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Structural Studies of Cytotoxic Marine Alkaloids: Synthesis of Novel Ring-E Analogues of Ascididemin and their in vitro and in vivo Biological Evaluation

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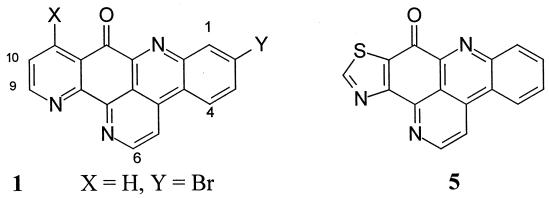
Abstract—The cytotoxic marine alkaloid ascididemin and various pyridine ring-E analogues have been synthesised in an attempt to determine the pharmaceutical utility and structure-activity requirements for the parent alkaloid. All compounds synthesised were evaluated in a wide range of biological screens for selective cytotoxicity, antiviral, antifungal and antimicrobial properties. Many analogues exhibited selective cytotoxicity to human solid tumour cell-lines in vitro, with one also exhibiting moderate antitumour activity in in vivo xenograft assays.

Introduction

Over the last 15 years, more than 35 alkaloids based upon the pyrido[2,3,4-*kl*]acridine skeleton have been reported from the marine environment.[1] Invariably these polycyclic aromatic alkaloids have been isolated due to the range of potentially useful biological activities they exhibit, which include antifungal, antiviral and antitumour properties. Some of the first examples of these compounds included the ascidian-derived pyridoacridone alkaloids 2-bromoleptoclinidinone (**1**)[2] and ascididemin (**2**),[3] with more recent examples including 11-hydroxyascididemin (**3**),[4] 9,10-dihydro-11-hydroxyascididemin (**4**)[5] and kuanoniamine A (**5**) (Fig. 1).[6] Numerous approaches to the synthesis of the pyridoacridone class of alkaloid have been reported[7] while only a limited number of biological studies of these compounds have been presented.[8]

Our interest in the ascididemin class of alkaloid was sparked by the 1990 report by Schmitz et al. that ascididemin inhibited the catalytic function of the DNA processing enzyme topoisomerase II.[9] As this enzyme is the molecular target of many current clinical anticancer drugs we became interested in assessing the human antitumour potential of ascididemin and related compounds. Our initial studies established the importance of N-8 in ring A, and a completed ring-E for the observed topoisomerase II enzyme inhibition, human tumour cytotoxicity and antifungal/antibacterial properties of ascididemin.[8b] We now report more complete studies on the chemistry of ring-E of the alkaloid ascididemin, whereby investigations of the chemistry of enamine (and related alkylamine) synthetic precursors have led to the synthesis of novel 5- and 6-substituted analogues.

To explore the structural features responsible for the biological activities of ascididemin and related compounds, all the derivatives in this study were tested for antiproliferative properties against a range of targets, including leukaemia cells, viruses and microorganisms. All compounds have also been tested in the US National Cancer Institute (NCI) in vitro primary screen, with several, including the natural product parent compound ascididemin, also being studied in Hollow Fibre and xenograft in vivo assays. The comprehensive testing by the NCI has shown that most of the compounds synthesised in the current study exhibited selective cytotoxicity against certain cancer types in vitro, and that a limited number of analogues also exhibited activity in vivo.



1 X = H, Y = Br
2 X = H, Y = H
3 X = OH, Y = H
4 9,10 dihydro, X = OH, Y = H

Figure 1.

Keywords: marine metabolites; structure-activity; antitumour compounds; alkaloids.

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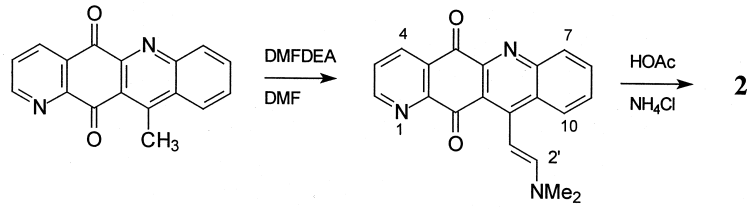


Scheme 1. Bracher's synthesis of ascididemin (**2**) from dione **6** via enamine **7**.[7b]

Chemistry

One of the more generally applicable procedures reported to date for the synthesis of ascididemin is Bracher's methodology which achieves a final ring-E annulation (i.e. **6** to **2** in Scheme 1) using dimethylformamide-diethylacetal (DMFDEA) in DMF under nitrogen to form an enamine (**7**), which is then cyclised to the pentacyclic product with ammonium chloride in acetic acid.[7b] As we, and others, have previously noted[8b,c] that dione **6** is essentially devoid of biological activity, we speculated that the more functionalised enamine **7** could exhibit interesting biological properties.

Reaction of quinone **6** with DMFDEA in DMF under nitrogen and subsequent removal of all volatiles in vacuo afforded the previously uncharacterised enamine **7** as a dark red solid. Introduction of an enamine functional group was confirmed by the observation of ^{1}H NMR signals at δ 7.50 and 7.15 (both 13 Hz doublets) and 3.08 (6H, s) and ^{13}C NMR signals at δ 94.4 (C-1′), 155.2 (C-2′) and 41.1 (NMe_2). As discussed in the Biological Activities section, **7** exhibited a wide range of potent biological activities including selective human tumour cytotoxicity, antiviral and antifungal properties. In order to extend the structure activity relationship of this finding, analogues **8**, **9**, **10**,[10] **11**,[10] and **12**[10] (Fig. 2) were synthesised in similar fashion (see Experimental section) and evaluated in the same range of biological assays (see Biological Activities section).

We next investigated β-alkylation of enamine **7** as a method with which to synthesise previously unrealised ring-E derivatives of ascididemin. It was presumed that reaction of **7** with Eschenmoser's salt (or the more classical Mannich reaction itself) would yield a difunctionalised side-chain product (i.e. **13**, which is a masked enal (**14**)). Both of these side-chain functional groups should be capable of undergoing further reaction with NH_3 to form pyridine ring-E, leaving the other non-reacting functional group as a substituent at the 5-position of ascididemin. Indeed, reaction of enamine **7** with Eschenmoser's salt yielded the desired but unstable enal **14** in low yield. The observation of an aldehydic (δ 10.01) and two olefinic (δ 6.77 and 6.37) protons in the ^{1}H NMR spectrum as well as carbon resonances at δ 190.8 (CH) and δ 133.1 (CH_2) supported the formation of **14**. In order to circumvent the instability of **14**, cyclisation chemistry was investigated using a three step-one pot reaction sequence. Thus dione **6** was reacted with DMFDEA to afford enamine **7**, that was in-turn reacted with Eschenmoser's salt in DMF to afford a product that was finally cyclised with NH_4Cl in HOAc. Subsequent work up and chromatography yielded 5-acetoxymethylascididemin (**15**) (27%). (Fig. 3)

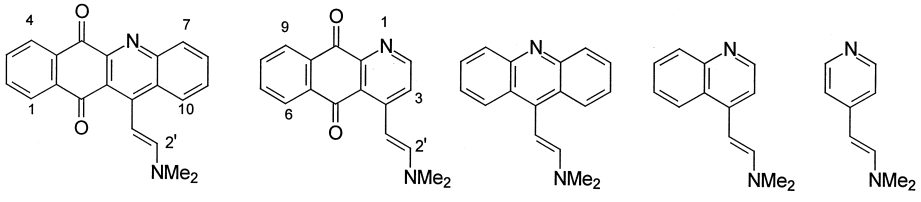


Figure 2.

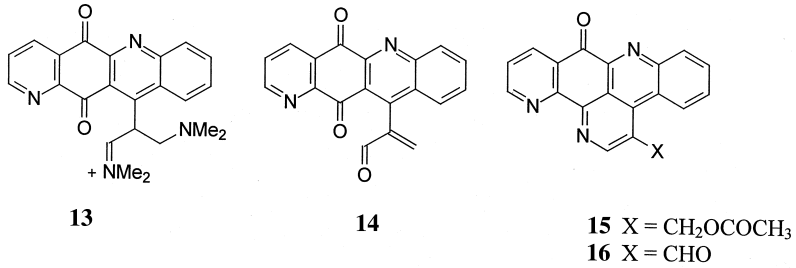
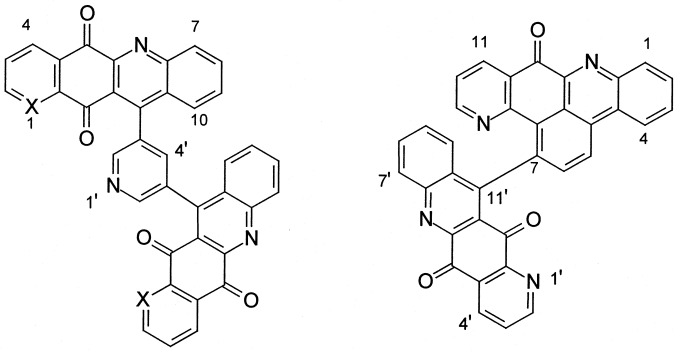


Figure 3.



17 X = N
18 X = CH

19

Figure 4.

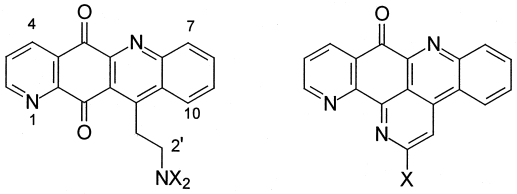
That the putative enal intermediate had undergone a Michael Reaction with acetate before final cyclisation was confirmed by extensive spectroscopic characterisation of **15**. Substitution at the 5-position was confirmed by the observation of H-6 (δ 9.35) as a singlet, while the observation of new ^{1}H (at δ 5.92 (2H, s) and 2.20 (3H, s)) and ^{13}C NMR resonances (δ 64.2, CH_2; 20.9, CH_3) confirmed the existence of an acetoxymethyl functional group. Further confirmation of its placement at C-5 was made by the observation of long range ^{1}H–^{13}C correlations from the methylene protons at 5.92 ppm to carbons C-4b, C-5, C-6 and the acetate carbonyl (δ 170.5). Trace quantities (<5%) of a product attributable to 5-formylascididemin (**16**) (δ_H 10.94 (CHO, s), 9.54 (H-6, s)) were also detected in the product mixture, however, due to insufficient quantities, this product was not isolated nor characterised.

Whilst exploring optimisation of the synthesis of **15**, we found that the relative quantity of acetic acid used in the final step was absolutely crucial in determining what reaction products were obtained. Thus using the established three step-one pot reaction but adding only half as much acetic acid solvent in the final step yielded the novel terpyridyl analogue **17** as the major product. HRFABMS indicated a molecular formula of $C_{37}H_{17}N_5O_4$, while the observation of 9 ^{1}H and 19 ^{13}C NMR resonances suggested the product was symmetrical. The ^{1}H NMR spectrum showed signals typical for the 11-substituted pyrido[2,3-*b*]-acridine-5,12-dione chromophore,[7b] while the observation of an additional aromatic three-proton spin system, at δ 7.73 (1H, t, J=2 Hz) and 8.69 (2H, d, J=2 Hz), was attributable to a 3,5-disubstituted pyridine ring. The deaza analogue **18** was synthesised in similar fashion, starting with 11-methyl-benzo[*b*]acridine-5,12-dione (Fig. 4).[11]

Further use of the three step-one pot reaction with an additional doubling of the final step concentration yielded no terpyridyl analogue **17** and only minor amounts of 5-acetoxymethyl- (**15**) and 5-formyl-ascididemin (**16**). The major product of the reaction was, however, deduced to be the unprecedented nonacycle alkaloid **19**. The structural elucidation of **19** relied on NMR spectroscopy and was supported by HRFAB mass spectrometry. HRFABMS determined the molecular formula to be $C_{35}H_{16}N_4O_3$, while inspection of the ^{1}H and ^{13}C NMR data suggested the presence of an essentially intact fragment of ascididemin, as well as an 11-substituted pyrido[2,3-*b*]acridine-5,12-dione fragment. The observation of benzenoid-type $^1J_{CH}$ coupling constants for C-5 (d, J=163 Hz) and particularly C-6 (d, J=163 Hz), as well as HMBC correlations from both H-5 and H-6 to a new aromatic carbon resonance (C-7, δ 137.7) supported the unusual non-pyridinoid nature of ring-E of the ascididemin fragment. Finally, interfragment connectivity between C-7 and C-11′ was established by the observation of an HMBC correlation between H-6 (δ 7.69) and C-11′ (δ 157.8) thus securing the structure of **19**.

Ethylamine analogues of enamine **7** were of interest as an extension of the structure-activity relationship of the biologically active enamines, and also because of their similarity to heterocyclic analogues of the human antitumor agent mitoxantrone.[12] Dimethylaminoethyl derivative **20** was prepared by reaction of dione **6** with Eschenmoser's salt in DMF at 120°C (95%). The observation of new ^{1}H NMR resonances at δ 4.09 and 3.06 (both br t, J=8 Hz) and δ 2.72 (6H, s) and new ^{13}C resonances at δ 56.8 and 26.0 (both CH_2) and δ 43.4 (CH_3) confirmed conversion of the methyl group in **6** to a dimethylaminoethyl group in **20**. The diethylamino derivative **21** was prepared by Mannich reaction with dione **6** utilising diethylamine as the base (Fig. 5).

It was found that **20** could be readily converted into ascididemin in 69% by heating in acetic acid with NH_4Cl. Presumably the initial reaction product is 5,6-dihydroascididemin[7h] but this is oxidised in situ to the more stable fully aromatic ascididemin. A natural extension of this reaction



20 X = CH_3
21 X = CH_2CH_3

22 X = CH_3
23 X = C_6H_5
24 X = CH=CH–C_6H_5

Figure 5.

Table 1. In vitro antiviral and antimicrobial activities of **2** and its analogues

Compound	Antiviral[a]			Antimicrobial[b]		
	HSV-1	PV1	Cyto (dose)	Ec	Bs	Ca
2	?	?	1+(10)	10 10^{20}	14 10^{20}	11 0^{20}
15	?	?	4+(80)	5^{20}	7^{20}	2^{20}
22	?	?	4+(20)	0^{20}	5^{20}	3^{20}
23	–	–	3+(20)	0^{20}	4^{20}	0^{20}
24	–	–	−(20)	0^{20}	5^{20}	0^{20}
6[c]	–	–	−(10)	0	10	1
7	4+	4+	1+(10)	12	12	10
8	2+	2+	−(10)	0	4	10
9	4+	?	2+(10)	2	12	21
10	4+	4+	2+(10)	4	9	11
11	?	?	2+(80)	0	1	4
12	?	?	1+(80)	0	0	0
20	2+	–	−(10)	5	9	2
21	–	–	−(10)	8	10	1
17	–	–	−(80)	0	4	0
18	3+	?	1+(80)	0	3	8
19	?	?	1+(80)	0	5	0

[a] Assay methods have been described elsewhere.[15a] Zones of cytotoxicity and antiviral activity were measured microscopically as excess radii from the disc and indicated by–, none detectable; 1+, 1–2 mm; 2+, 2–3.5 mm; 3+, 3.5–4.5 mm; 4+, greater than 4.5 mm; '?' indicates that cell death due to viral infection and compound toxicity has masked any antiviral activity.

[b] Zone of microbial inhibition against *E. coli*, *B. subtilis* and *C. albicans* for 120 μg of test compound (except where indicated by superscripted number, which is the loading in μg used) on a 6 mm paper disc. Incubation was for 18 h at 35°C. Zones measured as excess radii in mm.

[c] Data from Ref. 8b.

result was to then attempt a Mannich reaction of dione **6** with paraformaldehyde and NH_4Cl in acetic acid, which conveniently afforded ascididemin in 80% yield. As we have communicated, this reaction was found to be quite general for a range of analogues of dione **6**.[7j,m] We have now also found that the reaction is quite general for a range of aldehydes, allowing for simple insertion of carbon-based substituents at the 6-position of ascididemin. Thus reaction of dione **6** with NH_4Cl and acetaldehyde, benzaldehyde or cinnamaldehyde in acetic acid afforded the increasingly lipophilic ascididemin analogues **22**, **23** and **24** in yields of 90%, 81% and 72% respectively.

Biological Activities

All compounds were initially assayed for cytotoxicity against the BSC monkey kidney cell line, antiviral activity towards DNA (HSV-1) and RNA (PV-1) viruses and for antimicrobial activity against Gram positive and Gram negative bacteria and a fungus (Table 1). Ascididemin (**2**) exhibited more potent activity towards Gram positive and Gram negative bacteria than towards fungi and had no detectable antiviral activity due to cytotoxicity of the compound towards the viral host cells. Substitution at the 5 or 6 positions of ascididemin resulted in reduced antibacterial activities, while **15** and **22** exhibited a modest degree of antifungal activity towards the human pathogenic fungi *C. albicans*. Enamine functionalised diones exhibited substantially increased antimicrobial activities compared to the dione **6**, and also resulted in the observation of antiviral activities towards both DNA (HSV-1) and RNA (PV1) viruses. The saturated side-chain derivatives **20** and **21** both showed diminished antimicrobial and antiviral properties compared to enamine **7**.[13] As demonstrated by results for enamine **10**, the quinoid functional group is not required for the observation of antifungal or antiviral properties, but any reduction in chromophore size below three fused rings (i.e. **11** and **12**) resulted in less potent antiviral and antimicrobial activities. This chromophore size dependence of enamine biological activity is consistent with a mechanism action involving DNA intercalation.[14]

The biological properties of all compounds were further evaluated for cytotoxicity against the murine leukaemia cell line P388 and by the NCI in their in vitro disease-oriented primary antitumour screen (Table 2). Similar P388 activity was observed for ascididemin (**2**) and analogues **15**, **19**, **22**, **23** and **24** irrespective of the nature of the substituent, while all ring-E opened derivatives, with the exception of enamine **7**, were less cytotoxic than the parent compound. The NCI testing provides observations on the mean response parameters (GI_{50}, TGI, LC_{50}), differential cellular sensitivity and subpanel-specific patterns of sensitivity.[15b] In these primary assays ascididemin (**2**) was found to be selective for the melanoma and colo-rectal subpanels and to exhibit a panel average LC_{50} value of 6.5 μM.[16]

While the 5-acetoxymethyl (**15**) and 6-methyl (**22**) derivatives exhibited similar potency and subpanel selectivity to that observed for ascididemin, the more lipophilic derivatives **23** and **24** were less cytotoxic towards human tumor cell lines in vitro. Disruption of the pentacyclic structure of ascididemin generally resulted in derivatives that exhibited less potent cytotoxicity in the NCI assays, however enamines **7** and **8** and amine **20** exhibited sufficient potency and selectivity to warrant further in vivo evaluation.

COMPARE analysis of the NCI in vitro cytotoxicity profile observed for ascididemin indicated high similarity with those profiles observed for amine analogue **20** (Pairwise Correlation Coefficient=0.79) and enamine **7** (0.69), suggestive of similar mechanisms of cytotoxic action for the three compounds.[17] Given the propensity of *para*-quinone containing compounds to exhibit cellular toxicity via a mechanism involving redox cycling and the formation of reactive oxygen species (ROS)[18] we have undertaken studies to ascertain whether ascididemin is also capable of exhibiting toxicity via this mechanism. Our preliminary results suggest that ascididemin can indeed act to oxidatively damage DNA via a thiol-dependent conversion of oxygen to DNA-cleaving oxygen radicals.[19]

The potency and tumour cell line selectivity observed in vitro for ascididemin (**2**) and analogues **7**, **9**, **20**, **22**, **23** and **24** warranted further study in vivo. Preliminary in vivo evaluation was made at the NCI using the Hollow Fibre protocol,[15f] but of the seven compounds tested only three (**2**, **20** and **22**) exhibited significant reductions in cell viability. Ascididemin exhibited localised activity towards breast (MDA-MB-231 and -435) and CNS (SF-295 and U251) tumour cells, and more promisingly, towards the remote subcutaneously located melanoma (LOX IMVI) and non-small cell lung (NCI-H23) cell lines.

Table 2. In vitro antitumour activities (μM) of **2** and its analogues

Compound (NSC)[a]	P388 IC_{50}[b]	GI_{50}[c]	TGI	LC_{50}
2 (675670)	0.4	0.18 (2.6)	0.9 (2.7)	6.5 (2.5)
15 (694488)	0.5	0.05 (1.9)	0.6 (3.7)	8.0 (2.8)
22 (683787)	1.4	0.3 (2.3)	1.6 (3.8)	6.9 (2.4)
23 (686553)	1.4	4.1 (2)	16 (1.7)	47 (1.3)
24 (686555)	2.8	2.6 (1.7)	9 (1.8)	36 (1.3)
6 (659780)[d]	56	11	56	79
7 (680733)	0.4	0.6 (2.6)	3.3 (2.7)	24 (2.2)
8 (680732)	14	1.1 (2.1)	4.3 (2.3)	23 (1.9)
9 (686556)	12	7.7 (1.9)	29 (1.6)	66 (1.2)
10	24	n.t.[e]	n.t.	n.t.
11	69	n.t.	n.t.	n.t.
12 (690415)	71	98 (0.5)	100 (0)	100 (0)
20 (680734)	4	0.3 (3)	1.7 (3.5)	14 (2)
21 (683790)	7.6	1.4 (2)	6.9 (2)	42 (1.4)
17 (694492)	4	0.2 (2.3)	1.4 (2.6)	15.4 (2.2)
18 (694490)	8	4.3 (2.8)	30 (1.6)	75 (1.2)
19 (694491)	3.0	1.1 (2.8)	6.8 (2.4)	38 (1.3)

[a] NSC number is the NCI reference number for each compound. Search for this number at the NCI website[16] to view complete information of all in vitro assay profiles.
[b] IC_{50} against the P388 D1 murine leukaemia cell line.
[c] GI_{50} (50% growth inhibition), TGI (total growth inhibition) 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] P388 IC_{50} value from Ref. 8b, NCI data from Ref. 8c.
[e] n.t. Not tested.

6-Methylascididemin (**22**) exhibited a similar profile of activity, but also showed activity towards the colo-rectal tumour cell lines COLO 205 and SW-620, while amine **20** only showed moderate activity towards the CNS tumour cell line SF-295.

Based upon the observed Hollow Fibre assay results, more extensive subcutaneous xenograft in vivo assays were undertaken on ascididemin and amine **20**.[8c,15f] Ascididemin was found to exhibit no significant ($T/C < 40\%$)[8c] anti-tumour activity using non-toxic doses and standard NCI delivery schedules (data not shown).[20] The best result for the compound was observed against the colo-rectal tumour line HCT-116, with a *T*/*C* of 58% at 8 mg/kg/IP dose. A similar result has been reported for xenograft evaluation of 2-bromoleptoclinidinone (**1**, 2-bromoascididemin).[8c] Amine **20**, however, exhibited moderate antitumour activity (*T*/*C* 31% at 36 mg/kg/IP dose) towards the SF-295 (CNS) tumour cell line at non-toxic doses.

Conclusion

Although isolated as in vitro cytotoxic agents, it would appear from our results and those of others,[8c] that ascididemin and its 2-bromo analogue have limited in vivo anti-tumour activity. Increasing lipophilicity at the 6-position of ascididemin also results in further degradation of in vivo activity. A limiting factor in attempting more detailed in vivo evaluation (including dose and delivery route optimisation) of examples of the pyridoacridone family of alkaloids is the poor water solubility of the compounds, an issue we are currently attempting to address. The discovery however, of selective cytotoxicity for some ascididemin-related compounds against certain human-tumour cell lines is particularly encouraging and adds focus to the considerable synthetic interest in this class of alkaloid.

Experimental

General methods

Details of instrumental methods and general experimental procedures[7m] and biological assays[15] have been reported previously. NMR spectral assignments were made with the aid of the 2-dimensional NMR experiments, and $^{1}H-^{13}C$ coupling constants were determined by gated decoupled ^{13}C NMR experiments. The preparations of 11-methylpyrido[2,3-*b*]acridine-5,12-dione (dione **6**),[7b] 11-methylbenzo[*b*]acridine-5,12-dione,[11] cleistopholine,[21] enamines **10**, **11** and **12**[10] have been reported previously.

11-[2′-(Dimethylamino)vinyl]pyrido[2,3-*b*]acridine-5,12-dione (7). Dione **6** (200 mg, 0.73 mmol), dimethylformamide diethyl acetal (DMFDEA) (0.37 mL, 2.16 mmol) and DMF (4 mL), was heated to 120°C under N_2 for 1 h. The reaction mixture was allowed to cool and the solvent and unreacted DMFDEA was removed in vacuo to yield **7** as a blood red solid (228 mg, 95%). mp 230–231°C. IR (film) ν 1677, 1649, 1598, 1556, 1463, 1376, 1276, 1254, 1106 cm^{-1}. UV (CH_3OH): λ_{max} (log ϵ) 476 nm (4.0), 278 (4.4), 237 (4.4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (1H, dd, *J*=4.6, 1.7 Hz, H-2), 8.53 (1H, dd, *J*=7.9, 1.7 Hz, H-4), 8.13 (1H, d, *J*=8.7 Hz, H-10), 8.11 (1H, d, *J*=8.7 Hz, H-7), 7.62 (1H, t, *J*=7.6 Hz, H-8), 7.59 (1H, dd, *J*=7.9, 4.6 Hz, H-3), 7.50 (1H, d, *J*=12.9 Hz, H-1′), 7.38 (1H, t, *J*=7.6 Hz, H-9), 7.15 (1H, d, *J*=12.9 Hz, H-2′), 3.08 (6H, s, NMe_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.7 (d, *J*=3 Hz, C-5), 181.7 (C-5), 155.2 (d, *J*=163 Hz, C-2′), 155.1 (ddd, *J*=182, 7, 3 Hz, C-2), 153.7 (s, C-11), 150.4 (dd, *J*=11, 5 Hz, C-12a), 148.7 (t, *J*=8 Hz, C-6a), 148.1 (s, C-5a), 135.0 (dd, *J*=169, 6 Hz, C-4), 131.6 (dd, *J*=162, 9 Hz, C-8), 130.9 (dd, *J*=165, 8 Hz, C-7), 129.0 (d, *J*=7 Hz, C-4a), 128.8 (dd, *J*=163, 8 Hz, C-10), 127.2 (dd, *J*=163, 8 Hz, C-9), 127.2 (m, C-10a), 126.9 (dd, *J*=167, 9 Hz, C-3),

118.3 (s, C-11a), 94.4 (d, *J*=159 Hz, C-1′), 41.1 (br, NMe_2). EIMS *m/z* (%) 329 (M^+, 40). HREIMS (M^+) found 329.1157, $C_{20}H_{15}N_3O_2$ requires 329.1164.

11-[2′-(Dimethylamino)vinyl]benzo[*b*]acridine-5,12-dione (8). 11-methylbenzo[*b*]acridine-5,12-dione[11] (50 mg, 0.183 mmol), DMFDEA (63 μL, 0.37 mmol) in DMF (2 mL) were heated at 125°C under N_2 for 0.5 h. Solvent was removed in vacuo to yield **8** as a red solid (85 mg, 94%). mp 217–219°C ($CHCl_3$, cyclohexane); IR (film) ν 1678, 1634, 1596, 1557 cm^{-1}. UV (MeOH) λ_{max} (log ϵ) 241 nm (4.2), 277 (4.6), 464 (4.3). ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (2H, m), 8.18 (2H, d, *J*=8.6 Hz), 7.73 (1H, td, *J*=7.5, 1.4 Hz), 7.67 (2H, td, *J*=7.4, 1.2 Hz), 7.41 (1H, td, *J*=7.6, 1.2 Hz), 7.39 (1H, d, *J*=13.0 Hz), 7.06 (1H, d, *J*=13.0 Hz), 3.10 (6H, s, NMe_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.2 (d, *J*=4 Hz), 183.0 (d, *J*=3 Hz), 154.4 (d, *J*=165 Hz), 153.4 (m), 149.0 (m), 148.9 (m), 135.7 (d, *J*=7 Hz), 134.2 (dd, *J*=162, 8 Hz), 132.9 (dd, *J*=163, 8 Hz), 132.7 (t, *J*=6 Hz), 131.4 (ddd, *J*=160, 9, 2 Hz), 131.0 (dd, *J*=164, 8 Hz), 128.9 (dd, *J*=161, 8 Hz), 127.6 (m), 127.1 (dm, *J*=164 Hz), 127.0 (dm, *J*=164 Hz), 126.9 (dm, *J*=164 Hz), 118.6 (m), 94.2 (d, *J*=161 Hz). EIMS *m/z* (%) 278 (M^+, 70). HREIMS (M^+) found 328.1213, $C_{21}H_{16}N_2O_2$ requires 328.1212. Anal. calcd for $C_{21}H_{16}N_2O_2 \cdot H_2O$: C, 72.8; H, 5.2; N, 8.1%. Found: C, 73.1; H, 4.8; N, 7.9.

4-[2′-(Dimethylamino)vinyl]benzo[*b*]quinoline-5,10-quinone (9). Cleistopholine[21] (25 mg, 0.11 mmol), DMFDEA (77 μL, 0.45 mmol) in DMF (2 mL) were heated at 125°C under N_2 for 1.3 h. The red mixture was poured onto cold water (150 mL) and extracted with CH_2Cl_2 (3×50 mL). The red organic layer was washed with water (4×100 mL), then brine (2×150 mL) and dried ($MgSO_4$) and the solvent removed in vacuo. Recrystallisation of the red solid ($CHCl_3$, cyclohexane) afforded **9** (28 mg, 93%). mp 241–243°C. IR (smear) 1681, 1648, 1611, 1568, 1302, 1248, 1196, 1121 cm^{-1}. UV (MeOH) λ_{max} (log ϵ) 270 nm (5.6), 405 (4.4), 518 (3.4). ^1H NMR (400 MHz, $CDCl_3$) δ 8.48 (1H, d, *J*=5.7 Hz, H-2), 8.28 (1H, dd, *J*=7.8, 1.2 Hz, H-6), 8.23 (1H, dd, *J*=7.5, 1.3 Hz, H-9), 7.77 (1H, td, *J*=7.5, 1.5 Hz, H-8), 7.72 (1H, td, *J*=7.5, 1.5 Hz, H-7), 7.46 (1H, d, *J*=5.7 Hz, H-3), 7.42 (1H, d, *J*=13.4 Hz, H-2′), 7.12 (1H, d, *J*=13.4 Hz, H-1′), 3.07 (6H, s, NMe_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.3 (d, *J*=4 Hz, C-10), 182.9 (d, *J*=3 Hz, C-5), 151.5 (d, *J*=12 Hz, C-10a), 150.8 (m, C-4), 150.7 (d, *J*=181 Hz, C-2), 148.3 (dm, *J*=160 Hz, C-2′), 134.8 (m, C-9a), 134.2 (dd, *J*=163, 9 Hz, C-8), 133.2 (dd, *J*=164, 7 Hz, C-7), 132.4 (m, C-5a), 127.0 (dd, *J*=165, 7 Hz, C-9), 126.9 (dd, *J*=166, 8 Hz, C-6), 122.4 (m, C-4a), 119.7 (ddd, *J*=160, 8, 6 Hz, C-3), 93.2 (d, *J*=160 Hz, C-1′), 41.1 (br m, NMe_2). EIMS *m/z* (%) 278 (M^+, 70). HREIMS (M^+) found 278.1054, $C_{17}H_{14}N_2O_2$ requires 278.1055. Anal. calcd. for $C_{17}H_{14}N_2O_2 \cdot 0.25H_2O$: C, 72.2; H, 5.2; N, 9.9%. Found: C, 72.3; H, 5.0; N, 10.0.

5-Acetoxymethylascididemin (15). Dione **6** (46 mg, 0.17 mmol), DMFDEA (0.09 mL, 0.53 mmol) and DMF (1 mL) were heated to 120°C under N_2 for 1 h. The reaction mixture was allowed to cool and the solvent and unreacted DMFDEA removed under reduced pressure. The blood red residue, Eschenmoser's salt (54 mg, 0.29 mmol) and DMF (5.5 mL) were heated under N_2 at 115°C for 30 min, over which time the blood red coloration dissipated to orange/brown. The mixture was then allowed to cool and NH_4Cl (250 mg, 4.67 mmol) and acetic acid (25 mL *volume crucial*) were added, followed by continued heating at 115°C for 30 min. After cooling, the dark reaction mixture was poured onto ice, made basic with aqueous KOH (10%) and extracted exhaustively with $CHCl_3$. The combined organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed in vacuo. The product was purified by prep-TLC (SiO_2, CH_2Cl_2/MeOH/TEA 100:1:0.01) yielding **15** (16 mg, 27%) as a yellow powder. mp 257–259°C. IR (film) ν 2919, 1720, 1674, 1641, 1582, 1540, 1520, 1457, 1415, 1382, 1365, 1340, 1311 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.35 (1H, s, H-6), 9.19 (1H, dd, *J*=4.6, 1.8 Hz, H-9), 8.78 (1H, dd, *J*=7.9, 1.9 Hz, H-11), 8.68 (1H, dd, *J*=8.1, 1.4 Hz, H-1), 8.67 (1H, dd, *J*=8.1, 1.4 Hz, H-4), 8.04 (1H, ddd, *J*=8.1, 7.1, 1.2 Hz, H-2), 7.96 (1H, ddd, *J*=8.5, 7.1, 1.4 Hz, H-3), 7.68 (1H, dd, *J*=7.9, 4.6 Hz, H-10), 5.92 (2H, s, CH_2O), 2.20 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7 (C-12), 170.5 (O*C*(O)Me), 155.8 (C-9), 152.4 (C-6), 152.3 (C-7b), 150.0 (C-7a), 146.6 (C-13a), 145.9 (C-12a), 137.5 (C-4b), 136.4 (C-11), 133.9 (C-1), 131.7 (C-2), 131.0 (C-3), 128.5 (C-11a), 127.0 (C-5), 126.9 (C-4), 125.8 (C-10), 123.6 (C-4a), 118.7 (C-12b), 64.2 (CH_2O), 20.9 (CH_3). EIMS *m/z* (%) 355 (M^+, 95). HREIMS found 355.0955, $C_{21}H_{13}N_3O_3$ requires 355.0957.

3′,5′-Di(11-pyrido[2,3-*b*]acridine-5,12-dione)pyridine (17). Dione **6** (200 mg, 0.73 mmol), DMFDEA (0.40 mL, 2.34 mmol) and DMF (4 mL) were heated to 120°C under N_2 for 1 h. To the reaction mixture was then added Eschenmoser's salt (290 mg, 1.57 mmol) and DMF (4.0 mL) and heating continued at 120°C for 15 min under N_2. The mixture was then allowed to cool and NH_4Cl (1.000 g, 18.70 mmol) and acetic acid (50 mL *volume crucial*) were added followed by continued heating at reflux for 30 min. After cooling the dark reaction mixture was poured onto ice, made basic with aqueous KOH (10%) and extracted exhaustively with $CHCl_3$. The combined organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed in vacuo. Repeated silica gel chromatography afforded **17** (36 mg, 17%). mp >310°C. IR (film) ν 1688, 1638, 1582, 1496, 1460, 1374, 1329, 1263, 1223, 1101, 1066, 995, 752 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.16 (2H, dd, *J*=4.5, 1.7 Hz, H-2), 8.80 (2H, dd, *J*=7.9, 1.7 Hz, H-4), 8.69 (2H, d, *J*=2.1 Hz, H-2′), 8.54 (2H, d, *J*=8.3 Hz, H-7), 8.17 (2H, d, *J*=8.3 Hz, H-10), 8.00 (2H, ddd, *J*=8.2, 6.9, 1.2 Hz, H-8), 7.89 (2H, ddd, *J*=8.2, 6.9, 1.2 Hz, H-9), 7.81 (2H, dd, *J*=8.0, 4.6 Hz, H-3), 7.73 (1H, t, *J*=2.1 Hz, H-4′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6 (s, C-12), 181.3 (d, *J*=4 Hz, C-5), 155.8 (ddd, *J*=184, 7, 4 Hz, C-2), 149.6 (m, C-12a), 149.6 (m, C-6a), 149.2 (bs, C-11), 147.7 (ddd, *J*=183, 12, 6 Hz, C-2′), 147.2 (s, C-5a), 136.1 (dd, *J*=170, 6 Hz, C-4), 135.6 (dt, *J*=165, 6 Hz, C-4′), 133.7 (dd, *J*=163, 9 Hz, C-8), 132.5 (obsc, C-3′), 131.7 (dd, *J*=163, 6 Hz, C-7), 131.4 (dd, *J*=163, 9 Hz, C-9), 130.7 (d, *J*=8 Hz, C-4a), 129.8 (t, *J*=6 Hz, C-10a), 128.4 (dd, *J*=165, 7 Hz, C-10), 128.3 (dd, *J*=165, 9 Hz, C-3), 124.5 (s, C-11a). FABMS *m/z* (%) 620 ([$(M+Na)^+$ +2H], 45), 619 ([$(M+Na)^+$ +2H -H], 95), 618 ($(M+Na)^+$, 100), 598 ([$(M+H)^+$ +2H], 30), 597 ([$(M+H)^+$ +2H -H], 65), 596 ($(M+H)^+$, 85).

HRFABMS found 596.1356, $C_{37}H_{18}N_5O_4$ requires 596.1359.

3′,5′-Di(11-benzo[*b*]acridine-5,12-dione)pyridine (18). 11-Methylbenzo[*b*]acridine-5,12-dione[11] (200 mg, 0.73 mmol), DMFDEA (0.40 mL, 2.34 mmol) and DMF (4 mL) were heated to 120°C under N_2 for 30 min. To the reaction mixture were then added Eschenmoser's salt (277 mg, 1.50 mmol) and DMF (4.0 mL) and heating continued at 120°C for 15 min under N_2. The mixture was then allowed to cool and NH_4Cl (1.000 g, 18.70 mmol) and acetic acid (50 mL *volume crucial*) were added followed by continued heating at reflux for 30 min. After cooling the dark reaction mixture was poured onto ice, made basic with aqueous KOH (10%) and extracted exhaustively with $CHCl_3$. The combined organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed in vacuo. Repeated silica gel chromatography afforded **18** as a poorly soluble tan solid (20 mg, 9%). mp >310°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.69 (2H, d, *J*=2.0 Hz, H-2′), 8.55 (2H, d, *J*=8.4 Hz, H-7), 8.46 (2H, m, H-2 or H-3), 8.24 (2H, m, H-2 or H-3), 8.10 (2H, d, *J*=8.5 Hz, H-10), 7.99 (2H, ddd, *J*=8.4, 6.9, 1.2 Hz, H-8), 7.86 (2H, m, H-1 and H-4) 7.83 (2H, ddd, *J*=8.4, 6.9, 1.2 Hz, H-9), 7.64 (1H, t, *J*=2.0 Hz, H-4′). FABMS *m*/*z* (%) 594 ($(M+H)^+$, 10). HRFABMS found 594.1453 (MH^+), $C_{39}H_{20}N_3O_4$ requires 594.1454.

7-(11′-Pyrido[2′,3′-*b*]acridine-5′,12′-dione)-7-carbaascididemin (19). Enamine **7** (65 mg, 0.20 mmol), Eschenmoser's salt (70 mg, 0.38 mmol) and DMF (6 mL) were heated at 105°C for 10 min under N_2. The DMF was then removed under reduced pressure and the residue was dissolved in CH_2Cl_2/MeOH (8 mL, 4:1) and poured onto glacial acetic acid (5 mL), water (1.5 mL) and NH_4Cl (732 mg, 13.7 mmol). The mixture was stirred briskly at ambient temperature for 24 h, diluted with water, made basic with aqueous KOH (10%) and extracted exhaustively with $CHCl_3$. The combined organic extract was washed with brine, dried ($MgSO_4$) and the solvent removed in vacuo. Repeated silica gel chromatography afforded **19** as a tan solid (17 mg, 32%). mp (decomp.) >310°C. IR (film) ν 2924, 1689, 1667, 1650, 1581, 1461, 1375, 1328, 1259, 1065, 1035, 992, 764 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (1H, d, *J*=8.8 Hz, H-5), 8.97 (1H, dd, *J*=4.5, 1.7 Hz, H-2′), 8.79 (2H, dd, *J*=7.9, 1.7 Hz, H-4 and H-4′), 8.71 (1H, dd, *J*=8.1, 1.3 Hz, H-1), 8.64 (1H, dd, *J*=7.8, 1.9 Hz, H-11), 8.58 (1H, d, *J*=8.5 Hz, H-9′), 7.98 (1H, ddd, *J*=8.5, 7.0, 1.5 Hz, H-2), 7.97 (1H, ddd, *J*=8.5, 7.0, 1.5 Hz, H-3), 7.91 (1H, m, H-8′), 7.88 (1H, dd, *J*=4.6, 1.8 Hz, H-9), 7.72 (1H, dd, *J*=7.9, 4.5 Hz, H-3′), 7.69 (1H, d, *J*=8.8 Hz, H-6), 7.49 (2H, m, H-7′ and H-10′), 7.13 (1H, dd, *J*=7.8, 4.6 Hz, H-10). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.5 (d, *J*=4 Hz, C-12), 182.1 (d, *J*=4 Hz, C-5′), 181.2 (s, C-12′), 157.8 (t, *J*=5 Hz, C-11′), 155.6 (ddd, *J*=183, 8, 3 Hz, C-2′), 152.4 (ddd, *J*=181, 8, 3 Hz, C-9), 152.4 (dd, *J*=11, 6 Hz, C-7b), 149.4 (dd, *J*=10, 5 Hz, C-6a′), 149.3 (dd, *J*=12, 6 Hz, C-12a′), 147.9 (s, C-5a′ or C-12a), 145.8 (s, C-12a or C-5a′), 144.7 (t, *J*=8 Hz, C-13a), 137.7 (d, *J*=8 Hz, C-7), 136.3 (dd, *J*=167, 6 Hz, C-11), 135.9 (dd, *J*=170, 6 Hz, C-4′), 133.6 (obsc, C-4b), 133.0 (dd, *J*=171, 6 Hz, C-8′), 132.9 (dd, *J*=171, 6 Hz, C-1), 132.7 (d, *J*=163 Hz, C-6), 131.8 (dd, *J*=165, 5 Hz, C-9′), 130.6 (obsc, C-7a), 130.5 (dd, *J*=162, 9 Hz, C-3), 130.1 (dd, *J*=162, 8 Hz, C-2), 129.9 (dd, *J*=162, 8 Hz, C-7′), 128.9 (obsc, C-10a′), 128.0 (dd, *J*=167, 9 Hz, C-3′), 127.9 (d, *J*=7 Hz, 4a′), 127.5 (dd, *J*=163, 7 Hz, C-10′), 127.2 (obsc, C-11a), 125.2 (m, C-4a), 125.0 (d, *J*=163 Hz, C-5), 123.3 (dd, *J*=167, 8 Hz, C-10), 123.0 (d, *J*=7 Hz, C-12b), 122.9 (s, 11a′), 122.3 (dd, *J*=161, 8 Hz, C-4). FABMS *m*/*z* (%) 543 ([$(M+H)^+$ +2H], 15), 542 ([$(M+H)^+$ +2H -H], 30) 541 ($(M+H)^+$, 45). HRFABMS found 541.1301 (MH^+), $C_{35}H_{17}N_4O_3$ requires 541.1301.

11-[2′-(Dimethylamino)ethyl]pyrido[2,3-*b*]acridine-5,12-dione (20). Dione **6** (249 mg, 0.91 mmol), Eschenmoser's salt (246 mg, 1.33 mmol) and DMF (6 mL), were heated to 120°C under N_2 for 30 min. The orange/brown reaction mixture was cooled, poured onto ice-water (100 mL) and the resulting mixture made basic with aqueous KOH (10%). The product was extracted with CH_2Cl_2 (3×50 mL), washed with brine which had been made basic with a few drops of aqueous KOH (10%), dried ($MgSO_4$) and the solvent removed in vacuo to yield **20** as an orange brown residue (285 mg, 95%), mp 158–159°C. IR (film) ν 3060, 2955, 2919, 2861, 1690, 1643, 1614, 1584, 1502, 1467, 1414, 1373, 1331, 1267, 1220, 1102, 1067, 997, 967, 767, 732, 697 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (1H, dd, *J*=4.6, 1.8 Hz, H-2), 8.68 (1H, dd, *J*=8.0, 1.8 Hz, H-4), 8.57 (1H, dd, *J*=8.4, 0.9 Hz, H-10), 8.38 (1H, dd, *J*=8.4, 0.9 Hz, H-7), 7.91 (1H, ddd, *J*=8.3, 6.8 Hz, 1.4, H-8), 7.83 (1H, ddd, *J*=8.3, 6.8, 1.4 Hz, H-9), 7.75 (1H, dd, *J*=7.8, 4.6 Hz, H-3), 4.09 (2H, bt, *J*=7.5 Hz, H-1′), 3.06 (2H, bt, *J*=8.4 Hz, H-2′), 2.72 (6H, s, NMe_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.2 (s, C-12), 181.2 (d, *J*=4 Hz, C-5), 155.7 (ddd, *J*=182, 7, 3 Hz, C-2), 152.0 (m, C-11), 149.7 (dd, *J*=11, 5 Hz, C-12a), 149.0 (dd, *J*=9, 7 Hz, C-6a), 147.3 (s, C-5a), 135.9 (dd, *J*=169, 6 Hz, C-4), 133.3 (dd, *J*=162, 9 Hz, C-8), 132.3 (dd, *J*=165, 8 Hz, C-7), 130.9 (dd, *J*=163, 8 Hz, C-9), 130.1 (obsc, C-4a), 129.2 (m, C-10a), 128.2 (dd, *J*=167, 9 Hz, C-3), 125.7 (dd, *J*=163, 7 Hz, C-10), 124.9 (obsc, C-11a), 56.8 (t, *J*=139 Hz, C-2′), 43.4 (qd, *J*=139, 4 Hz, NMe_2) 26.0 (t, *J*=131 Hz, C-1′). EIMS *m*/*z* (%) 331 (M^+, 15). HREIMS found 331.1317 (M^+), $C_{20}H_{17}N_3O_2$ requires 331.1321. Anal. calcd for $C_{20}H_{17}N_3O_2 \cdot 0.75CH_2Cl_2$: C, 63.1; H, 4.7; N, 10.6%. Found: C, 63.4; H, 4.7; N, 10.8.

Conversion of 20 to ascididemin (2). Dione **20** (926 mg, 2.8 mmol) and NH_4Cl (2.9 g, 53.2 mmol) were heated at reflux in acetic acid (41 mL) for 30 min to afford, after workup and chromatography ascididemin (541 mg, 69%) identical in all respects with the literature data.[3]

11-[2′-(Diethylamino)ethyl]pyrido[2,3-*b*]acridine-5,12-dione (21). Diethylamine hydrochloride (108 mg, 0.99 mmol), paraformaldehyde (48 mg, 1.71 mmol) and glacial acetic acid (2 mL), were heated to 70°C for 5 min. Dione **6** (98 mg, 0.36 mmol) was then added to the reaction mixture and the resulting mixture was heated for 20 min at 110°C. The orange/brown reaction mixture was cooled, poured onto ice-water (50 mL) and the resulting mixture made basic with aq NH_3 and extracted with CH_2Cl_2 (3×30 mL). The orange brown CH_2Cl_2 extract was washed with aq NH_3 (3×100mL), then brine, dried ($MgSO_4$) and the solvent removed in vacuo to yield **21** as an orange brown residue (127 mg, 99%). IR (film) ν 3065, 2984, 1690, 1639,

1608, 1580, 1562, 1503, 1462, 1402, 1375, 1334, 1270, 1211, 1102, 1065, 1024, 1001, 965, 769, 732, 714 cm^{-1}. UV (CH_3OH) λ_{max} (log ϵ) 478 nm (3.4), 293 (4.2), 272 (4.2), 231 (4.3). ^1H NMR (400 MHz, $CDCl_3$) δ 9.09 (1H, dd, J=4.6, 1.7 Hz, H-2), 8.64 (1H, dd, J=7.9, 1.7 Hz, H-4), 8.35 (1H, dd, J=8.3, 1.0 Hz, H-10), 8.32 (1H, dd, J=8.3, 1.0 Hz, H-7), 7.85 (1H, ddd, J=8.2, 7.1, 1.2 Hz, H-8), 7.73 (1H, ddd, J=8.2, 7.1, 1.2 Hz, H-9), 7.71 (1H, dd, J=7.9, 4.6 Hz, H-3), 3.90 (2H, bt, J=7.5 Hz, H-1′), 2.88 (2H, bt, J=8.4 Hz, H-2′), 2.76 (4H, q, J=7.2 Hz, $N(CH_2CH_3)_2$), 1.09 (6H, t, J=7.2 Hz, $N(CH_2CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.8 (s, C-12), 181.5 (d, J=3 Hz, C-5), 155.6 (ddd, J=183, 7, 4 Hz, C-2), 154.7 (obsc, C-11), 149.9 (dd, J=11, 5 Hz, C-12a), 148.7 (dd, J=10, 6 Hz, C-6a), 147.5 (s, C-5a), 135.6 (dd, J=169, 6 Hz, C-4), 132.7 (dd, J=163, 9 Hz, C-8), 132.4 (dd, J=166, 8 Hz, C-7), 130.0 (dd, J=162, 8 Hz, C-9), 129.9 (d, J=6 Hz, C-4a), 129.3 (m, C-10a), 127.8 (dd, J=168, 9 Hz, C-3), 125.2 (dd, J=161, 7 Hz, C-10), 125.0 (t, J=2 Hz, C-11a), 52.9 (tm, J=135 Hz, C-2′), 46.7 (tq, J=132, 4 Hz, $N(CH_2CH_3)_2$), 26.7 (t, J=132 Hz, C-1′), 12.4 (q, J=125 Hz, $N(CH_2CH_3)_2$). EIMS m/z (%) 359 (M^+, 20). HREIMS found 359.1625 (M^+), $C_{22}H_{21}N_3O_2$ requires 359.1634.

6-Methylascididemin (22). Prepared following the general procedure that we have previously communicated[7j] in 90% yield. mp (decomp.) 266–268°C. IR (film) ν 2922, 2857, 1676, 1643, 1606, 1578, 1503, 1428, 1395, 1349, 1264, 1101, 1068, 1035, 947, 764, 736 cm^{-1}. UV (MeOH) λ_{max} (log ϵ) 198 nm (4.48), 221 (4.60), 248 (4.56), 273 (sh 4.35), 300 (4.14), 338 (3.85), 391 (3.91). ^1H NMR (400 MHz, $CDCl_3$) δ=9.18 (1H, dd, J=4.8, 1.8 Hz, H-9), 8.77 (1H, dd, J=7.9, 1.7 Hz, H-11), 8.63 (1H, bd, J=8.2 Hz, H-4), 8.56 (1H, dd, J=8.2, 1.0 Hz, H-1), 8.37 (1H, bs, H-5), 7.95 (1H, td, J=8.3, 1.4 Hz, H-2), 7.88 (1H, td, J=8.2, 1.3 Hz, H-3), 7.64 (1H, dd, J=7.9, 4.7 Hz, H-10), 3.05 (3H, bs, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.0 (d, J=4 Hz, C-12), 159.8 (qd, J=5, 1 Hz, C-6), 155.6 (ddd, J=182, 7, 3 Hz, C-9), 152.4 (dd, J=12, 5 Hz, C-7b), 149.2 (s, C-7a), 146.0 (s, C-12a), 145.7 (t, J=8 Hz, C-13a), 138.5 (d, J=3 Hz, C-4b), 136.6 (dd, J=167, 6 Hz, C-11), 133.0 (dd, J=165, 7 Hz, C-1), 131.6 (dd, J=163, 8 Hz, C-2), 130.5 (dd, J=163, 8 Hz, C-3), 129.0 (d, J=8 Hz, C-11a), 125.4 (dd, J=167, 8 Hz, C-10), 123.3 (m, C-4a), 122.8 (dd, J=160, 8 Hz, C-4), 116.4 (obsc, C-12b), 115.7 (dq, J=162, 4 Hz, C-5), 25.9 (qd, J=128, 3 Hz, CH_3). EIMS m/z (%) 297 (M^+, 100). HREIMS found 297.0904 (M^+), $C_{19}H_{11}N_3O$ requires 297.0902. Anal. calcd for $C_{19}H_{11}N_3O\cdot CH_2Cl_2$: C, 63.0; H, 3.4; N, 11.0%. Found: C, 63.1; H, 3.2; N, 11.1.

6-Phenylascididemin (23). Prepared following the general procedure[7j] in 81% yield. mp >310°C. IR (film) ν 1680, 1601, 1578, 1509, 1459, 1426, 1394, 1348, 1270, 1104, 1071, 1035, 947, 873, 813, 740, 694 cm^{-1}. UV (MeOH) λ_{max} (log ϵ) 202 nm (4.67), 248 (4.63), 272 (4.57), 303 (4.56), 407 (3.88). ^1H NMR (400 MHz, $CDCl_3$) δ 9.18 (1H, dd, J=4.6, 1.8 Hz, H-9), 8.80 (1H, s, H-5), 8.75 (1H, dd, J=7.9, 1.8 Hz, H-11), 8.69 (1H, dd, J=8.1, 1.5 Hz, H-4), 8.55 (1H, dd, J=8.2, 1.4 Hz, H-1), 8.31 (2H, dm, J=8.5 Hz, H-15), 7.94 (1H, td, J=8.3, 1.3 Hz, H-2), 7.87 (1H, td, J=7.2, 1.2 Hz, H-3), 7.63 (1H, dd, J=7.9, 4.7 Hz, H-10), 7.56 (2H, t, J=7.6 Hz, H-16), 7.51 (1H, t, J=7.1 Hz, H-17). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.0 (d, J=3 Hz, C-12), 157.8 (t, J=4 Hz, C-6), 155.5 (ddd, J=181, 8, 4 Hz, C-9), 152.4 (dd, J=11, 5 Hz, C-7b), 149.5 (s, C-7a), 145.9 (s, C-12a), 145.8 (m, 13a), 138.7 (d, J=3 Hz, C-4b), 138.5 (m, C-14), 136.5 (dd, J=169, 6 Hz, C-11), 133.1 (dd, J=165, 7 Hz, C-1), 131.7 (dd, J=162, 8 Hz, C-2), 130.6 (dd, J=162, 9 Hz, C-3), 130.0 (dt, J=160, 7 Hz, C-17), 129.1 (d, J=7 Hz, C-11a), 129.0 (dd, J=161, 7 Hz, C-16), 127.9 (dt, J=161, 7 Hz, C-15), 125.4 (dd, J=167, 9 Hz, C-10), 123.7 (obsc, C-4a), 122.8 (dd, J=160, 7 Hz, C-4), 116.9 (d, J=6 Hz, C-12b), 112.7 (d, J=163 Hz, C-5). EIMS m/z (%) 359 (M^+, 100). HREIMS found 359.1069 (M^+), $C_{24}H_{13}N_3O$ requires 359.1059. Anal. calcd. for $C_{24}H_{13}N_3O$: C, 80.2; H, 3.6; N, 11.7%. Found: C, 80.0; H, 3.5; N, 11.5.

6-Styrylascididemin (24). Prepared following the general procedure[7j] in 72% yield. mp 281–283°C. IR (film) ν 1682, 1636, 1595, 1577, 1499, 1449, 1431, 1399, 1349, 1271, 1184, 1102 cm^{-1}. UV (MeOH) λ_{max} (log ϵ) 203 nm (4.74), 253 (4.65), 279 (4.61), 332 (4.79), 430 (3.98). ^1H NMR (400 MHz, $CDCl_3$) δ 9.17 (1H, dd, J=4.7, 1.7 Hz, H-9), 8.72 (1H, dd, J=7.9, 1.7 Hz, H-11), 8.61 (1H, dd, J=8.2, 1.4 Hz, H-4), 8.52 (1H, dd, J=8.1, 1.4 Hz, H-1), 8.46 (1H, s, H-5), 7.96 (1H, d, J=16.1 Hz, H-15), 7.92 (1H, ddd, J=8.3, 6.9, 1.5 Hz, H-2), 7.85 (1H, ddd, J=8.1, 7.1, 1.3 Hz, H-3), 7.66 (2H, d, J=7.2 Hz, H-17), 7.61 (1H, dd, J=7.9, 4.6 Hz, H-10), 7.53 (1H, d, J=16.1 Hz, H-14), 7.41 (2H, t, J=7.6 Hz, H-18), 7.34 (1H, t, J=7.3 Hz, H-19). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.9 (d, J=4 Hz, C-12), 156.1 (d, J=5 Hz, C-6), 155.5 (ddd, J=181, 8, 4 Hz, C-9), 152.2 (dd, J=11, 5 Hz, C-7b), 149.4 (s, C-7a), 145.8 (s, C-12a), 145.8 (m, 13a), 138.5 (d, J=3 Hz, C-4b), 136.5 (dd, J=169, 7 Hz, C-11), 136.1 (dt, J=154, 5 Hz, C-15), 136.1 (m, C-16), 133.0 (dd, J=165, 7 Hz, C-1), 131.7 (dd, J=162, 9 Hz, C-2), 130.5 (dd, J=162, 9 Hz, C-3), 129.1 (d, J=6 Hz, C-11a), 129.0 (dt, J=161, 7 Hz, C-19), 128.8 (dd, J=160, 8 Hz, C-18), 127.6 (obsc, C-14), 127.6 (obsc, C-17), 125.4 (dd, J=167, 8 Hz, C-10), 123.5 (obsc, C-4a), 122.8 (dd, J=160, 8 Hz, C-4), 117.0 (d, J=6 Hz, C-12b), 113.4 (dd, J=163, 4 Hz, C-5). EIMS m/z (%) 385 (M^+, 85), 384 (100). HRFABMS found 386.1310 (MH^+), $C_{26}H_{16}N_3O$ requires 386.1293. Anal. calcd for $C_{26}H_{15}N_3O\cdot 0.75H_2O$: C, 78.3; H, 4.2; N, 10.5%. Found: C, 78.1; H, 4.1; N, 10.4%

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