Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 1127–1140

On the synthesis of pyrinodemin A. Part 1: The location of the olefin

Stuart P. Romeril, Victor Lee,* Jack E. Baldwin* and Timothy D. W. Claridge

Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford OX1 3TA, UK

Received 30 September 2004; revised 28 October 2004; accepted 18 November 2004

Available online 10 December 2004

Abstract—The elucidation of the structure of the cytotoxic marine sponge alkaloid pyrinodemin A by synthesis is described. Based on the ^{13}C NMR spectra of three double bond positional isomers and the natural product, it is concluded the C14′–C15′ isomer best represents the true structure of pyrinodemin A. In addition, the structural assignment of pyrinodemin C is evaluated.

1. Introduction

The bioassay-guided isolation of new compounds from marine sponges has led to the discovery of a diverse array of important biologically active alkaloids.[1] They are usually obtained in very small quantities, which makes accurate structural determination challenging, as attested by the instances of structural revisions in the literature.[2] With the unfeasible or unacceptable possibility of harvesting significant quantities from the natural source, it often falls to the synthetic chemist to confirm or revise the structure of these natural products.[3]

Pyrinodemin A was isolated from an unidentified marine sponge *Amphimedon* sp. collected off Nakijin, Okinawa, Japan by Kobayashi and co-workers.[4] The natural product exhibited cytotoxicity against murine leukaemia L1210 ($IC_{50}=0.058$ μg/mL) and KB epidermoid carcinoma cells ($IC_{50}=0.5$ μg/mL) in vitro, and the structure was proposed to be **1** based on electron impact mass spectrometry (EIMS) and NMR correlations. Although macrocyclic and oligomeric 3-alkylpyridines have been isolated,[1] the *cis*-cyclopent[*c*]-isoxazolidine structural motif is unique to the pyrinodemin family of alkaloids. Pyrinodemin A was isolated along with pyrinodemins B–D, cytotoxic bis-3-alkylpyridines containing the same bicyclic ring system showing minor structural variations in the right-hand side chain, and three monomeric 3-alkylpyridine alkaloids.[5]

As part of our ongoing studies into marine sponge alkaloids within the research group, we communicated the synthesis of three double bond positional (Δ-) isomers (**1**, **2**, **3**) of pyrinodemin A (Fig. 1).[6,7] The interesting structure of the natural product has attracted the attention of others and synthetic studies have also been reported by both the Snider group[8] and more recently Morimoto et al.[9] Herein we describe in full the syntheses and characterisation of these three molecules and give our rationalisation for the true identity of the natural product.

It was proposed that structure **1** could be biosynthetically formed via intramolecular cycloaddition of alkenyl-nitrone **4** (Scheme 1). This nitrone can be derived from the condensation of aldehyde **5** with hydroxylamine **6**, which in turn can be derived from aldehyde **7**, therefore the proposed structure of pyrinodemin A can be assembled from two 3-alkylpyridine aldehydes in a biomimetic synthesis. The retrosynthetic strategy towards aldehyde **5** (corresponding to the left-hand half common to all pyrinodemins) involved installation of the 3-alkylpyridine moiety using lithiated 3-picoline (Scheme 2). The alkyl chain derives from two further sections, a protected alkynol and an α,ω-dibromoalkane to act as electrophile. The modular nature of the convergent synthesis towards **1** allows the individual preparation of the left-hand and the right-hand sides, and thus potentially the synthesis of all the pyrinodemin alkaloids.

1.1. Synthesis of aldehyde 5

Commercially available 5-hexyn-1-ol **8** was protected as its *tert*-butyldiphenylsilyl ether **9** using *tert*-butyldiphenylchlorosilane and imidazole in 96% yield (Scheme 3). Deprotonation of **9** with *n*-butyllithium followed by

Keywords: Sponge; Pyrinodemin; Alkaloids; Nitrone; EIMS.
* Corresponding authors. Tel.: +44 1865 275693; fax: +44 1865 275632; e-mail addresses: victor.lee@chem.ox.ac.uk; jack.baldwin@chem.ox.ac.uk

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.051

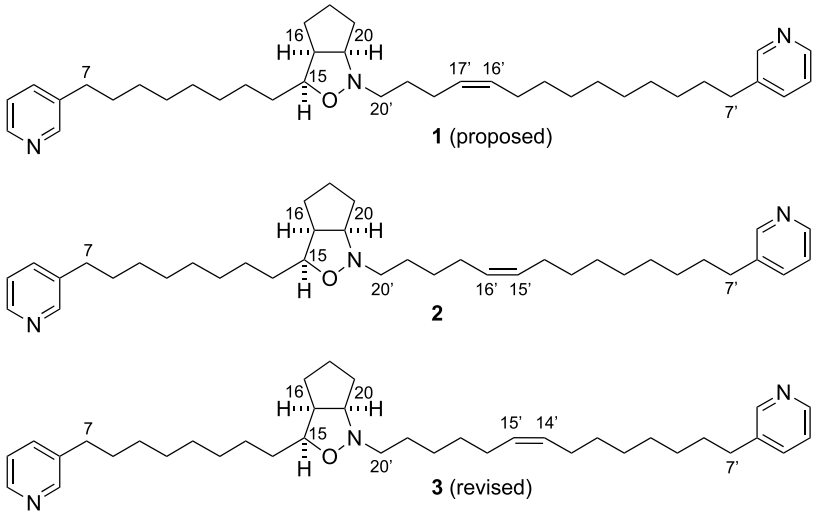
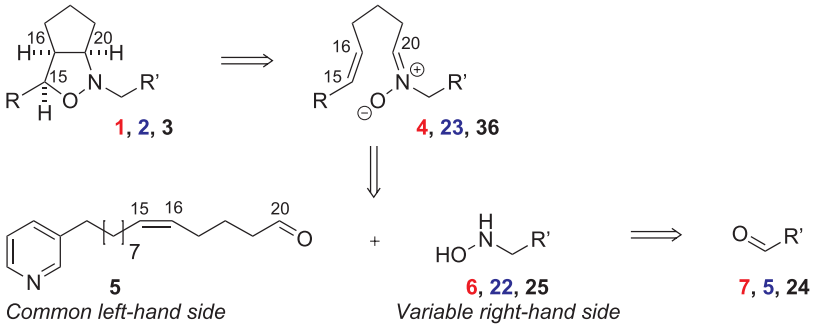
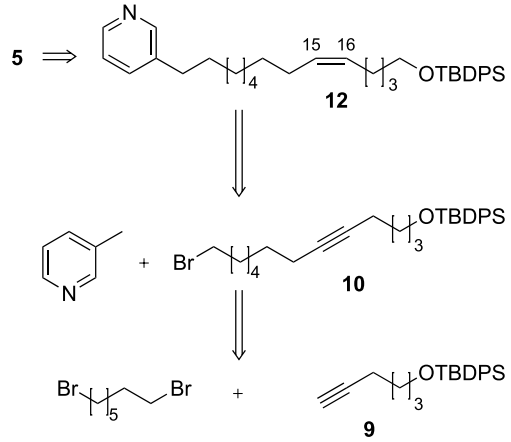


Figure 1. The three isomers of pyrinodemin A synthesised in this work.



Scheme 1. Retrosynthetic analysis of pyrinodemin A isomers **1**, **2** and **3**. For **1**, **4**, **6**, **7** R=Py$(CH_2)_8$ and R′=$(CH_2)_2$HC=CH$(CH_2)_9$Py; for **2**, **23**, **22**, **5** R′=$(CH_2)_3$HC=CH$(CH_2)_8$Py; for **3**, **36**, **25**, **24** R′=$(CH_2)_4$HC=CH$(CH_2)_7$Py.

addition of the acetylide anion to an excess of 1,7-dibromoheptane (prepared from 1,7-heptanediol and conc. HBr at reflux)[10] in 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1*H*)-pyrimidinone (DMPU)[11] gave **10** in 68% yield along with recovered starting material and dialkyation product (Section 1.5). Lindlar semi-hydrogenation revealed the *Z*-alkene **11** in 94% yield.[12] Displacement of the primary bromine using lithiated 3-picoline (generated using LDA in THF/DMPU)[13] gave **12** in 63% yield. Due to concerns over the work up and purification of the polar alcohol **13** in the presence of the tetra-*n*-butyl ammonium fluoride cation, ammonium fluoride in methanol[14] was used in preference to TBAF, and the desilylation proceeded in 97% yield. Oxidation using 2-iodoxybenzoic acid (IBX) in DMSO and THF delivered aldehyde **5** in 92% yield.[15]



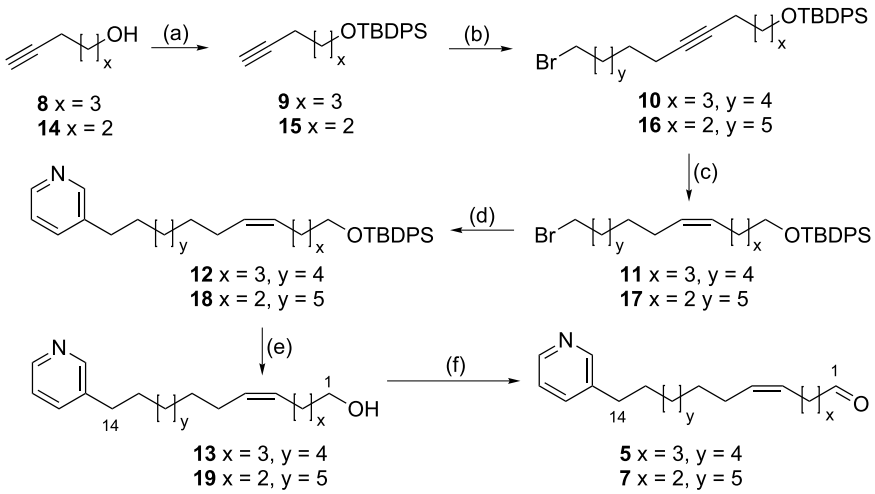
Scheme 2. Retrosynthetic analysis of aldehyde **5**.

1.2. Synthesis of aldehyde 7

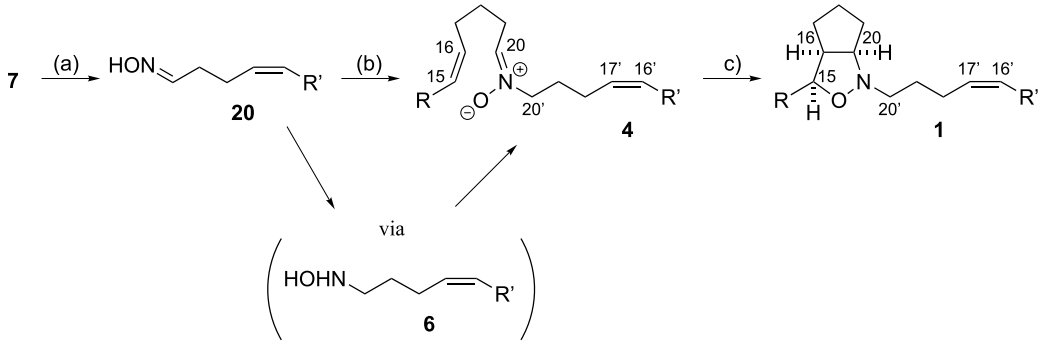
The synthesis commenced with the protection of the homologous 4-pentyn-1-ol **14** as its silyl ether **15** in 93% yield (Scheme 3). Deprotonation, followed by alkylation with excess 1,8-dibromooctane delivered **16** in 77% yield, which was subjected to Lindlar hydrogenation to give alkene **17** in 97% yield. Treatment with lithiated 3-picoline produced alkylpyridine **18** in 59% yield, deprotection with ammonium fluoride gave alcohol **19** in 97% yield, and subsequent IBX oxidation afforded the aldehyde **7** in 93% yield.

1.3. Synthesis of the C16′–C17′ isomer of pyrinodemin A (1)

With both aldehyde components in hand, the next step involved the biomimetic coupling of both aldehydes with a molecule of hydroxylamine. Aldehyde **7** was condensed



Scheme 3. Synthesis of aldehydes **5** and **7**. Reagents and conditions: (a) TBDPSCI, imidazole, THF, 96% for **9**, 93% for **15**; (b) *n*-BuLi, THF, then $Br(CH_2)_{y+3}Br$, DMPU, 68% for **10**, 77% for **16**; (c) Lindlar catalyst, quinoline, H_2, PhH, 94% for **11**, 97% for **17**; (d) 3-Picoline, LDA, THF, DMPU, 63% for **12**, 59% for **18**; (e) NH_4F, MeOH, 97% for **13**, 97% for **19**; (f) IBX, DMSO, THF, 92% for **5**, 93% for **7**.



Scheme 4. Synthesis of isomer **1**. $R=(CH_2)_8Py$, $R'=(CH_2)_9Py$. Reagents and conditions: (a) $NH_2OH\cdot HCl$, MeOH, NaOAc, 93%; (b) (i) $NaCNBH_3$, MeOH, pH 3; (ii) **5**, Na_2SO_4, DCM, 89% over two steps; (c) PhH, reflux, 41%.

with hydroxylamine hydrochloride using sodium acetate as base in methanol to give oxime **20** in 93% yield (Scheme 4). The product was identified as a ca. 1:1 mixture of *Z*:*E* oxime isomers from ^{1}H NMR, however this ratio was inconsequential as subsequent reduction with sodium cyanoborohydride delivered hydroxylamine **6**. 4-Ene-1-hydroxylamines have been shown to be thermally unstable,[16] decomposing via a reverse-Cope mechanism to give secondary hydroxylamines,[17] so the product was condensed immediately (without purification) with 1 equiv of aldehyde **5** in the presence of sodium sulphate to afford nitrone **4** in 89% yield over two steps. Heating nitrone **4** under reflux in benzene at high dilution (to promote intramolecular cyclisation) delivered a less-polar product in 41% yield. Mass-spectrometry indicated that the product was isomeric with nitrone **4** and ^{1}H NMR showed the disappearance of the distinctive triplet of the nitrone proton indicating that cyclisation had taken place.

The cyclised product of the reaction could be potentially any of four isomers of **1** (cycloaddition of the nitrone with either olefin and with either regioselectivity), therefore high-field (500 MHz) NMR experiments were required to confirm the structure (Fig. 2). HSQC-TOCSY[18] (τ_m=80 ms) indicated that C-20′ correlates with H-19′, H-18′ and H-17′ (in the olefinic region), thus locating the olefin between C-16′ and C-17′; HSQC-TOCSY and DQF-COSY confirmed the ring sizes in the bicyclic core; and 1D DPFGSE-NOESY[19] (τ_m= 400 ms) confirmed the *cis*-relationship of H-15 and the ring-junction protons H-16 and H-20. The product was thus assigned unambiguously as the C-16′, C-17′ isomer of pyrinodemin A (**1**).[20]

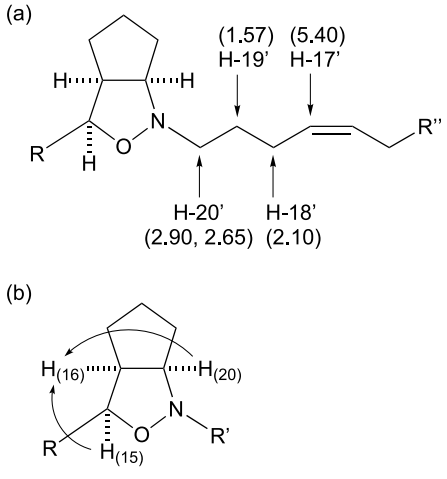
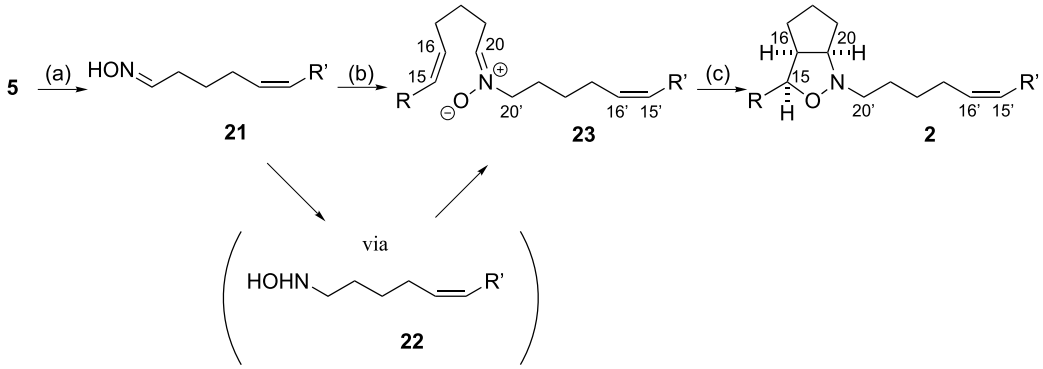


Figure 2. Structural determination of structure **1**.



Scheme 5. Synthesis of isomer **2**. R = $(CH_2)_8$Py, R′ = $(CH_2)_8$Py. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, MeOH, NaOAc, 84%; (b) (i) $NaCNBH_3$, MeOH, pH 3; (ii) **5**, Na_2SO_4, DCM, 71% over two steps; (c) PhH, heat, 63%.

The spectra of synthetic **1** and pyrinodemin A were similar and analysis of the ^1H and ^{13}C NMR spectra suggested that the gross structure (i.e., the central 5,5-bicyclic core and the 3-alkylpyridine chains) had been correctly assigned, however the olefinic and allylic regions showed some anomalies. Kobayashi reported that both the olefinic carbons and the allylic carbons appeared as coincidental peaks at 129.3 and 27.1 ppm, respectively. For synthetic **1** the olefinic carbons (C-16′ and C-17′) occur as distinct peaks at 129.2 and 130.3 ppm ($\Delta\delta = 1.1$), and the allylic carbons C-15′ and C-18′ at 27.1 and 24.8 ppm, respectively. The typical allylic carbon chemical shift for an *E*-olefin is ca. 31 ppm, and that for a *Z*-olefin is ca. 27 ppm.[21] Hence the observed spectral discrepancies did not originate from a mistakenly assigned C-16′-C17′ double bond geometry. The chemical shift deviation of C-18′ can be attributed to the proximity of the isoxazolidine nitrogen exerting a γ-shielding effect. Based on these data it was concluded that the proposed structure for the natural product was incorrect: structure **1** was not pyrinodemin A but rather a Δ-isomer. It was also concluded that the olefin must lie towards the centre of the chain, away from the isoxazolidine in a more magnetically symmetrical environment.

1.4. Synthesis of the C15′–C16′ isomer of pyrinodemin A (2)

The requirement for the olefin to reside further from the isoxazolidine would be satisfied by structure **2**, which derives from 2 equiv of aldehyde **5** (Scheme 1). This isomer is a more plausible candidate from a biosynthetic perspective, as it involves the coupling of two identical aldehyde units. This hypothesis was further encouraged by the isolation of oxime **21** from the same sponge extract as pyrinodemin A,[5] and we speculated that this oxime could be a biosynthetic precursor to pyrinodemin A. The proposed synthesis was put into practice and hydroxylamine hydrochloride was condensed with aldehyde **5** in the presence of sodium acetate to produce oxime **21** in 84% yield (Scheme 5). Reduction with sodium cyanoborohydride gave hydroxylamine **22** that was immediately condensed with a further equivalent of aldehyde **5** to form nitrone **23** in 71% yield over two steps. Thermal cyclisation of this nitrone gave a less-polar product in 63% yield: the structure of which was established as **2** by the same NMR experiments indicated above. The ^1H NMR and EIMS spectra were very similar to those of pyrinodemin A, however the ^{13}C NMR spectrum did not show coincidental olefinic or allylic carbon peaks: the olefinic carbons resonated at δ_C 130.0 and 130.4 ($\Delta\delta = 0.4$) and the allylic carbons at δ_C 27.0 and 27.1. In spite of the apparent similarities between pyrinodemin A and **2**, we observed two distinct and fully resolved peaks for both the allylic and olefinic carbons. Therefore, the critical regions of the spectra were inconsistent and we concluded that the C15′–C16′ isomer **2** was not the correct structure for pyrinodemin A.

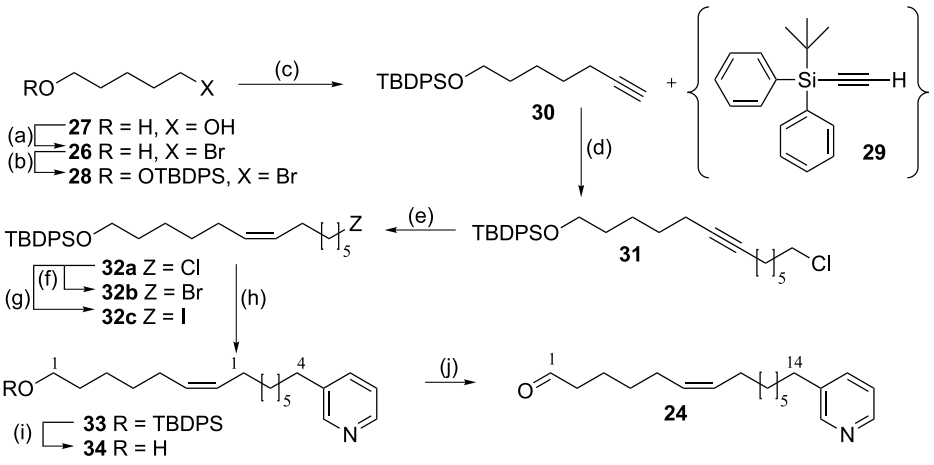
Independently and concurrently, Snider and Shi prepared structures **1** and **2**. Their results were identical (within a small experimental error) to those presented here (Table 1). They observed, but did not rationalise the non-coincident olefinic peaks and concluded that the double bond position in natural pyrinodemin A was at C15′–C16′. They described isomer **2** as 'probably' the structure of pyrinodemin A.[8]

1.5. Synthesis of aldehyde 24

We concluded that the magnitude of the difference in the chemical shifts of the olefinic carbons diminished as the olefin was moved further away from the central core, and therefore, set about the synthesis of the C14′–C15′ isomer of pyrinodemin A (**3**) This isomer can be derived from aldehyde **5** (as prepared above) and aldehyde **24** (via hydroxylamine **25**) according to our modular strategy (Scheme 1). It was envisaged that aldehyde **24** could be

Table 1. Comparison of the assignments of Snider and this work with pyrinodemin A

Isomer	δ_C Olefinic C (ppm)	$\Delta\delta$ (ppm)	δ_C Allylic C (ppm)
Pyrinodemin A[4]	129.3 (2C)	0	27.1 (2C)
C16′–C17′ **1** (Baldwin)[6]	129.2, 130.3	1.1	24.8, 27.1
C16′–C17′ **1** (Snider)[8]	129.3, 130.3	1.0	24.9, 27.0
C15′–C16′ **2** (Baldwin)[6]	129.5, 129.9	0.4	27.0, 27.1
C15′–C16′ **2** (Snider)[8]	129.6, 130.0	0.4	27.1, 27.2



Scheme 6. Synthesis of aldehyde **24**. Reagents and conditions: (a) $HBr_{(aq.)}$, toluene, reflux, 60%; (b)TBDPSCl, imidazole, THF, 96%; (c) LiC≡CH·eda, DMSO, THF, 84%; (d) *n*-BuLi, THF, DMPU, then $Cl(CH_2)_6I$, 85%; (e) Lindlar catalyst, H_2, quinoline, benzene, 83%; (f) NaI, acetone, reflux, 95%; (g) LiBr, butanone, reflux, 96%; (h) 3-picoline, LDA, DMPU, THF, 79% for **31a**, 63% for **31b**, 36% for **31c**; (i) NH_4F, MeOH, 95%; (j) IBX, DMSO, THF, 93%.

assembled using a similar synthetic route to the isomeric aldehydes **5** and **7**.

The corresponding alkynol (6-heptyn-1-ol) was not commercially available, so 5-bromopentan-1-ol **26** (obtained from 1,5-pentanediol **27** by selective monobromination)[22] was protected as its *tert*-butyldiphenylsilyl ether **28** in 96% yield, before conversion to the terminal alkyne (Scheme 6). The displacement of the bromide with lithium acetylide (stabilised as the ethylenediamine complex) at room temperature in DMSO was attempted,[23] however the yields were poor and inconsistent. Purification of the crude revealed the presence of *tert*-butyldiphenylsilyl ethyne **29**,[24] leading us to speculate that desilylation occurred due to the inefficient heat dissipation in the viscous liquid resulting in localised exotherms. In order to reduce the reaction temperature and decrease the viscosity, THF was added as a co-solvent. With precise temperature control at −5 °C and a slow addition of alkylbromide to the acetylide, the desilylation was avoided and the terminal alkyne **30** was delivered in 84% yield.

In the syntheses of the two previous fragments (aldehydes **5** and **7**), alkylation of a lithiated acetylene with a dibromide had been a key reaction. The starting material and the two products (mono and dialkylation of the dibromide) all had very similar R_f values and therefore the purification of the desired product was technically demanding. Although the reaction conditions were optimised, a small yet significant amount of dialkylation was observed (Table 2). It was decided to employ the commercially available 1-chloro-6-iodohexane instead, which gave a faster more efficient reaction. The alkylation was completely regioselective and no dialkylation product was detected. Deprotonation of acetylene **30** with *n*-butyllithium in THF/DMPU followed by addition of 2 equiv of 1-chloro-6-iodohexane afforded **31** in 85% yield, and subsequent Lindlar hydrogenation produced alkene **32a** in 83% yield.

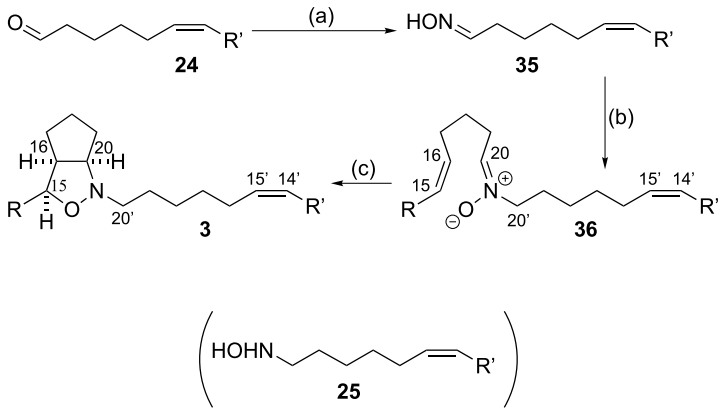
Table 2. Comparison of alkyne-anion alkylations

Alkynol	Electrophile	Alkynol (%)	Product (%)	Dialkylation (%)
TBDPSO (4, 5) **15**	$Br(CH_2)_8Br$ 4 equiv	4	77	4
TBDPSO (5, 6) **9**	$Br(CH_2)_7Br$ 4 equiv	9 (est.)[a]	68	4
TBDPSO (6, 7) **29**	$I(CH_2)_6Cl$ 2 equiv	5 (est.)[a]	85	—

[a] Yields of alkynol were estimated from the ^1H NMR of mixed fractions.

Table 3. Comparison of strategies towards 3-alkylpyridine **32**

Sequence X	Yield of Finkelstein reaction (%)	Yield of displacement reaction (%)	Overall yield (%)
Cl→I→CH_2Py	95	36	34
Cl→Br→CH_2Py	96	63	60
Cl→CH_2Py	—	79	79



Scheme 7. Synthesis of isomer **3**. R=$(CH_2)_8$Py, R′=$(CH_2)_7$Py. Reagents and conditions: (a) $NH_2OH\cdot HCl$, MeOH, NaOAc, 90%; (b) (i) $NaCNBH_3$, MeOH, pH 3; (ii) **5**, Na_2SO_4, DCM, 79% over two steps; (c) PhH, heat, 87%.

It had been proposed to transform the alkylchloride **32a** to the alkyliodide **32c** via a Finkelstein reaction[25] to improve the yield of the subsequent nucleophilic substitution with lithiated 3-picoline. Although halogen exchange was efficient, the yield of the subsequent S_N2 displacement reaction by lithiated 3-picoline was poor, proceeding at best in 36% yield, and typically in the range 10–20% yield. This was surprising given the efficient conversion observed by others for this reaction on similar substrates.[12,26] Conversion of **32a** to the alkylbromide **32b** and subsequent lithiated 3-picoline displacement gave moderate yields, consistent with an alkylation of this nature (vide supra), however using the original alkylchloride **32a** as a substrate the transformation proceeded cleanly in good yield, providing a superior route towards **33** (Table 3). Ammonium fluoride desilylation of **33** was achieved in 95% yield and subsequent oxidation with IBX in DMSO/THF delivered aldehyde **24** in 93% yield.

1.6. Synthesis of the C14′–C15′ isomer of pyrinodemin A (3)

Condensation of aldehyde **24** with hydroxylamine hydrochloride in the presence of sodium acetate in methanol gave oxime **35** in 90% yield (Scheme 7) Reduction with sodium cyanoborohydride in methanol at pH 3 gave hydroxylamine **25** and subsequent condensation with aldehyde **5** afforded nitrone **36** in 79% yield over two steps. Thermal cyclisation of nitrone **36** at high dilution delivered the C14′–C15′ isomer **3** in 87% yield. The compound was fully characterised:[27] the olefinic carbons were observed as two partially resolved peaks at 129.90 and 129.92 ppm ($\Delta\delta$ 0.02),[28] the allylic carbons exhibited a single resonance at 27.1 ppm and the signal distribution between 20 and 40 ppm appeared to be a better match with the published data than the two isomers previously prepared. As the available ^{13}C assignments for pyrinodemin A have been reported to only one decimal place, we conclude that this isomer is indistinguishable from the natural product. It is necessary to qualify that we cannot be absolutely certain that **3** is the true structure of pyrinodemin A until all other possible isomers have been discounted by synthesis or preferably by the re-isolation and characterisation of a pure authentic sample of the natural product using more reliable physical methods.

2. Morimoto's results

Morimoto et al. recently prepared isomers **2**, **3** and the C13′–C14′ isomer of pyrinodemin A, and based on the ^{13}C NMR $\Delta\delta$ values shown in Table 4, concluded that isomer **3** was the correct structure of pyrinodemin A.[9] Although the absolute ^{13}C NMR values do vary slightly, the $\Delta\delta$ values appear to be highly consistent between the synthetic groups. Morimoto's results rule-out the C13′–C14′ isomer as a candidate for the structure of pyrinodemin A, however it is curious that this isomer with the olefin in the exact centre of the chain should have such a high $\Delta\delta$ value. The result was not rationalised by the authors, however is presumably due to a conformational effect and it follows that other Δ-isomers (e.g., the C12′–C13′ isomer) could possess spectroscopic properties indistinguishable from the natural product.

Table 4. Morimoto's data in comparison to pyrinodemin A

Isomer	δ_C Olefinic C (ppm)	$\Delta\delta$	δ_C Allylic C (ppm)
Pyrinodemin A	129.3 (2C)	0	27.1 (2C)
C15′–C16′ (**2**)	129.6, 130.0	0.4	Unassigned
C14′–C15′ (**3**)	129.77, 129.79	0.02	Unassigned
C13′–C14′	129.6, 129.9	0.3	Unassigned

Data taken from Ref. 9.

3. EIMS analysis

Kobayashi assigned the location of the olefin in pyrinodemin A based on the EIMS fragmentation pattern shown in Figure 3.[4] The use of EIMS alone to ascertain the position of the olefin in an aliphatic chain is not a reliable technique (vide supra). It is known that alkene ions show a tendency to isomerise under EIMS conditions, and that the spectra of Δ-isomers are similar irrespective of double bond position.[29] The standard method to locate an olefin in an aliphatic chain is to functionalise the olefin, then examine the derivative using chemical ionisation.[30,31] More recently, collision activated decomposition in tandem mass spectrometry has been applied to natural products, without the

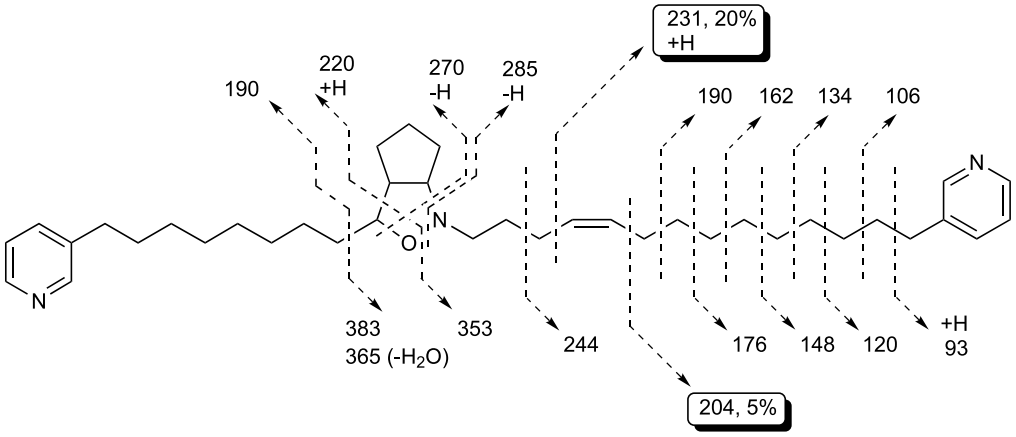


Figure 3. EIMS fragmentation pattern of the proposed structure of pyrinodemin A taken from Ref. 4. The fragments assigned as cleavage adjacent to the olefin are highlighted.

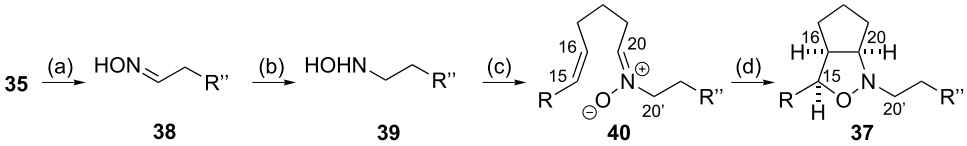
need for derivitisation.[32,33] In spite of this, the EIMS spectrum is the only other piece of indicative data available from the natural product, and it would be inadequate to neglect an analysis.

Kobayashi's EIMS spectrum of pyrinodemin A shows an $(M+2)^+$ peak at a higher intensity to the $(M+1)^+$ isotope peak.[34] This observation strongly suggested that the natural sample was contaminated with the dihydro analogue of pyrinodemin A **37**, possibly a natural product in its own right, inseparable from pyrinodemin A by the HPLC method used. In order to identify the contaminant peaks, and to provide a reference sample for the unsaturated isomers, analogue **37** was synthesised. It was hoped that the EIMS spectra of **37** and the three isomers already prepared would enable us to conduct a proper analysis of the EIMS data of the natural product.

The synthesis of **37** commenced from unsaturated oxime **35** which was selectively hydrogenated over palladium (5% on carbon) to give oxime **38** in 94% yield and subsequently reduced to the hydroxylamine **39** with sodium cyanoborohydride in 74% yield (Scheme 8). Condensation with aldehyde **5** gave nitrone **40** in 67% yield, and cyclisation produced the saturated analogue of pyrinodemin A (**37**) in 73% yield. High-resolution NMR experiments showed the presence of the 5,5-bicyclic isoxazolidine confirming the structure of the product as **37**.

The EIMS spectrum of any pyrinodemin A isomer is complicated by a contribution to intensity of peaks at $m/z \leq 190$ from fragmentation of the left-hand side of the molecule. Using the saturated analogue as a reference, it was possible to identify those peaks arising from the fragmentation of the right-hand side, and compare the intensities of these peaks for the different isomers (Table 5).

None of the EIMS spectra of the synthetic samples matched exactly that of the natural product but both the C15′–C16′ and the C14′–C15′ isomers (**2** and **3**) showed a reasonable fit. It should be noted that the natural product and synthetic samples were recorded on different instruments, which generates an experimental error. This is exemplified by the EIMS spectra of the C15′–C16′ isomer (**2**) recorded by Snider, Morimoto and ourselves where even significant peaks show a three-fold variation in intensity (e.g., *m/z* 231: Snider, 15%;[8] Morimoto, 48%;[9] this work, 29%). Given the experimental error inherent in this comparison, we conclude that anything more than this broad analysis is unjustified.



Scheme 8. Synthesis of saturated analogue **35**. R = $(CH_2)_8$Py, R″ = $(CH_2)_{12}$Py. Reagents and conditions: (a) Pd (5% on C), H_2, MeOH, 94%; (b) (i) $NaCNBH_3$, MeOH, pH 3, 74%; (ii) **5**, Na_2SO_4, DCM, 67% over two steps; (c) PhH, heat, 73%.

Table 5. EMIS data for pyrinodemin A and synthetically prepared Δ-isomers

m/z	Pyrinodemin A[a]	C16′–C17′ isomer (**1**)	C15′–C16′ isomer (**2**)	C14′–C15′ isomer (**3**)	C13′–C14′ isomer (Morimoto)[a]
231	20	2, (232) 6	29	30	0
244	25	16	43	18	12
259	10	15, (258) 40	6	27	37
270	17	11	18	23	17
285	42	43	49	82	75

[a] Data taken from Ref. 9.

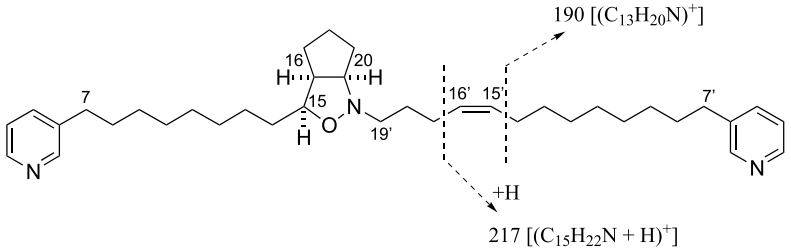


Figure 4. The EIMS fragmentation peaks used to assign the position of the olefin in pyrinodemin C (taken from Ref. 5).

4. A note on the structure of pyrinodemin C

Pyrinodemin C is a homologue of pyrinodemin A shorter by one-carbon in the right-hand alkyl chain. The position of the olefin was deduced by the application of the same EIMS analytical method (Fig. 4) as for pyrinodemin A (in this case from fragment ions *m*/*z* 190 and 217)[35] and assigned three methylenes from the isoxazolidine nitrogen. The only published ^{13}C NMR data for this compound are the allylic carbon chemical shifts (deduced from HMQC cross peaks), both of which are assigned as δ_C ca. 27 ppm. The results and rationalisation presented above suggest that the position of the olefin has been incorrectly assigned in pyrinodemin C and that a structural revision may be necessary for this natural product.

5. Conclusion

Prior to our communication on the synthesis of isomer **3**,[7] Snider proposed that the observed ^{13}C spectral discrepancy between natural pyrinodemin A and isomer **2** was unlikely to be due to the position of the olefin, but instead caused by concentration, pH or different parameters in the acquisition of the NMR spectra and maintained that isomer **2** was probably the correct structure of natural pyrinodemin A.[36] Experiments have shown that the olefinic ^{13}C NMR $\Delta\delta$ value of isomer **3** remains unchanged at low concentrations (1 mg), and furthermore our work on monomeric 3-alkylpyridine alkaloids has demonstrated that the chemical shifts of the pyridine aromatic protons are very sensitive to the presence of acid in the solution.[37] The spectra of pyrinodemin A and its synthetic isomers are not consistent with the presence of acid in the sample. If the olefinic ^{13}C chemical shifts of (natural) pyrinodemin A were sensitive to NMR acquisition parameters, then it would be difficult to explain the consistency of the olefinic $\Delta\delta^{13}$C as observed by the Baldwin, Morimoto and Snider groups for compounds **1** and **2**. In light of the smaller $\Delta\delta$ value observed with isomer **3** we believe that the ^{13}C spectral difference between natural pyrinodemin A and isomer **2** is due to the position of the olefin rather than the aforementioned factors or parameters.

In summary we have synthesised and characterised three Δ-isomers of pyrinodemin A. Based on comparisons of the ^{1}H and ^{13}C NMR data with those of the natural product, we believe that the C14′–C15′ isomer **3** is the best candidate for the structure of pyrinodemin A. There may however, be other isomers possessing spectroscopic properties indistinguishable from pyrinodemin A. In order to ascertain the true structure of the natural product all possible Δ-isomers would have to be synthesised or ideally, an authentic sample re-isolated and characterised using unambiguous techniques. Until these endeavours have been undertaken, we believe that the C14′–C15′ isomer (**3**) should be accepted as the structure of pyrinodemin A, and we have used this as a target for the asymmetric synthesis of the natural product.[38] In addition, analysis of the EIMS spectrum of pyrinodemin C also revealed that there may be a fundamental flaw in its structural assignment.

6. Experimental

6.1. General procedures

Proton magnetic resonance spectra were recorded on Brüker AC200 (200 MHz), Brüker DPX400 (400 MHz), Brüker DQX400 (400 MHz), Brüker AMX500 (500 MHz), and Brüker DRX500 (500 MHz), spectrometers at ambient temperature. Proton spectra assignments are supported by ^{1}H–^{1}H correlations (COSY) and by ^{1}H–^{13}C correlations (HMQC) where necessary. Chemical shifts (δ_H) are reported in parts per million (ppm) and are referenced to the residual solvent peak. Coupling constants (*J*) are reported to the nearest 0.5 Hz. Carbon magnetic resonance spectra were recorded on Brüker AC200 (50.3 MHz), Brüker DPX400 (100.6 MHz), Brüker DQX400 (100.6 MHz), Brüker AMX500 (125.8 MHz), and Brüker DRX500 (125.8 MHz), spectrometers at ambient temperature. Chemical shifts (δ_C) are reported in parts per million (ppm) and are referenced to the residual solvent peak. Carbon spectra assignments are supported by DEPT or APT analysis and ^{13}C–^{1}H correlations (HSQC) where necessary. The ^{13}C spectra of the 5,5-bicyclic isoxazolidine structures synthesised showed some broad peaks particularly those proximate to the ring nitrogen. The chemical shift δ_C of those assigned from HSQC and HSQC-TOCSY analysis are indicated with the symbol (≈) and are quoted to the nearest ppm, those not observed using either technique are indicated as ‘not observed’. Superscript ‘a’ denotes one of a pair of diastereotopic protons.

Infrared spectra were recorded on a Perkin–Elmer Paragon 1000 Fourier Transform spectrometer between NaCl plates. Absorption maxima (ν_{max}) of the major peaks are reported in wavenumbers (cm^{-1}). Low-resolution mass spectra were recorded using a TRIO-1 GCMS spectrometer, a Micromass Platform (APCI) spectrometer, Micromass Autospec spectrometer (CI^+) and a micromass ZAB spectrometer (CI^+, EI). Only molecular ions (M^+), protonated molecular ions (MH^+), fragments from molecular ions and other major peaks are reported. High-resolution mass spectra were recorded on a Micromass Autospec spectrometer and are

accurate to ±10 ppm. Electrospray mass spectra were recorded on a Waters Micromass LCT spectrometer. Microanalyses were carried out by Elemental Microanalysis Limited, and are reported to three significant figures. Melting points were measured using a Cambridge Instruments Gallen™ III hot stage melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed using Merck aluminium foil backed plates precoated with silica gel 60 F_{254} (1.05554). Visualisation was by the quenching of UV fluorescence ($\lambda_{max}=254$ nm); staining with 10% w/v ammonium molybdate in 1 M sulphuric acid, 20% w/v phosphomolybdic acid in ethanol, 3% w/v ninhydrin in 97:3 *n*-BuOH/AcOH, followed by heating; or iodine on silica. Retention factors (R_f) are reported to 2 decimal places. Flash chromatography was performed using ICN silica 32–63, 60 Å. All reactions were carried out under a positive atmosphere of argon at room temperature unless otherwise specified.

Anhydrous diethyl ether, and THF were obtained by distillation from sodium/benzophenone ketyl under nitrogen, anhydrous DCM was distilled from calcium hydride under nitrogen. PE refers to the fraction of light petroleum ether boiling between 40 and 60 °C, and was distilled before use. Benzene, diisopropylamine, hexane, 3-methylpyridine, pyridine, triethylamine, and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) were distilled from calcium hydride under argon or reduced pressure and stored over 4 Å molecular sieves under argon until used. Methanol was distilled from 4 Å molecular sieves under reduced pressure and stored over 4 Å molecular sieves under argon until used. Dimethyl sulphoxide was dried over 4 Å molecular sieves. Imidazole was dried under vacuum before use.

6.1.1. Preparation of the C16′–C17′ isomer of pyrinodemin A 1. A solution of nitrone **4** (0.286 g, 0.50 mmol) in benzene (250 mL) was heated at reflux for 24 h. The solvent was removed in vacuo, and the crude product was purified by flash chromatography (100% EtOAc) to yield **1** (0.118 g, 0.21 mmol, 41%) as a colourless oil; $R_f=0.13$ (100% EtOAc); ν_{max}/cm^{-1} (thin film) 2927 (s), 2854 (s), 1574 (m), 1478 (m), 1464 (m), 1422 (m), 1026 (m); *m/z* Probe APCI$^+$ (NH_3) 574 (MH^+, 100%); HRMS found 574.4735, $C_{38}H_{60}N_3O$ requires 574.4736; *m/z* Probe EIMS (+ve ion) 574 (1%), 556 (2%), 530 (9%), 515 (6%), 365 (28%), 355 (10%), 327 (9%), 315 (5%), 299 (15%), 285 (43%), 270 (11%), 259 (15%), 258 (40%), 244 (16%), 232 (6%), 231 (2%), 220 (20%), 218 (11%), 204 (6%), 190 (28%), 176 (44%), 162 (17%), 148 (20%), 136 (20%), 134 (14%), 120 (27%), 106 (100%), 93 (89%); δ_H (500 MHz, CD_3OD) 1.27–1.37 (24H, H-8 to H-12, H-8′ to H-14′), 1.33, 1.42 (2H, m, H-13), 1.46 (2H, m, H-18), 1.48 (1H, m, H-17), 1.50 (2H, m, H-14), 1.57 (2H, m, H-19′), 1.63 (1H, m, H-17), 1.65, 1.80 (2H, m, H-19), 2.03 (2H, m, H-15′), 2.10 (2H, m, H-18′), 2.65 (1H, m, H-20′), 2.65 (4H, t, $J=7.5$ Hz, H-7, H-7′), 2.85-2.95 (2H, m, H-16, H-20′^{a}), 3.50–3.60 (1H, m, H-20), 4.10–4.20 (1H, m, H-15), 5.40 (2H, m, H-17′, H-16′), 7.30–7.40 (2H, m, Py-*H*), 7.65–7.75 (2H, d, $J=8.0$ Hz, Py-*H*), 8.30–8.40 (4H, br, s, Py-*H*); δ_C (125.8 MHz, CD_3OD) 26.4, 27.8, 27.9, 28.7, 29.38, 30.8, 30.9, 31.0, 31.1, 31.2, 31.3, 31.4 (18CH_2), 32.8, 34.3 (4C, C-13, C-14, C13′, C14′), 51.5 (1C, C-16), 58.1 (1C, C-20′), 74.5 (1C, C-20), 79.6 (1C, C-15), 125.6 (2C, Py C-5*), 130.7, 131.9 (2CH, C-16′, C-17′), 138.4 (2C, Py C-4*), 140.6 (2C, Py C-3*), 148.0, 150.5 (4C, Py C-2*, C-6*).

δ_H (500 MHz, $CDCl_3$) 1.22–1.34 (20H, H-9 to H-12, H-9′ to H-14′), 1.34–1.49 (4H, m, H-13, H-18), 1.49–1.80 (12H, m, H-8, H-14, H-17, H-19, H-8′, H-19′), 1.91–2.16 (4H, m, H-15′, H-18′), 2.53–2.65 (1H, m, H-20′), 2.59 (4H, t, $J=7.7$ Hz, H-7, H-7′), 2.78–2.87 (2H, m, H-16, H-20′^{a}), 3.41–3.51 (1H, m, H-20), 4.01–4.08 (1H, m, H-15), 5.28–5.39 (2H, m, H-16′, H-17′), 7.16–7.22 (2H, m, Py-*H*), 7.43–7.50 (2H, m, Py-*H*), 8.38–8.50 (4H, br, s, Py-*H*); δ_C (125.8 MHz, $CDCl_3$) 24.8 (1C, C-18′), 26.2, 26.4 (2C, C-17, C-18), 26.9 (1C, C-13), 27.1 (1C, C-15′), 27.9, 28.7, 29.0, 29.2, 29.3, 29.4, 29.6 (13C, C-9 to C-14, C-9′ to C-14′, C-19′), 31.0 (2C, C-8, C-8′), 32.9 (2C, C-7, C-7′), 49.8 (1C, C-16), ≈57 (1C, C-20′), ≈73 (1C, C-20), ≈78 (1C, C-15), 123.1 (2C, Py C-5*), 129.2, 130.3 (2CH, C-16′, C-17′), 135.6 (2C, Py C-4*), 137.8 (2C, Py C-3*), 148.0, 149.8 (4C, Py C-2*, C-6*).

6.1.2. Preparation of the C15′–C16′ isomer of pyrinodemin A 2. A solution of nitrone **22** (0.142 g, 0.25 mmol) in benzene (100 mL) was heated at reflux for 18 h. The solvent was removed in vacuo to afford the crude product which was purified by flash chromatography (100% EtOAc) to yield **2** (0.089 g, 0.16 mmol, 63%) as a colourless oil; $R_f=0.30$ (100% EtOAc); ν_{max}/cm^{-1} (thin film) 2928 (s), 2855 (s), 1575 (m), 1478 (m), 1465 (m), 1422 (m), 1337 (w), 1189 (w), 1026 (m), 793 (w), 714 (s); *m/z* Probe APCI$^+$ (NH_3) 574.6 (MH^+, 100%); *m/z* Probe EIMS (+ve ion) 572 (2%), 555 (3%), 365 (55%), 355 (10%), 327 (8%), 315 (4%), 301 (9%), 285 (49%), 270 (18%), 259 (6%), 244 (43%), 231 (29%), 220 (38%), 204 (5%), 190 (21%), 176 (37%), 162 (16%), 148 (18%), 134 (13%), 120 (26%), 106 (100%), 93 (97%); HRMS found $MH^+=574.4721$, $C_{38}H_{60}N_3O$ requires 574.4736; δ_H (500 MHz, $CDCl_3$) 1.28–1.42 (18H, m, H-9 to H-12, and H-9′ to H-13′), 1.34 (1H, m, H-13), 1.44 (2H, m, H-18′), 1.46 (1H, m, H-18), 1.47 (1H, m, H-13′^{a}), 1.49 (1H, m, H-17), 1.49 (1H, m, H-14), 1.59 (2H, m, H-19′), 1.59 (1H, m, H-14′^{a}), 1.64 (4H, m, H-8, H-8′), 1.69 (1H, m, H-17′^{a}), 1.70 (1H, m, H-18′^{a}), 1.72 (1H, m, H-19), 1.80 (1H, m, H-19′^{a}), 2.04 (2H, m, H-14′), 2.07 (2H, m, H-17′), 2.60–2.72 (1H, m, H-20′), 2.60 (4H, t, $J=7.7$ Hz, H-7, H-7′), 2.78–2.89 (2H, m, H-16, H-20′^{a}), 3.40–3.51 (1H, m, H-20), 4.00–4.08 (1H, m, H-15), 5.28–5.38 (2H, m, H-15′, H-16′), 7.14–7.21 (2H, m, Py-*H*), 7.43–7.50 (2H, m, Py-*H*), 8.36–8.49 (4H, br, s, Py-*H*); δ_C (125.8 MHz, $CDCl_3$) 26.2, 26.3 (2C, C-17, C-18), 26.9 (1C, C-13), 27.0 (1C, C-17′), 27.1 (1C, C-14′), 27.4 (1C, C-18′), 27.7 (1C, C-19′), 28.6 (1C, C-14), 29.0, 29.1, 29.2, 29.3, 29.5, 29.6, (9C, C-9 to C-12 and C-9′ to C-13′), 31.0 (2C, C-8, C-8′), 32.9 (2C, C-7, C-7′), 34.1, (1C, C-19), 49.8 (1C, C-16), 56.8 (1C, C-20′), 72.5 (1C, C-20), 77.6 (1C, C-15), 123.1 (2C, Py C-5*), 129.5, 129.9 (2CH, C-15′, C-16′), 135.7 (2C, Py C-4*), 137.9 (2C, Py C-3*), 147.0, 149.8 (4C, Py C-2*, C-6*).

6.1.3. Preparation of 1-(*tert*-butyldiphenylsilyloxy)-5-bromopentane 28. To a solution of 5-bromopentan-1-ol **26** (3.00 g, 18.0 mmol) and imidazole (3.06 g, 45.0 mmol) in THF (40 mL) at 0 °C was added *tert*-butyldiphenylchlorosilane (5.20 mL, 19.9 mmol) dropwise. The mixture was allowed to warm to room temperature over 3 h, then was quenched with $NH_4Cl_{(aq)}$ (sat., 90 mL) and extracted

with 1:1 Et_2O:PE (4×30 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 30 mL) and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (15% benzene, 85% PE) to yield **28** (6.96 g, 17.2 mmol, 96%) as a colourless oil; R_f=0.18 (15% benzene, 85% PE); ν_{max}/cm^{-1} (thin film) 3071 (m), 2932 (s), 2858 (s), 1590 (w), 1473 (m), 1428 (s), 1390 (w), 1362 (w), 1253 (w), 1189 (w), 1112 (s), 1008 (w), 823 (m), 740 (m), 702 (s); *m/z* Probe CI^+ (NH_3) 424.2 ($M[^{81}Br]NH_4^+$, 100%), 407.2 ($M[^{81}Br]H^+$, 84%); HRMS found MH^+=405.1249, $C_{21}H_{30}OSi^{79}Br$ requires 405.1249; microanalysis found C, 62.3; H, 7.44; $C_{21}H_{29}OSiBr$ requires C, 62.2; H, 7.21; δ_H (200 MHz, $CDCl_3$) 1.20 (9H, s, $C(CH_3)_3$), 1.55–1.80 (4H, m, H-2, H-3), 1.95 (2H, quin., J=7.0 Hz, H-4), 3.45 (2H, t, J=7.0 Hz, H-5), 3.80 (2H, t, J=5.5 Hz, H-1), 7.40–7.57 (6H, m, Ph-*H*) 7.75–7.85 (4H, m, Ph-*H*); δ_C (50.3 MHz, $CDCl_3$) 19.3 (1C, $C(CH_3)_3$), 24.6 (1C, C-3), 27.0 (3C, $C(CH_3)_3$), 31.7 (1C, C-2), 32.6 (1C, C-5), 33.9 (1C, C-4), 63.6 (1C, C-1), 127.7 (4CH, Ph), 129.7 (2CH, Ph), 134.0 (2C, Ar*C*-Si), 135.7 (4CH, Ph).

6.1.4. Preparation of 1-(*tert*-butyldiphenylsilyloxy)-hept-6-yne 30. To a suspension of lithium acetylide-ethylene diamine complex (2.44 g, 90%, 23.9 mmol, 1.85 equiv) in DMSO (15 mL) and THF (5 mL) at −5 °C, was added a solution of 1-(*tert*-butyldiphenylsilyloxy)-5-bromopentane **28** (5.22 g, 12.9 mmol) in THF (5 mL) cooled to −40 °C via cannula dropwise over 30 min. The reaction was then stirred at room temperature for 1 h. The mixture was quenched with $NH_4Cl_{(aq)}$ (sat., 100 mL) and H_2O (20 mL), then extracted into 1:1 Et_2O:PE (3×100 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 2×50 mL), and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (20% benzene, 80% PE) to yield **30** (3.83 g, 10.9 mmol, 84%) as a colourless oil; R_f=0.34 (20% benzene, 80% PE); ν_{max}/cm^{-1} (thin film) 3309 (m), 3071 (w), 2933 (s), 2859 (s), 1459 (m), 1428 (s), 1371 (w), 1335 (w), 1112 (s), 823 (w), 740 (w), 702 (s); *m/z* Probe CI^+ (NH_3) 351.1 (MH^+, 100%); HRMS found MH^+= 351.2148, $C_{23}H_{31}OSi$ requires 351.2144; microanalysis found C, 78.9; H, 9.02; $C_{23}H_{30}OSi$ requires C, 78.8; H, 8.62; δ_H (200 MHz, $CDCl_3$) 1.10 (9H, s, $C(CH_3)_3$), 1.45–1.70 (6H, m, H-2 to H-4), 1.95 (1H, t, J=2.5 Hz, H-7), 2.15–2.27 (2H, m, H-5), 3.70 (2H, t, J=6.0 Hz, H-1), 7.35–7.50 (6H, m, Ph-*H*), 7.65–7.75 (4H, m, Ph-*H*); δ_C (50.3 MHz, $CDCl_3$) 18.4 (1C, C-5), 19.2 (1C, $C(CH_3)_3$), 25.0 (1C, C-3), 26.9 (3C, $C(CH_3)_3$), 28.2 (1C, C-4), 32.0 (1C, C-2), 63.7 (1C, C-1), 68.2 (1C, C-7), 84.6 (1C, C-6), 127.6 (4CH, Ph), 129.5 (2CH, Ph), 134.1 (2C, *C*-Si), 135.6 (4CH, Ph).

6.1.5. Preparation of 13-chloro-1-(*tert*-butyldiphenylsilyloxy)-tridec-6-yne 31. To a solution of 1-(*tert*-butyldiphenylsilyloxy)-hept-6-yne **30** (1.09 g, 3.11 mmol) in THF (10 mL) cooled to −16 °C was added *n*-BuLi (2.03M in hexanes, 1.54 mL, 3.13 mmol). After stirring at −16 °C for 1 h, the solution was cooled to −78 °C, and DMPU (3.77 mL, 31.2 mmol, 10 equiv) was added. After a further 15 min stirring, the yellow solution was transferred via cannula to a solution of 1-chloro-6-iodohexane (1.55 g, 6.29 mmol, 2 equiv) in THF (4 mL) at −78 °C. The solution was stirred for 3 h from −78 °C to room temperature. The mixture was quenched with H_2O (25 mL) and $NH_4Cl_{(aq)}$ (sat., 25 mL), then extracted into EtOAc (4×60 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 50 mL) and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (15% toluene, 85% PE) to yield **31** (1.24 g, 2.64 mmol, 85%) as a colourless oil; R_f= 0.13 (15% toluene, 85% PE); ν_{max}/cm^{-1} (thin film) 3071 (m), 2933 (s), 2858 (s), 1590 (w), 1472 (m), 1462 (m), 1428 (s), 1389 (w), 1361 (w), 1112 (s), 1009 (w), 824 (m), 740 (w), 702 (s); *m/z* Probe CI^+ (NH_3) 469.4 ($M[^{35}Cl]H^+$, 100%), 411.3 (21%), 213.1 (72%), 178.9 (29%); HRMS found MH^+=469.2698, $C_{29}H_{42}OSi^{35}Cl$ requires 469.2693; δ_H (200 MHz, $CDCl_3$) 1.15 (9H, s, $C(CH_3)_3$), 1.40–1.75 (12H, m, H-2 to H-4 and H-9 to H-11), 1.75–1.90 (2H, m, H-12), 2.15–2.30 (4H, m, H-5, H-8), 3.58 (2H, t, J=7.0 Hz, H-13), 3.78 (2H, t, J=6.0 Hz, H-1), 7.40-7.55 (6H, m, Ph-*H*), 7.70–7.85 (4H, m, Ph-*H*); δ_C (50.3 MHz, $CDCl_3$) 18.8 (2C, C-5, C-8), 19.3 (1C, $C(CH_3)_3$), 25.2, 26.5 ($2CH_2$), 27.0 (3C, $C(CH_3)_3$), 28.1, 29.0 ($3CH_2$), 32.2, 32.6 (2C, C-2, C-12), 45.0 (1C, C-13), 63.9 (1C, C-1), 80.0, 80.3 (2C, C-6, C-7), 127.7 (4CH, Ph), 129.6 (2CH, Ph), 134.1 (2C, *C*-Si), 135.6 0 (4CH, Ph).

6.1.6. Preparation of *Z*-13-chloro-1-(*tert*-butyldiphenylsilyloxy)-tridec-6-ene 32a. To a solution of 13-chloro-1-(*tert*-butyldiphenylsilyloxy)-tridec-6-yne **31** (2.13 g, 4.54 mmol) and benzene (30 mL) were added Lindlar catalyst (0.640 g) and quinoline (58 μL). The mixture was stirred under hydrogen gas (1 atm, balloon) for 1 h. The reaction had not reached completion, so additional Lindlar catalyst (0.410 g) was added, and the mixture stirred for a further 3 h. The mixture was filtered through cellulose and washed with benzene (150 mL). The organic phase was washed with $KHSO_{4(aq)}$ (1%, 30 mL), neutralised with $NaHCO_{3(aq)}$ (sat., 30 mL), then with $NaCl_{(aq)}$ (sat., 50 mL) and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (15% toluene, 85% PE) to yield **32a** (1.77 g, 3.76 mmol, 83%) as a colourless oil; R_f=0.20 (15% toluene, 85% PE); ν_{max}/cm^{-1} (thin film) 3071 (m), 3001 (m), 2931 (s), 2857 (s), 1656 (w), 1590 (w), 1472 (m), 1428 (m), 1390 (w), 1361 (w), 1188 (w), 1112 (s), 824 (m), 739 (w), 702 (s); *m/z* Probe CI^+ (NH_3) 471.4 ($M[^{35}Cl]H^+$, 100%), 256.2 (19%), 196.3 (33%), 179.9 (20%), 123.1 (21%), 95.1 (27%); HRMS found MH^+ 471.2848, $C_{29}H_{44}OSi^{35}Cl$ requires 471.2850; microanalysis found C, 73.8; H, 9.42; $C_{29}H_{43}OSiCl$ requires C, 73.9; H, 9.21; δ_H (200 MHz, $CDCl_3$) 1.10 (9H, s, $C(CH_3)_3$), 1.30–1.55 (10H, m, H-3, H-4, H-9 to H-11), 1.55-1.70 (2H, m, H-2), 1.80 (2H, quin., J= 7.0 Hz, H-12), 2.00–2.15 (4H, m, H-5, H-8), 3.55 (2H, t, J= 7.0 Hz, H-13), 3.72 (2H, t, J=6.5 Hz, H-1), 5.30–5.50 (2H, m, H-6, H-7), 7.35–7.55 (6H, m, Ph-*H*), 7.65–7.80 (4H, m, Ph-*H*); δ_C (50.3 MHz, $CDCl_3$) 19.3 (1C, $C(CH_3)_3$), 25.5, 26.8 ($2CH_2$), 26.9 (3C, $C(CH_3)_3$), 27.1, 27.2, 28.5, 29.5, 29.6 ($5CH_2$), 32.5, 32.6 (2C, C-2, C-12), 45.1 (1C, C-13), 64.0 (1C, C-1), 127.6 (4CH, Ph), 129.5 (2CH, Ph), 129.7, 130.0 (2C, C-6, C-7), 134.2 (2C, *C*-Si), 135.6 (4CH, Ph).

6.1.7. Preparation of *Z*-13-bromo-1-(*tert*-butyldiphenylsilyloxy)-tridec-6-ene 32b. A solution of *Z*-13-chloro-1-(*tert*-butyldiphenylsilyloxy)-tridec-6-ene **32a** (0.099 g,

0.21 mmol), and LiBr (0.623 g, 7.18 mmol) in butanone (2 mL) was heated at reflux for 24 h. The butanone was removed in vacuo, and H_2O (25 mL) was added. The product was extracted into EtOAc (3×30 mL). The organic phases were washed with $Na_2S_2O_{3(aq)}$ (sat., 20 mL), then $NaCl_{(aq)}$ (sat., 30 mL) and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (15% benzene, 85% PE) to yield **32b** (0.104 g, 0.20 mmol, 96%) as a colourless oil; R_f=0.20 (15% benzene, 85% PE); ν_{max}/cm^{-1} (thin film) 3071 (m), 3000 (m), 2932 (s), 2857 (s), 1590 (w), 1462 (m), 1428 (m), 1389 (w), 1361 (w), 1260 (w), 1112 (s), 824 (m), 740 (m), 702 (s); 534.5 (10%, MNH_3^+), 517.5 (100%, MH^+), 471.5 (18%), 455.4 (33%), 437.6 (30%), 375.5 (64%), 256.3 (35%), 196.2 (60%); HRMS found MH+ 515.2337 $C_{29}H_{44}OSiBr_{79}$ requires 515.2345 δ_H (200 MHz, $CDCl_3$) 1.10 (9H, br, s, $C(CH_3)_3$), 1.30–1.53 (10H, m, H-3, H-4, H-9 to H-11), 1.53–1.70 (2H, m, H-2), 1.88 (2H, quin, J=7.5 Hz H-12), 1.98-2.16 (4H, m, H-5, H-8), 3.42 (2H, t, J=7.0 Hz, H-13), 3.70 (2H, t, J=6.5 Hz, H-1), 5.30–5.48 (2H, m, H-6, H-7), 7.35–7.52 (6H, m, Ph-*H*), 7.67–7.75 (4H, m, Ph-*H*); δ_C (50.3 MHz, $CDCl_3$) 19.2 (1C, $C(CH_3)_3$), 25.5 ($1CH_2$), 26.9 (3C, $C(CH_3)_3$), 27.1, 27.2, 28.1, 28.4, 29.5, ($6CH_2$), 32.5, 32.8 (2C, C-2, C-12), 34.0 (1C, C-13), 64.0 (1C, C-1), 127.6 (4CH, Ph), 129.5 (2CH, Ph), 129.6, 130.0 (2C, C-6, C-7), 134.2 (2C, *C*-Si), 135.6 (4CH, Ph).

6.1.8. Preparation of *Z*-13-iodo-1-(*tert*-butyldiphenylsilyloxy)-tridec-6-ene 32c. A solution of *Z*-13-chloro-1-(*tert*-butyldiphenylsilyloxy)-tridec-6-ene **32a** (0.835 g, 1.77 mmol), and NaI (2.69 g, 17.9 mmol, 10 equiv), in acetone (15 mL), was heated at reflux for 24 h. The acetone was removed in vacuo, and H_2O (50 mL) was added. The product was extracted into EtOAc (3×50 mL). The organic phases were washed with $Na_2S_2O_{3(aq)}$ (sat., 30 mL), then $NaCl_{(aq)}$ (sat., 50 mL) and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (15% benzene, 85% PE) to yield **32c** (0.948 g, 1.68 mmol, 95%) as a colourless oil; R_f=0.32 (15% benzene, 85% PE); ν_{max}/cm^{-1} (thin film) 3071 (m), 3001 (m), 2930 (s), 2856 (s), 2362 (w), 2344 (w), 1655 (w), 1590 (w), 1472 (m), 1428 (m), 1389 (w), 1361 (w), 1190 (w), 1112 (s), 824 (m), 740 (m), 701 (s); *m/z* Probe CI^+ (NH_3) 580.3 (MNH_4^+, 67%), 563.2 (MH^+, 100%), 437.3 (35%), 256.1 (27%), 196.0 (30%); HRMS found MH^+ 563.2209, $C_{29}H_{44}OSiI$ requires 563.2206; microanalysis found C, 61.8; H, 7.62; $C_{29}H_{43}OSiI$ requires C, 61.9; H, 7.71; δ_H (200 MHz, $CDCl_3$) 1.08 (9H, s, $C(CH_3)_3$), 1.25–1.50 (10H, m, H-3, H-4, H-9 to H-11), 1.50–1.67 (2H, m, H-2), 1.83 (2H, quin, J=7.5 Hz, H-12), 1.95–2.13 (4H, m, H-5, H-8), 3.20 (2H, t, J=7.0 Hz, H-13), 3.68 (2H, t, J=6.5 Hz, H-1), 5.27–5.47 (2H, m, H-6, H-7), 7.35–7.50 (6H, m, Ph-*H*), 7.65–7.75 (4H, m, Ph-*H*); δ_C (50.3 MHz, $CDCl_3$) 7.3 (1C, C-13), 19.2 (1C, $C(CH_3)_3$), 25.5 ($1CH_2$), 26.9 (3C, $C(CH_3)_3$), 27.1, 27.2, 28.2, 29.5, 30.4 ($6CH_2$), 32.5, 33.5 (2C, C-2, C-12), 63.9 (1C, C-1), 127.6 (4CH, Ph), 129.5 (2CH, Ph), 129.6, 130.0 (2C, C-6, C-7), 134.1 (2C, *C*-Si), 135.6 (4CH, Ph).

6.1.9. Preparation of *Z*-1-(*tert*-butyldiphenylsilyloxy)-14-(pyridin-3-yl)-tetradec-6-ene 33. To a solution of diisopropylamine (0.111 mL, 0.79 mmol, 3 equiv) in THF (2 mL) at −10 °C was added *n*-BuLi (2.03 M in hexanes, 0.390 mL, 0.79 mmol, 3 equiv). The solution was stirred at −10 °C for 30 min then DMPU (0.094 mL, 0.78 mmol, 3 equiv) was added. After 15 min stirring, 3-methylpyridine (0.077 mL, 0.79 mmol) was added dropwise. After a further 60 minutes stirring at −10 °C, the solution was cooled to −78 °C. This solution was added via cannula to a solution of halide **32a**, **32b**, or **32c** (0.26 mmol) in THF (2 mL) cooled to −78 °C. The reaction was stirred for 22 h from −78 °C to room temperature. The reaction was quenched with $NH_4Cl_{(aq)}$ (sat., 10 mL) and H_2O (10 mL) and extracted with EtOAc (3×30 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 20 mL) and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (25% EtOAc, 75% benzene) to yield **33** (79% from **32a**, 63% from **32b**, 36% from **32c**) as a pale yellow oil; R_f=0.33 (25% EtOAc, 75% benzene); ν_{max}/cm^{-1} (thin film) 3000 (w), 2930 (s), 2856 (s), 1575 (w), 1462 (w), 1428 (m), 1389 (w), 1111 (s), 1026 (w), 824 (m), 740 (m), 702 (s); *m/z* Probe CI^+ (NH_3) 528.4 (MH^+, 100%), 470.3 (23%); HRMS found MH^+ 528.3668, $C_{35}H_{50}NOSi$ requires 528.3662; δ_H (200 MHz, $CDCl_3$) 1.13 (9H, s, $C(CH_3)_3$), 1.30–1.55 (12H, m, H-3, H-4, H-9 to H-12), 1.55–1.70 (4H, m, H-2, H-13), 2.00–2.20 (4H, m, H-5, H-8), 2.68 (2H, t, J=7.5 Hz, H-14), 3.74 (2H, t, J=6.5 Hz, H-1), 5.32–5.52 (2H, m, H-6, H-7), 7.22–7.32 (1H, m, Py H-5*), 7.40–7.60 (7H, m, 6H Ph-*H*, 1H Py H-4*), 7.70–7.80 (4H, m, Ph-*H*), 8.50–8.55 (2H, m, Py H-2*, H-6*); δ_C (50.3 MHz, $CDCl_3$) 19.7 (1C, $C(CH_3)_3$), 25.9 ($1CH_2$), 27.3 (3C, $C(CH_3)_3$), 27.7, 29.6, 29.7, 29.8, 30.0, 30.2, 31.6, 33.0, 33.5 ($10CH_2$), 64.4 (1C, C-1), 123.7 (1C, Py C-5*) 128.0 (4CH, Ph), 129.9 (2CH, Ph), 130.3 (2C, C-6, C-7), 134.6 (2C, Ar*C*-Si), 136.0 (4CH, Ph), 136.2 (1C, Py C-4*), 138.4 (1C, Py C-3*), 147.7, 150.4 (2C, Py C-2*, C-6*).

6.1.10. Preparation of *Z*-14-(pyridin-3-yl)-tetradec-6-en-1-ol 34. To a solution of **33** (1.44 g, 2.73 mmol) in MeOH (30 mL) was added NH_4F (1.43 g, 38.6 mmol, 14 equiv). The mixture was heated at 60 °C for 6.5 h. The reaction was quenched with $NaHCO_{3(aq)}$ (sat., 30 mL) and H_2O (30 mL) and extracted with EtOAc (3×100 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 50 mL) and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (30% Et_3N, 15% EtOAc, 55% PE) to yield **34** (0.754 g, 2.60 mmol, 95%) as a pale yellow oil; R_f=0.22 (30% Et_3N, 15% EtOAc, 55% PE); ν_{max}/cm^{-1} (thin film) 3339 (br, m), 3003 (m), 2928 (s), 2855 (s), 1578 (w), 1479 (w), 1461 (w), 1424 (m), 1065 (m), 713 (m); *m/z* Probe $APCI^+$ (NH_3) 290.4 (MH^+, 100%); HRMS found MH^+= 290.2496, $C_{19}H_{32}NO$ requires 290.2484; δ_H (200 MHz, $CDCl_3$) 1.15–1.45 (12H, m, H-3, H-4, and H-9 to H-12), 1.45–1.70 (4H, m, H-2, H-13), 1.85–2.10 (4H, m, H-5, H-8), 2.57 (2H, t, J=7.5 Hz, H-14), 2.90–3.30 (1H, br s, OH), 3.60 (2H, t, J=6.5 Hz, H-1), 5.20–5.42 (2H, m, H-6, H-7), 7.17 (1H, dd, J_1=8.0 Hz, J_2=5.0 Hz Py H-5*) 7.46 (1H, br, d, J=8.0 Hz Py H-4*), 8.34–8.45 (2H, m, Py H-2*, H-6*); δ_C (50.3 MHz, $CDCl_3$) 25.5, 27.1, 29.0, 29.1, 29.2, 29.5, 29.6, 31.0, 32.5, 32.7, 32.9 ($11CH_2$), 62.5 (1C, C-1), 123.3 (1C, Py C-5*), 129.7, 129.9 (2C, C-6, C-7), 135.9 (1C, Py C-4*), 138.0 (1C, Py C-3*), 146.9, 149.7 (2C, Py C-2*, C-6*).

6.1.11. Preparation of *Z*-14-(pyridin-3-yl)-tetradec-6-en-1-al 24. To a solution of IBX (0.454 g, 1.62 mmol,

1.5 equiv) in DMSO (5 mL) was added a solution of **34** (0.310 g, 1.07 mmol) in THF (1 mL) via cannula. The reaction mixture was stirred for 5 h after which time it was diluted by H_2O (100 mL), filtered and extracted with EtOAc (3×30 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 30 mL) and dried over Na_2SO_4. Filtration and removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (50% EtOAc, 50% PE) to yield **24** (0.285 g, 0.99 mmol, 93%) as a pale yellow oil; R_f=0.27 (50% EtOAc, 50% PE); ν_{max}/cm^{-1} (thin film) 3004 (w), 2928 (s), 2855 (s), 2718 (w), 1725 (s), 1575 (m), 1478 (m), 1461 (m), 1422 (m), 1190 (w), 1027 (m), 794 (w), 714 (m); *m/z* APCI$^+$ (NH_3) 288.3 (MH^+, 100%); HRMS found MH^+=288.2328, $C_{19}H_{30}NO$ requires 288.2327; δ_H (200 MHz, $CDCl_3$) 1.15–1.45 (10H, m, H-4 and H-9 to H-12), 1.45–1.70 (4H, m, H-3, H-13), 1.85–2.10 (4H, m, H-5, H-8), 2.38 (2H, dt, J_1=7.5 Hz, J_2=2.0 Hz, H-2), 2.55 (2H, t, J=7.5 Hz, H-14), 5.20–5.40 (2H, m, H-6, H-7), 7.15 (1H, dd, J_1=8.0 Hz, J_2=5.0 Hz, Py H-5*) 7.44 (1H, dd, J=8.0 Hz, J_2=2.0 Hz, Py H-4*), 8.39 (2H, apparent broad s, Py, H-2*, H-6*), 9.71 (0.5H, t, J=2.0 Hz, H-1); δ_C (50.3 MHz, $CDCl_3$) 22.0 (1C, C-3), 26.4, 27.2, 29.1, 29.2, 29.3, 29.6, 31.1, 33.0, (10CH_2, C-3 to C-5 and C-7 to C-14), 43.2 (1C, C-2), 123.2 (1C, Py C-5*), 128.2, 131.3 (2CH, C-5, C-6) 135.8 (1C, Py C-4*), 137.9 (1C, Py C-3*), 147.1, 149.9 (2C, Py C-2*, C-6*), 202.6 (1C, C-1).

6.1.12. Preparation of *Z*-14-(pyridin-3-yl)-tetradec-6-en-1-oxime 35. To a solution of **24** (0.138 g, 0.48 mmol) in MeOH (6 mL) were added NaOAc (0.119 g, 1.45 mmol, 3 equiv), then hydroxylamine hydrochloride (0.100 g, 1.44 mmol, 3 equiv). The mixture was stirred for 4.5 h at room temperature. The solvent was removed in vacuo and DCM (4 mL) and H_2O (4 mL) with $NaHCO_{3(s)}$ (0.146 g, 1.74 mmol, 3.5 equiv) were added to neutralise the acetic acid produced. The mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3×30 mL). The organic phases were successively washed with $NaHCO_{3(aq)}$ (sat., 20 mL), H_2O (20 mL), and $NaCl_{(aq)}$ (sat., 20 mL), and dried over Na_2SO_4. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (50% EtOAc, 50% PE) to yield **35** (0.131 g, 0.43 mmol, 90%) as a colourless oil; R_f=0.20, 0.29 (*E*/*Z* isomers, 50% EtOAc, 50% PE); ν_{max}/cm^{-1} (thin film) 3214 (br, m), 3086 (br, m), 3005 (m), 2927 (s), 2855 (s), 1655 (w), 1580 (m), 1462 (m), 1426 (m), 1334 (br, w), 1191 (w), 1030 (w), 903 (w), 795 (w), 712 (m); *m/z* Probe APCI$^+$ (NH_3) 303.3 (MH^+, 30%), 285.3 ($[MH-H_2O]^+$, 100%); HRMS found MH^+=303.2444, $C_{19}H_{31}N_2O$ requires 303.2436. The subscripts *E* and *Z* differentiate between the *E* and *Z* isomers.[39] δ_H (200 MHz, $CDCl_3$) 1.20–1.70 (14H, m, H-3, H-4 and H-9 to H-13), 1.90–2.10 (4H, m, H-5, H-8), 2.20 (1H, apparent q, J=7.0 Hz, 2H ×0.5H-2_E), 2.40 (1H, apparent q, J=6.5 Hz, 2H ×0.5H-2_Z), 2.59 (2H, t, J=7.5 Hz, H-14), 5.23–5.45 (2H, m, H-6, H-7), 6.70 (0.5H, t, J=5.5 Hz, H-1_Z), 7.21 (1H, dd, J_1=5.0 Hz, J_2=7.5 Hz, Py H-5*) 7.40–7.55 (1.5H, m, 0.5H H-1_E, 1H Py H-4*), 8.35–8.50 (2H, m, Py H-2*, H-6*); δ_C (50.3 MHz, $CDCl_3$) 24.9, 25.7, 26.3, 26.8, 27.1, 29.0, 29.1, 29.2, 29.4, 29.6, 31.0, 32.9 (11CH_2), 123.4, (1C, Py C-5*), 129.3, 130.2 (2C, C-6, C-7), 136.2 (1C, Py C-4*), 138.1 (1C, Py C-3*), 146.7, 149.6 (2C, Py C-2*, C-6*), 151.5 (1C, C-1 *Z*-oxime), 152.3 (1C, C-1 *E*-oxime).

6.1.13. Preparation of 1-[*N*-*Z*-14-(pyridin-3-yl)-tetradec-6-enylideneamino]-Z-14-(pyridin-3-yl)-tetradec-5-ene *N*-oxide 36. To a solution of **35** (0.0552 g, 0.18 mmol) in MeOH (10 mL) at 0 °C was added of methyl orange (indicator, 2 mg) and conc. HCl (6 M) turning the indicator red (approx. pH 3). $NaBH_3CN$ (0.0473 g, 0.75 mmol, 4 equiv) in MeOH (3 mL) was added dropwise with concurrent addition of conc. HCl to keep the mixture at pH 3. The mixture was stirred for 2 h at 0 °C. The mixture was basified (dropwise, tested with pH paper) with $NaOH_{(aq)}$ (6 N), and worked up using solvents chilled to 0 °C. The mixture was diluted with $NaCl_{(aq)}$ (sat., 20 mL) and H_2O (20 mL) and extracted with DCM (3×30 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 30 mL) and dried over Na_2SO_4. Concentration in vacuo (without heating) gave a solution of hydroxylamine **25** in DCM (ca. 50 mL). To the hydroxylamine solution, was added Na_2SO_4 (0.512 g), and aldehyde **5** (0.0524 g, 0.18 mmol, 1 equiv) in DCM (5 mL) via cannula. The flask was stirred for 18 h at room temperature. The crude reaction mixture was filtered, the solvent removed in vacuo and was purified by flash chromatography (30% Et_3N, 50% EtOAc, 20% PE) to yield nitrone **36** (0.0829 g, 0.14 mmol, 79%) as a yellow oil; R_f=0.12 (30% Et_3N, 50% EtOAc, 20% PE); ν_{max}/cm^{-1} (thin film) 3292 (br, m), 3003 (m), 2927 (s), 2855 (s), 2361 (m), 2342 (w), 1670 (m), 1575 (m), 1478 (m), 1463 (m), 1422 (m), 1027 (w), 794 (w), 714 (m); *m/z* Probe APCI$^+$ (NH_3) 574.6 (MH^+, 100%); HRMS found MH^+=574.4730, $C_{38}H_{60}N_3O$ requires 574.4736; δ_H (200 MHz, $CDCl_3$) 1.35 (22H, br, s, H-8 to H-12, and H-3′, 4′, H-9′ to H-12′), 1.50-1.80 (6H, m, H-3, H-13, H-13′), 1.80–2.20 (10H, m, H-4, H-7, H-2′, H-5′, H-8′), 2.45 (2H, apparent q, J=7.5 Hz, H-2), 2.62 (4H, t, J=7.5 Hz, H-14, H-14′), 3.76 (2H, t, J=7.0 Hz, H-1′), 5.25–5.50 (4H, m, H-5, H-6, H-6′, H-7′), 6.70 (1H, t, J=6.0 Hz, H-1), 7.22 (2H, dd, J_1 7.5 Hz, J_2 5.0 Hz, Py H-5*), 7.51 (2H, d, J=8.0 Hz, Py H-4*), 8.40–8.50 (4H, m, Py H-2*, H-6*); δ_C (50.3 MHz, $CDCl_3$) 26.1, 26.5, 26.7, 27.4, 27.6, 27.8, 29.5, 29.7, 29.8, 30.1, 31.5 (18CH_2), 33.4 (4CH_2, C-13, C-14, C-13′, C-14′), 65.7 (1C, C-1′), 123.6 (2C, Py C-5*), 128.8, 129.7, 130.7, 131.4 (4C, C-5, C-6, C-6′, C-7′), 136.2 (2C, Py C-4*), 138.3 (2C, Py C-3*), 139.4 (1C, C-1), 147.6, 150.3 (4C, Py C-2*, C-6*).

6.1.14. Preparation of the C14′–C15′ isomer of pyrinodemin A 3. A solution of nitrone **36** (0.0682 g, 0.12 mmol) in benzene (80 mL) was heated at reflux for 24 h. The solvent was removed in vacuo, to afford the crude product which was purified by flash chromatography (100% EtOAc) to yield **3** (0.0598 g, 0.10 mmol, 87%) as a pale yellow oil; R_f=0.16 (100% EtOAc); ν_{max}/cm^{-1} (thin film) 2928 (s), 2855 (s), 1575 (m), 1478 (m), 1465 (m), 1422 (m), 1339 (w), 1189 (w), 1026 (m), 794 (w), 714 (m); *m/z* Probe APCI$^+$ (NH_3) 574.4 (MH^+, 50%), 289.4 (67%), 286.4 (100%), 220.2 (67%); *m/z* Probe EIMS (+ve ion) 572 (3%), 555 (7%), 383 (14%), 365 (88%), 355 (15%), 327 (14%), 315 (9%), 299 (27%), 285 (82%), 270 (23%), 259 (27%), 244 (18%), 231 (30%), 220 (56%), 204 (6%), 190 (21%), 176 (34%), 162 (21%), 148 (19%), 134 (14%), 120 (28%), 106 (100%), 93 (84%); HRMS found 574.4729, $C_{38}H_{60}N_3O$ requires 574.4736; δ_H (500 MHz, $CDCl_3$) 1.22–1.38 (16H, m, H-9 to H-12, and H-9′ to H-12′), 1.41 (4H, m, H-17′, H-18′), 1.49 (2H, m, H-17, H-18), 1.51 (2H, m, H-13), 1.59 (2H, m, H-19′), 1.62 (2H, m, H-14), 1.68 (4H, m, H-8, H-8′),

1.72 (2H, m, H-17[a], H-18[a]), 1.73 (1H, m, H-19), 1.80 (1H, m, H-19[a]), 1.90–2.05 (4H, m, H-13′, H-16′), 2.53-2.61 (5H, m, H-7, H-7′, H-20′), 2.77–2.88 (2H, m, H-16, H-20′[a]), 3.40–3.50 (1H, m, H-20), 4.00–4.08 (1H, m, H-15), 5.30–5.38 (2H, m, H-14′, H-15′), 7.15–7.20 (2H, m, Py-*H*), 7.45–7.50 (2H, m, Py-*H*), 8.38–8.45 (4H, br, s, Py-*H*); δ_C (125.8 MHz, $CDCl_3$) 26.2 (1C, C-17), 26.4 (1C, C-18), 26.9 (2C, C-13, and C-17′ or C-18′), 27.1 (2C, C-13′, C-16′), 28.0 (1C, C-19′), 28.7 (1C, C-14), 29.0, 29.1, 29.2[†], 29.3, 29.5, 29.6[‡], (9C, C-9 to C-12 and C-9′ to C-12′ and C-18′ or C-17′), 31.0 (2C, C-8, C-8′), 32.9 (2C, C-7, C-7′), 34.5, (1C, C-19), 49.8 (1C, C-16), 57.4 (1C, C20′), 72.8 (1C, C-20), 77.5 (1C, C-15), 123.1 (2C, Py C-5[*]), 129.70, 129.72 (2CH, C-15′, C-16′), 135.6 (2C, Py C-4[*]), 137.8 (2C, Py C-3[*]), 147.1, 149.9 (4C, Py C-2[*], C-6[*]).

6.1.15. Preparation of 14-(pyridin-3-yl)-tetradecan-1-oxime 38. A solution of **35** (0.0492 g, 0.16 mmol) and catalyst (5% Pd on C, 5.9 mg) in MeOH (2 mL) was stirred under hydrogen (1 atm, balloon) for 5 h. Removal of solvent in vacuo afforded the crude which was purified by flash chromatography (60% EtOAc, 40% PE) to yield 14-(pyridin-3-yl)-tetradecan-1-oxime **38** (0.0465 g, 0.15 mmol, 94%) as a white waxy solid; R_f=0.23, 0.35 (*E*/*Z* isomers, 60% EtOAc, 40% PE); mp=81–85 °C; ν_{max}/cm^{-1} ($CHCl_3$) 3020 (br, w), 2929 (s), 2856 (s), 2361 (w), 2343 (w), 1578 (w), 1466 (w), 1425 (w), 1029 (w); *m*/*z* Probe APCI$^+$ (NH_3) 305.3 (MH^+, 44%), 287.3 ($[MH-H_2O]^+$, 100%); HRMS found MH^+=305.2607, $C_{19}H_{33}N_2O$ requires 305.2593; *m*/*z* Probe EIMS (+ve ion) 304.2 (5%), 287.2 (14%), 246.2 (62%), 232.2 (13%), 218.2 (16%), 204.1 (18%), 190.1 (14%), 176.1 (10%), 162.1 (13%), 148 (14%), 134.1 (8%), 120.1 (16%), 106.1 (100%), 93.0 (70%). The subscripts $_E$ and $_Z$ differentiate between the *E* and *Z* isomers.[39] δ_H (200 MHz, $CDCl_3$) 1.25 (18H, br, s, H-4 to H-12), 1.40–1.70 (4H, m, H-3, H-13), 2.11–2.27 (1H, m, 2H × 0.5H-2_E), 2.31–2.45 (1H, m, 2H ×0.5H-2_Z), 2.59 (2H, t, *J*=7.5 Hz, H-14), 6.67–6.76 (0.5H, m, H-1_Z), 7.16–7.29 (1H, m, Py H-5[*]) 7.39–7.55 (1.5H, m, 0.5H H-1_E, 1H Py H-4[*]), 8.38–8.51 (2H, m, Py H-2[*], H-6[*]); δ_C (50.3 MHz, $CDCl_3$) 25.0, 26.1, 26.7, 29.1, 29.3, 29.5 (11C, C-2 to C-12), 31.0, 33.0 (2C, C-13, C-14), 123.3, (1C, Py C-5[*]), 136.1 (1C, Py C-4[*]), 138.2 (1C, Py C-3[*]), 146.8, 149.6 (2C, Py C-2[*], C-6[*]), 151.8 (1C, C-1 *Z*-oxime), 152.5 (1C, C-1 *E*-oxime).

6.1.16. Preparation of 14-(pyridin-3-yl)-tetradecane-1-hydroxylamine 39. To a stirred solution of **38** (0.0453 g, 0.15 mmol) in MeOH (10 mL) at 0 °C was added of methyl orange (indicator, 2 mg) and conc. HCl (6 M) turning the indicator red (ca. pH 3). $NaBH_3CN$ (0.0354 g, 0.56 mmol, 4 equiv) in MeOH (1 mL) was added dropwise with concurrent addition of conc. HCl to keep the mixture at pH 3. The mixture was stirred for 3.5 h at room temperature. The mixture was basified (dropwise and tested with pH paper) with $NaOH_{(aq)}$ (6 N), diluted with $NaCl_{(aq)}$ (sat. 20 mL) and extracted with DCM (3×30 mL). The organic phases were washed with $NaCl_{(aq)}$ (sat., 20 mL) and dried over Na_2SO_4. Removal of solvent in vacuo to afford the crude product which was purified by flash chromatography (33.3% Et_3N, 33.3% EtOAc, 33.3% PE) to yield **39** (0.0341 g, 0.11 mmol, 74%) as a white waxy solid; R_f=0.13 (33.3% Et_3N, 33.3% EtOAc, 33.3% PE); mp=70–71 °C; *m*/*z* Probe CI$^+$ (NH_3) 307.2 (MH^+, 36%), 291.2 (100%, MH^+–H_2O); HRMS found MH^+=307.2754, $C_{19}H_{35}N_2O$ requires 307.2749; δ_H (200 MHz, $CDCl_3$) 1.24 (20H, br, s, H-3 to H-12), 1.43–1.70 (4H, m, H-2, H-13), 2.59 (2H, t, *J*=7.5 Hz, H-14), 2.93 (2H, t, *J*=7.0 Hz, H-1), 7.19 (1H, dd, J_1=5.0 Hz, J_2=7.5 Hz, Py H-5[*]) 7.42–7.52 (1H, m, Py H-4[*]), 8.40–8.45 (2H, m, Py H-2[*], H-6[*]); δ_C (50.3 MHz, $CDCl_3$) 27.0, 27.1, 29.1, 29.4, 29.6 (11C, C-2 to C-12), 31.1, 33.0 (2C, C-13, C-14), 54.0 (1C, C-1) 123.2, (1C, Py C-5[*]), 135.8 (1C, Py C-4[*]), 138.0 (1C, Py C-3[*]), 147.1, 149.9 (2C, Py C-2[*], C-6[*]).

6.1.17. Preparation of 1-[*N*-14-(pyridin-3-yl)-tetradecyl-ideneamino]-*Z*-14-(pyridin-3-yl)-tetradec-5-ene *N*-oxide 40. To a solution of **39** (0.0328 g, 0.11 mmol) in DCM (5 mL) was added Na_2SO_4 (1.16 g), and aldehyde **5** (0.0360 g, 0.13 mmol) in DCM (2 mL) via cannula. The reaction was stirred for 20 h at room temperature. The crude reaction mixture was filtered, the solvent removed in vacuo to afford the crude product which was purified by flash chromatography (40% Et_3N, 40% EtOAc, 20% PE) to yield nitrone **40** (0.0415 g, 0.072 mmol, 67%) as a pale yellow oil; R_f=0.28 (40% Et_3N, 40% EtOAc, 20% PE); ν_{max}/cm^{-1} ($CHCl_3$) 3306 (br, m), 2929 (s), 2856 (s), 1657 (m), 1578 (w), 1480 (w), 1464 (m), 1424 (m), 1028 (w), 713 (m); *m*/*z* Probe CI$^+$ (NH_3) 576.5 (MH^+, 100%), 367.2 (13%), 287.1 (5%); HRMS found MH^+=576.4897, $C_{38}H_{62}N_3O$ requires 576.4893; δ_H (200 MHz, $CDCl_3$) 1.10–1.40 (30H, m, H-8 to H-12 and H-3′ to H-12′), 1.40–1.70 (6H, m, H-3, H-13, H-13′), 1.80–2.12 (6H, m, H-4, H-7, H-2′), 2.48 (2H, apparent q, *J*=6.5 Hz, H-2), 2.58 (4H, t, *J*=7.5 Hz, H-14, H-14′), 3.71 (2H, t, *J*=7.0 Hz, H-1′), 5.22–5.46 (2H, m, H-5, H-6), 6.66 (1H, t, *J*=5.0 Hz, H-1), 7.18 (2H, dd, J_1=8.0 Hz, J_2=5.0 Hz, Py H-5[*]), 7.47 (2H, d, *J*=8.0 Hz, Py H-4[*]), 8.35–8.45 (4H, m, Py H-2[*], H-6[*]); δ_C (50.3 MHz, $CDCl_3$) 25.6, 26.3, 26.4, 27.0, 27.2, 27.4, 29.1, 29.4, 29.6, 31.1, 33.0, 34.4 (24CH_2), 65.4 (1C, C-1′), 123.2 (2C, Py C-5[*]), 128.4, 131.0 (2C, C-5, C-6), 135.8 (2C, Py C-4[*]), 138.0 (2C, Py C-3[*]), 139.1 (1C, C-1), 147.0, 149.8 (4C, Py C-2[*], C-6[*]).

6.1.18. Preparation of a saturated analogue of pyrinodemin A 37. A solution of nitrone **40** (0.0415 g, 0.072 mmol) in benzene (100 mL) was heated at reflux for 24 h. Removal of solvent in vacuo afforded the crude product which was purified by flash chromatography (100% EtOAc) to yield **37** (0.0304 g, 0.053 mmol, 73%) as a colourless oil; R_f=0.23 (100% EtOAc); ν_{max}/cm^{-1} (thin film) 2927 (s), 2854 (s), 1676 (w), 1575 (m), 1465 (m), 1422 (m), 1026 (m), 714 (s); *m*/*z* Probe APCI$^+$ (NH_3) 576.5 (MH^+, 100%), 291.4 (42%); EIMS (+ve ion) 575 (12%), 557 (10%), 483 (5%), 385 (24%), 367 (100%), 357 (24%), 329 (13%), 315 (16%), 301 (23%), 287 (80%), 274 (23%), 270 (20%), 246 (35%), 232 (13%), 231 (1%), 220 (59%), 218 (25%), 204 (12%), 190 (26%), 176 (30%), 162 (16%), 148 (18%), 134 (10%), 120 (21%), 106 (77%), 93 (73%); HRMS found 576.4897, $C_{38}H_{62}N_3O$ requires 576.4893; δ_H (400 MHz, $CDCl_3$) 1.18–1.84 (44H, m, H-8 to H-14, H-17, H-18, H-19, H-8′ to H-19′), 2.60–2.75 (1H, m, H-20′), 2.60 (4H, t, *J*=8.0 Hz, H-7, H-7′), 2.80–2.90 (2H, m, H-16, H-20′[a]), 3.42–3.51 (1H, m, H-20), 4.02–4.10 (1H, m, H-15),

[†] This data point can be resolved into two signals at 29.18 and 29.21 ppm.
[‡] This data point can be resolved into two signals at 29.58 and 29.63 ppm.

7.20 (2H, dd, $J_1=5.0$ Hz, $J_2=7.5$ Hz, Py H-5*), 7.48 (2H, d, $J=8.0$ Hz, Py H-4*), 8.40–8.48 (4H, m, Py H-2*, H-6*); δ_C (100.6 MHz, $CDCl_3$) 26.3, 26.5, 27.0, 27.4, 28.1, 28.8, 29.1[§], 29.3, 29.4, 29.5, 29.6[¶], 29.7, 31.1, 33.0 (CH_2), 49.9 (1C, C-16), 123.2 (2C, Py C-5*), 135.8 (2C, Py C-4*), 137.9 (2C, Py C-3*), 147.1, 149.9 (4C, Py C-2*, C-6*); C-15, C-20 and C-20′ were not observed.

Acknowledgements

We thank the States of Jersey Education Committee for a scholarship (to S. P. R.) and Professor Jun'ichi Kobayashi for providing us with the spectra of natural pyrinodemin A.

References and notes

1. Andersen, R. J.; Van Soest, R. W. M.; Kong, F. In Pelletier, S. W., Ed.; Alkaloids: Chemical and Biological Perspectives; Pergamon: Oxford, 1996; Vol. 10, pp 301–355.
2. (a) Ryo, G.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1994**, *50*, 13409–13416. (b) Fujita, M.; Nakao, Y.; Matsunaga, S.; Seiki, M.; Itoh, Y.; Van Soest, R. W. M.; Heubes, M.; Faulkner, D. J.; Fusetani, N. *Tetrahedron* **2001**, *57*, 3885–3890.
3. (a) Li, J.; Jeong, S.; Esser, L.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4765–4770. (b) Li, J.; Burgett, A. W. G.; Esser, L.; Amezcua, C.; Harran, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 4765–4770. (c) Song, H. Y.; Joo, J. W.; Kim, D.-S.; Jung, C.-K.; Kwak, H. S.; Park, J. H.; Lee, E.; Hong, C. Y.; Jeong, S. W.; Jeon, K.; Park, J. H. *J. Org. Chem.* **2003**, *68*, 8080–8087.
4. Tsuda, M.; Hirano, K.; Kubota, T.; Kobayashi, J. *Tetrahedron Lett.* **1999**, *40*, 4819–4820.
5. Hirano, K.; Kubota, T.; Tsuda, M.; Mikami, Y.; Kobayashi, J. *Chem. Pharm. Bull.* **2000**, *48*, 974–977.
6. Baldwin, J. E.; Romeril, S. P.; Lee, V.; Claridge, T. D. W. *Org. Lett.* **2001**, *3*, 1145–1148.
7. Romeril, S. P.; Lee, V.; Claridge, T. D. W.; Baldwin, J. E. *Tetrahedron Lett.* **2002**, *43*, 327–329.
8. Snider, B. B.; Shi, B. *Tetrahedron Lett.* **2001**, *42*, 1639–1642.
9. Morimoto, Y.; Kitao, S.; Okita, T.; Shoji, T. *Org. Lett.* **2003**, *5*, 2611–2614.
10. Coleman, W. R.; Bywater, W. G. *J. Am. Chem. Soc.* **1944**, *66*, 1821–1823.
11. Poulain, S.; Noiret, N.; Nugier-Chauvin, C.; Patin, H. *Liebigs Ann. Chem.* **1997**, 35–40.
12. Lindlar, H.; Dubuis, R., *Org. Syn. Coll.* 1973, Vol. V, 880–883.
13. Baldwin, J. E.; Melman, A.; Lee, V.; Firkin, C. R.; Whitehead, R. C. *J. Am. Chem. Soc.* **1998**, *120*, 8559–8560.
14. Zhang, W.; Robins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177–1180.
15. (a) Frigerio, M.; Santagostino, M.; Sputore, S.; Palmisano, G. *J. Org. Chem.* **1995**, *60*, 7272–7276. (b) Frigerio, M.; Santagostino, M. *Tetrahedron Lett.* **1994**, *35*, 8019–8022. (c) Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537–4538.
16. House, H. O.; Manning, D. T.; Melillo, D. G.; Lee, L. F.; Haynes, O. R.; Wilkes, B. E. *J. Org. Chem.* **1976**, *41*, 855–863.
17. Ciganek, E. *J. Org. Chem.* **1990**, *55*, 3007–3009.
18. John, B. K.; Plant, D.; Heald, S. L.; Hurd, R. E. *J. Magn. Reson.* **1991**, *94*, 664–669.
19. Stott, K.; Keeler, J.; Van, Q. N.; Shaka, A. J. *J. Magn. Reson.* **1997**, *125*, 302–324.
20. Throughout this article, we have used this terminology as a descriptor for the olefin position.
21. Vysotskii, M. V.; Imbs, A. B.; Popkov, A. A.; Latyshev, N. A.; Svetashev, V. I. *Tetrahedron Lett.* **1990**, *31*, 4367–4370.
22. Chong, J. M.; Heuft, M. A.; Rabbat, P. *J. Org. Chem.* **2000**, *65*, 5837–5838.
23. Novis Smith, W.; Beumel, O. F. Jr. *Synthesis* **1974**, 441–442.
24. δ_H (200 MHz, $CDCl_3$) 1.15 (9H, s, C(CH_3)$_3$), 2.75 (1H, s, C≡C*H*), 7.40–7.55 (6H, m, Ph-*H*) 7.75–7.85 (4H, m, Ph-*H*); δ_C (50.3 MHz, $CDCl_3$) 18.4 (1C, *C*(CH_3)$_3$), 26.9 (3C, C(*C*H_3)$_3$), 85.4, 97.3 (2C, *C*≡*C*), 127.8 (4CH, Ph), 129.7 (2CH, Ph), 132.7 (2C, Ar*C*-Si), 135.5 (4CH, Ph).
25. Lalonde, M.; Chan, T. H. *Synthesis* **1985**, 817–845.
26. Morimoto, Y.; Yokoe, C.; Kurihara, H.; Kinoshita, T. *Tetrahedron* **1998**, *54*, 12197–12214.
27. We have not been able to unambiguously assign C-17′ and C-18′ in the ^{13}C spectrum of **3**.
28. $\Delta\delta$ 0.02, exponential line broadening 0.50 Hz, referenced to $CDCl_3$ at 77.16 ppm. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512–7515.
29. Budzikiewicz, H.; Djerassi, C.; Williams, D. H. *Mass Spectrometry of Organic Chemistry*; Holden-Day: San Francisco, 1967.
30. Schmitz, B.; Klein, R. A. *Chem. Phys. Lipids* **1986**, *39*, 285–311.
31. Devijver, C.; Salmoun, M.; Braekman, J. C.; de Weerdt, W. H.; de Kluijver, M. J.; Gomez, R. *J. Nat. Prod.* **2000**, *63*, 978–980.
32. Tomer, K. B.; Crow, F. W.; Gross, M. L. *J. Am. Chem. Soc.* **1983**, *105*, 5478–5488.
33. Watanabe, K.; Tsuda, Y.; Yamane, Y.; Takashi, H.; Iguchi, K.; Naoki, H.; Fujita, T.; Van Soest, R. W. M. *Tetrahedron Lett.* **2000**, *41*, 9271–9276.
34. Kobayahsi's spectra of pyrinodemin A can be found in the supporting information of Ref. 6.
35. A typographical error appears in Ref. 5 where ion fragments *m/z* 190 and 217 were reported as $(C_{14}H_{22}N)^+$ and $(C_{16}H_{25}N)^+$, respectively. We presume that Kobaysahi et al. attempted to assign *m/z* 190 and 217 as $(C_{13}H_{20}N)^+$ and $(C_{15}H_{23}N)^+$, respectively, that is, cleavage on either side of the olefin as depicted in Figure 4.
36. Snider, B. B. *Chem. Eng. News* **2001**, *79*, 6.
37. (a) Goundry, W. R. F.; Lee, V.; Baldwin, J. E. *Tetrahedron Lett.* **2002**, *43*, 2745–2747. (b) Goundry, W. R. F.; Baldwin, J. E.; Lee, V. *Tetrahedron* **2003**, *59*, 1719–1729. (c) Romeril, S. P.; Lee, V.; Baldwin, J. E. *Tetrahedron Lett.* **2004**, *45*, 3273–3277.
38. Romeril, S. P.; Lee, V.; Baldwin, J. E.; Claridge, T. D. W.; Odell, B. *Tetrahedron Lett.* **2003**, *44*, 7757–7761.
39. For the assignment of ^1H and ^{13}C signals of *E* and *Z* oximes, see Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. Spectral Data for Structure Determination of Organic Compounds, 2nd ed.; Springer-Verlad: Berlin, 1981; p C195 and H175.

[§] This data point can be resolved into two signals at 29.09 and 29.13 ppm.
[¶] This data point can be resolved into two signals at 29.57 and 29.60 ppm.