms (FAB+): C<sub>32</sub>H<sub>34</sub>N<sub>4</sub>O<sub>7</sub>; m/z 586.2442, Δ 1.4 mmu. For <sup>1</sup>H and <sup>13</sup>C NMR spectra see Tables 1 and 2.

Tolyporphins G (7) and H (8): Amorphous red-brown solids, [α]<sub>D</sub> (mixture) -33 (c, 0.1, MeOH), CD (MeOH)  $\Delta\epsilon$  (nm) +0.22 (650), +0.50 ((558), +2.00 (528), +0.47 (460), +4.29 (410), +2.15 (395), +2.43 (391), +0.14 (382), +4.43 (374), 0 (371), -8.08 (364), -3.08 (360), -6.48 (356), -2.22 (324), -3.58 (309), 0 (291), +2.25 (276), +0.29 (263), +1.94 (243), UV (MeOH)  $\lambda_{max}$  286 nm ( $\epsilon$  13000), 312 (13000), 368 (18000), 482 (2500), 506 (3400), 548 (3200), 578 sh (1500), 628 (3100), 650 (3600), 686 (10000), ms (FAB<sup>+</sup>):  $C_{26}H_{24}N_4O_5$ ; m/z 472.1736  $\Delta$  2.3 mmu. For  $^{1}H$  and  $^{13}C$  NMR spectra see Tables 1 and 2.

Tolyporphin I (9): Amorphous red-pink solid,  $[\alpha]_D$  +64 (c, 0.13, MeOH), CD (MeOH) Δε (nm) +0.13 (600), +1.12 (527), +0.37 (440), +2.34 (408), +0.78 (390), +1.87 (387), 0 (380), +2.24 (373), 0 (371), -1.53 (357), -0.22 (320), -0.59 (305), 0 (283), -5.39 (261), UV (MeOH)  $\lambda_{max}$  280 nm sh (ε 12000), 306 sh (6000), 376 (14000), 504 (1900), 544 (1700), 624 (1500), 684 (7800), ms (FAB<sup>+</sup>): C<sub>28</sub>H<sub>26</sub>N<sub>4</sub>O<sub>6</sub>; m/z 514 .1848 Δ 0.8 mmu. For <sup>1</sup>H and <sup>13</sup>C NMR spectra see Tables 1 and 2.

Assays: [<sup>3</sup>H] azidopine photolabelling assays and [<sup>3</sup>H] vinblastine accumulation assays were performed by methods that we have described elsewhere.<sup>4</sup> Stock solutions of the tolyporphins in EtOH were kept in the dark at 4°C and solvent levels in these assays never exceeded 0.5 %.

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